Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 62 (2006) 9671–9680

Introduction of the Aib-Pro unit into peptides by means of the 'azirine/oxazolone method' on solid phase

Simon Stamm[†] and Heinz Heimgartner[*]

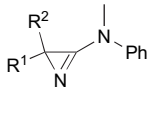
Institute of Organic Chemistry, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Received 19 May 2006; revised 13 July 2006; accepted 25 July 2006
Available online 21 August 2006

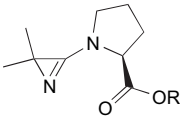
Abstract—A method for the direct introduction of Aib-Pro into peptides on solid phase was developed. The Aib-Pro unit was introduced by means of the 'azirine/oxazolone method' using allyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate as the synthon. After the reaction of the resin-bound amino or peptide acid with allyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate, the allyl protecting group of the resulting extended peptide could be removed by a mild Pd^0-promoted procedure. Cleavage of the peptide from the resin was performed with UV light at 352 nm and yielded C-terminal protected peptides. The method found a successful application in the syntheses of different Aib-Pro containing peptaibol segments. Furthermore, a protected derivative of the peptide antibiotic *Trichovirin I 1B* was prepared by segment condensation.

1. Introduction

2*H*-Azirin-3-amines are highly strained systems with versatile reactivity.[1] One very interesting and useful reaction is their application in peptide synthesis. In the 'azirine/oxazolone method', 2*H*-azirin-3-amines such as **1** or **2** are used as synthons for the introduction of sterically demanding α,α-disubstituted α-amino acids into peptides.[1–3] Thus, the reaction of 2*H*-azirin-3-amines, e.g., the α-aminoisobutyric acid (Aib) synthon **1a**, with amino or peptide acids leads to peptide amides, the terminal amide bonds of which can be hydrolyzed selectively to give extended peptide acids. In solution-phase chemistry, the 'azirine/oxazolone method' has proven to be successful for the introduction of a variety of sterically demanding α,α-disubstituted α-amino acids into oligopeptides,[4–13] endothiopeptides,[14–16] cyclic peptides,[17–18] and cyclic depsipeptides.[19–23]



1a $R^1 = R^2 = Me$
1b $R^1 - R^2 = -(CH_2)_4-$
1c $R^1 - R^2 = -(CH_2)_2O(CH_2)_2-$
1d $R^1 = Me$, $R^2 = PhCH_2$



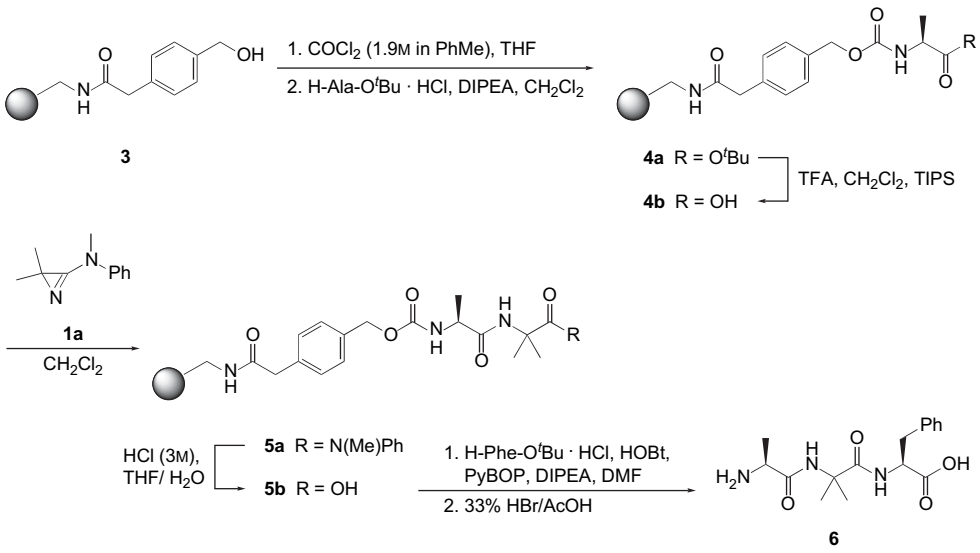
2a R = Me
2b $R = CH_2CHCH_2$
2c $R = PhCOCH_2$

Recently, we adapted the 'azirine/oxazolone method' to solid-phase conditions, in order to additionally benefit from their advantages,[24] e.g., the rapid access to peptides without the need for the isolation of the sometimes cumbersome peptide acid intermediates. In this method, the growing peptide was attached through a carbamate linker to a [4-(hydroxymethyl)phenyl]acetamidomethyl (PAM) polystyrene resin (**3**) (Scheme 1). After deprotection of ^{t}Bu ester **4a**, resin-bound amino acid **4b** was treated with a solution of **1a**. It is worth mentioning that unconsumed **1a** could easily be recovered and re-used. The terminal amide **5a** was selectively hydrolyzed with 3 M HCl in THF/H_2O to provide the resin-bound peptide acid **5b**. Further extension of the peptide chain could be achieved either with a ^{t}Bu-protected amino acid and a coupling reagent or with **1a**. Cleavage of the peptide from the resin was achieved with HBr (33%) in acetic acid, and yielded the tripeptide **6**. In a recent paper, we showed that the method is not restricted to the Aib synthon **1a**, and that it was successfully extended to the 1-aminocyclopentane-1-carboxylic acid synthon **1b**, the 4-amino-3,4,5,6-tetrahydro-2*H*-pyran-4-carboxylic acid synthon **1c**, and the α-methylphenylalanine synthon **1d**.[25]

Peptaibols are linear, amphiphilic oligopeptides from fungal sources with a high proportion of α,α-disubstituted α-amino acids, primarily, Aib.[26–27] Peptaibols show antibiotic properties due to self-association in lipid membranes forming ion channels.[28] Several peptaibols, or segments thereof, were synthesized by means of the 'azirine/oxazolone method'.[4,8–13] The 2*H*-azirin-3-amine **2a** played an important role in these syntheses, since **2a** allowed the direct

Keywords: α-Aminoisobutyric acid; Azirine/oxazolone method; Peptaibols; Peptide synthesis; Photocleavable linker.
Abbreviations: Aib, α-aminoisobutyric acid; DIPEA, *N*,*N*-diisopropylethylamine; HOBt, 1-hydroxybenzotriazole; PyBOP, (1*H*-benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate; TBTU, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; TIPS, triisopropylsilane; Z, benzyloxycarbonyl; Z-ONSu, *N*-[(benzyloxycarbonyl)oxy]succinimide.
* Corresponding author. Tel.: +41 44 6354282; fax: +41 44 6356836; e-mail: heimgart@oci.unizh.ch
† Part of the projected Ph.D. thesis of *S. Stamm*, Universität Zürich.

0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.07.082



Scheme 1.

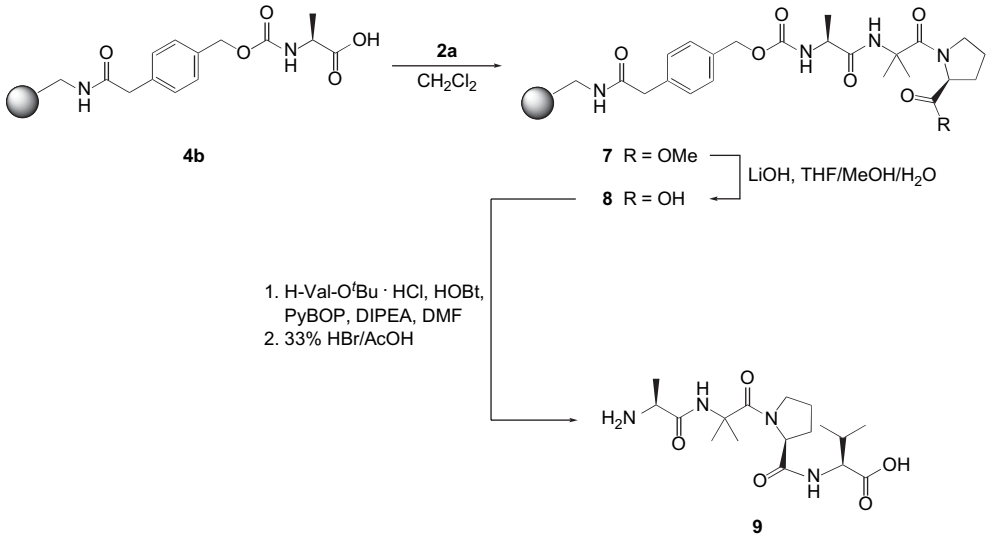
introduction of the frequently present, but relatively acid labile, Aib-Pro unit.[9] Unexpectedly, the use of **2a** on solid phase was not successful (vide infra).

Herein we report a method for the introduction of the Aib-Pro unit into peptides on solid phase, using a photolabile linker and a new Aib-Pro synthon (**2b**), which was especially developed for this purpose.[29]

2. Results and discussion

The most obvious approach to the introduction of the Aib-Pro unit was the use of synthon **2a** in analogy to the method outlined in Scheme 1. In doing so, the resin-bound amino acid **4b** was treated with a solution of **2a**, and the resulting resin-bound tripeptide methyl ester **7** was saponified with LiOH in a mixture of THF, MeOH, and H_2O (Scheme 2). After coupling with H-Val-O^{t}Bu by using PyBOP as the coupling reagent, the peptide was cleaved from the resin with HBr (33%) in acetic acid to give H-Ala-Aib-Pro-Val-OH (**9**) in 20% yield. In order to guarantee proper swelling of the resin during the saponification, the experiment was repeated with a Tentagel resin. However, the yield of 21% was still conspicuously lower in comparison with the introduction of the synthons **1a–d** (37–50%). The problem was recognized, when the synthesis of the model peptide H-Ala-Aib-Pro-Leu-Aib-Val-OH (on Tentagel resin) and the peptaibol segment (A8–A14 of *Trichovirin Ia*) H-Val-Aib-Gly-Aib-Aib-Pro-Leu-OH (on polystyrene resin) failed. Only peptide fragments, which are caused by Aib-Pro fissions, were detected. The analysis of the fragments revealed that the cleavage of the Aib-Pro amide bond occurred during the HBr-promoted cleavage of the peptide from the resin, and not during the hydrolysis of the terminal amide, which was necessary after the incorporation of **1a**.

Thus, a linker was required, which can be cleaved under milder conditions, but is still stable in TFA (50%) and HCl (3 M). Some years ago, Kunz introduced a Pd^0-labile allyl

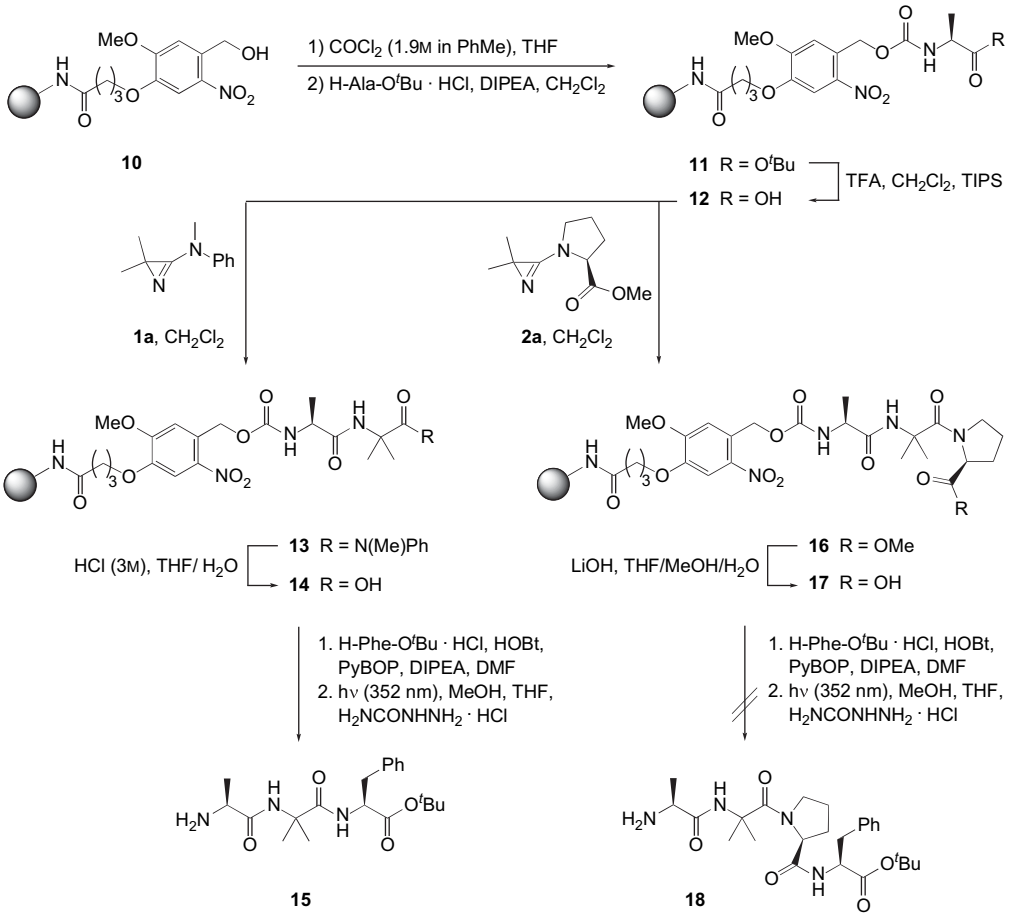


Scheme 2.

linker for the synthesis of peptide acids.[30] Therefore, we synthesized 2-{[(*Z*)-4-(triisopropylsilyloxy)but-2-enyl]oxy}-ethanoic acid[31] and attached its carboxy group via an alanine spacer to an aminomethyl polystyrene resin.[32] Additionally, (*E*)-7-hydroxyhept-5-enoic acid was prepared,[33] and its carboxy group was attached to an aminomethyl polystyrene resin. In both cases, the N-terminus of the first amino acid was immobilized through a carbamate group to the resin (in the first case after removing the TIPS-protecting group with TFA), and the resin-bound peptides **6** and **9** were synthesized analogously to the 'PAM/HBr-strategy'. Cleavage from the resin was performed with $Pd(Ph_3P)_4$ and $PhSiH_3$ in $DMSO/CH_2Cl_2$, but the desired peptides could only be obtained in low yield and after a painstaking purification of the Pd-containing crude product.

In 1995, Holmes introduced a photolabile nitroveratryl linker and immobilized peptides by coupling their C-terminus to the resin.[34–36] This photolinker is among the most effective ever described—it is stable to various chemical reactions, but can easily be cleaved with UV light with λ=365 nm, a wavelength that does not affect aromatic amino acids such as Trp or Tyr. The corresponding photocleavable resin **10** (Scheme 3) is commercially available from Novabiochem.

With the aim of testing the stability under acidic conditions, *N*-(4,5-dimethoxy-2-nitrobenzyloxy)carbonyl-L-alanine *tert*-butyl ester (NVOC-Ala-O^{t}Bu) was prepared by reaction of 4,5-dimethoxy-2-nitrobenzyl alcohol and $COCl_2$ in THF, and subsequently with H-Ala-O^{t}Bu·HCl under Schotten–Baumann conditions to give NVOC-Ala-O^{t}Bu, which was then subjected to acidic conditions. Apart from ^{t}Bu hydrolysis, neither in TFA (50%) nor in HCl (3 M) cleavage or decomposition of the linker was observed. Hence, the chloroformate of the photocleavable resin **10** was reacted with H-Ala-O^{t}Bu (Scheme 3). Deprotection of the ^{t}Bu ester **11** with TFA afforded the resin-bound amino acid **12**, which was treated with a solution of **1a**. Selective hydrolysis of the terminal amide of the resin-bound dipeptide **13** with 3 M HCl afforded the resin-bound peptide acid **14**, which was coupled with H-Phe-O^{t}Bu. Cleavage of the peptide from the resin was performed with UV light (16×8 W, λ_{max}=352 nm) in MeCN/H_2O, but the initial yields were disappointing. Most probably, the remaining nitrosoaldehyde functionalized resin captured the peptide **15** via imine formation with its amino group.[37] This assumption was supported by ^{1}H NMR experiments: the formyl signal of methyl 4-[(4-formyl-2-methoxy-5-nitrophenyl)oxy]butanoate disappeared within 20 min on reaction with isopropylamine, and a new signal at 8.65 ppm, typical for imines, appeared. To overcome the interception of the peptide, the cleavage from the resin was performed in a solution of semicarbazide hydrochloride in THF/MeOH.[37] Although **15** was now obtained in much better yield (ca. 30–40%), its purity was unsatisfactory. Therefore, the general procedures had to be slightly modified again. The reaction time for the ^{t}Bu ester hydrolysis was extended to 60 min to avoid the previously detected H-Ala-Phe-O^{t}Bu side product. Moreover, the synthesis was carried out on



Scheme 3.

a polystyrene instead of a Tentagel resin. The use of Tentagel resin suffered from (poly)ethylene glycol loss. Finally, model peptide **15** was prepared in high purity and in 33% yield (after prep. HPLC, based on resin loading).

After having established the protocol for the use of Aib synthon **1a**, the preparation of Aib-Pro containing peptides was attempted. But when dipeptide synthon **2a** was used to incorporate the Aib-Pro unit, the experiment failed again (Scheme 3). During the saponification of resin-bound methyl ester **16** to give **17**, we observed a considerable darkening of the resin, which prevented the subsequent photoinduced cleavage of the peptide from the resin.

This issue was addressed by the synthesis of the new Aib-Pro synthons allyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**2b**) and phenacyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**2c**), which contain easily removable carboxy-protecting groups.[29] The resin-bound amino acid **12** was reacted with a solution of **2c**, and the resulting resin-bound phenacyl ester was treated with tetrabutylammonium fluoride. Again, considerable darkening of the resin was observed. On the other hand, the allyl protecting group of resin-bound peptide **19**, obtained from the reaction of **12** and **2b**, was smoothly removed with $Pd(Ph_3P)_4$ and $PhSiH_3$[38] in CH_2Cl_2 to give the resin-bound peptide acid **17** (Scheme 4). The latter was then coupled with H-Phe-O^{t}Bu and PyBOP as the coupling reagent affording the corresponding resin-bound tetrapeptide. Cleavage from the resin was achieved with UV light (16×8 W, λ_{max}=352 nm) in a solution of semicarbazide hydrochloride in THF/MeOH and gave H-Ala-Aib-Pro-Phe-O^{t}Bu (**18**) in high purity and in 35% yield (after prep. HPLC, based on resin loading).

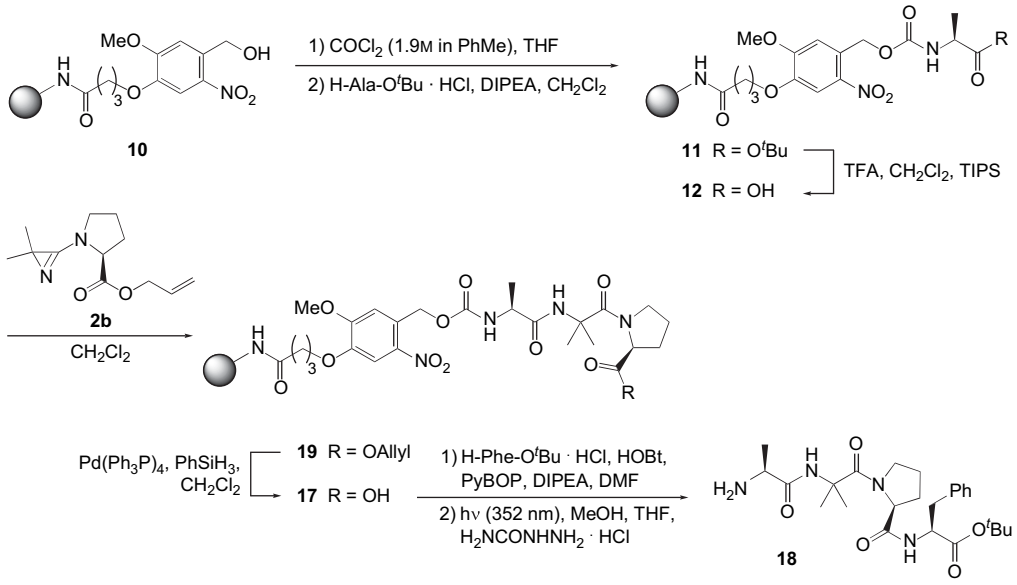
To evaluate the described protocol for the introduction of the Aib-Pro motif into peptides, the preparation of some peptaibol segments was attempted. The heptapeptide H-Val-Aib-Gly-Aib-Aib-Pro-Leu-O^{t}Bu (**20**, A8–A14 of *Trichovirin Ia*; Table 1), whose synthesis failed with the 'PAM/HBr-strategy' (see above), was prepared in 42% yield using the photocleavable resin **10**, the Aib synthon **1a**, and the allyl-protected Aib-Pro synthon **2b**. Gly and Leu were introduced with PyBOP as the coupling reagent. Analogously, the syntheses of the hexa- and octapeptides H-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-O^{t}Bu (**21**, A1–A6 of *Trichovirin I 1B*) and H-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu (**22**, A7–A14 of *Trichovirin I 1B*) were carried out. Sequence **21** was chosen to evaluate the introduction of non-aliphatic amino acids, while in the synthesis of **22**, an Aib-Pro unit had to be installed prior to an Aib residue and another Aib-Pro unit. Both peptides were prepared on the photocleavable resin **10** using the Aib synthon **1a** and the allyl-protected Aib-Pro synthon **2b**. All other amino acids were introduced with PyBOP as the coupling reagent. The side chain of Asn was not protected, while the hydroxy group of the serine side chain was masked as benzyl ether, a protecting group which is orthogonal to the ^{t}Bu protecting group. The peptides were obtained in 34% yield each (after prep. HPLC, based on resin loading), which is comparable to that of tetrapeptide **18** and therefore indicating a good linker stability.

In contrast to the 'PAM/HBr-strategy' (Scheme 1), the use of the photolinker allows the preparation of ^{t}Bu-protected peptides, which, in turn, offers the possibility of segment condensations. For that purpose, the C-terminal ^{t}Bu-protected peptides had to be transformed into N-terminal protected

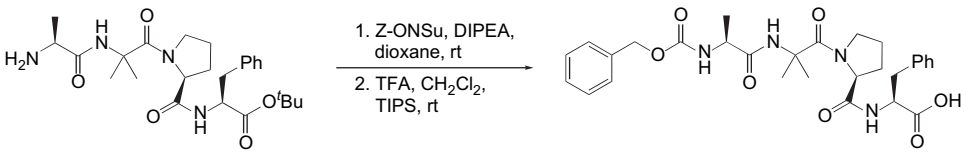
Table 1. Peptides synthesized by means of the 'azirine/oxazolone method' on solid phase

Sequence	Description	Yield [%][a]
H-Ala-Aib-Phe-O^{t}Bu (**15**)	Model peptide	33
H-Ala-Aib-Pro-Phe-O^{t}Bu (**18**)	Model peptide	35
H-Val-Aib-Gly-Aib-Aib-Pro-Leu-O^{t}Bu (**20**)	A8–A14 of *Trichovirin Ia*	42
H-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-O^{t}Bu (**21**)	A1–A6 of *Trichovirin I 1B*	34
H-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu (**22**)	A7–A14 of *Trichovirin I 1B*	34

[a] Based on resin loading, after prep. HPLC.

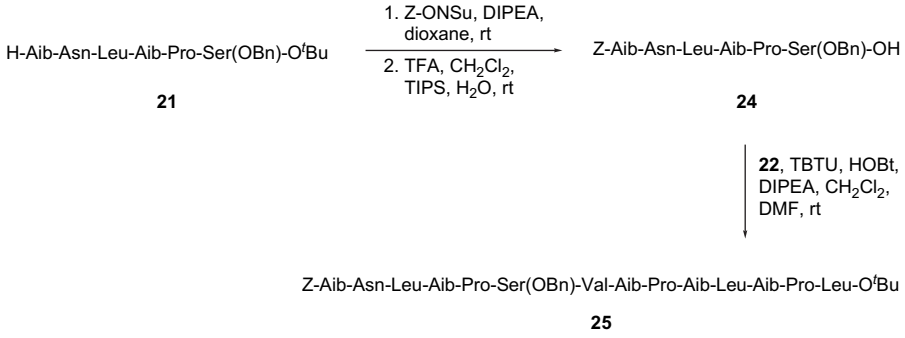


Scheme 4.



18 23

Scheme 5.



21 24 25

Scheme 6.

peptides. Therefore, the ^{t}Bu-protected peptide **18** was reacted with *N*-[(benzyloxycarbonyl)oxy]succinimide (Z-ONSu), and the corresponding ^{t}Bu protecting group was hydrolyzed to give the *N*-protected peptide **23** (Scheme 5).

Analogously, the heptapeptide **21** (A1–A6 of *Trichovirin I 1B*) was transformed into the *N*-protected peptide **24** (Scheme 6). Due to steric hindrance of Aib, the reaction time for the introduction of the *Z*-protecting group was extended to 8 h. However, the transformation was not complete, and **21** (20%) was partially recovered. The hydrolysis of the corresponding ^{t}Bu ester with TFA was accompanied by partial cleavage of the Aib-Pro amide bond. Most probably, the extent of this side reaction could be reduced by shortening the reaction time (HPLC/MS indicates complete deprotection already after 20 min). Finally, **24** was coupled with **22** (A7–A14 of *Trichovirin I 1B*) and TBTU as the coupling reagent, and the protected *Trichovirin I 1B* derivative (Z-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu; **25**) was obtained in 73% yield (Scheme 5).[39]

3. Conclusions

A method for the direct introduction of Aib-Pro into peptides via 'azirine coupling' on solid phase was developed. The protocol is based on a photocleavable resin (**10**) and the Aib-Pro synthon **2b**. After the coupling of the resin-bound peptide acid with **2b**, the allyl protecting group of the extended peptide was removed by a mild Pd^0-promoted procedure. In contrast to the previously described 'PAM/HBr-strategy', this protocol allows the isolation of peptides with a protected C-terminus. The method found a successful application in the syntheses of different Aib-Pro containing peptaibol segments. A subsequent segment condensation led to the *Trichovirin I 1B* derivative **25**, an oligopeptide containing three Aib-Pro units and two additional Aib residues.

4. Experimental

4.1. General

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Aminomethyl polystyrene resin (1% divinylbenzene, 100–200 mesh, loading 1.14 mmol/g) and aminomethyl Tentagel resin (90 μm, loading 0.28 mmol/g) from *Rapp Polymere* (*Rapp Polymere*, Tübingen, Germany). Hydroxymethyl-photolinker AM resin (**10**) (polystyrene, 1% divinylbenzene, 100–200 mesh, loading 0.75 mmol/g) from *Novabiochem* (*Calbiochem-Novabiochem*, Läufelfingen, Switzerland). *N*,2,2-Trimethyl-*N*-phenyl-2*H*-azirin-3-amine (**1a**) was synthesized according to the method of Villalgordo and Heimgartner.[40,41] Methyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**2a**), allyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**2b**), and phenacyl *N*-(2,2-dimethyl-2*H*-azirin-3-yl)-L-prolinate (**2c**) were synthesized according to Ref. 9 (**2a**) and Ref. 29 (**2b**, **2c**), respectively. H-Ser(OBn)-O^{t}Bu was prepared by treatment of Fmoc-Ser(OBn)-OH with *tert*-butyl trichloroacetamidate and removal of Fmoc with Et_2NH. The ^{1}H NMR spectra of H-Ser(OBn)-O^{t}Bu were in accordance with the data given in Ref. 42. Reaction vessels for solid-phase synthesis: Single fritted (20 μm) PE reservoir (15 ml) (*Separtis*, Grenzach-Wyhlen, Germany) was wrapped with aluminum foil and used on an *Advanced ChemTech PLS 4×6* Shaker (*Advanced ChemTech, Inc.*, Louisville, KY, USA) with a self-made adapter. The original *Advanced ChemTech* reaction vessels were used for reactions with $COCl_2$. Photolysis was performed in a quartz tube (13.5 cm, 13 mm i.d.) and irradiation with circularly arranged *sterilAir BLB8* lamps (16×8 W, λ_{max}=352 nm) (*sterilAir AG*, Weinfelden, Switzerland). High-performance liquid chromatography (HPLC) instrument: *Waters 600E* multisolvent delivery system equipped with a *Waters 996 PDA* (*Waters*, Milford, CA, USA); column: *Interchim Uptisphere WOD C18*, 300 Å, 10 μm, 250×21.2 mm (*Interchim*, Montluçon,

France); eluents: A=H_2O/TFA (0.1%), B=MeCN/TFA (0.1%); flow rate: 10 ml/min; various gradients. Column chromatography (CC): Silica gel *C-560* from *Chemie Uetikon* (*CU Chemie Uetikon GmbH*, Uetikon, Switzerland). IR spectra: *Perkin–Elmer*, *Spectrum one FT-IR* spectrophotometer (*Perkin–Elmer*, Wellesley, MA, USA), absorptions in cm^{-1}. NMR spectra: *Bruker AV-600* (*Bruker Biospin*, Karlsruhe, Germany). Chemical shifts in parts per million relative to tetramethylsilane as internal standard. 2D-NMR experiments were performed for assignment of the signals. The integer *n* in Xaa^n corresponds to the position of the amino acid within the peptide, but is only given if the amino acid was present more than once in the peptide and if the NMR signal could be assigned unambiguously. HPLC/MS: The system consists of a *Rheos 2000* pump, a *Rheos CPS-LC* degasser (*Flux Instruments*, Basel, Switzerland) and a *Thermo Finnigan Surveyor* photodiode array detector (*Thermo Finnigan*, San Jose, CA, USA). The HPLC system is equipped with a *HTS PAL* autosampler (*CTC Analytics*, Zwingen, Switzerland) and connected to a *Thermo Finnigan MSQ* linear quadrupole instrument. *Interchim Uptisphere C18-ODB*, 120 Å, 3 μm, 50×2.0 mm column; eluents: A=H_2O, B=MeCN, C=HCOOH (1%) in H_2O; flow rate: 0.2 ml/min, gradient (A/B/C): 0.0–15.0 min: 87:3:10 to 40:50:10. MS: *Bruker ESQUIRE-LC* quadrupole instrument (*Bruker Daltonik GmbH*, Bremen, Germany) or *Finnigan TSQ-700* triple quadrupole instrument (*Finnigan* MAT, San Jose, CA, USA). Direct infusion ESI-MS were performed with a syringe infusion pump at a flow rate of 5 μl/min.

4.2. General procedures 1–7 (GP 1–GP 7)

4.2.1. GP 1: attachment of the first amino acid. All manipulations were carried out under N_2. Hydroxymethyl-photolinker AM resin was swollen in THF. After filtration, a solution of $COCl_2$ (*CAUTION*) in toluene (ca. 20%, 10 equiv) and THF (ca. 0.7 ml/100 mg resin) were added to the resin, which was agitated at rt for 2 h, then washed with THF (2×) and CH_2Cl_2 (2×). In a separate vial, H-Xaa-O^{t}Bu·HCl (4 equiv) was dissolved in DIPEA (8 equiv) and CH_2Cl_2 (concn of H-Xaa-O^{t}Bu·HCl=ca. 0.2 M). This mixture was added to the resin and any ammonium salt that occurred was removed by filtration. The resin was agitated at rt overnight, then washed with DMF (3×) and CH_2Cl_2 (3×).

4.2.2. GP 2: removing the ^{t}Bu protecting group. The resin was swollen in CH_2Cl_2. TFA in CH_2Cl_2 (2 ml, 1×15 s, 25%; 2 ml, 1×60 min, 50%) and TIPS (5%, in each case) were added, and the resin was agitated at rt. Then, the resin was washed with CH_2Cl_2 (3×), DMF (2×), and CH_2Cl_2 (3×).

4.2.3. GP 3: coupling with 2*H*-azirin-3-amines 1a, 2a, and 2b. The corresponding resin was swollen in CH_2Cl_2. A solution of 2*H*-azirin-3-amine (4 equiv) in CH_2Cl_2 (concn of 2*H*-azirin-3-amine=ca. 0.2 M) was added, the resin was agitated at rt overnight, and then washed with CH_2Cl_2 (3×). Unconsumed 2*H*-azirin-3-amine could easily be recovered.

4.2.4. GP 4: hydrolysis of the terminal amide. The resin was swollen in THF. Aq HCl (ca. 2 ml/100 mg resin, 3 M in THF/H_2O, prepared from concd HCl and THF) was added, and the resin was agitated at rt overnight, then washed with THF (3×), DMF (3×), and CH_2Cl_2 (3×).

4.2.5. GP 5: coupling with H-Xaa-O^{t}Bu·HCl. The resin was swollen in DMF. HOBt (6 equiv) in DMF, PyBOP (4 equiv) in DMF, H-Xaa-O^{t}Bu·HCl (4 equiv) in DMF, and DIPEA (12 equiv) were added (concn of H-Xaa-O^{t}Bu·HCl=ca. 0.2 M), the resin was agitated at rt, and then washed with DMF (3×) and CH_2Cl_2 (3×).

4.2.6. GP 6: removal of the allyl protecting group. The resin was dried i.v. All manipulations were carried out under Ar. The resin was swollen in CH_2Cl_2. A mixture of $Pd(Ph_3P)_4$ (0.3 equiv) and $PhSiH_3$ (20 equiv) in CH_2Cl_2 (ca. 2 ml/100 mg resin) was added, the resin was agitated at rt for 1.5 h, and then washed with CH_2Cl_2 (3×), DMF, CH_2Cl_2 (2×), THF (1% H_2O) (2×), THF (1×), MeOH, and CH_2Cl_2 (2×).

4.2.7. GP 7: cleavage. The resin (ca. 0.075 mmol) was swollen in a solution of semicarbazide hydrochloride (24 mg) in MeOH/THF (2:1, 6 ml), and the mixture was degassed by bubbling Ar into the mixture. Under vigorous stirring, the resin was irradiated with 16×8 W (λ_{max}=352 nm) for 2 h, then the supernatant solution was removed, and the irradiation process was repeated two times. Afterwards, the resin was washed with MeOH/THF (2:1, 3×). All solutions were combined, concentrated under reduced pressure, and the crude product was purified by means of HPLC. The purified product was lyophilized.

4.3. Synthesis of peptides

4.3.1. H-Ala-Aib-Pro-Val-OH (9). Aminomethyl Tentagel resin (350 mg, 0.098 mmol) was swollen in DMF. 4-(Hydroxymethyl)phenylacetic acid (33 mg, 0.199 mmol), DIPEA (103 μl, 0.602 mmol), and PyBOP (104 mg, 0.200 mmol) in DMF (1.5 ml) were added, and the resin was agitated at rt for 30 min (negative Kaiser test), then washed with DMF (3×), CH_2Cl_2 (3×), and THF (3×). The resin was treated as described in GP 1–3. Then, the resin was swollen in a mixture of THF (1 ml) and MeOH (0.7 ml), and LiOH·H_2O (33 mg, 0.787 mmol) in H_2O (0.3 ml) was added. The resin was agitated at rt overnight, washed with THF/MeOH/H_2O (4:3:1, 3×), and DMF (3×). The resin was treated as described in GP 5 (2 h), then swollen in CH_2Cl_2. HBr in AcOH (33%, 3 ml) and two drops of H_2O were added, and the resin was agitated at rt for 4 h. The resin was separated by filtration and washed with AcOH/CH_2Cl_2 (1:1, 3×) and MeCN/CH_2Cl_2 (1:1, 3×). The solvents were evaporated under reduced pressure and the crude product was purified by means of HPLC. The purified product was lyophilized and yielded **9** (10 mg, 21%) as a colorless powder. IR (KBr): 3435s, 3271s, 3068s, 2969s, 2882s, 1674vs, 1630vs, 1548s, 1472m, 1422s, 1369m, 1338w, 1310w, 1269m, 1202vs, 1183vs, 1138vs, 1005w, 979w, 929w, 837w, 800w, 722m. ^{1}H NMR (DMSO-d_6, 600 MHz): ca. 10.0–7.0 (br s, NH_3(Ala)); 8.61 (s, NH(Aib)); 7.73 (d, *J*=8.2 Hz, NH(Val)); 4.42–4.41 (m, CH(α)(Pro)); 4.06–4.04 (m, CH(α)(Val)); 3.88–3.87 (m, CH(α)(Ala)); 3.57–3.54, 3.38–3.34 (2m, CH_2(δ)(Pro)); 2.04 (dsept., *J*=6.6, 6.6 Hz, CH(β)(Val)); 1.88–1.83, 1.81–1.76 (2m, CH_2(β)(Pro), CH_2(γ)(Pro)); 1.37, 1.34 (2s, 2 Me(Aib)); 1.36 (d, *J*=7.6 Hz, Me(Ala)); 0.90, 0.89 (2d, *J*=6.8 Hz, 2 Me(Val)). ^{13}C NMR (DMSO-d_6, 150 MHz): 173.0 (s, CO(Val)); 171.9 (s, CO(Pro)); 170.4 (s, CO(Aib)); 168.4

(s, CO(Ala)); 60.6 (d, CH(α)(Pro)); 57.2 (d, CH(α)(Val)); 56.0 (s, C(α)(Aib)); 47.9 (d, CH(α)(Ala)); 47.6 (t, CH_2(δ)(Pro)); 29.9 (d, CH(β)(Val)); 27.9, 25.2 (2t, CH_2(β)(Pro), CH_2(γ)(Pro)); 24.9, 24.5 (2q, 2 Me(Aib)); 19.1, 18.1 (2q, 2 Me(Val)); 17.3 (q, Me(Ala)). ESI-MS: 371 (100, $[M+H]^+$), 215 (34, $[Pro\text{-}Val]^+$).

4.3.2. H-Ala-Aib-Phe-O^{t}Bu (15). Aminomethyl polystyrene resin (62 mg, 0.071 mmol) was swollen in DMF. 4-[(4-Hydroxymethyl-2-methoxy-5-nitrophenyl)oxy]butanoic acid (40 mg, 0.140 mmol), PyBOP (72 mg, 0.138 mmol), and DIPEA (72 μl, 0.421 mmol) in DMF (1.5 ml) were added, and the resin was agitated at rt for 30 min, then washed with DMF (3×), CH_2Cl_2 (2×), and THF (2×). The resin was treated as described in GP 1–5 (overnight), and 7 to yield **15** (11.4 mg, 33%) as a colorless powder after prep. HPLC purification and lyophilization. IR (KBr): 3426s, 3399s, 3288s, 3065m, 3032m, 2983s, 2939s, 1724s, 1674vs, 1549s, 1516s, 1457m, 1440m, 1393m, 1368s, 1324w, 1259s, 1204vs, 1180vs, 1156vs, 1139vs, 1079w, 1030w, 1015w, 1003w, 948w, 929w, 881w, 839m, 800w, 754w, 739w, 722m, 700m. ^{1}H NMR (DMSO-d_6, 600 MHz): 8.31 (s, NH(Aib)); 8.01 (br s, NH_3(Ala)); 7.67 (d, J=7.8 Hz, NH(Phe)); 7.29–7.19 (m, 5 arom. H); 4.34 (ddd, J=8.2, 7.8, 6.4 Hz, CH(α)(Phe)); 3.84 (q, J=6.9 Hz, CH(α)(Ala)); 3.02 (dd, J=13.8, 6.4 Hz, 1 H of CH_2(Phe)); 2.95 (dd, J=13.8, 8.2 Hz, 1 H of CH_2(Phe)); 1.38, 1.37 (2s, 2 Me(Aib)); 1.33 (s, Me_3C); 1.32 (d, J=7.3 Hz, Me(Ala)). ^{13}C NMR (DMSO-d_6, 150 MHz): 172.9 (s, CO(Aib)); 170.4 (s, CO(Phe)); 168.8 (s, CO(Ala)); 137.4 (s, arom. C); 129.2, 128.1, 126.4 (3d, 5 arom. CH); 80.7 (s, Me_3C); 56.3 (s, C(α)(Aib)); 54.2 (d, CH(α)(Phe)); 48.3 (d, CH(α)(Ala)); 36.8 (t, CH_2(Phe)); 27.5 (q, Me_3C); 24.7, 24.6 (2q, 2 Me(Aib)); 17.1 (q, Me(Ala)). ESI-MS: 378 (53, $[M+H]^+$), 322 (100, $[M-{}^t\text{Bu}]^+$). HPLC/MS: t_R 10.3 min, m/z 378 (16, $[M+H]^+$), 322 (100, $[M-{}^t\text{Bu}]^+$), 157 (27, $[M-(\text{Phe-O}^t\text{Bu})]^+$).

4.3.3. H-Ala-Aib-Pro-Phe-O^{t}Bu (18). Hydroxymethyl-photolinker AM resin (101 mg, 0.075 mmol) was treated as described in GP 1–3, 6, 5 (2 h), and 7 to yield **18** (15.6 mg, 35%) as a colorless powder after prep. HPLC purification and lyophilization. IR (KBr): 3428s, 3293s, 3065s, 3032s, 2983s, 2940s, 1728s, 1674vs, 1548s, 1536s, 1499m, 1472m, 1456m, 1422m, 1415m, 1395m, 1369m, 1258m, 1203vs, 1177vs, 1156vs, 1135vs, 1052w, 1029w, 1004w, 928w, 879w, 836w, 800w, 740w, 721m, 701m. ^{1}H NMR (DMSO-d_6, 600 MHz): 8.65 (s, NH(Aib)); 8.08 (br s, NH_3(Ala)); 7.91 (d, J=7.4 Hz, NH(Phe)); 7.29–7.20 (m, 5 arom. H); 4.33–4.31 (m, CH(α)(Pro)); 4.26 (ddd, J=7.3, 7.3, 7.3 Hz, CH(α)(Phe)); 3.88–3.87 (m, CH(α)(Ala)); 3.52–3.50, 3.38–3.35 (2m, CH_2(δ)(Pro)); 2.97–2.95 (m, CH_2(Phe)); 1.86–1.76, 1.69–1.67 (2m, CH_2(β)(Pro), CH_2(γ)(Pro)); 1.36 (d, J=7.1 Hz, Me(Ala)); 1.35, 1.34 (2s, 2 Me(Aib)); 1.30 (s, Me_3C). ^{13}C NMR (DMSO-d_6, 150 MHz): 171.7 (s, CO(Pro)); 170.4 (s, CO(Phe)); 170.3 (s, CO(Aib)); 168.3 (s, CO(Ala)); 137.3 (s, arom. C); 129.2, 128.0, 126.3 (3d, 5 arom. CH); 80.3 (s, Me_3C); 60.7 (d, CH(α)(Pro)); 55.9 (s, C(α)(Aib)); 54.2 (d, CH(α)(Phe)); 47.8 (d, CH(α)(Ala)); 47.5 (t, CH_2(δ)(Pro)); 36.6 (t, CH_2(Phe)); 27.9 (t, CH_2(β)(Pro)); 27.4 (s, Me_3C); 25.0 (t, CH_2(γ)(Pro)); 24.7, 24.6 (2q, 2 Me(Aib)); 17.1 (q, Me(Ala)). ESI-MS: 497 (10, $[M+Na]^+$), 475 (100, $[M+H]^+$), 419 (17, $[M-{}^t\text{Bu}]^+$), 319 (7, $[M-(\text{Ala-Aib})]^+$). HPLC/MS: t_R 9.9 min, m/z 497 (10, $[M+Na]^+$), 475 (30, $[M+H]^+$), 419 (24, $[M-{}^t\text{Bu}]^+$), 319 (32, $[M-(\text{Ala-Aib})]^+$), 263 (100, $[M-(\text{Ala-Aib})-{}^t\text{Bu}]^+$).

4.3.4. H-Val-Aib-Gly-Aib-Aib-Pro-Leu-O^{t}Bu (20). Hydroxymethyl-photolinker AM resin (100 mg, 0.075 mmol) was treated as described in GP 1–5 (overnight), 2–4, 3, 6, 5 (2 h), and 7 to yield **20** (25.7 mg, 42%) as a colorless powder after prep. HPLC purification and lyophilization. IR (KBr): 3433vs, 3317vs, 3063m, 2978s, 2939s, 2877m, 1725m, 1671vs, 1543vs, 1537vs, 1469m, 1440m, 1416m, 1397m, 1384m, 1368m, 1333w, 1282m, 1248m, 1203vs, 1178vs, 1146s, 1018w, 947w, 878w, 837w, 801w, 722w. ^{1}H NMR (DMSO-d_6, 600 MHz): 8.82 (s, NH(Aib^2)); 8.20 (s, NH(Gly)); 8.08 (br s, NH_3(Val)); 7.81 (d, J=8.1 Hz, NH(Leu)); 7.72 (s, NH(Aib^4)); 7.43 (s, NH(Aib^5)); 4.37–4.35 (m, CH(α)(Pro)); 4.11–4.07 (m, CH(α)(Leu)); 3.61–3.59 (m, CH(α)(Val), 1 H of CH_2(Gly), 1 H of CH_2(δ)(Pro)); 3.55–3.51 (m, 1 H of CH_2(Gly)); 3.43–3.39 (m, 1 H of CH_2(δ)(Pro)); 2.12 (dsept., J=6.7, 6.7 Hz, CH(β)(Val)); 2.02–1.98 (m, 1 H of CH_2(β)(Pro)); 1.74–1.69 (m, 1 H of CH_2(β)(Pro), CH_2(γ)(Pro)); 1.63–1.57 (m, CH(γ)(Leu), 1 H of CH_2(β)(Leu)); 1.51–1.45 (m, 1 H of CH_2(β)(Leu)); 1.403, 1.398 (2s, 2 Me(Aib^2), 2 Me(Aib^4)); 1.38 (s, Me_3C); 1.35, 1.33 (2s, 2 Me(Aib^5)); 0.96, 0.94 (2d, J=7.0 Hz, 2 Me(Val)); 0.89, 0.82 (2d, J=6.3 Hz, 2 Me(Leu)). ^{13}C NMR (DMSO-d_6, 150 MHz): 174.6 (s, CO(Aib^2)); 174.3 (s, CO(Aib^4)); 171.7 (s, CO(Pro)); 171.1 (s, CO(Leu)); 171.0 (s, CO(Aib^5)); 168.4 (s, CO(Gly)); 168.0 (s, CO(Val)); 79.9 (s, Me_3C); 60.8 (d, CH(α)(Pro)); 57.5 (d, CH(α)(Val)); 56.13 (s, C(α)(Aib^4)); 56.09 (s, C(α)(Aib^2)); 56.0 (s, C(α)(Aib^5)); 50.9 (d, CH(α)(Leu)); 47.2 (t, CH_2(δ)(Pro)); 43.8 (t, CH_2(Gly)); 39.3 (t, CH_2(β)(Leu)); 29.5 (d, CH(β)(Val)); 28.4 (t, CH_2(β)(Pro)); 27.5 (q, Me_3C); 25.8 (q, 1 Me of 2 Me(Aib^4)); 25.6 (q, 1 Me of 2 Me(Aib^2)); 25.2 (q, 1 Me of 2 Me(Aib^5)); 25.0 (t, CH_2(γ)(Pro)); 24.5 (q, 1 Me of 2 Me(Aib^4)); 24.2 (d, CH(γ)(Leu)); 24.0 (q, 1 Me of 2 Me(Aib^5)); 23.7 (q, 1 Me of 2 Me(Aib^2)); 22.7, 21.2 (2q, 2 Me(Leu)); 18.4, 17.5 (2q, 2 Me(Val)). ESI-MS: 718 (17, $[M+Na]^+$), 696 (100, $[M+H]^+$). HPLC/MS: t_R 11.5 min, m/z 696 (100, $[M+H]^+$), 640 (27, $[M-{}^t\text{Bu}]^+$), 412 (24, $[M-(\text{Pro-Leu-O}^t\text{Bu})]^+$), 339 (16).

4.3.5. H-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-O^{t}Bu (21). Hydroxymethyl-photolinker AM resin (100 mg, 0.075 mmol) was treated as described in GP 1, 2, 5 (overnight), 2, 5 (90 min), 2, 3, 6, 5 (90 min), and 7 to yield **21** (21.7 mg, 34%) as a colorless powder after prep. HPLC purification and lyophilization. IR (KBr): 3427s, 3301s, 3066m, 2979m, 2961m, 2938m, 2875m, 1673vs, 1535s, 1470m, 1453m, 1424m, 1412m, 1369m, 1247m, 1203vs, 1182vs, 1138vs, 837w, 800w, 742w, 722m, 700w. ^{1}H NMR (DMSO-d_6, 600 MHz): 8.39–8.37 (m, NH(Asn)); 8.17–8.13 (m, NH(Leu), NH_3(Aib^1), NH(Aib^4)); 7.99 (d, J=8.0 Hz, NH(Ser)); 7.42–7.41 (m, 1 H of $CONH_2$(Asn)); 7.36–7.32, 7.29–7.27 (2m, 5 arom. H); 6.98 (s, 1 H of $CONH_2$(Asn)); 4.70 (ddd, J=7.5, 7.5, 7.5 Hz, CH(α)(Asn)); 4.49, 4.53 (AB, J=12.1 Hz, OCH_2Ph(Ser)); 4.42–4.40 (m, CH(α)(Pro)); 4.36–4.33 (m, CH(α)(Ser)); 4.29–4.25 (m, CH(α)(Leu)); 3.72 (dd, J=9.7, 5.8 Hz, 1 H of CH_2(β)(Ser)); 3.65 (dd, J=9.7, 4.4 Hz, 1 H of CH_2(β)(Ser)); 3.46–3.44, 3.42–3.38 (2m, CH_2(δ)(Pro)); 2.64–2.60, 2.43–2.39 (2m,

CH_2(β)(Asn)); 1.90–1.87, 1.77–1.72 (2m, CH_2(β)(Pro), CH_2(γ)(Pro)); 1.60–1.55 (m, CH(γ)(Leu)); 1.49–1.43 (m, CH_2(β)(Leu)); 1.45, 1.43 (2s, 2 Me(Aib[1])); 1.37 (s, Me_3C); 1.34, 1.30 (2s, 2 Me(Aib[4])); 0.86, 0.80 (2d, J=6.6 Hz, 2 Me(Leu)). ^{13}C NMR (DMSO-d_6, 150 MHz): 171.9 (s, CO(Pro)); 171.2 (s, CO(Leu), $CONH_2$(Asn)); 171.1 (s, CO(Aib[1])); 170.8 (s, CO(Aib[4])); 170.3 (s, CO(Asn)); 169.0 (s, CO(Ser)); 137.9 (s, arom. C); 128.1, 127.5, 127.4 (3d, 5 arom. CH); 80.5 (s, Me_3C); 72.1 (t, OCH_2Ph(Ser)); 69.3 (t, CH_2(β)(Ser)); 60.5 (d, CH(α)(Pro)); 56.2 (s, C(α)(Aib[1])); 55.6 (s, C(α)(Aib[4])); 52.8 (d, CH(α)(Ser)); 50.8 (d, CH(α)(Leu)); 49.8 (d, CH(α)(Asn)); 47.3 (t, CH_2(δ)(Pro)); 40.3 (t, CH_2(β)(Leu)); 36.8 (t, CH_2(β)(Asn)); 28.0 (t, CH_2(β)(Pro)); 27.5 (q, Me_3C); 25.0 (t, CH_2(γ)(Pro)); 24.9, 24.7 (2q, 2 Me(Aib[4])); 24.1 (d, CH(γ)(Leu)); 23.3, 23.1 (2q, 2 Me(Aib[1])); 22.9, 21.3 (2q, 2 Me(Leu)). ESI-MS: 768 (100, $[M+Na]^+$), 746 (48, $[M+H]^+$), 712 (28, $[M-{}^t\text{Bu}+Na]^+$), 622 (17). HPLC/MS: t_R 12.4 min, m/z 746 (100, $[M+H]^+$), 398 (34, [M−(Pro-Ser(OBn)-^{t}Bu)]$^+$).

4.3.6. H-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu (22). Hydroxymethyl-photolinker AM resin (100 mg, 0.075 mmol) was treated as described in GP 1–3, 6, 3–5 (overnight), 2, 3, 6, 5 (2 h), and 7 to yield **22** (24.8 mg, 34%) as a colorless powder after prep. HPLC purification and lyophilization. IR (KBr): 3440vs, 3330sh, 3058m, 2961s, 2874m, 1724sh, 1653vs, 1627vs, 1536vs, 1471s, 1451m, 1440m, 1416s, 1385m, 1368m, 1344w, 1249m, 1241m, 1203vs, 1178vs, 1146s, 839w, 833w, 800w, 722w. ^{1}H NMR (DMSO-d_6, 600 MHz): 9.00 (s, NH(Aib[2])); 8.13 (br s, NH_3(Val)); 7.77 (d, J=7.8 Hz, NH(Leu[8])); 7.72 (s, NH(Aib[4])); 7.69 (s, NH(Aib[6])); 7.38 (d, J=8.5 Hz, NH(Leu[5])); 4.37–4.35 (m, CH(α)(Pro[7])); 4.17–4.12 (m, CH(α)(Leu[5]), CH(α)(Pro[3])); 4.04–4.00 (m, CH(α)(Leu[8])); 3.76–3.73 (m, CH(α)(Val)); 3.60–3.55 (m, 3 H of 2 CH_2(δ)(Pro)); 3.40–3.36 (m, 1 H of 2 CH_2(δ)(Pro)); 2.21–2.18 (m, CH(β)(Val)); 2.12–2.08 (m, 1 H of 2 CH_2(β)(Pro)); 2.00–1.95 (m, 1 H of 2 CH_2(β)(Pro), 1 H of 2 CH_2(γ)(Pro)); 1.89–1.85 (m, 1 H of 2 CH_2(γ)(Pro)); 1.74–1.68 (m, 2 H of 2 CH_2(β)(Pro), 2 H of 2 CH_2(γ)(Pro)); 1.66–1.57 (m, 3 H of 2 CH_2(β)(Leu), 2 CH(γ)(Leu)); 1.48–1.44 (m, 1 H of 2 CH_2(β)(Leu)); 1.42, 1.40 (2s, 2 Me of 6 Me(Aib)); 1.38 (s, Me_3C, 1 Me of 6 Me(Aib)); 1.36, 1.35 (2s, 3 Me of 6 Me(Aib)); 1.00, 0.94 (2d, J=7.0 Hz, 2 Me(Val)); 0.91, 0.87, 0.82, 0.78 (4d, J=6.2 Hz, 4 Me(Leu)). ^{13}C NMR (DMSO-d_6, 150 MHz): 173.9 (s, CO(Aib[4])); 172.6 (s, CO(Pro[3])); 172.2 (s, CO(Aib[2])); 171.7 (s, CO(Leu[5]), CO(Pro[7])); 171.3 (s, CO(Leu[8])); 170.8 (s, CO(Aib[6])); 167.5 (s, CO(Val)); 79.9 (s, Me_3C); 63.2 (d, CH(α)(Pro[3])); 60.8 (d, CH(α)(Pro[7])); 56.9 (d, CH(α)(Val)); 56.2 (s, C(α)(Aib[4])); 56.1 (s, C(α)(Aib[2])); 55.6 (s, C(α)(Aib[6])); 51.1 (s, 2 CH(α)(Leu)); 48.0, 47.5 (2t, 2 CH_2(δ)(Pro)); 39.6, 39.2 (2t, 2 CH_2(β)(Leu)); 29.6 (d, CH(β)(Val)); 28.3, 27.6 (2t, 2 CH_2(β)Pro); 27.5 (q, Me_3C); 26.4 (q, 1 Me of 6 Me(Aib)); 25.5 (t, 1 CH_2 of 2 CH_2(γ)(Pro)); 25.4 (q, 1 Me of 6 Me(Aib)); 25.1 (t, 1 CH_2 of 2 CH_2(γ)(Pro)); 24.6, 24.32, 24.29 (3q, 3 Me of 6 Me(Aib)); 24.27, 24.2 (2d, 2 CH(γ)(Leu)); 23.9 (q, 1 Me of 6 Me(Aib)); 23.0, 22.9, 21.2, 20.6 (4q, 4 Me(Leu)); 18.6, 16.7 (2q, 2 Me(Val)). ESI-MS: 871 (19, $[M+Na]^+$), 849 (100, $[M+H]^+$). HPLC/MS: t_R 13.9 min, m/z 849 (100, $[M+H]^+$), 565 (93, [M−(Pro-Leu-O^{t}Bu)]$^+$).

4.3.7. Z-Ala-Aib-Pro-Phe-OH (23). Z-ONSu (4.3 mg, 17.3 μmol) and DIPEA (8.1 μl, 47.3 μmol) were added to a solution of **18** (9.3 mg, 15.8 μmol) in dioxane (2.5 ml) at rt, and the solution was stirred at rt for 3 h. The mixture was concentrated, and the crude product was purified by prep. HPLC. After lyophilization, Z-Ala-Aib-Pro-Phe-O^{t}Bu (HPLC/MS: t_R 16.5 min, m/z 631 (100, $[M+Na]^+$)) (8.5 mg, 89%) was obtained as a colorless powder, which was dissolved in CH_2Cl_2/TFA (1:1, 2 ml) and TIPS (100 μl). The solution was stirred at rt for 1 h, then it was concentrated under reduced pressure, and the crude product was purified by prep. HPLC. The purified product was lyophilized and yielded **23** (7.5 mg, 97%) as a colorless powder. IR (KBr): 3412vs, 3292vs, 3063m, 3032m, 2984s, 2940m, 2876m, 1722vs, 1650vs, 1535vs, 1499s, 1469m, 1454s, 1411s, 1380m, 1366m, 1340m, 1328m, 1317m, 1282m, 1243vs, 1215s, 1203s, 1177s, 1116m, 1072m, 1040m, 1028m, 1002w, 977w, 911w, 824w, 743m, 700s. ^{1}H NMR (DMSO-d_6, 600 MHz): ca. 13.2–11.2 (br s, COOH); 8.20 (s, NH(Aib)); 7.77 (d, J=8.0 Hz, NH(Phe)); 7.44 (d, J=7.6 Hz, NH(Ala)); 7.38–7.18 (m, 10 arom. H); 5.01, 5.06 (AB, J=12.6 Hz, CH_2(carbamate)); 4.37 (ddd, J=8.4, 8.4, 5.3 Hz, CH(α)(Phe)); 4.29–4.27 (m, CH(α)(Pro)); 4.10 (dq, J=7.2, 7.2 Hz, CH(α)(Ala)); 3.54–3.50, 3.35–3.31 (2m, CH_2(δ)(Pro)); 3.05 (dd, J=13.9, 5.1 Hz, 1 H of CH_2(Phe)); 2.93 (dd, J=13.9, 9.2 Hz, 1 H of CH_2(Phe)); 1.79–1.73 (m, 1 H of CH_2(β)(Pro)); 1.61–1.58 (m, CH_2(γ)(Pro)); 1.51–1.48 (m, 1 H of CH_2(β)(Pro)); 1.30, 1.29 (2s, 2 Me(Aib)); 1.21 (d, J=7.1 Hz, Me(Ala)). ^{13}C NMR (DMSO-d_6, 150 MHz): 172.6 (s, CO(Phe)); 171.9 (s, CO(Ala)); 171.7 (s, CO(Pro)); 170.9 (s, CO(Aib)); 155.6 (s, CO(carbamate)); 137.6, 137.0 (2s, 2 arom. C); 129.1, 128.2, 128.0, 127.7, 127.6, 126.2 (6d, 10 arom. CH); 65.2 (t, CH_2(carbamate)); 60.9 (d, CH(α)(Pro)); 55.5 (s, C(α)(Aib)); 53.2 (d, CH(α)(Phe)); 49.9 (d, CH(α)(Ala)); 47.2 (t, CH_2(δ)(Pro)); 36.6 (t, CH_2(Phe)); 27.8 (t, CH_2(β)(Pro)); 25.0 (q, 1 Me of 2 Me(Aib)); 24.9 (t, CH_2(γ)(Pro)); 24.6 (q, 1 Me of 2 Me(Aib)); 17.9 (q, Me(Ala)). ESI-MS: 591 (67, $[M+K]^+$), 575 (100, $[M+Na]^+$), 553 (44, $[M+H]^+$), 263 (96, [M−(Pro-Phe)−CO]$^+$, [Pro-Phe]$^+$). HPLC/MS: t_R 14.0 min, m/z 591 (51, $[M+K]^+$), 263 (100, [M−(Pro-Phe)−CO]$^+$, [Pro-Phe]$^+$).

4.3.8. Z-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-OH (24). Z-ONSu (6.0 mg, 24.1 μmol) and DIPEA (11.6 μl, 67.8 μmol) were added to a solution of **21** (19.4 mg, 22.6 μmol) in MeCN (5 ml) at rt, and the solution was stirred at rt for 6 h. Additional Z-ONSu (0.6 mg, 2.3 μmol) and DIPEA (3.9 μl, 22.6 μmol) were added, and the solution was stirred at rt for further 2 h. Then, the mixture was concentrated, and the crude product was purified by prep. HPLC. After lyophilization, Z-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-O^{t}Bu (HPLC/MS: t_R 16.6 min, m/z 880 (62, $[M+H]^+$)) (13.8 mg, 70%; additionally, **21** (3.8 mg, 20%) was isolated) was obtained as a colorless powder, which was dissolved in CH_2Cl_2/TFA (1:1, 2 ml), H_2O (1 drop), and TIPS (100 μl). The solution was stirred at rt for 45 min, then it was concentrated under reduced pressure, and the crude product was purified by prep. HPLC. The purified product was lyophilized and yielded **24** (8.9 mg, 67%) as a colorless powder. IR (KBr): 3418sh, 3308vs, 3063m, 3033m, 2957m, 2872m, 1660vs, 1532vs, 1469s, 1454s, 1412s, 1387m, 1366m, 1267s, 1203s, 1176s, 1094s, 1078m, 1028w, 740m, 698m.

^{1}H NMR (DMSO-d_6, 600 MHz): ca. 13.2–11.3 (br s, COOH); 8.49 (d, J=6.0 Hz, NH(Asn)); 8.06 (s, NH(Aib^1)); 7.96 (d, J=7.9 Hz, NH(Ser)); 7.74 (d, J=8.5 Hz, NH(Leu)); 7.49 (s, 1 H of $CONH_2$(Asn)); 7.48 (s, NH(Aib^4)); 7.40–7.26 (m, 10 arom. H); 7.04 (s, 1 H of $CONH_2$(Asn)); 5.08, 5.00 (AB, J=12.4 Hz, CH_2(carbamate)); 4.51 (s, OCH_2Ph(Ser)); 4.43–4.38 (m, CH(α)(Ser), CH(α)(Pro)); 4.32–4.29 (m, CH(α)(Asn)); 4.25–4.21 (m, CH(α)(Leu)); 3.75 (dd, J=9.8, 5.9 Hz, 1 H of CH_2(β)(Ser)); 3.68 (dd, J=9.8, 4.2 Hz, 1 H of CH_2(β)(Ser)); 3.45–3.42 (m, CH_2(δ)(Pro)); 2.67–2.63, 2.57–2.54 (2m, CH_2(β)(Asn)); 1.90–1.86, 1.75–1.56 (2m, CH_2(β)(Pro), CH_2(γ)(Pro), CH_2(β)(Leu), CH(γ)(Leu)); 1.36 (s, 1 Me of 2 Me(Aib^1)); 1.35, 1.332 (2s, 2 Me(Aib^4)); 1.325 (s, 1 Me of 2 Me(Aib^1)); 0.81, 0.74 (2d, J=6.3 Hz, 2 Me(Leu)). ^{13}C NMR (DMSO-d_6, 150 MHz): 175.3 (s, CO(Aib^1)); 172.1 (s, $CONH_2$(Asn)); 172.0 (s, CO(Pro)); 171.4 (s, CO(Ser)); 171.2 (s, CO(Leu)); 170.9 (s, CO(Asn)); 170.8 (s, CO(Aib^4)); 156.2 (s, CO(carbamate)); 138.1 (s, arom. C(Bn)); 136.4 (s, arom. C(Z)); 128.4, 128.2, 128.0, 127.7, 127.5, 127.4 (6d, 10 arom. CH); 72.2 (t, OCH_2Ph(Ser)); 69.4 (t, CH_2(β)(Ser)); 65.7 (t, CH_2(carbamate)); 60.7 (d, CH(α)(Pro)); 56.1 (s, C(α)(Aib^1)); 55.8 (s, C(α)(Aib^4)); 52.4 (d, CH(α)(Ser)); 51.6 (d, CH(α)(Asn)); 50.9 (d, CH(α)(Leu)); 47.5 (t, CH_2(δ)(Pro)); 39.4 (t, CH_2(β)(Leu)); 35.1 (t, CH_2(β)(Asn)); 27.9 (t, CH_2(β)(Pro)); 26.1 (q, 1 Me of 2 Me(Aib^1)); 25.1 (q, 1 Me of 2 Me(Aib^4)); 25.0 (t, CH_2(γ)(Pro)); 24.8 (q, 1 Me of 2 Me(Aib^4)); 24.0 (d, CH(γ)(Leu)); 23.9 (q, 1 Me of 2 Me(Aib^1)); 23.1, 20.7 (2q, 2 Me(Leu)). ESI-MS: 868 (45, [M−H+2Na]$^+$), 846 (100, [M+Na]$^+$). HPLC/MS: t_R 15.6 min, m/z 824 (11, [M+H]$^+$), 532 (100, [M−(Pro-Ser(OBn)-O^{t}Bu)]$^+$), 447 (40, [M−(Aib-Pro-Ser(OBn)-O^{t}Bu)]$^+$).

4.3.9. Z-Aib-Asn-Leu-Aib-Pro-Ser(OBn)-Val-Aib-Pro-Aib-Leu-Aib-Pro-Leu-O^{t}Bu (25). HOBt, TBTU, and DIPEA were taken from stock solutions. HOBt (1.7 mg, 11.1 μmol), TBTU (3.5 mg, 11.0 μmol), DIPEA (5.4 μl, 31.3 μmol), and **22** (10.6 mg, 11.0 μmol) were added to a solution of **24** (8.6 mg, 10.4 μmol) in CH_2Cl_2/DMF (1:1, 3 ml). The mixture was stirred at rt for 4 h, the solvent was evaporated under reduced pressure, and the crude product was purified by prep. HPLC. After lyophilization, **25** (12.5 mg, 73%) was obtained as a colorless powder. IR (KBr): 3435sh, 3306s, 2958m, 2872w, 1648vs, 1623vs, 1536vs, 1470s, 1454m, 1412s, 1385m, 1366m, 1269m, 1203m, 1172s, 1152m, 1094w, 1028w, 740w, 698w. ^{1}H NMR (DMSO-d_6, 600 MHz): 8.57 (d, J=5.5 Hz, NH(Asn)); 8.11 (s, 1 NH of 5 NH(Aib)); 7.90 (d, J=6.7 Hz, NH(Ser)); 7.82 (d, J=7.7 Hz, 1 NH of 3 NH(Leu)); 7.82, 7.79 (2s, 2 NH of 5 NH(Aib)); 7.74 (d, J=7.7 Hz, 1 NH of 3 NH(Leu)); 7.55, 7.54 (2s, 2 NH of 5 NH(Aib)); 7.53 (s, 1 H of CONH_2(Asn)); 7.39–7.30, 7.27–7.24 (2m, 10 arom. H, 1 NH of 3 NH(Leu), NH(Val)); 7.07 (s, 1 H of $CONH_2$(Asn)); 5.07, 5.00 (AB, J=12.3 Hz, CH_2(carbamate)); 4.55, 4.51 (AB, J=11.9 Hz, OCH_2Ph(Ser)); 4.38–4.36 (m, 1 H of 3 CH(α)(Pro)); 4.33–4.26 (m, CH(α)(Ser), CH(α)(Asn), 1 H of 3 CH(α)(Leu), 1 H of 3 CH(α)(Pro)); 4.19–4.14 (m, 1 H of 3 CH(α)(Leu), CH(α)(Val)); 4.08 (dd, J=8.4, 8.4 Hz, 1 H of 3 CH(α)(Pro)); 4.04–4.00 (m, 1 H of 3 CH(α)(Leu)); 3.86–3.83 (m, 1 H of CH_2(β)(Ser)); 3.77–3.68 (m, 1 H of CH_2(β)(Ser), 2 H of 3 CH_2(δ)(Pro)); 3.65–3.61, 3.52–3.48, 3.38–3.34, 3.30–3.25 (4m, 4 H of 3 CH_2(δ)(Pro)); 2.68–2.64, 2.60–2.56 (2m, CH_2(β)(Asn)); 2.24–2.19 (m, 2 H of 3 CH_2(β)(Pro)); 2.09 (dsept., J=6.8, 6.8 Hz, CH(β)(Val)); 2.01–1.98 (m, 1 H of 3 CH_2(β)(Pro)); 1.93–1.83 (m, 4 H of 3 CH_2(γ)(Pro)); 1.74–1.57 (m, 3 CH(γ)(Leu), 4 H of 3 CH_2(β)(Leu), 3 H of 3 CH_2(β)(Pro), 2 H of 3 CH_2(γ)(Pro)); 1.54–1.51 (m, 1 H of 3 CH_2(β)(Leu)); 1.48 (s, 1 Me of 10 Me(Aib)); ca. 1.44 (m, 1 H of 3 CH_2(β)(Leu)); 1.43, 1.42, 1.38, 1.37, 1.364, 1.358, 1.34 (7s, 9 Me of 10 Me(Aib), Me_3C); 0.91 (d, J=6.4 Hz, 1 Me of 6 Me(Leu)); 0.87, 0.83 (2d, J=6.8 Hz, 2 Me(Val)); 0.82 (d, J=6.4 Hz, 2 Me of 6 Me(Leu)); 0.749, 0.745 (2d, J=6.0 Hz, 2 Me of 6 Me(Leu)); 0.63 (d, J=6.2 Hz, 1 Me of 6 Me(Leu)). ^{13}C NMR (DMSO-d_6, 150 MHz): 175.7, 174.2, 173.0, 172.8, 172.74, 172.70, 172.6, 171.90, 171.86, 171.6, 171.3, 171.2, 171.1, 170.9, 169.6 (15s, 15 CO); 156.2 (s, CO(carbamate)); 137.9 (s, arom. C of CH_2Ph(Ser)); 136.2 (s, arom. C(Z)); 128.3, 128.1, 127.9, 127.6, 127.4, 127.2 (6d, 10 arom. CH); 79.8 (s, Me_3C); 72.0 (t, OCH_2Ph(Ser)); 68.6 (t, CH_2(β)(Ser)); 65.8 (t, CH_2(carbamate)); 63.9, 62.9, 60.8 (3d, 3 CH(α)(Pro)); 58.3 (d, CH(α)(Val)); 56.1, 56.0, 55.9, 55.8, 55.6 (5s, 5 C(α)(Aib)); 55.4, 51.9 (2d, CH(α)(Asn), CH(α)(Ser)); 51.1, 51.0 (2d, 3 CH(α)(Leu)); 48.3, 48.2, 47.6 (3t, 3 CH_2(δ)(Pro)); 39.5, 39.1, 39.1 (3t, 3 CH_2(β)(Leu)); 34.9 (t, CH_2(β)(Asn)); 29.6 (d, CH(β)(Val)); 28.4, 28.3, 28.1 (3t, 3 CH_2(β)(Pro)); 27.5 (q, Me_3C); 26.5, 26.0, 25.7 (3q, 3 Me of 10 Me(Aib)); 25.6 (t, 1 CH_2 of 3 CH_2(γ)(Pro)); 25.5 (q, 1 Me of 10 Me(Aib)); 25.4 (t, 1 CH_2 of 3 CH_2(γ)(Pro)); 25.3 (q, 1 Me of 10 Me(Aib)); 25.1 (t, 1 CH_2 of 3 CH_2(γ)(Pro)); 24.2 (q, d, 1 Me of 10 Me(Aib), 2 CH of 3 CH(γ)(Leu)); 23.81 (q, 1 Me of 10 Me(Aib)); 23.76 (d, 1 CH of 3 CH(γ)(Leu)); 23.3 (q, 2 Me of 10 Me(Aib)); 23.1 (q, 1 Me of 10 Me(Aib)); 23.0, 22.93, 22.89, 21.1, 20.3, 20.2 (6q, 6 Me(Leu)); 18.8, 17.9 (2q, 2 Me(Val)). ESI-MS: 1693 (17, [M+K]$^+$), 1677 (85, [M+Na]$^+$). HPLC/MS: t_R 18.1 min, m/z 1676 (2, [M+H]$^+$), 846 (100), 819 (35).

Acknowledgements

We thank Dr. Daniel Obrecht, Polyphor Ltd, Allschwil (Switzerland) for continuous advice and valuable discussions, as well as the analytical sections of our institute for 2D-NMR measurements and mass spectra. Financial support of the Swiss National Science Foundation and F. Hoffmann-La Roche AG, Basel is gratefully acknowledged.

References and notes

1. Heimgartner, H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 238–264.
2. Wipf, P. Ph.D. thesis, Universität Zürich, 1987.
3. Wipf, P.; Heimgartner, H. *Helv. Chim. Acta* **1988**, *71*, 140–154.
4. Wipf, P.; Heimgartner, H. *Helv. Chim. Acta* **1990**, *73*, 13–24.
5. Strässler, C.; Linden, A.; Heimgartner, H. *Helv. Chim. Acta* **1997**, *80*, 1528–1554.
6. Suter, G.; Stoykova, S. A.; Linden, A.; Heimgartner, H. *Helv. Chim. Acta* **2000**, *83*, 2961–2974.
7. Stamm, S.; Linden, A.; Heimgartner, H. *Helv. Chim. Acta* **2003**, *86*, 1371–1396.

8. Altherr, W.; Heimgartner, H. *Peptides 1990*; Giralt, E., Andreu, D., Eds.; ESCOM: Leiden, 1991; p 107.
9. Luykx, R.; Bucher, C. B.; Linden, A.; Heimgartner, H. *Helv. Chim. Acta* **1996**, *79*, 527–540.
10. Bucher, C. B.; Heimgartner, H. *Helv. Chim. Acta* **1996**, *79*, 1903–1915.
11. Pradeille, N.; Heimgartner, H. *J. Pept. Sci.* **2003**, *9*, 827–837.
12. Luykx, R.; Linden, A.; Heimgartner, H. *Helv. Chim. Acta* **2003**, *86*, 4093–4111.
13. Pradeille, N.; Zerbe, O.; Möhle, K.; Linden, A.; Heimgartner, H. *Chem. Biodivers.* **2005**, *2*, 1127–1152.
14. Lehmann, J.; Linden, A.; Heimgartner, H. *Tetrahedron* **1998**, *54*, 8721–8736.
15. Lehmann, J.; Heimgartner, H. *Helv. Chim. Acta* **1999**, *82*, 1899–1915.
16. Breitenmoser, R.; Linden, A.; Heimgartner, H. *Helv. Chim. Acta* **2002**, *85*, 990–1018.
17. Jeremic, T.; Linden, A.; Heimgartner, H. *Chem. Biodivers.* **2004**, *1*, 1730–1761.
18. Jeremic, T.; Linden, A.; Möhle, K.; Heimgartner, H. *Tetrahedron* **2005**, *61*, 1871–1883.
19. Villalgordo, J. M.; Heimgartner, H. *Helv. Chim. Acta* **1997**, *80*, 748–766.
20. Koch, K. N.; Linden, A.; Heimgartner, H. *Helv. Chim. Acta* **2000**, *83*, 233–257.
21. Koch, K. N.; Heimgartner, H. *Helv. Chim. Acta* **2000**, *83*, 1881–1900.
22. Iliev, B.; Linden, A.; Kunz, R.; Heimgartner, H. *Tetrahedron* **2006**, *62*, 1079–1094.
23. Köttgen, P.; Linden, A.; Heimgartner, H. *Helv. Chim. Acta* **2006**, *89*, 731–746.
24. Stamm, S.; Heimgartner, H. *Eur. J. Org. Chem.* **2004**, 3820–3827.
25. Stamm, S.; Linden, A.; Heimgartner, H. *Helv. Chim. Acta* **2006**, *89*, 1–15.
26. Brückner, H.; Kripp, T.; Kiess, M. *Peptides 1990*; Giralt, E., Andreu, D., Eds.; ESCOM: Leiden, 1991; p 387.
27. Auvin-Guette, C.; Rebuffat, S.; Prigent, Y.; Bodo, B. *J. Am. Chem. Soc.* **1992**, *114*, 2170–2174.
28. Das, M. K.; Raghothamma, S.; Balaram, P. *Biochemistry* **1986**, *25*, 7110–7117.
29. Stamm, S.; Heimgartner, H. *Helv. Chim. Acta*, in press.
30. Kunz, H.; Dombo, B. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 711–713.
31. Hiersemann, M. *Tetrahedron* **1999**, *55*, 2625–2638.
32. Kaljuste, K.; Undén, A. *Tetrahedron Lett.* **1996**, *37*, 3031–3034.
33. Nicolaou, K. C.; Petasis, N.; Seitz, S. *J. Chem. Soc., Chem. Commun.* **1981**, 1195–1196.
34. Holmes, C. P.; Jones, D. G. *J. Org. Chem.* **1995**, *60*, 2318–2319.
35. Holmes, C. P. *J. Org. Chem.* **1997**, *62*, 2370–2380.
36. For a review about photolabile protecting groups and linkers, see: Bochet, C. G. *J. Chem. Soc., Perkin Trans. 1* **2002**, 125–142.
37. Patchornik, A.; Amit, B.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 6333–6335.
38. Dessolin, M.; Guillerez, M.-G.; Thieriet, N.; Guibé, F.; Loffet, A. *Tetrahedron Lett.* **1995**, *36*, 5741–5744.
39. For solution phase syntheses of *Trichovirin I*, see: Brückner, H.; Koza, A. *Amino Acids* **2003**, *24*, 311–323, and Ref. 12.
40. Villalgordo, J. M.; Heimgartner, H. *Tetrahedron* **1993**, *49*, 7215–7222.
41. Villalgordo, J. M.; Heimgartner, H. *Helv. Chim. Acta* **1993**, *76*, 2830–2837.
42. Strazzolini, P.; Melloni, T.; Giumanini, A. G. *Tetrahedron* **2001**, *57*, 9033–9043.