(*R*)-*tert*-Butoxycarbonylamino-fluorenylmethoxycarbonyl-glycine from (*S*)-Benzyloxycarbonyl-serine or from Papain Resolution of the Corresponding Amide or Methyl Ester†

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The enantiospecific synthesis of (*R*)-Boc-(Fmoc)-aminoglycine **7** was achieved. (*S*)-Cbz-serine **1** was reacted with diphenylphosphoryl azide in the presence of triethylamine to yield cyclic (*S*) carbamate **2**. The ring nitrogen of **2** was protected with a Boc group (**3**). The cyclic carbamate of **3** was hydrolyzed with benzyltrimethylammonium hydroxide to yield the (*R*)-enantiomer of alcohol **4**. The oxidation of **4** with pyridinium dichromate yielded the enantiomerically pure (97% ee) (*R*)-Boc-(Cbz)-aminoglycine **5**, which was converted to **7** with retention of optical purity. Similarly, starting from (*S*)-Boc-serine **9**, cyclic (*S*) carbamate **10** was obtained. The ring nitrogen of **10** was protected with a Cbz group (**11**) with retention of configuration. The cyclic carbamate of **11** was base hydrolyzed to yield **12**, the (*S*)-enantiomer alcohol. Independently, Boc-(Fmoc)-aminoglycine amide **13** and Boc-(Fmoc)-aminoglycine methyl ester **14** were resolved using papain. The stereochemistry of the isolated acid was determined to be (*R*) by coelution on HPLC of its derivative with Marfey's reagent and that of an authentic sample (**7**) obtained by enantiospecific synthesis.

Introduction

Interest in monomeric building blocks that mimic the peptide backbone has dramatically increased with the coming of age of combinatorial chemistry. Peptoids,[1] azoles,[2] 2-isooxazoles,[3] oligosulfones and oligosulfoxides,[4] pyrrolidones,[5] vinylogous backbones,[6] β-methyl-amino acids,[7] and β-amino acids[8,9] are a few examples of such compounds. We have found aminoglycine (Agl) to offer great versatility and compatibility with biological systems when selectively alkylated and/or acylated to yield betidamino acids.[10,11] (Figure 1).

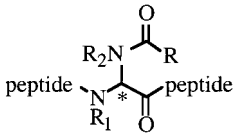

Figure 1. Representative structure of betidamino acids. *Indicates a chiral center.

Orthogonally protected α-(*N*-*tert*-butoxycarbonylamino)-N^{α}-(fluorenylmethoxycarbonyl)glycine, [Boc-(Fmoc)-Agl-OH] was first synthesized by Qasmi et al. as the smallest of the dibasic amino acids.[12] An advantageous property of the aminoglycine scaffold over many other building blocks is that it is a versatile precursor of a wide variety of betidamino acids that can be prepared directly during a solid-phase peptide synthesis. After incorporation of the orthogonally protected aminoglycine in the peptide chain and the deprotection of Boc, the amino function can be acylated ($R_1 = R_2 = H$), or alkylated[13] (R_1 and/or R_2 = alkyl group)[14] and acylated with the desired acyl derivative (R−CO-). Whereas betidamino acids ($R_1 = R_2 = H$) may assume conformational states reminiscent of amino

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† Abbreviations: Agl = aminoglycine, $(Boc)_2O$ = di-*tert*-butyl dicarbonate, *t*-BuOH = *tert*-butyl alcohol, DMAP = dimethylaminopyridine, DABCO = 1,4-diazabicyclo[2.2.2]octane, DPPA = diphenylphosphoryl azide, Et_3N = triethylamine, EtOAc = ethyl acetate, Fmoc-OSu = 9-fluorenylmethylsuccinimidyl carbonate, HOBt = *N*-hydroxybenzotriazole, Marfey's reagent = 1-fluoro-2,4-dinitrophenyl-5-alanine amide, MeCN = acetonitrile, PDC = pyridinium dichromate, Ph = phenyl.

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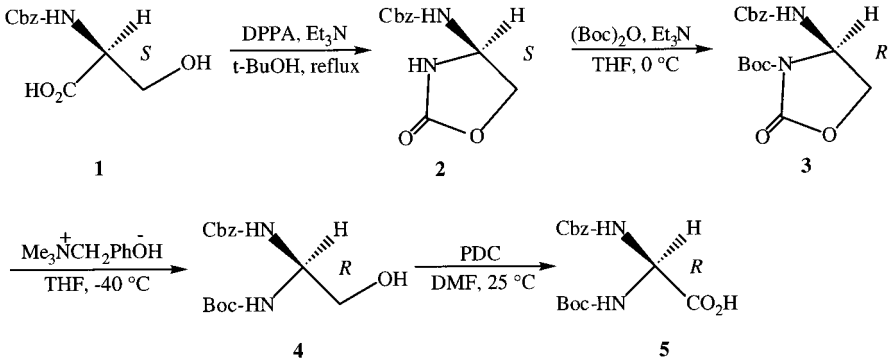
Scheme 1. Synthetic Scheme for (*R*)-Boc-(Cbz)-Agl-OH (5)



acids, methylation of either of the two nitrogens will induce unique conformational preferences difficult to mimic otherwise.[10] For example, betidamino acids methylated at R_2 will readily mimic β-methylated amino acids that are synthetically challenging to obtain in an optically pure form. Aminoglycine itself is achiral and unstable in its unprotected form due to the geminal diamino function. Mono-*N*-protected derivatives are more stable, chiral and can be handled or stored around 0 °C.[15] Di-*N*-protected derivatives of aminoglycine are stable at room temperature and are chiral when the N-substitutions are different. We describe the enantiospecific synthesis of (*R*)-Boc-(Fmoc)-Agl-OH from (*S*)-serine and the resolution of Boc-(Fmoc)-Agl-OMe and of Boc-(Fmoc)-Agl-NH_2 mediated by papain. The stereochemistry of the hydrolyzed acid (*R*) was determined by chromatographic comparison of Marfey's condensation products of authentic/synthetic and Boc-(Fmoc)-Agl-OH isolated after papain treatment.

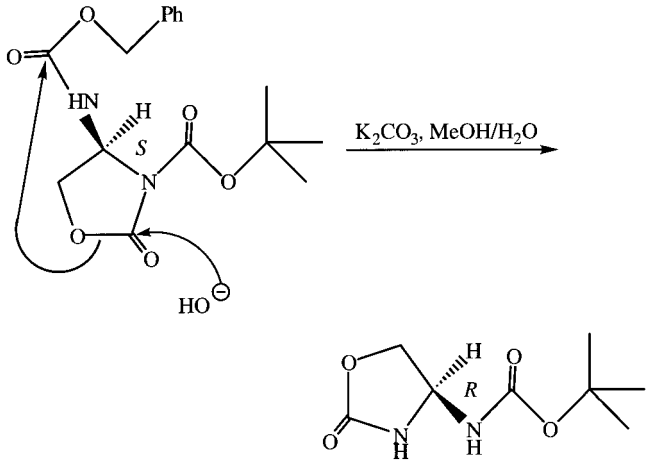
Results and Discussion

We focused on (*S*)-serine (L-serine) as a synthetic template for the enantiospecific synthesis of Boc-(Fmoc)-Agl-OH. Each of the three carbons of serine bears a functionality that can be selectively protected or modified with retention of configuration at the α-carbon as evidenced by an abundant literature on this topic.[16−19]

Our synthetic strategy (Scheme 1) took advantage of the carboxylic moiety to introduce the second amino function through its conversion to a carbamino group via the acyl azide−isocyanate pathway. The hydroxymethyl group was then oxidized to a carboxyl functionality.

More specifically, (*S*)-benzyloxycarbonyl-serine [(*S*)-Cbz-serine] **1** was converted into (*S*)-4-benzyloxycarbonylamino-2-oxazolidone **2** by refluxing with diphenylphosphoryl azide (DPPA) and triethylamine (Et_3N) in *t*-BuOH.[20] The ring nitrogen of **2** was protected with Boc using di-*tert*-butyl dicarbonate $(Boc)_2O$ and Et_3N in THF, at low temperature. The critical ring opening of the (*R*)-4-benzyloxycarbonylamino-3-*tert*-butoxycarbonyl-2-oxazolidone **3** was performed with benzyltrimethylammonium hydroxide in THF at −40 °C, yielding (*R*)-2-hydroxy-1-*tert*-butoxycarbonylaminoethylcarbamic acid benzyl ester **4**. These low-temperature conditions for base-induced ring opening of the oxazolidone turned out to be essential to avoid the undesired N-deprotection of the benzyloxycarbonyl group observed earlier by Ishizuka and Kunieda[21] in similar systems. We found in agreement with related examples of Ishizuka and Kunieda that **3**, treated with LiOH or K_2CO_3 in $MeOH/H_2O$, gave a mixture of the desired protected amino alcohol and a compound characterized as (*R*)-4-*tert*-butoxycarbonylamino-2-oxazolidone (the *R* enantiomer of **10**, (*R*)-**10**, see Schemes 2 and 5) as determined by identical physicochemical properties (^1H NMR, and melting point) except for their optical rotation of opposite signs.

Scheme 2. Proposed Mechanism for Base Elimination of Cbz Group



These observations led us to propose a mechanism for the elimination of the Cbz group whereby an alkoxylate is formed first (resulting in the opening of the oxazolidone) followed by an intramolecular nucleophilic displacement of the Cbz group resulting in a change in configuration Scheme 2. The same reaction was observed in preliminary experiments with (*S*)-3-benzyloxycarbonyl-4-*tert*-butoxycarbonylamino-2-oxazolidone **11** (see below).

The oxidation of **4** with pyridinium dichromate (PDC) in DMF at room temperature[22] led to (*R*)-benzyloxycarbonylamino-*tert*-butoxycarbonylaminoacetic acid [(*R*)-Boc-(Cbz)-Agl-OH, **5**].

The optical purity of **5** was measured after catalytic hydrogenation of the Cbz group and coupling of Marfey's reagent[23] in acetone in the presence of Et_3N. The dia-

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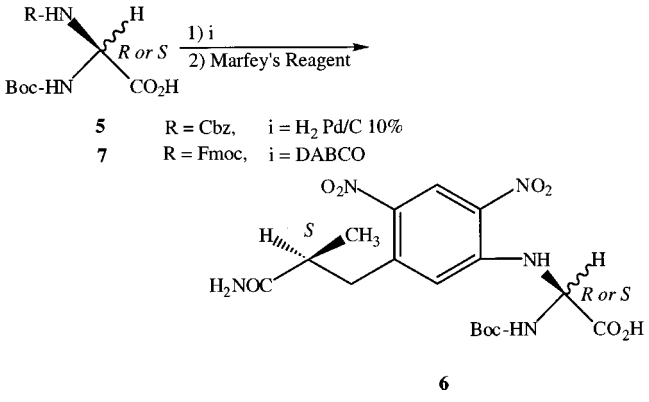
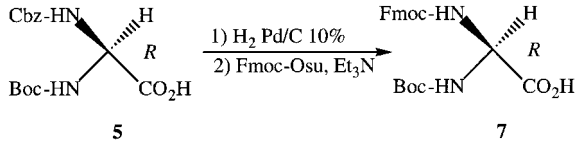
Scheme 3. Derivatization of Partially Protected Agl-Scaffolds for Determination of Optical Purity



Scheme 4: Conversion of (*R*)-Boc-(Cbz)-Agl-OH to (*R*)-Boc-(Fmoc)-Agl-OH



stereoisomeric mixture of Boc-aminoglycine derivatives **6** was then analyzed on reverse phase HPLC. The optical purity of (*R*)-Boc-(Cbz)-Agl-OH **5** (97% ee) was based on the integration of the absorbances corresponding to (*R*,*S*) and (*S*,*S*) diastereoisomers of **6**. Racemic Boc-(Cbz)-Agl-OH was prepared using the same protocol as that used to make racemic Boc-(Fmoc)-Agl-OH[10] and subjected to the same reactions producing a 1:1 mixture of (*R*,*S*) and (*S*,*S*) diastereoisomers of **6** (Scheme 3).

(*R*)-Boc-(Cbz)-Agl-OH **5** was converted to (*R*)-Boc-(Fmoc)-Agl-OH, **7** in two steps (Scheme 4): first the Cbz group was cleaved by catalytic hydrogenation in methanol at 0 °C and then, without isolation of the formed Boc-Agl-OH, excess Fmoc-OSu was added. This was followed by the slow addition of 1 equiv of Et_3N.

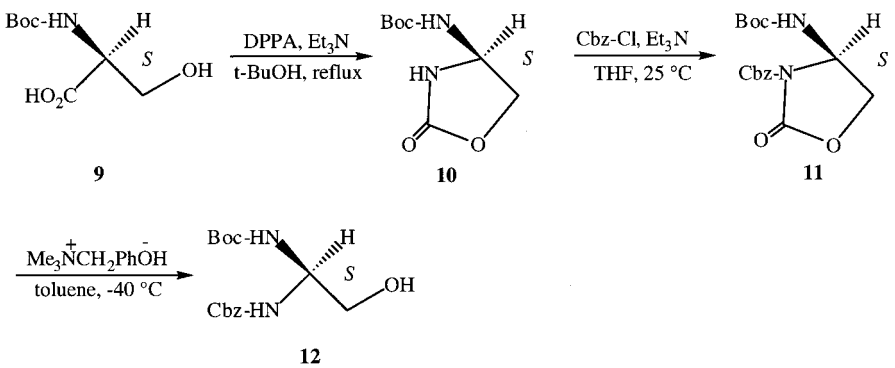
Determination of the optical purity of the (*R*)-Boc-(Fmoc)-Agl-OH **7** was carried out using the procedure described earlier for **5**. After cleavage of the Fmoc-group by DABCO, Boc-Agl-OH was coupled with Marfey's reagent, and the resulting mixture was analyzed by reverse-phase HPLC. Integration of the absorbances corresponding to (*R*,*S*) and (*S*,*S*) diastereoisomers of **6** showed 97% ee of the (*R*)-Boc-(Fmoc)-Agl-OH (Scheme 3).

Piperidine, which is the most widely used reagent for cleavage of the Fmoc-protecting group in solid-phase peptide synthesis protocol,[20] could not be used in our case. The excess would compete with Boc-Agl-OH for Marfey's reagent, and the separation of piperidine from labile Boc-Agl-OH would be very difficult. Other reagents known to cleave the Fmoc-protecting group, like tetrabutylammonium fluoride,[21] did not give satisfactory results in our case. Instead, we successfully used DABCO. Two equivalents of DABCO per 1 equiv of Fmoc-protected amine in DMF solution at room-temperature liberated the amino function within 30 min. As a tertiary base, DABCO does not react with Marfey's reagent, but provides the necessary basic conditions for coupling. To our knowledge this is the first report of the use of DABCO to cleave the Fmoc-protecting group. As was shown above, the reagent did not cause noticeable racemization of the chiral center of the protected aminoglycine.

We used (*S*)-Boc-serine **9** for the synthesis of **12**, the (*S*) enantiomer alcohol (Scheme 5). (*S*)-Boc-serine reacted with DPPA and Et_3N in *t*-BuOH to yield the (*S*)-4-*tert*-butoxycarbonylamino-2-oxazolidone **10**. The ring nitrogen of **10** was then reacted with an excess of benzyloxycarbonyl chloride in a THF/Et_3N solution at −40 °C to yield the (*S*)-3-benzyloxycarbonyl-4-*tert*-butoxycarbonylamino-2-oxazolidone **11**. Introduction of Fmoc on the ring nitrogen of **10** would have been convenient, but it would not have been stable under the basic conditions used for the ring opening. Compound **11** was then converted to the (*S*)-2-hydroxy-1-*tert*-butoxycarbonylaminoethylcarbamic acid benzyl ester **12** after hydrolysis with benzyltrimethylammonium hydroxide in toluene at −40 °C. It is noteworthy that the tendency for the undesired course of the reaction shown in Scheme 2 was greater with **11** than with **3**, hence the changes in experimental protocols. The alcohol **12** was the (*S*)-enantiomer of **4** with physical properties identical to that of **4** (mp, ^1H NMR spectrum, elemental composition) with the exception of the specific rotation which was of identical absolute value but opposite in sign. Oxidation of **12** to (*S*)-*tert*-butoxycarbonylamino-benzyloxycarbonylaminoacetic acid, [(*S*)-Boc-(Cbz)-Agl-OH] and conversion to (*S*)-*tert*-butoxycarbonylamino-fluorenylmethoxycarbonylaminoacetic acid [(*S*)-Boc-(Fmoc)-Agl-OH] can be achieved by the same steps that were described above for compound **4**.

It would have been evident to synthesize (*S*)-Boc-(Cbz)-Agl-OH from (*R*)-Cbz-serine using the synthetic protocol shown in Scheme 1. Alternatively, we identified modified synthetic protocols (changes of solvent and temperature as compared to those shown in Scheme 1) using (*S*)-Boc-serine for the synthesis of (*S*)-2-hydroxy-1-*tert*-butoxycarbonylaminoethylcarbamic acid benzyl ester **12** (Scheme

Scheme 5: Synthetic Scheme for (*S*)-2-Hydroxy-1-*tert*-butoxycarbonylaminoethylcarbamic Acid Benzyl Ester 12



5). In that case the introduction of the Cbz protecting group to the nitrogen of the oxazolidone maintained the order of priority of the groups around the chiral carbon atom resulting in retention of the (*S*) configuration.

We hypothesized that enzymatic resolution of amino acid derivatives could be more easily scaled up than the enantiospecific synthesis because of difficulties experienced during the oxidation step. Therefore, we sought conditions that would resolve (*R,S*)-Boc-(Fmoc)-Agl-OH. We found that both (*R,S*)-Boc-(Fmoc)-Agl-NH_2 **13** and (*R,S*)-Boc-(Fmoc)-Agl-OMe **14** were susceptible to papain hydrolysis, and detailed conditions are given in the Experimental Section. The extraction steps are delicate due to the fact that Boc-(Fmoc)-Agl-NH_2, Boc-(Fmoc)-Agl-OMe, and the resolved Boc-(Fmoc)-Agl-OH are relatively insoluble in most solvents. Despite these difficulties, significant quantities of (*R*)-Boc-(Fmoc)-Agl-OH (**7**) with high enantiomeric purity (>92%, >50 g) were obtained from racemic Boc-(Fmoc)-Agl-NH_2(**13**) or Boc-(Fmoc)-Agl-OMe (**14**). The stereochemistry of the isolated acid was determined to be (*R*) by coelution on HPLC of its adduct with Marfey's reagent and that of an authentic sample obtained by enantiospecific synthesis. Although we have focused our efforts toward the isolation of significant quantities of (*R*)-Boc-(Fmoc)-Agl-OH (**7**), the corresponding (*S*)-Boc-(Fmoc)-Agl-OMe and (*S*)-Boc-(Fmoc)-Agl-NH_2 were also isolated. In principle, both the methyl ester and amide could be hydrolyzed using a nonspecific hydrolase such as Pronase. This approach was not pursued because both betidamino-enantiomers are readily available from the same (*R*)-Boc-(Fmoc)-Agl-OH scaffold depending on whether the acylating agent used to mimic an amino acid side chain is built on the Boc- or Fmoc-bearing nitrogen of aminoglycine. It was hoped that the differences in solubility of either (*S*)-Boc-(Fmoc)-Agl-NH_2 or (*S*)-Boc-(Fmoc)-Agl-OMe and the desired (*R*)-Boc-(Fmoc)-Agl-OH during extractions at basic and acidic pHs would be such that one derivative could be favored over the other. This was not the case.

Conclusion

We describe the enantiospecific synthesis of optically enriched (97% ee) (*R*)-*tert*-butoxycarbonylamino-benzyloxycarbonylaminoacetic acid [(*R*)-Boc-(Cbz)-Agl-OH] **5** and (*R*)-*tert*-butoxycarbonylamino-fluorenylmethoxycarbonylaminoacetic acid [(*R*)-Boc-(Fmoc)-Agl-OH] **7**. We have also described synthetic protocols yielding the (*S*)-enantiomer of **4** precursor of **5** and **7**. In each case the chiral center of the protected aminoglycine is originated from commercially available (*S*)-serine derivatives. We have also described a practical, large-scale method for the enzymatic resolution of orthogonally protected Boc-(Fmoc)-Agl-methyl ester and amide.

Experimental Section

Melting points were determined on a capillary melting point apparatus and were not corrected. ^{1}H nuclear magnetic resonance spectra were recorded on a 500 MHz instrument. THF was distilled from potassium before use. Et_3N and *t*-BuOH were distilled from CaH_2. Other solvents were used without special purification. Papain was purchased from Acros, (Fisher Scientific, Pittsburgh, PA). Commercially available (*S*)-Cbz-serine and (*S*)-Boc-serine (Bachem, CA) were dried over P_2O_5 in a vacuum for 12 h before use. Benzyltrimethylammonium hydroxide (40% solution in methanol) was purchased from Aldrich (Milwaukee, WI) and was dried over CaH_2 for 12 h, centrifuged, and finally filtered before use. Other reagents were used without further purification. Analytical thin-layer chromatography was performed using Merck Kieselgel 60 F_{254} aluminum sheets (EM Science, Gibbstown, NJ). For the reverse-phase analytical HPLC, a Vydac (The Separations Group, Hesperia, CA) C_{18} column (0.46 × 25 cm, 5 μm particle size, 300 Å pore size) was used. Mobile phases A and B were 0.1% TFA in H_2O and 0.1% TFA in (60% MeCN/40% H_2O), respectively. Gradient shapes and flow rates are described in the text. UV detection was at 214 nm.

(*S*)-4-Benzyloxycarbonylamino-2-oxazolidone (2). (*S*)-Cbz-serine **1** (12.5 g, 52.3 mmol) and DPPA (15.8 g, 57.5 mmol) were dissolved in *t*-BuOH (200 mL). The reaction flask was flushed with argon, and Et_3N (5.82 g, 57.5 mmol) was added. The reaction mixture was refluxed under argon for 3 h and then cooled to room temperature. EtOAc (50 mL) was added, and solvents were evaporated under reduced pressure. A saturated solution of $NaHCO_3$ (500 mL) was then added to the residue, and the product was extracted with EtOAc (3 × 100 mL). The combined extracts were washed with saturated $NaHCO_3$ solution, 2% $NaHSO_4$ solution, water, and finally dried over anhydrous $MgSO_4$. After evaporation of the solvent under reduced pressure, the solid residue was subjected to crystallization from EtOAc to yield **2** (10.3 g, 84%): mp 169−171 °C (EtOAc); $[\alpha]^{25}_D = -87.3°$ (c = 1, MeOH); ^{1}H NMR (DMSO-d_6) 4.01−4.03 (m, 1H); 4.46−4.50 (m, 1H); 5.05 (s, 2H); 5.35−5.39 (m, 1H); 7.3−7.4 (m, 5H); 8.24 (d, 1H, J = 8.3 Hz); 8.30 (s, 1H). Elemental composition calculated for $C_{11}H_{12}N_2O_4$: C, 55.93%; H, 5.12%; N, 11.86%; found: C, 55.90%; H, 5.04%; N, 11.8.

(*R*)-4-Benzyloxycarbonylamino-3-*tert*-butoxycarbonyl-2-oxazolidone (3). Compound **2** (33.0 g, 0.14 mol), DMAP (360 mg), and Et_3N (17.0 g, 0.168 mol) were dissolved in dry THF (380 mL) and cooled in an ice bath. A solution of $(Boc)_2O$ (32.0 g, 0.147 mol) in THF (120 mL) was added to the stirred reaction mixture over 30 min. The temperature of the reaction mixture was kept between 5 and 10 °C, and the progress of the reaction was monitored by TLC. As soon as the starting material had been consumed, a solution of $NaHSO_4$ (20.13 g, 0.168 mmol) in water (150 mL) was added. The THF was evaporated under reduced pressure. The white suspension of the crude product was then dissolved in EtOAc, and the solution was washed with dilute $NaHSO_4$ solution, water, and finally dried with anhydrous $MgSO_4$. After evaporation of the solvent under reduced pressure, an oily residue was immediately dissolved in diethyl ether (200 mL). Within a few minutes, white crystals of the product started to precipitate. After filtration and concentration of the filtrate under reduced pressure, an additional amount of **3** was obtained (44.8 g, 95%): mp 128−130 °C; $[\alpha]^{20}_D = -8.7°$ (c = 1, MeOH); ^{1}H NMR (acetone) 1.44 (s, 9H); 4.19−4.21 (m, 1H); 4.59−4.63 (m, 1H); 5.09−5.13 (m, 2H); 5.93−5.96 (m, 1H); 7.30−7.40 (m, 5H); 7.51 (bs, 1H). Elemental composition calculated for $C_{16}H_{20}N_2O_6$: C, 57.14%; H, 5.99%; N, 8.33%; found: C, 57.46%; H, 5.90%; N, 8.36%.

(*R*)-2-Hydroxy-1-*tert*-butoxycarbonylaminoethylcarbamic Acid Benzyl Ester (4). Compound **3** (40.0 g, 0.119 mol) was dissolved in dry THF (1200 mL) and cooled to −40 °C, under argon atmosphere. Benzyltrimethylammonium hydroxide (59.3 mL of 40% solution in methanol, 0.13 mol) was then added over 20 min. After an additional 20 min of stirring at −40 °C, acetic acid (25 mL) and water (60 mL) were added. The reaction flask was transferred to a water bath (+35 °C) for 20 min followed by evaporation of the organic solvents. The white residue was dissolved in EtOAc (1.2 L), washed with 2% $NaHSO_4$ solution and water, dried with anhydrous $MgSO_4$, and evaporated. The solid **4** was dissolved in a small volume of hot EtOAc and precipitated with petroleum ether (yield was 30.4 g, 82%): mp 140−141 °C; $[\alpha]^{25}_D = +8.0°$ (c = 1, MeOH); ^{1}H NMR (DMSO) 1.37 (s, 9H); 3.30−3.37 (m, 2H); 4.79 (t, 1H, J = 6.0 Hz); 4.96−4.99 (m, 1H); 5.01 (s, 2H); 6.82 (bs, 1H); 7.26 (d, 1H, J = 6.1 Hz); 7.29−7.36 (m, 5H). Elemental composition calculated for $C_{15}H_{22}N_2O_5$: C, 58.05%; H, 7.15%; N, 9.03%; found: C, 58.14%; H, 6.98%; N, 8.96%.

(*R*)-Benzyloxycarbonylamino-*tert*-butoxycarbonylaminoacetic acid, [(*R*)-Boc-(Cbz)-Agl-OH] (5). Compound **4** (1.0 g, 3.22 mmol) was dissolved in DMF (3.2 mL). Solid PDC (3.64 g, 9.67 mmol) was then added, and the reaction mixture was stirred for 20 h at room temperature. The reaction mixture was then poured into water (100 mL), acidified to pH 3, and extracted with EtOAc (4 × 30 mL). The organic phase was washed with water. Upon the addition of a solution of saturated $NaHCO_3$ solution (3 × 30 mL), the product transferred to the aqueous phase which was washed once with EtOAc and then acidified with solid $NaHSO_4$ to pH 3. The precipitated product was then extracted with EtOAc (3 × 30 mL), and the combined extracts were washed with water, dried over anhydrous $MgSO_4$, and evaporated to yield **5** (596 mg, 59%): mp 149−150 °C; $[\alpha]^{25}_D = +5.5°$ ($c = 2$, DMF); ^{1}H NMR (DMSO) 1.38 (s, 9H); 5.04 (s, 2H); 5.26−5.28 (m, 1H); 7.30−7.36 (m, 6H); 7.83−7.85 (m, 1H), 12.95 (bs, 1H). Elemental composition calculated for $C_{15}H_{20}N_2O_6$: C, 55.55%; H, 6.22%; N, 8.64%; found: C, 55.62%; H, 6.04%; N, 8.54%.

Determination of the Optical Purity of 5. Palladium on activated carbon (10%) (1 mg) was added to a solution of **5** (12 mg) in methanol (0.5 mL). Hydrogenation was performed at 50 psi for 30 min. The above solution (5 μL) was then added to the solution of the Marfey's reagent (1 mg) in acetone (0.1 mL) and 10 μL of Et_3N. The reaction mixture was heated for 1 h at 40 °C to give a quantitative yield of **6**. Then the above solution (5 μL) was injected on the HPLC column. At a flow rate of 1 mL/min, and a gradient of MeCN from 0 to 60% in 25 min, retention times for the (*S*,*S*) diastereomer was 12.7 and 14.0 min for the (*R*,*S*) diastereomer. Integration of the two absorbances ($\lambda = 340$ nm) corresponding to the diastereoisomeric derivatives **6** of *tert*-butoxycarbonylaminoacetic acid showed 97% ee for **5**.

(*R*)-*tert*-Butoxycarbonylamino-fluorenylmethoxycarbonylaminoacetic Acid, [(*R*)-Boc-(Fmoc)-Agl-OH] (7). Compound **5** (324 mg, 1.0 mmol) was dissolved in methanol (7 mL), and palladium on activated carbon (10% Pd) (200 mg) was added. The reaction flask was flushed with hydrogen, and then hydrogenation was performed at 1 psi for 30 min at 0 °C with the reaction flask immersed in ice/water. A solution of Fmoc-OSu (505 mg, 1.5 mmol) was then dissolved in DMF (2 mL) and was added to the reaction mixture. The slow addition (20 min) of Et_3N (101 mg, 1 mmol) diluted with THF (0.5 mL) followed. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for an additional 1 h. The solvents were evaporated, and the residue was suspended in water and extracted with EtOAc (3 × 30 mL). The combined extracts were washed, dried, and evaporated similar to **4**. The solid residue containing **7** and fulvene was triturated with a small amount of diethyl ether, filtered, washed with diethyl ether over a filter, and dried under vacuum to yield **7** (363 mg, 88%): mp 190 °C dec (EtOAc); $[\alpha]^{25}_D = +10.5°$ ($c = 2$, DMF); ^{1}H NMR (DMSO) 1.39 (s, 9H); 4.22−4.28 (m, 3H); 5.26−5.29 (m, 1H); 7.30−7.35 (m, 3H); 7.40−7.43 (m, 2H); 7.72−7.74 (m, 2H)7.88−7.90 (m, 2H), 8.02 (d, 1H, $J = 7.3$ Hz). Elemental composition calculated for $C_{22}H_{24}N_2O_6$: C, 64.07%; H, 5.87%; N, 6.79%; found: C, 63.74%; H, 5.75%; N, 6.60%.

Determination of the Optical Purity of 7. Compound **7** (20 mg) was dissolved in DMF (0.1 mL), DABCO (18 mg) was added, and the reaction mixture was stirred at room temperature for 30 min. The above solution (3.5 μL) was then added to the solution of Marfey's reagent (1 mg) in acetone (0.1 mL) and treated as described earlier. Integration of the two absorbances corresponding to diastereoisomeric derivatives **6** of *tert*-butoxycarbonylaminoacetic acid showed a 97% ee for **7**.

(*S*)-4-*tert*-Butoxycarbonylamino-2-oxazolidone (10).[24] (*S*)-Boc-serine **9** (4.10 g, 20 mmol) and DPPA (6.05 g, 22 mmol) were dissolved in *t*-BuOH (80 mL). The reaction mixture was flushed with argon, and Et_3N (2.77 g, 22 mmol) was added. The reaction conditions and workup were the same as described for compound **2**. Compound **10** was dissolved in a small amount of hot EtOAc and precipitated with ether resulting in 2.45 g (61%) pure product: mp 187−189 °C (EtOAc/Et_2O); $[\alpha]^{25}_D = -107.1°$ ($c = 1$, MeOH); ^{1}H NMR (DMSO) 1.39 (s, 9H); 2.48−2.50 (m, 1H); 3.96−4.00 (m, 1H); 4.42−4.46 (m, 1H); 5.27−5.31 (m, 1H); 7.82 (d, 1H, $J = 8.4$ Hz); 8.21 (s, 1H). Elemental composition calculated for $C_8H_{14}N_2O_4$: C, 47.52%; H, 6.98%; N, 13.85%; found: C, 47.59%; H, 6.78%; N, 13.81%.

(*S*)-3-Benzyloxycarbonylamino-4-*tert*-butoxycarbonylamino-2-oxazolidone (11). Compound **10** (2.02 g, 10 mmol) was dissolved in THF (30 mL) and Et_3N (10 mL). The reaction mixture was cooled under argon to −40 °C. A solution of benzyl chloroformate, 95% pure (3.92 g, 20 mmol), in THF (3.3 mL) was slowly added at −40 °C. The stirred reaction mixture was allowed to warm to −15 °C within 40 min and then kept at −15 °C overnight. The reaction mixture was then quickly neutralized with a stoichiometric amount of cold 5% aqueous solution of $NaHSO_4$ and extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed, dried, and evaporated similarly to **4**. The solid residue was dissolved in a small amount of hot EtOAc and diluted with a large volume of ether. White crystals of the product started to precipitate within 10 min. The precipitate was cooled at 4 °C for 1 h before filtration of the crystals. The filtrate was concentrated under reduced pressure, and a second crop of product was isolated. The total yield of **11** was 3.17 g, 94%: mp 141−143 °C (EtOAc/Et_2O); $[\alpha]^{25}_D = +23.4°$ ($c = 1$, MeOH); ^{1}H NMR (DMSO) 1.35 (s, 9H); 4.03−4.06 (m, 1H); 4.49−4.53 (m, 1H); 5.21−5.26 (m, 2H), 5.70−5.80 (m, 1H); 7.32−7.41 (m, 5H); 8.06 (d, 1H, $J = 6.6$ Hz). Elemental composition calculated for $C_{16}H_{20}N_2O_6$: C, 57.14%; H, 5.99%; N, 8.33%; found: C, 57.40%; H, 5.96%; N, 8.36%.

(*S*)-2-Hydroxy-1-*tert*-butoxycarbonylaminoethylcarbamic Acid Benzyl Ester (12). Compound **11** (1.68 g, 5.0 mmol) was dissolved quickly in warm toluene (100 mL) and cooled to −45 °C. Benzyltrimethylammonium hydroxide (2.5 mL of 40% solution in methanol, 5.5 mmol) was added over 10 min to the suspension of **11** in toluene and stirred for an additional 45 min at −45 °C. Acetic acid (0.5 mL) was then added, the reaction mixture was warmed to room temperature, and the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc (50 mL), which was washed, dried, and evaporated similarly to **4**. The solid residue was subjected to crystallization from EtOAc to yield **12** (68%): mp 139−141 °C (EtOAc); $[\alpha]^{25}_D = -8.0°$ ($c = 1$, MeOH); ^{1}H NMR (DMSO) 1.37 (s, 9H); 3.30−3.37 (m, 2H); 4.79 (t, 1H, $J = 6.0$ Hz); 4.96−4.99 (m, 1H); 5.01 (s, 2H); 6.82 (bs, 1H); 7.26 (d, 1H, $J = 6.1$ Hz); 7.29−7.36 (m, 5H). Elemental composition calculated for $C_{15}H_{22}N_2O_5$: C, 58.05%; H, 7.15%; N, 9.03%; found: C, 58.26%; H, 6.86%; N, 8.90%.

Synthesis of (*R*,*S*)-*tert*-Butoxycarbonylamino-fluorenylmethoxycarbonyl-aminoacetamide [(*R*,*S*)-Boc-(Fmoc)-Agl-NH_2] (13). To a stirred solution of (*R*,*S*)-Boc-(Fmoc)-Agl-OH[10,12] (10.3 g, 25 mmol) in anhydrous DMF (40 mL) and EtOAc (100 mL) at 0 °C was added DCC (5.3 g, 25 mmol), followed by HOBt (3.5 g, 27 mmol) 5 min later. The mixture was slowly warmed to room temperature and stirred for an additional 30 min. After activation, 10 M cc. NH_3 solution in water (2.8 mL, ∼28 mmol) was added to the reaction mixture, with stirring, at room temperature for 30 min. The reaction mixture was then diluted to 400 mL with EtOAc. The precipitate (DCU) was filtered off, and the filtrate was washed with 5% $NaHSO_4$ solution, H_2O, 5% $KHCO_3$ solution, and H_2O. As DMF was removed from the EtOAc phase with aqueous washes, some precipitation of Boc-(Fmoc)-Agl-NH_2 occurred because of poor solubility in EtOAc. Boc-(Fmoc)-Agl-NH_2 was kept in solution by the addition of EtOAc and dried for a short time over anhydrous Na_2SO_4 to avoid precipitation of the product onto the Na_2SO_4 crystals. After filtration the solution was concentrated to 1/3 of its original volume; the suspension was allowed to stand at 4 °C overnight to yield 9.5 g (92%) of (*R*,*S*)-Boc-(Fmoc)-Agl-NH_2 as white powder: TLC R_f 0.7 ($CHCl_3$:MeOH:AcOH = 90:10:0.5); MS FAB: *m*/*e* 412.03 (M + H), calcd: 412.19 (M + H); $t_R = 3.3$ min for the amide and 4.0 min for the starting material (acid) determined by HPLC under

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isocratic condition (48% MeCN in 0.1% TFA) at a flow rate of 2.0 mL/min.

Papain Hydrolysis of 13.[25] Activation of papain: A mixture of EDTA (0.01 mol) in water (1000 mL; pH adjusted to 6.2 with 1 M NaOH), 0.05 mol cystein·HCl in water (1000 mL, pH adjusted to 6.2 with 1 M NaOH), H_2O (240 mL, MiliQ pure), and 0.06 mol of mercaptoethanol (10 mL) were used to dissolve and activate 1 g of papain at 25 °C for 2 h.

Enzymatic hydrolysis: To a filtered solution of **13** (50 g, 122 mmol) in DMF (850 mL) and MeCN (1400 mL, HPLC grade) was added the activated papain solution (2250 mL) which was gently shaken under argon atmosphere at 37 °C for 17−20 h. The hydrolysis was monitored by RP-HPLC. After 19 h, the mixture contained 50% amide and 50% acid according to HPLC. This ratio was not modified with either additional enzyme or reaction time. After evaporation of the MeCN, the pH of the reaction mixture was adjusted to 7.5−8.0 with 1 M NaOH, and the resulting solution was further diluted with water then left overnight at 0 °C, resulting in the precipitation of the original amide. Unhydrolyzed, chiral (*S*)-Boc-(Fmoc)-Agl-NH_2 (20.8 g, 42%) was collected by filtration: mp 162−6 °C (dec); $[\alpha]^{25}_D = -6.5°$ ($c = 2$, DMF), MS FAB: *m*/*e* 412.03 (M + H), calcd: 412.19 (M + H). The aqueous solution was extracted with EtOAc (to separate any remaining amide) and acidified to pH 2 with solid $NaHSO_4$. The solution was left standing at 0 °C overnight to yield the hydrolyzed chiral (*R*)-Boc-(Fmoc)-Agl-OH **7** (24 g, 48%) after filtration: mp 185−7 °C (dec), MS FAB: *m*/*e* 413.01 (M + H), calcd: 413.17 (M + H); $[\alpha]^{25}_D = +8.5°$ ($c = 2$, DMF). The optical purity of chiral (*R*)-Boc-(Fmoc)-Agl-OH **7** was tested as described earlier. Ratio of the integration of the two absorbances showed one of the two diastereomers of **7** to be in 92% enantiomeric excess (ee).

Synthesis of (*R,S*)-*tert*-Butoxycarbonylamino-fluorenylmethoxycarbonylacetic Acid Methyl Ester [(*R,S*)-Boc-(Fmoc)-Agl-OMe][15] (14). A mixture of (*R,S*)-Boc-(Fmoc)-Agl-OH[10,12] (21.0 g, 50 mmol) and powdered anhydrous K_2CO_3 (10.0 g, 100 mmol) in DMF (150 mL) was stirred in a tightly closed flask at room temperature for 10 min. Periodically the CO_2 that was produced was vented. The mixture was then cooled to 4 °C (ice bath), and CH_3I (9.35 mL, 150 mmol) was added to it and closed tightly. The stirring was continued in an ice bath for 2 h followed by 2 h at room temperature. The reaction was monitored by HPLC with a gradient running from 30% to 54% MeCN over 20 min at a flow rate of 1.0 mL/min. When the reaction was complete, the solid was filtered and washed with DMF. The filtrate was evaporated to dryness in a vacuum and crystallized from ether/petroleum ether to yield **14** (>90%): mp 104−106 °C, MS FAB: *m*/*e* 427.00 (M + H), calcd: 427.19 (M + H).

Papain Hydrolysis of 14.[25] Using conditions similar to those used for the papain hydrolysis of **13**, compound **14** (21.5 g, 50 mmol) was suspended and stirred in a phosphate buffer (pH = 6.2) consisting of 0.1 M $Na_2HPO_4·7H_2O$ and 0.1 M citric acid with 1.5 mM EDTA.Na_2 in water (300 mL) and 50% DMF/MeCN (80 mL). Activated aliquots of papain (100 mg) were added at regular intervals (12−24 h) until completion of hydrolysis monitored by HPLC. Solid (*S*)-Boc-(Fmoc)-Agl-OMe (9 g) was filtered after dilution of the above with water (900 mL). MS FAB: *m*/*e* 427.00 (M + H), calcd: 427.19 (M + H). Filtrate was treated as above (evaporation of MeCN, extraction with EtOAc and acidification) to yield (*R*)-Boc-(Fmoc)-Agl-OH **7** (10 g): mp 191−193 °C (dec); MS FAB: *m*/*e* 412.99 (M + H), calcd: 413.17 (M + H); $[\alpha]^{25}_D = +9.8°$ ($c = 2$, DMF). The optical purity of **7**, tested as earlier, showed (*R*)-Boc-(Fmoc)-Agl-OH to be in 95% enantiomeric excess (ee).

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