SYNTHESIS OF TRITIUM-LABELLED ISOCARBACYCLIN DERIVATIVES, RADIOLABELLED PROSTAGLANDIN I_1 ANALOGS(1)

Satoshi Sugiura, and Seizi Kurozumi*

Teijin Institute for Bio-Medical Research, Teijin Ltd.
4-3-2 Asahigaoka, Hino, Tokyo 191, Japan

Shiro Ikegami

Faculty of Pharmaceutical Science, Teikyo University
Sagamiko, Kanagawa 119-01, Japan

SUMMARY

Three tritiated isocarbacyclin [9(*O*)-metano-$\Delta^{6(9\alpha)}$-prostaglandin I_1] derivatives, [11-^{3}H]-isocarbacyclin methyl ester (**7a**), [11-^{3}H]-(17*S*)-17-methyl-20-homo-isocarbacyclin (**9**), and its methyl ester (**8a**) were synthesized. Tritium was introduced to the 11-position via [^{3}H]-sodium tetrahydroborate reduction of 11-dehydro-isocarbacyclin intermediates **10** and **11**.

Key Words: isocarbacyclin, prostacyclin analog, tritium labelling

INTRODUCTION

Prostacyclin (prostaglandin I_2) (**1**), an important autacoid with highly vasoactive properties, has been shown to be useful for treatment of cardiovascular diseases(2). Isocarbacyclin(3) [9(*O*)-methano-$\Delta^{6(9\alpha)}$-prostaglandin I_1] (**2**) is a chemically stable prostacyclin analog with potent biological activities. We have chosen methyl ester of isocarbacyclin (**3**) and methyl ester of (17*S*)-17-methyl-20-homoisocarbacyclin (**4**) as candidates for therapeutic agents.

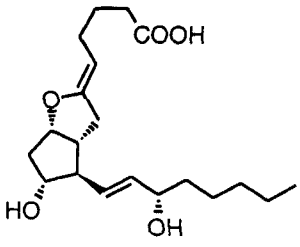
CCC 0362–4803/96/020129–10

Received 5 July 1995
Revised 1 September 1995

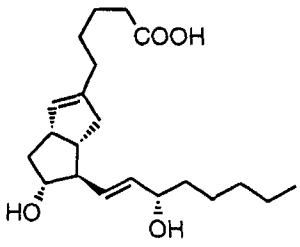
Tritium-labelled compounds were necessary for the pharmacokinetic and drug distribution studies. Recently, we have synthesized compounds **5** and **6**, which have di-tritiated ω-side chains(1b). The ring and the side chain-labelled isocarbacyclin derivatives may demonstrate different susceptibility of the labels at different positions to the metabolism *in vivo*. This article describes the preparation of compounds **7a** and **8a**, where 11-hydrogens of the compounds **3** and **4** were replaced by tritium, and the carboxylic acid **9** derived from **8a**.

RESULTS AND DISCUSSION

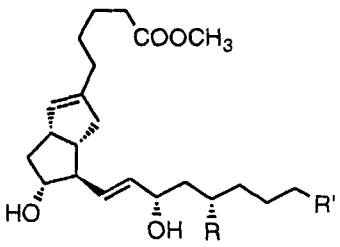
Tritium was introduced to the 11β-positions of compounds **3** and **4** via stereoselective reductive tritiation of the ketonic substrates **10** and **11**. Compound **10** was derived from bis-*t*-butyldimethylsilyl protected compound **12**(4) through a two-step process; a controlled treatment of **12** with tetrabutylammonium fluoride to give a partially deblocked product **13a**, followed by pyridinium chlorochromate oxidation of compound **13a**. The yield of **10** from **12** was 17%. Compound **11** was obtained from compound **14** by the same process in 18% yield. Treatment of **10** with sodium tetrahydroborate in isopropyl alcohol containing 10% diglyme provided **13a**, but raised multiple number of byproducts which included dienone compound **15**. The reproducibility of the yield of **13a** was poor. Addition of anhydrous cerium(III) chloride to the reaction system secured the reproducibility of **13a** production, but small amount of the 11β-hydroxy isomer (**13b**) (about 10% of **13a**) accompanied. The reaction condition with cerium(III) chloride was chosen for the [^{3}H]-labeling experiment of **10**. Treatment of the reaction mixture with tetrabutylammonium fluoride in tetrahydrofuran furnished compound **7a** accompanied by **7b**. HPLC separation provided **7a** (308mCi) of specific activity 18.4Ci/mmol and radiochemical purity 98%. A similar process provided compound **8a** (280mCi) of specific activity 17.7Ci/mmol and radiochemical purity 99%. Treatment of compound **8a** with lithium



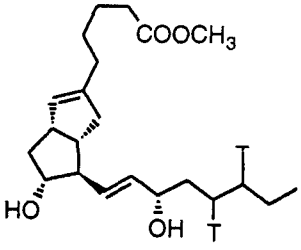
1



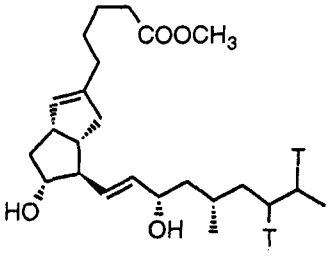
2



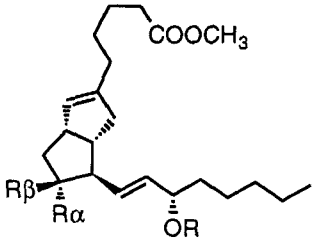
3 : R=H, R'=H
4 : R=CH_3, R'=CH_3

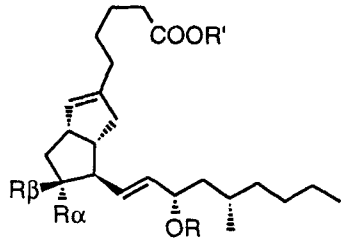


5

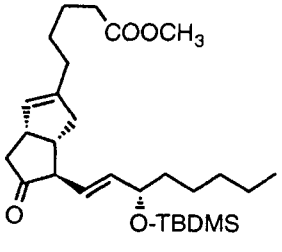


6

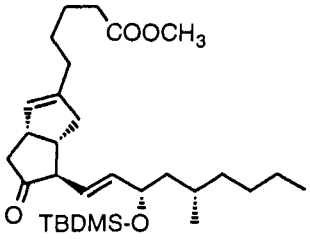




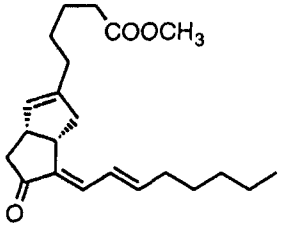
7a	:	Rα=OH,	Rβ=T,	R=H	**8a**	:	Rα=OH,	Rβ=T,	R=H, R'=CH_3
7b	:	Rα=T,	Rβ=OH,	R=H	**8b**	:	Rα=T,	Rβ=OH,	R=H, R'=CH_3
12	:	Rα=OTBDMS,	Rβ=H,	R=TBDMS	**9**	:	Rα=OH,	Rβ=T,	R=H, R'=H
13a	:	Rα=OH,	Rβ=H,	R=TBDMS	**14**	:	Rα=OTBDMS,	Rβ=H,	R=TBDMS, R'=CH_3
13b	:	Rα=H,	Rβ=OH,	R=TBDMS	**17**	:	Rα=OH,	Rβ=H,	R=TBDMS, R'=CH_3
16	:	Rα=OTBDMS,	Rβ=H,	R=H	**18**	:	Rα=OTBDMS,	Rβ=H,	R=H, R'=CH_3



10



11



15

hydroxide in methanol, followed by HPLC separation provided [11-^{3}H]-(17*S*)-17-methyl-20-homoisocarbacyclin (**9**) of radiochemical purity 97%.

EXPERIMENTAL

General

Radioactivity determinations were carried out using a Packard TRI-CARB 4530 liquid scintillation counter. IR spectra were recorded on a JASCO A102 spectrometer. ^{1}H-NMR spectra were obtained on a HITACHI R-90H (90MHz) and a JEOL JNM-GX 400 (400MHz) spectrometer and the chemical shift values are expressed in δ units relative to internal tetramethylsilane referenced at δ 0.00ppm. Mass spectra (MS) were taken on a LKB-9000 mass spectrometer. For high-peformance liquid chromatography (HPLC) separations and radiochemical purity determinations, a Shimazu LC-3A HPLC system equipped with ZORBAX-SIL (DuPont), LiChrosorb RP-18 (E.Merk), or YMC-PACK A-312 (YMC Co. Japan) column was used. Column chromatography was carried out on Daiso gel IR-60 silica gel (Daiso Co. Japan) and SEP-PAK (Millipore Co.). [^{3}H]-sodium tetrahydroborate was purchased from Amersham Japan Co.. All solvents were distilled prior to use. Diglyme and tetrahydrofuran were distilled from benzophenone ketyl. Pyridinium chlorochromate (Aldrich chemical Co.), 1M tetrabutylammonium fluoride solution in tetrahydrofuran (Aldrich Chemical Co.), and anhydrous cerium(III) chloride (Alfa Products) were used without futher purification.

Syntheses of 11-dehydro substrate

Methyl [(1*S*,5*S*,6*R*,7*R*)-7-hydroxy-6-[(3*S*,1*E*)-3-*t*-butyldimethylsilyloxy-1-octenyl]bicyclo[3.3.0]oct-2-en-3-yl]-pentanoate (**13a**)

To a stirred solution of **12** (1.09g) in tetrahydrofurane (40ml), 1M tetrabutylammonium fluoride tetrahydrofurane solution (1.2ml) was added and the resulting solution was stirred for 4hr. The reaction mixture was poured into saturated aqueous potassium hydrogen sulfate. The mixture was extracted with ethyl acetate. The organic

layer was washed with saturated aqueous sodium hydrogen carbonate and saturated aqueous sodium chloride, dried over anhydrous magnesium sulfate, and concentrated under vacuum to give a crude oil. The oil was separated on a silica gel column (silica gel, 40g; eluent, hexane - ethyl acetate with ratio varied from 50v/1v to 0v/1v) to give a mixture of **13a** and **16** (272mg), **3** (202mg, yield 30%), and **12** (297mg, recovery 27%). Separation of **13a** and **16** was accomplished by using HPLC (column, ZORBAX-SIL; eluent, 1% ethanol in hexane), and 188mg (yield 21%) of **13a** and 33mg (yield 4%) of **16** were obtained.

13a

IR (oil): ν_{max} 3466, 2953, 2930, 2859, 1742, 1254, 1090, 835, 775 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 0.04 (s, 3H), 0.06 (s, 3H), 0.90 (s, 9H), 0.75~0.95 (m, 3H), 1.2~1.7 (m, 15H), 1.8~2.10 (m, 4H), 2.2~2.5 (m, 4H), 2.9~3.05 (m, 1H), 3.67 (s, 3H), 3.6~3.8 (m, 1H), 3.95~4.15 (m, 1H), 5.30 (brs, 1H), 5.3~5.6 (m, 2H).

MS: (m/z) 478 (M^+), 421.

16

IR (oil): ν_{max} 3447, 2953, 2930, 2859, 1742, 1254, 1113, 837, 775 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 0.02 (s, 6H), 0.90 (s, 9H), 0.75~0.95 (m, 3H), 1.15~1.7 (m, 15H), 1.8~2.5 (m, 8H), 2.8~3.0 (m, 1H), 3.67 (s, 3H), 3.6~3.8 (m, 1H), 3.95~4.1 (m, 1H), 5.25 (brs, 1H), 5.4~5.6 (m, 2H).

MS: (m/z) 478 (M^+), 421.

Methyl [(1*S*,5*S*,6*R*)-7-oxo-6-[(3*S*,1*E*)-3-*t*-butyldimethylsilyloxy-1-octenyl]bicyclo[3.3.0]oct-2-en-3-yl]pentanoate (**10**)

To a stirred solution of **13a** (135mg) in dichloromethane (5ml) was added pyridinium chlorochromate (1.0g) and the mixture was stirred for 1hr. The mixture was diluted with diethyl ether (10ml), and filtrated through a layer of cerite, the filter-cake washed with ether. The combined filtrate was washed with aqueous potassium hydrogensulfate, aqueous sodium hydrogencarbonate, and aqueous sodium chloride. The organic layer was dried over anhydrous

magnesium sulfate. After concentration under vacuum, the crude oil was purified on a silica gel column (silica gel, 10g; eluent, hexane - ethyl acetate, 10v/1v) to give pure **10** (110mg, yield 82%).

10

IR (oil): ν_{max} 2930, 2857, 1742, 1254, 837, 777 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.88 (s, 9H), 0.8~0.95 (m, 3H), 1.2~1.7 (m, 13H), 2.0~2.8 (m, 9H), 3.25~3.4 (m, 1H), 3.67 (s, 3H), 4.0~4.15 (m, 1H), 5.28 (brs, 1H), 5.49 (dd, 1H, J=15.9, 6.1Hz), 5.57 (dd, 1H, J=15.9, 6.1Hz).

MS: (m/z) 476 (M^+), 419.

Methyl [(1*S*,5*S*,6*R*,7*R*)-7-hydroxy-6-[(3*S*,5*S*,1*E*)-3-*t*-butyldimethylsilyloxy-5-methyl-1-nonenyl]bicyclo[3.3.0]oct-2-en-3-yl]pentanoate (**17**)

To a stirred solution of **14** (1.74g) in tetrahydrofurane (35ml), 1M tetrabutylammonium fluoride tetrahydrofurane solution (5.62ml) was added and the resulting solution was stirred for 7hr. The reaction mixture was poured into saturated aqueous potassium hydrogen sulfate. The mixture was extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate and saturated aqueous sodium chloride, dried over anhydrous magunesium sulfate, and concentrated under vacuum to give a crude oil. The oil was separated on a silica gel column (silica gel, 120g; eluent, hexane - ethyl acetate with ratio varied from 20v/1v to 0v/1v) to give **17** (375mg, yield 26%), **18** (116mg, yield 8%), **4** (542mg, yield 48%), and **14** (355mg, recovery 20%).

17

IR (oil): ν_{max} 3455, 2955, 2928, 2859, 1742, 1252, 1088, 837, 775 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 0.04 (s, 3H), 0.06 (s, 3H), 0.89 (s, 9H), 0.75~0.95 (m, 6H), 1.0~1.7 (m, 16H), 1.8~2.15 (m, 3H), 2.2~2.55 (m, 5H), 2.9~3.15 (m, 1H), 3.67 (s, 3H), 3.7~3.85 (m, 1H), 4.0~4.25 (m, 1H), 5.29 (brs, 1H), 5.3~5.6 (m, 2H).

MS: (m/z) 506 (M^+), 449.

18

IR (oil): ν_{max} 3459, 2953, 2928, 2859, 1742, 1254, 1113, 837, 775 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 0.02 (s, 6H), 0.86 (s, 9H), 0.75~0.95 (m, 6H), 1.0~1.7 (m, 16H), 1.8~2.5 (m, 8H), 2.8~3.05 (m, 1H), 3.67 (s, 3H), 3.6~3.85 (m, 1H), 4.1~4.25 (m, 1H), 5.26 (brs, 1H), 5.35~5.65 (m, 2H).

MS: (m/z) 506 (M^+), 449.

Methyl [(1*S*,5*S*,6*R*)-7-oxo-6-[(3*S*,5*S*,1*E*)-3-*t*-butyldimethylsilyl-oxy-5-methyl-1-nonenyl]bicyclo[3.3.0]oct-2-en-3-yl]pentanoate (**11**)

To the stirred solution of **17** (170mg) in dichloromethane (5ml) was added pyridinium chlorochromate (900mg) and the mixture was stirred for 1.5hr. The mixture was diluted with ether (10ml), and filtered through a layer of cerite, the filter-cake washed with ether. The combined filtrate was washed with aqueous potassium hydrogensulfate, aqueous sodium hydrogencarbonate, and aqueous sodium chloride. The organic layer was dried over anhydrous magnesium sulfate. After concentration under vacuum, the crude oil was purified on a silica gel column (silica gel, 10g; eluent, hexane - ethyl acetate, 15v/1v) to give pure **11** (120mg, yield 71%).

11

IR (oil): ν_{max} 2945, 2865, 1746, 1253, 835, 774 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ 0.04 (s, 3H), 0.05 (s, 3H), 0.89 (s, 9H), 0.7~1.0 (m, 6H), 1.0~1.7 (m, 14H), 1.9~2.8 (m, 9H), 3.2~3.5 (m, 1H), 3.67 (s, 3H), 4.0~4.3 (m, 1H), 5.31 (brs, 1H), 5.4~5.65 (m, 2H).

MS: (m/z) 504 (M^+), 447.

Synthesis of tritium-labelled isocarbacyclin derivatives

All synthetic and analytical operations were performed with unlabelled compounds and the structures were confirmed by spectrometry and/or chromatographic comparisons with the authentic samples prior to the labelling experiments.

[11-^{3}H]-9(*O*)-methano-$\Delta^{6(9\alpha)}$-prostaglandin I_1 methyl ester (**7a**)

Powdered [^{3}H]-sodium tetrahydroborate (12.2μmol, 1Ci) of specific activity of 82Ci/mmol was taken up in diglyme (400μl) in a flask. Anhydrous cerium(III) chloride (29.6mg) suspended in isopropyl alcohol (3ml) and a solution of **10** (65mg) in isopropyl alcohol (3ml) were added. The mixture was stirred for 3hr, then 10% aqueous ammonium chloride (200μl) was added and the mixture was stirred for 10min. The mixture was concentrated under vacuum, then ether (10ml) and anhydrous magnesium sulfate (2g) were added. The solid was filtered and washed with ether (30ml). The combined filtrate was concentrated under vacuum to remove the diglyme, and 1.0M tetrabutylammonium fluoride solution in tetrahydrofurane (4ml) was added to the residue. After stirring for 2hr, 5% aqueous potassium hydrogensulfate (10ml) was added and the solution was extracted with ethyl acetate (3ml x 6). The combined organic layer was washed with 5% aqueous sodium hydrogencarbonate (10ml) and then with 5% aqueous sodium chloride (20ml), dried over anhydrous magnesium sulfate, and was concentrated under vacuum to give a residue of crude **7a**. The crude product was first roughly purified by silca gel (SEP-PAK) column chromatography (eluent, benzene 4ml, hexane - ethyl acetate 3v/1v 12ml, 2v/1v 16ml, 1v/1v 16ml) to give an oil of **7a** accompanied by **7b**. The oil dissolved in 2.0% ethanol in hexane (3ml) was purified by HPLC (column, ZORBAX SIL; eluent, 2.0% ethanol in hexane) to give pure **7a** (308mCi; specific activity, 18.4Ci/mmol; radiochemical purity, 98%). The overall radiochemical yield was 31%. Compound **7a** was stored as a solution of 5% ethanol-toluene.

[11-^{3}H]-(17*S*)-17-methyl-9(*O*)-methano-$\Delta^{6(9\alpha)}$-20-homoprostaglandin I_1 methyl ester (**8a**)

Powdered [^{3}H]-sodium tetrahydroborate (18.2μmol, 1Ci) of specific activity of 55Ci/mmol was taken up in diglyme (400μl) in a flask. Anhydrous cerium(III) chloride (29.6mg) suspended in

isopropyl alcohol (3ml) and a solution of **11** (50mg) in isopropyl alcohol (3ml) was added. The mixture was stirred for 1hr, then 10% aqueous ammonium chloride (200μl) was added and the mixture was stirred for 10min. The mixture was concentrated under vacuum, then ether (10ml) and anhydrous magnesium sulfate (2g) were added. The solid was filtered and washed with ether (30ml). The combined filtrate was concentrated under vacuum to remove the diglyme, and 1M tetrabutylammonium fluoride solution in tetrahydrofurane (4ml) was added to the residue. After stirring for 3hr, 5% aqueous potassium hydrogensulfate (10ml) was added and the solution was extracted with ethyl acetate (3ml x 6). The combined organic layer was washed with 5% aqueous sodium hydrogencarbonate (10ml) and then with 5% aqueous sodium chloride (20ml), dried over anhydrous magnesium sulfate, and was concentrated under vacuum to give a residue of crude **8a**. The crude product was first roughly purified by silca gel (SEP-PAK) column chromatography (eluent, benzene 4ml, hexane - ethyl acetate 3v/1v 12ml, 2v/1v 16ml, 1v/1v 16ml) to give an oil of **8a** accompanied by **8b**. The oil dissolved in 2.5% ethanol in hexane (3ml) was purified by HPLC (column, ZORBAX SIL; eluent, 3.0% ethanol in hexane) to give pure **8a** (280mCi; specific activity, 17.7Ci/mmol; radiochemical purity, 99%). The overall radiochemical yield was 28%. Compound **8a** was stored as a solution of 5% ethanol-toluene.

[11-^{3}H]-(17S)-17-methyl-9(*O*)-methano-$\Delta^{6(9\alpha)}$-20-homoprostaglandin I_1 (**9**)

The solution of **8a** (16.9mCi) was taken and its solvent was replaced with methanol (1ml) by evaporating the ethanol-toluene under vacuum. 4N lithium hydroxide (0.4ml) was added to the solution. The solution was stirred for 3hr at 40°C. The solvent was evaporated under vacuum, saturated aqueous potassium hydrogensulfate (3ml) was added to the residue and the reaction products were extracted with ethyl acetate (3ml x 4). The extract was dried over anhydrous magnesium sulfate. The solvent was replaced with 5%

ethanol in toluene (1ml) by evaporation under vacuum and the products were separated by HPLC (column, ZORBAX SIL; eluent, 5% ethanol in hexane containing 0.02% of acetic acid). Pure **9** (12.2mCi) of radiochemical purity 97% was obtaied with 72% radiochemical yield. The product **9** was store as a solution of ethanol (15ml).

Acknowledgement: We thank Dr. Yoshinori Kato of this Institute for discussion and help in preparing this manuscript.

REFERENCES

1. (a) Prostaglandin Chemistry Part XXXX; (b) For Part XXXIX: Manabe K., Tanaka T., Kurozumi S., and Kato Y.- J. Labelled Compds. Radiopharm. **29:** 1107 (1991)
2. Gryglewski R.J., Szczeklik A., and McGiff J.C.- Prtostaglandin; Clinical Trials, Raven Press, New York, 1985
3. Sibasaki M., Torisawa Y., and Ikegami S.- Tetrahedron Lett. **24**: 3493 (1983)
4. Hazato A., Tanaka T., Okamura N., Bannai K., Sugiura S., Manabe K., Tomimori K., Kurozumi S., and Noyori R.- 7th IUPAC Conference on Organic Synthesis, Abr. 4-A10 (1988), Nancy, France