Solid-Phase Syntheses of Peptoids using Fmoc-Protected *N*-Substituted Glycines: The Synthesis of (Retro)Peptoids of Leu-Enkephalin and Substance P

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Abstract: A particularly interesting class of oligomeric peptidomimetics is formed by the peptoids, which consist of *N*-substituted glycine residues. A solid-phase synthesis method for peptoids is presented in which these residues are introduced using their Fmoc derivatives. This "monomer" method allowed the monitored synthesis of relatively large quantities of pure peptoids as well as the translation of, in principle, any peptide into the corresponding peptoid. The required Fmoc-substituted glycines were accessible by convenient synthesis, and a number of monomers including those containing side chains with functional groups have been synthesized. The use of Fmoc monomers also allowed implementation of a solid-phase synthesis protocol on a commercial peptide synthesizer. The method was exemplified by the solid-phase syntheses of the (retro)peptoids of Leu-enkephalin and substance P. Mass spectrometric studies of (retro)peptoids were essential for their characterization, and the presence of the B- and Y″- type ions allows sequence analysis. Substance P (retro)-peptoids were biologically active. HPLC analysis showed an increased hydrophobicity, and pepsin treatment resulted in greatly reduced degradation compared with the corresponding peptide.

Keywords: mass spectrometry · peptidomimetics · peptoids · solid-phase synthesis · substance P

Introduction

Peptides and proteins play a crucial role in virtually all biological processes. In fact it is difficult, if not impossible, to name any biological process which does not involve these biopolymers. Peptides especially, which are usually smaller than proteins, are often important starting structures for the development of potential therapeutic agents.[1]

Since the application of peptides as drugs has been hampered, for example, by poor bioavailability and biodegradation by proteases, the development of peptidomimetics aiming at the remedy of these disadvantages while retaining biological activity has been spectacular. The oligomeric peptidomimetics are particularly interesting in this respect, since they provide access to an enormous molecular diversity by variation of the building blocks. The first and perhaps most well-known class of oligomeric peptidomimetics is composed of the peptoids, which consist of *N*-substituted glycine derivatives.[2, 3] The structural differences between a peptide and a peptoid are a consequence of the use of these building blocks and are readily apparent from the general structures of a peptide, peptoid, and retropeptoid depicted in Figure 1. Most striking is the absence of chirality in the *N*-substituted glycine residues, with the exception of proline. In a manner of speaking, one might consider peptoids as peptide mimics in which the side chain has been shifted from the chiral α-carbon atom in a peptide to the achiral nitrogen. Undoubtedly, this will have consequences for the biological activity of a (retro)peptoid compared with that of the parent peptide, but predictions as to the exact nature of these consequences will be difficult. For evaluation, several (retro)peptoid peptidomimetics of biologically active peptides have to be synthesized. The preparation of several biologically active peptoids has indeed shown that they are attractive and promising peptidomimetics in this respect.[2, 4, 5]

In contrast to the biological activity, direct consequences of the physical and chemical properties of peptoids can be more safely predicted. The absence of an amide N–H, which can act as a hydrogen-bond donor, is likely to decrease the solubility in a solvent capable of hydrogen-bond formation and increase the solubility in a more apolar solvent. The

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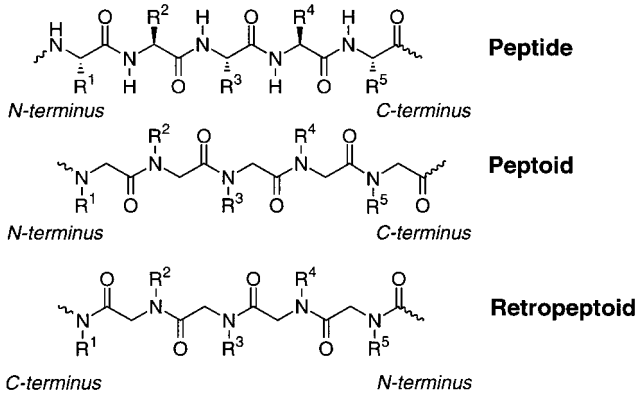
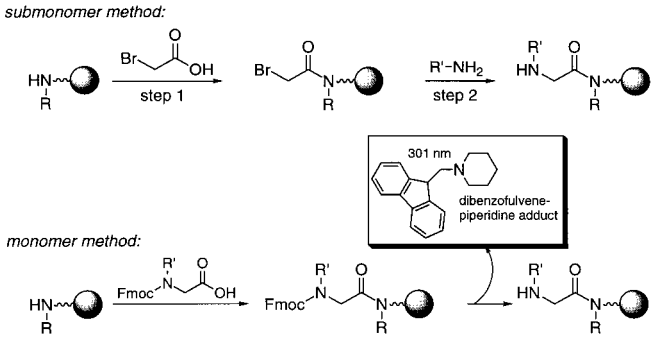


Figure 1. General structures of a peptide, peptoid, and retropeptoid.



Scheme 1. Submonomer vs. monomer approach to the synthesis of peptoids.

presence of tertiary amide bonds, which are also present in proline-containing peptides, will result in the presence of (many) *cis*/*trans* rotamers due to rotation about each tertiary amide bond in a peptoid, thereby severely complicating the NMR spectra.[6]

Finally, it is also realistic to assume that peptoids, because of the absence of a recurrent peptide–amide bond, will be less sensitive to proteolytic degradation. Indeed, this has been proven to be the case in the examples so far studied.[7]

As part of a program to study the structural characteristics (by X-ray, NMR, MS, and computer-assisted molecular modeling) as well as the biological properties of peptoids,[8, 9] we are interested in the synthesis of relatively large quantities of pure peptoids. In addition, we want to be able to translate in principle any peptide into the corresponding peptoid using a fully automated peptide synthesizer, with the ultimate goal of creating combinatorial oligomeric libraries for structure–activity relationship studies and drug discovery.

Results and Discussion

Zuckermann and colleagues have described the submonomer approach for the synthesis of peptoids (Scheme 1).[4a] Each cycle of monomer addition consists of an acylation step (step 1; backbone formation) and a nucleophilic displacement step (step 2; side-chain introduction). This method is very attractive because the separate monomers for building the peptoid do not have to be synthesized, and in principle any arbitrary amine can be employed in the synthesis of peptoids. While this approach may be the method of choice when small quantities of peptoids present in large libraries of relatively small peptoids (e.g. tripeptoids) are needed, it suffers from the disadvantage that large excesses of reagents (e.g. the amines) have to be used and that completion of the acylation and substitution reactions cannot be determined;[4a, b] this is essential for preparation of larger peptoids of reasonable purity.

On the basis of present-day peptide synthesis in which Fmoc-protected amino acids are often used,[10] employing Fmoc-protected *N*-substituted glycine derivatives seems a very attractive approach for synthesizing peptoids both in solution and on the solid phase (Scheme 1). With this monomer approach, which is analogous to peptide synthesis, considerable quantities of pure and relatively large peptoids should be accessible while progress of their synthesis on the solid phase can be monitored by quantifying the dibenzofulvene adduct obtained after cleavage of the Fmoc group (Scheme 1).[10] A both practical and important advantage of using the monomer approach is the easy implementation of the synthesis protocol for peptides on commercial automated peptide synthesizers. It is also possible to prepare peptide–peptoid hybrids as well as complete peptoids on these apparatus by means of essentially the same synthesis protocol, but with Fmoc-peptoid monomers and a more powerful coupling agent.

Our targets were the syntheses of both the peptoids (**2** and **5**) and retropeptoids (**3** and **6**) of Leu-enkephalin (**1**) and substance P (**4**; Figure 2). Leu-enkephalin (YGGFL) is representative of the endogenous opioid peptides, which act like opiates in biological systems. They display high affinity to and moderate selectivity for δ-opioid receptors.[11] The neuropeptide substance P, a member of the tachykinin family, functions as a neurotransmitter or neuromodulator in the central and peripheral nervous systems. Substance P (SP) is generally associated with physiological events leading to pain and inflammation.[12] These peptides were chosen in view of our interest in neuropeptides. We considered the relatively small Leu-enkephalin as the try-out sequence of our solid-phase synthesis method for the preparation of a (retro)peptoid. In contrast, the larger and more complicated sequence of Substance P was a particular challenge for translation to the corresponding (retro)peptoid peptidomimetics because it contains a considerable number of amino acids with side chains containing a functional group.

The required *N*-substituted glycine derivatives, denoted as peptoid monomers, for the solid-phase synthesis of Leu-enkephalin were NTyr, NPhe, and NLeu.[13] The syntheses of NPhe and NLeu as well as other peptoid monomers containing nonfunctional side chains are straightforward and are shown in Scheme 2. The amines which have to be used in the substitution reaction, involving ethyl bromoacetate, are often commercially available. Thus, appropriate amines **7a** and **7b** were alkylated with ethyl bromoacetate **8** to afford the *N*-substituted glycine ethyl esters **9a** and **9b**. Neither dialkylation nor aminolysis of the ester in the substitution reaction was observed. The use of ethyl bromoacetate **8** instead of the

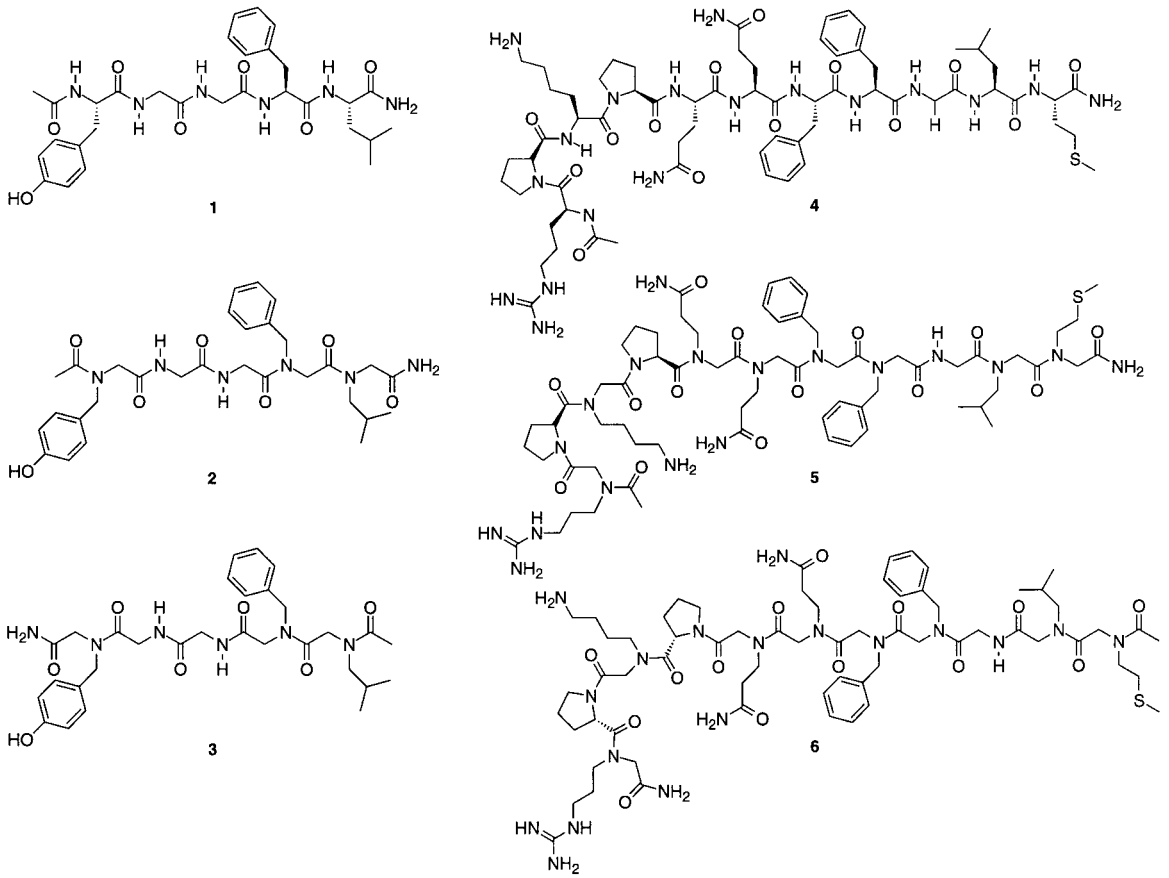
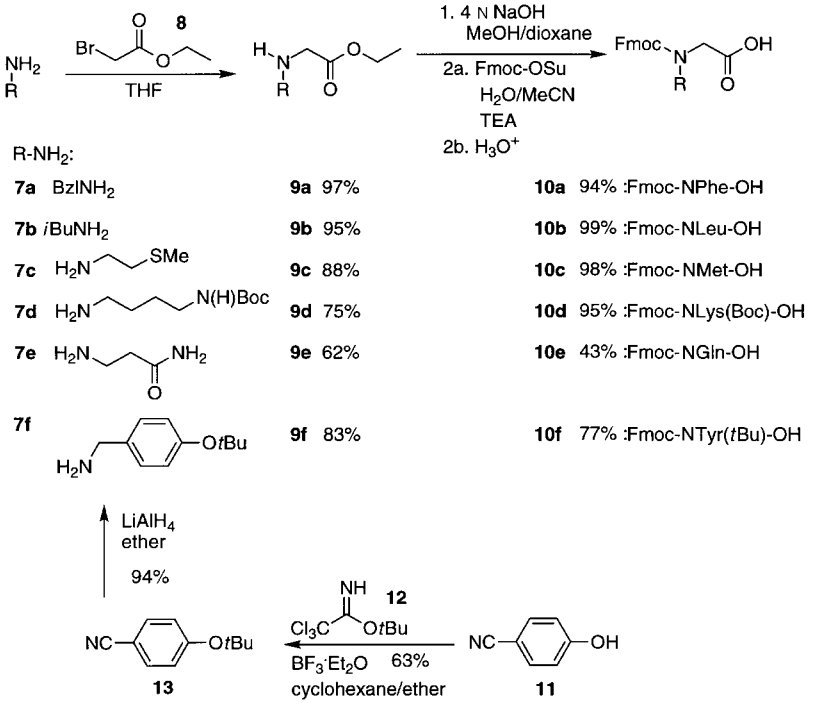


Figure 2. Acetylated Leu-enkephalin amide (**1**), its corresponding peptoid **2**, and retropeptoid **3**; acetylated substance P (**4**), its corresponding peptoid **5**, and retropeptoid **6**.

free acid allowed easy purification—if necessary—by column chromatography. Subsequently, esters **9a**,**b** were saponified with Tesser's base.[14] The amino groups of the resulting sodium salts were protected with a Fmoc group to provide the peptoid monomers **10a**,**b** required for the syntheses of the peptoid and retropeptoid of Leu-enkephalin.[15] However, the amine **7f** necessary for preparation of NTyr had to be synthesized (Scheme 2). Starting from 4-hydroxybenzonitrile **11** the OH-function was protected as a *tert*-butyl ether using *tert*-butyl trichloroacetimidate **12**,[16] after which the nitrile group was reduced to the desired benzylamine **7f**. Substitution, followed by saponification, and Fmoc-group attachment gave the NTyr peptoid monomer **10f** used in the solid-phase protocol.

In an earlier paper we showed that synthesis of peptoids in solution went well with PyBroP as a coupling reagent.[5a] Therefore, this coupling reagent was also used in the solid-phase syntheses of both the peptoid **2** and retropeptoid **3** of Leu-enkephalin **1** (Scheme 3). Starting with TentaGel SRAM resin, containing the acid-labile Rink-linker to be used for the preparation of a C-terminal amide,[17, 18] the peptoid was assembled in six consecutive Fmoc-cleavage-coupling cycles, the cleavage step of which could be monitored by UV. A more or less constant high level of Fmoc



Scheme 2. Preparation of monomers required for the syntheses of (retro)peptoids of Leu-enkephalin and substance P.

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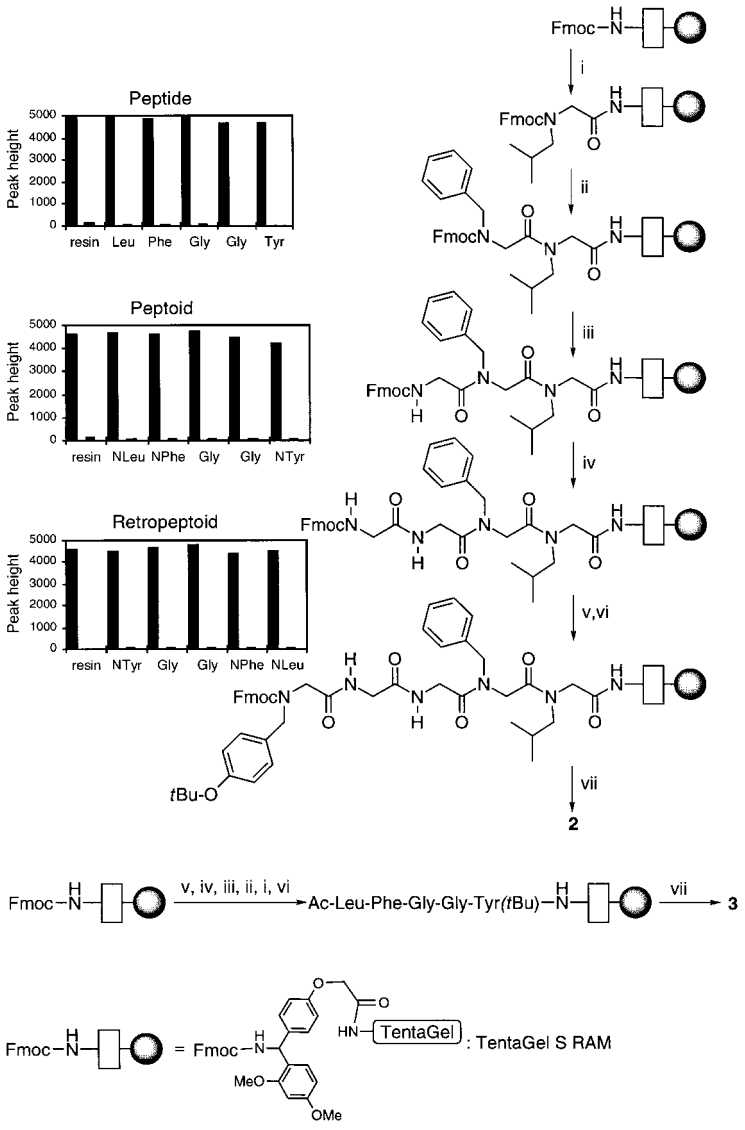
cleavage is a good indication for high-yielding coupling steps. These are shown in Scheme 3, indicating that the solid-phase peptoid synthesis went very well. For the purpose of comparison of mass spectra, the retropeptoid **3** was also synthesized starting from the peptoid monomer on the N-terminus of the peptoid **2** just synthesized. Peptoid **2** and retropeptoid **3** were obtained after deprotection and cleavage from the resin with TFA in the presence of water and triisopropylsilane (TIS). The mass spectra of both the peptoid **2** and the retropeptoid **3** as well as the parent peptide **1** have been previously discussed.[8a] Mass spectral studies are essential in the characterization of peptoids, since interpretation of NMR spectra is severely hampered by the presence of many conformations owing to rotation about the tertiary amide bonds in (retro)peptoids.



Scheme 3. Solid-phase synthesis of the peptoid **2** and retropeptoid **3** of Leu-enkephalin (**1**) and the solid-phase synthesis monitoring profiles. i) a) piperidine, b) Fmoc-NLeu-OH **10b**, PyBroP, DiPEA; ii) a) piperidine, b) Fmoc-NPhe-OH **10a**, PyBroP, DiPEA; iii) a) piperidine, b) Fmoc-Gly-OH, PyBroP, DiPEA; iv) a) piperidine, b) Fmoc-Gly-OH, PyBroP, DiPEA; v) a) piperidine, b) Fmoc-NTyr(*t*Bu)-OH (**10f**), PyBroP, DiPEA; vi) a) piperidine, b) Ac_2O, DiPEA, HOBt; vii) TFA/H_2O/TIS, 95/2.5/2.5 (*v*/*v*).

Having succeeded in the syntheses of both the peptoid **2** and retropeptoid **3** of Leu-enkephalin **1**, we moved on to the syntheses of both the peptoid **5** and retropeptoid **6** of Substance P (**4**). With regard to this, the synthesis of the peptoid monomer corresponding to the amino acid Arg was a particular challenge in view of the introduction of the guanidyl moiety.

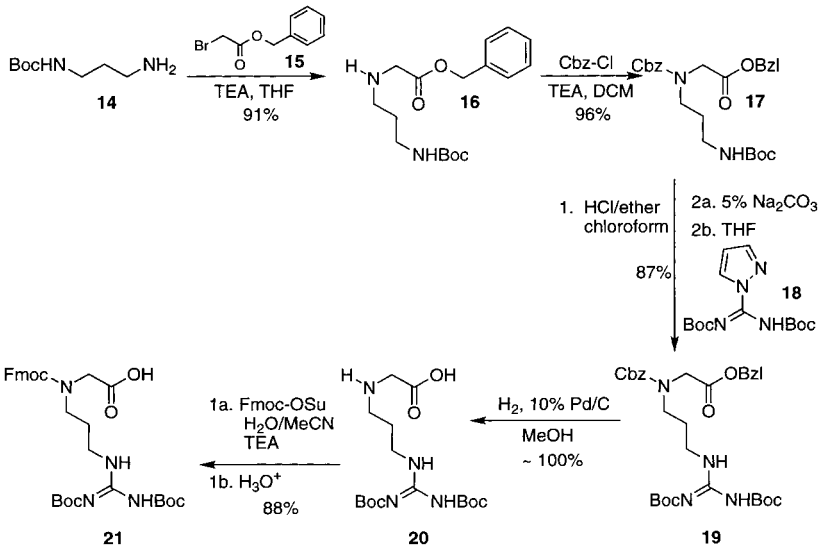
In principle, it should have been possible to prepare Fmoc-NArg(Boc)$_2$-OH (**21**) in a convenient way by deprotection of the side chain of Fmoc-NOrn(Boc)-OH and guanylation with *N*,*N*′-bis(Boc)-1-guanylpyrazole (**18**).[19] Unfortunately, the yield of this synthetic route was quite low (ca. 30%) and purification by column chromatography was tedious. Therefore, Fmoc-NArg(Boc)$_2$-OH (**21**) was synthesized starting from H-NOrn(Boc)-OBzl (**16**), depicted in Scheme 4.

H-NOrn(Boc)-OBzl (**16**) was prepared by treatment of *N*-Boc-1,3-diaminopropane (**14**) with benzyl bromoacetate (**15**) in the presence of TEA in THF. Benzyl bromoacetate was used instead of ethyl bromoacetate (**8**) because the α-amino group and the carboxyl group can then be deprotected simultaneously by hydrogenolysis. Treatment of **16** with benzyl chloroformate gave Cbz-NOrn(Boc)-OBzl (**17**). Removal of the Boc group of **17** with a saturated solution of HCl in ether and subsequent guanidylation of the free amine group with **18** afforded Cbz-NArg(Boc)$_2$-OBzl (**19**).[20] Removal of both the Cbz group and benzyl ester in **19** by hydrogenolysis over 10% Pd/C gave H-NArg(Boc)$_2$-OH (**20**). Subsequent protection of the α-amino group with Fmoc–OSu in the presence of TEA provided the protected crystalline Fmoc-NArg(Boc)$_2$-OH (**21**) in 88% yield.

The syntheses of the other required functional-group containing monomers of Substance P did not pose any particular problems (Scheme 2). The previously used Fmoc-NMet-OH (**10c**) was accessible using 2-amino ethyl methyl sulfide (**7c**).[5a, 21] Fmoc-NLys(Boc)-OH (**10d**) was accessible using *N*-Boc-1,4-diaminobutane (**7d**), and Fmoc-NGln-OH (**10e**) was accessible in a substitution reaction with the amide of β-alanine (**7e**) (Scheme 2).

After the preparation of all the required peptoid monomers, the stage was now set for the preparation of both the peptoid **5** and retropeptoid **6** of Substance P. For the purpose of comparison of mass spectra, and in order to illustrate that the solid-phase synthesis of (retro)peptoids is virtually identical to that of peptides, the solid-phase synthesis of Substance P is also shown.

Coupling and deprotection cycles for the solid-phase synthesis of (retro)peptoids are virtually identical to those for peptides and therefore can be implemented on a commercial solid-phase peptide synthesizer. For synthesis of the substance P peptide **4** (Scheme 5), the first monomer Fmoc-Met-OH was attached to the same resin used in the synthesis of the (retro)peptoid of Leu-enkephalin. After the last coupling cycle, the N-terminal Fmoc group was removed by a piperidine solution and the N-terminus



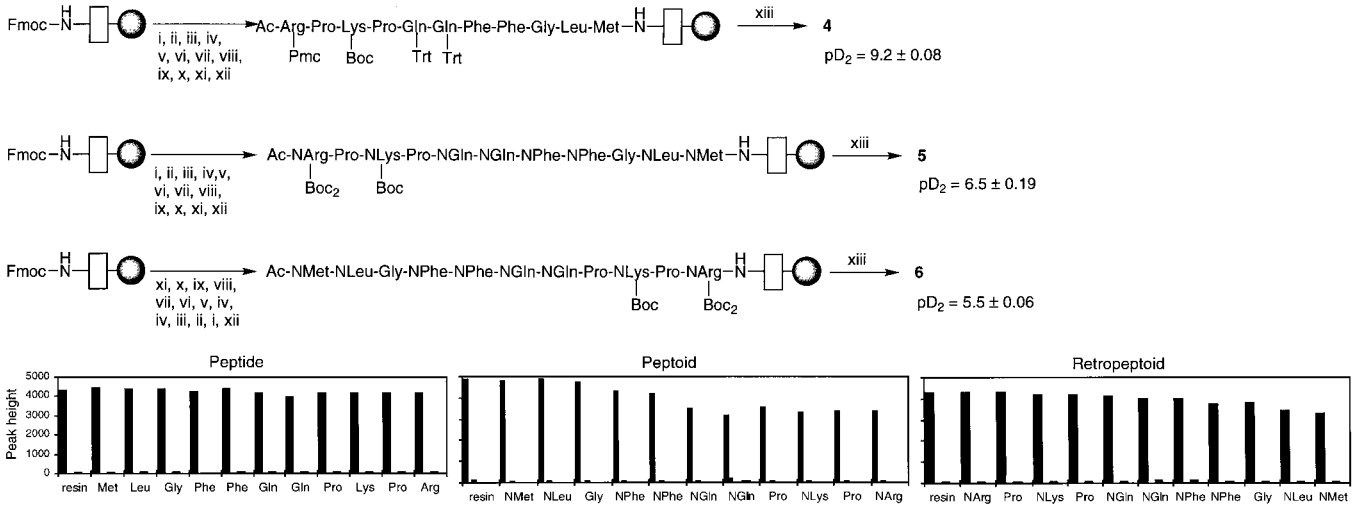
Scheme 4. Synthesis of peptoid monomer Fmoc-NArg(Boc)$_2$-OH (**22**) required for the solid-phase syntheses of the (retro)peptoids of substance P.

was acetylated.[22] Cleavage from the resin and simultaneous removal of the protecting groups using TFA with thioanisole, H_2O, ethanedithiol (EDT), and TIS as scavengers gave, after purification by Sephadex LH-20 and preparative HPLC, pure substance P (**4**). Peptoid **5** and retropeptoid **6** (Scheme 5), attached to the solid support, were prepared according to a protocol essentially identical to that described above for the substance P peptide, except that PyBroP was used as a coupling reagent instead of BOP—analogous to the synthesis of the (retro)peptoid of Leu-enkephalin. After deprotection, cleavage, and purification, 104 mg (26%) of peptoid **5** and 132 mg (37%) of retropeptoid **6** (Scheme 5) were obtained.

The structures of peptoid **5** and retropeptoid **6** of substance P were ascertained by FAB mass spectrometry (Figure 3).[8c]

Both the mass spectra of the peptide and peptoid exhibit B- and Y″-type sequence ions at identical *m/z* values. The presence of *N*-substituted glycine residues in a peptoid is further apparent from the presence of *N*-substituted immonium ions, which differ significantly in their fragmentation behavior from the corresponding immonium ions observed in the spectra of common oligopeptides.[8] Loss of the CH_2=NH imine molecule is the dominant fragmentation reaction in the collision induced dissociation (CID) spectra of all peptoid immonium ions investigated in this study. Thus, the $[M+H]^+$ CID spectra of the investigated peptoid peptidomimetics exhibit characteristic B- and Y″-type ions, which allow sequence analysis.

The biological activity of substance P (**4**) and its corresponding peptoid **5** and retropeptoid **6** was evaluated on isolated mouse trachea. It was found that **5** and **6** are agonists of substance P, displaying their activity in micro- to submicromolar concentrations (Scheme 5)![23] This is remarkable in view of the absence of chirality in most of the building blocks of the (retro)peptoid. The efficacy of the peptoid **5** and retropeptoid **6** of substance P is probably even greater since these peptoids are more lipophilic (Figure 4) than substance P itself and can therefore cross membrane barriers more easily. Moreover, degradation of (retro)peptoids by proteases is strongly reduced, as was shown by treatment of substance P with pepsin.[7] After 20 h peptoid **5** of substance P is still intact, whereas **4** is already largely



Scheme 5. Solid-phase syntheses of substance P (**4**), its corresponding peptoid **5**, and retropeptoid **6**, as well as the solid-phase synthesis monitoring profiles. For **4**: i) a) piperidine, b) Fmoc-Met-OH, BOP, HOBt, DiPEA; ii) a) piperidine, b) Fmoc-Leu-OH, BOP, HOBt, DiPEA; iii) a) piperidine, b) Fmoc-Gly-OH, BOP, HOBt, DiPEA; iv) a) piperidine, b) Fmoc-Phe-OH, BOP, HOBt, DiPEA; v) a) piperidine, b) Fmoc-Phe-OH, BOP, HOBt, DiPEA; vi) a) piperidine, b) Fmoc-Gln(Trt)-OH, BOP, HOBt, DiPEA; vii) a) piperidine, b) Fmoc-Gln(Trt)-OH, BOP, HOBt, DiPEA; viii) a) piperidine, b) Fmoc-Pro-OH, BOP, HOBt, DiPEA; ix) a) piperidine, b) Fmoc-Lys(Boc)-OH, BOP, HOBt, DiPEA; x) a) piperidine, b) Fmoc-Pro-OH, BOP, HOBt, DiPEA; xi) a) piperidine, b) Fmoc-Arg(Pmc)-OH, BOP, HOBt, DiPEA; xii) a) piperidine, b) Ac_2O, DiPEA, HOBt; xiii) TFA/thioanisole/H_2O/EDT/TIS, 86.5/5/5/2.5/1 (*v*/*v*). For **5** and **6**: i) a) piperidine, b) Fmoc-NMet-OH (**10c**), PyBroP, DiPEA; ii) a) piperidine, b) Fmoc-NLeu-OH (**10b**), PyBroP, DiPEA; iii) a) piperidine, b) Fmoc-Gly-OH, PyBroP, DiPEA; iv) a) piperidine, b) Fmoc-NPhe-OH **10a**), PyBroP, DiPEA; v) a) piperidine, b) **10a**, PyBroP, DiPEA; vi) a) piperidine, b) Fmoc-NGln-OH (**10e**), PyBroP, DiPEA; vii) a) piperidine, b) **10e**, PyBroP, DiPEA; viii) a) piperidine, b) Fmoc-Pro-OH, PyBroP, DiPEA; ix) a) piperidine, b) Fmoc-NLys(Boc)-OH (**10d**), PyBroP, DiPEA; x) a) piperidine, b) Fmoc-Pro-OH, PyBroP, DiPEA; xi) a) piperidine, b) Fmoc-NArg(Boc)$_2$-OH (**21**), PyBroP, DiPEA; xii) a) piperidine, b) Ac_2O, DiPEA, HOBt; xiii) TFA/thioanisole/H_2O/EDT/TIS, 86.5/5/5/2.5/1 (*v*/*v*).

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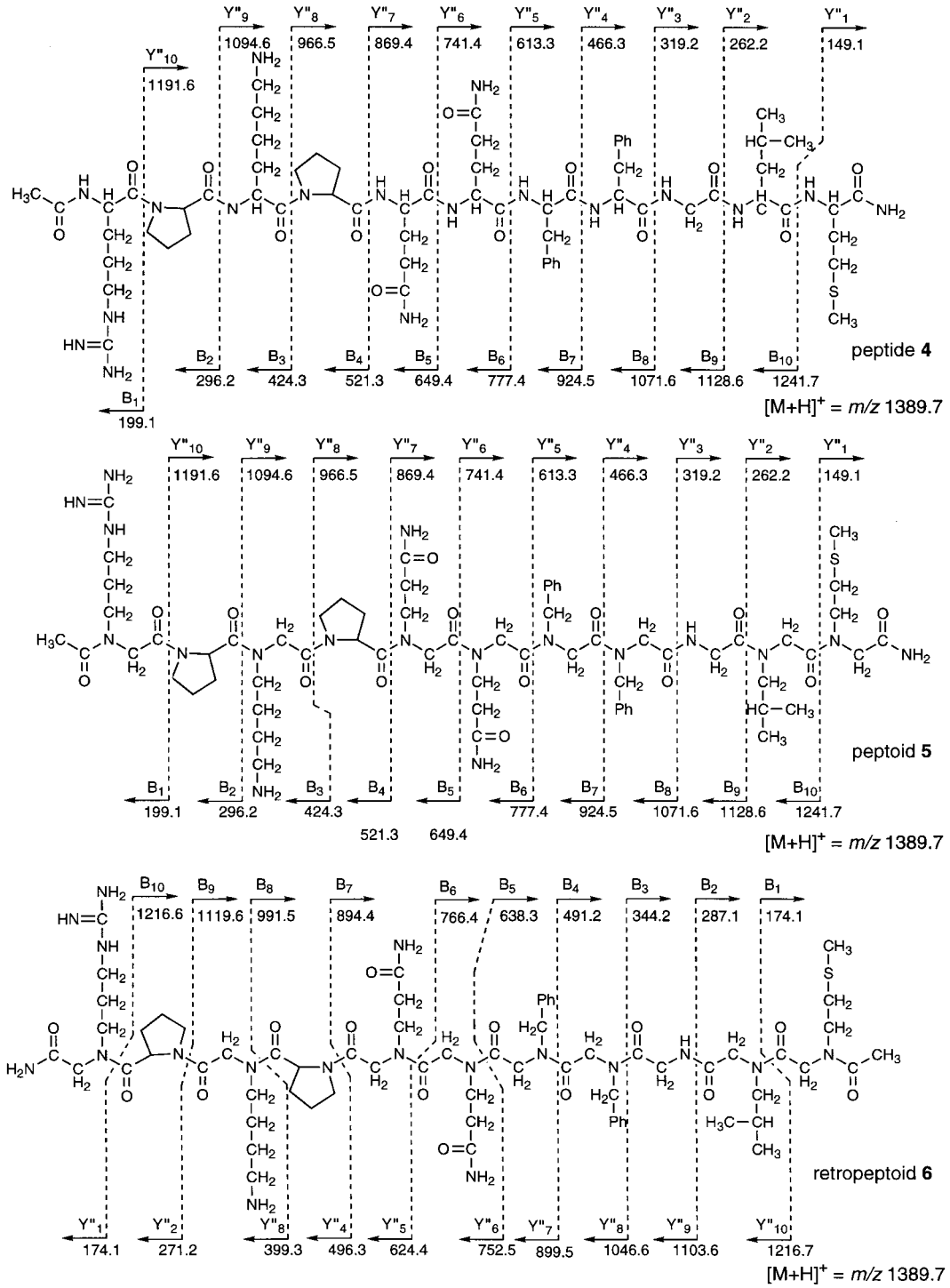

Figure 3. FAB mass spectra and mass fragments of substance P (**4**), its corresponding peptoid **5**, and retropeptoid **6**.

degraded after 30 min (Figure 5). This reduced degradation rate will also certainly contribute to a greater effectiveness of the (retro)peptoid.

Conclusions

We have developed an efficient synthetic strategy for the synthesis of peptoid monomers, that is, Fmoc-protected *N*-substituted glycines. It was shown that peptoid monomers can be conveniently prepared in high yields using economical chemistry. Only Fmoc-NGln-OH (**10e**) was obtained in a relatively low yield (overall yield of 26%), because poor solubility of the intermediates hampered the purification. When suitable protection groups are used, identical or similar side chains to those found in the proteinogenic amino acids, as well as other side chains, can be incorporated into peptoid monomers.

Furthermore, an efficient solid-phase methodology for the synthesis of peptoids using a fully automated peptide synthe-

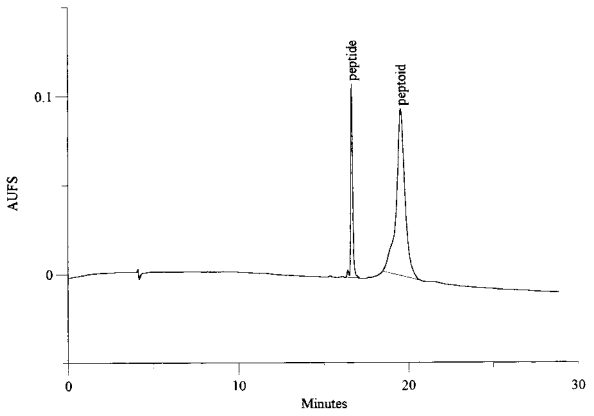

Figure 4. Relative hydrophobicity of substance P (**4**) and its corresponding peptoid **5**.

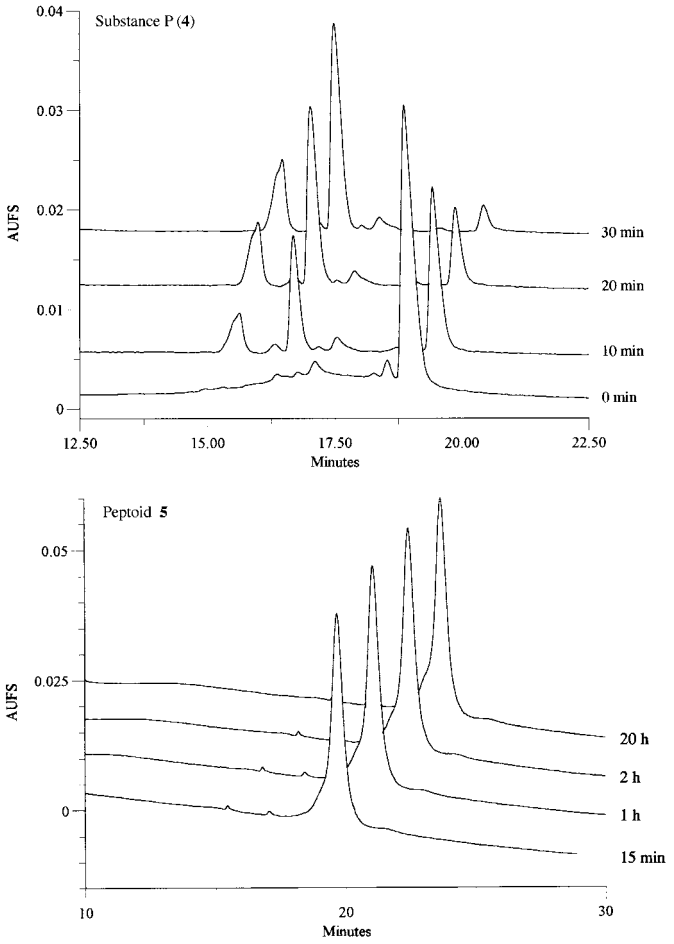

Figure 5. Pepsin treatment of Substance P (**4**) and its corresponding peptoid **5**.

sizer has been developed. It was shown that peptoids can be rapidly and conveniently synthesized from peptoid monomers, specifically, Fmoc-protected *N*-substituted glycines. With these peptoid monomers it was possible to follow the progress of the solid-phase synthesis on the ABI 433A peptide synthesizer with the UV monitoring system. This arrangement provides an adequate representation of the events which have taken place in the reaction vessel.

The synthesized substance P analogues, peptoid **5** and retropeptoid **6**, have been used to evaluate their biological activity compared with that of substance P derivative **4**. Recent results demonstrate agonist activity for the peptoid **5** and retropeptoid **6** analogues of substance P in vitro. Further evaluation of the biological properties of these peptoid peptidomimetics of substance P is currently being undertaken and will be published elsewhere.[23]

Experimental Section

General: Chemicals were obtained from commercial sources and used without further purification unless otherwise stated. TEA and DiPEA were distilled from ninhydrin and subsequently KOH. Dry solvents were distilled immediately prior to use from an appropriate drying agent. THF, ether, and cyclohexane were distilled from $LiAlH_4$. Ethanol-free DCM was distilled from CaH_2. Ethanol and methanol were refluxed over magnesium for 2 h, distilled, and stored on molecular sieves (3 Å). NMP, peptide grade, was purchased from Biosolve (The Netherlands). All protected amino acids were purchased from Advanced Chemtech (Belgium). BOP reagent was purchased from Richelieu Biotechnologies (Canada). Tentagel® SOH (130 μm) resin and Tentagel® SRAM (130 μm) resin were purchased from Rapp Polymere, Tübingen (Germany).

Reactions were run at ambient temperature unless otherwise noted. Reactions were monitored and R_f values were determined by thin-layer chromatography (TLC) on Merck precoated silica gel 60F-254 (0.25 mm) plates. Spots were visualized with UV light, ninhydrin, or Cl_2-TDM (*N,N,N′,N′*-tetramethyl-4,4′-diaminodiphenylmethane).[24] Solvents were evaporated under reduced pressure at 40 °C. Column chromatography was performed on Merck Kieselgel 60 (40–63 μm). Flash column chromatography was performed on Merck Kieselgel 60H (5–40 μm). Sephadex LH-20 (Pharmacia) was used for gel filtration. Fast atom bombardment (FAB) mass spectrometry was carried out with a Jeol JMS SX/SX102A four-sector mass spectrometer coupled with a HP-9000 data system. ^{1}H NMR spectra were recorded on a Jeol JNM-FX200 (200 MHz) or a Varian G-300 (300.1 MHz) spectrometer, and chemical shifts are given in ppm (δ) relative to TMS or TSP as internal standard. ^{13}C NMR spectra were recorded on a Jeol JNM-FX200 spectrometer or a Varian G-300 spectrometer operating at 50.1 and 75.5 MHz, respectively, and the chemical shifts are given in ppm (δ) relative to $CDCl_3$ ($\delta = 77.0$) or $(CD_3)_2SO$ ($\delta = 39.7$) as internal standard. ^{13}C NMR spectra were monitored by the attached proton test (APT) technique. The compounds were homogeneous according to NMR and TLC.

Solid-phase syntheses were carried out on an ABI 433A Peptide Synthesizer. Deprotection and coupling reactions were monitored at 301 nm. Analytical HPLC was performed on a semiautomatic HPLC system (Applied Biosystems) with an analytical reversed-phase column (Altech Econosphere C8, 5 μm, 250 × 4.6 mm), an UV detector operating at 214 nm (peptoids) or 220 nm (peptides), at a flow rate of 1 mL min^{-1}. Elution of peptoids was effected using an appropriate gradient from 10 mM $HClO_4$/100 mM $NaClO_4$ in water to 10 mM $HClO_4$/100 mM $NaClO_4$ in acetonitrile/water (90/10, *v*/*v*). Elution of peptides was effected using an appropriate gradient from 0.1 % TFA in water to 0.085 % TFA in acetonitrile/water (95/5, *v*/*v*). Preparative HPLC was performed on a semiautomatic HPLC system (Applied Biosystems) with a preparative reversed-phase column (Altech Econosphere C8, 10 μm, 250 × 22 mm), an UV detector operating at 220 nm, at a flow rate of 11.5 mL min^{-1}. Elution was effected using an appropriate gradient from 0.1 % TFA in water to 0.085 % TFA in acetonitrile/water (95/5, *v*/*v*).

H-NPhe-OEt (**9a**): Ethyl bromoacetate (**8**, 50 mmol, 5.54 mL) in THF (25 mL) was added dropwise to a cooled (ice bath) solution of benzylamine (**7a**, 110 mmol, 12.0 mL) in THF (25 mL). After stirring for 2.5 h at room temperature, the reaction mixture was concentrated in vacuo to remove THF and resuspended in ether. The mixture was filtered to remove benzylamine hydrobromide, the residue washed with ether, and the filtrate concentrated in vacuo. Column chromatography (silica, eluent: ether)

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afforded **9a** (9.36 g, 48.4 mmol) as an oil in 97% yield.[25] $R_f = 0.51$ (eluent: ether); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.28$ (t, 3H, OCH_2CH_3, $J = 7.2$ Hz), 1.96 (br s, 1H, NH), 3.41 (s, 2H, $NCH_2C(O)$), 3.81 (s, 2H, CH_2Ph), 4.19 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 7.27–7.35 (m, 5H, Ph); ^{13}C NMR (50.1 MHz, $CDCl_3$): $\delta = 13.8$ (OCH_2CH_3), 49.6 ($NCH_2C(O)$), 52.8 (CH_2Ph), 60.2 (OCH_2CH_3), 126.6, 127.8, 128.0, 139.2 (Ph), 171.9 ($NCH_2C(O)$).

Fmoc-NPhe-OH (**10a**): NaOH (4N, 2.50 mL) was added to a solution of H-*N*-Phe-OEt (**9a**, 10 mmol, 1.93 g) in dioxane (35 mL) and MeOH (12.5 mL). After stirring for 30 min at room temperature the reaction mixture was concentrated in vacuo to give H-*N*-Phe-ONa. ^{1}H NMR (200 MHz, D_2O): $\delta = 3.17$ (s, 2H, $NCH_2C(O)$), 3.73 (s, 2H, CH_2Ph), 7.37–7.41 (m, 5H, Ph).

The sodium salt was dissolved in water (10 mL) and the pH adjusted to 9–9.5 with concentrated hydrochloric acid. To this mixture a solution of Fmoc–OSu (10 mmol, 3.37 g) in acetonitrile (20 mL) was added in one portion. Stirring was continued for 30 minutes, and the pH was maintained at pH 8.5–9.0 by the addition of TEA. The reaction mixture was concentrated in vacuo to remove acetonitrile, and the residue was poured into 20% citric acid (60 mL). The aqueous layer was extracted with EtOAc (3×75 mL), and the combined organic layers were washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo to give Fmoc-NPhe-OH (**10a**) as an oil which was crystallized from EtOAc/hexanes to afford **10a** (3.65 g, 9.42 mmol) as a white solid in 94% yield. $R_f = 0.61$ (eluent: DCM/MeOH/HOAc, 90/10/0.5, *v*/*v*); the NMR spectra clearly show the presence of both rotamers; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.76$, 4.00 (two s, 2H, $NCH_2C(O)$), 4.22–4.30 (m, 1H, C*H*-Fmoc), 4.49, 4.56 (two s, 2H, CH_2Ph), 4.57 (d, 2H, CH_2-Fmoc, $J = 6.2$ Hz), 7.07–7.09 (m, 1H, Ph), 7.18–7.40 (m, 8H, Ar*H*-Fmoc, Ph), 7.49–7.56 (4 lines, 2H, Ar*H*-Fmoc), 7.74 (d, 2H, Ar*H*-Fmoc); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 46.8$, 47.8 ($NCH_2C(O)$), 47.2 (*C*H-Fmoc), 51.2, 51.4 (CH_2Ph), 67.7, 68.0 (CH_2-Fmoc), 120.0, 124.9, 127.1, 127.6, 127.7, 128.1, 128.7, 136.3, 141.3, 143.7 (Ar*C*-Fmoc, Ph), 156.2, 156.7 (Fmoc *C*=O), 174.9 ($NCH_2C(O)OH$).

H-NLeu-OEt (**9b**): H-*N*-Leu-OEt **9b** was prepared, analogously to the preparation of **9a**, from isobutylamine (**7b**, 110 mmol, 10.9 mL) and **8** (50 mmol, 5.54 mL). Column chromatography (silica, eluent: ether) gave **9b** (7.55 g, 47.4 mmol) as an oil in 95% yield. $R_f = 0.39$ (eluent: ether);[25] ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 0.93$ (d, 6H, $CH(CH_3)_2$, $J = 6.7$ Hz), 1.28 (t, 3H, OCH_2CH_3, $J = 7.2$ Hz), 1.56 (br s, 1H, NH), 1.74 (quintet, 1H, $CH_2CH(CH_3)_2$), 2.41 (d, 2H, $CH_2CH(CH_3)_2$, $J = 6.7$ Hz), 3.39 (s, 2H, $NCH_2C(O)$), 4.19 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz); ^{13}C NMR (50.1 MHz, $CDCl_3$): $\delta = 13.7$ (OCH_2CH_3), 20.0 ($CH_2CH(CH_3)_2$), 28.0 ($CH_2CH(CH_3)_2$), 50.6 ($NCH_2C(O)$), 57.1 ($CH_2CH(CH_3)_2$), 59.9 (OCH_2CH_3), 171.9 ($NCH_2C(O)$).

Fmoc-NLeu-OH (**10b**): Fmoc-NLeu-OH **10b** was prepared, analogous to the preparation of **10a**, from H-NLeu-OEt **9b** (10 mmol, 1.59 g). Crystallization (ether/hexanes) afforded Fmoc-NLeu-OH **10b** (3,39 g, 9.87 mmol) as a white solid in 99% yield. $R_f = 0.67$ (eluent: DCM/MeOH/HOAc, 90/10/0.5, *v*/*v*).

H-NLeu-ONa: ^{1}H NMR (200 MHz, D_2O): $\delta = 0.89$ (d, 6H, $CH(CH_3)_2$, $J = 6.7$ Hz), 1.74 (quintet, 1H, $CH_2CH(CH_3)_2$), 2.36 (d, 2H, $CH_2CH(CH_3)_2$, $J = 6.7$ Hz), 3.14 (s, 2H, $NCH_2C(O)$).

Fmoc-NLeu-OH **10b**: The NMR spectra clearly show the presence of both rotamers; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.72$, 0.87 (two d, 6H, $CH_2CH(CH_3)_2$, $J = 6.8$ Hz), 1.61, 1.82 (two septets, 1H, $CH_2CH(CH_3)_2$), 2.93, 3.13 (two d, 2H, $CH_2CH(CH_3)_2$, $J = 7.5$ Hz), 3.87, 3.99 (two s, 2H, $NCH_2C(O)$), 4.16–4.25 (m, 1H, C*H*-Fmoc), 4.46, 4.54 (two d, 2H, CH_2-Fmoc, $J = 6.0$ Hz), 7.23–7.41 (m, 4H, Ar*H*-Fmoc), 7.51–7.58 (4 lines, 2H, Ar*H*-Fmoc), 7.73 (t, 2H, Ar*H*-Fmoc); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 19.8$, 19.9 ($CH_2CH(CH_3)_2$), 27.2, 27.4 ($CH_2CH(CH_3)_2$), 47.3 (*C*H-Fmoc), 48.8, 49.6 ($NCH_2C(O)$), 55.7, 56.1 ($CH_2CH(CH_3)_2$), 67.4 (CH_2-Fmoc), 119.9, 124.8, 127.0, 127.6, 141.4, 143.9 (Ar*C*-Fmoc), 156.2, 157.1 (Fmoc *C*=O), 174.5, 174.7 ($NCH_2C(O)OH$).

H-NMet-OEt (**9c**): H-*N*-Met-OEt was prepared, analogously to the preparation of **9a**, from 2-aminoethyl methyl sulfide (**7c**, 30 mmol, 2.74 g) and **8** (15 mmol, 1.66 mL).[5b, 21] Column chromatography (silica, eluent: gradient of hexanes/EtOAc, 40/60 to hexanes/EtOAc, 20/80, *v*/*v*) gave **9c** (2.33 g, 13.2 mmol) as an oil in 88% yield.[25] $R_f = 0.56$ (eluent: DCM/MeOH/HOAc, 90/10/0.5, *v*/*v*); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.29$ (t, 3H, OCH_2CH_3, $J = 7.2$ Hz), 1.90 (br s, 1H, NH), 2.12 (s, 3H, SCH_3), 2.66 (t, 2H, CH_2SMe, $J = 6.2$ Hz), 2.84 (t, 2H, $HNCH_2$, $J = 6.2$ Hz), 3.44 (s, 2H, $NCH_2C(O)$), 4.20 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz); ^{13}C NMR (50.1 MHz, $CDCl_3$): $\delta = 13.6$ (OCH_2CH_3), 14.4 (SCH_3), 33.6 (CH_2SMe), 46.8 ($HNCH_2$), 49.9 ($NCH_2C(O)$), 60.0 (OCH_2CH_3), 171.6 ($NCH_2C(O)$).

Fmoc-NMet-OH (**10c**): Fmoc-NMet-OH was prepared, analogously to the preparation of **10a**, from H-NMet-OEt (**9c**, (10 mmol, 1.77 g). Crystallization (EtOAc/hexanes) afforded **10c** (3,65 g, 9.82 mmol) as a white solid in 98% yield. $R_f = 0.40$ (eluent: DCM/MeOH/HOAc, 90/10/0.5, *v*/*v*). H-*N*-Met-ONa: ^{1}H NMR (200 MHz, D_2O): $\delta = 2.10$ (s, 3H, SCH_3), 2.62–2.69 (m, 2H, CH_2SMe), 2.74–2.81 (m, 2H, $HNCH_2$), 3.18 (s, 2H, $NCH_2C(O)$). **10c**: The NMR spectra clearly show the presence of both rotamers; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.94$, 2.12 (two s, 3H, SCH_3), 2.35, 2.66 (two t, 2H, CH_2SMe, $J = 7.3$ Hz), 3.29, 3.51 (two t, 2H, $FmocNCH_2$, $J = 7.3$ Hz), 3.97, 4.07 (two s, 2H, $NCH_2C(O)$), 4.19–4.27 (m, 1H, C*H*-Fmoc), 4.48, 4.61 (two d, 2H, CH_2-Fmoc, $J = 6.2$ Hz), 7.29–7.43 (m, 4H, Ar*H*-Fmoc), 7.52–7.59 (4 lines, 2H, Ar*H*-Fmoc), 7.75 (t, 2H, Ar*H*-Fmoc); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 15.4$ (SCH_3), 32.0 (CH_2SMe), 47.2 (*C*H-Fmoc), 48.1, 48.3 ($FmocNCH_2$), 49.2, 49.7 ($NCH_2C(O)$), 67.3, 67.7 (CH_2-Fmoc), 119.9, 124.6, 127.1, 127.7, 141.3, 143.6 (Ar*C*-Fmoc), 155.6, 156.2 (Fmoc *C*=O), 174.6, 174.8 ($NCH_2C(O)OH$).

H-NLys(Boc)-OEt (**9d**): Ethyl bromoacetate (**8**, 50 mmol, 5.54 mL) in THF (30 mL) was added dropwise to a solution of *N*-Boc-1,4-diaminobutane (**7d**, 50 mmol, 9.41 g) and TEA (100 mmol, 13.94 mL) in THF (30 mL).[5b, 21] After stirring overnight at room temperature, the reaction mixture was concentrated in vacuo to remove THF and resuspended in ether. The reaction mixture was filtered to remove triethylamine hydrobromide, the residue was washed with ether and the filtrate concentrated in vacuo. Column chromatography (silica, eluent: gradient of hexanes/EtOAc, 1/1 to EtOAc, *v*/*v*) gave **9d** (10.31 g, 37.6 mmol) as an oil in 75% yield. $R_f = 0.69$ (eluent: DCM/MeOH/HOAc, 90/10/0.5, *v*/*v*); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.29$ (t, 3H, OCH_2CH_3, $J = 7.2$ Hz), 1.44 (s, 9H, $C(CH_3)_3$), 1.57 (m, 4H, CH_2CH_2), 2.70 (m, 2H, $HNCH_2$), 3.11 (m, 2H, $BocNCH_2$), 3.46 (s, 2H, $NCH_2C(O)$), 4.20 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 4.78 (br s, 1H, NH); ^{13}C NMR (50.1 MHz, $CDCl_3$): $\delta = 14.0$ (OCH_2CH_3), 27.0 ($BocNCH_2CH_2$), 27.5 ($HNCH_2CH_2$), 28.2 ($C(CH_3)_3$), 40.1 ($BocNCH_2$), 48.9 ($HNCH_2$), 50.6 ($NCH_2C(O)$), 60.4 (OCH_2CH_3), 78.6 ($C(CH_3)_3$), 155.8 (Boc *C*=O), 172.2 ($NCH_2C(O)$).

Fmoc-NLys(Boc)-OH (**10d**): Fmoc-NLys(Boc)-OH was prepared, analogously to the preparation of **10a**, from **9d** (10 mmol, 2.74 g). Crystallization (EtOAc/hexanes) afforded **10d** (4.46 g, 9.52 mmol) as a white solid in 95% yield. $R_f = 0.51$ (eluent: DCM/MeOH/HOAc, 90/10/0.5, *v*/*v*). H-NLys(Boc)-ONa: ^{1}H NMR (200 MHz, D_2O): $\delta = 1.42$ (s, 9H, $C(CH_3)_3$), 1.55 (m, 4H, CH_2CH_2), 2.79 (m, 2H, $HNCH_2$), 3.08 (m, 2H, $BocNCH_2$), 3.35 (s, 2H, $NCH_2C(O)$). **10d**: The NMR spectra clearly show the presence of both rotamers; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.2–1.4$, 1.4–1.6 (two m, 4H, CH_2CH_2), 1.45 (br s, 9H, $C(CH_3)_3$), 2.98 (m, 1H, $FmocNCH_2$), 3.09 (m, 2H, $FmocNCH_2$ and $BocNCH_2$), 3.34 (m, 1H, $BocNCH_2$), 3.89, 3.97 (two br s, 2H, $NCH_2C(O)$), 4.24 (m, 1H, C*H*-Fmoc), 4.45, 4.58 (two m, 2H, CH_2-Fmoc), 7.25–7.42 (m, 4H, Ar*H*-Fmoc), 7.56 (t, 2H, Ar*H*-Fmoc), 7.75 (m, 2H, Ar*H*-Fmoc); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 25.0$, 25.3 ($FmocNCH_2CH_2$), 27.1 ($BocNCH_2CH_2$), 28.4 ($C(CH_3)_3$), 40.1 ($BocNCH_2$), 47.2 (*C*H-Fmoc), 48.0 ($FmocNCH_2$), 48.4, 48.9 ($NCH_2C(O)$), 67.2, 67.7 (CH_2-Fmoc), 79.3 ($C(CH_3)_3$), 119.9, 124.7, 124.9, 127.0, 127.1, 127.6, 141.3, 141.4, 143.9 (Ar*C*-Fmoc), 156.0, 156.6 (Boc *C*=O and Fmoc *C*=O), 173.4 (broad, $NCH_2C(O)OH$).

H-NGln-OEt (**9e**): Ethyl bromoacetate (**8**, 41 mmol, 4.55 mL) in THF (40 mL) was added dropwise to a solution of H-β-Ala-NH_2 (**7e,** 41 mmol, 3.61 g) and TEA (82 mmol, 11.41 mL) in ethanol (60 mL),. After stirring overnight at room temperature, the reaction mixture was filtered, the residue washed with ether, and the filtrate concentrated in vacuo. Flash column chromatography (silica, eluent: MeOH/DCM, 10/90, *v*/*v*) gave **9e** (4.41 g, 25.3 mmol) as a light yellow oil in 62% yield. $R_f = 0.37$ (eluent: DCM/MeOH, 80/20, *v*/*v*); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.22$ (t, 3H, OCH_2CH_3, $J = 7.1$ Hz), 1.84 (br s, 1H, NH), 2.46 (t, 2H, $CH_2C(O)NH_2$, $J = 6.6$ Hz), 2.87 (t, 2H, $HNCH_2$, $J = 6.5$ Hz), 3.37 (s, 2H, $NCH_2C(O)$), 4.14 (q, 2H, OCH_2CH_3, $J = 7.1$ Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 14.1$ (OCH_2CH_3), 34.9 ($CH_2C(O)NH_2$), 44.7 ($HNCH_2$), 50.8 ($NCH_2C(O)$), 60.6 (OCH_2CH_3), 172.2 ($C(O)NH_2$), 172.3 ($C(O)OEt$).

Fmoc-NGln-OH (**10e**): NaOH (4N, 2.55 mL) was added to a solution of **9e** (10.2 mmol, 1.78 g) in dioxane (35.7 mL) and MeOH (12.8 mL). After stirring for 30 min at room temperature the reaction mixture was

concentrated in vacuo to afford H-NGln-ONa. ^{1}H NMR (300 MHz, D_2O): $\delta = 2.48$ (t, 2H, $CH_2C(O)NH_2$, $J = 6.8$ Hz), 2.84 (t, 2H, $HNCH_2$, $J = 6.8$ Hz), 3.19 (s, 2H, $NCH_2C(O)$).

The sodium salt was dissolved in water (50 mL), and the pH adjusted to 9–9.5 using 1N HCl. To this mixture a solution of Fmoc-OSu (11 mmol, 3.71 g) in acetone (40 mL) was added dropwise. Stirring was continued for 2 h and the pH was maintained at pH 9.5 by the addition of 1N NaOH. The reaction mixture was filtered, acidified to pH 2 using 2N HCl, and extracted with EtOAc (3×100 mL). The combined organic layers were concentrated in vacuo. Crystallization (EtOAc/hexanes) gave **10e** (1.62 g, 4.40 mmol) as a white solid in 43% yield. $R_f = 0.38$ (eluent: DCM/MeOH/HOAc, 90/10/0.5, *v*/*v*); the NMR spectra clearly show the presence of both rotamers; ^{1}H NMR (300 MHz, $(CD_3)_2SO$): $\delta = 2.33$ (q, 2H, $CH_2C(O)NH_2$), 3.46 (quintet, 2H, $HNCH_2$), 3.94, 4.01 (two s, 2H, $NCH_2C(O)$), 4.23–4.29 (m, 3H, CH_2-Fmoc, C*H*-Fmoc), 6.85 (br d, 1H, $C(O)NH_2$), 7.29–7.44 (m, 5H, ArH-Fmoc, $C(O)NH_2$), 7.61, 7.68 (two d, 2H, ArH-Fmoc), 7.88–7.91 (m, 2H, ArH-Fmoc); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$): $\delta = 33.8$, 34.3 ($CH_2C(O)NH_2$), 44.3, 44.9 (FmocNCH_2), 46.6 (*C*H-Fmoc), 48.9, 49.1 ($NCH_2C(O)$), 67.1 (CH_2-Fmoc), 120.1, 125.1, 126.7, 127.1, 127.7, 140.7, 143.7, 143.8 (Ar*C*-Fmoc), 155.2, 155.5 (Fmoc *C*=O), 171.0, 171.2 ($C(O)NH_2$), 172.4, 172.5 (*C*(O)OH).

4-*tert*-Butyloxybenzonitrile (**13**): 4-Hydroxybenzonitrile (**11**, 15 mmol, 1.79 g) was dissolved in ether (15 mL) and cyclohexane (30 mL). To this mixture, *tert*-butyltrichloroacetimidate **12** (60 mmol,[16] 10 mL) and a catalytic amount of $BF_3 \cdot Et_2O$ (300 μL) was added. After stirring for 22 h, solid $NaHCO_3$ was added and stirring was continued for 10 min. The reaction mixture was filtered and the filtrate concentrated in vacuo. Column chromatography (silica, eluent: hexanes/ether, 3/1, *v*/*v*) afforded **13** (1.66 g, 9.5 mmol) as a syrup in 63% yield. $R_f = 0.51$ (eluent: ether); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.42$ (s, 9H, $C(CH_3)_3$), 7.0–7.1 (m, 2H, ArC^{3}H, ArC^{5}H), 7.5–7.6 (m, 2H, ArC^{2}H, ArC^{6}H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 28.8 ($C(CH_3)_3$), 80.1 ($C(CH_3)_3$), 105.7 (CN), 119.0 (ArC^1), 122.9 (ArC^{3,5}), 133.3 (ArC^{2,6}), 159.9 (ArC^4).

4-*tert*-Butyloxybenzylamine (**7f**): A solution of 4-*tert*-butyloxybenzonitrile (**13,** 33.7 mmol, 5.90 g) in ether (20 mL) was added dropwise to a suspension of $LiAlH_4$ (51 mmol, 1.94 g) in ether (40 mL). The reaction mixture was refluxed for 3 h. The reaction mixture was then cooled to room temperature and water (1.96 mL), 15% NaOH (1.96 mL), and water (5.90 mL) were subsequently added. The mixture was filtered, the residue washed twice with ether, and the filtrate concentrated in vacuo. Water (100 mL) was added to the residue, and the pH adjusted to 2.7 with 1N $KHSO_4$. The aqueous layer was washed with ether (20 mL), the pH adjusted to 9–10 with 2N NaOH, and the layer extracted with DCM (150 mL). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to obtain 4-*tert*-butyloxybenzylamine **7f** (5.68 g, 31.7 mmol) in 94% yield. $R_f = 0.23$ (eluent: DCM/MeOH/TEA, 90/10/1, *v*/*v*). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.34$ (s, 9H, $C(CH_3)_3$), 1.82 (br s, 2H, CH_2NH_2), 3.84 (s, 2H, CH_2NH_2), 6.96 (m, 2H, ArC^{3}H, ArC^{5}H), 7.21 (d, 2H, ArC^{2}H, ArC^{6}H, $J = 8.4$ Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 28.7$ ($C(CH_3)_3$), 45.5 (CH_2NH_2), 78.2 ($C(CH_3)_3$), 124.1, 127.6, 137.1, 154.2 (Ar).

H-NTyr(*t*Bu)-OEt (**9f**): H-NTyr(*t*Bu)-OEt was prepared, analogously to the preparation of **7d**, from **7f** (19.5 mmol, 3.50 g), TEA (39 mmol, 5.43 mL), and ethyl bromoacetate (**8**, 19.5 mmol, 2.16 mL). Column chromatography (silica, eluent: hexanes/EtOAc, 40/60, *v*/*v*) gave **9f** (4.30 g, 16.2 mmol) as an oil in 83% yield. $R_f = 0.51$ (eluent: DCM/MeOH/HOAc, 90/10/0.5, *v*/*v*); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.28$ (t, 3H, OCH_2CH_3, $J = 7.2$ Hz), 1.33 (s, 9H, $C(CH_3)_3$), 1.88 (br s, 1H, NH), 3.41 (s, 2H, $NCH_2C(O)$), 3.74 (s, 2H, CH_2Ar), 4.20 (q, 2H, OCH_2CH_3, $J = 7.1$ Hz), 6.95 (m, 2H, ArC^{3}H, ArC^{5}H), 7.22 (m, 2H, ArC^{2}H, ArC^{6}H); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 14.2$ (OCH_2CH_3), 28.8 ($C(CH_3)_3$), 50.1 ($NCH_2C(O)$), 52.8 (CH_2Ar), 60.6 (OCH_2CH_3), 78.2 ($C(CH_3)_3$), 124.1 (ArC^3, ArC^5), 128.7 (ArC^2, ArC^6), 134.4 (ArC^1), 154.5 (ArC^4), 172.4 ($NCH_2C(O)$).

Fmoc-NTyr(*t*Bu)-OH (**10f**): Fmoc-NTyr(*t*Bu)-OH was prepared, analogously to the preparation of **10a**, from **9f** (10 mmol, 2.65 g). Crystallization (EtOAc/hexanes) afforded **10f** (3.55 g, 7.73 mmol) as a white solid in 77% yield. $R_f = 0.47$ (eluent: DCM/MeOH/HOAc, 90/10/0.5, *v*/*v*); the NMR spectra clearly show the presence of both rotamers. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.33$ (br s, 9H, $C(CH_3)_3$), 3.78, 3.97 (two s, 2H, $NCH_2C(O)$), 4.27 (m, 1H, C*H*-Fmoc), 4.43, 4.52 (two s, 2H, CH_2Ar), 4.57 (t, 2H, CH_2-Fmoc), 6.92 (m, 3H, ArC^{3}H, ArC^{5}H, ArC^{2}H, ArC^{6}H), 7.19 (d, 1H, ArC^{2}H, ArC^{6}H), 7.29 (m, 2H, Ar*H*-Fmoc), 7.38 (m, 2H, Ar*H*-Fmoc), 7.55 (d, 2H, Ar*H*-Fmoc), 7.74 (d, 2H, Ar*H*-Fmoc); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 28.8$ ($C(CH_3)_3$), 47.2 (*C*H-Fmoc), 47.5 ($NCH_2C(O)$), 50.6, 50.7 (CH_2Ar), 67.9, 68.2 (CH_2-Fmoc), 78.6 ($C(CH_3)_3$), 120.0, 124.9, 127.1, 127.7, 141.4, 143.7 (Ar*C*-Fmoc), 124.2 (ArC, ArC^5), 128.3, 128.8 (ArC^2, ArC^6), 131.0 (ArC^1), 155.1 (ArC^4), 156.6 (Fmoc *C*=O), 174.5 ($NCH_2C(O)$).

Ac-Tyr-Gly-Gly-Phe-Leu-NH$_2$ (Ac-YGGFL-NH$_2$) (**1**):

Resin preparation: Fmoc-Leu-TentaGel resin: Fmoc-Leu-OH was attached to the TentaGel® SOH resin (capacity 0.29 mmol/g) by the procedure of Sieber, yielding a resin with a loading of 0.24 mmol g^{-1} (83%) as was determined by Fmoc cleavage from a resin sample.[10, 26] With this resin (1.05 g), peptide **1** was synthesized on an automatic ABI 433A Peptide Synthesizer using the ABI FastMoc 0.25 mmol protocols,[27] except that the coupling time was 45 min instead of 20 min and BOP/HOBt activation was used instead of HBTU/HOBt. After cleavage of the Fmoc group by means of a 20% piperidine solution in NMP (2×3 min and 7.6 min), the resin was washed with NMP ($5 \times$). Subsequently, 4 equiv (1 mmol) of the appropriate amino acid were dissolved in NMP (2 mL), and BOP/HOBt (2 mL of 0.45M) in NMP was added. To this mixture DiPEA (1 mL, 2M) in NMP was added, and the activated amino acid was then transferred to the reaction vessel. After 45 min, the reaction vessel was drained and the resin was washed with NMP ($3 \times$). The deprotection and coupling reactions were followed by monitoring the dibenzofulvene–piperidine adduct at 301 nm.[27] The last coupling cycle was followed by removal of the Fmoc group by a piperidine solution, washing the resin with NMP, and acetylation of the N-terminus by treatment with an acetic anhydride capping solution (0.5M Ac_2O, 0.125M DiPEA, and 0.015M HOBt in NMP) for 15 min. Finally, the resin was washed with NMP ($5 \times$) and DCM ($6 \times$), removed from the reaction vessel, washed with ether, and dried in vacuo over P_2O_5. The anchored peptide thus obtained was cleaved from the solid support by treatment with a saturated solution of ammonia (20 mL) in MeOH for 12 h at room temperature. The mixture was then filtered and the residue washed thoroughly with MeOH. The total filtrate was concentrated in vacuo. Purification by flash column chromatography (silica 60H, eluent: MeOH/DCM, 10/90, *v*/*v*) gave a partially protected peptide (140 mg). R_f 0.79 (eluent: MeOH/DCM, 20/80, *v*/*v*). The *t*Bu group of Tyr(*t*Bu) in this peptide was removed by treatment with TFA/H_2O (95%, 5 mL) for 2 h at room temperature. The reaction mixture was concentrated in vacuo to a volume of approximately 1 to 2 mL, and then cold ether (50 mL) was added to precipitate the peptide. The precipitated peptide **1** was collected by filtration through a fritted glass funnel and dried in vacuo over P_2O_5 to give 96 mg peptide **1** (67%). $R_f = 0.56$ (eluent: MeOH/DCM, 20/80, *v*/*v*). The peptide **1** was pure according to analytical HPLC. FAB MS: $m/z = 597$ $[M+H]^+$.

Ac-NTyr-Gly-Gly-NPhe-NLeu-*N*H$_2$ (**peptoid 2**): Immobilized pentapeptoid **2** was assembled on an automatic ABI 433A Peptide Synthesizer using the ABI FastMoc 0.25 mmol protocols,[27] with the exceptions that the coupling time was 45 min instead of 20 min and PyBroP was used instead of HBTU/HOBt. TentaGel SRAM resin (1.04 g, capacity 0.24 mmol g^{-1}) was used, and for each coupling 1 mmol peptoid monomer was activated in the cartridge, with 1 mmol PyBroP, also present in the cartridge by addition of 2 mmol DiPEA and subsequent transfer to the reaction vessel. The anchored pentapeptoid **2** thus obtained was deprotected and cleaved from the solid support by treatment with TFA (10 mL) containing H_2O (0.25 mL) and TIS (0.25 mL) for 2.5 h at room temperature. The reaction mixture was filtered and the residue was washed thoroughly with TFA ($2 \times$ 3 mL). Work-up, described above for the preparation of **1**, and subsequent purification by flash column chromatography (silica 60H, eluent: MeOH/DCM, 10/90, *v*/*v*) gave peptoid **2** (109 mg, 76%), which was pure according to analytical HPLC. $R_f = 0.11$ (eluent: MeOH/DCM, 10/80, *v*/*v*); FAB MS: $m/z = 597$ $[M+H]^+$.

Ac-NLeu-NPhe-Gly-Gly-NTyr-NH$_2$ (**retropeptoid 3**): Synthesis, deprotection, cleavage from the resin and work-up of the immobilized retropeptoid **3** were carried out as described for the preparation of **2**. After purification by flash column chromatography (silica 60H, eluent: gradient of MeOH/DCM, 10/90 to MeOH/DCM, 20/80, *v*/*v*) and Sephadex LH-20 gel filtration (eluent: DCM/MeOH, 1/1, *v*/*v*), retropeptoid **3** was obtained in a yield of 14% (20 mg) which was pure according to analytical HPLC. $R_f = 0.48$ (eluent: MeOH/DCM, 20/80, *v*/*v*); FAB MS: $m/z = 597$ $[M+H]^+$.

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H-NOrn(Boc)-OBzl (**16**): Benzyl bromoacetate (**15**, 75 mmol, 11.9 mL) in THF (75 mL) was added dropwise to a solution of *N*-Boc-1,3-diaminopropane (**14**, 75 mmol, 13.07 g) and TEA (112.5 mmol, 15.7 mL) in THF (75 mL),[5b, 21]. After stirring overnight at room temperature, the reaction mixture was filtered to remove triethylamine hydrobromide; the residue was then washed with ether and the filtrate concentrated in vacuo. Column chromatography (silica, eluent: DCM) gave **16** (22.0 g, 68.3 mmol) as an oil in 91% yield. $R_f = 0.56$ (eluent: DCM/MeOH, 90/10, *v*/*v*). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.44$ (s, 9H, $C(CH_3)_3$), 1.65 (quintet, 2H, $HNCH_2CH_2$, $J = 6.6$ Hz), 1.73 (s, 1H, NH), 2.67 (t, 2H, $HNCH_2$, $J = 6.7$ Hz), 3.20 (br q, 2H, $BocNCH_2$), 3.44 (s, 2H, $NCH_2C(O)$), 4.92 (br s, 1H, NH), 5.17 (s, 2H, CH_2Ph), 7.29–7.38 (m, 5H, Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 28.0$ ($C(CH_3)_3$), 29.6 ($HNCH_2CH_2$), 38.4 ($BocNCH_2$), 46.7 ($HNCH_2$), 50.4 ($NCH_2C(O)$), 66.0 (CH_2Ph), 78.4 ($C(CH_3)_3$), 126.4, 126.7 127.9, 128.0, 128.2, 135.3 (Ph), 155.7 (Boc *C*=O), 171.9 ($NCH_2C(O)$).

Cbz-NOrn(Boc)-OBzl (**17**): Benzyl chloroformate (33 mmol, 4.71 mL) was added dropwise to a solution of **16** (30 mmol, 9.67 g) and TEA (33 mmol, 4.59 mL) at room temperature. After stirring for 3 h, the reaction mixture was concentrated in vacuo and dissolved in EtOAc. The organic layer was washed with 1N $KHSO_4$, water, and brine, dried over $MgSO_4$, and concentrated in vacuo. Column chromatography (silica, eluent: hexanes/ether, 1/1, *v*/*v*) gave **17** (13.16 g, 28.83 mmol) in 96% yield. $R_f = 0.36$ (eluent: hexanes/ether, 25/75, *v*/*v*); the NMR spectra clearly show the presence of both rotamers: ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.42$, 1.44 (two s, 9H, $C(CH_3)_3$), 1.67 (m, 2H, $CbzNCH_2CH_2$), 3.07–3.16 (m, 2H, $CbzNCH_2$), 3.35–3.45 (m, 2H, $BocNCH_2$), 3.99, 4.06 (two s, 2H, $NCH_2C(O)$), 5.10 (d, 2H, CH_2-Cbz), 5.18 (d, 2H, CH_2Ph), 7.28–7.36 (m, 10H, Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 27.9$, 28.5 ($CbzNCH_2CH_2$), 28.0 ($C(CH_3)_3$), 36.9, 37.4 ($BocNCH_2$), 45.6, 45.9 ($CbzNCH_2$), 49.0, 49.2 ($NCH_2C(O)$), 66.6 (CH_2Ph), 67.2, 67.4 (CH_2-Cbz), 78.6 ($C(CH_3)_3$), 126.6, 127.0, 127.4, 127.7, 127.9, 128.0, 128.1, 128.2, 128.3, 135.1, 135.2, 136.1 (Ph and Ph-Cbz), 155.7, 155.8, 156.1 (Boc *C*=O and Cbz *C*=O), 169.3 ($NCH_2C(O)$).

Cbz-NArg(Boc)$_2$-OBzl (**19**): A saturated solution of HCl in ether (ca. 20 mL) was added to a stirred solution of **17** (20 mmol, 9.13 g) in chloroform (150 mL). After stirring for 1.5 h at room temperature, the reaction mixture was concentrated in vacuo and dried in vacuo over KOH to yield Cbz-NOrn(HCl)-OBzl (7.8 g, 19.85 mmol) as a white solid in almost quantitative yield. $R_f = 0.27$ (eluent: DCM/MeOH/TEA, 80/20/1, *v*/*v*). The NMR spectra clearly show the presence of both rotamers. Cbz-NOrn(HCl)-OBzl: ^{1}H NMR (300 MHz, $(CD_3)_2SO$): $\delta = 1.80$ (m, 2H, $CbzNCH_2CH_2$), 2.78 (m, 2H, $CbzNCH_2$), 3.37 (m, 2H, H_2NCH_2), 4.11, 4.16 (two s, 2H, $NCH_2C(O)$), 5.02 (s, 1H, CH_2-Cbz), 5.12 (s, 2H, CH_2-Cbz and CH_2Ph), 5.16 (s, 1H, CH_2Ph), 7.24–7.37 (m, 10H, Ph), 7.91 (br s, 3H, $NH_2\cdot$HCl); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$): $\delta = 25.6$, 26.3 ($CbzNCH_2CH_2$), 36.4 (H_2NCH_2), 45.3, 45.8 ($CbzNCH_2$), 48.9, 49.2 ($NCH_2C(O)$), 65.9, 66.0 (CH_2Ph), 66.4, 66.5 (CH_2-Cbz), 127.1, 127.4, 127.7, 127.8, 128.0, 128.3, 128.4, 135.6, 135.7, 136.5, 136.6 (Ph and Ph-Cbz), 155.4, 155.6 (Cbz *C*=O), 169.5, 169.7 ($NCH_2C(O)$).

Subsequently Cbz-NOrn(HCl)-OBzl (15 mmol, 5.89 mmol) was dissolved in EtOAc (250 mL). This solution was washed with Na_2CO_3 (5%, 2 × 100 mL) and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue, Cbz-NOrn-OBzl, was redissolved in THF (100 mL), and *N,N′*-bis(Boc)-1-guanylpyrazole (**18**, 16.5 mmol, 5.12 g) was added to this solution.[19] After stirring for 24 h at room temperature, the reaction mixture was concentrated in vacuo. Column chromatography (silica, eluent: hexanes/ether, 1/1, *v*/*v*) afforded **19** (7.83 g, 13.08 mmol) as an oil in 87% yield. $R_f = 0.19$ (eluent: hexanes/ether, 1/1, *v*/*v*). The NMR spectra clearly show the presence of both rotamers; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (br s, 18H, two $C(CH_3)_3$), 1.81 (m, 2H, $CbzNCH_2CH_2$), 3.42 (m, 4H, $CbzNCH_2$ and $CbzNCH_2CH_2CH_2$), 4.07 (d, 2H, $NCH_2C(O)$), 5.09 (s, 2H, CH_2-Cbz), 5.18 (d, 2H, CH_2Ph), 7.25–7.35 (m, 10H, Ph and Ph-Cbz), 8.41 (br d, 1H, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 27.6$ ($CbzNCH_2CH_2$), 27.8 ($C(CH_3)_3$), 28.0 ($C(CH_3)_3$), 37.7, 37.8 ($CbzNCH_2CH_2CH_2$), 45.6, 45.6 ($CbzNCH_2$), 49.2 ($NCH_2C(O)$), 66.6 (CH_2Ph), 67.1, 67.3 (CH_2-Cbz), 78.8, 82.7, 82.8 ($C(CH_3)_3$), 127.4, 127.7, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 135.1, 135.2, 136.1, 136.3 (Ph and Ph-Cbz), 152.9, 153.0 (C=N), 155.8, 155.9, 156.0, 156.2 (Boc *C*=O and Cbz *C*=O), 163.3 (Boc *C*=O), 169.4 ($NCH_2C(O)$).

H-NArg(Boc)$_2$-OH (**20**): Cbz-NArg(Boc)$_2$-OBzl (**19**, 12 mmol, 7.18 g) was dissolved in MeOH (150 mL) and hydrogenated (1 atm) in the presence of 10% Pd/C (ca. 300 mg). After stirring for 5 h, the reaction mixture was filtered through Hyflo, and evaporation of the solvent in vacuo yielded **20** quantitatively (4.50 g, 12 mmol) as a white foam. $R_f = 0.30$ (DCM/MeOH/HOAc, 80/20/0.5, *v*/*v*). ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$): $\delta = 1.49$ (d, 18H, two $C(CH_3)_3$), 2.03 (m, 2H, $HNCH_2CH_2$), 3.02 (br t, 2H, $HNCH_2CH_2CH_2$, $J = 6.2$ Hz), 3.44 (s, 2H, $NCH_2C(O)$), 3.53 (m, 2H, $HNCH_2$); ^{13}C NMR (75.5 MHz, $CDCl_3/CD_3OD$): $\delta = 26.7$ ($HNCH_2CH_2$), 27.5 ($C(CH_3)_3$), 27.6 ($C(CH_3)_3$), 36.5 ($HNCH_2CH_2CH_2$), 44.1 ($HNCH_2$), 49.4 ($NCH_2C(O)$), 80.3 ($C(CH_3)_3$), 83.8 ($C(CH_3)_3$), 152.4 (C=N), 157.2 (Boc *C*=O), 161.8 (Boc *C*=O), 168.6 ($NCH_2C(O)$).

Fmoc-NArg(Boc)$_2$-OH (**21**): H-NArg(Boc)$_2$-OH (**20**, 10 mmol, 3.74 g) was dissolved in water (10 mL) and the pH adjusted to 9–9.5 with TEA. To this mixture a solution of Fmoc-OSu (10 mmol, 3.37 g) in acetonitrile (20 mL) was added in one portion. Stirring was continued for 30 minutes and the pH was maintained at pH 8.5–9.0 by the addition of TEA. The reaction mixture was concentrated in vacuo to remove acetonitrile, and the residue was poured into citric acid (20%, 60 mL). The aqueous layer was extracted with EtOAc (3 × 75 mL) and the combined organic layers were washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo to give an oil which was crystallized from ether/hexanes to afford **21** (5.26 g, 8.82 mmol) as a white solid in 88% yield. $R_f = 0.59$ (eluent: DCM/MeOH/HOAc, 90/10/0.5, *v*/*v*); the NMR spectra clearly show the presence of both rotamers; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.38$–1.52 (m, 19H, two $C(CH_3)_3$ and $FmocNCH_2CH_2$), 1.81 (m, 1H, $FmocNCH_2CH_2$), 3.16 (m, 2H, $FmocNCH_2CH_2CH_2$), 3.38 (m, 2H, $FmocNCH_2$), 3.99 (d, 2H, $NCH_2C(O)$), 4.19, 4.26 (two br t, 1H, *CH*-Fmoc), 4.43, 4.57 (two d, 2H, CH_2-Fmoc), 7.25–7.40 (m, 4H, Ar*H*-Fmoc), 7.55 (br t, 2H, Ar*H*-Fmoc), 7.74 (br t, 2H, Ar*H*-Fmoc); 8.33, 8.54 (two br s, 1H, NH); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 27.7$ ($FmocNCH_2CH_2$), 27.9 ($C(CH_3)_3$), 28.2 ($C(CH_3)_3$), 38.2 ($FmocNCH_2CH_2CH_2$), 46.0, 46.4 ($FmocNCH_2$), 47.2 (*CH*-Fmoc), 49.0, 49.3 ($NCH_2C(O)$), 67.4, 67.7 (CH_2-Fmoc), 79.6 ($C(CH_3)_3$), 83.2 ($C(CH_3)_3$), 119.8, 124.7, 124.9, 127.0, 127.1, 127.6, 141.2, 141.3, 143.8 (Ar*C*-Fmoc), 153.0, 153.1 (C=N) 156.1, 156.4 (Boc *C*=O and Fmoc *C*=O), 162.9, 163.1 (Boc C=O), 172.9, 173.3 (broad, $NCH_2C(O)OH$).

Ac-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH$_2$ (acetylated substance P, SP$_{1\text{-}11}$, 4): Immobilized undecapeptide **4** was assembled on an automatic ABI 433A Peptide Synthesizer using the ABI FastMoc 0.25 mmol protocols, as described for the preparation of **1.** The synthesis was carried out on TentaGel S RAM resin (1.09 g, capacity 0.23 mmol g^{-1}). The anchored undecapeptoid **4** thus obtained was deprotected and cleaved from the solid support by treatment with TFA (10 mL), thioanisole (0.5 mL), H_2O (0.5 mL), EDT (0.25 mL), and TIS (0.1 mL) for 2 h at room temperature. The reaction mixture was filtered and the residue was washed thoroughly with TFA (2 × 3 mL). The reaction mixture was concentrated in vacuo to a volume of approximately 1–2 mL and the residue was added dropwise to cold ether (60 mL). The precipitate was collected by centrifugation (2500 rpm, 10 min), the supernatant was decanted, and the pellet was resuspended in ether (60 mL) and centrifuged again. This was repeated twice. The pellet was then dissolved in HOAc (5%, ca. 15 mL) and lyophilized to give crude undecapeptide **4** (396 mg) as a white fluffy solid. Gel filtration over Sephadex LH-20 (eluent: MeOH/H_2O, 85/15, *v*/*v*) afforded almost pure peptide **4** (352 mg). An aliquot of the obtained peptide **4** (100 mg) was purified by preparative HPLC to obtain pure peptide **4** (84 mg) according to analytical HPLC. FAB MS: $m/z = 1389.7$ $[M+H]^+$.

Ac-NArg-Pro-NLys-Pro-NGln-NGln-NPhe-NPhe-Gly-NLeu-NMet-NH$_2$ (peptoid of SP$_{1\text{-}11}$; 5): Synthesis of immobilized undecapeptoid **5** was carried out using the same protocol as described above for the synthesis of **2**. The synthesis was carried out on TentaGel S RAM resin (1.09 g, capacity 0.23 mmol g^{-1}). Deprotection and cleavage from the resin was carried out as described for the preparation of **4**. Work-up was carried out as described for **4** and gave crude peptoid **5** (275 mg). After Sephadex LH-20 gel filtration (eluent: MeOH/H_2O, 85/15, *v*/*v*) and preparative HPLC, peptoid **5** was obtained in a yield of 26% (104 mg). The peptoid **5** was pure according to analytical HPLC. FAB MS: $m/z = 1389.7$ $[M+H]^+$.

Ac-NMet-NLeu-Gly-NPhe-NPhe-NGln-NGln-Pro-NLys-Pro-NArg-NH$_2$ (retropeptoid of SP$_{1\text{-}11}$; 6): Synthesis of immobilized retropeptoid **6** was carried out using the same protocol as described above for the synthesis of **2**. Deprotection and cleavage from the resin were carried out as described for the preparation of **4**. Work-up was carried out as described for **4** to afford crude retropeptoid **6** (335 mg). After Sephadex LH-20 gel filtration

(eluent: MeOH/H_2O, 85/15, *v*/*v*) and preparative HPLC, retropeptoid **6** was obtained in a yield of 33% (132 mg). The peptoid **6** was pure according to analytical HPLC. FAB MS: $m/z = 1389.7$ $[M+H]^+$.

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[1] A. Giannis, T. Kolter, *Angew. Chem.* **1993**, *105*, 1303 – 1326; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1244 – 1267.

[2] R. J. Simon, R. S. Kania, R. N. Zuckermann, V. D. Huebner, D. A. Jewell, S. Banville, S. Ng, L. Wang, S. Rosenberg, C. K. Marlowe, D. C. Spellmeyer, R. Tan, A. D. Frankel, D. V. Santi, F. E. Cohen, P. A. Bartlett, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9367 – 9371.

[3] H. Kessler, *Angew. Chem.* **1993**, *105*, 572 – 573; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 543 – 544.

[4] a) R. N. Zuckermann, J. M. Kerr, S. B. H. Kent, W. H. Moos, *J. Am. Chem. Soc.* **1992**, *114*, 10646 – 10647; b) R. N. Zuckermann, E. J. Martin, D. C. Spellmeyer, G. B. Stauber, K. R. Shoemaker, J. M. Kerr, G. M. Figliozzi, D. A. Goff, M. A. Siani, R. J. Simon, S. C. Banville, E. G. Brown, L. Wang, L. S. Richter, W. H. Moos, *J. Med. Chem.* **1994**, *37*, 2678 – 2685; c) R. J. Simon, E. J. Martin, S. M. Miller, R. N. Zuckermann, J. M. Blaney, W. H. Moos in *Techniques in Protein Chemistry V* (Ed.: J. W. Crabb), Academic Press, San Diego, CA, **1994**, 533 – 539; d) J. A. Gibbons, A. A. Hancock, C. R. Vitt, S. Knepper, S. A. Buckner, M. E. Brune, I. Milicic, J. F. Kerwin Jr., L. S. Richter, E. W. Taylor, K. L. Spear, R. N. Zuckermann, D. C. Spellmeyer, R. A. Braeckman, W. H. Moos, *J. Pharmacol. Exp. Ther.* **1996**, *277*, 885 – 899.

[5] a) J. A. W. Kruijtzer, R. M. J. Liskamp, *Tetrahedron Lett.* **1995**, *36*, 6969 – 6972; b) J. A. W. Kruijtzer, *Synthesis of Peptoid Peptidomimetics,* Thesis, Utrecht University, The Netherlands, **1996**.

[6] E. K. Bradley, *J. Magn. Reson. B* **1996**, *110*, 195 – 197.

[7] a) S. M. Miller, R. J. Simon, S. Ng, R. N. Zuckermann, J. M. Kerr, W. H. Moos, *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2657 – 2662; b) S. M. Miller, R. J. Simon, S. Ng, R. N. Zuckermann, J. M. Kerr, W. H. Moos, *Drug Dev. Res.* **1995**, *35*, 20 – 30.

[8] a) W. Heerma, C. Versluis, C. G. de Koster, J. A. W. Kruijtzer, I. Zigrovic, R. M. J. Liskamp, *Rapid Commun. Mass Spectrom.* **1996**, *10*, 459 – 464; b) W. Heerma, J. P. J. L. Boon, C. Versluis, J. A. W. Kruijtzer, L. J. F. Hofmeyer, R. M. J. Liskamp, *J. Mass Spectrom.* **1997**, *32*, 697 – 704; c) W. Heerma, J.-P. Boon, C. Versluis, *Tandem Mass Spectra of Peptides and Peptoids: Similarities and Differences*, Proc. 14th IMSC, Finland, **1997**, in press.

[9] G. J. Boks, *Structure and Conformational Behaviour of Peptoid Peptidomimetics. Towards an Understanding of the Mechanism of Action of Substance P and NK_1 Antagonists*, Thesis, Utrecht University, The Netherlands, **1997**.

[10] a) E. Atherton, R. C. Sheppard, *Solid-Phase Peptide Synthesis*, IRL, Oxford, England, **1989**; b) G. B. Fields, R. L. Noble, *Int. J. Pept. Protein Res.* **1990**, *35*, 161 – 214.

[11] a) J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, H. R. Morris, *Nature* **1975**, *258*, 577 – 579; b) A. L. Lomize, I. D. Pogozheva, H. I. Mosberg, *Biopolymers* **1996**, *38*, 221 – 234.

[12] a) K. J. Watling, S. Guard, *Neurotransmissions* **1992**, *VIII*, 1 – 6; b) N. Frossard, C. Advenier, *Life Sci.* **1991**, *49*, 1941 – 1953; c) J. E. Krause, Y. Takeda, A. D. Hershey, *J. Invest. Dermatol.* **1992**, *98 suppl.*, 2S – 7S; d) J. A. Lowe III, *Drugs Future* **1992**, *17*, 1115 – 1121; e) J. M. Lundberg, *Pharmacol. Rev.* **1996**, *48*, 113 – 178.

[13] The nomenclature is according to the proposed nomenclature by Simon et al. (ref. [2]). For the one letter code of amino acids, we have proposed the prefix n; thus for example nY, nF, and nL; see ref. [8a].

[14] G. I. Tesser, I. C. Balvert-Geers, *Int. J. Pept. Protein Res.* **1975**, *7*, 295 – 305.

[15] P. B. W. Ten Kortenaar, B. G. Van Dijk, J. M. Peeters, B. J. Raaben, P. J. H. M. Adams, G. I. Tesser, *Int. J. Pept. Protein Res.* **1986**, *27*, 398 – 400.

[16] A. Amstrong, I. Brackenridge, R. F. W. Jackson, J. M. Kirk, *Tetrahedron Lett.* **1988**, *29*, 2483 – 2486.

[17] a) H. Rink, *Tetrahedron Lett.* **1987**, *28*, 3787 – 3790; b) M. S. Bernatowicz, S. B. Daniels, H. Köster, *Tetrahedron Lett.* **1989**, *30*, 4645 – 4648.

[18] It was found that intramolecular aminolysis, that is, diketopiperazine formation was a serious by-reaction of a dipeptoid connected to TentaGel® S OH by an ester linkage. The use of Rink amide resin (TentalGel® S RAM), for the preparation of (peptoid) amides, entirely circumvented the problem of diketopiperazine formation.

[19] a) Y. Wu, G. R. Matsueda, M. Bernatowicz, *Synth. Commun.* **1993**, *23*, 3055 – 3060; b) M. Bernatowicz, Y. Wu, G. R. Matsueda, *Tetrahedron Lett.* **1993**, *34*, 3389 – 3392.

[20] a) This protection does appear to be sufficient to mask the nucleophilicity of the guanidino function. Ornithine formation through acylation of the unprotected ω-nitrogen during coupling and subsequent intramolecular decomposition during deprotection is completely avoided; b) A. S. Verdini, P. Lucietto, G. Fossati, C. Giordani, *Tetrahedron Lett.* **1992**, *33*, 6541 – 6542.

[21] $H_2N(CH_2)_4N(H)Boc$ (**7d**) and $H_2N(CH_2)_3N(H)Boc$ (**14**) were prepared according to: A. P. Krapcho, C. S. Kuell, *Synth. Commun.* **1990**, *20*, 2559 – 2564; $H_2N(CH_2)_2SCH_3$ (**7c**) was prepared by substitution of 2-bromoethylamine hydrobromide in ethanol with 2 equiv of $NaSCH_3$. The resulting 2-(methylthio)ethylamine was purified by vacuum distillation, b.p. 56 – 60 °C/20 mm Hg.

[22] The peptide was acetylated to facilitate purification.

[23] J. F. Westra-de Vlieger, L. J. F. Hofmeyer, J. A. W. Kruijtzer, W. Gommeren, J. Leysen, D. van Heuven-Nolsen, J. Wilting, F. P. Nijkamp, R. M. J. Liskamp, *Biochem. Pharmacol.*, submitted.

[24] E. Von Arx, M. Faupel, M. Bruggen, *J. Chromatogr.* **1976**, *120*, 224 – 228.

[25] For storage, the hydrochloride salt was prepared by dissolving the ester in ether followed by precipitation with a saturated solution of HCl in ether.

[26] P. Sieber, *Tetrahedron Lett.* **1987**, *28*, 6147 – 6150.

[27] *Applied Biosystems Model 433A Peptide Synthesizer User's Manual* **June 1993**, Version 1.0.

[28] *Applied Biosystems Research News* **June 1993**, Mode 433A Peptide Synthesizer, 1 – 12.

  0947-6539/98/0408-1580 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 8