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Solid-phase synthesis of *C*-terminally modified peptides

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Abstract: In this paper, a straightforward and generic protocol is presented to label the *C*-terminus of a peptide with any desired moiety that is functionalized with a primary amine. Amine-functional molecules included are polymers (useful for hybrid polymers), long alkyl chains (used in peptide amphiphiles and stabilization of peptides), propargyl amine and azido propyl-amine (desirable for 'click' chemistry), dansyl amine (fluorescent labeling of peptides) and crown ethers (peptide switches/hybrids). In the first part of the procedure, the primary amine is attached to an aldehyde-functional resin via reductive amination. To the secondary amine that is produced, an amino acid sequence is coupled via a standard solid-phase peptide synthesis protocol. Since one procedure can be applied for any given amine-functional moiety, a robust method for *C*-terminal peptide labeling is obtained. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: modified peptides; labeled peptides; solid-phase synthesis; reductive amination

INTRODUCTION

Since its development in the early sixties, solid-phase chemistry has become the main tool for peptide synthesis. The ability to force reactions to completion by the use of excess reagent, the prevention of solubility problems during synthesis, and especially the ease of purification have made this methodology a great synthetic success [1]. Because peptides are nowadays more and more incorporated as building blocks in a variety of molecular structures, with applications in the biomedical field as well as in nanoscience [2], there is a definite need for a generic strategy to efficiently modify peptides. Again, it is most convenient to perform these modifications on the solid phase. Moreover, since SPPS requires a build-up of the peptide from *C*- to *N*-terminus, peptide alterations on the solid phase are introduced at the *N*-terminus in the final step of the synthesis before cleavage. However, the ability to modify the *C*-terminus is sometimes more desirable and it also opens up the possibility to modify a peptide at both termini.

Here we describe a generic, straightforward and highly reproducible protocol to label the *C*-terminus of any peptide with a variety and, in principle, any desired primary amine with high yields. Modifications include polymers (useful for hybrid polymer systems [3]), long alkyl chains (used in peptide amphiphiles [4–6] and stabilization of peptides [7]), propargyl- and azide-functionalities (desirable for click chemistry [8–10]), dansyl and crown ether moieties (for fluorescent labeling or peptide switching, respectively). We think that the use of this straightforward protocol to modify peptides will be very useful for the peptide chemist, and for any scientist interested in using peptides as functional building blocks.

RESULTS AND DISCUSSION

In order to introduce moieties at the peptide *C*-terminus, a synthetic procedure was designed as depicted in Figure 1, based on a method developed by Barany *et al.* [11–17]. They demonstrated that with a commercially available aldehyde resin with a backbone amide linker (BAL) the first amino acid could be introduced via the primary amine instead of the C^{α}-carboxylic acid. Next, after reduction of the imine to a secondary amine, peptide synthesis could be continued using standard Fmoc peptide synthesis protocols [11–17]. The amino acids that were coupled via the primary amine were premodified at the *C*-terminus and could be introduced as esters [12], ethers [14], aldehydes [14,16], amides [12], thioesters [11], *p*-nitroanilides [11], hydrazines [12] and histydyl groups [13]. Barany *et al.* further showed that the introduction of simple amines like aniline, butenamine and the more complex glucosamine [12] was also possible; however, the yields were moderate to low (40–70%), and several different protocols were used.

In our modification of this procedure from the literature [11–13], one standard protocol was developed to couple, in principle, any desired amine to the aldehyde modified resin in very high yields [*vide infra*]. The desired amine was dissolved in a MeOH/DMF (1 : 1) mixture and added to the resin, either in its free form with an equivalent AcOH, or as HCl salt. After addition of the reducing agent, $NaBH_3CN$, the mixture

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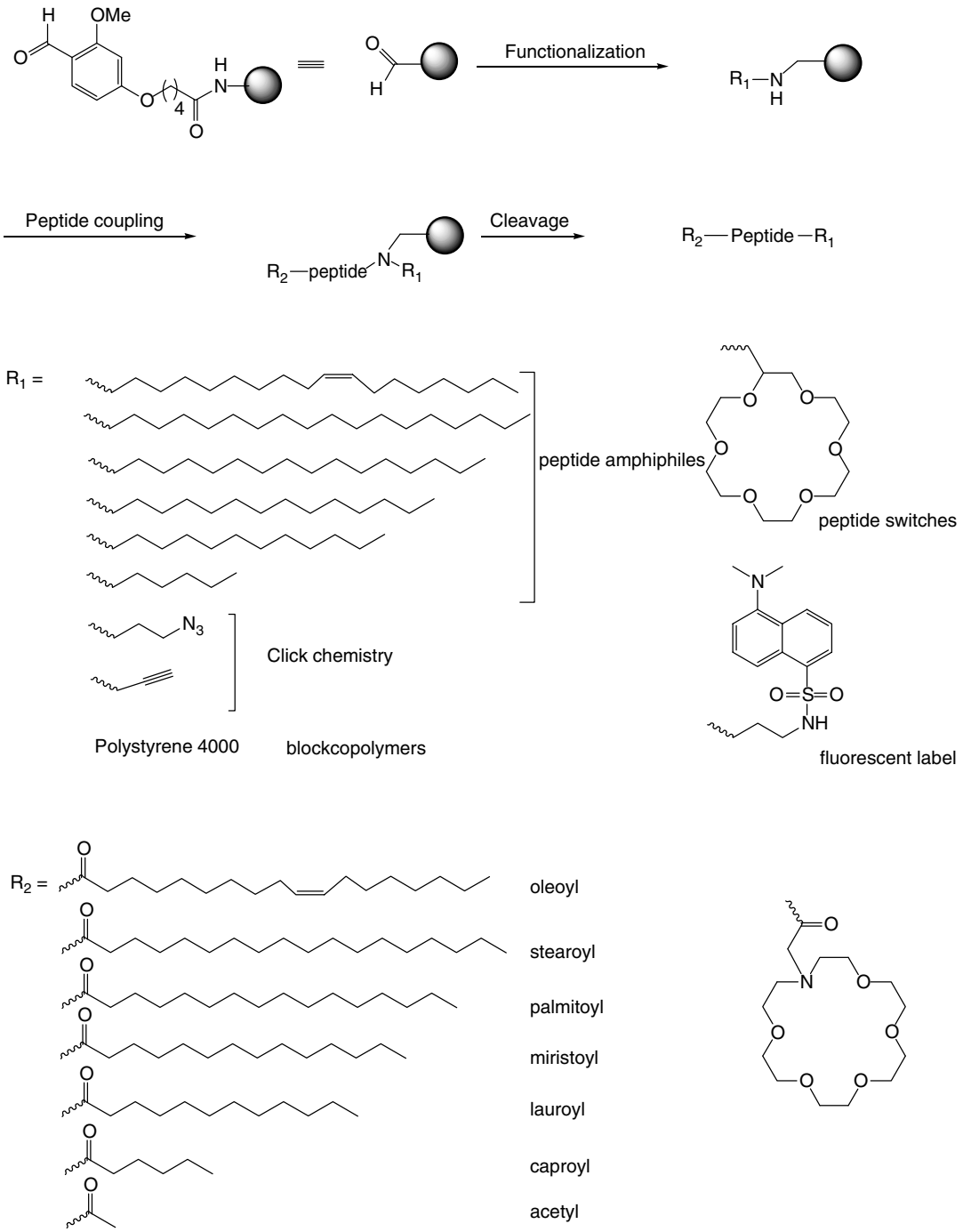

Figure 1 Solid-phase synthesis of *C*-terminally modified peptides.

was heated to 80 °C. Amines that did not dissolve at room temperature dissolved during heating. The elevated temperature therefore not only increased the speed and yield of the addition, but also allowed the use of less soluble amines. Since the Barany procedure for *C*-terminal modification was mostly applied to amino acids, coupling always had to be conducted at room temperature to minimize the amount of racemization [12]. Our approach was not limited by these difficulties.

A first series of amines that was tested on their coupling efficiency consisted of long *n*-alkyl amines ($C_nH_{2n+1}NH_2$): stearyl amine ($n = 18$), palmityl amine ($n = 16$), myristyl amine ($n = 14$), lauryl amine ($n = 12$), hexyl amine ($n = 6$) and unsaturated oleyl amine. After reductive amination the resin was washed with DMF, dichloromethane (DCM), and MeOH, and a chloranil test [18] was carried out to confirm that a secondary amine had formed.

The introduction of long hydrophobic moieties could sterically hinder the first amino acid coupling to the already less reactive secondary amine. These amide couplings were carried out using DIPCDI and HOBt as

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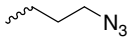
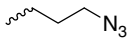
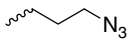
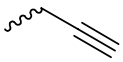
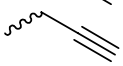
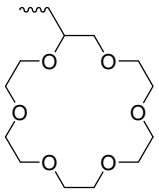
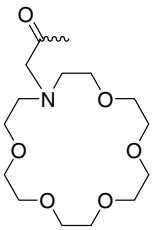
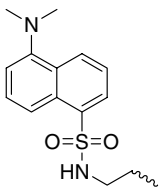
Table 1 Synthesis of *C*-terminally modified peptides

No.	R_1*C*-term[a]	R_2*N*-term	Peptide	Isolated yield [%]
1	$C_{18}H_{37}$	$C_5H_{11}C(O)$	GANPNAAG	61
2	$C_{18}H_{37}$	$C_{11}H_{23}C(O)$	GANPNAAG	60
3	$C_{18}H_{37}$	$C_{13}H_{27}C(O)$	GANPNAAG	55
4	$C_{18}H_{37}$	$C_{15}H_{31}C(O)$	GANPNAAG	55
5	$C_{18}H_{37}$	$C_{17}H_{35}C(O)$	GANPNAAG	70[b]
6	$C_{16}H_{33}$	$C_{15}H_{31}C(O)$	GANPNAAG	82
7	$C_{14}H_{29}$	$C_{13}H_{27}C(O)$	GANPNAAG	60
8	$C_{12}H_{25}$	$C_{11}H_{23}C(O)$	GANPNAAG	45
9	C_6H_{13}	$C_5H_{11}C(O)$	GANPNAAG	63
10	$C_{16}H_{33}$	$C_{15}H_{31}C(O)$	KGGGK	55
11	$C_{16}H_{33}$	$C_{15}H_{31}C(O)$	KGGK	62
12	$C_{16}H_{33}$	$C_{15}H_{31}C(O)$	KGK	73
13	$C_{16}H_{33}$	$C_{15}H_{31}C(O)$	KK	57
14	$C_{18}H_{35}$	$C_{17}H_{33}C(O)$	KGGK	61[b]
15	$C_{18}H_{35}$	$C_{17}H_{33}C(O)$	KGK	72[b]
16	$C_{18}H_{35}$	$C_{17}H_{33}C(O)$	KAAK	56[b]
17	$C_{16}H_{33}$	$C_{15}H_{31}C(O)$	ABAKABKAKABG	53[b]
18	$C_{16}H_{33}$	$CH_3C(O)$	ABAKABKAKABG	68[b]
19	$C_{16}H_{33}$	$CH_3C(O)$	KTVIIE[c]	50[b]
20	$C_{18}H_{37}$	$C_{17}H_{35}C(O)$	AGAGKGAGAG	54[b]
21	$C_{18}H_{37}$	$C_{17}H_{35}C(O)$	AGAGEGAGAG	52[b]
22		$CH_3C(O)$	GANPNAAG	75[b]
23		$C_{15}H_{31}C(O)$	GANPNAAG	40
24		$CH_3C(O)$	P_{33}[d]	71[b]
25		$CH_3C(O)$	GANPNAAG	78[b]
26		$C_{15}H_{31}C(O)$	GANPNAAG	43
27	PS_{20}	$PS_{35}C(O)$	GANPNAAG	15
28			GANPNAAG	82
29		H[e]	GGRGDSG	64[b]

[a] The success of the resin modification was established through an Fmoc determination after coupling of the first amino acid, and was found to be 90–99% of the theoretical maximal loading.
[b] Crude yield: yield after cleavage of the resin. The crude peptide had a purity of >90%.
[c] Resin loading after first amino acid was 75% of theoretical loading.
[d] P_{33} = Gly-Ala-Gln-Leu-Lys-Lys-Lys-Leu-Gln-Ala-Asn-Lys-Lys-Glu-Leu-Ala-Gln-Leu-Lys-Trp-Lys-Leu-Gln-Ala-Leu-Lys-Lys-Lys-Leu-Ala-Gln-Gly.
[e] Free amine.

coupling reagents in DMF. After 24 h, the chloranil [18] tests were negative, which was indicative of successful coupling of the first amino acid. The expected problems due to the presence of the alkyl chains were therefore not observed. With an Fmoc determination, the resin loading and yield of the reactions were calculated, and in most cases the yield was above 90%. The modified resins were employed in the preparation of

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a variety of peptides, e.g. GANPNAAG, AGAGEGAGAG, ABAKABKAKABKG, KGGGK, KGGK, KGK, KK and KAAK, using a standard peptide synthesis protocol (coupling: DIPCDI/HOBt/DMF, Fmoc cleavage: 20% piperidine/DMF). The peptide couplings proceeded smoothly and were not hindered by the long alkyl chains, and the peptides could be modified at the *N*-terminus with a second alkyl chain in an acylation with the appropriate carboxylic acid (Table 1). The peptides were cleaved from the resin in good yields. Characterization by mass spectrometry (Maldi-TOF or ESI+) and NMR confirmed the successful synthesis of these mono- or bialkyl peptides. Only in the case of oleyl amine did partial acid addition to the *double* bond occur under cleavage conditions.

In order to investigate the scope of this *C*-terminal modification method, the steric hindrance of the amines was increased by employing amine-functional polystyrene (2.5 kg/mol, polydispersity index (PDI) = 1.12, degree of polymerization (DP) 20) and an amine-functional crown ether in the coupling procedure (Figure 1). The reductive amination still proceeded smoothly using the same protocol and the first amino acid was coupled in high yield to the crown-ether modified resin (entry 28). The coupling of the first amino acid to the polystyrene-modified resin was more difficult and resulted in a lower yield (entry 27). The next seven amino acids in the GANPNAAG sequence were efficiently coupled to both resins, and the *N*-termini were modified either with a polystyrene chain (3.8 kg/mol, PDI = 1.04, DP 35) or another crown ether, forming a giant bio-hybrid amphiphile [3] or a peptide switch, respectively. Both the peptides were characterized with NMR and MALDI-TOF. From the results it is clear that sterically demanding primary amines can be coupled successfully to the aldehyde resin using a single protocol and one peptide coupling strategy.

A very useful peptide modification is the introduction of an azide or alkyne moiety, which would allow the use of the [2 + 3] cycloaddition reaction, also known as *click chemistry*. For this purpose, propargyl amine and 3-amino-propylazide were used in the reductive amination reaction and attached to the resin in almost quantitative yields (entry 22 to 27). Next, the GANPNAAG sequence and a Leucine zipper sequence [19] were coupled smoothly. These peptides could be modified on the solid phase at the *N*-terminus. After cleavage from the resin, the peptide has a 'click' moiety available at the *C*-terminus.

In many assays, it is useful to fluorescently label a peptide without compromising the side chains and *N*-terminus. Therefore, we coupled *N*-dansyl-1,3-diaminopropane to the aldehyde resin to fluorescently label an RGD sequence. After coupling the dansyl moiety the resin was fluorescent and the chloranil test positive, indicative of coupling. Subsequently, a GGRGDSG sequence was coupled to the resin and cleaved, yielding a fluorescently labeled peptide with a free *N*-terminus in good yield (entry 29). Though several fluorescently labeled resins are commercially available [20], and more elaborate routes than ours have been published to attach probes to a resin [21], our protocol is generic, straightforward, and any desired amine-functional fluorescent label can be chosen to be attached to a peptide.

CONCLUSIONS

We have presented a high yielding straightforward protocol to *C*-terminally modify peptides with a variety of functionalized and bulky amines. These peptides can be used in very diverse applications, ranging from click chemistry and biological assays to noncovalent stabilized peptides and giant amphiphiles. In our opinion, the ease of these protocols will make them very useful for any scientist to synthesize peptides as functional building blocks.

EXPERIMENTAL

Aldehyde functionalized resin was purchased from Novabiochem, amino acids were purchased from Bachem and Novabiochem, and all other chemicals were purchased from Baker, Fluka or Aldrich and were used as received. Mass spectra were recorded on a Bruker Biflex Maldi-TOF or a Thermofinnigan LCQ ESI-ion trap.

General Procedure for *C*-Terminally Labeled Peptides

Typical example: C_5H_{11}CO-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-NHC$_{18}H_{37}$ (1). To a commercially available aldehyde resin (4-(4-formyl-3-methoxyphenoxy)butyryl), (3 g, loading 0.74 mmol/g [20]) in 50 ml MeOH/DMF, 10 equivalents of both stearyl amine and $NaCNBH_3$ were added. Additionally, 10 equivalents of AcOH were added. Otherwise, when started with an amine HCl salt, no addition of AcOH is required. After gently stirring the mixture for 2.5 h at 80 °C, the resin was washed successively with DMF, DCM and EtOH or MeOH. The coupling was confirmed via a chloranil test. The first amino acid was coupled with 3.3 equiv. DIPCDI, 3.6 equiv. HOBt and 3 equiv. Fmoc glycine in DMF at room temperature. The coupling was carried out for 2 h and was repeated overnight, followed by washing the resin with DMF, DCM, i-PrOH and diethyl ether. After the glycine coupling procedure, any residual free amine was capped using 10 equiv. of acetic acid anhydride and 12 equiv. of pyridine. After shaking for 15 min, the resin was washed with DMF, DCM, i-PrOH, DCM and diethyl ether. The absence of free amines was examined with a chloranil test. The resin was dried *in vacuo* and an Fmoc determination was performed to calculate the yield.

Peptide couplings were carried out in DMF using 3.3 equiv. DIPCDI, 3.6 equiv. HOBt, and the following series of 3 equiv. Fmoc amino acids: Alanine, Alanine, Asparagine, Proline, Asparagine, Alanine, and Glycine. After every coupling the

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resin was washed with DMF, and a Kaiser test was performed. When the Kaiser test was negative, the Fmoc group was removed and the following coupling was started.

The Fmoc group was removed with 20% piperidine/DMF, 3 times during 6 min, followed by a DMF wash. In this particular case, from the point the first asparagine was incorporated, each deprotection step was performed using a piperidine solution containing 0.1 M HOBt, to minimize side products related to aspartimide formation. Lastly, 3 equiv. of hexanoic acid in DCM and 3.3 equiv. DIPCDI, 3.6 equiv. HOBt in DMF were added to the resin and shaken for 2 h. Before TFA-cleavage the resin was washed with DMF, DCM, i-PrOH, DCM and ether.

When the sequence was completed, the peptide was cleaved with 5% H_2O in TFA for 2–3 h. The free peptide was precipitated in ether, dissolved in AcOH and lyophilized. The crude peptide had a purity of >90%.

Resin loading: 0.51 mmol g^{-1} (97%). Maldi-TOF; Isolated yield (61%). Calculated: 1042.7 $(M + Na)^+$; Measured: 1042.6 $(M + Na)^+$, 1058.2 $(M + K)^+$; ^{1}H-NMR (Varian 400 MHz, DMSO-d_6) δ: 8.25 (d, 1H), 7.95 (t, 1H), 7.87 (t, 1H), 7.87 (d, 1H), 7.87 (d, 1H), 7.75 (d, 1H), 7.64 (s, 1H), 7.57 (d, 1H), 7.54 (t, 1H), 7.23 (s, 1H), 7.11 (s, 1H), 6.94 (s, 1H), 4.72 (q, 1H), 4.39 (q, 1H), 4.26 (qu, 1H), 4.22 (dd, 1H), 4.14 (qu, 1H), 4.12 (qu, 1H), 3.70 (m, 4H), 3.60 (m, 2H), 3.00 (q, 2H), 2.66 (d, 1H), 2.54 (d, 1H), 2.41 (d, 1H), 2.40 (d, 1H), 2.10 (t, 2H), 2.07 (m, 2H), 1.82 (m, 2H), 1.44 (qu, 2H), 1.35 (t, 2H), 1.22 (d, 6H), 1.20 (m, 40H), 1.12 (d, 3H), 0.82 (t, 6H).

$C_{11}H_{23}$CO-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-NHC$_{18}H_{37}$ (2). The compound was prepared analogously to **1**. Resin loading: 0.51 mmol g^{-1} (97%). Isolated yield (60%). Maldi-TOF; Calculated: 1126.8 $(M + Na)^+$; Measured: 1126.5 $(M + Na)^+$, 1152.6 $(M + K)^+$.

$C_{13}H_{27}$CO-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-NHC$_{18}$ H_{37} (3). The compound was prepared analogously to **1**. Resin loading: 0.51 mmol g^{-1} (97%). Isolated yield (55%). Maldi-TOF; Calculated: 1154.8 $(M + Na)^+$; Measured: 1155.2 $(M + Na)^+$, 11 705.2 $(M + K)^+$.

$C_{15}H_{31}$CO-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-NHC$_{18}$ H_{37} (4). The compound was prepared analogously to **1**. Resin loading: 0.51 mmol g^{-1} (97%). Isolated yield (55%). Maldi-TOF; Calculated: 1182.8 $(M + Na)^+$; Measured: 1182.5 $(M + Na)^+$, 1198.7 $(M + K)^+$.

$C_{17}H_{35}$CO-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-NHC$_{18}$ H_{37} (5). The compound was prepared analogously to **1**. Resin loading: 0.51 mmol g^{-1} (97%). Crude yield (70%). Maldi-Tof; Mass calculated: 1210.9 $(M + Na)^+$; measured: 1211.2 $(M + Na)^+$.

$C_{15}H_{31}$CO-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-NHC$_{16}$ H_{33} (6). The compound was prepared analogously to **1**. Resin loading: 0.64 mmol g^{-1} (99%). Isolated yield (82%). Maldi-Tof; Mass calculated: 1154.8 $(M + Na)^+$; measured: 1154.4 $(M + Na)^+$.

$C_{13}H_{27}$CO-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-NHC$_{14}H_{29}$ (7). The compound was prepared analogously to **1**. Resin loading: 0.65 mmol g^{-1} (97%). Isolated yield (60%). Maldi-Tof; Mass calculated: 1098.7 $(M + Na)^+$; measured: 1098.1 $(M + Na)^+$.

$C_{11}H_{23}$CO-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-NHC$_{12}$ H_{25} (8). The compound was prepared analogously to **1**. Resin loading: 0.64 mmol g^{-1} (93%). Isolated yield (45%). Maldi-Tof; Mass calculated: 1042.7 $(M + Na)^+$; measured: 1042.1 $(M + Na)^+$.

C_5H_{11}CO- Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-NHC$_6H_{13}$ (9). The compound was prepared analogously to **1**. Resin loading: 0.68 mmol g^{-1} (98%). Isolated yield (63%). Maldi-Tof; Mass calculated: 874.5 $(M + Na)^+$; measured: 873.9 $(M + Na)^+$.

$C_{15}H_{31}$CO-Lys-Gly-Gly-Gly-Lys-NHC$_{16}H_{33}$ (10). The compound was prepared analogously to **1**. Resin loading: 0.43 mmol g^{-1} (91%). Isolated yield (55%). Maldi-Tof; Mass calculated: 907.8 $(M + H)^+$; measured: 907.9 $(M + H)^+$, 930.00 $(M + Na)^+$.

$C_{15}H_{31}$CO-Lys-Gly-Gly-Lys-NHC$_{16}H_{33}$ (11). The compound was prepared analogously to **1**. Resin loading: 0.43 mmol g^{-1} (91%). Isolated yield (62%). Maldi-Tof; Mass calculated: 872.7 $(M + Na)^+$; measured: 872.9 $(M + Na)^+$.

$C_{15}H_{31}$CO-Lys-Gly-Lys-NHC$_{16}H_{33}$ (12). The compound was prepared analogously to **1**. Resin loading: 0.43 mmol g^{-1} (91%). Isolated yield (73%). Maldi-Tof; Mass calculated: 793.7 $(M + H)^+$; measured: 793.8 $(M + H)^+$, 816.04 $(M + Na)^+$.

$C_{15}H_{31}$CO-Lys-Lys-NHC$_{16}H_{33}$ (13). The compound was prepared analogously to **1**. Resin loading: 0.43 mmol g^{-1} (91%). Isolated yield (57%). Maldi-Tof; Mass calculated: 758.7 $(M + Na)^+$; measured: 758.9 $(M + Na)^+$.

$C_{17}H_{33}$CO-Lys-Gly-Gly-Lys-NHC$_{18}H_{35}$ (14). The compound was prepared analogously to **1**. Resin loading: 0.42 mmol g^{-1} (90%). Crude yield (61%). Maldi-Tof; Mass calculated: 924.7 $(M + Na)^+$; measured: 924.7 $(M + Na)^+$, 942.7 $(M + Na + H_2O)^+$, 960.8 $(M + Na + 2H_2O)^+$.

$C_{17}H_{33}$CO-Lys-Gly-Lys-NHC$_{18}H_{35}$ (15). The compound was prepared analogously to **1**. Resin loading: 0.42 mmol g^{-1} (90%). Crude yield (72%). Maldi-Tof; Mass calculated: 867.8 $(M + Na)^+$; measured: 867.8 $(M + Na)^+$, 885.8 $(M + Na + H_2O)^+$, 903.8 $(M + Na + 2H_2O)^+$.

$C_{17}H_{33}$CO-Lys-Ala-Ala-Lys-NHC$_{18}H_{35}$ (16). The compound was prepared analogously to **1**. Resin loading: 0.42 mmol g^{-1} (90%). Crude yield (56%). Maldi-Tof; Mass calculated: 952.8 $(M + Na)^+$; measured: 952.9 $(M + Na)^+$, 970.9 $(M + Na + H_2O)^+$, 988.9 $(M + Na + 2H_2O)^+$.

$C_{15}H_{31}$CO-Ala-Aib-Ala-Lys-Ala-Aib-Lys-Ala-Lys-Ala-Aib-Gly-NHC$_{16}H_{33}$ (17). The compound was prepared analogously to **1**. Resin loading: 0.54 mmol g^{-1} (97%). Crude yield (53%). Maldi-Tof; Mass calculated: 1554.1 $(M + Na)^+$; measured: 1553.2 $(M + Na)^+$.

Ac-Ala-Aib-Ala-Lys-Ala-Aib-Lys-Ala-Lys-Ala-Aib-Gly-NHC$_{16}H_{33}$ (18). The compound was prepared analogously to **1**. Resin loading: 0.54 mmol g^{-1} (97%). Crude yield (68%). Maldi-Tof; Mass calculated: 1357.9 $(M + Na)^+$; measured: 1357.2 $(M + Na)^+$.

Ac-Lys-Thr-Val-Ile-Ile-Glu-NHC$_{16}H_{33}$ (19). The compound was prepared analogously to **1**. Resin loading: 0.37 mmol g^{-1}

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(75%). Crude yield (50%). Maldi-Tof; Mass calculated: 990.7 $(M + Na)^+$; measured: 990.3 $(M + Na)^+$.

$C_{17}H_{35}$CO-Ala-Gly-Ala-Gly-Lys-Gly-Ala-Gly-Ala-Gly-$NHC_{18}H_{37}$ (20). The compound was prepared analogously to **1**. Resin loading: 0.68 mmol g^{-1} (99%). Crude yield (54%). Maldi-Tof; Mass calculated: 1256.8 $(M + Na)^+$; measured: 1256.5 $(M + Na)^+$.

$C_{17}H_{35}$CO-Ala-Gly-Ala-Gly-Glu-Gly-Ala-Gly-Ala-Gly-$NHC_{18}H_{37}$ (21). The compound was prepared analogously to **1**. Resin loading: 0.68 mmol g^{-1} (99%). Crude yield (52%). Maldi-Tof; Mass calculated: 1255.9 $(M + Na)^+$; measured: 1255.5 $(M + Na)^+$.

Ac-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-NH$(CH_2)_3$-N_3 (22). The compound was prepared analogously to **1**. Resin loading: 0.55 mmol g^{-1} (99%). Crude yield (75%). ESI-ion trap; Mass calculated $(M + Na)^+$: 817.4; measured: 817.4 $(M + Na)^+$.

$C_{15}H_{31}$-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-NH$(CH_2)_3$-N_3 (23). The compound was prepared analogously to **1**, using 3-amino-propylazide. Resin loading: 0.55 mmol g^{-1} (99%). Isolated yield (40%). Maldi-Tof; Mass calculated: 1013.6 $(M + Na)^+$; measured: 1013.6 $(M + Na)^+$.

Ac-P_{33}-NH$(CH_2)_3$-N_3 (24). P_{33} = Gly-Ala-Gln-Leu-Lys-Lys-Lys-Leu-Gln-Ala-Asn-Lys-Lys-Glu-Leu-Ala-Gln-Leu-Lys-Trp-Lys-Leu-Gln-Ala-Leu-Lys-Lys-Lys-Leu-Ala-Gln-Gly.

The compound was prepared analogously to **1**, using 3-amino-propylazide. Resin loading: 0.55 mmol g^{-1} (99%). Crude yield (71%). ESI-ion trap; Mass calculated: 537.3 $(M + 7H^+)$; 626.7 $(M + 6H^+)$; 751.9 $(M + 5H^+)$; 939.6 $(M + 5H^+)$; 1252.4 $(M + 6H^+)$; measured: 537.5(6+); 626.8(5+); 752.1 (4+); 939.6; 1253.0(3+).

Ac-Gly-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-$NHCH_2$- (25). The compound was prepared analogously to **1**, using propargyl amine. Resin loading: 0.55 mmol g^{-1} (99%). Crude yield (78%). ESI-ion trap; Mass calculated: 772.3 $(M + Na)^+$; measured: 772.5 $(M + Na)^+$.

$C_{15}H_{31}$- Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-$NHCH_2$- (26). The compound was prepared analogously to **1**, using propargyl amine. Resin loading: 0.55 mmol g^{-1} (99%). Isolated yield (43%). Maldi-Tof; Mass calculated: 968.6 $(M + Na)^+$; measured: 968.5 $(M + Na)^+$.

PS_{32}-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-PS_{20} (27). Preparation and characterization as described in Ref. 2

18-crown-6-CH_2C(O)-Gly-Ala-Asn-Pro-Asn-Ala-Ala-Gly-$NHCH_2$-1-aza-18-crown-6 (28). The compound was prepared analogously to **1**, using 3 equivalents of crown ether methylamine and 3 equivalents $NaCNBH_3$, followed by peptide coupling described for **1**. After deprotection of the final amino acid $BrCH_2COOH$ (3 equiv.), DIPCDI (3.3 equiv.), HOBt (3.6 equiv.) and DMF were added. After shaking for 3 h the resin was washed with DMF, 1-aza-18-crown-6 (3 equiv.) in DMF was added and the resin was shaken for 18 h. The peptide was cleaved with 5% H_2O in TFA for 2 h. The free peptide was precipitated in ether, dissolved in water and lyophilized.

Resin loading 0.58 mmol g^{-1} (90%). Crude yield (82%). Maldi-Tof; Mass calculated: 1250.4 $(M + H)^+$; measured: 1250.1 $(M + H)^+$, 1271.1 $(M + Na)^+$, 1287.0 $(M + K)^+$.

Gly-Gly-Arg-Gly-Asp-Ser-Gly-NH$(CH_2)_3$-NH-dansyl (29). The compound was prepared analogously to **1**, using 3 equivalents of *N*-dansyl-1,3-diaminopropane and 3 equivalents $NaCNBH_3$. Resin loading 0.45 mmol g^{-1} (88%). Crude yield (64%). ESI+; Calculated mass: 894.38$(M + H)^+$; Measured mass: 894.54 $(M + H)^+$. After the first amino acid, some product was cleaved with 5% H_2O in TFA. ESI+; Calculated mass 609.2147 $(M + Na)^+$, Measured mass: 609.2122 $(M + Na)^+$.

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