Tetrahedron 65 (2009) 5669–5675

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis and structural study of novel dimethylcyclobutyl β-peptides

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ARTICLE INFO

Article history:
Received 31 March 2009
Received in revised form 11 May 2009
Accepted 18 May 2009
Available online 23 May 2009

Keywords:
β-Peptides
Crystal structure
Hydrogen bonds
Cyclobutanes

ABSTRACT

The synthesis of some representative compounds of a new class of cyclobutane-containing β-peptides starting from (−)-verbenone as a chiral precursor is presented. In these products, the cyclobutane moiety is not a part of the peptide backbone but a bulky substituent at the β^3-position. These compounds have been carefully characterized and studied on the basis of the combined use of several experimental techniques together with molecular modeling by means of theoretical calculations. In the solid state, the non-cyclic β-peptides adopt a hairpin-like molecular folding ruled by intermolecular hydrogen bonds in the crystal packing.

1. Introduction

Designed peptides offer enormous possibilities for the preparation of materials with determined properties because they are chiral compounds that usually adopt well defined secondary and, in some cases, tertiary and quaternary structures.[1] Among them, β-peptides have revealed to be valuable elements in molecular architecture. Their propensity to give sheets, helices or strands, as the main foldings, has been established.[2] They present the advantage with respect to α-peptides that the number of residues usually needed to form foldamers is lower than that required in α-oligomers.

The control of chirality in the monomers combined with the use of carbocycles or other rigid rings, such as oxetanes, incorporated in the peptide backbone[3] has allowed to prepare β-peptides with interesting structural features that include the formation of nano sized fibrils and micelles.[4,5] The obtained results have permitted a better understanding of the combined influence of chirality and conformational flexibility on the molecular and supramolecular arrangement of these compounds. The acquired knowledge has been useful in the preparation of several materials such as nucleic acid mimics[6] and nanotubes.[7]

As the first step to understand this variety of structural features, the synthesis and investigation of small peptides is highly useful. Several studies have been reported on the behavior of β-peptides in solution,[2,3] but data related to their structure in the solid state are scarce. However, the knowledge of the crystal structure of peptides is very important regarding their incorporation in chiral materials. We decided, therefore, to investigate the crystal structures of small cyclobutane-containing β-peptides.

Previously, we had prepared two main types of cyclobutane β-peptides[8] corresponding to structures **1**–**4** in Chart 1. Products **1**[9] and **2**[10] are constituted by oligopeptides derived from (1*R*,2*S*)-2-aminocyclobutane-1-carboxylic acid, and β-alanine units that, in the case of tetrapeptide **2**, are joined in alternation. On the contrary, diastereomeric dipeptides **3** and **4** result from the direct coupling of two cyclobutane residues with the same or opposite chirality, respectively.[11] Tetrapeptide **2** presents a 14-helix type folding by formation of an intramolecular hydrogen bond as shown in Chart 1. Although this compound is a solid, unfortunately crystals were not good enough for X-ray structural study. Dipeptides **1**, **3**, and **4** presented intramolecular hydrogen bonds in solution giving cis-fused [4.2.0]octane structural units, in the same way that a recently described tetrapeptide constituted by four cyclobutane-containing residues.[5] In a clear contrast, these peptides in the solid state are intermolecularly hydrogen bonded yielding infinite antiparallel chains. The crystal packing determines a hairpin-like folding for these molecules.

In a recent publication,[12] we reported on the efficient and stereoselective addition of *N*-benzyl hydroxylamine to chiral alkenoates affording *N*-benzyl isoxazolidinones as single stereoisomers.

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doi:10.1016/j.tet.2009.05.039

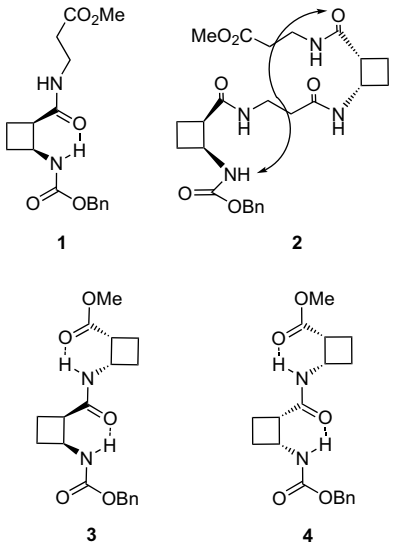


Chart 1. Representative features of cyclobutane β-peptides.

These compounds yield, upon hydrogenation, the corresponding β-amino acids in good yields.

In this work, we have designed and synthesized some β-peptides containing a dimethylcyclobutyl unit at the β^3-position, which is not incorporated in the peptide backbone. It is a bulk substituent that could afford rigidity to these cyclobutane-containing molecules. These new compounds have been synthesized from isoxazolidinones, prepared, in turn, from (−)-verbenone as a chiral precursor providing the dimethylcyclobutyl motif. We have also been successful in synthesizing the first cyclic cyclobutane-containing β-peptide in order to compare it with the open-chain products.

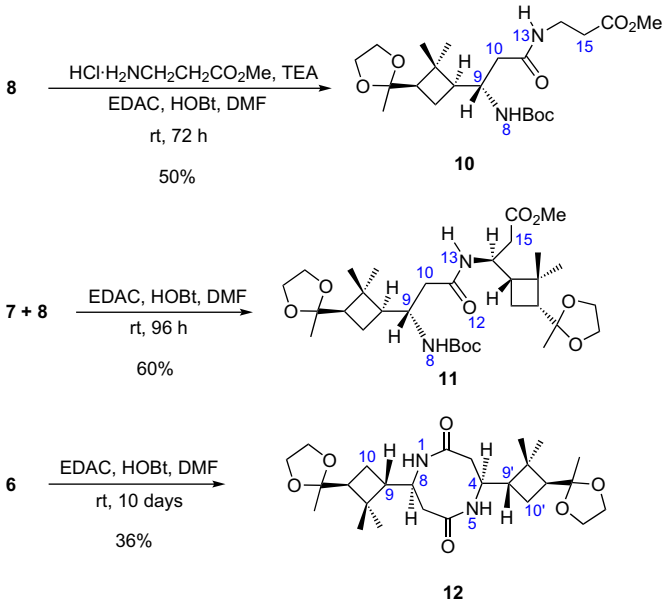
These β-dipeptides have been characterized and studied by means of NMR and IR spectroscopies,[13] circular dichroism (CD), X-ray structural analysis, and theoretical calculations. Determination of the molecular conformation and the crystal packing in the solid state has been also accomplished.

2. Results and discussion

Selectively protected β-amino acids were synthesized to be incorporated into the different β-peptides. For this purpose, we employed as a starting material the isoxazolidone **5** prepared from (−)-verbenone as previously described.[12] One-pot hydrogenolysis of the N–O and the *N*-benzyl bonds, in the presence of palladium hydroxide as a catalyst, gave free amino acid **6**. Interestingly, this compound could be chemoselectively methylated by reaction with diazomethane to provide amino ester **7** (Scheme 1).

Alternatively, catalytic hydrogenation of **5** in the presence of $(Boc)_2O$ afforded *tert*-butyl carbamate **8**. Both compounds **7** and **8** are suitably protected for their selective incorporation into oligomers. Furthermore, methylation of **8** led to the totally protected amino acid **9**.

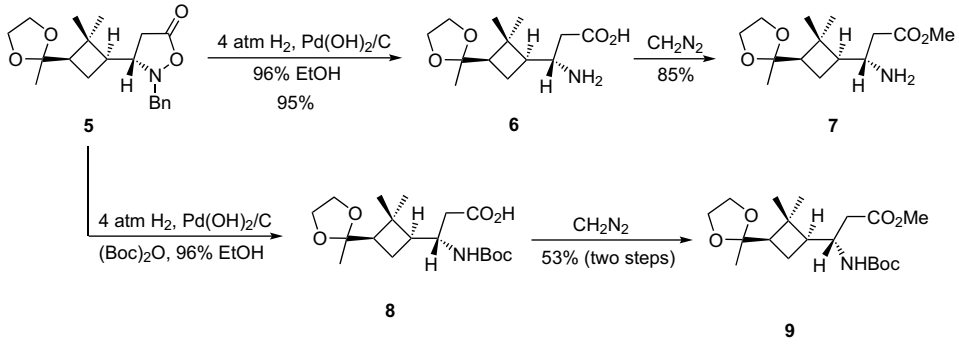
Then we prepared dipeptides **10**–**12** (Scheme 2). Presumably, compound **10** must display greater flexibility than the monomer **9**, the dipeptide **11** is intended to be conformationally restricted and cyclic dimer **12** must be highly rigid.



Scheme 2. Synthesis of β-peptides **10**–**12**.

The synthesis of the β-peptides was carried out as follows (Scheme 2). Acid **8** was coupled with β-Ala–OMe by treatment with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDAC) as dehydrating agent and 1 equiv of 1-hydroxybenzotriazole (HOBt) as a catalyst in anhydrous DMF solution at room temperature for 72 h. Dipeptide **10** was thus obtained in 50% yield after purification by column chromatography. In turn, bis(dimethylcyclobutyl) dipeptide **11** was prepared in 60% yield by coupling of acid **8** with amine **7** under similar conditions as before but for 4 days. The synthesis of cyclic **12** was accomplished in 36% yield by self-coupling of two molecules of free amino acid **6**. Longer reaction time (ca. 10 days) was required in this case to achieve the cyclization.

These new products are solid and were fully characterized. Circular dichroism (CD) in MeOH for dipeptide **10** showed a band at 208 nm while a positive Cotton effect, with bands at 205 and 225 nm, was observed for dipeptides **11** and **12** (see Supplementary data). IR spectra of the solids **10** and **12** displayed only one band centered at 3300 cm^{-1}. This band arises from the intermolecularly bonded



Scheme 1. Synthesis of conveniently protected β-amino acids.

amide and carbamate protons as can be established from X-ray studies (see below). Otherwise, IR spectra of dipeptides **10** and **12** at different concentrations in chloroform solution showed well defined bands at 3450 cm^{-1} and a shoulder at 3300 cm^{-1}. At 5 mM concentration, the existence of intermolecular aggregates can be excluded as shown by ^1H NMR. Then, the weak band at lower frequency can be attributed to intramolecularly bonded N*H* protons. The carbonyl region could not be investigated due to the signal overlap.

Complete ^1H and ^{13}C NMR chemical shift assignments for all dipeptides **10**–**12** were performed. In addition, we have considered the use of NOE data to account for the conformational bias in solution of the non-cyclic compounds **11** and **12**, as similarly reported by related peptides.[5,10,11] Theoretical calculations were also carried out to model the most stable conformations for **10** and **11**.

For both compounds, a conformational search afforded three representative structures, which were optimized at the B3LYP/6-31G(d) level of calculation. The geometries of the conformers of **10** were optimized both in the gas phase and in chloroform solution but only slight changes were observed for the two media. Therefore, geometries for **11** were optimized only in the gas phase. Figure 1 shows the structures of the conformers of **10** and Table 1 presents the relative energies of the most stable conformers of both dipeptides. The conformers of **11** are similar to those obtained for **10** and their structures can be found as Supplementary data.

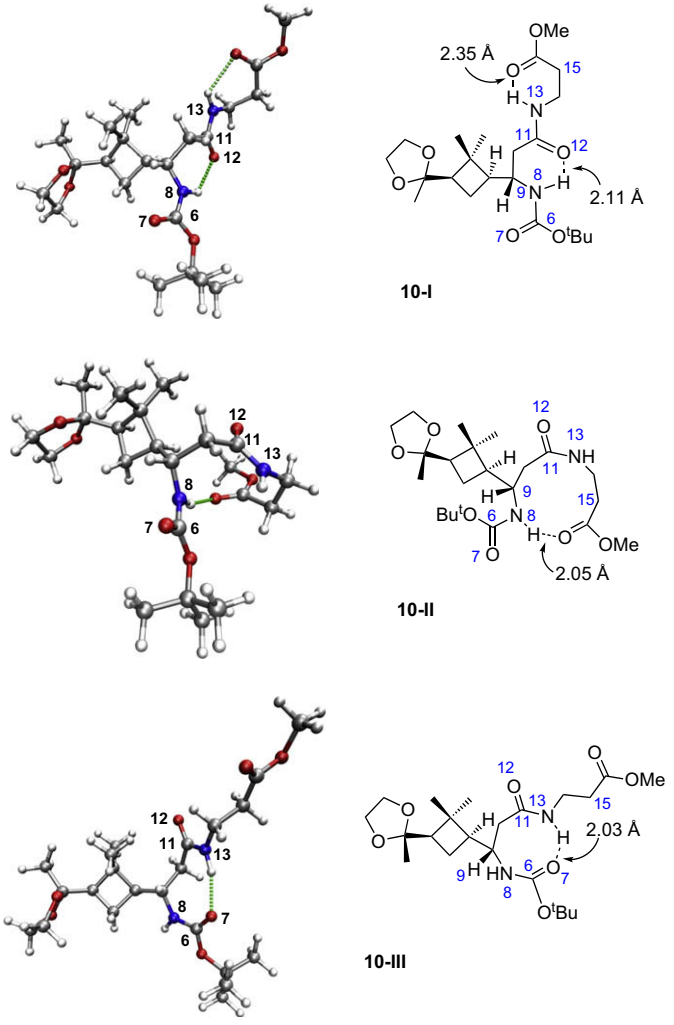


Figure 1. DFT calculated structures for the most stable conformers of dipeptide **10** in chloroform solution.

Table 1
Relative energies[a] obtained at B3LYP/6-31G(d) level of theory in the gas phase and in chloroform solution for conformers of dipeptides **10** and **11**

	Conformer	*E* (gas phase)	*E* (chloroform)[b]
10	**I**	0.0	0.0 (0.0)
	II	0.7	0.8 (0.7)
	III	2.4	2.5 (2.2)
11	**I**	0.0	0.0
	II	1.3	2.0
	III	2.9	4.0

[a] Relative to the most stable conformer. In kcal mol^{-1}.
[b] In parentheses, values obtained for geometries optimized in solution.

For dipeptide **10**, the most stable conformer, **10-I**, presents two hydrogen bonds. The strongest one, between N*H*8 and O12, was already present in **9** while the second one takes place between N*H*13 and O17. These hydrogen bonds lead two six-membered rings. The other two conformers present only one hydrogen bond. For **10-II** it involves N*H*8 and the carbonyl of the methyl ester group, leading to the formation of a 10-membered ring. Finally, in **10-III** we can observe an eight-membered ring due to the hydrogen bond between N*H*13 and O7.

The computed energy difference in chloroform solution between **10-I** and **10-II** is only 0.8 kcal mol^{-1}, while **10-III** lies 2.5 kcal mol^{-1} above **10-I** (see Table 1). This result is in good agreement with the results of Baquero et al. for other β-peptides.[14] In this case, the combined C10 and C6/C6 conformers made up about two-thirds of the population. Moreover, the predominance of C6/C6 confomers has been also predicted by theoretical calculations on some open-chain models.[3e]

Regarding dipeptide **11**, the energy differences between the most stable three conformers (4.0 kcal mol^{-1}) increase with respect to dipeptide **10** (2.5 kcal mol^{-1}). This result reflects a larger degree of conformational rigidity in **11** due to the presence of the second cyclobutane ring.

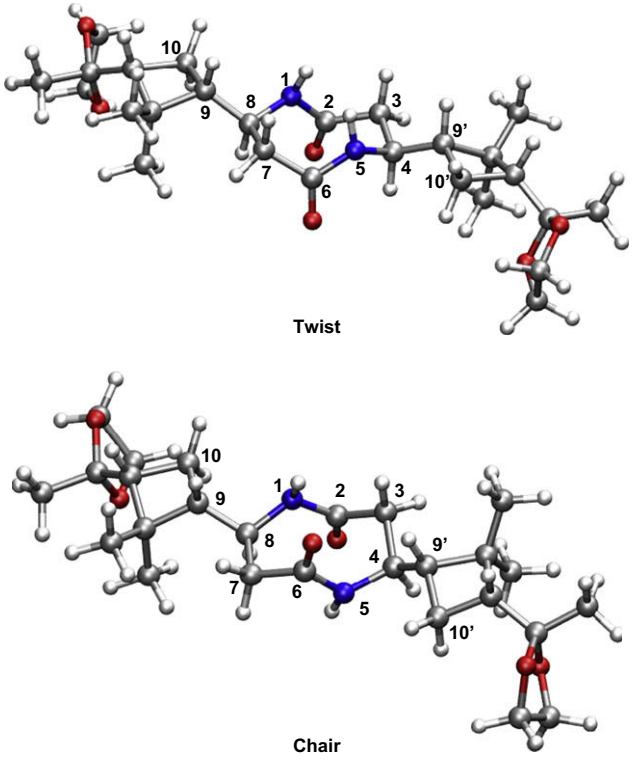


Figure 2. DFT calculated structures for the twist and chair conformers of dipeptide **12**.

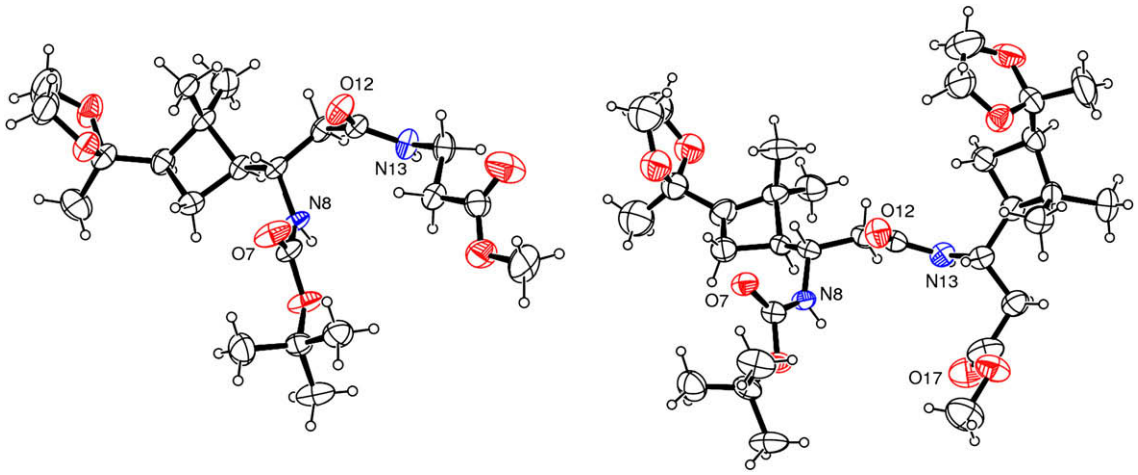


Figure 3. Molecular conformations of dipeptides **10** and **11** (from left to right) in the solid state.

The 2D NOESY spectrum of **11** was recorded in both $CDCl_3$ and benzene-d_6 solutions to disclose the chemical shifts and NOE contacts for all relevant protons (see Supplementary data). The most significant NOEs conclude that the same geometry is retained in both different solvents. The high diastereotopicity and the line-shape broadening shown with temperature increasing for the methylene protons H10a,b (δ=2.11 and 2.43 in $CDCl_3$) also accounts for the presence of some fluxional process in solution.

Conformational analysis of cyclic dimer **12** was also performed by ^{1}H NMR and theoretical calculations. The high rigidity of this cyclic C_2-symmetric molecule can be realized by the noticeable diastereotopicity shown by the α-carbonyl methylene protons, which appear at δ=2.33 and 2.89, respectively, in the ^{1}H NMR spectrum in $CDCl_3$, while the N*H* proton resonates at 6.48 ppm, which is well resolved as a large doublet. The 2D NOESY pointed out that protons H4/H8 are close to the methylene protons H3/H7 but NOE between N*H* and other any proton was not observed (see Scheme 2 for atom numeration). As mentioned above, the CD spectrum also accounts for the conformational restriction showing a strong Cotton effect. This spectrum is closely related to those of conformationally constrained 2,2-dimethylcyclobutane substituted α-dipeptides prepared in our laboratory and recently described.[15]

The macrocycle of dipeptide **12** may present two different conformations, which may be named as chair and twist by analogy with (*E*,*E*)-1,5-cyclooctadiene.[16] Figure 2 shows the optimized structures of the most stable twist and chair conformers. The twist conformer is more stable than the chair one by 2.0 kcal mol^{-1} in chloroform. In the twist conformer both N*H* protons are trans with respect to C*H*4 and C*H*8, respectively. On the other hand, in the chair structure N*H*1 is trans with respect to C*H*8, but N*H*5 is cis with respect to C*H*4. In the ^{1}H NMR spectrum of **12** both N*H*/C*H* coupling constants present the same value (10.8 Hz). This result is in good agreement with a twist conformation for the macrocycle.

As mentioned above, all three β-peptides are solids. For dipeptide **12**, although TEM analysis revealed the existence of microcrystals, the obtained crystals were not suitable for X-ray studies (see Supplementary data). A detailed study on **10** and **11** was carried out allowing us the determination of the molecular conformation in the solid state and the main features of the crystal packing for each dipeptide.

Crystal structure of compound **10** has been determined from single crystal X-ray diffraction data. Suitable crystals were obtained from dichloromethane–pentane solution. In the case of compound **11**, to obtain adequate single crystals was more difficult. Finally, its crystallization from methanol afforded middling quality single crystals whose structure proved to be a monohydrate of **11**.

These two peptides, as well as the previously described bis (cyclobutane) β-dipeptides,[11] adopt hairpin-like foldings in the

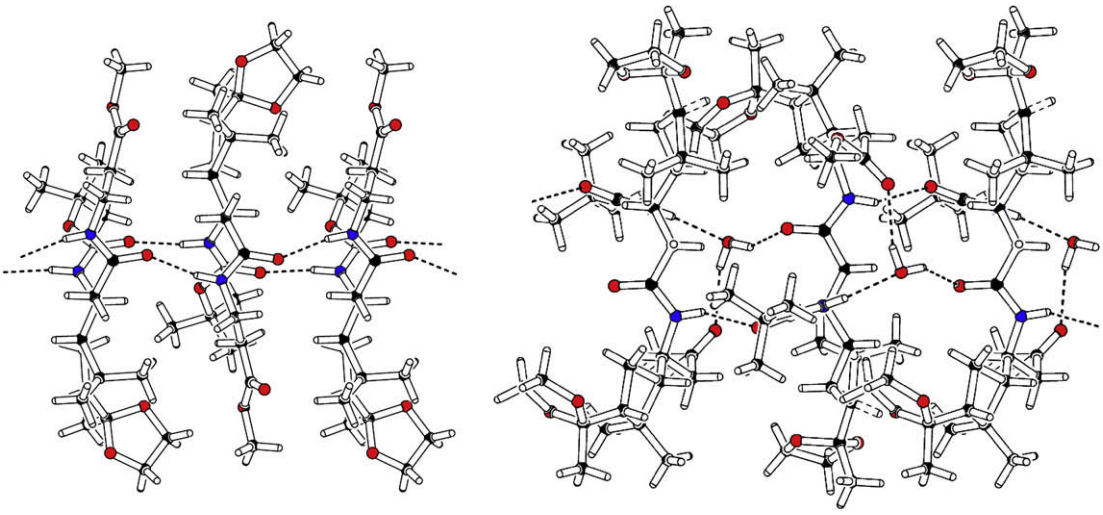


Figure 4. Intermolecular hydrogen bonds forming infinite chains in the crystal structure of dipeptides **10** and **11** (from left to right).

Table 2
Geometries of the intermolecular hydrogen bonds forming infinite chains in the crystal structures of dipeptides **10** and **11**[a]

Dipeptide **10**			
N13···O12(*s*)[b]	H13···O12(*s*)[b]	N13–H13···O12(*s*)[c]	*s*: *x*,−*y*+3/2,*z*−1/2
2.831(5)	1.83	164	
N8···O7(*s*)[b]	H8···O7(*s*)[b]	N8–H8···O7(*s*)[c]	*s*: *x*,−*y*+3/2,*z*−1/2
2.861(4)	1.84	169	
Dipeptide **11**			
N13···O7(*s*)[b]	H13···O7(*s*)[b]	N13–H13···O7(*s*)[c]	*s*: −*x*,*y*−1/2,−*z*+1/2
2.956(5)	2.06	144	
N8···Ow(*s*)[b]	H8···Ow(*s*)[b]	N8–H8···Ow(*s*)[c]	*s*: −*x*,*y*−1/2,−*z*+1/2
2.991(6)	1.99	164	
Ow···O17(*s*)[b]	Hw1···O17(*s*)[b]	Ow–Hw1···O17(*s*)[c]	*s*: −*x*,*y*+1/2,−*z*+1/2
2.956(7)	2.04	164	
Ow···O12[b]	Hw2···O12[b]	Ow–Hw2···O12[c]	
2.832(6)	2.01	146	

[a] *s* is the symmetry code of the neighbor molecule; w denotes that the atom belongs to a water molecule. N–H (1.03 Å), Ow–H (0.94 Å), and the rest of parameters involving hydrogens have been normalized.
[b] In angstroms.
[c] In degrees.

solid state (Fig. 3). These conformations are ruled by intermolecular hydrogen bonds in the crystal packing. In this way, crystal structure of **10** contains infinite parallel chains of molecules linked by hydrogen bonds involving amide groups (Fig. 4, left). Each amide group adopts a trans disposition and acts both as donor (N–H) and as acceptor group (C=O). So, each molecule is linked to its neighbor in the chain by two NH···O=C hydrogen bonds (Table 2).

In the hydrate of **11**, the crystal structure also contains infinite parallel chains of molecules linked by hydrogen bonds with the water molecules inserted in them in such a way that two neighbor molecules in the chain, M1 and M2, are bonded by a (M1)NH···O=C(M2) hydrogen bond and the water molecule is bonded to M1 by (M1)NH···Ow and OwH···O=C–OMe(M1) bonds, and to M2 by OwH···O=CNH(M2) bonds (Fig. 4, right). The parameters listed in Table 2 illustrate these features.

Although the number of crystal structures described for β-peptides is limited (about 30), the molecular and supramolecular arrangements described herein seems to be frequent in short β^3-substituted β-peptides.[17] In these cases, H-bonds point in the same direction along the chain axis and, therefore, chains are polar. This arrangement, i.e., folded conformers with no intramolecular H-bonds and linked by intermolecular NH···O=C hydrogen bonds into an infinite chain, has also been found in constrained β-peptides where Cα–Cβ are included in a cyclobutyl ring.[11] In these compounds, however, the two H-bonds of every conformer point in opposite directions and, as a result, chains are not polar.

3. Conclusion

In summary, we have synthesized and studied three new dimethylcyclobutyl β-dipeptides with different types of structures. Among them, we describe the first cyclic cyclobutane-containing β-peptide. The possibility to easily transform the side-chain of the cyclobutane ring in various functional groups[8a] gives access to a great diversity of substituted-cyclobutyl chiral β-peptides. The structural analysis of **10** and **11** has been achieved by experimental techniques in conjunction with molecular modeling. Although theoretical calculations predict intramolecularly hydrogen bonded conformations in solution, dipeptides **10** and **11** do not display a well defined preferential bias. The most rigid compound **12** preferentially adopts a twist conformation. In the solid state, dipeptides **10** and **11** adopt a hairpin-like conformation ruled by the intermolecular hydrogen bonds in the crystal packing.

4. Experimental section

4.1. Computational details

The Monte Carlo conformational search[18] was done using the MMFF force field[19] implemented in the Macromodel 7.0 program.[20] The solvent effect has been included using the GB/SA method[21] using chloroform as solvent. Within a range of 5 kcal mol^{-1} the number of conformers was 70 for **9**, 510 for peptide **10**, and 424 for peptide **11**. For peptide **12** both a twist and chair conformation of the macrocycle were considered. The number of conformers within a range of 5 kcal mol^{-1} was 26 and 47, respectively. Geometries of the most representative structures were optimized using the B3LYP density functional method[22] with the 6-31G(d) basis set.[23] Harmonic vibrational frequencies were calculated in order to verify that these structures correspond to energy minima (all frequencies are real). These calculations were carried out using the Gaussian-03 program.[24] Single point calculations in chloroform using the continuum model[25] implemented in the Jaguar 5.5 program[26] were also done for the structures optimized in the gas phase. The geometries of **9** and of the conformers of **10** were also optimized including the effect of the solvent.

4.2. Crystal structure analysis

CCDC-681292 (**10**) and CCDC-681293 (**11**·H_2O) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

4.3. NMR spectroscopy

NMR Data were collected on Bruker 250 MHz, 360 MHz, and 500 MHz spectrometers. All products were structurally characterized using 1D and 2D $^1H/^{13}C$ NMR methods. Variable-temperature experiments were also recorded when necessary. See text and Supplementary data for more experimental details.

4.4. Synthesis and purification of amino acids and peptides

4.4.1. (3S,1′S,3′R)-3-tert-Butoxycarbonylamino-3-[2′,2′-dimethyl-3′-(2-methyl-1,3-dioxolan-2-yl)cyclobutyl]propanoic acid, ***8***

A mixture of oxazolidinone **5**[12] (850 mg, 2.5 mmol), 20% $Pd(OH)_2$–C (214 mg), and $(BOC)_2O$ (700 mg, 3.2 mmol) in 96% EtOH (72 mL) was hydrogenated at room temperature under 4 atm of pressure. The reaction mixture was filtered through Celite, and the solvent was removed at reduced pressure to afford compound **8** (870 mg, 99% yield), which was pure enough to be used in the next steps without additional purification. For characterization purposes, a portion was chromatographed on Baker silica gel (1:6 hexane–EtOAc as eluent) to afford a colorless oil. $[\alpha]_D$ +6.1 (*c* 2.5, CH_2Cl_2); IR: 3424, 2981, 1718, 1709 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ=1.13 (s, 6H), 1.23 (s, 3H), 1.42 (s, 9H), 1.66–2.10 (complex absorption, 4H), 2.26–2.66 (complex absorption, 2H), 3.74–4.07 (m, 4H), 5.04 (d, *J*=10.8 Hz), 8.66 (br s, 1H); ^{13}C NMR (90 MHz, $CDCl_3$): δ=15.9, 21.6, 23.3, 27.93, 3.26, 40.6, 44.4, 47.8, 48.6, 62.9, 64.8, 78.6, 108.9, 140.6, 176.1; HRMS (ESI) calcd for M+Na: 380.2044, found: 380.2044.

4.4.2. Methyl (3S,1′S,3′R)-3-tert-butoxycarbonylamino-3-[2′,2′-dimethyl-3′-(2-methyl-1,3-dioxolan-2-yl)cyclobutyl]propanoate, ***9***

A solution of diazomethane (600 mg, 14.3 mmol) in CH_2Cl_2 (40 mL) was distilled onto a stirred solution of crude acid **8** (1 g, 2.8 mmol) in CH_2Cl_2 (10 mL). After stirring at room temperature for 1 h, excess diazomethane was destroyed by adding methanol and solvents were removed. The residue was chromatographed on

Baker silica gel (3:2 hexane–EtOAc as eluent) to afford product **9** as solid (480 mg, 53% yield for the two steps). Crystals, mp 93–95 °C (from ether–pentane); $[\alpha]_D$ +22.6 (*c* 0.7, CH_2Cl_2); IR 3432, 2978, 1721, 1701, 1500 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ=1.08 (complex absorption, 3H), 1.11 (complex absorption, 3H), 1.21 (s, 3H), 1.41 (s, 9H), 1.68–1.91 (complex absorption, 3H), 2.02 (dd, *J*=7.5 Hz, *J*′=11.4 Hz, 1H), 2.29 (dd, *J*=5.6 Hz, *J*′=15.7 Hz, 1H), 2.55 (dd, *J*=3.7 Hz, *J*′=15.7 Hz, 1H), 3.65 (s, 3H), 3.78–3.98 (m, 5H), 4.96 (d, *J*=10 Hz); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ=16.5, 22.2, 23.5, 28.1, 31.0, 38.2, 40.3, 44.9, 48.2, 48.7, 51.3, 63.4, 65.2, 78.7, 109.5, 155.3, 171.9; elemental analysis calcd for $C_{19}H_{33}NO_6$: C 61.43, H 8.95, N 3.77; found: C 61.47, H 9.01, N, 3.72.

4.4.3. Methyl (3*S*,1′*S*,3′*R*)-3-amino-3-[2′,2′-dimethyl-3′-(2-methyl-1,3-dioxolan-2-yl)cyclobutyl]propanoate, **7**

Following the same protocol described above for the preparation of ester **9**, amino ester **7** was quantitatively obtained from amino acid **6**[11] as yellow oil that decomposed under the usual chromatographic conditions. $[\alpha]_D$ −10.8 (*c* 2.5, CH_2Cl_2); IR: 3379, 2955, 1733 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$): δ=1.09–1.27 (complex absorption, 9H), 1.56–2.48 (complex absorption, 4H), 3.11 (m, 1H), 3.72 (s, 3H), 3.81–4.02 (complex absorption, 4H); ^{13}C NMR (90 MHz, $CDCl_3$): δ=16.5, 22.6, 23.4, 31.4, 40.2, 48.7, 49.3, 51.5, 63.6, 65.5, 109.3, 172.9; HRMS (ESI) calcd for M+Na: 294.1676; found: 294.1671.

4.5. General procedure for peptide coupling

A standard reaction is described as follows for the synthesis of **10**. β-Ala–OMe hydrochloride (320 mg, 2.3 mmol), DEC (1 g, 5.2 mmol), dry Et_3N (1 mL), and HOBt (162 mg, 1.2 mmol) were successively added to a solution of crude **8** (620 mg, 1.7 mmol) in dry DMF (65 mL). The mixture was stirred at room temperature under nitrogen atmosphere for 72 h. Then EtOAc (50 mL) was added and the resultant solution was washed with saturated aqueous $NaHCO_3$ (3×45 mL). The organic layers were dried over $MgSO_4$ and solvents were removed at reduced pressure. The residue was chromatographed through Baker silica gel (EtOAc as eluent) to afford peptide **10** (310 mg, 50%). Following the same procedure, peptide **11** was obtained in 60% (ca. 96 h). For peptide **12**, different portions of DEC (5.7 g, 30 mmol), dry Et_3N (4.2 mL) and HOBt (2 g, 15 mmol) were successively added to a solution of crude **6** (850 mg, 3.3 mmol) in dry DMF (200 mL). After 10 days, peptide **12** was obtained in 36% yield.

4.5.1. Dipeptide ***10***

Yield 50%. Crystals, mp 130–132 °C (from CH_2Cl_2–pentane); $[\alpha]_D$ +57.1 (*c* 0.31, CH_2Cl_2); IR: 3305, 2953, 1737, 1685, 1650, 1536 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ=1.07–1.08 (complex absorption, 6H), 1.2 (s, 3H), 1.4 (s, 9H), 1.63–2.03 (complex absorption, 4H), 2.14 (dd, *J*=5.3 Hz, *J*′=14.5 Hz, 1H), 2.41 (dd, *J*=2.6 Hz, *J*′=14.5 Hz, 1H), 2.51 (t, *J*=6 Hz, 2H), 3.46 (m, 2H), 3.66 (s, 3H), 3.73–3.96 (complex absorption, 5H), 5.21 (d, *J*=6.5 Hz, 1H), 6.45 (m, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ=16.5, 22.2, 23., 28.0, 31.3, 33.5, 34.9, 40.2, 40.2, 45.2, 48.5, 48.7, 51.4, 63.4, 65.3, 79.2, 109.9, 156.1, 170.7, 172.6; elemental analysis calcd for $C_{22}H_{38}N_2O_7$: C 59.71, H 8.65, N 6.33; found: C 59.69, H 8.83, N 6.29.

4.5.2. Dipeptide ***11***

Yield 60%. Crystals, mp 80–83 °C (from EtOH); $[\alpha]_D$ +91.0 (*c* 0.72, CH_2Cl_2); IR: 3314, 2950, 1693, 1658, 1493 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=1.09–1.11 (complex absorption, 12H), 1.21 (s, 6H), 1.41 (s, 9H), 1.62–2.02 (complex absorption, 8H), 2.11 (dd, *J*=4.35 Hz, *J*′=15.75 Hz, 1H), 2.34 (dd, *J*=5.25 Hz, *J*′=15.75 Hz, 1H), 2.43 (dd, *J*=3.45 Hz, *J*′=14.85 Hz, 1H), 2.52 (dd, *J*=3.5 Hz, *J*′=16.15 Hz, 1H), 3.66 (s, 3H), 3.75–3.98 (complex absorption, 9H), 4.24 (m, 1H), 5.30 (d, *J*=10.45 Hz, 1H), 6.26 (d, *J*=10.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=16.8, 22.7, 23.7, 28.3, 31.5, 38.0, 40.5, 44.5, 46.7, 49.0, 51.7, 63.7, 65.5; elemental analysis calcd for $C_{32}H_{54}N_2O_9$: C 62.93, H 8.91, N 4.59; found: C 62.22, H 8.84, N 4.45.

4.5.3. Dipeptide ***12***

Yield 36%. Crystals, mp 260–263 °C; $[\alpha]_D$ −10.14 (*c* 0.69, CH_2Cl_2); IR: 3337, 2968, 1656, 1461 cm^{-1}; 360-MHz ^1H NMR ($CDCl_3$) δ 1.13–1.23 (complex absorption, 18H), 1.57 (dd, *J*=9.72 Hz, *J*′=20.52 Hz, 2H), 1.80–1.87 (m, 2H), 1.97–2.05 (m, 2H), 2.13 (dd, *J*=7.5 Hz, *J*′=11.3 Hz, 2H), 2.33 (dd, *J*=10.8 Hz, *J*′=15.7 Hz, 2H), 2.89 (dd, *J*=6 Hz, *J*′=14.6 Hz, 2H), 3.66 (m, 1H), 3.78–4.01 (m, 8H), 6.18 (d, *J*=15.12 Hz, 1H); 90-MHz ^{13}C NMR ($CDCl_3$) δ 16.8, 22.9, 23.5, 31.1, 40.1, 42.9, 47.8, 48.4, 50.6, 63.3, 65.1, 109.2, 171.9. Anal. Calcd for $C_{32}H_{54}N_2O_9$: C, 65.25; H, 8.84; N, 5.85. Found: C, 64.98; H, 8.99; N, 5.22; HRMS (ESI) calcd for M+Na: 501.2935; found 501.2942.

Acknowledgements

Financial support from Ministerio de Educación y Ciencia (MEC) (CTQ2006-01080, CTQ2007-61704/BQU) and Generalitat de Catalunya (GC) (2005SGR-103) is gratefully acknowledged. We thank NMR and X-ray Services of the UAB for the use of their facilities. E.T. and C.A.-S. acknowledge to GC and MEC, respectively, for predoctoral fellowships.

Supplementary data

^1H and ^{13}C NMR spectra for new products **7**–**11**; NMR studies for compounds **9**–**11**; CD spectra of **9**–**12**; TEM images of **12**; computed structures of amino acid **9** and conformers of dipeptides **10**–**12**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.05.039.

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