N-Hydroxy and *N*-acyloxy peptides: synthesis and chemical modifications†

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Received 12th May 2006, Accepted 12th June 2006
First published as an Advance Article on the web 12th July 2006
DOI: 10.1039/b606677a

The preparation of a series of *N*-hydroxy peptides is described, along with their acylation on the oxygen of the pseudopeptide bond. Nineteen *N*-acyloxy peptides, first examples of this new class of pseudopeptides, were thus synthesized; they present a range of acyl groups, including *N*-protected amino acyl groups. Possibilities of elongation for these pseudopeptides were also investigated.

Introduction

We recently described an efficient method for the preparation of *N*-hydroxy peptides.[1] The hydroxamate groups in these pseudopeptides allow metal coordination, which can be used to preorganize peptide structures for molecular recognition by enzymes or receptors.[2] Moreover, the additional OH groups confers extra hydrogen bonding possibilities compared to native peptides, inducing unusual conformations[3,4] and/or allowing enhanced recognition towards biological receptors.[5]

Thus, tuning peptide bonds is a strategy that can be used to change not only the conformation of peptides, but also their sensitivity to enzymatic cleavage and/or their biological activity.[6] In connection with this, we considered that the oxygen of hydroxamates could serve to graft, through diverse O–X bonds, chains of various length and with different functionalities, in order to create a library of new pseudopeptides. *O*-Acylation of hydroxamates was first studied, to prepare *N*-acyloxy peptides (Fig. 1).

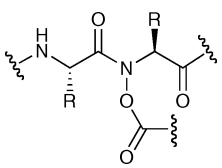


Fig. 1 *N*-Acyloxy peptide.

No example of this kind of modified peptides could be found in the literature. However, Mobashery *et al.* reported recently that an *O*-acylated amino acid–based hydroxamic acid (Fig. 2) was the prototype of a novel class of irreversible inhibitors for cathepsin B, an enzyme implicated in enhancing tumor invasiveness and metastasis.[7] The reactive Cys-29 thiolate of the enzyme is supposed to be acylated by this compound, and the resulting tetrahedral species would be stabilized by several hydrogen bonds within the enzyme pocket.

This single example suggests that *N*-acyloxy peptides also could interact favourably with biological targets such as proteases. It prompted us to investigate their preparation from *N*-hydroxy peptides.

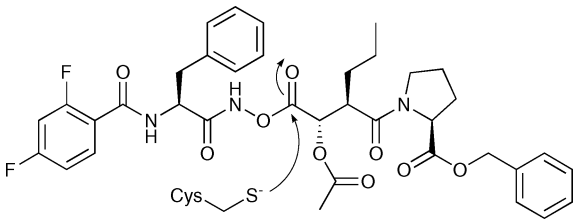
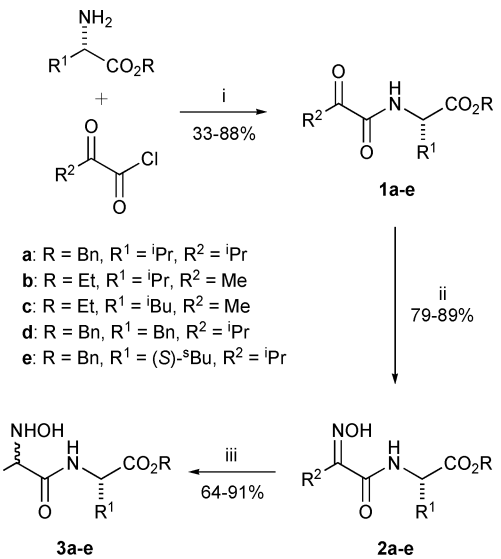


Fig. 2 Proposed mechanism for cathepsin B inhibition.[7]

Results and discussion

Synthesis of *N*-hydroxy peptides

N-Hydroxy peptides referred to in this study were prepared as described in Schemes 1 and 2, according to our previous work[1] (ethyl ester derivatives) or using a slightly modified procedure (benzyl ester derivatives). The first step involved the coupling of α-amino acids with α-keto acyl chlorides[8] to produce the



Scheme 1 Preparation of terminal *N*-hydroxy dipeptides. *Reagents and conditions*: i) *N*-methylmorpholine, CH_2Cl_2, 0 °C to rt. ii) $NH_2OH{\cdot}HCl$, $NaOAc{\cdot}3H_2O$ or NEt_3, THF or EtOH. iii) $BH_3{\cdot}NMe_3$, HCl, CH_2Cl_2 or EtOH.

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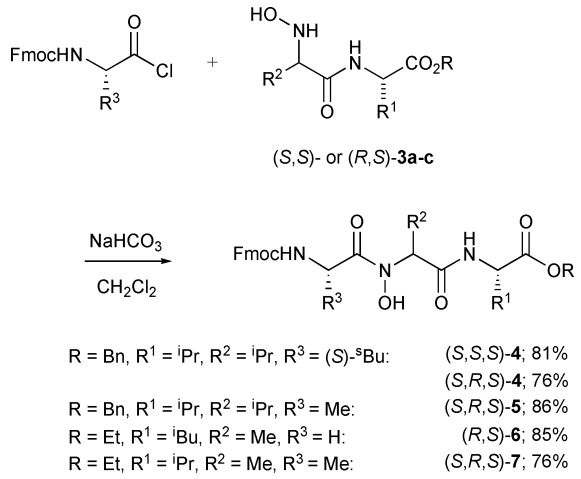
† Electronic supplementary information (ESI) available: Synthesis of α-keto-amides **1**, synthesis of α-hydroxyimino-amides **2** and assignment of configuration for compounds **3**. See DOI: 10.1039/b606677a



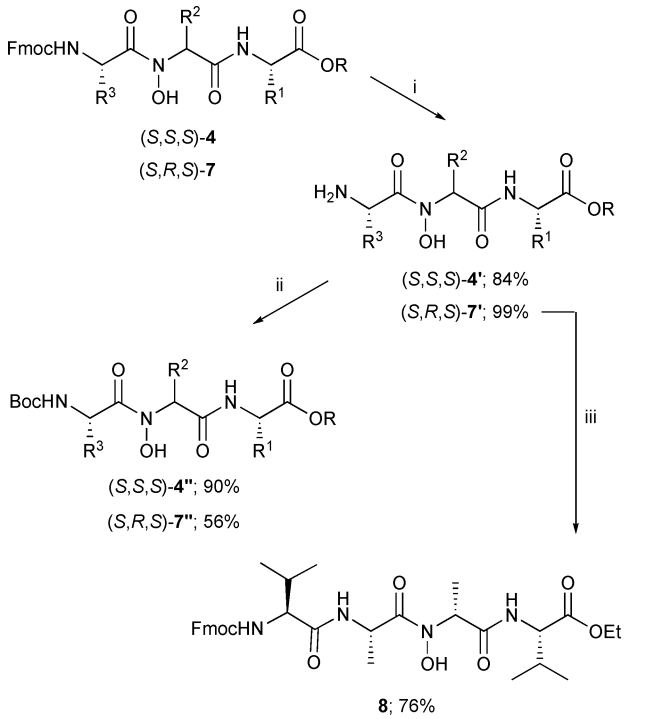
Scheme 2 Preparation of Fmoc-protected inner *N*-hydroxy tripeptides.

corresponding α-keto amides **1**. Condensation of **1** with hydroxylamine led to oximes **2**. Reduction of oximes to hydroxylamines is usually performed with sodium cyanoborohydride,[9] diborane[10] or borane adducts.[11] In our case, the trimethylamine–borane adduct[12] was found to be a reagent of choice. Compounds **3** were thus obtained in good yields as a 1 : 1 mixture of diastereomers which were separated by column chromatography. In order to assign the absolute configuration of each diastereomer, the N–O bond was reduced and the resulting dipeptide was compared with an authentic (*S*,*S*) sample prepared using standard peptide coupling methods (see ESI†).

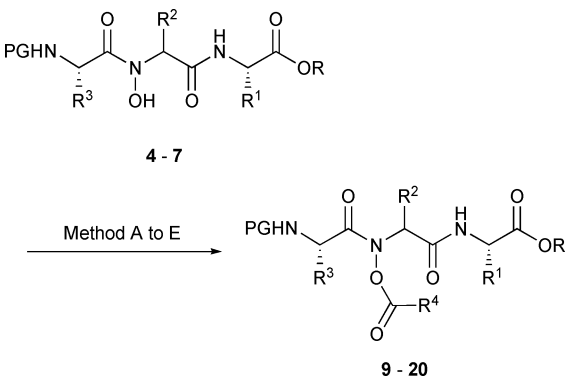
The selective *N*-acylation of **3** was achieved by reaction with Fmoc-protected amino acid chlorides[13] in the presence of sodium hydrogencarbonate (Scheme 2). The nature of the base was found to be important: for instance, the use of pyridine led to the *O*-acylated hydroxylamines as the major products.

The Fmoc-protecting groups of the obtained *N*-hydroxy tripeptides can be converted to Boc-protecting groups, as exemplified with (*S*,*S*,*S*)-**4** and (*S*,*R*,*S*)-**7** (Scheme 3). Elongation of *N*-hydroxy tripeptides *via* a classical peptide synthesis sequence was also accomplished, leading for instance to *N*-hydroxy tetrapeptide **8** (Scheme 3).

At this stage, we chose to investigate first the *O*-acylation of *N*-hydroxy tripeptides, in order to determine the scope and limitations of this acylation.



Scheme 3 *Reagents and conditions*: i) piperidine, THF. ii) Boc_2O, NEt_3, CH_2Cl_2, 0 °C to rt. iii) Fmoc–Val–OH, EDCI, HOBt, CH_2Cl_2, 0 °C to rt.



Scheme 4 *O*-Acylation of *N*-hydroxy peptides.

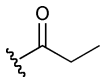
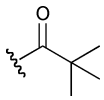
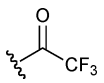
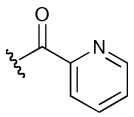
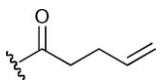
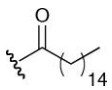
Synthesis of *N*-acyloxy peptides

Examples of *O*-acylation of hydroxamates found in the literature are limited to rather simple structures, for both the hydroxamate and the acyl group. For instance, *O*-benzoyl hydroxamic acids have been designed as precursors of amidyl radicals *via* homolytic cleavage of their N–O bond;[14,15] additionally, they were shown to undergo base-mediated rearrangement to give 2-acyloxyamides.[16]

We started our investigation with the acetylation of *N*-hydroxy peptide **4** (Scheme 4, $R^4 = CH_3$). Reaction of (*S*,*R*,*S*)-**4** with acetyl chloride (2 equiv.) in dichloromethane in the presence of pyridine (2 equiv.) led to *N*-acetyloxy peptide (*S*,*R*,*S*)-**9** in 71% yield. The use of acetic anhydride with pyridine as the solvent proved to be more effective, as shown in Table 1 (entry 1). The results were even better with the (*S*,*S*,*S*) diastereomer (entry 2) or the Boc-protected *N*-hydroxy tripeptide (*S*,*S*,*S*)-**4**″ (entry 3).

In order to diversify the nature of the acyl substituent (bulkiness, electronic effects), other commercially available anhydrides were reacted with *N*-hydroxy peptides **4** and **5** (Method A). *N*-Propanoyloxy and *N*-pivaloyloxy peptides **12–16** were obtained in generally good yields (entries 4 to 8). Reactions with propionic anhydride were completed in less than one day but, as could be expected, introduction of a bulky pivaloyl group required longer reaction times (up to 3 weeks). Even so, the reaction was only poorly efficient for one diastereomer of *N*-hydroxy peptide **4** (entry 8 *vs.* entry 7). Preparation of *N*-trifluoroacetyloxy peptide failed: purification of the crude material obtained after reaction of (*S*,*S*,*S*)-**4** with trifluoroacetic anhydride gave an inseparable mixture of the expected product and (*S*,*S*,*S*)-**4** (entry 9).

Table 1 Preparation of *N*-acyloxy peptides **9** to **20**

Entry	Tripeptide	Acyl group	Compound	Method[a] (Yield %)
1	(*S*,*R*,*S*)-**4**		**9**	A (86)
2	(*S*,*S*,*S*)-**4**	〃	**10**	A (94)
3	(*S*,*S*,*S*)-**4**″	〃	**11**	A (99)
4	(*S*,*R*,*S*)-**4**		**12**	A (99)
5	(*S*,*S*,*S*)-**4**	〃	**13**	A (97)
6	(*S*,*R*,*S*)-**5**	〃	**14**	A (80)
7	(*S*,*R*,*S*)-**4**		**15**	A (87)
8	(*S*,*S*,*S*)-**4**	〃	**16**	A (15)
9	(*S*,*S*,*S*)-**4**		—	A (inseparable mixture)
10	(*S*,*R*,*S*)-**5**		**17**	B (93)
				C (83)
11	(*S*,*R*,*S*)-**7**	〃	**18**	D (60)
12	(*S*,*R*,*S*)-**5**		—	B (starting material)
13	(*S*,*R*,*S*)-**5**		**19**	B (93)
14	(*S*,*R*,*S*)-**5**		**20**	C (90)

[a] Method A: anhydride (2 equiv.), pyridine. Method B: acid (4 equiv.), DCC (8 equiv.), CH_2Cl_2 then pyridine. Method C: acyl chloride (1.1 equiv.), DIEA (1.1 equiv.), CH_2Cl_2. Method D: acid (1.1 equiv.), BOP (1.1 equiv.), DIEA (2.2 equiv.), CH_2Cl_2.

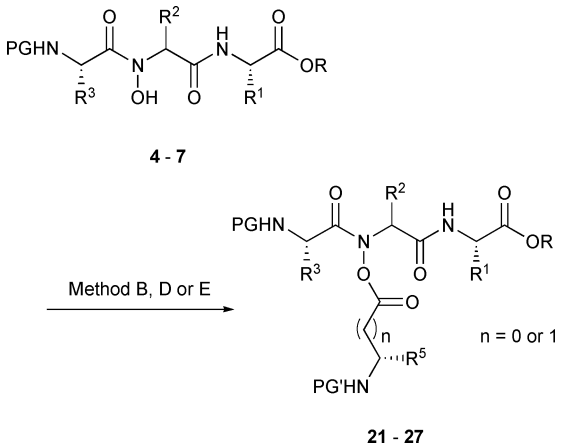
Other methods of acylation were investigated on a model reaction, with the purpose of widening the scope of acyl structures to be introduced. When direct coupling of benzoic acid and (*S*,*R*,*S*)-**5** was performed in the presence of the coupling agent DCC (1,3-dicyclohexylcarbodiimide), purification proved unsatisfying. The use of EDCI (1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide hydrochloride)[17] did not improve the yields of this transformation. However, when two equivalents of benzoic acid were first reacted with DCC in dichloromethane, and then the *N*-hydroxy peptide was added to the resultant anhydride, benzoylation of compound (*S*,*R*,*S*)-**5** was effective (Method B; entry 10). Benzoylation of (*S*,*R*,*S*)-**5** or (*S*,*R*,*S*)-**7** was also possible using benzoyl chloride (Method C; entry 10) or benzoic acid in the presence of the peptide coupling reagent BOP (benzotriazol-1-yloxytris-(dimethylamino)phosphonium hexafluorophosphate)[18] (Method D; entry 11). In the case of picolinic acid (Method B; entry 12), no coupling product was detected and only the *N*-hydroxy peptide (*S*,*R*,*S*)-**5** could be recovered, in 97% yield.

Finally, two hydrophobic groups were grafted on (*S*,*R*,*S*)-**5** in high yields, leading to compound **19** presenting an unsaturation (entry 13), and compound **20** exhibiting a palmitoyl side chain (entry 14).

Synthesis of *N*-aminoacyloxy peptides

Grafting amino acyl groups on the oxygen atom of *N*-hydroxy peptides would lead to diversified chemical structures (Scheme 5).

DCC-mediated coupling of Boc–Phe–OH with racemic 3-amino-1-hydroxypyrrolidin-2-one has been previously performed in order to resolve the racemate *via* two diastereomeric *N*,*O*-diacyl derivatives.[19] Unfortunately, the previously described acylation conditions turned out to be unfruitful when we tried to react Boc–Gly–OH with (*S*,*R*,*S*)-**5**: we failed to purify the obtained crude product. We then turned to the use of classical reagents for amino acid activation, such as the phosphonium reagent BOP and



Scheme 5 Preparation of *N*-aminoacyloxy peptides.

the aminium–uronium reagent HATU (*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate).[20]

Using these reagents (Table 2), a fourth amino acid residue could be grafted on pseudotripeptides (*R*,*S*)-**6** or (*S*,*R*,*S*)-**7**: Boc–Ala– (entries 1 and 2), Fmoc–Ala– (entry 3), Boc–Leu– (entry 4) and Boc-β-Ala– (entry 5). The corresponding *N*-aminoacyloxy peptides were obtained in good yields, HATU being generally the best coupling reagent.

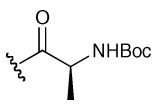
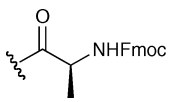
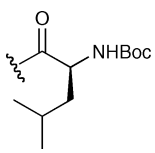
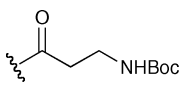
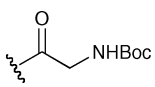
Surprisingly, BOP- or HATU-mediated coupling of (*S*,*R*,*S*)-**7** with Fmoc–Phe–OH or Boc–Gly–OH failed and only the starting material could be recovered. The same disappointing result was obtained for the reaction, carried out with BOP, of Fmoc–Gly–OH with the Fmoc-protected (*S*,*R*,*S*)-**7** or with the Boc-protected (*S*,*R*,*S*)-**7**″. On the other hand, compound **26** was isolated in 74% yield using the same conditions (entry 6).

Finally, as symmetrical anhydrides proved to be efficient for the synthesis of simple *N*-acyloxy peptides, we prepared the isolated anhydride $(Boc–Gly)_2O$[21] and reacted it successfully with *N*-hydroxy peptides (*S*,*S*,*S*)-**4** and (*S*,*R*,*S*)-**5** to give compounds **26** and **27** in high yields. The DCC-mediated formation of $(BocGly)_2O$ followed by filtration of the urea and direct use led to comparable results (entries 6 and 7).

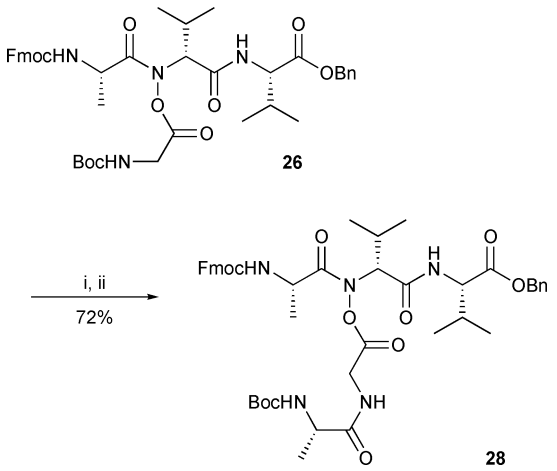
Chemical modifications of *N*-acyloxy peptides

Elongation of the side chain. When the acyl group grafted on the *N*-hydroxyl is a protected amino acid, this amino acid could in principle serve for elongating a second peptidic chain. Therefore, *N*-aminoacyloxy peptide **26** was treated with trifluoroacetic acid to remove the Boc group and the resulting salt was reacted with Boc–Ala–OH under standard coupling conditions (Scheme 6). Pentapeptidic compound **28** was thus isolated in 72% yield (two steps).

Table 2 Preparation of *N*-aminoacyloxy peptides **21** to **27**

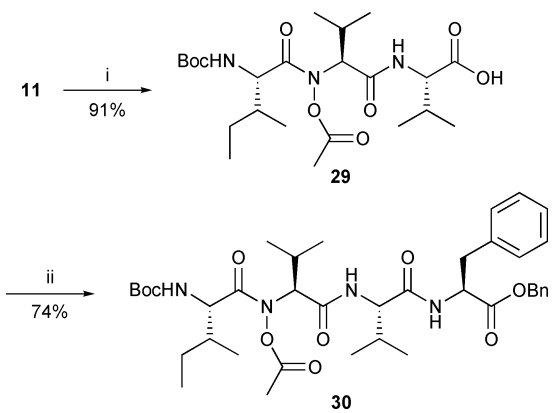
Entry	Tripeptide	Aminoacyl group	Compound	Method[a] (Yield %)
1	(*R*,*S*)-**6**		**21**	D (69) E (84)
2	(*S*,*R*,*S*)-**7**	″	**22**	D (86) E (56)
3	(*S*,*R*,*S*)-**7**		**23**	D (61) E (72)
4	(*S*,*R*,*S*)-**7**		**24**	D (67) E (77)
5	(*S*,*R*,*S*)-**7**		**25**	D (88)
6	(*S*,*R*,*S*)-**5**		**26**	B (88) D (74)
7	(*S*,*S*,*S*)-**4**	″	**27**	B (85)

[a] Method B: acid (4 equiv.), DCC (8 equiv.), CH_2Cl_2 then pyridine. Method D: acid (1.1 equiv.), BOP (1.1 equiv.), DIEA (2.2 equiv.), CH_2Cl_2. Method E: acid (1.1 equiv.), HATU (1.1 equiv.), DIEA (3.2 equiv.), DMF.



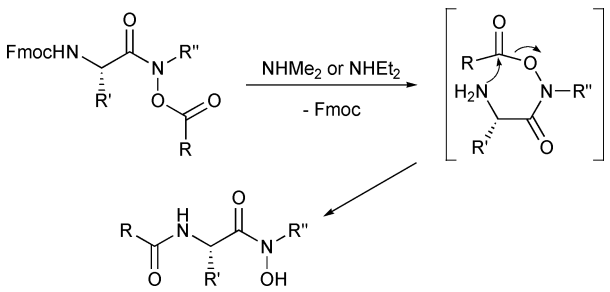
Scheme 6 Side chain elongation. *Reagents and conditions*: i) TFA. ii) Boc–Ala–OH, EDCI, HOBt, DIEA, CH_2Cl_2.

Elongation at C-terminal position. The possibility of synthesizing peptides presenting several *N*-acyloxy side chains of different nature was also considered. To cope with the issue of regioselectivity, the appropriate approach would be fragment condensation, involving short *N*-acyloxy peptides. Elongation at the C-terminal position is thus required and additives such as HOBt (1-hydroxybenzotriazole) have to be used together with DCC or EDCI, in order to avoid racemization.[22] This option was validated with the *N*-acetyloxy tripeptide **11** (Scheme 7). The N–O bond proved to be stable under the hydrogenolytic conditions used to remove the benzyl group, leading to the carboxylic acid **29**. Coupling with H–Phe–OBn gave the *N*-acyloxy tetrapeptide **30** in 74% yield.



Scheme 7 Elongation at C-terminal position. *Reagents and conditions*: i) H_2, Pd/C. ii) H–Phe–OBn·HCl, EDCI, HOBt, DIEA, CH_2Cl_2.

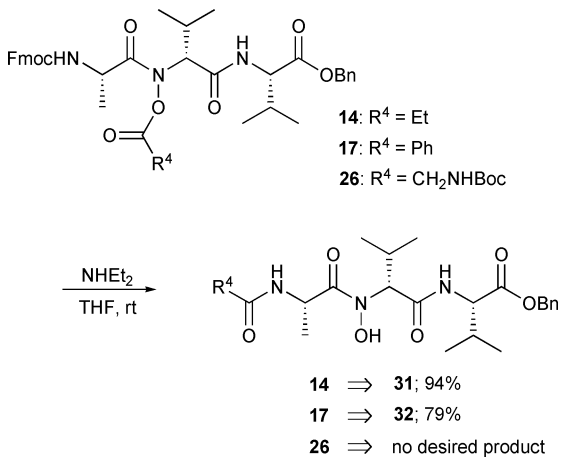
Elongation at N-terminal position and rearrangement. Finally, we investigated the elongation at the N-terminal position. In the case of Fmoc-protected *N*-acyloxy tripeptides, a *O*- to *N*-intramolecular acyl migration could be foreseen. Actually, working independently on *O*-acyl hydroxamic acids derived from Fmoc-protected amino acids, Braslau[23] and Phanstiel IV[24] simultaneously reported that the base-mediated cleavage of the Fmoc group resulted in a rearrangement through a six-membered transition state, leading to the formation of a new amide bond (Scheme 8).



Scheme 8 Rearrangement of *O*-acyl hydroxamic acids derived from amino acids.[22,23]

Recently, other *O*- to *N*-intramolecular acyl transfer reactions in peptides have been used to switch from soluble precursors to aggregative molecules, either for synthetic purposes[25] or for the study of conformational transitions relevant to degenerative diseases.[26] This exciting concept prompted us to investigate intramolecular acyl migration within our *N*-acyloxy peptides.

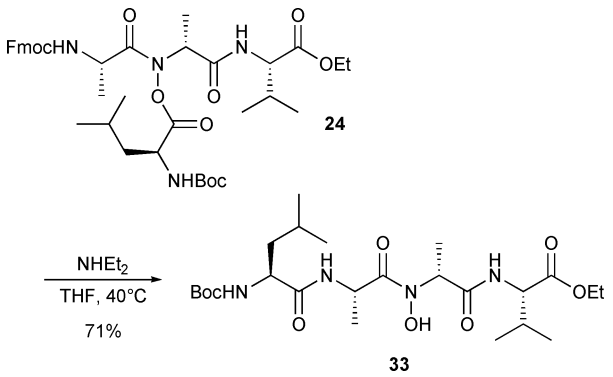
Compound **9** was treated with an excess of piperidine in order to remove the Fmoc group. However, even if the Fmoc group was indeed cleaved, piperidine reacted also on the carbonyl of the acetyl group and the obtained product was the *N*-hydroxy peptide H–Ile–Ψ[CO–N(OH)]–D-Val–Val–OBn (90% yield). On the other hand, when we used diethylamine (6 equiv.) to cleave the Fmoc group in compounds **14** and **17**, the expected rearrangement took place smoothly, to afford **31** and **32** in high yields (Scheme 9).



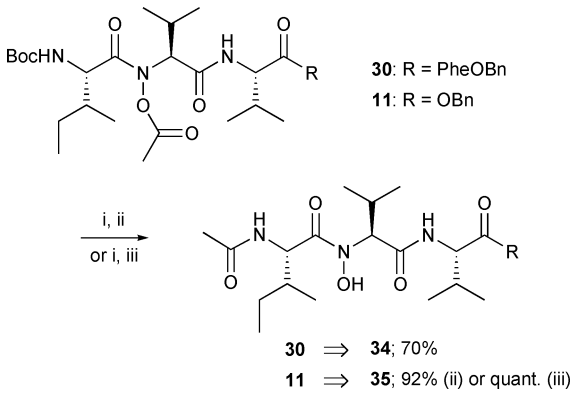
Scheme 9 Rearrangement of Fmoc-protected *N*-acyloxy peptides.

A similar migration of an aminoacyl group appeared even more interesting. When compound **26**, presenting a Boc–Gly-side chain, was treated with diethylamine (1.5 equiv.) the cleavage of the Fmoc group and that of the aminoacyl group were competitive (Scheme 9). A mixture of compound **5** (Fmoc-protected), its deprotected analog and Boc–Gly–NEt_2 was obtained. In the case of a more sterically hindered Boc–Leu-side chain (peptide **24**), no attack on the carbonyl group occurred and the rearranged product **33** was isolated in 71% yield (Scheme 10).

When a Boc-protected *N*-acyloxy peptide is the substrate, rearrangement can also be achieved (Scheme 11): treatment of compound **30** or **11** with trifluoroacetic acid followed by a basic treatment led to the formation in good yields of **34**



Scheme 10 Rearrangement-induced synthesis of *N*-hydroxy tetrapeptide.



Scheme 11 Rearrangement of Boc-protected *N*-acyloxy peptides. *Reagents and conditions*: i) TFA. ii) CH_2Cl_2–aq. $NaHCO_3$. iii) Boc–Ala–OH, EDCI, HOBt, (DIEA), CH_2Cl_2.

and **35** respectively. Moreover, when the intermediate TFA salt derived from **11** was reacted with Boc–Ala–OH in the presence of EDCI–HOBt, only the rearranged product **35** was obtained quantitatively, in the presence or absence of base.

Because of the rearrangement, a direct C → N elongation of *N*-acyloxy peptides with the *N*-acyloxy amino acid in the second position is not achievable. However, if the *N*-acyloxy amino acid is not in the second position, the six-membered transition state described in Scheme 8 cannot occur and elongation at the N-terminal position should be possible. In order to demonstrate this possibility, *N*-hydroxy tetrapeptide **33** was reacted with benzoyl anhydride, leading to **36** in quantitative yield. Cleavage of the Boc-group of **36** and coupling with Fmoc–Phe–OH allowed the formation of *N*-acyloxy pentapeptide **37** in 74% yield (Scheme 12).

Conclusion

A series of *N*-hydroxy peptides was synthesized and acyl groups were grafted on the oxygen of the hydroxamate under a variety of conditions. This allowed the introduction on the pseudopeptides of a range of acyl groups, including *N*-protected aminoacyl groups.

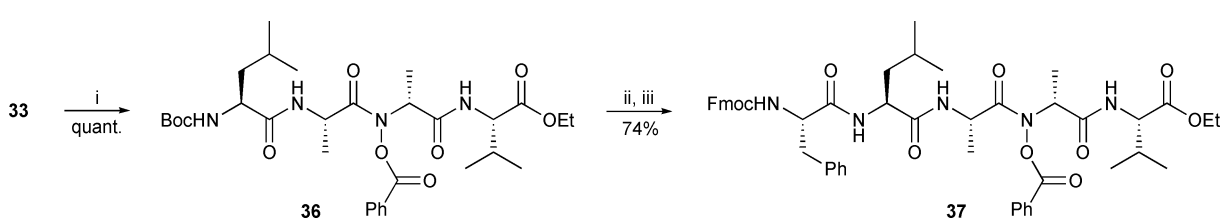
Elongation of the resulting *N*-acyloxy peptides is possible from the C-terminal amino acid, and from the aminoacyloxy side chain. Depending on the position of the *N*-acyloxy amino acid in the peptide, deprotection of the N-terminal nitrogen leads to a *O*- to *N*-intramolecular acyl migration or allows coupling with an additional amino acid.

The reactivity of other functionalized side chains is currently under study and results will be reported in due course.

Experimental (see also ESI†)

General procedures

All non-aqueous reactions were performed using oven-dried glassware under an atmosphere of argon. Standard inert atmosphere techniques were used in handling all air and moisture sensitive reagents. CH_2Cl_2 and EtOH were freshly distilled from CaH_2, and THF from sodium benzophenone ketyl. Unless otherwise stated, reagents were purchased from chemical companies and used without prior purification. Triethylamine and diethylamine were freshly distilled from CaH_2. Pyridine was distilled from CaH_2 and kept over KOH pellets. For chromatographic purification, reagent grade solvents were used as received. Reactions were monitored by thin layer chromatography (TLC) using aluminium-backed silica gel plates (Merck, Kieselgel 60 F_{254}). TLC spots were viewed under ultraviolet light and by heating the plate after treatment with a staining solution of $KMnO_4$ (300 mL water, 3 g $KMnO_4$, 20 g K_2CO_3, 0.3 g KOH), or ninhydrine (200 mL EtOH, 1 g ninhydrine), or TTC (for *N*-hydroxylamines; 100 mL EtOH, 0.5 g 2,3,5-triphenyl tetrazolium chloride). Product purification by flash chromatography was performed using Macherey Nagel Silica Gel 60 M (230–400 mesh). Melting points were determined with a Büchi B-540 apparatus and are given uncorrected. Optical rotations were measured on a Perkin Elmer 341 polarimeter; the corresponding concentration is reported in g 100 cm^{-3}. Infrared (IR) spectra were obtained either from neat films, from a thin film of a dichloromethane solution of the compound on sodium chloride discs, or from a dispersion of the compound in a KBr plate. IR spectra were recorded on a Nicolet Impact-400 FT-IR spectrometer and the data are reported as absorption maxima in cm^{-1}. ^1H NMR (200 or 300 MHz) and ^{13}C NMR (75 MHz) spectra were run on either a Bruker AC200 or Advance300 spectrometer, and obtained from $CDCl_3$ (δ_C 77.2 ppm; standard



Scheme 12 Elongation at the N-terminal position. *Reagents and conditions*: i) $(PhCO)_2O$, pyridine. ii) TFA. iii) Fmoc–Phe–OH, EDCI, HOBt, DIEA, CH_2Cl_2.

for ^{1}H spectra: tetramethylsilane δ_H 0.0 ppm). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quadruplet, h = heptuplet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, dq = doublet of quadruplet, br = broad; coupling constants J and $\delta_A - \delta_B$ (for AB spin system) are reported in Hz. Mass spectra (LRMS) were recorded on a Bruker Esquire 3000 plus (ESI) or a ThermoFinnigan PolarisQ EI/CI ion-trap spectrometer (DCI; ammonia–isobutane 62 : 38). Exact mass spectra (HRMS) were recorded at the "Service Central d'Analyses du CNRS", Vernaison, France, or at the "Centre Régional de Mesures Physiques de l'Ouest", Rennes, France. Elemental analyses were performed at the "Service Central d'Analyses du CNRS", Vernaison, France.

Synthesis of *N*-hydroxy-amino esters 3

(*S*,*S*)- and (*R*,*S*)–HN(OH)–Val$_1$–Val$_2$–OBn (3a). To a solution of **2a** (700 mg, 2.2 mmol) in CH_2Cl_2 (35 mL) at room temperature was added $BH_3{\cdot}NMe_3$ (320 mg, 4.00 mmol). HCl_g (prepared from H_2SO_4 and NH_4Cl) was bubbled through the mixture for 2 h. Na_2CO_3 (3 g) and MeOH (1 mL) were then added and the suspension was stirred overnight. Removal of the solvent under reduced pressure followed by purification by flash chromatography (silica gel, CH_2Cl_2–MeOH 100 : 0 to 98 : 2) of the residue allowed isolation as pale yellow oils, of, first, pure (*S*,*S*)-**3a** (166 mg, 0.51 mmol, 23%), second, a *circa* 1 : 1 mixture[27] of (*S*,*S*)- and (*R*,*S*)-**3a** (211 mg, 0.65 mmol, 30%), and third, (*R*,*S*)-**3a** (271 mg, 0.84 mmol, 38%). The latter could be crystallized into a white solid from CH_2Cl_2–cyclohexane.

*(*S,S*)-**3a***. R_f 0.25 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +22.5° (*c* 2.82 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.40–7.25 (5H, m, CH_{ar}), 7.04 (1H, d, *J* 9.4, NH), 5.14 (2H, ABq, *J* 12.2, $\delta_A - \delta_B$ 19.1, CH_2 –OBn), 4.71 (1H, dd, *J* 4.5 and 9.4, CH_α Val$_2$), 3.33 (1H, d, *J* 6.4, CH_α Val$_1$), 2.20–2.30 (1H, m, CH_{iPr} Val$_1$), 2.00–1.87 (1H, m, CH_{iPr} Val$_2$), 0.99 (3H, d, *J* 6.9, CH_3), 0.98 (3H, d, *J* 7.2, CH_3), 0.95 (3H, d, *J* 7.0, CH_3), 0.84 (3H, d, *J* 6.9, CH_3). δ_C (75 MHz; $CDCl_3$) 173.0, 172.8 (C=O), 135.3 (C_{ar}), 128.8, 128.7, 128.5 (CH_{ar}), 73.1 (CH_α Val$_1$), 67.5 (CH_2 –OBn), 56.8 (CH_α Val$_2$), 31.0 (CH_{iPr} Val$_2$), 29.2 (CH_{iPr} Val$_1$), 19.6, 19.4 (2 peaks), 17.5 (CH_3). IR: 3377, 2965, 1739, 1655, 1524, 1193, 752, 698. LRMS (DCI) *m*/*z*: 323.0 (M + H)$^+$, 339.8 (M + NH_4)$^+$. Anal.: found, C63.0; H8.3; N8.9. $C_{17}H_{26}N_2O_4$ requires C63.3; H8.1; N8.7%.

*(*R,S*)-**3a***. Mp: 84 °C. R_f 0.20 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +7.1° (*c* 3.21 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.37–7.32 (5H, m, CH_{ar}), 6.82 (1H, d, *J* 8.8, NH), 5.17 (2H, ABq, *J* 12.2, $\delta_A - \delta_B$ 26.8, CH_2 –OBn), 4.67 (1H, dd, *J* 4.7 and 8.8, CH_α Val$_2$), 3.31 (1H, d, *J* 6.4, CH_α Val$_1$), 2.30–2.16 (1H, m, CH_{iPr} Val$_1$), 2.06–1.92 (1H, m, CH_{iPr} Val$_2$), 0.99 (3H, d, *J* 6.9, CH_3), 0.96 (3H, d, *J* 6.3, CH_3), 0.94 (3H, d, *J* 6.8, CH_3), 0.84 (3H, d, *J* 6.9, CH_3). δ_C (75 MHz; $CDCl_3$) 172.7, 172.1 (C=O), 135.5 (C_{ar}), 128.8, 128.6 (2 peaks) (CH_{ar}), 72.4 (CH_α Val$_1$), 67.3 (CH_2 –OBn), 57.1 (CH_α Val$_2$), 31.4 (CH_{iPr} Val$_2$), 29.3 (CH_{iPr} Val$_1$), 19.8, 19.3, 19.0, 18.0 (CH_3). IR: 3362, 2965, 1717, 1675, 1535, 1281, 1006, 743. LRMS (DCI) *m*/*z*: 322.9 (M + H)$^+$, 339.7 (M + NH_4)$^+$. HRMS (ESI +) *m*/*z*: found, 345.1788; $C_{17}H_{26}N_2O_4Na$ requires 345.1790.

(*S*,*S*)- and (*R*,*S*)–HN(OH)–Ala–Val–OEt (3b). To a mixture of **2b** (1981 mg, 8.6 mmol) and $BH_3{\cdot}NMe_3$ (941 mg, 12.9 mmol) was added dropwise at 0 °C a 7 N ethanolic solution of HCl (137 mL) and the mixture was stirred at room temperature overnight. The solvent was then evaporated under reduced pressure and the colourless oil was taken up in CH_2Cl_2 (100 mL). Na_2CO_3 (8 g) was added and the suspension was stirred for 1 h, then filtered. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel, CH_2Cl_2–MeOH 99 : 1 to 93 : 7) allowed isolation of, first, pure (*S*,*S*)-**3b** (764 mg, 3.3 mmol, 38%), second, a mixture of (*S*,*S*)- and (*R*,*S*)-**3b** (678 mg, 2.9 mmol, 34%),[28] and third, pure (*R*,*S*)-**3b** (171 mg, 0.7 mmol, 9%), as colourless oils.

*(*S,S*)-**3b***. R_f 0.21 (CH_2Cl_2–MeOH 98 : 2). $[\alpha]_D^{25}$ −16.82 (*c* 1.76 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) 7.16 (1H, d, *J* 9.3, NH), 6.03 (1H, br s, NOH), 4.59 (1H, dd, *J* 4.9 and 9.3, CH_α Val), 4.32–4.09 (2H, m, CH_2 –OEt), 3.65 (1H, q, *J* 7.0, CH_α Ala), 2.32–2.10 (1H, m, CH_{iPr} Val), 1.29 (3H, t, *J* 7.2, CH_3 –OEt), 1.26 (3H, d, *J* 7.2, CH_3 Ala), 0.96 (3H, d, *J* 6.8, CH_3 Val), 0.92 (3H, d, *J* 6.8, CH_3 Val). δ_C (75 MHz, $CDCl_3$) 173.8, 172.4 (C=O), 61.6, 61.4 (CH_2 –OEt, CH_α Ala), 56.5 (CH_α Val), 31.0 (CH_{iPr} Val), 18.8, 17.5 (CH_3 Val), 15.3 (CH_3 Ala), 14.0 (CH_3 –OEt). IR: 3284, 2962, 1651, 1543, 1202, 1018. LRMS (DCI) *m*/*z*: 233.1 (M + H)$^+$.

*(*R,S*)-**3b***. R_f 0.20 (CH_2Cl_2–MeOH 98 : 2). $[\alpha]_D^{25}$ +26.05 (*c* 0.76 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.16 (1H, d, *J* 9.1, NH), 5.90 (1H, br s, NOH), 4.58 (1H, dd, *J* 5.1 and 9.1, CH_α Val), 4.27–4.13 (2H, m, CH_2 –OEt), 3.67 (1H, q, *J* 6.9, CH_α Ala), 2.26–2.15 (1H, m, CH_{iPr} Val), 1.28 (3H, t, *J* 7.2, CH_3 –OEt), 1.28 (3H, d, *J* 6.9, CH_3 Ala), 0.96 (3H, d, *J* 6.8, CH_3 Val), 0.93 (3H, d, *J* 6.8, CH_3 Val). δ_C (75 MHz, $CDCl_3$) 173.7, 172.2 (C=O), 61.2 (CH_2 –OEt, CH_α Ala), 56.8 (CH_α Val), 31.1 (CH_{iPr} Val), 18.9, 17.7 (CH_3 Val), 15.2 (CH_3 Ala), 14.1 (CH_3 –OEt). IR: 3329, 2962, 2917, 1730, 1651, 1516, 1022. HRMS (ESI +) *m*/*z*: found, 255.1321; $C_{10}H_{20}N_2O_4Na$ requires 255.1321.

(*S*,*S*)- and (*R*,*S*)–HN(OH)–Ala–Leu–OEt (3c). The title compounds were prepared as described for **3b** starting from **2c** (2443 mg, 10.0 mmol). Purification by flash chromatography (silica gel, CH_2Cl_2–MeOH 99 : 1 to 93 : 7) allowed isolation of, first, pure (*S*,*S*)-**3c** (641 mg, 2.6 mmol, 26%), second, a mixture of (*S*,*S*)- and (*R*,*S*)-**3c** (833 mg, 3.4 mmol, 34%),[28] and third, pure (*R*,*S*)-**3c** (491 mg, 2.0 mmol, 20%), as colourless oils.

*(*S,S*)-**3c***. R_f 0.20 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −20.4 (*c* 1.39 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) 6.95 (1H, d, *J* 9.6, NH), 4.75–4.65 (1H, m, CH_α Leu), 4.20 (2H, q, *J* 7.1, CH_2 –OEt), 3.65 (1H, q, *J* 7.1, CH_α Ala), 1.72–1.51 (3H, m, CH_2 Leu, CH_{iBu} Leu), 1.29 (3H, t, *J* 7.1, CH_3 –OEt), 1.24 (3H, d, *J* 7.1, CH_3 Ala), 0.96 (3H, d, *J* 5.8, CH_3 Leu), 0.95 (3H, d, *J* 6.2, CH_3 Leu). IR: 3288, 2962, 1741, 1673, 1516, 1190, 1029.

*(*R,S*)-**3c***. R_f 0.15 (CH_2Cl_2–MeOH 95 : 5). δ_H (200 MHz; $CDCl_3$) 6.91 (1H, d, *J* 8.9, NH), 4.72–4.60 (1H, m, CH_α Leu), 4.19 (2H, q, *J* 7.0, CH_2 –OEt), 3.64 (1H, q, *J* 6.9, CH_α Ala), 1.75–1.52 (3H, m, CH_2 Leu, CH_{iBu} Leu), 1.30 (3H, d, *J* 6.9, CH_3 Ala), 1.28 (3H, t, *J* 7.0, CH_3 –OEt), 0.96 (6H, d, *J* 5.2, CH_3 Leu). IR: 3288, 2962, 1741, 1673, 1516, 1190, 1029.

(*S*,*S*)- and (*R*,*S*)–HN(OH)–Val–Phe–OBn (3d). The title compounds were prepared as described for **3a** starting from **2d** (674 mg, 1.8 mmol). Purification by flash chromatography (silica gel, CH_2Cl_2–MeOH 99.75 : 0.25 to 96 : 4) allowed isolation of, first, pure (*S*,*S*)-**3d** (white solid, 163 mg, 0.4 mmol, 24%), second, a *circa* 1 : 1 mixture of (*S*,*S*)- and (*R*,*S*)-**3d** (189 mg, 0.5 mmol, 28%), and third, (*R*,*S*)-**3d** (350 mg) with an impurity. The last

fraction was recrystallized in CH_2Cl_2–cyclohexane to give 80 mg (0.2 mmol, 12%) of (*R*,*S*)-**3d** as a white solid.

*(*S,S*)-**3d**.* Mp: 103.5 °C. R_f 0.32 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −0.3° (*c* 1.00 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) 7.40–7.03 (10H, m, CH_{ar}), 6.85 (1H, d, *J* 8.9, NH), 5.16 (2H, ABq, *J* 12.2, $\delta_A - \delta_B$ 18.3, CH_2 –OBn), 5.06 (1H, m, CH_α Phe), 3.26 (1H, d, *J* 6.8, CH_α Val), 3.13 (2H, ABX, ^3J 6.2, ^2J 14.1, $\delta_A - \delta_B$ 14.6, CH_2 Phe), 1.90–1.70 (1H, m, CH_{iPr} Val), 0.88 (3H, d, *J* 6.8, CH_3 Val), 0.87 (3H, d, *J* 6.8, CH_3 Val). δ_C (75 MHz; $CDCl_3$) 172.4, 172.3 (C=O), 135.9, 135.1 (C_{ar}), 129.7 129.5, 129.4, 129.2, 128.8, 128.7 (2 peaks), 128.6, 127.2, 127.1 (CH_{ar}), 72.8 (CH_α Val), 67.6 (CH_2 –OBn), 52.6 (CH_α Phe), 38.1 (CH_2 Phe), 29.1 (CH_{iPr} Val), 19.5, 19.2 (CH_3 Val). IR: 3302, 2966, 1738, 1667, 1525, 1448, 1184, 739, 691. LRMS (DCI) *m*/*z*: 370.9 $(M + H)^+$, 387.8 $(M + NH_4)^+$. HRMS (ESI +): found, 393.1792; $C_{21}H_{26}N_2O_4Na$ requires 393.1790.

*(*R,S*)-**3d**.* Mp: 119–120 °C. R_f 0.24 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −0.17° (*c* 1.07 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) 7.40–7.05 (10H, m, CH_{ar}), 6.80 (1H, d, *J* 7.9, NH), 5.18 (2H, ABq, *J* 12.2, $\delta_A - \delta_B$ 18.7, CH_2 –OBn), 4.97 (1H, dt, *J* 6.5 and 7.9, CH_α Phe), 3.22 (1H, d, *J* 5.8, CH_α Val), 3.17 (2H, ABX, ^3J 6.4, ^2J 14.0, $\delta_A - \delta_B$ 38.5, CH_2 Phe), 2.00–1.80 (1H, m, CH_{iPr} Val), 0.92 (3H, d, *J* 6.3, CH_3 Val), 0.89 (3H, d, *J* 6.3, CH_3 Val). δ_C (75 MHz; $CDCl_3$) 172.1, 171.7 (C=O), 136.3, 135.3 (C_{ar}), 129.4, 128.8, 127.4 (CH_{ar}), 72.4 (CH_α Val), 67.5 (CH_2 –OBn), 52.8 (CH_α Phe), 37.9 (CH_2 Phe), 29.2 (CH_{iPr} Val), 19.6, 18.9 (CH_3 Val). IR: 3308, 2951, 1728, 1644, 1539, 1280, 753. LRMS (DCI): 371.1 $(M + H)^+$, 387.8 $(M + NH_4)^+$.

(*S*,*S*)- and (*R*,*S*)–HN(OH)–Val–Ile–OBn (3e). The title compounds were prepared as described for **3a** starting from **2e** (500 mg, 1.5 mmol). Purification by flash chromatography (silica gel, CH_2Cl_2–MeOH 99.75 : 0.25 to 96 : 4) of the residue allowed isolation of, first, pure (*S*,*S*)-**3e** (yellow oil, 41 mg, 0.12 mmol, 8%), second, a *circa* 1 : 1 mixture of (*S*,*S*)- and (*R*,*S*)-**3e** (233 mg, 0.69 mmol, 46%), and third, pure (*R*,*S*)-**3e** (yellow oil, 84 mg, 0.25 mmol, 17%).

*(*S,S*)-**3e**.* R_f 0.22 (CH_2Cl_2–MeOH 95 : 5). δ_H (200 MHz; $CDCl_3$) 7.25–7.45 (5H, m, CH_{ar}), 7.02 (1H, d, *J* 9.5, NH), 5.17 (2H, ABq, *J* 12.0, $\delta_A - \delta_B$ 24.6, CH_2 –OBn), 4.75 (1H, dd, *J* 4.5 and 9.5, CH_α Ile), 3.33 (1H, d, *J* 6.5, CH_α Val), 2.10–1.85 (2H, m, CH_{iPr} Val, CH_{sBu} Ile), 1.50–1.20 (1H, m, C*H*H Ile), 1.20–1.00 (1H, m, C*H*H Ile), 0.99 (3H, d, *J* 7.2, CH_3), 0.97 (3H, d, *J* 6.9, CH_3), 0.93 (3H, d, *J* 6.9, CH_3), 0.86 (3H, t, *J* 7.4, CH_3 Ile). δ_C (75 MHz; $CDCl_3$) 173.1, 172.8 (C=O), 135.3 (C_{ar}), 128.8, 128.7, 128.6, 128.5 (CH_{ar}), 73.0 (CH_α Val), 67.5 (CH_2 –OBn), 56.3 (CH_α Ile), 37.7 (CH_2 Ile), 29.2 (CH_{iPr} Val), 25.0 (CH_{sBu} Ile), 19.6, 19.3, 16.0, 11.7 (CH_3). IR: 3299, 2964, 1739, 1666, 1518, 1456, 1190, 1146, 996, 698.

*(*R,S*)-**3e**.* R_f 0.15 (CH_2Cl_2–MeOH 95 : 5). δ_H (200 MHz; $CDCl_3$) 7.38–7.30 (5H, m, CH_{ar}), 6.95 (1H, d, *J* 8.6, NH), 5.16 (2H, ABq, *J* 12.3, $\delta_A - \delta_B$ 20.6, CH_2 –OBn), 4.72 (1H, dd, *J* 4.8 and 8.6, CH_α Ile), 3.31 (1H, d, *J* 6.2, CH_α Val), 2.10–1.85 (2H, m, CH_{iPr} Val, CH_{sBu} Ile), 1.50–1.30 (1H, m, C*H*H Ile), 1.30–1.00 (1H, m, C*H*H Ile), 0.96 (3H, d, *J* 6.9, CH_3), 0.95 (3H, d, *J* 6.9, CH_3), 0.90 (3H, d, *J* 6.9, CH_3), 0.87 (3H, t, *J* 7.4, CH_3 Ile). δ_C (75 MHz; $CDCl_3$) 172.7, 172.1 (C=O), 135.4 (C_{ar}), 128.7, 128.6, 128.5 (CH_{ar}), 72.3 (CH_α Val), 67.2 (CH_2 –OBn), 56.5 (CH_α Ile), 38.0 (CH_2 Ile), 29.2 (CH_{iPr} Val), 25.3 (CH_{sBu} Ile), 19.7, 19.1, 15.7, 11.7 (CH_3). IR: 3319, 2964, 1738, 1666, 1462, 1188, 1002, 698.

Synthesis of *N*-hydroxy-peptides 4 to 7

Fmoc–Ile$_1$–Ψ[CO–N(OH)]–Val$_2$–Val$_3$–OBn ((*S*,*S*,*S*)-4). To a solution of (*S*,*S*)-**3a** (215 mg, 666 μmol) in CH_2Cl_2 (15 mL) at room temperature were added $NaHCO_3$ (168 mg, 1998 μmol) and Fmoc–Ile–Cl (301 mg, 854 μmol). The mixture was stirred overnight. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel, CH_2Cl_2–MeOH 100 : 0 to 95 : 5) gave (*S*,*S*,*S*)-**4** (353 mg, 0.54 mmol, 81% yield) as a white solid. Mp: 160–161 °C. R_f 0.41 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −18.28° (*c* 1.02 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) 8.73–8.39 (2H, m, NH Val_3, NOH), 7.72 (2H, d, *J* 7.4, CH_{ar} Fmoc), 7.64 (2H, d, *J* 7.3, CH_{ar} Fmoc), 7.45–7.10 (9H, m, CH_{ar}), 6.55 (1H, d, *J* 9.7, NH Ile_1), 5.30–5.20 (2H, m, C*H*H –OBn, CH_α Val_2), 5.10–4.95 (2H, m, C*H*H –OBn, CH_α Ile_1), 4.75 (1H, dd, *J* 4.7 and 8.8, CH_α Val_3), 4.52–4.07 (3H, m, CH Fmoc, CH_2 Fmoc), 2.50–2.29 (1H, m, CH_{iPr} Val_2), 2.26–2.06 (1H, m, CH_{iPr} Val_3), 1.92–1.73 (1H, m, CH_{sBu} Ile_1), 1.55–1.37 (1H, m, C*H*H Ile_1), 1.20–0.70 (19H, m, C*H*H Ile_1, CH_3 Ile_1, CH_3 Val_2, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 173.3, 171.9, 171.7 (C=O), 156.8 (C=O Fmoc), 144.2 (2 peaks), 141.4 (2 peaks) (C_{ar} Fmoc), 135.1 (C_{ar} –OBn), 128.7, 128.6, 128.4, 127.7, 127.0, 125.3, 120.0 (CH_{ar}), 67.5, 67.0 (CH_2 Fmoc, CH_2 –OBn), 63.1 (CH_α Val_2), 56.6 (CH_α Val_3), 55.3 (CH_α Ile_1), 47.4 (CH Fmoc), 37.6 (CH_{sBu} Ile_1), 32.0 (CH_{iPr} Val_3), 27.6 (CH_{iPr} Val_2), 24.5 (CH_2 Ile_1), 19.9, 19.1, 18.8, 17.6 (CH_3 Val), 15.5, 11.4 (CH_3 Ile_1). IR: 3386, 3302, 2964, 1719, 1629, 1522, 1236, 740. LRMS (DCI) *m*/*z*: 675.7 $(M + NH_4)^+$. HRMS (ESI +) *m*/*z*: found, 680.3311; $C_{38}H_{47}N_3O_7Na$ requires 680.3312.

Fmoc–Ile$_1$–Ψ[CO–N(OH)]–D-Val$_2$–Val$_3$–OBn ((*S*,*R*,*S*)-4). The title compound was prepared as described for (*S*,*R*,*S*)-**4** using (*R*,*S*)-**3a** (50 mg, 0.155 mmol). Purification by flash chromatography (silica gel, CH_2Cl_2–MeOH 98.5 : 1.5 to 98 : 2) followed by recrystallization form CH_2Cl_2–cyclohexane gave (*S*,*R*,*S*)-**4** (77 mg, 0.117 mmol, 76%) as a white solid. Mp: 164 °C. R_f 0.61 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +19.7 (*c* 1.20 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) 8.57 (1H, s, NOH), 7.73 (2H, d, *J* 7.2, CH_{ar} Fmoc), 7.57 (2H, d, *J* 7.2, CH_{ar} Fmoc), 7.44–7.18 (9H, m, CH_{ar}), 7.03 (1H, d, *J* 7.8, NH Val_3), 5.83 (1H, d, *J* 9.6, NH Ile_1), 5.05 (2H, ABq, *J* 12.2, $\delta_A - \delta_B$ 28.8, CH_2 –OBn), 4.98–4.85 (1H, m, CH_α Ile_1), 4.86 (1H, d, *J* 9.6, CH_α Val_2), 4.54 (1H, dd, *J* 4.8 and 7.8, CH_α Val_3), 4.44–4.32 (1H, m, C*H*H Fmoc), 4.26–4.11 (2H, m, CH Fmoc, C*H*H Fmoc), 2.62–2.34 (1H, m, CH_{iPr} Val_2), 2.26–2.07 (1H, m, CH_{iPr} Val_3), 2.04–1.80 (1H, m, CH_{sBu} Ile_1), 1.63–1.37 (1H, m, C*H*H Ile_1), 1.30–0.70 (19H, m, C*H*H Ile_1, CH_3 Ile_1, CH_3 Val_2, CH_3 Val_3). δ_C (75 MHz, $CDCl_3$) 172.1, 171.6 (C=O), 156.9 (C=O Fmoc), 144.2, 144.0, 141.4 (C_{ar} Fmoc), 135.2 (C_{ar} –OBn), 128.7, 128.6 (2 peaks), 127.8, 127.2, 125.4, 120.1 (CH_{ar}), 67.5, 67.3 (CH_2 Fmoc, CH_2 –OBn), 63.6 (CH_α Val_2), 57.7 (CH_α Val_3), 55.8 (CH_α Ile_1), 47.4 (CH Fmoc), 37.4 (CH_{sBu} Ile_1), 31.0 (CH_{iPr} Val_3), 27.9 (CH_{iPr} Val_2), 24.1 (CH_2 Ile_1), 19.7, 19.4, 19.1, 18.1 (CH_3 Val), 16.1, 11.6 (CH_3 Ile_1). IR: 3319, 2964, 1735, 1701, 1655, 1611, 1534, 1249, 740. LRMS (ESI +) *m*/*z*: 658.2 $(M + H)^+$, 680.3 $(M + Na)^+$, 696.2 $(M + K)^+$. Anal.: found, C69.2; H7.3; N6.4. $C_{38}H_{47}N_3O_7$ requires C69.4; H7.2; N6.4%.

Fmoc–Ala$_1$–Ψ[CO–N(OH)]–D-Val$_2$–Val$_3$–OBn ((*S*,*R*,*S*)-5). The title compound was prepared as described for (*S*,*R*,*S*)-**4** using (*R*,*S*)-**3a** (235 mg, 728 μmol). Purification by flash

chromatography (silica gel, pentane–EtOAc 9 : 1 to 1 : 1) gave (*S*,*R*,*S*)-**5** (379 mg, 626 μmol, 86%) as a white solid which can be recrystallized from CH_2Cl_2–cyclohexane. Mp: 184–185 °C. R_f 0.39 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +7.8° (*c* 1.00 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.6 (1H, s, NOH), 7.75 (2H, d, *J* 7.6, CH_{ar} Fmoc), 7.59 (2H, dd, *J* 7.0, 7.0, CH_{ar} Fmoc), 7.40–7.20 (9H, m, CH_{ar}), 6.86 (1H, d, *J* 8.2, NH Val_3), 5.95 (1H, d, *J* 8.2, NH Ala_1), 5.12 (2H, ABq, *J* 12.2, $\delta_A - \delta_B$ 22.8, CH_2 –OBn), 5.05–4.97 (1H, m, CH_α Ala_1), 4.78 (1H, d, *J* 9.6, CH_α Val_2), 4.55 (1H, dd, *J* 4.6 and 8.2, CH_α Val_3), 4.36–4.17 (3H, m, CH Fmoc, CH_2 Fmoc), 2.60–2.40 (1H, m, CH_{iPr} Val_2), 2.30–2.10 (1H, m, CH_{iPr} Val_3), 1.43 (3H, d, *J* 6.8, CH_3 Ala_1), 1.04 (3H, d, *J* 6.7, CH_3 Val_2), 0.98 (3H, d, *J* 6.7, CH_3 Val_2), 0.90 (3H, d, *J* 6.9, CH_3 Val_3), 0.85 (3H, d, *J* 6.8, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 175.8, 172.9, 171.6 (C=O), 156.0 (C=O Fmoc), 144.2, 144.0, 141.4 (C_{ar} Fmoc), 135.2 (C_{ar} –OBn), 128.8, 128.7, 127.8, 127.2, 125.4, 124.8, 120.1 (CH_{ar}), 67.6, 67.2 (CH_2 –OBn, CH_2 Fmoc), 63.7 (CH_α Val_2), 57.7 (CH_α Val_3), 47.6 (CH_α Ala_1), 47.3 (CH Fmoc), 31.1 (CH_{iPr} Val_3), 28.0 (CH_{iPr} Val_2), 19.7, 19.3, 19.2, 18.8, 18.0 (CH_3). IR: 3248, 3068, 2964, 1744, 1697, 1663,1610, 1551, 1105, 758, 739. HRMS (ESI +) *m*/*z*: found, 616.3028; $C_{35}H_{42}N_3O_7$ requires 616.3023.

Fmoc–Gly–Ψ[CO–N(OH)]–D-Ala–Leu–OEt ((*R*,*S*)-6). The title compound was prepared as described for (*S*,*R*,*S*)-**4** using (*R*,*S*)-**3c** (100 mg, 0.406 mmol) and Fmoc–Gly–Cl (132 mg, 0.418 mmol). Purification by flash chromatography (silica gel, pentane–EtOAc 1 : 1) gave (*R*,*S*)-**6** (181 mg, 0.345 mmol, 85%) as a white solid. Mp: 147–148 °C. R_f 0.55 (pentane–AcOEt 1 : 1). $[\alpha]_D^{25}$ +9.5 (*c* 1.94 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) 8.48 (1H, br s, NOH), 7.75 (2H, d, *J* 6.9, CH_{ar}), 7.60 (2H, d, *J* 7.2, CH_{ar}), 7.46–7.24 (4H, m, CH_{ar}), 6.80 (1H, d, *J* 8.2, NH Leu), 5.90–5.70 (1H, m, NH Gly), 5.40–5.10 (1H, m, CH_α Ala), 4.70–4.50 (1H, m, CH_α Leu), 4.50–4.05 (7H, m, CH_2 Gly, CH_2 Fmoc, CH Fmoc, CH_2 –OEt), 1.75–1.50 (3H, m, CH_2 Leu, CH_{iBu} Leu), 1.47 (3H, d, *J* 6.5, CH_3 Ala), 1.26 (3H, t, *J* 7.0, CH_3 –OEt), 1.0–0.8 (6H, m, CH_3 Leu). δ_C (75 MHz, $CDCl_3$) 173.88, 171.9, 170.5 (C=O), 157.0 (C=O Fmoc), 144.0 (2 peaks), 141.4 (C_{ar}), 127.8, 127.2, 125.3, 120.1 (CH_{ar}), 67.5 (CH_2 Fmoc), 62.0 (CH_2 –OEt), 54.7 (CH_α Ala), 51.1 (CH_α Leu), 47.2 (CH Fmoc), 42.6 (CH_2 Gly), 40.9 (CH_2 Leu), 25.0 (CH_{iBu} Leu), 22.9, 21.8 (CH_3 Leu), 14.2 (CH_3 –OEt), 13.9 (CH_3 Ala). IR: 3265, 2955, 1737, 1706, 1666, 1619, 1555, 1451, 1264, 737. LRMS (DCI) *m*/*z*: 542.7 $(M + NH_4)^+$. HRMS (ESI +) *m*/*z*: found, 548.2370; $C_{28}H_{35}N_3O_7Na$ requires 548.2373.

Fmoc–Ala_1–Ψ[CO–N(OH)]–D-Ala_2–Val_3–OEt ((*S*,*R*,*S*)-7). The title compound was prepared as described for (*S*,*R*,*S*)-**4** using (*R*,*S*)-**3b** (408 mg, 1.756 mmol) and Fmoc–Ala–Cl (608 mg, 1.844 mmol). Purification by flash chromatography (silica gel, pentane–EtOAc 1 : 1) gave (*S*,*R*,*S*)-**7** (698 mg, 1.328 mmol, 76%) as a white solid. Mp: 173 °C. R_f 0.38 (pentane–EtOAc 1 : 1). $[\alpha]_D^{25}$ +28.6 (*c* 1.00 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.46 (1H, br s, NOH), 7.75 (2H, d, *J* 7.6, CH_{ar}), 7.59 (2H, d, *J* 7.4, CH_{ar}), 7.39 (2H, dd, *J* 7.4 and 7.4, CH_{ar}), 7.30 (2H, dd, *J* 7.4 and 7.4, CH_{ar}), 6.84 (1H, d, *J* 9.1, NH Val_3), 5.82 (1H, d, *J* 7.6, NH Ala_1), 5.32 (1H, q, *J* 7.0, CH_α Ala_2), 4.99 (1H, dq, *J* 6.8 and 7.6, CH_α Ala_1), 4.52 (1H, dd, *J* 4.8 and 9.1, CH_α Val_3), 4.35–4.31 (2H, m, CH_2 Fmoc), 4.22–4.12 (3H, m, CH Fmoc, CH_2 –OEt), 2.20 (1H, dh, *J* 4.8 and 6.9, CH_{iPr} Val_3), 1.49 (3H, d, *J* 7.0, CH_3 Ala_2), 1.41 (3H, d, *J* 6.8, CH_3 Ala_1), 1.23 (3H, t, *J* 7.2, CH_3 –OEt), 0.93 (3H, d, *J* 6.9, CH_3 Val_3), 0.87 (3H, d, *J* 6.9, CH_3 Val_3). δ_C (75 MHz, $CDCl_3$) 173.6 (C=O Ala_1), 173.3 (C=O Val_3), 171.8 (C=O Ala_2), 156.4 (C=O Fmoc), 144.0, 141.4 (C_{ar}), 127.9, 127.2, 125.3, 120.1 (CH_{ar}), 67.4 (CH_2 Fmoc), 62.1 (CH_2 –OEt), 57.7 (CH_α Val_3), 54.5 (CH_α Ala_2), 47.2 (CH_α Ala_1, CH Fmoc), 30.7 (CH_{iPr} Val_3), 19.2, 17.8 (CH_3 Val_3), 17.6 (CH_3 Ala_1), 14.3 (CH_3 –OEt), 13.7 (CH_3 Ala_2). IR: 3302, 2965, 1739, 1708, 1670, 1618, 1540, 1251, 740. LRMS (DCI) *m*/*z*: 542.8 $(M + NH_4)^+$. Anal.: found, C64.2; H6.7; N8.0. $C_{28}H_{35}N_3O_7$ requires C64.0; H6.7; N8.0%.

H–Ile_1–Ψ[CO–N(OH)]–Val_2–Val_3–OBn ((*S*,*S*,*S*)-4′). To a solution of (*S*,*S*,*S*)-**4** (203 mg, 309 μmol) in THF (7 mL) at room temperature was added piperidine (1.5 mL). The mixture was stirred for 3 h. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel, pentane–EtOAc 1 : 1, EtOAc, then CH_2Cl_2–MeOH 90 : 10) gave (*S*,*S*,*S*)-**4′** (113 mg, 0.26 mmol, 84%) as a pale yellow solid. Mp: 116 °C. R_f 0.10 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −11.7° (*c* 1.00 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.83 (1H, d, *J* 8.9, NH Val_3), 7.40–7.30 (5H, m, CH_{ar}), 5.16 (2H, ABq, *J* 12.2, $\delta_A - \delta_B$ 34.1, CH_2 –OBn), 4.90 (1H, d, *J* 10.5, CH_α Val_2), 4.60 (1H, dd, *J* 5.3 and 8.9, CH_α Val_3), 3.87 (1H, d, *J* 6.3, CH_α Ile_1), 2.50–2.35 (1H, m, CH_{iPr} Val_2), 2.25–2.10 (1H, m, CH_{iPr} Val_3), 1.75–1.60 (1H, m, CH_{sBu} Ile_1), 1.60–1.45 (1H, m, CH*H* Ile_1), 1.20–1.05 (1H, m, C*H*H Ile_1), 0.98 (3H, d, *J* 6.5, CH_3 Val_2), 0.97 (3H, d, *J* 6.6, CH_3 Val_2), 0.90 (3H, d, *J* 7.0, CH_3), 0.88 (3H, d, *J* 7.4, CH_3), 0.84–0.78 (6H, m, CH_3). δ_C (75 MHz; $CDCl_3$) 175.0, 172.7, 171.4 (C=O), 135.4 (C_{ar}), 128.7 (CH_{ar}), 67.2 (CH_2 –OBn), 64.6 (CH_α Val_2), 57.1 (CH_α Val_3), 56.0 (CH_α Ile_1), 38.1 (CH_{sBu} Ile_1), 31.3 (CH_{iPr} Val_2), 27.8 (CH_{iPr} Val_3), 24.2 (CH_2 Ile_1), 19.8, 19.2, 17.8, 16.0, 11.4 (CH_3). IR: 3289, 3192, 2953, 1738, 1667, 1615, 1563, 1182, 704. LRMS (DCI) *m*/*z*: 436.1 $(M + H)^+$. HRMS (ESI +) *m*/*z*: found, 436.2811; $C_{23}H_{38}N_3O_5$ requires 436.2811.

H–Ala_1–Ψ[CO–N(OH)]–D-Ala_2–Val_3–OEt ((*S*,*R*,*S*)-7″). The title compound was prepared as described for (*S*,*S*,*S*)-**4″** using (*S*,*R*,*S*)-**7** (123 mg, 0.234 mmol). Purification by flash chromatography (silica gel, CH_2Cl_2–MeOH 9 : 1 to 8 : 2) followed by crystallization from pentane gave (*S*,*R*,*S*)-**7′** (70 mg, 0.231 mmol, 99%) as a white solid. Mp: 84 °C. R_f 0.17 (CH_2Cl_2–MeOH 8 : 2). $[\alpha]_D^{25}$ +49.0 (*c* 1.00 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) 7.50 (1H, d, *J* 8.6, NH Val_3), 5.50–4.50 (3H, br + q (5.07), *J* 7.0, CH_α Ala_2, NH_2 Ala_1), 4.50 (1H, dd, *J* 8.6, 6.5, CH_α Val_3), 4.27–4.09 (3H, m, CH_α Ala_1, CH_2 –OEt), 2.36–2.19 (1H, m, CH_{iPr} Val_3), 1.38 (3H, d, *J* 6.9, CH_3 Ala), 1.31 (3H, d, *J* 7.0, CH_3 Ala), 1.29 (3H, t, *J* 7.2, CH_3 –OEt), 0.97 (3H, d, *J* 6.9, CH_3 Val_3), 0.96 (3H, d, *J* 6.9, CH_3 Val_3). δ_C (75 MHz, $CDCl_3$) 176.6, 173.3, 170.8 (C=O), 61.4 (CH_2 –OEt), 58.1 (CH_α Ala_2), 55.7 (CH_α Val_3), 47.7 (CH_α Ala_1), 30.9 (CH_{iPr} Val_3), 21.2 (CH_3 Ala_1), 19.4, 18.4 (CH_3 Val_3), 14.3 (CH_3 –OEt), 13.5 (CH_3 Ala_2). IR: 3363, 3264, 2984, 1743, 1682, 1636, 1579, 1151. LRMS (DCI) *m*/*z*: 303.7 $(M)^+$, 304.9 $(M + H)^+$. Anal.: found, C51.5; H8.0; N13.4. $C_{13}H_{25}N_3O_5$ requires C51.5; H8.3; N13.8%.

Boc–Ile_1–Ψ[CO–N(OH)]–Val_2–Val_3–OBn ((*S*,*S*,*S*)-4″). A solution of (*S*,*S*,*S*)-**4′** (102 mg, 234 μmol) in CH_2Cl_2 (5 mL) was cooled to 0 °C. Di-*tert*-butyl dicarbonate (66 mg, 293 μmol) and triethylamine (38.0 μL, 270 μmol) were added. The solution was stirred for 30 min at 0 °C and for 1 h at room temperature. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel, pentane–EtOAc

9 : 1 then 1 : 1) gave (*S*,*S*,*S*)-**4**″ (113 mg, 211 μmol, 90%) as a white solid. Mp: 169 °C. R_f 0.52 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −25.0° (*c* 1.14 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.59 (1H, s, NOH), 8.07 (1H, d, *J* 9.1, NH Val_3), 7.40–7.25 (5H, m, CH_{ar}), 5.75 (1H, d, *J* 9.7, NH Ile_1), 5.16 (2H, ABq, *J* 12.1, $\delta_A - \delta_B$ 33.5, CH_2 –OBn), 5.11 (1H, d, *J* 10.4, CH_α Val_2), 4.80 (1H, dd, *J* 8.7 and 9.7, CH_α Ile_1), 4.65 (1H, dd, *J* 5.1 and 9.1, CH_α Val_3), 2.50–2.30 (1H, m, CH_{iPr} Val_2), 2.25–2.05 (1H, m, CH_{iPr} Val_3), 1.85–1.65 (1H, m, CH_{sBu} Ile_1), 1.60–1.45 (1H, m, CH*H* Ile_1), 1.42 (9H, s, CH_3 Boc), 1.20–1.05 (1H, m, C*H*H Ile_1), 0.96 (6H, d, *J* 6.7, CH_3 Val_2), 0.87 (3H, d, *J* 7.8, CH_3 Ile_1), 0.85 (3H, d, *J* 7.3, CH_3 Val_3), 0.81 (3H, t, *J* 7.6, CH_3 Ile_1), 0.76 (3H, d, *J* 6.9, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 172.7 (C=O Val_2), 172.1 (C=O Ile_1), 171.6 (C=O Val_3), 156.5 (C=O Boc), 135.4 (C_{ar} –OBn), 128.7 (2 peaks) (CH_{ar}), 79.4 (C_q Boc), 67.3 (CH_2 –OBn), 63.3 (CH_α Val_2), 56.8 (CH_α Val_3), 54.4 (CH_α Ile_1), 36.9 (CH_{sBu} Ile_1), 31.7 (CH_{iPr} Val_3), 28.5 (CH_3 Boc), 27.7 (CH_{iPr} Val_2), 24.7 (CH_2 Ile_1), 19.7 (2 peaks) (CH_3 Val_2), 19.1, 17.7 (CH_3 Val_3), 15.4, 11.2 (CH_3 Ile_1). IR: 3388, 3303, 2964, 1748, 1716, 1626, 1522, 1178, 752. LRMS (DCI +) *m*/*z*: 536.8 (M + H)$^+$, 553.0 (M + NH_4)$^+$. HRMS (ESI +) *m*/*z*: found, 558.3163; $C_{28}H_{45}N_3O_7Na$ requires 558.3155.

Boc–Ala_1–Ψ[CO–N(OH)]–D-Ala_2–Val_3–OEt ((*S*,*R*,*S*)-7″). The title compound was prepared as described for (*S*,*S*,*S*)-**4**″ using (*S*,*R*,*S*)-**7**′ (16 mg, 52.7 μmol). Purification by flash chromatography (silica gel, CH_2Cl_2–MeOH 95 : 5) gave (*S*,*R*,*S*)-**7**″ (12 mg, 29.7 μmol, 56%) as a white solid. Mp: 161 °C. R_f 0.49 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +17.7 (*c* 0.62 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.76 (1H, br s, NOH), 6.70 (1H, d, *J* 7.7, NH Val_3), 5.30–5.17 (2H, m, NH Ala_1, CH_α Ala_2), 4.90–4.80 (1H, m, CH_α Ala_1), 4.49 (1H, dd, *J* 5.1 and 7.7, CH_α Val_3), 4.27–4.16 (2H, m, CH_2 –OEt), 2.26–2.15 (1H, m, CH_{iPr} Val_3), 1.46 (3H, d, *J* 6.9, CH_3 Ala_2), 1.42 (9H, s, CH_3 Boc), 1.34 (3H, d, *J* 7.2, CH_3 Ala_1), 1.29 (3H, t, *J* 7.2, CH_3 –OEt), 0.96 (3H, d, *J* 6.9, CH_3 Val_3), 0.91 (3H, d, *J* 6.9, CH_3 Val_3). δ_C (75 MHz, $CDCl_3$) 176.3, 173.5, 171.7 (C=O), 156.0 (C=O Boc), 80.2 (C_q Boc), 62.1 (CH_2 –OEt), 57.8 (CH_α Val_3), 54.7 (CH_α Ala_2), 46.8 (CH_α Ala_1), 30.7 (CH_{iPr} Val_3), 28.5 (CH_3 Boc), 19.3, 18.0 (CH_3 Val_3), 17.5 (CH_3 Ala_1), 14.3 (CH_3 –OEt), 13.4 (CH_3 Ala_2). IR: 3281, 2966, 2921, 1745, 1670, 1614, 1524, 1164, 643. LRMS (DCI) *m*/*z*: 403.7 (M + H)$^+$.

Fmoc–Val_1–Ala_2–Ψ[CO–N(OH)]–D-Ala_3–Val_4–OEt (8). A mixture of (*S*,*R*,*S*)-**7**′ (23 mg, 75.8 μmol), Fmoc–Val–OH (26 mg, 75.8 μmol) and HOBt (10 mg, 75.8 μmol) in CH_2Cl_2 (2 mL) was cooled to 0 °C. EDCI (16 mg, 83.4 μmol) was added and the mixture was stirred for 1 h at 0 °C and for 1.5 h at room temperature. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel, pentane–EtOAc 1 : 2) gave (*S*,*S*,*R*,*S*)-**8** (36 mg, 57.6 μmol, 76%) as a white solid. Mp: 184 °C. R_f 0.21 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −3.1 (*c* 0.90 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.87 (1H, br s, NOH), 8.12 (1H, d, *J* 8.2, NH Ala_2), 7.75 (2H, d, *J* 7.4, CH_{ar}), 7.71 (1H, d, *J* 8.8, NH Val_4), 7.61 (1H, d, *J* 7.4, CH_{ar}), 7.57 (1H, d, *J* 7.4, CH_{ar}), 7.39 (2H, dd, *J* 7.4 and 7.4, CH_{ar}), 7.29 (2H, dd, *J* 7.4 and 7.4, CH_{ar}), 6.02 (1H, d, *J* 9.0, NH Val_1), 5.67 (1H, q, *J* 6.9, CH_α Ala_3), 5.40–5.25 (1H, m, CH_α Ala_2), 4.56 (1H, dd, *J* 5.8 and 9.0, CH_α Val_1), 4.40 (1H, dd, *J* 5.9 and 8.8, CH_α Val_4), 4.41–4.22 (3H, m, CH Fmoc, CH_2 Fmoc), 4.13 (2H, q, *J* 7.2, CH_2 –OEt), 2.11–1.95 (2H, m, CH_{iPr} Val_1, CH_{iPr} Val_4), 1.48 (3H, d, *J* 6.9, CH_3 Ala_3), 1.35 (3H, d, *J* 6.7, CH_3 Ala_2), 1.20 (3H, t, *J* 7.2, CH_3 –OEt), 0.90 (3H, d, *J* 6.9, CH_3 Val), 0.89 (3H, d, *J* 6.8, CH_3 Val), 0.87 (3H, d, *J* 6.9, CH_3 Val), 0.81 (3H, d, *J* 6.8, CH_3 Val). δ_C (75 MHz, $CDCl_3$) 174.1 (C=O Ala_3), 171.7 (C=O Ala_2), 171.2 (C=O Val_4), 170.6 (C=O Val_1), 156.8 (C=O Fmoc), 144.1, 144.0, 141.5, 141.4 (C_{ar}), 127.9, 127.3, 127.2, 125.4, 125.2, 120.2, 120.1 (CH_{ar}), 67.4 (CH_2 Fmoc), 61.3 (CH_2 –OEt), 59.4 (CH_α Val_1), 57.6 (CH_α Val_4), 52.8 (CH_α Ala_3), 47.2 (CH Fmoc), 45.9 (CH_α Ala_2), 32.8 (CH_{iPr} Val_1), 31.1 (CH_{iPr} Val_4), 19.2, 19.1 (CH_3 Val), 18.8 (CH_3 Ala_2), 18.2, 18.0 (CH_3 Val), 16.2 (CH_3 Ala_3), 14.3 (CH_3 –OEt). IR: 3310, 2966, 1736, 1707, 1670, 1626, 1534, 1250, 1030, 741. LRMS (DCI) *m*/*z*: 642.0 (M + NH_4)$^+$. Anal.: found, C63.6; H7.2; N8.8. $C_{33}H_{44}N_4O_8$ requires C63.4; H7.1; N9.0%.

Synthesis of *O*-acylated-*N*-hydroxy-peptides 9 to 27

Typical procedure for method A. To a solution of tripeptide (70 μmol) in pyridine (4 mL) at room temperature was added the anhydride (140 to 210 μmol). The reaction was monitored by TLC. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel) allowed isolation of pure *O*-acylated-*N*-hydroxy-peptide.

Typical procedure for method B. To a solution of acid (200 μmol) in CH_2Cl_2 (1.5 mL) at room temperature was added DCC (400 μmol). The mixture was stirred for 2 h and the resulting suspension was directly filtered onto the tripeptide (50 μmol). The solvent was evaporated, the residue taken up in pyridine (1.5 mL) and the mixture stirred overnight. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel) allowed isolation of pure *O*-acylated-*N*-hydroxy-peptide.

Typical procedure for method C. To a solution of tripeptide (70 μmol) in CH_2Cl_2 (4 mL) at room temperature were added DIEA (diisopropylethylamine; 77 μmol) and the acyl chloride (77 μmol). The mixture was stirred overnight. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel) allowed isolation of pure *O*-acylated-*N*-hydroxy-peptide.

Typical procedure for method D. To a solution of tripeptide (50 μmol) and acid (55 μmol) in CH_2Cl_2 (2 mL) at room temperature was added BOP (55 μmol), then DIEA (110 μmol). The reaction was monitored by TLC. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel) allowed isolation of pure *O*-acylated-*N*-hydroxy-peptide.

Typical procedure for method E. To a solution of tripeptide (50 μmol) in DMF (2 mL) at room temperature was added the acid (55 μmol), then HATU (55 μmol) and DIEA (160 μmol). The mixture was stirred for 5 h, then taken up in Et_2O and washed several times with a 5% aqueous solution of LiCl. The organic layer was dried over Na_2SO_4. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel) allowed isolation of pure *O*-acylated-*N*-hydroxy-peptide.

Fmoc–Ile_1–Ψ[CO–N(OAc)]–D-Val_2–Val_3–OBn (9). The title compound was prepared from (*S*,*R*,*S*)-**4** and acetic anhydride using method A. Eluent for flash chromatography: pentane–EtOAc 9 : 1 to 1 : 1; colourless oil, 86% yield. R_f 0.65 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +15.6° (*c* 2.40 in $CHCl_3$). δ_H (300 MHz;

$CDCl_3$) 7.74 (2H, d, *J* 7.4, CH_{ar} Fmoc), 7.55 (2H, dd, *J* 7.4 and 7.7, CH_{ar} Fmoc), 7.40–7.15 (10H, m, CH_{ar}, NH Val_3), 5.40 (1H, d, *J* 9.9, NH Ile_1), 5.05 (2H, br s, CH_2 –OBn), 4.50 (1H, dd, *J* 4.7 and 8.3, CH_α Val_3), 4.44–4.11 (5H, m, CH_α Ile_1, CH_α Val_2, CH Fmoc, CH_2 Fmoc), 2.50–2.35 (1H, m, CH_{iPr} Val_2), 2.25–2.06 (4H, m + s (2.20), CH_{iPr} Val_3, CH_3 Ac), 1.90–1.70 (1H, m, CH_{sBu} Ile_1), 1.57–1.41 (1H, m, CH*H* Ile_1), 1.16–0.76 (19H, m, C*H*H Ile_1, CH_3 Ile_1, CH_3 Val_2, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 172.9, 171.5 (C=O Ile_1, C=O Val_2), 168.8 (C=O Ac), 168.1 (C=O Val_3), 156.3 (C=O Fmoc), 144.2, 143.9, 141.5, 141.4 (C_{ar} Fmoc), 135.6 (C_{ar} –OBn), 128.6, 128.4, 127.8, 127.2, 125.3, 120.1 (CH_{ar}), 67.2, 67.0 (CH_2 Fmoc, CH_α Val_2, CH_2 –OBn), 57.5 (CH_α Val_3), 55.6 (CH_α Ile_1), 47.4 (CH Fmoc), 37.9 (CH_{sBu} Ile_1), 31.5 (CH_{iPr} Val_3), 26.4 (CH_{iPr} Val_2), 24.1 (CH_2 Ile_1), 19.8, 19.4, 19.2 (CH_3), 18.3 (CH_3 Ac), 17.7, 15.9 (CH_3), 11.4 (CH_3 Ile_1). IR: 3339, 2966, 1807, 1721, 1661, 1531, 1148, 741. HRMS (ESI +) *m/z*: found, 700.3591; $C_{40}H_{50}N_3O_8$ requires 700.3598.

Fmoc–Ile_1–Ψ[CO–N(OAc)]–Val_2–Val_3–OBn (10). The title compound was prepared from (*S*,*S*,*S*)-**4** and acetic anhydride using method A. Eluent for flash chromatography: CH_2Cl_2–MeOH 99 : 1 then 95 : 5; colourless oil, 94% yield. R_f 0.61 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −41.9° (*c* 1.12 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.75 (2H, d, *J* 7.6, CH_{ar} Fmoc), 7.56 (2H, d, *J* 7.4, CH_{ar} Fmoc), 7.45–7.20 (10H, m, CH_{ar}, NH Val_3), 5.35 (1H, d, *J* 9.9, NH Ile_1), 5.11 (2H, ABq, *J* 12.2, $\delta_A - \delta_B$ 26.2, CH_2 –OBn), 4.55 (1H, dd, *J* 7.7, 4.2, CH_α Val_3), 4.45–4.10 (5H, m, CH_α Ile_1, CH_α Val_2, CH Fmoc, CH_2 Fmoc), 2.65–2.50 (1H, m, CH_{iPr} Val_2), 2.32–2.11 (4H, m + s (2.23), CH_{iPr} Val_3, CH_3 Ac), 1.95–1.80 (1H, m, CH_{sBu} Ile_1), 1.60–1.45 (1H, m, CH*H* Ile_1), 1.20–0.70 (19H, m, C*H*H Ile_1, CH_3 Ile_1, CH_3 Val_2, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 172.5 (C=O Ile_1), 171.8 (C=O Val_3), 169.2, 168.0 (C=O), 156.3 (C=O Fmoc), 144.0, 143.9, 141.5 (C_{ar} Fmoc), 135.6 (C_{ar} –OBn), 128.7, 128.5, 127.9, 127.3, 125.2, 120.1 (CH_{ar}), 67.2, 67.1 (CH_2 Fmoc, CH_α Val_2, CH_2 –OBn), 57.4 (CH_α Val_3), 55.6 (CH_α Ile_1), 47.4 (CH Fmoc), 36.8 (CH_{sBu} Ile_1), 31.1 (CH_{iPr} Val_3), 27.8 (CH_{iPr} Val_2), 24.0 (CH_2 Ile_1), 20.0, 19.5, 19.0 (CH_3 Val), 18.6 (CH_3 Ac), 17.8 (CH_3 Val), 16.1, 11.3 (CH_3 Ile_1). IR: 3338, 2966, 1805, 1722, 1652, 1524, 1148, 758. HRMS (ESI +) *m/z*: found, 722.3417; $C_{40}H_{49}N_3O_8Na$ requires 722.3417.

Boc–Ile_1–Ψ[CO–N(OAc)]–Val_2–Val_3–OBn (11). The title compound was prepared from (*S*,*S*,*S*)-**4**″ and acetic anhydride using method A. Eluent for flash chromatography: CH_2Cl_2–MeOH 98 : 2; colourless oil, 99% yield. R_f 0.64 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −76.3° (*c* 1.60 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.55–7.24 (6H, m, CH_{ar}, NH Val_3), 5.16 (2H, ABq, *J* 12.2, $\delta_A - \delta_B$ 26.2, CH_2–OBn), 4.96 (1H, d, *J* 9.3, NH Ile_1), 4.54 (1H, dd, *J* 4.2 and 7.8, CH_α Val_3), 4.25 (1H, dd, *J* 8.7 and 9.3, CH_α Ile_1), 4.18–3.97 (1H, m, CH_α Val_2), 2.67–2.46 (1H, m, CH_{iPr} Val_2), 2.38–2.11 (4H, m + s (2.26), CH_{iPr} Val_3, CH_3 Ac), 2.00–1.73 (1H, m, CH_{sBu} Ile_1), 1.67–1.34 (10H, m + s (1.41), CH*H* Ile_1, CH_3 Boc), 1.26 − 0.77 (19H, m, C*H*H Ile_1, CH_3 Ile_1, CH_3 Val_2, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 172.7 (C=O Ile_1), 172.0 (C=O Val_3), 169.6 (C=O Ac), 168.1 (C=O Val_2), 155.7 (C=O Boc), 135.9 (C_{ar}–OBn), 128.9, 128.7 (CH_{ar}), 80.1 (C_q Boc), 67.2 (CH_α Val_2, CH_2–OBn), 57.7 (CH_α Val_3), 55.0 (CH_α Ile_1), 36.9 (CH_{sBu} Ile_1), 31.2 (CH_{iPr} Val_3), 28.6 (CH_3 Boc), 27.7 (CH_{iPr} Val_2), 24.2 (CH_2 Ile_1), 20.4, 19.6, 19.2 (CH_3 Val), 18.8 (CH_3 Ac), 18.0 (CH_3 Val), 16.3, 11.5 (CH_3 Ile_1). IR: 3341, 2967, 1806, 1716, 1656, 1518, 1367, 1175, 752, 698. LRMS (DCI +) *m/z*: 595.7 $(M + NH_4)^+$. HRMS (ESI +) *m/z*: found, 600.3264; $C_{30}H_{47}N_3O_8Na$ requires 600.3261.

Fmoc–Ile_1–Ψ[CO–N(OCOCH$_2$CH$_3$)]–D-Val_2–Val_3–OBn (12). The title compound was prepared from (*S*,*R*,*S*)-**4** and propionic anhydride using method A. Eluent for flash chromatography: pentane–EtOAc 9 : 1 to 1 : 1; colourless oil, 99% yield. R_f 0.67 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +24.4° (*c* 0.6 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.74 (2H, d, *J* 7.4, CH_{ar} Fmoc), 7.58–7.50 (2H, m, CH_{ar} Fmoc), 7.40–7.20 (10H, m, CH_{ar}, NH Val_3), 5.40 (1H, d, *J* 9.6, NH Ile_1), 5.06 (2H, br s, CH_2 –OBn), 4.50 (1H, dd, *J* 4.9 and 8.5, CH_α Val_3), 4.44–4.09 (5H, m, CH_α Ile_1, CH_α Val_2, CH Fmoc, CH_2 Fmoc), 2.54–2.39 (3H, m, CH_{iPr} Val_2, –COC*H*$_2$CH$_3$), 2.19–2.10 (1H, m, CH_{iPr} Val_3), 1.81–1.63 (1H, m, CH_{sBu} Ile_1), 1.54–1.40 (1H, m, CH*H* Ile_1), 1.21 (3H, t, *J* 7.5, –COCH$_2$C*H*$_3$), 1.15–0.80 (19H, m, C*H*H Ile_1, CH_3 Ile_1, CH_3 Val_2, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 173.0, 172.3, 171.5, 168.1 (C=O), 156.3 (C=O Fmoc), 144.2, 143.9, 141.5 (C_{ar} Fmoc), 135.7 (C_{ar} –OBn), 129.0, 128.6, 128.4, 127.9, 127.3, 125.3, 120.1 (CH_{ar}), 67.1, 67.0 (CH_2 Fmoc, CH_2 –OBn, CH_α Val_2), 57.6 (CH_α Val_3), 55.6 (CH_α Ile_1), 47.4 (CH Fmoc), 38.0 (CH_{sBu} Ile_1), 31.5 (CH_{iPr} Val_3), 26.3 (CH_{iPr} Val_2), 25.3 (–CO*C*H$_2$–), 24.2 (CH_2 Ile_1), 19.8, 19.4, 19.2, 17.8, 15.8 (CH_3), 11.4 (CH_3 Ile_1), 8.8 (–COCH$_2$*C*H$_3$). IR: 3339, 2964, 1805, 1721, 1661, 1528, 1239, 741. HRMS (ESI +) *m/z*: found, 714.3746; $C_{41}H_{52}N_3O_8$ requires 714.3754.

Fmoc–Ile_1–Ψ[CO–N(OCOCH$_2$CH$_3$)]–Val_2–Val_3–OBn (13). The title compound was prepared from (*S*,*S*,*S*)-**4** and propionic anhydride using method A. Eluent for flash chromatography: CH_2Cl_2–MeOH 95 : 5; colourless oil, 97% yield. R_f 0.65 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −36.5° (*c* 1.73 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.75 (2H, d, *J* 7.4, CH_{ar} Fmoc), 7.56 (2H, d, *J* 7.4, CH_{ar} Fmoc), 7.44–7.20 (10H, m, CH_{ar}, NH Val_3), 5.36 (1H, d, *J* 10.1, NH Ile_1), 5.10 (2H, ABq, *J* 12.3, $\delta_A - \delta_B$ 23.9, CH_2 –OBn), 4.54 (1H, dd, *J* 4.5 and 8.1, CH_α Val_3), 4.45–4.05 (5H, m, CH_α Ile_1, CH_α Val_2, CH Fmoc, CH_2 Fmoc), 2.63–2.45 (3H, m, CH_{iPr} Val_2, –COC*H*$_2$CH$_3$), 2.25–2.10 (1H, m, CH_{iPr} Val_3), 1.90–1.80 (1H, m, CH_{sBu} Ile_1), 1.60–1.45 (1H, m, CH*H* Ile_1), 1.23 (3H, t, *J* 7.2, –COCH$_2$C*H*$_3$,), 1.20–0.70 (19H, m, C*H*H Ile_1, CH_3 Ile_1, CH_3 Val_2, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 172.7 (2 peaks), 171.8, 168.0 (C=O), 156.3 (C=O Fmoc), 144.0, 143.9, 141.5 (C_{ar} Fmoc), 135.6 (C_{ar} –OBn), 129.3, 128.7, 128.5, 127.9, 127.2, 125.2, 120.1 (CH_{ar}), 67.2 (CH_2 Fmoc), 67.0 (2 peaks) (CH_2 –OBn, CH_α Val_2), 57.5 (CH_α Val_3), 55.6 (CH_α Ile_1), 47.4 (CH Fmoc), 36.8 (CH_{sBu} Ile_1), 31.1 (CH_{iPr} Val_3), 27.8 (CH_{iPr} Val_2), 25.4 (–CO*C*H$_2$–), 23.9 (CH_2 Ile_1), 20.0, 19.5, 19.0, 17.8 (CH_3 Val), 16.1 (CH_3), 11.2 (CH_3 Ile_1), 8.8 (–COCH$_2$*C*H$_3$). IR: 3338, 2964, 1800, 1723, 1654, 1525, 1236, 741. HRMS (ESI +) *m/z*: found, 736.3566; $C_{41}H_{51}N_3O_8Na$ requires 736.3574.

Fmoc–Ala_1–Ψ[CO–N(OCOCH$_2$CH$_3$)]–D-Val_2–Val_3–OBn (14). The title compound was prepared from (*S*,*R*,*S*)-**5** and propionic anhydride using method A. Eluent for flash chromatography: CH_2Cl_2–acetone 99 : 1 to 90 : 10; colourless oil, 80% yield. R_f 0.66 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +26.8° (*c* 1.58 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.74 (2H, d, *J* 7.4, CH_{ar} Fmoc), 7.57 (2H, dd, *J* 7.3 and 7.3, CH_{ar} Fmoc), 7.40–7.22 (9H, m, CH_{ar}), 7.21–7.12 (1H, br s, NH Val_3), 5.70 (1H, d, *J* 7.2, NH Ala_1), 5.13 (2H, ABq, *J* 12.3, $\delta_A - \delta_B$ 11.4, CH_2 –OBn), 4.52 (1H, dd, *J* 4.9 and 8.3,

CH_α Val_3), 4.50–4.27 (4H, m, CH_α Ala_1, CH_α Val_2, CH_2 Fmoc), 4.27–4.15 (1H, m, CH Fmoc), 2.60–2.40 (3H, m, CH_{iPr} Val_2, –$COCH_2CH_3$), 2.25–2.10 (1H, m, CH_{iPr} Val_3), 1.36 (3H, d, *J* 6.0, CH_3 Ala_1), 1.21 (3H, t, *J* 7.4, –$COCH_2CH_3$), 0.99 (6H, d, *J* 6.1, CH_3 Val_2), 0.91 (3H, d, *J* 6.8, CH_3 Val_3), 0.86 (3H, d, *J* 6.8, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 173.4, 172.3, 171.4, 167.9 (C=O), 155.5 (C=O Fmoc), 144.1, 143.9, 141.4 (2 peaks) (C_{ar} Fmoc), 135.6 (C_{ar} –OBn), 128.6, 128.4, 127.8, 127.2, 125.3, 125.2, 120.1 (CH_{ar}), 67.2 (CH_2 Fmoc), 67.1 (2 peaks) (CH_2 –OBn, CH_α Val_2), 57.5 (CH_α Val_3), 47.7 (CH_α Ala_1), 47.3 (CH Fmoc), 31.3 (CH_{iPr} Val_3), 27.0 (CH_{iPr} Val_2), 25.3 (–$COCH_2$–), 19.7, 19.1, 19.0, 17.8 (CH_3), 8.7 (–$COCH_2CH_3$). IR: 3338, 2966, 1802, 1724, 1664, 1529, 1451, 1245, 1060, 740. LRMS (DCI) *m*/*z*: 689.1 $(M + NH_4)^+$. HRMS (ESI +) *m*/*z*: found, 672.3292; $C_{38}H_{46}N_3O_8$ requires 672.3285.

Fmoc–Ile_1–Ψ[CO–N(OCO^tBu)]–D-Val_2–Val_3–OBn (15). The title compound was prepared from (*S*,*R*,*S*)-**4** and trimethylacetic anhydride using method A. Eluent for flash chromatography: CH_2Cl_2–MeOH 99.75 : 0.25 to 95 : 5; colourless oil, 87% yield. R_f 0.71 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +31.3° (*c* 3.08 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.73 (2H, d, *J* 7.2, CH_{ar} Fmoc), 7.57 (1H, d, *J* 7.2, CH_{ar} Fmoc), 7.51 (1H, d, *J* 7.2, CH_{ar} Fmoc), 7.43–7.14 (10H, m, CH_{ar}, NH Val_3), 5.47 (1H, d, *J* 9.1, NH Ile_1), 5.07 (2H, ABq, *J* 12.1, $\delta_A - \delta_B$ 26.9, CH_2 –OBn), 4.48 (1H, dd, *J* 4.7 and 7.7, CH_α Val_3), 4.43–4.03 (5H, m, CH_α Ile_1, CH_α Val_2, CH Fmoc, CH_2 Fmoc), 2.53–2.37 (1H, m, CH_{iPr} Val_2), 2.23–2.09 (1H, m, CH_{iPr} Val_3), 1.89–1.60 (1H, m, CH_{sBu} Ile_1), 1.59–1.41 (1H, m, CH*H* Ile_1), 1.34 (9H, s, CH_3 ^{t}Bu), 1.16–0.70 (19H, m, C*H*H Ile_1, CH_3 Ile_1, CH_3 Val_2, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 175.7, 173.1, 171.4, 167.9 (C=O), 156.2 (C=O Fmoc), 144.2, 143.9, 141.5 (C_{ar} Fmoc), 135.8 (C_{ar} –OBn), 128.6, 128.4, 127.8, 127.2, 125.4, 120.1 (CH_{ar}), 67.1, 66.9 (CH_α Val_2, CH_2 Fmoc, CH_2 –OBn), 57.6 (CH_α Val_3), 55.6 (CH_α Ile_1), 47.4 (CH Fmoc), 38.6, 38.4 (C ^{t}Bu, CH_{iPr} Val_2), 31.6 (CH_{iPr} Val_3), 27.2, 27.0 (CH_3 ^{t}Bu), 26.1 (CH_{sBu} Ile_1), 24.1 (CH_2 Ile_1), 19.9, 19.4, 19.2, 17.8, 15.7 (CH_3), 11.4 (CH_3 Ile_1). IR: 3342, 2966, 1795, 1732, 1661, 1520, 1228, 740. HRMS (ESI +) *m*/*z*: found, 742.4061; $C_{43}H_{56}N_3O_8$ requires 742.4067.

Fmoc–Ile_1–Ψ[CO–N(OCO^tBu)]–Val_2–Val_3–OBn (16). The title compound was prepared from (*S*,*S*,*S*)-**4** and trimethylacetic anhydride using method A. Eluent for flash chromatography: pentane–EtOAc 9 : 1 to 1 : 1; colourless oil, 15% yield. R_f 0.66 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −38.8° (*c* 2.46 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.74 (2H, d, *J* 7.2, CH_{ar} Fmoc), 7.56 (2H, d, *J* 6.8, CH_{ar} Fmoc), 7.45–7.15 (10H, m, CH_{ar}, NH Val_3), 5.42 (1H, d, *J* 9.1, NH Ile_1), 5.12 (2H, ABq, *J* 11.9, $\delta_A - \delta_B$ 21.9, CH_2 –OBn), 4.69–4.46 (1H, m, CH_α Val_3), 4.45–4.25 (3H, m, CH_α Ile_1, CH_2 Fmoc), 4.24–4.00 (2H, m, CH_α Val_2, CH Fmoc), 2.55–2.40 (1H, m, CH_{iPr} Val_2), 2.25–2.10 (1H, m, CH_{iPr} Val_3), 1.90–1.70 (1H, m, CH_{sBu} Ile_1), 1.64–1.48 (1H, m, CH*H* Ile_1), 1.36 (9H, s, CH_3 ^{t}Bu), 1.16–0.80 (19H, m, C*H*H Ile_1, CH_3 Ile_1, CH_3 Val_2, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 176.3, 172.6, 171.8, 167.5 (C=O), 156.2 (C=O Fmoc), 144.1, 143.9, 141.5 (C_{ar} Fmoc), 135.7 (C_{ar} –OBn), 128.8, 128.7, 128.6, 128.4, 127.9, 127.2, 125.3, 120.1 (CH_{ar}), 67.2, 67.0 (CH_α Val_2, CH_2 Fmoc, CH_2 –OBn), 57.6 (CH_α Val_3), 55.3 (CH_α Ile_1), 47.3 (CH Fmoc), 38.6 (C ^{t}Bu), 37.1 (CH_{sBu} Ile_1), 31.0 (CH_{iPr} Val_3), 27.6 (CH_{iPr} Val_2), 27.0 (CH_3 ^{t}Bu), 23.7 (CH_{sBu} Ile_1), 20.2 (CH_2 Ile_1), 19.4, 19.1, 19.0, 17.8 (CH_3 Val), 16.0, 11.2 (CH_3 Ile_1). IR: 3345, 2964, 1793, 1724, 1691, 1651, 1524, 1236, 1065, 741. HRMS (ESI +) *m*/*z*: found, 764.3894; $C_{43}H_{55}N_3O_8Na$ requires 764.3887.

Fmoc–Ala_1–Ψ[CO–N(OCOPh)]–D-Val_2–Val_3–OBn (17). The title compound was prepared from (*S*,*R*,*S*)-**5** and benzoic acid using method B (93% yield) or benzoic chloride using method C (83% yield). Eluent for flash chromatography: pentane–EtOAc 9 : 1 to 1 : 1; colourless oil. R_f 0.76 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +26.9° (*c* 1.06 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.12–8.00 (3H, m, CH_{ar}), 7.77–7.25 (16H, m, CH_{ar}, NH Val_3), 5.75 (1H, d, *J* 7.8, NH Ala_1), 5.15 (2H, ABq, *J* 12.6, $\delta_A - \delta_B$ 10.1, CH_2 –OBn), 4.63–4.45 (2H, m, CH_α Ala_1, CH_α Val_3), 4.39–4.26 (3H, m, CH_α Val_2, CH_2 Fmoc), 4.21–4.11 (1H, m, CH Fmoc), 2.70–2.55 (1H, m, CH_{iPr} Val_2), 2.30–2.15 (1H, m, CH_{iPr} Val_3), 1.37 (3H, d, *J* 6.4, CH_3 Ala_1), 1.08 (3H, d, *J* 6.7, CH_3 Val_2), 1.02 (3H, d, *J* 6.8, CH_3 Val_2), 0.94 (3H, d, *J* 6.8, CH_3 Val_3), 0.90 (3H, d, *J* 6.8, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 171.6 (C=O Ala_1), 171.0 (C=O Val_3), 168.0 (C=O Val_2), 164.9 (*C*OPh), 155.6 (C=O Fmoc), 144.1, 143.9, 141.4 (C_{ar} Fmoc), 135.6 (C_{ar} Ph), 135.0 (C_{ar} –OBn), 133.7, 130.5, 129.7, 129.1, 128.8, 128.7, 128.6, 128.5, 128.4, 127.9, 127.2, 126.2, 125.4, 125.3, 120.1 (CH_{ar}), 67.3, 67.1 (CH_α Val_2, CH_2 –OBn, CH_2 Fmoc), 57.6 (CH_α Val_3), 48.0 (CH_α Ala_1), 47.3 (CH Fmoc), 31.3 (CH_{iPr} Val_3), 27.2 (CH_{iPr} Val_2), 19.9, 19.5 (CH_3), 19.2 (CH_3 Ala_1), 18.9, 17.8 (CH_3). IR: 3334, 2965, 1773, 1718, 1666, 1539, 1451, 1236, 1006, 705. HRMS (ESI +) *m*/*z*: found, 742.3105; $C_{42}H_{45}N_3O_8Na$ requires 742.3104.

Fmoc–Ala_1–Ψ[CO–N(OCOPh)]–D-Ala_2–Val_3–OEt (18). The title compound was prepared from (*S*,*R*,*S*)-**7** and benzoic acid using method D. Eluent for flash chromatography: pentane–EtOAc 3 : 1; pale yellow oil, 60% yield. R_f 0.33 (pentane–EtOAc 3 : 1). $[\alpha]_D^{25}$ +24.75 (*c* 0.99 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.11 (2H, d, *J* 7.2, CH_{ar} Fmoc), 7.76 (2H, d, *J* 7.5, CH_{ar} Fmoc), 7.70–7.30 (10H, m, CH_{ar} Fmoc, Ph, NH Val_3), 5.63 (1H, d, *J* 7.5, NH Ala_1), 4.95 (1H, q, *J* 7.4, CH_α Ala_2), 4.65–4.45 (2H, m, CH_α Ala_1, CH_α Val_3), 4.40–4.00 (5H, m, CH_2 Fmoc, CH Fmoc, CH_2 –OEt), 2.35–2.10 (1H, m, CH_{iPr} Val_3), 1.52 (3H, d, *J* 7.2, CH_3 Ala_2), 1.42 (3H, d, *J* 6.8, CH_3 Ala_1), 1.35–1.10 (3H, m, CH_3 –OEt), 0.97 (3H, d, *J* 6.5, CH_3 Val_3), 0.94 (3H, d, *J* 6.8, CH_3 Val_3). δ_C (75 MHz, $CDCl_3$) 171.8, 169.3 (C=O), 165.6 (C=O Ph), 155.7 (C=O Fmoc), 144.0, 143.9, 141.4 (C_{ar} Fmoc), 135.2 (C_{ar} Ph), 130.6, 129.2, 127.9, 127.2, 125.3, 120.1 (CH_{ar}), 67.3 (CH_2 Fmoc), 61.2 (CH_2 –OEt), 59.2 (CH_α Ala_2), 57.8 (CH_α Val_3), 47.8 (CH_α Ala_1), 47.2 (CH Fmoc), 31.1 (CH_{iPr} Val_3), 19.2 (CH_3 Val_3), 18.2 (CH_3 Ala_1), 17.9 (CH_3 Val_3), 14.3 (CH_3 –OEt), 13.4 (CH_3 Ala_2). IR: 3350, 2957, 2928, 1765, 1683, 1534, 1447, 1232, 1009, 703. HRMS (ESI +) *m*/*z*: found, 652.2635; $C_{35}H_{39}N_3O_8Na$ requires 652.2635.

Fmoc–Ala_1–Ψ[CO–N($OCO(CH_2)_2CH{=}CH_2$)]–D-Val_2–Val_3–OBn (19). The title compound was prepared from (*S*,*R*,*S*)-**5** and 4-pentenoic acid using method B. Eluent for flash chromatography: pentane–EtOAc 9 : 1 to 1 : 1; colourless oil, 93%. R_f 0.73 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +12.9° (*c* 1.87 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.75 (2H, d, *J* 7.6, CH_{ar} Fmoc), 7.58 (2H, dd, *J* 7.3 and 7.3, CH_{ar} Fmoc), 7.45–7.25 (10H, m, CH_{ar}, NH Val_3), 5.95–5.72 (1H, m, –C*H*=CH_2), 5.69 (1H, d, *J* 7.7, NH Ala_1), 5.20–4.95 (4H, m, CH_2 –OBn, –CH=CH_2), 4.51 (1H, dd, *J* 5.0 and 8.3, CH_α Val_3), 4.49–4.25 (4H, m, CH_α Val_2, CH_α Ala_1, CH_2 Fmoc), 4.24–4.16 (1H, m, CH Fmoc), 2.65–2.35 (5H, m, CH_{iPr} Val_2, –$COCH_2CH_2$–), 2.25–2.00 (1H, m, CH_{iPr} Val_3), 1.34 (3H, d,

J 6.1, CH_3 Ala_1), 0.98 (6H, d, *J* 6.4, CH_3 Val_2), 0.91 (3H, d, *J* 6.8, CH_3 Val_3), 0.86 (3H, d, *J* 6.9, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 173.5 (C=O Ala_1), 171.4 (C=O Val_3), 171.1 (C=O), 168.0 (C=O Val_2), 155.6 (C=O Fmoc), 144.3, 144.1, 141.5 (C_{ar} Fmoc), 135.6 (–*C*H=CH_2, Car –OBn), 128.7, 128.5, 127.9, 127.2, 125.3, 120.1 (CH_{ar}), 116.7 (–CH=*C*H_2), 67.2 (2 peaks) (CH_2 –OBn, CH_2 Fmoc, CH_α Val_2), 57.6 (CH_α Val_3), 47.8 (CH_α Ala_1), 47.3 (CH Fmoc), 31.3 (CH_{iPr} Val_3), 31.1 (–CO*C*H_2–), 28.3 (–COCH_2*C*H_2–), 27.0 (CH_{iPr} Val_2), 19.7, 19.5, 19.2, 19.0, 17.8 (CH_3). IR: 3336, 2965, 1801, 1734, 1669, 1522, 1451, 1245, 1074, 741. HRMS (ESI +) *m*/*z*: found, 720.3255; $C_{40}H_{47}N_3O_8Na$ requires 720.3261.

Fmoc–Ala_1–Ψ[CO–N(OCO(CH_2)$_{14}$$CH_3$)]–D-$Val_2$–$Val_3$–OBn (20). The title compound was prepared from (*S*,*R*,*S*)-**5** and palmitoyl chloride using method C. Eluent for flash chromatography (using a mixture of silica gel and Na_2CO_3 (10%)): pentane–EtOAc 9 : 1 to 1 : 1; colourless oil, 90% yield. R_f 0.71 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +24.4° (*c* 1.64 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.68 (2H, d, *J* 7.4, CH_{ar} Fmoc), 7.51 (2H, dd, *J* 7.4 and 7.4, CH_{ar} Fmoc), 7.37–7.18 (9H, m, CH_{ar}), 7.14–7.00 (1H, m, NH Val_3), 5.58 (1H, d, *J* 7.2, NH Ala_1), 5.06 (2H, ABq, *J* 12.2, $\delta_A - \delta_B$ 12.6, CH_2 –OBn), 4.44 (1H, dd, *J* 4.9 and 8.5, CH_α Val_3), 4.41–4.19 (4H, m, CH_α Ala_1, CH_α Val_2, CH_2 Fmoc), 4.18–4.06 (1H, m, CH Fmoc), 2.52–2.30 (3H, m, CH_{iPr} Val_2, CH_2 α palmitoyl), 2.17–2.02 (1H, m, CH_{iPr} Val_3), 1.70–1.51 (2H, m, CH_2 β_β palmitoyl), 1.28 (3H, d, *J* 6.5, CH_3 Ala_1), 1.24–1.13 (24H, m, CH_2 palmitoyl), 0.91 (6H, d, *J* 6.4, CH_3 Val_2), 0.84 (3H, d, *J* 6.7, CH_3 Val_3), 0.79 (3H, d, *J* 7.6, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 173.5, 171.7, 171.5, 167.9 (C=O), 155.5 (C=O Fmoc), 144.1, 144.0, 141.5 (C_{ar} Fmoc), 135.6 (C_{ar} –OBn), 128.7, 128.5, 127.9, 127.3, 125.3, 120.1 (CH_{ar}), 67.2, 67.1 (CH_2 Fmoc, CH_α Val_2, CH_2 –OBn), 57.6 (CH_α Val_3), 47.8 (CH_α Ala_1), 47.3 (CH Fmoc), 32.1 (CH_2 palmitoyl), 31.8 (CH_2 palmitoyl), 31.4 (CH_{iPr} Val_3), 29.9, 29.8 (×3), 29.6, 29.5, 29.3, 29.2 (CH_2 palmitoyl), 26.9 (CH_{iPr} Val_2), 24.5, 22.9 (CH_2 palmitoyl), 19.8, 19.3, 19.2, 19.0, 17.8 (CH_3), 14.3 (CH_3 palmitoyl). IR: 3334, 2962, 1803, 1725, 1664, 1529, 1452, 1243, 1070, 740. LRMS (DCI) *m*/*z*: 871.6 $(M + NH_4)^+$. HRMS (ESI +) *m*/*z*: found, 876.5136; $C_{51}H_{71}N_3O_8Na$ requires 876.5139.

Fmoc–Gly_1–Ψ[CO–N(O–Ala_4–Boc)]–D-Ala_2–Leu_3–OEt (21). The title compound was prepared from (*R*,*S*)-**6** and Boc–Ala–OH using method D (69% yield) or method E (84% yield). Eluent for flash chromatography: CH_2Cl_2–MeOH 98 : 2; colourless oil. R_f 0.49 (CH_2Cl_2–MeOH 98 : 2). $[\alpha]_D^{25}$ −1.05 (*c* 2.09 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) 7.76 (2H, d, *J* 7.2, CH_{ar}), 7.60 (2H, d, *J* 7.2, CH_{ar}), 7.50–7.25 (4H, m, CH_{ar}), 7.03 (1H, d, *J* 7.2, NH Leu_3), 5.70–5.55 (1H, m, NH Gly_1), 5.27 (1H, d, *J* 7.9, NH Ala_4), 5.04 (1H, q, *J* 7.2, CH_α Ala_2), 4.65–4.30 (4H, m, CH_2 Fmoc, CH_α Ala_4, CH_α Leu_3), 4.30–4.00 (5H, m, CH Fmoc, CH_2 –OEt, CH_2 Gly_1), 1.75–1.35 (18H, m, CH_3 Ala_2, CH_3 Ala_4, CH_3 Boc, CH_2 Leu_3, CH_{iBu} Leu_3), 1.25 (3H, t, *J* 7.2, CH_3 –OEt), 0.93 (3H, d, *J* 5.4, CH_3 Leu_3), 0.91 (3H, d, *J* 5.8, CH_3 Leu_3). δ_C (75 MHz, $CDCl_3$) 172.9, 169.2 (C=O), 156.6, 155.3 (C=O Fmoc, C=O Boc), 143.9, 141.4 (C_{ar}), 127.8, 127.2, 125.2, 120.1 (CH_{ar}), 80.8 (C_q Boc), 67.5 (CH_2 Fmoc), 62.1 (CH_2 –OEt), 58.2 (CH_α Ala_2), 51.4, 51.2 (CH_α Ala_4, CH_α Leu_3), 47.2 (CH Fmoc), 40.8, 40.9 (CH_2 Gly_1, CH_2 Leu_3), 28.4 (CH_3 Boc), 25.0 (CH_{iBu} Leu_3), 22.9 (CH_3 Leu_3), 21.9, 21.7 (CH_3 Ala_4, CH_3 Leu_3), 14.2 (CH_3 –OEt, CH_3 Ala_2). IR: 3333, 2955, 2929, 1700, 1651, 1543, 1164, 1052, 741. HRMS (ESI +) *m*/*z*: found, 719.3272; $C_{36}H_{48}N_4O_{10}Na$ requires 719.3268.

Fmoc–Ala_1–Ψ[CO–N(O–Ala_4–Boc)]–D-Ala_2–Val_3–OEt (22). The title compound was prepared from (*S*,*R*,*S*)-**7** and Boc–Ala–OH using method D (86% yield) or method E (56% yield). Eluent for flash chromatography: CH_2Cl_2–MeOH 98 : 2; colourless oil. R_f 0.51 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −1.34 (*c* 1.66 in $CHCl_3$). δ_H (200 MHz; $CDCl_3$) 7.76 (2H, d, *J* 7.2, CH_{ar}), 7.58 (2H, d, *J* 7.2, CH_{ar}), 7.50–7.25 (5H, m, CH_{ar}, NH Val_3), 5.58 (1H, d, *J* 7.0, NH Ala_1), 5.30–5.10 (1H, m, NH Ala_4), 5.05–4.85 (1H, m, CH_α Ala_2), 4.70–4.00 (8H, m, CH Fmoc, CH_2 Fmoc, CH_α Ala_1, CH_α Val_3, CH_2 –OEt, CH_α Ala_4), 2.30–2.05 (1H, m, CH_{iPr} Val_3), 1.60–1.10 (21H, m + s (1.43) + t (1.24 *J* 7.1), CH_3 Ala_1, CH_3 Ala_2, CH_3 Ala_4, CH_3 Boc, CH_3 –OEt), 0.93 (3H, d, *J* 6.6, CH_3 Val_3), 0.90 (3H, d, *J* 6.8, CH_3 Val_3). δ_C (75 MHz, $CDCl_3$) 172.8, 171.8, 169.1 (C=O), 155.8, 155.3 (C=O Fmoc, C=O Boc), 143.9, 141.4 (C_{ar}), 127.9, 127.2, 125.2, 120.1 (CH_{ar}), 80.6 (C_q Boc), 67.4 (CH_2 Fmoc), 61.2 (CH_2 –OEt), 58.6, 58.0 (CH_α Val_3, CH_α Ala_2), 48.4, 47.7, 47.2 (CH_α Ala_4, CH_α Ala_1, CH Fmoc), 30.8 (CH_{iPr} Val_3), 28.4 (CH_3 Boc), 19.2 (CH_3 Val_3), 18.8 (CH_3 Ala_4), 18.1 (CH_3 Val_3), 17.5 (CH_3 Ala_1), 14.3 (CH_3 –OEt), 13.5 (CH_3 Ala_2). IR: 3334, 2972, 2920, 1793, 1695, 1524, 1250, 1049, 842, 738. HRMS (ESI +) *m*/*z*: found, 719.3266; $C_{36}H_{48}N_4O_{10}Na$ requires 719.3268.

Fmoc–Ala_1–Ψ[CO–N(O–Ala_4–Fmoc)]–D-Ala_2–Val_3–OEt (23). The title compound was prepared from (*S*,*R*,*S*)-**7** and Fmoc–Ala–OH using method D (61% yield) or method E (72% yield). Eluent for flash chromatography: CH_2Cl_2–MeOH 97 : 3; colourless oil. R_f 0.55 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −2.58 (*c* 3.18 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.74 (4H, d, *J* 7.4, CH_{ar}), 7.57 (4H, d, *J* 7.2, CH_{ar}), 7.45–7.25 (8H, m, CH_{ar}), 7.21 (1H, d, *J* 8.7, NH Val_3), 5.75–5.45 (2H, m, NH Ala_1, NH Ala_4), 5.04–4.91 (1H, m, CH_α Ala_2), 4.75–4.60 (1H, m, CH_α Ala_{1ou4}), 4.55–4.05 (7H, m, CH Fmoc, CH_2 Fmoc, CH_α Ala_{4ou1}, CH_α Val_3, CH_2 –OEt), 2.25–2.10 (1H, m, CH_{iPr} Val_3), 1.60–1.25 (9H, m, CH_3 Ala_1, CH_3 Ala_2, CH_3 Ala_4), 1.19 (3H, t, *J* 7.2, CH_3 –OEt), 0.93 (3H, d, *J* 6.9, CH_3 Val_3), 0.89 (3H, d, *J* 6.9, CH_3 Val_3). δ_C (75 MHz, $CDCl_3$) 172.0, 169.1 (C=O), 155.9 (2 peaks) (C=O Fmoc), 143.9 (2 peaks), 141.5 (C_{ar}), 127.9, 127.2, 125.2, 120.1 (CH_{ar}), 67.4 (2 peaks) (CH_2 Fmoc), 61.4 (CH_2 –OEt), 58.0 (CH_α Val_3, CH_α Ala_2), 48.8, 47.7, 47.2 (CH_α Ala_4, CH_α Ala_1, CH Fmoc), 30.7 (CH_{iPr} Val_3), 19.2 (CH_3 Val_3), 18.1, 17.8 (CH_3 Val_3, CH_3 Ala_4, CH_3 Ala_1), 14.3 (CH_3 –OEt), 13.6 (CH_3 Ala_2). IR: 3330, 2965, 1790, 1695, 1530, 1445, 1245, 1050, 740. HRMS (ESI +) *m*/*z*: found, 841.3435; $C_{46}H_{50}N_4O_{10}Na$ requires 841.3425.

Fmoc–Ala_1–Ψ[CO–N(O–Leu–Boc)]–D-Ala_2–Val_3–OEt (24). The title compound was prepared from (*S*,*R*,*S*)-**7** and Boc–Leu–OH using method D (67% yield) or method E (77% yield). Eluent for flash chromatography: pentane–EtOAc 2 : 1; colourless oil. R_f 0.53 (pentane–EtOAc 2 : 1). $[\alpha]_D^{25}$ −9.57 (*c* 1.4 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.76 (2H, d, *J* 6.8, CH_{ar}), 7.58 (2H, d, *J* 7.6, CH_{ar}), 7.50–7.20 (5H, m, CH_{ar}, NH Val_3), 5.57 (1H, d, *J* 7.8, NH Ala_1), 5.12 (1H, d, *J* 8.6, NH Leu), 5.00–4.80 (1H, m, CH_α Ala_2), 4.60–4.00 (8H, m, CH Fmoc, CH_2 Fmoc, CH_α Ala_1, CH_α Val_3, CH_2 –OEt, CH_α Leu), 2.30–2.10 (1H, m, CH_{iPr} Val_3), 1.90–1.60 (3H, m, CH_2 Leu_4, CH_{iBu} Leu), 1.60–1.20 (18H, m, CH_3 Ala_1, CH_3 Ala_2, CH_3 Boc, CH_3 –OEt), 1.10–0.80 (12H, m, CH_3 Val_3, CH_3 Leu). δ_C (75 MHz, $CDCl_3$) 172.7, 171.8, 171.3, 169.0 (C=O), 155.8, 155.6 (C=O Fmoc, C=O Boc), 144.0, 141.4 (C_{ar}), 127.9,

127.2, 125.2, 120.1 (CH_{ar}), 80.7 (C_q Boc), 67.4 (CH_2 Fmoc), 61.2 (CH_2 –OEt), 60.5 (CH_α Leu), 57.9 (CH_α Val_3), 51.4 (CH_α Ala_2), 47.6, 47.2 (CH_α Ala_1, CH Fmoc), 40.3 (CH_2 Leu), 30.9 (CH_{iPr} Val_3), 28.4 (CH_3 Boc), 25.0 (CH_{iBu} Leu), 23.0, 21.5, 21.2, 19.2, 18.1 (CH_3), 14.3 (CH_3 –OEt, CH_3 Ala_2). IR: 3345, 2931, 1695, 1535, 1447, 1250, 1162, 1028, 738. HRMS (ESI +) *m/z*: found, 761.3739; $C_{39}H_{54}N_4O_{10}Na$ requires 761.3738.

Fmoc–Ala_1–Ψ[CO–N(O–βAla–Boc)]–D-Ala_2–Val_3–OEt (25). The title compound was prepared from (*S*,*R*,*S*)-**7** and Boc–βAla–OH using method D (88% yield). Eluent for flash chromatography: CH_2Cl_2; colourless oil. R_f 0.38 (CH_2Cl_2). $[\alpha]_D^{25}$ +3.38 (*c* 1.42 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.75 (2H, d, *J* 7.5, CH_{ar}), 7.57 (1H, d, *J* 7.1, CH_{ar}), 7.56 (1H, d, *J* 6.8, CH_{ar}), 7.40–7.25 (5H, m, CH_{ar}, NH Val_3), 5.70–5.50 (2H, m, NH Ala_1, NH βAla), 4.99 (1H, q, *J* 7.1, CH_α Ala_2), 4.60–4.40 (2H, m, CH_α Val_3, CH_α Ala_1), 4.40–4.30 (2H, m, CH_2 Fmoc), 4.30–4.10 (3H, m, CH Fmoc, CH_2 –OEt), 3.55–3.45 (2H, m, C*H*$_2$NH), 2.80–2.70 (2H, m, C*H*$_2$$CH_2$NH), 2.30–2.10 (1H, m, CH_{iPr} Val_3), 1.55–1.20 (18H, m, CH_3 Ala_1, CH_3 Ala_2, CH_3 Boc, CH_3 –OEt), 0.94 (3H, d, *J* 6.9, CH_3 Val_3), 0.90 (3H, d, *J* 7.2, CH_3 Val_3). δ_C (75 MHz, $CDCl_3$) 172.0, 169.3 (C=O), 156.1, 155.8 (C=O Fmoc, C=O Boc), 143.9, 141.4 (C_{ar}), 127.9, 127.3, 125.3, 120.2 (CH_{ar}), 79.6 (C_q Boc), 67.4 (CH_2 Fmoc), 61.4 (CH_2 –OEt), 57.8 (CH_α Val_3), 47.6, 47.5, 47.3 (CH_α Ala_2, CH_α Ala_1, CH Fmoc), 36.4 (*C*H_2NH), 32.8 (*C*$H_2$$CH_2$NH), 29.9 ($CH_{iPr}$ Val_3), 28.5 (CH_3 Boc), 19.2 (CH_3 Val_3), 17.9, 17.8 (CH_3 Val_3, CH_3 Ala_1), 14.4 (CH_3 –OEt), 14.3 (CH_3 Ala_2). IR: 3347, 2963, 2923, 1793, 1694, 1515, 1246, 740. HRMS (ESI +) *m/z*: found, 719.3268; $C_{36}H_{48}N_4O_{10}Na$ requires 719.3268.

Fmoc–Ala_1–Ψ[CO–N(O–Gly–Boc)]–D-Val_2–Val_3–OBn (26). The title compound was prepared from (*S*,*R*,*S*)-**5** and Boc–Gly–OH using method B (88% yield) or method D (74% yield). Eluent for flash chromatography: pentane–EtOAc 9 : 1 to 1 : 1; white solid. Mp: 66 °C. R_f 0.35 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +21.0° (*c* 1.03 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.75 (2H, d, *J* 7.4, CH_{ar} Fmoc), 7.58 (2H, dd, *J* 6.1 and 6.1, CH_{ar} Fmoc), 7.42–7.18 (9H, m, CH_{ar}), 7.15–7.00 (1H, m, NH Val_3), 5.62 (1H, d, *J* 8.1, NH Ala_1), 5.20–5.00 (3H, m, CH_2 –OBn, NH Gly), 4.50 (1H, dd, *J* 4.7 and 8.3, CH_α Val_3), 4.45–4.27 (4H, m, CH_α Ala_1, CH_α Val_2, CH_2 Fmoc), 4.26–4.15 (1H, m, CH Fmoc), 4.05 (2H, d, *J* 8.1, CH_2 Gly), 2.58–2.40 (1H, m, CH_{iPr} Val_2), 2.26–2.10 (1H, m, CH_{iPr} Val_3), 1.43 (9H, s, CH_3 Boc), 1.36 (3H, d, *J* 5.8, CH_3 Ala_1), 0.98 (6H, d, *J* 6.5, CH_3 Val_2), 0.90 (3H, d, *J* 6.8, CH_3 Val_3), 0.86 (3H, d, *J* 6.8, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 173.6 (C=O), 171.4 (C=O Gly), 167.9 (C=O), 155.7, 155.6 (C=O Fmoc, C=O Boc), 144.0, 143.9, 141.4 (C_{ar} Fmoc), 135.5 (C_{ar} –OBn), 128.6, 128.5, 127.8, 127.2, 125.3, 120.1 (CH_{ar}), 80.5 (C_q Boc), 67.2, 67.1, (CH_2 Fmoc, CH_2 –OBn, CH_α Val_2), 57.6 (CH_α Val_3), 47.9 (CH_α Ala_1), 47.2 (CH Fmoc), 41.4 (CH_2 Gly), 31.1 (CH_{iPr} Val_3), 28.3 (CH_3 Boc), 28.0 (CH_{iPr} Val_2), 19.7, 19.1, 18.5, 17.8 (CH_3). IR: 3338, 2967, 1803, 1716, 1694, 1524, 1251, 740. HRMS (ESI +) *m/z*: found, 773.3786; $C_{42}H_{53}N_4O_{10}$ requires 773.3762.

Fmoc–Ile_1–Ψ[CO–N(O–Gly–Boc)]–Val_2–Val_3–OBn (27). The title compound was prepared from (*S*,*S*,*S*)-**4** and Boc–Gly–OH using method B (85% yield). Eluent for flash chromatography: pentane–EtOAc 9 : 1 to 1 : 1; white fluffy solid. Mp: 71–72 °C. R_f 0.50 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −42.5° (*c* 1.01 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.75 (2H, d, *J* 7.2, CH_{ar} Fmoc), 7.56 (2H, d, *J* 5.6, CH_{ar} Fmoc), 7.46–7.20 (10H, m, CH_{ar}, NH Val_3), 5.50–5.36 (1H, m, NH Ile_1), 5.29–5.24 (1H, m, NH Gly), 5.10 (2H, ABq, *J* 11.9, $\delta_A - \delta_B$ 24.7, CH_2 –OBn), 4.60–3.90 (8H, m, CH_α Ile_1, CH_α Val_2, CH_α Val_3, CH_2 Gly, CH Fmoc, CH_2 Fmoc), 2.60–2.40 (1H, m, CH_{iPr} Val_2), 2.27–2.07 (1H, m, CH_{iPr} Val_3), 1.96–1.73 (1H, m, CH_{sBu} Ile_1), 1.58–1.30 (10H, m + s (1.40), CH*H* Ile_1, CH_3 Boc), 1.17–0.75 (19H, m, C*H*H Ile_1, CH_3 Ile_1, CH_3 Val_2, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 172.6 (C=O), 171.9 (C=O Val_3), 169.4, 168.0 (C=O), 156.4, 155.7 (C=O Fmoc, C=O Boc), 143.8, 141.5 (C_{ar} Fmoc), 135.5 (C_{ar} –OBn), 128.8, 128.7, 128.5 127.9, 127.2, 125.2 (2 peaks), 120.1 (CH_{ar}), 80.4 (C_q Boc), 67.3, 67.1 (CH_2 Fmoc, CH_α Val_2, CH_2 –OBn), 57.4 (CH_α Val_3), 55.6 (CH_α Ile_1), 47.3 (CH Fmoc), 41.5 (CH_2 Gly), 36.3 (CH_{sBu} Ile_1), 31.1 (CH_{iPr} Val_3), 28.4 (CH_3 Boc), 27.8 (CH_{iPr} Val_2), 24.1 (CH_2 Ile_1), 19.8, 19.5, 19.0, 17.9 (CH_3 Val), 15.9, 11.1 (CH_3 Ile_1). IR: 3341, 2966, 1801, 1718, 1653, 1521, 1244, 1155, 1086, 741. LRMS (DCI) *m/z*: 832.4 (M + NH_4)$^+$. HRMS (ESI +) *m/z*: found, 837.4057; $C_{45}H_{58}N_4O_{10}Na$ requires 837.4051.

Chemical modifications

Fmoc–Ala_1–Ψ[CO–N(O–Gly_4–Ala_5–Boc)]–D-Val_2–Val_3–OBn (28). A solution of **26** (46 mg, 60 μmol) in TFA (1.6 mL) was stirred at room temperature for 1 h before volatile compounds were evaporated under reduced pressure.

A mixture of Boc–Ala–OH (15 mg, 78 μmol) and HOBt (11 mg, 78 μmol) in CH_2Cl_2 (4.5 mL) was stirred at room temperature. EDCI (15 mg, 78 μmol) and DIEA (31 μL, 180 μmol) were added and the resulting solution was transferred on the trifluoroacetic salt of deprotected **26**. The mixture was stirred overnight at room temperature. The solution was washed with brine and the organic layer dried over Na_2SO_4. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel, pentane–EtOAc 9 : 1) gave **28** (36 mg, 43 μmol, 72%) as a white solid. Mp: 73 °C. R_f 0.41 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +10.6° (*c* 1.02 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.75 (2H, d, *J* 7.3, CH_{ar} Fmoc), 7.62–7.53 (2H, m, CH_{ar} Fmoc), 7.44–7.24 (9H, m, CH_{ar}), 7.07 (1H, d, *J* 8.1, NH Val_3), 7.02–6.88 (1H, br, NH Ala_5), 5.64 (1H, d, *J* 8.1, NH Ala_1), 5.36–5.22 (1H, br, NH Gly_4), 5.13 (2H, ABq, *J* 12.2, $\delta_A - \delta_B$ 19.1, CH_2 –OBn), 4.55–4.43 (2H, m, CH_α Val_3, CH_α Ala), 4.40–4.27 (3H, m, CH_α Val_2, CH*H* Gly, CH*H* Fmoc), 4.27–4.15 (3H, m, CH Fmoc, C*H*H Fmoc, CH_α Ala), 4.08–3.97 (1H, m, C*H*H Gly), 2.57–2.41 (1H, m, CH_{iPr} Val_2), 2.26–2.11 (1H, m, CH_{iPr} Val_3), 1.40 (9H, s, CH_3 Boc), 1.38–1.30 (6H, m, CH_3 Ala_1, CH_3 Ala_5), 0.97 (6H, d, *J* 6.8, CH_3 Val_2), 0.90 (3H, d, *J* 6.9, CH_3 Val_3), 0.86 (3H, d, *J* 6.9, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 173.9, 173.5, 171.6, 168.5, 167.9 (C=O), 155.7 (2 peaks) (C=O Boc, C=O Fmoc), 144.0, 143.9, 141.5 (C_{ar} Fmoc), 135.5 (C_{ar}–OBn), 128.8, 128.7, 128.6 (2 peaks), 128.5, 127.9, 127.3, 125.3, 120.2 (CH_{ar}), 80.4 (C_q Boc), 67.3, 67.2 (CH_2 Fmoc, CH_α Val_2, CH_2 –OBn), 57.8 (CH_α Val_3), 50.0 (CH_α Ala_5), 47.9 (CH_α Ala_1), 47.3 (CH Fmoc), 40.2 (CH_2 Gly), 31.1 (CH_{iPr} Val_3), 28.5 (CH_3 Boc), 27.2 (CH_{iPr} Val_2), 19.8, 19.3, 19.2, 18.8, 17.9 (CH_3). IR: 3328, 2967, 2930, 1801, 1711, 1696, 1670, 1535, 1452, 1248, 741. LRMS (DCI) *m/z*: 861.5 (M + NH_4)$^+$. HRMS (ESI +) *m/z*: found, 882.3690; $C_{45}H_{57}N_5O_{11}K$ requires 882.3692.

Boc–Ile_1–Ψ[CO–N(OAc)]–Val_2–Val_3–OH (29). A mixture of **11** (21 mg, 37 μmol) and Pd/C (12 mg, 10%) in EtOH (2 mL) was placed under an atmosphere of hydrogen. The suspension

was stirred at room temperature for one hour, then filtered. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel, CH_2Cl_2–MeOH 95 : 5) gave **29** (16 mg, 34 μmol, 91%) as a colourless oil. R_f 0.04 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −52.4° (*c* 1.04 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.55–7.45 (1H, br, NH Val_3), 4.99 (1H, d, *J* 9.6, NH Ile_1), 4.55–4.40 (1H, m, CH_α Val_3), 4.35–4.20 (2H, m, CH_α Ile_1, CH_α Val_2), 2.66–2.43 (1H, m, CH_{iPr} Val_2), 2.39–2.10 (4H, m + s (2.26), CH_{iPr} Val_3, CH_3 Ac), 1.92–1.71 (1H, m, CH_{sBu} Ile_1), 1.66–1.37 (10H, m, CH_2 Ile_1, CH_3 Boc), 1.15–0.80 (19H, m, CH_2 Ile_1, CH_3 Ile_1, CH_3 Val_2, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 175.0, 172.9 (C=O), 169.5 (C=O Ac), 168.2 (C=O), 155.6 (C=O Boc), 80.1 (C_q Boc), 61.8 (CH_α Val_2), 57.6 (CH_α Val_3), 54.8 (CH_α Ile_1), 36.7 (CH_{sBu} Ile_1), 30.6 (CH_{iPr} Val_3), 28.4 (CH_3 Boc), 27.5 (CH_{iPr} Val_2), 24.0 (CH_2 Ile_1), 20.0, 19.2 (CH_3 Val), 18.9 (CH_3 Ac), 18.6, 17.9 (CH_3 Val), 16.1, 11.3 (CH_3 Ile_1). IR: 3333, 2966, 2931, 1807, 1717, 1656, 1524, 1392, 1368, 1173. LRMS (DCI) *m/z*: 504.9 $(M + NH_4)^+$. HRMS (ESI +) *m/z*: found, 510.2783; $C_{23}H_{41}N_3O_8Na$ requires 510.2791.

Boc–Ile_1–Ψ[CO–N(OAc)]–Val_2–Val_3–Phe_4–OBn (30). To a mixture of **29** (17 mg, 34 μmol), L-phenylalanine benzyl ester hydrochloride (11 mg, 37 μmol), EDCI (8 mg, 41 μmol) and HOBt (6 mg, 41 μmol) in CH_2Cl_2 (1.5 mL) was added DIEA (7 μL, 38 μmol). The solution was stirred overnight at room temperature, washed with brine and the organic layer dried over Na_2SO_4. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel, pentane–EtOAc 9 : 1 to 1 : 1) gave **30** (18 mg, 25 μmol, 74%) as a white-yellowish solid. Mp: 133 °C. R_f 0.37 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −28.5° (*c* 0.46 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 7.55–7.40 (1H, br, NH Val_3), 7.37–7.15 (8H, m, CH_{ar} Phe_4, –OBn), 7.10–7.00 (2H, m, CH_{ar} Phe_4), 6.52 (1H, d, *J* 5.9 Hz, NH Phe_4), 5.10 (2H, ABq, *J* 12.3 Hz, $\delta_A - \delta_B$ 11.8 Hz, CH_2 –OBn), 4.96 (1H, d, *J* 9.9 Hz, NH Ile_1), 4.90–4.80 (1H, m, CH_α Phe_4), 4.32–4.15 (2H, m, CH_α Ile_1, CH_α Val_3), 4.00–3.90 (1H, m, CH_α Val_2), 3.20–3.05 (2H, m, CH_2 Phe_4), 2.65–2.50 (1H, m, CH_{iPr} Val_2), 2.30–2.10 (4H, m + s (2.22), CH_{iPr} Val_3, CH_3 Ac), 1.90–1.70 (1H, m, CH_{sBu} Ile_1), 1.60–1.45 (1H, m, CH_2 Ile_1), 1.38 (9H, s, CH_3 Boc), 1.15–0.80 (19H, m, CH_2 Ile_1, CH_3 Ile_1, CH_3 Val_2, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 172.7, 171.2, 170.8, 169.5, 168.4 (C=O), 155.5 (C=O Boc), 136.1 (C_{ar} Phe_4), 135.4 (C_{ar} –OBn), 129.5, 128.7, 128.6, 127.1 (CH_{ar}), 80.0 (C_q Boc), 67.3 (CH_α Val_2, CH_2 –OBn), 58.9 (CH_α Val_3), 54.9 (CH_α Ile_1), 53.5 (CH_α Phe_4), 37.9 (CH_2 Phe_4), 36.8 (CH_{sBu} Ile_1), 30.1 (CH_{iPr} Val_3), 28.4 (CH_3 Boc), 27.4 (CH_{iPr} Val_2), 23.9 (CH_2 Ile_1), 20.0, 19.6, 19.4, 18.6, 17.7 (CH_3 Val_2, CH_3 Val_3, CH_3 Ac), 16.2, 11.3 (CH_3 Ile_1). IR: 3310, 2964, 2928, 1807, 1714, 1650, 1524, 1176, 698. LRMS (DCI) *m/z*: 742.3 $(M + NH_4)^+$. HRMS (ESI +) *m/z*: found, 747.3948; $C_{39}H_{56}N_4O_9Na$ requires 747.3945.

CH_3CH_2CO–Ala_1–Ψ[CO–N(OH)]–D-Val_2–Val_3–OBn (31). To a solution of **14** (56 mg, 83 μmol) in THF (1 mL) was added diethylamine (52 μL, 500 μmol). The mixture was stirred for 4 h at room temperature. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel, pentane–EtOAc 9 : 1 to 1 : 1) gave **31** (35 mg, 78 μmol, 94%) as a white solid. Mp: 190 °C. R_f 0.78 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +24.0° (*c* 1.28 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.96 (1H, s, NOH), 7.45–7.25 (5H, m, CH_{ar}), 6.98 (1H, d, *J* 8.3, NH Val_3), 6.55 (1H, d, *J* 7.3, NH Ala_1), 5.20–5.08 (1H, m, CH_α Ala_1), 5.15 (2H, ABq, *J* 12.2, $\delta_A - \delta_B$ 24.2, CH_2 –OBn), 4.74 (1H, d, *J* 9.6, CH_α Val_2), 4.54 (1H, dd, *J* 4.8 and 8.3, CH_α Val_3), 2.54–2.39 (1H, m, CH_{iPr} Val_2), 2.30–2.15 (3H, m + q (2.23, *J* 7.5), CH_{iPr} Val_3, $-COCH_2-$), 1.37 (3H, d, *J* 6.9, CH_3 Ala_1), 1.12 (3H, t, *J* 7.5, $-COCH_2CH_3$), 1.03 (3H, d, *J* 6.9, CH_3 Val_3), 0.97 (3H, d, *J* 6.8, CH_3 Val_2), 0.92 (3H, d, *J* 6.8, CH_3 Val_2), 0.88 (3H, d, *J* 6.9, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 173.9 (C=O Val_3), 173.0 ($-COCH_2-$), 172.0 (C=O Ala_1), 170.4 (C=O Val_2), 135.1 (C_{ar} –OBn), 128.5 (CH_{ar}), 67.3 (CH_2 –OBn), 64.4 (CH_α Val_2), 57.5 (CH_α Val_3), 45.4 (CH_α Ala_1), 30.7 (CH_{iPr} Val_3), 29.4 ($-COCH_2-$), 27.4 (CH_{iPr} Val_2), 19.6, 19.0, 17.8, 17.7, 17.6 (CH_3), 9.6 ($-CH_2CH_3$). IR: 3256, 3062, 2965, 2934, 2876, 1734, 1675, 1645, 1611, 1550, 1456, 1268, 735. LRMS (DCI) *m/z*: 466.4 $(M + NH_4)^+$. HRMS (ESI +) *m/z*: found, 472.2415; $C_{23}H_{35}N_3O_6Na$ requires 472.2424.

PhCO–Ala_1–Ψ[CO–N(OH)]–D-Val_2–Val_3–OBn (32). The title compound was prepared as described for **31** using **17** (26 mg, 37 μmol) and, after flash chromatography (silica gel, CH_2Cl_2–MeOH 99.5 : 0.5 to 98 : 2), was obtained as a white solid (17 mg, 34 μmol, 79%). Mp: 174 °C. R_f 0.45 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +57.2° (*c* 1.02 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 9.12 (1H, s, NOH), 7.86–7.80 (2H, m, CH_{ar}), 7.54–7.23 (9H, m, CH_{ar}, NH Ala_1), 7.10 (1H, d, *J* 8.3, NH Val_3), 5.45–5.32 (1H, m, CH_α Ala_1), 5.07 (2H, ABq, *J* 12.2, $\delta_A - \delta_B$ 25.4, CH_2 –OBn), 4.75 (1H, d, *J* 9.7, CH_α Val_2), 4.52 (1H, dd, *J* 4.9 and 8.3, CH_α Val_3), 2.50–2.31 (1H, m, CH_{iPr} Val_2), 2.27–2.10 (1H, m, CH_{iPr} Val_3), 1.49 (3H, d, *J* 6.9, CH_3 Ala_1), 1.00 (3H, d, *J* 6.8, CH_3 Val_2), 0.93 (3H, d, *J* 6.8, CH_3 Val_2), 0.91 (3H, d, *J* 6.9, CH_3 Val_3), 0.86 (3H, d, *J* 6.9, CH_3 Val_3). δ_C (75 MHz; $CDCl_3$) 173.0, 171.7, 171.1, 167.3 (C=O), 135.3 (C_{ar} –OBn), 134.2 (C_{ar} –Ph), 131.8, 128.8, 128.6 (2 peaks), 127.5 (CH_{ar}), 67.4 (CH_2 –OBn), 64.1 (CH_α Val_2), 57.8 (CH_α Val_3), 46.4 (CH_α Ala_1), 31.0 (CH_{iPr} Val_3), 27.7 (CH_{iPr} Val_2), 19.6, 19.2, 18.2, 18.0 (CH_3). IR: 3272, 3063, 2963, 2925, 1742, 1670, 1644, 1611, 1543, 1463, 1267, 1185, 694. LRMS (DCI) *m/z*: 497.7 $[M]^+$. HRMS (ESI +) *m/z*: found, 520.2424; $C_{27}H_{35}N_3O_6Na$ requires 520.2424.

Boc–Leu_1–Ala_2–Ψ[CO–N(OH)]–D-Ala_3–Val_4–OEt (33). To a solution of **24** (34 mg, 45 μmol) in THF (1 mL) was added diethylamine (29 μL, 278 μmol). The mixture was stirred overnight at 40 °C. After evaporation of the solvent under reduced pressure, the residue was taken up in CH_2Cl_2 and the solution was washed with brine. The organic layer was dried over Na_2SO_4. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel, CH_2Cl_2–MeOH 100 : 0 to 98 : 2) gave **33** (17 mg, 33 μmol, 71%) as a yellowish oil. R_f 0.21 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +5.7° (*c* 0.86 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.6 (1H, s, NOH), 7.35–7.22 (1H, m, NH Ala_2), 7.10–6.95 (1H, m, NH Val_4), 5.40–5.25 (1H, m, CH_α Ala_3), 5.15–4.95 (2H, m, NH Leu_1, CH_α Ala_2), 4.41 (1H, dd, *J* 5.4 and 8.3, CH_α Val_4), 4.30–4.20 (1H, m, CH_α Leu_1), 4.20–4.05 (2H, m, CH_2 –OEt), 2.20–2.06 (1H, m, CH_{iPr} Val_4), 1.65–1.48 (2H, m, CH_2 Leu_1), 1.43 (3H, d, *J* 6.5, CH_3 Ala_3), 1.38 (9H, s, CH_3 Boc), 1.29 (3H, d, *J* 6.7, CH_3 Ala_2), 1.23–1.15 (4H, m, CH_{iBu} Leu_1, CH_3 –OEt), 0.89 (3H, d, *J* 6.1, CH_3 Val_4), 0.86 (3H, d, *J* 6.9, CH_3 Val_4), 0.82 (6H, d, *J* 5.8, CH_3 Leu_1). δ_C (75 MHz; $CDCl_3$) 172.7, 172.4 (C=O), 155.8 (C=O Boc), 80.0 (C_q Boc), 61.8 (CH_2 –OEt), 57.7 (CH_α Val_4), 54.0 (CH_α Ala_3), 53.0 (CH_α Leu_1), 45.9 (CH_α Ala_2), 42.4 (CH_2 Leu_1), 31.0 (CH_{iPr} Val_4), 28.5 (CH_3 Boc), 24.8 (CH_{iBu} Leu_1), 23.0 (CH_3 Leu_1), 22.4 (CH_3 Leu_1), 19.2 (CH_3 Val_4), 18.2 (CH_3 Val_4), 17.9 (CH_3 Ala_2), 14.7 (CH_3 Ala_3), 14.3 (CH_3 –OEt). IR: 3310, 2964, 2929, 1736, 1670,

1630, 1528, 1170, 1025. HRMS (ESI +) m/z: found, 539.3046; $C_{24}H_{44}N_4O_8Na$ requires 539.3057.

Ac–Ile$_1$–Ψ[CO–N(OH)]–Val$_2$–Val$_3$–Phe$_4$–OBn (34). A solution of **30** (14 mg, 19 μmol) in TFA (1 mL) was stirred at room temperature for 2 h before volatile compounds were evaporated under reduced pressure. The residue was taken up in CH_2Cl_2 (5 mL) and an aqueous solution of $NaHCO_3$ (1 M, 5 mL) was added. The biphasic solution was vigorously stirred overnight at room temperature. The organic layer was dried over Na_2SO_4. Evaporation of the solvent under reduced pressure followed by purification by flash chromatography (silica gel, CH_2Cl_2–MeOH 100 : 0 to 95 : 5) gave **34** (8 mg, 13 μmol, 70%) as a white solid. Mp: 235–236 °C. R_f 0.16 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −23.5° (c 0.76 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.70–8.45 (2H, m + s (8.53), NH Val$_3$, NOH), 7.50–7.40 (1H, m, NH Ile$_1$), 7.38–7.15 (8H, m, CH_{ar}), 7.05–6.95 (2H, m, CH_{ar} Phe$_4$), 6.85–6.75 (1H, br, NH Phe$_4$), 5.25–5.05 (4H, m, CH_2 –OBn, CH_α Ile$_1$, CH_α Val$_2$), 4.95–4.85 (1H, m, CH_α Phe$_4$), 4.40–4.30 (1H, m, CH_α Val$_3$), 3.10–3.02 (2H, m, CH_2 Phe$_4$), 2.40–2.25 (1H, m, CH_{iPr} Val$_2$), 2.10–1.90 (4H, m + s (1.94), CH_{iPr} Val$_3$, CH_3 Ac), 1.85–1.75 (1H, m, CH_{sBu} Ile$_1$), 1.55–1.40 (1H, m, CH*H* Ile$_1$), 1.20–1.10 (1H, m, C*H*H Ile$_1$), 0.95–0.75 (18H, m, CH_3 Ile$_1$, CH_3 Val$_2$, CH_3 Val$_3$). δ_C (75 MHz; $CDCl_3$) 172.4, 171.8, 171.3, 171.0, 170.9 (C=O), 135.4 (C_{ar} Phe), 135.0 (C_{ar} –OBn), 129.3, 128.9, 128.8 (2 peaks), 127.5 (CH_{ar}), 67.7 (CH_2 –OBn), 63.7 (CH_α Val$_2$), 58.4 (CH_α Val$_3$), 53.5 (CH_α Ile$_1$), 53.2 (CH_α Phe$_4$), 38.1 (CH_2 Phe$_4$), 37.4 (CH_{sBu} Ile$_1$), 31.6 (CH_{iPr} Val$_3$), 27.7 (CH_{iPr} Val$_2$), 24.9 (CH_2 Ile$_1$), 22.9 (CH_3 Ac), 19.9, 19.2, 19.0 (CH_3 Val$_2$, CH_3 Val$_3$), 14.3, 11.3 (CH_3 Ile$_1$). IR: 3294, 3060, 2961, 2925, 2854, 1741, 1651, 1628, 1548, 1455, 1378, 1186, 698. HRMS (ESI +) m/z: found, 647.3416; $C_{34}H_{48}N_4O_7Na$ requires 647.3421.

Ac–Ile$_1$–Ψ[CO–N(OH)]–Val$_2$–Val$_3$–OBn (35). The title compound was prepared as described for **34** using **11** (9 mg, 16 μmol). Purification by flash chromatography (silica gel, $CHCl_3$–acetone 10 : 0 to 9 : 1) gave **34** (7 mg, 14 μmol, 92%) as a white solid. Mp: 137 °C. R_f 0.23 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ −25.4° (c 0.56 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.51 (1H, s, NOH), 8.40–8.23 (1H, m, NH Val$_3$), 7.40–7.30 (5H, br s, CH_{ar} –OBn), 7.05–6.90 (1H, m, NH Ile$_1$), 5.28–5.05 (4H, m, CH_2 –OBn, CH_α Ile$_1$, CH_α Val$_2$), 4.72 (1H, dd, J 4.6 and 9.2, CH_α Val$_3$), 2.45–2.30 (1H, m, CH_{iPr} Val$_2$), 2.25–2.10 (1H, m, CH_{iPr} Val$_3$), 2.00 (3H, s, CH_3 Ac), 1.85–1.72 (1H, m, CH_{sBu} Ile$_1$), 1.47–1.32 (1H, m, CH*H* Ile$_1$), 1.10–0.70 (19H, m, C*H*H Ile$_1$, CH_3 Ile$_1$, CH_3 Val$_2$, CH_3 Val$_3$). δ_C (75 MHz; $CDCl_3$) 172.9, 172.3, 171.4, 170.8 (C=O), 135.2 (C_{ar} –OBn), 128.9, 128.8, 128.5 (CH_{ar}), 67.7 (CH_2 –OBn), 63.5 (CH_α Val$_2$), 56.6 (CH_α Val$_3$), 53.3 (CH_α Ile$_1$), 37.4 (CH_{sBu} Ile$_1$), 32.0 (CH_{iPr} Val$_3$), 27.7 (CH_{iPr} Val$_2$), 24.7 (CH_2 Ile$_1$), 22.9 (CH_3 Ac), 19.8, 19.2, 18.9, 17.5 (CH_3 Val$_2$, CH_3 Val$_3$), 15.7, 11.4 (CH_3 Ile$_1$). IR: 3397, 3307, 3170, 3089, 2962, 2931, 2875, 1731, 1686, 1627, 1526, 1463, 1394, 1306, 1187, 1145, 751, 698. LRMS (DCI+) m/z: 477.9 $(M + H)^+$, 494.9 $(M + NH_4)^+$. HRMS (ESI +) m/z: found, 516.2482; $C_{25}H_{39}N_3O_6K$ requires 516.2476.

Boc–Leu$_1$–Ala$_2$–Ψ[CO–N(OCOPh)]–D-Ala$_3$–Val$_4$–OEt (36). The title compound was prepared from **33** (35 mg, 68 μmol) and benzoic acid using method B. Eluent for flash chromatography: pentane–EtOAc 9 : 1 to 1 : 1; yellowish oil (42 mg, 67 μmol, 99%). R_f 0.32 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +4.8° (c 1.15 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.13 (2H, d, J 7.6, CH_{ar}), 7.75–7.65 (2H, m, NH Val$_4$, CH_{ar}), 7.52 (2H, dd, J 7.7 and 7.7, CH_{ar}), 6.71 (1H, d, J 6.8, NH Ala$_2$), 4.95–4.76 (2H, m, NH Leu$_1$, CH_α Ala$_3$), 4.69 (1H, dq, J 6.8 and 6.8, CH_α Ala$_2$), 4.49 (1H, dd, J 5.1 and 8.5, CH_α Val$_4$), 4.27–4.02 (3H, m, CH_α Leu$_1$, CH_2 –OEt), 2.34–2.18 (1H, m, CH_{iPr} Val$_4$), 1.75–1.20 (21H, m, CH_{iBu} Leu$_1$, CH_2 Leu$_1$, CH_3 Ala$_2$ (1.51, d, J 6.8), CH_3 Boc (1.42, s), CH_3 Ala$_3$ (1.38, d, J 6.8), CH_3 –OEt), 0.98 (3H, d, J 6.4, CH_3 Val$_4$), 0.96 (3H, d, J 6.0, CH_3 Val$_4$), 0.92 (6H, d, J 6.2, CH_3 Leu$_1$). δ_C (75 MHz; $CDCl_3$) 173.7 (C=O Ala$_2$), 172.2 (C=O Ala$_3$), 171.7, 169.3 (C=O Leu$_1$, C=O Val$_4$), 165.6 (*C*OPh), 155.7 (C=O Boc), 135.2 (C_{ar}), 130.6, 129.2, 125.8 (CH_{ar}), 80.1 (C_q Boc), 61.1 (CH_2 –OEt), 59.4 (CH_α Ala$_3$), 57.9 (CH_α Val$_4$), 53.0 (CH_α Leu$_1$), 46.3 (CH_α Ala$_2$), 41.6 (CH_2 Leu$_1$), 31.0 (CH_{iPr} Val$_4$), 28.4 (CH_3 Boc), 24.8 (CH_{iBu} Leu$_1$), 23.2 (CH_3 Leu$_1$), 22.0 (CH_3 Leu$_1$), 19.2 (CH_3 Val$_4$), 18.0 (CH_3 Val$_4$), 17.9 (CH_3 Ala$_3$), 14.3 (CH_3 –OEt), 13.5 (CH_3 Ala$_2$). IR: 3314, 2962, 2929, 1771, 1741, 1655, 1534, 1452, 1368, 1238, 1170, 1016 710. HRMS (ESI +) m/z: found, 643.3315; $C_{31}H_{48}N_4O_9Na$ requires 643.3319.

Fmoc–Phe$_1$–Leu$_2$–Ala$_3$–Ψ[CO–N(OCOPh)]–D-Ala$_4$–Val$_5$–OEt (37). The title compound was prepared as described for **28** using **36** (21 mg, 34 μmol). Purification by flash chromatography (silica gel, pentane–EtOAc 9 : 1) gave **37** (22 mg, 25 μmol, 74%) as a white solid. Mp: 86–88 °C. R_f 0.33 (CH_2Cl_2–MeOH 95 : 5). $[\alpha]_D^{25}$ +1.48° (c 0.90 in $CHCl_3$). δ_H (300 MHz; $CDCl_3$) 8.13 (2H, d, J 7.6, CH_{ar} –COPh), 7.78–7.62 (4H, m, NH Val$_2$, CH_{ar}), 7.57–7.45 (4H, m, CH_{ar}), 7.42–7.10 (9H, m, CH_{ar}), 6.85–6.75 (1H, br s, NH Ala$_3$), 6.40–6.28 (1H, br s, NH Leu$_2$), 5.45–5.30 (1H, br s, NH Phe$_1$), 5.00–4.87 (1H, br, CH_α Ala$_4$), 4.70–4.57 (1H, m, CH_α Ala$_3$), 4.55–4.34 (3H, m, CH*H* Fmoc, CH_α Phe$_1$, CH_α Leu$_2$), 4.49 (1H, dd, J 5.2 and 8.4, CH_α Val$_5$), 4.34–4.23 (1H, m, CH Fmoc), 4.23–4.17 (3H, m, C*H*H Fmoc, CH_2 –OEt), 3.12–2.98 (2H, br, CH_2 Phe$_1$), 2.34–2.16 (1H, m, CH_{iPr} Val$_5$), 1.63–1.15 (12H, m, CH_{iBu} Leu$_2$, CH_2 Leu$_2$, CH_3 Ala$_4$, CH_3 Ala$_3$), 0.97 (3H, d, J 6.5, CH_3 Val$_5$), 0.95 (3H, d, J 6.5, CH_3 Val$_5$), 0.85 (6H, br s, CH_3 Leu$_2$). δ_C (125.9 MHz; $CDCl_3$) 173.6 (C=O), 171.8 (C=O Val$_5$), 171.0, 170.9, 169.3 (C=O), 165.6 (*C*OPh), 156.2 (C=O Fmoc), 143.8, 141.5 (CH_{ar} Fmoc), 136.3 (C_{ar} Phe$_1$), 135.3 (C_{ar} –COPh), 130.6, 129.5, 129.3, 128.9, 127.9, 127.3, 125.7, 125.3, 125.2, 120.2 (CH_{ar}), 67.3 (CH_2 Fmoc), 61.2 (CH_2 –OEt), 59.2 (CH_α Ala$_4$), 57.8 (CH_α Val$_5$), 56.2 (CH_α Phe$_1$), 51.7 (CH_α Leu$_2$), 47.3 (CH Fmoc), 46.4 (CH_α Ala$_3$), 41.3 (CH_2 Leu$_2$), 38.3 (CH_2 Phe$_1$), 31.0 (CH_{iPr} Val$_5$), 24.7 (CH_{iBu} Leu$_2$), 23.1 (CH_3 Leu$_2$), 22.0 (CH_3 Leu$_1$), 19.3, 18.1 (CH_3 Val$_5$), 17.8 (CH_3 Ala$_4$), 14.4 (CH_3 –OEt), 13.5 (CH_3 Ala$_3$). IR: 3295, 3063, 2959, 2927, 2868, 2855, 1736, 1647, 1535, 1451, 1237, 1038, 1015, 741, 706. HRMS (ESI +) m/z: found, 912.4159; $C_{50}H_{59}N_5O_{10}Na$ requires 912.4160.

Acknowledgements

Monique Lopez is thanked for technical assistance in the preparation of *N*-hydroxy peptides.

We are grateful to Université Joseph Fourier and the CNRS for financial support. This work is part of the project "Molecules and Therapeutic Targets" subsidized by the Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche.

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