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Stereoselective synthesis of pseudotripeptides incorporating uncommon bis-α-aminoacid derivatives and X-ray analysis. Part 3☆

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Abstract—Stereoselective synthesis of pseudotripeptides **4**, **5**, **6**, **8**, **9**, **13** and **14**, incorporating an uncommon bis(α-aminoacid) derivative, has been accomplished starting from the L-valine derived chiral synthon **1**. The configuration of the introduced stereogenic centres has been assigned on the basis of ^{1}H NMR spectroscopic data. The geometry of the tripeptides, deduced on the basis of ^{1}H NMR parameters and IR spectra, was confirmed by X-ray crystal structure analysis of **6**.

1. Introduction

In continuation of our program directed towards producing uncommon tripeptides, which are C-terminal at both ends of the chain,[1–3] we undertook the stereocontrolled synthesis of new pseudotripeptides **4**, **5**, **6**, **8**, **9**, **13** and **14** containing (*S*,*S*)-*ortho*-phenylene-bis-alanine, which can be considered as a cysteine isoster with L-valine units at the ends of the chain. Our interest in these unusual pseudopeptides arises from their potential biological activity as antibacterial[4] and/or herbicide agents.[5] In addition, short peptides can mimic some important aspect of protein structure or function. The strategy followed to accomplish the synthesis of these unusual tripeptides is based on the experience previously acquired on the stereoselective approach to similar substrates.[1–3]

2. Synthesis and stereochemical assignments

The stereoselective synthesis followed makes use of the chiral synthon **1**, a mono-lactim ether easily synthesized starting from L-valine, as reported previously.[1,3] Deprotonation of **1** with LHMDS followed by the alkylation with 0.5 equiv of α,α′-dibromo-*o*-xylene afforded **2** in chemical yield >90% and with a practically total double 1,4-*trans*-induction (de ⩾96%) with respect to the isopropyl group. In fact, as previously observed for similar substrates,[1–3] the ^{1}H NMR spectrum exclusively showed the *trans*-isomer. Diastereomer **2**, which has a C_2-axis of symmetry, was obtained pure by silica gel chromatography and its stereochemistry was established on the basis of the ^{1}H NMR spectra, as already reported.[1,3] The intermediate **2** was converted into **3** and then into the aminoester **4** by means of the Birch reaction and subsequent acid hydrolysis under mild conditions. The acetylation of **4** furnished the pseudotripeptide **5**, C-terminal at both ends of the chain, incorporating a bis(α-aminoacid) derivative cysteine isoster[6] (Scheme 1). The more complex pseudopeptide **6** was then obtained by treating **4** with the *t*-Boc-(L)-valine pentafluorophenylester (prepared from *t*-Boc-L-valine and pentafluorophenyltrifluoroacetate), as reported in Scheme 1.

The intermediate **2**, alkylated at both equivalent positions C-3, gave **7** in good yield and with a total *trans*-stereoselection induced by the isopropyl group, as already observed[3] (Scheme 2).

The *trans*-configuration of **7** was followed from the ^{1}H NMR and ^{13}C NMR spectra, which showed the existence of a symmetry element, that is, a C_2-axis. In fact, the signals of both methyl and isopropyl groups overlap as do the signals of the benzyl protons indicating their magnetic equivalence.[1–3] The stereochemistry was also

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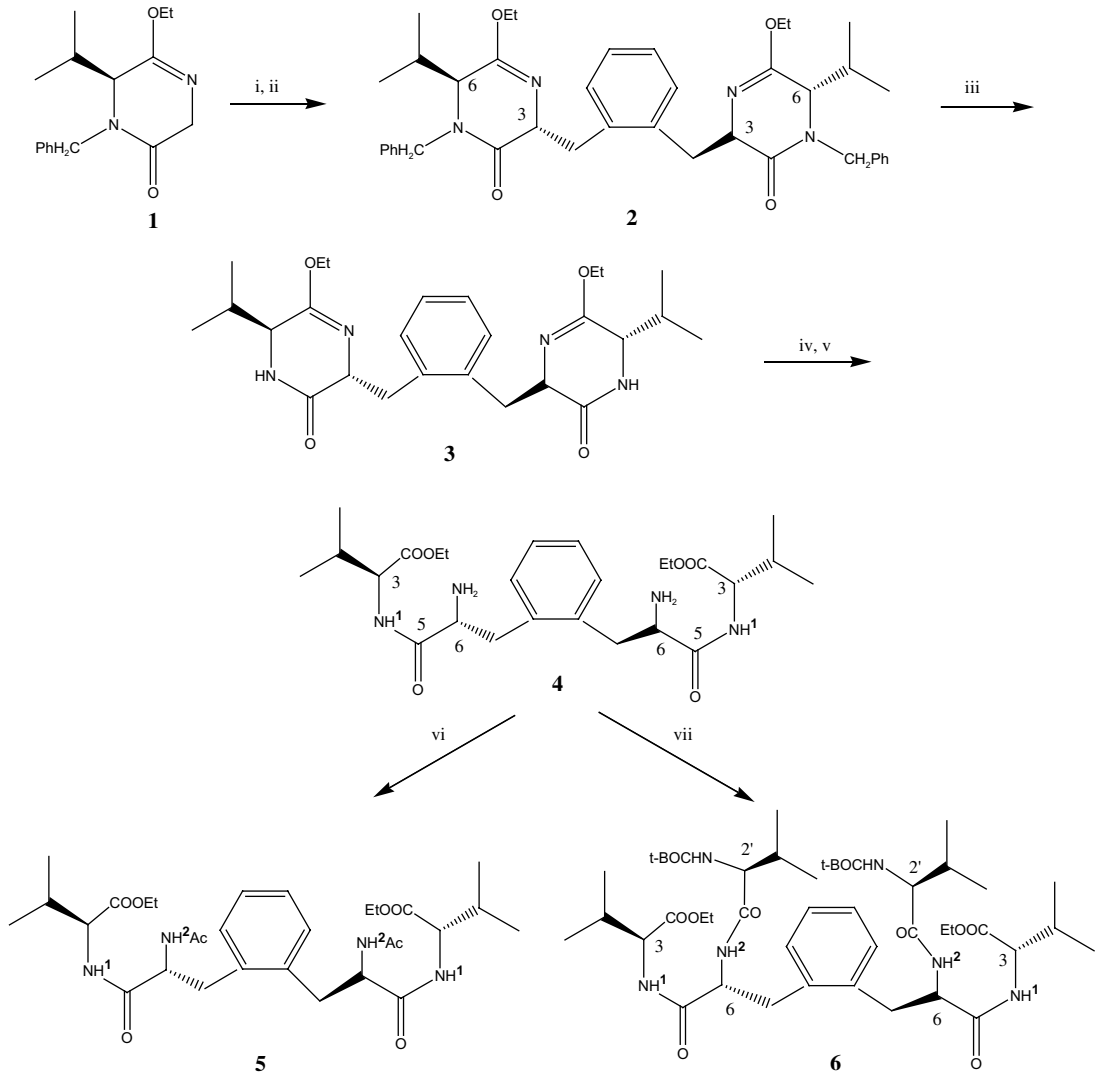
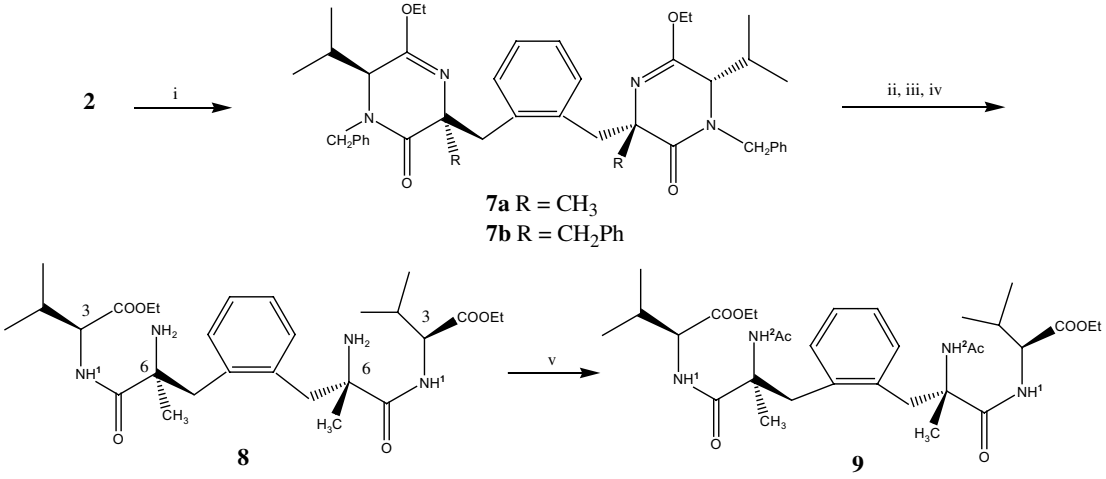


Scheme 1. Reagents and conditions: (i) 1 M LHMDS/THF; (ii) *o*,*o*-C_6H_4–$(CH_2Br)_2$; (iii) Li/NH_3; (iv) 0.5 M HCl at rt; (v) 1 M K_2CO_3; (vi) CH_3COCl/Et_3N, CH_2Cl_2; (vii) *t*-Boc-L-valine pentafluorophenylester, DMF at 50 °C.



Scheme 2. Reagents and conditions: (i) 1 M LHMDS/THF, then R–Br; (ii) Li/NH_3; (iii) 0.5 M HCl at rt; (iv) 1 M K_2CO_3; (v) CH_3COCl/Et_3N in CH_2Cl_2.

evident from the shielding effect observed in **7b** induced by the phenyl ring of (C-3)–CH_2Ph group on the (C-6)–H (see Experimental), the absolute configuration of stereocentre C-6 being known.[1–3] The intermediate **7a** was

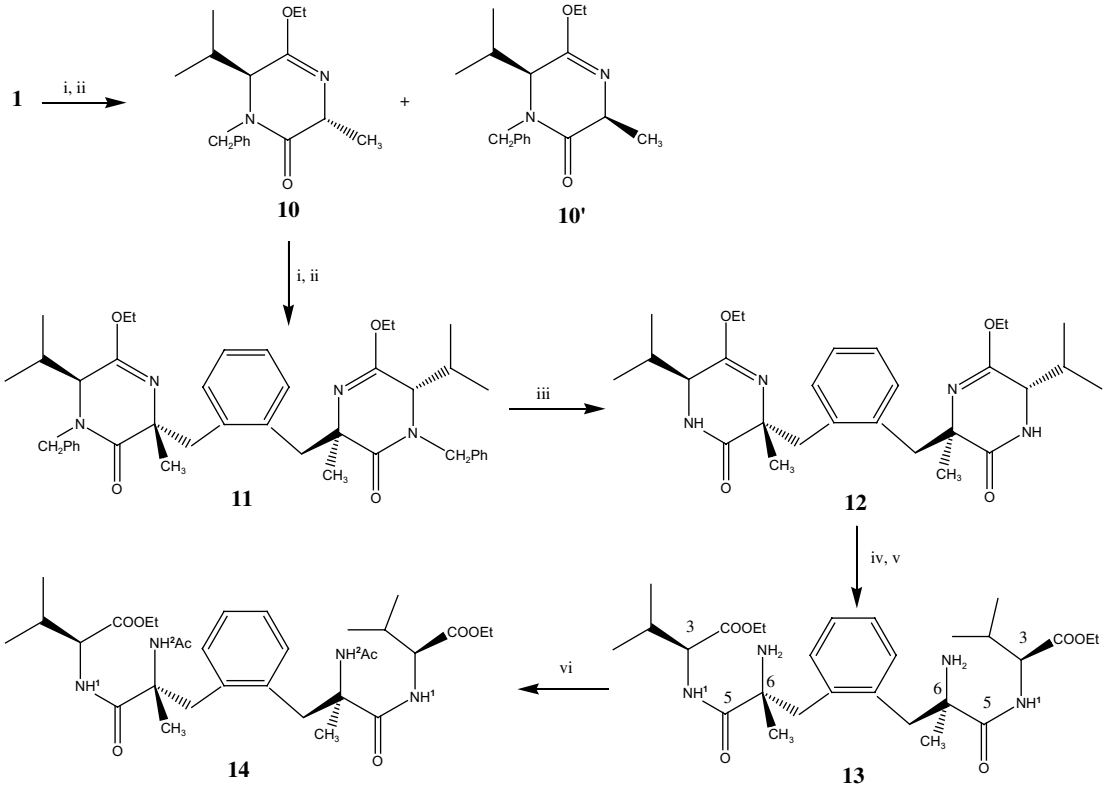
then converted into **9** (Scheme 2) by following the same procedure employed to obtain **5** (Scheme 1).

The synthesis of diastereomeric derivative **14**, with the opposite configuration at the C-6 stereocentres with respect to **9**, was accomplished on the basis of the stereocontrolled *trans*-induction well tested in the alkylation reaction. To this end, the strategy reported in Scheme 3 was followed, starting from diastereomer **10** obtained by the alkylation of the chiral synthon **1** with CH_3I. The reaction occurred in good chemical yield and with a *trans*/*cis* ratio ≅ 7:3 (measured by ^1H NMR or HPLC), the diastereomer **10** being easily separable by silica gel chromatography.

3. ^1H NMR and IR studies

The meaningful spectroscopic data of the unusual pseudopeptides investigated **4**, **5**, **6**, **8**, **9**, **13** and **14** are listed in Table 1 and their amide protons are labelled as $\mathbf{H^1}$ and $\mathbf{H^2}$ for clarity (see Schemes 1–3). As mentioned above, these compounds possess a C_2-axis of symmetry and then all the ^1H NMR and ^{13}C NMR signals overlap indicating their magnetic equivalence, as previously observed for similar derivatives.[1–3]

The spectroscopic studies of substrates with a free amine group, that is, **4**, **8** and **13** (Table 1), suggest that these nonclassical peptides form structures organized through



Scheme 3. Reagents and conditions: (i) 1 M LHMDS/THF; (ii) CH_3I; (iii) Li/NH_3; (iv) 0.5 M HCl at rt; (v) 1 M K_2CO_3; (vi) CH_3COCl/Et_3N, CH_2Cl_2.

Table 1. Significant ^1H NMR and IR data of pseudopeptides **4**, **5**, **6**, **8**, **9**, **13** and **14**

	δ_{NH} (ppm) (in 2 mM $CDCl_3$)		δ_{NH} (ppm) (in 2 mM DMSO)		$\delta_{NH}/\Delta T$ (ppb/°C) (in 2 mM $CDCl_3$)		IR (cm^{-1}) (2 mM $CHCl_3$)
	$\mathbf{H^1}$	$\mathbf{H^2}$	$\mathbf{H^1}$	$\mathbf{H^2}$	$\mathbf{H^1}$	$\mathbf{H^2}$	
4	7.7	—	8.1[a]	—	−2.3	—	3368 (broad)
5	8.4	6.6	8.5	8.15	−2.4	−0.8	3411 (sharp) 3307 (broad)
6	8.2–8.6	7.0	8.1	8.2	−4.4[b]	−4.0[b]	3386 (broad) 3308 (broad)
8	8.05	—	8.05	—	−2.0	–	3350 (broad)
9	7.3	6.4	7.65	7.7	−8.5	−2.4	3419 (sharp) 3370 (broad)
13	8.1	—	8.1	—	−1.7	—	3361 (broad)
14	6.8	6.1	7.65	7.75	−0.4	−0.2	3426 (sharp) 3381 (broad)

[a] δ_{NH} = 7.85 ppm in $CDCl_3$/DMSO = 80:20.
[b] Measured in DMSO.

intramolecular hydrogen bond between H^1 and (C-5)=O, giving rise to cyclic 11-membered conformations.[7,8] In fact, in all substrates the H^1 chemical shift is >7, the temperature coefficient $\Delta\delta_{NH}/\Delta T$ is <2.6 ppb and the IR spectra show only one broad band in the range 3350–3368 cm^{-1}, ascribable to hydrogen bonded NH amides. Most probably, in the case of **4**, the intramolecular hydrogen bond is not as strong as in **8** and **13**, because the H^1 signal displays a moderate downshift of 0.15 ppm by adding 20% DMSO. These findings lead us to deduce that the presence of the methyl group at both C-6 positions regardless of their absolute configurations, increases the tendency to assume conformations organized by a strong intramolecular hydrogen bond, which is resistant to a strongly competitive solvent in the formation of hydrogen bonds like DMSO.

From the spectroscopic data of **5** (Table 1) it is possible once again to deduce the presence of a strong intramolecular hydrogen bond between H^1 and (C-5)=O or CH_3C=O, inducing the formation of a cyclic 11- or 12-membered structure, respectively, while the acetamidic $-NH^2$ does not give rise to a hydrogen bond, as shown by its large downshift (1.55 ppm) registered on going from $CDCl_3$ to DMSO and the sharp band at 3411 cm^{-1}, ascribable to free acetamide $-NH^2$. Analogously to **5**, also in compounds **9** and **14** the acetamide $-NH^2$ does not form a hydrogen bond, while the amidic H^1 shows a different behaviour. In fact, in the pseudopeptide **9**, the chemical shift value (7.3 ppm), the consistent coefficient temperature (−8.5 ppb/°C), the downshift of 0.35 ppm observed on going from $CDCl_3$ to DMSO and the broad band at 3370 cm^{-1} lead us to argue that the H^1 probably exists in a dynamic equilibrium between the hydrogen-bonded and nonhydrogen-bonded states. Conversely, in the substrate **14** the H^1 shows very little tendency to form a hydrogen bond, as shown by the chemical shift value (6.8 ppm), the downshift of 0.85 ppm observed on going from $CDCl_3$ to DMSO and the presence of a broad band at 3381 cm^{-1}, largely weaker than the sharp one at 3426 cm^{-1} due to free acetamidic NH.

The ^1H NMR spectrum of the pseudopeptide **6** in $CDCl_3$ does not appear well resolved and in particular the signal ascribable to H^1, centred at 8.4 ppm, is severely broadened. However, after the addition of increasing quantities of DMSO the spectrum resolution is improved and just in the presence of 12% DMSO the signal at 8.1 ppm becomes a doublet and in 50% DMSO/$CDCl_3$ it suffers practically no further shift. The H^2, which resonates at 7 ppm in $CDCl_3$, is shifted to 8.2 ppm in DMSO and also the *t*-Boc-N*H* suffers a downshift of 1.2 ppm on going from $CDCl_3$ (a broad doublet centred at 5.3 ppm) to DMSO (a sharp doublet at 6.5 ppm). The temperature coefficient values in DMSO for H^1 and H^2 are borderline between the presence and the absence of an intramolecular hydrogen bonded state. Besides, the three amide protons show a different exchange rate in CD_3OD: after 60 min, H^2 was totally exchanged, while H^1 was about 70% and *t*-Boc-N*H* was about 30%. The slow exchange rate of the *t*-Boc-N*H* could be ascribed to the low acidity of the proton, which is furthermore sterically less accessible to the solvent. Besides, the IR spectrum in diluted $CHCl_3$ solution shows two broad bands of comparable intensity at 3308 and 3386 cm^{-1} ascribable to hydrogen bonded NHs. All these findings suggest that most probably in solution H^1 forms a stable intramolecular hydrogen bond, while H^2 is involved in a weak one and *t*-Boc-N*H* is in a nonhydrogen bonded state.

Thus, it can be inferred that in acetamide derivatives **5**, **9** and **14** the introduction of the methyl group at both C-6 stereocentres, regardless of their absolute configuration, considerably decreases the tendency to form intramolecular hydrogen bond between H^1 and (C-5)=O or CH_3C=O so much that in **14** the hydrogen bond can be considered barely present.

In conclusion, from the ^1H NMR and IR data it is reasonable to deduce that all the nonclassical peptides investigated, except **14**, are aggregated through the formation of intramolecular hydrogen bonds, between the carbonyl oxygens and the amide protons, to form a reverse-turn conformation, that is a 'U shaped structure'. However, such an intramolecularly compact conformation was firmly established by single crystal X-ray studies performed on pseudopeptide **6** (see below).

4. X-ray analysis

The solid state molecular structure of **6** is shown in Figure 1 together with the crystallographic numbering and the relevant bond parameters are listed in Table 2.

Two independent molecules are present in the asymmetric unit, which correspond to different conformers of the same enantiomer. The absolute configuration at the carbon atoms was assigned as *R* for C(8) and C(28) and *S* for C(10), C(20), C(30) and C(40). The stereogeometry of **6**, if the aromatic moiety is not considered, conforms

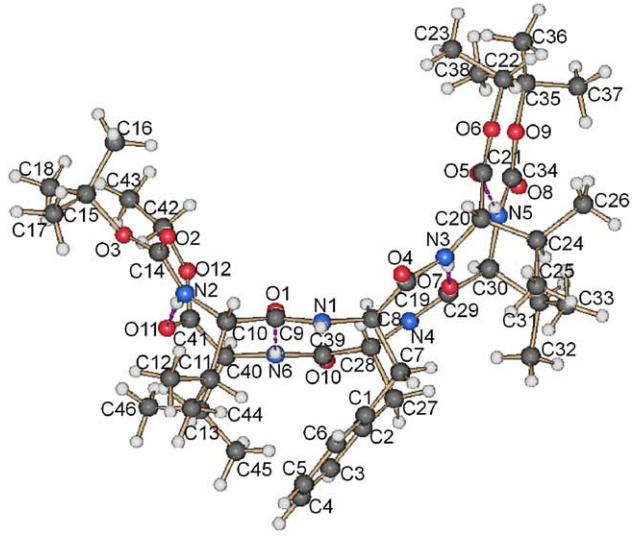


Figure 1. Molecular structure of **6** showing the atomic numbering. H-bonds are drawn in purple.

Table 2. Relevant bond parameters (Å) for **6**[a]

C(1)–C(7)	1.530(5)	C(2)–C(27)	1.531(6)
C(7)–C(8)	1.541(5)	C(27)–C(28)	1.534(6)
C(8)–N(1)	1.451(5)	C(28)–N(4)	1.459(5)
N(1)–C(9)	1.383(6)	N(4)–C(29)	1.370(6)
C(9)–O(1)	1.209(5)	C(29)–O(7)	1.212(5)
C(9)–C(10)	1.527(6)	C(29)–C(30)	1.527(6)
C(10)–C(11)	1.532(7)	C(30)–C(31)	1.543(6)
C(11)–C(12)	1.534(8)	C(31)–C(32)	1.519(7)
C(11)–C(13)	1.530(8)	C(31)–C(33)	1.515(7)
C(10)–N(2)	1.457(6)	C(30)–N(5)	1.445(5)
N(2)–C(14)	1.395(6)	N(5)–C(34)	1.390(6)
C(14)–O(2)	1.197(6)	C(34)–O(8)	1.194(6)
C(14)–O(3)	1.327(6)	C(34)–O(9)	1.335(5)
O(3)–C(15)	1.472(6)	O(9)–C(35)	1.475(6)
C(15)–C(16)	1.528(8)	C(35)–C(36)	1.546(7)
C(15)–C(17)	1.533(8)	C(35)–C(37)	1.521(7)
C(15)–C(18)	1.540(8)	C(35)–C(38)	1.534(7)
C(8)–C(19)	1.526(6)	C(28)–C(39)	1.530(6)
C(19)–O(4)	1.209(5)	C(39)–O(10)	1.209(5)
C(19)–N(3)	1.374(6)	C(39)–N(6)	1.384(6)
N(3)–C(20)	1.465(6)	N(6)–C(40)	1.464(6)
C(20)–C(21)	1.508(7)	C(40)–C(41)	1.510(7)
C(21)–O(5)	1.183(6)	C(41)–O(11)	1.192(6)
C(21)–O(6)	1.314(7)	C(41)–O(12)	1.326(6)
O(6)–C(22)	1.510(8)	O(12)–C(42)	1.504(8)
C(22)–C(23)	1.518(9)	C(42)–C(43)	1.488(9)
C(20)–C(24)	1.541(7)	C(40)–C(44)	1.528(9)
C(24)–C(25)	1.529(7)	C(44)–C(45)	1.520(8)
C(24)–C(26)	1.528(9)	C(44)–C(46)	1.535(7)

[a] Average on the two molecules.

to an approximate C_2-symmetry. The configurations of the stereogenic centres allows the *ortho*-substituents to orient their two chains in an antiparallel fashion, with the *t*-Boc moieties facing the COOEt ones of the opposite substituent and the valine side chains facing each other. As shown in Figure 1, the overall shape of the molecule is that of a reverse-turn or a 'U shaped structure' stabilized by intramolecular N–H···O interactions.

Two intramolecular hydrogen bonds between the amidic hydrogen and the carbonyl oxygen atom of the L-valine moieties from the opposite chain each form a 12-membered cycle, including the hydrogen atom, with the *ortho*-aromatic carbon atoms (Fig. 2), but they are quite asymmetrical.

One of them, N(3)–H(3)···O(7) with H(3)···O(7) 2.00(2), N(3)···O(7) 2.862(6) Å is in fact very linear, 176.2°, while the second one, N(6)–H(6)···O(1), with H(6)···O(1) 2.10(2) and N(6)···O(1) 2.894(6) Å, is 152.3°, probably distorted by the proximity of the phenyl ring. Moreover, two additional, although weaker, hydrogen bonding interactions involve the *t*-Boc-NH with N(2)–H(2)···O(11), H(2)···O(11) 2.28(6), N(2)···O(11) 3.133(7) Å and N–H···O angle of 172.1°; N(5)–H(5)···(5), H(5)···O(5) 2.29(3), N(5)···O(5) 3.141(8) Å and N–H···O angle of 168.6°.

Of the three amide protons present in each chain, only the ones labelled $\mathbf{H^2}$ in solution (Scheme 1), that is, N(1)–H(1) and N(4)–H(4) in the solid state, are not involved in intramolecular hydrogen bonding interactions

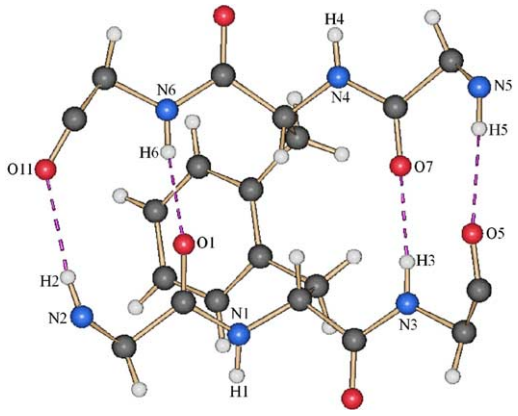


Figure 2. Intramolecular H-bonding interactions shown in detail. The L-valine side chains, *t*-Boc- and EtO-moieties have been omitted for clarity.

because of their outward orientation with respect to the anti-parallel chains (Fig. 2).

5. Experimental

5.1. General information

^{1}H and ^{13}C NMR spectra were recorded on a Gemini spectrometer at 300 MHz using $CDCl_3$ as the solvent, unless otherwise stated. Chemical shifts are reported in ppm relative to $CDCl_3$ and the coupling constants (*J*) are in Hz. IR spectra were recorded on a Nicolet 210 spectrometer. Optical rotation values were measured at 25 °C on a Perkin–Elmer 343 polarimeter. Melting points are uncorrected. Chromatographic separations were performed with silica gel 60 (230–400 mesh). Dry THF was distilled from sodium benzophenone ketyl.

5.2. (3*R*,6*S*)-*ortho*-Bis-(1-benzyl-3,6-dihydro-5-ethoxy-6-isopropyl-pirazin-2-one-3-methylene)benzene 2

To a solution of **1** (5.5 g, 20 mmol) in dry THF (50 mL) and cooled at −78 °C, a solution of 1 M LHMDS in THF (21 mL, 21 mmol) was dropped under stirring. After about 1 h, α,α′-dibromo-*o*-xylene (1.35 mL, 10 mmol) was added and the reaction monitored by TLC. When the reaction was complete, the mixture was allowed to warm up to room temperature under stirring. Water and ethyl acetate were added and after separation the organic solution was evaporated in vacuo. The residue was submitted to purification by silica gel chromatography eluting with hexane/ethyl acetate. The pure product was recovered as an oil in 90% yield. ^{1}H NMR δ: 0.89 (d, 6H, J = 6.8); 1 (d, 6H, J = 7); 1.18 (t, 6H, J = 6.8); 2.19 (m, 2H); 3.33 (dd, 2H, J = 7, 13.6); 3.55 (dd, 2H, J = 1.4, 3.6); 3.93 (d, 2H, J = 15); 3.97–4.2 (m, 6H); 4.38 (m, 2H); 5.5 (d, 2H, J = 15); 7–7.4 (m, 14ArH).^{13}C NMR δ: 14, 17.2, 19.8, 31.2, 35.7, 47, 59.6, 60.9, 61.4, 125.5, 127.2, 127.6, 128.4, 130.5, 135.9, 138.6, 158.3, 170. $[\alpha]_D$ = +64.2 (*c* 0.5, $CHCl_3$). Anal. Calcd for $C_{40}H_{50}N_4O_4$: C, 73.82; H, 7.74; N, 8.61. Found: C, 74.15; H, 7.77; N, 8.64.

5.3. (3*R*,6*S*)-*ortho*-Bis-(3,6-dihydro-5-ethoxy-6-isopropyl-pirazin-2-one-3-methylene)benzene 3

A solution of **2** (3.25 g, 5 mmol) in 50 mL of dry THF/*t*-butanol 9:1 was added to about 100 mL of liquid ammonia cooled at −50 °C and Li (0.07 g, 10 mmol) was then added to the substrate under stirring. The addition of Li, in small pieces, was controlled by the monitoring of the TLC in the presence of substrate (starting material) and was stopped as soon as the reaction mixture became blue. The reaction was then quenched with NH_4Cl and the cooling bath removed allowing the complete removal of NH_3. After addition of water, the aqueous solution was extracted with ethyl acetate and the organic solution evaporated to dryness under vacuum. The pure product was recovered as an oil in 85% yield after silica gel chromatography eluting with hexane/ethyl acetate. ^{1}H NMR δ: 0.79 (d, 6H, $J = 7$); 0.89 (d, 6H, $J = 7$); 1.23 (t, 6H, $J = 6.8$); 2.13 (m, 2H); 3.18 (dd, 2H, $J = 5.8, 13.6$); 3.28 (m, 2H); 3.7 (dd, 2H, $J = 4.8, 13.6$); 4.09 (m, 4H); 4.35 (m, 2H); 5.94 (br s, 2H); 7.08 (m, 4ArH). ^{13}C NMR δ: 14.2, 15.9, 18.1, 31.2, 36.1, 57.8, 59.8, 61.1, 126.1, 130.4, 137.5, 158.3, 171.8. $[\alpha]_D = +43.2$ (*c* 1.5, $CHCl_3$). Anal. Calcd for $C_{26}H_{38}N_4O_4$: C, 66.36; H, 8.14; N, 11.91. Found: C, 66.13; H, 8.15; N, 11.9.

5.4. (3*S*,6*R*)-*ortho*-Bis-(6-amino-4-aza-3-ethoxycarbonyl-2-methyl-5-oxa-hept-7-yl)benzene 4

To a solution of **3** (2.35 g, 5 mmol) in ethanol (50 mL), 2 M HCl (10 mL) was added and the reaction mixture, monitored by TLC and/or ^{1}H NMR, stirred at room temperature for about 12 h. The acid solution was evaporated in vacuo and the intermediate hydrochloride, isolated as a solid in practically quantitative yield, was dissolved in water (20 mL). The solution, after addition of K_2CO_3 (1.38 g, 10 mmol) was stirred for about 1 h, then the reaction product extracted with ethyl acetate and the organic solution washed with water. The organic solvent was completely removed in vacuo and the pure product was isolated as a wax in 90% yield after silica gel chromatography eluting with hexane/ethyl acetate. ^{1}H NMR: 0.91 (d, 12H, $J = 7$); 1.3 (t, 6H, $J = 7.2$); 2.08 (m, 2H); 2.78 (dd, 2H, $J = 8.8, 14.2$); 3.42 (dd, 2H, $J = 4.6, 14.2$); 3.7 (dd, 2H, $J = 4.8, 8.8$); 4.2 (q, 4H, $J = 7.2$); 4.49 (dd, 2H, $J = 4.6, 8.8$); 7.2 (s, 4ArH); 7.71 (d, 2H, $J = 8.8$). ^{13}C NMR δ: 13.8, 17.4, 18.5, 30.5, 37.7, 55.4, 56.6, 60.5, 126.5, 130.1, 136.4, 171.3, 174.1. $[\alpha]_D = +25.7$ (*c* 1, $CHCl_3$). Anal. Calcd for $C_{26}H_{42}N_4O_6$: C, 61.64; H, 8.36; N, 11.06. Found: C, 61.93; H, 8.4; N, 11.09.

5.5. (3*S*,6*R*)-*ortho*-Bis-(6-acetylamino-4-aza-3-ethoxycarbonyl-2-methyl-5-oxa-pent-7-yl)benzene 5

To a solution of **4** (2.53 g, 5 mmol) and triethylamine (2.1 mL, 15 mmol) in CH_2Cl_2 (30 mL) cooled at 0–5 °C was dropped acetyl chloride (1.07 mL, 15 mmol) under stirring. After about 2 h, 2 M HCl (3 mL) was added and the reaction product extracted by ethyl acetate. The organic solution was dried and the solvent was completely evaporated under vacuum. The residue was then submitted to purification by silica gel chromatography eluting with hexane/ethyl acetate. The pure product was recovered as a solid (mp 205.5–207 °C) in 80% yield. ^{1}H NMR δ: 0.88 (d, 6H, $J = 6.6$); 0.91 (d, 6H, $J = 6.6$); 1.28 (t, 6H, $J = 7.2$); 1.85 (s, 6H); 2.15 (m, 2H); 3.12 (m, 4H); 4.2 (q, 4H, $J = 7.2$); 4.3 (m, 2H); 5.28 (m, 2H); 6.64 (d, 2H, $J = 7.6$); 7 (m, 4ArH); 8.4 (br s, 2H). ^{13}C NMR δ: 14.2, 17.7, 19, 22.6, 30.1, 38.3, 54.2, 58.3, 60.8, 126.6, 131.5, 135.5, 169.4, 171.2, 171.7. $[\alpha]_D = +33$ (*c* 1, $CHCl_3$). Anal. Calcd for $C_{30}H_{46}N_4O_8$: C, 61.0; H, 7.85; N, 9.48. Found: C, 60.78; H, 7.82; N, 9.45.

5.6. *ortho*-Bis-[(6*R*)-((2′*S*)-*t*-butoxycarbonylamino-3′-methyl)butyrylamino-4-aza-(3*S*)-ethoxycarbonyl-2-methyl-5-oxa-hept-7-yl]benzene 6

It was obtained by treating **4** with the activated pentafluorophenylester of *N*-Boc-L-valine, prepared by stirring at 0 °C for about 2 h the *N*-Boc-L-valine (1.1 g, 5 mmol), dissolved in DMF (10 mL), with pentafluorophenyltrifluoroacetate (1.3 mL, 7.5 mmol) in the presence of pyridine (1.7 mL, 20 mmol). Water was then added, the ester extracted with ethyl acetate and after complete elimination of the organic solvent in vacuo, the residue was purified by silica gel chromatography eluting with hexane/ethyl acetate. The crude intermediate **4** (2 g, 4 mmol) was dissolved in DMF (10 mL) and stirred with pentafluorophenyl ester of *N*-Boc-(*S*)-valine (3.8 g, 10 mmol) at 50 °C. After 24 h, water was added and the reaction product extracted with ethyl acetate. After complete elimination of the organic solvent in vacuo, the residue was purified by silica gel chromatography eluting with hexane/ethyl acetate. The pure product was recovered as a solid (mp 208.5–209.5 °C) in 90% yield. ^{1}H NMR (DMSO) δ: 0.6 (d,6H, $J = 6.6$); 0.66 (d, 6H, $J = 7$); 0.78 (d, 12H, $J = 6$); 1.18 (t, 6H, $J = 6.2$); 1.38 (s, 18H); 1.75 (m, 2H); 1.93 (m, 2H); 2.95 (m, 4H); 3.86 (dd, 2H, $J = 6.2, 8.4$); 4.12 (m, 8H); 4.95 (m, 2H); 6.55 (d, 2H, $J = 8.6$); 7.06 (m, 4ArH); 8.1 (d, 2H, $J = 8$); 8.2 (d, 2H, $J = 8.8$). ^{13}C NMR δ: 14.2, 17.2, 18.2, 19, 19.3, 28.3, 31.1, 38.3, 54.3, 58, 59.2, 60.3, 61, 79.5, 127.1, 131.9, 135, 155.7, 170.9, 171.2. $[\alpha]_D = -49$ (*c* 1.1, $CHCl_3$). Anal. Calcd for $C_{46}H_{76}N_6O_{12}$: C, 61.04; H, 8.46; N, 9.28. Found: C, 61.3; H, 8.48; N, 9.25.

5.7. (3*R*,6*S*)-*ortho*-Bis-(1-benzyl-3,6-dihydro-5-ethoxy-6-isopropyl-3-methyl-pirazin-2-one-3-methylene)benzene 7a

It was obtained by alkylating **2**[1] with CH_3I and following the procedure already reported for an analogous derivative.[3] After gel chromatography eluting with hexane/ethyl acetate it was obtained pure in 85% yield ^{1}H NMR δ: 0.03 (d, 6H, $J = 7$); 0.83 (d, 6H, $J = 6.8$); 1.23 (t, 6H, $J = 7$); 1.5 (s, 6H); 1.65 (m, 2H); 3.28 (d, 2H, $J = 13.6$); 3.56 (d, 2H, $J = 2.4$); 3.78 (d, 2H, $J = 13.4$); 3.92 (d, 2H, $J = 15$); 4.11 (m, 4H); 5.41 (d, 2H, $J = 15$); 7.2 (m, 14ArH). ^{13}C NMR δ: 14.2, 15.1, 20.7, 29.9, 32.3, 42.6, 47.1, 60.2, 61.1, 63.5, 126.2, 127.3, 127.9, 128.5, 131.6, 136.6, 138.0, 155.1, 172.0. $[\alpha]_D = -34.4$ (*c* 0.2, $CHCl_3$). Anal. Calcd for

$C_{42}H_{54}N_4O_4$: C, 74.3; H, 8.02; N, 8.25. Found: C, 74.43; H, 8.05; N, 8.22.

5.8. (3*R*,6*S*)-*ortho*-Bis-(1,3-dibenzyl-3,6-dihydro-5-ethoxy-6-isopropyl-3-pirazin-2-one-3-methylene)benzene 7b

It was obtained by alkylating **2**[1] with $PhCH_2Br$ following the procedure already reported for an analogous derivative[3] and it was obtained pure as an oil in 80% yield after gel chromatography eluting with hexane/ethyl acetate. ^{1}H NMR δ: −0.2 (d, 6H, J = 6.9); 0.68 (d, 6H, J = 6.9); 1.33 (t, 6H, J = 7.2); 2.92 (d, 2H, J = 2.4); 3.04 (d, 2H, J = 12.3); 3.46 (d, 2H, J = 12.3); 3.5 (d, 2H, J = 12.9); 4 (d, 2H, J = 12.9); 4.13 (d, 2H, J = 15.3); 4.29 (m, 4H); 4.71 (d, 2H, J = 15.3); 6.7–7.36 (m, 24ArH). ^{13}C NMR δ: 14, 14.5, 20.8, 29.2, 42.9, 48.1, 49.5, 60.4, 61.4, 68.3, 126.4, 126.9, 127.8, 128.2, 130.8, 131.9, 136.1, 137.3, 137.9. $[\alpha]_D$ = −97 (c 1, $CHCl_3$). Anal. Calcd for $C_{54}H_{62}N_4O_4$: C, 78.04; H, 7.52; N, 6.74. Found: C, 78.68; H, 7.5; N, 6.75.

5.9. (3*S*,6*S*)-*ortho*-Bis-(6-amino-4-aza-3-ethoxycarbonyl-2,6-dimethyl-5-oxa-hept-7-yl)benzene 8

It was prepared starting from **7a** following the procedure reported to convert the derivative **2** into **4** and it was obtained pure as a wax in 70% overall yield after gel chromatography eluting with hexane/ethyl acetate. ^{1}H NMR δ: 0.83 (d, 6H, J = 6.8); 0.86 (d, 6H, J = 6.8); 1.28 (t, 6H, J = 7); 1.39 (s, 6H); 1.46 (br s, 4H); 2.15 (m, 2H); 3.05 (d, 2H, J = 13.8); 3.41 (d, 2H, J = 13.8); 4.2 (m, 4H); 4.42 (dd, 2H, J = 4.8, 8.8); 7.1–7.27 (m, 4ArH); 8.08 (d, 2H, J = 8.8).^{13}C NMR δ: 14.1, 17.7, 18.8, 30, 30.7, 41.7, 57.1, 59.2, 60.9, 126.9, 130.9, 136.2, 171.7, 176.5. $[\alpha]_D$ = −75.9 (c 0.6, $CHCl_3$). Anal. Calcd for $C_{28}H_{46}N_4O_6$: C, 62.9; H, 8.67; N, 10.48. Found: C, 62.78; H, 7.55; N, 10.45.

5.10. (3*S*,6*S*)-*ortho*-Bis-(6-acetylamino-4-aza-3-ethoxycarbonyl-2,6-dimethyl-5-oxa-hept-7-yl)benzene 9

It was obtained starting from **8** following the procedure reported for derivative **5** and it was obtained as a solid (mp 137–138.5 °C) in 80% yield after gel chromatography eluting with hexane/ethyl acetate ^{1}H NMR δ: 0.94 (d, 6H, J = 6.8); 0.97 (d, 6H, J = 6.8); 1.34 (t, 6H, J = 7.4); 1.73 (s, 6H); 1.94 (s, 6H); 2.25 (m, 2H); 3.37 (q_{AB}, 4H, J = 14.2); 4.26 (m, 4H); 4.7 (dd, 2H, J = 4.4, 9.2); 6.36 (s, 2H); 7.12 (s, 4ArH); 7.35 (d, 2H, J = 9.2). ^{13}C NMR δ: 14.1, 17.4, 19, 23.5, 24.4, 31.7, 37.4, 57.3, 61.1, 61.5, 126.3, 131.4, 135.3, 170.1, 172.2, 173.6. $[\alpha]_D$ = +13.9 (c 0.8, $CHCl_3$). Anal. Calcd for $C_{32}H_{50}N_4O_8$: C, 62.11; H, 8.14; N, 9.05. Found: C, 61.98; H, 8.15; N, 9.05.

5.11. Conversion of 1 into 10 and 10′

To a solution of **1** (2.25 g, 10 mmol) in dry THF (30 mL) and cooled at −78 °C, a solution of 1 M LHMDS in THF (10.5 mL, 10.5 mmol) was dropped under stirring. After about 1 h, CH_3I (0.6 mL, 10 mmol) was added and the reaction monitored by TLC. When the reaction was complete, the mixture was allowed to warm up to room temperature under stirring. Water and ethyl acetate were added and after separation the organic solution was evaporated in vacuo. The residue was submitted to silica gel chromatography eluting with hexane/ethyl acetate in order to separate the diastereomers **10** and **10′**.

5.12. (3*R*,6*S*)-1-Benzyl-3,6-dihydro-5-ethoxy-6-isopropyl-3-methyl-pirazin-2-one 10

It was recovered as an oil in 65% yield. ^{1}H NMR δ: 0.95 (d, 3H, J = 7); 1.06 (d, 3H, J = 7); 1.26 (t, 3H, J = 7.2); 1.57 (d, 3H, J = 7); 2.22 (m, 1H); 3.7 (dd, 1H, J = 1.4, 4); 3.95 (d, 1H, J = 15); 4.1 (m, 3H); 5.47 (d, 1H, J = 15); 7.17 (m, 5ArH). ^{13}C NMR δ: 14.2, 17.7, 20.1, 20.7, 31.6, 47.5, 53.9, 61.1, 62.4, 127.4, 127.7, 128.6, 136.3, 159.2, 171.3. $[\alpha]_D$ = +24.2 (c 1.7, $CHCl_3$). Anal. Calcd for $C_{17}H_{24}N_2O_2$: C, 70.8; H, 8.39; N, 9.71. Found: C, 71.1; H, 8.41; N, 9.65.

5.13. (3*S*,6*S*)-1-Benzyl-3,6-dihydro-5-ethoxy-6-isopropyl-3-methyl-pirazin-2-one 10′

It was recovered as an oil in 25% yield. ^{1}H NMR δ: 0.94 (d, 3H, J = 7); 1.09 (d, 3H, J = 7.2); 1.26 (t, 3H, J = 7); 2.18 (m, 1H); 3.73 (dd, 1H, J = 1.8, 3.4); 3.94 (d, 1H, J = 15); 4.02–4.29 (m, 2H); 5.49 (d, 1H, J = 15); 7.23–7.37 (m, 5ArH). ^{13}C NMR δ : 13.5, 17.1, 19.8, 20.8, 29.5, 46.2, 54.6, 60.3, 60.6, 127, 127.5, 128.1, 135.5, 156.7, 169.9. $[\alpha]_D$ = −12.1 (c 2.2, $CHCl_3$). Anal. Calcd for $C_{17}H_{24}N_2O_2$: C, 70.8; H, 8.39; N, 9.71. Found: C, 71.0; H, 8.43; N, 9.69.

5.14. (3*R*,6*S*)-*ortho*-Bis-(1-benzyl-3,6-dihydro-5-ethoxy-6-isopropyl-3-methyl-pirazin-2-one-3-methylene)benzene 11

It was obtained by alkylating **10** with α,α′-dibromo-*o*-xylene and following the procedure used for **2**. The pure product was recovered as an oil in 85% yield after purification by silica gel chromatography eluting with hexane/ethyl acetate. ^{1}H NMR δ: 0.81 (d, 6H, J = 6.6); 0.89 (d, 6H, J = 6.8); 1.2 (t, 6H, J = 7); 1.6 (s, 6H); 2.02 (m, 2H); 3.28 (d, 2H, J = 2.6); 3.48 (d, 2H, J = 13.6); 3.6 (d, 2H, J = 13.6); 3.84 (d, 2H, J = 15); 3.8–4.3 (m, 4H); 5.4 (d, 2H, J = 15); 6.8–7.25 (m, 14ArH). ^{13}C NMR δ: 14.1, 16.7, 20.1, 28.8, 29.9, 44.5, 46.1, 59.9, 60.2, 62.7, 125.6, 127.1, 128.1, 128.3, 130.6, 135.1, 137.7, 155, 171.7. $[\alpha]_D$ = −10.7 (c 1, $CHCl_3$). Anal. Calcd. for $C_{42}H_{54}N_4O_4$: C, 74.3; H, 8.02; N, 8.25. Found: C, 74.57; H, 8.03; N, 8.29.

5.15. (3*R*,6*S*)-*ortho*-Bis-(3,6-dihydro-5-ethoxy-6-isopropyl-3-methyl-pirazin-2-one-3-methylene)benzene 12

It was obtained submitting the intermediate **11** to the Birch reaction and following the procedure used for **3**. The pure product was recovered as an oil in 80% yield after purification by silica gel chromatography eluting with hexane/ethyl acetate.^{1}H NMR δ : 0.72 (d, 6H, J = 7); 0.8 (d, 6H, J = 7.2); 1.23 (t, 6H, J = 7.4); 1.6 (s,

6H); 2.08 (m, 2H); 2.89 (dd, 2H, $J = 1.2, 3$); 3.11 (d, 2H, $J = 13.2$); 3.49 (d, 2H, $J = 13.2$); 3.9–4.3 (m, 4H); 5.55 (br s, 2H); 7.1 (m, 4ArH). ^{13}C NMR δ: 14.2, 15.5, 18, 29.1, 30, 43.9, 57.4, 60.8, 62.8, 126.3, 130.2, 137, 155.8, 173.8. $[\alpha]_D = -30.3$ (c 1.1, $CHCl_3$). Anal. Calcd for $C_{28}H_{42}N_4O_4$: C, 67.44; H, 8.49; N, 11.24. Found: C, 67.67; H, 8.53; N, 11.29.

5.16. (3*S*,6*R*)-*ortho*-Bis-(6-amino-4-aza-3-ethoxycarbonyl-2,6-dimethyl-5-oxa-hept-7-yl)benzene 13

It was obtained from **12** and following the procedure used to convert **3** into **4**. The pure product was recovered as an oil in 80% yield after purification by silica gel chromatography eluting with hexane/ethyl acetate. ^{1}H NMR δ: 0.92 (d, 6H, $J = 7$); 0.96 (d, 6H, $J = 7$); 1.27 (t, 6H, $J = 7$); 1.37 (s, 6H); 2.2 (m, 2H); 3.22 (q_{AB}, 4H, $J = 14$); 4.18 (q, 4H, $J = 7$); 4.47 (dd, 2H, $J = 5.2, 9$); 7.2 (m, 4ArH); 8.11 (d, 2H, $J = 9$). ^{13}C NMR δ: 14.2, 17.7, 19, 28.2, 31.2, 41.5, 57, 59, 60.9, 126.9, 130.8, 136.3, 171.7, 176.8. $[\alpha]_D = +56$ (c 1.2, $CHCl_3$). Anal. Calcd for $C_{28}H_{46}N_4O_6$: C, 62.9; H, 8.67; N, 10.48. Found: C, 63.11; H, 8.63; N, 10.49.

5.17. (3*S*,6*R*)-*ortho*-Bis(6-acetylamino-4-aza-3-ethoxycarbonyl-2,6-dimethyl-5-oxa-hept-7-yl)benzene 14

It was obtained from **13** and following the procedure used to convert **4** into **5**. The pure product was recovered as a solid (mp 180–1 °C) in 80% yield, after purification by silica gel chromatography eluting with hexane/ethyl acetate. ^{1}H NMR δ: 0.87 (d, 6H, $J = 6.8$); 0.90 (d, 6H, $J = 6.8$); 1.28 (t, 6H, $J = 6.8$); 1.51 (s, 6H); 2.0 (s, 6H); 2.12 (m, 2H); 3.45 (q_{AB}, 4H, $J = 14.8$); 4.2 (m, 4H); 4.45 (dd, 2H, $J = 4.6, 8$); 6.2 (s, 2H); 6.87 (d, 2H, $J = 8$); 7.15 (m, 4ArH). ^{13}C NMR δ: 14, 17.7, 18.6, 23.4, 23.7, 31, 37, 57.5, 60.8, 61, 126.5, 130.8, 135.6, 170.3, 171.6, 173.5. $[\alpha]_D = +69.7$ (c 0.9, $CHCl_3$). Anal. Calcd for $C_{32}H_{50}N_4O_8$: C, 62.11; H, 8.14; N, 9.05. Found: C, 62.1; H, 8.15; N, 9.07.

5.18. X-ray crystallography

Transparent crystals of **6**, suitable for the X-ray diffraction studies, were obtained from a solution in ethyl acetate by slow evaporation of the organic solvent.

Diffraction intensities were collected on a Bruker AXS SMART 2000 CCD diffractometer using graphite monochromated Mo-Kα radiation ($\lambda = 0.71069$ Å). The data were collected using 0.3° wide ω scans, crystal-to-detector distance of 5.0 cm. The software SMART[9] was used for collecting frames of data, which were then processed for integration by software SAINT.[9] An empirical absorption correction was applied using the SADABS routine.[10] Data collections nominally covered a full sphere of reciprocal space with 30 s exposure time per frame. The structure was solved by direct methods (SIR97)[11] and refined on F^2 by full matrix least squares calculations using the SHELXTL/PC package.[12] Thermal vibrations were treated anisotropically. Some disorder, which could not be rationalized, affects the terminal atoms and is reflected in the higher thermal parameters. An attempt of a low temperature data collection was prevented from cracking of the crystal. The absolute configuration was assigned by using the Flack parameter (−0.05 for the correct absolute structure).[13] H atoms were geometrically positioned [C–H 0.93 and 0.97 Å for aromatic and aliphatic distances, N–H 0.86 Å] and refined 'riding' on their corresponding carbon or nitrogen atoms. Molecular graphics were prepared using SCHAKAL 97.[14]

5.19. Crystallographic data

$C_{46}H_{76}N_6O_{12}$, triclinic, $P1$ (No. 1), $a = 13.033(1)$, $b = 13.307(1)$, $c = 16.624(1)$ Å, $\alpha = 94.76(1)$, $\beta = 106.32(1)$, $\gamma = 94.79(1)$, $V = 2740(1)$ Å^3, $Z = 2$, $\mu = 0.079$ mm^{-1}. 26872 reflections were collected, 15414 unique, 8424 observed for $I > 2\sigma(I)$, which were used in all calculations. Final R factors: $R_1 = 0.0624$, $wR^2 = 0.177$.

Crystallographic data (excluding structure factors) for the structure of **6** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 249923. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)1223336033 or e-mail: deposit@ccdc.cam.ac.uk].

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