A GENERAL METHOD FOR THE PREPARATION OF ^{14}C-LABELED METABOLICALLY STABLE PROSTAGLANDINS

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SUMMARY

A method is described for the preparation of 5-bromopentanoic-5-^{14}C acid which is inserted *via* the Wittig ylid into the desired prostaglandin intermediate in the last carbon-carbon bond forming step of the Corey synthesis. Three different prostaglandins were prepared carbon-labeled (both ^{14}C and ^{13}C) in the metabolically stable position 5 to illustrate the general utility of this procedure.

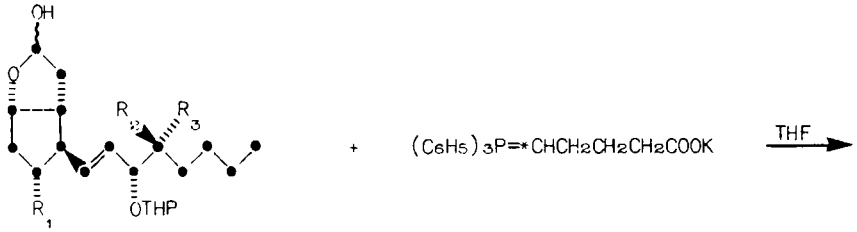
Key Words: Prostaglandins-5-^{14}C, 5-Bromopentanoic-5-^{14}C acid.

In vivo metabolism and absorption studies on a number of prostaglandin analogues required the preparation of several ^{14}C-labeled drugs with the label in a metabolically stable position. The need to label several prostaglandin analogues necessitated the development of a general synthetic method for the introduction of a ^{14}C label into this class of compounds. Existing routes to ^{14}C-labeled prostaglandins either introduce the label biochemically into the 1-^{14}C(1-7), 2-^{14}C(8,9), or 3-^{14}C(9) position, or synthetically into the 1-^{14}C position.(10) Since the major cleavage metabolic pathway of PGE_2(11) and $PGF_{2\alpha}$(12,13) in man involves loss of the first four carbon atoms *via* two β-oxidation steps,(11,12) these labeling procedures were unsatisfactory.(14)

0362-4803/85/020159-12$01.20

Received June 25, 1984
Revised August 6, 1984

The last carbon-carbon bond forming step in the Corey approach[15,16] to the synthesis of prostaglandins involves condensation of the Wittig ylid of 5-bromopentanoic acid (3) with the appropriate lactol (2). Since most prostaglandins have the same upper side



2a, R_1=R_2=R_3=CH_3
2b, R_1=R_2=H, R_3=F

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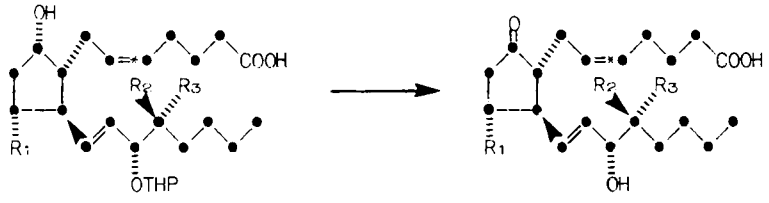


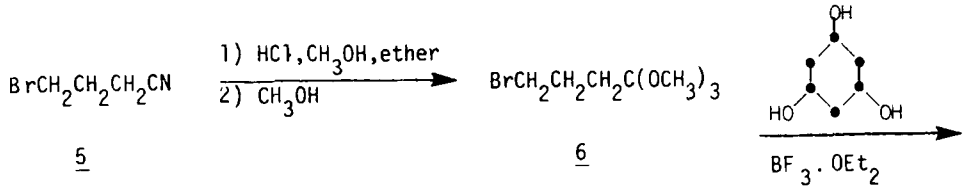
4a, R_1=R_2=R_3=CH_3
4b, R_1=R_2=H, R_3=F

1a, R_1=R_2=R_3=CH_3
1b, R_1=R_2=H, R_3=F

chain,[17] we decided to introduce a 5-^{14}C label via 5-bromopentanoic-5-^{14}C acid. In addition to introducing the label into a metabolically stable position, this approach conserves the number of radiochemical steps in the synthesis and avoids the custom synthesis of each particular lactol (2) in labeled form. Three different prostaglandins were then prepared carbon labeled using both 5-bromopentanoic-5-^{14}C acid and the 5-^{13}C derivative which illustrates the general utility of this procedure. In addition, prostacyclins[18] and thromboxanes[19] are also

prepared by use of the same five carbon Wittig reagent, so this route should be useful to label these compounds as well.

Terminally carbon labeled 5-bromopentanoic acid was prepared according to the scheme outlined below. Trimethyl ortho ester 6 was prepared from 4-bromobutyronitrile 5 via the iminoester



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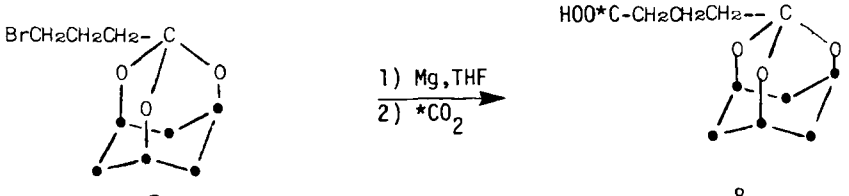

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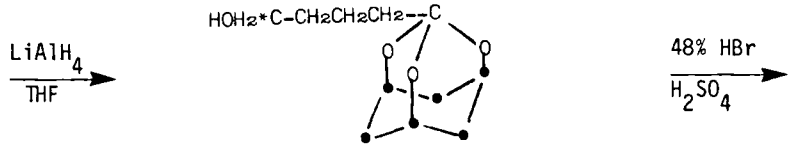

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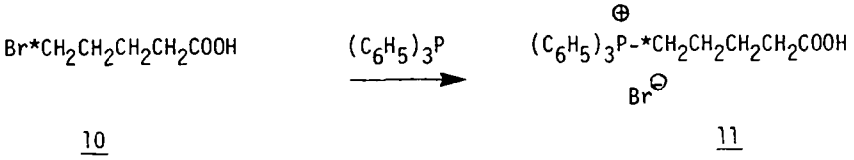

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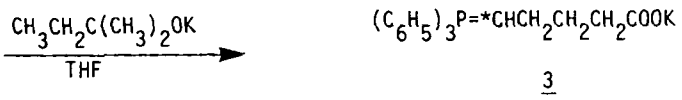
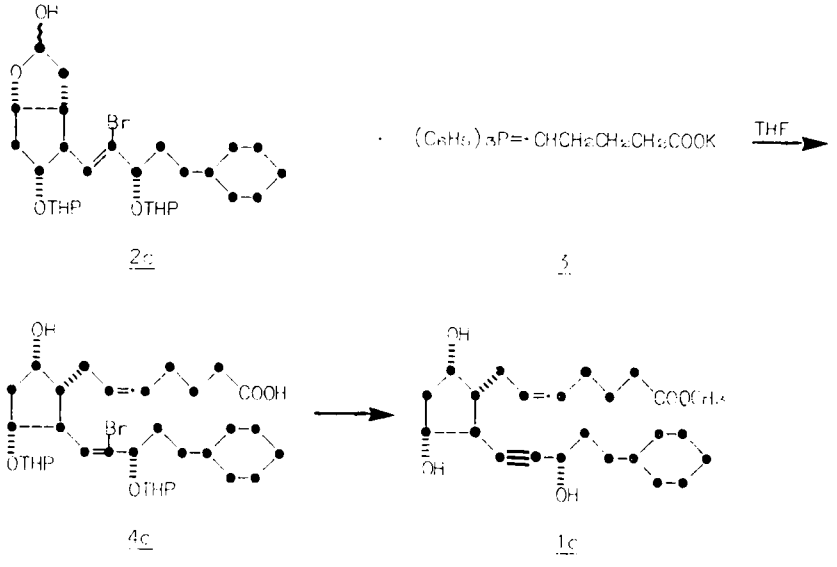


3

hydrochloride.[20] Ortho ester 6 was then transesterified[21] with cis-1,3,5-cyclohexantriol[22,23] using boron trifluoride etherate to form the desired oxaadamantane protected 7.[24] Ortho ester 7 was converted to its Grignard reagent and carboxylated with carbon dioxide-^{14}C (or-^{13}C) to give labeled acid 8, which was subsequently reduced with lithium aluminum hydride to alcohol 9. Deprotection of 9 and conversion of the alcohol to the bromide was conveniently carried out in one step using 48% hydrobromic acid with concentrated sulfuric acid at 130°[25,26] giving the desired 10. Bromo-acid 10 was then converted to the Wittig salt 11 by heating the neat acid with triphenylphosphine at 135°C.[27] Wittig salt 11 was converted to the ylid 3 using two equivalents of potassium t-amylate in tetrahydrofuran.[28]

Labeled ylid 3 was condensed with lactol 2a in tetrahydrofuran to give 4a after which Jones oxidation[29] and removal of the tetrahydropyranyl protecting group[30] gave the desired 1a-5-^{14}C or 1a-5-^{13}C.[31] Similarly, prostaglandins 1b-5-^{14}C and 1b-5-^{13}C were prepared from lactol 2b and labeled 3. A structurally different prostaglandin 1c was also prepared labeled at position 5. Lactol 2c[32] was condensed with labeled Wittig reagent 3 giving 4c which was



2c 3

4c 1c

dehydrobrominated with dimsyl anion, esterified, and deprotected to give 1c-5-^{13}C and 1c-5-^{14}C. The yield of Wittig phosphonium salt (11) from carbon dioxide-^{14}C was >70% and overall yield of the prostaglandins from carbon dioxide-^{14}C was > 20%.

EXPERIMENTAL

General. Spectra and microanalyses were made by the Physical Chemistry Department of Hoffmann-La Roche Inc. Radiochemical purity was determined on EM silica gel plates using an LB 2832 Berthold TLC Linear Analyzer System and radioactivity was measured by the liquid scintillation technique with a Packard Tricarb Model 2003/Automatic Control 2010 spectrometer. Tetrahydrofuran was distilled prior to use from benzophenone ketyl.

4-Bromo-1,1,1-trimethoxybutane (6) 4-Bromobutyronitrile (5, 30 g, 203 mmol, Aldrich) was esterified *via* the iminoester hydrochloride essentially according to the procedure of McElvain[20] to give 6 (11.1g, 48.8 mmol, 24% overall) as a colorless oil: bp 94-105°C (17 mm Hg); NMR ($CDCl_3$) δ 1.82 (br, 4, C_2H and C_3H), 3.25 (s, 9, CH_3O), and 3.47 (t, 2, J=6 Hz, CH_2Br); mass spectrum, m/z 195 (M-31).

1-(3-Bromopropyl)-2,8,9-trioxaadamantane (7) - Ortho ester 6 (6.35g, 28 mmol) was transesterified with cis-1,3,5-cyclohexanetriol[22,23] (3.3 g, 25 mmol) and boron trifluoride etherate (0.75 mL) in methanol (12 mL) essentially according to the method of Osbond[21] to give 7 (4.08 g, 15.5 mmol, 62%) as a colorless oil: bp 152-154°C (5 mm Hg); NMR ($CDCl_3$) δ1.6-1.9 (m, 5, cyclohexane axial and C_3H), 2.05 (m, 2, C_2H), 2.61 (br d, 3, cyclohexane equatorial, J=12 Hz), 3.45 (t, 3, CH_2Br, J=6 Hz), and 4.37 (br, 3, CHO); mass spectrum, m/z (262 M^+).

2,8,9-Trioxaadamantyl-1-butanoic-1-^{14}C acid (8) - Freshly ground magnesium (267 mg, 11 mmol) was added to a solution of 7 (2.63 g, 10 mmol) in 20 mL of freshly distilled tetrahydrofuran under nitrogen. After activated magnesium(33) (two small pieces), 1,2-dibromoethane (~ 2 drops) and iodine (~ 5 mg) were added to initiate the reaction, the solution was stirred for 1 h at 45°C. After being cooled, the solution was pipetted away from excess magnesium into a fresh dry flask. The solution was frozen in liquid nitrogen and degassed under high vacuum (freeze/thaw). Carbon dioxide-^{14}C (1.22 g, 6.1 mmol, 343 mCi; specific activity 56 mCi/mmol-generated from barium carbonate-^{14}C) was vacuum transferred into the solution flask which was subsequently stirred for 1.5h at -78°C. The solution was refrozen in liquid nitrogen (to recondense any unreacted $^{14}CO_2$) and stirred an additional 1 h at room temperature. Water (0.6 mL) was added to quench the reaction solution which was then concentrated under high vacuum. The residue was dissolved in water (50 mL), acidified with 6N hydrochloric acid to pH 3-4 and extracted with chloroform (5x20 mL). The combined chloroform extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was dissolved in 25 mL of 1N sodium hydroxide and washed with 25 mL of ether. The ether phase was back extracted with 5 mL of 1N sodium hydroxide. The combined aqueous alkaline phase was washed with 10 mL of ether, then acidified with 6N hydrochloric acid to pH 3-4 and extracted with chloroform (8x25 mL). The combined chloroform extracts were dried ($MgSO_4$) and concentrated in vacuo to give 1.40 g (6.1 mmol, 343 mCi, 100% from barium carbonate-^{14}C) of 8 as a white solid. An unlabeled sample of 8 was recrystallized from carbon tetrachloride to give mp 123-125°C: NMR ($CDCl_3$) δ 1.55-2.05 (m, 7, cyclohexane axial and C_2H, C_3H), 2.42 (t, 3, J=6 Hz, CH_2COOH), 2.58 (br d, 3, J=12 Hz), cyclohexane equatorial) and 4.37 (br, 3, -CHO-); mass spectrum, m/z 228 (M^+).

Anal. calcd. for $C_{11}H_{16}O_5$: C, 57.89; H, 7.07. Found: C, 57.46; H, 7.00.

1-(4-Hydroxybutyl-4-^{14}C)-2,8,9-trioxaadamantane (9) - Lithium aluminum hydride (474 mg, 12.5 mmol) was added to a solution of 8 (1.40g, 6.1 mmol) in 100 mL of ether and the suspension was stirred at room temperature for 2 h under nitrogen. The reaction was quenched with ethyl acetate (2 mL) followed by water (1.2 mL). The ether phase was washed with 2N sodium hydroxide (2x50 mL) and brine (25 mL). The combined aqueous washes were back extracted with ether (2x50 mL). The combined ether phase was dried twice ($MgSO_4$) and concentrated *in vacuo* to give 1.22 g (5.7 mmol, 315 mCi, 92%) of 9 as a colorless oil. An unlabeled sample exhibited the following spectra: NMR ($CDCl_3$) δ 1.52-2.0 (m, 10, axial, C_2H, C_3H, C_4H and hydroxyl), 2.57 (br d, 3, J=12 Hz, equatorial), 3.62 (m, 2, CH_2O), and 4.37 (br, 3, CHO); mass spectrum, m/z 214 (M^+).

5-Bromopentanoic-5-^{14}C acid (10) - A solution of 9 (1.22 g, 5.7 mmol) in 23 mL of 48% hydrobromic acid and 0.6 mL of concd sulfuric acid was heated at 130°C for 2.5 h.[25,26] The solution was diluted with 12 mL of water and extracted 5 times with 15 mL of chloroform. The combined organic extracts were washed with brine (5 mL), then dried ($MgSO_4$) and concentrated under vacuum to give 1.01 g (5.98 mmol, 98%) of crude 10. Distillation of the crude material (170°C/0.6 mmHg) yielded 900 mg (4.97 mmol, 278 mCi, 87% overall) of solid 10. A sample of unlabeled material was identical (NMR, MS, mp, TLC/silica gel-1% acetic acid in ethyl acetate) with reference material.[34]

(4-Carboxybutyl-1-^{14}C)triphenylphosphonium bromide (11-5-^{14}C) - A mixture of 10 (900 mg, 4.97 mmol) and triphenylphosphine (1.32 g, 5.04 mmol) was heated at 135-140°C in a sealed flask for 3 h[27] (crystallization occurred after 0.5 h). The solid mass was dissolved in 22 mL of refluxing chloroform and the solution was then concentrated to

8-10 mL. Ether was added to the cloud point (~1 mL), a seed crystal was added and then additional ether (10 mL) slowly added. The suspension was kept at room temperature for 0.5 h, then centrifuged and the mother liquor removed. The residue was mixed with ether (5 mL), the resulting suspension centrifuged and the mother liquor again removed. The residue was dried under high vacuum (60°C) giving 11 (1.98 g, 4.47 mmol, 250 mCi, 90%). An unlabeled sample was identical (NMR, MS, mp, TLC/silica gel-methanol) with reference material.[35]

(4-Carboxybutyl-1-^{13}C)triphenylphosphonium bromide (11-5-^{13}C) - Starting with barium carbonate-^{13}C, 11-5-^{13}C was prepared in the same manner described for 11-5-^{14}C.

(8R,11R,12S,15R,5Z,13E)-15-Hydroxy-11,16,16-trimethyl-9-oxoprosta-5,13-dien-1-oic-5-^{14}C acid (1a-5-^{14}C) - Potassium hydride (24.6% in mineral oil titrated for hydride content, 0.542 mL, 3 mmol) was injected into a dropping funnel connected to a 50 mL two neck flask (entire apparatus was flame dried and argon flushed). The potassium hydride suspension was washed twice with 5 mL portions of pentane and after removing the second pentane wash, t-amyl alcohol (265 mg, 0.327 mL, 3 mmol, distilled) in tetrahydrofuran (10 mL) was added. After the potassium hydride had dissolved, the solution was added dropwise to 11-5-^{14}C (443 mg, 1 mmol) in tetrahydrofuran (5 mL) at room temperature. After the red-orange solution was stirred 15 min, 2a (381 mg, 1 mmol)[36] in tetrahydrofuran (5 mL) was added and stirring continued 2h. The reaction was quenched with acetic acid (0.172 mL) and concentrated in vacuo. The residue was taken up in a mixture of water-methanol (10 mL, 1:1) and titrated to pH 2 with 4N sulfuric acid. Brine (2 mL) was added to the suspension which was then extracted with hexane (7x5 mL each, distilled). The combined hexane phase was washed with a mixture of water-methanol (1:1, 2x5 mL), brine (5 mL), then dried ($MgSO_4$) and concentrated in vacuo to give 4a (465 mg, 1 mmol, 100% crude).

The crude 4a was dissolved in ether-acetone (16 mL, 4:1) and titrated with Jones reagent[29] under nitrogen at 0° until the orange color persisted. The solution was stirred 1 h then quenched with 2-propanol (0.25 mL) and diluted with ether (50 mL). The solution was washed with water (4x5 mL), brine (5 mL), then dried ($MgSO_4$) and concentrated in vacuo to give 422 mg (91% crude) of ketone.

The crude ketone was deprotected by heating the residue in a mixture of tetrahydrofuran (6.3 mL)-acetic acid (23 mL)-water (12.6 mL) at 45° under argon for 5h. After concentrating the solution in vacuo, the residual oil was taken up in chloroform-hexane (1 mL, 65:35) and applied to a Sephadex LH-20 column (1.75x75 cm, 47 g, packed and eluted in the same solvent). Combination of the appropriate fractions (10 mL each) yielded 52 mg (0.14 mmol, 14%) of pure 1a plus impure fractions which after rechromatography on the Sephadex LH-20 yielded an additional 62 mg (0.16 mmol, 16%). Total overall yield of 1a-5-^{14}C was 114 mg (0.30 mmol, 16.8 mCi, 30%) which had a radiochemical purity of 97.8% (TLC: silica gel; hexane-isopropanol-methanol-acetic acid; 100:8:4:0.5).

(8R,11R,12S,15R,5Z,13E)-15-Hydroxy-11,16,16-trimethyl-9-oxoprosta-5,13-dien-1-oic-5-^{13}C acid (1a-5-^{13}C) - Starting with 11-5-^{13}C, 1a-5-^{13}C was prepared in the manner described for 1a-5-^{14}C.

(8R,12S,15R,16R,5Z,13E)-16-Fluoro-15-hydroxy-9-oxoprosta-5,13-dien-1-oic-5-^{14}C acid (1b-5-^{14}C). - Starting with Wittig salt 11-5-^{14}C and lactol 2b,[37] 1b-5-^{14}C was prepared in 37% overall yield essentially according to the procedure described for 1a-5-^{14}C.

(8R,12S,15R,16R,5Z,13E)-16-Fluoro-15-hydroxy-9-oxaprosta-5,13-dien-1-oic-5-^{13}C acid (1b-5-^{13}C). - Starting with Wittig salt 11-5-^{13}C and lactol 2b, 1b-5-^{13}C was prepared in the manner described for 1a-5-^{14}C.

[1R-[1α(Z),2β(S*),3α,5α]]-7-[2-(5-Cyclohexyl-3-hydroxy-1-pentynyl-3,5-dihydroxycyclopentyl]-5-heptenoic-5-^{14}C acid methyl ester(1c-5-^{14}C).

In the manner described for the preparation of 1a-5-^{14}C,11-5-^{14}C (4.21 mmole) was condensed with lactol 2c (0.84 mmol)[32] to give 4c.

Sodium hydride (14 mmol) was heated in dimethyl sulfoxide (10 mL) for 4h at 45°C under argon. Crude 4c from above in dimethyl sulfoxide (1 mL) was added and the solution stirred at room temperature overnight. The reaction solution was diluted with water (20 mL), acidified to pH∿ 5 with 2N sulfuric acid, then extracted with ether (4x20 mL). The combined ether layer was washed with water (2x10 mL), brine (10 mL) then dried ($MgSO_4$) and concentrated in vacuo.

The crude residue from above was esterified with diazomethane-ether solution and deprotected by heating the residue in acetic acid-water (15 mL, 2:1) at 40°C for 4h. The solution was concentrated in vacuo, then chromatographed on Sephadex LH-20 (1-3/4x70 cm column, 47 g packed and eluted in chloroform-hexane, 65:35). Combination of the appropriate fractions yielded 1c-5-^{14}C(32) (229 mg, 0.56 mmol, 31.5 mCi, 67% from lactol 1c, 13% from Wittig salt 11-5-^{14}C) having a purity of ∿99% as determined by TLC (silica gel: ethyl acetate, methanol, acetic acid; 100:2:0.1) and reverse phase HPLC (Dupont Zorbax ODS, 9.4 mm x 25 cm P.N.).

[1R-[1α(Z),2β(S*),3α,5α]]-7-[2-(5-Cyclohexyl-3-hydroxy-1-pentynyl)-3,5-dihydroxycyclopentyl]-5-heptenoic-5-^{13}C acid methyl ester (1c-5-^{13}C).

Starting with 11-5-^{13}C, 1c-5-^{13}C was prepared in the manner described for 1c-5-^{14}C.

Acknowledgment

We thank Dr. J. Blount and his staff in our Physical Chemistry Department, in particular, Dr. W. Benz for mass spectra, Dr. F. Scheidl for microanalyses, Dr. V. Toome for uv spectra, Mr. S. Traiman for ir spectra and Dr. T. Williams for nmr spectra. We also wish to thank Drs. G. Holland, R. Lucci and J.W. Scott for extremely helpful discussions and generous samples of many of the intermediates.

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