Pergamon

Tetrahedron 54 (1998) 10125–10152

TETRAHEDRON

Active Carbonate Resins: Application to the Solid-Phase Synthesis of Alcohol, Carbamate and Cyclic Peptides[1,2]

Jordi Alsina, Francesc Rabanal, Cristina Chiva, Ernest Giralt, and Fernando Albericio*

Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain

Received 31 March 1998; revised 11 June 1998; accepted 18 June 1998

Abstract

N,N'-disuccinimidyl carbonate (DSC) has been successfully used to generate carbonates and carbamates on conventional hydroxymethyl and aminomethyl based resins. This methodology extends the applicability of such linkers, which were initially designed for the anchoring of carboxylic acids. Thus, amino and hydroxy groups have been attached onto classical resins to give straightforward access to the solid-phase synthesis of alcohols, carbamates, and cyclic peptides with an evident pharmaceutical interest.

Keywords: combinatorial chemistry; *N,N'*-disuccinimidyl carbonate (DSC); handle; linker

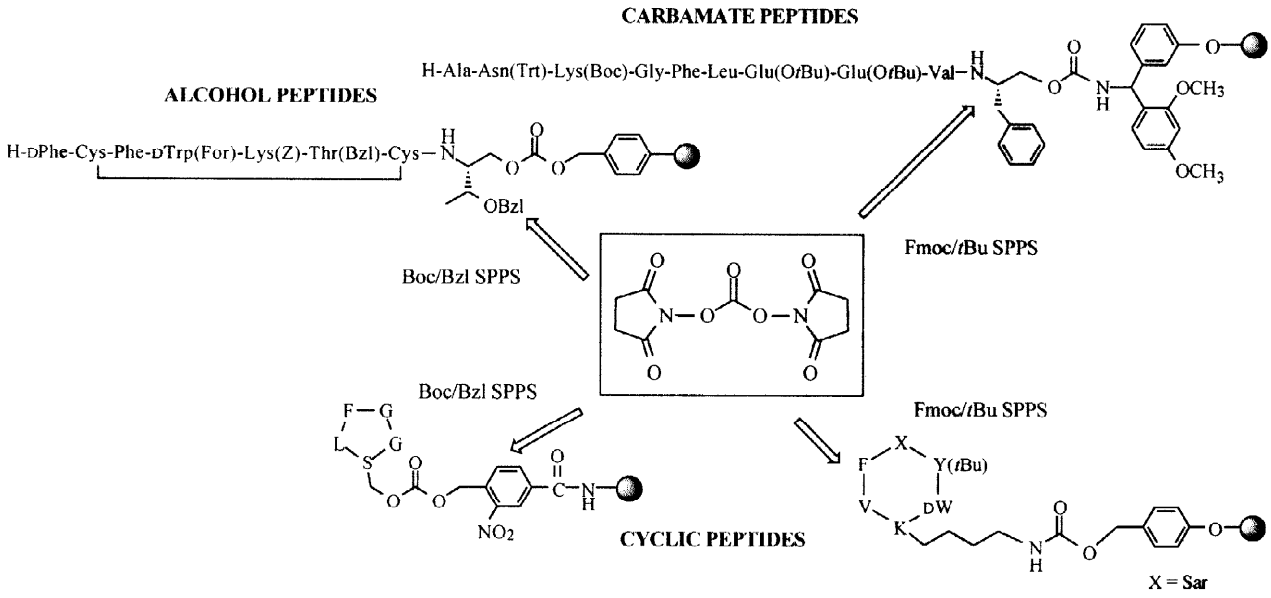
The increasing demand for new pharmaceutical products, together with the advent of combinatorial chemistry techniques to speed up the process of drug development, has made solid-phase synthesis a key methodology in the chemical and pharmaceutical fields.[3]

The solid-phase strategy as applied to peptide synthesis is a highly optimized process that generally commences with the attachment of the first amino acid *via* its carboxyl group to the solid support to form either an ester or an amide bond.[4] However, the need to prepare a wide variety of chemical compounds by solid-phase synthesis has fuelled the development of synthetic strategies to anchor functional groups other than the carboxyl unit. In this regard, the conversion of already existing bifunctional linkers, developed for carboxyl chemistry, into derivatized forms suitable for other organic functionalities is of the utmost importance since it would take advantage of the enormous effort invested in designing and chemically *fine-tuning* these molecules.

Pioneering efforts in this direction were undertaken independently some decades ago by the groups of Letsinger,[5] Merrifield,[6] and Matsueda.[7] In their investigations, benzylic alcohol resins were derivatized first as chloroformates and then as carbamates of α-amino acids to perform peptide syntheses in the $N \rightarrow C$ direction rather than in the $C \rightarrow N$ direction

0040-4020/98/$ - see front matter
PII: S0040-4020(98)00606-1

that is commonly used today. More recently, the growing interest in the solid-phase synthesis of compounds such as pseudopeptides, new biopolymers (i.e. based on carbamate and urea bonds), peptidomimetics, and combinatorial libraries of small organic compounds, has increased the scope of some bifunctional linkers that were conventionally used for peptide and oligonucleotide synthesis.[8] In this regard, our group has mainly concentrated on solid-phase synthesis of "head-to-tail" cyclic peptides, alcohol peptides, carbamate peptides, and peptidomimetics. The importance of cyclic peptides and peptidomimetics in the medicinal and pharmaceutical fields is well established and has been thoroughly reviewed elsewhere.[9] Thus, in this paper we will focus on the mild derivatisation of several linkers as succinimidyl active carbonates and the various strategies developed for the preparation of the above compounds (Scheme 1).



Scheme 1. Applications of DSC for SPPS

Results and discussion

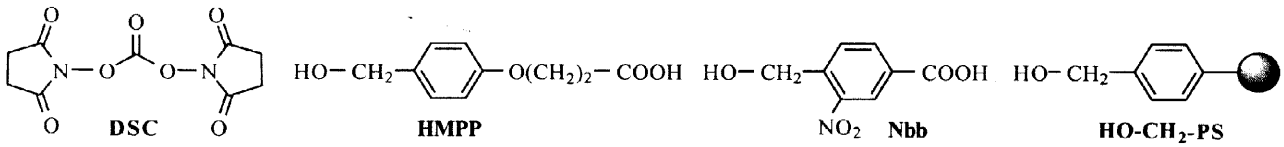
"Head-to-tail" cyclic peptides.

A convenient approach for the solid-phase synthesis (SPS) of "head-to-tail" cyclic peptides[10] involves the following general features: (i) *side-chain anchoring* of an initial partially protected amino acid residue to a polymeric support; (ii) *stepwise solid-phase* assembly of the linear sequence; (iii) *orthogonal* deprotection to selectively liberate a free α-carboxyl group for the subsequent cyclization step; (iv) efficient *activation* of the α-carboxyl group and its *condensation* with a free α-amino group to close the desired ring, using the *pseudo-dilution* phenomenon which favours intramolecular resin-bound reactions; and (v) final *deprotection* and *cleavage* to release the required free cyclic peptide into solution. This strategy was applied successfully to peptides containing Asp, Glu, Asn, and Gln by side-chain anchoring of the ω-carboxyl group of Asp or Glu to hydroxyl- or amine-resins.[11] Both

the Boc/Bzl[11a,d,f] and Fmoc/*t*Bu[11b,c,e] strategies have been used. In both cases, α-carboxyl groups can be orthogonally protected in form of allyl esters.[11c,e] These esters are completely stable under the conditions used for either Boc or Fmoc removal, TFA–CH_2Cl_2 and piperidine–DMF, respectively.[12] Furthermore, conditions used to remove allyl groups, i.e. Pd^0 in the presence of a nucleophile, are compatible with the side-chain protecting groups, Bzl and *t*Bu, respectively.[12]

For the extension of this approach to Lys/Orn-containing peptides, the obvious linkage between the ω-amino and the solid support is through a carbamate function, in a similar way to most common protecting groups for amines.[13] The carbamate function can be prepared by reaction of an active carbonate resin with the appropriate amino group.[14] Furthermore, the reaction of active carbonate resins with alcohols will provide carbonate resins that, at the end of the synthetic process, will render free alcohol-containing peptides. Thus, one of the key points in our approach has been the development of an efficient and reliable methodology to prepare active carbonate forms of hydroxymethyl linkers anchored onto solid supports. In early work, hydroxymethylated polystyrene resins were reacted with phosgene to yield the corresponding chloroformate derivatives.[5-7] However, this method is not devoid of problems. Phosgene is a highly toxic gas. In addition, loss of functionalisation in the resin has been reported and is probably a consequence of internal carbonate formation. Finally, phosgene is harsh and highly reactive and may be incompatible with certain acid labile linkers.

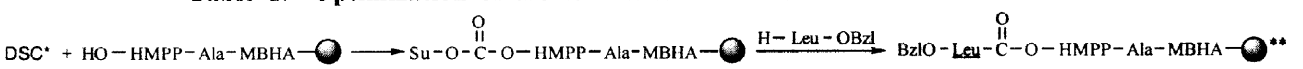
Several other active carbonate reagents have been described in the literature, such as *p*-nitrophenyl chloroformate,[15] carbonyldiimidazole (CDI),[16] and *N,N'*-disuccinimidyl carbonate (DSC).[17] The latter, introduced by Takeda and Ogura, has been used for the carbonyl insertion in the synthesis of ureas and heterocyclic compounds.[18] Furthermore, Ghosh *et al.* used DSC for the alkoxycarbonylation of amines in solution with excellent results.[19] This prompted us to use DSC for the preparation of carbamate- and carbonate-resins. Firstly, an optimization of the active carbonate formation on resin was carried out. For this purpose the linker 3-(4-hydroxymethyl)phenoxypropionic acid (HMPP)[20] was anchored to a conventional *p*-methylbenzhydrylamino-poly(styrene-co-1% divinylbenzene) (MBHA) resin, containing Phe as the internal reference amino acid (IRAA),[20,21] and this was used as a substrate for the study of the alkoxycarbonylation reaction. Once the succinimidyl carbonate was formed it was left to react with an amino acid derivative, *p*-Tos-OH·H-Leu-OBzl, to provide the corresponding carbamate resin. Amino acid analysis (AAA) of the acid hydrolisate (Leu *vs* Phe) gave the yield of both steps.



First of all the conditions of the formation of the active carbonate resin were fixed (entry 1, Table 1),[22] and the conditions of choice of the second step were investigated. The best results were obtained when the incorporation of the amino component was carried out for 5 h. Use

of either the salt in the presence of *N,N*-diisopropylethylamine (DIEA) (entry 1.2, Table 1), the free amine in *N,N*-dimethylformamide (DMF) (entry 1.4, Table 1), or pyridine (pyr) (entry 1.6, Table 1) gave similar results. Furthermore, longer reaction times (entries 1.3 and 1.7, Table 1) did not improve yields. Subsequently, the effect of changes in the first step was studied (entries 2 and 3, Table 1). When DSC–DMAP (10:1) in DMF was used, a practically quantitative yield was obtained for a time of 30–120 min (entries 2.1–2.3, Table 1). Furthermore, when DSC–DIEA (10:20) in DMF was used, optimal results, although slighlty inferior to the previous one, were obtained for a time of 90–150 min (entries 3.2–3.4, Table 1). Longer reaction times led to lower yields of carbonate formation (entries 2.4–2.6 and 3.4–3.6, Table 1).[23] As a comparison, a similar survey was performed with CDI (entries 4 and 5, Table 1). This reagent was not as effective as DSC since the yields of carbamate obtained hardly reached values above 20% (entry 5, Table 1).[24,25]

Table 1. Optimization of the formation of carbamate HMPP based resins



Entry	*Step 1*	t_1	*Step 2*	t_2	*Yield*
1.1	DSC–DMAP (10:1), DMF	6 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	2 h	58 %
1.2	DSC–DMAP (10:1), DMF	6 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	76 %
1.3	DSC–DMAP (10:1), DMF	6 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	8 h	76 %
1.4	DSC–DMAP (10:1), DMF	6 h	H-Leu-OBzl (10), DMF	5 h	80 %
1.5	DSC–DMAP (10:1), DMF	6 h	H-Leu-OBzl (10), pyr	2 h	57 %
1.6	DSC–DMAP (10:1), DMF	6 h	H-Leu-OBzl (10), pyr	5 h	75 %
1.7	DSC–DMAP (10:1), DMF	6 h	H-Leu-OBzl (10), pyr	8 h	76 %
2.1	DSC–DMAP (10:1), DMF	30 min	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	98 %
2.2	DSC–DMAP (10:1), DMF	1 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	100 %
2.3	DSC–DMAP (10:1), DMF	2 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	95 %
2.4	DSC–DMAP (10:1), DMF	4 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	87 %
2.5	DSC–DMAP (10:1), DMF	6 h 30 min	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	81 %
2.6	DSC–DMAP (10:1), DMF	9 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	75 %
3.1	DSC–DIEA (10:20), DMF	1 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	74 %
3.2	DSC–DIEA (10:20), DMF	1 h 30 min	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	88 %
3.3	DSC–DIEA (10:20), DMF	2 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	94 %
3.4	DSC–DIEA (10:20), DMF	2 h 30 min	Tos H-Leu-OBzl–DIEA (10:20), DMF	5 h	87 %
3.5	DSC–DIEA (10:20), DMF	3 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	77 %
3.6	DSC–DIEA (10:20), DMF	5 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	56 %
4.1	CDI–DMAP (10:1), DMF	6 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	14 %
4.2	CDI–DMAP (10:1), DMF	6 h	H-Leu-OBzl (10), DMF	5 h	11 %
4.3	CDI–DMAP (10:1), DMF	6 h	H-Leu-OBzl (10), pyr	5 h	13 %
5.1	CDI–DMAP (10:1), DMF	15 min	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	27 %
5.2	CDI–DMAP (10:1), DMF	30 min	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	25 %
5.3	CDI–DMAP (10:1), DMF	1 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	25 %
5.4	CDI–DMAP (10:1), DMF	2 h	Tos·H-Leu-OBzl–DIEA (10:20), DMF	5 h	27 %

*DSC was substituted by CDI in entries 4 and 5; **Leu signifies that it is anchored to the resin through the amino function

Once a satisfactory methodology for the activation of alcohols on the resin as succinimidyl carbonates had been achieved, we focused our efforts on anchoring trifunctional amino acids containing amino groups (Lys, Orn). The attachment of Fmoc-Lys-OAllyl or Fmoc-Orn-OAllyl through their ω-amino groups in the form of the TFA salt to the SuO-CO-O-HMPP-IRAA-MBHA-resin, employing the best conditions found previously, gave yields of carbamate formation comparable to those obtained with H-Leu-OBzl (83–100%, Table 3, entries 1–4). Subsequent elongation of the peptide chain using an Fmoc/*t*Bu strategy took place smoothly. At the end, removal of the allyl group was carried out by $Pd(PPh_3)_4$ in DMSO–THF–0.5 N aqueous HCl–morpholine (2:2:1:0.1, v/v/v/v) before removal of the last Fmoc group with piperidine. Removal firstly of the Fmoc group and then the allyl group may lead to the formation of some allylamine derivatives as by-products. Activation of the α-carboxyl group is preferably carried out with phosphonium salts, BOP or PyAOP,[26] because the use of uronium salts, HBTU or HATU, may produce guanidinium by-products and therefore a capping of the peptide chain.[27] Finally, cleavage with TFA in the presence of the corresponding scavengers released the free cyclic peptides. Four peptides were synthesized using this approach on active carbonate resins built up on polystyrene (entries 1–4, Tables 2 and 3): peptide **1**, an analogue of the somatostatin analogue peptide described by Veber and co-workers;[28] peptides **2** and **3**, gramicidin S and its Lys analogue;[29] and the antigenic peptide **4**.[30] All crude products corresponding to linear peptides gave a correct AAA and showed good purity by analytical HPLC. Cyclic peptides were also obtained with excellent purity for peptides **1–3**. On the other hand, peptide **4a** was not detected by either HPLC or MS.[31] The synthesis of this peptide was repeated on a PEG-PS-resin[32] (**4b**) and, in this case, the proper combination of the solid support and its lower degree of substitution afforded the correct peptide.

Table 2. Sequences of the peptides synthesized

Peptides
cyclo (Val-Phe-*N*(Me)Gly-Tyr-DTrp-Lys) (**1**)
cyclo (Leu-DPhe-Pro-Val-Orn)$_2$ (**2**)
cyclo (Leu-DPhe-Pro-Val-Lys)$_2$ (**3**)
cyclo (Leu-Arg-Met-Lys-Leu-Pro-Lys) (**4**)
cyclo (Leu-Phe-Gly-Gly-Ser) (**5**)
cyclo (Leu-Phe-Gly-Gly-Tyr) (**6**)
cyclo (Glu-Ala-Ala-Arg-DPhe-Pro-Glu-Asp-Asn-Ser) (**7**)
cyclo (Glu-Ala-Ala-Arg-DPhe-Pro-Glu-Asp-Asn-Tyr) (**8**)
cyclo (Cys^2,Cys^7)(H-DPhe-Cys-Phe-DTrp-Lys-Thr-Cys-Thr)-oh (**9**)
H-Ala-Asn-Lys-Gly-Phe-Leu-Glu-Glu-Val-Phe-oCONH$_2$ (**10**)
H-Tyr-Gly-Gly-Phe-oCO-Leu-NH$_2$ (**11**)

Table 3. Peptide synthesis

Peptide	*Anchoring aa (yield, %)*	*Handle (f)*	*Cleavage (yield %)*	*Lineal purity (%)*	*Cyclization method*	*Cyclic cleavage (%) purity (%)*
1	Fmoc-Lys-OAllyl (100)	HMPP (0.47)	TFA (96)	>85	BOP/HOBt	61 (>70)
2	Fmoc-Orn-OAllyl (93)	HMPP (0.48)	TFA (96)	>90	PyAOP/HOAt	27 (>70)
3	Fmoc-Lys-OAllyl (90)	HMPP (0.48)	TFA (94)	>70	PyAOP/HOAt	35 (>85)
4a	Fmoc-Lys-OAllyl (83)	HMPP (0.48)	TFA (70)	>80	PyAOP/HOAt	45 (0)
4b	Fmoc-Lys-OAllyl	HMPP-PEG-PS (0.19)	TFA (56)	>80	PyAOP/HOAt	31 (>55)
5	Boc-Ser-OAllyl (44)	Nbb (0.46)	hυ (75)	>70	PyAOP/HOAt	31 (>28)
6	Boc-Tyr-OAllyl (67)	Nbb (0.46)	hυ (72)	>75	PyAOP/HOAt	32 (>35)
7	Boc-Ser-OAllyl (38)	HO-CH_2-PS (1.35)	HF (85)	>80	PyAOP/HOAt	70 (>40)
8	Boc-Tyr-OAllyl (66)	HO-CH_2-PS (1.35)	HF (93)	>75	PyAOP/HOAt	71 (>30)
9	Boc-Thr(Bzl)-oh	HO-CH_2-PS (1.35)	HF (96)	>82	I_2	(>90)
10	Fmoc-Phe-o-CO-O-Su	H-AM-PEG-PS	TFA (62)	>85	-	-
11	Fmoc-Phe-o-CO-O-Su	H-Leu-PAL-PEG-PS	TFA (80)	>95	-	-

The incorporation of Ser and Tyr, as Fmoc-Ser-OAllyl and Fmoc-Tyr-OAllyl, was not as straightforward. Firstly, the yields were lower than for the Lys/Orn system, probably due to the reduced nucleophilicity of hydroxy functionalities in spite of the use of DMAP as a catalyst and the extended reaction times employed. Secondly, the corresponding carbonate bond generated proved not to be stable to piperidine, the reagent required for the removal of the Fmoc group. For instance, treatment with piperidine (20% in DMF, 3 x 1 min, 1 x 10 min) produced losses of 2–9% of amino acid in the case of Ser and *ca* 30% when the bound amino acid was Tyr.[33] For this reason a Boc/Bzl protection scheme was considered as a plausible alternative. In this approach a different set of hydroxymethyl linkers was used and the formation of the succinimidyl carbonate on the resin needed to be re-optimized. The most commonly used handle, the 4-(hydroxymethyl)phenylacetic acid (PAM) linker,[34] covalently bound to MBHA polystyrene resins gave very poor yields in the reaction with DSC and subsequent amino acid anchoring. Even with the best conditions employed, and using the model compound H-Leu-OBzl, yields of carbamate formed barely reached 10%. These results corroborate similar findings in our laboratory regarding the β-carboxyl esterification of Boc-Asp(OH)-OFm onto PAM-resins.[11f]

Photolabile linkers, such as 4-(hydroxymethyl)-3-nitrobenzoic acid (Nbb linker),[35] are also compatible with the Boc/Bzl protection scheme. Bearing this fact in mind, Nbb bound to MBHA was tested for succinimidyl carbonate and subsequent carbamate formation with H-Leu-OBzl with satisfactory results (yield 98%).[22] Incorporation of Boc-Tyr-OAllyl and Boc-Ser-OAllyl onto SuO-CO-Nbb-Ala-MBHA-resin were studied (Table 4). The best results were obtained in the presence of DMAP (0.2 equiv) in DMF for 17 h at 25 °C under an Ar atmosphere (entries 2.2 and 5.3, Table 4).[36] The use of either a different amount of DMAP or a shorter reaction time led to lower yields. After amino acid incorporation the resins were washed with DMF and CH_3OH, and treated with CH_3OH in the presence of 0.2 equiv of DMAP (30 min) to block any unreacted succinimidyl carbonate.

Table 4. Optimization of the formation of carbonate Nbb based resins

Entry	*Step 1*	t_1	*Step 2*	t_2	*Yield*
1.1	DSC–DMAP (10:1), DMF	2 h	Boc-Tyr-OAllyl–DMAP (10:1), DMF	21 h	52 %
1.2	DSC–DMAP (10:1), DMF	2 h	Boc-Tyr-OAllyl–DMAP (10:0.5), DMF	16 h	48 %
1.3	DSC–DMAP (10:1), DMF	2 h	Boc-Tyr-OAllyl–DMAP (10:0.2), DMF	15 h	59 %
2.1	DSC–DMAP (10:1), DMF	2 h	Boc-Tyr-OAllyl–DMAP (10:0.2), DMF	6 h	49 %
2.2	DSC–DMAP (10:1), DMF	2 h	Boc-Tyr-OAllyl–DMAP (10:0.2), DMF	17 h	67 %
2.3	DSC–DMAP (10:1), DMF	2 h	Boc-Tyr-OAllyl–DMAP (10:0.2), DMF	24 h	55 %
3.1	DSC–DMAP (10:1), DMF	2 h	Boc-Tyr-OAllyl–DMAP (10:0.1), DMF	16 h	59 %
4.1	DSC–DMAP (10:1), DMF	2 h	Boc-Ser-OAllyl–DMAP (10:0.1), DMF	16 h	42 %
4.2	DSC–DMAP (10:1), DMF	2 h	Boc-Ser-OAllyl–DMAP (10:0.1), DMF	41 h	27 %
5.1	DSC–DMAP (10:1), DMF	2 h	Boc-Ser-OAllyl–DMAP (10:0.2), DMF	6 h	26 %
5.2	DSC–DMAP (10:1), DMF	2 h	Boc-Ser-OAllyl–DMAP (10:0.2), DMF	15 h	44 %
5.3	DSC–DMAP (10:1), DMF	2 h	Boc-Ser-OAllyl–DMAP (10:0.2), DMF	17 h	46 %

Two peptides were synthesized using the Nbb linker (entries 5 and 6, Tables 2 and 3). Although acceptable yields and purities were obtained for the linear peptide sequences, the values for the cyclic analogues were substantially lower. In general terms, the Nbb linker was stable to the overall process of Boc/Bzl assembly, including the repetitive neutralization step with a tertiary base (DIEA–CH_2Cl_2 1:19, v/v). However, some losses were detected during the allyl group elimination due to the use of morpholine in the palladium treatment (overall losses after allyl removal and cyclization were 13% for the serine derivative and 21% for the tyrosine one). In addition, the photolytic treatment does not deprotect groups such as Bzl or Tos, and an additional acidolysis, in solution, with HF is required to produce the desired cyclic peptide.

Consequently, hydroxymethyl-co(polystyrene-divinylbenzene) (HO–CH_2–PS)[37] resins were considered as an alternative to overcome the drawbacks posed by Nbb linkers. In this case, anchoring yields for serine and tyrosine were similar to those obtained with Nbb resins. Use of this resin in combination with *N*-methylmorpholinium acetate instead of morpholine as the nucleophile led to a reduction in the premature losses of peptide chain (6% for Ser and 12% for Tyr). Finally, an acidolysis with anhydrous HF afforded the crude cyclic peptide (entries 7–8, Tables 2 and 3).

Head-to tail macrolactam formation in the present strategy is carried out on the resin. Hence, the cyclization step should take advantage of the *pseudo dilution* kinetic effect attributed to the solid-phase.[38] This phenomenon should favour intramolecular reactions over intermolecular ones, provided that the degree of functionalization of the resin is adequate. There is controversy in the literature regarding the role of the degree of substitution of resins on the ratio cyclomonomer/cyclooligomer. Thus, while Nishino *et al.* supports the idea that the extent of oligomerization depends mainly on the choice of *C*-terminal residue,[39] Plaué[40] and Ösapay *et al.*[41] described the effects of resin substitution on cyclization yields. They showed that the proportion of unwanted cyclodimers and cyclooligomers was usually higher in resins with high loading capacities. Although we have not performed a comparative study, we have found similar trends in the present macrolactamizations. Thus, when HO–CH_2–PS was

used (f = 1.35 mmol/g), the yields of desired cyclomonomer were usually lower than those achieved with the HMPP-MBHA resin (f = 0.47 mmol/g). However, relatively low yields of monomer were also obtained with Nbb resins (f = 0.46 mmol/g). An interesting case is that of peptide **4**. When HMPP-MBHA resin (f = 0.47 mmol/g) was used, only cyclooligomers were detected. It was necessary to use a PEG-PS resin with a substantially lower degree of substitution (f = 0.19 mmol/g) to achieve good yields of monomeric lactam peptides. It appears that, in addition to the functionalisation of the resin, other factors could also be highly influential on the yields of cyclic monomeric peptides. These factors include the type of resin and linker, peptide sequence, size of the ring, protecting groups, activating reagents, and solvents employed. Thus, it is reasonable to consider that the low purities of cyclic peptides **4**, **5**, **6** could be due to the fact that they are non-natural peptides, with the absence of residues that favour cyclization. Nevertheless, the results described here seem to indicate the convenience of using resins with a low degree of substitution to ensure optimal yields of the desired cyclomonomeric macrolactam peptides.

Alcohol peptides.

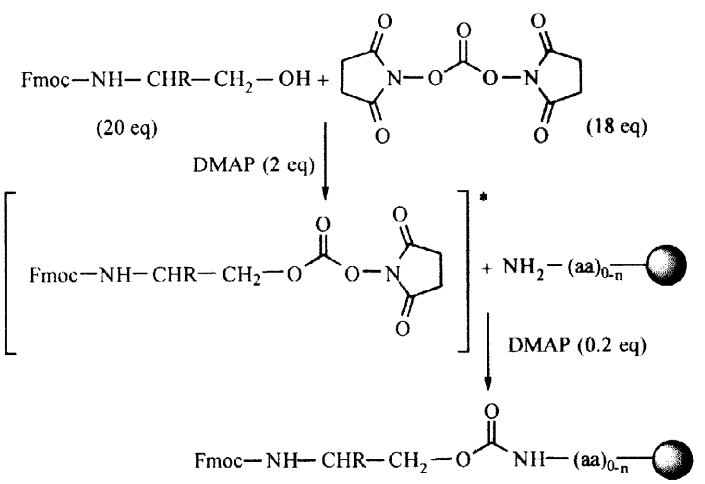
In addition to cyclic peptides, our group is also interested in the development of new, efficient methods for the synthesis of peptides with *C*-terminal and backbone modifications, such as alcohol and carbamate peptides. Such modifications confer increased metabolic stability to these compounds and have evident pharmaceutical implications. To the best of our knowledge, hydroxyl functions have only been anchored to the solid supports through: (i) phenylacetic acid chloride resins, which are labile to concentrated ammonia in dioxane (1:1);[42] (ii) chlorotrityl (ClTrt)-resins,[43] which are labile to dilute acid conditions (<1% TFA) and are therefore compatible with bases and nucleophiles, but not with acids; (iii) tetrahydropyranyl (THP)-based linkers,[44] which are labile to acid conditions [PPTS at 60 °C or TFA–H_2O (19:1)] and therefore exhibit a similar range of compatibilities to ClTrt-resins; (iv) hemi-succinate-linkers,[45] which are cleaved by base hydrolysis and, in principle, are compatible with Fmoc and Boc chemistries, but require a two step cleavage-deprotection protocol; (v) silane-based linkers,[46] which are cleaved by TBAF in CH_2Cl_2, followed by an aqueous treatment. Furthermore, reduction of conventional ester bonds between the peptide and the resin also affords the corresponding alcohol peptides.[47] Finally, phenol groups have also been attached to HMPP-resins through a Mitsunobu reaction.[48]

We have extended the strategy of carbonate bonding on resin to the preparation of alcohol peptides. In order to validate this approach we have synthesized Octreotide (Sandostatin®),[49] an inhibitor of the growth hormone (entry 9, Tables 2 and 3). In addition to the *C*-terminal hydroxy functionality, Octreotide contains a disulfide bridge between cysteines 2 and 7. Hence, it also provides the opportunity to verify the compatibility of carbonate resins to the oxidative conditions of disulfide cyclization. The procedure firstly involved the succinimidyl carbonate activation of the HO–CH_2–PS, to which *N*-Boc-*O*-Bzl-threoninol [Boc-Thr(Bzl)-oh] was anchored. The peptide sequence was then built up by conventional Boc/Bzl chemistry

using Acm protection for Cys, Bzl for Thr, 2-ClZ for Lys, and For for DTrp. Incorporation of Boc-Thr(Bzl)-oh occurred with a yield of 42%. This carbonate bond was completely stable to DIEA–CH_2Cl_2 (1:19) for 1 h at 25 °C (corresponding to 20 cycles of neutralization after TFA treatment for Boc removal) as shown by AAA of the peptide resin before and after the treatment. Disulfide bond formation was accomplished on solid-phase[10a,50] by I_2 (10 equiv/Acm) oxidation in HOAc–H_2O (4:1). The cyclic peptide was cleaved with anhydrous HF and, finally, the formyl group was eliminated from the indole moiety in solution with piperidine. Formyl deprotection was postponed until the peptide was cleaved from the resin due to the intrinsic lability of carbonate bonds to nucleophilic bases. The crude peptide showed an HPLC profile consisting of a major peak (>75% relative area) with the expected MS.[51]

Carbamate peptides.

Preparation of oligocarbamate peptides, which are more hydrophobic and more resistant to proteolytic degradation than their corresponding parent peptides, has been described starting from preformed *N*-protected amino *p*-nitrophenyl carbonates.[52] We describe here a more convenient method for the preparation of this type of polymer using *N*-Fmoc amino succinimidyl carbonate prepared *in situ* from the protected amino alcohol and DSC (Scheme 2).



Scheme 2. Synthesis of carbamate peptides

As an example, the synthesis of the nonapeptide protombin with a *C*-terminal carbamate unit was addressed (entry 10, Tables 2 and 3). In this case, the mixed succinimidyl carbonate of *N*-Fmoc-phenylalaninol (Fmoc-Phe-o-CO-OSu) from Fmoc-Phe-oh with DSC (0.9 equiv) in the presence of DMAP, without being isolated, was reacted with an aminomethyl resin (H-AM-Nle-PEG-PS). Standard Fmoc/*t*Bu assembly of the remaining sequence followed. Cleavage of the resin with TFA–H_2O (19:1) afforded the carbamate peptide in 62% yield and more than 85% purity. Similarly, carbamate bonds can be easily introduced in the central part of the polyamide backbone. The synthesis of the Leu-enkephalin analogue H-Tyr-Gly-Gly-

Phe-o-CO-Leu-NH_2 (entry 11, Tables 2 and 3) was carried out with a H-PAL-Nle-PEG-PS resin. The first amino acid, Fmoc-Leu-OH, was anchored onto the resin by standard Fmoc/*t*Bu protocols. The mixed active carbonate Fmoc-Phe-o-CO-OSu was then introduced to give the carbamate on resin. Completion of the sequence following Fmoc/*t*Bu procedures yielded the pseudopeptide in good yields (80% cleavage yield) and purity (>95% by analytical HPLC) (entry 11, Table 3).[53]

In summary, DSC has proven to be a useful reactant for the solid phase synthesis of alcohol peptides, carbamate peptides and cyclic peptides. The results and strategies described in this article illustrate the versatility of succinimidyl carbonate resins. Moreover, the carbonate and carbamate conversion of conventional hydroxy and aminomethyl linkers greatly extends their applications. The vast collection of existing bifunctional linkers covers a wide variety of reactivities designed for the carboxyl unit. DSC permits the adaptation of the pool of this type of compound to the chemistry of nucleophiles such as hydroxy and amino groups. We believe that succinimidyl carbonate linkers will also find widespread application in the field of synthetic libraries of small organic molecules due to the the mild preparation conditions and suitable reactivity.

Experimental procedures

General procedures:

Materials, solvents, instrumentation, and general methods were essentially as described in our previous publications.[1,11e,25,32,35b-d,50,54] Organic and peptide synthesis transformations and washes were performed at 25 °C unless indicated otherwise.

Boc- and Fmoc-protected amino acids, BOP, PyBOP, and MBHA resin (0.57 mmol/g) were purchased from Bachem Feinchemikalien AG (Bubendorf, Switzerland), Calbiochem-Novabiochem (Läufelfingen, Switzerland) or Advanced ChemTech (Louisville, USA). To accurately determine anchoring, coupling, and cleavage yields, resins were extended further with an IRAA,[20,21] introduced as its Boc derivative by standard coupling methods, at a point before the introduction of the handle. PyAOP, HOAt, and coupling reagents were obtained from the Biosearch division of PerSeptive Biosystems (Framingham, MA, USA), and used as received. Boc-Thr(Bzl)-oh and HO-CH_2-PS (1.35 mmol/g) were obtained from Advanced ChemTech. Nbb-Ala-MBHA was prepared from 4-hydroxymethyl-3-nitrobenzoic acid[35d] and MBHA-resin. All other chemicals were of the highest purity commercially available. Organic solvent extracts were dried over anhydrous $MgSO_4$, followed by solvent removal at reduced pressures and <40 °C. Solvents for peptide synthesis (DMF and CH_2Cl_2) and HPLC supplies (MeCN and Vydac or Nucleosil C_{18} reversed-phase columns, 4.6 x 250 mm, 5 and 10 μm) were obtained from Scharlau (Barcelona, Spain). DMF was bubbled with nitrogen to remove volatile contaminants and stored over activated 4 A molecular sieves.

Analytical HPLC was carried out on a Shimadzu instrument comprising two solvent delivery pumps (model LC-6A), automatic injector (model SIL-6B), variable wavelength

detector (model SPD-6A), system controller (model SCL-6B) and plotter (model C-R6A). Linear gradients of MeCN (+0.036% TFA) into H_2O (+0.045% TFA) were run at a flow rate of 1.0 mL/min, with UV detection at 220 nm. (Conditions A) from 1:9 to 1:0 over 30 min; (Conditions B) from 1:9 to 1:0 over 20 min; (Conditions C) from 1:9 to 4:6 over 30 min, and then from 4:6 to 1:0 over 5 min; (Conditions D) from 5:95 to 45:55 over 30 min; (Conditions E) from 5:95 to 65:35 over 30 min; (Conditions F) from 5:95 to 65:35 over 30 min, and then from 65:35 to 1:0 over 5 min; (Conditions G) from 1:9 to 4:6 over 40 min.

Peptide-resin samples were hydrolyzed in 12 N aqueous HCl–propionic acid (1:1), at 155 °C for 1–3 h (a drop of phenol was added when Tyr was present in the sequence). Subsequent amino acid analyses were performed on a Beckman System 6300 autoanalyzer. Thin-layer chromatography was performed on either Polygram SIL G/UV_{254} plates (0.250 mm, 40 x 80 mm, Macherey-Nagel) or Kieselgel 60 F_{254} (0.2 mm, 40 x 80 mm, EM Science). Spots were visualized using UV light. Melting points (expressed in °C) were taken on Büchi melting point apparatuses and are uncorrected.

Photolyses were carried out on a Rayonet RPR-100 photochemical reactor (Southern New England Ultraviolet Company). The reaction vessel was silylated before photolysis in order to prevent resin adhesion to the walls of the vessel. This was carried out by rinsing the reaction vessel 3 or 4 times with a 20% solution of Me_3SiCl in CH_2Cl_2, followed by washing with absolute EtOH and drying. Cleavage reactions with anhydrous HF were carried out in a teflon and Kel F reactor (Toho Kasei, Osaka, Japan).

^{1}H-NMR spectra were recorded at ambient temperature on a Varian Gemini XL-200 or on a Varian Unity XL-300 spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) downfield from tetramethylsilane (TMS). Coupling constants (J), in parentheses, are expressed in hertz (Hz).

MALDI-TOF mass spectrometry of peptide samples was performed on a Kompact Maldi I (Kratos Analytical, Manchester, UK) or on a Lasermat-2000 Mass Analyzer (Finnigan MAT) instrument using an α-cyano-4-hydroxycinnamic acid matrix. Electrospray mass spectra were obtained using a VG QUATTRO instrument (Fisons Instruments, Wythenshawe, UK) working in the positive mode, using nitrogen as the nebulizing and drying gas (10 and 450 L/h, respectively), with a source temperature of 80 °C, a capillary voltage of 3.5 kV and a focusing voltage of 60 V. The same instrument, fitted with a Cs gun, was used to obtain FAB spectra. CI mass spectra were acquired on a Hewlett Packard model HP-5988A. Molecular masses were calculated with the Sciex MacSpec 3.22 program.

Preparation of Amino Acid Derivatives

N^{α}-(9-Fluorenylmethoxycarbonyl) lysine allyl ester, Trifluoroacetate salt [Fmoc-Lys-OAllyl TFA salt]. DIEA (1.45 mL, 8.5 mmol, 2 equiv with respect to the Fmoc-derivative) was added to a solution of Fmoc-Lys-(Boc)-OH (2 g, 4.3 mmol) in allyl bromide (10 mL, 118.2 mmol, 5 mL/g of Fmoc-derivative) and the mixture was heated to 70–75 °C with magnetic stirring for 2 h. After this time complete esterification was indicated by TLC

[$CHCl_3$–MeOH–HOAc (95:5:3)]. The reaction mixture was diluted with EtOAc (200 mL) and washed with 0.1 N aqueous HCl (3 x 100 mL), 10% aqueous $NaHCO_3$ (3 x 100 mL), and saturated aqueous NaCl (3 x 100 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resultant oil was dissolved in TFA–CH_2Cl_2 (60 mL, 1:1, v/v) and the solution stirred at 25 °C for 50 min, when TLC monitoring [$CHCl_3$–MeOH–HOAc, 75:23:2, v/v/v)] indicated complete Boc removal. The solution was concentrated to dryness *in vacuo*, followed by repeated washings and evaporations with Et_2O to provide the NMR-pure title product as a white solid (2.1 g, 93% overall yield; R_f 0.40, $CHCl_3$–MeOH–HOAc, 75:23:2, v/v/v) which was used without further purification; mp 95–96 °C; ^1H NMR (DMSO-d_6) δ 7.28–7.93 (m, 9H), 5.87 (m, 1H), 5.29 (dd, J_1 = 17.2 Hz, J_2 = 1.7 Hz, 1H), 5.19 (dd, J_1 = 10.2 Hz, J_2 = 1.7 Hz, 1H), 4.57 (d, J = 5.2 Hz, 2H), 4.29 (t, J = 14.7 Hz, 1H), 4.28 (d, J = 14.7 Hz, 2H), 4.03 (m, 1H), 2.75 (m, 2H), 1.30–1.85 (m, 6H); CIMS (CH_4): calcd. for $C_{24}H_{28}N_2O_4$: 408. Found: *m/z* 409 $[M + H]^+$.

N^{α}-(9-Fluorenylmethoxycarbonyl) phenylalaninol [Fmoc-Phe-oh]. H-Phe-oh (2.0 g, 13.2 mmol) was suspended, with stirring, in 5% aqueous Na_2CO_3 (200 mL) and the pH was adjusted to 10. The suspension was cooled to 0 °C in an ice-bath, and Fmoc-OSu[55] (4.0 g, 11.9 mmol, 0.9 equiv) dissolved in dioxane (100 mL) was added dropwise to the suspension. Stirring was continued for 1 h while cooling in an ice-bath, and for another 3 h at 25 °C. The crude mixture was filtered to yield a solid which was dissolved in CH_2Cl_2 (300 mL). The organic phase was washed with 0.1 N aqueous HCl (3 x 75 mL), dried over $MgSO_4$, and concentrated to dryness *in vacuo* to provide the NMR-pure title product as a white solid (3.6 g, 81% yield); ^1H NMR ($CDCl_3$) δ 7.15–7.80 (m, 13H), 5.03 (d, J = 8.1 Hz, 1H), 4.30–4.50 (m, 2H), 4.18 (t, J = 6.6 Hz, 1H), 3.92 (broad s, 1H), 3.55–3.68 (m, 2H), 2.85 (d, J = 6.6 Hz, 2H); CIMS (CH_4), calcd. for $C_{24}H_{23}NO_3$ 373. Found: *m/z* 374 $[M + H]^+$.

N^{α}-tert-Butyloxycarbonyl serine allyl ester [Boc-Ser-OAllyl]. DIEA (2.5 mL, 14.6 mmol) was added to a solution of Boc-Ser-OH (3.0 g, 14.6 mmol) in CH_3CN–allyl bromide (2:1, 90 mL, 346.7 mmol allyl bromide, 10 mL/g Boc-derivative), and the reaction was allowed to proceed at 25 °C for 21 h, when TLC [$CHCl_3$–MeOH–HOAc (95:5:3)] indicated complete esterification. The reaction mixture was diluted with EtOAc (450 mL), washed successively with 10% aqueous Na_2CO_3 (3 x 150 mL), and saturated aqueous NaCl (3 x 100 mL), dried ($MgSO_4$) and concentrated to dryness *in vacuo* to provide the NMR-pure title product as a yellow oil (3.4 g, 95% yield; R_f 0.75, $CHCl_3$–MeOH–HOAc, 95:5:3, v/v/v); ^1H NMR ($CDCl_3$) δ 5.90 (m, 1H), 5.48 (broad s, 1H), 5.35 (ddt, J_1 = 10.6 Hz, J_2 = J_3 = 1.4 Hz, 1H), 5.27 (ddt, J_1 = 9.8 Hz, J_2 = J_3 = 1.4 Hz, 1H), 4.70 (dd, J_1 = J_2 = 1.4 Hz, 1H), 4.67 (dd, J_1 = J_2 = 1.4 Hz, 1H), 4.40 (m, 1H), 4.00 (dd, J_1 = 11.4 Hz, J_2 = 3.6 Hz, 1H), 3.91 (dd, J_1 = 11.4 Hz, J_2 = 3.6 Hz, 1H), 1.46 (s, 9H); CIMS (CH_4): calcd. for $C_{11}H_{19}NO_5$: 245. Found: *m/z* 246 $[M + H]^+$.

N^{α}-tert-Butyloxycarbonyl tyrosine allyl ester [Boc-Tyr-OAllyl]. DIEA (1.2 mL, 7.1 mmol) was added to a solution of Boc-Tyr-OH (2.0 g, 7.1 mmol) in CH_3CN-allyl bromide (3:2, 50 mL, 236.4 mmol allyl bromide, 10 mL/g Boc-derivative), and the reaction was allowed to proceed at 25 °C for 22 h, after which TLC monitoring [$CHCl_3$–MeOH–HOAc (95:5:3)]

indicated complete esterification. The reaction mixture was diluted with EtOAc (250 mL), washed successively with 10% aqueous Na_2CO_3 (3 x 100 mL), and saturated aqueous NaCl (3 x 100 mL), dried over $MgSO_4$ and concentrated to dryness *in vacuo* to provide the NMR-pure title product as a yellow solid (2.1 g, 92% yield; R_f 0.80, $CHCl_3$-MeOH-HOAc, 95:5:3, v/v/v); ^{1}H NMR ($CDCl_3$) δ 6.84 (AB, J = 8.0 Hz, 4H), 6.06 (broad s, 1H), 5.85 (m, 1H), 5.31 (d, J = 15.4 Hz, 1H), 5.25 (d, J = 9.4 Hz, 1H), 5.07 (d, J = 8.0 Hz, 1H), 4.60 (d, J = 5.4 Hz, 2H), 4.28 (m, 1H), 3.00 (m, 2H), 1.42 (s, 9H); CIMS (CH_4): calcd. for $C_{17}H_{23}NO_5$: 321. Found: *m/z* 322 $[M + H]^+$.

Active Carbonate Resins

Conversion of Conventional Hydroxymethyl Resins into Active Carbonate Resins and Subsequent Reaction with the Amino/Hydroxyl Component. MBHA resin (0.5 g, 0.57 mmol/g) was placed in a 20 mL polypropylene syringe fitted with a polyethylene filter disk. The resin was then washed with CH_2Cl_2 (5 x 0.5 min), treated with TFA–CH_2Cl_2 (2:3, v/v, 1 x 1 min, 1 x 20 min), and washed with CH_2Cl_2 (5 x 0.5 min). The trifluoroacetate salt was neutralized with DIEA–CH_2Cl_2 (1:19, v/v, 3 x 1 min), and the resin washed again with CH_2Cl_2 (5 x 0.5 min) and DMF (5 x 0.5 min). Boc-Ala-OH (270 mg, 1.43 mmol, 5 equiv) was coupled in the presence of equimolar amounts of DIPCDI and HOBt in DMF at 25 °C for 1 h (the resin was negative to the Kaiser ninhydrin test[56]). The resin was washed with DMF (5 x 0.5 min) and CH_2Cl_2 (3 x 0.5 min). The resultant Boc-Ala-MBHA-resin (Ala serving as IRAA[20,21]) was deprotected with TFA–CH_2Cl_2 (2:3, v/v, 1 x 1 min, 1 x 20 min), washed with CH_2Cl_2 (5 x 0.5 min), neutralized with DIEA–CH_2Cl_2 (1:19, v/v, 3 x 1min), and washed with CH_2Cl_2 (5 x 0.5 min) and DMF (5 x 0.5 min). The bifunctional spacer 3-(4-hydroxymethylphenoxy)propionic acid (168 mg, 0.86 mmol, 3 equiv) was then coupled in the presence of equimolar amounts of DIPCDI and HOBt in DMF at 25 °C for 3 h (negative to the Kaiser ninhydrin test). The resin was washed with DMF (5 x 0.5 min) and CH_2Cl_2 (3 x 0.5 min), and finally dried *in vacuo*. Aliquots of the resulting polymer (HMPP-Ala-MBHA) were taken to test the formation of the carbonate functionality on resin as described below.

HMPP-Ala-MBHA-resin (50 mg, 0.47 mmol/g) was swollen with CH_2Cl_2 (5 x 0.5 min) and DMF (5 x 0.5 min). DSC (60 mg, 10 equiv) and DMAP (2.9 mg, 1 equiv) in DMF (0.4 mL) were added to the resin under an Ar atmosphere and the reaction allowed to proceed at 25 °C for a time (t_1), with continuous mechanical shaking and occasional bubbling of Ar through the suspension. The resin was washed with DMF (8 x 0.5 min) and H-Leu-Obzl *p*-tosylate (92 mg, 10 equiv) and DIEA (80 μL, 20 equiv) in DMF (0.4 mL) were added to the resin under an Ar atmosphere at the reaction allowed to proceed at 25 °C for a time (t_2) with continuous mechanical shaking and occasional bubbling of Ar through the suspension. Finally, the resin was washed with DMF (5 x 0.5 min) and CH_2Cl_2 (5 x 0.5 min), and *dried in vacuo*. A sample (2–5 mg) of each resin was acid hydrolyzed (3 h), and evaluated by AAA. The ratio Leu/IRAA indicated the yield of formation of the active carbonate resin (see Table 1

for complete results), and thus the effectiveness of the particular conditions used. Similar protocols were used for Nbb-IRAA-MBHA-resin and HO-CH_2-resin.

Solid-Phase Peptide Synthesis[57]

cyclo (Val-Phe-Sar-Tyr-DTrp-Lys) (**1**). Side chain anchoring of Fmoc-Lys-OAllyl: Fmoc-Lys-OAllyl was coupled to HMPP-Ala-MBHA (100 mg, 0.47 mmol/g) as described above using DSC (120 mg, 10 equiv) and DMAP (5.7 mg, 1 equiv) in DMF (0.5 mL) under an Ar atmosphere in the first step (t_1 = 2 h), followed by later addition of the trifluoroacetate salt of Fmoc-Lys-OAllyl (245 mg, 10 equiv) with DIEA (0.16 mL, 20 equiv) in DMF (0.5 mL) under an Ar atmosphere (t_2 = 5 h). AAA of the resin acid hydrolysis (3 h) showed a quantitative yield of Lys incorporation.

Manual chain assembly was carried out in the $C \rightarrow N$ direction according to a standard Fmoc/*t*Bu protocol: Fmoc group removal with piperidine–DMF (1:4, v/v, 3 x 1 min, 2 x 5 min), DMF washes (5 x 0.5 min), amino acid coupling with Fmoc-amino acids (10 equiv) in the presence of HOAt[58] (10 equiv) and DIPCDI (10 equiv) in DMF for 30 min, and DMF washes (5 x 0.5 min). In the case of Fmoc-Phe-OH, a double coupling was carried out. Side-chain protection for Tyr was provided by *t*Bu. Couplings were monitored for completion by the Kaiser nynhidrin test. The amino acid composition of the acid-hydrolyzed (3 h) peptide-resin was: Val, 1.00; Phe, 1.01; Tyr, 0.95; Lys, 1.04; Ala, 1.05; Sar and Trp, not detected. An aliquot of the final protected peptide was cleaved from the resin by treatment with TFA–thioanisole–ethanedithiol–anisole (90:5:3:2, v/v/v/v) at 25 °C for 2 h (96% cleavage yield). The filtrate from the cleavage reaction was collected, and the peptide was precipitated with Et_2O. The crude product was homogeneous by analytical HPLC (t_R 21.5 min, > 85% purity; see Figure 1A); FABMS (nitrobenzyl alcohol matrix), calcd. for $C_{61}H_{70}N_8O_{10}$: 1075.3. Found: *m/z* 1075.9 $[M + H]^+$, 1097.8 $[M + Na]^+$.

Once the target sequence Fmoc-Val-Phe-Sar-Tyr(*t*Bu)-DTrp-Lys(-CO-O-HMPP-Ala-MBHA)-OAllyl was assembled, the *C*-terminal allyl ester was cleaved by treatment of the peptide-resin (20 mg, 0.29 mmol/g, 0.0058 mmol) with $Pd(PPh_3)_4$ (34 mg, 0.029 mmol, 5 equiv) in DMSO–THF–0.5 N aqueous HCl–morpholine (2:2:1:0.1, 1.02 mL) under Ar for 2 h 30 min at 25 °C.[54a] The peptidyl resin was then washed with THF (3 x 2 min), DMF (3 x 2 min), CH_2Cl_2 (3 x 2 min), DIEA–CH_2Cl_2 (1:19, 3 x 2 min), CH_2Cl_2 (3 x 2 min), diethyldithiocarbamic acid, sodium salt (0.03 M in DMF, 3 x 15 min),[59] DMF (5 x 2 min), CH_2Cl_2 (3 x 2 min), and DMF (3 x 1 min). The Fmoc group was removed with piperidine–DMF (1:4, v/v, 3 x 1 min, 2 x 5 min, 2 x 1min), followed by washings with DMF (5 x 0.5 min), and the resin-bound peptide cyclized by addition of a solution of BOP (13 mg, 5 equiv) and HOBt (4.4 mg, 5 equiv) in DMF (0.3 mL). The cyclization was iniciated by addition of DIEA (9.8 μL, 10 equiv). After 2 h, the peptide-resin was negative to the Kaiser ninhydrin test. Final cleavage of the anchoring linkage and *t*Bu side-chain protecting group of Tyr was performed with TFA–thioanisole–ethanedithiol–anisole (90:5:3:2, v/v/v/v) at 25 °C for 2 h (61% cleavage yield). The crude peptide was precipitated with Et_2O and the product obtained

showed a major peak (cyclic peptide) by analytical HPLC (t_R 13.7 min, >70% purity; see Figure 1C); FABMS (nitrobenzyl alcohol matrix), calcd. for $C_{43}H_{54}N_8O_7$: 794.9. Found: *m/z* 795.7 $[M + H]^+$, 817.6 $[M + Na]^+$.

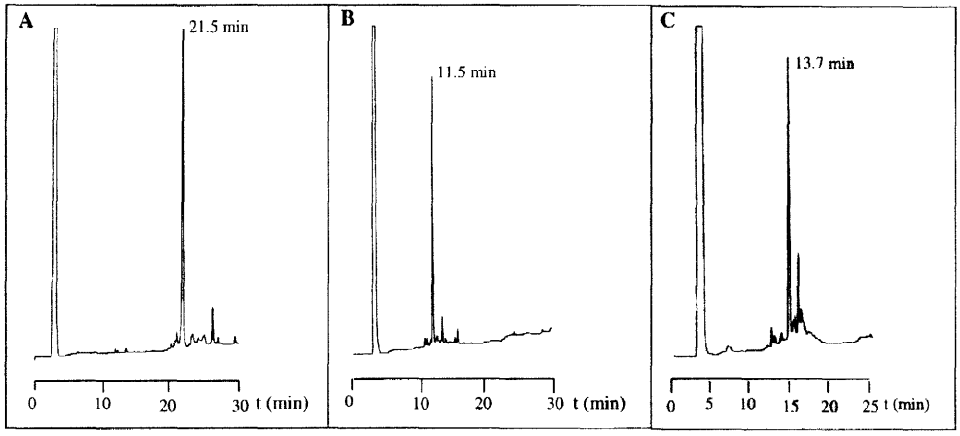


Figure 1. Analytical HPLC following Conditions A, as listed in General Procedures, of crude peptides related to (**1**). **A**: Fmoc-Val-Phe-Sar-Tyr-DTrp-Lys-OAllyl; **B**: H-Val-Phe-Sar-Tyr-DTrp-Lys-OH; **C**: *cyclo* (Val-Phe-Sar-Tyr-DTrp-Lys).

cyclo (Leu-Arg-Met-Lys-Leu-Pro-Lys) (**4a**). Side chain anchoring of Fmoc-Lys-OAllyl: Fmoc-Lys-OAllyl was coupled to HMPP-Ala-MBHA (600 mg, 0.47 mmol/g) as described above using DSC (722 mg, 10 equiv) and DMAP (34 mg, 1 equiv) in DMF (2 mL) under Ar atmosphere in the first step (t_1 = 2 h) and later addition of the trifluoroacetate salt of Fmoc-Lys-OAllyl (1.47 g, 10 equiv) with DIEA (0.96 mL, 20 equiv) in DMF (2 mL) under Ar atmosphere (t_2 = 5 h). AAA of the resin acid hydrolysis (3 h) showed a 83% yield of Lys incorporation.

Manual chain assembly was carried out in the $C \rightarrow N$ direction according to a standard Fmoc/*t*Bu protocol. Couplings were performed by dissolving the Fmoc-protected amino acid (5 equiv), PyAOP (5 equiv) and DIEA (10 equiv) in DMF. After 5 min, the solution was added to the resin and left for 1 h at 25 °C with occasional swirling. In the case of the first Fmoc-Leu-OH, a double coupling was carried out. Completion of couplings was assessed with the Kaiser ninhydrin test. Side-chain protection was provided by Pmc (Arg) and Boc (Lys). The amino acid composition of the acid-hydrolyzed (3 h) peptide-resin was: Pro, 1.04; Met, 0.94; Leu, 2.21; Lys, 1.81; Arg, 1.00. An aliquot of the final protected peptide was cleaved from the resin by treatment with TFA–phenol–triisopropylsilane–H_2O (88:5:2:5, v/v/v/v) at 25 °C for 2 h (70% cleavage yield). The filtrate from the cleavage reaction was collected, and the peptide was precipitated with Et_2O. Analytical HPLC revealed that the crude peptide was homogeneous (t_R 20.6 min, > 80% purity; Conditions A); MALDI-TOF MS, calcd. for $C_{58}H_{90}N_{12}O_{10}S$ 1147.5. Found: *m/z* 1148.7 $[M + H]^+$.

Once the target sequence Fmoc-Leu-Arg(Pmc)-Met-Lys(Boc)-Leu-Pro-Lys(-CO-O-HMPP-Ala-MBHA)-OAllyl was assembled, the *C*-terminal allyl ester was cleaved by treatment of the peptide-resin (200 mg, 0.20 mmol/g, 0.04 mmol) with $Pd(PPh_3)_4$ (231 mg, 0.2 mmol, 5 equiv) in DMSO–THF–0.5 N aqueous HCl–morpholine (2:2:1:0.1, 5.1 mL) under Ar for 3 h at 25 °C. The Fmoc group was removed as above and the resin-bound peptide cyclized by

addition of a solution of PyAOP[60] (104 mg, 5 equiv) and HOAt (27 mg, 5 equiv) in DMF (1 mL), and the cyclization was initiated by addition of DIEA (68 μL, 10 equiv). After 2 h the peptide-resin was negative to the Kaiser ninhydrin test. Final cleavage of the anchoring linkage and side-chain protecting groups was performed with TFA–phenol–triisopropylsilane–H_2O (88:5:2:5, v/v/v/v) at 25 °C for 2 h (45% cleavage yield). The crude peptide was precipitated with Et_2O and the product obtained showed three main peaks by analytical HPLC [>85% purity; total of the three peaks, see Figure 2A); t_R 30.8 min (19%) (cyclic dimer), t_R 32.7 min (21%) (cyclic trimer), and t_R 34.5 min (60%)]; MALDI-TOF MS, $C_{80}H_{148}N_{24}O_{14}S_2$ (cyclic dimer): 1734.4. Found: *m/z* 1735.1 $[M + H]^+$. MALDI-TOF MS, calcd. for $C_{120}H_{222}N_{36}O_{21}S_3$ (cyclic trimer): 2601.6. Found: *m/z* 2602.9 $[M + H]^+$.

cyclo (Leu-Arg-Met-Lys-Leu-Pro-Lys) (**4b**). Side-chain anchoring of Fmoc-Lys-OAllyl: Fmoc-Lys-OAllyl was coupled to HMPP-Ala-PEG-PS (1 g, 0.19 mmol/g) as described above using firstly DSC (486 mg, 10 equiv) and DMAP (23 mg, 1 equiv) in DMF (1.8 mL) under an Ar atmosphere (t_1 = 2 h), and secondly using the trifluoroacetate salt of Fmoc-Lys-OAllyl (992 mg, 10 equiv) and DIEA (0.65 mL, 20 equiv) in DMF (1.8 mL) under an Ar atmosphere (t_2 = 5 h).

Automated chain assembly was carried out in the *C* → *N* direction using a PerSeptive 9050 Plus PepSynthesizer according to a standard Fmoc/*t*Bu protocol. In this case, each Fmoc-protected amino acid (10 equiv) was activated with TBTU (10 equiv) and DIEA (20 equiv) in DMF for 1 h. Again, side-chain protection was provided by Pmc (Arg) and Boc (Lys). The amino acid composition of the acid-hydrolyzed (3 h) peptide-resin was: Pro, 0.90; Met, 1.00; Leu, 2.15; Lys, 2.01; Arg, 0.94. An aliquot of the final protected peptide was cleaved from the resin by treatment with TFA–phenol–triisopropylsilane–H_2O (88:5:2:5, v/v/v/v) at 25 °C for 3 h (56% cleavage yield). The filtrate from the cleavage reaction was collected and the peptide was precipitated with Et_2O. Analytical HPLC showed that the crude peptide was homogeneous (t_R 20.1 min, >80% purity; Conditions A); MALDI-TOF MS, calcd. for $C_{58}H_{90}N_{12}O_{10}S$ 1147.5. Found: *m/z* 1148.8 $[M + H]^+$.

Finally, the *C*-terminal allyl ester was cleaved by treatment of the peptide-resin (50 mg, 0.11 mmol/g, 0.0055 mmol) with $Pd(PPh_3)_4$ (32 mg, 0.0275 mmol, 5 equiv) in DMSO–THF–0.5 N aqueous HCl–morpholine (2:2:1:0.1, 1.53 mL) under Ar for 3 h at 25 °C. The Fmoc group was removed as above and the resin-bound peptide cyclized by addition of a solution of PyAOP (29 mg, 10 equiv) and HOAt (7.5 mg, 10 equiv) in DMF (0.4 mL), and the cyclization was initiated by addition of DIEA (19 μL, 20 equiv). After 2 h, the peptide-resin was negative to the Kaiser ninhydrin test. Final cleavage of the anchoring linkage and side-chain protecting groups was performed with TFA–phenol–triisopropylsilane–H_2O (88:5:2:5, v/v/v/v) at 25 °C for 3 h (31% cleavage yield). The crude peptide was precipitated with Et_2O and the product obtained showed two main peaks by analytical HPLC [>70% purity total of the two peaks, see Figure 2B); t_R 23.1 min (78%) (cyclic monomer), t_R 30.7 min (22%) (cyclic dimer)]; ESMS, calcd. for $C_{40}H_{74}N_{12}O_7S$ (cyclic monomer): 867.2. Found: *m/z* 434.7 $[M + 2H^+/2]$; MALDI-TOF MS, calcd. for $C_{80}H_{148}N_{24}O_{14}S_2$ (cyclic dimer): 1734.4. Found: *m/z* 1736.1 $[M + H]^+$.

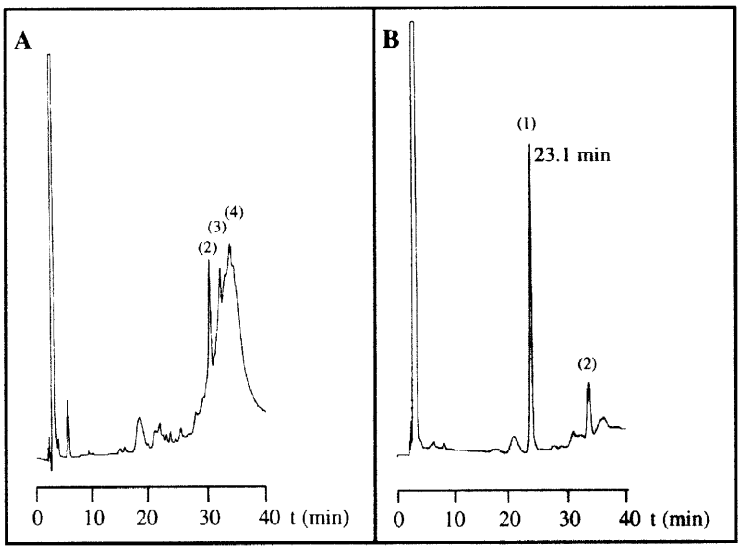


Figure 2. Analytical HPLC following Conditions G, as listed in General Procedures, of crude peptides (**4**). **A**: 4A; **B**: 4B; (1) cyclic monomer; (2) cyclic dimer; (3) cyclic trimer; (4) product not identified.

cyclo (Leu-Phe-Gly-Gly-Ser) (**5**). Side-chain anchoring of Boc-Ser-OAllyl: Boc-Ser-OAllyl was coupled to Nbb-Ala-MBHA (125 mg, 0.46 mmol/g) as described above. Firstly, DSC (147 mg, 10 equiv) and DMAP (7 mg, 1 equiv) in DMF (0.8 mL) were added under an Ar atmosphere (t_1 = 2 h) and secondly, Boc-Ser-OAllyl (141 mg, 10 equiv) and DMAP (1.4 mg, 0.2 equiv) in DMF (0.8 mL) were added onto the preformed succinimidyl carbonate resin under an Ar atmosphere (t_2 = 17 h). AAA acid hydrolysis (1 h) showed an minimum of Ser incorporation of 44%.[61]

Manual chain assembly was carried out in the $C \rightarrow N$ direction according to a standard Boc/Bzl protocol: Boc group removal with TFA–CH_2Cl_2 (2:3, v/v, 1 x 1 min, 1 x 30 min), CH_2Cl_2 washes (5 x 0.5 min), neutralization with DIEA–CH_2Cl_2 (1:19, v/v, 3 x 1 min), CH_2Cl_2 washes (5 x 0.5 min), amino acid coupling with Boc-amino acids (5 equiv) in the presence of HOBt (5 equiv) and DIPCDI (5 equiv) in DMF for 1 h, DMF washes (5 x 0.5 min) and CH_2Cl_2 washes (5 x 0.5 min). Couplings were monitored for completion by the Kaiser test. The amino acid composition of the acid-hydrolyzed (90 min) peptide-resin was: Leu, 1.01; Phe, 0.95; Gly, 2.04; Ser, 0.76; Ala 2.17. This result signifies that the incorporation of Ser was of 55%.

An aliquot of the final protected peptide was cleaved from the resin by photolysis at 350 nm. The general procedure for the photolytic cleavage was as follows: peptide-Nbb-resin was suspended in TFE–CH_2Cl_2 (1:4, v/v) in a cylindrical reaction vessel with a three-way stopcock. The suspension was blanketed under Ar by three successive cycles of degassing with a water pump and purging with the inert gas. The resin was photolysed for 8 h (75% cleavage yield) with vigorous magnetic stirring. The crude reaction mixture was filtered, combined with CH_2Cl_2 washes (2 mL) of the cleaved peptide-resin, and evaporated to dryness to give a material that showed a single major component by analytical HPLC (t_R 19.0 min, >70% purity; see Figure 3A); ESMS, calcd. for $C_{30}H_{45}N_5O_9$: 619.7. Found: *m/z* 620.3 $[M + H]^+$.

Once the target sequence Boc-Leu-Phe-Gly-Gly-Ser(CO-O-Nbb-Ala-MBHA)-OAllyl was assembled, the *C*-terminal allyl ester was cleaved by treatment of the peptide-resin (100 mg, 0.17 mmol/g, 0.017 mmol) with $Pd(PPh_3)_4$ (98 mg, 0.085 mmol, 5 equiv) in DMSO–THF–0.5 N aqueous HCl–morpholine (2:2:1:0.1, 2.04 mL) under Ar at 25 °C for 2 h 30 min.[54a] The Boc group was removed as above and the resin-bound peptide cyclized by addition of a solution of PyAOP (44 mg, 5 equiv) and HOAt (12 mg, 5 equiv) in DMF (0.5 mL), and the cyclization was initiated by addition of DIEA (29 μL, 10 equiv). After 2 h, the peptide-resin was slightly positive to the Kaiser ninhydrin test. The coupling was repeated again under the same conditions to yield a negative Kaiser ninhydrin test. The amino acid composition of the acid-hydrolyzed (90 min) peptide-resin was: Leu, 0.98; Phe, 0.97; Gly, 2.05; Ser, 0.63; Ala 2.50. Final cleavage of the anchoring linkage was performed by photolysis at 350 nm in TFE–CH_2Cl_2 (1:4, v/v) for 10 h (31% cleavage yield) and the crude product showed three main peaks by analytical HPLC [>80% purity, total of the three peaks, see Figure 3B: t_R: 14.4 min (28%) (cyclic peptide), t_R: 17.0 min (26%) (cyclic dimer), and t_R: 19.5 min (46%)]; ESMS, calcd. for $C_{22}H_{31}N_5O_6$ (cyclic peptide): 461.5. Found: *m/z* 462.0 $[M + H]^+$, 484.0 $[M + Na]^+$; ESMS, calcd. for $C_{44}H_{62}N_{10}O_{12}$ (cyclic dimer): 923.0. Found: *m/z* 923.8 $[2M + H]^+$, 945.8 $[2M + Na]^+$.

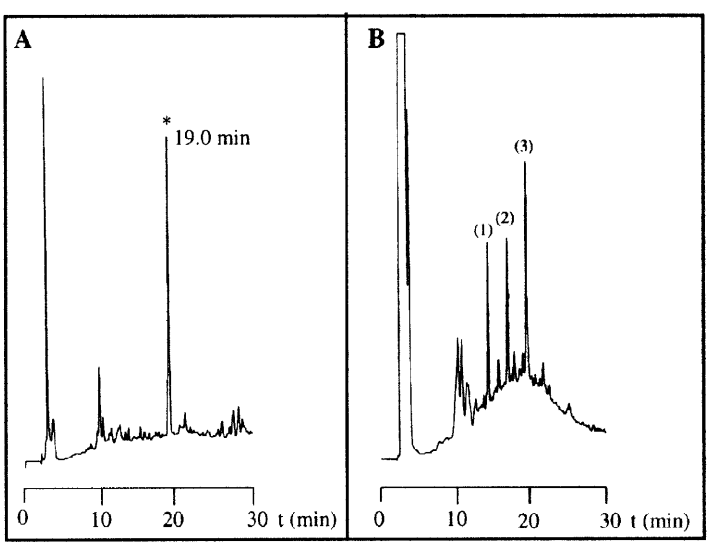


Figure 3. Analytical HPLC following Conditions A, as listed in General Procedures, of crude peptides related to (**5**). **A**: Boc-Leu-Phe-Gly-Gly-Ser-OAllyl; **B**: *cyclo* (Leu-Phe-Gly-Gly-Ser); (1) cyclic monomer; (2) cyclic dimer; (3) product not identified.

cyclo (Leu-Phe-Gly-Gly-Tyr) (**6**). Side-chain anchoring of Boc-Tyr-OAllyl. Boc-Tyr-OAllyl was coupled to HO-Nbb-Ala-MBHA (125 mg, 0.46 mmol/g) as described above using firstly DSC (147 mg, 10 equiv) and DMAP (7 mg, 1 equiv) in DMF (0.8 mL) under an Ar atmosphere (t_1 = 2 h) and, secondly, using Boc-Tyr-OAllyl (185 mg, 10 equiv) and DMAP (1.4 mg, 0.2 equiv) in DMF (0.8 mL) under an Ar atmosphere (t_2 = 17 h). AAA of the resin (hydrolysis with 6 N HCl in propionic acid with a drop of phenol at 155 °C for 3 h) showed a 67% minimum incorporation of Tyr.[61]

Manual chain assembly was carried out in the $C \rightarrow N$ direction according to a standard Boc/Bzl protocol with Boc-AA-OH/HOBt/DIPCDI (5:5:5, equiv/equiv/equiv) couplings in DMF (1 h per residue). The amino acid composition of the acid-hydrolyzed (90 min) peptide-resin was: Leu, 1.00; Phe, 0.96; Gly, 2.04; Tyr, 1.00; Ala 1.89. An aliquot of the final protected peptide was cleaved from the resin by photolysis at 350 nm in TFE–CH_2Cl_2 (1:4, v/v) for 8 h (72% cleavage yield). The crude cleaved peptide was homogeneous by analytical HPLC (t_R 21.0 min, >75% purity; see Figure 4A); ESMS, calcd. for $C_{36}H_{49}N_5O_9$: 695.8. Found: *m/z* 696.5 $[M + H]^+$.

Once the target sequence Boc-Leu-Phe-Gly-Gly-Tyr(-CO-O-Nbb-Ala-MBHA)-OAllyl was assembled, the *C*-terminal allyl ester was cleaved by treatment of the peptide-resin (100 mg, 0.19 mmol/g, 0.019 mmol) with $Pd(PPh_3)_4$ (110 mg, 0.095 mmol, 5 equiv) in DMSO–THF–0.5 N aqueous HCl-morpholine (2:2:1:0.1, 2.04 mL) under Ar at 25 °C for 2 h 30 min.[54a] The Boc group was removed, and the resin-bound peptide cyclized by addition of a solution of PyAOP (50 mg, 5 equiv) and HOAt (13 mg, 5 equiv) in DMF (0.5 mL), and cyclization was initiated by addition of DIEA (32 μL, 10 equiv). After 2 h at 25 °C, the peptide-resin was slightly positive to the Kaiser ninhydrin test. The coupling was repeated again under the same conditions (Kaiser ninhydrin test negative). The amino acid composition of the acid-hydrolyzed (90 min) peptide-resin was: Leu, 0.98; Phe, 0.93; Gly, 2.11; Tyr, 0.98; Ala 2.38. Final cleavage of the anchoring linkage was performed by photolysis at 350 nm in TFE-CH_2Cl_2 (1:4, v/v) for 10 h (31% cleavage yield) and the crude showed three main peaks by analytical HPLC [>70% purity, total of the three peaks, see Figure 4B: t_R: 16.6 min (35%) (cyclic peptide), t_R: 18.9 min (35%) (cyclic dimer) and t_R: 19.5 min (30%)]. ESMS, calcd. for $C_{28}H_{35}N_5O_6$ (cyclic peptide): 537.6. Found: *m/z* 538.0 $[M + H]^+$, 560.3 $[M + Na]^+$; ESMS, calcd. for $C_{56}H_{70}N_{10}O_{12}$ (cyclic dimer): 1075.2. Found: *m/z* 1076.0 $[2M + H]^+$, 1098.3 $[2M + Na]^+$.

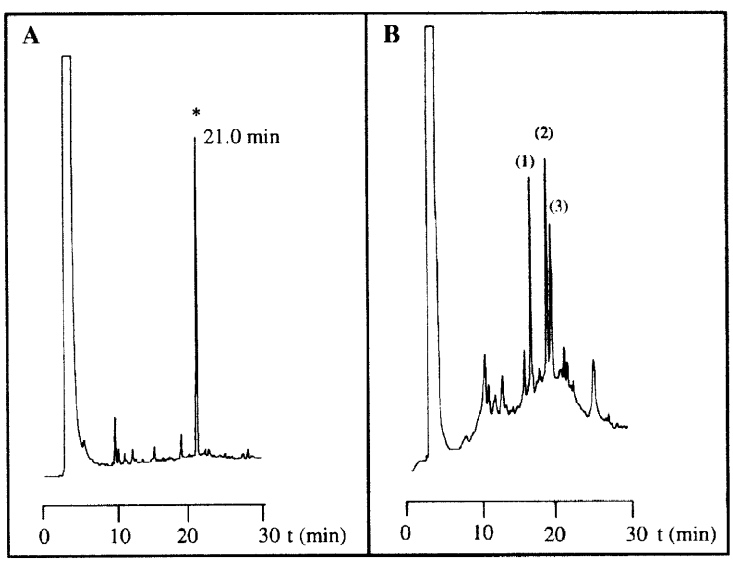


Figure 4. Analytical HPLC following Conditions A as listed in General Procedures of crude peptides related to (**6**). **A**: Boc-Leu-Phe-Gly-Gly-Tyr-OAllyl; **B**: *cyclo* (Leu-Phe-Gly-Gly-Tyr); (1) cyclic monomer; (2) cyclic dimer; (3) product not identified.

cyclo (Glu-Ala-Ala-Arg-DPhe-Pro-Glu-Asp-Asn-Ser) (7). Side-chain anchoring of Boc-Ser-OAllyl. Boc-Ser-OAllyl was coupled to HO-CH_2-PS (75 mg, nominative substitution of 1.35 mmol/g) as described above. AAA of the resin after acid hydrolysis (1 h) showed a minimum of 38% yield with respect to the theoretical substitution (final loading 0.37 mmol/g).

Manual chain assembly was carried out in the $C \rightarrow N$ direction according to a standard Boc/Bzl protocol using Boc-protected amino acids (5 equiv) and DIPCDI/HOBt-mediated couplings (5 equiv each) in DMF (1 h per residue). Boc-Asn-OH and Boc-Arg(Tos)-OH were coupled as follows: N^{α}-Boc-AA-OH was dissolved in DMF, and HOBt and DIPCDI were added. After 5 min, the solution was added to the resin and the coupling was allowed to proceed at 25 °C for 1 h.

Side-chain protection was provided by OChx (Glu and Asp) and Tos (Arg). The amino acid composition of the acid-hydrolyzed (90 min) peptide-resin was: Ala, 1.89; Phe, 0.98; Pro, 0.95; Glu, 2.13; Asp, 2.05; Ser, 0.82; Arg, not detected; final loading 0.26 mmol/g, which represents an overall yield of 64%. An aliquot of the final protected peptide was cleaved from the resin by treatment with HF–anisole (9:1, v/v) at 0 °C for 1 h. The peptide was precipitated with Et_2O, and dissolved in 10% aqueous HOAc. Analytical HPLC revealed that the crude peptide was homogeneous (t_R 17.0 min, >80% purity; see Figure 5A); MALDI-TOF MS, calcd. for $C_{47}H_{70}N_{14}O_{19}$: 1135.1 (unprotected linear peptide). Found: *m/z* 1136.0 $[M + H]^+$.

Once the target sequence Boc-Glu(OChx)-Ala-Ala-Arg(Tos)-DPhe-Pro-Glu(OChx)-Asp(OChx)-Asn-Ser(CO-O-CH_2-PS)-OAllyl was assembled, the *C*-terminal allyl ester was cleaved by treatment of the peptide-resin (50 mg, 0.26 mmol/g, 0.013 mmol) with $Pd(PPh_3)_4$ (75 mg, 0.065 mmol, 5 equiv) in $CHCl_3$–HOAc–NMM (37:2:1, v/v/v, 2 mL) under an Ar atmosphere at 25 °C for 2 h 30 min.[53b] The Boc group was removed, and the resin-bound peptide cyclized in DMF by treatment with PyAOP/HOAt/DIEA (5:5:10, equiv/equiv/equiv) at 25 °C for 2 h (resin negative to the Kaiser ninhydrin test). The amino acid composition of the acid-hydrolyzed (90 min) peptide-resin was: Ala, 1.97; Phe, 1.02; Pro, 0.95; Glu, 2.10; Asp, 1.96; Ser, 0.64; Arg, n.d; final loading 0.26 mmol/g; 60% overall synthesis yield. Final release of the peptide from the resin and concomitant removal of side-chain protecting groups was performed by treatment with HF–anisole (9:1, v/v) at 0 °C for 1 h. The cleaved peptide was precipitated with Et_2O, and dissolved in 10% aqueous HOAc, and showed a main peak by analytical HPLC (t_R 11.5 min; >40% purity, see Figure 5B); MALDI-TOF MS, calcd. for $C_{47}H_{68}N_{14}O_{18}$: 1117.1 (cyclic peptide). Found: *m/z* 1118.0 $[M + H]^+$.

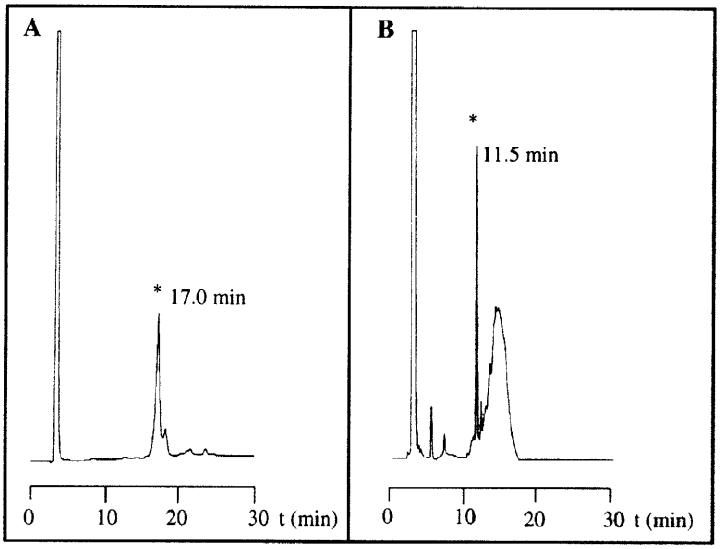


Figure 5. Analytical HPLC of crude peptides related to (7). **A**: H-Glu-Ala-Ala-Arg-DPhe-Pro-Glu-Asp-Asn-Ser-OH (Conditions D, as listed in General Procedures); **B**: *cyclo* (Glu-Ala-Ala-Arg-DPhe-Pro-Glu-Asp-Asn-Ser) (Conditions A, as listed in General Procedures).

cyclo (Glu-Ala-Ala-Arg-DPhe-Pro-Glu-Asp-Asn-Tyr) (**8**). Side-chain anchoring of Boc-Tyr-OAllyl. Boc-Tyr-OAllyl was coupled to HO-CH_2-PS (75 mg, 1.35 mmol/g) as described above. AAA of the resin after hydrolysis (6 N HCl in propionic acid with a drop of phenol, at 155 °C for 90 min) gave a 66% yield of Tyr incorporation (final loading 0.61 mmol/g).

Manual chain assembly was carried out in the $C \rightarrow N$ direction according to a standard Boc/Bzl protocol using Boc-protected amino acids (10 equiv) and DIPCDI/HOBt-mediated couplings (10 equiv each) in DMF (1 h per residue). The couplings of Boc-Asn-OH and Boc-Arg(Tos)-OH were carried out as described in the previous synthesis. Side-chain protection was provided by OChx (Glu and Asp) and Tos (Arg).

The amino acid composition of the acid-hydrolyzed (90 min) peptide-resin was: Ala, 1.95; Phe, 1.03; Pro, 0.93; Glu, 2.04; Asp, 2.05; Tyr, 1.63; Arg, n.d; final loading 0.20 mmol/g (51% synthesis yield). An aliquot of the final protected peptide was cleaved from the resin by treatment with HF–anisole (9:1, v/v) at 0 °C for 1 h (93% cleavage yield). The crude cleaved peptide was precipitated with Et_2O and dissolved in 10% aqueous HOAc. Evaluation of the purity by analytical HPLC showed that the crude peptide was fairly homogeneous (t_R 11.6 min, >75% purity; see Figure 6A); MALDI-TOF MS, calcd. for $C_{53}H_{74}N_{14}O_{19}$: 1211.2 (unprotected linear peptide). Found: *m/z* 1212.0 $[M + H]^+$.

Once the target sequence Boc-Glu(OChx)-Ala-Ala-Arg(Tos)-DPhe-Pro-Glu(OChx)-Asp(OChx)-Asn-Tyr(CO-O-CH_2-PS)-OAllyl was assembled, the *C*-terminal allyl ester was cleaved by treatment of the peptide-resin (75 mg, 0.20 mmol/g, 0.015 mmol) with $Pd(PPh_3)_4$ (87 mg, 0.075 mmol, 5 equiv) in $CHCl_3$–HOAc–NMM (37:2:1, v/v/v, 2 mL) under an Ar atmosphere at 25 °C for 2 h 30 min.[54b] The Boc group was removed, and the resin-bound peptide cyclized in DMF by treatment with PyAOP/HOAt/DIEA (5:5:10, equiv/equiv/equiv) at 25 °C for 2 h (negative Kaiser ninhydrin test). The amino acid composition of the acid-hydrolyzed (90 min) peptide-resin was: Ala, 1.89; Phe, 1.01; Pro, 0.94; Glu, 2.04; Asp, 2.12; Tyr, 1.43; Arg, not detected; final loading 0.19 mmol/g (45% synthesis yield). Final cleavage

of the anchoring linkage and concomitant removal of side-chain protecting groups was performed by treatment with HF–anisole (9:1, v/v) at 0 °C for 1 h (71% cleavage yield). The cleaved peptide was precipitated with Et_2O, and dissolved in 10% aqueous HOAc. Analytical HPLC revealed a main peak (t_R 19.5 min, >40% purity; see Figure 6B); MALDI-TOF MS, calcd. for $C_{53}H_{72}N_{14}O_{18}$: 1193.2 (cyclic peptide). Found: *m/z* 1194.8 $[M + H]^+$.

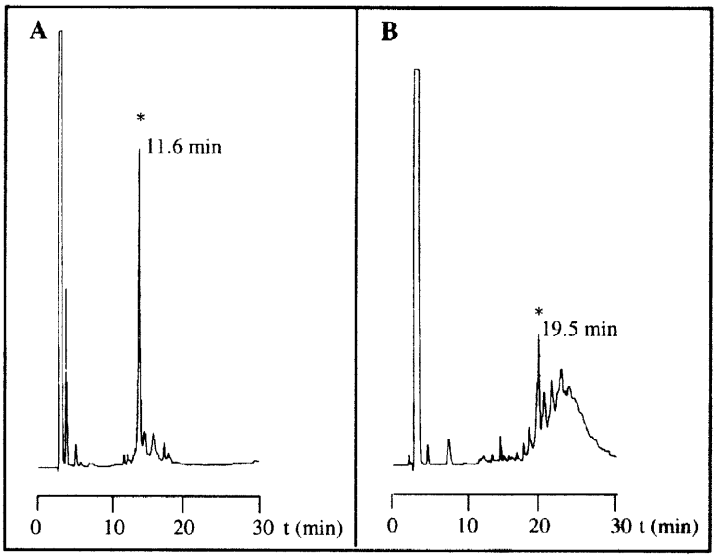


Figure 6. Analytical HPLC of crude peptides related to (**8**). **A**: H-Glu-Ala-Ala-Arg-DPhe-Pro-Glu-Asp-Asn-Tyr-OH (Conditions A, as listed in General Procedures); **B**: *cyclo* (Glu-Ala-Ala-Arg-DPhe-Pro-Glu-Asp-Asn-Tyr) (Conditions E, as listed in General Procedures).

Octreotide (Cys²-Cys⁷) H-DPhe-Cys-Phe-DTrp-Lys-Thr-Cys-Thr-oh (**9**). Boc-Thr(Bzl)-oh was coupled to HO-CH_2-PS (100 mg, 1.35 mmol/g) as described above for Boc-Ser-OAllyl and Boc-Tyr-OAllyl, firstly using DSC (346 mg, 10 equiv) and DMAP (16 mg, 1 equiv) in DMF (1.0 mL) under an Ar atmosphere (t_1 = 2 h) and, secondly, by addition of Boc-Thr(Bzl)-oh (399 mg, 10 equiv) and DMAP (3.3 mg, 0.2 equiv) in DMF (1.0 mL) also under an Ar atmosphere (t_2 = 17 h).

Manual chain assembly was carried out in the $C \rightarrow N$ direction according to a standard Boc/Bzl protocol. Couplings were performed by dissolving the Boc-protected amino acid (10 equiv), HOBt (10 equiv), and DIPCDI (10 equiv) in DMF. After 5 min, the solution was added to the resin and left for 1 h at 25 °C with occasional swirling. Completion of couplings was assessed with the Kaiser ninhydrin test. Side-chain protection was provided by Acm (Cys), Bzl (Thr), 2-ClZ (Lys) and For (DTrp). The amino acid composition of the acid-hydrolyzed (3 h) peptide-resin was: Phe, 2.11; Lys, 0.89; Cys, Thr and Trp n.d; the amino alcohol was not detected by AAA; final loading 0.174 mmol/g (42% synthesis yield). An aliquot of the final protected peptide was cleaved from the resin by treatment with HF–anisole (9:1, v/v) at 0 °C for 1 h (96% cleavage yield). The cleaved peptide was precipitated with Et_2O and dissolved in 10% aqueous HOAc. Analytical HPLC revealed that the crude was homogeneous (t_R 21.9 min, >82% purity; see Figure 7A); ESMS, calcd. for $C_{56}H_{78}N_{12}O_{13}S_2$ 1191.4 [H-DPhe-Cys(Acm)-Phe-DTrp(For)-Lys-Thr-Cys(Acm)-Thr-oh]. Found: *m/z* 1192.4 $[M + H]^+$, 596.9 $[M + 2H^+/2]$.

Once the target sequence Boc-DPhe-Cys(Acm)-Phe-DTrp(For)-Lys(2-ClZ)-Thr(Bzl)-Cys(Acm)-Thr(Bzl)-o-CO-O-CH_2-PS was assembled, the formation of the disulfide bridge was carried out in the solid-phase by washing the peptide-resin (20 mg, 0.174 mmol/g, 0.0035 mmol) with CH_2Cl_2 (5 x 0.5 min), DMF (5 x 0.5 min), and HOAc (2 x 1 min), and by treatment with I_2 (18 mg, 0.071 mmol, 20 equiv, 10 equiv/Acm) in 80% aqueous HOAc (2 mL) for 40 min at 25 °C with continuous mechanical shaking. The peptide-resin was washed with DMF (5 x 0.5 min), CH_2Cl_2 (5 x 0.5 min), saturated aqueous ascorbic acid solution (5 x 1 min), and CH_2Cl_2 (5 x 0.5 min). The Boc group was removed by treatment with TFA–CH_2Cl_2 (2:3, v/v, 1 x 1 min, 1 x 30 min), and the resin washed with CH_2Cl_2 (5 x 0.5 min). The release of the cyclic peptide from the resin and concomitant removal of Bzl side-chain protecting groups was carried out with HF–anisole (9:1, v/v) at 0 °C for 1 h. The cleaved peptide was precipitated with Et_2O, and dissolved in 10% aqueous HOAc. Analytical HPLC showed that the crude peptide was homogeneous (t_R 22.0 min, >85% purity; see Figure 7B); ESMS, calcd. for $C_{50}H_{66}N_{10}O_{11}S_2$ 1047.3 [(Cys^2-Cys^7) H-DPhe-Cys-Phe-DTrp(For)-Lys-Thr-Cys-Thr-oh]. Found: *m/z* 1048.8 $[M + H]^+$, 525.4 $[M + 2H^+/2]$.

Finally, removal of the For group of Trp was accomplished by treatment with piperidine–DMF (1:1, v/v, 0.4 mL) for 10 min at 25 °C with continuous stirring. The reaction was quenched with HOAc (0.8 mL), cooled in an ice-bath, and the solvent evaporated to dryness. The crude peptide showed one main peak by analytical HPLC (t_R 21.7 min, >75% purity; see Figure 7C); FABMS, calcd. for $C_{49}H_{66}N_{10}O_{10}S_2$ 1019.3 [(Cys^2-Cys^7] H-DPhe-Cys-Phe-DTrp-Lys-Thr-Cys-Thr-oh); Found: *m/z* 1019.2 $[M + H]^+$, 1041.2 $[M + Na]^+$.

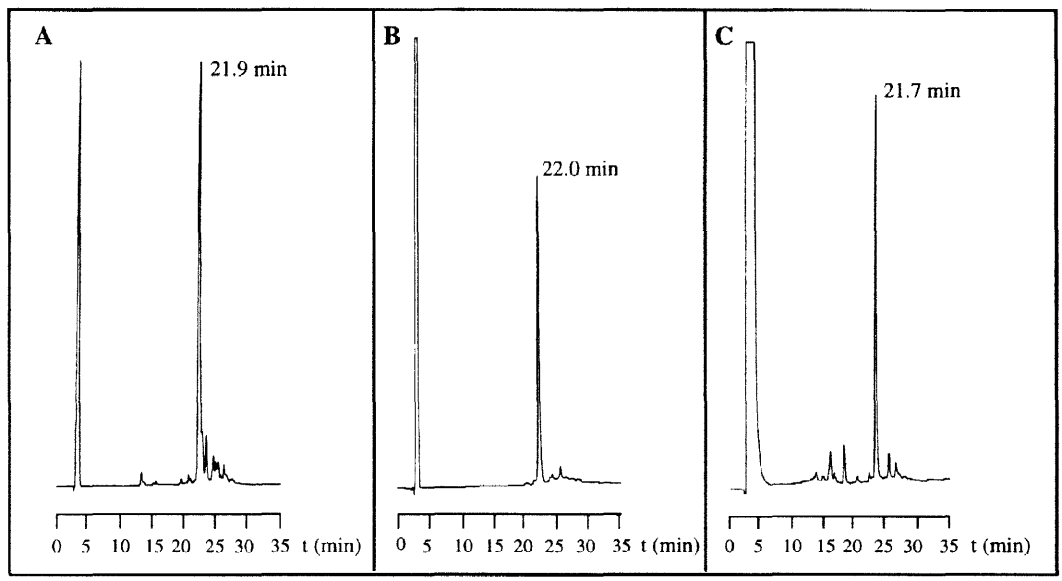


Figure 7. Analytical HPLC, following Conditions F as listed in General Procedures, of crude peptides related to (**9**). **A**: H-DPhe-Cys(Acm)-Phe-DTrp(For)-Lys-Thr-Cys(Acm)-Thr-oh; **B**: (Cys^2-Cys^7) H-DPhe-Cys-Phe-DTrp(For)-Lys-Thr-Cys-Thr-oh; C: (Cys^2-Cys^7) H-DPhe-Cys-Phe-DTrp-Lys-Thr-Cys-Thr-oh.

H-Ala-Asn-Lys-Gly-Phe-Leu-Glu-Glu-Val-Phe-o-CO-NH_2 (**10**) *(model of C-terminal carbamate peptide).* Preparation of Fmoc-Phe-o-CO-AM-Nle-PEG-PS. DSC (121 mg, 18 equiv) dissolved in CH_3CN (1.5 mL) and DMAP (6.4 mg, 2 equiv) dissolved in CH_2Cl_2 (0.3 mL) were added successively to a solution of Fmoc-Phe-oh (196 mg, 20 equiv) in CH_2Cl_2 (2

mL), and stirred at 25 °C for 4 h. The reaction mixture and DMAP (0.6 mg, 0.2 equiv) were added to the resin H-AM-Nle-PEG-PS and the suspension was continuously shaken under an Ar atmosphere at 25 °C for 12 h. Finally, the resin (Kaiser ninhydrin test was negative) was washed with DMF (5 x 0.5 min) and CH_2Cl_2 (5 x 0.5 min). H-AM-PEG-PS had previously been obtained by treatment of Fmoc-AM-Nle-PEG-PS (125 mg, 0.21 mmol/g) with piperidine-DMF (1:4, v/v, 3 x 1 min, 2 x 5 min), followed by washings with DMF (5 x 0.5 min) and CH_2Cl_2 (5 x 0.5 min).

Automated chain assembly was carried out in the $C \rightarrow N$ direction using an Abimed 422 PepSynthesizer according to a standard Fmoc/*t*Bu protocol. In this case, each Fmoc-protected amino acid (4 equiv) was activated with PyBOP (4 equiv) and NMM (8 equiv) in DMF for 45 min (systematic double coupling). Side-chain protection was provided by Trt (Asn), Boc (Lys) and O*t*Bu (Glu). The amino acid composition of the acid-hydrolyzed (3 h) peptide-resin was: Ala, 1.08; Asp, 1.06; Lys, 1.01; Gly, 1.01; Phe, 0.88; Leu, 1.01; Glu, 1.95; Val 0.79; H-Phe-oh, not detected. The fully protected peptide-resin was treated first with piperidine-DMF (1:4, v/v, 3 x 1 min, 2 x 5 min), washed with DMF (5 x 0.5 min), CH_2Cl_2 (5 x 0.5 min), and then cleaved with TFA-H_2O (19:1, v/v) at 25 °C for 1 h (62% cleavage yield). The filtrate from the cleavage reaction was collected, combined with TFA washes (2 x 2 mL) of the cleaved peptide-resin, and dried to give a material that showed a single major component by analytical HPLC (t_R 10.4 min, >85% purity; see Figure 8); FABMS (nitrobenzyl alcohol matrix), calcd. for $C_{55}H_{83}N_{13}O_{16}$: 1182.3. Found: ***m/z*** 1182.9 $[M + H]^+$, 1204.9 $[M + Na]^+$.

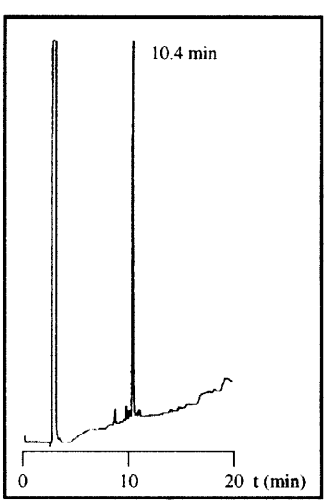


Figure 8. Analytical HPLC of crude peptide H-Ala-Asn-Lys-Gly-Phe-Leu-Glu-Glu-Val-Phe-o-CO-NH_2 (**10**). (Conditions B, as listed in General Procedures).

H-Tyr-Gly-Gly-Phe-o-CO-Leu-NH_2 (**11**) *(model of internal carbamate peptide).* Preparation of Fmoc-Phe-o-CO-Leu-PAL-Ile-Nle-PEG-PS. DSC (41 mg, 18 equiv) dissolved in CH_3CN (1.3 mL) and DMAP (9.9 mg, 9 equiv) dissolved in CH_2Cl_2 (0.3 mL) were added successively to a solution of Fmoc-Phe-oh (67 mg, 20 equiv) in CH_2Cl_2 (1.5 mL), and stirred for 4 h at 25 °C. The reaction mixture was added to the resin H-Leu-PAL-Ile-Nle-PEG-PS and the suspension was continuously shaken under an Ar atmosphere at 25 °C for 12 h. The resin,

which gave a negative Kaiser ninhydrin test, was washed with DMF (5 x 0.5 min) and CH_2Cl_2 (5 x 0.5 min). H-Leu-PAL-Ile-Nle-PEG-PS had previously been obtained by treatment of Fmoc-PAL-Ile-Nle-PEG-PS (50 mg, 0.009 mmol, 0.18 mmol/g) with piperidine–DMF (1:4, v/v, 3 x 1 min, 2 x 5 min), followed by DMF washes (5 x 0.5 min), coupling of Fmoc-Leu-OH (5 equiv) in the presence of HOBt (5 equiv) and DIPCDI (5 equiv) in DMF for 1 h at 25 °C, and washes with DMF (5 x 0.5 min).

Manual chain assembly was carried out in the $C \rightarrow N$ direction according to a standard Fmoc/*t*Bu protocol using Fmoc-AA-OH (5 equiv), HOBt (5 equiv), and DIPCDI (5 equiv) in DMF for 1 h per residue. Side-chain protection for Tyr was provided by *t*Bu. The amino acid composition of the acid-hydrolyzed (3 h) peptide-resin was: Gly, 1.95; Leu, 1.07; Tyr, 0.98; H-Phe-oh, not detected. Fmoc removal and cleavage with TFA–H_2O (19:1, v/v) at 25 °C for 90 min (80% cleavage yield), followed by the same work-up as for the peptide described above, gave the desired internal carbamate peptide, which was characterized by analytical HPLC (t_R 25.8 min, >95% purity; see Figure 9); MALDI-TOF MS, calcd. for $C_{29}H_{40}N_6O_7$: 584.7. Found: *m/z* 585.4 $[M + H]^+$.

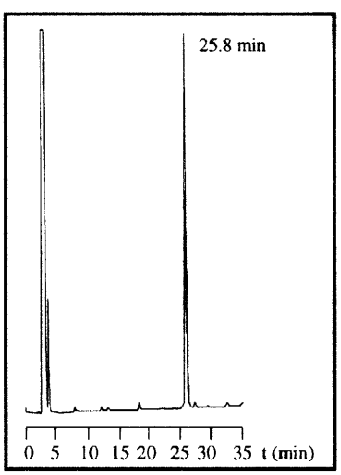


Figure 9. Analytical HPLC of crude peptide H-Tyr-Gly-Gly-Phe-o-CO-Leu-NH_2 (**11**). (Conditions C, as listed in General Procedures).

Acknowledgments. We thank Dr. Steven A. Kates (PerSeptive Biosystems, USA) for the generous gift of HOAt and PyAOP. This work was partially supported by CICYT (PB95-1131 and PB96-1490), Generalitat de Catalunya [Grup Consolidat (1995SGR 494) and Centre de Referència en Biotecnologia].

References and notes

[1]Portions of this work were reported in preliminary form: (a) Alsina, J.; Rabanal, F.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 9633–9636; (b) Alsina, J.; Chiva, C.; Ortiz, M.; Rabanal, F.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1997**, *38*, 883–886.

[2]The following abbreviations are used: AAA, amino acid analysis; AB, *p*-alkoxybenzyl; AM, *p*-[*R,S*]-α-[1-(9*H*-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxybenzyl]-phenoxyacetic acid; Acm, acetamidomethyl; Boc, *tert*-butyloxycarbonyl; BOP, benzotriazol-1-yl-*N*-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate; Bzl, benzyl; CDI, *N,N'*-carbonyldiimidazole; Chx, cyclohexyl; CIMS, chemical ionization mass spectrometry; ClTrt, chlorotrityl; 2-ClZ, 2-chlorobenzyloxycarbonyl; DIEA, *N,N*-diisopropylethylamine; DIPCDI, *N,N'*-diisopropylcarbodiimide; DMAP, *N,N*-dimethyl-4-aminopyridine; DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide; DSC, *N,N'*-disuccinimidyl carbonate; ESMS, electrospray mass spectrometry; FABMS, fast atom bombardment mass spectrometry; Fmoc, 9-fluorenylmethyloxycarbonyl; For, formyl; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBTU, *N*-[(1*H*-benzotriazol-1-yl) (dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HMFS, *N*-[(9-hydroxymethyl)-2-fluorenyl]succinamic acid; HMPP, 3-(4-hydroxymethyl)phenoxypropionic acid; HOAc, acetic acid; HOAt,

7-aza-1-hydroxybenzotriazole (3-hydroxy-3*H*-1,2,3-triazolo-[4,5-*b*]pyridine); HOBt, 1-hydroxybenzotriazole; HO–CH_2–PS, hydroxymethyl-co(polystyrene-divinylbenzene); H-Phe-oh, phenylalaninol; HPLC, high performance liquid chromatography; H-Thr-oh, threoninol; IRAA, "internal reference" amino acid; MALDI-TOF MS, matrix-assisted laser desorption ionization, time-of-flight mass spectroscopy; MBHA, *p*-methylbenzhydrylamine (derivatized polystyrene resin); MS, mass spectrometry; Nbb, nitrobenzamidobenzyl; NMM, *N*-methylmorpholine; PAC, *p*-alkoxybenzyl alcohol; PAL, 5-{[(4-amino)methyl]-3,5-dimethoxyphenoxy}valeric acid; PAM-resin, 4-(hydroxymethyl)phenylacetamido-resin; PEG-PS, polyethylene glycol-polystyrene (graft resin support); PPTS, pyridinium *p*-toluensulfonate; PS, copoly(styrene-1%-divinylbenzene) support; PyAOP, 7-aza-benzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)-phosphonium hexafluorophosphate; PyBOP, benzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)-phosphonium hexafluorophosphate; pyr, pyridine; SPPS, solid-phase peptide synthesis; Su, succinimidyl; *t*Bu, *tert*-butyl; TFA, trifluoroacetic acid; TFE, trifluoroethanol; THF, tetrahydrofuran; THP, tetrahydropyranyl; TMS, tetramethylsilane; Tos, tosyl.

[3](a) *Combinatorial Peptide and Nonpeptide Libraries*. Jung, G. Ed., VCH: Weinheim, 1996; (b) *Combinatorial Libraries. Synthesis, Screening and Application Potential*, Cortese, R. Ed., Walter de Gruyter & Co.: Berlin, 1996; (c) *Molecular Diversity and Combinatorial Chemistry. Libraries and Drug Discovery*, Chaiken, I.M.; Janda, K.D. Eds., ACS Books: Washington, 1996; (d) *Combinatorial Chemistry: Synthesis and Applications*, Wilson, S.R.; Czarnik, A.W. Eds., Wiley & Sons: New York, 1997; (e) *A Practical Guide to Combinatorial Chemistry*, DeWitt, S.H.; Czarnik, A.W. Eds., ACS Books: Washington, 1997.

[4](a) Merrifield, R.B. *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 799–810; (b) Fields, G.B.; Tian, Z.; Barany, G. In *Synthetic Peptides. A User's Guide*; Grant, G.A. Ed.; W. H. Freeman and Company: New York, 1992; pp 77–183; (c) Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Chemical Approaches to the Synthesis of Peptides and Proteins*; CRC: Boca Raton (FL), 1997.

[5]Letsinger, R.L.; Kornet, M.J. *J. Am. Chem. Soc.* **1963**, *85*, 3045–3046.

[6]Felix, A.M.; Merrifield, R.B. *J. Am. Chem. Soc.* **1970**, *92*, 1385–1391.

[7]Matsueda, R.; Maruyama, H.; Kitazawa, E.; Takahagi, H.; Mukaiyama, T. *Bull. Chem. Soc. Japan* **1973**, *46*, 3240–3247.

[8](a) *Oligonucleotide Synthesis. A Practical Approach.* Gait, M.J. Ed., IRL PRESS: Oxford (UK), 1985; (b) Aviño, A.; Güimil-García, R.; Díaz, A.; Albericio, F.; Eritja, R. *Nucleosides & Nucleotides* **1996**, *15*, 1871–1889; (c) Songster, M.F.; Barany, G. In *Methods in Enzymology, Solid-Phase Peptide Synthesis*; Fields, G.B. Ed., Academic Press: Orlando (FL), **1997**, *289*, 126–174.

[9](a) Ovchinnikov, Y.A.; Ivanov, V.T. In *The Proteins;* Neurath, H.; Hill, R.L. Eds., Academic Press: New York (NY), 1982, pp. 307–642; (b) Schmidt, U. *Pure & Appl. Chem.* **1986**, *58*, 295–301; (c) Rizo, J.; Gierasch, L.M. *Annu. Rev. Biochem.* **1992**, *61*, 387–418; (d) Giannis, A.; Kolter, T. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1244–1267; (e) Gante, J. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699–1720; (f) Liskamp, R.M.J. *Recl. Trav. Chim. Pays-Bas.* **1994**, *113*, 1–19.

[10](a) Kates, S.A.; Solé, N.A.; Albericio, F.; Barany, G. In *Peptides: Design, Synthesis and Biological Activity*; Basava, C.; Anantharamaiah, G.M. Eds., Birkhäuser: Boston (MA), 1994, pp. 39–58; (b) Blackburn, C.; Kates, S.A. In *Methods in Enzymology, Solid-Phase Peptide Synthesis*; Fields, G.B. Ed., Academic Press: Orlando (FL), **1997**, *289*, 175–198.

[11](a) Rovero, P.; Quartara, L.; Fabbri, G. *Tetrahedron Lett.* **1991**, *32*, 2639–2642; (b) McMurray, J.S. *Tetrahedron Lett.* **1991**, *32*, 7679–7682; (c) Trzeciak, A.; Bannwarth, W. *Tetrahedron Lett.* **1992**, *33*, 4557–4560; (d) Tromelin, A.; Fulachier, M-H.; Mourier, G.; Ménez, A. *Tetrahedron Lett.* **1992**, *33*, 5197–5200; (e) Kates, S.A.; Solé, N.A.; Johnson, C.R.; Hudson, D.; Barany, G.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 1549–1552; (f) Valero, M-L.; Giralt, E.; Andreu, D. *Tetrahedron Lett.* **1996**, *37*, 4229–4232.

[12]Guibé, F. *Tetrahedron*, **1998**, in press.

[13]Greene, T.W.; Wuts, P.G.M. In *Protective Groups in Organic Synthesis*; 2nd Ed., John Wiley & Sons, Inc., New York (NY), 1991.

[14]In recent years several carbamate resins have been described for the preparation of small molecule libraries and other peptide based polymers: (a) Burdick, D.J.; Struble, M.E.; Burnier, J.P. *Tetrahedron Lett.* **1993**, *34*, 2589–2592; (b) Hutchins, S.M.; Chapman, K.T. *Tetrahedron Lett.* **1994**, *35*, 4055–4058; (c) Hutchins, S.M.; Chapman, K.T. *Tetrahedron Lett.* **1995**, *36*, 2583–2586; (d) Kaljuste, K.; Undén, A. *Tetrahedron Lett.* **1995**, *36*, 9211–9214; (e) Zaragoza, F.; Vejle-Petersen, S. *Tetrahedron* **1996**, *52*, 10823–10826; (f) Dressman, B.A.; Spangle, L.A.; Kaldor, S.W. *Tetrahedron Lett.* **1996**, *37*, 937–940; (g) Kaljuste, K.; Undén, A. *Tetrahedron Lett.* **1996**, *37*, 3031–3034; (h) Gouilleux, L.; Fehrentz, J.A.; Winternitz, F.; Martinez, J. *Tetrahedron Lett.* **1996**, *37*, 7031–7034; (i) Marsh, I.A.; Smith, H.; Bradley, M. *Chem. Commun.* **1996**, pp. 941–942; (j) Ho, C.Y.; Kukla, M.J. *Tetrahedron Lett.* **1997**, *38*, 2799–2802; (k) Raju, B.; Kogan, T.P. *Tetrahedron Lett.* **1997**, *38*, 3373–3376. Work described in references (c) and (d) was based on similar approaches described previously. (l) Dixit, D.M.; Leznoff, C.C. *Chem. Commun.* **1977**, pp. 798–799; (m) Dixit, D.M.; Leznoff, C.C. *Isr. J. Chem.* **1978**, *17*, 248–252; (n) Breiphol, G.; Knolle, J.; Geiger, R. *Tetrahedron Lett.* **1987**, *28*, 5647–5650.

[15]Reiner, A. USA Patent 5030738.

[16]Staab, H.A. *Ann.* **1957**, *609*, 75–83.

[17]Ogura, H.; Kobayashi, T.; Shimizu, K.; Kawabe, K.; Takeda, K. *Tetrahedron Lett.* **1979**, *20*, 4745–4746.

[18]Takeda, K.; Ogura, H. *Synthetic Comm.* **1982**, *12*, 213–217.

[19]Ghosh, A.K.; Duong, T.T.; McKee, S.P.; Thompson, W.J. *Tetrahedron Lett.* **1992**, *33*, 2781–2784.

[20]Albericio, F.; Barany, G. *Int. J. Peptide Protein Res.* **1985**, *26*, 92–97. This is a Wang type resin (Wang, S-S. *J. Am. Chem. Soc.*

1973, *95*, 1328–1333) based linker and it is also known as PAC- or AB-linker.

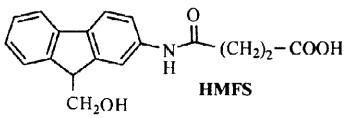
[21](a) Atherton, E.; Clive, D.L.; Sheppard, R.C. *J. Am. Chem. Soc.* **1975**, *97*, 6584–6585; (b) Matsueda, G.R.; Haber, E. *Anal. Biochem.* **1980**, *104*, 215–227; (c) Albericio, F.; Barany, G. *Int. J. Pept. Protein Res.* **1993**, *41*, 307–312.

[22]Yields were calculated taking as reference the incorporation of Fmoc-Leu-OH (10 equiv) on HMPP-Phe-MBHA-resin using a DIPCDI (10 equiv)-DMAP (1 equiv) coupling for 1 h at 25 °C. The coupling was repeated a further two times and the AAA gave Leu, 0.84; Phe, 1.00. Thus, a ratio of 0.84 for Leu/Phe was considered a quantitative yield. For Nbb-resins, the incorporation of Fmoc-Leu-OH took place with identical results.

[23]This can be interpreted by a Lossen rearrangement similar to the described recently by Zalipsky, S. Chem. Commun. **1998**, pp. 69-70.

[24]Recently, Hauske and Dorff have used CDI for the preparation of carbamate based HMPP-resins. The first step was carried out with CDI in THF for 2–4 h at 25 °C. The incorporation of the amino component was carried out in THF–NMP (1:1) for 4 h at 60 °C in the presence of excess of NMM. Hauske, J.R.; Dorff, P. *Tetrahedron Lett.* **1995**, *36*, 1589–1592.

[25]A similar strategy has been applied in the preparation of carbamate resins derived from the HMFS handle (Rabanal, F.; Giralt, E.; Albericio, F. *Tetrahedron Lett.* **1992**, *33*, 1775–1778; *Tetrahedron* **1995**, *51*, 1449–1458).



HMFS

For resins derived from this handle, control of both reactions is more critical since the presence of a good leaving group provides a favorable β-elimination side-reaction. Application of the identical conditions used for HMPP based resins led to a very low level of substitution (5%). Superior results were obtained when pyridinium hydrochloride (10 equiv) was used in place of DMAP and the reaction proceeded in DMF for 24 h at 25 °C. For the second reaction, the amino derivative (10 equiv) was dissolved in DMF and added to resin in the presence of DIEA (10 equiv) for 4 h at 25 °C to give an overall yield of 75%.

[26]For a review on coupling methods, see: Albericio, F.; Carpino, L.A. In *Methods in Enzymology, Solid-Phase Peptide Synthesis*; Fields, G.B. Ed., Academic Press: Orlando (FL), **1997**, *289*, 104–126.

[27](a) Gausepohl, H.; Pieles, U.; Frank, R.W. In *Peptides - Chemistry and Biology: Proceedings of the 12th American Peptide Symposium*. Smith, J.A.; Rivier, J.E. Ed.; ESCOM: Leiden (The Netherlands), 1992, pp. 523–524; (b) Story, S.C.; Aldrich, J.V. *Int. J. Peptide Protein Res.* **1994**, *43*, 292–296; (c) Arttamangkul, S.; Arbogast, B.; Barofsky, D.; Aldrich, J.V. *Lett. Pept. Sci.* **1996**, *3*, 357–370; (d) Albericio, F.; Frieden, A.; del Fresno, M.; Royo, M.; Alsina, J.; Jensen, K.J.; Kates, S.A.; Barany, G. In *Innovation and Perspectives in Solid Phase Synthesis & Combinatorial Chemical Libraries: Biomedical & Biological Applications*, Epton, R. Ed.; Mayflower Scientific: Kingswinford (United Kingdom), 1998, in press.

[28]Brady, S.F.; Paleveda, W.J.; Arison, B.H.; Freidinger, R.M.; Nutt, R.F.; Veber, D.F. In *Peptides, Proceedings of the 8th American Peptide Symposium*; Hruby, V.J.; Rich, D. Eds.; Pierce: Rockford, 1983, pp. 127–130.

[29]Consden, R.J.; Gordon, A.H.; Martin, A.J.P.; Synge, R.D.M. *Biochem. J.* **1947**, *28*, 596–602.

[30]Adams, S.; Albericio, F.; Alsina, J.; Smith, E.R.; Humphreys, R.E. *Arzneimittel-Forschung Drug Discovery* **1997**, *47(II)*, 1069–1077.

[31]This result could have been anticipated because the macrolactamization step is favoured by the presence of certain residues in the sequence, such as Gly, *N*-alkyl- and/or D- amino acids.

[32](a) Zalipsky, S.; Chang, J.L.; Albericio, F.; Barany, G. *Reactive Polym.* **1994**, *22*, 243–258; (b) Barany, G.; Albericio, F.; Kates, S.A.; Kempe, M. In *ACS Volume on Chemistry and Biological Applications of Polyethylene Glycol*, Harris, J.M.; Zalipsky, S. Eds.; ACS Books: Washington, DC, 1997, pp. 239–264.

[33]This instability in the presence of bases could be one of the reasons that longer reaction times did not improve the anchoring yield, because the protected amino acid linked on the resin can be release over the time in presence of DMAP.

[34](a) Mitchell, A.R.; Erickson, B.W.; Ryabtsev, M.N.; Hodges, R.S.; Merrifield, R.B. *J. Am. Chem. Soc.* **1976**, *98*, 7357–7362. (b) Mitchell, A.R.; Kent, S.B.H.; Engelhard, M.; Merrifield, R.B. *J. Org. Chem.* **1978**, *43*, 2845–2852.

[35](a) Rich, D.H.; Gurwara, S.K. *J. Am. Chem. Soc.* **1975**, *97*, 1575–1579; (b) Giralt, E.; Albericio, F.; Pedroso, E.; Granier, C.; Van Rietschoten, J. *Tetrahedron* **1982**, *38*, 1193–1208; (c) Barany, G.; Albericio, F. *J. Am. Chem. Soc.* **1985**, *107*, 4936–4942; (d) Lloyd-Williams, P.; Gairí, M.; Albericio, F.; Giralt, E. *Tetrahedron* **1991**, *47*, 9867–9880.

[36]These results were similar those obtained for the anchoring of the Fmoc-derivatives of Ser and Tyr onto HMPP-resins.

[37]Wang, S-S. *J. Org. Chem.* **1975**, *40*, 1235–1239.

[38](a) Mazur, S.; Jayalekshmy, P.J. *J. Am. Chem. Soc.* **1978**, *101*, 677–683; (b) Barany, G.; Merrifield, R.B. In *The Peptides* Vol 2; Gross, E.; Meienhofer, J. Eds.; Academic Press: New York (NY), 1979; pp.1–284.

[39]Nishino, N.; Xu, M.; Mihara, H.; Fujimoto, T.; Ueno, Y.; Kumagai, H. *Tetrahedron Lett.* **1992**, *33*, 1479–1482.

[40]Plaué, S. *Int. J. Peptide Protein Res.* **1990**, *35*, 510–517.

[41]Ösapay, G.; Profit, A.; Taylor, J.W. *Tetrahedron Lett.* **1990**, *31*, 6121–6124.

[42]Leznoff, C.C.; Wong, J.Y. *Can. J. Chem.* **1972**, *50*, 2892–2893.

[43](a) Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Peptide Protein Res.* **1991**, *37*, 513–520; (b) Wenschuh, H.; Beyermann, M.; Haber, H.; Seydel, J.K.; Krause, E.; Bienert, M.; Carpino, L.A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 405–410.
[44](a) Thompson, L.A.; Ellman, J.A. *Tetrahedron Lett.* **1994**, *35*, 9333–9336; (b) Liu, G.; Ellman, J.A. *J. Org. Chem.* **1995**, *60*, 7712–7713.
[45](a) Swistok, J.; Tilley, J.W.; Danho, W.; Wagner, R.; Mulkerins, K. *Tetrahedron Lett.* **1989**, *30*, 5045–5048; (b) Neugebauer, W.; Escher, E. *Helv. Chim. Acta* **1989**, *72*, 1319–1323; (c) Romanovskis, P.; Spatola, A.F. In *Peptides 1996. Proceedings of the 24th European Peptide Symposium* (Ramage, R.; Epton, R. eds.) Mayflower Scientific Ltd.: England (UK), 1997, in press.
[46](a) Farrall, M.J.; Fréchet, J.M.J. *J. Org. Chem.* **1976**, *41*, 3877–3882; (b) Chan, T-H.; Huang, W-Q. *J. Chem. Soc., Chem. Commun.* **1985**, 909–911.
[47](a) Stewart, J.M.; Morris, D.H. USA Patent 4254023, 1981; (b) Stewart, J.M.; Morris, D.H.; Chipkin, R.E. USA Patent 4254024, 1981; (c) Mergler, M.; Nyfeler, R. In *Peptides:Chemistry and Biology, Proceedings of the 22nd European Peptide Symposium,* Schneider, C.H.; Eberle, A.N, eds., ESCOM: Leiden (The Netherlands), 1993, pp. 177–178.
[48]Richter, L.S.; Gadek, T.R. *Tetrahedron Lett.* **1994**, *35*, 4705–4706.
[49]Pless, J. *Metabolism* **1992**, *41*, 5–6.
[50](a) García-Echeverría, C.; Albericio, F.; Pons, M.; Barany, G.; Giralt, E. *Tetrahedron Lett.* **1989**, *30*, 2441–2444; (b) Albericio, F.; Hammer, R.P.; García-Echeverría, C.; Molins, M.A.; Chang, J.L.; Munson, M.; Pons, M.; Giralt, E.; Barany, G. *Int. J. Peptide Protein Res.* **1991**, *37*, 402–413.
[51]The purity of intermediates was checked at different stages of the synthetic process (see Figure 7).
[52](a) Cho, C.Y.; Moran, E.J.; Cherry, S.R.; Stephans, J.C.; Fodor, S.P.A.; Adams, C.L.; Sundaram, A.; Jacobs, J.W.; Schultz, P.G. *Science* **1993**, *261*, 1303–1305; (b) Moran, E.J.; Wilson, T.E.; Cho, C.Y.; Cherry, S.R.; Schultz, P.G. *Biopolymers (Peptide Science)* **1995**, *37*, 213–219; (c) Paikoff, S.J.; Wilson, T.E.; Cho, C.Y.; Schultz, P.G. *Tetrahedron Lett.* **1996**, *37*, 5653–5656.
[53]Since our work was completed, other syntheses of several oligocarbamate peptides have been carried out. Thus, Jung *et al.* have described their preparation using either *N*-Fmoc amino benzotriazolyl carbonates previously isolated (Wiesmuller, K-H.; Teufel, B.; Brock, R.; Fruchtel, J.; Warrass, R.; Jung, G.; Walden, P. In *Peptides: Chemistry, Structure and Biology, Proceedings of the 14th American Peptide Symposium*, Kaumaya, P.T.P.; Hodges, R.S. Eds., Mayflower Scientific Ltd, Kingswinford, UK, 1996, pp 305–306) or prepared *in situ* (Warrass, R.; Walden, P.; Wiesmuller, K-H.; Jung, G. *Lett. Pept. Sci.* **1998**, *5*, in press). Furthermore, Halstrøm has synthesized, in solution, tripeptides containing a motif of carbamate from *N*-Fmoc amino succinimidyl carbonates previously isolated using our approach (Halstrøm, J.B., personal communication).
[54](a) Lloyd-Williams, P.; Jou, G.; Albericio, F.; Giralt. E. *Tetrahedron Lett.* **1991**, *32*, 4207–4210. (b) Kates, S.A.; Daniels, S.B.; Albericio, F. *Anal. Biochem.* **1993**, *212*, 303–310.
[55]Ten Kortenaar, P.B.W.; van Dijk, B.G.; Peeters, J.M.; Raaben, B.J.; Adams, P.J.H.M.; Tesser, G.I. *Int. J. Peptide Protein Res.* **1986**, *27*, 398–400.
[56]Kaiser, E.; Colescott, R.L.; Bossinger, C.D.; Cook, P.I. *Anal. Biochem.* **1970**, *34*, 595–598.
[57]Experimental protocols corresponding to syntheses of peptides **2** and **3** have been described elsewhere: Andreu, D.; Ruiz, S.; Carreño, C.; Alsina, J.; Albericio, F.; Jiménez, M.A.; de la Figuera, N.; Herranz, R.; García-López, M.T.; González-Muñiz, R. *J. Am. Chem. Soc.* **1997**, *119*, 10579–10586.
[58](a) Carpino, L.A. *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398; (b) Carpino, L.A.; El-Faham, A.; Minor, C.A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, pp. 201–203.
[59]These washing were carried out to remove traces of Pd (see ref. 54b).
[60]Albericio, F.; Cases, M.; Alsina, J.; Triolo, S.A.; Carpino, L.A.; Kates, S.A. *Tetrahedron Lett.* **1997**, *38*, 4853–4856.
[61]Ser and Tyr are partially destroyed during acid hydrolysis.