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Synthesis of the Protected 6–16 Segment of Zervamicin II-2, an Application of the Azirine/Oxazolone Method‡

NICOLAS PRADEILLE§ and HEINZ HEIMGARTNER*

Organisch-chemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

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Abstract: The protected 11 amino acid segment (6–16) of the peptaibol zervamicin II-2 was synthesized by using the 'azirine/oxazolone method' for the introduction of all Aib residues. Whereas a 2,2-dimethyl-2*H*-azirin-3-amine was used as the building block for Aib(7), methyl 2,2-dimethyl-2*H*-azirine-3-prolinate and -3-(3-hydroxyprolinate) proved to be ideally suited as dipeptide synthons for the introduction of Aib-Pro and Aib-Hyp, respectively. The coupling of *Z*-protected amino acids or peptide acids with the 2*H*-azirin-3-amines were performed in 75% to quantitative yield. Copyright © 2003 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: peptaibol; zervamicin; 2*H*-azirin-3-amine; azirine/oxazolone method; dipeptide synthon

INTRODUCTION

Peptaibols are membrane-active oligopeptides isolated from fungal sources. They have an antibacterial property due to their self-association in lipid membranes forming ion channels. They are linear amphiphilic oligopeptides containing 11–20 amino acids characterized by a content of up to 50% of the non-protein amino acid Aib (α-aminoisobutyric acid) and the presence of an acetylated *N*-terminus and a reduced *C*-terminus (e.g. Valol, Leuol, Pheol) [1,2]. They also often contain the Iva (α-ethyl alanine) residue [3,4]. Aib is the simplest α,α-disubstituted amino acid present in nature [5], and it is well known for its ability to induce helical folding of the peptide chain [6]. This effect still dominates when amino acids such as proline (Pro) and hydroxyproline (Hyp), known as 'helix breakers', are present [7].

The 16-residue peptaibols zervamicin are produced by *Emericellopsis salmosynnemata*. In 1981, Rinehart *et al.* reported the structure of 11 zervamicin structures [8], and the crystal structure of Leu[1]-Zervamicin IIB was established by Karle *et al.* in 1991 [9–11]. The solution phase synthesis of zervamicin IIB was performed by Ogrel *et al.* [12]. The highlighted protected 6–16 segment **1**, which is common to zervamicin II-2 and zervamicin II-5 in its unprotected form, containing valine in position 8 (Figure 1), was selected as a model for the demonstration of the use of the 'azirine/oxazolone method' in peptaibol synthesis [13,14].

In the past few years, the 'azirine/oxazolone method' has been shown to provide an efficient and elegant way to extend peptide chains by Aib, Aib-Pro and Aib-Hyp units by using the 2*H*-azirin-3-amine

Abbreviations: Amino acid and peptide nomenclature conforms to IUPAC-IUB rules (*J. Peptide Sci.* 2003, **9**: 1–8). Other abbreviations: Aib, α-aminoisobutyric acid; ESI-MS, electrospray ionization mass spectrometry; HOBt, 1-hydroxybenzotriazole; Hyp, L-*trans*-4-hydroxyproline; Leuol, L-leucinol ((*S*)-2-amino-4-methyl-1-pentanol); Me, methyl; OBn, benzyloxy; Pheol, L-phenylalaninol ((*S*)-2-amino-3-phenyl-1-propanol); PLC, preparative layer chromatography; TEA, triethylamine; TLC, thin layer chromatography; Valol, L-valinol ((*S*)-2-amino-3-methyl-1-butanol); *Z*, benzyloxycarbonyl.

* Correspondence to: Heinz Heimgartner, Organisch-chemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland; e-mail: heimgart@oci.unizh.ch
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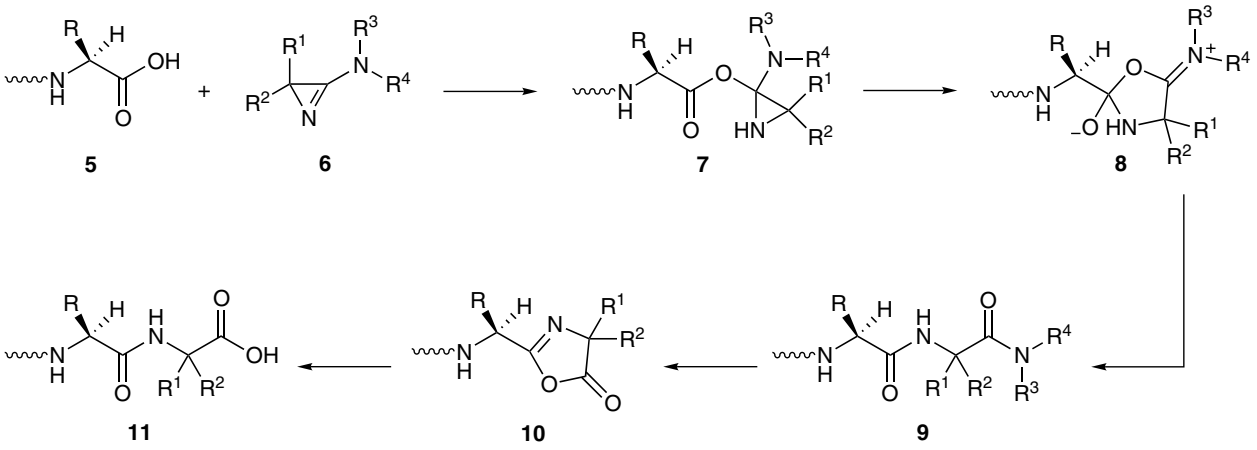
Z-Thr(OBn)-Aib-Val-Aib-Hyp(OBn)-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol
1

Ac-Trp-Ile-Gln-Aib-Ile-**Thr-Aib-Val-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Pheol**
Zervamicin II-2

Ac-Trp-Ile-Gln-Iva-Ile-**Thr-Aib-Val-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Pheol**
Zervamicin II-5

Figure 1 Zervamicines II-2 and II-5.



Scheme 1 Mechanism of the chain extension with 2*H*-azirin-3-amines.

derivatives **2**–**4** as building blocks (Figure 2). The preparation of these synthons has been described recently (**2** [15], **3** [16], **4** [17]).

The reaction mechanism of the coupling of an amino or peptide acid **5** with 2*H*-azirin-3-amines **6** is shown in Scheme 1. Protonation of N(1) of the azirine followed by nucleophilic addition of the carboxylate leads to aziridine intermediate **7**, which undergoes a ring enlargement to give zwitterion **8**.

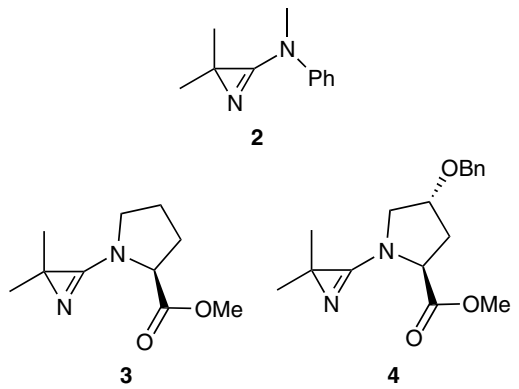


Figure 2 2*H*-Azirin-3-amines as building blocks for Aib, Aib-Pro and Aib-Hyp.

Ring opening of the latter then yields peptide **9**. Hydrolysis of **9** under mild conditions (3N HCl, THF/H_2O, r.t.) gives the peptide acid **11**. It has been shown that oxazolone **10** is an intermediate and can be prepared from **9** by treatment with gaseous HCl in non-nucleophilic solvents in high yield. The direct coupling of **10** with an amino component is also possible [14, 18].

By using this methodology, segments of the peptaibols alamethicin F30 [13], trichotoxin A-50(G) [19, 20], antiamoebin I [20–22] and trichovirin I 1B [16, 23] were prepared. Recently, the conventional solution phase synthesis of trichovirin I 4A was described [24].

The aim of the present synthesis was to show that the introduction of the residues Aib, Aib-Pro, and Aib-Hyp in the selected segment **1** can be done in an elegant way through the 'azirine/oxazolone method'. The successful coupling reactions with **2** and **3** have already been reported [13, 14, 16]. Their coupling potential is now illustrated in the reactions with Z-Thr(OBn)-OH and Z-Gln-Aib-Hyp(OBn)-OH, respectively. Furthermore, the reactions of **4** with Z-Val-OH and Z-Gln-OH show its potential as an Aib-Hyp synthon.

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MATERIALS AND METHODS

General

Amino acids and the amino alcohol Pheol were purchased from Novabiochem and Bachem, and were all in the L-configuration. Reagents and solvents were purchased from Acros, Fluka and Merck. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker ARX-300 spectrometer at 300 and 75.5 MHz respectively, employing CD_3OD and $CDCl_3$ as solvent at 300 K. Chemical shifts (δ) are given in ppm. COSY spectra were recorded either on a Bruker ARX-300 or Bruker DRX-600 spectrometer. HSQC and HMBC spectra were recorded on a Bruker DRX-600 spectrometer. Mass spectra were measured on a Finnigan TSQ-700 triple-stage quadrupole instrument for electrospray ionization mass spectrometry (ESI-MS). All the investigated compounds were dissolved in MeOH (HPLC grade) or in a 0.1% NaI solution in MeOH ($c \approx 5$ nmol/ml). ESI operating conditions in positive mode were: 16 scans average, capillary voltage 4500V, heated capillary temperature 210 °C, sheath gas N_2 (30 PSI). Melting points (Mp) were measured on Büchi B-540 apparatus. The IR spectra were recorded on a Perkin-Elmer Spectrum One spectrometer, absorption in cm^{-1}, using KBr. For the thin layer chromatography (TLC), Merck TLC aluminium sheets, silica gel 60 F_{254}, were used and the chromatograms were visualized with Schlittler reagent (composition: 1 g H_2PtCl_6 dissolved in 6 ml H_2O, addition of 20 ml 1N HCl, followed by 22.5 g KI dissolved in 225 ml H_2O, and H_2O to complete to 1 litre). The products were isolated either by means of preparative TLC, using Merck PLC plates (glass), silica gel 60 F_{254}, 40–63 μm, 2 mm, or by flash chromatography using Merck silica gel 60, 40–63 μm. Solvent systems used for the chromatographies were CH_2Cl_2/AcOEt (1 : 1) (A), AcOEt (B), AcOEt/MeOH (6 : 1) (C) and AcOEt/MeOH (3 : 1) (D). All solvents were purified using standard methods, and purchased chemicals were used without further purification.

Protocol A: Peptide Coupling

To a solution of an *N*-protected amino acid or *N*-protected peptide and 1 eq of a *C*-protected amino acid or *C*-protected peptide in MeCN were added 1 eq of TBTU and HOBt and 3 eq of TEA. The mixture was stirred overnight at room temperature (N_2 atmosphere) and checked by TLC. After completion of the reaction, the mixture was washed three times with brine. The organic layer was evaporated and the product was purified by flash chromatography, or preparative TLC.

Protocol B: Azirine Coupling

To a solution of the corresponding 2*H*-azirin-3-amine in THF or CH_2Cl_2, 1 eq of an *N*-protected amino acid or *N*-protected peptide was added, and the mixture was stirred at room temperature overnight (N_2 atmosphere). After completion of the reaction, the solvent was evaporated and the product was purified by flash chromatography, or preparative TLC.

Protocol C: Basic Hydrolysis of Peptide Esters

To a solution of the peptide ester in a mixture of THF, MeOH and H_2O (3 : 1 : 1), 3 eq of LiOH · H_2O was added, and the mixture was stirred at room temperature overnight (N_2 atmosphere). After completion of the reaction (checked by TLC), the mixture was acidified with 1N HCl to reach pH 1. The mixture was then extracted five times with CH_2Cl_2, the organic layers were gathered and evaporated. The product was purified by flash chromatography, or preparative TLC.

Protocol D: Hydrogenolytic Deprotection

The *N*-protected peptide was dissolved in MeOH, and 10% Pd/C (1/3 of the weight of the peptide) was added to the solution. The mixture was stirred overnight under H_2 atmosphere at room temperature. After completion of the reaction (checked by TLC), the solution was filtered through a celite path and the solvent was evaporated. The product was dried in high vacuum and used for the next reaction step without any further purification.

Protocol E: Acidic Hydrolysis of Peptide Amides

To a solution of the peptide amide in THF, the same volume of 6N HCl was added at 0 °C and the reaction was stirred overnight at room temperature (N_2 atmosphere). After completion of the reaction (checked by TLC), the solution was extracted with CH_2Cl_2. The organic layers were combined and dried over $MgSO_4$. After evaporation of the solvent, the product was dried in high vacuum and used for the next reaction step without any further purification.

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Synthesis of Z-Gln-Aib-Hyp(OBn)-OMe (12)

According to protocol B, the reaction was carried out with 50 mg (0.178 mmol) of Z-Gln-OH and 57.3 mg (0.190 mmol) of **4** dissolved in 3 ml of dry THF. After 48 h, the mixture was purified by flash chromatography (solvent systems (B) to (C)) and the product was dried in high vacuum to obtain 81.5 mg (78.7%) of **12** as a slightly yellowish foam.

ESI-MS (MeOH+NaI): **m/z** ***605.3 ((M+Na)$^+$).*** Mp 75°–78 °C. R_f(B) = 0.05, R_f(C) = 0.65. ^{1}H-NMR (CD_3OD) δ ppm: 1.448 ($2 \times 3H_{\beta Aib}$, s); 1.78–2.12 ($2H_{\beta Gln}$, $1H_{\beta Hyp}$, m); 2.12–2.25 ($1H_{\beta Hyp}$, m); 2.25–2.34 ($2H_{\gamma Gln}$, t); 3.683 ($3H_{OMe}$, s); 3.70–3.87 ($2H_{\delta Hyp}$, m); 4.17–4.27 ($1H_{\alpha Gln}$, $1H_{\gamma Hyp}$, m); 4.43–4.55 ($1H_{\alpha Hyp}$, $2H_{Hyp(OBn)}$, m); 4.89–5.03 ($2H_Z$, AB); 7.20–7.36 ($2 \times 5H_{arom.}$, m). ^{13}C-NMR (CD_3OD) δ ppm: 24.7, 25.4 ($2 \times 1C_{\beta Aib}$); 29.1 ($1C_{\beta Gln}$); 32.3 ($1C_{\gamma Gln}$); 34.0 ($1C_{\beta Hyp}$); 52.5 ($1C_{OMe}$); 53.7 ($1C_{\alpha Gln}$); 55.5 ($1C_{\alpha Aib}$); 57.4 ($1C_{\delta Hyp}$); 60.9 ($1C_{\alpha Hyp}$); 67.7 ($1C_Z$); 72.1 ($1C_{OBn}$); 78.3 ($1C_{\gamma Hyp}$); 128.7–129.3 ($2 \times 5CH_{arom.}$); 138.0, 139.4 ($2 \times 1C_{arom.}$); 158.2 ($1C_{OCONH}$); 173.0, 174.3, 174.4, 177.6 ($4 \times 1C_{CO}$). IR (KBr): 698s, 740s, 1028s, 1053s, 1083s, 1178s, 1207s, 1244s, 1363s, 1414s, 1454s, 1531s, 1625vs, 1667vs, 1720vs, 2950m, 2988m, 3033m, 3063m, 3308s.

Synthesis of Z-Gln-Aib-Hyp(OBn)-OH (13)

According to protocol C, the reaction was carried out with 893.4 mg (1.533 mmol) of **12** and 193.2 mg (4.600 mmol) of LiOH · H_2O dissolved in 56 ml of a mixture of THF, MeOH and H_2O (3 : 1 : 1). The mixture was stirred overnight, purified by flash chromatography (solvent systems (B) to (C)), and the product was dried in high vacuum to give 776 mg (89%) of **13** as a colourless foam.

ESI-MS (MeOH+NaI): **m/z** ***567.3 ((M—H)$^+$).*** Mp 110°–114 °C. R_f(C) = 0.05. ^{1}H-NMR (CD_3OD) δ ppm: 1.458 ($2 \times 3H_{\beta Aib}$, s); 1.81–2.13 ($1H_{\beta Hyp}$, $2H_{\beta Gln}$, m); 2.14–2.27 ($1H_{\beta Hyp}$, m); 2.26–2.35 ($2H_{\gamma Gln}$, t); 3.68–3.83 ($2H_{\delta Hyp}$, m); 4.16–4.26 ($1H_{\alpha Gln}$, $1H_{\gamma Hyp}$, m); 4.42–4.55 ($1H_{\alpha Hyp}$, $2H_{Hyp(OBn)}$, m); 4.91–5.05 ($2H_Z$, AB); 7.20–7.36 ($2 \times 5H_{arom.}$, m). ^{13}C-NMR (CD_3OD) δ ppm: 24.8, 25.2 ($2 \times 1C_{\beta Aib}$); 29.1 ($1C_{\beta Gln}$); 32.3 ($1C_{\gamma Gln}$); 34.4 ($1C_{\beta Hyp}$); 53.9 ($1C_{\alpha Gln}$); 55.6 ($1C_{\alpha Aib}$); 57.6 ($1C_{\delta Hyp}$); 61.4 ($1C_{\alpha Hyp}$); 67.7 ($1C_Z$); 72.0 ($1C_{OBn}$); 78.3 ($1C_{\gamma Hyp}$); 128.6–129.3 ($2 \times 5CH_{arom.}$); 139.4, 138.0 ($2 \times 1C_{arom.}$); 158.2 ($1C_{OCONH}$); 173.2, 174.1, 176.4, 177.7 ($4 \times 1C_{CO}$). IR (KBr): 640m, 698s, 739s, 1028s, 1054s, 1083s, 1178s, 1183s, 1248s, 1334s, 1366s, 1422s, 1454s, 1530s, 1621vs, 1667vs, 1712vs, 2940m, 2987m, 3033m, 3063m, 3324s.

Synthesis of Z-Gln-Aib-Hyp(OBn)-Aib-Pro-OMe (14)

According to protocol B, the reaction was carried out with 170.9 mg (0.300 mmol) of **13** and 64.7 mg (0.330 mmol) of **3** dissolved in 10 ml of dry THF. Five drops of MeOH was also added to the mixture. After stirring overnight, the solvent was carefully evaporated and the remaining white product was washed repeatedly with THF. After drying under vacuum, 174 mg (76.4%) of **14** was obtained as a white foam.

ESI-MS (MeOH+NaI): **m/z** ***787.5 ((M+Na)$^+$).*** Mp 110°–114 °C. R_f(C) = 0.35. ^{1}H-NMR (CD_3OD) δ ppm: 1.418, 1.458 ($2 \times 3H_{\beta Aib\ in\ Aib\text{-}Pro}$, 2s); 1.458, 1.508 ($2 \times 3H_{\beta Aib\ in\ Aib\text{-}Hyp}$, 2s); 1.74–2.02 ($2H_{\gamma Pro}$, $1H_{\beta Gln}$, $1H_{\beta Hyp}$, $1H_{\beta Pro}$, m); 2.02–2.18 ($1H_{\beta Gln}$, $1H_{\beta Pro}$, m); 2.31–2.40 ($2H_{\gamma Gln}$, t); 2.34–2.48 ($1H_{\beta Hyp}$, m); 3.46–3.56 ($1H_{\delta Hyp}$, d); 3.58–3.77 ($2H_{\delta Pro}$, m); 3.677 ($3H_{OMe}$, s); 3.84–3.93 ($1H_{\delta Hyp}$, d); 4.08–4.19 ($1H_{\alpha Gln}$, $1H_{\gamma Hyp}$, m); 4.38–4.45 ($1H_{\alpha Pro}$, m); 4.44–4.54 ($2H_{Hyp(OBn)}$, AB); 4.54–4.61 ($1H_{\alpha Hyp}$, dd); 5.01–5.15 ($2H_Z$, AB); 7.23–7.38 ($2 \times 5H_{arom.}$, m). ^{13}C-NMR (CD_3OD) δ ppm: 24.6, 24.7 ($2C_{\beta Aib\ in\ Aib\text{-}Hyp}$); 25.4, 26.1 ($2C_{\beta Aib\ in\ Aib\text{-}Pro}$); 26.8 ($1C_{\gamma Pro}$); 28.6 ($1C_{\beta Pro}$); 28.9 ($1C_{\beta Gln}$); 32.4 ($1C_{\gamma Gln}$); 34.9 ($1C_{\beta Hyp}$); 49.2 ($1C_{\delta Pro}$); 52.4 ($1C_{OMe}$); 54.9 ($1C_{\alpha Gln}$); 56.2 ($1C_{\delta Hyp}$); 57.6, 57.7 ($2C_{\alpha Aib}$); 61.8 ($1C_{\alpha Hyp}$); 62.1 ($1C_{\alpha Pro}$); 67.7 ($1C_Z$); 71.7 ($1C_{OBn}$); 78.4 ($1C_{\gamma Hyp}$); 128.6–129.5 ($2 \times 5CH_{arom.}$); 138.1, 139.4 ($2 \times 1C_{arom.}$); 158.4 ($1C_{OCONH}$); 173.7, 174.2, 174.6, 174.7, 177.5 ($6C_{CO}$). IR (KBr): 699s, 740s, 1028s, 1051s, 1093s, 1173s, 1206s, 1250s, 1365s, 1415s, 1454s, 1534s, 1654vs, 1718vs, 2946m, 2987m, 3033m, 3293s.

Synthesis of Z-Gln-Aib-Hyp(OBn)-Aib-Pro-OH (15)

According to protocol C, the reaction was carried out with 373.5 mg (0.488 mmol) of **14** and 61.5 mg (1.465 mmol) of LiOH · H_2O dissolved in 23 ml of a mixture of THF, MeOH and H_2O (3 : 1 : 1). The mixture was stirred overnight, and the product was purified by flash chromatography (solvent systems (B) to (C)) and dried in high vacuum to give 276.3 mg (75.4%) of **15** as a white foam.

ESI-MS (MeOH+NaI): **m/z** ***773.4 ((M+Na)$^+$).*** Mp 135°–140 °C. R_f(C) = 0.1. ^{1}H-NMR (CD_3OD) δ ppm: 1.417 ($3H_{\beta Aib\ in\ Aib\text{-}Pro}$, s); 1.465 ($3H_{\beta Aib\ in\ Aib\text{-}Pro}$,

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$3H_{\beta Aib\ in\ Aib\text{-}Hyp}$, s); 1.512 ($3H_{\beta Aib\ in\ Aib\text{-}Hyp}$, s); 1.80–2.02 ($2H_{\gamma Pro}$, $1H_{\beta Gln}$, $1H_{\beta Hyp}$, $1H_{\beta Pro}$, m); 2.02–2.17 ($1H_{\beta Gln}$, $1H_{\beta Pro}$, m); 2.31–2.40 ($2H_{\gamma Gln}$, t); 2.40–2.48 ($1H_{\beta Hyp}$, m); 3.47–3.56 ($1H_{\delta Hyp}$, d); 3.66–3.75 ($2H_{\delta Pro}$, m); 3.84–3.92 ($1H_{\delta Hyp}$, d); 4.09–4.18 ($1H_{\alpha Gln}$, $1H_{\gamma Hyp}$, m); 4.38–4.45 ($1H_{\alpha Pro}$, m); 4.45–4.54 ($2H_{Hyp(OBn)}$, AB); 4.54–4.60 ($1H_{\alpha Hyp}$, dd); 5.01–5.15 ($2H_Z$, AB); 7.20–7.37 ($2 \times 5H_{arom.}$, m). ^{13}C-NMR (CD_3OD) δ ppm: 24.6, 24.8 ($2 \times 1C_{\beta Aib\ in\ Aib\text{-}Hyp}$); 25.4, 26.1 ($2 \times 1C_{\beta Aib\ in\ Aib\text{-}Pro}$); 26.8 ($1C_{\gamma Pro}$); 28.7 ($1C_{\beta Pro}$); 29.1 ($1C_{\beta Gln}$); 32.4 ($1C_{\gamma Gln}$); 34.9 ($1C_{\beta Hyp}$); 49.2 ($1C_{\delta Pro}$); 54.9 ($1C_{\alpha Gln}$); 56.2 ($1C_{\delta Hyp}$); 57.7, 57.8 ($2 \times 1C_{\alpha Aib}$); 61.9 ($1C_{\alpha Hyp}$); 62.1 ($1C_{\alpha Pro}$); 67.7 ($1C_Z$); 71.7 ($1C_{OBn}$); 78.4 ($1C_{\gamma Hyp}$); 127.9–129.4 ($2 \times 5CH_{arom.}$); 138.1, 139.4 ($2 \times 1C_{arom.}$); 158.9 ($1C_{OCONH}$); 173.9, 174.1, 174.6, 176.0, 177.5 ($6C_{CO}$). IR (KBr): 698m, 721m, 741m, 800m, 1028m, 1088s, 1136s, 1204vs, 1342s, 1366s, 1427vs, 1536s, 1667vs, 2943m, 2988m, 3293s.

Synthesis of Z-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol (16)

According to protocol A, a mixture of 152.7 mg (0.203 mmol) of **15**, 30.8 mg (0.203 mmol) of Pheol, 65.3 mg (0.203 mmol) of TBTU, 31.3 mg (0.203 mmol) of HOBt, and 86 µl (0.610 mmol) of TEA in 15 ml of MeCN was stirred 24 h. The product was purified by flash chromatography (solvent systems (B) to (C)) and dried in high vacuum to give 119 mg (66.2%) of **16** as a white foam.

ESI-MS (MeOH+NaI): **m/z *906.6 ($(M+Na)^+$).*** Mp 92°–97 °C. R_f(C) = 0.2. ^{1}H-NMR (CD_3OD) δ ppm: 1.24–1.36 ($1H_{\beta Pro}$, m); 1.432, 1.447 ($2 \times 3H_{\beta Aib\ in\ Aib\text{-}Pro}$, 2s); 1.501 ($2 \times 3H_{\beta Aib\ in\ Aib\text{-}Hyp}$, s); 1.62–1.74 ($2H_{\gamma Pro}$, m); 1.76–1.96 ($1H_{\beta Gln}$, $1H_{\beta Hyp}$, m); 1.96–2.22 ($1H_{\beta Gln}$, $1H_{\beta Pro}$, m); 2.33–2.42 ($2H_{\gamma Gln}$, t); 2.45–2.57 ($1H_{\beta Hyp}$, m); 2.70–2.78 ($1H_{CH_2OH\ in\ Pheol}$, dd); 2.95–3.02 ($1H_{CH_2OH\ in\ Pheol}$, dd); 3.40–3.49 ($1H_{\delta Hyp}$, d); 3.57–3.63 ($2H_{CH_2Ph\ in\ Pheol}$, m); 3.63–3.79 ($2H_{\delta Pro}$, m); 3.91–3.99 ($1H_{\delta Hyp}$, d); 4.07–4.20 ($1H_{\alpha Gln}$, $1H_{\gamma Hyp}$, $1H_{\alpha Pheol}$, m); 4.28–4.35 ($1H_{\alpha Pro}$, m); 4.48–4.58 ($2H_{Hyp(OBn)}$, AB); 4.64–4.72 ($1H_{\alpha Hyp}$, dd); 5.02–5.19 ($2H_Z$, AB); 7.22–7.39 ($3 \times 5H_{arom.}$, m). ^{13}C-NMR (CD_3OD) δ ppm: 24.3, 24.7, 26.5 ($4C_{\beta Aib}$); 26.4 ($1C_{\gamma Pro}$); 28.7 ($1C_{\beta Pro}$); 30.0 ($1C_{\beta Gln}$); 32.7 ($1C_{\gamma Gln}$); 35.5 ($1C_{\beta Hyp}$); 37.9 ($1C_{CH_2OH\ in\ Pheol}$); 50.0 ($1C_{\delta Pro}$); 54.6 ($1C_{\alpha Gln}$); 55.4 ($1C_{\delta Hyp}$); 56.5 ($1C_{\alpha Pheol}$); 57.9, 58.0 ($2 \times 1C_{\alpha Aib}$); 62.2 ($1C_{\alpha Pro}$); 64.2 ($1C_{\alpha Hyp}$); 65.2 ($1C_{CH_2Ph\ in\ Pheol}$); 68.0 ($1C_Z$); 71.9 ($1C_{OBn}$); 78.8 ($1C_{\gamma Hyp}$); 127.3–130.8 ($3 \times 5CH_{arom.}$); 138.4, 139.6, 140.3 ($3 \times 1C_{arom.}$); 158.7 ($1C_{OCONH}$); 174.2, 174.4, 174.6, 175.0, 175.2, 177.7 ($6 \times 1C_{CO}$). IR (KBr): 700s, 743m, 1050s, 1081s, 1149m, 1176m, 1205m, 1248s, 1365s, 1409vs, 1454s, 1469s, 1543vs, 1646vs, 1720s, 2875m, 2940m, 2985m, 3031m, 3062m, 3289vs.

Synthesis of H-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol (17)

According to protocol D, the reaction was carried out with 528.7 mg (0.598 mmol) of **16** dissolved in 117 ml of MeOH, and 176.2 mg of Pd/C. After drying the product in high vacuum, 448.5 mg (quant.) of **17** was obtained as a yellowish foam.

ESI-MS (MeOH+NaI): **m/z *750.4 ($(M+H)^+$);* m/z *772.5 ($(M+Na)^+$).*** Mp 77°–82 °C. R_f(D) = 0.1. ^{1}H-NMR (CD_3OD) δ ppm: 1.23–1.36 ($1H_{\beta Pro}$, m); 1.436, 1.483 ($2 \times 3H_{\beta Aib\ in\ Aib\text{-}Pro}$, 2s); 1.526, 1.536 ($2 \times 3H_{\beta Aib\ in\ Aib\text{-}Hyp}$, 2s); 1.64–1.76 ($2H_{\gamma Pro}$, m); 1.76–1.91 ($1H_{\beta Hyp}$, $1H_{\beta Gln}$, m); 1.97–2.15 ($1H_{\beta Gln}$, $1H_{\beta Pro}$, m); 2.31–2.42 ($2H_{\gamma Gln}$, t); 2.50–2.60 ($1H_{\beta Hyp}$, dd); 2.70–2.80 ($1H_{CH_2OH\ in\ Pheol}$, dd); 2.95–3.03 ($1H_{CH_2OH\ in\ Pheol}$, dd); 3.35–3.45 ($1H_{\delta Hyp}$, m); 3.59–3.64 ($2H_{CH_2Ph\ in\ Pheol}$, m); 3.65–3.76 ($2H_{\delta Pro}$, m); 3.76–3.85 ($1H_{\delta Hyp}$, d); 4.05–4.14 ($1H_{\alpha Pheol}$, d); 4.11–4.18 ($1H_{\alpha Gln}$, m); 4.18–4.23 ($1H_{\gamma Hyp}$, m); 4.29–4.35 ($1H_{\alpha Pro}$, t); 4.51–4.62 ($2H_{Hyp(OBn)}$, AB); 4.66–4.74 ($1H_{\alpha Hyp}$, t); 7.22–7.34 ($2 \times 5H_{arom.}$, m). ^{13}C-NMR (CD_3OD) δ ppm: 24.3, 24.8 ($1C_{\beta Aib\ in\ Aib\text{-}Hyp}$, $1C_{\beta Aib\ in\ Aib\text{-}Pro}$); 26.4 ($1C_{\beta Aib\ in\ Aib\text{-}Hyp}$); 26.5 ($1C_{\beta Aib\ in\ Aib\text{-}Pro}$); 26.6 ($1C_{\gamma Pro}$); 30.8 ($1C_{\beta Pro}$); 32.0 ($1C_{\beta Gln}$); 32.8 ($1C_{\gamma Gln}$); 35.6 ($1C_{\beta Hyp}$); 37.9 ($1C_{CH_2OH\ in\ Pheol}$); 50.0 ($1C_{\delta Pro}$); 54.6 ($1C_{\alpha Gln}$); 55.5 ($1C_{\alpha Pheol}$, $1C_{\delta Hyp}$); 57.7, 57.9 ($2 \times 1C_{\alpha Aib}$); 62.3 ($1C_{\alpha Pro}$); 64.2 ($1C_{\alpha Hyp}$); 65.2 ($1C_{CH_2Ph\ in\ Pheol}$); 71.9 ($1C_{OBn}$); 78.9 ($1C_{\gamma Hyp}$); 127.3–130.8 ($2 \times 5CH_{arom.}$); 139.6, 140.2 ($2 \times 1C_{arom.}$); 174.4, 174.7, 175.1, 176.9, 178.2 ($6C_{CO}$). IR (KBr): 701m, 746m, 1049m, 1081m, 1148m, 1176m, 1204m, 1308m, 1364s, 1409vs, 1454s, 1470s, 1544vs, 1645vs, 2874m, 2939m, 2985m, 3029m, 3062m, 3289vs.

Synthesis of Z-Val-Aib-Hyp(OBn)-OMe (18)

According to protocol B, the reaction was carried out with 189.1 mg (0.752 mmol) of Z-Val-OH and 227.2 mg (0.752 mmol) of **4** dissolved in 10 ml of dry THF. The product was purified by flash chromatography (solvent systems (A) to (C)) and dried in high vacuum to give 321.1 mg (77%) of **18** as a white foam.

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ESI-MS (MeOH+NaI):* m/z *576.2 ($(M+Na)^+$). Mp 130°–134 °C. R_f(C) = 0.8. ^{1}H-NMR (CD_3OD) δ ppm: 0.90–0.94, 0.94–0.98 ($2 \times 3H_{\gamma Val}$, 2d); 1.445, 1.458 ($2 \times 3H_{\beta Aib}$, 2s); 1.93–2.07 ($1H_{\beta Val}$, $1H_{\beta Hyp}$, m); 2.07–2.20 ($1H_{\beta Hyp}$, m); 3.54–3.62 ($1H_{\delta Hyp}$, ABX); 3.684 ($3H_{OMe}$, s); 3.85–3.93 ($1H_{\delta Hyp}$, ABX); 3.98–4.02 ($1H_{\alpha Val}$, d); 4.18–4.27 ($1H_{\gamma Hyp}$, m); 4.43–4.54 ($2H_{Hyp(OBn)}$, $1H_{\alpha Hyp}$, m); 4.81–5.01 ($2H_Z$, AB); 7.20–7.37 ($2 \times 5H_{arom.}$, m). ^{13}C-NMR (CD_3OD) δ ppm: 18.6, 19.8 ($2 \times 1C_{\gamma Val}$); 24.8, 25.4 ($2 \times 1C_{\beta Aib}$); 33.2 ($1C_{\beta Val}$); 34.1 ($1C_{\beta Hyp}$); 52.6 ($1C_{OMe}$); 53.6 ($1C_{\delta Hyp}$); 57.5 ($1C_{\alpha Aib}$); 60.9 ($1C_{\alpha Hyp}$); 61.5 ($1C_{\alpha Val}$); 67.6 ($1C_Z$); 72.2 ($1C_{Hyp(OBn)}$); 78.1 ($1C_{\gamma Hyp}$); 128.7–129.4 ($2 \times 5CH_{arom.}$); 138.0, 139.4 ($2 \times 1C_{arom.}$); 158.3 ($1C_{OCONH}$); 173.0, 174.2, 174.4 ($3 \times 1C_{CO}$). IR (KBr): 697s, 738s, 755m, 788m, 996m, 1011m, 1028s, 1130vs, 1183vs, 1212vs, 1266s, 1280s, 1303m, 1333m, 1363vs, 1382m, 1418vs, 1455s, 1470s, 1498m, 1531vs, 1616vs, 1677vs, 1711vs, 1745vs, 2893m, 2962s, 3032m, 3064m, 3303m, 3367s.

Synthesis of Z-Val-Aib-Hyp(OBn)-OH (19)

According to protocol C, the reaction was carried out with 309.12 mg (0.558 mmol) of **18** and 70.3 mg (1.675 mmol) of LiOH · H_2O dissolved in 19 ml of a mixture of THF, MeOH and H_2O (3 : 1 : 1). The product was purified by preparative TLC (solvents system (D)) and dried in high vacuum to give 193.1 mg (64.1%) of **19** as a white powder.

ESI-MS (MeOH+NaI):* m/z *562.2 ($(M+Na)^+$). Mp 125°–130 °C. R_f(D) = 0.2. ^{1}H-NMR (CD_3OD) δ ppm: 0.90–0.94, 0.94–0.98 ($2 \times 3H_{\gamma Val}$, 2d); 1.475, 1.495 ($2 \times 3H_{\beta Aib}$, 2s); 1.95–2.09 ($1H_{\beta Val}$, $1H_{\beta Hyp}$, m); 2.13–2.26 ($1H_{\beta Hyp}$, m); 3.62–3.70 ($1H_{\delta Hyp}$, ABX); 3.77–3.85 ($1H_{\delta Hyp}$, ABX); 3.97–4.02 ($1H_{\alpha Val}$, d); 4.15–4.23 ($1H_{\gamma Hyp}$, m); 4.38–4.54 ($2H_{Hyp(OBn)}$, $1H_{\alpha Hyp}$, m); 4.90–5.03 ($2H_Z$, AB); 7.20–7.37 ($2 \times 5H_{arom.}$, m). ^{13}C-NMR (CD_3OD) δ ppm: 18.4, 19.7 ($2 \times 1C_{\gamma Val}$); 25.0 ($2 \times 1C_{\beta Aib}$); 32.1 ($1C_{\beta Val}$); 34.8 ($1C_{\beta Hyp}$); 54.0 ($1C_{\delta Hyp}$); 57.9 ($1C_{\alpha Aib}$); 61.9 ($1C_{\alpha Val}$); 63.0 ($1C_{\alpha Hyp}$); 67.6 ($1C_Z$); 71.9 ($1C_{Hyp(OBn)}$); 78.4 ($1C_{\gamma Hyp}$); 128.5–129.4 ($2 \times 5CH_{arom.}$); 138.1, 139.5 ($2 \times 1C_{arom.}$); 158.4 ($1C_{OCONH}$); 173.1, 173.8, 178.8 ($3 \times 1C_{CO}$). IR (KBr): 697s, 737s, 1028s, 1095s, 1181s, 1238s, 1270s, 1306s, 1367s, 1430vs, 1454vs, 1524vs, 1616vs, 1713vs, 2874m, 2935s, 2965s, 3033m, 3256s.

Synthesis of Z-Val-Aib-Hyp(OBn)-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol (20)

According to protocol A, a mixture of 150 mg (0.2 mmol) of **17**, 107.9 mg (0.2 mmol) of **19**, 64.2 mg (0.2 mmol) of TBTU, 30.7 mg (0.2 mmol) of HOBt, and 83.6 µl (0.6 mmol) of TEA in 12 ml of MeCN was stirred 24 h. The product was purified by preparative TLC (solvent system (C)) and dried in high vacuum to give 127.1 mg (50%) of **20** as a white foam.

ESI-MS (MeOH+NaI):* m/z *1293.8 ($(M+Na)^+$). Mp 117°–124 °C. R_f(C) = 0.4. ^{1}H-NMR (CD_3OD) δ ppm: 0.95–0.99, 0.99–1.02 ($2 \times 3H_{\gamma Val}$, 2d); 1.21–1.35 ($1H_{\beta Pro}$, m); 1.454, 1.477, 1.485, 1.516, 1.535, 1.566 ($6 \times 3H_{\beta Aib}$, 6s); 1.64–1.74 ($2H_{\gamma Pro}$, m); 1.82–1.94 ($2 \times 1H_{\beta Hyp}$, m); 1.96–2.10 ($1H_{\beta Pro}$, m); 2.10–2.28 ($2H_{\beta Gln}$, $1H_{\beta Val}$, m); 2.29–2.38 ($2H_{\gamma Gln}$, m); 2.46–2.61 ($2 \times 1H_{\beta Hyp}$, m); 2.70–2.80 ($1H_{CH_2OH\ in\ Pheol}$, ABX); 2.95–3.03 ($1H_{CH_2OH\ in\ Pheol}$, ABX); 3.45–3.53, 3.53–3.60 ($2 \times 1H_{\delta Hyp}$, 2ABX); 3.59–3.64 ($2H_{CH_2Ph\ in\ Pheol}$, m); 3.64–3.85 ($2 \times 1H_{\delta Pro}$, m); 3.85–3.93 ($1H_{\delta Hyp}$, AB); 4.01–4.06 ($1H_{\alpha Val}$, d); 4.09–4.21 ($2 \times 1H_{\gamma Hyp}$, $1H_{\alpha Pheol}$, $1H_{\delta Hyp}$, m); 4.28–4.36 ($1H_{\alpha Gln}$, $1H_{\alpha Hyp}$, m); 4.46–4.64 ($2 \times 2H_{Hyp(OBn)}$, $1H_{\alpha Pro}$, m); 4.66–4.74 ($1H_{\alpha Hyp}$, t); 5.05–5.23 ($2H_Z$, AB); 7.25–7.40 ($4 \times 5H_{arom.}$, m). ^{13}C-NMR (CD_3OD) δ ppm: 17.2, 19.8 ($2 \times 1C_{\gamma Val}$); 24.1, 24.9, 26.1, 26.3, 26.4, 26.9 ($6C_{\beta Aib}$, $1C_{\gamma Pro}$); 28.0 ($1C_{\beta Gln}$); 29.8 ($1C_{\beta Pro}$); 31.4 ($1C_{\gamma Gln}$); 33.0 ($1C_{\beta Val}$); 34.8 ($1C_{\beta Hyp}$); 35.9 ($1C_{\beta Hyp}$); 37.7 ($1C_{CH_2OH\ in\ Pheol}$); 49.7 ($1C_{\delta Pro}$); 54.5 ($1C_{\alpha Gln}$); 54.8 ($1C_{\alpha Pheol}$); 55.1 ($2C_{\delta Hyp}$); 57.7, 57.9 ($3C_{\alpha Aib}$); 62.2 ($1C_{\alpha Hyp}$); 62.9 ($1C_{\alpha Val}$, $1C_{\alpha Hyp}$); 63.9 ($1C_{\alpha Pro}$); 65.0 ($1C_{CH_2Ph\ in\ Pheol}$); 67.8 ($1C_Z$); 71.6, 71.7 ($2 \times 1C_{OBn}$); 78.5 ($2C_{\gamma Hyp}$); 127.0–130.5 ($4 \times 5CH_{arom.}$); 139.4, 140.0 ($4C_{arom.}$); 158.2 ($1C_{OCONH}$); 173.7, 174.2, 174.5, 174.8, 174.9, 175.5 ($9C_{CO}$). IR (KBr): 614m, 699s, 740m, 1028m, 1094s, 1176s, 1205m, 1265s, 1364s, 1411vs, 1454s, 1470s, 1543vs, 1647vs, 2875m, 2936m, 3031m, 3062m, 3291s.

Synthesis of H-Val-Aib-Hyp(OBn)-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol (21)

According to protocol D, the reaction was carried out with 128.3 mg (0.101 mmol) of **20** in 28.5 ml of MeOH, and 42.7 mg of Pd/C. After drying the product in high vacuum, 101.1 mg (88.1%) of **21** was obtained as a yellowish foam.

ESI-MS (MeOH):* m/z *1137.5 ($(M+H)^+$). Mp 107°–113 °C. R_f(C) = 0.15. ^{1}H-NMR (CD_3OD) δ ppm:

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0.88–0.94, 0.98–1.05 ($2 \times 3H_{\gamma Val}$, 2d); 1.21–1.34 ($1H_{\beta Pro}$, m); 1.454, 1.504, 1.516, 1.531, 1.552, 1.578 ($6 \times 3H_{\beta Aib}$, 6s); 1.64–1.75 ($2H_{\gamma Pro}$, m); 1.82–1.96 ($2 \times 1H_{\beta Hyp}$, m); 1.98–2.08 ($1H_{\beta Pro}$, m); 2.10–2.26 ($2H_{\beta Gln}$, $1H_{\beta Val}$, m); 2.26–2.44 ($2H_{\gamma Gln}$, m); 2.45–2.63 ($2 \times 1H_{\beta Hyp}$, m); 2.70–2.80 ($1H_{CH_2OH\ in\ Pheol}$, ABX); 2.94–3.03 ($1H_{CH_2OH\ in\ Pheol}$, ABX); 3.32–3.35 ($1H_{\alpha Val}$, d); 3.44–3.52, 3.52–3.58 ($2 \times 1H_{\delta Hyp}$, 2ABX); 3.59–3.64 ($2H_{CH_2Ph\ in\ Pheol}$, m); 3.64–3.86 ($2 \times 1H_{\delta Pro}$, m); 4.05–4.23 ($2 \times 1H_{\delta Hyp}$, $1H_{\gamma Hyp}$, $1H_{\alpha Pheol}$, m); 4.24–4.36 ($1H_{\gamma Hyp}$, $1H_{\alpha Gln}$, $1H_{\alpha Pro}$, m); 4.52–4.66 ($2 \times 2H_{Hyp(OBn)}$, $1H_{\alpha Hyp}$, m); 4.66–4.75 ($1H_{\alpha Hyp}$, t); 7.23–7.37 ($3 \times 5H_{arom.}$, m). ^{13}C-NMR (CD_3OD) δ ppm: 17.2, 19.8 ($2 \times 1C_{\gamma Val}$); 24.0, 24.9, 26.1, 26.3, 27.2 ($6C_{\beta Aib}$, $1C_{\gamma Pro}$); 27.9 ($1C_{\beta Pro}$); 29.8 ($1C_{\beta Gln}$); 32.9 ($1C_{\gamma Gln}$); 34.0 ($1C_{\beta Val}$); 35.0 ($1C_{\beta Hyp}$); 35.5 ($1C_{\beta Hyp}$); 37.7 ($1C_{CH_2OH\ in\ Pheol}$); 49.7 ($1C_{\delta Pro}$); 54.4 ($1C_{\alpha Gln}$); 54.8 ($1C_{\alpha Pheol}$); 55.1 ($2C_{\delta Hyp}$); 57.4, 57.7 ($3C_{\alpha Aib}$); 62.2 ($1C_{\alpha Hyp}$); 62.6 ($1C_{\alpha Hyp}$); 64.1 ($1C_{\alpha Pro}$); 65.0 ($1C_{CH_2Ph\ in\ Pheol}$); 68.8 ($1C_{\alpha Val}$); 71.6 ($2 \times 1C_{OBn}$); 78.5, 78.7 ($2 \times 1C_{\gamma Hyp}$); 127.1–130.5 ($3 \times 5CH_{arom.}$); 139.4, 140.0 ($3C_{arom.}$); 174.1, 174.5, 174.8, 177.2 ($9C_{CO}$). IR (KBr): 614m, 699m, 740m, 1028m, 1079s, 1149m, 1176s, 1204m, 1311m, 1364s, 1410vs, 1454s, 1470s, 1544vs, 1645vs, 2873m, 2932s, 3030m, 3062m, 3294vs.

Synthesis of Z-Thr(OBn)-Aib-N(Me)(Ph) (22)

According to protocol B, the reaction was carried out with 138 mg (0.402 mmol) of Z-Thr(OBn)-OH and 70 mg (0.402 mmol) of **2** dissolved in 16 ml of CH_2Cl_2. After 48 h, the mixture was purified by preparative TLC (solvent system CH_2Cl_2/AcOEt (1.5 : 1)) and dried in high vacuum to give 208.4 mg (quant.) of **22** as a white foam.

ESI-MS (MeOH+NaI):* m/z *540.6 ($(M+Na)^+$). Mp 134°–135 °C. R_f(A) = 0.7. ^{1}H-NMR ($CDCl_3$) δ ppm: 1.08–1.12 ($3H_{\gamma Thr}$, d); 1.384, 1.542 ($2 \times 3H_{\beta Aib}$, 2s); 3.241 ($3H_{N(Me)}$, s); 3.82–3.89 ($1H_{\alpha Thr}$, m); 3.94–4.03 ($1H_{\beta Thr}$, m); 4.42–4.58 ($2H_{OBn}$, AB); 5.09–5.23 ($2H_Z$, AB); 7.20–7.43 ($2 \times 5H_{arom.}$, m). ^{13}C-NMR ($CDCl_3$) δ ppm: 14.8 ($1C_{\gamma Thr}$); 26.1, 27.1 ($2 \times 1C_{\beta Aib}$); 41.2 ($1C_{N(Me)}$); 56.9 ($1C_{\alpha Thr}$); 57.9 ($1C_{\alpha Aib}$); 66.8 ($1C_Z$); 71.4 ($1C_{OBn}$); 74.4 ($1C_{\beta Thr}$); 127.5–129.2 ($3 \times 5CH_{arom.}$); 136.3, 137.7 ($2 \times 1C_{arom.}$); 144.6 ($1C_{arom.inN(Me)Ph}$); 156.0 ($1C_{OCONH}$); 169.6, 172.5 ($2 \times 1C_{CO}$). IR (KBr): 698s, 709s, 739s, 761s, 773s, 983s, 1027m, 1068s, 1095s, 1170m, 1214vs, 1286s, 1336m, 1360m, 1375m, 1397s, 1454s, 1467s, 1495vs, 1593s, 1630vs, 1676vs, 1721vs, 2896m, 2929m, 2969m, 2983m, 3027m, 3309s, 3421s.

Synthesis of Z-Thr(OBn)-Aib-OH (23)

According to protocol E, the reaction was carried out with 674.3 mg (1.304 mmol) of **22** in a mixture of 12 ml of 6N HCl and 12 ml of THF. The temperature was allowed to rise from 0 °C to room temperature overnight. After two extractions with CH_2Cl_2, the organic layer was dried with $MgSO_4$, filtered, the solvent was evaporated and the product was dried in high vacuum to give 560.2 mg (quant.) of **23** as a white foam.

ESI-MS (MeOH+NaI):* m/z *451.1 ($(M+Na)^+$). Mp 90°–92 °C. R_f(A) = 0.33. ^{1}H-NMR ($CDCl_3$) δ ppm: 1.14–1.19 ($3H_{\gamma Thr}$, d); 1.477, 1.492 ($2 \times 3H_{\beta Aib}$, 2s); 4.03–4.13 ($1H_{\alpha Thr}$, m); 4.34–4.41 ($1H_{\beta Thr}$, m); 4.52–4.64 ($2H_{OBn}$, AB); 5.094 ($2H_Z$, s); 7.22–7.34 ($2 \times 5H_{arom.}$, m). ^{13}C-NMR ($CDCl_3$) δ ppm: 14.9 ($1C_{\gamma Thr}$); 28.9, 29.7 ($2 \times 1C_{\beta Aib}$); 56.3 ($1C_{\alpha Aib}$); 57.4 ($1C_{\alpha Thr}$); 67.0 ($1C_Z$); 71.4 ($1C_{OBn}$); 74.6 ($1C_{\beta Thr}$); 127.7–128.4 ($2 \times 5CH_{arom.}$); 136.0, 137.7 ($2 \times 1C_{arom.}$); 156.2 ($1C_{OCONH}$); 169.1, 177.8 ($2 \times 1C_{CO}$). IR (KBr): 697vs, 737s, 1028s, 1069vs, 1174s, 1215vs, 1360vs, 1405vs, 1456vs, 1498vs, 1605vs, 1668vs, 1718vs, 2873m, 2933s, 2978s, 3033m, 3065m, 3349s.

Synthesis of Z-Thr(OBn)-Aib-Val-Aib-Hyp(OBn)-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol (1)

According to protocol A, the reaction was carried out with 94 mg (0.083 mmol) of **21**, 35.4 mg (0.083 mmol) of **23**, 26.5 mg (0.083 mmol) of TBTU, 12.7 mg (0.083 mmol) of HOBt and 34.9 µl (0.248 mmol) of TEA in 8 ml of MeCN. The product was purified by flash chromatography (solvent systems (B) to (D)) and dried in high vacuum to give 42 mg (32.8%) of **1** as a yellow foam.

ESI-MS (MeOH+NaI):* m/z *1569.9 ($(M+Na)^+$). Mp 115°–120 °C. R_f(D) = 0.7. ^{1}H-NMR (CD_3OD) δ ppm: 0.86–0.89, 0.89–0.92 ($2 \times 3H_{\gamma Val}$, 2d); 1.23–1.34 ($1H_{\beta Pro}$, $3H_{\gamma Thr}$, m); 1.409, 1.456, 1.499, 1.511, 1.513, 1.537, 1.562, 1.581 ($8 \times 3H_{\beta Aib}$, 8s); 1.63–1.74 ($2H_{\gamma Pro}$, m); 1.81–2.09 ($2 \times 1H_{\beta Hyp}$, $1H_{\beta Pro}$, $1H_{\beta Gln}$, m); 2.10–2.18 ($1H_{\beta Val}$, $1H_{\beta Gln}$, m); 2.20–2.38 ($2H_{\gamma Gln}$, m); 2.45–2.58 ($2 \times 1H_{\beta Hyp}$, m); 2.70–2.80 ($1H_{CH_2OH\ in\ Pheol}$, ABX); 2.94–3.03 ($1H_{CH_2OH\ in\ Pheol}$, ABX); 3.46–3.53 ($1H_{\delta Hyp}$, ABX); 3.54–3.66 ($1H_{\delta Hyp}$, $2H_{CH_2Ph\ in\ Pheol}$, m); 3.66–3.75, 3.75–3.86 ($2H_{\delta Pro}$, 2m); 4.00–4.08 ($1H_{\delta Hyp}$, AB); 4.10–4.36 ($1H_{\alpha Val}$, $1H_{\alpha Gln}$, $1H_{\alpha Pro}$, $2 \times 1H_{\gamma Hyp}$, $1H_{\alpha Pheol}$, $1H_{\delta Hyp}$, $1H_{\alpha Thr}$, $1H_{\beta Thr}$, m); 4.45–4.75 ($2 \times 1H_{\alpha Hyp}$, $2 \times 2H_{Hyp(OBn)}$, $2H_{Thr(OBn)}$, m); 5.10–5.24

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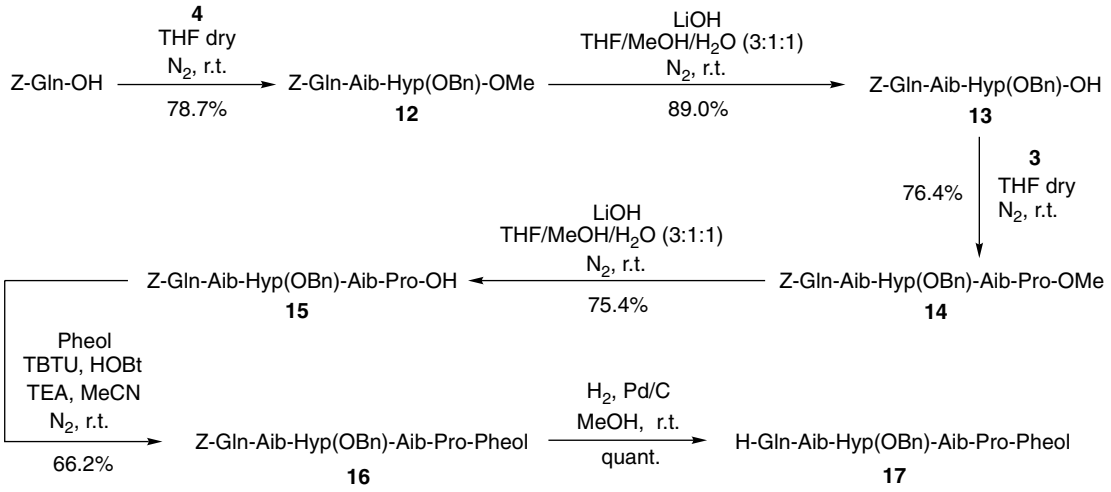

Scheme 2 Synthesis of the hexapeptide **17**.

($2H_Z$, AB); 7.23–7.40 ($5 \times 5H_{arom.}$, m). ^{13}C-NMR (CD_3OD) δ ppm: 16.6 ($1C_{\gamma Thr}$); 18.5, 19.7 ($2 \times 1C_{\gamma Val}$); 24.0, 24.1, 24.2, 24.9, 26.1, 26.3, 27.0, 27.6 ($8C_{\beta Aib}$, $1C_{\gamma Pro}$); 27.9 ($1C_{\beta Gln}$); 29.8 ($1C_{\beta Pro}$); 31.1 ($1C_{\gamma Gln}$); 32.7 ($1C_{\beta Val}$); 35.4 ($1C_{\beta Hyp}$); 35.8 ($1C_{\beta Hyp}$); 37.7 ($1C_{CH_2OH\ in\ Pheol}$); 49.7 ($1C_{\delta Pro}$); 54.4 ($1C_{\alpha Gln}$); 54.8 ($1C_{\alpha Pheol}$); 54.8, 55.1 ($2 \times 1C_{\delta Hyp}$); 57.7, 57.8, 58.1 ($4C_{\alpha Aib}$); 60.3 ($1C_{\alpha Hyp}$); 62.2 ($1C_{\alpha Hyp}$); 62.9 ($1C_{\alpha Pro}$); 63.3 ($1C_{\alpha Thr}$); 63.9 ($1C_{\alpha Val}$,); 65.0 ($1C_{CH_2Ph\ in\ Pheol}$); 68.0 ($1C_Z$); 71.6, 72.1 ($3C_{OBn}$); 75.0 ($1C_{\beta Thr}$); 78.4 ($2C_{\gamma Hyp}$); 127.1–130.5 ($5 \times 5CH_{arom.}$); 137.3, 139.3, 139.4, 140.0 ($5C_{arom.}$); 159.2 ($1C_{OCONH}$); 173.7, 174.1, 174.5, 174.8, 175.0, 175.7, 177.1 ($11C_{CO}$). IR (KBr): 699m, 740m, 1028m, 1078s, 1176s, 1207m, 1275m, 1364s, 1383s, 1410s, 1454s, 1469s, 1540vs, 1645vs, 2873m, 2934.83m, 2984m, 3031m, 3063m, 3293s.

RESULTS AND DISCUSSION

With the aim of introducing all Aib residues of the target undecapeptide **1** by the azirine coupling using the building blocks **2, 3** and **4**, it was decided to break **1** down into the three sub-segments Z-Gln-Aib-Hyp(OBn)-Aib-Pro-Pheol (**16**), Z-Val-Aib-Hyp(OBn)-OH (**19**) and Z-Thr(OBn)-Aib-OH (**23**). These three segments should then be coupled by using the well-known TBTU/HOBt method that should avoid epimerization during segment condensations. Indeed, no epimerization could be detected by NMR analysis. The protective groups of the amino groups and the side chain hydroxy groups were chosen to be stable under acidic and basic conditions [25]. Therefore, Z-protected amino acids were used, and the OH group of Thr was benzylated as was the OH group of the Aib-Hyp synthon.

The reaction of the Z-protected glutamine residue (Z-Gln-OH) with the Aib-Hyp dipeptide synthon **4** in THF at room temperature gave the tripeptide **12** in 79% yield (Scheme 2). Basic hydrolysis of the ester group of the latter with LiOH in THF/methanol/water led to **13**, which was coupled with azirine **3**, the Aib-Pro dipeptide synthon, to yield the pentapeptide **14**. This azirine coupling was performed with 76% yield. Another basic hydrolysis gave the pentapeptide acid **15**, which was coupled with L-Pheol by using the standard TBTU/HOBt method to give the *C*-terminal hexapeptide **16** in 66.2% yield. After deprotection of the *N*-terminus by hydrogenolysis, the glutamine segment **17** was obtained in quantitative yield. It is worth mentioning that the hydroxy benzyl group of the Hyp residue was stable under the chosen conditions (10% Pd/C).

Analogously, the reaction of the Z-protected valine residue (Z-Val-OH) with the Aib-Hyp dipeptide synthon **4** led to the tripeptide **18** in 77% yield (Scheme 3). The ester group of the latter was hydrolysed by treatment with LiOH in THF/methanol/water to give **19** in 64.1% yield. Using the TBTU/HOBt method, **19** was coupled with the glutamine segment **17** to give the *C*-terminal nonapeptide **20** in 50% yield. Deprotection of the *N*-terminus by hydrogenolysis gave the valine segment **21** in 88.1% yield.

The treatment of the benzyl and Z-protected threonine residue (Z-Thr(OBn)-OH) with the Aib synthon **2** in CH_2Cl_2 at room temperature gave the

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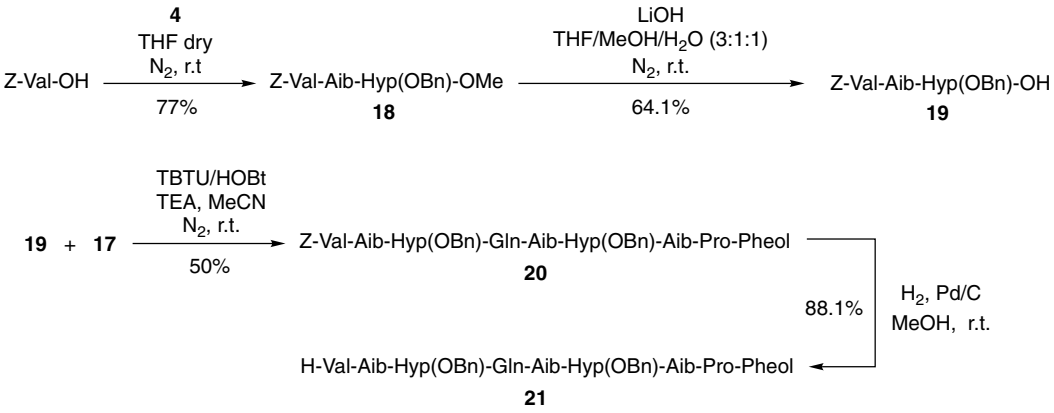
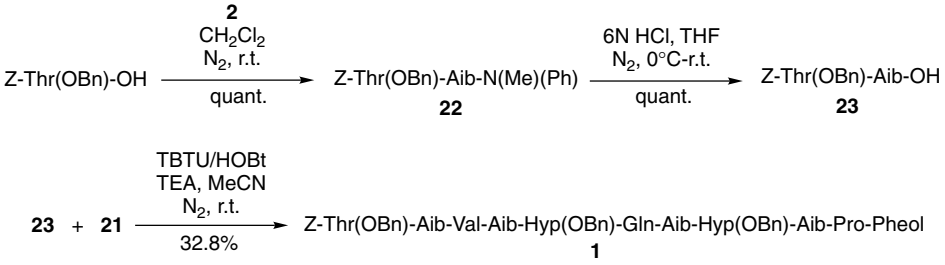


Scheme 3 Synthesis of the nonapeptide **21**.



Scheme 4 Synthesis of the undecapeptide **1**.

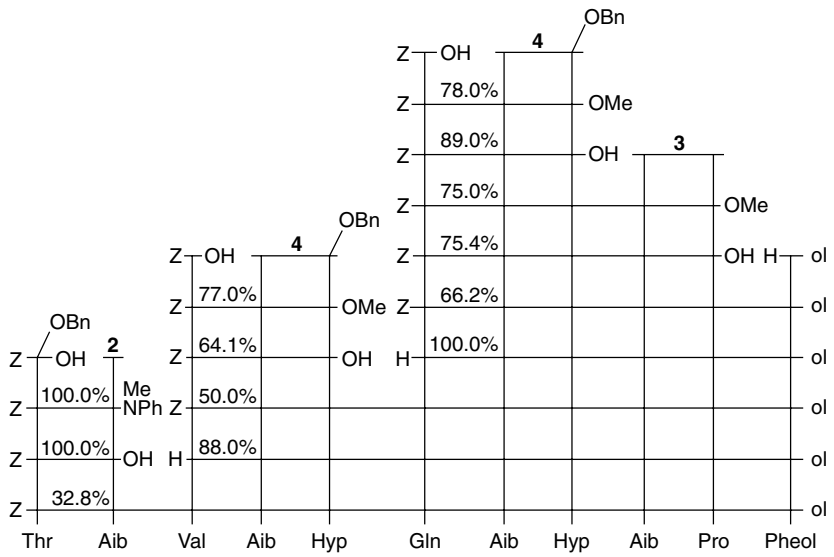


Figure 3 Synthesis of the protected 6–16 segment of zervamicin II-2.

tripeptide **22** in quantitative yield (Scheme 4). The latter was hydrolysed under the usual conditions, i.e. with 3N HCl in a 1 : 1 mixture of THF/water, the dipeptide **23** acid was obtained in quantitative yield. The final coupling of **23** with the segment **21** was achieved by using the TBTU/HOBt method, and the *C*-terminal nonapeptide **1** was obtained in 32.8% yield.

The whole synthesis of the protected 6–16 segment **1** is resumed in Figure 3. This overview

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shows that the yields were good to very good, except in some cases with the TBTU/HOBt coupling method. The introduction of all Aib residues using the 2*H*-azirin-3-amines **2, 3**, and **4** proved to be ideally suited and was performed to 75% in quantitative yield. The chosen protecting groups allowed the different conversions under acidic and basic conditions in the synthesis. The Z protecting group was removed smoothly, whereas the benzyl protecting groups of the threonine and the two hydroxy proline residues remained stable.

CONCLUSION

The introduction of the Aib, Aib-Pro and Aib-Hyp amino acid sequences in the synthesis of the 6–16 segment of zervamicin II-2 was successfully achieved by the azirine/oxazolone method with good to very good yields. The use of 2*H*-azirin-3-amines as synthons for α,α-disubstituted α-amino acids or dipeptides proved an elegant and efficient synthetic method. The combination of this method and the TBTU/HOBt coupling method has been shown to be a useful tool for the synthesis of Aib containing peptides.

The introduction of Aib into peptaibols was a difficult task for a long time (see [24]). During the past 10 years, these difficulties have been surmounted by the development of highly reactive coupling reagents and activated amino acids [12, 26, 27]. Furthermore, the fluoride activation of α,α-dialkylated α-amino acids and peptides has been shown to allow convenient peptaibol synthesis on the solid phase [28, 29].

Although the solid phase peptide synthesis is now the method of choice for routine, general and automated preparation of peptaibols, the solution phase synthesis also has some advantages. For example, any defined segment, not only the final product, can be obtained, e.g. for structure analysis by x-ray crystallography and for testing the bioactivity.

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