Example 28: 40-O-[2-(4',5'-dicarboethoxy-1',2',3'-triazol-1'-yl)-ethyl]-rapamycin

98 mg of 40-O-(2-azidoethyl)-rapamycin and 32 mg diethylacetylene dicarboxylate are suspended in 0.5 ml toluene and heated at 65 C for 5h. The reaction mixture is then cooled at room temperature, loaded on 10 g silica gel and eluted with hexane/ethyl acetate 1/1 to afford the title product: MS (FAB) m/z 1175 (20%,M+Na); 1121 (15%, M-MeOH); 1103 (60%, M-(MeOH+H2O))

H-NMR (CDCl3) d: 0.62 (1H, q, J=12 Hz); 1.40 (3H, t, J=8 Hz); 1.42 (3H, t, J=8 Hz); 3.13 (3H, s); 3.25 (3H, s); 3.33 (3H, s)

MBA (rel. IC50): 2.7

IL-6 dep. prol. (rel. IC50): 12

The previous examples may also be made using as starting material instead of rapamycin, 9-deoxo-rapamycin, 26-dihydro rapamycin, or 9-deoxo-, 26-dihydro-rapamycin. Alternatively, and preferably, as described e.g., in example 20, the rapamycin compounds of the above examples may be hydrogenated or reduced, using suitable protecting groups where necessary. The following novel methods for reducing the keto at C9, or hydrogenating the keto at C26 are provided:

Example 29: Removal of keto at C9

A stream of hydrogen sulfide is passed at room temperature through a stirred solution of 3.2 g (3.5 mmol) of rapamycin in 50 ml pyridine and 2.5 ml DMF. The solution turns from colorless to yellow. After two hours, the introduction of hydrogen sulfide is stopped and stirring is continued for five days, during which time the solution turns gradually orange. TLC and HPLC analysis verifies complete consumption of the starting material and the presence of a single new compound. The solution is purged with nitrogen for one hour and concentrated under reduced pressure. The residue is taken up in ethyl acetate, washed with cold 1N HCl solution (3x), saturated sodium bicarbonate solution and saturated brine. The organic layer is dried over anhydrous sodium sulfate and filtered and concentrated under reduced pressure. The residue is taken up in ether and the precipitated

sulfur is filtered off. Concentration of the ethereal solution followed by column chromatography on silica gel (10:4:1 CH_2Cl_2/i-Pr_2O/MeOH) yields 9-deoxorapamycin as a colorless foam. The identity of the product is confirmed by nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS), and/or infrared spectrosopy (IR). 9-deoxorapamycin is found to exhibit the following characteristic physical data: ^{1}H NMR ($CDCL_3$) δ 1.61 (3H,d,J = 1 Hz, C17-CH_3), 1.76 (3H,d,J = 1.2 Hz,C29-CH_3), 2.42 (1H,d,J = 14.5 Hz, H-9), 2.74 (1H,d,J = 14.5 Hz, H-9), 3.13 (3H,s,C16-OCH_3) 3.5 (3H,s,C27-OCH_3), 3.40 (3H,s,C39-OCH_3), 5.40 (1H,d,J = 10 Hz, H-30), 5.57 (1H,dd,J_1 = 8.6 Hz, J_2 = 15 Hz, H-22), 5.96 (1H,d,J = 9 Hz, H-18), 6.09 (1H,d,J = 1.7 Hz, 10-OH), 6.15 (1H,dd,J_1 = 10 Hz, J_2 = 15Hz, H-21), 6.37 (1H,dd,J_1 = 1.5 Hz, J_2 = 5 Hz, H-19), 6.38 (1H,J = 9.5 Hz, H-20). ^{13}C NMR ($CDCl_3$) δ 38.5 (C-9), 98.0 (C-10), 170.7 (C-1), 173.0 (C-8), 208.8 (C-32), 216.9 (C-26).

MS(FAB) m/z 922 8[M+Na^+]), 899 (M^+), 881 ([M-H_2O]$^+$), 868 ([M-OCH_3]$^+$), 850 ([M-(H_2O+OCH_3)]$^+$).

IR (major peaks)(cm^{-1}) 987, 1086, 1193, 1453, 1616, 1717, 1739, 3443.

MBA (rel. IC_{50}): 1

MLR (rel. IC_{50}): 14

IL-6 dep. prol. (rel. IC_{50}): 9

Example 30: Dihydrogenation of keto at C26

To a stirred solution of 421 mg (1.6 mmol) of tetramethylammonium triacetoxyborohydride in 2 ml of acetonitrile is added 2 ml of acetic acid. The resulting mixture is stirred for 30 minutes at room temperature and cooled to -35°C. At this temperature a solution of 180 mg (0.2 mmol) of 9-deoxo-rapamycin in 1 ml of acetonitrile is added and the resulting mixture is allowed to stir for 24 hours. The mixture is quenched with a saturated sodium potassium tartrate solution and allowed to warm to room temperature. Stirring is continued until both layers are clear and ethyl acetate is added. The layers are separated and the aqueous layer is extracted twice with ethyl acetate. The resulting organic solution is washed once with a 10% sodium bicarbonate solution and twice with

saturated brine, then dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue is purified by column chromatography on silica gel (90:10 AcOEt-hexane). As the starting material in this case was 9-deoxorapamycin, the final compound is 9-deoxorapamycin, 26-dihydrorapamycin is produced as a colorless foam, having the following characteristic spectroscopic data: ^{1}H NMR ($CDCl_3$) (major isomer) δ.9 (3H,d,J = 6.9 Hz, CHC<u>H</u>$_3$), 0.93 (3H,d,J = 6.9 Hz, CHC<u>H</u>$_3$), 1.00 (3H,d,J = 6.9 Hz CHC<u>H</u>$_3$), 1.07 (3H,d,J = 6.9 Hz, CHC<u>H</u>$_3$), 1.17 (3H,d,J = 6.9 Hz, CHC<u>H</u>$_3$), 1.61 (3H,d,J = 1Hz, C17-CH_3), 1.73 (3H,d,J = 1.2 Hz, C29-CH_3), 2.43 (1H,dd,J = 4.1 and 16.0 Hz, H-33), 2.46 (1H,d,J = 13.8 Hz, H-9), 2.58 (1H,m,H-25), 2.77 (1H,d,J = 13.8 Hz, H-9), 2.82 (1H,dd,J = 8.3 and 16.0 Hz, H-33), 3.17 (1H,dd,J = 4.1 and 9.2 Hz, H-27), 3.61 (2H,m, H-14 and H28), 5.19 (1H,ddd,J = 4.1, 4.6 and 8.3 Hz, H-34), 5.49 (1H, broad d,J = 5.0 Hz, H-2), 5.56 (1H,d,J = 9.1 Hz, H-30), 5.75 (1H,dd,J = 6.9 and 14.7 Hz, H-22), 5.76 (1H,s,10-OH), 5.99 (1H,broad d,J = 9.2 Hz, H-18), 6.10 (1H,m,H-21), 6.36 (2H,m,H-19 and H-20);
MS (FAB) m/z 924 ([M + Na]), 852 ([M-(H_2O + CH_3O)]$^+$).
MBA (rel. IC_{50}): 47
MLR (rel. IC_{50}): 134
IL-6 dep. prol. (rel. IC_{50}): 78

26-dihydrorapamycin is prepared in the same manner, using rapamycin in place of 9-deoxorapamycin. This product has the following characteristic spectroscopic data: ^{13}C-NMR ($CDCl_3$) (major isomer) d = 208.3 (C-32); 194.0 (C-9); 169.3 (C-1); 166.6 (C-8); 140.9 (C-22); 136.5 (C-29); 136.2 (C-17); 133.5 (C-20); 129.1 (C-21); 128.7 (C-18); 126.2 (C-30); 125.3 (C-19); 98.6 (C-10); 84.4 (C-39); 83.9 (C-16; 81.6 (C-27); 75.4 (C-34); 74.3 (C-28); 73.9 (C-40); 72.9 (C-26); 67.4 (C-14); 59.1 (27-O<u>C</u>H$_3$); 56.6 (39-O<u>C</u>H$_3$); 55.9 (16-O<u>C</u>H$_3$); 51.3 (C-2); 46.8 (C-31); 44.3 (C-6); 40.4 (C-33); 40.4 (C-25); 39.5 (C-24); 38.8 (C-15); 38.0 (C-36); 34.3 (C-23); 34.2 (C-38); 33.5 (C-11); 33.3 (C-37); 33.2 (C-35); 31.5 (C-42); 31.3 (C-41); 30.9 (C-13); 27.1 (C-12); 27.0 (C-3); 25.2 (C-5); 21.4 (23-<u>C</u>H$_3$); 20.7 (C-4); 17.3 (11-<u>C</u>H$_3$); 16.1 (31-<u>C</u>H3); 15.9 (35-<u>C</u>H$_3$); 14.4 (25-<u>C</u>H$_3$); 14.2 (29-<u>C</u>H$_3$); 10.3 (17-<u>C</u>H$_3$).

MS (FAB) m/z : 884 (M-OCH_3, 35%); 866 (M-[OCH_3 + H_2O], 100%; 848 (M-[OCH_3 + 2 H_2O], 40%).

MBA (rel. IC_{50}): 1.7

MLR (rel. IC_{50}): 1

IL-6 dep. prol. (rel. IC_{50}): 7.5

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APPLICA

CLAIMS

	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT											
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TOTAL DEP.	10						TOTAL DEP.									
TOTAL CLAIMS	11						TOTAL CLAIMS									

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