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Unusual peptides containing the 2,6-diaminopimelic acid framework: Stereocontrolled synthesis, X-ray analysis, and computational modelling. Part 2☆

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Abstract—The stereocontrolled synthesis of peptides **6**, **9** and **14**, structural variants of 2,6-diaminopimelic acid, was carried out starting from the chiral synthon **1**, easily obtained from L-valine. The configuration of the introduced stereogenic centres has been assigned on the basis of ^{1}H NMR spectroscopic data. X-Ray crystal structure and conformation analysis of **5** are also reported.

1. Introduction

In a recent paper[1] we described a new stereoselective approach to unusual tripeptides C-termini at both ends of the chain, containing the 2,6-diaminoipimelic acid (2,6-DAP) framework. We are interested in these structural derivatives of 2,6-DAP, which is biosynthesized in bacteria and higher plants, for their potential antibacterial and herbicide activity.[2] In fact, both (2*R*,6*R*)-DAP and (2*R*,6*S*)-DAP are involved in the biosynthetic conversion of pyruvate and L-aspartate to the amino acid L-lysine, necessary for the growth of Gram-(+) and many Gram-(−) bacteria. Both the L-lysine and (2*R*,6*S*)-DAP function as cross-linking constituents in the cell wall peptidoglycan of bacteria.[3] The structural analogues of 2,6-DAP can then function as inhibitors of biosynthetic formation or metabolism of this compound offering promising biological activity as antibacterial and herbicide agents. Furthermore, it is interesting to note that some tri- and tetrapeptides incorporating the 2,6-DAP skeleton conjugated with lauric or palmitic acid show biological activity as immuno-adjuvants.[4] Therefore, we undertook the asymmetric synthesis of peptides more complex than the 2,6-DAP derivatives previously described,[1] i.e. containing a proline residue fused to a diketopiperazine ring. The reason for our interest in the stereocontrolled synthesis of such peptide-like structures is because some natural products, containing a 6,5-fused ring system,[5] exhibit a wide range of biological activity (for instance, immunomodulators, antitumors, antibiotics).

This study has been complemented by X-ray analysis and extended molecular modelling conformational searches in solution and DFT computational investigations.

2. Synthesis and stereochemical assignments

Herein we have followed a strategy which makes use of the chiral synthon **1**, a mono-lactim ether easily synthesized from L-valine.[1] Deprotonation of **1** with LHMDS at −78°C, followed by alkylation with 0.5 equiv. of 1,3-diiodopropane, afforded, in 87% yield, the diastereomer **2** which was purified by silica gel chromatography, as previously described.[1] The largely prevalent diastereomer **2** was then converted into the corresponding enolate and then alkylated with 1,3-dichloropropane in good yield with a total 1,4-*trans* induction (>98%) with respect to the isopropyl group, as previously observed for similar sustrates.[1,6] By submitting the intermediate **3** to the Finkelstein reaction (NaI in refluxing acetone), the bicyclic derivative **4** was also isolated in good yield (Scheme 1).

The removal of the benzyl group from the intermediate **4** was achieved through the Birch reduction. However, by performing the reaction under the usual conditions (i.e. the addition of the substrate to an excess of Li

☆ For Part 1, see Reference 1.

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dissolved in liquid ammonia) the reaction gave in large prevalence compound **5′**. After some attempts complete transformation to product **5** was achieved by changing the usual Birch reaction methodology. The modification consisted of the addition of only 1 equiv. of Li to a solution of the substrate. It is noteworthy that even a slight excess of Li caused the formation of **5′** due to the further reduction of C=O group followed by the base catalysed dehydration (Scheme 1).

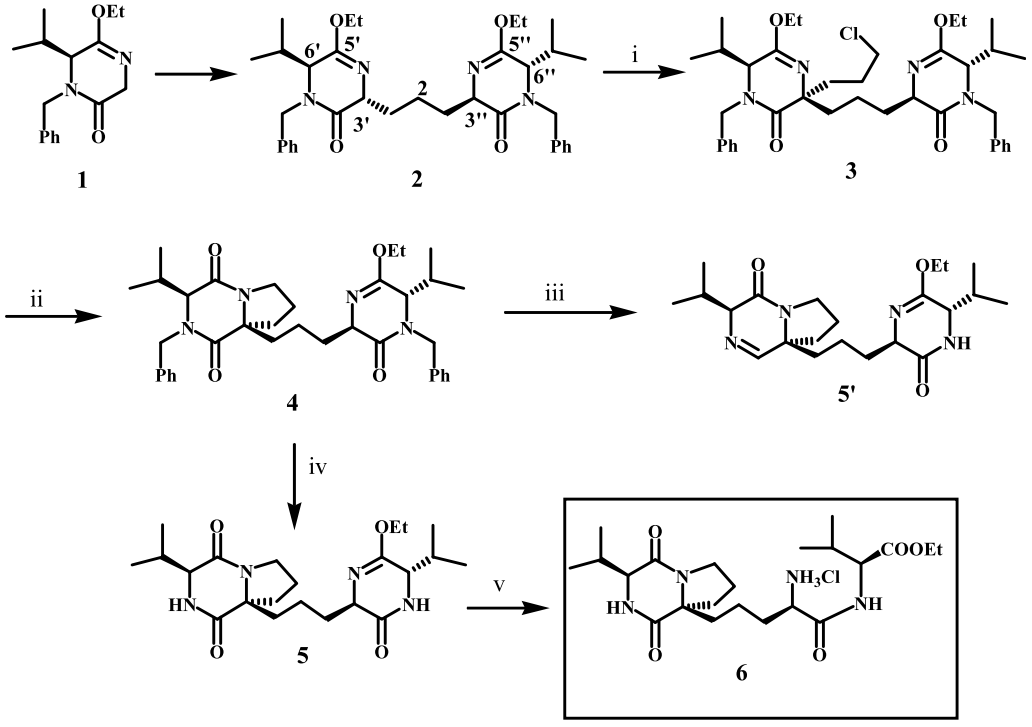
The proposed structure of the unexpected compound **5′** was ascertained by means of ^{13}C NMR, ^{1}H NMR and HPLC–MS spectra. Indeed, the following analytical data was available: a) In the low field region of the ^{13}C NMR spectrum, four signals were observed (158.5, 161.2, 167.5 and 171.8 ppm) attributable to either a C=O or a C=N group. The Attached Proton Test (APT) also showed that the signal at 161.2 ppm is due to a carbon atom bonded to an odd number of protons; b) the ^{1}H NMR showed a doublet proton signal at 7.9 ppm, split ($J=1.8$ Hz) by the H-6′ resonating at 3.99 ppm with the small J value being attributable to an allylic coupling constant; c) the HPLC–MS exhibited a molecular ion peak at mass 404, i.e. 16 unities lesser than the molecular ion showed by **5**. All these findings are consistent with the proposed structure of **5′**.

The acid cleavage of **5**, performed under mild conditions and in good yield, afforded the final product **6** which contained a bicyclic moiety analogous to that synthesized by Davies et al.[5] starting from a bis-lactim ethylether (Schollkopf's auxiliary).

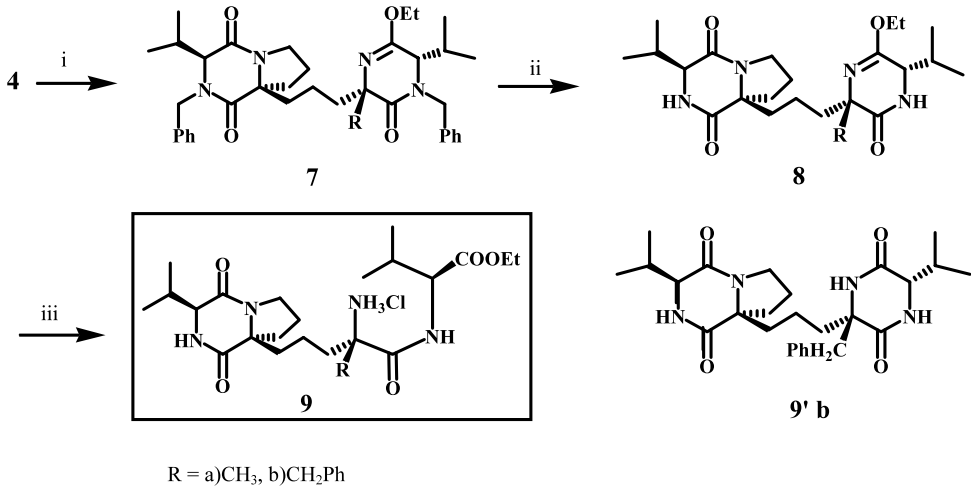
The intermediate **4**, alkylated with iodomethane or benzylbromide, gave **7** in a good yield and with total regio- and stereoselectivity (Scheme 2). The 1,4-*trans*-induction was demonstrated by comparing the ^{1}H NMR spectra of derivatives **7a** and **7b**, on the basis of the upfield shift induced on the H-6″ by the shielding effect of the phenyl ring of the benzyl group introduced on C-3″. Indeed, as already observed in analogous substrates,[7] the phenyl ring preferentially lies internal to the heterocyclic monolactim ether (i.e. the preferred 'aryl inside' arrangement is adopted) causing significant shielding on the *cis*-substituent at C-6″. This shielding of about 0.7 ppm seen for **7b**, with respect to the derivative **7a**, provided clear evidence of the *cis*-relationship between (C-3″)-CH_2Ph and (C-6″)-H. The absolute configuration of C-3″ follows on from the note (*S*)-configuration of the stereocentre at C-6″.

The monolactim ethers **7a**,**b** were submitted to the debenzylation affording **8a**,**b** in good yields. The final products **9a**,**b** were then obtained after acid cleavage in mild condition. It is noteworthy that while **9a** was isolated in good yield, the acid hydrolysis of **8b** furnished **9b** together with a not too negligible amount of **9′b** (Scheme 2).

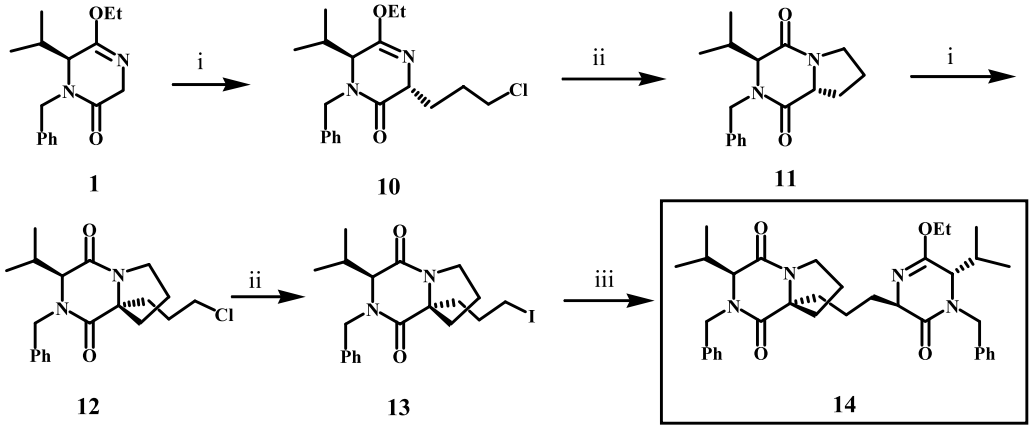
Diastereomeric derivatives with an opposite configuration at the stereocentre C-3′, in comparison to the compounds described above, were obtained by using the following alternative strategy. While still starting from synthon **1**, the protocol consisted of the formation of the fused ring **11**, followed by the alkylation with



Scheme 1. *Reagents and conditions*: (i) LHMDS, THF, at −78°C, then Cl-$(CH_2)_3$-Cl; (ii) NaI in refluxing acetone; (iii) Birch reduction by using a large excess of Li; (iv) Birch reduction by adding an equimolar amount of Li; (v) aqueous HCl 0.5 M, EtOH at room temperature.



Scheme 2. *Reagents and conditions*: (i) LHMDS, THF, at −78°C, then CH_3I or $PhCH_2Br$; (ii) Birch reduction using an equimolar amount of Li; (iii) aqueous HCl 0.5N, EtOH at room temperature.



Scheme 3. *Reagents and conditions*: (i) LHMDS, THF, at −78°C, then I-$(CH_2)_3$-Cl; (ii) NaI in refluxing acetone; (iii) Li-enolate of **1**, THF, at −78°C.

3-chloroiodopropane, which furnished **12**. This intermediate, after conversion into **13**, was reacted with the Li-enolate of **1** to give **14**, it being the epimer of **4** at the C-3′ stereocentre (Scheme 3).

The alkylation of bicyclic system **11** occurred with total regio- and diastereoselectivity giving the intermediate **12** in a good yield. The observed regioselection was due to the greater acidity of the (C-3)-H proton in comparison to the (C-6)-H one. In fact, computational investigation,[8] performed on the simple model **11′**, showed that the anion **11′A** was 1.5 kcal/mol more stable than the **11′B** one (Fig. 1).

3. ^{1}H NMR and IR studies

Compound **5** showed a linear relationship between the chemical shift of the amide protons (δ_{NH}) and the temperature: the temperature coefficients ($\Delta\delta_{NH}/\Delta T$) calculated for both amide protons in 1 mM $CDCl_3$ solution were substantially coincident, i.e. −12.8 and

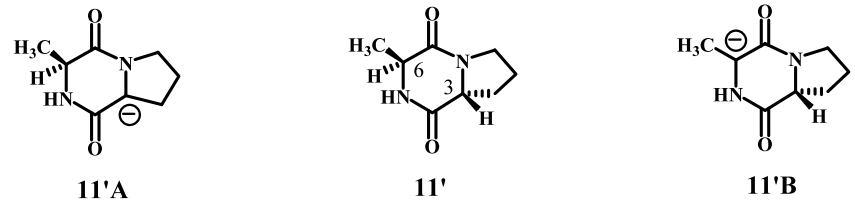


Figure 1.

−12.45 ppb/°C (see Fig. 2). The large temperature coefficients, the chemical shift values lower than 7 ppm for both amide protons in diluted solution at rt, and the decrease in chemical shifts observed from going from concentrated (30 mM, $\delta_{NH}=8.38$ and 8.42 ppm) to diluted (1 mM, $\delta_{NH}=6.1$ and 6.17 ppm) $CDCl_3$ solutions all suggested the existence of an equilibrium between a non hydrogen bonded and a hydrogen bonded state.[9] Thus, taking into account all the ^1H NMR parameters, it is reasonable to assume that the intermolecular aggregation, involving the amide proton and the carbonyl oxygen, is preferred to the intramolecular hydrogen bond which could produce a ten-membered ring, i.e. a non-peptide mimetic β-turn structure.

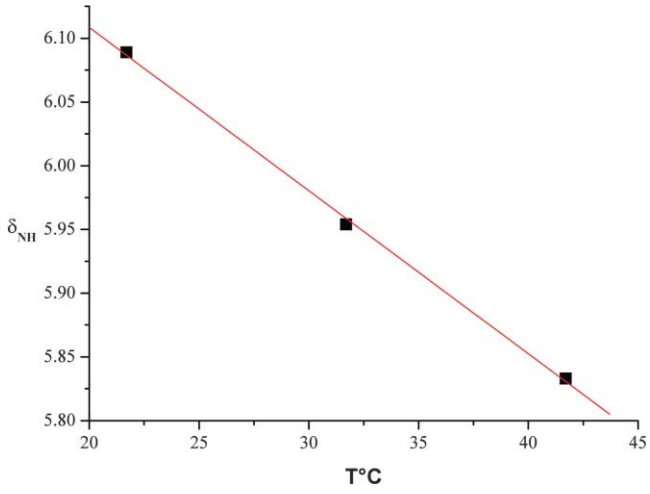


Figure 2. Linear relationship of the chemical shift of one amide proton (δ_{NH}) versus the temperature.

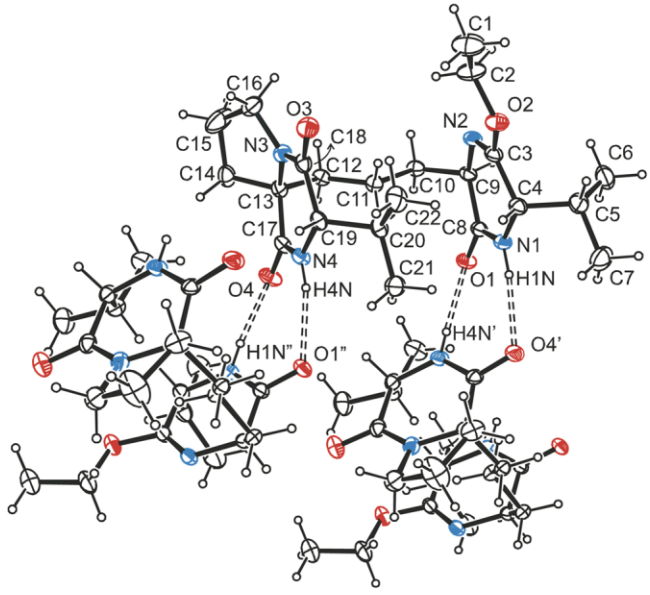


Figure 3. ORTEP drawing of **5** (thermal ellipsoids at 30% probability) showing the intermolecular N–H···O hydrogen bonds that each molecule establishes with two neighbours.

The absence of an intramolecular hydrogen bond was supported by the IR spectra of **5** in $CHCl_3$. In fact, a 30 mM solution displayed two different adsorptions for the NH stretching, indicating an equilibrium between a non hydrogen bonded and a hydrogen bonded structure: a narrow band at 3394 cm^{-1}, typical of a free hydrogen bond structure, and a broad band at 3197 cm^{-1} attributable to a hydrogen bonded stretching vibration. As the concentration was reduced to 3 mM, the band at 3197 cm^{-1} disappeared, thus showing that the hydrogen bonding was intermolecular rather than intramolecular.[9]

4. X-Ray analysis

The solid state molecular structure of **5** is shown in Figure 3 with the relevant bond lengths reported in Table 1. In this structure the diketopiperazine ring adopts an almost planar conformation (maximum deviation from planarity ca. 0.1 Å) which is different to what has been observed, for example, in the dipeptide *cyclo*(L-Phe-L-Pro)[10] in which the diketopiperazine ring is in a boat conformation, as it has been found in the majority of these heterocyclic systems. The proline ring adopts a slightly distorted envelope conformation. The main feature of compound **5** is the orientation of the six-membered rings that are facing each other being almost parallel (the angle between the rings being ca. 7°) despite the great degree of flexibility of the aliphatic bridge. The molecular conformation seems dictated by the minimisation of the molecular volume. The absolute configuration of the stereogenic centres at C(4) and C(19) is *S*, while at C(9) and C(13), it is *R* (see Section 2).

In the crystal packing each molecule is engaged in four intermolecular N–H···O hydrogen bonds N(1)–H(1N) 0.86(2), H(1N)···O(4) 2.06(2), N(1)···O(4) 2.907(2), N(4)–H(4N) 0.84(2), H(4N)···O(1) 2.04(2), N(1)···O(4) 2.871(2) Å, using the amidic hydrogen and the oxygen of the CO group (Fig. 3).

Table 1. Comparison between bond lengths (Å) of **5** obtained from the X-ray study and calculated (in square bracket)

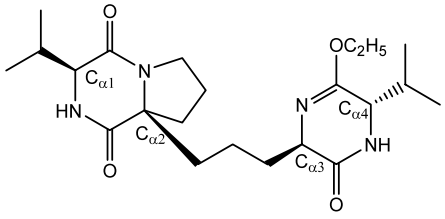
N(1)–C(8)	1.333(2) [1.37]	C(13)–N(3)	1.459(2) [1.48]
N(1)–C(4)	1.456(2) [1.46]	N(3)–C(18)	1.338(2) [1.36]
C(3)–C(4)	1.502(2) [1.52]	C(18)–O(3)	1.227(2) [1.23]
C(4)–C(5)	1.548(3) [1.56]	C(18)–C(19)	1.523(3) [1.53]
N(2)–C(3)	1.252(2) [1.27]	C(19)–N(4)	1.460(2) [1.46]
N(2)–C(9)	1.457(2) [1.46]	C(17)–N(4)	1.329(2) [1.36]
C(8)–C(9)	1.513(2) [1.53]	C(17)–O(4)	1.235(2) [1.23]
C(8)–O(1)	1.242(2) [1.23]	C(17)–C(13)	1.507(2) [1.53]
C(3)–O(2)	1.357(2) [1.35]	C(16)–N(3)	1.470(3) [1.47]
O(2)–C(2)	1.437(3) [1.44]	C(15)–C(16)	1.475(4) [1.55]
C(2)–C(1)	1.470(3) [1.52]	C(14)–C(15)	1.499(4) [1.54]
C(13)–C(14)	1.545(3) [1.55]	C(10)–C(11)	1.531(2) [1.53]
C(12)–C(13)	1.549(2) [1.55]	C(9)–C(10)	1.530(3) [1.54]
C(11)–C(12)	1.510(2) [1.53]	C(5)–C(6)	1.506(4) [1.54]
C(5)–C(7)	1.537(3) [1.54]	C(19)–C(20)	1.554(3) [1.56]
C(20)–C(21)	1.508(3) [1.54]	C(20)–C(22)	1.511(3) [1.54]

5. Molecular modelling: conformational analysis

A complete, extensive, unconstrained conformational analysis of compound **5** was performed by using an AMBER* force field[11] and the Monte Carlo[12] conformational search (MC/EM) varying all the degrees of freedom, including $CHCl_3$ as the solvent. A relatively non-polar solvent was chosen in order not to interfere with the hydrogen bonding of the amide protons. All the conformers within the energy gap of 6 kcal/mol were kept, and subsequently only those that lie below 3.6 kcal/mol were fully analysed (a total number of 123 conformers). The turn propensity of the minimum energy conformations was assessed by computing and analyzing geometric parameters, specifically the distance between the capping groups on the N- and C-termini (d_α), the virtual torsion angles β ($C_{\alpha i}$–$C_{\alpha i+1}$–$C_{\alpha i+2}$–N_{i+3}), and all the parameters indicative of hydrogen bonding (β- or γ-turns involve d_α <7 Å and $-60° < \beta < 60°$). Only 23 conformers (22.7%) satisfied these structural requisites, but none of them showed any evidence of intramolecular hydrogen bonds indicative of a classical β or γ turn. For these reasons, all these structures can be viewed as open turns. The most significant conformers found for compound **5** (i.e. all the conformers within $\Delta E = 1.5$ kcal/mol) are reported in Table 2. Among these the conformers 1, 3 and 10 represent open turns.

The low energy conformers were then compared to the X-ray structures observed. Most remarkably, the latter is very close to the modelled lowest energy structure (conformer 1 in Table 2), thus confirming the preference for this molecule to intermolecular hydrogen bonding. The geometry of this conformer was then refined via optimisation at the B3LYP/6-31G* level of theory in order to obtain a more accurate structure. The similarity with the observed X-ray structure is evident by the superposition plot of the calculated and X-ray determined geometry of **5** depicted in Figure 4 and by comparison of the bond distances (Table 1) in the calculated model and in the solid state structure. This agreement validates the joint MM/DFT computational approach used here for conformational investigation.

Table 2. Low energy conformations for compound **5**



Structural significative parameters, indexes of turns:

β	$C_{\alpha1}$-$C_{\alpha2}$-$C_{\alpha3}$-N(=)	$-60° < \beta < 60°$	d_α(1-4)<7 Å
β_1	$C_{\alpha1}$-$C_{\alpha2}$-$C_{\alpha3}$-N(H)	$-60° < \beta_1 < 60°$	d_α(1-3)<7 Å
β_2	$C_{\alpha4}$-$C_{\alpha3}$-$C_{\alpha2}$-N(H)	$-60° < \beta_2 < 60°$	d_α(2-4)<7 Å

Conformer	E (kcal/mol)	Population[a] (%)	d_α(1-4) (Å)	d_α(1-3) (Å)	d_α(2-4) (Å)	β	β_1	β_2
1	0.00	15.7	5.513	5.369	6.446	−53.2	36.4	−23.6
2	0.40	7.99	7.739	5.750	7.895	47.8	136.6	38.2
3	0.43	7.59	6.216	6.612	6.080	−54.1	35.7	−24.9
4	0.60	5.70	9.048	6.964	7.747	−64.0	22.0	−73.6
5	0.69	4.89	8.320	7.737	6.139	48.1	137.0	38.6
6	0.80	4.06	9.820	8.977	7.774	148.1	−122.7	38.6
7	0.92	3.31	8.605	6.973	6.678	−171.8	−81.1	−143.2
8	1.01	2.84	8.586	7.035	6.724	43.0	133.6	73.0
9	1.04	2.71	9.287	7.180	7.758	−60.7	25.7	−73.4
10	1.06	2.62	5.127	5.625	5.676	−53.6	36.2	−21.8
11	1.08	2.53	10.062	7.229	7.789	148.2	−121.9	140.0
12	1.08	2.53	7.155	5.563	6.782	67.3	57.2	25.9
13	1.10	2.45	8.322	6.175	7.030	−61.0	−86.2	−108.8
14	1.10	2.45	7.463	5.892	6.793	62.6	54.0	22.2
15	1.17	2.18	8.825	7.284	6.747	42.8	101.6	112.7
16	1.18	2.18	9.221	6.452	7.173	169.8	101.6	112.7
17	1.21	2.04	5.097	5.122	5.832	−102.5	−15.8	−71.0
18	1.25	1.90	8.031	6.903	6.219	−46.7	−52.3	−89.0
19	1.27	1.84	7.310	6.201	5.256	62.8	153.6	93.4
20	1.29	1.77	8.540	6.435	7.062	−55.6	−74.8	−103.9

[a] The population percentage was calculated according to the Boltzamn distribution $pop_2/pop_1 = e^{-\Delta E/RT}$ considering all the conformers which lie under 2.0 kcal/mol at $T = 298$ K.

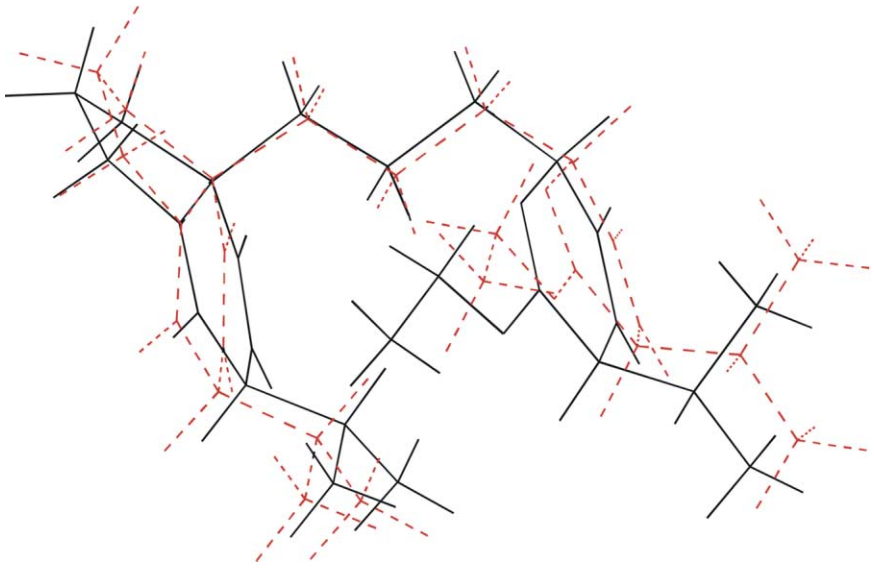


Figure 4. Superimposition of the DFT/optimized lowest energy conformer (dotted line) and X-ray (solid line) structure of **5**.

Table 3. Calculated and experimental ($CDCl_3$ solution) chemical shifts (δ in ppm) for the isolated monomer and the X-ray resolved trimer **5**. For atom labels, see Figure 3

Protons bonded to	Monomer		Trimer	
	calcd	exper.[a]	calcd	exper.[b]
C1[c]	1.25	1.3	1.21	1.27
C2[c]	4.08	4.15	4.03	4.1
C4[c]	3.86	3.88	3.93	3.78
C5 and C20	2.25 and 1.94	2.2	2.23 and 1.74	2.2
C6,C7,C21,C22[c]	0.95–1.04	0.87–1.12	1.12–1.41	0.82–1.13
C9	4.08	4.03	4.09	4.03
C10,C11,C12,C14,C15[c]	1.33–1.90	1.4–2.0	1.37–1.81	1.5–2.1
C16[c]	3.59	3.7	3.57	3.68
C19	3.88	3.66	3.15	3.61
N1 and N4	4.23 and 4.37	6.1 and 6.17	9.01 and 8.33	8.38 and 8.42

[a] 1 mM $CDCl_3$ solution.
[b] 30 mM $CDCl_3$ solution.
[c] Mean value for CH_3 or CH_2 protons.

To validate further the computed data and structures and provide a solid ground for the interpretation of the experiments, ^{1}H NMR chemical shift values were also simulated[13,14] for the B3LYP/6-31G* optimized structure of compound **5** (i.e. the lowest energy conformer) and compared to those observed in $CDCl_3$ solution at different concentrations. Proton chemical shifts were computed for both the isolated monomer (which idealistically represents the situation in dilute solution) and the trimer as resolved by X-ray crystallography (see Fig. 3, which approximately models the solid-phase environment or concentrate solution).[15] This data are reported in Table 3. Interestingly, the computed chemical shifts are in good agreement with the experimental chemical shift. In particular, the δ_{NH} values for the trimer show anomalous low-field values (8.33 and 9.01 ppm) in agreement with the observed ones ($\approx$8.4 ppm), supporting the hypothesis for intermolecular aggregation in concentrated solution with the absence of intramolecular hydrogen bonds.

On the other hand, the monomer displays much higher-field values for δ_{NH} (4.37 and 4.23 ppm) due to the lack of hydrogen bond interactions, which deshield the NH proton's signals. The same trend (i.e. decreasing in the δ_{NH} values) has been observed experimentally on going from concentrated (30 mM, $\delta_{NH} \approx 8.4$ ppm) to dilute (1 mM, $\delta_{NH} \approx 6.1$ ppm) solutions (see Section 3), supporting a loosening in intermolecular hydrogen bond interactions or, alternatively, suggesting an equilibrium between a hydrogen bonded and a non-hydrogen bonded structure, which shifts progressively towards the latter state as the solution is made more and more dilute. This hypothesis is in agreement with the IR spectra (see Section 3).

6. Conclusion

We have synthesized new optically active peptides containing the 2,6-DAP skeleton and a system based on a

proline residue fused to a diketopiperazine ring. This approach, accomplished by starting from L-valine, might also represent a new and simple synthetic path to brevianamides which are fungal metabolites, many of which show interesting biological activities.[16] The structures of these natural products incorporate a bicyclic system containing the diketopiperazine ring, already accomplished by us,[17] and diketopiperazine proline fused rings described herein. In the crystal state the compound **5** appears to be aggregated by the formation of intermolecular hydrogen bonds between the carbonyl oxygen and the amide proton. The computational conformational analysis is in good agreement with the X-ray analysis and also the ^{1}H NMR experiments in $CDCl_3$ (δ_{NH} and $\Delta\delta_{NH}/\Delta T$ values) and IR spectra are coherent with the formation of an intermolecular hydrogen bond.

We conclude that the experimental data (^{1}H NMR analysis, IR spectra, X-ray experiments), when complemented and supported by computational modelling, represents a valid tool to elucidate the structure and the intermolecular interactions of these molecules.

7. Experimental

7.1. 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, unless otherwise stated. Chemical shifts are reported in ppm relative to $CDCl_3$. 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. HPLC–MS spectra were recorded on a HP LC-MSD 1100 single quadrupole (with interface APCI-ES) spectrometer.

Dry THF was distilled from sodium benzophenone ketyl. Chromatographic separations were performed with silica gel 60 (230–400 mesh).

7.2. 1-[(3′*R*,6′*S*)-1′-Benzyl-3′-(3-chloropropyl)-6′-hydro-5′-ethoxy-6′-isopropyl-2-pirazinon-3′-yl]-3-[(3"*R*,6"*S*)-1"-benzyl-3",6"-dihydro-5"-ethoxy-6"-isopropyl-2″-pirazinon-3"-yl]propane, 3

To a solution of **2**[1] (5.9 g, 10 mmol) in dry THF (250 mL) cooled at −78°C was added 10 mmol of LHMDS (1 M solution in THF). After about one hour under stirring, 1-chloro-3-iodopropane (1.3 mL, 12 mmol) dissolved in 50 mL of dry THF was added and the reaction monitored by TLC. When the reaction was complete, water and ethylacetate was then added. The organic extract was dried over Na_2SO_4 and then evaporated to dryness in vacuo. The crude reaction product was purified by silica gel chromatography eluting with hexane/ethylacetate with the pure product isolated in about 80% yield. $[\alpha]_D = +2.8$ (c 0.7, $CHCl_3$); ^{1}H NMR δ: 0.88 (d, 3H, $J=7$); 0.92 (d, 3H, $J=7$); 1.05 (d, 3H, $J=6.8$); 1.08 (d, 3H, $J=6.8$); 1.22 (t, 3H, $J=7.2$); 1.25 (t, 3H, $J=7.2$); 1.5–2.3 (m, 12H); 3.45 (m, 2H); 3.67 (dd, 1H, $J=1.6$, 3.8); 3.76 (d, 1H, $J=3$); 3.9 (d, 1H, $J=15$); 3.98 (d, 1H, $J=15$); 3.95–4.2 (m, 5H); 5.46 (d, 1H, $J=15$); 5.48 (d, 1H, $J=15$); 7.3 (m, 10ArH). ^{13}C NMR δ: 14.1, 17.3, 17.5, 19.6, 19.9, 20.7, 28.0, 29.9, 31.5, 34.1, 37.6, 41.2, 44.9, 46.9, 47.1, 57.5, 60.6, 60.9, 61.1, 61.7, 62.9, 127.3, 127.4, 127.7, 128.2, 128.5, 136.1, 136.2, 156.0, 158.6, 170.3, 171.6. Anal. calcd for $C_{38}H_{53}N_4O_4Cl$: C, 68.6; H, 8.03; N, 8.42; Cl, 5.33. Found: C, 68.85; H, 8.05; N, 8.4; Cl, 5.31.

The doubly alkylated *1,3-bis[(3′R,6′S)-1′-benzyl-3′-(3-chloropropyl)-6′-hydro-5′-ethoxy-6′-isopropyl-2′-pirazinon-3′-yl]propane* was isolated as a by-product in about 10% yield. ^{1}H NMR δ: 0.91 (d, 6H, $J=7$); 1.09 (d, 6H, $J=6.8$); 1.26 (t, 6H, $J=7.4$); 1.4–2.3 (m, 16H); 3.4 (m, 4H); 3.77 (d, 2H, $J=3$); 3.9–4.2 (m, 6H); 5.43 (d, 2H, $J=15$); 7.3 (m, 10ArH). ^{13}C NMR δ: 13.9, 17.0, 18.0, 20.5, 26.6, 27.7, 29.6, 37.7, 41.6, 44.6, 46.7, 60.4, 61.0, 62.7, 127.2, 128.0, 128.3, 135.8, 155.9, 171.2.

7.3. 1-[(3′*S*,6′*R*)-4′-Benzyl-2′,5′-diketo-3′-isopropyl-1′,4′-diazabicyclo[4,3,0]-6′-nonyl]-3-[(3″*S*,6″*R*)-4″-benzyl-2″-ethoxy-3″,6″-dihydro-3″-isopropyl-5″-pirazinon-6″-yl]-propane, 4

A solution of the intermediate **3** (13.3 g, 20 mmol) and NaI (6 g, 40 mmol), dissolved in 50 mL of acetone, was refluxed for 36 hours. The organic solvent was evaporated, water added to the residue and the reaction product extracted with ethylacetate. The organic phase, having been dried over Na_2SO_4, was evaporated in vacuo to dryness and the residue purified by silica gel chromatography eluting with hexane/ethylacetate. The product was obtained pure in practically quantitative yield. $[\alpha]_D = +44.6$ (c 2.6, $CHCl_3$); ^{1}H NMR δ: 0.92 (d, 3H, $J=6.8$); 1.06 (d, 3H, $J=6.8$); 1.11 (d, 3H, $J=6.8$); 1.18 (d, 3H, $J=6.8$); 1.26 (t, 3H, $J=7$); 1.6–2.4 (m, 12H); 3.4 (m, 1H); 3.6 (d, 1H, $J=7.6$); 3.69 (dd, 1H, $J=1.6$, 4.2); 3.92 (d, 1H, $J=15$); 3.93 (d, 1H, $J=15$); 3.95–4.2 (m, 4H); 5.45 (d, 1H, $J=15$); 5.46 (d, 1H, $J=15$); 7.3 (m, 10ArH). ^{13}C NMR δ: 14.0, 17.4, 19.8, 19.9, 20.2, 20.5, 20.9, 31.5, 33.1, 33.7, 34.7, 38.2, 45.0, 47.3, 49.2, 57.3, 60.9, 61.9, 66.9, 67.1, 127.3, 127.4, 127.9, 128.5, 135.9, 136.0, 159.0, 164.7, 169.7, 170.0. Anal. calcd for $C_{36}H_{48}N_4O_4$: C, 71.97; H, 8.05; N, 9.33. Found: C, 71.9; H, 8.08; N, 9.36.

7.4. Birch reduction of 4

7.4.1. Usual procedure. Li (0.35 gr, 50 mmol) was dissolved in liquid ammonia (80 mL) cooled at −50°C under strirring. After one hour, the substrate **4** (2.1 g, 3.5 mmol), dissolved in 30 mL of dry THF/t-butanol 9:1, was added and the reaction mixture stirred for 5 minutes. The reaction was then quenced by the addition of NH_4Cl (2.7 gr, 50 mmol). After evaporation of the ammonia, to the crude reaction product was added water and ethylacetate. The organic extract was dried over Na_2SO_4 and evaporated under vacuum to dryness.

7.4.2. Modified procedure. A solution of the substrate **4** (2.1 g, 3.5 mmol) in 30 mL of dry THF/*t*-butanol 9:1 was added to about 80 mL of liquid ammonia cooled at −50°C. Li (0.05 gr, 7 mmol) was then added to the substrate under stirring. The addition of Li in small pieces, was controlled by the monitoring of the TLC in the presence of the substrate (starting material) and was stopped as soon as the reaction mixture became blue. The reaction was then rapidly quenced by the addition of NH_4Cl (0.43 gr, 8 mmol) and, after evaporation of ammonia, the residue was worked up as reported.

7.5. 1-[(3′*S*,6′*R*)-2′,5′-Diketo-3′-isopropyl-1′,4′-diazabicyclo[4,3,0]-6′-nonyl]-3-[(3″*S*,6″*R*)-2″-ethoxy-3″,6″-dihydro-3″-isopropyl-5″-pirazinon-6″-yl]propane, 5

Compound **5** was obtained with a 90% yield submitting the intermediate **4** to the modified Birch reaction. The product was a white solid melting at 169–171°C. In Section 7.19 is reported the X-ray resolved structure. ^{1}H NMR δ: 0.82 (d, 3H, J=6.6); 0.98 (d, 3H, J=6.6); 0.99 (d, 3H, J=6.6); 1.13 (d, 3H, J=7); 1.27 (t, 3H, J=7.2); 1.5–2.3 (m, 12H); 3.3 (m, 1H); 3.61 (dd, 1H, J=3.4, 8); 3.78 (m, 1H); 4–4.2 (m, 4H); 8.38 (d, 1H, J=2); 8.42 (d, 1H, J=3). ^{13}C NMR δ: 14.3, 16.0, 18.4, 19.1, 19.5, 19.9, 20.1, 32.6, 33.7, 33.9, 36.0, 36.6, 44.1, 57.1, 58.3, 61.2, 62.6, 67.5, 158.8, 164.9, 171.5, 172.4. $[\alpha]_D$=130.3 (*c* 1.1, $CHCl_3$).

7.6. 1-[(3′*S*,6′*R*)-2′-Keto-3′-isopropyl-1′,4′-diazabicyclo[4,3,0]-4′-nonen-6′-yl]-3-[(3″*S*,6″*R*)-2″-ethoxy-3″,6″-dihydro-3″-isopropyl-5″-pirazinon-6″-yl]propane, 5′

Compound **5′** was obtained in a practically quantitative yield starting from **4** and following the usual Birch procedure. $[\alpha]_D$=+78.6 (*c* 0.9, $CHCl_3$); ^{1}H NMR δ: 0.86 (d, 3H, J=6.6); 1.11 (d, 6H, J=7); 1.17 (d, 3H, J=6.6); 1.29 (t, 3H, J=7.2); 1.5–2.3 (m, 12H); 3.3 (m, 1H); 3.95 (m, 1H); 3.99 (dd, 1H, J=1.8, 6.6); 3.9–4.2 (m, 4H); 6.3 (bs, 1H); 7.9 (d, 1H, J=1.8). ^{13}C NMR δ: 14.2, 16.1, 18.2, 19.4, 20.3, 32.2, 33.2, 33.3, 33.9, 37.2, 43.1, 56.9, 58.6, 61.3, 66.6, 68.1, 158.5, 161.2, 167.5, 171.8. Anal. calcd for $C_{22}H_{36}N_4O_3$: C, 65.32; H, 8.97; N, 13.85. Found: C, 65.48; H, 9.01; N, 13.9.

7.7. 8-[(3′*S*,6′*R*)-2′,5′-Diketo-3′-isopropyl-1′,4′-diazabicyclo[4,3,0]non-6′-yl]-(2*S*,5*R*)-3-aza-5-amino-4-keto-2-isopropylethyloctanoate hydrochloride, 6

The intermediate **5** (0.42 g, 1 mmol) was dissolved in 10 mL of 50% EtOH/0.5N HCl and the solution stirred at room temperature. The reaction was monitored by TLC or ^{1}H NMR and when the starting material totally disappeared, the reaction mixture was evaporated in vacuo to dryness and the product recovered in practically quantitative yield. $[\alpha]_D$=−24 (*c* 0.8, 1 M HCl); ^{1}H NMR (CD_3OD) δ: 0.99 (d, 6H, J=7); 1.04 (d, 3H, J=6.8); 1.09 (d, 3H, J=6.8); 1.3 (t, 3H, J=7.4); 1.4–2.3 (m, 12H); 3.45 (m, 1H); 3.56 (d, 1H, J=8); 3.85 (m, 1H); 3.98 (t, 1H, J=6); 4.22 (q, 2H, J=7.4); 4.41 (d, 1H, J=5.4). ^{13}C NMR (CD_3OD) δ: 14.6, 18.6, 19.7, 19.9, 20.2, 20.5, 21.3, 31.7, 32.9, 34.5, 34.9, 38.9, 46.3, 54.1, 59.3, 62.3, 64.0, 68.1, 167.3, 170.1, 172.4. Anal. calcd for $C_{22}H_{39}N_4O_5Cl$: C, 55.63; H, 8.28; N, 11.79; Cl, 7.46. Found: C, 55.44; H, 8.25; N, 11.77; Cl, 7.44.

7.8. 1-[(3′*S*,6′*R*)-4′-Benzyl-2′,5′-diketo-3′-isopropyl-1′,4′-diazabicyclo[4,3,0]non-6′-yl]-3-[(3″*S*,6″*S*)-4″-benzyl-2″-ethoxy-3″-hydro-6″-methyl-3″-isopropyl-5″-pirazinon-6″-yl]propane, 7a

Compound **7a** was obtained in about 90% yield by alkylating **4** with methyl iodide and following the procedure reported in Section 7.2. $[\alpha]_D$=−27.6 (*c* 0.7, $CHCl_3$); ^{1}H NMR δ: 0.91 (d, 3H, J=6.6); 1.07 (d, 3H, J=6.6); 1.11 (d, 3H, J=6.6); 1.18 (d, 3H, J=6.6); 1.26 (t, 3H, J=7.4); 1.41 (s, 3H); 1.5–2.4 (m, 12H); 3.4 (m, 1H); 3.6 (d, 2H, J=8); 3.75 (d, 1H, J=2.6); 3.9–4.2 (m, 5H); 5.44 (d, 1H, J=15); 5.49 (d, 1H, J=15); 7.3 (m, 10ArH). ^{13}C NMR δ: 14.0, 17.0, 19.4, 19.9, 20.3, 20.4, 20.8, 29.3, 29.6, 33.0, 35.0, 39.0, 41.2, 45.2, 46.5, 49.2, 60.0, 60.4, 61.0, 66.9, 67.2, 127.5, 127.8, 127.4, 128.5, 128.6, 136.0, 136.2, 155.1, 165.0, 169.8, 172.7. Anal. calcd for $C_{37}H_{50}N_4O_4$: C, 72.28; H, 8.2; N, 9.11. Found: C, 72.02; H, 8.18; N, 9.15.

7.9. 1-[(3′*S*,6′*R*)-4′-Benzyl-2′,5′-diketo-3′-isopropyl-1′,4′-diazabicyclo[4,3,0]non-6′-yl]-3-[(3″*S*,6″*R*)-4″,6″-dibenzyl-2″-ethoxy-3″-hydro-3″-isopropyl-5″-pirazinon-6″-ylone]-propane, 7b

Compound **7b** was obtained in about 90% yield by alkylating **4** with benzylbromide and following the procedure reported in Section 7.2. $[\alpha]_D$=−40.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ: 0.82 (d, 3H, J=6.8); 0.91 (d, 3H, J=7.2); 1.16 (d, 3H, J=6.8); 1.22 (d, 3H, J=6.8); 1.33 (t, 3H, J=7); 1.7–2.4 (m, 12H); 3.01 (d, 1H, J=12.6); 3.17 (d, 1H, J=2.4); 3.31 (d, 1H, J=12.6); 3.4 (m, 1H); 3.63 (d, 1H, J=12.6); 3.96 (d, 1H, J=15); 3.98 (m, 1H); 4.02 (d, 1H, J=15); 4.1–4.4 (m, 2H); 5.06 (d, 1H, J=15); 5.48 (d, 1H, J=15); 6.6 (m, 2ArH); 7.3 (m, 13ArH). ^{13}C NMR δ: 14.2, 16.5, 19.6, 20.1, 20.4, 21.0, 29.9, 33.2, 35.2, 39.1, 42.0, 45.3, 46.7, 46.8, 49.4, 60.6, 60.9, 65.0, 67.1, 67.3, 126.3, 127.1, 127.7, 127.8, 127.9, 128.4, 128.8, 130.7, 135.3, 136.2, 137.2, 156.9, 165.2, 170.0, 170.4. Anal. calcd for $C_{43}H_{54}N_4O_4$: C, 74.75; H, 7.88; N, 9.26. Found: C, 74.55; H, 7.9; N, 9.3.

7.10. 1-[(3′*S*,6′*R*)-2′,5′-Diketo-3′-isopropyl-1′,4′-diazabicyclo[4,3,0]-6′-nonyl]-3-[(3″*S*,6″*S*)-2″-ethoxy-3″,6″-dihydro-3″-isopropyl-6″-methyl-5″-pirazinon-6″-yl]propane, 8a

Compound **8a** was obtained in 90% yield by submitting **7a** to the Birch reaction. $[\alpha]_D$=+12.7 (*c* 1.2, $CHCl_3$); ^{1}H NMR δ: 0.86 (d, 3H, J=7); 1 (d, 3H, J=7); 1.02 (d, 3H, J=6.8); 1.1 (d, 3H, J=6.8); 1.27 (t, 3H, J=7.4); 1.32 (s, 3H); 1.4–2.4 (m, 12H); 3.35 (m, 1H); 3.62 (dd, 1H, J=3.8, 7.8); 3.9–4.2 (m, 4H); 6.14 (bs, 1H); 6.58 (d, 1H, J=3.8). ^{13}C NMR δ: 14.2, 16.1, 18.3, 19.1, 19.7, 19.8, 20, 28.4, 30.4, 33.2, 34.2, 39.2, 40.9, 45.1, 58, 59.9, 61.1, 63.1, 66.9, 156.1, 165, 171.4, 174.3. Anal. calcd for $C_{23}H_{38}N_4O_4$: C, 63.57; H, 8.81; N, 12.89. Found: C, 63.75; H, 8.85; N, 12.86.

7.11. 1-[(3′*S*,6′*R*)-2′,5′-Diketo-3′-isopropyl-1′,4′-diazabicyclo[4,3,0]-6′-nonyl]-3-[(3″*S*,6″*R*)-6″-benzyl-2″-ethoxy-3″,6″-dihydro-3″-isopropyl-5″-pirazinon-6″-yl]propane, 8b

Compound **8b** was obtained in 85% yield by submitting **7b** to the Birch reaction. $[\alpha]_D = -25.2$ (*c* 1, $CHCl_3$); ^1H NMR δ: 0.75 (d, 3H, *J* = 7); 0.78 (d, 3H, *J* = 7); 1.05 (d, 3H, *J* = 7); 1.12 (d, 3H, *J* = 6.8); 1.3 (t, 3H, *J* = 7); 1.35–2.3 (m, 12H); 2.7 (bs, 1H); 2.74 (d, 1H, *J* = 14.6); 3.18 (d, 1H, *J* = 14.6); 3.38 (m, 1H); 3.62 (dd, 1H, *J* = 4, 7.6); 3.95 (m, 1H); 4.1 (m, 2H); 5.6 (bs, 1H); 6.3 (d, 1H, *J* = 3.2); 7.25 (m, 5ArH). ^{13}C NMR δ: 14.2, 15.7, 17.9, 19.1, 19.6, 19.8, 19.9, 29.6, 33.2, 33.9, 39.3, 40.6, 45.2, 47.2, 57.3, 61.1, 63.2, 65.1, 66.8, 126.5, 127.6, 130.3, 136.3, 157.7, 165.2, 171.5, 172.4. Anal. calcd for $C_{29}H_{42}N_4O_4$: C, 68.21; H, 8.29; N, 10.97. Found: C, 67.98; H, 8.31; N, 10.95.

7.12. 8-[(3′*S*,6′*R*)-2′,5′-Diketo-3′-isopropyl-1′,4′-diazabicyclo[4,3,0]non-6′-yl]-(2*S*,5*S*)-3-aza-5-amino-4-keto-2-isopropyl-5-methylethyloctanoate hydrochloride, 9a

Compound **9a** was obtained in practically quantitative yield starting from **8a** and following the procedure as described in Section 7.7. $[\alpha]_D = +10$ (*c* 1.4, $CHCl_3$); ^1H NMR δ: 0.93 (d, 3H, *J* = 6.6); 0.96 (d, 3H, *J* = 6.6); 1.00 (d, 3H, *J* = 7); 1.17 (d, 3H, *J* = 7); 1.3 (t, 3H, *J* = 7); 1.34 (s, 3H); 1.4–2.4 (m, 12H); 3.3 (m, 1H); 3.99 (dd, 1H, *J* = 3.8, 7.6); 4.2 (m, 3H); 6.4 (bs, 1H); 4.5 (dd, 1H, *J* = 4.8, 8.8); 7.87 (d, 1H, *J* = 1.8); 8.02 (d, 1H, *J* = 8.8). ^{13}C NMR δ: 14.2, 18.2, 19, 19.1, 19.4, 19.6, 19.7, 23.2, 30.8, 33.1, 33.7, 37.2, 37.9, 44.9, 58, 60.5, 61.6, 63.3, 66.6, 165, 170.8, 171.1, 171.7. Anal. calcd for $C_{23}H_{41}N_4O_5Cl$: C, 56.49; H, 8.45; N, 11.46; Cl, 7.25. Found: C, 56.68; H, 8.43; N, 11.5; Cl, 7.27.

7.13. 8-[(3′*S*,6′*R*)-2′,5′-Diketo-3′-isopropyl-1′,4′-diazabicyclo[4,3,0]non-6′-yl]-(2*S*,5*R*)-3-aza-5-amino-5-benzyl-4-keto-2-isopropylethyloctanoate hydrochloride, 9b

Compound **9b** was obtained from **8b** following the procedure described in Section 7.7. After chromatographic purification by elution with hexane/ethylacetate, the product was isolated with a 50% yield. However it was not obtained in a sufficiently pure form to measure the specific rotation. ^1H NMR (CD_3OD) δ: 1.04 (d, 3H, *J* = 7); 1.06 (d, 3H, *J* = 7);1.08 (d, 3H, *J* = 7); 1.09 (d, 3H, *J* = 7); 1.34 (t, 3H, *J* = 7.4); 1.4–2.4 (m, 12H); 3.3 (q_{AB}, 1H, *J* = 14.4); 3.46 (m, 1H); 3.58 (d, 1H, *J* = 8.4); 3.9 (m, 1H); 4.28 (m, 2H); 4.4 (d, 1H, *J* = 6.6); 7.4 (m, 5ArH). ^{13}C NMR (CD_3OD) δ: 14.7, 19.4, 19.7, 20.0, 20.7, 31.4, 34.6, 35.0, 36.4, 39.2, 43.1, 46.4, 60.2, 62.4, 64.2, 65.2, 68.2, 129, 129.9, 131.6, 134, 167.4, 171.5, 172.6, 172.7.

From the reaction bis-diketopiperazine **9′b** was also obtained in about a 50% yield. ^1H NMR δ: 0.77 (d, 3H, *J* = 7); 0.81 (d, 3H, *J* = 6.8); 0.98 (d, 3H, *J* = 7); 1.1 (d, 3H, *J* = 6.8); 1.3–2.3 (m, 12H); 2.4 (d, 1H, *J* = 1.2); 2.78 (d, 1H, *J* = 13.2); 3.2 (d, 1H, *J* = 13.2); 3.33 (m, 1H); 3.68 (dd, 1H, *J* = 3.4, 7.8); 4.1 (m, 1H); 6.1 (bs, 1H); 7.2 (m, 5ArH); 8.2 (bs, 1H); 8.6 (d, 1H, *J* = 3.4).

7.14. (3*R*,6*S*)-1-Benzyl-5-ethoxy-6-isopropyl-3-(3-chloropropyl)-6-hydro-2-pirazinone, 10

Compound **10** was obtained with an 85% yield by alkylating **1** with 1-chloro-3-iodopropane and following the procedure as described in Section 7.2. $[\alpha]_D = +50.5$ (*c* 2.0, $CHCl_3$); ^1H NMR δ: 0.91 (d, 3H, *J* = 7); 1.03 (d, 3H, *J* = 6.8); 1.23 (t, 3H, *J* = 6.8); 1.8–2.3 (m, 5H); 3.58 (t, 2H, *J* = 6.6); 3.68 (dd, 1H, *J* = 1.6, 4); 3.91 (d, 1H, *J* = 15.2); 4.1 (m, 3H); 5.46 (d, 1H, *J* = 15.2); 7.3 (m, 5ArH). ^{13}C NMR δ: 14.0; 17.4; 19.8; 28.7; 31.0; 31.5; 44.9; 47.2; 57.1; 61.0; 61.8; 127.3; 127.5; 128.4; 136.0; 159.2; 169.7. Anal. calcd for $C_{19}H_{27}N_2O_2Cl$: C, 65.04; H, 7.76; N, 7.98; Cl, 10.1. Found: C, 65.3; H, 7.77; N, 7.95; Cl, 10.12.

7.15. (3*S*,6*R*)-4-Benzyl-2,5-diketo-3-isopropyl-1,4-diazabicyclo[4,3,0]nonane, 11

Compound **11** was obtained in practically quantitative yield from **10** following the procedure as described in Section 7.3. The product was not obtained in a sufficiently pure form to measure the specific rotation. ^1H NMR δ: 1.07 (d, 3H, *J* = 6.9); 1.15 (d, 3H, *J* = 6.9); 1.8–2.4 (m, 5H); 3.52 (m, 1H); 3.6 (m, 1H); 3.71 (d, 1H, *J* = 6.2); 3.72 (m, 1H); 3.98 (d, 2H, *J* = 15); 4.2 (m, 2H); 5.4 (d, 2H, *J* = 15); 7.3 (m, 5ArH). ^{13}C NMR δ: 18.1; 19.7; 22.0; 29.7; 31.4; 45.2; 48.3; 58.5; 67.1; 127.4; 127.5; 128.5; 135.7; 164.2; 167.5.

7.16. (3*S*,6*R*)-4-Benzyl-2,5-diketo-3-isopropyl-6-(3-chloropropyl)-1,4-diazabicyclo[4,3,0]nonane, 12

Compound **12** was obtained in 80% yield by alkylating **11** with 1-chloro-3-iodopropane and following the procedure as described in Section 7.2. $[\alpha]_D = -55.3$ (*c* 1.5, $CHCl_3$); ^1H NMR δ: 0.83 (d, 3H, *J* = 6.9); 1.18 (d, 3H, *J* = 6.9); 1.5–2.4 (m, 9H); 3.4 (m, 3H); 3.87 (d, 1H, *J* = 2.4); 3.9 (m, 1H); 3.99 (d, 1H, *J* = 14.7); 5.4 (d, 1H, *J* = 14.7); 7.3 (m, 5ArH). ^{13}C NMR δ: 15.5; 19.7; 19.9; 26.8; 30.4; 35.1; 35.4; 43.3; 44.1; 47.1; 64.0; 66.5; 128.0; 128.7; 135.4; 163.5; 168.6. Anal. calcd for $C_{20}H_{27}N_2O_2Cl$: C, 66.19; H, 7.5; N, 7.72; Cl, 9.77. Found: C, 66.38; H, 7.53; N, 7.75; Cl, 9.8.

7.17. (3*S*,6*R*)-4-Benzyl-2,5-diketo-3-isopropyl-6-(3-iodopropyl)-1,4-diazabicyclo[4,3,0]nonane, 13

Compound **13** was obtained in practically quantitative yield from **12** and following the procedure as described in Section 7.3. The product was not obtained in a sufficiently pure form to measure the specific rotation. ^1H NMR δ: 0.82 (d, 3H, *J* = 6.8); 1.16 (d, 3H, *J* = 6.8); 1.5–2.4 (m, 9H); 3 (m, 2H); 3.35 (m, 1H); 3.85 (d, 1H, *J* = 1.8); 3.9 (m, 1H); 3.97 (d, 1H, *J* = 14.4); 5.4 (d, 2H, *J* = 14.4); 7.3 (m, 5ArH). ^{13}C NMR δ: 5.0; 15.1; 19.2; 19.5; 27.3; 29.9; 34.7; 38.4; 42.8; 46.6; 63.4; 65.8; 127.3; 128.2; 134.9; 162.7; 167.8.

7.18. 1-[(3′*S*,6′*S*)-4′-Benzyl-2′,5′-diketo-3′-isopropyl-1′,4′-diazabicyclo[4,3,0]-6′-nonyl]-3-[(3″*S*,6″*R*)-4″-benzyl-2″-ethoxy-3″,6″-dihydro-3″-isopropyl-5″-pirazinon-6″-yl]-propane, 14

To a solution of **1** (2.74 g, 10 mmol) in dry THF (100 mL) cooled at −78°C was added 10 mmol of LHMDS (1 M solution in THF). After about one hour under stirring, the intermediate **13** (4.5 g, 10 mmol) was added and the reaction monitored by TLC. When the starting material disappeared, water and ethylacetate were then added. The organic extract was dried over Na_2SO_4 and then evaporated to dryness in vacuo. The crude reaction product after purification by silica gel chromatography (eluting with hexane/ethylacetate) was obtained in about a 70% yield. However, the product was not recovered in a sufficiently pure form to measure the specific rotation. ^{1}H NMR δ: 0.78 (d, 3H, J=6.6); 0.91 (d, 3H, J=6.6); 1.04 (d, 3H, J=7); 1.09 (d, 3H, J=7); 1.12 (t, 3H, J=7); 1.4–2.4 (m, 12H); 3.35 (m, 1H); 3.67 (dd, 1H, J=1.4, 4); 3.74 (d, 1H, J=2.6); 3.8–4.1 (m, 6H); 5.38 (d, 1H, J=15); 5.45 (d, 1H, J=15); 7.3 (m, 10ArH). ^{13}C NMR δ: 13.7, 13.8, 14.9, 17.3, 19.4, 19.6, 29.5, 31.2, 32.9, 35.1, 37.7, 42.9, 46.6, 46.8, 57.1, 60.8, 61.3, 63.0, 66.7, 127.1, 127.2, 127.4, 127.6, 128.1, 128.3, 134.8, 135.8, 158.8, 163.0, 168.7, 169.5.

7.19. X-Ray crystallography of 5

The diffraction experiments were carried out at room temperature on a Bruker AXS SMART 2000 CCD based diffractometer using graphite monochromated Mo–Kα radiation (λ=0.71073 Å). Intensity data were measured over full diffraction spheres using 0.3° wide ω scans, crystal-to-detector distance 5.0 cm. The software SMART[18] was used for collecting frames of data, indexing reflections and determination of lattice parameters. The collected frames were then processed for integration by software SAINT[18] and an empirical absorption correction applied with SADABS.[19] The structure was solved by direct methods (SIR97)[20] and subsequent Fourier syntheses, and refined by full-matrix least-squares calculations on F^2 (SHELXTL),[21] attributing anisotropic thermal parameters to the non-hydrogen atoms. The methyl and methylene hydrogen atoms were placed in calculated positions and refined with idealized geometry, whereas the other H atoms were located in the Fourier map and refined isotropically.

7.19.1. Crystallographic data. $C_{22}H_{36}N_4O_4$, orthorhombic, $P2_12_12_1$ (No. 19), a=9.2158(7), b=9.6257(7), c=26.205(2) Å, V=2324.6(3) Å^3, Z=4, d_{calcd}=1.202 Mg m^{-3}, μ=0.083 mm^{-1}. 30453 reflections were collected, 6781 unique, 5062 observed for $I>2\sigma(I)$, which were used in all calculations. Final R factors: R_1=0.0486 [$I>2\sigma(I)$], wR_2=0.1516 (all data).

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 212112. Copies of the data can be obtained free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)1223336033 or e-mail: deposit@ccdc.cam.ac.uk].

7.20. Computational methods

All calculations were carried out on SGI IRIX 6.5 workstations. Molecular mechanic calculations were performed using the implementation of the Amber all-atom force field (AMBER*) within the framework of Macromodel version 5.5.[22] The AMBER* force field in MMOD 5.5 contained a new set of parameters for proline containing peptides, recently developed on the basis of high-level ab initio calculations.[23] The solvent effect was included by the implicit chloroform GB/SA solvation model of Still et al.[24] 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 minimized 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 5 kcal/mol above the global minimum were discarded.

All DFT calculations (i.e. geometry optimizations and chemical shift simulations) were carried out using the standard tools available in the Gaussian 98 package,[25] with the DFT/B3LYP functional (i.e. Becke's three parameter hybrid functional with the Lee-Yang-Parr correlation functional)[26] and the 6-31G(d) basis set. This functional and basis set have been shown to properly describe both standard hydrogen bonds,[27] as well as non-classical, weakly bound hydrogen bonds (such as C–H···O=C interactions),[28–30] and to provide reliable results for the protons chemical shifts.[13,14,29] However, the computed data does not directly yield the chemical shift value, but only a value for the isotropic magnetic tensor. The chemical shift value was obtained from the equation $\delta_H = 32.18 - \sigma_H$, where 32.18 is the calculated isotropic magnetic tensor for the protons in tetramethylsilane and σ_H is the calculated isotropic magnetic tensor for the investigated proton. This procedure has been recently validated and applied in other molecular systems.[29,30]

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