Synthesis of [Phenyl-2-³H]-travoprost: Isopropyl Ester Prodrug of a Selective Prostaglandin FP Receptor Agonist

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Summary

A method for the preparation of tritium labeled travoprost, a new ocular hypotensive prostaglandin, is described. A highly selective catalytic deiodination has been identified which provides [phenyl-2-³H]-travoprost in a single synthetic step from 2'-iodo-travoprost.

Keywords: travoprost, prostaglandin $F_{2\alpha}$ agonist, catalytic deiodination, tritium

Introduction

Travoprost [**1a**, (+)-fluprostenol isopropyl ester], an ester prodrug form of a potent and selective prostaglandin FP receptor agonist, has been shown to be very effective in decreasing intraocular pressure in non-human primates (1). This compound is currently undergoing late-stage clinical evaluation for the control of ocular
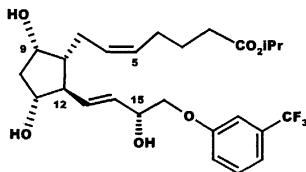
Travoprost, **1a**

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hypertension in the treatment of glaucoma (2, 3). To support absorption, tissue distribution, metabolism, and excretion (ADME) studies we undertook the synthesis of radiolabeled travoprost. The clinical dose of travoprost was anticipated to be approximately 2 μg per day, and since preclinical ADME studies would utilize a correspondingly low dose, it was critical to incorporate a label with high specific activity into a metabolically stable position of the molecule. The preparation of $[^{14}C]$ *rac*-fluprostenol has been reported (4); however, the length of this synthesis and the low specific activity of the labeled compound prepared by this procedure made it unsuitable for our purpose.

Previous studies had demonstrated that tritium atoms attached at either the C-9 or C-15 position of travoprost were not sufficiently metabolically stable to be useful for the desired ADME studies (5). Incorporation of tritium into the phenyl ring of travoprost was anticipated to afford the desired metabolic stability. The most direct approach for the incorporation of a tritium label into the phenyl ring late in the synthesis appeared to be through catalytic dehalogenation. Such an approach would require conditions that would not affect the 5,6-*cis* double bond or the 13,14-*trans* double bond of the allylic alcohol. To our knowledge, aryl substituted prostaglandins with a tritium label on the aromatic ring have not been previously reported. Selective debromination of an aryl bromide in the presence of an olefin has been observed (6); and further, selectivity has been observed for the deiodination of an aryl iodide in the presence of an allylic alcohol (7). Based on these observations, we examined the feasibility of preparing tritium labeled travoprost by using a catalytic dehalogenation approach; this report describes an efficient synthesis of [phenyl-2-^{3}H]-travoprost (**1b**) by that route.

Results and Discussion

Preparation of the desired aryliodide substrate, 2'-iodo-travoprost (**14**), was achieved by a synthetic sequence similar to that previously reported for the preparation of selected 16-phenoxy analogs of $PGF_{2\alpha}$ (8), but with incorporation of the 2-iodo-5-trifluoromethylphenoxy moiety. The requisite aryliodophosphonate intermediate (**4**)

was readily prepared from 2-iodo-5-trifluoromethylphenol (**2**) as shown in Scheme 1. Reaction of Corey aldehyde benzoate (**6**, single stereoisomer) with phosphonate **4**

Scheme 1. Synthesis of phenoxyketophosphonates.
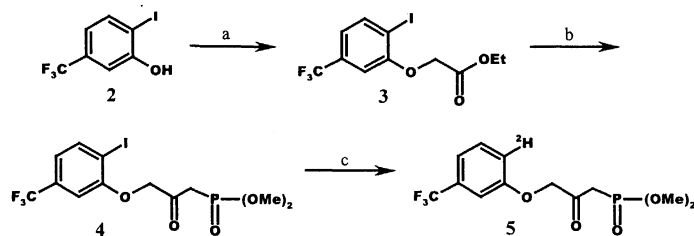
Reagents: a) BrCH$_2$CO$_2$Et, K$_2$CO$_3$, acetone; b) (MeO)$_2$P(=O)Me, *n*-BuLi, THF; c) Et$_3$N, K$_2$CO$_3$, 10% Pd/C, deuterium gas, EtOAc.

provided the C-15 keto intermediate **7** which was reduced to the C-15 hydroxyl compound **8** using (-)-chlorodiisopinocampheylborane (Scheme 2). Though the diastereomeric ratio of product **8** was not determined, this reaction sequence was anticipated to give predominately the desired C-15 epimer based on diasteromeric ratios ranging from 4:1 to 9:1 observed when closely related compounds were reduced under similar conditions (9). Due to the inability to separate the epimers at this point, the mixture was carried forward and separation was achieved later in the sequence. Removal of the benzoyl protective group from **8** provided the diol **9**. The two hydroxyl groups were converted to THP ethers (**10**), and the alpha chain was incorporated into the molecule. This was accomplished by reduction of lactone **10** to the lactol **11** followed by Wittig coupling of **11** with 4-carboxybutyl triphenylphosphonium bromide to afford the acid **12**. Conversion of the derived acid to the isopropyl ester **13** was followed by cleavage of the THP ether protective groups, and the chromatographic separation of the mixture of C-15 epimers was readily achieved to provide the desired 2'-iodo-travoprost (**14**).

Identification of conditions suitable for the catalytic deiodination of **3** was pursued initially; it was reasoned that these conditions would serve as a basis from which to evaluate the deiodination of **9** and ultimately **14**. Reaction of a solution of **3** in EtOAc with hydrogen in the presence of 10% Pd/C did not lead to deiodination. The addition

of base (K$_2$CO$_3$, CaCO$_3$, or NEt$_3$) to the reaction mixture was also not productive. Changing the solvent to EtOH and including K$_2$CO$_3$ provided complete deiodination in 30 minutes. However, since the non-protic solvent EtOAc was preferred for conducting the tritiation, this approach was evaluated further, focusing on identifying a suitable base to include in the reaction. This effort ultimately lead to the

Scheme 2. Synthesis of [phenyl-2-^{3}H]travoprost.
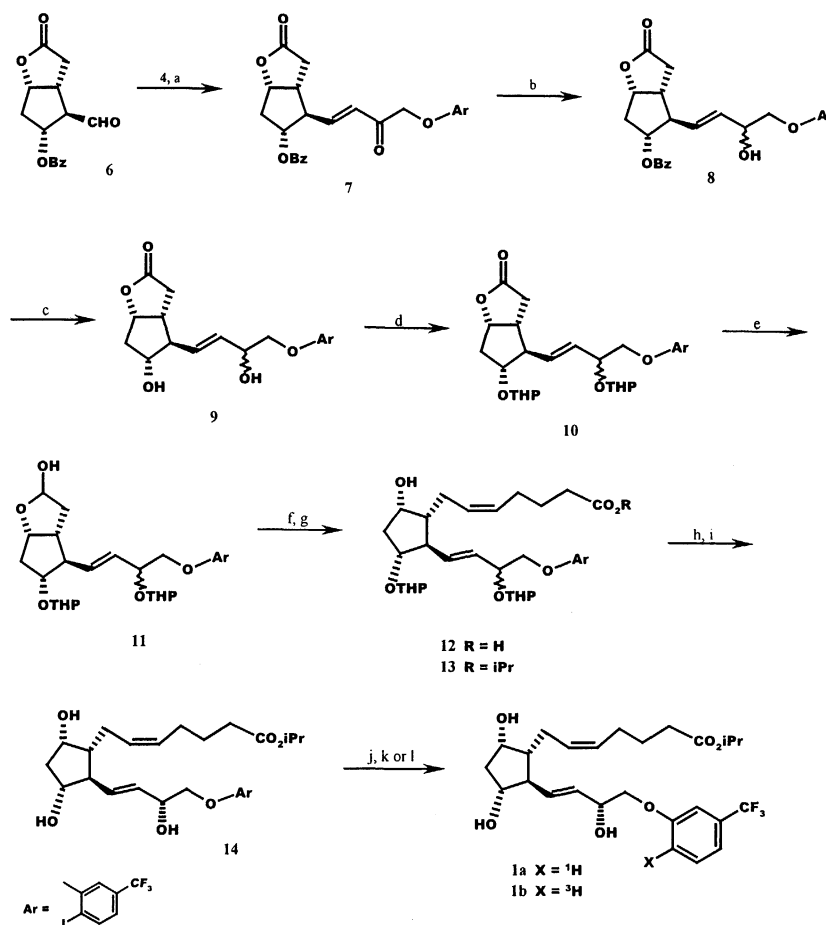
Reagents: a) LiCl, Et$_3$N, THF; b) (-)-DIP-Cl, THF; c) K$_2$CO$_3$, MeOH; d) DHP, *p*-TsOH, CH$_2$Cl$_2$; e) DIBAL-H, THF; f) Ph$_3$P-(CH$_2$)$_4$CO$_2$H, *t*-BuOK, THF; g) (CH$_3$)$_2$CHI, DBU, acetone; h) HCl, MeOH/H$_2$O; i) chromatography; j) 10% Pd/C, K$_2$CO$_3$, Et$_3$N, EtOAc; k) hydrogen; l) tritium.

identification of conditions that provided complete deiodination of **3**, namely EtOAc containing Et$_3$N (1-2 equiv), K$_2$CO$_3$ and 10% Pd/C in an equivalent mass ratio, H$_2$,

2 h. These conditions were equally effective in achieving deiodination of phosphonate **4**, exemplified by reaction in the presence of deuterium gas to provide dimethyl [3-(2-deuterio-5-trifluoromethyl-phenoxy)-2-oxo-propyl]-phosphonate (**5**). When the lactone intermediate **9** was treated with hydrogen gas under these same conditions (30 min) deiodination occurred with no apparent reduction of the double bond to give the 16-trifluoromethylphenoxy product, which was found to be identical to an authentic sample (TLC, NMR, MS) (10). Finally, when **14** was reacted under similar catalytic dehalogenation conditions, the desired deiodination was observed with no apparent reduction of either of the double bonds in the molecule, to give **1a** and a small amount (<5%) of the product arising from *cis*-to-*trans* isomerization at the C-5 double bond. The undesired *trans*-isomer was removed by chromatography to provide **1a**, travoprost, which was identical to an authentic sample. The identity of the 5,6-*trans*-isomer byproduct was confirmed by comparison with an authentic sample of this compound (11). It is important to note that numerous chromatography columns were evaluated for their ability to provide separation of **1a** from 5,6-*trans*-**1a**. Of these, only the Beckman Ultrasphere ODS column provided consistent and reproducible separation.

Incorporation of the tritium label proceeded essentially as described above. However, to further minimize the potential for any reduction of the double bonds, the deiodination reaction was terminated prior to completion. Tritium gas (50 Ci) was used and the reaction was stopped at 20 min when 85% of the theoretical uptake of tritium gas was achieved. Evaluation of the crude product showed the presence of 5,6-*trans*-**1b** (~3%) which was separated by chromatography to provide pure **1b**, [phenyl-2-³H]-travoprost, with a radiochemical purity of 99% [55 mCi, specific activity 23 Ci/mmol]. The utility of these catalytic deiodination conditions for the incorporation of multiple tritium atoms into travoprost has not yet been evaluated.

Experimental

Organic extracts were dried over $MgSO_4$ (unless otherwise noted) which was removed by filtration and washed with the appropriate dry solvent. Chromatography refers to

low pressure column chromatography conducted on 230 - 400 mesh silica gel from E. Merck. Evaporations were performed under reduced pressure on a rotary evaporator at 40°C. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 200 MHz and 50.3 MHz, respectively, with a Varian Model VXR-200 spectrometer. Spectra were recorded in CDCl$_3$ and chemical shifts are reported in parts per million (δ) relative to tetramethylsilane as the internal standard. Mass spectra were recorded on either a Finnigan LQ LCMS or a PerSeptive Biosystems Voyager RP time-of-flight mass spectrometer. The high-resolution spectrum was obtained by Analytical Instrument Group, Inc., Raleigh, North Carolina. The elemental analysis was performed by Atlantic Microlabs, Norcross, Georgia. TLC plates (EM Sciences, Silica 60) were developed in the indicated solvent mixtures and spots visualized by UV light and phosphomolybdic acid spray. Purification of the radiolabeled material was performed using a HPLC system that consisted of a Waters M6000 pump system, a Beckman Ultrasphere ODS semi-preparative column, a Lambda-Max model 480 UV detector, and a NEN in-house model radioactivity detector built using Canberra components. Radioactive samples were counted on a Beckman LS6000TA counter. Palladium-on-carbon was purchased from Aldrich Chemical Co. Compound **6** [(3aR)-(3aα,4ß,5α, 6aα)-5-(benzoyloxy)-hexahydro-2-oxo-2*H*-cyclopenta[*b*]furan-4-carboxaldehyde, Corey aldehyde benzoate] is available as the single stereoisomer from The Upjohn Co., Fine Chemical Division.

2-Iodo-5-trifluoromethyl-phenol (2). To a stirred suspension of NaH (60% dispersion in oil, 9.2 g, 0.23 mol,) in toluene (800 mL) was added 3-trifluoromethyl-phenol (26.6 g, 0.164 mol). After 5 min, iodine (41.6 g, 0.164 mol) was added and the suspension stirred for 16 h at room temperature, diluted with 3 N HCl (600 mL), and extracted with ether (3 x 200 mL). The combined extracts were washed with brine (200 mL), dried, and evaporated to a residue which was purified by chromatography (gradient, 10% to 20% EtOAc in hexane) to give an oil (40 g, 86%): lit. mp 36°C (12); ^{1}H NMR (CDCl$_3$) δ 7.78 (d, J = 8.0 Hz, 1H), 7.21 (s, 1H), 6.92 (d, J = 8.0 Hz, 1H), 4.89 (s, 1H); MS (CI) *m/z* 289 (M+1).

Ethyl (2-Iodo-5-trifluoromethyl-phenoxy)acetate (3). To a solution of **2** (39.8 g, 0.138 mol) in acetone (250 mL) was added K$_2$CO$_3$ (28.6 g, 0.207 mol) and ethyl bromoacetate (27.8 g, 0.166 mol); this mixture was heated at reflux temperature for 16 h. The reaction mixture was filtered and the filtrate evaporated to a residue which was dissolved in ether (300 mL) and washed with 5% aqueous NaHCO$_3$ (100 mL) and brine (100 mL), dried, and evaporated to a syrup which was purified by chromatography (gradient, 5% to 10% EtOAc in hexane) to give a solid (25 g, 48%): mp 43-45°C; ^{1}NMR (CDCl$_3$) δ 7.93 (d, J = 8.2 Hz, 1H), 7.00 (dd, J = 1.4 and 8.2 Hz, 1H), 6.89 (d, J = 1.4 Hz, 1H), 4.73 (s, 2H), 4.30 (q, J = 7.2 Hz, 2H), 1.30 (t, J = 7.2 Hz, 3H); MS (CI) *m/z* 375 (M+1). Analysis. Calculated for C$_{11}$H$_{10}$F$_3$IO$_3$: C, 35.32; H, 2.69; I, 33.92. Found: C, 35.41; H, 2.65; I, 33.84.

Dimethyl [3-(2-iodo-5-trifluoromethyl-phenoxy)-2-oxo-propyl]-phosphonate (4). A solution of dimethyl methylphosphonate (7.9 mL, 0.07 mol) in THF (250 mL)

under nitrogen was cooled to -78°C and *n*-BuLi (32 mL of a 2.5 M solution in hexane, 0.08 mol) was added slowly. This mixture was stirred at -78°C for 30 min and a solution of **3** (24 g, 0.06 mol) in THF (250 mL) was added followed by stirring for 2 h during which the mixture was allowed to warm to -40°C. The reaction mixture was poured into water (500 mL) and extracted with ether (4 x 100 mL). The aqueous layer was acidified to pH 2 with 1 N HCl and extracted with chloroform (4 x 200 mL). The combined extracts were washed with water (2 x 100 mL) and brine (2 x 100 mL), dried, and evaporated to a residue which was purified by chromatography (EtOAc) to give a viscous syrup (6.2 g, 22%): R_f 0.3 (30% EtOAc in hexane); ^{1}H NMR (CDCl$_3$) δ 7.94 (d, J = 8.0 Hz, 1H), 7.03 (d, J = 8.0 Hz, 1H), 6.94 (s, 1H), 4.82 (s, 2H), 3.84 (s, 3H), 3.79 (s, 3H), 3.43 (d, J = 22.0 Hz, 2H).

Dimethyl [3-(2-deuterio-5-trifluoromethyl-phenoxy)-2-oxo-propyl]-phosphonate (5).

To a solution of **4** (50 mg, 0.11 mmol) in dry EtOAc (5 mL) was added Et$_3$N (15 µL, 0.11 mmol) followed by dry solid K$_2$CO$_3$ (50 mg, 0.36 mmol) and 10% Pd/C (50 mg). The mixture was stirred under nitrogen for 5 min and the nitrogen line was replaced with a deuterium-filled balloon. The reaction mixture was monitored by TLC and after 2 h only a single new compound was present [R_f 0.66; R_f of **4** is 0.5 (40% hexane in EtOAc)]. The reaction mixture was filtered through a filter aid and the filtrate evaporated to provide a viscous syrup (35 mg, 96%): ^{1}H NMR (CDCl$_3$) δ 7.42 (d, J = 8.0 Hz, 1H), 7.28 (d, J = 8.0 Hz, 1H), 7.15 (s, 1H), 4.80 (s, 2H), 3.78 (s, 3H), 3.75 (s, 3H), 3.25 (d, J = 24.0 Hz, 2H); MS (CI) *m/z* 328 (M+1).

(3aR)(3aα,4ß,5α,6aα)-5-Hydroxy-4-[(E)-3-hydroxy-4-(2-iodo-5-trifluoromethyl-phenoxy)-but-1-enyl]-hexahydro-cyclopenta[*b*]furan-2-one (9).

Step A: A mixture of **4** (4.40 g, 9.7 mmol), Corey aldehyde benzoate (**6**) (2.20 g, 8.08 mmol), and LiCl (0.37 g, 9.7 mmol) in THF (400 mL) was cooled to 0°C and Et$_3$N (1.4 mL, 9.7 mmol) was added. The yellow slurry that formed was stirred at -5°C for 20 min and then allowed to warm to ambient temperature (3 h); dilute KH$_2$PO$_4$ (100 mL) and EtOAc (100 mL) were added. The organic layer was separated and the aqueous extracted with EtOAc (3 x 50 mL). The combined extracts were washed with brine (2 x 50 mL), dried, and evaporated to provide a residue which was purified by chromatography (50% EtOAc in hexane) to give the α,ß-unsaturated ketone (**7**) as a colorless syrup (2.93 g, 60%): R_f 0.41 (50% EtOAc in hexane); ^{1}H NMR (CDCl$_3$) δ 7.95 (m, 3H), 7.50 (m, 3H), 6.95 (m, 4H), 5.38 (q, 1H), 5.12 (t, 1H), 4.73 (s, 2H), 2.65 (m, 4H), 2.31 (m, 2H).

Step B: A solution of (-)-chlorodiisopinocampheylborane (3.10 g, 9.8 mmol) in THF (150 mL) was cooled to 0°C under nitrogen and a solution of **7** (2.93 g, 4.9 mmol) in THF (150 mL) was slowly added. The mixture was stirred at 0°C for 2 h and then cooled to -20°C for 18 h. Methanol (20 mL) was added and the mixture allowed to warm to room temperature (30 min); a saturated solution of KH$_2$PO$_4$ (50 mL) and EtOAc (50 mL) were added. The organic layer was separated and the aqueous extracted with EtOAc (3 x 50 mL). The combined extracts were washed with brine (2 x 50 mL), dried, and evaporated to provide a residue which was purified by

chromatography (gradient, 30% to 50% EtOAc in hexane) to give an enantioenriched mixture of diastereomers (8) as a colorless viscous syrup (2.88 g, 97%): R_f 0.17 (50% EtOAc in hexane); ^{1}H NMR (CDCl$_3$) δ 7.98 (d, J = 7.4 Hz, 2H), 7.86 (d, J = 8.0 Hz, 1H), 7.52 (m, 1H), 7.40 (m, 2H), 7.00 (d, J = 8.0 Hz, 1H), 6.92 (s, 1H), 5.83 (m, 2H), 5.30 (q, 1H), 5.07 (t, 1H), 4.60 (m, 1H), 4.05 (m, 1H), 3.91 (m, 1H), 2.9-2.2 (m, 7H).

Step C: A solution of **8** (2.88 g, 4.8 mmol) in MeOH (50 mL) containing K$_2$CO$_3$ (0.8 g) at room temperature was stirred for 2 h. The reaction mixture was filtered and the filtrate was evaporated to a residue which was dissolved in water (100 mL) and extracted with a solution of 10% Et$_2$O in hexane (4 x 50 mL). The aqueous solution was acidified to pH 2 with 1 N HCl and extracted with CHCl$_3$ (5 x 50 mL). The combined extracts were washed with water (2 x 50 mL), brine (2 x 50 mL), dried (Na$_2$SO$_4$), and evaporated to a residue which was purified by chromatography (60% EtOAc in hexane to EtOAc containing 1% EtOH) to give the diol **9** as a colorless syrup (1.87 g, 77%): R_f 0.10 (60% EtOAc in hexane); ^{1}H NMR (CDCl$_3$) δ 7.91 (d, J = 8.0 Hz, 1H), 7.02 (d, J = 8.0 Hz, 1H), 7.00 (s, 1H), 5.76 (m, 2H), 4.93 (m, 1H), 4.61 (s, 1H), 4.05 (m, 3H), 2.9-2.0 (m); MS (CI) *m/z* 521 (M+Na).

[1R-[1α(Z),2ß(1E),3α,5α]]-7-[5-Hydroxy-2-[4-(2-iodo-5-trifluoromethyl)phenoxy]-3-[(tetrahydro-2*H*-pyran-2-yl)oxy]-1-butenyl]-3-[(tetrahydro-2*H*-pyran-2-yl)oxy]cyclopentyl]-5-heptenoic acid 1-methylethyl ester (13).

Step A: A solution of **9** (2.09 g, 4.2 mmol) and dihydropyran (1.2 mL, 12.6 mmol) in CH$_2$Cl$_2$ (100 mL) and THF (20 mL) was cooled to 0°C and *p*-toluenesulfonic acid (25 mg) was added in one portion. The reaction mixture was warmed to ambient temperature and stirred for 1.5 h followed by the addition of 5% aqueous NaHCO$_3$ (50 mL). The organic layer was removed and the aqueous extracted with dichloromethane (2 x 25 mL). The combined extracts were dried and evaporated to a residue which was purified by chromatography (gradient, 30% to 60% EtOAc in hexane) to give the protected diol **10** as a colorless syrup (2.76 g, 98%): R_f 0.31 (50% EtOAc in hexane); ^{1}H NMR (CDCl$_3$) δ 7.89 (d, J = 8.0 Hz, 1H), 7.03 (d, J = 8.0 Hz, 1H), 6.97 (s, 1H), 5.65 (m, 2H), 4.95 (m, 2H), 4.65 (m, 2H), 4.05 (m, 4H), 3.5 (m, 2H), 2.8-1.5 (m).

Step B: A solution of **10** (2.76 g, 4.1 mmol) in THF (50 mL) was cooled to -78°C and a solution of DIBAL-H (6.2 mmol, 4.2 mL of a 1.5 M solution in toluene) was added. The mixture was stirred at this temperature for 1 h and then quenched by adding methanol (5 mL) and a saturated aqueous solution of potassium sodium tartrate tetrahydrate (50 mL) followed by extraction with EtOAc (3 x 50 mL). The combined extracts were washed with brine (50 mL), dried, and evaporated to a residue which was purified by chromatography (gradient, 30% to 60% EtOAc in hexane) to give the lactol **11** as a colorless syrup (2.34 g, 85%): R_f 0.21 (50% EtOAc in hexane); ^{1}H NMR (CDCl$_3$) δ 7.88 (d, J = 8.0 Hz, 1H), 7.16 (m, 6H), 7.65 (m, 3H), 4.6 (m, 3H), 4.0 (m, 5H), 3.5 (m, 2H), 2.0-1.5 (m).

Step C: A suspension of 4-carboxybutyl triphenylphosphonium bromide (4.7 g, 10.5 mmol) in THF (50 mL) was cooled to 0°C and a solution of potassium *t*-butoxide (21 mL of a 1.0 M solution in THF) was added slowly. The resultant reddish solution was stirred for 10 min followed by the slow addition of a solution of **11** (2.34 g, 3.5 mmol) in THF (50 mL). After stirring for 15 min at 0°C, the reaction mixture was warmed to ambient temperature (30 min) followed by the addition of a 50% solution of KH_2PO_4 (100 mL) and EtOAc (100 mL). The organic layer was separated and the aqueous extracted with EtOAc (3 x 50 mL). The combined extracts were washed with brine (2 x 50 mL), dried, and evaporated to provide crude acid **12**. A solution of **12** in acetone (40 mL) was cooled to 0°C and DBU (6.3 mL, 42 mmol) was added; after stirring for 10 min, 2-iodopropane (3.5 mL, 35 mmol) was added. This mixture was stirred for 18 h, gradually warming to ambient temperature. Solvent was evaporated and the residue partitioned between dilute aqueous KH_2PO_4 (50 mL) and EtOAc (50 mL). The organic layer was separated and the aqueous extracted with EtOAc (3 x 50 mL). The combined extracts were washed with brine (2 x 50 mL), dried, and evaporated to provide a residue which was purified by chromatography (gradient, 30% to 50% EtOAc in hexane) to give the isopropyl ester **13** as a pale yellow oil (2.36 g, 85% from **11**): R_f 0.57 (40% hexane in EtOAc); ^1H NMR ($CDCl_3$) δ 7.89 (d, J = 8.0 Hz, 1H), 7.08 (s, 1H), 6.97 (d, J = 8.0 Hz, 1H), 5.5 (m, 4H), 5.1 (m, 2H), 4.6 (m, 3H), 4.0 (m, 7H), 3.4 (m, 2H), 2.5-1.3 (m), 1.25 (d, J = 6.0 Hz, 6H).

[1R-[1α(Z),2β(1E,3R*),3α,5α]]-7-[3,5-Dihydroxy-2-[3-hydroxy-4-[2-iodo-5-(trifluoromethyl)phenoxy]-1-butenyl]cyclopentyl]-5-heptenoic acid, 1-methyl ethyl ester (14). A solution of **13** (2.36 g, 3.0 mmol) in a mixture of MeOH (50 mL) and water (50 mL) was cooled to 0°C and 12 N HCl (0.5 mL) was added dropwise. After maintaining this temperature for 15 min, the ice bath was removed and the reaction mixture was stirred for 1.5 h. A 5% solution of $NaHCO_3$ (50 mL) was added and the mixture extracted with EtOAc (3 x 50 mL). The combined extracts were washed with brine (50 mL), dried, and evaporated to a residue which was purified by chromatography (gradient, 40% to 80% EtOAc in hexane containing 1% EtOH) to give a mixture of **14** and the C15 epimer (R_f 0.23) followed by pure **14** as a pale yellow oil (0.98 g, 49%): R_f 0.16, (40% hexane in EtOAc); ^{1}NMR ($CDCl_3$) δ 7.89 (d, J = 8.0 Hz, 1H), 7.02 (s, 1H), 7.00 (d, J = 8.0 Hz, 1H), 5.74 (m, 2H), 5.40 (m, 2H), 4.99 (m, 1H), 4.61 (m, 1H), 4.07 (m, 4H), 2.83 (d, J = 4.0 Hz, 1H), 2.65 (d, J = 4.0 Hz, 1H), 2.2 (m), 1.7 (m, 6H), 1.22 (d, J = 6.0 Hz, 6H); ^{13}C ($CDCl_3$) δ 173.5 (C), 157.3 (C), 145.2 (C), 139.9 (CH), 135.3 (CH), 132.5 (C), 131.8 (C), 130.0 (CH), 129.1 (CH), 129.0 (CH), 119.6, 108.8, 78.1 (CH), 73.4 (CH_2), 73.1 (CH), 70.6 (CH), 67.7 (CH), 56.1 (CH), 50.6 (CH), 42.9 (CH_2), 34.0 (CH_2), 26.7 (CH_2), 25.7 (CH_2), 24.9 (CH_2), 21.9 (2 CH_3); MS (CI) *m/z* 649 (M+Na); HRMS: (fab), (M+H); Calculated for $C_{26}H_{35}F_3IO_6$: 627.1430. Found: 627.1427.

[1R-[1α(Z),2β(1E,3R*),3α,5α]]-7-[3,5-Dihydroxy-2-[3-hydroxy-4-[3-(trifluoromethyl)phenoxy]-1-butenyl]cyclopentyl]-5-heptenoic acid, 1-methyl ethyl ester (1a, travoprost). A solution of **14** (62.6 mg, 0.1 mmol) in EtOAc (8 mL) was stirred vigorously under nitrogen for 5 min. K_2CO_3 (50 mg, 0.3 mmol), Et_3N

(30 μL, 0.2 mmol) and 10% Pd/carbon (50 mg) were added sequentially to the reaction mixture and the nitrogen line was replaced with a hydrogen-filled balloon. The system was flushed with hydrogen for 3 min and the reaction proceeded at room temperature under a positive pressure of hydrogen. After stirring for 30 min the reaction mixture was filtered through a 0.45 μ nylon membrane and the filter cake washed with EtOAc (2 mL). The filtrate was concentrated to an oil which was purified by chromatography (gradient: 20% hexane in EtOAc containing 1% EtOH to EtOAc containing 1% EtOH) to give 1a as an oil (35 mg, 70%): R_f 0.21 (EtOAc); ^{1}H NMR (CDCl$_3$) δ 7.39 (m, 1H), 7.21 (m, 3H), 5.64 (m, 2H), 5.41 (m, 2H), 4.99 (m, 1H), 4.55 (m, 1H), 4.20 (m, 3H), 2.85 (bs, 2H), 2.4-2.1 (m, 8H), 1.8-1.3 (m, 6H), 1.21 (d, J = 6.0 Hz, 6H). HPLC analysis showed the presence of 5,6-*trans*-1a (5%), which could be readily separated using a Beckman semi-preparative Ultrasphere ODS column (4.6 x 150 mm, 5 μ) eluted with CH$_3$CN /H$_2$O (40:60) for 30 min, then linear gradient in 10 minutes to CH$_3$CN/H$_2$O (70:30), 0.75 mL/min, UV detector at 220 nM. Elution times: 1a, 36 min; 5,6-*trans*-1a, 38 min; 14, 46 min.

[1R-[1α(Z),2ß(1E,3R*),3α,5α]]-7-[3,5-Dihydroxy-2-[3-hydroxy-4-[2-tritio-5-(trifluoromethyl)phenoxy]-1-butenyl]cyclopentyl]-5-heptenoic acid, 1-methyl ethyl ester (1b, [phenyl-2-^{3}H]travoprost). To a stirred solution of 14 (62.6 mg, 0.1 mmol) in EtOAc (8 mL) and Et$_3$N (30 μL) was added K$_2$CO$_3$ (50 mg) and 10% Pd/C (50 mg). The reaction flask was cooled to -78°C and the tritiation system was evacuated using a vacuum pump. Tritium gas (50 Ci) was introduced into the reaction flask and the reaction mixture was stirred at room temperature for 20 min, at which point 85% of the theoretical uptake of tritium gas was reached. The volatile material was removed and the residue was co-evaporated with methanol three times and the crude product (270 mCi) dissolved in ethanol (5 mL). Evaluation of the crude product by analytical HPLC showed the presence of approximately 3% of the 5,6-*trans* isomer in addition to the desired 5,6-*cis* isomer. This material was purified by HPLC using the Beckman semi-preparative Ultrasphere ODS column monitored with a UV detector set at 214 nm. The column was eluted with water/acetonitrile (60:40, v/v) at a flow rate of 2 mL/min. Those fractions containing only the desired product were combined and evaporated to an oil, which was dissolved in ethanol (11 mL) to provide 1b: 55 mCi; specific activity 23 Ci/mmol; radiochemical purity, 99%, as determined by HPLC on a Beckman analytical Ultrasphere ODS column using a mobile phase of water/acetonitrile (60:40) with a flow rate of 1 mL/min. No attempt was made to recover unreacted 14.

References

1. Hellberg, M., Sallee, V., McLaughlin, M. A., Sharif, N. A., DeSantis, L., Dean, T. R., Zinke, P. W. *Invest. Opthal. Vis. Sci.* **39**, S1961 (1998).

2. Garadi, R., Silver, L., Landry, T., Turner, F. D. *Invest. Opthal. Vis. Sci.* **40**, S4378 (1999).

3. Kothe, A. C., Ripp, K. M., Sharma, V., Von Tress, M. S., DeSantis, L., Bergamini, M. V. W., Robertson, S. M. *Invest. Opthal. Vis. Sci.* **41**, S1483 (2000).

4. White, D. F. *J. Labelled Compd. Radiopharm.* **13**, 23 (1977).

5. Sanders, M. Pharmacokinetics Unit, Alcon Research, Ltd., personal communication.

6. Bloxsidge, J. P., Elvidge, J. A., Gower, M., Jones, J. R., Evans, E. A., Kitcher, J. P., Warrell, D. C. *J. Labelled Compd. Radiopharm.* **18**, 1141 (1981).

7. Seltzman, H. H., Roche, M. J., Laudeman, C. P., Wyrick, C. D., Carroll, F. I. *J. Labelled Compd. Radiopharm.* **41**, 811 (1998).

8. Klimko, P. G., Bishop, J. E., Sallee, V. L., Zinke, P. W. U.S. Patent 5,889,052 (1999).

9. Zinke, P. W. Medicinal Chemistry Unit, Alcon Research Ltd., personal communication.

10. Bowler, J., Crossley, N. S. U.S. Patent 4,321,275 (1982).

11. Dean, W. D. Chemical Preparations Research Unit, Alcon Research, Ltd., personal communication.

12. Carson, J. R. U.S. Patent 4,652,584 (1987).