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3-AMINO-2H-AZIRINES IN THE SYNTHESIS OF PEPTIDES: TETRAPEPTIDES WITH α,α-DISUBSTITUTED α-AMINO ACIDS

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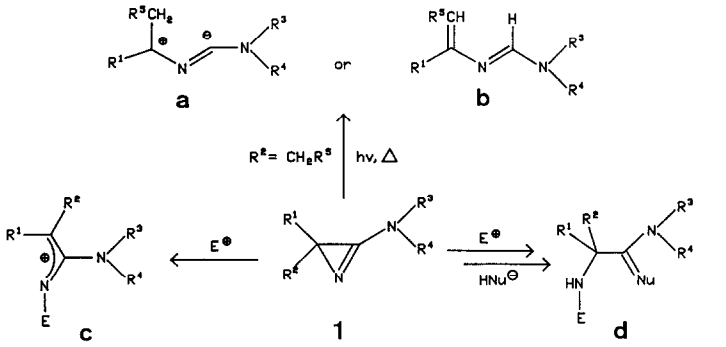
Abstract - 3-Amino-2,2-dialkyl-2H-azirines **1** are synthons for α,α-disubstituted α-amino acids. Tetrapeptides **15**, containing two such amino acids, were synthesized in high yield by application of the "azirine/oxazolone-method". In this versatile approach for the incorporation of disubstituted amino acids into the peptide chain, N-protected amino acids or peptides are coupled with 3-amino-2H-azirines **1**. After selective hydrolysis of the newly formed C-terminal amide, condensation with valine-benzylester was achieved via in situ generated oxazole-5(4H)-ones in the presence of hydroxybenzotriazole.
The conformational properties of tetrapeptides **15** are discussed on the basis of NMR- and CD-spectra. It is shown that α,α-disubstitution favours the formation of β-turns even in very short oligopeptides.
The "azirine/oxazolone-method" offers an efficient strategy for the synthesis of peptide-models used for conformational studies.

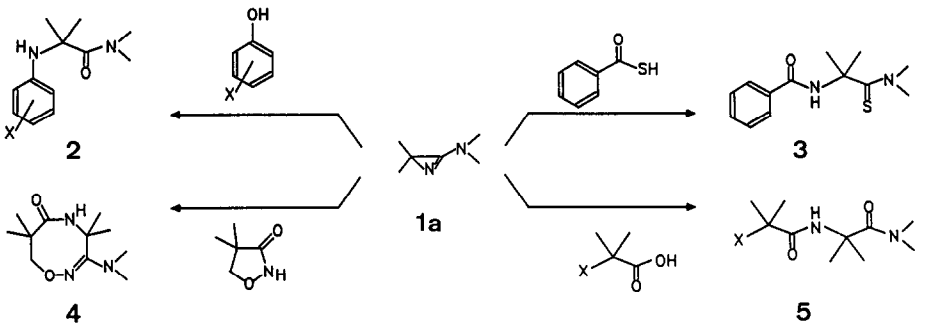
Introduction

The synthesis of 3-amino-2H-azirines **1** had been described by Rens and Ghosez in 1970.[1] Since then they have become readily available and have proved to be reactive molecules [2-5] and versatile building blocks in the synthesis on N-heterocycles.[5-11] It is of particular importance that all three types of azirine ring cleavage are known. Whereas photolysis of **1** with UV-light and thermolysis are accompanied by cleavage of the C,C-bond to give nitrile ylides **a** [5] and 2-azabutadienes **b** [8], respectively, treatment of **1** with electrophilic reagents (e.g. sulfonic acids [12], acyl chlorides [13], aryl halides [14], etc.) leads to the formation of 1-azallyl cations **c** as reactive intermediates via N(1),C(2)-cleavage. The reaction of aminoazirines **1** with electrophiles in the presence of reasonable nucleophiles yields aziridine intermediates which rearrange with cleavage of the former N(1),C(3)-azirine bond.[6,15,16]



Scheme 1

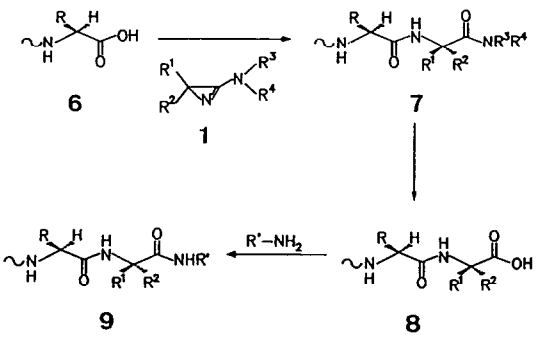
During the last few years a large variety of OH- [14,17,18], SH- [11], and NH-acidic compounds [6,7,19] have been reacted with aminoazirines **1**. In all cases the ring opening of the azirine occured between N(1) and C(3) (cf.[20]), i.e. α-amino acid derivatives of type **d** are formed. Some relevant examples are shown in Scheme 2. All the products **2-5** contain the amino azirine **1a** as an α-amino-isobutyric acid (Aib)-fragment. The reaction with bifunctional carboxylic acids, especially with α-amino acids and peptides, was carefully investigated as a potential method for the synthesis of peptides containing α,α-disubstituted α-amino acids.[18,21-25] The extension of peptide chains by Aib-units via the coupling reaction with 3-amino-2,2-dimethyl-2H-azirine (**1a**) already takes place at 0°C, usually with excellent yield.



Scheme 2

The crucial step in the application of **1a** as an Aib-equivalent in peptide synthesis is the conversion of the terminal amide group in compounds **5** to a carboxylic acid or ester group. We have found that this amide can be hydrolyzed selectively under remarkable mild conditions (Scheme 3).[10,18,22,26] Thus, treating a suspension or solution of diamides of type **7** with HCl in the presence of water leads to the formation of the corresponding N-acyl amino acids **8**, via intermediate 1,3-oxazol-5(4H)-ones. In the case of terminal N,N-dimethylamides, this selective hydrolysis

is achieved with 3N HCl in THF/H_2O (1:1) at 35°C; hydrolysis of the corresponding N-methylanilides is even faster by a factor of 10. The astonishing mild and selective cleavage of the terminal amide group in **7** proceeds with excellent yield and without any epimerization at the second to last amino acid.[22] Together with the efficient coupling of **8** and another amino component via *in situ* generated 1,3-oxazol-5(4H)-ones in the presence of additives like $ZnCl_2$ or camphor-10-sulfonic



Scheme 3

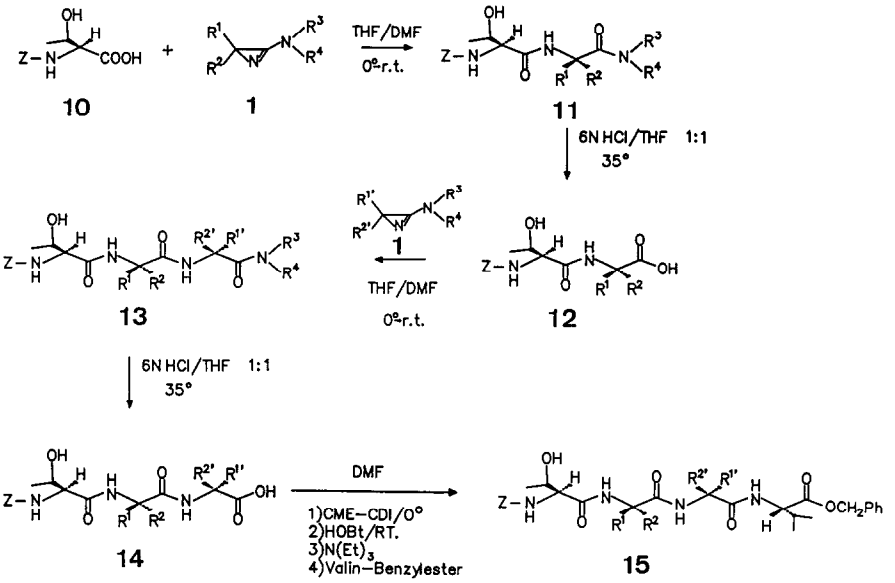
acid [27], the reaction of a peptide with **1** followed by hydrolysis represents an attractive strategy for the synthesis of peptides containing α,α-disubstituted α-amino acids. This procedure which we call "azirine/oxazolone method" has some advantages over conventional methods.

Recently, we have demonstrated the ability of this method in the synthesis of the C-terminal nonapeptide (Z-Leu-Aib-Pro-Aib-Aib-Glu(OBzl)-Gln-Pheol) of the ionophore Alamethicin [25,28] where all three Aib-units stem from **1a**.

Results and Discussion

Synthesis of Oligopeptides - Oligopeptides containing α,α-disubstituted α-amino acids are of particular interest with respect to their conformational properties.[29-32] The "azirine/oxazolone-method" offers a versatile approach for the synthesis of peptide models which differ in α,α-disubstitution.[23] In the present paper, the synthesis of tetrapeptides of type **15** is described (Scheme 4).[33]

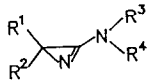
The coupling reaction of Z-protected L-threonine (**10**) with 3-amino-2H-azirines **1a-e** (Table 1) occured at 0°C → r.t. in THF- or DMF-solution. After a reaction time of 2-8 h the dipeptide amide **11** was precipitated with ether, filtered, and dried *in vacuo*. In all cases, colourless solids of **11** were obtained in excellent yields (cf. Table 2) and analytically pure. It is also worth mentioning that the Thr-hydroxy group was not protected in this coupling reaction. Selective hydrolysis of the terminal amide group was achieved in a 1:1-mixture of 6N HCl and THF at 35°C within 1-24 h. The reaction time for the anilides **11c-e** lies at the lower range of this interval, whereas for the dimethylamides **11a,b** 11-24 h are typical. The yields of dipeptides **12** are given in Table 2; neither cleavage of the "inner" amide bond nor of the Z-group was observed. In addition one should stress the fact that no epimerization of threonine could be detected in this hydrolysis.



Scheme 4

The dipeptides of type **12** with deprotected carboxyl group were again treated with a 3-amino-2H-azirine **1** in THF or DMF at 0°C → r.t. to give tripeptide amides **13** in very good yields (Table 2). Smooth hydrolysis with 6N HCl/THF at 35°C led to spectroscopically pure tripeptides **14** in 80-95% yield. These acyl components in DMF-solution were transformed into 1,3-oxazol-5(4H)-ones by means of the water-soluble N-cyclohexyl-N'-[2-(4-methylmorpholin-4-ylium)ethyl]carbodiimid-p-toluenesulfonate (CME-CDI). *In situ* coupling with L-valine-benzylester in the presence of 1-hydroxybenzotriazole (HOBt) gave the tetrapeptide benzylesters **15** in moderate yields (52-72%, Table 2). The relatively poor yield of this last coupling step with a conventional method [34] is a consequence of steric hindrance, a well known fact in peptide synthesis with α,α-disubstituted α-amino acids (cf. [35-37]).

Table 1. 3-Amino-2H-azirines **1** used in the synthesis of the tetrapeptides **15**.



1	R^1, R^2	R^3	R^4	Amino Acid Code
a	CH_3, CH_3	CH_3	CH_3	Aib
b	$CH_3CH_2CH_2$, $CH_3CH_2CH_2$	CH_3	CH_3	Dpg
c	$-(CH_2)_4-$	CH_3	Ph	Acp
d	$-(CH_2)_5-$	CH_3	Ph	Ach
e	$-(CH_2)_3-$	CH_3	Ph	Acb

Table 2. Chemical yields of the syntheses of di-, tri-, and tetrapeptides **11-15**

Dipeptides Z-Thr-A-X				Tripeptides Z-Thr-A-B-X			Tetrapeptides Z-Thr-A-B-Val-OBzl		
11	A-X	**12**	A-OH	**13**	A-B-X	**14** A-B-OH	**15**	A-B	
a	Aib-$N(CH_3)_2$ 95%	Aib	93%	**a**	Aib-Aib-$N(CH_3)_2$ 95%	Aib-Aib 91%	**a**	Aib-Aib	52%
				b	Aib-Dpg-$N(CH_3)_2$ 99%	Aib-Dpg 80%	**b**	Aib-Dpg	57%
b	Dpg-$N(CH_3)_2$ 98%	Dpg	98%	**c**	Dpg-Aib-$N(CH_3)_2$ 87%	Dpg-Aib 95%	**c**	Dpg-Aib	60%
				d	Dpg-Dpg-$N(CH_3)_2$ 77%	Dpg-Dpg 92%	**d**	Dpg-Dpg	57%
c	Acp-$N(CH_3)$Ph 94%	Acp	95%	**e**	Acp-Acp-$N(CH_3)$Ph 94%	Acp-Acp 92%	**e**	Acp-Acp	59%
d	Ach-$N(CH_3)$Ph 94%	Ach	89%	**f**	Ach-Ach-$N(CH_3)$Ph 87%	Ach-Ach 72%	**f**	Ach-Ach	72%
e	Acb-$N(CH_3)$Ph 75%	Acb	98%	**g**	Acb-Dpg-$N(CH_3)_2$ 78%	Acb-Dpg 94%	**g**	Acb-Dpg	59%

The structure of all peptides has been elucidated by spectral methods, i.e. IR, ^{1}H- and ^{13}C-NMR, and mass spectra; elemental analyses were all within the usual range ($\pm$ 0.3%).

Conformational Studies of Tetrapeptides 15 - Peptides containing α,α-disubstituted α-amino acids are currently of interest because of their stereochemistry.[29,32,38-39] The disubstitution of the α-carbon induces a considerable constraint on the conformational freedom.[29,40-42] As a consequence, certain secondary structures are stabilized selectively. While for conformations of alanine ca. 15% of the surface of a Ramachandran-plot [43] are available, less than 0.5% remains for Aib.[30] Therefore the introduction of Aib in a peptide chain increases for example the helical domain [31,32] and promotes formation of specific β-turns (cf. [44,45]).

The temperature coefficient of the NH-absorption in ^{1}H-NMR spectra (dδ/dT) and the CD-spectra made it possible to get a first insight into conformational properties of tetrapeptides **15**.[44] Chemical shifts of the amide protons of **15a-g** in DMSO were measured at 22, 50, 80, and 110°C (Fig. 1 and Table 3) and their signals were attached to NH_{Thr} and NH_{Val} by means of proton double resonance and 2D-NMR experiments.

As shown for **15a** (Fig. 1), the chemical shifts are linear functions of the temperature in all tetrapeptides. In **15a**, **c**, **e**, and **f**, the NH of valine showed a coefficient dδ/dT below or close to 1.0×10^{-3} ppm/°C indicating participation in an intramolecular 4→1 H-bond.[44] The three tetrapeptides **15b**, **d**, and **g** containing Dpg at position 3 showed different features without clear indication of intramolecularly binded NH. This behavior is an indication of a more extended structure because the NH of valine is solvent exposed. The relative small dδ/dT-coefficient of one Gly(2,2-dialkyl)-NH can be explained with its position within the β-turn, shielded from the solvent by the α-substituent of the α,α-disubstituted α-amino acid.

Figure 1. NH-Absorptions of **15a** at different temperatures in DMSO (c = 0.02mM).

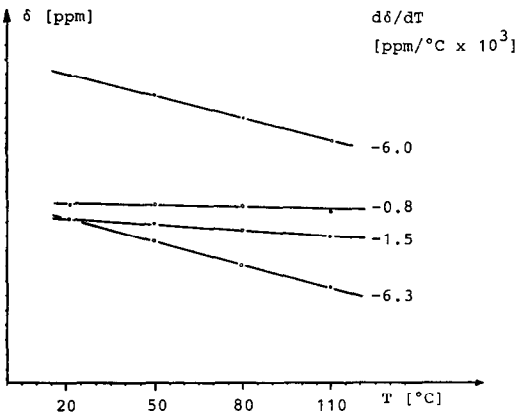


Figure 2. CD-spectrum of **15a** in CF_3CH_2OH (c = 0.6 mg/ml)

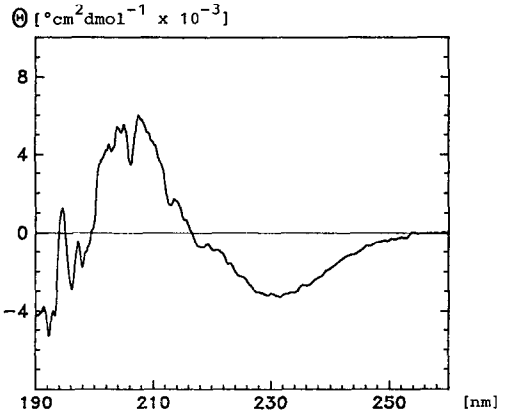


Table 3. NH-Temperature coefficients dδ/dT [ppm/°C x 10^3] of **15a-g** in DMSO.

Tetrapeptide **15**	^{NH}Thr	^{NH}Val	^{NH}Gly(2,2-dialkyl)
a	-6.3	-0.8	-6.0, -1.5
b	-6.0	-3.1	-5.0, -1.2
c	-5.4	-0.9	-3.3, -1.8
d	-6.5	-4.7	-3.0, -1.6
e	-5.6	-0.8	-6.5, -2.0
f	-6.0	-1.0	-5.8, -1.1
g	-6.5	-4.8	-3.9, -1.7

Further information on the preferred conformation of **15a-g** in solution was gained from the CD-spectra in trifluoroethanol (c = ca. 0.6 mg/ml). Again the Cotton-effects of **15a**, **c**, **e**, and **f** are in very good agreement with each other. In Figure 2 the typical spectrum of **15a** is shown from which the presence of a type-II β-turn may be concluded.[25,44,46-47] The spectra of **15b** and **g** still are well comparable with that of **15a**, but they show increasing amounts of another secondary structure (i.e. an "extended" structure). The influence of two Dpg-units in **15d** on the conformation is obvious from the CD-spectrum, indicating a largely extended structure (β-sheet).

Conclusion

The synthesis of tetrapeptides **15a-g** demonstrates the advantageous use of 3-amino-2H-azirines **1** as equivalents of α,α-disubstituted α-amino acids in peptide synthesis. The difficulties of the coupling reaction with these sterically crowded amino acids, which in conventional procedures occurs with low yield, are overcome with the "azirine-method". The selective hydrolysis of the terminal amide group of resulting peptide amides is achieved under very mild conditions without epimerization. For further coupling with amino acids or peptide fragments the reaction via intermediate 1,3-oxazol-5(4H)-ones is successfully applied. In conclusion the "azirine/oxazolone-method" offers a potent and attractive strategy for the synthesis of peptides using 3-amino-2H-azirines **1** as synthons.

Acknowledgements

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Experimental Section

For general remarks see [23]. Being representative for all tetrapeptides **15a-g** only the synthesis of **15a** is described in detail. Complete spectral data for all intermediates are given in [33].

1. **Z-Thr-Aib-N(CH_3)$_2$ (11a)**: To a solution of 1.0 g Z-Thr (**10**) in 8 ml THF were added 663 mg 3-dimethylamino-2,2-dimethyl-2H-azirine (**1a**) at 0°C. After stirring for 2 h at r.t. the dipeptide was precipitated by addition of 50 ml ether, washed with ether, and dried *in vacuo*: 1.36 g (95%) colourless **11a**; mp 153-155°C; $[\alpha]_D^{22°}$ = -29.3° (c = 0.987, EtOH). IR (KBr) 3400m (br), 3250m, 1715s, 1665s, 1620s, 1550m, 1280m, 1250m, 1130m; ^{1}H-NMR ($CDCl_3$) 7.3-7.2 (m, 6H), 5.81 (d, NH), 5.16, 5.09 (AB, 2H), 4.4-4.3 (m, 1H), 4.1-4.0 (m, 1H), 2.97 (s, 6H), 2.9-2.8 (s, OH), 1.58 (s, 3H), 1.51 (s, 3H), 1.17 (d, 3H). ^{13}C-NMR ($CDCl_3$) 172.3 (s), 169.7 (s), 156.8 (s), 136.1 (s), 128.4 (d), 128.2 (d), 128.0 (d), 67.1 (t), 66.7 (d), 58.8 (d), 56.9 (s), 37.9 (q), 25.3 (q), 25.2 (q), 18.3 (q). CI-MS (m/z) 366. Anal. calc. for $C_{18}H_{27}N_3O_5$ (365.43): C 59.16, H 7.44, N 11.49; found: C 59.06, H 7.38, N 11.58.

2. **Z-Thr-Aib-OH (12a)**: A solution of 652 mg **11a** in 16 ml 3N HCl (THF/H_2O 1:1) was stirred at 35°C for 24 h. After addition of 5 ml 2N HCl the reaction mixture was thoroughly extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and mixed with ether/petroleum ether. The precipitate was dried *in vacuo*: 565 mg (93%) colourless **12a**; mp 83.5-86.5°C. IR (KBr) 3370m (br), 3320m, 3230m, 1720s, 1685s, 1665s, 1560s, 1290m, 1260m, 1170m, 1135m. ^{1}H-NMR (CD_3OD) 7.4-7.3 (m, 5H), 5.11 (s, 2H), 4.1-4.0 (m, 2H), 1.48 (s, 6H), 1.18 (d, 3H). ^{13}C-NMR (CD_3OD) 177.7 (s), 172.1 (s), 158.4 (s), 138.0 (s), 129.4 (d), 129.0 (d), 128.8 (d), 68.6 (d), 67.8 (t), 61.8 (d), 57.1 (s), 25.3 (q), 25.0 (q), 19.7 (q).

3. **Z-Thr-Aib-Aib-N(CH_3)$_2$ (13a)**: According to experiment 1, 500 mg **12a** and 173 mg **1a** in THF were reacted for 70 min: 634 mg (95%) colourless **13a**; mp 183.5-186.5°C; $[\alpha]_D^{22°}$ = -3.6° (c = 0.585, EtOH). IR (KBr) 3380m, 3310m, 1770s, 1665s, 1630s, 1555m, 1275m, 1245m, 1130m. ^{1}H-NMR ($CDCl_3$) 7.75 (s, NH), 7.35 (s, 5H), 6.69 (s, NH), 5.9-5.8 (m, NH), 5.14 (s, 2H), 4.4-4.3 (m, 1H), 4.1-4.0 (m, 1H), 3.04 (s, 6H), 1.76 (s, OH), 1.62 (s, 6H), 1.53 (s, 3H), 1.45 (s, 3H), 1.21 (d, 3H). ^{13}C-NMR (CD_3OD) 175.8 (s), 174.9 (s), 172.6 (s), 158.6 (s), 138.2 (s), 129.5 (d), 129.0 (d), 128.5 (d), 68.4 (d), 67.7 (t), 62.8 (d), 58.2 (s), 57.7 (s), 38.4 (br s), 26.3 (q), 26.0 (q), 24.9 (q), 20.1 (q). CI-MS (m/z) 451. Anal. calc. for $C_{22}H_{34}N_4O_6$ (450.53): C 58.65, H 7.60, N 12.43; found: C 58.90, H 7.52, N 12.70.

4. **Z-Thr-Aib-Aib-OH (14a)**: According to experiment 2, 634 mg **13a** were hydrolyzed at 35°C (15 h) to yield 543 mg (91%) colourless **14a**; mp 230-232°C. ^{1}H-NMR (CD_3OD) 7.48 (s, NH), 7.4-7.3 (m, 5H), 5.12 (s, 2H), 4.1-3.9 (m, 2H), 1.43 (s, 6H), 1.42 (s, 3H), 1.40 (s, 3H), 1.20 (d, 3H).

5. **Z-Thr-Aib-Aib-Val-OBzl (15a)**: A stirred solution of 250 mg **14a** in 1.2 ml DMF was mixed with 250 mg N-cyclohexyl-N'-[2-(4-methylmorpholin-4-ylium)ethyl]carbodiimid-p-toluene sulfonate (CME-CDI) at 0°C. After 7 min 159 mg 1-hydroxybenzotriazole and a solution of 224 mg Val-OBzl p-toluene sulfonate and 59.7 mg NEt_3 in 0.5 ml DMF were added and stirred over night. After addition of CH_2Cl_2 the organic layer was extracted with 2N HCl, 2N NaOH, and brine and dried with Na_2SO_4. The tetrapeptide was precipitated with ether/petroleum ether and dried *in vacuo*: 188 mg (52%) colourless **15a**; mp 144.5-147°C; $[\alpha]_D^{22°}$ = -33.5° (c = 0.840, EtOH). IR ($CHCl_3$) 3420s, 3340m, 1730s, 1695s, 1670s, 1500s, 1455m, 1385m, 1230m, 1190m. ^{1}H-NMR ($CDCl_3$) 7.4-7.3 (m, 10H), 7.20 (m, NH), 6.86 (s, NH), 6.37 (s, NH), 5.82 (d, NH), 5.22, 5.12 (AB, 2H), 5.20, 5.07 (AB, 2H), 4.6-4.4 (m, 2H), 4.07 (dd, 1H), 3.85 (s, OH), 2.3-2.1 (m, 1H), 1.57 (s, 3H), 1.49 (s, 3H), 1.38 (s, 3H), 1.31 (s, 3H), 1.21 (d, 3H), 0.94, 0.93 (2d, 6H). ^{13}C-NMR ($CDCl_3$) 174.8 (s), 172.6 (s), 172.4 (s), 171.5 (s), 157.0 (s), 135.9 (s), 135.4 (s), 128.5 (d), 128.4 (d), 128.3 (d), 128.2 (d), 128.1 (d), 128.0 (d), 67.3 (t), 66.7 (t), 66.7 (d), 60.2 (d), 58.1 (d), 57.2 (s), 56.9 (s), 30.8 (d), 27.0 (q), 26.6 (q), 23.8 (q), 23.4 (q), 19.2 (q), 19.0 (q), 18.1 (q). FAB-MS (m/z) 613. Anal. calc. for $C_{32}H_{44}N_4O_8$ (612.72): C 62.72, H 7.23, N 9.14; found: C 62.82, H 7.34, N 9.15.

6. **Z-Thr-Aib-Dpg-Val-OBzl (15b)**: Colourless solid; $[\alpha]_D^{22°}$ = -30.0° (c = 0.890, EtOH). IR ($CHCl_3$) 3420m, 1730s, 1680s, 1610s, 1500s, 1470m, 1455m, 1385w, 1310m, 1230m, 1190m. ^{1}H-NMR ($CDCl_3$) 7.3-7.2 (m, 11H), 6.86 (s, NH), 6.81 (d, NH), 5.78 (d, NH), 5.19, 5.08 (AB, 4H), 4.6-4.55 (m, 1H), 4.4-4.35 (m, 1H), 4.05 (d, 1H), 2.2-1.65 (m, 9H), 1.52 (s, 3H), 1.41 (s, 3H), 1.17 (d, 3H), 0.9-0.75 (m, 12H). ^{13}C-NMR ($CDCl_3$) 173.5 (s), 172.2 (s), 172.0 (s), 171.1 (s), 154.8 (s), 136.1 (s), 135.1 (s), 128.5 (d), 128.4 (d), 128.3 (d), 128.2 (d), 128.0 (d), 67.3 (t), 67.1 (t), 66.2 (d), 63.7 (s), 59.4 (d), 57.7 (d), 57.4 (s), 38.8 (t), 37.1 (t), 31.1 (d), 26.5 (q), 23.9 (q), 19.0 (q), 18.8 (q), 17.6 (q), 17.0 (t), 16.6 (t), 14.0 (q). FAB-MS (m/z) 669. Anal. calc. for $C_{36}H_{52}N_4O_6$ (668.83): C 64.65, H 7.84, N 8.38; found: C 64.43, H 7.97, N 8.29.

7. **Z-Thr-Dpg-Aib-Val-OBzl (15c)**: Colourless crystals; mp 138.7-139.6°C; $[\alpha]_D^{22°}$ = -41.5° (c = 0.887, EtOH). IR (KBr) 3440m, 3360s, 3270m, 2970m, 2960m, 1745s, 1715s, 1675s, 1660s, 1515s, 1470m, 1455m, 1385m, 1235s, 1215m, 1190m, 1150m. ^{1}H-NMR ($CDCl_3$) 7.4-7.25 (m, 10H), 7.13 (d, NH), 6.85 (s, NH), 6.36 (s, NH), 5.85 (d, NH), 5.23, 5.10 (AB, 2H), 5.21, 5.08 (AB, 2H), 4.65-4.3 (m, 2H), 4.07 (dd, 1H), 2.3-2.0 (m, 1H), 1.9-1.0 (m, 8H), 1.56 (s, 3H), 1.34 (s, 3H), 1.21 (d, 3H), 1.0-0.85 (m, 12H). ^{13}C-NMR ($CDCl_3$) 174.5 (s), 172.4 (s), 171.5 (s), 171.1 (s), 157.0 (s), 135.9 (s), 135.3 (s), 128.5 (d), 128.4 (d), 128.3 (d), 128.2 (d), 128.1 (d), 128.0 (d), 67.3 (t), 66.8 (t), 66.3 (d), 63.1 (s), 59.7 (d), 57.8 (d), 57.0 (s), 38.2 (t), 34.3 (t), 31.0 (d), 26.9 (q), 23.8 (q), 19.2 (q), 19.0 (q), 17.9 (q), 16.6 (t), 16.4 (t), 14.1 (q). FAB-MS (m/z) 669. Anal. calc. for $C_{36}H_{52}N_4O_8$ (668.83): C 64.65, H 7.84, N 8.38; found: C 64.60, H 8.08, N 8.53.

8. **Z-Thr-Dpg-Dpg-Val-OBzl (15d)**: The crude product was purified by chromatography (SiO_2, ether/CH_2Cl_2 1:1). Colourless solid; $[\alpha]_D^{22°}$ = -26.4° (c = 1.12, EtOH). IR ($CHCl_3$) 3430m, 1725s, 1680s, 1505s, 1455m, 1370m, 1350w, 1310w, 1240m, 1150m. ^{1}H-NMR ($CDCl_3$) 7.4-7.25 (m, 10H), 7.15 (br s, NH), 6.90 (s, NH), 6.00 (m, NH), 5.74 (d, NH), 5.21, 5.11 (AB, 2H), 5.13, 5.00 (AB, 2H), 4.55 (m, 1H), 4.45-4.3 (br s, OH), 4.1-4.05 (m, 1H), 3.65-3.55 (m, 1H), 2.6-1.9, 1.85-1.5 (2m, 17H), 1.20 (d, 3H), 0.9-0.85 (m, 18H). ^{13}C-NMR ($CDCl_3$) 173.3 (s), 171.8 (s), 171.3 (s), 170.4 (s), 156.9 (s), 136.1 (s), 135.1 (s), 128.6 (d), 128.5 (d), 128.4 (d), 128.2 (d), 128.0 (d), 127.9 (d), 67.2 (2t), 66.5 (d), 64.2 (s), 64.1 (s), 59.2 (d), 57.5 (d), 38.8 (t), 38.4 (t), 37.4 (t), 36.2 (t), 31.2 (q), 19.0 (q), 17.6 (q), 17.1 (t), 17.0 (t), 16.9 (t), 16.7 (t), 14.0 (q), 13.9 (q), 13.8 (q). FAB-MS (m/z) 725.

9. **Z-Thr-Acp-Acp-Val-OBzl (15e)**: Colourless crystals; mp 208.8-210.2°C; $[\alpha]_D^{22°}$ = -55.6° (c = 0.947, CH_3COOH). IR (KBr) 3420w, 3360m, 3280m, 2960m, 1735m, 1725s, 1665s, 1540m, 1510m, 1455w, 1385w, 1280m, 1240m, 1180w, 1150w. ^{1}H-NMR (DMSO-d_6, 60°C) 8.21 (br s, NH), 7.3-7.25 (m, 10H), 7.23 (m, NH), 6.95 (br s, NH), 5.13, 5.05 (AB, 2H), 5.08, 4.97 (AB, 2H), 4.87 (s, NH), 4.15-4.10 (m, 1H), 3.95-3.9 (m, 2H), 3.15-3.05 (br s, OH), 2.3-1.5 (m, 17H), 1.08 (d, 3H), 0.88 (t, 6H). ^{13}C-NMR (DMSO-d_6, 70°C) 173.5 (s), 172.6 (s), 170.8 (s), 170.7 (s), 155.8 (s), 136.5 (s), 135.7 (s), 127.8 (d), 127.5 (d), 127.4 (d), 127.3 (d), 127.1 (d), 66.4 (d), 66.1 (t), 65.9 (t), 65.5 (s), 65.2 (s), 60.8 (d), 57.8 (d), 37.0 (t), 35.1 (t), 34.8 (t), 29.5 (d), 23.8 (t), 23.6 (t), 19.1 (q), 18.4 (q), 17.7 (q). FAB-MS (m/z) 665.

Anal. calc. for $C_{36}H_{48}N_4O_8$ (664.79): C 65.04, H 7.28, N 8.43; found: C 64.78, H 7.50, N 8.39.

10. **Z-Thr-Ach-Ach-Val-OBzl (15f)**: Colourless crystals; mp 89.6-91.5°C; $[\alpha]_D^{22°}$ = -53.6° (c = 1.04, EtOH). IR (KBr) 3420s, 2930m, 2860m, 1730s, 1670s, 1530s, 1465m, 1450m, 1390w, 1375w, 1255m, 1230m, 1170w, 1150w. ^{1}H-NMR ($CDCl_3$) 7.4-7.25 (m, 10H), 7.22 (m, NH), 6.95 (br s, NH), 6.35 (br s, NH), 5.85 (d, NH), 5.24, 5.11 (AB, 2H), 5.18, 5.07 (AB, 2H), 4.55-4.45 (m, 2H), 4.11 (dd, 1H), 3.83 (br s, OH), 2.5-1.4 (m, 21H), 1.20 (d, 3H), 0.95-0.85 (m, 6H). ^{13}C-NMR ($CDCl_3$) 174.7 (s), 172.8 (s), 172.6 (s), 171.8 (s), 157.2 (s), 135.8 (s), 135.4 (s), 128.5 (d), 128.4 (d), 128.2 (d), 67.5 (t), 66.7 (t), 66.2 (d), 57.9 (d), 34.6 (t), 34.2 (t), 30.8 (d), 29.1 (t), 25.1 (t), 25.0 (t), 21.5 (t), 21.4 (t), 21.1 (t), 20.9 (t), 19.4 (q), 19.1 (q), 18.1 (q). FAB-MS (m/z) 693. Anal. calc. for $C_{38}H_{52}N_4O_8$ (692.85): C 65.87, H 7.56, N 8.08; found: C 65.61, H 7.38, N 8.36.

11. **Z-Thr-Acb-Dpg-Val-OBzl (15g)**: Colourless crystals; mp 97.4-99.7°C; $[\alpha]_D^{22°}$ = -29.3° (c = 1.085, EtOH). IR (KBr) 3400m, 2960m, 2870m, 1725s, 1670s, 1520s, 1470m, 1455m, 1380m, 1290m, 1260m, 1230m, 1180m. ^{1}H-NMR (DMSO-d_6) 8.65 (br s, NH), 7.69 (d, NH), 7.4-7.25 (m, 10H), 7.20 (s, NH), 7.00 (m, NH), 5.15-4.8 (m, 4H), 4.7-4.65 (m, 1H), 4.15-3.95 (m, 1H), 3.9-3.85 (m, 1H + OH), 2.5-1.15 (m, 15H), 1.07 (d, 3H), 1.0-0.7 (m, 12H). ^{13}C-NMR (DMSO-d_6) 172.7 (s), 170.6 (s), 170.5 (s), 169.8 (s), 155.4 (s), 136.3 (s), 135.2 (s), 127.8 (d), 127.7 (d), 127.5 (d), 127.4 (d), 127.1 (d), 126.9 (d), 66.1 (d), 65.2 (t), 65.0 (t), 62.0 (s), 60.4 (d), 58.5 (s), 57.5 (d), 36.4 (t), 34.6 (t), 30.1 (t), 29.9 (t), 28.9 (d), 19.3 (q), 18.4 (q), 17.8 (q), 15.6 (t), 15.4 (t), 14.7 (t), 13.5 (q). FAB-MS (m/z) 681. Anal. calc. for $C_{37}H_{52}N_4O_8$ (680.84): C 65.27, H 7.69, N 8.22; found: C 64.99, H 7.56, N 8.45.

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