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Collagen cross-links: a convenient synthesis of *tert*-butyl-(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-(2-oxiranyl)butanoate

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Abstract

An efficient synthesis of *tert*-butyl-(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-(2-oxiranyl) butanoate (**5**), the key intermediate for preparation of collagen cross-links (+)-pyridinoline (Pyd, **1**) and (+)-deoxypyridinoline (Dpd, **2**) was described from (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**6**) in six steps. Also, an improved synthesis of iodide (2*S*)-(−)-**4b** was presented.

1. Introduction

The cross-links, pyridinoline (Pyd, **1**)[1] and deoxypyridinoline (Dpd, **2**)[2] (Fig. 1), which are formed during the maturation of collagen, play an important role in governing the biochemical properties and functional integrity of this tissue.[3] During the process of bone resorption, cross-links **1**,**2** are released into the serum and excreted in the urine either in free form or linked to the different peptide fragments of collagen.[3] Therefore, increased levels of pyridinium cross-links **1**,**2** are present in the urine of patients with osteoporosis and other metabolic diseases. Thus, quantification of cross-links **1**,**2** is important for diagnosis of osteoporosis.[4–6] Unfortunately, Pyd (**1**) and Dpd (**2**) are isolated from natural sources such as animal bones (e.g., sheep, ox) in a very low yield by acid hydrolysis followed by several tedious purification steps,[7] and hence the cost of these biochemical markers **1**,**2** is extremely high.[8] Therefore, development of an efficient synthetic method for **1**,**2**, which are needed for immunoassay development, is critically important.[9,10] Recently, we described[9] (Fig. 1) the synthesis of natural (+)-Pyd (**1**) and (+)-Dpd (**2**), by quaternization of (*S*,*S*)-(−)-**3** with iodides (2*S*,5*R*)-(+)-**4a** or (2*S*)-(−)-**4b** followed by aqueous trifluoroacetic acid hydrolysis. The 3-hydroxypyridine derivative, (*S*,*S*)-(−)-**3** was synthesized from the epoxide (2*S*)-**5**, which in turn was prepared from (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**6**). Although our five-step synthesis of epoxide (2*S*)-**5** from acid (4*S*)-**6** enabled

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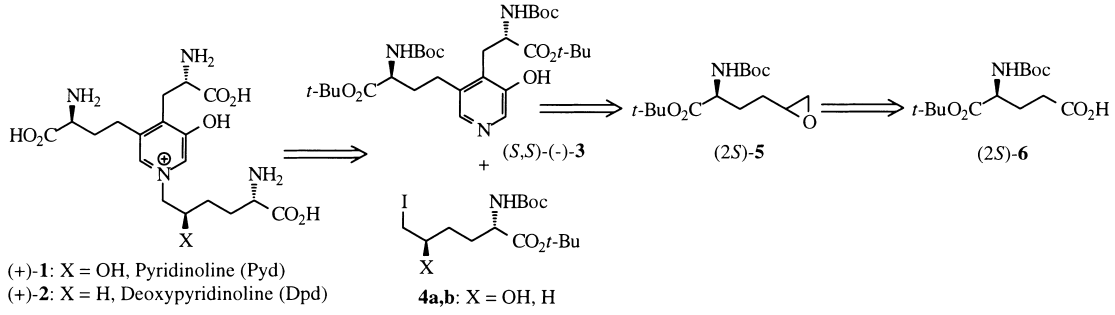
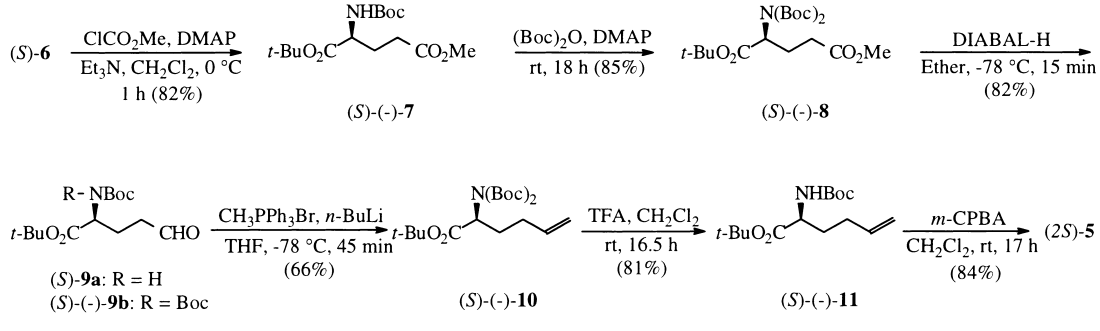


Figure 1.

us to produce the cross-links **1**,**2**, the method required the use of hazardous diazomethane and therefore poses problems for large scale preparation of **1** and **2**.[9] In this paper, we describe a convenient synthesis of the key synthon, *tert*-butyl-(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-(2-oxiranyl)butanoate (**5**) from a chiral pool starting material, (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**6**). An improved synthesis of (2*S*)-(−)-*tert*-butyl-2-[(*tert*-butoxycarbonyl)amino]-6-iodohexanoate (**4b**) required for preparation of (+)-Dpd (**2**) is also presented.

2. Results and discussion

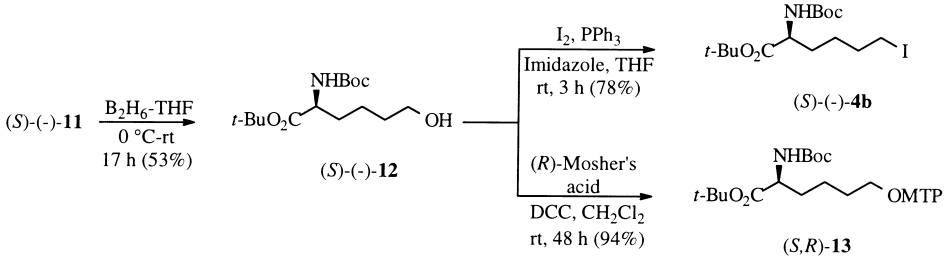
Our new strategy for synthesis of epoxide (2*S*)-**5** was based on the feasibility of extending aldehyde (*S*)-**9a** via a Wittig reaction to an olefin followed by oxidation. The commercially available L-glutamic acid derivative, (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**6**) was chosen as a starting material because, both C1-acid and amino groups were appropriately protected as dictated by the total synthesis of (+)-**1** and (+)-**2**.[9] Thus, esterification of (*S*)-**6** using methyl chloroformate in the presence of triethylamine and a catalytic amount of DMAP in methylene chloride gave (*S*)-(−)-1-*tert*-butyl-5-methyl-[(2-*tert*-butoxycarbonyl)amino]pentanedioate (**7**) in 85% yield (Scheme 1). The direct reduction of methyl ester in (*S*)-(−)-**7** to the corresponding aldehyde (*S*)-**9a** with DIBAL-H under various conditions produced a complex mixture of products. The difficulty in isolation of aldehyde (*S*)-**9a**, presumably due to the interference of nitrogen,[11] led us to introduce a second Boc group. Therefore, (*S*)-(−)-**7** was treated with $(Boc)_2O$ in the presence of DMAP in acetonitrile[12] followed by silica gel column chromatography to give bis-Boc compound (*S*)-(−)-**8** in excellent yield (85%). In contrast to the reduction of (*S*)-(−)-**7**, the bis-Boc compound (*S*)-(−)-**8** with DIBAL-H in ether at −78°C, underwent smooth conversion to aldehyde (*S*)-(−)-**9b**. The aldehyde (*S*)-(−)-**9b** was isolated as thick oil in 65% yield after purification by silica gel column chromatography.



Scheme 1.

The next step in the synthesis of *tert*-butyl-(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-(2-oxiranyl)butanoate (**5**) was to extend aldehyde (*S*)-(−)-**9b** to olefin (*S*)-(−)-**10**. Thus, Wittig reaction of aldehyde (*S*)-(−)-**9b** with the ylide generated from methyl triphenylphosphonium bromide and *n*-BuLi in THF at −78°C gave olefin (*S*)-(−)-**10** after silica gel column chromatography in 66% yield as thick oil. Selective removal of one of the Boc groups in (*S*)-(−)-**10** was accomplished by treatment with 1.5 equiv. of trifluoroacetic acid[13] in CH_2Cl_2 at room temperature. The desired mono-Boc compound (*S*)-(−)-**11** was isolated by silica gel column chromatography in excellent yield (81%).[14] Interestingly, no noticeable hydrolysis of the *tert*-butyl ester in (*S*)-(−)-**11** was observed under these mild reaction conditions. Finally, oxidation of olefin (*S*)-(−)-**11** with *m*-CPBA in CH_2Cl_2 at room temperature followed by silica gel column chromatography produced the desired epoxide (2*S*)-**5** in 91% yield as a 1:1 diastereomeric mixture.[9] Thus, the key synthon (2*S*)-**5** required for synthesis of cross-links **1**,**2** was prepared in six steps from (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**6**). The present method is suitable for preparation of large amounts of (2*S*)-**5** by avoiding the use of hazardous diazomethane.

The (*S*)-(−)-*tert*-butyl-6-2-[(*tert*-butoxycarbonyl)amino]-6-iodohexanoate (**4b**) required for quaternization of (*S*,*S*)-(−)-**3g** in the synthesis of (+)-Dpd (**2**) was previously prepared[9] from an L-lysine derivative, (2*S*)-(−)-6-amino-2-[(*tert*-butoxycarbonyl)amino]hexanoic acid. The reported synthesis[9] gave a moderate yield of (*S*)-(−)-**4b** involving the conversion of the C6-amino group in (2*S*)-(−)-6-amino-2-[(*tert*-butoxycarbonyl)amino]hexanoic acid to the hydroxyl group using sodium nitroprusside and aqueous sodium hydroxide as a key step. Alternatively, the iodide (*S*)-(−)-**4b** was realized (Scheme 2) from the olefin (*S*)-(−)-**11** in good overall yield, hence making (*S*)-(−)-**11** an extremely valuable common intermediate for both components of the (+)-Dpd (**2**) synthesis. Thus, hydroboration of the olefin (*S*)-(−)-**11** using diborane–THF complex and purification by silica gel column chromatography gave the alcohol (*S*)-(−)-**12** in 53% yield. The hydroxyl group in (*S*)-(−)-**12** was converted to the iodide (*S*)-(−)-**4b** using triphenylphosphine, iodine and imidazole in THF in 78% yield.[9] In order to confirm if any racemization had occurred to the (2*S*) chiral center during the synthesis of (2*S*)-**5** and (*S*)-(−)-**4b**, the hydroxy compound (*S*)-(−)**12** was converted (Scheme 2) to the corresponding MTP ester (*S*,*R*)-**13**. Thus, treatment of (*S*)-(−)**12** with 2.0 equiv. of (*R*)-(+)-α-methoxy-α-trifluoromethyl phenylacetic acid in the presence of DCC in methylene chloride at room temperature afforded the MTP ester (*S*,*R*)-**13** in 94% yield.[15] Analysis of (*S*,*R*)-**13** by ^1H and ^{19}F NMR clearly established that it was a single isomer (ee: >98%) and thus confirmed that the stereochemistry of (2*S*) chiral center was not affected throughout the synthesis.



Scheme 2.

In summary, an efficient synthesis of *tert*-butyl-(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-(2-oxiranyl)-butanoate (**5**), the key synthon for the preparation of collagen cross-links **1**,**2**, was described starting from a commercially available (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**6**) in six steps. An improved synthesis of (*S*)-(−)-*tert*-butyl-2-[(*tert*-butoxycarbonyl)amino]-6-iodohexanoate (**4b**) was achieved from the olefin (*S*)-(−)-**11** which is also a key component for prepara-

tion of (+)-deoxypyridinoline (**2**). The hydroxy compound (*S*)-(−)**12** was converted to the corresponding MTP ester (*S*,*R*)-**13** for confirmation of the stereochemistry of the (2*S*) chiral center.

3. Experimental

3.1. General methods and materials

^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz), the chemical shifts (δ) were reported in ppm relative to TMS, and coupling constants (*J*) were reported in hertz. Electrospray ionization mass spectrometry (ESI/MS) were carried using a Perkin–Elmer (Norwalk, CT) Sciex API 100 Benchtop system employing Turbo IonSpray ion source and the HRMS obtained using a Nermang 3010 MS-50, JEOL SX102-A. Thin layer chromatography was performed on pre-coated Whatman MK6F silica gel 60 Å plates (layer thickness: 250 μm) and visualized with UV light and/or using a $KMnO_4$ solution [$KMnO_4$ (1.0 g), NaOH (8.0 g) in water (200 mL)] or phosphomolybdic acid reagent (20 wt% solution in ethanol). Column chromatography was performed on silica gel, Merck grade 60 (230–400 mesh). Anhydrous solvents were freshly distilled [(THF from a purple solution of sodium and benzophenone) and (CH_2Cl_2 from CaH_2)] under nitrogen. 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 reverse phase (RP) HPLC was performed using a Waters μBondapak RCM C18 10μ (8×100 mm) column (solvents ratio v/v reported) unless otherwise stated. Optical rotations were measured on Autopol III polarimeter from Rudolph Research, Flanders, NJ.

3.2. (S)-(−)-1-tert-Butyl-5-methyl-[(2-tert-butoxycarbonyl)amino]pentanedioate (7)

Triethylamine (1.4 mL, 9.99 mmol, 1.5 equiv.) was added to a solution of (4*S*)-5-(*tert*-butoxy)-4-[(*tert*-butoxycarbonyl)amino]-5-oxopentanoic acid (**6**, 2.00 g, 6.59 mmol) dissolved in CH_2Cl_2 (30 mL) under nitrogen. The mixture was cooled to 0°C, 4-dimethylaminopyridine (DMAP, 0.081 g, 0.66 mmol, 0.1 equiv.) followed by methyl chloroformate (0.610 mL, 7.90 mmol, 1.2 equiv.) were added sequentially and the mixture stirred for 1 h. The reaction mixture was then diluted with CH_2Cl_2 (200 mL) and washed with aq. 1 M $NaHCO_3$ solution (200 mL). The organic layer was dried (Na_2SO_4) and the solvent removed on a rotary evaporator. Purification of the crude compound by silica gel column chromatography (30% EtOAc in hexanes) afforded 1.71 g of (*S*)-(−)-**7** in 82% yield as a thick oil. R_f: 0.48 (30% EtOAc in hexanes); analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid=60:40, 2.0 mL min at 215 nm, t_R: 4.54 min, >99%; $[\alpha]_D^{20}$ −28.2 (*c* 1.52, MeOH); ^{1}H NMR ($CDCl_3$): δ 5.07 (d, 1H, *J*=8.1 Hz), 4.24–4.16 (m, 1H), 3.68 (s, 3H), 2.48–2.29 (m, 2H), 2.22–2.08 (m, 1H), 1.97–1.85 (m, 1H), 1.46 (s, 9H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 173.3, 171.3, 155.3, 82.2, 79.7, 53.3, 51.7, 30.1, 28.3, 28.1, 28.0; ESI/MS (m/z): 318 $(M+H)^+$, 335 $(M+NH_4)^+$, 635 $(2\times M+H)^+$, 652 $(2\times M+NH_4)^+$.

3.3. (S)-(−)-1-tert-Butyl-5-methyl-2-[bis-(tert-butoxycarbonyl)amino]pentanedioate (8)

DMAP (0.127 g, 1.04 mmol, 0.2 equiv.) was added to a solution of (*S*)-(−)-**7** (1.65 g, 5.20 mmol) dissolved in acetonitrile (20 mL) under nitrogen. To this mixture, a solution of di-*tert*-butyldicarbonate (2.27 g, 10.4 mmol, 2.0 equiv.) in acetonitrile (10 mL) was added at room temperature via a double ended

needle and stirred for 18 h. The solvent was removed on a rotary evaporator below 40°C bath temperature and dried on a vacuum pump. The residue was purified by silica gel column chromatography (20% EtOAc in hexanes) to afford 1.84 g of (*S*)-(−)-**8** in 85% yield as a colorless thick oil. R_f: 0.5 (20% EtOAc in hexanes); $[\alpha]_D^{20}$ −24.5 (*c* 1.46, MeOH); analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid=60:40, 2.0 mL min at 215 nm, t_R: 10.17 min, 98%; ^{1}H NMR ($CDCl_3$): δ 4.80–4.75 (m, 1H), 3.66 (s, 3H), 2.43–2.35 (m, 3H), 2.19–2.11 (m, 1H), 1.49 (s, 18H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 173.4, 169.4, 152.4, 82.9, 81.4, 58.1, 51.6, 30.8, 27.9, 27.8, 24.6; ESI/MS (m/z): 418 $(M+H)^+$, 852 $(2\times M+NH_4)^+$.

3.4. (S)-(−)-1-tert-Butyl-2-[bis-(tert-butoxycarbonyl)amino]-5-oxopentanoate (9b)

Diisobutylaluminum hydride (DIBAL-H, 1.0 M soln in toluene, 15.0 mL, 15.0 mmol, 1.1 equiv.) was added dropwise to a −78°C cooled solution of (*S*)-(−)-**8** (5.568 g, 13.3 mmol) in anhydrous ether (165 mL) under nitrogen over 3 min. The reaction mixture was stirred for 15 min then quenched with water (3.3 mL) and allowed to warm to room temperature. The resulting white thick solution was filtered through celite powder and washed with ether (3×100 mL). The filtrate was concentrated and remaining trace amount of water was removed azeotropically using toluene (2×100 mL) on a rotary evaporator. The crude compound was purified by silica gel column chromatography (15% EtOAc in hexanes) to afford 4.24 g of (*S*)-(−)-**9b** in 82% yield as a thick oil. R_f: 0.37 (20% EtOAc in hexanes); $[\alpha]_D^{20}$ −23.1 (*c* 0.805, $CHCl_3$); analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid=70:30; 2.0 mL min at 225 nm, t_R: 2.94 min, 99%; ^{1}H NMR ($CDCl_3$): δ 9.67 (t, 1H, *J*=1.2 Hz), 4.73 (dd, 1H, *J*=9.6, 5.1 Hz), 2.62–2.37 (m, 3H), 2.19–2.04 (m, 1H), 1.50 (s, 18H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 201.5, 169.4, 152.5, 83.1, 81.5, 58.1, 40.6, 27.9, 27.8, 21.9; ESI/MS (m/z): 388 $(M+H)^+$, 405 $(M+NH_4)^+$.

3.5. (S)-(−)-1-tert-Butyl-2-[bis-(tert-butoxycarbonyl)amino]-5-hexenoate (10)

n-BuLi (2.5 M soln in hexane, 2.2 mL, 5.5 mmol, 2.0 equiv.) was added dropwise to a suspension of methyl triphenylphosphonium bromide (1.97 g, 5.5 mmol, 2.0 equiv.) in THF (40 mL) at room temperature under nitrogen. After stirring the mixture for 30 min, the resulting orange ylide solution was cooled to 0°C and a solution of (*S*)-(−)-**9b** (1.07 g, 2.76 mmol, 1.0 equiv.) in THF (10 mL) was added via a double ended needle. The reaction was stirred for 45 min at 0°C then quenched with saturated aq. NH_4Cl solution (10 mL). The mixture was diluted with water (50 mL) and extracted with EtOAc (3×50 mL). The combined organic layers were then dried (Na_2SO_4) and the solvent removed on a rotary evaporator. The crude compound was purified by silica gel column chromatography (10% EtOAc in hexanes) to afford 0.704 g of (*S*)-(−)-**10** in 66% yield as a thick oil. R_f: 0.62 (15% EtOAc in hexanes); $[\alpha]_D^{20}$ −16.19 (*c* 1.48, MeOH); analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid=70:30; 2.0 mL min at 225 nm, t_R: 4.90 min, 98%; ^{1}H NMR ($CDCl_3$): δ 5.88–5.74 (m, 1H), 5.07–4.95 (m, 2H), 4.75–4.70 (m, 1H), 2.22–2.06 (m, 3H), 2.00–1.89 (m, 1H), 1.50 (s, 18H), 1.44 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 170.0, 152.6, 137.6, 115.4, 82.7, 81.1, 58.3, 30.4, 28.6, 27.9, 29.8; ESI/MS (m/z): 386 $(M+H)^+$; 788 $(2\times M+NH_4)^+$.

3.6. (S)-(−)-1-tert-Butyl-2-[(tert-butoxycarbonyl)amino]-5-hexenoate (11)

Trifluoroacetic acid (0.170 mL, 2.2 mmol, 1.5 equiv.) was added to a solution of (*S*)-(−)-**10** (0.571 g, 1.48 mmol) dissolved in CH_2Cl_2 (15 mL) at room temperature and stirred for 16.5 h. The mixture was then diluted with ether (60 mL) and washed with 10% aq. NaOH (30 mL) and brine (30 mL). The organic layer was dried (Na_2SO_4) and the solvent was removed on a rotary evaporator. The crude compound was

purified by silica gel column chromatography (10% EtOAc in hexanes) to afford 0.343 g of (*S*)-(−)-**11** in 81% yield as a thick oil. R_f: 0.39 (15% EtOAc in hexanes); $[\alpha]_D^{20}$ −20.7 (*c* 1.41, MeOH); analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid=70:30, 2.0 mL min at 215 nm, t_R: 4.35 min, 98%; ^{1}H NMR ($CDCl_3$): δ 5.87–5.74 (m, 1H), 5.08–4.97 (m, 3H), 4.19 (q, 1H, *J*=12.9, 7.5 Hz), 2.18–2.03 (m, 2H), 1.93–1.81 (m, 1H), 1.74–1.62 (m, 1H), 1.46 (s, 9H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 172.1, 155.5, 137.4, 115.5, 81.8, 79.6, 53.5, 32.2, 29.3, 28.2, 27.9; ESI/MS (m/z): 286 $(M+H)^+$; 303 $(M+NH_4)^+$, 571 $(2\times M+H)^+$, 588 $(2\times M+NH_4)^+$.

*3.7. (2S)-*tert*-Butyl-2-[(*tert*-butoxycarbonyl)amino]-4-(2-oxiranyl)-hexanoate (**5**)*

m-Chloroperoxybenzoic acid (57–58%, 0.121 g, 0.7 mmol, 2.0 equiv.) was added to a solution (*S*)-(−)-**11** (0.100 g, 0.350 mmol) in CH_2Cl_2 (2 mL) at room temperature and stirred for 17 h. The reaction was quenched with 10% aq. sodium sulfite solution (2.0 mL) and stirred for 30 min. The mixture was diluted with water (10 mL) and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were washed with 4% aq. $NaHCO_3$ soln (3×10 mL), dried (Na_2SO_4) and the solvent removed on a rotary evaporator. The crude product was purified by silica gel column chromatography (20% EtOAc in hexanes) to afford 0.090 g of (2*S*)-**5g** in 86% yield as a mixture of diastereomers in 1:1 ratio.[9] R_f: 0.55 (30% EtOAc in hexanes); analytical RP HPLC: MeCN:0.1% aqueous trifluoroacetic acid=50:50, 2.0 mL min at 215 nm, t_R: 8.81 min, 98%; IR (KBr): 3348, 3079, 1730, 1711, 1523, 1357, 1158 cm^{-1}, ^{1}H NMR ($CDCl_3$): δ 5.12–5.00 (m, 1H), 4.26–4.16 (m, 1H), 2.96–2.90 (m, 1H), 2.78–2.74 (m, 1H), 2.50–2.46 (m, 1H), 2.02–1.62 (m, 4H), 1.46 (s, 9H), 1.44 (s, 9H); ESI/MS: 302 $(M+H)^+$, 319 $(M+NH_4)^+$; 324 $(M+Na)^+$, 620 $(2\times M+NH_4)^+$.

*3.8. (S)-(−)-*tert*-Butyl-2-[(*tert*-butoxycarbonyl)amino]-6-hydroxyhexanoate (**12**)*

(*S*)-(−)-1-*tert*-Butyl-2-[(*tert*-butoxycarbonyl)amino]-5-hexenoate (**11,** 0.233 g, 0.817 mmol) in THF (4 mL) was cooled to 0°C and a solution of borane–THF complex (1 M soln in THF, 1.1 mL, 1.1 mmol, 1.3 equiv.) was added under nitrogen. The cooling bath was removed, the mixture allowed to warm to room temperature and stirred for 17 h. The reaction was then cooled to 0°C, 1 N aq. NaOH (1.2 mL, 1.2 mmol, 1.5 equiv.) added followed by 30% H_2O_2 (1.0 mL), and stirred for 30 min. The mixture was diluted with water (10 mL) and extracted with EtOAc (3×10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated on a rotary evaporator. The crude product was purified by silica gel column chromatography (40% EtOAc in hexanes) to afford 0.132 g of (*S*)-(−)-**12** in 53% yield as a colorless oil. R_f: 0.23 (40% EtOAc in hexanes); analytical RP HPLC: MeCN:0.1% aqueous acetic acid=40:60, 2.0 mL min at 215 nm, t_R: 8.26 min, 99%; $[\alpha]_D^{20}$ −29.2 (*c* 1.32, MeOH) {lit.[9] $[\alpha]_D^{20}$ −27.5 (*c* 1.28, MeOH)}; ^{1}H NMR ($CDCl_3$): δ 5.05 (d, 1H, *J*=8.1 Hz), 4.20–4.09 (m, 1H), 3.62 (q, 2H, *J*=11.4, 5.1 Hz), 1.80–1.60 (m, 6H), 1.45 (s, 9H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 172.1, 155.6, 81.7, 79.6, 62.4, 53.7,32.6, 32.0, 28.2, 27.9, 21.2; ESI/MS (m/z): 303 $(M+H)^+$, 321 $(M+NH_4)^+$.

*3.9. MTP ester of (S)-(−)-*tert*-butyl-2-[(*tert*-butoxycarbonyl)amino]-6-hydroxyhexanoate (**13**)*

(*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid 0.075 g, 0.32 mmol, 2.0 equiv.), DCC (0.066 g, 0.32 mmol, 2.0 equiv.) and a catalytic amount of DMAP (0.010 g) were added sequentially to a solution of (*S*)-(−)-**12** (0.050 g, 0.16 mmol) dissolved in CH_2Cl_2 (2 mL) at room temperature under nitrogen. After stirring the reaction for 17 h, the mixture was filtered and the filtrate concentrated on a rotary evaporator. The crude product was purified by silica gel column chromatography (20% EtOAc in hexanes) to afford

0.078 g of (*S*,*R*)-**13** in 94% yield. ^{1}H NMR ($CDCl_3$): δ 7.53–7.49 (m, 2H), 7.43–7.39 (m, 3H), 4.98 (d, 1H, *J*=8.4 Hz), 4.35–4.27 (m, 2H), 4.18–4.12 (m, 1H), 3.54 (s, 3H), 1.78–1.54 (m, 6H), 1.44 (s, 9H), 1.43 (s, 9H); ^{19}F NMR ($CDCl_3$ and α,α,α-trifluorotoluene as an internal standard): δ −8.89 (s, CF_3); ESI/MS (m/z): 520 $(M+H)^+$, 537 $(M+NH_4)^+$.

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