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SYNTHESIS OF [^{14}C]- AND [^{3}H]-LABELLED (+)-[1R-[1α,2α(Z),3β,4α]]-7-[3-[(PHENYLSULFONYL)AMINO]BICYCLO[2.2.1]HEPT-2-YL]-5-HEPTENOIC ACID, ((+)-S-145) AND ITS CALCIUM SALT (S-1452)

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SUMMARY

(+)-S-145 and S-1452, thromboxane A_2 receptor antagonists, were labelled with ^{3}H and ^{14}C for studies on the metabolic fate and characterization of receptor binding. ^{3}H and ^{14}C were incorporated into the benzene ring in the phenylsulfonylamide side chain with specific radioactivities of 26.4 Ci/mmol and 8.73 mCi/mmol, respectively. The common key intermediate, amine 12a was synthesized in eight steps from 4a for facile labelling.

Key words: S-145, S-1452, ^{3}H, ^{14}C, thromboxane A_2 receptor antagonist

INTRODUCTION

A number of sulfonyl derivatives of (±)-[1α,2α(Z),3β,4α]-7-(3-aminobicyclo-[2.2.1]hept-2-yl)-5-heptenoic acid have been synthesized in our laboratory by Narisada et al. (1). Among such compounds, (±)-[1α,2α(Z),3β,4α]-7-[3-[(phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-yl]-5-heptenoic acid, S-145 (1), was found to be a potent and selective thromboxane A_2 (TXA_2)-receptor antagonist which efficiently suppresses platelet aggregation and vascular, respiratory smooth muscle constriction both *in vitro* and *in vivo* (2). By further investigation, Narisada et al. clarified that optically active (+)-S-145 (1a) is more than ten times stronger than its enantiomer, (−)-S-145 (1b), in all of its biological activities. S-1452 (3), the calcium salt of (+)-S-145 (1a), was chosen as a candidate for a drug because of its chemical stability. Considerable research effort has been directed toward its development for use in the chemotherapy of various TXA_2-

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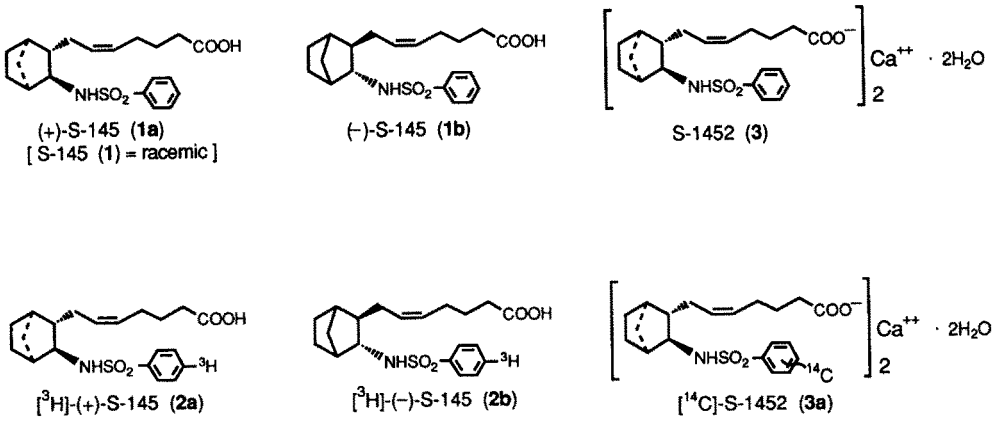


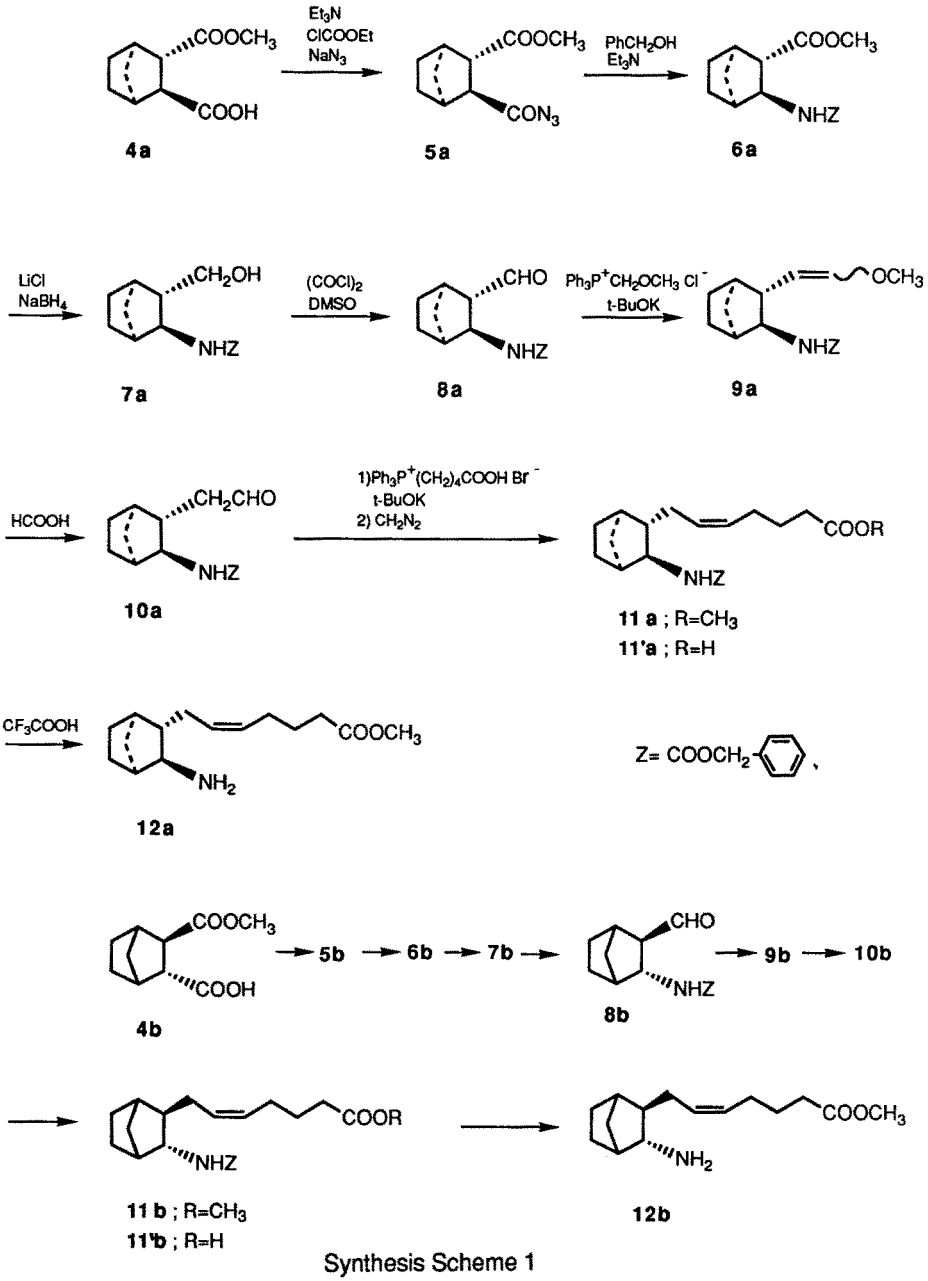
Fig. 1

and/or PGH_2-mediated disorders, such as angina pectoris, asthma, myocardial infarction and other circulatory disorders. Thus, [^{3}H]-(+)-S-145 (2a), [^{3}H]-(−)-S-145 (2b) and [^{14}C]-S-1452 (3a) were required for studies on the characterization of TXA_2-receptor binding and metabolic fates.

SYNTHESIS

Carbon atoms in the benzene ring of the phenylsulfonylamide side chain of S-1452 (3) and the hydrogen atom of position 4 in the benzene ring of the phenylsulfonylamide side chain of (+)- and (−)-S-145 (1a, 1b) were chosen for ^{14}C and ^{3}H labelling, respectively, because they are stable to metabolic loss and enable facile preparation of 2a, 2b and 3a. These labelled compounds were synthesized as shown in the synthesis scheme in which the labels were introduced near the end of a sequence of reactions in order to avoid the difficulties of multi-step hot micro synthesis and decomposition caused by self radiolysis. Therefore, unlike the syntheses of unlabelled (+)-S-145 (1a), (−)-S-145 (1b) and S-1452 (3) (3), the protected heptenoic acid side chain was constructed before the labelling steps (sulfonation). Compound 2a and 2b were synthesized by coupling of [^{3}H]benzenesulfonyl chloride (4) with the corresponding amine 12a or 12b, followed by saponification into the sodium salt in micro synthesis. [^{14}C]-S-1452 (3a) was synthesized from [^{14}C]benzene (13) (5) *via* [^{14}C]benzenesulfonic acid and [^{14}C]benzenesulfonyl chloride (14), which was coupled with amine 12a, followed by saponification and conversion into calcium salt. The common key inter-

mediate, amine 12a, was synthesized for facile labelling from a chiral half ester 4a (3) in eight steps. The carboxyl group of half ester 4a was changed with ethyl chloroformate and sodium azide into azide 5a, which was converted into carbobenzoxy amine 6a. Transformation of the methoxycarbonyl group in 6a to the heptenoic acid side chain was accomplished as follows. Reduction of 6a with sodium borohydride followed by Swern oxidation with oxalyl chloride/triethylamine/dimethylsulfoxide afforded aldehyde 8a,



4a **5a** **6a**

7a **8a** **9a**

10a

11 a ; R=CH_3
11'a ; R=H

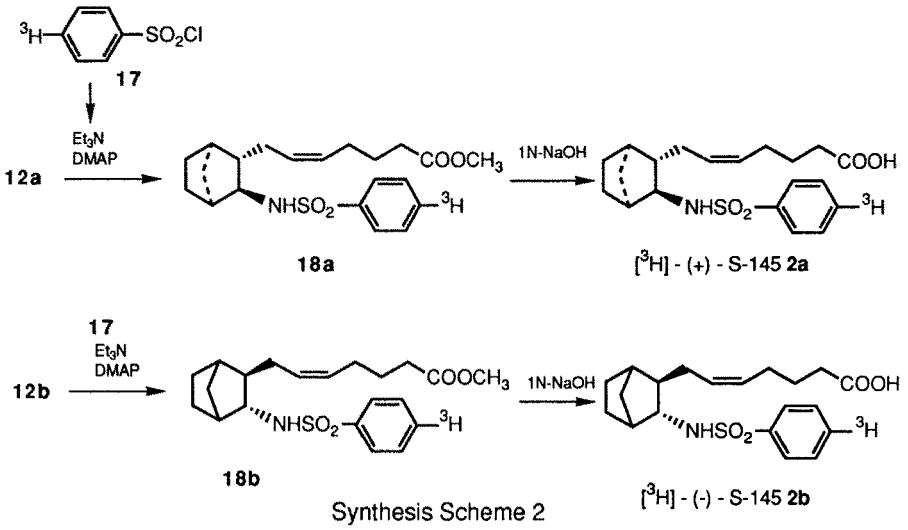
12a

4b **8b**

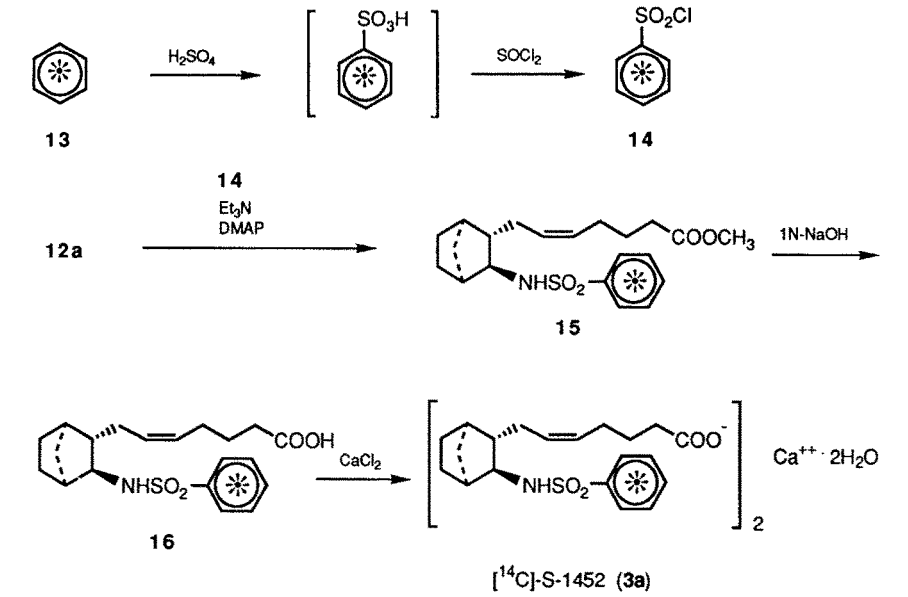
11 b ; R=CH_3
11'b ; R=H

12b

Synthesis Scheme 1



Synthesis Scheme 2



Synthesis Scheme 3

which upon treatment with (methoxymethyl)triphenylphosphoran, yielded methoxyethylene compound 9a. Acid treatment of 9a with 90% formic acid followed by Wittig reaction with (4-carboxybutyl)triphenylphosphoran gave heptenoic acid derivative 11'a *via* aldehyde 10a. After usual esterification of the acid 11'a with diazomethane into

methyl ester 11a, deprotection of the amino group of 11a with trifluoroacetic acid in anisole gave the desired amine 12a in 16.8% overall yield from half ester 4a. 12b was prepared from 4b (3) in the same manner as the synthesis of 12a.

EXPERIMENTAL

The radiochemical purity of every labelled compound was determined by TLC autoradiogram followed by liquid scintillation counting, using Aloka LSC-672 and HPLC with a radioactivity flow monitor, Packard Trace II 7150. The labelled compounds 2a, 2b and 3a and labelled intermediates 15, 16, 18a and 18b were identified with the corresponding unlabelled authentic sample by comparison of TLC (R_f value), HPLC (retention time) and IR or NMR spectral data. Optical rotation was determined with a Perkin-Elmer Model 241 polarimeter using a 1-dm microcell. IR spectra were recorded on a JASCO A702 spectrometer. Proton NMR spectra were obtained with a Varian VXR-200 and Gemini-200 spectrometers using deuteriochloroform, unless otherwise stated, with tetramethylsilane as the internal reference. The compounds were chromatographed on Merck precoated thin silica gel plate (0.25 mm) in the solvent described. The results (data on TLC and HPLC) are shown in Tables 1 and 2.

(+)-[1R-(2-*exo*,3-*endo*)]-3-(Azidocarbonyl)bicyclo[2.2.1]heptane-2-carboxylic acid methyl ester (5a)

To a stirred solution of (+)-[1R-(2-*exo*,3-*endo*)]bicyclo[2.2.1]heptane-2,3-dicarboxylic acid 2-methyl ester (4a) (3) (20 g, 100 mmol) in acetone (170 ml) were added dropwise triethylamine (18.2 ml, 131 mmol) and ethylchloroformate (12.5 ml, 131 mmol) at 0°C under nitrogen atmosphere. After stirring for 15 min, a solution of sodium azide (19.7 g, 303 mmol) in water (57 ml) was added dropwise to the mixture, which was then stirred for 25 min. Next, the reaction mixture was poured into a mixture of 2 N hydrochloric acid (200 ml) and ethyl acetate (300 ml) with stirring at room temperature. The organic layer was separated from the mixture, washed with 5% sodium bicarbonate and water, dried with magnesium sulfate and evaporated *in vacuo* below 35°C , leaving a viscous oil (*ca.* 23 g, 100 mmol) as crude 5a. 5b was prepared from 4b in the same manner. Crude 5a and 5b were used immediately for the next reactions without any purification, because of their instability. 5a and 5b: IR ν cm^{-1} (film): 2136, 1731, 1715, 1702.

(+)-[1R-(2-*exo*,3-*endo*)]-3-[[(Phenylmethoxy)carbonyl]amino]bicyclo[2.2.1]heptane-2-carboxylic acid methyl ester (**6a**)

Crude **5a** (22.3 g, 100 mmol) obtained above was dissolved in anhydrous benzene (140 ml) and stirred for 1 hr at 80°C. To this solution, benzyl alcohol (12.5 ml, 121 mmol) and triethylamine (18.2 ml, 131 mmol) were added dropwise and then stirred under reflux for 1.5 hr. After cooling, the reaction mixture was poured into a mixture of 2 N-hydrochloric acid (200 ml) and ethyl acetate (200 ml) with stirring. The organic layer was separated from the mixture, washed with 5% sodium bicarbonate and water, dried with magnesium sulfate and evaporated *in vacuo* below 35°C, leaving a viscous oil as a residue (*ca.* 35 g) which was chromatographed on silica gel (Merck No. 7734, 250 g; elution with toluene-ethyl acetate = 9:1 ~ 4:1). The fractions containing the desired compound were combined and evaporated *in vacuo* at 35°C, leaving a crystalline residue (*ca.* 30 g) which was purified by recrystallization from petroleum ether to give pure **6a** (23.5 g, 77.6 mmol) as colorless needles in 76.6% yield. **6b** was prepared from **5b** in the same manner. **6a**: m.p. 60-60.5°C. $[\alpha]_D^{24}$ +38.6 ± 0.8 (c 1.003, $CHCl_3$). Anal. Calcd for $C_{17}H_{21}NO_4$: C, 67.31; H, 6.98; N, 4.62. Found: C, 67.23; H, 7.06; N, 4.72. IR ν cm^{-1} ($CHCl_3$): 3445, 1727, 1505. **6a** and **6b**: NMR δ ppm ($CDCl_3$): 1.20-1.80 (m, 7H), 1.88-1.95 (m, 1H), 1.46 (br s, 2H), 3.40-3.80 (m, 1H), 3.70 (s, 3H), 4.16-4.30 (m, 1H), 4.78-4.98 (m, 1H), 5.09 (s, 1H), 7.35 (s, 5H). **6b**: m.p. 61.5-62°C. Anal. Calcd for $C_{17}H_{21}NO_4$: C, 67.31; H, 6.98; N, 4.62. Found: C, 67.51; H, 7.04; N, 4.88. $[\alpha]_D^{24}$ -40 ± 0.8 (c 1.008, $CHCl_3$). IR ν cm^{-1} ($CHCl_3$): 3450, 1730, 1507.

(+)-[1S-(2-*endo*,3-*exo*)]-[3-(Hydroxymethyl)bicyclo[2.2.1]hept-2-yl]carbamic acid phenylmethyl ester (**7a**)

To a stirred solution of ester **6a** (54.7 g, 180 mmol) in anhydrous tetrahydrofuran (378 ml) were added portionwise lithium chloride (23 g, 541 mmol) and sodium borohydride (20 g, 540 mmol) at 0°C under nitrogen atmosphere. Next, ethanol (760 ml) was added, and the mixture was stirred for 7 hr at room temperature. After quenching the excess reagents by addition of 2 N hydrochloric acid to pH 7, the reaction mixture was concentrated to about a third of the initial volume and poured into a mixture of water (1500 ml) and ethyl acetate (700 ml) with stirring at room temperature. The organic layer was separated from the mixture, washed with 5% sodium bicarbonate and water, dried with magnesium sulfate and evaporated *in vacuo* at 35°C, leaving a viscous

oil as a residue (50 g) which was chromatographed on silica gel (Merck No. 7734, 180 g; elution with n-hexane-dichloromethane-ethyl acetate = 8:1:1 ~ ethyl acetate). The fractions containing the desired compound were combined and evaporated *in vacuo*, leaving a crystalline residue which was purified by recrystallization from dichloromethane-ethyl ether to give pure 7a (34.3 g, 125 mmol) as colorless needles in 69.2% yield. 7b was prepared from 6b in the same manner. 7a: m.p. 120-120.5°C. $[\alpha]_D^{24}$ (*c* 1.015, MeOH). Anal. Calcd for $C_{16}H_{21}NO_3$: C, 69.79; H, 7.69; N, 5.09. Found: C, 69.76; H, 7.62; N, 5.06. IR ν cm^{-1} ($CHCl_3$): 3615, 3440, 3385, 1700, 1509. 7a and 7b: NMR δ ppm ($CDCl_3$): 1.21-1.76 (m, 7H), 2.03 (br s, 1H), 2.39 (s, 1H), 2.24-2.86 (br, 1H), 3.44 (d of ABq, A-part, J = 10.2, 10.7 Hz, 1H), 3.58 (d of ABq, B-part, J = 5.5, 10.7 Hz, 1H), 3.47-3.62 (m, 1H), 5.08 (ABq, A-part, J = 12.1 Hz, 1H), 5.12 (ABq, B-part, J = 12.1 Hz, 1H), 7.36 (s, 5H). 7b: m.p. 120-121.5°C. Anal. Calcd for $C_{16}H_{21}NO_3$: C, 69.79; H, 7.69; N, 5.09. Found: C, 69.74; H, 7.77; N, 5.12. $[\alpha]_D^{24}$ -27.3 ± 0.7 (*c* 1.020, $CHCl_3$). IR ν cm^{-1} ($CHCl_3$): 3620, 3455, 3400, 1700, 1511.

(+)-[1S-(2-*endo*,3-*exo*)]-[3-Formylbicyclo[2.2.1]hept-2-yl]carbamic acid phenylmethyl ester (8a)

To a stirred solution of oxalyl chloride (16 ml, 184 mmol) in anhydrous dichloromethane (280 ml) was added dropwise a solution of dimethylsulfoxide (26 ml, 367 mmol) in anhydrous dichloromethane (60 ml) over a period of 10 min at -78°C. After stirring for 20 min, a solution of 7a (38.9 g, 141 mmol) in dichloromethane-dimethylsulfoxide (5:2, 140 ml) was added dropwise over a period of 15 min to the mixture at -78°C. After stirring for 20 min, triethylamine (98 ml, 705 mmol) was added over a period of 3 min, and the resulting mixture was allowed to come to room temperature gradually during 50 min with stirring. The mixture was washed successively with 2 N hydrochloric acid, water, 5% sodium bicarbonate and water, dried with magnesium sulfate and evaporated *in vacuo*, leaving a viscous oil as a residue which was chromatographed on silica gel (Merck No. 7734, 250 g; elution with n-hexane-ethyl acetate = 4:1 ~ 2:1). The fractions containing the desired compound were combined and evaporated *in vacuo* to obtain almost pure 8a (42.0 g, 140 mmol) as an oil in nearly quantitative yield. 8b was prepared from 7b in the same manner. 8a: Anal. Calcd for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.13. Found: C, 70.40; H, 7.17; N, 5.22. 8a and 8b: IR ν cm^{-1} ($CHCl_3$): 3440, 1715, 1504, 1218, 1022. NMR δ ppm ($CDCl_3$): 1.20-1.80 (m, 6H), 2.01 (d, J = 5.0 Hz,

1H), 2.49 (s, 1H), 2.53 (s, 1H), 4.14-4.27 (m, 1H), 4.85-5.16 (m, 1H), 5.09 (s, 2H), 7.34 (s, 5H), 9.77 (s, 1H).

(+)-[1S-2-*endo*,3-*exo* (E)]]-[3-(2-Methoxyethenyl)bicyclo[2.2.1]hept-2-yl]carbamic acid phenylmethyl ester (**9a**)

To a stirred suspension of (methoxymethyl)triphenylphosphonium chloride (111 g, 325 mmol) in anhydrous tetrahydrofuran (400 ml) was added portionwise potassium tert-butoxide (34.9 g, 311 mmol) at 0°C under nitrogen atmosphere. After stirring for 1 hr, a solution of **8a** (40 g, 140 mmol) in anhydrous tetrahydrofuran (100 ml) was added dropwise over a period of 5 min to the mixture and stirred for 30 min at 0°C. After quenching the excess reagent by addition of water, the mixture was concentrated *in vacuo* to about 100 ml, poured into water and extracted with toluene. The extract was washed with water, dried with magnesium sulfate and evaporated *in vacuo*, leaving a viscous oil as a residue which was chromatographed on silica gel (Merck No. 7734, 300 g; elution with n-hexane-ethyl acetate = 9:1 ~ 4:1). The fractions containing the desired compound were combined and evaporated *in vacuo*, leaving a viscous oil as a mixture (41.6 g, 138 mmol) of E and Z isomers at the double bond. The ratio of E and Z of the mixture (**9a**) was 3:1 according to the data from proton NMR determination. The mixture was used for the next reaction without further purification. **9b** was prepared from **8b** in the same manner. **9a**: Anal. Calcd for $C_{18}H_{23}NO_3$: C, 71.73; H, 7.69; N, 4.65. Found: C, 71.99; H, 7.81; N, 4.73. **9a** and **9b**: IR ν cm^{-1} ($CHCl_3$): 3438, 1709, 1649, 1504, 1212. NMR δ ppm ($CDCl_3$): 1.16-1.69 (m, 7H), 1.90-2.06 (m, 1H), 2.47 (s, 1H), 3.49 (s, 3H, E-isomer), 3.53 (s, 3H Z-isomer), 3.60 (br s, 1H), 4.34 (dd, J = 10.0, 6.0 Hz, 1H Z-isomer), 4.70 (dd, J = 8.6, 12.6, 1H E-isomer), 5.08 (s, 2H), 5.81 (d, J = 6.0 Hz, 1H Z-isomer), 6.30 (d, J = 12.6 Hz, 1H E-isomer), 7.27-7.40 (m, 5H).

(+)-[1S-(2-*endo*,3-*exo*)]-3-(2-Oxoethyl)bicyclo[2.2.1]hept-2-yl-carbamic acid phenylmethyl ester (**10a**)

A mixture of **9a** (41.6 g, 138 mmol) and 90% formic acid (42 ml) was stirred for 1.5 hr at room temperature. The mixture was poured into a stirred mixture of sodium bicarbonate (80 g, 950 mmol), water (100 ml) and ethyl acetate (200 ml). The stirring was continued until the evolution of carbon dioxide ceased. The organic layer was separated from the mixture, washed with water, dried with magnesium sulfate and evaporated *in vacuo* below 35°C, leaving a viscous oil as a residue (38 g) which was

purified by chromatography on silica gel (Merck No. 7734, 300 g; elution with n-hexane-ethyl acetate = 9:1 - 4:1) to obtain almost pure **10a** (34.5 g, 120 mmol) as a viscous oil in 86.9% yield. **10b** was prepared from **9b** in the same manner. **10a**: Anal. Calcd for $C_{17}H_{21}NO_3$: C, 71.05; H, 7.37; N, 4.87. Found: C, 71.13; H, 7.25; N, 4.90. **10a** and **10b**: IR ν cm^{-1} ($CHCl_3$); 3438, 1714, 1504, 1218, 1023. NMR δ ppm ($CDCl_3$): 1.12-1.74 (m, 7H), 1.92 (s, 1H), 2.30-2.56 (m, 2H), 2.72 (d of ABq, 1 part, J = 5.2, 17.4 Hz, 1H), 3.36-3.59 (m, 1H), 4.91-5.18 (m, 1H), 5.08 (s, 2H), 7.28-7.40 (m, 5H), 9.73 (s, 1H).

(+)-[1S-[1α,2α(Z),3β,4α]]-7-[3-[[(Phenylmethoxy)carbonyl]amino]bicyclo[2.2.1]hept-2-yl]-5-heptenoic acid methyl ester (**11a**)

To a stirred suspension of (4-carboxybutyl)triphenylphosphonium bromide (73.1 g, 165 mmol) in anhydrous tetrahydrofuran (1500 ml) was added portionwise potassium tert-butoxide (33.3 g, 297 mmol) at 0°C under nitrogen atmosphere. After stirring for 1 hr, a solution of aldehyde **10a** (15.8 g, 55 mmol) in anhydrous tetrahydrofuran (80 ml) was added dropwise over a period of 8 min at -15°C, and the mixture was stirred for 30 min under nitrogen atmosphere. The reaction mixture was adjusted to pH 7 by addition of 2 N hydrochloric acid and concentrated *in vacuo* below 30°C to about 500 ml. The resulting mixture was diluted with water (2000 ml), acidified to pH 2 by addition of diluted hydrochloric acid and extracted with ethyl acetate (500 ml). The extract was washed with water, dried with magnesium sulfate and evaporated *in vacuo*, leaving a viscous oil as a residue which was dissolved in n-hexane (100 ml) and passed through a column of silica gel (Merck No. 7734, 140 g; elution with n-hexane-ethyl acetate 1:1). The eluent was evaporated *in vacuo* below 30°C, leaving crude (**11'a**) (18.4 g, 49.5 mmol, ratio of Z and E = 96.7:3.3) as a colorless viscous oil which was esterified with diazomethane in the usual manner to give crude methyl ester **11a** (18.9 g, 49 mmol). The crude methyl ester was chromatographed on silica gel (Merck No. 7734, 250 g; elution with n-hexane-ethyl acetate = 4:1). The fractions containing the ester were combined and evaporated *in vacuo* below 30°C, leaving an ester **11a** (17.3 g, 45 mmol, purity 96.7%) in 81.8% yield.

Further purification of ester **11a** (Elimination of E-isomer)

For further purification of **11a**, the ester **11a** (purity, 96.7%; impurity = E-isomer 3.3%) obtained above was changed into an acid by saponification and purified by preparative HPLC as follows. To a stirred solution of **11a** (10 g, 26 mmol, purity 96.7%)

in methanol (200 ml) was added dropwise 1 N potassium hydroxide (52 ml, 52 mmol) and the resulting mixture was stirred for 5 hr at room temperature. After being concentrated *in vacuo* below 30°C to about 60 ml, the mixture was poured into water (100 ml), and washed with ethyl ether to remove neutral impurities, then acidified to pH 2 and extracted with ethyl acetate (200 ml). The extract was washed with aqueous sodium chloride, dried with magnesium sulfate and evaporated *in vacuo* below 30°C, leaving a viscous oil (9.9 g) which was purified by preparative HPLC (column, YMC ODS-15, $\phi 30 \times 250$ mm; mobile phase, CH_3CN-MeOH-H_2O-AcOH = 300:200:300:1; 37 ml/min; detector UV 220 nm). The fractions containing pure acid **11'a** were combined, concentrated *in vacuo* below 30°C to about half the original volume and extracted with ethyl acetate. The extract was washed with water, dried with magnesium sulfate and evaporated *in vacuo* below 30°C, leaving a crystalline residue which was purified by recrystallization from n-hexane-ethyl ether to give **11'a** (6.70 g, 18 mmol, purity 99.5%), as prisms in 65% yield. Purified acid **11'b** (3.5 g, 9.43 mmol) was esterified again with diazomethane to give **11a** (3.63 g, 9.43 mmol, purity 99.5%) as a viscous oil. **11b** was prepared from **10b** and purified in the same manner described above. **11'a**: m.p. 71-72°C. Anal. Calcd for $C_{22}H_{29}NO_4$: C, 71.13; H, 7.87; N, 3.77. Found: C, 71.07; H, 7.95; N, 3.95. $[\alpha]_D^{24}$ +29.2 ± 0.7 (c = 1.006, $CHCl_3$). **11'a** and **11'b**: IR ν cm^{-1} ($CHCl_3$): 3455, 3400-2400 (br), 1710, 1508. NMR δ ppm ($CDCl_3$): 0.82-1.04 (m, 1H), 1.07-1.34 (m, 2H), 1.34-1.80 (m, 6H), 1.88-2.23 (m, 5H), 2.24-2.48 (m, 3H), 3.32-3.58 (m, 1H), 4.74-4.96 (m, 1H), 5.09 (s, 2H), 5.26-5.46 (m, 2H), 6.50-9.80 (br, 1H), 7.28-7.43 (m, 5H). **11'b**: m.p. 71.5-72°C. Anal. Calcd for $C_{22}H_{29}NO_4$: 71.13; H, 7.87; N, 3.77. Found: C, 70.89; H, 7.86; N, 3.97. $[\alpha]_D^{24}$ -29.6 ± 0.7 (c = 1.004, $CHCl_3$). **11a**: Anal. Calcd. for $C_{23}H_{31}NO$: C, 71.66; H, 8.11; N, 3.63. Found: C, 71.72; H, 8.08; N, 3.83. **11a** and **11b**: IR ν cm^{-1} ($CHCl_3$): 3440, 1717, 1504, 1221, 1020. NMR δ ppm ($CDCl_3$): 0.84-0.99 (m, 1H), 1.08-1.30 (m, 2H), 1.35-1.77 (m, 6H), 1.90-2.15 (m, 5H), 2.31 (t, J = 7.3 Hz, 2H), 2.44 (s, 1H), 3.40-3.58 (m, 1H), 3.65 (s, 3H), 4.80-4.95 (m, 1H), 4.80-4.95 (m, 1H), 5.08 (ABq A-part, J = 13 Hz, 1H), 5.09 (ABq A-part, J = 13 Hz, 1H), 5.09 (ABq B-part, J = 13 Hz, 1H), 5.27-5.47 (m, 1H), 7.30-7.44 (m, 5H).

(+)-[1R-[1α,2α(Z),3β,4α]]-7-(3-Aminobicyclo[2.2.1]hept-2-yl)-5-heptenoic acid methyl ester (**12a**)

A mixture of ester **11a** (3.63 g, 9.43 mmol), anisole (15 ml) and trifluoroacetic acid

(50 ml) was stirred for 5.5 hr at 45°C. After removal of volatiles *in vacuo* below 35°C, the resulting mixture was diluted with ether (100 ml) and then extracted with 2 N sulfuric acid (100 ml). The aqueous solution was washed with ethyl ether and made alkaline at 0°C by addition of potassium carbonate and extracted with ethyl acetate (100 ml). The extract was washed with aqueous sodium chloride, dried with magnesium sulfate and evaporated *in vacuo* below 30°C to obtain almost pure 12a (1.76 g, 7.01 mmol) as a colorless viscous oil in 74.3% yield. IR ν cm^{-1} ($CHCl_3$): 3200 (br), 1726, 1435, 1210. NMR δ ppm ($CDCl_3$): 0.73-0.88 (m, 1H), 1.19-1.80 (m, 10H), 1.84-2.18 (m, 6H), 2.33 (t, J = 7.4 Hz, 2H), 2.66-2.79 (m, 1H), 3.67 (s, 3H), 5.30-5.55 (m, 2H).

[U-^{14}C]Benzenesulfonyl chloride (14)

Cold fuming sulfuric acid (30% fuming sulfuric acid-conc sulfuric acid 1:5, 0.6 ml) was added to [U-^{14}C]benzene (100 mCi, 130 mg, 1.67 mmol) (5) in a small test tube at 0°C and stirred for 2.5 hr at 60°C. Next, thionyl chloride (1.44 ml, 19.8 mmol) was added dropwise, through a capillary tube, over a period of 10 min to the mixture at room temperature. The stirring was continued at 60°C until the initial turbidity cleared and gas evolution ceased. After being cooled to -50°C, cold water (3.5 ml) and ether (4 ml) were added to the mixture, which was allowed to come to 0°C with stirring in an ice bath. The mixture was extracted with ether (15 ml×2). The extracts were washed with cold water (3 ml), cold 2.5% sodium bicarbonate aqueous solution (5 ml×2), and cold aqueous sodium chloride (5 ml) successively, dried with sodium sulfate and evaporated *in vacuo* (15 mmHg) below 30°C, leaving a colorless mobile oil (100 mCi, 297 mg) as almost pure 14. 14 was identified with unlabelled benzenesulfonyl chloride by comparison of IR spectra. The residual oil was used for the next reaction without further purification.

(+)-[1R-[1α,2α(Z),3β,4α]]-7-[3-[([U-^{14}C]Phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-yl]-5-heptenoic acid methyl ester (15)

To a stirred solution of 12a (604 mg, 2.5 mmol), triethylamine (170 mg, 1.68 mmol) and dimethylaminopyridine (0.3 mg) in anhydrous dichloromethane (2.5 ml) was added dropwise a solution of [U-^{14}C]benzenesulfonyl chloride (14) (100 mCi, 297 mg, 1.67 mmol) in anhydrous dichloromethane (1.5 ml) at 0°C. After stirring for 2 hr at room temperature, the mixture was poured into cold 2% sulfuric acid (20 ml) and extracted with ethyl ether (20 ml×2). The extracts were washed successively with water (10 ml),

cold 2% sodium bicarbonate (10 ml), water (10 ml) and aqueous sodium bicarbonate (10 ml), then dried with sodium sulfate and evaporated *in vacuo* below 30°C, leaving a colorless viscous oil as a residue (727 mg), which was chromatographed on silica gel (Merck Lobar column size B; elution with benzene-ethyl acetate) to obtain almost pure sulfonamide 15 (96 mCi, 627 mg, 1.60 mmol, radiochemical purity 99.6%) in 96% radiochemical yield. 15 was identified using the unlabelled authentic sample by comparison of the HPLC and TLC data shown in Tables 1 and 2.

(+)-[1R-[1α,2α(Z),3β,4α]]-7-[3-[([U-^{14}C]Phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-yl]-5-heptenoic acid (16)

To a stirred solution of methyl ester 15 (96 mCi, 627 mg, 1.60 mmol) in methanol (7.0 ml) was added 1 N sodium hydroxide (3.2 ml). After stirring for 21 hr at room temperature, the mixture was concentrated *in vacuo* below 25°C to about 3.5 ml, poured into water (10 ml) and washed with ethyl ether (20 ml×2) in order to remove neutral impurities. To the resulting aqueous alkaline solution, unlabelled 16 (2.16 g, 5.74 mmol, purity 99%) was added as a carrier. The mixture was acidified to pH 2.5 by addition of 1 N sulfuric acid and extracted with ether (30 ml×2). The extracts were washed with water (15 ml ×2), dried with sodium sulfate and evaporated *in vacuo* below 25 °C to give free acid 16 (85.8 mCi, 2.7 g, 7.17 mmol, radiochemical purity 99.6%, 12 mCi/mmol) as a crystalline residue. In order to minimize the decomposition caused by self radiolysis, the specific radioactivity of 16 obtained above was adjusted to 8.73 mCi/mmol by addition of the carrier (1.0 g, 2.65 mmol) and an equivalent molar amount of 1 N sodium hydroxide (9.82 ml). 16 was stored at -20°C as the sodium salt methanol-water (4:1) solution. This 16 (sodium salt: 85.8 mCi, 3.92 g, 9.82 mmol, 8.73 mCi/mmol, 1.72 mCi/ml, 78.4 mg/ml) was identified using the unlabelled authentic sample by comparison of the HPLC and TLC data shown in Tables 1 and 2.

(+)-[1R-[1α,2α(Z),3β,4α]]-7-[3-[([U-^{14}C]Phenylsulfonyl)amino]bicyclo[2.21]hept-2-yl]-5-heptenoic acid calcium salt (2:1) dihydrate (3a)

A solution of sodium salt (24 mCi, 1.17 g, 2.94 mmol) of 16 in methanol-water (4:1, 15 ml) obtained above was evaporated *in vacuo* below 20°C almost to dryness, leaving a white crystalline residue. To the solution of the residue in water (13 ml), a solution of calcium chloride (327 mg, 2.95 mmol) in water (4 ml) was added dropwise at room temperature with vigorous stirring. The stirring was continued for 2 hr at room

temperature for complete salt exchange. After being cooled to 0°C, the precipitated calcium salts were collected by filtration. The calcium salts were washed with water (2 ml×3), ethyl ether (5 ml×3) and dried *in vacuo* over phosphorus pentoxide at room temperature to a constant weight and allowed to stand under atmospheric air to have two equivalent mol of water finally give almost pure **3a** (23.2 mCi, 1.126 g, 1.42 mmol, radiochemical purity 96.7%, 20.55 μCi/mg, 17.0 mCi/mmol), d.p. above 300°C as a white powder in 99% yield. **3a** was identified using the unlabelled authentic sample by comparison of the HPLC and TLC data shown in Tables 1 and 2 and IR spectra determination.

(+)-[1R-[1α,2α(Z),3β,4α]]-7-[3-[([4-^{3}H]Phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-yl]-5-heptenoic acid methyl ester (**18a**)

To a stirred solution of amine **12a** (27 mg, 0.108 mmol) in anhydrous benzene (2 ml) were added triethylamine (42 μl, 0.30 mmol), dimethylaminopyridine (0.3 mg), and a solution of [4-^{3}H]benzenesulfonyl chloride (**17**) (100 mCi, 29 Ci/mmol, 0.6 mg, 0.00345 mmol) (4) in benzene (20 ml). The mixture was concentrated *in vacuo* (70 mmHg) at 35°C to 2 ml by distillation and stirred for 4.0 hr at room temperature. Next, the reaction mixture was evaporated *in vacuo* below 25°C, leaving a viscous oil as a residue which was purified by chromatography on silica gel (Merck No. 7734, 900 mg; elution with benzene-ethyl acetate 9:1). The fractions containing the desired pure [^{3}H]-labelled compound were combined and evaporated *in vacuo* below 30°C to obtain pure amide **18a** (34 mCi, 0.5 mg, 0.00129 mmol) as an oil in 34% radiochemical yield. The product **18a** was identified with unlabelled authentic sample by comparison of the HPLC and TLC data shown in Tables 1 and 2.

(+)-[1R-[1α,2α(Z),3β,4α]]-7-[3-[([4-^{3}H]Phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-yl]-5-heptenoic acid, [^{3}H]-(+)-S-145 (**2a**)

A mixture of **18a** (34 mCi, 0.5 mg, 0.00129 mmol) and 1 N-sodium hydroxide (0.1 ml, 0.1 mmol) in methanol (0.8 ml) was stirred for 15 hr at room temperature. The mixture was evaporated *in vacuo* below 25°C, leaving crude sodium salt of **2a** as a crystalline solid which was purified by chromatography (Waters SEP PAK C-18; elution with water-ethanol = 4:1). The fractions containing the desired pure [^{3}H]-labelled compound were combined and evaporated *in vacuo* to dryness below 25°C, leaving **2a** (31.9 mCi, 0.483 mg, 26.35 Ci/mmol, radiochemical purity 99.2%) as a sodium salt in

93.8% yield. In order to minimize the decomposition caused by self radiolysis, the product was dissolved in 95% ethanol, and the concentration of 2a was adjusted to 1.0 mCi/ml. 2a was identified using the unlabelled authentic sample by comparison of the TLC and HPLC data shown in the Tables 1 and 2.

(−)-[1S-[1α,2α(Z),3β,4α]]-7-[3-[([4-^{3}H]Phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-yl]-5-heptenoic acid methyl ester (18b)

To a stirred solution of (−)-[1S-[1α,2α(Z),3β,4α]]-7-(3-aminobicyclo[2.2.1]hept-2-yl)-5-heptenoic acid methyl ester (12b) (40 mg, 0.18 mmol) in anhydrous benzene (2 ml) were added triethylamine (20 mg, 0.2 mmol) and a solution of [4-^{3}H]benzenesulfonyl chloride (17) (100 mCi, 23.7 Ci/mmol) (4) in anhydrous benzene (11.5 ml). After being concentrated *in vacuo* (60 mmHg) at 35°C to 2 ml. The mixture was stirred for 2.5 h at room temperature. The reaction mixture was evaporated *in vacuo* below 25°C, leaving a viscous oil, which was purified by chromatography on silica gel (Merck No. 7734, 900 mg; elution with benzene-ethylacetate 9:1) to give pure 18b (80 mCi, 1.2 mg, 0.0033 mmol) as a viscous oil in 80% radiochemical yield. 18b was identified using the unlabelled authentic sample by comparison of the HPLC and TLC data shown in Tables 1 and 2.

(−)-[1S-[1α,2α(Z),3β,4α]]-7-[3-[([4-^{3}H]Phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-yl]-5-heptenoic acid, [^{3}H]-(−)-S-145 (2b)

Table 1. TLC Data

Compound No.	Solvent system	Rf value
1a, 1b, 2a, 2b, 3a, 16 and authentic sample (St.)	Cf : MeOH = 95 : 5	0.31
5a and 5b	unstable on TLC plate	
6a and 6b	n-Hexane : AcOEt = 4 : 1	0.30
7a and 7b	n-Hexane : AcOEt = 1 : 1	0.46
8a and 8b	n-Hexane : AcOEt = 4 : 1	0.24
9a and 9b	n-Hexane : AcOEt = 4 : 1	0.42
10a and 10b	n-Hexane : AcOEt = 4 : 1	0.22
11a and 11b	n-Hexane : AcOEt = 4 : 1	0.35
11'a and 11'b	CH_2Cl_2 : MeOH = 50 : 1	0.22
12a and 12b	CH_3CN : AcOH : H_2O = 15 : 1 : 1	0.46
14 and 17	unstable on TLC plate	
15, 18a, 18b and St.	C_6H_6 : AcOEt = 7 : 1	0.35

St. = corresponding authentic sample, Cf = chloroform.

Table 2. HPLC Data

Compound No.	Column	Mobile phase $CH_3CN : MeOH : H_2O : AcOH$	Retention time (min)
1, 1a and 2a	A	300 : 200 : 500 : 1 (1.5 ml/min)	11.80
1b and 2b	A	300 : 200 : 500 : 1 (1.5 ml/min)	11.80
3 and 3a	A	300 : 200 : 500 : 1 (1.5 ml/min)	11.80
E-isomers of S-145	A	300 : 200 : 500 : 1 (1.5 ml/min)	14.30
11'a and its unlabelled authentic sample (St.)	B	300 : 200 : 350 : 1 (2.0 ml/min)	11.30
E-isomer of 11'a	B	300 : 200 : 350 : 1 (1.5 ml/min)	12.42
15 and St.	A	300 : 200 : 300 : 1 (1.5 ml/min)	7.36
E-isomer of 15	A	300 : 200 : 300 : 1 (1.5 ml/min)	8.31
16 and St.	A	300 : 200 : 500 : 1 (1.5 ml/min)	11.80
18a, 18b and St.	A	300 : 200 : 300 : 1 (1.5 ml/min)	7.36

Column A = Nucleosil $5C_{18}$, ϕ4.6 mm×150 mm, Column B = Cosmosil $5C_{18}$, ϕ4.6 mm×150 mm, UV: 220 nm. St. = corresponding authentic sample.

A mixture of 18b (80 mCi, 1.21 mg, 0.0033 mmol) and 1 N-sodium hydroxide (0.1 ml, 0.1 mmol) in methanol (0.8 ml) was stirred for 15 hr at room temperature. The mixture was evaporated *in vacuo* below 25°C, leaving a crude sodium salt as a crystalline residue, which was purified by chromatography (Waters SEP PAK C-18; elution with ethanol-water 1:4). The fractions containing the desired pure compound were combined and evaporated *in vacuo* to dryness at 25°C to give 2b as the sodium salt (71.5 mCi, 1.09 mg, 0.00297 mmol, 24.07 Ci/mmol, radiochemical purity 99.9%) in 89.3% radiochemical yield. In order to minimize the decomposition caused by self radiolysis, the product was dissolved in 95% ethanol, and the concentration of 2b was adjusted to 1.0 mCi/ml. 2b was identified using the unlabelled authentic sample by comparison of the HPLC and TLC data shown in the Tables 1 and 2.

REFERENCES AND NOTES

1. Narisada M., Ohtani M., Watanabe F., Uchida K., Arita H., Doteuchi M., Hanasaki K., Kakushi H., Otani K. and Hara S. — J. Med. Chem. *31*: 1847 (1988).

2. Narisada M., Ohtani M., Watanabe F., Uchida K., Arita H., Doteuchi M., Ueda M., Hanasaki K., Kakushi H., Otani K., Hara S. and Nakajima M. — Advances in Prostaglandin, Thromboxane and Leukotriene Research *19*: 659 (1989). Kurosawa A., Arita H., Doteuchi M., Ueda M. and Uchida K. — Proceedings of the International Symposium on Biosignalling in Cardiac and Vascular Systems, 5-7 September 1988, Kyoto, Japan, Edited by Fujiwara M., Narumiya S. and Miwa S. (Pergamon Press) Section 8, 410.
3. Ohtani M., Matsuura T., Watanabe F. and Narisada M. — J. Org. Chem. *56*: 2122 (1991).
4. [4-^{3}H]Benzenesulfonyl chloride benzene solution was purchased from Amersham International plc. (Lot No. TRQ 5265 and TRQ 5437).
5. [U-^{14}C]Benzene was purchased from NEN/DuPont (Lot No. 2558-250) for 12a.