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Inhibition of HIV Replication: Synthesis of [4-^{14}C]-5α-Androstan-16α-bromo-3β-ol-17-one

Mingcheng Han[†]*, Brendan A. Hayes[†]*,
Patrick T. Prendergast[‡], Surendra Gupta[†]

[†]American Radiolabeled Chemicals, Inc., 11624
Bowling Green Drive, St. Louis, MO 63146, and
[‡]Edenland Inc., Baybush, Straffan, Co. Kildare, Ireland

SUMMARY

An efficient synthesis of [4-^{14}C]-5α-Androstan-16α-bromo-3β-ol-17-one (**1**) via the intermediate [4-^{14}C] testosterone (**8**) is described. This compound targets cellular metabolic pathways and clinical trials to HIV-viral pathogenicity are promising.

Key word: 5α-Androstan-16α-bromo-3β-ol-17-one [4-^{14}C], antiviral.

INTRODUCTION

Strategies aimed at combating acquired immune deficiency syndrome (AIDS) are many[1] and generally target aspects of viral replication unique to HIV. Alternatively cellular metabolism might be modified to reduce viral pathogenicity: metabolic pathways for nucleotide synthesis and cell proliferation mandatory for efficient viral replication present attractive therapeutic targets. Recent reports by Henderson[2], Schwartz and Gordon test this hypothesis and find that

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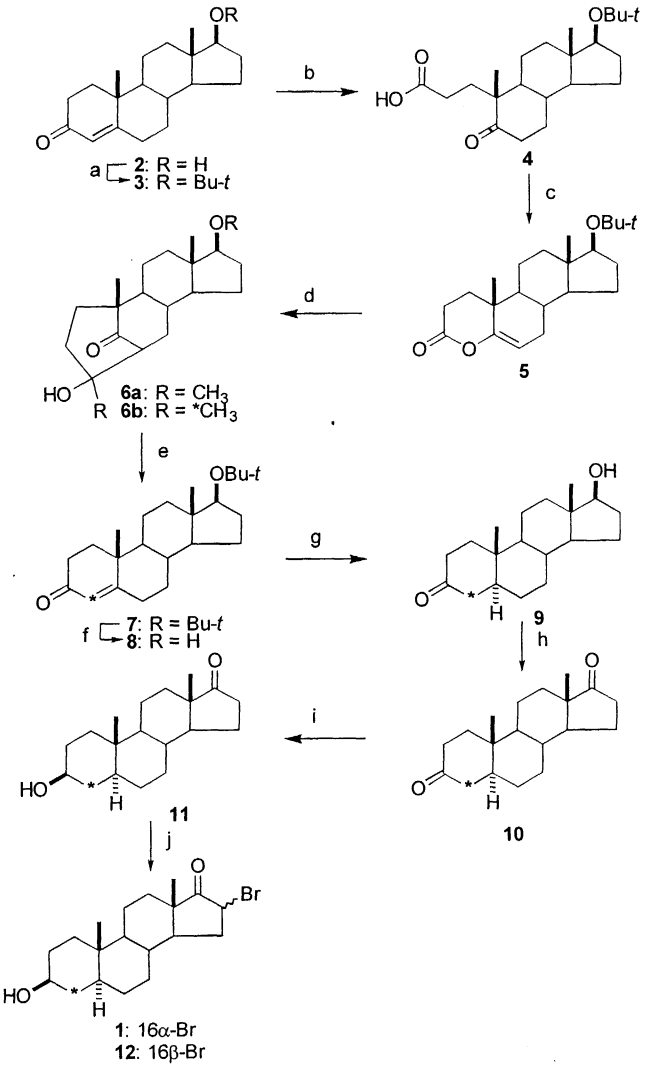
dehydroepiandrosterone (DHEA), 16α-fluoro-5-androsten-17-one and 3β-hydroxy-16α-fluoro-5α-androstan-17-one have a modest down regulative effect on HIV replication and proliferation in phytohemagglutinin -stimulated peripheral blood lymphocytes as measured by syncytia formation, release of p24 antigen, and accumulation of reverse transcriptase activity. Recent clinical trial results by Prendergast[3] using the title compound, 5α-Androstan-16α-bromo-3β-ol-17-one (EpiBr), are dramatic. Epi-Br has been shown to have a potent down regulative effect on HIV replication and proliferation. Since Epi-Br is neither toxic in mice nor mutagenic in bacteria and has been shown to down regulate HIV replication and proliferation, it is potentially a valuable therapeutic modality in AIDS treatment.

DISCUSSION

The synthesis of [4-^{14}C] Epi-Br herein follows Turner's[4] method and transforms unlabeled testosterone **2** into [4-^{14}C] labeled testosterone **9**. The hydroxyl group at the 17-position is first protected as the *t*-butyl ether, **3**, by condensation of **2** with isobutylene[5]. Ozone scission of the 4-C double bond gives keto-acid **4**, which is cyclised to the lactone **5** by heating with a mixture of sodium acetate/acetic anhydride. Subjection of lactone **6** to standard Grignard reaction with ^{14}C-methyl magnesium iodide in ether afforded compound **6b** in 75% yield. This structure was confirmed by comparison with unlabeled compound **6a**, which was formed under the same reaction conditions in 95% yield by reaction of lactone **6** with an excess of unlabeled methyl magnesium iodide. TLC behaviour of **6a** and **6b** was identical. The structural assignment was confirmed by NMR: among other signals the ^{13}C spectrum shows one carbonyl group (220.09 ppm) and two carbons bonded to oxygen [80.32 ppm (CHBu-*t*), 78.69 ppm (CHOH)]. Treatment of **6b** with methanolic sodium hydroxide at room temperature overnight gave compound **7** (95%). Exposure of the *t*-Butyl ether, **7**, to 6N HCl afforded [4-^{14}C] testosterone **8** (93%). Careful Birch reduction[6] of the enone **8** produced the keto alcohol **9** (88%), which contains the 5α-A/B ring junction. Jones oxidation of **9** at room temperature in acetone yielded the 3,17-dione **10**, in which the 3-keto

Synthetic Path[a]



[a]a) $F_3CSO_3H/CH_2{=}C_3H_6$-*i*, CH_2Cl_2, 95%; b) (i) O_3, EtOAc/MeCOOH; (ii) H_2O/H_2O_2, overnight, 87%; c) Ac_2O/AcONa, 90%; d) *CH_3MgI, ether, 75%; e) MeOH/H_2O/NaOH, 95%; f) 6N HCl/MeOH, 93%; g) Li/Liq. NH_3,THF/Toluene, 88%; h) Jones oxidation, 93%; i) LiAl[t-butoxide]$_3$H, THF, 91%; j) $CuBr_2$, MeOH, 75%.
*Radiolabeled position

group can be selectively reduced with lithium tri-*t*-butoxy aluminohydride to the 3β-hydroxy compound **11** (91%). Selective bromination[7] of **11** with $CuBr_2$ in methanol gave an HPLC separable mixture of the target compound **1** (75%) and the 16β-bromo isomer **12** (20%).

RESULTS

High specific activity radiolabeled [4-^{14}C]-5α-Androstan-16α-bromo-3β-ol-17-one (**1**), a potent inhibitor of HIV replication has been synthesized by a versatile ten step sequence from testosterone (**2**) in ca 28% overall yield.

EXPERIMENTAL

General Methods: Melting points were determined on a Kofler micro hot stage and uncorrected. NMR spectra were recorded at ambient temperature in $CDCl_3$ with a 5 mm probe on either a Varian Gemini-300 operating at 300 MHz (^{1}H) or 75 MHz (^{13}C). For ^{1}H NMR and ^{13}C NMR spectra, the internal references were TMS (δ 0.00) and $CDCl_3$ (δ 77.00), respectively. Solvents were used either as purchased or dried and purified by standard methodology. Flash chromatography was performed using silica gel (32-63 microns) purchased from Scientific Absorbents, Atlanta, GA. 4-Androsten-17β-ol-3-one was purchased from Steraloids Inc. Pre-HPLC column [Econosil, 10 um silica column, 250 mm × 20 mm) was purchased from Econosil Inc. Standard workup denotes solvent extraction, brine washing and sodium sulfate drying prior to solvent removal. [^{14}C] Methyl iodide was made at American Radiolabeled Chemicals, Inc.

4-Androsten-17β-(1,1-dimethylethoxy)-3-one (3)

Isobutylene (10 ml) and F_3CSO_3H (0.25 ml) were added to a stirred solution of testosterone **2**(3.3 g, 11.6 mmol) in methylene chloride (35 mL) at –78 ^{0}C. The reaction mixture was stirred for 15 min while the temperature rose to –10 ^{0}C, then cooled to –18 ^{0}C for 48 h. After adding triethylamine (1 mL), the solution was evaporated and the oily residue was purified by chromatography (silica gel, 15 %

EtOAc in hexane, v / v) to give the crystalline compound **3** (3.7 g, 95%): mp 160 – 162 ^{0}C from EtOAc / hexane; IR 2966, 2880, 2864, 1676, 1616, 1455, 1435, 1393, 1329, 1364, 1357, 1269, 1228, 1189, 1132, 1116, 1080, 1026, 1037, 1012 cm^{-1}; ^{1}H NMR 5.71 (s, 1H, 4-CH=C), 3.37 (t, 1H, J = 7.8 Hz, 17-C*H*O), 1.19(s, 3H, 19-CH_3), 1.13(s, 9H, OC$(CH_3)_3$), 0.76(s, 3H, 18-CH_3); ^{13}C NMR 199.12 (C=O), 171.14 (C=), 123.55 (=C), 80.35 (COBu-*t*), 71.95 (*C*$(CH_3)_3$), 28.53 (C(*C*$H_3)_3$), 11.39 (CH_3), 53.91, 50.22, 42.10, 38.45, 36.68, 35.53, 35.34, 33.75, 32.65, 31.42, 30.91, 23.52, 20.44, 17.20.

17β-(1,1-Dimethylethoxy)-5-oxo-3,5-seco-4-norandrosta-3-carboxylic acid (4)

Ozone was introduced into a solution of enone **3** (3.6 g, 32 mmol) in acetic acid (30 ml) and EtOAc (88 mL) at –(10 ~ 20) ^{0}C with stirring for 6 h. After expelling excess ozone by bubbling O_2 for 5 min, water (15 mL) and 30% H_2O_2 (3 mL) were added at -15 ^{0}C. After stirring at room temperature overnight, the reaction mixture was poured into brine (100 mL), and extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with brine (2 × 100 mL) and extracted with saturated $NaHCO_3$ (3 × 100 mL). The combined basic extracts were acidified with 1 N HCl at ice bath temperature until pH < 3. The white solids were extracted with EtOAc (3 × 100 mL). Solvent removal gave the keto-acid **4** (3.3 g, 87%) as crystalline solid: mp 87 – 89 ^{0}C from EtOAc / hexane; IR 2972, 2872, 1707, 1424, 1456, 1446, 1390, 1362, 1312, 1268, 1299, 1253, 1229, 1197, 1076, 1022 cm^{-1}; ^{1}H NMR 3.38 (t, 1H, J = 7.7 Hz, 17-C*H*O), 1.13 (s, 12H, 19-CH_3, OC$(CH_3)_3$), 0.78 (s, 3H, 18-CH_3); ^{13}C NMR 214.91 (C=O), 179.24 (COOH), 80.34 (COBu-*t*), 72.20 (*C*$(CH_3)_3$), 28.50 (C(*C*$H_3)_3$), 11.43 (CH_3), 50.22, 50.14, 47.99, 42.25, 37.77, 36.43, 34.61, 30.73, 30.66, 29.08, 28.93, 23.55, 20.85, 20.28.

17β-(1,1-Dimethylethoxy)-5-oxo-3,5-seco-4-nor-5-androstene (5)

A mixture of keto-acid **4** (3.2 g, 8.7 mmol) in CH_3COONa (1.7 g, 20 mmol) and $(CH_3CO)_2O$ (34 mL) was heated at 135 – 140 ^{0}C for 3 h. Solvent removal under reduced pressure gave a residue, which was purified by chromatography (silica gel, 10% EtOAc in hexane, v / v) to yield **5** (2.7 g, 90%) as a crystalline

solid: mp 116 –118 ^{0}C from EtOAc / hexane; IR 2971, 2871, 2847, 1687, 1461, 1377, 1389, 1342, 1331, 1361, 1262, 1218, 1200, 1161, 1137, 1097, 1066, 1082, 1020 cm^{-1}; ^{1}H NMR 5.25 (dd, J = 3.54 Hz, J = 2.19, 1H, =CH), 3.38 (t, 1H, J = 7.8 Hz, 17-C*H*O), 1.14 (s, 9H, OC(CH$_3$)$_3$), 1.12 (s, 3H, 19-CH$_3$), 0.76 (s, 3H, 18-CH$_3$); ^{13}C NMR 167.95 (C=O), 154.18 (C=), 104.91 (=C), 80.21 (COBu-*t*), 71.84 (*C*(CH$_3$)$_3$), 28.44 (C(*C*H$_3$)$_3$), 11.27 (CH$_3$), 50.49, 48.71, 42.10, 36.29, 34.34, 31.34, 30.77, 30.71, 28.49, 27.25, 23.43, 20.31, 18.60.

17β-(1,1-Dimethylethoxy)-3-hydroxy-3-methyl-5-oxo-3(5→6βH)abeo-A-androstane (6a)

Methyl magnesium iodine (3.0 mmol, 1.0 ml, 3.0 M solution in diethyl ether) was added to a solution of lactone **5** (200 mg, 0.58 mmol) in ether (20 mL) at 0^{0}C with stirring under nitrogen. After 30 min, 1N HCl (5 mL) was added to the reaction and the mixture was poured into brine (50 mL). Standard workup though ethyl acetate gave **6a** (200 mg, 95%) as crystalline solid: mp 181-183 ^{0}C from EtOAc / hexane; IR 3408, 3054, 2975, 2869, 1703, 1459, 1390, 1377, 1361, 1265, 1237, 1200, 1171, 1084, 1061, 1037, 967, 910 cm^{-1}; ^{1}H 3.34 (t, 1H, J = 7.8 Hz, 17-C*H*OBu-*t*), 1.27 (s, 3H, 19-CH$_3$), 1.11 (s, 9H, C(CH$_3$)$_3$), 0.98 (s, 3H, 3-CH$_3$), 0.65 (s, 3H, 18-CH$_3$); ^{13}C NMR 220.09 (C=O), 80.32 (COBu-*t*),), 78.69 3-CHOH), 72.05 (*C*(CH$_3$)$_3$), 28.51 (C(*C*H$_3$)$_3$), 11.41 (CH$_3$), 56.79, 50.88, 50.21, 46.18, 42.10, 38.35, 36.92, 35.36, 32.64, 30.77, 29.26, 27.59, 24.05, 23.28, 17.97.

17β-(1,1-Dimethylethoxy)-3-hydroxy-3-[^{14}C]methyl-5-oxo-3(5→6βH)abeo-A-androstane (6b)

A solution of lactone **5** (1.87 g, 5.4 mmol) in ether (25 ml) was added to a solution of [^{14}C] methyl magnesium iodine, prepared from methyl iodide (250 mCi, 4.5 mmol, S.A.:55.5 mCi / mmol) and magnesium turning (131 mg) in ether (15 mL), at –10 ^{0}C with stirring under a nitrogen atmosphere. After 1 h at 0 ^{0}C, 1N HCl (5 mL) was added to the reaction and the mixture was poured into brine. Standard workup though ethyl acetate gave **6b** (1.3 g, 187 mCi, S.A. 52.0 mCi/mmol, 75%) as crystalline solid: mp 180-182 ^{0}C from EtOAc / hexane.

[4-^{14}C]-Androsten-17β-(1,1-dimethylethoxy)-3-one (7)

A mixture of compound **6b** (0.91 g, 2.5 mmol, 130 mCi, 52.0 mCi/mmol) and 30% NaOH (10 mL) in EtOH (70 mL) was stirred at room temperature over night. The reaction mixture was concentrated under reduced pressure and poured into brine. Standard workup though ethyl acetate gave a solid, which was purified by chromatography (silica gel, 15 % EtOAc in hexane, v / v) to give compound **7** (785 mg, 123 mCi, S.A. 54.3 mCi, 95%) as a crystalline solid: mp 158-160 ^{0}C from EtOAc / hexane.

[4-^{14}C]-4-Androsten-17β-ol-3-one (8)

A solution of enone **7** (785 mg, 123 mCi) and 6 N HCl (14 ml) in EtOH (30 mL) was heated under reflux for 2 h. After cooling to room temperature, the resulting solution was poured into brine and standard workup though ethyl acetate gave a solid, which was dissolved in methanol and treated with activated charcoal. Charcoal filtration followed by solvent removal gave a solid (650 mg, 115 mCi, S.A. 51.0 mCi/mmol, 93 %), mp 154-155 ^{0}C from EtOAc / hexane [lit.[8] 155 ^{0}C].

[4-^{14}C]-5α-Androstan-17β-ol-3-one (9)

A solution of [4-^{14}C] testosterone **8** (500 mg, 1.73 mmol, 95.7 mCi, S.A. 55 mCi/mmol) in THF/toluene (1:1, 20 mL) was added dropwise to a Li / liq. NH_3 / THF / toluene solution, prepared from Li (100 mg) dissolved in liq. NH_3 (50 mL), THF (10mL) and toluene (10 mL), with stirring (ca 2 min). After 3 min, the excess lithium was destroyed by addition of 1,2-dibromoethane until the blue color was discharged. The reaction was allowed to stand at room temperature overnight. EtOAc (200 mL) was added, and standard workup a solid which was purified by chromatography (silica gel, 25% EtOAc in hexane, v/v) to yield **9** (440 mg, 88%, 84.2 mCi, S.A. 55.5 mCi/mmol)as a crystalline solid: mp 179-180 ^{0}C from EtOAc / hexane [lit.[8] 180-181^{0}C].

[4-^{14}C]-5α-Androstan-3,17-dione (10)

Jones reagent was added dropwise to a stirred solution of keto-alcohol **9** (400 mg, 1.4 mmol, 77.7 mCi) in acetone (10 mL) at 0 ^{0}C until persistence of an

orange coloration. After 30 min, isopropanol was added to discharge the color and the reaction mixture was poured into brine (100 mL). Extraction with EtOAc (3 × 100 mL) and standard workup gave dione **10** (370 mg, 70 mCi, S.A. 54.2 mCi/mmol, 93%) as a crystalline solid: mp 127-129 ^{0}C from EtOAc / hexane [lit.[8] 129-131 ^{0}C].

[4-^{14}C]-5α-Androstan-3β-ol-17-one (11)

LiAl[(OBu-*t*)$_3$]H (1.8 mmol, 3.6 mL, 0.5 M in glyme) was added dropwise to a stirred solution of dione **10** (350 mg, 1.2 mmol, 66 mCi) in THF (10 mL) at -78 ^{0}C and the mixture was allowed to warm -20 ^{0}C overnight. Saturated NH_4Cl (3 mL) was added and the reaction mixture was allowed to warm to room temperature. Standard workup though ethyl acetate gave **11** (315 mg, 60 mCi, S.A. 54.5 mCi/mmol, 91%) as a crystalline solid: mp: 173-175 ^{0}C from EtOAc / hexane [lit.[8] 173-174 ^{0}C].

[4-^{14}C]-5α-Androstan-16α-bromo-3β-ol-17-one (1)

A solution of the keto alcohol **11** (200 mg, 0.69 mmol, 37.5 mCi in methanol (20 ml) was heated at 65 ^{0}C with cupric bromide (400 mg) overnight. After solvent removal, water (50 mL) was added to the dark gum and the mixture extracted with chloroform (5 × 50 mL). The combined organic layers were washed with water (3 × 50 mL), brine (100 ml) and dried over Na_2SO_4. The solvent removal gave a solid which was purified by HPLC [Econosil, 10 um silica column, 250 mm × 20 mm, EtOAc/hexane (10:90, v/v), 15 ml/min] to give the target compound **1**(191 mg, 28.4 mCi, S.A. 55.1 mCi, 75%) and compound **12** (64 mg, 9.5 mCi, 25%). The target compound **1** was obtained as a crystalline solid: mp 159-161 ^{0}C from EtOAc / hexane [lit[8]. 160-161 ^{0}C].

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