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THE SYNTHESIS OF 2,3-DINORPROSTACYCLIN METABOLITES—A NEW APPROACH TO SPIROLACTONE HEMIACETALS

G. L. BUNDY,* C. H. LIN and J. C. SIH
Experimental Chemistry Research, The Upjohn Company, Kalamazoo, MI 49001, U.S.A.

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Abstract—The major human urinary metabolites of prostacyclin and 6-keto-$PGF_{1\alpha}$ have been synthesized by a direct route, involving three-carbon homologation of bicyclic lactone intermediates and spontaneous spirolactonization of the products. The fact that these 2,3-dinor-6-oxo metabolites exist almost exclusively as spirolactone hemiacetals under acidic conditions (pH 5 and below) may explain the reported difficulties in derivatizing samples of biological origin. Several 19,19,20,20-d_4 metabolites have also been synthesized.

Prostacyclin (**1**),[1,2] the major product formed from arachidonic acid-derived endoperoxide (PGH_2) in mammalian blood vessel walls, is a vasodilator and an extremely potent inhibitor of platelet aggregation. It appears to play a significant role as a hemostatic agent[3] and may function as a circulating hormone,[4] counterbalancing the vasoconstrictor, pro-aggregatory effects of thromboxane A_2 (TXA_2), formed from endoperoxide PGH_2 in the platelets. Prostacyclin appears to have clinical potential for a variety of platelet-related applications,[3,5] e.g. prevention of platelet loss during cardiopulmonary by-pass and hemodialysis, and treatment of peripheral vascular disease, unstable angina and stroke.

Prostacyclin (PGI_2, **1**) possesses only limited chemical stability and is rapidly ($t_{1/2} \sim 3$ min) transformed into 6-keto-$PGF_{1\alpha}$ (**2**) at physiological pH and 37°C.[1] Like other naturally occurring prostaglandins, PGI_2 and 6-keto-$PGF_{1\alpha}$ are also metabolically unstable and undergo rapid oxidative degradation via the usual pathways, i.e. oxidation at C-15, reduction at C-13,14, β-oxidation, and ω and ω-1 oxidation. Recently, the major human urinary metabolites of both PGI_2 and 6-keto-$PGF_{1\alpha}$ have been isolated[6,7] and have been assigned structures **3–6** (Fig. 1) based on mass spectral characterization. In the rat,[8–10] several additional metabolites have been isolated, which have been hydroxylated at C-19 or C-20 (**7**, **8**) and which have undergone β-oxidation of the alkyl side chain as well (**9**). It is metabolites such as **3–6** which will have to be measured to determine endogenous levels of PGI_2 (and 6-keto-$PGF_{1\alpha}$) or, in conjunction with clinical trials, to measure blood levels following PGI_2 administration.

We report herein the first chemical synthesis of the major urinary metabolites of PGI_2.[11] These compounds will serve as chromatographic standards for comparision with biologically-derived samples, and will facilitate development of efficient derivatization procedures to be used in routine assay work. This last point was deemed especially important in view of reported difficulties in derivatizing the PGI_2 metabolites via standard procedures.[9,11]

RESULTS AND DISCUSSION

Dinorprostacyclin metabolites from dinorprostaglandin intermediates

Our first approach to the most abundant PGI_2 human metabolite **3** began with 2,3-dinorprostaglandin intermediate **10** (previously used in the synthesis of dinor prostaglandin metabolites)[12] and is summarized in Fig. 2. With all of the requisite carbons already in place, the remaining synthetic problem was a straightforward one, viz. regioselective hydration of the C-5,6 double bond. This three-stage transformation (**10** → **11**) was carried out by the well-established procedures[13] (a) iodoetherification (b) dehydrohalogenation (c) mild acid hydrolysis, thereby affording 6-keto intermediate **11** in 68% overall yield from **10**. It is noteworthy that the success of the dehydrohalogenation step depended on C-1 being at the alcohol oxidation level. When C-1 was a carboxyl group, dehydrohalogenation of the intermediate iodo ether yielded an α,β-unsaturated ester rather than the desired enol ether. Although hydroxyketone **11** existed predominantly as the cyclic 6,9-hemiacetal (very weak 1710 cm^{-1} absorption in **11**), acetylation under standard conditions (acetic anhydride, pyridine) afforded the 9α-acetate cleanly. Desilylation (tetrabutylammonium fluoride), followed by Jones oxidation to the C-1 acid, and cleavage of the tetrahydropyranyl ethers afforded key intermediate **12** (m.p. 100–103°). Base hydrolysis of crystalline acetate **12** (sodium hydroxide, aqueous t-butyl alcohol) led to the sodium salt of the desired triol keto acid **3**, which, upon acidification (pH 5 or below), afforded spirolactone hemiacetal **13a** in essentially quantitative yield. Under conditions acidic enough to protonate the caboxyl group, it was impossible to avoid the spirolactonization. The spirolactone hemiacetal **13a** is partially extractable out of ethyl acetate into aqueous sodium bicarbonate or aqueous dibasic sodium phosphate (pH 9) (but not appreciably into water at pH 7), presumably via partial hydrolysis of the lactone.

Although the overall yield of metabolite **13a** from 2,3-dinor intermediate **10** was 55%, this route required six steps and presumed the availability of dinor intermediate **10**.[12] Application of this route to other series (e.g. the ω-carboxy metabolites) would first require the three-carbon Wittig homologation of the appropriate lactone intermediate, a reaction which proceeded in only 40% yield in the parent series.[12] Hence, an alternate, more direct approach was sought.

Dinorprostacyclin metabolites from bicyclic lactone intermediates

A wide variety of approaches have been investigated for the three-carbon homologation of ketones (i → ii,

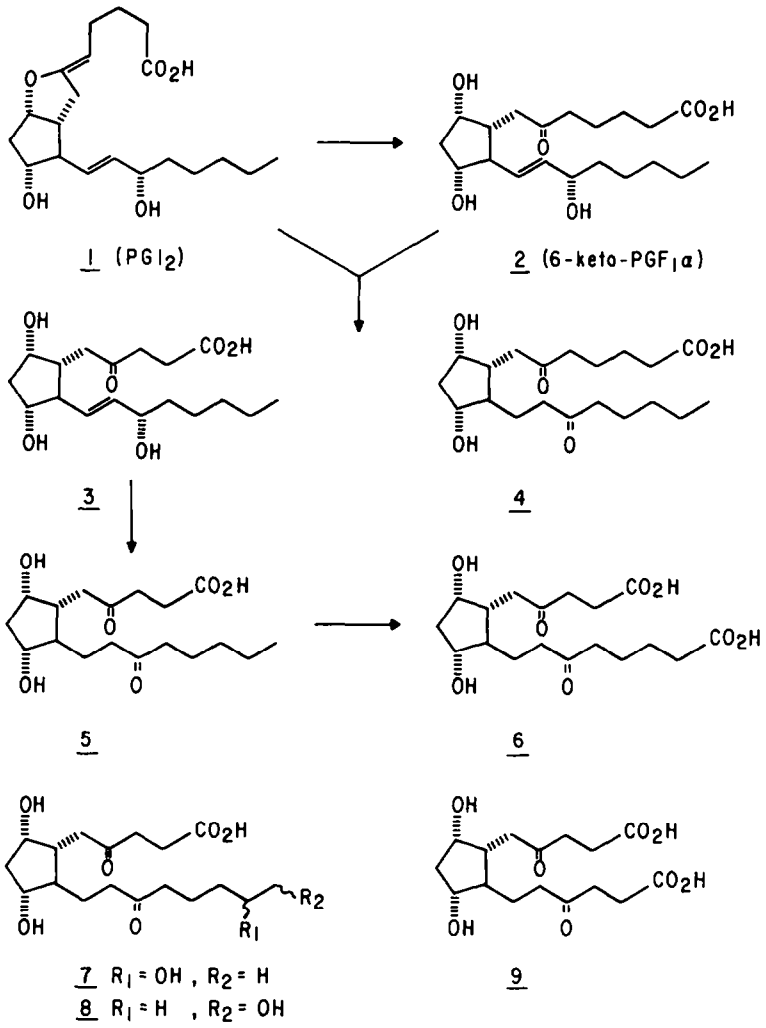
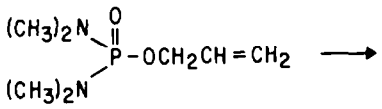


Fig. 1. Metabolism of prostacyclin and 6-keto-PGF$_{1\alpha}$.[6-10]

Y = CH_2)—an operation requiring a homoenolate anion or its synthetic equivalent.[14] However, most of these approaches did not appear promising for the analogous conversion of lactones to spirolactone hemiacetals (i→ii, Y = O). Some of the requisite reagents were not sufficiently reactive toward lactones, some of the routes required additional manipulation of the initial adducts to obtain the desired oxidation state of the added carbons, some required regioselectivity of attack by an ambident nucleophile, while others utilized reagents incompatible with the usual acid-sensitive hydroxyl protecting groups.

i ii



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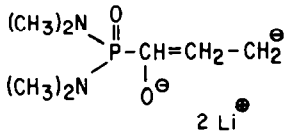


iv

The method of choice for the direct conversion of bicyclic lactone **14a** to spirolactone hemiacetal **13a** (Fig. 3) was a homologation sequence developed for ketones by Sturtz *et al.*[15,16] who used it to synthesize steroidal 17-spirolactones. Addition of dianion **iv** (generated from allyl tetramethylphosphorodiamidate **iii** with butyllithium) to bicyclic lactone **14a** at −25° gave an unstable adduct (not characterized) which, upon mild acidification (pH 1–3 with sodium bisulfate, 0°), afforded spirolactone hemiacetal **13a** [as the 11,15-bis(t-butyldimethylsilyl)derivative]. Removal of the silyl groups either under acidic conditions or with fluoride gave metabolite **13a** in 30–35% isolated yield. Modest atempts to improve this yield by varying reagent ratios and reaction temperature were unsuccessful. Although the most abundant by-product in this reaction was starting lactone **14a**,

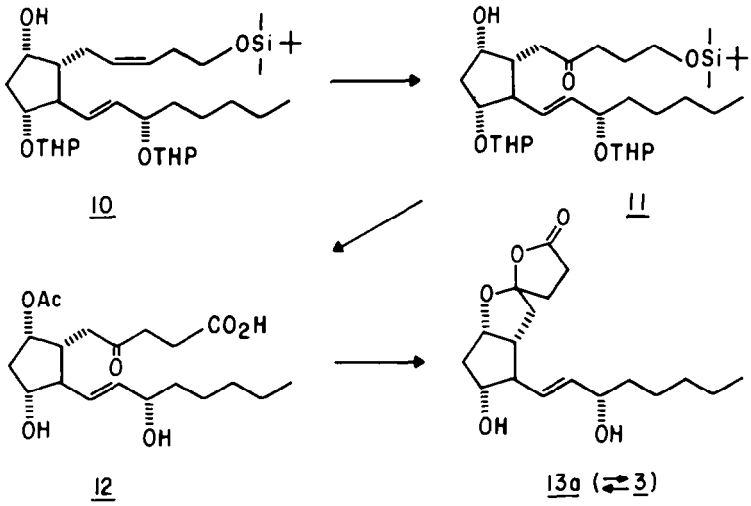


Fig. 2. 2,3-Dinorprostacyclin metabolites from 2,3-dinorprostaglandin intermediates.

use of relatively more dianion iv led to even higher recovery of **14a**, suggesting that lactone enolate formation may be competitive with addition to the lactone carbonyl. Noteworthy is the fact that conversion of the initial adduct to the spirolactone hemiacetal was possible under conditions sufficiently mild that a tetrahydropyranyl ether in the same molecule (Fig. 4 below) could survive.

2,3-Dinorprostacyclin metabolite **13a** produced by this direct route was identical spectrally and by tlc to the material obtained via the more circuitous route **10** → **13a** and to that derived from the microbiological oxidation.[11] Although metabolite **13a** exhibited a symmetrical round spot in a wide variety of tlc solvent systems, the presence of two closely spaced signals of equal intensity in the ^{13}C-NMR spectrum (176.31, 175.93) indicated it was a mixture of C-6 epimers. The ratio of isomers depended to some extent upon which solvent combination had been used for the final chromatographic purification.

The spirolactone hemiacetal portion of this dinor metabolite is apparently in equilibrium with the hydroxy keto acid form (i.e. **13a**⇄**3**) under some conditions. The ratio of low-field ^{13}C-NMR signals changed on prolonged exposure to the NMR solvent, deuterochloroform, unless the solvent had first been filtered through basic alumina. This equilibration also occurred on silica, shown by the fact that the spots of **13a** were very streaky in the absence of 0.1–1.0% acetic acid. Chromatographic purification of **13a** was effectively performed using either acid-washed silica gel (neutral solvents) or standard silica gel with 0.5% acetic acid (easily removed afterward) in the eluting solvent.

It is worth noting that, under the conditions used by most of the biochemical investigators during their isolation work (usually pH 3), all of the 2,3-dinorprostacyclin and 6-keto-PGF$_{1\alpha}$ metabolites will be entirely in the form of their lactone hemiacetal. Hence, special attention must

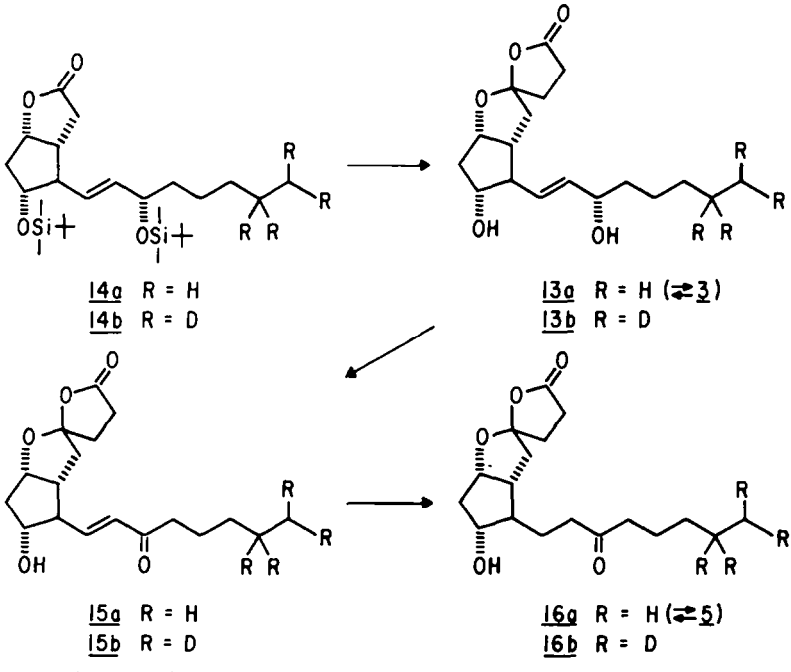


Fig. 3. 2,3-Dinorprostacyclin metabolites from bicyclic lactone intermediates.

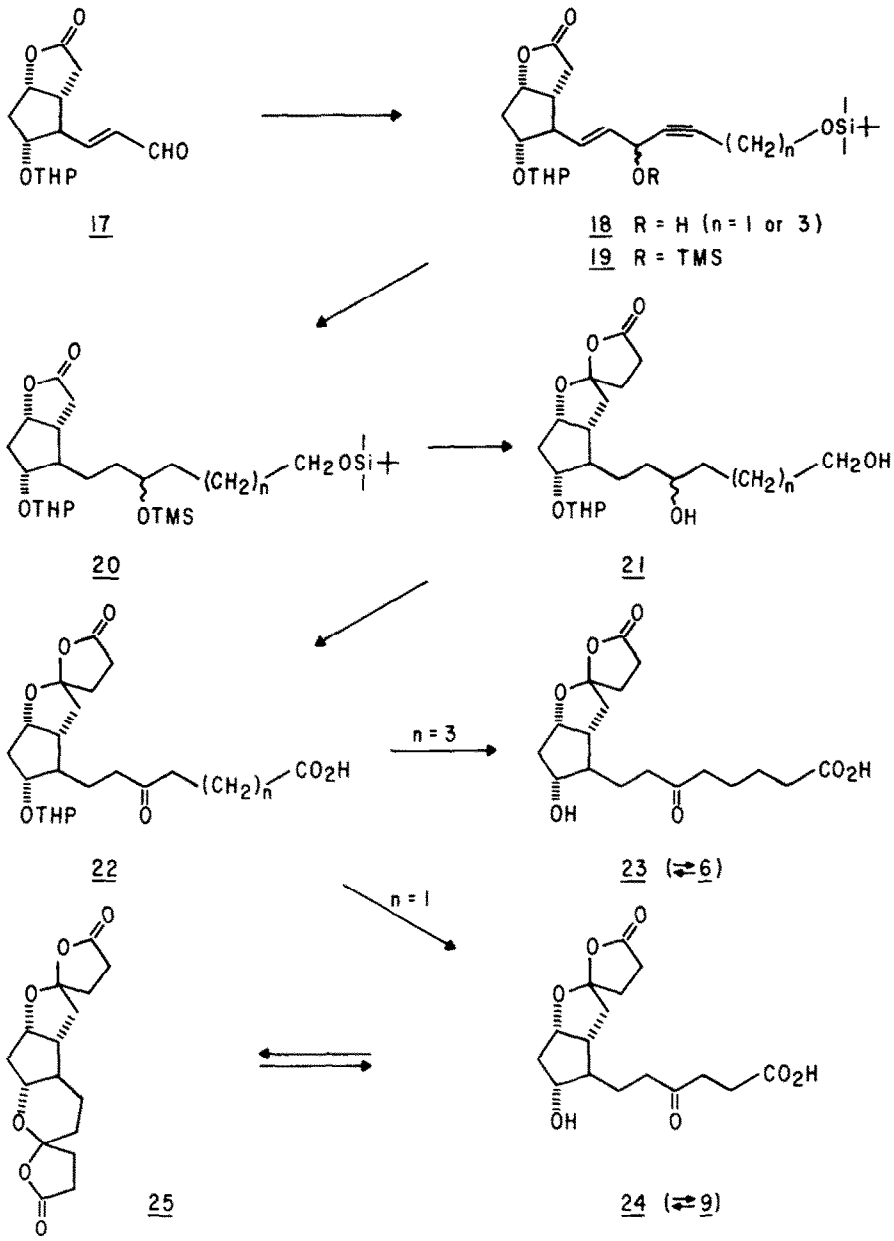


Fig. 4. ω-Carboxy 2,3-dinorprostacyclin metabolites.

be given to the appropriate derivatization sequence for mass spec sample preparation. For example, spirolactone **13a** does not react with diazomethane in ether/methanol at 0°. The methoxime of **13a** could be prepared using methoxyamine hydrochloride in either pyridine or aqueous methanol (with sodium acetate). This procedure trapped the metabolite in its open form (syn/anti methoxime mixture), which then reacted routinely with diazomethane. The mass spectrum of the methoxime methyl ester of **13a** (after silylation) was identical to published spectra of biologically derived material.[6-8] The problems of preparing derivatives for mass spectrometric study became even more complex with some of the ω-carboxy metabolites described below (especially **24**).

15-Keto- and 13,14 - dihydro - 15 - keto metabolites **15a** and **16a** were obtained by selective low temperature oxidation of **13a** with Jones reagent (−78°C; →**15a**; 75%), followed by hydrogenation (H_2, PtO_2, ethyl acetate; →**16a**). 13,14 - Dihydro - 15 - keto metabolite **16a** was identical spectrally and by tlc to the material obtained microbiologically[11] (as a minor byproduct), and by mass spectrometry, after appropriate derivatization, to that obtained by Sun[9] and Frölich.[7]

ω - Carboxy - 2,3 - dinorprostacyclin metabolites. The synthesis of 19 - carboxy - 2,3,20 - trinor - PGI_2 metabolite **23** is outlined in Fig. 4. The required 20-hydroxy intermediate **20** (n = 3) was obtained by the addition of 1-t-butyldimethylsilyloxy-4-pentynyllithium[17] to unsaturated aldehyde **17**,[18] followed by silylation[19] and hydrogenation. Conversion of lactone **20** (n = 3) to spirolactone **21** (n = 3), accomplished by the direct route described above, proceeded in 33% yield. Jones oxidation (−25°), followed by tetrahydropyranyl ether hydrolysis yielded ω-carboxy metabolite **23** (⇄**6**) an inseparable mixture of C-6 epimers. Equilibration of the spirolactone hemiacetal ring system of **21** (n = 3) (with the open form)

was sufficiently slow at −25° that no appreciable oxidation occurred at C-9. In contrast to ω-dinor metabolite **24** discussed below, hydroxy keto acid **23** showed no tendency to form a spirolactone hemiacetal between C-11,15 and 20 under mild acidic conditions.

An analogous sequence, also outlined in Fig. 4, starting with aldehyde **17** and 1 - t - butyldimethylsilyloxy - 2 - propynyllithium, led to intermediate **22** (n = 1). In this case, the spirolactonization/desilylation procedure (**20**→**21**, n = 1) proceeded in unusually low yield (13%); however, attempts to simply desilylate intermediate **20** (n = 1) also proceeded in abnormally low yield (40%), for reasons which were not certain (possibly water solubility of the product).

In contrast to the reasonably clean deprotection of intermediate **22** (n = 3; →**23**), acidic removal of the tetrahydropyranyl ether of **22** (n = 1) under mild conditions (1:7:8 phosphoric acid, water, tetrahydrofuran, 40°, 3 hr) gave a 55:45 mixture of keto acid **24** and bis(spirolactone hemiacetal) **25** (a mixture of epimers at C-6 and C-15, although homogeneous by tlc), as well as two minor unidentified products of intermediate polarity. Under more strongly acidic conditions (0.1 M hydrochloric acid in tetrahydrofuran) pure **24** and **25** each yielded the same 1:8 equilibrium mixture, favoring bis-lactone **25**. Both **24** and **25** are stable for at least seven days in ethyl acetate solution at 25°. The facile, acid catalyzed interconversion of **24** and **25** will have to be taken into consideration in the design of separation and derivatization techniques to be used for biologically derived material. These problems are currently under investigation.

Deuterated dinorprostacyclin metabolites. In order to facilitate absorption, distribution, metabolism, and excretion (ADME) studies with prostacyclin in humans and in laboratory animals, it was desirable to have access to tetradeuterated (d_4) metabolites. The deuterated metabolites, labelled in chemically stable positions, would serve as carriers for the corresponding biologically derived (*non*-deuterated) materials through the isolation, purification, derivatization sequences and also act as an internal standard during the final mass spectrometric analysis.[20]

Labeling with deuterium in the carboxyl side chain, as had been done with the classical prostaglandins,[20] was obviously not satisfactory for the 2,3-dinorprostacyclin metabolites. In terms of both synthetic ease and chemical stability of the deuterium in the product, the optimum location for incorporation of the four deuterium atoms appeared to be C-19 and C-20, i.e. the ω-end of the alkyl side chain. The straightforward synthetic route to the required intermediates is outlined in Fig. 5. 4 - Pentyn - 1 - ol was first converted to the corresponding mesylate **27**, and addition of deuterium gas (Wilkinson's homogeneous catalyst in benzene)[21] then cleanly led to d_4-mesylate **28**. Neither d_4-mesylate **28** nor d_4-bromide **29** (lithium bromide, acetone, 25°, 18 hr) was satisfactory for quantitative mass spectral analysis of deuterium incorporation, due to intramolecular loss of MsOD and DBr respectively in the mass spectrometer. d_4-Bromide **29** was converted to the Grignard reagent and added to aldehyde **17** (with *t*-butyldimethylsilyl in place of THP) (ether/tetrahydrofuran, −78°, 1 hr). Chromatographic separation of C-15 epimers (~60:40 favoring the desired 15S isomer), followed by silylation, afforded key 19,19,20,20-d_4 intermediate **14b** [m.p. 85–88°, ν_{max} 2213, 2187, 2150, 2106 cm^{-1} (C–D stretch)].

Conversion of silyl derivative **14b** to deuterated metabolite **13b** was accomplished in 29% overall yield via the allyl tetramethylphosphorodiamidate process described earlier for the all-protium case (**13a**). Mass spectral analysis of the silylated methoxime methyl ester corresponding to **13b** in the *m/e* 570–574 region (M^+–OCH_3) indicated the following deuterium incorporation: 0% d_0, 0% d_1, 0.1% d_2, 1.53% d_3, 98.46% d_4, >d_4, 0%. Deuterated 15-keto- and 13,14 - dihydro - 15 - keto - metabolites **15b** and **16b** were obtained by low temperature Jones oxidation and catalytic hydrogenation as described earlier.

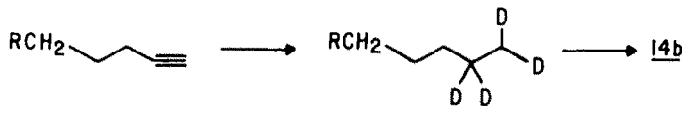
Summary. The most abundant human urinary metabolites of prostacyclin and 6-keto-$PGF_{1\alpha}$ have been synthesized, as well as three 19,19,20,20-d_4 derivatives. The key step, addition of the dianion from allyl tetramethylphosphorodiamidate to lactone intermediates affording homologated (+ three C) spirolactone hemiacetals after acidification, appears to be compatible with a variety of other functional groups and should prove a generically useful process.

EXPERIMENTAL

General methods. Melting points were obtained with a Fisher–Johns melting point apparatus and are uncorrected. Infrared spectra were recorded on either a Perkin–Elmer Model 137 or a Digilab Model TFS-14D spectrophotometer. The proton NMR spectra were obtained on a Varian A-60A spectrometer as solutions in deuterochloroform, or when specifically noted, carbon tetrachloride. Chemical shifts are reported in ppm downfield from tetramethylsilane as internal standard. The ^{13}C NMR spectra were obtained with a Varian CFT-20 spectrometer. High resolution mass spectra and deuterium analyses were obtained with a CEC 21-110B spectrometer. Burdick and Jackson "distilled in glass" solvents were used throughout. Thin layer chromatographic analyses were performed on Analtech Silica Gel GF (250 μ) plates. The phrase "standard acidic workup" below refers to the following procedure: The reaction mixture was poured into a mixture of ice and brine, acidified to about pH 2 with aqueous sodium bisulfate, and extracted thoroughly with ethyl acetate. The extracts were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated on a Buchi rotary evaporator.

t-Butyldimethylsilyl intermediate **10**

To a magnetically stirred suspension of lithium aluminum hydride (2.30 g, 6.50 mmol) in 100 ml of tetrahydrofuran, cooled in a 0–5°C bath, was added dropwise over a 5 min period a solution of 11,15 - *bis* - tetrahydropyranyl - 2,3 - dinor - $PGF_{2\alpha}$[12] (6.95 g) in 50 ml of ether. Stirring was continued at ambient temperature for



26 R = OH
27 R = OMs
28 R = OMs
29 R = Br

Fig. 5. Intermediates for d_4-metabolites.

18 hr. The reaction vessel was cooled in an ice bath and saturated sodium sulfate solution (10–15 ml) was added cautiously dropwise until the evolution of hydrogen ceased. The gelatinous aluminum salts were coagulated with anhydrous sodium sulfate diluted with 800 ml of ether and filtered with suction through celite–sodium sulfate (1:1). Chromatography of the crude product (6.05 g) on 210 g of silica gel (Silica Gel 60, 70–230 mesh, EM Reagents), eluting with 1:1 ethyl acetate/hexane yielded 3.92 g of the primary alcohol corresponding to **10**: R_f 0.38 (2:1 ethyl acetate/hexane); NMR: 5.50 (m, 4H), 4.67 (m, 2H), 4.30–3.10 (m, 11H; includes hydroxyl), 2.80–1.10 (m, 28H), 0.88 (t, 3H).

A portion of the above diol (2.641 g, 5.50 mmol) was placed in 25 ml of dimethylformamide, cooled to −78°, and treated with 0.910 g (6.05 mmol) of t-butyldimethylsilyl chloride and 0.524 g (7.70 mmol) of imidazole.[19] After addition, the solution was kept at −20° to −15° for 3 hr. Crushed ice (5–10 g) was added, the contents stirred for 5 min, and diluted with 200 ml of ether. The ether solution was washed 3× with water, saturated brine and dried over anhydrous sodium sulfate. Chromatographic purification of the crude product (3.17 g) on 200 g of silica gel (3:1 hexane/ethyl acetate) afforded 2.61 g ofpure silyl derivative **10**, a colorless oil: R_f 0.35 (3:1 hexane/ethyl acetate); NMR: 5.45 (m, 4H), 4.68 (m, 2H), 4.18–3.17 (m, 8H, includes hydroxyl), 3.65 (t, J = 6 Hz, 2H), 2.70–1.10 (m, 28H), 0.88 (s, 12H), 0.05 (s, 6H).

2,3-Dinor-6-oxo intermediate **11**

(a) *Iodoetherification.* To a magnetically stirred solution of **10** (2.610 g, 4.39 mmol) in 50 ml of methylene chloride and 50 ml of saturated sodium bicarbonate solution, cooled in an ice-water bath, was added dropwise over a 12 min period 1.23 g (4.82 mmol) of iodine in 90 ml of methylene chloride. Stirring was continued in a melting ice bath (5° to 10°) for 45 min. The contents were diluted with 250 ml of methylene chloride, the organic phase separated and successively washed 2× with 5% sodium thiosulfate solution (total ~350–400 ml), saturated brine and dried through anhydrous sodium sulfate. The crude iodo ether (3.04 g) was chromatographed on 220 g of silica gel (7:1 hexane/ethyl acetate) and yielded 2.4 g of pure iodo ether as a colorless oil: R_f 0.42, 0.39 (4:1 hexane/ethyl acetate; starting material exhibited R_f 0.23 on the same plate); NMR: (CCl_4 solvent) 5.50 (m, 2H), 4.65 (m, 2H), 4.15–3.20 (m, 11H), 2.70–1.10 (m, 28H), 0.89 (s, 12H), 0.09 (s, 6H).

(b) *Dehydrohalogenation.* The preceding iodo ether (1.982 g, 2.75 mmol) was dissolved in 80 ml of benzene and 2.75 ml of DBN and warmed under nitrogen in a 45° oil bath for 20 hr. The solution was diluted with 350 ml of ether, the ether washed twice with water, saturated brine and dried through anhydrous sodium sulfate. Removal of the ether *in vacuo* yielded 1.543 g of a pale yellow oil. The crude enol ether, already containing 5–10% of 6-oxo product **11** by tlc, was hydrolyzed immediately without further purification.

(c) *Hydrolysis.* The crude enol ether from part (b) above was placed in 60 ml of ether and 5 ml of 2N potassium bisulfate solution. Stirring was maintained at room temperature for 4.5 hr. The reaction solution was diluted with 200 ml of ether, washed with saturated brine and the ether dried through anhydrous sodium sulfate. Removal of the solvent *in vacuo* gave 1.498 g of 6-oxo intermediate **11**, a colorless oil: R_f 0.29 (2:1 hexane/ethyl acetate; intermediate enol ether exhibited R_f 0.67, 0.62 on the same plate); IR: ν_{max} (neat) 3420, 2950, 1710, 1250, 1020, 980, 840 and 780 cm^{-1}. The 6-oxo intermediate **11** was homogeneous by tlc and was used in subsequent steps without further purification.

9-Acetoxy-6-oxo-11,15-diol **12**

(a) *Acetylation.* Alcohol **11** (1.498 g, 2.46 mmol) was dissolved in 17 ml of pyridine, 3 ml of acetic anhydride and 30 mg of N,N - dimethyl - 4 - aminopyridine. The solution was stirred at room temperature for 1.5 hr. The reaction was quenched with 5–10 g of crushed ice, stirred 10 min and diluted with 350 ml of ether. The ether solution was washed with ice water, twice with ice-cold 1N potassium bisulfate (total 200 ml), ice water, saturated brine and dried through anhydrous sodium sulfate. Removal of the solvent *in vacuo* yielded 1.520 g of the 9α-acetate corresponding to **11**, about 95% pure by tlc. R_f 0.55 (2:1 hexane/ethyl acetate; alcohol **11** had R_f 0.29 on the same plate); IR: ν_{max} (neat) 2950, 1740, 1720, 1240, 1020, 975, 840, 780 cm^{-1}; NMR: (CCl_4) 5.50 (m, 2H), 5.05 (m, 1H), 2.65 (m, 2H), 4.15–3.20 (m, 5H), 3.65 (t, 2H, J = 6 Hz), 2.70–1.10 (m, 30H), 2.00 (s, 3H), 0.88 (s, 12H), 0.01 (s, 6H).

(b) *Silyl ether hydrolysis.* The crude acetate from part (a) immediately above was dissolved in 5 ml of tetrahydrofuran, treated under nitrogen with 5 ml of (n-Bu)$_4$NF (0.75 M in THF), and stirred at ambient temperature for 5.5 hr. The reaction was diluted with 350 ml of ether, and washed successively with saturated brine, water, saturated brine and the ether dried through anhydrous sodium sulfate. The crude primary alcohol product, 1.35 g, was about 95% pure by tlc and was not purified further at this stage. R_f 0.28 (2:1 ethyl acetate/hexane; the starting silyl ether had R_f 0.75 on the same plate); IR: ν_{max} (neat) 3450, 2950, 1740, 1720, 1240, 1020, 980 cm^{-1}.

(c) *C-1 Oxidation, THP ether hydrolysis.* Jones reagent (2.67 M, 2.50 ml, 6.68 mmol) was added over a 1 min period to a magnetically stirred solution of the crude product (part b) in 50 ml of acetone cooled in a −30° acetone-dry ice bath. Stirring was maintained in a −20° to −16° bath for 1 hr. Isopropanol (4 ml) was added, the contents stirred at room temperature for 3 min, and diluted with 400 ml of ethyl acetate. The EtOAc solution was washed five times with saturated brine (total 850 ml), dried through anhydrous sodium sulfate, and evaporated *in vacuo*.

The crude acid (>90% pure by tlc) was dissolved in 40 ml of acetic acid/water/tetrahydrofuran (20:10:3) and warmed in a 45° oil bath for 3.5 hr. The reaction solution was diluted with 500 ml of EtOAc–hexane (1:1), washed three times with saturated brine (total 500 ml) and dried through anhydrous sodium sulfate. Removal of the solvent *in vacuo* yielded 1.020 g of crude keto acid **12**, which was chromatographed on a Merck B Lobar pre-packed silica column. Elution with 3:1 methylene chloride/acetone, followed by 3:1 methylene chloride/acetone containing 0.5% acetic acid, afforded 493 mg of pure keto acid **12**, which crystallized on standing (overall yield from iodo ether was 55% of theory). Keto acid **12**, m.p. 100–103°; R_f 0.30 (2:25:75 acetic acid/acetone/methylene chloride, developed twice); NMR: 6.00 (broad s, OH), 5.50 (m, 2H), 5.05 (m, 1H), 4.00 (m, 2H), 2.80–1.10 (m, 18H), 2.02 (s, 3H), 0.88 (t, 3H). MS (TMS derivative): M^+ (obs) 600.3300; calc. for $C_{29}H_{56}O_7Si_3$, 600.3334.

2,3-Dinor-6-oxo-PGF$_1\alpha$ 6,9-hemiacetal 1,6-lactone **13a**

To a magnetically stirred solution of acetate **12** (35 mg) in 1 ml of t-butyl alcohol was added 0.90 ml of 1N aqueous sodium hydroxide. Stirring was maintained at ambient temperature for 1.5 hr. At the end of this period, standard acidic workup (see General Methods) afforded 30 mg of pure 2,3 - dinor - 6 - oxo - PGF$_1\alpha$, 6,9-hemiacetal, 1,6-lactone **13**. This product was identical by tlc, IR and NMR to material prepared via the more direct route described below (full spectral characterization in that experiment) and also to material prepared via the microbiological oxidation of 6-oxo-PGF$_1\alpha$ reported earlier.[11]

Allyl tetramethylphosphorodiamidate

Following the procedure of Sturtz *et al.*[16] a one liter, 3-necked, round-bottomed flask, fitted with a nitrogen inlet and an air stirrer, was charged with 200 g (about 130 ml) of commercial 50% aqueous sodium hydroxide. To the *vigorously* stirred aqueous base was added a solution of 50 g of bis(dimethylamino)phosphorochloridate (Aldrich; 0.264 mole assuming the 90% purity indicated), 18 g of allyl alcohol (0.31 mole, 17% excess), and 3.68 g of triethylbenzylammonium chloride in 100 ml of methylene chloride. An additional 100 ml of methylene chloride was added to facilitate stirring, which had been rendered nearly impossible by the immediate and voluminous precipitation of sodium chloride. The reaction mixture was stirred vigorously in a nitrogen atmosphere for an additional 90 min at 25°, then poured into ice water and extracted with methylene chloride. The extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated *in vacuo*.

Distillation of the crude product afforded 38 g (75%) of pure allyl tetramethylphosphorodiamidate: B.p. 70–72°/0.6 mm; IR ν_{max} (neat) 3060, 1640, 1450, 1290, 1220, 1180, 1040, 1000, 915, 840,

810, 750 and 670 cm^{-1}; NMR: 6.35–5.05 (m, 3H), 4.65–4.25 (m, 2H), 2.66 (d, J = 9.5 Hz, 12H); ^{13}C-NMR: 134.04, 133.69, 116.96, 65.77, 65.53, 36.71, 36.52 ppm.

2,3-Dinor-6-oxo-PGF$_{1\alpha}$ 6,9-hemiacetal 1,6-lactone **13a** *from lactone* **14a**

A 50 ml, 2-necked round-bottomed flask, fitted with a 25 ml addition funnel, magnetic stirrer and septum inlet, was flame-dried, then cooled in an atmosphere of nitrogen. Butyllithium (12.5 ml; 1.6M; 20 mmoles) in hexane was transferred to the flask via syringe and cooled to −50° under nitrogen. To the stirred butyllithium at −50° was added dropwise over 5 min a solution of 1.92 g (10 mmoles) of allyl tetramethylphosphorodiamidate in 5 ml of anhydrous tetrahydrofuran (cloudy precipitate at first, then a homogeneous dark yellow–orange solution which lightened to pale yellow when the addition was complete). The cooling bath was allowed to warm to −25°, and the reaction mixture was stirred at that temperature for 45 min. Then tetramethylethylenediamine (3.01 ml, 20 mmoles) was added, followed (still at −25° to −20°) by a solution of 2.48 g (5 mmoles) of bis (*t*-butyldimethylsilyl ether) **14a** in 10 ml of tetrahydrofuran (added dropwise over 15 min). The reaction mixture was then stirred at 25° for 4 hr, after which the tetrahydrofuran was removed on the rotary evaporator. The residue was diluted with ethyl acetate, poured into brine containing 30 ml of 2N aqueous sodium bisulfate, and extracted with ethyl acetate. The extracts were washed with brine, dried over anhydrous magnesium sulfate and concentrated *in vacuo*, thereby affording a crude product weighing 3.69 g.

Desilylation with acid. Exactly one-half of the crude product from the preceding paragraph was dissolved in 10 ml of tetrahydrofuran, diluted with 20 ml of water and 50 ml of acetic acid, and then stirred at 45° for 60 hr. The reaction mixture was concentrated to an oil, using a high vacuum pump on the rotary evaporator, and the residue was taken up in 200 ml of ethyl acetate. The ethyl acetate solution was washed with brine, water and more brine (all containing a trace of potassium bisulfate to keep the spirolactone intact and out of the aqueous layer), then dried over anhydrous magnesium sulfate and evaporated. Chromatographic purification of the crude product (905 mg) on 285 g of 40–60 μ silica gel (20:80:0.5 isopropyl alcohol/hexane/acetic acid) yielded 270 mg of pure hemiacetal lactone **13a** (33% of theory from lactone **14a**).

Desilylation with fluoride. The second half of the crude product from the first paragraph of this experiment was dissolved in 50 ml of tetrahydrofuran, treated with 50 ml of 0.7M tetrabutylammonium fluoride (in tetrahydrofuran), and stirred for 60 hr at 25° (16 hr was sufficient). The tetrahydrofuran was removed *in vacuo* and the residue was taken up in 200 ml of ethyl acetate. This solution was washed with brine, water and brine (all containing a trace of potassium bisulfate), dried over anhydrous magnesium sulfate, and evaporated. Chromatographic purification of the crude product, exactly as described in the preceding paragraph, afforded 263 mg (32%) of pure hemiacetal lactone **13a**. In each case small amounts of lactone diol corresponding to **14a** were recovered from later fractions of these chromatograms. The hemiacetal lactone **13a** synthesized by this route was identical to that prepared from dinor intermediate **10** and to that from the microbiological oxidation of 6-oxo-PGF$_{1\alpha}$: IR: ν_{max} (neat) 3380, 1770, 1310, 1270, 1200, 1190, 1070, 1040, 1000, 965, 900 and 790 cm^{-1}; NMR: 5.70–5.40 (m, 2H), 4.85–4.40 (m, 1H), 4.25–3.40 (m, 4H, incl. OH); ^{13}C-NMR: 176.31, 175.93, 136.66, 135.67, 131,75, 131.54, 117.73, 83.25, 82.88, 78.01, 76.53, 72.97, 57.70, 54.88, 45.48, 42.42, 41.85, 40.74, 39.35, 37.30, 37.08, 32.22, 31.79, 29.16, 28.52, 25.15, 22.61, 14.01 (the ratio of the two carbonyl carbon signals varied depending upon which solvents were used for the chromatographic purification and with the length of time in the NMR solvent (in this case $CDCl_3$ filtered through basic alumina). Mass spectrum (TMS derivative): M^+-CH_3 (found) 453.2505; Calc. for $C_{23}H_{41}Si_2O_5$, 453.2492; other ions at *m/e* 397, 378, 367, 341, 307, 281, 263, 243, 225. R_f 0.32 (30:70 isopropyl alcohol/hexane), 0.27 (AIX),[22] 0.22 (ethyl acetate), 0.23 (35:65 acetone/methylene chloride), 0.60 (75:25 acetone/hexane). The presence of 0.5–1.0% of acetic acid in the tlc solvents minimized tailing but was not necessary.

2,3-Dinor-6-oxo-PGF$_{1\alpha}$ methoxime methyl ester

A stirred solution of 270 mg of spirolactone **13a** in 10 ml of dry pyridine was treated with 270 mg of methoxyamine hydrochloride, and the resulting solution was stirred for 16 hr at 25°. Standard acidic workup, followed by esterification with excess ethereal diazomethane (0°, 5 min), afforded the crude methoxime methyl ester (341 mg). Chromatographic purification on 85 g of 40–60 μ silica gel (30:70 acetone/methylene chloride) yielded 236 mg (74%) of pure 2,3 - dinor - 6 - oxo - PGF$_{1\alpha}$ methoxime methyl ester, a viscous colorless oil (syn/anti mixture): R_f 0.20, 0.16 (40:60 acetone/methylene chloride), 0.16 (ethyl acetate), 0.26 (AIX);[22] IR: ν_{max} (neat) 3380, 1735, 1640, 1440, 1200, 1170, 1050, 975, 890 cm^{-1}; NMR: 5.65–5.35 (m, 2H), 4.30–3.40 (s at 3.80, 3.76, 3.66 superimposed on m, 12H total); mass spec (TMS derivative): M^+ (obs) 601.3671; calc for $C_{29}H_{59}NO_6Si_3$, 601.3650; other ions at *m/e* 586, 570, 530, 511, 480, 440, 421, 390, 350, 294, 217, 205. This mass spectrum is identical to the published[7,9] spectrum of the major human urinary metabolite of prostacyclin and 6-keto-PGF$_{1\alpha}$.

2,3-Dinor-6,15-dioxo-PGF$_{1\alpha}$ 6,9-hemiacetal 1,6-lactone **15a**

A solution of 162 mg (0.50 mmole) of spirolactone diol **13a** in 10 ml of acetone was cooled to −78° and treated with 0.15 ml of Jones reagent. The mixture was stirred at −78° for 1 hr, then treated with 0.5 ml of isopropyl alcohol and allowed to warm gradually to room temperature. Following removal of the acetone *in vacuo* and standard acidic workup, chromatographic purification of the crude product on 18 g of Mallinckrodt CC-4 acid-washed silica gel (ethyl acetate) yielded 120 mg of pure 2,3 - dinor - 6,15 - diketone **15a**, a colorless, semi-viscous oil: R_f 0.48 (AIX), 0.52 (99:1 ethyl acetate/acetic acid), 0.57, 0.59 (C-6 epimers, 25:75:1 acetone/methylene chloride/acetic acid); IR: ν_{max} (neat) 3440, 1770, 1690, 1665, 1625, 1440, 1310, 1270, 1240, 1190, 1080, 1040, 985, 900, 790, 640 cm^{-1}; NMR: 7.05–6.0 (m, 2H), 4.95–4.45 (m, 1H), 4.35–3.60 (m, 1H), 3.35 (broad s, 1H) and 0.90 (t, J = 5 Hz, 3H); mass spectrum (TMS derivative):M^+ (obs) 394.2171; calc. for $C_{21}H_{34}O_5Si$, 394.2175.

13,14 - Dihydro - 2,3 - dinor - 6,15 - dioxo - PGF$_{1\alpha}$ 6,9 - hemiacetal 1,6 - lactone **16a**

A Solution of 60 mg of enone **15a** in 15 ml of ethyl acetate was hydrogenated over 30 mg of platinum oxide catalyst at 25° in a standard atmospheric hydrogenation apparatus. The mixture was then filtered through Celite (CO_2 atmosphere) and concentrated *in vacuo*. Chromatographic purification of the crude product on 20 g of silica gel (elution with AIX solvent)[22] afforded 42 mg of clean saturated ketone **16a**, a semiviscous, colorless oil: R_f 0.48 (AIX), 0.47, 0.50 (C-6 epimers, 18:82:1 acetone/methylene chloride/acetic acid); IR: ν_{max} neat) 3450, 1770, 1705, 1445, 1410, 1310, 1240, 1200, 1090, 1040, 1000, 900, 790, 640 cm^{-1}; NMR: 4.90–4.35 (m, 1H), 4.15–3.30 (m, 1H), 2.64 (s, 1H, hydroxyl), 0.90 (t, J = 5 Hz, 3H); mass spectrum (TMS derivative): M^+ (obs) 396.2346; calc'd for $C_{21}H_{36}O_5Si$, 396.2332.

Alkynyl intermediate **18** ($n = 3$)

A solution of 4.16 g (21.0 mmoles) of 1 - t - butyldimethylsilyloxy - 4 - pentyne[17] in 25 ml of tetrahydrofuran was cooled to between −20° and −10° and treated dropwise over 5 min with methyllithium (12.0 ml, 21.0 mmole) in ether. After stirring an additional 5 min the gas evolution ceased. This solution was then added to a THF (20 ml) solution containing aldehyde **17**[18] (5.6 g, 20 mmole) at −70 ~ −60°C over a period of 10 min. After stirring an additional 10 min, the reaction was stopped by adding aqueous sodium bisulfate. The reaction mixture was allowed to warm to room temperature and extracted with ether (1 l). The ether layer was washed with sodium bisulfate, sodium bicarbonate, brine and dried over anhydrous sodium sulfate. Concentration *in vacuo* gave the crude product (9.4 g) which was purified by column chromatography using 250 g of silica gel (1:1 ethyl acetate/hexane).

The purified adduct **18** (n = 3) was completely clean by tlc and weighed 7.2 g (76% of theory): IR: ν_{max} (neat) 3420, 3220, 1775, 975, 835, 780 cm^{-1}; NMR (CCl_4) 5.72–5.52 (m, 2H), 5.14–4.52 (m, 3H), 4.20–3.75 (m, 1H), 0.86 (s, 9H), 0.02 (s, 6H); mass spec (TMS derivative): M^+ not observed; weak M-CH_3 at *m/e* 535; M-C_4H_9 (obs) 493.2433; calc. for $C_{25}H_{41}Si_2O_6$, 493.2442.

Bis(silyl) intermediate **19** (*n* = 3)

A mixture of 37.0 g (77.5 mmol) of alcohol **18** and 55.5 ml of hexamethyldisilazane - trimethylchlorosilane - pyridine (6:3:10) was stirred at room temperature under nitrogen for 10 min. About 50 ml toluene was then added and the excess reagent along with toluene was removed *in vacuo*. The residue was treated with 500 ml toluene and the mixture was filtered through a layer of Celite. The filtrate was concentrated *in vacuo*. Repeated addition and removal of toluene afforded a pyridine-free yellow oil **19** (42.6 g), homogeneous by tlc. The crude TMS ether **19** (n = 3) was used immediately in the next step without purification.

Hydrogenation of silyl intermediate **19** (→**20**, *n* = 3)

The crude product from the preceding experiment was dissolved in 210 ml ethyl acetate and 4.2 g 10% palladium on carbon was added. The mixture was hydrogenated in a Parr shaker hydrogenation apparatus. After 6 hr at room temperature the mixture was filtered through a layer of Celite. The filtrate was concentrated *in vacuo* to give a colorless oil (38.8 g). This oil can be either used directly in the next step without purification or purified by column chromatography using silica gel – 60 (63–200 μ E. Merck), eluting with hexane–ethyl acetate (4:1, with 0.01% triethylamine added). The purified silyl intermediate **20** was homogeneous by tlc: R_f 0.55 (2:1 hexane/ethyl acetate, trace of triethylamine); IR ν_{max} 2950, 2860, 1780, 1460, 1360, 1250, 1180, 1100, 1080, 1040, 1020, 840 and 780 cm^{-1}; NMR (CCl_4, –$SiCH_3$ as internal standard) 5.10–4.70 (m, 1H), 4.70–4.5 (m, 1H), 4.10–3.28 (m, 6H), 0.86 (s, 9H); mass spectrum: no M^+ obs., M-C_4H_9 (obs) 499.2930; calc. for $C_{25}H_{47}O_4Si_2$, 499.2911.

2,3-*Dinor*-6,9-*hemiacetal* 1,6-*lactone* **21** (*n* = 3)

Butyllithium/hexane (25.0 ml, 40 mmoles) was transferred to a 100 ml two-necked round-bottomed flask (50 ml addition funnel, septum, nitrogen inlet, magnetic stirrer) and cooled under nitrogen to −50°. To the stirred butyllithium at −50° was added dropwise a solution of 3.84 g (20 mmole) of allyl tetramethylphosphorodiamidate in 10 ml of tetrahydrofuran. The reaction mixture was then warmed to −25°, stirred at that temperature for 1 hr, and finally treated with 6.02 ml (40 mmoles) of tetramethylethylenediamine, followed dropwise by a solution of 5.56 g (10 mmoles) of lactone **20** in 20 ml of tetrahydrofuran. The pale yellow solution was warmed to room temperature, stirred at 25° for 4 hr, and then concentrated *in vacuo*. The residue was diluted with ethyl acetate, cooled to 0°, poured into ice/brine/60 ml of 2M sodium bisulfate, and extracted with ethyl acetate. The extracts were washed with brine, dried over anhydrous magnesium sulfate and concentrated.

The crude product from the preceding paragraph was dissolved in 160 ml tetrahydrofuran, treated with 160 ml of 0.7M tetrabutylammonium fluoride (in tetrahydrofuran), and stirred at 25° for 18 hr in an atmosphere of nitrogen. The tetrahydrofuran was removed on the rotary evaporator, and the residue was partitioned between ethyl acetate and brine (containing enough potassium bisulfate that the pH was 1–2). The ethyl acetate layer was dried over anhydrous magnesium sulfate and evaporated. The crude product (18 g) was purified by chromatography on 1 kg of CC-4 acid-washed silica gel (35:65 acetone/methylene chloride, rapid elution) and afforded 1.40 g (33%) of pure spirolactone **21** (n = 3): R_f 0.21 (99:1 ethyl acetate/acetic acid), 0.33 (35:65:1 acetone/methylene chloride/acetic acid); IR ν_{max} (neat) 3430, 1775, 1370, 1245, 1200, 1140, 1080, 1040, 1020, 900, 870, 850, 820 and 790 cm^{-1}.

Metabolite **23**

(a) *Oxidation.* To a stirred −25° solutin of 1.4 g (3.286 mmoles) of diol **21** (n = 3) in 75 ml of acetone was added 4.2 ml of Jones reagent. The dark reaction mixture was stirred at −25 to −20° for 1 hr, then treated with 1 ml of 2-propanol and stirred 15 min longer at −20°. Ethyl acetate (75 ml) was added, and the cold reaction mixture was filtered through Celite. The filtrate was concentrated to remove the acetone, and the residue was partitioned between ethyl acetate and brine. The ethyl acetate layer was washed with brine (containing a little sodium bisulfate), dried over anhydrous magnesium sulfate, and evaporated. The crude keto acid **22** (n = 3) weighted 1.4 g. R_f 0.41 (30:70:1 isopropyl alcohol/hexane/acetic acid) 0.56 (AIX),[22] 0.50 (75:25:1 ethyl acetate/hexane/acetic acid).

(b) *THP hydrolysis.* The crude product from part (a) above was dissolved in a mixture of 80 ml of tetrahydrofuran, 60 ml of water and 10 ml of commercial 85% phosphoric acid, and stirred at 40° for 16 hr. The mixture was diluted with 100 ml more water and the tetrahydrofuran was removed on the rotary evaporator. Solid sodium chloride was added to fully saturate the aqueous layer, and the product was isolated by extraction with ethyl acetate. Enough aqueous sodium bisulfate was added to all the brine washes to keep the pH of the aqueous layer at 1–2; required about 1 ml (2N) per 300 ml of brine. The ethyl acetate extracts were washed with brine (three times), dried over anhydrous magnesium sulfate, and evaporated. The crude product (1.3 g) was purified by chromatography on 150 g of 40–60 μ silica gel. Elution with the organic layer from the mixture 1350:300:450:1500 ethyl acetate/acetic acid/isooctane/water gave 510 mg of pure metabolite **23** (44% of theory based on diol **21**). R_f 0.18 (50:50:1 acetone/hexane/acetic acid), 0.21 (AIX), 0.32 (40:60:1 isopropyl alcohol/hexane/acetic acid); IR ν_{max} (neat) 3430, 2650, 1765, 1700, 1410, 1200, 1090, 1040, 1000, 895, 790, 640 cm^{-1}; NMR 6.20–5.80 (broad s, 2H, OH), 4.85–4.40 (m, 1H), 4.20–3.50 (m, 1H); mass spec (TMS derivative): M^+ (obs) 498.2469, calc. for $C_{24}H_{42}O_7Si_2$ 498.2469, other ions at *m/e* 480, 455, 408, 382, 365, 301, 283, 255, 201, 175, 73.

1-*t*-*Butyldimethylsilyloxy*-2-*propyne*

A round-bottomed flask equipped with a magnetic stirring bar was charged with 56 g (1 mol) propargyl alcohol (Aldrich), 180 g (1.2 mol) *t*-butyldimethylchlorosilane, 163 g (2.4 mol) imidazole, and 100 ml DMF. The mixture was stirred under nitrogen at room temperature for 24 hr. After the mixture was treated with 20 ml water with stirring (1 hr), 500 ml water was added and the mixture was extracted with ether (1 l). The ether layer was washed with water, 10% sodium bisulfate, saturated sodium bicarbonate, brine, and dried over anhydrous magnesium sulfate. After filtration, the ether was removed by distillation using a Vigreux column. A small amount of potassium carbonate powder was added to prevent the decomposition of the material during the distillation. The distillation under reduced pressure gave a colorless oil (b.p. 104–105°/170 mm, 165 g, 97%). IR ν_{max} (neat) 3300, 2960, 2930, 2900, 2850, 2120, 1460, 1390, 1360, 1260, 1100, 1000, 940, 920, 840, 780, 660 and 630 cm^{-1}; NMR (CCl_4, $SiCH_3$ as internal standard) 4.16 (d, J = 2.5 Hz, 2H), 2.14 (t, J = 2.5 Hz, 1H), 0.82 (s, 9H).

Alkynyl intermediate **18** (*n* = 1)

A two-neck round-bottomed flask equipped with a magnetic stirring bar, a rubber septum and a nitrogen inlet tube was charged with 10.2 g (60 mmol) of 1 - t - butyldimethylsilyloxy - 2 - propyne and 60 ml of tetrahydrofuran under nitrogen. The colorless solution was cooled to −78° and 37.5 ml (60 mmol) of n-butylithium in hexane (1.6M) was added dropwise over a period of 10 min. The mixture was stirred at −78° for 0.5 hr. Then 14 g (50 mmol) of lactone-aldehyde **17** dissolved in 100 ml of tetrahydrofuran was added *via* syringe over a period of 10 min. The resulting light brown solution was stirred at −78° for one hour. The mixture was then quenched with saturated ammonium chloride and extracted with ether. The ether layer was washed with water, brine and dried over anhydrous magnesium sulfate. Filtration and concentration afforded a light yellow oil. Column chromatography using 1 kg silica gel (1:1 ethyl acetate/hexane) yielded 18.2 g (80%) of pure alkynyl intermediate **18** (n = 1). R_f 0.49 (1:2 hexany/ethyl acetate); IR ν_{max} (neat) 3400, 2920, 2850, 1870, 1460, 1405, 1360, 1250, 1130, 1070, 1020, 970, 905, 870, 835, 815, 775, 720 and 660 cm^{-1}; NMR (CCl_4, $SiCH_3$ as internal standard) 5.72–5.45 (m, 2H), 4.98–4.32 (m, 3H), 4.20 (d, J = 1.5 Hz, 2H), 0.80 (s, 9H); mass spectrum (low resolution): M^+ not

observed, peaks present at *m/e* 307, 289, 271, 243, 225, 215, 199, 159, 125, 115 and 101.

Saturated bis(silyl) intermediate **20** (*n* = 1)

Using a procedure identical to that described above for the preparation of **19** (n = 3), alcohol **18** (n = 1) from the preceding experiment (18 g) was converted to C-15 trimethylsilyl derivative **19** (n = 1). Hydrogenation of this crude product exactly as described earlier afforded 17 g of crude saturated bis(silyl) intermediate **20** (n = 1). Chromatography of the crude product on 1 kg of silica gel (0.01:4:1 triethylamine/hexane/ethyl acetate) afforded pure **20** (n = 1), a semi-mobile, colorless oil: R_f 0.35 (3:1 hexane/ethyl acetate, trace triethylamine); IR ν_{max} (neat) 2950, 2870, 1870, 1460, 1350, 1250, 1170, 1280, 1230, 870, 840, 780 and 750 cm^{-1}; NMR (CCl_4, $-SiCH_3$ as the internal standard) 5.08–4.70 (m, 1H), 4.78–4.52 (m, 1H), 4.22–3.22 (m, 6H), 0.88 (s, 9H); mass spec: M^+ not obs, $M-C_4H_9$ (obs) 471.2574; calc. for $C_{23}H_{43}O_6Si_2$, 471.2598.

Spirolactone **21** (*n* = 1)

A 100 ml, 3-necked, round-bottomed flask fitted with nitrogen inlet, septum, 50 ml addition funnel, and magnetic stirrer, was flame dried and cooled in an atmosphere of nitrogen. To 25.0 ml of butyllithium (1.6 M, 40 mmoles), well-stirred at −50°, was added a solution of 3.84 g (20 mmoles) of allyl tetramethylphosphorodiamidate in 10 ml of tetrahydrofuran. The resulting light yellow solution was warmed to −25°, stirred at that temperature for 1 hr, then treated with 6.02 ml (40 mmoles) of tetramethylenediamine, followed dropwise by a solution of 5.28 g (10 mmoles) of lactone **20** in 20 ml of tetrahydrofuran. The reaction mixture was stirred for 4 hr at 25°, then concentrated *in vacuo*. The residue was diluted with ethyl acetate (200 ml), cooled to 0°, poured into ice/brine/60 ml 2M sodium bisulfate, and extracted with ethyl acetate. The extracts were washed with brine, dried over anhydrous magnesium sulfate and evaporated.

The crude product from the preceding paragraph was dissolved in 160 ml of tetrahydrofuran, treated with 160 ml of tetrabutylammonium fluoride (0.7 M in tetrahydrofuran) and stirred for 18 hr at 25°. Following removal of the tetrahydrofuran on the rotary evaporator, the residue was diluted with ethyl acetate, poured into (ice/brine/enough sodium bisulfate to make pH ~ 1–2) and extracted with ethyl acetate. The extracts were washed with brine (containing a trace of sodium bisulfate), dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The crude product (16 g) was chromatographed on a column containing 1.1 kg of CC-4 acid-washed silica gel. Elution with 1:1 acetone/methylene chloride yielded 520 mg (13%) of clean spirolactone diol **21** (n = 1), a viscous, pale yellow oil: R_f 0.16 (AIX);[22] IR ν_{max} (neat) 3420, 1770, 1360, 1200, 1130, 1070, 1030, 1020, 900, 870, 810 and 790 cm^{-1}.

Metabolites **24** *and* **25**

(a) *Oxidation.* A solution of 520 mg of spirolactone diol **21** (n = 1) in 35 ml of acetone was cooled to −25° and treated with 0.5 ml of Jones reagent. The reaction mixture was stirred at −25 to −20°, and an additional 0.5 ml of Jones reagent was added at 15 min and 30 min (i.e. 1.5 ml total). After 30 min longer at −25°, isopropyl alcohol (1 ml) was added, and stirring at −25° was continued 15 min longer. The mixture was then poured into ice/brine and extracted with ethyl acetate. The extracts were washed with brine (containing a little sodium bisulfate), dried over anhydrous magnesium sulfate, and concentrated *in vacuo*. The crude keto acid **22** (520 mg) was used below without further purification. R_f 0.35 (AIX).[22]

(b) *THP hydrolysis.* The crude oxidation product **22** (n = 1) was dissolved in a mixture of 25 ml of tetrahydrofuran, 20 ml of water, and 3 ml of 85% phosphoric acid, and the resulting solution was stirred at 40° for 3 hr. The mixture was then diluted with 50 ml more water and concentrated *in vacuo* to remove the tetrahydrofuran. The aqueous residue was fully saturated by adding solid sodium chloride, then extracted thoroughly with ethyl acetate. The extracts were washed with brine, dried over anhydrous magnesium sulfate, and evaporated, thereby yielding 410 mg of a viscous yellow oil. The crude product was chromatographed on 100 g of CC-4 acid-washed silica gel. Elution with 25:75 acetone/methylene chloride provided 43 mg of clean bis(spirolactone) **25**, a viscous, pale yellow oil: R_f 0.28 (35:65:1 isopropyl alcohol/hexane/acetic acid), 0.45 (AIX), 0.71 (25:75:1 acetone/methylene chloride/acetic acid); IR ν_{max} (neat) 1775, 1445, 1360, 1300, 1260, 1240, 1200, 1190, 1140, 1090, 1040, 1000, 900, 790, 690 and 630 cm^{-1}; mass spectrum: M^+ (found), 308.1266; calc. for $C_{16}H_{20}O_6$, 308.1260; other ions at *m/e* 294, 264, 250, 208, 193, 180, 166, 149, 111, 98, 84.

Elution of the above chromatogram with 1:1 acetone/methylene chloride afforded 55 mg of pure spirolactone hydroxy keto acid **24**: R_f 0.17 (35:65:1 isopropyl alcohol/hexane/acetic acid), 0.14 (AIX), 0.16 (25:75:1 acetone/methylene chloride/acetic acid); IR ν_{max} (neat) 3420, 3300, 2650, 1760, 1705, 1200, 1090, 1040, 1000, 900 and 890 cm^{-1}; mass spectrum (TMS derivative): M^+ (obs), 470.2184; calc'd for $C_{22}H_{38}O_7Si_2$, 470.2156; other ions at *m/e* 455, 427, 380, 354, 337, 290, 264, 227, 208, 175, 147, 129, 111, 73.

Also obtained from this chromatogram were two minor products with tlc mobility between **24** and **25**. These products, as yet unidentified, were not transformed into **24** or **25** under acidic conditions.

Interconversion of **24** *and* **25**

A 1 mg sample of pure keto acid **24** (from the previous experiment) was dissolved in 0.2 ml of tetrahydrofuran, treated with 0.02 ml of aqueous 1M hydrochloric acid, and allowed to stand at 25° in a stoppered vial. Samples (5 μl) were removed directly from the reaction mixture and analyzed by tlc (25/75/1 acetone/methylene chloride/acetic acid) at 5 min, 1.5 hr, 5 hr and 72 hr. The ratio **24**:**25**, estimated by tlc, was 2:1 (5 min), 1:1 (1.5 hr), 1:8 (5 hr). No significant change in the ratio occurred after about 5 hr, and no additional new products were observed even after 72 hr.

The same reaction was repeated using pure bis(spirolactone ketal) **25** as starting material. In this case, the same 1:8 ratio favoring **25** was observed, but the equilibration was complete in 5 min or less. Again there was no further change in tlc profile even after 72 hr. In neither case was any of unknown structures from the previous experiment formed.

4-Pentyn-1-ol methanesulfonate **27**

A solution of 30 g (0.357 mole) of 4 - pentyn - 1 - ol (Farchan) in 1000 ml of methylene chloride was cooled to −20° and treated with 75 ml (0.537 mole) of triethylamine (added in one portion), followed by 30 ml (0.395 mole) of methanesulfonyl chloride, added dropwise via addition funnel with good stirring over 15 min. After an additional 20 min at −20°, 100 ml of ice/water was added, and stirring was continued 5 min longer. Standard acidic workup afforded 57.8 g (100%) of pure mesylate **27**, a mobile colorless oil: IR ν_{max} (neat) 3280, 3025, 2945, 2850, 2120, 1340, 1170, 1085, 1010, 970, 930, 835, 775, 660 cm^{-1}; NMR 4.37 (t, J = 5.5 Hz, 2H), 3.03 (s, 3H), 2.70–1.70 (m, 5H); ^{13}C-NMR 82.13, 69.80, 68.27, 37.35, 27.95, 14.76; R_f 0.72 (10:90 acetone/methylene chloride; 4 - pentyn - 1 - ol has R_f 0.39 in this solvent).

4,4,5,5 - d_4 - Pentan - 1 - ol, methanesulfonate **28**

A solution of 500 mg of tris(triphenylphosphine)rhodium(I) chloride (Wilkinson's catalyst, Aldrich) in 160 ml of benzene was shaken with deuterium gas (40 psi) in a Parr hydrogenation apparatus at 25° for 20 min. To this solution was added 29 g of mesylate **27**. The deuterium atmosphere was re-introduced (40 psi), and the mixture was shaken at 25° for 1 hr, by which time deuterium uptake had ceased. Following removal of the benzene on the rotary evaporator, NMR analysis of the crude product showed that approximately 10% of the olefin intermediate remained (5.1 ppm). The crude product was resubjected to the same deuterium addition conditions for 1 hr longer, then re-isolated as above. The resulting dark oil (29 g), containing the catalyst and a little benzene could be used directly in the next transformation. **28**: IR ν_{max} (neat) 3030, 2945, 2850, 2210, 2150, 2100, 1350, 1175, 1020, 980, 950, 840 cm^{-1}; NMR 7.36 (benzene), 4.24 (t, J = 6 Hz,

2H), 2.98 (s, 3H), 2.05–1.15 (m, 4H), 1.00–0.78 (m, 1H); ^{13}C-NMR 128.39, 70.28, 69.39, 37.32, 24–18 (m), 16–11 (m).

4,4,5,5-d$_4$-1-bromopentane **29**

A solution of 28 g of crude mesylate **28** and 30 g of anhydrous lithium bromide in 200 ml of acetone was stirred at 25° in a nitrogen atmosphere for 18 hr. The mixture was then filtered through a medium porosity sintered glass funnel, and the acetone was distilled from the filtrate through a 12 in glass helix-packed column at atmospheric pressure. The residue was partitioned between 1:1 ether/pentane and water. The organic extracts were washed twice with water, once with brine, dried over anhydrous sodium sulfate, and finally distilled through the 12 in glass helix column. Vacuum distillation of the residue afforded 16.7 g (60%) of pure 4,4,5,5 - d$_4$ - 1 - bromopentane **29**. B.p. 54–55°/56 mm; NMR 3.42 (t, J = 6.5 Hz, 2H), 2.20 1.20 (m, 4H), 1.02–0.77 (m, 1H); ^{13}C-NMR 33.68, 32.67, 30.21, 21 (pent), 13.2 (pent).

d$_4$-Lactone, bis(t-butyldimethylsilyl) intermediate **14b**

Magnesium turnings (1.39 g) were flame-dried, then cooled in an atmosphere of nitrogen. Anhydrous ether 25 ml was added in one portion, followed by a solution of 8.98 g (57.94 mmoles) of 4,4,5,5 - d$_4$ - 1 - bromopentane in 15 ml of ether, added dropwise via addition funnel at a rate which maintained gentle reflux of the ether. The Grignard formation was allowed to proceed for 30 min at 25° after the refluxing ceased. The reaction mixture was then diluted with 80 ml of dry tetrahydrofuran, cooled to −78° and treated dropwise with a solution of 8.98 g (28.97 mmoles) of aldehyde **17** (with t-butyldimethylsilyl in place of THP) in 80 ml of tetrahydrofuran. After 1 hr at −78°, the mixture was poured into cold aqueous ammonium chloride and extracted with ethyl acetate. The extracts were washed with brine, dried over anhydrous sodium sulfate and concentrated *in vacuo*. Chromatographic purification of the crude product (11.1 g) on 1.2 kg of silica gel (3% acetone/methylene chloride) yielded 6.0 g of the pure (15S)-alcohol (PG-numbering): R_f 0.39 (5:95 acetone/methylene chloride; starting silyl aldehyde had R_f 0.53 on the same plate). Further elution of the above chromatogram, with 5% acetone/methylene chloride gave 3.8 g of pure (15R)-alcohol (R_f 0.28). That the less polar epimer at this stage was indeed the desired (15S)-epimer was demonstrated by silyl ether hydrolysis of each isomer and tlc comparison with authentic lactone diol.[23]

A solution of 6 g of the (15S)-alcohol from the preceding paragraph, 4.5 g of t-butyldimethylsilyl chloride, and 4.0 g of imidazole in 60 ml of dimethylformamide was stirred at 25° for 72 hr (longer than necessary). The mixture was then poured into cold water and extracted with 40:60 ethyl acetate/hexane. The extracts were washed with water, dried over anhydrous sodium sulfate and concentrated. Chromatographic purification of the crude product (7.0 g) on 1.6 kg of 40–60 μg silica gel (8:92 ethyl acetate/hexane) afforded 5.4 g of pure d$_4$ - bis(t - butyldimethylsilyl) derivative **14b**, which crystallized spontaneously upon removal of the solvent: R_f 0.58 (10:90 ethyl acetate/hexane); IR ν_{max} 2213, 2187, 2150, 2106, 1755, 1250, 1165, 1120, 975, 835, 775 cm^{-1}; NMR 5.56–5.36 (m, 2H), 5.15–4.80 (m, 1H), 4.24–3.80 (m, 2H), 0.88 (s, 18H), 0.04 (s, 12H); mass spec: M$^+$ (obs) 443.2949; calc. for $C_{23}H_{39}D_4O_4Si_2$ 443.2951; other ions at *m/e* 425, 353, 311, 293, 267, 219, 191, 177, 147, 131, 117, 91. M.p. 85–88°.

2,3 - *Dinor* - 6 - *oxo* - 19,19,20,20 - d$_4$ - *PGF*$_{1\alpha}$ 6,9-hemiacetal 1,6-lactone **13b**

Using exactly procedures and amounts as described earlier herein for the conversion of **14a→13a**, 2.5 g (5 mmoles) of d$_4$-lactone **14b** was converted into 480 mg (29%) of pure d$_4$-spirolactone **13b**: R_f 0.32 (30:70 isopropyl alcohol/hexane); IR ν_{max} (neat) 3380, 2200, 2150, 2100, 1770, 1310, 1270, 1200, 1190, 1090, 1040, 1000, 970, 900, 790 cm^{-1}; NMR 5.70–5.40 (m, 2H), 4.85–4.40 (m, 1H), 4.25–3.40 (m, 4H, includes hydroxyl), 1.00–0.75 (m, 1H); mass spectrum (as the methoxime methyl ester, prepared as described in an earlier experiment): ions at *m/e* 605 (M$^+$), 590, 574, 530, 484, 440, 425, 394, 384, 350, 294, 217, 191, 177, 147, 73; comparison of relative intensities of peaks in the 572–575 region with those from the all-protium material indicated 0% d$_0$, 0% d$_1$, 0.1% d$_2$, 1.53% d$_3$, 98.46% d$_4$, >d$_4$ 0%.

2,3 - *Dinor* - 6,15 - *dioxo* - 19,19,20,20 - d$_4$ - *PGF*$_{1\alpha}$ 6,9-*hemiacetal* 1,6-*lactone* **15b**

Using procedures and amounts identical to those described above for the preparation of enone **15a**, 19,19,20,20-d$_4$ derivative **15b** (130 mg) was obtained, a pale yellow, viscous oil: R_f 0.48 (AIX), 0.52 (99:1 ethyl acetate/acetic acid); IR ν_{max} (neat) 3440, 2200, 2150, 2100, 1770, 1690, 1665, 1625, 1440, 1310, 1270, 1190, 1080, 1040, 985, 900, 790, 640 cm^{-1}; NMR 7.05–6.00 (m, 2H), 4.95–4.45 (m, 1H), 4.35–3.60 (m, 1H), 3.55 (broad s, 1H), 0.96–0.70 (m, 1H); mass spec (TMS derivative): M$^+$ (obs) 398.2420; calc. for $C_{21}H_{30}D_4O_5Si$, 398.2426, other ions at *m/e* 383, 355, 326, 282, 245, 207, 193, 173, 155, 145, 133, 103, 73, 55.

13,14 - *Dihydro* - 2,3 - *dinor* - 6,15 - *dioxo* - 19,19,20,20 - d$_4$ - *PGF*$_{1\alpha}$ 6,9 - *hemiacetal* 1,6-*lactone* **16b**

Using the same procedures as described earlier for **16a**, hydrogenation of 75 mg of d$_4$-enone **15b** afforded, after chromatographic purification 55 mg of pure saturated ketone **16b**, a colorless, viscous oil: R_f 0.48 (AIX), 0.47, 0.50 (18:82:1 acetone/methylene chloride/acetic acid); IR 3450, 2200, 2150, 2100, 1770, 1705, 1445, 1410, 1310, 1200, 1090, 1040, 1000, 900, 790, 640 cm^{-1}; NMR 4.90–4.35 (m, 1H), 4.15–3.30 (m, 1H), 2.65 (s, 1H, hydroxyl), 0.95–0.75 (m, 1H); mass spec (TMS derivative): M$^+$ (obs) 400.2579; calc. for $C_{21}H_{32}D_4O_5Si$, 400.2583, other ions at *m/e* 385, 355, 285, 265, 247, 207, 193, 173, 145, 129, 103, 93, 73, 55, 45.

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