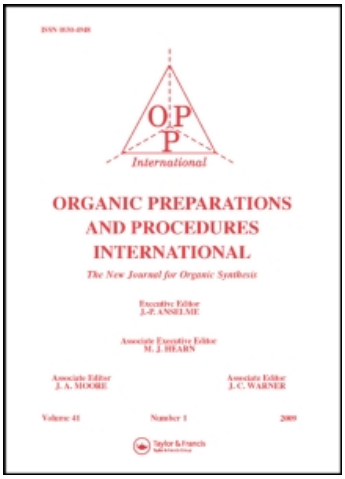

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SYNTHESIS OF (*S*)-(-)-1-[(9-FLUORENYLMETHOXYCARBONYL)AMINO-5-CARBOXYPENTYL]-3-HYDROXYPYRIDINIUM TRIFLUOROACETATE

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OPPI BRIEFS

SYNTHESIS OF (*S*)-(–)-1-[(9-FLUORENYLMETHOXYCARBONYL)AMINO-5-CARBOXYPENTYL]-3-HYDROXYPYRIDINIUM TRIFLUOROACETATE

Submitted by (02/10/00)

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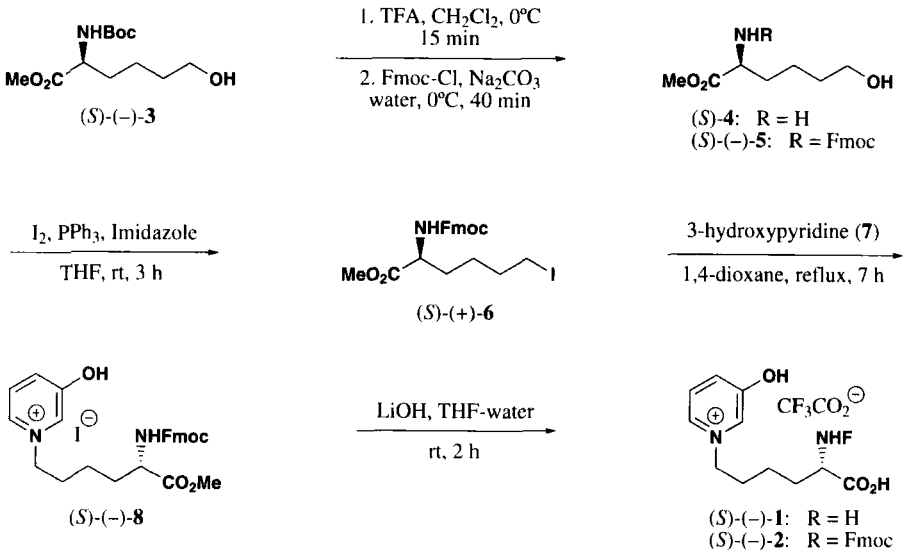
Derivatives of (*S*)-(–)-1-(amino-5-carboxypentyl]-3-hydroxypyridinium salt **1** are present in bone collagen and play an important role in maintaining the integrity of this tissue.[1,2] The synthesis of racemic (±)-**1** starting from *tert*-butyl-(2-*tert*-butoxycarbonyl)amino acetate has been reported previously.[3] We present here the preparation of an important chiral building block, (*S*)-(–)-Fmoc derivative of **1** (compound **2**), needed for the synthesis of peptides comprising collagen, *via* solid phase technology.

Accordingly, (*S*)-(–)-methyl 2-[(*tert*-butoxycarbonyl)amino]-6-hydroxypentanoate (**3**), which was prepared from *N*-α-*tert*-Boc-lysine,[4] was hydrolyzed using trifluoroacetic acid in methylene chloride to give the (*S*)-amine (**4**) which without purification, was treated with 9-fluorenylmethyl chloroformate[5] to afford (*S*)-(–)-methyl 2-[(9-fluorenylmethoxycarbonyl)amino]-6-hydroxypentanoate (**5**) in 65% yield. The hydroxy functionality in (*S*)-**5** was transformed to the corresponding iodide (*S*)-(+)-**6** using iodine, triphenylphosphine and imidazole in 81% yield. The iodide (*S*)-(+)-**6** was then treated with 2.0 equiv. of 3-hydroxypyridine (**7**) in refluxing acetonitrile[3] to form the pyridinium compound (*S*)-(–)-**8** in 90% yield after purification by preparative reversed phase HPLC. Finally, the methyl ester was hydrolyzed using lithium hydroxide in THF-water to the corresponding acid (*S*)-(–)-**1**. Since, partial hydrolysis of Fmoc group was also observed under these conditions, as determined HPLC and ESI-MS, the crude product of LiOH hydrolysis was further treated with Fmoc-Cl. The resulting material was purified by preparative reversed phase HPLC to afford the desired (*S*)-(–)-1-[(9-fluorenylmethoxycarbonyl)amino-5-carboxypentyl]-3-hydroxypyridinium trifluoroacetate (**2**) in 48% yield.



Scheme 1

In summary, the chiral synthesis of (*S*)-(−)-1-[(9-fluorenylmethoxycarbonyl)amino-5-carboxypentyl]-3-hydroxypyridinium trifluoroacetate (**2**) was achieved starting from (*S*)-(−)-methyl 2-[(*tert*-butoxycarbonyl)amino]-6-hydroxypentanoate (**3**).

EXPERIMENTAL SECTION

^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz). Electrospray ionization mass spectrometry (ESI-MS) was carried on a Perkin-Elmer (Norwalk, CT) Sciex API 100 Benchtop system employing Turbo Ionspray ion source and HRMS were obtained on a Nermang 3010 MS-50, JEOL SX102-A mass spectrometers. All reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Sigma Chemical Co. (St. Louis, MO) and used without purification, except where noted. All the solvents employed were of HPLC grade purchased from EM Science (Gibbstown, NJ) and used as received. Analytical reversed phase (RP) HPLC was performed using a Waters μBondapak RCM C18 10μ (8 x 100 mm) column (solvents ratio v/v reported). Preparative reversed phase (RP) HPLC was performed using a Waters μBondapak RCM C18 10μ (40 x 100 mm) column (solvents ratio v/v reported). Virtis Freezemobile-25SL purchased from The Virtis Company, Gardiner, NY was used for lyophilization. Optical rotations were measured on Autopol III polarimeter from Rudolph Research, Flanders, NJ.

(*S*)-(+)-Methyl 2-[(9-fluorenylmethoxycarbonyl)amino]-6-hydroxyhexanoate (5).- Trifluoroacetic acid (10.0 mL) was added to a solution (*S*)-(−)-methyl 2-[(*tert*-butoxycarbonyl)amino]-6-hydroxyhexanoate (**3**, 3.44 g, 13.18 mmol) in CH_2Cl_2 (20 mL) at 0°. After stirring for 30 min, the mixture was concentrated using a rotary evaporator. Toluene (2 x 20 mL) was added to the residue and the trace amounts of trifluoroacetic acid were removed by azeotropic distillation on a rotary evaporator. The resulting crude amine (*S*)-**4** was dissolved in THF (26 mL), and the solution was cooled to 0-5°. To

this mixture, was added solution of sodium carbonate (4.15 g, 39.54 mmol, 3.0 equiv.) in water (26 mL), followed by 9-fluorenylmethyl chloroformate (Fmoc-Cl, 3.74 g, 14.49 mmol, 1.1 equiv.) in portionwise over a 10 min period. After the addition was complete, the mixture was stirred for an additional 30 min and diluted with water (100 mL) and EtOAc (150 mL). The organic layer was separated and the aqueous layer extracted with EtOAc (2 x 150 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$) and concentrated on a rotary evaporator. The crude product was purified by silica gel column chromatography (60% EtOAc in hexanes) to afford 3.256 g of (*S*)-(+)-**5** in 65% yield as a colorless viscous oil. R_f: 0.42 (60% EtOAc in hexanes). Analytical RP HPLC: MeCN: 0.1% aqueous trifluoroacetic acid/50:50, 2.0 mL/min at 225 nm, R_t: 6.65 min, >99%; $[\alpha]_D^{23}$ +6.1° (c 0.59, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.76 (d, 2 H, *J* = 7.2 Hz), 7.60 (d, 2 H, *J* = 6.9 Hz), 7.44 – 7.29 (m, 4 H), 5.33 (d, 1 H, *J* = 11.1 Hz), 4.44 – 4.38 (m, 3 H), 4.22 (t, 1 H, *J* = 7.2 Hz), 3.76 (s, 3 H), 3.65 (t, 2 H, *J* = 5.1 Hz), 1.94 – 1.30 (m, 6 H); ^{13}C NMR ($CDCl_3$): δ 172.9, 155.9, 143.9, 143.7, 141.3, 127.7, 127.1, 125.1, 120.0, 77.2, 67.0, 62.5, 53.7, 52.4, 47.1, 32.5, 32.1, 21.5; ESI-MS (*m/z*): 384 $(M + H)^+$, 401 $(M + NH_4)^+$, 406 $(M + Na)^+$, 789 $(2 \times M + Na)^+$; HRMS (FAB, m/z): calcd for $C_{22}H_{26}NO_5$, 384.1811 $(M + H)^+$; observed, 384.1819.

(*S*)-(+)-Methyl 2-[(9-Fluorenylmethoxycarbonyl)amino]-6-iodohexanoate (6).- Triphenylphosphine (3.29 g, 12.57 mmol, 1.5 equiv.), imidazole (0.911 g, 13.41 mmol, 1.6 equiv.) and iodine (3.15 g, 12.57 mmol, 1.5 equiv.) were added sequentially to a solution of (*S*)-(+)-**5** (3.21 g, 8.38 mmol) in THF (84 mL) at room temperature under nitrogen. After stirring for 3 h, the solvent was removed on a rotary evaporator to dryness and the crude product was purified by silica gel column chromatography (10-25% EtOAc in hexanes) to afford 3.326 g of iodide (*S*)-(+)-**6** in 81% yield as a white solid. R_f: 0.32 (20% EtOAc in hexanes), mp. 121-123°. Analytical RP HPLC: MeCN: 0.1% aqueous trifluoroacetic acid/80:20, 2.0 mL/min at 225 nm, R_t: 3.93 min, >99%; $[\alpha]_D^{23}$ +11.9° (c 0.85, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.77 (d, 2 H, *J* = 7.5 Hz), 7.60 (d, 2 H, *J* = 7.2 Hz), 7.43 – 7.29 (m, 4 H), 5.31 (d, 1 H, *J* = 8.1 Hz), 4.44 – 4.34 (m, 3 H), 4.23 (t, 1 H, *J* = 6.9 Hz), 3.77 (s, 3 H), 3.18 (t, 2 H, *J* = 6.6 Hz), 1.92 – 1.32 (m, 6 H); ^{13}C NMR ($CDCl_3$): δ 172.7, 155.8, 143.8, 143.7, 141.3, 127.7, 127.1, 125.1, 120.0, 119.9, 77.2, 67.0, 53.5, 52.5, 47.2, 32.6, 31.5, 26.0, 6.1; ESI-MS (*m/z*): 494 $(M + H)^+$, 511 $(M + NH_4)^+$, 1004 $(2 \times M + NH_4)^+$; HRMS (FAB, m/z): calcd for $C_{22}H_{25}NO_4I$, 494.0828 $(M + H)^+$; observed, 494.0837.

Anal. Calcd for $C_{22}H_{25}INO_4$: C, 53.56; H, 4.90; N, 2.84; I, 25.72
Found: C, 53.47; H, 4.80; N, 2.69; I, 25.39

(*S*)-(–)-Pyridinium Compound (8).- A mixture of iodide (*S*)-(+)-**6** (3.295 g, 6.684 mmol) and 3-hydroxypyridine (**7**, 1.90 g, 20.05 mmol, 3.0 equiv.) in anhydrous 1,4-dioxane (134 mL) was gently refluxed for 7 h under nitrogen. The solvent was removed on a rotary evaporator to dryness and the crude product was purified by preparative RP HPLC (MeCN: 0.1% aqueous trifluoroacetic acid/45:55, 45 mL/min at 225 nm). The solvent was removed on a rotary evaporator to about 200 mL and lyophilized to afford 2.749 g of (*S*)-(–)-**8** in 90% yield as a pale yellow viscous oil. Analytical RP HPLC: MeCN: 0.1% aqueous trifluoroacetic acid/50:50, 2.0 mL/min at 225 nm, R_t: 4.55 min, >99%;

$[\alpha]_D^{23}$ –17.1 (c 0.69, MeOH); ^{1}H NMR (CD_3OD): δ 8.47 (s, 1 H), 8.40 (d, 1 H, *J* = 5.4 Hz), 7.90 – 7.79 (m, 4 H), 7.65 (t, 2 H, *J* = 7.2 Hz), 7.40 (t, 2 H, *J* = 7.2 Hz), 7.33 – 7.28 (m, 2 H), 4.56 - 4.49 (m, 2 H), 4.44 – 4.29 (m, 2 H), 4.23 – 4.16 (m, 2 H), 3.70 (s, 3 H), 2.08 – 1.94 (m, 2 H), 1.94 – 1.82 (m, 1 H), 1.79 – 1.66 (m, 1 H), 1.50 – 1.36 (m, 2 H); ^{13}C NMR ($CDCl_3$): δ 174.2, 159.2, 158.7, 145.3, 145.2, 142.6, 136.6, 134.1, 132.5, 129.8, 128.8, 128.2, 128.1, 126.2, 126.1, 121.0, 67.9, 62.7, 54.9, 52.8, 31.9, 31.6, 23.5; ESI-MS (*m/z*): 881 $(M)^+$; HRMS (FAB, m/z): calcd for $C_{27}H_{29}N_2O_5$, 461.2076 $(M)^+$; observed, 461.2079.

(*S*)-(–)-1-[(9-Fluorenylmethoxycarbonyl)amino-5-carboxypentyl]-3-hydroxypyridinium Trifluoroacetate (2).- Lithium hydroxide monohydrate (0.707 g, 17.245 mmol, 3.0 equiv.) was added to a solution of pyridinium compound (*S*)-(–)-**8** (2.65 g, 5.75 mmol) in THF (40 mL) at room temperature. To this mixture, water (20 mL) was added and the reaction mixture was stirred for 1.5 h. Analysis of the reaction mixture by analytical RP HPLC (MeCN: 0.1% aqueous trifluoroacetic acid/50:50, 2.0 mL/min at 225 nm) and by ESI-MS indicated the disappearance of starting ester (*S*)-(–)-**8**, but in addition to the desired Fmoc-acid (*S*)-(–)-**2**, some corresponding free amino acid (*S*)-(–)-**1** was also present. The solvent was evaporated to dryness on rotary evaporator, the residue dissolved in water (25 mL) and the pH adjusted to 3.5 using 6N HCl. The solvent was removed on a rotary evaporator, the resulting crude mixture of (*S*)-(–)-**2** and (*S*)-(–)-**1** was dissolved in THF (20 mL). The mixture was cooled in an ice bath and a solution of sodium carbonate (2.41 g, 23.0 mmol, 4.0 equiv.) in water (20 mL) was added. To this mixture, Fmoc-Cl (1.63 g, 6.3 mmol, 1.1 equiv.) was added portionwise over a 5 min period and the mixture was stirred for 30 min. The solvent was removed on a rotary evaporator, water (20 mL) added to the residue and the pH was adjusted to 6.0 using 6N HCl. The crude product was purified by preparative RP HPLC (MeCN:0.1% aq trifluoroacetic acid/38:62, 45 mL/min at 225 nm). The solvent was concentrated on a rotary evaporator to about 200 mL and lyophilized in a 250 mL round bottom flask, which was fitted in a 1200 mL Fast-Freeze flask, Labconco, to give 1.95 g of (*S*)-(–)-acid (**2**) as a pale yellow gummy material. This gummy (*S*)-(–)-acid (**2**) was dissolved in MeCN (10 mL) and azeotroped on a rotary evaporator using toluene (3 x 25 mL) followed by CH_2Cl_2 (3 x 25 mL). The product was dried *in vacuo* (0.5 mm Hg) at room temperature for 48 h to give 1.55 g of (*S*)-(–)-acid-TFA salt (**2**) in 48% yield as a pale yellow glassy material. Analytical RP HPLC: MeCN: 0.1% aqueous trifluoroacetic acid/40:60; 2.0 mL/min at 225 nm, R_t: 6.22 min, >99%; $[\alpha]_D^{23}$ –7.3° (c 0.51, MeOH); ^{1}H NMR (CD_3OD): δ 8.47 (s, 1 H), 8.40 (d, 1 H, *J* = 5.4 Hz), 7.88 – 7.78 (m, 4 H), 7.66 (t, 2 H, *J* = 8.1 Hz), 7.40 (t, 2 H, *J* = 7.8 Hz), 7.33 – 7.28 (m, 2 H), 4.57 – 4.48 (m, 2 H), 4.42 – 4.28 (m, 2 H), 4.23 – 4.16 (m, 2 H), 2.10 – 1.86 (m, 3 H), 1.80 – 1.68 (m, 1 H), 1.52 – 1.38 (m, 2 H); ^{13}C NMR (CD_3OD): δ 175.5, 159.2, 158.7, 145.3, 145.1, 142.6, 136.6, 134.1, 132.5, 129.7, 128.8, 128.2, 128.1, 126.3, 126.2, 121.0, 68.0, 62.8, 54.7, 32.0, 31.6, 23.5; ESI-MS (m/z): 447 $(M)^+$; HRMS (FAB, m/z): calcd for $C_{26}H_{27}N_2O_5$, 447.1920; observed: 447.1910.

Anal. Calcd for $C_{28}H_{28}F_3N_2O_7 \cdot H_2O$: C, 58.08; H, 5.22; N, 4.83. Found: C, 57.98; H, 4.98; N, 4.69

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MICROWAVE-ASSISTED SYNTHESIS OF TRIARYL PHOSPHATES

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(08/30/99)

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Triaryl phosphates are of considerable interest because of their utility as plasticizers, flame retardants, lubricating oil additives and hydraulic fluids.[1] They are synthesized by reaction of phosphoryl chloride with phenols at elevated temperature (160-250°) in the presence of a Lewis acid catalyst,[2] at room temperature using a phase transfer catalyst[3] or by using polymer supported phenoxides.[4] Patented procedures are also available in the literature[5,6] for the synthesis of triaryl phosphates. Some of the reported methods have limitations such as (i) drastic reaction conditions,[2] (ii) liberation of hydrogen chloride which may cause corrosion problems in industrial reactors, (iii) tedious work-up, and (iv) long reaction times. In certain cases, pyridine or dialkylanilines have been used to neutralize the liberated hydrogen chloride. High temperatures often lead to many by-products. Plasticizer grade triaryl phosphates must be of very high purity and the products obtained have to be distilled at low pressure for purification. Distillation at low pressures on an industrial scale raises serious problems.