used as a model because of cost and availability factors.

The results in Table I clearly show that the vesicles incubated with the IgG at the 1-mg/mL concentrated level contain significantly greater quantities of bound protein than vesicles incubated with 100 μg/mL of protein. For example, with the derivative IX, the vesicles had 230 μg of bound protein at the higher concentration but only 35 μg at the lower concentration. Thus the method enables the labeling of ca. 6 mg of lipid vesicles with a significant quantity of antibody. This method can be extended 23 to the conjugation of monoclonal antibodies to vesicles. Such conjugates have potential utility as immunodirected therapeutic and diagnostic agents.

Registry No. III, 68354-84-7; IV, 103024-61-9; V, 103003-22-1; VI, 68354-85-8; VII, 103003-23-2; VIII, 103003-24-3; IX, 103003-25-4; X, 68354-86-9; XI, 73960-67-5; XII, 103003-26-5; XIII, 103003-27-6; XIV, 79360-09-1; XV, 103003-28-7; XVI, 103003-29-8; XVII, 103003-30-1; α-bromo-*p*-toluylic acid, 6232-88-8; *N*-hydroxysuccinimide, 6066-82-6; *m*-maleimidobenzoic acid succinimido ester, 58626-38-3.

Structure–Activity Studies of 16-Methoxy-16-methyl Prostaglandins

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The synthesis of the pure diastereoisomer of 16-methoxy-16-methyl-$PGF_{2\alpha}$, -PGE_2, and -PGE_1 is described. The absolute configuration of C-16 was established by chemical methods, while the absolute C-15 configurations of the diastereoisomers were assigned tentatively on the basis of their chromatographic behavior and NMR spectra. The synthetic prostaglandin analogues were evaluated for antisecretory, antifertility, and diarrheogenic effects. Both the C-15 and C-16 configurations were found to be critical for the biological activities. These studies indicate that the introduction of the methyl and methoxy groups at C-16 into the prostaglandin analogues markedly increases the ratio of antisecretory to diarrheogenic action. One of the PGE_1 derivatives, **9f**(15α,16*R*) (MDL 646, mexiprostil), was selected for further pharmacological evaluation and is currently under clinical investigation.

In a previous paper[1] we reported the synthesis of 16-methoxy prostaglandins and that some of the 16-methoxy-PGE_2 analogues, in preliminary screening, were shown to have antisecretory effects in the Heidenhain gastric pouch test in dogs.[2] These compounds had weak activity after parenteral administration and no effect when administered orally. An additional methyl group was introduced on C-16 to make the molecule more resistant to metabolic oxidation of the C-15 hydroxyl group, following a strategy reported by many investigators (e.g., 16,16-dimethyl-PGs).[3] The synthesis and structure–activity relationship of 16-methoxy-16-methyl-$PGF_{2\alpha}$, -PGE_2, and -PGE_1 analogues are the subject of this paper.

Chemistry. Wittig reaction of the aldehydes **1a**,**b**[4] (Scheme I) with the racemic phosphonate **2a** gave the enones **4a–d**, and the diastereoisomers were separated by preparative layer chromatography.

The absolute C-16 configuration of enones **4a–d** was established by resolving the acid **10** with (*S*)-(+)-amphetamine into the (+) isomer **11a** and the partially resolved (−) isomer **11b** (Scheme II) and by comparing the circular dichroism (CD) curves of **11a** and **11b** with that of **3b**[5] [CD (c = 13.1 g L^{-1}, cyclohexane), θ_{226} +330; $[\alpha]_D$ +32.4° (c 1, $CHCl_3$)], a similar compound of known configuration *R* (Figure 1). The latter was prepared from resolved (*R*)-(−)-atrolactic acid[6,7] by treating it with NaH/CH_3I in THF and hydrogenation with 5% Rh/Al_2O_3 as catalyst according to Stocker[8] (Scheme III). From the *R*-(+) isomer **11a** the optically active phosphonate **2b** was prepared (Scheme II). Reaction of **2b** with **1a** and **1b** gave (Scheme I) **4a** and **4c**, thus establishing the configuration for **4a–d**. Reduction of **4a–d** with $NaBH_4$ gave the four pairs of the epimeric diols **5e**,**f**, **5g**,**h**, **6e**,**f**, and **6g**,**h**. The epimeric diols were separated by column chromatography, and in each case the less polar diols (TLC, Et_2O) (**5e**,**g**, **6e**,**g**) were obtained in greater amounts than the more polar compounds (**5f**,**h**, **6f**,**h**), the relative rates varying from 2:1 to 4:1, depending on the C-16 configurations and on the presence or absence of the double bond in the upper side chain.

Deacetylation of **5e–h** with K_2CO_3 in dry MeOH gave the related $PGF_{2\alpha}$ analogues **7e–h**. The PGE_2 and PGE_1 analogues **8e–h** and **9e–h** were produced by protecting **5e–h** and **6e–h** with dihydropyran (i), deacetylating with K_2CO_3 in dry MeOH (ii), oxidizing the C-9 hydroxy derivative with Collins reagent (iii), and acid hydrolysis of the tetrahydropyranyl ethers (iv).

The configurations of the C-15 isomers were assigned tentatively on the basis of their chromatographic behavior and NMR spectra, and both methods resulted in the same conclusions. With the first technique, the β(*S*) configurations were assigned to the less polar epimers **5e**,**g–9e**,**g**, and the α(*R*) to the more polar epimers, **5f**,**h–9f**,**h**, in analogy with the chromatographic behavior of the natural prostaglandins, following a rule accepted by many investigators.[9] With the second method, the chemical shift of

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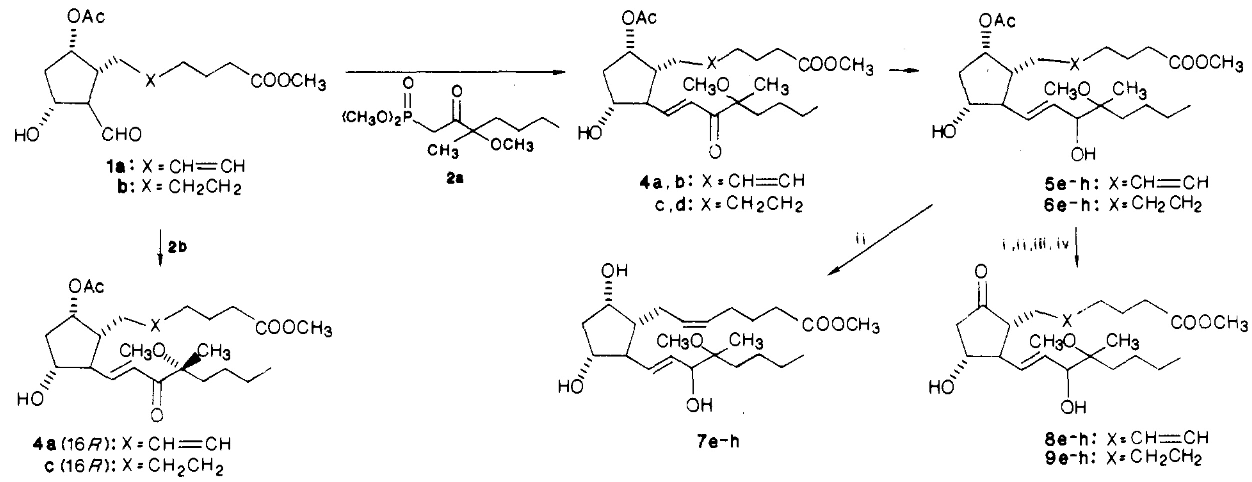
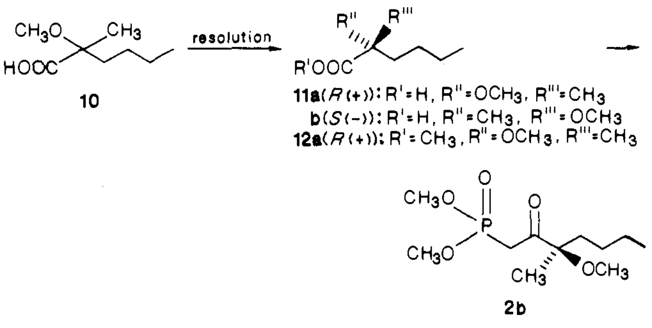
Scheme I. Synthesis of 16-Methoxy-16-methyl-PGE_2, -PGE_1, and -$PGF_{2\alpha}$[a]

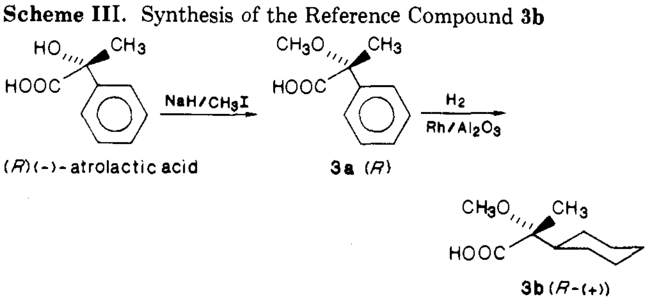


[a] i, DHP/PTSA; ii, K_2CO_3/MeOH; iii, $Pyr_2\cdot CrO_3$; iv, CH_3COOH, H_2O, THF (19:11:3).

Scheme II. Synthesis of the Optically Active Phosphonate **2b**



Scheme III. Synthesis of the Reference Compound **3b**



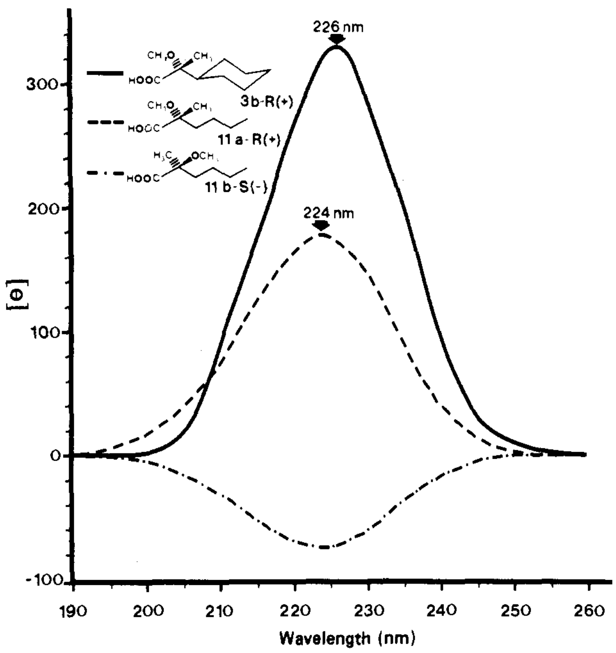
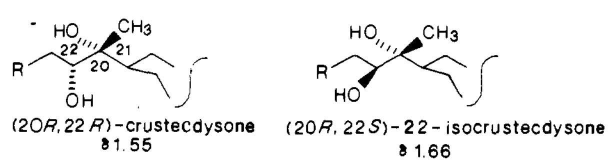


Figure 1. CD curves of **3b**, **11a**, and **11b**.

the C-16 methyl group appeared to be influenced only by the relative configurations of the C-15 and C-16 chiral centers and was independent of the other parts of the prostaglandin. Comparison of the chemical shift values, related to the C-16 methyl groups of **5e–h–9e–h** (Figure 2), with that of C-21 methyl groups, related to crust-



ecdysone and 22-isocrustecdysone reported by Kerb and colleagues,[10] enabled us to assign the configuration to **5e–h–9e–h**. In fact, for the conformations depicted, compounds with the two oxygenated functions in threo position **5e,h–9e,h** and 22-isocrustecdysone) displayed the largest chemical shifts, while those with the two oxygenated functions in erythro position (**5f,g–9f,g** and crustecdysone) had the smallest.

Pharmacology. Since natural prostaglandins have many biological effects,[11] the aim of these studies was to see whether or not C-16 substitution could dissociate the antisecretory from the antifertility and diarrheogenic effects. These latter two effects may be considered as undesirable consequences of the use of PGs[12] and therefore to be minimized as much as possible.

Results and Discussion

The antisecretory activity, diarrheogenic, and antifertility effects of all the 16-methoxy-16-methyl-PGE_2, -PGE_1,

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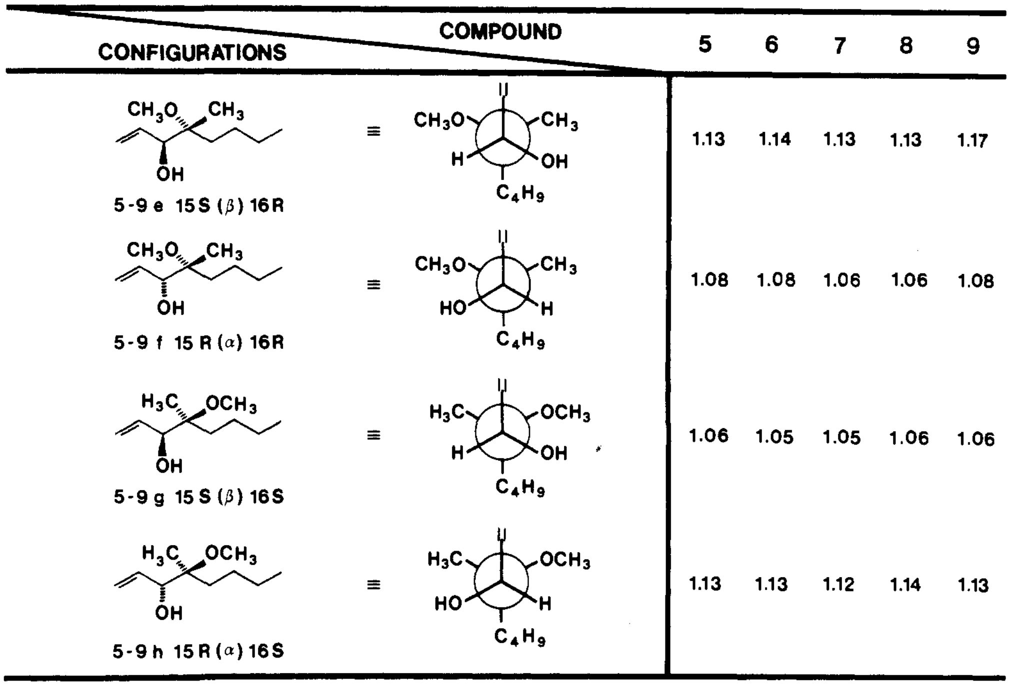

Figure 2. Chemical shifts (δ) of **5e**–h–**9e**–h C-16 methyl groups.

Table I. Comparative Antisecretory, Diarrheal, and Antifertility Effects of 16-Methoxy-16-methyl Prostaglandin Analogues

compound	antisecretory,[a] rat, iv: ID_{50}, μg/kg	diarrhea,[b] mouse, po: ED_{50}, μg/kg	ED_{50} diarrhea/ ID_{50} antisecretory	antifertility,[c] hamster, sc: ED_{50}, μg/kg
$PGF_{2\alpha}$	>300[d]	570		60
7e(β,*R*)[e]	>2000	>4000		>4000
7f(α,*R*)	1500	888		600
7g(β,*S*)	>4000	>4000		>4000
7h(α,*S*)	>2000	1815		1500
PGE_2	26	1500	58	700
	>100 ig			
16-methoxy-PGE_2 (α,*R*)	>50			
8e(β,*R*)	NT[f]	NT[f]		>1000
8f(α,*R*)	7.2	757	105	>1000
8g(β,S)	1500	912		>1000
8h(α,*S*)	500	479		>1000
PGE_1	28.5	1024	36	1800
16-methoxy-PGE_1 (α,*R*)	>100 ig			
9e(β,*R*)	>1000	>4000		>2500
9f(α,*R*)	4.5	1350	300	>2500
	15 ig[g]			
9g(β,*S*)	>3000	>4000		>2500
9h(α,*S*)	100 (-35%)	774		>2500
	300 (-40%)[h]			

[a] At least four animals per dose (three doses) were used. [b] Mice fasted for 24 h before the test were given logarithmically graded doses (100 μg/kg–4000 μg/kg) of PG orally (po). Diarrhea was assessed after 1 h, with stools being scored from 0 to 4. [c] Determined in hamsters (two to six dose levels, 6–18 animals per group) treated subcutaneously (sc) from day 4 to day 6 of gestation. [d] At the dose of 500 μg/kg, some animals died. [e] Parenthetical α,β and *R*,*S* designations indicate the absolute stereochemistry. The first letter in the grouping refers to the C-15 configuration and the second letter to the C-16 configuration. [f] NT indicates not tested. [g] See ref. 15. [h] Blood was noted in urine.

and -$PGF_{2\alpha}$ were evaluated. The results are reported in Table I, with the antisecretory activities of 16-methoxy-PGE_2 and -PGE_1. The absolute configurations of both C-15 and C-16 are important for the biological activities. For compounds with the same C-16 configuration, the isomers with the C-15α configuration were always more active than the corresponding C-15β epimers, as normally observed for natural prostaglandins (**7f**–**9f** vs. **7e**–**9e** and **7**–**9h** vs. **7g**–**9g**). Among the PGE_2 and PGE_1 analogues, the most potent antisecretory effects were observed with the C-16*R* isomers (**8f**,**9f**), while the C-16*S* configuration was associated with an increase in the diarrheogenic effect (**8h**, **9h**). These results seem to indicate a selectivity of action depending on the C-16 configuration that is not found among $PGF_{2\alpha}$ analogues.

The ratios for ED_{50} diarrhea to ID_{50} antisecretory effects, which were 58 and 36 for natural PGE_2 and PGE_1, were 105 for the 16-methoxy-16-methyl-PGE_2 analogue **8f**(α,*R*) and 300 for the PGE_1 analogue **9f**(α,*R*). Increases in this ratio have been observed by others[13] after the same change in structure.

In the antifertility test, PGE_2 and PGE_1 analogues were inactive at the doses tested, and among the $PGF_{2\alpha}$ derivatives, **7f**(α,*R*) was one-tenth as potent as the natural $PGF_{2\alpha}$. The decrease in the antifertility effect is a further indication of the selectivity of action that seems to have been induced by the introduction of the methoxy and

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methyl groups at C-16 of PGs.

In conclusion, addition of a methyl group at C-16 of 16-methoxy-PGEs has produced the desired potentiation of the antisecretory effect of the parent compounds. Furthermore, this double substitution gives a compound **9f**, (α,*R*)-MDL 646 (international nonproprietary name (WHO): mexiprostil), that has high antisecretory effect, both iv and ig, and a marked selectivity of action. This last product has been selected for further pharmacological evaluation[14,15] and is currently under clinical investigation.

Experimental Section

Melting points were taken in open capillary tubes and are uncorrected. NMR spectra were obtained on a Bruker WH-270 spectrometer or on a Varian A-60 (VA60) spectrometer, when specified. Spectra were recorded in $CDCl_3$, and data are reported as δ values with respect to Me_4Si. IR spectra, obtained on a Perkin-Elmer 580 spectrophotometer, were recorded in $CDCl_3$ unless otherwise noted, and data are reported in reciprocal centimeters. Low-resolution mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6L mass spectrometer at 17–70 eV, with use of the direct insertion system and an ion source temperature of 200 °C. CD curves were recorded on a Jobin-Yvon Dichrographe III Mark II. Optical activities, determined on a Perkin-Elmer 241 polarimeter, were recorded in $CHCl_3$ at 25 °C. Column chromatography was carried out on Merck 60 silica gel (70–230 mesh). Acidic silica gel was prepared by washing Merck 60 silica gel (70–230 mesh) with 10% aqueous HCl, water till neutral, and MeOH. Acidic SiO_2 was dried in vacuo and successively activated in an oven at 120 °C for 2 h.

Preparative layer chromatography was performed on 20 × 40 cm plates coated with Merck 60 PF_{254} silica gel (0.6-mm thick). Thin-layer chromatography (TLC) was used to monitor reactions and column fractions and to establish the homogeneity of products. TLC was carried out on Merck 60 F_{254} silica gel plates (0.25-mm thick). Spots were visualized by spraying with concentrated H_2SO_4 followed by heating at 120 °C. When elemental analyses are indicated only by symbols of the elements, the analytical results obtained were within ±0.4% of the theoretical values. When necessary, the reactions were carried out under an atmosphere of dry nitrogen. Anhydrous $MgSO_4$ was used to dry organic extracts.

(±)-(3-Methoxy-3-methyl-2-oxoheptyl)phosphonic Acid Dimethyl Ester (2a). To a vigorously stirred mixture of NaCN (27.5 g, 0.56 mol), water (10 mL), crushed ice (65 g, and freshly distilled 2-hexanone (50 g, 0.5 mol) was added dropwise a warm (40–50 °C) solution of $Na_2S_2O_5$ (63.5 g, 0.33 mol) in water (85 mL), while the temperature of the reaction was maintained below 40 °C. After being stirred for 30 min, the mixture was cooled to 0 °C and maintained at this temperature for 2.0 h without stirring. The two liquid phases were decanted, and the insoluble material was washed with water (5 mL). The combined liquid phases were separated in a separatory funnel, and the lower aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried ($CaCl_2$), filtered, dried ($MgSO_4$) again, and concentrated to yield 57.5 g (90%) of crude 2-hydroxy-2-methylhexyl cyanide containing a small amount of starting material: IR (neat) 3460 (OH), 2250 (C≡N), 1710 (C=O) cm^{-1}.

A solution of the crude cyanohydrin prepared above (57.2 g, 0.45 mol) in dry MeOH (45 mL) was cooled to 0 °C, saturated with dry HCl, kept in the refrigerator (0–5 °C) overnight, and then cautiously poured into ice water (90 mL). The solution was immediately extracted with petroleum ether, which was discarded, and after 5 h at room temperature, the solution was saturated with NaCl and extracted with CH_2Cl_2. The organic extracts were washed with water, saturated $NaHCO_3$, and brine, dried, and concentrated. The residue was distilled under reduced pressure [bp 78 °C (18 mmHg)] to give 49 g (68%) of 2-hydroxy-2-methylhexanoic acid methyl ester: IR (neat) 3500 (OH), 1740 (C=O) cm^{-1}; NMR δ 0.88 (3 H, br t, *J* = 4.5 Hz, CH_3), 0.90–1.90 (6 H, m, 3 CH_2), 1.40 (1 H, s, OH), 3.80 (3 H, s, $COOCH_3$).

To a stirred suspension of 81.8% NaH in mineral oil (9.1 g, 0.31 mol) in THF (120 mL), a solution of the 2-hydroxy-2-methyl-hexanoic acid methyl ester prepared above (48.07 g, 0.3 mol) in THF (120 mL) was added dropwise to maintain a gentle evolution of hydrogen. The solution was refluxed and CH_3I (189 g, 83 mL, 1.33 mol) was added over a period of 30 min. After being refluxed for 30 min, the reaction mixture was cooled and then quenched by addition of MeOH (10 mL). The suspension was poured into ice-cooled 10% HCl (200 mL) and saturated with NaCl, and the organic layer was separated. The organic phase was concentrated under reduced pressure to give a residue, and the aqueous phase was extracted with Et_2O. The organic extracts and the residue were combined, washed with saturated $Na_2S_2O_3$ and brine, dried, and concentrated. The residue was distilled under reduced pressure [bp 85–90 °C (18 mmHg)] to give 48 g (92%) of 2-methoxy-2-methylhexanoic acid methyl ester: IR (neat) 1730 (C=O) cm^{-1}; NMR δ 0.90 (3 H, br t, *J* = 4.5 Hz, CH_2CH_3), 1.1–2 (6 H, m, 3 CH_2), 1.40 (3 H, s, CCH_3), 3.30 (3 H, s, OCH_3), 3.45 (3 H, s, $COOCH_3$).

To a stirred solution, cooled to −78 °C, of dimethyl methylphosphonate (77 g, 0.621 mol) in THF (300 mL) was added dropwise a 1.6 M solution of *n*-butyllithium in hexane (375 mL, 0.6 mol). The reaction mixture was stirred for 15 min at −78 °C, and then a solution of 2-methoxy-2-methylhexanoic acid methyl ester (47.04 g, 0.27 mol) in THF (75 mL) was added dropwise over a period of 30 min. The reaction mixture was stirred at −78 °C for 2 h and then cautiously poured into a saturated solution of NaH_2PO_4. The two layers were separated, and the aqueous phase was extracted with Et_2O. The organic phases were combined, dried, and concentrated. The residue was distilled under reduced pressure [bp 110–115 °C (0.3 mmHg)] to give 60.4 g (84%) of **2a** as a colorless oil: NMR δ 0.92 (3 H, br t, CH_2CH_3), 1.27 (3 H, s, CCH_3), 1.1–1.9 (6 H, m, 3 CH_2), 2.97–3.6 (2 H, 2 dd, J_{CH_2-P} = 24 Hz, J_{gem} = 6 Hz, CH_2P), 3.22 (3 H, s, OCH_3), 3.79 (6 H, d, *J* = 12 Hz, $PO(OCH_3)_2$).

(*R*)-(+)-(3-Methoxy-3-methyl-2-oxoheptyl)phosphonic Acid Dimethyl Ester (2b). To a solution of the 2-methoxy-2-methylhexanoic acid methyl ester (100 g, 0.5 mol) prepared as previously described in 95° EtOH (375 mL) was added a 2 N NaOH solution (375 mL), and the mixture was refluxed for 3 h. Ethanol was distilled at atmospheric pressure, and the cooled aqueous solution was extracted with petroleum ether, acidified with 6 N HCl (150 mL), saturated with NaCl, and extracted with EtOAc. The organic phase was washed with brine, dried, and concentrated. The crude residue was distilled under reduced pressure [bp 80 °C (0.5 mmHg)] to give 67 g (83.7%) of **10**: IR (neat) 3600–2300 (OH), 1710 (acid C=O) cm^{-1}; NMR δ 0.92 (3 H, br t, *J* = 4.5 Hz, CH_2CH_3), 1.1–2.1 (6 H, m, 3 CH_2), 1.42 (3 H, s, CCH_3), 3.35 (3 H, s, OCH_3), 7.2–8.4 (1 H, br, COOH). To a stirred solution of **10** (64.1 g, 0.4 mol) in petroleum ether (270 mL), a solution of (*S*)-(+)-amphetamine (54.1 g, 0.4 mol) in petroleum ether (270 mL) was added. Stirring was stopped, the mixture was left overnight at room temperature, and the resultant salt was collected by decantation. Recrystallization of this salt from acetone (four times) to constant rotation and melting point gave 22.4 g (19%) of the resolved salt: mp 136 °C; $[\alpha]_D$ +18.7° (*c* 1.0). A solution of the resolved salt (22.16 g, 75 mmol) in water (60 mL) was acidified with 2 N HCl (40 mL), saturated with NaCl, and extracted with Et_2O. The combined extracts were washed with brine, dried, and concentrated. The residue was distilled [bp 80 °C (0.5 mmHg)] to give 11.2 g (93%) of (*R*)-(+)-2-methoxy-2-methylhexanoic acid (**11a**): $[\alpha]_D$ +12.9° (*c* 1.07); CD (*c* = 5.24 g L^{-1}, cyclohexane), θ_{224} +177.

The mother liquors from each crystallization were pooled and concentrated. Recrystallization of the resultant salt from acetone (two times) to constant rotation gave only a partially resolved salt: mp 98–100 °C; $[\alpha]_D$ +8.4° (*c* 1.0). Acidification of this salt produced the partially resolved acid **11b**: $[\alpha]_D$ −5.2° (*c* 1.07); CD (*c* = 5.94 g L^{-1}, cyclohexane), θ_{244} −73. The resolved acid **11a** (11 g, 68.6 mmol) was esterified (MeOH, HCl) to give 11 g (92%) of **12a**: $[\alpha]_D$ +26.5° (*c* 1.08). The resultant ester **12a** (11 g, 63.1 mmol) was converted to 14.1 g (84%) of the phosphonate **2b**, $[\alpha]_D$

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+39.6° (*c* 1.00), as described above for **2a**.

15-Dehydro-16-methoxy-16-methyl-PGF$_{2\alpha}$ 9-Acetate Methyl Esters (4a,b). To a stirred suspension, cooled in ice, of 81.8% NaH in mineral oil (2.64 g, 0.09 mol) in DME (105 mL) was added dropwise a solution of **2a** (27.69 g, 0.104 mol) in DME (135 mL). The ice bath was removed, the mixture was stirred for 1 h, and then a solution of the aldehyde **1a** (14.05 g, 0.045 mol) in DME (170 mL) was added dropwise. The reaction mixture was stirred for 5 h and then poured into a saturated solution of NaH_2PO_4. The two layers were separated, and the aqueous phase was extracted with Et_2O. The organic phases were combined, washed with brine, dried, and concentrated to give a crude product. The mixture was separated by preparative layer chromatography on SiO_2 with use of 7:3 Et_2O–hexane, yielding 6.15 g (30.2%) of the less polar compound **4a**(16*R*) as a colorless oil: IR 3600 (OH), 1730 (ester C=O), 1690 (ketone C=O) cm^{-1}; NMR (VA-60) δ 0.90 (3 H, t, CH_3-20), 1.30 (3 H, s, CH_3-16), 2.10 (3 H, s, $OCOCH_3$), 3.24 (3 H, s, OCH_3), 3.70 (3 H, s, $COOCH_3$), 4.13 (1 H, m, H-11), 5.17 (1 H, m, H-9), 5.39 (2 H, m, cis CH=CH), 6.86 (2 H, m, trans CH=CH); MS, *m/z* 453 (M^{+1}); $[\alpha]_D$ +58.7° (*c* 0.98). Anal. ($C_{25}H_{40}O_7$) C, H. The procedure also yielded 8.6 g (42.2%) of the more polar compound **4b**(16*S*) as a colorless oil: IR 3605 (OH), 1730 (ester C=O), 1690 (ketone C=O) cm^{-1}; NMR (VA-60) δ 0.90 (3 H, t, CH_3-20), 1.29 (3 H, s, CH_3-16), 2.10 (3 H, s, $OCOCH_3$), 3.22 (3 H, s, OCH_3), 3.70 (3 H, s, $COOCH_3$), 4.05 (1 H, m, H-11), 5.16 (1 H, m, H-9), 5.38 (2 H, m, cis CH=CH), 6.96 (2 H, m, trans CH=CH); MS, *m/z* 453 (M^{+1}); $[\alpha]_D$ +26.8° (*c* 0.86). Anal. ($C_{25}H_{40}O_7$) C, H.

15-Dehydro-16-methoxy-16-methyl-PGF$_{1\alpha}$ 9-Acetate Methyl Esters (4c,d). With use of the procedure described above, with **2a** and **1b** as starting materials, separation of the mixture of the two isomers gave a similar yield of the less polar compound **4c**(16*R*) as a colorless oil: IR 3600 (OH), 1730 (ester C=O), 1690 (ketone C=O) cm^{-1}; NMR δ 0.89 (3 H, t, CH_3-20), 1.29 (3 H, s, CH_3-16), 2.08 (3 H, s, $OCOCH_3$), 3.21 (3 H, s, OCH_3), 3.68 (3 H, s, $COOCH_3$), 4.11 (1 H, m, H-11), 5.23 (1 H, m, H-9), 6.87 (2 H, m, trans CH=CH); MS, *m/z* 455 (M^{+1}); $[\alpha]_D$ +53.8° (*c* 0.81). Anal. ($C_{25}H_{42}O_7$) C, H. The procedure also gave a similar yield of the more polar compound **4d**(16*S*) as a colorless oil: IR 3605 (OH), 1730 (ester C=O), 1690 (ketone C=O) cm^{-1}; NMR δ 0.88 (3 H, t, CH_3-20), 1.29 (3 H, s, CH_3-16), 2.08 (3 H, s, $OCOCH_3$), 3.21 (3 H, s, OCH_3), 3.67 (3 H, s, $COOCH_3$), 4.11 (1 H, m, H-11), 5.23 (1 H, m, H-9), 6.87 (2 H, m, trans CH=CH); MS, *m/z* 455 (M^{+1}); $[\alpha]_D$ +19.1° (*c* 1.16). Anal. ($C_{25}H_{42}O_7$) C, H.

(15*S*,16*R*)-16-Methoxy-16-methyl-*epi*-PGF$_{2\alpha}$ 9-Acetate Methyl Ester (5e) and (16*R*)-16-Methoxy-16-methyl-PGF$_{2\alpha}$ 9-Acetate Methyl Ester (5f). To a stirred solution, cooled to −78 °C, of **4a** (1.9 g, 4.2 mmol) in a 7:3 mixture of MeOH–water (150 mL) was added solid $NaBH_4$ (4 g) in three portions at 15-min intervals. The reaction mixture was stirred at −78 °C for 2 h and then cautiously poured into a saturated solution of NaH_2PO_4 and extracted with Et_2O. The organic extracts were concentrated under reduced pressure, and the aqueous residue was extracted with CH_2Cl_2. The organic extracts were washed with brine, dried, and concentrated. Purification of the residue by column chromatography with 4:6 hexane–Et_2O as eluent gave 1.0 g (52.4%) of the 15β-alcohol **5e** as a viscous oil: IR 3610, 3550 (OH), 1730 (ester C=O) cm^{-1}; NMR δ 0.90 (3 H, t, CH_3-20), 1.13 (3 H, s, CH_3-16), 2.06 (3 H, s, $OCOCH_3$), 3.24 (3 H, s, OCH_3), 3.66 (3 H, s, $COOCH_3$), 3.93 (1 H, m, H-11), 4.12 (1 H, br d, *J*(H14–H15) = 5.5 Hz, H-15), 5.12 (1 H, m, H-9), 5.34 (2 H, m, cis CH=CH), 5.60–5.71 (2 H, 2 m, trans CH=CH); $[\alpha]_D$ +19.2° (*c* 1); TLC (Et_2O). Purification with 3:7 hexane–Et_2O as eluent gave 0.5 g (26.2%) of the 15α-alcohol **5f** as a viscous oil: IR 3600, 3550, 3420 (OH), 1730 (ester C=O) cm^{-1}; NMR δ 0.91 (3 H, t, CH_3-20), 1.08 (3 H, s, CH_3-16), 2.04 (3 H, s, $OCOCH_3$), 3.22 (3 H, s, OCH_3), 3.67 (3 H, s, $COOCH_3$), 3.93 (1 H, m, H-11), 4.10 (1 H, d, *J*(H14–H15) = 5.5 Hz, H-15), 5.10 (1 H, m, H-9), 5.23 (2 H, m, cis CH=CH), 5.60 (2 H, m, trans CH=CH); $[\alpha]_D$ +52.2° (*c* 1.01); TLC (Et_2O).

(15*S*,16*S*)-16-Methoxy-16-methyl-*epi*-PGF$_{2\alpha}$ 9-Acetate Methyl Ester (5g) and (16*S*)-16-Methoxy-16-methyl-PGF$_{2\alpha}$ 9-Acetate Methyl Ester (5h). The enone **4b** (2.69 g, 5.94 mmol) was reduced as described above to give 1.8 g (66.7%) of the 15β-alcohol **5g**: IR 3605 (OH), 1730 (ester C=O) cm^{-1}; NMR δ 0.92 (3 H, t, CH_3-20), 1.06 (3 H, s, CH_3-16), 2.05 (3 H, s, $OCOCH_3$), 3.22 (3 H, s, OCH_3), 3.67 (3 H, s, $COOCH_3$), 3.94 (1 H, m, H-11), 4.15 (1 H, br d, *J*(H14–H15) = 3.5 Hz, H-15), 5.12 (1 H, m, H-9), 5.34 (2 H, m, cis CH=CH), 5.63 (2 H, m, trans CH=CH); $[\alpha]_D$ +16.7° (*c* 0.99); TLC (Et_2O). The procedure also gave 0.59 g (21.8%) of the 15α-alcohol **5h**: IR 3610, 3540 (OH), 1730 (ester C=O) cm^{-1}; NMR δ 0.90 (3 H, t, CH_3-20), 1.13 (3 H, s, CH_3-16), 2.06 (3 H, s, $OCOCH_3$), 3.24 (3 H, s, OCH_3), 3.66 (3 H, s, $COOCH_3$), 3.94 (1 H, m, H-9), 4.09 (1 H, dd, *J*(CH–OH) = 3 Hz, *J*(H14–H15) = 6.5 Hz, H-15), 5.10 (1 H, m, H-9), 5.33 (2 H, m, cis CH=CH), 5.57–5.71 (2 H, 2 m, trans CH=CH); $[\alpha]_D$ +46.6° (*c* 1.04); TLC (Et_2O).

(15*S*,16*R*)-16-Methoxy-16-methyl-*epi*-PGF$_{1\alpha}$ 9-Acetate Methyl Ester (6e) and (16*R*)-16-Methoxy-16-methyl-PGF$_{1\alpha}$ 9-Acetate Methyl Ester (6f). The enone **4c** (4.58 g, 10.07 mmol) was reduced as described above to give 2.94 g (63.9%) of the 15β-alcohol **6e**: IR 3605, 3540 (OH), 1730 (ester C=O) cm^{-1}; NMR δ 0.90 (3 H, t, CH_3-20), 1.14 (3 H, s, CH_3-16), 2.07 (3 H, s, $OCOCH_3$), 3.25 (3 H, s, OCH_3), 3.67 (3 H, s, $COOCH_3$), 3.95 (1 H, m, H-11), 4.15 (1 H, br d, *J*(H15–H14) = 2.5 Hz, H-15), 5.20 (1 H, m, H-9), 5.65 (2 H, m, trans CH=CH); $[\alpha]_D$ +21.5° (*c* 1); TLC (7:3 EtOAc–hexane). The procedure also gave 0.69 g (15%) of the 15α-alcohol **6f**: IR 3600, 3540 (OH), 1730 (C=O) cm^{-1}; NMR δ 0.91 (3 H, t, CH_3-20), 1.08 (3 H, s, CH_3-16), 2.06 (3 H, s, $OCOCH_3$), 3.23 (3 H, s, OCH_3), 3.68 (3 H, s, $COOCH_3$), 3.88 (1 H, m, H-11), 4.05 (1 H, d, *J*(H15–H14) = 7 Hz, H-15), 5.18 (1 H, m, H-9), 5.60 (2 H, m, trans CH=CH); $[\alpha]_D$ +30.7° (*c* 1.24); TLC (EtOAc).

(15*S*,16*S*)-16-Methoxy-16-methyl-*epi*-PGF$_{1\alpha}$ 9-Acetate Methyl Ester (6g) and (16*S*)-16-Methoxy-16-methyl-PGF$_{1\alpha}$ 9-Acetate Methyl Ester (6h). The enone **4d** (0.9 g, 1.98 mmol) was reduced as described above to give 0.53 g (58.6%) of the 15β-alcohol **6g**: IR 3600, 3560 (OH), 1730 (C=O) cm^{-1}; NMR δ 0.92 (3 H, t, CH_3-20), 1.05 (3 H, s, CH_3-16), 2.07 (3 H, s, $OCOCH_3$), 3.23 (3 H, s, OCH_3), 3.68 (3 H, s, $COOCH_3$), 3.93 (1 H, m, H-11), 4.15 (1 H, d, *J*(H14–H15) = 2.5 Hz, H-15), 5.19 (1 H, m, H-9), 5.63 (2 H, m, trans CH=CH); $[\alpha]_D$ +17.3° (*c* 0.95); TLC (7:3 EtOAc–hexane). The procedure also gave 0.13 g (14.4%) of the 15α-alcohol **6h**: IR 3600, 3540, 3420 (OH), 1730 (C=O) cm^{-1}; NMR δ 0.91 (3 H, t, CH_3-20), 1.13 (3 H, s, CH_3-16), 2.07 (3 H, s, $OCOCH_3$), 3.25 (3 H, s, OCH_3), 3.68 (3 H, s, $COOCH_3$), 3.89 (1 H, m, H-11), 4.15 (1 H, d, *J*(H14–H15) = 7 Hz, H-15), 5.17 (1 H, m, H-9), 5.53 (1 H, dd, *J*(H13–H14) = 15 Hz, *J*(H12–H13) = 9 Hz, H-13), 5.71 (1 H, dd, H-14); $[\alpha]_D$ +45.3° (*c* 0.76); TLC (EtOAc).

(15*S*,16*R*)-16-Methoxy-16-methyl-*epi*-PGF$_{2\alpha}$ Methyl Ester (7e). To a stirred solution of **5e** (0.21 g, 0.46 mmol) in dry MeOH (10 mL) was added anhydrous K_2CO_3 (0.15 g). The reaction mixture was stirred at room temperature for 5 h, quenched by the addition of acidic Amberlite CG 120, filtered, and concentrated. Purification of the residue on a column of acidic SiO_2, prepared with 9:1 hexane–Et_2O, gave, at 3:7 hexane–Et_2O, 0.14 g (73.8%) of **7e** as a viscous oil: IR 3610, 3540 (OH), 1730 (C=O) cm^{-1}; NMR δ 0.91 (3 H, t, CH_3-20), 1.13 (3 H, s, CH_3-16), 3.24 (3 H, s, OCH_3), 3.68 (1 H, s, $COOCH_3$), 3.98 (1 H, m, H-11), 4.10 (1 H, br d, *J*(H14–H15) = 2.5 Hz, H-15), 4.21 (1 H, m, H-9), 5.41 (2 H, m, cis CH=CH), 5.63 (2 H, m, trans CH=CH); MS, *m/z* 394 ($M^+ - H_2O$); $[\alpha]_D$ +9.9° (*c* 2.2); TLC (3:7 acetone–hexane). Anal. ($C_{23}H_{40}O_6$) C, H.

(16*R*)-16-Methoxy-16-methyl-PGF$_{2\alpha}$ Methyl Ester (7f). The 15α-alcohol **5f** (0.27 g, 0.59 mmol) was reacted as described above to give 0.20 g (82.1%) of **7f**: IR 3610, 3540 (OH), 1730 (C=O) cm^{-1}; NMR δ 0.91 (3 H, t, CH_3-20), 1.06 (3 H, s, CH_3-16), 3.21 (3 H, s, OCH_3), 3.67 (1 H, s, $COOCH_3$), 4.00 (1 H, m, H-11), 4.09 (1 H, d, *J*(H14–H15) = 5.5 Hz, H-15), 4.18 (1 H, m, H-9), 5.39 (2 H, m, cis CH=CH), 5.58 (2 H, m, trans CH=CH); MS, *m/z* 394 ($M^+ - H_2O$); $[\alpha]_D$ +23.3° (*c* 1.33); TLC (3:7 acetone–hexane). Anal. ($C_{23}H_{40}O_6$) C, H.

(15*S*,16*S*)-16-Methoxy-16-methyl-*epi*-PGF$_{2\alpha}$ Methyl Ester (7g). The 15β-alcohol **5g** (0.22 g, 0.48 mmol) was reacted as described above to give 0.13 g (65.4%) of **7g**: IR 3600, 3530 (OH), 1730 (C=O) cm^{-1}; NMR δ 0.92 (3 H, t, CH_3-20), 1.05 (3 H, s, CH_3-16), 3.21 (3 H, s, OCH_3), 3.67 (3 H, s, $COOCH_3$), 3.97 (1 H, m, H-11), 4.12 (1 H, br d, *J*(H14–H15) = 5.5 Hz, H-15), 4.20 (1 H, m, H-9), 5.40 (2 H, m, cis CH=CH), 5.53–5.66 (2 H, 2 m, trans CH=CH); MS, *m/z* 394 ($M^+ - H_2O$); $[\alpha]_D$ +6.4° (*c* 2.67); TLC (3:7 acetone–hexane). Anal. ($C_{23}H_{40}O_6$) C, H.

(16*S*)-16-Methoxy-16-methyl-PGF$_{2\alpha}$ Methyl Ester (7h). The 15α-alcohol **5h** (0.48 g, 1.06 mmol) was reacted as described above to give 0.23 g (52.6%) of **7h**: IR 3610, 3540 (OH), 1730 (C=O) cm^{-1}; NMR δ 0.90 (3 H, t, CH_3-20), 1.12 (3 H, s, CH_3-16), 3.24 (3 H, s, OCH_3), 3.67 (3 H, s, $COOCH_3$), 3.99 (1 H, m, H-11), 4.06 (1 H, br d, *J*(H14–H15) = 5.5 Hz, H-15), 4.19 (1 H, m, H-9), 5.40 (2 H, m, cis CH=CH), 5.60 (2 H, m, trans CH=CH); MS, *m/z* 394 (M^+ − H_2O); $[\alpha]_D$ +50° (*c* 0.89); TLC (3:7 acetone–hexane). Anal. ($C_{23}H_{40}O_6$) C, H.

(15*S*,16*R*)-16-Methoxy-16-methyl-*epi*-PGE$_2$ Methyl Ester (8e). To a solution of **5e** (2.3 g, 5.06 mmol) in anhydrous benzene (200 mL) at room temperature were added 3,4-dihydro-2*H*-pyran (10 mL) and anhydrous *p*-toluenesulfonic acid (96 mg, 0.506 mmol) in benzene (30 mL). After 30 min, the reaction mixture was poured into a dilute solution of $NaHCO_3$, and the two layers were separated. The organic phase was washed with water, dried, and concentrated. Purification of the residue by column chromatography with hexane and increasing amounts of Et_2O as eluent gave 3 g (95%) of the 11,15-dipyranyl derivative: IR 1630 (C=O) cm^{-1}; NMR δ 0.90 (3 H, t, CH_3-20), 1.09, 1.10, 1.13, 1.14 (3 H, 4 s, CH_3-16), 1.2–2.7 (30 H, m, 14 CH_2 + 2 CH), 2.04 (3 H, s, $COOCH_3$), 3.22–3.27 (3 H, 2 s, OCH_3), 3.4–4.1 (6 H, m, 2 CH_2O + 2 CHO), 3.67 (3 H, s, $COOCH_3$), 4.6–4.8 (2 H, m, 2 OCHO), 5.06 (1 H, m, H-9), 5.3–5.7 (4 H, m, 2 CH=CH); TLC (Et_2O).

To a stirred solution of the 11,15-dipyranyl derivative (3 g, 3.82 mmol) prepared above in dry MeOH (70 mL) was added anhydrous K_2CO_3 (0.69 g). The reaction mixture was stirred at room temperature for 24 h, poured into a saturated solution of NaH_2PO_4, and extracted with Et_2O. The organic extracts were concentrated under reduced pressure, and the aqueous residue was extracted with Et_2O. The organic phase was washed with water, dried, and concentrated to give 2.6 g (93%) of 9-hydroxy-11,15-dipyranyl derivative: IR 3605, 3520 (OH), 1730 (C=O) cm^{-1}; NMR δ 0.90 (3 H, t, CH_3-20), 1.10–1.13 (3 H, 2 s, CH_3-16), 1.2–2.6 (30 H, m, 14 CH_2 + 2 CH), 3.22–3.28 (3 H, 2 s, OCH_3), 3.4–4.2 (7 H, m, 2 CH_2O + 3 CHO), 3.67 (3 H, s, $COOCH_3$), 4.6–4.8 (2 H, m, 2 OCHO), 5.3–5.7 (4 H, m, 2 CH=CH); TLC (Et_2O).

To a stirred suspension of Collins reagent[16] ($Pyr_2 \cdot CrO_3$) (7 g), Celite (3.5 g), and anhydrous CH_2Cl_2 (350 mL) was added a solution of the 9-hydroxy-11,15-dipyranyl derivative (2.6 g, 4.48 mmol) prepared above in anhydrous CH_2Cl_2 (20 mL). After 1 h, the reaction mixture was poured into Et_2O (450 mL) and filtered on a Celite bed. The filtrate was washed with water, dried, and concentrated. Purification of the residue by column chromatography with hexane and increasing amounts of Et_2O gave 2 g (77%) of 9-keto-11,15-dipyranyl derivative: IR 1735 (ester, ketone C=O) cm^{-1}; NMR δ 0.91 (3 H, t, CH_3-20), 1.10, 1.11, 1.14, 1.15 (3 H, 4 s, CH_3-16), 1.2–2.9 (30 H, m, 14 CH_2 + 2 CH), 3.22–3.28 (3 H, 2 s, OCH_3), 3.5–4.3 (6 H, m, 2 CH_2O + 2 CHO), 3.67 (3 H, s, $COOCH_3$), 4.6–4.8 (2 H, m, 2 OCHO), 5.2–5.8 (4 H, m, 2 CH=CH); TLC (7:3 Et_2O–hexane).

A stirred solution of CH_3COOH, water, THF[17] (19:11:3) (33 mL), and the 9-keto-11,15-dipyranyl derivative (2 g, 3.45 mmol) prepared above was heated at 45 °C for 2 h, cooled, diluted with cold water (50 mL), alkalinized with solid $NaHCO_3$ at pH 7.2, and extracted with Et_2O. The organic phase was dried and concentrated. Purification of the residue by column chromatography on acidic SiO_2 with hexane and increasing amounts of Et_2O gave 0.37 g (32%) of pure **8e** as a viscous oil: IR 3600, 3540 (OH), 1735 (ester and ketone C=O), 1670 (C=C) cm^{-1}; NMR δ 0.90 (3 H, t, CH_3-20), 1.13 (3 H, s, CH_3-16), 3.24 (3 H, s, OCH_3), 3.67 (3 H, s, $COOCH_3$), 4.14 (2 H, m, H-11 + H-15), 5.48 (2 H, m, cis CH=CH), 5.74 (2 H, m, trans CH=CH); MS, *m/z* 410 (M^+); $[\alpha]_D$ −62° (c 1); TLC (7:3 EtOAc–CH_2Cl_2). Anal. ($C_{23}H_{38}O_6$) C, H.

(16*R*)-16-Methoxy-16-methyl-PGE$_2$ Methyl Ester (8f). The 15α-alcohol **5f** was reacted as described above to give a similar yield of **8f** as a viscous oil: IR 3600, 3540, 3400 (OH), 1740 (ester and ketone C=O), 1670 (C=C) cm^{-1}; NMR δ 0.91 (3 H, t, CH_3-20), 1.06 (3 H, s, CH_3-16), 3.25 (3 H, s, OCH_3), 3.69 (3 H, s, $COOCH_3$), 4.11 (2 H, m, H-11 + H-15), 5.40 (2 H, m, cis CH=CH), 5.72 (2 H, m, trans CH=CH); MS, *m/z* 410 (M^+); $[\alpha]_D$ −52.2° (c 1.02); TLC (7:3 EtOAc-CH_2Cl_2). Anal. ($C_{23}H_{38}O_6$) C, H.

(15*S*,16*S*)-16-Methoxy-16-methyl-*epi*-PGE$_2$ Methyl Ester (8g). The 15β-alcohol **5g** was reacted as described above to give a similar yield of **8g** as a viscous oil: IR 3600, 3540 (OH), 1740 (ester, ketone C=O) cm^{-1}; NMR δ 0.92 (3 H, t, CH_3-20), 1.06 (3 H, s, CH_3-16), 3.23 (3 H, s, OCH_3), 3.65 (3 H, s, $COOCH_3$), 4.13 (1 H, m, H-11), 4.17 (1 H, m, H-15), 5.40 (2 H, m, cis CH=CH), 5.71 (2 H, m, trans CH=CH); MS, *m/z* 392 (M^+ − H_2O); $[\alpha]_D$ −60.6° (c 1.1); TLC (6:4 acetone–hexane). Anal. ($C_{23}H_{38}O_6$) C, H.

(16*S*)-16-Methoxy-16-methyl-PGE$_2$ Methyl Ester (8h). The 15α-alcohol **5h** was reacted as described above to give a similar yield of 8h as a viscous oil: IR 3605, 3540, 3420 (OH), 1740 (ester, ketone C=O) cm^{-1}; NMR δ 0.91 (3 H, t, CH_3-20), 1.14 (3 H, s, CH_3-16), 3.25 (3 H, s, OCH_3), 3.68 (3 H, s, $COOCH_3$), 4.11 (2 H, m, H-11 + H-15), 5.38 (2 H, m, cis CH=CH), 5.71 (2 H, m, trans CH=CH); MS, *m/z* 392 (M^+ − H_2O); $[\alpha]_D$ −57.2° (*c* 1); TLC (6:4 acetone–hexane). Anal. ($C_{23}H_{38}O_6$) C, H.

(15*S*,16*R*)-16-Methoxy-16-methyl-*epi*-PGE$_1$ Methyl Ester (9e). The 15β-alcohol **6e** was reacted as described above to give a similar yield of **9e** as a viscous oil: IR 3600, 3540 (OH), 1735 (ester and ketone C=O) cm^{-1}; NMR δ 0.92 (3 H, t, CH_3-20), 1.17 (3 H, s, CH_3-16), 3.26 (3 H, s, OCH_3), 3.69 (3 H, s, $COOCH_3$), 4.10 (1 H, m, H-11), 4.17 (1 H, br d, *J*(H14–H15) = 3 Hz, H-15), 5.75 (2 H, m, trans CH=CH); MS, *m/z* 412 (M^+); $[\alpha]_D$ −83.2° (c 1.0); TLC (8:2 EtOAc–CH_2Cl_2). Anal. ($C_{23}H_{40}O_6$) C, H.

(16*R*)-16-Methoxy-16-methyl-PGE$_1$ Methyl Ester (9f). The 15α-alcohol **6f** was reacted as described above to give a similar yield of **9f** as white crystals: mp 49 °C (Et_2O–hexane); IR 3590, 3550, 3390 (OH), 1735 (ester and ketone C=O), 1670 (C=C) cm^{-1}; NMR δ 0.90 (3 H, t, CH_3-20), 1.08 (3 H, s, CH_3-16), 3.23 (3 H, s, OCH_3), 3.68 (3 H, s, $COOCH_3$), 4.08 (1 H, m, H-11), 4.14 (1 H, d, *J*(H15–H14) = 6.5 Hz, H-15), 5.70 (2 H, m, trans CH=CH); MS, *m/z* 412 (M^+); $[\alpha]_D$ −50.3° (*c* 0.96); TLC (8:2 EtOAc–CH_2Cl_2). Anal. ($C_{23}H_{40}O_6$) C, H.

(15*S*,16*S*)-16-Methoxy-16-methyl-*epi*-PGE$_1$ Methyl Ester (9g). The 15β-alcohol **6g** was reacted as described above to give a similar yield of **9g** as a viscous oil: IR 3605, 3560 (OH), 1740 (ester and ketone C=O), 1670 (C=C) cm^{-1}; NMR δ 0.94 (3 H, t, CH_3-20), 1.06 (3 H, s, CH_3-16), 3.24 (3 H, s, OCH_3), 3.69 (3 H, s, $COOCH_3$), 4.11 (1 H, m, H-11), 4.19 (1 H, d, *J*(H14–H15) = 3.5 Hz, H-15), 5.75 (2 H, m, trans CH=CH); MS, *m/z* 394 (M^+ − H_2O); $[\alpha]_D$ −87.2° (*c* 1.00); TLC (8:2 EtOAc-CH_2Cl_2). Anal. ($C_{23}H_{40}O_6$) C, H.

(16*S*)-16-Methoxy-16-methyl-PGE$_1$ Methyl Ester (9h). The 15α-alcohol **6h** was reacted as described above to give a similar yield of **9h** as a viscous oil: IR 3600, 3530, 3400 (OH), 1740 (ester and ketone C=O), 1670 (C=C) cm^{-1}; NMR δ 0.91 (3 H, t, CH_3-20), 1.13 (3 H, s, CH_3-16), 3.26 (3 H, s, OCH_3), 3.67 (3 H, s, $COOCH_3$), 4.07 (1 H, m, H-11), 4.11 (1 H, d, *J*(H14–H15) = 7 Hz, H-15), 5.66 (1 H, dd, *J*(H13–H14) = 15 Hz, *J*(H13–H12) = 9 Hz, H-13), 5.80 (1 H, dd, H-14); MS, *m/z* 394 (M^+ − H_2O); $[\alpha]_D$ −57.7° (*c* 1.01); TLC (8:2 EtOAc–CH_2Cl_2). Anal. ($C_{23}H_{40}O_6$) C, H.

Gastric Antisecretory, Antifertility, and Diarrheal Studies. Gastric secretion was evaluated in the stomach perfusion test of the anesthetized rat according to the method of Ghosch and Schild as previously described.[15] Gastric hypersecretion was induced by continuous intravenous (iv) infusion of histamine (1.5 mg/kg per hour). In some cases the effect of spontaneous secretion of conscious rats with a chronic gastric cannula was also evaluated, with the compound administered intragastrically (ig).[15] The doses inhibiting secretion by 50% (ID_{50}) were estimated from the graph of the response, plotted as percentage of control values, vs. the log of the dose. The antifertility test was carried out in Syrian Golden hamsters by a procedure previously described.[18] Briefly, the animals were treated for three consecutive days, starting on the fourth day of gestation. The animals were killed on the tenth day of gestation, and the numbers of implantation sites and live fetuses were counted. The ED_{50} value is the dose required to induce pregnancy arrest in 50% of the animals. The diarrheal

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effect was evaluated by the method of Randall and Baruth.[19] Charles River male CD_1 mice weighing 20–22 g were fasted for 24 h before the test. The mice, 10 animals per dose, were given orally logarithmically graded doses of each prostaglandin. Immediately after administration, the animals were put into individual cages with blotting paper in the bottom for 1 h, and diarrhea was scored from the size of the spots of dampness produced by stools, with use of the arbitrary scale from 0 to 4 as described.[19] The ED_{50} values were calculated by the method of Miller and Tainter.[20]

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Registry No. **1a**, 61408-40-0; **1b**, 76817-33-9; (±)-**2a**, 61408-83-1; **2b**, 76831-72-6; **4a**, 61408-57-9; **4b**, 61408-58-0; **4c**, 76817-34-0; **4d**, 76817-35-1; **5e**, 103456-75-3; **5e** (dipyranyl deriv.), 103530-63-8; **5f**, 103456-76-4; **5f** (dipyranyl deriv.), 103530-64-9; **5g**, 103456-77-5; **5g** (dipyranyl deriv.), 103530-65-0; **5h**, 103456-78-6; **5h** (dipyranyl deriv.), 103530-66-1; **6e**, 103456-79-7; **6e** (dipyranyl deriv.), 76817-44-2; **6e** (9-hydroxy; 11,15-dipyranyl), 76817-48-6; **6f**, 103456-80-0; **6f** (dipyranyl deriv.), 76817-42-0; **6f** (9-hydroxy-11,15-dipyranyl deriv.), 76817-46-4; **6g**, 103456-81-1; **6g** (dipyranyl deriv.), 76817-45-3; **6g** (9-hydroxy-11,15-dipyranyl deriv.), 76817-49-7; **6h**, 103456-82-2; **6h** (dipyranyl deriv.), 76817-43-1; **6h** (9-hydroxy-11,15-dipyranyl deriv.), 76817-47-5; **7e**, 61408-62-6; **7e** (11,15-dipyranyl deriv.), 103530-67-2; **7f**, 61408-61-5; **7f** (11,15-dipyranyl deriv.), 103530-68-3; **7g**, 61408-59-1; **7g** (11,15-dipyranyl deriv.), 103530-69-4; **7h**, 61408-60-4; **7h** (11,15-dipyranyl deriv.), 103530-70-7; **8e**, 61408-67-1; **8e** (dipyranyl deriv.), 103530-71-8; **8f**, 61408-68-2; **8f** (dipyranyl deriv.), 103530-72-9; **8g**, 61408-65-9; **8g** (dipyranyl deriv.), 103530-73-0; **8h**, 61408-66-0; **8h** (dipyranyl deriv.), 103530-74-1; **9e**, 76817-54-4; **9e** (dipyranyl deriv.), 76817-52-2; **9f**, 76822-56-5; **9f** (dipyranyl deriv.), 76817-50-0; **9g**, 76817-55-5; **9g** (dipyranyl deriv.), 76817-53-3; **9h**, 76817-56-6; **9h** (dipyranyl deriv.), 76817-51-1; (±)-**10**, 70908-63-3; **11a**, 103456-74-2; **11a** (*S*-amphetamine salt), 103456-83-3; **11b**, 103456-84-4; **11b** (*S*-amphetamine salt), 103456-85-5; **12a**, 103530-62-7; 2-hexanone, 591-78-6; (±)-2-hydroxy-2-methylhexanenitrile, 103456-72-0; (±)-2-hydroxy-2-methylhexanoic acid methyl ester, 103530-61-6; (±)-2-methoxy-2-methylhexanoic acid methyl ester, 103456-73-1; dimethyl methylphosphonate, 756-79-6.

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Dihydropyridazinone Cardiotonics: The Discovery and Inotropic Activity of 1,3-Dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2*H*-indol-2-one[1,2]

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We discovered that **6** (*N*-[4-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)phenyl]acetamide) is a potent positive inotrope in dogs, and we have prepared several lactam analogues of this agent. These included **16** (1,3-dihydro-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2*H*-indol-2-one), **32** (the analogous quinolin-2-one), and **37** (the analogous benzazepin-2-one). The inotropic ED_{50}'s of these compounds were 24, 3.3, and 5.2 μg/kg, respectively, after iv administration to pentobarbital-anesthetized dogs. Compound **20** (LY195115, 1,3-dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2*H*-indol-2-one), the geminal dimethyl analogue of **16**, was 3.5-fold more potent than **16** when administered iv (ED_{50} = 6.8 μg/kg). However, the most profound effect of the geminal alkyl substitution was on oral activity. The approximate ED_{50}'s of **20** and **16** after oral administration to conscious dogs were 25 and 400 μg/kg, respectively. The increase in contractility produced by 25 μg/kg of **20** was maximally sustained in excess of 8 h. Thus, **20** is one of the most potent and long-acting oral inotropes described to date.

Although several new drugs for the treatment of congestive heart failure (CHF) have been introduced in recent years, many patients remain symptomatically compromised and mortality continues to be high.[3] The most salient pathophysiological features of CHF are a diminution of ventricular contractility and profound, sympathetically mediated vasoconstriction. Thus, both peripheral vasodilators[4] and positive inotropes[5] have salubrious effects in CHF patients; the hemodynamic effects of these two classes of drugs are similar.[6]

Development of a new generation of cardiotonics with combined inotropic and vasodilator activities for the chronic management of CHF has engendered considerable interest. Such agents with dual activities ameliorate the symptoms of CHF by simultaneously exerting a direct positive inotropic effect on the failing myocardium and reducing impedance to ventricular ejection. Several of these dual-activity cardiotonics, from diverse chemical

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(2) A note on nomenclaure: For most of the compounds described in this paper (e.g., **6**, **7**, and **20**) the dihydropyridazinone moiety is systematically named as a 1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl substituent. Hence, a methyl substituent β to the dihydropyridazinone carbonyl is termed a 4-methyl substituent. However, some of the compounds mentioned in this paper are systematically named as 6-aryl-4,5-dihydro-3(2*H*)-pyridazinones (e.g., **38** and **39**). For these compounds a methyl substituent β to the dihydropyridazinone carbonyl is termed a 5-methyl substituent (note ref 40).

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