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Stereoselective approach to uncommon tripeptides incorporating a 2,6-diaminopimelic acid framework: X-ray analysis and conformational studies. Part 4☆

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Abstract—Stereoselective synthesis of pseudopeptides **4**, **5**, **8**, **9**, **13** and **14**, incorporating 2,6-diamino-4-methylen-1,7-heptanedioic acid residue, has been accomplished starting from the L-valine derived chiral synthon **1**. The absolute configuration of new stereocentres was assigned on the basis of ^{1}H NMR spectra. The geometry of these unnatural tripeptides was deduced on the basis of ^{1}H NMR parameters and IR spectra. X-ray analysis of the unusual peptide **18** and conformational studies of **5**, **9** and **14** are also reported.

1. Introduction

The present article is a continuation of our program directed towards producing uncommon tripeptides C-terminal at both ends of the chain.[1–4] For this purpose, we undertook the stereoselective synthesis of new and complex pseudopeptides, as **5**, **9** and **14**, incorporating the 4-methylen-derivative of the 2,6-diaminopimelic acid (pointed out in red in Schemes 1 and 2) and the L-valine units at the ends of the chain.

Our interest in these 'unnatural' tripeptides arises from their potential biological activity as antibacterial[5] and/or herbicide agents.[6] In fact, it can be supposed that mimetics of 2,6-diaminopimelic acid (2,6-DAP) can function as inhibitors of biosynthetic formation or metabolism of L-lysine and *meso*-2,6-DAP. Furthermore, short peptides, able to mimic some important aspect of protein structure or their function, can behave as bioactive molecules.[7] Finally, such compounds are interesting because in the literature it has been reported that some tri- and tetrapeptides incorporating the 2,6-DAP skeleton, conjugated with lauric or palmitic acid, show biological activity as immuno-adjuvants.[8] 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.[1–4]

2. Synthesis and stereochemical assignments

The stereoselective synthesis, makes use of chiral synthon **1**, a mono-lactim ether easily synthesised starting from L-valine, as reported previously.[1–4] Deprotonation of **1** with LHMDS followed by the alkylation with 0.5 equiv of 2-iodomethyl-3-iodopropene (obtained from 2-chloromethyl-3-chloropropene and NaI) afforded the diastereomer (3′*R*,6′*S*,3″*R*,6″*S*)-**2** in good yield and with a practically total double 1,4-*trans* induction with respect to the isopropyl group (ds $\geqslant$96%).[1–4]

Isomer **2**, which has a C_2-symmetry axis, was obtained pure by silica gel chromatography with its stereochemistry well established on the basis of the ^{1}H NMR spectra, as already reported.[1,3,4] Intermediate **2**, submitted to the Birch reduction to remove the benzyl groups and to the acid hydrolysis in mild conditions, was converted into hydrochloride **3** and then into aminoester **4**. The acylation of **4** gave the 'non-classical tripeptide' **5**, C-terminal

☆ Refs. 1–3 are considered to be Parts 1–3.
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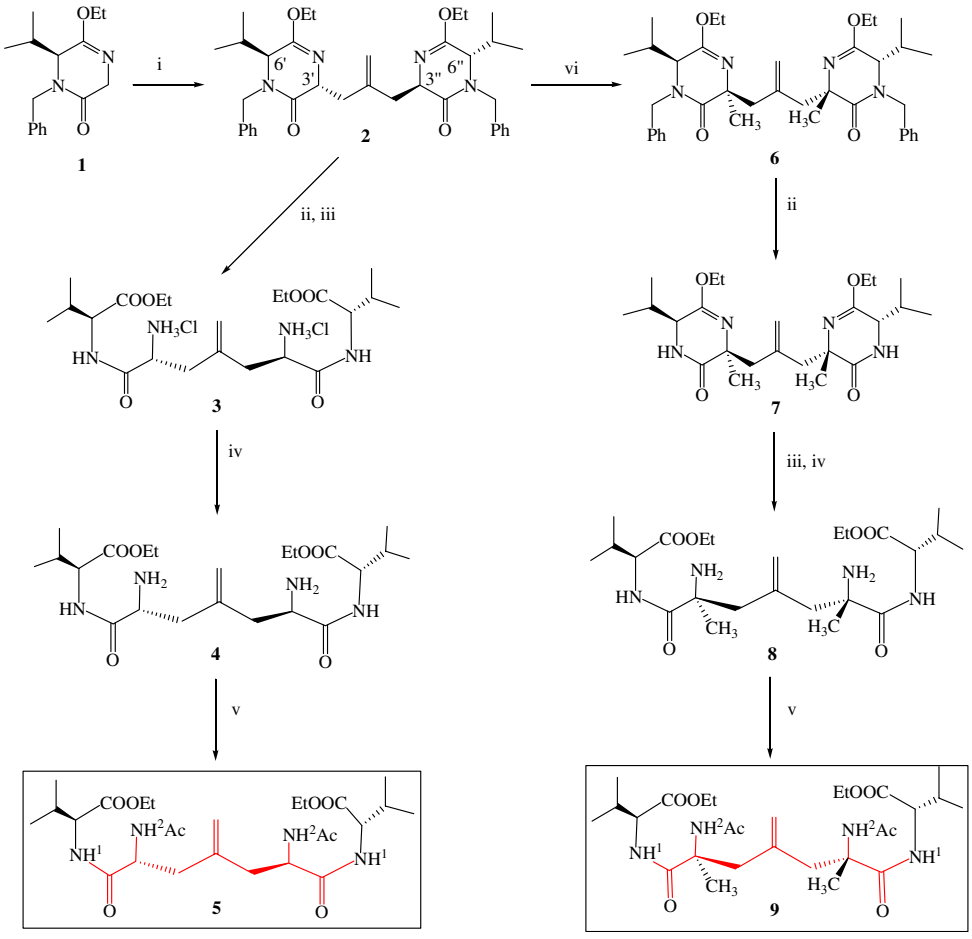
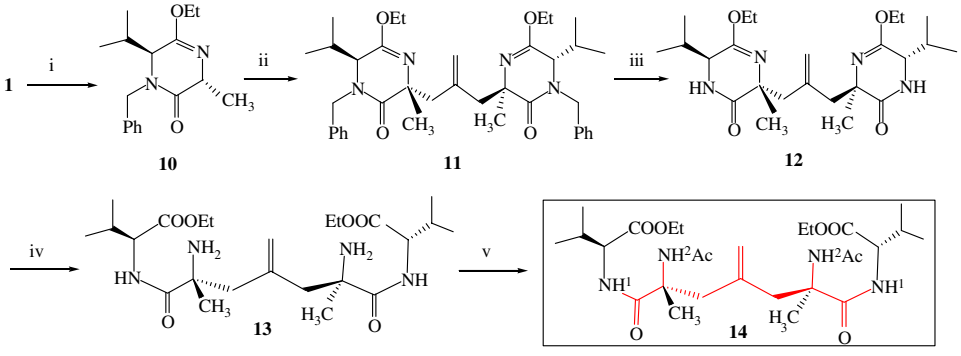


Scheme 1. Reagents and conditions: (i) 1 M LHMDS/THF, then 0.5 equiv of $(CH_2I)_2C{=}CH_2$; (ii) Li/NH_3; (iii) 0.5 M HCl at rt; (iv) 1 M K_2CO_3; (v) CH_3COCl/Et_3N, CH_2Cl_2; (vi) 1 M LHMDS/THF, then 2 equiv of CH_3I.



Scheme 2. Reagents and conditions: (i) LHMDS/THF, then CH_3I; (ii) LHMDS/THF, then 0.5 equiv of $(ICH_2)_2C{=}CH_2$; (iii) Li/NH_3; (iv) 0.5 M HCl at rt, then 1 M K_2CO_3; (v) CH_3COCl/Et_3N, CH_2Cl_2.

at both ends of the chain, which can also be considered as a 2,6-DAP derivative (Scheme 1). Intermediate **2**, alkylated with CH_3I at both positions 3′ and 3″, gave **6** in good yield and with a practically total *trans*-induction (de $\geqslant$96%) induced by the isopropyl group, as previously observed for similar substrates[1] (Scheme 1). The *trans*-stereoselection was evident from the ^1H NMR and ^{13}C NMR spectra, which showed the molecules possessing a C_2-axis of symmetry. In fact, in compound **6** the signals for both methyl and isopropyl groups overlap as do the signals for benzylic protons indicating their magnetic equivalence. The overlapping signals also occurred between (C-3′)-H and (C-3″)-H, (C-6′)-H and (C-6″)-H and between (C-5′)-OC_2H_5 and (C-5″)-OC_2H_5 protons. However, the stereochemistry of **6** was confirmed by the NOE registered between the (C-3′)-CH_3 and (C-6′)-H, the absolute configuration of stereocentre C-6′ being known. Intermediate **6** was then converted into **9** following the same procedure used to obtain **5** (Scheme 1).

The synthesis of diastereomeric derivatives **12**, **13** and **14**, with an opposite configuration at the stereocentres C-3′ and C-3″, in comparison to **6**, **7** and **9**, respectively, was accomplished on the basis of the practically total stereocontrolled *trans*-induction observed in the alkylation reaction. Thus, the strategy described in Scheme 2 was followed, starting from **10** synthesised as previously reported.[1,3]

3. ^{1}H NMR and IR studies

Firstly, it is important to underline that 'no classical' tripeptides **5**, **9** and **14** possess a symmetry element, that is, a C_2-axis. In fact, as previously observed for similar derivatives,[1,3,4] the ^{1}H NMR and ^{13}C NMR spectra show half of the expected signals (see above).

The simple peptides **15**, **16** and **17**, **18**, synthesised starting from **10** and **1**, respectively, (Scheme 2) have been investigated as models in order to acquire spectroscopic data useful for deducing the geometry of the more complex tripeptides **5**, **9** and **14** through ^{1}H NMR and IR informations. In fact, unnatural peptides **16**, **17** and **18** can be considered as monomeric structures of **14**, **5** and **9**, respectively. To determine the structural features of the uncommon peptides synthesised, we performed conformational investigations by both ^{1}H NMR and IR spectroscopies, as well as by molecular modelling studies.[2–4,9]

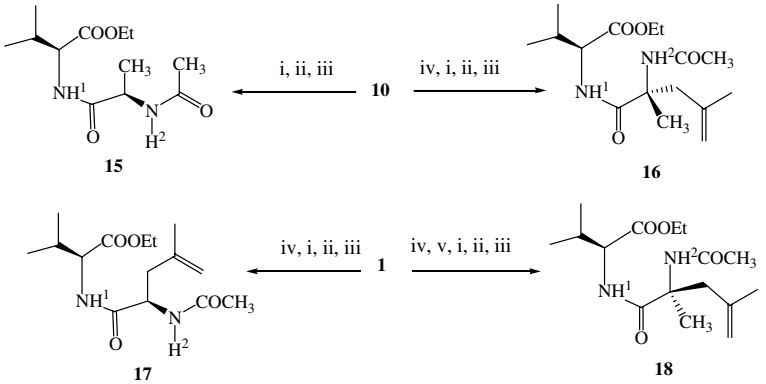
The meaningful ^{1}H NMR and IR data of substrates submitted to the investigation are listed in Table 1 and, for clarity their amide protons are labelled as H^1 and H^2 (see Schemes 1–3).

In a 20 mM $CDCl_3$ solution of **15**, H^1 and H^2 protons resonate at 6.9 and 6.35 ppm, respectively, and in a 2 mM solution they are shifted upfield to 6.6 and 6.1 ppm, respectively. By adding only 4% of DMSO (a competitive solvent in the formation of hydrogen bonds), both protons suffer a significant downshift to 7.25 and 6.9 ppm, respectively. Besides, the IR spectrum in the 2 mM $CHCl_3$ solution shows only one intense sharp band at 3428 cm^{-1}, ascribable to the free amidic NHs. These data and the small temperature coefficient, $\Delta\delta_{NH}/\Delta T$, suggest that dipeptide **15** most probably forms intermolecular hydrogen bonds, which are broken by the addition of just 4% of DMSO. This hypothesis is strengthened by the spectroscopic data of peptide **17**, which exists in two conformations in a ratio of ≈1/3, as evidenced by the ^{1}H NMR spectrum. In $CDCl_3$ solution, the H^1 and the H^2 of the less abundant conformer resonate at 8.5 and at 6.2 ppm, respectively, while in the more abundant one they resonate at 6.6 and at 5.9 ppm, respectively. In DMSO, the H^1 doublet of the minor conformer does not change while the H^2 undergoes a relevant downfield shift. In addition, the less abundant conformer shows a temperature coefficient value of

Table 1. Meaningful ^{1}H NMR and IR data of substrates **5**, **9**, **14**, **15**, **16**, **17** and **18**

Product	δ_{NH} (ppm) (in 2 mM $CDCl_3$)		δ_{NH} (ppm) (in 2 mM $CDCl_3$/DMSO)		$\Delta\delta_{NH}/\Delta T$ (ppb/°C) (in $CDCl_3$)		IR (cm^{-1}) (2 mM $CHCl_3$)
	H^1	H^2	H^1	H^2	H^1	H^2	
5	7.5	7.0	7.9[a]	7.4[a]	−2.0	−2.1	3415 (sharp) 3303 (broad)
9	8.1	7.1	8.0[a]	7.2[a]	−3.8	−1.0	3370 (broad) 3425 (sharp)
14	7.4	6.95	7.5[a]	7.5[a]	−1.9	−2.2	3376 (broad) 3281 (broad)
15	6.6	6.1	7.25[b]	6.9[b]	−2.1	−2.1	3428 (sharp)
16	6.95	6.3	7.4[c]	7.0[c]	−0.9	−1.5	3429 (sharp) 3387 (sharp)
17 [d]	6.6	5.9	8.4[f]	7.9[f]	−1.5	−1.5	3430 (sharp)
17 [e]	8.5	6.2	8.5[f]	8.0[f]	−6.0	−1.5	3299 (broad)
18	6.9	6.4	7.3[g]	7.0[g]	−0.4	−1.9	3437 (sharp) 3388 (sharp)

[a] In $CDCl_3$/DMSO = 80/20.
[b] In $CDCl_3$/DMSO = 96/4.
[c] In $CDCl_3$/DMSO = 88/12.
[d] More abundant conformer.
[e] Less abundant conformer.
[f] In 100% DMSO.
[g] In $CDCl_3$/DMSO = 92/8.



Scheme 3. Reagents and conditions: (i) Li/NH_3; (ii) 0.5 M HCl at rt, then 1 M Na_2CO_3; (iii) CH_3COCl/Et_3N in CH_2Cl_2; (iv) LHMDS/THF, then $CH_2{=}C(CH_3)CH_2Cl$; (v) LHMDS/THF, then CH_3I.

−6.0 ppb/°C for H^1 and −1.5 ppb/°C for H^2. Conversely, both H^1 and H^2 of the prevalent conformer suffer a large downfield shift in DMSO and show the same small value of the temperature coefficient. The IR spectrum in 2 mM $CHCl_3$ solution shows one sharp band at 3430 cm^{-1}, attributable to the free amidic NH, and the less intense broad band at 3299 cm^{-1} attributable to a hydrogen bonding structure. These findings lead us to believe that the minor conformer most probably forms an intramolecular hydrogen bond between H^1 and the acetamidic carbonyl oxygen giving rise to a cyclic seven-membered structure, while in the major conformer any hydrogen bond is present, analogous to that observed for **15**. We believe that the value of −6.0 ppb/°C, calculated for the H^1 in the less abundant conformer, is probably due to the existence of a dynamic equilibrium between the intramolecularly hydrogen-bonded and non-hydrogen-bonded structure, the latter increasing with the temperature.

In tripeptide **5**, which can be considered a dimeric structure of **17**, both H^2 protons, which do not display concentration dependence, absorb as a doublet at 7 ppm in $CDCl_3$ ($\Delta\delta_{NH}/\Delta T = -2.1$ ppb/°C) and the signal shifted to 7.4 ppm by adding 20% DMSO. The H^1 protons, which absorb as doublet at 7.5 ppm ($\Delta\delta_{NH}/\Delta T = -2$ ppb/°C) and display no concentration dependence, are downfield shifted by 0.4 ppm on the addition of 20% DMSO, as exhibited by the H^2 protons. Besides, the IR spectrum shows a broad band at 3303 cm^{-1}, ascribable to a hydrogen bonded NH amide, and a sharp band at 3415 cm^{-1} characteristic of a free NH amide.

By comparing these data with those reported for **17** and **15**, it is possible to argue that tripeptide **5** exists in a conformation in which both H^1 and H^2 are engaged in intramolecular hydrogen bonds.

Dipeptide **18** in $CDCl_3$ shows a doublet at 6.9 ppm for H^1 and a singlet at 6.4 ppm for H^2, which suffer a significant downfield shift by adding only 8% of DMSO and show a temperature coefficient of −0.4 and −1.9 ppb/°C, respectively. The IR spectrum shows two sharp bands at 3437 and 3388 cm^{-1} of comparable intensity ascribable to two different free NHs amide. In fact, the structure resolved by X-ray crystallography (see later) shows the presence of an intermolecular hydrogen bond between H^1 and the acetamidic carbonyl oxygen and a weak intramolecular hydrogen bond between H^2 and (C-4)═O which gives rise to a five-membered cyclic structure.

In tripeptide **9**, the magnetically equivalent H^2 protons absorb as a singlet at 7.1 ppm in 2 mM $CDCl_3$ solution and are shifted to 7.2 ppm by adding 20% of DMSO. Additionally, these protons show no concentration dependence and a small temperature coefficient ($\Delta\delta_{NH}/\Delta T = -1$ ppb/°C). The H^1 protons, which do not display concentration dependence, absorb as a doublet at 8.1 ppm, are shifted to 8.0 ppm after the addition of 20% DMSO and exhibit a temperature coefficient $\Delta\delta_{NH}/\Delta T = -3.8$ ppb/°C in 2 mM $CDCl_3$ solution. In addition, the IR spectrum of **9** shows a very broad band centred at 3370 cm^{-1} attributable to the hydrogen bonded NH's amide. These findings suggest that both H^1 and H^2 probably form intramolecular hydrogen bonds. Thus, it is possible to infer that the presence of CH_3 at C-5 and C-9 positions favours the formation of intramolecular hydrogen bonds between the amide NHs and the carbonyl groups, making the structure very compact.

In tripeptide **16**, the H^1 protons resonate at 6.95 and 6.3 ppm, respectively, which are shifted to a lower field after addition of 12% DMSO, and show a temperature coefficient of −0.9 ppb/°C and −1.5 ppb/°C, respectively. The IR spectrum shows two sharp bands of comparable intensity at 3429 and 3387 cm^{-1} ascribable to free amidic NHs. Thus, it is reasonable to deduce that **16**, analogously to **15**, does not form hydrogen bonds.

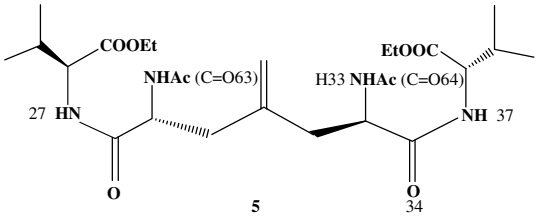
The H^2 protons of the tripeptide **14** absorb as a singlet at 6.95 ppm, in 2 mM $CDCl_3$ solution, which is shifted downfield to 7.5 ppm after addition of 20% DMSO; their temperature coefficient in $CDCl_3$ solution is −2.2 ppb/°C. Neither H^1 protons (doublet at 7.4 ppm, $\Delta\delta_{NH}/\Delta T = -1.9$ ppb/°C) display concentration dependence in $CDCl_3$ nor do they undergo an appreciable shift downfield by adding 20% of DMSO ($\delta_{NH} = 7.5$ ppm). The IR spectrum exhibits two broad bands at 3281 and 3376 cm^{-1}, ascribable to the two different hydrogen bonded –NHs amide, and one sharp band at 3425 cm^{-1} characteristic of a free –NH amide. From these data it can be believed that H^1 probably forms a strong intramolecular hydrogen bond, while H^2 a weaker one. Therefore, from the comparison between **9** and **14**, it is possible deduce that the absolute configurations of C-5 and C-9 affect the conformation of the tripeptide. In fact, from the spectroscopic data we can deduce that, owing to the formation of the two strong intramolecular hydrogen bonds, tripeptide **9** possesses a more organised structure than **14**.

4. Molecular modelling: conformational analysis

A complete, extensive unconstrained conformational analysis of **5**, **9** and **14** was performed by using AMBER* force field[10] and the Monte Carlo conformational search[11] (MC/EM), all degrees of freedom being varied. A relative nonpolar solvent, such as $CHCl_3$, was chosen in order to not interfere in hydrogen bonding of the amide protons and to compare these results with the ^1H NMR data. All the conformers within the energy gap of 6 kcal/mol were kept and subsequently only the ones below 3.6 kcal/mol were fully analysed.

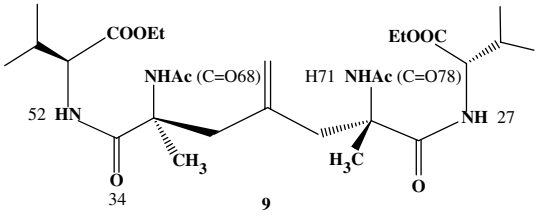
The investigated compounds show a preference to assume very compact conformations in which both amidic protons H^1 and H^2 form intramolecular hydrogen bonds, according to that deduced from spectroscopic data. Among the conformers significantly populated, that is, population >3.0%, at 298 K (Tables 2–4) a relevant role is played by the two most populated conformations.

Table 2. Most significant low energy conformers of **5** (438 within 6 kcal/mol, 14 within 2.0 kcal/mol)



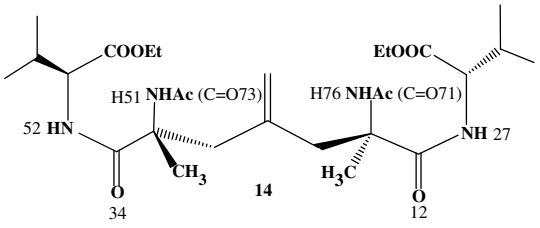
Conformers	Energy (kcal/mol)	Number of H-bonds	Population (%)
1	0.0	3	37.0
2	0.54	3	14.8
3	0.84	3	8.9
4	0.87	3	8.5
5	0.90	3	8.1
6	0.95	3	7.0
7	1.46	3	3.0

Table 3. Most significant low energy conformers of **9** (399 within 6 kcal/mol, 15 within 2.0 kcal/mol)



Conformers	Energy (kcal/mol)	Number of H-bonds	Population (%)
1	0.0	3	36.6
2	0.71	3	11.0
3	0.79	3	9.6
4	0.88	3	8.4
5	1.06	3	6.2
6	1.08	3	5.9
7	1.11	3	5.1
8	1.25	2	4.4

Table 4. Most significant low energy conformers of **14** (291 within 6 kcal/mol, 14 within 2.0 kcal/mol)



Conformers	Energy (kcal/mol)	Number of H-bonds	Population (%)
1	0.0	3	45.3
2	0.55	3	19.5
3	1.13	3	6.8
4	1.43	3	4.1
5	1.51	2	3.6
6	1.53	2	3.6
7	1.57	3	3.2

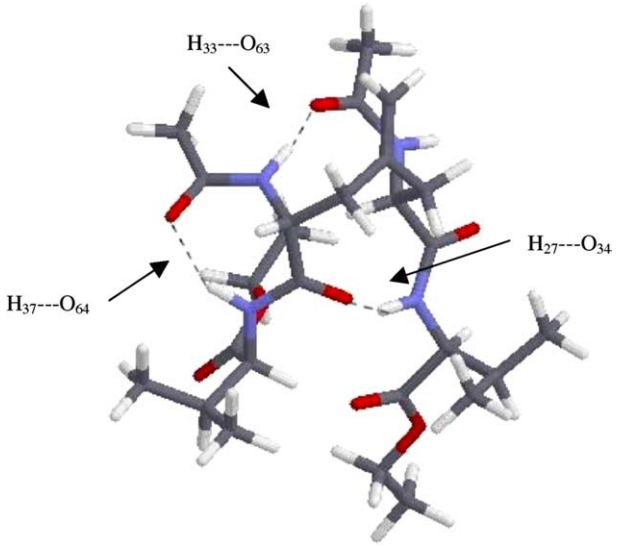


Figure 1. Lowest energy conformation for compound **5** (the arrows point out the hydrogen bonds).

In molecule **5**, all conformers show the existence of intramolecular amidic hydrogen bonds. In particular, in the lowest energy conformers (1 and 2 in Table 2) the two strongest intramolecular hydrogen bonds (1.81 Å $H_{33}\cdots O_{63}$ and 1.93 Å $H_{27}\cdots O_{34}$) form two rings of 10 terms (Fig. 1), while the third one (1.86 Å $H_{37}\cdots O_{64}$) gives rise to a seven-membered ring. It is noteworthy that the major difference among the most populated conformations lies in the orientation of the lateral chains.

From the examination of the lowest energy conformations of **9** (Table 3), it can be observed that conformer 1 shows three intramolecular amidic hydrogen bonds (1.80 Å $H_{52}\cdots O_{68}$, 1.86 Å $H_{27}\cdots O_{78}$ and 1.73 Å $H_{71}\cdots O_{34}$) forming two seven-membered rings and a nine-membered one (Fig. 2). In conformations 2 and 3 both H_{52} and H_{71} form hydrogen bonds with the carbonyl oxygen O_{68} giving rise to both a seven- and a ten-membered ring (1.78 Å $H_{71}\cdots O_{68}$ and 1.86 Å $H_{52}\cdots O_{68}$), the third one being the same of conformer 1 (1.86 Å $H_{27}\cdots O_{78}$).

In the lowest energy conformation the molecule **14** (Table 4) shows three amidic intramolecular hydrogen bonds (1.86 Å $H_{27}\cdots O_{71}$, 1.73 Å $H_{52}\cdots O_{73}$ and 1.80 Å $H_{51}\cdots O_{12}$), which give rise to two seven-membered rings and one of 9 terms (Fig. 3). Also in conformers 2 and 3, the hydrogen bonds produce two seven-membered rings and one of 9 terms. Conformer 4, analogously to 1, shows the existence of three hydrogen bonds (1.73 Å $H_{27}\cdots O_{71}$, 1.80 Å $H_{52}\cdots O_{73}$ and 1.73 Å $H_{76}\cdots O_{34}$) forming two seven-membered rings and a nine-membered one.

In the conformation with reduced hydrogen bonds, we can observe the presence of two rings, one of 10 and one of 7 terms (1.82 Å $H_{51}\cdots O_{71}$, 1.82 Å $H_{52}\cdots O_{73}$), confirming the importance of the configuration of C-5 and C-9 stereocentres, as suggested by the ^{1}H NMR data.

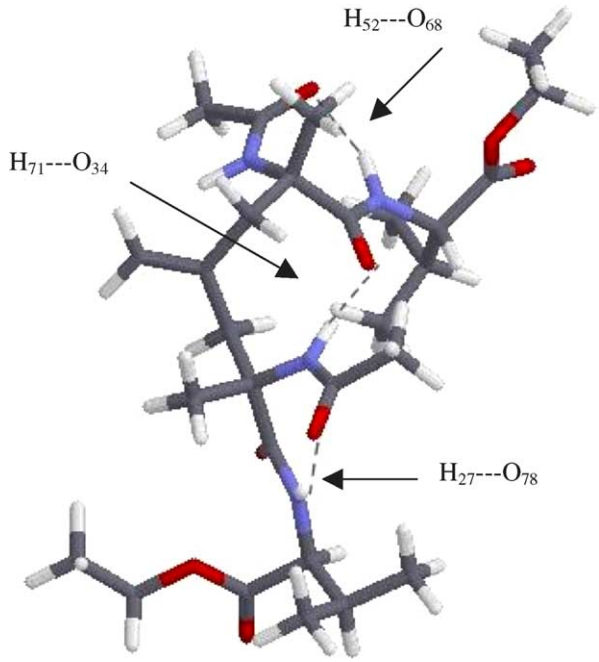


Figure 2. Lowest energy conformation for compound **9** (the arrows point out the hydrogen bonds).

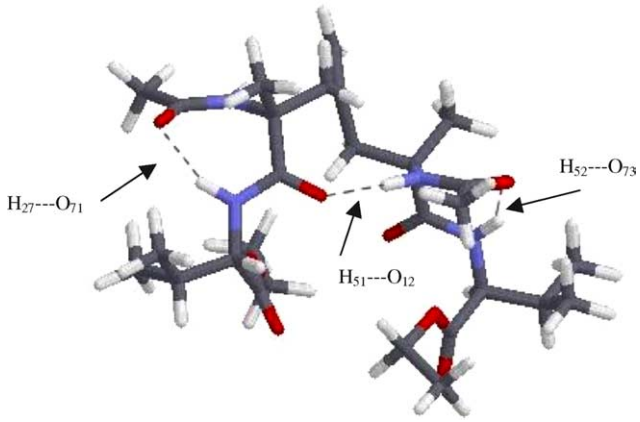


Figure 3. Lowest energy conformation for compound **14** (the arrows point out the hydrogen bonds).

5. X-ray analysis

In the solid state, two conformers of **18** are present in the asymmetric unit, but only one is shown in Figure 4 because the sole difference between them is in the orientation of the C_2H_5 group belonging to the ester moiety

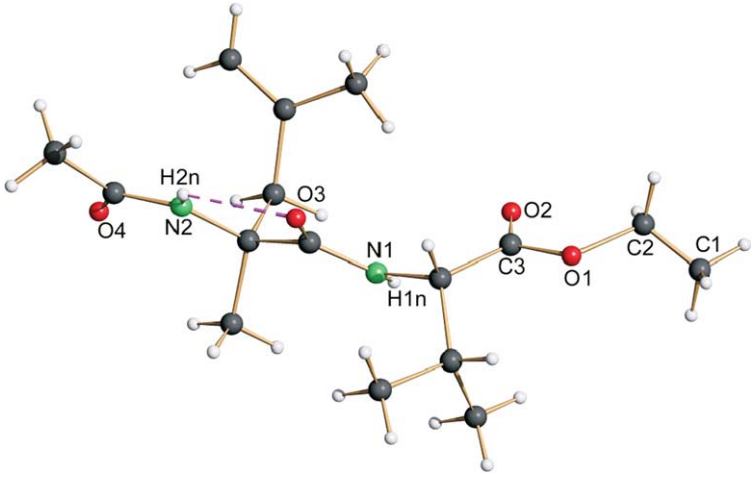


Figure 4. Molecular structure (SCHAKAL drawing) of **18**, the intramolecular N(2)–H(2n)···O(3) hydrogen bond being outlined in purple.

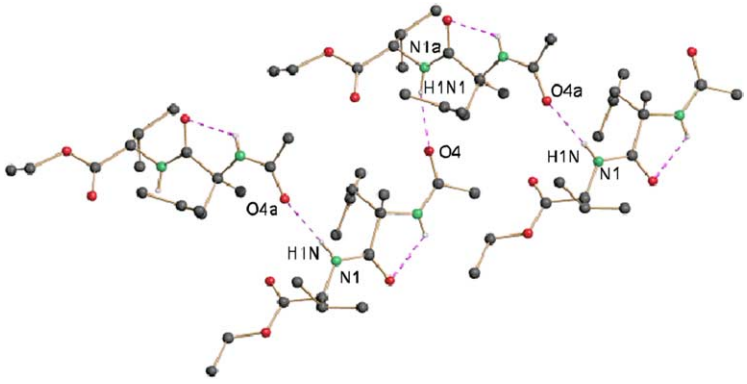


Figure 5. Crystal packing of **18** (hydrogen bonds are outlined in purple).

[the angles O(1)C(3)O(2) and O(1)C(2)C(1) being 43.7(8)° and 6.0(6)°, respectively]. A weak intramolecular hydrogen bond connects the carbonyl oxygen O(3) with the amidic hydrogen H(2n): N(2)–H(2n)···O(3), 112(2)°, H(2n)···O(3) 2.22(3) Å.

The two conformers are linked together through an intermolecular hydrogen bond between the amidic hydrogen and the carbonyl oxygen of the amidic units not engaged in the intramolecular hydrogen bond [N(1)–H(1N)···O(4a), 162(2)°, H(1N)···O(4a) 2.24(3) Å, N(1a)–H(1N1)···O(4), 177(3)°, H(1N1)···O(4) 2.11(3) Å]. In the crystal packing (Fig. 5), each conformer is engaged in two intermolecular hydrogen bonds using both the amidic NHs and the carbonyl oxygen, thus generating a zig-zag chain.

6. Experimental

6.1. General information

Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Gemini spectrometer at 300 MHz using $CDCl_3$ as the solvent. 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. Dry THF was distilled from sodium benzophenone ketyl. Chromatographic separations were performed with silica gel 60 (230–400 mesh). Synthesis and spectroscopic data of compounds **1**, **2**, **3** and **10** are reported in Refs. 1 and 3.

6.2. (2*S*,5*R*,9*R*,12*S*)-5,9-Diamino-3,11-diaza-2,12-diisopropyl-4,10-dioxa-7-methylentridecane-1,13-dicarboxylic acid diethylester, 4

A solution of **3** (2.65 g, 5 mmol) and K_2CO_3 (1.4 g, 10 mmol) in water (20 mL) was stirred for 1 h, the reaction product was then extracted with ethyl acetate and the organic solution washed with water. After complete removal of the organic solvent in vacuo, the product was purified by silica gel chromatography eluting with hexane/ethyl acetate and the pure product was isolated in 95% yield. ^{1}H NMR δ 0.90 (d, 6H, J = 6.6); 0.93 (d, 6H, J = 6.6); 1.26 (t, 6H, J = 7); 1.62 (br s, 4H); 2.15 (m, 4H); 2.73 (dd, 2H, J = 3.4, 14.2); 3.58 (dd, 2H, J = 3.6, 11); 4.18 (m, 4H); 4.44 (dd, 2H, J = 5.2, 9.2); 4.99 (s, 2H); 7.94 (d, 2H, J = 9.2). ^{13}C NMR δ 14.2, 17.7, 19, 31, 40.2, 52.3, 56.9, 61.1, 117.8, 142.1, 171.9, 176.6. $[\alpha]_D$ = +22.5 (c 1, $CHCl_3$). Anal. Calcd for $C_{22}H_{40}N_4O_6$: C, 57.87; H, 8.83; N, 12.27. Found: C, 57.94; H, 8.85; N, 12.25.

6.3. (2*S*,5*R*,9*R*,12*S*)-5,9-Diacetyldiamino-3,11-diaza-2,12-diisopropyl-4,10-dioxa-7-methylentridecane-1,13-dicarboxylic acid diethylester, 5

To a solution of **4** (2.28 g, 5 mmol) and triethylamine (2.8 mL, 20 mmol) in CH_2Cl_2 (20 mL) cooled at 0–5 °C was added acetyl chloride (1.1 mL, 15 mmol) under stirring. After about 2 h, 2 M HCl (5 mL) was added and the reaction product extracted by ethyl acetate. The organic solution was dried over Na_2SO_4 and the solvent 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 144.5–146 °C) in 80% yield. ^{1}H NMR δ 0.97 (d, 6H, J = 6.6); 1 (d, 6H, J = 6.6); 1.31 (t, 6H, J = 6.9); 2.05 (s, 6H); 2.24 (m, 2H); 2.46 (dd, 2H, J = 8.1, 14.7); 2.61 (dd, 2H, J = 5.4, 14.7); 4.23 (q, 4H, J = 6.9); 4.47 (dd, 2H, J = 5.1, 8.4); 4.85 (s, 2H); 4.93 (m, 2H); 7.05 (d, 2H, J = 7.8); 7.54 (d, 2H, J = 8.4). ^{13}C NMR δ 14.1, 17.6, 19, 22.8, 30.7, 40.5, 51.3, 57.5, 61, 115.3, 140.4, 170.6, 171.4, 172. $[\alpha]_D$ = +40.7 (c 0.7, $CHCl_3$). Anal. Calcd for $C_{26}H_{44}N_4O_8$: C, 57.76; H, 8.2; N, 10.36. Found: C, 57.88; H, 8.2; N, 10.35.

6.4. 1-[(3′*S*,3″*S*,6′*S*,6″*S*)-1′Benzyl-6′-hydro-5′-hetoxy-6′-isopropyl-pirazin-3′-methyl-3′-yl-2′-one]-3-[1″-benzyl-3″,6″-dihydro-5″-hetoxy-6″-isopropyl-3″-methyl-pirazin-3″-yl-2″one]-2-methylenpropane, 6

To a stirred solution of **2** (1.2 g, 2 mmol) in dry THF (50 mL) and cooled at −78 °C, a solution of LHMDS in THF (1 M, 4 mL) was added under an inert atmosphere. After about 1 h, CH_3I (0.25 mL, 4 mmol) was added and the reaction monitored by TLC. When the reaction reached completion, the mixture was allowed to warm up to room temperature under stirring. Water and ethyl acetate were added and after separation, the organic solvent was completely removed in vacuo. The residue was submitted to silica gel chromatography eluting with hexane/ethyl acetate and the pure product obtained as an oil in 85% yield. ^{1}H NMR δ 0.93 (d, 6H, J = 6.6); 1.01 (d, 6H, J = 7.2); 1.28 (t, 6H, J = 6.9); 1.43 (s, 6H); 2.2 (m, 2H); 2.64 (d, 2H, J = 12.9); 2.79 (d, 2H, J = 12.9); 3.73 (d, 2H, J = 3); 3.97 (d, 2H, J = 15); 4.14 (m, 4H); 5.12 (s, 2H); 5.49 (d, 2H, J = 15); 7.23–7.36 (m, 10ArH). ^{13}C NMR δ 14.2, 17.2, 20.6, 29.9, 30.7, 46.8, 47.3, 60.5, 61.1, 61.8, 119.9, 127.4, 128.0, 128.6, 136.6, 141.7, 155.0, 172.5. $[\alpha]_D$ = +38 (c 1, $CHCl_3$). Anal. Calcd for $C_{38}H_{52}N_4O_4$: C, 72.58; H, 8.33; N, 8.91. Found: C, 72.55; H, 8.3; N, 8.9.

6.5. 1-[(3′*S*,3″*S*,6′*S*,6″*S*)-6′-Hydro-5′-hetoxy-6′-isopropyl-pirazin-3′-methyl-3′-yl-2′-one]-3- [3″,6″-dihydro-5″-hetoxy-6″-isopropyl-3″-methyl-pirazin-3″-yl-2″one]-2-methylenpropane, 7

Intermediate **6** (3.14 g, 5 mmol), dissolved in dry THF/*tert*-butanol 9:1 (20 mL), was added to a solution of Li (0.07 g, 10 mmol) in liquid ammonia (ca. 50 mL), cooled at about −50 °C and stirred under an inert atmosphere. After 5 min, the reaction mixture was quenched with 1 g of 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 80% yield after silica gel chromatography by eluting with hexane/ethyl acetate. ^{1}H NMR δ 0.82 (d, 6H, J = 6.6);

0.97 (d, 6H, $J = 7.4$); 1.29 (t, 6H, $J = 7.4$); 1.35 (s, 6H); 2.15 (m, 2H); 2.43 (d, 2H, $J = 12.8$); 2.76 (d, 2H, $J = 12.8$); 3.92 (dd, 2H, $J = 1.4$, 3.2); 4.17 (q, 4H, $J = 7.4$); 4.96 (s, 2H); 6 (s, 2H). ^{13}C NMR δ 14.5, 16.2, 18.6, 29.6, 30.5, 47.3, 58.2, 61.1, 62.2, 119.0, 142.2, 155.4, 173.9. $[\alpha]_D = +93.2$ (c 0.5, $CHCl_3$). Anal. Calcd for $C_{24}H_{40}N_4O_4$: C, 64.26; H, 8.99; N, 12.49. Found: C, 64.48; H, 8.98; N, 12.5.

6.6. (2*S*,5*S*,9*S*,12*S*)-5,9-Diamino-5,9-dimethyl-3,11-diaza-2,12-diisopropyl-4,10-dioxa-7-methylentridecane-1,13-dicarboxylic acid diethylester, 8

To a solution of **7** (2.24 g, 5 mmol) in ethanol (40 mL) was added 0.5 M HCl (20 mL) and the reaction mixture, monitored by TLC, stirred at room temperature for about 12 h. The acid solution was evaporated in vacuo and the crude product stirred for 2 h with K_2CO_3 (2.1 g, 15 mmol) in water (5 mL). The reaction product was extracted with ethyl acetate and the organic solution washed with water. The organic solvent was then evaporated in vacuo and the product was purified by silica gel chromatography eluting with hexane/ethyl acetate. The product was isolated as an oil in 90% yield ^{1}H NMR δ 0.92 (d, 12H, $J = 7$); 1.27 (t, 6H, $J = 7$); 1.29 (s, 6H); 2.18 (m, 2H); 2.19 (d, 2H, $J = 13.8$); 2.75 (d, 2H, $J = 13.8$); 4.17 (m, 4H); 4.38 (dd, 2H, $J = 5.2$, 9.2); 5 (s, 2H); 8.32 (d, 2H, $J = 9.2$). ^{13}C NMR δ 13.9, 17.5, 18.8, 27.7, 30.4, 45.9, 56.9, 57, 60.7, 119, 141.5, 171.6, 177.1. $[\alpha]_D = -43.7$ (c 1.6, $CHCl_3$). Anal. Calcd for $C_{24}H_{44}N_4O_6$: C, 59.48; H, 9.15; N, 11.56. Found: C, 59.62; H, 9.17; N, 11.52.

6.7. (2*S*,5*S*,9*S*,12*S*)-5,9-Diacetyldiamino-5,9-dimethyl-3,11-diaza-2,12-diisopropyl-4,10-dioxa-7-methylentridecan-1,13-dicarboxylic acid diethylester, 9

It was obtained in 80% yield following the procedure used for the preparation of **5**. ^{1}H NMR δ 0.92 (d, 6H, $J = 7$); 0.98 (d, 6H, $J = 7$); 1.34 (t, 6H, $J = 7$); 1.75 (s, 6H); 1.97 (s, 6H); 2.28 (d, 2H, $J = 14$); 2.3 (m, 2H); 3.18 (d, 2H, $J = 14$); 4.26 (q, 4H, $J = 7$); 4.85 (m, 4H); 7.05 (s, 2H); 8.08 (d, 2H, $J = 9.6$). ^{13}C NMR δ 14, 17.3, 19.1, 24.1, 24.6, 31.3, 39.4, 57.2, 60.1, 61.9, 120.3, 137.9, 169.1, 173.8, 174.2. $[\alpha]_D = +21.6$ (c 0.4, $CHCl_3$). Mp 195.5–197 °C. Anal. Calcd for $C_{28}H_{48}N_4O_8$: C, 59.13; H, 8.51; N, 9.85. Found: C, 59.25; H, 8.5; N, 9.88.

6.8. 1-[(3′*R*,3″*R*,6′*S*,6″*S*)-1′Benzyl-6′-hydro-5′-hetoxy-6′-isopropyl-pirazin-3′-methyl-3′-yl-2′-one]-3-[1″-benzyl-3″,6″-dihydro-5″-hetoxy-6″-isopropyl-3″-methyl-pirazin-3″-yl-2″one]-2-methylenpropane, 11

It was obtained as a solid in 85% yield by alkylation of **10**[1] with 2-chloromethyl-3-chloropropene and following the procedure already reported for an analogous derivative.[1] ^{1}H NMR δ 0.84 (d, 6H, $J = 6.6$); 1 (d, 6H, $J = 6.9$); 1.25 (t, 6H, $J = 7.2$); 1.44 (s, 6H); 2.17 (m, 2H); 2.41 (d, 2H, $J = 12.6$); 2.79 (d, 2H, $J = 12.6$); 3.65 (d, 2H, $J = 2.4$); 3.93 (d, 2H, $J = 15.3$); 4.04–4.3 (m, 4H); 4.81 (s, 2H); 5.42 (d, 2H, $J = 15.3$); 7.18–7.38 (m, 10ArH). ^{13}C NMR δ 14.1, 16.8, 20.2, 29.1, 29.2, 46.4, 49.2, 60.3, 60.6, 62.5, 117.5, 127.3, 128.3, 128.4, 135.5, 142.8, 155.0, 171.5. $[\alpha]_D = -107.6$ (c 1.1, $CHCl_3$). Mp 237–240 °C (dec.). Anal. Calcd for $C_{38}H_{52}N_4O_4$: C, 72.58; H, 8.33; N, 8.91. Found: C, 72.35; H, 8.35; N, 8.92.

6.9. 1-[(3′*R*,3″*R*,6′*S*,6″*S*)-6′-Hydro-5′-hetoxy-6′-isopropyl-pirazin-3′-methyl-3′-yl-2′-one]-3-[3″,6″-dihydro-5″-hetoxy-6″-isopropyl-3″-methyl-pirazin-3″-yl-2″one]-2-methylenpropane, 12

It was obtained in 80% yield following the procedure used for the preparation of **7**. ^{1}H NMR δ 0.83 (d, 6H, $J = 6.6$); 0.96 (d, 6H, $J = 7.4$); 1.28 (t, 6H, $J = 7.2$); 1.28 (s, 6H); 2.25 (m, 2H); 2.27 (d, 2H, $J = 13.2$); 2.73 (d, 2H, $J = 13.2$); 3.87 (dd, 2H, $J = 1.8$, 2.4); 4.16 (m, 4H); 4.83 (s, 2H); 6.1 (br s, 2H). ^{13}C NMR δ 14.3, 16.0, 18.2, 28.9, 30.8, 48.3, 58.4, 60.9, 61.8, 116.7, 142.5, 155.9, 173.9. $[\alpha]_D = -95.5$ (c 1.1, $CHCl_3$). Anal. Calcd for $C_{24}H_{40}N_4O_4$: C, 64.26; H, 8.99; N, 12.49. Found: C, 64.24; H, 9.02; N, 12.47.

6.10. (2*S*,5*R*,9*R*,12*S*)-5,9-Diamino-5,9-dimethyl-3,11-diaza-2,12-diisopropyl-4,10-dioxa-7-methylentridecane-1,13-dicarboxylic acid diethylester, 13

It was obtained in 85% yield following the procedure used for the preparation of **8**. ^{1}H NMR δ 0.91 (d, 6H, $J = 7.2$); 0.94 (d, 6H, $J = 7.2$); 1.26 (t, 6H, $J = 7$); 1.30 (s, 6H); 1.70 (br s, 4H); 2.08 (d, 2H, $J = 14.7$); 2.21 (m, 2H); 2.83 (d, 2H, $J = 14.7$); 4.20 (m, 4H); 4.42 (dd, 2H, $J = 4.8$, 9.3); 4.93 (s, 2H); 8.29 (d, 2H, $J = 9.3$).^{13}C NMR δ 14.1, 17.4, 18.9, 28.9, 30.9, 46.7, 56.5, 56.7, 60.8, 117.1, 141.9, 171.8, 176.9. $[\alpha]_D = +17.2$ (c 1, $CHCl_3$). Anal. Calcd for $C_{24}H_{44}N_4O_6$: C, 59.48; H, 9.15; N, 11.56. Found: C, 59.7; H, 9.12; N, 11.59.

6.11. (2*S*,5*R*,9*R*,12*S*)-5,9-Diacetyldiamino-5,9-dimethyl-3,11-diaza-2,12-diisopropyl-4,10-dioxa-7-methylentridecane-1,13-dicarboxylic acid diethylester, 14

It was obtained in 85% yield following the procedure used for the preparation of **9**. ^{1}H NMR δ 0.94 (d, 6H, $J = 7$); 0.99 (d, 6H, $J = 7$); 1.3 (t, 6H, $J = 7.4$); 1.61 (s, 6H); 2.08 (s, 6H); 2.22 (m, 2H); 2.67 (q_{AB}, 4H, $J = 14.6$); 4.21 (m, 4H); 4.46 (dd, 2H, $J = 4.8$, 8.2); 5.06 (s, 2H); 6.98 (s, 2H); 7.44 (d, 2H, $J = 8.2$). ^{13}C NMR δ 14.1, 17.7, 18.9, 23.9, 31.0, 43.4, 57.7, 59.6, 61.1, 121.1, 139.9, 170.9, 171.8, 174.2. $[\alpha]_D = +69.7$ (c 0.9, $CHCl_3$). Anal. Calcd for $C_{28}H_{48}N_4O_8$: C, 59.13; H, 8.51; N, 9.85. Found: C, 59.05; H, 8.52; N, 9.85.

6.12. (2*S*,5*R*)-5-Acetylamino-3-aza-2-isopropyl-5-methyl-4-oxa-hexanoic acid ethyl ester, 15

The product was obtained starting from **10** and following the procedure reported in Scheme 3. ^{1}H NMR δ 0.94 (d, 3H, $J = 6.9$); 0,98 (d, 3H, $J = 6.9$); 1.3 (t, 3H, $J = 7.2$); 1.42 (d, 3H, $J = 6.9$); 2.04 (s, 3H); 2.22 (m,

1H); 4.22 (m, 2H); 4.55 (dd, 1H, $J = 5.1, 9$); 4.6 (m, 1H); 6.1 (d, 1H, $J = 6.9$); 6.6 (d, 1H, $J = 10.2$). ^{13}C NMR δ 14, 17.5, 18.6, 18.8, 22.7, 30.9, 48.6, 57.2, 61, 170.4, 171.5, 173.1. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.13. (2*S*,5*R*)-5-Acetylamino-3-aza-2-isopropyl-5,7-dimethyl-4-oxa-7-octenoic acid ethyl ester, 16

The product was obtained starting from **10** and following the procedure reported in Scheme 3. ^{1}H NMR δ 0.92 (d, 3H, $J = 6.9$); 0.96 (d, 3H, $J = 6.9$); 1.28 (t, 3H, $J = 7.5$); 1.65 (s, 3H); 1.71 (s, 3H); 2.01 (s, 3H); 2.21 (m, 1H); 2.76 (q_{AB}, 2H, $J = 14.4$); 4.19 (m, 2H); 4.47 (dd, 1H, $J = 4.5, 8.4$); 4.78 (s, 1H); 4.93 (s, 1H); 6.3 (s, 1H); 6.95 (d, 1H, $J = 8.4$). ^{13}C NMR δ 14.2, 17.7, 18.9, 23.3, 24.2, 24.5, 31.1, 45, 57.6, 59.7, 61.2, 115.8, 141.4, 170.1, 171.8, 173.8. $[\alpha]_D = +42.9$ (c 1.4, $CHCl_3$). Anal. Calcd for $C_{16}H_{28}N_2O_4$: C, 61.5; H, 9.03; N, 8.97. Found: C, 61.31; H, 8.98; N, 8.94.

6.14. (2*S*,5*R*)-5-Acetylamino-3-aza-2-isopropyl-7-methyl-4-oxa-7-octenoic acid ethyl ester, 17

The product was obtained starting from **1** and following the procedure reported in Scheme 3. ^{1}H NMR (mixture of conformers A+B) δ 0.96 (m, 6H); 1.31 (m, 3H); 1.8 (s, 3H); 2.04 (m, 3H); 2.2 (m, 1H); 2.5 (m, 2H); 4.2 (m, 2H); 4.48 (m, 1H); 4.61 and 5.34 (m, 1H); 4.88 (m, 2H); 5.9 (d, H_A, $J = 6.9$); 6.2 (d, H_B, $J = 9$); 6.6 (d, H_A, $J = 7.8$); 8.5 (d, H_B, $J = 10.2$). ^{13}C NMR δ 14, 17.6, 18.8, 21.9, 22.7, 30.8, 40.3, 51.2, 57.2, 60.9, 113.8, 140.8, 170.3, 171.5, 171.8. $[\alpha]_D = +30$ (c 1.1, $CHCl_3$). Anal. Calcd for $C_{15}H_{26}N_2O_4$: C, 60.38; H, 8.78; N, 9.39. Found: C, 60.12; H, 8.8; N, 9.42.

6.15. (2*S*,5*S*)-5-Acetylamino-3-aza-2-isopropyl-5,7-dimethyl-4-oxa-7-octenoic acid ethyl ester, 18

The product was obtained starting from **1** and following the procedure reported in Scheme 3. ^{1}H NMR δ 0.93 (d, 3H, $J = 6.9$); 0.96 (d, 3H, $J = 6.9$); 1.31 (t, 3H, $J = 7.2$); 1.66 (s, 3H); 1.7 (s, 3H); 2.02 (s, 3H); 2.2 (m, 1H), 2.83 (q_{AB}, 2H, $J = 14$); 4.22 (m, 2H); 4.5 (dd, 1H, $J = 4.6, 8.4$); 4.76 (s, 1H); 4.9 (s, 1H); 6.4 (s, 1H); 6.9 (d, 1H, $J = 9.2$). ^{13}C NMR δ 14.1, 17.6, 18.9, 23.3, 24, 24.1, 31.2, 43.7, 57.4, 59.9, 61.2, 115.7, 140.9, 170.2, 171.7, 174. Mp: 109–110 °C. $[\alpha]_D = -4.2$ (c 0.8, $CHCl_3$). Anal. Calcd for $C_{16}H_{28}N_2O_4$: C, 61.5; H, 9.03; N, 8.97. Found: C, 61.71; H, 9.05; N, 8.95.

6.16. Computational method

Molecular mechanics calculations were performed on SGI IRIX 6.5 workstations using the implementation of Amber all-atom force field (AMBER*) within the framework of Macromodel version 5.5.[12] The solvent effect was included by the implicit chloroform GB/SA solvation model of Still et al.[13] The torsional space of each molecule was randomly varied with the usage-directed Monte Carlo conformational search of Chang–Guida–Still. For each search, at least 1000 starting structures for each variable torsion angle was generated and minimised until the gradient was less than 0.05 kJ/A mol. The cyclic moieties containing the amide bonds were also included into the search. Duplicate conformations and those with an energy in excess of 6 kcal/mol above the global minimum were discarded.

6.17. Crystallographic data

Single crystal X-ray diffraction data of **18**: $C_{16}H_{28}N_2O_4$, Fw 312.40, colourless parallelepiped, size: $0.35 \times 0.27 \times 0.20$ mm, Orthorhombic, space group $C222_1$, $a = 12.446$ (1) Å, $b = 15.880(1)$ Å, $c = 36.796(3)$ Å, $V = 7272.5(9)$ Å^3, theta range for data collection 1.11–30.04°, $Z = 16$, $F(000) = 2720$, $D_x = 1.141$ mg/m^3, $\mu = 0.082$ mm^{-1}, data collected on a Bruker AXS CCD diffractometer (Mo-Kα radiation, $\lambda = 0.71073$ Å) at 293(2) K, total of 47,712 reflections, of which 10,643 unique [$R_{(int)} = 0.0675$]. Empirical absorption correction was applied, initial structure model by direct methods. Anisotropic full-matrix least-squares refinement on F^2 for all non-hydrogen atoms yielded $R_1 = 0.0508$ and $wR_2 = 0.1265$ for [$I > 2\sigma(I)$] and $R_1 = 0.1158$ and $wR_2 = 0.1511$ for all intensity data. All hydrogen atoms were added in calculated position except the amidic hydrogens that were located in the Fourier map. Goodness-of-fit = 0.911. CCDC number is 252649.

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