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DIMETHYL 3,4,5,6-[$^2H_4$] -2-OXOHEPTYLPHOSPHONATE : A READILY AVAILABLE REAGENT FOR THE PREPARATION OF DEUTERATED PROSTANOIDS- APPLICATION TO THE SYNTHESIS OF $^2H_4$-LABELLED (±)-PROSTAGLANDIN D_2

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SUMMARY: *Dimethyl 3,4,5,6-[$^2H_4$]-2-oxoheptylphosphonate (**1a**) was conveniently prepared in two steps from ethyl sorbate and used in a total synthesis of low-blank (≲ 0.2% ^2H_o) 16,17,18,19-[$^2H_4$]-prostaglandin D_2 (**2a**). The required allylic alcohol 15S-**8** was isolated by fractional crystallisation of the mixture of* R/S *epimers and then transformed to a key intermediate, 15S-silyloxy compound* **10**, *by regiocontrolled monosilylation of diol* **9**.

Key words: Deuteration, prostaglandins, prostaglandin D_2, dimethyl 3,4,5,6-[$^2H_4$]-2-oxoheptylphosphonate, nuclear magnetic resonance (^{13}C) spectroscopy.

INTRODUCTION

It is well established that deuterated analogues of eicosanoids (biologically relevant C_{20} carboxylic acids) are indispensable tools in tracer studies concerning biochemical transformations and metabolism. If used as internal standards, they are also extremely valuable in facilitating ultratrace analysis of the endogeneous unlabelled fatty acids in biological material by use of mass spectrometry. Amongst the various synthetic strategies employed in the preparation of deuterated eicosanoids, the approach of chemical total synthesis has been shown to be most effective in terms of versatility of deuterium introduction and convenience for bulk preparation[1]. In particular, the well elaborated "classical" prostaglandin methodology developed by *Corey* and coworkers[2,3] provides ready access to various prostanoids (prostanoic acid derivatives) as well as their deuterated analogues[4-7]. Along these lines an extremely convenient two-step synthesis of a new deuterated phosphonate reagent (**1a**) has been developed and will be described in this paper. The application of **1a** to the first chemical total synthesis of deuterated prostaglandin D_2 (PGD_2,**2a**) is also demonstrated.

RESULTS

As reported for other esters of saturated carboxylic acids[8,9], ethyl sorbate (**3**) readily reacts with the lithium salt of dimethyl methanephosphonate to give the known phosphonate **4**[10] in 64% yield after distillation (Scheme 1). Deuteration of diene **4** with molecular deuterium gas in the presence of Wilkinson's catalyst **5** accomplished the convenient two-step synthesis of the desired title compound dimethyl 3,4,5,6-[$^2H_4$] -2-oxoheptylphosphonate (**1a**). As judged from the mass spectra (see Experimental) the isotopic composition of the tetradeuterated product depended on the amount of catalyst used. The unlabelled species **1b** was below the instrumental detection threshold (≲0.1% ^2H_o referred to $^2H_4$).

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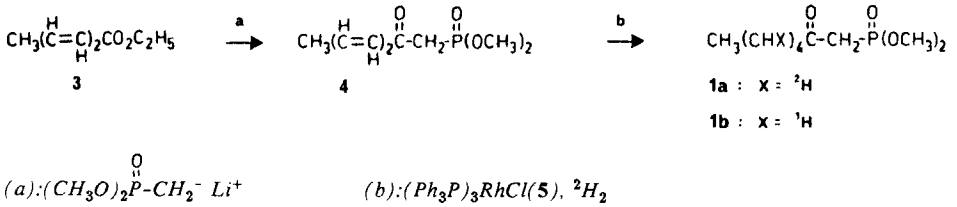
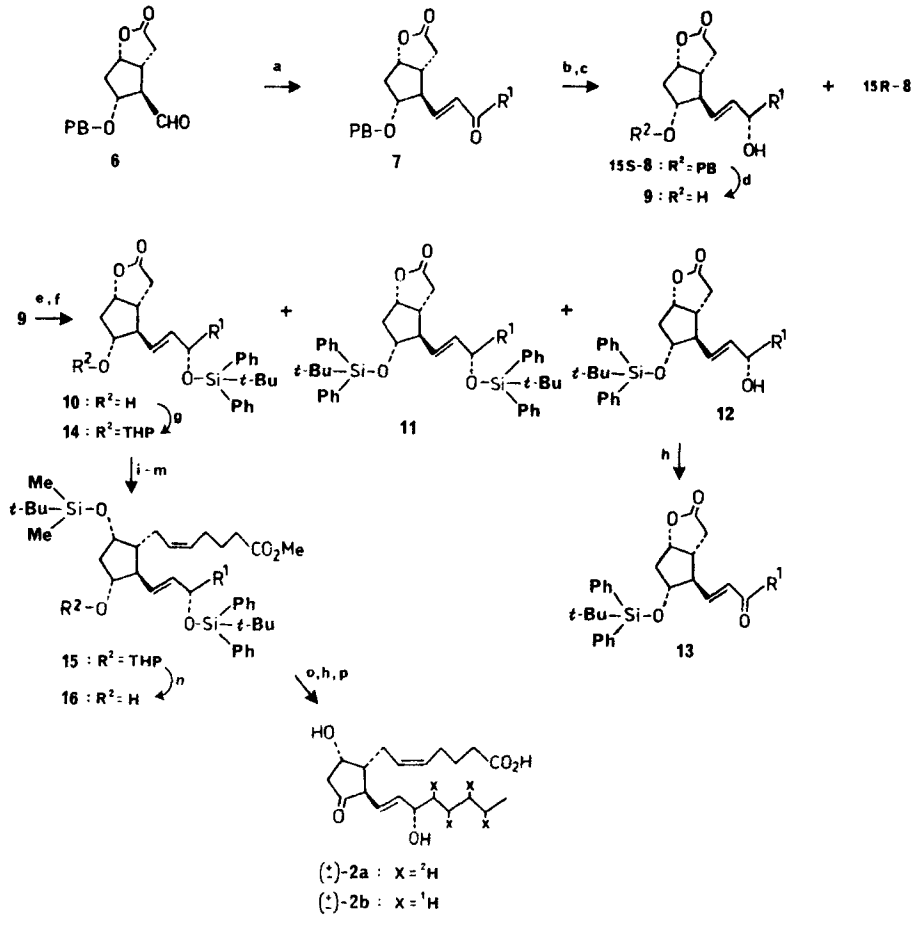
Scheme 1: *Synthesis of dimethyl 3,4,5,6-[$^2H_4$]-2-oxoheptylphosphonate (***1a***)*



Following the well elaborated prostaglandin methodology[2,4,6,11] the phosphonate reagent **1a** was condensed with Corey aldehyde **6** (Scheme 2). The resultant enone **7** was then reduced with sodium borohydride to give a ca. 1:1 mixture of epimeric allylic alcohols. The required 15*S* isomer of **8**[14] was either obtained by silica gel column chromatography or, more conveniently, by fractional crystallisation. Removal of the 4-phenylbenzoyl protective group ($MeOH/K_2CO_3$) afforded diol **9**, a valuable labelled key intermediate in general prostaglandin syntheses[2].
Diol **9** was then treated with 1 to 1.5 equivalents of *tert.*-butyldiphenylchlorosilane to yield a mixture of lactones **10**,**11**, and **12** in 69-97 % total yield. Due to their completely different chromatographic mobility, these new products were conveniently separated on a preparative scale by column chromatography using silica gel. However, while the disilyl compound **11** (oil) was readily identified by spectroscopic methods (not shown), a conclusive distinction between the two 11- and 15-monosilyl derivatives was not possible. Thus both the regioisomers **10** and **12** were then separately treated with manganese dioxide or excess pyridinium chlorochromate in dichloromethane. Whereas the major monosilyl species **10** which had been obtained in 38-42 % yield in the above mentioned silylation reactions ((e), Scheme 2) remained unchanged upon exposure to the oxidation reagent, the minor monosilyl derivative **12** (isolated in crystalline state, m.p. 112.5°C, and 4-6 % yield) was completely converted into a new and less polar compound which was readily identified as being **13** by ^{1}H-NMR spectroscopy on the basis of its characteristic olefinic enone signal resonance pattern. The major regioisomer therefore was the 15-silyl ether **10** which gave **14**, the starting material after protection with 3,4-dihydro-2*H*-pyran for the subsequent PGD_2 synthesis.

The following sequence of reaction steps leading to dextrorotatory PGD_2 was in complete analogy with the synthetic route of *Ogawa* et al. which has recently been published[15]. Briefly, reduction of the fully protected lactone **14** using di*iso*butylaluminium hydride, Wittig olefination ($Ph_3P{=}CH(CH_2)_3CO_2^-$) of the intermediate lactol followed by protection of the carboxylic acid (CH_2N_2) and hydroxy group (*tert.*-butyldimethylchlorosilane) led to the derivatised F-prostaglandin **15**. Removal of the 11-THP protective group proceeded with a degree of difficulty. Although it has been reported that anhydrous magnesium bromide is capable of cleaving THP ethers in the presence of different silyloxy groups[16], employing this reaction on **15** gave the desired alcohol **16** in only about 20 % yield. Consequently, the tedious procedure of thermal cleavage[15] was used (135°C, acetonitrile, 14 to 20 d). Thus, selective removal of the 11-tetrahydropyranyl group of **15**, saponification of methyl ester **16**, oxidation of the resultant

Scheme 2: *Synthesis of (±)-16,17,18,19-[$^2H_4$]-prostaglandin D_2,***2a**. *All structures shown refer to racemic material*[14].



PB: *4-$C_6H_5C_6H_4CO$-* R^1: *$CH_3(CH^2H)_4$-* THP: *tetrahydropyran-2-yl*

(a) *reagent* **1a** */ NaH*
(b) *BH_4^- / Ce^{3+}*
(c) *separation of 15*S- *and 15*R-**8**
(d) *MeOH/K_2CO_3*
(e) *ClSi(*t.-*Bu)Ph_2 / imidazole*
(f) *MPLC separation*
(g) *3,4-dihydro-2*H*-pyran / H^+*
(h) *pyridinium chlorochromate*
(i) *DIBAH /-78°C*
(k) *$Ph_3P{=}CH(CH_2)_3CO_2^-$*
(l) *CH_2N_2*
(m) *ClSi(*t.-*Bu)Me_2*
(n) *135°C / 14-20 d*
(o) *OH^-/H_2O/MeOH*
(p) *HF / H_2O / CH_3CN*

hydroxy acid, and final removal of the remaining silyl protecting groups (HF, acetonitrile, water) accomplished the thirteen-step synthesis of $^2H_4$ -labelled, crystalline prostaglandin D_2(**2a**). The isotopic composition of **2a** exhibited 4% $^2H_2$, 24% $^2H_3$, 62% $^2H_4$, 10% $^2H_5$ and less then 0.2% ^2H_o (referred to $^2H_4$).

DISCUSSION

In line with the above illustration of *Corey's* approach to prostaglandin total synthesis, two alternative routes to synthesise deuterated analogues of dimethyl 2-oxoheptylphosphonate have been previously described [4,17]. However, although these reagents exhibit remarkably high isotopic homogeneity (low blank, marginal scrambling), the deuterium is introduced at an early stage of the synthetic route and the final product is obtained in several steps. Thus, the more economical of two-step synthesis of the desired title compound **1a** depicted in Scheme 1 was developed which is particularly suitable for large-scale preparations. Mass spectrometric analysis of the isotopic composition of **1a** revealed that homogeneous catalytic saturation of the double bonds of **4** gave rise to the introduction of more than four deuterium atoms. Furthermore, inspection of the four methylene triplets in the ^{13}C-NMR spectra of reagent **1a** and the subsequent labelled prostanoids revealed low-field ($\Delta\delta \sim 0.4$ ppm) singlets of low intensity which are indicative of the presence of small amounts of unlabelled CH_2 groups.

Although various members of the arachidonic acid cascade have been prepared as their deuterated analogues[1], no chemical synthesis of deuterated prostaglandin D_2 is presently available[18]. Among the synthetic variants known to lead to unlabelled D-prostaglandins, e.g. rearrangement of endoperoxides[19], ring expansion of appropriate cyclobutanes[20], interconversion of prostaglandin F_{2a}[21-23], and total syntheses from Corey lactone or related starting materials[13,15,24-29], the latter rationale seemed most promising. Thus, a mixture of the epimeric alcohols 15*S*-**8** and 15*R*-**8**[14] was conveniently prepared in two steps after Wittig-Horner condensation of Corey lactone **6** with reagent **1a** followed by borohydride reduction of **7** in the presence of cerium-(III)[30]. In order to circumvent the tedious large-scale chromatographic separation of the epimeric allylic alcohols **8**, a simple and novel method of fractional crystallisation from suitable solvent mixtures was developed. Deprotection of 15*S*-**8** provided the labelled diol **9**. Taking advantage of the different reactivities and steric requirements of the 11- and 15-hydroxy groups, diol **9** was then subjected to regiocontrolled silylation to afford a mixture of unequivocally (see above) identified silyl compounds. Chromatographic isolation of **10** and protection with the tetrahydropyranyl group completed the novel and alternate synthesis of **14**, a prerequisite in *Ogawa's* route[15] to unlabelled prostaglandin D_2 (**2b**). Further transformations of the protected lactone **14** were essentially as described for the unlabelled species[15] and resulted in the first chemical total synthesis of deuterated PGD_2 (**2a**). The mass spectrum of a suitable derivative of **2a** proved marginal loss of deuterium in the course of the synthesis. Finally, in comparison with previous analyses of the ^{13}C-NMR spectra of other deuterated[31] or unlabelled[32] prostaglandins all carbon-13 shifts could be completely assigned.

EXPERIMENTAL SECTION

TLC (thin-layer chromatography): SiO_2 60 F_{254} (E.Merck, 5 x 7.5 cm, precoated aluminium plates). The TLC spots were visualised first by UV light then by spraying with either alcoholic phosphomolybdic acid (3.5%) or anisaldehyde followed by heating at 140°C. The latter reagent induces characteristically coloured spots[33]. -**MPLC** (medium-pressure liquid chromatography) : separations were performed on a Labomatic system at 10-20 bar using columns charged with 15-25 μm silica gel LiChroprep^R Si 60 (E.Merck) which were coupled with a combined refractive

index/UV detector. Solvent systems used for TLC and MPLC (v/v): (I), EtOAc/*n*-hexane (2:3); (II), EtOAc/*n*-hexane(1:3);(III), EtOAc; (IV), EtOAc/*n*-hexane (3:1); (V), Et_2O; (VI), EtOAc/*n*-hexane (1:5); (VII), EtOAc/*n*-hexane (1:10); (VIII), 2 vol % AcOH in system (II); (IX), 0.5 vol % AcOH in system (IV); (X), 2 vol % AcOH in system (IV); (XI), EtOAc/AcOH (98:2).- **Melting points (m.p.)**, uncorrected (Electrothermal).- **IR**(KBr/Film, cm^{-1}): Perkin-Elmer spectrophotometer model 283, only intense or characteristic absorptions are reported.- **NMR**(80 MHz/^{1}H, 20 MHz/^{13}C proton-decoupled) :Bruker Fourier-Transform instrument WP 80(24°C) unless otherwise indicated, solvent $CDCl_3$, int.std.TMS, shifts (δ scale) in ppm.- **MS**: Unless otherwise indicated a Hewlett-Packard model 5985A was used, fragments reported as *m/z* (rel.abund.,%). Samples were introduced either by direct inlet (DIP-MS) or *via* coupled capillary gas chromatograph (GC/MS). Ionization was induced by electron impact (PI/EI, 70 eV) or electron capture-chemical ionization (NI/CI, 160 eV) using methane as reactant gas. The isotopic composition (corrected for natural abundance) was determined by selected ion monitoring (SIM).

Dimethyl (*E,E*)-2-oxohepta-3,5-dienylphosphonate, 4[10]

To a stirred solution of distilled dimethyl methanephosphonate (204.5 g, 1.65 mol) in 800 mL of dry tetrahydrofuran was slowly (4 h) added at -78°C 1000 mL (1.60 mol) of 1.6 *M* *n*-butyllithium in *n*-hexane under an atmosphere of argon. The mixture was stirred at the same temperature for 1 h, then a solution of 107.9 g (0.77 mol) of freshly distilled (b.p. 77°C/17 mm) ethyl sorbate (**3**, *trans,trans*-2,4-hexadienoic acid ethyl ester) in 100 mL of dry tetrahydrofuran was added over 45 min. The resultant mixture was stirred for 18 h at -78°C and then warmed to 0°C for 1 h. The reaction was then quenched at the same temperature with 20% aqueous hydrochloric acid (300 mL). The organic phase was separated and the aqueous phase was first saturated with solid sodium chloride and then extracted (2x100 mL) with dichloromethane. The combined extracts were dried (Na_2SO_4), evaporated, and fractional under vacuum. Pure **4** was obtained as a colourless liquid (107.2 g, 64%, Ref.[10]: 52% after column chromatography) at b.p.129°C/0.015 mbar; n_D^{20} 1.5221. Calc. for $C_9H_{15}O_4P$ (218.2) C 49.54%, H 6.93%, P 14.19%; found C 49.46%, H 7.18%, P 14.19% .-**TLC** :R_f 0.33(III), brown spot with iodine vapour .- **IR**(film):2970,2860,1690,1670, 1650,1600,1270,1030.-^{1}**H-NMR**:1.89(d,J=2.4 Hz,3H,*H-7*), 3.22(d, J=22.5 Hz,2H,*H-1*),3.78(d,J=10.9 Hz,6H,OCH_3), 6.0-6.5(m,3H) and 7.05-7.4(m,1H,*olefinic hydrogens*). -^{13}**C-NMR**: 14.3(s,*C-7*), 39.6(d,J=129.4 Hz,*C-1*),53.1(d,J=7.3 Hz,OCH_3), 127.4,130.4,142.3,145.5(s,*C-3/4/5/6*),191.4(d,J=6.1 Hz, *C*-2).- Compound **4** decomposes slowly upon storage.

Dimethyl 3,4,5,6-[$^2H_4$]-2-oxoheptylphosphonate, 1a

To a clear, vigorously stirred pre-deuterated solution of 6.0 g(6.48 mmol) of tris(triphenylphosphine)rhodium(I)-chloride (**5**,Wilkinson's catalyst) in 900 mL of acetone/benzene (3:2,v/v) was added at room temperature under a deuterium atmosphere a ca. 100 mL portion of a solution of **4** (100.0 g,0.458 mol) in 100 mL of acetone/benzene(3:2,v/v). After 15h the deuterium uptake had ceased considerably and the remaining solution of **4** was added. The deuteration was completed after 24h. The mixture was concentrated to about 200 mL, diluted with 2000 mL of *n*-hexane, filtered, evaporated, and the residual oil was finally distilled under vacuum to afford pure **1a** (86.6 g, 83%),b.p.96°C/0.015 mbar; $n_D^{19.5}$ 1.4439. Calc.for

$C_9{}^1H_{15}{}^2H_4O_4P$ (226.2) C 47.78%, $^1H+^2H$ 10.24%, P 13.69%; found C 47.75%, $^1H+^2H$ 10.30%, P 13.52% .-**TLC**: R_f 0.38(III), brown spot with iodine vapour.- **^{1}H-NMR**: 0.87(broad d,J=6.6 Hz, 3H,*H-7*), 1.26(broad m, 2H,*H-5/6*),1.57 (broad m,1H,*H-4*),2.60(broad m,1H,*H-3*),3.09(d, J=22.7 Hz, 2H,*H-1*),3.79(d,J=11.2 Hz,6H,OCH_3).- **^{13}C-NMR**:13.8(s,*C-7*), 21.9 (t,J=19.5 Hz,*C-6*),22.6(t,J=19.5 Hz,*C-4*),30.6(t,J=19.5 Hz,*C-5*),41.4(d,J=128.2 Hz,*C-1*),43.7(t, J=19.5 Hz,*C-3*), 53.0 (d,J=6.1 Hz, OCH_3),202.4(d,J=6.1 Hz,*C-2*).-

MS (NI/CI(CH_4)): The isotopic composition taken from SIM tracings of the O-2',3',4',5',6'-pentafluorobenzyloxime derivative of **1a** (*m/z* 401,$[M-20]^{-\cdot}$) and corrected for natural abundance was calculated in comparison to the corresponding derivative of **1b** (MW 417): 3% $^2H_2$, 22% $^2H_3$, 62% $^2H_4$, 11% $^2H_5$, 2% $^2H_6$; ^2H_o (referred to ^2H_o) $\leq$ 0.1%. This batch was used in the next step. The rate of the deuteration and final isotopic composition are both highly dependent on the amount of solvent and catalyst used (not shown).

(dl)-3α,5α-Dihydroxy-2ß-([4',5',6',7'-$^2H_4$]-3'-oxo-*trans*-1'-octenyl)-3-(4-phenylbenzoyl)-1α -cyclopentane acetic acid γ-lactone, 7

Enone 7 was prepared from technical (ca. 85-90% pure) racemic Corey aldehyde **6** and the sodium salt of phosphonate **1a** as previously described for another isotopomer[4]. Recrystallisation from EtOAc/*n*-hexane afforded pure 7 in 63-65% yield, m.p. 125.5°C. Calc. for $C_{28}{}^1H_{26}{}^2H_4O_5$ (450.5) C 74.64%, $^1H+^2H$ 7.61%; found C 74.56 %, $^1H+^2H$ 7.80.- **TLC**:R_f 0.36(I),0.77(II). **^{13}C-NMR**[14,34] : 13.7*(C-20)*,21.9(t,J=19.5 Hz,*C-19)*, 23.2(t,J=19.5 Hz,*C-17)*, 30.8(t,J=19.5 Hz,*C-18)*,35.1*(C-7)*, 38.0*(C-10)*,40.7(t,J=19.5 Hz,*C-16*),42.7*(C-8)*,54.3*(C-12)*,80.5*(C-11)*,83.3*(C-9)*,131.6 *(C-14)*, 142.6*(C-13)*,175.9*(C-6)*,200.1*(C-15)*.-

(dl)-3α,5α-Dihydroxy-2ß-([4',5',6',7'-$^2H_4$]-3'α,ß-hydroxy-*trans*-1'-octenyl)-3-(4-phenylbenzoyl)-1α-cyclopentaneacetic acid γ-lactone, *15S*-8 and *15R*-8

Solid sodium borohydride (2.8 g,74 mmol,0.5 equiv.) was slowly (1 h) added in portions to a stirred and cooled (0°C) mixture of 7 (66.7 g,0.148 mol), tetrahydrofuran (800 mL), methanol (400 mL) and a 0.4 *M* solution of cerium (III)-chloride pentahydrate in methanol (750 mL) . The mixture was quenched with 15% aqueous acetic acid, and then diluted with 2000 mL of water. After removal of most of the organic solvents the product was isolated by extraction with ethyl acetate. Rotoevaporation of the dried (Na_2SO_4) extracts left essentially pure **8** as a ca. 1:1 mixture[6] of epimeric 15*S* , 15*R* allylic alcohols in quantitative yield (70.8g).

Isolation of (dl)-3α,5α-Dihydroxy-2ß-([4',5',6',7'-$^2H_4$]-3'α-hydroxy-*trans*-1'-octenyl)-3-(4-phenylbenzoyl)-1α-cyclopentaneacetic acid γ-lactone, *15S*-8.

The chromatographic separation of the 15*R* and 15*S* epimers of **8** was efficiently achieved on silica gel using a mixture of ethyl acetate/*n*-hexane (3:1) as mobile phase[2,6].

TLC: *15S*-**8**,R_f 0.77(III),0.57(IV),0.37(V);*15R*-**8**,R_f 0.71(III),0.45(IV).-*15S*-**8**: m.p.96.5°C, calc. for $C_{28}{}^1H_{28}{}^2H_4O_5$ (452.6) C 74.31 %, $^1H+^2H$ 8.02 %; found C 74.42 %, $^1H+^2H$ 8.12 %.-

In order to enable a facile large-scale separation of 15*R*- and 15*S*-**8** the following fractional crystallisation procedure was developed. A mixture of 15*R*-**8** and 15*S*-**8** (8.0 g) was dissolved in warm ethyl acetate (50 mL) and then diluted with *n*-hexane (100 mL). Seed crystals of pure 15*R*-**8** were then added and the solution was stored at +4°C, for 18 h. The precipitated crystals were

collected, washed with a minimum of cold solvent mixture, and recrystallised twice from EtOAc /*n*-hexane (1:1) to give 15*R*-**8** (2.51 g, 98 % pure). The combined mother liquors were evaporated to dryness and the residue was redissolved in 20 mL of hot methanol. After the addition of seed crystals of pure 15*S*-**8** and cooling to -28°C for 18h, a precipitation was obtained which was separated, washed with a minimum of cold methanol and then recrystallised twice from methanol to provide 15*S*-**8** (2.91 g). Evaporation of the combined mother liquors and purification by MPLC gave a further crop of the epimers of **8**. Finally, 4.0 g of 15*S*-**8** and 3.27 g of 15*R*-**8** were obtained (total yield : 91 %).

(dl)-3α,5α-Dihydroxy-2ß-([4',5',6',7'-$^2H_4$]-3'α-hydroxy-*trans*-1'-octenyl)-1α-cyclopentane-acetic acid γ-lactone, 9

Protective group removal by use of potassium carbonate/methanol[2,6,13] afforded diol **9** as an oil in 85-90% yield.- **TLC:** R_f 0.28 (III).- **^{13}C-NMR**[14]: 13.9(*C-20*),22.1(t,J=19.5 Hz,*C-19*),24.6(t, J=19.5 Hz,*C-17*),31.1(t,J=19.5 Hz,*C-18*),34.2(*C-7*),36.7(t,J=19.5 Hz,*C-16*),39.9(*C-10*),42.6(*C-8*), 56.3 (*C-12*),72.9(*C-15*),76.6(*C-11*),82.8(*C-9*),130.4(*C-13*),137.4(*C-14*),177.3(*C-6*).- The isotopic composition of **9** was determined by mass spectrometry (PI/EI,SIM) of the bis(*tert.*-butyldimethylsilyl)ether derivative (m.p. 65-6°C,R_f 0.38(VI))[6,35] recording the abundant ion cluster around $[M-57]^+$ at *m/z* 443 : 3%$^2H_2$,25%$^2H_3$,60%$^2H_4$,12%$^2H_5$; $^2H_0$ referred to $^2H_4$ ≤0.1%.-

Silylation of 9

A solution of diol **9** (1 equiv., 5.30 g,19.5mmol), imidazole (3 equiv.,3.98g), and freshly distilled *tert.*-butyldiphenylchlorosilane (1.5 equiv.,7.48 mL) was prepared in a minimum of dry dimethyl formamide (45 mL) at 4°C and left at the same temperature for 18 h. Excess water was then slowly added and the products were isolated by extraction with *n*-hexane. Evaporation of the combined extracts, column chromatography on silica gel(mobile phase: (I)), collection of the appropriate UV-absorbing fractions, evaporation and drying under vacuum, afforded the desired silyl ethers **10** (4.14 g, 42%), **11**(7.46 g, 51%), and **12**(0.38 g, 4%) in a total yield of 97%. Repetition of the reaction at -20°C for 18 h and use of one equiv. of chlorosilane (referred to 9) gave **10**(38%),**11**(25%), and **12**(6%) in 69% yield after chromatography (1 mmol scale).

(dl)-3α,5α-Dihydroxy-2ß-([4',5',6',7'-$^2H_4$]-3'α-(*tert.*-butyldiphenylsilyl)oxy-*trans*-1'-octenyl)-1α-cyclopentaneacetic acid γ-lactone, 10.-TLC:R_f 0.39(I).- **^{13}C-NMR**[14,34]: 13.8(*C-20*), 22.1(t,J=19.5 Hz,*C-19*), 24.0(t,J=19.5 Hz,*C-17*), 31.1(t,J=19.5 Hz,*C-18*),34.2(*C-7*),37.4(t,J=19.5 Hz, *C-16*), 39.7 (*C-10*),42.6(*C-8*), 56.2(*C-12*),74.4(*C-15*),76.6(*C-11*),82.6(*C-9*),129.3(*C-13*),137.2(*C-14*), 177.3(*C-6*).-

(dl)-3α-(*tert.*-butyldiphenylsilyl)oxy-5α-hydroxy-2ß-([4',5',6',7'-$^2H_4$]-3'α-(*tert.*-butyldiphenylsilyl)oxy-*trans*-1'-octenyl)-1α-cyclopentaneacetic acid γ-lactone, 11, colourless oil, **TLC:** R_f 0.76(I).- **^{13}C-NMR**[14,34] : 13.9(*C-20*), 35.4(*C-7*), 40.3(*C-10*), 42.2(*C-8*), 56.7(*C-12*), 74.0 (*C-15*), 79.1(*C-11*), 84.1(*C-9*), 129.6(*C-13*), 135.5(*C-14*), 177.4(*C-6*); *C-16/17/18/19* not detected.-

(dl)-3α-(*tert.*-butyldiphenylsilyl)oxy-5α-hydroxy-2ß-([4',5',6',7'-$^2H_4$]-3'α-hydroxy-*trans*-1'-octenyl)-1α-cyclopentaneacetic acid γ-lactone, 12, colourless crystals, m.p. 112.5°C.-**TLC:** R_f 0.28(I).-Calc. for $C_{31}{}^1H_{38}{}^2H_4O_4Si$ (510.7) C 72.90%,$^1H+^2H$ 9.07%; found C 73.00%,$^1H+^2H$ 8.83%.-

^{13}C-NMR[14,34]: 13.8(*C-20*), 34.6(*C-7*), 40.5(*C-10*), 42.3(*C-8*), 56.8(*C-12*), 72.5(*C-15*), 79.1(*C-11*), 83.1(*C-9*), 129.8(*C-13*), 136.1(*C-14*), 176.9(*C-6*); *C-16/17/18/19:* not detected.-

Oxidation of the monosilyl ethers 10 and 12

(dl)-3α-(*tert.*-butyldiphenylsilyl)oxy-5α-hydroxy-2ß-([4',5',6',7'-$^{2}H_4$]-*trans*-1'-octen-3'-onyl)-1α-cyclopentaneacetic acid γ-lactone, 13

Two suspensions of neutral alumina (90 mg), sodium acetate (30 mg), and pyridinium chlorochromate (84 mg, 5 equiv.) were prepared separately in 2 mL of dichloromethane. After stirring under an argon atmosphere for 3h solutions of monosilyl ethers **10** and **12** (40 mg, 78 µmol) in each 0.5 mL CH_2Cl_2 were added to the mixtures and stirring was continued for 1h. Addition of diethyl ether (5 mL), filtration through a short column of silica gel, and final purification by column chromatography (mobile phase (I)) afforded in the case of **10** 38 mg of starting material. Oxidation of **12** and work-up as described yielded a new product which was identified as enone **13** (31 mg,oil, 78% yield).-Calc. for $C_{31}{}^{1}H_{36}{}^{2}H_4O_4Si$ (508.8) C 73.19%, $^{1}H+^{2}H$ 8.71%; found C 72.93%, $^{1}H+^{2}H$ 8.91%.- **TLC**: R_f 0.49(I).- **^{1}H-NMR**(selected resonances, shifts calculated for an AB spin system of *H-13/14*): 5.99(d,J_{AB}=15.6 Hz, *H-14*), 6.37(dd, J_{AB}=15.6 Hz, $J_{H\text{-}12/13}$=7.5 Hz,*H-13*).- **^{13}C-NMR**[14,34]: 13.8(*C-20*), 34.6(*C-7*), 40.8(*C-10*), 41.9(*C-8*), 56.8(*C-12*), 78.7(*C-11*), 82.9(*C-9*), 131.5(*C-14*), 144.0(*C-13*), 176.3(*C-6*); *C-16/17/18/19* not detected.- Similar results were obtained using manganese dioxide as an oxidising agent.

3α-(Tetrahydropyran-2-yloxy)-5α-hydroxy-2ß-([4',5',6',7'-$^{2}H_2$]-3'α-(*tert.*-butyldiphenylsilyl)oxy-*trans*-1'-ocetenyl)-1α-cyclopentaneacetic acid γ-lactone, 14

4.0 g(7.8 mmol) of the 15-monosilyl ether **10**[14] was dissolved in a mixture of dry dichloromethane (85 mL) and 10 equiv. (7.1 mL, 78 mmol) of freshly distilled 3,4-dihydro-2*H*-pyran. After cooling at 0°C, 4-toluenesulphonic acid monohydrate (99 mg,0.5 mmol) was added and stirring was continued for 1 h. Excess saturated aqueous sodium hydrogen carbonate was then added and the organic phase was separated, evaporated and the residue was subjected to column chromatography (mobile phase (II)). Evaporation of the appropriate fractions left 3.9 g(85%) of **14** as an oil.-**TLC**:R_f 0.63(I),0.39(II).-

Methyl (dl)-16,17,18,19-[$^{2}H_4$]-(5*Z*,13*E*)-9α-((*tert.*-butyldimethylsilyl)oxy)-11α-(tetrahydropyran-2-yloxy)-15α-((*tert.*-butyldiphenylsilyl)oxy)prosta-5,13-dienoate[14],15

The protected F-prostaglandin **15** was prepared essentially as described previously for the unlabelled compound[15] in four steps with 64% total yield. Briefly, reduction of lactone **14** (3.9 g, 6.5 mmol) with di*iso*butylaluminium hydride (DIBAH) at -78°C and work-up afforded a lactol as a colourless oil (**TLC**: R_f 0.55(I)) which was used immediately in the following reaction. Wittig olefination with excess ylide (prepared from 4-carboxybutyltriphenylphosphonium bromide and dimsyl sodium in dry dimethyl sulphoxide) at 15°C for 1 h gave a carboxylic acid which was subsequently converted into its methyl ester (**TLC**: R_f 0.61(I)) by use of excess ethereal diazomethane. The purified (MPLC, mobile phase (I)) methyl ester (6.1 g) was then silylated (0°C,3 h) to provide **15** (3.4 g) after column chromatography (mobile phase (VII).-**TLC**: R_f 0.64(VI),0.41(VII).-

Methyl (dl)-16,17,18,19-[$^2H_2$]-(5*Z*,13*E*)-9α-((*tert.*-butyldimethylsilyl)oxy)-11α-hydroxy-15α-((*tert.*-butyldiphenylsilyl)oxy)-prosta-5,13-dienoate, 16

Removal of the tetrahydropyranyl protective group of **15** under the conditions described for other substrates[16] by use of either commercial (Aldrich) or in situ prepared (from 1,2-dibromoethane and magnesium metal) anhydrous magnesium dibromide proceeded with difficulty. As judged from TLC, numerous side-products were formed and the required hydroxy ester **16** (**TLC**: R_f 0.59(VI)) was isolated in only about 20% yield after column chromatography (mobile phase (VII)). Thermal cleavage of **15** exactly as described[15] afforded **16** as an oil in 45-46% isolated yield.

(dl)-16,17,18,19-[$^2H_4$]-(5*Z*,13*E*)-9α,15α-Dihydroxy-11-oxo-prosta-5,13-dienoic acid, 16,17,18,19- [$^2H_4$]-PGD$_2$, 2a

The methyl ester **16** was hydrolysed (excess aqueous 30% sodium hydroxyde in methanol, 0°C, 2h) to give the corresponding hydroxy acid (**TLC**: R_f 0.43(VIII)) as an oil in 77% yield after MPLC (mobile phase (I)). The purified product was then oxidised according to known general procedures[11,15,25] using 5 equiv. of each pyridinium chlorochromate and anhydrous sodium acetate in dichloromethane (20°C,1h). The resultant crude carboxy ketone (83%) was dissolved in acetonitrile, transferred to a Polythene container, cooled to -20°C, and then treated with excess 48% aqueous hydrofluoric acid[15,25,36] for 4 d. Work-up, chromatographic purification (mobile phase (IX)), and recrystallisation from a mixture of diethyl ether, ethyl acetate and *n*-hexane afforded pure, crystalline **2a** in 40% yield from **16**; m.p. 84-85°C(loc.cit[27]: m.p. 85-87°C).Calc. for $C_{20}{}^1H_{28}{}^2H_4O_5$ (356.5) C 67.38%, $^1H+^2H$ 10.18%; found C 67.17%, $^1H+^2H$ 10.07%.-**TLC**: R_f 0.48 (X),0.70(XI).- **^{1}H-NMR**(300 MHz):[37] 0.87(d,J=6.7 Hz,3H,*H-20*),1.96(m,1H,*H-8*),2.83(dd, J=7.5 / 12.1 Hz, 1H,*H-12*),4.16(t,J=6.3 Hz,1H,*H-15*),4.51(m,1H,*H-9*), 5.43-5.67(m,4H,*H-5/6/13/14*).- **^{13}C-NMR**(75.4 MHz)[37]:13.9(*C-20*), 22.1(t,J=19.2 Hz,*C-19*),24.5(t,J=19.2 Hz,*C-17*),24.5(*C-3*),25.4 (*C-7*),26.3(*C-4*),31.1(t,J=19.1 Hz,*C-18*),32.7(*C-2*),36.4(t,J=19.3 Hz,*C-16*), 47.8(*C-10*),48.5(*C-8*), 53.9(*C-12*),67.9(*C-9*),72.7(*C-15*),125.9(*C-13*),127.4(*C-6*),130.8(*C-5*),137.1(*C-14*),176.5(*C-1*),216.1 (*C-11*).-**MS**(70 eV,EI obtained on a MAT 711 instrument, resolution 13000, [M-H_2O]$^+$·recorded):calc. 338.2395;found 338.2392.- MS(DI,132°C,70 eV):338(2%, [M-18]$^+$·),320(40%, [M-2x18]$^+$·),245(100%, [M-2x18-75]$^+$,194(59%),135(42%),134(44%),119(48%).-

The NI/CI(CH_4) mass spectrum and isotopic composition of **2a** in comparison with **2b** were determined from the O-methyloxime 2',3',4',5',6'-pentafluorobenzyl ester 9,15-bis(trimethylsilylether) derivative (MW 709): 528(100%,[M-181]$^-$),438(38%,[M-181-90]$^-$). Deuterium distribution of the derivative of **2a**: 4% $^2H_2$,24% $^2H_3$, 62% $^2H_4$,10% $^2H_5$; $^2H_0$ (referred to $^2H_4$) $\leq$ 0.2%.-

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REFERENCES AND NOTES

1. Review on deuterated eicosanoids: Meese C.O.-*J.Lab.Comp.Radiopharm.* **23**: 295(1986)
2. Corey E.J., Schaaf T.K., Huber W., Koelliker U. and Weinshenker N.M.-*J.Am.Chem.Soc.***92**:397(1970)
3. For a review covering the different synthetic routes to unlabelled prostanoids see: Roberts S.M. and Scheinmann F.- *"New Synthetic Routes to Prostaglandins and Thromboxanes"*, Academic Press, London (1982); Beck G.- in *"Arzneimittel, Fortschritte 1972 bis 1985"* (Eds. A.Kleemann, E.Lindner, J.Engel), VCH Verlagsgesellschaft mbH, Weinheim, p.838(1987)
4. Meese C.O., Borstel B. and Beck G.-*J.Lab.Comp.Radiopharm.* **19**:491(1982)
5. Meese C.O., Fischer C., Thalheimer P. and Fürst O.- *Biomed.Mass Spectrom.***12**: 544 (1985)
6. Fischer C. and Meese C.O..-*Biomed.Mass Spectrom.***12**: 399 (1985)
7. Meese C.O., Fürst O. and Borstel B.-*J.Lab.Comp.Radiopharm.***23**:175(1986)
8. Corey E.J. and Kwiatkowski G.T.-*J.Am.Chem.Soc.***88**: 5654 (1966)
9. Corey E.J., Vlattas I., Andersen N.H. and Harding K.- *J.Am.Chem.Soc.***90**: 3247(1968)
10. Corey E.J., Ohuchida S. and Hahl R.- *J.Am.Chem.Soc.***106**: 3875 (1984)
11. The general procedures of prostaglandin methodology were used: loc.cit.[12,13]
12. Pike J.E., Lincoln F.H. and Schneider W.P.- *J.Org.Chem.***34**: 3552 (1969)
13. Andersen N.H., Imamoto S. and Picker D.H.-*Prostaglandins* **14**: 61 (1977)
14. Prostanoic acid numbering used: Nelson N.A.-*J.Med.Chem.* **17**: 911(1974)
15. Ogawa Y., Nunomoto M. and Shibasaki M.-*J.Org.Chem.***51**: 1625 (1986)
16. Kim S. and Park J.H.-*Tetrahedron Lett.* **28**: 439 (1987)
17. Taber D.F. and Lee C.H.-*J.Lab.Comp.Radiopharm.* **14**:599 (1978)
18. An enzymatic preparation of crude labelled PGD_2 from $^2H_8$-arachidonic acid has been reported: Barrow S.E., Heavey D.J., Ennis M., Chappell C.G., Blair I.A. and Dollery C.T.-*Prostaglandins* **28**: 743 (1984)
19. Hamberg M. and Samuelsson B.-*Proc.Natl.Acad.Sci.USA* **70**: 899 (1973)
20. Crossland N.M., Kelly D.R., Roberts S.M., Reynolds D.P. and Newton R.F.-*J.C.S.Chem.Commun.* 681 (1979)
21. Hayashi M. and Tanouchi T.-*J.Org.Chem.***38**: 2115 (1973)
22. Nishizawa E.E., Miller W.L., Gorman R.R. and Bundy G.L.- *Prostaglandins* **9**: 109 (1975)
23. Hart T.W., Metcalfe D.A. and Scheinmann F.-*J.C.S.Chem.Commun.*156 (1979)
24. Jenny E.F., Schäublin P., Fritz H. and Fuhrer H.-*Tetrahedron Lett.*2235 (1974)
25. Newton R.F., Reynolds D.P., Webb C.F. and Roberts S.M.-*J.C.S.Chem.Commun.*2055 (1981)
26. Bundy G.L., Morton D.R., Peterson D.C., Nishizawa E.E. and Miller W.L.-*J.Med.Chem.***26**: 790 (1983)
27. Collington E.W., Wallis C.J. and Waterhouse I.-*Tetrahedron Lett.***24**: 3125 (1983)
28. Suzuki M., Yanagisawa A. and Noyori R.-*Tetrahedron Lett.***25**: 1383 (1984)
29. Cainelli G., Giacomini D., Panunzio M., Martelli G. and Spunta G.-*Tetrahedron* **41**: 1385 (1985)
30. Trivalent lanthanide salts are known to generally suppress unwanted 1,4-reduction of enones: Luche J.-L.-*J.Am.Chem.Soc.***100**: 2226 (1978)
31. Meese C.O.-*J.Lab.Comp.Radiopharm.***20**: 817(1983) and references cited therein.

32. Pehk T., Välimäe T., Samel N., Lopp M., Lille Ü. and Lippmaa E.- *Eesti NSV Tead.Acad.Toim.,Keem* **31**:85(1982); *Chem.Abstr.***97**: 72140w (1982)
33. Ubatuba F.B.-*J.Chromatogr.***161**: 165 (1978)
34. Shift data of protective group(s) omitted.
35. Lin C.H. and Stein S.J.-*Synth.Commun.* **6**: 503 (1976)
36. Newton R.F., Reynolds D.P., Finch M.A.W., Kelly D.R. and Roberts S.M.-*Tetrahedron Lett.* 3981 (1979)
37. For ^{1}H-NMR data of **2b** see loc.cit.[24,29]; ^{13}C-NMR assignment according to related prostanoids, loc.cit.[31,32]. Spectra were recorded on a Bruker CXP 300 instrument at 300 MHz (^{1}H) and 75.4 MHz(^{13}C).