

(SEQ ID NO: 6871 ) Cys Glu Tyr Cys His Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 6872 ) Cys Glu Tyr Cys His Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 6873 ) Cys Glu Tyr Cys His Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 6874 ) Cys Glu Tyr Cys His Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 6875 ) Cys Glu Tyr Cys His Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 6876 ) Cys Glu Tyr Cys Ile Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 6877 ) Cys Glu Tyr Cys Ile Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 6878 ) Cys Glu Tyr Cys Ile Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 6879 ) Cys Glu Tyr Cys Ile Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 6880 ) Cys Glu Tyr Cys Ile Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 6881 ) Cys Glu Tyr Cys Ile Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 6882 ) Cys Glu Tyr Cys Ile Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 6883 ) Cys Glu Tyr Cys Ile Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 6884 ) Cys Glu Tyr Cys Ile Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 6885 ) Cys Glu Tyr Cys Ile Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 6886 ) Cys Glu Tyr Cys Ile Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 6887 ) Cys Glu Tyr Cys Ile Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 6888 ) Cys Glu Tyr Cys Ile Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 6889 ) Cys Glu Tyr Cys Ile Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 6890 ) Cys Glu Tyr Cys Ile Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 6891 ) Cys Glu Tyr Cys Ile Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 6892 ) Cys Glu Tyr Cys Ile Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 6893 ) Cys Glu Tyr Cys Ile Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 6894 ) Cys Glu Tyr Cys Ile Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 6895 ) Cys Glu Tyr Cys Ile Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 6896 ) Cys Glu Tyr Cys Ile Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 6897 ) Cys Glu Tyr Cys Ile Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 6898 ) Cys Glu Tyr Cys Ile Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 6899 ) Cys Glu Tyr Cys Ile Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 6900 ) Cys Glu Tyr Cys Leu Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 6901 ) Cys Glu Tyr Cys Leu Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 6902 ) Cys Glu Tyr Cys Leu Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 6903 ) Cys Glu Tyr Cys Leu Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 6904 ) Cys Glu Tyr Cys Leu Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 6905 ) Cys Glu Tyr Cys Leu Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 6906 ) Cys Glu Tyr Cys Leu Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 6907 ) Cys Glu Tyr Cys Leu Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 6908 ) Cys Glu Tyr Cys Leu Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 6909 ) Cys Glu Tyr Cys Leu Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 6910 ) Cys Glu Tyr Cys Leu Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 6911 ) Cys Glu Tyr Cys Leu Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 6912 ) Cys Glu Tyr Cys Leu Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 6913 ) Cys Glu Tyr Cys Leu Asn Gly Ala Cys Thr Ala Cys

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(SEQ ID NO: 6914 ) Cys Glu Tyr Cys Leu Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 6915 ) Cys Glu Tyr Cys Leu Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 6916 ) Cys Glu Tyr Cys Leu Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 6917 ) Cys Glu Tyr Cys Leu Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 6918 ) Cys Glu Tyr Cys Leu Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 6919 ) Cys Glu Tyr Cys Leu Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 6920 ) Cys Glu Tyr Cys Leu Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 6921 ) Cys Glu Tyr Cys Leu Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 6922 ) Cys Glu Tyr Cys Leu Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 6923 ) Cys Glu Tyr Cys Leu Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 6924 ) Cys Glu Tyr Cys Lys Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 6925 ) Cys Glu Tyr Cys Lys Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 6926 ) Cys Glu Tyr Cys Lys Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 6927 ) Cys Glu Tyr Cys Lys Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 6928 ) Cys Glu Tyr Cys Lys Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 6929 ) Cys Glu Tyr Cys Lys Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 6930 ) Cys Glu Tyr Cys Lys Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 6931 ) Cys Glu Tyr Cys Lys Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 6932 ) Cys Glu Tyr Cys Lys Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 6933 ) Cys Glu Tyr Cys Lys Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 6934 ) Cys Glu Tyr Cys Lys Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 6935 ) Cys Glu Tyr Cys Lys Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 6936 ) Cys Glu Tyr Cys Lys Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 6937 ) Cys Glu Tyr Cys Lys Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 6938 ) Cys Glu Tyr Cys Lys Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 6939 ) Cys Glu Tyr Cys Lys Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 6940 ) Cys Glu Tyr Cys Lys Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 6941 ) Cys Glu Tyr Cys Lys Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 6942 ) Cys Glu Tyr Cys Lys Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 6943 ) Cys Glu Tyr Cys Lys Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 6944 ) Cys Glu Tyr Cys Lys Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 6945 ) Cys Glu Tyr Cys Lys Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 6946 ) Cys Glu Tyr Cys Lys Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 6947 ) Cys Glu Tyr Cys Lys Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 6948 ) Cys Glu Tyr Cys Met Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 6949 ) Cys Glu Tyr Cys Met Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 6950 ) Cys Glu Tyr Cys Met Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 6951 ) Cys Glu Tyr Cys Met Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 6952 ) Cys Glu Tyr Cys Met Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 6953 ) Cys Glu Tyr Cys Met Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 6954 ) Cys Glu Tyr Cys Met Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 6955 ) Cys Glu Tyr Cys Met Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 6956 ) Cys Glu Tyr Cys Met Asn Pro Thr Cys Val Gly Cys

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(SEQ ID NO: 6957 ) Cys Glu Tyr Cys Met Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 6958 ) Cys Glu Tyr Cys Met Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 6959 ) Cys Glu Tyr Cys Met Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 6960 ) Cys Glu Tyr Cys Met Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 6961 ) Cys Glu Tyr Cys Met Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 6962 ) Cys Glu Tyr Cys Met Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 6963 ) Cys Glu Tyr Cys Met Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 6964 ) Cys Glu Tyr Cys Met Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 6965 ) Cys Glu Tyr Cys Met Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 6966 ) Cys Glu Tyr Cys Met Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 6967 ) Cys Glu Tyr Cys Met Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 6968 ) Cys Glu Tyr Cys Met Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 6969 ) Cys Glu Tyr Cys Met Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 6970 ) Cys Glu Tyr Cys Met Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 6971 ) Cys Glu Tyr Cys Met Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 6972 ) Cys Glu Tyr Cys Phe Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 6973 ) Cys Glu Tyr Cys Phe Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 6974 ) Cys Glu Tyr Cys Phe Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 6975 ) Cys Glu Tyr Cys Phe Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 6976 ) Cys Glu Tyr Cys Phe Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 6977 ) Cys Glu Tyr Cys Phe Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 6978 ) Cys Glu Tyr Cys Phe Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 6979 ) Cys Glu Tyr Cys Phe Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 6980 ) Cys Glu Tyr Cys Phe Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 6981 ) Cys Glu Tyr Cys Phe Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 6982 ) Cys Glu Tyr Cys Phe Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 6983 ) Cys Glu Tyr Cys Phe Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 6984 ) Cys Glu Tyr Cys Phe Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 6985 ) Cys Glu Tyr Cys Phe Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 6986 ) Cys Glu Tyr Cys Phe Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 6987 ) Cys Glu Tyr Cys Phe Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 6988 ) Cys Glu Tyr Cys Phe Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 6989 ) Cys Glu Tyr Cys Phe Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 6990 ) Cys Glu Tyr Cys Phe Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 6991 ) Cys Glu Tyr Cys Phe Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 6992 ) Cys Glu Tyr Cys Phe Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 6993 ) Cys Glu Tyr Cys Phe Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 6994 ) Cys Glu Tyr Cys Phe Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 6995 ) Cys Glu Tyr Cys Phe Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 6996 ) Cys Glu Tyr Cys Pro Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 6997 ) Cys Glu Tyr Cys Pro Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 6998 ) Cys Glu Tyr Cys Pro Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 6999 ) Cys Glu Tyr Cys Pro Asn Pro Ala Cys Val Ala Cys

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(SEQ ID NO: 7000 ) Cys Glu Tyr Cys Pro Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7001 ) Cys Glu Tyr Cys Pro Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7002 ) Cys Glu Tyr Cys Pro Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7003 ) Cys Glu Tyr Cys Pro Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7004 ) Cys Glu Tyr Cys Pro Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7005 ) Cys Glu Tyr Cys Pro Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7006 ) Cys Glu Tyr Cys Pro Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7007 ) Cys Glu Tyr Cys Pro Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7008 ) Cys Glu Tyr Cys Pro Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7009 ) Cys Glu Tyr Cys Pro Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7010 ) Cys Glu Tyr Cys Pro Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7011 ) Cys Glu Tyr Cys Pro Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7012 ) Cys Glu Tyr Cys Pro Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7013 ) Cys Glu Tyr Cys Pro Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7014 ) Cys Glu Tyr Cys Pro Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7015 ) Cys Glu Tyr Cys Pro Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7016 ) Cys Glu Tyr Cys Pro Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7017 ) Cys Glu Tyr Cys Pro Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7018 ) Cys Glu Tyr Cys Pro Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7019 ) Cys Glu Tyr Cys Pro Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7020 ) Cys Glu Tyr Cys Ser Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7021 ) Cys Glu Tyr Cys Ser Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7022 ) Cys Glu Tyr Cys Ser Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7023 ) Cys Glu Tyr Cys Ser Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7024 ) Cys Glu Tyr Cys Ser Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7025 ) Cys Glu Tyr Cys Ser Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7026 ) Cys Glu Tyr Cys Ser Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7027 ) Cys Glu Tyr Cys Ser Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7028 ) Cys Glu Tyr Cys Ser Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7029 ) Cys Glu Tyr Cys Ser Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7030 ) Cys Glu Tyr Cys Ser Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7031 ) Cys Glu Tyr Cys Ser Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7032 ) Cys Glu Tyr Cys Ser Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7033 ) Cys Glu Tyr Cys Ser Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7034 ) Cys Glu Tyr Cys Ser Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7035 ) Cys Glu Tyr Cys Ser Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7036 ) Cys Glu Tyr Cys Ser Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7037 ) Cys Glu Tyr Cys Ser Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7038 ) Cys Glu Tyr Cys Ser Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7039 ) Cys Glu Tyr Cys Ser Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7040 ) Cys Glu Tyr Cys Ser Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7041 ) Cys Glu Tyr Cys Ser Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7042 ) Cys Glu Tyr Cys Ser Asn Gly Thr Cys Gly Gly Cys

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(SEQ ID NO: 7043 ) Cys Glu Tyr Cys Ser Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7044 ) Cys Glu Tyr Cys Thr Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7045 ) Cys Glu Tyr Cys Thr Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7046 ) Cys Glu Tyr Cys Thr Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 7047 ) Cys Glu Tyr Cys Thr Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 7048 ) Cys Glu Tyr Cys Thr Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7049 ) Cys Glu Tyr Cys Thr Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7050 ) Cys Glu Tyr Cys Thr Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7051 ) Cys Glu Tyr Cys Thr Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7052 ) Cys Glu Tyr Cys Thr Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 7053 ) Cys Glu Tyr Cys Thr Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 7054 ) Cys Glu Tyr Cys Thr Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7055 ) Cys Glu Tyr Cys Thr Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7056 ) Cys Glu Tyr Cys Thr Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7057 ) Cys Glu Tyr Cys Thr Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7058 ) Cys Glu Tyr Cys Thr Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 7059 ) Cys Glu Tyr Cys Thr Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 7060 ) Cys Glu Tyr Cys Thr Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7061 ) Cys Glu Tyr Cys Thr Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7062 ) Cys Glu Tyr Cys Thr Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7063 ) Cys Glu Tyr Cys Thr Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7064 ) Cys Glu Tyr Cys Thr Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 7065 ) Cys Glu Tyr Cys Thr Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 7066 ) Cys Glu Tyr Cys Thr Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7067 ) Cys Glu Tyr Cys Thr Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7068 ) Cys Glu Tyr Cys Trp Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7069 ) Cys Glu Tyr Cys Trp Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7070 ) Cys Glu Tyr Cys Trp Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 7071 ) Cys Glu Tyr Cys Trp Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 7072 ) Cys Glu Tyr Cys Trp Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7073 ) Cys Glu Tyr Cys Trp Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7074 ) Cys Glu Tyr Cys Trp Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7075 ) Cys Glu Tyr Cys Trp Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7076 ) Cys Glu Tyr Cys Trp Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 7077 ) Cys Glu Tyr Cys Trp Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 7078 ) Cys Glu Tyr Cys Trp Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7079 ) Cys Glu Tyr Cys Trp Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7080 ) Cys Glu Tyr Cys Trp Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7081 ) Cys Glu Tyr Cys Trp Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7082 ) Cys Glu Tyr Cys Trp Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 7083 ) Cys Glu Tyr Cys Trp Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 7084 ) Cys Glu Tyr Cys Trp Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7085 ) Cys Glu Tyr Cys Trp Asn Gly Ala Cys Gly Ala Cys

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(SEQ ID NO: 7086 ) Cys Glu Tyr Cys Trp Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7087 ) Cys Glu Tyr Cys Trp Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7088 ) Cys Glu Tyr Cys Trp Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 7089 ) Cys Glu Tyr Cys Trp Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 7090 ) Cys Glu Tyr Cys Trp Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7091 ) Cys Glu Tyr Cys Trp Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7092 ) Cys Glu Tyr Cys Tyr Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7093 ) Cys Glu Tyr Cys Tyr Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7094 ) Cys Glu Tyr Cys Tyr Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 7095 ) Cys Glu Tyr Cys Tyr Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 7096 ) Cys Glu Tyr Cys Tyr Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7097 ) Cys Glu Tyr Cys Tyr Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7098 ) Cys Glu Tyr Cys Tyr Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7099 ) Cys Glu Tyr Cys Tyr Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7100 ) Cys Glu Tyr Cys Tyr Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 7101 ) Cys Glu Tyr Cys Tyr Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 7102 ) Cys Glu Tyr Cys Tyr Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7103 ) Cys Glu Tyr Cys Tyr Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7104 ) Cys Glu Tyr Cys Tyr Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7105 ) Cys Glu Tyr Cys Tyr Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7106 ) Cys Glu Tyr Cys Tyr Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 7107 ) Cys Glu Tyr Cys Tyr Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 7108 ) Cys Glu Tyr Cys Tyr Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7109 ) Cys Glu Tyr Cys Tyr Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7110 ) Cys Glu Tyr Cys Tyr Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7111 ) Cys Glu Tyr Cys Tyr Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7112 ) Cys Glu Tyr Cys Tyr Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 7113 ) Cys Glu Tyr Cys Tyr Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 7114 ) Cys Glu Tyr Cys Tyr Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7115 ) Cys Glu Tyr Cys Tyr Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7116 ) Cys Glu Tyr Cys Val Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7117 ) Cys Glu Tyr Cys Val Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7118 ) Cys Glu Tyr Cys Val Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 7119 ) Cys Glu Tyr Cys Val Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 7120 ) Cys Glu Tyr Cys Val Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7121 ) Cys Glu Tyr Cys Val Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7122 ) Cys Glu Tyr Cys Val Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7123 ) Cys Glu Tyr Cys Val Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7124 ) Cys Glu Tyr Cys Val Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 7125 ) Cys Glu Tyr Cys Val Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 7126 ) Cys Glu Tyr Cys Val Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7127 ) Cys Glu Tyr Cys Val Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7128 ) Cys Glu Tyr Cys Val Asn Gly Ala Cys Thr Gly Cys

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(SEQ ID NO: 7129 ) Cys Glu Tyr Cys Val Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7130 ) Cys Glu Tyr Cys Val Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 7131 ) Cys Glu Tyr Cys Val Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 7132 ) Cys Glu Tyr Cys Val Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7133 ) Cys Glu Tyr Cys Val Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7134 ) Cys Glu Tyr Cys Val Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7135 ) Cys Glu Tyr Cys Val Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7136 ) Cys Glu Tyr Cys Val Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 7137 ) Cys Glu Tyr Cys Val Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 7138 ) Cys Glu Tyr Cys Val Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7139 ) Cys Glu Tyr Cys Val Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7140 ) Cys Glu Tyr Cys --- Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7141 ) Cys Glu Tyr Cys --- Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7142 ) Cys Glu Tyr Cys --- Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 7143 ) Cys Glu Tyr Cys --- Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 7144 ) Cys Glu Tyr Cys --- Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7145 ) Cys Glu Tyr Cys --- Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7146 ) Cys Glu Tyr Cys --- Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7147 ) Cys Glu Tyr Cys --- Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7148 ) Cys Glu Tyr Cys --- Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 7149 ) Cys Glu Tyr Cys --- Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 7150 ) Cys Glu Tyr Cys --- Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7151 ) Cys Glu Tyr Cys --- Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7152 ) Cys Glu Tyr Cys --- Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7153 ) Cys Glu Tyr Cys --- Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7154 ) Cys Glu Tyr Cys --- Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 7155 ) Cys Glu Tyr Cys --- Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 7156 ) Cys Glu Tyr Cys --- Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7157 ) Cys Glu Tyr Cys --- Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7158 ) Cys Glu Tyr Cys --- Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7159 ) Cys Glu Tyr Cys --- Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7160 ) Cys Glu Tyr Cys --- Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 7161 ) Cys Glu Tyr Cys --- Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 7162 ) Cys Glu Tyr Cys --- Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7163 ) Cys Glu Tyr Cys --- Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7164 ) Cys Glu Trp Cys Ala Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7165 ) Cys Glu Trp Cys Ala Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7166 ) Cys Glu Trp Cys Ala Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 7167 ) Cys Glu Trp Cys Ala Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 7168 ) Cys Glu Trp Cys Ala Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7169 ) Cys Glu Trp Cys Ala Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7170 ) Cys Glu Trp Cys Ala Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7171 ) Cys Glu Trp Cys Ala Asn Pro Thr Cys Thr Ala Cys

FIG. 3  
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(SEQ ID NO: 7172 ) Cys Glu Trp Cys Ala Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7173 ) Cys Glu Trp Cys Ala Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7174 ) Cys Glu Trp Cys Ala Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7175 ) Cys Glu Trp Cys Ala Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7176 ) Cys Glu Trp Cys Ala Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7177 ) Cys Glu Trp Cys Ala Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7178 ) Cys Glu Trp Cys Ala Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7179 ) Cys Glu Trp Cys Ala Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7180 ) Cys Glu Trp Cys Ala Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7181 ) Cys Glu Trp Cys Ala Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7182 ) Cys Glu Trp Cys Ala Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7183 ) Cys Glu Trp Cys Ala Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7184 ) Cys Glu Trp Cys Ala Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7185 ) Cys Glu Trp Cys Ala Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7186 ) Cys Glu Trp Cys Ala Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7187 ) Cys Glu Trp Cys Ala Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7188 ) Cys Glu Trp Cys Arg Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7189 ) Cys Glu Trp Cys Arg Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7190 ) Cys Glu Trp Cys Arg Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7191 ) Cys Glu Trp Cys Arg Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7192 ) Cys Glu Trp Cys Arg Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7193 ) Cys Glu Trp Cys Arg Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7194 ) Cys Glu Trp Cys Arg Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7195 ) Cys Glu Trp Cys Arg Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7196 ) Cys Glu Trp Cys Arg Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7197 ) Cys Glu Trp Cys Arg Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7198 ) Cys Glu Trp Cys Arg Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7199 ) Cys Glu Trp Cys Arg Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7200 ) Cys Glu Trp Cys Arg Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7201 ) Cys Glu Trp Cys Arg Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7202 ) Cys Glu Trp Cys Arg Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7203 ) Cys Glu Trp Cys Arg Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7204 ) Cys Glu Trp Cys Arg Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7205 ) Cys Glu Trp Cys Arg Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7206 ) Cys Glu Trp Cys Arg Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7207 ) Cys Glu Trp Cys Arg Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7208 ) Cys Glu Trp Cys Arg Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7209 ) Cys Glu Trp Cys Arg Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7210 ) Cys Glu Trp Cys Arg Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7211 ) Cys Glu Trp Cys Arg Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7212 ) Cys Glu Trp Cys Asn Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7213 ) Cys Glu Trp Cys Asn Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7214 ) Cys Glu Trp Cys Asn Asn Pro Ala Cys Val Gly Cys

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(SEQ ID NO: 7215 ) Cys Glu Trp Cys Asn Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7216 ) Cys Glu Trp Cys Asn Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7217 ) Cys Glu Trp Cys Asn Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7218 ) Cys Glu Trp Cys Asn Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7219 ) Cys Glu Trp Cys Asn Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7220 ) Cys Glu Trp Cys Asn Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7221 ) Cys Glu Trp Cys Asn Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7222 ) Cys Glu Trp Cys Asn Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7223 ) Cys Glu Trp Cys Asn Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7224 ) Cys Glu Trp Cys Asn Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7225 ) Cys Glu Trp Cys Asn Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7226 ) Cys Glu Trp Cys Asn Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7227 ) Cys Glu Trp Cys Asn Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7228 ) Cys Glu Trp Cys Asn Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7229 ) Cys Glu Trp Cys Asn Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7230 ) Cys Glu Trp Cys Asn Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7231 ) Cys Glu Trp Cys Asn Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7232 ) Cys Glu Trp Cys Asn Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7233 ) Cys Glu Trp Cys Asn Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7234 ) Cys Glu Trp Cys Asn Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7235 ) Cys Glu Trp Cys Asn Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7236 ) Cys Glu Trp Cys Asp Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7237 ) Cys Glu Trp Cys Asp Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7238 ) Cys Glu Trp Cys Asp Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7239 ) Cys Glu Trp Cys Asp Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7240 ) Cys Glu Trp Cys Asp Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7241 ) Cys Glu Trp Cys Asp Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7242 ) Cys Glu Trp Cys Asp Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7243 ) Cys Glu Trp Cys Asp Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7244 ) Cys Glu Trp Cys Asp Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7245 ) Cys Glu Trp Cys Asp Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7246 ) Cys Glu Trp Cys Asp Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7247 ) Cys Glu Trp Cys Asp Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7248 ) Cys Glu Trp Cys Asp Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7249 ) Cys Glu Trp Cys Asp Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7250 ) Cys Glu Trp Cys Asp Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7251 ) Cys Glu Trp Cys Asp Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7252 ) Cys Glu Trp Cys Asp Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7253 ) Cys Glu Trp Cys Asp Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7254 ) Cys Glu Trp Cys Asp Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7255 ) Cys Glu Trp Cys Asp Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7256 ) Cys Glu Trp Cys Asp Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7257 ) Cys Glu Trp Cys Asp Asn Gly Thr Cys Val Ala Cys

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(SEQ ID NO: 7258 ) Cys Glu Trp Cys Asp Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7259 ) Cys Glu Trp Cys Asp Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7260 ) Cys Glu Trp Cys Gln Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7261 ) Cys Glu Trp Cys Gln Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7262 ) Cys Glu Trp Cys Gln Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 7263 ) Cys Glu Trp Cys Gln Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 7264 ) Cys Glu Trp Cys Gln Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7265 ) Cys Glu Trp Cys Gln Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7266 ) Cys Glu Trp Cys Gln Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7267 ) Cys Glu Trp Cys Gln Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7268 ) Cys Glu Trp Cys Gln Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 7269 ) Cys Glu Trp Cys Gln Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 7270 ) Cys Glu Trp Cys Gln Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7271 ) Cys Glu Trp Cys Gln Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7272 ) Cys Glu Trp Cys Gln Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7273 ) Cys Glu Trp Cys Gln Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7274 ) Cys Glu Trp Cys Gln Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 7275 ) Cys Glu Trp Cys Gln Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 7276 ) Cys Glu Trp Cys Gln Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7277 ) Cys Glu Trp Cys Gln Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7278 ) Cys Glu Trp Cys Gln Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7279 ) Cys Glu Trp Cys Gln Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7280 ) Cys Glu Trp Cys Gln Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 7281 ) Cys Glu Trp Cys Gln Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 7282 ) Cys Glu Trp Cys Gln Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7283 ) Cys Glu Trp Cys Gln Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7284 ) Cys Glu Trp Cys Glu Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7285 ) Cys Glu Trp Cys Glu Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7286 ) Cys Glu Trp Cys Glu Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 7287 ) Cys Glu Trp Cys Glu Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 7288 ) Cys Glu Trp Cys Glu Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7289 ) Cys Glu Trp Cys Glu Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7290 ) Cys Glu Trp Cys Glu Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7291 ) Cys Glu Trp Cys Glu Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7292 ) Cys Glu Trp Cys Glu Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 7293 ) Cys Glu Trp Cys Glu Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 7294 ) Cys Glu Trp Cys Glu Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7295 ) Cys Glu Trp Cys Glu Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7296 ) Cys Glu Trp Cys Glu Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7297 ) Cys Glu Trp Cys Glu Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7298 ) Cys Glu Trp Cys Glu Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 7299 ) Cys Glu Trp Cys Glu Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 7300 ) Cys Glu Trp Cys Glu Asn Gly Ala Cys Gly Gly Cys

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(SEQ ID NO: 7301 ) Cys Glu Trp Cys Glu Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7302 ) Cys Glu Trp Cys Glu Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7303 ) Cys Glu Trp Cys Glu Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7304 ) Cys Glu Trp Cys Glu Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 7305 ) Cys Glu Trp Cys Glu Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 7306 ) Cys Glu Trp Cys Glu Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7307 ) Cys Glu Trp Cys Glu Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7308 ) Cys Glu Trp Cys Gly Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7309 ) Cys Glu Trp Cys Gly Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7310 ) Cys Glu Trp Cys Gly Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 7311 ) Cys Glu Trp Cys Gly Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 7312 ) Cys Glu Trp Cys Gly Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7313 ) Cys Glu Trp Cys Gly Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7314 ) Cys Glu Trp Cys Gly Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7315 ) Cys Glu Trp Cys Gly Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7316 ) Cys Glu Trp Cys Gly Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 7317 ) Cys Glu Trp Cys Gly Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 7318 ) Cys Glu Trp Cys Gly Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7319 ) Cys Glu Trp Cys Gly Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7320 ) Cys Glu Trp Cys Gly Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7321 ) Cys Glu Trp Cys Gly Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7322 ) Cys Glu Trp Cys Gly Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 7323 ) Cys Glu Trp Cys Gly Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 7324 ) Cys Glu Trp Cys Gly Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7325 ) Cys Glu Trp Cys Gly Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7326 ) Cys Glu Trp Cys Gly Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7327 ) Cys Glu Trp Cys Gly Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7328 ) Cys Glu Trp Cys Gly Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 7329 ) Cys Glu Trp Cys Gly Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 7330 ) Cys Glu Trp Cys Gly Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7331 ) Cys Glu Trp Cys Gly Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7332 ) Cys Glu Trp Cys His Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7333 ) Cys Glu Trp Cys His Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7334 ) Cys Glu Trp Cys His Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 7335 ) Cys Glu Trp Cys His Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 7336 ) Cys Glu Trp Cys His Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7337 ) Cys Glu Trp Cys His Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7338 ) Cys Glu Trp Cys His Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7339 ) Cys Glu Trp Cys His Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7340 ) Cys Glu Trp Cys His Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 7341 ) Cys Glu Trp Cys His Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 7342 ) Cys Glu Trp Cys His Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7343 ) Cys Glu Trp Cys His Asn Pro Thr Cys Gly Ala Cys

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(SEQ ID NO: 7344 ) Cys Glu Trp Cys His Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7345 ) Cys Glu Trp Cys His Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7346 ) Cys Glu Trp Cys His Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7347 ) Cys Glu Trp Cys His Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7348 ) Cys Glu Trp Cys His Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7349 ) Cys Glu Trp Cys His Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7350 ) Cys Glu Trp Cys His Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7351 ) Cys Glu Trp Cys His Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7352 ) Cys Glu Trp Cys His Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7353 ) Cys Glu Trp Cys His Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7354 ) Cys Glu Trp Cys His Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7355 ) Cys Glu Trp Cys His Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7356 ) Cys Glu Trp Cys Ile Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7357 ) Cys Glu Trp Cys Ile Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7358 ) Cys Glu Trp Cys Ile Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7359 ) Cys Glu Trp Cys Ile Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7360 ) Cys Glu Trp Cys Ile Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7361 ) Cys Glu Trp Cys Ile Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7362 ) Cys Glu Trp Cys Ile Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7363 ) Cys Glu Trp Cys Ile Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7364 ) Cys Glu Trp Cys Ile Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7365 ) Cys Glu Trp Cys Ile Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7366 ) Cys Glu Trp Cys Ile Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7367 ) Cys Glu Trp Cys Ile Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7368 ) Cys Glu Trp Cys Ile Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7369 ) Cys Glu Trp Cys Ile Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7370 ) Cys Glu Trp Cys Ile Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7371 ) Cys Glu Trp Cys Ile Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7372 ) Cys Glu Trp Cys Ile Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7373 ) Cys Glu Trp Cys Ile Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7374 ) Cys Glu Trp Cys Ile Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7375 ) Cys Glu Trp Cys Ile Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7376 ) Cys Glu Trp Cys Ile Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7377 ) Cys Glu Trp Cys Ile Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7378 ) Cys Glu Trp Cys Ile Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7379 ) Cys Glu Trp Cys Ile Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7380 ) Cys Glu Trp Cys Leu Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7381 ) Cys Glu Trp Cys Leu Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7382 ) Cys Glu Trp Cys Leu Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7383 ) Cys Glu Trp Cys Leu Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7384 ) Cys Glu Trp Cys Leu Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7385 ) Cys Glu Trp Cys Leu Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7386 ) Cys Glu Trp Cys Leu Asn Pro Thr Cys Thr Gly Cys

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(SEQ ID NO: 7387 ) Cys Glu Trp Cys Leu Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7388 ) Cys Glu Trp Cys Leu Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7389 ) Cys Glu Trp Cys Leu Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7390 ) Cys Glu Trp Cys Leu Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7391 ) Cys Glu Trp Cys Leu Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7392 ) Cys Glu Trp Cys Leu Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7393 ) Cys Glu Trp Cys Leu Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7394 ) Cys Glu Trp Cys Leu Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7395 ) Cys Glu Trp Cys Leu Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7396 ) Cys Glu Trp Cys Leu Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7397 ) Cys Glu Trp Cys Leu Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7398 ) Cys Glu Trp Cys Leu Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7399 ) Cys Glu Trp Cys Leu Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7400 ) Cys Glu Trp Cys Leu Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7401 ) Cys Glu Trp Cys Leu Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7402 ) Cys Glu Trp Cys Leu Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7403 ) Cys Glu Trp Cys Leu Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7404 ) Cys Glu Trp Cys Lys Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7405 ) Cys Glu Trp Cys Lys Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7406 ) Cys Glu Trp Cys Lys Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7407 ) Cys Glu Trp Cys Lys Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7408 ) Cys Glu Trp Cys Lys Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7409 ) Cys Glu Trp Cys Lys Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7410 ) Cys Glu Trp Cys Lys Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7411 ) Cys Glu Trp Cys Lys Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7412 ) Cys Glu Trp Cys Lys Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7413 ) Cys Glu Trp Cys Lys Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7414 ) Cys Glu Trp Cys Lys Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7415 ) Cys Glu Trp Cys Lys Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7416 ) Cys Glu Trp Cys Lys Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7417 ) Cys Glu Trp Cys Lys Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7418 ) Cys Glu Trp Cys Lys Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7419 ) Cys Glu Trp Cys Lys Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7420 ) Cys Glu Trp Cys Lys Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7421 ) Cys Glu Trp Cys Lys Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7422 ) Cys Glu Trp Cys Lys Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7423 ) Cys Glu Trp Cys Lys Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7424 ) Cys Glu Trp Cys Lys Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7425 ) Cys Glu Trp Cys Lys Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7426 ) Cys Glu Trp Cys Lys Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7427 ) Cys Glu Trp Cys Lys Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7428 ) Cys Glu Trp Cys Met Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7429 ) Cys Glu Trp Cys Met Asn Pro Ala Cys Thr Ala Cys

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(SEQ ID NO: 7430 ) Cys Glu Trp Cys Met Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7431 ) Cys Glu Trp Cys Met Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7432 ) Cys Glu Trp Cys Met Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7433 ) Cys Glu Trp Cys Met Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7434 ) Cys Glu Trp Cys Met Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7435 ) Cys Glu Trp Cys Met Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7436 ) Cys Glu Trp Cys Met Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7437 ) Cys Glu Trp Cys Met Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7438 ) Cys Glu Trp Cys Met Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7439 ) Cys Glu Trp Cys Met Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7440 ) Cys Glu Trp Cys Met Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7441 ) Cys Glu Trp Cys Met Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7442 ) Cys Glu Trp Cys Met Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7443 ) Cys Glu Trp Cys Met Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7444 ) Cys Glu Trp Cys Met Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7445 ) Cys Glu Trp Cys Met Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7446 ) Cys Glu Trp Cys Met Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7447 ) Cys Glu Trp Cys Met Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7448 ) Cys Glu Trp Cys Met Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7449 ) Cys Glu Trp Cys Met Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7450 ) Cys Glu Trp Cys Met Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7451 ) Cys Glu Trp Cys Met Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7452 ) Cys Glu Trp Cys Phe Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7453 ) Cys Glu Trp Cys Phe Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7454 ) Cys Glu Trp Cys Phe Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7455 ) Cys Glu Trp Cys Phe Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7456 ) Cys Glu Trp Cys Phe Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7457 ) Cys Glu Trp Cys Phe Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7458 ) Cys Glu Trp Cys Phe Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7459 ) Cys Glu Trp Cys Phe Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7460 ) Cys Glu Trp Cys Phe Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7461 ) Cys Glu Trp Cys Phe Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7462 ) Cys Glu Trp Cys Phe Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7463 ) Cys Glu Trp Cys Phe Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7464 ) Cys Glu Trp Cys Phe Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7465 ) Cys Glu Trp Cys Phe Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7466 ) Cys Glu Trp Cys Phe Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7467 ) Cys Glu Trp Cys Phe Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7468 ) Cys Glu Trp Cys Phe Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7469 ) Cys Glu Trp Cys Phe Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7470 ) Cys Glu Trp Cys Phe Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7471 ) Cys Glu Trp Cys Phe Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7472 ) Cys Glu Trp Cys Phe Asn Gly Thr Cys Val Gly Cys

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(SEQ ID NO: 7473 ) Cys Glu Trp Cys Phe Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7474 ) Cys Glu Trp Cys Phe Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7475 ) Cys Glu Trp Cys Phe Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7476 ) Cys Glu Trp Cys Pro Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7477 ) Cys Glu Trp Cys Pro Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7478 ) Cys Glu Trp Cys Pro Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7479 ) Cys Glu Trp Cys Pro Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7480 ) Cys Glu Trp Cys Pro Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7481 ) Cys Glu Trp Cys Pro Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7482 ) Cys Glu Trp Cys Pro Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7483 ) Cys Glu Trp Cys Pro Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7484 ) Cys Glu Trp Cys Pro Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7485 ) Cys Glu Trp Cys Pro Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7486 ) Cys Glu Trp Cys Pro Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7487 ) Cys Glu Trp Cys Pro Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7488 ) Cys Glu Trp Cys Pro Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7489 ) Cys Glu Trp Cys Pro Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7490 ) Cys Glu Trp Cys Pro Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7491 ) Cys Glu Trp Cys Pro Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7492 ) Cys Glu Trp Cys Pro Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7493 ) Cys Glu Trp Cys Pro Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7494 ) Cys Glu Trp Cys Pro Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7495 ) Cys Glu Trp Cys Pro Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7496 ) Cys Glu Trp Cys Pro Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7497 ) Cys Glu Trp Cys Pro Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7498 ) Cys Glu Trp Cys Pro Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7499 ) Cys Glu Trp Cys Pro Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7500 ) Cys Glu Trp Cys Ser Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7501 ) Cys Glu Trp Cys Ser Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7502 ) Cys Glu Trp Cys Ser Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7503 ) Cys Glu Trp Cys Ser Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7504 ) Cys Glu Trp Cys Ser Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7505 ) Cys Glu Trp Cys Ser Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7506 ) Cys Glu Trp Cys Ser Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7507 ) Cys Glu Trp Cys Ser Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7508 ) Cys Glu Trp Cys Ser Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7509 ) Cys Glu Trp Cys Ser Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7510 ) Cys Glu Trp Cys Ser Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7511 ) Cys Glu Trp Cys Ser Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7512 ) Cys Glu Trp Cys Ser Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7513 ) Cys Glu Trp Cys Ser Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7514 ) Cys Glu Trp Cys Ser Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7515 ) Cys Glu Trp Cys Ser Asn Gly Ala Cys Val Ala Cys

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(SEQ ID NO: 7516 ) Cys Glu Trp Cys Ser Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7517 ) Cys Glu Trp Cys Ser Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7518 ) Cys Glu Trp Cys Ser Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7519 ) Cys Glu Trp Cys Ser Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7520 ) Cys Glu Trp Cys Ser Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7521 ) Cys Glu Trp Cys Ser Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7522 ) Cys Glu Trp Cys Ser Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7523 ) Cys Glu Trp Cys Ser Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7524 ) Cys Glu Trp Cys Thr Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7525 ) Cys Glu Trp Cys Thr Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7526 ) Cys Glu Trp Cys Thr Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7527 ) Cys Glu Trp Cys Thr Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7528 ) Cys Glu Trp Cys Thr Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7529 ) Cys Glu Trp Cys Thr Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7530 ) Cys Glu Trp Cys Thr Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7531 ) Cys Glu Trp Cys Thr Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7532 ) Cys Glu Trp Cys Thr Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7533 ) Cys Glu Trp Cys Thr Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7534 ) Cys Glu Trp Cys Thr Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7535 ) Cys Glu Trp Cys Thr Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7536 ) Cys Glu Trp Cys Thr Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7537 ) Cys Glu Trp Cys Thr Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7538 ) Cys Glu Trp Cys Thr Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7539 ) Cys Glu Trp Cys Thr Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7540 ) Cys Glu Trp Cys Thr Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7541 ) Cys Glu Trp Cys Thr Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7542 ) Cys Glu Trp Cys Thr Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7543 ) Cys Glu Trp Cys Thr Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7544 ) Cys Glu Trp Cys Thr Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7545 ) Cys Glu Trp Cys Thr Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7546 ) Cys Glu Trp Cys Thr Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7547 ) Cys Glu Trp Cys Thr Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7548 ) Cys Glu Trp Cys Trp Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7549 ) Cys Glu Trp Cys Trp Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7550 ) Cys Glu Trp Cys Trp Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7551 ) Cys Glu Trp Cys Trp Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7552 ) Cys Glu Trp Cys Trp Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7553 ) Cys Glu Trp Cys Trp Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7554 ) Cys Glu Trp Cys Trp Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7555 ) Cys Glu Trp Cys Trp Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7556 ) Cys Glu Trp Cys Trp Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7557 ) Cys Glu Trp Cys Trp Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7558 ) Cys Glu Trp Cys Trp Asn Pro Thr Cys Gly Gly Cys

FIG. 3  
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(SEQ ID NO: 7559 ) Cys Glu Trp Cys Trp Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7560 ) Cys Glu Trp Cys Trp Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7561 ) Cys Glu Trp Cys Trp Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7562 ) Cys Glu Trp Cys Trp Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 7563 ) Cys Glu Trp Cys Trp Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 7564 ) Cys Glu Trp Cys Trp Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7565 ) Cys Glu Trp Cys Trp Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7566 ) Cys Glu Trp Cys Trp Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7567 ) Cys Glu Trp Cys Trp Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7568 ) Cys Glu Trp Cys Trp Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 7569 ) Cys Glu Trp Cys Trp Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 7570 ) Cys Glu Trp Cys Trp Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7571 ) Cys Glu Trp Cys Trp Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7572 ) Cys Glu Trp Cys Tyr Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7573 ) Cys Glu Trp Cys Tyr Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7574 ) Cys Glu Trp Cys Tyr Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 7575 ) Cys Glu Trp Cys Tyr Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 7576 ) Cys Glu Trp Cys Tyr Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7577 ) Cys Glu Trp Cys Tyr Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7578 ) Cys Glu Trp Cys Tyr Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7579 ) Cys Glu Trp Cys Tyr Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7580 ) Cys Glu Trp Cys Tyr Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 7581 ) Cys Glu Trp Cys Tyr Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 7582 ) Cys Glu Trp Cys Tyr Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7583 ) Cys Glu Trp Cys Tyr Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7584 ) Cys Glu Trp Cys Tyr Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7585 ) Cys Glu Trp Cys Tyr Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7586 ) Cys Glu Trp Cys Tyr Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 7587 ) Cys Glu Trp Cys Tyr Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 7588 ) Cys Glu Trp Cys Tyr Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7589 ) Cys Glu Trp Cys Tyr Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7590 ) Cys Glu Trp Cys Tyr Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7591 ) Cys Glu Trp Cys Tyr Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7592 ) Cys Glu Trp Cys Tyr Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 7593 ) Cys Glu Trp Cys Tyr Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 7594 ) Cys Glu Trp Cys Tyr Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7595 ) Cys Glu Trp Cys Tyr Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7596 ) Cys Glu Trp Cys Val Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7597 ) Cys Glu Trp Cys Val Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7598 ) Cys Glu Trp Cys Val Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 7599 ) Cys Glu Trp Cys Val Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 7600 ) Cys Glu Trp Cys Val Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7601 ) Cys Glu Trp Cys Val Asn Pro Ala Cys Gly Ala Cys

**FIG. 3**  
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(SEQ ID NO: 7602 ) Cys Glu Trp Cys Val Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7603 ) Cys Glu Trp Cys Val Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7604 ) Cys Glu Trp Cys Val Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 7605 ) Cys Glu Trp Cys Val Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 7606 ) Cys Glu Trp Cys Val Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7607 ) Cys Glu Trp Cys Val Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7608 ) Cys Glu Trp Cys Val Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7609 ) Cys Glu Trp Cys Val Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7610 ) Cys Glu Trp Cys Val Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 7611 ) Cys Glu Trp Cys Val Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 7612 ) Cys Glu Trp Cys Val Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7613 ) Cys Glu Trp Cys Val Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7614 ) Cys Glu Trp Cys Val Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7615 ) Cys Glu Trp Cys Val Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7616 ) Cys Glu Trp Cys Val Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 7617 ) Cys Glu Trp Cys Val Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 7618 ) Cys Glu Trp Cys Val Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7619 ) Cys Glu Trp Cys Val Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7620 ) Cys Glu Trp Cys --- Asn Pro Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7621 ) Cys Glu Trp Cys --- Asn Pro Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7622 ) Cys Glu Trp Cys --- Asn Pro Ala Cys Val Gly Cys  
 (SEQ ID NO: 7623 ) Cys Glu Trp Cys --- Asn Pro Ala Cys Val Ala Cys  
 (SEQ ID NO: 7624 ) Cys Glu Trp Cys --- Asn Pro Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7625 ) Cys Glu Trp Cys --- Asn Pro Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7626 ) Cys Glu Trp Cys --- Asn Pro Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7627 ) Cys Glu Trp Cys --- Asn Pro Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7628 ) Cys Glu Trp Cys --- Asn Pro Thr Cys Val Gly Cys  
 (SEQ ID NO: 7629 ) Cys Glu Trp Cys --- Asn Pro Thr Cys Val Ala Cys  
 (SEQ ID NO: 7630 ) Cys Glu Trp Cys --- Asn Pro Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7631 ) Cys Glu Trp Cys --- Asn Pro Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7632 ) Cys Glu Trp Cys --- Asn Gly Ala Cys Thr Gly Cys  
 (SEQ ID NO: 7633 ) Cys Glu Trp Cys --- Asn Gly Ala Cys Thr Ala Cys  
 (SEQ ID NO: 7634 ) Cys Glu Trp Cys --- Asn Gly Ala Cys Val Gly Cys  
 (SEQ ID NO: 7635 ) Cys Glu Trp Cys --- Asn Gly Ala Cys Val Ala Cys  
 (SEQ ID NO: 7636 ) Cys Glu Trp Cys --- Asn Gly Ala Cys Gly Gly Cys  
 (SEQ ID NO: 7637 ) Cys Glu Trp Cys --- Asn Gly Ala Cys Gly Ala Cys  
 (SEQ ID NO: 7638 ) Cys Glu Trp Cys --- Asn Gly Thr Cys Thr Gly Cys  
 (SEQ ID NO: 7639 ) Cys Glu Trp Cys --- Asn Gly Thr Cys Thr Ala Cys  
 (SEQ ID NO: 7640 ) Cys Glu Trp Cys --- Asn Gly Thr Cys Val Gly Cys  
 (SEQ ID NO: 7641 ) Cys Glu Trp Cys --- Asn Gly Thr Cys Val Ala Cys  
 (SEQ ID NO: 7642 ) Cys Glu Trp Cys --- Asn Gly Thr Cys Gly Gly Cys  
 (SEQ ID NO: 7643 ) Cys Glu Trp Cys --- Asn Gly Thr Cys Gly Ala Cys  
 (SEQ ID NO: 7644 ) Cys Glu Phe Cys Ala Asn Pro Ala Cys Thr Gly Cys

FIG. 3  
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(SEQ ID NO: 7645 ) Cys Glu Phe Cys Ala Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7646 ) Cys Glu Phe Cys Ala Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7647 ) Cys Glu Phe Cys Ala Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7648 ) Cys Glu Phe Cys Ala Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7649 ) Cys Glu Phe Cys Ala Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7650 ) Cys Glu Phe Cys Ala Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7651 ) Cys Glu Phe Cys Ala Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7652 ) Cys Glu Phe Cys Ala Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7653 ) Cys Glu Phe Cys Ala Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7654 ) Cys Glu Phe Cys Ala Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7655 ) Cys Glu Phe Cys Ala Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7656 ) Cys Glu Phe Cys Ala Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7657 ) Cys Glu Phe Cys Ala Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7658 ) Cys Glu Phe Cys Ala Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7659 ) Cys Glu Phe Cys Ala Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7660 ) Cys Glu Phe Cys Ala Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7661 ) Cys Glu Phe Cys Ala Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7662 ) Cys Glu Phe Cys Ala Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7663 ) Cys Glu Phe Cys Ala Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7664 ) Cys Glu Phe Cys Ala Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7665 ) Cys Glu Phe Cys Ala Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7666 ) Cys Glu Phe Cys Ala Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7667 ) Cys Glu Phe Cys Ala Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7668 ) Cys Glu Phe Cys Arg Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7669 ) Cys Glu Phe Cys Arg Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7670 ) Cys Glu Phe Cys Arg Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7671 ) Cys Glu Phe Cys Arg Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7672 ) Cys Glu Phe Cys Arg Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7673 ) Cys Glu Phe Cys Arg Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7674 ) Cys Glu Phe Cys Arg Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7675 ) Cys Glu Phe Cys Arg Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7676 ) Cys Glu Phe Cys Arg Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7677 ) Cys Glu Phe Cys Arg Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7678 ) Cys Glu Phe Cys Arg Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7679 ) Cys Glu Phe Cys Arg Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7680 ) Cys Glu Phe Cys Arg Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7681 ) Cys Glu Phe Cys Arg Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7682 ) Cys Glu Phe Cys Arg Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7683 ) Cys Glu Phe Cys Arg Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7684 ) Cys Glu Phe Cys Arg Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7685 ) Cys Glu Phe Cys Arg Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7686 ) Cys Glu Phe Cys Arg Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7687 ) Cys Glu Phe Cys Arg Asn Gly Thr Cys Thr Ala Cys

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(SEQ ID NO: 7688 ) Cys Glu Phe Cys Arg Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7689 ) Cys Glu Phe Cys Arg Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7690 ) Cys Glu Phe Cys Arg Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7691 ) Cys Glu Phe Cys Arg Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7692 ) Cys Glu Phe Cys Asn Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7693 ) Cys Glu Phe Cys Asn Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7694 ) Cys Glu Phe Cys Asn Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7695 ) Cys Glu Phe Cys Asn Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7696 ) Cys Glu Phe Cys Asn Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7697 ) Cys Glu Phe Cys Asn Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7698 ) Cys Glu Phe Cys Asn Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7699 ) Cys Glu Phe Cys Asn Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7700 ) Cys Glu Phe Cys Asn Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7701 ) Cys Glu Phe Cys Asn Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7702 ) Cys Glu Phe Cys Asn Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7703 ) Cys Glu Phe Cys Asn Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7704 ) Cys Glu Phe Cys Asn Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7705 ) Cys Glu Phe Cys Asn Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7706 ) Cys Glu Phe Cys Asn Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7707 ) Cys Glu Phe Cys Asn Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7708 ) Cys Glu Phe Cys Asn Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7709 ) Cys Glu Phe Cys Asn Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7710 ) Cys Glu Phe Cys Asn Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7711 ) Cys Glu Phe Cys Asn Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7712 ) Cys Glu Phe Cys Asn Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7713 ) Cys Glu Phe Cys Asn Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7714 ) Cys Glu Phe Cys Asn Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7715 ) Cys Glu Phe Cys Asn Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7716 ) Cys Glu Phe Cys Asp Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7717 ) Cys Glu Phe Cys Asp Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7718 ) Cys Glu Phe Cys Asp Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7719 ) Cys Glu Phe Cys Asp Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7720 ) Cys Glu Phe Cys Asp Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7721 ) Cys Glu Phe Cys Asp Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7722 ) Cys Glu Phe Cys Asp Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7723 ) Cys Glu Phe Cys Asp Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7724 ) Cys Glu Phe Cys Asp Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7725 ) Cys Glu Phe Cys Asp Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7726 ) Cys Glu Phe Cys Asp Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7727 ) Cys Glu Phe Cys Asp Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7728 ) Cys Glu Phe Cys Asp Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7729 ) Cys Glu Phe Cys Asp Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7730 ) Cys Glu Phe Cys Asp Asn Gly Ala Cys Val Gly Cys

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(SEQ ID NO: 7731 ) Cys Glu Phe Cys Asp Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7732 ) Cys Glu Phe Cys Asp Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7733 ) Cys Glu Phe Cys Asp Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7734 ) Cys Glu Phe Cys Asp Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7735 ) Cys Glu Phe Cys Asp Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7736 ) Cys Glu Phe Cys Asp Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7737 ) Cys Glu Phe Cys Asp Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7738 ) Cys Glu Phe Cys Asp Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7739 ) Cys Glu Phe Cys Asp Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7740 ) Cys Glu Phe Cys Gln Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7741 ) Cys Glu Phe Cys Gln Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7742 ) Cys Glu Phe Cys Gln Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7743 ) Cys Glu Phe Cys Gln Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7744 ) Cys Glu Phe Cys Gln Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7745 ) Cys Glu Phe Cys Gln Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7746 ) Cys Glu Phe Cys Gln Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7747 ) Cys Glu Phe Cys Gln Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7748 ) Cys Glu Phe Cys Gln Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7749 ) Cys Glu Phe Cys Gln Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7750 ) Cys Glu Phe Cys Gln Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7751 ) Cys Glu Phe Cys Gln Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7752 ) Cys Glu Phe Cys Gln Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7753 ) Cys Glu Phe Cys Gln Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7754 ) Cys Glu Phe Cys Gln Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7755 ) Cys Glu Phe Cys Gln Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7756 ) Cys Glu Phe Cys Gln Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7757 ) Cys Glu Phe Cys Gln Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7758 ) Cys Glu Phe Cys Gln Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7759 ) Cys Glu Phe Cys Gln Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7760 ) Cys Glu Phe Cys Gln Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7761 ) Cys Glu Phe Cys Gln Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7762 ) Cys Glu Phe Cys Gln Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7763 ) Cys Glu Phe Cys Gln Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7764 ) Cys Glu Phe Cys Glu Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7765 ) Cys Glu Phe Cys Glu Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7766 ) Cys Glu Phe Cys Glu Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7767 ) Cys Glu Phe Cys Glu Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7768 ) Cys Glu Phe Cys Glu Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7769 ) Cys Glu Phe Cys Glu Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7770 ) Cys Glu Phe Cys Glu Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7771 ) Cys Glu Phe Cys Glu Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7772 ) Cys Glu Phe Cys Glu Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7773 ) Cys Glu Phe Cys Glu Asn Pro Thr Cys Val Ala Cys

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(SEQ ID NO: 7774 ) Cys Glu Phe Cys Glu Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7775 ) Cys Glu Phe Cys Glu Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7776 ) Cys Glu Phe Cys Glu Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7777 ) Cys Glu Phe Cys Glu Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7778 ) Cys Glu Phe Cys Glu Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7779 ) Cys Glu Phe Cys Glu Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7780 ) Cys Glu Phe Cys Glu Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7781 ) Cys Glu Phe Cys Glu Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7782 ) Cys Glu Phe Cys Glu Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7783 ) Cys Glu Phe Cys Glu Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7784 ) Cys Glu Phe Cys Glu Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7785 ) Cys Glu Phe Cys Glu Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7786 ) Cys Glu Phe Cys Glu Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7787 ) Cys Glu Phe Cys Glu Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7788 ) Cys Glu Phe Cys Gly Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7789 ) Cys Glu Phe Cys Gly Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7790 ) Cys Glu Phe Cys Gly Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7791 ) Cys Glu Phe Cys Gly Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7792 ) Cys Glu Phe Cys Gly Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7793 ) Cys Glu Phe Cys Gly Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7794 ) Cys Glu Phe Cys Gly Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7795 ) Cys Glu Phe Cys Gly Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7796 ) Cys Glu Phe Cys Gly Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7797 ) Cys Glu Phe Cys Gly Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7798 ) Cys Glu Phe Cys Gly Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7799 ) Cys Glu Phe Cys Gly Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7800 ) Cys Glu Phe Cys Gly Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7801 ) Cys Glu Phe Cys Gly Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7802 ) Cys Glu Phe Cys Gly Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7803 ) Cys Glu Phe Cys Gly Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7804 ) Cys Glu Phe Cys Gly Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7805 ) Cys Glu Phe Cys Gly Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7806 ) Cys Glu Phe Cys Gly Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7807 ) Cys Glu Phe Cys Gly Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7808 ) Cys Glu Phe Cys Gly Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7809 ) Cys Glu Phe Cys Gly Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7810 ) Cys Glu Phe Cys Gly Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7811 ) Cys Glu Phe Cys His Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7812 ) Cys Glu Phe Cys His Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7813 ) Cys Glu Phe Cys His Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7814 ) Cys Glu Phe Cys His Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7815 ) Cys Glu Phe Cys His Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7816 ) Cys Glu Phe Cys His Asn Pro Ala Cys Gly Gly Cys

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(SEQ ID NO: 7817 ) Cys Glu Phe Cys His Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7818 ) Cys Glu Phe Cys His Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7819 ) Cys Glu Phe Cys His Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7820 ) Cys Glu Phe Cys His Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7821 ) Cys Glu Phe Cys His Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7822 ) Cys Glu Phe Cys His Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7823 ) Cys Glu Phe Cys His Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7824 ) Cys Glu Phe Cys His Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7825 ) Cys Glu Phe Cys His Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7826 ) Cys Glu Phe Cys His Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7827 ) Cys Glu Phe Cys His Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7828 ) Cys Glu Phe Cys His Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7829 ) Cys Glu Phe Cys His Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7830 ) Cys Glu Phe Cys His Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7831 ) Cys Glu Phe Cys His Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7832 ) Cys Glu Phe Cys His Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7833 ) Cys Glu Phe Cys His Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7834 ) Cys Glu Phe Cys His Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7835 ) Cys Glu Phe Cys His Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7836 ) Cys Glu Phe Cys Ile Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7837 ) Cys Glu Phe Cys Ile Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7838 ) Cys Glu Phe Cys Ile Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7839 ) Cys Glu Phe Cys Ile Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7840 ) Cys Glu Phe Cys Ile Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7841 ) Cys Glu Phe Cys Ile Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7842 ) Cys Glu Phe Cys Ile Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7843 ) Cys Glu Phe Cys Ile Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7844 ) Cys Glu Phe Cys Ile Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7845 ) Cys Glu Phe Cys Ile Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7846 ) Cys Glu Phe Cys Ile Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7847 ) Cys Glu Phe Cys Ile Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7848 ) Cys Glu Phe Cys Ile Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7849 ) Cys Glu Phe Cys Ile Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7850 ) Cys Glu Phe Cys Ile Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7851 ) Cys Glu Phe Cys Ile Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7852 ) Cys Glu Phe Cys Ile Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7853 ) Cys Glu Phe Cys Ile Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7854 ) Cys Glu Phe Cys Ile Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7855 ) Cys Glu Phe Cys Ile Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7856 ) Cys Glu Phe Cys Ile Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7857 ) Cys Glu Phe Cys Ile Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7858 ) Cys Glu Phe Cys Ile Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7859 ) Cys Glu Phe Cys Ile Asn Gly Thr Cys Gly Ala Cys

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(SEQ ID NO: 7860 ) Cys Glu Phe Cys Leu Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7861 ) Cys Glu Phe Cys Leu Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7862 ) Cys Glu Phe Cys Leu Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7863 ) Cys Glu Phe Cys Leu Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7864 ) Cys Glu Phe Cys Leu Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7865 ) Cys Glu Phe Cys Leu Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7866 ) Cys Glu Phe Cys Leu Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7867 ) Cys Glu Phe Cys Leu Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7868 ) Cys Glu Phe Cys Leu Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7869 ) Cys Glu Phe Cys Leu Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7870 ) Cys Glu Phe Cys Leu Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7871 ) Cys Glu Phe Cys Leu Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7872 ) Cys Glu Phe Cys Leu Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7873 ) Cys Glu Phe Cys Leu Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7874 ) Cys Glu Phe Cys Leu Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7875 ) Cys Glu Phe Cys Leu Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7876 ) Cys Glu Phe Cys Leu Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7877 ) Cys Glu Phe Cys Leu Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7878 ) Cys Glu Phe Cys Leu Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7879 ) Cys Glu Phe Cys Leu Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7880 ) Cys Glu Phe Cys Leu Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7881 ) Cys Glu Phe Cys Leu Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7882 ) Cys Glu Phe Cys Leu Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7883 ) Cys Glu Phe Cys Leu Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7884 ) Cys Glu Phe Cys Lys Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7885 ) Cys Glu Phe Cys Lys Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7886 ) Cys Glu Phe Cys Lys Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7887 ) Cys Glu Phe Cys Lys Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7888 ) Cys Glu Phe Cys Lys Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7889 ) Cys Glu Phe Cys Lys Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7890 ) Cys Glu Phe Cys Lys Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7891 ) Cys Glu Phe Cys Lys Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7892 ) Cys Glu Phe Cys Lys Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7893 ) Cys Glu Phe Cys Lys Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7894 ) Cys Glu Phe Cys Lys Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7895 ) Cys Glu Phe Cys Lys Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7896 ) Cys Glu Phe Cys Lys Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7897 ) Cys Glu Phe Cys Lys Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7898 ) Cys Glu Phe Cys Lys Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7899 ) Cys Glu Phe Cys Lys Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7900 ) Cys Glu Phe Cys Lys Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7901 ) Cys Glu Phe Cys Lys Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7902 ) Cys Glu Phe Cys Lys Asn Gly Thr Cys Thr Gly Cys

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(SEQ ID NO: 7903 ) Cys Glu Phe Cys Lys Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7904 ) Cys Glu Phe Cys Lys Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7905 ) Cys Glu Phe Cys Lys Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7906 ) Cys Glu Phe Cys Lys Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7907 ) Cys Glu Phe Cys Lys Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7908 ) Cys Glu Phe Cys Met Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7909 ) Cys Glu Phe Cys Met Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7910 ) Cys Glu Phe Cys Met Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7911 ) Cys Glu Phe Cys Met Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7912 ) Cys Glu Phe Cys Met Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7913 ) Cys Glu Phe Cys Met Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7914 ) Cys Glu Phe Cys Met Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7915 ) Cys Glu Phe Cys Met Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7916 ) Cys Glu Phe Cys Met Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7917 ) Cys Glu Phe Cys Met Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7918 ) Cys Glu Phe Cys Met Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7919 ) Cys Glu Phe Cys Met Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7920 ) Cys Glu Phe Cys Met Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7921 ) Cys Glu Phe Cys Met Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7922 ) Cys Glu Phe Cys Met Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7923 ) Cys Glu Phe Cys Met Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7924 ) Cys Glu Phe Cys Met Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7925 ) Cys Glu Phe Cys Met Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7926 ) Cys Glu Phe Cys Met Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7927 ) Cys Glu Phe Cys Met Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7928 ) Cys Glu Phe Cys Met Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7929 ) Cys Glu Phe Cys Met Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7930 ) Cys Glu Phe Cys Met Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7931 ) Cys Glu Phe Cys Met Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7932 ) Cys Glu Phe Cys Phe Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7933 ) Cys Glu Phe Cys Phe Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7934 ) Cys Glu Phe Cys Phe Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7935 ) Cys Glu Phe Cys Phe Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7936 ) Cys Glu Phe Cys Phe Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7937 ) Cys Glu Phe Cys Phe Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7938 ) Cys Glu Phe Cys Phe Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7939 ) Cys Glu Phe Cys Phe Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7940 ) Cys Glu Phe Cys Phe Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7941 ) Cys Glu Phe Cys Phe Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7942 ) Cys Glu Phe Cys Phe Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7943 ) Cys Glu Phe Cys Phe Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7944 ) Cys Glu Phe Cys Phe Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7945 ) Cys Glu Phe Cys Phe Asn Gly Ala Cys Thr Ala Cys

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(SEQ ID NO: 7946 ) Cys Glu Phe Cys Phe Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7947 ) Cys Glu Phe Cys Phe Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7948 ) Cys Glu Phe Cys Phe Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7949 ) Cys Glu Phe Cys Phe Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7950 ) Cys Glu Phe Cys Phe Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7951 ) Cys Glu Phe Cys Phe Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7952 ) Cys Glu Phe Cys Phe Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7953 ) Cys Glu Phe Cys Phe Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7954 ) Cys Glu Phe Cys Phe Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7955 ) Cys Glu Phe Cys Phe Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7956 ) Cys Glu Phe Cys Pro Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7957 ) Cys Glu Phe Cys Pro Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7958 ) Cys Glu Phe Cys Pro Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7959 ) Cys Glu Phe Cys Pro Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7960 ) Cys Glu Phe Cys Pro Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7961 ) Cys Glu Phe Cys Pro Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7962 ) Cys Glu Phe Cys Pro Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7963 ) Cys Glu Phe Cys Pro Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7964 ) Cys Glu Phe Cys Pro Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 7965 ) Cys Glu Phe Cys Pro Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7966 ) Cys Glu Phe Cys Pro Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7967 ) Cys Glu Phe Cys Pro Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7968 ) Cys Glu Phe Cys Pro Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7969 ) Cys Glu Phe Cys Pro Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7970 ) Cys Glu Phe Cys Pro Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7971 ) Cys Glu Phe Cys Pro Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7972 ) Cys Glu Phe Cys Pro Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7973 ) Cys Glu Phe Cys Pro Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7974 ) Cys Glu Phe Cys Pro Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7975 ) Cys Glu Phe Cys Pro Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 7976 ) Cys Glu Phe Cys Pro Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 7977 ) Cys Glu Phe Cys Pro Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 7978 ) Cys Glu Phe Cys Pro Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 7979 ) Cys Glu Phe Cys Pro Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 7980 ) Cys Glu Phe Cys Ser Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 7981 ) Cys Glu Phe Cys Ser Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 7982 ) Cys Glu Phe Cys Ser Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 7983 ) Cys Glu Phe Cys Ser Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 7984 ) Cys Glu Phe Cys Ser Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 7985 ) Cys Glu Phe Cys Ser Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 7986 ) Cys Glu Phe Cys Ser Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 7987 ) Cys Glu Phe Cys Ser Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 7988 ) Cys Glu Phe Cys Ser Asn Pro Thr Cys Val Gly Cys

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(SEQ ID NO: 7989 ) Cys Glu Phe Cys Ser Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 7990 ) Cys Glu Phe Cys Ser Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 7991 ) Cys Glu Phe Cys Ser Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 7992 ) Cys Glu Phe Cys Ser Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 7993 ) Cys Glu Phe Cys Ser Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 7994 ) Cys Glu Phe Cys Ser Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 7995 ) Cys Glu Phe Cys Ser Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 7996 ) Cys Glu Phe Cys Ser Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 7997 ) Cys Glu Phe Cys Ser Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 7998 ) Cys Glu Phe Cys Ser Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 7999 ) Cys Glu Phe Cys Ser Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8000 ) Cys Glu Phe Cys Ser Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8001 ) Cys Glu Phe Cys Ser Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8002 ) Cys Glu Phe Cys Ser Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8003 ) Cys Glu Phe Cys Ser Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8004 ) Cys Glu Phe Cys Thr Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8005 ) Cys Glu Phe Cys Thr Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8006 ) Cys Glu Phe Cys Thr Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8007 ) Cys Glu Phe Cys Thr Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8008 ) Cys Glu Phe Cys Thr Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8009 ) Cys Glu Phe Cys Thr Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8010 ) Cys Glu Phe Cys Thr Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8011 ) Cys Glu Phe Cys Thr Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8012 ) Cys Glu Phe Cys Thr Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8013 ) Cys Glu Phe Cys Thr Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8014 ) Cys Glu Phe Cys Thr Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8015 ) Cys Glu Phe Cys Thr Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8016 ) Cys Glu Phe Cys Thr Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8017 ) Cys Glu Phe Cys Thr Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8018 ) Cys Glu Phe Cys Thr Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8019 ) Cys Glu Phe Cys Thr Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8020 ) Cys Glu Phe Cys Thr Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8021 ) Cys Glu Phe Cys Thr Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8022 ) Cys Glu Phe Cys Thr Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8023 ) Cys Glu Phe Cys Thr Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8024 ) Cys Glu Phe Cys Thr Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8025 ) Cys Glu Phe Cys Thr Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8026 ) Cys Glu Phe Cys Thr Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8027 ) Cys Glu Phe Cys Thr Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8028 ) Cys Glu Phe Cys Trp Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8029 ) Cys Glu Phe Cys Trp Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8030 ) Cys Glu Phe Cys Trp Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8031 ) Cys Glu Phe Cys Trp Asn Pro Ala Cys Val Ala Cys

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(SEQ ID NO: 8032 ) Cys Glu Phe Cys Trp Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8033 ) Cys Glu Phe Cys Trp Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8034 ) Cys Glu Phe Cys Trp Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8035 ) Cys Glu Phe Cys Trp Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8036 ) Cys Glu Phe Cys Trp Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8037 ) Cys Glu Phe Cys Trp Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8038 ) Cys Glu Phe Cys Trp Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8039 ) Cys Glu Phe Cys Trp Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8040 ) Cys Glu Phe Cys Trp Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8041 ) Cys Glu Phe Cys Trp Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8042 ) Cys Glu Phe Cys Trp Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8043 ) Cys Glu Phe Cys Trp Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8044 ) Cys Glu Phe Cys Trp Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8045 ) Cys Glu Phe Cys Trp Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8046 ) Cys Glu Phe Cys Trp Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8047 ) Cys Glu Phe Cys Trp Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8048 ) Cys Glu Phe Cys Trp Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8049 ) Cys Glu Phe Cys Trp Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8050 ) Cys Glu Phe Cys Trp Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8051 ) Cys Glu Phe Cys Trp Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8052 ) Cys Glu Phe Cys Tyr Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8053 ) Cys Glu Phe Cys Tyr Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8054 ) Cys Glu Phe Cys Tyr Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8055 ) Cys Glu Phe Cys Tyr Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8056 ) Cys Glu Phe Cys Tyr Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8057 ) Cys Glu Phe Cys Tyr Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8058 ) Cys Glu Phe Cys Tyr Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8059 ) Cys Glu Phe Cys Tyr Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8060 ) Cys Glu Phe Cys Tyr Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8061 ) Cys Glu Phe Cys Tyr Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8062 ) Cys Glu Phe Cys Tyr Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8063 ) Cys Glu Phe Cys Tyr Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8064 ) Cys Glu Phe Cys Tyr Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8065 ) Cys Glu Phe Cys Tyr Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8066 ) Cys Glu Phe Cys Tyr Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8067 ) Cys Glu Phe Cys Tyr Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8068 ) Cys Glu Phe Cys Tyr Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8069 ) Cys Glu Phe Cys Tyr Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8070 ) Cys Glu Phe Cys Tyr Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8071 ) Cys Glu Phe Cys Tyr Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8072 ) Cys Glu Phe Cys Tyr Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8073 ) Cys Glu Phe Cys Tyr Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8074 ) Cys Glu Phe Cys Tyr Asn Gly Thr Cys Gly Gly Cys

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(SEQ ID NO: 8075 ) Cys Glu Phe Cys Tyr Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8076 ) Cys Glu Phe Cys Val Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8077 ) Cys Glu Phe Cys Val Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8078 ) Cys Glu Phe Cys Val Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8079 ) Cys Glu Phe Cys Val Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8080 ) Cys Glu Phe Cys Val Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8081 ) Cys Glu Phe Cys Val Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8082 ) Cys Glu Phe Cys Val Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8083 ) Cys Glu Phe Cys Val Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8084 ) Cys Glu Phe Cys Val Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8085 ) Cys Glu Phe Cys Val Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8086 ) Cys Glu Phe Cys Val Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8087 ) Cys Glu Phe Cys Val Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8088 ) Cys Glu Phe Cys Val Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8089 ) Cys Glu Phe Cys Val Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8090 ) Cys Glu Phe Cys Val Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8091 ) Cys Glu Phe Cys Val Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8092 ) Cys Glu Phe Cys Val Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8093 ) Cys Glu Phe Cys Val Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8094 ) Cys Glu Phe Cys Val Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8095 ) Cys Glu Phe Cys Val Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8096 ) Cys Glu Phe Cys Val Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8097 ) Cys Glu Phe Cys Val Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8098 ) Cys Glu Phe Cys Val Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8099 ) Cys Glu Phe Cys Val Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8100 ) Cys Glu Phe Cys --- Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8101 ) Cys Glu Phe Cys --- Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8102 ) Cys Glu Phe Cys --- Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8103 ) Cys Glu Phe Cys --- Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8104 ) Cys Glu Phe Cys --- Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8105 ) Cys Glu Phe Cys --- Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8106 ) Cys Glu Phe Cys --- Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8107 ) Cys Glu Phe Cys --- Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8108 ) Cys Glu Phe Cys --- Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8109 ) Cys Glu Phe Cys --- Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8110 ) Cys Glu Phe Cys --- Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8111 ) Cys Glu Phe Cys --- Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8112 ) Cys Glu Phe Cys --- Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8113 ) Cys Glu Phe Cys --- Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8114 ) Cys Glu Phe Cys --- Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8115 ) Cys Glu Phe Cys --- Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8116 ) Cys Glu Phe Cys --- Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8117 ) Cys Glu Phe Cys --- Asn Gly Ala Cys Gly Ala Cys

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(SEQ ID NO: 8118 ) Cys Glu Phe Cys --- Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8119 ) Cys Glu Phe Cys --- Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8120 ) Cys Glu Phe Cys --- Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8121 ) Cys Glu Phe Cys --- Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8122 ) Cys Glu Phe Cys --- Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8123 ) Cys Glu Phe Cys --- Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8124 ) Cys Glu Leu Cys Ala Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8125 ) Cys Glu Leu Cys Ala Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8126 ) Cys Glu Leu Cys Ala Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8127 ) Cys Glu Leu Cys Ala Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8128 ) Cys Glu Leu Cys Ala Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8129 ) Cys Glu Leu Cys Ala Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8130 ) Cys Glu Leu Cys Ala Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8131 ) Cys Glu Leu Cys Ala Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8132 ) Cys Glu Leu Cys Ala Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8133 ) Cys Glu Leu Cys Ala Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8134 ) Cys Glu Leu Cys Ala Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8135 ) Cys Glu Leu Cys Ala Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8136 ) Cys Glu Leu Cys Ala Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8137 ) Cys Glu Leu Cys Ala Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8138 ) Cys Glu Leu Cys Ala Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8139 ) Cys Glu Leu Cys Ala Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8140 ) Cys Glu Leu Cys Ala Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8141 ) Cys Glu Leu Cys Ala Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8142 ) Cys Glu Leu Cys Ala Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8143 ) Cys Glu Leu Cys Ala Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8144 ) Cys Glu Leu Cys Ala Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8145 ) Cys Glu Leu Cys Ala Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8146 ) Cys Glu Leu Cys Ala Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8147 ) Cys Glu Leu Cys Ala Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8148 ) Cys Glu Leu Cys Arg Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8149 ) Cys Glu Leu Cys Arg Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8150 ) Cys Glu Leu Cys Arg Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8151 ) Cys Glu Leu Cys Arg Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8152 ) Cys Glu Leu Cys Arg Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8153 ) Cys Glu Leu Cys Arg Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8154 ) Cys Glu Leu Cys Arg Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8155 ) Cys Glu Leu Cys Arg Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8156 ) Cys Glu Leu Cys Arg Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8157 ) Cys Glu Leu Cys Arg Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8158 ) Cys Glu Leu Cys Arg Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8159 ) Cys Glu Leu Cys Arg Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8160 ) Cys Glu Leu Cys Arg Asn Gly Ala Cys Thr Gly Cys

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(SEQ ID NO: 8161 ) Cys Glu Leu Cys Arg Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8162 ) Cys Glu Leu Cys Arg Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8163 ) Cys Glu Leu Cys Arg Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8164 ) Cys Glu Leu Cys Arg Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8165 ) Cys Glu Leu Cys Arg Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8166 ) Cys Glu Leu Cys Arg Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8167 ) Cys Glu Leu Cys Arg Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8168 ) Cys Glu Leu Cys Arg Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8169 ) Cys Glu Leu Cys Arg Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8170 ) Cys Glu Leu Cys Arg Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8171 ) Cys Glu Leu Cys Arg Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8172 ) Cys Glu Leu Cys Asn Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8173 ) Cys Glu Leu Cys Asn Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8174 ) Cys Glu Leu Cys Asn Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8175 ) Cys Glu Leu Cys Asn Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8176 ) Cys Glu Leu Cys Asn Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8177 ) Cys Glu Leu Cys Asn Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8178 ) Cys Glu Leu Cys Asn Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8179 ) Cys Glu Leu Cys Asn Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8180 ) Cys Glu Leu Cys Asn Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8181 ) Cys Glu Leu Cys Asn Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8182 ) Cys Glu Leu Cys Asn Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8183 ) Cys Glu Leu Cys Asn Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8184 ) Cys Glu Leu Cys Asn Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8185 ) Cys Glu Leu Cys Asn Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8186 ) Cys Glu Leu Cys Asn Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8187 ) Cys Glu Leu Cys Asn Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8188 ) Cys Glu Leu Cys Asn Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8189 ) Cys Glu Leu Cys Asn Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8190 ) Cys Glu Leu Cys Asn Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8191 ) Cys Glu Leu Cys Asn Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8192 ) Cys Glu Leu Cys Asn Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8193 ) Cys Glu Leu Cys Asn Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8194 ) Cys Glu Leu Cys Asn Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8195 ) Cys Glu Leu Cys Asn Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8196 ) Cys Glu Leu Cys Asp Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8197 ) Cys Glu Leu Cys Asp Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8198 ) Cys Glu Leu Cys Asp Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8199 ) Cys Glu Leu Cys Asp Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8200 ) Cys Glu Leu Cys Asp Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8201 ) Cys Glu Leu Cys Asp Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8202 ) Cys Glu Leu Cys Asp Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8203 ) Cys Glu Leu Cys Asp Asn Pro Thr Cys Thr Ala Cys

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(SEQ ID NO: 8204 ) Cys Glu Leu Cys Asp Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8205 ) Cys Glu Leu Cys Asp Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8206 ) Cys Glu Leu Cys Asp Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8207 ) Cys Glu Leu Cys Asp Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8208 ) Cys Glu Leu Cys Asp Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8209 ) Cys Glu Leu Cys Asp Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8210 ) Cys Glu Leu Cys Asp Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8211 ) Cys Glu Leu Cys Asp Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8212 ) Cys Glu Leu Cys Asp Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8213 ) Cys Glu Leu Cys Asp Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8214 ) Cys Glu Leu Cys Asp Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8215 ) Cys Glu Leu Cys Asp Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8216 ) Cys Glu Leu Cys Asp Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8217 ) Cys Glu Leu Cys Asp Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8218 ) Cys Glu Leu Cys Asp Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8219 ) Cys Glu Leu Cys Asp Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8220 ) Cys Glu Leu Cys Gln Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8221 ) Cys Glu Leu Cys Gln Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8222 ) Cys Glu Leu Cys Gln Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8223 ) Cys Glu Leu Cys Gln Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8224 ) Cys Glu Leu Cys Gln Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8225 ) Cys Glu Leu Cys Gln Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8226 ) Cys Glu Leu Cys Gln Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8227 ) Cys Glu Leu Cys Gln Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8228 ) Cys Glu Leu Cys Gln Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8229 ) Cys Glu Leu Cys Gln Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8230 ) Cys Glu Leu Cys Gln Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8231 ) Cys Glu Leu Cys Gln Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8232 ) Cys Glu Leu Cys Gln Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8233 ) Cys Glu Leu Cys Gln Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8234 ) Cys Glu Leu Cys Gln Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8235 ) Cys Glu Leu Cys Gln Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8236 ) Cys Glu Leu Cys Gln Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8237 ) Cys Glu Leu Cys Gln Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8238 ) Cys Glu Leu Cys Gln Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8239 ) Cys Glu Leu Cys Gln Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8240 ) Cys Glu Leu Cys Gln Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8241 ) Cys Glu Leu Cys Gln Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8242 ) Cys Glu Leu Cys Gln Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8243 ) Cys Glu Leu Cys Gln Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8244 ) Cys Glu Leu Cys Glu Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8245 ) Cys Glu Leu Cys Glu Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8246 ) Cys Glu Leu Cys Glu Asn Pro Ala Cys Val Gly Cys

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(SEQ ID NO: 8247 ) Cys Glu Leu Cys Glu Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8248 ) Cys Glu Leu Cys Glu Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8249 ) Cys Glu Leu Cys Glu Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8250 ) Cys Glu Leu Cys Glu Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8251 ) Cys Glu Leu Cys Glu Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8252 ) Cys Glu Leu Cys Glu Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8253 ) Cys Glu Leu Cys Glu Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8254 ) Cys Glu Leu Cys Glu Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8255 ) Cys Glu Leu Cys Glu Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8256 ) Cys Glu Leu Cys Glu Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8257 ) Cys Glu Leu Cys Glu Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8258 ) Cys Glu Leu Cys Glu Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8259 ) Cys Glu Leu Cys Glu Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8260 ) Cys Glu Leu Cys Glu Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8261 ) Cys Glu Leu Cys Glu Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8262 ) Cys Glu Leu Cys Glu Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8263 ) Cys Glu Leu Cys Glu Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8264 ) Cys Glu Leu Cys Glu Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8265 ) Cys Glu Leu Cys Glu Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8266 ) Cys Glu Leu Cys Glu Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8267 ) Cys Glu Leu Cys Glu Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8268 ) Cys Glu Leu Cys Gly Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8269 ) Cys Glu Leu Cys Gly Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8270 ) Cys Glu Leu Cys Gly Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8271 ) Cys Glu Leu Cys Gly Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8272 ) Cys Glu Leu Cys Gly Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8273 ) Cys Glu Leu Cys Gly Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8274 ) Cys Glu Leu Cys Gly Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8275 ) Cys Glu Leu Cys Gly Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8276 ) Cys Glu Leu Cys Gly Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8277 ) Cys Glu Leu Cys Gly Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8278 ) Cys Glu Leu Cys Gly Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8279 ) Cys Glu Leu Cys Gly Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8280 ) Cys Glu Leu Cys Gly Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8281 ) Cys Glu Leu Cys Gly Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8282 ) Cys Glu Leu Cys Gly Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8283 ) Cys Glu Leu Cys Gly Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8284 ) Cys Glu Leu Cys Gly Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8285 ) Cys Glu Leu Cys Gly Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8286 ) Cys Glu Leu Cys Gly Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8287 ) Cys Glu Leu Cys Gly Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8288 ) Cys Glu Leu Cys Gly Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8289 ) Cys Glu Leu Cys Gly Asn Gly Thr Cys Val Ala Cys

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(SEQ ID NO: 8290 ) Cys Glu Leu Cys Gly Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8291 ) Cys Glu Leu Cys Gly Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8292 ) Cys Glu Leu Cys His Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8293 ) Cys Glu Leu Cys His Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8294 ) Cys Glu Leu Cys His Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8295 ) Cys Glu Leu Cys His Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8296 ) Cys Glu Leu Cys His Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8297 ) Cys Glu Leu Cys His Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8298 ) Cys Glu Leu Cys His Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8299 ) Cys Glu Leu Cys His Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8300 ) Cys Glu Leu Cys His Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8301 ) Cys Glu Leu Cys His Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8302 ) Cys Glu Leu Cys His Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8303 ) Cys Glu Leu Cys His Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8304 ) Cys Glu Leu Cys His Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8305 ) Cys Glu Leu Cys His Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8306 ) Cys Glu Leu Cys His Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8307 ) Cys Glu Leu Cys His Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8308 ) Cys Glu Leu Cys His Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8309 ) Cys Glu Leu Cys His Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8310 ) Cys Glu Leu Cys His Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8311 ) Cys Glu Leu Cys His Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8312 ) Cys Glu Leu Cys His Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8313 ) Cys Glu Leu Cys His Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8314 ) Cys Glu Leu Cys His Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8315 ) Cys Glu Leu Cys His Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8316 ) Cys Glu Leu Cys Ile Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8317 ) Cys Glu Leu Cys Ile Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8318 ) Cys Glu Leu Cys Ile Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8319 ) Cys Glu Leu Cys Ile Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8320 ) Cys Glu Leu Cys Ile Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8321 ) Cys Glu Leu Cys Ile Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8322 ) Cys Glu Leu Cys Ile Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8323 ) Cys Glu Leu Cys Ile Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8324 ) Cys Glu Leu Cys Ile Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8325 ) Cys Glu Leu Cys Ile Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8326 ) Cys Glu Leu Cys Ile Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8327 ) Cys Glu Leu Cys Ile Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8328 ) Cys Glu Leu Cys Ile Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8329 ) Cys Glu Leu Cys Ile Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8330 ) Cys Glu Leu Cys Ile Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8331 ) Cys Glu Leu Cys Ile Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8332 ) Cys Glu Leu Cys Ile Asn Gly Ala Cys Gly Gly Cys

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(SEQ ID NO: 8333 ) Cys Glu Leu Cys Ile Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8334 ) Cys Glu Leu Cys Ile Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8335 ) Cys Glu Leu Cys Ile Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8336 ) Cys Glu Leu Cys Ile Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8337 ) Cys Glu Leu Cys Ile Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8338 ) Cys Glu Leu Cys Ile Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8339 ) Cys Glu Leu Cys Ile Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8340 ) Cys Glu Leu Cys Leu Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8341 ) Cys Glu Leu Cys Leu Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8342 ) Cys Glu Leu Cys Leu Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8343 ) Cys Glu Leu Cys Leu Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8344 ) Cys Glu Leu Cys Leu Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8345 ) Cys Glu Leu Cys Leu Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8346 ) Cys Glu Leu Cys Leu Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8347 ) Cys Glu Leu Cys Leu Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8348 ) Cys Glu Leu Cys Leu Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8349 ) Cys Glu Leu Cys Leu Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8350 ) Cys Glu Leu Cys Leu Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8351 ) Cys Glu Leu Cys Leu Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8352 ) Cys Glu Leu Cys Leu Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8353 ) Cys Glu Leu Cys Leu Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8354 ) Cys Glu Leu Cys Leu Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8355 ) Cys Glu Leu Cys Leu Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8356 ) Cys Glu Leu Cys Leu Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8357 ) Cys Glu Leu Cys Leu Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8358 ) Cys Glu Leu Cys Leu Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8359 ) Cys Glu Leu Cys Leu Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8360 ) Cys Glu Leu Cys Leu Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8361 ) Cys Glu Leu Cys Leu Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8362 ) Cys Glu Leu Cys Leu Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8363 ) Cys Glu Leu Cys Leu Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8364 ) Cys Glu Leu Cys Lys Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8365 ) Cys Glu Leu Cys Lys Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8366 ) Cys Glu Leu Cys Lys Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8367 ) Cys Glu Leu Cys Lys Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8368 ) Cys Glu Leu Cys Lys Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8369 ) Cys Glu Leu Cys Lys Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8370 ) Cys Glu Leu Cys Lys Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8371 ) Cys Glu Leu Cys Lys Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8372 ) Cys Glu Leu Cys Lys Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8373 ) Cys Glu Leu Cys Lys Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8374 ) Cys Glu Leu Cys Lys Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8375 ) Cys Glu Leu Cys Lys Asn Pro Thr Cys Gly Ala Cys

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(SEQ ID NO: 8376 ) Cys Glu Leu Cys Lys Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8377 ) Cys Glu Leu Cys Lys Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8378 ) Cys Glu Leu Cys Lys Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8379 ) Cys Glu Leu Cys Lys Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8380 ) Cys Glu Leu Cys Lys Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8381 ) Cys Glu Leu Cys Lys Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8382 ) Cys Glu Leu Cys Lys Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8383 ) Cys Glu Leu Cys Lys Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8384 ) Cys Glu Leu Cys Lys Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8385 ) Cys Glu Leu Cys Lys Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8386 ) Cys Glu Leu Cys Lys Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8387 ) Cys Glu Leu Cys Lys Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8388 ) Cys Glu Leu Cys Met Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8389 ) Cys Glu Leu Cys Met Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8390 ) Cys Glu Leu Cys Met Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8391 ) Cys Glu Leu Cys Met Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8392 ) Cys Glu Leu Cys Met Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8393 ) Cys Glu Leu Cys Met Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8394 ) Cys Glu Leu Cys Met Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8395 ) Cys Glu Leu Cys Met Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8396 ) Cys Glu Leu Cys Met Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8397 ) Cys Glu Leu Cys Met Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8398 ) Cys Glu Leu Cys Met Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8399 ) Cys Glu Leu Cys Met Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8400 ) Cys Glu Leu Cys Met Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8401 ) Cys Glu Leu Cys Met Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8402 ) Cys Glu Leu Cys Met Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8403 ) Cys Glu Leu Cys Met Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8404 ) Cys Glu Leu Cys Met Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8405 ) Cys Glu Leu Cys Met Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8406 ) Cys Glu Leu Cys Met Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8407 ) Cys Glu Leu Cys Met Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8408 ) Cys Glu Leu Cys Met Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8409 ) Cys Glu Leu Cys Met Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8410 ) Cys Glu Leu Cys Met Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8411 ) Cys Glu Leu Cys Met Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8412 ) Cys Glu Leu Cys Phe Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8413 ) Cys Glu Leu Cys Phe Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8414 ) Cys Glu Leu Cys Phe Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8415 ) Cys Glu Leu Cys Phe Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8416 ) Cys Glu Leu Cys Phe Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8417 ) Cys Glu Leu Cys Phe Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8418 ) Cys Glu Leu Cys Phe Asn Pro Thr Cys Thr Gly Cys

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(SEQ ID NO: 8419 ) Cys Glu Leu Cys Phe Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8420 ) Cys Glu Leu Cys Phe Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8421 ) Cys Glu Leu Cys Phe Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8422 ) Cys Glu Leu Cys Phe Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8423 ) Cys Glu Leu Cys Phe Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8424 ) Cys Glu Leu Cys Phe Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8425 ) Cys Glu Leu Cys Phe Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8426 ) Cys Glu Leu Cys Phe Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8427 ) Cys Glu Leu Cys Phe Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8428 ) Cys Glu Leu Cys Phe Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8429 ) Cys Glu Leu Cys Phe Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8430 ) Cys Glu Leu Cys Phe Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8431 ) Cys Glu Leu Cys Phe Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8432 ) Cys Glu Leu Cys Phe Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8433 ) Cys Glu Leu Cys Phe Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8434 ) Cys Glu Leu Cys Phe Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8435 ) Cys Glu Leu Cys Phe Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8436 ) Cys Glu Leu Cys Pro Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8437 ) Cys Glu Leu Cys Pro Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8438 ) Cys Glu Leu Cys Pro Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8439 ) Cys Glu Leu Cys Pro Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8440 ) Cys Glu Leu Cys Pro Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8441 ) Cys Glu Leu Cys Pro Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8442 ) Cys Glu Leu Cys Pro Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8443 ) Cys Glu Leu Cys Pro Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8444 ) Cys Glu Leu Cys Pro Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8445 ) Cys Glu Leu Cys Pro Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8446 ) Cys Glu Leu Cys Pro Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8447 ) Cys Glu Leu Cys Pro Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8448 ) Cys Glu Leu Cys Pro Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8449 ) Cys Glu Leu Cys Pro Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8450 ) Cys Glu Leu Cys Pro Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8451 ) Cys Glu Leu Cys Pro Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8452 ) Cys Glu Leu Cys Pro Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8453 ) Cys Glu Leu Cys Pro Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8454 ) Cys Glu Leu Cys Pro Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8455 ) Cys Glu Leu Cys Pro Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8456 ) Cys Glu Leu Cys Pro Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8457 ) Cys Glu Leu Cys Pro Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8458 ) Cys Glu Leu Cys Pro Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8459 ) Cys Glu Leu Cys Pro Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8460 ) Cys Glu Leu Cys Ser Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8461 ) Cys Glu Leu Cys Ser Asn Pro Ala Cys Thr Ala Cys

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(SEQ ID NO: 8462 ) Cys Glu Leu Cys Ser Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8463 ) Cys Glu Leu Cys Ser Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8464 ) Cys Glu Leu Cys Ser Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8465 ) Cys Glu Leu Cys Ser Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8466 ) Cys Glu Leu Cys Ser Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8467 ) Cys Glu Leu Cys Ser Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8468 ) Cys Glu Leu Cys Ser Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8469 ) Cys Glu Leu Cys Ser Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8470 ) Cys Glu Leu Cys Ser Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8471 ) Cys Glu Leu Cys Ser Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8472 ) Cys Glu Leu Cys Ser Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8473 ) Cys Glu Leu Cys Ser Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8474 ) Cys Glu Leu Cys Ser Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8475 ) Cys Glu Leu Cys Ser Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8476 ) Cys Glu Leu Cys Ser Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8477 ) Cys Glu Leu Cys Ser Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8478 ) Cys Glu Leu Cys Ser Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8479 ) Cys Glu Leu Cys Ser Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8480 ) Cys Glu Leu Cys Ser Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8481 ) Cys Glu Leu Cys Ser Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8482 ) Cys Glu Leu Cys Ser Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8483 ) Cys Glu Leu Cys Ser Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8484 ) Cys Glu Leu Cys Thr Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8485 ) Cys Glu Leu Cys Thr Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8486 ) Cys Glu Leu Cys Thr Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8487 ) Cys Glu Leu Cys Thr Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8488 ) Cys Glu Leu Cys Thr Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8489 ) Cys Glu Leu Cys Thr Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8490 ) Cys Glu Leu Cys Thr Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8491 ) Cys Glu Leu Cys Thr Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8492 ) Cys Glu Leu Cys Thr Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8493 ) Cys Glu Leu Cys Thr Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8494 ) Cys Glu Leu Cys Thr Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8495 ) Cys Glu Leu Cys Thr Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8496 ) Cys Glu Leu Cys Thr Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8497 ) Cys Glu Leu Cys Thr Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8498 ) Cys Glu Leu Cys Thr Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8499 ) Cys Glu Leu Cys Thr Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8500 ) Cys Glu Leu Cys Thr Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8501 ) Cys Glu Leu Cys Thr Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8502 ) Cys Glu Leu Cys Thr Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8503 ) Cys Glu Leu Cys Thr Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8504 ) Cys Glu Leu Cys Thr Asn Gly Thr Cys Val Gly Cys

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(SEQ ID NO: 8505 ) Cys Glu Leu Cys Thr Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8506 ) Cys Glu Leu Cys Thr Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8507 ) Cys Glu Leu Cys Thr Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8508 ) Cys Glu Leu Cys Trp Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8509 ) Cys Glu Leu Cys Trp Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8510 ) Cys Glu Leu Cys Trp Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8511 ) Cys Glu Leu Cys Trp Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8512 ) Cys Glu Leu Cys Trp Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8513 ) Cys Glu Leu Cys Trp Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8514 ) Cys Glu Leu Cys Trp Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8515 ) Cys Glu Leu Cys Trp Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8516 ) Cys Glu Leu Cys Trp Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8517 ) Cys Glu Leu Cys Trp Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8518 ) Cys Glu Leu Cys Trp Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8519 ) Cys Glu Leu Cys Trp Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8520 ) Cys Glu Leu Cys Trp Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8521 ) Cys Glu Leu Cys Trp Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8522 ) Cys Glu Leu Cys Trp Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8523 ) Cys Glu Leu Cys Trp Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8524 ) Cys Glu Leu Cys Trp Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8525 ) Cys Glu Leu Cys Trp Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8526 ) Cys Glu Leu Cys Trp Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8527 ) Cys Glu Leu Cys Trp Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8528 ) Cys Glu Leu Cys Trp Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8529 ) Cys Glu Leu Cys Trp Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8530 ) Cys Glu Leu Cys Trp Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8531 ) Cys Glu Leu Cys Trp Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8532 ) Cys Glu Leu Cys Tyr Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8533 ) Cys Glu Leu Cys Tyr Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8534 ) Cys Glu Leu Cys Tyr Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8535 ) Cys Glu Leu Cys Tyr Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8536 ) Cys Glu Leu Cys Tyr Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8537 ) Cys Glu Leu Cys Tyr Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8538 ) Cys Glu Leu Cys Tyr Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8539 ) Cys Glu Leu Cys Tyr Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8540 ) Cys Glu Leu Cys Tyr Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8541 ) Cys Glu Leu Cys Tyr Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8542 ) Cys Glu Leu Cys Tyr Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8543 ) Cys Glu Leu Cys Tyr Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8544 ) Cys Glu Leu Cys Tyr Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8545 ) Cys Glu Leu Cys Tyr Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8546 ) Cys Glu Leu Cys Tyr Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8547 ) Cys Glu Leu Cys Tyr Asn Gly Ala Cys Val Ala Cys

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(SEQ ID NO: 8548 ) Cys Glu Leu Cys Tyr Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8549 ) Cys Glu Leu Cys Tyr Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8550 ) Cys Glu Leu Cys Tyr Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8551 ) Cys Glu Leu Cys Tyr Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8552 ) Cys Glu Leu Cys Tyr Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8553 ) Cys Glu Leu Cys Tyr Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8554 ) Cys Glu Leu Cys Tyr Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8555 ) Cys Glu Leu Cys Tyr Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8556 ) Cys Glu Leu Cys Val Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8557 ) Cys Glu Leu Cys Val Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8558 ) Cys Glu Leu Cys Val Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8559 ) Cys Glu Leu Cys Val Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8560 ) Cys Glu Leu Cys Val Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8561 ) Cys Glu Leu Cys Val Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8562 ) Cys Glu Leu Cys Val Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8563 ) Cys Glu Leu Cys Val Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8564 ) Cys Glu Leu Cys Val Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8565 ) Cys Glu Leu Cys Val Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8566 ) Cys Glu Leu Cys Val Asn Pro Thr Cys Gly Gly Cys  
(SEQ ID NO: 8567 ) Cys Glu Leu Cys Val Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8568 ) Cys Glu Leu Cys Val Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8569 ) Cys Glu Leu Cys Val Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8570 ) Cys Glu Leu Cys Val Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8571 ) Cys Glu Leu Cys Val Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8572 ) Cys Glu Leu Cys Val Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8573 ) Cys Glu Leu Cys Val Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8574 ) Cys Glu Leu Cys Val Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8575 ) Cys Glu Leu Cys Val Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8576 ) Cys Glu Leu Cys Val Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8577 ) Cys Glu Leu Cys Val Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8578 ) Cys Glu Leu Cys Val Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8579 ) Cys Glu Leu Cys Val Asn Gly Thr Cys Gly Ala Cys  
(SEQ ID NO: 8580 ) Cys Glu Leu Cys --- Asn Pro Ala Cys Thr Gly Cys  
(SEQ ID NO: 8581 ) Cys Glu Leu Cys --- Asn Pro Ala Cys Thr Ala Cys  
(SEQ ID NO: 8582 ) Cys Glu Leu Cys --- Asn Pro Ala Cys Val Gly Cys  
(SEQ ID NO: 8583 ) Cys Glu Leu Cys --- Asn Pro Ala Cys Val Ala Cys  
(SEQ ID NO: 8584 ) Cys Glu Leu Cys --- Asn Pro Ala Cys Gly Gly Cys  
(SEQ ID NO: 8585 ) Cys Glu Leu Cys --- Asn Pro Ala Cys Gly Ala Cys  
(SEQ ID NO: 8586 ) Cys Glu Leu Cys --- Asn Pro Thr Cys Thr Gly Cys  
(SEQ ID NO: 8587 ) Cys Glu Leu Cys ---- Asn Pro Thr Cys Thr Ala Cys  
(SEQ ID NO: 8588 ) Cys Glu Leu Cys --- Asn Pro Thr Cys Val Gly Cys  
(SEQ ID NO: 8589 ) Cys Glu Leu Cys --- Asn Pro Thr Cys Val Ala Cys  
(SEQ ID NO: 8590 ) Cys Glu Leu Cys --- Asn Pro Thr Cys Gly Gly Cys

FIG. 3  
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(SEQ ID NO: 8591 ) Cys Glu Leu Cys --- Asn Pro Thr Cys Gly Ala Cys  
(SEQ ID NO: 8592 ) Cys Glu Leu Cys --- Asn Gly Ala Cys Thr Gly Cys  
(SEQ ID NO: 8593 ) Cys Glu Leu Cys --- Asn Gly Ala Cys Thr Ala Cys  
(SEQ ID NO: 8594 ) Cys Glu Leu Cys --- Asn Gly Ala Cys Val Gly Cys  
(SEQ ID NO: 8595 ) Cys Glu Leu Cys --- Asn Gly Ala Cys Val Ala Cys  
(SEQ ID NO: 8596 ) Cys Glu Leu Cys --- Asn Gly Ala Cys Gly Gly Cys  
(SEQ ID NO: 8597 ) Cys Glu Leu Cys --- Asn Gly Ala Cys Gly Ala Cys  
(SEQ ID NO: 8598 ) Cys Glu Leu Cys --- Asn Gly Thr Cys Thr Gly Cys  
(SEQ ID NO: 8599 ) Cys Glu Leu Cys --- Asn Gly Thr Cys Thr Ala Cys  
(SEQ ID NO: 8600 ) Cys Glu Leu Cys --- Asn Gly Thr Cys Val Gly Cys  
(SEQ ID NO: 8601 ) Cys Glu Leu Cys --- Asn Gly Thr Cys Val Ala Cys  
(SEQ ID NO: 8602 ) Cys Glu Leu Cys --- Asn Gly Thr Cys Gly Gly Cys  
(SEQ ID NO: 8603 ) Cys Glu Leu Cys --- Asn Gly Thr Cys Gly Ala Cys

**FIG. 3**  
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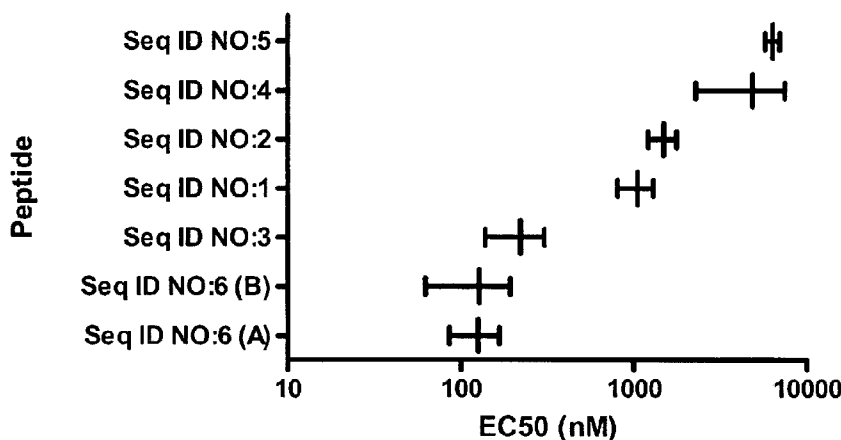
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Figure 1. EC<sub>50</sub> of peptides in *in vitro* cGMP assay



(57) Abstract: Peptides that act as GC-C receptor agonists and contain at least one D-cys and are useful for the treatment of diuresis and heart disease as well as other disorders are described.

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# Methods and Compositions for the Treatment of Heart Failure and other Disorders

## TECHNICAL FIELD

- 5 This disclosure relates to methods and compositions for the treatment of heart failure, gastrointestinal disorders and other disorders.

## BACKGROUND

Heart failure is a hemodynamic disorder resulting from impairment of the ability of the  
10 ventricle to fill with and/or eject blood. The disorder is commonly characterized by shortness of breath, fatigue, limited exercise tolerance, and fluid retention (both pulmonary congestion and peripheral edema). Heart failure is generally progressive and can result in Class IV heart failure (NYHA Heart Failure Classification) in which any physical activity brings on symptoms such as shortness of breath, and symptoms can  
15 occur even when the patient is at rest. Patients with symptoms of advanced heart failure are treated by tightly controlling fluid status and are often administered intravenous peripheral vasodilators and/or positive inotropic agents. Patients suffering Class IV heart failure should be at complete rest (confined to a bed or chair). Among the agents that are intravenously administered for treatment of advanced heart failure are dobutamine (beta  
20 receptor antagonist), milrinone (phosphodiesterase inhibitor), and nesiritide. Nesiritide is a cardiac derived peptide hormone (human natriuretic peptide B) that is thought to bind to and activate guanylate cyclase A (GC-A) receptor.

The guanylate cyclase-C (GC-C) receptor (reviewed by Lucas et al. 2000 Pharmacol. Rev  
25 52:375-414 and Vaandrager et al. 2002 Molecular and Cellular Biochemistry 230:73-83) is a key regulator in mammals of intestinal function (although low levels of GC-C have been detected in other tissues). GC-C responds to the endogenous hormones, guanylin

and uroguanylin, and to enteric bacterial peptides from the heat stable enterotoxin family (ST peptides). When agonists bind to GC-C, there is an elevation of the second messenger, cyclic GMP, and an increase in chloride and bicarbonate secretion, resulting in an increase in intestinal fluid secretion.

5

## SUMMARY

Described herein are methods for treating other disorders such as congestive heart failure and benign prostatic hyperplasia by administering a peptide or small molecule (parenterally or orally) that acts as an agonist of the GC-C receptor. Such agents can be used in combination with natriuretic peptides (e.g., atrial natriuretic peptide, brain natriuretic peptide or C-type natriuretic peptide), a diuretic, or an inhibitor of angiotensin converting enzyme.

The peptides described herein can be used alone or in combination therapy to prevent and/or treat disorders associated with fluid and sodium retention, e.g., diseases of the electrolyte-water/electrolyte transport system within the kidney, gut and urogenital system, heart failure (e.g., congestive heart failure or acute heart failure), hypertension, salt dependent forms of high blood pressure, hepatic edema, and liver cirrhosis. In addition they can be used to facilitate diuresis or control intestinal fluid. The peptides and agonists described herein can also be used to treat disorders where there is abnormal proliferation of epithelial cells within the kidney (e.g. as in the case of renal cancer).

The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat kidney disease. "Kidney disease" includes renal failure (including acute renal failure), renal insufficiency, nephrotic edema, glomerulonephritis, pyelonephritis, kidney failure, chronic renal failure, nephritis, nephrosis, azotemia, uremia, immune renal disease, acute nephritic syndrome, rapidly progressive nephritic syndrome, nephrotic syndrome, Berger's Disease, chronic nephritic/proteinuric syndrome,

tubulointerstitial disease, nephrotoxic disorders, renal infarction, atheroembolic renal disease, renal cortical necrosis, malignant nephroangiosclerosis, renal vein thrombosis, renal tubular acidosis, renal glucosuria, nephrogenic diabetes insipidus, Bartter's Syndrome, Liddle's Syndrome, polycystic kidney disease, medullary cystic disease, 5 medullary sponge kidney, hereditary nephritis, and nail-patella syndrome, along with any disease or disorder that relates to the renal system and related disorders, as well as symptoms indicative of, or related to, renal or kidney disease and related disorders.

The peptides and agonists described herein can be used alone or in combination therapy 10 to prevent or treat polycystic kidney disease. Polycystic kidney disease "PKD" (also called "polycystic renal disease") refers to a group of disorders characterized by a large number of cysts distributed throughout dramatically enlarged kidneys. The resultant cyst development leads to impairment of kidney function and can eventually cause kidney failure. "PKD" specifically includes autosomal dominant polycystic kidney disease 15 (ADPKD) and recessive autosomal recessive polycystic kidney disease (ARPKD), in all stages of development, regardless of the underlying cause.

The peptides and agonists described herein can be used for treating heart failure, including heart failure at any of stages I-IV according to New York Heart Association 20 (NYHA) Functional Classification.

The peptides can also be used for treating IBS and other gastrointestinal disorders and conditions (e.g., gastrointestinal motility disorders, chronic intestinal pseudo-obstruction, colonic pseudo-obstruction, Crohn's disease, duodenogastric reflux, dyspepsia, functional dyspepsia, nonulcer dyspepsia, a functional gastrointestinal disorder, functional 25 heartburn, gastroesophageal reflux disease (GERD), gastroparesis, irritable bowel syndrome (IBS, e.g., constipation predominant-IBS, diarrhea predominant-IBS, and/or alternating-IBS)), post-operative ileus, ulcerative colitis, chronic constipation, and disorders and conditions associated with constipation (e.g. constipation associated with

use of opiate pain killers, post-surgical constipation, and constipation associated with neuropathic disorders as well as other conditions and disorders are described herein

Without being bound by any particular theory, in the case of heart failure, salt retention,  
5 fluid retention disorders and combinations thereof the peptides are also useful because  
they may elicit one or more of diuresis, naturesis and/or kaliuresis. Thus, the peptides  
described herein may be diuretics.

Without being bound by any particular theory, in the case of IBS and other  
10 gastrointestinal disorders the peptides are useful because they may increase  
gastrointestinal motility. The peptides may also decrease inflammation and may decrease  
gastrointestinal pain, visceral pain, chronic visceral hypersensitivity, or hypersensitivity  
to colorectal distension.

15 Described herein are pharmaceutical compositions comprising certain peptides that are  
capable of activating the guanylate-cyclase C (GC-C) receptor. Also described herein are  
pharmaceutical compositions comprising a peptide or GC-C agonist described herein and  
one or more additional therapeutic agents including, without limitation, the agents  
described herein. The other agents can be administered with the peptides described  
20 herein (simultaneously or sequentially). They can also be linked to a peptide described  
herein to create therapeutic conjugates.

Described herein are methods for treating various disorders by administering a peptide  
that acts as a partial or complete agonist of the GC-C receptor. In certain embodiments,  
25 the peptide includes at least six cysteines that can form three disulfide bonds. In certain  
embodiments the disulfide bonds are replaced by other covalent cross-links and in some  
cases the cysteines are substituted by other residues to provide for alternative covalent  
cross-links. The peptides may also include at least one trypsin or chymotrypsin cleavage

site and/or an amino or carboxy-terminal analgesic peptide or small molecule, e.g., AspPhe or some other analgesic peptide. When present within the peptide, the analgesic peptide or small molecule may be preceded by a chymotrypsin or trypsin cleavage site that allows release of the analgesic peptide or small molecule. Certain peptides include a  
5 functional chymotrypsin or trypsin cleavage site located so as to allow inactivation of the peptide upon cleavage. Certain peptides having a functional cleavage site undergo cleavage and gradual inactivation in the digestive tract, and this is desirable in some circumstances. In certain peptides, a functional chymotrypsin site is altered, increasing the stability of the peptide *in vivo*.

10

The methods described herein include a method for increasing intestinal motility comprising administering a GC-C receptor agonist, e.g., a peptide described herein, to a patient in need thereof; a method for treating a disorder associated with reduced gastrointestinal transit rates or reduced gastrointestinal motility comprising administering  
15 a GC-C receptor agonist, e.g., a peptide described herein, to a patient in need thereof; a method for treating a gastrointestinal hypomotility disorder comprising administering a GC-C receptor agonist, e.g., a peptide described herein, to a patient in need thereof; a method for treating a non-inflammatory gastrointestinal disorder comprising administering a GC-C receptor agonist, e.g., a peptide described herein, to a patient in  
20 need thereof; a method for treating a gastrointestinal disorder other than Crohn's disease and ulcerative colitis comprising administering a GC-C receptor agonist to a patient in need thereof; and methods and compositions for increasing intestinal motility comprising administering a GC-C receptor agonist to a patient in need thereof. The disorders which can be treated by administering a GC-C receptor agonist include, for example,  
25 constipation, constipation dominant irritable bowel syndrome and pelvic floor dyssynergia. In certain embodiments the patient has been diagnosed as suffering from IBS according to the Rome criteria. In certain embodiments the patient is female.

In certain embodiments the peptides include either one or two or more contiguous negatively charged amino acids (e.g., Asp or Glu) or one or two or more contiguous positively charged residues (e.g., Lys or Arg) or one or two or more contiguous positively or negatively charged amino acids at the carboxy terminus. In these embodiments all of the flanking amino acids at the carboxy terminus are either positively or negatively charged. In other embodiments the carboxy terminal charged amino acids are preceded by a Leu. For example, any of the following amino acid sequences can be added to the carboxy terminus of the peptide: Asp; Asp Lys; Lys Lys Lys Lys Lys Lys; Asp Lys Lys Lys Lys Lys Lys; Leu Lys Lys; and Leu Asp. It is also possible to simply add Leu at the carboxy terminus.

Described herein is a peptide or a pharmaceutically acceptable salt thereof comprising the amino acid sequence:

Xaa<sub>1</sub> Xaa<sub>2</sub> Xaa<sub>3</sub> Cys Glu Xaa<sub>6</sub> Xaa<sub>7</sub> Cys Xaa<sub>9</sub> Pro Ala Cys Thr Gly Xaa<sub>15</sub> Xaa<sub>16</sub> (SEQ ID NO:7) or a pharmaceutically acceptable salt thereof,

wherein

Xaa<sub>1</sub> is any amino acid or is missing;

Xaa<sub>2</sub> is Ala, Gly, Lys, Ser, Val or is missing;

Xaa<sub>3</sub> is Cys or D-Cys;

Xaa<sub>6</sub> is any amino acid;

Xaa<sub>7</sub> is Cys or D-Cys;

Xaa<sub>9</sub> is Asn or Thr;

Xaa<sub>15</sub> is Cys or D-Cys;

Xaa<sub>16</sub> is Lys, Tyr or is missing;

provided that:

- (a) one or more of Xaa<sub>3</sub>, Xaa<sub>7</sub> and Xaa<sub>15</sub> is D-Cys when Xaa<sub>16</sub> is other than Lys; and  
 (b) the peptide does not consist of the sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys.



In various embodiments: Xaa<sub>3</sub> is D-Cys; Xaa<sub>7</sub> is D-Cys; Xaa<sub>15</sub> is D-Cys; Xaa<sub>3</sub> is D-Cys; Xaa<sub>7</sub> is Cys; Xaa<sub>15</sub> is D-Cys; Xaa<sub>6</sub> is Val, Ile, Leu Tyr, Phe, or Trp; Xaa<sub>6</sub> is Val, Ile, or Leu; Xaa<sub>6</sub> is Val; Xaa<sub>6</sub> is Ile; Xaa<sub>6</sub> is Leu; Xaa<sub>6</sub> is Tyr, Phe, Trp; Xaa<sub>1</sub> is any amino acid; Xaa<sub>1</sub> is Gly or Ala; Xaa<sub>1</sub> is Gly; Xaa<sub>1</sub> is Ala; Xaa<sub>1</sub> is missing; Xaa<sub>6</sub> is Tyr; Xaa<sub>6</sub> is Phe; Xaa<sub>6</sub> is Trp; at least one of Xaa<sub>3</sub>, Xaa<sub>7</sub> and Xaa<sub>15</sub> is D-Cys; at least two of Xaa<sub>3</sub>, Xaa<sub>7</sub> and Xaa<sub>15</sub> are D-Cys; Xaa<sub>3</sub>, Xaa<sub>7</sub> and Xaa<sub>15</sub> are all D-Cys; Xaa<sub>9</sub> is Asn; Xaa<sub>9</sub> is Thr; Xaa<sub>16</sub> is Lys; Xaa<sub>16</sub> is Tyr; Xaa<sub>16</sub> is missing; the peptide is a peptide in any of Figures 3a and 3b; and the peptide is purified.

10 Also described is a pharmaceutical composition comprising any of the aforementioned peptides and a pharmaceutically acceptable carrier.

Also described is a method for reducing fluid retention, the method comprising administering the pharmaceutical composition comprising any of the aforementioned peptides and a pharmaceutically acceptable carrier or a pharmaceutical composition  
15 comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys.

Also described is a method of treating a disorder selected from: heart failure,  
20 hypertension, salt dependent forms of high blood pressure, hepatic edema, or liver cirrhosis comprising administering a pharmaceutical composition comprising any of the aforementioned peptides and a pharmaceutically acceptable carrier or a pharmaceutical composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys.

25 The peptides can be used to treat chronic or acute heart failure. In acute heart failure the patient appears to be in good health, but suddenly develops a large myocardial infarction or rupture of a cardiac valve. The acute heart failure is usually largely systolic and the sudden reduction in cardiac output often results in systemic hypotension without

peripheral edema. Chronic heart failure is typically observed in patients with dilated cardiomyopathy or multivalvular heart disease that develops or progresses slowly. In chronic heart failure, arterial pressure tends to be well maintained until very late in the course, but there is often accumulation of peripheral edema

5

Also described is a method for increasing naturesis comprising administering the pharmaceutical composition comprising any of the aforementioned peptides and a pharmaceutically acceptable carrier or a pharmaceutical composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala  
10 Cys Thr Gly Cys.

Also described is a method for increasing diuresis comprising administering the pharmaceutical composition comprising any of the aforementioned peptides and a pharmaceutically acceptable carrier or a pharmaceutical composition comprising a  
15 peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys.

Also described is a method of treating a gastrointestinal disorder comprising administering the pharmaceutical composition comprising any of the aforementioned  
20 peptides and a pharmaceutically acceptable carrier or a pharmaceutical composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys.

In various embodiments the gastrointestinal disorder is selected from: a gastrointestinal  
25 motility disorder, chronic intestinal pseudo-obstruction, colonic pseudo-obstruction, Crohn's disease, duodenogastric reflux, dyspepsia, functional dyspepsia, nonulcer dyspepsia, a functional gastrointestinal disorder, functional heartburn, gastroesophageal reflux disease (GERD), gastroparesis, irritable bowel syndrome, post-operative ileus,

inflammatory bowel disorder, ulcerative colitis, constipation, chronic constipation, chronic idiopathic constipation.

Also described is a method for treating obesity comprising administering the  
5 pharmaceutical composition comprising any of the aforementioned peptides and a  
pharmaceutically acceptable carrier or a pharmaceutical composition comprising a  
peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala  
Cys Thr Gly Cys.

10 Also described is a method for treating benign prostatic hyperplasia comprising  
administering the pharmaceutical composition comprising any of the aforementioned  
peptides and a pharmaceutically acceptable carrier or a pharmaceutical composition  
comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys  
Asn Pro Ala Cys Thr Gly Cys.

15 Also described is a method for treating constipation comprising administering the  
pharmaceutical composition comprising any of the aforementioned peptides and a  
pharmaceutically acceptable carrier or a pharmaceutical composition comprising a  
peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala  
20 Cys Thr Gly Cys.

In various embodiments: the constipation is idiopathic constipation; the constipation is  
chronic idiopathic constipation; the gastrointestinal disorder is irritable bowel syndrome;  
the irritable bowel syndrome is diarrhea-predominant irritable bowel syndrome; the  
25 irritable bowel syndrome is constipation-predominant irritable bowel syndrome; the  
irritable bowel syndrome is alternating-irritable bowel syndrome; the gastrointestinal  
disorder is inflammatory bowel disorder; the gastrointestinal disorder is Crohn's disease;  
and the gastrointestinal disorder is ulcerative colitis.

Also described is a method for increasing gastrointestinal motility comprising administering the pharmaceutical composition comprising any of the aforementioned peptides and a pharmaceutically acceptable carrier or a pharmaceutical composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys  
5 Asn Pro Ala Cys Thr Gly Cys.

Also described is a method for decreasing gastrointestinal pain or visceral pain comprising administering the pharmaceutical composition comprising any of the aforementioned peptides and a pharmaceutically acceptable carrier or a pharmaceutical  
10 composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys.

Also described is a method of preventing or treating a side-effect associated with opioid administration, the method comprising administering to a patient that is being treated  
15 with an opioid a pharmaceutical composition comprising any of the aforementioned peptides and a pharmaceutically acceptable carrier or a pharmaceutical composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys. In various embodiments: the patient is being treated with an opioid selected from the group consisting of alfentanil, buprenorphine, butorphanol,  
20 codeine, dezocine, dihydrocodeine, fentanyl, hydrocodone, hydromorphone, levorphanol, meperidine (pethidine), methadone, morphine, nalbuphine, oxycodone, oxymorphone, pentazocine, propiram, propoxyphene, sufentanil and tramadol; the patient is being treated with an opioid selected from the group consisting of: morphine, codeine, oxycodone, hydrocodone, dihydrocodeine, propoxyphene, fentanyl and tramadol; the side  
25 effect is selected from the group consisting of constipation, nausea and vomiting; and the method further comprises administering an opioid antagonist (e.g., naloxone or naltrexone).

Also described is a pharmaceutical composition comprising an opioid and any forgoing peptide or a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys. In various embodiments: the opioid is selected from the group consisting of alfentanil, buprenorphine, butorphanol, codeine, dezocine,  
5 dihydrocodeine, fentanyl, hydrocodone, hydromorphone, levorphanol, meperidine (pethidine), methadone, morphine, nalbuphine, oxycodone, oxymorphone, pentazocine, propiram, propoxyphene, sufentanil and tramadol; and the opioid is selected from the group consisting of: morphine, codeine, oxycodone, hydrocodone, dihydrocodeine, propoxyphene, fentanyl and tramadol.

10

Also described is a pharmaceutical kit comprising:

- (a) a first container containing pharmaceutical dosage units comprising an effective amount of an opioid; and
- (b) a second container containing pharmaceutical dosage units comprising an  
15 effective amount of a forgoing peptide or a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys. In various embodiments: the opioid is selected from the group consisting of alfentanil, buprenorphine, butorphanol, codeine, dezocine, dihydrocodeine, fentanyl, hydrocodone, hydromorphone, levorphanol, meperidine (pethidine), methadone, morphine, nalbuphine, oxycodone, oxymorphone,  
20 pentazocine, propiram, propoxyphene, sufentanil and tramadol; and the opioid is selected from the group consisting of: morphine, codeine, oxycodone, hydrocodone, dihydrocodeine, propoxyphene, fentanyl and tramadol.

Also described is a method for preparing a pharmaceutical composition comprising  
25 admixing a forgoing peptide and a pharmaceutically acceptable carrier.

Also described herein are purified peptides comprising, consisting of, or consisting essentially of the amino acid sequence of SEQ ID NO: 7 and those peptides depicted in Figures 3a and Figure 3b.

Also described herein are pharmaceutical compositions comprising peptides comprising, consisting of, or consisting essentially of the amino acid sequence of SEQ ID NO:7 and those peptides depicted in Figure 3a and Figure 3b.

5

In certain embodiments, for example, when fully folded, the peptide includes disulfide bonds between Cys<sub>3</sub> and Cys<sub>8</sub>, between Cys<sub>4</sub> and Cys<sub>12</sub> and between Cys<sub>7</sub> and Cys<sub>15</sub>. In other embodiments, the peptide is a reduced peptide having no disulfide bonds. In still other embodiments the peptide has one or two disulfide bonds chosen from: a disulfide  
10 bond between Cys<sub>3</sub> and Cys<sub>8</sub>, a disulfide bond between Cys<sub>4</sub> and Cys<sub>12</sub> and a disulfide bond between Cys<sub>7</sub> and Cys<sub>15</sub>. In other embodiments, one or more of Cys<sub>3</sub>, Cys<sub>7</sub>, or Cys<sub>15</sub> is a D-Cys residue and the D-Cys residues can form disulfide bonds in the same manner as the Cys residues. Thus, the peptide may include, for example, one or more disulfide bonds between D-Cys<sub>3</sub> and Cys<sub>8</sub>, between Cys<sub>4</sub> and Cys<sub>12</sub>, between D-Cys<sub>7</sub> and Cys<sub>15</sub>,  
15 between Cys<sub>7</sub> and D-Cys<sub>15</sub>, between D-Cys<sub>7</sub> and D-Cys<sub>15</sub>.

In some embodiments the peptide is 13, 14, 15, or 16 amino acids long.

In certain embodiments, one or more amino acids can be replaced by a non-naturally  
20 occurring amino acid or a naturally or non-naturally occurring amino acid analog. In certain embodiments, one or more L-amino acids can be substituted with a D-amino acid. There are many amino acids beyond the standard 20 amino acids (Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val). Some are naturally-occurring others are not (see, for example, Hunt, *The Non-Protein Amino*  
25 *Acids: In Chemistry and Biochemistry of the Amino Acids*, Barrett, Chapman and Hall, 1985). For example, an aromatic amino acid can be replaced by 3,4-dihydroxy-L-phenylalanine, 3-iodo-L-tyrosine, triiodothyronine, L-thyroxine, phenylglycine (Phg) or nor-tyrosine (norTyr). Phg and norTyr and other amino acids including Phe and Tyr can be substituted by, e.g., a halogen, -CH<sub>3</sub>, -OH, -CH<sub>2</sub>NH<sub>3</sub>, -C(O)H, -CH<sub>2</sub>CH<sub>3</sub>, -CN, -

CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, -SH, or another group. Any amino acid can be substituted by the D-form of the amino acid. Thus, for example, a cysteine residue can be substituted by a D-cysteine residue.

- 5 With regard to non-naturally occurring amino acids or naturally and non-naturally occurring amino acid analogs, a number of substitutions in the peptide of SEQ ID NO:7 or the peptides of Figure 3a and Figure 3b are possible alone or in combination.

Glu can be replaced by *gamma*-Hydroxy-Glu or *gamma*-Carboxy-Glu.

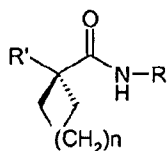
10

Ala can be replaced by an alpha substituted amino acid such as *L-alpha*-methylphenylalanine or by analogues such as: 3-Amino-Tyr; Tyr(CH<sub>3</sub>); Tyr(PO<sub>3</sub>(CH<sub>3</sub>)<sub>2</sub>); Tyr(SO<sub>3</sub>H); *beta*-Cyclohexyl-Ala; *beta*-(1-Cyclopentenyl)-Ala; *beta*-Cyclopentyl-Ala; *beta*-Cyclopropyl-Ala; *beta*-Quinolyl-Ala; *beta*-(2-Thiazolyl)-Ala; *beta*-(Triazole-1-yl)-Ala; *beta*-(2-Pyridyl)-Ala; *beta*-(3-Pyridyl)-Ala; Amino-Phe; Fluoro-Phe; Cyclohexyl-Gly; *t*Bu-Gly; *beta*-(3-benzothienyl)-Ala; *beta*-(2-thienyl)-Ala; 5-Methyl-Trp; and 4-Methyl-Trp.

15

Pro can be an N(alpha)-C(alpha) cyclized amino acid analogues with the structure:

20



$n = 0, 1, 2, 3$  . Pro can also be homopro (L-pipecolic acid); hydroxy-Pro; 3,4-Dehydro-Pro; 4-fluoro-Pro; or *alpha*-methyl-Pro.

Val or Leu can also be an alpha-substituted or N-methylated amino acid such as *alpha*-amino isobutyric acid (*aib*), *L/D-alpha*-ethylalanine (*L/D*-isovaline), *L/D*-methylvaline, or *L/D-alpha*-methylleucine or a non-natural amino acid such as *beta*-fluoro-Ala.

25

Gly can be *alpha*-amino isobutyric acid (*aib*) or *L/D-alpha*-ethylalanine (*L/D-isovaline*).

Further examples of unnatural amino acids include: an unnatural analogue of tyrosine; an  
5 unnatural analogue of glutamine; an unnatural analogue of phenylalanine; an unnatural  
analogue of serine; an unnatural analogue of threonine; an alkyl, aryl, acyl, azido, cyano,  
halo, hydrazine, hydrazide, hydroxyl, alkenyl, alkynyl, ether, thiol, sulfonyl, seleno, ester,  
thioacid, borate, boronate, phospho, phosphono, phosphine, heterocyclic, enone, imine,  
aldehyde, hydroxylamine, keto, or amino substituted amino acid, or any combination  
10 thereof; an amino acid with a photoactivatable cross-linker; a spin-labeled amino acid; a  
fluorescent amino acid; an amino acid with a novel functional group; an amino acid that  
covalently or noncovalently interacts with another molecule; a metal binding amino acid;  
an amino acid that is amidated at a site that is not naturally amidated, a metal-containing  
amino acid; a radioactive amino acid; a photocaged and/or photoisomerizable amino acid;  
15 a biotin or biotin-analogue containing amino acid; a glycosylated or carbohydrate  
modified amino acid; a keto containing amino acid; amino acids comprising polyethylene  
glycol or polyether; a heavy atom substituted amino acid (e.g., an amino acid containing  
deuterium, tritium, <sup>13</sup>C, <sup>15</sup>N, or <sup>18</sup>O); a chemically cleavable or photocleavable amino  
acid; an amino acid with an elongated side chain; an amino acid containing a toxic group;  
20 a sugar substituted amino acid, e.g., a sugar substituted serine or the like; a carbon-linked  
sugar-containing amino acid; a redox-active amino acid; an  $\alpha$ -hydroxy containing acid;  
an amino thio acid containing amino acid; an  $\alpha$ ,  $\alpha$  disubstituted amino acid; a  $\beta$ -amino  
acid; a cyclic amino acid other than proline; an O-methyl-L-tyrosine; an L-3-(2-  
naphthyl)alanine; a 3-methyl-phenylalanine; a *p*-acetyl-L-phenylalanine; an O-4-allyl-L-  
25 tyrosine; a 4-propyl-L-tyrosine; a tri-O-acetyl-GlcNAc $\beta$ -serine; an L-Dopa; a fluorinated  
phenylalanine; an isopropyl-L-phenylalanine; a *p*-azido-L-phenylalanine; a *p*-acyl-L-  
phenylalanine; a *p*-benzoyl-L-phenylalanine; an L-phosphoserine; a phosphoserine; a  
phosphonotyrosine; a *p*-iodo-phenylalanine; a 4-fluorophenylglycine; a *p*-  
bromophenylalanine; a *p*-amino-L-phenylalanine; an isopropyl-L-phenylalanine; L-3-(2-



naphthyl)alanine; an amino-, isopropyl-, or O-allyl-containing phenylalanine analogue; a dopa, O-methyl-L-tyrosine; a glycosylated amino acid; a p-(propargyloxy)phenylalanine; dimethyl-Lysine; hydroxy-proline; mercaptopropionic acid; methyl-lysine; 3-nitro-tyrosine; norleucine; pyro-glutamic acid; Z (Carbobenzoxyl); ε-Acetyl-Lysine; β-alanine; 5 aminobenzoyl derivative; aminobutyric acid (Abu); citrulline; aminohexanoic acid; aminoisobutyric acid; cyclohexylalanine; d-cyclohexylalanine; hydroxyproline; nitro-arginine; nitro-phenylalanine; nitro-tyrosine; norvaline; octahydroindole carboxylate; ornithine; penicillamine; tetrahydroisoquinoline; acetamidomethyl protected amino acids and pegylated amino acids. Further examples of unnatural amino acids and amino acid 10 analogs can be found in U.S. 20030108885, U.S. 20030082575, US20060019347 (paragraphs 410-418) and the references cited therein. The peptides described herein can include further modifications including those described in US20060019347, paragraph 589.

15 In some embodiments, an amino acid can be replaced by a naturally-occurring, non-essential amino acid, e.g., taurine.

Methods to manufacture peptides containing unnatural amino acids can be found in, for example, US20030108885, US20030082575, US20060019347, Deiters et al., J Am 20 Chem Soc. (2003) 125:11782-3, Chin et al., Science (2003) 301:964-7, and the references cited therein.

Peptides that include non-natural amino acids can also be prepared using the methods described in WO02086075

25

The peptides described herein can have one or more conventional peptide bonds replaced by an alternative bond. Such replacements can increase the stability of the peptide. For example, replacement of the peptide bond between D-Cys<sub>15</sub> or Cys<sub>15</sub> and Xaa<sub>16</sub> with an alternative bond can reduce cleavage by carboxy peptidases and may increase half-life in

the digestive tract. Bonds that can replace peptide bonds include: a retro-inverso bonds (C(O)-NH instead of NH-C(O)); a reduced amide bond (NH-CH<sub>2</sub>); a thiomethylene bond (S-CH<sub>2</sub> or CH<sub>2</sub>-S); an oxomethylene bond (O-CH<sub>2</sub> or CH<sub>2</sub>-O); an ethylene bond (CH<sub>2</sub>-CH<sub>2</sub>); a thioamide bond (C(S)-NH); a trans-olefine bond (CH=CH); an fluoro substituted trans-olefine bond (CF=CH); a ketomethylene bond (C(O)-CHR or CHR-C(O) wherein R is H or CH<sub>3</sub>); and a fluoro-ketomethylene bond (C(O)-CFR or CFR-C(O) wherein R is H or F or CH<sub>3</sub>).

The peptides described herein can be modified using standard modifications.

10 Modifications may occur at the amino (N-), carboxy (C-) terminus, internally or a combination of any of the preceding. In one aspect described herein, there may be more than one type of modification of the peptide. Modifications include but are not limited to: acetylation, amidation, biotinylation, cinnamoylation, farnesylation, formylation, myristoylation, palmitoylation, phosphorylation (Ser, Tyr or Thr), stearoylation, 15 succinylation, sulfurylation and cyclisation (via disulfide bridges or amide cyclisation), and modification by Cy3 or Cy5. The peptides described herein may also be modified by 2, 4-dinitrophenyl (DNP), DNP-lysine, modification by 7-Amino-4-methyl-coumarin (AMC), fluorescein, NBD (7-Nitrobenz-2-Oxa-1,3-Diazole), p-nitro-anilide, rhodamine B, EDANS (5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid), dabcy1, dabsyl, 20 dansyl, Texas Red, Fmoc, and Tamra (Tetramethylrhodamine). The peptides described herein may also be conjugated to, for example, polyethylene glycol (PEG); alkyl groups (e.g., C1-C20 straight or branched alkyl groups); fatty acid radicals; combinations of PEG, alkyl groups and fatty acid radicals (see U.S. Patent 6,309,633; Soltero et al., 2001 Innovations in Pharmaceutical Technology 106-110); BSA and KLH (Keyhole Limpet Hemocyanin). The addition of PEG and other polymers which can be used to modify 25 peptides described herein is described in US2006019347 section IX.

The peptides and agonists described herein can be chemically modified to increase therapeutic activity by synthetically adding sugar moieties (WO 88/02756; WO

89/09786; DE 3910667 A1, EP 0 374 089 A2; and U.S. 4,861,755), adding cationic anchors (EP0363589), lipid moieties (WO91/09837; U.S. 4,837,303) or the substituents described as compounds I, II, and III in US5552520.

- 5 The peptides described herein bear some sequence similarity to ST peptides. However, they include amino acid changes and/or additions that improve functionality. These changes can, for example, increase or decrease activity (e.g., increase or decrease the ability of the peptide to stimulate intestinal motility), alter the ability of the peptide to fold correctly, alter the stability of the peptide, alter the ability of the peptide to bind the  
10 GC-C receptor and/or decrease toxicity. In some cases the peptides may function more desirably than wild-type ST peptide. For example, they may limit undesirable side effects such as diarrhea and dehydration.

- In some embodiments one or both members of one or more pairs of Cys residues  
15 (including where a Cys residue has been substituted with a D-cys residue) which normally form a disulfide bond can be replaced by homocysteine, penicillamine, 3-mercaptoproline (Kolodziej et al. 1996 Int J Pept Protein Res 48:274);  $\beta$ ,  $\beta$  dimethylcysteine (Hunt et al. 1993 Int J Pept Protein Res 42:249) or diaminopropionic acid (Smith et al. 1978 J Med Chem 21:117) to form alternative internal cross-links at the  
20 positions of the normal disulfide bonds.

- In addition, one or more disulfide bonds can be replaced by alternative covalent cross-links, e.g., an amide linkage (-CH<sub>2</sub>CH(O)NHCH<sub>2</sub>- or -CH<sub>2</sub>NHCH(O)CH<sub>2</sub>-), an ester linkage, a thioester linkage, a lactam bridge, a carbamoyl linkage, a urea linkage, a  
25 thiourea linkage, a phosphonate ester linkage, an alkyl linkage (-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-), an alkenyl linkage(-CH<sub>2</sub>CH=CHCH<sub>2</sub>-), an ether linkage (-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>- or -CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>-), a thioether linkage (-CH<sub>2</sub>CH<sub>2</sub>SCH<sub>2</sub>- or -CH<sub>2</sub>SCH<sub>2</sub>CH<sub>2</sub>-), an amine linkage (-CH<sub>2</sub>CH<sub>2</sub>NHCH<sub>2</sub>- or -CH<sub>2</sub>NHCH<sub>2</sub>CH<sub>2</sub>-) or a thioamide linkage (-CH<sub>2</sub>CH(S)HNHCH<sub>2</sub>- or -CH<sub>2</sub>NHCH(S)CH<sub>2</sub>-). For example, Ledu et al. (Proc Nat'l

Acad. Sci. 100:11263-78, 2003) describe methods for preparing lactam and amide cross-links. Schafmeister et al. (J. Am. Chem. Soc. 122:5891, 2000) describes stable, hydrocarbon cross-links. Hydrocarbon cross links can be produced via metathesis (or methathesis followed by hydrogenation in the case of saturated hydrocarbons cross-links) using one or another of the Grubbs catalysts (available from Materia, Inc. and Sigma-Aldrich and described, for example, in U.S. Patent No. 5,831,108 and 6,111,121). In some cases, the generation of such alternative cross-links requires replacing the Cys residues with other residues such as Lys or Glu or non-naturally occurring amino acids. In addition the lactam, amide and hydrocarbon cross-links can be used to stabilize the peptide even if they link amino acids at positions other than those occupied by Cys. Such cross-links can occur between two amino acids that are separated by two amino acids or between two amino acids that are separated by six amino acids (see, e.g., Schafmeister et al. (J. Am. Chem. Soc. 122:5891, 2000)).

The peptide may contain additional carboxyterminal or amino terminal amino acids or both. For example, the peptide can include an amino terminal sequence that facilitates recombinant production of the peptide and is cleaved prior to administration of the peptide to a patient. The peptide can also include other amino terminal or carboxyterminal amino acids. In some cases the additional amino acids protect the peptide, stabilize the peptide or alter the activity of the peptide. In some cases some or all of these additional amino acids are removed prior to administration of the peptide to a patient. The peptide can include 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 80, 90, 100 or more amino acids at its amino terminus or carboxy terminus or both. The number of flanking amino acids need not be the same. For example, there can be 10 additional amino acids at the amino terminus of the peptide and none at the carboxy terminus.

The peptides can be co-administered with or linked, e.g., covalently linked to any of a variety of other peptides or compounds including analgesic peptides or analgesic compounds including, without limitation, the agents described herein.

Amino acid, non-amino acid, peptide and non-peptide spacers can be interposed between a peptide that is a GC-C receptor agonist and a peptide that has some other biological function, e.g., an analgesic peptide or a peptide used to treat obesity. The linker can be one that is cleaved from the flanking peptides *in vivo* or one that remains linked to the flanking peptides *in vivo*. For example, glycine, beta-alanine, glycyl-glycine, glycyl-beta-alanine, gamma-aminobutyric acid, 6-aminocaproic acid, L-phenylalanine, L-tryptophan and glycil-L-valil-L-phenylalanine can be used as spacers (Chaltin et al. 2003 Helvetica Chimica Acta 86:533-547; Caliceti et al. 1993 FARMCO 48:919-32) as can polyethylene glycols (Butterworth et al. 1987 J. Med. Chem 30:1295-302) and maleimide derivatives (King et al. 2002 Tetrahedron Lett. 43:1987-1990). Various other linkers are described in the literature (Nestler 1996 Molecular Diversity 2:35-42; Finn et al. 1984 Biochemistry 23:2554-8; Cook et al. 1994 Tetrahedron Lett. 35:6777-80; Brokx et al. 2002 Journal of Controlled Release 78:115-123; Griffin et al. 2003 J. Am. Chem. Soc. 125:6517-6531; Robinson et al. 1998 Proc. Natl. Acad. Sci. USA 95:5929-5934). Linkers are also described in US20050171014, for example, amino acid linkers such as FALA, VLALA, ALAL, ALALA, 2-cyclohexyl-L-alanine-LALA, 2-cyclohexyl-L-alanine-2-cyclohexyl-L-alanine-LAL, 1-naphtyl-alanine-ChaLAL and 1-naphtyl-alanine-LALA. Peptides and agonists described herein can also be conjugated to: an affinity tag (such as (histidine 6) H6), a HIV tat peptide residues 49-57, HIV tat peptide residues 49-56, the tat sequence YGRKKRRQRRR, a polyarginine peptide having from 6 to 20 residues (such as R6) and the following peptide sequences: YARKARRQARR, YARAAARQARA, YARAARRAARR, YARAARRAARA, ARRRRRRRRR, and YAAARRRRRRR, which are disclosed in WO 99/29721 and in US patent No. 6,221,355 (seq. id. nos. 3-8).

The peptides described herein can be attached to one, two or more different moieties each providing the same or different functions. For example, the peptide can be linked to a molecule that is an analgesic and to a peptide that is used to treat obesity. The peptide and various moieties can be ordered in various ways. For example, a peptide described herein can have an analgesic peptide linked to its amino terminus and an anti-obesity

peptide linked to its carboxy terminus. The additional moieties can be directly covalently bonded to the peptide or can be bonded via linkers.

The peptides described herein can be a cyclic peptide or a linear peptide. In addition, multiple copies of the same peptide can be incorporated into a single cyclic or linear  
5 peptide.

The peptides can include the amino acid sequence of a peptide that occurs naturally in a vertebrate (e.g., mammalian) species or in a bacterial species. In addition, the peptides can be partially or completely non-naturally occurring peptides. Also within the disclosure are peptidomimetics corresponding to the peptides described herein.

10

Described herein is a method for treating congestive heart failure, the method comprising administering to a patient a pharmaceutical composition comprising a purified peptide comprising, consisting of or consisting essentially of the amino acid sequence of SEQ ID NO:7 (e.g., a peptide in Figure 3a or Figure 3b). The peptide can be administered alone  
15 or in combination with another agent for treatment of congestive heart failure, for example, a natriuretic peptide such as atrial natriuretic peptide, brain natriuretic peptide or C-type natriuretic peptide, an inhibitor of angiotensin converting enzyme, a diuretic (e.g. furesomide (Lasix), bumetanide (Bumex), ethacrynic acid (Edecrin), torsemide (Demadex), amiloride (Midamor), spironolactone (Aldactone), chorthiazide (Diuril),  
20 metolazone (Zaroxilyn)), an Angiotension-Converting Enzyme (ACE) inhibitor (e.g. captopril (Capoten), enalapril (Vasotec), lisinopril (Prinivil, Zestril), ramipril (Altace)), a Beta blocker (e.g. carvedilol (Coreg) or an inotropes (e.g. digoxin, dobutamine, dopamine Milrinone). In various embodiments the congestive heart failure is categorized as Class II congestive heart failure; the congestive heart failure is categorized as Class III  
25 congestive heart failure; and the congestive heart failure is categorized as Class IV congestive heart failure. The New York Heart Association (NYHA) functional classification system relates congestive heart failure symptoms to everyday activities and the patient's quality of life. The NYHA defines the classes of patient symptoms relating

to congestive heart failure as: Class II-slight limitation of physical activity, comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea; Class III-marked limitation of physical activity, comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea and Class IV- unable to carry out any physical activity without discomfort, symptoms of cardiac insufficiency at rest, if any physical activity is undertaken, discomfort is increased. Heart failure treatment using the peptides and methods described herein can also be classified according to the ACC/AHA guidelines (Stage A: At risk for developing heart failure without evidence of cardiac dysfunction; Stage B: Evidence of cardiac dysfunction without symptoms; Stage C: Evidence of cardiac dysfunction with symptoms; and Stage D: Symptoms of heart failure despite maximal therapy).

Described herein is a method for treating a gastrointestinal disorder, the method comprising administering a peptide comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO:7, e.g., a peptide in Figure 3a or Figure 3b. In various embodiments: the patient is suffering from a disorder selected from the group consisting of: gastrointestinal motility disorders, chronic intestinal pseudo-obstruction, colonic pseudo-obstruction, Crohn's disease, duodenogastric reflux, dyspepsia, functional dyspepsia, nonulcer dyspepsia, a functional gastrointestinal disorder, functional heartburn, gastroesophageal reflux disease (GERD), gastroparesis, irritable bowel syndrome, post-operative ileus, ulcerative colitis, chronic constipation, and disorders and conditions associated with constipation (e.g. constipation associated with use of opiate pain killers, post-surgical constipation, and constipation associated with neuropathic disorders as well as other conditions and disorders are described herein); the patient is suffering from a gastrointestinal motility disorder, chronic intestinal pseudo-obstruction, colonic pseudo-obstruction, Crohn's disease, duodenogastric reflux, dyspepsia, functional dyspepsia, nonulcer dyspepsia, a functional gastrointestinal disorder, functional heartburn, gastroesophageal reflux disease (GERD), gastroparesis, inflammatory bowel disease, irritable bowel syndrome (e.g. d-IBS, c-IBS, and/or a-IBS), post-operative ileus,

ulcerative colitis, chronic constipation, and disorders and conditions associated with constipation (e.g. constipation associated with use of opiate pain killers, post-surgical constipation, and constipation associated with neuropathic disorders as well as other conditions and disorders are described herein); the patient has been diagnosed with a functional gastrointestinal disorder according to the Rome Criteria (e.g. Rome II), the patient has been diagnosed with irritable bowel syndrome (e.g. (e.g. diarrhea predominant-IBS, constipation predominant-IBS, and/or alternating-IBS), according to the Rome Criteria (e.g. Rome II); the composition is administered orally; the peptide comprises 30 or fewer amino acids, the peptide comprises 20 or fewer amino acids, the peptide comprises no more than 5 amino acids prior to Cys<sub>6</sub>; the peptide comprises 14 amino acids, the peptide comprises 13 amino acids; the peptide comprises 150, 140, 130, 120, 110, 100, 90, 80, 70, 60, 50, 40, or 30 or fewer amino acids. In other embodiments, the peptide comprises 20 or fewer amino acids. In other embodiments the peptide comprises no more than 20, 15, 10, or 5 peptides subsequent to Cys<sub>18</sub>. In certain embodiments Xaa<sub>19</sub> is a chymotrypsin or trypsin cleavage site and an analgesic peptide is present immediately following Xaa<sub>19</sub>.

Described herein is a method for treating a patient suffering from constipation. Clinically accepted criteria that define constipation include the frequency of bowel movements, the consistency of feces and the ease of bowel movement. One common definition of constipation is less than three bowel movements per week. Other definitions include abnormally hard stools or defecation that requires excessive straining (Schiller 2001, *Aliment Pharmacol Ther* 15:749-763). Constipation may be idiopathic (functional constipation or slow transit constipation) or secondary to other causes including neurologic, metabolic or endocrine disorders. These disorders include diabetes mellitus, hypothyroidism, hyperthyroidism, hypocalcaemia, Multiple Sclerosis, Parkinson's disease, spinal cord lesions, Neurofibromatosis, autonomic neuropathy, Chagas disease, Hirschsprung's disease and Cystic fibrosis. Constipation may also be the result of surgery (postoperative ileus) or due to the use of drugs such as analgesics (like opioids),



antihypertensives, anticonvulsants, antidepressants, antispasmodics and antipsychotics. Also disclosed are methods for increasing gastrointestinal motility in a patient, the method comprising administering to a patient a pharmaceutical composition comprising a purified peptide comprising, consisting of or consisting essentially of the amino acid sequence of SEQ ID NO:7, e.g., a peptide in Figure 3a or Figure 3b.

Also disclosed are methods for increasing the activity of (activating) an intestinal guanylate cyclase (GC-C) receptor in a patient, the method comprising administering to a patient a pharmaceutical composition comprising a purified peptide comprising, consisting of or consisting essentially of the amino acid sequence of SEQ ID NO:7, e.g., a peptide in Figure 3a or Figure 3b.

Also disclosed is an isolated nucleic acid molecule comprising a nucleotide sequence encoding a peptide comprising, consisting of or consisting essentially of SEQ ID NO:7, e.g., a peptide in Figure 3a or Figure 3b.

Also described is a method for treating obesity, the method comprising administering to a patient a pharmaceutical composition comprising or consisting essentially of a purified peptide comprising, consisting of or consisting essentially of the amino acid sequence of SEQ ID NO:7, e.g., a peptide in Figure 3a or Figure 3b.

Also described is a method for treating benign prostatic hyperplasia, the method comprising administering to a patient a pharmaceutical composition comprising a purified peptide comprising, consisting of or consisting essentially of the amino acid sequence of SEQ ID NO:7, e.g., a peptide in Figure 3a or Figure 3b. The peptide can be administered alone or in combination with another agent for treatment of BPH, for example, a 5-alpha reductase inhibitor (e.g., finasteride) or an alpha adrenergic inhibitor (e.g., doxazosine).

Also described is a method for treating or reducing pain, including visceral pain, pain associated with a gastrointestinal disorder or pain associated with some other disorder, the method comprising administering to a patient a pharmaceutical composition comprising or consisting essentially of a purified peptide comprising, consisting of or  
5 consisting essentially of the amino acid sequence of SEQ ID NO:7, e.g., a peptide in Figure 3a or Figure 3b.

Also described is a method for treating inflammation, including inflammation of the gastrointestinal tract, e.g., inflammation associated with a gastrointestinal disorder or  
10 infection or some other disorder, the method comprising administering to a patient a pharmaceutical composition comprising a purified peptide comprising, consisting of or consisting essentially of the amino acid sequence of SEQ ID NO: 7, e.g., a peptide in Figure 3a or Figure 3b.

15 In prevention and/or treatment disorders associated with fluid and sodium retention (e.g., heart failure, congestive heart failure, kidney disease, etc), the agents described herein can be administered, for example, via a parenteral route, intravenously, and/or subcutaneously. Intravenous administration may comprise, for example, (1) one or more successive rounds of a bolus followed by an infusion or an infusion followed by a bolus,  
20 (2) infusion, and (3) bolus administration. The dosage may vary depending on the administration schedule. Thus, bolus administrations may involve dosing from about 0.1-1000 ug/kg, from about 1-100 ug/kg, or from about 10, 15, 20, 25, or 30 ug/kg. Infusion administrations may involve dosing from about 0.1-1000 ug/kg/hour, from about 1-100 ug/kg/hour, about 10 ug/kg/hour. During the duration of an infusion administration, the  
25 dosage may vary (for example, decreasing over time, increasing over time, and combinations thereof) or may remain constant. Subcutaneous administration may involve dosing from about 0.1-1000 ug/kg, from about 1-100 ug/kg, from about 10, 15, 20, 25, or 30 ug/kg.

As noted above, isolated nucleic acid molecules comprising a sequence encoding a peptide described herein are described. Also described are vectors, e.g., expression vectors that include such nucleic acid molecules and can be used to express a peptide described herein in a cultured cell (e.g., a eukaryotic cell or a prokaryotic cell). The vector can further include one or more regulatory elements, e.g., a heterologous promoter or elements required for translation operably linked to the sequence encoding the peptide. In some cases the nucleic acid molecule will encode an amino acid sequence that includes the amino acid sequence of a peptide described herein. For example, the nucleic acid molecule can encode a preprotein or a preproprotein that can be processed to produce a peptide described herein. In cases where unnatural amino acids are present in the peptides described herein, selector codons can be utilized in the synthesis of such peptides similar to that described in US20060019347 (for example, paragraphs 398-408, 457-499, and 576-588) herein incorporated by reference.

A vector that includes a nucleotide sequence encoding a peptide described herein or a peptide or peptide comprising a peptide described herein may be either RNA or DNA, single- or double-stranded, prokaryotic, eukaryotic, or viral. Vectors can include transposons, viral vectors, episomes, (e.g., plasmids), chromosomes inserts, and artificial chromosomes (e.g. BACs or YACs). Suitable bacterial hosts for expression of the encode peptide or peptide include, but are not limited to, *E. coli*. Suitable eukaryotic hosts include yeast such as *S. cerevisiae*, other fungi, vertebrate cells, invertebrate cells (e.g., insect cells), plant cells, human cells, human tissue cells, and whole eukaryotic organisms. (e.g., a transgenic plant or a transgenic animal). Further, the vector nucleic acid can be used to transfect a virus such as vaccinia or baculovirus (for example using the Bac-to-Bac® Baculovirus expression system (Invitrogen Life Technologies, Carlsbad, CA)).

As noted above the disclosure includes vectors and genetic constructs suitable for production of a peptide described herein or a peptide or peptide comprising such a

peptide. Generally, the genetic construct also includes, in addition to the encoding nucleic acid molecule, elements that allow expression, such as a promoter and regulatory sequences. The expression vectors may contain transcriptional control sequences that control transcriptional initiation, such as promoter, enhancer, operator, and repressor  
5 sequences. A variety of transcriptional control sequences are well known to those in the art and may be functional in, but are not limited to, a bacterium, yeast, plant, or animal cell. The expression vector can also include a translation regulatory sequence (e.g., an untranslated 5' sequence, an untranslated 3' sequence, a poly A addition site, or an internal ribosome entry site), a splicing sequence or splicing regulatory sequence, and a  
10 transcription termination sequence. The vector can be capable of autonomous replication or it can integrate into host DNA.

Also described are isolated host cells harboring one of the forgoing nucleic acid molecules and methods for producing a peptide by culturing such a cell and recovering  
15 the peptide or a precursor of the peptide. Recovery of the peptide or precursor may refer to collecting the growth solution and need not involve additional steps of purification. Proteins of the present disclosure, however, can be purified using standard purification techniques, such as, but not limited to, affinity chromatography, thermoprecipitation, immunoaffinity chromatography, ammonium sulfate precipitation, ion exchange  
20 chromatography, filtration, electrophoresis and hydrophobic interaction chromatography.

The peptides can be purified. Purified peptides are peptides separated from other proteins, lipids, and nucleic acids or from the compounds from which it is synthesized. The peptide can constitute at least 10, 20, 50, 70, 80 or 95% by dry weight of the purified  
25 preparation.

Also described is a method of increasing the level of cyclic guanosine 3'-monophosphate (cGMP) in an organ, tissue (e.g., the intestinal mucosa), or cell (e.g., a cell bearing GC-A receptor) by administering to a patient a composition comprising or consisting essentially

of a purified peptide comprising, consisting of or consisting essentially of the amino acid sequence of SEQ ID NO:7 (e.g., a peptide in Figure 3a or Figure 3b).

Also described is a method for treating hypertension. The method comprises:  
5 administering to the patient a pharmaceutical composition comprising, consisting essentially of, or consisting of a peptide or agonist described herein and a pharmaceutically acceptable carrier. The composition can be administered in combination with another agent for treatment of hypertension, for example, a diuretic, an ACE inhibitor, an angiotensin receptor blocker, a beta-blocker, or a calcium channel  
10 blocker.

Also described is a method for treating secondary hyperglycemias in connection with pancreatic diseases (chronic pancreatitis, pancreatectomy, hemochromatosis) or endocrine diseases (acromegaly, Cushing's syndrome, pheochromocytoma or  
15 hyperthyreosis), drug-induced hyperglycemias (benzothiadiazine saluretics, diazoxide or glucocorticoids), pathologic glucose tolerance, hyperglycemias, dyslipoproteinemias, adiposity, hyperlipoproteinemias and/or hypotensions is described. The method comprises: administering to the patient a pharmaceutical composition comprising, consisting essentially of, or consisting of a peptide or agonist described herein and a  
20 pharmaceutically acceptable carrier.

The peptides described herein can be present with a counterion. Useful counterions include salts of: acetate, benzenesulfonate, benzoate, calcium edetate, camsylate, carbonate, citrate, edetate (EDTA), edisylate, embonate, esylate, fumarate, gluceptate,  
25 gluconate, glutamate, glycolylarsanilate, hexylresorcinate, iodide, bromide, chloride, hydroxynaphthoate, isethionate, lactate, lactobionate, estolate, maleate, malate, mandelate, mesylate, mucate, napsylate, nitrate, pantothenate, phosphate, salicylate, stearate, succinate, sulfate, tartarate, tartrate, hydrochlorate, theoclate, acetamidobenzoate, adipate, alginate, aminosalicylate, anhydromethylenecitrate,

ascorbate, aspartate, camphorate, caprate, caproate, caprylate, cinnamate, cyclamate, dichloroacetate, formate, gentisate, glucuronate, glycerophosphate, glycolate, hippurate, fluoride, malonate, napadisylate, nicotinate, oleate, orotate, oxalate, oxoglutarate, palmitate, pectinate, pectinate polymer, phenylethylbarbiturate, picrate, propionate, 5 pidolate, sebacate, rhodanide, tosylate, and tannate.

Also described are methods for producing any of the forgoing peptides comprising providing a cell harboring a nucleic acid molecule encoding the peptide, culturing the cell under conditions in which the peptide is expressed, and isolating the expressed peptide.

10

Also described are methods for producing any of the forgoing peptides comprising chemically synthesizing the peptide and then purifying the synthesized peptide.

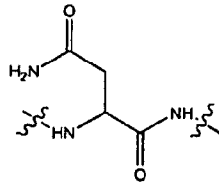
Also described are pharmaceutical compositions comprising the forgoing peptides.

Also described are nucleic acid molecules encoding any of the forgoing peptides, vectors (e.g., expression vectors) containing such nucleic acid molecules and host cells harboring 15 the nucleic acid molecules or vectors.

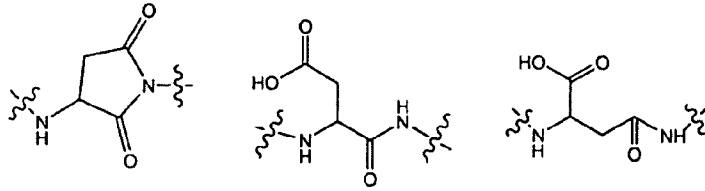
#### **Metabolites of Asparagine**

In some cases an asparagine (Asn) of a peptide described herein can be metabolized to 20 have a different structure and the GC receptor agonist containing such a metabolite of Asn may retain activity. Peptides where one or more Asn, e.g., one or more Asn of an embodiment of SEQ ID NO:7, e.g., a peptide in Figure 3a or Figure 3b described herein are replaced by a metabolite of Asn can be useful in the methods described herein and can be present in a pharmaceutical composition that optionally contains one or more 25 additional active ingredients.

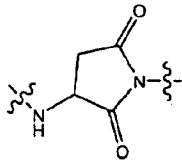
For example, one or more Asn of a peptide and the peptide bond carboxy terminal thereto having the structure:



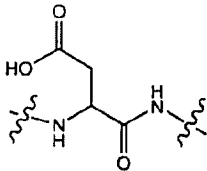
can be replaced by a group having a structure selected from:



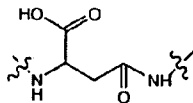
- 5 Thus, the Asn and the peptide bond carboxy terminal there to can be replaced by a cyclic imide:



Asp:

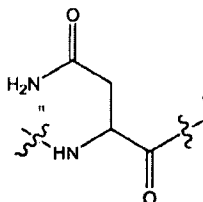


10



The Asp can be L-Asp or D-Asp. The isoAsn can be D-isoAsn or L-isoAsn.

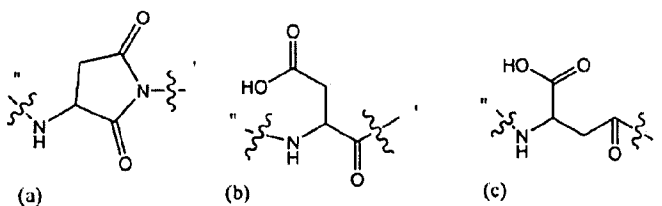
Considering the asparagine only, one or more Asn having the structure:



5

can be optionally replaced by a group having a structure selected from (a), (b) and

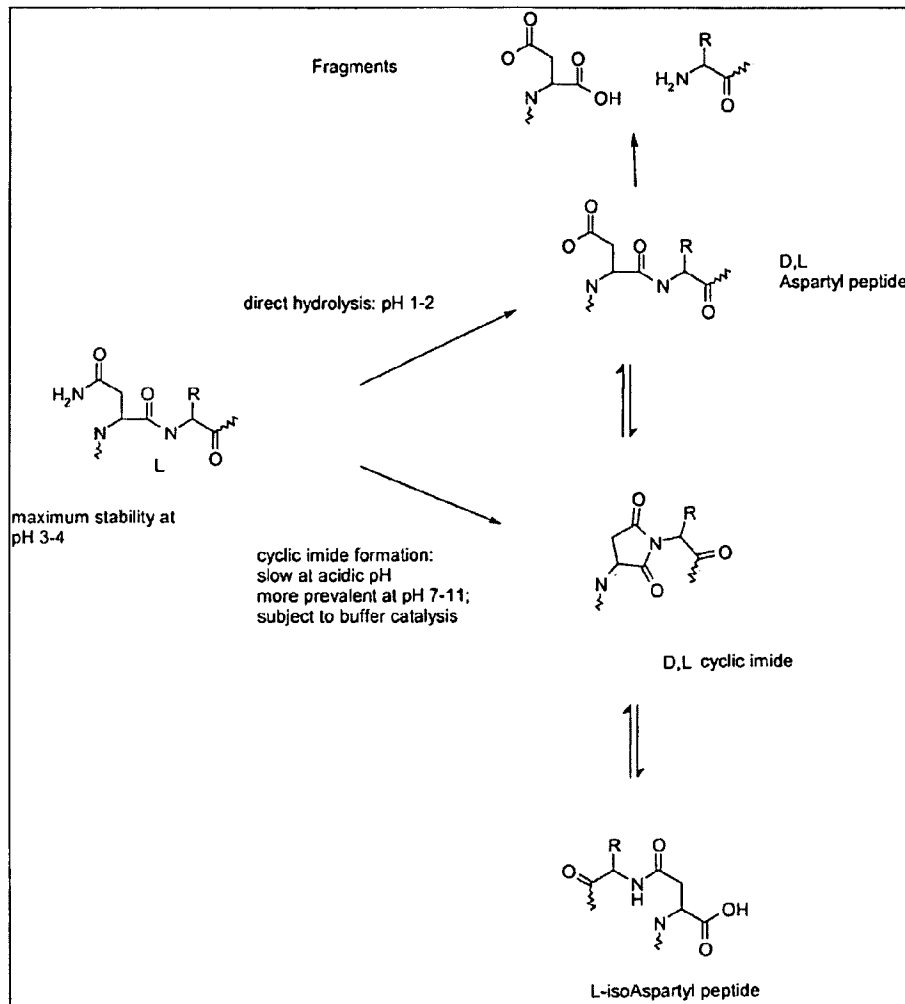
(c):



provided that an Asn at the carboxy terminus is not replaced by structure (a) or structure  
 10 (c). When the Asn is at the carboxy terminus of the peptide, structure (a) cannot form.  
 Since structure (c) is formed through structure (a), structure (c) cannot be formed when  
 the Asn is at the carboxy terminus.

The formation of the various metabolites of Asp is depicted below.





The details of one or more embodiments described herein are set forth in the accompanying description. All of the publications, patents and patent applications are hereby incorporated by reference.

5

## FIGURES

Figure 1 depicts the results of studies on certain peptides tested in the intestinal GC-C receptor activity assay.

10 

Figure 2 depicts the results of studies on certain peptides tested in the rat diuresis assay.

Figures 3a and 3b depict certain peptides within SEQ ID NO:7.

15 

Figure 4 depicts various pre, pro, N-terminal non-core, and C-terminal non-core sequences that can be included in a peptide comprising SEQ ID NO:7.

## DETAILED DESCRIPTION

The peptides described herein bind to the intestinal guanylate cyclase (GC-C) receptor, a  
20 regulator of fluid and electrolyte balance. The apical membrane of the intestinal epithelial surface is a major site of GC-C receptor expression. Activation of the GC-C receptor in the intestine leads to an increase in intestinal epithelial cyclic GMP (cGMP). This increase in cGMP is believed to cause a decrease in water and sodium absorption and an increase in chloride and potassium ion secretion, leading to changes in intestinal  
25 fluid and electrolyte transport and increased intestinal motility. The intestinal GC-C receptor possesses an extracellular ligand binding region, a transmembrane region, an intracellular protein kinase-like region and a cyclase catalytic domain. Proposed functions for the GC-C receptor are fluid and electrolyte homeostasis, the regulation of

epithelial cell proliferation and the induction of apoptosis (Shalubhai 2002 *Curr Opin Drug Dis Devel* 5:261-268).

In addition to being expressed in the intestine by gastrointestinal epithelial cells, GC-C is  
5 expressed in extra-intestinal tissues including kidney, lung, pancreas, pituitary, adrenal, developing liver and gall bladder (reviewed in Vaandrager 2002 *Mol Cell Biochem* 230:73-83, Kulaksiz et al. 2004, *Gastroenterology* 126:732-740) and male and female reproductive tissues (reviewed in Vaandrager 2002 *Mol Cell Biochem* 230:73-83). This suggests that the GC-C receptor agonists can be used in the treatment of disorders outside  
10 the GI tract, for example, congestive heart failure and benign prostatic hyperplasia.

In humans, the GC-C receptor is activated by guanylin (Gn) (U.S. 5,969,097), uroguanylin (Ugn) (U.S. 5,140,102) and lymphoguanylin (Forte et al. 1999 *Endocrinology* 140:1800-1806). Interestingly, these agents are 10-100 fold less potent  
15 than a class of bacterially derived peptides, termed ST (reviewed in Gianella 1995 *J Lab Clin Med* 125:173-181). ST peptides are considered super agonists of GC-C and are very resistant to proteolytic degradation.

ST peptide is capable of stimulating the enteric nervous system (Rolfe et al., 1994, *J*  
20 *Physiol* 475: 531-537; Rolfe et al. 1999 *Gut* 44: 615-619; Nzegwu et al. 1996 *Exp Physiol* 81: 313-315). Also, cGMP has been reported to have antinociceptive effects in multiple animal models of pain (Lazaro Ibanez et al. 2001 *Eur J Pharmacol* 426: 39-44; Soares et al. 2001 *British J Pharmacol* 134: 127-131; Jain et al. 2001 *Brain Res* 909:170-178; Amarante et al. 2002 *Eur J Pharmacol* 454:19-23). Thus, GC-C agonists may have  
25 both an analgesic as well an anti-inflammatory effect.

In bacteria, ST peptides are derived from a preproprotein that generally has at least 70 amino acids. The pre and pro regions are cleaved as part of the secretion process, and the

resulting mature protein, which generally includes fewer than 20 amino acids, is biologically active.

Among the known bacterial ST peptides are: *E. coli* ST Ib (Moseley et al. 1983 Infect. Immun. 39:1167) having the mature amino acid sequence Asn Ser Ser Asn Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr (SEQ ID NO:\_\_); *E. coli* ST Ia (So and McCarthy 1980 Proc. Natl. Acad. Sci. USA 77:4011) having the mature amino acid sequence Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys Tyr (SEQ ID NO:7); *E. coli* ST I\* (Chan and Giannella 1981 J. Biol. Chem. 256:7744) having the mature amino acid sequence Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Tyr Pro Ala Cys Ala Gly Cys Asn (SEQ ID NO:\_\_); *C. freundii* ST peptide (Guarino et al. 1989b Infect. Immun. 57:649) having the mature amino acid sequence Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys Tyr (SEQ ID NO:\_\_); *Y. enterocolitica* ST peptides, Y-ST(Y-STa), Y-STb, and Y-STc (reviewed in Huang et al. 1997 Microb. Pathog. 22:89) having the following pro-form amino acid sequences: Gln Ala Cys Asp Pro Pro Ser Pro Pro Ala Glu Val Ser Ser Asp Trp Asp Cys Cys Asp Val Cys Cys Asn Pro Ala Cys Ala Gly Cys (SEQ ID NO:\_\_) (as well as a Ser-7 to Leu-7 variant of Y-STa (SEQ ID NO:\_\_), (Takao et al. 1985 Eur. J. Biochem. 152:199)); Lys Ala Cys Asp Thr Gln Thr Pro Ser Pro Ser Glu Glu Asn Asp Asp Trp Cys Cys Glu Val Cys Cys Asn Pro Ala Cys Ala Gly Cys (SEQ ID NO:\_\_); Gln Glu Thr Ala Ser Gly Gln Val Gly Asp Val Ser Ser Ser Thr Ile Ala Thr Glu Val Ser Glu Ala Glu Cys Gly Thr Gln Ser Ala Thr Thr Gln Gly Glu Asn Asp Trp Asp Trp Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Phe Gly Cys (SEQ ID NO:\_\_), respectively; *Y. kristensenii* ST peptide having the mature amino acid sequence Ser Asp Trp Cys Cys Glu Val Cys Cys Asn Pro Ala Cys Ala Gly Cys (SEQ ID NO:\_\_); *V. cholerae* non-01 ST peptide (Takao et al. (1985) *FEBS lett.* 193:250) having the mature amino acid sequence Ile Asp Cys Cys Glu Ile Cys Cys Asn Pro Ala Cys Phe Gly Cys Leu Asn (SEQ ID NO:\_\_); and *V. mimicus* ST peptide (Arita et al. 1991 FEMS Microbiol. Lett. 79:105) having the mature amino acid sequence Ile Asp Cys Cys Glu Ile Cys Cys Asn Pro Ala Cys Phe Gly Cys Leu Asn (SEQ ID

NO: \_\_). The immature (including pre and pro regions) form of *E. coli* ST-1A (ST-P) protein has the sequence:

mkkmlaifsvlsvfsfsqstesldsskekitletkkcdvkvknsekksenmntfyccelccnpacagcy (SEQ ID NO: \_\_; see GenBank® Accession No. P01559 (gi:123711)). The pre sequence extends

5 from aa 1-19. The pro sequence extends from aa 20-54. The mature protein extends from 55-72. The immature (including pre and pro regions) form of *E. coli* ST-1B (ST-H) protein has the sequence:

mkkilfiflsvlsvfpaqadkpvesskekiteskkcniakksnksqpsmnsnyccelccnpactgcy (SEQ ID NO: \_\_; see GenBank® Accession No. P07965 (gi:3915589)). The immature (including

10 pre and pro regions) form of *Y. enterocolitica* ST protein has the sequence:

mkkivfvlvmlssfgafgqetvsgqfsdalstpitaevyqacdpplpaevsdwdccdvccnpacagc

(SEQ ID NO: \_\_; see GenBank® Accession No. S25659 (gi:282047)). The peptides described herein, e.g., a peptide comprising SEQ ID NO:7 7 (e.g., a peptide of Figure 3a or Figure 3b) can include all or part of such pre and/or pro sequences.

15

Figure 4 depicts various pre, pro, N-terminal non-core, and C-terminal non-core sequences that can be included in a peptide comprising SEQ ID NO:7 (e.g., a peptide of Figure 3a or Figure 3b). Thus, a peptide can include the amino acid sequence:

A'-B'-C'-D'-E' wherein:

20 A' is an amino acid sequence comprising a pre sequence depicted in Figure 4 or is missing;

B' is an amino acid sequence comprising a pro sequence depicted in Figure 4 or is missing;

25 C' is an amino acid sequence comprising an N-terminal non-core sequence depicted in Figure 4 or is missing;

D' is an amino acid sequence comprising a GC-C receptor agonist peptide amino acid sequence (e.g., SEQ ID NO:7, a peptide in Figure 3a or a peptide in Figure 3b); and

E' is an amino acid sequence comprising a C-terminal non-core sequence depicted in Figure 4 or is missing;

The peptides described herein, like the bacterial ST peptides, have six Cys (or D-Cys) residues. These six residues form three disulfide bonds in the mature and active form of the peptide. If the six Cys (or D-Cys) residues are identified, from the amino to carboxy terminus of the peptide, as A, B, C, D, E, and F, then the disulfide bonds form as follows: A-D, B-E, and C-F. The formation of these bonds is thought to be important for GC-C receptor binding.

Certain of the peptides described herein may include analgesic or antinociceptive tags such as the carboxy-terminal sequence AspPhe immediately following a Trp, Tyr or Phe that creates a functional chymotrypsin cleavage site or following Lys or Arg that creates a functional trypsin cleavage site. Chymotrypsin in the intestinal tract can potentially cleave such peptides immediately carboxy terminal to the Trp, Phe or Tyr residue, releasing the dipeptide, AspPhe. This dipeptide has been shown to have analgesic activity in animal models (Abdikkahi et al. 2001 *Fundam Clin Pharmacol* 15:117-23; Nikfar et al 1997, 29:583-6; Edmundson et al 1998 *Clin Pharmacol Ther* 63:580-93). In this manner such peptides can treat both pain and inflammation. Other analgesic peptides can be present at the amino or carboxy terminus of the peptide (e.g., following a functional cleavage site) including: endomorphin-1, endomorphin-2, nocistatin, dalargin, lupron, and substance P.

In some cases, the peptides described herein are produced as a prepro protein that includes the amino terminal leader sequence:  
mkksilfiflsvlfsfpfaqdakpvesskekitleskkcniakksnksgpesmn. Where the peptide is produced by a bacterial cell, e.g., *E. coli*, the forgoing leader sequence will be cleaved and the mature peptide will be efficiently secreted from the bacterial cell. U.S. Patent No. 5,395,490 describes vectors, expression systems and methods for the efficient production of ST peptides in bacterial cells and methods for achieving efficient secretion

of mature ST peptides. The vectors, expression systems and methods described in U.S. Patent No. 5,395,490 can be used to produce the ST peptides and variant ST peptides of the present disclosure

5 Variant Peptides

The disclosure includes variant peptides which can include one, two, three, four, five, six, seven, eight, nine, or ten (in some embodiments fewer than 5 or fewer than 3 or 2 or fewer) amino acid substitutions and/or deletions compared to the sequences of SEQ ID NO:7 (e.g., a sequence in Figure 3a or Figure 3b) The substitution(s) can be conservative or non-conservative. The naturally-occurring amino acids can be substituted by D-  
10 isomers of any amino acid, non-natural amino acids, natural and natural amino acid analogs and other groups. A conservative amino acid substitution results in the alteration of an amino acid for a similar acting amino acid, or amino acid of like charge, polarity, or hydrophobicity. At some positions, even conservative amino acid substitutions can alter  
15 the activity of the peptide. A conservative substitution can substitute a naturally-occurring amino acid for a non-naturally-occurring amino acid. The amino acid substitutions among naturally-occurring amino acids are listed in Table II.

Table II

For Amino Acid	Code	Replace with any of
Alanine	Ala	Gly, Cys, Ser
Arginine	Arg	Lys, His
Asparagine	Asn	Asp, Glu, Gln,
Aspartic Acid	Asp	Asn, Glu, Gln
Cysteine	Cys	Met, Thr, Ser
Glutamine	Gln	Asn, Glu, Asp
Glutamic Acid	Glu	Asp, Asn, Gln
Glycine	Gly	Ala
Histidine	His	Lys, Arg
Isoleucine	Ile	Val, Leu, Met
Leucine	Leu	Val, Ile, Met
Lysine	Lys	Arg, His
Methionine	Met	Ile, Leu, Val
Phenylalanine	Phe	Tyr, His, Trp
Proline	Pro	
Serine	Ser	Thr, Cys, Ala
Threonine	Thr	Ser, Met, Val
Tryptophan	Trp	Phe, Tyr
Tyrosine	Tyr	Phe, His
Valine	Val	Leu, Ile, Met

In some circumstances it can be desirable to treat patients with a variant peptide that  
 5 binds to and activates intestinal GC-C receptor, but is less active than the non-variant  
 form the peptide. This reduced activity can arise from reduced affinity for the receptor or  
 a reduced ability to activate the receptor once bound or reduced stability of the peptide.

#### Production of peptides

10 Useful peptides can be produced either in bacteria including, without limitation, *E. coli*,  
 or in other existing systems for peptide or protein production (e.g., *Bacillus subtilis*,  
 baculovirus expression systems using *Drosophila* Sf9 cells, yeast or filamentous fungal  
 expression systems, mammalian cell expression systems), or they can be chemically  
 15 synthesized.



If the peptide or variant peptide is to be produced in bacteria, e.g., *E. coli*, the nucleic acid molecule encoding the peptide will preferably also encode a leader sequence that permits the secretion of the mature peptide from the cell. Thus, the sequence encoding  
5 the peptide can include the pre sequence and the pro sequence of, for example, a naturally-occurring bacterial ST peptide. The secreted, mature peptide can be purified from the culture medium.

The sequence encoding a peptide described herein is preferably inserted into a vector  
10 capable of delivering and maintaining the nucleic acid molecule in a bacterial cell. The DNA molecule may be inserted into an autonomously replicating vector (suitable vectors include, for example, pGEM3Z and pcDNA3, and derivatives thereof). The vector nucleic acid may be a bacterial or bacteriophage DNA such as bacteriophage lambda or M13 and derivatives thereof. Construction of a vector containing a nucleic acid  
15 described herein can be followed by transformation of a host cell such as a bacterium. Suitable bacterial hosts include but are not limited to, *E. coli*, *B. subtilis*, *Pseudomonas*, *Salmonella*. The genetic construct also includes, in addition to the encoding nucleic acid molecule, elements that allow expression, such as a promoter and regulatory sequences. The expression vectors may contain transcriptional control sequences that control  
20 transcriptional initiation, such as promoter, enhancer, operator, and repressor sequences. A variety of transcriptional control sequences are well known to those in the art. The expression vector can also include a translation regulatory sequence (c.g., an untranslated 5' sequence, an untranslated 3' sequence, or an internal ribosome entry site). The vector can be capable of autonomous replication or it can integrate into host DNA to ensure  
25 stability during peptide production.

The protein coding sequence that includes a peptide described herein can also be fused to a nucleic acid encoding a peptide affinity tag, e.g., glutathione S-transferase (GST), maltose E binding protein, protein A, FLAG tag, hexa-histidine, myc tag or the influenza

HA tag, in order to facilitate purification. The affinity tag or reporter fusion joins the reading frame of the peptide of interest to the reading frame of the gene encoding the affinity tag such that a translational fusion is generated. Expression of the fusion gene results in translation of a single peptide that includes both the peptide of interest and the affinity tag. In some instances where affinity tags are utilized, DNA sequence encoding a protease recognition site will be fused between the reading frames for the affinity tag and the peptide of interest.

Genetic constructs and methods suitable for production of immature and mature forms of the peptides and variants described herein in protein expression systems other than bacteria, and well known to those skilled in the art, can also be used to produce peptides in a biological system.

Mature peptides and variants thereof can be synthesized by the solid-phase chemical synthesis. For example, the peptide can be synthesized on Cyc(4-CH<sub>2</sub> Bxl)-OCH<sub>2</sub>-4-(oxymethyl)-phenylacetamidomethyl resin using a double coupling program. Protecting groups must be used appropriately to create the correct disulfide bond pattern. For example, the following protecting groups can be used: t-butyloxycarbonyl (alpha-amino groups); acetamidomethyl (thiol groups of Cys residues B and E); 4-methylbenzyl (thiol groups of Cys residues C and F); benzyl (gamma-carboxyl of glutamic acid and the hydroxyl group of threonine, if present); and bromobenzyl (phenolic group of tyrosine, if present). Coupling is effected with symmetrical anhydride of t-butoxycarbonylamino acids or hydroxybenzotriazole ester (for asparagine or glutamine residues), and the peptide is deprotected and cleaved from the solid support in hydrogen fluoride, dimethyl sulfide, anisole, and p-thiocresol using 8/1/1/0.5 ratio (v/v/v/w) at 0°C for 60 min. After removal of hydrogen fluoride and dimethyl sulfide by reduced pressure and anisole and p-thiocresol by extraction with ethyl ether and ethyl acetate sequentially, crude peptides are extracted with a mixture of 0.5M sodium phosphate buffer, pH 8.0 and N, N-dimethylformamide using 1/1 ratio, v/v. The disulfide bond for Cys residues B and E is

the formed using dimethyl sulfoxide (Tam et al. (1991) *J. Am. Chem. Soc.* 113:6657-62). The resulting peptide is purified by reverse-phase chromatography. The disulfide bond between Cys residues C and F is formed by first dissolving the peptide in 50% acetic acid in water. Saturated iodine solution in glacial acetic acid is added (1 ml iodine solution per 100 ml solution). After incubation at room temperature for 2 days in an enclosed glass container, the solution is diluted five-fold with deionized water and extracted with ethyl ether four times for removal of unreacted iodine. After removal of the residual amount of ethyl ether by rotary evaporation the solution of crude product is lyophilized and purified by successive reverse-phase chromatography.

10

Peptides can also be synthesized by many other methods including solid phase synthesis using traditional Fmoc protection (i.e., coupling with DCC-HOBt and deprotection with piperidine in DMF). Cys thiol groups can be trityl protected. Treatment with TFA can be used for final deprotection of the peptide and release of the peptide from the solid-state resin. In many cases air oxidation is sufficient to achieve proper disulfide bond formation.

15

#### **Example 1: Preparation of peptides**

Peptides can be recombinantly produced in bacteria as follows. T7 expression vectors, pET26b(+) (Novagen) expressing the peptide of interest are constructed using standard molecular biology techniques and are transformed into *E. coli* bacterial host BL21  $\lambda$  DE3 (Invitrogen). A single colony is inoculated and grown shaking overnight at 30°C in L broth + 25 mg/l kanamycin. The overnight culture is added to 3.2 L of batch medium (Glucose 25 g/l, Casamino Acids 5 g/l, Yeast Extract 5 g/l,  $\text{KH}_2\text{PO}_4$  13.3 g/l,  $(\text{NH}_4)_2\text{HPO}_4$  4 g/l,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  1.2 g/l, Citric Acid 1.7 g/l, EDTA 8.4 mg/l,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  2.5 mg/l,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  15 mg/l,  $\text{CuCl}_2 \cdot 4\text{H}_2\text{O}$  1.5 mg/l,  $\text{H}_3\text{BO}_3$  3 mg/l,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  2.5 mg/l, Zn Acetate- $2\text{H}_2\text{O}$  13 mg/l, Ferric Citrate 100 mg/l, Kanamycin 25 mg/l, Antifoam DF<sub>2</sub>0<sub>4</sub> 1 ml/l) and fermented using the following process parameters : pH 6.7 - control with base only (28%  $\text{NH}_4\text{OH}$ ), 30°C, aeration : 5 liters per minute. After the

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initial consumption of batch glucose (based on monitoring dissolved oxygen (DO) levels), 1.5 L of feed medium (Glucose 700 g/l, Casamino Acids 10 g/l, Yeast Extract 10 g/l, MgSO<sub>4</sub>-7H<sub>2</sub>O 4 g/l, EDTA 13 mg/l, CoCl<sub>2</sub>-6H<sub>2</sub>O 4 mg/l, MnCl<sub>2</sub>-4H<sub>2</sub>O 23.5 mg/l, CuCl<sub>2</sub>-4H<sub>2</sub>O 2.5 mg/l, H<sub>3</sub>BO<sub>3</sub> 5 mg/l, Na<sub>2</sub>MoO<sub>4</sub>-2H<sub>2</sub>O 4 mg/l, Zn Acetate-2H<sub>2</sub>O 16 mg/l, 5 Ferric Citrate 40 mg/l, Antifoam DF<sub>2</sub>O<sub>4</sub> 1 ml/l) is added at a feed rate controlled to maintain 20% DO. IPTG is added to 0.2 mM 2 hours post feed start. The total run time is approximately 40-45 hours (until feed exhaustion).

Cells are collected by centrifugation at 5,000 g for 10 minutes. The cell pellet is 10 discarded and the supernatant is passed through a 50 Kd ultrafiltration unit. The 50 Kd filtrate (0.6 liters) is loaded onto a 110 ml Q-Sepharose fast Flow column (Amersham Pharmacia, equilibrated with 20 mM Tris-HCl pH 7.5) at a flow rate of 400 ml/hour. The column is washed with six volumes of 20 mM Tris-HCl pH 7.5 and proteins are eluted with 50 mM acetic acid collecting 50 ml fractions. Fractions containing peptide are 15 pooled and the solvent is removed by rotary evaporation. The dried proteins are resuspended in 10 ml of 8% acetic acid, 0.1% trifluoroacetic acid (TFA) and loaded onto a Varian Polaris C18-A column (250 X 21.2 mm 10 µm, equilibrated in the same buffer) at a flow rate of 20 ml/min. The column is washed with 100 ml of 8% methanol, 0.1% TFA and developed with a gradient (300 ml) of 24 to 48% methanol, 0.1% TFA, 20 collecting 5-ml fractions. Fractions containing peptide are pooled and the solvent is removed by rotary evaporation. The peptides are dissolved in 0.1%TFA and lyophilized.

Peptide fractions are analyzed by standard LCMS and HPLC. Peptides can also be chemically synthesized by a commercial peptide synthesis company.

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#### **Example 2: Activation of the intestinal GC-C receptor by peptides**

The ability of peptides to activate the intestinal GC-C receptor was assessed in an assay employing the T84 human colon carcinoma cell line (American Type Culture Collection (Bethesda, Md)). For the assays cells were grown to confluency in 24-well culture plates

with a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's medium (DMEM), supplemented with 5% fetal calf serum and were used at between passages 54 and 60.

5 Briefly, monolayers of T84 cells in 24-well plates were washed twice with 1 ml/well DMEM, then incubated at 37°C for 10 min with 0.45 ml DMEM containing 1 mM isobutylmethylxanthine (IBMX), a cyclic nucleotide phosphodiesterase inhibitor. Test peptides (50µl) were then added and incubated for 30 minutes at 37°C. The media was aspirated and the reaction was then terminated by the addition of ice cold 0.5 ml of 0.1N  
10 HCl. The samples were held on ice for 20 minutes and then evaporated to dryness using a heat gun or vacuum centrifugation. The dried samples were resuspended in 0.5ml of phosphate buffer provided in the Cayman Chemical Cyclic GMP EIA kit (Cayman Chemical, Ann Arbor, MI). Cyclic GMP was measured by EIA according to procedures outlined in the Cayman Chemical Cyclic GMP EIA kit. Figure 1 shows the activity of  
15 chemically synthesized peptide variants (depicted below) in the GC-C receptor activity assay. EC<sub>50</sub> is defined as the concentration by which 50% of the maximal activity is seen. Maximum cGMP level in assay is determined as the activity of the positive ST control, Cys-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Tyr SEQ ID NO:6 and set to 100%. The positive control was tested twice in this assay.

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### **Example 3. Diuresis and Natriuresis Assays**

#### Effect on Diuresis and Natriuresis

The effect of peptides/GC-agonists described herein on diuresis and natriuresis can be determined using methodology similar to that described in WO06/001931 (examples 6 (p.  
25 42) and 8 (p.45)). Briefly, a peptide described herein (180-pmol) is infused for 60 min into a group of 5 anesthetized rats. Given an estimated rat plasma volume of 10 mL, the infusion rate is approximately 3 pmol/mL/min. Blood pressure, urine production, and sodium excretion are monitored for approximately 40 minutes prior to the infusion, during the infusion, and for approximately 50 minutes after the infusion to measure the

effect of the peptide/GC-C agonist on diuresis and natriuresis. For comparison, a control group of five rats is infused with regular saline. Urine and sodium excretion can be assessed. Dose response can also be determined. A peptide/GC-C agonist described herein is infused intravenously into rats over 60 minutes. Urine is collected at 30 minute intervals up to 180 minutes after termination of peptide/GC-C agonist infusion, and urine volume, sodium excretion, and potassium excretion are determined for each collection interval. Blood pressure is monitored continuously. For each dose a dose-response relationship for urine volume, sodium and potassium excretion can be determined. Plasma concentration of the peptide/GC-agonist is also determined before and after iv infusion.

#### Rat Diuresis Experiment

Female Sprague-Dawley rats (> 170 g, 2-8 per group) are given 3.0mL of isotonic saline perorally, and then anesthetized with isoflurane /oxygen. Once an appropriate level of anesthesia has been achieved, a sterile polyurethane catheter (~16 cm, 0.6mm ID, 0.9mm OD) is inserted 1.5-2.0 cm into the urethra and secured using 1 – 2 drops of veterinary bond adhesive applied to urethra/catheter junction. Rats are then dosed with either vehicle or test article via the intravenous or intraperitoneal route. Rats are then placed in appropriately sized rat restraint tubes, with the catheter protruding out of the restraint tube into a 10 mL graduated cylinder. Rats are allowed to regain consciousness, and the volume of urine excreted over a 1-5 hour duration is recorded periodically for each rat. Figure 2 shows the results of several peptides described herein tested in the rat diuresis assay.

#### **Example 4: Kd determination and binding assays**

A competition binding assay can be performed using rat intestinal epithelial cells to determine the affinity of a peptide for intestinal GC-G receptor. Epithelial cells from the small intestine of rats are obtained as described by Kessler et al. (*J. Biol. Chem.* 245: 5281-5288 (1970)). Briefly, animals are sacrificed and their abdominal cavities exposed.

The small intestine is rinsed with 300 ml ice cold saline or PBS. 10 cm of the small intestine measured at 10 cm from the pylorus is removed and cut into 1 inch segments. Intestinal mucosa is extruded from the intestine by gentle pressure between a piece of parafilm and a P-1000 pipette tip. Intestinal epithelial cells are placed in 2 ml PBS and pipetted up and down with a 5 ml pipette to make a suspension of cells. Protein concentration in the suspension is measured using the Bradford method (*Anal. Biochem.* 72: 248-254 (1976)).

A competition binding assay can be performed based on the method of Giannella et al. (*Am. J. Physiol.* 245: G492-G498) between [<sup>125</sup>I] labeled peptide. The assay mixture contains: 0.5 ml of DME with 20 mM HEPES-KOH pH 7.0, 0.9 mg of the cell suspension listed above, 21.4 fmol [<sup>125</sup>I]-SEQ ID NO:4 (42.8 pM), and different concentrations of competitor peptide (0.01 to 1000 nM). The mixture is incubated at room temperature for 1 hour, and the reaction is stopped by applying the mixture to GF/B glass-fiber filters (Whatman). The filters are washed with 5 ml ice-cold PBS and radioactivity is measured. Giannella et al. (*Am. J. Physiol.* 245: G492-G498) observed that the K<sub>d</sub> for wild-type ST peptide in this same assay is ~13 nM.

Similar competition binding assays can be performed in intestinal epithelial cells from wild-type and guanylate cyclase C knockout (GC-C KO; Mann et al. 1997 *Biochem and Biophysical Research Communications* 239:463) mice. Mouse intestinal epithelial cells are prepared as described above for rat intestinal epithelial cells except the cells are homogenized with an Omni homogenizer for 20 seconds on the maximum setting to make a suspension of cells.

The binding of peptides to GC-C receptors on the cell surface of human colonic cells (T84 cells; ATCC Catalog No. CCL-248) can be characterized in a competitive radioligand-binding assay at pH conditions of 5, 7 and 8. The radiolabeled tracer used in these experiments is <sup>125</sup>I- labeled control peptide. To determine binding constants,

competitive inhibition of binding is used. T84 cells are cultured in T-150 plastic flasks in DMEM and Ham's F-12 medium containing 5% fetal bovine serum. Monolayers at 60-70% confluency (approximately  $10^7$  cells) are collected by gentle scraping followed by centrifugation, and washed twice in 50 mL of phosphate-buffered saline (PBS). The cells are resuspended in 1 mL DMEM containing 20 mM *N*-(2-hydroxymethyl)piperazine-*N'*-(2-ethanesulfonic acid) (Hepes), pH 7.0 and 0.5% bovine serum albumin (BSA). T84 cells are incubated with a constant amount of  $^{125}\text{I}$ - control peptide containing various concentrations of cold competitor. Free  $^{125}\text{I}$ - control peptide is separated from bound tracer by rapid suction filtration. The binding reactions are carried out in 1.5 mL microfuge tubes in 0.24 mL of DMEM/20 mM Hepes pH 7.0/0.5% BSA containing:  $2.5 \times 10^5$  T84 cells (0.25 mg protein), 200,000 cpm  $^{125}\text{I}$ - control peptide (41 fmol, 170 pM), and 0.01 to 1,000 nM competitor. Binding assays at pH 5.0 are done in DMEM/20 mM 2-(*N*-morpholino) ethanesulfonic acid (Mes), pH 5.0. Binding assays in pH 8.0 are done in DMEM/20 mM Hepes/50 mM sodium bicarbonate pH 8.0. One sample contains no competitor ( $B_0$ ) and another contained no cells. After incubation at 37 °C for 1 h, the reaction mixtures are applied to Whatman GF/B glass-fiber filters by suction filtration. The filters are then rinsed with 10 mL ice-cold PBS buffer, inserted into plastic tubes, and added to 2 mL scintillation fluid. Radioactivity is measured in a LS 6500 liquid scintillation counter (Beckman-Coulter). The percent bound in each sample is calculated by the equation:

$$\% B/B_0 = \frac{(\text{sample cpm} - \text{no cells cpm}) \times 100}{(B_0 \text{ cpm} - \text{no cells cpm})}$$

Nonlinear regression analysis of the binding data is used to calculate the concentration of competitor that resulted in 50% radioligand bound ( $IC_{50}$ ). The apparent dissociation equilibrium constant ( $K_i$ ) for each competitor is obtained from the  $IC_{50}$  values and the previously reported estimate of the dissociation constant for the radioligand,  $K_d \cong 15$  nM (Hamra et al. 1997 PNAS 2705-10) and the method of Cheng and Prusoff 1973 Biochem Pharmacol 22:3099-108. Using a two site model, high and low affinity-binding sites are identified on T84 cells ( $K_{i1}$  and  $K_{i2}$ ) for all the test agents.



**Example 5: Pharmacokinetic properties of peptides**

To study the pharmacokinetics of peptides, absorbability studies in mice are performed by administering a peptide intravenously via tail vein injection or orally by gavage to 8-week-old CD1 mice. Serum is collected from the animals at various time points and tested for the presence of peptide using a competitive enzyme-linked immunoabsorbent assay.

A similar bioavailability study can be performed in which LCMS rather than ELISA is used to detect peptide. Initially, serum samples are extracted from the whole blood of exposed and control mice, then injected directly (10 $\mu$ L) onto an in-line solid phase extraction (SPE) column (Waters Oasis HLB 25 $\mu$ m column, 2.0 x 15mm direct connect) without further processing. The sample on the SPE column is washed with a 5% methanol, 95% dH<sub>2</sub>O solution (2.1 mL/min, 1.0 minute), then loaded onto an analytical column using a valve switch that places the SPE column in an inverted flow path onto the analytical column (Waters Xterra MS C8 5 $\mu$ m IS column, 2.1 x 20mm). The sample is eluted from the analytical column with a reverse phase gradient (Mobile Phase A: 10 mM ammonium hydroxide in dH<sub>2</sub>O, Mobile Phase B: 10 mM ammonium hydroxide in 80% acetonitrile and 20% methanol; 20% B for the first 3 minutes then ramping to 95% B over 4 min. and holding for 2 min., all at a flow rate of 0.4 mL/min.). At 9.1 minutes, the gradient returns to the initial conditions of 20%B for 1 min. Peptide is eluted from the analytical column, and it is detected by triple-quadrupole mass spectrometry. Instrument response is converted into concentration units by comparison with a standard curve using known amounts of chemically synthesized peptide prepared and injected in mouse serum using the same procedure.

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Similarly, oral bioavailability is determined in rats using LCMS methodology. Rat plasma samples containing peptide are extracted using a Waters Oasis MAX 96 well solid phase extraction (SPE) plate. A 200  $\mu$ L volume of rat plasma is mixed with 200  $\mu$ L of <sup>13</sup>C, <sup>15</sup>N - peptide in the well of a prepared SPE plate. The samples are drawn through the

stationary phase with 15 mm Hg vacuum. All samples are rinsed with 200  $\mu$ L of 2% ammonium hydroxide in water followed by 200  $\mu$ L of 20% methanol in water. The samples are eluted with consecutive 100  $\mu$ L volumes of 5/20/75 formic acid/water/methanol and 100  $\mu$ L 5/15/80 formic acid/water/methanol. The samples are  
5 dried under nitrogen and resuspended in 100  $\mu$ L of 20% methanol in water. Samples are analyzed by a Waters Quattro Micro mass spectrometer coupled to a Waters 1525 binary pump with a Waters 2777 autosampler. A 40  $\mu$ L volume of each sample is injected onto a Thermo Hypersil GOLD C18 column (2.1x50 mm, 5  $\mu$ m). Peptides are eluted by a gradient over 3 minutes with acetonitrile and water containing 0.05% trifluoroacetic acid.

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Oral bioavailability can also be determined using a radioimmunoassay (RIA) detection method. Female CD-1 mice (Charles River, Wilmington, MA) weighing approximately 25 g (7-8 weeks old) or female CD rats (Charles River, Wilmington, MA) weighing approximately 153 g are included in this study. Monoclonal antibody recognizing the  
15 peptide and  $^{125}$ I labeled- peptide, a labeled tracer, are used in these experiments. Animals are fasted overnight before administration of compounds. Blood is drawn from all dosed animals by retro-orbital eye bleeding at specific intervals and test compound levels are analyzed by radioimmunoassay (RIA). Samples (80  $\mu$ L) are first diluted to 0.5 mL with start buffer (8% methanol, 0.095% TFA in water) and applied to C18 columns previously  
20 conditioned with 1 mL methanol and equilibrated with 2 mL of start buffer. After washing with 1 mL start buffer, peptide is eluted with 0.8 mL of 80% methanol, 0.05% TFA and dried down in a centrifugal evaporator. Samples are reconstituted in 0.194 mL assay buffer (PBS buffer, pH 7.4, containing 10% fetal bovine serum). Standard dilutions of peptide are made in rat plasma. To perform RIA analysis, samples from  
25 dosed animal and standards are mixed with 5  $\mu$ L diluted antibody (in RIA wash buffer: phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA), 1:40,000 final dilution, 0.0022  $\mu$ g), and incubated 1 to 4 h at 4  $^{\circ}$ C. One tube contains the zero standard ( $B_0$ ) and another no standard and no antibody (non-specific binding, NSB). Labeled tracer (0.018  $\mu$ Ci, diluted in RIA wash buffer) is then added and incubated at 4

°C for 12 to 18 h. The antibody bound fraction containing peptide is collected by magnetic separation using 10  $\mu$ L of sheep anti-mouse IgG beads previously washed twice in 10 volumes RIA assay buffer. The beads are then washed twice with 1 mL of RIA wash buffer, collected by magnetic separation, resuspended in 0.1 mL of RIA wash buffer, and added to 2 mL scintillation fluid. Radioactivity is measured in a LS 6500 scintillation counter (Beckman-Coulter). The binding efficiency is defined as the percent radioactivity in the  $B_0$  sample compared to the input counts. The percent bound in each sample is calculated by the equation:

$$10 \quad \% B/B_0 = \frac{(\text{sample cpm} - \text{NSB cpm}) \times 100}{(B_0 \text{ cpm} - \text{NSB cpm})}$$

A standard curve is prepared by plotting % B/B<sub>0</sub> as a function of the log peptide concentration. A concentration vs. time plot is generated from the data in GraphPad Prism or Summit Software PK Solutions 2.0 to generate oral and i.v. PK curves. The area under the curve from T = 0 to 4 hours (AUC<sub>0-4h</sub>) is calculated by the software for both p.o. and i.v. dosed animals. If the values are below the lower limit of detection (LOD) than the LOD is used to estimate the value (in this experiment 2 nM). Oral Bioavailability (F) is calculated using the equation:

$$20 \quad F = \frac{(\text{AUC}_{\text{p.o.},(0-4\text{h})} * D_{\text{i.v.}})}{(\text{AUC}_{\text{i.v.},(0-4\text{h})} * D_{\text{p.o.}})}$$

where  $D_{\text{i.v.}}$  and  $D_{\text{p.o.}}$  equal the intravenous and oral dose, respectively.

#### **Example 6: In vitro proteolytic stability of peptides**

The stability of peptides in the presence of several mammalian digestive enzymes can be determined. Peptides are exposed to a variety of *in vitro* conditions including digestive enzymes and low pH environments designed to simulate gastric fluid. Peptides are incubated with chymotrypsin, trypsin, pepsin, aminopeptidase, carboxypeptidase A, or simulated gastric fluid (sgf) at pH 1.0. Samples are collected at 0, 3, and 24 h for all conditions except pepsin digestion and the SGF. For the latter two conditions, samples

are obtained at 0, 1, and 3 h. Negative control samples are prepared for initial and final time points. A separate, positive activity control is run in parallel to test peptide. All samples are analyzed by LC/MS

5 Chymotrypsin

500  $\mu$ l samples of 0.01 mg/mL peptide and guanylin (Sigma-Aldrich, G116; positive control) are prepared in the chymotrypsin reaction buffer (100 mM Tris-HCl, 10 mM CaCl<sub>2</sub>, pH 7.5) in 2 mL eppendorf tubes. Zero and 24 h control samples are prepared by adding 5  $\mu$ L of a 10 mM chymostatin (Sigma-Aldrich, C7268; a chymotrypsin inhibitor) stock for a final concentration of 100  $\mu$ M. All samples are incubated at 37 °C for 5 min. 20  $\mu$ L of a 0.01 mg/mL chymotrypsin stock ( $\alpha$ -chymotrypsin from bovine pancreas; Sigma-Aldrich, C6423) are added to each sample for a 0.0004 mg/mL final concentration. Samples are returned to the 37° C water bath. The reaction is quenched with 5  $\mu$ L of a 10 mM chymostatin stock at each time point for a final concentration of 15 100  $\mu$ M. No extra chymostatin is added to the control samples as they already had inhibitor. Samples are subsequently flash frozen in liquid nitrogen, and stored at -80 °C until analysis. Upon analysis, samples are thawed and transferred to a 1 mL 96-well plate. Standards of peptide and guanylin are prepared in chymotrypsin reaction buffer at 0.625, 1.25, 2.50, 5.00, and 10.00  $\mu$ g/mL concentrations. These standards are used to 20 generate a standard curve for quantification of samples. When necessary, the standard curves are also used to calculate the concentration of the corresponding digestion product. 10  $\mu$ L injections are made of each sample and standard.

Trypsin

25 500  $\mu$ L samples of 0.01 mg/mL peptide and BAEE (N<sub>alpha</sub> Benzoyl-L-arginine ethyl ester hydrochloride; Sigma-Aldrich, B4500; positive control) are prepared with trypsin reaction buffer (100 mM Tris-HCl, pH 7.5) in 2 mL eppendorf tubes. Zero and 24 h time point control samples are prepared (N = 1) with 5  $\mu$ L of a 100 mg/mL AEBSF (4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride; a trypsin inhibitor) stock for a final

concentration of 1 mg/mL. All control and test samples (0, 3, and 24 h) are incubated at 37 °C for 5 min. Twenty (20)  $\mu$ L of a 0.01 mg/mL trypsin (Sigma-Aldrich, T6467) stock are added to each sample for a final concentration of 0.0004 mg/mL. Samples are returned to the 37°C water bath. The reaction is quenched with 5  $\mu$ L of a 100 mg/mL

5 AEBSF stock, which is added to each sample at the indicated timepoint, for a final concentration of 1 mg/mL. No extra AEBSF is added to the control samples as they already had inhibitor. Samples are subsequently flash frozen in liquid nitrogen, and stored at -80 °C until analysis. Upon analysis, samples are thawed and transferred to a 1 mL 96-well plate. Standards of peptide and BAEE are prepared in trypsin reaction buffer

10 at 0.625, 1.25, 2.50, 5.00, and 10.00  $\mu$ g/mL concentrations. These standards are used to generate a standard curve for quantification of samples. When necessary, the standard curves are also used to calculate the concentration of the corresponding digestion product. Ten (10)  $\mu$ L injections are made of each sample and standard.

15 Pepsin

500  $\mu$ L samples of 100 U/mL pepsin (Pepsin porcine gastric mucosa; Sigma-Aldrich, P68871; U = release of 0.01 absorbance at 280 nM (A280) as TCA soluble hydrolysis products per min at 37 °C of hemoglobin) are prepared in the pepsin reaction buffer (100 mM HCl-KCl, pH 2.0) in 5 mL polystyrene round bottom tubes. To the control samples

20 (0 and 24 h), 500  $\mu$ L of a 1 M ammonium acetate (pepsin inhibitor) stock are added, for a final concentration of 0.5 M. All control and test samples (0, 1, and 3 h) are incubated at 37 °C for 5 min, while shaking. Fifty (50)  $\mu$ L of 0.1 mg/mL peptide and Insulin B chain, oxidized (Sigma-Aldrich, I6383; positive control), stocks are added to the respective tubes. Samples are returned to the 37 °C shaking water bath. Reactions are quenched by

25 the addition of 500  $\mu$ L of 1 M ammonium acetate for a final concentration of 0.5 M (except to the control samples, which already contained 0.5 M ammonium acetate). Samples are cooled on ice and stored at 4 °C until analysis. Upon analysis, samples are transferred to a 1 mL 96-well plate. Standards of peptide and Insulin B chain, oxidized, are prepared in 25 mM Tris-hydrochloric acid, 500 mM sodium chloride, pH 7.5 buffer at

0.625, 1.25, 2.50, 5.00, and 10.00  $\mu\text{g}/\text{mL}$  concentrations. These standards are used to generate a standard curve for quantification of samples. Ten (10)  $\mu\text{L}$  injections are made of each sample and standard.

5 Aminopeptidase

500  $\mu\text{L}$  samples of 0.01 mg/mL peptide and chemically synthesized wild type ST (positive control) are prepared in the aminopeptidase reaction buffer (5 mM Tris-HCl, 5 mM  $\text{MgCl}_2$ , pH 7.5) in 2 mL eppendorf tubes. 5  $\mu\text{L}$  of a 5 mg/mL Bestatin hydrochloride (BioChemika, 08170; an aminopeptidase inhibitor) stock is added to each control sample (0 and 24 h), for a final concentration of 0.05 mg/mL. All control and test samples (0, 3, and 24 h) are incubated at 37 °C for 5 min. 0.02 U aminopeptidase (Aminopeptidase M, amino acid aryl amidase (Roche, 102768; U = hydrolysis of 1.0  $\mu\text{mol}$  of L-leucinamide to leucine and  $\text{NH}_3$  per min at pH 8.5 at 25 °C) are added to each sample. Samples are returned to the 37 °C water bath. The reaction is quenched with 5  $\mu\text{L}$  of a 5 mg/mL Bestatin hydrochloride stock at the proper time point. No extra Bestatin hydrochloride is added to the control samples since they already had inhibitor present. Samples are subsequently flash frozen in liquid nitrogen, and stored at -80 °C until analysis. Upon analysis, samples are thawed and transferred to a 1 mL 96-well plate. Standards of peptide are prepared in aminopeptidase reaction buffer at 0.625, 1.25, 2.50, 5.00, and 10.00  $\mu\text{g}/\text{mL}$  concentrations. These standards are used to generate a standard curve for quantification of samples. When necessary, the standard curves are also used to calculate the concentration of the corresponding digestion product. Ten (10)  $\mu\text{L}$  injections are made of each sample and standard.

25 Carboxypeptidase A

500  $\mu\text{L}$  samples of 0.01 mg/mL peptide and N-CBZ-Glycine-Glycine-Leucine (Z-Gly-Gly-Leu; Sigma-Aldrich, C8501; positive control) are prepared in the carboxypeptidase A reaction buffer (25 mM Tris-HCl, 500 mM NaCl, pH 7.5) in 2 mL eppendorf tubes. Five (5)  $\mu\text{L}$  of a 40  $\mu\text{g}/\text{mL}$  carboxypeptidase inhibitor (carboxypeptidase inhibitor from potato

tuber (Sigma-Aldrich, C0279) stock is added to each control sample (0 and 24 h), for a final concentration of 0.4  $\mu\text{g}/\text{mL}$ . All control and test (0, 3 and 24 h) samples are incubated at 37 °C for 5 min. Twenty (20)  $\mu\text{L}$  of a 0.01 mg/mL carboxypeptidase A (Carboxypeptidase A from human pancreas; Sigma-Aldrich, C5358) stock is added to each sample. The samples are returned to the 37 °C water bath. The reaction is quenched with 5  $\mu\text{L}$  of a 40  $\mu\text{g}/\text{mL}$  carboxypeptidase inhibitor at the proper time point. No extra carboxypeptidase inhibitor is added to the control samples since there is already inhibitor present. Samples are subsequently flash frozen in liquid nitrogen, and stored at -80 °C until analysis. Upon analysis, samples are thawed and transferred to a 1 mL 96-well deep microtiter plate. Standards of peptide and Z-Gly-Gly-Leu are prepared in carboxypeptidase A reaction buffer at 0.625, 1.25, 2.50, 5.00, and 10.00  $\mu\text{g}/\text{mL}$  concentrations. These standards are used to generate a standard curve for quantification of samples. When necessary, the standard curves are also used to calculate the concentration of the corresponding digestion product. Ten (10)  $\mu\text{L}$  injections are made of each sample and standard.

#### Carboxypeptidase A - Identification of Proteolysis Product

To analyze carboxypeptidase A digestion product, samples of 0.01 mg/mL peptide are prepared in the carboxypeptidase A reaction buffer at a total volume of 500  $\mu\text{L}$  in 2 mL eppendorf tubes. Triplicate samples are prepared for the following time points: 0, 15, 30, 60, 120, 180 and 240 min. The samples are incubated at 37 °C for 5 min. Twenty (20)  $\mu\text{L}$  of a 0.01 mg/mL carboxypeptidase A stock are added to each sample and returned to the 37 °C water bath. The reactions are quenched with 5  $\mu\text{L}$  of a 40  $\mu\text{g}/\text{mL}$  carboxypeptidase inhibitor at the proper time points. Samples are subsequently flash frozen in liquid nitrogen, and stored at -80 °C until analysis. Upon analysis, samples are thawed and transferred to a 1 mL 96-well plate. Standards of peptide prepared in carboxypeptidase A reaction buffer at 0.625, 1.25, 2.50, 5.00, and 10.00  $\mu\text{g}/\text{mL}$  concentrations. These standards are used to generate a standard curve for quantification of samples. When necessary, the standard curves are also used to calculate the

concentration of the corresponding digestion product. Ten (10)  $\mu\text{L}$  injections are made of each sample and standard. If the formation of a digestion product is evident, then a spectral analysis used to determine the mass of the digestion product, and predict its possible identity.

5

#### Simulated Gastric Fluid (SGF)

Samples of peptide are prepared in the simulated gastric fluid buffer (0.2% NaCl (w/v), 0.7% HCl (v/v), pH 1) to a total volume of 500  $\mu\text{L}$  in 2 mL eppendorf tubes. The reference control and test samples (0, 1 and 3 h) are incubated at 37 °C for the time point indicated. The reference control sample is diluted 10-fold (1000  $\mu\text{L}$  volume) in distilled water for a final concentration of 10  $\mu\text{M}$  and chilled on ice. At each time point, samples are diluted 10-fold (1000  $\mu\text{L}$  volume) in distilled water for an expected concentration of 10  $\mu\text{M}$ , and chilled on ice, until analysis. Upon analysis, samples are transferred to a 1 mL 96-well plate. Standards of peptide are prepared in distilled water at 0.625, 1.25, 15 2.50, 5.00, and 10.00  $\mu\text{M}$  concentrations. These standards are used to generate a standard curve for quantification of samples. Ten (10)  $\mu\text{L}$  injections are made of each sample and standard.

#### **Example 7: Rodent intestinal transit assays**

20 In order to determine whether a peptide increases the rate of gastrointestinal transit, the peptide and controls are tested using a murine gastrointestinal transit (GIT) assay (Moon et al. *Infection and Immunity* 25:127, 1979). In this assay, charcoal, which can be readily visualized in the gastrointestinal tract, is administered to mice after the administration of a test compound. The distance traveled by the charcoal is measured and expressed as a  
25 percentage of the total length of the colon.

Mice are fasted with free access to water for 12 to 16 hours before the treatment with peptide or control buffer. The peptides are orally administered at 1  $\mu\text{g}/\text{kg}$  – 1  $\text{mg}/\text{kg}$  of peptide in buffer (20mM Tris pH 7.5) 7 minutes before being given an oral dose of 5%



Activated Carbon (Aldrich 242276-250G). Control mice are administered buffer only before being given a dose of Activated Carbon. After 15 minutes, the mice are sacrificed and their intestines from the stomach to the cecum are dissected. The total length of the intestine as well as the distance traveled from the stomach to the charcoal front is  
5 measured for each animal and the results are expressed as the percent of the total length of the intestine traveled by the charcoal front. All results are reported as the average of 10 mice  $\pm$  standard deviation. A comparison of the distance traveled by the charcoal between the mice treated with peptide versus the mice treated with vehicle alone is performed using a Student's t test and a statistically significant difference is considered  
10 for  $P < 0.05$ . P-values are calculated using a two-sided T-Test assuming unequal variances. Controls include vehicle alone (e.g. Tris buffer) and Zelnorm®.

An identical experiment can be performed to determine if peptides are effective in a chronic dosing treatment regimen. Briefly, 8 week old CD1 female mice are dosed orally  
15 once a day for 5 days with either peptide (0.06mg/kg or 0.25mg/kg in 20mM Tris pH 7.5) or vehicle alone (20mM Tris pH 7.5). On the 5<sup>th</sup> day, a GIT assay is performed identical to that above except 200 $\mu$ l of a 10% charcoal solution is administered.

The gastrointestinal transit assay can also performed in male and female CD rats (Charles  
20 River; Wilmington, MA). The assay is performed as described above for mice except an average of 5-8 animals are used for each test group and test peptide and 5% activated carbon are administered simultaneously (versus 7 minutes apart). In addition, the animals are sacrificed 10 minutes after the administration of peptide and test compound. The experiment can be performed in male and female rats.

25

The gastrointestinal transit assay can also performed in wild-type mice and mice lacking the guanylate cyclase C receptor (GC-C KO; Mann et al 1997 Biochem and Biophysical Research Communications 239:463). Wild type and GC-C KO mice are fasted overnight and test peptide or vehicle alone are orally administered 10 minutes prior to an oral dose

of a 10% Activated Carbon/10% Gum Arabic suspension. Animals are sacrificed 5 minutes after peptide or vehicle administration. is is

**Example 8: Intestinal secretion assay in suckling mice (SuMi assay)**

5 Peptides are tested for their ability to increase intestinal secretion using a suckling mouse model of intestinal secretion. In this model a test compound is administered to suckling mice that are between 7 and 9 days old. After the mice are sacrificed, the gastrointestinal tract from the stomach to the cecum is dissected (“guts”). The remains (“carcass”) as well as the guts are weighed and the ratio of guts to carcass weight is calculated. If the  
10 ratio is above 0.09, one can conclude that the test compound increases intestinal secretion. Wild type ST peptide can be used as a control in this assay.

**Example 9: Colonic hyperalgesia animal models**

Hypersensitivity to colorectal distension is common in patients with IBS and may be  
15 responsible for the major symptom of pain. Both inflammatory and non-inflammatory animal models of visceral hyperalgesia to distension have been developed to investigate the effect of compounds on visceral pain in IBS.

I. Trinitrobenzenesulphonic acid (TNBS)-induced rectal allodynia in two rodent  
20 models

TNBS visceral hypersensitivity rat model

Male Wistar rats (220-250 g) are premedicated with 0.5 mg/kg of acepromazine injected intraperitoneally (IP) and anesthetized by intramuscular administration of 100 mg/kg of ketamine. Pairs of nichrome wire electrodes (60 cm in length and 80 µm in diameter) are  
25 implanted in the striated muscle of the abdomen, 2 cm laterally from the white line. The free ends of electrodes are exteriorized on the back of the neck and protected by a plastic tube attached to the skin. Electromyographic (EMG) recordings are started 5 days after surgery. Electrical activity of abdominal striated muscle is recorded with an

electroencephalograph machine (Mini VIII, Alvar, Paris, France) using a short time constant (0.03 sec.) to remove low-frequency signals (<3 Hz).

Ten days post surgical implantation, trinitrobenzenesulphonic acid (TNBS) is  
5 administered to induce rectal inflammation. TNBS (80 mg kg<sup>-1</sup> in 0.3 ml 50 % ethanol) is administered intrarectally through a silicone rubber catheter introduced at 3 cm from the anus under light diethyl-ether anesthesia, as previously described (Morteau et al. 1994 Dig Dis Sci 39:1239). Following TNBS administration, rats are placed in plastic tunnels where they are severely limited in mobility for several days before colorectal  
10 distension (CRD). Experimental compound is administered one hour before CRD which is performed by insertion into the rectum, at 1 cm of the anus, a 4 cm long balloon made from a latex condom (Gue et al, 1997 *Neurogastroenterol. Motil.* 9:271). The balloon is fixed on a rigid catheter taken from an embolectomy probe (Fogarty). The catheter attached balloon is fixed at the base of the tail. The balloon, connected to a barostat, is  
15 inflated progressively by steps of 15 mmHg, from 0 to 60 mmHg, each step of inflation lasting 5 min. Evaluation of rectal sensitivity, as measured by EMG, is performed before (1-2 days) and 3 days following rectal instillation of TNBS.

The number of spike bursts that corresponds to abdominal contractions is determined per  
20 5 min periods. Statistical analysis of the number of abdominal contractions and evaluation of the dose-effects relationships is performed by a one way analysis of variance (ANOVA) followed by a post-hoc (Student or Dunnett tests) and regression analysis for ED50 if appropriate.

25 TNBS visceral hypersensitivity model in wild-type (WT) mice and mice lacking the guanylate-cyclase C receptor (GC-C KO)

TNBS induced visceral hypersensitivity can be assessed in WT and GC-C KO mice. Two groups (WT and GC-C KO) of male mice (22-25g) are surgically prepared for electromyographic (EMG) recordings. Three electrodes are implanted in the striated

muscles of the abdomen for EMG recording of abdominal contractions. Colorectal distension (CRD) is performed with a balloon inflated by 10s steps of 0.02 ml from 0 to 0.12ml. Under basal conditions mice are submitted to control CRD (time 0) followed by oral administration of test peptide (0.01 and 0.3  $\mu\text{g}/\text{kg}$ ) or vehicle only (distilled water, 1 ml) at 3 hours. One hour post dosing the CRD procedure is repeated. Abdominal EMG contractile response to colorectal distension in basal conditions in both WT and GC-C KO mice (12-14 mice per group) is determined in the absence of vehicle and test peptide, and the mean  $\pm$  standard error of the mean (SEM) are determined.

10 For TNBS induced visceral hypersensitivity conditions, mice are submitted to control CRD (time 0) and TNBS (20 mg/kg) is administered at 3 days. Three days post intracolonic TNBS-induction animals are orally administered test peptide (0.01 and 0.3  $\mu\text{g}/\text{kg}$ ) or vehicle (distilled water, 1ml) 1 hour before CRD. The effect of test peptide (0.01  $\mu\text{g}/\text{kg}$ ) on abdominal response to colorectal distension after TNBS in WT and GC-C  
15 KO mice (12-14 per group) at a volume distension of 0.8 ml is determined and the mean  $\pm$  standard error of the mean (SEM) is determined.

## II. Partial restraint stress-induced hyperalgesia model

Five groups of female Wistar rats (weighing 200-250g each), are surgically prepared for electromyography as described (Mortcau et al. 1994 Dig Dis Sci 39:1239-48) and can be  
5 used to evaluate the effects of a test peptide on colorectal sensitivity and compliance after a 2 hour partial restraint stress session. Partial restraint stress (PRS), a relatively mild stress, is induced as previously described (Mortcau et al. 1994 Dig Dis Sci 39:1239-48). Female rats are lightly anesthetized with diethyl ether and their shoulders, upper forelimbs and thoracic trunk are wrapped in a confining harness of paper tape to restrict,  
10 but not prevent body movements. Control sham-stress animals are anesthetized but not wrapped. Animals receive isobaric colorectal distensions (CRD) directly prior to (control CRD) and 15 minutes after two hours of partial restraint induced stress. Rats are treated orally with test peptide (0.3, 3, 30 ug/kg) or vehicle only (distilled water 1 mL) one hour before the CRD procedure. For the CRD procedure, rats are acclimatized to restraint in  
15 polypropylene tunnels (diameter: 7 cm; length: 20 cm) periodically for several days before CRD in order to minimize recording artifacts. The balloon used for distension is 4 cm long and made from a latex condom. It is fixed on a rigid catheter taken from an embolectomy probe (Fogarty). CRD is performed by insertion of the balloon in the rectum at 1 cm from the anus. The tube is fixed at the base of the tail. Isobaric  
20 distensions are performed from 0 to 60 mmHg, with each distension step lasting 5 minutes. The first distension is performed at a pressure of 15 mmHg and an increment of 15 mmHg is added at each following step, until a maximal pressure of 60 mmHg is attained. Electromyographic recordings commence 5 days after surgery. Electrical activity is recorded with an electroencephalograph (Mini VIII, Alvar, Paris, France) using  
25 a short time constant (0.03 sec.) to remove low-frequency signals (<3 Hz) and a paper speed of 3.6 cm/minute. Isobaric distensions of the colon are performed by connecting the balloon to a computerized barostat. Colonic pressure and balloon volume are continuously monitored on a potentiometric recorder (L6514, Linseis, Selb, Germany) with a paper speed of 1.0 cm/minute. The number of spike bursts, corresponding to

abdominal contractions, is evaluated per 5-minute period. Colorectal volumes are determined as the maximal volume obtained for each stage of distension using the potentiometric recorder. Statistical analysis of these two parameters is performed using a one way analysis of variance (ANOVA) followed by an unpaired two-tailed Student's *t* test using GraphPad Prism 4.0. *p* values < 0.05 are considered significantly different. The values are expressed as mean ± SEM.

### III. Water avoidance stress-induced hyperalgesia model

The effect of peptides on basal visceral nociception can be tested using a model of water avoidance stress-induced visceral hyperalgesia in adult male Wistar rats. The stress involves confining rats to a platform surrounded by water for a period of 1 hour and then measuring their visceromotor response to colonic distension using electromyography (EMG).

At least 7 days prior to stress measurements, animals are deeply anesthetized with pentobarbital sodium (45 mg/kg) and equipped with electrodes implanted into the external oblique musculature, just superior to the inguinal ligament. Electrode leads are then tunneled subcutaneously and externalized laterally for future access. Following surgery, rats are housed in pairs and allowed to recover for at least 7 days. On the day of the experiment, animals are lightly anesthetized with halothane, and a lubricated latex balloon (6 cm) is inserted intra-anally into the descending colon. Animals are allowed to recover for 30 minutes, and colorectal distension (CRD) is initiated. The CRD procedure consists of graded intensities of phasic CRD (10, 20, 40, 60 mmHg; 20 s duration; 4 min inter-stimulus interval). Visceromotor response (VMR) to CRD is quantified by measuring EMG activity.

To determine the effect of a test peptide in a model of water avoidance stress-induced visceral hyperalgesia, a baseline CRD is recorded and then the animals are subjected to 1 hour of water avoidance stress. For water avoidance stress, the test apparatus consists of a Plexiglas tank with a block affixed to the center of the floor. The tank is filled with fresh room temperature water (25°C) to within 1 cm of the top of the block. The animals

are placed on the block for a period of 1 h. The sham water avoidance stress entails placing the rats on the same platform in a waterless container.

#### Phenylbenzoquinone-induced writhing model

5 The PBQ-induced writhing model can be used to assess pain control activity of the peptides and GC-C receptor agonists. This model is described by Siegmund et al. (1957 Proc. Soc. Exp. Bio. Med. 95:729-731). Briefly, one hour after oral dosing with a test compound, e.g., a peptide, morphine or vehicle, 0.02% phenylbenzoquinone (PBQ) solution (12.5 mL/kg) is injected by intraperitoneal route into the mouse. The number of  
10 stretches and writhings are recorded from the 5<sup>th</sup> to the 10<sup>th</sup> minute after PBQ injection, and can also be counted between the 35<sup>th</sup> and 40<sup>th</sup> minute and between the 60<sup>th</sup> and 65<sup>th</sup> minute to provide a kinetic assessment. The results are expressed as the number of stretches and writhings (mean  $\pm$  SEM) and the percentage of variation of the nociceptive threshold calculated from the mean value of the vehicle-treated group. The statistical  
15 significance of any differences between the treated groups and the control group is determined by a Dunnett's test using the residual variance after a one-way analysis of variance ( $P < 0.05$ ) using SigmaStat Software.

#### **Example 10: Measuring the effect of peptides on bowel habits**

20 Single doses of 30, 100, 300, 1000 or 3000  $\mu$ g of peptide are given to healthy males and postmenopausal females. At each dose level (peptide or placebo (vehicle) is administered orally in 5.0 mL 50 mM phosphate buffer (pH 6.0) plus 3x20mL water rinses and 175 mL water after at least a 10-hour fast. In each dosing group, subjects are  
25 randomized to receive either placebo or peptide. Bowel habits (including Bristol Stool Form Scale score (BSFS), stool frequency, and stool weight) are evaluated for each collected bowel movement 48 hours prior to dose and up to approximately 48 hours postdose. The BSFS scale is as follows: (1) Separate hard lumps, like nuts; (2) Sausage-shaped but lumpy, (3) Like a sausage or snake but with cracks on its surface, (4) Like a

sausage or snake, smooth and soft, (5) Soft blobs with clear-cut edges, (6) Fluffy pieces with ragged edges, a mushy stool, and (7) Watery, no solid pieces.

5 **Example 11: Examination of the effect of peptides on the consistency and timing of bowel movements in humans after a seven-day dosing period.**

Seven daily doses of 30, 100, 300, or 1000 µg of peptide are given to healthy subjects. Peptide or placebo (vehicle) is administered orally in 5.0 mL 50 mM phosphate buffer (pH 6.0) plus 3x20mL water rinses and 175 mL water after at least a 10-hour fast. In each  
10 dosing group, subjects are randomized to receive peptide or receive placebo. Daily mean BSFS scores, mean stool weight and mean ease of passage for the different dosing groups during the seven days prior to and the seven days during dosing with peptide are collected. The Mean Ease of Passage Scale is as follows: (1) Manual disimpaction, (2) Enema needed, (3) Straining needed, (4) Normal, (5) Urgent without pain, (6) Urgent  
15 with pain, and (7) Incontinent.

**Example 12: Peptide effects in a rat model of postoperative ileus.**

Female CD rats are used to test the effect of peptide on delayed transit induced by abdominal surgery and manual manipulation of the small intestine. Groups of at least  
20 nine rats undergo abdominal surgery under isoflurane anesthesia. Surgery consists of laparotomy and 5 minutes of gentle manual intestinal massage. Following recovery from anesthesia, rats are dosed orally with either 10 µg/kg peptide 3 or vehicle (20mM Tris) in a volume of 300µl. 1 hour after dosing, intestinal transit rate is measured. Animals are again dosed with 300µl of the peptide followed immediately by 500µl of a charcoal meal  
25 (10% charcoal, 10% gum arabic in water). To calculate the distance of the small intestine traveled by the charcoal front, after 20 minutes, the total length of the intestine as well as the distance traveled from the stomach to the charcoal front are measured for each animal.



**Example 13: Peptide effect on cGMP levels and secretion in ligated loops rodent models**

The effect of peptide on cGMP levels and secretion are studied by injecting peptide  
5 directly into an isolated loop in either wild-type or GC-C KO mice. This done by  
surgically ligating a loop in the small intestine of the mouse. The methodology for  
ligated loop formation is a similar to that described in London et al. 1997 Am J Physiol  
p.G93-105. The loop is roughly centered and is a length of 1-3 cm. The loops are  
injected with 100 $\mu$ l of either peptide (5 $\mu$ g) or vehicle (20 mM Tris, pH 7.5 or Krebs  
10 Ringer, 10mM Glucose, HEPES buffer (KRGH)). Following a recovery time of 90  
minutes the loops are excised. Weights are recorded for each loop before and after  
removal of the fluid contained therein. The length of each loop is also recorded. A  
weight to length ratio (W/L) for each loop is calculated to determine the effects of  
peptide on secretion.

15

To determine the effect of peptide on cGMP activity, fluid from the loop is collected in  
ice-cold trichloroacetic acid (TCA) and stored at  $-80^{\circ}\text{C}$  for use in an assay to measure  
cGMP levels in the fluid. Intestinal fluid samples are TCA extracted, and cyclic GMP is  
measured by EIA according to procedures outlined in the Cayman Chemical Cyclic GMP  
20 EIA kit (Cayman Chemical, Ann Arbor, MI) to determine cyclic GMP levels in the  
intestinal fluid of the mouse in the presence of either peptide or vehicle. The effects of  
peptide on cGMP levels and secretion in ligated loops in female CD rats can also be  
determined using protocols similar to those described above. In the case of the rat,  
however four loops of intestine are surgically ligated. The first three loops are distributed  
25 equally in the small intestine and the fourth loop is located in colon. Loops are 1 to 3  
centimeters, and are injected with 200 $\mu$ L of either peptide (5 $\mu$ g) or vehicle (Krebs  
Ringer, 10mM glucose, HEPES buffer (KRGH)).

**Example 14. Peptide effects on opioid induced constipation**

The effect of peptide on opioid induced constipation is studied by dosing female rats  
5 (~160g each) with 300µl of the opiate, morphine (2.5mg/kg) via intra-peritoneal  
injection. Thirty minutes post dosing, animals are treated with 300 µl of SEQ ID NO:3  
or vehicle only. Ten minutes later, the animals are orally dosed with 500µl 10%  
charcoal, 10% gum arabic meal. After ten minutes, the animals are sacrificed and  
gastrointestinal transit is measured as in Example 3 above.

10

**Example 15. Mass Spectrometry Characterization of Disulfide Bonds in peptide**

The position of disulfide bonds in a test peptide can be determined as follows. To  
identify the optimal conditions required to partially reduce a test peptide, chemically  
synthesized peptide is alkylated with iodoacetamide after TCEP (tris(2-carboxyethyl)  
15 phosphine) treatment (0.1 to 10 mM for 20 minutes at room temperature). After TCEP  
reduction, the reaction is adjusted to pH 8.0 with Tris and iodoacetamide is added to 50  
mM. The reaction products are analyzed by LC-MS. Partially reduced peptide is then  
cyanylated, cleaved with base and completely reduced to separate fragments. After  
partial reduction, both cyanylation and cleavage of peptide are performed either in a test  
20 tube or in an HPLC column. A modified method of Wu and Watson ((2002) *Methods Mol.*  
*Biol.* 194: 1-22) is used to determine the position of the disulfide bonds. The steps are  
carried out manually, with isolation of the alkylation products by solid phase extraction  
(SPE), or in-line (automated), with reactions occurring in an SPE column. Briefly, the  
manual procedure comprised the following. Chemically synthesized peptide is partially  
25 reduced with 1 mM tris(2-carboxyethyl) phosphine (TCEP) at pH 3. The sulfhydryl  
groups of partially reduced peptide are cyanylated with 2.1 µmoles of 1-cyano-4-  
dimethylamino-pyridinium tetrafluoroborate (CDAP) for 15 minutes. The reaction  
mixture is then diluted to 0.5 mL with 10 mM ammonium acetate pH 5.8 and applied to  
an Amprep octadecyl C18 minicolumn (100 mg, GE HealthTech). The minicolumn is

washed with 1 mL of 10 mM ammonium acetate pH 5.8 and peptides eluted with 0.6 mL methanol. After drying, the peptides are cleaved in 1 M NH<sub>4</sub>OH and fully reduced with 0.1 M TCEP. After drying, the peptide fragments are reconstituted in 0.1% formic acid and analyzed by LC-MS. Briefly, the automated procedure comprised the following.

- 5 Peptide is loaded onto an Oasis HLB 2 X 15 mm column (Waters). Reactions are carried out by filling a 5 mL sample loop with 1.2 mM TCEP, 2.4 mM CDAP, 2 M NH<sub>4</sub>OH or 6 mM TCEP and pushing each reagent through the column with 0.1% formic acid in 5% methanol at a flow rate of 0.3 mL/min. The column is then back-flushed and the cleaved peptides analyzed by LC-MS.

10

- LC-MS analysis can be conducted using an Atlantis dC18 2.1 X 50 mm column (Waters) equilibrated in 98% buffer A (0.1 % formic acid), 2% buffer B (0.1% formic acid: 85% methanol, 15% CH<sub>3</sub>CN) at a flow rate of 0.3 mL/min. After a 4 min wash with the same buffers, peptides are eluted with a linear gradient of 2% buffer B to 40% buffer B over 38
- 15 min with a constant flow rate of 0.3 mL/min. Cleaved peptide masses are determined using a Micromass Q-ToF II instrument equipped with an electrospray ionization (ESI) source operating in positive ion mode. The instrument is programmed to scan in the mass range of *m/z* 100 to 1000. Molecular weight predictions and data analysis are carried out with MassLynx version 4.0 software. Based on the method of Wu and Watson (*supra*), a
- 20 list of possible fragments resulting from CN-induced cleavage of singly reduced and cyanylated species of peptide with all possible disulfide linkage combinations is generated. The list included the signature fragments for each possible structure and is used to predict the disulfide bonding pattern of the test peptide.

## 25 PEGylated Peptides

The *in vivo* half-life of peptides can be extended by conjugating the peptide to a water soluble polymer, such as polyethylene glycol (PEG) to create a PEGylated peptide. The polyethylene glycol molecules are usually connected to the peptide via a reactive group

found on the peptide, e.g., an amino group found within a lysine or at the amino terminus of the peptide.

5 Various methods are known for attaching polyethylene glycol to a peptide (see, for example, U.S. Pat. No. 4,002,531; U.S. Pat. No. 4,904,584; U.S. Pat. No. 5,834,594; U.S. Pat. No. 5,824,784 and U.S. Pat. No. 5,985,265).

#### Administration of peptides and GC-C receptor agonists

10 For therapeutic and preventive treatment of disorders described herein, the peptides and agonists described herein can be administered orally, e.g., as a tablet or cachet containing a predetermined amount of the active ingredient, pellet, gel, paste, syrup, bolus, electuary, slurry, sachet; capsule; powder; lyophilized powder; granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion, via a liposomal formulation (see, e.g., EP  
15 736299) or in some other form. Orally administered compositions can include binders, lubricants, inert diluents, lubricating, surface active or dispersing agents, flavoring agents, and humectants. Orally administered formulations such as tablets may optionally be coated or scored and may be formulated so as to provide sustained, delayed or controlled release of the active ingredient therein. The peptides and agonists can be co-  
20 administered with other agents used to treat gastrointestinal disorders including but not limited to the agents described herein. The peptides and agonists can also be administered by rectal suppository. For the treatment of disorders outside the gastrointestinal tract such as congestive heart failure and benign prostatic hypertrophy, peptides and agonists are preferably administered parenterally or orally.

25

The peptides described herein can be administered alone or in combination with other agents. For example, the peptides can be administered together with an analgesic peptide or compound. The analgesic peptide or compound can be covalently attached to a

peptide described herein or it can be a separate agent that is administered together with or sequentially with a peptide described herein in a combination therapy.

Combination therapy can be achieved by administering two or more agents, e.g., a peptide described herein and an analgesic peptide or compound, each of which is formulated and administered separately, or by administering two or more agents in a single formulation. Other combinations are also encompassed by combination therapy. For example, two agents can be formulated together and administered in conjunction with a separate formulation containing a third agent. While the two or more agents in the combination therapy can be administered simultaneously, they need not be. For example, administration of a first agent (or combination of agents) can precede administration of a second agent (or combination of agents) by minutes, hours, days, or weeks. Thus, the two or more agents can be administered within minutes of each other or within 1, 2, 3, 6, 9, 12, 15, 18, or 24 hours of each other or within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14 days of each other or within 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks of each other. In some cases even longer intervals are possible. While in many cases it is desirable that the two or more agents used in a combination therapy be present in within the patient's body at the same time, this need not be so.

Combination therapy can also include two or more administrations of one or more of the agents used in the combination. For example, if agent X and agent Y are used in a combination, one could administer them sequentially in any combination one or more times, e.g., in the order X-Y-X, X-X-Y, Y-X-Y, Y-Y-X, X-X-Y-Y, etc.

Combination therapy can also include the administration of two or more agents via different routes or locations. For example, (a) one agent is administered orally and another agent is administered intravenously or (b) one agent is administered orally and another is administered locally. In each case, the agents can either simultaneously or sequentially. Approximated dosages for some of the combination therapy agents described herein are found in the "BNF Recommended Dose" column of tables on pages 11-17 of WO01/76632 (the data in the tables being attributed to the March 2000 British

National Formulary) and can also be found in other standard formularies and other drug prescribing directories. For some drugs, the customary prescribed dose for an indication will vary somewhat from country to country.

The agents, alone or in combination, can be combined with any pharmaceutically acceptable carrier or medium. Thus, they can be combined with materials that do not produce an adverse, allergic or otherwise unwanted reaction when administered to a patient. The carriers or mediums used can include solvents, dispersants, coatings, absorption promoting agents, controlled release agents, and one or more inert excipients (which include starches, polyols, granulating agents, microcrystalline cellulose (e.g. 5 celphere, Celphere beads®), diluents, lubricants, binders, disintegrating agents, and the like), etc. If desired, tablet dosages of the disclosed compositions may be coated by standard aqueous or nonaqueous techniques.

Compositions of the present disclosure may also optionally include other therapeutic 15 ingredients, anti-caking agents, preservatives, sweetening agents, colorants, flavors, desiccants, plasticizers, dyes, glidants, anti-adherents, anti-static agents, surfactants (wetting agents), anti-oxidants, film-coating agents, and the like. Any such optional ingredient must be compatible with the compound described herein to insure the stability of the formulation.

20 The composition may contain other additives as needed, including for example lactose, glucose, fructose, galactose, trehalose, sucrose, maltose, raffinose, maltitol, melezitose, stachyose, lactitol, palatinite, starch, xylitol, mannitol, myoinositol, and the like, and hydrates thereof, and amino acids, for example alanine, glycine and betaine, and peptides and proteins, for example albumen.

25

Examples of excipients for use as the pharmaceutically acceptable carriers and the pharmaceutically acceptable inert carriers and the aforementioned additional ingredients include, but are not limited to binders, fillers, disintegrants, lubricants, anti-microbial agents, and coating agents such as:

BINDERS: corn starch, potato starch, other starches, gelatin, natural and synthetic gums such as acacia, xanthan, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (*e.g.*, ethyl cellulose, cellulose acetate, 5 carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone (*e.g.*, povidone, crospovidone, copovidone, etc), methyl cellulose, Methocel, pre-gelatinized starch (*e.g.*, STARCH 1500® and STARCH 1500 LM®, sold by Colorcon, Ltd.), hydroxypropyl methyl cellulose, microcrystalline cellulose (*e.g.* AVICEL™, such as, AVICEL-PH-101™, -103™ and -105™, sold by FMC Corporation, 10 Marcus Hook, PA, USA), or mixtures thereof,

FILLERS: talc, calcium carbonate (*e.g.*, granules or powder), dibasic calcium phosphate, tribasic calcium phosphate, calcium sulfate (*e.g.*, granules or powder), microcrystalline cellulose, powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, 15 pre-gelatinized starch, dextrose, fructose, honey, lactose anhydrate, lactose monohydrate, lactose and aspartame, lactose and cellulose, lactose and microcrystalline cellulose, maltodextrin, maltose, mannitol, microcrystalline cellulose & guar gum, molasses, sucrose, or mixtures thereof,

DISINTEGRANTS: agar-agar, alginic acid, calcium carbonate, microcrystalline 20 cellulose, croscarmellose sodium, crospovidone, polacrillin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, clays, other algins, other celluloses, gums (like gellan), low-substituted hydroxypropyl cellulose, or mixtures thereof,

25 LUBRICANTS: calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, sodium stearyl fumarate, vegetable based fatty acids lubricant, talc, hydrogenated vegetable oil (*e.g.*, peanut oil, cottonseed oil, sunflower oil, sesame oil,

olive oil, corn oil and soybean oil), zinc stearate, ethyl oleate, ethyl laurate, agar, syloid silica gel (AEROSIL 200, W.R. Grace Co., Baltimore, MD USA), a coagulated aerosol of synthetic silica (Deaussa Co., Plano, TX USA), a pyrogenic silicon dioxide (CAB-O-SIL, Cabot Co., Boston, MA USA), or mixtures thereof,

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ANTI-CAKING AGENTS: calcium silicate, magnesium silicate, silicon dioxide, colloidal silicon dioxide, talc, or mixtures thereof,

ANTIMICROBIAL AGENTS: benzalkonium chloride, benzethonium chloride, benzoic acid, benzyl alcohol, butyl paraben, cetylpyridinium chloride, cresol, chlorobutanol, dehydroacetic acid, ethylparaben, methylparaben, phenol, phenylethyl alcohol, phenoxyethanol, phenylmercuric acetate, phenylmercuric nitrate, potassium sorbate, propylparaben, sodium benzoate, sodium dehydroacetate, sodium propionate, sorbic acid, thimersol, thymo, or mixtures thereof, and

15

COATING AGENTS: sodium carboxymethyl cellulose, cellulose acetate phthalate, ethylcellulose, gelatin, pharmaceutical glaze, hydroxypropyl cellulose, hydroxypropyl methylcellulose (hypromellose), hydroxypropyl methyl cellulose phthalate, methylcellulose, polyethylene glycol, polyvinyl acetate phthalate, shellac, sucrose, titanium dioxide, carnauba wax, microcrystalline wax, gellan gum, maltodextrin, methacrylates, microcrystalline cellulose and carrageenan or mixtures thereof.

The formulation can also include other excipients and categories thereof including but not limited to L-histidine, Pluronic®, Poloxamers (such as Lutrol® and Poloxamer 188), ascorbic acid, glutathione, permeability enhancers (e.g. lipids, sodium cholate, acylcarnitine, salicylates, mixed bile salts, fatty acid micelles, chelators, fatty acid, surfactants, medium chain glycerides), protease inhibitors (e.g. soybean trypsin inhibitor, organic acids), pH lowering agents and absorption enhancers effective to promote bioavailability (including but not limited to those described in US6086918 and

25



US5912014), creams and lotions (like maltodextrin and carrageenans); materials for chewable tablets (like dextrose, fructose, lactose monohydrate, lactose and aspartame, lactose and cellulose, maltodextrin, maltose, mannitol, microcrystalline cellulose and guar gum, sorbitol crystalline); parenterals (like mannitol and povidone); plasticizers  
5 (like dibutyl sebacate, plasticizers for coatings, polyvinylacetate phthalate); powder lubricants (like glyceryl behenate); soft gelatin capsules (like sorbitol special solution); spheres for coating (like sugar spheres); spheronization agents (like glyceryl behenate and microcrystalline cellulose); suspending/gelling agents (like carrageenan, gellan gum, mannitol, microcrystalline cellulose, povidone, sodium starch glycolate, xanthan gum);  
10 sweeteners (like aspartame, aspartame and lactose, dextrose, fructose, honey, maltodextrin, maltose, mannitol, molasses, sorbitol crystalline, sorbitol special solution, sucrose); wet granulation agents (like calcium carbonate, lactose anhydrous, lactose monohydrate, maltodextrin, mannitol, microcrystalline cellulose, povidone, starch), caramel, carboxymethylcellulose sodium, cherry cream flavor and cherry flavor, citric  
15 acid anhydrous, citric acid, confectioner's sugar, D&C Red No. 33, D&C Yellow #10 Aluminum Lake, disodium edetate, ethyl alcohol 15%, FD& C Yellow No. 6 aluminum lake, FD&C Blue #1 Aluminum Lake, FD&C Blue No. 1, FD&C blue no. 2 aluminum lake, FD&C Green No.3, FD&C Red No. 40, FD&C Yellow No. 6 Aluminum Lake, FD&C Yellow No. 6, FD&C Yellow No.10, glycerol palmitostearate, glyceryl  
20 monostearate, indigo carmine, lecithin, manitol, methyl and propyl parabens, mono ammonium glycyrrhizinate, natural and artificial orange flavor, pharmaceutical glaze, poloxamer 188, Polydextrose, polysorbate 20, polysorbate 80, polyvidone, pregelatinized corn starch, pregelatinized starch, red iron oxide, saccharin sodium, sodium carboxymethyl ether, sodium chloride, sodium citrate, sodium phosphate, strawberry  
25 flavor, synthetic black iron oxide, synthetic red iron oxide, titanium dioxide, and white wax.

Solid oral dosage forms may optionally be treated with coating systems (e.g. Opadry® fx film coating system, for example Opadry® blue (OY-LS-20921), Opadry® white (YS-2-7063), Opadry® white (YS-1-7040), and black ink (S-1-8106).

- 5 The agents either in their free form or as a salt can be combined with a polymer such as polylactic-glycolic acid (PLGA), poly-(l)-lactic-glycolic-tartaric acid (P(l)LGT) (WO 01/12233), polyglycolic acid (U.S. 3,773,919), polylactic acid (U.S. 4,767,628), poly( $\epsilon$ -caprolactone) and poly(alkylene oxide) (U.S. 20030068384) to create a sustained release formulation. Such formulations can be used to implants that release a peptide or another
- 10 agent over a period of a few days, a few weeks or several months depending on the polymer, the particle size of the polymer, and the size of the implant (see, e.g., U.S. 6,620,422). Other sustained release formulations and polymers for use in are described in EP 0 467 389 A2, WO 93/24150, U.S. 5,612,052, WO 97/40085, WO 03/075887, WO 01/01964A2, U.S. 5,922,356, WO 94/155587, WO 02/074247A2, WO 98/25642, U.S.
- 15 5,968,895, U.S. 6,180,608, U.S. 20030171296, U.S. 20020176841, U.S. 5,672,659, U.S. 5,893,985, U.S. 5,134,122, U.S. 5,192,741, U.S. 5,192,741, U.S. 4,668,506, U.S. 4,713,244, U.S. 5,445,832 U.S. 4,931,279, U.S. 5,980,945, WO 02/058672, WO 9726015, WO 97/04744, and. US20020019446. In such sustained release formulations microparticles (Delie and Blanco-Prieto 2005 Molecule 10:65-80) of peptide are
- 20 combined with microparticles of polymer. One or more sustained release implants can be placed in the large intestine, the small intestine or both. U.S. 6,011,011 and WO 94/06452 describe a sustained release formulation providing either polyethylene glycols (i.e. PEG 300 and PEG 400) or triacetin. WO 03/053401 describes a formulation which may both enhance bioavailability and provide controlled release of the agent within the GI
- 25 tract. Additional controlled release formulations are described in U.S. 6,734,188, WO 02/38129, EP 326 151, U.S. 5,236,704, WO 02/30398, WO 98/13029; U.S. 20030064105, U.S. 20030138488A1, U.S. 20030216307A1, U.S. 6,667,060, WO 01/49249, WO 01/49311, WO 01/49249, WO 01/49311, and U.S. 5,877,224.

The agents can be administered, e.g., by intravenous injection, intramuscular injection, subcutaneous injection, intraperitoneal injection, topical, sublingual, intraarticular (in the joints), intradermal, buccal, ophthalmic (including intraocular), intranasally (including using a cannula), intraspinally, intrathecally, or by other routes. The agents can be administered orally, e.g., as a tablet or cachet containing a predetermined amount of the active ingredient, gel, pellet, paste, syrup, bolus, electuary, slurry, capsule, powder, lyophilized powder, granules, sachet, as a solution or a suspension in an aqueous liquid or a non-aqueous liquid, as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion, via a micellar formulation (see, e.g. WO 97/11682) via a liposomal formulation (see, e.g., EP 736299, WO 99/59550 and WO 97/13500), via formulations described in WO 03/094886, via bilosome (bile-salt based vesicular system), via a dendrimer, or in some other form. Orally administered compositions can include binders, lubricants, inert diluents, lubricating, surface active or dispersing agents, flavoring agents, and humectants. Orally administered formulations such as tablets may optionally be coated or scored and may be formulated so as to provide sustained, delayed or controlled release of the active ingredient therein. The agents can also be administered transdermally (i.e. via reservoir-type or matrix-type patches, microneedles, thermal poration, hypodermic needles, iontophoresis, electroporation, ultrasound or other forms of sonophoresis, jet injection, or a combination of any of the preceding methods (Prausnitz et al. 2004, Nature Reviews Drug Discovery 3:115-124)). The agents can be administered using high-velocity transdermal particle injection techniques using the hydrogel particle formulation described in U.S. 20020061336. Additional particle formulations are described in WO 00/45792, WO 00/53160, and WO 02/19989. An example of a transdermal formulation containing plaster and the absorption promoter dimethylisobornide can be found in WO 89/04179. WO 96/11705 provides formulations suitable for transdermal administration. The agents can be administered in the form a suppository or by other vaginal or rectal means. The agents can be administered in a transmembrane formulation as described in WO 90/07923. The agents can be administered non-invasively via the dehydrated particulates described in U.S. 6,485,706. The agent can be administered in an enteric-

coated drug formulation as described in WO 02/49621. The agents can be administered intranasally using the formulation described in U.S. 5,179,079. Formulations suitable for parenteral injection are described in WO 00/62759. The agents can be administered using the casein formulation described in U. S. 20030206939 and WO 00/06108. The agents can be administered using the particulate formulations described in U.S. 20020034536.

The agents, alone or in combination with other suitable components, can be administered by pulmonary route utilizing several techniques including but not limited to intratracheal instillation (delivery of solution into the lungs by syringe), intratracheal delivery of liposomes, insufflation (administration of powder formulation by syringe or any other similar device into the lungs) and aerosol inhalation. Aerosols (e.g., jet or ultrasonic nebulizers, metered-dose inhalers (MDIs), and dry-powder inhalers (DPIs)) can also be used in intranasal applications. Aerosol formulations are stable dispersions or suspensions of solid material and liquid droplets in a gaseous medium and can be placed into pressurized acceptable propellants, such as hydrofluoroalkanes (HFAs, i.e. HFA-134a and HFA-227, or a mixture thereof), dichlorodifluoromethane (or other chlorofluorocarbon propellants such as a mixture of Propellants 11, 12, and/or 114), propane, nitrogen, and the like. Pulmonary formulations may include permeation enhancers such as fatty acids, saccharides, chelating agents, enzyme inhibitors (e.g., protease inhibitors), adjuvants (e.g., glycocholate, surfactin, span 85, and nafamostat), preservatives (e.g., benzalkonium chloride or chlorobutanol), and ethanol (normally up to 5% but possibly up to 20%, by weight). Ethanol is commonly included in aerosol compositions as it can improve the function of the metering valve and in some cases also improve the stability of the dispersion. Pulmonary formulations may also include surfactants which include but are not limited to bile salts and those described in U.S. 6,524,557 and references therein. The surfactants described in U.S. 6,524,557, e.g., a C8-C16 fatty acid salt, a bile salt, a phospholipid, or alkyl saccharide are advantageous in that some of them also reportedly enhance absorption of the peptide in the formulation. Also suitable in the disclosure are

dry powder formulations comprising a therapeutically effective amount of active compound blended with an appropriate carrier and adapted for use in connection with a dry-powder inhaler. Absorption enhancers which can be added to dry powder formulations of the present disclosure include those described in U.S. 6,632,456. WO 5 02/080884 describes new methods for the surface modification of powders. Aerosol formulations may include U.S. 5,230,884, U.S. 5,292,499, WO 01/78694, WO 01/78696, U.S. 2003019437, U. S. 20030165436, and WO 96/40089 (which includes vegetable oil). Sustained release formulations suitable for inhalation are described in U.S. 20010036481A1, 20030232019A1, and U.S. 20040018243A1 as well as in WO 10 01/13891, WO 02/067902, WO 03/072080, and WO 03/079885. Pulmonary formulations containing microparticles are described in WO 03/015750, U.S. 20030008013, and WO 00/00176. Pulmonary formulations containing stable glassy state powder are described in U.S. 20020141945 and U.S. 6,309,671. Other aerosol formulations are described in EP 1338272A1 WO 90/09781, U. S. 5,348,730, U.S. 15 6,436,367, WO 91/04011, and U.S. 6,294,153 and U.S. 6,290,987 describes a liposomal based formulation that can be administered via aerosol or other means. Powder formulations for inhalation are described in U.S. 20030053960 and WO 01/60341. The agents can be administered intranasally as described in U.S. 20010038824. The agents can be incorporated into microemulsions, which generally are 20 thermodynamically stable, isotropically clear dispersions of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules (Encyclopedia of Pharmaceutical Technology (New York: Marcel Dekker, 1992), volume 9). For the preparation of microemulsions, surfactant (emulsifier), co-surfactant (co-emulsifier), an oil phase and a water phase are necessary. Suitable surfactants include any surfactants 25 that are useful in the preparation of emulsions, e.g., emulsifiers that are typically used in the preparation of creams. The co-surfactant (or "co-emulsifier") is generally selected from the group of polyglycerol derivatives, glycerol derivatives and fatty alcohols. Preferred emulsifier/co-emulsifier combinations are generally although not necessarily selected from the group consisting of: glyceryl monostearate and polyoxyethylene

stearate; polyethylene glycol and ethylene glycol palmitostearate; and caprylic and capric triglycerides and oleoyl macroglycerides. The water phase includes not only water but also, typically, buffers, glucose, propylene glycol, polyethylene glycols, preferably lower molecular weight polyethylene glycols (e.g., PEG 300 and PEG 400), and/or glycerol, and the like, while the oil phase will generally comprise, for example, fatty acid esters, modified vegetable oils, silicone oils, mixtures of mono- di- and triglycerides, mono- and di-esters of PEG (e.g., oleoyl macroglycerides), etc.

The agents described herein can be incorporated into pharmaceutically-acceptable nanoparticle, nanosphere, and nanocapsule formulations (Delie and Blanco-Prieto 2005 Molecule 10:65-80). Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland et al., 1987; Quintanar-Guerrero et al., 1998; Douglas et al., 1987). To avoid side effects due to intracellular polymeric overloading, ultrafine particles (sized around 0.1  $\mu\text{m}$ ) can be designed using polymers able to be degraded in vivo (e.g. biodegradable polyalkyl-cyanoacrylate nanoparticles). Such particles are described in the prior art (Couvreur et al, 1980; 1988; zur Muhlen et al., 1998; Zambaux et al. 1998; Pinto-Alphandry et al., 1995 and U.S. Pat. No. 5,145,684).

The agents described herein can be formulated with pH sensitive materials which may include those described in WO04041195 (including the seal and enteric coating described therein) and pH-sensitive coatings that achieve delivery in the colon including those described in US4910021 and WO9001329. US4910021 describes using a pH-sensitive material to coat a capsule. WO9001329 describes using pH-sensitive coatings on beads containing acid, where the acid in the bead core prolongs dissolution of the pH-sensitive coating. U. S. Patent No. 5,175, 003 discloses a dual mechanism polymer mixture composed of pH-sensitive enteric materials and film-forming plasticizers capable of conferring permeability to the enteric material, for use in drug-delivery systems; a matrix pellet composed of a dual mechanism polymer mixture permeated with a drug and sometimes covering a pharmaceutically neutral nucleus; a membrane-coated pellet

comprising a matrix pellet coated with a dual mechanism polymer mixture envelope of the same or different composition; and a pharmaceutical dosage form containing matrix pellets. The matrix pellet releases acid-soluble drugs by diffusion in acid pH and by disintegration at pH levels of nominally about 5.0 or higher. The agents described herein

5 may be formulated in the pH triggered targeted control release systems described in WO04052339. The agents described herein may be formulated according to the methodology described in any of WO03105812 (extruded hydratable polymers); WO0243767 (enzyme cleavable membrane translocators); WO03007913 and WO03086297 (mucoadhesive systems); WO02072075 (bilayer laminated formulation

10 comprising pH lowering agent and absorption enhancer); WO04064769 (amidated peptides); WO05063156 (solid lipid suspension with pseudotropic and/or thixotropic properties upon melting); WO03035029 and WO03035041 (erodible, gastric retentive dosage forms); US5007790 and US5972389 (sustained release dosage forms); WO04112711 (oral extended release compositions); WO05027878, WO02072033, and

15 WO02072034 (delayed release compositions with natural or synthetic gum); WO05030182 (controlled release formulations with an ascending rate of release); WO05048998 (microencapsulation system); US Patent 5,952, 314 (biopolymer); US5108758 (glassy amylose matrix delivery); US 5840860 (modified starch based delivery). JP10324642 (delivery system comprising chitosan and gastric resistant material

20 such as wheat gliadin or zein); US5866619 and US6368629 (saccharide containing polymer); US 6531152 (describes a drug delivery system containing a water soluble core (Ca pectinate or other water-insoluble polymers) and outer coat which bursts (eg hydrophobic polymer-Eudragit)); US 6234464; US 6403130 (coating with polymer containing casein and high methoxy pectin; WO0174175 (Maillard reaction product);

25 WO05063206 (solubility increasing formulation); WO04019872 (transferring fusion proteins). The agents described herein may be formulated using gastrointestinal retention system technology (GIRES; Merrion Pharmaceuticals). GIRES comprises a controlled-release dosage form inside an inflatable pouch, which is placed in a drug capsule for oral administration. Upon dissolution of the capsule, a gas-generating system inflates the

pouch in the stomach where it is retained for 16-24 hours, all the time releasing agents described herein.

The agents described herein can be formulated in an osmotic device including the ones  
5 disclosed in US4503030, US5609590 and US5358502. US4503030 discloses an osmotic device for dispensing a drug to certain pH regions of the gastrointestinal tract. More particularly, the disclosure relates to an osmotic device comprising a wall formed of a semi-permeable pH sensitive composition that surrounds a compartment containing a drug, with a passageway through the wall connecting the exterior of the device with the  
10 compartment. The device delivers the drug at a controlled rate in the region of the gastrointestinal tract having a pH of less than 3.5, and the device self- destructs and releases all its drug in the region of the gastrointestinal tract having a pH greater than 3.5, thereby providing total availability for drug absorption. U. S. Patent Nos. 5,609, 590 and 5, 358,502 disclose an osmotic bursting device for dispensing a beneficial agent to an  
15 aqueous environment. The device comprises a beneficial agent and osmagent surrounded at least in part by a semi-permeable membrane. The beneficial agent may also function as the osmagent. The semi-permeable membrane is permeable to water and substantially impermeable to the beneficial agent and osmagent. A trigger means is attached to the semi-permeable membrane (e. g., joins two capsule halves). The trigger means is  
20 activated by a pH of from 3 to 9 and triggers the eventual, but sudden, delivery of the beneficial agent. These devices enable the pH-triggered release of the beneficial agent core as a bolus by osmotic bursting.

The agents described herein may be formulated based on the disclosure described in U. S. Patent No. 5,316, 774 which discloses a composition for the controlled release of an  
25 active substance comprising a polymeric particle matrix, where each particle defines a network of internal pores. The active substance is entrapped within the pore network together with a blocking agent having physical and chemical characteristics selected to modify the release rate of the active substance from the internal pore network. In one embodiment, drugs may be selectively delivered to the intestines using an enteric



- material as the blocking agent. The enteric material remains intact in the stomach but degrades under the pH conditions of the intestines. In another embodiment, the sustained release formulation employs a blocking agent, which remains stable under the expected conditions of the environment to which the active substance is to be released. The use of
- 5 pH-sensitive materials alone to achieve site-specific delivery is difficult because of leaking of the beneficial agent prior to the release site or desired delivery time and it is difficult to achieve long time lags before release of the active ingredient after exposure to high pH (because of rapid dissolution or degradation of the pH-sensitive materials).
- 10 The agents may also be formulated in a hybrid system which combines pH-sensitive materials and osmotic delivery systems. These hybrid devices provide delayed initiation of sustained-release of the beneficial agent. In one device a pH-sensitive matrix or coating dissolves releasing osmotic devices that provide sustained release of the beneficial agent see U. S. Patent Nos. 4,578, 075, 4,681, 583, and 4,851, 231. A second
- 15 device consists of a semipermeable coating made of a polymer blend of an insoluble and a pH-sensitive material. As the pH increases, the permeability of the coating increases, increasing the rate of release of beneficial agent see U. S. Patent Nos. 4,096, 238,4, 503,030, 4, 522, 625, and 4,587, 117.
- 20 The agents described herein may be formulated in terpolymers according to U. S. Patent No. 5,484, 610 which discloses terpolymers which are sensitive to pH and temperature which are useful carriers for conducting bioactive agents through the gastric juices of the stomach in a protected form. The terpolymers swell at the higher physiologic pH of the intestinal tract causing release of the bioactive agents into the intestine. The terpolymers
- 25 are linear and are made up of 35 to 99 wt % of a temperature sensitive component, which imparts to the terpolymer LCST (lower critical solution temperature) properties below body temperatures, 1 to 30 wt % of a pH sensitive component having a pKa in the range of from 2 to 8 which functions through ionization or deionization of carboxylic acid groups to prevent the bioactive agent from being lost at low pH but allows bioactive

agent release at physiological pH of about 7.4 and a hydrophobic component which stabilizes the LCST below body temperatures and compensates for bioactive agent effects on the terpolymers. The terpolymers provide for safe bioactive agent loading, a simple procedure for dosage form fabrication and the terpolymer functions as a protective carrier  
5 in the acidic environment of the stomach and also protects the bioactive agents from digestive enzymes until the bioactive agent is released in the intestinal tract.

The agents described herein may be formulated in pH sensitive polymers according to those described in U. S. Patent No. 6,103, 865. U. S. Patent No. 6,103, 865 discloses  
10 pH-sensitive polymers containing sulfonamide groups, which can be changed in physical properties, such as swellability and solubility, depending on pH and which can be applied for a drug-delivery system, bio-material, sensor, and the like, and a preparation method therefore. The pH-sensitive polymers are prepared by introduction of sulfonamide groups, various in pKa, to hydrophilic groups of polymers either through coupling to the  
15 hydrophilic groups of polymers, such as acrylamide, N, N- dimethylacrylamide, acrylic acid, N-isopropylacrylamide and the like or copolymerization with other polymerizable monomers. These pH-sensitive polymers may have a structure of linear polymer, grafted copolymer, hydrogel or interpenetrating network polymer.

20 The agents described herein may be formulated according U. S. Patent No. 5, 656, 292 which discloses a composition for pH dependent or pH regulated controlled release of active ingredients especially drugs. The composition consists of a compactable mixture of the active ingredient and starch molecules substituted with acetate and dicarboxylate residues. The preferred dicarboxylate acid is succinate. The average substitution degree  
25 of the acetate residue is at least 1 and 0. 2-1.2 for the dicarboxylate residue. The starch molecules can have the acetate and dicarboxylate residues attached to the same starch molecule backbone or attached to separate starch molecule backbones. The present disclosure also discloses methods for preparing said starch acetate dicarboxylates by transesterification or mixing of starch acetates and starch dicarboxylates respectively.

The agents described herein may be formulated according to the methods described in U. S. Patent Nos. 5,554, 147,5, 788, 687, and 6,306, 422 which disclose a method for the controlled release of a biologically active agent wherein the agent is released from a hydrophobic, pH-sensitive polymer matrix. The polymer matrix swells when the environment reaches pH 8.5, releasing the active agent. A polymer of hydrophobic and weakly acidic comonomers is disclosed for use in the controlled release system. Also disclosed is a specific embodiment in which the controlled release system may be used. The pH-sensitive polymer is coated onto a latex catheter used in ureteral catheterization. A ureteral catheter coated with a pH-sensitive polymer having an antibiotic or urease inhibitor trapped within its matrix will release the active agent when exposed to high pH urine.

The agents described herein may be formulated in/with bioadhesive polymers according to US Patent No. 6,365, 187. Bioadhesive polymers in the form of, or as a coating on, microcapsules containing drugs or bioactive substances which may serve for therapeutic, or diagnostic purposes in diseases of the gastrointestinal tract, are described in US6365187. The polymeric microspheres all have a bioadhesive force of at least 11 mN/cm<sup>2</sup> (110 N/m<sup>2</sup>) Techniques for the fabrication of bioadhesive microspheres, as well as a method for measuring bioadhesive forces between microspheres and selected segments of the gastrointestinal tract in vitro are also described. This quantitative method provides a means to establish a correlation between the chemical nature, the surface morphology and the dimensions of drug-loaded microspheres on one hand and bioadhesive forces on the other, allowing the screening of the most promising materials from a relatively large group of natural and synthetic polymers which, from theoretical consideration, should be used for making bioadhesive microspheres. Solutions of medicament in buffered saline and similar vehicles are commonly employed to generate an aerosol in a nebulizer. Simple nebulizers operate on Bernoulli's principle and employ a stream of air or oxygen to generate the spray particles. More complex nebulizers

employ ultrasound to create the spray particles. Both types are well known in the art and are described in standard textbooks of pharmacy such as Sprowls' American Pharmacy and Remington's The Science and Practice of Pharmacy. Other devices for generating aerosols employ compressed gases, usually hydrofluorocarbons and chlorofluorocarbons, which are mixed with the medicament and any necessary excipients in a pressurized container, these devices are likewise described in standard textbooks such as Sprowls and Remington.

The agents can be a free acid or base, or a pharmacologically acceptable salt thereof.

10 Solids can be dissolved or dispersed immediately prior to administration or earlier. In some circumstances the preparations include a preservative to prevent the growth of microorganisms. The pharmaceutical forms suitable for injection can include sterile aqueous or organic solutions or dispersions which include, e.g., water, an alcohol, an organic solvent, an oil or other solvent or dispersant (e.g., glycerol, propylene glycol, polyethylene glycol, and vegetable oils). The formulations may contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

Pharmaceutical agents can be sterilized by filter sterilization or by other suitable means.

20 The agent can be fused to immunoglobulins or albumin, albumin variants or fragments thereof, or incorporated into a liposome to improve half-life. Thus the peptides described herein may be fused directly or via a peptide linker, water soluble polymer, or prodrug linker to albumin or an analog, fragment, or derivative thereof. Generally, the albumin proteins that are part of the fusion proteins of the present disclosure may be derived from

25 albumin cloned from any species, including human. Human serum albumin (HSA) consists of a single non-glycosylated peptide chain of 585 amino acids with a formula molecular weight of 66,500. The amino acid sequence of human HSA is known [See Meloun, et al. (1975) FEBS Letters 58:136; Behrens, et al. (1975) Fed. Proc. 34:591; Lawn, et al. (1981) Nucleic Acids Research 9:6102-6114; Minghetti, et al. (1986) J. Biol.

Chem. 261:6747, each of which are incorporated by reference herein]. A variety of polymorphic variants as well as analogs and fragments of albumin have been described. [See Weitkamp, et al., (1973) Ann. Hum. Genet. 37:219]. For example, in EP 322,094, various shorter forms of HSA. Some of these fragments of HSA are disclosed, including

5 HSA(1-373), HSA(1-388), HSA(1-389), HSA(1-369), and HSA(1-419) and fragments between 1-369 and 1-419. EP 399,666 discloses albumin fragments that include HSA(1-177) and HSA(1-200) and fragments between HSA(1-177) and HSA(1-200). Methods related to albumin fusion proteins can be found in US 7,056,701, US 6,994,857, US 6,946,134, US6,926,898, and US 6,905,688 and the related priority documents and

10 references cited therein. The agent can also be conjugated to polyethylene glycol (PEG) chains. Methods for pegylation and additional formulations containing PEG-conjugates (i.e. PEG-based hydrogels, PEG modified liposomes) can be found in Harris and Chess, Nature Reviews Drug Discovery 2: 214-221 and the references therein. Peptides can also be modified with alkyl groups (e.g., C1-C20 straight or branched alkyl groups); fatty acid

15 radicals; and combinations of PEG, alkyl groups and fatty acid radicals (see U.S. Patent 6,309,633; Soltero et al., 2001 Innovations in Pharmaceutical Technology 106-110). The agent can be administered via a nanocochleate or cochleate delivery vehicle (BioDelivery Sciences International). The agents can be delivered transmucosally (i.e. across a mucosal surface such as the vagina, eye or nose) using formulations such as that

20 described in U.S. 5,204,108. The agents can be formulated in microcapsules as described in WO 88/01165. The agent can be administered intra-orally using the formulations described in U.S. 20020055496, WO 00/47203, and U.S. 6,495,120. The agent can be delivered using nanoemulsion formulations described in WO 01/91728A2.

25 Controlled release formulations

In general, one can provide for controlled release of the agents described herein through the use of a wide variety of polymeric carriers and controlled release systems including erodible and non-erodible matrices, osmotic control devices, various reservoir devices, enteric coatings and multiparticulate control devices.

Matrix devices are a common device for controlling the release of various agents. In such devices, the agents described herein are generally present as a dispersion within the polymer matrix, and are typically formed by the compression of a polymer/drug mixture or by dissolution or melting. The dosage release properties of these devices may be dependent upon the solubility of the agent in the polymer matrix or, in the case of porous matrices, the solubility in the sink solution within the pore network, and the tortuosity of the network. In one instance, when utilizing an erodible polymeric matrix, the matrix imbibes water and forms an aqueous-swollen gel that entraps the agent. The matrix then gradually erodes, swells, disintegrates or dissolves in the GI tract, thereby controlling release of one or more of the agents described herein. In non-erodible devices, the agent is released by diffusion through an inert matrix.

Agents described herein can be incorporated into an erodible or non-erodible polymeric matrix controlled release device. By an erodible matrix is meant aqueous-erodible or water-swallowable or aqueous-soluble in the sense of being either erodible or swallowable or dissolvable in pure water or requiring the presence of an acid or base to ionize the polymeric matrix sufficiently to cause erosion or dissolution. When contacted with the aqueous environment of use, the erodible polymeric matrix imbibes water and forms an aqueous-swollen gel or matrix that entraps the agent described herein. The aqueous-swollen matrix gradually erodes, swells, disintegrates or dissolves in the environment of use, thereby controlling the release of a compound described herein to the environment of use.

The erodible polymeric matrix into which an agent described herein can be incorporated may generally be described as a set of excipients that are mixed with the agent following its formation that, when contacted with the aqueous environment of use imbibes water and forms a water-swollen gel or matrix that entraps the drug form. Drug release may occur by a variety of mechanisms, for example, the matrix may disintegrate or dissolve

from around particles or granules of the agent or the agent may dissolve in the imbibed aqueous solution and diffuse from the tablet, beads or granules of the device. One ingredient of this water-swollen matrix is the water-swollable, erodible, or soluble polymer, which may generally be described as an osmopolymer, hydrogel or water-swollable polymer. Such polymers may be linear, branched, or crosslinked. The polymers may be homopolymers or copolymers. In certain embodiments, they may be synthetic polymers derived from vinyl, acrylate, methacrylate, urethane, ester and oxide monomers. In other embodiments, they can be derivatives of naturally occurring polymers such as polysaccharides (e.g. chitin, chitosan, dextran and pullulan; gum agar, gum arabic, gum karaya, locust bean gum, gum tragacanth, carrageenans, gum ghatti, guar gum, xanthan gum and scleroglucan), starches (e.g. dextrin and maltodextrin), hydrophilic colloids (e.g. pectin), phosphatides (e.g. lecithin), alginates (e.g. ammonium alginate, sodium, potassium or calcium alginate, propylene glycol alginate), gelatin, collagen, and cellulosics. Cellulosics are cellulose polymer that has been modified by reaction of at least a portion of the hydroxyl groups on the saccharide repeat units with a compound to form an ester-linked or an ether-linked substituent. For example, the cellulosic ethyl cellulose has an ether linked ethyl substituent attached to the saccharide repeat unit, while the cellulosic cellulose acetate has an ester linked acetate substituent. In certain embodiments, the cellulosics for the erodible matrix comprises aqueous-soluble and aqueous-erodible cellulosics can include, for example, ethyl cellulose (EC), methylethyl cellulose (MEC), carboxymethyl cellulose (CMC), CMEC, hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), cellulose acetate (CA), cellulose propionate (CP), cellulose butyrate (CB), cellulose acetate butyrate (CAB), CAP, CAT, hydroxypropyl methyl cellulose (HPMC), HPMCP, HPMCAS, hydroxypropyl methyl cellulose acetate trimellitate (HPMCAT), and ethylhydroxy ethylcellulose (EHEC). In certain embodiments, the cellulosics comprises various grades of low viscosity (MW less than or equal to 50,000 daltons, for example, the Dow Methocel™ series E5, E15LV, E50LV and K100LY) and high viscosity (MW greater than 50,000 daltons, for example,

E4MCR, E10MCR, K4M, K15M and K100M and the Methocel™ K series) HPMC. Other commercially available types of HPMC include the Shin Etsu Metolose 90SH series.

The choice of matrix material can have a large effect on the maximum drug concentration attained by the device as well as the maintenance of a high drug concentration. The  
5 matrix material can be a concentration-enhancing polymer, for example, as described in WO05/011634.

Other materials useful as the erodible matrix material include, but are not limited to, pullulan, polyvinyl pyrrolidone, polyvinyl alcohol, polyvinyl acetate, glycerol fatty acid  
10 esters, polyacrylamide, polyacrylic acid, copolymers of ethacrylic acid or methacrylic acid (EUDRAGIT, Rohm America, Inc., Piscataway, New Jersey) and other acrylic acid derivatives such as homopolymers and copolymers of butylmethacrylate, methylmethacrylate, ethylmethacrylate, ethylacrylate, (2-dimethylaminoethyl) methacrylate, and (trimethylaminoethyl) methacrylate chloride.

15

The erodible matrix polymer may contain a wide variety of the same types of additives and excipients known in the pharmaceutical arts, including osmopolymers, osmagens, solubility-enhancing or-retarding agents and excipients that promote stability or processing of the device.

20

Alternatively, the agents of the present disclosure may be administered by or incorporated into a non-erodible matrix device. In such devices, an agent described herein is distributed in an inert matrix. The agent is released by diffusion through the inert matrix. Examples of materials suitable for the inert matrix include insoluble plastics (e.g  
25 methyl acrylate-methyl methacrylate copolymers, polyvinyl chloride, polyethylene), hydrophilic polymers (e.g. ethyl cellulose, cellulose acetate, crosslinked polyvinylpyrrolidone (also known as crospovidone)), and fatty compounds (e.g. carnauba wax, microcrystalline wax, and triglycerides). Such devices are described further in Remington: The Science and Practice of Pharmacy, 20th edition (2000).



Matrix controlled release devices may be prepared by blending an agent described herein and other excipients together, and then forming the blend into a tablet, caplet, pill, or other device formed by compressive forces. Such compressed devices may be formed using any of a wide variety of presses used in the fabrication of pharmaceutical devices.

5 Examples include single-punch presses, rotary tablet presses, and multilayer rotary tablet presses, all well known in the art. See for example, Remington: The Science and Practice of Pharmacy, 20th Edition, 2000. The compressed device may be of any shape, including round, oval, oblong, cylindrical, or triangular. The upper and lower surfaces of the compressed device may be flat, round, concave, or convex.

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In certain embodiments, when formed by compression, the device has a strength of at least 5 Kiloponds (Kp)/cm<sup>2</sup> (for example, at least 7 Kp/cm<sup>2</sup>). Strength is the fracture force, also known as the tablet hardness required to fracture a tablet formed from the materials, divided by the maximum cross-sectional area of the tablet normal to that force.

15 The fracture force may be measured using a Schleuniger Tablet Hardness Tester, Model 6D. The compression force required to achieve this strength will depend on the size of the tablet, but generally will be greater than about 5 kP/cm<sup>2</sup>. Friability is a well-known measure of a device's resistance to surface abrasion that measures weight loss in percentage after subjecting the device to a standardized agitation procedure. Friability values of from 0.8 to 1.0% are regarded as constituting the upper limit of acceptability.  
20 Devices having a strength of greater than 5 kP/cm<sup>2</sup> generally are very robust, having a friability of less than 0.5%. Other methods for forming matrix controlled-release devices are well known in the pharmaceutical arts. See for example, Remington: The Science and Practice of Pharmacy, 20th Edition, 2000.

25

As noted above, the agents described herein may also be incorporated into an osmotic control device. Such devices generally include a core containing one or more agents as described herein and a water permeable, non-dissolving and non-eroding coating surrounding the core which controls the influx of water into the core from an aqueous

environment of use so as to cause drug release by extrusion of some or all of the core to the environment of use. In certain embodiments, the coating is polymeric, aqueous-permeable, and has at least one delivery port. The core of the osmotic device optionally includes an osmotic agent which acts to imbibe water from the surrounding environment via such a semi-permeable membrane. The osmotic agent contained in the core of this device may be an aqueous-swellaible hydrophilic polymer or it may be an osmogen, also known as an osmagent. Pressure is generated within the device which forces the agent(s) out of the device via an orifice (of a size designed to minimize solute diffusion while preventing the build-up of a hydrostatic pressure head).

5

10 Osmotic agents create a driving force for transport of water from the environment of use into the core of the device. Osmotic agents include but are not limited to water-swellaible hydrophilic polymers, and osmogens (or osmagens). Thus, the core may include water-swellaible hydrophilic polymers, both ionic and nonionic, often referred to as osmopolymers and hydrogels. The amount of water-swellaible hydrophilic polymers present in the core may range from about 5 to about 80 wt% (including for example, 10 to 50 wt%). Nonlimiting examples of core materials include hydrophilic vinyl and acrylic polymers, polysaccharides such as calcium alginate, polyethylene oxide (PEO), polyethylene glycol (PEG), polypropylene glycol (PPG), poly (2-hydroxyethyl methacrylate), poly (acrylic) acid, poly (methacrylic) acid, polyvinylpyrrolidone (PVP) and crosslinked PVP, polyvinyl alcohol (PVA), PVA/PVP copolymers and PVA/PVP copolymers with hydrophobic monomers such as methyl methacrylate, vinyl acetate, and the like, hydrophilic polyurethanes containing large PEO blocks, sodium croscarmellose, carrageenan, hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), hydroxypropyl methyl cellulose (HPMC), carboxymethyl cellulose (CMC) and

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25 carboxyethyl cellulose (CEC), sodium alginate, polycarbophil, gelatin, xanthan gum, and sodium starch glycolat. Other materials include hydrogels comprising interpenetrating networks of polymers that may be formed by addition or by condensation polymerization, the components of which may comprise hydrophilic and hydrophobic monomers such as those just mentioned. Water-swellaible hydrophilic polymers include but are not limited to

PEO, PEG, PVP, sodium croscarmellose, HPMC, sodium starch glycolate, polyacrylic acid and crosslinked versions or mixtures thereof.

The core may also include an osmogen (or osmagent). The amount of osmogen present in  
5 the core may range from about 2 to about 70 wt% (including, for example, from 10 to 50  
wt%). Typical classes of suitable osmogens are water-soluble organic acids, salts and  
sugars that are capable of imbibing water to thereby effect an osmotic pressure gradient  
across the barrier of the surrounding coating. Typical useful osmogens include but are not  
limited to magnesium sulfate, magnesium chloride, calcium chloride, sodium chloride,  
10 lithium chloride, potassium sulfate, sodium carbonate, sodium sulfite, lithium sulfate,  
potassium chloride, sodium sulfate, mannitol, xylitol, urea, sorbitol, inositol, raffinose,  
sucrose, glucose, fructose, lactose, citric acid, succinic acid, tartaric acid, and mixtures  
thereof. In certain embodiments, the osmogen is glucose, lactose, sucrose, mannitol,  
xylitol, sodium chloride, including combinations thereof.

15 The core may include a wide variety of additives and excipients that enhance the  
performance of the dosage form or that promote stability, tableting or processing. Such  
additives and excipients include tableting aids, surfactants, water-soluble polymers, pH  
modifiers, fillers, binders, pigments, disintegrants, antioxidants, lubricants and flavorants.  
Nonlimiting examples of additives and excipients include but are not limited to those  
20 described elsewhere herein as well as microcrystalline cellulose, metallic salts of acids  
(e.g. aluminum stearate, calcium stearate, magnesium stearate, sodium stearate, zinc  
stearate), pH control agents (e.g. buffers, organic acids, organic acid salts, organic and  
inorganic bases), fatty acids, hydrocarbons and fatty alcohols (e.g. stearic acid, palmitic  
acid, liquid paraffin, stearyl alcohol, and palmitol), fatty acid esters (e.g. glyceryl (mono-  
25 and di-) stearates, triglycerides, glyceryl (palmiticstearic) ester, sorbitan esters (e.g.  
sorbitan monostearate, saccharose monostearate, saccharose monopalmitate, sodium  
stearyl fumarate), polyoxyethylene sorbitan esters), surfactants (e.g. alkyl sulfates (e.g.  
sodium lauryl sulfate, magnesium lauryl sulfate), polymers (e.g. polyethylene glycols,  
polyoxyethylene glycols, polyoxyethylene, polyoxypropylene ethers, including

copolymers thereof), polytetrafluoroethylene), and inorganic materials (e.g. talc, calcium phosphate), cyclodextrins, sugars (e.g. lactose, xylitol), sodium starch glycolate). Nonlimiting examples of disintegrants are sodium starch glycolate (e. g., Explotab™ CLV, (microcrystalline cellulose (e. g., Avicel™), microcrystalline silicified cellulose (e.g., ProSolv™), croscarmellose sodium (e. g., Ac-Di-Sol™). When the agent described herein is a solid amorphous dispersion formed by a solvent process, such additives may be added directly to the spray-drying solution when forming an agent described herein/concentration-enhancing polymer dispersion such that the additive is dissolved or suspended in the solution as a slurry, Alternatively, such additives may be added following the spray-drying process to aid in forming the final controlled release device.

A nonlimiting example of an osmotic device consists of one or more drug layers containing an agent described herein, such as a solid amorphous drug/polymer dispersion, and a sweller layer that comprises a water-swellaible polymer, with a coating surrounding the drug layer and sweller layer. Each layer may contain other excipients such as tableting aids, osmagents, surfactants, water-soluble polymers and water-swellaible polymers.

Such osmotic delivery devices may be fabricated in various geometries including bilayer (wherein the core comprises a drug layer and a sweller layer adjacent to each other), trilayer (wherein the core comprises a sweller layer sandwiched between two drug layers) and concentric (wherein the core comprises a central sweller agent surrounded by the drug layer). The coating of such a tablet comprises a membrane permeable to water but substantially impermeable to drug and excipients contained within. The coating contains one or more exit passageways or ports in communication with the drug-containing layer(s) for delivering the drug agent. The drug-containing layer(s) of the core contains the drug agent (including optional osmagents and hydrophilic water-soluble polymers), while the sweller layer consists of an expandable hydrogel, with or without additional osmotic agents.

When placed in an aqueous medium, the tablet imbibes water through the membrane, causing the agent to form a dispensable aqueous agent, and causing the hydrogel layer to expand and push against the drug-containing agent, forcing the agent out of the exit  
5 passageway. The agent can swell, aiding in forcing the drug out of the passageway. Drug can be delivered from this type of delivery system either dissolved or dispersed in the agent that is expelled from the exit passageway.

The rate of drug delivery is controlled by such factors as the permeability and thickness  
10 of the coating, the osmotic pressure of the drug-containing layer, the degree of hydrophilicity of the hydrogel layer, and the surface area of the device. Those skilled in the art will appreciate that increasing the thickness of the coating will reduce the release rate, while any of the following will increase the release rate: increasing the permeability of the coating; increasing the hydrophilicity of the hydrogel layer; increasing the osmotic  
15 pressure of the drug-containing layer; or increasing the device's surface area.

Other materials useful in forming the drug-containing agent, in addition to the agent described herein itself, include HPMC, PEO and PVP and other pharmaceutically acceptable carriers. In addition, osmagents such as sugars or salts, including but not  
20 limited to sucrose, lactose, xylitol, mannitol, or sodium chloride, may be added. Materials which are useful for forming the hydrogel layer include sodium CMC, PEO (e.g. polymers having an average molecular weight from about 5,000,000 to about 7,500,000 daltons), poly (acrylic acid), sodium (polyacrylate), sodium croscarmellose, sodium starch glycolat, PVP, crosslinked PVP, and other high molecular weight hydrophilic  
25 materials.

In the case of a bilayer geometry, the delivery port(s) or exit passageway(s) may be located on the side of the tablet containing the drug agent or may be on both sides of the tablet or even on the edge of the tablet so as to connect both the drug layer and the

sweller layer with the exterior of the device. The exit passageway(s) may be produced by mechanical means or by laser drilling, or by creating a difficult-to-coat region on the tablet by use of special tooling during tablet compression or by other means.

- 5 The osmotic device can also be made with a homogeneous core surrounded by a semipermeable membrane coating, as in US3845770. The agent described herein can be incorporated into a tablet core and a semipermeable membrane coating can be applied via conventional tablet-coating techniques such as using a pan coater. A drug delivery passageway can then be formed in this coating by drilling a hole in the coating, either by
- 10 use of a laser or mechanical means. Alternatively, the passageway may be formed by rupturing a portion of the coating or by creating a region on the tablet that is difficult to coat, as described above. In one embodiment, an osmotic device comprises: (a) a single-layer compressed core comprising: (i) an agent described herein, (ii) a hydroxyethylcellulose, and (iii) an osmagent, wherein the hydroxyethylcellulose is
- 15 present in the core from about 2.0% to about 35% by weight and the osmagent is present from about 15% to about 70% by weight; (b) a water-permeable layer surrounding the core; and (c) at least one passageway within the water-permeable layer (b) for delivering the drug to a fluid environment surrounding the tablet. In certain embodiments, the device is shaped such that the surface area to volume ratio (of a water-swollen tablet) is
- 20 greater than  $0.6 \text{ mm}^{-1}$  (including, for example, greater than  $1.0 \text{ mm}^{-1}$ ). The passageway connecting the core with the fluid environment can be situated along the tablet band area. In certain embodiments, the shape is an oblong shape where the ratio of the tablet tooling axes, i.e., the major and minor axes which define the shape of the tablet, are between 1.3 and 3 (including, for example, between 1.5 and 2.5). In one embodiment, the combination
- 25 of the agent described herein and the osmagent have an average ductility from about 100 to about 200 Mpa, an average tensile strength from about 0.8 to about 2.0 Mpa, and an average brittle fracture index less than about 0.2. The single-layer core may optionally include a disintegrant, a bioavailability enhancing additive, and/or a pharmaceutically acceptable excipient, carrier or diluent.

In certain embodiments, entrainment of particles of agents described herein in the extruding fluid during operation of such osmotic device is desirable. For the particles to be well entrained, the agent drug form is dispersed in the fluid before the particles have an opportunity to settle in the tablet core. One means of accomplishing this is by adding a disintegrant that serves to break up the compressed core into its particulate components. Nonlimiting examples of standard disintegrants include materials such as sodium starch glycolate (e. g. , Explotab™ CLV), microcrystalline cellulose (e. g., Avicel™), microcrystalline silicified cellulose (e. g., ProSolV™) and croscarmellose sodium (e. g., Ac-Di-Sol™), and other disintegrants known to those skilled in the art. Depending upon the particular formulation, some disintegrants work better than others. Several disintegrants tend to form gels as they swell with water, thus hindering drug delivery from the device. Non-gelling, non-swelling disintegrants provide a more rapid dispersion of the drug particles within the core as water enters the core. In certain embodiments, non-gelling, non-swelling disintegrants are resins, for example, ion-exchange resins. In one embodiment, the resin is Amberlite™ IRP 88 (available from Rohm and Haas, Philadelphia, PA). When used, the disintegrant is present in amounts ranging from about 50-74% of the core agent.

Water-soluble polymers are added to keep particles of the agent suspended inside the device before they can be delivered through the passageway(s) (e.g., an orifice). High viscosity polymers are useful in preventing settling. However, the polymer in combination with the agent is extruded through the passageway(s) under relatively low pressures. At a given extrusion pressure, the extrusion rate typically slows with increased viscosity. Certain polymers in combination with particles of the agent described herein form high viscosity solutions with water but are still capable of being extruded from the tablets with a relatively low force. In contrast, polymers having a low weight-average, molecular weight (< about 300,000) do not form sufficiently viscous solutions inside the tablet core to allow complete delivery due to particle settling. Settling of the particles is a

problem when such devices are prepared with no polymer added, which leads to poor drug delivery unless the tablet is constantly agitated to keep the particles from settling inside the core. Settling is also problematic when the particles are large and/or of high density such that the rate of settling increases.

5

In certain embodiments, the water-soluble polymers for such osmotic devices do not interact with the drug. In certain embodiments the water-soluble polymer is a non-ionic polymer. A nonlimiting example of a non-ionic polymer forming solutions having a high viscosity yet still extrudable at low pressures is Natrosol™ 250H (high molecular weight hydroxyethylcellulose, available from Hercules Incorporated, Aqualon Division, Wilmington, DE; MW equal to about 1 million daltons and a degree of polymerization equal to about 3,700). Natrosol 250H™ provides effective drug delivery at concentrations as low as about 3% by weight of the core when combined with an osmagent. Natrosol 250H™ NF is a high-viscosity grade nonionic cellulose ether that is soluble in hot or cold water. The viscosity of a 1% solution of Natrosol 250H using a Brookfield LVT (30 rpm) at 25°C is between about 1,500 and about 2,500 cps.

In certain embodiments, hydroxyethylcellulose polymers for use in these monolayer osmotic tablets have a weight-average, molecular weight from about 300,000 to about 1.5 million. The hydroxyethylcellulose polymer is typically present in the core in an amount from about 2.0% to about 35% by weight.

Another example of an osmotic device is an osmotic capsule. The capsule shell or portion of the capsule shell can be semipermeable. The capsule can be filled either by a powder or liquid consisting of an agent described herein, excipients that imbibe water to provide osmotic potential, and/or a water-swellaable polymer, or optionally solubilizing excipients. The capsule core can also be made such that it has a bilayer or multilayer agent analogous to the bilayer, trilayer or concentric geometries described above.



Another class of osmotic device useful in this disclosure comprises coated swellable tablets, for example, as described in EP378404. Coated swellable tablets comprise a tablet core comprising an agent described herein and a swelling material, preferably a hydrophilic polymer, coated with a membrane, which contains holes, or pores through  
5 which, in the aqueous use environment, the hydrophilic polymer can extrude and carry out the agent. Alternatively, the membrane may contain polymeric or low molecular weight water-soluble porosigens. Porosigens dissolve in the aqueous use environment, providing pores through which the hydrophilic polymer and agent may extrude.

Examples of porosigens are water-soluble polymers such as HPMC, PEG, and low  
10 molecular weight compounds such as glycerol, sucrose, glucose, and sodium chloride. In addition, pores may be formed in the coating by drilling holes in the coating using a laser or other mechanical means. In this class of osmotic devices, the membrane material may comprise any film-forming polymer, including polymers which are water permeable or impermeable, providing that the membrane deposited on the tablet core is porous or  
15 contains water-soluble porosigens or possesses a macroscopic hole for water ingress and drug release. Embodiments of this class of sustained release devices may also be multilayered, as described, for example, in EP378404.

When an agent described herein is a liquid or oil, such as a lipid vehicle formulation, for  
20 example as described in WO05/011634, the osmotic controlled-release device may comprise a soft-gel or gelatin capsule formed with a composite wall and comprising the liquid formulation where the wall comprises a barrier layer formed over the external surface of the capsule, an expandable layer formed over the barrier layer, and a semipermeable layer formed over the expandable layer. A delivery port connects the  
25 liquid formulation with the aqueous use environment. Such devices are described, for example, in US6419952, US6342249, US5324280, US4672850, US4627850, US4203440, and US3995631.

The osmotic controlled release devices of the present disclosure can also comprise a coating. In certain embodiments, the osmotic controlled release device coating exhibits

one or more of the following features: is water-permeable, has at least one port for the delivery of drug, and is non-dissolving and non-eroding during release of the drug formulation, such that drug is substantially entirely delivered through the delivery port(s) or pores as opposed to delivery primarily via permeation through the coating material itself. Delivery ports include any passageway, opening or pore whether made  
5 mechanically, by laser drilling, by pore formation either during the coating process or *in situ* during use or by rupture during use. In certain embodiments, the coating is present in an amount ranging from about 5 to 30 wt% (including, for example, 10 to 20 wt%) relative to the core weight.

10

One form of coating is a semipermeable polymeric membrane that has the port(s) formed therein either prior to or during use. Thickness of such a polymeric membrane may vary between about 20 and 800  $\mu\text{m}$  (including, for example, between about 100 to 500  $\mu\text{m}$ ). The diameter of the delivery port (s) may generally range in size from 0.1 to 3000  $\mu\text{m}$  or  
15 greater (including, for example, from about 50 to 3000  $\mu\text{m}$  in diameter). Such port(s) may be formed post-coating by mechanical or laser drilling or may be formed *in situ* by rupture of the coatings; such rupture may be controlled by intentionally incorporating a relatively small weak portion into the coating. Delivery ports may also be formed *in situ* by erosion of a plug of water-soluble material or by rupture of a thinner portion of the  
20 coating over an indentation in the core. In addition, delivery ports may be formed during coating, as in the case of asymmetric membrane coatings of the type disclosed in US5612059 and US5698220. The delivery port may be formed *in situ* by rupture of the coating, for example, when a collection of beads that may be of essentially identical or of a variable agent are used. Drug is primarily released from such beads following rupture  
25 of the coating and, following rupture, such release may be gradual or relatively sudden. When the collection of beads has a variable agent, the agent may be chosen such that the beads rupture at various times following administration, resulting in the overall release of drug being sustained for a desired duration.

Coatings may be dense, microporous or asymmetric, having a denser region supported by a thick porous region such as those disclosed in US5612059 and US5698220. When the coating is dense the coating can be composed of a water-permeable material. When the coating is porous, it may be composed of either a water-permeable or a water-

5 impermeable material. When the coating is composed of a porous water-impermeable material, water permeates through the pores of the coating as either a liquid or a vapor. Nonlimiting examples of osmotic devices that utilize dense coatings include US3995631 and US3845770. Such dense coatings are permeable to the external fluid such as water and may be composed of any of the materials mentioned in these patents as well as other

10 water-permeable polymers known in the art.

The membranes may also be porous as disclosed, for example, in US5654005 and US5458887 or even be formed from water-resistant polymers. US5120548 describes another suitable process for forming coatings from a mixture of a water-insoluble polymer and a leachable water-soluble additive. The porous membranes may also be

15 formed by the addition of pore-formers as disclosed in US4612008. In addition, vapor-permeable coatings may even be formed from extremely hydrophobic materials such as polyethylene or polyvinylidene difluorid that, when dense, are essentially water-impermeable, as long as such coatings are porous. Materials useful in forming the coating include but are not limited to various grades of acrylic, vinyls, ethers,

20 polyamides, polyesters and cellulosic derivatives that are water-permeable and water-insoluble at physiologically relevant pHs, or are susceptible to being rendered water-insoluble by chemical alteration such as by crosslinking. Nonlimiting examples of suitable polymers (or crosslinked versions) useful in forming the coating include plasticized, unplasticized and reinforced cellulose acetate (CA), cellulose diacetate,

25 cellulose triacetate, CA propionate, cellulose nitrate, cellulose acetate butyrate (CAB), CA ethyl carbamate, CAP, CA methyl carbamate, CA succinate, cellulose acetate trimellitate (CAT), CA dimethylaminoacetate, CA ethyl carbonate, CA chloroacetate, CA ethyl oxalate, CA methyl sulfonate, CA butyl sulfonate, CA p-toluene sulfonate, agar acetate, amylose triacetate, beta glucan acetate, beta glucan triacetate, acetaldehyde

dimethyl acetate, triacetate of locust bean gum, hydroxiated ethylene-vinylacetate, EC, PEG, PPG, PEG/PPG copolymers, PVP, HEC, HPC, CMC, CMEC, HPMC, HPMCP, HPMCAS, HPMCAT, poly (acrylic) acids and esters and poly- (methacrylic) acids and esters and copolymers thereof, starch, dextran, dextrin, chitosan, collagen, gelatin,  
5 polyalkenes, polyethers, polysulfones, polyethersulfones, polystyrenes, polyvinyl halides, polyvinyl esters and ethers, natural waxes and synthetic waxes. In various embodiments, the coating agent comprises a cellulosic polymer, in particular cellulose ethers, cellulose esters and cellulose ester-ethers, i.e., cellulosic derivatives having a mixture of ester and ether substituents, the coating materials are made or derived from poly (acrylic) acids and  
10 esters, poly (methacrylic) acids and esters, and copolymers thereof, the coating agent comprises cellulose acetate, the coating comprises a cellulosic polymer and PEG, the coating comprises cellulose acetate and PEG.

Coating is conducted in conventional fashion, typically by dissolving or suspending the  
15 coating material in a solvent and then coating by dipping, spray coating or by pan-coating. In certain embodiments, the coating solution contains 5 to 15 wt% polymer. Typical solvents useful with the cellulosic polymers mentioned above include but are not limited to acetone, methyl acetate, ethyl acetate, isopropyl acetate, n-butyl acetate, methyl isobutyl ketone, methyl propyl ketone, ethylene glycol monoethyl ether, ethylene glycol  
20 monoethyl acetate, methylene dichloride, ethylene dichloride, propylene dichloride, nitroethane, nitropropane, tetrachloroethane, 1,4-dioxane, tetrahydrofuran, diglyme, water, and mixtures thereof. Pore-formers and non- solvents (such as water, glycerol and ethanol) or plasticizers (such as diethyl phthalate) may also be added in any amount as long as the polymer remains soluble at the spray temperature. Pore-formers and their use  
25 in fabricating coatings are described, for example, in US5612059. Coatings may also be hydrophobic microporous layers wherein the pores are substantially filled with a gas and are not wetted by the aqueous medium but are permeable to water vapor, as disclosed, for example, in US5798119. Such hydrophobic but water-vapor permeable coatings are typically composed of hydrophobic polymers such as polyalkenes, polyacrylic acid

derivatives, polyethers, polysulfones, polyethersulfones, polystyrenes, polyvinyl halides, polyvinyl esters and ethers, natural waxes and synthetic waxes. Hydrophobic microporous coating materials include but are not limited to polystyrene, polysulfones, polyethersulfones, polyethylene, polypropylene, polyvinyl chloride, polyvinylidene fluoride and polytetrafluoroethylene. Such hydrophobic coatings can be made by known phase inversion methods using any of vapor-quench, liquid quench, thermal processes, leaching soluble material from the coating or by sintering coating particles. In thermal processes, a solution of polymer in a latent solvent is brought to liquid-liquid phase separation in a cooling step. When evaporation of the solvent is not prevented, the resulting membrane will typically be porous. Such coating processes may be conducted by the processes disclosed, for example, in US4247498, US4490431 and US4744906. Osmotic controlled-release devices may be prepared using procedures known in the pharmaceutical arts. See for example, Remington: The Science and Practice of Pharmacy, 20th Edition, 2000.

As further noted above, the agents described herein may be provided in the form of microparticulates, generally ranging in size from about 10 $\mu$ m to about 2mm (including, for example, from about 100 $\mu$ m to 1mm in diameter). Such multiparticulates may be packaged, for example, in a capsule such as a gelatin capsule or a capsule formed from an aqueous-soluble polymer such as HPMCAS, HPMC or starch; dosed as a suspension or slurry in a liquid ; or they may be formed into a tablet, caplet, or pill by compression or other processes known in the art. Such multiparticulates may be made by any known process, such as wet- and dry-granulation processes, extrusion/spheronization, roller-compaction, melt-congealing, or by spray-coating seed cores. For example, in wet-and dry- granulation processes, the agent described herein and optional excipients may be granulated to form multiparticulates of the desired size. Other excipients, such as a binder (e. g., microcrystalline cellulose), may be blended with the agent to aid in processing and forming the multiparticulates. In the case of wet granulation, a binder such as microcrystalline cellulose may be included in the granulation fluid to aid in

forming a suitable multiparticulate. See, for example, Remington : The Science and Practice of Pharmacy, 20 Edition, 2000. In any case, the resulting particles may themselves constitute the therapeutic composition or they may be coated by various film-forming materials such as enteric polymers or water-swellaible or water-soluble polymers, or they may be combined with other excipients or vehicles to aid in dosing to patients. Suitable pharmaceutical compositions in accordance with the disclosure will generally include an amount of the active compound(s) with an acceptable pharmaceutical diluent or excipient, such as a sterile aqueous solution, to give a range of final concentrations, depending on the intended use. The techniques of preparation are generally well known in the art, as exemplified by Remington's Pharmaceutical Sciences (18th Edition, Mack Publishing Company, 1995).

### **Kits**

The agents described herein and combination therapy agents can be packaged as a kit that includes single or multiple doses of two or more agents, each packaged or formulated individually, or single or multiple doses of two or more agents packaged or formulated in combination. Thus, one or more agents can be present in first container, and the kit can optionally include one or more agents in a second container. The container or containers are placed within a package, and the package can optionally include administration or dosage instructions. A kit can include additional components such as syringes or other means for administering the agents as well as diluents or other means for formulation.

Thus, the kits can comprise: a) a pharmaceutical composition comprising a compound described herein and a pharmaceutically acceptable carrier, vehicle or diluent; and b) a container or packaging. The kits may optionally comprise instructions describing a method of using the pharmaceutical compositions in one or more of the methods described herein (e.g. disorders associated with fluid and sodium retention (such as diseases of the electrolyte-water/electrolyte transport system within the kidney, gut and

urogenital system, heart failure (e.g. congestive heart failure including heart failure at any of stages I-IV according to New York Heart Association (NYHA) Functional Classification), hypertension, hypotension, salt dependent forms of high blood pressure, hepatic edema, liver cirrhosis, kidney disease, polycystic kidney disease) and

5 gastrointestinal disorders (e.g. gastrointestinal motility disorders, chronic intestinal pseudo-obstruction, colonic pseudo-obstruction, Crohn's disease, duodenogastric reflux, dyspepsia, functional dyspepsia, nonulcer dyspepsia, a functional gastrointestinal disorder, functional heartburn, gastroesophageal reflux disease (GERD), gastroparesis, irritable bowel syndrome, post-operative ileus, ulcerative colitis, chronic constipation,

10 and disorders and conditions associated with constipation (e.g. constipation associated with use of opiate pain killers, post-surgical constipation, and constipation associated with neuropathic disorders as well as other conditions and disorders described herein)).

The kit may optionally comprise a second pharmaceutical composition comprising one or more additional agents including but not limited to those including analgesic peptides and

15 compounds, an agent used to treat heart failure (Diuretics (e.g. furesomide (Lasix), bumetanide (Bumex), ethacrynic acid (Edecrin), torsemide (Demadex), amiloride (Midamor), spironolactone (Aldactone), chorthiazide (Diuril), metolazone (Zaroxilyn)), Angiotension-Converting Enzyme (ACE) inhibitors (e.g. captopril (Capoten), enalapril (Vasotec), lisinopril (Prinivil, Zestril), ramipril (Altace)), Beta blockers (e.g. carvedilol

20 (Coreg) and Inotropes (e.g. digoxin, dobutamine, dopamine Milrinone)), a phosphodiesterase inhibitor, an agent used to treat gastrointestinal and other disorders (including those described herein), an agent used to treat constipation, an antidiarrheal agent, an insulin or related compound (including those described herein), an anti-hypertensive agent, an agent useful in the treatment of respiratory and other disorders, an

25 anti-obesity agent, an anti-diabetic agents, an agent that activates soluble guanylate cyclase and a pharmaceutically acceptable carrier, vehicle or diluent. The pharmaceutical composition comprising the compound described herein and the second pharmaceutical composition contained in the kit may be optionally combined in the same pharmaceutical composition.

A kit includes a container or packaging for containing the pharmaceutical compositions and may also include divided containers such as a divided bottle or a divided foil packet. The container can be, for example a paper or cardboard box, a glass or plastic bottle or jar, a re-sealable bag (for example, to hold a "refill" of tablets for placement into a different container), or a blister pack with individual doses for pressing out of the pack according to a therapeutic schedule. It is feasible that more than one container can be used together in a single package to market a single dosage form. For example, tablets may be contained in a bottle which is in turn contained within a box.

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An example of a kit is a so-called blister pack. Blister packs are well known in the packaging industry and are being widely used for the packaging of pharmaceutical unit dosage forms (tablets, capsules, and the like). Blister packs generally consist of a sheet of relatively stiff material covered with a foil of a preferably transparent plastic material. During the packaging process, recesses are formed in the plastic foil. The recesses have the size and shape of individual tablets or capsules to be packed or may have the size and shape to accommodate multiple tablets and/or capsules to be packed. Next, the tablets or capsules are placed in the recesses accordingly and the sheet of relatively stiff material is sealed against the plastic foil at the face of the foil which is opposite from the direction in which the recesses were formed. As a result, the tablets or capsules are individually sealed or collectively sealed, as desired, in the recesses between the plastic foil and the sheet. Preferably the strength of the sheet is such that the tablets or capsules can be removed from the blister pack by manually applying pressure on the recesses whereby an opening is formed in the sheet at the place of the recess. The tablet or capsule can then be removed via said opening.

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It maybe desirable to provide a written memory aid containing information and/or instructions for the physician, pharmacist or subject regarding when the medication is to be taken. A "daily dose" can be a single tablet or capsule or several tablets or capsules to



be taken on a given day. When the kit contains separate compositions, a daily dose of one or more compositions of the kit can consist of one tablet or capsule while a daily dose of another one or more compositions of the kit can consist of several tablets or capsules. A kit can take the form of a dispenser designed to dispense the daily doses one at a time in the order of their intended use. The dispenser can be equipped with a memory-aid, so as to further facilitate compliance with the regimen. An example of such a memory-aid is a mechanical counter which indicates the number of daily doses that have been dispensed. Another example of such a memory-aid is a battery-powered micro-chip memory coupled with a liquid crystal readout, or audible reminder signal which, for example, reads out the date that the last daily dose has been taken and/or reminds one when the next dose is to be taken.

Methods to increase chemical and/or physical stability of the agents the described herein are found in U.S. 6,541,606, U.S. 6,068,850, U.S. 6,124,261, U.S. 5,904,935, and WO 00/15224, U.S. 20030069182 (via the additon of nicotinamide), U.S. 20030175230A1, U.S. 20030175230A1, U.S. 20030175239A1, U.S. 20020045582, U.S. 20010031726, WO 02/26248, WO 03/014304, WO 98/00152A1, WO 98/00157A1, WO 90/12029, WO 00/04880, and WO 91/04743, WO 97/04796 and the references cited therein.

Methods to increase bioavailability of the agents described herein are found in U.S. 6,008,187, U.S. 5,424,289, U.S. 20030198619, WO 90/01329, WO 01/49268, WO 00/32172, and WO 02/064166. Glycyrrhizinate can also be used as an absorption enhancer (see, e.g., EP397447). WO 03/004062 discusses Ulex europaeus I (UEAI) and UEAI mimetics which may be used to target the agents described herein to the GI tract. The bioavailability of the agents described herein can also be increased by addition of oral bioavailability-enhancing agents such as those described in U.S. 6,818,615 including but not limited to: cyclosporins (including cyclosporins A through Z as defined in Table 1 of U.S. 6,818,615), for example, cyclosporin A (cyclosporin), cyclosporin F, cyclosporin D, dihydro cyclosporin A, dihydro cyclosporin C, acetyl cyclosporin A, PSC-833, (Me-Ile-

4)-cyclosporin (SDZ-NIM 811) (both from Sandoz Pharmaceutical Corp.), and related oligopeptides produced by species in the genus *Topycladium*); antifungals including but not limited to ketoconazole; cardiovascular drug including but not limited to MS-209 (BASF), amiodarone, nifedipine, reserpine, quinidine, nicardipine, ethacrynic acid, propafenone, reserpine, amiloride; anti-migraine natural products including but not limited to ergot alkaloids; antibiotics including but not limited to cefoperazone, tetracycline, chloroquine, fosfomycin; antiparasitics including but not limited to ivermectin; multi-drug resistance reversers including but not limited to VX-710 and VX-853 (Vertex Pharmaceutical Incorporated); tyrosine kinase inhibitors including but not limited to genistein and related isoflavonoids, quercetin; protein kinase C inhibitors including but not limited to calphostin; apoptosis inducers including but not limited to ceramides; and agents active against endorphin receptors including but not limited to morphine, morphine congeners, other opioids and opioid antagonists including (but not limited to) naloxone, naltrexone and nalmefene).

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The agents described herein can be fused to a modified version of the blood serum protein transferrin. U.S. 20030221201, U.S. 20040023334, U.S. 20030226155, WO 04/020454, and WO 04/019872 discuss the manufacture and use of transferrin fusion proteins. Transferrin fusion proteins may improve circulatory half life and efficacy, decrease undesirable side effects and allow reduced dosage.

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The peptides and agonists described herein can be recombinantly expressed in bacteria. Bacteria expressing the peptide or agonists can be administered orally, rectally, mucosally or in via some other mode of administration including but not limited to those described herein. Bacterial hosts suitable for such administration include but are not limited to certain *Lactobacteria* (e.g. *Lactococcus lactis*, *Lactobacillus plantarum*, *Lact. rhamnosus* and *Lact. paracasei ssp. Paracasei* and other species found in normal human flora (Ahme et al. Journal of Applied Microbiology 1998 85:88)), certain *Streptococcus sp.* (e.g. *S. gordonii*), and certain *B. subtilis* strains (including pSM539 described in

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Porzio et al. BMC Biotechnology 2004 4:27). The peptides and agonists described herein can be administered using the Heliobacter based preparation methods described in WO06/015445.

5 Dosage

The dose range for adult humans is generally from 0.005 mg to 10 g/day orally. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount of compound described herein which is effective at such dosage or as  
10 a multiple of the same, for instance, units containing 5 mg to 500 mg, usually around 10 mg to 200 mg. The precise amount of compound administered to a patient will be the responsibility of the attendant physician. However, the dose employed will depend on a number of factors, including the age and sex of the patient, the precise disorder being treated, and its severity.

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A dosage unit (e.g. an oral dosage unit) can include from, for example, 1 to 30  $\mu\text{g}$ , 1 to 40  $\mu\text{g}$ , 1 to 50  $\mu\text{g}$ , 1 to 100  $\mu\text{g}$ , 1 to 200  $\mu\text{g}$ , 1 to 300  $\mu\text{g}$ , 1 to 400  $\mu\text{g}$ , 1 to 500  $\mu\text{g}$ , 1 to 600  $\mu\text{g}$ , 1 to 700  $\mu\text{g}$ , 1 to 800  $\mu\text{g}$ , 1 to 900  $\mu\text{g}$ , 1 to 1000  $\mu\text{g}$ , 10 to 30  $\mu\text{g}$ , 10 to 40  $\mu\text{g}$ , 10 to 50  $\mu\text{g}$ , 10 to 100  $\mu\text{g}$ , 10 to 200  $\mu\text{g}$ , 10 to 300  $\mu\text{g}$ , 10 to 400  $\mu\text{g}$ , 10 to 500  $\mu\text{g}$ , 10 to 600  
20  $\mu\text{g}$ , 10 to 700  $\mu\text{g}$ , 10 to 800  $\mu\text{g}$ , 10 to 900  $\mu\text{g}$ , 10 to 1000  $\mu\text{g}$ , 100 to 200  $\mu\text{g}$ , 100 to 300  $\mu\text{g}$ , 100 to 400  $\mu\text{g}$ , 100 to 500  $\mu\text{g}$ , 100 to 600  $\mu\text{g}$ , 100 to 700  $\mu\text{g}$ , 100 to 800  $\mu\text{g}$ , 100 to 900  $\mu\text{g}$ , 100 to 1000  $\mu\text{g}$ , 100 to 1250  $\mu\text{g}$ , 100 to 1500  $\mu\text{g}$ , 100 to 1750  $\mu\text{g}$ , 100 to 2000  $\mu\text{g}$ , 100 to 2250  $\mu\text{g}$ , 100 to 2500  $\mu\text{g}$ , 100 to 2750  $\mu\text{g}$ , 100 to 3000  $\mu\text{g}$ , 200 to 300  $\mu\text{g}$ , 200 to 400  $\mu\text{g}$ , 200 to 500  $\mu\text{g}$ , 200 to 600  $\mu\text{g}$ , 200 to 700  $\mu\text{g}$ , 200 to 800  $\mu\text{g}$ , 200 to 900  $\mu\text{g}$ , 200  
25 to 1000  $\mu\text{g}$ , 200 to 1250  $\mu\text{g}$ , 200 to 1500  $\mu\text{g}$ , 200 to 1750  $\mu\text{g}$ , 200 to 2000  $\mu\text{g}$ , 200 to 2250  $\mu\text{g}$ , 200 to 2500  $\mu\text{g}$ , 200 to 2750  $\mu\text{g}$ , 200 to 3000  $\mu\text{g}$ , 300 to 400  $\mu\text{g}$ , 300 to 500  $\mu\text{g}$ , 300 to 600  $\mu\text{g}$ , 300 to 700  $\mu\text{g}$ , 300 to 800  $\mu\text{g}$ , 300 to 900  $\mu\text{g}$ , 300 to 1000  $\mu\text{g}$ , 300 to 1250  $\mu\text{g}$ , 300 to 1500  $\mu\text{g}$ , 300 to 1750  $\mu\text{g}$ , 300 to 2000  $\mu\text{g}$ , 300 to 2250  $\mu\text{g}$ , 300 to 2500  $\mu\text{g}$ , 300 to 2750  $\mu\text{g}$ , 300 to 3000  $\mu\text{g}$ , 400 to 500  $\mu\text{g}$ , 400 to 600  $\mu\text{g}$ , 400 to 700  $\mu\text{g}$ , 400 to 800  $\mu\text{g}$ ,

400 to 900  $\mu\text{g}$ , 400 to 1000  $\mu\text{g}$ , 400 to 1250  $\mu\text{g}$ , 400 to 1500  $\mu\text{g}$ , 400 to 1750  $\mu\text{g}$ , 400 to 2000  $\mu\text{g}$ , 400 to 2250  $\mu\text{g}$ , 400 to 2500  $\mu\text{g}$ , 400 to 2750  $\mu\text{g}$ , 400 to 3000  $\mu\text{g}$ , 500 to 600  $\mu\text{g}$ , 500 to 700  $\mu\text{g}$ , 500 to 800  $\mu\text{g}$ , 500 to 900  $\mu\text{g}$ , 500 to 1000  $\mu\text{g}$ , 500 to 1250  $\mu\text{g}$ , 500 to 1500  $\mu\text{g}$ , 500 to 1750  $\mu\text{g}$ , 500 to 2000  $\mu\text{g}$ , 500 to 2250  $\mu\text{g}$ , 500 to 2500  $\mu\text{g}$ , 500 to 2750  $\mu\text{g}$ , 500 to 3000  $\mu\text{g}$ , 600 to 700  $\mu\text{g}$ , 600 to 800  $\mu\text{g}$ , 600 to 900  $\mu\text{g}$ , 600 to 1000  $\mu\text{g}$ , 600 to 1250  $\mu\text{g}$ , 600 to 1500  $\mu\text{g}$ , 600 to 1750  $\mu\text{g}$ , 600 to 2000  $\mu\text{g}$ , 600 to 2250  $\mu\text{g}$ , 600 to 2500  $\mu\text{g}$ , 600 to 2750  $\mu\text{g}$ , 600 to 3000  $\mu\text{g}$ , 700 to 800  $\mu\text{g}$ , 700 to 900  $\mu\text{g}$ , 700 to 1000  $\mu\text{g}$ , 700 to 1250  $\mu\text{g}$ , 700 to 1500  $\mu\text{g}$ , 700 to 1750  $\mu\text{g}$ , 700 to 2000  $\mu\text{g}$ , 700 to 2250  $\mu\text{g}$ , 700 to 2500  $\mu\text{g}$ , 700 to 2750  $\mu\text{g}$ , 700 to 3000  $\mu\text{g}$ , 800 to 900  $\mu\text{g}$ , 800 to 1000  $\mu\text{g}$ , 800 to 1250  $\mu\text{g}$ , 800 to 1500  $\mu\text{g}$ , 800 to 1750  $\mu\text{g}$ , 800 to 2000  $\mu\text{g}$ , 800 to 2250  $\mu\text{g}$ , 800 to 2500  $\mu\text{g}$ , 800 to 2750  $\mu\text{g}$ , 800 to 3000  $\mu\text{g}$ , 900 to 1000  $\mu\text{g}$ , 900 to 1250  $\mu\text{g}$ , 900 to 1500  $\mu\text{g}$ , 900 to 1750  $\mu\text{g}$ , 900 to 2000  $\mu\text{g}$ , 900 to 2250  $\mu\text{g}$ , 900 to 2500  $\mu\text{g}$ , 900 to 2750  $\mu\text{g}$ , 900 to 3000  $\mu\text{g}$ , 1000 to 1250  $\mu\text{g}$ , 1000 to 1500  $\mu\text{g}$ , 1000 to 1750  $\mu\text{g}$ , 1000 to 2000  $\mu\text{g}$ , 1000 to 2250  $\mu\text{g}$ , 1000 to 2500  $\mu\text{g}$ , 1000 to 2750  $\mu\text{g}$ , 1000 to 3000  $\mu\text{g}$ , 2 to 500  $\mu\text{g}$ , 50 to 500  $\mu\text{g}$ , 3 to 100  $\mu\text{g}$ , 5 to 20  $\mu\text{g}$ , 5 to 100  $\mu\text{g}$ , 10  $\mu\text{g}$ , 20  $\mu\text{g}$ , 30  $\mu\text{g}$ , 40  $\mu\text{g}$ , 50  $\mu\text{g}$ , 60  $\mu\text{g}$ , 70  $\mu\text{g}$ , 75  $\mu\text{g}$ , 80  $\mu\text{g}$ , 90  $\mu\text{g}$ , 100  $\mu\text{g}$ , 150  $\mu\text{g}$ , 200  $\mu\text{g}$ , 250  $\mu\text{g}$ , 300  $\mu\text{g}$ , 350  $\mu\text{g}$ , 400  $\mu\text{g}$ , 450  $\mu\text{g}$ , 500  $\mu\text{g}$ , 550  $\mu\text{g}$ , 600  $\mu\text{g}$ , 650  $\mu\text{g}$ , 700  $\mu\text{g}$ , 750  $\mu\text{g}$ , 800  $\mu\text{g}$ , 850  $\mu\text{g}$ , 900  $\mu\text{g}$ , 950  $\mu\text{g}$ , 1000  $\mu\text{g}$ , 1050  $\mu\text{g}$ , 1100  $\mu\text{g}$ , 1150  $\mu\text{g}$ , 1200  $\mu\text{g}$ , 1250  $\mu\text{g}$ , 1300  $\mu\text{g}$ , 1350  $\mu\text{g}$ , 1400  $\mu\text{g}$ , 1450  $\mu\text{g}$ , 1500  $\mu\text{g}$ , 1550  $\mu\text{g}$ , 1600  $\mu\text{g}$ , 1650  $\mu\text{g}$ , 1700  $\mu\text{g}$ , 1750  $\mu\text{g}$ , 1800  $\mu\text{g}$ , 1850  $\mu\text{g}$ , 1900  $\mu\text{g}$ , 1950  $\mu\text{g}$ , 2000  $\mu\text{g}$ , 2050  $\mu\text{g}$ , 2100  $\mu\text{g}$ , 2150  $\mu\text{g}$ , 2200  $\mu\text{g}$ , 2250  $\mu\text{g}$ , 2300  $\mu\text{g}$ , 2350  $\mu\text{g}$ , 2400  $\mu\text{g}$ , 2450  $\mu\text{g}$ , 2500  $\mu\text{g}$ , 2550  $\mu\text{g}$ , 2600  $\mu\text{g}$ , 2650  $\mu\text{g}$ , 2700  $\mu\text{g}$ , 2750  $\mu\text{g}$ , 2800  $\mu\text{g}$ , 2850  $\mu\text{g}$ , 2900  $\mu\text{g}$ , 2950  $\mu\text{g}$ , 3000  $\mu\text{g}$ , 3250  $\mu\text{g}$ , 3500  $\mu\text{g}$ , 3750  $\mu\text{g}$ , 4000  $\mu\text{g}$ , 4250  $\mu\text{g}$ , 4500  $\mu\text{g}$ , 4750  $\mu\text{g}$ , 5000  $\mu\text{g}$  of a peptide or agonist described herein. In various embodiments, the dosage unit is administered with food at anytime of the day, without food at anytime of the day, with food after an overnight fast (e.g. with breakfast), at bedtime after a low fat snack. In various embodiments, the dosage unit is administered once a day, twice a day, three times a day, four times a day, five times a day, six times a day. The dosage unit can optionally comprise other agents.

A dosage unit (e.g. an oral dosage unit) can include, for example, from 1 to 30  $\mu\text{g}$ , 1 to 40  $\mu\text{g}$ , 1 to 50  $\mu\text{g}$ , 1 to 100  $\mu\text{g}$ , 1 to 200  $\mu\text{g}$ , 1 to 300  $\mu\text{g}$ , 1 to 400  $\mu\text{g}$ , 1 to 500  $\mu\text{g}$ , 1 to 600  $\mu\text{g}$ , 1 to 700  $\mu\text{g}$ , 1 to 800  $\mu\text{g}$ , 1 to 900  $\mu\text{g}$ , 1 to 1000  $\mu\text{g}$ , 10 to 30  $\mu\text{g}$ , 10 to 40  $\mu\text{g}$ , 10 to 50  $\mu\text{g}$ , 10 to 100  $\mu\text{g}$ , 10 to 200  $\mu\text{g}$ , 10 to 300  $\mu\text{g}$ , 10 to 400  $\mu\text{g}$ , 10 to 500  $\mu\text{g}$ , 10 to 600  $\mu\text{g}$ , 10 to 700  $\mu\text{g}$ , 10 to 800  $\mu\text{g}$ , 10 to 900  $\mu\text{g}$ , 10 to 1000  $\mu\text{g}$ , 100 to 200  $\mu\text{g}$ , 100 to 300  $\mu\text{g}$ , 100 to 400  $\mu\text{g}$ , 100 to 500  $\mu\text{g}$ , 100 to 600  $\mu\text{g}$ , 100 to 700  $\mu\text{g}$ , 100 to 800  $\mu\text{g}$ , 100 to 900  $\mu\text{g}$ , 100 to 1000  $\mu\text{g}$ , 100 to 1250  $\mu\text{g}$ , 100 to 1500  $\mu\text{g}$ , 100 to 1750  $\mu\text{g}$ , 100 to 2000  $\mu\text{g}$ , 100 to 2250  $\mu\text{g}$ , 100 to 2500  $\mu\text{g}$ , 100 to 2750  $\mu\text{g}$ , 100 to 3000  $\mu\text{g}$ , 200 to 300  $\mu\text{g}$ , 200 to 400  $\mu\text{g}$ , 200 to 500  $\mu\text{g}$ , 200 to 600  $\mu\text{g}$ , 200 to 700  $\mu\text{g}$ , 200 to 800  $\mu\text{g}$ , 200 to 900  $\mu\text{g}$ , 200 to 1000  $\mu\text{g}$ , 200 to 1250  $\mu\text{g}$ , 200 to 1500  $\mu\text{g}$ , 200 to 1750  $\mu\text{g}$ , 200 to 2000  $\mu\text{g}$ , 200 to 2250  $\mu\text{g}$ , 200 to 2500  $\mu\text{g}$ , 200 to 2750  $\mu\text{g}$ , 200 to 3000  $\mu\text{g}$ , 300 to 400  $\mu\text{g}$ , 300 to 500  $\mu\text{g}$ , 300 to 600  $\mu\text{g}$ , 300 to 700  $\mu\text{g}$ , 300 to 800  $\mu\text{g}$ , 300 to 900  $\mu\text{g}$ , 300 to 1000  $\mu\text{g}$ , 300 to 1250  $\mu\text{g}$ , 300 to 1500  $\mu\text{g}$ , 300 to 1750  $\mu\text{g}$ , 300 to 2000  $\mu\text{g}$ , 300 to 2250  $\mu\text{g}$ , 300 to 2500  $\mu\text{g}$ , 300 to 2750  $\mu\text{g}$ , 300 to 3000  $\mu\text{g}$ , 400 to 500  $\mu\text{g}$ , 400 to 600  $\mu\text{g}$ , 400 to 700  $\mu\text{g}$ , 400 to 800  $\mu\text{g}$ , 400 to 900  $\mu\text{g}$ , 400 to 1000  $\mu\text{g}$ , 400 to 1250  $\mu\text{g}$ , 400 to 1500  $\mu\text{g}$ , 400 to 1750  $\mu\text{g}$ , 400 to 2000  $\mu\text{g}$ , 400 to 2250  $\mu\text{g}$ , 400 to 2500  $\mu\text{g}$ , 400 to 2750  $\mu\text{g}$ , 400 to 3000  $\mu\text{g}$ , 500 to 600  $\mu\text{g}$ , 500 to 700  $\mu\text{g}$ , 500 to 800  $\mu\text{g}$ , 500 to 900  $\mu\text{g}$ , 500 to 1000  $\mu\text{g}$ , 500 to 1250  $\mu\text{g}$ , 500 to 1500  $\mu\text{g}$ , 500 to 1750  $\mu\text{g}$ , 500 to 2000  $\mu\text{g}$ , 500 to 2250  $\mu\text{g}$ , 500 to 2500  $\mu\text{g}$ , 500 to 2750  $\mu\text{g}$ , 500 to 3000  $\mu\text{g}$ , 600 to 700  $\mu\text{g}$ , 600 to 800  $\mu\text{g}$ , 600 to 900  $\mu\text{g}$ , 600 to 1000  $\mu\text{g}$ , 600 to 1250  $\mu\text{g}$ , 600 to 1500  $\mu\text{g}$ , 600 to 1750  $\mu\text{g}$ , 600 to 2000  $\mu\text{g}$ , 600 to 2250  $\mu\text{g}$ , 600 to 2500  $\mu\text{g}$ , 600 to 2750  $\mu\text{g}$ , 600 to 3000  $\mu\text{g}$ , 700 to 800  $\mu\text{g}$ , 700 to 900  $\mu\text{g}$ , 700 to 1000  $\mu\text{g}$ , 700 to 1250  $\mu\text{g}$ , 700 to 1500  $\mu\text{g}$ , 700 to 1750  $\mu\text{g}$ , 700 to 2000  $\mu\text{g}$ , 700 to 2250  $\mu\text{g}$ , 700 to 2500  $\mu\text{g}$ , 700 to 2750  $\mu\text{g}$ , 700 to 3000  $\mu\text{g}$ , 800 to 900  $\mu\text{g}$ , 800 to 1000  $\mu\text{g}$ , 800 to 1250  $\mu\text{g}$ , 800 to 1500  $\mu\text{g}$ , 800 to 1750  $\mu\text{g}$ , 800 to 2000  $\mu\text{g}$ , 800 to 2250  $\mu\text{g}$ , 800 to 2500  $\mu\text{g}$ , 800 to 2750  $\mu\text{g}$ , 800 to 3000  $\mu\text{g}$ , 900 to 1000  $\mu\text{g}$ , 900 to 1250  $\mu\text{g}$ , 900 to 1500  $\mu\text{g}$ , 900 to 1750  $\mu\text{g}$ , 900 to 2000  $\mu\text{g}$ , 900 to 2250  $\mu\text{g}$ , 900 to 2500  $\mu\text{g}$ , 900 to 2750  $\mu\text{g}$ , 900 to 3000  $\mu\text{g}$ , 1000 to 1250  $\mu\text{g}$ , 1000 to 1500  $\mu\text{g}$ , 1000 to 1750  $\mu\text{g}$ , 1000 to 2000  $\mu\text{g}$ , 1000 to 2250  $\mu\text{g}$ , 1000 to 2500  $\mu\text{g}$ , 1000 to 2750  $\mu\text{g}$ , 1000 to 3000  $\mu\text{g}$ , 2 to 500  $\mu\text{g}$ , 50 to 500  $\mu\text{g}$ , 3 to 100  $\mu\text{g}$ , 5 to 20  $\mu\text{g}$ , 5 to 100  $\mu\text{g}$ , 10  $\mu\text{g}$ , 20  $\mu\text{g}$ , 30  $\mu\text{g}$ , 40  $\mu\text{g}$ , 50  $\mu\text{g}$ , 60  $\mu\text{g}$ , 70  $\mu\text{g}$ , 75  $\mu\text{g}$ , 80  $\mu\text{g}$ , 90  $\mu\text{g}$ , 100  $\mu\text{g}$ , 150

5  $\mu\text{g}$ , 200  $\mu\text{g}$ , 250  $\mu\text{g}$ , 300  $\mu\text{g}$ , 350  $\mu\text{g}$ , 400  $\mu\text{g}$ , 450  $\mu\text{g}$ , 500  $\mu\text{g}$ , 550  $\mu\text{g}$ , 600  $\mu\text{g}$ , 650  $\mu\text{g}$ , 700  $\mu\text{g}$ , 750  $\mu\text{g}$ , 800  $\mu\text{g}$ , 850  $\mu\text{g}$ , 900  $\mu\text{g}$ , 950  $\mu\text{g}$ , 1000  $\mu\text{g}$ , 1050  $\mu\text{g}$ , 1100  $\mu\text{g}$ , 1150  $\mu\text{g}$ , 1200  $\mu\text{g}$ , 1250  $\mu\text{g}$ , 1300  $\mu\text{g}$ , 1350  $\mu\text{g}$ , 1400  $\mu\text{g}$ , 1450  $\mu\text{g}$ , 1500  $\mu\text{g}$ , 1550  $\mu\text{g}$ , 1600  $\mu\text{g}$ , 1650  $\mu\text{g}$ , 1700  $\mu\text{g}$ , 1750  $\mu\text{g}$ , 1800  $\mu\text{g}$ , 1850  $\mu\text{g}$ , 1900  $\mu\text{g}$ , 1950  $\mu\text{g}$ , 2000  $\mu\text{g}$ , 2050  $\mu\text{g}$ , 2100  $\mu\text{g}$ ,  
 2150  $\mu\text{g}$ , 2200  $\mu\text{g}$ , 2250  $\mu\text{g}$ , 2300  $\mu\text{g}$ , 2350  $\mu\text{g}$ , 2400  $\mu\text{g}$ , 2450  $\mu\text{g}$ , 2500  $\mu\text{g}$ , 2550  $\mu\text{g}$ ,  
 2600  $\mu\text{g}$ , 2650  $\mu\text{g}$ , 2700  $\mu\text{g}$ , 2750  $\mu\text{g}$ , 2800  $\mu\text{g}$ , 2850  $\mu\text{g}$ , 2900  $\mu\text{g}$ , 2950  $\mu\text{g}$ , 3000  $\mu\text{g}$ ,  
 3250  $\mu\text{g}$ , 3500  $\mu\text{g}$ , 3750  $\mu\text{g}$ , 4000  $\mu\text{g}$ , 4250  $\mu\text{g}$ , 4500  $\mu\text{g}$ , 4750  $\mu\text{g}$ , 5000  $\mu\text{g}$  of a peptide  
 or agonist described herein and from 10 mg to 600 mg (e.g. 10 mg, 20 mg, 30 mg, 40 mg,  
 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 120 mg, 140 mg, 160 mg, 180 mg, 200  
 10 mg, 220 mg, 240 mg, 260 mg, 280 mg, 300 mg, 320 mg, 340 mg, 360 mg, 380 mg, 400  
 mg, 420 mg, 440 mg, 460 mg, 480 mg, 500 mg, 520 mg, 540 mg, 560 mg, 580 mg, 600  
 mg) of furosemide (Lasix).

A dosage unit (e.g. an oral, intravenous or intramuscular dosage unit) can include,  
 15 for example, from 1 to 30  $\mu\text{g}$ , 1 to 40  $\mu\text{g}$ , 1 to 50  $\mu\text{g}$ , 1 to 100  $\mu\text{g}$ , 1 to 200  $\mu\text{g}$ , 1 to 300  
 $\mu\text{g}$ , 1 to 400  $\mu\text{g}$ , 1 to 500  $\mu\text{g}$ , 1 to 600  $\mu\text{g}$ , 1 to 700  $\mu\text{g}$ , 1 to 800  $\mu\text{g}$ , 1 to 900  $\mu\text{g}$ , 1 to 1000  
 $\mu\text{g}$ , 10 to 30  $\mu\text{g}$ , 10 to 40  $\mu\text{g}$ , 10 to 50  $\mu\text{g}$ , 10 to 100  $\mu\text{g}$ , 10 to 200  $\mu\text{g}$ , 10 to 300  $\mu\text{g}$ , 10 to  
 400  $\mu\text{g}$ , 10 to 500  $\mu\text{g}$ , 10 to 600  $\mu\text{g}$ , 10 to 700  $\mu\text{g}$ , 10 to 800  $\mu\text{g}$ , 10 to 900  $\mu\text{g}$ , 10 to 1000  
 20  $\mu\text{g}$ , 100 to 200  $\mu\text{g}$ , 100 to 300  $\mu\text{g}$ , 100 to 400  $\mu\text{g}$ , 100 to 500  $\mu\text{g}$ , 100 to 600  $\mu\text{g}$ , 100 to  
 700  $\mu\text{g}$ , 100 to 800  $\mu\text{g}$ , 100 to 900  $\mu\text{g}$ , 100 to 1000  $\mu\text{g}$ , 100 to 1250  $\mu\text{g}$ , 100 to 1500  $\mu\text{g}$ ,  
 100 to 1750  $\mu\text{g}$ , 100 to 2000  $\mu\text{g}$ , 100 to 2250  $\mu\text{g}$ , 100 to 2500  $\mu\text{g}$ , 100 to 2750  $\mu\text{g}$ , 100 to  
 3000  $\mu\text{g}$ , 200 to 300  $\mu\text{g}$ , 200 to 400  $\mu\text{g}$ , 200 to 500  $\mu\text{g}$ , 200 to 600  $\mu\text{g}$ , 200 to 700  $\mu\text{g}$ , 200  
 to 800  $\mu\text{g}$ , 200 to 900  $\mu\text{g}$ , 200 to 1000  $\mu\text{g}$ , 200 to 1250  $\mu\text{g}$ , 200 to 1500  $\mu\text{g}$ , 200 to 1750  
 $\mu\text{g}$ , 200 to 2000  $\mu\text{g}$ , 200 to 2250  $\mu\text{g}$ , 200 to 2500  $\mu\text{g}$ , 200 to 2750  $\mu\text{g}$ , 200 to 3000  $\mu\text{g}$ , 300  
 25 to 400  $\mu\text{g}$ , 300 to 500  $\mu\text{g}$ , 300 to 600  $\mu\text{g}$ , 300 to 700  $\mu\text{g}$ , 300 to 800  $\mu\text{g}$ , 300 to 900  $\mu\text{g}$ ,  
 300 to 1000  $\mu\text{g}$ , 300 to 1250  $\mu\text{g}$ , 300 to 1500  $\mu\text{g}$ , 300 to 1750  $\mu\text{g}$ , 300 to 2000  $\mu\text{g}$ , 300 to  
 2250  $\mu\text{g}$ , 300 to 2500  $\mu\text{g}$ , 300 to 2750  $\mu\text{g}$ , 300 to 3000  $\mu\text{g}$ , 400 to 500  $\mu\text{g}$ , 400 to 600  $\mu\text{g}$ ,  
 400 to 700  $\mu\text{g}$ , 400 to 800  $\mu\text{g}$ , 400 to 900  $\mu\text{g}$ , 400 to 1000  $\mu\text{g}$ , 400 to 1250  $\mu\text{g}$ , 400 to  
 1500  $\mu\text{g}$ , 400 to 1750  $\mu\text{g}$ , 400 to 2000  $\mu\text{g}$ , 400 to 2250  $\mu\text{g}$ , 400 to 2500  $\mu\text{g}$ , 400 to 2750

5  $\mu\text{g}$ , 400 to 3000  $\mu\text{g}$ , 500 to 600  $\mu\text{g}$ , 500 to 700  $\mu\text{g}$ , 500 to 800  $\mu\text{g}$ , 500 to 900  $\mu\text{g}$ , 500 to 1000  $\mu\text{g}$ , 500 to 1250  $\mu\text{g}$ , 500 to 1500  $\mu\text{g}$ , 500 to 1750  $\mu\text{g}$ , 500 to 2000  $\mu\text{g}$ , 500 to 2250  $\mu\text{g}$ , 500 to 2500  $\mu\text{g}$ , 500 to 2750  $\mu\text{g}$ , 500 to 3000  $\mu\text{g}$ , 600 to 700  $\mu\text{g}$ , 600 to 800  $\mu\text{g}$ , 600 to 900  $\mu\text{g}$ , 600 to 1000  $\mu\text{g}$ , 600 to 1250  $\mu\text{g}$ , 600 to 1500  $\mu\text{g}$ , 600 to 1750  $\mu\text{g}$ , 600 to 2000  $\mu\text{g}$ ,  
600 to 2250  $\mu\text{g}$ , 600 to 2500  $\mu\text{g}$ , 600 to 2750  $\mu\text{g}$ , 600 to 3000  $\mu\text{g}$ , 700 to 800  $\mu\text{g}$ , 700 to 900  $\mu\text{g}$ , 700 to 1000  $\mu\text{g}$ , 700 to 1250  $\mu\text{g}$ , 700 to 1500  $\mu\text{g}$ , 700 to 1750  $\mu\text{g}$ , 700 to 2000  $\mu\text{g}$ ,  
700 to 2250  $\mu\text{g}$ , 700 to 2500  $\mu\text{g}$ , 700 to 2750  $\mu\text{g}$ , 700 to 3000  $\mu\text{g}$ , 800 to 900  $\mu\text{g}$ , 800 to 1000  $\mu\text{g}$ , 800 to 1250  $\mu\text{g}$ , 800 to 1500  $\mu\text{g}$ , 800 to 1750  $\mu\text{g}$ , 800 to 2000  $\mu\text{g}$ , 800 to 2250  $\mu\text{g}$ ,  
800 to 2500  $\mu\text{g}$ , 800 to 2750  $\mu\text{g}$ , 800 to 3000  $\mu\text{g}$ , 900 to 1000  $\mu\text{g}$ , 900 to 1250  $\mu\text{g}$ , 900 to 1500  $\mu\text{g}$ , 900 to 1750  $\mu\text{g}$ , 900 to 2000  $\mu\text{g}$ , 900 to 2250  $\mu\text{g}$ , 900 to 2500  $\mu\text{g}$ , 900 to 2750  $\mu\text{g}$ ,  
900 to 3000  $\mu\text{g}$ , 1000 to 1250  $\mu\text{g}$ , 1000 to 1500  $\mu\text{g}$ , 1000 to 1750  $\mu\text{g}$ , 1000 to 2000  $\mu\text{g}$ , 1000 to 2250  $\mu\text{g}$ , 1000 to 2500  $\mu\text{g}$ , 1000 to 2750  $\mu\text{g}$ , 1000 to 3000  $\mu\text{g}$ , 2 to 500  $\mu\text{g}$ , 50  
to 500  $\mu\text{g}$ , 3 to 100  $\mu\text{g}$ , 5 to 20  $\mu\text{g}$ , 5 to 100  $\mu\text{g}$ , 10  $\mu\text{g}$ , 20  $\mu\text{g}$ , 30  $\mu\text{g}$ , 40  $\mu\text{g}$ , 50  $\mu\text{g}$ , 60  $\mu\text{g}$ ,  
70  $\mu\text{g}$ , 75  $\mu\text{g}$ , 80  $\mu\text{g}$ , 90  $\mu\text{g}$ , 100  $\mu\text{g}$ , 150  $\mu\text{g}$ , 200  $\mu\text{g}$ , 250  $\mu\text{g}$ , 300  $\mu\text{g}$ , 350  $\mu\text{g}$ , 400  $\mu\text{g}$ , 450  
15  $\mu\text{g}$ , 500  $\mu\text{g}$ , 550  $\mu\text{g}$ , 600  $\mu\text{g}$ , 650  $\mu\text{g}$ , 700  $\mu\text{g}$ , 750  $\mu\text{g}$ , 800  $\mu\text{g}$ , 850  $\mu\text{g}$ , 900  $\mu\text{g}$ , 950  $\mu\text{g}$ ,  
1000  $\mu\text{g}$ , 1050  $\mu\text{g}$ , 1100  $\mu\text{g}$ , 1150  $\mu\text{g}$ , 1200  $\mu\text{g}$ , 1250  $\mu\text{g}$ , 1300  $\mu\text{g}$ , 1350  $\mu\text{g}$ , 1400  $\mu\text{g}$ ,  
1450  $\mu\text{g}$ , 1500  $\mu\text{g}$ , 1550  $\mu\text{g}$ , 1600  $\mu\text{g}$ , 1650  $\mu\text{g}$ , 1700  $\mu\text{g}$ , 1750  $\mu\text{g}$ , 1800  $\mu\text{g}$ , 1850  $\mu\text{g}$ ,  
1900  $\mu\text{g}$ , 1950  $\mu\text{g}$ , 2000  $\mu\text{g}$ , 2050  $\mu\text{g}$ , 2100  $\mu\text{g}$ , 2150  $\mu\text{g}$ , 2200  $\mu\text{g}$ , 2250  $\mu\text{g}$ , 2300  $\mu\text{g}$ ,  
2350  $\mu\text{g}$ , 2400  $\mu\text{g}$ , 2450  $\mu\text{g}$ , 2500  $\mu\text{g}$ , 2550  $\mu\text{g}$ , 2600  $\mu\text{g}$ , 2650  $\mu\text{g}$ , 2700  $\mu\text{g}$ , 2750  $\mu\text{g}$ ,  
20 2800  $\mu\text{g}$ , 2850  $\mu\text{g}$ , 2900  $\mu\text{g}$ , 2950  $\mu\text{g}$ , 3000  $\mu\text{g}$ , 3250  $\mu\text{g}$ , 3500  $\mu\text{g}$ , 3750  $\mu\text{g}$ , 4000  $\mu\text{g}$ ,  
4250  $\mu\text{g}$ , 4500  $\mu\text{g}$ , 4750  $\mu\text{g}$ , 5000  $\mu\text{g}$  of a peptide or agonist described herein and from  
0.2 mg to 10 mg (e.g. 0.2 mg, 0.4 mg, 0.5 mg, 0.75 mg, 1 mg, 1.5 mg, 2 mg, 2.5 mg, 3  
mg, 3.5 mg, 4 mg, 4.5 mg, 5 mg, 5.5 mg, 6 mg, 6.5 mg, 7 mg, 7.5 mg, 8 mg, 8.5 mg, 9  
mg, 9.5 mg, 10 mg) of bumetanide (Bumex®).

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The precise amount of each of the two or more active ingredients in a dosage unit will depend on the desired dosage of each component. Thus, it can be useful to create a dosage unit that will, when administered according to a particular dosage schedule (e.g., a dosage schedule specifying a certain number of units and a particular timing for

administration), deliver the same dosage of each component as would be administered if the patient was being treated with only a single component. In other circumstances, it might be desirable to create a dosage unit that will deliver a dosage of one or more components that is less than that which would be administered if the patient was being treated only with a single component. Finally, it might be desirable to create a dosage unit that will deliver a dosage of one or more components that is greater than that which would be administered if the patient was being treated only with a single component. The pharmaceutical composition can include additional ingredients including but not limited to the excipients described herein. In certain embodiments, one or more therapeutic agents of the dosage unit may exist in an extended or control release formulation and additional therapeutic agents may not exist in extended release formulation. For example, a peptide or agonist described herein may exist in a controlled release formulation or extended release formulation in the same dosage unit with another agent that may or may not be in either a controlled release or extended release formulation. Thus, in certain embodiments, it may be desirable to provide for the immediate release of one or more of the agents described herein, and the controlled release of one or more other agents.

In certain embodiments the dosage unit and daily dose are equivalent. In certain embodiments the dosage unit and the daily dose are not equivalent. In various embodiments, the dosage unit is administered twenty minutes prior to food consumption, twenty minutes after food consumption, with food at anytime of the day, without food at anytime of the day, with food after an overnight fast (e.g. with breakfast), at bedtime after a low fat snack. In various embodiments, the dosage unit is administered once a day, twice a day, three times a day, four times a day, five times a day, six times a day.

When two or more active ingredients are combined in single dosage form, chemical interactions between the active ingredients may occur. For example, acidic and basic active ingredients can react with each other and acidic active ingredients can facilitate the



degradation of acid labile substances. Thus, in certain dosage forms, acidic and basic substances can be physically separated as two distinct or isolated layers in a compressed tablet, or in the core and shell of a press-coated tablet. Additional agents that are compatible with acidic as well as basic substances, have the flexibility of being placed in either layer. In certain multiple layer compositions at least one active ingredient can be enteric-coated. In certain embodiments thereof at least one active ingredient can be presented in a controlled release form. In certain embodiments where a combination of three or more active substances are used, they can be presented as physically isolated segments of a compressed multilayer tablet, which can be optionally film coated.

10

The therapeutic combinations described herein can be formulated as a tablet or capsule comprising a plurality of beads, granules, or pellets. All active ingredients including the vitamins of the combination are formulated into granules or beads or pellets that are further coated with a protective coat, an enteric coat, or a film coat to avoid the possible chemical interactions. Granulation and coating of granules or beads is done using techniques well known to a person skilled in the art. At least one active ingredient can present in a controlled release form. Finally these coated granules or beads are filled into hard gelatin capsules or compressed to form tablets.

20 The therapeutic combinations described herein can be formulated as a capsule comprising microtablets or minitables of all active ingredients. Microtablets of the individual agents can be prepared using well known pharmaceutical procedures of tablet making like direct compression, dry granulation or wet granulation. Individual microtablets can be filled into hard gelatin capsules. A final dosage form may comprise one or more microtablets of each individual component. The microtablets may be film coated or enteric coated.

25

The therapeutic combinations described herein can be formulated as a capsule comprising one or more microtablets and powder, or one or more microtablets and granules or beads. In order to avoid interactions between drugs, some active ingredients of a said

combination can be formulated as microtablets and the others filled into capsules as a powder, granules, or beads. The microtablets may be film coated or enteric coated. At least one active ingredient can be presented in controlled release form.

5 The therapeutic combinations described herein can be formulated wherein the active ingredients are distributed in the inner and outer phase of tablets. In an attempt to divide chemically incompatible components of proposed combination, few interacting components are converted in granules or beads using well known pharmaceutical procedures in prior art. The prepared granules or beads (inner phase) are then mixed with  
10 outer phase comprising the remaining active ingredients and at least one pharmaceutically acceptable excipient. The mixture thus comprising inner and outer phase is compressed into tablets or molded into tablets. The granules or beads can be controlled release or immediate release beads or granules, and can further be coated using an enteric polymer in an aqueous or non-aqueous system, using methods and materials  
15 that are known in the art.

The therapeutic combinations described herein can be formulated as single dosage unit comprising suitable buffering agent. All powdered ingredients of said combination are mixed and a suitable quantity of one or more buffering agents is added to the blend to  
20 minimize possible interactions.

The agents described herein, alone or in combination, can be combined with any pharmaceutically acceptable carrier or medium. Thus, they can be combined with materials that do not produce an adverse, allergic or otherwise unwanted reaction when  
25 administered to a patient. The carriers or mediums used can include solvents, dispersants, coatings, absorption promoting agents, controlled release agents, and one or more inert excipients (which include starches, polyols, granulating agents, microcrystalline cellulose, diluents, lubricants, binders, disintegrating agents, and the

like), etc. If desired, tablet dosages of the disclosed compositions may be coated by standard aqueous or nonaqueous techniques.

#### **Treatment of the side-effects of opioid administration**

- 5 GGC receptor agonists, e.g., GCC receptor agonist peptides described herein, may be useful in the treatment of one or more side effects of opioid administration, e.g., opioid induced constipation, nausea and/or vomiting. In the case of constipation, the GCC receptor agonist peptide can be administered at a dosage to induce laxation within a desired time (e.g., within 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7  
10 hours, 8 hours, 9 hours, 10 hours, 12 hours, 18 hours or 24 hours).

The the GCC receptor agonist peptide can be administered to maintain regular bowel movements in a patient who is a chronic opioid user (e.g., a terminally-ill patient). The administration can be via any convenient route (e.g., sublingual, parenteral, intravenous, subcutaneous).

15

- Thus, the peptides described herein can be administered to a patient that is taking one or more of the following opioids: Acetorphine, Acetyldihydrocodeine, Acetylmorphine, Alfentanil, Allylprodine, Anileridine, Bemidone, Benzylmorphine, Bezitramide, Buprenorphine, Butorphanol, Carfentanil/Carfentanyl, Clonitazene, Codeine, Codeine-N-  
20 Oxide, Codeinone, Cyclazocine, Cyclophorphan, Desomorphine, Dextromoramide, Dextropropoxyphene, Dezocine, Diacetyldihydromorphine, Diamorphine/Diacetylmorphine (Heroin), Diethylthiambutene, Difenoxin, Dihydrocodeine, Dihydrocodeinone Enol Acetate, Dihydroetorphine, Dihydroisocodeine, Dihydromorphine, Dimethylthiambutene, Diphenoxylate, Dipropanoylmorphine,  
25 Drobetabol, Ethylketocyclazocine, Ethylmorphine, Etonitazene, Etorphine, Fentanyl, Hydrocodone, Hydromorphone, Isomethadone, Ketobemidone, Laudanum, Lefetamine, Levallorphan, Levo-Alphaacetylmethadol (LAAM), Levomethorphan, Levorphanol, Loperamide, Meptazinol, Metazocine, Methadone, Monoacetylmorphine, Morphine,

Morphine-6-Glucuronide, Morphine-N-Oxide, Morphinone, MPPP (1-Methyl 4-Phenyl 4-Propionoxypiperidine), Myorphine, Nalbuphine/Nalbufine, Nicocodeine, Nicodicodeine, Nicomorphine, Norcodeine, Ohmefentanyl, Oxycodone, Oxymorphone, Pentazocine, PEPAP (1-Phenethyl-4-Phenyl-4-Piperidinol Acetate (Ester)), Pethidine  
5 (Meperidine), Phenadoxone, Phenazocine, Phenoperidine, Pholcodeine, Piminodine, Piritramide, Prodine, Propiram, Propoxyphene, Racemethorphan, Remifentanyl, Sufentanyl, Thebaine, Thiofentanyl/Thiofentanyl, Tilidine, and Tramadol. The peptide can be co-administered with or co-formulated with any of the preceding peptides.

Where the GCC receptor agonist is co-formulated with an opioid the composition may  
10 further include one or more other active ingredients that may be conventionally employed in analgesic and/or cough-cold-antitussive combination products. Such conventional ingredients include, for example, aspirin, acetaminophen, phenylpropanolamine, phenylephrine, chlorpheniramine, caffeine, and/or guaifenesin. Typical or conventional ingredients that may be included in the opioid component are described, for example, in  
15 the Physicians' Desk Reference, 1999, the disclosures of which are hereby incorporated herein by reference, in their entirety.

In addition, the composition may further include one or more compounds that may be designed to enhance the analgesic potency of the opioid and/or to reduce analgesic  
20 tolerance development. Such compounds include, for example, dextromethorphan or other NMDA antagonists (Mao, M. J. et al., Pain 1996, 67, 361), L-364,718 and other CCK antagonists (Dourish, C. T. et al., Eur J Pharmacol 1988, 147, 469), NOS inhibitors (Bhargava, H. N. et al., Neuropeptides 1996, 30, 219), PKC inhibitors (Bilsky, E. J. et al., J Pharmacol Exp Ther 1996, 277, 484), and dynorphin antagonists or antisera (Nichols,  
25 M. L. et al., Pain 1997, 69, 317). The disclosures of each of the foregoing documents are hereby incorporated herein by reference, in their entireties.

The combination products, such as pharmaceutical compositions comprising opioids in combination with a GCC agonist may be in any dosage form, such as those described

herein, and can also be administered in various ways, as described herein. In a preferred embodiment, the combination products of the disclosure are formulated together, in a single dosage form (that is, combined together in one capsule, tablet, powder, or liquid, etc.). When the combination products are not formulated together in a single dosage  
5 form, the opioid compounds and the GCC agonists may be administered at the same time (that is, together), or in any order. When not administered at the same time, preferably the administration of an opioid and a GCC agonist occurs less than about one hour apart, less than about 30 minutes apart, less than about 15 minutes apart, and less than about 5 minutes apart. Administration of the combination of an opioid and a GCC agonist can be,  
10 for example, oral, although other routes of administration, as described above, are contemplated to be within the scope of the present disclosure. Although it is the opioids and GCC agonists may both be administered in the same fashion (that is, for example, both orally), if desired, they may each be administered in different fashions (that is, for example, one component of the combination product may be administered orally, and  
15 another component may be administered intravenously). The dosage of the combination products of the disclosure may vary depending upon various factors such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration, the age, health and weight of the recipient, the nature and extent of the symptoms, the kind of concurrent treatment, the frequency of treatment, and the effect  
20 desired.

Although the proper dosage of the combination products of this disclosure will be readily ascertainable by one skilled in the art, by way of general guidance, where an opioid compounds is combined with a GCC agonist, for example, typically a daily dosage may  
25 range from about 0.01 to about 100 milligrams, 0.1 to about 10 milligrams of the opioid, 15 to about 200 milligrams, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 milligrams of opioid per kilogram of patient body weight. The opioid-GCC agonist combination product can include, for example, from 1 to 30  $\mu\text{g}$ , 1 to 40  $\mu\text{g}$ , 1 to 50  $\mu\text{g}$ , 1 to 100  $\mu\text{g}$ , 1 to 200  $\mu\text{g}$ , 1 to 300  $\mu\text{g}$ , 1 to 400  $\mu\text{g}$ , 1 to 500  $\mu\text{g}$ , 1 to 600  $\mu\text{g}$ , 1 to 700  $\mu\text{g}$ , 1 to 800  $\mu\text{g}$ , 1 to 900  $\mu\text{g}$ , 1 to 1000  $\mu\text{g}$ ,

10 to 30  $\mu\text{g}$ , 10 to 40  $\mu\text{g}$ , 10 to 50  $\mu\text{g}$ , 10 to 100  $\mu\text{g}$ , 10 to 200  $\mu\text{g}$ , 10 to 300  $\mu\text{g}$ , 10 to 400  
 $\mu\text{g}$ , 10 to 500  $\mu\text{g}$ , 10 to 600  $\mu\text{g}$ , 10 to 700  $\mu\text{g}$ , 10 to 800  $\mu\text{g}$ , 10 to 900  $\mu\text{g}$ , 10 to 1000  $\mu\text{g}$ ,  
100 to 200  $\mu\text{g}$ , 100 to 300  $\mu\text{g}$ , 100 to 400  $\mu\text{g}$ , 100 to 500  $\mu\text{g}$ , 100 to 600  $\mu\text{g}$ , 100 to 700  
 $\mu\text{g}$ , 100 to 800  $\mu\text{g}$ , 100 to 900  $\mu\text{g}$ , 100 to 1000  $\mu\text{g}$ , 100 to 1250  $\mu\text{g}$ , 100 to 1500  $\mu\text{g}$ , 100 to  
5 1750  $\mu\text{g}$ , 100 to 2000  $\mu\text{g}$ , 100 to 2250  $\mu\text{g}$ , 100 to 2500  $\mu\text{g}$ , 100 to 2750  $\mu\text{g}$ , 100 to 3000  
 $\mu\text{g}$ , 200 to 300  $\mu\text{g}$ , 200 to 400  $\mu\text{g}$ , 200 to 500  $\mu\text{g}$ , 200 to 600  $\mu\text{g}$ , 200 to 700  $\mu\text{g}$ , 200 to  
800  $\mu\text{g}$ , 200 to 900  $\mu\text{g}$ , 200 to 1000  $\mu\text{g}$ , 200 to 1250  $\mu\text{g}$ , 200 to 1500  $\mu\text{g}$ , 200 to 1750  $\mu\text{g}$ ,  
200 to 2000  $\mu\text{g}$ , 200 to 2250  $\mu\text{g}$ , 200 to 2500  $\mu\text{g}$ , 200 to 2750  $\mu\text{g}$ , 200 to 3000  $\mu\text{g}$ , 300 to  
400  $\mu\text{g}$ , 300 to 500  $\mu\text{g}$ , 300 to 600  $\mu\text{g}$ , 300 to 700  $\mu\text{g}$ , 300 to 800  $\mu\text{g}$ , 300 to 900  $\mu\text{g}$ , 300  
10 to 1000  $\mu\text{g}$ , 300 to 1250  $\mu\text{g}$ , 300 to 1500  $\mu\text{g}$ , 300 to 1750  $\mu\text{g}$ , 300 to 2000  $\mu\text{g}$ , 300 to 2250  
 $\mu\text{g}$ , 300 to 2500  $\mu\text{g}$ , 300 to 2750  $\mu\text{g}$ , 300 to 3000  $\mu\text{g}$ , 400 to 500  $\mu\text{g}$ , 400 to 600  $\mu\text{g}$ , 400 to  
700  $\mu\text{g}$ , 400 to 800  $\mu\text{g}$ , 400 to 900  $\mu\text{g}$ , 400 to 1000  $\mu\text{g}$ , 400 to 1250  $\mu\text{g}$ , 400 to 1500  $\mu\text{g}$ ,  
400 to 1750  $\mu\text{g}$ , 400 to 2000  $\mu\text{g}$ , 400 to 2250  $\mu\text{g}$ , 400 to 2500  $\mu\text{g}$ , 400 to 2750  $\mu\text{g}$ , 400 to  
3000  $\mu\text{g}$ , 500 to 600  $\mu\text{g}$ , 500 to 700  $\mu\text{g}$ , 500 to 800  $\mu\text{g}$ , 500 to 900  $\mu\text{g}$ , 500 to 1000  $\mu\text{g}$ ,  
15 500 to 1250  $\mu\text{g}$ , 500 to 1500  $\mu\text{g}$ , 500 to 1750  $\mu\text{g}$ , 500 to 2000  $\mu\text{g}$ , 500 to 2250  $\mu\text{g}$ , 500 to  
2500  $\mu\text{g}$ , 500 to 2750  $\mu\text{g}$ , 500 to 3000  $\mu\text{g}$ , 600 to 700  $\mu\text{g}$ , 600 to 800  $\mu\text{g}$ , 600 to 900  $\mu\text{g}$ ,  
600 to 1000  $\mu\text{g}$ , 600 to 1250  $\mu\text{g}$ , 600 to 1500  $\mu\text{g}$ , 600 to 1750  $\mu\text{g}$ , 600 to 2000  $\mu\text{g}$ , 600 to  
2250  $\mu\text{g}$ , 600 to 2500  $\mu\text{g}$ , 600 to 2750  $\mu\text{g}$ , 600 to 3000  $\mu\text{g}$ , 700 to 800  $\mu\text{g}$ , 700 to 900  $\mu\text{g}$ ,  
700 to 1000  $\mu\text{g}$ , 700 to 1250  $\mu\text{g}$ , 700 to 1500  $\mu\text{g}$ , 700 to 1750  $\mu\text{g}$ , 700 to 2000  $\mu\text{g}$ , 700 to  
20 2250  $\mu\text{g}$ , 700 to 2500  $\mu\text{g}$ , 700 to 2750  $\mu\text{g}$ , 700 to 3000  $\mu\text{g}$ , 800 to 900  $\mu\text{g}$ , 800 to 1000  $\mu\text{g}$ ,  
800 to 1250  $\mu\text{g}$ , 800 to 1500  $\mu\text{g}$ , 800 to 1750  $\mu\text{g}$ , 800 to 2000  $\mu\text{g}$ , 800 to 2250  $\mu\text{g}$ , 800 to  
2500  $\mu\text{g}$ , 800 to 2750  $\mu\text{g}$ , 800 to 3000  $\mu\text{g}$ , 900 to 1000  $\mu\text{g}$ , 900 to 1250  $\mu\text{g}$ , 900 to 1500  
 $\mu\text{g}$ , 900 to 1750  $\mu\text{g}$ , 900 to 2000  $\mu\text{g}$ , 900 to 2250  $\mu\text{g}$ , 900 to 2500  $\mu\text{g}$ , 900 to 2750  $\mu\text{g}$ , 900  
to 3000  $\mu\text{g}$ , 1000 to 1250  $\mu\text{g}$ , 1000 to 1500  $\mu\text{g}$ , 1000 to 1750  $\mu\text{g}$ , 1000 to 2000  $\mu\text{g}$ , 1000  
25 to 2250  $\mu\text{g}$ , 1000 to 2500  $\mu\text{g}$ , 1000 to 2750  $\mu\text{g}$ , 1000 to 3000  $\mu\text{g}$ , 2 to 500  $\mu\text{g}$ , 50 to 500  
 $\mu\text{g}$ , 3 to 100  $\mu\text{g}$ , 5 to 20  $\mu\text{g}$ , 5 to 100  $\mu\text{g}$ , 10  $\mu\text{g}$ , 20  $\mu\text{g}$ , 30  $\mu\text{g}$ , 40  $\mu\text{g}$ , 50  $\mu\text{g}$ , 60  $\mu\text{g}$ , 70  $\mu\text{g}$ ,  
75  $\mu\text{g}$ , 80  $\mu\text{g}$ , 90  $\mu\text{g}$ , 100  $\mu\text{g}$ , 150  $\mu\text{g}$ , 200  $\mu\text{g}$ , 250  $\mu\text{g}$ , 300  $\mu\text{g}$ , 350  $\mu\text{g}$ , 400  $\mu\text{g}$ , 450  $\mu\text{g}$ ,  
500  $\mu\text{g}$ , 550  $\mu\text{g}$ , 600  $\mu\text{g}$ , 650  $\mu\text{g}$ , 700  $\mu\text{g}$ , 750  $\mu\text{g}$ , 800  $\mu\text{g}$ , 850  $\mu\text{g}$ , 900  $\mu\text{g}$ , 950  $\mu\text{g}$ , 1000  
 $\mu\text{g}$ , 1050  $\mu\text{g}$ , 1100  $\mu\text{g}$ , 1150  $\mu\text{g}$ , 1200  $\mu\text{g}$ , 1250  $\mu\text{g}$ , 1300  $\mu\text{g}$ , 1350  $\mu\text{g}$ , 1400  $\mu\text{g}$ , 1450  $\mu\text{g}$ ,

1500  $\mu\text{g}$ , 1550  $\mu\text{g}$ , 1600  $\mu\text{g}$ , 1650  $\mu\text{g}$ , 1700  $\mu\text{g}$ , 1750  $\mu\text{g}$ , 1800  $\mu\text{g}$ , 1850  $\mu\text{g}$ , 1900  $\mu\text{g}$ ,  
1950  $\mu\text{g}$ , 2000  $\mu\text{g}$ , 2050  $\mu\text{g}$ , 2100  $\mu\text{g}$ , 2150  $\mu\text{g}$ , 2200  $\mu\text{g}$ , 2250  $\mu\text{g}$ , 2300  $\mu\text{g}$ , 2350  $\mu\text{g}$ ,  
2400  $\mu\text{g}$ , 2450  $\mu\text{g}$ , 2500  $\mu\text{g}$ , 2550  $\mu\text{g}$ , 2600  $\mu\text{g}$ , 2650  $\mu\text{g}$ , 2700  $\mu\text{g}$ , 2750  $\mu\text{g}$ , 2800  $\mu\text{g}$ ,  
2850  $\mu\text{g}$ , 2900  $\mu\text{g}$ , 2950  $\mu\text{g}$ , 3000  $\mu\text{g}$ , 3250  $\mu\text{g}$ , 3500  $\mu\text{g}$ , 3750  $\mu\text{g}$ , 4000  $\mu\text{g}$ , 4250  $\mu\text{g}$ ,  
5 4500  $\mu\text{g}$ , 4750  $\mu\text{g}$ , 5000  $\mu\text{g}$  of a GCC agonist described herein.

When provided as a single dosage form, the potential exists for a chemical interaction between the combined active ingredients (for example, an opioid and a GCC agonist). For this reason, the preferred dosage forms of the combination products of this disclosure are formulated such that although the active ingredients are combined in a single dosage form, the physical contact between the active ingredients is minimized (that is, reduced).  
10

In order to minimize contact, one embodiment of this disclosure where the product is orally administered provides for a combination product wherein one active ingredient is enteric coated. By enteric coating one or more of the active ingredients, it is possible not only to minimize the contact between the combined active ingredients, but also, it is possible to control the release of one of these components in the gastrointestinal tract such that one of these components is not released in the stomach but rather is released in the intestines. Another embodiment of this disclosure where oral administration is desired provides for a combination product wherein one of the active ingredients is coated with a sustained-release material which effects a sustained-release throughout the  
15  
20 gastrointestinal tract and also serves to minimize physical contact between the combined active ingredients. Furthermore, the sustained-released component can be additionally enteric coated such that the release of this component occurs only in the intestine. Still another approach would involve the formulation of a combination product in which the one component is coated with a sustained and/or enteric release polymer, and the other component is also coated with a polymer such as a low-viscosity grade of hydroxypropyl methylcellulose (HPMC) or other appropriate materials as known in the art, in order to  
25 further separate the active components. The polymer coating serves to form an additional

barrier to interaction with the other component.

Dosage forms of the combination products include those wherein one active ingredient is enteric coated can be in the form of tablets such that the enteric coated component and  
5 the other active ingredient are blended together and then compressed into a tablet or such that the enteric coated component is compressed into one tablet layer and the other active ingredient is compressed into an additional layer. Optionally, in order to further separate the two layers, one or more placebo layers may be present such that the placebo layer is between the layers of active ingredients. In addition, dosage forms of the present  
10 disclosure can be in the form of capsules wherein one active ingredient is compressed into a tablet or in the form of a plurality of microtablets, particles, granules or non-perils, which are then enteric coated. These enteric coated microtablets, particles, granules or non-perils are then placed into a capsule or compressed into a capsule along with a granulation of the other active ingredient.

15 These as well as other ways of minimizing contact between the components of combination products of the present disclosure, whether administered in a single dosage form or administered in separate forms but at the same time by the same manner, will be readily apparent to those skilled in the art in light of the present disclosure.

20

#### Analgesic Agents in combitherapy

The peptides and agonists described herein can be used in combination therapy with an analgesic agent, e.g., an analgesic compound or an analgesic peptide. These peptides and compounds can be administered with the peptides described herein (simultaneously or  
25 sequentially). They can also be optionally covalently linked or attached to an agent described herein to create therapeutic conjugates. Among the useful analgesic agents are: Ca channel blockers, 5HT receptor antagonists (for example 5HT3, 5HT4 and 5HT1 receptor antagonists), opioid receptor agonists (loperamide, fedotozine, and fentanyl),



NK1 receptor antagonists, CCK receptor agonists (e.g., loxiglumide), NK1 receptor antagonists, NK3 receptor antagonists, norepinephrine-serotonin reuptake inhibitors (NSRI), vanilloid and cannabinoid receptor agonists, and sialorphan. Analgesics agents in the various classes are described in the literature.

5

Among the useful analgesic peptides are sialorphan-related peptides, including those comprising the amino acid sequence QHNPR (SEQ ID NO: ), including: VQHNPR (SEQ ID NO: ); VRQHNPR (SEQ ID NO: ); VRGQHNPR (SEQ ID NO: ); VRGPQHNPR (SEQ ID NO: ); VRGPRQHNPR (SEQ ID NO: ); VRGPRRQHNPR (SEQ ID NO: ); and RQHNPR (SEQ ID NO: ). Sialorphan-related peptides bind to neprilysin and inhibit neprilysin-mediated breakdown of substance P and Met-enkephalin. Thus, compounds or peptides that are inhibitors of neprilysin are useful analgesic agents which can be administered with the peptides described herein in a co-therapy or linked to the peptides described herein, e.g., by a covalent bond. Sialorphan and related peptides are described in U.S. Patent 6,589,750; U.S. 20030078200 A1; and WO 02/051435 A2.

10  
15

Opioid receptor antagonists and agonists can be administered with the peptides described herein in co-therapy or linked to the agent described herein, e.g., by a covalent bond. For example, opioid receptor antagonists such as naloxone, naltrexone, methyl naloxone, nalmefene, cypridime, beta funaltrexamine, naloxonazine, naltrindole, and nor-binaltorphimine are thought to be useful in the treatment of IBS. It can be useful to formulate opioid antagonists of this type in a delayed and sustained release formulation such that initial release of the antagonist is in the mid to distal small intestine and/or ascending colon. Such antagonists are described in WO 01/32180 A2. Enkephalin pentapeptide (HOE825; Tyr-D-Lys-Gly-Phe-L-homoserine) is an agonist of the mu and delta opioid receptors and is thought to be useful for increasing intestinal motility (*Eur. J. Pharm.* 219:445, 1992), and this peptide can be used in conjunction with the peptides described herein. Also useful is trimebutine which is thought to bind to mu/delta/kappa

20  
25

opioid receptors and activate release of motilin and modulate the release of gastrin, vasoactive intestinal peptide, gastrin and glucagons. Kappa opioid receptor agonists such as fedotozine, asimadoline, and ketocyclazocine, and compounds described in WO 03/097051 and WO05/007626 can be used with or linked to the peptides described  
5 herein. In addition, mu opioid receptor agonists such as morphine, diphenyloxybate, frakefamide (H-Tyr-D-Ala-Phe(F)-Phe-NH<sub>2</sub>; WO 01/019849 A1) and loperamide can be used.

Tyr-Arg (kyotorphin) is a dipeptide that acts by stimulating the release of met-enkephalins to elicit an analgesic effect (*J. Biol. Chem* 262:8165, 1987). Kyotorphin can  
10 be used with or linked to the peptides described herein.

Chromogranin-derived peptide (CgA 47-66; see, e.g., Ghia et al. 2004 *Regulatory Peptides* 119:199) can be used with or linked to the peptides described herein.

15 CCK receptor agonists such as caerulein from amphibians and other species are useful analgesic agents that can be used with or linked to the peptides described herein.

Conotoxin peptides represent a large class of analgesic peptides that act at voltage gated  
20 Ca channels, NMDA receptors or nicotinic receptors. These peptides can be used with or linked to the peptides described herein.

Peptide analogs of thymulin (FR Application 2830451) can have analgesic activity and can be used with or linked to the peptides described herein.

25 CCK (CCKa or CCKb) receptor antagonists, including loxiglumide and dexloxiglumide (the R-isomer of loxiglumide) (WO 88/05774) can have analgesic activity and can be used with or linked to the peptides described herein.

Other useful analgesic agents include 5-HT<sub>4</sub> agonists such as tegaserod (Zelnorm®), mosapride, metoclopramide, zacopride, cisapride, renzapride, benzimidazolone derivatives such as BIMU 1 and BIMU 8, and lorexapride. Such agonists are described in: EP1321142 A1, WO 03/053432A1, EP 505322 A1, EP 505322 B1, US 5,510,353, EP  
5 507672 A1, EP 507672 B1, and US 5,273,983.

Calcium channel blockers such as ziconotide and related compounds described in, for example, EP625162B1, US 5,364,842, US 5,587,454, US 5,824,645, US 5,859,186, US 5,994,305, US 6,087,091, US 6,136,786, WO 93/13128 A1, EP 1336409 A1, EP 835126  
10 A1, EP 835126 B1, US 5,795,864, US 5,891,849, US 6,054,429, WO 97/01351 A1, can be used with or linked to the peptides described herein.

Various antagonists of the NK-1, NK-2, and NK-3 receptors (for a review see Giardina et al. 2003 *Drugs* 6:758) can be used with or linked to the peptides described herein.  
15

NK1 receptor antagonists such as: aprepitant (Merck & Co Inc), vofopitant, ezlopitant (Pfizer, Inc.), R-673 (Hoffmann-La Roche Ltd), SR-48968 (Sanofi Synthelabo), CP-122,721 (Pfizer, Inc.), GW679769 (Glaxo Smith Kline), TAK-637 (Takeda/Abbot), SR-14033, and related compounds described in, for example, EP 873753 A1, US  
20 20010006972 A1, US 20030109417 A1, WO 01/52844 A1, can be used with or linked to the peptides described herein.

NK-2 receptor antagonists such as nepadutant (Menarini Ricerche SpA), saredutant (Sanofi-Synthelabo), GW597599 (Glaxo Smith Kline), SR-144190 (Sanofi-Synthelabo)  
25 and UK-290795 (Pfizer Inc) can be used with or linked to the peptides described herein.

NK3 receptor antagonists such as osanetant (SR-142801; Sanofi-Synthelabo), SSR-241586, talnetant and related compounds described in, for example, WO 02/094187 A2, EP 876347 A1, WO 97/21680 A1, US 6,277,862, WO 98/11090, WO 95/28418, WO

97/19927, and Boden et al. (*J Med Chem.* 39:1664-75, 1996) can be used with or linked to the peptides described herein.

5 Norepinephrine-serotonin reuptake inhibitors (NSRI) such as milnacipran and related compounds described in WO 03/077897 A1 can be used with or linked to the peptides described herein.

Vanilloid receptor antagonists such as arvanil and related compounds described in WO 01/64212 A1 can be used with or linked to the peptides described herein.

10

The analgesic peptides and compounds can be administered with the peptides and agonists described herein (simultaneously or sequentially). The analgesic agents can also be covalently linked to the peptides and agonists described herein to create therapeutic conjugates. Where the analgesic is a peptide and is covalently linked to an agent described herein the resulting peptide may also include at least one trypsin cleavage site. When present within the peptide, the analgesic peptide may be preceded by (if it is at the carboxy terminus) or followed by (if it is at the amino terminus) a trypsin cleavage site that allows release of the analgesic peptide.

20 In addition to sialorphin-related peptides, analgesic peptides include: AspPhe, endomorphin-1, endomorphin-2, nocistatin, dalargin, lupron, ziconotide, and substance P.

#### Diabetes, Obesity and other disorders

25 Pharmaceutical compositions comprising at least two of: 1) an agent that stimulates the production of cAMP (e.g., glucagon-like peptide 1 (GLP-1)); 2) an agent that inhibits the degradation of a cyclic nucleotide (e.g., a phosphodiesterase inhibitor); and 3) a peptide or agonist described herein useful for treating diabetes and obesity. Such compositions may also be useful for treating secondary hyperglycemias in connection with pancreatic diseases (chronic pancreatitis, pancreatectomy, hemochromatosis) or endocrine diseases

(acromegaly, Cushing's syndrome, pheochromocytoma or hyperthyreosis), drug-induced hyperglycemias (benzothiadiazine saluretics, diazoxide or glucocorticoids), pathologic glucose tolerance, hyperglycemias, dyslipoproteinemias, adiposity, hyperlipoproteinemias and/or hypotensions.

5

The phosphodiesterase inhibitor can be specific for a particular phosphodiesterase (e.g., Group III or Group IV) or a non-specific phosphodiesterase inhibitor, such as papaverine, theophylline, enprofyllines and/or IBMX. Specific phosphodiesterase inhibitors which inhibit group III phosphodiesterases (cGMP-inhibited phosphodiesterases), including  
10 indolidane (LY195115), cilostamide (OPC 3689), lixazinone (RS 82856), Y-590, imazodane (CI914), SKF 94120, quazinone, ICI 153,110, cilostazole, bemorandane (RWJ 22867), siguzodane (SK&F 94-836), adibendane (BM 14,478), milrinone (WIN 47203), enoximone (MDL 17043), pimobendane (UD-CG 115), MCI-154, saterinone (BDF 8634), sulmazole (ARL 115), UD-CG 212, motapizone, piroximone, and ICI  
15 118233 can be useful. In addition, phosphodiesterase inhibitors which inhibit group IV phosphodiesterases (cAMP-specific phosphodiesterases), such as rolipram ZK 62711; pyrrolidone), imidazolidinone (RO 20-1724), etazolate (SQ 65442), denbufylline (BRL 30892), ICI63197, and RP73401 can be used.

#### 20 Other Agents for Use in Combitherapy

Also within the disclosure are pharmaceutical compositions comprising a peptide or agonists described herein and a second therapeutic agent. The second therapeutic agent can be administered to treat any condition for which it is useful, including conditions that are not considered to be the primary indication for treatment with the second therapeutic  
25 agent. The second therapeutic agent can be administered simultaneously or sequentially. The second therapeutic agent can be covalently linked to the peptides and agonists described herein to create a therapeutic conjugate. When the second therapeutic agent is another peptide, a linker including those described herein may be used between the peptide described herein and the second therapeutic peptide.

Examples of therapeutic agents used to treat heart failure include diuretics (e.g. furosemide (Lasix), bumetanide (Bumex), ethacrynic acid (Edecrin), torsemide (Demadex), amiloride (Midamor), spironolactone (Aldactone), chorthiazide (Diuril),  
5 metolazone (Zaroxylyn)), Angiotension-Converting Enzyme (ACE) inhibitors (e.g. captopril (Capoten), enalapril (Vasotec), lisinopril (Prinivil, Zestril), ramipril (Altace)), Beta blockers (e.g. carvedilol (Coreg) and Inotropes (e.g. digoxin, dobutamine, dopamine Milrinone).

10           Examples of additional therapeutic agents to treat gastrointestinal and other disorders include:

agents to treat constipation (e.g., a chloride channel activator such as the bicyclic fatty acid, Lubiprostone (formerly known as SPI-0211; Sucampo Pharmaceuticals, Inc.;  
15 Bethesda, MD), a laxative (eg. a bulk-forming laxative (e.g. nonstarch polysaccharides, Colonel Tablet (polycarbophil calcium), Plantago Ovata®, Equalactin® (Calcium Polycarbophil)), fiber (e.g. FIBERCON® (Calcium Polycarbophil), an osmotic laxative, a stimulant laxative (such as diphenylmethanes (e.g. bisacodyl), anthraquinones (e.g. cascara, senna), and surfactant laxatives (e.g. castor oil, docusates), an  
20 emollient/lubricating agent (such as mineral oil, glycerine, and docusates), MiraLax (Braintree Laboratories, Braintree MA), dexloiglumide (Forest Laboratories, also known as CR 2017 Rottapharm (Rotta Research Laboratorium SpA)), saline laxatives, enemas, suppositories, and CR 3700 (Rottapharm (Rotta Research Laboratorium SpA));  
25 acid reducing agents such as proton pump inhibitors (e.g., omeprazole (Prilosec®), esomeprazole (Nexium®), lansoprazole (Prevacid®), pantoprazole (Protonix®) and rabeprazole (Aciphex®)) and Histamine H<sub>2</sub>-receptor antagonist (also known as H<sub>2</sub> receptor blockers including cimetidine, ranitidine, famotidine and nizatidine);

prokinetic agents including itopride, octreotide, bethanechol, metoclopramide (Reglan®), domperidone (Motilium®), erythromycin (and derivatives thereof) or cisapride (propulsid®);

5 prokineticin peptides homologs, variants and chimeras thereof including those described in US 7,052,674 which can be used with or linked to the peptides described herein; pro-motility agents such as the vasostatin-derived peptide, chromogranin A (4-16) (see, e.g., Ghia et al. 2004 Regulatory Peptides 121:31) or motilin agonists (e.g., GM-611 or mitemincinal fumarate) or nociceptin/Orphanin FQ receptor modulators (US20050169917);

10

other peptides which can bind to and/or activate GC-C including those described in US20050287067;

complete or partial 5HT (e.g. 5HT1, 5HT2, 5HT3, 5HT4) receptor agonists or antagonists  
15 (including 5HT1A antagonists (e.g. AGI-001 (AGI therapeutics), 5HT2B antagonists (e.g. PGN1091 and PGN1164 (Pharmagene Laboratories Limited), and 5HT4 receptor agonists (such as tegaserod (ZELNORM®), prucalopride, mosapride, metoclopramide, zacopride, cisapride, renzapride, benzimidazolone derivatives such as BIMU 1 and BIMU 8, and lorexapride). Such agonists/modulators are described in: EP1321142 A1,  
20 WO 03/053432A1, EP 505322 A1, EP 505322 B1, US 5,510,353, EP 507672 A1, EP 507672 B1, US 5,273,983, and US 6,951,867); 5HT3 receptor agonists such as MKC-733; and 5HT3 receptor antagonists such as DDP-225 (MCI-225; Dynogen Pharmaceuticals, Inc.), cilansetron (Calmactin®), alosetron (Lotronex®), Ondansetron HCl (Zofran®), Dolasetron (ANZEMET®), palonosetron (Aloxi®), Granisetron  
25 (Kytril®), YM060(ramosetron; Astellas Pharma Inc.; ramosetron may be given as a daily dose of 0.002 to 0.02 mg as described in EP01588707) and ATI-7000 (Aryx Therapeutics, Santa Clara CA);

30

muscarinic receptor agonists;

anti-inflammatory agents;

antispasmodics including but not limited to anticholinergic drugs (like dicyclomine (e.g. Colimex®, Formulex®, Lomine®, Protylol®, Viscerol®, Spasmoban®, Bentyl®,  
 5 Bentylol®), hyoscyamine (e.g. IB-Stat®, Nulev®, Levsin®, Levbid®, Levsinex  
 Timecaps®, Levsin/SL®, Anaspaz®, A-Spas S/L®, Cystospaz®, Cystospaz-M®,  
 Donnamar®, Colidrops Liquid Pediatric®, Gastroed®, Hyco Elixir®, Hyosol®,  
 Hyospaz®, Hyosyne®, Losamine®, Medispaz®, Neosol®, Spacol®, Spasdel®,  
 Symax®, Symax SL®), Donnatal (e.g. Donnatal Extentabs®), clidinium (e.g. Quarzan, in  
 10 combination with Librium = Librax), methantheline (e.g. Banthine), Mepenzolate (e.g.  
 Cantil), homatropine (e.g. hycodan, Homapin), Propantheline bromide (e.g. Pro-  
 Banthine), Glycopyrrolate (e.g. Robinul®, Robinul Forte®), scopolamine (e.g.  
 Transderm-Scop®, Transderm-V®), hyosine-N-butylbromide (e.g. Buscopan®),  
 Pirenzepine (e.g. Gastrozepin®) Propantheline Bromide (e.g. Propanthel®),  
 15 dicycloverine (e.g. Merbentyl®) , glycopyrronium bromide (e.g. Glycopyrrolate®) ,  
 hyoscine hydrobromide , hyoscine methobromide , methanthelinium, and octatropine);  
 peppermint oil; and direct smooth muscle relaxants like cimetropium bromide,  
 mebeverine (DUSPATAL®, DUSPATALIN®, COLOFAC MR®, COLOTAL®),  
 otilonium bromide (octilonium), pinaverium (e.g. Dicetel® (pinaverium bromide; Solvay  
 20 S.A.)), Spasfon® (hydrated phloroglucinol and trimethylphloroglucinol)and trimebutine  
 (including trimebutine maleate (Modulon®);

antidepressants, including but not limited to those listed herein, as well as tricyclic  
 antidepressants like amitriptyline (Elavil®), desipramine (Norpramin®), imipramine  
 25 (Tofranil®), amoxapine (Asendin®), nortriptyline; the selective serotonin reuptake  
 inhibitors (SSRI's) like paroxetine (Paxil®), fluoxetine (Prozac®), sertraline (Zoloft®),  
 and citalopram (Celexa®); and others like doxepin (Sinequan®) and trazodone  
 (Desyrel®);



centrally-acting analgesic agents such as opioid receptor agonists, opioid receptor antagonists (e.g., naltrexone);

agents for the treatment of Inflammatory bowel disease;

5

agents for the treatment of Crohn's disease and/or ulcerative colitis (e.g., alequel (Enzo Biochem, Inc.; Farmingsale, NY), the anti-inflammatory peptide RDP58 (Genzyme, Inc.; Cambridge, MA), and TRAFICET-EN<sup>TM</sup> (ChemoCentryx, Inc.; San Carlos, CA);

10 agents that treat gastrointestinal or visceral pain;

agents that increase cGMP levels (as described in US20040121994) like adrenergic receptor antagonists, dopamine receptor agonists and PDE (phosphodiesterase) inhibitors including but not limited to those disclosed herein;

15

purgatives that draw fluids to the intestine (e.g., VISICOL®, a combination of sodium phosphate monobasic monohydrate and sodium phosphate dibasic anhydrate);

20 Corticotropin Releasing Factor (CRF) receptor antagonists (including NBI-34041 (Neurocrine Biosciences, San Diego, CA), CRH9-41, astressin, R121919 (Janssen Pharmaceutica), CP154,526, NBI-27914, Antalarmin, DMP696 (Bristol-Myers Squibb) CP-316,311 (Pfizer, Inc.), SB723620 (GSK), GW876008 (Neurocrine/Glaxo Smith Kline), ONO-2333Ms (Ono Pharmaceuticals), TS-041 (Janssen), AAG561 (Novartis) and those disclosed in US 5,063,245, US 5,861,398, US20040224964, US20040198726,  
25 US20040176400, US20040171607, US20040110815, US20040006066, and US20050209253);

glucagon-like peptides (glp-1) and analogues thereof (including exendin-4 and GTP-010 (Gastrotech Pharma A)) and inhibitors of DPP-IV (DPP-IV mediates the inactivation of glp-1);

5 tofisopam, enantiomerically-pure R-tofisopam, and pharmaceutically-acceptable salts thereof (US 20040229867);

tricyclic anti-depressants of the dibenzothiazepine type including but not limited to Dextofisopam® (Vela Pharmaceuticals), tianeptine (Stablon®) and other agents  
10 described in US 6,683,072;

(E)-4 (1,3bis(cyclohexylmethyl)-1,2,3,4,-tetrahydro-2,6-diono-9H-purin-8-yl)cinnamic acid nonaethylene glycol methyl ether ester and related compounds described in WO  
02/067942;

15 the probiotic PROBACTRIX® (The BioBalance Corporation; New York, NY) which contains microorganisms useful in the treatment of gastrointestinal disorders;

antidiarrheal drugs including but not limited to loperamide (Imodium, Pepto Diarrhea),  
20 diphenoxylate with atropine (Lomotil, Lomocot), cholestyramine (Questran, Cholybar), atropine (Co-Phenotrope, Diarsed, Diphenoxylate, Lofene, Logen, Lonox, Vi-Atro, atropine sulfate injection) and Xifaxan® (rifaximin; Salix Pharmaceuticals Ltd), TZP-201(Tranzyme Pharma Inc.), the neuronal acetylcholine receptor (nAChR) blocker AGI-004 (AGI therapeutics), and bismuth subsalicylate (Pepto-bismol);

25 anxiolytic drugs including but not limited to Ativan (lorazepam), alprazolam (Xanax®), chlordiazepoxide/clidinium (Librium®, Librax®), clonazepam (Klonopin®), clorazepate (Tranxene®), diazepam (Valium®), estazolam (ProSom®), flurazepam (Dalmane®),

oxazepam (Serax®), prazepam (Centrax®), temazepam (Restoril®), triazolam (Halcion®);

Bedelix® (Montmorillonite beidellitic; Ipsen Ltd), Solvay SLV332 (ArQule Inc), YKP  
5 (SK Pharma), Asimadoline (Tioga Pharmaceuticals/Merck), AGI-003 (AGI Therapeutics);

neurokinin antagonists including those described in US20060040950;

10 potassium channel modulators including those described in US7,002,015;

the serotonin modulator AZD7371 (AstraZeneca Plc);

15 M3 muscarinic receptor antagonists such as darifenacin (Enablex; Novartis AG and zamifenacin (Pfizer);

herbal and natural therapies including but not limited to acidophilus, chamomile tea, evening primrose oil, fennel seeds, wormwood, comfrey, and compounds of Bao-Ji-Wan  
20 (magnolol, honokiol, imperatorin, and isoimperatorin) as in US6923992; and

compositions comprising lysine and an anti-stress agent for the treatment of irritable bowel syndrome as described in EP01550443.

The peptides and agonists described herein can be used in combination therapy  
25 with insulin and related compounds including primate, rodent, or rabbit insulin including biologically active variants thereof including allelic variants, more preferably human insulin available in recombinant form. Sources of human insulin include pharmaceutically acceptable and sterile formulations such as those available from Eli Lilly (Indianapolis, Ind. 46285) as Humulin™ (human insulin rDNA origin). See the

THE PHYSICIAN'S DESK REFERENCE, 55<sup>sup.th</sup> Ed. (2001) Medical Economics, Thomson Healthcare (disclosing other suitable human insulins). The peptides and agonists described herein can also be used in combination therapy with agents that can boost insulin effects or levels of a subject upon administration, e.g. glipizide and/or  
5 rosiglitazone. The peptides and agonists described herein can be used in combination therapy with SYMLIN® (pramlintide acetate) and Exenatide® (synthetic exendin-4; a 39 aa peptide).

The peptides and agonists described herein can also be used in combination  
10 therapy with agents (e.g., Entereg™ (alvimopan; formerly called adolor/ ADL 8-2698), conivaptan and related agents describe in US 6,645,959) used for the treatment of postoperative ileus and other disorders.

The peptides and agonists described herein can be used in combination therapy  
15 with an anti-hypertensive agent including but not limited to:

(1) diuretics, such as thiazides, including chlorthalidone, chlorthiazide, dichlorophenamide, hydroflumethiazide, indapamide, polythiazide, and hydrochlorothiazide; loop diuretics, such as bumetanide, ethacrynic acid, furosemide, and torsemide; potassium sparing agents, such as amiloride, and triamterene; carbonic  
20 anhydrase inhibitors, osmotics (such as glycerin) and aldosterone antagonists, such as spironolactone, eplerenone, and the like; (2) beta-adrenergic blockers such as acebutolol, atenolol, betaxolol, bevantolol, bisoprolol, bopindolol, carteolol, carvedilol, celiprolol, esmolol, indenolol, metoprolol, nadolol, nebivolol, penbutolol, pindolol, propanolol, sotalol, tertatolol, tilisolol, and timolol, and the like;

(3) calcium channel blockers such as amlodipine, aranidipine, azelnidipine, barnidipine, benidipine, bepridil, cinaldipine, clevidipine, diltiazem, efonidipine, felodipine, gallopamil, isradipine, lacidipine, lemildipine, lercanidipine, nicardipine, nifedipine, nilvadipine, nimodipine, nisoldipine, nitrendipine, manidipine, pranidipine, and verapamil, and the like;

- (4) angiotensin converting enzyme (ACE) inhibitors such as benazepril; captopril; ceranapril; cilazapril; delapril; enalapril; enalapril; fosinopril; imidapril; lisinopril; losinopril; moexipril; quinapril; quinaprilat; ramipril; perindopril; perindopril; quanipril; spirapril; tenocapril;trandolapril, and zofenopril, and the like;
- 5 (5) neutral endopeptidase inhibitors such as omapatrilat, cadoxatril and ecadotril, fosidotril, sampatrilat, AVE7688, ER4030, and the like;
- (6) endothelin antagonists such as tezosentan, A308165, and YM62899, and the like;
- (7) vasodilators such as hydralazine, clonidine, minoxidil, and nicotinyln  
10 alcohol, and the like;
- (8) angiotensin II receptor antagonists such as aprosartan, candesartan, eprosartan, irbesartan, losartan, olmesartan, prazosartan, tasosartan, telmisartan, valsartan, and EXP-3137, F16828K, and RNH6270, and the like;
- (9)  $\alpha/\beta$  adrenergic blockers such as nipradilol, arotinolol and amosulalol, and  
15 the like;
- (10) alpha 1 blockers, such as terazosin, urapidil, prazosin, tamsulosin, bunazosin, trimazosin, doxazosin, naftopidil, indoramin, WHP 164, and XEN010, and the like;
- (11) alpha 2 agonists such as lofexidine, tiamenidine, moxonidine, rilmenidine  
20 and guanobenz, and the like;
- (12) aldosterone inhibitors, and the like; and
- (13) angiopoietin-2-binding agents such as those disclosed in WO03/030833.

Specific anti-hypertensive agents that can be used in combination with  
25 peptides and agonists described herein include, but are not limited to:

diuretics, such as thiazides (e.g., chlorthalidone, cyclothiazide (CAS RN 2259-96-3), chlorothiazide (CAS RN 72956-09-3, which may be prepared as disclosed in US2809194), dichlorophenamide, hydroflumethiazide, indapamide, polythiazide,

bendroflumethazide, methyclothazide, polythiazide, trichlormethazide, chlorthalidone, indapamide, metolazone, quinethazone, althiazide (CAS RN 5588-16-9, which may be prepared as disclosed in British Patent No. 902,658), benzthiazide (CAS RN 91-33-8, which may be prepared as disclosed in US3108097), buthiazide (which may be prepared  
5 as disclosed in British Patent Nos. 861,367), and hydrochlorothiazide), loop diuretics (e.g. bumetanide, ethacrynic acid, furosemide, and torasemide), potassium sparing agents (e.g. amiloride, and triamterene (CAS Number 396-01-0)), and aldosterone antagonists (e.g. spironolactone (CAS Number 52-01-7), epirenone, and the like);  $\beta$ -adrenergic blockers such as Amiodarone (Cordarone, Pacerone), bunolol hydrochloride (CAS RN  
10 31969-05-8, Parke-Davis), acebutolol ( $\pm$ N-[3-Acetyl-4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]-butanamide, or ( $\pm$ )-3'-Acetyl-4'-[2-hydroxy-3-(isopropylamino) propoxy] butyranilide), acebutolol hydrochloride (e.g. Sectral<sup>®</sup>, Wyeth-Ayerst), alprenolol hydrochloride (CAS RN 13707-88-5 see Netherlands Patent Application No. 6,605,692), atenolol (e.g. Tenormin<sup>®</sup>, AstraZeneca), carteolol  
15 hydrochloride (e.g. Cartrol<sup>®</sup> Filmtab<sup>®</sup>, Abbott), Celiprolol hydrochloride (CAS RN 57470-78-7, also see in US4034009), cetamolol hydrochloride (CAS RN 77590-95-5, see also US4059622), labetalol hydrochloride (e.g. Normodyne<sup>®</sup>, Schering), esmolol hydrochloride (e.g. Brevibloc<sup>®</sup>, Baxter), levobetaxolol hydrochloride (e.g. Betaxon<sup>™</sup> Ophthalmic Suspension, Alcon), levobunolol hydrochloride (e.g. Betagan<sup>®</sup> Liquifilm<sup>®</sup>  
20 with C CAP<sup>®</sup> Compliance Cap, Allergan), nadolol (e.g. Nadolol, Mylan), practolol (CAS RN 6673-35-4, see also US3408387), propranolol hydrochloride (CAS RN 318-98-9), sotalol hydrochloride (e.g. Betapace AF<sup>™</sup>, Berlex), timolol (2-Propanol, 1-[(1,1-dimethylethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-, hemihydrate, (S)-, CAS RN 91524-16-2), timolol maleate (S)-1-[(1,1-dimethylethyl) amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl] oxy]-2-propanol (Z)-2-butenedioate (1:1) salt, CAS  
25 RN 26921-17-5), bisoprolol (2-Propanol, 1-[4-[[2-(1-methylethoxy)ethoxy]-methyl]phenoxy]-3-[(1-methylethyl)amino]-, ( $\pm$ ), CAS RN 66722-44-9), bisoprolol fumarate (such as ( $\pm$ )-1-[4-[[2-(1-Methylethoxy) ethoxy]methyl]phenoxy]-3-[(1-

methylethylamino]-2-propanol (E) -2-butenedioate (2:1) (salt), e.g., Zebeta™, Lederle  
 Consumer), nebivolol (2H-1-Benzopyran-2-methanol,  $\alpha\alpha'$ -[iminobis(methylene)]bis[6-  
 fluoro-3,4-dihydro-, CAS RN 99200-09-6 see also U.S. Pat. No. 4,654,362), cicloprolol  
 hydrochloride, such 2-Propanol, 1-[4-[2-(cyclopropylmethoxy)ethoxy]phenoxy]-3-[1-  
 5 methylethylamino]-, hydrochloride, A.A.S. RN 63686-79-3), dexpropranolol  
 hydrochloride (2-Propanol, 1-[1-methylethylamino]-3-(1-naphthalenyloxy)-  
 hydrochloride (CAS RN 13071-11-9), diacetolol hydrochloride (Acetamide, N-[3-acetyl-  
 4-[2-hydroxy-3-[(1-methyl-ethyl)amino]propoxy][phenyl]-, monohydrochloride CAS RN  
 69796-04-9), dilevalol hydrochloride (Benzamide, 2-hydroxy-5-[1-hydroxy-2-[1-methyl-  
 10 3-phenylpropyl)amino]ethyl]-, monohydrochloride, CAS RN 75659-08-4), exaprolol  
 hydrochloride (2-Propanol, 1-(2-cyclohexylphenoxy)-3-[(1-methylethyl)amino]-,  
 hydrochloride CAS RN 59333-90-3), fleistolol sulfate (Benzoic acid, 2-fluro-, 3-[[2-  
 [aminocarbonyl)amino]-dimethylethyl]amino]-2-hydroxypropyl ester, ( $\pm$ )- sulfate (1:1)  
 (salt), CAS RN 88844-73-9; metalol hydrochloride (Methanesulfonamide, N-[4-[1-  
 15 hydroxy-2-(methylamino)propyl]phenyl]-, monohydrochloride CAS RN 7701-65-7),  
 metoprolol 2-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[1-methylethylamino]-; CAS  
 RN 37350-58-6), metoprolol tartrate (such as 2-Propanol, 1-[4-(2-  
 methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, e.g., Lopressor®, Novartis),  
 pamatolol sulfate (Carbamic acid, [2-[4-[2-hydroxy-3-[(1-  
 20 methylethyl)amino]propoxyl]phenyl]-ethyl]-, methyl ester, ( $\pm$ ) sulfate (salt) (2:1), CAS  
 RN 59954-01-7), penbutolol sulfate (2-Propanol, 1-(2-cyclopentylphenoxy)-3-[1,1-  
 dimethylethylamino]1, (S)-, sulfate (2:1) (salt), CAS RN 38363-32-5), practolol  
 (Acetamide, N-[4-[2-hydroxy-3-[(1-methylethyl)amino]-propoxy]phenyl]-, CAS RN  
 6673-35-4;) tiprenolol hydrochloride (Propanol, 1-[(1-methylethyl)amino]-3-[2-  
 25 (methylthio)-phenoxy]-, hydrochloride, ( $\pm$ ), CAS RN 39832-43-4), tolamolol  
 (Benzamide, 4-[2-[[2-hydroxy-3-(2-methylphenoxy)-propyl]amino]ethoxyl]-, CAS RN  
 38103-61-6), bopindolol, indenolol, pindolol, propanolol, tertatolol, and tilisolol, and the  
 like; calcium channel blockers such as besylate salt of amlodipine (such as 3-ethyl-5-  
 methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-

pyridinedicarboxylate benzenesulphonate, e.g., Norvasc®, Pfizer), clentiazem maleate (1,5-Benzothiazepin-4(5H)-one, 3-(acetyloxy)-8-chloro-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-(2S-cis)-, (Z)-2-butenedioate (1:1), see also US4567195), isradipine (3,5-Pyridinedicarboxylic acid, 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-, methyl 1-methylethyl ester, (±)-4(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate, see also US4466972); nimodipine (such as isopropyl (2-methoxyethyl) 1, 4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylate, e.g. Nimotop®, Bayer), felodipine (such as ethyl methyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate-, e.g. Plendil® Extended-Release, AstraZeneca LP), nilvadipine (3,5-Pyridinedicarboxylic acid, 2-cyano-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-3-methyl 5-(1-methylethyl) ester, also see US3799934), nifedipine (such as 3,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester, e.g., Procardia XL® Extended Release Tablets, Pfizer), diltiazem hydrochloride (such as 1,5-Benzothiazepin-4(5H)-one, 3-(acetyloxy)-5[2-(dimethylamino)ethyl]-2,3-dihydro-2(4-methoxyphenyl)-, monohydrochloride, (+)-cis-, e.g., Tiazac®, Forest), verapamil hydrochloride (such as benzeneacetonitrile, (alpha)-[[3-[[2-(3,4-dimethoxyphenyl) ethyl]methylamino]propyl]-3,4-dimethoxy-(alpha)-(1-methylethyl) hydrochloride, e.g., Isoptin® SR, Knoll Labs), teludipine hydrochloride (3,5-Pyridinedicarboxylic acid, 2-[(dimethylamino)methyl]4-[2-[(1E)-3-(1,1-dimethylethoxy)-3-oxo-1-propenyl]phenyl]-1,4-dihydro-6-methyl-, diethyl ester, monohydrochloride) CAS RN 108700-03-4), belfosdil (Phosphonic acid, [2-(2-phenoxyethyl)-1,3-propane-diyl]bis-, tetrabutyl ester CAS RN 103486-79-9), fostedil (Phosphonic acid, [[4-(2-benzothiazolyl)phenyl]methyl]-, diethyl ester CAS RN 75889-62-2), aranidipine, azelnidipine, barnidipine, benidipine, bepridil, cinaldipine, clevidipine, efonidipine, gallopamil, lacidipine, lemildipine, lercanidipine, monatepil maleate (1-Piperazinebutanamide, N-(6,11-dihydrodibenzo(b,e)thiepin-11-yl)<sub>4</sub>-(4-fluorophenyl)-, (±)-, (Z)-2-butenedioate (1:1) (±)-N-(6,11-Dihydrodibenzo(b,e)thiepin-11-yl)-4-(p-fluorophenyl)-1-piperazinebutyramide maleate (1:1) CAS RN 132046-06-1), nicardipine, nisoldipine, nitrendipine, manidipine, pranidipine, and the like;



T-channel calcium antagonists such as mibefradil; angiotensin converting enzyme (ACE) inhibitors such as benazepril, benazepril hydrochloride (such as 3-[[1-(ethoxycarbonyl)-3-phenyl-(1S)-propyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H -1-(3S)-benzazepine-1-acetic acid monohydrochloride, e.g., Lotrel®, Novartis), captopril (such as 1-[(2S)-3-mercapto-  
5 2-methylpropionyl]-L-proline, e.g., Captopril, Mylan, CAS RN 62571-86-2 and others disclosed in US4046889), ceranapril (and others disclosed in US4452790), cetapril (alacepril, Dainippon disclosed in Eur. Therap. Res. 39:671 (1986); 40:543 (1986)), cilazapril (Hoffman-LaRoche) disclosed in J. Cardiovasc. Pharmacol. 9:39 (1987), indalapril (delapril hydrochloride (2H-1,2,4-Benzothiadiazine-7-sulfonamide, 3-  
10 bicyclo[2.2.1]hept-5-en-2-yl-6-chloro-3,4-dihydro-, 1,1-dioxide CAS RN 2259-96-3); disclosed in US4385051), enalapril (and others disclosed in US4374829), enalapril, enalaprilat, fosinopril, ((such as L-proline, 4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy)propoxy](4-phenylbutyl) phosphinyl]acetyl]-, sodium salt, trans—, e.g., Monopril, Bristol-Myers Squibb and others disclosed in US4168267), fosinopril sodium (L-Proline,  
15 4-cyclohexyl-1-[[[R)-[(1S)-2-methyl-1-(1-oxopropoxy)propox), imidapril, indolapril (Schering, disclosed in J. Cardiovasc. Pharmacol. 5:643, 655 (1983)), lisinopril (Merck), losinopril, moexipril, moexipril hydrochloride (3-Isoquinolinecarboxylic acid, 2-[(2S)-2-[[[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,- 2,3,4-tetrahydro-6,7-dimethoxy-, monohydrochloride, (3S)- CAS RN 82586-52-5), quinapril, quinaprilat,  
20 ramipril (Hoechst) disclosed in EP 79022 and Curr. Ther. Res. 40:74 (1986), perindopril erbumine (such as 2S,3aS,7aS-1-[(S)-N-[(S)-1-Carboxybutyl]alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1), e.g., Aceon®, Solvay), perindopril (Servier, disclosed in Eur. J. clin. Pharmacol. 31:519 (1987)), quanipril (disclosed in US4344949), spirapril (Schering, disclosed in Acta.  
25 Pharmacol. Toxicol. 59 (Supp. 5):173 (1986)), tenocapril, trandolapril, zofenopril (and others disclosed in US4316906), rentiapril (fentiapril, disclosed in Clin. Exp. Pharmacol. Physiol. 10:131 (1983)), pivopril, YS980, teprotide (Bradykinin potentiator BPP9a CAS RN 35115-60-7), BRL 36,378 (Smith Kline Beecham, see EP80822 and EP60668), MC-838 (Chugai, see C.A. 102:72588v and Jap. J. Pharmacol. 40:373 (1986), CGS 14824

(Ciba-Geigy, 3-([1-ethoxycarbonyl-3-phenyl-(1S)-propyl]amino)-2,3,4,5-tetrahydro-2-oxo-*o*-1-(3S)-benzazepine-1 acetic acid HCl, see U.K. Patent No. 2103614), CGS 16,617 (Ciba-Geigy, 3(S)-[[[(1S)-5-amino-1-carboxypentyl]amino]-2,3,4,- 5-tetrahydro-2-oxo-1H-1-benzazepine-1-ethanoic acid, see US4473575), Ru 44570 (Hoechst, see  
5 Arzneimittelforschung 34:1254 (1985)), R 31-2201 (Hoffman-LaRoche see FEBS Lett. 165:201 (1984)), CI925 (Pharmacologist 26:243, 266 (1984)), WY-44221 (Wyeth, see J. Med. Chem. 26:394 (1983)), and those disclosed in US2003006922 (paragraph 28), US4337201, US4432971 (phosphoramidates); neutral endopeptidase inhibitors such as omapatrilat (Vanlev®), CGS 30440, cadoxatril and ecadotril, fasidotril (also known as  
10 aladotril or alatriopril), sampatrilat, mixanpril, and gemopatrilat, AVE7688, ER4030, and those disclosed in US5362727, US5366973, US5225401, US4722810, US5223516, US4749688, US5552397, US5504080, US5612359, US5525723, EP0599444, EP0481522, EP0599444, EP0595610, EP0534363, EP534396, EP534492, EP0629627; endothelin antagonists such as tezosentan, A308165, and YM62899, and the like;  
15 vasodilators such as hydralazine (apresoline), clonidine (clonidine hydrochloride (1H-Imidazol-2-amine, N-(2,6-dichlorophenyl)4,5-dihydro-, monohydrochloride CAS RN 4205-91-8), catapres, minoxidil (loniten), nicotiny alcohol (roniacol), diltiazem hydrochloride (such as 1,5-Benzothiazepin-4(5H)-one,3-(acetyloxy)-5[2-(dimethylamino)ethyl]-2,-3-dihydro-2(4-methoxyphenyl)-, monohydrochloride, (+)-cis,  
20 e.g., Tiazac®, Forest), isosorbide dinitrate (such as 1,4:3,6-dianhydro-D-glucitol 2,5-dinitrate e.g., Isordil® Titradose®, Wyeth-Ayerst), sosorbide mononitrate (such as 1,4:3,6-dianhydro-D-glucito- 1,5-nitrate, an organic nitrate, e.g., Ismo®, Wyeth-Ayerst), nitroglycerin (such as 2,3 propanetriol trinitrate, e.g., Nitrostat® Parke-Davis), verapamil hydrochloride (such as benzeneacetonitrile, (±)-(alpha)[3-[[2-(3,4  
25 dimethoxyphenyl)ethyl]methylamino]propyl]-3,4-dimethoxy-(alpha)- (1-methylethyl) hydrochloride, e.g., Covera HS® Extended-Release, Searle), chromonar (which may be prepared as disclosed in US3282938), clonitate (Annalen 1870 155), droprenilamine (which may be prepared as disclosed in DE2521113), lidoflazine (which may be prepared as disclosed in US3267104); prenylamine (which may be prepared as disclosed in

US3152173), propatyl nitrate (which may be prepared as disclosed in French Patent No. 1,103,113), mioflazine hydrochloride (1-Piperazineacetamide, 3-(aminocarbonyl)<sub>4</sub>-[4,4-bis(4-fluorophenyl)butyl]-N-(2,6-dichlorophenyl)-, dihydrochloride CAS RN 83898-67-3), mixidine (Benzeneethanamine, 3,4-dimethoxy-N-(1-methyl-2-pyrrolidinylidene)-

5 Pyrrolidine, 2-[(3,4-dimethoxyphenethyl)imino]-1-methyl-1-Methyl-2-[(3,4-dimethoxyphenethyl)imino]pyrrolidine CAS RN 27737-38-8), molsidomine (1,2,3-Oxadiazolium, 5-[(ethoxycarbonyl)amino]-3-(4-morpholinyl)-, inner salt CAS RN 25717-80-0), isosorbide mononitrate (D-Glucitol, 1,4:3,6-dianhydro-, 5-nitrate CAS RN 16051-77-7), erythrityl tetranitrate (1,2,3,4-Butanetetrol, tetranitrate, (2R,3S)-rel-CAS

10 RN 7297-25-8), clonitrate(1,2-Propanediol, 3-chloro-, dinitrate (7CI, 8CI, 9CI) CAS RN 2612-33-1), dipyridamole Ethanol, 2,2',2'',2'''-[(4,8-di-1-piperidinylpyrimido[5,4-d]pyrimidine-2,6-diyl)dinitrilo]tetrakis- CAS RN 58-32-2), nicorandil (CAS RN 65141-46-0 3-), pyridinecarboxamide (N-[2-(nitrooxy)ethyl]-Nisoldipine<sub>3,5</sub>-

Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, methyl 2-

15 methylpropyl ester CAS RN 63675-72-9), nifedipine<sub>3,5</sub>-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester CAS RN 21829-25-4),

perhexiline maleate (Piperidine, 2-(2,2-dicyclohexylethyl)-, (2Z)-2-butenedioate (1:1) CAS RN 6724-53-4), oxprenolol hydrochloride (2-Propanol, 1-[(1-methylethyl)amino]-3-

[2-(2-propenyloxy)phenoxy]-, hydrochloride CAS RN 6452-73-9), pentrinitrol (1,3-

20 Propanediol, 2,2-bis[(nitrooxy)methyl]-, mononitrate (ester) CAS RN 1607-17-6),

verapamil (Benzeneacetonitrile,  $\alpha$ -[3-[[2-(3,4-dimethoxyphenyl)ethyl]-methylamino]propyl]-3,4-dimethoxy- $\alpha$ -(1-methylethyl)- CAS RN 52-53-9) and the like;

angiotensin II receptor antagonists such as, aprosartan, zolasartan, olmesartan, prazosartan, FI6828K, RNH6270, candesartan (1 H-Benzimidazole-7-carboxylic acid, 2-

25 ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]4-yl]methyl]- CAS RN 139481-59-7),

candesartan cilexetil ((+/-)-1-(cyclohexylcarbonyloxy)ethyl-2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]-1H-benzimidazole carboxylate, CAS RN 145040-37-5, US5703110 and US5196444), eprosartan (3-[1-4-carboxyphenylmethyl]-2-n-butyl-imidazol-5-yl)-(2-thienylmethyl) propenoic acid, US5185351 and US5650650), irbesartan (2-n-butyl-3-

[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]1,3-diazaspiro[4,4]non-1-en-4-one,  
 US5270317 and US5352788), losartan (2-N-butyl-4-chloro-5-hydroxymethyl-1-[(2'-(1H-  
 tetrazol-5-yl)biphenyl-4-yl)-methyl]imidazole, potassium salt, US5138069, US5153197  
 and US5128355), tasosartan (5,8-dihydro-2,4-dimethyl-8-[(2'-(1H-tetrazol-5-yl)[1,1'-  
 5 biphenyl]4-yl)methyl]-pyrido[2,3-d]pyrimidin-7(6H)-one, US5149699), telmisartan (4'-  
 [(1,4-dimethyl-2'-propyl-(2,6'-bi-1H-benzimidazol)-1'-yl)]-[1,1'-biphenyl]-2-carboxylic  
 acid, CAS RN 144701-48-4, US5591762), milfasartan, abitesartan, valsartan (Diovan®  
 (Novartis), (S)-N-valeryl-N-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]valine,  
 US5399578), EXP-3137 (2-N-butyl-4-chloro-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-  
 10 methyl]imidazole-5-carboxylic acid, US5138069, US5153197 and US5128355), 3-(2'-  
 (tetrazol-5-yl)-1,1'-biphen-4-yl)methyl-5,7-dimethyl-2-ethyl-3H-imidazo[4,5-b]pyridine,  
 4'[[2-ethyl-4-methyl-6-(5,6,7,8-tetrahydroimidazo[1,2-a]pyridin-2-yl)-benzimidazol-1-  
 yl]-methyl]-1,1'-biphenyl]-2-carboxylic acid, 2-butyl-6-(1-methoxy-1-methylethyl)-2-  
 [[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]guinazolin-4(3H)-one, 3-[2'-carboxybiphenyl-  
 15 4-yl)methyl]-2-cyclopropyl-7-methyl-3H-imidazo[4,5-b]pyridine, 2-butyl-4-chloro-1-  
 [(2'-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-carboxylic acid, 2-butyl-4-chloro-1-  
 [[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl)methyl]-1H-imidazole-5-carboxylic acid-1-  
 (ethoxycarbonyloxy)ethyl ester potassium salt, dipotassium 2-butyl-4-(methylthio)-1-  
 [[2-[[[(propylamino)carbonyl]amino]-sulfonyl](1,1'-biphenyl)-4-yl)methyl]-1H-  
 20 imidazole-5-carboxylate, methyl-2-[[4-butyl-2-methyl-6-oxo-5-[[2'-(1H-tetrazol-5-yl)-  
 [1,1'-biphenyl]-4-yl)methyl]-1-(6H)-pyrimidinyl)methyl]-3-thiophencarboxylate, 5-[(3,5-  
 dibutyl-1H-1,2,4-triazol-1-yl)methyl]-2-[2-(1H-tetrazol-5-ylphenyl)]pyridine, 6-butyl-2-  
 (2-phenylethyl)-5[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-methyl]pyrimidin-4-(3H)-one  
 D,L lysine salt, 5-methyl-7-n-propyl-8-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-  
 25 [1,2,4]-triazolo[1,5-c]pyrimidin-2(3H)-one, 2,7-diethyl-5-[[2'-(5-tetrazolyl)biphenyl-4-  
 yl)methyl]-5H-pyrazolo[1,5-b][1,2,4]triazole potassium salt, 2-[2-butyl-4,5-dihydro-4-  
 oxo-3-[2'-(1H-tetrazol-5-yl)-4-biphenylmethyl]-3H-imidazol[4,5-c]pyridine-5-  
 ylmethyl]benzoic acid, ethyl ester, potassium salt, 3-methoxy-2,6-dimethyl-4-[[2'-(1H-  
 tetrazol-5-yl)-1,1'-biphenyl-4-yl]methoxy]pyridine, 2-ethoxy-1-[[2'-(5-oxo-2,5-dihydro-

1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-1H-benzimidazole-7-carboxylic acid, 1-[N-(2'-(1H-tetrazol-5-yl)biphenyl-4-yl-methyl)-N-valerolylaminomethyl)cyclopentane-1-carboxylic acid, 7-methyl-2n-propyl-3-[[2'1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-3H-imidazo[4,5-6]pyridine, 2-[5-[(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridine-3-yl)methyl]-2-quinolinyl]sodium benzoate, 2-butyl-6-chloro-4-hydroxymethyl-5-methyl-3-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]pyridine, 2-[[[2-butyl-1-(4-carboxyphenyl)methyl]-1H-imidazol-5-yl)methyl]amino]benzoic acid tetrazol-5-yl)biphenyl-4-yl)methyl]pyrimidin-6-one, 4(S)-[4-(carboxymethyl)phenoxy]-N-[2(R)-[4-(2-sulfobenzamido)imidazol-1-yl]octanoyl]-L-proline, 1-(2,6-dimethylphenyl)-4-butyl-1,3-dihydro-3-[[6-[2-(1H-tetrazol-5-yl)phenyl]-3-pyridinyl)methyl]-2H-imidazol-2-one, 5,8-ethano-5,8-dimethyl-2-n-propyl-5,6,7,8-tetrahydro-1-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-1H,4H-1,3,4a,8a-tetrazacyclopentanaphthalene-9-one, 4-[1-[2'-(1,2,3,4-tetrazol-5-yl)biphen-4-yl)methylamino]-5,6,7,8-tetrahydro-2-triflylquinazoline, 2-(2-chlorobenzoyl)imino-5-ethyl-3-[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-1,3,4-thiadiazoline, 2-[5-ethyl-3-[2-(1H-tetrazole-5-yl)biphenyl-4-yl)methyl]-1,3,4-thiazoline-2-ylidene]aminocarbonyl-1-cyclopentencarboxylic acid dipotassium salt, and 2-butyl-4-[N-methyl-N-(3-methylcrotonoyl)amino]-1-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-1H-imidazole-5-carboxylic acid 1-ethoxycarbonyloxyethyl ester, those disclosed in patent publications EP475206, EP497150, EP539086, EP539713, EP535463, EP535465, EP542059, EP497121, EP535420, EP407342, EP415886, EP424317, EP435827, EP433983, EP475898, EP490820, EP528762, EP324377, EP323841, EP420237, EP500297, EP426021, EP480204, EP429257, EP430709, EP434249, EP446062, EP505954, EP524217, EP514197, EP514198, EP514193, EP514192, EP450566, EP468372, EP485929, EP503162, EP533058, EP467207 EP399731, EP399732, EP412848, EP453210, EP456442, EP470794, EP470795, EP495626, EP495627, EP499414, EP499416, EP499415, EP511791, EP516392, EP520723, EP520724, EP539066, EP438869, EP505893, EP530702, EP400835, EP400974, EP401030, EP407102, EP411766, EP409332, EP412594, EP419048, EP480659, EP481614, EP490587, EP467715, EP479479, EP502725, EP503838, EP505098, EP505111

EP513,979 EP507594, EP510812, EP511767, EP512675, EP512676, EP512870,  
EP517357, EP537937, EP534706, EP527534, EP540356, EP461040, EP540039,  
EP465368, EP498723, EP498722, EP498721, EP515265, EP503785, EP501892,  
EP519831, EP532410, EP498361, EP432737, EP504888, EP508393, EP508445,  
5 EP403159, EP403158, EP425211, EP427463, EP437103, EP481448, EP488532,  
EP501269, EP500409, EP540400, EP005528, EP028834, EP028833, EP411507,  
EP425921, EP430300, EP434038, EP442473, EP443568, EP445811, EP459136,  
EP483683, EP518033, EP520423, EP531876, EP531874, EP392317, EP468470,  
EP470543, EP502314, EP529253, EP543263, EP540209, EP449699, EP465323,  
10 EP521768, EP415594, WO92/14468, WO93/08171, WO93/08169, WO91/00277,  
WO91/00281, WO91/14367, WO92/00067, WO92/00977, WO92/20342, WO93/04045,  
WO93/04046, WO91/15206, WO92/14714, WO92/09600, WO92/16552, WO93/05025,  
WO93/03018, WO91/07404, WO92/02508, WO92/13853, WO91/19697, WO91/11909,  
WO91/12001, WO91/11999, WO91/15209, WO91/15479, WO92/20687, WO92/20662,  
15 WO92/20661, WO93/01177, WO91/14679, WO91/13063, WO92/13564, WO91/17148,  
WO91/18888, WO91/19715, WO92/02257, WO92/04335, WO92/05161, WO92/07852,  
WO92/15577, WO93/03033, WO91/16313, WO92/00068, WO92/02510, WO92/09278,  
WO9210179, WO92/10180, WO92/10186, WO92/10181, WO92/10097, WO92/10183,  
WO92/10182, WO92/10187, WO92/10184, WO92/10188, WO92/10180, WO92/10185,  
20 WO92/20651, WO93/03722, WO93/06828, WO93/03040, WO92/19211, WO92/22533,  
WO92/06081, WO92/05784, WO93/00341, WO92/04343, WO92/04059, US5104877,  
US5187168, US5149699, US5185340, US4880804, US5138069, US4916129,  
US5153197, US5173494, US5137906, US5155126, US5140037, US5137902,  
US5157026, US5053329, US5132216, US5057522, US5066586, US5089626,  
25 US5049565, US5087702, US5124335, US5102880, US5128327, US5151435,  
US5202322, US5187159, US5198438, US5182288, US5036048, US5140036,  
US5087634, US5196537, US5153347, US5191086, US5190942, US5177097,  
US5212177, US5208234, US5208235, US5212195, US5130439, US5045540,  
US5041152, and US5210204, and pharmaceutically acceptable salts and esters thereof;

$\alpha/\beta$  adrenergic blockers such as nipradilol, arotinolol, amosulalol, bretylium tosylate (CAS RN: 61-75-6), dihydroergtamine mesylate (such as ergotaman-3', 6', 18-trione, 9, 10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'( $\alpha$ ))-, monomethanesulfonate, e.g., DHE 45® Injection, Novartis), carvedilol (such as ( $\pm$ )-1-(Carbazol-4-yloxy)-3-[[2-  
5 (o-methoxyphenoxy)ethyl]amino]-2-propanol, e.g., Coreg®, SmithKline Beecham), labetalol (such as 5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl) amino] ethyl]salicylamide monohydrochloride, e.g., Normodyne®, Schering), bretylium tosylate (Benzenemethanaminium, 2-bromo-N-ethyl-N,N-dimethyl-, salt with 4-methylbenzenesulfonic acid (1:1) CAS RN 61-75-6), phentolamine mesylate (Phenol, 3-  
10 [[(4,5-dihydro-1H-imidazol-2-yl)methyl](4-methylphenyl)amino]-, monomethanesulfonate (salt) CAS RN 65-28-1), solypertine tartrate (5H-1,3-Dioxolo[4,5-f]indole, 7-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-, (2R,3R)-2,3-dihydroxybutanedioate (1:1) CAS RN 5591-43-5), zolertine hydrochloride (Piperazine, 1-phenyl-4-[2-(1H-tetrazol-5-yl)ethyl]-, monohydrochloride (8Cl, 9Cl) CAS RN 7241-94-3)  
15 and the like;  
 $\alpha$  adrenergic receptor blockers, such as alfuzosin (CAS RN: 81403-68-1), terazosin, urapidil, prazosin (Minipress®), tamsulosin, bunazosin, trimazosin, doxazosin, naftopidil, indoramin, WHP 164, XEN010, fenspiride hydrochloride (which may be prepared as disclosed in US3399192), proroxxan (CAS RN 33743-96-3), and labetalol hydrochloride  
20 and combinations thereof;  $\alpha$  2 agonists such as methyldopa, methyldopa HCL, lofexidine, tiamenidine, moxonidine, rilmenidine, guanobenz, and the like;  
aldosterone inhibitors, and the like; renin inhibitors including Aliskiren (SPP100; Novartis/Speedel); angiotensin-2-binding agents such as those disclosed in WO03/030833;  
25 anti-angina agents such as ranolazine (hydrochloride 1-Piperazineacetamide, N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-, dihydrochloride CAS RN 95635-56-6), betaxolol hydrochloride (2-Propanol, 1-[4-[2 (cyclopropylmethoxy)ethyl]phenoxy]-3-[(1-methylethyl)amino]-, hydrochloride CAS RN 63659-19-8), butoprozine hydrochloride (Methanone, [4-

[3(dibutylamino)propoxy]phenyl](2-ethyl-3-indoliziny)-, monohydrochloride CAS RN 62134-34-3), cinepazet maleate-1-Piperazineacetic acid, 4-[1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]-, ethyl ester, (2Z)-2-butenedioate (1:1) CAS RN 50679-07-7), tosisfen (Benzenesulfonamide, 4-methyl-N-[[[(1S)-1-methyl-2-phenylethyl]amino]carbonyl]- CAS RN 32295-184), verapamilhydrochloride (Benzeneacetonitrile,  $\alpha$ -[3-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]propyl]-3,4-dimethoxy- $\alpha$ -(1-methylethyl)-, monohydrochloride CAS RN 152-114), molsidomine (1,2,3-Oxadiazolium, 5-[(ethoxycarbonyl)amino]-3-(4-morpholinyl)-, inner salt CAS RN 25717-80-0), and ranolazine hydrochloride (1-Piperazineacetamide, N-(2,6-dimethylphenyl)<sub>4</sub>-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-, dihydrochloride CAS RN 95635-56-6); tosisfen (Benzenesulfonamide, 4-methyl-N-[[[(1S)-1-methyl-2-phenylethyl]amino]carbonyl]- CAS RN 32295-184); adrenergic stimulants such as guanfacine hydrochloride (such as N-amidino-2-(2,6-dichlorophenyl) acetamide hydrochloride, e.g., Tenex® Tablets available from Robins); methyl dopa hydrochlorothiazide (such as levo-3-(3,4-dihydroxyphenyl)-2-methylalanine) combined with Hydrochlorothiazide (such as 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, e.g., the combination as, e.g., Aldoril® Tablets available from Merck), methyl dopa-chlorothiazide (such as 6-chloro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide and methyl dopa as described above, e.g., Aldoclor®, Merck), clonidine hydrochloride (such as 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride and chlorthalidone (such as 2-chloro-5-(1-hydroxy-3-oxo-1-isoindoliny) benzenesulfonamide), e.g., Combipres®, Boehringer Ingelheim), clonidine hydrochloride (such as 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, e.g., Catapres®, Boehringer Ingelheim), clonidine (1H-Imidazol-2-amine, N-(2,6-dichlorophenyl)4,5-dihydro-CAS RN 4205-90-7), Hyzaar (Merck; a combination of losartan and hydrochlorothiazide), Co-Diovan (Novartis; a combination of valsartan and hydrochlorothiazide), Lotrel (Novartis; a combination of benazepril and amlodipine) and Caduet (Pfizer; a combination of amlodipine and atorvastatin), and those agents disclosed in US20030069221.



The peptides and agonists described herein can be used in combination therapy with one or more of the following agents useful in the treatment of respiratory and other disorders including but not limited to:

- 5 (1)  $\beta$ -agonists including but not limited to: albuterol (PROVENTIL®, SALBUTAMOL®, VENTOLIN®), bambuterol, bitoterol, clenbuterol, fenoterol, formoterol, isoetharine (BRONKOSOL®, BRONKOMETER®), metaproterenol (ALUPENT®, METAPREL®), pirbuterol (MAXAIR®), reproterol, rimiterol, salmeterol, terbutaline (BRETHAIRE®, BRETHINE®, BRICANYL®), adrenalin, 10 isoproterenol (ISUPREL®), epinephrine bitartrate (PRIMATENE®), ephedrine, orciprenline, fenoterol and isoetharine;
- (2) steroids, including but not limited to beclomethasone, beclomethasone dipropionate, betamethasone, budesonide, budesonide, butixocort, dexamethasone, flunisolide, fluocortin, fluticasone, hydrocortisone, methyl prednisone, mometasone, 15 predonisolone, predonisone, tipredane, tixocortal, triamcinolone, and triamcinolone acetone;
- (3)  $\beta$ 2-agonist-corticosteroid combinations [e.g., salmeterol-fluticasone (ADVAIR®), formoterol-budesonid (SYMBICORT®)];
- (4) leukotriene D4 receptor antagonists/leukotriene antagonists/LTD4 20 antagonists (i.e., any compound that is capable of blocking, inhibiting, reducing or otherwise interrupting the interaction between leukotrienes and the Cys LTI receptor) including but not limited to: zafirlukast, montelukast, montelukast sodium (SINGULAIR®), pranlukast, iralukast, pobilukast, SKB-106,203 and compounds described as having LTD4 antagonizing activity described in U.S. Patent No. 5,565,473;
- 25 (5) 5-lipoxygenase inhibitors and/or leukotriene biosynthesis inhibitors [e.g., zileuton and BAY1005 (CA registry 128253-31-6)];
- (6) histamine H1 receptor antagonists/antihistamines (i.e., any compound that is capable of blocking, inhibiting, reducing or otherwise interrupting the interaction between histamine and its receptor) including but not limited to: astemizole, acrivastine,

antazoline, azatadine, azelastine, astemizole, brompheniramine, brompheniramine maleate, carbinoxamine, carebastine, cetirizine, chlorpheniramine, chlorpheniramine maleate, cimetidine, clemastine, cyclizine, cyproheptadine, descarboethoxyloratadine, dexchlorpheniramine, dimethindene, diphenhydramine, diphenylpyraline, doxylamine succinate, doxylamine, ebastine, efletirizine, epinastine, farnotidine, fexofenadine, hydroxyzine, hydroxyzine, ketotifen, levocabastine, levocetirizine, levocetirizine, loratadine, meclizine, mepyramine, mequitazine, methdilazine, mianserin, mizolastine, noberastine, norastemizole, noraztemizole, phenindamine, pheniramine, picumast, promethazine, pynlamine, pyrilamine, ranitidine, temelastine, terfenadine, trimeprazine, tripelenamine, and triprolidine;

(7) an anticholinergic including but not limited to: atropine, benztropine, biperiden, flutropium, hyoscyamine (e.g. Levsin®; Levbid®; Levsin/SL®, Anaspaz®, Levsinex timecaps®, NuLev®), ilutropium, ipratropium, ipratropium bromide, methscopolamine, oxybutinin, rispenzepine, scopolamine, and tiotropium;

(8) an anti-tussive including but not limited to: dextromethorphan, codeine, and hydromorphone;

(9) a decongestant including but not limited to: pseudocphedrine and phenylpropanolamine;

(10) an expectorant including but not limited to: guaifenesin, guaicolsulfate, terpin, ammonium chloride, glycerol guaicolate, and iodinated glycerol;

(11) a bronchodilator including but not limited to: theophylline and aminophylline;

(12) an anti-inflammatory including but not limited to: fluribiprofen, diclophenac, indomethacin, ketoprofen, S-ketroprophen, tenoxicam;

(13) a PDE (phosphodiesterase) inhibitor including but not limited to those disclosed herein;

(14) a recombinant humanized monoclonal antibody [e.g. xolair (also called omalizumab), rhuMab, and talizumab];

(15) a humanized lung surfactant including recombinant forms of surfactant proteins SP-B, SP-C or SP-D [e.g. SURFAXIN®, formerly known as dsc-104 (Discovery Laboratories)],

(16) agents that inhibit epithelial sodium channels (ENaC) such as amiloride  
5 and related compounds;

(17) antimicrobial agents used to treat pulmonary infections such as acyclovir, amikacin, amoxicillin, doxycycline, trimethoprin sulfamethoxazole, amphotericin B, azithromycin, clarithromycin, roxithromycin, clarithromycin, cephalosporins( ceffoxitin, cefmetazole etc), ciprofloxacin, ethambutol, gentimycin, ganciclovir, imipenem,  
10 isoniazid, itraconazole, penicillin, ribavirin, rifampin, rifabutin, amantadine, rimantidine, streptomycin, tobramycin, and vancomycin;

(18) agents that activate chloride secretion through Ca<sup>++</sup> dependent chloride channels (such as purinergic receptor (P2Y(2) agonists);

(19) agents that decrease sputum viscosity, such as human recombinant DNase  
15 1, (Pulmozyme®);

(20) nonsteroidal anti-inflammatory agents (acemetacin, acetaminophen, acetyl salicylic acid, alclofenac, alminoprofen, apazone, aspirin, benoxaprofen, bezpiperylon, bucloxic acid, carprofen, clidanac, diclofenac, diclofenac, diflunisal, diflusinal, etodolac, fenbufen, fenbufen, fenclofenac, fenclozic acid, fenoprofen, fentiazac, feprazone,  
20 flufenamic acid, flufenisal, flufenisal, fluprofen, flurbiprofen, flurbiprofen, furofenac, ibufenac, ibuprofen, indomethacin, indomethacin, indoprofen, isoxepac, isoxicam, ketoprofen, ketoprofen, ketorolac, meclofenamic acid, meclofenamic acid, mefenamic acid, mefenamic acid, miroprofen, mofebutazone, nabumetone oxaprozin, naproxen, naproxen, niflumic acid , oxaprozin, oxpinac, oxyphenbutazone, phenacetin,  
25 phenylbutazone, phenylbutazone, piroxicam, piroxicam, pirprofen, pranoprofen, sudoxicam, tenoxicam , sulfasalazine, sulindac, sulindac, suprofen, tiaprofenic acid, tiopinac, tiroxaprofen, tolfenamic acid, tolmetin, tolmetin, zidometacin, zomepirac, and zomepirac); and

(21) aerosolized antioxidant therapeutics such as S-Nitrosoglutathione.

The peptides and agonists described herein can be used in combination therapy with an anti-obesity agent. Suitable such agents include, but are not limited to:

- 11 $\beta$  HSD-1 (11-beta hydroxy steroid dehydrogenase type 1) inhibitors, such as BVT  
5 3498, BVT 2733, 3-(1-adamantyl)-4-ethyl-5-(ethylthio)-4H-1,2,4-triazole, 3-(1-  
adamantyl)-5-(3,4,5-trimethoxyphenyl)-4-methyl-4H-1,2,4-triazole, 3- adamantanyl-  
4,5,6,7,8,9,10,11,12,3a-decahydro-1,2,4-triazolo[4,3-a][1,1]annulene, and those  
compounds disclosed in WO01/90091, WO01/90090, WO01/90092 and WO02/072084;
- 5HT antagonists such as those in WO03/037871, WO03/037887, and the like;
- 10 5HT1a modulators such as carbidopa, benserazide and those disclosed in US6207699,  
WO03/031439, and the like;
- 5HT2c (serotonin receptor 2c) agonists, such as BVT933, DPCA37215, IK264, PNU  
22394, WAY161503, R-1065, SB 243213 (Glaxo Smith Kline) and YM 348 and those  
disclosed in US3914250, WO00/77010, WO02/36596, WO02/48124, WO02/10169,  
15 WO01/66548, WO02/44152, WO02/51844, WO02/40456, and WO02/40457;
- 5HT6 receptor modulators, such as those in WO03/030901, WO03/035061,  
WO03/039547, and the like;
- acyl-estrogens, such as oleoyl-estrone, disclosed in del Mar-Grasa, M. et al., Obesity  
Research, 9:202-9 (2001) and Japanese Patent Application No. JP 2000256190;
- 20 anorectic bicyclic compounds such as 1426 (Aventis) and 1954 (Aventis), and the  
compounds disclosed in WO00/18749, WO01/32638, WO01/62746, WO01/62747, and  
WO03/015769;

CB 1 (cannabinoid-1 receptor) antagonist/inverse agonists such as rimonabant (Acomplia; Sanofi), SR-147778 (Sanofi), SR-141716 (Sanofi), BAY 65-2520 (Bayer), and SLV 319 (Solvay), and those disclosed in patent publications US4973587, US5013837, US5081122, US5112820, US5292736, US5532237, US5624941, 5 US6028084, US6509367, US6509367, WO96/33159, WO97/29079, WO98/31227, WO98/33765, WO98/37061, WO98/41519, WO98/43635, WO98/43636, WO99/02499, WO00/10967, WO00/10968, WO01/09120, WO01/58869, WO01/64632, WO01/64633, WO01/64634, WO01/70700, WO01/96330, WO02/076949, WO03/006007, WO03/007887, WO03/020217, WO03/026647, WO03/026648, WO03/027069, 10 WO03/027076, WO03/027114, WO03/037332, WO03/040107, WO03/086940, WO03/084943 and EP658546;

CCK-A (cholecystokinin-A) agonists, such as AR-R 15849, GI 181771 (GSK), JMV-180, A-71378, A-71623 and SR146131 (Sanofi), and those described in US5739106;

CNTF (Ciliary neurotrophic factors), such as GI-181771 (Glaxo-SmithKline), SR146131 15 (Sanofi Synthelabo), butabindide, PD170,292, and PD 149164 (Pfizer);

CNTF derivatives, such as Axokine® (Regeneron), and those disclosed in WO94/09134, WO98/22128, and WO99/43813;

dipeptidyl peptidase IV (DP-IV) inhibitors, such as isoleucine thiazolidide, valine pyrrolidide, NVP-DPP728, LAF237, P93/01, P 3298, TSL 225 (tryptophyl-1,2,3,4- 20 tetrahydroisoquinoline-3-carboxylic acid; disclosed by Yamada et al, Bioorg. & Med. Chem.Lett. 8 (1998) 1537-1540), TMC-2A/2B/2C, CD26 inhibitors, FE 999011, P9310/K364, VIP 0177, SDZ 274-444, 2-cyanopyrrolidides and 4-cyanopyrrolidides as disclosed by Ashworth et al, Bioorg. & Med. Chem. Lett., Vol. 6, No. 22, pp 1163-1166 and 2745-2748 (1996) and the compounds disclosed patent publications. WO99/38501, 25 WO99/46272, WO99/67279 (Probiodrugs), WO99/67278 (Probiodrugs), WO99/61431 (Probiodrugs), WO02/083128, WO02/062764, WO03/000180, WO03/000181, WO03/000250, WO03/002530, WO03/002531, WO03/002553, WO03/002593,

WO03/004498, WO03/004496, WO03/017936, WO03/024942, WO03/024965,  
WO03/033524, WO03/037327 and EP1258476;

5 growth hormone secretagogue receptor agonists/antagonists, such as NN703, hexarelin,  
MK-0677 (Merck), SM-130686, CP-424391 (Pfizer), LY 444,711 (Eli Lilly), L-692,429  
and L-163,255, and such as those disclosed in USSN 09/662448, US provisional  
application 60/203335, US6358951, US2002049196, US2002/022637, WO01/56592 and  
WO02/32888;

10 H3 (histamine H3) antagonist/inverse agonists, such as thioperamide, 3-(1H-imidazol-4-  
yl)propyl N-(4-pentenyl)carbamate, clobenpropit, iodophenpropit, imoproxifan, GT2394  
(Gliatech), and A331440, O-[3-(1H-imidazol-4-yl)propanol]carbamates (Kiec-  
Kononowicz, K. et al., Pharmazie, 55:349-55 (2000)), piperidine-containing histamine  
H3-receptor antagonists (Lazewska, D. et al., Pharmazie, 56:927-32 (2001),  
benzophenone derivatives and related compounds (Sasse, A. et al., Arch.  
Pharm.(Weinheim) 334:45-52 (2001)), substituted N-phenylcarbamates (Reidemeister, S.  
15 et al., Pharmazie, 55:83-6 (2000)), and proxifan derivatives (Sasse, A. et al., J. Med.  
Chem.. 43:3335-43 (2000)) and histamine H3 receptor modulators such as those  
disclosed in WO02/15905, WO03/024928 and WO03/024929;

leptin derivatives, such as those disclosed in US5552524, US5552523, US5552522,  
US5521283, WO96/23513, WO96/23514, WO96/23515, WO96/23516, WO96/23517,  
20 WO96/23518, WO96/23519, and WO96/23520;

leptin, including recombinant human leptin (PEG-OB, Hoffman La Roche) and  
recombinant methionyl human leptin (Amgen);

lipase inhibitors, such as tetrahydrolipstatin (orlistat/Xenical®), Triton WR1339,  
RHC80267, lipstatin, teasaponin, diethylumbelliferyl phosphate, FL-386, WAY-121898,  
25 Bay-N-3176, valilactone, esteracin, ebelactone A, ebelactone B, and RHC 80267, and

those disclosed in patent publications WO01/77094, US4598089, US4452813, USUS5512565, US5391571, US5602151, US4405644, US4189438, and US4242453;

lipid metabolism modulators such as maslinic acid, erythrodiol, ursolic acid uvaol, betulinic acid, betulin, and the like and compounds disclosed in WO03/011267;

- 5 Mc4r (melanocortin 4 receptor) agonists, such as CHIR86036 (Chiron), ME-10142, ME-10145, and HS-131 (Melacure), and those disclosed in PCT publication Nos. WO99/64002, WO00/74679, WO01/991752, WO01/25192, WO01/52880, WO01/74844, WO01/70708, WO01/70337, WO01/91752, WO02/059095, WO02/059107, WO02/059108, WO02/059117, WO02/06276, WO02/12166, WO02/11715,  
10 WO02/12178, WO02/15909, WO02/38544, WO02/068387, WO02/068388, WO02/067869, WO02/081430, WO03/06604, WO03/007949, WO03/009847, WO03/009850, WO03/013509, and WO03/031410;

Mc5r (melanocortin 5 receptor) modulators, such as those disclosed in WO97/19952, WO00/15826, WO00/15790, US20030092041;

- 15 melanin-concentrating hormone 1 receptor (MCHR) antagonists, such as T-226296 (Takeda), SB 568849, SNP-7941 (Synaptic), and those disclosed in patent publications WO01/21169, WO01/82925, WO01/87834, WO02/051809, WO02/06245, WO02/076929, WO02/076947, WO02/04433, WO02/51809, WO02/083134, WO02/094799, WO03/004027, WO03/13574, WO03/15769, WO03/028641,  
20 WO03/035624, WO03/033476, WO03/033480, JP13226269, and JP1437059;

mGluR5 modulators such as those disclosed in WO03/029210, WO03/047581, WO03/048137, WO03/051315, WO03/051833, WO03/053922, WO03/059904, and the like;

- serotonergic agents, such as fenfluramine (such as Pondimin® (Benzeneethanamine, N-ethyl-alpha-methyl-3-(trifluoromethyl)-, hydrochloride), Robbins), dexfenfluramine  
25

(such as Redux® (Benzencethanamine, N-ethyl-alpha-methyl-3-(trifluoromethyl)-, hydrochloride), Interneuron) and sibutramine ((Meridia®, Knoll/Reductil™) including racemic mixtures, as optically pure isomers (+) and (-), and pharmaceutically acceptable salts, solvents, hydrates, clathrates and prodrugs thereof including sibutramine  
5 hydrochloride monohydrate salts thereof, and those compounds disclosed in US4746680, US4806570, and US5436272, US20020006964, WO01/27068, and WO01/62341;

NE (norepinephrine) transport inhibitors, such as GW 320659, despiramine, talsupram, and nomifensine;

NPY 1 antagonists, such as BIBP3226, J-115814, BIBO 3304, LY-357897, CP-671906,  
10 GI-264879A, and those disclosed in US6001836, WO96/14307, WO01/23387, WO99/51600, WO01/85690, WO01/85098, WO01/85173, and WO01/89528;

NPY5 (neuropeptide Y Y5) antagonists, such as 152,804, GW-569180A, GW-594884A, GW-587081X, GW-548118X, FR235208, FR226928, FR240662, FR252384, 1229U91, GI-264879A, CGP71683A, LY-377897, LY-366377, PD-160170, SR- 120562A, SR-  
15 120819A, JCF-104, and H409/22 and those compounds disclosed in patent publications US6140354, US6191160, US6218408, US6258837, US6313298, US6326375, US6329395, US6335345, US6337332, US6329395, US6340683, EP01010691, EP-01044970, WO97/19682, WO97/20820, WO97/20821, WO97/20822, WO97/20823, WO98/27063, WO00/107409, WO00/185714, WO00/185730, WO00/64880,  
20 WO00/68197, WO00/69849, WO/0113917, WO01/09120, WO01/14376, WO01/85714, WO01/85730, WO01/07409, WO01/02379, WO01/23388, WO01/23389, WO01/44201, WO01/62737, WO01/62738, WO01/09120, WO02/20488, WO02/22592, WO02/48152, WO02/49648, WO02/051806, WO02/094789, WO03/009845, WO03/014083, WO03/022849, WO03/028726 and Norman et al., J. Med. Chem. 43:4288-4312 (2000);



opioid antagonists, such as nalmefene (REVEX®), 3-methoxynaltrexone, methylnaltrexone, naloxone, and naltrexone (e.g. PT901; Pain Therapeutics, Inc.) and those disclosed in US6734188, US20050004155 and WO00/21509;

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orexin antagonists, such as SB-334867-A and those disclosed in patent publications  
5 WO01/96302, WO01/68609, WO02/44172, WO02/51232, WO02/51838, WO02/089800,  
WO02/090355, WO03/023561, WO03/032991, and WO03/037847;

PDE inhibitors (e.g. compounds which slow the degradation of cyclic AMP (cAMP) and/or cyclic GMP (cGMP) by inhibition of the phosphodiesterases, which can lead to a relative increase in the intracellular concentration of cAMP and cGMP; possible PDE  
10 inhibitors are primarily those substances which are to be numbered among the class consisting of the PDE3 inhibitors, the class consisting of the PDE4 inhibitors and/or the class consisting of the PDE5 inhibitors, in particular those substances which can be designated as mixed types of PDE3/4 inhibitors or as mixed types of PDE3/4/5 inhibitors) such as those disclosed in patent publications DE1470341, DE2108438,  
15 DE2123328, DE2305339, DE2305575, DE2315801, DE2402908, DE2413935, DE2451417, DE2459090, DE2646469, DE2727481, DE2825048, DE2837161, DE2845220, DE2847621, DE2934747, DE3021792, DE3038166, DE3044568, EP000718, EP0008408, EP0010759, EP0059948, EP0075436, EP0096517, EP0112987, EP0116948, EP0150937, EP0158380, EP0161632, EP0161918, EP0167121, EP0199127,  
20 EP0220044, EP0247725, EP0258191, EP0272910, EP0272914, EP0294647, EP0300726, EP0335386, EP0357788, EP0389282, EP0406958, EP0426180, EP0428302, EP0435811, EP0470805, EP0482208, EP0490823, EP0506194, EP0511865, EP0527117, EP0626939, EP0664289, EP0671389, EP0685474, EP0685475, EP0685479, JP92234389, JP94329652, JP95010875, US4963561, US5141931, WO9117991, WO9200968,  
25 WO9212961, WO9307146, WO9315044, WO9315045, WO9318024, WO9319068, WO9319720, WO9319747, WO9319749, WO9319751, WO9325517, WO9402465, WO9406423, WO9412461, WO9420455, WO9422852, WO9425437, WO9427947, WO9500516, WO9501980, WO9503794, WO9504045, WO9504046, WO9505386,

WO9508534, WO9509623, WO9509624, WO9509627, WO9509836, WO9514667,  
WO9514680, WO9514681, WO9517392, WO9517399, WO9519362, WO9522520,  
WO9524381, WO9527692, WO9528926, WO9535281, WO9535282, WO9600218,  
WO9601825, WO9602541, WO9611917, DE3142982, DE1116676, DE2162096,  
5 EP0293063, EP0463756, EP0482208, EP0579496, EP0667345 US6331543,  
US20050004222 (including those disclosed in formulas I-XIII and paragraphs 37-39, 85-  
0545 and 557-577), WO9307124, EP0163965, EP0393500, EP0510562, EP0553174,  
WO9501338 and WO9603399, as well as PDE5 inhibitors (such as RX-RA-69, SCH-  
51866, KT-734, vesnarinone, zaprinast, SKF-96231, ER-21355, BF/GP-385, NM-702  
10 and sildenafil (Viagra<sup>TM</sup>)), PDE4 inhibitors (such as etazolate, ICI63197, RP73401,  
imazolidinone (RO-20-1724), MEM 1414 (R1533/R1500; Pharmacia Roche),  
denbufylline, rolipram, oxagrelate, nitraquazone, Y-590, DH-6471, SKF-94120,  
motapizone, lixazinone, indolidan, olprinone, atizoram, KS-506-G, dipamfylline, BMY-  
43351, atizoram, arofylline, filaminast, PDB-093, UCB-29646, CDP-840, SKF-107806,  
15 piclamilast, RS-17597, RS-25344-000, SB-207499, TIBENELAST, SB-210667, SB-  
211572, SB-211600, SB-212066, SB-212179, GW-3600, CDP-840, mopidamol,  
anagrelide, ibudilast, amrinone, pimobendan, cilostazol, quazinone and N-(3,5-  
dichloropyrid-4-yl)-3-cyclopropylmethoxy-4-difluoromethoxybenzamide, PDE3  
inhibitors (such as ICI153, 100, bemorandane (RWJ 22867), MCI-154, UD-CG 212,  
20 sulmazole, ampizone, cilostamide, carbazeran, piroximone, imazodan, CI-930,  
siguazodan, adibendan, saterinone, SKF-95654, SDZ-MKS-492, 349-U-85, emoradan,  
EMD-53998, EMD-57033, NSP-306, NSP-307, revizinone, NM-702, WIN-62582 and  
WIN-63291, enoximone and milrinone, PDE3/4 inhibitors (such as benafentrine,  
trequinsin, ORG-30029, zardaverine, L-686398, SDZ-ISQ-844, ORG-20241, EMD-  
25 54622, and tolafentrine) and other PDE inhibitors (such as vinpocetin, papaverine,  
enprofylline, cilomilast, fenoximone, pentoxifylline, roflumilast, tadalafil(Cialis®),  
theophylline, and vardenafil(Levitra®);

Neuropeptide Y2 (NPY2) agonists include but are not limited to: peptide YY and  
fragments and variants thereof (e.g. YY3-36 (PYY3-36) (N. Engl. J. Med. 349:941,

2003; IKPEAPGE DASPEELNRY YASLRHYLNL VTRQRY (SEQ ID NO:XXX)) and PYY agonists such as those disclosed in WO02/47712, WO03/026591, WO03/057235, and WO03/027637;

serotonin reuptake inhibitors, such as, paroxetine, fluoxetine (Prozac™), fluvoxamine, 5 sertraline, citalopram, and imipramine, and those disclosed in US6162805, US6365633, WO03/00663, WO01/27060, and WO01/162341;

thyroid hormone  $\beta$  agonists, such as KB-2611 (KaroBioBMS), and those disclosed in WO02/15845, WO97/21993, WO99/00353, GB98/284425, U.S. Provisional Application No. 60/183,223, and Japanese Patent Application No. JP 2000256190;

10 UCP-1 (uncoupling protein-1), 2, or 3 activators, such as phytanic acid, 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (TTNPB), retinoic acid, and those disclosed in WO99/00123;

$\beta$ 3 (beta adrenergic receptor 3) agonists, such as AJ9677/TAK677 (Dainippon/Takeda), L750355 (Merck), CP331648 (Pfizer), CL-316,243, SB 418790, BRL-37344, L-796568, 15 BMS-196085, BRL-35135A, CGP12177A, BTA-243, GW 427353, Trecadrine, Zeneca D7114, N-5984 (Nisshin Kyorin), LY-377604 (Lilly), SR 59119A, and those disclosed in US5541204, US5770615, US5491134, US5776983, US488064, US5705515, US5451677, WO94/18161, WO95/29159, WO97/46556, WO98/04526 and WO98/32753, WO01/74782, WO02/32897, WO03/014113, WO03/016276, 20 WO03/016307, WO03/024948, WO03/024953 and WO03/037881;

noradrenergic agents including, but not limited to, diethylpropion (such as Tenuate® (1-propanone, 2-(diethylamino)-1-phenyl-, hydrochloride), Merrell), dextroamphetamine (also known as dextroamphetamine sulfate, dexamphetamine, dexedrine, Dexampex, Ferndex, Oxydess II, Robese, Spancap #1), mazindol ((or 5-(p-chlorophenyl)-2,5- 25 dihydro-3H-imidazo[2,1-a]isoindol-5-ol) such as Sanorex®, Novartis or Mazanor®, Wyeth Ayerst), phenylpropanolamine (or Benzenemethanol, alpha-(1-aminoethyl)-,

hydrochloride), phentermine ((or Phenol, 3-[[4,5-duhydro-1H-imidazol-2-yl)ethyl](4-methylphenyl-amino), monohydrochloride) such as Adipex-P®, Lemmon, FASTIN®, Smith-Kline Beecham and Ionamin®, Medeva), phendimetrazine ((or (2S,3S)-3,4-Dimethyl-2-phenylmorpholine L-(+)-tartrate (1:1)) such as Metra® (Forst) , Plegine®  
5 (Wyeth-Ayerst), Prelu-2® (Boehringer Ingelheim), and Statobex® (Lemmon), phendamine tartrate (such as Thephorin® (2,3,4,9-Tetrahydro-2-methyl-9-phenyl-1H-indenol[2,1-c]pyridine L-(+)-tartrate (1:1)), Hoffmann-LaRoche), methamphetamine (such as Desoxyn®, Abbot ((S)-N, (alpha)-dimethylbenzeneethanamine hydrochloride)), and phendimetrazine tartrate (such as Bontril® Slow-Release Capsules,  
10 Amarin (-3,4-Dimethyl-2-phenylmorpholine Tartrate);

fatty acid oxidation upregulator/inducers such as Famoxin® (Genset);

monamine oxidase inhibitors including but not limited to befloxatone, moclobemide, brofaromine, phenoxathine, esuprone, befol, toloxatone, pirlindol, amiflamine, sercloremin, bazinaprine, lazabemide, milacemide, caroxazone and other certain  
15 compounds as disclosed by WO01/12176; and

other anti-obesity agents such as 5HT-2 agonists, ACC (acetyl-CoA carboxylase) inhibitors such as those described in WO03/072197, alpha-lipoic acid (alpha-LA), AOD9604, appetite suppressants such as those in WO03/40107, ATL-962 (Alizyme PLC), benzocaine, benzphetamine hydrochloride (Didrex), bladderwrack (focus  
20 vesiculosus), BRS3 (bombesin receptor subtype 3) agonists, bupropion, caffeine, CCK agonists, chitosan, chromium, conjugated linoleic acid, corticotropin-releasing hormone agonists, dehydroepiandrosterone, DGAT1 (diacylglycerol acyltransferase 1) inhibitors, DGAT2 (diacylglycerol acyltransferase 2) inhibitors, dicarboxylate transporter inhibitors, ephedra, exendin-4 (an inhibitor of glp-1) FAS (fatty acid synthase) inhibitors (such as  
25 Cerulenin and C75), fat resorption inhibitors (such as those in WO03/053451, and the like), fatty acid transporter inhibitors, natural water soluble fibers (such as psyllium, plantago, guar, oat, pectin), galanin antagonists, galega (Goat's Rue, French Lilac),

garcinia cambogia, germander (teucrium chamaedrys), ghrelin antibodies and ghrelin antagonists (such as those disclosed in WO01/87335, and WO02/08250), peptide hormones and variants thereof which affect the islet cell secretion, such as the hormones of the secretin/gastric inhibitory peptide (GIP)/vasoactive intestinal peptide (VIP)/pituitary adenylate cyclase activating peptide (PACAP)/glucagon-like peptide II (GLP-II)/glicentin/glucagon gene family and/or those of the adrenomedullin/amylin/calcitonin gene related peptide (CGRP) gene family including GLP-1 (glucagon-like peptide 1) agonists (e.g. (1) exendin-4, (2) those GLP-1 molecules described in US20050130891 including GLP-1(7-34), GLP-1(7-35), GLP-1(7-36) or GLP-1(7-37) in its C-terminally carboxylated or amidated form or as modified GLP-1 peptides and modifications thereof including those described in paragraphs 17-44 of US20050130891, and derivatives derived from GLP-1-(7-34)COOH and the corresponding acid amide are employed which have the following general formula:



wherein R=H or an organic compound having from 1 to 10 carbon atoms. Preferably, R is the residue of a carboxylic acid. Particularly preferred are the following carboxylic acid residues: formyl, acetyl, propionyl, isopropionyl, methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, tert-butyl.) and glp-1 (glucagon-like peptide-1), glucocorticoid antagonists, glucose transporter inhibitors, growth hormone secretagogues (such as those disclosed and specifically described in US5536716), interleukin-6 (IL-6) and modulators thereof (as in WO03/057237, and the like), L-carnitine, Mc3r (melanocortin 3 receptor) agonists, MCH2R (melanin concentrating hormone 2R) agonist/antagonists, melanin concentrating hormone antagonists, melanocortin agonists (such as Melanotan II or those described in WO 99/64002 and WO 00/74679), nomame herba, phosphate transporter inhibitors, phytopharm compound 57 (CP 644,673), pyruvate, SCD-1 (stearoyl-CoA desaturase-1) inhibitors, T71 (Tularik, Inc., Boulder CO), Topiramate (Topimax®, indicated as an anti-convulsant which has been shown to increase weight loss), transcription factor modulators (such as those disclosed in WO03/026576),  $\beta$ -hydroxy

steroid dehydrogenase-1 inhibitors ( $\beta$ -HSD-1),  $\beta$ -hydroxy- $\beta$ -methylbutyrate, p57 (Pfizer), Zonisamide (Zonegran™, indicated as an anti-epileptic which has been shown to lead to weight loss), and the agents disclosed in US20030119428 paragraphs 20-26.

- 5 The peptides and agonists described herein can be used in therapeutic combination with one or more anti-diabetic agents, including but not limited to:
- PPAR $\gamma$  agonists such as glitazones (e.g., WAY-120,744, AD 5075, balaglitazone, ciglitazone, darglitazone (CP-86325, Pfizer), englitazone (CP-68722, Pfizer), isaglitazone (MIT/J&J), MCC-555 (Mitsubishi disclosed in US5594016), pioglitazone (such as such as
- 10 Actos™ pioglitazone; Takeda), rosiglitazone (Avandia™;Smith Kline Beecham), rosiglitazone maleate, troglitazone (Rezulin®, disclosed in US4572912), rivoglitazone (CS-011, Sankyo), GL-262570 (Glaxo Wellcome), BRL49653 (disclosed in WO98/05331), CLX-0921, 5-BTZD, GW-0207, LG-100641, JJT-501 (JPNT/P&U), L-895645 (Merck), R-119702 (Sankyo/Pfizer), NN-2344 (Dr. Reddy/NN), YM-440
- 15 (Yamanouchi), LY-300512, LY-519818, R483 (Roche), T131 (Tularik), and the like and compounds disclosed in US4687777, US5002953, US5741803, US5965584, US6150383, US6150384, US6166042, US6166043, US6172090, US6211205, US6271243, US6288095, US6303640, US6329404, US5994554, W097/10813, W097/27857, W097/28115, W097/28137, W097/27847, WO00/76488,
- 20 WO03/000685, WO03/027112, WO03/035602, WO03/048130, WO03/055867, and pharmaceutically acceptable salts thereof;
- biguanides such as metformin hydrochloride (N,N-dimethylimidodicarbonimidic diamide hydrochloride, such as Glucophage™, Bristol-Myers Squibb); metformin hydrochloride with glyburide, such as Glucovance™, Bristol-Myers Squibb); buformin
- 25 (Imidodicarbonimidic diamide, N-butyl-); etoformine (1-Butyl-2-ethylbiguanide, Schering A. G.); other metformin salt forms (including where the salt is chosen from the group of, acetate, benzoate, citrate, flumarate, embonate, chlorophenoxyacetate, glycolate, palmoate, aspartate, methanesulphonate, maleate, parachlorophenoxyisobutyrate, formate, lactate, succinate, sulphate, tartrate, cyclohexanecarboxylate, hexanoate,

octanoate, decanoate, hexadecanoate, octodecanoate, benzenesulphonate, trimethoxybenzoate, paratoluenesulphonate, adamantanecarboxylate, glycoxylate, glutamate, pyrrolidonecarboxylate, naphthalenesulphonate, 1-glucosephosphate, nitrate, sulphite, dithionate and phosphate), and phenformin;

5 protein tyrosine phosphatase-1B (PTP-1B) inhibitors, such as A-401,674, KR 61639, OC-060062, OC-83839, OC-297962, MC52445, MC52453, ISIS 113715, and those disclosed in WO99/585521, WO99/58518, WO99/58522, WO99/61435, WO03/032916, WO03/032982, WO03/041729, WO03/055883, WO02/26707, WO02/26743, JP2002114768, and pharmaceutically acceptable salts and esters thereof;

10 sulfonyleureas such as acetohexamide (e.g. Dymelor, Eli Lilly), carbutamide, chlorpropamide (e.g. Diabinese®, Pfizer), gliamilide (Pfizer), gliclazide (e.g. Diamcron, Servier Canada Inc), glimepiride (e.g. disclosed in US4379785, such as Amaryl™, Aventis), glipentide, glipizide (e.g. Glucotrol or Glucotrol XL Extended Release, Pfizer), gliquidone, glisolamide, glyburide/glibenclamide (e.g. Micronase or Glynase Prestab, Pharmacia & Upjohn and Diabeta, Aventis), tolazamide (e.g. Tolinase), and tolbutamide (e.g. Orinase), and pharmaceutically acceptable salts and esters thereof;

15 meglitinides such as repaglinide (e.g. Prandin®, Novo Nordisk), KAD1229 (PF/Kissei), and nateglinide (e.g. Starlix®, Novartis), and pharmaceutically acceptable salts and esters thereof;

20  $\alpha$  glucoside hydrolase inhibitors (or glucoside inhibitors) such as acarbose (e.g. Precose™, Bayer disclosed in US4904769), miglitol (such as GLYSET™, Pharmacia & Upjohn disclosed in US4639436), camiglibose (Methyl 6-deoxy-6-[(2R,3R,4R,5S)-3,4,5-trihydroxy-2-(hydroxymethyl)piperidino]- $\alpha$ -D-glucopyranoside, Marion Merrell Dow), voglibose (Takeda), adiposine, emiglitate, pradimicin-Q, salbostatin, CKD-711, MDL- 25,637, MDL-73,945, and MOR 14, and the compounds disclosed in US4062950, US4174439, US4254256, US4701559, US4639436, US5192772, US4634765, US5157116, US5504078, US5091418, US5217877, US51091 and WO01/47528 (polyamines);

25  $\alpha$ -amylase inhibitors such as tendamistat, trestatin, and A1-3688, and the compounds

disclosed in US4451455, US4623714, and US4273765;  
SGLT2 inhibitors including those disclosed in US6414126 and US6515117;  
an  $\alpha_2$  inhibitor such as disclosed in US6548529;  
insulin secretagogues such as linoglide, A-4166, forskolin, dibutyl cAMP,  
5 isobutylmethylxanthine (IBMX), and pharmaceutically acceptable salts and esters  
thereof;  
fatty acid oxidation inhibitors, such as clomoxir, and etomoxir, and pharmaceutically  
acceptable salts and esters thereof;  
A<sub>2</sub> antagonists, such as midaglizole, isaglidole, deriglidole, idazoxan, earoxan, and  
10 fluparoxan, and pharmaceutically acceptable salts and esters thereof;  
insulin and related compounds (e.g. insulin mimetics) such as biota, LP-100, novarapid,  
insulin detemir, insulin lispro, insulin glargine, insulin zinc suspension (lente and  
ultralente), Lys-Pro insulin, GLP-1 (1-36) amide, GLP-1 (73-7) (insulintropin, disclosed  
in US5614492), LY-315902 (Lilly), GLP-1 (7-36)-NH<sub>2</sub>, AL-401 (AutoImmune), certain  
15 compositions as disclosed in US4579730, US4849405, US4963526, US5642868,  
US5763396, US5824638, US5843866, US6153632, US6191105, and WO 85/05029, and  
primate, rodent, or rabbit insulin including biologically active variants thereof including  
allelic variants, more preferably human insulin available in recombinant form (sources of  
human insulin include pharmaceutically acceptable and sterile formulations such as those  
20 available from Eli Lilly (Indianapolis, Ind. 46285) as Humulin™ (human insulin rDNA  
origin), also see the THE PHYSICIAN'S DESK REFERENCE, 55<sup>sup</sup>.th Ed. (2001)  
Medical Economics, Thomson Healthcare (disclosing other suitable human insulins);  
non-thiazolidinediones such as JT-501 and farglitazar (GW-2570/GI- 262579), and  
pharmaceutically acceptable salts and esters thereof;  
25 PPAR $\alpha/\gamma$  dual agonists such as AR-HO39242 (Astrazeneca), GW-409544 (Glaxo-  
Wellcome), BVT-142, CLX-0940, GW-1536, GW-1929, GW-2433, KRP-297 (Kyorin  
Merck; 5-[(2,4-Dioxo thiazolidinyl)methyl] methoxy-N-[[4-(trifluoromethyl)phenyl]  
methyl]benzamide), L-796449, LR-90, MK-0767 (Merck/Kyorin/Banyu), SB 219994,  
muraglitazar (BMS), tesaglitazar (Astrazeneca), reglitazar (JTT-501) and those disclosed



in WO99/16758, WO99/19313, WO99/20614, WO99/38850, WO00/23415,  
WO00/23417, WO00/23445, WO00/50414, WO01/00579, WO01/79150,  
WO02/062799, WO03/004458, WO03/016265, WO03/018010, WO03/033481,  
WO03/033450, WO03/033453, WO03/043985, WO 031053976, U.S. application Ser.  
5 No. 09/664,598, filed Sep. 18, 2000, Murakami et al. Diabetes 47, 1841-1847 (1998), and  
pharmaceutically acceptable salts and esters thereof;  
other insulin sensitizing drugs;  
VPAC2 receptor agonists;  
GLK modulators, such as those disclosed in WO03/015774;  
10 retinoid modulators such as those disclosed in WO03/000249;  
GSK 3 $\beta$ /GSK 3 inhibitors such as 4-[2-(2-bromophenyl)-4-(4-fluorophenyl)-1H-  
imidazol-5-yl]pyridine and those compounds disclosed in WO03/024447, WO03/037869,  
WO03/037877, WO03/037891, WO03/068773, EP1295884, EP1295885, and the like;  
glycogen phosphorylase (HGLPa) inhibitors such as CP-368,296, CP-316,819,  
15 BAYR3401, and compounds disclosed in WO01/94300, WO02/20530, WO03/037864,  
and pharmaceutically acceptable salts or esters thereof;  
ATP consumption promoters such as those disclosed in WO03/007990;  
TRB3 inhibitors;  
vanilloid receptor ligands such as those disclosed in WO03/049702;  
20 hypoglycemic agents such as those disclosed in WO03/015781 and WO03/040114;  
glycogen synthase kinase 3 inhibitors such as those disclosed in WO03/035663  
agents such as those disclosed in WO99/51225, US20030134890, WO01/24786, and  
WO03/059870;  
insulin-responsive DNA binding protein-1 (IRDBP-1) as disclosed in WO03/057827, and  
25 the like;  
adenosine A2 antagonists such as those disclosed in WO03/035639, WO03/035640, and  
the like;  
PPAR $\delta$  agonists such as GW 501516, GW 590735, and compounds disclosed in  
JP10237049 and WO02/14291;

dipeptidyl peptidase IV (DP-IV) inhibitors, such as isoleucine thiazolidide, NVP-DPP728A (1-[[[2-[(5-cyanopyridin-2-yl)amino]ethyl]amino]acetyl]-2-cyano-(S)-pyrrolidine, disclosed by Hughes et al, *Biochemistry*, 38(36), 11597-11603, 1999), P32/98, NVP-LAF-237, P3298, TSL225 (tryptophyl-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid, disclosed by Yamada et al, *Bioorg. & Med. Chem. Lett.* 8 (1998) 1537-1540), valine pyrrolidide, TMC-2A/2B/2C, CD-26 inhibitors, FE999011, P9310/K364, VIP 0177, DPP4, SDZ 274-444, 2-cyanopyrrolidides and 4-cyanopyrrolidides as disclosed by Ashworth et al, *Bioorg. & Med. Chem. Lett.*, Vol. 6, No. 22, pp 1163-1166 and 2745-2748 (1996), and the compounds disclosed in US6395767, US6573287, US6395767 (compounds disclosed include BMS-477118, BMS-471211 and BMS 538,305), WO99/38501, WO99/46272, WO99/67279, WO99/67278, WO99/61431 WO03/004498, WO03/004496, EP1258476, WO02/083128, WO02/062764, WO03/000250, WO03/002530, WO03/002531, WO03/002553, WO03/002593, WO03/000180, and WO03/000181;

15 GLP-I agonists such as exendin-3 and exendin-4 (including the 39 aa peptide synthetic exendin-4 called Exenatide®), and compounds disclosed in US2003087821 and NZ 504256, and pharmaceutically acceptable salts and esters thereof; peptides including amlintide and Symlin® (pramlintide acetate); and glyco kinase activators such as those disclosed in US2002103199 (fused heteroaromatic compounds) and WO02/48106 (isoindolin-1-one-substituted propionamide compounds).

20

The peptides and agonists described herein useful in the treatment of obesity can be administered as a cotherapy with electrostimulation (US20040015201).

25 The peptides and agonists described herein can be used in combination therapy with agents that activate soluble guanylate cyclase, for example those described in US20040192680.

The peptides and agonists described herein can be used in combination therapy with a phosphodiesterase inhibitor. PDE inhibitors are those compounds which slow the degradation of cyclic AMP (cAMP) and/or cyclic GMP (cGMP) by inhibition of the phosphodiesterases, which can lead to a relative increase in the intracellular concentration of cAMP and/or cGMP. Possible PDE inhibitors are primarily those substances which are to be numbered among the class consisting of the PDE3 inhibitors, the class consisting of the PDE4 inhibitors and/or the class consisting of the PDE5 inhibitors, in particular those substances which can be designated as mixed types of PDE3/4 inhibitors or as mixed types of PDE3/4/5 inhibitors. By way of example, those PDE inhibitors may be mentioned such as are described and/or claimed in the following patent applications and patents: DE1470341, DE2108438, DE2123328, DE2305339, DE2305575, DE2315801, DE2402908, DE2413935, DE2451417, DE2459090, DE2646469, DE2727481, DE2825048, DE2837161, DE2845220, DE2847621, DE2934747, DE3021792, DE3038166, DE3044568, EP000718, EP0008408, EP0010759, EP0059948, EP0075436, EP0096517, EP0112987, EP0116948, EP0150937, EP0158380, EP0161632, EP0161918, EP0167121, EP0199127, EP0220044, EP0247725, EP0258191, EP0272910, EP0272914, EP0294647, EP0300726, EP0335386, EP0357788, EP0389282, EP0406958, EP0426180, EP0428302, EP0435811, EP0470805, EP0482208, EP0490823, EP0506194, EP0511865, EP0527117, EP0626939, EP0664289, EP0671389, EP0685474, EP0685475, EP0685479, JP92234389, JP94329652, JP95010875, U.S. Pat. Nos. 4,963,561, 5,141,931, WO9117991, WO9200968, WO9212961, WO9307146, WO9315044, WO9315045, WO9318024, WO9319068, WO9319720, WO9319747, WO9319749, WO9319751, WO9325517, WO9402465, WO9406423, WO9412461, WO9420455, WO9422852, WO9425437, WO9427947, WO9500516, WO9501980, WO9503794, WO9504045, WO9504046, WO9505386, WO9508534, WO9509623, WO9509624, WO9509627, WO9509836, WO9514667, WO9514680, WO9514681, WO9517392, WO9517399, WO9519362, WO9522520, WO9524381, WO9527692, WO9528926, WO9535281, WO9535282, WO9600218, WO9601825, WO9602541, WO9611917, DE3142982, DE1116676, DE2162096, EP0293063, EP0463756, EP0482208,

EP0579496, EP0667345 US6,331,543, US20050004222 (including those disclosed in formulas I-XIII and paragraphs 37-39, 85-0545 and 557-577) and WO9307124, EP0163965, EP0393500, EP0510562, EP0553174, WO9501338 and WO9603399. PDE5 inhibitors which may be mentioned by way of example are RX-RA-69, SCH-51866, KT-5 734, vesnarinone, zaprinast, SKF-96231, ER-21355, BF/GP-385, NM-702 and sildenafil (Viagra®). PDE4 inhibitors which may be mentioned by way of example are RO-20-1724, MEM 1414 (R1533/R1500; Pharmacia Roche), DENBUFYLLINE, ROLIPRAM, OXAGRELATE, NITRAQUAZONE, Y-590, DH-6471, SKF-94120, MOTAPIZONE, LIXAZINONE, INDOLIDAN, OLPRINONE, ATIZORAM, KS-506-G, 10 DIPAMFYLLINE, BMY-43351, ATIZORAM, AROFYLLINE, FILAMINAST, PDB-093, UCB-29646, CDP-840, SKF-107806, PICLAMILAST, RS-17597, RS-25344-000, SB-207499, TIBENELAST, SB-210667, SB-211572, SB-211600, SB-212066, SB-212179, GW-3600, CDP-840, MOPIDAMOL, ANAGRELIDE, IBUDILAST, AMRINONE, PIMOBENDAN, CILOSTAZOL, QUAZINONE and N-(3,5-15 dichloropyrid-4-yl)-3-cyclopropylmethoxy4-difluoromethoxybenzamide. PDE3 inhibitors which may be mentioned by way of example are SULMAZOLE, AMPIZONE, CILOSTAMIDE, CARBAZERAN, PIROXIMONE, IMAZODAN, CI-930, SIGUAZODAN, ADIBENDAN, SATERINONE, SKF-95654, SDZ-MKS-492, 349-U-85, EMORADAN, EMD-53998, EMD-57033, NSP-306, NSP-307, REVIZINONE, NM-20 702, WIN-62582 and WIN-63291, ENOXIMONE and MILRINONE. PDE3/4 inhibitors which may be mentioned by way of example are BENAFENTRINE, TREQUINSIN, ORG-30029, ZARDAVERINE, L-686398, SDZ-ISQ-844, ORG-20241, EMD-54622, and TOLAFENTRINE. Other PDE inhibitors include: cilomilast, pentoxifylline, roflumilast, tadalafil(Cialis®), theophylline, and vardenafil(Levitra®), zaprinast (PDE5 25 specific).

The peptides and agonists described herein can be used in combination therapy (for example, in order to decrease or inhibit uterine contractions) with a tocolytic agent

including but not limited to beta-adrenergic agents, magnesium sulfate, prostaglandin inhibitors, and calcium channel blockers.

The peptides and agonists described herein can be used in combination therapy  
5 with an anti-neoplastic agents including but not limited to alkylating agents,  
epipodophyllotoxins, nitrosoureas, antimetabolites, vinca alkaloids, anthracycline  
antibiotics, nitrogen mustard agents, and the like. Particular anti-neoplastic agents may  
include tamoxifen, taxol, etoposide and 5-fluorouracil. The peptides and agonists  
described herein can be used in combination therapy (for example as in a  
10 chemotherapeutic composition) with an antiviral and monoclonal antibody therapies.

The peptides and agonists described herein can be used in combination therapy  
(for example, in prevention/treatment of congestive heart failure or another method  
described herein) with the partial agonist of the nociceptin receptor ORL1 described by  
15 Dooley et al. (The Journal of Pharmacology and Experimental Therapeutics, 283 (2):  
735-741, 1997). The agonist is a hexapeptide having the amino acid sequence Ac- RYY  
(RK) (WI) (RK)-NH<sub>2</sub> ("the Dooley peptide"), where the brackets show allowable  
variation of amino acid residue. Thus Dooley peptide can include but are not limited to  
KYYRWR, RYYRWR, KWRYYR, RYYRWK, RYYRWK (all-D amino acids),  
20 RYYRIK, RYYRIR, RYYKIK, RYYKIR, RYYKWR, RYYKWK, RYYRWR,  
RYYRWK, RYYRIK, RYYKWR, RYYKWK, RYYRWK and KYYRWK, wherein the  
amino acid residues are in the L-form unless otherwise specified. The peptides and  
agonists described herein can also be used in combination therapy with peptide conjugate  
modifications of the Dooley peptide described in WO0198324.

25

#### Methods of Treatment

A number of disorders might be prevented or treated with GC-C receptor agonists and agents that increase cGMP levels including the peptides and agonists described herein.

The peptides and agonists described herein can be used alone or in combination therapy for the treatment or prevention of congestive heart failure. Such agents can be used in combination with natriuretic peptides (e.g., atrial natriuretic peptide, brain natriuretic peptide or C-type natriuretic peptide), a diuretic, or an inhibitor of angiotensin converting  
5 enzyme.

The peptides and agonists described herein can be used alone or in combination therapy for the treatment or prevention of benign prostatic hyperplasia (BPH). Such agents can be used in combination with one or more agents for treatment of BPH, for example, a 5-  
10 alpha reductase inhibitor (e.g., finasteride) or an alpha adrenergic inhibitor (e.g., doxazosine).

The peptides and agonists described herein can be used alone or in combination therapy for the treatment, prevention or reduction of visceral pain associated with a  
15 gastrointestinal disorder or pain associated with another disorder.

The peptides and agonists described herein can be used alone or in combination therapy for the treatment or prevention of obesity-related disorders (e.g. disorders that are associated with, caused by, or result from obesity). Examples of obesity-related disorders  
20 include overeating and bulimia, hypertension, diabetes, elevated plasma insulin concentrations and insulin resistance, dyslipidemias, hyperlipidemia, endometrial, breast, prostate and colon cancer, osteoarthritis, obstructive sleep apnea, cholelithiasis, gallstones, heart disease, abnormal heart rhythms and arrhythmias, myocardial infarction, congestive heart failure, coronary heart disease, sudden death, stroke, polycystic ovarian  
25 disease, craniopharyngioma, the Prader-Willi Syndrome, Frohlich's syndrome, GH-deficient subjects, normal variant short stature, Turner's syndrome, and other pathological conditions showing reduced metabolic activity or a decrease in resting energy expenditure as a percentage of total fat-free mass, e.g., children with acute lymphoblastic leukemia. The agents described herein may be used to reduce or control body weight (or

fat) or to prevent and/or treat obesity or other appetite related disorders related to the excess consumption of food, ethanol and other appetizing substances. The agents may be used to modulate lipid metabolism, reduce body fat (e.g. via increasing fat utilization) or reduce (or suppress) appetite (e.g. via inducing satiety). Further examples of obesity-related disorders are metabolic syndrome, also known as syndrome X, insulin resistance syndrome, sexual and reproductive dysfunction, such as infertility, hypogonadism in males and hirsutism in females, gastrointestinal motility disorders, such as obesity-related gastroesophageal reflux, respiratory disorders, such as obesity-hypoventilation syndrome (Pickwickian syndrome), cardiovascular disorders, inflammation, such as systemic inflammation of the vasculature, arteriosclerosis, hypercholesterolemia, hyperuricaemia, lower back pain, gallbladder disease, gout, and kidney cancer. The agents of the present disclosure are also useful for reducing the risk of secondary outcomes of obesity, such as reducing the risk of left ventricular hypertrophy.

The peptides and agonists described herein can be used alone or in combination therapy for the treatment or prevention of gastrointestinal related disorders including: chronic intestinal pseudo-obstruction (Ogilvie's syndrome), colonic pseudoobstruction, Crohn's disease, dyspepsia (including functional dyspepsia or nonulcer dyspepsia), duodenogastric reflux, functional bowel disorder, functional gastrointestinal disorders, functional heartburn, gastroesophageal reflux disease (GERD), gastrointestinal motility disorders, gastroparesis (e.g. idiopathic gastroparesis), hypertrophic pyloric stenosis, Inflammatory bowel disease, irritable bowel syndrome (IBS), post-operative ileus, and ulcerative colitis. The peptides and agonists described herein can be used alone or in combination therapy to patient suffering from or susceptible to GI disorders relating to damage to the GI tract stemming from impact or surgical intervention. The peptides and agonists described herein can be used alone or in combination therapy to patients at risk for or having particular diseases associated with hypomotility (e.g. colonic inertia) or stasis in the GI tract. For example, diabetic neuropathy, anorexia nervosa, and achlorhydria are frequently accompanied by gastric hypomotility. Damage to the GI tract

following surgical intervention, for instance, can result in substantial gastric stasis. The peptides and agonists described herein can be administered alone or in combination therapy to patients susceptible to or having a GI disorder associated with diabetes (e.g. diabetic gastropathy). The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat GI disorders characterized by at least one of nausea, vomiting, heartburn, postprandial discomfort, diarrhea, constipation, indigestion or related symptoms. The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat GI disorders associated with at least one of diabetes, anorexia nervosa, bulimia, achlorhydria, achalasia, anal fissure, haemorrhoids, irritable bowel syndrome, intestinal pseudoobstruction, scleroderma and gastrointestinal damage.

The peptides and agonists described herein can be used to prevent and/or treat constipation. Constipation can be used to describe bowel patterns which include one or more of hard, small, infrequent stools; the sensation of difficulty in passing stool, specifically excessive or ineffectual straining; the sensation of incomplete evacuation. Constipation has also been described as the passage of stool less than a certain number (e.g. 3) of times per week. A number of conditions can be associated with constipation. Constipation can be associated with numerous disorders and conditions. For example, constipation can be (1) associated with the use of a therapeutic agent (e.g. antihypertensives, anticonvulsants, antispasmodics, analgesics, anticholinergics, antidepressants, antipsychotics, cation-containing agents, anticonvulsants, ganglion blockers, vinca alkaloids); (2) associated with a muscular, neuropathic, metabolic or endocrine disorder (including but not limited to myotonic dystrophy, dermatomyositis, systemic sclerosis, scleroderma, amyloidosis (neurologic or muscular), ischemia, tumor of the central nervous system, autonomic neuropathy, Chagas disease, cystic fibrosis, diabetes mellitus, Hirschsprung disease, hyperthyroidism, hypocalcaemia, hypothyroidism, Multiple Sclerosis, neurofibromatosis, Parkinson's disease, and spinal cord lesions (for example, related to sacral nerve damage related to trauma or a tumor or



the enteric nervous system)); (3) post-surgical constipation (postoperative ileus); (4) associated with a structural colon alteration (for example that associated with Neoplasm, stricture, volvulus, anorectal, inflammation, prolapse, rectocele, or fissure); (5) associated with the a gastrointestinal disorder; (6) associated with a systemic illness or disorder (for example, electrolyte abnormalities, thyroid disease, diabetes mellitus, 5 panhypopituitarism, Addison's disease, pheochromocytoma, uremia, porphyria); (7) chronic constipation; (8) associated with the use of analgesic drugs (e.g. opioid induced constipation); (9) associated with megacolon; and (10) idiopathic constipation (functional constipation). Functional constipation can be associated with normal transit, slow transit 10 (e.g. one or fewer bowel movements per week) and pelvic floor dyssynergia. Pelvic floor dyssynergia is considered a disorder of the rectum and anus although these patients also have abnormal contractions throughout the colon. Patients with pelvic floor dyssynergia have abnormal colonic pressure waves prior to defecation and present with symptoms that may include a sensation of incomplete evacuation, excessive straining, a need for 15 digital disimpaction, perianal heaviness, and tenesmus. Constipation can be associated with bloating and abdominal pain. The peptides and agonists described herein can be used to prevent and/or treat low stool frequency or poor stool consistency.

The peptides and agonists described herein can be used to treat decreased intestinal 20 motility, slow digestion or slow stomach emptying. The peptides and agonists can be used to relieve one or more symptoms of IBS (bloating, pain, constipation), GERD (acid reflux into the esophagus), duodenogastric reflux, functional dyspepsia, or gastroparesis (nausea, vomiting, bloating, delayed gastric emptying) and other disorders described herein. The peptides and agonists described herein can be used to treat flatulence.

25

The peptides and agonists described herein can be used to increase intestinal motility, slow colonic transit, and to prevent and/or treat gastrointestinal immotility and other conditions calling for laxative or stool softener therapy. Gastrointestinal immotility can include constipation, and also includes delayed oral cecal transit time, irregular Taxation,

and other related gastrointestinal motility dysfunction including impaction. Impaction is a condition where a large mass of dry, hard stool develops in the rectum, often due to chronic constipation. This mass may be so hard that it cannot be excreted. The subjects affected by constipation or gastrointestinal immotility can be refractory to laxative  
5 therapy and/or stool softener therapy.

The peptides and agonists described herein can be used for the treatment or prevention of cancer, pre-cancerous growths, or metastatic growths. For example, they can be used for the prevention or treatment of: colorectal/local metastasized colorectal cancer, intestinal  
10 polyps, gastrointestinal tract cancer, lung cancer, cancer or pre-cancerous growths or metastatic growths of epithelial cells, polyps, breast, colorectal, lung, ovarian, pancreatic, prostatic, renal, stomach, bladder, liver, esophageal and testicular carcinoma, carcinoma (e.g., basal cell, basosquamous, Brown-Pearce, ductal carcinoma, Ehrlich tumor, Krebs, Merkel cell, small or non-small cell lung, oat cell, papillary, bronchiolar, squamous cell,  
15 transitional cell, (Walker), leukemia (e.g., B-cell, T-cell, HTLV, acute or chronic lymphocytic, mast cell, myeloid), histiocytosis, histiocytosis, Hodgkin's disease, non-Hodgkin's lymphoma, plasmacytoma, reticuloendotheliosis, adenoma, adeno-carcinoma, adenofibroma, adenolymphoma, ameloblastoma, angiokeratoma, angiolymphoid hyperplasia with eosinophilia, sclerosing angioma, angiomatosis, apudoma, branchionia,  
20 malignant carcinoid syndrome, carcinoid heart disease, carcinosarcoma, cementoma, cholangioma, cholesteatoma, chondrosarcoma, chondroblastoma, chondrosarcoma, chordoma, choristoma, craniopharyngioma, chondroma, cylindroma, cystadenocarcinoma, cystadenoma, cystosarcoma phyllodes, dysgenninoma, ependymoma, Ewing sarcoma, fibroma, fibrosarcoma, giant cell tumor, ganglioneuroma,  
25 glioblastoma, glomangioma, granulosa cell tumor, gynandroblastoma, hamartoma, hemangioendothelioma, hemangioma, hemangio-pericytoma, hemangiosarcoma, hepatoma, islet cell tumor, Kaposi sarcoma, leiomyoma, leiomyosarcoma, leukosarcoma, Leydig cell tumor, lipoma, liposarcoma, lymphangioma, lymphangiomyoma, lymphangiosarcoma, medulloblastoma, meningioma, mesenchymoma, mesonephroma,

mesothelioma, myoblastoma, myoma, myosarcoma, myxoma, myxosarcoma, neurilemmoma, neuroma, neuroblastoma, neuroepithelioma, neurofibroma, neurofibromatosis, odontoma, osteoma, osteosarcoma, papilloma, paraganglioma, paraganglionia, nonchromaffin, pinealoma, rhabdomyoma, rhabdomyosarcoma, Sertoli  
5 cell tumor, teratoma, theca cell tumor, and other diseases in which cells have become dysplastic, immortalized, or transformed.

The peptides and agonists described herein can be used for the treatment or prevention of: Familial Adenomatous Polyposis (FAP) (autosomal dominant syndrome) that precedes  
10 colon cancer, hereditary nonpolyposis colorectal cancer (HNPCC), and inherited autosomal dominant syndrome.

For treatment or prevention of cancer, pre-cancerous growths and metastatic growths, the peptides and agonists described herein can be used in combination therapy with radiation  
15 or chemotherapeutic agents, an inhibitor of a cGMP-dependent phosphodiesterase or a selective cyclooxygenase-2 inhibitor. A number of selective cyclooxygenase-2 inhibitors are described in US20010024664, U.S. Pat. No. 5,380,738, U.S. Pat. No. 5,344,991, U.S. Pat. No. 5,393,790, U.S. Pat. No. 5,434,178, U.S. Pat. No. 5,474,995, U.S. Pat. No. 5,510,368, WO02/062369, WO 96/06840, WO 96/03388, WO 96/03387, WO 96/19469,  
20 WO 96/25405, WO 95/15316, WO 94/15932, WO 94/27980, WO 95/00501, WO 94/13635, WO 94/20480, and WO 94/26731, the disclosures of which are herein incorporated by reference. [Pyrazol-1-yl]benzenesulfonamides have also been described as inhibitors of cyclooxygenase-2.

25 The peptides and agonists described herein can be used in the treatment or prevention of inflammation. Thus, they can be used alone or in combination with an inhibitor of cGMP-dependent phosphodiesterase or a selective cyclooxygenase-2 inhibitor for treatment of: organ inflammation, IBD (e.g. Crohn's disease, ulcerative colitis), asthma, nephritis, hepatitis, pancreatitis, bronchitis, cystic fibrosis, ischemic bowel diseases,

intestinal inflammations/allergies, coeliac disease, proctitis, eosinophilic gastroenteritis, mastocytosis, and other inflammatory disorders. The peptides and agonists described herein can be used alone or in combination therapy in the treatment or prevention of gastrointestinal tract inflammation (e.g. inflammation associated with a gastrointestinal disorder, gastrointestinal tract infection, or another disorder). They can be used alone or in combination therapy with phenoxyalkylcarboxylic acid derivatives for the treatment of interstitial cystitis, irritable bowel syndrome, ulcerative colitis, and other inflammatory conditions, as mentioned in US20050239902A1.

10 The peptides and agonists described herein can also be used to treat or prevent insulin-related disorders, for example: II diabetes mellitus, hyperglycemia, obesity, disorders associated with disturbances in glucose or electrolyte transport and insulin secretion in cells, or endocrine disorders. They can be also used in insulin resistance treatment and post-surgical and non-post surgery decrease in insulin responsiveness.

15 The peptides and agonists described herein can be used to prevent and/or treat pulmonary and respiratory related disorders, including, inhalation, ventilation and mucus secretion disorders, pulmonary hypertension, chronic obstruction of vessels and airways, acute respiratory failure, and irreversible obstructions of vessels and bronchi. One may administer an agent described herein for treating bronchospasm, for inducing bronchodilation, for treating chronic obstructive pulmonary disease (including chronic bronchitis with normal airflow), for treating asthma (including bronchial asthma, intrinsic asthma, extrinsic asthma, acute asthma, chronic or inveterate asthma (e.g. late asthma and airways hyper-responsiveness), dust-induced asthma, allergen-induced asthma, viral-induced asthma, cold-induced asthma, pollution-induced asthma and exercise-induced asthma) and for treating rhinitis (including acute-, allergic, atrophic rhinitis or chronic rhinitis (such as rhinitis caseosa, hypertrophic rhinitis, rhinitis purulenta, rhinitis sicca), rhinitis medicamentosa, membranous rhinitis (including croupous, fibrinous and pseudomembranous rhinitis), scrofulous rhinitis, perennial allergic rhinitis, seasonal

rhinitis (including rhinitis nervosa (hay fever) and vasomotor rhinitis). The peptides described herein may also be useful in the treatment of dry eye disease and chronic sinusitis. The peptides described herein may also be used to prevent and/or treat disorders characterized by acute pulmonary vasoconstriction such as may result from pneumonia, traumatic injury, aspiration or inhalation injury, fat embolism in the lung, acidosis inflammation of the lung, adult respiratory distress syndrome, acute pulmonary edema, acute mountain sickness, post-cardiac surgery, acute pulmonary hypertension, persistent pulmonary hypertension of the newborn, perinatal aspiration syndrome, hyaline membrane disease, acute pulmonary thromboembolism, herapin-protamine reactions, sepsis, status asthmaticus or hypoxia (including iatrogenic hypoxia) and other forms of reversible pulmonary vasoconstriction. Such pulmonary disorders also are also characterized by inflammation of the lung including those associated with the migration into the lung of nonresident cell types including the various leucocyte subclasses. Also included in the respiratory disorders contemplated are: bullous disease, cough, chronic cough associated with inflammation or iatrogenic induced, airway constriction, pigeon fancier's disease, eosinophilic bronchitis, asthmatic bronchitis, chronic bronchitis with airway obstruction (chronic obstructive bronchitis), eosinophilic lung disease, emphysema, farmer's lung, allergic eye diseases (including allergic conjunctivitis, vernal conjunctivitis, vernal keratoconjunctivitis, and giant papillary conjunctivitis), idiopathic pulmonary fibrosis, cystic fibrosis, diffuse pan bronchiolitis and other diseases which are characterized by inflammation of the lung and/or excess mucosal secretion. Other physiological events which are contemplated to be prevented, treated or controlled include platelet activation in the lung, chronic inflammatory diseases of the lung which result in interstitial fibrosis, such as interstitial lung diseases (ILD) (e.g., idiopathic pulmonary fibrosis, or ILD associated with rheumatoid arthritis, or other autoimmune conditions), chronic obstructive pulmonary disease (COPD)(such as irreversible COPD), chronic sinusitis, fibroid lung, hypersensitivity lung diseases, hypersensitivity pneumonitis, idiopathic interstitial pneumonia, nasal congestion, nasal polyposis, and otitis media.

The peptides and agonists described herein can be used alone or in combitherapy to prevent or treat: retinopathy, nephropathy, diabetic angiopathy, and edema formation

5 The peptides and agonists described herein can be used alone or in combitherapy to prevent or treat neurological disorders, for example, headache, tension-type headache, migraines, anxiety, stress, cognitive disorders, cerebral ischemia, brain trauma, movement disorders, aggression, psychosis, seizures, panic attacks, hysteria, sleep disorders, depression, schizoaffective disorders, sleep apnea, attention deficit syndromes,  
10 memory loss, dementia, memory and learning disorders as discussed in Moncada and Higgs 1995 FASEB J. 9:1319-1330; Severina 1998 Biochemistry 63:794; Lee et al. 2000 PNAS 97: 10763-10768; Hobbs 1997 TIPS 18:484-491; Murad 1994 Adv. Pharmacol. 26:1-335; and Denninger et al. 1999 Biochim. Biophys. Acta 1411:334-350 and narcolepsy. They may also be used as a sedative.

15

The peptides and detectably peptides and agonists described herein can be used as markers to identify, detect, stage, or diagnosis diseases and conditions of small intestine, including, without limitation: Crohn's disease, colitis, inflammatory bowel disease, tumors, benign tumors, such as benign stromal tumors, adenoma, angioma, adenomatous  
20 (pedunculated and sessile) polyps, malignant, carcinoid tumors, endocrine cell tumors, lymphoma, adenocarcinoma, foregut, midgut, and hindgut carcinoma, gastrointestinal stromal tumor (GIST), such as leiomyoma, cellular leiomyoma, leiomyoblastoma, and leiomyosarcoma, gastrointestinal autonomic nerve tumor, malabsorption syndromes, celiac diseases, diverticulosis, Meckel's diverticulum, colonic diverticula, megacolon,  
25 Hirschsprung's disease, irritable bowel syndrome, mesenteric ischemia, ischemic colitis, colorectal cancer, colonic polyposis, polyp syndrome, intestinal adenocarcinoma, Liddle syndrome, Brody myopathy, infantile convulsions, and choreoathetosis

The peptides and agonists described herein can be conjugated to another molecule (e.g., a diagnostic or therapeutic molecule) to target cells bearing the GC-C receptor, e.g., cystic fibrosis lesions and specific cells lining the intestinal tract. Thus, they can be used to target radioactive moieties or therapeutic moieties (active moieties like a radionuclide, an enzyme, a fluorescent label, a metal chelating group, a chemiluminescent label, a bioluminescent label, a chemotherapeutic, a toxin, an inactive prodrug, a radiosensitizing agent, a photodynamic agent) to the intestine to aid in imaging and diagnosing or treating colorectal/metastasized or local colorectal cancer. In addition, they can be used to deliver antisense molecules or nucleic acid molecules (like normal copies of the p53 tumor suppressor gene) to the intestinal tract. The peptides and agonists described herein can also be used to increase the number of GC-C molecules on the surface of a cell. In some embodiments the cell is a metastasized colorectal cancer cell. In one embodiment the peptide or agonist described herein is therapeutically conjugated to a second agent. In certain embodiments, the second agent can be radioactive or radiostable. In certain embodiments the second agent can be selected from the group consisting of a compound that causes cell death, a compound that inhibits cell division, a compound that induces cell differentiation, a chemotherapeutic, a toxin and a radiosensitizing agent. In certain embodiments the second agent can be selected from the group consisting of: methotrexate, doxorubicin, daunorubicin, cytosinarabioside, etoposide, 5-4 fluorouracil, melphalan, chlorambucil, cis-platin, vindesine, mitomycin, bleomycin, purothionin, macromomycin, 1,4-benzoquinone derivatives, trenimon, ricin, ricin A chain, Pseudomonas exotoxin, diphtheria toxin, Clostridium perfringens phospholipase C, bovine pancreatic ribonuclease, pokeweed antiviral protein, abrin, abrin A chain, cobra venom factor, gelonin, saporin, modeccin, viscumin, volkensin, nitroimidazole, metronidazole and misonidazole. In certain embodiments the second agent can be a cytotoxic agent selected from the group consisting of cemadotin, a derivative of cemadotin, a derivative of hemiasterlin, esperamicin C, neocarzinostatin, maytansinoid DM1, 7-chloromethyl-10,11 methylenedioxy-camptothecin, rhizoxin, and the halichondrin B analog, ER-086526.

The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat inner ear disorders, e.g., to prevent and/or treat Meniere's disease (including symptoms thereof such as vertigo, hearing loss, tinnitus, sensation of fullness  
5 in the ear), Mal de débarquement syndrome, otitis externa, otitis media, otorrhea, acute mastoiditis, otosclerosis, otic pain, otic bleeding, otic inflammation, Lermoyez's syndrome, vestibular neuronitis, benign paroxysmal positional vertigo (BPPV), herpes zoster oticus, Ramsay Hunt's syndrome, herpes, labyrinthitis, purulent labyrinthitis, perilymph fistulas, presbycusis, ototoxicity (including drug-induced ototoxicity),  
10 neuromas (including acoustic neuromas), aerotitis media, infectious myringitis, bullous myringitis, squamous cell carcinoma, basal cell carcinoma, pre-cancerous otic conditions, nonchromaffin paragangliomas, chemodectomas, glomus jugulare tumors, glomus tympanicum tumors, perichondritis, aural eczematoid dermatitis, malignant external otitis, subperichondrial hematoma, ceruminomas, impacted cerumen, sebaceous cysts,  
15 osteomas, keloids, otalgia, tinnitus, tympanic membrane infection, tympanitis, otic furuncles, petrositis, conductive and sensorineural hearing loss, epidural abscess, lateral sinus thrombosis, subdural empyema, otitic hydrocephalus, Dandy's syndrome, bullous myringitis, diffuse external otitis, foreign bodies, keratosis obturans, otic neoplasm, otomycosis, trauma, acute barotitis media, acute eustachian tube obstruction, postsurgical  
20 otalgia, cholesteatoma, infections related to an otic surgical procedure, and complications associated with any of said disorders. The peptides and agonists described herein can be used alone or in combination therapy to maintain fluid homeostasis in the inner ear.  
neuronitis (including viral neuronitis), ganglionitis, geniculate

25 The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat disorders associated with bicarbonate secretion, e.g., Cystic Fibrosis.



The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat disorders associated with bile secretion. In addition, they can be used to facilitate or control chloride and bile fluid secretion in the gall bladder.

5 The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat disorders associated with liver cell regeneration. This may include administration of the peptides and agonists to liver transplant recipients and to patients with drug or alcohol induced-liver damage. Furthermore, the peptides and agonists may be useful to treat liver damage as in the case of viral mediated hepatitis. The peptides and  
10 agonists described herein may be used alone or in combination to prevent and/or treat liver abscess, liver cancer (either primary or metastatic), cirrhosis (such as cirrhosis caused by the alcohol consumption or primary biliary cirrhosis), amebic liver abscess, autoimmune hepatitis, biliary atresia, coccidioidomycosis disseminated,  $\delta$  agent (hepatitis  $\delta$ ), hemochromatosis, hepatitis a, hepatitis b, hepatitis c, or any other acute, subacute,  
15 fulminant or chronic hepatitis of viral, metabolic or toxic etiology, hepatocellular carcinoma, pyogenic liver abscess, Reye's syndrome, sclerosing cholangitis, Wilson's disease, drug induced hepatotoxicity, or fulminant or acute liver failure. The peptides and agonists may be used in stimulating hepatic regeneration after surgical hepatectomy.

20

The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat myocardial infraction, coronary artery disease, nitrate-induced tolerance, nitrate tolerance, diastolic dysfunction, angina pectoris, stable, unstable and variant (Prinzmetal) angina, atherosclerosis, thrombosis, endothelial dysfunction, cardiac  
25 edema, stroke, conditions of reduced blood vessel patency, e.g., postpercutaneous transluminal coronary angioplasty (post-PTCA), and peripheral vascular disease.

The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat glaucoma.

The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat immunodeficiency.

The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat bladder outlet obstruction and incontinence.

5 The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat male (e.g. erectile dysfunction) or female sexual dysfunction, dysmenorrhea, endometriosis, polycystic ovary syndrome, vaginal dryness, uterine pain, or pelvic pain. These peptides and agonists described herein can be utilized as tocolytic agents that decrease or arrest uterine contractions. The peptides and agonists described  
10 herein can be used to prevent/treat premature/preterm labor. Premature or preterm labor can be associated with, for example, an illness/disorder/condition of the mother (such as pre-eclampsia, high blood pressure or diabetes, abnormal shape or size of the uterus, weak or short cervix, hormone imbalance, vaginal infection that spreads to the uterus, abnormalities of the placenta, such as placenta previa, and excessive amniotic fluid),  
15 premature rupture of the amniotic membranes ("water breaks"), large fetus, and more than one fetus. The peptides or agonists described herein can be used to prevent uterine rupture. The peptides or agonists described herein can be used treat rapid uterine contractions (for example, associated with placental abruption wherein the placental abruption is associated with hypertension, diabetes, a multiply pregnancy, an unusually  
20 large amount of amniotic fluid, numerous previous deliveries, or advanced maternal age (e.g. >40 years old). In certain embodiments they can be used in combination with a phosphodiesterase inhibitor. The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat infertility, for example, male infertility due to poor sperm quality, decreased sperm motility or low sperm count.

25

The peptides and agonists described herein can be used alone or in combination therapy to prevent and/or treat osteopenia disorders (bone loss disorders). "Bone loss disorders" include conditions and diseases wherein the inhibition of bone loss and/or the promotion

of bone formation is desirable. Among such conditions and diseases are osteoporosis, osteomyelitis, Paget's disease (osteitis deformans), periodontitis, hypercalcemia, osteonecrosis, osteosarcoma, osteolytic metastases, familial expansile osteolysis, prosthetic loosening, periprosthetic osteolysis, bone loss attendant rheumatoid arthritis, and cleidocranial dysplasia (CCD). Osteoporosis includes primary osteoporosis, endocrine osteoporosis (hyperthyroidism, hyperparathyroidism, Cushing's syndrome, and acromegaly), hereditary and congenital forms of osteoporosis (osteogenesis imperfecta, homocystinuria, Menkes' syndrome, and Rile-Day syndrome) and osteoporosis due to immobilization of extremities, osteomyelitis, or an infectious lesion in bone leading to bone loss. The peptides and agonists can be used alone or in combination therapy to stimulating bone regeneration. The bone regeneration may be following reconstruction of bone defects in cranio-maxillofacial surgery, or following an implant into bone, for example a dental implant, bone supporting implant, or prosthesis. The bone regeneration may also be following a bone fracture.

The peptides and agonists described herein may be used alone or in combination therapy (for example, with other agents that increase cGMP) to prevent or treat disorders related to an alteration in cGMP including, but not limited to Alzheimer's disease, psoriasis, skin necrosis, scarring, fibrosis, baldness, Kawasaki's Disease, nutcracker oesophagus (US20050245544), septic shock, NSAID-induced gastric disease or disorder, ischemic renal disease or disorder, peptic ulcer, sickle cell anemia, epilepsy, and a neuroinflammatory disease or disorder (for example as described in WO05105765).

The peptides described herein can be used as immunogens to create antibodies for immunoassays. The peptides described herein that have homology to ST peptides can be used as immunogens to treat and/or prevent one or more disease symptoms associated with traveler's diarrhea and for vaccination against pathogens, including but not limited to enterotoxigenic *E. coli* (ETEC). They may also be used in vaccines which also comprise interleukin 18 and either saponin adjuvant or CpG adjuvant for example as described in WO05039634 and WO05039630. The methods described in US20040146534, US4220584, US4285391, US5182109, US4603049, US4545931, US4886663, US4758655, WO08402700, FR2525592, and FR2532850 can be similarly used to create immunogens comprising the peptides described herein. US6043057, US5834246, US5268276, and EP368819, specifically describe an expression system containing CTB (cholera toxin Beta subunit) fused to an ST-like peptide under a foreign promoter for use as a vaccine. The nucleic acids that encode the peptides described herein may be use as genetic vaccines as described in US20050260605 and WO0148018. The nucleic acid molecules may also be used for the manufacture of a functional ribonucleic acid, wherein the functional ribonucleic acid is selected from the group comprising ribozymes, antisense nucleic acids and siRNA (as described in WO05103073).

What is claimed is:

1. A peptide or a pharmaceutically acceptable salt thereof comprising the amino acid sequence:

Xaa<sub>1</sub> Xaa<sub>2</sub> Xaa<sub>3</sub> Cys Glu Xaa<sub>6</sub> Xaa<sub>7</sub> Cys Xaa<sub>9</sub> Pro Ala Cys Thr Gly Xaa<sub>15</sub> Xaa<sub>16</sub>

5 (SEQ ID NO:7) or a pharmaceutically acceptable salt thereof,

wherein

Xaa<sub>1</sub> is any amino acid or is missing;

Xaa<sub>2</sub> is Ala, Gly, Lys, Ser, Val or is missing;

Xaa<sub>3</sub> is Cys or D-Cys;

10 Xaa<sub>6</sub> is any amino acid;

Xaa<sub>7</sub> is Cys or D-Cys;

Xaa<sub>9</sub> is Asn or Thr;

Xaa<sub>15</sub> is Cys or D-Cys;

Xaa<sub>16</sub> is Lys, Tyr or is missing;

15 provided that:

(a) one or more of Xaa<sub>3</sub>, Xaa<sub>7</sub> and Xaa<sub>15</sub> is D-Cys when Xaa<sub>16</sub> is other than Lys;

and

(b) the peptide does not consist of the sequence D-Cys Cys Glu Leu Cys Cys Asn  
Pro Ala Cys Thr Gly Cys.

20

2. The peptide of claim 1 wherein Xaa<sub>3</sub> is D-Cys

3. The peptide of any of the forgoing claims wherein Xaa<sub>7</sub> is D-Cys.

25 4. The peptide of any of the forgoing claims wherein Xaa<sub>15</sub> is D-Cys.

5. The peptide of any of claims 1, 3 and 4 wherein Xaa<sub>3</sub> is Cys

6. The peptide of any claims 1, 2, 4, and 5 wherein Xaa<sub>7</sub> is Cys.
7. The peptide of any of claims 1-3, 5 and 6 wherein Xaa<sub>15</sub> is Cys.
- 5 8. The peptide of any of the forgoing claims wherein Xaa<sub>6</sub> is Val, Ile, Leu  
Tyr, Phe, or Trp.
9. The peptide of any of the forgoing claims wherein Xaa<sub>6</sub> is Val, Ile, or Leu
- 10 10. The peptide of any of the forgoing claims wherein Xaa<sub>6</sub> is Val.
11. The peptide of any of claims 1-9 wherein Xaa<sub>6</sub> is Ile.
12. The peptide of any of claims 1-9 wherein Xaa<sub>6</sub> is Leu.
- 15 13. The peptide of any claims 1-8 wherein Xaa<sub>6</sub> is Tyr, Phe, Trp.
14. The peptide of any of the forgoing claims wherein Xaa<sub>1</sub> is any amino acid.
- 20 15. The peptide of any of the forgoing claims wherein Xaa<sub>1</sub> is Gly or Ala.
16. The peptide of any of the forgoing claims wherein Xaa<sub>1</sub> is Gly.
17. The peptide of any of claims 1-15 wherein Xaa<sub>1</sub> is Ala.
- 25 18. The peptide of any of claims 1-13 wherein Xaa<sub>1</sub> is missing.
19. The peptide of claim 13 wherein Xaa<sub>6</sub> is Tyr.

20. The peptide of claim 13 wherein Xaa<sub>6</sub> is Phe.
21. The peptide of claim 13 wherein Xaa<sub>6</sub> is Trp.
- 5 22. The peptide of any of the forgoing claims wherein at least one of Xaa<sub>3</sub>, Xaa<sub>7</sub> and Xaa<sub>15</sub> is D-Cys.
23. The peptide of claim 1 wherein at least two of Xaa<sub>3</sub>, Xaa<sub>7</sub> and Xaa<sub>15</sub> are D-Cys.
- 10 24. The peptide of any of claim 1 wherein Xaa<sub>3</sub>, Xaa<sub>7</sub> and Xaa<sub>15</sub> are all D-Cys.
25. The peptide of any of the forgoing claims wherein Xaa<sub>9</sub> is Asn
- 15 26. The peptide of any of claims 1-24 wherein Xaa<sub>9</sub> is Thr.
27. The peptide of any of the forgoing claims wherein Xaa<sub>16</sub> is Lys
28. The peptide of any of claims 1-26 wherein Xaa<sub>16</sub> is Tyr
- 20 29. The peptide of any of claims 1-26 wherein Xaa<sub>16</sub> is missing.
30. The peptide of any of Figures 3a and 3b or a pharmaceutically acceptable salt thereof.
- 25 31. A peptide comprising SEQ ID NO:1 or a pharmaceutically acceptable salt thereof.

32. A peptide comprising SEQ ID NO:2 or a pharmaceutically acceptable salt thereof.
33. A peptide comprising SEQ ID NO:3 or a pharmaceutically acceptable salt thereof.
34. A peptide comprising SEQ ID NO:4 or a pharmaceutically acceptable salt thereof.
35. A peptide comprising SEQ ID NO:5 or a pharmaceutically acceptable salt thereof.
36. A peptide consisting of SEQ ID NO:1 or a pharmaceutically acceptable salt thereof.
37. A peptide consisting of SEQ ID NO:2 or a pharmaceutically acceptable salt thereof.
38. A peptide consisting of SEQ ID NO:3 or a pharmaceutically acceptable salt thereof.
39. A peptide consisting of SEQ ID NO:4 or a pharmaceutically acceptable salt thereof.
40. A peptide consisting of SEQ ID NO:5 or a pharmaceutically acceptable salt thereof.
41. A PEGylated peptide comprising the peptide of any of claims 1-40.



42. The PEGylated peptide of claim 41 wherein the peptide is PEGylated at the amino terminus.
43. The peptide of any of the forgoing claims wherein the peptide is purified.
- 5
44. A pharmaceutical composition comprising the peptide of any claims 1-43 and a pharmaceutically acceptable carrier.
45. A method for reducing fluid retention, the method comprising  
10 administering the pharmaceutical composition of claim 44 or a pharmaceutical composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys.
46. A method of treating a disorder selected from: heart failure, hypertension,  
15 salt dependent forms of high blood pressure, hepatic edema, or liver cirrhosis comprising administering to a patient in need thereof an effective amount of the pharmaceutical composition of claim 44 or a pharmaceutical composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr  
20 Gly Cys.
47. The method of claim 46 wherein the disorder is heart failure.
48. The method of claim 47 wherein the heart failure is acute heart failure.
- 25
49. The method of claim 47 wherein the heart failure is chronic heart failure
50. The method of claim 46 wherein the disorder is hypertension.

51. The method of claim 46 wherein the disorder is salt-dependent high blood pressure.

52. The method of claim 46 wherein the disorder is hepatic edema.

5

53. The method of claim 46 wherein the disorder is liver cirrhosis

54. A method for increasing naturesis comprising administering an effective amount of the pharmaceutical composition of claim 44 or a pharmaceutical composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys to a patient in need thereof.

55. A method for increasing diuresis comprising administering an effective amount of the pharmaceutical composition of claim 44 or a pharmaceutical composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys to a patient in need thereof.

56. A method of treating a gastrointestinal disorder comprising administering an effective amount of the pharmaceutical composition of claim 44 or a pharmaceutical composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys to a patient in need thereof.

57. The method of claim 56 wherein the gastrointestinal disorder is selected from: a gastrointestinal motility disorder, chronic intestinal pseudo-obstruction, colonic pseudo-obstruction, Crohn's disease, duodenogastric reflux, dyspepsia, functional dyspepsia, nonulcer dyspepsia, a functional gastrointestinal disorder, functional heartburn, gastroesophageal reflux disease (GERD), gastroparesis, irritable bowel syndrome, post-operative ileus, inflammatory bowel disorder, ulcerative colitis, constipation, chronic constipation, and chronic idiopathic constipation.

58. A method for treating obesity comprising administering an effective amount of the pharmaceutical composition of claim 44 or a pharmaceutical composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys to a patient in need thereof.

59. A method for treating benign prostatic hyperplasia comprising administering the pharmaceutical composition of claim 44 or a pharmaceutical composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys.

60. The method of claim 57 wherein the disorder is constipation.

61. The method of claim 60 wherein the constipation is idiopathic constipation.

62. The method of claim 60 wherein the constipation is chronic idiopathic constipation.

63. The method of claim 57 wherein the gastrointestinal disorder is irritable bowel syndrome.

64. The method of claim 63 wherein the irritable bowel syndrome is diarrhea-predominant irritable bowel syndrome.

65. The method of claim 63 wherein the irritable bowel syndrome is constipation-predominant irritable bowel syndrome.

66. The method of claim 63 wherein the irritable bowel syndrome is alternating-irritable bowel syndrome.

67. The method of claim 57 wherein the gastrointestinal disorder is  
5 inflammatory bowel disorder.

68. The method of claim 57 wherein the gastrointestinal disorder is Crohn's disease.

69. The method of claim 57 wherein the gastrointestinal disorder is ulcerative  
10 colitis.

70. A method for increasing gastrointestinal motility comprising  
administering an effective amount of the pharmaceutical composition of claim 44 or a  
15 pharmaceutical composition comprising a peptide consisting of the amino acid sequence  
D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys to a patient in need thereof.

71. A method for treating gastrointestinal pain or visceral pain comprising  
administering an effective amount of the pharmaceutical composition of claim 44 or a  
20 pharmaceutical composition comprising a peptide consisting of the amino acid sequence  
D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys to a patient in need thereof.

72. A method of treating a side-effect associated with opioid administration,  
the method comprising administering to a patient that is being treated with an opioid an  
25 effective amount of the pharmaceutical composition of claim 44 or a pharmaceutical  
composition comprising a peptide consisting of the amino acid sequence D-Cys Cys Glu  
Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys.

73. The method of claim 72 wherein the patient is being treated with an opioid selected from the group consisting of alfentanil, buprenorphine, butorphanol, codeine, dezocine, dihydrocodeine, fentanyl, hydrocodone, hydromorphone, levorphanol, meperidine (pethidine), methadone, morphine, nalbuphine, oxycodone, oxymorphone, pentazocine, propiram, propoxyphene, sufentanil and tramadol.

74. The method of claim 73 wherein the patient is being treated with an opioid selected from the group consisting of: morphine, codeine, oxycodone, hydrocodone, dihydrocodeine, propoxyphene, fentanyl and tramadol.

10

75. The method of claim 72 wherein the side effect is selected from the group consisting of constipation, nausea and vomiting.

76. The method of any of claim 72-75 further comprising administering an opioid antagonist.

15

77. The method of claim 76 wherein the opioid antagonist is naloxone or naltrexone.

78. A pharmaceutical composition comprising an opioid and a peptide of any of claims 1-43 or a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys.

20

79. The pharmaceutical composition of claim 78 wherein the opioid is selected from the group consisting of alfentanil, buprenorphine, butorphanol, codeine, dezocine, dihydrocodeine, fentanyl, hydrocodone, hydromorphone, levorphanol, meperidine (pethidine), methadone, morphine, nalbuphine, oxycodone, oxymorphone, pentazocine, propiram, propoxyphene, sufentanil and tramadol.

25

80. The pharmaceutical composition of claim 78 wherein the opioid is selected from the group consisting of: morphine, codeine, oxycodone, hydrocodone, dihydrocodeine, propoxyphene, fentanyl and tramadol.

5 81. A pharmaceutical kit comprising:

(a) a first container containing pharmaceutical dosage units comprising an effective amount of an opioid; and

(b) a second container containing pharmaceutical dosage units comprising an effective amount of a peptide according to any of claims 1-43 or a peptide consisting of the amino acid sequence D-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys.

10

82. The pharmaceutical kit of claim 81 wherein the opioid is selected from the group consisting of: alfentanil, buprenorphine, butorphanol, codeine, dezocine, dihydrocodeine, fentanyl, hydrocodone, hydromorphone, levorphanol, meperidine (pethidine), methadone, morphine, nalbuphine, oxycodone, oxymorphone, pentazocine, propiram, propoxyphene, sufentanil and tramadol.

15

83. The pharmaceutical kit of claim 82 wherein the opioid is selected from the group consisting of: morphine, codeine, oxycodone, hydrocodone, dihydrocodeine, propoxyphene, fentanyl and tramadol.

20

84. A method for preparing a pharmaceutical composition comprising admixing a peptide according to any of claims 1-43 and a pharmaceutically acceptable carrier.

25

Figure 1. EC<sub>50</sub> of peptides in *in vitro* cGMP assay

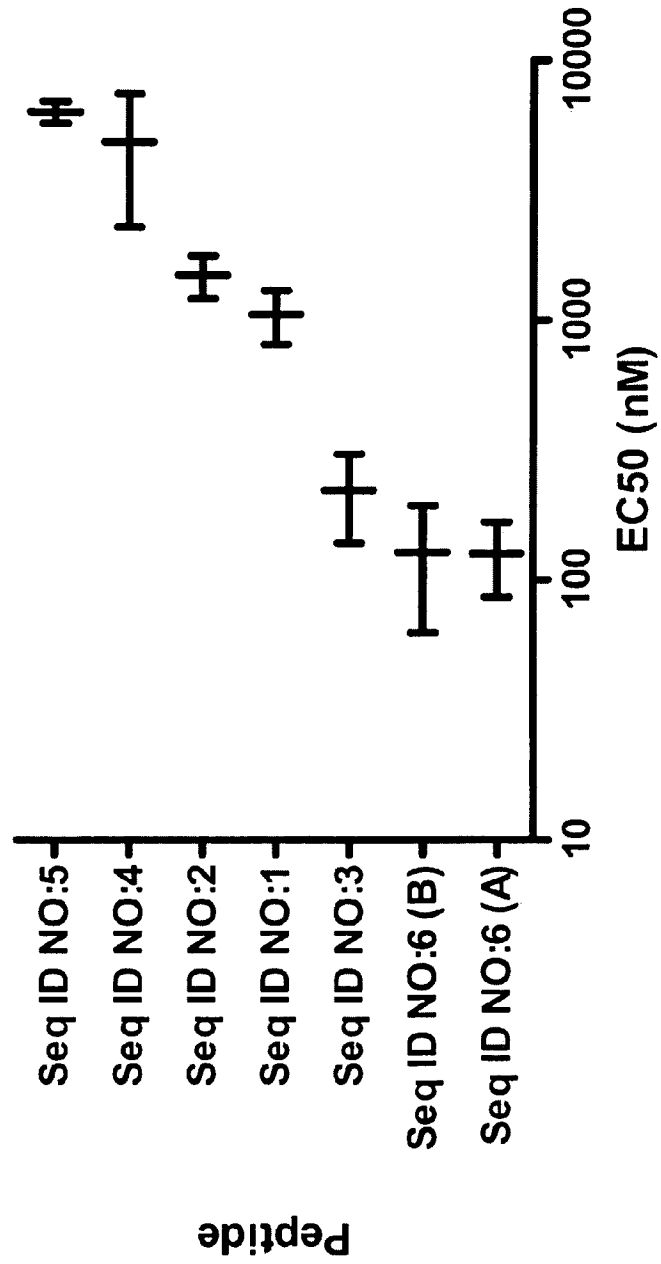
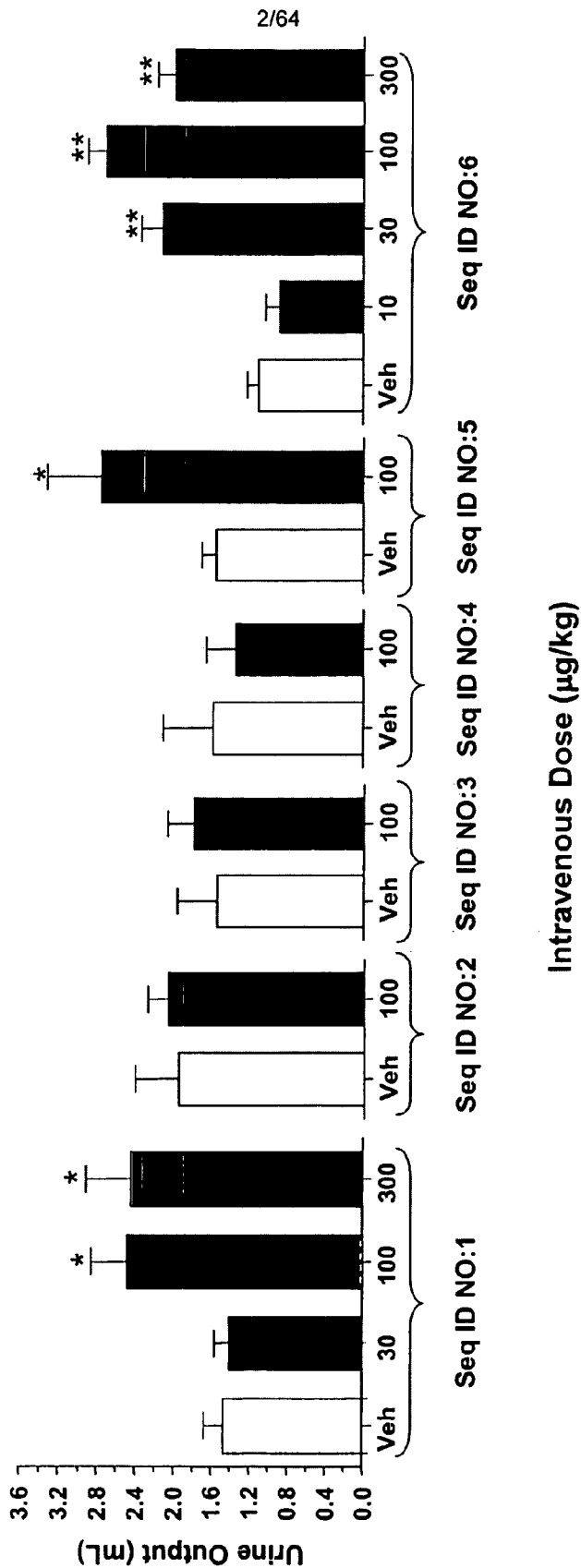


Figure 2. *In vivo* effect of peptides on urine output in rodents



Veh-Vehicle only  
 \* p < 0.05  
 \*\* p < 0.01







FIGURE 3A (sheet 3 of 58)

---	---	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
---	---	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
---	---	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
---	---	Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
---	---	Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
---	---	Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
---	---	Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
---	---	Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
---	---	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
---	---	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
---	---	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
---	---	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
---	---	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
---	---	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
---	---	Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
---	---	Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
---	---	Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
---	---	Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
---	---	Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
---	---	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
---	---	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
---	---	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
---	---	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
---	---	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
---	---	D-Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
---	---	D-Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
---	---	D-Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
---	---	D-Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
---	---	D-Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
---	---	D-Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
---	---	D-Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
---	---	D-Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
---	---	D-Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
---	---	D-Cys	Cys	Glu	Val	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
---	---	D-Cys	Cys	Glu	Val	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
---	---	D-Cys	Cys	Glu	Val	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
---	---	D-Cys	Cys	Glu	Val	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
---	---	D-Cys	Cys	Glu	Val	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
---	---	D-Cys	Cys	Glu	Ile	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
---	---	D-Cys	Cys	Glu	Ile	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
---	---	D-Cys	Cys	Glu	Ile	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
---	---	D-Cys	Cys	Glu	Ile	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
---	---	D-Cys	Cys	Glu	Ile	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
---	---	D-Cys	Cys	Glu	Ile	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
---	---	D-Cys	Cys	Glu	Ile	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
---	---	D-Cys	Cys	Glu	Ile	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
---	---	D-Cys	Cys	Glu	Ile	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
---	---	D-Cys	Cys	Glu	Ile	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
---	---	D-Cys	Cys	Glu	Ile	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
---	---	D-Cys	Cys	Glu	Ile	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
---	---	D-Cys	Cys	Glu	Ile	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
---	---	D-Cys	Cys	Glu	Ile	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr



















FIGURE 3A (sheet 12 of 58)

--	Gly	Cys	Cys	Glu	Tyr	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Gly	Cys	Cys	Glu	Tyr	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Gly	Cys	Cys	Glu	Tyr	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Gly	Cys	Cys	Glu	Tyr	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Gly	Cys	Cys	Glu	Tyr	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Gly	Cys	Cys	Glu	Tyr	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
--	Gly	Cys	Cys	Glu	Tyr	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Gly	Cys	Cys	Glu	Tyr	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Gly	Cys	Cys	Glu	Tyr	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
--	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
--	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
--	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
--	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
--	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
--	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr





































FIGURE 3A (sheet 29 of 58)

--	Val	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Val	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Val	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Val	D-Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Val	D-Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Val	D-Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
--	Val	D-Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Val	D-Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
--	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
--	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
--	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
--	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
--	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	--
--	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
--	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
--	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	--
--	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
--	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	--	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	--
Ala	--	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	--	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	--	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	--
Ala	--	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	--	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	--	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	--
Ala	--	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	--	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	--	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	--
Ala	--	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	--	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	--	Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	--
Ala	--	Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	--	Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr











FIGURE 3A (sheet 34 of 58)

Ala	---	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	---	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	---	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	---	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	---	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	---	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	---	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	---	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	---	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	---	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	---	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	---	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	---	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	---	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	---	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	---	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	---	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	---	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	---	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	---	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	---	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	---	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	---	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	---	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	---	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	---	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	---	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	---	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Ala	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Ala	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Ala	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Ala	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Ala	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Ala	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Ala	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Ala	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Ala	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Ala	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Ala	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Ala	Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Ala	Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Ala	Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Ala	Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Ala	Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Ala	Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Ala	Cys	Cys	Glu	Val	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Ala	Cys	Cys	Glu	Val	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Ala	Cys	Cys	Glu	Val	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Ala	Cys	Cys	Glu	Val	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Ala	Cys	Cys	Glu	Val	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Ala	Cys	Cys	Glu	Val	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Ala	Cys	Cys	Glu	Ile	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Ala	Cys	Cys	Glu	Ile	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Ala	Cys	Cys	Glu	Ile	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr









FIGURE 3A (sheet 39 of 58)

Ala	Ala	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Ala	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Ala	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Ala	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Ala	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Ala	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Ala	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Ala	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Ala	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Ala	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Ala	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Ala	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Ala	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Ala	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Ala	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Ala	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Ala	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Ala	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Ala	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Ala	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Ala	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Ile	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Ile	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Ile	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Ile	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Ile	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Ile	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Ile	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Ile	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Ile	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Ile	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Ile	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Ile	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr

FIGURE 3A (sheet 40 of 58)

Ala	Gly	Cys	Cys	Glu	Ile	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Ile	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Ile	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Ile	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Ile	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Ile	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Ile	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Ile	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Ile	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Leu	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Leu	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Leu	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Leu	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Leu	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Leu	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Leu	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Leu	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Leu	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Leu	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Leu	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Leu	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Leu	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr



FIGURE 3A (sheet 41 of 58)

Ala	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Phe	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Val	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	D-Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Val	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr



FIGURE 3A (sheet 43 of 58)

Ala	Gly	D-Cys	Cys	Glu	Tyr	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	D-Cys	Cys	Glu	Tyr	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Tyr	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Tyr	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	D-Cys	Cys	Glu	Tyr	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Tyr	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Tyr	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	D-Cys	Cys	Glu	Tyr	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Tyr	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Tyr	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	D-Cys	Cys	Glu	Tyr	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Tyr	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Tyr	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	D-Cys	Cys	Glu	Tyr	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Tyr	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Tyr	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	D-Cys	Cys	Glu	Tyr	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Tyr	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Phe	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	---
Ala	Gly	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Gly	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	---
Ala	Gly	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Gly	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr

































FIGURE 3A (sheet 58 of 58)

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Ala	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	--
Ala	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	--
Ala	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	--
Ala	Val	D-Cys	Cys	Glu	Phe	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	--
Ala	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	--
Ala	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	--
Ala	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	--
Ala	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Val	D-Cys	Cys	Glu	Trp	Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	--
Ala	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	--
Ala	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Asn	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr
Ala	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	--
Ala	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Lys
Ala	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	Cys	Tyr
Ala	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	--
Ala	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Lys
Ala	Val	D-Cys	Cys	Glu	Trp	D-Cys	Cys	Thr	Pro	Ala	Cys	Thr	Gly	D-Cys	Tyr

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FIGURE 3B

Seq ID NO:	Sequence
SEQ ID NO. 1	(D-Cys)-Cys-Glu-Tyr-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Tyr
SEQ ID NO. 2	Cys-Cys-Glu-Tyr-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Lys
SEQ ID NO. 3	Cys-Cys-Glu-Phe-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Tyr
SEQ ID NO. 4	Cys-Cys-Glu-Trp-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Tyr
SEQ ID NO. 5	Cys-Cys-Glu-Tyr-Cys-Cys-Thr-Pro-Ala-Cys-Thr-Gly-Cys-Tyr
SEQ ID NO. 6	Cys-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Tyr



Pro sequence	Pro sequence	pre-pro sequence	Processed Active (includes leader)	Pre-processed Active (without pro)	Pre-processed Active (without the pro)
N/A	QSDPTSTPANTV	N/A	SDSDSDSDSDSD	N/A	SDSDSDSDSDSD
RELIHVAIVAD PISCTID	QSTASLADLQTL RQPLAIRRRA CQVYRSPS	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS	SDSDSDSDSDSD	N/A	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS
N/A	RSQDQVTRPS	N/A	SDSDSDSDSDSD	N/A	SDSDSDSDSDSD
RELIHVAIVAD PISCTID	QSTASLADLQTL RQPLAIRRRA CQVYRSPS	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS	SDSDSDSDSDSD	N/A	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS
N/A	QSTASLADLQTL RQPLAIRRRA CQVYRSPS	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS	SDSDSDSDSDSD	N/A	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS
N/A	QSTASLADLQTL RQPLAIRRRA CQVYRSPS	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS	SDSDSDSDSDSD	N/A	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS
N/A	QSTASLADLQTL RQPLAIRRRA CQVYRSPS	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS	SDSDSDSDSDSD	N/A	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS
N/A	QSTASLADLQTL RQPLAIRRRA CQVYRSPS	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS	SDSDSDSDSDSD	N/A	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS
N/A	QSTASLADLQTL RQPLAIRRRA CQVYRSPS	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS	SDSDSDSDSDSD	N/A	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS
N/A	QSTASLADLQTL RQPLAIRRRA CQVYRSPS	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS	SDSDSDSDSDSD	N/A	RELIHVAIVADPISCTID RQPLAIRRRA CQVYRSPS

FIG. 4  
(sheet 2 of 3)

Pro sequence	Pro sequence	pro-pro sequence	Processed Active (with)	Pro-processed Active (without pro)	Pro-processed Active (without the pro)
<p>                     MDDLVAVLQV                      RSDVQ                      MDDLVAVLQV                      RSDVQ                 </p>	<p>                     QVHSHKQVVA                      PDIHSHKQVVA                      LSHKQVVA                      HSHKQVVA                 </p>	<p>                     MDDLVAVLQV                      RSDVQ                      MDDLVAVLQV                      RSDVQ                 </p>	<p>                     HSHKQVVA                      PDIHSHKQVVA                      LSHKQVVA                      HSHKQVVA                 </p>	<p>                     MDDLVAVLQV                      RSDVQ                      MDDLVAVLQV                      RSDVQ                 </p>	<p>                     HSHKQVVA                      PDIHSHKQVVA                      LSHKQVVA                      HSHKQVVA                 </p>
<p>                     MDDLVAVLQV                      RSDVQ                      MDDLVAVLQV                      RSDVQ                 </p>	<p>                     QVHSHKQVVA                      PDIHSHKQVVA                      LSHKQVVA                      HSHKQVVA                 </p>	<p>                     MDDLVAVLQV                      RSDVQ                      MDDLVAVLQV                      RSDVQ                 </p>	<p>                     HSHKQVVA                      PDIHSHKQVVA                      LSHKQVVA                      HSHKQVVA                 </p>	<p>                     MDDLVAVLQV                      RSDVQ                      MDDLVAVLQV                      RSDVQ                 </p>	<p>                     HSHKQVVA                      PDIHSHKQVVA                      LSHKQVVA                      HSHKQVVA                 </p>

FIG. 4  
(sheet 3 of 3)

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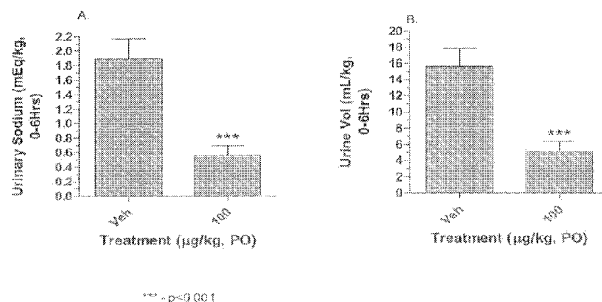
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(54) **Title:** COMPOSITIONS AND METHODS FOR TREATING DISORDERS ASSOCIATED WITH SALT OR FLUID RETENTION

Figure 1. The Effect of Lubiprostone (100µg/kg, PO) on (A) Urinary Sodium and (B) Urine Volume Measured Over 6 Hours in Female Sprague-Dawley Rats



(57) **Abstract:** Methods for reducing the risk of or treating a disorder associated with fluid and/or salt retention in a patient are described. The methods include administering to the patient an agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

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**COMPOSITIONS AND METHODS FOR TREATING  
DISORDERS ASSOCIATED WITH SALT OR FLUID RETENTION**

**BACKGROUND**

5 Excessive retention of fluid or salt is associated with various disorders, including cardiovascular disorders such as congestive heart failure, hypertension, cardiac hypertrophy and stroke. Excessive retention of fluid or salt can also cause or be associated with renal disorders and can lead to ascites build up in the abdomen, for example, in the case of liver disease.

**SUMMARY**

10 Described herein as a method of reducing the risk of or treating a disorder associated with fluid and/or salt retention in a patient, the method comprising administering to the patient an agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in  
15 the intestine and increases anion secretion in the intestine. In certain cases the method comprises administering two or more agents selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine. In various embodiments: the agent reduces sodium absorption in the intestine; the  
20 agent increases anion secretion in the intestine; and the agent both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

In certain embodiments of the method: the agent is selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator (preferably an agonist of soluble guanylate  
25 cyclase), c) a prostanoid, d) a chloride channel activator, e) a 5HT<sub>4</sub> agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels (preferably an agent that increases cAMP levels in the body or a tissue), k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin. In certain  
30 embodiments the method comprises administering two or more agents selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator (preferably an



agonist of soluble guanylate cyclase), c) a prostanoid, d) a chloride channel activator, e) a 5HT<sub>4</sub> agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels (preferably an agent that increases cAMP levels in the body or a tissue), k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin.

In various embodiments: the chloride channel activator is lubiprostone; the sodium transport inhibitor is amiloride; the sodium transport inhibitor is an NHE3 inhibitor; the 5HT<sub>4</sub> agonist is Zelnorm; the prostanoid is selected from: the compound represented by CAS Registry No.

333963-40-9, the compound represented by CAS Registry No. 136790-76-6, (–)-7-

[(2R,4aR,5R,7aR)-2-(1,1-difluoropentyl)-2-hydroxy-6-oxooctahydrocyclopenta[b]pyran-5-yl]heptanoic acid; and the 13, 14-dihydro-15-keto prostaglandins E disclosed in US5284858

including 13,14-dihydro-15-keto-PGE<sub>2</sub> alkyl ester, 13,14-dihydro-15-keto-PGE<sub>2</sub> cycloalkyl ester; 13,14-dihydro-15-keto-PGE<sub>2</sub> hydroxy alkyl ester, 13,14-dihydro-15-keto-PGE<sub>2</sub> benzyl

ester, 13,14-dihydro-15-keto-PGE<sub>1</sub> alkyl ester, 13,14-dihydro-6,15-diketo-PGE<sub>1</sub> alkyl ester,

13,14-dihydro-15-keto-18-methoxy-19, 20-dinor-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-

15-keto-18-methoxy-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto- $\Delta^2$ -PGE<sub>2</sub> or an alkyl

ester thereof, 13,14-dihydro-15-keto-20-methoxy- $\Delta^2$ -PGE<sub>2</sub> or an alkyl ester thereof, 13,14-

dihydro-15-keto-3R,S-methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-3R,S-

methyl-20-methoxy-PGE<sub>2</sub> or an alkyl ester thereof, 13, 14-dihydro-15-keto-11-dehydroxy-11R-

methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-fluoro-11-dehydroxy-11R-

methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-hydroxy-PGE<sub>2</sub> or an alkyl

ester thereof, 13,14-dihydro-15-keto-16R,S-fluoro-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-

15-keto-16R,S-methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-16,16-dimethyl-

PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-16,16-dimethyl-20-methoxy-PGE<sub>2</sub> or an

alkyl ester thereof, 13,14-dihydro-15-keto-17S-methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-

dihydro-15-keto-19-methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-20-

isopropylidene PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-20-ethyl-PGE<sub>2</sub> or an

alkyl ester thereof, 13,14-dihydro-15-keto-20-ethyl-11-dehydroxy-11R-methyl-PGE<sub>2</sub> or an alkyl

ester thereof, 13,14-dihydro-15-keto-20-n-propyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-

15-keto-20-ethyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16R,S-fluoro-PGE<sub>1</sub>

or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16R,S-fluoro-11-dehydroxy-11R-methyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16R,S-methyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16,16-dimethyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-19-methyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-20-methyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-11-dehydroxy-11R-methyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-11-dehydroxy-11R-hydroxymethyl-PGE<sub>1</sub> alkyl ester, 13,14-dihydro-15-keto-20-methyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto- $\Delta^2$ -PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-fluoro-20-methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-16,16-difluoro-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-5,6-dehydro-20-methoxy-PGE<sub>2</sub> or an alkyl ester thereof, and 13,14-dihydro-6,15-diketo-16R,S-fluoro-PGE<sub>1</sub> or an alkyl ester thereof; the prostanoid is misoprostol; the prostanoid is the free acid of the compound associated with CAS registry NO. 59122-49-5; the prostanoid comprises a mixture of stereoisomers; only a single isomer of a prostanoid is administered; the laxative is selected from: a CCK-1 antagonist, a stimulant, a bulk-producing agent and a stool softener; the laxative is selected from dexloxiglumide, psyllium husk, docusate sodium, bisacodyl, and phenolphthalein; the polymer resin is selected from psyllium, lipid lowering polymers, nonabsorbed polymer resins, and sodium binding polymers; the lipid lowering polymer is selected from: Colesevelam, Sevalmer, Cholestyramine; the nonabsorbed polymer resin is selected from: hyaluronic acid, polycarbophil calcium, polyvinyl acetate, polyvinyl pyrrolidone, polystyrene sulfate; the sodium-binding polymer is selected from: crosslinked polyvinylsulfamate polymer, N-(bis-phosphonic-ethyl) polyvinylamine polymer, poly- $\alpha$ -acrylic acid polymer, poly- $\alpha$ -fluoroacrylic acid polymer, polyvinylphosphoramidic polymer, polyvinylsulfamate polymer, polyvinylsulfamate/vinylsulfate copolymer, polyvinylsulfate polymer, polyvinylsulfonate polymer, polyvinylsulfonate polymer, vinylphosphonate/ $\alpha$ -fluoroacrylic acid copolymer, vinylphosphonate/ $\alpha$ -fluoroacrylic acid copolymer, or vinylphosphonate/acrylic acid copolymer; the sodium-binding polymer is administered as core-shell composition which further comprises a semi-permeable shell; the semi-permeable shell comprises at least one of a poly-11 trimethylammoniumundecylmethacrylate polymer, a styrene-vinylpyridine polymer, 11-dimethyl-aminodecylmethacrylate/laurylmethacrylate copolymer, or a polyallylamine/polystyrene sulfonate polymer.

In certain embodiments the method includes: administering an anti-diabetic agent; administering an anti-obesity agent; administering potassium or a salt thereof; administering a PDE inhibitor (e.g., PDE5-specific PDE inhibitor, a cGMP-specific PDE inhibitor or a cAMP-specific PDE inhibitor); administering a polymer resin (e.g., psyllium, a nonabsorbed polymer resin, a nonabsorbed polymer resin is selected from hyaluronic acid, polycarbophil calcium, polyvinyl acetate, and polyvinyl pyrrolidone; a polymer resin that is a lipid lowering polymer, a lipid lowering polymer is selected from: colesevelam or sevalmer); administering an anti-hypertensive agent (e.g., an antihypertensive agent is selected from: a diuretic, an inhibitor of angiotensin converting enzyme, an angiotensin II receptor antagonist, a calcium channel blocker, a beta-adrenergic antagonist, alpha-adrenergic antagonist, a renin inhibitor, and an aldosterone antagonist); administering two or more anti-hypertensive agents wherein the two or more antihypertensive agents are independently selected from: a diuretic, an inhibitor of angiotensin converting enzyme, an angiotensin II receptor antagonist, a calcium channel blocker, a beta-adrenergic antagonist, alpha-adrenergic antagonist, a renin inhibitor, and an aldosterone antagonist. In some cases: the anti-hypertensive agent is a diuretic; the diuretic is selected from the group consisting of: a loop diuretic, a thiazide, a potassium sparing agent, and an osmotic diuretic; the diuretic is a loop diuretic; the diuretic is furosemide, bumetanide, ethacrynic or torsemide; the diuretic is a thiazide; the thiazide is bendroflumethiazide, hydrochlorothiazide, indapamide, chlortalidone or metolazone; the diuretic is a potassium sparing agent; the potassium sparing agent is amiloride or triamterene; the diuretic is an osmotic diuretic; the osmotic diuretic is glucose or mannitol; the antihypertensive agent is an angiotensin converting enzyme inhibitor; the angiotensin converting enzyme inhibitor is selected from: Benazepril (Lotensin), Captopril (Capoten), Enalapril/Enalaprilat (Vasotec), Fosinopril (Monopril), Lisinopril (Zestril and Prinivil), Moexipril (Univasc), Perindopril (Aceon), Quinapril (Accupril), Ramipril (Altace), and Trandolapril (Mavik); the antihypertensive agent is an angiotensin II receptor antagonist; the angiotensin II receptor antagonist is selected from: Candesartan, Eprosartan, Irbesartan, Losartan, Olmesartan, Telmisartan, Valsartan; the antihypertensive agent is a calcium channel blocker; the calcium channel blocker is selected from: Amlodipine (Norvasc), Felodipine (Plendil), Nicardipine (Cardene), Nifedipine (Procardia, Adalat), Nimodipine (Nimotop), Nisoldipine (Sular), Nitrendipine (Cardif, Nitrepin), and Lacidipine

- (Motens), Lercanidipine (Zanidip), Verapamil (Calan, Isoptin), Gallopamil (D600), Diltiazem (Cardizem), and Menthol (mint oil); the calcium channel blocker is Amlodipine; the antihypertensive agent is a beta-adrenergic antagonist; the beta-adrenergic antagonist is selected from: Dichloroisoprenaline, Practolol, Pronethalol, Alprenolol, Carteolol, Levobunolol,
- 5 Mepindolol, Metipranolol, Nadolol, Oxprenolol, Penbutolol, Pindolol, Propranolol, Sotalol, Timolol, Acebutolol, Atenolol, Betaxolol, Bisoprolol, Esmolol, Metoprolol, Nebivolol, Carvedilol, Celiprolol, Labetalol, and Butoxamine; the antihypertensive agent is an alpha-adrenergic antagonist; the alpha-adrenergic antagonist is selected from:
- 10 Doxazosin (Cardura), Prazosin (Minipress), Phenoxybenzamine, Phentolamine (Regitine), Tamsulosin (Flomaxtra/Flomax), Alfuzosin (Uroxatral), and Terazosin (Hytrin); the antihypertensive agent is a renin inhibitor; the renin inhibitor is selected from: Tekturna® (Rasilez and Aliskiren) and SPP635; the antihypertensive agent is an aldosterone antagonist; and the aldosterone antagonist is Spironolactone, Canrenone, or Eplerenone.
- 15 In some case the method includes: administering a lipid altering agent. In certain cases: the lipid altering agent is a cholesterol lowering agent; the agent lowers low density cholesterol; the lipid altering agent is selected from the group consisting of: a statin; a fibrate; niacin; a CETP inhibitor; a MTP inhibitor; a cholesterol absorption inhibitor; a squalene synthesis inhibitor; and a bile acid sequestrant; the lipid altering agent is a statin; the statin is chosen from simvastatin,
- 20 rosuvastatin and atorvastatin; the lipid altering agent is a cholesterol absorption inhibitor; the cholesterol absorption inhibitor is ezetimibe; the lipid altering agent is a bile acid sequestrant; the bile acid sequestrant is chosen from cholestyramine, colesevelam and colestipol; the lipid altering agent is a fibrate; and the fibrate is fenofibrate.
- 25 In some cases the method includes: administering misoprostol and psyllium; administering methyl 7-[(1R,2R,3R)-3-hydroxy-2-[(E,4S)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate; administering psyllium and a peptide that activates the guanylate cyclase C receptor. In some cases the peptides is selected from:
- 30 Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;

- Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 5 d-Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 d-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 d-Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 d-Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 d-Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 10 d-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 d-Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 d-Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;  
 Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;  
 15 Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;  
 Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;  
 Val Tyr Ile Gln Tyr Gln Gly Phe Arg Val Gln Leu Glu Ser Met Lys Lys Leu Ser Asp Leu Glu Ala Gln Trp Ala Pro;  
 Ser Pro Arg Leu Gln Ala Gln Ser Leu Leu Pro Ala Val Cys His His Pro Ala Leu Pro Gln Asp Leu Gln Pro Val Cys;  
 Ala Ser Gln Glu Ala Ser Ser Ile Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala  
 20 Cys Thr Gly Cys Leu;  
 Val Tyr Ile Gln Tyr Gln Gly Phe Arg Val Gln Leu Glu Ser Met Lys Lys Leu Ser Asp Leu Glu Ala Gln Trp Ala Pro;  
 Ser Pro Arg Leu Gln Ala Gln Ser Leu Leu Pro Ala Val Cys His His Pro Ala Leu Pro Gln Asp Leu Gln Pro Val Cys;  
 Ala Ser Gln Glu Ala Ser Ser Ile Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala  
 Cys Thr Gly Cys Leu;  
 25 Pro Gly Thr Cys Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys;  
 Val Thr Val Gln Asp Gly Asn Phe Ser Phe Ser Leu Glu Ser Val Lys Lys Leu Lys Asp Leu Gln Glu Pro Gln Glu  
 Pro;  
 Arg Val Gly Lys Leu Arg Asn Phe Ala Pro Ile Pro Gly Glu Pro Val Val Pro Ile Leu Cys Ser Asn Pro Asn Phe Pro;  
 Glu Gln Leu Lys Pro Leu Cys Lys Glu Pro Asn Ala Gln Glu Ile Leu Gln Arg Leu Glu Glu Ile Ala Glu Asp Pro Gly  
 30 Thr Cys Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys;  
 Met Pro Ser Thr Gln Tyr Ile Arg Arg Pro Ala Ser Ser Tyr Ala Ser Cys Ile Trp Cys Thr Thr Ala Cys Ala Ser Cys His  
 Gly Arg Thr Thr Lys Pro Ser Leu Ala Thr;  
 Ala Asp Leu Cys Glu Ile Cys Ala Phe Ala Ala Cys Thr Gly Cys Leu;  
 Val Gln Val Glu Glu Gly Gly Phe Ser Phe Pro Leu Asp Ala Val Lys Lys Leu Glu Glu Leu Met Gly Val Asp Met;  
 35 Thr Val Lys Gln Ser Pro Arg Leu Ala Lys Thr Ser Thr Thr Ala Val Cys Thr Asn Pro Asp Leu Pro Ala Val Phe Leu;  
 Pro Leu Cys Lys Ser Lys Gly Ala Ala Asn Ser Phe Phe Arg Leu Gly Phe Val Ala Ala Arg Ala Asp Leu Cys Glu Ile  
 Cys Ala Phe Ala Ala Cys Thr Gly Cys Leu;

Gln Glu Glu Cys Glu Leu Cys Ile Asn Met Ala Cys Thr Gly Tyr;  
 Val Tyr Ile Gln Tyr Glu Gly Phe Gln Val Asn Leu Asp Ser Val Lys Lys Leu Asp Lys Leu Leu Glu Gln Leu Arg  
 Gly;  
 Phe His His Gln Met Gly Asp Gln Arg Asp Pro Ser Ile Leu Cys Ser Asp Pro Ala Leu Pro Ser Asp Leu Gln Pro Val;  
 5 and  
 Cys Glu Asn Ser Gln Ala Val Asn Ile Phe Arg Ala Leu Arg Tyr Ile Asn Gln Glu Glu Cys Glu Leu Cys Ile Asn Met  
 Ala Cys Thr Gly Tyr

In some cases: the disorder is associated with fluid retention; the disorder is associated with salt  
 10 retention; the disorder is a cardiovascular disorder; the cardiovascular disorder is  
 cardiomyopathy; the cardiomyopathy is associated with Chagas disease; the cardiovascular  
 disorder is hypertension; the hypertension is salt-sensitive hypertension; the cardiovascular  
 disorder is congestive heart failure; the cardiovascular disorder is cardiac hypertrophy; the  
 cardiovascular disorder is a heart attack; the cardiovascular disorder is stroke; the patient is  
 15 suffering from salt-sensitive hypertension; the patient is suffering from congestive heart failure;  
 the patient is suffering from cardiac hypertrophy; the patient has suffered a heart attack; the  
 patient has suffered a stroke; the patient is salt sensitive; the patient is suffering from  
 hypertension; the disorder is a renal disorder; the renal disorder is selected from chronic renal  
 failure or acute renal failure; the disorder is associated with ascites; the disorder is cirrhosis; the  
 20 patient is suffering from alcoholism; the disorder hepatitis; the hepatitis is chronic hepatitis; the  
 hepatitis is severe alcoholic hepatitis without cirrhosis; the disorder is Budd-Chiari syndrome;  
 and the disorder is constrictive pericarditis.

Also described herein is a pharmaceutical composition comprising: a first agent that is an anti-  
 25 hypertensive agent and a second agent selected from: a) an agent that reduces sodium absorption  
 in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both  
 reduces sodium absorption in the intestine and increases anion secretion in the intestine.

In various embodiments: the second agent reduces sodium absorption in the intestine; the second  
 30 agent increases anion secretion in the intestine; the second agent both reduces sodium absorption  
 in the intestine and increases anion secretion in the intestine; the second agent is selected from:  
 a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator, c) a

prostanoid, d) a chloride channel activator, e) a 5HT4 agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels, k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin;

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Also described herein is a pharmaceutical composition comprising: a first agent that is an anti-diabetic agent and a second agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

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Also described herein is a pharmaceutical composition comprising: an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine. In various embodiments: the second agent reduces sodium absorption in the intestine; the second agent increases anion secretion in the intestine; the second agent both reduces sodium absorption in the intestine and increases anion secretion in the intestine; the second agent is selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator, c) a prostanoid, d) a chloride channel activator, e) a 5HT4 agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels, k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin.

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Also disclosed is a pharmaceutical composition comprising: potassium or a salt thereof and a second agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine. In various embodiments: the second agent reduces sodium absorption in the intestine; the second agent increases anion secretion in the intestine; the second agent both reduces sodium absorption in the intestine and increases anion secretion in the intestine; the second agent is selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator, c) a prostanoid,

d) a chloride channel activator, e) a 5HT4 agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels, k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin.

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Also disclosed is a pharmaceutical composition comprising: a first agent that is a PDE inhibitor and a second agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine. In various

10 embodiments: the second agent reduces sodium absorption in the intestine; the second agent increases anion secretion in the intestine; the second agent both reduces sodium absorption in the intestine and increases anion secretion in the intestine; the second agent is selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator, c) a prostanoid, d) a chloride channel activator, e) a 5HT4 agonist, f) a cyclic nucleotide, g) a sodium transport  
15 inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels, k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin.

Some salt sensitive individuals may have a greater than 5-mm Hg decrease in mean blood  
20 pressure when daily salt intake is reduced from 260 mmol to 20 mmol. There are various tests for assessing salt sensitivity (see, for example, de la Sierra et al. 2002 Journal of Human Hypertension 16:256).

In various embodiments: the method comprises administering to the patient an agent that  
25 reduces sodium absorption in the intestine or increases anion secretion in the intestine; the agent comprises at least one GCC receptor agonist; the GCC receptor agonist is a peptide (e.g., the peptide comprises guanylin or uroguanylin; the peptide comprises ST peptide; the peptide comprises the amino acid sequence Cys Cys Glu Xaa Cys Cys Asn Pro Ala Cys Thr Gly Cys wherein Xaa is selected from Phe, Trp and Tyr (SEQ ID NO:A); the peptide comprises the  
30 amino acid sequence Cys Cys Glu Xaa Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr wherein Xaa is selected from Phe, Trp and Tyr (SEQ ID NO:B)); the agent comprises a prostanoid,



prostaglandin E1 or a prostaglandin E1 derivative; the prostaglandin E1 derivative is misoprostol; the prostaglandin E1 derivative is lubiprostone; the agent comprises at least one 5HT4 receptor agonist; the 5HT4 receptor agonist is Zelnorm; the agent comprises at least one cyclic nucleotide; the cyclic nucleotide is cGMP; the cyclic nucleotide is cAMP.

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In other embodiments: the disorder is a cardiovascular disorder; the cardiovascular disorder is cardiomyopathy; the cardiomyopathy is associated with Chagas disease; the cardiovascular disorder is hypertension; the hypertension is salt-sensitive hypertension; the cardiovascular disorder is congestive heart failure; the cardiovascular disorder is cardiac hypertrophy; the cardiovascular disorder is a heart attack; the cardiovascular disorder is stroke; the patient has been diagnosed with a cardiovascular disorder; the cardiovascular disorder is cardiomyopathy; the cardiomyopathy is associated with Chagas disease; the patient is suffering from a cardiovascular disorder; the patient is suffering from salt-sensitive hypertension; the patient is suffering from congestive heart failure; the patient is suffering from cardiac hypertrophy; the patient has suffered a heart attack; the patient has suffered a stroke; the patient is salt sensitive and/or the patient is suffering from hypertension; the disorder is a renal disorder (e.g., chronic renal failure or acute renal failure); the patient has been diagnosed with a renal disorder; the disorder is associated with ascites; the disorder is cirrhosis; the patient is suffering from alcoholism; the disorder hepatitis; the hepatitis is chronic hepatitis; the hepatitis is severe alcoholic hepatitis without cirrhosis; the disorder is congestive heart failure; the disorder is Budd-Chiari syndrome; the disorder is constrictive pericarditis; and the patient is suffering from congestive heart failure.

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In various embodiments the method further comprises administering an agent that reduces sodium absorption in the intestine and/or increases anion secretion and an agent useful in the treatment of congestive heart failure. For example, the agent useful in the treatment of congestive heart failure is selected from: Nesiritide, Dobutamine, Milrinone, Levosimendan (Simdax®), Adenosine, an adenosine analog, an agent which increases cellular availability of adenosine, an adenosine A<sub>2</sub> receptor agonist, an adenosine transport inhibitor, and an adenosine deaminase inhibitor; and the adenosine analog is selected from: N<sup>6</sup>-[(1,2-dihydro-1-acenaphthyl)enyl)methyl]adenosine, Dipyridamole and iodotulericidin

In various embodiments the method further comprises administering an anti-hypertensive agent. For example: the antihypertensive agent is selected from: a diuretic, an inhibitor of angiotensin converting enzyme, an angiotensin II receptor antagonist; a calcium channel blocker; a beta-adrenergic antagonist; alpha-adrenergic antagonist; a renin inhibitor; and an aldosterone antagonist; the method comprises administering two or more anti-hypertensive agents wherein the two or more antihypertensive agents are independently selected from: a diuretic, an inhibitor of angiotensin converting enzyme, an angiotensin II receptor antagonist; a calcium channel blocker; a beta-adrenergic antagonist; alpha-adrenergic antagonist; a renin inhibitor; and an aldosterone antagonist; the agent that reduces sodium absorption in the intestine or increases anion secretion is co-administered with the antihypertensive agent; the co-administered agents are combined in a unit dosage form; the diuretic is selected from the group consisting of: a loop diuretic, a thiazide, a potassium sparing agent, and an osmotic diuretic; the loop diuretic is furosemide, bumetanide, ethacrynic or torsemide; the diuretic is a thiazide (e.g., bendroflumethiazide, hydrochlorothiazide, indapamide, chlortalidone or metolazone); the diuretic is a potassium sparing agent (e.g., amiloride or triamterene); the diuretic is an osmotic diuretic; the osmotic diuretic is glucose or mannitol; the antihypertensive agent is an angiotensin converting enzyme inhibitor; angiotensin converting enzyme inhibitor is selected from: Benazepril (Lotensin), Captopril (Capoten), Enalapril/Enalaprilat (Vasotec), Fosinopril (Monopril), Lisinopril (Zestril and Prinivil), Moexipril (Univasc), Perindopril (Aceon), Quinapril (Accupril), Ramipril (Altace), and Trandolapril (Mavik); the antihypertensive agent is an angiotensin II receptor antagonist; the angiotensin II receptor antagonist is selected from: Candesartan, Eprosartan, Irbesartan, Losartan, Olmesartan, Telmisartan, Valsartan; the antihypertensive agent is a calcium channel blocker; the calcium channel blocker is selected from: Amlodipine (Norvasc), Felodipine (Plendil), Nifedipine (Cardene), Nifedipine (Procardia, Adalat), Nimodipine (Nimotop), Nisoldipine (Sular), Nitrendipine (Cardif, Nitrepin), and Lacidipine (Motens), Lercanidipine (Zanidip), Verapamil (Calan, Isoptin), Gallopamil (D600), Diltiazem (Cardizem), and Menthol (mint oil); the calcium channel blocker is Amlodipine; the antihypertensive agent is a beta-adrenergic antagonist; the beta-adrenergic antagonist is selected from: Dichloroisoprenaline, Practolol, Pronethalol, Alprenolol, Carteolol, Levobunolol, Mepindolol, Metipranolol, Nadolol, Oxprenolol, Penbutolol, Pindolol, Propranolol, Sotalol,

Timolol, Acebutolol, Atenolol, Betaxolol, Bisoprolol, Esmolol, Metoprolol, Nebivolol, Carvedilol, Celiprolol, Labetalol, and Butoxamine; the antihypertensive agent is an alpha-adrenergic antagonist; the alpha-adrenergic antagonist is selected from: Doxazosin (Cardura), Prazosin (Minipress), Phenoxybenzamine, Phentolamine (Regitine), Tamsulosin (Flomaxtra/Flomax), Alfuzosin (Uroxatral), and Terazosin (Hytrin); the antihypertensive agent is a renin inhibitor; the renin inhibitor is selected from: Tekturna® (Rasilez and Aliskiren) and SPP635; the antihypertensive agent is an aldosterone antagonist; and the aldosterone antagonist is Spironolactone, Canrenone, or Eplerenone.

10 In some embodiments the method further comprises administering a lipid altering agent. For example: the lipid altering agent is a cholesterol lowering agent, the agent lowers low density cholesterol; the lipid altering agent is selected from the group consisting of: a statin; a fibrate; niacin; a CETP inhibitor; a MTP inhibitor; a cholesterol absorption inhibitor; a squalene synthesis inhibitor; and a bile acid sequestrant; the lipid altering agent is a statin; the statin is  
15 chosen from simvastatin, rosuvastatin and atorvastatin; the lipid altering agent is a cholesterol absorption inhibitor; the cholesterol absorption inhibitor is ezetimibe; the lipid altering agent is a bile acid sequestrant; the bile acid sequestrant is chosen from cholestyramine, colesevelam and colestipol; the lipid altering agent is a fibrate; and the fibrate is fenofibrate.

20 Also featured is a pharmaceutical composition comprising an agent that reduces sodium absorption in the intestine and/or increases anion secretion and an anti-hypertensive agent. In various embodiments: the antihypertensive agent is selected from: a diuretic, an inhibitor of angiotensin converting enzyme, an angiotensin II receptor antagonist; a calcium channel blocker; a beta-adrenergic antagonist; alpha-adrenergic antagonist; a renin inhibitor; and an aldosterone  
25 antagonist; the pharmaceutical composition comprises administering two or more anti-hypertensive agents wherein the two or more antihypertensive agents are independently selected from: a diuretic, an inhibitor of angiotensin converting enzyme, an angiotensin II receptor antagonist; a calcium channel blocker; a beta-adrenergic antagonist; alpha-adrenergic antagonist; a renin inhibitor; and an aldosterone antagonist; the diuretic is selected from the group consisting  
30 of: a loop diuretic, a thiazide, a potassium sparing agent, and an osmotic diuretic; the diuretic is a loop diuretic; the loop diuretic is furosemide, bumetanide, ethacrynic or torsemide; the diuretic is

a thiazide (e.g., the thiazide is bendroflumethiazide, hydrochlorothiazide, indapamide, chlortalidone or metolazone); the diuretic is a potassium sparing agent; the potassium sparing agents is amiloride or triamterene; the diuretic is an osmotic diuretic; osmotic diuretic is glucose or mannitol; the antihypertensive agent is an angiotensin converting enzyme inhibitor; the  
5 angiotensin converting enzyme inhibitor is selected from: Benazepril (Lotensin), Captopril (Capoten), Enalapril/Enalaprilat (Vasotec), Fosinopril (Monopril), Lisinopril (Zestril and Prinivil), Moexipril (Univase), Perindopril (Accon), Quinapril (Accupril), Ramipril (Altace), and Trandolapril (Mavik); the antihypertensive agent is an angiotensin II receptor antagonist; the  
10 angiotensin II receptor antagonist is selected from: Candesartan, Eprosartan, Irbesartan, Losartan, Olmesartan, Telmisartan, Valsartan; the antihypertensive agent is a calcium channel blocker.; the calcium channel blocker is selected from: Amlodipine (Norvasc), Felodipine (Plendil), Nifedipine (Cardene), Nifedipine (Procardia, Adalat), Nimodipine (Nimotop), Nisoldipine (Sular), Nitrendipine (Cardif, Nitrepin), and Lacidipine (Motens), Lercanidipine (Zanidip), Verapamil (Calan, Isoptin), Gallopamil (D600), Diltiazem (Cardizem), and Menthol  
15 (mint oil); the calcium channel blocker is Amlodipine; the antihypertensive agent is a beta-adrenergic antagonist; the beta-adrenergic antagonist is selected from: Dichloroisoprenaline, Practolol, Pronethalol, Alprenolol, Carteolol, Levobunolol, Mepindolol, Metipranolol, Nadolol, Oxprenolol, Penbutolol, Pindolol, Propranolol, Sotalol, Timolol, Acebutolol, Atenolol, Betaxolol, Bisoprolol, Esmolol Metoprolol, Nebivolol, Carvedilol, Celiprolol, Labetalol, and  
20 Butoxamine; the antihypertensive agent is an alpha-adrenergic antagonist; the alpha-adrenergic antagonist is selected from: Doxazosin (Cardura), Prazosin (Minipress), Phenoxybenzamine, Phentolamine (Regitine), Tamsulosin (Flomaxtra/Flomax), Alfuzosin (Uroxatral), and Terazosin (Hytrin); the antihypertensive agent is a renin inhibitor; the renin inhibitor is selected from: Tekturna® (Rasilez and Aliskiren) and SPP635; the antihypertensive agent is an aldosterone  
25 antagonist; and the aldosterone antagonist is Spironolactone, Canrenone, or Eplerenone.

Also featured is a pharmaceutical composition comprising an agent that reduces sodium absorption in the intestine and/or increases anion secretion and a lipid altering agent. In various  
embodiments: the lipid altering agent is a cholesterol lowering agent; the agent lowers low  
30 density cholesterol; the lipid altering agent is selected from the group consisting of: a statin; a fibrate; niacin; a CETP inhibitor; a MTP inhibitor; a cholesterol absorption inhibitor; a squalene

synthesis inhibitor; and a bile acid sequestrant; the lipid altering agent is a statin; the statin is chosen from simvastatin, rosuvastatin and atorvastatin; the lipid altering agent is a cholesterol absorption inhibitor; the cholesterol absorption inhibitor is ezetimibe; the lipid altering agent is a bile acid sequestrant; the bile acid sequestrant is chosen from cholestyramine, colesevelam and colestipol; the lipid altering agents is a fibrate; and the fibrate is fenofibrate.

Also featured is a pharmaceutical composition comprising an agent that reduces sodium absorption in the intestine and/or increases anion secretion and an agent useful in the treatment of congestive heart failure. In various embodiments: the agent useful in the treatment of congestive heart failure is selected from: Nesiritide, Dobutamine, Milrinone, Levosimendan (Simdax®), Adenosine, an adenosine analog, an agent which increases cellular availability of adenosine, an adenosine A<sub>2</sub> receptor agonist, an adenosine transport inhibitor, and an adenosine deaminase inhibitor; the adenosine analog is selected from: N<sup>6</sup>-[(1,2-dihydro-1-acenaphthyl)enyl]methyl]adenosine, Dipyridamole and iodotulercidin

In the various pharmaceutical compositions: the agent that reduces sodium absorption in the intestine or increases anion secretion comprises at least one GCC receptor agonist; the GCC receptor agonist is a peptide; the peptide comprises guanylin or uroguanylin; the peptide comprises ST peptide; the peptide comprises the amino acid sequence Cys Cys Glu Xaa Cys Cys Asn Pro Ala Cys Thr Gly Cys wherein Xaa is selected from Phe, Trp and Tyr (SEQ ID NO:A); the peptide comprises the amino acid sequence Cys Cys Glu Xaa Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr wherein Xaa is selected from Phe, Trp and Tyr (SEQ ID NO:B); the peptide comprises an amino acid sequence selected from:

Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 d-Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 d-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;

- d-Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
d-Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
d-Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
d-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
5 d-Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
d-Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;  
Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;  
Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;  
10 Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;  
Val Tyr Ile Gln Tyr Gln Gly Phe Arg Val Gln Leu Glu Ser Met Lys Lys Leu Ser Asp Leu Glu Ala Gln Trp Ala Pro;  
Ser Pro Arg Leu Gln Ala Gln Ser Leu Leu Pro Ala Val Cys His His Pro Ala Leu Pro Gln Asp Leu Gln Pro Val Cys;  
Ala Ser Gln Glu Ala Ser Ser Ile Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala  
Cys Thr Gly Cys Leu;  
15 Val Tyr Ile Gln Tyr Gln Gly Phe Arg Val Gln Leu Glu Ser Met Lys Lys Leu Ser Asp Leu Glu Ala Gln Trp Ala Pro;  
Ser Pro Arg Leu Gln Ala Gln Ser Leu Leu Pro Ala Val Cys His His Pro Ala Leu Pro Gln Asp Leu Gln Pro Val Cys;  
Ala Ser Gln Glu Ala Ser Ser Ile Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala  
Cys Thr Gly Cys Leu;  
Pro Gly Thr Cys Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys;  
20 Val Thr Val Gln Asp Gly Asn Phe Ser Phe Ser Leu Glu Ser Val Lys Lys Leu Lys Asp Leu Gln Glu Pro Gln Glu  
Pro;  
Arg Val Gly Lys Leu Arg Asn Phe Ala Pro Ile Pro Gly Glu Pro Val Val Pro Ile Leu Cys Ser Asn Pro Asn Phe Pro;  
Glu Glu Leu Lys Pro Leu Cys Lys Glu Pro Asn Ala Gln Glu Ile Leu Gln Arg Leu Glu Glu Ile Ala Glu Asp Pro Gly  
Thr Cys Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys;  
25 Met Pro Ser Thr Gln Tyr Ile Arg Arg Pro Ala Ser Ser Tyr Ala Ser Cys Ile Trp Cys Thr Thr Ala Cys Ala Ser Cys His  
Gly Arg Thr Thr Lys Pro Ser Leu Ala Thr;  
Ala Asp Leu Cys Glu Ile Cys Ala Phe Ala Ala Cys Thr Gly Cys Leu;  
Val Gln Val Glu Glu Gly Gly Phe Ser Phe Pro Leu Asp Ala Val Lys Lys Leu Glu Glu Leu Met Gly Val Asp Met;  
Thr Val Lys Gln Ser Pro Arg Leu Ala Lys Thr Ser Thr Thr Ala Val Cys Thr Asn Pro Asp Leu Pro Ala Val Phe Leu;  
30 Pro Leu Cys Lys Ser Lys Gly Ala Ala Asn Ser Phe Phe Arg Leu Gly Phe Val Ala Ala Arg Ala Asp Leu Cys Glu Ile  
Cys Ala Phe Ala Ala Cys Thr Gly Cys Leu;  
Gln Glu Glu Cys Glu Leu Cys Ile Asn Met Ala Cys Thr Gly Tyr;  
Val Tyr Ile Gln Tyr Glu Gly Phe Gln Val Asn Leu Asp Ser Val Lys Lys Leu Asp Lys Leu Leu Gln Gln Leu Arg  
Gly;  
35 Phe His His Gln Met Gly Asp Gln Arg Asp Pro Ser Ile Leu Cys Ser Asp Pro Ala Leu Pro Ser Asp Leu Gln Pro Val;  
and

Cys Glu Asn Ser Gln Ala Val Asn Ile Phe Arg Ala Leu Arg Tyr Ile Asn Gln Glu Glu Cys Glu Leu Cys Ile Asn Met  
Ala Cys Thr Gly Tyr.

In the various pharmaceutical compositions: the agent that reduces sodium absorption in the  
5 intestine and/or increases anion secretion comprises a prostanoid, prostaglandin E1 or a  
prostaglandin E1 derivative; the prostaglandin E1 derivative is misoprostol; the prostaglandin E1  
derivative is lubiprostone; the agent that reduces sodium absorption in the intestine and/or  
increases anion secretion comprises at least one 5HT4 receptor agonist; the 5HT4 receptor  
agonist is Zelnorm; the agent that reduces sodium absorption in the intestine and/or increases  
10 anion secretion comprises at least one cyclic nucleotide; the cyclic nucleotide is cGMP; and the  
cyclic nucleotide is cAMP.

In various cases the method comprises administering an agent that reduces sodium absorption in  
the intestine and/or increases anion secretion and an agent useful in the treatment of congestive  
15 heart failure. For example, the agent useful in the treatment of congestive heart failure is  
selected from: Nesiritide, Dobutamine, Milrinone, Levosimendan (Simdax®), Adenosine, an  
adenosine analog, an agent which increases cellular availability of adenosine, an adenosine A<sub>2</sub>  
receptor agonist, an adenosine transport inhibitor, and an adenosine deaminase inhibitor; and the  
adenosine analog is selected from: N<sup>6</sup>-[(1,2-dihydro-1-acenaphthyl)enyl]methyl]adenosine,  
20 Dipyridamole and iodotulercidin

#### DESCRIPTION OF DRAWINGS

Figure 1 depicts the results of a study of the effect of lubiprostone of urinary sodium and urine  
volume in rats.

25 Figure 2 depicts the results of a study on the effect of demonstrate ST peptide (SEQ ID NO: 1:  
CCELCCNPACTGCY) at 300 µg/kg on urine sodium and urine volume in rats.

Figure 3 depicts the results of a study on the effect of demonstrate ST peptide (SEQ ID NO: 1:  
CCELCCNPACTGCY) at 1000 µg/kg on urine sodium and urine volume in rats.

**DETAILED DESCRIPTION**

A major function of salt is to regulate blood volume and pressure including the flexibility of the blood vessels. When blood volume increases or the blood vessel walls don't expand enough, blood pressure increases. Blood pressure is an indicator of the incidence of cardiovascular events like heart attacks and strokes. Salt sensitivity is a measure of how blood pressure responds to a decrease in salt intake. Salt-sensitive individuals experience an exaggerated blood pressure elevation when they are given a salt load. Among those who already have hypertension, salt sensitivity increases the risk of developing cardiovascular conditions such as heart attack, left ventricular hypertrophy and kidney problems. Recent studies by the National Institutes of Health suggest that salt sensitivity increases the risk of death even for those with normal blood pressure. In the absence of hypertension, salt may increase the reactivity of platelets, thus causing abnormal blood clot formation which in turn leads to cardiovascular events like stroke, heart attack, and kidney disease directly. Although global initiatives have been undertaken to reduce dietary salt intake voluntary compliance is thus far, unachievable. Therefore, agents that reduce sodium absorption (e.g. dietary sodium) or increase anion secretion in the intestine are useful in the treatment of cardiovascular disease and other disorders associated with fluid and/or sodium retention.

The agents described herein can be used alone or in combination therapy to prevent and/or treat cardiovascular disorders, including: hypertension (for example, including salt sensitive hypertension), cardiomyopathy (for example, associated with Chagas disease), cardiac hypertrophy, heart attack, stroke, congestive heart failure (including heart failure at any of stages I-IV according to New York Heart Association (NYHA) Functional Classification), and bone wasting (e.g. bone wasting associated with cardiovascular disorders). Congestive heart failure is a hemodynamic disorder resulting from impairment of the ability of the ventricle to fill with and/or eject blood. The disorder is commonly characterized by shortness of breath, fatigue, limited exercise tolerance, and fluid retention (both pulmonary congestion and peripheral edema). Congestive heart failure is generally progressive and can result in Class IV heart failure (NYHA Heart Failure Classification) in which any physical activity brings on symptoms such as shortness of breath, and symptoms can occur even when the patient is at rest. Patients with symptoms of advanced heart failure are treated by tightly controlling fluid status and are often



administered intravenous peripheral vasodilators and/or positive inotropic agents. Patients suffering Class IV heart failure should be at complete rest (confined to a bed or chair).

5 The agents described herein can be used alone or in combination therapy to prevent and/or treat renal disorders associated with fluid or salt retention. Renal disorders includes renal failure (including acute and chronic renal failure), renal insufficiency, nephrotic edema, glomerulonephritis, pyelonephritis, kidney failure, chronic renal failure, nephritis, nephrosis, uremia, immune renal disease, acute nephritic syndrome, rapidly progressive nephritic syndrome, nephrotic syndrome, chronic nephritic/proteinuric syndrome, tubulointerstitial disease,  
10 nephrotoxic disorders, renal cortical necrosis, polycystic kidney disease, and hereditary nephritis, along with any disease or disorder that relates to the renal system and related disorders, as well as symptoms indicative of, or related to, renal or kidney disease and related disorders.

The agents described herein can be used alone or in combination therapy to prevent and/or treat  
15 disorders associated with ascites. Ascites (also known as peritoneal cavity fluid, peritoneal fluid excess, hydroperitoneum or abdominal dropsy) is an accumulation of fluid in the peritoneal cavity. Currently, salt restriction is a baseline step in therapy for ascites related disorders including liver disorders such as cirrhosis (e.g. cirrhosis associated with alcoholism, viral hepatitis, or cryptogenic cirrhosis), chronic hepatitis, severe alcoholic hepatitis without cirrhosis,  
20 Budd-Chiari syndrome (a veno-occlusive disease involving obstruction of the hepatic vein), and cardiovascular disorders such as heart failure and constrictive pericarditis.

Without being bound by any particular theory, in the case of disorders associated with fluid or salt retention, the methods may elicit one or more of decreased sodium absorption and/or  
25 increased anion absorption.

#### Useful Agents

Agents that reduce sodium absorption in the intestine or increases anion secretion in the intestine include, but are not limited to, guanylate cyclase receptor C agonists, soluble guanylate cyclase modulators, prostanoids including prostaglandin E and derivatives thereof, chloride channel  
30 activators (e.g. Amitiza® (lubiprostone)), 5HT4 agonists, cyclic nucleotides, laxatives, CFTR

(cystic fibrosis transmembrane conductance regulator) modulators, agents which affect cAMP levels, sodium transport inhibitors (e.g. sodium channel inhibitors such as amiloride), phosphodiesterase inhibitors, renin inhibitors and aldosterone antagonists, potassium, polymer resins, combinations thereof.

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#### Guanylate cyclase-C agonists

The guanylate cyclase-C (GC-C) receptor (reviewed by Lucas et al. 2000 *Pharmacol. Rev* 52:375-414 and Vaandrager et al. 2002 *Molecular and Cellular Biochemistry* 230:73-83) is a key regulator in mammals of intestinal function (although low levels of GC-C have been detected in  
10 other tissues). GC-C responds to the endogenous hormones, guanylin and uroguanylin, and to enteric bacterial peptides from the heat stable enterotoxin family (ST peptides). When agonists bind to GC-C, there is an elevation of the second messenger, cyclic GMP, and an increase in chloride and bicarbonate secretion, resulting in an increase in intestinal fluid secretion.

15 In addition to being expressed in the intestine by gastrointestinal epithelial cells, GC-C is expressed in extra-intestinal tissues including kidney, lung, pancreas, pituitary, adrenal, developing liver and gall bladder (reviewed in Vaandrager 2002 *Mol Cell Biochem* 230:73-83, Kulaksiz et al. 2004, *Gastroenterology* 126:732-740) and male and female reproductive tissues (reviewed in Vaandrager 2002 *Mol Cell Biochem* 230:73-83). This suggests that the GC-C  
20 receptor agonists can be used in the treatment of disorders outside the GI tract, for example, congestive heart failure and benign prostatic hyperplasia.

In humans, the GC-C receptor is activated by guanylin (Gn) (U.S. 5,969,097), uroguanylin (Ugn) (U.S. 5,140,102) and lymphoguanylin (Forte et al. 1999 *Endocrinology* 140:1800-1806).

25 Interestingly, these agents are 10-100 fold less potent than a class of bacterially derived peptides, termed ST (reviewed in Gianella 1995 *J Lab Clin Med* 125:173-181). ST peptides are considered super agonists of GC-C and are very resistant to proteolytic degradation. In bacteria, ST peptides are derived from a preproprotein that generally has at least 70 amino acids. The pre and pro regions are cleaved as part of the secretion process, and the resulting mature protein,  
30 which generally includes fewer than 20 amino acids, is biologically active.

Among the known bacterial ST peptides are: *E. coli* ST Ib (Moseley et al. 1983 *Infect. Immun.* 39:1167) having the mature amino acid sequence Asn Ser Ser Asn Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr (SEQ ID NO: \_\_\_\_); *E. coli* ST Ia (So and McCarthy 1980 *Proc. Natl. Acad. Sci. USA* 77:4011) having the mature amino acid sequence Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys Tyr (SEQ ID NO:7). *E. coli* ST I\* (Chan and Giannella 1981 *J. Biol. Chem.* 256:7744) having the mature amino acid sequence Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Tyr Pro Ala Cys Ala Gly Cys Asn (SEQ ID NO: \_\_\_\_); *C. freundii* ST peptide (Guarino et al. 1989b *Infect. Immun.* 57:649) having the mature amino acid sequence Asn Thr Phe Tyr Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Ala Gly Cys Tyr (SEQ ID NO: \_\_\_\_); *Y. enterocolitica* ST peptides, Y-ST(Y-STa), Y-STb, and Y-STc (reviewed in Huang et al. 1997 *Microb. Pathog.* 22:89) having the following pro-form amino acid sequences: Gln Ala Cys Asp Pro Pro Ser Pro Pro Ala Glu Val Ser Ser Asp Trp Asp Cys Cys Asp Val Cys Cys Asn Pro Ala Cys Ala Gly Cys (SEQ ID NO: \_\_\_\_ ) (as well as a Ser-7 to Leu-7 variant of Y-STa (SEQ ID NO: \_\_\_\_), (Takao et al. 1985 *Eur. J. Biochem.* 152:199)); Lys Ala Cys Asp Thr Gln Thr Pro Ser Pro Ser Glu Glu Asn Asp Asp Trp Cys Cys Glu Val Cys Cys Asn Pro Ala Cys Ala Gly Cys (SEQ ID NO: \_\_\_\_); Gln Glu Thr Ala Ser Gly Gln Val Gly Asp Val Ser Ser Ser Thr Ile Ala Thr Glu Val Ser Glu Ala Glu Cys Gly Thr Gln Ser Ala Thr Thr Gln Gly Glu Asn Asp Trp Asp Trp Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Phe Gly Cys (SEQ ID NO: \_\_\_\_), respectively; *Y. kristensenii* ST peptide having the mature amino acid sequence Ser Asp Trp Cys Cys Glu Val Cys Cys Asn Pro Ala Cys Ala Gly Cys (SEQ ID NO: \_\_\_\_); *V. cholerae* non-01 ST peptide (Takao et al. (1985) *FEBS lett.* 193:250) having the mature amino acid sequence Ile Asp Cys Cys Glu Ile Cys Cys Asn Pro Ala Cys Phe Gly Cys Leu Asn (SEQ ID NO: \_\_\_\_); and *V. mimicus* ST peptide (Arita et al. 1991 *FEMS Microbiol. Lett.* 79:105) having the mature amino acid sequence Ile Asp Cys Cys Glu Ile Cys Cys Asn Pro Ala Cys Phe Gly Cys Leu Asn (SEQ ID NO: \_\_\_\_). The immature (including pre and pro regions) form of *E. coli* ST-1A (ST-P) protein has the sequence: mkkllmlaifsvlsfpsfsqstesldsskekitletkkedvkvknnsekksennmntfyceelcnpacagcy (SEQ ID NO: \_\_\_\_; see GenBank® Accession No. P01559 (gi:123711)). The pre sequence extends from aa 1-19. The pro sequence extends from aa 20-54. The mature protein extends from 55-72. The immature (including pre and pro regions) form of *E. coli* ST-1B (ST-H) protein has the sequence: mkkailfifsvlsfspaqaqkpvesskekitletkkcniaakksnksgpesmnsnyceelcnpactgcy (SEQ ID NO: \_\_\_\_; see GenBank® Accession No. P07965 (gi:3915589)). The immature (including pre

and pro regions) form of *Y. enterocolitica* ST protein has the sequence:  
 mkkivfvlvlnlssfgafgqetvagqfsdalstpitaevykqacdpplppaevssdwdecdvccnpackgc  
 (SEQ ID NO:.....; see GenBank® Accession No. S25659 (gi:282047)).

- 5 GC-C agonist peptides of particular interest include peptides consisting, consisting essentially of  
 or comprising:
- Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 10 Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 15 d-Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 d-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 d-Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 d-Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;  
 d-Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 20 d-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 d-Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 d-Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys;  
 Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;  
 Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;  
 25 Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;  
 Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;  
 Val Tyr Ile Gln Tyr Gln Gly Phe Arg Val Gln Leu Glu Ser Met Lys Lys Leu Ser Asp Leu Glu Ala Gln Trp Ala Pro;  
 Ser Pro Arg Leu Gln Ala Gln Ser Leu Leu Pro Ala Val Cys His His Pro Ala Leu Pro Gln Asp Leu Gln Pro Val Cys;  
 Ala Ser Gln Glu Ala Ser Ser Ile Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala  
 30 Cys Thr Gly Cys Leu;  
 Val Tyr Ile Gln Tyr Gln Gly Phe Arg Val Gln Leu Glu Ser Met Lys Lys Leu Ser Asp Leu Glu Ala Gln Trp Ala Pro;  
 Ser Pro Arg Leu Gln Ala Gln Ser Leu Leu Pro Ala Val Cys His His Pro Ala Leu Pro Gln Asp Leu Gln Pro Val Cys;  
 Ala Ser Gln Glu Ala Ser Ser Ile Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala  
 Cys Thr Gly Cys Leu;  
 35 Pro Gly Thr Cys Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys;

Val Thr Val Gln Asp Gly Asn Phe Ser Phe Ser Leu Glu Ser Val Lys Lys Leu Lys Asp Leu Gln Glu Pro Gln Glu  
Pro;

Arg Val Gly Lys Leu Arg Asn Phe Ala Pro Ile Pro Gly Glu Pro Val Val Pro Ile Leu Cys Ser Asn Pro Asn Phe Pro;  
Glu Glu Leu Lys Pro Leu Cys Lys Glu Pro Asn Ala Gln Glu Ile Leu Gln Arg Leu Glu Glu Ile Ala Glu Asp Pro Gly

5 Thr Cys Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys;

Met Pro Ser Thr Gln Tyr Ile Arg Arg Pro Ala Ser Ser Tyr Ala Ser Cys Ile Trp Cys Thr Thr Ala Cys Ala Ser Cys His  
Gly Arg Thr Thr Lys Pro Ser Leu Ala Thr;

Ala Asp Leu Cys Glu Ile Cys Ala Phe Ala Ala Cys Thr Gly Cys Leu;

Val Gln Val Glu Glu Gly Gly Phe Ser Phe Pro Leu Asp Ala Val Lys Lys Leu Glu Glu Leu Met Gly Val Asp Met;

10 Thr Val Lys Gln Ser Pro Arg Leu Ala Lys Thr Ser Thr Thr Ala Val Cys Thr Asn Pro Asp Leu Pro Ala Val Phe Leu;  
Pro Leu Cys Lys Ser Lys Gly Ala Ala Asn Ser Phe Phe Arg Leu Gly Phe Val Ala Ala Arg Ala Asp Leu Cys Glu Ile  
Cys Ala Phe Ala Ala Cys Thr Gly Cys Leu;

Gln Glu Glu Cys Glu Leu Cys Ile Asn Met Ala Cys Thr Gly Tyr;

Val Tyr Ile Glu Tyr Glu Gly Phe Gln Val Asn Leu Asp Ser Val Lys Lys Leu Asp Lys Leu Leu Glu Gln Leu Arg

15 Gly;

Phe His His Gln Met Gly Asp Gln Arg Asp Pro Ser Ile Leu Cys Ser Asp Pro Ala Leu Pro Ser Asp Leu Gln Pro Val;  
and

Cys Glu Asn Ser Gln Ala Val Asn Ile Phe Arg Ala Leu Arg Tyr Ile Asn Gln Glu Glu Cys Glu Leu Cys Ile Asn Met  
Ala Cys Thr Gly Tyr.

20

The peptides which act as GC-C receptor agonists include peptides related to guanylin,  
uroguanylin, ST peptide and other peptides described in US 2004/0266989; US 2005/0020811;  
US 2006/0094658; US 2006/0258593; US 2006/0281682; and US 2007/0010450, all of which  
are hereby incorporated by reference in their entirety.

25 Described herein are pharmaceutical compositions comprising certain peptides that are capable  
of activating the guanylate-cyclase C (GC-C) receptor and one or more additional therapeutic  
agents including, without limitation, the agents described herein. The other agents can be  
administered with the peptides described herein (simultaneously or sequentially). They can also  
be linked to a peptide described herein to create therapeutic conjugates.

### 30 Variant GC-C agonist Peptides

The disclosure includes variant peptides which can include one, two, three, four, five, six, seven,  
eight, nine, or ten (in some embodiments fewer than 5 or fewer than 3 or 2 or fewer) amino acid  
substitutions and/or deletions compared to the sequence of a peptide described herein. In

certain embodiments, the cysteine residues are neither substituted nor deleted. The substitution(s) can be conservative or non-conservative. The naturally-occurring amino acids can be substituted by D-isomers of any amino acid, non-natural amino acids, natural and natural amino acid analogs and other groups. A conservative amino acid substitution results in the alteration of an amino acid for a similar acting amino acid, or amino acid of like charge, polarity, or hydrophobicity. At some positions, even conservative amino acid substitutions can alter the activity of the peptide. A conservative substitution can substitute a naturally-occurring amino acid for a non-naturally-occurring amino acid. The amino acid substitutions among naturally-occurring amino acids are listed in Table I.

10

Table I

For Amino Acid	Code	Replace with any of
Alanine	Ala	Gly, Cys, Ser
Arginine	Arg	Lys, His
Asparagine	Asn	Asp, Glu, Gln,
Aspartic Acid	Asp	Asn, Glu, Gln
Cysteine	Cys	Met, Thr, Ser
Glutamine	Gln	Asn, Glu, Asp
Glutamic Acid	Glu	Asp, Asn, Gln
Glycine	Gly	Ala
Histidine	His	Lys, Arg
Isoleucine	Ile	Val, Leu, Met
Leucine	Leu	Val, Ile, Met
Lysine	Lys	Arg, His
Methionine	Met	Ile, Leu, Val
Phenylalanine	Phe	Tyr, His, Trp
Proline	Pro	
Serine	Ser	Thr, Cys, Ala
Threonine	Thr	Ser, Met, Val
Tryptophan	Trp	Phe, Tyr
Tyrosine	Tyr	Phe, His
Valine	Val	Leu, Ile, Met

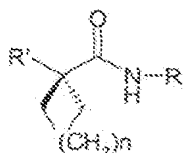
In some circumstances it can be desirable to treat patients with a variant peptide that binds to and activates intestinal GC-C receptor, but is less active than the non-variant form the peptide. This reduced activity can arise from reduced affinity for the receptor or a reduced ability to activate the receptor once bound or reduced stability of the peptide.

In certain embodiments, one or more amino acids can be replaced by a non-naturally occurring amino acid or a naturally or non-naturally occurring amino acid analog. In certain embodiments, one or more L-amino acids can be substituted with a D-amino acid. There are many amino acids  
 5 beyond the standard 20 amino acids (Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val). Some are naturally-occurring others are not (see, for example, Hunt, *The Non-Protein Amino Acids: In Chemistry and Biochemistry of the Amino Acids*, Barrett, Chapman and Hall, 1985). For example, an aromatic amino acid can be replaced  
 10 by 3,4-dihydroxy-L-phenylalanine, 3-iodo-L-tyrosine, triiodothyronine, L-thyroxine, phenylglycine (Phg) or nor-tyrosine (norTyr). Phg and norTyr and other amino acids including Phe and Tyr can be substituted by, e.g., a halogen, -CH<sub>3</sub>, -OH, -CH<sub>2</sub>NH<sub>3</sub>, -C(O)H, -CH<sub>2</sub>CH<sub>3</sub>, -CN, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, -SH, or another group. Any amino acid can be substituted by the D-form of the amino acid. Thus, for example, a cysteine residue can be substituted by a D-cysteine residue.

15 Glu can be replaced by *gamma*-Hydroxy-Glu or *gamma*-Carboxy-Glu.

Ala can be replaced by an alpha substituted amino acid such as L-*alpha*-methylphenylalanine or by analogues such as: 3-Amino-Tyr; Tyr(CH<sub>3</sub>); Tyr(PO<sub>3</sub>(CH<sub>3</sub>)<sub>2</sub>); Tyr(SO<sub>3</sub>H); *beta*-Cyclohexyl-Ala; *beta*-(1-Cyclopentenyl)-Ala; *beta*-Cyclopentyl-Ala; *beta*-Cyclopropyl-Ala; *beta*-Quinoly-  
 20 Ala; *beta*-(2-Thiazolyl)-Ala; *beta*-(Triazole-1-yl)-Ala; *beta*-(2-Pyridyl)-Ala; *beta*-(3-Pyridyl)-Ala; Amino-Phe; Fluoro-Phe; Cyclohexyl-Gly; *t*Bu-Gly; *beta*-(3-benzothienyl)-Ala; *beta*-(2-thienyl)-Ala; 5-Methyl-Trp; and 4-Methyl-Trp.

Pro can be an N(*alpha*)-C(*alpha*) cyclized amino acid analogues with the structure:



25  $n = 0, 1, 2, 3$

Pro can also be homopro (L-pipecolic acid); hydroxy-Pro; 3,4-Dehydro-Pro; 4-fluoro-Pro; or *alpha*-methyl-Pro.

Val or Leu can also be an alpha-substituted or N-methylated amino acid such as *alpha*-amino isobutyric acid (*aib*), *L/D-alpha*-ethylalanine (*L/D-isovaline*), *L/D-methylvaline*, or *L/D-alpha*-methylleucine or a non-natural amino acid such as *beta*-fluoro-Ala.

5

Gly can be *alpha*-amino isobutyric acid (*aib*) or *L/D-alpha*-ethylalanine (*L/D-isovaline*).

Further examples of unnatural amino acids include: an unnatural analogue of tyrosine; an unnatural analogue of glutamine; an unnatural analogue of phenylalanine; an unnatural analogue  
10 of serine; an unnatural analogue of threonine; an alkyl, aryl, acyl, azido, cyano, halo, hydrazine, hydrazide, hydroxyl, alkenyl, alkynyl, ether, thiol, sulfonyl, seleno, ester, thioacid, borate, boronate, phospho, phosphono, phosphine, heterocyclic, enone, imine, aldehyde, hydroxylamine, keto, or amino substituted amino acid, or any combination thereof; an amino acid with a photoactivatable cross-linker; a spin-labeled amino acid; a fluorescent amino acid; an amino acid  
15 with a novel functional group; an amino acid that covalently or noncovalently interacts with another molecule; a metal binding amino acid; an amino acid that is amidated at a site that is not naturally amidated, a metal-containing amino acid; a radioactive amino acid; a photocaged and/or photoisomerizable amino acid; a biotin or biotin-analogue containing amino acid; a glycosylated or carbohydrate modified amino acid; a keto containing amino acid; amino acids  
20 comprising polyethylene glycol or polyether; a heavy atom substituted amino acid (e.g., an amino acid containing deuterium, tritium, <sup>13</sup>C, <sup>15</sup>N, or <sup>18</sup>O); a chemically cleavable or photocleavable amino acid; an amino acid with an elongated side chain; an amino acid containing a toxic group; a sugar substituted amino acid, e.g., a sugar substituted serine or the like; a carbon-linked sugar-containing amino acid; a redox-active amino acid; an  $\alpha$ -hydroxy  
25 containing acid; an amino thio acid containing amino acid; an  $\alpha$ ,  $\alpha$  disubstituted amino acid; a  $\beta$ -amino acid; a cyclic amino acid other than proline; an O-methyl-L-tyrosine; an L-3-(2-naphthyl)alanine; a 3-methyl-phenylalanine; a *p*-acetyl-L-phenylalanine; an O-4-allyl-L-tyrosine; a 4-propyl-L-tyrosine; a tri-O-acetyl-GlcNAc $\beta$ -serine; an L-Dopa; a fluorinated phenylalanine; an isopropyl-L-phenylalanine; a *p*-azido-L-phenylalanine; a *p*-acyl-L-phenylalanine; a *p*-  
30 benzoyl-L-phenylalanine; an L-phosphoserine; a phosphoserine; a phosphonotyrosine; a *p*-iodo-phenylalanine; a 4-fluorophenylglycine; a *p*-bromophenylalanine; a *p*-amino-L-



phenylalanine; an isopropyl-L-phenylalanine; L-3-(2-naphthyl)alanine; an amino-, isopropyl-, or O-allyl-containing phenylalanine analogue; a dopa, O-methyl-L-tyrosine; a glycosylated amino acid; a p-(propargyloxy)phenylalanine; dimethyl-Lysine; hydroxy-proline; mercaptopropionic acid; methyl-lysine; 3-nitro-tyrosine; norleucine; pyro-glutamic acid; Z (Carbobenzoxyl);  $\epsilon$ -Acetyl-Lysine;  $\beta$ -alanine; aminobenzoyl derivative; aminobutyric acid (Abu); citrulline; aminohexanoic acid; aminoisobutyric acid; cyclohexylalanine; d-cyclohexylalanine; hydroxyproline; nitro-arginine; nitro-phenylalanine; nitro-tyrosine; norvaline; octahydroindole carboxylate; ornithine; penicillamine; tetrahydroisoquinoline; acetamidomethyl protected amino acids and pegylated amino acids. Further examples of unnatural amino acids and amino acid analogs can be found in U.S. 20030108885, U.S. 20030082575, US20060019347 (paragraphs 410-418) and the references cited therein. The peptides described herein can include further modifications including those described in US20060019347

In some embodiments, an amino acid can be replaced by a naturally-occurring, non-essential amino acid, e.g., taurine.

Methods to manufacture peptides containing unnatural amino acids can be found in, for example, U.S. 20030108885, U.S. 20030082575, US20060019347, Deiters et al., *J Am Chem Soc.* (2003) 125:11782-3, Chin et al., *Science* (2003) 301:964-7, and the references cited therein.

Peptides that include non-natural amino acids can also be prepared using the methods described in WO02086075

The peptides described herein can have one or more conventional peptide bonds replaced by an alternative bond. Such replacements can increase the stability of the peptide. For example, replacement of the peptide bond between D-Cys<sub>15</sub> or Cys<sub>15</sub> and Xaa<sub>16</sub> with an alternative bond can reduce cleavage by carboxy peptidases and may increase half-life in the digestive tract.

Bonds that can replace peptide bonds include: a retro-inverso bonds (C(O)-NH instead of NH-C(O)); a reduced amide bond (NH-CH<sub>2</sub>); a thiomethylene bond (S-CH<sub>2</sub> or CH<sub>2</sub>-S); an oxomethylene bond (O-CH<sub>2</sub> or CH<sub>2</sub>-O); an ethylene bond (CH<sub>2</sub>-CH<sub>2</sub>); a thioamide bond (C(S)-NH); a trans-olefine bond (CH=CH); an fluoro substituted trans-olefine bond (CF=CH); a ketomethylene bond (C(O)-CHR or CHR-

C(O) wherein R is H or CH<sub>3</sub>; and a fluoro-ketomethylene bond (C(O)-CFR or CFR-C(O) wherein R is H or F or CH<sub>3</sub>).

The peptides described herein can be modified using standard modifications. Modifications may occur at the amino (N-), carboxy (C-) terminus, internally or a combination of any of the preceding. In one aspect described herein, there may be more than one type of modification of the peptide. Modifications include but are not limited to: acetylation, amidation, biotinylation, cinnamoylation, farnesylation, formylation, myristoylation, palmitoylation, phosphorylation (Ser, Tyr or Thr), stearylation, succinylation, sulfurylation and cyclisation (via disulfide bridges or amide cyclisation), and modification by Cy3 or Cy5. The peptides described herein may also be modified by 2, 4-dinitrophenyl (DNP), DNP-lysine, modification by 7-Amino-4-methyl-coumarin (AMC), fluorescein, NBD (7-Nitrobenz-2-Oxa-1,3-Diazole), p-nitro-anilide, rhodamine B, EDANS (5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid), dabcyf, dabsyl, dansyl, texas red, FMOC, and Tamra (Tetramethylrhodamine). The peptides described herein may also be conjugated to, for example, polyethylene glycol (PEG); alkyl groups (e.g., C1-C20 straight or branched alkyl groups); fatty acid radicals; combinations of PEG, alkyl groups and fatty acid radicals (see U.S. Patent 6,309,633; Soltero et al., 2001 Innovations in Pharmaceutical Technology 106-110); BSA and KLH (Keyhole Limpet Hemocyanin). The addition of PEG and other polymers which can be used to modify peptides described herein is described in US2006019347 section IX.

The peptides and agonists described herein can be chemically modified to increase therapeutic activity by synthetically adding sugar moieties (WO 88/02756; WO 89/09786; DE 3910667 A1, EP 0 374 089 A2; and U.S. 4,861,755), adding cationic anchors (EP0363589), lipid moieties (WO91/09837; U.S. 4,837,303) or the substituents described as compounds I, II, and III in US5552520.

Certain GCC agonist peptides described herein bear some sequence similarity to ST peptides. However, they include amino acid changes and/or additions that improve functionality. These changes can, for example, increase or decrease activity (e.g., increase or decrease the ability of the peptide to stimulate intestinal motility), alter the ability of the peptide to fold correctly, alter

the stability of the peptide, alter the ability of the peptide to bind the GC-C receptor and/or decrease toxicity. In some cases the peptides may function more desirably than wild-type ST peptide. For example, they may limit undesirable side effects such as diarrhea and dehydration.

5 In some embodiments one or both members of one or more pairs of Cys residues (including where a Cys residue has been substituted with a D-cys residue) which normally form a disulfide bond can be replaced by homocysteine, penicillamine, 3-mercaptoproline (Kolodziej et al. 1996 Int J Pept Protein Res 48:274);  $\beta$ ,  $\beta$  dimethylcysteine (Hunt et al. 1993 Int J Pept Protein Res 42:249) or diaminopropionic acid (Smith et al. 1978 J Med Chem 21:117) to form alternative  
10 internal cross-links at the positions of the normal disulfide bonds.

In addition, one or more disulfide bonds can be replaced by alternative covalent cross-links, e.g., an amide linkage (-CH<sub>2</sub>CH(O)NHCH<sub>2</sub>- or -CH<sub>2</sub>NHCH(O)CH<sub>2</sub>-), an ester linkage, a thioester linkage, a lactam bridge, a carbamoyl linkage, a urea linkage, a thiourea linkage, a phosphonate  
15 ester linkage, an alkyl linkage (-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-), an alkenyl linkage(-CH<sub>2</sub>CH=CHCH<sub>2</sub>-), an ether linkage (-CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>- or -CH<sub>2</sub>OCH<sub>2</sub>CH<sub>2</sub>-), a thioether linkage (-CH<sub>2</sub>CH<sub>2</sub>SCH<sub>2</sub>- or -CH<sub>2</sub>SCH<sub>2</sub>CH<sub>2</sub>-), an amine linkage (-CH<sub>2</sub>CH<sub>2</sub>NHCH<sub>2</sub>- or -CH<sub>2</sub>NHCH<sub>2</sub>CH<sub>2</sub>-) or a thioamide linkage (-CH<sub>2</sub>CH(S)HNHCH<sub>2</sub>- or -CH<sub>2</sub>NHCH(S)CH<sub>2</sub>-). For example, Ledu et al. (Proc Nat'l Acad. Sci. 100:11263-78, 2003) describe methods for preparing lactam and amide cross-links.  
20 Schafmeister et al. (J. Am. Chem. Soc. 122:5891, 2000) describes stable, hydrocarbon cross-links. Hydrocarbon cross links can be produced via metathesis (or methathesis followed by hydrogenation in the case of saturated hydrocarbons cross-links) using one or another of the Grubbs catalysts (available from Materia, Inc. and Sigma-Aldrich and described, for example, in U.S. Patent No. 5,831,108 and 6,111,121). In some cases, the generation of such alternative  
25 cross-links requires replacing the Cys residues with other residues such as Lys or Glu or non-naturally occurring amino acids. In addition the lactam, amide and hydrocarbon cross-links can be used to stabilize the peptide even if they link amino acids at positions other than those occupied by Cys. Such cross-links can occur between two amino acids that are separated by two amino acids or between two amino acids that are separated by six amino acids (see, e.g.,  
30 Schafmeister et al. (J. Am. Chem. Soc. 122:5891, 2000)).

The peptide may contain additional carboxyterminal or amino terminal amino acids or both. For example, the peptide can include an amino terminal sequence that facilitates recombinant production of the peptide and is cleaved prior to administration of the peptide to a patient. The peptide can also include other amino terminal or carboxyterminal amino acids. In some cases the additional amino acids protect the peptide, stabilize the peptide or alter the activity of the peptide. In some cases some or all of these additional amino acids are removed prior to administration of the peptide to a patient. The peptide can include 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 80, 90, 100 or more amino acids at its amino terminus or carboxy terminus or both. The number of flanking amino acids need not be the same. For example, there can be 10 additional amino acids at the amino terminus of the peptide and none at the carboxy terminus.

The peptides can be co-administered with or linked, e.g., covalently linked to any of a variety of other peptides or compounds including analgesic peptides or analgesic compounds including, without limitation, the agents described herein.

Amino acid, non-amino acid, peptide and non-peptide spacers can be interposed between a peptide that is a GC-C receptor agonist and a peptide that has some other biological function, e.g., an analgesic peptide or a peptide used to treat obesity. The linker can be one that is cleaved from the flanking peptides *in vivo* or one that remains linked to the flanking peptides *in vivo*. For example, glycine, beta-alanine, glycyL-glycine, glycyL-beta-alanine, gamma-aminobutyric acid, 6-aminocaproic acid, L-phenylalanine, L-tryptophan and glycyL-L-valil-L-phenylalanine can be used as spacers (Chaltin et al. 2003 Helvetica Chimica Acta 86:533-547; Caliceti et al. 1993 FARMCO 48:919-32) as can polyethylene glycols (Butterworth et al. 1987 J. Med. Chem 30:1295-302) and maleimide derivatives (King et al. 2002 Tetrahedron Lett. 43:1987-1990). Various other linkers are described in the literature (Nestler 1996 Molecular Diversity 2:35-42; Finn et al. 1984 Biochemistry 23:2554-8; Cook et al. 1994 Tetrahedron Lett. 35:6777-80; Brokx et al. 2002 Journal of Controlled Release 78:115-123; Griffin et al. 2003 J. Am. Chem. Soc. 125:6517-6531; Robinson et al. 1998 Proc. Natl. Acad. Sci. USA 95:5929-5934). Linkers are also described in US20050171014, for example, amino acid linkers such as FALA, VLALA, ALAL, ALALA, 2-cyclohexyl-L-alanine-LALA, 2-cyclohexyl-L-alanine-2-cyclohexyl-L-alanine-LAL, 1-naphtyl-alanine-ChaLAL and 1-naphtyl-alanine-LALA. Peptides and agonists

described herein can also be conjugated to: an affinity tag (such as (histidine 6) H6), a HIV tat peptide residues 49-57, HIV tat peptide residues 49-56, the tat sequence YGRKKRRQRRR, a polyarginine peptide having from 6 to 20 residues (such as R6) and the following peptide sequences: YARKARRQARR, YARAAARQARA, YARAARRAARR, YARAARRAARA, 5 ARRRRRRRRRR, and YAAARRRRRRR, which are disclosed in WO 99/29721 and in US patent No. 6,221,355 (seq. id. nos. 3-8).

The peptides described herein can be attached to one, two or more different moieties each providing the same or different functions. For example, the peptide can be linked to a molecule that is an analgesic and to a peptide that is used to treat obesity. The peptide and various 10 moieties can be ordered in various ways. For example, a peptide described herein can have an analgesic peptide linked to its amino terminus and an anti-obesity peptide linked to its carboxy terminus. The additional moieties can be directly covalently bonded to the peptide or can be bonded via linkers.

The peptides described herein can be a cyclic peptide or a linear peptide. In addition, multiple 15 copies of the same peptide can be incorporated into a single cyclic or linear peptide.

The peptides can include the amino acid sequence of a peptide that occurs naturally in a vertebrate (e.g., mammalian) species or in a bacterial species. In addition, the peptides can be partially or completely non-naturally occurring peptides. Also within the disclosure are peptidomimetics corresponding to the peptides described herein.

20

#### Soluble Guanylate Cyclase modulators

Soluble guanylate cyclase (sGC) is a nitric oxide (NO) sensing haemprotein that has been described in many eukaryotes. In response to various stimuli sGC converts GTP into the cyclic cGMP. sGC is a heterodimeric protein consisting of homologous alpha and beta subunits. Each 25 subunit consists of an N-terminal domain which may bind haem-nitric oxide and/or oxygen, a central domain of unknown function, and a C-terminal consensus nucleotide cyclase domain. sGC can be activated via both nitric oxide (NO) dependent and independent manners. When NO binds to the haem prosthetic group in the beta subunit of sGC, catalysis is accelerated by 2-3 orders of magnitude.

Agents that function as sGC modulators useful alone and in combination in the compositions and methods of the present disclosure include but are not limited to: NO donors, eNOS transcriptional enhancers, haem-dependent sGC stimulators, haem-independent sGC activators and NOS substrates.

Nitric oxide (NO) donors are pharmacologically active substances that release NO *in vivo* or *in vitro*. There are different classes of NO donors, which include organic nitrates (e.g., nitroglycerin), isosorbides (e.g. isosorbide dinitrate, isosorbide mononitrate, isosorbide 5-mononitrate, isosorbide 2-mononitrate, CA Registry no. 16051-77-7), S-nitrosothiols, iron-nitrosyl complexes (e.g., sodium nitroprusside), sydnonimines, C-nitroso compounds, and secondary amine/NO complex ions. Specific examples of some of the classes of NO donors named above include: amyl nitrite, Isosorbide (Dilatrate®-SR, Imdur®, Ismo®, Isordil®, Isordil® Titrados®), Monoker®), FK 409 (NOR-3); FR 144420 (NOR-4); 3-morpholinosydnonimine; Linsidomine chlorohydrate ("SIN-1"); S-nitroso-N-acetylpenicillamine ("SNAP"), AZD3582 (CINOD lead compound), NCX 4016 (an m-nitroxymethyl phenyl ester of acetyl salicylic acid), NCX 701, NCX 1022, HCT 1026, NCX 1015, NCX 950, NCX 1000, NCX 1020, AZD 4717, NCX 1510/NCX 1512, NCX 2216, and NCX 4040 (all available from NicOx S.A.), S-nitrosoglutathione (GSNO), S-nitrosoglutathione mono-ethyl-ester (GSNO-ester), diethylenetriamine/NO (DETA/NO, a compound of nitric oxide covalently linked to diethylenetriamine), 6-(2-hydroxy-1-methyl-nitrosohydrazino)-N-methyl-1-hexanamine (NOC-9) or diethylamine NONOate, S-nitrosothiol, a nitrite, a sydnonimine, a NONOate, a N-nitrosoamine, a N-hydroxyl nitrosamine, a nitrosimine, a diazetine dioxide, an oxatriazole 5-imine, an oxime, a hydroxylamine, a N-hydroxyguanidine, a hydroxyurea or a furoxan. Nitric oxide donors are also as disclosed in U.S. Pat. Nos. 5,155,137, 5,366,997, 5,405,919, 5,650,442, 5,700,830, 5,632,981, 6,290,981, 5,691,423, 5,721,365, 5,714,511, 6,511,911, and 5,814,666, Chrisselis et al. (2002) *J Med Chem.* 45:5406-9 (such as NO donors 14 and 17), and *Nitric Oxide Donors for Pharmaceutical and Biological Research*, Eds: Peng George Wang, Tingwei Bill Cai, Naoyuki Taniguchi, Wiley, 2005.

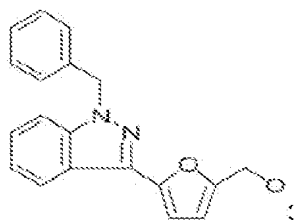
Endothelial NO synthase is subject to physiological and pathophysiological regulation both at the transcriptional and at the post-transcriptional level. Compounds which enhance eNOS transcription are described in WO 02/064146, WO 02/064545, WO 02/064546 and WO 02/064565, and corresponding patent documents such as US2003/0008915, US2003/0022935, 5 US2003/0022939 and US2003/0055093 for example. Other eNOS transcriptional enhancers include those described in US20050101599 (e.g. 2,2-difluorobenzo[1,3]dioxol-5-carboxylic acid indan-2-ylamide, and 4-fluoro-N-(indan-2-yl)-benzamide), and Sanofi-Aventis compounds AVE3085 and AVE9488 (CA Registry NO. 916514-70-0; Schäfer et al., *Journal of Thrombosis and Haemostasis* 2005; Volume 3, Supplement 1: abstract number P1487).

10

Evgenov et al. (2006) *Nature Reviews-Drug Discovery* 5:755-768 review a novel class of haem-dependent sGC-stimulators which share several characteristics including crucial dependency on the presence of the reduced prosthetic haem moiety and strong synergistic enzyme activation when combined with NO. Haem-dependent sGC stimulators include but are not limited to:

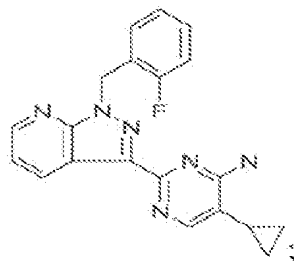
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YC-1 (see patent publications EP667345 and DE 19744026)

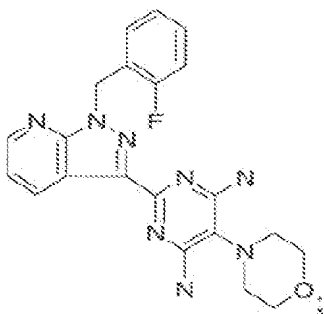


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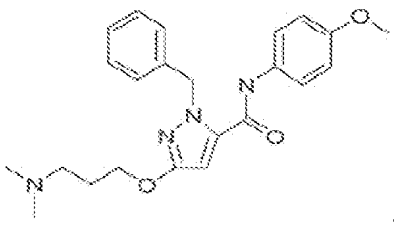
BAY 41-2272 (see patent publications DE19834047 and DE19942809)



BAY 41-8543 (see patent publication DE19834044)

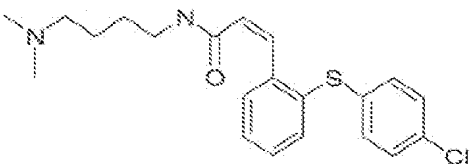


CFM-1571 (see patent publication WO2000027394)



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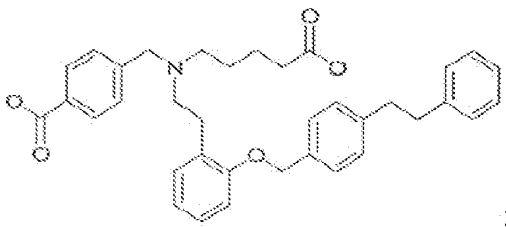
A350-619



10 and other compounds disclosed in Tetrahedron Letters (2003), 44(48): 8661-8663.

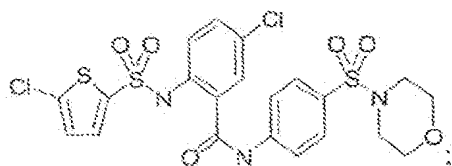
sGC can also be activated in a NO- and haem-independent manner by haem-independent sGC activators which include but are not limited to:

15 BAY 58-2667 (see patent publication DE19943635)

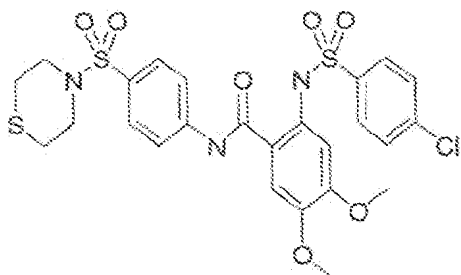


HMR-1766 (ataciguat sodium, see patent publication WO2000002851)





S 3448 (2-(4-chloro-phenylsulfonylamino)-4,5-dimethoxy-N-(4-(thiomorpholine-4-sulfonyl)-  
 5 phenyl)-benzamide; see patent publications DE19830430 and WO2000002851)



; and

HMR-1069 (Sanofi-Aventis).

10

L-arginine acts as the endogenous substrate of NOS. Other NOS substrates which can be converted to NO may be useful in the methods and compositions described herein. NOS substrates include but are not limited to L-arginine, n-hydroxyguanidine based analogs (such as N[G]-hydroxy-L-arginine (NOHA), (1-(3, 4-dimethoxy-2-chlorobenzylideneamino)-3-  
 15 hydroxyguanidine), and PR5 (1-(3, 4-dimethoxy-2-chlorobenzylideneamino)-3-hydroxyguanidine); L-arginine derivatives (such as homo-Arg, homo-NOHA, N-tert-butyloxy- and N-(3-methyl-2-butenyl)oxy-L-arginine, canavanine, epsilon guanidine-carpoic acid, agmatine, hydroxyl-agmatine, and L-tyrosyl-L-arginine); N-alkyl-N'-hydroxyguanidines (such as N-cyclopropyl-N'-hydroxyguanidine and N-butyl-N'-hydroxyguanidine), N-aryl-N'-  
 20 hydroxyguanidines (such as N-phenyl-N'-hydroxyguanidine and its para-substituted derivatives which bear -F, -Cl, -methyl, -OH substituents, respectively); guanidine derivatives such as 3-(trifluormethyl) propylguanidine; and others reviewed in Cali et al. (2005) Current Topics in Medicinal Chemistry 5:721-736) and disclosed in the references cited therein.

25 Prostanoids, Prostaglandin E1 (PGE1), Prostaglandin E2 (PGE2), and derivatives thereof

Prostanoids are a class of eicosanoids which include prostaglandins, thromboxanes and prostacyclins. Prostaglandin E<sub>1</sub> and its derivatives have been implicated in multiple functions including anion secretion and the regulation of urinary sodium excretion (Lifschitz et al. (1978) Prostaglandins 16:607-19). Agents useful in the methods described herein include dinoprostone, gemeprost, alprostadil, limaprost, butaprost, 11-deoxy PGE<sub>1</sub>, AH23848, AH13205, or a 19-hydroxy PGE, the prostaglandin E agonists described in patent publications EP1097922 and EP1114816, the 19-hydroxy prostaglandin analogues described in US4,127,612, Enprostil (a synthetic prostaglandin), PGE<sub>2</sub>, PGE<sub>1</sub> and PGE<sub>1</sub> derivatives (such as those described in US5,219,885, the chloride channel activator lubiprostone (Amitiza®) and derivatives thereof, and misoprostol) as well as 6-keto prostaglandin E<sub>1</sub> (6-k PGE<sub>1</sub>) and derivatives thereof as disclosed in patent publications US4,205,178 and DE2840032. Misoprostol, a synthetic prostaglandin E<sub>1</sub> (PGE<sub>1</sub>) analogue sold as Cytotec®, is a 1:1:1:1 mixture of 11R, 16S; 11S, 16R; 11R, 16R; and 11S, 16S isomers. In certain embodiments, misoprostol comprises all 4 isomers. In other embodiments, misoprostol comprises, consists essentially of or consists of the single isomer (SC 30249) which is associated with CAS registry NO. 59122-49-5. In other embodiments, misoprostol comprises, consists essentially of or consists of the free acid of the single isomer (SC 30249).

Prostaglandin E derivatives also include the compounds represented by CAS Registry Nos. 333963-40-9 and 136790-76-6, (-)-7-[(2R,4aR,5R,7aR)-2-(1,1-difluoropentyl)-2-hydroxy-6-oxooctahydrocyclopenta[b]pyran-5-yl]heptanoic acid; and the 13, 14-dihydro-15-keto prostaglandins E disclosed in US5284858 including 13,14-dihydro-15-keto-PGE<sub>2</sub> alkyl ester, 13,14-dihydro-15-keto-PGE<sub>2</sub> cycloalkyl ester; 13,14-dihydro-15-keto-PGE<sub>2</sub> hydroxy alkyl ester, 13,14-dihydro-15-keto-PGE<sub>2</sub> benzyl ester, 13,14-dihydro-15-keto-PGE<sub>1</sub> alkyl ester, 13,14-dihydro-6,15-diketo-PGE<sub>1</sub> alkyl ester, 13,14-dihydro-15-keto-18-methoxy-19, 20-dinor-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-18-methoxy-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto- $\Delta^2$ -PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-20-methoxy- $\Delta^2$ -PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-3R,S-methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-3R,S-methyl-20-methoxy-PGE<sub>2</sub> or an alkyl ester thereof, 13, 14-dihydro-15-keto-11-dehydroxy-11R-methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-fluoro-11-dehydroxy-11R-methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-

16R,S-hydroxy-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-fluoro-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-16,16-dimethyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-16,16-dimethyl-20-methoxy-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-17S-methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-19-methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-20-isopropylidene PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-20-ethyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-20-ethyl-11-dehydroxy-11R-methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-20-n-propyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-20-ethyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16R,S-fluoro-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16R,S-fluoro-11-dehydroxy-11R-methyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16R,S-methyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16,16-dimethyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-19-methyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-20-methyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-11-dehydroxy-11R-methyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-11-dehydroxy-11R-hydroxymethyl PGE<sub>1</sub> alkyl ester, 13,14-dihydro-15-keto-20-methyl-PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto- $\Delta^2$ -PGE<sub>1</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-fluoro-20-methyl-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-16,16-difluoro-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-15-keto-5,6-dehydro-20-methoxy-PGE<sub>2</sub> or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16R,S-fluoro-PGE<sub>1</sub> or an alkyl ester thereof, the compounds disclosed in examples 1-60, and the prostaglandin E's as described in claims 1-6.

#### SHT4 receptor agonists

The SHT4 receptor (i.e. 5-hydroxy-tryptamine receptor or serotonin-4 receptor) is a seven transmembrane-spanning G-protein which mediates many cellular functions both in the central nervous system and at the periphery. SHT4 receptor agonists including Cisapride (and analogs thereof) and Zelnorm® (Tegaserod) which is known to increase intestinal fluid secretion, and the compounds and pharmaceutically acceptable salts thereof disclosed in patent publications WO2005080389, US2005228014, US2006100426, US2006100236, US 2006135764, US2006183901, WO2006183901, WO2006094063, WO2006094063, WO2006108127, WO2006127815, US2006276482 and US200711796 including TD-5108, CAS Registry No.

866933-46-2 (disclosed in WO2006108127) and CAS Registry No. 863248-59-3 (disclosed in US20060183901) are useful in the methods and compositions described herein.

### Cyclic nucleotides

5 Cyclic nucleotides and variants thereof useful in the methods described herein include but are not limited to cyclic AMP (cAMP) and cyclic GMP (cGMP) and analogues and variants thereof. Cyclic nucleotides include those discussed in F. Schwede et al. (2000) *Pharmacology & Therapeutics* 87:199–226, including, for example, 2-Cl-cAMP, 6-MBC-cAMP (N<sup>6</sup>-mono-tert-butylcarbamoyl-cyclic AMP), 7-deaza-cAMP, 8-aminocAMP, 8-Br-cAMP (8-bromo-cyclic  
10 AMP), 8-Br-cGMP (8-bromo-cyclic GMP), 8-Br-PET-cGMP, 8-Cl-cAMP (8-chloro-cAMP), 8-methylamino-cAMP, 8-pCPT-cAMP (8-para-chlorophenylthio-cyclic AMP), 8-pCPT-cGMP (8-para-chlorophenylthio-cyclic GMP), 8-piperidino-cAMP, 8-thioisopropyl-cAMP, 8-thiomethyl-cAMP, dibutyryl-cyclic AMP, Bt<sub>2</sub>-cAMP (N<sup>6</sup>, O<sup>2'</sup>-dibutyryl-cyclic AMP), Bt<sub>2</sub>-cAMP/AM (Bt<sub>2</sub>-cAMP acetoxymethyl ester), Bt<sub>2</sub>-cGMP (N<sup>2</sup>, O<sup>2'</sup>-dibutyryl-cyclic GMP), cAMP, cGMP,  
15 cGMP/AM, N<sup>2</sup>-Bt-cGMP (N<sup>2</sup>-monobutyryl-cyclic GMP), N<sup>6</sup>-benzoyl-cAMP, N<sup>6</sup>-benzyl-cAMP, N<sup>6</sup>-Bt-cAMP (N<sup>6</sup>-monobutyryl-cyclic AMP), N<sup>6</sup>-carbamoylphenyl-cAMP, N<sup>6</sup>-phenyl-8-pCPT-cAMP, N<sup>6</sup>-phenyl-cAMP, PET-cGMP ( $\beta$ -phenyl-1 (N<sup>2</sup>-etheno-cyclic GMP), Rp-8-Br-cAMPS (Rp-8-bromo-adenosine 3',5'-cyclic monophosphorothioate), Rp-8-Br-cGMPS, Rp-8-Br-PET-cGMPS (Rp-8-bromo- $\beta$ -phenyl-1 (N<sup>2</sup>-etheno-guanosine 3', 5'- cyclic monophosphorothioate),  
20 Rp-8-Cl-cAMPS, Rp-8-Cl-cGMPS, Rp-8-pCPT-cAMPS, Rp-8-pCPT-cGMPS, Rp-cAMPS (Rp-diastereomer of adenosine 3', 5'-cyclic monophosphorothioate), Rp-cGMPS (Rp-diastereomer of guanosine 3', 5'-cyclic monophosphorothioate), Sp-5,6-Cl<sub>2</sub>-cBIMPS (Sp-5,6-dichloro-1- $\beta$ -D-ribofuranosyl-benzimidazole-3', 5'-cyclic monophosphorothioate), Sp-8-Br-PET-cGMPS (Sp-8-bromo- $\beta$ -phenyl-1 (N<sup>2</sup>-etheno-guanosine 3', 5'-cyclic monophosphorothioate), Sp-8-Cl-cGMPS,  
25 Sp-8-pCPT-cGMPS, Sp-cAMPS (Sp-diastereomer of adenosine 3', 5'- cyclic monophosphorothioate), and Sp-cGMPS (Sp-diastereomer of guanosine 3', 5'-cyclic monophosphorothioate).

cGMP can include a product created by exposure of cGMP to one or more enzymes present in  
30 the digestive tract, e.g., the dephosphorylated cGMP (e.g., riboguanosine or guanosine or deoxyriboguanosine or deoxyguanosine), phosphorylated forms of GMP and GMP derivatives

(e.g., guanylate monophosphate or riboguanilate monophosphate or ribodeoxyguanylate monophosphate or deoxyriboguanilate monophosphate whether as the 5'-monophosphate, the 2'-monophosphate, the 3'-monophosphate or the 2', 3'-monophosphate intermediate form); the hydroxylated or deoxy forms of the ribose sugar of the nucleotide (e.g., ribose, deoxyribose, ribose monophosphate, or deoxyribose monophosphate); guanine; and methylated guanine and cGMP such N2-methylguanine, N7-methylguanine, cGMP methylated at N2 or cGMP methylated at N7. Thus analogues of cGMP include, but are not limited to, those that have modifications to the purine ring system, to the ribose, or to the phosphate group. In some cases the metabolic end products of cGMP such as xanthine and uric acid might be useful in the methods and compositions described herein. The analogue of cGMP may be cell membrane permeable.

cGMP and analogs thereof useful in the methods and compositions described herein also include, but are not limited to: 8-(4-chlorophenylthio)guanosine 3',5'-cyclic monophosphate (Menshikov et al. 1993 *Eur J Pharmacol.* 245:281-4), dibutyryl guanosine 3',5'-cyclic monophosphate (db cGMP), 8-bromo-guanosine 3',5'-cyclic monophosphate (8-bromo cGMP), 8-(4-chlorophenylthio)-guanosine 3',5'-cyclic monophosphate (8-(4, chlorophenylthio) cGMP, Rp-guanosine 3',5'-cyclic monophosphate (Rp-cGMP) and Sp-guanosine 3',5'-cyclic monophosphate (Sp-cGMPS) (the S isomer of cGMP), cyclic guanosine-3',5'-triphosphate, cyclic guanosine-3',5'-diphosphate, cyclic guanosine-3',5'-triphosphate, cyclic deoxyguanosine-3',5'-monophosphate, cyclic deoxyguanosine-3',5'-diphosphate, cyclic deoxyguanosine-3',5'-triphosphate, cyclic guanosine-2',3'-monophosphate, cyclic guanosine-2',3'-diphosphate, cyclic guanosine-2',3'-triphosphate, cyclic 2-(N-methyl)-guanosine-3',5'-monophosphate, cyclic 2-(N-methyl)-guanosine-3',5'-diphosphate, cyclic 2-(N-methyl)-guanosine-3',5'-triphosphate, cyclic 2-(N-methyl)-deoxyguanosine-3',5'-monophosphate, cyclic 2-(N-methyl)-doxyguanosine-3',5'-diphosphate, cyclic 2-(N-methyl)-deoxyguanosine-3',5'-triphosphate, cyclic 2-(N-methyl)-guanosine-2',3'-monophosphate, cyclic 2-(N-methyl)-guanosine-2',3'-diphosphate, cyclic 2-(N-methyl)-guanosine-2',3'-triphosphate, cyclic 7-(N-methyl)-guanosine-3',5'-monophosphate, cyclic 7-(N-methyl)-guanosine-3',5'-diphosphate, cyclic 7-(N-methyl)-guanosine-3',5'-triphosphate, cyclic 7-(N-methyl)-deoxyguanosine-3',5'-monophosphate, cyclic 7-(N-methyl)-deoxyguanosine-3',5'-diphosphate, cyclic 7-(N-methyl)-deoxyguanosine-3',5'-triphosphate,

cyclic 7-(N-methyl)-guanosine-2',3'-monophosphate, cyclic 7-(N-methyl)-guanosine-2',3'-diphosphate, cyclic 7-(N-methyl)-guanosine-2',3'-triphosphate, cyclic 2,7-(N,N'-dimethyl)-guanosine-3',5'-monophosphate, cyclic 2,7-(N,N'-dimethyl)-guanosine-3',5'-diphosphate, cyclic 2,7-(N,N'-dimethyl)-guanosine-3',5'-triphosphate, cyclic 2,7-(N,N'-dimethyl)-deoxyguanosine-3',5'-monophosphate, cyclic 2,7-(N,N'-dimethyl)-deoxyguanosine-3',5'-diphosphate, cyclic 2,7-(N,N'-dimethyl)-deoxyguanosine-3',5'-triphosphate, cyclic 2,7-(N,N'-dimethyl)-guanosine-2',3'-monophosphate, cyclic 2,7-(N,N'-dimethyl)-guanosine-2',3'-diphosphate, and cyclic 2,7-(N,N'-dimethyl)-guanosine-2',3'-triphosphate; cGMP analogs available from TWC Biosearch International (Hong Kong, China) including but not limited to: Rp-8-pCPT-cGMPS (CN-206), Sp-8-pCPT-cGMPS (CN-207), 8-Bromoguanosine-3',5'-cyclic monophosphate, sodium salt (CN-205), Rp-8-Bromoguanosine-3',5'-cyclic monophosphorothioate, sodium salt (CN-216), Sp-8-Bromoguanosine-3',5'-cyclic monophosphorothioate, sodium salt (CN-217), and N2,2'-O-Dibutyrylguanosine-3',5'-cyclic monophosphate, sodium salt (CN-215); cGMP analogs disclosed in Corbin et al. 1986 Journ. Biol. Chem. 261:1208 including but not limited to: 5'-NH-cGMP, 3'-NH-cGMP, cGMPS(R<sub>p</sub>), cGMPS(S<sub>p</sub>), cGMP-N(CH<sub>3</sub>)<sub>2</sub>(S<sub>p</sub>), cGMP-N(CH<sub>3</sub>)<sub>2</sub>(R<sub>p</sub>), 8-BR-cGMP, β-H<sub>5</sub>C<sub>6</sub>-1-N<sup>2</sup>-etheno-cGMP, 8-S(4-Cl)-C<sub>6</sub>H<sub>4</sub>-cGMP, 7-Deaza-cGMP, 8-H<sub>5</sub>C<sub>6</sub>H<sub>2</sub>CS-cGMP, 6-HS-cGMP, 1-H<sub>3</sub>C-cGMP, 8-HS-cGMP, N<sup>2</sup>-nH<sub>13</sub>C<sub>6</sub>-cGMP, 8-H<sub>5</sub>C<sub>6</sub>(O)C-cGMP, 8-HO-cGMP, N<sup>2</sup>-nH<sub>7</sub>C<sub>3</sub>(O)C-cGMP, 8-H(2-HO-iH<sub>7</sub>C<sub>3</sub>)<sub>c</sub>GMP, 8-H<sub>3</sub>C(O)C-cGMP, 8-(H<sub>5</sub>C<sub>2</sub>)<sub>2</sub>N-cGMP, N<sup>2</sup>-[2,4-(O<sub>2</sub>N)<sub>2</sub>-H<sub>3</sub>C<sub>6</sub>]<sub>c</sub>GMP, 8-H<sub>2</sub>N-cIMP, and 2'-Deoxy-cGMP; cGMP analogs available from Biolog Life Science Institute (Hayward, CA) including but not limited to : N<sup>2</sup>-MB-cGMP (cIMP), 3-deaza cGMP, 2- Aminopurine riboside- 3', 5'- cyclic monophosphate (2-NH<sub>2</sub>-cPuMP), 2'- Deoxyguanosine- 3', 5'- cyclic monophosphate (2'-cdGMP), 2'- O- (N-Methylantraniloyl)guanosine- 3', 5'- cyclic monophosphate (MANT-cGMP), 2'-O-Me-cGMP, and cGMP-AM; 8-bromoadenosine-cGMP, N2,2'-O-Dibutyrylguanosine-3',5'-cyclic monophosphate, sodium salt (available from Biomol; Plymouth, PA), and cGMP analogs available from other commercial supplier including Sigma Aldrich and Boehringer Mannheim).

### Laxatives

Laxatives useful alone and in combination in the methods described herein include but are not limited to bulk-producing agents, stool softeners, hydrating agents, stimulants, and other laxatives including dexloxiglumide/ CR 2017 (a CCK-1 antagonist, Forest Laboratories and

Rottapharm - Rotta Research Laboratorium SpA) and CR 3700 (Rottapharm (Rotta Research Laboratorium SpA).

5 Bulk-producing agents (i.e., bulk-forming or bulking agents) cause the stool to be bulkier and to retain more water, as well as forming an emollient gel. Bulk-producing agents have the gentlest of effects among laxatives and can also be taken just for maintaining regular bowel movements. Bulk-producing agents include but are not limited to dietary fiber, bran, psyllium husk (e.g., Metamucil), methylcellulose (e.g., Citrucel), and polycarbophil including calcium polycarbophil (e.g., Fibercon).

10 Stool softeners (i.e. surfactants) cause water & fats to penetrate the stool, making it easier to move along. Stool softeners include anionic surfactants such as docusate and salts and derivatives thereof. Perfusion studies suggest that docusates may inhibit fluid absorption or stimulate secretion in jejunum. Docusates include docusate sodium (e.g., Colace, Diocto) and Docusate calcium (e.g., Surfak).

15 Hydrating agents (i.e., osmotics) cause the intestines to concentrate more water within, softening the stool. There are two principal types of hydrating agents, saline and hyperosmotic. Hydrating agents include but are not limited to magnesium hydroxide (e.g., milk of magnesia), magnesium citrate, magnesium sulfate, and epsom salt.

20 Stimulants (i.e., irritants) alter water and electrolyte secretion and can stimulate peristaltic action. Stimulants include but are not limited to the diphenylmethane derivative Bisacodyl (e.g., Dulcolax, Fleet, Alophen, Correctol, Carter's Little Pills), phenolphthalein (e.g., yellow or white phenolphthalein) which inhibits re-absorption of digestive secretions, and naturally derived laxatives including senna (derived from the senna plant, may be sold as Senokot or Sennoside), aloe vera, and Cascara sagrada which comprises as active ingredients, anthraquinones, ricinoleic acid, and castor oil. Castor oil acts directly on intestinal mucosa or nerve plexus and alters water and electrolyte secretion. It is converted into ricinoleic acid (the active component) in the gut.

25

CFTR (cystic fibrosis transmembrane conductance regulator) modulators

The cystic fibrosis transmembrane conductance regulator protein (CFTR) is a cAMP- activated chloride (Cl) channel expressed in epithelial cells in mammalian airways, intestine, pancreas and testis. CFTR is the chloride-channel responsible for cAMP-mediated Cl secretion. Hormones, such as a  $\beta$ -adrenergic agonist, or toxins, such as cholera toxin, lead to an increase in cAMP, activation of cAMP-dependent protein kinase, and phosphorylation of the CFTR Cl channel, which causes the channel to open. CFTR is predominantly located in epithelia where it provides a pathway for the movement of Cl ions across the apical membrane and a key point at which to regulate the rate of transepithelial salt and water transport.

CFTR modulators may function for example, by increasing protein kinase regulated chloride channel gating of wild-type or mutant CFTR ("direct CFTR regulators"), by correcting folding or cellular processing of a mutant CFTR proteins ("correctors) or by activating molecules such as G-protein coupled P2Y and P2X purinergic receptors which regulate CFTR ("P2Y and P2X receptor agonists").

Direct CFTR regulators useful alone and in combination in the methods and compositions described herein include but are not limited to the pyrrolo[2,3-b]pyrazines derivatives described in Noel et. al. (2006) J Pharmacol Exp Ther 319:349-359 (e.g. compounds represented by CAS. Nos. 913748-30-8, 655239-23-9, 655239-22-8, 655239-21-7, 496864-17-6, 496864-16-5, 496864-15-4, 496864-06-3, 496864-05-2, 479551-48-9, 913748-29-5, 496864-10-9, 496864-09-6, 139962-78-0, and 139962-77-9, specifically those including the compounds described as RP107 [7-n-butyl-6-(4-hydroxyphenyl)[5H]-pyrrolo[2,3-b]pyrazine], RP108 [7-n-butyl-6-(4-chlorophenyl)[5H]pyrrolo[2,3-b]pyrazine], and those compounds comprising 4-hydroxyphenyl and 7-n-butyl moieties); isoflavones such as genistein; 5,6-dichloro-1-ethyl-1,3-dihydro-2H-benzimidazol-2-one(DCEBIO); 1-ethyl-2-benzimidazolone (1-EBIO); chlorzoxazone; zoxazolamine (2-amino-5-chlorzoxazole); 4-Chlorobenzo[F]isoquinoline (CBIQ); the phenylglycine and sulfonamide derivatives including 2-[(2-1H-indol-3-yl-acetyl)-methylamino]-N-(4-isopropylphenyl)-2-phenylacetamide and 6-(ethylphenylsulfamoyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid cycloheptylamide which are described in Pedemonte et al. (2005) Molecular Pharmacology 67: 1797-1807; the compounds disclosed in Yang et al. (2003) Journal of Biological chemistry 278:35079-85 which have tetrahydrobenzothiophene, benzofuran, pyrimidinetrione, dihydropyridine, or anthraquinone core structures and which



activate defective Delta F508-CFTR chloride channel gating; the benzoquinolizinium compounds disclosed in Mariving-Mounir et al. (2004) *J Med Chem* 47:962-972 including 5-butyl-7-chloro-6 hydroxybenzo[c]quinolizinium chloride (8u, MPB-104); the thiazolidinone compounds disclosed in Ma et al. (2002) *Clin Invest* 110:1651-1658 including CFTR(inh)-172, compound Vri-532 (CAS Registry No. 38214-71-0) and the xanthine derivatives disclosed in 5 Chappe et al. (1998) *Br J Pharmacol.* 123:683-93 including 3,7-dimethyl-1-propyl xanthine and 3,7-dimethyl-1-isobutyl xanthine.

Correctors useful alone and in combination in the methods described herein include but are not limited to 4-phenylbutyrate (4-PBA); the 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid 10 diamides described in Hirth et al. (2005) *Bioorg Med Chem* 15:2087-2091 including compound 1 and the compounds with reasonable activity disclosed in tables 1, 2, and 3; curcumin; the "corrector" compounds disclosed in patent publication WO2006101740 including the compounds disclosed in paragraph 2, figure 3a (compounds corr-1a, corr-3a, corr-2a, and corr-4a (CAS Registry No. 421580-53-2)), claim 1 (compounds of formula I), claim 4 (compounds 15 having formula Ia), claim 7 (compounds having formula Ib), claim 12 (compounds having formula II), claim 19 (compounds having formula III), claim 30 (compounds having formula IV), claim 36 (compounds having formula V), and the individual compounds disclosed in claims 11, 18, 29, 35, and 44; the 1,4-dihydropyridines compounds which are CFTR correctors disclosed in Pedemonte et al. (2005) *Molecular Pharmacology* 68:1736-1746 including methyl-1,4-dihydro- 20 2,6-dimethyl-3-nitro-4-2-(trifluoromethylphenyl)pyridine-5-carboxylate (BayK-8644); the phenylglycine and sulfonamide comprising "corrector" compounds disclosed in patent publication WO2005120497 including 2-[(2-1H-Indol-3-yl-acetyl)-methyl-amino]-N-(4-isopropyl-phenyl)-2-phenyl-acetamide, 2-[(2-1H-Indol-3-yl-acetyl)-methyl-amino]-N-(4-isopropyl-phenyl)-2-(4-methoxy-phenyl)-acetamide, 2-[(2-1H-Indol-3-yl-acetyl)-methyl-amino]- 25 N-(4-methoxy-phenyl)-2-phenyl-acetamide, 2-[(2-1H-Indol-3-yl-acetyl)-methyl-amino]-2,N-bis-(4-methoxy-phenyl)-acetamide, N-(2,3-Dihydro-benzo[1,4]dioxin-6-yl)-2-(2-1H-indol-2-yl-acetylamino)-2-p-tolyl-acetamide, N-(2,3-Dihydro-benzo[1,4]dioxin-6-yl)-2-[(2-1H-indol-3-yl-acetyl)-methyl-amino]-2-(4-methoxy-phenyl)-acetamide, 2-(2-1H-Indol-3-yl-acetylamino)-N-(4-isopropyl-phenyl)-2-phenyl-acetamide, N-Benzo[1,3]dioxol-5-yl-2-[(2-1H-indol-3-yl-acetyl)- 30 methyl-amino]-2-p-tolyl-acetamide, 2-[(2-Acetylamino-acetyl)-methyl-amino]-N-(2,3-dihydro-benzo [1,4]dioxin-6-yl)-2-phenyl-acetamide, 6-[(2-Ethoxy-phenyl)-methyl-sulfamoyl]-4-oxo-

1,4-dihydro-quinoline-3-carboxylic acid allylamide, 6-(Ethyl-phenyl-sulfamoyl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (3-methoxy-propyl)-amide, 6-(Methyl-m-tolyl-sulfamoyl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (pyridin-2-ylmethyl)-amide, 6-(Methyl-m-tolyl-sulfamoyl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid(2-cyclohex-1-enyl-ethyl)-amide, 6-  
5 (1,4-Dioxa-8-aza-spiro[4.5]decane-8-sulfonyl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (3-methyl-butyl)-amide, 6-[Ethyl-(4-fluoro-phenyl)-sulfamoyl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid cyclopentylamide, 6-(Methyl-o-tolyl-sulfamoyl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (3-methyl-butyl)-amide, 6-[(2,6-Dimethyl-phenyl)-methyl-sulfamoyl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid butylamide, 6-(Allyl-phenyl-sulfamoyl)-4-oxo-1,4-dihydro-  
10 quinoline-3-carboxylic acid (furan-2-ylmethyl)-amide, 6-[Ethyl-(4-fluoro-phenyl)-sulfamoyl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid(tetrahydro-furan-2-ylmethyl)-amide, 6-(Methyl-m-tolyl-sulfamoyl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid sec-butylamide, and 6-(2,3-Dihydro-indole-1-sulfonyl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid cyclohexylamide; the aminoarylthiazoles, quinazolinyaminopyrimidinones, and bisaminomethylbithiazoles  
15 compounds disclosed as correctors of defective DeltaF508-CFTR cellular processing disclosed in Pedemonte et al. (2005) *Journal of Clinical Investigation* 115:2564-2571; the compounds disclosed in Van Goor et al. (2006) *Am J Physiol Lung Cell Mol Physiol* 290:L1117-30 including VRT-325 (CAS Registry No. 815592-21-3) and VRT-422(CAS Registry No. 815589-62-9); and the pyrimidine derivatives disclosed as modulators of ATP-binding cassette  
20 transporters in WO2004111014.

P2Y and P2X receptor agonists useful alone and in combination in the methods and compositions described herein include but are not limited to Denufosol, Diquafosol, the dinucleotide compounds disclosed as selective agonists of P2Y receptors in Shaver et al. (2005) *Purinergic Signalling* 1:183-191.

#### 25 Agents which affect cAMP levels

Agents that modulate (e.g. increase cAMP levels) are useful in the methods and compositions described herein. Agents that modulate cAMP levels useful alone and in combination in the methods described herein include but are not limited to vanadate, cholera toxin, heat labile toxin, and adenylate cyclase agonists. Adenylate cyclase is a transmembrane protein that catalyzes the

conversion of ATP to cAMP and pyrophosphate. Thus, adenylate cyclase agonists may increase cAMP levels. Adenylate cyclase agonists include but are not limited to forskolin and isoproterenol (e.g. isoprenaline).

#### Sodium Transport Inhibitors

5 Agents that inhibit the transport of sodium are useful in the methods and combinations described herein. These agents may function, for example, by inhibiting the activity of molecules involved in  $\text{Na}^+/\text{H}^+$  exchange (NHE inhibitors) or sodium glucose transport (SGLT1 inhibitors).

NHE inhibitors useful alone and in combination in the methods described herein include but are not limited to Amiloride and NHE3 inhibitors such as Aventis compounds AVE-0657, S1611  
10 (disclosed in Wiemann M et al. (1999). Pflugers Arch 438:255-262.), S8218, and S3226 (CAS Registry No. 215183-03-2); Cariporide (HOE642, CAS Registry No. 159138-81-5), Zoniporide (CAS Registry No. 241800-98-6), T-162559 (CAS Registry No. 339532-12-6), HOE 694 (3-methanesulfonyl-4-piperidino-benzoyl)guanidine methanesulfonate, CAS Registry No. 149725-40-6), and the compounds disclosed in patent publications DE102005044817, WO2006074813,  
15 WO2005117977, WO2005026173, DE10312963, DE10304294, WO2003101984, WO2003053434, DE10163992, WO2003051866, WO2003048129, WO2002024637, WO2002020496, WO2002046169, and WO2001079186.

Taurine is an example of a sodium glucose transport inhibitor.

#### Phosphodiesterase Inhibitors

20 Phosphodiesterase inhibitors, which block one or more of the five subtypes of the enzyme phosphodiesterase (PDE) and therefore prevent the inactivation of cAMP and cGMP, are useful alone and in combination in the methods and compositions described herein. Certain PDEs exhibit cyclic nucleotide specificity, for example, PDE5 is cGMP specific; thus PDE5 inhibitors specifically prevent the inactivation of cGMP.

25 PDE inhibitors include PDE3 inhibitors, PDE4 inhibitors, PDE5 inhibitors, and inhibitors with multiple specificity including PDE3/4 and PDE3/4/5 inhibitors. Specific PDE inhibitors include but are not limited to those disclosed in patent publications DE1470341, DE2108438, DE2123328, DE2305339, DE2305575, DE2315801, DE2402908, DE2413935, DE2451417, DE2459090, DE2646469, DE2727481, DE2825048, DE2837161, DE2845220, DE2847621,

DE2934747, DE3021792, DE3038166, DE3044568, EP000718, EP0008408, EP0010759,  
EP0059948, EP0075436, EP0096517, EP0112987, EP0116948, EP0150937, EP0158380,  
EP0161632, EP0161918, EP0167121, EP0199127, EP0220044, EP0247725, EP0258191,  
EP0272910, EP0272914, EP0294647, EP0300726, EP0335386, EP0357788, EP0389282,  
5 EP0406958, EP0426180, EP0428302, EP0435811, EP0470805, EP0482208, EP0490823,  
EP0506194, EP0511865, EP0527117, EP0626939, EP0664289, EP0671389, EP0685474,  
EP0685475, EP0685479, JP92234389, JP94329652, JP95010875, US4963561, US5141931,  
WO9117991, WO9200968, WO9212961, WO9307146, WO9315044, WO9315045,  
WO9318024, WO9319068, WO9319720, WO9319747, WO9319749, WO9319751,  
10 WO9325517, WO9402465, WO9406423, WO9412461, WO9420455, WO9422852,  
WO9425437, WO9427947, WO9500516, WO9501980, WO9503794, WO9504045,  
WO9504046, WO9505386, WO9508534, WO9509623, WO9509624, WO9509627,  
WO9509836, WO9514667, WO9514680, WO9514681, WO9517392, WO9517399,  
WO9519362, WO9522520, WO9524381, WO9527692, WO9528926, WO9535281,  
15 WO9535282, WO9600218, WO9601825, WO9602541, WO9611917, DE3142982, DE1116676,  
DE2162096, EP0293063, EP0463756, EP0482208, EP0579496, EP0667345 US6331543,  
US20050004222 (including those disclosed in formulas I-XIII and paragraphs 37-39, 85-0545  
and 557-577), WO9307124, EP0163965, EP0393500, EP0510562, EP0553174, WO9501338  
and WO9603399, as well as PDE5 inhibitors (such as RX-RA-69, SCH-51866, KT-734,  
20 vesnarinone, zaprinast, SKF-96231, ER-21355, BF/GP-385, NM-702, vardenafil (LEVITRA®);  
and tadalafil (CIALIS® and sildenafil (Viagra™)), PDE4 inhibitors (such as etazolate,  
ICI63197, RP73401, imazolidinone (RO-20-1724), MEM 1414 (R1533/R1500; Pharmacia  
Roche), denbutylline, rolipram, oxagrelate, nitraquazone, Y-590, DH-6471, SKF-94120,  
motapizone, lixazinone, indolidan, olprinone, atizoram, KS-506-G, dipamfylline, BMY-43351,  
25 atizoram, arofylline, filaminast, PDB-093, UCB-29646, CDP-840, SKF-107806, piclamilast, RS-  
17597, RS-25344-000, SB-207499, TIBENELAST, SB-210667, SB-211572, SB-211600, SB-  
212066, SB-212179, GW-3600, CDP-840, mopidamol, anagrelide, ibudilast, amrinone,  
pimobendan, cilostazol, quazinone and N-(3,5-dichloropyrid-4-yl)-3-cyclopropylmethoxy4-  
difluoromethoxybenzamide, PDE3 inhibitors (such as ICI153, 100, bemorandane (RWJ 22867),  
30 MCI-154, UD-CG 212, sulmazole, ampizone, cilostamide, carbazeran, piroximone, imazodan,  
CI-930, signazodan, adibendan, saterinone, SKF-95654, SDZ-MKS-492, 349-U-85, emoradan,

EMD-53998, EMD-57033, NSP-306, NSP-307, revizinone, NM-702, WIN-62582 and WIN-63291, enoximone and milrinone, PDE3/4 inhibitors (such as benafentrine, trequinsin, ORG-30029, zardaverine, L-686398, SDZ-ISQ-844, ORG-20241, EMD-54622, and tolafentrine) and other PDE inhibitors (such as vinpocetin, papaverine, enprofylline, cilomilast, fenoximone, pentoxifylline, roflumilast, and theophylline.

#### Renin inhibitors and aldosterone antagonists

Renin functions in the Renin Angiotensin System (RAS) and is a key regulator of blood pressure. Suppression of the RAS has been shown to treat hypertension and reduce cardiovascular events.

10 Renin inhibitors useful alone and in combination in the methods and compositions described herein include but are not limited to Tekturna® (Rasilez and Aliskiren) and SPP635.

Aldosterone is a steroid hormone which functions in the regulation of sodium and potassium balance in the blood. Aldosterone antagonists useful alone and in combination in the methods and compositions described herein include but are not limited to Spironolactone, Canrenone, and Eplerenone.

#### Polymer Resins

Polymer resins useful alone and in combination in the methods and compositions described herein include but are not limited to the dietary fiber psyllium seed husk (psyllium), lipid lowering polymers (e.g. Colesevelam, Sevalmer, Cholestyramine), nonabsorbed polymer resins (e.g. hyaluronic acid, polycarbophil calcium, polyvinyl acetate, polyvinyl pyrrolidone, polystyrene sulfate (alone and in combination with yellow phenolphthalein and including potassium salt derivatives thereof)), and sodium binding polymers.

Sodium-binding polymers (also called cation exchange cores) include but are not limited to those disclosed in US20050220750 and US20060024265 including crosslinked polyvinylsulfamate polymer, N-(bis-phosphonic-ethyl) polyvinylamine polymer, poly- $\alpha$ -acrylic acid polymer, poly- $\alpha$ -fluoroacrylic acid polymer, polyvinylphosphoramidic polymer, polyvinylsulfamate polymer, polyvinylsulfamate/vinylsulfate copolymer, polyvinylsulfate polymer, polyvinylsulfonate polymer, polyvinylsulfonate polymer, vinylphosphonate/ $\alpha$ -fluoroacrylic acid copolymer, vinylphosphonate/ $\alpha$ -fluoroacrylic acid copolymer, or vinylphosphonate/acrylic acid copolymer

and combinations thereof. In certain embodiments, the sodium-binding polymer is administered as core-shell composition which further comprises a semi-permeable shell, said semi-permeable shell comprising at least one of a poly-11 trimethylammoniumundecylmethacrylate polymer, a styrene-vinylpyridine polymer, 11-dimethyl-aminodecylmethacrylate/laurylmethacrylate copolymer, or a polyallylamine/polystyrene sulfonate polymer. An example of a core-shell composition useful alone and the combination in the methods and compositions described herein is ILY101 (Ilypsa).

#### Example 1: Preparation of peptides

10 Peptides can be recombinantly produced in bacteria as follows. T7 expression vectors, pET26b(+) (Novagen) expressing the peptide of interest are constructed using standard molecular biology techniques and are transformed into *E. coli* bacterial host BL21  $\lambda$  DE3 (Invitrogen). A single colony is inoculated and grown shaking overnight at 30°C in L broth + 25 mg/l kanamycin. The overnight culture is added to 3.2 L of batch medium (Glucose 25 g/l, 15 Casamino Acids 5 g/l, Yeast Extract 5 g/l,  $\text{KH}_2\text{PO}_4$  13.3 g/l,  $(\text{NH}_4)_2\text{HPO}_4$  4 g/l,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  1.2 g/l, Citric Acid 1.7 g/l, EDTA 8.4 mg/l,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  2.5 mg/l,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  15 mg/l,  $\text{CuCl}_2 \cdot 4\text{H}_2\text{O}$  1.5 mg/l,  $\text{H}_3\text{BO}_3$  3 mg/l,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  2.5 mg/l, Zn Acetate- $2\text{H}_2\text{O}$  13 mg/l, Ferric Citrate 100 mg/l, Kanamycin 25 mg/l, Antifoam DF<sub>2</sub>O<sub>4</sub> 1 ml/l) and fermented using the following process parameters : pH 6.7 - control with base only (28%  $\text{NH}_4\text{OH}$ ), 30°C, aeration : 5 liters per 20 minute. After the initial consumption of batch glucose (based on monitoring dissolved oxygen (DO) levels), 1.5 L of feed medium (Glucose 700 g/l, Casamino Acids 10 g/l, Yeast Extract 10 g/l,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  4 g/l, EDTA 13 mg/l,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  4 mg/l,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  23.5 mg/l,  $\text{CuCl}_2 \cdot 4\text{H}_2\text{O}$  2.5 mg/l,  $\text{H}_3\text{BO}_3$  5 mg/l,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  4 mg/l, Zn Acetate- $2\text{H}_2\text{O}$  16 mg/l, Ferric Citrate 40 mg/l, Antifoam DF<sub>2</sub>O<sub>4</sub> 1 ml/l) is added at a feed rate controlled to maintain 20% DO. IPTG is 25 added to 0.2 mM 2 hours post feed start. The total run time is approximately 40-45 hours (until feed exhaustion).

Cells are collected by centrifugation at 5,000 g for 10 minutes. The cell pellet is discarded and the supernatant is passed through a 50 Kd ultrafiltration unit. The 50 Kd filtrate (0.6 liters) is 30 loaded onto a 110 ml Q-Sepharose fast Flow column (Amersham Pharmacia, equilibrated with

20 mM Tris-HCl pH 7.5) at a flow rate of 400 ml/hour. The column is washed with six volumes of 20 mM Tris-HCl pH 7.5 and proteins are eluted with 50 mM acetic acid collecting 50 ml fractions. Fractions containing peptide are pooled and the solvent is removed by rotary evaporation. The dried proteins are resuspended in 10 ml of 8% acetic acid, 0.1% trifluoroacetic acid (TFA) and loaded onto a Varian Polaris C18-A column (250 X 21.2 mm 10 µm, equilibrated in the same buffer) at a flow rate of 20 ml/min. The column is washed with 100 ml of 8% methanol, 0.1% TFA and developed with a gradient (300 ml) of 24 to 48% methanol, 0.1% TFA, collecting 5-ml fractions. Fractions containing peptide are pooled and the solvent is removed by rotary evaporation. The peptides are dissolved in 0.1%TFA and lyophilized.

Peptide fractions are analyzed by standard LCMS and HPLC. Peptides can also be chemically synthesized by a commercial peptide synthesis company.

#### Example 2: Activation of the intestinal GC-C receptor by peptides

The ability of peptides to activate the intestinal GC-C receptor can be assessed in an assay employing the T84 human colon carcinoma cell line (American Type Culture Collection (Bethesda, Md)). For the assays cells are grown to confluency in 24-well culture plates with a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's medium (DMEM), supplemented with 5% fetal calf serum and were used at between passages 54 and 60.

Briefly, monolayers of T84 cells in 24-well plates are washed twice with 1 ml/well DMEM, then incubated at 37°C for 10 min with 0.45 ml DMEM containing 1 mM isobutylmethylxanthine (IBMX), a cyclic nucleotide phosphodiesterase inhibitor. Test peptides (50µl) are then added and incubated for 30 minutes at 37°C. The media is aspirated and the reaction was then terminated by the addition of ice cold 0.5 ml of 0.1N HCl. The samples are held on ice for 20 minutes and then evaporated to dryness using a heat gun or vacuum centrifugation. The dried samples are resuspended in 0.5ml of phosphate buffer provided in the Cayman Chemical Cyclic GMP EIA kit (Cayman Chemical, Ann Arbor, MI). Cyclic GMP is measured by EIA according to procedures outlined in the Cayman Chemical Cyclic GMP EIA kit. EC<sub>50</sub> is defined as the concentration by which 50% of the maximal activity is seen. Maximum cGMP level in assay is