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(+)- and (−)-2-Aminocyclobutane-1-carboxylic Acids and Their Incorporation into Highly Rigid β-Peptides: Stereoselective Synthesis and a Structural Study

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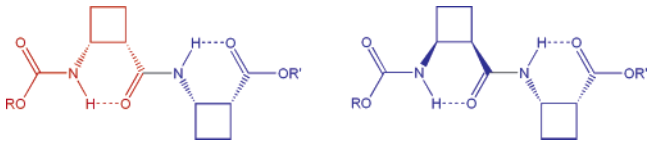

Several derivatives of (+)- and (−)-2-aminocyclobutane-1-carboxylic acid, **1**, have been prepared through enantiodivergent synthetic sequences. The stereoselective synthesis of free amino acid (+)-**1** has been achieved, and this product has been fully characterized for the first time. Stereocontrolled alternative synthetic methodologies have been developed for the preparation of bis(cyclobutane) β-dipeptides in high yields. Among them, enantio and diastereomers have been synthesized. β,β- and β,δ-Dimers resulting from the coupling of a cyclobutane residue and a linear amino acid have also been prepared. The ability of the cyclobutane ring as a structure-promoting unit both in the monomers and in the dimers has been manifested. The NMR structural study and DFT theoretical calculations evidence the formation of strong *intramolecular* hydrogen bonds giving rise to cis-fused [4.2.0]octane structural units that confer high rigidity on these molecules both in solution and in the gas phase. The contribution of a cis−trans conformational equilibrium derived from the rotation around the carbamate N−C(O) bond has also been observed, the trans form being the major conformer. In the solid state, this equilibrium does not exist, and moreover, *intermolecular* hydrogen bonds are present.

Introduction

Cyclobutane amino acids (CBAAs) have been found in nature isolated or incorporated into peptides.[1a,b] This is the case, for instance, of the antibiotic X-1092 produced by the Streptomyces species X-1092.[1c,d] Furthermore, the incorporation of the cyclobutane ring into conformationally restricted peptidomimetics sometimes results in the preparation of biologically active products such as the tuftsine analogue Thr-[Mom[2]]-Pro-Arg with increased resistance toward enzimatic hydrolysis with respect to tuftsine.[2] Small oligomers of cycloalkane β-amino acids have shown a great propensity to adopt preferred conformations leading to secondary structures.[1b,3] Regarding cyclobutane-containing β-peptides,[4] we have recently shown that tetrapeptide **3**, formed by two units of (−)-2-aminocyclobutane-1-carboxylic acid, (−)-**1**, and two β-alanine residues joined in alternance, presents a 14-helical folding in solution.[5] In contrast, oligomers of β-alanine do not show any defined conformational bias, neither in the crystal state[6a] nor in aqueous solution.[6b]

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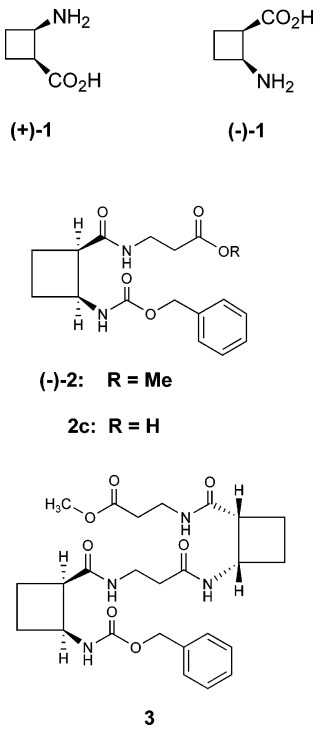
CHART 1



Otherwise, dipeptide (−)-**2** (Chart 1) adopts a hairpin-like conformation in the solid state.[7] In addition, Claridge et al. reported that a hexamer formed by oxetane residues forms a 10-helix.[8] Moreover, interesting biological activities have been displayed by β-foldamers. This finding has prompted the research in the synthesis and study of conformationally constrained β-amino acids[9] and β-oligomers[10] allowing the production of novel antibiotics and nonhaemolytic agents[11a−d] as well as mimics of somatostatin[11e] among other pharmaceutically active products.[1b,11f]

(−)-2-Aminocyclobutane-1-carboxylic acid, (−)-**1**, has been very recently synthesized by Bolm et al. via the desymmetrization of a cyclic meso anhydride through its quinine-mediated opening in the presence of benzyl alcohol.[12] Gauzy et al. have reported on the synthesis of both (+)- and (−)-**1** by [2 + 2]-photocycloaddition of ethylene to a chiral uracyl derivative to afford a 60:40 mixture of diastereomers. Isolation of each isomer by chromatography allowed the pursuit of the synthesis of both enantiomeric amino acids.[13] Previously to these works, we reported on the stereodivergent synthesis of the orthogonally protected derivatives (−)-**6a**[7] and **11**[14] with opposite chirality. These compounds were prepared from chiral half-ester **4** (Scheme 1), which results from the enzymatic desymmetrization of meso cyclobutane 1,2-dicarboxylic acid methyl ester,[15] and some aspects on their use in the preparation of bis(cyclobutane) β-dipeptides (bisCBDPs) were described in a preliminary communication.[14]

Now in this article, we show the efficiency and the versatility of our stereodivergent synthetic approach to cyclobutane β-amino acids and β-peptides. Thus, we describe full details on the synthesis of both enantiomeric CBAAs (+)- and (−)-**1** and several useful derivatives for the preparation of oligomers. β,β- and β,δ-Dipeptides containing a cyclobutane residue and a linear fragment are representative instances. We also provide and discuss improved methodology for the preparation of the bisCBDPs formally resultant from self-coupling of the conveniently protected enantiomeric amino acids, as well as one with another. Finally, we present a structural study made on the basis of X-ray diffraction analysis, NMR experiments, and DFT theoretical calculations, all of them accounting for the ability of the cyclobutane ring to induce secondary structures both in the monomers and in different types of cyclobutane-β-peptides.

Results and Discussion

1. Synthesis of Amino Acids and Peptides. The general strategy to synthesize the enantiomers (+)- and (−)-**1** and some conveniently protected derivatives is depicted in Scheme 1. Half-ester **4** could be transformed into (−)-**6a** or **6b** through a Curtius rearrangement in the presence of benzyl alcohol or *tert*-butyl alcohol, respectively. Saponification of the methyl ester and subsequent hydrogenolysis of the benzyl carbamate (−)-**7a** allowed us to achieve the first synthesis of the free amino acid (−)-**1**.[7] The synthetic way to CBAA (+)-**1** passes through the asymmetric diester **8** prepared in 90% yield by treatment of **4** with *tert*-butyl trichloroacetimidate.[16] Selective saponification of the methyl ester under mild conditions (0.25 M NaOH, 0 °C, 1 h) to avoid epimerization led quantitatively to **9**, which is a pseudoenantiomer of **4**. The acyl azide **10** was prepared in 91% yield by reaction of **9** with ethyl chloroformate, followed by treatment with sodium azide. Subsequent Curtius rearrangement was accomplished by heating **10** in the presence of benzyl alcohol in refluxing toluene to afford fully protected β-amino acid **11**. The intermediate carboxylic acid (+)-**7a** was quantitatively prepared by reaction of **11** with trifluoroacetic acid and triethylsi-

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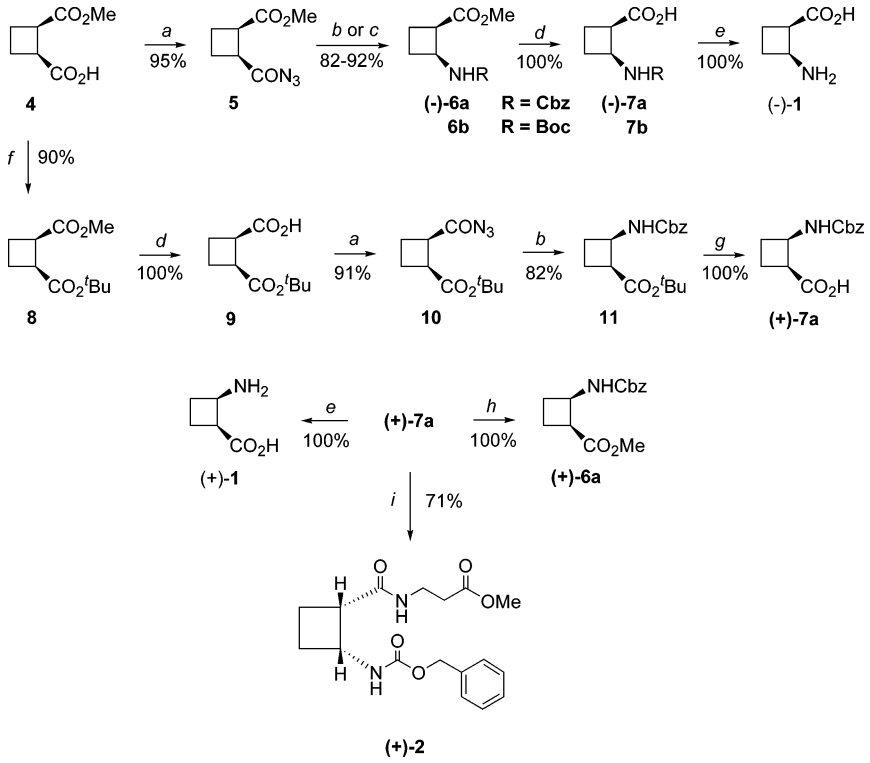
SCHEME 1[a]



[a] Reagents and conditions. (a) (i) $ClCO_2Et$, Et_3N, acetone, 0 °C, 3 h. (ii) NaN_3, H_2O, rt, 1.5 h. (b) BnOH, refluxing toluene, 3.5 h. (c) refluxing *t*-BuOH, overnight. (d) 0.25 M NaOH, 1:10 THF−H_2O, 0 °C, 1 h. (e) H_2, 10% Pd/C, MeOH, 1 atm, rt, 1.5 h. (f) $Cl_3C-C(NH)OtBu$, CH_2Cl_2, rt, overnight. (g) TFA, Et_3SiH, CH_2Cl_2, rt, 2 h. (h) CH_2N_2, ether. (i) $H_2N(CH_2)_2CO_2Me$, DEC, HOBt, DMF, rt, 20 h.

lane.[17] Esterification with diazomethane provided the methyl ester (+)-**6a** whose specific rotation magnitude compared well with that of the enantiomer (−)-**6a** showing that optical purity is respected throughout the synthetic sequence.

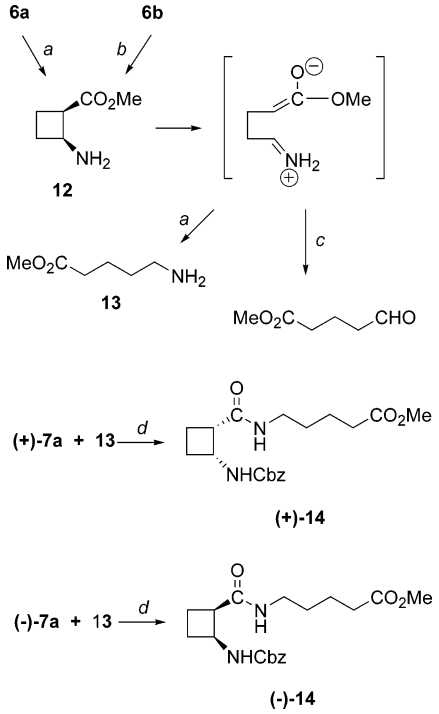
Moreover, catalytic hydrogenolysis (Pd/C) of the benzyl carbamate in (+)-**7a** led quantitatively to the enantiomeric free amino acid (+)-**1** whose melting point, specific rotation, and ^1H NMR spectrum compared well with those corresponding to (−)-**1**.

Dipeptide (+)-**2** could also be prepared, in 71% yield, by coupling acid (+)-**7a** with *β*-Ala-OMe.

The synthesis of bisCBDPs was first tried by coupling acids (+)- or (−)-**7a** with the amines resulting from the hydrogenolysis of benzyl carbamates (+)-or (−)-**6a**. Revising the results of several batches, we detected a serious problem derived from the instability of the cyclobutane ring under the hydrogenation conditions. Amino ester **12**, resulting from the hydrogenation of (−)-**7a** (Scheme 2), is a captodative system that can evolve to an open-chain iminium salt, which is reduced in situ to the achiral *δ*-amino ester **13**.

Then, we studied the hydrogenation under several conditions related to the catalyst (Pd/C, $Pd(OH)_2$) and reaction time. We found that conditions needed to produce the free amine without ring-opening were difficult to reproduce. Reactivity was slightly increased when $Pd(OH)_2$ was used. With this catalyst, at short reaction times, a mixture of starting material, cyclobutylamine **12** and linear amino ester **13**, was detected on some occasions showing that reduction of the iminium salt competes with the hydrogenolysis of the benzyl

SCHEME 2[a]



[a] Reagents and conditions. (a) H_2, catalyst. (b) TFA, Et_3SiH, CH_2Cl_2, rt, 2 h. (c) H_3O^+. (d) DEC, HOBt, DMF, rt, 20 h.

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carbamate. Obviously, longer reaction times favored the production of **13**. This amino acid was condensed with (+)- and (−)-**7a** to provide dipeptides (+)- and (−)-**14**, respectively, which are constituted of a β- and a δ-residue (Scheme 2).[18]

To avoid this difficulty, we then prepared the N-Boc derivative **6b** as described above (Scheme 1). Deprotection of the amine was carried out under treatment with trifluoroacetic acid and triethylsilane affording **12**. When this product was submitted to lyophilization before peptide coupling to remove acid traces that diminish the reaction yield, the aldehyde shown in Scheme 2 was detected by ^{1}H NMR along with amine **12**.

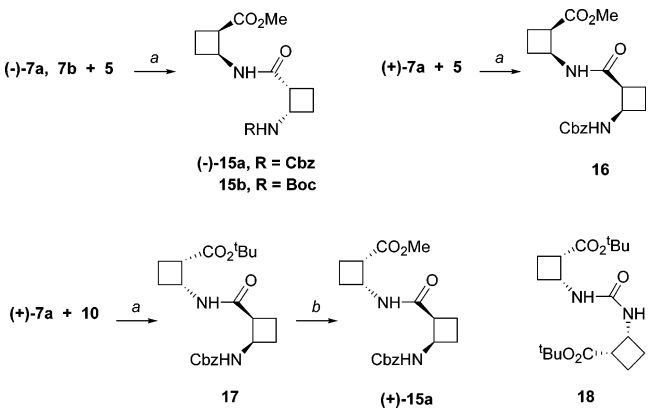
Other authors have also given accounts on the ease of some cyclobutane derivatives to give open-chain products under several conditions.[19] Fortunately, the ring-opening is not observed when a less electrophilic amido group, instead of an ester, is placed in a vicinal position with respect to a free cyclobutylamine, allowing us to synthesize peptides such as **3** (Chart 1).

To circumvent this difficulty and, at the same time, to shorten the synthetic sequence, we performed the in situ reactions of carboxylic acids (+)- and (−)-**7a** and **7b**, respectively, with the isocyanates resulting from Curtius rearrangement of acyl azides **5** or **10**, in refluxing toluene for 6 h (Scheme 3). Under these conditions, dipeptides (−)-**15a**, **15b**, **16**, and **17** resulted in 40−50% yield, after purification by flash chromatography.

Urea derivatives such as **18** were often obtained in 30−35% yield along with the peptides. Similar results were found by Hibbs et al. in the preparation of peptides incorporating an *endo*-5-norbornene residue following a similar methodology.[20] The formation of these byproducts could be avoided by using a molecular sieve pad, intercalated between the condenser and the reaction flask, to secure a water-free medium. In this way, pure dipeptides (−)-**15a** and **15b** were obtained in 66−70% yield and no urea trace was detected in any case. The structures of the peptides and the ureas were elucidated by X-ray diffraction analysis of compounds (−)-**15a**, **16**, **17**, and **18**. The structure of the already known acid (−)-**7a**[7] has also been elucidated by X-ray diffraction analysis in this work. Dipeptide **17** was converted into (+)-**15a** to asses the enantiomeric relationship with (−)-**15a** by removal of the *tert*-butyl ester as usual, followed by methylation with diazomethane. All compounds **15**−**17** are suitably protected for their later incorporation into longer oligomers.

2. X-ray Diffraction Analysis. The conformation and interactions in the solid state for the bisCBDPs (−)-**15a**, **16**, and **17** have been determined from single-crystal X-ray data. Suitable crystals were obtained from methanol−pentane or from ethyl acetate−pentane solutions. Crystal structures of the three dipeptides have, as a common feature, the existence of infinite chains of molecules linked by hydrogen bonds involving amide groups. Every chain comprises two antiparallel ···(H−N−C=O···)$_n$ patterns (Figure 1, Table 1) where all amide groups adopt a trans disposition. This packing description is common to the three dipeptides and can be related to a similar biased conformation. In this way, a hairpin-like folding can be considered, where the second residue is placed in the middle of the hairpin (Figure 2). The amide planes are orthogonal to the mean plane of the hairpin in order to form the above-mentioned intermolecular hydrogen bonds. Conformational differences are mainly involved in the disposition of terminal groups. For example, the bulky *tert*-butyl ester of compound **17** is placed outside of the hairpin, whereas the smaller methyl ester can be inside (**16**) or outside (**15a**). Moreover, some structural disorder is present in the phenyl and *tert*-butyl groups (not shown in the figures, see the Supporting Information). Crystal structures of (−)-**7a**, (−)-**15a**, **16**, **17**, and **18** have been deposited with the Cambridge Crystallographic Data Center and allocated deposition numbers CCDC 272745, CCDC 272746, CCDC 272747, CCDC 272748, and CCDC 272749, respectively.

SCHEME 3[a]



[a] Reagents and conditions. (a) Et_3N, toluene, 110 °C, 6 h. (b) (i) TFA, Et_3SiH, rt, 4 h. (ii) CH_2N_2.

3. NMR Studies. Complete ^{1}H and ^{13}C resonance assignments were performed using 1D and 2D NMR techniques (COSY, HSQC, and HMBC experiments). Moreover, variable-temperature 2D NOESY spectra were recorded at several temperatures, and temperature coefficients (Table 2) were measured on the synthesized molecules in order to state the conformational bias in solution. Although the ^{1}H NMR spectra were recorded in several deuterated solvents such as $CDCl_3$, acetonitrile, acetone, and methanol, most experiments were preferably carried out in $CDCl_3$ solution. As a general trend, it can be stated that in all studied compounds the presence of two different conformational structures was observed due to the cis−trans amide-bond isomerization in the benzyl carbamate fragment (Figure 3). The major conformation was assigned to the trans rotamer based on the above X-ray crystal structures and DFT theoretical calculations (vide infra). The amount of the minor cis rotamer was in the range of 5−10% as previously reported for tetrapeptide **3**.[5] The determination of the exact temperature and the energy barrier involved in the cis−trans interconversion is hampered due to peak broadening and chemical-shift dependence on temperature.

(18) Latter to publication, we have realized that measures of specific rotations described for dipeptides (+)- and (−)-**15**, and **17** in ref 14 were made on contaminated samples and are, therefore, wrong.

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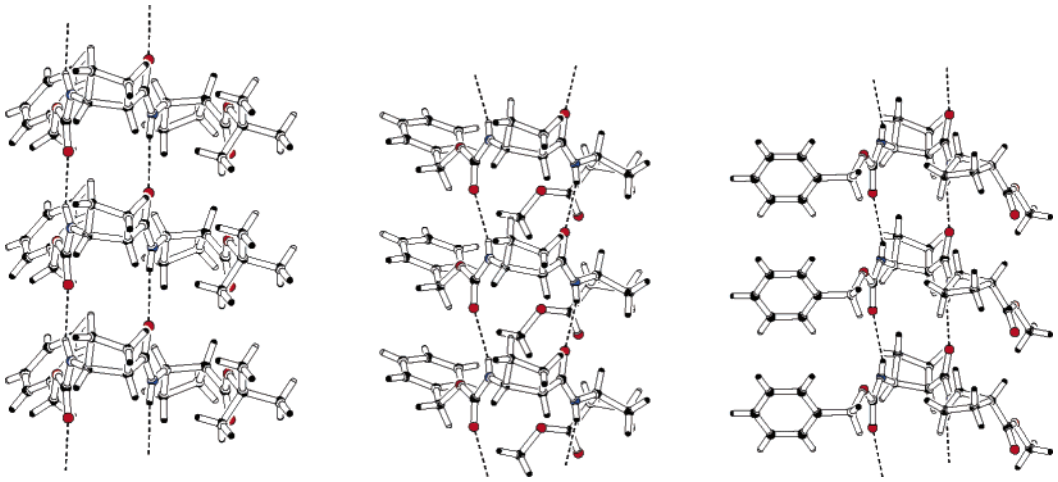

FIGURE 1. Intermolecular hydrogen bonds forming infinite chains in the crystal structures of dipeptides (−)-**15a**, **16**, and **17** (from left to right).

TABLE 1. Geometries of the Intermolecular Hydrogen Bonds Forming Infinite Chains in the Crystal Structures of Dipeptides (−)-15a, 16, and 17[a]

compound	N11···O10(*s*)[b]	H11···O10(*s*)[b]	N11-H11···O10(*s*)[c]	*s*
15a	2.982 (2)	2.014	155.41	$x, y+1, z$
16	3.049 (4)	2.049	163.07	$x+1, y, z$
17	3.019 (6)	2.031	159.71	$x+1, y, z$
	N18···O17(*s*)[b]	H18···O17(*s*)[b]	N18-H18···O17(*s*)[c]	*s*
15a	3.035 (2)	2.038	162.25	$x, y-1, z$
16	2.997 (4)	2.009	159.76	$x-1, y, z$
17	3.164 (6)	2.145	169.96	$x-1, y, z$

[a] N11−H11 and N18−H18 (1.030 Å) and the rest of parameters involving H11 and H18 have been normalized;[21] *s* is the symmetry code of the neighbor molecule. [b] In angstroms. [c] In degrees.

At room temperature, the NMR signals appeared as broad multiplets confirming the contribution of dynamic processes experimentally evidenced by the presence of strong negative exchange cross peaks in selective 1D NOESY and 2D NOESY spectra. When the temperature is lowered, all ^{1}H resonances became sharper and the particular variation of the N*H* and $H_{a,a'}$ chemical shifts could be used to monitor hydrogen-bonding properties by measurement of temperature chemical-shift coefficients. These data are valuable to establish, in a qualitative way, the apparent strength of possible intermolecular hydrogen bonding with carbonyl groups. For the monomers, the N*H* chemical shift usually shows a considerable upfield effect of about −0.25/−0.35 ppm in the minor conformation, probably due to the anisotropic properties of the carbonyl group.

However, a complicating factor for the proper analysis of this minor conformation is that tiny chemical exchange cross peaks are also visible even at 230−250 K. The carbamate N*H* resonance in the major conformation always shows a large coupling-constant value with the vicinal H_a proton (between 8 and 9 Hz) confirming its preferred anti disposition.

For the highly rigid dicyclobutane derivatives **16** and **17**, similar trends are also observed despite of their diastereomeric relationship (Figure 3b). Whereas N*H*11 protons experience a general upfield effect in the minor conformation around −0.3 ppm, the other amide N*H*18 proton feels a smaller but observable downfield effect between +0.05/+0.12 ppm confirming a minor variation of its surroundings. In the case of the *t*-Bu-derivative **17**, the N*H*11 proton shows a milder hydrogen bonding than N*H*18 clearly evidenced by the broad resonance peaks of N*H*11, whereas N*H*18 displays a well-resolved doublet at room temperature. The strong N*H*11-residual HDO exchange cross peak analysis in the NOESY spectra and temperature coefficients also account for the exchange differences between both N*H* protons (Table 2, Figure 4). Moreover, the trans disposition of the N−H18 bond is assessed by the strong NOE observed between N*H*18 and Hb.

Finally, for dimer **2c**[5] (Figure 3c), the ethylene fragment offers a more flexible motion allowing more free rotations around the peptide CO−N bond. Thus, although in acetonitrile and acetone this molecule displays the same behavior as that described above, more flexibility for the CO−NH18 bond is evidenced in $CDCl_3$ by simultaneous NOE between H_b and both the N*H*18 and diastereotopic α-NCH_2 protons. This effect is also highlighted from the analysis of the minor conformation in which the N*H*11 proton shows a different reversal downfield shift of +0.18 ppm, whereas a very strong upfield shift of −0.62 ppm is observed for N*H*18.

4. Theoretical Calculations. DFT theoretical calculations were done to account for the conformational features of (−)-**6a**, (−)-**15a**, and **16** as representative instances. The corresponding structures for the cis−trans rotamers related to the carbamate group in (−)-**6a** are

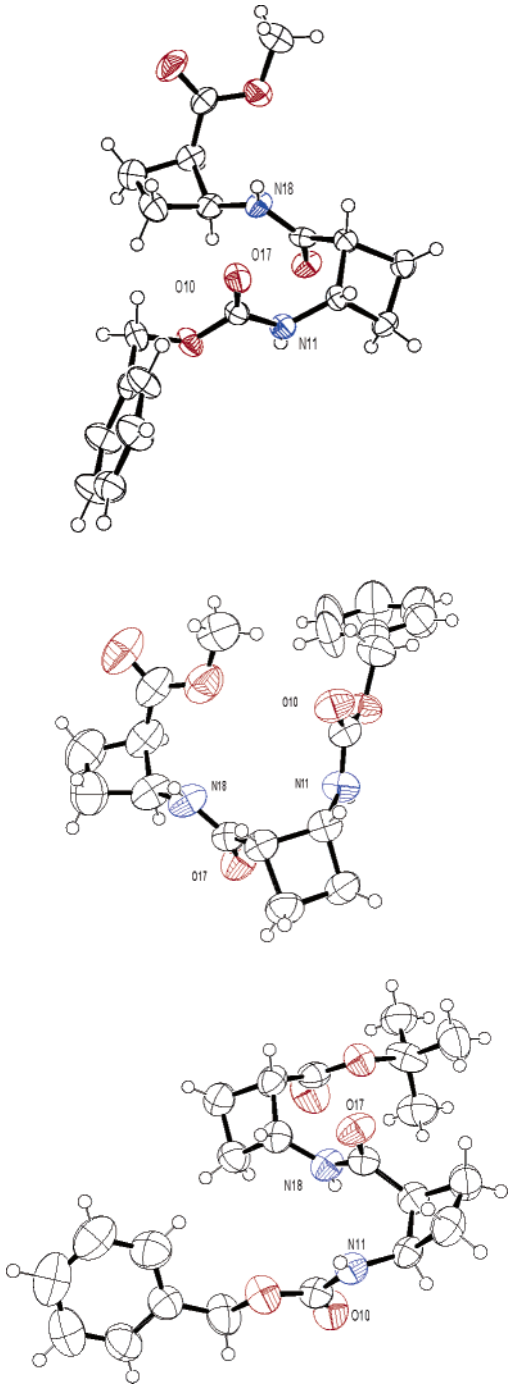

FIGURE 2. Molecular conformations of dipeptides (−)-**15a**, **16**, and **17** (from top to bottom) in the solid state.

TABLE 2. Temperature Coefficients[a] and, in Parentheses, Chemical Shifts[b] Measured for Several Cyclobutane Derivatives in $CDCl_3$ Solution

compound	NH11	NH18
2c	−1.3 (5.77)	−3.3 (6.74)
(−)-**6a**	−0.8 (5.74)	
16	−1.1 (5.79)	−0.7 (6.42)
17	−1.3 (5.82)	−0.6 (6.58)

[a] In ppb/°C. [b] In ppm at 298 K.

shown in Figure 5. The trans rotamer is the most stable one, and its rearrangement toward the cis one involves

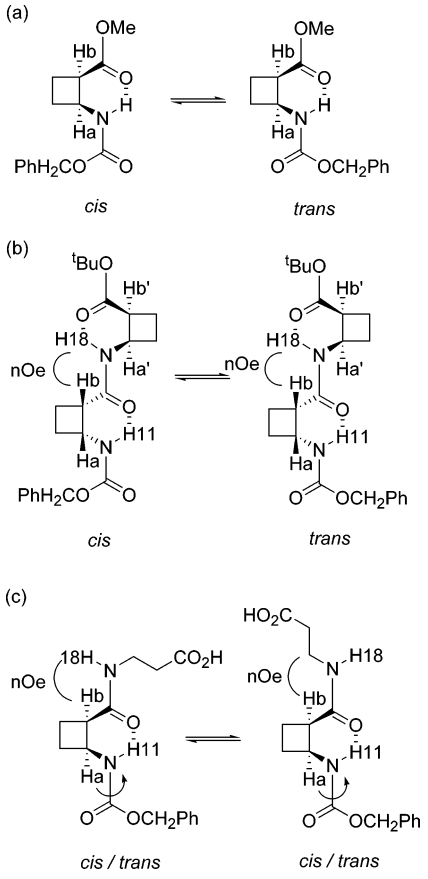

FIGURE 3. Intramolecular hydrogen bonds, cis−trans rotamers, and significant NOEs for (a) monomer (−)-**6a**, (b) dimer **17**, and (c) dimer **2c**.

a ΔG^0 of 2.3 kcal mol^{-1} and a $\Delta G^{\ddagger}$ of 17.9 kcal mol^{-1} in the gas phase. In chloroform solution these values are 2.6 and 17.6 kcal mol^{-1}, respectively.

In both structures, we can observe the presence of an intramolecular hydrogen bond. Other type of conformers due to the flexibility of the benzyl carbamate also emerge. Thus, the C(carbonyl)−O−C−C(phenyl) dihedral angle of the absolute minimum is −93°, but another conformer with a dihedral angle of −172° has been located as being only 0.3 kcal mol^{-1} higher in Gibbs energy. The chemical shifts for the N*H* proton in both conformers have been computed, the obtained values being δ 5.8 ppm (trans) and 5.4 ppm (cis). The difference $\Delta\delta$ of 0.4 ppm is in excellent agreement with the observed differences between the major and minor conformers.

Taking into account the general conformational bias of the bisCBDPs, Figure 6 shows the most stable structures obtained for (−)-**15a** and **16**.

These structures can be compared with those obtained from X-ray diffraction (Figure 2). The main difference between the solid state and the gas-phase optimized structures is that, in the latter, there are intramolecular CO···HN hydrogen bonds. The comparison with the structure optimized for the monomer (−)-**6a** (Figure 5) shows that dimerization strengthens the CO···HN hydrogen bonds. Moreover, N*H*18 displays a stronger hydrogen bonding than N*H*11 in good accordance with NMR results.

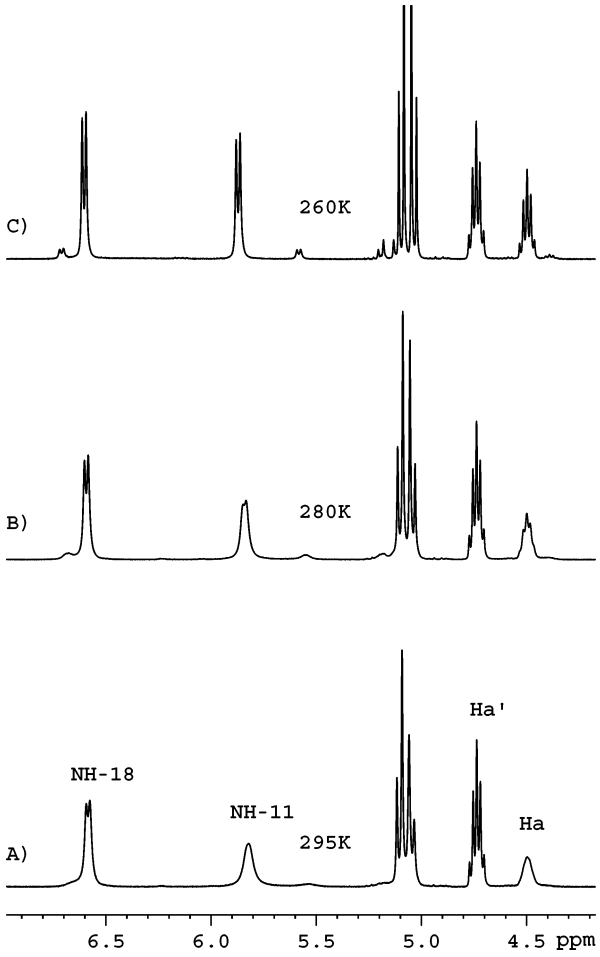

FIGURE 4. Expansions of the ^{1}H NMR spectra for dimer **17** (in $CDCl_3$) at several temperatures: (A) 295 K; (B) 280 K; (C) 260 K.

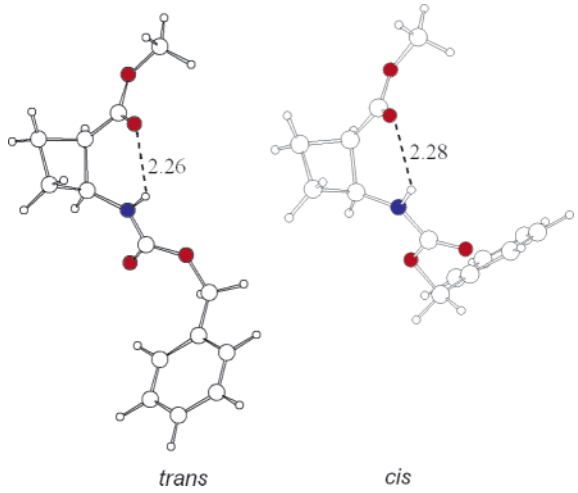

FIGURE 5. Structures of the trans and cis conformers of amino acid (−)-**6a**. Selected interatomic distances in Å.

Concluding Remarks

The high versatility and efficiency of the enantiodivergent synthetic approach to cyclobutane amino acids and derivatives presented herein has been shown with the synthesis of the enantiomeric CBAAs (+)- and (−)-**1** and several types of oligomers. These include β,β- and

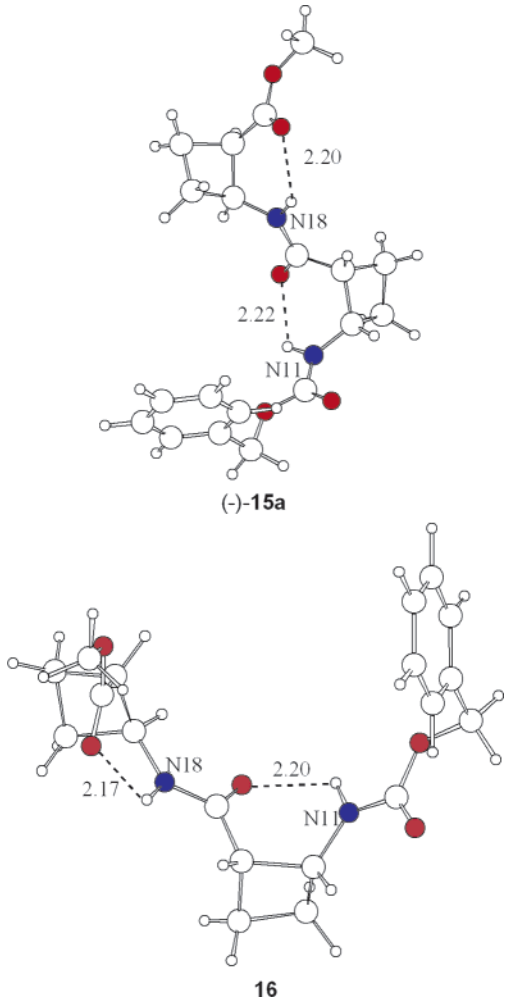

FIGURE 6. Structures of dipeptides (−)-**15a** and **16**. Selected interatomic distances in Å.

β,δ-dipeptides, some of them with an enantiomeric or diastereomeric relationship. Two synthetic ways have been explored for the preparation of bisCBDPs. The way involving reaction of a carboxylic acid with an acyl azide shortens the synthetic sequence and circumvents the difficulties in the deprotection of amines derived from the instability of the cyclobutane amino esters under several conditions. From a structural point of view, the ability of the cyclobutane ring to induce conformational bias has been evidenced. The formation of intramolecular hydrogen bonds is a general trend both in solution and in the gas phase (from DFT calculations), giving rise to the formation of highly rigid bicyclic structural units both in the monomers and in the dimers. For dipeptides **15**−**17** in the solid state, both the amide C17(O)−N18 bond and the carbamate group are disposed in a perpendicular plane with respect to the mean cyclobutane ring of the first residue, in such a way that infinite antiparallel intermolecular hydrogen bond chains are formed. Moreover, a cis−trans equilibrium associated to the rotation of the carbamate group is observed in solution, with the trans conformer being the major one. In the solid state, only the trans conformation is observed.

The potential of these and other related compounds in the search of novel therapeutic agents is the object of intense investigation in our laboratories.

Experimental Section

Computational Details. All calculations have been done using the Gaussian 03 program.[22] A preliminary exploration of the conformational space has been done using the semiempirical AM1 method.[23] The most stable structures have been fully optimized using the B3LYP[24] density functional method with the 6-31G(d) basis set. Harmonic vibrational frequencies of all the structures have been computed to verify that they are energy minima or transition states. Gibbs energies of these structures have been computed at 1 atm and 298.15 K. The effect of solvation by chloroform ($\epsilon = 4.9$) in the cis−trans conformational rearrangement of (−)-**6** has been taken into account using the CPCM method.[25] For the cis and trans conformers of (−)-**6** we have computed the ^{1}H chemical shifts using the gauge-independent atomic orbital (GIAO) method[26] at the B3LYP level of calculation with the 6-31+G(d,p) basis set.

Methyl-(1*R*,2*S*)-2-*tert*-butyloxycarbonylaminocyclobutane-1-carboxylate, 6b. A solution of azide **5** (522 mg, 2.8 mmol) in *t*-BuOH (9 mL) was heated to reflux overnight. The solvent was removed to give compound **6b** as a solid (616 mg, 94% yield) that was purified by crystallization. Crystals, mp 76.1 °C (EtOAc−hexane). $[\alpha]_D$ −131.0 (*c* 0.62, CH_2Cl_2). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.38 (s, 9H), 1.87−1.97 (m, 2H), 2.25 (m, 2H), 3.35 (m, 1H), 3.67 (s, 3H), 4.41 (m, 1H), 5.33 (m, 1H). ^{13}C NMR (62.5 MHz, CDCl3): δ 18.1, 27.8, (3C), 29.1, 44.9, 45.6, 51.3, 79.0, 154.4, 174.4. Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.75; H, 8.44; N, 6.13.

(1*R*,2*S*)-2-*tert*-Butyloxycarbonylaminocyclobutane-1-carboxylic Acid, 7b. A mixture of **6b** (168 mg, 0.7 mmol) in 10:1 H_2O−THF (7 mL) and 0.25 M NaOH (6 mL) was stirred at 0 °C for 3 h. Then the reaction mixture was extracted with CH_2Cl_2, and 4 M HCl was added to the aqueous layer to reach pH 2. Then the acid aqueous phase was extracted with EtOAc, and the solvent was removed at reduced pressure to afford acid **2** as a solid (129 mg, 82% yield). Crystals, mp 117 °C (ether−hexane). $[\alpha]_D$ −48.6 (*c* 0.74, CH_2Cl_2). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.45 (s, 9H), 1.72 (m, 1H), 2.04 (m, 1H), 2.32 (m, 2H), 3.35 (m, 1H), 4.35 (m, 1H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 27.3, 27.8 (3C), 29.4, 45.6, 47.0, 81.0, 157.3, 176.9. Anal. Calcd for $C_{10}H_{17}NO_4$: C, 55.80; H, 7.96; N, 6.51. Found: C, 55.45; H, 7.95; N, 6.35.

***tert*-Butyl-(1*S*,2*R*)-2-methoxycarbonylcyclobutane-1-carboxylate, 8.** *tert*-Butyl trichloroacetimidate (0.7 mL, 4.0 mmol) was added to a solution of half-ester **4**[7] (316 mg, 2.0 mmol) in dry dichloromethane (20 mL), and the mixture was stirred at room-temperature overnight. The solvent was removed at reduced pressure, and the residue was purified by column chromatography (3:1 hexanes−EtOAc as eluent) affording diester **8** (386 mg, 90% yield). Oil. $[\alpha]_D$ +4.0 (*c* 0.50, MeOH). IR (film): 1733 cm^{-1}. ^{1}H NMR (250 MHz, acetone-d_6): δ 1.42 (s, 9H), 2.16 (m, 2H), 2.24 (m, 2H), 3.36 (m, 2H), 3.64 (s, 3H). ^{13}C NMR (62.5 MHz, acetone-d_6): δ 21.5, 21.7, 28.3 (3C), 40.1, 41.4, 50.7, 79.6, 172.0, 173.1. Anal. Calcd for $C_{11}H_{18}O_4$: C, 61.60; H, 8.50. Found: C, 61.53; H, 8.48.

(1*S*,2*R*)-2-*tert*-Butyloxycarbonylcyclobutane-1-carboxylic Acid, 9. NaOH (0.25 M, 7.5 mL) was added to a solution of diester **8** (200 mg, 0.9 mmol) in 1:10 THF−H_2O (17.6 mL). The mixture was stirred at 0 °C for 1 h, and then the aqueous solution was extracted with dichloromethane. After acidification to pH 2 with 2 M HCl, the aqueous phase was extracted with ethyl acetate. The solvent was evaporated under vacuo, and the residue was chromatographed (EtOAc as eluent) to give quantitatively (187 mg) half-ester **9**. Oil. $[\alpha]_D$ +9.3 (*c* 1.08, MeOH). IR (film): 1798, 1690 cm^{-1}. ^{1}H NMR (250 MHz, acetone-d_6): δ 1.42 (s, 9H), 2.14 (m, 2H), 2.28 (m, 2H), 3.35 (m, 2H). ^{13}C (NMR (62.5 MHz, acetone-d_6): δ 21.3 (2C), 27.1 (3C), 40.0, 41.0, 79.2, 171.7, 173.5. Anal. Calcd for $C_{10}H_{16}O_4$: C, 60.00; H, 8.58. Found: C, 60.16; H, 8.43.

***tert*-Butyl-(1*S*,2*R*)-2-azidocarbonylcyclobutanecarboxylate, 10.** A mixture of half-ester **9** (600 mg, 3.0 mmol), ethyl chloroformate (0.6 mL, 3.8 mmol), freshly distilled Et_3N (0.8 mL, 3.5 mmol), and dry acetone (19 mL) was stirred at 0 °C for 3 h. Then a solution of sodium azide (429 mg, 6.6 mmmol) in water (10 mL) was added, and the resultant mixture was stirred at room temperature for 1.5 h. The reaction mixture was then extracted with dichloromethane. The layers were separated, the organic phase was dried over $MgSO_4$, and the solvents were removed at reduced pressure to afford azide **10** as a yellow oil that was used in the next step without further purification (615 mg, 91% yield). IR (film): 2134, 1714 cm^{-1}. ^{1}H NMR (250 MHz, acetone-d_6): δ 1.42 (s, 9H), 2.14 (m, 2H), 2.28 (m, 2H), 3.35 (m, 2H). ^{13}C NMR (62.5 MHz, acetone-d_6): δ 22.0 (2C), 27.7 (3C), 42.4, 43.4, 80.5, 172.0, 180.2.

***tert*-Butyl-(1*S*,2*R*)-2-benzyloxycarbonylaminocyclobutane-1-carboxylate, 11.** A solution of azide **10** (530 mg, 2.4 mmol) and benzyl alcohol (0.5 mL, 4.9 mmol) in dry toluene (14 mL) was heated to reflux for 3.5 h. The solvent was evaporated at reduced pressure, and the residue was chromatographed (dichloromethane as eluent) to provide pure **11** (590 mg, 82% yield). Oil. $[\alpha]_D$ +41 (*c* 0.42, $CHCl_3$). IR (film): 3330, 1738 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.40 (s, 9H), 1.93 (m, 2H), 2.29 (m, 2H), 3.25 (m, 1H), 4.47 (m, 1H), 5.06 (s, 2H), 5.64 (broad s, 1H), 7.32 (m, 5H). ^{13}C (NMR) (62.5 MHz, $CDCl_3$): δ 19.0 (2C), 26.6 (3C), 46.4, 46.6, 67.1, 81.5, 128.6, 129.0, 137.0, 155.8, 174.0.

Methyl-(1*S*,2*R*)-2-benzyloxycarbonylaminocyclobutane-1-carboxylate, (+)-6a through Acid (+)-7a. Trifluoroacetic acid (0.7 mL, 9.1 mmol) and Et_3SiH (0.15 mL, 1.8 mmol) were successively added to a solution of **11** (200 mg, 0.7 mmol) in dry dichloromethane (1.5 mL). The mixture was stirred at room temperature for 2 h, and the volatiles were removed under reduced pressure to afford the crude acid (+)-**7a** as a dense oil whose spectroscopic data compared well with those described for its enantiomer (−)-**7a**.[7] This product was used in the next steps without further purification.

An ethereal solution of excess diazomethane was distilled onto acid (+)-**7a** (200 mg, 0.8 mmol) in 5 mL of ether at 0 °C, and the resultant solution was stirred at room temperature for 20 min. Excess diazomethane was destroyed by the addition of $CaCl_2$, and the solvent was removed to give (+)-**6a** quantitatively (210 mg). Oil. $[\alpha]_D$ +83 (*c* 0.70, $CHCl_3$). IR (film): 3354, 1732 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.97 (m, 2H), 2.31 (m, 2H), 3.35 (m, 1H), 3.63 (s, 3H), 4.52 (m, 1H), 5.06 (s, 2H), 5.60 (broad s, 1H), 7.32 (m, 5H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 19.1, 28.3, 45.5, 46.3, 52.2, 67.2, 128.6, 129.0, 137.0, 155.8, 173.5. 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.

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General Procedure for the Hydrogenation of Compounds (+)- and (−)-7a. The synthesis of (−)-**1** is described. A solution of the N-protected amino acid (−)-**7a** (100 mg, 0.4 mmol) in dry methanol was hydrogenated over 10% Pd/C (9 mg) at room temperature and at 1 atm for 1.5 h. The catalyst was removed by filtration through Celite, washed with dichloromethane, and the filtrate was evaporated to afford quantitatively (48 mg) the free amino acid (−)-**1**.

(1*R*,2*S*)-2-Aminocyclobutane-1-carboxylic Acid, (−)-1. Crystals, mp 130 °C (dec) (from MeOH−H_2O), $[\alpha]_D$: −83 (*c* 0.70, H_2O), (lit.[12] mp 130 °C (dec), $[\alpha]_D$ −80 (*c* 1.00, H_2O). IR (film): 3354, 1732 cm^{-1}. ^1H NMR (250 MHz, D_2O): δ 2.05 (m, 2H), 2.26 (m, 2H), 3.21 (m, 1H), 3.93 (m, 1H). ^{13}C NMR (62.5 MHz, D_2O): δ 21.1, 25.0, 41.3, 45.5, 180.8.

(1*S*,2*R*)-2-Aminocyclobutane-1-carboxylic Acid, (+)-1. Crystals, mp 131−133 °C (from MeOH−H_2O), $[\alpha]_D$ +80 (*c* 0.75, H_2O). Spectroscopic ^1H and ^{13}C NMR data are in total agreement with those described above for the enantiomer.

General Procedures for the Synthesis of Pepetides. Method A: Peptide Coupling. Hydroxybenzotriazole (HOBt) (1.0 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (DEC) hydrochloride (3.4 mmol) were successively added to a solution containing the acid (1.2 mmol) and the amine (1.1 mmol) in freshly distilled dry DMF (14 mL). The light-protected mixture was stirred at room temperature under argon atmosphere. Total conversion of the reaction was monitored by TLC. After completion, ethyl acetate was added, and the solution was washed with five portions of saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$, and the solvent was removed at reduced pressure to afford the corresponding peptide that was purified by column chromatography (3:2 hexanes−EtOAc).

Method B: Reaction between a Carboxylic Acid and an Azide. Carboxylic acids (−)-**7a** or **7b** (5 mmol) were heated in boiling anhydrous toluene (20 mL) for 40 min in such a way that the refluxing solvent passed through a molecular-sieve pad before returning to the flask. Azide **5** (4 mmol) was then added, and the mixture was heated for 6 h. The solvent was removed at reduced pressure, and the residue was purified by column chromatography.

2-Benzyloxycarbonylamino-(1*S*,2*R*)-cyclobutane-1-carboxylic Acid *N*-Methoxycarbonylethyl Amide, (+)-2. This was prepared according to Method A. 71% yield. Crystals, mp 94−95 °C (EtOAc) $[\alpha]_D$: +72 (*c* 0.8, MeOH), (lit.[7] mp 91−93 °C, $[\alpha]_D$ −67 (*c* 2.9, MeOH) for the enantiomer). ^1H and ^{13}C NMR spectroscopic data are in good agreement with those described for the enantiomer.[7] Anal. Calcd for $C_{17}H_{22}N_2O_5$: C, 61.05; H, 6.64; N, 8.38. Found: C, 60.95; H, 6.82; N, 8.59.

Dipeptide (−)-15a. Method B. 529 mg, 70% yield. Crystals, mp 79−80 °C (EtOAc−pentane). $[\alpha]_D$ −167 (*c* 1.06, MeOH). IR (solid): 3304, 1714, 1655 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.94 (m, 2H), 2.05 (m, 2H), 2.16 (m, 1H), 2.31 (m, 3H), 3.17 (m, 1H), 3.39 (m, 1H), 3.70 (s, 3H), 4.48 (q, J = 8.7 Hz, 1H), 4.74 (q, J = 8.5 Hz, 1H), 5.03 (d, J = 12 Hz, 1H), 5.11(d, J = 12 Hz, 1H), 5.90 (d, J = 8.7 Hz, 1H), 6.54 (d, J = 8.7 Hz, 1H), 7.35 (m, 5H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 18.5, 19.1, 29.2, 29.5, 44.0, 44.4, 46.1, 46.4, 51.7, 66.5, 127.9, 128.0, 128.4, 136.4, 155.6, 172.2, 174.6. Anal. Calcd for $C_{19}H_{24}N_2O_5$: C, 63.30; H, 6.72; N, 7.77. Found: C, 63.41; H, 6.89; N, 7.49.

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Supporting Information Available: Full description of products (+)-**14**, (−)-**14**, (+)-**15a**, **15b**, **16**, **17**, and **18**; ^1H and ^{13}C NMR spectra of (+)- and (−)-**1**, **10**, **11**, (+)- and (−)-**14**, (+)-**15a**; NMR studies for **2c, 6a**, **16**, and **17**; cartesian coordinates and total energies for all computed structures; drawings of models for the structural disorder found in the crystals of dipeptides **16** and **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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