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SYNTHESES OF ^{3}H- AND ^{2}H-LABELED (16*S*)-15-DEOXY-16-HYDROXY-16-METHYL-5-THIAPROSTAGLANDIN E_1 METHYL ESTERS[1]

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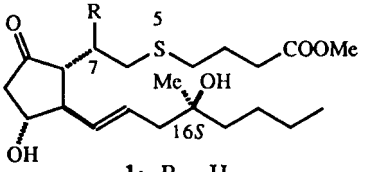
SUMMARY

The syntheses of [7-^{2}H]-, [7-^{3}H]-, and [2,2,3,3,4,4-$^{2}H_6$]-(16*S*)-15-deoxy-16-hydroxy-16-methyl-5-thiaprostaglandin E_1 methyl ester (**2**, **3**, and **4**) are described. Both 7-labeled compounds, **2** and **3**, were prepared from the Δ^{7}-precursor (**11**) by treatment with *in-situ* generated tributyltin deuteride and [^{3}H]hydride, respectively. The hexa-deuterated compound **4** was prepared starting from tetrahydrofuran-d_8

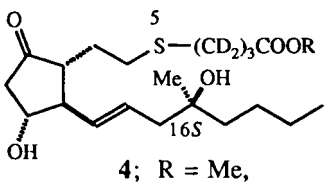
KEYWORDS: 7-tritium-labeled prostaglandin E_1, 7-deuterium-labeled prostaglandin E_1, hexadeuteroprostaglandin E_1

INTRODUCTION

Some prostaglandin E_1 and E_2 analogues are known to show both antisecretory and cytoprotective activities.[2] (16*S*)-15-Deoxy-16-hydroxy-16-methyl-5-thiaprostaglandin E_1 methyl ester[3] (**1**) is an example among those ones. It was necessary to obtain labeled compounds by either deuterium or tritium for preclinical studies of **1**. We report here the syntheses of [7-^{2}H]-, [7-^{3}H]-, and [2,2,3,3,4,4-$^{2}H_6$]-(16*S*)-15-deoxy-16-hydroxy-16-methyl-5-thiaprostaglandin E_1 methyl ester (**2**, **3**, and **4**)



1; R = H,
2; R = **D**,
3; R = **T**



4; R = Me,
5; R = H,

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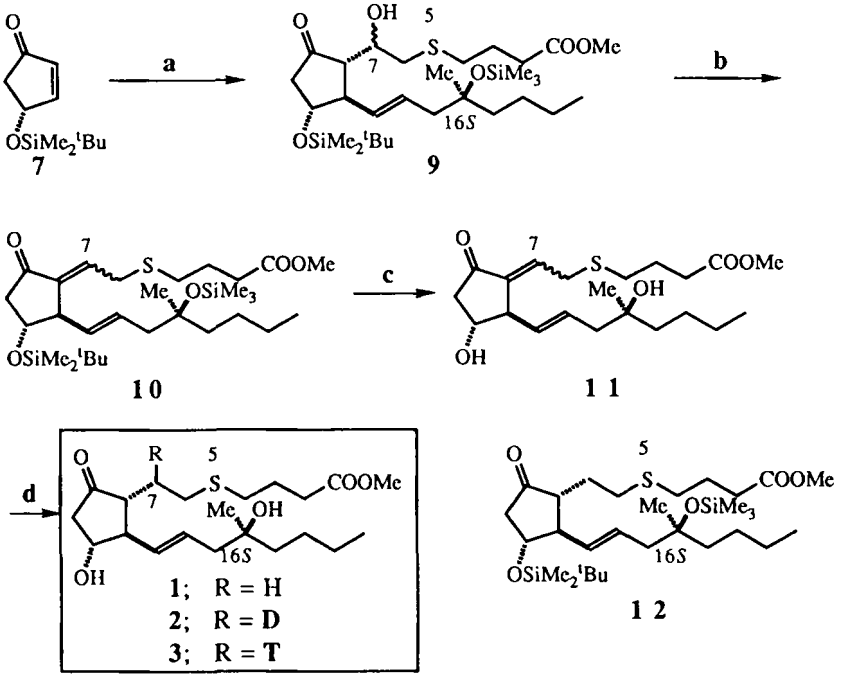
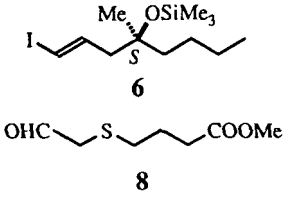
SYNTHESIS

Compound **3**, in which the hydrogen atom at the 7-position is substituted by a tritium atom, was synthesized from the Δ^7 olefinic precursor **11** by conjugate reduction of the enone function with *in-situ* generated tributyltin [^{3}H]hydride in the presence of palladium(0) catalyst (Scheme 1), and was used for the pharmacokinetics and metabolism studies of the compound **1**. Deuteration of the precursor **11** with tributyltin deuteride was also successful as a cold run to produce the [7-^{2}H] derivative **2**.



a) **6**—^{t}BuLi, 1-pentynylcopper(I)—$(Me_2N)_3P$, then **8** ; **b)** MsCl, DMAP ; **c)** $^n Bu_4N^+F^-$—pyridine ; **d)** $Pd(Ph_3P)_4$, Bu_3SnCl—$NaBH(D\ or\ T)_4$

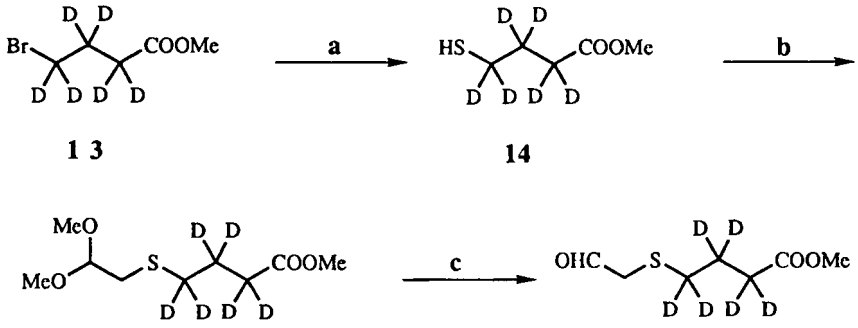


[MsCl = methanesulfonyl chloride
DMAP = 4-dimethylaminopyridine]

Scheme 1

Conjugate addition of the mixed cuprate generated from the vinyl iodide[4] **6**, to the chiral enone **7** followed by trapping of the resulting enolate intermediate with the aldehyde[3] **8** gave the three-component coupling adduct[3,5] **9** (81%). Treatment[3] of the adduct **9** with methanesulfonyl chloride and 4-dimethylaminopyridine afforded the dehydrated product **10** in 87% yield. Desilylation of the product **10** with hydrogen fluoride provided the precursor **11** (88%) as a mixture of the 7*E*- and 7*Z*-isomer. Conjugate reduction of **11** with tributyltin deuteride,[6] *in-situ* formed from tributyltin chloride and sodium borodeuteride, in the presence of palladium(0) catalyst[7] yielded the mono-deuterium labeled product **2** (44%) after preparative HPLC purification. Deuterium content of **2** was estimated to be *ca.* 85% by mass spectrometric measurement. A similar reduction[6] of the precursor **11** was carried out using tributyltin chloride, sodium boro[^{3}H]hydride (1 Ci; 58.9 Ci / mmol), and palladium(0) catalyst to furnish the radioactive product (146.8 mCi) in 14.7% yield. Purification of the obtained product by preparative HPLC provided 103 mCi of **3** with 95.1% radiochemical purity, whose specific activity was calculated to be 13.8 Ci / mmol.

Compound **4**, in which six hydrogen atoms at 2,3,4-positions are substituted by deuterium atoms, was synthesized from tetrahydrofuran-d_8 (Scheme 2 and 3), and was used for the internal standard of GC-MS quantitative analysis.

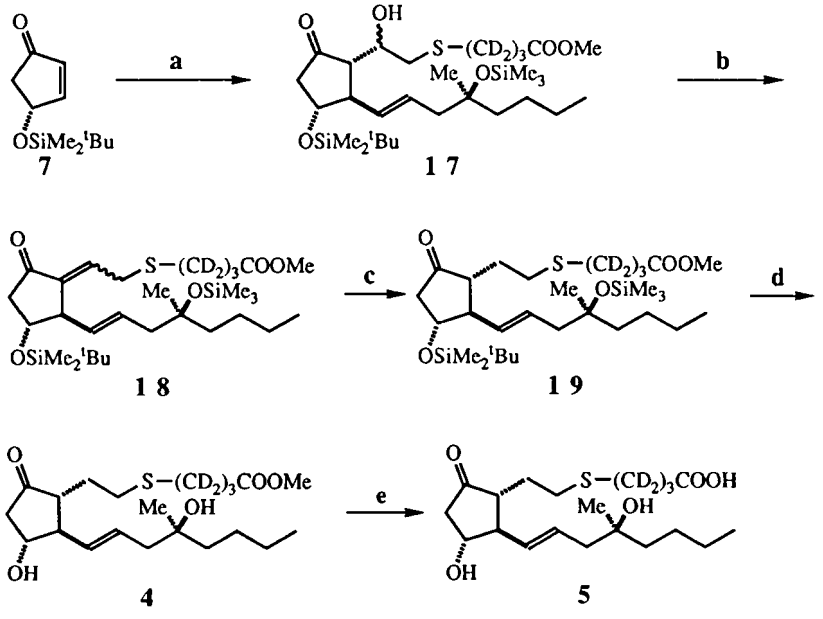


13 **14** **15** **16**

a) (1) $(NH_2)_2C=S$, then aq. NaOH, (2) MeOH, H_2SO_4 ;
b) NaH, $(MeO)_2CHCH_2Br$; **c)** aq. acetic acid

Scheme 2

Reaction of the hexa-deuterated bromide[8] **13** with thiourea afforded the mercapto ester **14** (84%) after alkaline work-up and re-esterification. Treatment of the mercapto ester **14** with 1-bromo-2,2-dimethoxyethane in the presence of sodium hydride gave the sulfide **15** in 67% yield. Acidic treatment of the acetal **15** with aq. acetic acid provided the aldehyde **16** (89%) as an enolate trapping agent (Scheme 2). Similar three-component coupling procedure[3,5] yielded the coupling adduct **17** (56%) which was similarly converted into the dehydrated enone **18** (70%). Conjugate reduction of the deuterated enone **18** with tributyltin hydride in the presence of palladium(0) catalyst gave the reduced product **19** in 89% yield. Desilylation of the product **19** with hydrogen fluoride furnished the deuterated product **4** (93%) which was further purified by



a) 6—^{t}BuLi, 1-pentynylcopper(I)—$(Me_2N)_3P$, then **16** ; b) MsCl, DMAP ; c) $Pd(Ph_3P)_4$, Bu_3SnH ; d) hydrogen fluoride—pyridine ; e) porcine liver esterase

Scheme 3

preparative HPLC. Hydrolysis of the deuterated methyl ester **4** with porcine liver esterase[9] produced the deuterated carboxylic acid **5** in 89% yield. Neither hexadeuterated aliquot of **4** nor **5** was enough to use for the internal standard of GC-MS quantitative analysis.

EXPERIMENTAL

IR spectra were recorded on a JASCO A 102 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were 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 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 (16*S*)-15-deoxy-16-hydroxy-16-methyl-5-thia[7-^{2}H]- and [7-^{3}H]prostaglandin E_1 methyl ester (2 and 3)

(16*S*)-11-*O*-*t*-Butyldimethylsilyl-15-deoxy-7-hydroxy-16-methyl-16-trimethylsilyloxy-5-thiaprostaglandin E_1 methyl ester (9)

A solution of (*S*,*E*)-1-iodo-4-methyl-4-trimethylsilyloxy-1-octene (**6**; 10.8 g, 31.5 mmol) in ether (35 ml) was added at -78°C to a 2.0 M pentane solution of *t*-butyllithium (31.5 ml, 63.0 mmol), and the mixture was stirred at -78°C for 2 h. To this mixture was added at -78°C a solution of 1-pentynylcopper(I) (4.31 g, 33 mmol) and hexamethylphosphorous triamide (11.8 g, 72.6 mmol) in ether (30 ml), and the mixture was stirred at -78°C for 30 min. Then, a solution of (*R*)-4-*t*-butylsilyloxy-2-cyclopentenone (**7**; 6.37 g, 30.0 mmol) in tetrahydrofuran (THF; 100 ml) was added at -78°C during 20 min, and the resulting mixture was stirred at -78C for 20 min, then at -40°C for 1.5 h. To the mixture was added at -78°C a solution of methyl 4-formylmethylthiobutanoate (**8**; 5.55 g, 31.5 mmol) in ether (50 ml), and the whole mixture was continued to stir at -78°C for 1 h. The reaction mixture was poured into a 4.0 M acetate buffer solution (300 ml), and the resulting mixture was vigorously stirred at r. t. for 15 min. The obtained mixture was taken up in hexane (400 ml), and the mixture was stirred for additional 45 min. The mixture was filtered off through Celite. The separated aqueous (aq.) layer was extracted with hexane (300 ml). The combined extracts were washed successively with aq. acetate buffer solution, aq. ammoniacal ammonium chloride solution, aq. ammonium chloride solution, and then brine. The obtained organic layer was dried over magnesium sulfate, filtered, and evaporated to leave a crude product, which was purified by column chromatography on silica gel (600 g) with 10 : 1 up to 3 : 1 mixtures of hexane and ethyl acetate as eluants to yield the three-component coupling adduct **9** (14.6 g, 24.3 mmol, 81%) as a diastereomeric mixture; R_f 0.35 (hexane : ethyl acetate = 4 : 1); ^{1}H-NMR ($CDCl_3$): δ 0.06 (s, 6H), 0.10 (s, 9H), 0.92 (s+t, 12H), 1.14 (s, 3H), 1.1-3.0 (m, 21H), 3.73 (s, 3H), 3.7-4.4 (m, 2H), 5.2-6.0 (m, 2H).

(16*S*)-11-*O*-*t*-Butyldimethylsilyl-15-deoxy-16-methyl-16-trimethylsilyloxy-5-thia-Δ^7-prostaglandin E_1 methyl ester (10)

Methanesulfonyl chloride (2.70 ml, 4.0 g, 35 mmol) was added at 0°C to a stirred solution of 4-dimethylaminopyridine (8.51 g, 70 mmol) in dichloromethane (110 ml), and the mixture was stirred at 0°C for 30 min. To the mixture was added a solution of the adduct **9** (14.0 g, 23.3 mmol) in dichloromethane (50 ml), and the reaction mixture was stirred at r. t. for 2 h. The reaction mixture was quenched with water (35 ml), and the mixture was taken up in hexane (500 ml). The separated aq. layer was extracted with hexane (300 ml). The combined extracts were washed with aq. potassium bisulfate solution, aq. sodium bicarbonate solution, and brine. The organic layer was dried over magnesium sulfate, filtered, and concentrated under vacuum to leave a crude product, which was chromatographed on silica gel (500 g) with a 19 : 1 mixture of hexane and ethyl acetate to provide the dehydrated product **10** (11.82 g, 20.2 mmol, 87%) as a 7*E* and 7*Z* mixture; R_f 0.40 (hexane : ethyl acetate = 4 : 1); ^{1}H-NMR ($CDCl_3$): δ 0.05 (s, 6H), 0.09 (s, 9H), 0.90 (s+t, 12H), 1.13 (s, 3H), 1.0-3.6 (m, 19H), 3.67 (s, 3H), 4.0-4.3 (m, 1H), 5.3-5.6 (m, 2H), 6.70 (dt, 1H, J = 13 & 3 Hz).

(16*S*)-15-Deoxy-16-hydroxy-16-methyl-5-thia-Δ^7-prostaglandin E_1 methyl ester (11)

To a stirred solution of the bis(silyl) ether **10** (4.74 g, 8.09 mmol) in acetonitrile (150 ml) were added at room temperature (r. t.) pyridine (7.5 ml) and a 1.0 M THF solution of tetrabutylammonium fluoride (30 ml, 30 mmol), and the resulting mixture was stirred at r. t. for 3 h. The reaction mixture was quenched with aq. ammonium chloride solution, and extracted with ethyl acetate (3 x 150 ml). The separated organic layers were washed with aq. potassium bisulfate solution, aq. sodium bicarbonate, and then brine, dried over magnesium sulfate, filtered, and evaporated *in vacuo*. The residual oil was purified by column chromatography on silica gel (300 g) using an 1 : 2 mixture of hexane and ethyl acetate to yield the desilylated product **11** (2.85 g, 7.12 mmol, 88%) as a mixture of 7*E* and 7*Z* isomers. The obtained mixture (367 mg) was separated by preparative HPLC (Zorbax SIL column, 25 cm x 20 mm I.D.) eluted with a 19 : 1 mixture of hexane and ethyl acetate (flow rate; 9.9 ml / min, UV detection 210 nm) to give the 7*E*-**11** (275 mg, 75%); R_f 0.33 (hexane : ethyl acetate = 1 : 4); ^{1}H-NMR ($CDCl_3$): δ 0.90 (t, 3H, J = 7 Hz), 1.13 (s, 3H), 1.0-3.6 (m, 21H), 3.67 (s, 3H), 4.0-4.3 (m, 1H), 5.3-5.6 (m, 2H), 6.70 (dt, 1H, J = 13 & 3 Hz).

(16*S*)-15-Deoxy-16-hydroxy-16-methyl-5-thia[7-^{2}H]prostaglandin E_1 methyl ester (2)

Tributyltin chloride (5.4 μl, 20 μmol) was added at r. t. to a stirred mixture of sodium borodeuteride (0.8 mg, 17.4 μmol) in 1,2-dimethoxyethane (200 μl), and the mixture was stirred at r. t. for 45 min. To the mixture was added ethanol (200 μl) and the mixture was stirred for additional 30 min. To this solution were added at r. t. a solution of the Δ^7 precursor **11** (10.0 mg, 25.1 μmol) in ethanol (1 ml), and then a solution of tetrakis-(triphenylphosphine)palladium(0) (1.0 mg, 0.87 μmol) in 1,2-dimethoxyethane (500 μl) and the resulting mixture was stirred at r. t. for 3 h. The reaction mixture was quenched with aq. ammonium chloride solution, and the mixture was extracted with ethyl acetate. The separated organic layer was washed with brine, dried over magnesium sulfate, filtered, and evaporated *in vacuo*. The residual oil was subjected to silica gel column chromatography using a Sep-Pak C18 cartridge with 2 : 1 (10 ml), 1 : 1 (20 ml), 1 : 2 (20 ml), and then 1 : 3 (20 ml) mixtures of hexane and ethyl acetate as eluants to give a crude product (10.2 mg), which was subjected to HPLC purification (OH-120 column, 25 cm x 4.6 mm I.D.) eluting with a 19 : 1 mixture of hexane and ethanol (flow rate; 1.0 ml / min, UV detection 220 nm) to provide the deuterated product **2** (3.1 mg, 7.7 μmol, 44%). The product **2** was identical (HPLC) with the non-deuterated authentic sample of **1**, prepared by conjugate reduction of the same aldol product **10** and desilylation of the resulting intermediate **12** in a similar procedure. **2**; R_t 13.2 min (OH-120 column, 25 cm x 4.6 mm I.D.; hexane : ethanol = 19 : 1; flow rate; 1.5 ml / min, UV detection 210 nm); IR (neat): 3420, 2220, 2120, 1740, 1435, 1215, 1170, 1155, 1080, 975 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.93 (t, 3H, J = 6 Hz), 1.16 (s, 3H), 1.1-3.0 (m, 17H), 3.67 (s, 3H), 3.9-4.3 (m, 1H), 5.3-6.0 (m, 2H).

(16*S*)-15-Deoxy-16-hydroxy-16-methyl-5-thia[7-^{3}H]-prostaglandin E_1 methyl ester (3)

Sodium boro[^{3}H]hydride (1 Ci, 58.9 Ci / mmol) was placed in a 5 ml flask with a stirring bar and dissolved in 1,2-dimethoxyethane (200 µl) under nitrogen atmosphere. In a similar procedure to the preparation of the above **2**, tributyltin chloride (5.4 µl, 20 µmol) was added, and the mixture was stirred at r. t. for 40 min. To the mixture was added ethanol (200 µl) and the mixture was stirred for additional 30 min. To this solution were added at r. t. a solution of the Δ^7 precursor **11** (10.0 mg, 25.1 µmol) in ethanol (1 ml), and then a solution of tetrakis(triphenylphosphine)palladium(0) (1.0 mg, 0.87 µmol) in 1,2-dimethoxyethane (500 µl) and the resulting mixture was stirred at r. t. for 3 h. The reaction mixture was quenched with aq. ammonium chloride solution (2 ml), and the mixture was extracted with ethyl acetate (4 x 1 ml). The combined organic layers were dried over magnesium sulfate, filtered, and evaporated *in vacuo*. The residual oil was subjected to silica gel column chromatography using a Sep-Pak C18 cartridge with benzene (5 ml) then 2 : 1 (7 ml), 1 : 1 (8 ml), 1 : 2 (8 ml), and then 1 : 3 (12 ml) mixtures of hexane and ethyl acetate as eluants collecting each 4 ml fraction to give a radioactive 5 to 9 fractions (146.8 mCi, 14.7%), which were combined and evaporated *in vacuo* to leave a crude oil (7.0 mg). The crude residue was subjected to HPLC purification (OH-120 column, 25 cm x 4.6 mm I.D.) eluting with a 19 : 1 mixture of hexane and ethanol (flow rate; 1.0 ml / min, UV detection 220 nm) to provide the tritiated product **3** (103 mCi) with 95.1% radiochemical purity, whose specific activity was calculated to be 13.8 Ci / mmol by weight and radioassay. The product **3** was identical (HPLC) with the above-mentioned authentic sample of **1**.

Synthesis of (16*S*)-15-deoxy-16-hydroxy-16-methyl-5-thia[2,2,3,3,4,4-$^2H_6$]prostaglandin E_1 methyl ester (4) and its carboxylic acid (5)

Methyl 4-mercapto[2,2,3,3,4,4-$^2H_6$]butanoate (14)

To a mixture of thiourea (3.25 g, 42 8 mmol) in ethanol (20 ml) was added the hexadeuterated bromo ester **13** (6.51 g, 34.8 mmol), prepared from tetrahydrofuran-d_8 in four steps according to the cited procedure.[7] The mixture was heated to reflux for 3 h, and a 4.0 M sodium hydroxide solution (33 ml, 130 mmol) was added to the cooled reaction mixture. After the resulting mixture was refluxed for 2 h, ice-water and then sulfuric acid (10 ml) were added, and the mixture was extracted twice with ethyl acetate (100 ml). The separated organic layers were washed with brine, dried over magnesium sulfate, filtered, and evaporated *in vacuo*. The obtained crude mercapto acid (5.54 g) was dissolved in a mixture of methanol (15 ml), dichloromethane (50 ml), and sulfuric acid (0.5 ml), and the resulting mixture was stirred at r. t. for 20 h. Aq. sodium bicarbonate solution was added and the mixture was extracted twice with ethyl acetate (100 ml). Usual work-up (drying, filtration, and concentration) afforded a crude residue (4.5 g), which was chromatographed on silica gel (200 g) with a 9 : 1 mixture of hexane and ethyl acetate to yield the mercapto ester **14** (4.10 g, 29.3 mmol, 84%). This mercapto ester **14** was identical with a non-deuterated authentic sample corresponding to the compound **14**; R_f 0.50 (hexane : ethyl acetate = 4 : 1); IR (neat): 2570, 2220, 2120, 1740, 1200, 1150 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.30 (s, 1H), 3.60 (s, 3H).

Methyl 4-(2,2-dimethoxyethylthio)[2,2,3,3,4,4-$^2H_6$]butanoate (15)

A solution of the obtained mercapto ester **14** (4.10 g, 29.3 mmol) in *N*,*N*-dimethylformamide (DMF; 15 ml) to a suspension of sodium hydride (60% content; 1.17 g, 29.3 mmol) in DMF (30 ml), and the mixture was stirred at r. t. for 1 h. Then, 1-bromo-2,2-dimethoxyethane (5.24 g, 31.0 mmol) was added at r. t. to this mixture, and the resulting mixture was stirred at r. t. for 2 h. The reaction mixture was poured into ice-water, and the mixture was extracted twice with ethyl acetate (100 ml). The separated organic layers were washed with brine, dried over magnesium sulfate, filtered, and evaporated to leave a crude product (6.04 g), which was purified by column chromatography on silica gel (200 g) using hexane and ethyl acetate (15 : 1 up to 6 : 1) to produce the acetal ester **15** (4.48 g, 19.6 mmol, 67%) coinciding with an unlabeled

authentic sample **15**; R_f 0.17 (hexane : ethyl acetate = 4 : 1); ^{1}H-NMR ($CDCl_3$): δ 3.35 (d, 2H, J = 9 Hz), 3.43 (s, 6H), 3.69 (s, 3H), 4.70 (t, 1H, J = 9 Hz).

Methyl 4-formylmethylthio[2,2,3,3,4,4-$^2H_6$]butanoate (16)

A solution of the deuterated acetal **15** (922 mg, 4.04 mmol) in acetic acid (2 ml) and water (3 ml) was heated at 80°C for 30 min, and the reaction mixture was poured into ice-cooled aq. sodium bicarbonate solution. The mixture was extracted with ethyl acetate (3 x 50 ml). The separated extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residual oil (754 mg) was distilled to give the pure aldehyde **16** (652 mg, 3.58 mmol, 89%) coinciding with an unlabeled authentic sample **16**; bp: 95-96°C / 0.35 mmHg; R_f 0.23 (hexane : ethyl acetate = 4 : 1); ^{1}H-NMR ($CDCl_3$): δ 3.16 (d, 2H, J = 3.7 Hz), 3.61 (s, 3H), 9.39 (t, 1H, J = 3.7 Hz).

(16*S*)-11-*O*-*t*-Butyldimethylsilyl-15-deoxy-7-hydroxy-16-methyl-16-trimethylsilyloxy-5-thia[2,2,3,3,4,4-$^2H_6$]prostaglandin E_1 methyl ester (17)

In a similar procedure to the preparation of the three-component adduct **9** from the aldehyde **8**, a solution of the vinyl iodide **6** (1.34 g, 3.94 mmol) in ether (5 ml) was allowed to react with a 1.7 M pentane solution of *t*-butyllithium (4.64 ml, 7.88 mmol) at -78°C for 2 h. A solution of 1-pentynylcopper(I) (514 mg, 3.94 mmol) and hexamethylphosphorous triamide (1.28 g, 7.88 mmol) in ether (5 ml) was added at -78°C, and the mixture was stirred at -78°C for 1 h. Then, a solution of the chiral enone **7** (760 mg, 3.58 mmol) in THF (15 ml) was added at -78°C, and the resulting mixture was stirred at -78°C for 15 min, then at -40°C for 30 min. To the mixture was added at -78°C a solution of the deuterated aldehyde **16** (652 mg, 3.58 mmol), and the whole mixture was continued to stir at -78°C for 30 min. Acetic acid (1.18 g, 19.7 mmol) was added at -78°C and the mixture was stirred at the same temperature for additional 15 min. The whole mixture was poured into an aq. 4.0 M acetate buffer solution (35 ml), and the mixture was extracted with hexane (3 x 100 ml). A similar work-up gave a crude residue, which was chromatographed on silica gel (100 g) with 9 : 1, 6 : 1, then 4 : 1 mixtures of hexane and ethyl acetate to yield the coupling adduct **17** (1.223 g, 2.00 mmol, 56%) compatible with an unlabeled authentic sample **9**; R_f 0.27 (hexane : ethyl acetate = 4 : 1); ^{1}H-NMR ($CDCl_3$): δ 0.05 (s, 6H), 0.09 (s, 9H), 0.95 (s+t, 12H), 1.14 (s, 3H), 1.1-3.0 (m, 15H), 3.70 (s, 3H), 3.7-4.4 (m, 2H), 5.2-6.0 (m, 2H).

(16*S*)-11-*O*-*t*-Butyldimethylsilyl-15-deoxy-16-methyl-16-trimethylsilyloxy-5-thia-Δ^7-[2,2,3,3,4,4-$^2H_6$]prostaglandin E_1 methyl ester (18)

In a similar manner to the preparation of the compound **10** by dehydration of the aldol product **9**, methanesulfonyl chloride (586 mg, 400 μl, 5.1 mmol) was added at 0°C to a solution of 4-dimethylaminopyridine (1.44 g, 11.8 mmol) in dichloromethane (20 ml), and the mixture was stirred at 0°C for 30 min. To the mixture was added at 0°C a solution of the coupling adduct **17** (1.223 g, 2.00 mmol) in dichloromethane (10 ml), and the resulting mixture was stirred at 0°C for 20 h. A similar work-up afforded a crude oil (1.09 g), which was purified by column chromatography on silica gel (100 g) with 19 : 1, 9 : 1, then 6 : 1 mixtures of hexane and ethyl acetate to furnish the dehydrated enone **18** (836 mg, 1.41 mmol, 70%) compatible with an unlabeled authentic sample **10**; R_f 0.45 (hexane : ethyl acetate = 4 : 1); ^{1}H-NMR ($CDCl_3$): δ 0.05 (s, 6H), 0.07 (s, 9H), 0.89 (s+t, 12H), 1.13 (s, 3H), 1.1-1.6 (m, 6H), 2.0-2.75 (m, 4H), 3.1-3.3 (m, 2H), 3.35-3.50 (m, 1H), 3.67 (s, 3H), 4.1-4.3 (m, 1H), 5.35-5.55 (m, 2H), 6.74 (dt, 1H, J = 7.5 & 2 Hz).

(16*S*)-11-*O*-*t*-Butyldimethylsilyl-15-deoxy-16-methyl-16-trimethylsilyloxy-5-thia[2,2,3,3,4,4-$^2H_6$]prostaglandin E_1 methyl ester (19)

In an analogous manner to the preparation of the products, **2** and **3**, tributyltin hydride (3.4 g, 3.2 ml, 11.7 mmol) was added dropwise during 2 h to a stirred mixture of the dehydrated product **18** (686 mg, 1.16 mmol) and tetrakis(triphenylphosphine)-palladium(0) (67 mg, 0.058 mmol), and the mixture was stirred at r. t. for 5 h. Purification of the reaction mixture by column chromatography on silica gel (100 g) with 30 : 1, 20 : 1, then 10 : 1 mixtures of hexane and ethyl acetate yielded the reduced product **19** (614 mg, 1.03 mmol, 89%) identical with an unlabeled authentic sample **12**; R_f 0.26 (hexane : ethyl acetate = 2 : 1); ^{1}H-NMR ($CDCl_3$): δ 0.05 (s, 6H), 0.12 (s, 9H), 0.8-1.0

(s+t, 12H), 1.16 (s, 3H), 1.1-1.6 (m, 6H), 1.7-2.9 (m, 10H), 3.67 (s, 3H), 4.03 (m, 1H), 5.15-5.85 (m, 2H).

(16*S*)-15-Deoxy-16-hydroxy-16-methyl-5-thia[2,2,3,3,4,4-$^2H_6$]prostaglandin E_1 methyl ester (4)

To a stirred solution of the obtained silyl ether **19** (150 mg, 0.253 mmol) in acetonitrile (5 ml) were added at r. t. pyridine (0.25 ml) and then a 1.0 M THF solution of tetrabutylammonium fluoride (1.0 ml, 1.0 mmol), and the resulting mixture was stirred at r. t. for 3 h. Similar work-up gave a crude product, which was chromatographed on silica gel (20 g) with a 1 : 3 and then 1 : 4 mixture of hexane and ethyl acetate to furnish the desired hexa-deuterated product **4** (96 mg, 0.235 mmol, 93%). The obtained compound **4** (96 mg, 0.235 mmol) was further purified by preparative HPLC (YMC SH-430 S-15 SIL column) eluted with a mixture of hexane containing 5% ethanol to produce the 99.5% pure sample **4** (88 mg, 0.216 mmol, 92%), which was identical with a unlabeled authentic sample **1**; R_f 0.25 (hexane : ethyl acetate = 1 : 4); IR (neat): 3420, 2220, 2120, 1740, 1435, 1215, 1170, 1155, 1080, 975 cm^{-1}; ^1H-NMR ($CDCl_3$): δ 0.93 (t, 3H, J = 6 Hz), 1.19 (s, 3H), 1.2-3.0 (m, 18H), 3.70 (s, 3H), 3.9-4.3 (m, 1H), 5.3-6.0 (m, 2H); ^{13}C-NMR ($CDCl_3$): δ 14.1, 23.3, 26.2, 27.1, 27.7, 29.2, 41.3, 45.0, 45.9, 51.6, 53.0, 55.1, 71.9, 72.6, 130.0, 133.2, 173.6, 214.4; EI-MS (m/z): 388 (M^+-18), 370, 288, 230, 204, 173, 148, 101, 77, 43.

(16*S*)-15-Deoxy-16-hydroxy-16-methyl-5-thia[2,2,3,3,4,4-$^2H_6$]prostaglandin E_1 (5)

A 0.1 M phosphate buffer solution (pH 8, 10 ml) and then porcine liver esterase[7] (0.1 ml, Sigma Chemical Co.) were added at r. t. to a solution of the hexa-deuterated ester **4** (44 mg, 0.108 mmol) in acetone (1 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 (4 x 50 ml). The separated extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Purification of the crude residue by silica gel (10 g) column chromatography (ethyl acetate : methanol = 95 : 5) furnished the deuterated carboxylic acid **5** (38 mg, 0.096 mmol, 89%), which was identical with a corresponding unlabeled authentic sample **5**; R_f 0.23 (ethyl acetate); IR (neat): 3380, 3050, 2220, 2120, 1720, 1150, 1075, 970, 900, 735 cm^{-1}; ^1H-NMR ($CDCl_3$): δ 0.93 (t, 3H, J = 6 Hz), 1.19 (s, 3H), 1.1-3.0 (m, 16H), 3.85-4.35 (m, 1H), 4.54 (bs, 3H), 5.25-6.0 (m, 2H); EI-MS (m/z): 318, 303, 275, 239, 215, 181, 149, 121, 83, 43.

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