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Design and Concise Synthesis of Fully Protected Analogues of L-γ-Carboxyglutamic Acid

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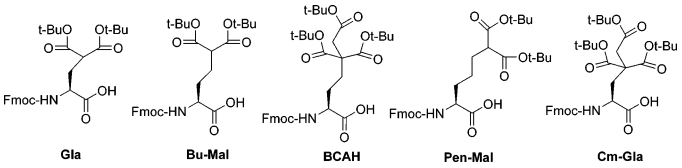

The design and synthesis of four nonnaturally occurring amino acid analogues of L-γ-carboxyglutamic acid (Gla), appropriately protected for Fmoc-based solid-phase peptide synthesis (SPPS), is described. These amino acids are Bu-Mal **2**, BCAH **3**, Pen-Mal **4**, and Cm-Gla **5**. These Gla analogues have been designed to replace the glutamic acid of position 1 in the cyclic decapeptide G1TE, which is a potent inhibitor of tyrosine kinase, to further enhance binding to the Grb2-SH2 domain of signal transduction receptors. In the new amino acids, the propionic acid side chain of Glu has been replaced by a malonyl or a carboxymethylmalonyl moiety located at different distances from the α-carbon to optimize interactions in the phosphotyrosine-binding cavity of the Grb2-SH2 domain. Additionally, a direct and efficient synthetic route for the preparation of Fmoc-protected L-γ-carboxyglutamic acid, which is amenable to large-scale production, has been developed to provide this important and unique amino acid[1] in 55% overall yield.

Introduction

L-γ-Carboxyglutamic acid (L-Gla) is formed in proteins via the posttranslational modification of L-glutamic acid by vitamin K carboxylase.[1] This amino acid has been found in several vertebrate calcium-binding proteins such as osteocalcin and contryphan.[2] L-Gla was also found unexpectedly in some neuroactive peptides such as conantoxin GV and conantoxin T.[3] Inhibition of the signal transduction pathways of tyrosine kinase growth factor receptors represents a new approach under intensive investigation in cancer therapy research.[4] Particularly, blocking the interaction between the phosphotyrosine (pTyr) containing activated receptors and the Src homology 2 (SH2) domain of the growth factor receptor-bound protein-2 (Grb2) constitutes an attractive strategy to develop new antitumor agents due to their potential to shut down the mitogenically important *Ras* activation pathway.[5] Unlike other SH2 domains,[6] the Grb2-SH2 domain requires a peptide ligand to bind to it in a β-turn conformation with a minimal recognition motif of pY-X-N.[7] However, the poor cellular permeability and phosphatase lability of phosphotyrosine (pTyr) provides somewhat limited use of pTyr-containing inhibitors in whole cell systems. To overcome these problems and enhance improved binding specificity, we

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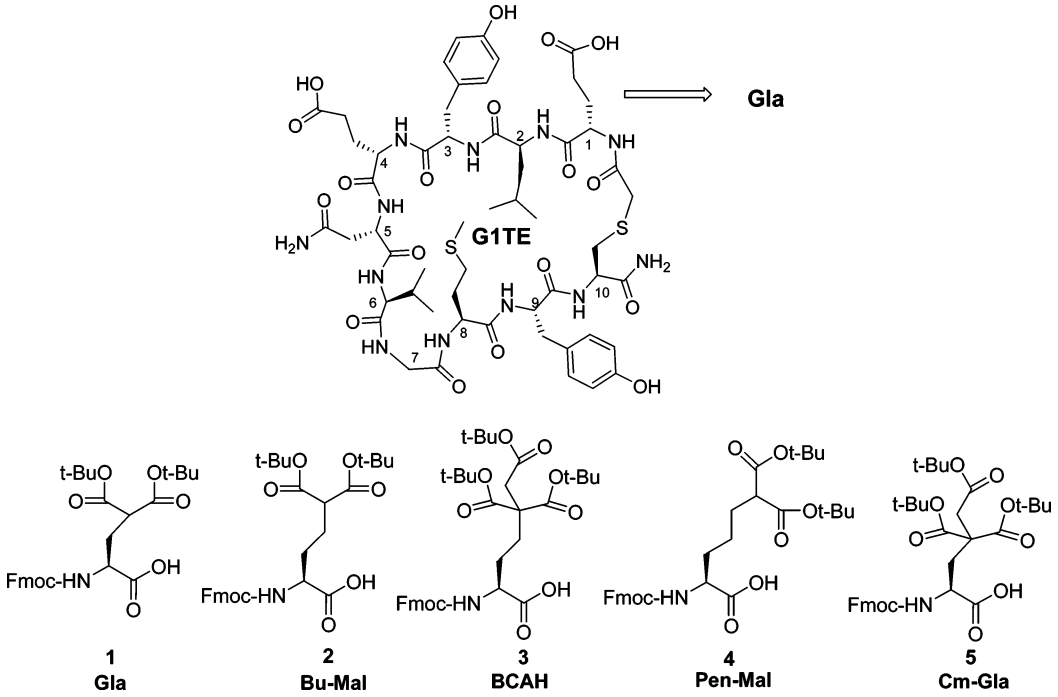

FIGURE 1. Incorporation of various analogues of G1TE in position 1.

discovered earlier a disulfide-bridged cyclic peptide termed G1 by phage-display library.[8] This cyclic undecapeptide, which does not contain a phosphotyrosine moiety, can specifically bind to the Grb2-SH2 domain with an affinity of 10−25 μM. To enhance its stability in vivo, we further designed a thioether-bridged cyclic peptide termed G1TE (Figure 1), which is redox stable.[8,9]

In our previous studies,[10] we found that the Glu[1] side chain in G1TE compensates for the absence of Tyr[3] phosphorylation in retaining effective binding to the Grb2-SH2 domain. Replacement of Glu[1] with γ-carboxyglutamic acid (Gla) greatly improves binding affinity. On the basis of these findings, we undertook the design and synthesis of the Gla analogues Bu-Mal (**2**), BCAH (**3**), Pen-Mal (**4**), and Cm-Gla (**5**), as shown in Figure 1, to optimize the H-bond interactions of the acidic side chain of the amino acid in position 1 of G1TE analogues with the positively charged arginines within the pTyr-binding pocket of the protein. Molecular modeling indicated that the acidic side chain of Gla[1] was not long enough, so Bu-Mal and Pen-Mal were designed to optimize the best length of the amino acid side chain in position 1, thus allowing for the terminal malonyl group to be optimally positioned within the pTyr-binding cavity.[10]

We report herein concise routes to the syntheses of fully protected Bu-Mal (**2**), BCAH (**3**), Pen-Mal (**4**), and Cm-Gla (**5**), which are suitable for Fmoc-chemistry based SPPS. Considering that the fully Fmoc-protected Gla has been used in a number of peptide syntheses,[11] and this compound is relatively expensive,[12] we have also developed a concise and economical route to the preparation of Fmoc-protected L-γ-carboxyglutamic acid for easier availability.

Results and Discussion

Synthesis of Bu-Mal (2) and BCAH (3). Treatment of L-homoserine with allyl chloroformate and $NaHCO_3$ followed by allyl bromide yielded the *N*-Alloc, allyl ester **6** (Scheme 1).[13] The diallyl protected homoserine **6** was reacted with MsCl in CH_2Cl_2, followed by NaI in acetone to produce the pure iodide derivative **7** in 77% overall yield from L-homoserine. Nucleophilic displacement of the iodine on compound **7** with the anion (generated by NaH) of di-*tert*-butyl malonate proceeded cleanly to afford the common intermediate **8** in 83% yield. At this point, what remained was to convert the Alloc to the Fmoc derivative and deprotect the carboxylic acid. We removed the Alloc and the allyl ester simultaneously with a catalytic amount of $Pd(PPh_3)_4$. After attempting a variety of nonbasic allyl scavengers, including *N*-methylaniline, pyrrolidine,[13] and morpholine, we found diethylamine[14] to be the best additive to effect the deprotection cleanly and rapidly. Since any residual diethylamine would block subsequent Fmoc protection, we needed to completely remove residual diethylamine. Because this residual diethylamine might be complexed as the diethylamine salt of the carboxylic acid, we employed the methods of Carrasco et

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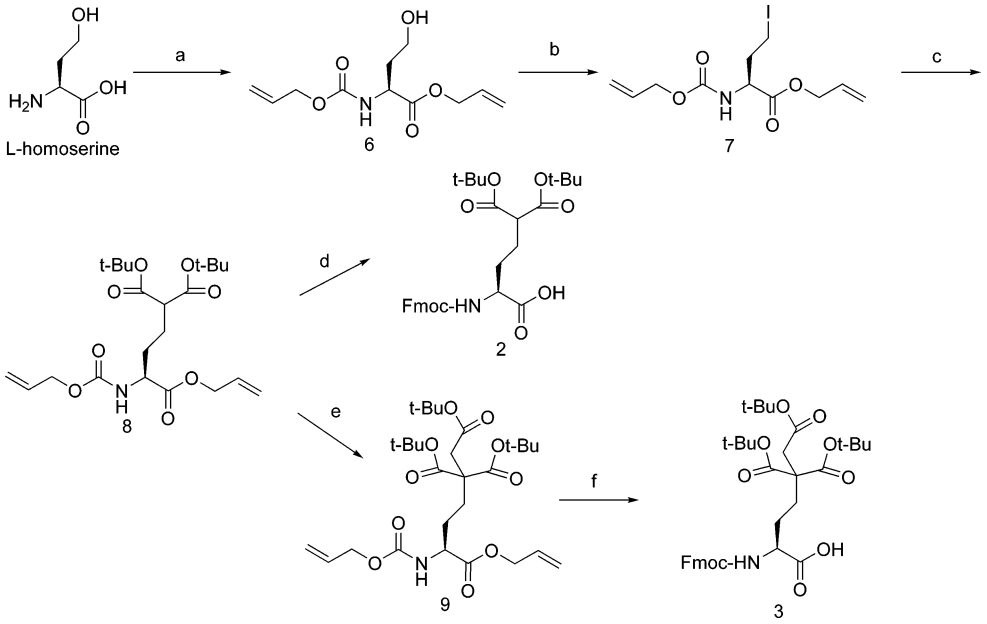
SCHEME 1[a]



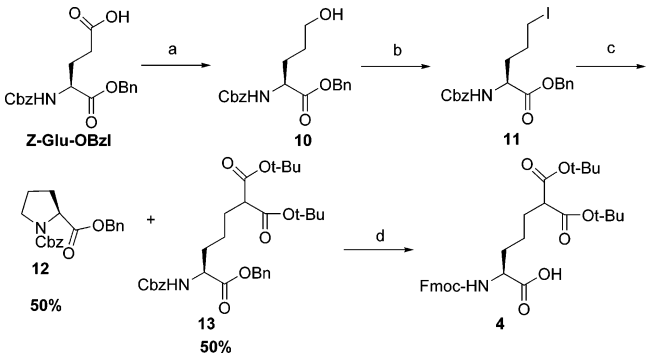
[a] Reagents and conditions: (a) (i) allyl chloroformate, H_2O-CH_3CN, Na_2CO_3, rt, (ii) allyl bromide, $NaHCO_3$, DMF, 86% for two steps; (b) (i) MsCl, Et_3N, CH_2Cl_2, 100%, (ii) NaI, acetone, rt, 12 h, 90%; (c) NaH, di-*tert*-butyl malonate, DMF, 83%; (d) (i) $Pd(Ph_3)_4$, Et_2NH, DCM, (ii) Fmoc-OSu, $NaHCO_3$, DMF/H_2O (1/1), 80%; (e) NaHMDS, *tert*-butyl bromoacetate, THF, 80%; (f) (i) $Pd(Ph_3)_4$, Et_2NH, DCM, (ii) Fmoc-OSu, $NaHCO_3$, DMF/H_2O (1/1), 76%.

al.[13] and treated the reaction mixture with 1 equiv of $NaHCO_3$ to ensure the free-base form of all the amines. Subsequent removal of all the solvents under vacuum for 2 h served to effectively remove the remaining diethylamine and the majority of the *N*-allyldiethylamine.

The resulting residue was treated directly with Fmoc-OSu to provide the desired protected amino acid **2** in 80% yield. The overall yield of **2** from L-homoserine was 51%. Treatment of **8** with NaHMDS at −78 °C followed by alkylation with *tert*-butyl bromoacetate provided the fully protected amino acid **9** in 80% yield. Using the same method as before to deprotect the Alloc group and the allyl ester simultaneously, followed by Fmoc-protection of the free amine group, we were able to generate **3** in 76% yield. The overall yield of fully protected BCAH (**3**) from L-homoserine was 39%.

Synthesis of Pen-Mal (4). The α-benzyl ester of *N*-Cbz-protected Glu was converted into alcohol **10** (Scheme 2) by reduction of the corresponding mixed anhydride with $NaBH_4$.[15] The free alcohol of **10** was then iodinated with I_2 in the presence of imidazole and triphenyl phosphite.[16] Treatment of di-*tert*-butyl malonate with NaHMDS at −78 °C followed by alkylation with **11** generated only the lactam **12**[17] when conducted at −78 °C. However, addition of **11** to NaHMDS treated di-*tert*-butyl malonate that had been maintained at −78 °C for 30 min, followed by an increase of the temperature of the reaction mixture to ambient produced both lactam **12** and the malonate derivative **13** in 50% yield, respectively, after 2 h. Removal of the Cbz moiety and the benzoate ester simultaneously by hydrogenation with a catalytic amount of 10% Pd−C, followed by Fmoc protection gave **4** in a 33% overall yield via four steps.

SCHEME 2[a]



[a] Reagents and conditions: (a) (i) $ClCO_2Et$, *N*-methylmorpholine, THF, −10 °C, (ii) $NaBH_4$, MeOH, 0 °C to rt, 88% for two steps; (b) PPh_3, imidazole, I_2, dry THF, 0 °C, 90%; (c) NaHMDS, di-*tert*-butyl malonate, THF; (d) (i) H_2, 10% Pd−C, MeOH, (ii) Fmoc-OSu, $NaHCO_3$, CH_3CN/H_2O (1/1), 86%.

Synthesis of Cm-Gla (5) and Gla (1). The starting material, D-Garner's alcohol **14**, can be easily prepared from D-serine on a large scale.[18] This primary alcohol was oxidized by using Swern oxidation and used to prepare **16** via aldol condensation.[19] Hydrogenation of **16** by using H_2/10% Pd−C gave **17** in 90% yield, but surprisingly, no cleavage of the Cbz group was observed. We assume that compound **16** may have still contained a small amount of dimethyl sulfide that poisoned the Pd−C catalyst. Treatment of **17** with NaHMDS at −78 °C followed by alkylation with *tert*-butyl iodioacetate provided **18** in 90% yield. For this substance, the yield of alkylation of **17** is very low if we use *tert*-butyl bromoacetate. Use of bismuth(III) bromide to selectively deprotect the acetonide group provided

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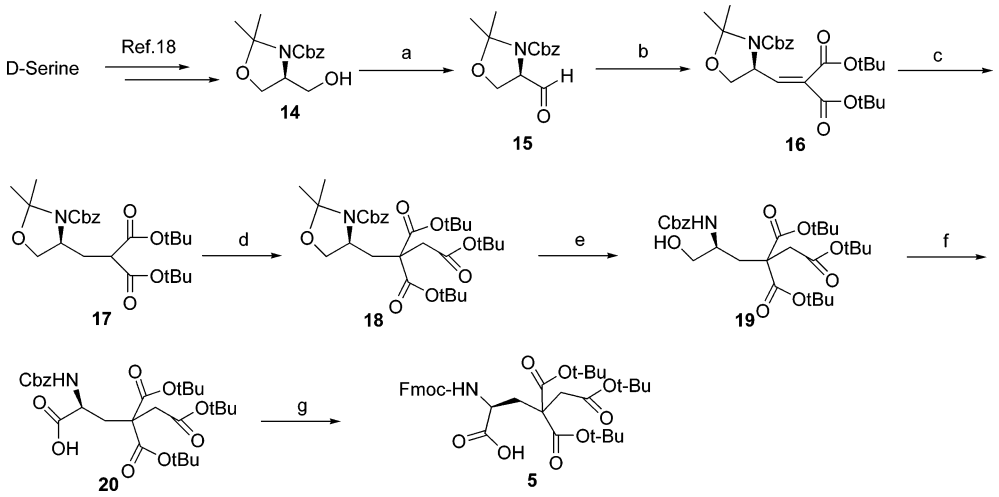
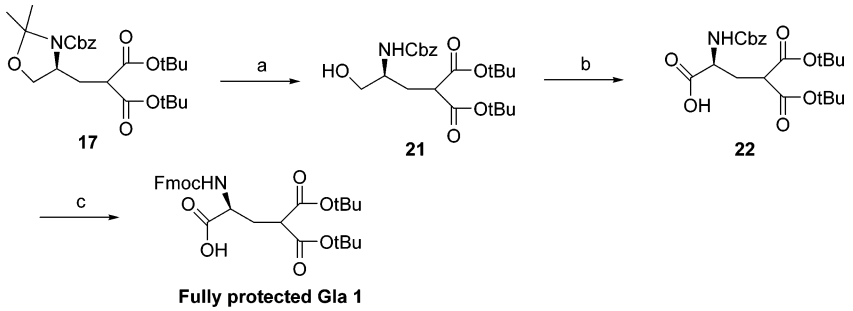
SCHEME 3[a]



[a] Reagents and conditions: (a) $(CO)_2Cl_2$, DMSO, CH_2Cl_2, −78 °C, 90%; (b) (i) LDA, THF−HMPA, di-*tert*-butyl malonate, −78 °C, (ii) Ac_2O, DMAP, pyridine or $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, 80% for two steps; (c) H_2, 10% Pd−C, 90%; (d) NaHMDS, *tert*-butyl iodioacetate, THF, 90%; (e) $BiBr_3$ (10 mol %), 1.2 equiv of H_2O, anhydrous CH_3CN, rt, 10 h, 93%; (f) (i) Dess−Martin, CH_2Cl_2, 92%, (ii) $NaClO_2$, 2-methyl-2-butene, *t*-BuOH/H_2O, 90%; or NaClO, $NaClO_2$, TEMPO, CH_3CN/H_2O, 90%; (g) (i) 10% Pd−C, H_2, 2.5 bar, rt, overnight, MeOH, (ii) Fmoc-Su, $NaHCO_3$, CH_3CN/H_2O (1/1), 80%.

SCHEME 4[a]



[a] Reagents and conditions: (a) $BiBr_3$ (10 mol %), 1.2 equiv of H_2O, anhydrous CH_3CN, rt, 8 h, 92%; (b) NaClO, $NaClO_2$, TEMPO, CH_3CN/H_2O, 92% (c) (i) H_2, 10% Pd−C, MeOH, (ii) Fmoc-OSu, $NaHCO_3$, CH_3CN/H_2O (1/1), 90%.

compound **19** in 93% yield.[20] The purity of the resulting product was very high without the need for further purification. Subsequent oxidation of the primary hydroxyl of **19** to **20** involved a two-step reaction sequence: (i) alcohol **19** was treated with Dess−Martin periodinane[21] to afford aldehyde in 92% yield, then (ii) the aldehyde was oxidized with sodium chlorite[22] to afford the acid **20** in 90% yield. We also oxidized the primary hydroxyl group of **19** to the carboxylic acid **20** in one step in 90% yield, by using sodium chlorite catalyzed by TEMPO and bleach.[23] We then removed the Cbz by hydrogenation with a catalytic amount of 10% Pd−C under 2.5 bar H_2 atmosphere at room temperature, followed by Fmoc protection to give **5** in a 42% overall yield via six steps from D-Garner's aldehyde (Scheme 3).

The cyclic N,O-aminal **17** was also used as a starting platform for a more efficient synthesis of a fully protected Gla. The acetonide group was selectively removed, to yield compound **21** in 92% yield by using bismuth bromide.[20] The purity of the resulting product is very high, without the need for further purification. Subsequent oxidation of the primary hydroxyl **21** with sodium chlorite catalyzed by TEMPO and bleach[23] gave carboxylic acid **22** in 92% yield. Then the α-amino group was deprotected by catalytic reduction with H_2/10% Pd−C, yielding the enantiomerically pure L-Gla. Then the L-Gla was reacted without purification with Fmoc-OSu to yield the final product. The overall yield of fully protected Gla (**1**) from D-Garner's aldehyde **15** was 55% (Scheme 4).

Determination of Enantiomeric Purity. To determine the enantiomeric purity of final products **1**−**5**, phenylalanine amide dipeptides were prepared by solid-phase techniques, and the resulting diastereomers were separated by RHPLC (Scheme 5). Analysis of the racemic D,L-phenylalanine-containing dipeptides (**23**) indicated good separation of diastereomers (diastereomeric retention time difference of 1.5 min). Next, the corresponding dipeptide **24** was prepared with enantiomerically pure L-phenylalanine, and shown to have less than 3% diastereomeric contamination resulting from D-isomers, except for compound **3**. The ee values for **1**, **2**, **3**, **4**, and **5** were 99%, 95%, 85%, 98%, and 98%, respectively.

In summary, four analogues of Gla have been prepared in good yield with use of stereoselective syntheses. We have also developed a new synthetic route to efficiently prepare fully

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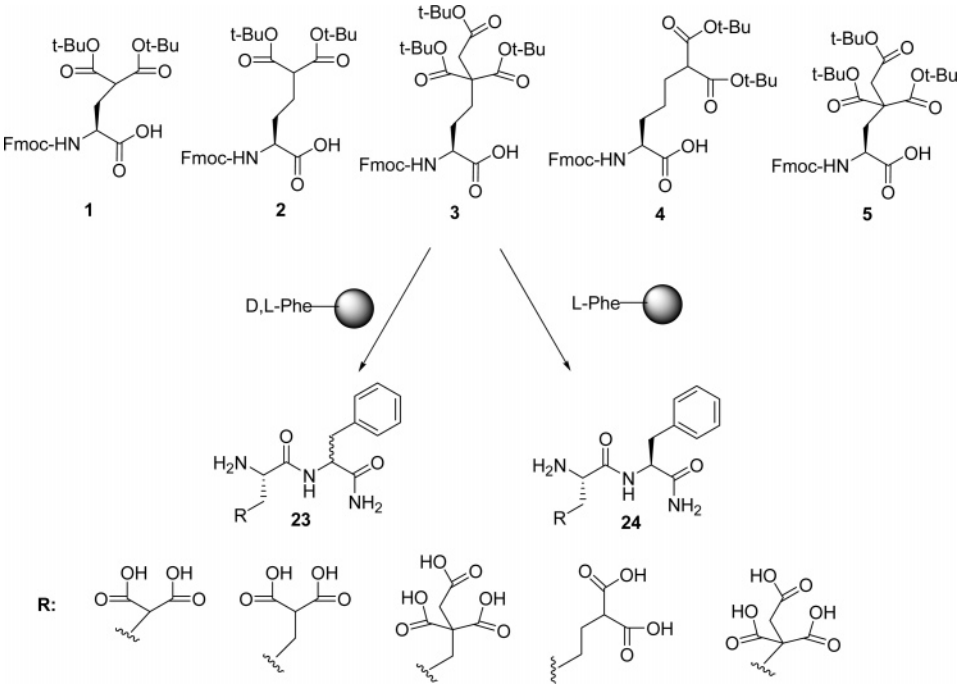
SCHEME 5



protected Gla in 55% overall yield and high enantiomeric purity (99% ee). Our approach offers the advantage of an inexpensive starting material D-serine, and inexpensive, nontoxic reagents, two factors which may be of value for commercial production. The incorporation of these amino acids into the G1TE analogues for Grb2-SH2 domain antagonist is currently in progress.

Experimental Section

(*S*)-2-Alloxycarbonylamino-4-iodobutyric Acid Allyl Ester (7). *N*-Alloc, allyl ester **6** (3.10 g, 12.76 mmol) was dissolved in dry CH_2Cl_2 (45 mL) under Ar, and the solution was cooled to 0 °C. Triethylamine (2.15 mL, 15.31 mmol) and methanesulfonyl chloride (3.08 mL, 15.31 mmol) were added, the ice bath removed, and the mixture stirred for 4 h. The reaction mixture was diluted with DCM (30 mL) and washed with ice-cold water (3 × 50 mL) and then brine (3 × 50 mL). The organic layer was dried over anhydrous sodium sulfate, and removal of the solvent yielded a colorless liquid (4.09 g, 100%). A solution of methanesulfonyl ester (4.09 g, 12.76 mmol) in acetone (35 mL) was added to a solution of sodium iodide (20.80 g, 127.6 mmol, 10 equiv) in dry acetone (35 mL) under argon. The mixture was stirred for 12 h at 25 °C, and the resulting yellow-brown slurry was then concentrated in vacuo. Ether (150 mL) was added, and sodium thiosulfate (10% aq w/v, approximately 8 mL) was then slowly added, dissolving the solids and substantially decolorizing the organic layer to afford a pale yellow solution. The organic layer was separated and dried over anhydrous sodium sulfate, and the solvent was removed in vacuo to give a yellow oil. This was purified by flash column chromatography to give **7** as an oil (4.21 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.91 (2H, m), 5.38−5.22 (5H, m), 4.66 (2H, dd, J = 1.2, 5.6 Hz), 4.59 (2H, d, J = 5.6 Hz), 4.44 (1H, m), 3.19 (2H, t, J = 7.6 Hz), 2.45 (1H, m), 2.23 (1H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.9, 156.7, 133.3, 132.2, 120.3, 119.0, 67.3, 67.0, 55.7, 37.9 ppm. IR (neat): 3329, 3082, 2944, 1699, 1523, 1267, 1180, 1042, 988, 928 cm^{-1}. FAB-MS *m*/*z* (rel intensity): 354 (MH^+, 100), 268 [MH^+ − (C_3H_6 + CO_2), 20], 226 (MH^+ − HI, 11). HRMS (FAB): calcd for $C_{11}H_{17}INO_4$ [MH^+] 354.0202, found 354.0220. $[\alpha]^{25}_D$ +22.29 (*c* 1.17, $CHCl_3$).

(*S*)-2-Alloxycarbonylamino-5-*tert*-butoxycarbonylhexanedioic Acid 1-Allylester 6-*tert*-Butyl Ester (8). The *tert*-butyl malonate (0.370 g, 1.71 mmol) was added to a suspension of 60% sodium hydride (68 mg 1.71 mmol) in THF (4.5 mL) at −30 °C, and the mixture was stirred for 30 min at room temperature. The suspension gradually became a clear solution. A solution of iodide **7** (0.598 g, 1.63 mmol) in THF (4 mL) was added slowly, and the mixture was stirred for 12 h at room temperature. The reaction was quenched with saturated aqueous ammonium chloride, and the product was extracted twice with ethyl acetate (2 × 25 mL). The combined organic layers were washed three times with saturated aqueous ammonium chloride (3 × 10 mL) and were dried over sodium sulfate. After evaporation under reduced pressure the product was purified by silica gel flash chromatography giving **8** as an oil (0.498 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.91 (2H, m), 5.36−5.20 (5H, m), 4.64 (2H, dt, J = 1.2, 5.6 Hz), 4.57 (2H, d, J = 5.6 Hz), 4.39 (1H, m), 3.14 (1H, t, J = 7.2 Hz), 1.92−1.73 (4H, m), 1.45−1.47 (18H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7, 168.3, 155.7, 132.5, 131.4, 118.9, 117.8, 81.6, 66.0, 65.8, 53.5, 53.1, 29.9, 27.8, 24.2 ppm. IR (neat): 3361, 2978, 2935, 1723, 1521, 1368, 1247, 1137, 988, 847 cm^{-1}. FAB-MS *m*/*z* (rel intensity): 442 (MH^+, 4), 386 (MH^+ − C_4H_8, 16), 330 (MH^+ − $2C_4H_8$, 100). HRMS (FAB): calcd for $C_{22}H_{36}NO_8$ [MH] $^+$ 442.2441, found 442.2413. $[\alpha]^{25}_D$ +5.70 (*c* 0.865, $CHCl_3$).

(*S*)-2-*tert*-Butoxycarbonyl-5-(9*H*-fluoren-9-ylmethoxycarbonylamino)hexanedioic-1-*tert*-butyl Ester (2). A solution of **8** (0.368 g, 1.0 mmol), Et_2NH (3.1 mL, 30.0 mmol), and $Pd(PPh_3)_4$ (0.116 g, 0.1 mmol) in 10 mL of methylene chloride was stirred for 1 h at 25 °C. The solvent was evaporated, and the residue was redissolved in CH_3CN (10 mL). A solution of $NaHCO_3$ (84 mg, 1.0 mmol) in H_2O (4.5 mL) was added and the mixture was stirred for 30 min. The solvents were evaporated, and the resulting mixture was dried 5 h under vacuum at 50 °C. The resulting solids were dissolved in DMF (30 mL) and H_2O (30 mL), treated with $NaHCO_3$ (168 mg, 2.0 mmol) and Fmoc-OSu (377 mg, 1.10 mmol), and stirred for 24 h. The solvents were removed, and the residue was dissolved in EtOAc (150 mL) and washed with 0.1 M $KHSO_4$ (4 × 50 mL), H_2O (4 × 50 mL), and brine (100 mL). After drying and removal of the solvent, the residue was purified by chromatography to yield **2** as a white foam (431 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (2H, d, J = 7.6 Hz), 7.61 (2H, m), 7.40 (2H, dd, J = 7.2 Hz), 7.31 (2H, dd, J = 7.4 Hz), 5.52 (1H, d, J = 7.6 Hz), 4.45−4.35 (3H, m), 4.24 (1H, t, J = 7.2 Hz), 3.19 (1H, t,

J = 6.8 Hz), 1.94−1.80 (4H, m), 1.46−1.47 (18H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.2, 143.7, 141.3, 127.7, 127.1, 125.1, 119.9, 81.9, 67.3, 64.8, 53.1, 47.1, 27.9, 24.2 ppm. IR (neat): 3371, 2977, 2359, 1707, 1688, 1524, 1365, 1217, 1142, 759 cm^{-1}. FAB-MS (negative ion)-*m*/*z* (rel intensity): 538 (M − H, 100), 342 (M − H − $C_{13}H_9CH_2OH$, 63), 316 [M − H − ($C_{13}H_{10}$ + CO_2), 58]. HRMS (FAB): calcd for $C_{30}H_{36}NO_8$ [M − H] 538.244, found 538.244. $[\alpha]^{25}_D$ +14.28 (*c* 0.16, $CHCl_3$).

(*S*)-2-Allyloxycarbonylamino-5,5-bis-*tert*-butoxycarbonylheptanedioic-1-allylester-7-*tert*-butyl Ester (9). A solution of NaHMDS (1.98 mL, 1.0 M in THF, 1.98 mmol) was added to a solution of **8** (0.694 g 1.883 mmol) in THF (13 mL) at −30 °C, and the mixture was stirred for 40 min at room temperature. A solution of *tert*-butyl bromoacetate (0.367 g, 3.77 mmol) in THF (4 mL) was added slowly at 0 °C, and the mixture was stirred for 2 h at room temperature. The reaction was quenched with saturated aqueous ammonium chloride, and the product was extracted twice with ethyl acetate (3 × 30 mL). The combined organic layers were washed three times with saturated aqueous ammonium chloride (3 × 10 mL) and were dried over sodium sulfate. After evaporation under reduced pressure the product was purified by silica gel flash chromatography and gave **9** as an oil (0.837 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.89 (2H, m), 5.35−5.19 (5H, m), 4.63 (2H, dd, J = 1.2, 4.8 Hz), 4.55 (2H, d, J = 5.6 Hz), 4.36 (1H, m), 2.77 (2H, d, J = 1.2 Hz), 2.03−1.67 (4H, m), 1.45−1.42 (27H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 168.3, 167.8, 154.4, 131.3, 130.2, 117.5, 116.3, 115.8, 80.5, 79.9, 65.1, 64.7, 64.4, 59.1, 54.6, 52.5, 50.5, 37.1,, 26.7, 26.6, 26.5, 25.9, 19.7, 12.9 ppm. IR (neat): 3369, 2978, 2935, 1725, 1368, 1247, 1146, 846 cm^{-1}. FAB-MS *m*/*z* (rel intensity): 556 (MH^+, 0.9), 500 ($MH^+ - C_4H_8$, 2.6), 388 ($MH^+ - 3C_4H_{18}$, 51). HRMS (FAB): calcd for $C_{28}H_{45}NKO_{10}$ [M + K^+] 594.268, found 594.267. $[\alpha]^{25}_D$ +18.58 (*c* 0.485, $CHCl_3$).

(*S*)-5,5-Bis-*tert*-butoxycarbonyl-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)heptanedioic Acid 7-*tert*-Butyl Ester (3). A solution of **9** (0.54 g, 0.97 mmol), Et_2NH (3.0 mL, 29.0 mmol), and $Pd(PPh_3)_4$ (0.112 g, 0.097 mmol) in 10 mL of methylene chloride was stirred for 1 h at 25 °C. The solvent was evaporated, and the residue was redissolved in CH_3CN (10 mL). A solution of $NaHCO_3$ (81.5 mg, 0.97 mmol) in H_2O (4.0 mL) was added and the mixture was stirred for 30 min. The solvents were evaporated, and the resulting mixture was dried 5 h under vacuum at 50 °C. The resulting solids were dissolved in DMF (28 mL) and H_2O (28 mL), treated with $NaHCO_3$ (163 mg, 1.94 mmol) and Fmoc-OSu (343 mg, 1.0 mmol), and stirred for 24 h. The solvents were removed, and the residue was dissolved in EtOAc (150 mL) and washed with 0.1 M $KHSO_4$ (4 × 40 mL), H_2O (4 × 40 mL), and brine (70 mL). After drying and removal of the solvent, the residue was purified by chromatography to yield **3** as a white foam (482 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (2H, d, J = 7.6 Hz), 7.62 (2H, d, J = 7.6 Hz), 7.39 (2H, dd, J = 7.2 Hz), 7.30 (2H, dt, J = 1.2, 7.2 Hz), 5.67 (1H, d, J = 7.6 Hz), 4.40 (2H, m), 4.32 (1H, m), 4.22 (1H, t, J = 7.4 Hz), 2.80 (2H, s), 2.10−1.78 (4H, m), 1.45−1.41 (27H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 169.1, 156.2, 143.8, 143.7, 141.2, 127.7, 127.6, 127.1, 125.2, 119.9, 82.0, 81.4, 67.3, 55.9, 53.6, 47.0, 38.4, 27.9, 27.7, 26.5 ppm. IR (neat): 3135, 2978, 1725, 1682, 1367, 1146, 841 cm^{-1}. FAB-MS (negative ion)-*m*/*z* (rel intensity): 652 (M − H, 28), 456 (M − H − $C_{13}H_9CH_2OH$, 100), 430 [M − H − ($C_{13}H_{10}$ + CO_2), 60). HRMS (FAB): calcd for $C_{36}H_{46}NO_{10}$ [M − H] 652.312, found 652.316 $[\alpha]^{25}_D$ +10.46 (*c* 0.375, $CHCl_3$).

(*S*)-Benzyloxycarbonylamino-5-hydroxypentanoic Acid Benzyl Ester (10). To a stirred solution of *N*-protected amino acid Z-Glu-OBzl (5.571 g, 15.0 mmol) in THF (75 mL) at −10 °C was added *N*-methylmorpholine (1.65 mL, 15 mmol) followed by ethyl chloroformate (1.434 mL, 15 mmol). After 10 min, $NaBH_4$ (1.70 g, 45 mmol) was added in one portion. MeOH (150 mL) was then added dropwise to the mixture over a period of 20 min at 0 °C. The solution was stirred for an additional 30 min and then neutralized with 1 M $KHSO_4$. The organic solvents were removed, and the product was extracted with EtOAc (3 × 60 mL). The combined organic phases were washed consecutively with 1 M $KHSO_4$, H_2O, 5% aqueous $NaHCO_3$, and H_2O and dried (Na_2SO_4), and the solvent was evaporated. The residue was purified by column chromatography to give **10** as a white solid (4.718 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28−7.24 (10H, m), 5.68 (1H, d, J = 8.4 Hz), 5.08−5.15 (4H, m), 4.42 (1H, m), 3.56 (2H, t, J = 6.0 Hz), 2.31 (1H, br s, OH), 1.89 (1H, m), 1.74 (1H, m), 1.53 (2H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.4, 156.1, 136.2, 135.2, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 67.1, 67.0, 61.8, 53.7, 29.1, 28.1 ppm. IR (neat): 3316, 3033, 2922, 2870, 1730, 1686, 1530, 1254, 1203, 1058 cm^{-1}. FAB-MS *m*/*z* (rel intensity): 358 (MH^+, 15), 314 ($MH^+ - CO_2$, 15), 91 ($C_7H_7^+$, 100). HRMS (FAB): calcd for $C_{20}H_{24}NO_5$ [MH^+] 358.1654, found 358.1647. $[\alpha]^{25}_D$ −2.96 (*c* 1.00, $CHCl_3$).

(*S*)-2-Benzyloxycarbonylamino-5-iodopentanoic Acid Benzyl Ester (11). To a 100 mL flask was added **10** (3.561 g, 9.96 mmol), imidazole (1.085 g, 15.94 mmol), Ph_3P (3.93 g, 14.95 mmol), and anhydrous THF (109 mL) under a nitrogen atmosphere. Then iodine (3.79 g, 14.95 mmol) was added in portions at 0 °C. The reaction mixture was stirred at room temperature for 2 h. Excess iodine was removed by the addition of aqueous sodium thiosulfate. The mixture was transferred to a separating funnel. The organic layer was diluted with ether, washed with water, and dried ($MgSO_4$). The solid was filtered and the organic solution was concentrated. The residue was treated with diethyl ether and precipitated triphenylphosphine oxide was removed by filtration. The filtrate was concentrated and purified by column chromatography to afford **11** as a white solid (4.19 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36−7.31 (10H, m), 5.37 (1H, d, J = 7.6 Hz), 5.19−5.10 (4H, m), 4.45 (1H, m), 3.13 (2H, m), 1.98−1.75 (4H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 155.8, 136.1, 135.1, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 67.3, 67.1, 53.0, 33.6, 29.0 ppm. IR (neat): 3312, 3032, 2968, 2358, 1723, 1688, 1530, 1273, 1251, 729 cm^{-1}. FAB-MS *m*/*z* (rel intensity): 468 (MH^+, 14), 424 ($MH^+ - CO_2$, 6.5), 91 ($C_7H_7^+$, 100). HRMS (FAB): calcd for $C_{20}H_{23}INO_4$ [MH^+] 468.0672, found 468.0700. $[\alpha]^{25}_D$ +2.56 (*c* 0.86, $CHCl_3$).

(*S*)-2-Benzyloxycarbonylamino-6-*tert*-butoxycarbonylheptanedioic Acid 1-Benzyl Ester 7-*tert*-Butyl Ester (13). The *tert*-butyl malonate (1.47 g, 6.78 mmol) was added to a solution of NaHMDS (6.78 mL, 1.0 M in THF, 6.78 mmol) in THF (15 mL) at −78 °C, and the mixture was stirred 30 min at the same temperature. A solution of iodide **11** (3.17 g, 6.78 mmol) in THF (15 mL) was added slowly, and the mixture was stirred for 20 min at −78 °C. Then the reaction mixture was stirred at room temperature for 2 h. The reaction was quenched with saturated aqueous ammonium chloride, and the product was extracted twice with ethyl acetate (2 × 35 mL). The combined organic layers were washed three times with saturated aqueous ammonium chloride (3 × 10 mL) and were dried over sodium sulfate. After evaporation under reduced pressure the product was purified by silica gel flash chromatography to give **12** as a white solid (1.15 g, 50%) and **13** as a oil (1.886 g, 50%).

Compound **12**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37−7.21 (10H, m), 5.22−4.98 (4H, m), 4.44 and 4.37 (each dd, J = 3.6, 8.8 Hz, 0.5H), 3.63 (1H, m), 3.49 (1H, m), 3.33 (1H, m), 2.02−1.83 (3H, m) ppm.

Compound **13**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.29 (10H, m), 5.30 (1H, d, J = 8.4 Hz), 5.15 (2H, s), 5.09 (2H, s), 4.41 (1H, m), 3.06 (1H, t, J = 7.4 Hz), 1.91−1.65 (4H, m), 1.49−1.44 (18H, m), 1.35 (2H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.1, 168.6, 155.8, 136.2, 135.2, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 81.4, 67.1, 66.9, 53.8, 53.6, 32.3, 28.1, 27.9, 22.9 ppm. IR (neat): 3359, 2977, 2934, 1721, 1368, 1248, 1163, 1136, 696 cm^{-1}. FAB-MS with KCl-*m*/*z* (rel intensity): 594 (M + K^+, 3.2), 500 ($MH^+ - C_4H_8$, 5), 91 ($C_7H_7^+$, 100). HRMS (FAB): calcd for $C_{31}H_{41}NO_8K$ [M + K^+] 594.247, found 594.248. $[\alpha]^{25}_D$ −1.42 (*c* 1.465, $CHCl_3$).

(*S*)-2-*tert*-Butoxycarbonyl-6-(9*H*-fluoren-9-ylmethoxycarbonylamino)heptanedioic Acid 1-*tert*-Butyl Ester (4). A mixture of **13** (1.383 g, 2.49 mmol), CH_3OH (20 mL), and 10% Pd−C (0.138 g) was stirred vigorously under a H_2 atmosphere until hydrogen was absorbed to saturation. The mixture was filtered and concentrated to give a crude oil. A mixture of the above oil, *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (0.84 g, 2.49 mmol), and $NaHCO_3$ (0.628 g, 7.47 mmol) in water and acetonitrile (1:1, 20 mL) was stirred at room temperature overnight. Acetonitrile was evaporated under reduced pressure and the resulting solution was adjusted to pH 4 with 10% citric acid. The mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, concentrated, and purified by flash chromatography to afford **4** as a white solid (1.19 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (2H, d, J = 7.6 Hz), 7.59 (2H, d, J = 6.4 Hz), 7.38 (2H, dd, J = 7.4 Hz), 7.31 (2H, dd, J = 7.4 Hz), 5.35 (1H, d, J = 8.0 Hz), 4.44−4.35 (3H, m), 4.21 (1H, t, J = 6.8 Hz), 3.14 (1H, t, J = 7.4 Hz), 1.95−1.72 (4H, m), 1.45−1.39 (20H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.8, 143.6, 141.3, 127.7, 127.0, 125.1, 119.9, 81.6, 67.2, 53.5, 47.1, 31.8, 27.9, 22.9 ppm. IR (neat): 3327, 2977, 2934, 2362, 2341, 1719, 1247, 1137, 846, 738 cm^{-1}. FAB-MS (negative ion)-*m*/*z* (rel intensity): 552 (M − H, 59), 356 (M − H − $C_{13}H_9CH_2OH$, 100), 330 [M − H − ($C_{13}H_{10}$ + CO_2), 76]. HRMS (FAB): calcd for $C_{31}H_{38}NO_8$ [M − H] 552.260, found 552.261. $[\alpha]^{25}_D$ +10.94 (*c* 0.32, $CHCl_3$).

(4*S*)-2-(3-Benzyloxycarbonyl-2,2-dimethyloxazolidin-4-ylmethyl)malonic Acid Di-*tert*-butyl Ester (17). A mixture of **16** (1.0 g, 2.17 mmol), CH_3OH (10 mL), and 10% Pd−C (0.10 g) was stirred vigorously under a H_2 atmosphere until hydrogen was absorbed to saturation. The mixture was then filtered and concentrated to give a crude oil that was purified by flash chromatography to afford **17** as a oil (0.9 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36−7.30 (5H, m), 5.14 (2H, m), 4.11−3.93 (2H, m), 3.81 (1H, m), 3.22 (1H, m), 2.24 (1H, m), 2.12 (1H, m), 1.56−1.41 (24H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3, 128.5, 127.9, 94.4, 81.6, 67.2, 66.6, 55.4, 51.2, 32.3, 27.9, 27.8, 26.6, 23.0 ppm. IR (neat): 2979, 2935, 1724, 1706, 1367, 1135, 846 cm^{-1}. FAB-MS *m*/*z* (rel intensity): 464 (MH^+, 3.8), 408 ($MH^+ - C_4H_8$, 2.2), 91 ($C_7H_7^+$, 100). HRMS (FAB): calcd for $C_{25}H_{38}NO_7$ [MH^+] 464.2648, found 464.2635. $[\alpha]^{25}_D$ +20.94 (*c* 1.62, $CHCl_3$).

(4*S*)-2,2-Dimethyl-4-(2,2,3-tris-*tert*-butoxycarbonylpropyl)oxazolidine-3-carboxylic Acid Benzyl Ester (18). To a 100 mL flask was added NaI (5.49 g, 36.6 mmol) and anhydrous acetone (50 mL) under nitrogen atmosphere. Then *tert*-butyl bromoacetate (4.76 g, 24.4 mmol) was added slowly at room temperature. The reaction mixture was stirred at room temperature for 2 h. The solid was filtered and the organic solution was concentrated. The residue was treated with hexane and precipitated white solid was removed by filtration. The filtrate was concentrated and gave *tert*-butyl iodoacetate (5.313 g, 90%).

A solution of NaHMDS (1.79 mL, 1.0 M in THF, 1.98 mmol) was added to a solution of **17** (0.754 g 1.63 mmol) in THF (11 mL) at −78 °C, and the mixture was stirred for 40 min at −78 °C. A solution of *tert*-butyl iodoacetate (0.787 g, 3.25 mmol) in THF (4 mL) was added slowly at −78 °C, and the mixture was stirred for 5 h at −78 °C. The reaction was quenched with saturated aqueous ammonium chloride, and the product was extracted twice with ethyl acetate (3 × 30 mL). The combined organic layers were washed three times with saturated aqueous ammonium chloride (3 × 10 mL) and were dried over sodium sulfate. After evaporation under reduced pressure the product was purified by silica gel flash chromatography and gave **18** as a oil (0.845 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31−7.23 (5H, m), 5.05 (2H, m), 3.94−3.75 (3H, m), 2.98−2.80 (2H, m), 2.35 (2H, m), 1.55−1.38 (33H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.7, 169.1, 128.4, 127.9, 82.1, 81.7, 80.9, 67.7, 66.9, 55.0, 54.7, 39.1, 37.9, 36.0, 35.5, 28.0, 27.7, 26.7, 24.6, 23.1 ppm. IR (neat): 2979, 2934, 1726, 1367, 1140, 1075, 846 cm^{-1}. FAB-MS *m*/*z* (rel intensity): 578 (MH^+, 3.5), 522 ($MH^+ - C_4H_8$, 2.3), 57 ($C_4H_9^+$, 100). HRMS (FAB): calcd for $C_{31}H_{48}NO_9$ [MH^+] 578.333, found 578.335. $[\alpha]^{25}_D$ +4.27 (*c* 2.78, $CHCl_3$).

(5*S*)-Benzyloxycarbonylamino-3,3-bis-*tert*-butyoxycarbonyl-6-hydroxyhexanoic Acid *tert*-Butyl Ester (19). To a solution of cyclic *N*,*O*-aminal **18** (0.804 g, 1.39 mmol) in anhydrous CH_3CN (13.9 mL) was added bismuth(III) bromide (62 mg, 0.139 mmol) at room temperature. After 30 min, water (0.03 mL, 1.67 mmol) was added. The reaction mixture was stirred at room temperature until all starting material had disappeared (about 10 h), and was then quenched by adding saturated aqueous $NaHCO_3$ (1 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated to afford the pure product **19** as a solid (0.695 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29−7.24 (5H, m), 5.39 (1H, d, J = 8.0 Hz), 5.02 (2H, s), 3.74 (1H, m), 3.54 (2H, br s), 2.86 (2H, d, J = 2.4 Hz), 2.77 (OH, br s), 2.18 (2H, d, J = 6.0 Hz), 1.39−1.30 (27H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 169.4, 169.3, 156.4, 136.4, 128.4, 128.0, 82.3, 81.4, 66.7, 66.3, 55.4, 50.3, 39.1, 33.2, 28.0, 27.8, 27.7 ppm. IR (neat): 3382, 2978, 2934, 1724, 1368, 1245, 1142, 845 cm^{-1}. FAB-MS *m*/*z* (rel intensity): 538 (MH^+, 3.5), 482 ($MH^+ - C_4H_8$, 7.5), 91 ($C_7H_7^+$, 100). HRMS (FAB): calcd for $C_{28}H_{43}NO_9K$ [M + K^+] 576.257, found 576.255. $[\alpha]^{25}_D$ −10.15 (*c* 0.5, $CHCl_3$).

(2*S*)-Benzyloxycarbonylamino-4,4-bis-*tert*-butyoxycarbonylhexanedioic Acid 6-*tert*-Butyl Ester (20). To a solution of **19** (0.408 g, 0.759 mmol) in DCM (6.5 mL) was added Dess−Martin periodinane (0.398, 97%, 0.91 mmol) at room temperature. After 40 min, the reaction was quenched by adding saturated aqueous Na_2SO_3 (3 mL). The layers were separated, and the aqueous layer was extracted with DCM. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to afford the aldehyde as a oil (0.375 g, 92%). The aldehyde was placed in a flask with 14 mL of *tert*-butyl alcohol to which was added 2-methyl-2-butene (2.0 M in THF, 7.6 mL, 15.2 mmol) followed by a solution of sodium chlorite (0.618 g, 6.83 mmol) and monobasic sodium phosphate (0.733 g, 5.31 mmol) in 6.2 mL of water. As the oxidant was added the solution became bright yellow and evolved heat. The reaction was stirred overnight, changing from yellow to colorless. It was then concentrated under vacuum, diluted with saturated $NaHCO_3$, and extracted once with hexanes. The aqueous phase was then acidified with 2 M HCl and extracted twice with ether, the combined ether extracts then dried over Na_2SO_4. The product was concentrated in vacuo affording the product as a white solid (0.347 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34−7.26 (5H, m), 5.67 (1H, d, J = 8.0 Hz), 5.11 (2H, s), 4.40 (1H, t, J = 8.4 Hz), 2.92 (2H, d, J = 4.4 Hz), 2.60 (1H, d, J = 15.2 Hz), 2.39 (1H, dd, J = 12, 15.2 Hz), 1.45−1.41 (27H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0, 169.0, 156.4, 136.0, 128.4, 128.1, 128.0, 82.8, 82.7, 81.8, 67.2, 55.4, 51.2, 38.4, 33.7, 27.9, 27.7 ppm. IR (neat): 3330, 2979, 2935, 1724, 1368, 1243, 1143, 844 cm^{-1}. FAB-MS (negative ion)-*m*/*z* (rel intensity): 550 (M − H, 59), 442 (M − H − $C_6H_5CH_2OH$, 100), 342 [M − H − ($C_6H_5CH_2OH$ + C_3H_6O + CO_2), 29]. HRMS (FAB): calcd for $C_{28}H_{40}NO_{10}$ [M − H] 550.265, found 550.263. $[\alpha]^{25}_D$ −0.49 (*c* 3.60, $CHCl_3$).

(2*S*)-4,4-Bis-*tert*-butyoxycarbonyl-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)hexanedioic Acid 6-*tert*-Butyl Ester (5). A mixture of **20** (0.619 g, 1.123 mmol), CH_3OH (30 mL), and 10% Pd−C (50 mg) was stirred vigorously under 2.5 bar H_2 atmosphere at room temperature overnight. The mixture was filtered and concentrated to give a crude oil. A mixture of the above oil, *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (0.379 g, 1.123 mmol), and $NaHCO_3$ (0.283 g, 3.369 mmol) in water and acetonitrile (1:1, 15 mL) was stirred at room temperature overnight. The acetonitrile was evaporated under reduced pressure, and the resulting solution was adjusted to pH 4 with 10% citric acid. The mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, concentrated, and purified by flash chromatography to afford **5** as a white solid (0.575 g, 80%). ^{1}H

NMR (400 MHz, $CDCl_3$): δ 7.75 (2H, d, J = 7.2 Hz), 7.60 (2H, t, J = 7.2 Hz), 7.38 (2H, dd, J = 7.4 Hz), 7.31 (2H, m), 5.99 (1H, d, J = 7.6 Hz), 4.46 (1H, m), 4.36 (1H, m), 4.24 (1H, m), 4.22 (1H, t, J = 7.0 Hz), 2.93 (2H, q, J = 16.2 Hz), 2.60 (1H, dd, J = 2.6, 15.4 Hz), 2.46 (1H, dd, J = 11.6, 15.4 Hz), 1.47−1.43 (27H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.0, 168.9, 156.5, 143.8, 143.6, 141.2, 127.6, 127.0, 125.2, 125.1, 119.9, 82.8, 82.7, 81.9, 67.3, 55.6, 51.3, 47.0, 38.7, 33.5, 28.0, 27.7 ppm. IR (neat): 3134, 2944, 2832, 1449, 1024, 914, 732 cm^{-1}. FAB-MS (negative ion)-*m*/*z* (rel intensity): 638 (M − H, 48), 442 (M − H − $C_{13}H_9CH_2OH$, 100), 342 [M − H − ($C_{13}H_9CH_2OH$ + C_4H_8 + CO_2), 92]. HRMS (FAB): calcd for $C_{35}H_{44}NO_{10}$ [M − H] 638.296, found 638.293. $[\alpha]^{25}_D$ +1.29 (*c* 0.63, $CHCl_3$).

(2*S*)-2-(2-Benzyloxycarbonylamino-3-hydroxypropyl)malonic Acid Di-*tert*-butyl Ester (21). To a solution of cyclic *N*,*O*-aminal **17** (55 mg, 0.119 mmol) in anhydrous MeCN (1.2 mL) was added bismuth(III) bromide (5.3 mg, 0.0119 mmol) at room temperature. After 30 min, water (0.003 mL, 0.142 mmol) was added. The reaction mixture was stirred at room temperature until all starting material had disappeared (about 8 h), and then was quenched by the addition of saturated aqueous $NaHCO_3$ (0.1 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated to afford product **21** as a pure oil (46 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31−7.28 (5H, m), 5.15 (1H, d, J = 8.4 Hz), 5.05 (2H, s), 3.73−3.52 (3H, m), 3.24 (1H, t, J = 6.0 Hz), 2.49 (OH, s), 2.10−1.92 (2H, m), 1.45−1.39 (18H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.9, 156.4, 136.3, 128.5, 128.4, 128.1, 82.0, 66.8, 64.9, 51.7, 51.1, 29.7, 27.8 ppm. IR (neat): 3381, 2979, 2883, 2934, 2361, 2341, 1723, 1523, 1255, 1143, 1055 cm^{-1}. FAB-MS *m*/*z* (relative intensity): 424 (MH^+, 4.8), 368 (MH^+ − C_4H_8, 9.6), 91 ($C_7H_7^+$, 100). HRMS (EI, *m*/*z*): calcd for $C_{22}H_{34}NO_7$ [MH^+] 424.2335, found 424.2310. $[\alpha]^{25}_D$ −8.45 (*c* 1.75, $CHCl_3$).

(2*S*)-Benzyloxycarbonylamino-4-*tert*-butoxycarbonylpentanedioic Acid 5-*tert*-Butyl Ester (22). Alcohol **21** (44 mg, 0.104 mmol) was dissolved in 2 mL of 3:2 CH_3CN:pH 6.7 NaH_2PO_4 and Na_2HPO_3 aq buffer (0.67M phosphate) and warmed to 40 °C. TEMPO (2.0 mg, 0.014 mmol) was added to the reaction followed by 135 μL of a 2 M solution of 80% sodium chlorite (31 mg, 270 μmol) in H_2O. A diluted solution of bleach (8.0 μL) in 160 μL of H_2O was then added gradually over 1 h, and the reaction was stirred at 40 °C for 18 h. The reaction was cooled to room temperature and quenched with saturated aqueous Na_2SO_3 until the mixture became colorless. The CH_3CN was removed under reduced pressure and the aqueous mixture was acidified to pH <3 with 1 M HCl and extracted with Et_2O. The organic layers were dried over $MgSO_4$ and evaporated to give 66 mg of **22** as an oil (41.8 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36−7.31 (5H, m), 5.45 (1H, d, J = 8.4 Hz), 5.11 (2H, d, J = 2.0 Hz), 4.46 (1H, m), 3.35 (1H, t, J = 6.4 Hz), 2.46 (1H, m), 2.18 (1H, m), 1.46−1.43 (18H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.6, 168.3, 168.1, 156.1, 128.5, 128.2, 128.1, 82.3, 67.2, 52.4, 50.8, 30.7, 27.8 ppm. IR (neat): 3329, 2979, 2935, 2342, 1720, 1519, 1368, 1250, 1139, 844 cm^{-1}. FAB-MS (negative ion)-*m*/*z* (rel intensity): 436 (M − H, 100), 362 (M − H − C_4H_9OH, 17), 328 (M − H − $C_6H_5CH_2OH$, 59). HRMS (EI, *m*/*z*): calcd for $C_{22}H_{30}NO_8$ [M − H] 436.1971, found 436.1967. $[\alpha]^{25}_D$ +6.40 (*c* 2.1, $CHCl_3$).

***N*-α-Fmoc-L-γ-carboxyglutamic Acid γ,γ-*tert*-Butyl Ester (1).** The mixture of **22** (30 mg, 0.069 mmol), CH_3OH (2 mL), and 10% Pd−C (5 mg) was stirred vigorously under a H_2 atmosphere until hydrogen was absorbed to saturation. The mixture was filtered and concentrated to give a crude oil (21 mg, 100%). A mixture of the above oil, *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (69.4 mg, 0.206 mmol), and $NaHCO_3$ (51.9 mg, 0.617 mmol) in water and acetonitrile (1:1, 5 mL) was stirred at room temperature overnight. Acetonitrile was evaporated under reduced pressure, and the resulting solution was adjusted to pH 4 with 10% citric acid. The mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, concentrated, and purified by flash chromatography to afford **1** as a solid (32.4 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (2H, d, J = 7.6 Hz), 7.59 (2H, dd, J = 3.2, 7.2 Hz), 7.40 (2H, dd, J = 7.2 Hz), 7.40 (2H, ddd, J = 1.2, 7.6 Hz), 5.58 (1H, d, J = 8.0 Hz), 4.45 (2H, m), 4.35 (1H, m), 4.22 (1H, t, J = 6.8 Hz), 3.41 (1H, t, J = 7.2 Hz), 2.46 (1H, m), 2.21 (1H, m), 1.46−1.48 (18H, m) ppm. FAB-MS *m*/*z* (rel intensity) 526 (MH^+, 1), 470 (MH^+ − *t*-Bu, 4), 414 (MH^+ − 2*t*-Bu, 31). $[\alpha]^{25}_D$ −8.7 (*c* 1.0, MeOH)

Determination of Enantiomeric Purity. Dipeptides **23** and **24** were prepared from **1**, **2**, **3**, **4**, and **5**, using Rink amide-AM resin (0.51 mequiv/g) with Fmoc-protocols similar to those described previously. Fmoc-D,L-Phe- and Fmoc-L-Phe-Rink amide resins were prepared by coupling the appropriate Fmoc-protected amino acids to Rink resin, with the resulting Fmoc-protected resins (12.5 mg) then being washed well with several 1 mL portions of DMF. Fmoc amino protection was removed by treatment with 20% piperidine in DMF (0.5 mL, 1 min followed by 0.5 mL, 20 min). Resins were washed well with DMF (10 × 1 mL) then coupled overnight with a solution of active ester formed by reacting 12.5 μmol each of Fmoc-Gla-OH (**2**, **3**, **4** and **5**), HOAt, and PyAOP and 25 μmol of DIPEA in DMF (1.0 mL, 2 h). Resins were washed with DMF (10 × 1 mL), and *N*-terminal Fmoc-protection was removed by treatment with 20% piperidine in NMP (0.5 mL, 5 min). Deblocked resins were first washed with DMF (10 × 1 mL) and dichloromethane (10 × 2 mL), then dipeptides were cleaved from the resin by treating the resin with a mixture of trifluoroacetic acid (TFA, 1.85 mL), H_2O (50 μL) and triethylsilane (50 μL) for 2 h. TFA was evaporated by a stream of dry N_2, and the residue was triturated with ether. The resulting crude peptide was analyzed by HPLC. Retention times of diastereomeric peaks, as determined with dipeptide **23**, prepared from racemic D,L-phenylalanine, indicated diastereomers eluting at 16.97 min and at 18.44 min. Enantiomeric contamination of L-Fmoc-Gla(*t*Bu$_2$)-OH (**1**) was then determined by similar analysis of dipeptide **24**, where diastereomeric contamination accounted for an area less than 1% of that observed for the major diastereomer. These results indicated greater than 99% enantiomeric purity. The ee values of **2**, **3**, **4**, and **5** were 95%, 85%, 98%, and 99%, respectively.

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Supporting Information Available: ^{1}H NMR spectra of all unknown compound and compound **1**, and ^{13}C NMR spectra of unknown compounds **2**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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