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A new efficient synthesis of enantiopure diastereomeric 3′-aminocyclopentylglycines

Maria Luisa Gelmi, Francesca Clerici, Raffaella Gandolfi and Sara Pellegrino*

Istituto di Chimica Organica 'A. Marchesini', Facoltà di Farmacia, Università di Milano, Via Venezian 21, I-20133 Milano, Italy

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Abstract—A new synthesis of enantiopure (1′*S*,3′*R*,2*R*)- and (1′*R*,3′*S*,2*R*)-3′-aminocyclopentylglycines (−)-**12a** and (−)-**12b** was performed by taking advantage of (±)-2-amino-3-oxo-norbornane-2-carboxylic acid derivative *exo*-**2** as the starting material. The use of an acylase from *Aspergillus melleus* in phosphate buffer allowed the 'one-pot' transformation of the β-ketoester (±)-*exo*-**2** into 3′-carboxycyclopentylglycine (±)-**3a** and (±)-**3b**, via a retro-Dieckman reaction, which, by direct kinetic resolution, were isolated as compounds (−)-**3a** and (−)-**3b**.
Starting from a mixture of (−)-**3a** and (−)-**3b**, enantiopure 3′-aminocyclopentylglycines (−)-**12a** and (−)-**12b** as well as differently substituted 3-amino derivatives were prepared efficiently using a very simple synthetic protocol that requires a single chromatographic purification.

1. Introduction

3′-Aminocyclopentylglycine derivatives **1** (Fig. 1) are constrained amino acid in which both the skeleton of lysine and ornithine are included.

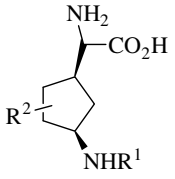

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Figure 1. 3′-Aminocyclopentylglycine derivatives.

The interest towards these nonnatural amino acids is related to their different biological activities depending on the substitution pattern on the ring, on 3′-amino group and on the stereochemistry of the three stereocentres. As examples, the inhibition of the NO-synthase enzymes (NOS), responsible for the transformation of arginine into citrulline,[1] is reported for the above compound, where the amino group was transformed into an amidino group. The *N*-acetyl-*N*-hydroxyl derivative has been synthesized for its potential activity as antibiotic drug, an inhibitor of the biosynthesis of amino acid based siderophores.[2] Amino acid **1**, functionalized at C-3 with a guanidino group, has been inserted into a peptide chain instead of the natural arginine and tested in the prevention and treatment of conditions characterized by abnormal thrombosis in mammals.[3] Other activities are reported such as the inhibitor activity of metalloprotease[4] and of influenza neuraminidase.[5] Recently, functionalized 3′-amino cyclopentylglycines were tested as dipeptidyl peptidase IV inhibitors.[6] Finally, many efforts were devoted to the preparation of polyhydroxylated compounds, which mimics the sugar portion of polioxines and nikkomicynes, compounds characterized by antifungal activity.[7]

Owing to the general biological importance of the amino acids of class **1**, we planned the preparation of a couple of diastereomeric 3′-aminocyclopentylglycines following a different synthetic reaction path with respect to those reported in the literature[1,2,6,7a–c,8] by taking advantage of the 3-oxygen substituted 2-amino-norbornane-2-carboxylic acid derivatives. Recent studies in our group revealed that norbornane amino acids are interesting key starting materials to obtain new constrained amino acids with control of the stereochemistry of several centres.[9] In particular, 3-hydroxy substituted compounds allowed us to synthesize two epimeric cyclopentylglycine derivatives by disconnection of the C_2–C_3 bond.[9c]

* Corresponding author. E-mail: sara.pellegrino@unimi.it

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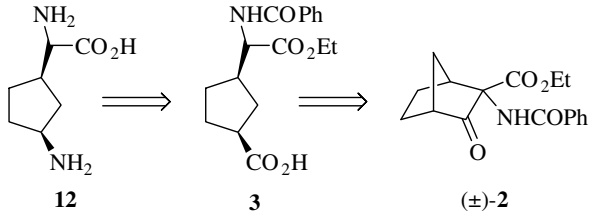
Following this synthetic strategy, we planned the preparation of diastereomeric 3′-aminocyclopentylglycines **12** starting from the 2-amino-3-oxo-norbornane-2-carboxylic acid derivative **2**. As shown in the retrosynthetic Scheme 1, by performing a retro-Dieckman reaction on ketone (±)-**2**, it is possible to obtain two 3′-carboxycyclopentylglycines **3** (see also Scheme 4), epimeric at the amino acid stereocentre and characterized by a *cis*-relationship between the two carbon substituents on the ring. Their transformation into the targeted compounds **12** can be achieved by the way of a Curtius transposition.



Scheme 1. Retrosynthesis of **12**.

The above transformation was efficiently realized without isolation of the different intermediates obtaining both functionalized or unfunctionalized derivatives at the 3′-amino group (Schemes 2, 3, 5, and 6).

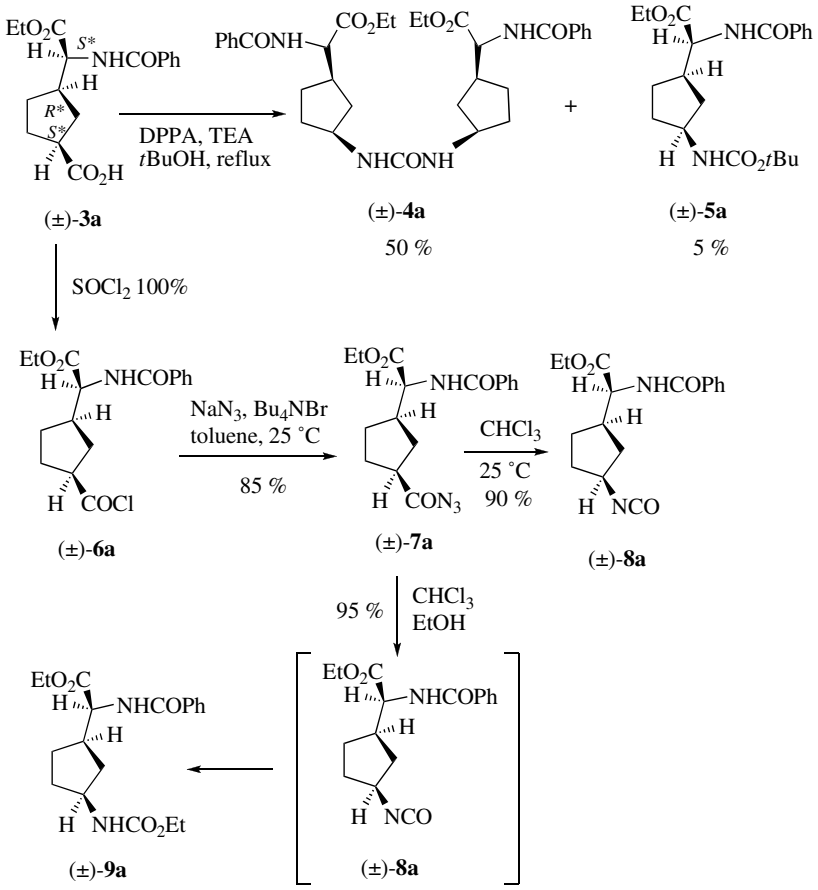
Furthermore, by starting from racemic (±)-**2**, and using a very efficient protocol consisting of a 'one-pot' retro-Dieckman reaction-chemoenzymatic resolution of each pair of epimeric 3-carboxycyclopentylglicine derivatives, followed by a 'one-pot' transformation of the 3′-carboxylic acid group into the amino one, enantiopure 3′-aminocyclopentylglycine derivatives were generated (Scheme 4).

It should be emphasized that the whole synthetic protocol required only one chromatographic purification.

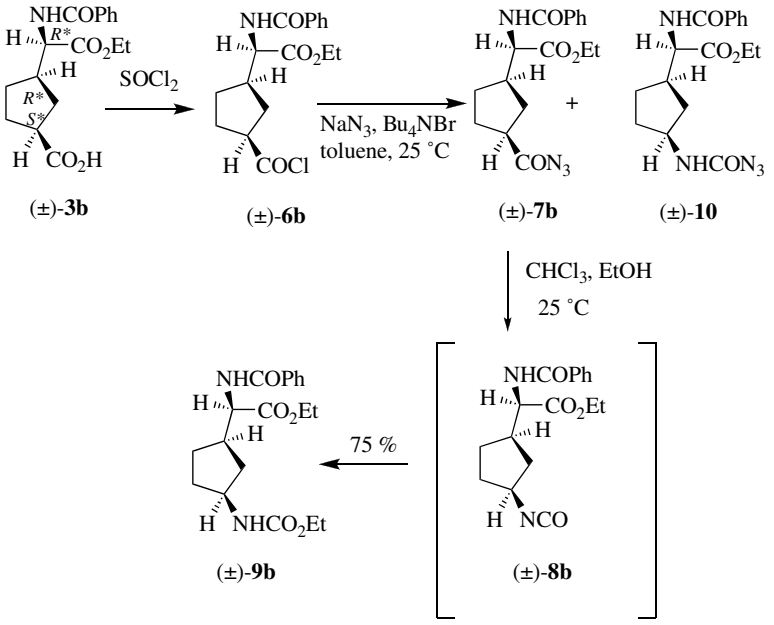
2. Results and discussion

The two epimeric cyclopentylglycines (±)-**3a**,**b**[9b] were the key starting materials for the preparation of the corresponding 3′-amino compounds **12**.

A modified Curtius reaction was first used with the aim of transforming the 3′-carboxy group into the 3′-amino one. According to the Ninomiya method,[10] acid **3a** was made to react with diphenylphosphoryl azide (DPPA) operating in *t*-butanol and triethylamine. The reaction gave a mixture of compounds in which urea **4a** was the main product (50%). The expected *N*-Boc-carbamate **5a** was isolated in only 5% yield (Scheme 2). In principle, starting from racemic compound **3a** a mixture of diastereomers of symmetric urea **4a** was expected to be formed. Since any attempt to



Scheme 2. Curtius transposition of **3a**.



Scheme 3. Curtius transposition of **3b**.

separate the isomers (HPLC technique) failed, and ^{1}H and ^{13}C NMR analyses presented unitary signals, we were unable to determine if a single diastereomer or a mixture of diastereomers was obtained.

The goal of the preparation of N-protected amino derivatives was achieved performing the classical Curtius reaction (Scheme 2). Since the intermediates of the above transformation were not stable and could not be isolated in pure form, a step-by-step study was first carried out on the crude reaction mixtures, characterizing all intermediates by IR and ^{1}H NMR analyses.

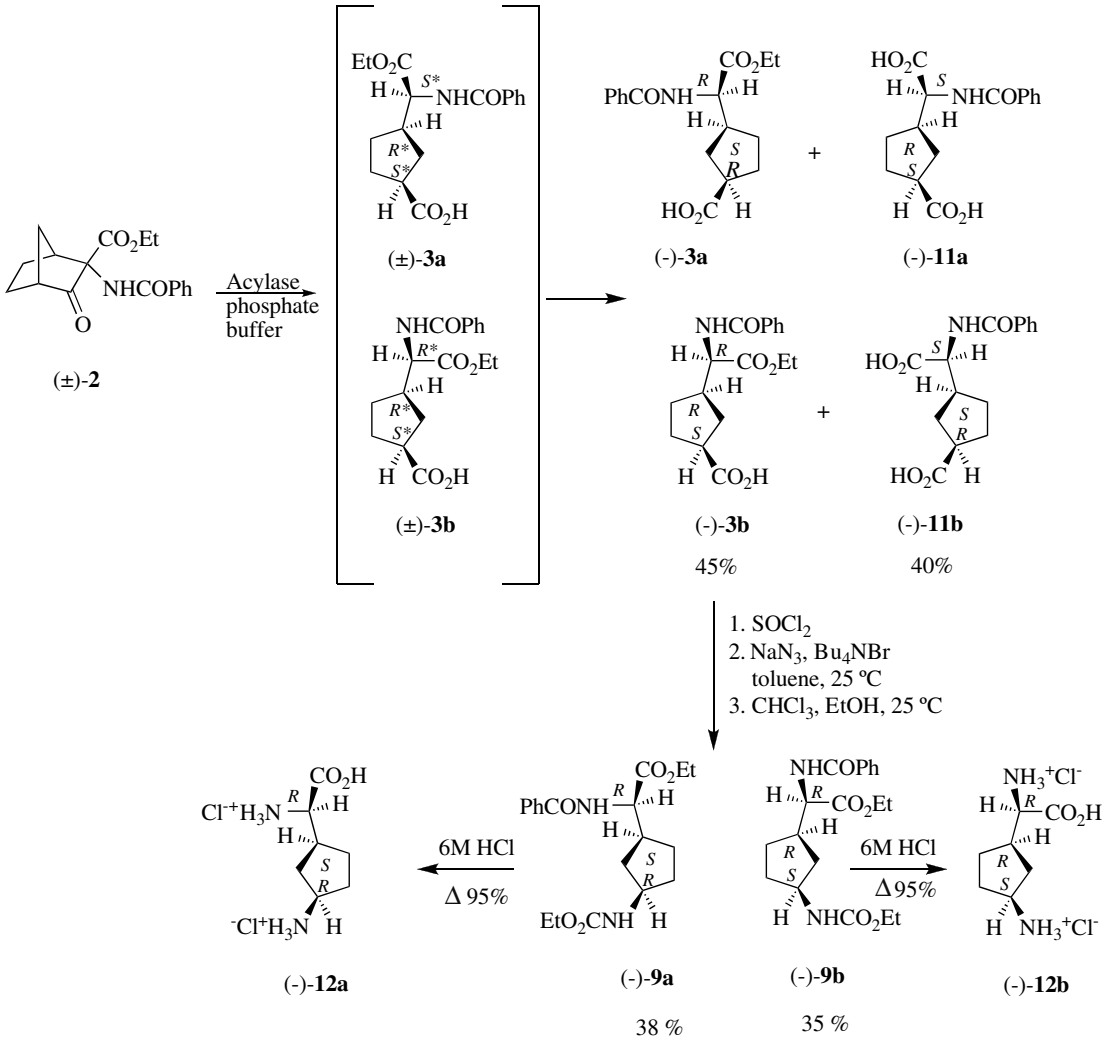
The reaction of acid **3a** with thionyl chloride (10 °C, 2 h) gave the corresponding acyl chloride **6a** in quantitative yields (IR: 1791 cm^{-1}, COCl; ^{1}H NMR: δ 3.32–3.25, H-3′). Any attempt to purify this compound gave the starting material. According to a reported procedure,[11] the crude compound **6a** was then treated with sodium azide operating in a mixture of H_2O/acetone at 0 °C; however, these reaction conditions hydrolyzed the acyl chloride group to the corresponding starting acid **3a**. This problem was overcome by operating under solid liquid phase transfer catalysis conditions using anhydrous toluene, sodium azide (1.1 equiv) and tetrabutylammonium bromide as the catalyst, operating at 25 °C. Acyl azide **7a** was obtained from **6a** after 4 h in 85% yield (crude compound). The structure of the acyl azide was confirmed by IR (2163 cm^{-1}, CON_3) and ^{1}H NMR (δ 2.99–2.83, H-3′). Interestingly, it was found that when **7a** was stood in $CHCl_3$ (24 h), the acyl azide was not stable and was transformed into the corresponding isocyanate **8a** (90%, crude compound; IR: 2258 cm^{-1}; ^{1}H NMR: δ 3.97–3.88, H-3′). Instead, the use of $CHCl_3$ stabilized with EtOH gave the 'one-pot' transformation of the acyl azide **7a** into the ethyl carbamate **9a** (12 h, 95%), via isocyanate **8a**, which could not be isolated. This behaviour can be ascribed to the acidity of the medium, which probably catalyzed the transformation of the acyl azide into isocyanate from which, by addition of EtOH, the corresponding ethyl carbamate was formed.

With the stability and reactivity of the above intermediates established, we planned a very quick synthesis, without purification and in some cases isolation of the intermediates testing the single transformation step by step with IR and ^{1}H NMR techniques starting from both **3a** and **3b**.

The ethyl carbamate **9a** was obtained from **3a** in 80% overall yield.

The same transformations were made for epimer **3b** (Scheme 3). According to the above procedure, acyl chloride **6b** was first prepared, which was then transformed into acyl azide **7b** using NaN_3 (1.1 equiv, 4 h). The ^{1}H NMR analysis revealed the presence of a mixture of compounds corresponding to the expected acyl azide **7b** (δ 2.93–2.80, H-3′) together with isocyanate **8b** (δ 3.90–3.60, H-3′) and trace amounts of a new compound (δ 6.0 d, $J = 6.9$ Hz, exch.; δ 4.18–4.03, H-3′). The mixture was treated directly with $CHCl_3$ containing EtOH (12 h) to give complete transformation of isocyanate **8b** into ethyl carbamate **9b**, which was isolated in 75% overall yield after column chromatography A trace amount of byproduct **10** (5%) corresponding to a carbamoylazide was also isolated.

With a good protocol to obtain the epimeric 3′-aminocyclopentylglycine derivatives in hand, we planned their preparation in an enantiopure form. Recently, we reported on the preparation of enantiopure 3′-carboxy-cyclopentylglycines **3** using an enzymatic resolution starting from the corresponding racemic compounds.[9b] Acylase from *Aspergillus melleus* is able to selectively hydrolyze the (*S*)-enantiomer of both pure racemic substrates (±)-**3a** and



Scheme 4. 'One-pot' retro-Dieckmann reaction/enzymatic resolution/Curtius transposition.

(±)-**3b** to give bicarboxylic acid (−)-(1*S*)-**12a** and ester (−)-(1*R*)-**3a** from (±)-**3a** and (−)-(1*S*)-**12b** and ester (−)-(1*R*)-**3b** from (±)-**3b** with an ee >99% and 50% molar conversion.

Here, we report on an improvement of this enzymatic procedure using the same enzyme and, as a starting material for this kinetic resolution, the β-ketoester (±)-*exo*-**2** (2 g) in the phosphate buffer (0.1 M, pH 7) was first transformed, via a retro-Dieckman reaction, into the epimeric mixture of (±)-**3a** and (±)-**3b**. Their direct enzymatic resolution allowed us to obtain a mixture of esters (−)-**3a** and (−)-**3b** and bicarboxylic acids (−)-**11a** and (−)-**11b**. Bicarboxylic acids **11** were easily separated from esters **3** by crystallization in dichloromethane. The mother liquor containing the mixture of esters (−)-**3a** and (−)-**3b** was isolated in 45% yield and used for further transformation into the 3′-amino compounds (Scheme 4).

According to the above procedures, the mixture of enantiopure esters (−)-**3a** and (−)-**3b** was first transformed into the acyl chlorides (compounds **6a**,**b**). Their reaction with NaN_3 (1.1 equiv, 3 h) afforded acyl azides **7a**,**b**, which were then stood in $CHCl_3$ stabilized with EtOH (12 h, 25 °C). The corresponding ethyl carbamates **9a**,**b** were formed and efficiently separated by column chromatography. Compounds (−)-**9a** and (−)-**9b** were isolated in pure form in 38% and 35% overall yields, respectively (Scheme 4).

The hydrolysis of (−)-**9a** and (−)-**9b** with 6 M HCl at 90 °C for 24 h afforded (−)-**12a** and (−)-**12b** in 95% yield. The ^1H NMR analysis of **12a** (δ 3.76, d, $J = 7.6$ Hz, H-2) revealed the presence of a trace amount of a second epimer (95:5 ratio), deriving from partial epimerization at an amino acid stereocentre. The same behaviour was observed when starting from **12b** (95:5 ratio; δ 3.84, d, $J = 6.2$ Hz, H-2) (Scheme 4).

Since carbamates and asymmetric ureas of cyclopentylglycine derivatives are compounds of biological interest,[6] we planned their preparation using the above synthetic way, which represents a valuable and faster synthetic procedure with respect to those reported in the literature. Starting from pure (−)-**3a**, the corresponding enantiopure isocyanate **8a** was obtained in 90%. Different selective transformations of isocyanate were performed.

Its reaction with *t*-BuOH and in presence of a catalytic amount of $SnCl_4$ gave *t*-butylcarbamate (−)-**5a** (60%) (Scheme 5).

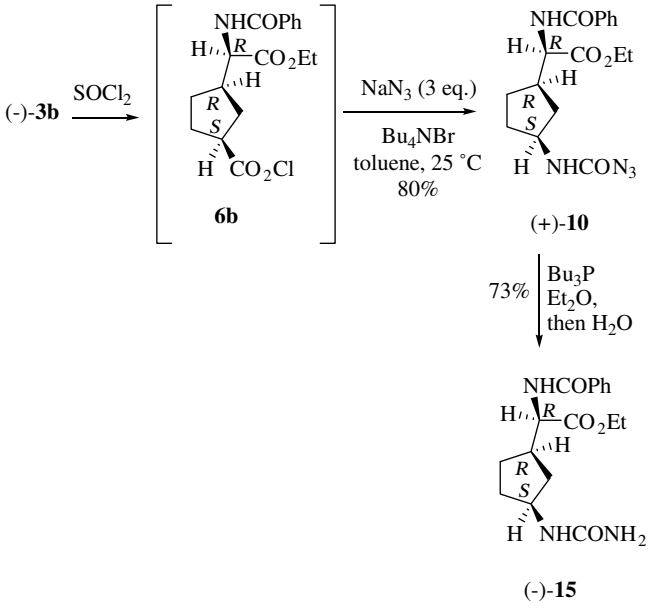
When isocyanate **8a** was made to react with the *p*-methoxybenzylamine, the asymmetric urea (−)-**13a** was isolated in 80% yield (Scheme 5).

Finally, the transformation of isocyanate group in the free amine was studied. Compound **8a** was treated with pyridine (1 equiv) in water. The reaction is very fast (10 min), and urea **4a** was isolated in 70% yield (Scheme 5). The NMR spectra of enantiopure compound **4a** are superimposable to those of **4a** prepared as reported in synthetic Scheme 2.

Instead, when the reaction was performed in $CHCl_3$ and in the presence of *p*-toluensulfonic acid (1 equiv), *p*-toluensulfonate (+)-**14a** was formed in quantitative yield from enantiopure **8a** (Scheme 5).

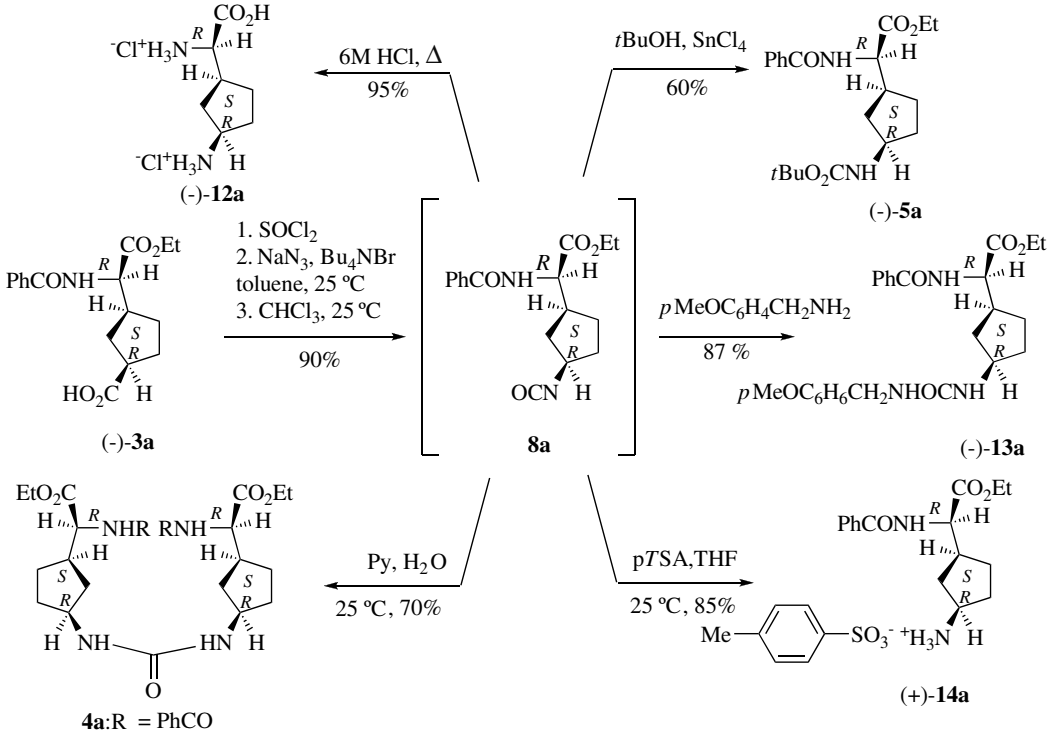
Furthermore, it is possible to obtain the free amino acid (−)-**12a** (95%), via direct hydrolysis of isocyanate **8a** with 6 N HCl at reflux.

Since the transformation of racemic acyl chloride **6b** in the presence of NaN_3 gave the functionality as a byproduct, we evaluated the possibility of increasing the yield of compound **10**. Enantiopure acyl chloride **6b**, obtained from (−)-**3b**, was made to react with an excess of NaN_3 (3 equiv) in toluene in the presence of a catalytic amount of TBABr (25 °C, 12 h). The purification of the crude reaction mixture by chromatography on silica gel gave carbamoylazide (+)-**10** (80%). Its reduction was performed according to Staudinger procedure[12] using Bu_3P in Et_2O operating at room temperature for 6 h. The corresponding urea (−)-**15** was isolated in 73% yield (Scheme 6).



Scheme 6. Synthesis of ureido derivative (−)-**15**.

It should be noted that when starting from epimer **3a** and using an excess of NaN_3 (3 equiv), the formation of the corresponding carbamoyl azide was never detected by NMR. It can be concluded that isocyanate **8b** is more reactive with respect to isocyanate **8a**.



Scheme 5. Synthesis of 3′-amino derivatives.

3. Conclusions

In conclusion, the synthesis of a series of diastereomeric 3′-amino cyclopentylglycine derivatives was performed starting from 2-amino-3-oxo-norbornane-2-carboxylic acid derivative (±)-*exo*-**2** taking advantage of the retro-Dieckman and Curtius reactions as key steps. Even if different synthetic steps are involved in the synthesis of amino acids **12**, the whole synthetic procedure does not require the isolation of the intermediates and a single chromatographic purification step is needed to separate the mixture of diastereomers.

4. Experimental

4.1. General

Melting points were measured with a Büchi B-540 heating unit and are uncorrected. ^{1}H NMR spectra were recorded on 200 or 500 MHz spectrometers. Thin Layer Chromatography (TLC) analyses were performed on ready-to-use silica gel carbamoyl azide **10**, which could be transformed into the corresponding urea derivative by reduction of the azido plates. Column chromatography was performed on silica gel [Kieselgel 60–70 230 ASTM] with the solvents indicated. IR spectra were taken with a FT-IR spectrophotometer. If not specified, ethanol free $CHCl_3$ was used in all experiments. Compounds **2**, (−)-**3a** and (−)-**3b** are known compounds.[9b]

4.2. 'One-pot' retro-condensation reaction/enantiomer resolution

Ketone (±)-*exo*-**2** (2 g, 6.65 mmol) was suspended in phosphate buffer (400 mL, 0.1 M, pH 7) and Acylase from *A. melleus* (10 g/L) was added. The biotransformation system was incubated at 30 °C with magnetic stirring. After 48 h (HPLC analysis: Chiralcel OD column, hexane/*i*-PrOH/TFA: 85:15:1, flow: 0.5 mL/min, $\lambda = 230$ nm), the reaction mixture was extracted with ethyl acetate. The aqueous phase was treated with 5% HCl (pH 2) and extracted with a mixture of ethyl acetate/THF (1:1, 3 × 20 mL). All the combined organic phases were dried over Na_2SO_4 and the solvent removed under vacuum. A mixture of compounds (−)-**11a** and (−)-**11b** (770 mg, 40%) was separated from the mixture of esters (−)-**3a** and (−)-**3b** (955 mg, 45%) by crystallization with CH_2Cl_2. It is possible to isolate compound (−)-**11a** from (−)-**11b** and compound (−)-**3a** from (−)-**3b** by column chromatography.[9b]

4.3. 'One-pot' synthesis of ethyl benzoylamino-(3′-ethoxycarbonylamino-cyclopentyl)-acetates 9a and 9b

Operating at 10 °C with stirring, compound (±)-**3a**, or (±)-**3b**, or (−)-**3a** or (−)-**3b** or the 1:1 mixture of (−)-**3a**/(−)-**3b** (200 mg, 0.63 mmol) was dissolved in $SOCl_2$ (2 mL). After 2 h (^{1}H NMR analysis), $SOCl_2$ was evaporated. The crude reaction mixture was treated with anhydrous toluene (3 × 10 mL) and evaporated under vacuum to give acyl chloride, **6a** and/or **6b**. Compound **6** was then dissolved in anhydrous toluene (5 mL) operating under nitrogen. A catalytic amount of TBABr (20 mg, 0.063 mmol) and NaN_3 (55 mg, 0.75 mmol) was added to the solution, which was stirred at room temperature for 3 h (^{1}H NMR and IR analyses). The reaction mixture was taken up in H_2O (5 mL) and the phases were separated. The aqueous solution was extracted with AcOEt (3 × 10 mL) and the combined organic layers were dried over Na_2SO_4. After evaporation of the solvent, crude acyl azide **7a** and/or **7b** was obtained. The residue was taken up with $CHCl_3$ stabilized with EtOH (10 mL) and the mixture was stirred for 12 h (TLC: cyclohexane/AcOEt, 1:1). The solvent was removed under vacuum and the crude mixture was purified by flash chromatography (cyclohexane/AcOEt, 2:1) to obtain pure compound **9** [from **3a**, **9a** (180 mg, 80%); from mixture (−)-**3a**/(−)-**3b**, **9a** (85 mg, 38%) and **9b** (80 mg, 35%) from pure **3b**, **9b** (170 mg, 75%)]. Trace amount of **10** (10 mg, 5%) was detected when **9b** was prepared (see below).

4.3.1. Ethyl (1′*S*,3′*R*,2*R*)-2-benzoylamino-(3′-chlorocarbonyl-cyclopentyl)-acetate 6a. Crude compound. IR (Nujol) ν_{max} 1791, 1731, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.84–7.40 (m, 5H), 6.77 (d, $J = 8.0$ Hz, 1H, exch.), 4.90 (dd, $J = 8.0$, 5.5 Hz, 1H), 4.24 (q, $J = 7.0$ Hz, 2H), 3.32–3.25 (m, 1H), 2.62–2.55 (m, 1H), 2.21–1.56 (m, 6H), 1.31 (t, $J = 7.0$ Hz, 3H).

4.3.2. Ethyl (1′*R*,3′*S*,2*R*)-2-benzoylamino-(3′-chlorocarbonyl-cyclopentyl)acetate 6b. Crude compound. IR (Nujol) ν_{max} 1790, 1728, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.81–7.40 (m, 5H), 6.73 (d, $J = 8.5$ Hz, 1H, exch.), 4.90 (dd, $J = 8.5$, 6.2 Hz, 1H), 4.26 (q, $J = 7.4$ Hz, 2H), 3.35–3.20 (m, 1H), 2.59–2.42 (m, 1H), 2.32–1.52 (m, 6H), 1.30 (t, $J = 7.4$ Hz, 3H).

4.3.3. Ethyl (1′*S*,3′*R*,2*R*)-2-benzoylamino-(3′-azidocarbonyl-cyclopentyl)acetate 7a. Crude compound. IR (Nujol): ν_{max} 2163, 1700, 1665 cm^{-1}; ^{1}H NMR δ (ppm) ($CDCl_3$) δ 7.88–7.40 (m, 5H), 6.80 (d, $J = 8.1$ Hz, 1H, exch.), 4.89 (dd, $J = 8.1$, 5.1 Hz, 1H), 4.25 (q, $J = 7.0$ Hz, 2H), 2.99–2.83 (m, 1H), 2.78–2.53 (m, 1H), 2.30–1.60 (m, 6H), 1.32 (t, $J = 7.0$ Hz, 3H).

4.3.4. Ethyl (1′*R*,3′*S*,2*R*)-2-benzoylamino-(3′-azidocarbonyl-cyclopentyl)acetate 7b. (Mixture with **15**) IR (Nujol) 2163, 1700, 1665 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.85–7.42 (m, 5H), 6.86 (d, $J = 7.7$ Hz, 1H, exch.), 4.88 (dd, $J = 7.7$, 5.9 Hz, 1H), 4.26 (q, $J = 7.0$ Hz, 2H), 2.93–2.80 (m, 1H), 2.80–2.45 (m, 1H), 2.30–1.60 (m, 6H), 1.33 (t, $J = 7.0$ Hz, 3H).

4.3.5. Ethyl (1′*S*,3′*R*,2*R*)-2-benzoylamino-(3′-ethoxycarbonylamino-cyclopentyl)acetate 9a. $[\alpha]_D^{25} = -5.3$ (*c* 0.3, $CHCl_3$); mp 175 °C (CH_2Cl_2/*i*-Pr_2O); IR (Nujol) ν_{max} 1728, 1689, 1641 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.83–7.44 (m, 5H), 6.73 (d, $J = 8.4$ Hz, 1H, exch.), 4.82 (dd, $J = 8.4$, 7.0 Hz, 1H), 4.65 (br s, 1H, exch.), 4.23 (q, $J = 7.0$ Hz, 2H), 4.07 (q, $J = 7.0$ Hz, 2H), 4.10–3.97 (m, 1H), 2.55–2.38 (m, 1H), 2.35–2.13 (m, 1H), 2.10–1.95 (m, 1H), 1.80–1.27 (m, 4H), 1.30 (t, $J = 7.0$ Hz, 3H), 1.21 (t, $J = 7.0$ Hz, 3H), ^{13}C NMR ($CDCl_3$) δ 172.3, 167.6, 156.3, 134.1, 132.1, 128.8, 127.3, 61.8, 60.8, 55.5, 52.2, 41.2, 36.7, 32.5, 25.9, 14.8, 14.4. MS (ESI) *m*/*z* 363.6

$(M+H)^+$. Anal. Calcd: C, 62.97; H, 7.23; N, 7.73; Found: C, 62.95; H, 7.27; N, 7.70.

4.3.6. Ethyl (1′*R*,3′*S*,2*R*)-2-benzoylamino-(3′-ethoxycarbonylamino-cyclopentyl)acetate 9b. $[\alpha]_D^{25} = -21.2$ (*c* 1, $CHCl_3$); mp 140 °C (CH_2Cl_2/*i*-Pr_2O); IR (Nujol) ν_{max} 1725, 1689 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.82–7.40 (m, 5H), 6.75 (d, $J = 8.4$ Hz, 1H, exch.), 4.86 (br s, 1H, exch.), 4.81 (dd, $J = 8.4$, 7.8 Hz, 1H), 4.24 (q, $J = 7.0$ Hz, 2H), 4.09 (q, $J = 6.9$ Hz, 2H), 4.03–3.94 (m, 1H), 2.50–2.34 (m, 1H), 2.20–1.35 (m, 6H), 1.29 (t, $J = 7.0$ Hz, 3H),1.21 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.3, 167.7, 156.5, 134.1, 132.1, 128.8, 127.3, 61.8, 60.9, 55.5, 52.0, 41.6, 35.9, 32.8, 26.9, 14.8, 14.4. MS (ESI) *m*/*z* 363.6 $(M+H)^+$. Anal. Calcd: C, 62.97; H, 7.23; N, 7.73; Found: C, 62.93; H, 7.28; N, 7.69.

4.4. Ethyl (1′*S*,3′*R*,2*R*)-benzoylamino-(3′-isocyanate-cyclopentyl)acetate 8a

Operating at 10 °C under stirring, (−)-**3a** (200 mg, 0.63 mmol) was dissolved in $SOCl_2$ (2 mL). The reaction was monitored by ^1H NMR analysis. After 2 h, $SOCl_2$ was evaporated. The crude reaction mixture was treated with anhydrous toluene (3 × 10 mL) and evaporated under vacuum to obtain acyl chloride **6a**, which was then dissolved in anhydrous toluene (5 mL) operating under nitrogen. A catalytic amount of TBABr (20 mg, 0.063 mmol) and NaN_3 (55 mg, 0.75 mmol) was added to the solution, which was stirred at room temperature for 3 h (IR, NMR analyses). The reaction mixture was taken up with H_2O (5 mL) and the phases were separated. The aqueous solution was extracted with AcOEt (3 × 5 mL). All the organic layers were dried over Na_2SO_4 and evaporated to obtain acyl azide **7a**, which was stood in ethanol free $CHCl_3$ (10 mL) for 24 h under stirring until complete transformation of **7a** into isocyanate **8a** (NMR analysis). Crude isocyanate **8a** (85%) can be obtained after solvent evaporation. IR (Nujol) ν_{max} 3334, 2258, 1715, 1662 cm^{-1}; ^1H NMR δ ($CDCl_3$) 7.84–7.40 (m, 5H), 6.77 (d, $J = 8.4$ Hz, 1H, exch.), 4.86 (dd, $J = 8.4$, 6.3 Hz, 1H), 4.22 (q, $J = 7.0$ Hz, 2H), 3.97–3.88 (m, 1H), 2.57–2.44 (m, 1H), 2.26–2.04 (m, 1H), 1.98–1.54 (m, 5H), 1.30 (t, $J = 7.0$ Hz, 3H).

4.5. Ethyl (1′*S*,3′*R*,2*R*)-2-benzoylamino-(3′-*t*-butoxycarbonylamino-cyclopentyl)acetate (−)-5a

t-BuOH (5 mL) and a catalytic amount of $SnCl_4$ were added to a solution of isocyanate **8a** in $CHCl_3$ (obtained from 0.63 mmol of (−)-**3a**, see above). After 24 h (TLC: CH_2Cl_2/Et_2O, 2:1), the solvent was evaporated and the crude reaction mixture was chromatographed on silica gel (CH_2Cl_2/Et_2O, 20:1 to 5:1). Pure compound (−)-**5a** was obtained after crystallization (180 mg, 60%). $[\alpha]_D^{25} = -10.3$ (*c* 1, $CHCl_3$); mp 200 °C (CH_2Cl_2/Et_2O); IR (Nujol) ν_{max} 3340, 1728, 1700, 1665 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.80–7.42 (m, 5H), 6.74 (d, $J = 8.1$ Hz, 1H, exch.), 4.79 (t, $J = 8.1$, 3.0 Hz, 1H), 4.55 (d, $J = 6.2$ Hz, 1H, exch.), 4.20 (q, $J = 7.0$ Hz, 2H), 3.95–3.85 (m, 1H), 2.43–2.35 (m, 1H), 2.29–2.12 (m, 1H), 2.11–1.91 (m, 1H), 1.87–1.24 (m, 4H), 1.28 (s, 9H), 1.15 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR δ (ppm) ($CDCl_3$) 172.4, 167.6, 155.6, 134.1, 132.0, 128.8, 127.3, 85.4, 61.8, 55.4, 52.0, 41.4, 36.7, 32.5, 28.6, 26.0, 14.4. MS (ESI) *m*/*z* 413.1 $(M+Na)^+$. Anal. Calcd: C, 64.59; H, 7.74; N, 7.17; Found: C, 64.55; H, 7.78; N, 7.14.

4.6. (1′*S*,3′*R*,2*R*)-*N*,*N*′-Bis-[3-(benzoylamino-ethoxycarbonylmethyl)-cyclopentyl]urea 4a

Method a: Pure compound (±)-**3a** (100 mg, 0.3 mmol) was dissolved in *t*-BuOH. TEA (188 μL, 0.3 mmol) and DPPA (64 μL, 0.3 mmol) were then added. The mixture was heated at reflux for 3 h (TLC: CH_2Cl_2/Et_2O 2:1). The solvent was removed and the mixture was taken up with AcOEt and extracted with a solution of citric acid (5%, 3 × 5 mL), with H_2O (3 × 5 mL) and with brine (3 × 5 mL). The organic layer was dried over Na_2SO_4 and evaporated under vacuum. The residue was chromatographed on silica gel (CH_2Cl_2/Et_2O 10:1) affording compound **4a** (79 mg, 50%) and a trace amount of **5a** (7 mg, 5%). *Method b*: A solution of isocyanate **8a** in $CHCl_3$ (obtained from 0.63 mmol of (−)-**3a**, see above) was dissolved in H_2O (2 mL) and pyridine (50 μL, 0.63 mmol). The mixture was stirred for 10 min after which a solid was separated and filtered. The solid was washed with THF and dried under vacuum and pure compound **4a** was isolated (235 mg, 70%). Any attempt to completely dissolve compound **4a** in order to determine the specific rotation failed. Mp 200 °C; IR (Nujol) ν_{max} 3340, 1728, 1700, 1665 cm^{-1}; ^1H NMR (DMSO) δ 8.67 (d, $J = 7.3$ Hz, 2H, exch.), 7.86–7.41 (m, 10H), 5.75 (d, $J = 7.7$ Hz, 2H, exch.), 4.26 (t, $J = 7.3$ Hz, 2H), 4.13–4.06 (m, 4H), 3.92–3.82 (m, 2H), 2.39–2.30 (m, 2H), 2.26–2.06 (m, 2H), 1.82–1.34 (m, 10H), 1.16 (t, $J = 7.0$ Hz, 6H). ^{13}C NMR δ (DMSO) 172.5, 167.5, 157.9, 134.5, 132.1, 128.4, 128.3, 61.0, 58.0, 51.2, 42.1, 38.2, 32.6, 27.7, 14.8. MS (ESI) *m*/*z* 605.0 $(M-H)^+$. Anal. Calcd: C, 65.33; H, 6.98; N, 9.23; Found: C, 65.30; H, 7.01; N, 9.20.

4.7. Synthesis of ethyl (1′*S*,3′*R*,2*R*)-2-benzoylamino-[3′-(*N*′-*p*-methoxybenzyl)ureido-cyclopentyl]acetate (−)-13a

p-Methoxybenzylamine (80 μL, 0.62 mmol) was added to a solution of isocyanate **8a** in $CHCl_3$ (obtained from 0.63 mmol of (−)-**3a**, see above). The mixture was stirred overnight. A white solid was separated, filtered and washed with CH_2Cl_2. The solid was dried under vacuum obtaining compound (−)-**13a** (230 mg, 87%). $[\alpha]_D^{25} = -6.9$ (*c* 0.4, DMSO) Mp 165 °C; IR (Nujol) ν_{max} 3344, 3264, 1745, 1632 cm^{-1}; ^1H NMR (DMSO) δ 8.68 (d, $J = 7.7$ Hz, 1H, exch.), 7.87–7.40 (m, 5H), 6.87, 6.82 (AA′XX′ system, $J = 8.8$ Hz, 4H), 6.06 (t, $J = 5.5$ Hz, 1H, exch.), 5.98 (d, $J = 7.3$ Hz, 1H, exch.), 4.26 (dd, $J = 7.7$, 3.9 Hz, 1H), 4.22–4.02 (m, 4H), 3.88–3.85 (m, 1H), 3.70 (s, 3H), 2.46–2.30 (m, 1H), 2.06–1.93 (m, 1H), 1.82–1.75 (m, 2H), 1.46–1.20 (m, 3H), 1.16 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR δ (ppm) (DMSO) 172.5, 167.5, 158.7, 158.3, 134.5, 133.5, 132.1, 129.0, 128.3, 114.3, 61.0, 58.1, 55.7, 51.4, 43.0, 41.5, 37.4, 32.6, 27.8, 14.8. Anal. Calcd: C, 66.21; H, 6.89; N, 9.27; Found C, 66.18; H, 6.93; N, 9.25.

4.8. Synthesis of ethyl (1′*S*,3′*R*,2*R*)-2-benzoylamino-(3′-amino-cyclopentyl)acetate *p*-toluensulfonate (+)-14a

p-Toluensulfonic acid (87 μL, 0.62 mmol) was added to a solution of isocyanate **8a** in $CHCl_3$ (obtained from 0.63 mmol of (−)-**3a**, see above). The mixture was stirred overnight. A white solid was separated, filtered and washed with CH_2Cl_2. The solid was dried under vacuum obtaining compound (+)-**14a** (240 mg, 85%). $[\alpha]_D^{25} = +8.9$ (*c* 0.4, DMSO) Mp 192–193 °C; IR (Nujol) ν_{max} 3347, 3060, 1736, 1642 cm^{-1}; ^1H NMR (DMSO) δ 8.67 (d, $J = 7.3$ Hz, 1H, exch.), 7.86–7.41 (m, 7H), 7.09 (d, $J = 7.7$ Hz, 2H), 4.37 (t, $J = 7.3$ Hz, 1H), 4.18–4.02 (m, 2H), 3.47–3.13 (m, 1H), 2.50–2.16 (m, 1H), 2.26 (s, 3H), 2.24–2.06 (m, 1H), 1.82–1.34 (m, 8H), 1.16 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR δ (ppm) (DMSO) 172.2, 167.5, 146.2, 138.4, 134.4, 132.1, 128.9, 128.8, 128.3, 126.1, 61.1, 57.2, 55.6, 51.3, 39.8, 35.2, 30.1, 27.7, 21.5, 14.8. Anal. Calcd: C, 59.72; H, 6.54; N, 6.06; Found C, 59.66; H, 6.60; N, 6.00.

4.9. Ethyl (1′*R*,3′*S*,2*R*)-benzoylamino-(3′-carbamoyl-cyclopentyl)acetate 10

Operating at 10 °C under stirring, (−)-**3b** (200 mg, 0.63 mmol) was dissolved in $SOCl_2$ (2 mL). The reaction was monitored by ^1H NMR analysis. After 2 h, $SOCl_2$ was evaporated. The crude reaction mixture was treated with anhydrous toluene (3 × 10 mL) and evaporated under vacuum obtaining acyl chloride **6b**, which was then dissolved in anhydrous toluene (5 mL) operating under nitrogen. A catalytic amount of TBABr (20 mg, 0.063 mmol) and NaN_3 (165 mg, 1.9 mmol) was added to the solution, which was stirred at room temperature for 3 h (IR analysis). The reaction mixture was taken up with H_2O (5 mL) and the phases were separated. The aqueous solution was extracted with AcOEt (3 × 5 mL). The combined organic layers were dried over Na_2SO_4 and evaporated obtaining carbamoyl azide (+)-**10** (180 mg, 80%). Mp 167 °C (CH_2Cl_2/*i*-Pr_2O); $[\alpha]_D^{25} = +3.0$ (*c* 1, $CHCl_3$); IR (Nujol) ν_{max} 3324, 2137, 1741, 1674 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.82–7.40 (m, 5H), 6.85 (d, $J = 8.4$ Hz, 1H, exch.), 6.0 (d, $J = 6.9$ Hz, 1H, exch.), 4.76 (dd, $J = 8.4$, 5.9 Hz, 1H), 4.24 (q, $J = 7.0$ Hz, 2H), 4.18–4.03 (m, 1H), 2.46–2.43 (m, 1H), 2.39–2.00 (m, 2H), 1.99–1.45 (m, 4H), 1.30 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR δ ($CDCl_3$) 172.2, 167.9, 156.2, 133.8, 132.2, 128.9, 127.3, 62.0, 55.7, 52.0, 42.1, 35.4, 32.7, 27.3, 14.4. MS (ESI) *m*/*z* 382.2 $(M+Na)^+$. Anal. Calcd: C, 56.82; H, 5.89; N, 19.49; Found: C, 56.78; H, 5.91; N, 19.47.

4.10. Ethyl (1′*R*,3′*S*,2*R*)-benzoylamino-(3′-ureido-cyclopentyl)acetate 15

Carbamoyl azide (+)-**10** (150 mg, 0.417 mmol) was dissolved in anhydrous Et_2O operating under nitrogen. Ph_3P (85 μL, 0.834 mmol) was added to the solution and the reaction was stirred for 6 h at room temperature (TLC: CH_2Cl_2/Et_2O 2:1). The solvent was removed under vacuum; H_2O (3 mL) and THF (3 mL) were added to the residue. The mixture was stirred at reflux for 14 h. After the removal of H_2O and crystallization, compound (−)-**15** (108 mg, 73%) was obtained. Mp 190 °C (AcOEt/CH_2Cl_2/*i*-Pr_2O); $[\alpha]_D^{25} = -10.2$ (*c* 1, MeOH); IR (Nujol) ν_{max} 3240, 1730, 1700, 1665 cm^{-1}; ^1H NMR (CD_3OD) δ 7.85–7.41 (m, 5H), 4.48 (d, $J = 9.5$ Hz, 1H), 4.25 (q, $J = 7.0$ Hz, 2H), 4.02–3.92 (m, 1H), 2.52–2.39 (m, 1H), 2.36–2.19 (m, 1H), 2.02–1.44 (m, 5H), 1.27 (t, $J = 7.0$ Hz, 3H); MS (ESI) *m*/*z* 356.2 $(M+H)^+$. ^{13}C NMR δ (CD_3OD) 172.1, 169.4, 160.6, 134.1, 131.7, 128.4, 127.4, 61.1, 57.9, 50.8, 39.8, 37.2, 32.2, 26.9, 13.3. Anal. Calcd: C, 61.25; H, 6.95; N, 12.60; Found: C, 61.20; H, 6.98; N, 12.55.

4.11. General procedure for the preparation of amino acids 12

Operating in a sealed tube, ethylcarbamate (−)-**9a** or (−)-**9b** (70 mg, 0.19 mmol) or isocyanate **6a** (55 mg, 0.19 mmol) was suspended in HCl (6N, 0.6 mL). The mixture was heated at 90 °C for 24 h (TLC: CH_2Cl_2/Et_2O, 2:1). The mixture was cooled at 0 °C. A solid was separated, and filtered. The aqueous layer was washed with Et_2O (3 × 5 mL) and then was evaporated to dryness under reduced pressure affording amino acid (−)-**12a** or (−)-**12b** (40 mg, 95%) and trace amount of its epimer (+)-**12b** or (+)-**12a** (5 mg, 5%).

4.11.1. (1′*S*,3′*R*,2*R*)-2,3′-Diamino-cyclopentyl-acetic acid dihydrochloride 12a. Oil, $[\alpha]_D^{25} = -17.7$ (*c* 1, MeOH); IR (Nujol): 3340, 2520, 1700, 1631 cm^{-1}; ^1H NMR δ (D_2O) 3.76 (d, $J = 7.6$ Hz, 1H), 3.75–3.50 (m, 1H), 2.45–2.20 (m, 2H), 2.10–1.30 (m, 5H), ^{13}C NMR δ (D_2O) 173.5, 57.9, 51.2, 39.7, 34.3, 29.2, 27.0. Anal. Calcd: C, 36.38; H, 6.98; N, 12.12; Found: C, 36.35; H, 7.02; N, 12.10.

4.11.2. (1′*R*,3′*S*,2*R*)-2,3′-Diamino-cyclopentyl-acetic acid dihydrochloride 12b. Oil, $[\alpha]_D^{25} = -15.2$ (*c* 1, MeOH); IR (Nujol): 3340, 2523, 1700, 1631 cm^{-1}; ^1H NMR δ (D_2O) 3.84 (d, $J = 6.2$ Hz, 1H), 3.65–3.51 (m, 1H), 2.45–2.18 (m, 2H), 2.05–1.30 (m, 5H); ^{13}C NMR δ (D_2O) 172.6, 56.8, 50.8, 39.5, 34.3, 29.4, 25.9. Anal. Calcd: C, 36.38; H, 6.98; N, 12.12; Found: C, 36.34; H, 7.03; N, 12.10.

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