SYNTHESES OF ^{3}H- AND ^{2}H-LABELED (17*R*)-17,20-DIMETHYL-7-THIAPROSTAGLANDIN E_1 METHYL ESTER[1]

Toshio Tanaka,* Kiyoshi Bannai, Kenji Manabe, and Seizi Kurozumi

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

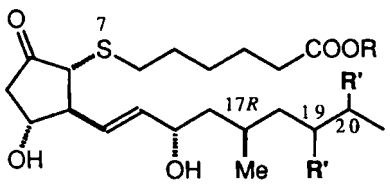
SUMMARY

The syntheses of [19,20-$^{3}H_2$]- and [3,3,4,4,5,5,6,6-$^{2}H_8$]-(17*R*)-17,20-dimethyl-7-thiaprostaglandin E_1 methyl esters (**3** and **5**) are described. The di-tritiated compound **3** was prepared from its protected (Z)-Δ^{19}-precursor (**21**) *via* catalytic reduction with tritium gas having a specific activity of 49 Ci / mmol. The octa-deuterated compound **5** was prepared from tetrahydrofuran-d_8.

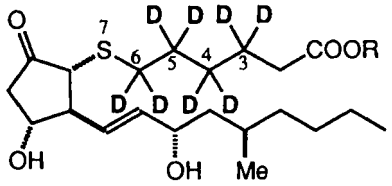
KEYWORDS: tritium-labeled 7-thiaprostaglandin E_1, catalytic hydrogenation, deuterated 7-thiaprostaglandin E_1

INTRODUCTION

Prostaglandin E_1 and its analogues are now clinically used for treatment of peripheral vascular disease and so on.[2] (17*R*)-17,20-Dimethyl-7-thiaprostaglandin E_1 methyl ester[3] (**1**) is one of orally active prostaglandin E_1 analogues. We report here the syntheses of (17*R*)-17,20-dimethyl-7-thia[19,20-$^{3}H_2$]prostaglandin E_1 methyl ester (**3**) and (17*R*)-17,20-dimethyl-7-thia[3,3,4,4,5,5,6,6-$^{2}H_8$]prostaglandin E_1 methyl ester (**5**) as pharmaceutical tools.



1; R = Me, R' = H
2; R = H, R' = H
3; R = Me, R' = T
4; R = H, R' = T



5; R = Me
6; R = H

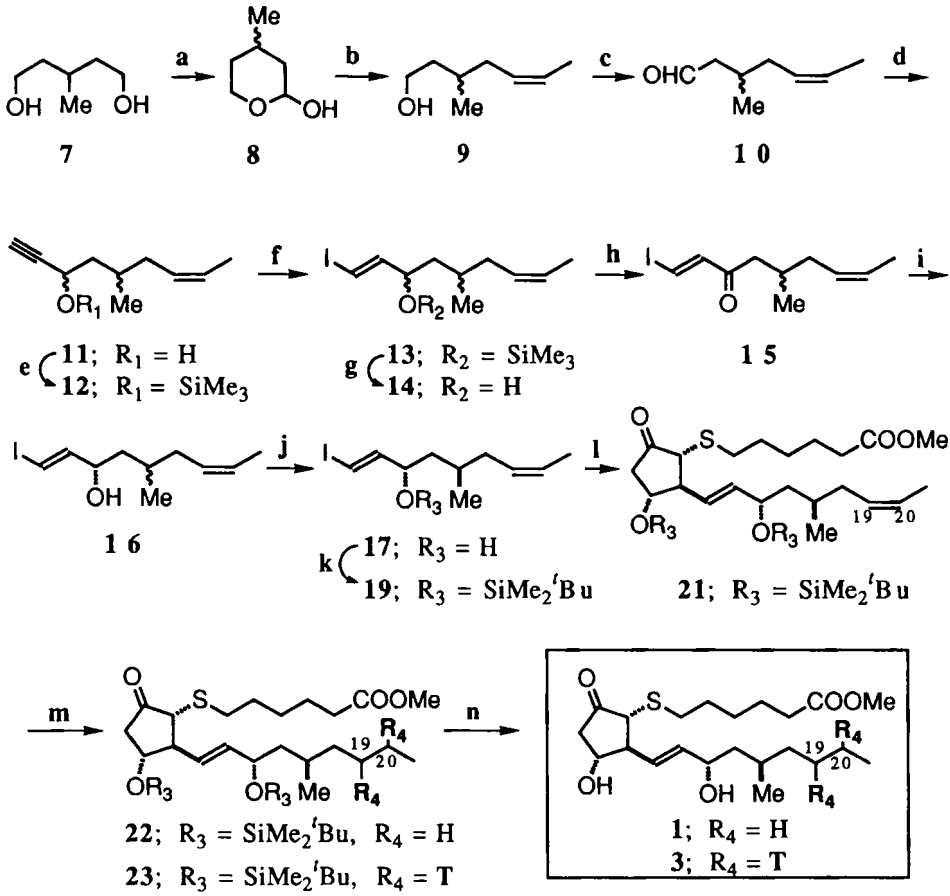
0362–4803/91/080933–12$06.00

Received 17 April 1991
Revised 19 April 1991

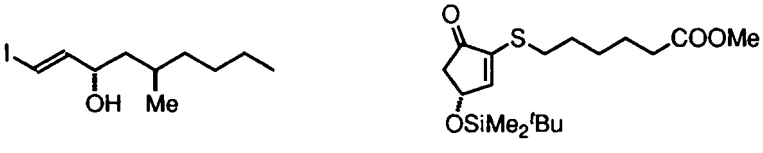
SYNTHESIS

The tritium-labeled compound is the [19,20-$^3H_2$] derivative **3**, where two hydrogen atoms on the ω-side chain of prostaglandin E_1 analogue **1** are substituted by tritium atoms. The tritiated **3** was synthesized from the (*Z*)-Δ^{19} olefinic precursor **21** by catalytic hydrogenation with tritium gas (Scheme 1), and was used for the pharmacokinetics and metabolism studies of the substance **1**.

Selective oxidation of the starting diol **7** with pyridinium chlorochromate (PCC) resulted in the cyclic hemiacetal formation of **8** as an oxidized product (57%). Reaction of the product **8** with ethylidenetriphenylphosphorane gave the (*Z*)-olefinic alcohol **9** in 67% yield, which was accompanied by *ca.* 15% (*E*)-isomer of **9**. Because the (*E*)-olefin is known to be less active toward catalytic hydrogenation than the (*Z*)-olefin, the following reactions are carried out using a mixture of the (*Z*)-olefinic substance concomitant with a small amount of the (*E*)-olefin. Oxidation of the resulting alcohol **9** with PCC yielded(52%) the aldehyde **10**, which was allowed to react with lithium acetylide-ethylenediamine complex to provide the acetylenic alcohol **11** (58%). After protection (87%) of **11** with trimethylsilyl group, the resulting product **12** was converted into the vinyl iodide **13** (37%) accompanied by its desilylated vinyl iodide **14** (41%) by subsequent treatment with disiamylborane, trimethylamine oxide, and then iodine under alkaline condition.[4] Desilylation of **13** with *p*-toluenesulfonic acid in methanol also gave the iodovinyl alcohol **14** (95%). Oxidation of **14** with PCC yielded the ketone **15** (95%). Treatment of **15** with (*S*)-BINAL-H[5] produced the chiral iodovinyl alcohol **16** (93%) as a diastereomeric mixture, whose optical purity was determined to be 99.6 : 0.4 by high-performance liquid chromatography (HPLC) analysis of its 3,5-dinitrophenylcarbamate derivative. Separation of a diastereomeric mixture **16** by preparative HPLC furnished the optically pure **17** (40%). The structure of **17** was proved by partial reduction of the (*Z*)-olefin with platinum oxide under hydrogen atmosphere leading to the authentic (3*S*,5*R*) alcohol **18**.[3a] The alcohol **17** was converted into the protected **19** (98%) by use of *t*-butylchlorodimethylsilane. An organocopper reagent[3a] prepared from the (*Z*)-olefinic **19** in the presence of 1-pentynylcopper(I) was coupled with the (*R*)-4-protected 2-substituted 2-cyclopentenone intermediate **20** to form the coupling adduct **21** (82%) as a precursor to be labeled. Catalytic



a) pyridinium chlorochromate (PCC) ; b) $C_2H_5P^+Ph_3Br^-$, NaH ; c) PCC ; d) lithium acetylide, ethylenediamine ; e) Me_3SiCl, imidazole ; f) disiamylborane / Me_3N-O / I_2 (NaOH) ; g) *p*-TsOH ; h) PCC ; i) (*S*)-(-)-binaphthol-$LiAlH_4$-EtOH ; j) HPLC separation ; k) ^{t}BuMe$_2$SiCl, imidazole ; l) ^{t}BuLi, 1-pentynylcopper(I),**20** ; m) T_2, Pd / C ; n) HF-pyridine or aq. CH_3COOH



18 **20**

Scheme 1

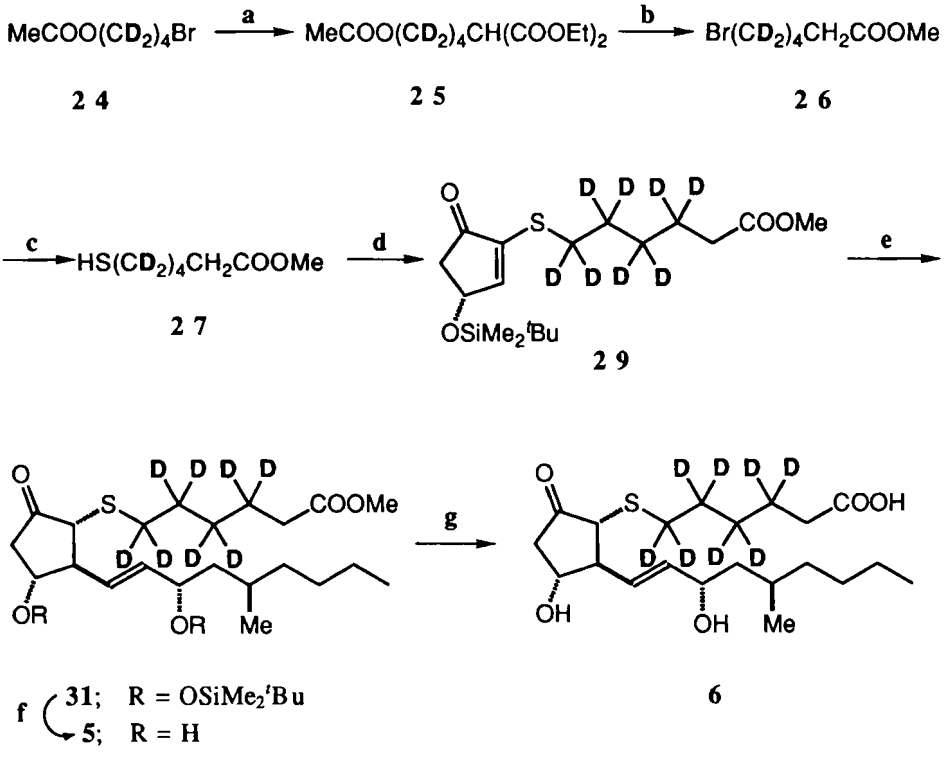
hydrogenation of the (Z)-Δ^{19} substrate **21** with 10% palladium on activated carbon under hydrogen atmosphere gave the (Z)-Δ^{19}-reduced product **22**, which was desilylated with tetrabutylammonium fluoride to furnish the product **2** after preparative HPLC purification. A similar catalytic reduction of the same precursor **21** under tritium gas afforded the di-tritiated product **23**, which was converted into the desired substance **3** after a similar preparative HPLC purification. The specific activity of the obtained **3** was found to be 49 Ci / mmol. Hydrolysis of the methyl ester **3** with porcine liver esterase[6] gave the tritiated carboxylic acid **4**.

The deuterium-labeled compound is the [3,3,4,4,5,5,6,6-$^2H_8$] derivative **5**, where eight hydrogen atoms on the α-side chain of prostaglandin E_1 analogue **1** are substituted by deuterium atoms. The deuterated **5** was synthesized from tetrahydrofuran-d_8 for the use as an internal standard in GC-MS quantitative analysis (Scheme 2).

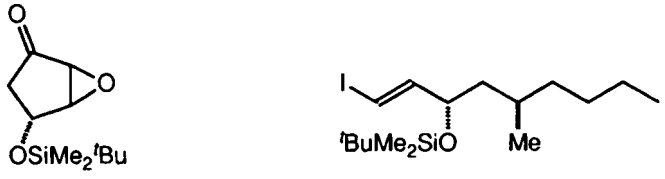
Alkylation of diethyl malonate with the octa-deuterated bromide[7] **24** afforded the alkylated malonate **25** (84%), which was subsequently hydrolyzed, brominated, decarboxylated, and re-esterified in three steps to provide the deuterated bromo ester **26** in 45% yield as shown in Scheme 2. Treatment of the bromide **26** with thiourea gave the mercapto ester **27** (78%) after alkaline hydrolysis.[3a] Reaction[3a] of the thiol **27** with the chiral cyclopentenone epoxide **28** in the presence of basic alumina yielded the deuterated enone **29** (72%). The 7-thiaprostaglandin E_1 derivative **31** was obtained in 65% yield as coupling product by conjugate addition[3a] of the mixed cuprate, prepared from the vinyl iodide **30** corresponding to the ω-side chain of the prostaglandin E_1 derivatives **1**, to the enone **29**. Desilylation of the adduct **31** with hydrogen fluoride-pyridine furnished the desired octa-deuterated product **5** (68%). The corresponding deuterated carboxylic acid **6** was obtained by treatment of the ester **5** with porcine liver esterase[6] in 68% yield. The obtained deuterated products, **5** and **6**, were confirmed by their mass spectrometric analysis to contain eight deuterium atoms.

EXPERIMENTAL

IR spectra were recorded on a JASCO A 102 spectrometer. ^{1}H-NMR spectra was obtained on a HITACHI R-90H (90 MHz) and a JEOL JNM-GX 400 (400 MHz) spectrometer in $CDCl_3$, respectively. Chemical shifts and coupling constants (J) are given in δ (ppm) relative to internal tetramethylsilane and Hz, respectively. The following abbreviation



a) $CH_2(COOEt)_2$, NaH ; b) 1) MeCOOH, HBr, 2) HBr, H_2SO_4, 3) MeOH, H_2SO_4 ;
c) 1) $(NH_2)_2C=S$, then NaOH, 2) MeOH, H_2SO_4 ; d) **28**, alumina ;
e) **30**, ^{t}BuLi, PhSCu ; f) HF-pyridine ; g) porcine liver esterase



28 **30**

Scheme 2

are used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), b (broad). Mass spectra (MS) were taken at 70 eV on a LKB-9000 mass spectrometer. For high-performance liquid chromatography (HPLC) analysis, a Shimadzu Model LC-6A equipped with a Shimadzu SPD-6A UV detector (210 nm) and a Shimadzu C-R3A chromatopac was employed. Thin-layer chromatography (TLC) was performed using

Merck silica gel (Kiesel gel 60 F_{254}) analytical plate. The plates were sprayed with a solution of 2% *p*-anisaldehyde in 5% ethanolic sulfuric acid and then heated until the spots became clearly visible. Column chromatography was carried out on Daiso gel IR-60 silica gel. All reactions were performed under argon or nitrogen. Solvents for reactions were purified if necessary before use by distillation from suitable drying agents. Solvents for extraction and chromatography were GR grades.

Synthesis of (17*R*)-17,20-dimethyl-7-thia[19,20-$^3H_2$]prostaglandin E_1 methyl ester (3) and its carboxylic acid (4)

(1*RS*,3*RS*)-1-Hydroxy-3-methyltetrahydropyran (8)

Pyridinium chlorochromate (PCC; 100 g, 0.46 mol) was added stepwise at 0°C to a stirred mixture of 3-methyl-1,5-pentanediol (7; 68 g, 0.576 mol) and dichloromethane (500 ml). After the mixture was stirred at room temperature (r. t.) for 30 min, ether (600 ml) was added to the mixture. The resulting mixture was filtrared through Celite. The obtained filtrate was evaporated under vacuum to give a crude residue, which was chromatographed on silica gel (1.2 kg) with hexane-ethyl acetate (2 : 1) to afford the oxidized product **8** (38 g, 0.328 mol, 57%) in a cyclized form as a diastereomeric mixture; ^{1}H-NMR ($CDCl_3$): δ 0.90 (t, 3H, J = 6 Hz), 1.0-2.2 (m, 5H), 3.2-4.3 (m, 2H), 4.4-4.8 (m, 1H), 5.1-5.35 (m, 1H).

(±)-(Z)-3-Methyl-5-heptenol (9)

Dry dimethyl sulfoxide (DMSO; 300 ml) was added at r. t. to sodium hydride (60% content; 42.6 g, 1.07 mol), and the suspension was heated at 75-80°C for 50 min. After the reaction mixture was cooled at 0°C for 15 min, a solution of ethyltriphenylphosphonium bromide (198 g, 0.532 mol) in DMSO (300 ml) was added at 0°C to the mixture. After stirring at r. t. for 1 h, the mixture was cooled again at 0°C. To the resulting mixture was added at 0°C a solution of **8** (32.6 g, 0.281 mol) in DMSO (60 ml), and the reaction mixture was stirred at r. t. for 18 h. Saturated aqueous (aq.) ammonium chloride solution (1, 000 ml) was added at 0°C and the resulting mixture was extracted twice with ether (1,000 ml). The combined organic layers were washed with aq. ammonium chloride solution, aq. sodium bicarbonate solution, and then brine. The separated organic layers were dried over magnesium sulfate, and concentrated under vacuum to give a crude residue (97 g). After depositing precipitate was filtered off through Celite, the resulting product was purified on silica gel (500 g) column chromatography with hexane-ethyl acetate (10 : 1) to yield the titled compound **9** (24.1 g, 0.188 mol, 67%). This product was accompanied by *ca.* 15% (*E*)-isomer of **9** judged by ^{1}H-NMR measurement (δ 1.65; d, 3H, J = 6 Hz). Contamination with this (*E*)-isomer continued throughout the following all reaction sequence as described later; IR (neat): 3350, 3040, 1655, 1060, 1005, 965, 690 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.90 (d, 3H, J = 6 Hz), 1.35-1.45 (m, 1H), 1.55 (d, 3H, J = 6 Hz), 1.8-2.1 (m, 2H), 3.33 (bs, 1H), 3.6-3.75 (m, 2H), 5.35-5.57 (m, 2H); EI-MS (m/z): 128 (M^+), 110, 95, 82, 71, 55, 41.

(±)-(Z)-3-Methyl-5-heptenal (10)

A solution of the above-obtained product (21.8 g, 0.171 mol) of **9** and its (*E*)-isomer in dichloromethane (90 ml) was added dropwise at 0°C to a stirred suspension of pyridinium chlorochromate (62.5 g, 0.29 mol), and the mixture was stirred at r. t. for 4 h. The above-mentioned work-up with ether gave an oily product, which was chromatographed on silica gel (500 g) using hexane-ethyl acetate (19 : 1) as an eluant to yield the aldehyde **10** (11.2 g, 0.089 mol, 52%); IR (neat): 3040, 1710, 1165, 965, 680 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.90 (d, 3H, J = 6 Hz), 1.57 (d, 3H, J = 6 Hz), 1.8-2.5 (m, 5H), 5.3-5.6 (m, 2H), 9.77 (t, 1H, J = 3 Hz).

(3*RS*,5*RS*,*Z*)-3-Hydroxy-5-methyl-7-nonen-1-yne (11)

A solution of the aldehyde **10** (14.0 g, 0.111 mol) in DMSO (50 ml) was added dropwise at r. t. to a solution of lithium acetylide-ethylenediamine complex (17.5 g, 0.190 mol) in DMSO (250 ml), and the resulting mixture was stirred at r. t. for 1 h. Aq. ammonium chloride solution was added and the mixture was extracted with ether (2 x 500 ml). The separated organic layers were washed with aq. ammonium chloride

solution and brine, dried over magnesium sulfate, and then concentrated *in vacuo* to leave a crude residue. Its column chromatography on silica gel (500 g) using hexane-ethyl acetate (19 : 1) gave the acetylated **11** (9.79 g, 0.064 mol, 58%).

(3*RS*,5*RS*,*Z*)-5-Methyl-3-trimethylsilyloxy-7-nonen-1-yne (12)

To a solution of **11** (4.44 g, 29.0 mmol) in *N*,*N*-dimethylformamide (DMF; 20 ml) was added at 0°C imidazole (4.38 g, 64.4 mmol) and then chlorotrimethylsilane (4.10 ml, 3.51 g, 32.3 mmol). The resulting mixture was stirred at r. t. for 18 h, and poured into ice-water. The organic layer was taken up in hexane (200 ml). The separated aq. layer was extracted with hexane (200 ml). The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and evaporated to afford a crude product (6 g), which was purified by silica gel (150 g) column chromatography with a 19 : 1 mixture of hexane and ethyl acetate as an eluant to yield the silylated **12** (5.65 g, 25.2 mmol, 87%); IR (neat): 3340, 3040, 2120, 1255, 1085, 840, 750 cm^{-1}; ^1H-NMR ($CDCl_3$): δ 0.17 (s, 9H), 0.90 (d, 3H, J = 6 Hz), 1.67 (d, 3H, J = 6 Hz), 1.5-2.3 (m, 5H), 2.36 (d, 1H, J = 6 Hz), 4.43 (m, 1H), 5.2-5.9 (m, 2H).

(3*RS*,5*RS*,1*E*,7*Z*)-3-Hydroxy-1-iodo-5-methyl-1,7-nonadiene (14) through (3*RS*,5*RS*,1*E*,7*Z*)-1-iodo-5-methyl-3-trimethylsilyloxy-1,7-nonadiene (13)

A 1.0 M tetrahydrofuran (THF) solution of diborane (13.8 ml, 13.8 mmol) was added at 0°C to a stirred solution of 2-methyl-2-butene (3.30 ml, 2.18 g, 31.2 mmol), and the mixture was stirred at 0°C for 20 min. To the reaction mixture was added at r. t. a solution of the alkyne **12** (2.68 g, 12.0 mmol) in THF (15 ml), and then the mixture was stirred at r. t. for 1 h. Anhydrous trimethylamine oxide (3.76 g, 50 mmol) was added at 0°C by portions, and the reaction mixture was stirred at r. t. for 40 min. The resulting mixture was poured into a cooled 4.0 N sodium hydroxide solution (125 ml) at 0°C. Then, a solution of iodine (6.1 g, 24.0 mmol) in THF (30 ml) was added to the mixture, and the whole mixture was stirred at r. t. for 1 h. The organic layer was extracted twice with ether (500 ml). The organic layers were washed with aq. sodium thiosulfate solution, then brine, and dried over magnesium sulfate. Evaporation of the solvent left a crude product (5.83 g), which was separated by column chromatography on silica gel (270 g) with a 19 : 1 mixture of hexane and ethyl acetate to give the vinyl iodide **13** (1.55 g, 4.4 mmol, 37%) and its desilylated vinyl iodide **14** (1.37 g, 4.9 mmol, 41%). **13**; ^1H-NMR ($CDCl_3$): δ 1.0 (s, 9H), 0.89 (d, 3H, J = 6 Hz), 1.55 (d, 3H, J = 6 Hz), 1.1-2.1 (m, 5H), 4.0-4.4 (m, 1H), 5.3-5.6 (m, 2H), 6.33 (d, 1H, J = 15 Hz), 6.66 (dd, 1/2H, J = 15 & 5 Hz), 6.69 (dd, 1/2H, J = 15 & 5 Hz). **14**; IR (neat): 3350, 3040, 1605, 1170, 1050, 1010, 950, 700 cm^{-1}; ^1H-NMR ($CDCl_3$): δ 0.89 (d, 3H, J = 6 Hz), 1.55 (d, 3H, J = 6 Hz), 1.1-2.1 (m, 5H), 2.2 (d, 1H, J = 6 Hz), 4.0-4.4 (m, 1H), 5.3-5.6 (m, 2H), 6.33 (d, 1H, J = 15 Hz), 6.66 (dd, 1/2H, J = 15 & 5 Hz), 6.69 (dd, 1/2H, J = 15 & 5 Hz).

A small amount of *p*-toluenesulfonic acid was added at r. t. to a solution of the above **13** (1.55 g, 4.4 mmol) in methanol (15 ml), and the mixture was stirred at r. t. for 40 min. After addition of aq. sodium bicarbonate solution and evaporation of methanol, the residual mixture was extracted with ether (500 ml). The separated organic layer was washed twice with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to leave a crude product, which was purified by silica gel (40 g) column chromatography with hexane-ethyl acetate (19 : 1) to give **14** (1.17 g, 4.2 mmol, 95%).

(±)-(1*E*,7*Z*)-1-Iodo-5-methyl-3-oxo-1,7-nonadiene (15)

A solution of **14** (2.52 g, 9.0 mmol) in dichloromethane (40 ml) was added dropwise at r. t. to a stirred suspension of pyridinium chlorochromate (3.82 g, 17.7 mmol), and the resulting mixture was stirred at r. t. for 2 h. Usual work-up with ether and column chromatography on silica gel (70 g) with hexane-ethyl acetate (19 : 1) gave the oxidized **15** (2.38 g, 8.6 mmol, 95%); ^1H-NMR ($CDCl_3$): δ 0.89 (d, 1H, J = 6 Hz), 1.54 (d, 3H, J = 6 Hz), 1.7-2.7 (m, 5H), 5.0-5.9 (m, 2H), 7.13 (d, 1H, J = 16 Hz), 7.81 (d, 1H, J = 16 Hz).

(3*S*,5*RS*,1*E*,7*Z*)-3-Hydroxy-1-iodo-5-methyl-1,7-nonadiene (16)

A solution of **15** (1.94 g, 7.0 mmol) in THF (20 ml) was treated at -100°C for 1.5 h with a solution of (*S*)-BINAL-H in THF prepared by mixing lithium aluminium hydride ($LiAlH_4$; 796 mg, 21.0 mmol), ethanol (1.15 ml, 19.6 mmol), and (*S*)-(-)-binaphthol (5.87 g, 20.5 mmol) in THF (60 ml).[5] After methanol (6 ml) was added at -78°C to decompose the excess reducing agent, saturated aq. sodium sulfate solution (6 ml) and then ethyl

acetate (50 ml) was added at r. t.. The resulting mixture was treated with magnesium sulfate (15 g), and filtered. The filtrate was evaporated and diluted with hexane to precipitate binaphthol as crystals. After filtration, the resulting organic solution was evaporated to give a crude product, which was purified by column chromatography on silica gel (40 g) using an 18 : 1 mixture of hexane and ethyl acetate to yield the reduced product **16** (1.82 g, 6.5 mmol, 93%) as a diastereomeric mixture; IR (neat): 3350, 3040, 1605, 1170, 1055, 1010, 950, 700 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.89 (d, 3H, J = 6 Hz), 1.55 (d, 3H, J = 6 Hz), 1.1-2.1 (m, 6H), 3.9-4.4 (m, 1H), 5.0-5.7 (m, 2H), 6.25 (d, 1H, J = 15 Hz), 6.53 (dd, 1/2H, J = 15 & 5 Hz), 6.56 (dd, 1/2H, J = 15 & 5 Hz).

To determine the optical purity of the product, **16** was converted into its 3,5-dinitrophenylcarbamate derivative. To a solution of **16** (10 mg) in toluene (0.5 ml) and pyridine (0.1 ml) was added at r. t. 3,5-dinitrophenylisocyanate (10 mg), and the resulting mixture was stirred at r. t. for 1 h. The reaction mixture was subjected to preparative thin-layer chromatography (hexane : ethyl acetate = 4 : 1) to give a diastereomeric mixture of 3,5-dinitrophenylcarbamate of **16**. The optical purity of this asymmetric reduction product was determined to be 99.6 : 0.4 by HPLC analysis for (3*S*)-diastereomers (R_t = 24.6 and 26.8 min) and (3*R*)-diastereomers (R_t = 20.8 and 22.9 min) (YMC-AK03 column; 25 cm x 20 mm I.D.; flow rate, 0.5 ml / min; detection, UV 254 nm, hexane : dichloromethane : ethanol = 70 : 30 : 2).

(3*S*,5*R*,1*E*,7*Z*)-3-Hydroxy-1-iodo-5-methyl-1,7-nonadiene (17)

A diastereomeric mixture of **16** (1.82 g, 6.5 mmol) was separated with preparative HPLC (TOSOH silica column; 30 cm x 100 mm I.D.; flow rate, 300 ml / min; detection, UV 254 nm, hexane : ethyl acetate = 16 : 1) to give the desired (3*S*,5*R*)-diastereomer **17** (R_t = 47.7 min; 720 mg, 2.57 mmol, 40%) and the (3*S*,5*S*)-diastereomer **S-17** (R_t = 54.4 min; 875 mg, 3.13 mmol, 48%). **17**; $[\alpha]_D^{23}$ +10.0° (*c* 0.52, MeOH); IR (neat): 3350, 3040, 1608, 1170, 1058, 1010, 950, 700, 678 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.89 (d, 3H, J = 6 Hz), 1,54 (d, 3H, J = 6 Hz), 1.0-2.3 (m, 6H), 3.9-4.4 (m, 1H), 5.1-5.8 (m, 2H), 6.26 (d, 1H, J = 15 Hz), 6.56 (dd, 1H, J = 15 & 5 Hz). **S-17**; $[\alpha]_D^{23}$ +3.9° (*c* 0.54, MeOH); IR (neat): 3350, 3040, 1608, 1170, 1060, 1010, 950, 700, 678 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.88 (d, 3H, J = 6 Hz), 1,53 (d, 3H, J = 6 Hz), 1.0-2.2 (m, 5H), 3.0 (bs, 1H), 3.8-4.3 (m, 1H), 5.0-5.8 (m, 2H), 6.23 (d, 1H, J = 15 Hz), 6.57 (dd, 1H, J = 15 & 5 Hz).

The less polar **17** (10 mg) was dissolved in a mixture of ethyl acetate (0.5 ml) and acetic acid (0.5 ml). To the mixture was added a catalytic amount of platinum oxide, and the resulting mixture was stirred at r. t. for 45 min under hydrogen atmosphere. After filtration of the catalyst, the filtrate was concentrated under reduced pressure to give an almost pure residue as a partially reduced product, which was identical (TLC, HPLC, IR, and NMR) with the authentic sample of (3*S*,5*R*,1*E*)-3-hydroxy-1-iodo-5-methyl-1-nonene (**18**).[3a]

(3*S*,5*R*,1*E*,7*Z*)-1-Iodo-5-methyl-3-*t*-butyldimethylsilyloxy-1,7-nonadiene (19)

Imidazole (329 mg, 4.8 mmol) and *t*-butylchlorodimethylsilane (363 mg, 2.4 mmol) were added at r. t. to a stirred solution of **17** (520 mg, 1.86 mmol) in DMF (10 ml), and the mixture was stirred at r. t. for 4 h. Usual work-up and purification by silica gel (40 g) column chromatography using hexane gave the silylated **19** (718 mg, 1.82 mmol, 98%); $[\alpha]_D^{24}$ -40.1° (*c* 0.55, MeOH); IR (neat): 3040, 1608, 1258, 1082, 950, 835, 805, 775, 700 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.03 (s, 6H), 0.88 (s+t, 12H), 1.57 (d, 3H, J = 6 Hz), 1.0-2.2 (m, 5H), 4.12 (dt, 1H, J = 8 & 6 Hz), 5.1-5.8 (m, 1H), 6.18 (d, 1H, J = 15 Hz), 6.58 (dd, 1H, J = 15 & 5 Hz);.

(17*R*,*Z*)-Δ^{19}-11,15-*O*-Bis(*t*-butyldimethylsilyl)-17,20-dimethyl-7-thia-prostaglandin E_1 methyl ester (21)

A 1.51 M pentane solution of *t*-butyllithium (2.90 ml, 4.38 mmol) was added at -78°C to a stirred solution of **19** (718 mg, 1.82 mmol) in ether (12 ml), and the mixture was stirred at -78°C for 2 h. To this mixture, a solution of 1-pentynylcopper(I) (285 mg, 2.18 mmol) and hexamethylphosphorous triamide (715 mg, 4.38 mmol) in ether (12 ml) was added at -78°C, and the reaction mixture was stirred at -78°C for 50 min. Then, to this mixture was added at -78°C a solution of (*R*)-4-*t*-butyldimethylsilyloxy-2-(5-methoxycarbonylpentylthio)-2-cyclopentenone[3a] (**20**; $[\alpha]_D^{23}$ -23.2° (*c* 0.60, MeOH); 712 mg, 1.91 mmol) in THF (15 ml), and the resulting mixture was stirred at -78°C for 2 h, then at

-50°C for 1.5 h. The reaction mixture was poured into an aq. 4.0 M acetate buffer solution (75 ml), and the whole mixture was continued to stir at r. t. for 30 min. The organic layer was taken up to hexane (300 ml), and the separated aq. layer was extracted with hexane (300 ml). The combined organic solutions were washed with aq. ammonium chloride solution, aq. ammoniacal ammonium chloride solution, aq. ammonium chloride solution and brine, and then dried over magnesium sulfate. Removal of the solvent under vacuum afforded 1.24 g of a crude product, which was subjected to silica gel (50 g) column chromatography with a 19 : 1 mixture of hexane and ethyl acetate as an eluant to yield the adduct **21** (955 mg, 1.49 mmol, 82%); $[\alpha]_D^{19}$ -28.1° (*c* 0.52, MeOH); IR (neat): 3040, 1740, 1255, 1110, 1080, 965, 880, 835, 775, 665 cm^{-1}; ^1H-NMR ($CDCl_3$): δ 0.06 (s, 12H), 0.90 (bs, 24H), 1.55 (d, 3H, J = 6 Hz), 1.0-3.0 (m, 19H), 3.60 (s, 3H), 3.7-4.4 (m, 2H), 5.1-5.6 (m, 4H); EI-MS (m/z): 583 (M^+-57), 441, 410, 381, 347, 289, 241, 185, 129; FD-MS (m/z): 640 (M^+), 583 (M^+-57), 115, 57; High-resolution MS for $C_{30}H_{55}O_5SSi_2$ (M^+-^{t}Bu): Calcd m/z: 583.3306; Found 583.3288.

(17*R*)-17,20-Dimethyl-7-thiaprostaglandin E_1 methyl ester (1) *via* (17*R*)-11,15-*O*-bis(*t*-butyldimethylsilyl)-17,20-dimethyl-7-thia-prostaglandin E_1 methyl ester (22)

Palladium on activated carbon (10%; 18 mg) was added to a solution of the (*Z*)-Δ^{19} olefin **21** (13 mg) in ethyl acetate (1 ml), and the mixture was stirred at r. t. for 50 min under hydrogen atmosphere. The catalyst was filtered off through Celite and the separated catalyst was washed with dichloromethane. The filtrate and washings were concentrated *in vacuo* to leave a crude residue, which contained the desired partially reduced product **22** coinciding (TLC and HPLC) with the authentic sample of **22**.[3a]

To a solution of the above crude product **22** in acetonitrile (0.5 ml) were added at r. t. pyridine (15 μl) and then hydrogen fluoride-pyridine (30 μl). The resulting mixture was stirred at the same temperature for 3 h. Aq. sodium bicarbonate solution was poured into the reaction mixture, and the resulting mixture was extracted twice with ethyl acetate (10 ml). The separated organic layers were washed with aq. pottasium bisulfate solution, aq. sodium bicarbonate solution, and then brine. After drying over magnesium sulfate and filtration, the obtained organic layer was evaporated under vacuum to provide a crude residue (7 mg).

The obtained crude product including **1** was separated by preparative HPLC (Zorbax SIL column; 25 cm x 4.6 mm I.D.) eluting with a 91 : 9 mixture of hexane and isopropyl alcohol (flow rate; 1.0 ml / min, UV detection; 210 nm) to furnish the desired product **1** (R_t 23.2 min) along with its 8-epimer (R_t 15.4 min) as a tautomer.[3a] The final product **1** was identical (TLC, HPLC, NMR, and MS) with the authentic sample of **1** as well as its 8-epimer.

Hot Synthesis

(17*R*)-17,20-Dimethyl-7-thia[19,20-$^3H_2$]prostaglandin E_1 methyl ester (3) *via* (17*R*)-11,15-*O*-bis(*t*-butyldimethylsilyl)-17,20-dimethyl-7-thia[19,20-$^3H_2$]prostaglandin E_1 methyl ester (23)

The silylated precursor **21** (33 mg, 0.06 mmol) was added to 10% palladium on charcoal (62 mg) in a hydrogenation vessel and ethanol (2.5 ml) was added. The mixture was stirred under 10 Ci of tritium gas. Gas uptake was very rapid and the reaction was ceased after 10 min by rapid filtration through a milex-SR (0.5 μm) filter, washing with ethanol (20 ml), and then rotary evaporated at 20°C. Labile tritium was removed by adding ethanol (1 ml) to the residue and removing the solvent by rotary evaporation as before. This was repeated twice more and the residue (3.35 Ci) was dissolved in ethanol (10 ml). The obtained crude material was rotary evaporated at 20°C to dryness, and glacial acetic acid (3 ml) and water (1 ml) was added. The resulting mixture was stirred at 20°C for 17 h under nitrogen. Analytical thin-layer chromatography on Whatman KC_{18} reverse-phase plates in acetonitrile : 0.01% acetic acid containing water (45 : 55) showed *ca.* 31% isomeric mixture of the desired di-tritiated product **3** and its 8-epimer as a tautomer. After concentration of the reaction mixture, HPLC purification of the crude product was performed on a Macro Dynamax ODS column (35 cm x 22 mm I.D.) eluted with a mixture of acetonitrile and 0.01% acetic acid containing water (45 : 55) (flow rate; 8 ml / min, UV detection; 210 nm). The main

peaks were pooled and analysis by a similar thin-layer chromatography showed the fraction containing the product **3** (*ca.* 200 mCi) to be about 53% radiochemical purity. The obtained partially purified product (*ca.* 150 mCi) was further purified by HPLC on Hypersil Si column (25 cm x 4.6 mm I.D., flow rate; 1.5 ml / min; UV detection; 210 nm) eluted with a mixture of toluene and ethanol (98.6 : 1.4) to furnish the di-tritiated product **3**. The radiochemical purity of the final product **3** was estimated to be 95% by HPLC analysis on Lichrosphere Si 60 column (25 cm x 4.6 mm I.D.) The specific activity of **3** was found to be 49 Ci / mmol by weight and radioassay.

(17*R*)-17,20-Dimethyl-7-thia[19,20-$^3H_2$]prostaglandin E_1 (4)

A solution of the di-tritiated methyl ester **3** (100 μCi) in acetone (0.2 ml) and porcine liver esterase[6] (20 μl, Sigma Chemical Co.) were added at r. t. to a 0.1 M phosphate buffer solution (2 ml, pH 8). After stirring at r. t. for 1 h, the mixture was acidified to pH 4 with 0.1 N HCl, and the mixture was extracted with ethyl acetate. The separated organic layer was dried over magnesium sulfate, and evaporated to give a crude carboxylic acid **4**, which was identical (TLC and HPLC) with the cold authentic sample of **2**. HPLC purification of the crude **4** on Lichrosorb RP-18 column (25 cm x 4.6 mm I.D.) eluting with a 38 : 62 mixture of acetonitrile and water containing 0.1% acetic acid provided the di-tritiated acid **4** containing its 8-epimer (40.6 μCi, 40.6%), whose radiochemical purity was found to be over 95%.

Synthesis of (17*R*)-17,20-dimethyl-7-thia[3,3,4,4,5,5,6,6-$^2H_8$]-prostaglandin E_1 methyl ester (5) and its carboxylic acid (6)

Ethyl 6-acetoxy-2-ethoxycarbonyl[3,3,4,4,5,5,6,6-$^2H_8$]hexanoate (25)

A solution of diethyl malonate (8.95 g, 56 mmol) in DMF (10 ml) was added at r. t. to a mixture of sodium hydride (50% content; 2.8 g, 56 mmol) in DMF (10 ml), and the mixture was stirred at the same temperature for 30 min. To this mixture was added at r. t. 4-bromo[1,1,2,2,3,3,4,4-$^2H_8$]butyl acetate (**24**; 7.3 g, 36 mmol), prepared from tetrahydrofuran-d_8 (99.5 atom % D) according to the cited procedure.[7] The resulting mixture was stirred at r. t. for 30 min and then quenched with aq. ammonium chloride solution. The mixture was extracted with hexane (3 x 100 ml). The separated organic layers were combined, washed with water, then brine, and dried over magnesium sulfate. After filtration, removal of the solvent *in vacuo* left a crude residue, which was chromatographed on silica gel (300 g) with a 3 : 1 mixture of hexane and ethyl acetate to give the alkylated product **25** (8.53 g, 30.2 mmol, 84%). This product was identical with a non-deuterated authentic sample corresponding to the compound **25**.

Methyl 6-bromo[3,3,4,4,5,5,6,6-$^2H_8$]hexanoate (26)

A solution of the product **25** (8.53 g, 30.2 mmol) in a mixture of acetic acid (18 ml) and 48% hydrobromic acid (10 ml) stirred at r. t. for 18 h to hydrolyze esters. After concentration of the mixture under reduced pressure, the residual crude dioic acid was dissolved in a mixture of 48% hydrobromic acid (10 ml) and sulfuric acid (3 ml), and the reaction mixture was heated at 110°C for 3 h for decarboxylation. Water and then ammonium sulfate were added and the mixture was extracted with ethyl acetate (3 x 100 ml). Usual work-up (washing, drying, and evaporation) gave a crude decarboxylated product, which was treated with methanol (250 ml) in the presence of a few drops of sulfuric acid for 3 h under reflux to provide a crude ester of **26** (5.0 g) after usual work-up. Purification of crude **26** by column chromatography on silica gel (250 g) with 9 : 1 and then 4 : 1 mixtures of hexane and ethyl acetate yielded the octa-deuterated bromo ester **26** (2.98 g, 13.7 mmol, 45%); ^{1}H-NMR ($CDCl_3$): δ 2.33 (s, 2H), 3.73 (s, 3H); EI-MS (m/z): 218 (M^+), 216 (M^+), 137, 89.

Methyl 6-mercapto[3,3,4,4,5,5,6,6-$^2H_8$]hexanoate (27)

A mixture of the bromide **26** (2.94 g, 13.5 mmol) and thiourea (1.34 g, 17.6 mmol) in ethanol (20 ml) was heated to reflux for 2 h. After addition of 5.4 N sodium hydroxide solution (10 ml, 54 mmol), the mixture was refluxed for 2 h. Ice-water and then sulfuric

acid were added and the mixture was extracted with ethyl acetate (3 x 100 ml). The separated organic layers were washed with brine, dried over magnesium sulfate, filtered, and evaporated *in vavuo* to leave a crude mercaptocarboxylic acid, which was dissolved in a mixture of methanol (500 ml), dichloromethane (190 ml), and a few drops of sulfuric acid. After the mixture was refluxed for 5 h, removal of the solvents *in vacuo* afforded to give a crude reaction mixture. The residual oil was taken up in dichloromethane (100 ml), and the organic layer was washed with aq. sodium bicarbonate solution, and then brine. After usual drying over magnesium sulfate and evaporation, the residual oil was chromatographed on silica gel (150 g) with 6 : 1 and then 1 : 1 mixtures of hexane and ethyl acetate to yield the mercapto ester **27** (1.78 g, 10.5 mmol, 78%). This ester was identical with a non-deuterated authentic sample corresponding to the compound **27**.

(*R*)-4-*t*-Butyldimethylsilyloxy-2-(5-methoxycarbonyl[1,1,2,2,3,3,4,4-$^2H_8$]pentylthio)-2-cyclopentenone (29)

Basic alumina (Woelm activity I; 12.5 g) was added at 0°C to a solution of the mercapto ester **27** (1.78 g, 10.5 mmol) and (*R*)-4-*t*-butyldimethylsilyloxy-2,3-epoxy-2-cyclopentenone[3a] **28** (2.63 g, 11.5 mmol) in hexane (50 ml), and the mixture was stirred at the same temperature for 30 min. After filtration of the alumina and washing with dichloromethane (100 ml), fitrate and washings were concentrated *in vacuo* to leave a crude adduct, which was recrystallized from methanol to produce the adduct **29** (2.86 g, 7.52 mmol, 72%); IR (neat): 3410, 3050, 2860, 2210, 2100, 1735, 1710, 1185, 1160, 1080, 955, 905, 835, 770 cm^{-1}; ^1H-NMR ($CDCl_3$): δ 0.13 (s, 6H), 0.90 (s, 9H), 2.33 (s, 2H), 2.1-3.2 (dd x 2, 2H, J = 19 & 6, 19 & 2 Hz), 3.74 (s, 3H), 5.06 (m, 1H), 6.97 (d, 1H, J = 3.5 Hz); EI-MS (m/z): 380 (M^+), 349, 323, 291, 243, 217, 189, 153, 104, 75; FD-MS (m/z): 380 (M^+), 323 (M^+-57).

(17*R*)-11,15-*O*-Bis(*t*-butyldimethylsilyl)-17,20-dimethyl-7-thia[3,3,4,4,5,5,6,6-$^2H_8$]prostaglandin E_1 methyl ester (31)

According to the reported procedure,[3a] a 2.0 M pentane solution of *t*-butyllithium (1.16 ml, 2.33 mmol) was added at -78°C to a stirred solution of (3*S*,5*R*,1*E*)-3-*t*-butyldimethylsilyloxy-1-iodo-5-methyl-1-nonene[3a] **30** (461 mg, 1.16 mmol) in ether (5 ml), and the resulting mixture was stirred at -78°C for 2 h. A solution of phenylthiocopper (201 mg, 1.16 mmol) and hexamethylphosphorous triamide (380 mg, 2.33 mmol) in ether (5 ml) was then added at -78°C to the mixture, and the resulting mixture was stirred at the same temperature for 1 h. To the reaction mixture was added a solution of the deuterated enone **29** (368 mg, 0.97 mmol) in ether (5 ml), and the whole mixture was continued to stir at -78°C for 15 min, then at -40°C for 1 h. The reaction mixture was poured into a 4.0 M acetate buffer solution, and the organic layer was taken up in hexane (200 ml). The separated organic layer was washed with aq. ammonium chloride solution then brine, dried over magnesium sulfate, and evaporated under vacuum. The residual oil was chromatographed on silica gel (30 g) with hexane-ethyl acetate (19 : 1) to produce the adduct **31** (405 mg, 0.625 mmol, 65%); ^1H-NMR ($CDCl_3$): δ 0.03 (s, 12H), 0.7-1.0 (m, 24H), 1.1-1.9 (m, 9H), 2.31 (s, 2H), 2.3-3.3 (m, 4H), 3.73 (s, 3H), 3.9-4.5 (m, 2H), 5.65-5.90 (m, 2H).

(17*R*)-17,20-Dimethyl-7-thia[3,3,4,4,5,5,6,6-$^2H_8$]prostaglandin E_1 methyl ester (5)

A solution of the adduct **31** (81 mg, 0.125 mmol), pyridine (0.2 ml), and hydrogen fluoride-pyridine (0.4 ml) in acetonitrile (5 ml) was stirred at r. t. for 6 h. The mixture was neutralized with aq. sodium bicarbonate solution, and extracted twice with ethyl acetate (50 ml). The combined extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. Purification of the residual oil (61 mg) by silica gel (20 g) column chromatography using hexane-ethyl acetate (1 : 2) furnished the desired product **5** (32 mg, 0.086 mmol, 68%); ^1H-NMR ($CDCl_3$): δ 0.7-1.0 (m, 6H), 1.1-1.9 (m, 9H), 2.31 (s, 2H), 2.3-3.3 (m, 6H), 3.73 (s, 3H), 3.9-4.5 (m, 2H), 5.65-5.90 (m, 2H); EI-MS (m/z): 422 (M^+), 404 (M^+-18), 386, 373, 360, 333, 235, 127, 76, 57, 43.

(17*R*)-17,20-Dimethyl-7-thia[3,3,4,4,5,5,6,6-$^2H_8$]prostaglandin E_1 (6)

A 0.1 M phosphate buffer solution (pH 8, 3 ml) and then porcine liver esterase[6] (30 μl) were added at r. t. to a solution of the product **5** (18 mg, 0.043 mmol) in acetone (0.3

ml), and the resulting mixture was stirred at r. t. for 20 h. The mixture was acidified to pH 4 with 0.1 N HCl solution, saturated with ammonium sulfate, and extracted with ethyl acetate (3 x 50 ml). The combined extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Purification of a crude product by silica gel column chromatography (10 g; hexane : ethyl acetate : acetic acid = 20 : 80 : 0.1) gave the deuterated carboxylic acid **6** (12 mg, 0.029 mmol, 68%); EI-MS (m/z): 390 (M^+-18), 372, 245, 218, 133, 107, 76, 43.

ACKNOWLEDGMENT

We are indebted to Amersham International plc. for the tritiation experiments to prepare the compound **3**.

REFERENCES

1. Prostaglandin Chemistry Part XXXVII. For Part XXXVI: ref. 7
2. Kurozumi S—"Kohza Prostaglandin, 7. Iyakuhin," ed. by Yamamoto S., Murota S., Tokyo Kagaku Dohjin, Tokyo (1988), Chap. 3, pp 96-98, and references cited therein.
3. (a) Tanaka T., Okamura N., Bannai K., Hazato A., Sugiura S., Manabe K., Kamimoto F., and Kurozumi S—*Chem. Pharm. Bull.* **33**: 2359 (1985), (b) Yasuda H., Sonobe M., Hatanaka I., Yamashita M., Miyamoto Y., Terada M., Amenomori M., Kikkawa R., Shigeta Y., Motoyama Y., and Saito N.—*Biochem. Biophys. Res. Commun.* **150**: 225 (1988), (c) Nagamatsu T., Kojima J., Ito M., Kondo N., and Suzuki Y.—*Japan J. Pharmacol.* **51**: 521 (1989), (d) Suzuki K., Saito N., Sakata Y., Toyota T., and Goto Y.—*Prostaglandins* **40**: 463 (1990), (e) Motoyama Y., Sakata Y., Seki J., Asada T., Namikawa Y., Horiai H., and Ono T.—*Thromb. Res.*, submitted for publication, (f) Motoyama Y., Sakata Y., Seki J., Sato M., Namikawa Y., Horiai H., and Ono T.—*Thromb. Res.*, submitted for publication,
4. (a) Zweifel G. and Brown H. C.—*Org. React.* **13**: 1 (1963), (b) Wolfe S. and Rauk A.—*Can. J. Chem.* **44**: 2591 (1966) (c) Skotnicki J. S., Schaub R. E., Bernardy K. F., Siuta G. J., Poletto J. F., Weiss M. J., and Dessy F.—*J. Med. Chem.* **20**: 1551 (1977)
5. Noyori R., Tomino I., Tanimoto Y., and Nishizawa M.—*J. Amer. Chem. Soc.* **106**: 6709 (1984)
6. (a) Ito Y., Shibata T., Sawai H., and Ohno M—*J. Amer. Chem. Soc.* **103**: 6739 (1981), (b) Hazato A., Tanaka T., Toru T., Okamura N., Bannai K., Sugiura S., Manabe K., and Kurozumi S.—*Nippon Kagaku Kaishi*, 1390 (1983)
7. Tanaka T., Bannai., Hazato A., Manabe., and Kurozumi S—*J. Labelled Compds. Radiopharm.*, in press (1991)