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Stereoselective synthesis and conformational analysis of unnatural tetrapeptides. Part 2☆

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Abstract—Stereoselective synthesis of unnatural tetrapeptides **20a** and **20b**, **21a** and **21b** and **30** and **31**, containing two L-valine units and two unnatural α-amino acids (ornithine and modified aspartic acid), has been accomplished starting from the L-valine derived chiral synthon **1**. Structural investigations of these non-proteinogenic peptides have been carried out on the acetamido derivatives using ^{1}H NMR, IR spectroscopic techniques and a conformational analysis based on molecular dynamics (MD) and cluster analysis.

1. Introduction

To follow up our programme directed towards the stereoselective synthesis of unnatural peptides, we have recently focused our attention on new peptidomimetic structures incorporating non-proteinogenic α-aminoacids and L-valine units at both ends of the chain. We have lately reported the stereoselective synthesis of pseudotetrapeptides having a C-terminus at both ends of the chain containing the L-valine unit and two modified α-aminoacids, as proline and aspartic acid.[1] The aim of this research is to investigate the capability of these pseudopeptides to mimic the biological properties exhibited by some natural peptides with the advantage of metabolic stability.

Herein we report a simple stereoselective synthesis of unnatural tetrapeptides involving the L-valine unit at the two ends of the chain and two unnatural α-aminoacids selected between (2*R*)-methyl-aspartic acid, a (2*R*)- or (2*S*)-methyl derivative of 2,4-diaminobutyric acid and (*R*)-ornithine or its (2*R*)- or (2*S*)-methyl derivative. The synthetic approach to accomplish the asymmetric synthesis of the title pseudotetrapeptides is similar to that followed in the previous stereoselective synthesis and is based on the chiral monolactim ether **1**, a synthon easily obtained from L-valine.[1–3]

The non-proteinogenic peptides, such as diacetylderivatives, **20a** and **20b**, **21a** and **21b** and **30** and **31** have been obtained in satisfactory overall yields. Spectroscopic analysis using ^{1}H NMR and IR techniques has been performed to ascertain the presence of intramolecular hydrogen bonds. Furthermore, a theoretical investigation based on molecular dynamics (MD) and cluster analysis has been carried out to verify the possible formation of β- or γ-turns, which is responsible for the solidly packed conformations.

2. Synthesis

The stereoselective synthesis of tetrapeptides **20a** and **21a** was performed using the chiral synthon **1**, a monolactim ether easily synthesized from L-valine,[1–5] while pseudopeptides **21a** and **21b** were obtained starting from synthon **2**, obtained from **1** in a diastereomeric mixture (see Ref. 4 for detailed description) and following the same stereoselective approach previously employed.[1–5]

Intermediates **10a** or **11a**, along with diastereomer **12** or **13**, respectively (Scheme 1), were obtained in de's of 50% and 40% by alkylation of the chiral synthon **1** with *N*,*N*-dibenzyl-2-iodo-ethylamine **5** or *N*,*N*-dibenzyl-3-iodo-propylamine **6**, which were prepared starting from 2- or 3-aminopropanol, respectively (Scheme 2). Conversely, the reaction occurred with a practically total regio- and diastereoselectivity (de >98%) by alkylating the diastereomeric mixture of the chiral synthon **2** with only diastereomer

☆ Ref. 1 is considered to be Part 1.

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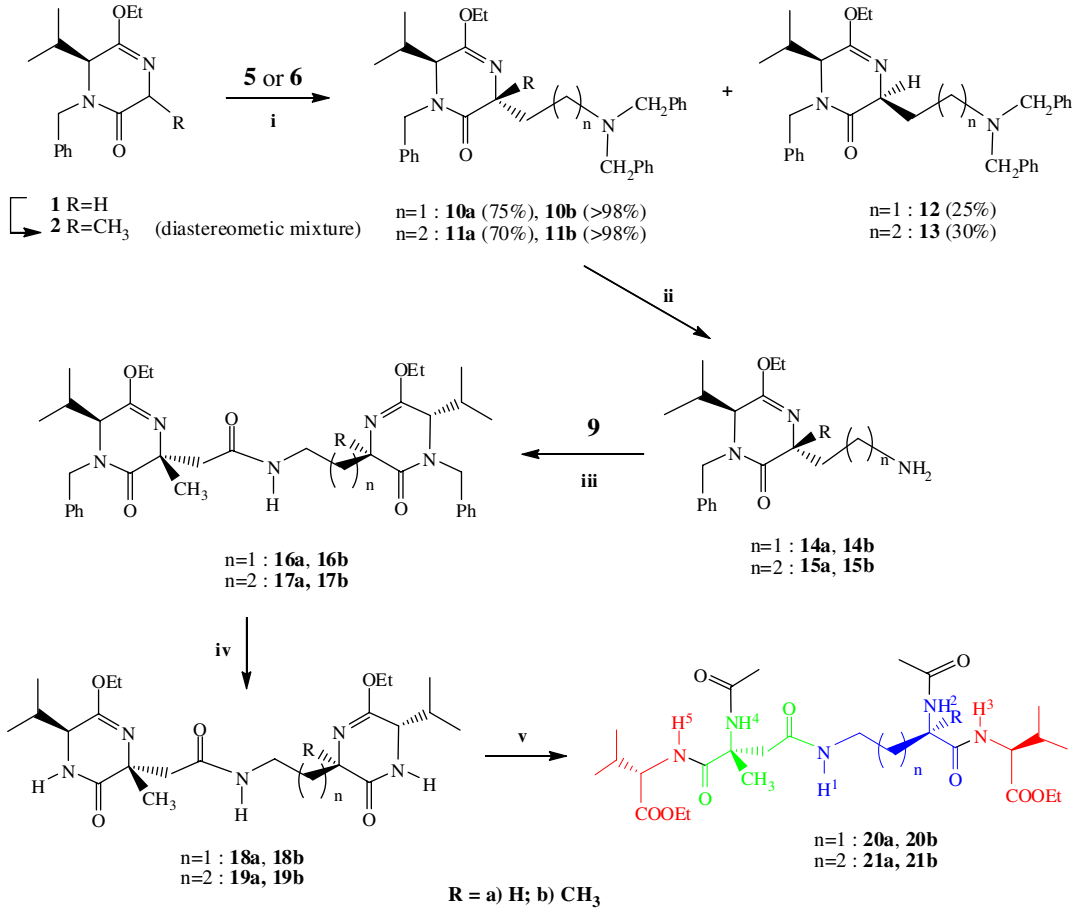
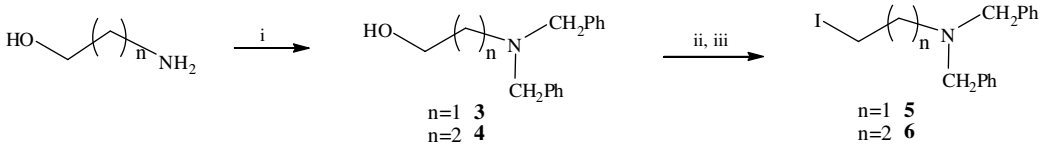


Scheme 1. Reagents and conditions: (i) 1 M LHMDS in dry THF, then **5** or **6**; (ii) $H_2/Pd(OH)_2$ in MeOH; (iii) **9** in dry THF at rt; (iv) Li/NH_3 at −78 °C in dry THF/*t*-butanol = 10:1; (v) 0.5 M HCl in EtOH at rt, then CH_3COCl in CH_2Cl_2/Et_3N.



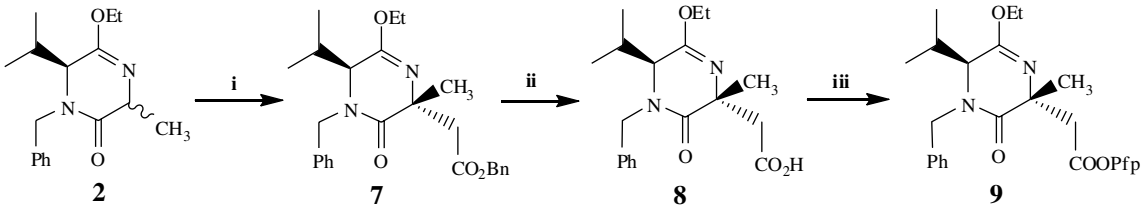
Scheme 2. Reagents and conditions: (i) $PhCH_2Br$ in acetone at rt in the presence of K_2CO_3; (ii) $SOCl_2$ in CH_2Cl_2; (iii) NaI in acetone at rt.

10b or **11b** being obtained, as had been already observed for similar substrates.[2–4]

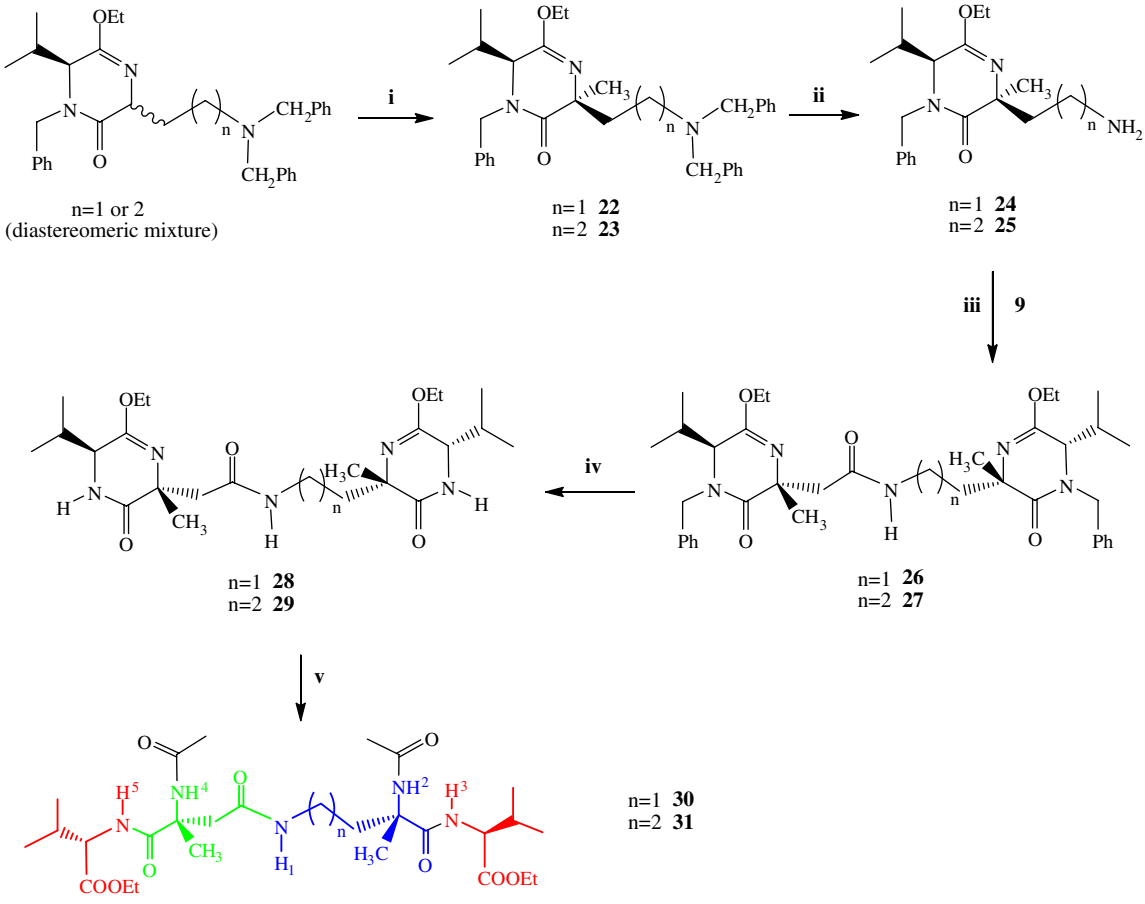
The hydrogenolysis of **10a** and **10b** or **11a** and **11b**, performed in the presence of $Pd(OH)_2$, afforded the masked cyclic pseudodipeptides **14a** and **14b** or **15** and **15b**, respectively. Such cyclic unnatural dipeptides can be regarded as formed from (*S*)-*N*-benzylvaline and the following diaminoacids: (2*R*)-2,4-diaminobutyric acid **14a** or (2*R*)-2-methyl-2,4-diaminobutyric acid **14b** or (2*R*)-ornithine **15a** or (2*R*)-methyl ornithine **15b** (Scheme 1). Then, the cyclic pseudodipeptides **14a** and **14b** and **15a** and **15b** reacted with the activated ester **9**, another masked dipeptide containing (*S*)-*N*-benzyl-valine and (2*R*)-methyl-aspartic acid, which was obtained from the diastereomeric mixture of **2** (Scheme 3), as already described.[2] Debenzylation by the Birch reaction was carried out on the intermediates **16a** and **16b** and **17a** and **17b** to obtain **18a** and **18b** and **19a** and **19b**, respectively. These intermediates, after acid hydrolysis under mild conditions, were converted into the corresponding non-proteinogenic tetrapeptides **20a** and **20b** and **21a** and **21b**, respectively, as diacetylderivatives. These pseudotetrapeptides are formed by two L-valine units (red), (2*R*)-methyl-aspartic acid (green) and a diaminoacid (blue) (Scheme 1).

To synthesize pseudopeptides **30** and **31** (Scheme 4), which are diastereomers with respect to **20b** and **21b**, we employed the masked dipeptide **24** or **25** as a nucleophile. These were obtained by alkylating the diastereomeric mixture **10a** + **12** or **11a** + **13**, respectively, with CH_3I. The reaction was characterized by a nearly total 1,4-*trans* induction with respect to the isopropyl group, the de being larger than 98%.[2]

After hydrogenolysis, performed in the presence of $Pd(OH)_2$, the amine derivative **24** or **25** obtained was matched with the electrophile **9** to give **26** or **27** which



Scheme 3. Reagents and conditions: (i) LHMDS/THF, $BrCH_2COOBn$ (Ref. 4); (ii) H_2/Pd on charcoal in CH_3OH; (iii) pentafluorophenyl trifluoroacetate ($CF_3COOPfp$) in CH_2Cl_2/pyridine (Ref. 4).



Scheme 4. Reagents and conditions: (i) 1 M LHMDS in dry THF, then CH_3I; (ii) H_2/$Pd(OH)_2$ in MeOH; (iii) **9** in dry THF at rt; (iv) Li/NH_3 at −78 °C in dry THF/*t*-butanol = 10:1; (v) 0.5 M HCl in EtOH at rt then CH_3COCl in CH_2Cl_2/Et_3N.

when submitted to the reaction sequence described above in Scheme 1, furnished tetrapseudopeptides **30** and **31** in satisfactory overall yield (Scheme 4).

3. ^{1}H NMR and IR studies

The six tetrapseudopeptides synthesized were examined by means of ^{1}H NMR and IR spectroscopic techniques to elucidate the main structural features. It is well known that (a) the chemical shift values of the amidic protons (δ_{NH}), (b) the temperature coefficient ($\Delta\delta_{NH}/\Delta T$) values and (c) the δ_{NH} variations after the addition of a good hydrogen bond forming solvent, such as DMSO, can provide useful information with regards to the existence of hydrogen bonds. Further information can be obtained from IR spectra in dilute solution. More precisely a broad band in the range of 3300–3370 cm^{-1} indicates that the amidic NH bond gives rise to an intra-molecular hydrogen bond, while a sharp band higher than 3400 cm^{-1} is usually due to a non-hydrogen bonded NH amide group.[1–7] However, since spectroscopic parameters do not always provide unambiguous responses, additional studies such as theoretical conformational analysis based on molecular dynamics are useful for obtaining more reliable information.

Numbers 1–5 have been assigned to the various amide protons (see Schemes 1,4 and Tables 1,2). The ^{1}H NMR spec-

Table 1. Selected NOE data for substrates **20a** and **20b**, **21** and **21b** and **30** and **31**

	Proton irradiated	NOE registered on the proton italic	
20a	NH^2 (d) at 6.66 ppm	CH_3–CO (s) at 2.03 ppm	
	NH^3 (d) at 7.65 ppm	C*H*–NH^2 (dd) at 4.42 ppm	C*H*–NH^3 (m) at 4.5 ppm
	NH^5 (d) at 8.23 ppm	CH_2–CNH^4 doublets at 2.64 ppm and 2.95 ppm	CH_3–CNH^4 (s) at 1.7 ppm
20b	NH^2 (s) at 7.41 ppm	CH_3–CO (s) at 2.06 ppm	CH_3–CNH^2 (s) at 1.61 ppm
	CH_2–CNH^4 (d) at 2.58 or (d) at 2.95 ppm	NH^4 (s) at 7.61 ppm	NH^5 (d) at 8.34 ppm
	NH^5 (d) at 8.34 ppm	CH_2–CNH^4 doublets at 2.58 ppm and 2.95 ppm	CH_3–CNH^4 (s) at 1.67 ppm
21a	NH^2 (d) at 6.59 ppm	CH_3–CO (s) at 2.03 ppm	C*H*–NH^2 (m) at 4.62 ppm
	NH^3 (d) at 6.91 ppm	C*H*–NH^3 (dd) at 4.42 ppm	C*H*–NH^2 (m) at 4.62 ppm
	NH^5 (d) at 8.35 ppm	CH_2–CNH^4 (d) at 2.62 ppm	CH_3–CNH^4 (s) at 1.70 ppm
21b	NH^2 (s) at 6.68 ppm	CH_3–CNH^2 (s) at 1.57 ppm	CH_3–$CONH^2$ (s) at 2.01 ppm
	NH^3 (d) at 7.04 ppm	CH_3–CNH^2 (s) at 1.57 ppm	CH_2–CNH^2 (m) at 1.78 ppm
	NH^4 (s) at 7.79 ppm	CH_2–NH^4 (d) at 2.52 ppm and (d) at 2.92 ppm	CH_3–CNH^4 (s) at 1.66 ppm
	NH^5 (d) at 8.36 ppm	CH_2–NH^4 (d) at 2.52 ppm and (d) at 2.92 ppm	CH_3–CNH^4 (s) at 1.66 ppm
30	NH^3 (d) at 7.39 ppm	CH_3–CNH^2 (s) at 1.6 ppm	
	NH^4 (s) at 7.72 ppm	CH_2–CNH^4 (d) at 2.49 ppm and (d) at 2.91 ppm	CH_3–CNH^4 (s) at 1.67 ppm
	NH^5 (d) at 8.33 ppm	CH_2–CNH^4 (d) at 2.49 ppm and (d) at 2.91 ppm	CH_3–CNH^4 (s) at 1.67 ppm
31	NH^3 (d) at 6.98 ppm	CH_3–CNH^2 (s) at 1.66 ppm	
	NH^4 (s) at 7.60 ppm	CH_2–CNH^4 (d) at 2.81 ppm and (d) at 2.99 ppm	CH_3–$CONH^4$ (s) at 2.04 ppm
	NH^5 (d) at 8.09 ppm	CH_2–CNH^4 (d) at 2.81 ppm and (d) at 2.99 ppm	

tra assignments of these protons were achieved on the basis of the signal multiplicity as well as NOE experiments. This analysis takes advantage of the CH_2 protons adjacent to the carbonyl group, which resonate as two doublets: one in the range of 2.5–2.8 ppm and the other in the range of 2.9–3.0 ppm (Fig. 1 and Table 1). The meaningful ^1H NMR and IR data for the substrates **20a** and **20b**, **21a** and **21b** and **30** and **31** recorded in diluted solution are shown in Table 2.

In all the pseudopeptides investigated herein only the amide proton $\mathbf{H}^1$ is characterized by a multiplet, while in pseudopeptides **20a** and **21a** the amide proton showing a singlet can be only ascribed to $\mathbf{H}^4$ resonating at 7.47 and 7.61 ppm, respectively.

The data reported in Table 2 suggest that the $\mathbf{H}^1$ proton is probably involved in the intramolecular hydrogen bonds in all the pseudopeptides investigated here. In fact, despite a δ value less than 7 ppm (from 6.43 to 6.93 ppm) and the relatively large downfield shifts (from 0.65 to 1.29 ppm) displayed upon addition of DMSO, the temperature coefficients values (in the range of 3.2–6.0 ppb/°C) suggest the existence of a hydrogen bonded structure in equilibrium with a non-hydrogen bonded one.

Although in pseudopeptide **21b,** it was not possible to measure the temperature coefficient for $\mathbf{H}^1$, we hypothesized that the behaviour of this amide proton is analogous to that observed in pseudopeptide **20b**. The data also indicate that while a dynamic equilibrium between a hydrogen bonded and a non-hydrogen bonded structure characterizes the amide proton $\mathbf{H}^2$ in **20b** and **30**, the same does not probably occur in **20a** and **31**.

Furthermore, in spite of the chemical shifts being lower than 7 ppm registered for **21a** and **21b** (6.59 and 6.68 ppm), the temperature coefficient values (3.7 and 4.8 ppb/°C) may be indicative of the existence of an equilibrium probably shifted towards the non-hydrogen bonded state.

The chemical shift significantly higher than 7 ppm, the small $\Delta\delta_{NH}$ values upon addition of the competitive solvent DMSO, and the low temperature dependence registered for the $\mathbf{H}^3$ proton in **20a** and **20b** again suggest the existence of an intramolecular hydrogen bond. The temperature coefficient value (6.7 ppb/°C) registered for **20a** suggests that the $\mathbf{H}^3$ proton is involved in a dynamic equilibrium between a hydrogen bonded and a non-hydrogen bonded structure. The relatively small high shifts (0.23–0.4 ppm) registered upon the addition of DMSO and the temperature coefficient (3.3–3.6 ppb/°C) observed in **30** and **31** suggest that also in these compounds the $\mathbf{H}^3$ proton probably participates to a dynamic equilibrium.

The amide proton $\mathbf{H}^4$ almost certainly forms intramolecular hydrogen bonds since it shows high chemical shifts values (in the range of 7.47–7.79 ppm) in all tetrapseudopeptides.

Similarly to $\mathbf{H}^4$, the $\mathbf{H}^5$ proton also most likely contributes to intramolecular hydrogen bonds in all unnatural tetrapeptides, its chemical shifts being in the range of 8.09–8.36 ppm. It is worth mentioning that upon addition of DMSO, the amide proton $\mathbf{H}^5$ is characterized by a low shift instead of a high shift, as generally observed. This unexpected behaviour, also reported by. Fernandez et al.[7] (without providing any explanation), could be caused by a local magnetic field change due to a different conformation of the molecule upon the addition of DMSO.

From the chemical shift values we can infer that pseudotetrapeptides **20a** and **20b** and **30** are more prone to form intramolecular hydrogen bonds than **21a** and **21b** and **31**. In fact, while the $\mathbf{H}^4$ and $\mathbf{H}^5$ chemical shifts are signifi-

Table 2. Meaningful ^{1}H NMR and IR data of substrates **20a** and **20b**, **30**, **21a** and **21b** and **31**

	δ_{NH} (ppm) (2 mM $CDCl_3$)	δ_{NH} (ppm) ($CDCl_3$ + 20% DMSO)	$\lvert\Delta\delta_{NH}/\Delta T\rvert$ (ppb/°C) (in $CDCl_3$)	ν_{NH} (cm^{-1}) (2 mM $CHCl_3$)
	$\mathbf{H}^1$ 6.93 (m)	$\mathbf{H}^1$ 7.71	$\mathbf{H}^1$ 4.2	
	$\mathbf{H}^2$ 6.66 (d)	$\mathbf{H}^2$ 7.46	$\mathbf{H}^2$ 2.1	
20a	$\mathbf{H}^3$ 7.65 (d)	$\mathbf{H}^3$ 8.04	$\mathbf{H}^3$ 6.7	3300, 3418
	$\mathbf{H}^4$ 7.47 (s)	$\mathbf{H}^4$ 7.92	$\mathbf{H}^4$ 0.6	
	$\mathbf{H}^5$ 8.23 (d)	$\mathbf{H}^5$ 7.74	$\mathbf{H}^5$ 3.1	
	$\mathbf{H}^1$ 6.68 (m)	$\mathbf{H}^1$ 7.70	$\mathbf{H}^1$ 3.5	
	$\mathbf{H}^2$ 7.41 (s)	$\mathbf{H}^2$ 7.71	$\mathbf{H}^2$ 4.0	
20b	$\mathbf{H}^3$ 7.66 (d)	$\mathbf{H}^3$ 7.53	$\mathbf{H}^3$ 2.7	3280, 3371, 3433
	$\mathbf{H}^4$ 7.61 (s)	$\mathbf{H}^4$ 7.87	$\mathbf{H}^4$ 1.3	
	$\mathbf{H}^5$ 8.34 (d)	$\mathbf{H}^5$ 7.74	$\mathbf{H}^5$ 3.0	
	$\mathbf{H}^1$ 6.66 (m)	$\mathbf{H}^1$ 7.70	$\mathbf{H}^1$ 6.0	
	$\mathbf{H}^2$ 7.36 (s)	$\mathbf{H}^2$ 7.69	$\mathbf{H}^2$ 5.8	
30	$\mathbf{H}^3$ 7.39 (d)	$\mathbf{H}^3$ 7.62	$\mathbf{H}^3$ 3.3	3290, 3372, 3433
	$\mathbf{H}^4$ 7.72 (s)	$\mathbf{H}^4$ 7.94	$\mathbf{H}^4$ 2.6	
	$\mathbf{H}^5$ 8.33 (d)	$\mathbf{H}^5$ 7.95	$\mathbf{H}^5$ 4.0	
	$\mathbf{H}^1$ 6.50 (m)	$\mathbf{H}^1$ 7.79	$\mathbf{H}^1$ 3.2	
	$\mathbf{H}^2$ 6.59 (d)	$\mathbf{H}^2$ 7.39	$\mathbf{H}^2$ 3.7	
21a	$\mathbf{H}^3$ 6.91 (d)	$\mathbf{H}^3$ 7.77	$\mathbf{H}^3$ 2.8	3331, 3429
	$\mathbf{H}^4$ 7.61 (s)	$\mathbf{H}^4$ 8.02	$\mathbf{H}^4$ 1.3	
	$\mathbf{H}^5$ 8.35 (d)	$\mathbf{H}^5$ 7.95	$\mathbf{H}^5$ 1.8	
	$\mathbf{H}^1$ 6.43 (m)	$\mathbf{H}^1$ 7.42	$\mathbf{H}^1$ [a]	
	$\mathbf{H}^2$ 6.68 (s)	$\mathbf{H}^2$ 7.36	$\mathbf{H}^2$ 4.8	
21b	$\mathbf{H}^3$7.06 (d)	$\mathbf{H}^3$ 7.39	$\mathbf{H}^3$ 2.0	3300, 3371, 3428
	$\mathbf{H}^4$ 7.79 (s)	$\mathbf{H}^4$ 8.00	$\mathbf{H}^4$ 2.6	
	$\mathbf{H}^5$ 8.36 (d)	$\mathbf{H}^5$ 7.97	$\mathbf{H}^5$ 2.1	
	$\mathbf{H}^1$ 6.70 (m)	$\mathbf{H}^1$ 7.35	$\mathbf{H}^1$ 4.6	
	$\mathbf{H}^2$ 6.80 (s)	$\mathbf{H}^2$ 7.31	$\mathbf{H}^2$ 0.3	
31	$\mathbf{H}^3$ 6.98 (d)	$\mathbf{H}^3$ 7.38	$\mathbf{H}^3$ 3.6	3302, 3372, 3435
	$\mathbf{H}^4$ 7.60 (s)	$\mathbf{H}^4$ 7.93	$\mathbf{H}^4$ 0.6	
	$\mathbf{H}^5$ 8.09 (d)	$\mathbf{H}^5$ 7.93	$\mathbf{H}^5$ 0.6	

[a] It was not possible to measure the temperature coefficient, $\Delta\delta_{NH}/\Delta T$, because in dilute solution the $\mathbf{H}^1$ signal becomes broad.

cantly higher than 7 ppm in all substrates, protons $\mathbf{H}^2$ and $\mathbf{H}^3$ in **20a** and **20b** and **30** exhibit a non-negligible shift with respect to **21a** and **21b** and **31**. It is conceivable to believe that this behaviour is due to a greater structural flexibility of the substrates **21a** and **21b** and **31** where the carbon chain includes one additional CH_2 unit with respect to **20a** and **20b** and **30**. Also, the introduction of a second methyl group [(especially in the case of a resulting (*S*)-configuration, as in **20b** and **21b**)], apparently increases, although to a smaller extent, the tendency to give intramolecular hydrogen bonds.

Finally, we must outline that the above discussion concerning the presence of intramolecular hydrogen bonds, deduced from ^{1}H NMR spectroscopic investigations, is enforced by the IR data collected in Table 2.

4. Molecular modelling and conformational analysis

A computational strategy based on a two step protocol has been employed to explore the conformational space of pseudotetrapeptides **20a**, **20b**, **21a**, **21b**, **30** and **31** and to obtain structural information useful to rationalize the spectroscopic data.

In the first step, a high-temperature quenched molecular dynamics (QMD) has been carried out to identify the most populated regions of the conformational space (phase space). This step has been followed by (i) a molecular dynamics at room temperature that provides statistical indications about the hydrogen bond lifetimes and (ii) a cluster analysis of the trajectory, which represents a useful method to identify the most common hydrogen bond patterns. The distribution of the clusters in the PCA1/PCA2 space using principal component analysis[8] is represented in Figure 2.

Representative conformations of the six peptides, obtained from cluster analysis, are depicted in Figures 3–8. These conformations correspond to the most populated structures within different sets of clusters. These sets have been obtained by grouping together the original clusters on the basis of the similarity of the hydrogen bond pattern (see Computational details in Section 7). A list of hydrogen bond lifetimes for each compound is given in Table 3. From Figures 2 and 3 we can easily recognize that peptide **20a** is characterized by two rather stable conformations that interconvert one to the other.

One conformation **Cl1** has an S-type folded structure (population of 34.3%) and the other **Cl3** a C-type folded structure (population of 23.9%). The remaining population is distributed between structures that are intermediate between these two minima. The two conformers maintain the three hydrogen bonds H4–O1, H5–O4 and H5–O1 that are characterized by the largest life-times (94.8%, 76.3% and 60.2%, respectively). However, while in **Cl1** the $\mathbf{H}^1$ does not participate in any hydrogen bond and $\mathbf{H}^2$ and $\mathbf{H}^3$ interact with O1 (lifetimes are 43.5% and 28.2% for H2–O1 and H3–O1, respectively), in **Cl3** all three hydrogen atoms H1, H2 and H3 interact with O7, the corresponding lifetimes being 16.1%, 45.4% and 21.4%, respectively.

Peptide **21a** (see Figs. 2 and 4) has a carbon chain, which contains an additional CH_2 unit with respect to **20a**. This chain lengthening increases the structural flexibility and, consequently, **21a** is characterized by four minima: one S-type folded structure **Cl7** (population of 24.5%) and three different C-type folded structures, **Cl3**, **Cl4** and **Cl6** (populations of 15.2%, 25.8% and 11.4%, respectively).

These minima can be thought to formally originate from the minimum **Cl1** found for **20a**. The structural features of **21a** make possible the existence of three different minima (in place of only one minimum **Cl1**) characterized by the same hydrogen pattern, but a different relative orientation of the two limbs of the peptide. In all conformations of peptide **21a**, the H4–O1 and H5–O4 hydrogen bonds can be detected. These are in fact characterized by highest lifetimes (96.5% and 89.5%, respectively). However, while in the S-type conformation H1 is not involved in any hydrogen bond and H2 and H3 interact with O1 (H2–O1 and H3–O4 have lifetimes of 23.4% and 21.9%, respectively), in the C-type conformations H1 interacts with O5

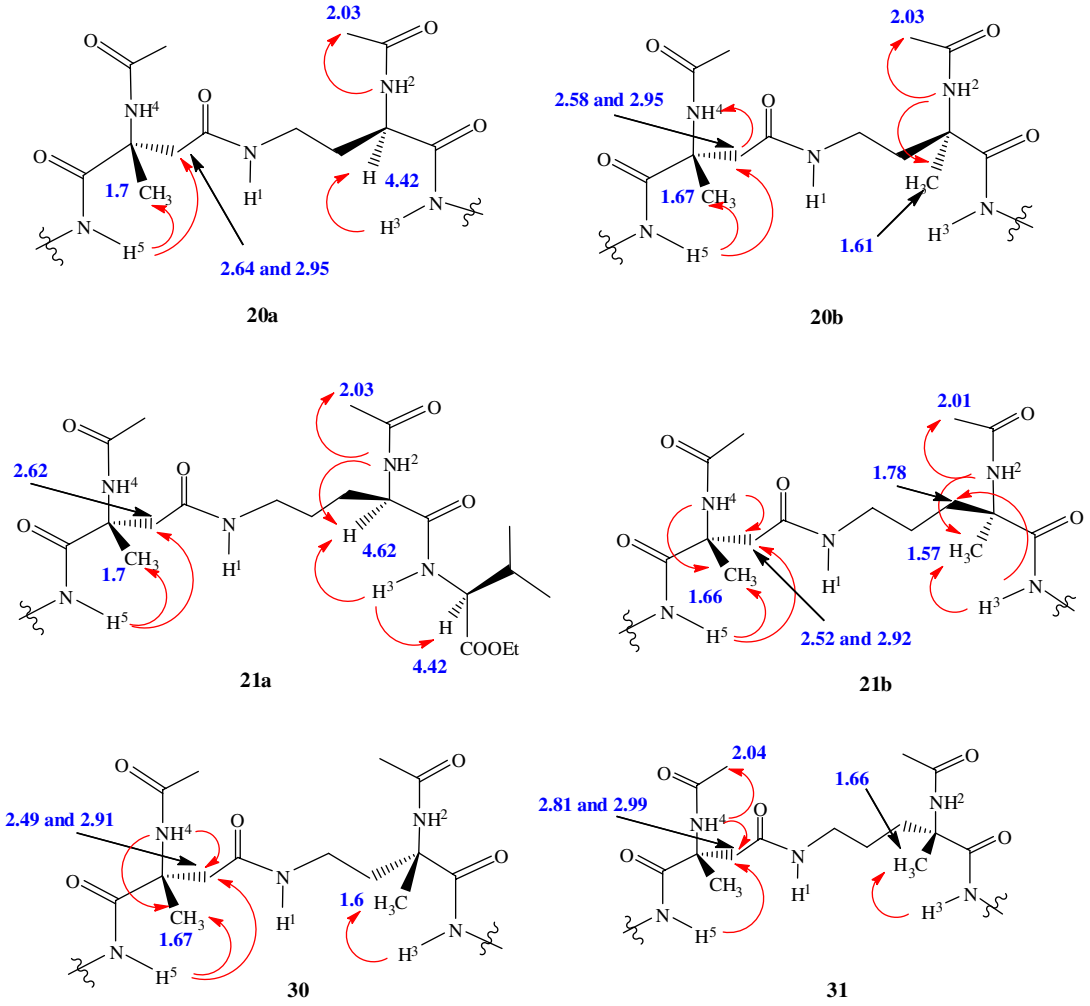


Figure 1. Selected NOE enhancement for substrates **20a** and **20b**, **21a** and **21b** and **30** and **31**.

(lifetime of 73.4%), H2 with O7 (lifetime of 27.8%) and H3 with O2 (lifetime of 39.7%). Pseudopeptide **20b** (Figs. 2 and 5) can be obtained from **20a** after substitution of a hydrogen atom with a methyl group to give an (*R*)-configuration. The effect of the methyl group is that of destabilizing the S-type folded structure **Cl1**. As a consequence, even if two stable minima still exist, the C-type conformer **Cl2** becomes highly populated (populations are 76.8% and 11.7% for **Cl2** and **Cl1**, respectively). The high population of **Cl2** can be easily understood. In this conformer, in addition to the strong hydrogen bonds H4–O1 and H5–O4 (lifetimes are 95.4% and 86.2%, respectively) already observed in **Cl1**, two additional interactions are characterized by considerable lifetimes (H3–O2 92% and H2–O7 72.2%). In particular, H2–O7 is responsible for the formation in **Cl2** of a rather stable 14-membered cyclic structure.

The chain lengthening in **20b** (inclusion of an additional CH_2 unit) causes a strong destabilization of the structure. In the resulting compound **21b** (see Figs. 2 and 6) a plethora of minima appears, each one characterized by a different orientation of the two limbs of the peptide. If we consider the four most stable conformers **Cl4**, **Cl5**, **Cl7** and **Cl10** (populations of 20.6%, 5.6%, 18.7% and 15.4%, respectively), the most steady hydrogen bonds are H3–O2 (lifetime 89.0%), H5–O4 (lifetime 83.1%) and H1–O5 (lifetime 68.9%). The hydrogen bonds H2–O5 and H4–O1 have a lifetime of 18.7% and 50.5%, respectively, and are involved in an equilibrium between a non-hydrogen bonded and a hydrogen bonded structure.

Pseudopeptide **30** (Figs. 2 and 7) can be considered to formally originate from **20a** after substitution of the hydrogen atom with a methyl group to give an (*S*)-configuration. This corresponds to the most stable of the six pseudopeptides examined here. We have detected in this case only one representative conformation **Cl2**, with a population of 94.0%.

Seven rather strong hydrogen bonds determine the high stability of this structure characterized by a large population. These are H1–O5 (lifetime 78.0%), H2–O7 (lifetime 87.0%), H3–O2 (lifetime 78.8%), H3–O6 (lifetime 51.2%), H4–O1 (lifetime 98.6%), H5–O4 (lifetime 86.6%) and H5–O1 (lifetime 52.2%). Again, the inclusion of an additional CH_2 unit in the carbon chain causes a decrease in the stability of this structure and, consequently, the resulting pseudopeptide **31** (see Figs. 2 and 8) is characterized by four minima **Cl1**, **Cl2**, **Cl3** and **Cl4** with populations of 28.4%, 16.0%, 13.1% and 32.1%, respectively.

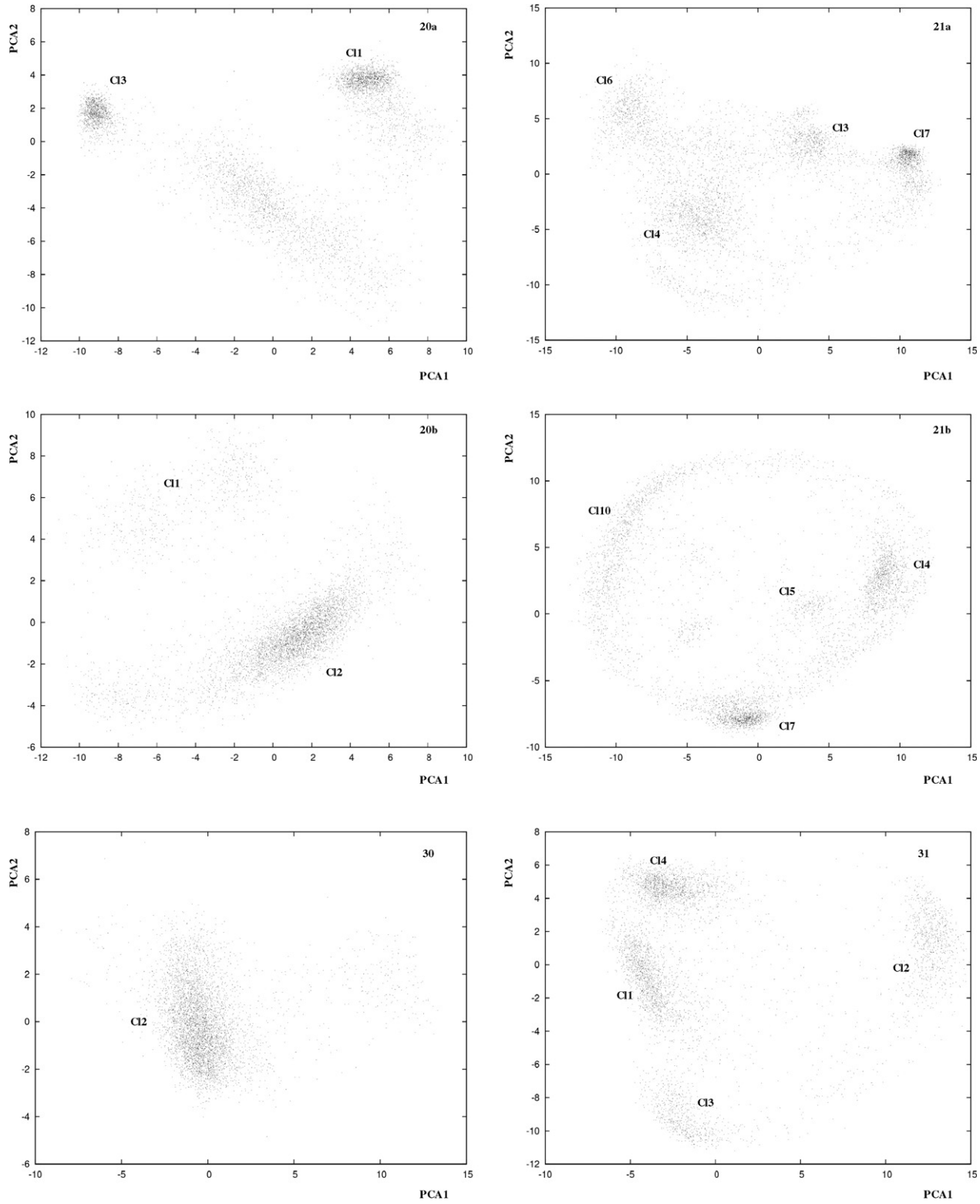


Figure 2. Principal component analysis for peptides **20a**, **20b**, **21a**, **21b**, **30** and **31.** PCA1 and PCA2 are linear combinations of internal coordinates describing the two most important conformational motions.

The H3–O2 (lifetime 92.8%) and H5–O4 (lifetime 90.5%) hydrogen bonds can be detected in all four conformations. Our computations indicate that the H1 proton can interact with either O5 or O3, thus leading to an equilibrium between H1–O5 and H1–O3 hydrogen bonds (corresponding lifetimes are 68.3% and 23.3%, respectively). Similarly, H4 can form a hydrogen bond either with O1 (lifetime 81.1%) or O3 (lifetime 15.7%). Also an equilibrium exists between a non-hydrogen bonded state and a structure characterized by the H2–O7 bond (lifetime 14.4%). These data support

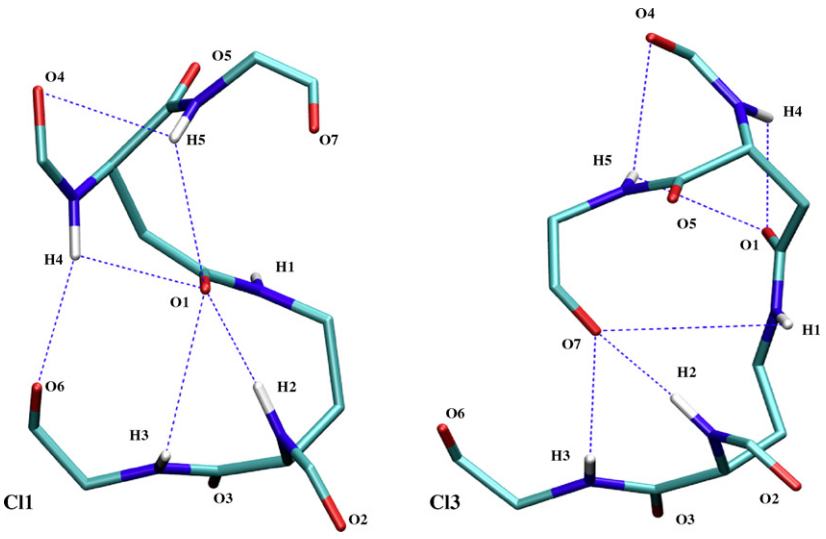


Figure 3. Representative conformations for pseudopeptide **20a**.

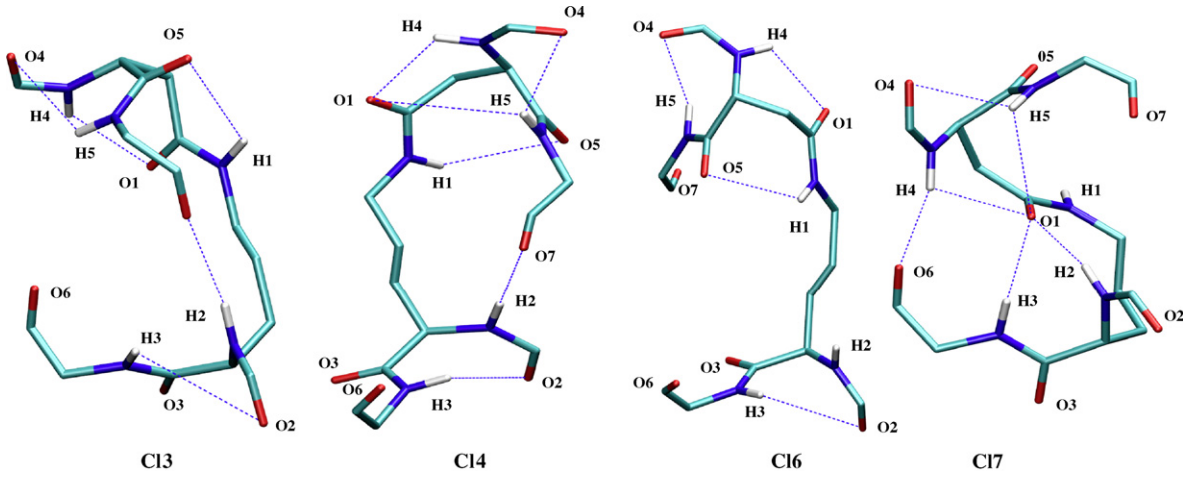


Figure 4. Representative conformations for pseudopeptide **21a**.

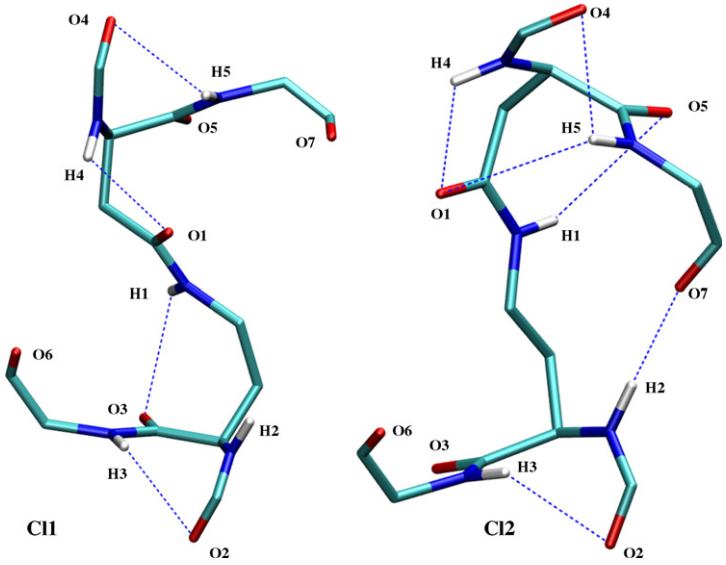


Figure 5. Representative conformations for pseudopeptide **20b**.

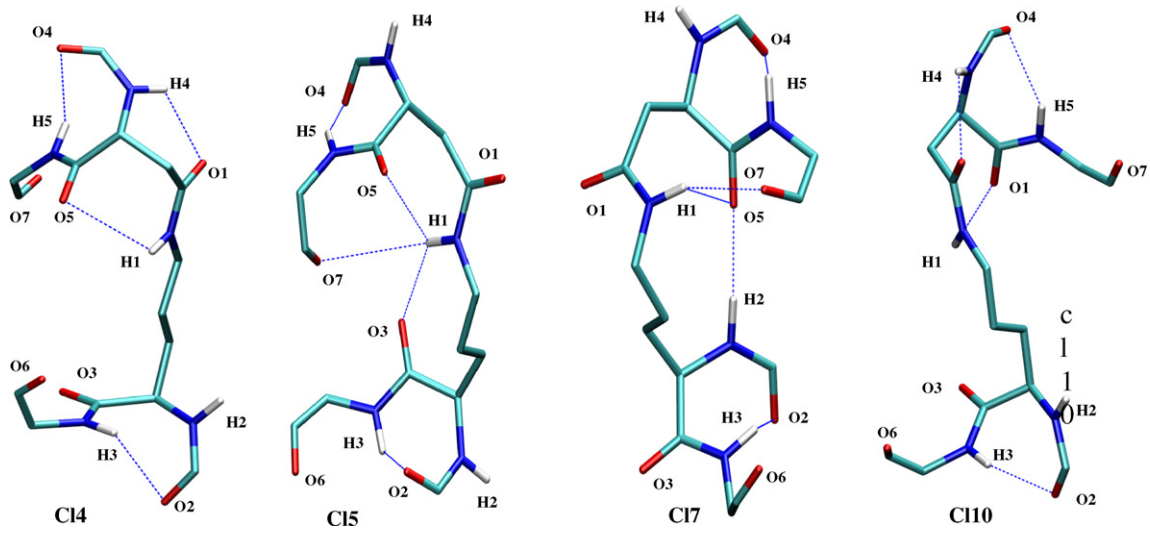


Figure 6. Representative conformations for pseudopeptide **21b**.

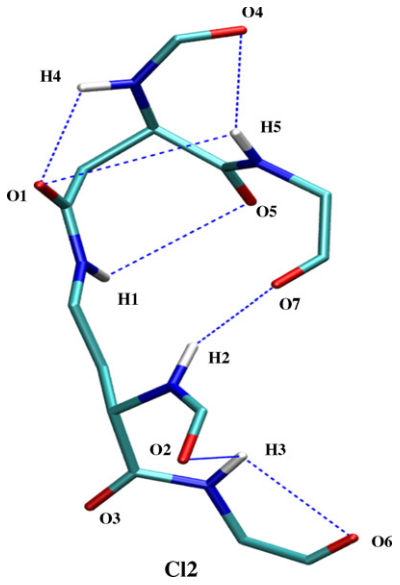


Figure 7. Representative conformation for pseudopeptide **30**.

the idea that pseudotetrapeptides **20a** and **20b** and **30** are more prone to form steady intramolecular hydrogen bonds leading to stable structures in comparison to **21a** and **21b** or **31** where the chain lengthening gives a greater structural flexibility. Also the introduction of a second methyl group especially in the (*S*)-configuration increases the stability of the global peptide folding, as suggested by the experimental data.

5. Conclusions

Spectroscopic investigation using ^{1}H NMR and IR techniques combined with conformational analysis based on molecular dynamics (MD) and cluster analysis has been demonstrated to be an effective tool in elucidating the structures of various pseudotetrapeptides and to analyze the features of intramolecular hydrogen bonds involving carbonyl oxygens and amide protons. The MD analysis which provides conformer populations and hydrogen bond lifetimes is in good agreement with the ^{1}H NMR and IR data. The results indicate that pseudotetrapeptides **20a** and **20b** and **30** are more prone to form intramolecular hydrogen bonds with respect to **21a** and **21b** and **31**. Most probably this behaviour is due to the greater structural flexibility of substrates **21a** and **21b** and **31**, which include in the carbon chain one additional CH_2 unit with respect to **20a** and **20b** and **30**. Also, the introduction of a second methyl group [(especially in the case of the resulting (*S*)-configuration, as in **20b** and **21b**)] apparently increases, although to a smaller extent, the tendency to give intramolecular hydrogen bonds.

6. Experimental

6.1. General information

^{1}H and ^{13}C NMR spectra were recorded on a Gemini spectrometer at 300 MHz (in about 15 mM solutions) 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 FT 380 spectrometer. Optical rotations were measured at 25 °C on a Perkin–Elmer 343 polarimeter. Dry THF was distilled from sodium benzophenone ketyl and chromatographic separations were performed with Silica Gel 60 (230–400 mesh).

Synthesis and spectroscopic data of compounds **1** and **2** are reported in Ref. 5b while in Ref. 4 are reported the data of derivative **7**.

6.2. 2-(*N*,*N*-Dibenzylamino)ethanol 3

A mixture of benzyl bromide (12.5 mL, 105 mmol), 3-aminoethanol (3 mL, 50 mmol) and K_2CO_3 (13.8 g, 100 mmol) in 100 mL of acetone was stirred at rt for 12 h. The reaction

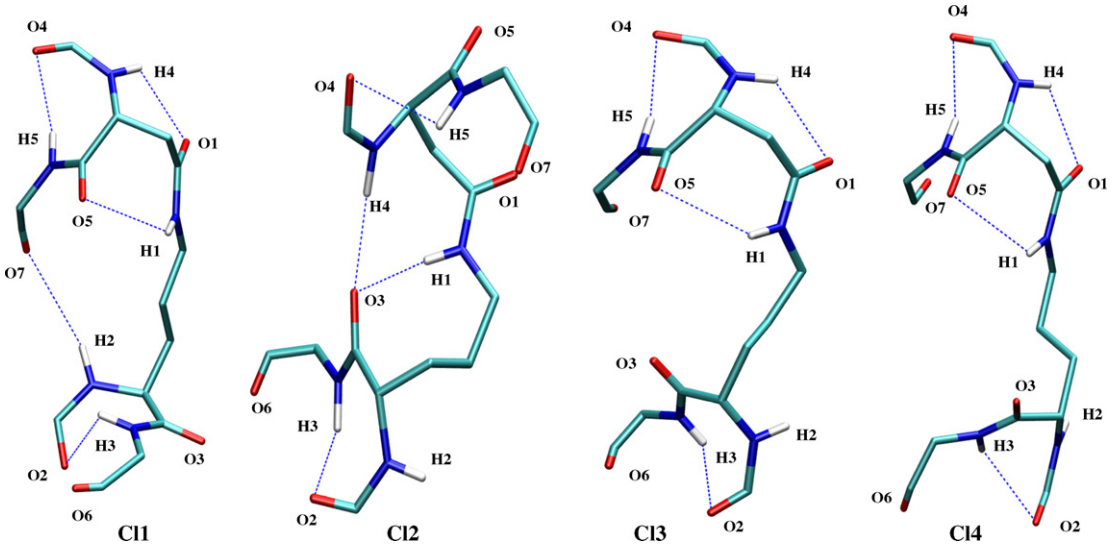


Figure 8. Representative conformations for pseudopeptides **31**.

mixture was filtered off and the organic solution evaporated in vacuo to dryness. The residue was submitted to purification by silica gel chromatography eluting with cyclohexane/ethyl acetate and the oily product was recovered as a wax in 90% yield. ^{1}H NMR: δ 2.5–2.6 (br s, ^{1}H); 2.73 (t, 2H, $J = 5.4$), 3.6–3.7 (m, 6H), 7.4 (m, 10ArH). ^{13}C NMR: δ 54.6, 58.0, 58.4, 127.1, 128.3, 128.8, 138.6.

6.3. 2-(*N*,*N*-Dibenzylamino)propanol 4

Compound **4** was synthesized starting from 3-aminopropanol and following the procedure reported for **3.** After purification by silica gel chromatography eluting with cyclohexane/ethyl acetate the oily product was recovered in 90% yield. ^{1}H NMR: δ 1.79 (m, 2H); 2.67 (t, 2H, $J = 5.6$); 3.50–3.75 (m, 6H); 4.70 (br s, 1H); 7.3 (m, 10ArH). ^{13}C NMR: δ 27.9, 52.7, 58.3, 63.4, 127.0, 128.2, 128.9, 138.1.

6.4. (*N*,*N*-Dibenzylamino)-2-iodoethane 5

To a stirred solution of **3** (4.8 g, 20 mmol) in triethylamine (5.6 mL, 40 mmol) and chloroform (20 mL), thionyl chloride (3 mL, 40 mmol) was added and the reaction mixture was stirred at rt After about 1 h, water was added and the organic extract dried on $CaCl_2$ and then evaporated in vacuo to dryness. To the crude reaction product dissolved in acetone (100 mL) was added NaI (9 g, 60 mmol) and the mixture stirred at rt After about 24 h, the reaction mixture was filtered off and the organic solution was evaporated in vacuo. The residue was dissolved in ethyl acetate and the organic solution was washed twice with water. The product was recovered as a wax in 80% overall yield after purification by silica gel chromatography eluting with cyclohexane/ethyl acetate. ^{1}H NMR: δ 2.88 (t, 2H, $J = 7.8$); 3.21 (t, 2H, $J = 7.8$); 3.68 (s, 4H); 7.32 (m, 10ArH). ^{13}C NMR: δ 4.03, 26.7, 55.7, 57.7, 126.9, 128.1, 128.6, 138.7.

6.5. (*N*,*N*-Dibenzylamino)-3-iodopropane 6

Compound **6** was synthesized starting from **4** and following the procedure reported for **5**. The product was recovered as a wax in 80% overall yield after purification by silica gel chromatography eluting with cyclohexane/ethyl acetate. ^{1}H NMR: δ 2.04 (m, 2H); 2.56 (t, 2H, $J = 6.6$); 3.20 (t, 2H, $J = 7$); 3.60 (s, 4H); 7.32 (m, 10ArH). ^{13}C NMR: δ 4.3, 31.4, 53.6, 58.4, 126.8, 128.1, 128.7, 139.3.

6.6. (3*R*,6*S*)-(1-Benzyl-5-ethoxy-6-isopropyl-3-methyl-1,6-dihydro-pyrazin-2-yl)-acetic acid 8

Compound **7**[4] (2.18 g, 5 mmol) dissolved in methanol (20 mL) was submitted to hydrogenolysis in a Parr apparatus in the presence of Palladium on charcoal under 5 atm of hydrogen pressure. After about 24 h, the catalyst was filtered off and the organic solution evaporated in vacuo. The pure product was obtained as an oil in practically quantitative yield. ^{1}H NMR: δ 0.99 (d, 3H, $J = 7$); 1.12 (d, 3H, $J = 7$); 1.31 (t, 3H, $J = 7$); 2.30 (m, 1H); 2.82 (m, 1H); 3.05 (m, 1H); 3.83 (d, 1H, $J = 3$); 3.99 (d, 1H, $J = 15.3$); 4.25 (m, 2H); 5.53 (d, 1H, $J = 15.3$); 7.30 (m, 5ArH). ^{13}C NMR: δ 13.6, 17.0, 20.2, 28.4, 29.0, 46.2, 58.2, 60.7, 60.8, 127.2, 128.1, 128.3, 135.1, 157.1, 171.3, 174.9. $[\alpha]_D = -14.8$ (c 0.5, $CHCl_3$). Anal. Calcd for $C_{19}H_{26}N_2O_4$: C, 65.87; H, 7.56; N, 8.09. Found: C, 65.95; H, 7.56; N, 8.1.

6.7. (3*R*,6*S*)-(1-Benzyl-5-ethoxy-6-isopropyl-3-methyl-1,6-dihydro-pyrazin-2-yl)-acetic acid pentafluorophenyl ester 9

Compound **9** was synthesized following the procedure reported in Ref. 4 for an analogous derivative. The pure product was obtained as an oil in 90% yield after purification by silica gel chromatography eluting with hexane/ethyl acetate. ^{1}H NMR: δ 0.96 (d, 3H, $J = 7$); 1.11 (d, 3H, $J = 7$); 1.23 (t, 3H, $J = 7$); 1.61 (s, 3H); 2.30 (m, 1H); 2.95 (d, 1H, $J = 15.8$); 3.70 (d, 1H, $J = 15.8$); 3.86 (d,

Table 3. Lifetimes for the various hydrogen bonds in substrates **20a**, **20b**, **21a**, **21b**, **30** and **31**

Structure	Hydrogen bond	Lifetime (%)
20a	H4–O1	94.8
	H5–O4	76.3
	H5–O1	60.2
	H1–O5	48.2
	H2–O7	45.4
	H2–O1	43.5
	H3–O6	38.7
	H3–O2	35.2
	H3–O1	28.2
	H3–O7	21.4
	H1–O7	16.1
21a	H4–O1	96.5
	H5–O4	89.5
	H1–O5	73.4
	H3–O6	47.1
	H3–O2	39.7
	H5–O1	34.4
	H5–O7	30.1
	H2–O7	27.8
	H2–O1	23.4
	H3–O1	21.9
20b	H4–O1	95.4
	H3–O2	92.0
	H5–O4	86.2
	H1–O5	73.0
	H2–O7	72.2
	H5–O1	44.2
	H2–O5	18.7
21b	H3–O2	89.0
	H5–O4	83.1
	H1–O5	68.9
	H4–O1	50.5
	H5–O7	37.1
	H3–O6	27.8
30	H4–O1	98.6
	H2–O7	87.0
	H5–O4	86.6
	H3–O2	78.8
	H1–O5	78.0
	H5–O1	52.4
	H3–O6	51.2
31	H3–O2	92.8
	H5–O4	90.5
	H4–O1	81.1
	H1–O5	68.3
	H3–O6	45.1
	H5–O7	37.8
	H5–O1	29.3
	H1–O3	23.3
	H4–O3	15.7
	H2–O7	14.4

1H, $J = 2.6$); 4.0 (m, 3H); 5.48 (d, 1H, $J = 14.6$); 7.20 (m, 5ArH). ^{13}C NMR: δ 13.7, 17.1, 20.3, 28.7, 29.5, 46.0, 46.7, 58.9, 61.1, 61.3, 127.3, 128.1, 128.7, 135.3, 157.7, 166.4, 170.9. $[\alpha]_D = -31.5$ (*c* 1, $CHCl_3$). Anal. Calcd for $C_{25}H_{25}F_5N_2O_4$: C, 58.59; H, 4.92; N, 5.47. Found: C, 58.55; H, 4.93; N, 5.45.

6.8. (3*R*,6*S*)-1-Benzyl-3-(2-dibenzylaminoethyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 10a

To a solution of lactim **1**[5b] (8.22 g, 30 mmol) in dry THF (100 mL) and cooled at −78 °C, a solution of 1 M LHMDS in THF (30 mL, 30 mmol) was added dropwise under stirring. After about 1 h, the electrophile **5** (10.5 g, 30 mmol) was added, then the reaction mixture was allowed to warm up to room temperature and kept stirring until the reaction was practically complete (overnight). After the addition of water and ethyl acetate, the organic solution was separated and then evaporated in vacuo. The reaction product was submitted to silica gel chromatography, eluting with hexane/ethyl acetate and diastereomers **10a** and **12** (obtained in the ratio ~ 75:25, respectively) were separated. The pure product **10a** was recovered as an oil in about 60% yield. ^{1}H NMR: δ 0.92 (d, 3H, $J = 7$); 1,03 (d, 3H, $J = 7$); 1.05 (d, 3H, $J = 7$); 1.9 (m, 1H); 2.2 (m, 1H); 2.40–2.83 (m, 3H); 3.45–3.82 (m, 7H); 3.9 (d, 1H, $J = 15$); 4.16 (m, 1H); 5.46 (d, 1H, $J = 15$); 7.34–7.45 (m, 15ArH). ^{13}C NMR: δ 14.3, 17.9, 20.3, 31.1, 31.7, 47.7, 49.6, 56.0, 58.4, 61.2, 62.1, 126.7, 127.4, 128.1, 128.2, 128.3, 128.5, 128.9, 129.1, 136.6, 140.2, 159.1, 171.0. $[\alpha]_D = +39.8$ (*c* 1, $CHCl_3$). Anal. Calcd for $C_{32}H_{39}N_3O_2$: C, 77.23; H, 7.90; N, 8.44. Found: C, 77.02; H, 7.9; N, 8.42.

6.9. (3*R*,6*S*)-1-Benzyl-3-methyl-3-(3-dibenzylaminopropyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 10b

The product was obtained by alkylating lactim **2**[5b] with the electrophile **5** and following the procedure described for **10a**. The product was recovered as an oil in 80% yield after silica gel chromatography eluting with hexane/ethyl acetate. ^{1}H NMR: δ 0.86 (d, 3H, $J = 7$); 1.03 (d, 3H, $J = 7$); 1.12 (t, 3H, $J = 7$); 1.48 (s, 3H); 1.93 (m, 1H), 2.03–2.55 (m, 4H); 3.38–3.58 (m, 6H); 5.45 (d, 1H, $J = 15$); 7.22 (m, 15ArH). ^{13}C NMR: δ 14.0, 17.2, 20.4, 28.9, 29.5, 39.7, 46.5, 48.4, 58.0, 59.5, 60.5, 61.0, 126.5, 128.0, 128.5, 128.6, 128.7, 136.1, 139.7, 155.7, 172.0. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.10. (3*R*,6*S*)-1-Benzyl-3-(3-dibenzylaminobutyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 11a

The product was obtained by alkylating lactim **1** with electrophile **6** and following the procedure described for **10a**. The reaction product was submitted to silica gel chromatography, eluting with hexane/ethyl acetate and diastereomers **11a** and **13** (obtained in the ratio ~ 70:30, respectively) were separated. The pure diastereomer **11a** was recovered as an oil in about 60% yield. ^{1}H NMR: δ 0.92 (d, 3H, $J = 7$); 1.06 (d, 3H, $J = 7$); 1.24 (t, 3H, $J = 7.2$); 1.60 (m, 2H); 1.90 (m, 1H); 2.09 (m, 1H); 2.22 (m, 1H); 2.50 (t, 2H, $J = 7.2$); 3.58 (s, 4H); 3.70 (dd, 1H, $J = 1.8, 3.9$); 3.91 (d, 1H, $J = 15$); 4.05 (m, 3H); 5.50 (d, 1H, $J = 15$); 7.20–7.50 (m, 15ArH). ^{13}C NMR: δ 14.0, 17.3, 19.8, 22.2, 31.2, 31.4, 47.1, 52.9, 57.3, 57.9, 60.8, 61.7, 126.4, 127.3, 127.6, 127.8, 128.5, 128.6, 136.3, 139.7, 158.7, 170.1. $[\alpha]_D = +41.0$ (*c* 0.9, $CHCl_3$). Anal. Calcd for $C_{33}H_{41}N_3O_2$: C, 77.46; H, 8.08; N, 8.21. Found: C, 77.67; H, 8.09; N, 8.15.

6.11. (3*R*,6*S*)-1-Benzyl-3-methyl-3-(3-dibenzylaminobutyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 11b

The compound was obtained by alkylating lactim **2** with electrophile **6** and following the procedure described for **10a**. The product was recovered as an oil in 85% yield after silica gel chromatography eluting with hexane/ethyl acetate. ^{1}H NMR: δ 0.94 (d, 3H, $J = 7$); 1.11 (d, 3H, $J = 7$); 1.26 (t, 3H, $J = 7$); 1.32 (m, 1H); 1.48 (s, 3H); 1.54 (m, 2H); 2.07 (m, 1H); 2.27 (m, 1H), 2.40 (t, 2H, $J = 6.9$); 3.51 (q_{AB}, 4H, $J = 13.8$); 3.80 (d, 1H, $J = 2.7$); 3.91 (d, 1H, $J = 15$); 3.94–4.18 (m, 2H); 5.55 (d, 1H, $J = 15$); 7.30 (m, 15ArH). ^{13}C NMR: δ 14.1, 17.2, 20.4, 21.8, 29.6, 41.0, 46.4, 53.2, 57.9, 60.4, 60.5, 61.0, 126.5, 127.5, 127.9, 128.4, 128.6, 136.1, 139.7, 155.7, 172.2. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.12. (3*S*,6*S*)-1-Benzyl-3-(3-dibenzylaminopropyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 12

The oily product, obtained in diastereomeric mixture with **10a**, was recovered in about 20% yield after separation by silica gel chromatography eluting with hexane/ethyl acetate. ^{1}H NMR: δ 0.93 (d, 3H, $J = 7$); 1,04 (d, 3H, $J = 7$); 1.06 (d, 3H, $J = 7$); 1,9 (m, 1H); 2.2 (m, 1H); 2.40–2.80 (m, 3H); 3.5–3.8 (m, 7H); 3.9 (d, 1H, $J = 15$); 4.15 (m, 1H); 5.44 (d, 1H, $J = 15$); 7.34–7.45 (m, 15ArH). ^{13}C NMR: δ 14.4, 18, 20.4, 31, 31.8, 47.9, 49.5, 56.1, 58.2, 61.4, 62.3, 126.8, 127.5, 128, 128.2, 128.3, 128.5, 128.9, 129.1, 136.8, 140.3, 159.3, 171.0. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.13. (3*S*,6*S*)-1-Benzyl-3-(3-dibenzylaminobutyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 13

The product, obtained in a diastereomeric mixture with **11a**, was recovered as an oil in about 25% yield after separation by silica gel chromatography eluting with hexane/ethyl acetate. ^{1}H NMR: δ 0.92 (d, 3H, $J = 7$); 1.10 (d, 3H, $J = 7$); 1.25 (t, 3H, $J = 7.2$); 1.6 (m, 1H); 1.85 (m, 1H); 1.98 (m, 1H); 2.13 (m, 2H); 2.52 (t, 2H, $J = 6.9$); 3.60 (q_{AB}, 4H, $J = 13.5$); 3.71 (dd, 1H, $J = 1.8, 3.3$); 3.96 (d, 1H, $J = 15$); 4.10 (m, 3H); 5.44 (d, 1H, $J = 15$); 7.35 (m, 15ArH). ^{13}C NMR: δ 14.0, 17.5, 19.9, 21.6, 31.1, 31.5, 47.4, 52.3, 57.3, 57.4, 61.0, 62.0, 127.4, 127.7, 128.2, 128.6, 129.2, 130.6, 136.3, 159.1, 170.3. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.14. (3*R*,6*S*)-1-Benzyl-3-(3-aminopropyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 14a

To a solution of **10a** (5 g, 10 mmol) in methanol (20 mL) was added 0.5 g of $Pd(OH)_2$ and submitted to hydrogenation in a Parr apparatus under 5 atm of pressure. After about 24 h the catalyst was filtered off and the organic solution was evaporated in vacuo. The oily product was recovered in practically quantitative yield. ^{1}H NMR: δ 0.96 (d, 3H, $J = 7$); 1.08 (d, 3H, $J = 7$); 1.28 (t, 3H, $J = 7$); 2.10 (m, 1H); 2.23 (m, 1H); 2.43 (m, 1H); 3.17 (m, 2H); 3.71 (m, 1H); 3.97 (d, 1H, $J = 15$); 4.10 (m, 3H); 4.41 (br s, 2H); 5.45 (d, 1H, $J = 15$); 7.18–7.43 (m, 5ArH). ^{13}C NMR: δ 13.8, 17.5, 19.7, 31.3, 33.7, 38.4, 47.5, 56.6, 61.4, 61.9, 127.3, 127.4, 128.5, 135.7, 159.4, 169.8. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.15. (3*R*,6*S*)-1-Benzyl-3-methyl-3-(3-aminopropyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 14b

The compound was obtained as an oil in practically quantitative yield starting from **10b** and following the procedure described for **14a**. ^{1}H NMR: δ 0.95 (d, 3H, $J = 7$); 1.07 (d, 3H, $J = 7$); 1.27 (t, 3H, $J = 7$); 1.62 (m, 2H); 1.90–2.35 (m, 5H); 2.79 (t, 2 H, $J = 6.9$); 3.72 (m, 1H); 3.94 (d, 1H, $J = 15$); 4.12 (m, 3H); 5.50 (d, 1H, $J = 15$); 7.30 (m, 5ArH). ^{13}C NMR: δ 13.7; 17.0; 19.5; 31.1; 46.9; 48.8; 53.4; 57.1; 60.6; 60.7; 61.4; 127.3; 127.8; 128.2; 135.8; 158.9; 169.9. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.16. (3*R*,6*S*)-1-Benzyl-3-(3-aminobutyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 15a

The compound was obtained from **11a** and following the procedure used for **14a**. The oily product was recovered in practically quantitative yield. ^{1}H NMR: δ 0.95 (d, 3H, $J = 7$); 1.07 (d, 3H, $J = 7$); 1.27 (t, 3H, $J = 7$); 1.62 (m, 2H); 1.90–2.35 (m, 5H); 2.79 (t, 2H, $J = 6.9$); 3.72 (m, 1H); 3.94 (d, 1H, $J = 15$); 4.12 (m, 3H); 5.50 (d, 1H, $J = 15$); 7.30 (m, 5ArH). ^{13}C NMR: δ 13.7, 17.0, 19.5, 31.1, 46.9, 48.8, 53.4, 57.1, 60.6; 60.7, 61.4, 127.3, 127.8, 128.2, 135.8, 158.9, 169.9. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.17. (3*R*,6*S*)-1-Benzyl-3-methyl-3-(3-aminobutyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 15b

The compound was obtained as an oil in practically quantitative yield starting from **11b** and following the procedure described for **14a**. ^{1}H NMR: δ 0.90 (d, 3H, $J = 7$); 1.07 (d, 3H, $J = 7$); 1.25 (t, 3H, $J = 7.2$); 1.47 (s, 3H); 1.20–1.70 (m, 3H); 2.0–2.3 (m, 2H); 2.63 (m, 2H); 3.77 (d, 1H, $J = 3$); 3.92 (d, 1H, $J = 15$); 4.05 (m, 2H); 5.46 (d, 1H, $J = 15$); 7.3 (m, 5ArH). ^{13}C NMR: δ 13.6, 16.8, 19.9, 28.0, 28.3, 29.2, 40.0, 41.5, 46.3, 59.8, 60.1, 60.8, 127.1, 127.9, 128.1, 135.6, 155.4, 171.8. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.18. 2-[(2*R*,5*S*)-4-Benzyl-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl]-*N*-[2-((2*R*,5*S*)-4-benzyl-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-3-oxopyrazin-2-yl)ethyl]acetamide 16a

A solution of **14a** (1.6 g, 5 mmol) and the activated ester **9** (2.56 g, 5 mmol) in dry THF (15 mL) was stirred at rt for 12 h and the reaction monitored by TLC. Water was added and the reaction product was extracted with ethyl acetate. The organic phase was evaporated in vacuo and the residue submitted to purification by silica gel chromatography eluting with hexane/ethyl acetate. The oily product was recovered pure in 85% yield. ^{1}H NMR: δ 0.92 (t, 6H,

$J = 7$); 1.05 (d, 6H, $J = 7$); 1.27 (m 4H); 1.53 (s, 3H); 1.8–2.1 (m, 2H); 2.1–2.42 (m, 3H); 2.57 (d, 1H, $J = 14.2$); 2.97 (d, 1H, $J = 14.2$); 3.33 (m, 1H); 3.57 (m, 1H); 3.69 (dd, 1H, $J = 1.8$, 4); 3.83 (d, 1H, $J = 3$); 3.96 (d, 1H, $J = 15$); 4.16 (m, 6H); 5.32 (d, 1H, $J = 15$); 5.44 (d, 1H, $J = 15$); 6.9 (m, 1H); 7.2 (m, 10ArH). ^{13}C NMR: δ 13.9, 14.0, 17.2, 17.6, 19.9, 20.4, 28.4, 29.6, 31.5, 32.6, 37.1, 47.2, 47.6, 48.6, 57.1, 59.0, 60.9, 61.4, 61.6, 62.0, 127.3, 127.6, 128.0, 128.5, 128.7, 136.0, 136.1, 156.8, 159.8, 169.6, 170.0, 171.8. $[\alpha]_D = +28.4$ (*c* 0.9, $CHCl_3$). Anal. Calcd for $C_{37}H_{51}N_5O_5$: C, 68.81; H, 7.96; N, 10.84. Found: C, 68.95; H, 7.94; N, 10.82.

6.19. 2-[(2*R*,5*S*)-4-Benzyl-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl]-*N*-[2-((2*R*,5*S*)-4-benzyl-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl)ethyl]acetamide 16b

The compound was obtained starting from **14b** following the procedure described for **16a**. ^{1}H NMR: δ 0.90 (d, 6H, $J = 7$); 1.05 (d, 3H, $J = 7$); 1.06 (d, 3H, $J = 7$); 1.25 (m, 6H); 1.48 (s, 3H); 1.52 (s, 3H); 1.90 (m, 1H); 2.18 (m, 3H); 2.59 (d, 1H, $J = 14.2$); 2.89 (d, 1H, $J = 14.2$); 3.08 (m, 1H); 3.30 (m, 1H); 3.76 (d, 1H; $J = 2.8$); 3.80 (d, 1H, $J = 2,8$); 4.10 (m, 6H); 5.35 (d, 2H, $J = 15$); 6.59 (m, 1H); 7.23 (m, 10 ArH). ^{13}C NMR: δ 13.7, 13.8, 17.1, 17.2, 20.2, 28.1, 28.2, 29.4, 29.6, 35.3, 41.5, 46.8, 48.3, 58.8, 56.0, 60.7, 61.3, 127.1, 127.3, 127.7, 127.8, 128.3, 128.5, 135.7, 135.8, 156.3, 156.6, 169.3, 171.5, 171.8. $[\alpha]_D = +7.1$ (*c* 0.7, CH_3OH). Anal. Calcd for $C_{38}H_{35}N_5O_5$: C, 69.17; H, 8.10; N, 10.61. Found: C, 69.42; H, 8.1; N, 10.57.

6.20. 2-[(2*R*,5*S*)-4-Benzyl-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl]-*N*-[2-((2*R*,5*S*)-4-benzyl-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-3-oxopyrazin-2-yl)propyl]acetamide 17a

The compound was obtained starting from **15a** and following the procedure described for **16a**. ^{1}H NMR: δ 0.92 (m, 6H); 1.05 (m, 6H); 1.21 (t, 3H, $J = 7.2$); 1.23 (t, 3H, $J = 7.2$); 1.58 (s, 3H); 1.60 (m, 1H); 1.8–2.4 (m, 5H); 2.68 (d, 1H, $J = 14.4$); 2.95 (d, 1H, $J = 14.4$); 3.25 (m, 2H); 3.68 (m, 1H); 3.82 (d, 1H, $J = 2.6$); 3.91 (d, 1H, $J = 15.4$); 4.1 (m, 5H); 5.34 (d, 1H, $J = 15.4$); 5.47 (d, 1H, $J = 15.4$); 6.58 (m, 1H); 7.3 (m, 10ArH). ^{13}C NMR: δ 13.8, 13.9, 17.1, 17.4, 19.8, 20.3, 25.0, 28.0, 29.6, 30.6, 31.4, 38.9, 47.2, 48.4, 57.2, 59.0, 60.9, 61.0, 61.5, 61.8, 127.3, 127.4, 127.6, 127.9, 128.4, 128.5, 135.9, 136.0, 156.7, 159.3, 169.7, 170.1, 171.6. $[\alpha]_D = +31.2$ (*c* 2.2, $CHCl_3$). Anal. Calcd for $C_{38}H_{53}N_5O_5$: C, 69.17; H, 8.10; N, 10.61. Found: C, 69.5; H, 8.08; N, 10.6.

6.21. 2-[(2*R*,5*S*)-4-benzyl-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl]-*N*-[2-((2*R*,5*S*)-4-benzyl-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl)propyl]acetamide 17b

The compound was obtained starting from **15b** following the procedure described for **16a**. ^{1}H NMR: δ 0.87 (d, 6H, $J = 7$); 1.03 (d, 6H, $J = 7$); 1.20 (m, 6H); 1.42 (s, 3H); 1.52 (s, 3H); 1.10–1.60 (m, 3H); 1.98–2.24 (m, 1H); 2.18 (m, 2H); 2.77 (q_{AB}, 2H, $J = 14.4$); 3.10 (m, 2H); 3.71 (m, 1H); 3.80 (m, 1H); 3.85–4.15 (m, 6H); 5.26 (d, 1H, $J = 15$); 5.43 (d, 1H, $J = 15$); 6.42 (m, 1H); 7.18–7.35 (m, 10ArH). ^{13}C NMR: δ 13.8, 14.0, 17.2, 20.3, 20.4, 24.3, 28.1, 28.7, 29.6, 29.7, 38.9, 40.4, 46.5, 47.2, 48.5, 59.1, 60.1, 60.6, 60.9, 61.1, 61.5, 127.5, 127.9, 128.2, 128.5, 128.6, 135.9, 156.0, 156.8, 170.0, 171.6, 172.1. $[\alpha]_D = +9.7$ (*c* 1.2, $CHCl_3$). Anal. Calcd for $C_{39}H_{55}N_5O_5$: C, 69.51; H, 8.23; N, 10.39. Found: C, 69.66; H, 8.24; N, 10.4.

6.22. 2-[(2*R*,5*S*)-6-Ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl]-*N*-[2-((2*R*,5*S*)-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-3-oxopyrazin-2-yl)ethyl]acetamide 18a

A solution of **16a** (6.45 g, 10 mmol) in 20 mL of dry THF/*t*-butanol 9:1 was added to about 100 mL of liquid ammonia cooled at −50 °C. Then, Li (0.14 g, 20 mmol) was added. The addition of Li was stopped as soon as the reaction mixture became blue, the starting material having disappeared. The reaction was then quenched with NH_4Cl and the cooling bath removed to allow the complete evaporation of NH_3. After addition of water and ethyl acetate, the aqueous solution was acidified to pH 4 with diluted HCl and the organic solution evaporated to dryness under vacuum. The product was recovered as a wax in 94% yield. ^{1}H NMR: δ 0.86 (d, 6H, $J = 7$); 0.98 (d, 6H, $J = 7$); 1.27 (m, 6H); 1.41 (s, 3H); 1.90 (m, 1H); 2.20 (m, 3H), 2.47 (d, 1H, $J = 14.4$); 2.80 (d, 1H, $J = 14.4$); 3.40 (m, 2H); 3.86 (d, 1H, $J = 2.6$); 3.97–4.23 (m, 5H); 6.78 (br s, 1H); 6.84 (br s, 1H); 7.04 (br s, 1H). ^{13}C NMR: δ 14.0, 14.1, 16.3, 16.5, 18.3, 18.4, 28.1, 31.0, 32.3, 32.7, 36.5, 47.3, 56.6, 58.5, 58.6, 61.2, 61.4, 157.3, 159.4, 170.0, 172.1, 174.0. $[\alpha]_D = +9.2$ (*c* 0.2, $CHCl_3$). Anal. Calcd for $C_{23}H_{39}N_5O_5$: C, 59.33; H, 8.44; N, 15.04. Found: C, 59.43; H, 8.46; N, 15.1.

6.23. 2-[(2*R*,5*S*)-6-Ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl]-*N*-[2-((2*R*,5*S*)-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl)ethyl]-acetamide 18b

The compound was obtained starting from **16b** following the procedure described for **18a** ^{1}H NMR: δ 0.87 (t, 6H, $J = 7$); 0.99 (d, 3H, $J = 7$); 1.01 (d, 3H, $J = 7$); 1.78 (m, 1H); 2.20 (m, 3H); 2.59 (q_{AB}, 2H, $J = 14.8$); 3.10 (m, 1H); 3.38 (m, 1H); 3.90 (m, 2H); 4.15 (m, 4H); 6.60 (m, 1H) 7.17 (br s, 1H); 7.23 (br s, 1H). ^{13}C NMR: δ 14.1, 14.2, 16.3, 16.8, 18.4, 18.6, 27.4, 29.2, 30.9, 31.4, 35.0, 40.4, 46.8, 58.2, 58.4, 58.6, 61.1, 61.3, 157.4, 157.5, 169.8, 173.8, 174.7. $[\alpha]_D = +12.0$ (*c* 0.9, $CHCl_3$). Anal. Calcd for $C_{24}H_{41}N_5O_5$: C, 60.10; H, 8.62; N, 14.60. Found: C, 60.32; H, 8.64; N, 14.57.

6.24. 2-[(2*R*,5*S*)-6-Ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl]-*N*-[2-((2*R*,5*S*)-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-3-oxopyrazin-2-yl)propyl]acetamide 19a

The compound was obtained starting from **17a** following the procedure described for **18a**. ^{1}H NMR: δ 0.92 (m,

12H); 1.26 (m, 6H); 1.46 (s, 3H); 1.50–2.10 (m, 4H); 2.28 (m, 2H); 2.46 (d, 1H, $J = 14.2$); 2.97 (d, 1H, $J = 14.2$); 3.1 (m, 1H); 3.37 (m, 1H); 3.90 (m, 1H); 3.92–4.20 (m, 6H); 6.16 (m, 1H); 6.23 (s, 1H); 6.36 (s, 1H). ^{13}C NMR: δ 14.0, 14.1, 16.1, 16.2, 18.2, 18.4, 25.1, 28.7, 30.7, 31.2, 32.1, 32.8, 38.8, 47.6, 57.1, 58.5, 58.6, 58.9, 61.2, 61.3, 157.1, 158.5, 169.8, 172.2, 173.8. $[\alpha]_D = +33.8$ (c 0.9, CH_3OH). Anal. Calcd for $C_{24}H_{41}N_5O_5$: C, 60.1; H, 8.62; N, 14.6. Found: C, 60.22; H, 8.64; N, 14.58.

6.25. 2-[(2*R*,5*S*)-6-Ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl]-*N*-[2-((2*R*,5*S*)-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-2-methyl-3-oxopyrazin-2-yl)propyl]acetamide 19b

The compound was obtained starting from **17b** following the procedure described for **18a**. ^{1}H NMR: δ 0.88 (m, 6H); 1.02 (m, 6H); 1.29 (m, 6H); 1.40 (s, 3H); 1.45 (s, 3H); 1.40–1.80 (m, 2H); 1.96 (m, 1H); 2.10 (s, 1H); 2.30 (m, 2H) 2.47 (d, 1H, $J = 14.7$); 2.98 (d, 1H, $J = 14.7$); 3.15 (m, 1H); 3.25 (m, 1H); 3.95 (m, 1H); 4.15 (m, 5H); 6.10 (m, 1H); 6.48 (s, 1H); 6.59 (s, 1H). ^{13}C NMR: δ 14.0, 14.1, 16.1, 16.2, 18.3, 18.4, 24.5, 28.6, 28.8, 30.7, 31.0, 38.9, 39.1, 47.5, 58.5, 58.6, 58.8, 59.8, 61.0, 61.2, 156.3, 157.1, 169.8, 173.9, 174.3. $[\alpha]_D = +5.5$ (c 0.9, $CHCl_3$). Anal. Calcd for $C_{25}H_{43}N_5O_5$: C, 60.83; H, 8.78; N, 14.19. Found: C, 61.1; H, 8.72; N, 14.2.

6.26. (2*S*,5*R*,11*R*,14*S*)-5,11-Diacetyldiamino-3,8,13-triazo-2,14-diisopropyl-5-methyl-4,7,12-trioxo-pentadecan-1,15-dioic acid diethylester 20a

HCl 0.5 M (30 mL) was added to a solution of **18a** (2.8 g, 6 mmol) dissolved in ethanol (50 mL) and the reaction mixture was stirred at room temperature, monitored by TLC. After about 12 h, ethanol was evaporated, the residue extracted with CH_2Cl_2 and the organic solution dried over $CaCl_2$. After filtration, triethylamine (0.84 mL, 6 mmol) was added to the organic solution cooled to −10 °C. Acetyl chloride (0.45 mL, 6.3 mmol) was added and after 10–15 min the cooling bath was removed. The reaction mixture, monitored by TLC, was stirred for 2–3 h and then the organic solvent evaporated under vacuum. The residue was dissolved in ethyl acetate, the organic solution washed with 2 M HCl and then dried over Na_2SO_4. After evaporation in vacuo to dryness, the residue was submitted to purification by silica gel chromatography eluting with hexane/ethyl acetate. The pure product was obtained as a wax in 90% yield. ^{1}H NMR: δ 0.96 (m, 12H); 1.24 (m, 6H); 1.70 (s, 3H); 1.71–298 (m, 2H) 2.03 (s, 6H); 2.22 (m, 2H); 2.64 (d, 1H, $J = 14$); 2.95 (d, 1H, $J = 14$); 3.10 (m, 1H); 3.65 (m, 1H); 4.18 (m, 5H); 4.50 (m, 2H); 6.66 (d, 1H, $J = 5.4$); 6.93 (m, 1H); 7.47 (s, 1H); 7.65 (d, 1H, $J = 6.8$); 8.23 (d, 1H, $J = 7.8$). ^{13}C NMR: δ 14.1, 17.7, 17.8, 19.0, 19.1, 23.0, 23.8, 30.8, 30.9, 33.6, 36.1, 42.8, 43.2, 50.6, 57.6, 57.8, 59.1, 61.0, 170.5, 171.2, 171.4, 171.6, 173.6. $[\alpha]_D = +38.5$ (c 0.7, $CHCl_3$). Anal. Calcd for $C_{27}H_{47}N_5O_9$: C, 55.37; H, 8.09; N, 11.96. Found: C, 55.55; H, 8.11; N, 11.95.

6.27. (2*S*,5*R*,11*R*,14*S*)-5,11-Diacetyldiamino-3,8,13-triazo-2,14-diisopropyl-5,11-dimethyl-4,7,12-trioxo-pentadecan-1,15-dioic acid diethylester 20b

The compound was obtained starting from **18b** following the procedure described for **20a**. ^{1}H NMR: δ 0.94 (d, 6H, $J = 7$); 0.98 (d, 6H, $J = 7$); 1.27 (t, 6H, $J = 7$); 1.61 (s, 3H); 1.67 (s, 3H); 2.06 (s, 3H); 2.08 (s, 3H); 2.22 (m, 4H); 2.58 (d, 1H, $J = 14.1$); 2.95 (d, 1H, $J = 14.1$); 3.23 (m, 2H); 4.18 (m, 4H); 4.43 (m, 2H); 6.68 (m, 1H); 7.41 (s, 1H); 7.61 (s, 1H); 7.66 (d, 1H, $J = 8.4$); 8.34 (d, 1H, $J = 8.1$). ^{13}C NMR: δ 14.1, 17.6, 19.0, 19.1, 23.0, 23.4, 23.9, 24.1, 30.9, 35.6, 37.8, 42.4, 57.6, 57.8, 59.0, 60.3, 61.1, 171.0, 171.1, 171.5, 171.6, 171.8, 173.6, 173.7. $[\alpha]_D = +53.3$ (c 0.4, CH_3Cl). Anal. Calcd for $C_{28}H_{49}N_5O_9$: C, 56.08; H, 8.24; N, 11.68. Found: C, 56.22; H, 8.27; N, 11.65.

6.28. (2*S*,5*R*,12*R*,15*S*)-5,12-Diacetyldiamino-3,8,14-triazo-2,15-diisopropyl-5-methyl-4,7,13-trioxo-hexadecan-1,16-dioic acid diethylester 21a

The compound was obtained starting from **19a** following the procedure described for **20a**. ^{1}H NMR: δ 0.98 (m, 12H); 1.30 (t, 6H, $J = 7.5$); 1.70 (s, 3H); 1.50–1.78 (m, 3H); 1.88 (m, 1H); 2.03 (s, 3H); 2.04 (s, 3H); 2.20 (m, 2H); 2.62 (d, 1H, $J = 14.1$); 2.95 (d, 1H, $J = 14.1$); 3.30 (m, 2H); 4.20 (m, 4H); 4.42 (dd, 1H, $J = 4.8$, 8.1); 4.48 (dd, 1H, $J = 5.1$, 8.4); 4.62 (m, 1H); 6.50 (m, 1H); 6.59 (d, 1H, $J = 8.1$); 6.91 (d, 1H, $J = 8.4$); 7.61 (s, 1H); 8.35 (d, 1H, $J = 8.1$). ^{13}C NMR: δ 14.0, 17.5, 17.7, 18.9, 22.6, 22.9, 23.9, 25.4, 29.9, 30.9, 38.8, 42.4, 52.4, 57.2, 57.6, 58.9, 61.0, 170.6, 170.9, 171.0, 171.5, 172.0, 173.7. $[\alpha]_D = +34.1$ (c 0.6, $CHCl_3$). Anal. Calcd for $C_{28}H_{49}N_5O_9$: C, 56.08; H, 8.24; N, 11.68. Found: C, 55.92; H, 8.21; N, 11.68.

6.29. (2*S*,5*R*,12*R*,15*S*)-5,12-Diacetyldiamino-3,8,14-triazo-2,15-diisopropyl-5,12-dimethyl-4,7,13-trioxo-hexadecan-1,16-dioic acid diethylester 21b

The compound was obtained starting from **19b** following the procedure described for **20a**. ^{1}H NMR: δ 0.98 (m, 12H); 1.23 (m, 6H); 1.40–1.80 (m, 3H); 1.57 (s, 3H); 1.66 (s, 3H); 2.00 (s, 3H); 2.05 (s, 3H); 2.20–2.50 (m, 3H); 2.52 (d, 1H, $J = 14.1$); 2.92 (d, 1H, $J = 14.1$); 3.20 (m, 1H); 3.35 (m, 1H); 4.20 (m, 5H); 4.44 (m, 1H); 6.43 (m, 1H); 6.68 (s, 1H); 7.04 (d, 1H, $J = 8.4$); 7.79 (s, 1H); 8.36 (d, 1H, $J = 8.4$). ^{13}C NMR: δ 14.0, 17.4, 17.7, 19.0, 20.9, 22.9, 23.3, 23.6, 23.8, 23.9, 30.7, 30.9, 34.2, 38.9, 42.5, 57.5, 59.0, 60.2, 60.3, 61.1, 61.2, 170.3, 170.9, 171.1, 171.5, 171.9, 173.7, 173.8. $[\alpha]_D = +28.3$ (c 0.9, $CHCl_3$). Anal. Calcd for $C_{29}H_{51}N_5O_9$: C, 56.75; H, 8.38; N, 11.41. Found: C, 56.66; H, 8.35; N, 11.45.

6.30. (3*S*,6*S*)-1-Benzyl-3-methyl-3-(3-dibenzylaminopropyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 22

The compound was obtained by alkylating the diastereomeric mixture **10a** + **12** with CH_3I and following the proce-

dure above reported for the alkylation of lactim **1**. After silica gel chromatography, the product was isolated as an oil in 90% yield. ^{1}H NMR: δ 0.82 (d, 3H, $J = 7$); 1,05 (d, 3H, $J = 7$); 1.11 (t, 3H, $J = 7$); 1.38 (s, 3H); 2.05 (m, 1H); 2.20 (m, 2H); 2.70 (m, 2H); 3.62 (s, 4H); 3.69 (d, 1H, $J = 2.4$); 3.77 (m, 2H); 3.94 (d, 1H, $J = 15$); 5.50 (d, 1H, $J = 15$); 7.18–7.45 (m, 15ArH). ^{13}C NMR: δ 14.0, 17.1, 20.6, 26.8, 28.9, 29.9, 38.0, 46.5, 47.9, 58.2, 59.3, 60.5, 60.9, 126.5, 127.5, 128.0, 128.6, 136.3, 139.9, 155.0, 173.0. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.31. (3*S*,6*S*)-1-Benzyl-3-methyl-3-(3-dibenzylaminobutyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 23

The compound was obtained starting from the diastereomeric mixture **11a** + **13** and following the procedure described for the alkylation of lactim **1**. After silica gel chromatography, the product was isolated as an oil in 90% yield. ^{1}H NMR: δ 0.86 (d, 3H, $J = 6.9$); 1.06 (d, 3H, $J = 6.9$); 1.21 (t, 3H, $J = 7.2$); 1.38 (s, 3H); 1.60–1.90 (m, 4H); 2.20 (m, 1H); 2.42 (t, 1H, $J = 6.9$); 3.54 (s, 4H); 3.71 (m, 1H); 3.96 (d, 1H, $J = 16.5$); 4.05 (m, 2H); 4.48 (d, 1H, $J = 16.5$); 7.30 (m, 15ArH). ^{13}C NMR: δ 13.9, 17.1, 20.5, 21.4, 29.0, 29.7, 38.7, 46.5, 53.3, 57.9, 60.0, 60.4, 60.9, 126.5, 127.3, 127.7, 127.9, 128.5, 136.3, 139.6, 154.9, 173.0. $[\alpha]_D = +5.3$ (*c* 1.2, $CHCl_3$). Anal. Calcd for $C_{34}H_{43}N_3O_2$: C, 77.68; H, 8.24; N, 7.99. Found: C, 77.98; H, 8.27; N, 7.95.

6.32. (3*S*,6*S*)-1-Benzyl-3-methyl-3-(3-aminopropyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 24

The product, isolated as an oil, was obtained in practically quantitative yield starting from **22** following the procedure used for **14a**. ^{1}H NMR: δ 0.93 (d, 3H, $J = 7$); 1.09 (d, 3H, $J = 7$); 1.26 (t, 3H, $J = 7$); 1.45 (s, 3H); 1.93 (br s, 2H); 2.0 (m, 2H); 2.25 (m, 1H); 2.95 (m, 2H); 3.75 (d, 1H, $J = 2.4$); 3.95 (d, 1H, $J = 15$); 4.07 (m, 2H); 5,50 (d, 1H, $J = 15$); 7.18–7.42 (m, 5ArH). ^{13}C NMR: δ 13.7, 16.8, 20.1, 28.9, 29.4, 37.6, 43.7, 46.2, 59.4, 60.2, 60.7, 127.1, 127.5, 128.3, 135.9, 154.9, 172.4. $[\alpha]_D = +13.2$ (*c* 1.2, $CHCl_3$). Anal. Calcd for $C_{19}H_{29}N_3O_2$: C, 68.85; H, 8.82; N, 12.68. Found: C, 68.91; H, 8.8; N, 12.64.

6.33. (3*S*,6*S*)-1-Benzyl-3-methyl-3-(3-aminobutyl)-5-ethoxy-1,6-dihydro-6-isopropylpyrazin-2-(3*H*)-one 25

The oily product, synthesized starting from **23** following the procedure used for **14a**, was recovered in practically quantitative yield. ^{1}H NMR: δ 0.91 (d, 3H, $J = 7$); 1.08 (d, 3H, $J = 7$); 1.25 (t, 3H, $J = 7$); 1.42 (s, 3H); 1.4–2.0 (m, 6H); 2.20 (m, 1H); 2.73 (t, 2H, $J = 7$); 3.75 (m, 1H); 3.94 (d, 1H, $J = 15$); 4.10 (m, 2H); 5.50 (d, 1H, $J = 15$); 7.15–7.40 (m, 5ArH). ^{13}C NMR: δ 13.7, 16.7, 20.2, 27.7, 28.9, 29.4, 38.1, 41.9, 46.2, 59.7, 60.2, 60.7, 127.1, 127.5, 128.2, 135.9, 154.8, 172.6. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.34. 2-[(2*R*,5*S*)-4-Benzyl-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl]-*N*-[2-((2*S*,5*S*)-4-benzyl-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl)ethyl]acetamide 26

The compound was obtained by reacting **24** with the activated ester **9** following the procedure reported for **16a**. The product was recovered as an oil in 85% yield after silica gel chromatography eluting with hexane/ethyl acetate. ^{1}H NMR: δ 0.9 (d, 6H, $J = 7$); 1.05 (d, 3H, $J = 7$); 1.08 (d, 3H, $J = 7$); 1.23 (t, 3H, $J = 7$); 1.26 (t, 3H, $J = 7$); 1.44 (s, 3H); 1.54 (s, 3H); 1.9 (m, 1H); 2.2 (m, 3H); 2.58 (d, 1H, $J = 14.6$); 3.0 (d, 1H, $J = 14.6$); 3.42 (m, 2H); 3.76 (d, 1H, $J = 2.5$); 3.83 (d, 1H, $J = 2.5$); 3.94 (d, 1H, $J = 15$); 4.12 (m, 4H); 4.18 (d, 1H, $J = 15$); 5.34 (d, 1H, $J = 15$); 5.5 (d, 1H, $J = 15$); 6.8 (m, 1H); 7.32 (m, 10ArH). ^{13}C NMR: δ 13.7, 13.8, 16.8, 16.9, 20.1, 20.2, 28.3, 29.3, 29.5, 35.0, 39.4, 46.4, 46.9, 48.4, 58.8, 59.4, 60.5, 60.8, 61.3, 127.0, 127.3, 127.6, 127.8, 128.3, 128.4, 135.7, 135.8, 155.5, 156.5, 169.3, 171.5, 172.3. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.35. 2-[(2*R*,5*S*)-4-Benzyl-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl]-*N*-[2-((2*S*,5*S*)-4-benzyl-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl)propyl]acetamide 27

The compound was obtained by reacting **25** with the activated ester **9** and following the procedure reported for **16a**. 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 0.91 (m, 6H); 1.09 (d, 6H, $J = 7$); 1.26 (m, 6H); 1.40 (s, 3H); 1.57 (s, 3H); 1.5–2.0 (m, 4H); 2.21 (m, 2H); 2.84 (q_{AB}, 2H, $J = 14.4$); 3.18 (m, 1H); 3.24 (m, 1H); 3.75 (m, 1H); 3.83 (m, 1H); 3.93 (d, 1H, $J = 15$); 3.98–4.22 (m, 5H); 5.39 (d, 1H, $J = 15$); 5.52 (d, 1H, $J = 15$); 6.55 (m, 1H); 7.3 (m, 10ArH). ^{13}C NMR: δ 13.9, 14.0, 17.0, 17.2, 20.4, 24.2, 28.1, 29.0, 29.6, 29.7, 38.3, 39.3, 46.3, 47.1, 48.5, 59.1, 59.8, 60.5, 60.8, 60.9, 61.5, 127.3, 127.4, 127.8, 127.9, 128.5, 128.6, 135.9, 136.0, 155.1, 156.7, 169.7, 171.6, 172.7. $[\alpha]_D = +5.4$ (*c* 0.8, $CHCl_3$). Anal. Calcd for $C_{39}H_{55}N_5O_5$: C, 69.51; H, 8.23; N, 10.39. Found: C, 69.75; H, 8.26; N, 10.4.

6.36. 2-[(2*R*,5*S*)-6-Ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl]-*N*-[2-((2*S*,5*S*)-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl)-ethyl]acetamide 28

The product was obtained starting from **26** following the procedure used for **18a**. ^{1}H NMR: δ 0.83 (d, 3H, $J = 7$); 0.87 (d, 3H, $J = 7$); 0.98 (d, 3H, $J = 7$); 1.06 (d, 3H, $J = 7$); 1.26 (t, 3H, $J = 7$); 1.29 (t, 3H, $J = 7$); 1.35 (s, 3H); 1.43 (s, 3H); 1.98 (m, 2H); 2.35 (m, 3H); 2.98 (d, 1H, $J = 14.6$); 3.18 (m, 1H); 3.35 (m, 1H); 3.98 (m, 1H); 4.11 (m, 5H); 5.85 (br s, 1H); 6.40 (br s, 1H). ^{13}C NMR: δ 13.9, 14.0, 15.9, 16.1, 18.1, 18.3, 27.8, 28.6, 30.4, 30.5, 35.5, 38.9, 47.3, 58.0, 58.4, 58.5, 59.3, 61.0, 61.3, 156.8, 157.2, 169.6, 173.9, 174.4. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.37. 2-[(2*R*,5*S*)-6-Ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-3-oxopyrazin-2-yl]-*N*-[2-((2*S*,5*S*)-6-ethoxy-2,3,4,5-tetrahydro-5-isopropyl-2-methyl-2-methyl-3-oxopyrazin-2-yl)propyl]acetamide 29

The product was obtained starting from **27** and following the procedure used for **18a**. ^{1}H NMR: δ 0.85 (m, 6H); 1.0 (m, 6H); 1.27 (m, 3H); 1.34 (s, 6H); 1.44 (s, 3H); 1.40–1.70 (m, 2H); 2.0 (m, 2H); 2.30 (m, 2H); 2.47 (d, 1H, $J = 14.6$); 2.94 (d, 1H, $J = 14.6$); 3.08 (m, 1H); 3.28 (m, 1H); 3.90–4.30 (m, 6H); 6.05 (s, 1H); 6.10 (m, 1H); 6.48 (br s, 1H). ^{13}C NMR: δ 13.8, 13.9, 16.0, 16.1, 18.0, 18.1, 24.4, 28.0, 28.5, 30.1, 30.6, 37.6, 38.8, 47.1, 57.7, 58.3, 59.5, 60.0, 60.7, 60.8, 156.1, 157.1, 169.6, 173.7, 174.5. $[\alpha]_D = -19.9$ (*c* 0.7, $CHCl_3$). Anal. Calcd for $C_{25}H_{43}N_5O_5$: C, 60.83; H, 8.78; N, 14.19. Found: C, 60.95; H, 8.77; N, 14.18.

6.38. (2*S*,5*R*,11*S*,14*S*)-5,11-Diacetyldiamino-3,8,13-triazo-2,14-diisopropyl-5,11-dimethyl-4,7,12-trioxo-pentadecan-1,15-dioic acid diethylester 30

The product was obtained starting from **28** following the procedure used for **20a** ^{1}H NMR: δ 0.95 (m, 12H); 1.27 (t, 3H, $J = 7$); 1.28 (t, 3H, $J = 7$); 1.60 (s, 3H); 1.67 (s, 3H); 2.05 (s, 6H); 2.10–2.37 (m, 3H); 2.49 (d, 1H, $J = 14.1$); 2.91 (d, 1H, $J = 14.1$); 3.15 (m, 2H); 3.35 (m, 1H); 4.19 (m, 4H); 4.42 (m, 2H); 6.66 (m, 1H); 7.36 (s, 1H); 7.39 (d, 1H, $J = 8.2$); 7.72 (s, 1H); 8.33 (d, 1H, $J = 8.7$). ^{13}C NMR: δ 13.9, 17.4, 17.6, 18.8, 20.6, 22.7, 23.1, 23.4, 23.7, 30.5, 30.7, 35.2, 35.9, 41.9, 45.8, 57.6, 58.8, 59.6, 60.9, 60.9, 170.9, 171.0, 171.2, 171.3, 171.6, 173.6, 173.8, 173.9. The product was not isolated in sufficiently pure form to measure the specific rotation.

6.39. (2*S*,5*R*,12*S*,15*S*)-5,12-Diacetyldiamino-3,8,14-triazo-2,15-diisopropyl-5,12-dimethyl-4,7,13-trioxo-hexadecan-1,16-dioic acid diethylester 31

The product was obtained starting from **29** and following the procedure used for **20a**. ^{1}H NMR: δ 1.00 (m, 12H); 1.31 (m, 6H); 1.66 (s, 3H); 1.40–1.90 (m, 3H); 2.04 (s, 3H); 2.06 (s, 3H); 2.25 (m, 2H); 2.50 (m, 1H); 2.81 (d, 1H, $J = 14.7$); 2.99 (d, 1H, $J = 14.7$); 3.18 (m, 1H); 3.35 (m, 1H); 4.25 (m, 4H); 4.45 (dd, 1H, $J = 4.8$, 8.1); 4.49 (dd, 1H, $J = 5.1$, 8.4); 6.7 (m, 1H); 6.80 (s, 1H); 6.98 (d, 1H, $J = 8.4$Hz); 7.60 (s, 1H); 8.09 (d, 1H, $J = 8.1$). ^{13}C NMR: δ 14.0, 17.6, 17.8, 18.9, 19.0, 23.0, 23.3, 23.4, 23.0, 24.0, 30.7, 30.9, 33.1, 38.9, 42.2, 57.4, 57.7, 58.7, 60.2, 61.1, 61.4, 170.1, 170.8, 170.9, 171.6, 172.3, 173.7, 174.2. $[\alpha]_D = +26.1$ (*c* 0.5, $CHCl_3$). Anal. Calcd for $C_{29}H_{51}N_5O_9$: C, 56.75; H, 8.38; N, 11.41. Found: C, 56.87; H, 8.39; N, 11.38.

7. Computational details

The 3D molecular structures were built using the CORINA package.[9] All calculations were performed at the molecular mechanics (MM) level with the AMBER 8.0 program.[10] Simulations were carried out using the Gaff force field.[11] The AM1-BCC method,[12] as implemented in the Antechamber package, was employed to assign charges to atoms.[13] Solvation effects were taken into account using the Generalized Born Model.[14] Thus, dynamics were carried out with a dielectric constant $\varepsilon = 4.9$ to simulate the electrostatic effects of chloroform (the solvent where ^{1}H NMR data have been recorded). To locate the lowest energy structure, without being trapped in local minima, we first employed a preliminary QMD simulation where the molecules were heated from 0 to 600 K in 100 ps and then, a trajectory of 10 ns was carried out at constant temperature (600 K) and constant pressure (1 atm) with an integration step of 2 fs. The SHAKE algorithm[15] was used to constrain the stretching of bonds involving hydrogen atoms. The coordinates of the pseudopeptides were saved on a trajectory file every 10 ps, giving a total of 1000 conformations for further analysis. Each of the so obtained structures was energy minimized till the root mean square of the Cartesian elements of the gradient was less than 0.001 kcal mol^{-1} using a full conjugate gradient minimization and the GB/SA model.[14,16] This preliminary simulation provided the best starting structure for a new molecular dynamics at 300 K to identify intramolecular hydrogen bonds and examine the conformational space of the pseudopeptides at ambient temperature. These dynamics were carried out for 5 ns using a time step of 0.002 ps (using SHAKE algorithm[15] to constrain the stretching of bonds involving hydrogen atoms) and writing the coordinates every 1 ps on a trajectory file. We analyzed this file with the 'ptraj' package (an AMBER module)[10] to obtain an estimate of the lifetime of every H-bond during the simulation. The lifetime is expressed as a percentage of the existence of the H-bond during the whole simulation (distance between acceptor and donor shorter than 4 Å, and a bond angle larger than 90°). To identify and visualize the most important conformations of the peptides, we carried out a cluster analysis. For this purpose, the MMTSB toolset was used.[17] We clustered the conformations obtained from the dynamics on the basis of structural similarity (using kclust and a fixed radius clustering of 2.0 Å on Cartesian coordinate RMSD of heavy atoms and hydrogen atoms involved in hydrogen bonds). Clusters were grouped together, if possible, on the basis of the similarity of the hydrogen bond pattern. For each set of clusters the most populated structures have been selected as the representative conformations of each pseudopeptide.

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