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A practical synthesis of fully protected L-γ-carboxyglutamic acid

Sheng Jiang, Christopher C. Lai, James A. Kelley and Peter P. Roller*

Laboratory of Medicinal Chemistry, CCR, National Cancer Institute–Frederick, NIH, Frederick, MD 21702, USA

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Abstract—We have developed a new synthetic route for the preparation of Fmoc protected L-γ-carboxyglutamic acid in 60% overall yield (>99% ee) via a six-step synthesis from D-Garner's aldehyde. An aldol condensation and the selective cleavage of the acetonide protective group are key steps.
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1. Introduction

L-γ-Carboxyglutamic acid (L-Gla) is formed in proteins via the post-translational modification of L-glutamic acid by vitamin K carboxylase.[1] It has been found in several vertebrate calcium-binding proteins such as osteocalcin.[2,3] L-Gla has also been identified unexpectedly in some of the neuroactive peptides such as conantoxin GV and conantoxin T.[4,5] We have found that replacement of L-Glu with L-Gla in cyclic peptides, such as G1TE, substantially improves the latter's binding affinity to the Grb2–SH2 domain protein, thereby improving its cellular signaling inhibitory properties.[6–8] Numerous syntheses of L-Gla or its derivatives have appeared in the literature, including both racemic syntheses[9–11] and chiral syntheses.[12–17] In these approaches, the overall yields of resolved L-Gla[9–16] have been on the order of 6–13%, except for Hiskey's method[17] whereby they obtained a 60% yield of the L-Gla product. In this method, the preparation of chiral L-Gla was achieved by using expensive starting materials. Considering that the fully Fmoc protected L-Gla has been used in a number of peptide synthesis,[4,6–8,18] and this substance is considerably more expensive,[19] we developed, and present here a practical, concise and economical route to the preparation of Fmoc protected L-γ-carboxyglutamic acid for easier availability.

Fmoc protected L-γ-carboxyglutamic acid was synthesized using the procedure described in Scheme 1. The starting material, D-Garner's aldehyde, can be easily prepared from D-serine on a large scale.[20] The latter intermediate **2** was reacted with di-*tert*-butyl malonate by using aldol condensation conditions, and then the β-hydroxy group was eliminated by treatment with $(CF_3CO)_2O$ and Et_3N.[21,22] Using Yao's procedure[23] to selectively deprotect the acetonide group with bismuth bromide provided compound **4** in 93% yield. The purity of the resulting product was very high and without the need for further purification. We also found that changing the reaction conditions by addition of 1 equiv water in anhydrous acetonitrile enhanced selectivity with no deprotection of *tert*-butyl ester being observed. Subsequent transformation of aminoalcohol **4** to amino acid **1** involved a three-step reaction sequence: (i) selective oxidation using PDC as an oxidant,[24] (ii) α-amino group deprotection by catalytic reduction using H_2/10% Pd–C, yielding the enantiomerically pure L-Gla **6**, and (iii) the L-Gla amino acid was reacted with Fmoc–OSu without purification to yield the final product. The structures of final product **1** and intermediates **3** and **4** were fully characterized and confirmed by ^{1}H NMR, ^{13}C NMR, optical rotation, IR, and MS. The enantiomeric purity was determined by measurement of optical rotation.

In summary, we developed a new synthetic route to efficiently prepare the Fmoc protected L-γ-carboxyglutamic acid in 60% overall yield (>99% ee) via a six-step procedure. In this synthesis route, only the intermediate **3** and the final product **1**, need a flash column chromatography purification. The key synthetic steps involving aldol condensation and selective cleavage of the acetonide protecting group have been optimized for yield and selectivity.

Keywords: L-γ-Carboxyglutamic acid; Aldol condensation; D-Garner's aldehyde; Stereoselective synthesis.
* Corresponding author. Tel.: +1 301 846 5904; fax: +1 301 846 6033; e-mail: proll@helix.nih.gov

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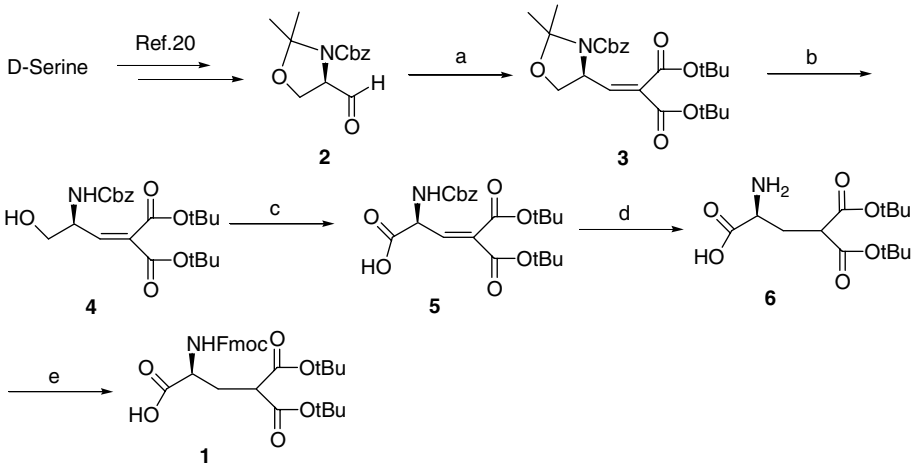

Scheme 1. The synthesis of Fmoc protected L-γ-carboxyglutamic acid. Reagents and conditions: (a) (i) LDA, THF–HMPA, di-*tert*-butyl malonate, −78 °C; (ii) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, 80% for two steps; (b) $BiBr_3$ (10 mol %), 1 equiv H_2O, anhydrous acetonitrile, rt, 12 h, 93%; (c) PDC, DMF, 83%; (d) H_2, 10% Pd–C, methanol, 100%; (e) Fmoc–Su, $NaHCO_3$, CH_3CN/H_2O (1/1), 96%.

Advantages of this route include the simplicity of the procedure, the use of inexpensive, nontoxic reagents and use of an inexpensive D-serine as a starting material, which may strengthen industrial interest in this route.

2. Experimental

2.1. Synthesis of 2*S*-(3-benzyloxycarbonyl-2,2-dimethyl-oxazolidin-4-ylmethylene)-malonic acid di-*tert*-butyl ester (3)

To a solution of diisopropylamine (14.1 mL, 0.12 mol) in anhydrous THF (150 mL) was added *n*-BuLi (42.2 mL, 1.6 M in hexane, 0.068 mol) at 0 °C under N_2, and the mixture were stirred for 30 min. After stirring for an additional 30 min at −78 °C, anhydrous HMPA (22.6 mL, 0.13 mol) was added, and the mixture was stirred for 30 min. A solution of di-*tert*-butyl malonate (14.7 g, 0.068 mol) in THF (50 mL) was injected into the above mixture. After 30 min, a solution of D-Garner's aldehyde (14.92 g, 0.057 mol) in THF (100 mL) was introduced, and the reaction mixture was stirred for 2 h at −78 °C. The mixture was quenched with saturated aqueous NH_4Cl and extracted with ether. The organic layer was washed with brine and dried (Na_2SO_4). Removal of the solvents afforded a crude oil. To the mixture of the above oil and Et_3N (36 mL, 0.256 mol) in CH_2Cl_2 (80 mL) at 0 °C was added $(CF_3CO)_2O$ (18 mL, 0.128 mmol). The reaction was stirred at 0 °C for 12 h and at rt for 6 h, quenched with saturated NH_4Cl, and extracted with CH_2Cl_2. After drying (Na_2SO_4), the extracts were filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel to afford pure **3** (20.9 g, 80%). ^1H NMR (400 MHz, $CDCl_3$): 7.26–7.36 (5H, m), 6.73 (1H, d, $J = 8.8$ Hz), 5.13 (2H, m), 4.80 (1H, m), 4.22 (1H, m), 3.86 (1H, dd, $J =$ 3.2, 9.2 Hz), 1.43–1.56 (24H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 163.9, 163.1, 152.1, 146.7, 136.3, 131.3, 128.3, 128.1, 127.7, 127.6, 95.1, 82.2, 81.9, 68.4, 66.6, 55.7, 28.0, 26.1, 23.9 ppm. IR (neat): 2980, 2938, 2878, 2360, 2341, 1708, 1403, 1367, 1348, 1161, 733 cm^{-1}. $[\alpha]_D^{25}$ −50.8 (*c* 17.45, $CHCl_3$). FAB-MS *m*/*z* (relative intensity) 462 ($M+H^+$, 5), 406 ($M+H^+-t$-Bu, 12), 350 ($M+H^+-2t$-Bu, 49). HRMS (FAB): calcd for $C_{25}H_{36}NO_7$ $[M+H]^+$ 462.249, found 462.248.

2.2. Synthesis of 2*S*-(2-benzyloxycarbonylamino-3-hydroxy-propylidene)-malonic acid di-*tert*-butyl ester (4)

To a solution of cyclic *N*,*O*-aminal **3** (4.62 g, 10 mmol) in anhydrous MeCN (100 mL) was added bismuth(III) bromide (450 mg, 1.0 mmol) at room temperature. After 30 min, water (0.18 mL, 10 mmol) was added. The reaction mixture was stirred at room temperature for 12 h. When all starting material had disappeared (about 12 h), the reaction mixture was quenched by adding saturated aqueous $NaHCO_3$ (5 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 **4** (3.92 g, 93%). ^1H NMR (400 MHz, $CDCl_3$): 7.33 (5H, br s), 6.70 (1H, d, $J = 8.4$ Hz), 5.44 (1H, d, $J = 8.0$ Hz), 5.09 (2H, d, $J = 4.4$ Hz), 4.65 (1H, br s), 3.75 (2H, m), 2.70 (1H, t, $J = 6.0$ Hz), 1.49–1.51 (18H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): 164.7, 155.9, 142.3, 136.1, 132.8, 128.5, 128.1, 82.9, 82.3, 67.0, 64.3, 52.0, 27.9 ppm. IR (neat): 3371, 2979, 2883, 2359, 1707, 1368, 1254, 1160, 754 cm^{-1}. $[\alpha]_D^{25}$ − 19.35 (*c* 5.85, $CHCl_3$). FAB-MS *m*/*z* (relative intensity) 422 ($M+H^+$, 3), 366 ($M+H^+-t$-Bu, 11), 310 ($M+H^+-2t$-Bu, 53). HRMS (FAB): calcd for $C_{22}H_{32}NO_7$ $[M+H]^+$ 422.218, found 422.220.

2.3. Synthesis of *N*-α-Fmoc-L-γ-carboxy-glutamic acid-γ,γ-*tert*-butyl ester (1)

To a stirred mixture of alcohol **4** (1.79 g, 4.24 mmol), in dry DMF (18 mL) at room temperature under argon was added pyridinium dichromate (6.38 g, 16.96 mmol). After stirring overnight, another portion of pyridinium dichromate (1.595 g, 4.24 mmol) was added and the resulting mixture was stirred for additional 4 h. The

reaction mixture was diluted with ethyl ether (100 mL) and washed with 1 N HCl (5×100 mL). The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to give a crude oil **5** (1.54 g, 83%). To the mixture of the above oil, CH_3OH (20 mL), and 10% Pd–C (0.3 g) was stirred vigorously under a H_2 atmosphere until hydrogen was absorbed to saturation. The mixture was filtrated and concentrated to give a crude oil **6** (1.02 g, 100%). A mixture of the above oil, *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (1.43 g, 4.24 mmol) and $NaHCO_3$ (1.43 g, 16.96 mmol) in water and acetonitrile (1:1, 20 mL) was stirred at room temperature overnight. Acetonitrile was evaporated under reduced pressure, the resulting solution was adjusted to pH = 4 using 10% citric acid. The mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated and purified by flash chromatography to afford **1** as a solid (1.79 g, 96% from **6**). ^{1}H NMR (400 MHz, $CDCl_3$): 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. ^{13}C NMR (100 MHz, $CDCl_3$): 175.3, 168.4, 168.2, 156.2, 143.8, 143.6, 141.2, 127.7, 127.1, 125.1, 119.9, 82.4, 82.3, 67.9, 67.3, 52.4, 50.8, 47.0, 30.7, 27.8, 27.7 ppm. IR (film): 3397, 3182, 2978, 1723, 1670, 1531, 1150, 1050 cm^{-1}. $[\alpha]_D^{25} - 8.7$ (*c* 1.0, MeOH) [lit.[25] −8.6 (*c* 0.173, MeOH)]. FAB-MS *m*/*z* (relative intensity) 526 ($M+H^+$, 1), 470 ($M+H^+-t$-Bu, 4), 414 ($M+H^+-2t$-Bu, 31). HRMS (FAB): calcd for $C_{29}H_{36}NO_8$ $[M+H]^+$ 526.244, found 526.247.

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