Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 3569–3584

TETRAHEDRON: *ASYMMETRY*

Enantioselective synthetic approaches to cyclopropane and cyclobutane β-amino acids: synthesis and structural study of a conformationally constrained β-dipeptide

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Received 21 July 2000; accepted 28 July 2000

Abstract

Synthetic approaches to carbocyclic compounds, namely cyclopropane and cyclobutane β-amino acids, are presented. One of them is based on enzymatic desymmetrization of meso diesters, leading to the enantioselective production of *cis*-hemiesters, which afforded β-amino acids through Curtius rearrangements. The enantiomeric excess for the cyclobutane derivatives was 91% whereas the cyclopropanes were obtained in 63% ee. According to another strategy, an enantiomerically pure cyclopropane *trans*-β-amino acid, bearing a quaternary center, has been synthesized from a homochiral precursor easily available from D-glyceraldehyde. The preparation and structural investigation of the first synthesized cyclobutane containing dipeptide is also described. A hairpin-like conformation of this molecule in the solid state has been demonstrated by X-ray structural analysis, showing crystal packing induced by the presence of the rigid cyclobutane moiety and the formation of intermolecular hydrogen bonds. NMR experiments confirmed that these molecules also tend to produce aggregates in solution. On the contrary, theoretical calculations suggest that intramolecular interactions are important in the gas phase, as expected. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

β-Amino acids are a widespread class of non-proteinogenic amino acids found in nature in free form or as a part of peptidic products with antibiotic, antifungal, cytotoxic and other pharmacological properties. Among the non-peptidic compounds, β-lactams are prominent and

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PII: S0957-4166(00)00297-4

include antibiotics and other medicinal agents. β-Amino acids are also key structural components of important products such as the potent enzyme inhibitors statins and the anticancer agent taxol (Paclitaxel®).[1]

These amino acids have recently been the object of a renewed interest since the pioneer works of Seebach[2] and Gellman[3] showing that polymers composed of β-amino acids, i.e. β-peptides, can fold into a stable helical secondary structure analogous to the α-helix in proteins.[4] Thus, in the last 4 years, it has been shown that cyclic as well as acyclic peptides composed of β-amino acid residues adopt turn, helical, and sheet-like conformations.[5] Theoretical studies of β-peptide models have also been reported.[6] Moreover, some peptides consisting of β-amino acids display interesting biological properties, such as high stability against degradation by proteases,[2,7] conferring on them a great potential as drug candidates.[8]

Consequently, the excitement about the promising biological and pharmacokinetic properties of β-peptides has stimulated a strong synthetic activity in the field of the corresponding building blocks, i.e. the β-amino acids.[9]

Conformationally constrained α- and β-amino acids have been incorporated into peptide surrogates to be used in structural and biomechanistic investigations as well as to obtain peptides with new or improved properties.[10] With this purpose in mind, in contrast to α-amino acids, the use of carbocyclic β-amino acids is very limited and there are only a few examples on the incorporation of cyclopropane,[11] cyclopentane[4b] or cyclohexane[3] residues in peptidic chains and, as far as we know, there are no examples of cyclobutane containing β-peptides. Furthermore, among the large number of general synthetic methods used to prepare β-amino acids,[12] 2-aminocyclopropane-[11,13a] and 2-aminocyclobutane-1-carboxylic acids[13b] have been synthesized only in racemic form.

As part of our research program on the synthesis and structural investigation of carbocyclic amino acids and related peptidomimetics,[14] we envisaged the synthesis of cyclopropane and cyclobutane β-amino acids[15] as well as their incorporation into new β-oligomers to be studied from structural and biological points of view.

In this paper, we report two synthetic approaches to such amino acids. The first one is based on enzymatic desymmetrization of carbocyclic meso diesters and affords *cis*-derivatives. The use of a chiral precursor in the synthesis of a cyclopropane β-amino acid with *trans* stereochemistry is also described. The enantio- and diastereomeric purity of the synthesized products has been determined by NMR and by HPLC using non-commercial chiral stationary phases. These amino acids are conveniently protected for their latter incorporation into peptidic chains. As an illustrative example, we describe herein the synthesis and the structural determination, using NMR and CD spectroscopies and by X-ray crystallography, of the first cyclobutane containing β-peptide which results from the coupling between (1*R*,2*S*)-2-(*N*-Cbz-amino)cyclobutane-1-carboxylic acid with β-Ala-OMe. Results of theoretical calculations simulating its structural features in the gas phase are also reported.

2. Results and discussion

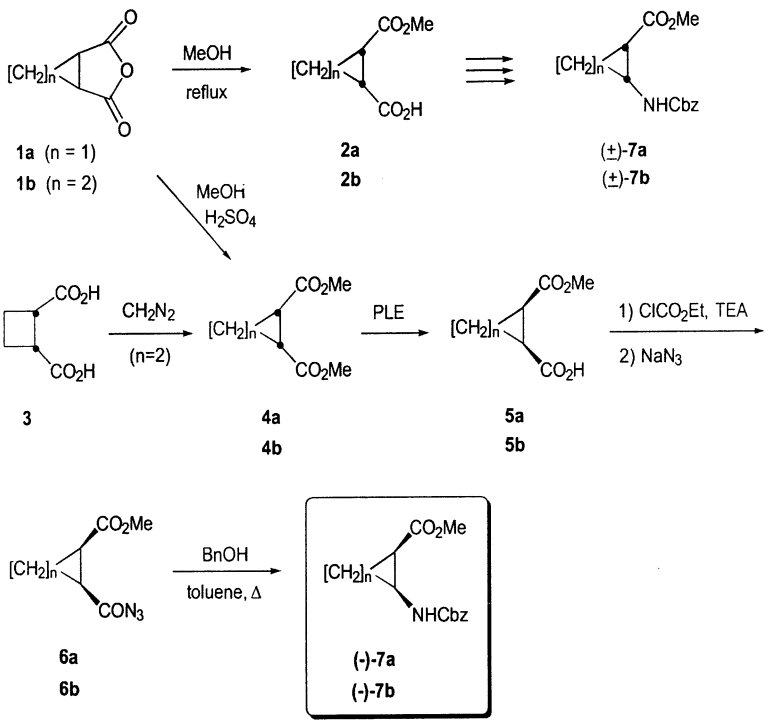
2.1. Synthesis of cyclopropane or cyclobutane amino acids and dipeptide **16**

The enzymatic synthesis of chiral building blocks from prochiral substrates has been applied to the production of enantiopure bioactive molecules,[16] and hydrolytic enzymes, such as pig

leaver esterase (PLE), play a prominent role in such biotransformations. Jones et al.[17] described the enantioselective hydrolysis of cyclopropane or cyclobutane meso diesters to afford the corresponding hemiesters in high ee. We have used these compounds as the synthetic precursors to carbocyclic β-amino acids via Curtius rearrangements. Alternatively, we have also prepared cyclopropane derivatives from optically active starting materials, obtained from D-glyceraldehyde as a source of chirality.

*2.1.1. Synthesis of 2-amino-(1*R,*2*S*)-cyclopropane- and 2-amino-(1*R,*2*S*)-cyclobutane-1-carboxylic acid derivatives (–)-**7a** and (–)-**7b** through a chemoenzymatic approach*

The synthetic sequences leading to compounds (–)-**7a** and (–)-**7b** are depicted in Scheme 1. Maleic anhydride derivatives **1a** and **1b** were the precursors providing cyclopropane and cyclobutane *meso* diesters **4a** and **4b**, respectively, through Fisher esterification. Compound **1a** is commercially available but **1b** is not. This product could be prepared, according to the literature, by photochemical [2+2] cycloaddition of ethylene to maleic anhydride,[18] but the reaction was very slow and afforded **1b** in modest yield (35–40%) after several days. Commercial cyclobutane-1,2-dicarboxylic diacid **3** is quite expensive but it is a more convenient starting material which provided diester **4b** quantitatively upon reaction with diazomethane.



Scheme 1.

Dimethyl diesters **4a**,**b** were hydrolyzed chemoselectively by PLE to afford hemiesters **5a**,**b** in 91% yield and >97% ee.[17] The next synthetic steps involve the degradative conversion of the carboxyl into an amino group via an intermediate acyl azide. Thus, hemiester **5a** was reacted with ethyl chloroformate in the presence of triethylamine followed by reaction with sodium

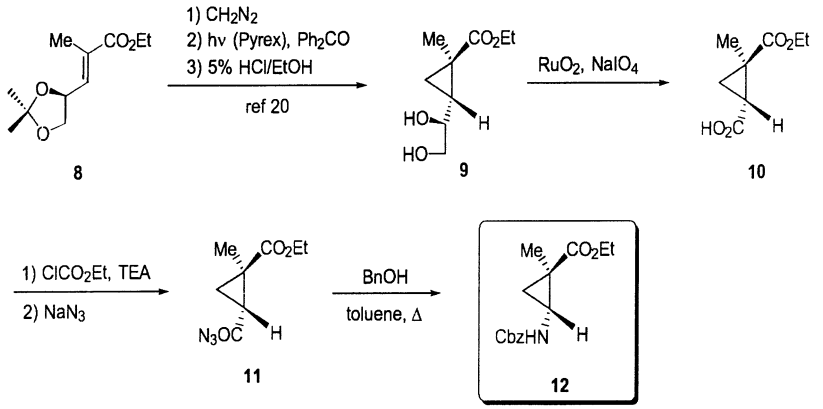
azide to produce **6a**. In a parallel way, hemiester **5b** was converted into **6b**. Curtius rearrangement of these compounds was carried out by heating respective toluene solutions of **6a** and **6b** in the presence of benzyl alcohol in order to produce the corresponding *N*-Cbz protected amines. Temperature was crucial in order to obtain carbamate (–)-**7a** in good ee. When a solution containing the cyclobutane derivative **6b** was heated to reflux for 16 h, (–)-**7b** was produced in 91% ee, as determined by HPLC (vide infra). However, heating of **6a** under similar conditions afforded **7a** as a racemate. In contrast, compound (–)-**7a** was obtained in 63% ee when a toluene solution of **6a** and benzyl alcohol was heated at 50°C for 3 h. The observed racemization can be attributed to the labile proton at C1 and to the presence of an electron-withdrawing group and an electron-donor function in the cyclopropane 1,2-relative positions. This capto-dative system shows the ability to isomerize through open-chain species produced under a variety of conditions.[19] These features also account for the isomerization observed during the Curtius rearrangement of related molecules.[20] On the other hand, diastereomerical homogeneity of compounds (–)-**7a** and (–)-**7b** was assessed by ^{1}H and ^{13}C NMR, since only one set of signals was observed in each case.

Racemic **7a**,**b** were prepared from racemic **2a**,**b** following the same transformations as those described above for the optically active products, and were used as the reference standards in HPLC ee measurements. Hemiesters **2a**,**b** resulted, in turn, from the reaction of **1a**,**b** with methanol.

Although chemical overall yields for (–)-**7a** and (–)-**7b** were very satisfactory (89 and 57% from **1a** and **1b**, respectively), the modest enantiomeric excess accomplished for the cyclopropane derivative (–)-**7a** prompted us to explore another synthetic route leading to cyclopropane β-amino acids.

*2.1.2. Synthesis of ethyl (1*R,*2*R*)-2-*N*-(benzyloxycarbonylamino)-2-methyl-1-cyclopropane carboxylate, **12**, from a chiral precursor*

Unsaturated ester **8** (Scheme 2) is easily prepared from D-glyceraldehyde by Wittig–Horner condensation with a suitable phosphonate.[21] In a recent report,[22] we described the highly stereoselective cyclopropanation (85% de) of **8** by means of the 1,3-dipolar cycloaddition of diazomethane and photolysis of the produced pyrazoline. Subsequent hydrolysis of the acetonide afforded the cyclopropane derivative **9** in 60% overall yield for the three steps. Diol was

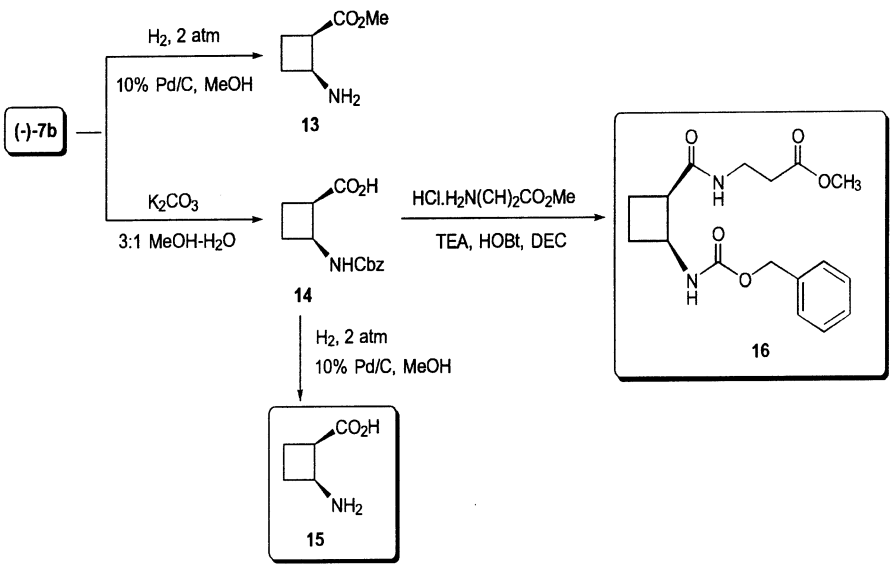


Scheme 2.

oxidatively cleaved by RuO_2 affording carboxylic acid **10**. Curtius rearrangement of acyl azide **11**, promoted by heating a mixture of this compound and benzyl alcohol in toluene to reflux for 1.5 h, afforded compound **12** in 53% yield from diol **9** and in enantiomerically pure form as deduced from the HPLC analysis described below. In this case, the presence of the quaternary center at C1 must prevent isomerization of these molecules.

2.1.3. Selective functional group manipulations in ***7b****: syntheses of free amino acid* ***15*** *and dipeptide* ***16***

The orthogonal protecting groups in (−)-**7b** are convenient for the selective deprotection of the carboxylic acid or the amine function in order to provide derivatives suitable for their incorporation into peptides (Scheme 3).



Scheme 3.

Thus, benzyl carbamate was removed through Pd/C catalyzed hydrogenation of (−)-**7b** affording amino ester **13** in 84% yield. Alternatively, methyl ester in (−)-**7b** was hydrolyzed by treatment with potassium carbonate in a 3:1 methanol/water mixture at room temperature for 4 h, furnishing compound **14**. A long reaction time and the use of strong bases was precluded in order to prevent *cis–trans* epimerization. The *cis*-configuration of **14** was assured by NOE experiments.[15] The amine protection was removed by hydrogenation, as above, to give the free amino acid **15**, as a very hygroscopic solid, in 40% overall yield from diacid **3**.

Acid **14** was condensed with β-Ala-OMe hydrochloride by using excess 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide as a dehydratating agent and 1 equivalent of 1-hydroxybenzotriazole (HOBT) as a catalyst, in the presence of TEA to liberate the amine, in anhydrous DMF at room temperature for 20 h. In this way, fully protected dipeptide **16** was obtained as a crystalline solid in 86% yield.

2.2. HPLC determinations

Several chiral stationary phases based on immobilized polysaccharide derivatives[23] were tested in order to develop an analytical method to asses the enantiomeric content of **7a**,**b** and **12**. The low wavelength of maximal absorption (210 nm) and the low absorptivity of these compounds prevented the use of solvents other than heptane and 2-propanol, mixtures of which were used as the mobile phase. Refractive index detection was not possible because of the low response of the sample.

Compound (±)-**7b** was the first to be analyzed and several conditions were tried (Table 1). A slight splitting of peaks was observed when using columns based on amylose 3,5-dimethylphenylcarbamate or chitosan 3,5-dichlorophenylcarbamate. Cellulose 3,5-dimethylphenylcarbamate and amylose 4-chlorophenylcarbamate were unable to distinguish between the two enantiomers of (±)-**7b**. Resolved peaks were obtained with cellulose 3,5-dichlorophenylcarbamate, chitosan 3,5-dimethylphenylcarbamate and amylose 3,5-dichlorophenylcarbamate. However, completely resolved peaks were only obtained with the latter two phases when using a 95:5 heptane/2-propanol mixture as the eluent. When (–)-**7b** was chromatographed on both columns, an enantiomeric excess of 91% was determined.

Table 1
HPLC data for compounds **7a**, **7b**, and **12** on chiral stationary phases (CSP)[a,b]

Compound	Chiral selector in the CSP	k'_1	α	R_s	Mobile phase heptane/2-PrOH
7b	Cellulose 3,5-dimethylphenylcarbamate	2.50	1.00		95:5
7b	Amylose 3,5-dimethylphenylcarbamate	1.85	1.08		95:5
7b	Amylose 4-chlorophenylcarbamate	2.34	1.00		90:10
7b	Chitosan 3,5-dichlorophenylcarbamate	2.86	1.17		95:5
7b	Cellulose 3,5-dichlorophenylcarbamate	2.23	1.32	1.29	90:10
7b	Chitosan 3,5-dimethylphenylcarbamate	1.90	2.17	3.07	95:5
7b	Amylose 3,5-dichlorophenylcarbamate	5.33	1.47	2.55	95:5
7a	Amylose 4-chlorophenylcarbamate	8.91	1.08		95:5
7a	Chitosan 3,5-dimethylphenylcarbamate	4.75	1.09		95:5
7a	Cellulose 3,5-dichlorophenylcarbamate	20.57	1.21	1.08	98:2
7a	Amylose 3,5-dichlorophenylcarbamate	28.23	1.24	1.55	98:2
12	Chitosan 3,5-dimethylphenylcarbamate	7.00			95:5
12	Amylose 3,5-dichlorophenylcarbamate	8.30			95:5

[a] See Section 4 for a definition of the chromatographic parameters k'_1, α and R_s.
[b] All chromatographic runs were performed on 15×0.46 cm ID columns. Flow: 1 mL/min. UV detection: **7a**, 208 nm; **7b**, 254 nm; **12**, 211 nm.

Similarly, even if several chiral selectors showed a certain enantioselectivity for the two enantiomers of (±)-**7a**, this compound was only fully resolved when the column containing amylose 3,5-dichlorophenyl carbamate was employed. Using the chromatographic conditions determined for the racemic mixture, an enantiomeric excess of 63% was determined for (–)-**7a**.

In contrast, only one peak was observed when **12** was chromatographed on both amylose 3,5-dichlorophenyl carbamate- and chitosan 3,5-dimethylphenylcarbamate-based columns eluting with 95:5 heptane/2-propanol, suggesting the presence of only one enantiomer. Polarimetric detection confirmed this hypothesis.

2.3. Structure analysis

Several experiments were carried out to investigate the secondary stucture of **16** both in solution and in the solid state. Circular dichroism shows a negative band, at 222 nm, of difficult interpretation. Otherwise, in order to evidence hydrogen bonds in solution, the NMR chemical shift of the N*H* protons was investigated at different concentrations in CD_2Cl_2 or in CD_2Cl_2 after the addition of small amounts of DMSO. The fact that δ_{NH} was shown to be dilution dependent, at low sample concentration, suggests a strong tendency to produce molecular aggregates whereas the non-dependence of the DMSO concentration agrees with the presence of strong hydrogen bonds.

A single crystal X-ray diffraction study revealed the molecular features and the intermolecular interactions present in the solid state. Some selected data are collected in Table 2 (see Fig. 1 for

Table 2
Selected distances (Å) and angles (°) with e.s.d. values in parentheses for **16**

Bond lengths				
N1–C4	1.440(5)		C9–C8	1.525(6)
C5–N1	1.340(5)		C8–C7	1.528(6)
C5–O3	1.232(5)		N2–C10	1.342(5)
C6–C5	1.506(5)		C10–O4	1.216(5)
C6–C7	1.536(5)		C10–O5	1.342(5)
C6–C9	1.554(5)		O5–C11	1.448(5)
C9–N2	1.426(4)			
Bond angles				
C4–N1–H1	121(4)		N2–C9–C8	117.2(3)
C5–N1–C4	121.9(4)		N2–C9–C6	120.2(3)
C5–N1–H1	117(4)		C8–C9–C6	89.4(3)
O3–C5–N1	122.2(4)		C9–N2–H2	120(3)
O3–C5–C6	123.0(4)		C10–N2–H2	115(3)
N1–C5–C6	114.7(4)		C10–N2–C9	121.9(4)
C5–C6–C7	114.8(4)		O4–C10–N2	124.2(4)
C5–C6–C9	110.4(3)		O4–C10–O5	123.5(4)
C7–C6–C9	87.3(3)		N2–C10–O5	112.3(4)
C8–C7–C6	89.9(3)		C10–O5–C11	115.7(3)
C9–C8–C7	88.7(3)			
Torsion angles				
C6–C5–N1–C4	−179.2(4)		C9–C6–C7–C8	16.2(3)
O3–C5–N1–C4	−2.5(6)		C7–C6–C9–C8	−16.2(3)
C9–N2–C10–O4	−11.4(7)		C6–C9–C8–C7	16.3(3)
C9–N2–C10–O5	169.8(3)		C9–C8–C7–C6	−16.5(3)
O5–C11–C12–C17	72.2(5)		C5–C6–C9–N2	−22.2(5)
Hydrogen bonds				
N1–H1	N1···O3[i]	H1···O3[i]	N1–H1···O3[i]	
0.82(6)	2.993(5)	2.20(6)	165(6)	
N2–H2	N2···O4[ii]	H2···O4[ii]	N2–H2···O4[ii]	
0.90(6)	2.849(5)	1.97(6)	168(5)	

[i] $x, y+1, z$.
[ii] $x, y-1, z$.

atom numeration). Both the carbamate and amide groups are planar with a null pyramidalization of nitrogen atoms. Cyclobutane is twisted and, therefore, its substituents are not synperiplanar but are almost synclinal. On the whole, the molecule shows a hairpin-like conformation as can be observed in Fig. 1. In the crystal structure, molecules are linked by intermolecular hydrogen bonds forming infinite chains parallel to crystallographic vector b, as shown in Fig. 2. Two N–H···O hydrogen bonds are present between every pair of neighboring molecules in the chain.

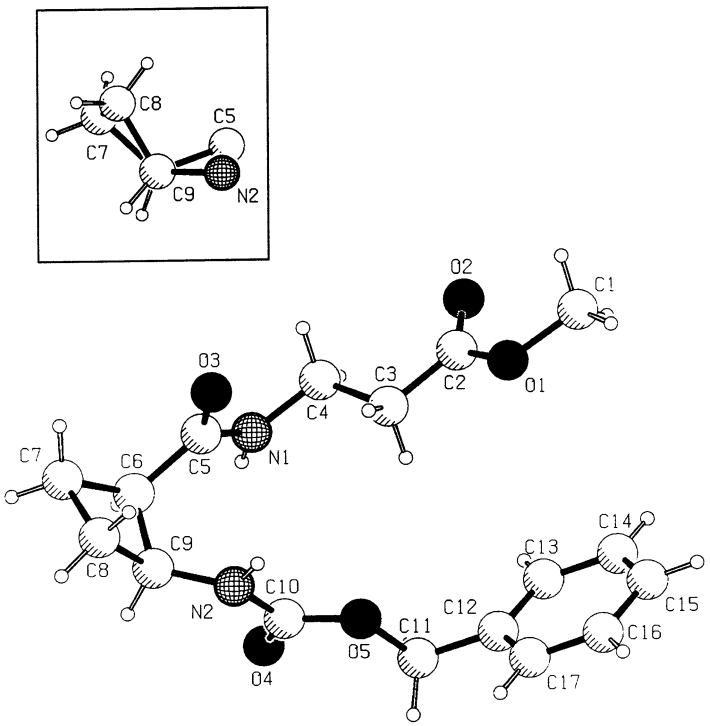


Figure 1. Structure of dipeptide **16** as determined by X-ray structural analysis. Inset: Projection view of the cyclobutane ring showing the almost synclinal disposition of the two chains

In contrast, intramolecular interactions are important in the gas phase, as suggested by theoretical calculations on several structures of **16**.[24–28] In the first calculation, the X-ray structure has been taken as the starting point and it is similar to the final geometry, the main difference being the presence of an intramolecular hydrogen bond involving N1–*H* and O4 with an H···O bond length of 2.30 Å. An exploration of the potential energy surface using the semiempirical PM3 method[29] leads to different alternative conformations. Those having the lowest energies have been reoptimized at the BPW91 level of calculation. The structure presented in Fig. 3 is the most stable one obtained by this procedure. It is 1.7 kcal mol^{-1} lower in energy than the former structure and presents an intramolecular N2–H···O hydrogen bond.

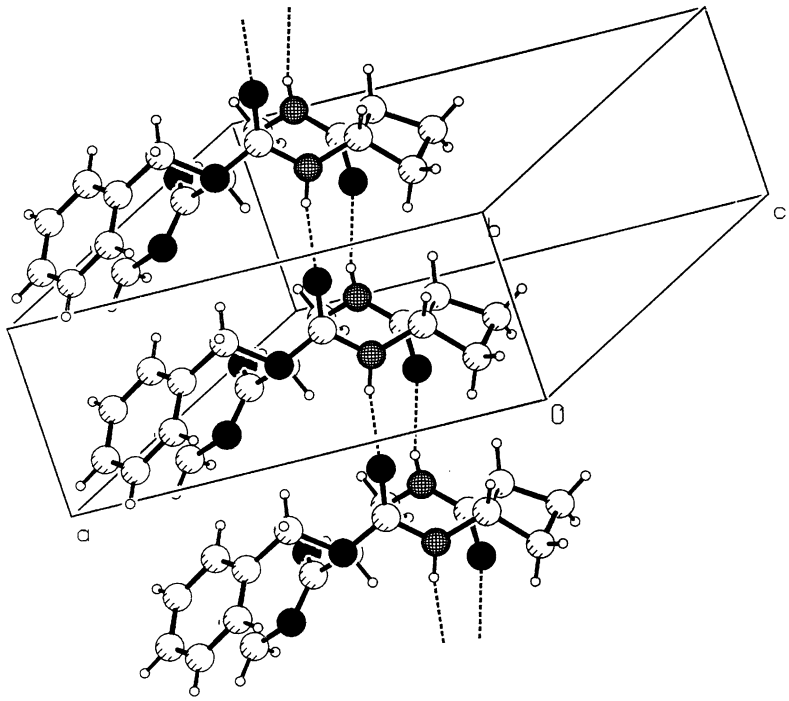


Figure 2. Crystal packing for dipeptide **16** showing the intermolecular hydrogen bonds

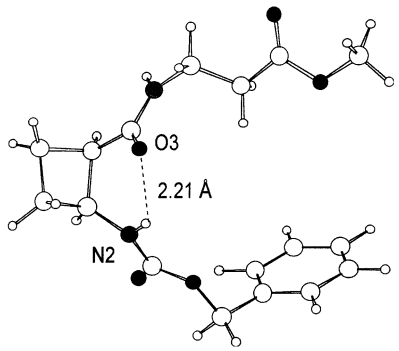


Figure 3. Optimized (BPW91) structure of dipeptide **16** showing an intramolecular hydrogen bond

3. Concluding remarks

Optically active carbocyclic β-amino acids have been synthesized in high ee and good yields. A cyclobutane derivative has been incorporated for the first time into a β-dipeptide, which shows a hairpin-like conformation in the solid state. This secondary structure is determined both by the rigid cyclobutane moiety and by the presence of intermolecular hydrogen bonds in the crystal packing. The strong tendency of these molecules to form aggregates also in solution has been confirmed by NMR. Otherwise, theoretical calculations show the presence of intramolecular hydrogen bonds in the gas phase.

4. Experimental

4.1. General

Flash column chromatography was carried out on silica gel (240–400 mesh) unless otherwise stated. Baker-silica® (40 μm) was used for the chromatography of acid-sensitive products. Melting points were determined on a hot stage and are uncorrected. Distillation of small amounts of material was effected in a bulb-to-bulb distillation apparatus, with oven temperatures (ot) being reported. ^{1}H and ^{13}C NMR spectra were recorded at 250 and 62.5 MHz, respectively.

4.2. HPLC determinations

Columns of 150×4.6 mm were used. The chiral stationary phases are listed in Table 1. Heptane/2-propanol mixtures were used as the mobile phase. Flow rate: 1 mL/min; UV detection: 210 nm. Chromatographic parameters are defined as follows. *Selectivity factor*, $\alpha = t_2 - t_0/t_1 - t_0$, where t_i is the retention time for each enantiomer and t_0 the dead time of the column. *Resolution factor*, R_s, calculated as $R_s = 2(t_2 - t_1)/w_2 + w_1$, where w_i is the peak width at the baseline. An R_s value over 1.5 implies the complete resolution of peaks. *Capacity factor* for the first eluted enantiomer, $k'_1 = t_1 - t_0/t_0$.

4.3. Crystal structure analysis of **16**

Data: $C_{17}H_{22}N_2O_5$, $M_r = 334.37$, colorless prism, crystallized from ethyl acetate, crystal size 0.58×0.11×0.04, $a = 12.368(3)$ Å, $b = 4.930(1)$ Å, $c = 14.734(3)$ Å, $\beta = 96.50(2)°$, $V = 892.6(3)$ Å^3, $T = 293$ K, monoclinic, space group $P2_1$ (No. 4), $Z = 2$, $D_c = 1.244$ g cm^{-3}, $\mu = 0.92$ cm^{-1}, Enraf–Nonius CAD4 diffractometer, graphite monochromated Mo Kα ($\lambda = 0.71069$ Å), data collected range $2° < 2\theta < 50°$, ω–2θ scan. The structure was resolved by direct methods[30] and refined by full matrix least squares[31] on F^2 for all reflections, 1766 unique reflections, 1007 observed [$I > 2\sigma(I)$], number of variables 223, non-hydrogen atoms were refined anisotropically, H atoms bonded to carbon atoms were placed in calculated positions with isotropic displacement parameters fixed at 1.5 (methyl H) or 1.2 times (the rest) U_{eq} of the corresponding carbon atoms, $R(F) = 0.046$ for the observed reflections, $R_w(F^2) = 0.118$ for all data, max. shift/error 0.001, and residual ρ_{max} 0.176 e Å^{-3}.

4.4. Reaction of **1a**,**b** with methanol: diesters **4a**,**b** and racemic hemiesters **2a**,**b**

Typical experiments were run as follows.

4.4.1. Acid-catalyzed reaction

A mixture of compound **1b** (200 mg, 1.6 mmol), concentrated H_2SO_4 (two drops) and anhydrous methanol (10 mL) was heated to reflux for 2 h. Then methanol was removed at reduced pressure, the residue was dissolved in water and the resulting solution was extracted with dichloromethane. The combined extracts were dried over $MgSO_4$ and the solvent was removed to afford dimethyl *cis*-cyclobutane-1,2-dicarboxylate, **4b** (240 mg, 87% yield), as an oil. In a similar manner, dimethyl *cis*-cyclopropane-1,2-dicarboxylate, **4a**, was prepared from **1a**.

Spectroscopic data for these compounds (IR, ^{1}H and ^{13}C NMR) are in good agreement with those described in Ref. 15 for the same products.

4.4.2. Uncatalyzed reaction

A solution of anhydride **1b** (200 mg, 1.6 mmol) in anhydrous methanol was heated to reflux for 7 h. The solvent was removed affording methyl hydrogen *cis*-cyclobutane-1,2-dicarboxylate, **2b** (253 mg), quantitatively. Methyl hydrogen *cis*-cyclopropane-1,2-dicarboxylate, **2a**, was prepared from **1a** following the same protocol. Spectroscopic data for **2a**,**b** agree with those data reported for optically pure compounds in this work and in Ref. 17.

*4.5. Synthesis of diester **4b** through reaction of diacid **3** with diazomethane*

An ethereal solution of excess diazomethane was distilled onto **3** (500 mg, 3.5 mmol) in 10 mL of ether, at 0°C, and the resultant solution was stirred at 0°C for 20 min. Excess diazomethane was destroyed by the addition of $CaCl_2$, and the solvent was removed to give **4b** (600 mg) quantitatively.

*4.6. PLE-catalyzed hydrolyses of diesters **5a**,**b**: hemiesters (–)-**5a** and (–)-**5b***

These compounds were prepared according to the procedure described by Jones et al.[17] Methyl hydrogen (–)-(1*R*,2*S*)-cyclopropane-1,2-dicarboxylate, **5a**: yield, 577 mg (91%); crystals, mp 78–81°C (from EtOAc); $[\alpha]_D$ −11.2 (*c* 1.2, MeOH), −23.5 (*c* 1.1, $CHCl_3$) (lit[17] mp 81–83°C (solvent not mentioned); $[\alpha]_D$ −13.4 (*c* 0.97, $CHCl_3$)). Methyl hydrogen (–)-(1*R*,2*S*)-cyclobutane-1,2-dicarboxylate, **5b**: yield, 500 mg (90%); oil, $[\alpha]_D$ −3.6 (*c* 2.4, $CHCl_3$) (lit[17] $[\alpha]_D$ −3.0 (*c* 2.1, $CHCl_3$)). The spectroscopic data for these products are in good agreement with those described in Ref. 17.

*4.7. Synthesis of protected amino acids (–)-**7a** and (–)-**7b** through acyl azides **6a** and **6b***

4.7.1. Synthesis of the acyl azides

A typical experiment is described for the preparation of **6a**. Ethyl chloroformate (200 μL, 2.4 mmol) was added to an ice-cold solution of hemiester (–)-**5a** (230 mg, 1.6 mmol) and freshly distilled TEA (0.3 mL, 2.1 mmol) in dry acetone (10 mL). After stirring at 0°C for 2 h, sodium azide (167 mg, 2.6 mmol) in 5 mL of water was added, and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was extracted with ether and the combined organic extracts were dried over $MgSO_4$. The solvents were removed under reduced pressure to afford a yellowish oil identified by the spectroscopic data as methyl 2-azidocarbonyl-(1*R*,2*S*)-cyclopropane-1-carboxylate, **6a** (257 mg, 95% yield); IR (film) 2130, 1736, 1170, 1110 cm^{-1}; 250 MHz ^{1}H NMR (acetone-d_6) 1.35 (m, 1H), 1.54 (m, 1H), 2.24 (m, 2H), 3.63 (s, 3H); 62.5 MHz ^{13}C NMR (acetone-d_6) 12.21, 22.68, 23.27, 51.50, 169.34, 176.34.

In a similar manner, methyl 2-azidocarbonyl-(1*R*,2*S*)-cyclobutane-1-carboxylate, **6b**, was prepared. Yield, 692 mg (90%); oil; IR (film) 2137, 1736, 1173, 1110 cm^{-1}; 250 MHz ^{1}H NMR (acetone-d_6) 2.16 (m, 4H), 3.38 (m, 2H), 3.59 (s, 3H); 62.5 MHz ^{13}C NMR (acetone-d_6) 23.11 (2C), 42.35, 44.07, 52.66, 175.34, 182.08.

4.7.2. Curtius rearrangement

*4.7.2.1. Synthesis of methyl 2-benzyloxycarbonylamino-(1*R,*2*S*)-cyclopropane-1-carboxylate, (–)-**7a***. A solution of azide **6a** (150 mg, 0.9 mmol) and benzyl alcohol (0.2 mL, 1.9 mmol) was heated at 50°C for 3 h. The solvent was evaporated at reduced pressure and the residue was chromatographed on Baker-silica® (dichloromethane). The solvent was removed to afford (–)-**7a** (140 mg, 89% yield) in 63% ee, as determined by HPLC by using chitosan 3,5-dimethylphenylcarbamate as the chiral stationary phase and 95:5 heptane/2-propanol as eluent. Crystals, 74–77°C (from EtOAc–hexane); $[\alpha]_D$ −91 (*c* 1.1, MeOH); IR (film) 3412, 1710, 1687, 1110 cm^{-1}; 250 MHz ^1H NMR ($CDCl_3$) 1.13–1.33 (m, 2H), 1.90 (m, 1H), 3.34 (m, 1H), 3.66 (s, 3H), 5.09 (s, 2H), 5.50 (broad s, 1H), 7.33 (m, 5H); 62.5 MHz ^{13}C NMR ($CDCl_3$) 14.56, 19.21, 32.09, 52.44, 67.29, 128.56, 128.94, 136.91, 157.05, 172.93. Anal. calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.59; H, 5.91; N, 5.71.

*4.7.2.2. Synthesis of methyl 2-benzyloxycarbonylamino-(1*R,*2*S*)-cyclobutane-1-carboxylate, (–)-**7b***. A solution of azide **6b** (300 mg, 1.6 mmol) and benzyl alcohol (0.4 mL, 3.6 mmol) in toluene (8 mL) was heated to reflux for 16 h. The solvent was evaporated at reduced pressure and the residue was chromatographed on Baker-silica® (dichloromethane). The solvent was removed to afford (–)-**7b** (320 mg, 70% yield) in 91% ee, as determined by HPLC by using chitosan 3,5-dimethylphenylcarbamate or amilose 3,5-dichlorophenylcarbamate as chiral stationary phases and 95:5 heptane/2-propanol as eluent. Dense oil, ot 155°C (0.05 torr); $[\alpha]_D$ −83 (*c* 2.05, $CHCl_3$); IR (film) 3355, 1723, 1687, 1110 cm^{-1}; 250 MHz ^1H NMR ($CDCl_3$) 1.97 (m, 2H), 2.31 (m, 2H), 3.35 (m, 1H), 3.63 (s, 3H), 4.52 (m, 1H), 5.06 (s, 2H), 5.65 (broad s, 1H), 7.32 (m, 5H); 62.5 MHz ^{13}C NMR ($CDCl_3$) 19.12, 30.10, 45.53, 46.56, 52.18, 67.15, 128.58, 128.97, 136.97, 155.79, 175.53. Anal. calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 64.04; H, 6.30; N, 5.31.

4.8. Ethyl 2-carboxy-1-methyl-(1R,2R)-cyclopropane-1-carboxylate, *10*

A mixture of diol **9** (300 mg, 1.6 mmol), prepared according to Ref. 20, $RuO_2 \cdot xH_2O$ (80 mg) and $NaIO_4$ (1.7 g, 8.0 mmol) in 2:2:3 $CCl_4/CH_3CN/H_2O$ (14 mL) was stirred at room temperature for 1 h. Ether (2 mL) was added and the layers were separated. The aqueous layer was extracted with ether and the combined organic phases were dried over $MgSO_4$. The solvents were removed at reduced pressure and the residue was filtered through Celite® in vacuo, to afford a brownish oil (220 mg, 80% yield) that was impossible to purify using the usual chromatographic techniques and unsuitable for $[\alpha]_D$ determination and for microanalysis. IR (film) 3600–2600 (broad), 1729, 1708 cm^{-1}; 250 MHz ^1H NMR ($CDCl_3$) 1.22 (t, $J=7.3$, 3H), 1.30 (dd, $J=6.6$, $J=4.4$, 1H), 1.41 (s, 3H), 1.59 (dd, $J=8.8$, $J'=4.4$, 1H), 2.30 (dd, $J=8.8$, $J'=6.6$, 1H), 4.11 (q, $J=7.3$, 2H); 62.5 MHz ^{13}C NMR ($CDCl_3$) 13.00, 14.06, 21.47, 27.42, 28.12, 61.41, 173.17, 176.70.

4.9. Synthesis of ethyl 2-benzyloxycarbonylamino-1-methyl-(1R,2R)-cyclopropane-1-carboxylate, *12*, through acyl azide *11*

Ethyl chloroformate (170 μL, 1.8 mmol) was added to an ice-cold solution of acid **10** (210 mg, 1.2 mmol) and freshly distilled TEA (220 μL, 1.6 mmol) in dry acetone (8 mL). The mixture was

stirred at 0°C for 1 h, then a solution of NaN_3 (133 mg, 2.0 mmol) in 3 mL of water was added and the resultant solution was stirred at room temperature for 2 h. Water (7 mL) was added and the reaction mixture was extracted with ether. The combined organic phases were dried over $MgSO_4$ and the solvents were removed affording an oil which was identified as ethyl 2-azidocarbonyl-1-methyl-(1*R*,2*R*)-cyclopropane-1-carboxylate, **11**, by its spectroscopic data. IR (film) 2137, 1729, 1708, 1279 cm^{-1}; 250 MHz ^1H NMR ($CDCl_3$) 1.22 (t, $J=7.3$, 3H), 1.35 (dd, $J=6.6$, $J'=4.4$, 1H), 1.36 (s, 3H), 1.61 (dd, $J=8.8$, $J'=4.4$, 1H), 2.28 (dd, $J'=8.8$, $J'=6.6$, 1H), 4.12 (q, $J=7.3$, 2H); 62.5 MHz ^{13}C NMR ($CDCl_3$) 15.49, 14.09, 24.58, 32.54, 32.73, 64.31, 175.26, 179.75.

A solution of compound **11** (250 mg, 1.2 mmol) and benzyl alcohol (300 μL, 2.8 mmol) in toluene (5 mL) was heated to reflux for 1.5 h. The solvent was removed at reduced pressure and the residue was chromatographed on silica gel (5:1 hexane/ethyl acetate). Traces of benzyl alcohol were removed by crystallization to afford 243 mg of pure **12** (70% yield). Crystals, mp 101–103°C (from EtOAc–pentane); $[\alpha]_D$ +27.9 (*c* 0.70, $CHCl_3$); IR (KBr) 3325, 1715, 1687 cm^{-1}; 250 MHz ^1H NMR ($CDCl_3$) 0.70 (m, 1H), 1.21 (t, $J=7.0$, 3H), 1.25 (s, 3H), 1.59 (m, 1H), 3.18 (m, 1H), 4.09 (q, $J=6.6$, 2H), 5.03 (s, NH), 5.10 (s, 2H), 7.32 (s, 5H); 62.5 MHz ^{13}C NMR ($CDCl_3$) 13.06, 14.09, 21.65, 36.24, 60.76, 66.94, 128.14, 128.47, 136.17, 156.73, 173.88. Anal. calcd for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05. Found: C, 65.03, H, 6.82; N, 4.86.

*4.10. Methyl 2-amino-(1*R,*2*S*)-cyclobutane-1-carboxylate*, ***13***

A stirred mixture of (−)-**7b** (140 mg, 0.5 mmol) and 10% Pd/C (14 mg) in methanol (10 mL) was hydrogenated overnight under 2 atmospheres of pressure. The catalyst was removed by filtration through Celite® and the solvent was evaporated to afford amine **13** (61 mg, 84% yield) as a hygroscopic solid unsuitable for microanalysis; mp 77–80°C (from EtOH); $[\alpha]_D$ −19 (*c* 1.4, MeOH); IR (film) 2951, 1734, 1104 cm^{-1}; 250 MHz ^1H NMR (methanol-d_4) 1.65 (m, 4H), 2.42 (m, 1H), 3.01 (m, 1H), 3.66 (s, 3H), 4.79 (broad s, 3H); 62.5 MHz ^{13}C NMR (methanol-d_4) 24.63, 28.37, 36.39, 50.52, 55.63, 180.13.

*4.11. 2-Benzyloxycarbonylamino-(1*R,*2*S*)-cyclobutane-1-carboxylic acid*, ***14***

Ester (−)-**7b** (130 mg, 0.5 mmol) was added to a solution of K_2CO_3 (340 mg, 2.5 mmol) in a 3:1 methanol/water mixture and the solution was stirred at room temperature for 6 h. Methanol was evaporated under reduced pressure and the resultant aqueous solution was washed with ether and 5% HCl was added until the pH reached 2. The acid aqueous solution was extracted with ether and the combined organic extracts were dried over $MgSO_4$. The solvent was evaporated to afford acid **14** (102 mg, 86% yield) as a pasty solid unsuitable for microanalysis; $[\alpha]_D$ −98 (*c* 1.1, MeOH); IR (film) 3433, 3353, 1711, 1687, 1433, 1155 cm^{-1}; 250 MHz ^1H NMR (acetone-d_6) 2.11 (m, 2H), 2.36 (m, 2H), 3.22 (broad s, 1H), 3.42 (m, 1H), 4.56 (m, 1H), 5.10 (s, 2H), 6.57 (broad s, 1H), 7.41 (m, 5H); 62.5 MHz ^{13}C NMR (acetone-d_6) 17.92, 28.36, 44.9, 46.48, 67.74, 127.58, 128.19, 137.06, 155.24, 174.58.

*4.12. 2-Amino-(1*R,*2*S*)-cyclobutane-1-carboxylic acid*, ***15***

A stirred mixture of **14** (140 mg, 0.6 mmol) and 10% Pd/C (15 mg) in methanol (10 mL) was hydrogenated overnight under 2 atmospheres of pressure. The catalyst was removed by filtration

through Celite®, the solvent was evaporated and the residue was washed with methanol to give amino acid **15** (60 mg, 85% yield) as a highly hygroscopic solid unsuitable for microanalysis; mp 130°C (dec) (from MeOH–H_2O); $[\alpha]_D$ −9 (*c* 1.5, H_2O); IR (KBr) 3414, 3390, 1714 cm^{-1}; 250 MHz ^1H NMR (D_2O) 1.59 (m, 4H), 2.20 (m, 1H), 2.98 (m, 1H), 4.79 (broad s, 3H); 62.5 MHz ^{13}C NMR (D_2O) 23.31, 23.40, 36.95, 48.28, 182.86.

*4.13. 2-Benzyloxycarbonylamino-(1*R,*2*S*)-cyclobutane-1-carboxylic acid* N-*methoxycarbonyl-ethyl amide*, ***16***

To a solution of acid **14** (120 mg, 0.5 mmol) in dry DMF (3 mL) under a nitrogen atmosphere, freshly distilled TEA (70 μL, 0.5 mmol), methyl β-alaninate hydrochloride (100 mg, 0.7 mmol), and 1-hydroxybenzotriazole (32 mg, 0.2 mmol) were added successively. The mixture was stirred until all reagents were dissolved and then 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (276 mg, 1.5 mmol) was added. The light-protected resultant solution was stirred at room temperature for 20 h, ethyl acetate (10 mL) was added and then the solution was washed with saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$, the solvents were removed at reduced pressure and the residue was chromatographed on Baker-silica® (1:1 dichloromethane/EtOAc) to afford dipeptide **16** (142 mg, 86% yield) as a solid. Crystals, mp 91–93°C (from EtOAc); $[\alpha]_D$ −67 (*c* 2.9, MeOH); IR (KBr) 3304, 1738, 1687, 1643 cm^{-1}; 400 MHz ^1H NMR (acetone-d_6) 1.94 (m, 2H, H_7), 2.25 (m, 2H, H_8), 2.46 (m, 2H, H_3), 3.24 (m, 1H, H_6), 3.41 (m, 2H, H_4), 3.60 (s, 3H, H_1), 4.39 (m, 1H, H_9), 5.03 (s, 2H, H_{11}), 6.53 (broad s, 1H, N_2–*H*), 7.09 (broad s, 1H, N_1–*H*), 7.30 (m, 5H, Ph); 100 MHz ^{13}C NMR (acetone-d_6) 18.68 (C_7), 29.03 (C_8), 34.80 (C_3), 36.12 (C_4), 47.18 (C_6), 48.32 (C_9), 52.15 (C_1), 67.44 (C_{11}), 128.88 ($C_{13}/C_{14}/C_{15}$), 128.99 ($C_{13}/C_{14}/C_{15}$), 129.44 ($C_{13}/C_{14}/C_{15}$), 138.17 (C_{12}), 157.70 (C_{10}), 173.73 (C_5), 174.73 (C_2). Anal. calcd for $C_{17}H_{22}N_2O_5$: C, 61.07; H, 6.63; N, 8.38. Found: C, 61.08; H, 6.52; N, 8.34.

5. Supporting information

A complete list of crystallographic data for dipeptide **16** is available on request from the Director of the Cambridge Crystallographic Data Centre. Any request should be accompanied by a full literature citation of this paper.

Acknowledgements

G.P.A., E.M. and M.M.-V. thank the Ministerio de Educación for respective predoctoral fellowships. Financial support from DGESIC through the project PB97-0214 is gratefully acknowledged.

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