Stereoconservative Synthesis of Orthogonally Protected γ-Functionalized Amino Acids Using *N*-Tritylserine Derivatives

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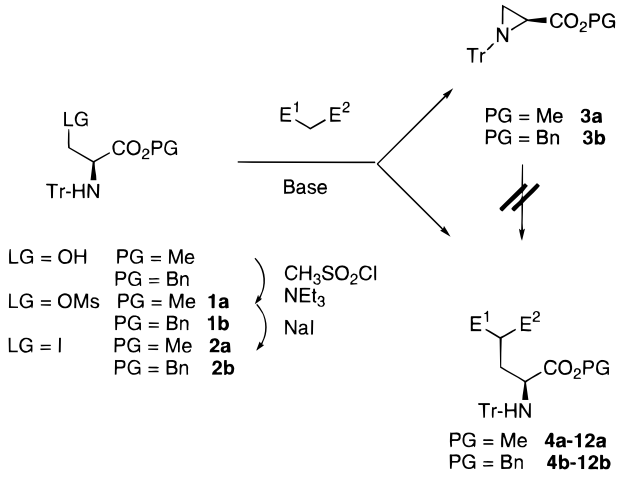
Use of commercial optically pure α-amino acids as a "chiral pool" is a conceptually attractive approach that has known much progress and applications for the elaboration of unusual and non-natural amino acids[1] and many other interesting compounds.[2] Several synthetic equivalents for β-alanyl cation have been developed for this goal. However, the ring-opening of cyclic electrophiles such as serine β-lactone,[3] aziridines,[4] or serine sulfamidate[5] by soft basic nucleophiles such as malonates was unfruitful or difficult.[6] Despite interesting applications with organocuprates,[7] attempted substitution on to β-haloalanines by malonates failed and gave the corresponding dehydroalanine.[8] One way to overcome these difficulties may be to use open-chain electrophilic serine derivatives and to block the amine moiety with an appropriate bulky protecting group such as *N*-(triphenylmethyl)[9] or *N*-(phenylfluorenyl).[10] Very recently (1996), Cherney and Wang demonstrated that the Mitsunobu reaction can be performed efficiently with *N*-trityl and *N*-(phenylfluorenyl)serine esters.[9b] The *N*-trityl group prevents α-proton abstraction by non-nucleophilic bases and protects the α-ester from saponification.[11] Moreover, this group is resistant toward strong bases and most reductive agents but can be easily removed under mildly acidic conditions or by hydrogenolysis. Thus, the well-known functional diversity of malonate compounds could be readily exploited for generating unusual and non-natural amino acids especially in the glutamate series. We report herein on the synthesis of a variety of orthogonally protected α-amino acids, functionalized at the γ-position, using *N*-tritylserine derivatives.

Scheme 1. Nucleophilic Substitution of the Leaving Group (LG) by a Selection of Malonate-Related Nucleophiles



The nucleophilic displacement on mesylate **1a** and iodides **2a** and **2b** by malonate related anions was investigated. The starting compounds **1a** and **2a/b** were prepared in almost quantitative yields from the easily available *N*-tritylserine methyl and benzyl esters (Scheme 1). These reagents can be stored at −18 °C for several months, without detectable degradation.[12] Initially, we studied the reactivity of these compounds toward sodium dimethylmalonate under anhydrous conditions (THF−HMPA). Sterically hindered mesylate **1a** did not react with sodium dimethylmalonate below 50 °C. At higher temperatures, it cyclized and gave aziridine-2-carboxylate **3a** as the only product. On the other hand, all attempts to use the corresponding triflate (prepared *in situ*) at low temperatures failed and led to complex mixtures of unidentified products. In contrast, compound **2a** reacted with sodium malonates at 30−40 °C and provided an easily separable mixture of aziridine **3a** (30%) and triester **4a** (65%) (Scheme 1, Table 1).

To check that basic conditions and heating did not epimerize the α-carbon, an aliquot of compound **4a** was totally hydrolyzed and decarboxylated (4 h in refluxing 6 N HCl) to glutamic acid. The hydrolysate was quantitatively derivatized using (+)-FLEC as previously reported,[13] and the enantiomeric excess was determined by HPLC to be 97% relative to authentic samples of D, L, and DL-glutamate. Compound **3a** was deprotected under mild

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(12) Traces of aziridine carboxylate **3a/b** were detected after 6 months at −18 °C. ^{1}H and ^{13}C NMR spectra of compounds **2a** and **2b** showed considerable doubling of several signals, which suggests the existence of at least two distinct rotamers. This was not observed for the more hindered corresponding mesylates **1a/b**. See: Son, J.-K.; Kalvin, D.; Woodard, R. W. *Tetrahedron Lett.* **1988**, *29*, 4045−4048.

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Table 1. Products of the Nucleophilic Attack of Compounds 2a/b by Malonate-Related Anions[a]

entry	$E^1CH_2E^2$	Methods[b]	**3** (**a**/**b** %)	compd (**a**/**b** %)	ee (**a**/**b**)
1	$CH_2(CO_2Me)_2$	A[c]	30/22	**4a/b** (65/74)	97/97
2	$CH_2(CO_2tBu)_2$	A	16	**5b** (78)	99[d]
3	MeO_2CCH_2CN	A	20	**6a** (74)	98
4	t-BuO_2CCH_2CN	A	12	**7a** (86)	98
5	$CH_2(CN)_2$	A[e]	11	**8a** (77)	98
6	$CH_3COCH_2CO_2tBu$	A	40	**9b** (57)	ND
7	PhO_2SCH_2CN	A	50	**10a** (40)	ND
8	$Ph_2C{=}NCH_2CO_2Et$	B	<5	**11a** (30)	f
9	$Ph_2C{=}NCH_2CN$	C[16]	8	**12a** (90)	f

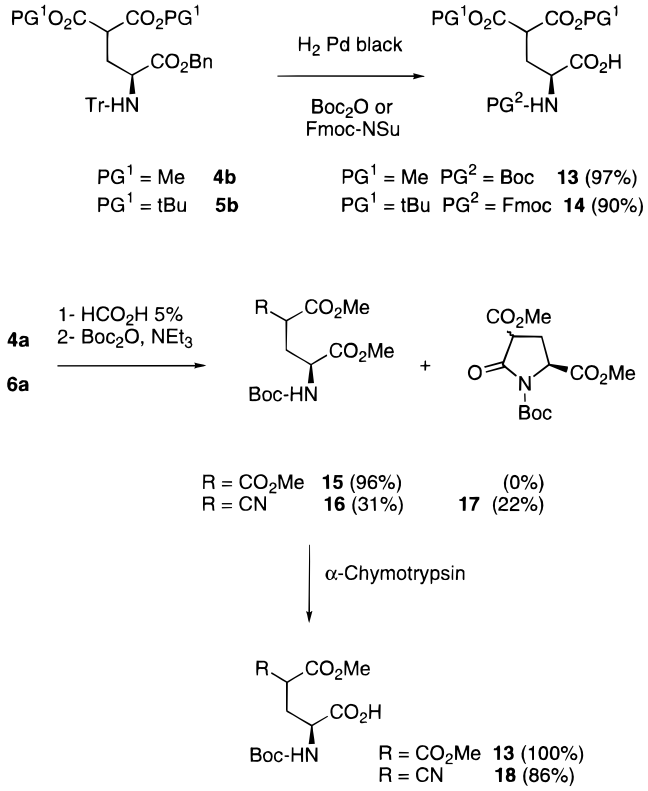
[a] All experiments were carried out on a 1 mmol scale. Yields are given for silica gel flash chromatography purified products; ee were calculated from HPLC analysis of FLEC derivatives. [b] Methods: A (NaH/THF−HMPA 80:20, 40 °C, 12 h); B (LDA/THF−HMPA 80−20, 40 °C, 12 h); C (Bu_4NHSO_4/10% NaOH−CH_2Cl_2, rt 4 h). [c] Dibenzyl, *tert*-butyl ethyl, and benzyl methyl malonates gave similar results. [d] >99% after recrystallization. [e] At 30 °C, 4 h. [f] Decomposed in refluxing 6 N HCl.

acidic conditions (5% formic acid in dichloroethane)[14] and saponified and was analyzed as described above. For higher temperatures and prolonged times of reaction, a serious decrease of yields and enantiomeric excess was observed, whereas the proportion of optically pure aziridine increased (e.g., **3a**, 51% yield, ee = 100%; **4a**, 45% yield, ee = 58% after 24 h at 60 °C). This indicates that substitution and cyclization are competitive reactions and racemization occurs after the substitution step. Moreover, we confirmed that compound **3a** is not an intermediate in a cyclization/ring-opening process since it did not react at 65 °C (2 days) with a 10-fold excess of reagent. No significant difference in reactivity was observed between methyl and benzyl esters.

Our study was extended to a series of malonate-related anions and some other nucleophiles (Table 1). Thus, malonic-branched amino acids were isolated in moderate to good yields (30−90%) and typically 97−99% enantiomeric excess, except for too rigid or too bulky nucleophiles such as Meldrum's acid, bis(phenylsulfonyl)methane, and tetraethylmethylene diphosphonate (data not shown). With most malonates, the use of a slight excess of sodium hydride led to traces of the corresponding lactams. As anticipated, the marked tendency of the malonic center to epimerize precluded the separation of the diastereomers obtained from unsymmetrical nucleophiles (Table 1, entries 3, 4, 6−9).[15] Diaminoglutarate derivative **12a** was obtained in high yield (90%) as a 1:1 mixture of diastereomers (determined by NMR). Although deprotection resulted in a complete degradation of the product, we assume that the very mild conditions employed (phase transfer at room temperature for 4 h)[16] did not epimerize the chiral center.[17]

As a preliminary application of the method, the natural but unusual amino acid 4-carboxyglutamate (Gla)[18] was readily prepared. Boc-Gla$(OMe)_2$-OH (**13**) and Fmoc-Gla$(OtBu)_2$-OH (**14**) were obtained from compounds **4b** and **5b** in 97% and 90% yields, respectively, and each in >98% ee as monitored by HPLC analysis of the FLEC-Glu derivatives (Scheme 2). Following a different approach,

Scheme 2. Synthesis of Boc- and Fmoc-Protected l-γ-Carboxyglutamate and L-γ-Cyanoglutamate from *N*-Trityl Compounds 4a/b, 5b, and 6a



acidic detritylation[14] of triester **4a**, protection with di-*tert*-butyl dicarbonate and regioselective hydrolysis of the α-methyl ester by α-Chymotrypsin[19] (Scheme 2) afforded compound **13** (ee > 99%). Although many preparations of optically pure Gla have been reported in the literature, most of them require either resolution of racemates[20] or asymetric induction.[21] Multistep synthesis of Gla derivatives starting from glutamate,[9a] pyroglutamate,[22] or proline[23] are difficult or relatively more complex than the route we describe here. In a similar way the 4-cyanoglutamate **18**, which has shown interesting pharmacological properties,[24] was synthesized from compound **6a**. However, in this latter case, diester **16** was isolated in a disappointing yield (31%) along with the corresponding lactam **17** (22%) resulting from an intramolecular addition of the intermediate free amine to the cyano group.

In summary, the nucleophilic substitution of *N*-trityl-3-iodoalanine esters **2a/b** by malonate, related anions is an appropriate approach for synthesizing various γ-functionalized amino acids in reasonable yields and almost without epimerization of the α-center. Moreover, it

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allows an orthogonality of protecting groups and hence complements previous methods for synthesizing unusual amino acids.

Experimental Section

General Procedures. All reagents employed were of analytical grade and were purchased from Aldrich Chemical Co. and Lancaster Synthesis Ltd. Serine derivatives were purchased from Aldrich Chemical Co. and Bachem. THF was distilled before use from sodium−benzophenone, and DMF was dried over activated 4 Å molecular sieves. HMPA was distilled from sodium and kept over 4 Å molecular sieves. All other solvents were of analytical grade and were used without further purification. Flash chromatography was performed on 40−60 μm (230−400 mesh) Merck silica gel. NMR δ and J values are given in ppm and Hz, respectively. Melting points were measured on a hot stage Kofler apparatus and are uncorrected. Mass combustion analyses were carried out by the "Service de Microanalyse" of the ICSN (Gif-sur-Yvette, France).

(*S*)-1-Methyl-*N*-(triphenylmethyl)-3-iodoalaninate (2a)/(*S*)-1-Benzyl-*N*-(triphenylmethyl)-3-iodoalaninate (2b). Compound **1a/b** (30 mmol) was treated with sodium iodide (49 g, 300 mmol) in dry acetone (300 mL) under argon for 24 h at room temperature. The volume was concentrated *in vacuo* at room temperature. The product, dissolved in diethyl ether, was washed with 10% sodium thiosulfate until the color faded and with brine. The organic layer was dried over sodium sulfate, and the solvent was evaporated under reduced pressure. **2a** (pale yellow paste, 13.51 g, quantitative): $[\alpha]^{25}_D = +21°$ (c = 1.0, chloroform); ^{1}H NMR ($CDCl_3$) (two rotamers 1:1) δ 7.52−7.43 (m, 6H), 7.32−7.19 (m, 9H), 4.39 (dd, J = 6.2, J' = 8.3) + 3.48 (dd, J = 3.5, J' = 7.0) (1H), 3.76 (s) + 3.30 (s) (3H), 3.35 (dd, J = 3.5, J' = 6.2) + 3.21 (dd, J = 7.0, J' = 9.8) (1H), 2.70 (dd, J = 8.3, J' = 13.0) + 2.54 (dd, J = 6.2, J' = 13.0) (1H), 2.3 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 172.7 + 171.3, 145.6 + 145.5, 128.6−126.5 (complex), 71.1 + 70.8, 56.3, 52.9, 52.0, 48.4, 20.2, 9.6; MS (DCI, NH_3) m/z 472 (MH^+, 1.5), 344 (MH^+ − HI, 0.75), 243 (Tr^+, 100), 230 (M − Tr^+, 10). **2b** (pale yellow paste, 15.61 g, 95%): $[\alpha]^{25}_D = +5°$ (c = 1.0, chloroform); ^{1}H NMR ($CDCl_3$) (two rotamers 2:1) δ 7.50 (bd, J = 7.1, 3H), 7.42−7.35 (m, 3H), 7.33−7.14 (m, 14H), 5.22 (d, J = 13.3) + 4.69 (AB, J_{AB} = 12.2, δ_A = 4.79, δ_B = 4.60) (2H), 4.41 (m) + 3.53 (m) (1H), 3.33−3.15 (m, 1H), 2.95−2.6 (bm, 1H), 1.57 + 2.24 (2bs, 1H); ^{13}C NMR ($CDCl_3$) δ 172.0 + 170.6, 145.6 + 145.4, 135.2 + 135.1, 129.5−126.5 (complex), 71.0 + 70.8, 67.4 + 67.1, 56.1, 53.4, 48.5, 20.3, 9.7; MS (DCI, NH_3) m/z 548 (MH^+, 1), 420 (MH^+ − HI, 0.75), 306 (M − Tr^+, 15), 243 (Tr^+, 100).

Enantiomeric Excesses Determination.[13] **Deprotection of Aziridines.** Compounds **3a/b** (1 mg) were treated with TFA−CH_2Cl_2 50:50 (2 mL) for 5 min at room temperature. After removal of the solvent under reduced pressure, the product was stirred for 1 h with 1 N lithium hydroxide (2 mL). **Hydrolysis of Compounds 4−9.** Compounds **4**−**9** (1 mg) suspended in 6 N hydrogen chloride (500 μL) were heated for 4 h at 100 °C. **Derivatization.** After neutralization with 1 N acetic acid, the product was freeze-dried. The residue was dissolved in a 1 M borate buffer pH 6.35 (100 μL), and the solution was treated with an 18 mM solution of (+)-1-(9-fluorenyl)ethyl chloroformate in acetone ((+)-FLEC-Cl) (500 μL) for 4 min at room temperature. The aqueous layer, washed twice with pentane, was analyzed by HPLC using a spherisorb octyl 3 μm analytical column (150 × 4.6 mm); linear gradient **3a/b**, acetonitrile−THF−50 mM acetic acid pH 4.35 (0.5 mL/min, 150−200 bars): 0−8 min (8:17:75), 8−22 min (8:17:75 to 0:25:75), 22−45 min (0:25:75); **4a/b**−**9a/b**, acetonitrile−THF−50 mM acetic acid pH 4.35 (0.5 mL/min, 150−200 bars): 0−8 min (8:17:75), 8−22 min (8:17:75 to 0:30:70), 22−45 min (0:30:70 to 0:40:60); detection at 265 nm detection at 265 nm. Retention times (rt): **3a/b**, 20.24 ± 0.18 min ((+)-FLEC-D-Azy), 21.02 ± 0.26 min ((+)-FLEC-L-Azy); **4a/b**−**9a/b**, 21.67 ± 0.57 min ((+)-FLEC-D-Glu), 23.06 ± 0.52 min ((+)-FLEC-L-Glu).

Method A. All reactions were carried out in dried glassware under an argon atmosphere. All solvents used were dried by the usual procedures. The malonate (1 mmol) was added to a suspension of 80% sodium hydride (1.0 mmol) in THF−HMPA 60−40 (5 mL), and the mixture was stirred 30 min at room temperature. A solution of iodide **2a/b** (1 mmol) in THF (5 mL) was added, and the mixture was stirred for 12 h at 40 °C. The reaction was quenched with saturated aqueous ammonium chloride, and the product was extracted twice with diisopropyl ether (2 × 25 mL). The combined organic layers were washed six times with saturated aqueous ammonium chloride (6 × 10 mL) and were dried over sodium sulfate. After evaporation under reduced pressure the product was purified by silica gel flash chromatography.

Method B. *N*-(Diphenylmethylene)glycine ethyl ester (278 mg, 1 mmol) was treated with freshly prepared LDA in a mixture of dry THF−HMPA 60:40 (5 mL) for 1 h at −78 °C. After dropwise addition of compound **2a** (451 mg, 1 mmol) dissolved in THF (5 mL), the mixture was stirred overnight at room temperature. The reaction was quenched with saturated aqueous ammonium chloride, and the product was extracted as described for method A.

Method C. A mixture of compound **2a** (451 mg, 1 mmol), *N*-(diphenylmethylene)acetonitrile (225 mg, 1 mmol), and tetrabutylammonium hydrogen sulfate (410 mg, 1.2 mmol) dissolved in dichloromethane (14 mL) was treated with 10% sodium hydroxide (6 mL) for 4 h at room temperature. The organic layer was dried over sodium sulfate, and the volume was reduced *in vacuo*.

(*S*)-1,5,5′-Trimethyl-*N*-(triphenylmethyl)-4-carboxyglutamate (4a) (Method A). Purification by silica gel flash chromatography (solvent: ethyl acetate−cyclohexane 10:90) gave **3a** (103 mg, 30%) and **4a** (yellowish solid, 311 mg, 65%): mp = 97−99 °C; $[\alpha]^{25}_D = +56°$ (c = 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.46 (bd, J = 6.7, 6H), 7.30−7.14 (m, 9H), 3.77 (s, 3H), 3.70 (s, 3H), 3.65 (d, J = 7.1, 1H), 3.45 (t, J = 5.9, 1H), 3.15 (s, 3H), 2.71 (b, 1H), 2.38 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 174.4, 169.5, 169.3, 145.5, 128.7, 127.8, 126.4, 71.2, 54.3, 52.8 + 52.7, 51.7, 48.1, 34.2; MS (DCI, NH_3) m/z 476 (MH^+, 25), 243 (Tr^+, 100), 234 ($M2H^+$ − Tr^+, 10). Anal. Calcd for $C_{28}H_{29}NO_6$: C, 70.72; H, 6.15; N, 2.95. Found: C, 70.71; H, 6.18; N, 3.11.

(*S*)-1-Benzyl-5,5′-dimethyl-*N*-(triphenylmethyl)-4-carboxyglutamate (4b) (Method A). Purification by silica gel flash chromatography (solvent: ethyl acetate−cyclohexane 10:90) gave **3b** (82 mg, 22%) and **4b** (white gum, 409 mg, 74%): $[\alpha]^{25}_D = +20°$ (c = 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.46 (dd, J = 1.4, J' = 8.4, 2H), 7.38−7.34 (m, 8H), 7.27−7.15 (m, 10H), 5.15 (ds, 2H), 4.00 (m, 1H), 3.67 (s, 3H), 3.54 (s, 3H), 3.21 (m, 1H), 2.50−2.31 (m, 2H), 1.83 (b, 1H); ^{13}C NMR ($CDCl_3$) δ 172.4, 170.9, 166.2, 145.6 + 145.3, 135.4 + 135.1, 128.7−126.3 (complex), 71.2, 66.9, 52.7, 51.8, 45.5 + 45.3, 34.1; MS (DCI, NH_3) m/z 552 (MH^+, 80), 310 ($M2H^+$ − Tr^+), 243 (Tr^+, 100). Anal. Calcd for $C_{34}H_{33}NO_6$: C, 74.03; H, 6.03; N, 2.54. Found: C, 74.28; H, 6.27; N, 2.33.

(*S*)-1-Benzyl-5,5′-di-*tert*-butyl-*N*-(triphenylmethyl)-4-carboxyglutamate (5b) (Method A). Purification by silica gel flash chromatography (solvent: ethyl acetate-cyclohexane 5−95) gave **3b** (67 mg, 16%) and **5b** (yellowish crystals, 497 mg, 78%): mp 121−122 °C; $[\alpha]^{25}_D = +7°$ (c = 1.0, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.49 (bd, J = 7.2) + 7.38 (bd, J = 7.2) + 7.34−7.10 (m) (20H), 5.22 (s, 2H), 3.69 (m, 1H), 3.29 (m, 1H), 2.39 (m) + 2.28 (b) (3H), 1.39 (s, 9H), 1.24 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.9, 167.0, 166.8, 146.8, 145.7 + 145.5, 135.6, 129.3, 128.8−126.2 (complex), 81.5 + 81.4, 71.1, 66.7, 54.4, 50.3, 45.4, 27.7 + 27.6; MS (DCI, NH_3) m/z 636 (MH^+, 35), 394 ($M2H^+$ − Tr^+, 4), 243 (Tr^+, 100). Anal. Calcd for $C_{40}H_{45}NO_6$: C, 75.56; H, 7.13; N, 2.20. Found: C, 75.89; H, 7.21; N, 1.93.

(2*S*,4*RS*)-1,5-Dimethyl-*N*-(triphenylmethyl)-4-cyanoglutamate (6a) (Method A). Purification by silica gel flash chromatography (solvent: ethyl acetate−cyclohexane 20−80) gave **3a** (70 mg, 20%) and **6a** (yellow gum, 328 mg, 74%): IR ($CHCl_3$) 2260; ^{1}H NMR ($CDCl_3$) δ 7.48−7.41 (m, 6H), 7.36−7.16 (m, 9H), 4.22 (dd, J = 7.2, J' = 8.3, 1H), 3.82 + 3.80 (2s, 3H), 3.69 (s, 3H), 3.21 (m, 1H), 2.73−2.60 (m, 2H), 2.03 (b, 1H); ^{13}C NMR ($CDCl_3$) δ 171.2, 170.4, 165.7 + 165.6, 145.1, 128.6−126.6 (complex), 115.0 + 114.7, 71.0, 53.8 + 53.7, 46.1 + 45.4, 42.7 + 42.5, 37.4 + 37.1; MS (DCI, NH_3) m/z 443 (MH^+, 4), 243 (Tr^+, 100), 201 ($M2H^+$ − Tr^+, 5). Anal. Calcd for $C_{27}H_{26}N_2O_4$: C, 73.28; H, 5.92; N, 6.33. Found: C, 73.01; H, 6.06; N, 5.97.

(*S*)-1,5-Dimethyl-*N*-(*tert*-butyloxycarbonyl)-4-carboxyglutamate 13 from 4b. A mixture of compound **4b** (954 mg, 1.5 mmol) and diterbutyl dicarbonate (242 mg, 1.65 mmol) was vigorously stirred with palladium black (90 mg, 10% w/w) in a

mixture of ethyl acetate−methanol 50:50 (75 mL) under hydrogen (2.5 bars) for 4 h at room temperature. Filtration of the catalyst, elimination of the solvent under reduced pressure, and silica gel flash chromatography afforded **13** (colorless oil, 310 mg, 97%): $[\alpha]^{25}_D = -1.9°$ ($c = 1.0$, methanol); ^{1}H NMR ($CDCl_3$) δ 7.98 (b, 1H), 5.26 (bd, 1H), 4.40 (bm, 1H), 3.76 (s, 3H), 3.75 (s, 3H), 3.58 (t, $J = 6.8$, 1H), 2.58 (m, 1H), 2.25 (m, 1H), 1.44 (s 9H); ^{13}C NMR ($CDCl_3$) δ 175.1, 169.5, 169.1, 155.5, 80.4, 52.8, 51.6, 48.3, 39.6, 31.1, 28.2; MS (DCI, NH_3) *m/z* 337 (MNH_4^+, 40), 320 (MH^+, 100), 281 ($MNH_4^+ - (CH_3)_2C{=}CH_2$, 60), 264 ($MH^+ - (CH_3)_2C{=}CH_2$, 55), 220 ($MH^+ - (CH_3)_2C{=}CH_2 - CO_2$, 15). Anal. Calcd for $C_{12}H_{21}NO_8$: C, 46.90; H, 6.89; N, 4.56. Found: C, 46.74; H, 7.35; N, 4.20.

(*S*)-5,5′-Di-*tert*-butyl-*N*-(9-fluorenylmethyloxycarbonyl)-4-carboxyglutamate (14). Compound **5b** (954 mg, 1.5 mmol) was vigorously stirred with palladium black (90 mg, 10% w/w) in a mixture of ethyl acetate−methanol 50:50 (75 mL) under hydrogen (2.5 bars) for 8 h at room temperature. The solvent was evaporated under reduced pressure, and the residue was treated with Fmoc-*N*-hydroxysuccinimide (580 mg, 1.65 mmol) and diisopropylethylamine (295 μL, 1.65 mmol after neutrality) in dry THF for 2 h at room temperature. The reaction was quenched by addition of 10% citric acid, the product was extracted twice with diethyl ether, and the organic layer was dried over sodium sulfate. Evaporation of the solvent under reduced pressure and silica gel flash chromatography afforded **14** (colorless paste, 713 mg, 90%): $[\alpha]^{25}_D = 0°$ ($c = 1$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.75 (d, $J = 7.4$, 2H), 7.59 (d, $J = 5.7$, 2H), 7.42−7.16 (m, 4H), 5.56 (bd, 1H), 4.44 (dd, $J = 7.0$, $J' = 10.2$, 2H), 4.33 (t, $J = 10.2$, 1H), 4.21 (t, $J = 7.0$, 1H), 2.54−2.43 (m, 1H), 2.27−2.15 (m, 1H), 1.46 (s 18H); ^{13}C NMR ($CDCl_3$) δ 175.8, 168.4, 168.2, 156.2, 143.8, 143.6, 141.2, 127.7, 127.1, 125.2, 125.1, 119.0, 82.4, 67.3, 52.4, 50.8, 47.0, 39.6, 30.7, 27.8; MS (DCI, NH_3) *m/z* 543 (MNH_4^+, 55), 526 (MH^+, 45), 487 ($MNH_4^+ - (CH_3)_2C{=}CH_2$, 20), 470 ($MH^+ - (CH_3)_2C{=}CH_2$, 30), 443 ($MNH_4^+ - (CH_3)_2C{=}CH_2 - CO_2$, 6), 426 ($MH^+ - (CH_3)_2C{=}CH_2 - CO_2$, 7), 414 ($MH^+ - 2\ (CH_3)_2C{=}CH_2$, 10), 387 ($MNH_4^+ - 2\ (CH_3)_2C{=}CH_2 - CO_2$, 10), 370 ($MH^+ - 2\ (CH_3)_2C{=}CH_2 - CO_2$, 55), 179 ($Fl^+$, 100). Anal. Calcd for $C_{29}H_{35}NO_8$: C, 66.27; H, 6.71; N, 2.61. Found: C, 66.71; H, 7.19; N, 2.39.

(*S*)-1,5,5′-Trimethyl-*N*-(*tert*-butyloxycarbonyl)-4-carboxyglutamate (15). Compound **4a** (142.5 mg, 0.3 mmol) was stirred for 15 min in a mixture of dichloroethane−formic acid 95:5 (12 mL) at room temperature. After evaporation of the solvent *in vacuo*, the residue was dissolved in DMF (10 mL) and was neutralized with triethylamine. The product was treated with di-*tert*-butyl dicarbonate (75 mg, 0.33 mmol) and triethylamine (46 μL, 0.33 mmol) overnight at room temperature. The solvent was evaported under reduced pressure, and the product was dissolved in ethyl acetate and washed with 10% citric acid. Drying of the organic layer over sodium sulfate, evaporation of the solvent, and purification by silica gel flash chromatography (solvent: ethyl acetate−cyclohexane 25:75) afforded **15** (colorless oil, 96 mg, 96%): $[\alpha]^{25}_D = -17°$ ($c = 1.0$, chloroform); ^{1}H NMR ($CDCl_3$) δ 5.02 (b, 1H), 4.39 (bm, 1H), 3.76 (s, 3H), 3.75 + 3.74 (ds, 3H), 3.53 (t, $J = 7.0$, 1H), 2.53 (m, 1H), 2.20 (m, 1H), 1.44 (s 9H); ^{13}C NMR ($CDCl_3$) δ 172.2, 169.4, 169.0, 155.2, 80.2, 52.8 + 52.5, 52.4, 51.7, 48.3, 31.5, 28.2. MS (DCI, NH_3) *m/z* 351 (MNH_4^+, 100), 334 (MH^+, 95), 295 ($MNH_4^+ - (CH_3)_2C{=}CH_2$, 55), 278 ($MH^+ - (CH_3)_2C{=}CH_2$, 25), 234 ($MH^+ - (CH_3)_2C{=}CH_2 - CO_2$, 15), 174 ($MH^+ - (CH_3)_2C{=}CH_2 - CO_2 - HCO_2Me$, 10). Anal. Calcd for $C_{14}H_{23}NO_8$: C, 50.44; H, 6.95; N, 4.20. Found: C, 50.58; H, 7.27; N, 3.90.

(*S*)-5,5′-Dimethyl-*N*-(*tert*-butyloxycarbonyl)-4-carboxyglutamate (13) from 15. Compound **32** (333 mg, 1 mmol) dissolved in DMSO (10 mL) was added to a 0.1M KH_2PO_4 buffer, and the pH was adjusted to 7.6. α-Chymotrypsin (100 mg, type II 54 u/mg) was added, and the mixture was stirred at 37 °C. The reaction was monitored by TLC. After complete consumption of the starting compound, the reaction was quenched with 10% citric acid and the product was extracted four times with dichloromethane. The organic layer was dried over sodium sulfate, and the volume was evaporated under reduced pressure. Silica gel flash chromatography (solvent: chloroform−methanol−acetic acid 94:5:1) gave **30** (colorless oil, 318 mg, quantitative): $[\alpha]^{25}_D = -1.8°$ ($c = 1.0$, methanol); NMR data were identical to those described above.

(2*S*,4*RS*)-1,5-Dimethyl-*N*-(*tert*-butyloxycarbonyl)-4-cyanoglutamate (16). Compound **6a** (2.2 g, 5 mmol) was treated by formic acid as reported above. Silica gel flash chromatography (solvent: ethyl−acetate−hexane 25:75) yielded **16** (amorphous solid, 465 mg, 31%) and **17** (colorless oil, 332 mg, 27%). **16**: mp = 83−84 °C; IR ($CHCl_3$) 2250; ^{1}H NMR ($CDCl_3$) δ 5.22 (b, 1H), 4.51 (bm, 1H), 3.85 + 384 (2s, 3H), 3.80 + 3.79 (2s, 3H), 3.78−3.67 (m, 1H), 2.68−2.47 (m, 1H), 2.42−2.19 (m, 1H), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 173.0, 171.7, 171.1, 152.0, 83.3, 52.3, 50.1, 30.1, 27.9, 27.3, 26.8; MS (DCI, NH_3) *m/z* 301 (MH^+, 100), 245 ($MH^+ - (CH_3)_2C{=}CH_2$, 35), 201 ($MH^+ - (CH_3)_2C{=}CH_2 - CO_2$, 30). Anal. Calcd for $C_{13}H_{20}N_2O_6$: C, 51.99; H, 6.71; N, 9.33. Found: C, 51.56; H, 6.43; N, 9.69.

(2*S*,4*RS*)-5-Methyl-*N*-(*tert*-butyloxycarbonyl)-4-cyanoglutamate (18). Compound **16** (150 mg, 1 mmol) was treated as reported for compound **13** from **15**. Silica gel flash chromatography (solvent: ethyl acetate−acetic acid 99:1) yielded **18** (colorless oil, 123 mg, 86%): IR ($CHCl_3$) 2250; ^{1}H NMR ($CDCl_3$) δ 5.25 (b, 1H), 4.46 (bm, 1H), 3.85 + 3.84 (ds, 3H), 3.74 (dd, $J =$ 5.7, 8.5, 1H), 2.62 (m, 1H), 2.39 (m, 1H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 174.2, 173.5, 155.6, 115.7, 81.1, 53.8, 33.9, 32.0, 28.2, 24.5; MS (DCI, NH_3) *m/z* = 304 (MNH_4^+, 55), 287 (MH^+, 20), 260 ($MNH_4^+ - CO_2$, 85), 243 ($MH^+ - CO_2$, 100). Anal. Calcd for $C_{12}H_{18}N_2O_6$: C, 50.35; H, 6.34; N, 9.78. Found: C, 49.87; H, 6.78; N, 9.36.

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Supporting Information Available: Description of ^{1}H and ^{13}C NMR spectra and characterization of compounds **1**, **3**, and **7**−**12** (4 pages). This material is contained in librairies on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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