(αMe)Hyv: chemo-enzymatic synthesis, and preparation and preferred conformation of model depsipeptides †

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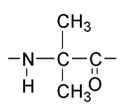
By a chemo-enzymatic approach we performed a large-scale, stereoselective synthesis of the C^{α}-methylated α-hydroxy acid L-(αMe)Hyv. We also prepared model depsipeptides based on this sterically demanding residue in combination with the α-amino acids L-Ala, L-Val, and Aib. From solution (FT-IR absorption and ^{1}H NMR) and crystal-state (X-ray diffraction) conformational analyses we found that L-(αMe)Hyv forces depsipeptides to fold into right-handed β-turn/helical structures by analogy with the reported propensity of L-(αMe)Val, its α-amino acid counterpart.

Introduction

Increasing effort is currently being devoted to the synthesis and conformational analysis of a variety of cyclic and linear compounds characterized by the presence of α-hydroxy acids. More specifically, these compounds include oligo- and poly-esters as biodegradable and biocompatible materials,[1–3] and depsipeptides and depsiproteins (in which amide and ester groups are concomitantly present in the main chain) to mimic naturally-occurring ion carriers[4,5] or to check the influence of specific H-bonds on peptide or protein bioactivity and conformation with little effect on other structural parameters.[6–16]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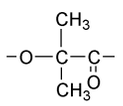
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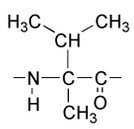
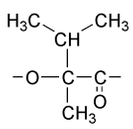

(αMe)Val


(αMe)Hyv

In recent years we have focused on the study of the 3D-structure and applications of peptides based on conformationally constrained α-amino acids, in particular those characterized by C^{α}-methylation. For example, Aib (α-aminoisobutyric acid)[17,18] and (αMe)Val (C^{α}-methyl valine)[18–22] are among the strongest known inducers of β-turn[23–25] and 3_{10}/α-helical[26] conformations. These and related C^{α}-methylated α-amino acids have been shown to represent excellent tools for the construction of rigid spacers,[27] templates[28] and catalysts.[29] Sometime ago, we expanded the arsenal of structurally restricted building blocks by designing and synthesizing a β-bend ribbon spiral structure (a variant of the 3_{10}-helix) characterized by the $(Aib\text{-}Hib)_n$ sequence, where Hib (α-hydroxyisobutyric acid) is the rigid, C^{α}-methylated α-hydroxy acid related to Aib.[8,9]

In this paper we describe a large-scale, chemo-enzymatic synthesis of L- (or *S*)-(αMe)Hyv (C^{α}-methyl, C^{α}-hydroxyisovaleric acid or 2-hydroxy-2,3-dimethylbutanoic acid),[30–32] the α-hydroxy analogue of (αMe)Val and the second member of the family of 3D-structurally restricted, C^{α}-methylated α-hydroxy acids, the preferred conformation of which has already been investigated. We have incorporated (αMe)Hyv either in position 1 or in an internal position of a large set of depsipeptides using specific solution methods which allowed us to overcome the severe problems generated by its extreme steric bulkiness. A solution and crystal-state conformational investigation clearly showed that L-(αMe)Hyv, in analogy to its α-amino counterpart L-(αMe)Val, supports right-handed β-turns and helical structures of depsipeptides.

Experimental

Chemo-enzymatic synthesis of L-(*S*)-α-hydroxyisovaleric acid

13.8 g (160 mmol) of isopropyl methyl ketone were dissolved in 48 mL of methyl *tert*-butyl ether and the solution was cooled to 0 °C. Then, 30 mL of an aqueous HbHNL (hydroxynitrile lyase from *Hevea brasilensis*) enzyme solution[32] (5300 units mL^{-1}) were mixed with 34 mL of water and the pH of the solution was adjusted to 4.0. This enzyme preparation was added to the ketone solution and 7.6 g of sorbitol were added. The reaction mixture was vigorously stirred for 10 min until a stable emulsion had formed. Freshly prepared hydrogen cyanide (30 mL, 760 mmol) was added and the reaction vessel was tightly sealed. After the emulsion had been stirred for another 15 min, the reaction mixture was dissolved in 100 mL of organic solvent and 5 g of Celite were added. The mixture was stirred for 10 min and after filtration the phases were separated. The aqueous layer was extracted twice with methyl *tert*-butyl ether. The combined organic phases were dried over anhydrous sodium

† Electronic supplementary information (ESI) available: analytical data. See http://www.rsc.org/suppdata/p2/b1/b107691b/

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sulfate and the solvent was removed. The yield of the cyanohydrin was 77%. For the determination of the enantiomeric excess, a sample of the residual was analysed by gas chromatography using a 25 m × 0.32 mm Chirasil-Dex-CB capillary column. The experimentally determined enantiomeric excess was 82%.

The (*S*)-cyanohydrin was hydrolysed using concentrated hydrochloric acid according to the literature procedure[31] (yield 63%). To improve the enantiomeric excess of (*S*)-α-hydroxyisovaleric acid, the crude acid was crystallized as its diastereomeric salt with (*S*)-α-phenylethylamine using a 9 : 1 mixture of ethyl acetate–ethanol.[30] The enantiomeric excess of the (*S*)-α-hydroxyacid was analysed by gas chromatography on its derivative 1,3-dioxolan-4-one using a 25 m × 0.32 mm Chirasil-Dex-CB capillary column and found to be 93%.

Details of the procedure for the most challenging depsipeptide coupling reaction are given below.

Synthesis of Boc-L-Ala-L-(αMe)Hyv-OBzl

To a stirred solution of H-L-(αMe)Hyv-OBzl (1.3 mmol, 287 mg) in dry CH_2Cl_2 (3 mL) were added scandium triflate (0.78 mmol, 384 mg), Boc-L-Ala-OH (3.9 mmol, 738 mg) and 4-(dimethylamino)pyridine (DMAP) (3.9 mmol, 476 mg), and the mixture was cooled to −10 °C for 30 min. Then, *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide (EDC) (3.9 mmol, 748 mg) was added and stirring was continued for 30 min. The resulting mixture was allowed to warm up to room temperature over a period of 16 h, stirred at 40 °C for an additional 8 h and at room temperature for 40 h. The reaction mixture was diluted with CH_2Cl_2 (15 mL). The organic layer was washed with 0.1 M HCl (2 × 10 mL), an aqueous saturated solution of $NaHCO_3$ (2 × 10 mL) and brine (1 × 10 mL), dried over sodium sulfate, and concentrated. A pure product was obtained after silica gel chromatography (a cyclohexane–ethyl acetate 95 : 5 mixture as eluant) in 75% yield (383 mg).

FT-IR absorption spectra

FT-IR absorption spectra were recorded using a Perkin-Elmer model 1720X FT-IR spectrophotometer (Norwalk, CT), nitrogen flushed, equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Solvent (baseline) spectra were obtained under the same conditions. Cells with path lengths of 0.1, 1.0 and 10 mm (with CaF_2 windows) were used. Spectrograde [^{2}H]chloroform (99.8% ^{2}H) was purchased from Merck (Darmstadt, Germany).

^{1}H NMR spectra

^{1}H NMR spectra were recorded with a Bruker model AM 400 spectrometer (Karlsruhe, Germany). Measurements were carried out in [^{2}H]chloroform (99.96% ^{2}H; Merck) and in [$^2H_6$]DMSO ([$^2H_6$]dimethyl sulfoxide) (99.96% $^2H_6$; Fluka, Buchs, Switzerland) with tetramethylsilane as the internal standard.

Crystallographic data for H-L-(αMe)Hyv-L-Val-OMe (OMe, methoxy)

$C_{12}H_{23}NO_4$, M = 245.3. Monoclinic, a = 5.900(2), b = 10.151(3), c = 12.088(3) Å, β = 100.64(3)°, U = 711.5(4) Å^3, T = 293 K, space group $P2_1$, Z = 2, D_c = 1.145 g cm^{-3}, μ = 0.698 mm^{-1} (Cu-Kα), 1179 reflections measured, 1126 unique (R_{int} = 0.014) which were used in all calculations, final R value 0.043 [on $F \geq 4\sigma(F)$].

Crystallographic data for Ac-L-(αMe)Hyv-L-Val-OMe (Ac, acetyl)

$C_{14}H_{25}NO_4$, M = 287.4. Monoclinic, a = 11.287(3), b = 17.451(4), c = 13.315(3) Å, β = 105.59(8)°, U = 2526(1) Å^3, T = 293 K, space group $P2_1$, Z = 6, D_c = 1.133 g cm^{-3}, μ = 0.705 mm^{-1} (Cu-Kα), 4335 reflections measured, 4158 unique (R_{int} = 0.068) which were used in all calculations, final R value 0.064 [on $F \geq 4\sigma(F)$].

Crystallographic data for Ac-L-(αMe)Hyv-(Aib)$_4$-OMe

$C_{25}H_{44}N_4O_8$, M = 528.6. Triclinic, a = 8.281(2), b = 9.539(3), c = 10.417(3) Å, α = 99.18(5), β = 96.34(4), γ = 109.03(5)°, U = 756.2(4) Å^3, T = 293 K, space group $P1$, Z = 1, D_c = 1.161 g cm^{-3}, μ = 0.714 mm^{-1} (Cu-Kα), 2258 reflections measured, 2255 unique which were used in all calculations, final R value 0.081 [on $F \geq 4\sigma(F)$].

Crystallographic data for Ac-(Aib)$_5$-OMe

$C_{23}H_{41}N_5O_7$, M = 499.6. Monoclinic, a = 18.687(4), b = 8.326(2), c = 19.443(4) Å, β = 105.99(7)°, U = 2908(1) Å^3, T = 293 K, space group $P2_1/n$, Z = 4, D_c = 1.141 g cm^{-3}, μ = 0.700 mm^{-1} (Cu-Kα), 5107 reflections measured, 4306 unique (R_{int} = 0.076) which were used in all calculations, final R value 0.083 [on $F \geq 4\sigma(F)$].

X-Ray crystal structure determinations

Colourless crystals (0.4 × 0.3 × 0.3 mm, 0.6 × 0.5 × 0.4 mm, 0.4 × 0.3 × 0.1 mm and 0.30 × 0.15 × 0.08 mm, respectively) of H-L-(αMe)Hyv-L-Val-OMe, Ac-L-(αMe)Hyv-L-Val-OMe, Ac-L-(αMe)Hyv-(Aib)$_4$-OMe and Ac-(Aib)$_5$-OMe were grown from methanol solution (slow evaporation), methanol solution (slow evaporation), acetonitrile solution (slow evaporation) and a chloroform–ethyl acetate solvent mixture–petroleum ether (vapour diffusion), respectively. Data collection was performed on a Philips PW1100 four-circle diffractometer. The four structures were solved by direct methods, using the SHELXS 97[33] program. Refinement was performed using the SHELXL 97[34] program. H-atoms were calculated at idealized positions and refined as riding. Fractional atomic coordinates, tables of hydrogen atoms coordinates, anisotropic displacement parameters, bond lengths, bond angles, and torsion angles for the four structures are available from the Cambridge Crystallographic Data Centre.‡

Results and discussion

Synthesis and characterization

L-(*S*)-(αMe)Hyv was produced on a large scale *via* acid hydrolysis of the corresponding (*S*)-cyanohydrin,[31] which was in turn synthesized from isopropyl methyl ketone and hydrogen cyanide according to the procedure of Griengl *et al.*[32] This chemo-enzymatic method yielded the (*S*)-cyanohydrin with 82% enantiomeric excess. It is evident that it is difficult for the enzyme (hydroxynitrile lyase from *Hevea brasilensis*, HbHNL) used in this synthetic step to clearly stereodifferentiate between the methyl and the isopropyl groups in the ketone substrate. To increase the enantiopurity to a more acceptable degree (93%), the (*S*)-hydroxy acid was further purified by preferential crystallisation of its diastereomeric salt with (*S*)-α-phenylethylamine according to the method of Mori *et al.*[30]

Peptide synthesis was performed in solution by established procedures. The Z (benzyloxycarbonyl)/OMe protected L-Val homo-oligomers (from dimer to tetramer) were prepared in 75–91% yield using Z-L-Val-OH,[35] H-L-Val-OMe[36] and the EDC–1-hydroxy-1,2,3-benzotriazole (HOBt) method.[37] Removal of the Z N^{α}-protection was achieved by catalytic hydrogenation. Acetylation of H-(L-Val)$_4$-OMe and H-(Aib)$_5$-OMe[38] was achieved with an excess of acetic anhydride.

‡ CCDC reference numbers 172174–172177. See http://www.rsc.org/suppdata/p2/b1/b107691b/ for crystallographic files in .cif or other electronic format.

The L-(αMe)Hyv residue was easily incorporated (55–89% yield) at the *N*-terminus of a peptide chain, *i.e.* of (H-L-Val)$_{1–3}$-OMe and H-(Aib)$_4$-OMe,[38] without protection of the α-hydroxy function, using the EDC–1-hydroxy-7-aza-1,2,3-benzotriazole (HOAt) method.[39] Acetylation of the *O*-terminal L-(αMe)Hyv residue in the depsipeptides was achieved in 55–87% yield using an excess of acetic anhydride in the presence of DMAP.[40]

Incorporation of an L-(αMe)Hyv residue in an *internal* position of the peptide chain proved to be very challenging. In our hands, all classical methods for C-activation of N^{α}-protected α-amino acids, including EDC–HOBt,[37] EDC–HOAt,[39] acyl fluoride[41] and symmetrical anhydride (in the presence/absence of DMAP) failed. Eventually, we succeeded in the preparation of Boc-L-Ala-L-(αMe)Hyv-OBzl (Boc, *tert*-butyloxycarbonyl; OBzl, benzyloxy) in 75% yield using Boc-L-Ala-OH[42] and a recently published general procedure for acylation of hindered tertiary alcohols which involves EDC and the unique combination of catalysts scandium triflate–DMAP[43,44] (see Experimental section). It is worth noting that Z-L-Ala-OH did not survive the scandium triflate treatment, affording in part Z-L-Ala-OBzl. The H-L-(αMe)Hyv-OBzl derivative was obtained in 95% yield from H-L-(αMe)Hyv-OH and benzyl bromide in the presence of triethylamine. Selective cleavage of the *C*-terminal benzyl ester function of the fully protected didepsipeptide by catalytic hydrogenation proceeded smoothly. Finally, the resulting N^{α}-protected didepsipeptide free acid Boc-L-Ala-L-(αMe)Hyv-OH was satisfactorily coupled to H-L-Val-OMe[36] by the EDC–HOBt procedure to afford the tridepsipeptide Boc-L-Ala-L-(αMe)Hyv-L-Val-OMe with an *internal* L-(αMe)Hyv residue. If lengthening of the tridepsipeptide chain from the N-terminus is required, methods are available for the selective deprotection of the Boc group in the presence of an ester from a tertiary alcohol [such as that characterizing the L-Ala-L-(αMe)-Hyv sequence].[45]

The final compounds and intermediate sequences were obtained in a chromatographically homogeneous state and were characterized by melting point determination (if solid), polarimetry, mass spectrometry, and solid-state IR absorption (Table 1). Additional analytical data (thin-layer chromatography R_f values in three different solvent systems and ^{1}H/^{13}C NMR results) have been deposited as electronic supplementary information.

Solution conformational analysis

The preferred conformation adopted by the L-(αMe)Hyv containing depsipeptides was assessed in the structure supporting solvent $CDCl_3$ by FT-IR absorption and ^{1}H NMR techniques. Figs. 1 and 2 illustrate the FT-IR absorption spectra in the informative N–H stretching region, while Figs. 3 and 4 show the ^{1}H NMR titrations of NH proton chemical shifts.

The FT-IR absorption curve of Ac-L-(αMe)Hyv-(L-Val)$_3$-OMe (Fig. 1) is characterized by two bands in the 3445–3425 cm^{-1} region (free, solvated NH groups) and one band of comparable intensity at 3385 cm^{-1} (weakly H-bonded NH groups).[46,47] A 10-times dilution does not alter the spectral pattern. In striking contrast, the FT-IR spectrum of the related Ac-(L-Val)$_4$-OMe peptide (Fig. 1) changes dramatically from 1×10^{-3} to 1×10^{-4} mol dm^{-3} concentration, showing, in particular, a remarkable decrease in the intensity of the 3275 cm^{-1} band (associated with the extremely strongly H-bonded NH groups typical of the β-sheet conformation) with a concomitant significant increase of the band assigned to free NH groups at 3430 cm^{-1}. From this analysis it is evident that the L-(αMe)-Hyv residue disrupts the strongly self-associated species formed by the L-Val homo-peptide, without being able, however, to promote a substantial folding in the molecule. Indeed, it is reasonable to associate the band at 3385 cm^{-1} exhibited by the L-(αMe)Hyv/L-Val depsipeptide with intramolecularly H-bonded, fully extended (C_5) conformers.[48]

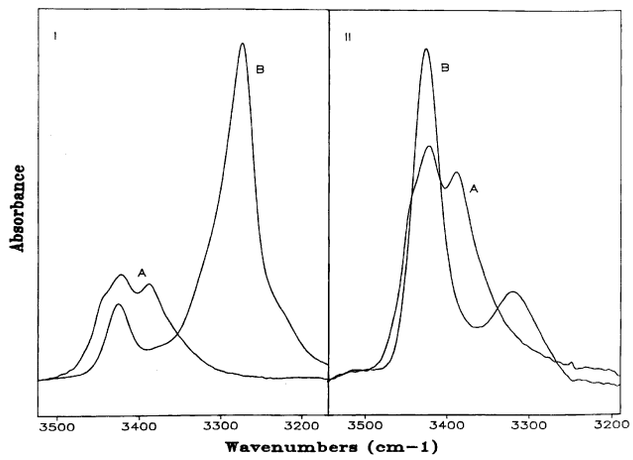

Fig. 1 FT-IR absorption spectra (3500–3200 cm^{-1} region) of Ac-L-(αMe)Hyv-(L-Val)$_3$-OMe (**A**) and Ac-(L-Val)$_4$-OMe (**B**) in $CDCl_3$ solution. Peptide concentrations: 1×10^{-3} mol dm^{-3} (**I**) and 1×10^{-4} mol dm^{-3} (**II**).

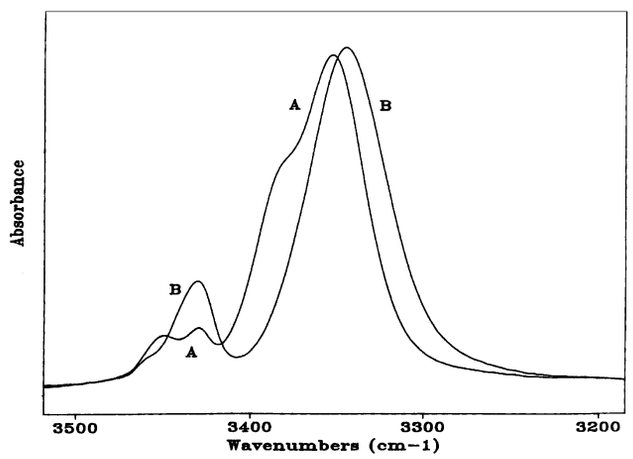

Fig. 2 FT-IR absorption spectra (3500–3200 cm^{-1} region) of Ac-L-(αMe)Hyv-(Aib)$_4$-OMe (**A**) and Ac-(Aib)$_5$-OMe (**B**) in $CDCl_3$ solution. Peptide concentration: 1×10^{-3} mol dm^{-3}.

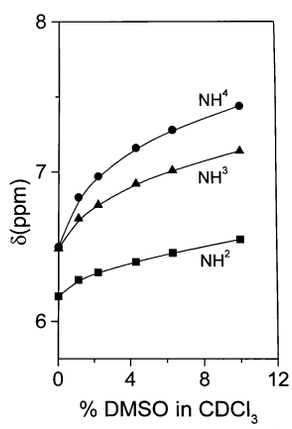

Fig. 3 Plot of NH proton chemical shifts in the ^{1}H NMR spectrum of Ac-L-(αMe)Hyv-(L-Val)$_3$-OMe as a function of increasing percentages of DMSO (v/v) added to the $CDCl_3$ solution. Peptide concentration: 1×10^{-3} mol dm^{-3}.

Fig. 2 shows that a L-(αMe)Hyv residue can be hosted in an (Aib)$_n$ homo-peptide chain without a marked perturbation of its highly folded conformation. Indeed, the intense band at 3345 cm^{-1} of Ac-(Aib)$_5$-OMe, typical of intramolecularly H-bonded, helical peptides,[46,49] is still largely preserved in the depsipeptide analogue Ac-L-(αMe)Hyv-(Aib)$_4$-OMe. However, the presence of a band at 3385 cm^{-1} in the spectrum of the latter indicates the co-existence of fully-extended forms to some degree.

Table 1 Physical properties and analytical data for the newly synthesized derivatives and peptides

Compound	Yield (%)	Mp/°C[a]	Recrystallization solvent[b]	$[\alpha]_D^{20}$[c]	MS (*m*/*z*)[e] Calculated	Observed	Species	IR (KBr) *ν*/cm^{-1}[g]
H-L-(αMe)Hyv-OBzl	95	Oil	—	−6.2	245.115	245.117	$[M + Na]^+$	1729[h]
Boc-L-Ala-L-(αMe)Hyv-OBzl	75	Oil	—	−37.1	416.205	416.214[f]	$[M + Na]^+$	3382, 1746, 1717, 1503[h]
Boc-L-Ala-L-(αMe)Hyv-OH	99	Oil	—	−34.4	304.176	304.190[f]	$[M + H]^+$	3320, 1748, 1720, 1510[h]
Boc-L-Ala-L-(αMe)Hyv-L-Val-OMe	83	Oil	—	−67.3	417.260	417.278	$[M + H]^+$	3326, 1745, 1693, 1524[h]
H-L-(αMe)Hyv-L-Val-OMe	70	72–74	CH_2Cl_2–PE	−48.8	246.170	246.181	$[M + H]^+$	3414, 3329, 1749, 1655, 1514
H-L-(αMe)Hyv-L-$(Val)_2$-OMe	78	184–185	EtOAc–PE	−72.4	345.238	345.255	$[M + H]^+$	3362, 3283, 1750, 1645, 1536
H-L-(αMe)Hyv-L-$(Val)_3$-OMe	79	220–221	EtOAc–PE	−96.4	444.307	444.308	$[M + H]^+$	3300, 1747, 1641, 1546
Ac-L-(αMe)Hyv-L-Val-OMe	55	56–58	EtOAc–PE	−28.8	288.180	288.188	$[M + H]^+$	3340, 3322, 1741, 1662, 1535
Ac-L-(αMe)Hyv-L-$(Val)_2$-OMe	68	126–127	EtOAc–PE	−49.6	387.249	387.256	$[M + H]^+$	3312, 1751, 1650, 1544
Ac-L-(αMe)Hyv-L-$(Val)_3$-OMe	87	200–202	EtOAc–PE	−71.6	486.317	486.330	$[M + H]^+$	3292, 1744, 1642, 1534
Z-L-$(Val)_2$-OMe	91	106–107	EtOAc–PE	−36.0	365.207	365.216	$[M + H]^+$	3300, 1743, 1691, 1652, 1538
Z-L-$(Val)_3$-OMe	90	212–214	EtOAc–PE	−62.2	464.276	464.282	$[M + H]^+$	3291, 1744, 1693, 1643, 1540
Z-L-$(Val)_4$-OMe	75	267–268	EtOAc–PE	−80.0[d]	563.344	563.355	$[M + H]^+$	3283, 1743, 1704, 1641, 1539
Ac-L-$(Val)_4$-OMe	75	310–312	MeOH–PE	−130.6[d]	471.312	471.322	$[M + H]^+$	3279, 1746, 1637, 1546
H-L-(αMe)Hyv-$(Aib)_4$-OMe	55	208–209	CH_2Cl_2–PE	−2.8	487.313	487.317	$[M + H]^+$	3450, 3396, 3316, 1729, 1672, 1652, 1524
Ac-L-(αMe)Hyv-$(Aib)_4$-OMe	73	203–204	CH_2Cl_2–PE	+18.8	529.323	529.337	$[M + H]^+$	3399, 3355, 3307, 1739, 1730, 1687, 1658, 1521
Ac-$(Aib)_5$-OMe	44	278–279	CH_2Cl_2–PE	—	500.308	500.317	$[M + H]^+$	3434, 3305, 1733, 1666, 1538

[a] Determined on a Leitz model Laborlux apparatus (Wetzlar, Germany). [b] PE, petroleum ether bp 40–60 °C; EtOAc, ethyl acetate; MeOH, methanol; DE, diethyl ether. [c] Determined on a Perkin-Elmer model 241 polarimeter (Norwalk, CT) equipped with a Haake model L thermostat (Karlsruhe, Germany); $c = 0.5$ (MeOH); reported in units of 10^{-1} deg cm^2 g^{-1}. [d] $c = 0.5$ (TFE). [e] Determined on a PerSeptive Biosystems model Mariner API-TOF mass spectrometer. A 1% HCOOH in a H_2O–CH_3CN 1 : 1 solvent mixture was used for dissolving and injecting the samples. [f] The reported *m*/*z* peak shows little intensity since the largest peak observed corresponds to the $[M + H - Boc]^+$ fragment. [g] Determined in KBr pellets on a Perkin-Elmer model 580 B spectrophotometer equipped with a Perkin-Elmer model 3600 IR data station and a model 660 printer (only bands in the 3500–3200 cm^{-1} and 1800–1500 cm^{-1} regions are reported). [h] Determined as a film.

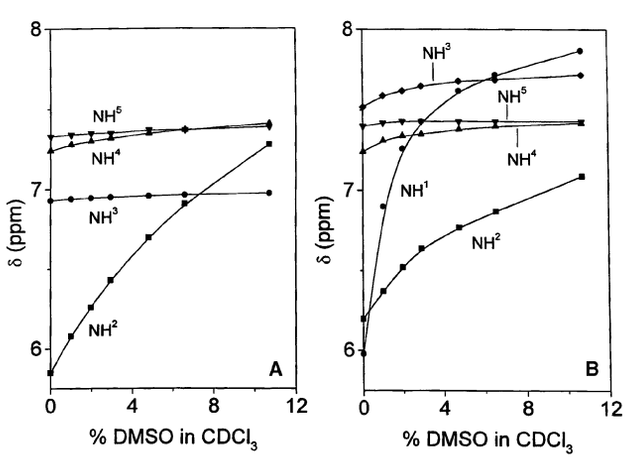

Fig. 4 Plot of NH proton chemical shifts in the ^{1}H NMR spectrum of Ac-L-(αMe)Hyv-$(Aib)_4$-OMe (**A**) and Ac-$(Aib)_5$-OMe (**B**) as a function of increasing percentages of DMSO (v/v) added to the $CDCl_3$ solution. Peptide concentration: 1×10^{-3} mol dm^{-3}.

More detailed information on the conformational preferences of the L-(αMe)Hyv depsipeptides in $CDCl_3$ solution was extracted from a 400 MHz ^{1}H NMR investigation (Figs. 3 and 4). The delineation of inaccessible (presumably intramolecularly H-bonded) NH groups by ^{1}H NMR was performed by using the solvent dependence of the NH chemical shifts by adding increasing amounts of the H-bonding acceptor DMSO to the $CDCl_3$ solution.[50,51] Unambiguous assignments for all NH proton signals were obtained by ROESY and TOCSY experiments.

From an inspection of Fig. 3 it is clear that all three NH protons of Ac-L-(αMe)Hyv-$(L\text{-}Val)_3$-OMe are sensitive to the addition of DMSO, although this phenomenon is less significant for the NH^2 proton. These results, taken together with the corresponding FT-IR absorption data, strongly support the view that the predominant 3D-structure of this tetradepsipeptide in $CDCl_3$ solution is not characterized by intramolecularly H-bonded, folded forms. Rather, it is reasonable to assume that an intramolecularly H-bonded, fully extended (C_5) conformer, involving the Val^2 residue, might in part populate the equilibrium mixture.

In both parts **A** and **B** of Fig. 4 two classes of NH protons were observed. Class (i) [NH^2 proton for Ac-L-(αMe)Hyv-$(Aib)_4$-OMe, and NH^1 and NH^2 protons for Ac-$(Aib)_5$-OMe] includes protons whose chemical shifts are remarkably sensitive to the addition of DMSO. Class (ii) (NH^3 to NH^5 protons of both compounds) includes those displaying behaviour characteristic of shielded protons (extremely modest sensitivity of chemical shifts to solvent composition). These ^{1}H NMR results are in agreement with the FT-IR absorption data discussed above, allowing us to conclude that both Ac-$(Aib)_5$-OMe[46,49]

and its related depsipeptide Ac-L-(αMe)Hyv-(Aib)$_4$-OMe are largely folded in a 3_{10}-helical conformation where the first two residues in the main chain are not involved in the intramolecularly H-bonded scheme.

Crystal-state conformational analysis

By X-ray diffraction we determined the molecular and crystal structures of the didepsipeptides H-L-(αMe)Hyv-L-Val-OMe and Ac-L-(αMe)Hyv-L-Val-OMe, the pentadepsipeptide Ac-L-(αMe)Hyv-(Aib)$_4$-OMe and the related pentapeptide Ac-(Aib)$_5$-OMe. The molecular structures with the atomic numbering schemes are illustrated in Figs. 5–8. Backbone and side-chain

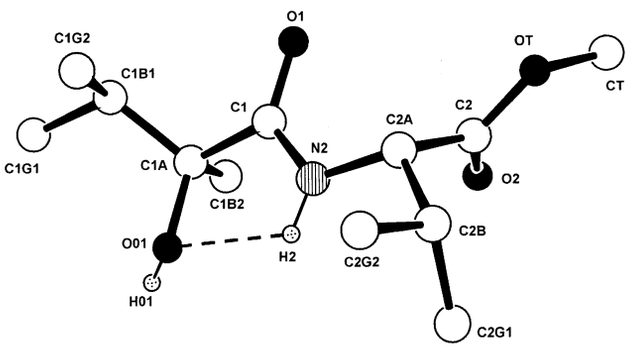

Fig. 5 X-Ray diffraction structure of H-L-(αMe)Hyv-L-Val-OMe with numbering of the atoms. The intramolecular H-bond is indicated by a dashed line.

torsion angles[52] are given in Table 2. In Table 3 the intra- and intermolecular H-bond parameters are listed. The X-ray diffraction structures of two other Aib homo-pentapeptides, namely Tos-(Aib)$_5$-OMe (Tos, tosyl)[53] and Z-(Aib)$_5$-O*t*Bu (O*t*Bu, *tert*-butoxy)[54] have already been reported.

Bond lengths and bond angles are in general agreement with previously reported values for the geometry of the ester[55] and amide[56] groups, the peptide unit,[57,58] and the Aib residue.[59,60]

In H-L-(αMe)Hyv-L-Val-OMe (Fig. 5) the ψ_1 angle is close to the *cis* conformation, thereby preventing the occurrence of the intramolecularly H-bonded O01–H01 ··· O1=C1 form (oxy-analogue of the C_5 conformation),[24] but favouring the formation of a different pseudocyclic C_5 species involving the (peptide) N2–H2 ··· O01 (alcohol) intramolecular H-bond. The *C*-terminal Val residue is *semi*-extended.

The only relevant backbone difference seen among the three independent molecules in the asymmetric unit of Ac-L-(αMe)Hyv-L-Val-OMe (Fig. 6) is found in the conformation of the Val^2 residue, right-handed (distorted) helical in molecules **A** and **B**, but *semi*-extended in molecule **C**. In all three molecules the conformation of the L-(αMe)Hyv residue is right-handed helical.

The pentadepsipeptide Ac-L-(αMe)Hyv-(Aib)$_4$-OMe is folded in a right-handed 3_{10}-helical structure[26] stabilized by three 1⟵4 C=O ··· H–N intramolecular H-bonds (Fig. 7). All O ··· N distances are well within the accepted range for such H-bonds.[61–63] Interestingly, one of the H-bond acceptors is the acetoxy ester carbonyl. The usual inversion of the handedness of the *C*-terminal helical residue with respect to that of the preceding ones[64] is also found in this 3_{10}-helical peptide ester. Overall, the conformation adopted by the pentapeptide Ac-(Aib)$_5$-OMe (Fig. 8) strictly parallels those published for Tos-(Aib)$_5$-OMe[53] and Z-(Aib)$_5$-O*t*Bu[54] and that discussed above for Ac-L-(αMe)Hyv-(Aib)$_4$-OMe. These findings are strongly in favour of the conclusion that neither the nature of the *N*- and *C*-protecting groups nor the Aib⟶(αMe)Hyv replacement are effective in inducing a significant alteration in the global architecture of the -(Aib)$_5$- homopeptide sequence.

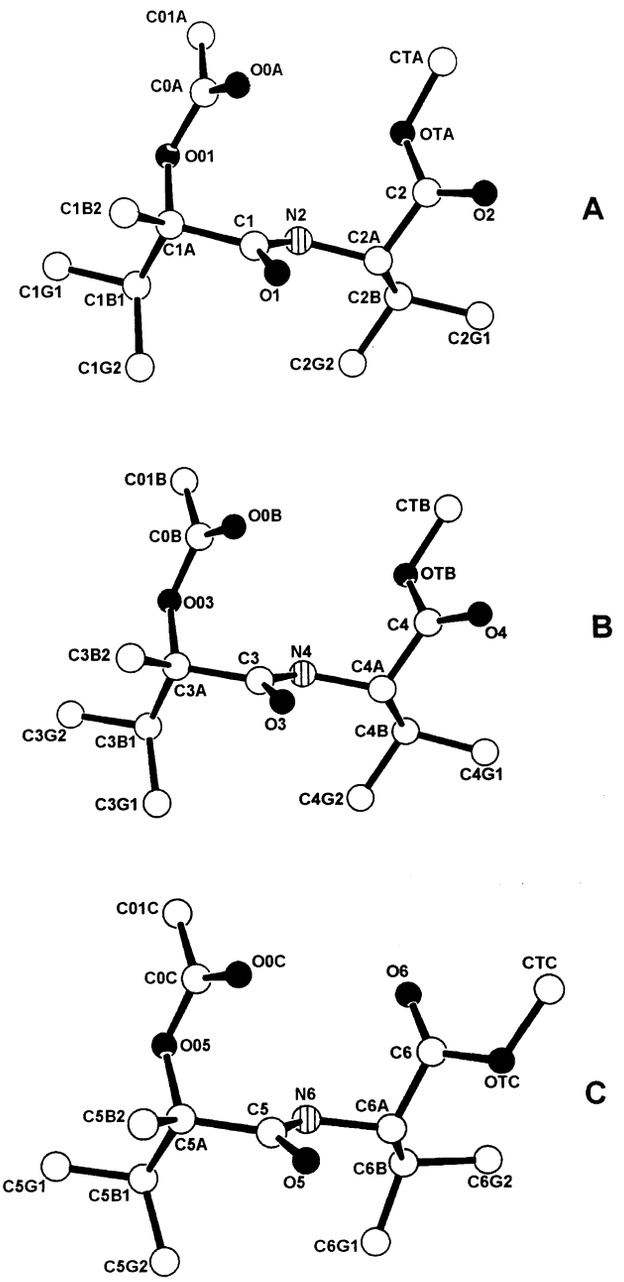

Fig. 6 X-Ray diffraction structures of the three independent molecules in the asymmetric unit of Ac-L-(αMe)Hyv-L-Val-OMe with numbering of the atoms.

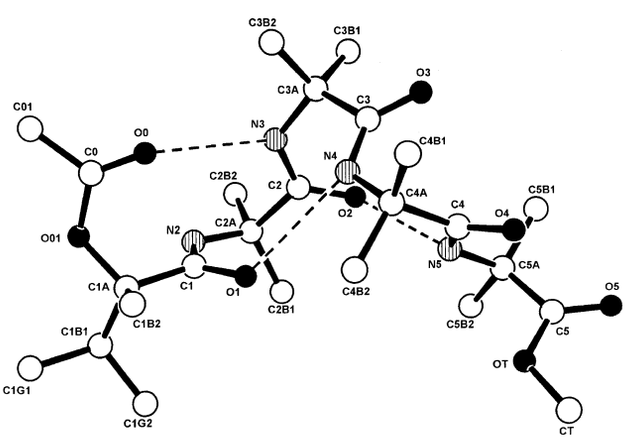

Fig. 7 X-Ray diffraction structure of Ac-L-(αMe)Hyv-(Aib)$_4$-OMe with numbering of the atoms. The three intramolecular H-bonds are indicated by dashed lines.

Table 2 Selected torsion angles (deg)[52] for the four X-ray diffraction structures solved in this work

		Ac-L-(αMe)Hyv-L-Val-OMe				
Torsion angle	H-L-(αMe)Hyv-L-Val-OMe	Mol. **A**	Mol. **B**	Mol. **C**	Ac-L-(αMe)Hyv-$(Aib)_4$-OMe	Ac-$(Aib)_5$-OMe
ω_0		174.2(3)	−178.2(4)	176.5(5)	−167.1(7)	−168.0(5)
φ_1		−54.3(4)	−54.8(4)	−55.9(4)	−55.6(7)	−56.8(6)
ψ_1	13.9(3)	−43.8(4)	−45.0(4)	−47.3(4)	−48.5(8)	−33.6(6)
ω_1	173.7(2)	−178.0(3)	−177.0(4)	179.7(3)	−169.8(6)	−177.2(4)
φ_2	−66.3(4)	−91.5(4)	−98.5(4)	−95.1(4)	−55.3(9)	−51.2(6)
ψ_2	150.2(2)[a]	−44.5(4)[c]	−48.7(5)[e]	138.6(3)[g]	−36.5(9)	−36.4(6)
ω_2	179.7(3)[b]	178.1(6)[d]	−178.0(6)[f]	176.8(4)[h]	−172.7(6)	−175.1(4)
φ_3					−58.0(9)	−55.7(3)
ψ_3					−34.8(9)	−32.5(6)
ω_3					−173.8(6)	−177.2(4)
φ_4					−61.2(8)	−61.4(5)
ψ_4					−35.4(9)	−30.9(6)
ω_4					175.5(7)	177.3(4)
φ_5					47.2(10)	47.2(6)
ψ_5					46.0(9)[i]	50.3(5)[i]
ω_5					175.4(9)[j]	172.7(4)[j]
$\chi_1^{1,1}$	−62.9(3)	−60.5(4)	173.6(3)	−60.9(5)	−57.8(8)	
$\chi_1^{1,2}$	62.4(3)	173.1(3)	−59.7(4)	169.4(4)	176.8(7)	
$\chi_2^{1,1}$	61.6(4)	179.3(4)	−173.7(4)	−68.6(6)		
$\chi_2^{1,2}$	−65.3(4)	−58.3(5)	−53.5(5)	170.0(6)		

[a] N2–C2A–C2–OT. [b] C2A–C2–OT–CT. [c] N2–C2A–C2–OTA. [d] C2A–C2–OTA–CTA. [e] N4–C4A–C4–OTB. [f] C4A–C4–OTB–CTB. [g] N6–C6A–C6–OTC. [h] C6A–C6–OTC–CTC. [i] N5–C5A–C5–OT. [j] C5A–C5–OT–CT.

Table 3 Intra- and intermolecular H-bond parameters for the four X-ray diffraction structures solved in this work

				Distance/Å		
Compound	Donor D–H	Acceptor A	Symmetry operations of A	D ··· A	H ··· A	Angle/deg D–H ··· A
H-L-(αMe)Hyv-L-Val-OMe	N2–H2	O01	x, y, z	2.558(3)	2.15	109
	O01–H01	O1	$x + 1, y, z$	2.771(3)	1.95	175
Ac-L-(αMe)Hyv-L-Val-OMe	N2–H2	O5	$x + 1, y, z + 1$	3.075(5)	2.22	171
	N4–H4	O1	x, y, z	2.999(4)	2.16	166
	N6–H6	O3	x, y, z	2.979(4)	2.13	170
Ac-L-(αMe)Hyv-$(Aib)_4$-OMe	N3–H3	O0	x, y, z	3.147(9)	2.42	143
	N4–H4	O1	x, y, z	3.123(7)	2.35	150
	N5–H5	O2	x, y, z	3.083(8)	2.44	132
	N2–H2	O4	$x, y, z - 1$	2.998(7)	2.32	136
Ac-$(Aib)_5$-OMe	N3–H3	O0	x, y, z	3.029(5)	2.22	156
	N4–H4	O1	x, y, z	2.987(5)	2.20	152
	N5–H5	O2	x, y, z	3.012(5)	2.28	144
	N1–H1	O4	$x - ½, -y + ½, z - ½$	2.817(5)	1.97	168
	N2–H2	O5	$x - ½, -y + ½, z - ½$	3.198(5)	2.44	147

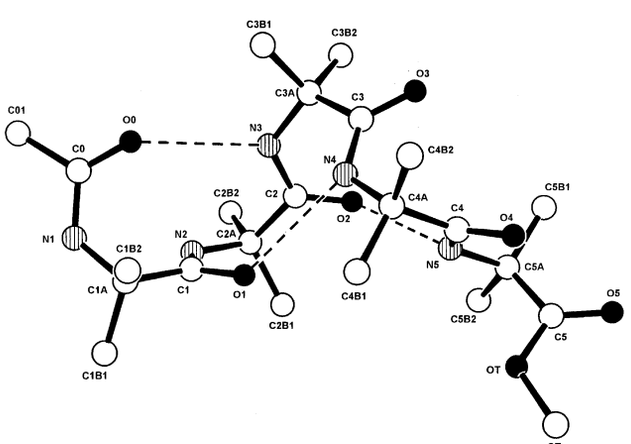

Fig. 8 X-Ray diffraction structure of Ac-$(Aib)_5$-OMe with numbering of the atoms. The three intramolecular H-bonds are indicated by dashed lines.

All amide, peptide and ester groups of the four structures are *trans* (ω torsion angles) with no deviation >7.3° from planarity, except for the ω_0 and ω_1 torsion angles of Ac-L-(αMe)Hyv-$(Aib)_4$-OMe and the ω_0 torsion angle of Ac-$(Aib)_5$-OMe, which deviate from *trans* planarity by 10.2–12.9°. The methyl ester group adopts a conformation with respect to the *C*-terminal CA–N bond between the *antiperiplanar* and *anticlinal* conformations for molecules **A** and **B** of Ac-L-(αMe)Hyv-L-Val-OMe, Ac-L-(αMe)Hyv-$(Aib)_4$-OMe and Ac-$(Aib)_5$-OMe, but between the *synperiplanar* and *synclinal* conformations for H-L-(αMe)Hyv-L-Val-OMe and molecule **C** of Ac-L-(αMe)Hyv-L-Val-OMe.[65] The L-(αMe)Hyv isopropyl side chain ($\chi_1^{1,1}$ and $\chi_1^{1,2}$ torsion angles) is found in the *t*, g^- conformation in all structures except in H-L-(αMe)Hyv-L-Val-OMe where it adopts the g^+, g^- conformation. The same conclusions apply for the L-Val isopropyl side chain ($\chi_2^{1,1}$ and $\chi_2^{1,2}$ torsion angles) of the didepsipeptide.[66]

The molecules of the *O*-unprotected didepsipeptide ester H-L-(αMe)Hyv-L-Val-OMe pack in the unit cell *via* intermolecular (alcohol) O01–H01 ··· O1=C1 (peptide) H-bonds, forming rows in the *a* direction. The O ··· O distance is normal.[67,68]

The three molecules in the asymmetric unit of the fully blocked didepsipeptide Ac-L-(αMe)Hyv-L-Val-OMe are linked together in the crystal through intermolecular H-bonds involving exclusively the peptide carbonyl groups as acceptors. More specifically, the three H-bonds, (molecule **A** peptide) N2–H2 ··· O5=C5 (molecule **C** peptide), (molecule **B** peptide)

N4–H4 ··· O1=C1 (molecule **A** peptide), and (molecule **C** peptide) N6–H6 ··· O3=C3 (molecule **B** peptide) give rise to **B**–**A**–**C**–**B**–**A**–**C** chains of molecules along the *a*,*c* direction.

The crystal packing mode for the pentadepsipeptide Ac-L-(αMe)Hyv-$(Aib)_4$-OMe is characterized by (peptide) N2–H2 ··· O4=C4 (peptide) intermolecular H-bonds, generating rows of molecules along the *c* direction. In the crystals of the related pentapeptide Ac-$(Aib)_5$-OMe two different types of intermolecular H-bonds link the molecules in a head-to-tail fashion along the *a*,*c* direction, a strong (amide) N1–H1 ··· O4=C4 (peptide) H-bond and a weak (peptide) N2–H2 ··· O5=C5 (ester) H-bond.[61–63]

Conclusions

In this paper we have reported the stereospecific synthesis of the α-hydroxy acid L-(αMe)Hyv by a combined chemical and enzymatic approach. Due to the extremely poor reactivity of its hydroxy function, we successfully incorporated L-(αMe)Hyv in an *internal* position of the peptide chain only by taking advantage of a recently proposed method for the acylation of sterically hindered tertiary alcohols which concomitantly exploits the Lewis acid scandium(III) triflate and the tertiary amine DMAP as catalysts.[43,44] For the same reason, addition of L-(αMe)Hyv at the *N*-terminus of a peptide chain is particularly straightforward as this reaction does not even require *O*-protection.

In the X-ray diffraction structures of all four molecules of the two depsipeptides containing an *O*-acylated L-(αMe)Hyv residue that were solved in this work, this first chiral C^{α}-tetrasubstituted α-hydroxy acid studied to date is right-handed helical (with average φ, ψ torsion angles −55.1, −46.1°) as expected on the basis of the well known *gem*-dialkyl effect.[69] This structural property, making it ideally suited for the stabilization of β-turn and 3_{10}/α-helices in depsipeptides, strictly reflects the published propensity of the parent Hib α-hydroxy acid.[8,9] The conformational tendency of L-(αMe)Hyv also closely resembles that of the related chiral, C^{α}-tetrasubstituted α-amino acid L-(αMe)Val.[18–22] It is also worth noting that replacement with L-(αMe)Hyv of a single residue in the intermolecular β-sheet forming sequence -$(L\text{-Val})_4$- is sufficient to disrupt this ordered self-associated secondary structure. Taken together, these results support the view that the L-(αMe)Hyv residue represents an additional valuable tool for the design and synthesis of conformationally constrained, folded depsipeptides.

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