CLAIMS

1. A method of preparing an isolated peptide comprising a lipophilically modified lysine side chain, comprising the steps of:

(i) assembling the amino acid sequence of said peptide with protected reactive functional groups in the side chains in a step-wise manner using solid phase peptide synthesis (SPPS), wherein the side chain of lysine to be modified is protected by a trityl-based protecting group, particularly monomethoxytrityl (Mmt) or 4-methyltrityl (Mtt);

(ii) drying the solid phase resin after the assembling of the amino acid sequence has been completed;

(iii) treating the dried resin several times with a solution of 0.5 to 1.5% (v/v) trifluoroacetic acid (TFA) in dichloromethane (DCM) in order to deprotect the lysine side chain to be modified, wherein the number of TFA in DCM treatments is adjusted such that the yield of modified lysine side chain in step (ix) is at least 85% as compared to peptide without modified lysine side chain;

(iv) neutralizing the resin;

(v) coupling at least one activated 9-fluorenylmethyloxycarbonyl (Fmoc)-bound linker moiety to the deprotected lysine side chain;

(vi) deprotecting the terminal functional group of the linker coupled to the lysine side chain in step (v);

(vii) coupling an activated lipophilic moiety, particularly an activated fatty acid, to the deprotected terminal functional group of the linker of step (vi);

(viii) drying the resin; and

(ix) cleaving the peptide from the resin,

wherein the isolated peptide is an exendin-4 derivative having a length of between 30 and 44, particularly between 38 and 40 amino acids, wherein the sequence identity to wild-type exendin-4 (SEQ ID NO.: 3) in the region corresponding to amino acids 1-13 of wild-type exendin is at least 65%.

2. The method of claim 1, wherein

(i) the sequence identity to wild-type exendin-4 in the region corresponding to amino acids 22-39 of wild-type exendin is at least 70%, and/or

(ii) the lipophilically modified lysine side chain is at position 14 (Lys(14)) with respect to the amino acid positions of wild-type exendin-4.

3. The method of claim 1 or 2, wherein, in step (iii), the dried resin is treated at least seven times for at least 5 minutes, particularly nine times for at least 10 minutes or nine times for at least 15 min, with a solution of about 1% (v/v) TFA in DCM.

4. The method of any one of the preceding claims, further comprising the steps of:
(viii-a) analyzing the deprotection yield of step (iii) by cleaving a test sample of the resin bound peptide; and
(viii-b) optionally repeating steps (iii) to (viii-a) until the content of cleaved peptide containing the modified lysine side chain is at least 85%, particularly at least 90%, more particularly at least 95% compared to peptide without modified lysine side chain.

5. The method of any one of the preceding claims, wherein the isolated peptide has the amino acid sequence
H-dS-Q-G-T-F-T-S-D-L-S-K-Q-K(γE-Palm)-E-S-K-A-A-Q-D-F-I-E-W-L-K-A-G-G-P-S-S-G-A-P-P-P-S-NH_2 (SEQ ID NO.: 1).

6. The method of any one of the preceding claims, wherein the cleavage of step (ix) is performed with a cleavage cocktail comprising at least 90% (v/v) trifluoroacetic acid and at least 1% (v/v) ethanedithiol, which is free of thioanisole and ethylmethylsulfide.

7. The method of claim 6, wherein the cleavage cocktail further comprises a suitable indole compound selected from indoles carrying a C_1-C_3 alkyl or a C_1-C_3 acyl subsitutent at the carbon atom C3 or at the nitrogen atom, particularly 3-methylindole.

8. The method of any one of claims 1-5, wherein the cleavage of step (ix) is performed with a cleavage cocktail comprising at least 80% (w/w) trifluoroacetic acid and at least 1% (w/w) 1,2-ethanedithiol, which is free of thioanisole and ethylmethylsulfide.

9. The method of claim 8, wherein the cleavage cocktail consists of 88.2-98.3% (w/w) trifluoroacetic acid, 1-4.3% (w/w) 1,2-ethanedithiol and 0.7-7.5% (w/w) 3-methylindole.

10. The method of any one of the preceding claims, further comprising the steps of:
(x) filtering the peptide solution after cleavage;
(xi) distilling the filtered peptide solution under vacuum;
(xii) adding the residual fraction from the distillation to an antisolvent comprising a dialkyl ether and a heptane;

(xiii) stirring the precipitated solution

(xiv) filtering the precipitated solution;

(xv) washing the precipitate; and

(xvi) drying the wet peptide.

11. The method of claim 10, wherein in step (xii), the antisolvent is a mixture consisting of diisopropyl ether (DIPE) and n-heptane, particularly a mixture of DIPE and n-heptane in a ratio of from 25:75 (v/v) to 35:65 (v/v).

12. The method of any one of the preceding claims, comprising the steps of:

(i) assembling the amino acid sequence of a peptide of SEQ ID NO.: 1 with protected reactive functional groups in the side chains in a step-wise manner using solid phase peptide synthesis (SPPS), wherein the side chain of lysine 14 is protected by monomethoxytrityl (Mmt);

(ii) drying the solid phase resin for at least 5 hours at room temperature after the assembling of the amino acid sequence has been completed;

(iii) treating the dried resin nine times for 10 minutes each with a solution of 1% (v/v) trifluoroacetic acid (TFA) in dichloromethane (DCM) in order to deprotect the lysine side chain at position 14;

(iv) neutralizing the resin with a solution of 3% diisopropyl ethylamine (DIPEA) in DCM, until the pH of the solution remains at ≥8;

(v) coupling an activated Fmoc-Glu-OtBu linker moiety to the deprotected lysine side chain under basic conditions;

(vi) cleaving the Fmoc group of the linker coupled to the lysine side chain in step (v) with 20% (v/v) piperidine in DMF;

(vii) coupling activated palmitic acid to the deprotected terminal functional group of the linker of step (vi);

(viii) drying the resin;

(viii-a) analyzing the deprotection yield of step (iii) by cleaving a test sample of the resin bound peptide;

(viii-b) repeating steps (iii) to (viii-a) until the content of cleaved peptide containing the modified lysine side chain is at least 85%; and

(ix) cleaving the peptide from the resin at 25°C using a cleavage cocktail comprising at least 90% (v/v) trifluoroacetic acid and at least 1% (v/v) ethanedithiol, which is free of thioanisole and ethylmethylsulfide.

13. The method of any one of the preceding claims, comprising the steps of:

(i) assembling the amino acid sequence of a peptide of SEQ ID NO.: 1 with protected reactive functional groups in the side chains in a step-wise manner using solid phase peptide synthesis (SPPS), wherein the side chain of lysine 14 is protected by monomethoxytrityl (Mmt);

(ii) drying the solid phase resin for at least 5 hours at room temperature after the assembling of the amino acid sequence has been completed;

(iii) treating the dried resin nine times for 10 minutes each with a solution of 1% (v/v) trifluoroacetic acid (TFA) in dichloromethane (DCM) in order to deprotect the lysine side chain at position 14;

(iv) neutralizing the resin with a solution of 3% diisopropyl ethylamine (DIPEA) in DCM, until the pH of the solution remains at ≥8;

(v) coupling an activated Fmoc-Glu-OtBu linker moiety to the deprotected lysine side chain under basic conditions;

(vi) cleaving the Fmoc group of the linker coupled to the lysine side chain in step (v) with 20% (v/v) piperidine in DMF;

(vii) coupling activated palmitic acid to the deprotected terminal functional group of the linker of step (vi);

(viii) drying the resin;

(viii-a) analyzing the deprotection yield of step (iii) by cleaving a test sample of the resin bound peptide;

(viii-b) repeating steps (iii) to (viii-a) until the content of cleaved peptide containing the modified lysine side chain is at least 85%;

(ix) cleaving the peptide from the resin using a cleavage cocktail consisting of 96.0-97.5% (w/w) TFA, 1.7-2.6% (w/w) EDT and 0.7-1.5% (w/w) 3-methylindole;

(x) filtering the peptide solution after cleavage at room temperature;

(xi) distilling the filtered peptide solution under vacuum at ≤30°C;

(xii) adding the residual fraction from the distillation to an antisolvent consisting of DIPE and n-heptane in a ratio of from 25:75 (v/v) to 35:65 (v/v);

(xiii) stirring the precipitated solution at 10-15°C for 1-18 hours;

(xiv) filtering the precipitated solution;

(xv) washing the precipitate 4-5 times by (xv-a) resuspending the precipitate in ethyl acetate, (xv-b) stirring the resulting suspension for 30-60 minutes and (xv-c) filtering off ethyl acetate; and

(xvi) drying the wet peptide under vacuum.