Improved Synthesis of the Boc and Fmoc Derivatives of 4-(2′-Aminoethyl)-6-dibenzofuranpropionic Acid: An Unnatural Amino Acid That Nucleates β-Sheet Folding

Haimanot Bekele, Carey L. Nesloney, Kurt W. McWilliams, Niki M. Zacharias, Penchit Chitnumsub, and Jeffery W. Kelly*

Department of Chemistry, Texas A&M University, College Station, Texas 77843-3255

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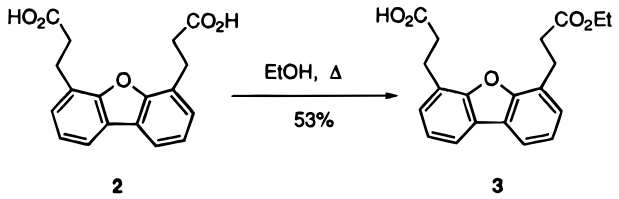
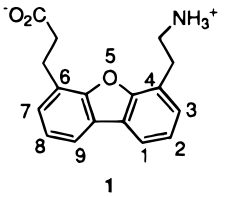
Introduction

Our laboratory's approach toward achieving β-sheet folding in relatively small peptides has focused on the use of conformationally restricted templates that serve to nucleate β-hairpin and β-sheet formation.[1−10] These templates are unnatural aromatic amino acids that are designed to replace the backbone of the $i + 1$ and $i + 2$ residues of the β-turn region required for chain reversal. The majority of our efforts have centered on the dibenzofuran-based amino acid template, 4-(2′-aminoethyl)-6-dibenzofuranpropionic acid (**1**), synthesized within.[1−6,9,10] An NMR structural evaluation of heptapeptides incorporating **1** reveals intramolecular hydrogen bonding between the α-amino acids flanking **1** as well as hydrophobic cluster formation involving the aromatic ring of **1** and the side chains of the flanking hydrophobic α-amino acid residues as indicated by observed NOEs.[3,5] These heptapeptides have a partial β-sheet structure in the sequence flanking **1**, with fraying at the ends of the strands. In appropriate tridecapeptides, the hydrogen-bonded hydrophobic cluster nucleates the formation of a β-hairpin structure that subsequently self-associates into a cross-β fibril.[10] Unlinking the intramolecular folding and self-association equilibria was accomplished by strategically replacing two of the exterior amide protons in the tridecapeptide with methyl groups. These N-methylated tridecapeptides incorporating **1** have been characterized by analytical equilibrium ultracentrifugation, far-UV CD, FT-IR, and a variety of NMR experiments that support a β-hairpin-like structure.[7] Interestingly, these peptides exhibit an increase in β-sheet structure with increasing temperature that may prove to be general for β-sheets stabilized by hydrophobic interactions. Considering the dependence of our laboratory on the dibenzofuran-based amino acid as well as growing interest from the pharmaceutical industry, it was necessary to improve the synthesis of this compound. The original synthesis was not well suited to producing large quantities of **1**.



2 **3**

Figure 1. Previous strategy for monoesterfication of C_2-symmetrical diacid.



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Results and Discussion

Previous synthetic strategies for the preparation of template **1** have been limited by the inefficient conversion of the C_2-symmetrical diacid **2** to the monoacid monoester compound **3** (Figure 1).[1,2,11] The geometry of the diacid **2** prohibits differentiation of the propionic acid side chains using the classical anhydride approach.[12,13] The transformation of **2** to the unsymmetrical monoacid monoester **3** generally provides a nearly statistical distribution of products (e.g., 1:2:1 starting diacid: monoacid−monoester:diester) employing a variety of esterification methods. Although the diacid and diester compounds can be recycled, separation of the three compounds is laborious when the reaction is performed on a large scale.

We set out to develop a new synthesis of **1** that would desymmetrize the dibenzofuran molecule at a very early stage in the synthetic sequence, taking advantage of selective metalation reactions. Dibenzofuran undergoes regioselective metalation at the 4 and 6 positions presumably due to the *ortho*-directing effect of oxygen.[14−16] However, dimetalation is somewhat difficult to achieve, requiring either strongly basic organosodium or potassium reagents[15] or the addition of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) to *s*-BuLi.[17,18] An alternative approach to reacting the 4,6-dianion with a single electrophile to generate a symmetrical product takes advantage of an early observation by our laboratory that two sequential deprotonation−silylation reactions employing *n*-BuLi and Me_3SiCl can be performed with high selectivity and in excellent yield.[11] It follows that a

* To whom correspondence should be addressed. Phone: (409) 845-4242. Fax: (409) 845-9452. E-mail: kelly@chemvx.tamu.edu.

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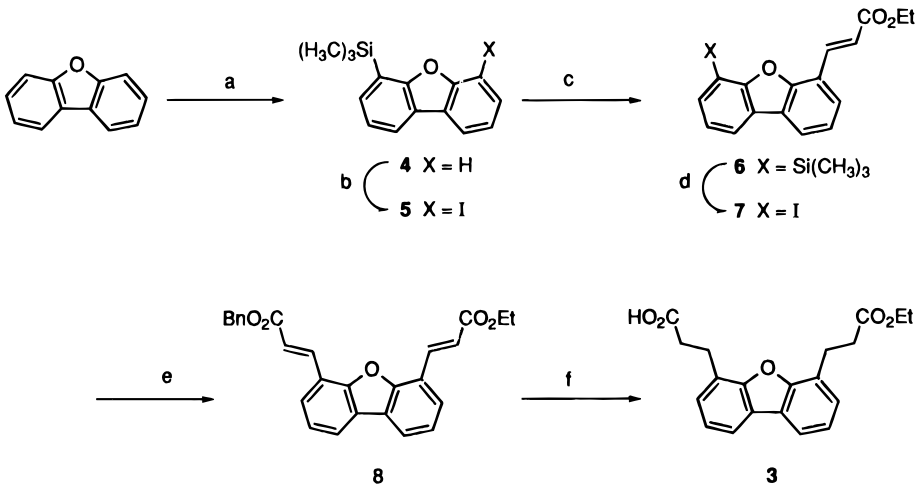

Figure 2. Outline of the synthesis of ethyl 6-(3-carboxypropyl)-4-dibenzofuranpropionate (**3**). Reaction conditions: (a) (i) 1.2 equiv of *n*-BuLi in THF initially at 0 °C and then at room temperature, 5 h, (ii) 2.0 equiv of TMSCl in THF at 0 °C, room temperature 20 min, reflux 2 h, room temperature 12 h, 99%; (b) (i) 1.6 equiv of *n*-BuLi, 1.6 equiv of TMEDA, initially at 0 °C and then at room temperature, 5.5 h, (ii) 2.3 equiv of I_2 in Et_2O, initially at −78 °C and then at room temperature, 12 h, 75%; (c) 3.0 equiv of ethyl acrylate, 3.0 equiv of Et_3N, 2.1 mol % of $Pd(OAc)_2$, 5.1 mol % of P(*o*-tol)$_3$, CH_3CN, heated to 85 °C for 3 h, 93%; (d) 2.8 equiv of ICl, 3.0 equiv of K_2CO_3, CCl_4, initially at 0 °C and then at room temperature, 95%; (e) 2.0 equiv of benzyl acrylate, 2.0 equiv of Et_3N, 2.2 mol % of $Pd(OAc)_2$, 5.0 mol % of P(*o*-tol)$_3$, CH_3CN, heated to 85 °C for 5 h, 75%; (f) 55 psi H_2, 5 mol% 10% Pd/C, 1:1 HOAc: EtOH, and ethyl acetate, 24 h, 95%.

similar sequential metalation procedure could be used to obtain an appropriately substituted unsymmetrical 4,6-disubstituted dibenzofuran skeleton. The monoanion was reacted with trimethylsilyl chloride, and the resulting TMS derivative **4** was deprotonated again and reacted with iodine to yield **5** (Figure 2). This approach desymmetrizes dibenzofuran at an early stage of the synthesis of **1**, differentiating the 4 and 6 positions.

Monometalation of dibenzofuran was readily accomplished by treatment with 1.2 equiv of *n*-butyllithium in THF first at 0 °C for 50 min and then at rt for 5 h. Addition of trimethylsilyl chloride at 0 °C, followed by heating at reflux for 2 h, afforded 4-(trimethylsilyl)-dibenzofuran (**4**) in nearly quantitative yield. Metalation of **4** using 1.6 equiv each of *n*-butyllithium and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) afforded the anion in the 6 position. Exposure to an ethereal solution of I_2 at −78 °C afforded 4-iodo-6-(trimethylsilyl)dibenzofuran (**5**) in 75% overall yield. This is a considerable improvement over the differentiation obtained at the 4 and 6 positions in the esterification of symmetrical diacid **2** (50% yield).

Reaction of **5** with ethyl acrylate in the presence of palladium acetate, tri-*o*-tolylphosphine, and triethylamine in acetonitrile provided the α,β-unsaturated ester **6** in 93% yield. Iododesilylation of **6** with ICl in carbon tetrachloride in the presence of potassium carbonate afforded the corresponding iodide **7** in 95% isolated yield. A second Heck reaction using benzyl acrylate afforded the unsaturated compound **8** in 75% yield. Hydrogenation of **8** was accomplished in 1:1 ethanol:acetic acid with a minimal amount of ethyl acetate added to achieve solubility using 10% Pd/C as a catalyst to provide ethyl 4-(3-carboxypropyl)-6-dibenzofuran propionate (**3**) in 95% isolated yield. Hence, the unsymmetrical monoacid monoester **3** was obtained from dibenzofuran in six steps with an overall yield of 46%. Intermediate **3** can then be converted to the Boc-derivative **11** as reported previously[1,2,11] in an overall yield of 35% from dibenzofuran (Figure 3). The Fmoc-N-terminally protected derivative can be prepared directly from **3**; however, the overall

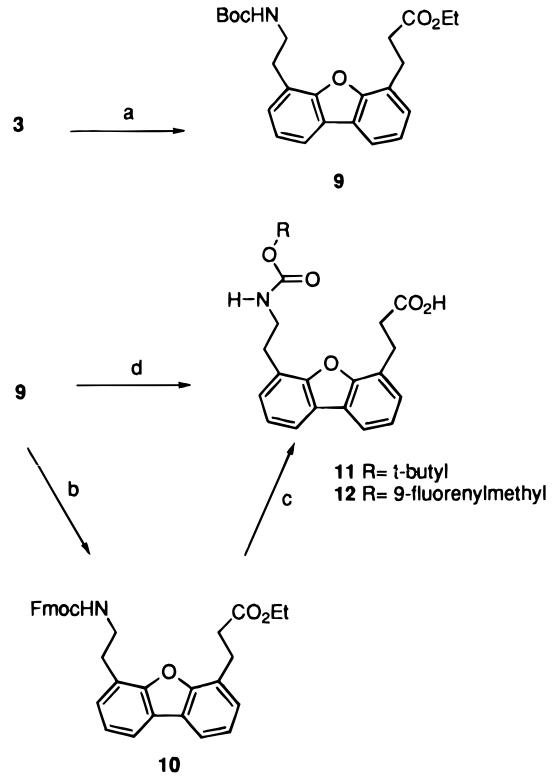

Figure 3. Outline of the synthesis of Fmoc- or Boc-N-terminally protected amino acid from **3**. Reaction conditions: (a) 2.0 equiv of diphenyl phosphorazidate, TEA, *t*-BUOH, reflux, 75%; (b) (i) 30% TFA CH_2Cl_2, 50 min, (ii) CH_2Cl_2, Na_2CO_3, Fmoc-OSu, 77%; (c) 5% HCl/AcOH, reflux, 99%; (d) NaOH, EtOH, reflux, 95%.

yield and ease of product isolation were improved by proceeding from the Boc derivative **9** (Figure 3). TFA mediated removal of the Boc group in **9**, and subsequent carbamylation with 9-(fluorenylmethyloxycarbonyl)-*N*-hydroxysuccinimide (Fmoc-OSu) afforded **10** in a 78% yield. Hydrolysis of the ethyl ester was achieved with 5% HCl in acetic acid to yield **12** in an overall isolated yield of 30% from dibenzofuran.[19] Purification of the

intermediates in this sequence is significantly easier than for the previous synthesis, primarily because starting material and product have quite different polarities.

Conclusion

The sequential metalation of dibenzofuran provides a method for differentiation of the 4 and 6 positions at the beginning of the synthesis of the 4-(2′-aminoethyl)-6-dibenzofuranpropionic acid β-sheet nucleator. The desymmetrizing metalation reactions in combination with sequential Heck reactions contribute to a new practical preparative synthesis of both the Boc- and Fmoc-derivatives of 4-(2-aminoethyl)-6-dibenzofuranpropionic acid. Purification of the intermediates is easier in the new route, and the overall yields from dibenzofuran for the Boc derivative (35%) and the new Fmoc derivative (30%) are respectable.

Experimental Section

General Methods. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium/benzophenone ketyl under nitrogen (N_2). The concentration of *n*-BuLi was determined by titration with isobutyl alcohol in the presence of 1,10-phenanthroline as an indicator. Trimethylsilyl chloride (TMSCl) was distilled from calcium hydride under N_2. Triethylamine (TEA) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) were refluxed over ninhydrin, distilled, and then redistilled from calcium hydride or potassium hydroxide. Column chromatography was performed as described by Still.[20] Preparative HPLC was carried out on Waters 600 preparative HPLC using C_4 column. Solvent A was composed of 95% water, 5% acetonitrile, and 0.2% TFA. Solvent B was composed of 5% water, 95% acetonitrile, and 0.2% TFA. Unless otherwise noted, all reactions were run under argon or nitrogen. Coupling constants (J) were reported in Hz.

4-(Trimethylsilyl)dibenzofuran (4). To a solution of dibenzofuran (21.8 g, 129.6 mmol) in THF (260 mL) at 0 °C was added *n*-BuLi (97.5 mL of a 1.6 M hexanes solution, 156 mmol) dropwise over 50 min. The resulting dark orange solution was stirred at rt for 5 h and then cooled to 0 °C. Addition of TMSCl (32.8 mL, 258 mmol) in THF (33 mL) over 5 min resulted in the solution becoming yellow. The reaction mixture was stirred at room temperature for 20 min (after this interval it became cloudy), refluxed for 2 h, and then stirred at rt overnight. The reaction mixture was poured into 250 g of crushed ice and stirred. The mixture was then transferred to a separatory funnel and diluted with ether (100 mL). The aqueous layer was extracted with ether (3 × 75 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to afford 31.6 g (99%) of a pale yellow oil that was determined by GCMS and HPLC to contain a 95:5 ratio of 4-(trimethylsilyl)-dibenzofuran:4,6-bis(trimethylsilyl)dibenzofuran. The mixture was used in the subsequent iodination reaction without further purification: ^{1}H NMR for the 95:5 mixture (acetone-d_6, 200 MHz) δ 8.13−8.07 (m, 2 H), 7.69−7.32 (m, 5 H), 0.46 (s, 8 H); ^{13}C NMR (acetone-d_6, 50 MHz) δ 133.79, 128.43, 124.00, 123.11, 122.02, 112.70, −0.07; EIMS *m*/*z* 240.0972, M^+ calcd for $C_{15}H_{16}OSi$ 240.0970.

4-Iodo-6-(trimethylsilyl)dibenzofuran (5). To a solution of 4-(trimethylsilyl)dibenzofuran **(4)** (7.93g, 33.0 mmol, including 5% 4,6-bis(trimethylsilyl)dibenzofuran in Et_2O (70 mL) and TMEDA (7.97 mL, 52.8 mmol) at 0 °C was slowly added *n*-BuLi (33.0 mL of 1.6 M solution in hexanes, 52.8 mmol) via syringe. The resulting dark brown solution was then stirred at room temperature for 5.5 h. The solution of dibenzofuran anion was then slowly added via cannula to a stirred solution of I_2 (19.2 g, 75.9 mmol) in Et_2O (70 mL) at −78 °C. The resulting brown mixture was allowed to warm to rt while being stirred overnight. The mixture was transferred to a cold (0 °C) solution of 20% $NaHSO_3$ (∼500 mL) and stirred vigorously. It was diluted with CH_2Cl_2 (∼200 mL), and additional solid $NaHSO_3$ was added until the mixture became pale yellow. Vigorous stirring was continued for 2.5 h. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were extracted with 20% $NaHSO_3$ (2 × 150 mL), dried over magnesium sulfate, and concentrated under reduced pressure to afford a pale yellow solid. Dissolving the yellow solid in hexanes allowed for separation of a brown, gummy, insoluble impurity. Evaporation of the hexanes in vacuo and silica gel chromatography (100% hexanes) gave **5** (8.57g) as a cream-colored solid (75% based on 95% monosilylated dibenzofuran in the starting material). An analytical sample could be obtained after purification by preparative C_4 HPLC as a white solid: mp 74−77 °C; ^{1}H NMR ($CDCl_3$, 200MHz) δ 7.92 (dd, J_o = 7.6, J_m = 1.4, 1 H), 7.90 (dd, J_o = 7.6, J_m = 1.5, 1 H), 7.79 (dd, J_o = 7.8, J_m = 1.1, 1 H), 7.56 (dd, J_o = 7.2, J_m = 1.4, 1 H), 7.35 (t, J_o = 7.6, 1 H), 7.09 (t, J_o = 7.7, 1 H), 0.50 (s, 7 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 156.33, 150.04, 135.47, 132.91, 124.39, 124.12, 123.50, 122.94, 122.88, 122.03, 120.47, −1.06; EIMS *m*/*z* 365.9877, M^+ calcd for $C_{15}H_{15}OISi$ 365.9939 and 350.9676, $[M - CH_3]^+$ calcd 350.9704.

Ethyl 4-(Trimethylsilyl)-6-dibenzofuranpropenoate (6). A round-bottomed flask containing 4-iodo-6-(trimethylsilyl)-dibenzofuran **(5)** (3.82 g, 10.4 mmol), palladium acetate (49.2 mg, 2.1 mol %), and tri-*o*-tolylphosphine (0.162 g, 5.1 mol %) was fitted with a condenser, and the system was alternately evacuated under high vacuum and flushed with Ar (four cycles). Acetonitrile (16.5 mL), triethylamine (4.36 mL, 31.1 mmol), and ethyl acrylate (3.39 mL, 31.3 mmol) were added through the condenser, and the mixture was heated to 85 °C in an oil bath. The reaction mixture was cooled to rt when the palladium precipitated after 2 h. The mixture was concentrated under reduced pressure to give a yellow-brown solid that was then partitioned between CH_2Cl_2 (120 mL) and H_2O (150 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 75 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by flash chromatography (95:5 hexanes:ethyl acetate) to afford the pure compound (3.27 g, 93%) as an oil that solidified upon standing to afford a beige solid: mp 48−52 °C; ^{1}H NMR (acetone-d_6, 200 MHz) δ 8.16 (d, J_o = 7.6, 2 H), 7.98 (d, J = 16.2, 1 H), 7.77 (dd, J_o = 7.0, J_p = 0.6, 1 H), 7.64 (dd, J_o = 7.2, J_m = 1.3, 1 H), 7.44 (td, J_o = 7.6, J_m = 1.6, 2 H), 7.20 (d, J = 16.2, 1 H), 4.28 (q, J = 7.1, 2 H), 1.34 (t, J = 7.1, 3 H), 0.52 (s, 8 H); ^{13}C NMR (acetone-d_6, 50 MHz) δ 167.51, 139.99, 134.60, 134.32, 129.78, 126.14, 124.59, 124.55, 124.08, 123.89, 123.36, 123.25, 122.98, 120.74, 61.27, 14.99, −0.56; EIMS *m*/*z* 338.1349, M^+ calcd for $C_{20}H_{22}O_3Si$ 338.1338.

Ethyl 4-Iodo-6-dibenzofuranpropenoate (7). A 1 L round-bottomed flask charged with ethyl 4-(trimethylsilyl)-6-dibenzofuranpropenoate **(6)** (11.37 g, 33.6 mmol), anhydrous K_2CO_3 (14.22 g, 102.9 mmol), and anhydrous CCl_4 (230 mL) was cooled to 0 °C. Then a solution of ICl (5.0 mL, 95.5 mmol) in anhydrous CCl_4 (34 mL) was added to the mixture via a Teflon cannula at 0 °C. The resulting 2.8 M solution was then transferred to the cold reaction flask via the Teflon cannula. The red wine colored solution was stirred at rt in darkness overnight. The reaction mixture was poured into 500 mL of a 20% $Na_2S_2O_3$ solution and vigorously stirred. Additional solid $Na_2S_2O_3$ was added, and the mixture was stirred until a very pale yellow color persisted. The mixture was transferred to a separatory funnel, and the aqueous layer was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were washed with 20% $Na_2S_2O_3$ (3 × 500 mL), 10% HCl (2 × 500 mL), 1 N NaOH (3 × 500 mL), and H_2O (1 × 500 mL). The colorless organic layer was then dried over $MgSO_4$ (solution became pale pink) and concentrated in vacuo to give 16.60 g of a beige solid. After further drying under high vacuum, the solid was crushed and stirred in a minimal amount of hexanes and ethyl acetate solution (95:5) for 2 h. This resulted in the removal of the pink color after filtration 12.5 g (95%) of white solid: mp 114−116 °C; ^{1}H NMR (acetone-d_6, 200 MHz) δ 8.15 (dd, J_o = 7.7, J_m = 1.1, 1 H), 8.14 (dd, J_o = 7.7, J_m = 1.1, 1 H), 7.96 (d, J = 16.1, 1 H), 7.95 (d, J_o = 7.8, 1 H), 7.82 (dt, J_o = 7.7, J_p = 0.6, 1 H), 7.48 (t, J_o = 7.6, 1 H), 7.25 (t, J_o = 7.7, 1 H), 7.15 (d, J = 16.1, 1 H), 4.29 (q, J = 7.1, 2 H), 1.34 (t, J =

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7.1, 3 H); ^{13}C NMR (acetone-d_6, 50 MHz) δ 167.52, 150.05, 139.50, 137.77, 130.41, 126.59, 126.48, 125.21, 125.03, 124.50, 123.40, 122.41, 121.02, 76.29, 69.67, 61.38, 15.04; MS $^+$FAB (NBA) *m/z* 393.0001, $[M + H]^+$ calcd for $C_{17}H_{13}O_3I$ 392.9988.

Ethyl 4-[3-(Benzyloxycarbonyl)propenyl]-6-dibenzofuranpropenoate (8). An oven-dried, 50 mL round-bottomed flask was charged with ethyl 4-iodo-6-dibenzofuranpropenoate **(7)** (5.00 g, 12.75 mmol), palladium acetate (62.9 mg, 2.2 mol %), and tri-*o*-tolylphosphine (194 mg, 5.0 mol %), and the flask was fitted with a condenser. The system was alternately evacuated under high vacuum and purged with Ar (four cycles). Acetonitrile (40 mL), triethylamine (3.5 mL, 25.5 mmol), and benzyl acrylate (3.9 mL, 25.5 mmol) were added via syringe through the condenser. The resulting dark brown mixture was heated to 85 °C in an oil bath. The reaction mixture was cooled to rt after 5 h when the palladium precipitated. After removal of solvent under reduced pressure, the mixture was taken up in CH_2Cl_2 (200 mL) and washed with H_2O (150 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. Purification by flash chromatography (70:30 hexanes:ethyl acetate) afforded 4.05 g (75%) of a yellowish white solid: mp 184−187 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.06 (d, J = 16.2, 1H), 8.02 (d, J = 16.2, 1 H), 7.97 (d, J = 7.5, 2H), 7.61 (dd J_o = 7.5, J_m = 3.6, 2 H), 7.49−7.33 (m, 7 H), 7.08 (d, J = 16.2, 1H), 7.00 (d, J = 16.2, 1 H), 5.34 (s, 2H), 4.60 (q, J = 7.2, 2 H), 1.32 (t, J = 7.1, 3 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.85, 151.08, 136.23, 135.32, 133.10, 125.44, 125.05, 121.35, 120.49, 119.22, 118.69, 118.259, 116.80, 63.28, 57.52, 11.24; MS $^+$FAB (NBA) *m/z* 427.1554, $[M + H]^+$ calcd for $C_{27}H_{22}O_5$ 427.1545.

Ethyl 4-(3-Carboxypropyl)-6-dibenzofuranpropionate (3). A 500 mL hydrogenation bottle was charged with ethyl 4-[3-(benzyloxycarbonyl)propenyl]-6-dibenzofuranpropenoate **(8)** (2.44 g, 5.75 mmol) in a 1:1 mixture of ethanol and acetic acid (150 mL) and a minimal amount of ethyl acetate to help with the solublization. Then 10% Pd/C catalyst (300 mg, 5 mol %) was added, and the mixture was hydrogenated at 55 psi H_2 for 24 h. The catalyst was removed by filtration through a nylon membrane, and the solvents were removed under reduced pressure. The resulting pale yellow solid was dissolved in ether (50 mL) and washed with H_2O (2 × 30 mL). The organic layer was dried over $MgSO_4$, concentrated under reduced pressure, and further dried under high vacuum to afford the saturated monoacid monoester (1.85 g, 95%) as a white solid: mp 92−93 °C, ^{1}H NMR (acetone-d_6, 200 MHz) δ 10.65 (bs, 1H), 7.92 (dd, J_o = 7.4, J_m = 1.5, 2 H), 7.39 (m, 2 H), 7.30 (m, 2 H), 4.07 (q, J = 7.1, 2 H), 3.31 (t, J = 7.6, 4 H), 2.85 (m, 4 H), 1.16 (t, J = 7.1, 3 H); ^{13}C NMR (CD_3Cl, 50 MHz) δ 178.91, 173.28, 154.38, 127.17, 127.12, 124.47, 124.30, 124.18, 122.89, 119.01, 118.90, 80.63, 34.28, 33.95, 25.48, 25.23, 14.19; MS $^+$FAB (NBA) *m/z* 340.1333, $[M + H]^+$ calcd for $C_{20}H_{20}O_5$ 340.1311.

Ethyl 4-[2-[(*tert*-butyloxy)carbamyl]ethyl]-6-dibenzofuranpropionate (9). Compound **9** was synthesized exactly as previously described in ref 3. Briefly, a solution containing the monoacid monoester **3**, *tert*-butyl alcohol, triethylamine, and diphenyl phosphorazidate was heated at reflux for 24 h. The solvent was removed under reduced pressure, and the crude was dissolved in ether and washed with 2 M citric acid, 5% sodium bicarbonate, and water. The organic layer was dried with Na_2SO_4 and the solvent removed to afford the pale yellow solid **9**. The crude was recrystallized from 3:1 EtOH/H_2O to afford the Curtius product **9** (75%) (see ref 3 for physical and spectroscopic data).

Ethyl 4-[2-(9-Fluorenylmethyloxycarbamyl)ethyl]-6-dibenzofuranpropionate (10). A 50 mL round-bottomed flask was charged with 0.18 g (0.44 mmol) of **9** and evacuated under high vacuum for 2 h and back-filled with Ar. A 30% v/v solution of trifluoroacetic acid in dichloromethane (20 mL) was added and the resulting mixture stirred for 50 min at rt. The solvent was removed in vacuo and the residue redissolved in 20 mL of CH_2Cl_2 to which 0.500 g of Na_2CO_3 was added. The mixture was stirred at rt for 10 min to consume any remaining TFA, and FMOC-OSu (0.200 g 0.60 mmol) was added as a solid. This mixture was stirred for 12 h, followed by removal of Na_2CO_3 by filtration through a 45 μm nylon membrane. Removal of CH_2Cl_2 under reduced pressure yields an oil that was purified by flash chromatography (70:30, hexanes:ethyl acetate) to give 0.180 g (77% yield) of a white solid: mp 157−159 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.18 (t, J = 7, 3H), 2.79 (t, J = 6.3, 2H), 3.22 (t, J = 6.0, 2H), 3.31 (t, J = 6.2, 2H), 3.68 (t, J = 6.5, 2H), 4.1 (m, 3H), 4.36 (d, J = 7.4, 2H), 5.05 (t, J = 7, 1H), 7.24 (m, 6H), 7.37 (t, J = 7.5, 2H), 7.51 (d, J = 7.5, 2H), 7.73 (d, J = 7.6, 2H), 7.82 (m, 2H); MS(FAB-NBA/CH_2Cl_2) $[M + H]$ = 534.2287, calcd for $C_{34}H_{31}NO_5$ $[M + H]$ 534.2281.

Hydrolysis of 9 To Afford 11. The Boc amino acid was stored as **9** and hydrolyzed just prior to use. The ethyl ester in **9** was hydrolyzed exactly as described in detail previously in ref 3. Briefly, a 50 mL round-bottomed flask charged with 4 g (9.7 mmol) of **9**, 30 mL of absolute ethanol, and 0.36 g (14.6 mmol) of NaOH was heated at reflux for 2 h. The solvent was removed under reduced pressure, affording a solid that was partitioned between 50 mL of CH_2Cl_2 and 100 mL of 0.5 M citric acid. The organic layer was separated, and the aqueous layer was washed with CH_2Cl_2 (2 × 50 mL). The combined organic layers were dried with $MgSO_4$, and the solvent was removed to afford 3.52 g (95%) of **11**, which is used directly in solid-phase peptide synthesis: ^{1}H NMR ($(CD_3)CO$, 200 MHz) δ 1.35 (s, 9H), 2.84 (t, J = 7.7, 2H), 3.20 (t, J = 7.7, 2H), 3.32 (t, J = 7.7, 2H), 3.56 (q, J = 7.7, 2H), 6.16 (bs, 0.8H), 7.33 (m, 4H), 7.90 (m, 1H), 7.94 (d, J = 1.6, 1H), 10.8 (bs, 0.5H) (see ref 3 for more details).

Hydrolysis of 10 To Afford 12. The FMOC amino acid was stored as **10** and hydrolyzed just prior to use, adapting a published procedure by Carpino et al.[19] Briefly, a 100 mL round-bottomed flask fitted with a reflux condensor was charged with **10** (0.75 g, 1.4 mmol) and 25 mL of 8% HCl in acetic acid (23.5 mL of acetic acid:1.5 mL of cond HCl) that was heated at reflux for 12 h. The reaction mixture was poured into 50 mL of distilled H_2O. The resulting precipitate was extracted with CH_2Cl_2 (3 × 50 mL), dried, and concentrated to yield 0.73 g (99% yield) of **12** as a white solid: ^{1}H($CDCl_3$ 300 MHz) δ 2.79 (t, J = 6.0, 2H), 3.22 (t, J = 6.3, 2H), 3.37 (t, J = 6.5, 2H), 3.68 (q, J = 7.0, 2H), 4.08 (m, 3H), 4.40 (d, J = 6.4, 2H), 5.05 (br s,1H), 7.24 (m, 6H), 7.43 (m, 2H), 7.51 (m, 2H), 7.77 (m, 2H), 7.83 (m, 2H); $C_{32}H_{27}NO_5$ MS (FAB$^-$ NBA/acetone) $[M + Na]^+$ = 528.1793, calcd $[M + Na]^+$ = 528.1787.

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Supporting Information Available: ^{1}H NMR spectra for new compounds **4**−**8** and **10** (6 pages). This material is contained in libraries 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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