Protective group strategies in carbohydrate and peptide chemistry

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A two-step fluorous capping procedure in solid phase peptide synthesis

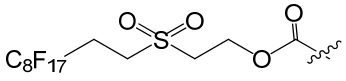
CHAPTER 5

Introduction:

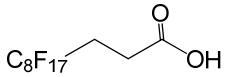
The synthesis of peptides by solid phase procedures has reached a high level of efficiency and oligopeptides up to a length of 50 amino acids are routinely prepared[1]. Nonetheless, present state of the art in solid phase peptide synthesis (SPPS) dictates that the outcome of a specific peptide synthesis cannot always be predicted. Although in a standard SPPS protocol the reagents and protected amino acid building blocks are used in five or more fold excess, the required near quantitative yields in the corresponding reactions are not always attained. In the elongation cycle of SPPS, comprising the removal of the terminal amino-protecting group and coupling of a suitably protected amino acid to the

growing peptide chain, the condensation step is most often incomplete. Consequently the solid phase synthesis of an oligopeptide is accompanied by the formation of truncated and deletion sequences. The accumulation of these unwanted sequences, in particular the (n-1) peptides frequently complicates the purification of the target full-length product[2].

Figure 1: The fluorous capping reagent PFD-OH and the FMsc protecting group.



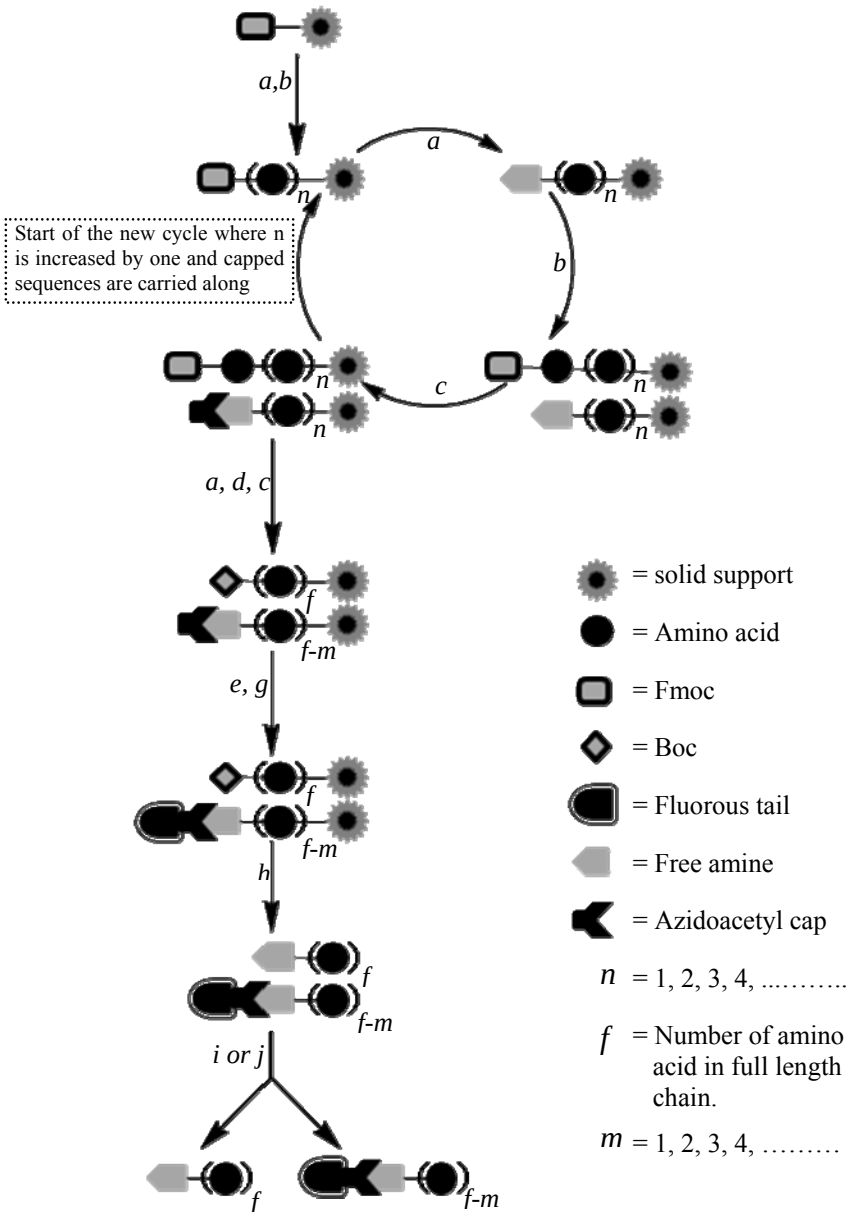
1, FMsc



2, PFD-OH

The advent of light-fluorous techniques, in which compounds differing in fluorine content are separated by chromatography using a fluorous stationary phase, offers an opportunity to improve the purification of oligopeptides obtained by SPPS[3]. Two approaches can be distinguished. In the first approach a suitable fluorous protecting group is developed and installed at the N-terminal end of the target oligopeptide in the final stage of the solid phase synthesis. The fluorous protecting group survives the subsequent cleavage of the peptide from the solid support and the fluorous target peptide is separated from the non-fluorous impurities by fluorous HPLC or fluorous solid phase extraction (F-SPE) [4]. Final steps of this approach entail the removal of the fluorous protecting group and the isolation of the target oligopeptide. Several examples of fluorous protecting groups are reported[5]. An example of such a protecting group is the [1*H*,1*H*,2*H*,2*H*]-perfluorodecylsulfonylethoxycarbonyl (FMsc, Figure 1) group, the application of which in carbohydrate chemistry is described in chapter 3[6]. In the second approach the intermediate and immobilized oligopeptides that resist elongation en route to the target peptide are capped after every condensation step in the SPPS cycle with a fluorous reagent, making the truncated and deletion sequences fluorous and leaving the target compound untouched[7]. The final step of the second approach entails the separation of the non-fluorous target peptide from the fluorous impurities by fluorous HPLC or fluorous solid phase extraction (F-SPE).

Figure 2: General elongation cycle of the SPPS approach, using a two step fluorous capping procedure and ensuing isolation of the target peptide using fluorous techniques.



Reagents and conditions; *a*) Piperdine (20% in NMP), 15 min.; *b*) Fmoc-amino acid, HCTU, Dipea, NMP; *c*) Azidoacetic acid, HCTU, NMP, 20 min; *d*) Boc_2O, Dipea, NMP, 30 min; *e*) PMe_3, H_2O, dioxane, 2h; *g*) PFD-OH, HCTU, Dipea, NMP, 1h; *h*) TFA, TIS, H_2O, 45 min; *i*) F-HPLC; *j*) FSPE.

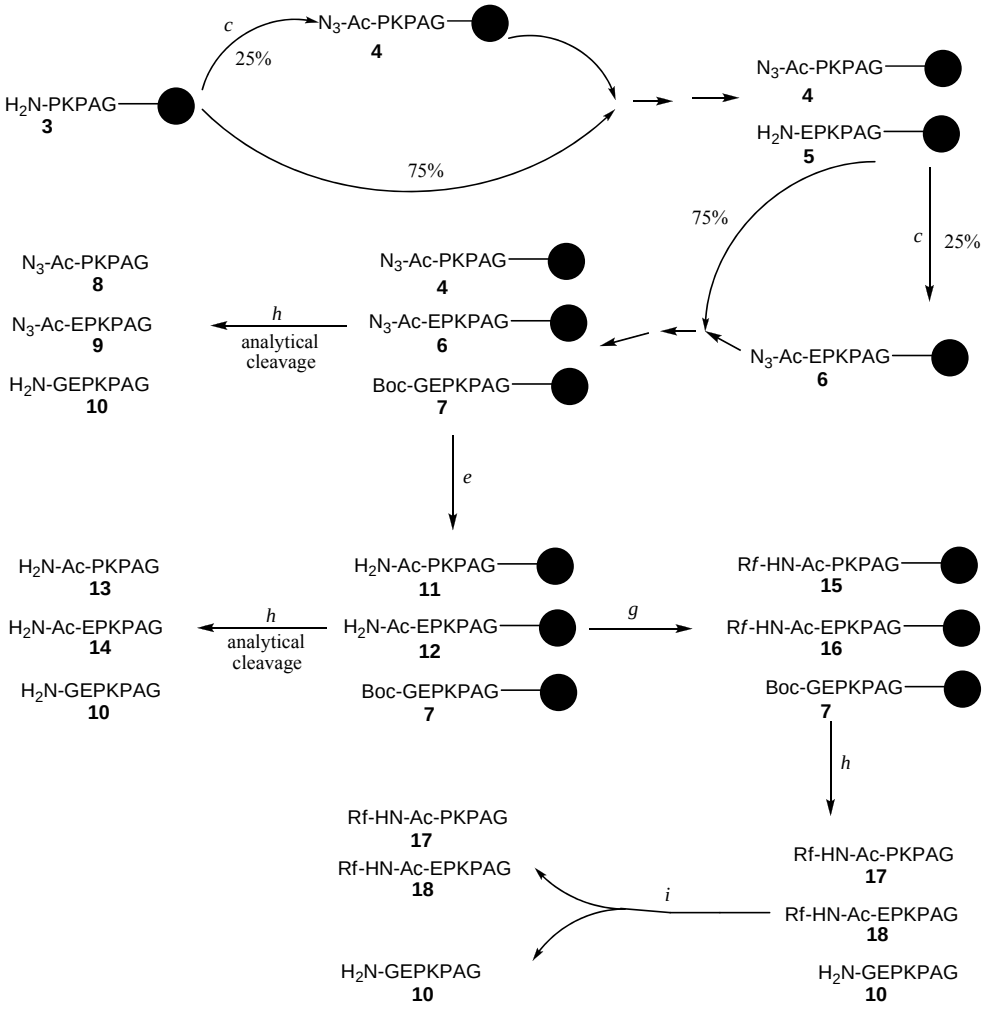
The present chapter describes the exploration of a fluorous capping approach in SPPS. Capping of incomplete sequences in standard SPPS is commonly done with acetic anhydride and it seems obvious to replace acetic anhydride with a suitable fluorous anhydride. However anhydrides with sufficient fluorine content are not commercially available and the corresponding acids are rather expensive. Therefore a new SPPS protocol endowed with a two-step fluorous capping procedure was designed (Figure 2). Each elongation cycle of this SPPS protocol entails (*a*) Fmoc removal, (*b*) coupling of the next suitably protected amino acid and (*c*) capping with azidoacetic acid under influence of HCTU. In this way the azidoacetyl group is introduced at the amino functions of intermediate sequences that failed to react in the condensation steps en route to the oligopeptide target. After completion of the SPPS the Fmoc protecting group in the target peptide is replaced with the Boc group (*d*). In the next event the azides in the immobilized incomplete sequences are reduced to amino functions (*e*) and made fluorous by condensation with (1H,1H,2H,2H)-perfluoroundecanoic acid (PFD-OH, **2**, Figure 1, *g*). Finally the immobilized oligopeptides are deprotected, cleaved from the solid support (*h*) and purified by fluorous HPLC (*i*) or fluorous solid phase extraction (F-SPE, *j*).

Results and discussion:

The feasibility of the two-step capping strategy was examined with the aid of the synthesis of model peptide GEPKPAG (**10**). The manual SPPS of this heptamer was executed in such a manner, that deletion sequences were deliberately obtained (Figure 3). In the SPPS protocol the Fmoc group was removed with piperidine and 5 equivalents of the required amino acids building blocks were used in presence of the activator HCTU and Dipea as base. When the SPPS to **10** had reached the stage of the pentamer **3** with a free amino function, 25% of the resin **3** was taken out and the immobilized pentamer PKPAG **3** was condensed with azidoacetic acid under influence of HCTU and Dipea for 20 minutes (Figure 3). The reaction went to completion, as determined by the chloranil test and the resulting azidoacetyl capped immobilized pentamer **4** was remixed with the untreated resin **3** (Figure 3). The next amino acid was coupled and the terminal amino function was unmasked to give resin **5** (Figure 3). Next, 25% of the resin containing immobilized hexamer **5** and azidoacetyl capped **4** was taken aside as described above, capped with an

azidoacetyl group to give **6** and remixed with the rest of the resin still containing **4** and **5** (Figure 3). The last Boc-protected amino acid Gly was coupled to give full length protected peptide **7** (Figure 3). The Boc group was used instead of Fmoc protection because the Fmoc group is not stable during the forthcoming reduction with trimethylphosphine[8].

Figure 3: Synthesis and purification of heptamer GEPKPAG.



Reagents and conditions; *c*) Azidoacetic acid, HCTU, NMP, 20 min; *e*) PMe_3, H_2O, dioxane, 2h; *g*) PFD-OH, HCTU, Dipea, NMP, 1h; *h*) TFA, TIS, H_2O, 45 min; *i*) FSPE.

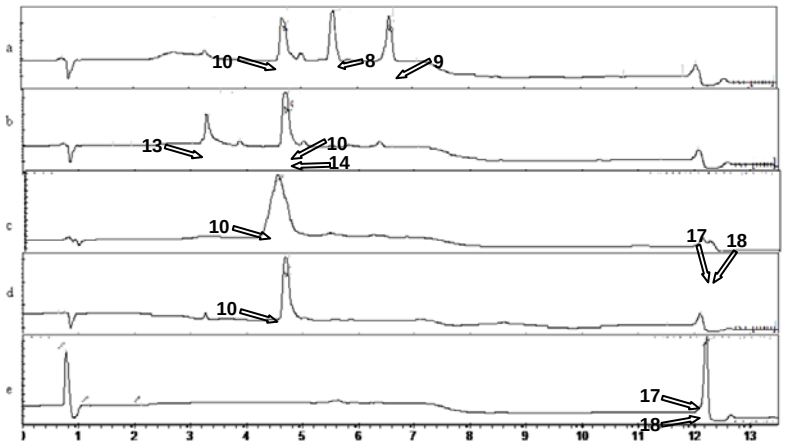
To analyze the product distribution a small part of the thus obtained resin mixture was subjected to standard deblocking and cleavage conditions. The resin mixture containing Boc protected heptamer together with the deliberately introduced azidoacetyl capped deletion sequences pentamer and hexamer was treated with TFA/TIS/H_2O (95:2.5:2.5) to remove the Boc group, the amino acid side chain protecting groups and to release the peptides from the resin[9]. Analysis by LC-MS showed the presence of three major peaks which correspond to the target heptamer (**10**) and the azidoacetyl capped pentamer (**8**) and hexamer (**9**) (Figure 4a).

Next, conditions were explored that allow complete reduction of the azides to amino functions. The resin was treated with trimethylphosphine in a mixture of H_2O and 1,2-dioxane for 2 hours. The reduction of azide to amine was ensured by repetition of the reductive treatment[10]. The result was substantiated by LC-MS analysis of the crude mixture, obtained by subjecting a small amount of resin to the standard deprotection/cleavage conditions (Figure 4b). The remaining resin **7**, **11** and **12**, having deletion sequences with free amine functions was treated with (1H,1H,2H,2H)-perfluoroundecanoic acid (PFD-OH, **1**), the condensing agent HCTU and Dipea in NMP (Figure 3). After completion of the reaction (**11** to **15** and **12** to **16**), as monitored by the TNBS test[11], the peptides were released from the resin and the crude mixture containing **10**, **17** and **18** was analyzed by LC-MS which showed two peaks, representing target heptamer and the fluorous peptides, respectively Figure 4c). The crude mixture was conveniently purified by fluorous solid phase extraction (FSPE) using a stepwise gradient of acetonitrile in H_2O with 0.1%TFA. Mixtures of acetonitrile/H_2O/TFA (20/79.9/0.1) and acetonitrile/H_2O/TFA (50/49.9/0.1) were used to elute the target peptide **10** (Figure 4d). The fluorous penta- and hexamer were eluted with acetonitrile/H_2O/TFA (90/9.9/0.1) and acetonitrile/TFA (99.9/0.1), respectively (Figure 4e). The overall yield of **10** obtained after FSPE was 17% (Table 1, Entry 1).

To further ascertain the feasibility of the two-step capping procedure in oligopeptide synthesis, the decapeptide VEAAIDYIDA (**19**), a peptide derived from acyl carrier protein, was selected as a suitable model[5b,12]. Following the manual SPPS procedure described above tetrameric and nonameric deletion sequences were deliberately produced

by taking out 20% of the resin at the corresponding stage, capping it with the azidoacetyl group and remixing it with the rest of the resin. After completion of the sequence, the Fmoc-group on the last amino acid residue was removed and the Boc group was installed by treatment with di-*tert*-butyl-dicarbonate (Boc_2O) and Dipea[13]. A small portion of the resin was subjected to standard deblocking and cleavage conditions and the obtained mixture was analyzed (Figure 5a)[14]. The resin mixture containing the crude mixture of target peptide and two azido capped tetrameric and nonameric deletion sequences was treated with trimethylphosphine (PMe_3) and the resulting free amines in the deletion sequences were coupled with fluorous PFD-OH (**1**). The peptide was cleaved from the resin and analyzed (Figure 5b). Target peptide **19** could be conveniently purified by FSPE (Figure 5c) and was isolated in 7% overall yield (Table 1, Entry 2).

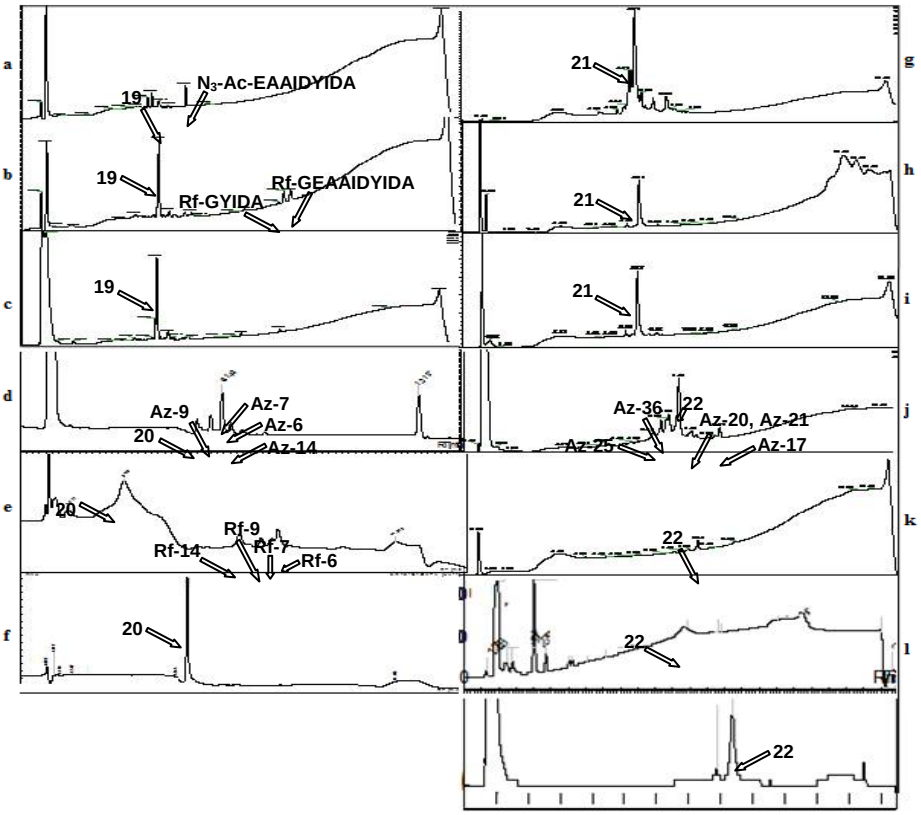
Figure 4: LC Chromatograms of GEPKPAG. a) after synthesis; b) after reduction; c) after fluorous capping; d) after FSPE (target peptide); e) after FSPE (fluorous capped fragments).



To investigate whether the two-step capping procedure could be transferred to automatic SPPS, the assembly of nonadecamer SSKKSGSYSGSKGSKRRIL **20** using an automated peptide synthesizer and Fmoc chemistry was undertaken. To ensure the acquirement of deletion sequences, 2 eq. instead of the usual 5 eq. of protected amino acid building blocks were employed in the coupling step and the coupling time was reduced

from the usual 45 minutes to 30 minutes. After each coupling, the unreacted resin bound amino functions were capped by reaction with azidoacetic acid in combination with HCTU and Dipea. After the completion of the target sequence, the Fmoc at the N-terminal end was quantitatively replaced by the Boc group, as shown by the TNBS test. A small portion of the resin was taken out, subjected to standard deblocking and cleavage conditions. The

Figure 5: LC Chromatograms. a) crude peptide **19** after synthesis; b) crude peptide **19** after fluorous capping; c) peptide **19** after FSPE; d) crude peptide **20** after synthesis; e) crude peptide **20** after fluorous capping; f) peptide **20** after FSPE; g) crude peptide **21** after synthesis; h) crude peptide **21** after fluorous capping; i) peptide **21** after FSPE; j) crude peptide **22** after synthesis; k) peptide **22** after fluorous capping; l) peptide **22**, F-HPLC-trace; m) peptide **22** HPLC (C_{18}) trace).



mixture comprising target peptide and deletion sequences was analyzed (Figure 5d). The remaining resin was subjected to reducing conditions and the obtained amino functions on the unwanted sequences were provided with a fluorous tail by condensation with PFD-OH (**1**). The peptide was released from the resin and analyzed (Figure 5e). The crude mixture was easily purified by FSPE to give target peptide **20** (Figure 5f) in 9% overall yield (Table 1, Entry 3).

Similarly peptide EPLTSLTPR-Abu-NTAWNRLKL **21**, part of an envelope protein of the Moloney virus, containing a 2-aminobutyric acid residure (Abu) as an isosteric replacement for Cysteine (Cys) was prepared on an automated peptide synthesizer using Fmoc chemistry combined with azidoacetic acid capping (Fmoc/AzidoAc protocol)[15]. This time the usual excess of amino acid building blocks and the coupling time were employed, minimizing the formation of deletion sequences. At the final stage of the SPPS the Fmoc group was replaced with the Boc group. A small portion of the resin was subjected to standard deblocking and cleavage conditions and the obtained mixture was analyzed (Figure 5g). The crude target oligopeptide **21** (Figure 5h) was obtained by the same sequence of events as described for the preparation of oligopeptide **20**. Finally, FSPE purification gave target peptide **21** (Figure 5i) in 21% overall yield (Table 1, Entry 4).

As a last example BDSTLRLBVQSTHVDIRTLEDLLMGTLGIVPBIBSQKP **22**, a peptide derived from E7 protein of HPV16 was assembled using the Fmoc/AzidoAc protocol on automated peptide synthesizer.[16] Again 2-aminobutyric acid residue was introduced as the isostere of Cys. The LC chromatograms of the non-fluorous reaction mixture and the crude reaction mixture containing the fluorous deletion peptides are shown in figures 5j and 5k respectively. Pure target peptide **22** (Figure 5l) was obtained by F-HPLC purification in 7% overall yield (Table 1, Entry 5).

Table 1: Yields of different peptides.

Entry	Peptide	Yield
1	GEPKPAG (**10**)	17%
2	VEAAIDYIDA (**19**)	7%
3	SSKKSGSYSGSKGSKRRIL (**20**)	9%
4	EPLTSLTPRBNTAWNRLKL (**21**)	21%
5	BDSTLRLBVQSTHVSIRTLEDLLMGTLGIVPSIBSQKP (**22**)	7%

Conclusion:

This chapter described the successful implementation of a two-step fluorous capping procedure in an otherwise standard SPPS protocol allowing the improved isolation of five natural and artificially designed oligopeptides. In the elongation cycle of the SPPS protocol amino functions of unwanted sequences were provided with the azidoacetyl group and in the final stage of the oligopeptide synthesis the thus appended azides were reduced to amine functions and subsequently capped with a fluorous tail. The fluorous deletion sequences were conveniently separated from target peptides using FSPE or F-HPLC.

Experimental:

General: All chemicals used in the solid phase peptide synthesis, with the exception of the HCTU were from Biosolve (The Netherlands) and used as received. HCTU was purchased IRIS Biotech GmbH (Germany). Resins were bought at Rapp Polymere GmbH (Germany). (1H,1H,2H,2H)-perfluoroundecanoic acid was bought at Fluorous Technologies Inc. Fmoc amino acids were from SENN Chemicals. Fmoc amino acids used in the synthesis were: Fmoc-Abu-OH, Fmoc-Ala-OH, Fmoc-Arg-(Pmc)-OH, Fmoc-Asn-(Trt)-OH, Fmoc-Asp-(tBu)-OH, Fmoc-Gln-(Trt)-OH, Fmoc-Glu-(tBu)-OH, Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-His-(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys-(Boc)-OH, Fmoc-Met-OH, Fmoc-Pro-OH, Fmoc-Ser-(tBu)-OH, Fmoc-Thr-(tBu)-OH, Fmoc-Trp-

(Boc)-OH, Fmoc-Tyr-(tBu)-OH, Fmoc-Val-OH. Azidoacetic acid was prepared according to the literature procedure[17]. Analytical LC/MS was conducted on a JASCO system using an Alltima C_{18} analytical column (5 μm particle size, flow 1.0 ml/min). Absorbance was measured at 214 nm and 254 nm. Solvent system: A: 100% H_2O, B: 100% acetonitrile, C: 1% $TFA_{(aq)}$. Gradients of B in 10% C were applied over 13 minutes.

General cycle of peptide syntheis: The solid phase peptide synthesis was performed on ABI 433A (Applied Biosystems) applying Fmoc based protocol starting either from Rink amide MBHA resin or Tentagel preloaded with Fmoc-Leu-OH (Wang linker). Alternatively a mechanical shaker was used and the reagents were introduced manually (manual peptide synthesis). The synthesis was performed on 50 μmol according to established methods. The consecutive steps performed in each cycle were:
1) Deprotection of the Fmoc group with 20% piperdine in NMP for 15 minutes; 2) DMF wash; 3) Coupling of the appropriate amino acid applying a five-fold excess. Generally the Fmoc amino acid (0.25 mmol) were dissolved in 0.25 M HCTU in NMP (1 ml), the resulting solution was transferred to the reaction vessel followed by 0.5 ml of 1 M DIPEA in NMP to initiate the coupling; 4) DMF wash; 5) Capping with azidoacetic acid as described in the general procedure for azide capping; 6) DMF wash

General procedure for azide capping: To the resin bound peptide (1 eq) was added azidoacetic acid (0.2 M/NMP, 5 eq), HCTU (0.25 M/NMP, 5 eq) and DIPEA (1 M/NMP, 10 eq). The mixture was shaken for 20 minutes. Next, solvents were drained off and the resin was subsequently washed with DMF.

General procedure for reduction of azide: To the suspension of resin bound peptide (1 eq) in dioxane was added trimethylphosphine (1 M /toluene, 8 eq) and H_2O (222 eq). The mixture was shaken for 2 hours. Next, the solvents were drained off and the resin was washed with NMP. The procedure was repeated once more and the solvents were drained off and the resin was subsequently washed with NMP and DCM.
General procedure for fluorous capping: To the suspension of resin bound peptide (1 eq) in NMP was added (1H,1H,2H,2H)-perfluoroundecanoic acid (5 eq), HCTU (5 eq) and DIPEA (10 eq). The mixture was shaken for 1 hour. Next, solvents were drained off and the resin was subsequently washed with NMP and DCM.

General procedure for introduction of Boc: To the suspension of resin bound peptide (1 eq) in NMP was added di-*tert*-butyl-dicarbonate (Boc_2O, 5 eq) and DIPEA (5 eq), the mixture was shaken for 30 minutes. Next, solvents were drained off and the resin was subsequently washed with NMP and DCM.

General procedure for cleaving the peptide from the resin: To the resin bound peptide (10 μmol, 1 eq) in a cartridge with a filter was added a cocktail of TFA/TIS/H_2O (95/2.5/2.5). The mixture was kept for 1 hour with occasion swirling. Next, the solvents were filtered into cold diethyl ether. The mixture was centrifuged, the diethyl ether was removed, and precipitates were washed with diethyl ether and air dried.

General procedure for fluorous solid phase extraction (FSPE): A FSPE cartridge preloaded with 2 g of fluorous silica gel was eluted with acetonitrile (10 ml) and acetonitrile/H_2O/TFA (20/79.9/0.1, 15 ml). The crude peptide was dissolved in the minimum amount of water and acetonitrile was added to help in dissolution if necessary, followed by loading to the cartridge. The cartridge was eluted with acetonitrile/H_2O/TFA (20/79.9/0.1, 10 ml) and acetonitrile/H_2O/TFA (50/49.9/0.1, 15 ml) to elute the target peptide. Next, fluorous capped truncated sequences were eluted with acetonitrile/H_2O/TFA (90/9.9/0.1, 15 ml) and acetonitrile/TFA (99.9/0.1, 10 ml).

Gly-Glu-Pro-Lys-Pro-Ala-Gly-NH_2 (10): Resin bound peptide **10** was prepared using ABI 433A (Applied Biosystems) according to general cycle of peptide synthesis. At pentamer stage after deprotection, 25% of the resin was taken out and capped with azidoacetyl according to the general procedure as described above. The two resins were remixed, and next amino acid was coupled and Fmoc was deprotected. Again 25% of the resin was taken out and capped with azidoacetyl. The two resins were remixed, and synthesis was continued according to the general procedure. Boc-Gly-OH was coupled as the last amino acid residue. The crude peptide was washed with NMP and DCM and air dried. A small amount of the peptide was cleaved according to the general procedure for the cleavage of peptide from the resin described above and subjected to the LCMS. The remainder of the resin was reduced, coupled with PFD-OH and cleaved from the resin according to the respective general procedures described above. The peptide was cleaved (40 mg, Initial loading = 0.34 mmol/g, final loading 0.30 mmol/g, 11.9 μmol) according to the general procedure for the cleavage of peptide from the resin described above and small amount was subjected to the LCMS. The crude peptide was purified by FSPE according to the general procedure as described above to afford peptide **10** (1300 μg, 1.99 μmol, 17%). LCMS: 00-20% B, RT = 4.51 min; ESI-MS: $[M+H]^+$ 654.6 (calc. 654.7).

Val-Glu-Ala-Ala-Ile-Asp-Tyr-Ile-Asp-Ala-NH_2 (19): Resin bound peptide **19** was prepared manually using peptide synthetic cycle as described above. At tetramer stage after deprotection, 20% of the resin was taken out and capped with azidoacetyl according to the general procedure as described above. The two resins were remixed, and standard cycle was followed. Next amino acid was coupled and Fmoc was deprotected. At nonamer stage after deprotection, again 20% of the resin was taken out and capped with azidoacetyl. The two resins were remixed, and synthesis was continued according to the general procedure. Next, the Boc group was introduced to the full length peptide according to the general procedure as described above. The crude peptide was washed with NMP and DCM and air dried. A small amount of the peptide was cleaved according to the general procedure for the cleavage of peptide from the resin described above and subjected to the LCMS. The remainder of the resin was reduced, coupled with PFD-OH and cleaved from the resin according to the respective general procedures described above. The peptide was cleaved (30 mg, Initial loading = 0.58 mmol/g, final loading 0.37 mmol/g, 11.2 μmol) according to the general procedure for the cleavage of peptide from the resin described above and small amount was subjected to the LCMS. The crude peptide was purified by FSPE according to the general procedure as described above to afford peptide **19** (900 μg, 0.84 μmol, 7%). LCMS: 00-50% B, RT = 4.59 min; ESI-MS: $[M+H]^+$ 1078.5 (calc. 1078.2).

Ser-Ser-Lys-Lys-Ser-Gly-Ser-Tyr-Ser-Gly-Ser-Lys-Gly-Ser-Lys-Arg-Arg-Ile-Leu-OH (20): Resin bound peptide **20** was prepared using ABI 433A (Applied Biosystems) according to general cycle of peptide synthesis. Next, the Boc group was introduced to the full length peptide according to the general procedure as described above. The crude peptide was washed with NMP and DCM and air dried. A small amount of the peptide was cleaved according to the general procedure for the cleavage of peptide from the resin described above and subjected to the LCMS. The remainder of the resin was reduced, coupled with PFD-OH and cleaved from the resin according to the respective general procedures described above. The peptide was cleaved (25 mg, Initial loading = 0.26 mmol/g, final loading 0.18 mmol/g, 4.54 μmol) according to the general procedure for the cleavage of peptide from the resin described above and small amount was subjected to the LCMS. The crude peptide was purified by FSPE according to the general procedure as described above to afford peptide **20** (800 μg, 0.4 μmol, 9%). LCMS: 00-90% B, RT = 3.88 min; ESI-MS: $[M+H]^{2+}$ 1007.7 (calc. 1007.7), $[M+H]^{3+}$ 672.1 (calc. 672.1).

Glu-Pro-Leu-Thr-Ser-Leu-Thr-Pro-Agr-Abu-Asn-Thr-Ala-Trp-Asn-Agr-Leu-Lys-Leu-OH (21): Resin bound peptide **21** was prepared using ABI 433A (Applied Biosystems) according to general cycle of peptide synthesis. Next, the Boc group was introduced to the full length peptide according to the general procedure as described above. The crude peptide was washed with NMP and DCM and air dried. A small amount of the peptide was cleaved according to the general procedure for the cleavage of peptide from the resin described above and subjected to the LCMS. The remainder of the resin was reduced, coupled with PFD-OH and cleaved from the resin according to the respective general procedures described above. The peptide was cleaved (17 mg, Initial loading = 0.26 mmol/g, final loading 0.18 mmol/g, 3.0 μmol) according to the general procedure for the cleavage of peptide from the resin described above and small amount was subjected to the LCMS. The crude peptide was purified by FSPE according to the general procedure as described above to afford peptide **21** (1400 μg, 0.64 μmol, 21%). LCMS: 00-90% B, RT = 4.55 min; ESI-MS: $[M+H]^{2+}$ 1099.0 (calc. 1098.8), $[M+H]^{3+}$ 733.2 (calc. 732.9).

Abu-Asp-Ser-thr-Leu-Arg-Leu-Abu-Val-Gln-Ser-Thr-His-Val-Asp-Ile-Arg-Thr-Leu-Glu-Asp-Leu-Leu-Met-Gly-Thr-Leu-Gly-Ile-Val-Pro-Abu-Ile-Abu-Ser-Gln-Lys-Pro-OH (22): Resin bound peptide **22** was prepared using ABI 433A (Applied Biosystems) according to general cycle of peptide synthesis. Next, the Boc group was introduced to the full length peptide according to the general procedure as described above. The crude peptide was washed with NMP and DCM and air dried. A small amount of the peptide was cleaved according to the general procedure for the cleavage of peptide from the resin described above and subjected to the LCMS. The remainder of the resin was reduced, coupled with PFD-OH and cleaved from the resin according to the respective general procedures described above. The peptide was cleaved according to the general procedure for the cleavage of peptide from the resin described above and small amount was subjected to the LCMS. The crude peptide was purified by FSPE according to the general procedure as described above to afford peptide **22** in 7% yield. LCMS: 10-90% B, RT = 8.36 min; ESI-MS: $[M+H]^{3+}$ 1363.3 (calc1363.4).

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