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A stereoselective synthesis of 7α-(3′-carboxypropyl)estradiol from a noncontrolled substance

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*Alkylation of 3,17β-bis(2-trimethylsilyl)ethoxymethyl-1,3,5(10) estratriene-6-one (**2**) with 5-bromo-1-pentene using NaHMDS in THF afforded 3,17β-bis(2-trimethylsilyl)ethoxymethyl-7-α-(4′-pentenyl)-1,3,5(10)estratriene-6-one (**3**) in excellent stereoselectivity (>95% epimeric excess). Functionalization of the side chain in compound **3** was accomplished via ozonolysis, oxidation and esterification to give **5** in 72% yield. The reduction of ester (**5**) using $NaBH_4$ in MeOH afforded the corresponding 6α-hydroxy compound (**6**) as a single isomer in 72% yield. The hydroxyl group in **6** was removed by converting to the corresponding xanthate (**7**) followed by reduction using n-Bu_3SnH to afford **8** in good yield. Finally, the SEM protective groups in **8** were removed, after which the ester function was hydrolyzed with LiOH to give 7α-(3′-carboxypropyl)estradiol (**10**), in 10.6% overall yield from **3**. (Steroids **62**:771–775, 1997) © 1997 by Elsevier Science Inc.*

Introduction

Estradiol (E_2, **1**) is a hormone which controls differentiation, growth and function of the reproductive system in females. It also plays an important role in the etiology of estrogen-dependent tumors and other endocrinological disorders.[1–3] Therefore, it is critical to understand the phenomena of regulation of E_2 (**1**) within the organism for the diagnosis and treatment of estrogen related illness.[4–7] A variety of estradiol (**1**) derivatives have been prepared via functionalization mainly at C2,[8–9] C6,[10–12] C7,[13–18] C11,[19–21] and C16 positions[22] for use as probes to study the estrogen binding proteins [i.e. estrogen receptors (ER) or antibodies] and for quantification of estradiol (**1**). Compounds prepared via functionalization at C7-position of estradiol (**1**) showed high binding affinity for estrogen binding proteins and exhibited significant antiestrogenic activity.[13–18] Recently, for our program[23] on the studies of estrogen binding proteins and for quantification of E_2 (**1**), we needed probes derived from 7α-(3′-carboxypropyl)estradiol (**10**).[13] Previously, the acid (**10**) and related C7-substituted estradiol derivatives were prepared by Bucourt *et al.*[13] from 17β-hydroxyestra-4,6-dien-3-one and its derivatives and recently, from a testosterone derivative.[18] These literature methods[13,18] for the preparation of C7-substituted estradiol derivatives involved the use of controlled substances as starting materials and hence require adherence to stringent regulatory procedures during synthesis as well as in the subsequent handling and accountability for the resulting probes. Therefore, the use of noncontrolled substances such as estradiol (**1**) in the synthetic process, is critically important. Recently, Kunzer *et al.*[24] prepared 7-alkylestradiols via conjugate addition of the corresponding organolithium reagents to a vinylsulfone derivative of estradiol. However, this method gave a mixture of epimers at the C7 position, and therefore required chromatographic separation of the stereoisomers. In this paper, we describe a stereoselective synthesis of 7α-(3′-carboxypropyl)estradiol (**10**) from 3,17β-bis(2-trimethylsilyl)ethoxymethyl-1,3,5(10) estratriene-6-one (**2**), a noncontrolled substance.

Experimental

General methods

IR spectra were recorded on Perkin–Elmer 1600 spectrometer using sodium chloride plates for liquids and potassium bromide disks for solids. ^1H and ^{13}C NMR spectra were recorded on a varian Gemini- spectrometer (300 MHz). Mass spectra were obtained on a Nermang 3010 MS-50 or JEOL SX102-A mass spectrometers or Perkin–Elmer Sciex API III electrospray mass spectrometer. Thin layer chromatography was performed on pre-coated Watman MK6F silica gel 60 Å plates (layer thickness: 250 μm) and were visualized with UV light and/or using $KMnO_4$ solution [$KMnO_4$ (1 g), NaOH (8 g) in water (200 mL)] or phosphomolybdic acid reagent (20 wt.% solution in ethanol), unless otherwise noted. Column chromatography was performed on silica gel, Merck grade 60 (230–400 mesh). THF was freshly distilled from a purple solution of sodium and benzophenone and CH_2Cl_2 was freshly distilled from CaH_2 under nitrogen. All reagents were purchased from Aldrich Chemical Co., (Milwaukee, Wisconsin, USA) or Sigma Chemical Co. (St. Louis, Missouri, USA) and used without further purification, except where noted. All the solvents employed were of HPLC grade and purchased from EM Science

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(Gibbstown, New Jersey, USA) and used as received. Reverse phase (RP) analytical HPLC was performed using a Waters μBondapak C18 10 μ (8 mm × 100 mm) column eluting at 2 mL/min with MeCN:0.1% aqueous trifluoroacetic acid [ratio v/v reported] with UV at 225 nm unless otherwise stated. Preparative RP HPLC was performed using a Waters μBondapak C18 10 μ (40 mm × 100 mm) column eluting at 45 mL/min with MeCN: 0.1% aqueous trifluoroacetic acid [ratio v/v reported] with UV at 225 nm unless otherwise stated.

*3,17β-Bis(2-trimethylsilyl)ethoxymethyl-7α-(4'-pentenyl)-1,3,5(10)estratriene-6-one (**3**)*

In a dry single necked round bottom flask equipped with magnetic stir bar and nitrogen inlet, 3,17β-bis(2-trimethylsilyl)ethoxymethyl-1,3,5(10)estratriene-6-one[23] (**2**, 3.07 g, 5.61 mmol) was dissolved in anhydrous THF (80 mL), and cooled to 0°C. A solution of NaHMDS (1.5 M solution in THF, 16.8 mL, 16.8 mmol, 3 equiv.) was added dropwise. The resulting solution was stirred for 30 min at 0–5°C, cooled −78°C (dry ice-acetone bath) and added HMPA (2.92 mL, 16.8 mmol, 3.0 equiv.). After 10 min, 5-bromo-1-pentene (2.65 mL, 22.4 mmol, 4 equiv.) was added and stirred for 40 min at −78°C. The cooling bath was then removed, allowed the mixture to warm to room temperature and stirred for 17 h. The mixture was cooled to −78°C, quenched with NH_4Cl (8 mL) and diluted with ethyl acetate (300 mL) and water (20 mL). The aqueous layer was separated and re-extracted with ethyl acetate (240 mL). The combined organic layers were washed with brine (120 mL), dried (Na_2SO_4) and the solvent was removed on a rotary evaporator. The crude product was purified by silica gel column chromatography (7% ethyl acetate in hexane) to afford 0.728 g of 3,17β-bis(2-trimethylsilyl)ethoxymethyl-7α-(4'-pentenyl)-1,3,5(10)estratriene-6-one (**3**) in 21% yield. ^{1}H NMR ($CDCl_3$: δ 7.65 (d, 1 H, J = 3.0 Hz), 7.31 (d, 1 H, J = 8.7 Hz), 7.18 (d, 1 H, J = 3.0, 8.4 Hz), 5.66–5.80 (m, 1 H), 5.23 (dd, 2 H, J = 1.5, 8.4 Hz), 4.88–5.00 (m, 2 H), 4.69 (s, 2 H), 3.55–3.77 (m, 5 H), 1.22–2.75 (m, 18 H), 0.92–0.98 (m, 4 H), 0.79 (s, 3 H), 0.02 (s, 9 H), 0.02 (s, 9 H); ^{13}C NMR ($CDCl_3$): δ 200.8, 156.06, 139.5, 138.5, 132.3, 127.2, 122.3, 114.7, 114.1, 94.3, 92.9, 85.9, 66.3, 64.8, 48.5, 45.2, 42.9, 42.2, 37.3, 36.9, 33.5, 27.7, 26.5, 26.3, 22.9, 22.1, 18.0, 17.9, 11.4, −1.6; ES–MS: 615 $(M)^+$, 632 $(M + NH_4)^+$).

Starting material [3,17β-bis(2-trimethylsilyl)ethoxymethyl-1,3,5(10)estratriene-6-one (**2**)[23], 0.445 g, 14%) was recovered. Also, 1.47 g of 3,17β-bis(2-trimethylsilyl) ethoxymethyl-[6-*O*-(4'-pentenyl)-6-ene]1,3,5(10)estratriene (**4**) was isolated (42%) as a colorless thick oil. ^{1}H NMR ($CDCl_3$): δ 7.26 (d, 1 H, J = 2.7 Hz), 7.15 (d, 1 H, J = 8.4 Hz), 6.94 (dd, 1 H, J = 2.7, 8.4 Hz), 5.80–5.94 (m, 1 H), 5.22 (q, 2 H, J = 6.9, 8.4 Hz), 4.98–5.12 (m, 2 H), 4.86 (s, 1 H), 4.71 (s, 2 H), 3.56–3.83 (m, 5 H), 1.25–2.34 (m, 18 H), 0.92–0.98 (m, 4 H), 0.80 (s, 3 H), 0.03 (s, 9 H), 0.02 (s, 9 H); ^{13}C NMR ($CDCl_3$): δ 156.1, 151.8, 138.2, 133.7, 133.4, 124.1, 115.1, 114.7, 110.6, 99.7, 94.3, 93.2, 86.1, 66.1, 66.0, 64.7, 49.1, 43.3, 42.6, 37.6, 36.7, 30.3, 28.26, 27.7, 23.9, 23.1, 18.0, 17.9, 11.5, −1.6; ES–MS: 616 $(M H)^+$, 632 $(M + NH_4)^+$.

*Hydrolysis of 3,17β-Bis(2-trimethylsilyl)ethoxymethyl-[6-O-(4'-pentenyl)-6-ene]1,3,5(10)estratriene (**4**)*

3,17β-Bis(2-trimethylsilyl) ethoxymethyl-[6-O-(4'-pentenyl)-6-ene]1,3,5(10)estratriene (**4**, 0.091 g, 0.15 mmol) was dissolved in acetone (8 mL) and added 5% aq. HCl (8 mL) at room temperature. The progress of the reaction of was monitored by TLC (20% ethyl acetate in hexane) and after 25 min, toluene (20 mL) was added. The solvent was removed on a rotary evaporator and the residue was dried on vacuum pump. The crude product was purified by silica gel column chromatography (20% ethyl acetate in hexane) to afford 0.069 g of 3,17β-bis(2-trimethylsilyl)ethoxymethyl-1,3,5(10) estratriene-6-one (**2**) in 85% yield which was identical with the compound prepared previously in this laboratory.[23] ^{1}H NMR ($CDCl_3$): δ 7.70 (d, 1 H, J = 3 Hz); 7.36 (d, 1 H, J = 8.7 Hz); 7.21 (dd, 1 H, J = 3, 8.7 Hz); 5.25 (s, 2 H); 4.71 (s, 2 H); 3.78–3.60 (m, 5 H); 2.73 (dd, 1 H, J = 3.3, 16.8 Hz); 2.46–1.32 (m, 12 H); 0.99–0.93 (m, 4 H); 0.81 (s, 3 H); 0.03 (s, 9 H); 0.02 (s, 9 H); ES–MS: 547 $(M)^+$.

*3,17β-Bis(2-trimethylsilyl)ethoxymethyl-7α-(3'-methoxycarbonylpropyl)-1,3,5(10)estratriene-6-one (**5**)*

3,17β-Bis(2-trimethylsilyl)ethoxymethyl-7α-(4'-pentenyl)-1,3,5(10)-estratriene-6-one (3) (0689 g, 4.07 mmol) was dissolved in a mixture of CH_2Cl_2-MeOH (2:1, 60 mL) in a 250 mL round bottom flask and pyridine (0.590 mL, 7.29 mmol, 6.5 equiv.) was added followed by Sudan III indicator solution (0.1% in ethanol, 3 drops). The resulting light pink solution was cooled to −78°C (dry ice-acetone) with stirring and passed a stream of ozone (generated at 90 V, 7.5 psi of O_2 and 0.2 standard liter per min of flow rate) through an inlet just below the surface of the solution. After the disappearance of Sudan III indicator color (about 30–40 min, solution became light blue color) the ozone flow was stopped and nitrogen was bubbled gently into the solution to displace the excess ozone present. Dimethyl sulfide (1.15 mL, 15.7 mmol, 14 equiv.) was added, the cooling bath was removed and the mixture was allowed to warm to room temperature. After stirring the mixture over night, the solvent was removed on a rotary evaporator and residue was dried on vacuum pump.

The resulting crude aldehyde was dissolved in *t*-BuOH (20 mL) and 2-methyl-2-butene (4 mL). To this mixture, a freshly prepared sodium chlorite solution [prepared by dissolving (0.190 g, 1.68 mmol) of sodium chlorite in phosphate buffer solution (pH 3.3, 0.2 M, 2.6 mL)] at room temperature. After stirring the mixture for 30 min, the solvent was removed to dryness on a rotary evaporator, the residue was dissolved in ethyl acetate (120 mL) and added brine (120 mL). The solution was adjusted to pH 3 with 1M HCl. The organic layer was separated, washed with 2% aq. sodium sulfite solution (pH 4, 120 mL) and dried (Na_2SO_4). The solvent was removed on a rotary evaporator. The crude acid was dissolved in a mixture of ethyl acetate (10 mL) and ether (10 mL), cooled with ice bath and treated with a freshly prepared ethereal diazomethane solution (4 mL). After stirring the mixture for 30 min at 0–5°C, the solvent was carefully removed on a rotary evaporator. Purification of the crude product by silica gel column chromatography (10–20% ethyl acetate in hexane) afforded 0.725 g of 3,17β-bis(2-trimethylsilyl)ethoxymethyl-7α-(3'-methoxycarbonylpropyl)-1,3,5(10)estratriene-6-one (**5**) in 72% yield as colorless thick oil. R_f: 0.52 (25% ethyl acetate in hexane); Analytical RP HPLC [MeCN/0.1% aqueous AcOH (90:10), 1 mL/min at 230 nm] R_t 11.51 min, 99.1%; IR (neat): 2951, 2927, 2875, 1739, 1683, 1607, 1492, 1248, 1059, 998, 860, 835 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.65 (d, 1 H, J = 2.7 Hz); 7.31 (d, 1 H, J = 8.7 Hz); 7.18 (dd, 1 H, J = 2.4, 8.4 Hz); 5.23 (s, 2 H); 4.69 (s, 2 H); 3.54–3.77 (m, 5 H); 3.62 (s, 3 H); 2.22–2.70 (m, 18 H); 0.84–0.97 (m, 4 H); 0.78 (s, 3H); 0.02 (s, 9H); 0.02 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 200.5, 173.8, 156.1, 139.5, 132.2, 127.2, 122.4, 114.1, 94.3, 92.9, 85.9, 65.3, 64.8, 51.4, 48.1, 45.1, 42.9, 42.1, 37.3, 36.9, 33.6, 27.7, 26.5, 22.9, 22.5, 21.9, 18, 17.9, 11.4, −1.6; ES–MS: 647, $(M)^+$, 664 $(M + NH_3)^+$.

*3,17β-Bis(2-trimethylsilyl)ethoxymethyl-7α-(3'-methoxycarbonyl propyl)-1,3,5(10)estratriene (**6**)*

$NaBH_4$ (0.123 g, 3.26 mmol, 3.0 equiv.) was added to 3,17β-bis (2-trimethylsilyl)ethoxymethyl-7α-(3'-methoxycarbonylpropyl)-1,3,5 (10)estratriene-6-one (**5**, 0.704 g, 1.088 mmol) dissolved in anhydrous methanol (8 mL) at 0–5°C (ice bath) under nitrogen atmosphere. After 30 min, the cooling bath was removed and the mixture was stirred at room temperature for 6 h. The reaction was quenched with

water (2 mL) and the solvent was removed on a rotary evaporator. The residue was dissolved in ethyl acetate (50 mL) and water (5 mL), organic layer was separated and the acqueous layer was re-extracted with ethyl acetate (20 mL). The combined organic layers were washed with brine (10 mL), dried ($MgSO_4$ and the solvent was removed on a rotary evaporator. The crude compound was purified by silica gel column chromatography (20–40% ethyl acetate in hexane) to afford 0.495 g of 3,17β-bis(2-trimethylsilyl)ethoxymethyl-6α-hydroxy-7α-(3′-methoxycarbonyl propyl)-1,3,5(10) estratriene (**6**) in 70% yield as a colorless thick oil. R_f: 0.35 (35% ethyl acetate in hexane); Analytical RP HPLC: [MeCN/0.1% aqueous TFA (90:10), 2.0 mL/min at 225 nm], R_t: 3.28 min, 98.1%; ^{1}H NMR ($CDCl_3$): δ 7.35 (s, 1 H, J = 1.8 Hz), 7.16 (d, 1 H, J = 8.4 Hz); 6.90 (dd, 1 H, J = 2.7, 8.4 Hz); 5.21 (q, 2 H, J = 3, 9.9 Hz); 4.87 (dd, 1 H, J = 5.4, 9 Hz) 3.59–3.68 (m, 5 H); 3.62 (s, 3 H), 2.55 (d, 1 H, J = 9 Hz); 1.08–2.38 (m, 18 H); 0.91–0.99 (m, 4 H); 0.79 (s, 3 H); 0.02 (s, 9 H); 0.2 (s, 9H); ES–MS: 666 $(M + NH_4)^+$, 1314 $(2 \times M + NH_4)^+$.

3,17β-Bis(2-trimethylsilyl)ethoxymethyl-6α-(imidazolylthiocarbonyl)-7α-(3′-methoxycarbonylpropyl)-1,3,5(10)estratriene (**7**)

1,1-Thiocarbonyldiimidazole (0.404 g, 2.7 mmol, 3 equiv.) was added to the solution of 3,17β-bis(2-trimethylsilyl)ethoxymethyl-6α-hydroxy-7α-(3′-methoxycarbonylpropyl)-1,3,5(10)estratriene (**6**, 0.491 g, 0.757 mmol) in anhydrous THF (8.0 mL) at room temperature under nitrogen. After refluxing the mixture for 7 h, it was cooled and the solvent was removed on rotary evaporator. The crude compound was purified by silica gel column chromatography (20–30% ethyl acetate in hexane) to afford 0.335 g of 3,17β-bis(2-trimethylsilyl)ethoxymethyl-6α-(imidazolylthiocarbonyl)-7α-(3′-methoxycarbonylpropyl)-1,3,5(10)estratriene (**7**) in 59% yield as a colorless thick oil. R_f: 0.35 (35% ethyl acetate in hexane); Analytical RP HPLC: [MeCN/0.1% aqueous TFA, (90/10), 2.0 mL/min at 225 nm] R_t 4.34 min, 97.7%. ^{1}H NMR ($CDCl_3$): δ 8.28 (s, 1 H), 7.56–7.55 (m, 1 H); 7.13–7.26 (m, 3 H); 6.94 (dd, 1 H, J = 2.7, 8.1 Hz); 5.31 9d, 1 H, J = 4.5 Hz) 5.16 9s, 2 H); 4.69 (s, 2 H); 3.57–3.74 (m, 5 H); 3.63 9s, 3 H); 1.24–2.56 (m, 18 H); 0.88–0.97 (m, 4 H); 0.83 (s, 3 H); 0.02 (s, 9 H); 0.02 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 173.7, 166.1, 155.9, 135.7, 133.9, 133.8, 131.1, 127.8, 116.8, 116.1, 115.2, 94.2, 93.1, 85.9, 66.2, 64.7, 53.5, 51.5, 46.5, 43.5, 43.3, 39.5, 38.2, 37.1, 34.3, 27.8, 27.3, 26.1, 25.5, 23, 18, 17.9, 11.6, −1.6; ES–MS: 759 $(M + H)^+$.

3,17β-Bis(2-trimethylsilyl)ethoxymethyl-7α-(3′-methoxycarbonylpropyl)-1,3,5(10)estratriene (**8**)

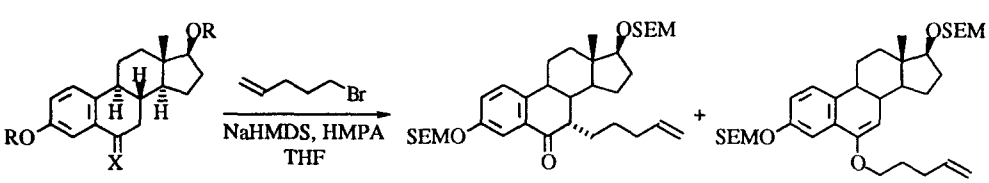
The xanthate (**7**, 0.328 g, 0.433 mmol) was dissolved in anhydrous toluene (8 mL) in a dry single necked round bottom flask and added a catalytic amount of 2,2′-azabisisobutyronitrile (AIBN, 0.032 g). The mixture was heated to 100°C and added tri-*n*-butyltin hydride (0.28 mL, 1.038 mmol, 2.4 equiv.) under nitrogen. After refluxing the mixture for 4.0 h, the solvent was removed on a rotary evaporator. The crude product was purified by silica gel column chromatography (10% ethyl acetate in hexane) to afford 0.258 g of 3,17**8**) in 95% yield as colorless thick oil. Analytical RP HPLC: [MeCN/0.1% aqueous TFA (90/10), 2.0 mL/min at 225 nm] R_t: 5.21 min, 98.6%; IR (neat): 2951, 2894, 1741, 1609, 1499, 1248, 1059, 1019, 859, 835 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.20 (d, 1 H, J = 9.0 Hz); 6.82 (dd, 1 H, J = 2.4, 8.4 Hz); 6.74 (d, 1 H, J = 2.4 Hz); 5.19 (s, 2 H); 4.69 (s, 2 H); 3.58–3.77 (m, 5 H); 3.64 (s, 3H); 2.90 (dd, 1H, J = 6, 17.9 Hz); 2.74 (d, 1 H, J = 17.1 Hz); 1.02–2.36 (m, 18 H); 0.91–0.99 (m, 4H); 0.79 (s, 3 H); 0.02 (s, 9H); 0.02 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 174.3, 155.5, 136.6, 132.9, 126.9, 117.1, 113.9, 94.2, 93.0, 86.3, 66.1, 64.7, 51.4, 46.4, 43.1, 41.5, 38.0, 37.4, 34.5, 34.2, 32.9, 27.9, 27.1, 25.1, 23.4, 22.4, 18.1, 17.9, 11.6, −1.6; ES–MS: 650 $(M + NH_4)^+$.

7α-(3′-Carboxypropyl)-3,17β-dihydroxy-1,3,5(10)estratriene(**10**)

Forty-eight percent aqueous HF (0.030 mL) was added to the solution 3,17β-bis(2-trimethylsilyl)ethoxymethyl-7α-(3′-methoxycarbonylpropyl)-1,3,5(10)estratriene (**8**, 0.009 g, 0.0142 mmol) in MeCN (2.0 mL) at room temperature and stirred for 3.0 h. The solvent was removed on a rotary evaporator and the residue was dried on vacuum pump. The resulting crude ester (**9**) was dissolved in THF (1 mL) and added LiOH (0.002 g, 0.0426 mmol, 3.0 equiv.) followed by H_2O (0.5 mL) at room temperature. After stirring the mixture for 3.0 h, the solvent was removed to dryness on a rotary evaporator and the crude product was dissolved in water (2.0 mL) and acidified to pH 4.0 with 5% aq. HCl. Purification of the crude product by preparative RP HPLC [MeCN/0.1% aqueous TFA (25/75), 45 mL/min at 225 nm] and lyophilization afforded 0.0046 g of 7α-(3′-carboxypropyl)-3,17β-dihydroxy-1,3,5(10)estratriene [**10**, 7α-(3′-carboxypropyl)estradiol] in 89% yield as a colorless amorphous powder.[13] Mp: >245°C (with decomposition); Analytical RP HPLC: [MeCN: 0.1% aqueous TFA (25/75), 2 mL/min at 225 nm] R_t: 13.79 min, 99.9%. ^{1}H NMR (CD_3OD): δ 7.08 (d, 1 H, J = 8.7 Hz); 6.52 (dd, 1 H, J = 2.7, 8.4 Hz); 6.47 (1 H, J = 2.7 Hz); 3.66 (1 H, J = 8.4 Hz); 2.84 (dd, 1 H, J = 5.1, 16.5 Hz); 2.69 (d, 1 H, J = 16.5 Hz); 2.36–0.99 (m, 18 H); 0.77 (s, 3 H); ^{13}C NMR (CD_3OD): δ 156.2, 137.6, 131.9, 128, 117.1, 114.1, 82.7, 47.8, 44.5, 43.6, 39.6, 38.2, 35.6, 34.5, 30.7, 28.6, 26.2, 24.6, 23.5, 11.6; ES–MS: 376 $(M + NH_4)^+$, 734 $(2 \times M + NH_4)^+$; HRMS (FAB) calcd for $C_{22}H_{31}O_4$ $(M + H)^+$, 359.2222; found 359.2219.

Results and discussion

Our strategy for the preparation of 7α-(3′-carboxypropyl)estradiol (**10**) (Scheme 1, 2) involves the stereoselective alkylation of 3, 17β-bis(2-trimethylsilyl)ethoxymethyl-1,3,5(10)-estratriene-6-one (**2**)[23] with 5-bromo-1-pentene in the presence of a base, followed by functionalization of the side chain and removal of C6-keto group. Thus, 3, 17β-bis(2-trimethylsilyl)ethoxymethyl-1,3,5(10)-estratriene-6-one (**2**) in THF was treated with NaHMDS, HMPA and 4.0 equiv. of



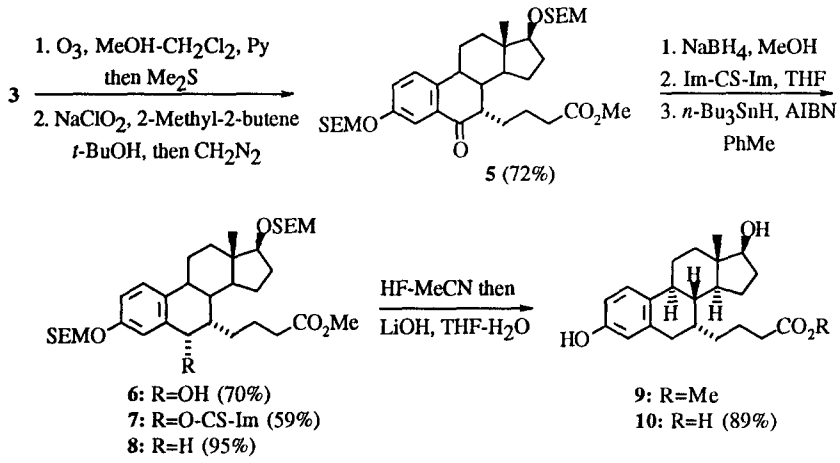
1: R=H; X=H_2

2: R=SEM; X=O

3 (21%)

4 (42%)

Scheme 1



Scheme 2

5-bromo-1-pentene in THF at −78°C. Since there was no reaction at −78°C, the mixture was slowly warmed to room temperature and the progress of the reaction was monitored by TLC. After stirring for 16–18 h, it was then quenched with sat. NH_4Cl solution at −78°C and the crude product was purified by silica gel column chromatography to afford 3,17β-bis(2-trimethylsilyl)ethoxymethyl-7α-(4′-pentenyl)-1,3,5(10)estratriene-6-one (**3**) in 21% yield and >95 epimeric excess along with 14% of the recovered starting material **2**. The alkylation took place from the sterically less hindered α-face of the enolate of **2**, and thus gave the compound (**3**) in excellent stereoselectivity. In addition to the desired product **3**, a nonpolar *O*-alkylated compound (**4**) was also isolated (up to 42% yield). The structure of compound (**4**) was assigned by spectroscopic data. ^1H NMR spectrum of **4** showed a singlet for C7-olefinic proton at δ 4.71 and the ^{13}C NMR clearly indicated the absence of carbonyl function and the presence of an additional two olefinic carbons. While our efforts to improve the yield of the desired 3,17β-bis(2-trimethylsilyl)ethoxymethyl-7α-(4′-pentenyl)-1,3,5(10)estratriene-6-one (**3**) using different bases (e.g. LDA, LiHMDS, KHMDS) and conditions were not successful, further modification to the alkylation step are being pursued. Functionalization of the side chain in the byproduct (**4**) to the corresponding acid by ozonolysis-oxidation sequence, led to the hydrolysis of ether linkage to give the ketone (**2**) in low yield (<20%). Nevertheless, the byproduct (**4**) was easily hydrolyzed with 5% HCl in acetone to afford the ketone (**2**) in 85% yield which was recycled in the alkylation step. Thus, the yield of the desired *C*-alkylated product (**3**) was 43% when corrected for starting material (**2**) recovered directly from the reaction or obtained by hydrolysis of byproduct (**4**).

The next step in the synthesis of 7α-(3′-carboxypropyl)estradiol (**10**) was to functionalize the side chain in **3**. Accordingly, the olefin (**3**) (Scheme 2) was first subjected to ozonolysis in MeOH–CH_2Cl_2 at −78°C. The resulting crude aldehyde was oxidized with sodium chlorite to the corresponding acid and esterified with ethereal diazomethane to afford methyl ester (**5**) in 72% overall yield. The keto functionality in **5** was reduced with $NaBH_4$ in methanol to give the alcohol (**6**) in 72% yield as an α-isomer. The 6-β-hydrogen in compound (**6**) in ^1H NMR spectrum appeared as doublet of a doublet at δ 4.87 with coupling constant 5.4 and 9.0 Hz, which is consistent with the proposed structure.[25] The hydroxy functionality in **6** was converted to the modified xanthate (**7**)[26] in 59% yield which upon deoxygenation using tri *n*-butyltin hydride[27] afforded **8** in excellent yield (91%). Finally, the SEM protective groups in **8** were hydrolyzed using 48% HF in acetonitrile[23] and the resulting crude ester (**9**) was treated with LiOH in THF-H_2O to afford 7α-(3′-carboxypropyl)estradiol (**10**)[13] in 89% yield after purification by preparative HPLC. The α-stereochemistry for the C7-substituent in estradiol was established based on comparison with the known stereochemistry at carbons C-8, C-9, C-13, C-14 and C-17 by spectroscopic studies.[18,28] In the ^1H NMR of acid (**10**), the C6-benzylic protons were clearly evident at δ 2.84 and 2.69 with coupling constants 5.1, 16.5 Hz and 16.5 Hz, which is consistent with the structure.

In summary, a convenient method was developed for the preparation of 7α-(3′-carboxypropyl)estradiol (**10**) from 3, 17β-bis(2-trimethylsilyl)ethoxymethyl-1,3,5(10)-estratriene-6-one (**2**) via alkylation with 5-bromo-1-pentene and further transformations. The method afforded compound (**3**) in excellent stereoselectivity (>95% epimeric excess) and the byproduct (**4**) was hydrolyzed and recycled to improve the overall yield (10.6%) of **10**. The acid (**10**) is the key building block for preparation of various estradiol probes and affinity materials which are critical for the development of estradiol immunoassays. The use of noncontrolled substance (2) as starting material, eliminates the Drug Enforcement Agency's (DEA) regulatory requirements, which otherwise would have to be imposed if the probes were prepared from controlled substances such as 17β-hydroxyestra-4,6-dien-3-one or testosterone derivatives.

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