ARTICLE

OBC
www.rsc.org/obc

β-Pseudopeptide foldamers. The homo-oligomers of (4*R*)-(2-oxo-1,3-oxazolidin-4-yl)-acetic acid (D–Oxac)

Gianluigi Luppi,[a] Roberta Galeazzi,[b] Marco Garavelli,[a] Fernando Formaggio[c] and Claudia Tomasini*[a]

[a] *Department of Chemistry "G. Ciamician", Alma Mater Studiorum, University of Bologna, 40126 Bologna, Italy. E-mail: claudia.tomasini@unibo.it; Fax: +39 0512099456; Tel: +39 0512099511*

[b] *Università Politecnica delle Marche, Department of Material and Earth Sciences, 60131 Ancona, Italy. E-mail: roberta@mta01.univpm.it; Fax: +39 0712204714; Tel: +39 0712204724*

[c] *Institute of Biomolecular Chemistry, CNR, Department of Chemistry, University of Padova, 35131 Padova, Italy. E-mail: fernando.formaggio@unipd.it; Fax: +39 0498275239; Tel: +39 0498275277*

Received 20th April 2004, Accepted 10th June 2004
First published as an Advance Article on the web 14th July 2004

A total synthesis in solution and a conformational analysis of the homo-oligomers of (4*R*)-(2-oxo-1,3-oxazolidin-4-yl)-acetic acid (D–Oxac) to the tetramer level are described. As the D–Oxac building block contains both an oxazolidin-2-one and a β-amino acid group, it may represent a new type of conformationally constrained tool for the construction of β-pseudopeptide foldamers. A conformational investigation using NMR and an extensive, unconstrained analysis with a Monte Carlo search to the octamer level, both in water and in chloroform, showed that these homo-oligomers tend to fold in a regular helical structure in a competitive solvent, such as water. Since aqueous solutions are of major relevance for biological systems, these molecules are good candidates for application to these environments.

Introduction

In the last few years the design and synthesis of oligomers based on β-amino acids have been extensively carried out, both in the presence and absence of stabilising hydrogen bonds.[1] Non-hydrogen bonded secondary structures, for example, poly(Pro)$_n$ helices, occur occasionally in proteins and in short peptides. Individual strands within the collagen triple helix are folded in a poly(Pro)$_n$ II (PPII) conformation. Short PPII helices play important roles in protein-protein recognition. Seebach and coworkers[2] have studied oligomers of β-HPro-peptides and have demonstrated that this class of β-peptides may still adopt distinct folding patterns due to the high intrinsic folding propensity of the β-peptide backbone. More recently, Gellman and coworkers[3] have studied the oligomers of 2,2-disubstituted-pyrrolidine-4-carboxylic acid (2,2-DPCA) and have demonstrated that these compounds display well defined conformational preferences, although they cannot form hydrogen bonds.

We have recently introduced pseudopeptides based on an α-amino acid cyclic derivatives (Pro analogues) as building blocks where the classical amide bonds are replaced by imide bonds. More specifically, oligomers of substituted 1,3-oxazolidin-2-ones (L–Oxd)[4] and γ-lactams (L–pGlu)[5] have been examined. These studies have demonstrated that these oligomers adopt a *semi*-extended secondary structure similar to that of poly(Pro)$_n$ II even in the dimer and that conformational equilibria are not operative. In these molecules hydrogen bonds are missing and the stabilizing effect is due to the imide moiety, which always adopts a conformation with the two carbonyls in the *anti* disposition (Fig. 1).

In this article, we describe our synthetic and conformational results obtained with oligoimides where the single unit is a β-amino acid cyclic derivative, (4*R*)-(2-oxo-1,3-oxazolidin-4-yl)-acetic acid (D–Oxac). These homo-oligomers are expected to adopt more flexible structures than those characteristic of their α-amino acid counterparts, although they still contain imide moieties (Fig. 2). Nevertheless, we will show here that these molecules fold in ordered helical 3D-structures, in polar solvents.

These oligomers afford similar results to what observed with β-peptides,[6] which surprisingly have exhibited greater conformational stability than α-peptides, although they provide an additional "rotatable" bond in the backbone. Indeed it has been demonstrated that β-peptides can form stable helices with only four to six residues, whereas an α-peptide of that length would be disordered.[3]

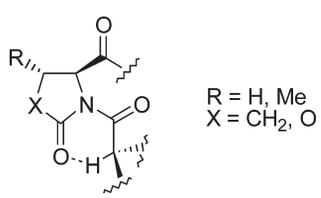


Fig. 1 Preferred conformation of the side chain of the acylated L–pGlu (R = H, X = CH_2) and L–Oxd (R = Me, X = O) rings. The disposition of the imide moiety, which is due to the tendency of the two carbonyls to repel each other, is further stabilized by formation of a C=O···H–C hydrogen bond, with an energetic contribution of approximately 1.4 kcal mol^{-1}.[4,5]

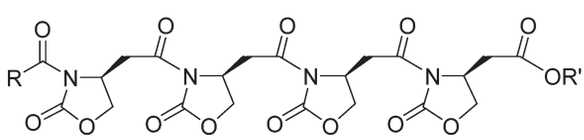


Fig. 2 Chemical structure of the longest D–Oxac homo-oligomer investigated in this work.

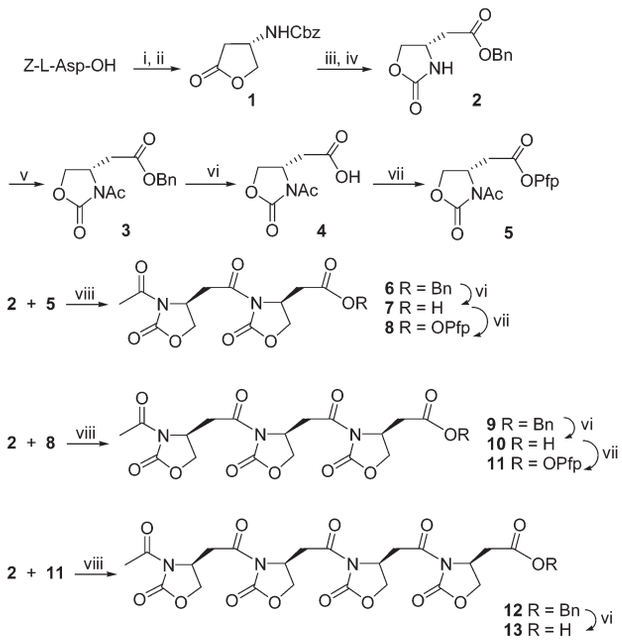
Results and discussion

Synthesis of oligomers

The synthesis of the monomer H–D–Oxac–OBn (**2**) (Scheme 1) has already been described.[7,8] It involves the transformation of Z–L–Asp–OH into its corresponding internal anhydride by reaction with neat acetic anhydride under microwave irradiation. The product was obtained in quantitative yield and was subsequently reduced to the lactone **1** with $NaBH_4$ in dry THF. This reaction can afford a mixture of **1** and the corresponding γ-hydroxy acid, which however are both starting materials for the cyclization reaction to the oxazolidin-2-one **2**. Therefore, either pure **1** or a mixture of **1** and its hydroxy acid was treated with Cs_2CO_3 (3 equivalents) in water and acetone (3 : 1 ratio) for 4 h at reflux to obtain the caesium salt of the oxazolidin-2-one acetate (H–D–Oxac–O$^-$Cs$^+$), with formation of benzyl alcohol

DOI: 10.1039/b405856a

as a side product. The solvents were removed under reduced pressure, DMF and benzyl bromide were added and the mixture was stirred at room temperature overnight.[9] After flash chromatography H–D–Oxac–OBn **2** was obtained pure in 45% overall yield from Z–L–Asp–OH. We chose the acetyl as an appropriate blocking group for the N-terminal D–Oxac unit, because of its high polarity. Indeed, our aim was the synthesis of significantly polar and hopefully water soluble β-pseudopeptide oligomers.



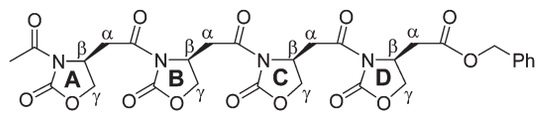
Scheme 1 Reagents and conditions: (i) Ac_2O (3 equiv.), microwave (1 min.); (ii) $NaBH_4$ (1.1 equiv.), THF, room temperature, 16 h; (iii) Cs_2CO_3 (2 equiv.), acetone/water 1 : 3, reflux, 3 h; (iv) BnBr (1.1 equiv.), room temperature, 2 h; (v) AcCl (1.1 equiv.), DIEA (3 equiv.), DMAP (0.5 equiv.), dry DMF, room temperature, 24 h; (vi) Pd/C 5% (10% w/w), H_2 (2 atm.), MeOH, room temperature, 1 h; (vii) CF_3CO_2Pfp (1.3 equiv.), pyridine (2 equiv.), dry DMF, room temperature, 1 h; (viii) DIEA (4 equiv.), DMAP (0.5 equiv.), dry DMF, room temperature, 24 h.

The synthesis of the homo-oligomers was achieved in liquid phase, by transformation of the acids **4, 7** and **10** into the corresponding pentafluorophenyl esters **5, 8** and **11**, following a well established procedure.[10] The activated esters were then coupled with H–D–Oxac–OBn **2** in the presence of DIEA and DMAP in DMF. A variety of other bases were tested in this coupling reaction (including DBU and triethylamine), but the best results were obtained with a mixture of DIEA (3 equivalents) and DMAP (0.5 equivalents). Using this approach, dimer Ac–(D–Oxac)$_2$–OR, trimer Ac–(D–Oxac)$_3$–OR and tetramer Ac–(D–Oxac)$_4$–OR (R = Bn or H) were obtained, both as benzyl esters and as free acids, by repeating the following reactions: benzyl group removal, transformation of the free acid into the activated ester, and coupling with H–D–Oxac–OBn **2**. Upon formation of the longest oligomers, we obtained compounds of progressively higher water solubility.

Conformational analysis of oligomers

An experimental investigation of the preferred conformation assumed by Ac–(D–Oxac)$_n$–OBn (n = 1–4) oligomers in $CDCl_3$ solution was performed using ^{1}H NMR. The chemical shifts of the α-protons of the four oligomers are reported in Table 1. We can see that the α-protons of rings A, B and C of compounds **(6), (9)** and (**12**) have chemical shifts that are more deshielded of about 0.5 ppm if compared to those of ring D. Clearly, this is a general effect as it has been previously observed in all of the pseudopeptides containing oxazolidin-2-ones or γ-lactam rings.[4,5] We have previously demonstrated that this unusual shift is due to the presence of an endocyclic carbonyl in a close proximity to the αCH protons.[4,5] Indirectly, from the present data we deduce that the two carbonyls of the D–Oxac oligomers are forced to adopt an *anti* disposition. This

Table 1 αCH proton chemical shifts of the Ac–(D–Oxac)$_n$–OBn (n = 1–4) homo-oligomers in $CDCl_3$ solution



		Chemical shifts in $CDCl_3$ solution (400 MHz)			
Entry	Compound	α-CH_2 ring A	α-CH_2 ring B	α-CH_2 ring C	α-CH_2 ring D
1	**2**	—	—	—	2.74 and 3.14
2	**6**	—	—	3.20 and 3.63	2.74 and 3.14
3	**9**	—	3.27 and 3.52–3.64		2.74 and 3.10
4	**12**	3.25–3.38 and 3.50–3.68			2.74 and 3.12

Table 2 βCH proton chemical shifts of the Ac–(D–Oxac)$_n$–OBn (n = 1–4) homo-oligomers in $CDCl_3$ solution

		Chemical shifts in $CDCl_3$ solution (400 MHz)			
Entry	Compound	β-CH ring A	β-CH ring B	β-CH ring C	β-CH ring D
1	**2**	—	—	—	4.78
2	**6**	—	—	4.85	4.74
3	**9**	—	4.84	4.84	4.74
4	**12**	4.84	4.84	4.84	4.76

αC–H···O=C interaction is clearly absent for the C-terminal αCH proton, that therefore resonates in the expected spectral region.

A similar effect has been also observed for the β-protons (Table 2). However, in this case it is much weaker as a variation of only about 0.1 ppm is found.

This different behaviour of the C-terminal residue cannot be ascribed to a mere stereoelectronic effect, due to the C-terminal carboxyl derivative being an ester rather than an imide (as in the case of the first three residues). Indeed, the αCH protons of residue D should be more deshielded than the αCH protons of residues A–C in view of the highest electrowithdrawing power of the oxygen (ester) as compared to that of the nitrogen (imide). For instance the methyl group of *N*-ethyl acetamide resonates as a singlet at 1.98 ppm, in $CDCl_3$, while the methyl group of ethyl acetate resonates in $CDCl_3$ at 2.04 ppm. Moreover, we have recently synthesized two tetrapeptides, containing the D–Oxac ring, that assume preferentially a β-turn conformation: Boc–L–Val–D–Oxac–Gly–L–Ala–OBn and Boc–L–Val–D–Oxac–Gly–L–Ala–OBn.[7] In both cases the αCH protons of the D–Oxac moiety resonate between 2.65 and 2.85 ppm, so they are nearly 1 ppm more shielded in comparison with the αCH proton of rings A, B and C.

In conclusion, both α- and β-protons of each D–Oxac oligomer are affected by the presence of the *endo*cyclic carbonyl. Thus, a reasonable preferred conformation should invoke an *anti* disposition for the two carbonyls.

With the aim of confirming these experimental results and in particular to obtain a better insight into the conformational preference of the Ac/OBn D–Oxac tetramer in a 3D-structure supporting solvents (chloroform) and in a competitive solvent (water) as well, an extensive unconstrained conformational analysis was performed by varying all of the degrees of freedom using the Monte Carlo[11] conformational search (MC/EM), the OPLS* force field[12] for energy calculation and the GB/SA both in water and chloroform to include the solvent effect.[13] All of the conformers within the energy range of 25 kJ mol^{-1} (about 6 kcal mol^{-1}) were considered, but sub-

Table 3 Calculated energy (in kcal mol^{-1}) and "ϕ"/"ψ" (in °) values for all of the conformers of Ac–(D–Oxac)$_4$–OBn **12** within 2 kcal mol^{-1} in water

Conformer entry	Energy /kcal mol^{-1}	"ϕ_1"	"ψ_1"	"ϕ_2"	"ψ_2"	"ϕ_3"	"ψ_3"	"ϕ_4"	"ψ_4"
1	0.00	71.9	8.0	71.5	11.9	74.3	8.9	101.5	−6.6
2	0.66	70.9	4.5	70.7	3.7	70.5	14.5	61.3	−67.1
3	0.69	72.0	8.1	69.4	2.3	70.6	2.5	99.3	−26.8
4	0.73	71.8	7.4	71.3	2.2	72.4	9.6	102.5	−11.6
5	0.85	68.7	−8.1	63.9	−7.9	70.1	5.1	66.5	−60.8
6	1.04	71.9	8.0	70.7	16.0	72.0	1.3	99.2	−1.70
7	1.16	76.1	4.0	63.2	−13.0	58.8	56.7	65.9	−10.4
8	1.22	72.1	8.5	72.9	8.9	71.7	8.1	73.4	25.0
9	1.56	71.8	7.7	72.3	13.1	67.2	−14.8	49.0	0.7
10	1.60	71.9	8.1	71.8	7.0	73.0	−126.7	106.7	−1.5
11	1.83	72.3	9.9	69.1	3.2	71.8	9.9	66.1	−74.4
12	1.86	71.1	5.7	67.0	−16.5	63.2	83.3	101.1	−6.5
13	1.94	71.8	7.8	71.8	8.3	71.7	7.0	72.6	92.5
14	1.95	74.5	−1.7	64.2	−16.2	63.1	60.5	67.3	−94.4

Table 4 Calculated energy (in kcal mol^{-1}) and "ϕ"/"ψ" (in °) values for all of the conformers of Ac–(D–Oxac)$_4$–OBn **12** within 2 kcal mol^{-1} in chloroform

Conformer entry	Energy/kcal mol^{-1}	"ϕ_1"	"ψ_1"	"ϕ_2"	"ψ_2"	"ϕ_3"	"ψ_3"	"ϕ_4"	"ψ_4"
1	0.00	72.3	3.8	72.5	11.7	76.0	6.7	102.0	−8.0
2	0.28	59.7	−20.4	75.6	−162.9	45.5	−10.7	72.7	22.5
3	0.51	72.4	4.5	70.1	4.1	71.7	−1.1	99.0	−27.3
4	0.78	73.7	7.6	55.4	13.0	74.8	−3.6	97.0	−19.8
5	0.80	72.5	4.9	71.7	4.4	71.8	5.0	74.2	21.6
6	0.94	68.4	2.2	70.8	−6.8	68.8	91.2	99.6	−9.6
7	1.37	73.7	8.8	56.5	9.5	80.0	11.0	102.5	−12.2
8	1.38	90.3	−157.2	50.4	−8.4	70.4	0.5	97.4	−32.2
9	1.44	75.4	10.0	55.1	9.8	79.5	8.3	101.6	−4.9
10	1.54	69.0	−22.3	79.8	98.3	74.1	9.9	53.6	21.9
11	1.59	77.9	5.0	63.0	−131	58.9	54.5	67.4	−10.3
12	1.65	90.6	−157.7	50.5	−10.5	72.7	7.1	102.1	−12.4
13	1.77	89.6	−159.5	50.3	−7.8	73.1	7.2	102.1	−17.7
14	1.83	72.5	4.6	72.0	4.9	72.0	5.5	74.2	21.5
15	1.86	72.1	4.9	72.2	9.8	44.1	48.0	104.5	−6.3
16	1.93	59.2	−112.7	72.1	6.9	72.1	3.2	102.7	−6.1
17	1.94	68.5	−3.9	58.9	19.7	75.7	−7.7	85.4	102.0

sequently only those lying below 15 kJ mol^{-1} (about 3.6 kcal mol^{-1}) were fully analysed.

We obtained different results, upon switching the analysis from structure-supporting solvents (*e. g.* chloroform) to competitive solvents (*e. g.* water). Nevertheless, all of the conformations, both in water and in chloroform, show an *anti* orientation for the two carbonyl groups of each imide moiety. This result is in agreement with the variations of chemical shifts observed for Ac–(D–Oxac)$_n$–OBn (n = 2–4) reported in Tables 1 and 2. A similar effect was also noticed for the L–Oxd[4] and pyroglutamic acids[5] homo-oligomers.

To describe in detail the results of our computations, we defined "ϕ" and "ψ" torsion angles, considering the following "virtual" bonds for each residue: "ϕ" = —N—C$_\beta$—C(=O)—N— and "ψ" = —C(=O)—N—C$_\beta$—C(=O)— (Fig. 3).

Fig. 3 The "ψ" and "ϕ" "virtual" torsion angles (in red) used in this work to describe the computed preferred conformations of the D–Oxac units.

In water there are 14 calculated conformers within 2 kcal mol^{-1} of the minimum energy conformer that was located at 0 kcal mol^{-1} (Table 3) *versus* 17 within 2 kcal mol^{-1} in chloroform (Table 4). If we exclude the irrelevant C-terminal D–Oxac ester residue, the main difference between the two solvents is that in water the six most stable conformations are regular helices, whereas in chloroform the second, the fourth and sixth conformations are irregularly folded. This different behaviour is not unexpected as it can be ascribed to a competitive effect induced by water which stabilizes the helical forms where all of the polar carbonyl groups are accessible to solvation. On the contrary, in a non-polar solvent (chloroform) this stabilization is missing, so that the molecules can assume both helical and irregularly geometries. The most stable helical conformation in water has average "ψ" and "ϕ" torsion angles 72.6 °, 9.6 ° and 3 D–Oxac residue per turn. The helical pitch (axial translation per helical turn) is 9.12 Å. This result is good agreement with the data reported for β-proline helices[14] and for poly(Pro)n II helices,[15] which both have 3.0 residues per turn.

Fig. 4 shows a superimposition of the six most stable conformations for the D–Oxac tetramer in water and in chloroform, respectively.

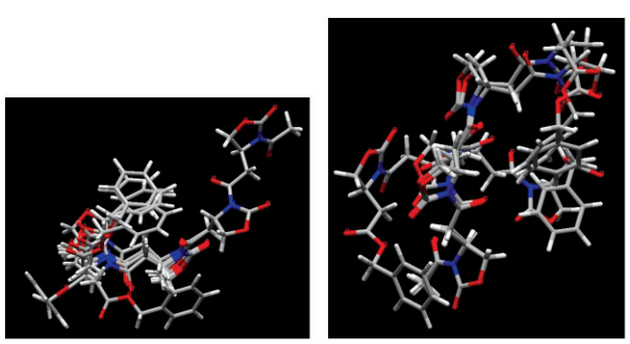


Fig. 4 Superposition of the six most stable conformations for Ac–(D–Oxac)$_4$–OBn in water (left) and in chloroform (right). The conformers were superimposed with "RMS Fit", which allows to overlay two or more molecules by minimizing the distance between corresponding atoms in the two target molecules.

In order to asses the stability of the extended lowest energy conformer in water in a longer foldamer, a detailed conformational analysis was conducted on Ac–(D–Oxac)$_8$–OMe containing eight units by using the same computational protocol described for Ac–(D–Oxac)$_4$–OBn **12**. The results confirmed the existence of this helix-like structure even in a longer structure. Indeed only two

Table 5 Calculated energy (in kcal mol^{-1}) and "ϕ"/"ψ" (in °) values for all of the conformers of Ac–(D–Oxac)$_8$–OBn within 6 kcal mol^{-1} in water. For clarity only the minima, maxima and average values are reported

Conformer entry	Energy/kcal mol^{-1}	min "ϕ"	max "ϕ"	ave "ϕ"	min "ψ"	max "ψ"	ave "ψ"
1	0.00	68.0	72.7	70.5	1.4	8.1	4.7
2	4.84	67.9	72.0	70.3	3.4	10.2	6.3

stable conformers were found within 6 kcal mol^{-1} with a geometry in accordance with what found for Ac–(D–Oxac)$_4$–OBn **12** (Table 5 and Fig. 5). The most stable helical conformation in water has average "ϕ" and "ψ" torsion angles 70.5°, 4.7° and three D–Oxac residues per turn. The helical pitch (axial translation per helical turn) is 8.95 Å. This result is in agreement with the data reported for **12** in water.

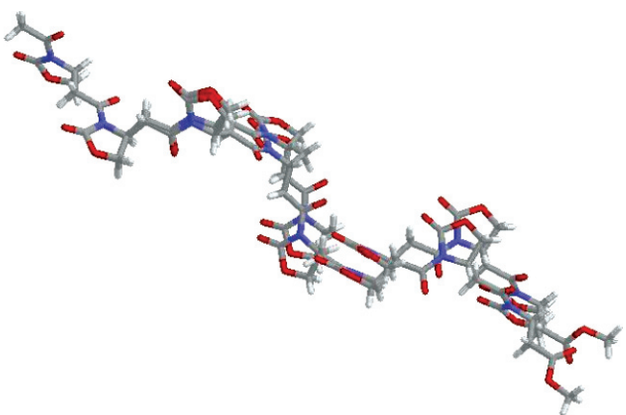


Fig. 5 Superposition of the two most stable conformations for Ac–(D–Oxac)$_8$–OMe in water.

As in water the six preferred conformations are very similar and regular helices and since the water environment is of major importance in biological systems, the lowest energy conformation in water was used as the starting structure for high level DFT calculations (Fig. 6) which enabled us also to simulate the ^{1}H[16] and ^{13}C NMR[17] chemical shift (δ) values for the B3LYP/6-31G* optimized structure of Ac–(D–Oxac)$_4$–OBn (**12**). On the other hand, a comparison of the calculated and measured values of ^{1}H and ^{13}C NMR chemical shifts in chloroform is not significant, because in this case the calculated conformation is only one out of a complex mixture of conformations, which have similar energies and are in a fast equilibrium. On the contrary, the ^{1}H NMR data will not describes a single conformation, but an average over all the accessible conformations.

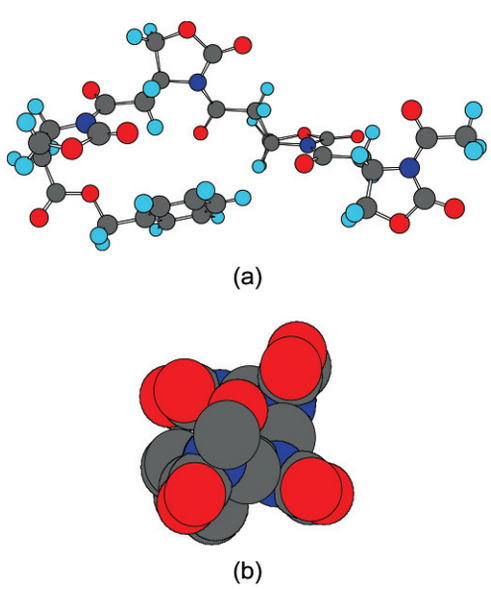


Fig. 6 (a) Side view of the DFT optimized structure for the preferred conformer of tetramer (12) in water. (b) Top view. We chose the space filling model display to show the symmetrical disposition of the oxazolidin-2-ones rings.

The ^{1}H and ^{13}C chemical shifts in water obtained by means of DFT calculations have been compared to those observed in CD_3OD solution (a polar protic solvent similar to water).[18] The results are shown in Table 6, where the ^{1}H and ^{13}C chemical shifts of the atoms in the α, β and γ positions of rings A, B, C, and D have been compared. NOESY1D experiments (mixing time = 1.0 s) enabled us to assign all of the protons belonging to the same D–Oxac unit. Moreover, by means of HSQC experiments, we were able to assign the signals of all of the carbon atoms. A comparison of the calculated and measured chemical shifts indicated that in most cases a good agreement was obtained. In particular, by comparison of the ^{1}H chemical shifts, the experimental data are in general quite close to the simulated values, except for a 0.3–0.5 ppm shift to higher field consistently seen. A particularly good agreement was also obtained with the measured and calculated values for the ^{13}C signals, where the difference between the calculated and measured values is not larger than ± 2 ppm.

Conclusions

A total synthesis and a conformational analysis of the homo-oligomers of (4*R*)-(2-oxo-1,3-oxazolidin-4-yl)-acetic acid (D–Oxac) to the tetramer level has been described. D–Oxac is a building block which contains both an oxazolidon-2-one and a β-amino acid group. Based on these characteristics we designed a new type of conformationally constrained β-pseudopeptide foldamers. The synthesis was carried out in the liquid phase in good yield and the 3D structure of the oligomers was analysed by NMR. Furthermore, an extensive, unconstrained conformational analysis was performed with a Monte Carlo search on Ac–(D–Oxac)$_4$–OBn, both in water and in chloroform to the octamer level. The conformational analysis clearly showed that this molecule fold in an ordered helix in competitive solvents such as water. This outcome was further validated by DFT ^{1}H and ^{13}C NMR simulations that furnished chemical shifts which were successfully compared with the experimental values. Since the water environment is of major importance in biological systems, the molecules described in this paper are good candidates for biological application.

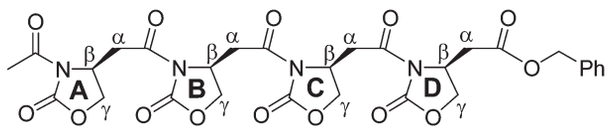
Experimental

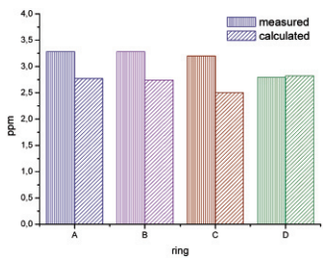
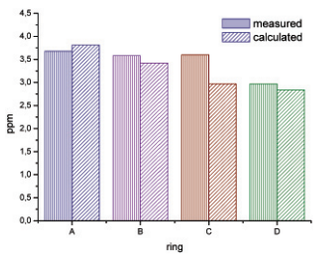
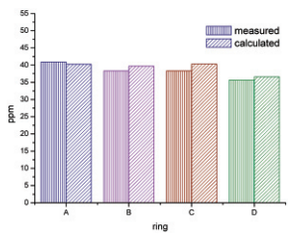
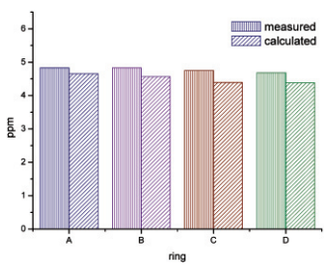
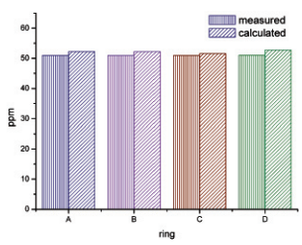
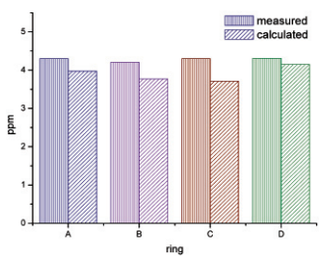
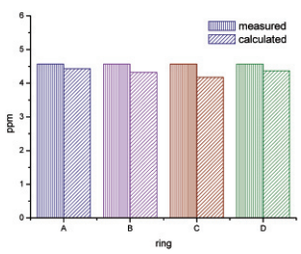
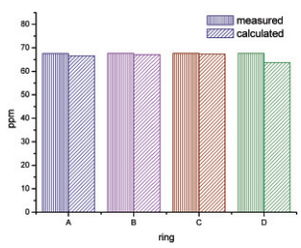
Synthesis and characterization

Materials and reagents were of the highest commercially available grade and were used without further purification. Reaction were monitored by thin-layer chromatography using Merck 60 F254 silica gel covered plastic plates. Compounds were visualized by use of UV light and ceric ammonium molybdate. Flash chromatography was performed using a Merck a 60 silica gel stationary phase. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova 300, a Varian Mercury 400 or a Varian Inova 600 spectrometer. Chemical shifts are reported in δ values relative to the solvent peak of $CHCl_3$, set at 7.27 ppm. The experimental chemical shifts were assigned by means of gCOSY experiments. Infrared absorption spectra were recorded with a Nicolet 210 FT-IR absorption spectrometer. Melting points were determined in open capillaries and are uncorrected. Microwave-assisted reactions were performed in a Prolabo Synthewave 402 apparatus (ν = 33 MHz, P_{max} = 300 W). Flash chromatography was performed with Merck 60 silica gel (230–400 mesh).

(5-Oxo-tetrahydrofuran-3-yl)-carbamic acid benzyl ester (1). A mixture of Z–L–Asp–OH (10 mmol, 2.68 g) and Ac_2O (30 mmol, 2.84 mL) was irradiated with microwaves at 70%

Table 6 Comparison of the experimental (left) and calculated (right) chemical shifts for the homo-oligomer **12**



Position	^{1}H	^{1}H	^{13}C
α			
β			
γ			

power for 1 min, then the mixture was concentrated. The Z–L–Asp internal anhydride, crystallized from ethyl acetate, was obtained pure in 95% yield (2.37 g),[19] and was immediately reduced to the corresponding lactone. A solution of this anhydride (9.5 mmol, 2.37 g) in THF (20 mL) was added dropwise to a suspension of $NaBH_4$ (10.5 mmol, 0.40 g) in THF (15 mL) at 0 °C. Then, the suspension was stirred for 16 hr at room temperature, followed by addition of 6N aqueous HCl to pH 2. THF was evaporated under reduced pressure, and water (50 mL) was added to the residue. The mixture was extracted with diethyl ether (4 × 50 mL) and compound (**1**) was obtained pure after flash chromatography (cyclohexane/ethyl acetate 1 : 1 as eluant) in 80% yield (1.79 g). The yield can vary, because the lactone (**1**) can partially or totally be recovered as the corresponding γ-hydroxy acid. This side reaction does not affect the yield of H–D–Oxac–OBn, as both (**1**) and the corresponding hydroxy acid are good starting materials for the following reaction. M.p. = 74 °C; $[\alpha]_D$ − 18.6 (c 1, DCM); IR (film): ν = 3309, 1780, 1685 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ = 2.45 (dd, 1 H, J = 2.8, 18.0 Hz), 2.80 (dd, 1 H, J = 7.6, 18.0 Hz), 4.12–4.24 (m, 1 H), 4.36–4.55 (m, 2 H), 5.09 (m, 2 H), 5.44 (bs, 1 H), 7.27–7.32 (m, 5H, Ph); ^{13}C-NMR ($CDCl_3$): δ = 31.8, 35.1, 67.5, 73.8, 128.5, 128.6, 128.9, 136.1, 156.0, 175.3. Elemental analysis for $C_{12}H_{13}NO_4$ (235.2): calcd. C 61.27, H 5.57, N5.95; found C 61.23, H 5.61, N 5.99.

H–D–Oxac–OBn (2). Solid caesium carbonate (27 mmol, 3.06 g) was added to a stirred solution of **1** (9 mmol, 2.12 g) in acetone (5 mL) and water (15 mL). The mixture was refluxed for 3 h, then water and acetone were removed under reduced pressure and the resulting H–D–Oxac–O$^-$ caesium salt was directly transformed into its benzyl ester. DMF (3 mL) was added to the residue and benzyl bromide (10 mmol, 1.19 mL), and the mixture was stirred for 24 h. Then, ethyl acetate was added (50 mL) and the organic layer was washed with 1N HCl (3 × 20 mL), dried over sodium sulfate, and concentrated. The product **2** was obtained pure after flash chromatography (cyclohexane/ethyl acetate 1 : 1 as eluant) in 60% yield (3.81 g). mp = 82–83 °C; $[\alpha]_D$ = −32.5 (c 1.0, CH_2Cl_2); IR (CH_2Cl_2): ν 3240, 1731, 1706 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.64 (dd, 1 H, J = 6.2, 17.2 Hz, C*H*HCO), 2.74 (dd, 1 H, J = 7.8, 17.2 Hz, CH*H*CO), 4.05 (dd, 1 H, J = 5.4, 8.4 Hz, C*H*HO), 4.17–4.31 (m, 1 H, CHN), 4.55 (t, 1 H, J = 8.4 Hz, CH*H*O), 5.15 (s, 2 H, OCH_2Ph), 5.96 (bs, 1 H, NH), 7.37 (s, 5 H, Ph); ^{13}C-NMR ($CDCl_3$, 200 MHz) δ 39.7, 49.0, 67.2, 69.5, 128.5, 128.7, 128.8, 135.2, 159.0, 170.4. Elemental analysis for $C_{12}H_{13}NO_4$ (235.24): calcd. C 61.27, H 5.57, N 5.95; found C 61.30, H 5.61, N 5.98.

Ac–D–Oxac–OBn (3). A solution of acetyl chloride (5.5 mmol, 0.40 mL) in DMF (0.5 mL) was added dropwise to a stirred solution of oxazolidin-2-one (**2**) (5 mmol, 1.18 g), DIEA (15 mmol, 0.6 mL) and DMAP (2.5 mmol, 0.31 g) in dry DMF (3 mL) The mixture was stirred for 24 h under nitrogen at room temperature, then was diluted with ethyl acetate (50 mL), washed with 1 M aqueous HCl (3 × 30 mL) and 5% aqueous $NaHCO_3$ (1 × 30 mL), dried over sodium sulfate, and concentrated *in vacuo*. The fully protected oxazolidin-2-one (**3**) was obtained pure in 95% yield (0.32 g) as an oil after silica gel chromatography (cyclohexane/ethyl acetate 8 : 2 as eluant). $[\alpha]_D$ = +126.2 (c 1.2, CH_2Cl_2); IR (film): ν 1779, 1733, 1699 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.51 (s, 3 H, $COCH_3$), 2.70 (dd, 1 H, J = 9.3, 16.5 Hz, C*H*HCO), 3.14 (dd, 1 H, J = 3.0, 16.5 Hz, CH*H*CO), 4.21 (dd, 1 H, J = 3.3, 9.2 Hz, C*H*HO), 4.52 (t, 1 H, J = 9.2 Hz, CH*H*O), 4.70–4.78 (m, 1 H, CHN), 5.15

(s, 2 H, OCH_2Ph), 7.29–7.41 (s, 5 H, Ph); ^{13}C-NMR ($CDCl_3$, 200 MHz) δ 23.5, 36.3, 50.4, 66.7, 67.1, 128.1, 128.2, 128.3, 134.9, 153.1, 169.5, 170.0. Elemental analysis for $C_{14}H_{15}NO_5$ (277.27): calcd. C 60.64, H 5.45, N 5.05; found C 60.61, H 5.50, N 5.08.

Ac–D–Oxac–OH (4). To a solution of the fully protected oxazolidin-2-one (**3**) (4 mmol, 1.11 g) in ethyl acetate (20 mL) was added 10% palladium on charcoal (0.10 g) and the mixture was stirred in a Parr apparatus under 3 atm of hydrogen for 1 h. Then, the catalyst was filtered on a celite pad and the mixture was concentrated. The carboxylic acid (**4**) was obtained pure in quantitative yield (0.74 g) without any further purification.

$[\alpha]_D$ = +110.6 (c 0.2, acetone); IR (CH_2Cl_2): ν 3231, 1779, 1719, 1673 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.54 (s, 3 H, $COCH_3$), 2.74 (dd, 1 H, J = 9.6, 17.2 Hz, C*H*HCO), 3.19 (dd, 1 H, J = 2.6, 17.2 Hz, CH*H*CO), 4.23 (dd, 1 H, J = 3.4, 9.2 Hz, C*H*HO), 4.56 (t, 1 H, J = 9.2 Hz, CH*H*O), 4.69–4.84 (m, 1 H, CHN); ^{13}C-NMR (acetone d_6, 200 MHz) δ 23.7, 36.4, 51.5, 68.0, 154.2, 170.5, 171.7. Elemental analysis for $C_7H_9NO_5$ (187.15): calcd. C 44.92, H 4.85, N 7.48; found C 44.96, H 4.78, N 7.50.

Ac–D–Oxac–OPfp (5). To a stirred solution of carboxylic acid (**4**) (2 mmol, 0.37 g) in dry DMF (1 mL) pyridine (2.2 mmol, 0.17 mL) was added, followed by pentafluorophenyl trifluoroacetate (2.5 mmol, 0.52 mL). The reaction was allowed to stir for 45 min at room temperature, then it was diluted with ethyl acetate (50 mL), washed with 0.1 M aqueous HCl (2 × 30 mL) and 5% aqueous $NaHCO_3$ (1 × 30 mL), dried over sodium sulfate, and concentrated *in vacuo*. The pentafluorophenyl ester **5** was obtained in quantitative yield, but it could not be purified by silica gel chromatography. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.58 (s, 3 H, $COCH_3$), 3.08 (dd, 1 H, J = 9.3, 17.4 Hz, C*H*HCO), 3.49 (dd, 1 H, J = 3.3, 17.4 Hz, CH*H*CO), 4.28 (dd, 1 H, J = 3.6, 9.3 Hz, C*H*HO), 4.61 (t, 1 H, J = 9.3 Hz, CH*H*O), 4.80–4.92 (m, 1 H, CHN).

Ac–(D–Oxac)$_2$–OBn (6). Ac–D–Oxac–OPfp (**5**) (2 mmol, 0.82 g) in dry DMF (2 mL) was added in one portion to a stirred solution of H–D–Oxac–OBn (**2**) (1.9 mmol, 0.45 g), DIEA (3.8 mmol, 1.11 mL) and DMAP (0.2 mmol, 24 mg) in dry DMF (3 mL). The reaction was allowed to stir for 16 h at room temperature, then it was diluted with ethyl acetate (50 mL), washed with 0.1 M aqueous HCl (2 × 30 mL) and 5% aqueous $NaHCO_3$ (1 × 30 mL), dried over sodium sulfate and concentrated *in vacuo*. The di-oxazolidin-2-one (**6**) was obtained pure in 70% yield (0.58 g) as a white solid after silica gel chromatography (cyclohexane/ethyl acetate 9 : 1 as eluant). mp = 82–84 °C; $[\alpha]_D$ = +108.3 (c 1.0, CH_2Cl_2); IR (CH_2Cl_2): ν 1790, 1734, 1702 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.53 (s, 3 H, $COCH_3$), 2.74 (dd, 1 H, J = 9.6, 17.2 Hz, C*H*HCO), 3.14 (dd, 1 H, J = 4.0, 17.2 Hz, CH*H*CO), 3.20 (dd, 1 H, J = 8.8, 17.8 Hz, C*H*HCO), 3.63 (dd, 1 H, J = 3.6, 17.8 Hz, CH*H*CO), 4.08 (dd, 1 H, J = 3.6, 9.2 Hz, C*H*HO), 4.26 (dd, 1 H, J = 4.0, 9.6 Hz, C*H*HO), 4.55 (t, 1 H, J = 9.2 Hz, CH*H*O), 4.62 (t, 1 H, J = 9.6 Hz, CH*H*O), 4.71–4.79 (m, 1 H, CHN), 4.82–4.89 (m, 1 H, CHN), 5.15 (AB, 2 H, J = 12.0 Hz, OCH_2Ph), 7.31–7.42 (m, 5 H, Ph); ^{13}C-NMR ($CDCl_3$, 300 MHz) δ 23.6, 36.3, 38.7, 50.5, 50.7, 67.0, 67.6, 67.8, 128.4, 128.5, 128.6, 135.0, 153.0, 153.3, 169.6, 169.9, 170.5. Elemental analysis for $C_{21}H_{28}N_2O_8$ (436.46): calcd. C 57.79, H 6.47, N 6.42; found C 57.76, H 6.51, N 6.45.

Ac–(D–Oxac)$_2$–OH (7). For the synthetic procedure from (**6**) see above the preparation of Ac–D–Oxac–OH (**4**). Yield: 92%; mp = 179–181 °C (dec.); $[\alpha]_D$ = +161.3 (c 1.0, acetone); IR (nujol): ν 3297, 1772, 1752, 1726, 1706, 1686 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.53 (s, 3 H, $COCH_3$), 2.77 (dd, 1 H, J = 8.8, 16.8 Hz, C*H*HCO), 3.08–3.30 (m, 2 H, CH*H*CO + C*H*HCO), 3.67 (dd, 1 H, J = 4.8, 18.4 Hz, CH*H*CO), 4.09 (dd, 1 H, J = 3.6, 9.2 Hz, C*H*HO), 4.28 (dd, 1 H, J = 43.6, 9.0 Hz, C*H*HO), 4.57 (t, 1 H, J = 9.2 Hz, CH*H*O), 4.61 (t, 1 H, J = 9.0 Hz, CH*H*O), 4.68–4.80 (m, 1 H, CHN), 4.82–4.93 (m, 1 H, CHN); ^{13}C-NMR (acetone d_6, 300 MHz) δ 23.7, 36.5, 39.0, 51.5, 51.7, 68.3, 68.5, 154.4, 170.8, 171.2, 172.1. Elemental analysis for $C_{14}H_{22}N_2O_8$ (346.33): calcd. C 48.55, H 6.40, N 8.09; found C 48.52, H 6.43, N 8.12.

Ac–(D–Oxac)$_2$–OPfp (8). For the synthetic procedure from (**7**) see above the preparation of Ac–D–Oxac–OPfp (**5**). Yield: 95%; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.57 (s, 3 H, $COCH_3$), 3.13 (dd, 1 H, J = 9.2, 17.2 Hz, C*H*HCO), 3.23 (dd, 1 H, J = 8.0, 17.6 Hz, C*H*HCO), 3.51 (dd, 1 H, J = 3.2, 17.2 Hz, C*H*HCO), 3.67 (dd, 1 H, J = 4.8, 17.6 Hz, C*H*HCO), 4.11 (dd, 1 H, J = 5.8, 9.0 Hz, C*H*HO), 4.31 (dd, 1 H, J = 3.6, 9.4 Hz, C*H*HO), 4.58 (t, 1 H, J = 9.0 Hz, CH*H*O), 4.66 (t, 1 H, J = 9.4 Hz, CH*H*O), 4.80–4.94 (m, 1 H, CHN).

Ac–(D–Oxac)$_3$–OBn (9). For the synthetic procedure from (**2**) and (**8**) see above the preparation of Ac–(D–Oxac)$_2$–OBn (**6**). Yield 50%; mp = 186–188 °C; $[\alpha]_D$ = +130.2 (c 1.0, CH_2Cl_2); IR (nujol): ν 1772, 1706, 1686 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.52 (s, 3 H, $COCH_3$), 2.74 (dd, 1 H, J = 9.6, 17.2 Hz, C*H*HCO), 3.10 (dd, 1 H, J = 3.9, 17.0 Hz, CH*H*CO), 3.27 (dd, 2 H, J = 8.1, 17.7 Hz, 2 × C*H*HCO), 3.52–3.64 (m, 2 H, 2 × CH*H*CO), 4.03–4.15 (m, 2 H, 2 × C*H*HO), 4.25 (dd, 1 H, J = 3.3, 9.3 Hz, C*H*HO), 4.48–4.62 (m, 3 H, 3 × CH*H*O), 4.72–4.88 (m, 3 H, 3 × CHN), 5.15 (AB, 2 H, J = 12.0 Hz, OCH_2Ph), 7.28–7.42 (m, 5 H, Ph); ^{13}C-NMR ($CDCl_3$, 300 MHz) δ 23.7, 36.4, 38.5, 38.6, 50.5, 50.6, 50.8, 67.1, 67.5, 67.8, 68.0, 128.5, 128.6, 128.7, 135.1, 153.1, 169.6, 169.7, 170.0, 170.1, 170.4, 170.5. Elemental analysis for $C_{27}H_{37}N_3O_{11}$ (579.60): calcd. C 55.95, H 6.43, N 7.25; found C 55.91, H 6.46, 7.21.

Ac–(D–Oxac)$_3$–OH (10). For the synthetic procedure from (**9**) see above the preparation of Ac–D–Oxac–OH (**4**). Yield: 92%. mp = 192–195 °C; $[\alpha]_D$ = +187.5 (c 1.0, CH_2Cl_2); IR (nujol): ν 3510, 1786, 1733, 1693 cm^{-1}; ^{1}H NMR (acetone d_6, 300 MHz): δ 2.41 (s, 3 H, $COCH_3$), 2.87 (dd, 1 H, J = 9.0, 17.1 Hz, C*H*HCO), 3.06 (dd, 1 H, J = 2.7, 17.1 Hz, CH*H*CO), 3.30 (dd, 1 H, J = 9.0, 18.0 Hz, 2 × C*H*HCO), 3.34 (dd, 1 H, J = 9.0, 18.0 Hz, C*H*HCO), 3.56 (dd, 1 H, J = 3.3, 18.0 Hz, 2 × CH*H*CO), 3.58 (dd, 2 H, J = 3.3, 18.0 Hz, 2 × CH*H*CO), 4.19 (dd, 1 H, J = 3.6, 9.0 Hz, C*H*HO), 4.23 (dd, 1 H, J = 3.9, 9.0 Hz, C*H*HO), 4.32 (dd, 1 H, J = 4.5, 9.0 Hz, C*H*HO), 4.52–4.63 (m, 3 H, 3 × CH*H*O), 4.70–4.86 (m, 3 H, 3 × CHN); ^{13}C-NMR (acetone d_6, 300 MHz) δ 23.7, 36.5, 38.9, 39.0, 51.5, 51.6, 51.7, 68.3, 68.5, 68.7, 154.4, 154.6, 170.8, 171.2, 171.4, 172.1. Elemental analysis for $C_{21}H_{28}N_2O_8$ (489.20): calcd. C 49.08, H 6.38, N 8.58; found C 49.11, H 6.35, N 8.62.

Ac–(D–Oxac)$_3$–OPfp (11). For the synthetic procedure from (**10**) see above the preparation of Ac–D–Oxac–OPfp (**5**). Yield: 95%; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.54 (s, 3 H, $COCH_3$), 3.15 (dd, 1 H, J = 8.8, 17.2 Hz, C*H*HCO), 3. 31 (dd, 1 H, J = 8.2, 17.6 Hz, C*H*HCO), 3.36 (dd, 1 H, J = 7.8, 17.6 Hz, C*H*HCO), 3.48 (dd, 1 H, J = 3.4, 17.2 Hz, CH*H*CO), 3.60 (dd, 1 H, J = 4.8, 17.6 Hz, 2 × CH*H*CO), 3.63 (dd, 1 H, J = 4.2, 17.6 Hz, 2 × CH*H*CO), 4.09–4.21 (m, 2 H, 2 × C*H*HO), 4.32 (dd, 1 H, J = 3.6, 9.4 Hz, C*H*HO), 4.51–4.74 (m, 3 H, CH*H*O), 4.81–4.93 (m, 3 H, 3 × CHN).

Ac–(D–Oxac)$_4$–OBn (12). For the synthetic procedure from (**2**) and (**11**) see above the preparation of Ac–(D–Oxac)$_2$–OBn (**5**). Yield 50%. mp = 206–210 °C; $[\alpha]_D$ = +73.3 (c 0.3, CH_2Cl_2); IR (CH_2Cl_2): ν 1791, 1732, 1699 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.52 (s, 3 H, $COCH_3$), 2.75 (dd, 1 H, J = 9.3, 16.5 Hz, C*H*HCO), 3.12 (dd, 1 H, J = 3.0, 16.5 Hz, CH*H*CO), 3.22–3.38 (m, 3 H, 3 × C*H*HCO), 3.50–3.68 (m, 3 H, 3 × CH*H*CO), 4.06–4.18 (m, 3 H, 3 × C*H*HO), 4.25 (dd, 1 H, J = 3.6, 9.3 Hz, C*H*HO), 4.51–4.63 (m, 4 H, 3 × CH*H*O), 4.72–4.90 (m, 4 H, 4 × CHN), 5.15 (AB, 2 H, J = 12.0 Hz, OCH_2Ph), 7.30–7.42 (m, 5 H, Ph); ^{13}C-NMR ($CDCl_3$, 300 MHz) δ 23.7, 36.4, 38.4, 38.6, 38.7, 50.6, 50.7, 50.8, 50.9, 67.1, 67.2, 67.8, 67.9, 68.1, 128.5, 128.6, 128.7, 135.1, 153.1, 153.4, 169.5, 169.6, 169.7, 169.8, 170.0, 170.2, 170.5. Elemental analysis for $C_{33}H_{46}N_4O_{14}$ (722.74): calcd. C 54.84, H 6.42, N 7.75; found C 54.80, H 6.47, N 7.70.

Ac–(D–Oxac)$_4$–OH (13). For the synthetic procedure from (**12**) see above the preparation of Ac–D–Oxac–OH (**4**). Yield: 92%. mp = 149–150 °C (dec.); $[\alpha]_D$ = +117.2 (c 0.1, acetone); IR (nujol): ν 3610, 3523, 1747, 1739, 1735, 1730, 1727, 1687, 1679 cm^{-1}; ^{1}H NMR (acetone d_6, 400 MHz): δ 2.40 (s, 3 H, $COCH_3$), 2.81–2.91

(m, 1 H, C*H*HCO), 3.01–3.08 (m, 1 H, CH*H*CO), 3.20–3.41 (m, 3 H, 3 × C*H*HCO), 3.51–3.63 (m, 3 H, CH*H*CO), 4.10–4.23 (m, 3 H, 3 × C*H*HO), 4.25–4.36 (m, 1 H, C*H*HO), 4.53–4.63 (m, 4 H, 4 × CH*H*O), 4.70–4.78 (m, 1 H, CHN), 4.79–4.85 (m, 3 H, 3 × CHN); ^{13}C-NMR (acetone d_6, 400 MHz) δ 23.1, 35.9, 38.2, 38.3, 38.4, 50.8, 50.9, 51.0, 51.1, 67.6, 67.7, 67.9, 68.0, 153.7, 153.9, 154.0, 170.1, 170.2, 170.6, 170.7, 170.8, 171.0, 171.5. Elemental analysis for $C_{26}H_{40}N_4O_{14}$ (436.46): calcd. C 49.36, H 6.37, N 8.86; found C 49.33, H 6.38, N 8.85.

Computational methods

All calculations were carried out on SGI IRIX 6.5 workstations. Molecular mechanics calculations were performed using the implementation of OPLS force field (OPLS*)[12] within the framework of Macromodel version 5.5.[20] The solvent effect was included by the implicit chloroform GB/SA solvation model of Still *et al.*[13] The torsional space of each molecule was randomly varied with the usage-directed Monte Carlo conformational search of Chang, Guida, and Still.[11] For each search, at least 1000 starting structures for each variable torsion angle were generated and minimized until the gradient was less than 0.05 kJ Å^{-1} mol^{-1}. The cyclic moieties containing the urethane bonds were also included into the search. Duplicate conformations and those with an energy in excess of 25 kJ mol^{-1} 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,[21] with the DFT/B3LYP functional (*i.e.*, the Becke's three parameter hybrid functional with the Lee-Yang-Parr correlation functional)[22] and the 6–31G(d) basis set. This functional and basis set have been shown to properly describe both standard hydrogen bonds,[23] as well as non-classical, weak hydrogen bonds (such as C–H···O=C interactions),[24] and to provide reliable results for the chemical shifts.[16,17] It should be noted that computed data do not directly yield the chemical shift value, but only a value for the isotropic magnetic tensor. The chemical shift values are obtained from the following equations:

$$\delta_H = 32.18 - \sigma_H$$

$$\delta_c = 189.73 - \sigma_c$$

where 32.18 and 189.73 are the calculated isotropic magnetic tensors for the protons and carbons in tetramethylsilane, respectively, and σ_H and σ_c are the calculated isotropic magnetic tensors for the investigated protons and carbons.

Acknowledgements

The authors are grateful to prof. Claudio Toniolo (Department of Chemistry, University of Padova) for stimulating discussions. C. T. and G. L. thank MURST Cofin 2002 ("Sintesi di peptidi ciclici o lineari contenenti amminoacidi inusuali analoghi di arginina, glicina o acido aspartico (RGD) come nuovi inbitori di integrine"), FIRB 2001 ("Eterocicli azotati come potenziali inbitori enzimatici") and 60% (Ricerca Fondamentale Orientata), and the University of Bologna (funds for selected topics "Sintesi e Caratterizzazione di Biomolecole per la Salute Umana") for financial support.

References

1 (*a*) S. H. Gellman, *Acc. Chem. Res.*, 1998, **31**, 173; (*b*) D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes and J. S. Moore, *Chem. Rev.*, 2001, **101**, 3893; (*c*) R. P. Cheng, S. H. Gellman and W. F. DeGrado, *Chem. Rev.*, 2001, **101**, 3219.
2 S. Abele, K. Vögtli and D. Seebach, *Helv. Chim. Acta*, 1999, **82**, 1539.
3 B. R. Huck, J. D. Fisk, I. A. Guzei, H. A. Carlson and S. H. Gellman, *J. Am. Chem. Soc.*, 2003, **125**, 9035.
4 (*a*) S. Lucarini and C. Tomasini, *J. Org. Chem.*, 2001, **66**, 727; (*b*) C. Tomasini, V. Trigari, S. Lucarini, F. Bernardi, M. Garavelli, C. Peggion, F. Formaggio and C. Toniolo, *Eur. J. Org. Chem.*, 2003, 259.
5 F. Bernardi, M. Garavelli, M. Scatizzi, C. Tomasini, V. Trigari, M. Crisma, F. Formaggio, C. Peggion and C. Toniolo, *Chem. Eur. J.*, 2002, **8**, 2516.
6 (*a*) D. Seebach and J. L. Matthews, *J. Chem Soc. Chem. Commun.*, 1997, 2015; (*b*) W. F. DeGrado, J. P. Schneider and Y. Hamuro, *J. Peptide Res.*, 1999, **54**, 206; (*c*) R. P. Cheng, S. H. Gellman and W. F. DeGrado, *Chem. Rev.*, 2001, **101**, 3219.
7 G. Luppi, M. Villa and C. Tomasini, *Org. Biomol. Chem.*, 2003, **1**, 247.
8 (*a*) W. B. Lutz, C. Ressler and D. E. Jr. Nettleton, *J. Am. Chem. Soc.*, 1959, **81**, 167; (*b*) C. W. Jefford and J. Wang, *Tetrahedron Lett.*, 1993, **34**, 1111; (*c*) J.-I. Park, G. R. Tian and D. H. Kim, *J. Org. Chem.*, 2001, **66**, 3696.
9 I. Shin, M. Lee, J. Lee, M. Jung, W. Lee and J. Yoon, *J. Org. Chem.*, 2000, **65**, 7667.
10 M. Green and J. Berman, *Tetrahedron Lett.*, 1990, **31**, 5851.
11 G. Chang, W. C. Guida and W. C. Still, *J. Am. Chem. Soc.*, 1989, **111**, 4379.
12 (*a*) W. L. Jorgensen and J. Tirado-Rives, *J. Am. Chem. Soc.*, 1988, **110**, 1657; (*b*) J. Pranata, S. G. Wierschke and W. L. Jorgensen, *J. Am. Chem. Soc.*, 1991, **113**, 2810.
13 W. C. Still, A. Tempczyk, R. C. Hawley and T. Hendrickson, *J. Am. Chem. Soc.*, 1990, **112**, 6127.
14 L. M. Sandvoss and H. A. Carlson, *J. Am. Chem. Soc.*, 2003, **125**, 15855.
15 (*a*) B. J. Stapley and T. P. Creamer, *Protein Sci.*, 1999, **8**, 587; (*b*) R. Z. Kramer, L. Vitagliano, J. Bella, R. Berisio, L. Mozzarella, B. Brodsky, A. Zagari and H. M. Barman, *J. Mol. Biol.*, 1998, **280**, 623; (*c*) E. Beausoleil and W. D. Lubell, *Biopolymers*, 2000, **53**, 249.
16 (*a*) K. Wolinski, J. F. Hilton and P. Pulay, *J. Am. Chem. Soc.*, 1990, **112**, 8251; (*b*) J. L. Dodds, R. McWeeny and A. J. Sadlej, *Mol. Phys.*, 1980, **41**, 1419; (*c*) R. Ditchfield, *Mol. Phys.*, 1974, **27**, 789; (*d*) R. McWeeny, *Phys. Rev.*, 1962, **126**, 1028; (*e*) F. London, *J. Phys. Radium*, 1937, **8**, 397; (*f*) A. Bagno, *Chem. Eur. J.*, 2001, **7**, 1652.
17 (*a*) A. Bagno, F. Rastrelli and G. Saielli, *J. Phys. Chem. A*, 2003, **107**, 9964; (*b*) C. Bassarello, P. Cimino, L. Gomez-Paloma, R. Riccio and G. Bifulco, *Tetrahedron*, 2003, **59**, 9555; (*c*) J. A. Vila, H. A. Baldoni, D. R. Ripoll and H. A. Scheraga, *J. Biomol. NMR*, 2003, **26**, 113; (*d*) O. Walker, P. Mutzenhardt, P. Tekely and D. Canet, *J. Am. Chem. Soc.*, 2002, **124**, 865; (*e*) M. Ragazzi, P. Carbone and D. R. Ferro, *Int. J. Quant. Chem.*, 2002, **88**, 663; (*f*) M. Buhl, J. Gauss, M. Hofmann and P. V. Schleyer, *J. Am. Chem. Soc.*, 1993, **115**, 12385; (*g*) P. V. Schleyer, J. Gauss, M. Buhl, R. Greatrex and M. A. Fox, *J. Chem. Soc., Chem. Commun.*, 1993, 1766.
18 The experimental chemical shifts were measured in CD_3OD at 600 MHz and were assigned by means of NOESY1D and HSQC experiments.
19 T. Munegumi, Y.-Q. Meng and K. Harada, *Chem. Lett.*, 1989, **62**, 2748.
20 F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caulfield, G. Chang, T. Hendrickson and W. C. Still, *J. Comput. Chem.*, 1990, **11**, 440.
21 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millan, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Patersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malik, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzales, M. Head-Gordon, E. S. Replogle, J. Pople, *Gaussian 98, Revision A.9,* Gaussian Inc., Pittsburgh, PA, 1998.
22 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648.
23 (*a*) L. Gonzalez, O. Mo and M. Yanez, *J. Comput. Chem.*, 1997, **18**, 1124; (*b*) R. A. Klein, *J. Comput. Chem.*, 2002, **23**, 585; (*c*) J. J. Novoa and C. Sosa, *J. Phys. Chem.*, 1995, **99**, 15837.
24 L. Brunel, F. Carre', S. G. Dutremez, C. Guerin, F. Dahan, O. Eisenstein and G. Sini, *Organometallics*, 2001, **20**, 47.