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Synthesis of Nonproteinogenic Amino Acids To Probe Lantibiotic Biosynthesis

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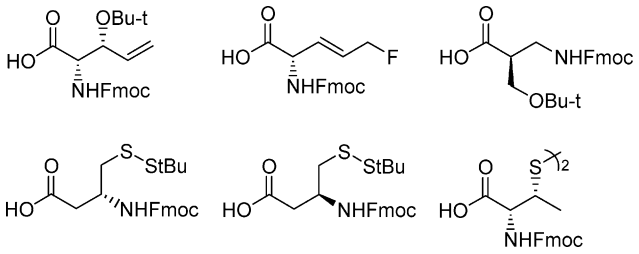

The synthesis of six nonproteinogenic amino acids appropriately protected for Fmoc-based solid-phase peptide synthesis is described. These amino acids are (2*S*,3*R*)-vinylthreonine, (2*S*)-(*E*)-2-amino-5-fluoro-pent-3-enoic acid (fluoroallylglycine), (*S*)-β^2-homoserine, (*S*) and (*R*)-β^3-homocysteine, and (2*R*,3*R*)-methylcysteine. Once incorporated into peptides, these compounds were envisioned to serve as alternative substrates for the lantibiotic synthases that dehydrate serine and threonine residues in their peptide substrates and catalyze the subsequent intramolecular Michael-type addition of cysteines to the dehydroamino acids.

Introduction

Nonproteinogenic amino acids with highly functionalized side chains are frequently found as constituents of biologically important peptides. As a result, there has been significant interest in the synthesis, biosynthesis, and biological activities of these compounds.[1] In addition, β-amino acids have gained recent attention both for their natural occurrence and as building blocks for oligomers with well-defined folding behavior.[2] The so-called lantibiotics are a group of ribosomally synthesized and post-translationally modified peptide antibiotics.[3] These modifications involve dehydrations of Ser and Thr residues followed by intramolecular Michael-type additions of Cys residues to the dehydroamino acids in a regio- and stereoselective fashion (Scheme 1). The enzymatic system that carries out these modifications during the biosynthesis of lacticin 481 has been recently reconstituted in vitro.[4] The enzyme was shown to display a high degree of substrate promiscuity, opening the possibility of introducing nonproteinogenic amino acids into its peptide

SCHEME 1

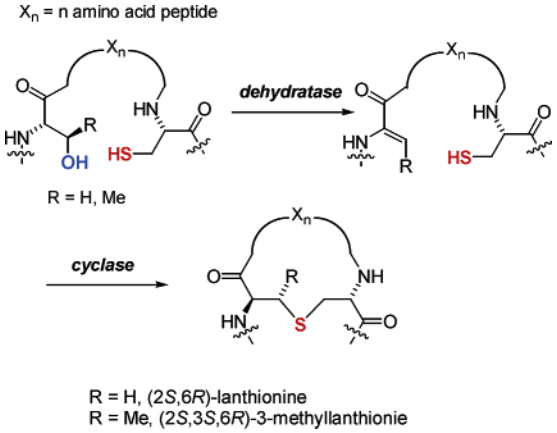

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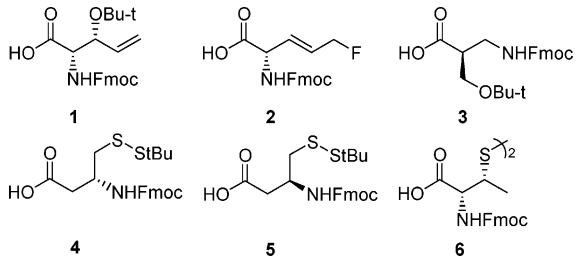
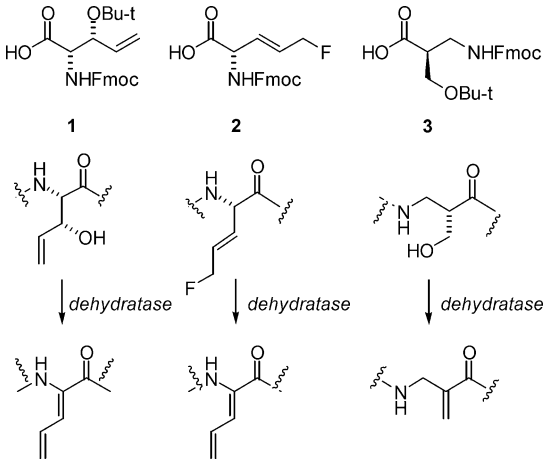
CHART 1



SCHEME 2



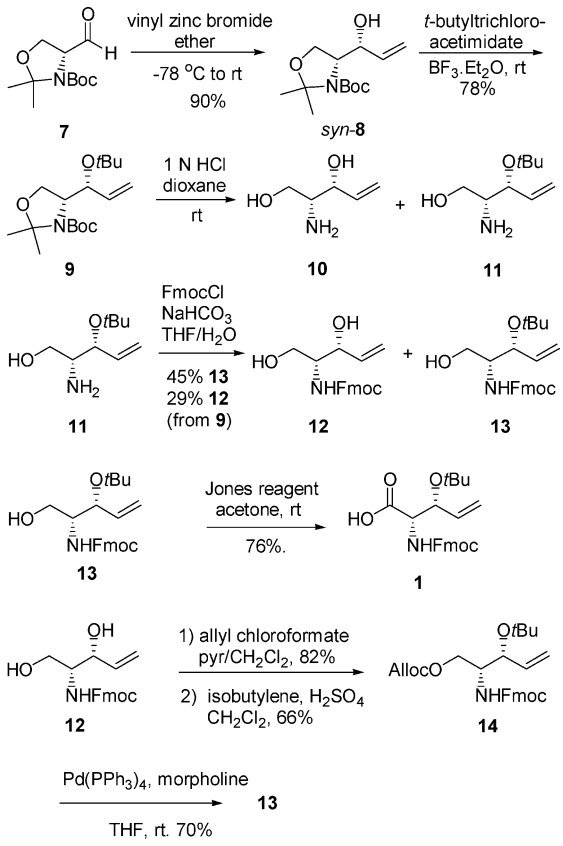
substrate. Since the ribosomal origin of the lantibiotics limits in vivo engineering of their structures to the 20−22 proteinogenic amino acids, a synthetic approach would greatly expand the functional and structural diversity that can be incorporated into lantibiotics via the biosynthetic enzymes. We report here the preparation of a series of nonproteinogenic amino acids designed to investigate this premise. These compounds are the vinylthreonine derivative **1**, fluoroallylglycine **2**, the β-amino acids **3**−**5**, and cysteine derivative **6** (Chart 1). All compounds were appropriately protected for use in Fmoc-based solid-phase peptide synthesis (SPPS).

Once incorporated into the peptide substrate for the dehydratase, amino acids **1**−**3** were envisioned as possible Ser or Thr analogues that would serve to further investigate the substrate specificity of the dehydration reaction (Scheme 2). Similarly, the β^3-homocysteines **4** and **5** and 3-methylcysteine **6** were anticipated as potential alternative nucleophiles for the cyclase catalyzed Michael-type addition. In the case of **6** this would provide (2*R*,3*R*,6*S*)-3-methyllanthionine instead of (2*S*,3*S*,6*R*)-3-methyllanthionine, which has been found in all lantibiotics characterized to date.[3]

Results and Discussion

Synthesis of Vinylthreonine 1, (*E*)-2-Amino-5-fluoro-pent-3-enoic Acid 2, and β^2-Homoserine 3. A number of elegant approaches have been described for the asymmetric synthesis of various β-hydroxy α-amino acids, but the previous methods for the asymmetric synthesis of derivatives of vinylthreonine produced moderate yields and/or stereoselectivity and utilized protecting groups that are not directly amenable for SPPS.[5] Hence, we explored alternative routes toward the synthesis of **1**. Protected amino acids **1** and **2** were both accessed from the D-Garner aldehyde[6] **7**. Vinyllithium was combined with anhydrous zinc bromide in diethyl ether followed by addition of aldehyde **7** to produce alcohol **8** as a solid with 5:1 *syn*-diastereoselectivity in 90% yield (Scheme 3).[7c] Recrystallization of the crude reaction mixture produced *syn*-**8** in 24:1 dr. The recrystallized allylic alcohol was converted to the corresponding *tert*-butyl ether **9** in 78% yield by treatment with *tert*-butyl trichloroacetimidate and a catalytic amount of boron trifluoride etherate in cyclohexane at room temperature. Initial attempts to deprotect the isopropylidene and Boc groups of compound **9** with 10% HCl, followed by protection of the resulting free amino group with FmocCl, afforded compound **13** in low yield (32%). Byproduct **10** resulting from cleavage of the *tert*-butyl ether was also formed in the deprotection step. A survey of a series of reaction conditions showed that treatment with 1 N HCl−dioxane at room temperature for 24 h maximized the desired product, although significant amounts of **10** were still formed. The crude mixture of

SCHEME 3



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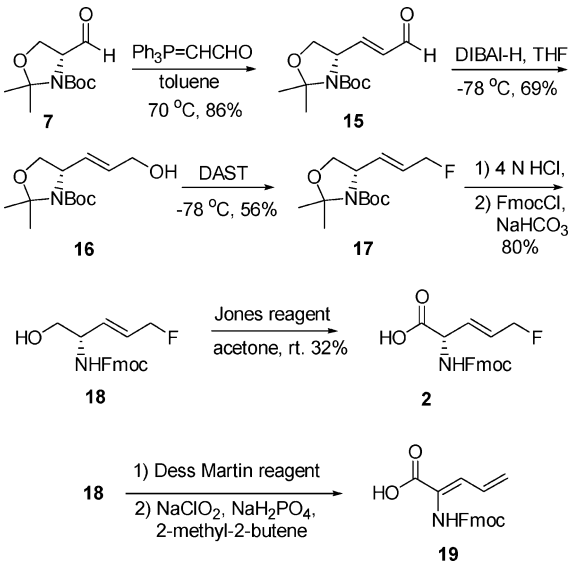
SCHEME 4

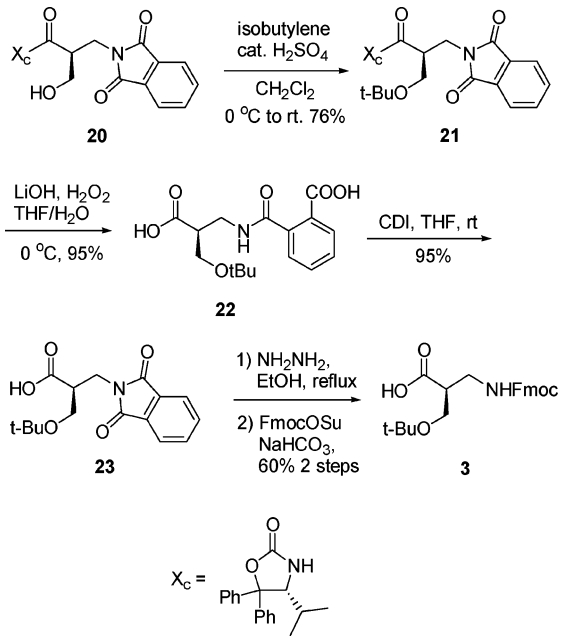


compounds **10** and **11** was treated with FmocCl and $NaHCO_3$ to provide compound **13** in 45% yield (2 steps), along with 29% (2 steps) of compound **12**. Compounds **12** and **13** were separated by silica gel chromatography. To increase the overall yield, compound **12** was transformed into **13** in three steps (Scheme 3). Subsequent oxidization of alcohol **13** with Jones reagent afforded the protected target amino acid **1** in 76% yield.

For the preparation of fluorinated amino acid **2**, the (*E*)-α,β-unsaturated aldehyde **15** was prepared by Wittig reaction of aldehyde **7** with (triphenyl-phosphanylidene)-acetaldehyde in 86% yield (Scheme 4).[8] DIBAL-H reduction of aldehyde **15** provided allylic alcohol **16** in 69% yield. With this key intermediate in hand we turned our attention to the introduction of fluorine. A wide variety of fluorinating reagents are available.[9] We opted for the use of diethylaminosulfur trifluoride (DAST), as it is a highly effective reagent for the direct, one-step, and high-yielding conversion of alcohols into fluorides under mild conditions. Treatment of allylic alcohol **16** with DAST afforded fluorinated compound **17** in 56% yield. The moderate yield is probably due to competitive S_N2' reaction,[10] although this product was not isolated. Deprotection of the isopropylidene and Boc groups of compound **17** with 4 N HCl−dioxane, followed by protection of the free amino group with FmocCl and $NaHCO_3$, afforded compound **18** in 80% yield (2 steps). Oxidization of **18** with Jones reagent afforded the target amino acid **2** in low yield 32%. Attempts to improve the yield using either PDC or the Dess−Martin reagent failed to give the desired target compound **2**. Under the latter conditions, the conjugated product **19** was formed instead of protected amino acid **2**.

For the preparation of Fmoc-β^2-homoserine(But)-OH **3**, the common approach of conjugate addition of nitrogen nucleophiles to α,β-unsaturated carboxylic acid derivatives is not practical. Several years ago, Seebach and co-workers developed an asymmetric synthesis method to access β^2-homoserine derivatives.[11] In this method, a

SCHEME 5



modified Evans auxiliary was used and excellent stereoselectivity was obtained. We applied this methodology to prepare the β^2-homoserine derivative **20**. The hydroxyl group of compound **20** was protected as the *tert*-butyl ether typically used for Fmoc-based solid-phase peptide synthesis in the presence of isobutylene and catalytic H_2SO_4 in CH_2Cl_2. Removal of the chiral auxiliary from compound **21** with LiOH and H_2O_2 proceeded smoothly (Scheme 5), but as previously observed,[12] the phthaloyl group is not stable under the reaction conditions and ring opening took place to give compound **22** in 95% yield. Rather than opting for other deprotection conditions that leave the phthaloyl group intact but that lead to an erosion in er,[11c] we chose to reinstall the phthaloyl group with CDI in THF[12] to afford compound **23** in 95% yield. Subsequent hydrazinolysis of the phthaloyl group proceeded smoothly, and the product mixture was carried on without further purification. Protection of the resulting amino group with FmocOSu and $NaHCO_3$ in THF and H_2O afforded amino acid **3** in 60% yield (2 steps).

Synthesis of β^3-Homocysteines 4 and 5 and (2*R*,3*R*)-Methylcysteine 6. Since the stereochemical requirement for acceptance of β-amino acids by the lantibiotic synthases is unknown, both enantiomers of β^3-homocysteine were prepared. The preparation of the (*R*)-isomer followed the Arndt−Eistert homologation protocol developed by Seebach and co-workers[13a] using the *S*-*tert*-butyl disulfide protecting group for the thiol functionality of L-Fmoc-cysteine (Scheme 6). After formation of diazoketone **24**, silver-mediated Wolf rearrange-

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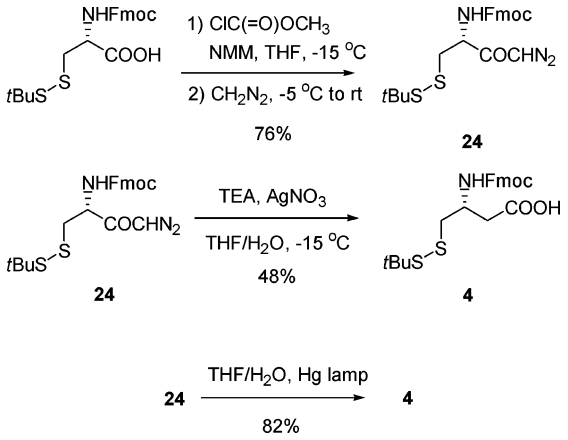
SCHEME 6

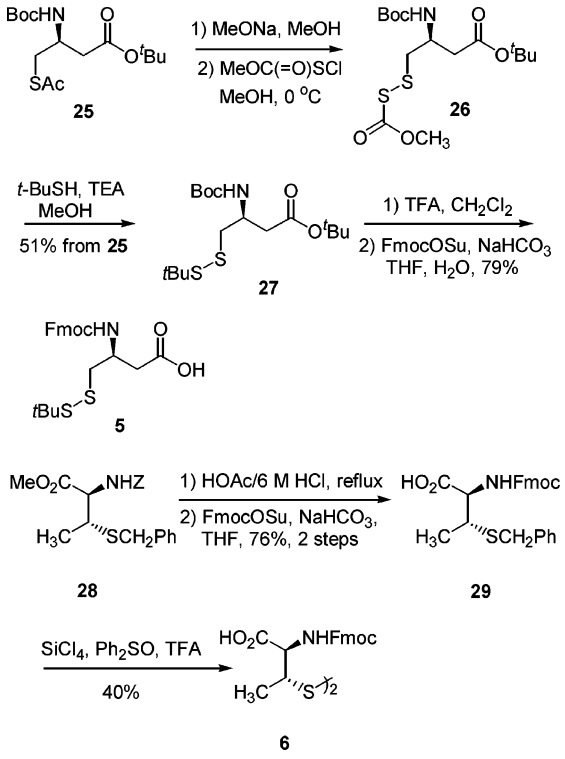


ment[14] resulted in the desired product but in disappointing yield (48%). However, when the rearrangement was induced by photolysis, the protected β^3-homocysteine derivative **4** was obtained in excellent yield (82%). This protected β^3-amino acid can be incorporated into the N-terminal position of peptides by Fmoc-based SPPS and provides, after global deprotection, a disulfide-masked N-terminal thiol that can be used immediately for expressed protein ligation[4,15] to provide the desired 51-mer substrate analogues for LctM.

The same protocol can in principle be used for the preparation of (*S*)-β^3-homocysteine, but this route suffers from the high cost of Fmoc-D-Cys(S*t*Bu) (1 g, $225, BACHEM 2005 catalog). Hence an alternative route was developed starting from L-aspartic acid. The advanced intermediate **25** was obtained in three steps[16] from commercially available *N*-α-Boc-L-aspartic acid *β*-*tert*-butyl ester *N*-hydroxysuccinimide ester (Boc-Asp(O*t*Bu)-OSu, Scheme 7). Treatment of **25** with sodium methoxide in methanol quantitatively yielded the corresponding thiol,[17] which was converted to intermediate **26** (Scheme 7). Without further purification, treatment of activated compound **26** with *tert*-butylthiol in the presence of triethylamine gave the *tert*-butyl disulfide **27** in 51% yield from **25**. Subsequent removal of the Boc group with TFA in methylene chloride followed by protection of the primary amine using Fmoc-OSu yielded the target compound **5** in 79% yield.

For the preparation of disulfide **6**, benzyl-protected methylcysteine derivative **28** was prepared from L-threonine using previously developed aziridine methodology.[18] Acidic removal of both the carboxybenzyl group and hydrolysis of the methyl ester, followed by protection of the primary amine with Fmoc, provided compound **29** in

SCHEME 7



76% yield (Scheme 7). The benzyl group was subsequently removed using silicon tetrachloride in the presence of diphenylsulfoxide and trifluoro acetic acid[19] to provide the desired (2*R*,3*R*)-methylcystine for immediate use in solid-phase peptide synthesis[19] and subsequent expressed protein ligation.

In summary, nonproteinogenic amino acids **1**−**6** were prepared using stereoselective syntheses. The incorporation of these amino acids into the 51-mer peptide substrate for lacticin 481 synthase is currently in progress.

Experimental Section

(4*R*,1′*R*)-4-(1-Hydroxy-allyl)-2,2-dimethyl-oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (*syn*-8). MeLi (1.6 M in Et_2O, 42.36 mL, 67.77 mmol) was added to a solution of tetravinyltin (3.05 mL, 16.94 mmol) in Et_2O (127 mL) at 0 °C and the mixture was stirred for 15 min at the same temperature. Then $ZnBr_2$ (15.24 g, 67.77 mmol) was added to the mixture and stirred for 1 h at room temperature. The resulting solution of vinyl zinc bromide in Et_2O was slowly added to a suspension of **7** (3.88 g, 16.94 mmol) and $ZnBr_2$ (3.82 g, 16.94 mmol) in Et_2O (43 mL) at −78 °C. Then the reaction mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was cooled to 0 °C, and saturated aqueous NH_4Cl was added. The organic layer was separated and the aqueous layer was extracted with ether (40 mL × 3). The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 3:1, R_f = 0.3) to give a mixture of *syn* and *anti* **8** (*syn*:*anti* = 5:1) (3.90 g, 90%) as a white solid. Recrystallization of the solid from a solution of hexane/EtOAc (5:1) gave *syn*-**8** (2.89 g, 74 %) as a white solid: mp 80−81 °C (lit. 80−80.5 °C); $[\alpha]^{20}_D$ = 47.07 (*c* 1.25, $CHCl_3$) (lit. 48.9 (c 1.25, $CHCl_3$)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80 (m, 1H), 5.32 (d, J = 17.2 Hz, 1H), 5.21 (d, J =

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10.0 Hz, 1H), 4.34 (br, 1H), 4.20 (br, 1H), 3.96 (m, 1H), 3.91−3.87 (m, 2H), 1.56 (s, 3H), 1.48 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.1, 137.7, 117.9, 94.3, 81.4, 76.1, 64.5, 61.8, 28.3, 27.1, 24.2; MS *m/z* 258 ($M^+ + H^+$, 0.24), 202 (5.9), 144 (9.1), 100 (27.2), 57 (100).

(4*R*,1′*R*)-4-(1-*tert*-Butoxy-allyl)-2,2-dimethyl-oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (9). To a solution of *syn*-**8** (2.60 g, 10.12 mmol) in cyclohexane (20 mL) was added *tert*-butyl-2,2,2-trichloroacetimide (4.09 mL, 23.16 mmol), followed by $BF_3{\cdot}Et_2O$ (202 μL) at room temperature. After stirring for 2.5 days, the reaction was quenched with $NaHCO_3$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 10:1, R_f = 0.4) to give product **9** (1.341 g) as a clear oil. Elution of the column with hexane/EtOAc 2:1 recovered reactant *syn*-**8** (1.993 g). The recovered compound *syn*-**8** was dissolved in cyclohexane (15.5 mL) again. *tert*-Butyl-2,2,2-trichloroacetimide (2.74 mL) and $BF_3{\cdot}Et_2O$ (155 uL) were added to the reaction mixture. After stirring for 2.5 days at room temperature, the reaction was quenched with $NaHCO_3$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 10:1) to give product **9** (523 mg) as a clear oil and recovered reactant *syn*-**8** (1.257 g) (hexane/EtOAc = 2:1). The recovered compound *syn*-**8** was dissolved in cyclohexane (8 mL) once more. *tert*-Butyl-2,2,2-trichloroacetimide (2.2 mL) and $BF_3{\cdot}Et_2O$ (83 uL) were added to the reaction mixture. After stirring for 2.5 days at room temperature, the reaction was quenched with $NaHCO_3$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 12:1, R_f = 0.3) to give product **9** (605 mg) as a clear oil. The overall product **9** from this procedure was 3.121 g (yield 78%): $[\alpha]^{20}_D$ = 43.86 (*c* 0.93, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.85 (m, 1H), 5.23−5.13 (m, 2H), 4.51 and 4.38 (t, *J* = 5.6 Hz, 1H), 4.06 (ddd, *J* = 17.2, 9.6, 1.6 Hz, 1H), 3.93 and 3.81 (m, 1H), 3.88 (dd, *J* = 8.8, 6.4 Hz, 1H), 1.53−1.39 (m, 15 H), 1.17 and 1.14 (s, 9H); note that this compound exists as different rotamers at 25 °C; ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.6 and 152.1, 137.3 and 137.1, 116.7 and 116.4, 94.6 and 93.9, 79.9 and 79.8, 74.5 and 74.3, 71.4 and 70.6, 63.6 and 63.3, 61.1 and 60.5, 28.7, 28.4, 26.4 and 25.5, 24.4 and 23.0; IR (thin film, cm^{-1}) 3076, 1704, 1694, 1642; MS *m/z* (ESI) 336.4 ($M^+ + Na^+$), 314.5 ($M^+ + H^+$). Anal. Calcd for $C_{17}H_{31}NO_4$: C, 65.14; H, 9.97; N, 4.47. Found: C, 65.07; H, 10.12; N, 4.63

(2*R*,3*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-*tert*-butyl-pent-4-enoic Acid (13). Method A. A solution of compound **9** (107 mg, 0.342 mmol) in MeOH (6 mL) and aqueous 10% HCl (3 mL) was heated to 40 °C. After stirring for 3 h, the reaction mixture was diluted with CH_2Cl_2. The organic layers were separated, and the aqueous layer was extracted with EtOAc (20 mL × 3). The combined organic layers were washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated to give crude amino alcohol **11** (38 mg) as a clear oil, which was used without further purification. The crude amino alcohol was dissolved in THF/H_2O (2:1, 3 mL). FmocCl (72 mg, 0.257 mmol) was added, followed by $NaHCO_3$ (42 mg, 0.5 mmol) at room temperature. After stirring for 24 h, the reaction mixture was diluted with ether. The organic layers were separated and the aqueous layer was extracted with ether (20 mL × 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 3:1, R_f = 0.24) to give compound **13** (43 mg, 32%, two steps) as a foam.

Method B. Compound **9** (1.785 g, 5.702 mmol) was dissolved in 1 N HCl−dioxane (60 mL) at room temperature. The reaction mixture was stirred overnight. Volatiles were removed, the residue was diluted with THF and concentrated again. The residue was dissolved in THF/H_2O (2:1, 75 mL). FmocCl (2.246 g, 8.017 mmol) was added, followed by $NaHCO_3$ (1.106 g, 13.167 mmol) at room temperature. After stirring for 24 h, the reaction mixture was diluted with EtOAc. The organic layers were separated and the aqueous layer was extracted with ether (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 3:1) to give compound **13** (1.020 g, 45%, two steps, R_f = 0.24) as a foam and compound **12** (551 mg, 28.5%, two steps, R_f = 0.1) as a solid.

Compound **13**: $[\alpha]^{20}_D$ = −3.72 (*c* 0.835, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.60 (d, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.32 (t, *J* = 7.6 Hz, 2H), 5.89−5.85 (m, 1H), 5.29 (d, *J* = 17.2 Hz, 1H), 5.26 (m, 1H), 5.18 (d, *J* = 10.8 Hz, 1H), 4.44 (dd, *J* = 10.4, 6.8 Hz, 1H), 4.36 (dd, *J* = 10.4, 6.8 Hz, 1H), 4.28−4.22 (m, 2H), 3.71 (br, 3H), 2.71 (br, 1H), 1.22 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.9, 144.1, 141.5, 139.0, 127.9, 127.3, 125.3, 120.2, 116.6, 75.4, 72.6, 67.0, 63.3, 56.6, 47.5, 28.8; IR (thin film) 3437, 3377, 1713, 1605, 1505 cm^{-1}; MS *m/z* (ESI) 418 ($M^+ + Na^+$), 396 ($M^+ + H^+$); HRMS calcd for $C_{24}H_{29}NO_4$ ($M^+ + Na^+$) 418.1994, found 418.2010.

Compound **12**: mp 79−82 °C; $[\alpha]^{20}_D$ = 3.06 (*c* 0.82, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.58 (d, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.30 (t, *J* = 7.2 Hz, 2H), 5.91−5.84 (m, 1H), 5.46 (d, *J* = 8.4 Hz, 1H), 5.35 (d, *J* = 17.2 Hz, 1H), 5.22 (d, *J* = 10.4 Hz, 1H), 4.46−4.42 (m, 2H), 4.36 (m, 1H), 4.20 (t, *J* = 6.8 Hz, 1H), 3.81 (br, 2H), 3.74−3.73 (m, 1H), 3.14 (br, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.2, 144.0, 141.5, 137.6, 128.0, 127.3, 125.3, 120.2, 116.8, 72.8, 67.1, 63.9, 55.7, 47.4; IR (thin film) 3400, 3067, 1694 cm^{-1}; MS *m/z* (ESI) 340.1 ($M^+ + H^+$); HRMS calcd for $C_{20}H_{21}NO_4$ ($M^+ + Na^+$) 362.1368, found 362.1348.

Carbonic Acid Allyl Ester (2*S*,3*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-pent-4-enyl Ester (12a). To a solution of compound **12** (100 mg, 0.295 mmol) in CH_2Cl_2 (4 mL) and dry pyridine (72 μL, 3.0 equiv) was added allyl chloroformate (57 μL) at 0 °C. Then the reaction was warmed to room temperature and stirred for 24 h. Water was added, the organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 2:1, R_f = 0.4) to give compound **12a** (102 mg, 82%) as a white solid: mp 102−105 °C; $[\alpha]^{20}_D$ = 5.61 (*c* 0.425, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.57 (d, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.31 (td, *J* = 7.6, 0.8 Hz, 2H), 5.96−5.86 (m, 2H), 5.36 (m, 2H), 5.29−5.24 (m, 3H), 4.63 (d, *J* = 6.0 Hz, 2H), 4.45−4.34 (m, 3H), 4.30−4.20 (m, 3H), 3.98 (m, 1H), 2.51 (d, *J* = 3.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.7, 155.3, 144.0, 141.5, 137.0, 131.5, 128.0, 127.3, 125.3, 120.2, 119.5, 117.3, 71.0, 69.1, 67.2, 66.8, 53.8, 47.4; IR (thin film) 3400, 3067, 1747, 1723, 1701, 1522 cm^{-1}; MS *m/z* (ESI) 446.2 ($M^+ + Na^+$), 430.2 ($M^+ + Li^+$), 424.2 ($M^+ + H^+$); HRMS calcd for $C_{24}H_{26}NO_6$ ($M^+ + H^+$) 424.1760, found 424.1761.

Carbonic Acid Allyl Ester (2*S*,3*R*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-pent-4-enyl Ester (14). To a solution of compound **12a** (54 mg, 0.128 mmol) in CH_2Cl_2 (2 mL) was added a catalytic amount H_2SO_4 (5 μL) at 0 °C. Then isobutylene was bubbled through the solution for 15 min. The reaction was sealed and stirred overnight. The reaction mixture was diluted with ethyl acetate and washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 4:1, R_f = 0.6) to give compound **14** (40 mg, 66%): $[\alpha]^{20}_D$ = −4.20 (*c* 0.58, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.2 Hz, 2H), 7.58 (d, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 2H), 7.31 (td, *J* = 7.6, 3.2 Hz, 2H), 5.91−5.80 (m, 2H), 5.35 (dd, *J* = 16.8, 1.2 Hz, 1H), 5.28 (dt, *J* = 12.9 Hz, 1.2 Hz, 1H), 5.26 (dd, *J* = 10.4, 0.8 Hz, 1H), 5.17 (dt, *J* = 10.4, 1.2 Hz, 1H), 5.12 (d, *J* = 8.8 Hz, 1H), 4.63 (dt, *J* = 6.0 Hz, 1.2 Hz, 2H), 4.44−4.34 (m, 2H), 4.24−4.17 (m, 4H), 3.94−3.93 (m, 1H), 1.19 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 154.7, 143.9, 141.3, 138.4, 131.4, 127.7,

127.0, 125.1, 120.0, 119.1, 116.6, 74.9, 70.4, 68.6, 66.8, 66.3, 54.1, 47.2, 28.5; IR (thin film) 3424, 3339, 3068, 1748, 1731, 1715, 1651 cm^{-1}; MS *m/z* (ESI) 502 ($M^+ + Na^+$), 480 ($M^+ + H^+$); HRMS calcd for $C_{28}H_{34}NO_6$ ($M^+ + H^+$) 480.2386, found 480.2362.

(2*R*,3*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-*tert*-butyl-pent-4-enoic Acid (13). To a solution of compound **14** (15 mg, 0.031 mmol) and $(Ph_3P)_4Pd$ (2 mg) in THF (0.5 mL) was added morpholine (3 μL, 0.037 mmol) at room temperature under N_2. The reaction mixture was stirred for 30 min. Ethyl acetate was added, the resulting mixture was washed with 1 N HCl, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with saturated $NaHCO_3$, dried over Na_2SO_4, concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 3:1, $R_f = 0.2$) to give compound **13** (8 mg, 70%) as a foam.

(2*S*,3*R*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-pent-4-enoic Acid (1). To a solution of compound **13** (0.79 g, 2.01 mmol) in acetone (56 mL) was added Jones reagent (1.0 M in H_2O, 6.02 mL) at 0 °C. After stirring for 1 h, the reaction mixture was warmed to room temperature and stirred for an additional 5 h. Then the reaction was quenched with 2-propanol (1.2 mL). After the resulting mixture was stirred for 2 h, water was added. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by preparative TLC (MeOH/MeOH = 20:1, $R_f = 0.1$) to give amino acid **1** (624 mg, 76%) as a foam: mp 64 °C (foam); $[\alpha]^{20}_D = 9.12$ (*c* 1.05, MeOH); ^1H NMR (400 MHz, MeOH-d_4) δ 7.78 (d, $J = 7.6$ Hz, 2H), 7.65 (m, 2H), 7.38 (t, $J = 7.2$ Hz, 2H), 7.29 (td, $J = 7.2$, 0.8 Hz, 2H), 6.62 (d, $J = 9.6$ Hz, 1H), 5.91−5.85 (m, 1H), 5.34 (dd, $J = 17.2$, 1.2 Hz, 1H), 5.16 (dd, $J = 10.8$, 1.2 Hz, 1H), 4.62 (m, 1H), 4.36−4.28 (m, 2H), 4.24−4.20 (m, 2H), 1.18 (s, 9H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 174.0, 158.6, 145.3, 145.1, 142.6, 140.2, 128.8, 128.2, 126.3, 126.0, 120.9, 116.8, 76.0, 74.2, 68.2, 60.7, 28.8; IR (thin film) 3429, 3294, 3073, 1724, 1715, 1607 cm^{-1}; MS *m/z* (ESI) 410.2 ($M^+ + H^+$); HRMS calcd for $C_{24}H_{27}NO_5$ ($M^+ + Na^+$) 432.1787, found 432.1796.

(*S*)-2,2-Dimethyl-4-((1*E*)-3-oxo-propenyl)-oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (15). Triphenylphosphoranylidene-acetaldehyde (320 mg, 1 mmol) was added to a solution of D-Garner aldehyde **7** (229 mg, 1 mmol) in dry toluene (12 mL). The reaction mixture was heated to 70 °C. After stirring for 24 h, the solution was concentrated and the residue was purified by column chromatography on nutralized silica gel (hexane/EtOAc/Et_3N = 70/20/1, $R_f = 0.3$) to give α,β-unsaturated aldehyde **15** (220 mg, 86%) as an ample oil: ^1H NMR (400 MHz, $CDCl_3$) δ 9.55 (d, $J = 17.2$ Hz, 1H), 6.71 (td, $J = 15.6$, 5.7 Hz, 1H), 6.16 (m, 1H), 4.66−4.51 (m, 1H), 4.15−4.05 (m, 1H), 3.85−3.82 (m, 1H), 1.63−1.40 (m, 15H).

(*S*)-((1*E*)-3-Hydroxy-propenyl)-2,2-dimethyl-oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (16). To a solution of aldehyde **15** (220 mg, 0.863 mmol) in dry THF (4 mL) was added DIBAl-H (1.2 mL, 1.2 mmol, 1.3 equiv, 1.0 M in hexanes) within 10 min at −75 °C. After the reaction mixture was stirred at the same temperature for 2 h, it was slowly warmed to −40 °C and stirred for 1 h. Then the resulting reaction mixture was cooled to −75 °C, and 0.2 mL of MeOH was slowly added so as to keep the internal temperature below −70 °C. The resulting mixture was poured into 0.1 M HCl, the organic layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 2:1, $R_f = 0.3$) to give alcohol **16** (154 mg, 69%) as a yellow oil: ^1H NMR (400 MHz, $CDCl_3$) δ 5.78−5.64 (m, 2H), 4.40−4.28 (m, 1H), 4.13 (t, $J = 4.8$ Hz, 2H), 4.02 (dd, $J = 8.8$, 6.0 Hz, 1H), 3.72 (dd, $J = 8.8$, 2.0 Hz, 1H), 1.59−1.41 (m, 15H); IR (thin film) 3435, 1696 cm^{-1}.

(*S*)-((1*E*)-3-Fluoro-propenyl)-2,2-dimethyl-oxazolidine-3-carboxylic Acid *tert*-Butyl Ester (17). To a cooled solution (−78 °C) of DAST (0.8 mL, 6 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise a solution of alcohol **16** (514 mg, 2 mmol) in dry CH_2Cl_2 (20 mL) over a period of 1 h. After the reaction mixture was stirred at −78 °C for 3 h, water was added. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 7:1, $R_f = 0.3$) to give compound **17** (292 mg, 56%) as a yellow oil: $[\alpha]^{20}_D = 4.53$ (*c* 0.99, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.78 (m, 2H), 4.85 (dd, $J = 48.6$, 4.4 Hz, 2H), 4.44−4.30 (m, 1H), 4.04 (dd, $J = 8.4$, 6.8 Hz, 1H), 3.75 (d, $J = 8.4$ Hz, 1H), 1.61−1.38 (m, 15H); ^{13}C NMR (100 MHz, acetone-d_6) δ 152.3, 135.0 (d, $J = 11.4$ Hz), 127.2 (d, $J = 16.7$ Hz), 94.3, 83.2 (d, $J = 160.0$ Hz), 79.7, 68.5, 59.2, 28.5, 26.8, 23.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ −212.9 and −214.1 (t, $J = 48.6$ Hz), note that this compound exists as different rotamers at 25 °C; IR (thin film) 2981, 1694, 1386, 1367, 1100 cm^{-1}; MS *m/z* (ESI) 282.3 ($M^+ + Na^+$), 260.2 ($M^+ + H^+$); HRMS calcd for $C_{13}H_{22}NO_3F$ ($M^+ + Na^+$) 282.1481, found 282.1490.

(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-5-fluoro-pent-3-enoic Acid (18). Compound **17** (287 mg, 1.108 mmol) was dissolved in 4 N HCl−dioxane (2.5 mL) at room temperature. After stirring for 45 min, the reaction mixture was concentrated. The residue was dissolved in THF and evaporated again to give crude amino alcohol, which was used without further purification. The crude amino alcohol was dissolved in dioxane/H_2O (1:1, 16 mL). FmocCl (434 mg, 1.551 mmol) was added, followed by $NaHCO_3$ (215 mg, 2.659 mmol). The reaction mixture was stirred overnight. Ethyl acetate was added, the organic 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, filtered, and concentrated. The residue was purified by column chromatography on silica gel (hexane/EtOAc = 1:1, $R_f = 0.3$) to give compound **18** (302 mg, 80%) as a white solid: mp 137−139 °C; $[\alpha]^{20}_D = -14.94$ (*c* 0.29, MeOH); ^1H NMR (400 MHz, MeOH-d_4) δ 7.77 (d, $J = 7.6$ Hz, 2H), 7.64 (d, $J = 6.8$ Hz, 2H), 7.37 (t, $J = 7.6$ Hz, 2H), 7.29 (td, $J = 7.6$, 1.2 Hz, 2H), 5.81 (br, 2H), 4.82 (dd, $J = 47.6$, 3.2 Hz, 2H), 4.42−4.35 (m, 2H), 4.20−4.17 (m, 2H), 3.54−3.52 (m, 2H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 157.2, 144.1 (d, $J = 10.9$ Hz), 141.4, 131.9 (d, $J = 10.9$ Hz), 127.6, 126.9, 126.6 (d, $J = 17.1$ Hz), 125.0, 119.7, 82.4 (d, $J = 162.6$ Hz), 66.5, 63.7 (d, $J = 2.3$ Hz), 54.5, 47.3; ^{19}F NMR (376 MHz, MeOH-d_4) δ −215.5 (t, $J = 47.6$ Hz); IR (thin film) 3315, 1684, 1589, 1542 cm^{-1}; MS *m/z* (ESI) 364.1 ($M^+ + Na^+$), 348.2 ($M^+ + Li^+$), 342.1 ($M^+ + H^+$); HRMS calcd for $C_{20}H_{20}NO_3F$ ($M^+ + Na^+$) 364.1325, found 364.1328. Anal. Calcd for $C_{20}H_{20}NO_3F$: C, 70.37; H, 5.91; N, 4.10; Found: C, 70.06; H, 5.96; N, 4.18.

(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-5-fluoro-pent-3-enoic Acid (2). Jones Oxidation. To a solution of compound **18** (58 mg, 0.170 mmol) in acetone (4.7 mL) was added Jones reagent (1.0 M, 0.51 mL, 0.510 mmol) at 0 °C. Then the reaction was warmed to room temperature and stirred overnight. The reaction was quenched with 0.5 mL of 2-propanol. After the resulting mixture was stirred for 2 h, water was added. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 4:1, $R_f = 0.4$) to give compound **2** (19 mg, 32%) as a white solid: mp 129−132 °C; $[\alpha]^{20}_D = -5.67$ (*c* 0.35, $CHCl_3$); ^1H NMR (400 MHz, MeOH-d_4) δ 7.76 (d, $J = 7.6$ Hz, 2H), 7.64 (t, $J = 6.4$ Hz, 2H), 7.36 (t, $J = 7.6$ Hz, 2H), 7.28 (t, $J = 7.6$ Hz, 2H), 6.01 (dt, $J = 15.6$ Hz, 4.0 Hz, 1H), 5.86 (m, 1H), 4.82 (dd, $J = 47.2$, 5.6 Hz, 2H), 4.67 (br, 1H), 4.40−4.31 (m, 2H), 4.19 (t, $J = 6.8$ Hz, 1H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 176.4, 156.8, 144.1 (d, $J = 23.5$ Hz), 141.4, 131.6 (d, $J = 11.3$ Hz), 127.6, 127.0, 125.6 (d, $J = 15.9$ Hz), 125.0, 119.7, 82.4 (d,

J = 161.6 Hz), 66.6, 57.6, 47.2; ^{19}F NMR (376 MHz, MeOH-d_4) δ −215.9 (td, J = 47.2, 12.0 Hz); IR (thin film) 3392, 3073, 3035, 1694, 1682, 1586 cm^{-1}; MS *m/z* (ESI) 378.1 ($M^+ + Na^+$), 362.2 ($M^+ + Li^+$); HRMS calcd for $C_{20}H_{18}NO_4F$ ($M^+ + Na^+$) 378.1118, found 378.1124.

2-[(2*S*)-2-*tert*-Butoxymethyl-3-((4*R*)-4-isopropyl-2-oxo-5,5-diphenyl-oxazolidin-3-yl)-3-oxo-propyl]-isoindole-1,3-dione (21). To a solution of compound **20** (631 mg, 1.23 mmol) in CH_2Cl_2 (20 mL) was added a catalytic amount of H_2SO_4 (41 μL). The resulting mixture was cooled to 0 °C, isobutylene was bubbled into the solution for 15 min, and then the reaction mixture was allowed to warm to room temperature and stirred overnight. The mixture was diluted with CH_2Cl_2 and was washed with water and brine successively, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc = 2:1, R_f = 0.5) to give product **21** (532 mg, 76%) as a white solid: mp 151−153 °C; $[\alpha]^{20}_D$ = 128.8 (*c* 0.945, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75−7.73 (m, 2H), 7.69−7.66 (m, 2H), 7.42−7.25 (m, 7H), 7.21−7.12 (m, 3H), 5.49 (d, J = 2.8 Hz, 1H), 4.38−4.33 (m, 1H), 3.96−3.85 (m, 2H), 3.78 (dd, J = 8.8 Hz, 4.4 Hz, 1H), 3.67 (dd, J = 8.8 Hz, 5.6 Hz, 1H), 1.96−1.89 (m, 1H), 1.12 (s, 9H), 0.93 (d, J = 7.2 Hz, 3H), 0.74 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 167.8, 152.5, 142.1, 138.4, 133.7, 131.9, 128.7, 128.33, 128.26, 127.7, 125.8, 125.6, 123.2, 89.0, 73.3, 64.0, 61.4, 43.5, 36.5, 30.2, 27.3, 21.7, 15.8; IR (thin film, cm^{-1}) 3057, 1779, 1716, 1610; MS *m/z* (ESI) 591.2 ($M^+ + Na^+$), 569.6 ($M^+ + H^+$). Anal. Calcd for $C_{34}H_{36}N_2O_6$: C, 71.81; H, 6.38; N,4.93. Found: C, 71.75; H, 6.43; N, 5.06.

(*S*)-*N*-(3-*tert*-Butoxy-2-carboxy-propyl)-phthalamic Acid (22). To a solution of compound **21** (1.32 g, 2.32 mmol) in THF/H_2O (125 mL, 2.4:1) was added H_2O_2 (30% aqueous 955 μL, 0.4 mmol) at 0 °C. After the mixture was stirred for 5 min, LiOH·H_2O (198 mg, 4.65 mmol) was added. The mixture was stirred at the same temperature for 3 h. Na_2SO_3 (1.16 g) was added at 0 °C, and the reaction mixture was stirred for 30 min. THF was evaporated and 16 mL of Et_2O was added. The suspension was stirred for about 15 min and the solid was filtered and washed with 1 mL of 1 N NaOH (16 mL), H_2O (16 mL), Et_2O (10 mL), and pentane (10 mL) successively to give an auxiliary 600 mg (recovery 92%). The filtrate was diluted with EtOAc and the aqueous layer was acidified to pH 2 with 1 M HCl, which was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated to give compound **22** (713 mg, 95%) as a sticky oil: ^{1}H NMR (400 MHz, CD_3OD) δ 7.95 (dt, J = 8.0 Hz, 1H), 7.59 (td, J = 7.6 Hz, 1.2 Hz, 1H), 7.52 (td, J = 7.6 Hz, 1.6 Hz, 1H), 7.42 (dd, J = 7.6 Hz, 1.2 Hz, 1H), 3.72−3.67 (m, 2H), 3.61−3.58 (m, 2H), 3.61−3.58 (m, 2H), 2.95−2.94 (m, 1H), 1.19 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4, 173.0, 169.2, 139.9, 133.0, 131.3, 130.7, 130.5, 128.8, 74.2, 62.1, 46.9, 39.9, 27.7; IR (thin film) 3569−2620 (br), 1772, 1716, 1557; MS *m/z* (ESI) 346.1 ($M^+ + Na^+$), 324.1 ($M^+ + H^+$); HRMS calcd for $C_{16}H_{22}NO_6 + H^+$ 324.1447, found 324.1446.

(*S*)-2-*tert*-Butoxymethyl-3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propionic Acid (23). To a solution of compound **22** (375 mg, 1.162 mmol) in dry THF (23 mL) was added 1,1-carbonyl diimidazole (CDI, 570 mg, 3.486 mmol) at room temperature. The reaction mixture was stirred for 47 h and then was concentrated. The residue was redissolved in 10% $NaHCO_3$ (16 mL). Then the aqueous solution was acidified to pH 1 with 1 N HCl. The resulting precipitate was extracted with EtOAc, and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 12:1, R_f = 0.4) to give product **23** (337 mg, 95%) as a white solid: mp 121−124 °C; $[\alpha]^{20}_D$ = −2.04 (*c* 0.595, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86−7.24 (m, 2H), 7.72−7.70 (m, 2H), 4.09 (dd, J = 14.4 Hz, 6.8 Hz, 1H), 3.95 (dd, J = 14.4 Hz, 8.0 Hz, 1H), 3.62 (d, J = 5.6 Hz, 2H), 3.20−3.16 (m, 1H), 1.12 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9, 168.4, 134.3, 132.2, 123.6, 74.3, 60.6, 44.5, 37.0, 27.4; IR (thin film) 3574, 3472, 3138, 1774, 1715, 1610; MS *m/z* (ESI) 328.1 ($M^+ + Na^+$), 306.1 ($M^+ + H^+$); HRMS calcd for $C_{16}H_{20}NO_5 + H^+$ 306.1341, found 306.1338. Anal. Calcd for $C_{34}H_{36}N_2O_6$: C, 62.94; H, 6.27; N,4.59. Found: C, 62.44; H, 6.15; N, 4.58.

(*S*)-2-*tert*-Butoxymethyl-3-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionic Acid (3). Compound **23** (335 mg, 1.1 mmol) was dissolved in EtOH (13 mL). Hydrazine (140 μL) was added and the reaction mixture was heated to reflux. After being stirred for 30 min under reflux, the reaction was cooled to room temperature and the solvent was evaporated to give a white solid. Then the solid was suspended in CH_2Cl_2, and the solvent was filtered. The organic layer was discarded, and the solid (370 mg) was used to do the next step without purification. The solid was dissolved in THF/H_2O (1/1, 40 mL), the reaction mixture was cooled to 0 °C, and FmocOSu (450 mg, 1.32 mmol) was added, followed by $NaHCO_3$ (914 mg, 11 mmol). After the reaction mixture was stirred overnight, the solvent was removed, the residue was dissolved in 3 mL of 10% $NaHCO_3$, and the resulting solution was washed with Et_2O. The aqueous layer was acidified to pH = 1 with 1 N HCl and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified with silica gel column chromatography (hexane/EtOAc = 2:1 then CH_2Cl_2/MeOH = 16:1, R_f = 0.1) to give product **3** (262 mg, 60%, two steps) as a foam: $[\alpha]^{20}_D$ = 7.32 (*c* 0.68, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 7.2 Hz, 2H), 7.64 (d, J = 8.0 Hz, 2H), 7.38 (t, J = 7.2 Hz, 2H), 7.30 (t, J = 7.2 Hz, 2H), 7.10 (t, J = 5.6 Hz, 1H), 4.33 (d, J = 7.0 Hz, 2H), 4.20 (t, J = 7.0 Hz, 1H), 3.58 (d, J = 5.6 Hz, 2H), 3.41 −3.32 (m, 2H), 2.72 (m, 1H), 1.16 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 158.7, 145.3, 142.6, 128.8, 128.1, 162.2, 120.9, 74.2, 67.7, 61.9, 48.4, 48.0, 41.0, 27.7; IR (thin film, cm^{-1}) 339, 3073, 1718, 1708, 1525; MS *m/z* (ESI) 420.2 ($M^+ + Na^+$), 398.2 ($M^+ + H^+$); HRMS calcd for $C_{23}H_{28}NO_5 + H^+$ 398.1967, found 398.1985.

(*R*)-Diazomethyl Ketone 24. To a solution of FmocCys(S*t*Bu)OH (86 mg, 0.2 mmol) in THF (−15 °C) was added NMM (15.6 μL, 0.2 mmol), followed by the addition of methyl chloroformate (22 μL, 0.2 mmol). The mixture was stirred under a N_2 atmosphere at −15 °C for 2 h. Filtration gave the crude anhydride, which was slowly treated with diazo methane in ether solution at −5 °C. The reaction mixture was allowed to warm to room temperature and left overnight. A couple of drops of HOAc were added to quench the reaction. The mixture was washed with saturated aqueous $NaHCO_3$ (15 mL), NH_4Cl (15 mL), dried over $MgSO_4$, and concentrated under reduced pressure. Purification over silica gel gave the desired product **24** (EtOAc/Hexane = 1:1, R_f = 0.65) as a white solid (74 mg, 76%): $[\alpha]^{20}_D$ = −48.1 (*c* 0.86, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30 (s, 9 H), 3.06 (m, 2H), 4.21 (t, 1 H, J = 6.5 Hz), 4.44−4.52 (m, 3 H), 5.45 (br, 1 H), 5.76 (d, 1 H, J = 8.0), 7.29−7.40 (m, 4H), 7.60 (m, 2H), 7.75 (d, 2 H, J = 7.5 Hz); ^{13}C NMR (125 MHz $CDCl_3$) δ 29.7, 41.9, 47.7, 48.3, 54.8, 57.1, 60.3, 66.8, 119.9, 119.9, 125.0, 127.0, 127.6, 141.2, 141.3, 143.5, 143.6, 155.7, 191.9; IR (thin film, cm^{-1}) 3306, 2959, 2108, 1362; MS *m/z* (ESI) 427.9 ($M^+ - N_2 + H$), 478.0 ($M^+ + Na$); HRMS (ESI^+) calcd for ($M^+ - N_2 + H$) $C_{23}H_{25}N_3NaS_2$ 478.1235, found 478.1219.

***(R)*-4-*tert*-Butyldisulfanyl-3-(9*H*-fluoren-9-ylmethoxycarbonylamino)-butyric Acid (4). Method A.** A mixture of $AgNO_3$ (3.3 mg, 0.02 mmol) and compound **24** (81.1 mg, 0.18 mmol) was dissolved in 9.6 mL of THF/H_2O (5:1) with the exclusion of light at −15 °C, followed by the addition of TEA (78 μL, 0.52 mmol). The mixture was stirred overnight at −15 °C, and the reaction mixture was acidified by addition of 10% aqueous citric acid (2 mL). The reaction mixture was diluted with EtOAc (30 mL), washed with brine, and concentrated. Purification over silica gel gave the desired product **4** (CH_3OH/DCM = 1:10, R_f = 0.3) as a foamlike white solid.

Method B. A solution of diazoketone **24** (74 mg, 0.17 mmol) in 9.6 mL of degassed THF/H_2O (5:1) was irradiated with long wavelength UV light (Hg lamp) under nitrogen for 3 h. The

reaction mixture was diluted with EtOAc (30 mL), washed with brine and concentrated. Purification over silica gel gave the desired product **4** (CH_3OH/DCM = 1:10, R_f = 0.3) as a foamlike white solid (56 mg, 82%): $[\alpha]^{20}_D$ = −8.80 (*c* 0.8, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.32 (s, 9 H), 2.77−3.2.99 (m, 4 H), 4.22−4.40 (m, 4 H), 5.45(br, 1 H), 7.26−7.41 (m, 4H), 7.41 (d, 2H, *J* = 7.6 Hz), 7.75 (d, 2 H, *J* = 7.6 Hz); ^{13}C NMR (100 MHz $CDCl_3$) δ 29.7, 36.8, 43.5, 47.1, 47.7, 66.9, 119.9, 125.1, 127.0, 127.6, 141.2, 143.8, 155.6, 176.2; IR (thin film, cm^{-1}) 3324, 3066, 2961, 1713, 1517, 1451, 1248; MS *m/z* (ESI) 446.1 (M^+ + H); HRMS (ESI^+) calcd for (M^+ + H) $C_{23}H_{28}NO_4S_2$ 446.1460, found 446.1445.

(*S*)-3-*tert*-Butoxycarbonylamino-4-mercapto-butyric Acid *tert*-Butyl Ester (25a). Compound **25** (261 mg, 0.783 mmol) was dissolved in methanol solution (0.1 M $NaOCH_3$ in 54 mL CH_3OH) and the solution was stirred at room temperature under nitrogen for 15 min. The mixture was acidified with acetic acid and extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried over $MgSO_4$, and concentrated, and the product was dried under reduced pressure to give the crude thiol product **25a**, which was used immediately in the next step reaction without further purification: ^1H NMR (500 MHz, $CDCl_3$) δ 1.43 and 1.42 (2 s, 18 H), 2.48−2.68 (m, 4 H), 4.02 (m, 1H), 5.28 (br, 1 H).

(*S*)-(Methoxycarbonylsulfenyl)-3-*tert*-butoxycarbonylamino-4-mercapto-butyric Acid *tert*-Butyl Ester (26). To a stirred ice-cold solution of methoxycarbonylsulfenyl chloride (0.14 mL, 1.56 mmol) in methanol (5 mL) was added dropwise a solution of the thiol **25a** (0.783 mmol) in methanol (4 mL) that was prepared as described above. The clear solution was stirred for 2 h at 0 °C. Then the methanol was evaporated under reduced pressure and a white crystalline residue **26** was obtained: ^1H NMR (500 MHz, $CDCl_3$) δ 1.43 and 1.49 (2 s, 18 H), 2.61 (m, 1 H), 2.70 (m, 1 H), 2.96−3.08 (m, 2 H), 3.88 (s, 3 H), 4.05 (m, 1H), 5.26 (m, 1 H). Compound **26** was used immediately in the next step without further purification.

(1-*tert*-Butyldisulfanylmethyl-5,5-dimethyl-3-oxo-hexyl)-carbamic Acid *tert*-Butyl Ester (27). To a solution of *tert*-butyl mercaptan (0.17 mL, 0.16 mmol) and TEA (0.1 mL, 0.72 mmol) in methanol (13 mL) was added a solution of compound **26** in methanol (10 mL). The mixture was stirred at room temperature for 20 min and concentrated. The residue was dissolved in EtOAc and washed with water, brine, dried over $MgSO_4$ and concentrated. Purification over silica gel gave desired product **27** (EtOAc/hexane = 1:2, R_f = 0.6) as colorless oil in 51% yield over three steps (from compound **25** to compound **27**): ^1H NMR (500 MHz, $CDCl_3$) δ 1.31 (s, 9 H), 1.39 (s, 9 H), 1.40 (s, 9 H), 2.55−2.62 (m, 2 H), 2.85−2.98 (m, 2 H), 4.10 (m, 1 H), 5.09−5.11 (m, 1 H); ^{13}C NMR (125 MHz $CDCl_3$) δ 27.8, 27.9, 28.1, 28.2, 29.3, 29.5, 29.7, 29.8, 38.1, 44.3, 47.5, 47.8, 79.2, 80.9, 154.8, 170.6; IR (thin film, cm^{-1}) 3366, 2972, 2932, 1717, 1500, 1366, 1248, 1161; HRMS (ESI^+) calcd for (M^+ + H) $C_{17}H_{34}NO_4S_2$ 380.1929, found 380.1927.

4-*tert*-Butyldisulfanyl-3-(9*H*-fluoren-9-ylmethoxycarbonylamino)-butyric Acid *tert*-Butyl Ester (5). Compound **27** (0.105 g, 0.28 mmol) was dissolved in a solution of TFA/CH_2Cl_2 (2 mL/2 mL) and stirred at room temperature for 4 h. After removal of solvent, the residue was dissolved in a solution of THF/H_2O (2 mL/2 mL), followed by the addition of FmocOSu (0.104 g, 0.28 mmol) and $NaHCO_3$ (237 mg, 2.8 mmol). The resulting mixture was stirred overnight and was adjusted to ∼pH 3 with 6 N HCl. The mixture was diluted with EtOAc and the layers were separated. The aqueous phase was extracted further with EtOAc. The combined EtOAc extracts were concentrated. Purification over silica gel gave desired product **5** (CH_3OH/CH_2Cl_2 = 1:10, R_f = 0.3) as a white solid (60 mg, 79%): $[\alpha]^{20}_D$ = +7.17 (*c* 0.8, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 1.33 (s, 9 H), 2.77−3.02 (m, 4 H), 4.20−4.87 (m, 4 H), 5.47−5.49 (m, 1 H), 7.29−7.41 (m, 4H), 7.41 (d, 2H, *J* = 7.6 Hz), 7.75 (d, 2 H, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 29.8, 36.8, 43.4, 47.1, 47.7, 48.2, 66.9, 1199, 125.1, 127.0, 127.6, 141.1, 143.8, 155.6, 176.4; IR (thin film, cm^{-1}) 3323, 3066, 2961, 1713, 1518, 1450, 1248, 1044; MS *m/z* (ESI) 446.1 (M^+ + H); HRMS (ESI^+) calcd for (M^+ + H) $C_{23}H_{28}NO_4S_2$ 446.1460, found 446.1460.

(2*R*,3*R*)-3-Benzylsulfanyl-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-butyric Acid (29). Compound **28** (0.965 g, 2.69 mmol) was dissolved in HOAc (9.5 mL) and HCl (6 M, 9.5 mL). The reaction mixture was refluxed at 100 °C for 9 h and concentrated. The residue was lyophilized to give a white solid. The solid was washed twice with EtOAc. The resulting salt was dissolved in a mixture of THF (9.5 mL) and H_2O (9.5 mL), and to this solution was added FmocOSu (1.0 g, 2.96 mmol), followed by the addition of $NaHCO_3$ (2.26 g, 16.9 mmol). The reaction mixture was stirred at room temperature overnight and extracted with Et_2O. The basic aqueous layer was acidified to pH around 2 with 3 N HCl and extracted times with EtOAc (3 × 100 mL). The combined EtOAc layers were washed with brine, dried over $MgSO_4$, and concentrated. Purification by silica gel chromatography (CH_3OH/CH_2Cl_2 = 1:10, R_f = 0.3) afforded the desired product **29** (0.9015 g, 76%) as a white solid: $[\alpha]^{20}_D$ = +41.2, (*c* 2.0, $CHCl_3$); mp 64−66 °C; ^1H NMR (500 MHz, CD_3OD + $CDCl_3$) δ 1.26 (d, 3 H, *J* = 7.0 Hz), 2.82 (m, 1 H), 3.18 (d, 1 H, *J* = 6.4 Hz), 3.76 (Abq, 2H), 4.23 (t, 1H, *J* = 7.0 Hz), 4.29−4.44 (m, 3H), 7.20−7.39 (m, 9H), 7.64−7.78 (m, 4H); IR (thin film, cm^{-1}) 3307, 3064, 3030, 2967, 2927, 1718, 1513, 1449, 1233; ^{13}C NMR ($CDCl_3$) δ 18.5, 34.8, 41.6, 46.3, 58.1, 66.3, 119.1, 124.3, 126.3, 126.9, 127.6, 128.1, 137.4, 140.5, 143.0, 143.2, 156.4, 172.8; HRMS (ESI^+) calcd for (M^+ + Na) $C_{26}H_{25}NO_4NaS$ 470.1402, found 410.1387.

(2*R*,3*R*)-3-[2-Carboxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-1-methyl-ethyl disulfanyl]-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-butyric Acid (6). $SiCl_4$ (1.6 mL, 13.5 mmol) and Ph_2SO (0.683 g, 3.38 mmol) in 3 mL of TFA were added to compound **29** (0.318 g, 0.677 mmol) at 0 °C. The resulting mixture was stirred for 1 h and diluted with Et_2O (30 mL). The solution was treated with saturated aqueous $NaHCO_3$ (50 mL). The aqueous layer was then acidified to pH ∼1 and extracted with EtOAc (3 × 100 mL). The combined EtOAc layers was dried over $MgSO_4$ and concentrated. Purification by silica gel chromatography (CH_3OH/CH_2Cl_2 = 1:2, R_f = 0.3) gave title compound (97 mg, 40%) as a white solid: $[\alpha]^{20}_D$ = −27.3, (*c* 1.0, $CHCl_3$); mp 180−182 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 1.31 (d, 3 H, *J* = 7.2 Hz), 3.62 (m, 1 H), 4.23 (t, *J* = 7.0 Hz), 4.32−4.65 (m, 3H), 7.20−7.39 (m, 4H), 7.50−7.78 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 17.5, 46.6, 47.6, 58.0, 66.0, 120.2, 124.3, 125.4, 125.5, 126.1, 127.2, 129.6, 129.8, 130.8, 131.2, 131.4, 131.9, 132.6, 140.8, 143.8, 156.1, 171.7; IR (thin film, cm^{-1}) 3306, 3066, 2965, 2926, 1693, 1517, 1449, 1210; LRMS (ESI^+) calcd for (M^+) $C_{38}H_{36}N_2O_8S_2$ 712.2, found 712.9; HRMS (ESI^+) calcd for (M^+ + H) $C_{38}H_{37}N_2O_8S_2$ 713.1991, found 713.1975.

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

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