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(54) **SOLID PHASE SYNTHESIS OF H(GLY2)GLP-2**

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(57) **ABSTRACT**

The present invention relates to a method of preparing a peptide comprising the amino acid sequence His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp (SEQ ID NO:1). In particular, the method comprises the steps of providing a first peptide fragment having a first protection group, which peptide fragment is conjugated to a support; providing a second peptide fragment having a second protection group; removing the first protection group from the first peptide fragment; and coupling the second peptide fragment to the N-terminally deprotected, support-conjugated first peptide fragment. The present invention further relates to a method of preparing a pharmaceutical composition containing said peptide.

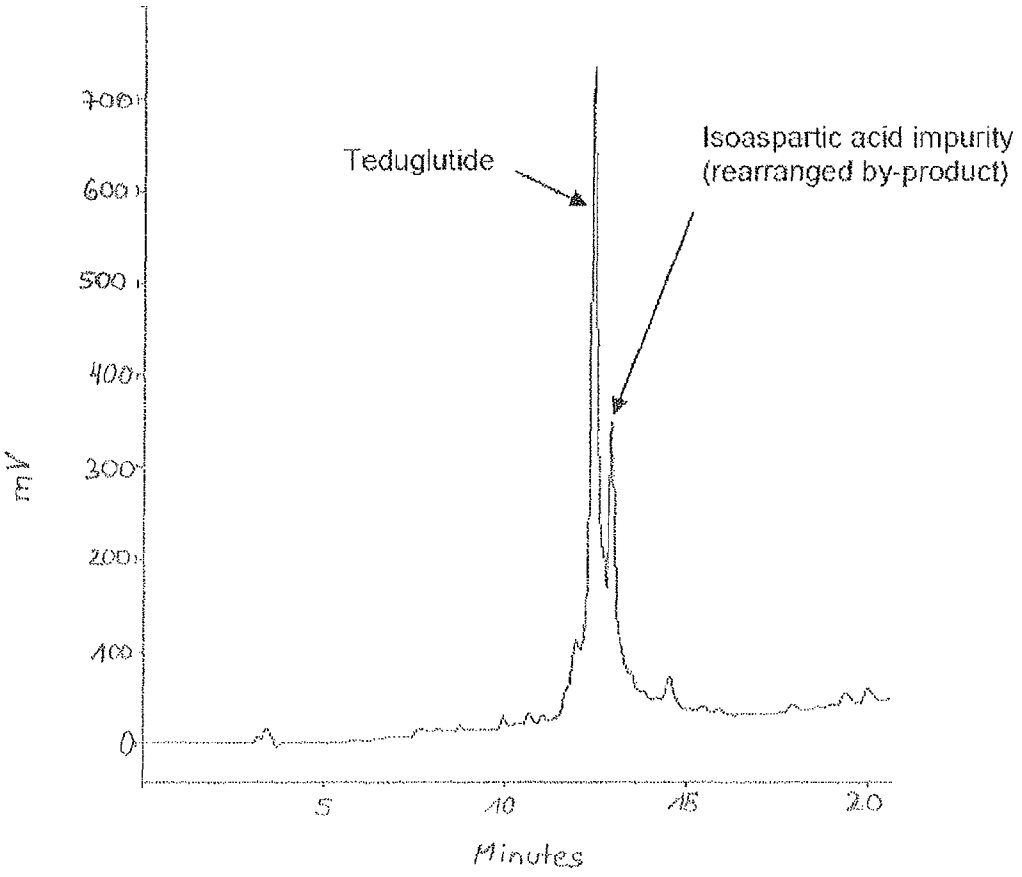

Figure 1

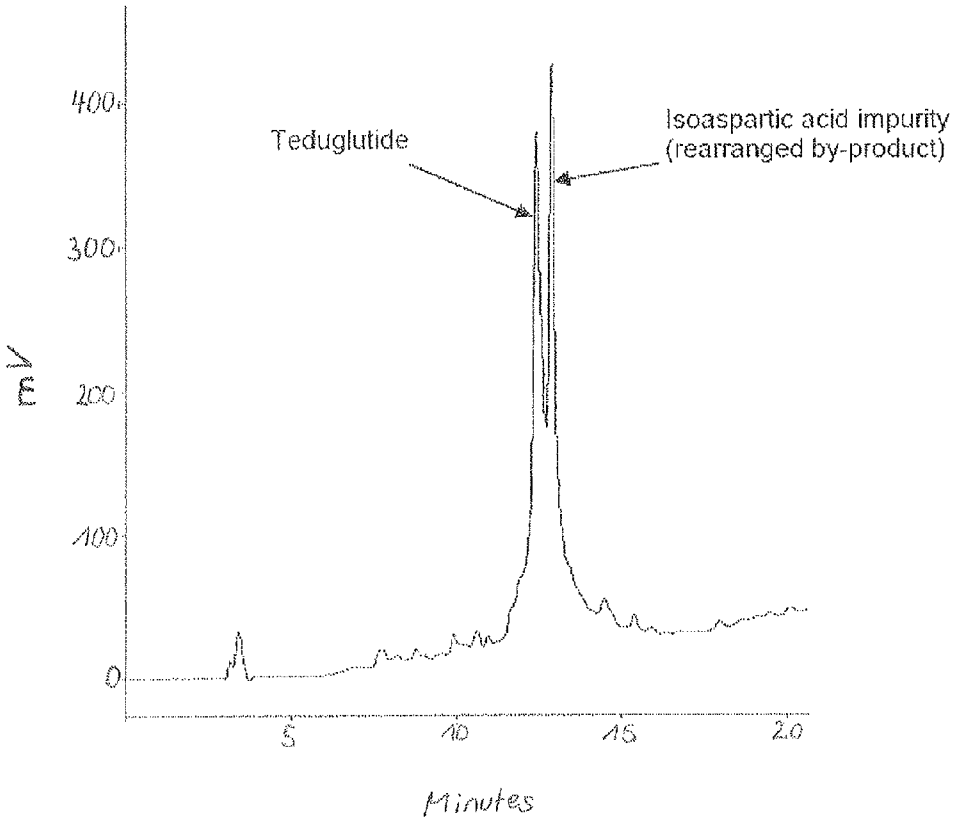

Figure 2

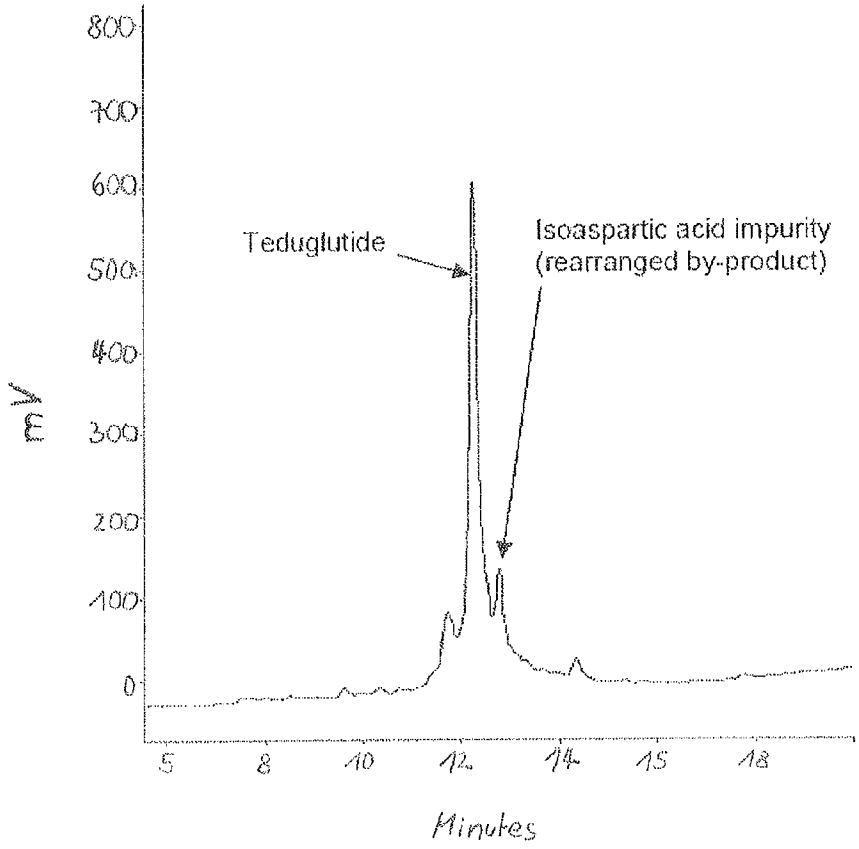

Figure 3

SOLID PHASE SYNTHESIS OF H(GLY2)GLP-2

SUBJECT OF THE INVENTION

[0001] The present invention relates to a method of preparing a peptide comprising the amino acid sequence His-Gly-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp (SEQ ID NO:1). In particular, the method comprises the steps of providing a first peptide fragment having a first protection group, which peptide fragment is conjugated to a support; providing a second peptide fragment having a second protection group; removing the first protection group from the first peptide fragment; and coupling the second peptide fragment to the N-terminally deprotected, support-conjugated first peptide fragment. The present invention further relates to a method of preparing a pharmaceutical composition containing said peptide.

BACKGROUND OF THE INVENTION

[0002] Glucagon-like peptide-2 (GLP-2) is a 33 amino acid peptide having therapeutic applications in the treatment of diseases of the gastrointestinal tract. This naturally occurring hormone has been shown to regulate the growth, proliferation and maintenance of cells lining the gastrointestinal tract. In particular, it has been determined that GLP-2 and analogs thereof act as trophic agents to enhance and maintain the functioning of the gastrointestinal tract and to promote growth of intestinal tissue (see, e.g., European patent application EP 1 246 639 A2).

[0003] Teduglutide, hereinafter also referred to as "h[Gly2]GLP-2", is a single-chain and non-glycosylated 33-amino acid peptide having the following sequence:

$$His^{1}-Gly^{2}-Asp^{3}-Gly^{4}-Ser^{5}-Phe^{6}-Ser^{7}-Asp^{8}-Glu^{9}-Met^{10}-Asn^{11}-Thr^{12}-Ile^{13}-Leu^{14}-Asp^{15}-Asn^{16}-Leu^{17}-Ala^{18}-Ala^{19}-Arg^{20}-Asp^{21}-Phe^{22}-Ile^{23}-Asn^{24}-Trp^{25}-Leu^{26}-Ile^{27}-Gln^{28}-Thr^{29}-Lys^{30}-Ile^{31}-Thr^{32}-Asp^{33}$$ (SEQ ID NO:1)

[0004] This analog of GLP-2 differs from native GLP-2 by a change in one amino acid, i.e. alanine is replaced by glycine in position 2. This change has been determined to result in a peptide with a longer half-life. In particular, animal studies indicate that administration of this peptide produces a significant increase in both the mass and absorptive surface area of the epithelium lining the intestine, and moreover has a pronounced effect on reducing gut permeability.

[0005] As many other therapeutic peptides, this GLP-2 analog can be manufactured recombinantly by expression in *E. coli*. However, in order to increase the production yield and to eliminate the need for some animal-derived raw materials in production, there was a need to provide alternative methods of preparing teduglutide.

[0006] In the prior art, several solutions have been sought for chemically synthesizing peptides in general.

[0007] Solid-phase peptide synthesis (SPPS) is a method introduced by Merrifield in 1963 (J. Amer. Chem. Soc. 1963, 85: 2149-2154). An overview of solid-phase synthesis of peptides and proteins is provided by S. B. H. Kent (Annu. Rev. Biochem. 1988, 57: 957-989).

[0008] In general, one strategy for the synthesis of peptide chains by solid-phase synthesis is the stepwise solid-phase synthesis. In stepwise SPPS, the C-terminal amino acid in the form of an N-[alpha]-protected, if necessary side-chain protected reactive derivative is covalently coupled either directly or by means of a suitable linker to a solid support, e.g. a polymeric resin, which is swollen in an organic solvent. The N-[alpha]-protection group is removed, and the subsequent protected amino acids are added in a stepwise fashion. When the desired peptide chain length has been obtained, the side-chain protection groups are removed, and the peptide is cleaved from the support. Over the years, two major coupling strategies have been developed based on the use of different N-[alpha]-protection groups and matching side-chain protection groups. Merrifield used t-butyloxycarbonyl (Boc) as the N-[alpha] protection group, while 9-fluorenylmethyloxy-carbonyl (Fmoc) was introduced by Carpino and Han (J. Org. Chem. 1972, 37: 3404-3409).

[0009] A general synthesis method for the preparation of GLP-2 molecules including teduglutide is described, e.g., in international patent applications WO 2006/117565 and WO 2008/056155. According to these applications, peptides were synthesized batchwise in a polyethylene vessel equipped with a polypropylene filter for filtration using 9-fluorenylmethyloxycarbonyl (Fmoc) as N-[alpha]-amino protection group and suitable common protection groups for side-chain functionalities. The amino acids were coupled as in situ generated N-hydroxybenzotriazole (HOBt) or 1-hydroxy-7-aza-benzotriazole (HOAt) esters made from appropriate N-[alpha]-protected amino acids and HOBt or HOAt by means of diisopropylcarbodiimide (DIC) in DMF. These substances can react with O-acylurea formed by the reaction of DIC and the carboxylic acid group of the amino acid to form an active ester. Deprotection of the Fmoc group was performed by treatment with piperidine in DMF. Subsequently, the peptides were cleaved from the resins by treatment with 95% trifluoroacetic acid (TFA). The crude freeze-dried product was analyzed by high-performance liquid chromatography (HPLC) and identified by mass spectrometry (MS).

[0010] According to the prior art, GLP-2 molecules are being considered as candidates for standard chemical synthesis by the Fmoc-solid phase approach. It appeared to be a common understanding that GLP-2 molecules are probably best assembled in a linear fashion by solid phase chemistry due to the relative ease of assembly and the ultimate manufacturing scale. However, numerous side reactions can occur during solid phase synthesis, some of which are specific to the chemistries employed using Fmoc methodology.

[0011] In particular, it has been found that one particular problem in the synthesis of teduglutide by Fmoc-solid phase chemistry involves rearrangement of the -Asp-Gly-bond at position 3-4 in the molecule resulting in the formation of the [beta]-Asp analogue (so-called "aspartimide by-product formation"). The [beta]-isomerization of -Asp-Gly- bonds involves the carboxy side-chain group from the aspartic acid forming a peptide bond with the [alpha]-amino group of the adjacent glycine via a succinimide intermediate. The main cause of this reaction is the treatment of the teduglutide-solid phase with piperidine, or other bases during the Fmoc removal stage. This reaction resulting in the undesired by-

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