Syntheses and CD-Spectroscopic Investigations of Longer-Chain β-Peptides: Preparation by Solid-Phase Couplings of Single Amino Acids, Dipeptides, and Tripeptides

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The synthesis and CD-spectroscopic analysis of eleven water-soluble β-peptides composed of all-β^3 or alternating β^2- and β^3-amino acids is described. Different approaches for the efficient syntheses of longer-chain β-peptides (> 9 residues) were investigated. They were synthesized on solid phase with Fmoc-protected amino acids or Fmoc-protected di- or tripeptide fragments (assembled using solution-phase synthesis). The use of preformed fragments significantly increased the purity of the crude peptides and facilitated purification. Especially, the use of Fmoc-protected β^2/β^3-dipeptides for the synthesis of a 'mixed' β^2/β^3-nonapeptide proved to be remarkably effective, yielding the crude peptide in 95% purity and without detectable epimerization of the β^2-amino acid residues. This is a significant improvement over previously reported procedures for the solid-phase synthesis of β-peptides, and foreshadows that the field of β-peptide research will now switch from synthesis to the design and study of complex functional 'β-proteins'.

1. Introduction. – Solid-phase peptide synthesis (SPPS) has advanced tremendously since the pioneering work of *Merrifield*, *Sheppard*, and others [1]. In fact, milestone achievements, such as the total synthesis of bradykinin and bovine insuline [2], as well as more-recent developments in combinatorial chemistry and solid-phase reagents [3] imply that this is, indeed, a very mature field. Yet, many standard SPPS protocols fail, mainly due to sequence-specific problems, such as folding and aggregation of the growing peptide chain [4]. Thus, large efforts are still made on improving the methods for SPPS, as witnessed from recent developments, such as chaotropic salt [5] and pseudo-prolines [6], invented to prevent folding during synthesis, and by several contributions on this topic at international conferences and proceedings.

Considering the obstacles related to α-peptide synthesis, it is not surprising that the solid-phase synthesis of unnatural oligomers, such as β-peptides, can be particularly cumbersome. β-Peptides, as well as other oligomeric organic molecules, *e.g.*, oligoureas, aza-peptides, and oligomers of α-aminoxy acids, have recently received much attention due to their propensity to form well-defined structures similar to those found in nature

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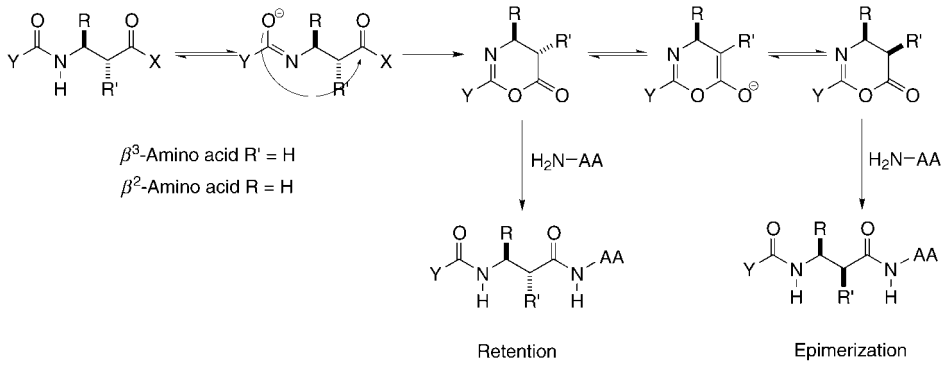
[7]. Hence, β-peptides may be designed to fold into defined secondary structures, like helices, turns, tubes, and sheets, with as few as six residues [8]. Furthermore, β-peptides displaying interesting biological properties, *e.g.*, antibacterial [9], antiproliferative [10], and somatostatin-mimicking [11] activities have been described and proven to be stable towards proteolytic [12] and metabolic [13] degradation.

While potentially pharmaceutically useful β-peptides are likely to be short and of low molecular weight [14], the natural expansion of our fundamental research on the folding and function of β-peptides is to assemble larger sequences capable of forming tertiary structures, *i.e.*, 'β-proteins'. Recently, we paved the way to this goal by reporting the first native chemical ligation between two unprotected β-peptide strands [15]. Concurrently, *Raines* and co-workers reported on 'hybrid' proteins, where a small β-dipeptide was introduced into RNase A by means of expressed-protein ligation [16].

Unifying for both these achievements is the need for effective syntheses of medium-to-long sequences of β-peptides by SPPS. The solid-phase synthesis of β-peptides differs from that of α-peptides in several aspects: *i*) increased folding during synthesis (the high propensity of β-peptides to form stable secondary structures is expected to influence the coupling and/or deprotection reactions), *ii*) expensive building blocks (although many β^3-amino acids are now commercially available, the high price or the labor spent on their preparation mandates that as little material as possible be used in each coupling), *iii*) lack of racemization (β^3-amino acids cannot racemize during activation and coupling, while β^2-amino acids can; *Scheme 1*). These differences suggest that certain variations of the typical SPPS protocol are needed to synthesize longer-chain β-peptides in an efficient and economic way.

Scheme 1. *Possible Racemization Pathway for β-Amino Acids During Coupling.* Activated β^2-amino acids may undergo racemization *via* cyclization to a dihydro-oxazinone, similar to α-amino acids, while β^3-amino acids are configurationally stable.

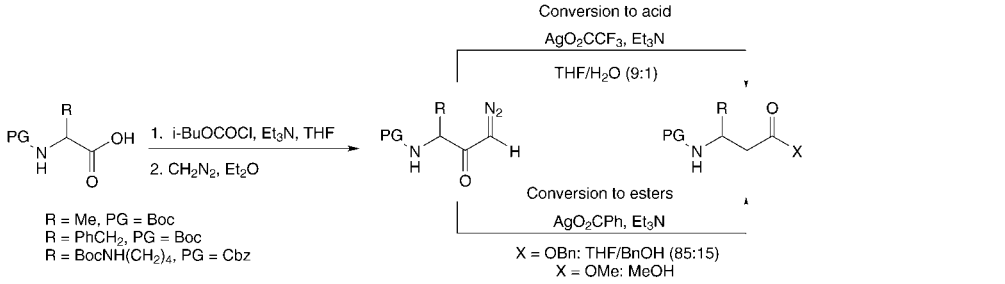


We have previously established that the standard protocol for α-peptide synthesis, as implemented on an automated peptide synthesizer, gave the Fmoc-protected derivative of H-β^3-HVal-β^3-HGlu-β^3-HOrn-β^3-HVal-β^3-HOrn-β^3-HGlu-β^3-HVal-OH as a by-product in the synthesis [8k]. Use of the stronger base DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) for Fmoc-deprotection overcomes this limitation, as demonstrated by the successful SPPS of a 24 amino acid β-peptide *via* single amino acid

couplings [17]. In this report, we wish to outline the strategies and methods used to prepare a large collection of longer-chain β-peptides [18], some of which have been used in previously reported biological studies.

2. Syntheses. – 2.1. *Synthesis of β-Amino Acids.* The required β^3-substituted amino acids were prepared from the commercially available α-amino acids by the well-established *Arndt–Eistert* homologation (**1**–**3** → **4**–**6**; *Scheme 2*).

Scheme 2. *Synthesis of β^3-Amino Acid Derivatives* **4**–**6**. The *Arndt–Eistert* homologation of protected α-amino acids yields β^3-amino acids. All carbamate protecting groups important for peptide chemistry are tolerated, and products with both protected and free COOH functions may be obtained.



(*S*)-**1a** R = Me, PG = Boc
(*R*)-**1b** R = Me, PG = Fmoc
(*S*)-**2a** R = $PhCH_2$, PG = Boc
(*S*)-**2b** R = $PhCH_2$, PG = Fmoc
(*R*)-**2b** R = $PhCH_2$, PG = Fmoc
(*S*)-**3a** R = $BocNH(CH_2)_4$, PG = Cbz
(*R*)-**3b** R = $BocNH(CH_2)_4$, PG = Fmoc

(*S*)-**4a** R = Me, PG = Boc, X = OH
(*R*)-**4b** R = Me, PG = Fmoc
(*S*)-**5a** R = $PhCH_2$, PG = Boc, X = OBn
(*S*)-**5b** R = $PhCH_2$, PG = Fmoc, X = OH
(*R*)-**5b** R = $PhCH_2$, PG = Fmoc, X = OH
(*S*)-**6a** R = $BocNH(CH_2)_4$, PG = Cbz, X = OMe
(*R*)-**6b** R = $BocNH(CH_2)_4$, PG = Fmoc, X = OH

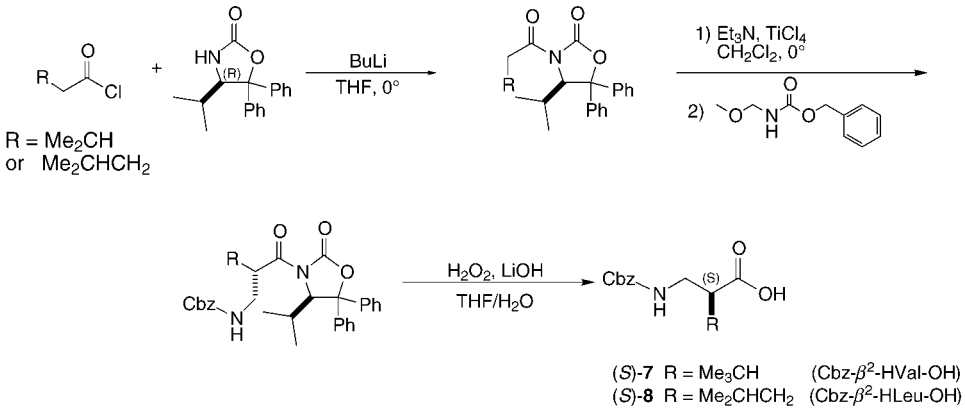
As previously shown by our group, this procedure is compatible with a large variety of *N*-protecting groups and allows the preparation of either the free acid or the corresponding ester-protected derivative [19].

The β^2-substituted amino acids **7** and **8** were obtained by diastereoselective amidomethylation of the titanium enolates formed from acylated oxazolidinone auxiliaries following a previously reported procedure [20] (*Scheme 3*).

2.2. *Synthesis of a Homologous Series of Amphiphilic β-Peptides by Coupling Tripeptide Building Blocks in the Solid Phase.* β-Peptides capable of forming amphiphilic 3_{14}-helices have been shown to have interesting biological properties. Our group reported in 1999 that a nine-residue-long β-peptide with the sequence H-(β^3-HAla-β^3-HLys-β^3-HPhe)$_3$-OH inhibits uptake of small-intestinal cholesterol and lipids by inhibiting the SR-BI protein [21]. Similar peptides with slightly different aliphatic side chains and peptide lengths have also been shown to have antibacterial and/or hemolytic activity [9][3]). The need for further biological investigations of this class of amphiphilic peptides prompted us to synthesize a series of β-peptides with the sequence H-(β^3-HAla-β^3-HLys-β^3-HPhe)$_n$-OH ($n = 3–6$). We intended to use these peptides for the following studies: *i*) pharmacokinetic investigations of the distribution of β-

[3]) Both the activity and selectivity of such amphiphilic peptides for mammalian *vs.* bacterial cells strongly depends upon the nature of the side chains [9d–f].

Scheme 3. *Synthesis of β²-Amino Acid Derivatives* **7** *and* **8**. Diastereoselective amidomethylation of the Ti enolate formed from an acylated oxazolidinone auxiliary yields Cbz-protected β^2-amino acids.



peptides in body tissue[4]), *ii*) investigations of antibacterial and hemolytic activities, of the TFA and HCl salts of the peptides with $n = 3-6$[5]), and *iii*) *in-vitro* and *in-vivo* studies of the cholesterol-inhibitory activity of the longer members of this series, *i.e.*, $n = 4-6$. The 15-residue peptide ($n = 5$), to be used for *in-vivo* cholesterol-inhibition studies, had to be prepared on a relatively large scale (*ca.* 1 g)[6]), and the nonapeptide ($n = 3$) needed for the pharmacokinetic study had to contain one ^{14}C-labeled amino acid.

These prerequisites, together with the repeating pattern of the sequence, made us decide to use a combined solution- and solid-phase synthesis[7]). We, thus, prepared the tripeptide unit Fmoc-(β^3-HAla-β^3-HLys(Boc)-β^3-HPhe)-OH in solution and then continued to couple this fragment on the solid support by Fmoc-based SPPS. We figured that this strategy would simplify the purification of the desired pentadecapeptide. Furthermore, the lack of complete coupling and deprotection would not be a serious obstacle since this would generate the shorter derivatives in the series, *i.e.*, the nonamer ($n = 3$) and the dodecamer ($n = 4$).

2.2.1. *Solution-Phase Synthesis of the Protected Tripeptide Fmoc-(β^3-HAla-β^3-HLys(Boc)-β^3-HPhe)-OH*. To follow an orthogonal protecting-group strategy throughout the synthesis and to be able to use Boc-protection[8]) of the Lys side chain during Fmoc SPPS, we chose the (benzyloxy)carbonyl (Cbz) protecting group for the N-terminus during tripeptide assembly. As outlined in *Scheme 4*, Cbz-β^3-HLys(Boc)-

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[4]) A pharmacokinetic study on the distribution of the ^{14}C-labelled derivative of the nonapeptide ($n = 3$) in rat has been reported recently [13].

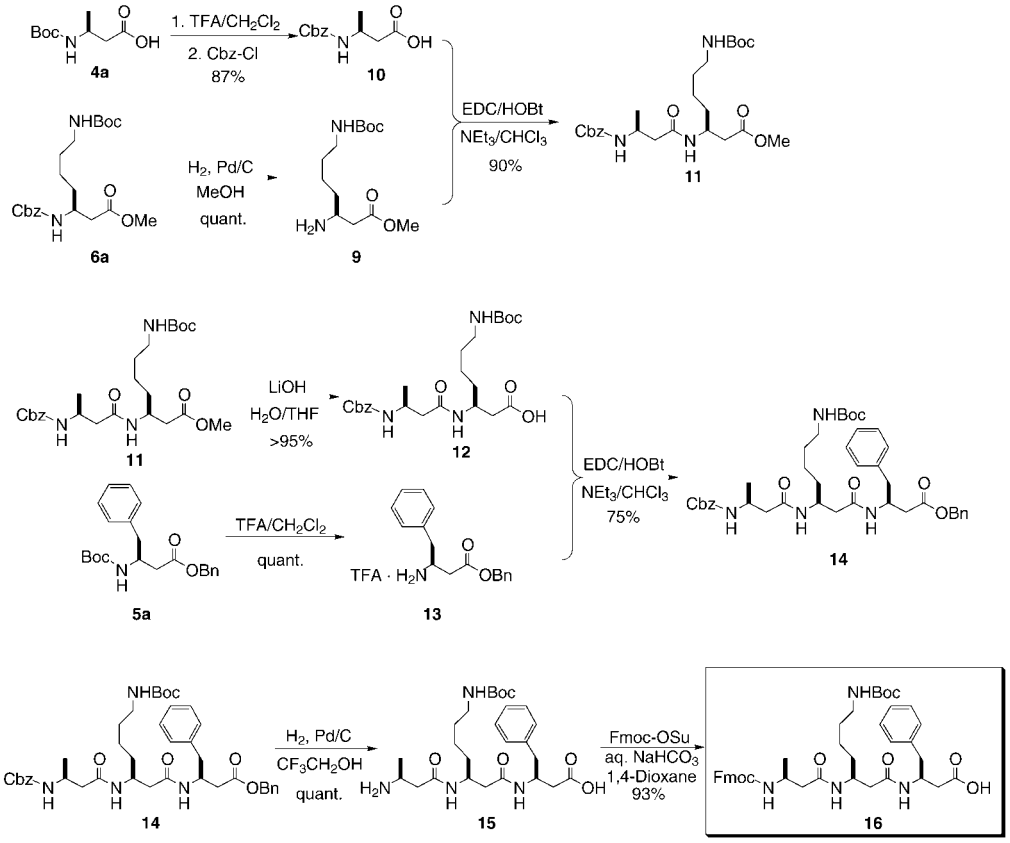
[5]) The weak antibacterial and low hemolytic activities of these peptides have been reported elsewhere [9c].

[6]) Any peptide chemist would consider this a huge amount of material, especially for a β-peptide.

[7]) *DeGrado* and co-workers reported an alternative method [9a]; in this case the Fmoc-protected tripeptide building block was prepared by SPPS on a highly acid-labile resin and then cleaved. This peptide was then used for solid-phase synthesis on *Rink* amide resin. This procedure is advantageous when smaller quantities of the peptide are to be prepared.

[8]) For full systematic names and specification of the abbreviations familiar to peptide chemists, see *Sect. 1* in the *Exper. Part*.

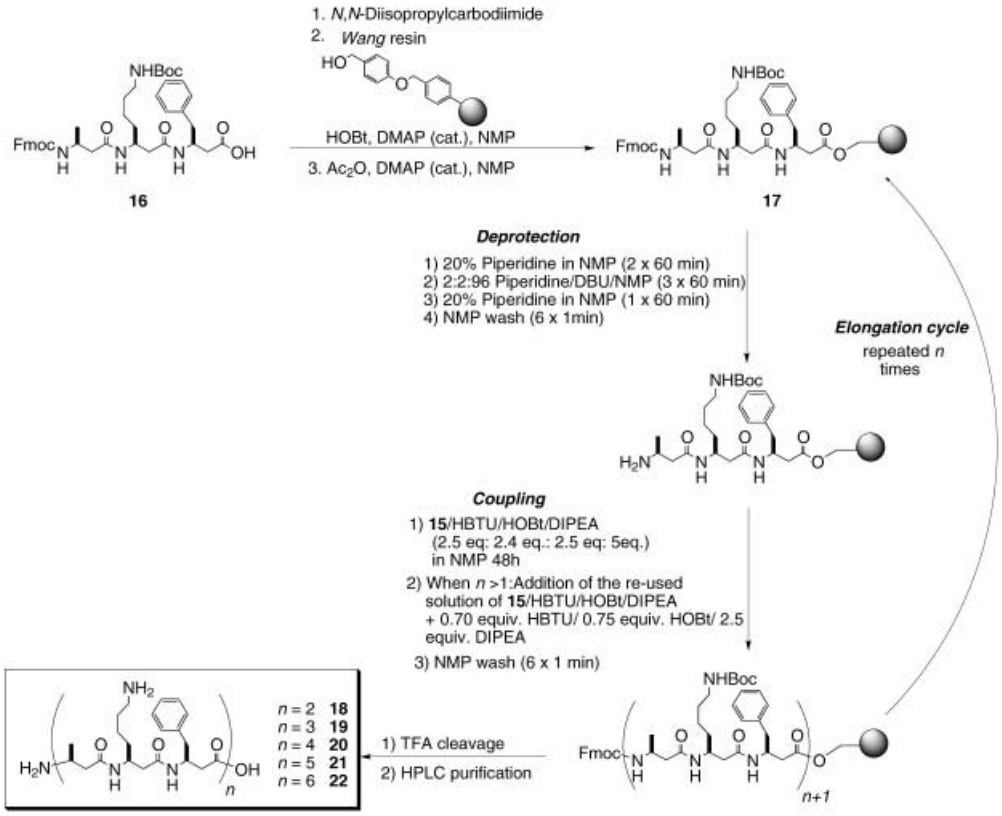
Scheme 4. *Solution-Phase Synthesis of the Fmoc-Protected Tripeptide* **16** (overall yield: 60%). For abbrev., see *Sect. 1* in the *Exper. Part.*



OMe (**6a**) was deprotected to **9** by means of catalytic hydrogenation, and the latter was coupled to Cbz-β^3-HAla-OH (**10**) by standard coupling procedures (EDC/HOBt)[8]). The dipeptide **11** was obtained in *ca.* 90% yield after column chromatography. Saponification of the ester by LiOH in THF afforded the free acid **12** in $>95\%$ yield. Coupling of the dipeptide acid to H-β^3-HPhe-OBn (**13**) gave the fully protected tripeptide **14** in *ca.* 75% yield. Due to the poor solubility of **14**, the catalytic hydrogenation had to be performed in trifluoroethanol; nevertheless, both terminal protecting groups were smoothly removed in quantitative yield leading to **15**. The Fmoc-group could be introduced with Fmoc-OSu in a dioxane/H_2O mixture in up to 93% yield. The total yield of the tripeptide **16** from the corresponding β^3-amino acids was 60% on a 5-g scale.

2.2.2. *Solid-Phase Synthesis of H-(β^3-HAla-β^3-HLys-β^3-HPhe)$_n$-OH* ($n=2-6$). The Fmoc-protected tripeptide **16** was anchored to *Wang* resin (initial loading: 0.99 mmol/g) with *N,N*-diisopropylcarbodiimide (DIC), HOBt, and a catalytic amount of DMAP[8]) (*Scheme 5*). The degree of anchoring (**17**), as determined by UV-spectro-

Scheme 5. *SPPS of H-(β^3-HAla-β^3-HLys-β^3-HPhe)$_n$-OH* (**18**–**22**, $n = 2-6$). For abbrev., see *Sect. 1* in the *Exper. Part*.



scopic analysis, was determined to be 0.50 mmol/g. This poor loading was probably due to the relatively low excess (3 equiv.) of the tripeptide (3 equiv.) employed.

The remaining free OH functional groups of the resin were capped with Ac_2O and a catalytic amount of DMAP, and the Fmoc group was removed with 20% piperidine in NMP and piperidine/DBU/NMP (2:2:96). The use of DBU is essential to achieve an efficient Fmoc deprotection when the growing peptide chain contains more than *ca.* 6 β-amino acid residues. The use of β^3-amino acids eliminates the risk of racemization and allowed us to develop a special procedure for chain elongation (*Scheme 5*). Initially, the resin was treated with 2.5 equiv. of tripeptide **16**, 2.4 equiv. of HBTU, 2.5 equiv. of HOBt, and 5 equiv. of DIPEA[8]). Invariably, when n was >1, the coupling reaction was not finished after 48 h, as determined by subjecting a small amount of the beads to the TNBS[8]) test. To keep the consumption of the tripeptide to a minimum, we then re-introduced the coupling mixture from the previous step. This solution, containing the activated tripeptide and DIPEA, was stored at $-18°$ when not used. To compensate for the probable hydrolysis of some of the activated peptide ester, we also added additional coupling reagents and DIPEA. This procedure of elongation and

deprotection was then repeated until the desired chain length ($n = 2-6$) was reached. Standard TFA cleavage and precipitation with Et_2O afforded the crude peptide as its TFA salt.

2.2.3. *Characterization, HPLC Analysis, and Purification of H-(β^3-HAla-β^3-HLys-β^3-HPhe)$_n$-OH* ($n = 2-6$). Initially, the synthesis was stopped at the hexapeptide level (**18**, $n = 2$), and part of the beads was removed for subsequent use in the preparation of the ^{14}C-labeled derivative [13]. A fraction of these beads was subjected to standard TFA cleavage, and the purity of the crude peptide was determined by HPLC (analytical reversed-phase C_{18} column). As expected, the crude hexapeptide was relatively pure (> 90%). Purification by preparative HPLC yielded 163 mg of pure **18** without major problems.

Next, the synthesis was stopped at the 15-mer stage, (**19**, $n = 3$) and the majority of the beads were subjected to TFA cleavage. In this case, the analytical HPLC chromatogram showed three major peaks with several minutes difference in retention times. Purification of this mixture by preparative HPLC and subsequent analysis by analytical HPLC revealed the 9-mer (**19**), 12-mer (**20**), and 15-mer (**21**) in a ratio of 1:2:15[9]). This estimation corresponds well with 34 mg of isolated 9-mer, 58 mg of 12-mer, and 359 mg of 15-mer. Notably, in total, > 800 mg pure pentadecapeptide **21** was isolated to be used in subsequent *in-vivo* experiments (this required repetition of the described solid-phase synthesis and *ca.* 20 injections on the HPLC system at our disposal!).

The remaining fraction of beads was subjected to one final coupling to produce the 18-mer **22** ($n = 6$). Analysis of this peptide by analytical HPLC revealed the presence of the shorter analogs **19**, **20**, and **21**. Again, the different peptides were easily separated by preparative HPLC, yielding 91 mg of the pure octadecapeptide **22**. To our surprise, a small fraction with a longer retention time was also seen in the chromatogram, which was shown by ESI-MS to correspond to the 21-mer. Clearly, the longer reaction times must have caused partial deprotection of the Fmoc group. This problem was painstakingly obvious in the synthesis of peptide **19** by the conventional 'one-amino-acid-at-a-time' procedure described below.

2.3. *Solid-Phase Synthesis of β^3-Peptides by Single-Amino-Acids Couplings.* – 2.3.1. *Solid-Phase Synthesis of all-(R)-H-(β^3-HAla-β^3-HLys-β^3-HPhe)$_3$-OH*. The enantiomer *ent*-**19**, composed solely of (*R*)-β^3-amino acids[10]), was prepared according to the improved solid-phase procedure[11]). In this case, the first amino acid was anchored to *Wang* resin (0.99 mmol/g initial substance, 0.63 mmol/g loading), and SPPS was completed through standard 'one-amino-acid-at-a-time' coupling. Positive TNBS tests indicating *incomplete* couplings after 3 h were experienced after coupling residues number five and eight. Prolonged reaction time or surcharge of additional amino acid and activating agents were then needed in order to complete the reactions. Notably, crude *ent*-**19** had a purity of only 12%, *i.e.*, much lower than the one produced by fragment coupling. Furthermore, several peaks had similar retention times, which made

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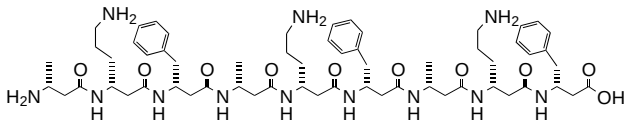
[9]) The area under each peak was divided by the number of chromophores present in each molecule.

[10]) The proteolytic stability of this *all-*(*R*)-β^3-peptide has previously been reported [12c].

[11]) The peptide was assembled under the same conditions as those given in *Scheme 4*, with the exception that 4 equiv. of the activated amino acid was used.

the preparative HPLC purification less straightforward than the fragment-coupling approach. Nevertheless, 15 mg of the nonapeptide *ent*-**19** could be obtained in 95% purity.



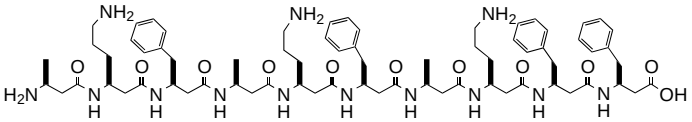
ent-**19**

The low efficiency of the above synthesis agrees with our general experience that the main difficulty with solid-phase synthesis of β-peptides is faced when the growing peptide chain is between six and ten residues long. Despite improvements with Fmoc deprotection, problems associated with the coupling steps, as judged from TNBS tests, are frequently encountered at this stage. These problems are probably related to the pronounced folding tendency of β-peptides, especially those composed of six to ten residues.

2.3.2. *Solid-Phase Synthesis of H-(β^3-HAla-β^3-HLys-β^3-HPhe)$_3$-(β^3-HPhe)$_n$-OH* ($n = 1-3$). Following the synthesis of **21** *via* the tripeptide-condensation approach described above, we attempted the synthesis of a larger quantity of the nonapeptide **19** using the standard 'one-amino-acid-at-a-time' method. Believing that all hurdles for successful SPPS of this kind of β-peptide had been overcome, we initiated the synthesis by anchoring the first amino acid to the *Wang* resin using 4 equiv. of amino acid, DIC, and a catalytic amount of DMAP for 24 h. The resin was then carefully washed, shrunk, and dried *in vacuo*. The degree of substitution, as determined spectrophotometrically after Fmoc deprotection, was found to be extraordinarily low (< 0.1 mmol/g). Thus, the loading reaction was repeated once more – now with 3 equiv. of amino acid – which resulted in a loading of 0.25 mmol/g. A third attempt was made with 3 equiv. of amino acid. This time, the degree of substitution was found to be 0.5 mmol/g. Finally, a fourth loading with only 0.5 equiv. of amino acid was performed before the resin was capped with Ac_2O.

The solid-phase assembly of the peptide was continued despite the low initial loading. No further complications were observed during the chain elongation, as judged from TNBS tests after each coupling step. The peptide was cleaved from the resin and the crude product analyzed by analytical HPLC. However, to our great surprise, a complex mixture of peptides appeared to be present. Furthermore, none of the signals had a retention time identical to that of the previously characterized nonapeptide **19**. Three main peaks in a ratio of *ca.* 1:3:1 separated by *ca.* 4 min were observed, each with a longer retention time than **19**. The sample was purified by preparative HPLC, and three fractions were collected. Analysis by ESI-MS showed that these products corresponded to H-(β^3-HAla-β^3-HLys-β^3-HPhe)$_3$-(Phe)$_n$-OH ($n = 1$, 2, and 3), *i.e.*, **23**, **24**, and **25**, respectively.

Obviously, the C-terminal part of these peptides (two to four HPhe residues) was formed during the repeated loading of the resin. The low degree of substitution must have been caused by the loss of the Fmoc group during the coupling or washing steps. A careful analysis of the solvents used revealed no inconsistencies. Thus, the uncontrolled



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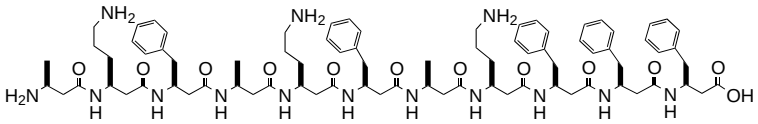

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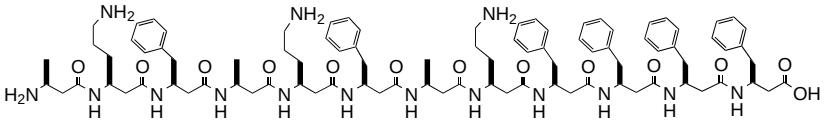

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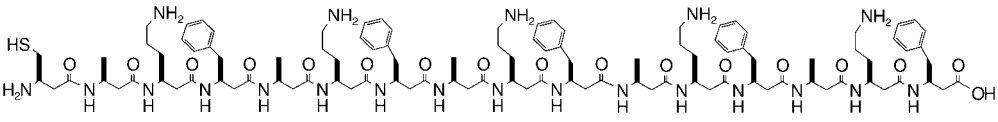
Fmoc deprotection must have taken place during the loading steps themselves[12]). Most likely, catalytic amounts of DMAP caused this deprotection during the long reaction times, since it seems unlikely that 1,3-diisoproylcarbodiimide (DIC) or the corresponding urea compound are basic enough in DMF to promote Fmoc deprotection[13])[14]).

2.3.3. *Solid-Phase Synthesis of the Hexapeptide H-β³-HCys-(β³-HAla-β³-HLys-β³-HPhe)₅-OH.* Part of the beads remaining from the 15-mer synthesis (*cf. Sect. 2.2.3*) were used to attach an *N*-terminal β³-HCys residue, producing **26** (for possible thioligation [15] or dimerization to the corresponding disulfide-linked peptide containing 32 β-amino acids).



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Hexadecapeptide **26** was obtained by adding Boc-β³-HCys(Trt)-OH[15]) to the resin containing bound **19**–**21**. The addition of this single amino acid proceeded smoothly and provided the crude homocystein-substituted derivatives after cleavage and

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[12]) This observation also explains why small quantities of the corresponding 21-mer peptide H-(β³-HAla-β³-HLys-β³-HPhe)₇-OH were isolated in the synthesis described in *Sect. 2.2.3*.

[13]) A 10% solution of DMAP is known to affect Fmoc-deprotection with $t_{1/2} = 85$ min [22]; however, in this case, only a catalytic amount of DMAP was used.

[14]) Partial deprotection of the Fmoc group during the normal peptide coupling would not be completely surprising since DIPEA is known to cause gradual Fmoc deprotection after prolonged reaction times [22].

[15]) The decision to use a Boc-protected amino acid was rationalized by the availability of this amino acid [23a,b] and the fact that this should be the *N*-terminal amino acid.

deprotection. Although the whole series of peptides could again be obtained after HPLC purification, only the hexadecamer **26** was collected and characterized.

In summary, a number of hurdles may be encountered when using the conventional 'single-amino-acid-at-a-time' procedure for the preparation of longer-chain β-peptides. It was assumed that longer reaction times during the loading and coupling steps could compensate for the high price of β-amino acids, especially since β^3-amino acids do not racemize. However, as evidenced by these observations, *care must be taken regarding the reaction times as well*, especially during the loading step (Fmoc deprotection!).

2.4. *Synthesis of a Water-Soluble β-Peptide Capable of Forming a 12/10-Helix, by Solid-Phase Fragment Couplings of β^2/β^3-Dipeptides.* The synthesis of peptides containing β^2-amino acids represents a special challenge, since β^2-amino acids may, in contrast to their β^3-analogs, can racemize during activation (*via* oxazinone formation, *cf. Scheme 1*). This racemization may be suppressed by means of less-strongly activating agents and by keeping the temperature low. However, these factors, especially the temperature, are difficult to control during SPPS; in fact, partial racemization was reported in our previous syntheses of β^2-amino acid containing peptides [19d].

Bearing these obstacles in mind, we figured that our combined solution- and solid-phase approach would be especially attractive for the preparation of peptides containing β^2-amino acids. To demonstrate this, we set out to prepare the nonapeptide *all*-(*S*)-H-β^3-HPhe-β^2-HVal-β^3-HPhe-β^2-HLeu-β^3-HLys-β^2-HLeu-β^3-HLys-β^2-HLeu-β^3-HPhe-OH (**34**) (*Schemes 6–8*).

Initially, the dipeptide building blocks Fmoc-β^2-HVal-β^3-HPhe-OH (**28**), Fmoc-β^2-HLeu-β^3-HPhe-OH (**30**), and Fmoc-β^2-HLeu-β^3-HLys(Boc)-OH (**33**) were prepared in solution, carbodiimide-mediated couplings (*Schemes 6* and *7*)[16]). The couplings were run at 0° in the presence of HOBt to avoid racemization. Typically, the fully protected dipeptides **27**, **29**, and **31** were obtained in >90% diastereoisomer purity[17]), which could be increased by recrystallization to >98%, as judged from NMR and HPLC analysis. *C*-Terminal deprotection and exchange of the *N*-terminal protection from Boc- or Cbz groups for Fmoc (**27** → **28**, **29** → **30**, and **31** → **32** → **33**) provided the desired dipeptide building blocks.

Having the necessary building blocks at hand, we started the solid-phase synthesis. Prudent by our accumulated experiences, we again looked for possible improvements in the procedures used. In order to avoid the presence of DMAP during the loading, we instead turned to MSNT[8]) [24]. This procedure worked amazingly well. Despite the fact that only 3 equiv. of dipeptide were employed, the ester formation with the *Wang* resin proceeded in 82% yield. This is far more efficient than our previous syntheses on *Wang* resins, even when a larger excess of amino acid or peptide was used. All couplings proceeded smoothly and were finished within 24 h, as judged by TNBS tests. The high coupling efficiency is remarkable, considering that 2 equiv. of the Fmoc-protected dipeptide was used at most. In fact, the crude peptide **34** was found to be *ca.* 95% pure

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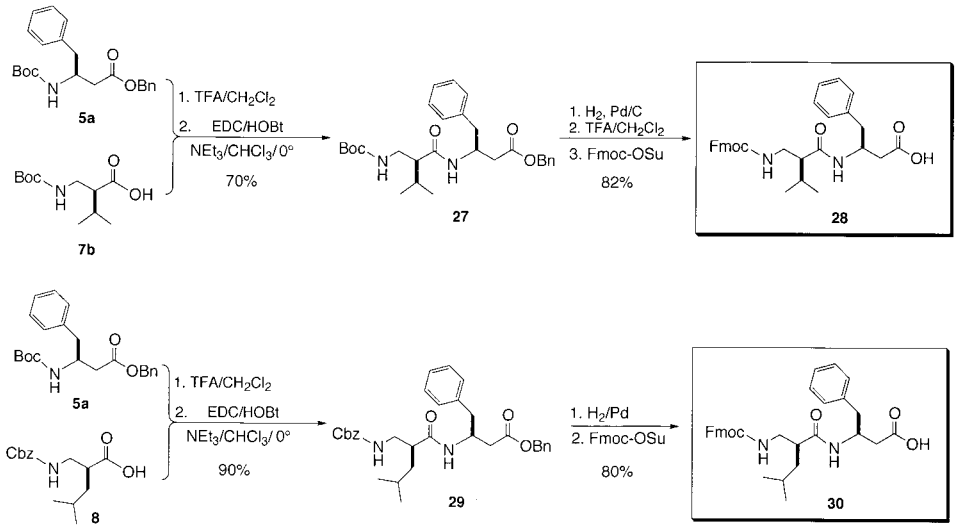
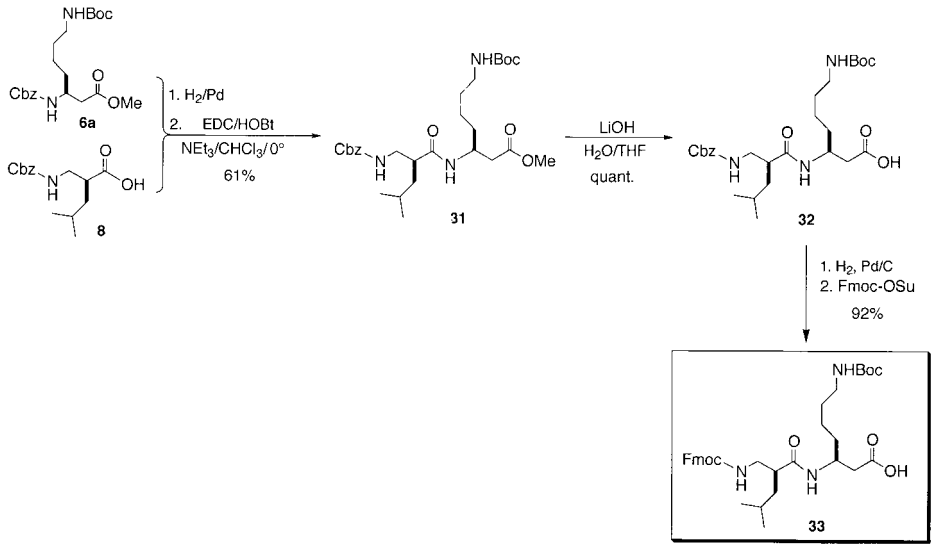
[16]) The Boc-protected H-β^2-HVal-OH (*Scheme 6*) was prepared from the Cbz-protected derivative (*Scheme 3*). The only reason for using the Boc-protected amino acid was the fact that a sample was available in our laboratory [23c].

[17]) The peptide coupling is expected to proceed without notable racemization under these conditions. The main source of configurational impurity is associated with the lack of complete stereoselectivity in the preparation of the β^2-amino acid.

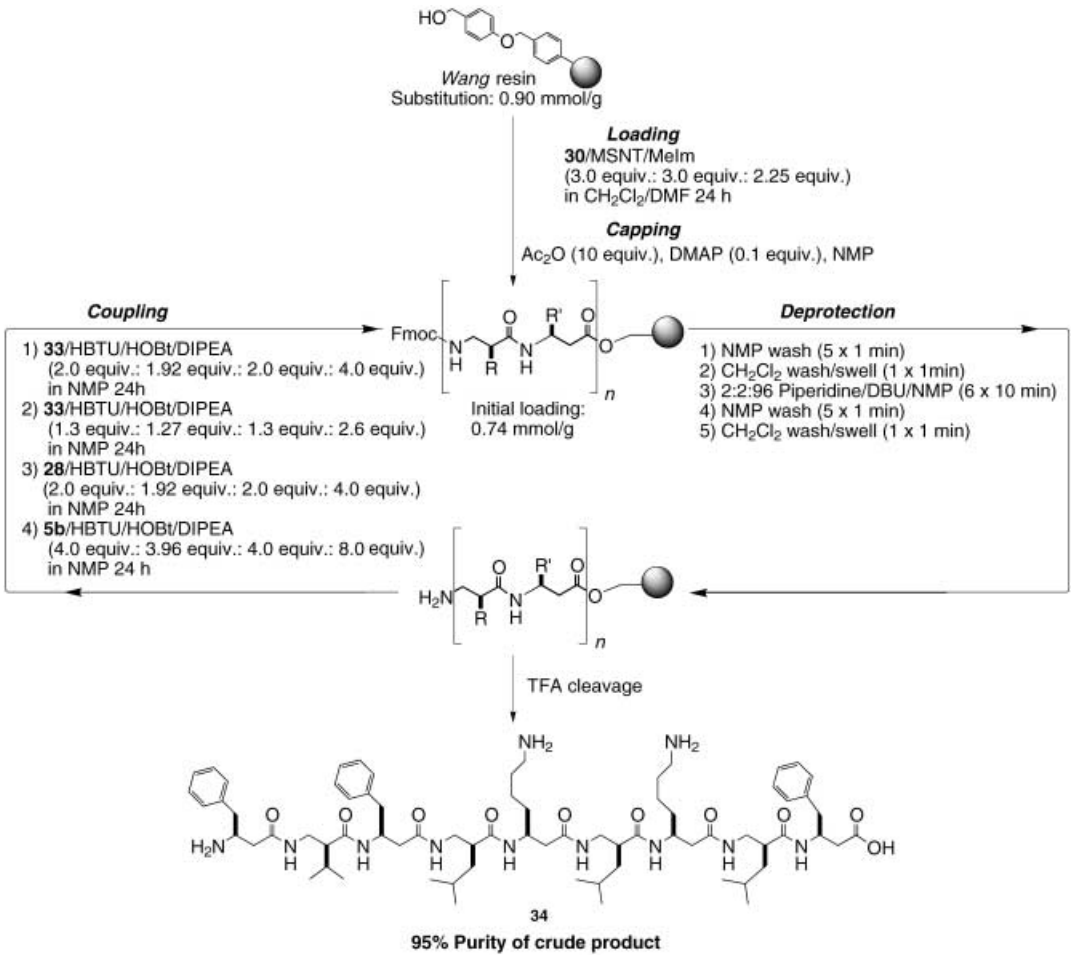
Scheme 6. *Solution-Phase Synthesis of the Fmoc-Protected Dipeptide Building Blocks* **28** *and* **30**. Carbodiimide mediated coupling at 0° in the presence of HOBt efficiently suppresses racemization of the β^2-amino acids. Recrystallization of the fully protected dipeptides yields highly diasteroisomerically enriched materials (> 95% pure) for SPPS. For abbrev., see *Sect. 1* in the *Exper. Part*.



Scheme 7. *Solution-Phase Synthesis of the Fmoc-Protected Dipeptide Building Block* **33** (in diasteroisomerically enriched form, > 98%). For abbrev., see *Sect. 1* in *Exper. Part*.



Scheme 8. *Solid-Phase Synthesis of the 'Mixed' β-Peptide* **34**. The MSNT/MeIm protocol greatly increases the efficiency of loading of the first dipeptide building block **30** onto the *Wang* resin. Subsequent chain elongation with only 2 equiv. of the building blocks proceeded surprisingly well, yielding crude **34** in 95% purity. For abbrev., see *Sect. 1* in the *Exper. Part*.



after TFA cleavage! This is, so far, the most-effective solid-phase synthesis of a β-peptide ever conducted in our laboratories.

The reason(s) for the high efficiency of this synthesis is probably related to the slight modifications of the protocol in *Scheme 8* and to the β^2/β^3-sequence, which might be less prone to folding during synthesis, and which is known to give rise to better solubility [8d][18]).

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[18]) The sequence of alternating β^2- and β^3-amino acids is expected to fold into a *12/10*-helix [8d,e,o]. This helix lacks a net dipole moment, which might affect its folding propensities under the conditions used for peptide synthesis. β^2/β^3-Peptides with aliphatic side chains are highly soluble in organic solvents ($CHCl_3$, AcOEt), in contrast to their β^3-counterparts [8d].

3. Circular-Dichroism Studies. – Circular dichroism (CD) is a tool widely used for structural investigations of peptides and proteins. In contrast to α-peptides, where an established correlation between CD spectra and secondary structures exists, such reliable correlations are only beginning to accumulate for unnatural oligomers [25]. Thus, care should be taken when interpreting the CD spectra of β-peptides, at least until more structures are unambiguously determined by alternative methods, such as NMR spectroscopy and X-ray diffraction. Nevertheless, CD spectra are useful for obtaining a first hint of the conformational preference of β-peptides. The CD spectra of **19**–**22**, **23**–**26**, and **34** are presented in *Figs. 1–5*.

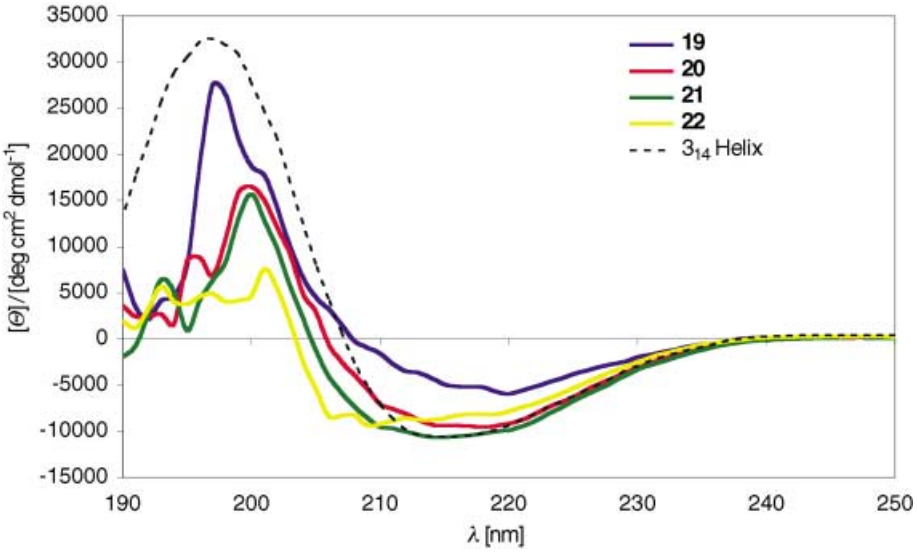

Fig. 1. *CD Spectra of the β-Peptides* **19**–**22** (TFA salts). The spectra were recorded at 20.0° in MeOH at a conc. of 0.2 mM. The spectrum of **19** resembles that of a 'typical' 3_{14}-helix (dotted line), while the appearance of the spectra of **20**–**22** are different.

The normalized CD spectrum of a β-heptapeptide adopting an (*M*)-3_{14}-helix in MeOH solution, as confirmed by NMR spectroscopy, is shown in *Fig. 1* [8k]. This CD pattern (maximum near 200 nm, zero crossing between 205 and 210 nm, minimum between 215 and 220 nm) has been observed in all cases where a 3_{14}-helical conformation was established [8a,h,i,k]. Although recent theoretical studies have shown that similar CD spectra may result from different conformations [26], our practical experience suggests the following about the CD spectra of β-peptides adopting the 3_{14}-helical conformation: *i*) a 'stable' 3_{14}-helical conformation usually displays both a positive and a negative band, *ii*) the intensity of the minimum between 215 and 220 nm correlates with the stability of the 3_{14}-helical conformation, and *iii*) a low intensity of the maximum near 200 nm is often associated with both a broadening of

the minimum and a hypsochromic shift interpreted as a loss of secondary structure[19]). For the peptides **19**–**22** shown in *Fig. 1*, these guidelines would suggest that the β-nonapeptide **19** adopts a 3_{14}-helical conformation in MeOH (the overall shape of the spectrum), while the longer analogs **20**–**22** adopt alternative conformations to larger extents when the chain lengths increase (less-intense maxima, broader minima at shorter wavelength). This suggestion agrees with the fact that there are no indications (CD, NMR, X-ray), so far, that β-peptides longer than 12 residues and containing proteinogenic amino acid side chains adopt a 3_{14}-helical conformation in the absence of special additives[20]).

The CD spectra of the two enantiomeric β-nonapeptides (**19** and *ent*-**19**) composed of *all*-(*S*)-β^3- and *all*-(*R*)-β^3-amino acids, respectively, are shown in *Fig.* 2. Clearly, these β-peptides adopt 3_{14}-helices of different handedness, *i.e.*, **19** an (*M*)-3_{14}-helix and *ent*-**19** a (*P*)-3_{14}-helix.

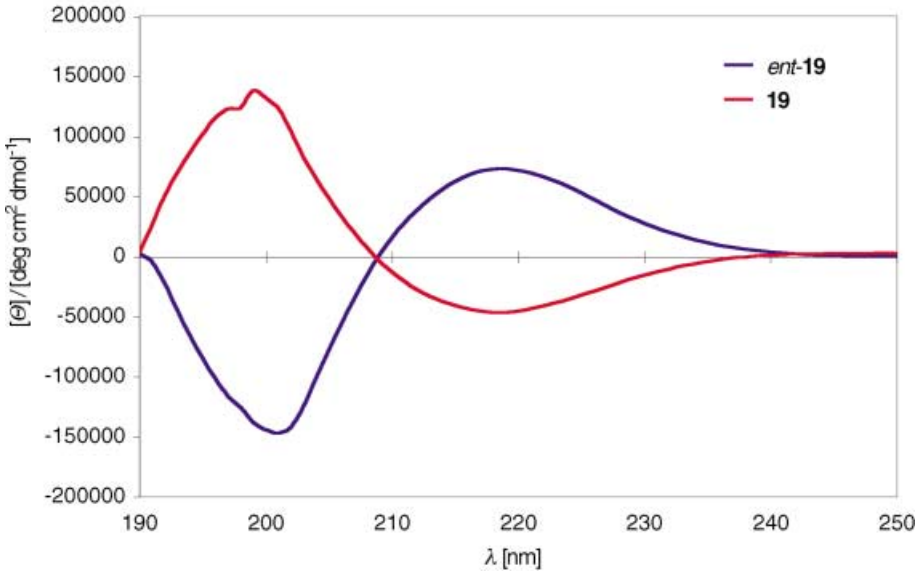

Fig. 2. *CD Spectra of the enantiomeric* β*-peptides* **19** *and* ent-**19**. The spectra were recorded at 20.0° in MeOH at a conc. of 0.2 mM. Peptide **19** was present as the HCl salt[24]), *ent*-**19** as the TFA salt. Both peptides show spectra characteristic for the 3_{14}-helical conformation; the slight difference in intensity is probably a result of the different salts.

In *Fig. 3*, the normalized CD spectra of β-decapeptide **23**, β-undecapeptide **24**, and β-dodecapeptide **25** are shown together with the spectrum of the 'parent' β-peptide **19**. Obviously, the minimum around 215 nm intensifies when the chain length is increased

[19]) Similar changes in the CD spectra were observed for a β-dodecapeptide when the solvent was changed from MeOH to H_2O [27a]. Subsequent NMR spectroscopic analysis established that this β-peptide has a 3_{14}-helical conformation in MeOH, but is disordered in H_2O [27b]. Salt bridges may be used to stabilize the 3_{14}-helix [8k].

[20]) CD Measurements in the presence of phospholipid micelles and vesicles indicate formation of an amphiphilic 3_{14}-helix for **21** (data not shown) and similar β-peptides [9a,d].

by β^3-HPhe residues, (*cf.* **19**, **23**, and **24**). This trend is not observed for the dodecamer **25** (loss of intensity of the minimum at 215 nm and of the maximum at 200 nm). Our experience would suggest that the stability of the 3_{14}-helical conformation increases up to the undecapeptide stage, while longer peptides appear to prefer this conformation to a lesser extent.

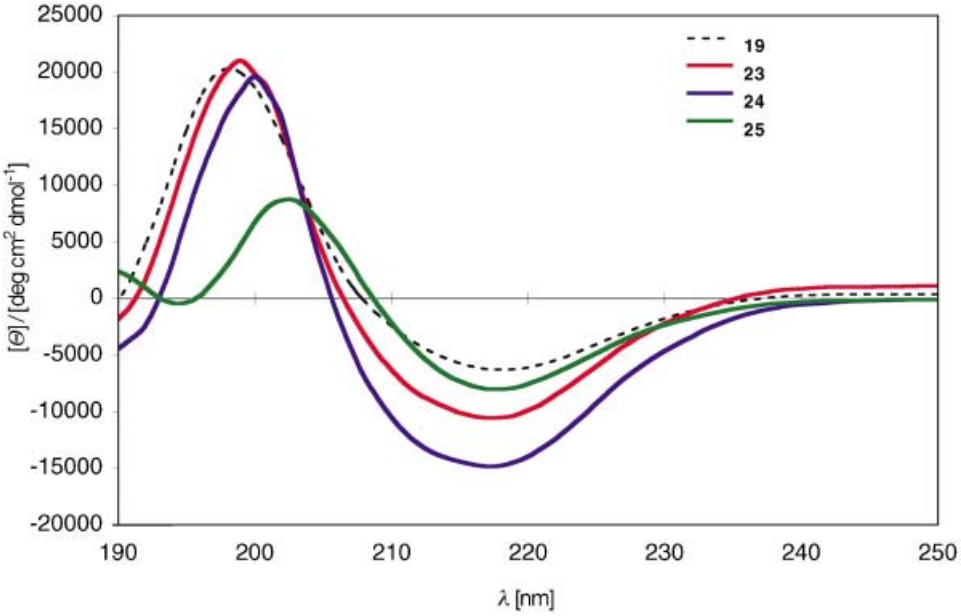

Fig. 3. *CD Spectra of β-peptides* **23**–**25** compared to **19**. All spectra were recorded at 20.0° in MeOH at a conc. of 0.2 mM. All peptides were measured as their TFA salts. The minimum near 215 nm intensifies with increasing chain length (addition of β^3-HPhe residues; *cf.* **19**, **23**, and **24**). The dodecamer **25** differs from this trend, and the overall shape of the spectrum changes, perhaps indicating that the hydrophobic part of this molecule tends to adopt a 3_{14}-helical conformation.

The CD spectrum of the homocysteine-containing β-peptide **26** is shown in *Fig. 4*, together with the spectrum of the 'parent' compound **21**. Again, comparison suggests that the β-peptide composed of more than twelve residues is less prone to adopt a 3_{14}-helical conformation. In this case, the additional residue leads to a CD spectrum with an even broader minimum at shorter wavelength. These spectra closely resemble those obtained for a β-tetracosapeptide in MeOH and H_2O [17][21]).

The 'mixed' β-peptide **34**, composed of alternating β^2- and β^3-amino acid residues, is expected to adopt the so-called 12/10-helix. High-resolution methods have established that the CD spectrum of molecules adopting this conformation is characterized by a very strong maximum near 205 nm [8d,e,o]. As can be seen in *Fig. 5*, the CD spectrum of the nonapeptide **34** shows this typical pattern. Therefore, this peptide most likely adopts the 12/10-helical conformation in MeOH solution[22]).

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[21]) In this case, the dispersion of the NH region in ^{1}H-NMR spectra points to a disordered structure [17] (see also [27]).

[22]) A detailed NMR-spectroscopic analysis of **34** is in progress and will be reported in due course.

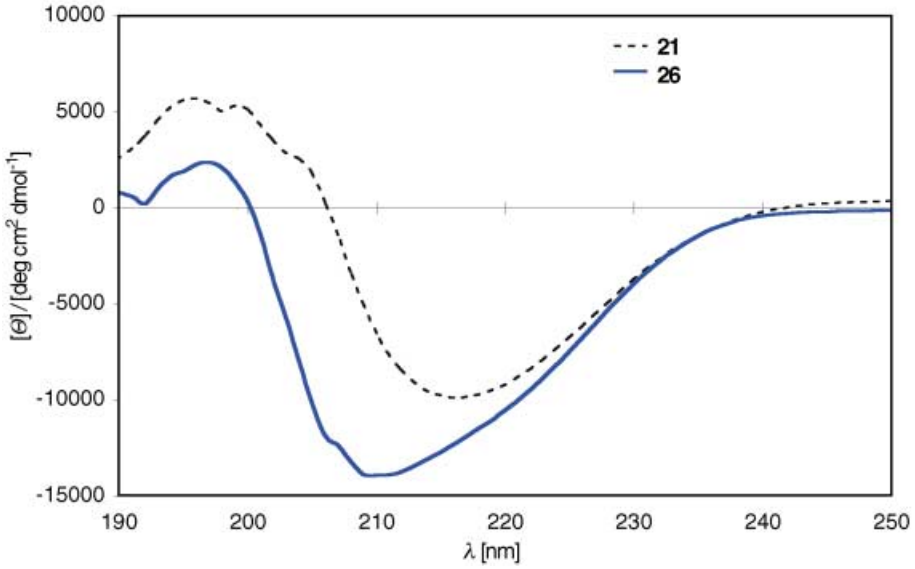

Fig. 4. *CD Spectrum of the homocysteine-containing β-hexadecapeptide* **26** *relative to the pentadecamer* **21**. Both spectra were recorded at 20.0° in MeOH at a conc. of 0.2mM. Both peptides were present as their TFA salts. The minimum observed near 215 nm for **21** is shifted hypsochromically in the case of **26**. Similar spectra have been observed before and correspond, most likely, to less-ordered conformations.

4. Conclusions. – In this report, we have tried to impart our experiences on the SPPS of longer-chain β-peptides. From the various approaches and protocols investigated, we conclude that the initial loading of the first building block to *Wang* resin is best performed using the MSNT/MeIm procedure[8]), which is efficient even when a small excess of the amino acid partner is used in the reaction. Alternatively, other resins with different anchoring groups may be used to increase the loading, which clearly is the most material-consuming step. Amide-bond formation during chain assembly may be executed with less than 2 equiv. of the amino acid or peptide to be coupled since the coupling time may be substantially longer than that commonly used in α-peptide synthesis. A longer coupling time is allowed when the COOH group of a β^3-amino acid is activated, since such amino acids do not racemize through the oxazinone pathway. However, coupling times longer than 48 h should be avoided since the DIPEA may cause partial Fmoc deprotection.

The most-critical step during chain elongation is encountered when the growing β-peptide chain is between seven and ten residues long, as witnessed by incomplete Fmoc-deprotection and longer coupling times. This may indicate that the optimal stability of the 3_{14}-helical conformation of β-peptides is found with six to twelve *all*-β^3-amino acid residues (even in solvents like DMF and NMP). For the synthesis of longer-chain β-peptides, it is, thus, advantageous to decrease the number of couplings in this range by using preformed, Fmoc-protected di- or tripeptide building blocks. The use of preformed β^2/β^3-dipeptides as building blocks for the synthesis of 'mixed' β^2/β^3-peptides is particularly valuable, since this procedure completely eliminates epimerization at the

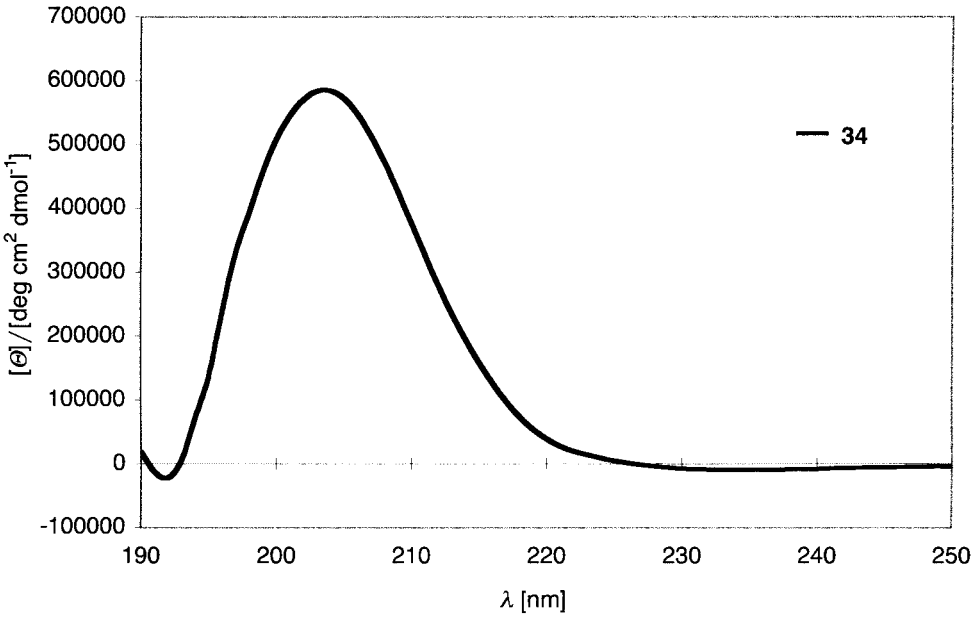

Fig. 5. *CD Spectrum of the mixed β^2/β^3-nonapeptide* **34** (TFA salt). The spectrum was recorded at 20.0° in MeOH at a conc. of 0.2mM. The very strong maximum observed near 205 nm is characteristic for the 12/10-helical conformation.

β^2-amino acid moiety. This approach is, therefore, recommended for the incorporation of β^2-amino acids in solid-phase (peptide) synthesis. The high overall efficiency of the protocol (purity of crude product $>95\%$) is surprising and could be explained by the stability of the 12/10-helical conformation in solvents like DMF and NMP.

Finally, the CD spectroscopic studies, although not as reliable as high-resolution methods, suggest that β-peptides with a chain length exceeding twelve residues composed of *all-*β^3-amino acids might have a reduced tendency to adopt the 3_{14}-helical conformation. This implies that special precautions and detailed design are required before longer elements of β-peptidic secondary (or even tertiary) structures are prepared. Yet, the lessons learned from the present investigation have taken us several steps closer to the ultimate goal of production of *de novo* designed 'β-proteins'!

We thank the *Swedish Foundation for International Cooperation in Research and Higher Education* (*STINT*) (*P.I.A.*) and the *Deutscher Akademischer Austauschdienst (Hochschulsonderprogramm III)* (*J. F.*) for postdoctoral fellowships. *Dr. S. Abele*, *Dr. A. Jacobi*, and *Dr. A. Böhm* kindly supplied samples of various amino acid derivatives. Continuing generous support from *Novartis Pharma AG* (Basel) is gratefully acknowledged.

Experimental Part

1. *General.* Abbreviations: Boc = (*tert*-butoxy)carbonyl, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DIC = 1,3-diisopropylcarbodiimide, DIPEA = *N*,*N*-diisopropyl(ethyl)amine, DMAP = 4-(dimethylamino)pyridine, DMF = dimethylformamide, EDC = 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide hydrochloride, FC = flash chromatography, Fmoc-OSu = *N*-{[(9*H*-fluoren-9-yl-methoxy)carbonyl]oxy}-succinimide, GP = general procedure, HBTU = *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate,

HOBt = 1-hydroxy-1*H*-benzotriazole hydrate, MeIm = 1-methyl-1*H*-imidazole, MSNT = 1-(mesitylsulfonyl)-3-nitro-1*H*-1,2,4-triazole, NMM = *N*-methylmorpholine, NMP = *N*-methylpyrrolidin-2-one, PE = petroleum ether (40/60), TFA = trifluoroacetic acid, TFE = 2,2,2-trifluoroethanol, TIS = triisopropylsilane, TNBS = 2,4,6-trinitrobenzenesulfonic acid; three-letter amino acid abbreviations are used for α-amino acids (Xaa), β-HXaa = β-homoamino acid [8a][28].

THF was freshly distilled over Na/benzophenone under Ar before use. $CHCl_3$ for optical rotation measurements (ORD) was filtered over basic Al_2O_3 (*Woelm N*, actvity I) to remove EtOH. Solvents for chromatography and workup procedures were distilled from *Sikkon* (hexane, AcOEt, MeOH), P_2O_5 (CH_2Cl_2, $CHCl_3$), and KOH/$FeSO_4$ (Et_2O). Et_3N was distilled from CaH_2 and stored over KOH. *i*-BuOCOCl was distilled before use and stored at 4° under Ar. α-Amino-acid derivatives were purchased from *Bachem*, *Senn*, *Degussa*, or *Fluka*. *Fmoc-(*R*)-β^3-HAla-OH* (**4b**) and *Fmoc (*R*)-β^3-HPhe-OH* (**5b**) were provided by *S. Abele* [19d] and were recrystallized freshly before use. *Cbz-(*S*)-β^2-HVal-OH* (**7**) was provided by *A. Böhm*, and Boc-(*R*)-β^3-HCys(Trt)-OH by *A. Jacobi*. *Wang* resin (100–200 mesh) was purchased from *Novabiochem*. HOBt Was purchased from *Fluka* (containing 20% H_2O) or from *Senn* (anh.). Ar was purchased from *PanGas*. All other chemicals were used as received from *Fluka* or *Novabiochem*. Temps. were monitored with an internal digital thermometer (*Ebro TTX-690*). Reactions carried out under the exclusion of light were performed in flasks completely wrapped in aluminum foil. **Caution**: The generation and handling of diazomethane requires special precautions [29]. Agitation in SPPS was achieved by bubbling N_2 gas through the mixture. TLC: *Merck* silica gel *60 F_{254}* plates; UV detection and dipping into solutions of *a*) ninhydrin (300 mg), AcOH (3 ml), and 1-butanol (100 ml) or *b*) anisaldehyde (9.2 ml), AcOH (3.75 ml), conc. H_2SO_4 (12.5 ml), and EtOH (338 ml), followed by heating with a heat gun. FC: *Fluka* silica gel *60* (40–63 μm) at r.t. with a pressure of *ca.* 0.3 bar. Anal. HPLC: *Merck LaChrom* HPLC system (*L-7150* pump, *L-7400* UV detector with variable-wavelength monitor), *Merck Lichrospher-100* column (CRP-18, 100 × 4.6 mm); unless otherwise stated, binary solvent system (*A* = MeCN/TFA 99.9 : 0.1, *B* = H_2O/TFA 99.9 : 0.1), flow rate 1.2 ml/min, UV detection at 220 nm. Prep. HPLC: *Macherey-Nagel* column (*Nucleosil 100-7 C_{18}*, 250 × 21 mm), binary solvent system *A*/*B* (see *anal. HPLC*), flow rate 24 ml/min, UV detection at 220 nm. M.p.: determined in open-end glass capillary tubes on a *Büchi 510* apparatus, uncorrected. ORD: $[\alpha]_D$ determined on a *Perkin-Elmer 241* polarimeter (10 cm, 1 ml cell) at r.t.; solvent and the conc. (in g/100 ml) are indicated. CD Spectra: on a *Jasco J-710*, from 190 to 250 nm at 25.0°, 1-mm rectangular cell, average of five baseline-corrected, smoothed scans, peptide conc. 0.2 mM or 0.02 mM; molar ellipticity Θ in $deg \cdot cm^2 \cdot dmol^{-1}$ (λ in nm). IR Spectra: on a *Perkin-Elmer 782* spectrophotometer, as film, 1–2% $CHCl_3$ soln., or KBr-pellets, maxima are classified as *s* (strong), *m* (medium), and *w* (weak); in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: on a *Bruker AMX-500* (500/125 MHz), *AMX-400* (400/100 MHz), *ARX-300* (300/75 MHz), or *Varian Gemini 300* (300/75 MHz). Chemical shifts δ in ppm relative to Me_4Si ($\delta = 0$ ppm) as internal standard, *J* values in Hz. MS: *ZAB2 SEQ* (FAB; 3-nitrobenzylalcohol matrix), on *Finnigan MAT TSQ 700* (ESI), or on *IonSpec Ultima 4.7-T FT* ion cyclotron resonance (ICR) mass spectrometers; MALDI (matrix-assisted laser-desorption ionization) in a 2,5-dihydroxybenzoic acid matrix; *m*/*z* in % of base peak. Elemental analyses were performed by the *Microanalytical Laboratory* of the *Laboratorium für Organische Chemie*, ETH Zürich. All peptides were characterized by high-resolution (HR) electrospray MS, and their purity was checked by running samples on an anal. HPLC column using two different gradients.

2. *General Procedures* (GP) *for the Preparation of β-Amino Acids and the β-Tripeptide Building Block* **16**. 2.1. *Synthesis of* N-*Boc- and* N-*Cbz-Protected Diazo Ketones* (*GP 1a*). Similarly to the reported procedures [19a,b,d], the corresponding α-amino acid was dissolved in THF (0.2M) under Ar and cooled to −25°. After addition of Et_3N (1.05 equiv.) and i-BuOCOCl (1.05 equiv.), the mixture was stirred at −25° for 30 min. A soln. of CH_2N_2 (2.60 equiv.) in Et_2O was added to the resulting white suspension, and stirring was continued for 16 h. Excess CH_2N_2 was destroyed by addition of AcOH, and the solvent was removed. The yellow residue was dissolved in AcOEt and washed with sat. aq. $NaHCO_3$, sat. aq. NH_4Cl, and sat. aq. NaCl soln. The org. layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Finally, FC and/or recrystallization afforded the desired pure diazo ketone.

2.2. *Synthesis of* N-*Fmoc-Protected Diazo Ketones* (GP 1b). The pertinent *N*-Fmoc-protected α-amino acids were transformed to the corresponding diazo ketones following an optimized procedure [19d] using NMM (1.05 equiv.) instead of Et_3N.

2.3. *Rearrangement of α-Diazo Ketones to* N-*Boc-protected β^3-Amino Acids* (GP 2a). Similarly to the reported procedures [19a,b,d], the diazo ketone was dissolved in a 9 : 1 mixture (0.25M) of THF and H_2O and then cooled to −25° under Ar and under exclusion of light. After 15 min at −25°, a homogeneous soln. of F_3CCO_2Ag (0.11 equiv.) in Et_3N (2.9 equiv.) was added, and the resulting mixture was allowed to warm to r.t. within 5 h in the dark. After filtration through *Celite*, the THF was removed under reduced pressure. The brown

residue was diluted with sat. aq. $NaHCO_3$ soln. and extracted with Et_2O. The aq. layer was adjusted to pH 1–2 with 1N HCl at 0° and then extracted with AcOEt. The org. layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Finally, FC and/or recrystallization furnished the pure *N*-protected β^3-amino acids.

2.4. *Rearrangement of α-Diazo Ketones to* N-*Fmoc-Protected β³-Amino Acids* (GP 2b). The diazo ketone was dissolved in THF (0.25M) containing 10% H_2O and cooled to −25° under Ar in the dark. A soln. of CF_3CO_2Ag (0.11 equiv.) in NMM (2.8 equiv.) was added, and the mixture was stirred at r.t. for 4 h under exclusion of light. Workup as in *GP 2a*, FC, and recrystallization afforded the pure *N*-Fmoc-protected β-amino acids.

2.5. *Rearrangement of α-Diazo Ketones to* N-*Boc-Protected β³-Amino Acid Benzyl Ester Derivatives* (GP 2c). Similar to the reported procedure [30], the diazo ketone was dissolved in a mixture of anh. THF and anh. BnOH (85:15, 0.25M) and cooled to −25° under Ar and exclusion of light. A homogeneous soln. of silver benzoate (0.11 equiv.) in Et_3N (2.8 equiv.) was added, and the resulting mixture was stirred at r.t. for 5 h in the dark. Subsequent to filtration through *Celite*, the mixture was concentrated under reduced pressure, and BnOH was removed by vacuum distillation. The residue was dissolved in AcOEt. After workup with sat. aq. $Na_2S_2O_3$, $NaHCO_3$, NH_4Cl, and NaCl soln. and drying (Na_2SO_4), the resulting crude product was purified by FC and recrystallized to yield the desired β^3-amino acid benzyl ester.

2.6. *Rearrangement of α-Diazo Ketones to* N-*Protected β³-Amino Acid Methyl Ester Derivatives* (GP 2d). Similar to the reported procedure [19a,b,d], the diazo ketone was dissolved in anh. MeOH (0.25M) under Ar at r.t., cooled to −25° under exclusion of light, and treated with a soln. of silver benzoate (0.11 equiv.) in anh. Et_3N (2.8 equiv.). The resulting mixture was stirred at r.t. for 5 h in the dark and filtered through *Celite*. The solvent was removed under reduced pressure, and the residue was taken up in AcOEt. Workup by washing the org. layer with sat. aq. $Na_2S_2O_3$, sat. aq. $NaHCO_3$, sat. aq. NH_4Cl, and sat. aq. NaCl soln., drying (Na_2SO_4), concentration *in vacuo,* and FC afforded the desired *N*-protected β^3-amino acid methyl ester.

2.7. *Ultrasound-Mediated Rearrangement of α-Diazo Ketones to* N-*Fmoc-Protected β³-Amino Acids* (GP 2e). Following [19e], the diazo ketone was dissolved in a mixture of dioxane and H_2O (5:1, 50 ml/mmol) under Ar at r.t. Then, CF_3CO_2Ag (0.11 equiv.) was added, and the mixture was sonicated for 2 h under exclusion of light. After filtration through *Celite*, the solvent was removed under reduced pressure, and the residue was taken up in AcOEt. Workup by washing the org. layer with sat. aq. $Na_2S_2O_3$, sat. aq. $NaHCO_3$, sat. aq NH_4Cl, and sat. aq. NaCl soln., drying (Na_2SO_4), concentration *in vacuo*, FC, and recrystallization afforded the desired Fmoc-protected β^3-amino acid.

2.8. *Boc-Deprotection of Amino Acids or Peptides* (GP 3). The Boc-protected amino acid or peptide was dissolved in CH_2Cl_2 (1M) at r.t. under Ar. After cooling to 0°, 1 equiv. of TFA was added, and the resulting homogeneous mixture was stirred for 2 h at r.t. The solvent was removed under reduced pressure, and the resulting oily residue was stripped with Et_2O (3×). Finally, the residue was dried *in vacuo* overnight.

2.9. *Cbz-Deprotection of Amino Acids or Peptides* (GP 4). The Cbz-protected amino acid or peptide was dissolved in MeOH or TFE (0.1M), and Pd/C (10%; 0.1 equiv.) was added after careful degassing and saturation of the solvent with Ar [32]. The flask was evacuated, flushed with H_2 several times, and the mixture was stirred under H_2 (1 bar) for 16 h. Subsequent filtration through *Celite* and concentration under reduced pressure afforded the desired product, which was used in the following peptide coupling without further purification.

2.10. *Peptide Coupling with β³-Amino Acids, EDC, and HOBt* (GP 5a). The corresponding free acid and unprotected amine derivatives were dissolved in anh. $CHCl_3$ (0.5M) at r.t. under Ar. Then, Et_3N (1.2 equiv. without and 5 equiv. with TFA salts present) was added, and the mixture was cooled to 0°. After stirring for 15 min at 0°, HOBt (1.2 equiv.) was added, and stirring was continued for another 15 min. Then, EDC (1.2 equiv.) was added, and the resulting mixture was stirred at r.t. for 14 h. Dilution with $CHCl_3$ was followed by thorough washing with 1N HCl, sat. aq. $NaHCO_3$, and sat. aq. NaCl soln. The org. layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The resulting pale yellow product was purified by FC or precipitation.

2.11. *Peptide Coupling with β²-Amino Acids, EDC, and HOBt* (GP 5b). The free acid and the corresponding unprotected amine derivative were dissolved in anh. $CHCl_3$ (0.5M) at r.t. After cooling to 0°, NMM (1.2 equiv. without and 3 equiv. with TFA salts present) was added, and the mixture was stirred for 15 min at 0° under Ar. Then, HOBt (1.2 equiv.) was added, and stirring was continued for 15 min. Finally, EDC (1.2 equiv.) was added, and the mixture was stirred for 24 h at 0°. Subsequent dilution with $CHCl_3$ was followed by thorough washing with 1N HCl (3×), sat. aq. $NaHCO_3$ (2×), and sat. aq. NaCl soln. (1×). The org. layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The resulting pale yellow product was purified by FC and/or recrystallization.

2.12. *(*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-1-diazobutan-2-one* (Boc-(*S*)-Ala-CHN_2; (*S*)-**1**). Transformation of Boc-Ala-OH (18.9 g, 100.00 mmol) according to *GP 1a* and crystallization (Et_2O/pentane) afforded (*S*)-**1** as yellow crystals (13.75 g, 65%). Spectroscopic data: [19b].

2.13. *(*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-1-diazo-4-phenylbutan-2-one* (Boc-(*S*)-Phe-CHN_2; (*S*)-**2a**). Transformation of Boc-Phe-OH (26.53 g, 100.00 mmol) according to *GP 1a*, FC (pentane/AcOEt 3:1), and crystallization (Et_2O/pentane) afforded (*S*)-**2a** as pale yellow crystals (20.30 g, 70%). Spectroscopic data: [19b].

2.14. *(*S*)-1-Diazo-3-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino)-4-phenylbutan-2-one* (Fmoc-(*S*)-Phe-CHN_2; (*S*)-**2b**). Transformation of Fmoc-Phe-OH (8.00 g, 20.65 mmol) according to *GP 1b*, FC (CH_2Cl_2/Et_2O 6:1 → 1:1), and crystallization (Et_2O/pentane) afforded diazoketone (*S*)-**2b** as pale yellow crystals (5.60 g, 66%). Spectroscopic data: [19c].

2.15. *(*S*)-3-{[(Benzyloxy)carbonyl]amino}-7-{[(*tert*-butoxy)carbonyl]amino}-1-diazoheptan-2-one* (Cbz-(*S*)-Lys(Boc)-CHN_2; (*S*)-**3a**). Transformation of Cbz-Lys(Boc)-OH (38.05 g, 100.00 mmol) according to *GP 1a*, FC (pentane/AcOEt 5:1 → 2:1), and crystallization (Et_2O/pentane) afforded (*S*)-**3a** as yellow crystals (29.70 g, 74%). Spectroscopic data were in accordance with those obtained for Fmoc-(*S*)-Lys(Boc)-CHN_2 [31].

2.16. *(*R*)-7-{[(*tert*-Butoxy)carbonyl]amino}-1-diazo-3-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino)heptan-2-one* (Fmoc-(*R*)-Lys(Boc)-CHN_2; (*R*)-**3b**). Transformation of Fmoc-Lys(Boc)-OH (5.00 g, 10.67 mmol) according to *GP 1b*, FC (CH_2Cl_2/Et_2O 6:1 → 1:1), and crystallization (CH_2Cl_2/pentane) yielded (*R*)-**3b** as pale yellow crystals (4.40 g, 84%). For spectroscopic data of the (*S*)-enantiomer, see [31].

2.17. *(*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}butanoic Acid* (Boc-(*S*)-β^3-HAla-OH; (*S*)-**4a**). Rearrangement of (*S*)-**1** (13.75, 64.55 mmol) according to *GP 2a* and recrystallization (AcOEt/pentane) yielded (*S*)-**4a** (11.50 g, 56.60 mmol, 88%) as a colorless powder. Spectroscopic data were in agreement with those of *ent*-**4a** [19b].

2.18. *Benzyl(*S*)-3-{[(*tert*-Butoxy)carbonyl]amino}-4-phenylbutanoate* (Boc-(*S*)-β^3-HPhe-OBn; (*S*)-**5a**). Rearrangement of (*S*)-**2a** (20.30 g, 70.24 mmol) according to *GP 2c*, FC (AcOEt/pentane 1:6), and recrystallization (AcOEt/pentane) furnished (*S*)-**5a** (21.50 g, 58.19 mmol, 83%) as a colorless powder. Spectroscopic data: [26a].

2.19. *(*S*)-3-({[(9*H*-Fluoren-9-yl)methoxy]carbonyl}amino)-4-phenylbutanoic Acid* (Fmoc-(*S*)-β^3-HPhe-OH; (*S*)-**5b**). Rearrangement of (*S*)-**2b** (1.40 g, 3.41 mmol) according to *GP 2e*, FC (AcOEt/pentane), and recrystallization (AcOEt/pentane) furnished (*S*)-**5b** (1.31 g, 3.27 mmol, 96%) as a colorless powder. Spectroscopic data: [31].

2.20. *Methyl (*S*)-3-{[(Benzyloxy)carbonyl]amino}-7-{[(*tert*-butoxy)carbonyl]amino}heptanoate* (Cbz-(*S*)-β^3-HLys(Boc)-OMe; (*S*)-**6a**). Rearrangement of (*S*)-**3a** (29.70 g, 73.52 mmol) according to *GP 2d*, FC (AcOEt/pentane 1:5), and recrystallization (CH_2Cl_2/pentane) afforded (*S*)-**6a** (24.00 g, 58.82 mmol, 80%) as a colorless powder. M.p. 72°. $[\alpha]_D^{r.t.} = -18.9$ ($c = 1.095$, $CHCl_3$). IR ($CHCl_3$): 3451*m*, 3008*m*, 2942*m*, 1711*s*, 1509*s*, 1455*m*, 1438*m*, 1393*w*, 1367*m*, 1169*m*, 864*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.35–1.59 (*m*, 3 CH_2); 1.43 (*s*, *t*-Bu); 2.53–2.55 (*m*, CH_2CO); 2.95–3.07 (*m*, CH_2N); 3.66 (*s*, MeO); 3.92–4.03 (*m*, NC*H*(R)CH_2); 4.56 (br. *s*, NH); 5.09 (*s*, PhCH_2); 5.24 (*d*, $J = 8.6$, NH); 7.29–7.40 (*m*, Ph). ^{13}C-NMR (100 MHz, $CDCl_3$): 23.19 (CH_2); 28.43 (Me); 29.66 (CH_2); 33.99 (CH_2); 38.97 (CH_2); 40.17 (CH_2); 47.97 (CH); 51.69 (Me); 66.68 (CH_2); 79.11 (C); 128.11 (CH); 128.51 (CH); 136.55 (C); 156.0 (C); 156.07 (C); 171.95 (C). HR-MALDI-MS: 431.2153 (100, $[M + Na]^+$, $C_{21}H_{32}N_2O_6^+$; calc. 431.2150), 309.1811 (98.50, $[M - Boc + 2H]^+$, $C_{16}H_{25}N_2O_4^+$; calc. 309.1815). Anal. calc. for $C_{21}H_{32}N_2O_6$ (408.59): C 61.75, H 7.90, N 6.86; found: C 61.62, H 7.97, N 6.80.

2.21. *(*R*)-7-{[(*tert*-Butoxy)carbonyl]amino}-3-({[(9*H*-fluoren-9-yl)methoxy]carbonyl}amino)heptanoic Acid* (Fmoc-(*R*)-β^3-HLys(Boc)-OH; **6b**). Rearrangement of (*R*)-**3b** (4.40 g, 8.93 mmol) according to *GP 2b*, FC (AcOEt/pentane/AcOH 10:10:1) and recrystallization ($CHCl_3$/pentane) afforded Fmoc-(*R*)-β^3-HLys-(Boc)-OH (**6b**) (3.57 g, 7.41 mmol, 83%) as a colorless powder. Spectroscopic data corresponded to those of *ent*-**6b** [19d].

2.22. *(*S*)-2-{[(*Benzyloxycarbonyl*)amino]methyl}-3-methylbutanoic Acid* (Cbz-(*S*)-β^2-HVal-OH; (*S*)-**7**). This material [23c] was prepared according to [19].

2.23. *(*S*)-2-{[(Benzyloxycarbonyl)amino]methyl}-4-methylpentanoic Acid* (Cbz-(*S*)-β^2-HLeu-OH; (*S*)-(**8**). This material was prepared according to [19].

2.24. *Methyl (*S*)-3-Amino-7-{[(*tert*-butoxy)carbonyl]amino}heptanoate* ((*S*)-β^3-HLys(Boc)-OMe; (*S*)-(**9**). Compound (*S*)-**6a** (24.00 g, 58.82 mmol, 1 equiv.) was deprotected according to *GP 4* to afford (*S*)-**9** as a colorless oil (15.79 g, 57.64 mmol, 98%), which was used in the following peptide coupling without further purification. $[\alpha]_D^{r.t.} = +6.54$ ($c = 1.055$, $CHCl_3$). IR ($CHCl_3$): 3454*w*, 3008*m*, 2936*m*, 1709*s*, 1507*s*, 1438*m*, 1392*w*, 1367*m*, 1168*s*, 1016*w*, 862*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.15–1.38 (*m*, 3 CH_2); 1.42 (*s*, *t*-Bu); 2.10–2.15 (*dd*, $J = 15.3, 6.3$, 1 H, CH_2CO); 2.20–2.24 (*dd*, $J = 15.3, 4.4$, 1 H, CH_2CO); 3.02–3.10 (*m*, CH_2NH_2, NC*H*(R)CH_2);

3.64 (*s*, MeO); 4.58 (br. *s*, NH). ESI-MS: 571.5 (9, $[2M+Na]^+$), 549.5 (35, $[2M+H]^+$), 297.3 (23, $[M+Na]^+$), 275.3 (100, $[M+H]^+$).

2.25. *(*S*)-3-{[(Benzyloxy)carbonyl]amino}butanoic Acid* (Cbz-(*S*)-β^3-HAla-OH; (*S*)-**10**). Compound (*S*)-**4a** (11.50 g, 56.60 mmol) was deprotected following *GP 3*. The crude product was dissolved in 2M NaOH and cooled to 5°. Then, 4M NaOH and benzyl chloroformate (1.2 equiv.) were added simultaneously under vigorous stirring at 5° over 1 h [33]. The mixture was stirred for 1 h at 5° and was washed with Et_2O. The aq. layer was acidified to pH 1 with conc. HCl, saturated with NaCl, and extracted with AcOEt (3×). The combined org. layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to furnish a pale oil. The crude product was crystallized from AcOEt/pentane to yield (*S*)-**10** (11.70 g, 49.37 mmol, 87%) as a colorless solid. $[\alpha]_D^{r.t.} = -15.7$ ($c = 1.04$, $CHCl_3$). IR ($CHCl_3$): 3437*w*, 3032*m*, 1713*s*, 1511*s*, 1454*m*, 1342*m*, 1248*m*, 1061*m*, 912*w*, 600*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.22 (*d*, $J = 6.8$, Me); 2.53 (*d*, $J = 4.2$, CH_2CO); 4.11 (br. *s*, CH); 5.08 (*s*, $PhCH_2$); 5.24 (*m*, NH); 7.28–7.36 (*m*, Ph), 8.75 (br. *s*, CO_2H). ^{13}C-NMR (100 MHz, $CDCl_3$): 20.36 (Me); 40.43 (CH_2); 43.84 (CH); 66.77 (CH_2); 128.05 (CH); 128.13 (CH); 128.50 (CH); 136.27 (C); 155.77 (C); 176.37 (C). ESI-MS. 495.1 (4, $[2M+Na-2H]^-$), 473.2 (26, $[2M-H]^-$), 236.1 (100, $[M-H]^-$). Anal. calc. for $C_{12}H_{15}NO_4$ (237.25): C 60.75, H 6.37, N 5.90; found: C 60.79, H 6.30, N 5.71.

2.26. *Cbz-(*S*)-β^3-HAla-(*S*)-β^3-HLys(Boc)-OMe* (**11**). Coupling of (*S*)-**10** (10.70 g, 45.15 mmol, 1.0 equiv.) and (*S*)-**9** (12.37 g, 45.15 mmol, 1.0 equiv.) with Et_3N (7.54 ml, 54.18 mmol, 1.2 equiv.) according to *GP 5a* followed by FC (AcOEt/pentane 60:40 → AcOEt) afforded **11** (18.78 g, 38.09 mmol, 84%) as a colorless solid. On a smaller scale (4.22 mmol), the yield could be increased to 91%. M.p.: 109–111°. $[\alpha]_D^{r.t.} = -22.7$ ($c = 1.02$, $CHCl_3$). IR ($CHCl_3$): 3432*w*, 3007*m*, 2979*m*, 1710*s*, 1505*s*, 1455*m*, 1367*m*, 1170*s*, 1105*m*, 1059*m*, 651*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.23–1.25 (*d*, $J = 6.7$, Me); 1.26–1.36 (*m*, CH_2); 1.39–1.55 (*m*, 2 CH_2); 1.43 (*s*, *t*-Bu); 2.32 (*dd*, $J = 14.5, 5.7$, 1 H, CH_2CO); 2.47–2.40 (*m*, 1 H, CH_2CO); 2.50 (*d*, $J = 5.5$, CH_2CO); 2.51 (*d*, $J = 5.3$, 1 H, CH_2CO); 3.06 (*m*, CH_2NH); 3.66 (*s*, MeO); 4.01–4.07 (*m*, NHC*H*(R)CH_2); 4.19–4.26 (*m*, NHC*H*(R)CH_2); 4.63 (br. *s*, NH), 5.08 (*s*, $PhCH_2$), 5.68 (br. *s*, NH); 6.25 (br. *s*, NH); 7.28–7.35 (*m*, Ph). ^{13}C-NMR (100 MHz, $CDCl_3$): 20.39 (Me); 23.10 (CH_2); 28.40 (Me); 29.56 (CH_2); 33.51 (CH_2); 38.53 (CH_2); 40.07 (CH_2); 42.28 (CH_2); 44.74 (CH); 45.81 (CH); 51.70 (Me); 66.46 (CH_2); 79.06 (C); 127.98 (CH); 128.44 (CH); 129.27 (CH); 136.58 (C); 155.73 (C); 156.09 (C); 170.27 (C); 172.09 (C). HR-MALDI-MS: 516.2685 (36, $[M+Na]^+$, $C_{25}H_{39}N_3O_7^+$; calc. 516.2681); 416.2162 (35, $[M-Boc+H+Na]^+$, $C_{20}H_{31}N_3O_5^+$; calc. 416.2169); 394.2345 (100, $[M-Boc+2H]^+$, $C_{20}H_{32}N_3O_5^+$; calc. 394.2348). Anal. calc. for $C_{25}H_{39}N_3O_7$ (493.28): C 60.83, H 7.96, N 8.51; found: C 60.97, H 8.01, N 8.50.

2.27. *Cbz-(*S*)-β^3-HAla-(*S*)-β^3-HLys(Boc)-OH* (**12**). The dipeptide **11** (12.00 g, 24.34 mmol, 1.0 equiv.) was dissolved in THF (250 ml) and stirred for 15 min at r.t. Then, a 1.0M soln. of $LiOH \cdot H_2O$ (1.53 g, 36.51 mmol, 1.5 equiv.) in H_2O (36.5 ml) was added. The yellow mixture was stirred for 16 h at r.t., treated with 1N HCl, and extracted with AcOEt and $CHCl_3$. The combined org. layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to yield **12** (11.43 g, 23.85 mmol, 98%) as a colorless solid, which was used in the following coupling without further purification.

2.28. *Benzyl-(*S*)-3-Amino-4-phenylbutanoate* (H-(*S*)-β^3-HPhe-OBn; (*S*)-**13**). Compound (*S*)-**5a** (9.27 g, 25.05 mmol) was deprotected according to *GP 3*. Concentration under reduced pressure and drying of the residue *in vacuo* for 24 h yielded (*S*)-**13** (9.59 g, 25.05 mmol, quant.) as its TFA salt and was used without further purification.

2.29. *Cbz-(*S*)-β^3-HAla-(*S*)-β^3-HLys(Boc)-(*S*)-β^3-Phe-OBn* (**14**). Dipeptide **12** (12.00 g, 25.05 mmol, 1.0 equiv.) and (*S*)-**13** (6.74 g, 25.05 mmol, 1.0 equiv.) were subjected to EDC-mediated coupling with Et_3N (17.43 ml, 125.05 mmol, 5.0 equiv.) according to *GP 5a*. After workup, the crude product was dissolved in $CHCl_3$/MeOH 3:1 and precipitated with MeCN to afford **14** (13.45 g, 18.42 mmol, 74%) as a colorless solid. R_f 0.74 (CH_2Cl_2/MeOH 9:1). M.p. 184–185°. $[\alpha]_D^{r.t.} = -2.62$ ($c = 0.935$, $CHCl_3$/MeOH 3:1). IR (KBr): 3308*s*, 3063*w*, 2974*m*, 2934*m*, 2858*w*, 1734*s*, 1682*s*, 1642*s*, 1538*s*, 1457*m*, 1366*m*, 1312*m*, 1267*s*, 1198*m*, 1170*s*, 1115*m*, 1062*m*, 980*m*, 908*w*, 867*w*, 750*s*, 698*s*. ^{1}H-NMR (400 MHz, CD_3OD): 1.15–1.30 (*m*, CH_2); 1.19–1.21 (*d*, $J = 6.6$, Me); 1.35–1.48 (*m*, 2 CH_2); 1.43 (*s*, *t*-Bu); 2.15–2.20 (*dd*, $J = 14.1, 6.3$, 1 H, CH_2CO); 2.26–2.33 (*m*, 3 H, CH_2CO); 2.46–2.51 (*dd*, $J = 15.7, 7.1$, 1 H, CH_2CO); 2.54–2.59 (*dd*, $J = 15.7, 5.6$, 1 H, CH_2CO); 2.76–2.81 (*dd*, $J = 13.7, 7.5$, 1 H, $PhCH_2$); 2.84–2.89 (*dd*, $J = 13.7, 6.9$, 1 H, $PhCH_2$); 3.02 (*m*, CH_2NH); 3.95–4.03 (*m*, 2 NHC*H*(R)CH_2); 4.44–4.46 (*m*, NHC*H*(R)CH_2); 5.02 (br. *s*, NH), 5.08 (*s*, $PhCH_2O$); 5.11 (*s*, $PhCH_2O$); 5.52 (br. *s*, NH); 6.25 (br. *s*, NH); 6.25 (br. *s*, NH); 7.12–7.40 (*m*, 3 Ph). ^{13}C-NMR (100 MHz, $CDCl_3$): 20.66 (Me); 23.29 (CH_2); 28.51 (Me); 29.54 (CH_2); 30.91 (CH_2); 33.40 (CH_2); 38.19 (CH_2); 40.22 (CH_2); 41.24 (CH_2); 42.82 (CH_2); 45.22 (CH); 47.39 (CH); 47.91 (CH); 66.77 (CH_2); 66.94 (CH_2); 79.45 (C); 126.88 (CH); 128.06 (CH); 128.26 (CH); 128.51 (CH); 128.59 (CH); 128.68 (CH); 128.72 (CH); 128.81 (CH); 129.47 (CH); 135.89 (C); 136.81 (C); 137.79 (C); 156.58 (C); 157.12 (C); 171.28 (C); 171.36 (C); 171.66 (C). HR-MALDI-MS: 753.3829 (9, $[M+Na]^+$,

$C_{41}H_{54}N_4O_8^+$; calc. 753.3834); 653.3333 (100, $[M-Boc+Na]^+$, $C_{36}H_{46}N_4O_6^+$; calc. 653.3338); 631.3478 (6, $[M-Boc+H]^+$, $C_{36}H_{46}N_4O_6^+$; calc. 631.3472). Anal. calc. for $C_{41}H_{54}N_4O_8$ (730.90): C 67.38, H 7.45, N 7.67; found: C 67.28, H 7.65, N 7.64.

2.30. *H-(*S*)-β³-HAla-(*S*)-β³-HLys(Boc)-(*S*)-β³-Phe-OH* (**15**). Tripeptide **14** (5.80 g, 7.95 mmol) was dissolved in TFE (320 ml) and deprotected according to *GP 4*. Filtration through *Celite* and concentration under reduced pressure yielded **15** (4.23 g, 8.36 mmol; quant.) as a colorless powder. M.p. 196–198°. R_f 0.05 (CH_2Cl_2/MeOH 9:1). $[\alpha]_D^{r.t.} = -1.84$ ($c = 0.89$, $CHCl_3$). IR (KBr): 3299*s*, 3007*m*, 2934*s*, 2876*m*, 1711*s*, 1646*s*, 1540*s*, 1454*m*, 1392*s*, 1366*m*, 1276*m*, 1251*m*, 1170*s*, 1032*m*, 865*w*, 747*m*, 701*m*. ^{1}H-NMR (400 MHz, CD_3OD): 1.15–1.28 (*m*, CH_2); 1.25 (*d*, $J = 6.7$, Me); 1.30–1.49 (*m*, 2 CH_2); 1.35 (*s*, *t*-Bu); 1.99–2.08 (*m*, 1 H, CH_2CO); 2.23–2.36 (*m*, 3 H, CH_2CO); 2.61–2.77 (*m*, 1 H, CH_2CO, $PhCH_2$); 2.91–3.02 (*m*, CH_2NH); 3.47–3.56 (*m*, $NHCH(R)CH_2$); 4.19–4.26 (*m*, $NHCH(R)CH_2$); 4.30–4.40 (*m*, $NHCH(R)CH_2$); 5.40 (br. *s*, NH), 7.07–7.19 (*m*, Ph). ^{13}C-NMR (100 MHz, $CDCl_3$): 18.81 (Me); 23.20 (CH_2); 28.50 (Me); 29.61 (CH_2); 33.31 (CH_2); 39.36 (CH_2); 40.33 (CH_2); 41.84 (CH_2); 42.42 (CH_2); 44.74 (CH); 46.92 (CH); 48.34 (CH); 79.39 (C); 126.67 (CH); 128.53 (CH); 129.57 (CH); 138.15 (C); 156.97 (C); 170.14 (C); 171.46 (C); 178.65 (C). ESI-MS: 1013 (4, $[2M+H]^+$); 529 (8, $[M+Na]^+$); 507 (100, $[M+H]^+$); 407 (58, $[M-Boc+2H]^+$).

2.31. *Fmoc-(*S*)-β³-HAla-(*S*)-β³-HLys(Boc)-(*S*)-β³-Phe-OH* (**16**). Tripeptide **15** (3.81 g, 7.53 mmol, 1 equiv.) was suspended in a 1:1 mixture of dioxane (60.99 ml) and H_2O (60.99 ml) [34]. After adding powdered Na_2CO_3 (1.59 g, 15.07 mmol, 2 equiv.), the mixture was sonicated for 30–60 min (solubilization). The homogeneous soln. was cooled to 0°, Fmoc-OSu [35] (2.54 g, 7.53 mmol, 1 equiv.) was added, and the resulting mixture was stirred for 24 h at r.t. The pH was adjusted to 1–2, the soln. was filtered (*N4* filter), and the colorless precipitate was dried *in vacuo*. The crude product was redissolved in $CHCl_3$/MeOH 3:1, precipitated with MeCN, filtered, and dried *in vacuo* to yield **16** (5.10 g, 7.00 mmol, 93%) as a colorless powder. M.p. 207–209°. R_f 0.52 (CH_2Cl_2/MeOH 9:1). Anal. HPLC (20–80% *A* in 20 min): t_R 18.2 min (> 98%). $[\alpha]_D^{r.t.} = -2.06$ ($c = 1.02$, $CHCl_3$). IR (KBr): 3334*s*, 3064*m*, 2970*m*, 2956*m*, 1710*s*, 1684*s*, 1636*s*, 1598*m*, 1540*s*, 1449*m*, 1365*m*, 1259*s*, 1168*m*, 1116*m*, 1060*m*, 983*w*, 901*w*, 867*w*, 756*m*, 737*s*, 702*m*, 622*m*. ^{1}H-NMR (500 MHz, DMSO): 0.95–1.10 (*m*, CH_2); 1.20 (*d*, $J = 6.4$, Me); 1.12–1.35 (*m*, 2 CH_2); 1.37 (*s*, *t*-Bu); 2.02–2.18 (*m*, 2 CH_2CO); 2.24–2.28 (*m*, 1 H, CH_2CO); 2.33 (*d*, $J = 7.9$, 1 H, CH_2CO); 2.68–2.75 (*m*, $PhCH_2$); 2.82–2.84 (*m*, CH_2NH); 3.82–3.86 (m, $NHCH(R)CH_2$); 3.88–3.94 (*m*, OCH_2CH); 4.16–4.23 (*m*, $NHCH(R)CH_2$); 4.24–4.30 (*m*, $NHCH(R)CH_2$, OCH_2CH); 6.68–6.73 (*m*, NH); 7.16–7.29 (*m*, 5 arom. H); 7.31–7.34 (*m*, 2 arom. H); 7.39–7.43 (*m*, 2 arom. H); 7.67 (*d*, $J = 7.4$, 2 arom. H), 7.88 (*d*, $J = 7.5$, 2 arom. H); 12.22 (*s*, CO_2H). ^{13}C-NMR (125 MHz, DMSO): 20.16 (Me); 22.74 (CH_2); 28.25 (Me); 29.31 (CH_2); 32.98 (CH_2); 37.78 (CH_2); 39.83 (CH_2); 40.26 (CH_2); 41.13 (CH_2); 42.51 (CH_2); 44.22 (CH); 45.89 (CH); 46.74 (CH); 47.19 (CH); 65.13 (CH_2); 77.25 (C); 120.08 (CH); 125.12 (CH); 126.07 (CH); 127.02 (CH); 127.57 (CH); 128.09 (CH); 129.07 (CH); 138.59 (C); 140.71 (C); 143.86 (C); 143.93 (C); 155.15 (C); 155.52 (C); 169.16 (C); 169.24 (C); 172.34 (C). HR-MALDI-MS: 751.3672 (11, $[M+Na]^+$, $C_{41}H_{52}N_4O_8^+$; calc. 751.3677); 651.3151 (58, $[M-Boc+Na]^+$, $C_{36}H_{44}N_4O_6^+$; calc. 651.3155); 629.3319 (8, $[M-Boc+H]^+$, $C_{36}H_{44}N_4O_6^+$; calc. 629.3322). ESI-MS: 1456 (4, $[2M-H]^-$); 727 (100, $[M-H]^-$); 505 (69, $[M-Fmoc]^-$). Anal. calc. for $C_{41}H_{52}N_4O_8$ (728.88): C 67.56, H 7.19, N 7.69; found: C 65.79, H 7.02, N 7.40.

3. *Solid-Phase Synthesis of Long-Chain β-Peptides* via *Coupling of Tripeptide Building Blocks.* 3.1. *Anchoring of Fmoc-(*S*)-β³-HAla-(*S*)-β³-HLys(Boc)-(*S*)-β³-Phe-OH* (**16**) *on* Wang *Resin*. *Wang* resin (2 g, initial loading 0.99 mmol/g, 1 equiv.) was dried *in vacuo* for 1 h and swelled in NMP (20 ml) for 45 min. Peptide **16** (4.32 g, 5.94 mmol, 3 equiv.) was dissolved in NMP (30 ml) at 50°. The homogeneous soln. was added to the resin, followed by DIC (0.92 ml, 5.94 mmol, 3 equiv.), HOBt (0.46 g, 5.94 mmol, 3 equiv.), and a cat. amount of DMAP. The suspension was agitated for 48 h. The resin was filtered, washed with NMP (40 ml, 3 × 5 min) and CH_2Cl_2 (40 ml, 5 × 5 min), and dried *in vacuo* overnight to afford **17**. The peptide loading was determined by measuring the absorbance of the dibenzofulvene piperidine adduct as follows: a defined amount of resin (*ca.* 5 mg) was suspended in piperidine/DMF 20:80 (10.0 ml) in a measuring flask, and the Fmoc-deprotection was allowed to continue for 1 h. The mixture was transferred to a UV cell, and the absorbance of the resulting soln. was measured at 290 nm. The concentration (c in [mM]) of the benzofulvene adduct in soln. was determined by means of a calibration curve[23]). The loading (Subst, in [mmol/g resin]) was then calculated according to *Eqn. 1* [27a]. For **16**, the loading (average of two measurements) was 0.50 mmol/g.

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[23]) This calibration curve was obtained by plotting the absorbance of a series of benzofulvene piperidine adducts, prepared by deprotection of Fmoc-Gly, as a function of concentration (0–1 mM).

$$\text{Subst}^n = c^n \cdot V^n / \{m^n_{\text{resin}} - [c^n \cdot V^n \cdot (M_W - 18.0) / 1000]\} \quad (1)$$

3.2. *Solid-Phase Syntheses of Peptides* **19**–**22**. The loaded resin (**17**) (0.5 g, 0.25 mmol of **16**) was put in a solid-phase reactor and covered with NMP (5 ml). Unreacted OH groups were capped with Ac_2O (0.5 ml, 5.0 mmol, 20 equiv.) and a cat. amount of DMAP (50 mg, 0.40 mmol) for 2.5 h under intensive N_2 bubbling. The resin was filtered and washed with NMP 2:2:96 (5 ml, 5 × 1 min). Solid-phase synthesis was continued by removing the Fmoc group with *1*) 20% piperidine in NMP (5 ml, 2 × 60 min), *2*) DBU/piperidine/NMP (5 ml, 3 × 60 min), and *3*) 20% piperidine in NMP (5 ml, 1 × 60 min). The resin was washed with NMP (5 ml, 6 × 3 min), and the elongation sequence was initiated by addition of a homogeneous soln. of **16** (0.455 g, 0.625 mmol, 2.5 equiv.), HBTU (0.228 g, 0.600 mmol, 2.4 equiv.), and HOBt (0.101 g, 0.625 mmol, 2.5 equiv.) in NMP (5 ml), followed by addition of DIPEA (0.214 ml, 1.25 mmol, 5.0 equiv.). The slightly yellow suspension was agitated by N_2 bubbling for 72 h. Monitoring of the coupling reaction was performed with TNBS [36]. In the case of a positive TNBS test (indicating incomplete coupling), the suspension was allowed to react for another 10 h with **16** (0.5 equiv.) and coupling reagents. The resin was filtered off, the filtrate was collected, dried, and stored at −20°. The dry resin was washed with NMP (5 ml, 6 × 5 min) prior to Fmoc deprotection using the following solutions: piperidine/NMP 1:4 (5 ml, 2 × 60 min), DBU/piperidine/NMP 1:1:48 (5 ml, 3 × 60 min), and piperidine/NMP 1:4 (5 ml, 1 × 60 min). After filtration, the resin was washed with NMP (5 ml, 6 × 3 min), and SPPS was continued by repeating the following sequence. In each coupling step, a homogeneous soln. of **16** (0.455 g, 0.625 mmol, 2.5 equiv.), HBTU (0.228 g, 0.600 mmol, 2.4 equiv.) and HOBt (0.101 g, 0.625 mmol, 2.5 equiv.) in NMP (5 ml) was added. Subsequent to initial mixing by N_2 bubbling for 5 min, DIPEA (0.214 ml, 1.25 mmol, 5.0 equiv.) was added, and the resulting suspension was allowed to react for 48 h. A soln. of an additional amount of coupling reagents was added, *i.e.*, HBTU (0.142 g, 1.5 equiv.), HOBt (0.061 g, 1.5 equiv.), and DIPEA (0.128 ml, 3.0 equiv.) when the 're-use' soln. (2 ml) was added. Thereafter, mixing was continued for 48 h. When the desired sequence of the peptide was obtained, the resin was filtered off and washed with NMP (5 ml, 6 × 3 min). Final Fmoc deprotection was performed with mixtures of piperidine/NMP 1:4 (8 ml, 2 × 60 min), DBU/piperidine/NMP 1:1:48 (8 ml, 3 × 60 min), and piperidine/NMP 1:4 (8 ml, 1 × 60 min). The resin was filtered again and washed extensively with NMP (5 ml, 6 × 4 min) and CH_2Cl_2 (8 ml, 10 × 1 min), shrunk with MeOH (5 ml, 6 × 3 min) and dried over KOH *in vacuo* for 24 h. The peptides were cleaved from the resin by exposure to TFA/H_2O 95:5 (40 ml) for 2 h. The resin was removed by filtration, washed with TFA, and the org. layer was concentrated under reduced pressure. The oily residue was precipitated by addition of cold Et_2O, and the crude product was dried *in vacuo*. This methodology was used to prepare the 15-mer **21** and the 18-mer **22**. In the case of the 15-mer, 642 mg of crude product were obtained (59%), whereas the 18-mer synthesis yielded 168 mg of crude product (54%). Due to incomplete coupling, both crude products also contained the lower homologs (9-mer **19**, 12-mer **20**, and, in the second synthesis, the 15-mer **21**.

3.3. *Solid-Phase Synthesis of* **18**. Following the coupling procedure described in *Sect. 3.2.*, **17** (378 mg resin, 0.189 mmol) was coupled with **16** (344 mg, 0.47 mmol, 2.5 equiv.) to afford 220 mg of crude product (92% purity) after TFA-mediated cleavage and precipitation with cold Et_2O.

3.4. *HPLC Analysis and Purification of β-Peptides* **18**–**22**. RP-HPLC Analysis of the crude products were performed as described in *Sect. 1* using a linear gradient (10–100% *A* in 30 min). Crude products were purified by prep. RP-HPLC using a gradient of *A* and *B* (20–55% *A* in 40 min, then 55–80% *A* in 10 min). The purified, lyophilized peptides **18**–**22** were analyzed again by anal. RP-HPLC with a linear gradient of *A* and *B* (40–60% *A* in 20 min).

3.5. *H-(*S*)-β³-HAla-(*S*)-β³-HLys-(*S*)-β³-Phe-(*S*)-β³-HAla-(*S*)-β³-HLys-(*S*)-β³-Phe-OH* (**18**). Purification of the crude product resulting from the SPPS of **18** (220 mg) gave 163 mg of a colorless lyophilizate. Anal. HPLC (15–50% MeCN in 20 min; 50–80% MeCN in 5 min; 80–98% MeCN in 5 min): t_R 6.8 min, (> 98%). ^{1}H-NMR (500 MHz, CD_3OD): 1.11 (*d*, *J* = 6.7, Me); 1.28–1.47 (*m*, 4 CH_2); 1.34 (*d*, *J* = 6.7, Me); 1.52–1.73 (*m*, 2 CH_2); 2.25–2.61 (*m*, 6 CH_2CO); 2.69–2.90 (*m*, 2 C*H*$_2$Ph, 2 C*H*$_2$NH$_2$); 3.66–3.71 (*m*, NHC*H*(R)CH$_2$); 4.17–4.22 (*m*, NHC*H*(R)CH$_2$); 4.26–4.35 (*m*, 2 NHC*H*(R)CH$_2$); 4.39–4.45 (*m*, NHC*H*(R)CH$_2$); 4.50–4.56 (*m*, NHC*H*(R)CH$_2$); 7.17–7.27 (*m*, 2 Ph); 7.79 (*d*, *J* = 8.9, NH); 7.98 (*d*, *J* = 8.9, NH); 8.09 (*d*, *J* = 8.9, NH). ^{13}C-NMR (125 MHz, $CDCl_3$): 18.84, 20.92 (Me); 23.89, 23.98 (CH_2); 28.09, 28.37 (CH_2); 35.16, 35.29 (CH_2); 39.47, 39.53 (CH_2); 40.62, 40.65 (CH_2); 41.28, 41.76 (CH_2); 42.20, 42.26 (CH_2); 43.56 (CH_2); 44.22 (CH); 46.57 (CH); 47.35, 47.46 (CH); 49.59, 49.67 (CH); 127.65, 127.68 (CH); 129.49, 129.53 (CH); 130.48, 130.54 (CH); 139.46, 139.53 (C); 171.55 (C); 171.83 (C); 172.04 (C); 172.35 (C); 172.80 (C); 175.03 (C). HR-MALDI-MS: 817.4948 (19, $[M+Na]^+$, $C_{42}H_{66}N_8O_7^+$; calc. 817.4947); 800.4684 (100, $[M-OH+Na]^+$, $C_{42}H_{65}N_8O_6^+$; calc. 800.4688); 795.5118 (5, $[M+H]^+$, $C_{42}H_{67}N_8O_7^+$; calc. 795.5116). ESI-MS: 817.6 (2, $[M+Na]^+$); 795.6 (4, $[M+H]^+$); 398.5 (100, $[M+2H]^{2+}$).

3.6. *H-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-OH* (**19**). Purification of the crude product resulting from SPPS of **21** by RP-HPLC also afforded **19** as its TFA salt (34 mg). Anal. HPLC (10–100% *A* in 30 min): t_R 11.5 min (> 95%). ^{1}H-NMR (500 MHz, D_2O): 1.02–1.30 (*m*, 6 CH_2); 1.07 (*d*, *J* = 6.6, 3 Me); 1.26 (*d*, *J* = 6.7, Me); 1.52–1.73 (*m*, 3 CH_2); 2.12–2.20 (*m*, CH_2CO); 2.21–2.36 (*m*, 5 CH_2CO); 2.38–2.55 (*m*, 3 CH_2CO); 2.60–2.72 (*m*, 2 PhCH_2); 2.82–2.93 (*m*, PhCH_2, 3 CH_2NH_2); 3.61–3.66 (*m*, NHCH(R)CH_2); 3.88–3.97 (*m*, 3 NHCH(R)CH_2); 4.07–4.15 (*m*, 2 NHCH(R)CH_2); 4.32–4.46 (*m*, 3 NHCH(R)CH_2); 7.20–7.32 (*m*, 3 Ph); 7.79 (*d*, *J* = 8.9, NH); 7.98 (*d*, *J* = 8.7, NH); 8.09 (*d*, *J* = 8.7, NH). ^{13}C-NMR (125 MHz, D_2O): 20.11 (Me); 22.09, 22.19, 22.28 (CH_2); 24.67, 24.69, 24.73 (CH_2); 28.57, 28.85, 28.91 (CH_2); 35.02, 35.16 (CH_2); 41.55, 41.62, 41.80 (CH_2); 42.39 (CH_2); 43.57 (CH_2); 43.95 (CH_2); 44.99, 46.05, 46.07, 47.59, 49.05, 49.19, 50.56, 51.14, 51.24 (CH); 129.43 (CH); 131.24, 131.27 (CH); 131.94, 132.03, 132.23 (CH); 140.51, 140.56, 140.72 (C); 173.65, 174.53, 174.55, 174.63, 174.70, 175.18, 175.36, 175.52, 177.89 (C). ESI-MS: 1207.0 (12, $[M+Na]^+$); 1184.0 (34, $[M+H]^+$); 592.4 (93, $[M+2H]^{2+}$); 395.5 (100, $[M+3H]^{3+}$).

3.7. *H-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³- Phe-OH* (**20**). Purification of the crude product resulting from the SPPS of **21** by RP-HPLC also afforded **20** as its TFA salt (58 mg). Anal. HPLC (10–100% *A* in 30 min): t_R 14.1 min (> 98%). ^{1}H-NMR (500 MHz, CD_3OD): 1.13 (*d*, *J* = 6.8, Me); 1.14 (*d*, *J* = 6.8, 2 Me); 1.30–1.60 (*m*, 8 CH_2); 1.36 (*d*, *J* = 6.8, Me); 1.61–1.75 (*m*, 4 CH_2); 2.22–2.50 (*m*, 6 CH_2CO); 2.51–2.72 (*m*, 6 CH_2CO); 2.73–3.05 (*m*, 4 PhCH_2, 4 NH_2CH_2); 3.68–3.76 (*m*, NHCH(R)CH_2); 4.28–4.53 (*m*, 8 NHCH(R)CH_2); 4.62–4.69 (*m*, 2 NHCH(R)CH_2); 4.72–4.81 (*m*, NHCH(R)CH_2); 6.80 (*m*, NH); 7.13–7.28 (*m*, 4 Ph); 7.53 (*d*, *J* = 8.5, NH); 7.77 (*d*, *J* = 8.5, NH); 7.98 (*d*, *J* = 8.6, NH); 8.15 (*d*, *J* = 9.6, NH); 8.27 (*m*, NH); 8.36 (*m*, NH); 8.46 (*d*, *J* = 8.8, NH). ^{13}C-NMR (125 MHz, CD_3OD): 18.79, 20.93 (Me); 21.21, 21.40, 23.91, 24.04, 24.41, 24.59, 28.02, 28.12, 28.69, 28.84, 35.89, 35.94, 36.64, 36.74, 39.17, 40.63, 40.69, 41.12, 41.51, 41.55, 41.62, 41.81, 42.19, 43.19, 43.25, 43.32, 43.52, 43.61 (CH_2); 43.69, 43.81, 44.47, 46.63, 46.97, 47.17, 47.45, 47.55, 48.66, 49.28, 49.62, 49.78 (CH); 127.59, 127.63, 127.66 (CH); 129.28, 129.44, 129.49, 129.52 (CH); 130.48, 130.59, 130.62, 130.66 (CH); 139.30, 139.50, 139.72, 139.86 (C); 171.23, 171.32, 171.43, 171.59, 171.62, 171.82, 171.89, 171.98, 172.04, 172.13, 173.09, 175.05 (C). ESI-MS: 1573.4 (5, $[M+H]^+$); 786.6 (62, $[M+2H]^{2+}$); 524.9 (100, $[M+3H]^{3+}$); 394.1 (40, $[M+4H]^{4+}$).

3.8. *H-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-OH* (**21**). Purification of the crude product resulting from the SPPS of **21** by RP-HPLC afforded **21** as its TFA salt (359 mg). Anal. HPLC (10–100% MeCN in 30 min; C_{18}): t_R 16.6 min (> 96%). ^{1}H-NMR (500 MHz, CD_3OD): 1.12 (*m*, 3 Me); 1.32–1.78 (*m*, 36 H, CH_2 and Me); 2.20–2.49 (m, 8 CH_2CO); 2.52–2.75 (m, 7 CH_2CO); 2.78–3.02 (*m*, 5 PhCH_2, 5 NH_2CH_2); 3.66–3.75 (*m*, NHCH(R)CH_2); 4.25–4.52 (*m*, 10 NHCH(R)CH_2); 4.57–4.78 (*m*, 4 NHCH(R)CH_2); 7.08–7.26 (*m*, 5 Ph); 7.54 (*d*, *J* = 8.6, NH); 7.76 (*d*, *J* = 8.5, NH); 7.99 (*d*, *J* = 8.7, NH); 8.16 (*d*, *J* = 9.5, NH); 8.23–8.40 (*m*, NH); 8.46 (*d*, *J* = 8.9, NH). ESI-MS: 1983.7 (8, $[M+Na]^+$); 1961.7 (23, $[M+H]^+$); 981.3 (52, $[M+2H]^{2+}$); 654.2 (48, $[M+3H]^{3+}$); 491.0 (100, $[M+4H]^{4+}$); 395.4 (12, $[M+5H]^{5+}$).

3.9. *H-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-OH* (**22**). Purification of the crude product resulting from the SPPS of **22** by RP-HPLC afforded its TFA salt (91 mg). Anal. HPLC (10–100% *A* in 30 min): t_R 18.8 min (> 98%). ESI-MS: 2349.7 (2, $[M+H]^+$); 1175.5 (4, $[M+2H]^{2+}$); 783.9 (47, $[M+3H]^{3+}$); 588.1 (100, $[M+4H]^{4+}$); 470.7 (58.6, $[M+5H]^{5+}$).

3.10. *Counter-Ion Replacement of β-Peptides.* The multiply charged peptides **18**–**22** were isolated as the corresponding TFA salts (one TFA per basic site) after HPLC purification and lyophilization. Replacement of these counter ions by Cl^- [24]) was accomplished by dissolving the peptide in 0.1M HCl (> 100 equiv.) followed by re-lyophilization. This step was repeated once. The oily residue was then lyophilized 3 × from pure H_2O or until a fluffy white lyophilizate was obtained.

4. *Solid-Phase Synthesis of β-Peptides by Coupling Single Fmoc-Protected β³-Amino Acids.* 4.1. all-(R)-*H-(β³-HAla-β³-HLys-β³-HPhe)₃-OH* (*ent*-**19**). 4.1.1. *Anchoring. Wang* resin (0.3 g, initial loading 0.99 mmol/g, 1 equiv.) was dried *in vacuo* for 1 h and swelled in CH_2Cl_2 (10 ml) for 45 min. Fmoc-(*R*)-β³-HPhe-OH (*R*)-**5b** (477 mg, 1.19 mmol, 4 equiv.), DIC (0.23 ml, 1.48 mmol, 5 equiv.), and DMAP (3.6 mg, 0.03 mmol, 0.1 equiv.) were dissolved in DMF (3 ml). The homogeneous soln. was added to the resin, and the suspension was mixed for

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[24]) Replacement of the counter ions was done in order to avoid possible interference of TFA in antimicrobial assays [9c].

24 h. The resin was filtered, washed with DMF (5 ml, 3 × 5 min), CH_2Cl_2 (5 ml, 6 × 5 min), and MeOH (5 ml, 3 × 5 min) and dried *in vacuo* overnight. The peptide loading (*cf. Sect. 3.1*) was found to be 0.33 mmol/g. A second loading was performed with (*R*)-**5b** (240 mg, 0.59 mmol, 2 equiv.), DIC (0.12 ml, 0.74 mmol, 2.5 equiv.), DMAP (3.6 mg, 0.03 mmol, 0.1 equiv.), and HOBt (106 mg, 0.65 mmol, 2.2 equiv.) in DMF (3 ml). After washing, shrinking, and drying of the resin, the loading was determined to be 0.63 mmol/g.

4.1.2. *Solid-Phase Synthesis.* The resin with the anchored amino acid (*R*)-**5b** (300 mg, 0.63 mmol/g substitution, 0.19 mmol) was put in a solid-phase reactor and covered with DMF (3 ml). Unreacted OH groups were capped with Ac_2O (0.28 ml, 2.7 mmol) and a cat. amount of DMAP (33 mg, 0.27 mmol) overnight under intensive N_2 bubbling. The resin was filtered and washed with DMF (3 ml, 10 × 1 min). The solid-phase synthesis was continued by removing the Fmoc group with 20% piperidine in DMF (3 ml, 5 × 30 min). The resin was washed with DMF (3 ml, 6 × 3 min), and the elongation sequence was initiated by addition of a homogeneous soln. of the Fmoc-protected (*R*)-amino acid (0.567 mmol, 3 equiv.), HBTU (208 mg, 0.548 mmol, 2.9 equiv.), and HOBt (92 mg, 0.567 mmol, 3 equiv.) in DMF (3 ml), followed by addition of $EtN(i\text{-}Pr)_2$ (0.188 ml, 1.1 mmol, 5.8 equiv.). The resulting slightly yellow suspension was mixed by N_2 bubbling, and the coupling reactions were monitored by withdrawing a small aliquot of the beads and subjecting them to the TNBS test. Typically, 3 h of coupling were sufficient. A positive TNBS test (incomple coupling) was observed at residue six (from the *C*-terminus), *i.e.*, at the hexamer stage. In this case, another 1.5 equiv. of Fmoc-β^3-HAla-OH together with the corresponding quantities of coupling reagents were added, and the coupling was allowed to proceed for another 1.5 h (still positive TNBS test). The Fmoc-deprotection protocol was then switched to *1*) 20% piperidine in DMF (3 ml, 2 × 30 min), *2*) DBU/piperidine/DMF 1:1:48 (3 ml, 3 × 30 min), and *3*) 20% piperidine in DMF (3 ml, 1 × 30 min); this protocol was maintained through the rest of the synthesis. Similarly, coupling of residue eight (Fmoc-β^3-HLys(Boc)-OH) required 4 h. However, in this case, no additional amino acid had to be added. After completing the synthesis and performing the final deprotection, the resin was washed with DMF (5 ml, 6 × 4 min) and CH_2Cl_2 (5 ml, 10 × 1 min), shrunk with MeOH (5 ml, 6 × 3 min), and dried *in vacuo* for 24 h. Final cleavage was achieved with 40 ml of aq. TFA (95%). The resin was removed by filtration and washed with TFA. The org. layer was concentrated under reduced pressure. The oily residue was precipitated with cold Et_2O, and the peptide was dried *in vacuo* to yield 238 mg of the crude product as its TFA salt.

4.1.3. *HPLC Analysis and Purification of* ent-**19**. As in *Sect. 3.4*. Anal. HPLC (10–100% *A* in 30 min): t_R 11.4 min (95%). ESI-MS (pos. mode): 1184.0 (10, $[M+H]^+$); 592.5 (90, $[M+2H]^{2+}$); 395.5 (100, $[M+3H]^{3+}$). ESI-MS (neg. mode): 1182.0 (100, $[M-H]^-$). Other data identical to those reported for **19**.

4.2. *Solid-Phase Synthesis of H-(β^3-HAla-β^3-HLys-β^3-HPhe)$_3$-(β^3-HPhe)$_n$-OH* ($n = 1-3$; **23**–**25**). *Wang* resin (500 mg, initial loading 0.99 mmol/g, 1 equiv.) and all glassware used were dried *in vacuo* overnight. Fmoc-(*S*)-β^3-HPhe-OH (800 mg, 1.98 mmol, 4 equiv.), DIC (0.39 ml, 2.5 mmol, 5 equiv.), and DMAP (10 mg, 0.08 mmol, 0.15 equiv.) were dissolved in DMF (5 ml) and added to the resin. The suspension was allowed to react for 24 h. The resin was filtered, washed with DMF (10 ml, 3 × 5min), CH_2Cl_2 (10 ml, 6 × 5 min), and MeOH (10 ml, 3 × 5 min), and dried *in vacuo* overnight. The loading was determined as described in *Sect. 3.1*. Due to an extraordinarily low loading of < 0.1 mmol/g, two more loadings were performed using Fmoc-(*S*)-β^3-HPhe-OH (600 mg, 1.5 mmol, 3 equiv.), DIC (0.29 ml, 1.9 mmol, 3.8 equiv.), and DMAP (10 mg, 0.08 mmol, 0.15 equiv.) in DMF (5 ml) for 24 h. The degree of substitution was raised in this way to 0.25 and 0.50 mmol/g, resp. A fourth attempt to increase the loading was performed using Fmoc-(*S*)-β^3-HPhe-OH (400 mg, 1.0 mmol, 2 equiv.), DIC (0.20 ml, 1.25 mmol, 2.5 equiv.), and DMAP (5 mg, 0.05 mmol, 0.1 equiv.) in DMF (5 ml) overnight. The mixture was allowed to further react for 1 h at elevated temp. (gentle heating with a heat-gun) and 6 h at r.t. The resin was washed with DMF (10 ml, 6 × 5 min) and capped with Ac_2O (0.5 ml, 5.3 mmol) and a cat. amount of DMAP (64 mg, 0.5 mmol) overnight. A final analysis after washing, shrinking, and drying indicated a lowered (!) loading of 0.26 mmol/g. Despite these puzzling observations (see discussion), we decided to continue with the solid-phase synthesis by removing the Fmoc group with DBU/piperidine/DMF 1:1:48 (5 ml, 6 × 30 min) followed by washing with DMF (5 ml, 6 × 3 min). The elongation sequence was initiated by addition of a homogeneous soln. of the Fmoc-protected amino acid (0.625 mmol, 2.5 equiv.), HBTU (230 mg, 0.606 mmol, 2.4 equiv.), and HOBt (101 mg, 0.62 mmol, 2.5 equiv.) in DMF (5 ml), followed by addition of DIPEA (0.21 ml, 1.25 mmol, 5.0 equiv.). The resulting slightly yellow suspension was mixed by N_2 bubbling. The coupling reactions were monitored by withdrawing a small aliquot of the beads and subjecting them to the TNBS test. Typically, 3 h of coupling were sufficient. No particular difficulties were encountered during the chain elongation. After completing the synthesis and performing the final Fmoc deprotection, the resin was washed carefully with DMF (10 ml, 6 × 4 min) and CH_2Cl_2 (10 ml, 10 × 1 min), shrunk with MeOH (10 ml, 6 × 3 min), and finally dried *in vacuo* for 24 h. The dried resin was treated for 2.5 h with TFA/H_2O 95:5

(80 ml). The resin was removed by filtration, washed with TFA, and the org. layer was concentrated under reduced pressure. The product was precipitated with cold Et_2O and dried *in vacuo* to yield 417 mg of crude product (TFA salt). The peptides were purified by prep. RP-HPLC (30–70% *A* in 40 min) and were lyophilized to yield 18, 58 and 10 mg of **23**, **24**, and **25**, resp., as TFA salts.

*Data of H-(*S*)-β³-HAla-(*S*)-β³-HLys-(*S*)-β³-Phe-(*S*)-β³-HAla-(*S*)-β³-HLys-(*S*)-β³-Phe-(*S*)-β³-HAla-(*S*)-β³-HLys-(*S*)-β³-Phe-(*S*)-β³-Phe-OH* (**23**). Anal. HPLC (20–80% *A* in 20 min): t_R 11.3 min (>98%). ^{1}H-NMR (500 MHz, CD_3OD): 1.06–1.57 (*m*, 6 CH_2); 1.09 (*d*, *J* = 6.5, CH_3); 1.21 (*d*, *J* = 6.6, Me); 1.37 (*d*, *J* = 6.6, Me); 1.61–1.82 (*m*, 3 CH_2); 2.25–2.75 (*m*, 10 CH_2CO, 3 PhCH_2); 2.82–2.93 (*m*, PhCH_2, 3 NH_2CH_2); 3.14–3.18 (*m*, NHC*H*(R)CH_2); 3.70–3.78 (*m*, NHC*H*(R)CH_2); 4.30–4.48 (*m*, 6 NHC*H*(R)CH_2); 4.52–4.60 (*m*, NHC*H*(R)CH_2); 4.63–4.68 (*m*, NHC*H*(R)CH_2); 7.06–7.28 (*m*, 4 Ph); 7.48 (*d*, *J* = 9.0, NH); 7.78 (*d*, *J* = 9.3, NH); 7.91 (*d*, *J* = 9.2, NH); 8.16 (*d*, *J* = 8.5, NH); 8.23 (*d*, *J* = 9.5, NH); 8.26 (*m*, 3 NH); 8.42 (*m*, 2 NH). ^{13}C-NMR (125 MHz, CD_3OH): 18.99, 21.27, 21.53 (Me); 22.70, 22.84, 23.13 (CH_2); 23.59, 23.89, 23.94 (CH_2); 24.54 (CH_2); 28.22, 28.92, 29.03 (CH_2); 35.58, 36.04, 36.09 (CH_2); 40.96, 41.00, 41.34 (CH_2); 42.57 (CH_2); 43.36 (CH_2); 43.95 (CH_2); 45.94, 46.95, 47.10, 47.55, 48.00, 48.58, 49.45, 49.79, 49.93 (CH); 127.62 (CH); 129.32, 129.49, 129.52, 129.58 (CH); 130.51, 130.59, 130.62, 130.64 (CH); 139.35, 139.42, 139.52, 139.64 (C); 171.19, 171.36, 171.56, 171.76, 171.93, 172.10, 172.23, 172.31, 173.26, 175.45 (C). ESI-MS: 1367.0 (11, $[M+Na]^+$); 1345.1 (42, $[M+H]^+$); 672.8 (47, $[M+2H]^{2+}$); 449.1 (100, $[M+3H]^{3+}$).

*Data of H-(*S*)-β³-HAla-(*S*)-β³-HLys-(*S*)-β³-Phe-(*S*)-β³-HAla-(*S*)-β³-HLys-(*S*)-β³-Phe-(*S*)-β³-HAla-(*S*)-β³-HLys-(*S*)-β³-Phe-(*S*)-β³-Phe-(*S*)-β³Phe-OH* (**24**). Anal. HPLC (20–80% *A* in 20 min): t_R 14.6 min (>98%). ^{1}H-NMR (500 MHz, CD_3OD): 1.14 (*d*, *J* = 6.6, Me); 1.21 (*d*, *J* = 6.6, Me); 1.38 (*d*, *J* = 6.7, Me); 1.39–1.75 (*m*, 9 CH_2); 2.22–2.77 (*m*, 11 CH_2CO, 3 CH_2Ph); 2.79–2.97 (*m*, 2 CH_2Ph, 3 NH_2CH_2); 3.70–3.75 (*m*, 2 NHC*H*(R)CH_2); 4.32–4.37 (*m*, 2 NHC*H*(R)CH_2); 4.38–4.47 (*m*, 4 NHC*H*(R)CH_2); 4.52–4.70 (*m*, 4 NHC*H*(R)CH_2); 4.63–4.68 (*m*, NHC*H*(R)CH_2); 7.08–7.29 (*m*, 5 Ph); 7.54 (*d*, *J* = 9.6, NH); 7.71 (*d*, *J* = 9.3, NH); 7.74 (*m*, 2 NH); 7.91 (*d*, *J* = 8.9, NH); 8.04 (*d*, *J* = 8.8, NH); 8.17 (*d*, *J* = 9.5, NH); 8.23 (*d*, *J* = 8.9, 2 NH); 8.27 (*d*, *J* = 9.0, NH); 8.46 (*d*, *J* = 8.9, 2 NH). ^{13}C-NMR (125 MHz, CD_3OD): 18.99, 21.29, 21.67 (Me); 23.89, 24.61, 24.64, 28.17, 29.07, 29.09, 36.03, 36.84, 36.97, 39.19, 40.72, 40.95, 40.99, 41.01, 41.36, 41.56, 41.71, 41.89, 42.26, 42.44, 42.85, 43.18, 43.41, 43.50, 43.64 (CH_2); 44.00, 44.07, 46.94, 47.04, 47.55, 48.55, 49.45, 49.63, 49.69, 49.79, 49.94 (CH); 127.59, 127.69, 127.69, 127.75 (CH); 129.27, 129.38, 129.50, 129.56 (CH); 130.49, 130.52, 130.57, 130.61, 130.67 (CH); 139.19, 139.40, 139.52, 139.54, 139.61 (C); 171.05, 171.20, 171.54, 171.62, 171.76, 172.09, 172.34, 172.38, 173.28, 175.24 (C). ESI-MS: 1529.2 (20, $[M+Na]^+$); 1507.2 (37, $[M+H]^+$); 753.4 (34, $[M+2H]^{2+}$); 502.8 (100, $[M+3H]^{3+}$).

*Data of H-(*S*)-β³-HAla-(*S*)-β³-HLys-(*S*)-β³-Phe-(*S*)-β³-HAla-(*S*)-β³-HLys-(*S*)-β³-Phe-(*S*)-β³-HAla-(*S*)-β³-HLys-(*S*)-β³-Phe-(*S*)-β³-Phe-(*S*)-β³-Phe-(*S*)-β³-Phe-OH* (**25**). Anal. HPLC (20–80% *A* in 20 min.): t_R 18.0 min (>98%). ^{1}H-NMR (500 MHz, CD_3OD): 1.13–1.77 (*m*, 9 CH_2); 1.14 (*d*, *J* = 6.0, Me); 1.21 (*d*, *J* = 6.0, Me); 1.38 (*d*, *J* = 6.0, Me); 2.22–2.82 (*m*, 12 CH_2CO, 4 PhCH_2); 2.83–3.00 (*m*, 2 PhCH_2, 3 NH_2CH_2); 3.74 (*m*, NHC*H*(R)CH_2); 4.34–4.50 (*m*, 6 NHC*H*(R)CH_2); 4.51–4.73 (*m*, 5 NHC*H*(R)CH_2); 7.06–7.28 (*m*, 6 Ph); 7.47 (*d*, *J* = 9.6, NH); 7.71 (*d*, *J* = 9.3, NH); 7.92 (*d*, *J* = 9.0, NH); 8.05–8.15 (*m*, 2 NH); 8.19 (*d*, *J* = 9.0, NH); 8.24 (*m*, 3 NH); 8.28 (*d*, *J* = 9.0, NH); 8.48 (*m*, 2 NH). ^{13}C-NMR (125 MHz, CD_3OD): 19.00, 21.30, 21.67 (Me); 23.89, 24.65, 24.76, 28.15, 29.09, 30.78, 36.02, 36.87, 37.18, 39.42, 40.96, 41.01, 41.37, 41.69, 42.07, 42.24, 42.68, 43.19, 43.40, 43.64 (CH_2); 44.04, 46.94, 47.56, 47.67, 48.37, 48.66, 49.46, 49.63, 49.79 (CH); 127.67 (CH); 129.32, 129.50, 129.54 (CH); 130.51, 130.60 (CH); 139.32, 139.51 (C); 171.29, 171.51, 171.77, 172.12, 172.37, 173.29, 175.31 (C). ESI-MS: 1690.3 (15, $[M+Na]^+$); 1667.2 (42, $[M+H]^+$); 834.3 (80, $[M+2H]^{2+}$); 556.4 (100, $[M+3H]^{3+}$).

4.3. *Solid-Phase Synthesis and Purification of the Cys-Containing β-hexadecapeptide* **26**. A part of the resin remaining from the SPPS of **21** (*cf. Sect. 3*) was used for the synthesis of a β³-HCys-containing analog of this peptide. The resin (160 mg; *ca.* 0.064 mmol) containing the bound peptides **19**–**21** was placed in a reactor and allowed to swell in CH_2Cl_2 for 1 h. The Fmoc group was removed with DBU/piperidine/DMF 1:1:48 (3 ml, 6 × 10 min). After washing with NMP (3 ml, 9 × 1 min) and CH_2Cl_2 (3 ml, 1 × 1 min), a soln. of Boc-β³-HCys(Trt)-OH (148 mg, 0.32 mmol, 5 equiv.), HBTU (116 mg, 0.31 mmol, 4.8 equiv.), and HOBt (52 mg, 0.32 mmol, 5 equiv.) in NMP (3 ml) was added to the resin, followed by DIPEA (0.11 ml, 0.64 mmol, 10 equiv.). The mixture was allowed to react overnight (negative TNBS test). The resin was washed with NMP (5 ml, 10 × 1 min) and CH_2Cl_2 (5 ml, 10 × 1 min), shrunk with MeOH (5 ml, 5 × 1 min), and dried *in vacuo*. The peptide was cleaved from the resin and simultaneously deprotected with a mixture of TFA/H_2O/EDT/TIS 94.5:2.5:2.5:1 (20 ml) for 3 h to yield – after evaporation, precipitation (Et_2O), washing (Et_2O), and drying – 170 mg of crude product. The crude material was found to contain mainly three components: the β³-HCys derivative of **19** (t_R 12.5 min, 8.6%) and **20** (t_R 20.0 min, 15%), resp., and the desired peptide **26** (t_R 25.5 min, 47%).

Data for H-(S)-β³-Cys-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-(S)-β³-HAla-(S)-β³-HLys-(S)-β³-Phe-OH (**26**). Anal. HPLC (20–80% *A* in 20 min): t_R 16.5 min (> 98%). ESI-MS: 2076.6 (5, $[M+H]^+$); 1039.8 (13, $[M+2H]^{2+}$); 693.2 (67, $[M+3H]^{3+}$); 520.2 (100, $[M+4H]^{4+}$); 416.6 (17.4, $[M+5H]^{5+}$).

5. *Preparation of β²/β³-Dipeptide Building Blocks* **28**–**33**. 5.1. *Boc-(S)-β²-HVal-(S)-β³-HPhe-OBn* (**27**). Boc-(*S*)-β³-HPhe-OBn ((*S*)-**5a**) (320 mg, 0.865 mmol, 1.0 equiv.) was deprotected according to *GP 3*. The resulting oily residue was coupled to Boc-(*S*)-β²-HVal-OH[16]) (200 mg, 0.86 mmol, 1.0 equiv.) according to *GP 5b*. FC (AcOEt/pentane 2:11 → 1:1) Afforded **27** (408 mg, 0.845 mmol, 98%) as a colorless solid. NMR Spectroscopic analysis showed that the product was a 85:15 mixture of diastereoisomers due to the low enantiomeric purity of the starting Boc-(*S*)-β²-HVal-OH. Recrystallization from AcOEt/hexane enhanced this ratio to 94:6 (70% yield). M.p. 166–168°. R_f 0.33 (AcOEt/pentane 1:2). $[\alpha]_D^{r.t.} = +16.2$ ($c = 0.5$, $CHCl_3$). IR ($CHCl_3$): 3650*w*, 2900*s*, 1730*s*, 1470*s*, 1380*s*, 1230*s*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.74 (*d*, *J* = 6.6, Me); 0.86 (*d*, *J* = 6.7, Me); 1.40 (*s*, *t*-Bu); 1.72–1.81 (*m*, C*H*(C*H*Me$_2$)$_2$); 1.90–1.98 (*m*, C*H*(R)CO); 2.52–2.57 (*m*, PhCH_2); 2.79–2.84 (*dd*, *J* = 13.7, 7.5, C*H*HCO); 2.87–2.93 (*dd*, *J* = 13.6, 7.4, 1 H, CH_2CO); 3.11–3.20 (*m*, 1 H, BocHNCH_2); 3.30–3.38 (*m*, 1 H, BocHNCH_2); 4.51–4.58 (*m*, NHC*H*(R)CH_2); 5.03 (br. *s*, NH); 5.09–5.12 (*d*, *J* = 12.2, 1 H, PhCH_2O); 5.09–5.12 (*d*, *J* = 12.2, 1 H, PhCH_2O); 6.05 (br. *s*, NH); 7.12–7.15 (*m*, 2 arom. H); 7.18–7.23 (*m*, 1 arom. H); 7.25–7.30 (*m*, 2 arom. H); 7.34–7.40 (*m*, 5 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 19.97 (Me); 20.67 (Me); 23.59 (CH); 28.42 (Me); 37.66 (CH_2); 40.01(CH_2); 40.61 (CH_2); 47.35 (CH); 54.07 (CH); 66.64 (CH_2); 79.09 (C); 128.80 (CH), 128.46 (CH); 128.50 (CH); 128.65 (CH); 128.68 (CH); 129.58 (CH); 135.55 (C); 137.36 (C); 156.63 (C); 173.69 (C); 174.42 (C). HR-MALDI-MS: 505.2673 (95, $[M+Na]^+$, $C_{28}H_{38}N_2O_5Na$; calc. 505.6016); 405.2 (80, $[M-t\text{-Bu}+Na]^+$, $C_{24}H_{37}N_2O_7Na$; calc. 502.2524); 383.2 (100, $[M-t\text{-Bu}]^+$. Anal. calc. for $C_{28}H_{38}N_2O_5$ (482.62): C 69.86, H 7.94, N 5.80; found: C 69.83, H 7.95, N 5.98.

5.2. *Fmoc-(S)-β²-HVal-(S)-β³-HPhe-OH* (**28**). Boc-(*S*)-β²-HVal-(*S*)-β³-HPhe-OBn (**27**) (0.292 g, 0.60 mmol) was deprotected according to *GP 4* and *GP 3*, which afforded the free dipeptide in quantitative yield. The residue was directly dissolved in a 1:1 mixture of dioxane/H_2O (0.062M) before Na_2CO_3 (0.136 g, 1.21 mmol, 2 equiv.) was added. Then, Fmoc-OSu [35] (0.204 g, 0.60 mmol, 1 equiv.) was added, the mixture was sonicated for 60 min and then allowed to react for 24 h at r.t. The pH was adjusted to 1–2, and AcOEt was added to dissolve the white precipitate formed. The org. layer was washed with H_2O (4 × 20 ml), dried (Na_2SO_4), and evaporated to a white solid. The solid was dissolved in a soln. of 5% MeOH in CH_2Cl_2 (insoluble materials being removed by filtration), and precipitated by addition of pentane. Filtration and drying *in vacuo* afforded 261 mg (82%) of **28** as a colorless powder. This material was pure enough to be used in SPPS. An anal. sample was obtained by FC (MeOH/CH_2Cl_2 1:10, 1% AcOH). M.p. 226°. R_f 0.10 (MeOH/CH_2Cl_2 1:20, 1% AcOH). Anal. HPLC (20–80% *A* in 20 min): t_R 17.5 min (> 98%). $[\alpha]_D^{r.t.} = +4.2$ ($c = 0.2$, DMF). IR (KBr): 3314*m*, 3066*w*, 2962*s*, 1705s, 1646*s*, 1548*s*, 1449*w*, 1264*m*, 1140*w*, 738*m*. ^{1}H-NMR (400 MHz, CD_3OD): 0.59 (*d*, *J* = 6.7, Me); 0.83 (*d*, *J* = 6.7, Me); 1.58–1.70 (*m*, C*H*Me$_2$); 2.02–2.08 (*m*, C*H*(R)CO); 2.48–2.55 (*m*, PhCH_2); 2.72–2.79 (*m*, 1 H, CH_2CO); 2.88–2.96 (*m*, 1 H, CH_2CO); 3.13–3.20 (*m*, FmocHNC*H*H); 3.34–3.40 (*m*, FmocHNCH_2); 4.19–4.23 (*m*, C*H*CH_2O); 4.24–4.29 (*m*, 1 H, CHCH_2O); 4.31–4.35 (*m*, 1 H, CHCH_2O); 4.48–4.61 (*m*, NHC*H*(R)CH_2); 6.13 (br. *s*, NH); 6.78 (*m*, NH); 7.12–7.18 (*m*, 1 arom. H); 7.21–7.25 (*m*, 3 arom. H); 7.29–7.34 (*m*, 2 arom. H); 7.36–7.41 (*m*, 2 arom. H); 7.62–7.66 (*m*, 2 arom. H); 7.78–7.80 (*d*, *J* = 7.6, 2 arom. H); 7.93–7.95 (*d*, *J* = 8.6, arom. H). ^{13}C-NMR (100 MHz, CD_3OD): 20.57 (Me); 21.02 (Me); 29.65 (CH); 40.76 (CH_2); 41.20 (CH_2); 42.49 (CH_2); 48.41 (CH); 48.83 (CH); 55.38 (CH); 68.01 (CH_2); 120.94 (CH); 126.27 (CH); 127.51 (CH), 128.21 (CH); 128.82 (CH); 129.44 (CH); 130.39 (CH); 139.74 (C); 142.60 (C); 145.39 (C); 158.81 (C); 175.69 (C); 175.94 (C). HR-MALDI-MS: 537.2366 (37, $[M+Na]^+$, $C_{31}H_{34}N_2O_5Na$; calc. 537.2369); 515.2545 (21, $[M+H]^+$, $C_{31}H_{35}N_2O_5$); 497.2440 (30.6, $[M-OH]^+$, $C_{31}H_{33}N_2O_4$); 273.0401 (100). Anal. calc. for $C_{31}H_{34}N_2O_5$ (482.62): C 69.86, H 7.94, N 5.80; found: C 69.83, H 7.95, N 5.98.

5.3. *Cbz-(S)-β²-HLeu-(S)-β³-HPhe-OBn* (**29**). Boc-(*S*)-β³-HPhe-OBn ((*S*)-**5a**) (0.99 g, 2.68 mmol, 1.0 equiv.) was deprotected according to *GP 3*. The resulting oily residue was coupled to Cbz-(*S*)-β²-HLeu-OH ((*S*)-(**8**) (0.75 g, 2.68 mmol, 1.0 equiv.) according to *GP 5b*. FC (AcOEt/pentane 2:1 → 1:1) afforded **29** (1.28 mg, 2.41 mmol, 90%) as a colorless powder. NMR Spectroscopic analysis showed no presence of the unwanted diastereoisomer. M.p. 135–137°. R_f 0.30 (AcOEt/pentane 1:2). $[\alpha]_D^{r.t.} = +26.2$ ($c = 0.5$, $CHCl_3$). IR ($CHCl_3$): 3650*w*, 3600*m*, 3000*s*, 1710*s*, 1660*m*, 1510*m*, 1420*m*, 1200*s*, 1140*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.77–0.83 (*m*, 2 Me); 1.06–1.15 (*m*, CH_2CHMe_2); 1.24–1.42 (*m*, CH_2CHMe$_2$); 2.26–2.34 (*m*, C*H*(R)CO); 2.43–2.49 (*dd*, *J* = 15.8, 6.2, 1 H, CH_2CO); 2.52–2.59 (*dd*, *J* = 15.8, 5.0, 1 H, CH_2CO); 2.83 (*d*, *J* = 7.4, PhCH_2); 3.09–3.17 (*m*, 1 H, BocHNCH_2); 3.28–3.36 (*m*, 1 H, BocHNCH_2); 4.50–4.59 (*m*, NHC*H*(R)CH_2); 5.02–5.13 (*m*, 2 PhCH_2O); 5.33 (br. *s*, NH); 5.91–5.94 (br. *d*, *J* = 8.7, NH); 7.11–7.14 (*d*, *J* = 6.7, 2 arom. H); 7.19–7.40 (*m*, 13 arom. H). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.23 (Me); 22.94 (Me); 25.61 (CH); 37.97 (CH_2); 38.96 (CH_2);

40.06 (CH_2); 43.36 (CH_2); 45.23 (CH); 47.21 (CH); 66.56 (CH_2); 66.67 (CH_2); 77.02 (C); 126.82 (CH), 128.00 (CH); 128.04 (CH); 128.43 (CH); 128.46 (CH); 128.48 (CH); 128.62 (CH); 128.65 (CH); 129.16 (CH); 135.55 (C); 136.67 (C); 137.21 (C); 156.56 (C); 171.56 (C); 173.93 (C). HR-MALDI-MS: 553.2668 (100, $[M+Na]^+$, $C_{32}H_{38}N_2O_5Na^+$; calc. 553.2673); 531.2849 (18, $[M+H]^+$, $C_{32}H_{39}N_2O_5$; calc. 531.2846); 423.2279 (34.55, $[M-Cbz+Na]^+$, $C_{24}H_{31}N_2O_3Na^+$; calc. 423.2281). Anal. calc. for $C_{32}H_{38}N_2O_5$ (530.66): C 72.43, H 7.22, N 5.28; found: C 72.61, H 7.41, N 5.15.

5.4. *Fmoc-(S)-β²-HLeu-(S)-β³-HPhe-OH* (**30**). Peptide **29** (1.2 g, 2.26 mmol) was deprotected according to *GP 1b*. The resulting dried filtrate was dissolved in a 1:1 mixture of dioxane/H_2O (0.062M) and Na_2CO_3 (0.479 g, 4.52 mmol, 2 equiv.). Fmoc-OSu (0.762 g, 2.26 mmol, 1 equiv.) was added, the mixture was sonicated for 60 min and left to react for 24 h at r.t. The pH was adjusted to 1–2, and AcOEt was added to dissolve the white precipitate formed. The org. layer was washed with H_2O (4 × 20 ml), dried (Na_2SO_4), and evaporated to a white solid. The solid was dissolved in a soln. of 5% MeOH in CH_2Cl_2 (insoluble materials being removed by filtration), and precipitated by addition of pentane. Filtration and drying *in vacuo* afforded **30** (0.95 g, 80%) as a colorless powder pure enough for SPPS. An anal. sample was obtained by FC ($MeOH/CH_2Cl_2$ 1:10, 1% AcOH). M.p. 218°. R_f 0.10 ($MeOH/CH_2Cl_2$ 1:20, 1% AcOH). Anal. HPLC (20–80% *A* in 20 min): t_R 19.0 min (> 98%). $[\alpha]_D^{r.t.} = +2.2$ ($c = 0.5$, DMF). IR ($CHCl_3$): 3323*s*, 3064*s*, 2954*s*, 1697s, 1646*s*, 1541*s*, 1450*m*, 1258*s*, 1152*m*, 1032*m*. ^{1}H-NMR (500 MHz, CD_3OD): 0.72–0.74 (*m*, 2 Me); 0.95–1.08 (*m*, CH_2CHMe_2); 1.32–1.40 (*m*, CH_2CHMe_2); 2.38–2.43 (*m*, CH(R)CO); 2.46–2.53 (*m*, $PhCH_2$); 2.67–2.72 (*dd*, $J = 13.3$, 9.5, 1 H, CH_2CO); 2.92–2.98 (*m*, 1 H, CH_2CO); 3.11–3.14 (*m*, $FmocHNCH_2$); 4.17–4.23 (*m*, $CHCH_2O$); 4.25–4.28 (*m*, 1 H, $CHCH_2O$); 4.32–4.36 (*m*, 1 H, $CHCH_2O$); 4.51–4.58 (*m*, $NHCH(R)CH_2$); 6.25 (br. *s*, NH); 6.93 (*m*, NH); 7.14–7.18 (*m*, 1 arom. H); 7.20–7.26 (*m*, 3 arom. H); 7.30–7.33 (*m*, 2 arom. H); 7.37–7.41 (*m*, 2 arom H); 7.60–7.66 (*m*, 2 arom. H); 7.78–7.80 (*d*, $J = 7.5$, 2 arom. H); 7.96–7.97 (*d*, $J = 8.7$, arom. H). ^{13}C-NMR (125 MHz, CD_3OD): 22.19 (Me); 23.72 (Me); 26.46 (CH); 39.86 (CH_2); 40.58 (CH_2); 41.02 (CH_2); 44.77 (CH_2); 46.53 (CH); 48.23 (CH); 48.83 (CH); 67.81 (CH_2); 120.78 (CH), 126.14 (CH); 127.29 (CH); 128.04 (CH); 128.65 (CH); 129.22 (CH); 130.19 (CH); 139.49 (C); 142.43 (C); 145.18 (C); 158.71 (C); 175.26 (C); 176.07 (C). HR-MALDI-MS: 551.2516 (100, $[M+Na]^+$, $C_{32}H_{36}N_2O_5Na^+$; calc. 551.2519); 529.2697 (26, $[M+H]^+$, $C_{32}H_{37}N_2O_5^+$; calc. 529.2690); 511.26 (51, $[M-OH]^+$, $C_{32}H_{35}N_2O_4^+$); 333.18 (32, $[M-C_{14}H_{11}O]^+$, $C_{18}H_{25}N_2O_4^+$); 307.20 (53, $[M-Fmoc+H]^+$, $C_{17}H_{27}N_2O_3$).

5.5. *Cbz-(S)-β²-HLeu-(S)-β³-HLys(Boc)-OMe* (**31**). Cbz-(*S*)-β³-HLys-OMe ((*S*)-**6a**) (3.00 g, 7.35 mmol, 1.0 equiv.) was deprotected according to *GP 4*. A portion of the resulting oily residue (737 mg, 2.9 mmol, 1.0 equiv.) was coupled to Cbz-(*S*)-β²-HLeu-OH (**8**) (750 mg, 2.69 mmol, 1.0 equiv.) according to *GP 5b*. FC (AcOEt/pentane 4:1 → 1:1) afforded **31** (869 mg, 1.62 mmol, 61%) as a colorless solid. NMR spectroscopic analysis did not show the presence of the undesired diastereoisomer. M.p. 68–69°. R_f 0.46 (AcOEt/pentane 1:2). $[\alpha]_D^+ = +5.32$ ($c = 0.555$, $CHCl_3$). IR ($CHCl_3$): 3453*w*, 3028*w*, 3009*m*, 2956*m*, 2878*m*, 1710*s*, 1671*m*, 1507*m*, 1439*m*, 1410*w*, 1365*m*, 1229*m*, 1216*s*, 1168*m*, 1089*w*, 645*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.89 (*d*, $J = 5.6$, Me); 0.90 (*d*, $J = 5.7$, Me); 1.19–1.37 (*m*, $CHMe_2$, CH_2); 1.43 (*s*, *t*-Bu); 1.44–1.61 (*m*, 3 CH_2); 2.32–2.47 (*m*, CH_2CO); 2.49–2.55 (*m*, CHCO); 3.07 (*m*, CH_2NHBoc); 3.13–3.22 (*m*, 1 H, $CbzHNCH_2$); 3.30–3.41 (*m*, $CbzHNCH_2$); 3.62 (*m*, MeO); 4.21 (*m*, $NHCH(R)CH_2$); 4.56 (br. *s*, NH); 5.08 (*s*, $PhCH_2$); 5.51 (br. *s*, NH); 5.98 (*d*, $J = 8.7$, NH); 7.30–7.40 (*m*, Ph). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.31 (Me); 22.95 (Me); 23.23 (CH_2); 25.89 (CH); 28.43 (Me); 29.51, 33.85, 38.87, 39.00, 40.23, 43.47 (CH_2); 45.38, 46.02 (CH); 51.86 (Me); 66.57 (CH_2); 79.14 (C); 128.01, 128.05, 128.48 (CH); 136.67 (C); 156.04, 156.61 (C); 172.30, 174.17 (C). HR-MALDI-MS: 558.3143 (77, $[M+Na]^+$, $C_{28}H_{45}N_3O_7^+$; calc. 558.3146); 502.2522 (29, $[M-t\text{-}Bu+Na]^+$, $C_{24}H_{37}N_3O_7^+$; calc. 502.2524); 458.2623 (57, $[M-Boc+Na]^+$, $C_{23}H_{37}N_3O_5^+$; calc. 458.2627); 436.2803 (100, $[M-Boc+H]^+$, $C_{23}H_{37}N_3O_5^+$; calc. 436.2805). Anal. calc. for $C_{28}H_{45}N_3O_7$ (535.68): C 62.78, H 8.47, N 7.84; found: C 62.74, H 8.33, N 7.66.

5.6. *Cbz-(S)-β²-HLeu-(S)-β³-HLys(Boc)-OH* (**32**). Dipeptide **31** (837 mg, 1.56 mmol, 1.0 equiv.) was dissolved in THF (7.8 ml) under Ar and stirred for 15 min at r.t. Then, a 1.0M soln. of $LiOH \cdot H_2O$ (328 mg, 7.81 mmol, 5 equiv.) in H_2O (7.8 ml) was added. The yellow mixture was stirred for 20 h at 0°, treated with 1N HCl, and extracted with AcOEt and $CHCl_3$. The combined org. layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to yield **32** (815 mg, 1.56 mmol; quant) as a colorless solid, which was used without further purification. IR ($CHCl_3$): 3450*w*, 3029*w*, 3008*m*, 2953*s*, 2874*m*, 1711*s*, 1671*m*, 1548*m*, 1501*m*, 1434*m*, 1410*w*, 1363*m*, 1227*m*, 1220*s*, 1168*m*, 1080*w*, 819*m*, 645*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 0.81–0.83 (*m*, 2 Me); 1.08–1.17 (*m*, $CHMe_2$); 1.20–1.32 (*m*, CH_2); 1.37 (*s*, *t*-Bu); 1.38–1.53 (*m*, 3 CH_2); 2.25–2.38 (*m*, CHCO); 2.41–2.52 (*m*, CH_2CO); 2.98–3.05 (*m*, CH_2NHBoc); 3.06–3.17 (*m*, 1 H, $CbzHNCH_2$); 3.20–3.29 (*m*, 1 H, $CbzHNCH_2$); 4.17 (*m*, $NHCH(R)CH_2$); 4.58 (br. *s*, NH); 4.99–5.05 (*m*, $PhCH_2$); 5.65 (br. *s*, NH); 6.26 (*m*, NH); 7.21–7.32 (*m*, Ph). ^{13}C-NMR (100 MHz, $CDCl_3$): 22.21 (Me); 23.02 (Me); 23.22 (CH_2); 25.61 (CH); 25.92 (CH_2); 28.43 (Me); 29.49, 33.76, 38.89, 40.19, 43.51 (CH_2); 45.15, 46.26 (CH); 67.97 (CH_2); 79.11 (C); 128.04,

128.51 (CH); 136.92 (C); 156.16, 156.87 (C); 172.35, 174.59 (C). HR-MALDI-MS: 544.2999 (39, $[M+Na]^+$, $C_{27}H_{43}N_3O_7^+$; calc. 544.3002); 488.2378 (16, $[M-t\text{-Bu}+Na]^+$, $C_{23}H_{35}N_3O_7^+$; calc. 488.2379); 444.2474 (54, $[M-Boc+Na]^+$, $C_{22}H_{35}N_3O_5^+$; calc. 444.2470); 422.2657 (100, $[M-Boc+H]^+$, $C_{22}H_{35}N_3O_5^+$; calc. 422.2658).

5.7. *H-(S)-β²-HLeu-(S)-β³-HLys(Boc)-OH* (**32b**). Peptide **32** (815 mg, 1.56 mmol, 1.0 equiv.) was deprotected according to *GP 4*. The resulting viscous oil (696 mg, 1.56 mmol; quant.) was used without further purification.

5.8. *Fmoc-(S)-β²-HLeu-(S)-β³-HLys(Boc)-OH* (**33**). Peptide **32b** (696 mg, 1.56 mmol, 1 equiv.) was suspended in dioxane (13 ml) and H_2O (13 ml) [34]. Powdered Na_2CO_3 (334 mg, 3.13 mmol, 2 equiv.) was added, and the mixture was sonicated for 45 min until the dipeptide was entirely dissolved. The homogeneous soln. was cooled to 0°, Fmoc-OSu [35] (527 mg, 1.56 mmol, 1 equiv.) was added, and the resulting mixture was stirred for 24 h at r.t. Then, the pH was adjusted to 1–2, the soln. was filtered (*N4* filter), and the colorless precipitate was dried *in vacuo*. The crude product was dissolved in a 3:1 mixture of $CHCl_3$ and MeOH, precipitated with MeCN, filtered, and dried *in vacuo* to yield **33** (877 mg, 1.44 mmol; 92%) as a colorless powder. M.p. 135–137°. Anal. HPLC (20–80% *A* in 20 min) t_R 18.0 min (> 98%). $[\alpha]_D^{rt} = +15.51$ ($c = 0.555$, $CHCl_3$). IR ($CHCl_3$): 3458*w*, 3022*w*, 3008*m*, 2972*m*, 2954*m*, 2878*m*, 1712*s*, 1671*m*, 1649*s*, 1508*m*, 1431*m*, 1410*w*, 1362*m*, 1220*m*, 1214*s*, 1161*m*, 1080*w*, 870*m*. ^{1}H-NMR (400 MHz, CD_3OD): 0.89–0.93 (*m*, 2 Me); 1.14–1.19 (*m*, C*H*Me$_2$); 1.28–1.40 (*m*, CH_2); 1.41–1.63 (*m*, 3 CH_2); 1.42 (*s*, *t*-Bu); 2.40–2.58 (*m*, CH_2CO, C*H*(R)CO); 2.98–3.02 (*m*, CH_2NHBoc); 3.20 (m, 1 H, CbzHNCH_2); 3.32 (*m*, 1 H, CbzHNCH_2); 4.18–4.30 (*m*, NHC*H*(R)CH_2, OCH_2C*H*); 4.32–4.39 (*m*, OCH_2CH); 7.03 (*m*, NH); 7.28 (*m*, 2 arom. H); 7.34 (*m*, 2 arom. H); 7.63 (*m*, 2 arom. H); 7.78 (*d*, $J = 7.5$, 2 arom. Ph). ^{13}C-NMR (100 MHz, CD_3OD): 22.38 (Me); 23.90 (Me); 24.44 (CH_2); 27.25 (CH); 28.84 (Me); 30.55, 34.90, 40.10, 40.82, 41.29, 44.88 (CH_2); 46.75, 47.63, 48.42 (CH); 67.99 (CH_2); 79.88 (C); 120.96, 126.24, 128.20, 128.82 (CH); 142.61, 145.37 (C); 158.14, 158.83 (C); 175.15, 176.42 (C). HR-MALDI-MS: 632.3305 (45, $[M+Na]^+$, $C_{34}H_{47}N_3O_7^+$; calc. 632.3309); 576.2692 (15, $[M-t\text{-Bu}+Na]^+$, $C_{30}H_{39}N_3O_7^+$; calc. 576.2963); 532.2795 (65, $[M-Boc+Na]^+$, $C_{29}H_{39}N_3O_5^+$; calc. 532.2794); 510.2963 (100, $[M-Boc+H]^+$, $C_{29}H_{39}N_3O_5^+$; calc. 510.2966).

6. *Solid-Phase Synthesis of H-(S)-β³-HPhe-(S)-β²-HVal-(S)-β³-Phe-(S)-β²-HLeu-(S)-β³-HLys-(S)-β²-Leu-(S)-β³-HLys-(S)-β²-HLeu-(S)-β³-Phe-OH* (**34**) via *Partial Fragment Condensation*. *Wang* resin (362 mg, initial loading 0.90 mmol/g, 1 equiv.), Fmoc-(*S*)-β²-HLeu-(*S*)-β³-HPhe-OH (**30**; 516 mg, 0.977 mmol, 3 equiv.) and all glassware used were dried *in vacuo* overnight. The flask containing **30** was capped with a septum and kept under Ar. A minimum amount of 20% DMF in anh. CH_2Cl_2 was added to dissolve the dipeptide, and MSNT (289 g, 0.977 mmol, 3 equiv.) and MeIm (58 µl, 0.733 mmol, 2.25 equiv.) were introduced. After 10 min, this soln. was transferred to a sealed flask containing the resin, and the mixture was left to react for 2 d under Ar with gentle agitation. Then, the resin was filtered off, washed with DMF (10×), CH_2Cl_2 (10×), and shrunk with MeOH (5×). After drying *in vacuo* for 24 h, the degree of loading was found to be 0.74 mmol/g (*cf. Sect. 3.1*) [26a].

The resin with the anchored dipeptide (531 mg, 0.391 mmol) was put in a solid-phase reactor and swelled for 30 min by addition of CH_2Cl_2. Capping was performed twice with Ac_2O (0.25 ml, 2.65 mmol, 10 equiv.) and a cat. amount of DMAP (3.2 mg, 0.02 mmol) in CH_2Cl_2 for 1 h under intensive N_2 bubbling. The resin was filtered and washed with NMP (5 ml, 10 × 1 min). The Fmoc group was removed with DBU/piperidine/NMP 2:2:96 (5 ml, 6 × 60 min). The resin was washed with NMP (5 ml, 9 × 1 min) and CH_2Cl_2 (5 ml, 1 × 1 min). The elongation sequence was initiated by addition of a homogeneous soln. of **33** (477 mg, 0.78 mmol, 2.0 equiv.), HBTU (285 mg, 0.75 mmol, 1.92 equiv.), HOBt (127 mg, 0.78 mmol, 2.0 equiv.), and DIPEA (0.27 ml, 1.56 mmol, 4.0 equiv.) in NMP (5 ml). The slightly yellow suspension was agitated by N_2 bubbling for 24 h. This procedure (deprotection and coupling of **33**) was repeated once more to yield the resin-bound hexamer. At this stage, the resin was shrunk and dried *in vacuo*. SPPS was continued with one third of the material (*ca.* 0.130 mmol) by addition of **28** (138 mg, 0.26 mmol, 2.0 equiv.), HBTU (95 mg, 0.25 mmol, 1.92 equiv.), HOBt (42 mg, 0.26 mmol, 2.0 equiv.), and DIPEA (0.09 ml, 0.52 mmol, 4.0 equiv.) in NMP (5 ml), and the reaction was allowed to proceed for 24 h. The last residue was introduced by reacting with **5b** (209 mg, 0.52 mmol, 4.0 equiv.) in the presence of HBTU (195 mg, 0.51 mmol, 3.96 equiv.), HOBt (84 mg, 0.52 mmol, 4.0 equiv.), and DIPEA (0.18 ml, 1.04 mmol, 8.0 equiv.) in NMP (5 ml) for 24 h. The resin was washed with NMP (5 ml, 10 × 1 min) and CH_2Cl_2 (5 ml, 10 × 1 min), shrunk with MeOH (5 ml, 5 × 1 min), and dried *in vacuo*. The resulting peptide was cleaved from the resin and deprotected by exposure to TFA/H_2O 95:5 for 3 h to yield – after evaporation, precipitation (Et_2O), washing (Et_2O), and drying *in vacuo* – crude **34** (100 mg). Purification of the crude product by RP-HPLC afforded **34** as its TFA salt (78 mg, colorless lyophilizate). Anal. HPLC (20–90% *A* in 30 min): t_R 22.3 min (> 98%). ^{1}H-NMR (500 MHz, CD_3OD): 0.52 (*d*, $J = 6.7$, Me); 0.72 (*d*, $J = 6.6$, 2 Me); 0.83 (*d*, $J = 6.7$, Me); 0.87 (*d*, $J = 6.0$, 2 Me); 0.88–1.02 (*m*, 2 CH_2C*H*Me$_2$); 0.92 (*d*, $J = 6.6$, Me); 0.93 (*d*, $J = 6.5$, Me); 1.09–1.14 (*m*, CH_2C*H*Me$_2$); 1.30–1.71 (*m*, C*H*Me$_2$, 3 CH_2CHMe$_2$, 6 CH_2), 1.94–1.99 (*m*, C*H*(R)CO); 2.00–

2.05 (t, J = 12.3, CH(R)CO); 2.11–2.16 (t, J = 11.8, CH(R)CO); 2.21–2.26 (t, J = 12.1, CH(R)CO); 2.33–2.42 (m, 1 H, CH_2CO); 2.45–2.72 (m, 9 H, CH_2CO, PhCH_2); 2.75–2.98 (m, 3 CH_2CO, 2 NH$_2$CH_2); 3.04–3.09 (m, CH_2NHR); 3.30–3.32 (m, NH$_2$); 3.37–3.41 (m, 1 H, CH_2NHR); 3.43–3.51 (m, 1 H, CH_2NHR); 3.58–3.66 (m, 1 H, CH_2NHR); 3.74–3.82 (m, 3 H, 2 CH_2NHR); 4.31–4.38 (m, NHCH(R)CH$_2$); 4.42–4.50 (m, NHCH(R)CH$_2$); 4.58–4.67 (m, 3 NHCH(R)CH$_2$); 7.14–7.31 (m, 13 arom. H); 7.34–7.38 (m, 2 arom. H); 7.83–7.86 (m, NH); 8.17–8.20 (d, J = 9.2, NH); 8.51–8.56 (m, 3 NH); 8.58–8.60 (d, J = 8.9, NH); 8.70–8.72 (d, J = 9.3, NH); 8.73–8.76 (d, J = 9.2, NH). ^{13}C-NMR (125 MHz, CD$_3$OD): 20.65 (Me); 20.83 (Me); 21.00 (Me); 22.34 (Me); 22.46 (Me); 22.50 (Me); 23.90 (Me); 23.93 (Me); 24.07 (CH$_2$); 24.28 (CH$_2$); 26.68 (CH); 27.34 (CH); 28.40 (CH$_2$); 28.49 (CH$_2$); 29.99 (CH); 35.89 (CH$_2$); 35.98 (CH$_2$); 37.24 (CH$_2$); 39.95 (CH$_2$); 39.99 (CH$_2$); 40.02 (CH$_2$); 40.11 (CH$_2$); 40.50 (CH$_2$); 40.59 (CH$_2$); 40.94 (CH$_2$); 41.22 (CH$_2$); 42.34 (CH$_2$); 43.56 (CH$_2$); 43.71 (CH$_2$); 44.21 (CH$_2$); 44.45 (CH$_2$); 37.24 (CH$_2$); 45.18 (CH$_2$); 45.23 (CH); 45.53 (CH); 45.65 (CH); 48.54 (CH); 49.22 (CH); 49.33 (CH$_2$); 49.50 (CH); 49.67 (CH); 51.62 (CH); 51.85 (CH); 55.36 (CH); 127.58 (CH); 127.67 (CH); 128.63 (CH); 129.45 (CH); 129.46 (CH); 130.14 (CH); 130.47 (CH); 130.49 (CH); 130.58 (CH); 136.91 (C); 139.54 (C); 139.65 (C); 171.95 (C); 173.59 (C); 173.74 (C); 173.87 (C); 175.68 (C); 175.82 (C); 176.31 (C); 176.39 (C). ESI-MS (pos. mode): 1281.0 (30, $[M+H]^+$); 640.8 (45, $[M+2H]^{2+}$); 427.8 (100, $[M+3H]^{3+}$). ESI-MS (neg. mode): 1280.0 (100, $[M-H]^-$).

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