DIMETHYL 3,3,4,4,5,5,6,6-($^2H_8$)-6-METHOXYCARBONYL-2-OXO--HEXANEPHOSPHONATE, A VERSATILE REAGENT FOR THE SYNTHESIS OF DEUTERATED ω-CARBOXY PROSTANOIDS

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SUMMARY

An efficient preparation of the title compound 1a starting from propargyl alcohol and proceeding via deuterated monomethyl adipate (8a) is described. As demonstrated by the first total synthesis of deuterated ω-carboxy-thromboxane B_2 (13a), 1a may serve as a versatile starting compound for the synthesis of various ω-carboxy prostanoic acid metabolites.

Key Words: catalytic deuteration, prostaglandins, thromboxanes, adipic acid monomethyl-ester

INTRODUCTION

Studies on the in vivo transformation of infused prostaglandin F_{1a} (1,2), prostaglandin $E_{1,2}$ (3-5), prostaglandin D_2(6,7), prostacyclin I_2 (8,9), and thromboxane B_2(10) have shown that these prostanoids are metabolized in man (as well as in various animal species) to their corresponding ω-carboxy metabolites I-V (Scheme 1) which occur in plasma or are excreted predominantly in urine.

Deuterium labelled major metabolites of prostanoids were required as internal standards for the gas chromatographic-mass spectrometric quantification of endogenous prostanoids in biological samples (11). To enable the chemical synthesis of these metabolites, a deuterated phosphonate such as 1a is required which permits the introduction of the lower side chain. The requisite compound 1a could serve as a common reagent for the Wittig-Horner condensation with the corresponding aldehydes (Y=CHO, Scheme 1) to provide a variety of deuterated ω-carboxy metabolites of prostanoids.

RESULTS

The synthesis of 1a starts from the readily available diynediol 2 (Scheme 2) (12). The hydroxyl groups of 2 were protected by tetrahydropyranyl groups (13), the resultant

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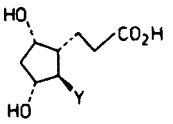

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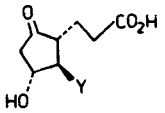

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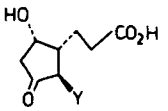

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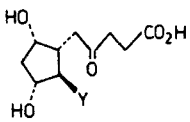

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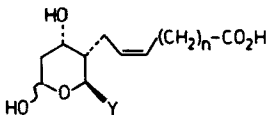
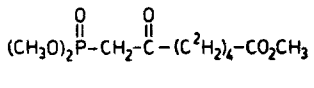
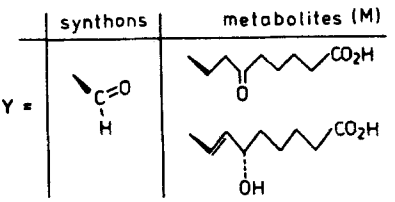


V (TXB_2-M)
n=1,3

Scheme 1



1a



compound 3 was saturated using molecular deuterium gas and Wilkinson's catalyst (14), and the protective groups were then removed by treatment with methanol in the presence of a polymer supported sulphonic acid (15). Subsequent oxidation with nitric acid (16) yielded pure octadeuterated adipic acid 6a in 71% overall yield (based on 2). A portion of the acid 6a was converted to the diester 7a(methanol, 2,2-dimethoxypropane, acid catalyst). The desired labelled monoester 8a was obtained in 85% yield by simple heating of a 2:1 mixture of 6a and 7a for four days at about 210°C. The title phosphonate 1a was prepared from 8a in two steps. Chemoselective cleavage of the methyl ester of 8a with excess lithium salt of dimethyl methanephosphonate followed by esterification of intermediate 9a with ethereal diazomethane completed the synthesis of 1a.

$R\text{-}O\text{-}CH_2\text{-}C{\equiv}C\text{-}C{\equiv}C\text{-}CH_2\text{-}O\text{-}R$

2 : R=H

3 : R=THP

$R^1O_2C\text{-}(CX_2)_4\text{-}CO_2R^2$

6a,b : $R^1{=}R^2{=}H$

7a,b : $R^1{=}R^2{=}CH_3$

8a,b : $R^1{=}H, R^2{=}CH_3$

$R\text{-}O\text{-}CH_2(CX_2)_4\text{-}CH_2\text{-}O\text{-}R$

4a,b : R=THP

5a,b: R=H

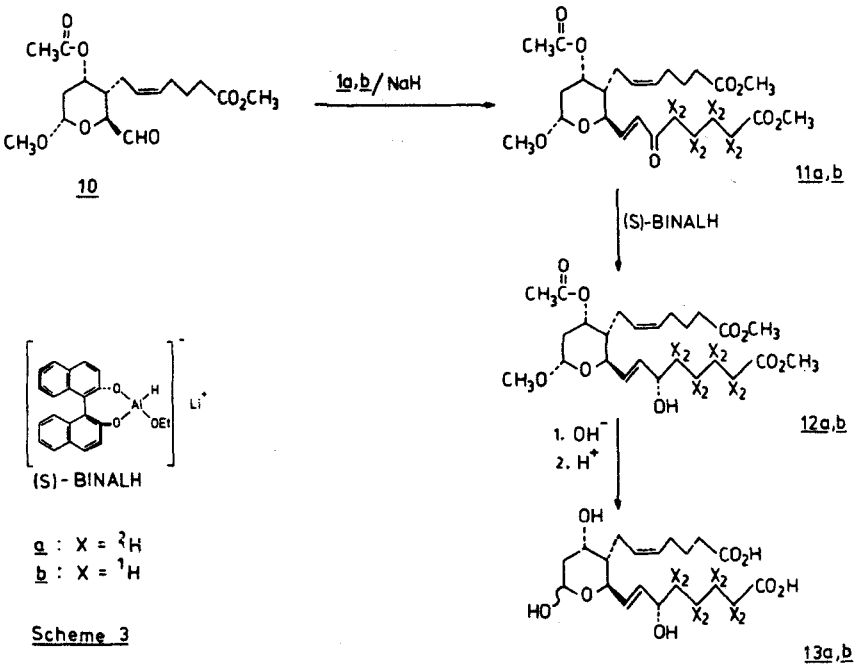
$(CH_3O)_2\overset{O}{\overset{\|}{P}}\text{-}CH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}(CX_2)_4\text{-}CO_2R$

9a,b : R=H

1a,b : $R{=}CH_3$

Scheme 2, a : X = ^2H ; b : X = ^1H

The use of the new labelled building block 1a in a new total synthesis of a thromboxane metabolite is depicted in Scheme 3.



Scheme 3

In analogy to previously described syntheses of other thromboxanes (17-21), the chiral aldehyde 10 (22) was condensed with the sodium salt of 1a. Highly selective 1,2-reduction of the enone 11 with the chiral reducing agent S-BINALH (23,24), yielded the allylic alcohol 12a with the natural 15S configuration (25) (90% yield, 98% 15S). Subsequent hydrolysis of the ester and acetoxy groups ($LiOH/H_2O$) and cleavage of the methoxy group (H_3PO_4) furnished the product 13a which was purified by chromatography and characterized spectroscopically. The above sequence represents the first chemical total synthesis (12) of the labelled (26) ω-carboxy metabolite 13a of thromboxane B_2 (ω-carboxy-TXB_2 or (25) 20-nor-19-carboxy-TXB_2).

DISCUSSION

Acetylenic compounds have been shown to be useful precursors for the introduction of the deuterated lower (27-29) or upper (30) side chains of primary prostaglandins. However, as a result of allenic rearrangements, the attempted direct (heterogeneous or homogeneous) deuteration of 2 led to a number of carbonyl-containing side products and gave only poor yields of 5a. Excellent results were obtained when 2 was first protected as its tetrahydropyranyl (THP) ether. After chromatographic removal of some trace impurities which were found to poison the catalyst, homogeneous deuteration (31,32) using Wilkinson's catalyst $(Ph_3P)_3RhCl$ (14) essentially as described recently (29) and simple protective group cleavage afforded highly labelled 5a (14% $^2H_7$, 85% $^2H_8$). A crucial step in the synthesis of

1a was the selective functionalization of the dicarboxylic acid 6a (33a,b). Although various methods have been described for the preparation of monoalkyl adipates (e.g. selective esterification of 6b or partial saponification of 7b), the published procedures (34-38) could not be reproduced in the present small-scale runs. Finally, autocatalytic transesterification of a suitable mixture of 6a and 7a led to the desired monoester 8a. Thus, the useful intermediate 8a (39) was obtained in a 50% total yield over six steps from 2.

In the course of the chemical total synthesis of some unlabelled ω-carboxy prostaglandins, the lower side chain was introduced by means of appropriate phosphonates (41,42) or phosphoranes (43). The newly developed method for the preparation of the title compound 1a seems to be more suitable and convenient with respect to yield, economy of the deuterium introduced, and reactivity in the subsequent step (44). Further transformations of the enone 11a and protective group removal finally resulted in the first chemical total synthesis of the desired metabolite 13 (12). The unlabelled analogue has recently been identified after incubation of TXB_2 with lung and liver microsomes from pregnant rabbits (46).

As a consequence of the different and, in part, rather drastic reaction conditions employed in the synthesis of 1a, an inevitable loss of deuterium is detected in 13a by mass spectrometry (1.2 % $^2H_4$, 5.2 % $^2H_5$, 22.4 % $^2H_6$, 34.2 % $^2H_7$,33.8 % $^2H_8$, 3.2 % $^2H_9$). However, after suitable derivatization of 13a and by using appropriate recording techniques, a mass spectrum is obtained which exhibits complete retention of the introduced deuterium in high-mass fragments. As shown by selected ion monitoring (SIM) in the range of the molecular ion the amount of unlabelled material ($^2H_0$) as compared to the $^2H_8$ species is less than 0.2 %. Thus, 13a can serve as a low-blank internal standard in the mass spectrometric stable isotope dilution trace analysis of ω-carboxy-thromboxane B_2 (13b) in biological samples (47).

ACKNOWLEDGEMENT

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EXPERIMENTAL SECTION

TLC: SiO_2 60 F_{254} (E.Merck "Fertigplatten"); solvent systems for TLC and column chromatography: (I), EtOAc/n-hexane (2:3); (II), EtOAc/n-hexane (1:4); (III) EtOAc/MeOH (8.5:1.5); (IV), EtOAc; (V) EtOAc/n-hexane (3:1); (VI), EtOAc/AcOH (98:2); (VII), EtOAc/MeOH/AcOH(90:10:2); (VIII), EtOAc/n-hexane (1:5); (IX), EtOAc/n-hexane (1:2); (X), EtOAc/n-hexane (1:1).- Melting points (m.p.), uncorrected (Electrothermal).-IR(KBr/Film, cm^{-1}): Perkin-Elmer 283, only intense or characteristic absorptions are reported.-NMR(80

MHz/^{1}H,20 MHz/^{13}C-proton-decoupled): Bruker WP 80(24°C), *unless otherwise indicated* solvent $CDCl_3$, int.std. TMS, shifts in ppm (δ scale).- MS : Hewlett-Packard 5985A, fragments reported as m/z(rel.abund.,%).

2,4-Hexadiyne-1,6-diol, 2

Although it is commercially available (Fluka, CH-Buchs) this starting compound is readily prepared from propargyl alcohol as described (48-53), and obtained in 71-85% yield after recrystallization from boiling EtOAc under argon, m.p. 114°C (108-109°C(53),111-112°C (51)), TLC :R_f 0.21(I).
^{1}H-NMR($CDCl_3$):1.57(t,J=6.2Hz,1H,OH),4.36(d,J=6.2 Hz, 2H, OCH_2).-^{13}C-NMR($CDCl_3$):51.0 (C-1/6),69.2(C-3/4),79.7(C-2/5), assignment based on ref. (52). IR(KBr):1443, 1352, 1034, 912.

Bis-1,6-(tetrahydropyran-2-yloxy)-2,4-hexadiyne,3

To a mixture of 2(16.3g,0.15 mol, dried over P_2O_5), dry tetrahydrofuran (200 ml), and 35g(0.42 mol) of freshly distilled 2,3-dihydropyran were added at 0°C 0.1 ml of an anhydrous 10%(w/v) solution of p-toluenesulphonic acid in tetrahydrofuran. Stirring was continued first for 1 h at 0°C then for 24 h at room temperature. Excess solid anhydrous potassium carbonate was then added and stirring was continued for 7 h. The salts were filtered off, and the filtrate was evaporated to dryness. The yellow oily residue was dissolved in 50 ml of mobile phase (I) and filtered (protected from light) through a short column charged with 40g alumina (basic, TSC Woelm, lower layer) and 20 g SiO_2(TSC Woelm, upper layer). Elution with the same solvent system, collection of the appropriate fractions, evaporation and drying in vacuo (1 h, 50°C, 0.1 mm) left essentially pure (TLC,NMR) 3 in quantitative yield and suitable for the next step; 41.4 g, n_D^{20} 1.5170. The yellow oil solidifies after some weeks in the refrigerator, three crystallizations from n-pentane provide yellow crystals, m.p.53-56°C.TLC : R_f 0.78(I),R_f 0.47(II). Found C 68.31%, H 7.94%; calc. for $C_{16}H_{22}O_4$(278.3) C 69.04%, H 7.97%. The compound decomposes on exposure to light and upon attempted vacuum distillation.
^{1}H-NMR($CDCl_3$): 1.4-2.0(m,6H),3.3-4.0(m,2H),4.33(s,2H,OCH_2), 4.81(broad s,1H).-^{13}C-NMR ($CDCl_3$):acetylenic chain,54.5(C-1/6),70.2(C-3/4),75.5(C-2/5); THP-ether,19.1(C-3),25.4(C-4), 30.3(C-2),62.2(C-5),97.2(C-1), assignment based on ref.(29,54).IR(film): 2950,1445,1345,1205, 1120,1025,900,870,815.

2,2,3,3,4,4,5,5-($^{2}H_8$)-Bis-1,6-(tetrahydropyran-2-yloxy)-hexane, 4a

To a clear, vigorously stirred pre-deuterated solution of 5.1g tris(triphenylphosphine)-rhodium (I) chloride in 300 ml of acetone/benzene (3:2,v/v) was added at room temperature under a deuterium atmosphere in one portion 3 (20.2g, 72.5 mmol), dissolved in 50 ml of the same solvent mixture. After 20 h the solvents were distilled off, 100 ml of n-hexane was added and the resulting suspension was filtered through alumina (TSC Woelm, ca.50g). Elution with n-hexane, evaporation and drying (1 h, 50°C, 0.1 mm) left pure 4a, 20.2g, 95%, colourless oil, n_D^{20} 1.4633. TLC: R_f 0.80(I). Found C 65.41%, ^{1}H 7.53%, 2H 5.74%; calc. for $C_{16}H_{22}{}^{2}H_8O_4$(294.4)C 65.26%,^{1}H 7.53%,^{2}H 5.47%.-

^{1}H-NMR($CDCl_3$):1.3-2.0(m,6H),3.2-4.0(m,4H),4.49(broad s,1H).- ^{13}C-NMR($CDCl_3$):hexane chain, 67.5(C-1),CD_2 not detected; THP-ether,19.7(C-3),25.6(C-4),30.9(C-2),63.3(C-5),99.0 (C-1);unlabelled 4b:two additional signals at 25.7ppm and 29.8 ppm (C-2/5 and C-3/4).- IR(film):2950,2200,2100,1355,1200,1125,1035.-

2,2,3,3,4,4,5,5-($^2H_8$)-Hexane-1,6-diol, 5a.

A mixture of 4a(17.0g, 57.8 mmol), methanol (300 ml), and cation exchange resin (Dowex AG 50W-X8, 5 g) was stirred for 12h at room temperature. Filtration, evaporation, and drying (1 h, 0.1 mm) gave an oil (7.5 g) which gradually solidified in the refrigerator. Recrystallization from EtOAc/n-hexane (-40°C) provided colourless crystals, 6.7g (89%), m.p. 42-44°C. TLC: R_f 0.59 (III). Found C 57.04%, ^{1}H 4.77 %, ^{2}H 12.71%; calc. for $C_6H_6{}^2H_8O_2$(126.2) C 57.09%, ^{1}H 4.79%, ^{2}H 12.76%.-^{1}H-NMR($CDCl_3$): 1.37(broad s, 1H), 3.65 (broad s,2H).- ^{13}C-NMR($CDCl_3$):62.6(C-1/6); CD_2 not detected.- Unlabelled 5b: two additional signals at 25.6 ppm and 32.8 ppm. IR(KBr): 2900,2195,2080,1345,1035,960,930.-

The isotopic purity of 5a was determined from the mass spectra (EI/DIP) of the 1,6-bis-(dimethyl-tert.-butylsilyl) derivatives of 5a(M=354) and 5b (M=346) using the abundant $M^{+\cdot}$-57 fragment; 5a:$^2H_0$-$^2H_5$, not detected, $^2H_6 \leqslant 1\%$, $^2H_7$ 14%, $^2H_8$ 85%, $> {}^2H_8$ not detected. For comparison, the corresponding silyl derivative, prepared from heterogeneous deuteration (5% Rh/C,EtOAc) showed marked scrambling: $^2H_0$-$^2H_4$ 3%,$^2H_5$ 3%,$^2H_6$ 8%, $^2H_7$ 24%, $^2H_8$ 32%, $^2H_9$ 21%, $^2H_{10}$ 8%, $^2H_{11} \sim 1\%$.

2,2,3,3,4,4,5,5,-($^2H_8$)-Adipic acid, 6a

A solution of 5a (38.0g,0.30 mol) in 38 ml of distilled water was slowly added with stirring to 220g of 65% aqueous nitric acid. During the addition and for further 3h the temperature of the reaction was carefully maintained between 5°C and 10°C. The mixture was heated at 50-60°C for 15 min and then stored at -4°C for 12h. The precipitated crystals were collected, washed with a minimum of icewater, and recrystallized after the addition of charcoal from 80 ml of boiling water to give 6a (39.0g) in 84% yield, m.p. 148-150°C (ref. (33) m.p. 140-141°C). Found C 46.82%, ^{1}H+^{2}H 11.68; calc. for $C_6H_2{}^2H_8O_4$(154.2) C 46.74, ^{1}H+^{2}H 11.75%.-^{13}C-NMR(D_2O/NaOH, int. std. sodium 3-(trimethylsilyl)-propanoate-d_4): weak complex multiplets centered at 27.4 and 39.3,186.6($-CO_2^-$) (unlabelled 6b: two additional signals at 28.5 ppm(C-3/4) and 40.2 ppm (C-2/5)).- IR(KBr):1700,1410,1305,1180, 950,655.

Dimethyl 2,2,3,3,4,4,5,5-($^2H_8$)-adipate, 7a

A mixture of 15.0g (97.2 mmol) 6a, 2,2-dimethoxypropane (60ml), resin Dowex AG 50W-X8 (6.6g) and methanol (60ml) was stirred at room temperature. As the rate of esterification depends on the activity of the resin and the rate of stirring, the time course was monitored.

Briefly, mg-aliquots were withdrawn, filtered, evaporated, and redissolved in ethereal diazoethane (55). After evaporation the residue was dissolved in deuterochloroform and the ratio of methyl esters versus ethyl esters was determined by integration of the OCH_3 and OCH_2 resonance signals in the ^{1}H-NMR spectra. After 16-24 h the reaction was complete. Filtration, evaporation (at 30°C/17 mm) and finally vacuum distillation afforded pure 7a, 14.5g(82%), $n_D^{24.5}$ 1.4249, b.p. 55-58°C/0.01 mm, m.p. +7°C. Found C 52.49%, ^{1}H 3.30%, ^{2}H 8.90%; calc. for $C_8H_6{}^2H_8O_4$ (182.2) C 52.72%, ^{1}H 3.32%, ^{2}H 8.84%.- ^{1}H-NMR($CDCl_3$) 3.67(s,OCH_3).-^{13}C-NMR($CDCl_3$): weak multiplets centered at 23 ppm and 33 ppm, 51.6 (OCH_3),174.1 ($\underline{C}O_2CH_3$).-IR(film):2960,2220,2110,1740,1435,1270,1145,1080,1000.

Monomethyl 2,2,3,3,4,4,5,5-($^2H_8$)-adipate, 8a

11.7 g (75.9 mmol) of dry (P_2O_5) adipic acid 6a and 6.9 g (37.8 mmol) of dimethyl ester 7a were heated for 4 days at 205-215°C under protection from atmospheric moisture. The mixture was cooled to 0°C and the brown solidified mass was extracted with 100 ml of carbon tetrachloride. The crystals were filtered off, washed with cold carbon tetrachloride(2x20 ml) and dried in vacuo to yield 8.2 g (53 mmol) of essentially pure recovered 6a. The organic phase was evaporated (30°C/17 mm) to leave an oil (10.2 g) which consisted mainly of the desired monoester 8a (estimated by ^{1}H-NMR spectroscopy as described for 7a). Pure 8a (8.7 g, 52 mmol) was obtained after fractionation in vacuo, 85% (based on recovered 6a), $n_D^{24.2}$ 1.4373, b.p. 105-6°C,/0.02 mm, m.p. + 9°C. Found C 50.17%, ^{1}H 2.43%, ^{2}H 9.74%; calc. for $C_7H_4{}^2H_8O_4$(168.2) C 49.98, ^{1}H 2.40 %, ^{2}H 9.58%.- IR(film): 2945, 2210, 2100, 1733, 1703, 1430, 1272.- ^{1}H-NMR($CDCl_3$):3.67(s,OCH_3). - ^{13}C-NMR($CDCl_3$): weak multiplets centered at 23 ppm and 33 ppm; 51.7 (OCH_3), 174.4 ($\underline{C}O_2CH_3$),179.3($\underline{C}O_2H$). GC/MS (NI/CI;2,3,4,5,6-pentafluorobenzyl ester derivative, M=348): the isotopic composition taken from SIM tracings and corrected for natural abundance was calculated in comparison to the $M^{-\cdot}$-181 fragments of 8b (M=340), $^2H_0$-$^2H_4$ $\leqslant$ 0.2%; $^2H_5$ 1.7%; $^2H_6$ 6.3%; $^2H_7$ 19.7%; $^2H_8$ 72.1%; $^2H_9 \leqslant 0.1\%$; $^2H_0$ (referred to $^2H_8$) $\leqslant$ 0.1%.

Dimethyl 3,3,4,4,5,5,6,6,-($^2H_8$)-6-methoxycarbonyl-2-oxo-hexanephosphonate,1a

To a stirred solution of distilled dimethyl methanephosphonate (50g, 0.4 mol) in 800 ml of dry tetrahydrofuran was slowly (3 h) added at -78°C 250 ml(0.4 mol) of 1.6M n-butyllithium in n-hexane under an atmosphere of argon. The mixture was stirred at the same temperature for 2 h, then a solution of 16.8g(0.1 mol) of 8a in 150 ml of dry tetrahydrofuran was added. The resultant mixture was stirred for 16h at -78°C and then quenched at -10°C with 0.4 mol(100 ml) of 14% aqueous hydrochloric acid. The organic phase was separated and the aqueous phase was first saturated with solid sodium chloride and then extracted (5x200 ml) with dichloromethane. The combined extracts and organic phase were dried (Na_2SO_4) and evaporated to give 51.2g of a crude brown oil. 30.2g of this oil were dissolved in 50ml of methanol and treated with excess ethereal diazomethane at 0°C. The solvents were removed

on a rotary evaporator and the residual oil was distilled in vacuum (bath temperature 150-175°C). Pure product 1a was obtained as a pale yellow oil (12.8g,79%) at b.p. 138-140°C/0.02mm; $n_D^{24.5}$ 1.4527, found C 43.65%, $^1H + ^2H$ 10.16%, P 11.39%; calc. for $C_{10}H_{11}{}^2H_8O_6P$(274.3) C 43.80%, $^1H+^2H$ 9.91%, P 11.29%. IR(film): 2955,2205,2100,1725,1710,1433,1255,1025,810.-TLC : R_f 0.20(IV).- ^{1}H-NMR($CDCl_3$): 3.09(d,J=22.7 Hz,2H,PO-CH_2), 3.67 (s,3H,CO_2CH_3), 3.79(d,J=11.2 Hz,6H,$(CH_3O)_2PO$).-^{13}C-NMR($CDCl_3$): weak multiplets centered at 23,33,43 ppm, 41.4(d,J=126.9 Hz,C-1), 51.5 (CO_2CH_3), 53.1(d,J=6.1Hz, $(CH_3O)_2PO$),174.0(CO_2 CH_3),210.9(d,J=6.1 Hz, C-2); the carbons unlabelled in 1b resonate at 22.8,24.2,33.8, and 43.7 ppm.- MS(70eV,PI/EI,DIP). The mass spectra (m/z 100-350) and isotopic composition in comparison with 1b were determined from the O-tert.-butyloxime derivatives. 1a(M=354, clusters of fragments, most intense fragments reported): 289(30%), 271(28%),258(14%),239(27%), 229(30%),211(84%), 184(71%),167(25%),166(31%),151(59%),125(70%),109(100%). Deuterium distribution of 1a($M^{+\cdot}$-56,m/z 289):2.5% $^2H_5$, 9.9% $^2H_6$, 27.2% $^2H_7$,56.3% $^2H_8$, 3.8% $^2H_9$, $^2H_0$-$^2H_4$ $\leqslant$ 0.5%, $^2H_0$ (referred to $^2H_8$) $\leqslant$ 0.1%. 1b(M=337):281(32%),264(57%),250(15%),232(33%),221(28%), 204(100%), 181(97%), 164(46%), 150(53%), 124(85%), 109(68%).-

16,16,17,17,18,18,19,19-($^2H_8$)-20-nor-19-carboxy-thromboxane B_2,13a

The aldehyde 10(0.65g,1.9mmol) (20) was dissolved in dry 1,2-dimethoxyethane (DME,20ml) under argon and added rapidly at 0-6°C to a stirred solution of the sodium salt of 1a (prepared from 1.31 g 1a(4.8 mmol) and 200 mg NaH(55-60%, 4.6 mmol) in 60 ml of dry DME). After 1 h the reaction was quenched by slow addition of 2 M phosphate buffer (pH 7). The mixture was extracted with n-hexane and the dried (Na_2SO_4) organic phase rotoevaporated. The residual oil was then redissolved in a minimum of solvent mixture (VIII) and subjected to column chromatography on silica gel (Merck SiO_2-60,300g). Successive elution with solvent mixtures (VIII) (1 liter), (IX) (2 liters), and (X) (2 liters), collection of the appropriate UV-absorbing fractions, evaporation and drying in vacuum afforded the desired oily enone 11a (729mg, R_f 0.71 (V)) in 78% yield (based on 10), found C 61.03%, $^1H+^2H$ 9.43%; calc. for $C_{25}H_{30}{}^2H_8O_9$ (490.6) C 61.21%, $^1H+^2H$ 9.44%.

The chiral hydride reagent (S)-BINALH (23,24) was prepared under an argon atmosphere as follows. To 3ml 1 M $LiAlH_4$ in dry THF were added at 20°C during 5 min 3 ml of 1 M EtOH in dry THF. A solution of 859mg (3 mmol) (S)-(-)-2,2'-dihydroxy-1,1'-binapthyl in 7ml of dry THF was then slowly (20 min) added and stirring was continued for 45 min. The mixture was then cooled to -100°C and a solution of 299mg (0.61 mmol) 11a in 8ml of dry THF was added dropwise to the chiral hydride reagent. After 1h the temperature was raised to about -85°C, and 2.5 h later excess reagent was destroyed by addition of 3 ml of dry MeOH. After dilution with CH_2Cl_2(50ml) the mixture was poured in 400ml 0.2M phosphate buffer. The crude product and recovered binaphthol were isolated by extraction with CH_2Cl_2. After evaporation to about 10-20ml the bulk of the binaphthol was precipitated by addition of excess n-hexane and removed by filtration. Column chromatography (SiO_2-60, 130g) using first 700 ml

of a mixture of EtOAc/n-hexane (1:3, containing 0.3% Et_3N) and then 2 liters of EtOAc/n-hexane (2:3, containing 0.3% Et_3N) afforded complete separation of the 15-hydroxy epimers. The main fraction contained 266 mg of pure oily 12a (R_f 0.37(V), found C60.74%, $^1H+^2H$ 10.11%; calc. for $C_{25}H_{32}{}^2H_8O_9$(492.6) C 60.95%, $^1H+^2H$ 9.82%) together with 5.5mg of the epimeric 15R-alcohol (R_f 0.45(V)), the yield at this step was 90 %.

133mg(0.27mmol) of S-alcohol 12a was dissolved under argon in a mixture of MeOH (10ml) and 0.2 N aqueous LiOH (10ml). After 17 h at room temperature the reaction mixture was cooled (0°C) and then acidified to pH 2 with concentrated phosphate buffer (10ml), and extracted thoroughly with CH_2Cl_2. The combined extracts were washed with 80% aqueous NaCl, dried (Na_2SO_4) and evaporated to give 103 mg (91%) of essentially pure (TLC: R_f 0.38 (VI)) 11-O-methyl-ω-carboxy-thromboxane B_2 as an oil. 88 mg (0.21mmol) of the glycoside were dissolved in a mixture of THF(6ml), 85% H_3PO_4(0.5ml), and water (4.5ml). After stirring for 24 h under argon at 40°C, saturated aqueous NaCl (10ml) was added to the clear solution and the mixture was extracted several times with EtOAc. The combined extracts were washed with 80% aqueous NaCl, dried (Na_2SO_4), and evaporated to yield 75mg (88%, 80% yield from 12a) of chromatographically pure 13a as a colourless oil which gradually solidified, m.p. 65-69°C. TLC: R_f 0.18(VI), R_f 0.45(VII). In spite of several low-pressure column chromatographic runs (Merck Lobar SiO_2-60, solvent system (VI)) and careful drying (P_2O_5), elemental analysis of 13a (and 13b) suggested the presence of variable amounts of water. Nevertheless, mass spectra of various derivatives of 13a (and analogously prepared 13b) were in accordance with the data published for the corresponding derivative of biosynthetic 13b (46).

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