123883-17-0; **17b**·HCl, 123883-18-1; (*R*)-**18**, 23357-47-3; (*S*)-**18**, 85977-52-2; (*R*)-**19**, 123883-19-2; (*R*)-**19**·HCl, 123883-20-5; (*S*)-**19**, 123883-21-6; **20**, 123883-22-7; **21**, 123883-23-8; **21**·HCl, 123883-24-9; **22**, 123883-25-0; **23**, 123883-26-1; **23**·HCl, 123883-27-2; **24**, 123883-28-3; **25**, 123883-29-4; **25**·HCl, 123883-30-7; **26a**, 30933-66-5; **26b**, 123883-31-8; **27a**, 16176-73-1; **27b**, 123883-32-9; **27** (X = Cl, R = CHO), 123883-33-0; **28a**, 94980-84-4; **28b**, 123883-34-1; **29**, 39830-66-5; **30**, 123883-35-2; **31**, 38073-22-2; **32**, 38073-26-6; **32**·HCl, 38073-29-9; $AcO(CH_2)_2NO_2$, 18942-89-7; $MeNH(CH_2)_2OH$, 109-83-1; $MeNHCH_2CH(OMe)_2$, 122-07-6; 8-(4-chlorobutyl)-8-azaspiro[4.5]decane-7,9-dione, 21098-11-3; 2,3-dihydro-1*H*-indene-1-carboxylic acid, 14381-42-1; 6-methoxyindan-1-carboxylic acid, 62956-62-1; 5-methoxy-2-methyl-2,3,4,8,9,9a-hexahydro-1*H*-indeno[1,7-*cd*]azepin-1-one, 123883-36-3; benzocycloheptene-1-carboxylic acid, 14378-56-4; 2-cyano-1,2,3,4,8,9,10,10a-octahydronaphth[1,8-*cd*]azepine, 123883-37-4; 5-chloro-1,2,3,4,8,9,10,10a-octahydronaphth[1,8-*cd*]azepine, 123883-38-5; *N*-(2-hydroxyethyl)-*N*-methyl-1-tetralincarboxamide, 123883-39-6; 7-methoxy-1,2,3,4-tetrahydro-1-naphthaleneacetic acid, 27559-29-1; 10-methoxy-3,4,4a,5,6,7-hexahydronaphth[1,8-*cd*]azepine, 123883-40-9; 7-methoxy-1,2,3,4-tetrahydronaphthalene-1-methanamine, 57314-45-1; 4-methoxy-1-(*p*-tolylsulfonyl)-2,3,7,8,9,9a-hexahydro-1*H*-benzo[*de*]quinoline, 123883-41-0; 4-methoxy-2,3,7,8,9,9a-hexahydro-1*H*-benzo[*de*]quinoline, 123883-42-1; *N*-tosyl-5-chloro-4-(cyanomethyl)indole, 99696-42-1; 1-tetralincarboxylic acid, 1914-65-4; 7-chloro-1-tetralincarboxylic acid, 91193-16-7; 7-methoxy-1-tetralincarboxylic acid, 85858-95-3; 6-chloro-1-indancarboxylic acid, 52651-15-7; 2,3,4,5-tetrahydro-1*H*-3-benzazepine, 4424-20-8; 6-chloro-2,3,4,5-tetrahydro-1*H*-3-benzazepine, 26232-35-9.

Total Synthesis of the Four Stereoisomers of Dihexadecanoyl Phosphatidylinositol and the Substrate Stereospecificity of Human Erythrocyte Membrane Phosphatidylinositol 4-Kinase

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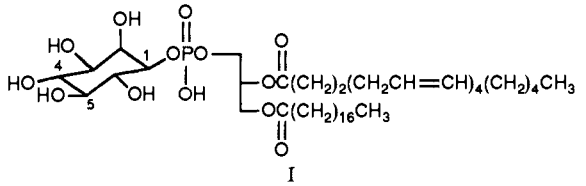
A new and convenient method for the preparation of the four stereoisomers of dihexadecanoyl phosphatidylinositol has been developed. An enantiomeric pair of acid-labile, pentaprotected *myo*-inositol building blocks was synthesized in high yield and coupled with chiral phenyl dihexadecanoylglyceryl phosphates to give the fully protected phosphatidylinositols. These were subsequently deprotected by hydrogenolysis and self-hydrolysis in aqueous ethanol to give the desired pure products. Comparison of these compounds as potential substrates for a partially purified phosphatidylinositol 4-kinase (EC 2.7.1.67) derived from human erythrocyte membranes revealed that the chirality of the inositol ring is crucial for efficient phosphorylation, whereas the chirality of the glycerol moiety is relatively unimportant. Moreover, the similarity in phosphorylation rates of the naturally occurring mammalian phospholipid, I, and its synthetic stereochemical counterpart, compound **10a**, suggests that the enzyme is relatively tolerant to changes in fatty acid composition.

The identification of D-*myo*-inositol 1,4,5-tris(phosphate)[1] and diacylglycerol[2] as intracellular second messengers has led to wide interest in enzymes that regulate their synthesis and metabolism. Their precursor is the minor membrane phospholipid phosphatidylinositol 4,5-bis(phosphate) (PIP_2), which is cleaved by a receptor-coupled phospholipase C upon stimulation by a range of neurotransmitters, hormones, and growth factors.[3]

In order to maintain the supply of PIP_2, rapid sequential phosphorylation of the more abundant phospholipid phosphatidylinositol (PI, I) by specific kinases occurs.



I

Initially, the 4-hydroxyl group of PI is phosphorylated by a PI 4-kinase to give phosphatidylinositol 4-phosphate (PIP), followed by a PIP 5-kinase-catalyzed phosphorylation of the 5-hydroxyl group.[3] PI is also a substrate for phospholipases A[4] and C[5] and a 3-kinase.[6] The relative importance of these different enzymes in modulating the production of second messengers is thus far unclear, and more detailed information about their specificity and kinetics requires supplies of pure, chemically and stereochemically well-defined PI analogues. Although various methods have been reported for the synthesis of chiral PI analogues[7] following the pioneering work of Shvets et al.[8] in 1970, many stages, including the resolution of the inositol building block, have been hampered by low yields and lack of reproducibility. Here, we describe an unambiguous route for the preparation of the four stereoisomers of the dihexadecanoyl analogue of I in good yield and high chemical purity, starting from readily available materials, and their use as probes to study the stereochemical requirements for binding to a partially purified PI 4-kinase derived from human erythrocyte membranes.

Chemistry

The observation that 2,3(1):5,6(4)-di-*O*-isopropylidene-D-*myo*-inositol could be regioselectively silylated at the

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§ Research and Development Division, SK&F U.S.A.

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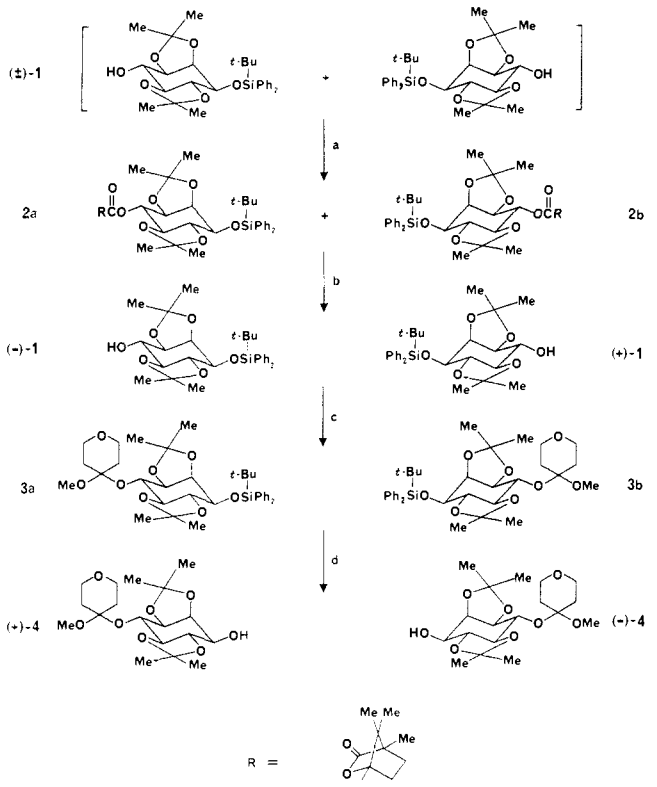
Scheme I[a]



[a] Reagents: a, (−)-camphanic acid chloride, pyridine, dioxane; b, KOH, EtOH; c, 5,6-dihydro-4-methoxy-2*H*-pyran, pyridinium *p*-toluenesulfonate, CH_2Cl_2; d, Bu_4NF, THF.

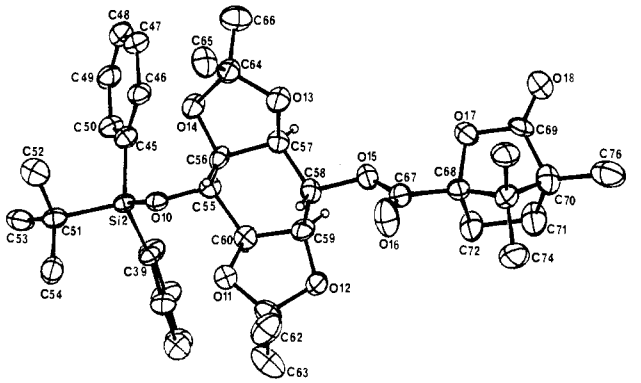


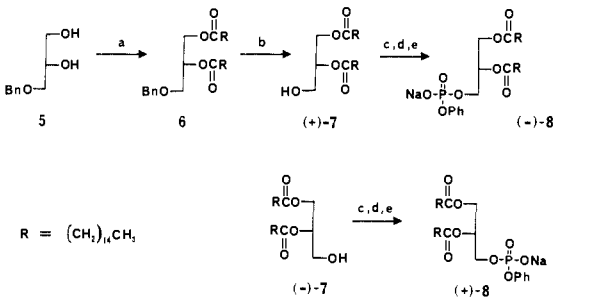
Figure 1. Absolute configuration of 1-*O*-(*tert*-butyldiphenylsilyl)-4-*O*-camphanyl-2,3:5,6-di-*O*-isopropylidene-D-*myo*-inositol (**2a**).

1(3)-position with *tert*-butyldiphenylsilyl chloride[9] offered the product ((±)-1) as a suitable starting point in the synthetic route.[10] Initially, (±)-1 was separated into its enantiomers via the diastereoisomeric pair of camphanyl derivatives, **2a** and **2b**, prepared from (−)-camphanyl chloride[11] (Scheme I), and separated by HPLC. The absolute stereochemistry of the less polar ester, **2a**, was established as the 1-*O*-silyl-D-*myo*-inositol derivative by single-crystal X-ray analysis (see Figure 1). Esters **2a** and **2b** were subsequently hydrolyzed with alcoholic potassium hydroxide to give the resolved silyl derivatives (+)-1 and (−)-1 and converted to the 4-methoxytetrahydropyran-4-yl derivatives[12] **3a** and **3b**. Finally, desilylation of **3a** and **3b** with tetrabutylammonium fluoride furnished the chiral, pentaprotected alcohol building blocks (+)-4 and (−)-4.

The chiral glycerol building blocks employed were the novel phenyl esters of 1,2-di-*O*-hexadecanoyl-*sn*-glycer-3-yl phosphate ((+)-8) and 2,3-di-*O*-hexadecanoyl-*sn*-glycer-1-yl phosphate ((−)-8). These were prepared from known starting materials as shown in Scheme II, by using standard methods (see the Experimental Section).

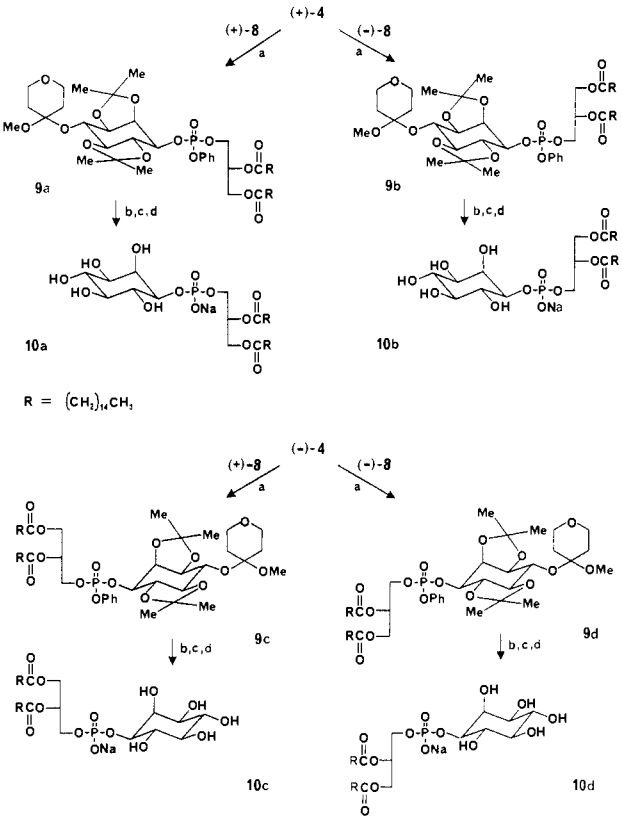
Coupling of the inositol and glycerol building blocks was accomplished rapidly and in reasonable yields using the condensing agent 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) in pyridine,[13] as shown in Scheme III.

Scheme II[a]



[a] Reagents: a, $H_3C(CH_2)_{14}COCl$, pyridine, CH_2Cl_2; b, H_2, Pd/C (5%), EtOH; c, $PhOP(O)Cl_2$, pyridine; d, H_2O; e, Amberlite IRC-50 (Na form), aqueous EtOH.

Scheme III[a]



[a] Reagents: a, MSNT, pyridine; b, H_2, PtO_2, EtOH; c, H_2O, EtOH; d, Amberlite IRC-50 (Na form), aqueous EtOH.

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Table I. Comparison of **10a–d** and I as Substrates for Human Erythrocyte PI 4-Kinase[a]

compound	formation of PIP	
	pmol min^{-1}	relative rate, %
I	23.5 ± 0.74	100
10a	24.9 ± 1.41	106
10b	23.5 ± 1.57	100
10c	1.01 ± 0.09	4.3
10d	0.76 ± 0.05	3.2

[a] Partially purified human erythrocyte PI 4-kinase was incubated with mammalian PI (I) or analogues at a final assay concentration of 200 μM as described in the Experimental Section. The data represent the mean ± standard error for a representative assay conducted in triplicate.

Sequential deprotection of the products (**9a–d**) was performed first by removing the phenyl group by hydrogenolysis, followed by hydrolysis of the inositol protecting groups in aqueous ethanol using the acidity of the phosphate diester thus produced to catalyze the reaction. On partial concentration and centrifugation, the phosphatidylinositols were obtained as white solids, which were subsequently converted to the stable sodium salts (**10a–d**) by ion exchange.

High-field proton NMR spectroscopic analysis of the products showed them to be pure and essentially similar in the inositol and glycerol resonances to a sample of the naturally occurring phosphatidylinositol D-*myo*-inositol 1-(1-*O*-octadecanoyl-2-*O*-(5,8,11,14-eicosatetraenoyl)-*sn*-glycer-3-yl phosphate) (**I**) derived from bovine liver. A comparison of the NMR spectra of these two compounds is available as supplementary material (Figure 2).

Results and Discussion

Phosphatidylinositol analogues possess two asymmetric centers. All naturally occurring analogues, whether extracted from mammalian or plant tissues, contain a *myo*-inositol system substituted at the D-1 position and a glycerol moiety acylated at the *sn*-1- and 2-positions, although the fatty acid composition is variable.[14] Whether this stereochemical arrangement is important for these phospholipids to be accepted as substrates for the membrane-bound PI 4-kinase is a question of fundamental importance for furthering our understanding of intracellular signaling mechanisms and also as a basis for rational substrate-based inhibitor design. We have therefore compared the rates of phosphorylation of the four synthetic dihexadecanoyl PI stereoisomers (**10a–d**) with that of the mammalian PI (I) using a partially purified human erythrocyte membrane PI 4-kinase, and the results are presented in Table I.

The mammalian phospholipid I and its synthetic stereochemical counterpart, **10a**, were found to phosphorylate at very similar rates. Moreover, the diastereoisomer of **10a**, having the opposite configuration in the glycerol moiety (**10b**), is accepted equally well as a substrate. Conversely, compounds **10c** and **10d**, in which the inositol moiety is substituted at the L-1 (D-3) position, are very poor substrates for the kinase, phosphorylating at only some 3–4% of the rate of the analogues having a D-1-substituted inositol. These two compounds, which differ only in glycerol configuration, are phosphorylated at similar rates and show a lack of stereospecificity in this part of the molecule, as was found in **10a** and **10b**.

It is therefore evident that neither the structure nor the configuration of the diacylglycerol portion of I is essential for efficient phosphorylation to the corresponding PIP derivative, and it is likely that this part of the molecule serves only to anchor the lipid to the membrane, or in the in vitro assay described here, to the mixed substrate/detergent micelle. That the chirality of the inositol ring is crucial for efficient phosphorylation by the kinase suggests that the axial hydroxyl group in the 2-position plays a key role in recognition by the enzyme active site.

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Experimental Section

Chemistry. ^1H NMR spectra were recorded with a Bruker AM250 (250 MHz) or a Bruker AM360 (360 MHz) spectrometer, and ^{31}P spectra were recorded at 145.8 MHz on a Bruker AM360 spectrometer. EI mass spectra were recorded on a VG analytical 70-70F double-focusing mass spectrometer employing 70-eV electron ionization and a trap current of 200 mA. FAB spectra were obtained on a VG analytical 70-250 SEQ hybrid mass spectrometer. Ionization was effected with xenon as bombarding gas at 8 kV. Glycerol/thioglycerol 1:1 was used as matrix, with acidification using dilute HCl. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. Microanalytical data, where determined, are within 0.4% of theoretical values unless stated, and melting points are uncorrected.

Mammalian PI (I, containing predominantly D-*myo*-inositol 1-(1-*O*-octadecanoyl-2-*O*-(5,8,11,14-eicosatetraenoyl)-*sn*-glycer-3-yl phosphate) as the ammonium salt) was purchased from Sigma Chemical Co Ltd., adenosine 5′-[γ-^{32}P]triphosphate (3000 Ci $mmol^{-1}$) was obtained from Amersham International Plc, and dithiothreitol, Triton X-100, and ATP were supplied by Boehringer Mannheim Ltd.

1-*O*-(*tert*-Butyldiphenylsilyl)-4-*O*-camphanyl-2,3:5,6-di-*O*-isopropylidene-D-*myo*-inositol (2a) and 3-*O*-(*tert*-Butyldiphenylsilyl)-6-*O*-camphanyl-1,2:4,5-di-*O*-isopropylidene-D-*myo*-inositol (2b). Solid (−)-camphanic acid chloride (5.86 g, 27.0 mmol) was added in one portion to a solution of 1(3)-*O*-(*tert*-butyldiphenylsilyl)-2,3(1):5,6(4)-di-*O*-isopropylidene-D-*myo*-inositol ((±)-1,[10] 9.00 g, 18.0 mmol) and dry pyridine (2.2 mL, 27.3 mmol) in dry dioxane (100 mL). The mixture was then stirred at room temperature for 4.5 h. Further (−)-camphanic acid chloride (2.00 g, 9.23 mmol) and dry pyridine (0.75 mL, 9.30 mmol) were added, and stirring was continued as before. The mixture was allowed to stand overnight and was diluted to ca. 5 times its volume with Et_2O. The solution was washed with aqueous NaCl and then H_2O, dried ($MgSO_4$), and evaporated to a gum. This was chromatographed (SiO_2, C_6H_{14}/Et_2O) to give the required mixture of diastereoisomers **2a** and **2b** as a white solid (11.9 g, 97%). Separation of the diastereoisomers was effected by preparative HPLC on a 25 × 2.25 cm Spherisorb silica column (SiO_2 10 μm, C_6H_{14}/THF 93:7). Both isomers were colorless crystalline solids. The less polar isomer (**2a**) was obtained as large prisms from C_6H_{14}: mp 179–180 °C; $[\alpha]^{20}_D$ +4.4° (*c* 1.54 in MeCN); ^1H NMR (250 MHz, $CDCl_3$) δ 0.93 (3 H, s), 1.01 (3 H, s), 1.08 (9 H, s), 1.09 (3 H, s), 1.27 (3 H, s), 1.34 (3 H, s), 1.46 (3 H, s), 1.60 (3 H, s), 1.65 (1 H, m), 1.89 (1 H, m), 2.03 (1 H, m), 2.44 (1 H, m), 3.25 (1 H, dd, *J* = 9.2, 11.1 Hz), 3.87–3.95 (2 H, m), 4.00 (1 H, dd, *J* = 3.9, 9.9 Hz), 4.15, (1 H, dd, *J* = 9.2, 9.9 Hz), 5.33 (1 H, dd, *J* = 6.4, 11.1 Hz), 7.34–7.47 (6 H, m), 7.78–7.84 (4 H, m); MS *m/e* 678 (M^+) (not observed), 663 (5), 621 (10), 563 (69), 365 (100), 279 (98). Anal. ($C_{38}H_{50}O_9Si$) C, H.

The more polar isomer (**2b**) was also obtained as prisms from C_6H_{14}: mp 192.5–193.5 °C; $[\alpha]^{20}_D$ −9.0° (*c* 0.62 in MeCN); ^1H NMR (250 MHz, $CDCl_3$) δ 0.94 (3 H, s), 1.00 (3 H, s), 1.08 (9 H, s), 1.09 (3 H, s), 1.26 (3 H, s), 1.37 (3 H, s), 1.46 (3 H, s), 1.60 (3 H, s), 1.65 (1 H, m), 1.89 (1 H, m), 2.04 (1 H, m), 2.41 (1 H, m), 3.25 (1 H, dd, *J* = 9.2, 11.2 Hz), 3.84 (1 H, dd, *J* = 4.7, 6.9 Hz), 3.91 (1 H, dd, *J* = 4.0, 4.7 Hz), 4.00 (1 H, dd, *J* = 4.0, 10.0 Hz), 4.16 (1 H, dd, *J* = 9.2, 10.0 Hz), 5.36 (1 H, dd, *J* = 6.9, 11.2 Hz), 7.33–7.47 (6 H, m), 7.78–7.85 (4 H, m).

1-*O*-(*tert*-Butyldiphenylsilyl)-2,3:5,6-di-*O*-isopropylidene-D-*myo*-inositol ((−)-1). A solution of KOH (5.60 g, 100 mmol) in EtOH (200 mL) was added to a solution of **2a** (2.00 g, 2.95 mmol) in EtOH (200 mL). After the resulting mixture was stirred at room temperature for 30 min, glacial AcOH (5.7 mL) was added and the EtOH solution was evaporated to dryness. The resulting material was partitioned between Et_2O and aqueous

$NaHCO_3$, and the Et_2O layer was washed with H_2O, dried ($MgSO_4$), and evaporated to a gum. Flash chromatography of this material (SiO_2, C_6H_{14}/Et_2O) afforded (−)-1 as a white solid foam (1.38 g, 94%). A small sample was subsequently crystallized from C_6H_{14}: mp 127–128 °C; $[\alpha]^{20}_D$ −2.5° (*c* 2.02 in MeCN); ^1H NMR (250 MHz, $CDCl_3$) δ 1.07 (9 H, s), 1.27 (3 H, s), 1.42 (3 H, s), 1.49 (3 H, s), 1.55 (3 H, s), 2.35 (1 H, d, *J* = 2.9 Hz), 3.13 (1 H, m), 3.72 (1 H, dd, *J* = 4.8, 6.6 Hz), 3.82–3.94 (2 H, m), 3.98–4.10 (2 H, m), 7.33–7.47 (6 H, m), 7.78–7.86 (4 H, m). Anal. ($C_{28}H_{38}O_6Si$) C, H.

3-*O*-(*tert*-Butyldiphenylsilyl)-1,2:4,5-di-*O*-isopropylidene-D-*myo*-inositol ((+)-1). Camphanate ester **2b** (0.726 g, 1.07 mmol) was saponified with KOH (2.03 g, 36.2 mmol) in EtOH (140 mL) in an identical manner with that described for **2a** above. The product alcohol (+)-1 was obtained after chromatography as a solid foam (0.496 g, 93%). A sample was crystallized from C_6H_{14}: mp 127–129 °C; $[\alpha]^{20}_D$ +2.3° (*c* 2.10 in MeCN); ^1H NMR (250 MHz, $CDCl_3$) δ 1.07 (9 H, s), 1.27 (3 H, s), 1.42 (3 H, s), 1.49 (3 H, s), 1.55 (3 H, s), 1.63 (3 H, s), 2.46 (1 H, d, *J* = 2.9 Hz), 3.13 (1 H, m), 3.71 (1 H, dd, *J* = 4.8, 6.6 Hz), 3.82–3.93 (2 H, m), 3.98–4.10 (2 H, m), 7.33–7.44 (6 H, m), 7.78–7.86 (4 H, m). Anal. ($C_{28}H_{38}O_6Si$) C, H.

1-*O*-(*tert*-Butyldiphenylsilyl)-4-*O*-(4-methoxytetrahydropyran-4-yl)-2,3:5,6-di-*O*-isopropylidene-D-*myo*-inositol (3a). Compound (−)-1 (0.396 g, 0.794 mmol) was stirred at room temperature with 5,6-dihydro-4-methoxy-2*H*-pyran (0.920 g, 8.06 mmol) and pyridinium *p*-toluenesulfonate (0.048 g, 0.190 mmol) over ground, activated 4-Å molecular sieves (0.359 g) in dry CH_2Cl_2 (3.0 mL). After 1 h, further 5,6-dihydro-4-methoxy-2*H*-pyran (0.920 g, 8.06 mmol) was added and the resulting mixture was stirred at room temperature overnight. The reaction mixture was then filtered and the filtrate was diluted with Et_2O and washed, first with saturated aqueous $NaHCO_3$ solution and then with H_2O. The organic phase was dried ($MgSO_4$) and evaporated to a gum which was flash chromatographed (SiO_2, C_6H_{14}/Et_2O), giving **3a** as a white solid foam (0.403 g, 87%), which appeared by TLC and NMR to contain a trace contaminant. This material was desilylated without further purification.

3-*O*-(*tert*-Butyldiphenylsilyl)-6-*O*-(4-methoxytetrahydropyran-4-yl)-1,2:4,5-di-*O*-isopropylidene-D-*myo*-inositol (3b). This compound was prepared from (+)-1 (0.459 g, 0.920 mmol) and 5,6-dihydro-4-methoxy-2*H*-pyran (1.53 g, 13.4 mmol) in CH_2Cl_2 (3.5 mL) in the presence of ground activated molecular sieves (0.416 g) and pyridinium *p*-toluenesulfonate (0.056 g, 0.220 mmol) exactly as described in the preparation of **3a** above. The product, **3b**, was obtained as a white solid foam (0.539 g, 96%), containing a trace contaminant. Again this material was desilylated without further purification.

4-*O*-(4-Methoxytetrahydropyran-4-yl)-2,3:5,6-di-*O*-isopropylidene-D-*myo*-inositol ((+)-4). Compound **3a** (0.400 g, 0.650 mmol) in THF (4.0 mL) was treated with tetrabutylammonium fluoride (1.0 M solution in THF; 4.0 mL) and the mixture was stirred at room temperature for 16 h. The reaction mixture was separated by chromatography (SiO_2, C_6H_{14}/Et_2O) and the product, (+)-4, obtained as a gum was crystallized from C_6H_{14} as fine white needles (0.180 g, 70%): mp 122–122.5 °C; $[\alpha]^{20}_D$ +34.9° (c 0.53 in MeCN); $(M + H)^+$ = 375.2078 (FAB), $C_{18}H_{31}O_8$ requires 375.2019; ^1H NMR (250 MHz, $CDCl_3$) δ 1.35 (3 H, s), 1.41 (3 H, s), 1.43 (3 H, s), 1.53 (3 H, s), 1.70–2.00 (4 H, m), 2.41 (1 H, d, *J* = 8.7 Hz), 3.30 (3 H, s), 3.33 (1 H, dd, *J* = 9.4, 10.4 Hz), 3.59–3.85 (5 H, m), 3.93–4.05 (2 H, m), 4.13 (1 H, dd, *J* ≃ 5.6, 5.6 Hz), 4.46 (1 H, dd, *J* ≃ 4.9, 4.9 Hz).

6-*O*-(4-Methoxytetrahydropyran-4-yl)-1,2:4,5-di-*O*-isopropylidene-D-*myo*-inositol ((−)-4). Treatment of **3b** (0.509 g, 0.830 mmol) in THF (5 mL) with tetrabutylammonium fluoride (1.0 M solution in THF; 5.0 mL) followed by workup and chromatography as described for (+)-4 above furnished the product (−)-4 as fine white needles from C_6H_{14} (0.212 g, 55%): mp 122–123 °C; $[\alpha]^{20}_D$ −38.7° (*c* 0.284 in MeCN). No molecular ion was observed in either high-resolution EI or FAB MS. In the EI spectrum $(M - CH_3)^+$ = 359.171, $C_{17}H_{27}O_8$ requires 359.1706; ^1H NMR (250 MHz, $CDCl_3$) δ 1.35 (3 H, s), 1.41 (3 H, s), 1.43 (3 H, s), 1.53 (3 H, s), 1.70–2.00 (4 H, m), 2.45 (1 H, d, *J* = 8.7 Hz), 3.30 (3 H, s), 3.33 (1 H, dd, *J* = 9.4, 10.4), 3.60–3.85 (5 H, m), 3.93–4.06 (2 H, m), 4.13 (1 H, dd, *J* ≃ 5.6, 5.6 Hz), 4.46 (1 H, dd, *J* ≃ 4.9, 4.9 Hz).

1-*O*-Benzyl-2,3-di-*O*-hexadecanoyl-*sn*-glycerol (6). To a stirred, ice-cooled solution of **5**[15] (3.43 g, 18.8 mmol) in dry CH_2Cl_2 (60 mL) was added dry pyridine (3.13 g, 39.5 mmol) followed by slow addition of hexadecanoyl chloride (10.9 g, 39.5 mmol) over 30 min. Stirring was continued at room temperature overnight, after which the mixture was diluted with Et_2O and treated with H_2O. The Et_2O layer was washed with more H_2O, dried ($MgSO_4$), and evaporated to an oil, which solidified. Recrystallization from MeOH gave **6** as a white powder (11.4 g, 92%): mp 41–42 °C; $[\alpha]^{20}_D$ −6.1° (c 2.0 in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.88 (6 H, t, *J* = 6.6 Hz), 1.25 (48 H, br s), 1.60 (4 H, br m), 2.30 (4 H, m), 3.59 (2 H, d, *J* = 5.2 Hz), 4.19 (1 H, dd, *J* = 6.4, 11.9 Hz), 4.35 (1 H, dd, *J* = 3.8, 11.9 Hz), 4.54 (2 H, m), 5.24 (1 H, m), 7.24–7.38 (5 H, m). Anal. ($C_{42}H_{74}O_5$) C, H.

2,3-Di-*O*-hexadecanoyl-*sn*-glycerol ((+)-7). Compound **6** (9.06 g, 13.8 mmol) was dissolved in EtOH (600 mL) at 40 °C and hydrogenolyzed over Pd/C (2.0 g, 5%) at 50 psi pressure for 2.5 h. The resulting solution was cooled, filtered, and evaporated to dryness to give a white solid. A further batch of solid was obtained by extracting the catalyst residue with hot MeOH to give a total of 7.00 g of (+)-7 (90%). A sample of this was recrystallized from EtOH to give colorless crystals: mp 67.5–68.0 °C; $[\alpha]^{20}_D$ +1.9° (*c* 0.98 in $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.88 (6 H, t, *J* = 6.6 Hz), 1.25 (48 H, br s), 1.63 (4 H, br m), 2.13 (1 H, br s), 2.33 (4 H, m), 3.73 (2 H, d, *J* = 4.8 Hz), 4.28 (2 H, m), 5.08 (1 H, m). Anal. ($C_{35}H_{68}O_5$) C, H.

Sodium 2,3-Di-*O*-hexadecanoyl-*sn*-glycer-1-yl Phenyl Phosphate ((−)-8). To a stirred, ice-cooled solution of phenyl dichlorophosphate (0.560 g, 2.65 mmol) in dry pyridine (10 mL) under dry N_2 was added a solution of (+)-7 in pyridine (10 mL) and CH_2Cl_2 (10 mL) over 45 min. After addition was complete, the cooling bath was removed and the mixture was stirred for a further 1.5 h. Excess H_2O was added and stirring continued for 1 h, after which the mixture was diluted with CH_2Cl_2, washed with aqueous HCl (0.25 M, 3 × 400 mL), dried ($MgSO_4$), and evaporated at 30 °C to a gum (1.24 g). The crude free acid was dissolved in a mixture of EtOH (500 mL) and H_2O (100 mL) at 40 °C and passed down a column of Amberlite IRC-50 (Na form). Eluate was concentrated to about 100 mL and left to precipitate at 4 °C overnight to yield (−)-8 as a white powder (0.48 g, 37%): $[\alpha]^{20}_D$ −3.2° (*c* 1.04 in $CHCl_3$); ^1H NMR (250 MHz, DMSO-d_6/CD_2Cl_2 1:1) δ 0.87 (6 H, t, *J* = 6.6 Hz), 1.25 (48 H, br s), 1.52 (4 H, br m), 2.21 (4 H, m), 3.90 (2 H, m), 4.07 (1 H, dd, *J* = 6.8, 12.0 Hz), 4.27 (1 H, dd, *J* = 3.3, 12.0 Hz), 5.10 (1 H, m), 6.91–6.97 (1 H, m), 7.13–7.23 (4 H, m). Anal. ($C_{41}H_{72}O_8PNa$) C, H.; the compound is hygroscopic and contained 1.0% H_2O.

Sodium 1,2-Di-*O*-hexadecanoyl-*sn*-glycer-3-yl Phenyl Phosphate ((+)-8). Treatment of (−)-7[16] (1.50 g, 2.63 mmol) in dry pyridine (15 mL) and CH_2Cl_2 (15 mL) with phenyl dichlorophosphate (0.800 g, 3.82 mmol) in pyridine (15 mL) by the method described for (−)-8 and subsequent conversion to the sodium salt gave a white powder of (+)-8 (0.93 g, 49%) which softened gradually above 65 °C: $[\alpha]^{20}_D$ +4.6° (*c* 2.0 in $CHCl_3$); ^1H NMR (250 MHz, DMSO-d_6/CD_2Cl_2 1:1) δ 0.87 (6 H, t, *J* = 6.6 Hz), 1.25 (48 H, br s), 1.51 (4 H, br m), 2.21 (4 H, m), 3.88 (2 H, m), 4.07 (1 H, dd, *J* = 6.8, 12.0 Hz), 4.27 (1 H, dd, *J* = 3.2, 12.0 Hz), 5.09 (1 H, m), 6.90–6.96 (1 H, m), 7.13–7.23 (4 H, m); MS *m/e* (negative FAB) 723 ((M − H)$^-$, 100), 467 (11), 255 (52), 213 (15), 169 (20), 107 (30). Anal. ($C_{41}H_{72}O_8PNa$) C, H.

2,3:5,6-Di-*O*-isopropylidene-4-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol 1-(1,2-Di-*O*-hexadecanoyl-*sn*-glycer-3-yl phenyl phosphate) (9a). To a solution of (+)-4 (0.170 g, 0.454 mmol) and (+)-8 (0.510 g, 0.683 mmol) in dry pyridine (3 mL) was added 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT)[13] (0.510 g, 1.721 mmol). The mixture (initially a clear yellow solution) was stirred at room temperature. Over about 15–20 min the solution gradually became cloudy and then opaque. After stirring for a further 40 min, the mixture was partitioned between aqueous NaCl and Et_2O, and the Et_2O layer was washed with H_2O, dried ($MgSO_4$), and evaporated to a gum. This material was flash chromatographed (SiO_2, C_6H_{14}/Et_2O), giving the product (**9a**) as a colorless glass (0.394 g, 80%); a

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mixture of two diastereoisomers. ^{1}H NMR (250 MHz, CD_2Cl_2) δ inter alia 2.28 (4 H, m), 3.25 and 3.26 (3 H, 2 s), 3.36 (1 H, dd, $J \simeq$ 10.1, 10.1 Hz), 3.50–3.64 (2 H, m), 3.65–3.76 (2 H, m), 3.98–4.20 (4 H, m), 4.27–4.35 (3 H, m), 4.43 and 4.58 (1 H, 2 dd, in the former, $J \simeq$ 4.5, 4.5 Hz), and in the latter, $J \simeq$ 4.6, 4.6 Hz), 4.69–4.82 (1 H, m), 5.22 (1 H, m), 7.18–7.38 (5H, m).

2,3:5,6-Di-*O*-isopropylidene-4-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol 1-(2,3-Di-*O*-hexadecanoyl-*sn*-glycer-1-yl phenyl phosphate) (9b). Reaction of (+)-4 (0.140 g, 0.374 mmol) and (−)-8 (0.420 g, 0.562 mmol) in the presence of MSNT (0.420 g, 1.417 mmol) and subsequent workup as described for the preparation of **9a** gave **9b** as a colorless glass (0.253 g, 63%): ^{1}H NMR (250 MHz, CD_2Cl_2) δ inter alia 2.28 (4 H, m), 3.25 and 3.26 (3 H, 2 s), 3.37 (1 H, dd, $J \simeq$ 10.0, 10.0 Hz), 3.50–3.63 (2 H, m), 3.63–3.75 (2 H, m), 3.96–4.20 (4 H, m), 4.23–4.37 (3 H, m), 4.44 and 4.57 (1 H, 2 dd, $J \simeq$ 4.6, 4.6 Hz in each case), 4.68–4.83 (1 H, m), 5.25 (1 H, m), 7.17–7.38 (5 H, m).

1,2:4,5-Di-*O*-isopropylidene-6-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol 3-(1,2-Di-*O*-hexadecanoyl-*sn*-glycer-3-yl phenyl phosphate) (9c). Reaction of (−)-4 (0.171 g, 0.456 mmol) and (+)-8 (0.511 g, 0.685 mmol) in the presence of MSNT (0.514 g, 1.74 mmol) followed by the workup described for **9a** gave **9c** as a glass (0.371 g, 75%): ^{1}H NMR (250 MHz, CD_2Cl_2) δ inter alia 2.28 (4 H, m), 3.25 and 3.26 (3 H, 2 s), 3.38 (1 H, dd, $J \simeq$ 9.6, 9.6 Hz), 3.51–3.65 (2 H, m), 3.65–3.77 (2 H, m), 3.97–4.17 (4 H, m), 4.26–4.37 (3 H, m), 4.44 and 4.57 (1 H, 2 dd, in the former $J \simeq$ 4.5, 4.5 Hz, in the latter $J \simeq$ 4.6, 4.6 Hz), 4.70–4.83 (1 H, m), 5.25 (1 H, m), 7.18–7.38 (5 H, m).

1,2:4,5-Di-*O*-isopropylidene-6-*O*-(4-methoxytetrahydropyran-4-yl)-D-*myo*-inositol 3-(2,3-Di-*O*-hexadecanoyl-*sn*-glycer-1-yl phenyl phosphate) (9d). Reaction of (−)-4 (0.100 g, 0.267 mmol) and (−)-8 (0.300 g, 0.402 mmol) in the presence of MSNT (0.300 g, 1.01 mmol) followed by the workup described for **9a** gave **9d** as a glass (0.170 g, 59%): ^{1}H NMR (250 MHz, CD_2Cl_2) δ inter alia 2.28 (4 H, m), 3.25 and 3.26 (3 H, 2 s), 3.36 (1 H, dd, $J \simeq$ 10.1, 10.1 Hz), 3.50–3.64 (2 H, m), 3.64–3.77 (2 H, m), 3.98–4.20 (4 H, m), 4.27–4.35 (3 H, m), 4.43 and 4.58 (1 H, 2 dd, J = 4.6, 4.6 Hz in each case), 4.68–4.83 (1 H, m), 5.23 (1 H, m), 7.15–7.41 (5 H, m).

D-*myo*-Inositol 1-(Sodium 1,2-di-*O*-hexadecanoyl-*sn*-glycer-3-yl phosphate) (10a). A solution of **9a** (0.250 g, 0.231 mmol) in EtOH (60 mL) was hydrogenolyzed for 3.5 h over PtO_2 (0.25 g) at room temperature and 50 psi pressure. Complete removal of the phenyl group was demonstrated by NMR of an aliquot. After filtration to remove the catalyst, the solution was diluted with EtOH (40 mL) and H_2O (50 mL) and stirred at 40 °C for 6 h and then at room temperature for 36 h. The solution (containing some suspended white solid) was concentrated to approximately one-third of its volume in vacuo at 30 °C, and the resulting white precipitate was collected by centrifugation and dried at ca. 40 °C over P_2O_5 in vacuo, to give a white powder (0.128 g). This was dissolved in aqueous EtOH (66% v/v EtOH; 200 mL) with vigorous stirring at 40 °C and the resulting clear solution passed down a column of Amberlite IRC-50 (Na form). The column was washed through with further aqueous EtOH of the same composition (100 mL), and the combined eluates were concentrated at 30 °C in vacuo to about 40% of their initial volume. The resulting white precipitate was again collected by centrifugation and dried as before to give **10a** as a white powder (0.092 g). A small sample was further purified by reprecipitation, by dissolving in aqueous EtOH (66% v/v; 40 mL) at 45 °C and concentrating the filtered solution slowly in vacuo at 30 °C. The solid was collected and dried as before: mp 214–217 °C dec (begins to soften at ca. 125 °C); ^{1}H NMR (360 MHz; 72 °C; DMSO-d_6) δ (one signal apparently obliterated by the water peak) 0.85 (6 H, t, J = 6.8 Hz), 1.24 (48 H, br s), 1.52 (4 H, br m), 2.26 (4 H, m), 2.93 (1 H, dd, $J \simeq$ 8.8, 8.8 Hz), 3.39 (1 H, dd, $J \simeq$ 9.3, 9.3 Hz), 3.56 (1 H, m), 3.62 (1 H, dd, $J \simeq$ 9.2, 9.2 Hz), 3.72–3.87 (3 H, m), 3.95 (1 H, br s, O*H*), 4.10 (1 H, dd, J = 7.1, 12.0 Hz), 4.17 (2 H, br s, O*H*), 4.30 (1 H, dd, J = 3.1, 12.0 Hz), 4.95 (1 H, br s, O*H*), 5.08 (1 H, m), 6.27 (1 H, br s, O*H*); ^{31}P NMR (145 MHz; 64 °C; DMSO-d_6) δ 2.30.

D-*myo*-Inositol 1-(Sodium 2,3-di-*O*-hexadecanoyl-*sn*-glycer-1-yl phosphate) (10b). Compound **9b** (0.163 g, 0.150 mmol) was deprotected by hydrogenolysis and hydrolysis as described for the preparation of **10a**, to yield the free acid of **10b** as a white powder (0.081 g). This was converted to the sodium salt (0.064 g), a sample of which was reprecipitated from aqueous EtOH to give **10b** as a white powder: mp 218–219 °C (softens above 205 °C); ^{1}H NMR (250 MHz; 55 °C; DMSO-d_6) δ inter alia 0.87 (6 H, t, J = 6.6 Hz), 1.26 (48 H, br s), 1.53 (4 H, br m), 2.27 (4 H, m), 2.94 (1 H, m), 3.41 (1 H, m), 3.53–3.69 (2 H, m), 3.74–3.90 (3 H, m), 4.03 (1 H, d, $J_{CH\text{-}OH}$ = 6.6 Hz, O*H*), 4.10 (1 H, dd, J = 7.2, 12.1 Hz), 4.21 (1 H, s, O*H*), 4.23 (1 H, s, O*H*), 4.30 (1 H, dd, J = 3.4, 12.2 Hz), 4.96 (1 H, d, $J_{CH\text{-}OH}$ = 2.4 Hz, O*H*) 5.08 (1 H, m), 6.22 (1 H, s, O*H*); ^{31}P NMR (145 MHz; 64 °C; DMSO-d_6) δ 2.14.

D-*myo*-Inositol 3-(Sodium 1,2-di-*O*-hexadecanoyl-*sn*-glycer-3-yl phosphate) (10c). Compound **9c** (0.291 g, 0.269 mmol) was deprotected as described for **9a** to give the free acid of **10c** as a white powder (0.180 g). This was converted to the sodium salt (0.135 g). A sample of this product was reprecipitated from aqueous EtOH to give **10c** as a white powder: mp 228–238 °C (softening at 138 °C); ^{1}H NMR (250 MHz; 55 °C; DMSO-d_6) δ inter alia 0.85 (6 H, t, J = 6.5 Hz), 1.24 (48 H, br s), 1.51 (4 H, br m), 2.25 (4 H, m), 2.92 (1 H, m), 3.38 (1 H, m), 3.50–3.66 (2 H, m), 3.73–3.87 (3 H, m), 4.00 (1 H, d, $J_{CH\text{-}OH}$ = 5.9 Hz, O*H*), 4.08 (1 H, dd, J = 6.9, 11.9 Hz), 4.20 (2 H, m, O*H*), 4.29 (1 H, dd, J = 3.1, 12.00 Hz), 4.96 (1 H, d, $J_{CH\text{-}OH}$ = 2.4 Hz, O*H*), 5.07 (1 H, m), 6.25 (1 H, s, O*H*); ^{31}P NMR (145 MHz; 64 °C; DMSO-d_6) δ 2.32.

D-*myo*-Inositol 3-(Sodium 2,3-di-*O*-hexadecanoyl-*sn*-glycer-1-yl phosphate) (10d). Compound **9d** (0.162 g, 0.150 mmol) was deprotected as described for **9a** to give the free acid of **10d** as a white powder (0.066 g). This was converted to the sodium salt (0.037 g), mp 175 °C (softens above 135 °C): ^{1}H NMR (250 MHz; 55 °C; DMSO-d_6) δ inter alia 0.87 (6 H, t, J = 6.6 Hz), 1.26 (48 H, br s), 1.54 (4 H, br m), 2.28 (4 H, m), 2.94 (1 H, m), 3.39 (1 H, m), 3.52–3.68 (2 H, m), 3.73–3.85 (3 H, m), 4.01 (1 H, d, $J_{CH\text{-}OH}$ = 6.4 Hz, O*H*), 4.11 (1 H, dd, J = 7.0, 11.9 Hz), 4.19 (1 H, s, O*H*), 4.21 (1 H, s, O*H*), 4.32 (1 H, dd, J = 3.2, 11.9 Hz), 5.01 (1 H, d, $J_{CH\text{-}OH}$ = 2.7 Hz, O*H*), 5.08 (1 H, m), 6.32 (1 H, s, O*H*); ^{31}P NMR (145 MHz; 64 °C; DMSO-d_6) δ 2.34.

X-ray Crystal Analysis. Single crystals of **2a** suitable for X-ray crystal structure analysis were grown from C_6H_{14}. Crystal data for **2a**: $C_{38}H_{50}O_9Si$, M_r = 678.91, cell parameters (obtained from a least-squares fit to 25 reflections with 60° < 2θ < 70°); a = 11.601 (5) Å, b = 24.699 (5) Å, c = 12.815 (4) Å, β = 97.84 (4)°, V = 3637.5 (38) Å^3, monoclinic, $P2_1$; Z = 4, ρ = 1.240 g cm^{-3}, μ = 9.803 cm^{-1}, $F(000)$ = 1456, T = 188 K. There were 6493 unique reflections collected in a range of 2° < 2θ < 132° on an Enraf-Nonius CAD4 diffractometer with graphite monochromated Cu Kα radiation, λ = 1.541 84 Å. Data were corrected for Lorentz and polarization effects and for absorption. The absorption correction was taken from PSI scans of nine reflections with χ > 80°. The structure was solved with use of MULTAN80.[17] Observed data, 6211 with $I > 3\sigma(I)$, were employed in a least-squares refinement of 433 variables (on F) which converged to the conventional crystallographic residuals R = 0.063, R_w = 0.083, GOF = 3.077. An extinction coefficient of the type described by Zachariasen[18] was included in the latter stages. Convergence was indicated by max (Δ/σ) = 0.01. A final difference Fourier map showed no excursions of heights beyond ±0.496 e Å^{-3}. The two crystallographically independent molecules differ principally in the rotameric disposition of the *tert*-butyldiphenylsilyl group. A complete list of bond distances and angles, torsion angles, and positional parameters are available as supplementary material (Tables II–V).

PI 4-Kinase Assay. Highly purified human erythrocyte membranes were prepared as outlined by Hawkins et al.[19] The PI 4-kinase associated with these membranes was extracted with Triton X-100 and purified approximately 90-fold through a combination of chromatographic steps, namely DEAE-Sephadex,

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hydroxyapatite, and gel filtration (details to be published elsewhere). The K_m values for ATP and PI for this PI kinase preparation were 47 and 105 μM, respectively, and the specific activity was 25 nmol of PIP formed min^{-1} mg^{-1}.

PI 4-kinase was assayed in a buffer containing 0.3% (w/v) Triton X-100, 100 μM [γ-^{32}P]ATP (50 000 cpm $nmol^{-1}$), 200 μM PI, 1 mM dithiothreitol, 15 mM $MgCl_2$, 1 mM EGTA, 0.1 M NaCl, and 50 mM HEPES (pH 7.4, 30 °C) in a total volume of 0.2 mL. Reactions were linear over the time period used. The incubations were terminated by the addition of 0.75 mL of $CHCl_3$/MeOH/concentrated HCl (40:80:1). Two phases were obtained by the addition of 0.25 mL of $CHCl_3$ and 0.1 M HCl, and the organic phase was washed twice with 0.5 mL of $CHCl_3$/MeOH/0.1 M HCl (3:48:47). A 200-μL portion of the resulting organic phase was then counted for ^{32}P radioactivity. To confirm that the assay monitored specifically the formation of PI 4[^{32}P]P, the organic phase obtained as described above was dried under vacuum, the phospholipids were deacylated with $MeNH_2$, and the products were analyzed by ion-exchange HPLC on a partisphere wax column as described previously.[6] At least 95% of the ^{32}P was found to co-migrate with authentic 1-(*sn*-glycer-3-ylphospho)-D-*myo*-inositol 4-phosphate.

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Supplementary Material Available: X-ray crystal data on compound **2a** (Tables II–V) and NMR spectral comparison of compounds I and **10a** (Figure 2) (14 pages). Ordering information is given on any current masthead page.

Design of an Antithrombotic–Antihypertensive Agent (Wy 27569). Synthesis and Evaluation of a Series of 2-Heteroaryl-Substituted Dihydropyridines

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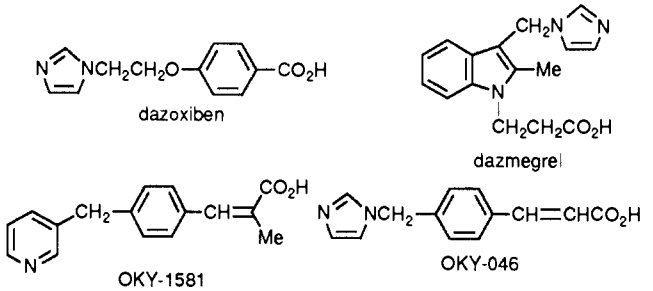
An approach to the design of potential combined antithrombotic–antihypertensive agents is described. A series of 1,4-dihydropyridines bearing a 1*H*-imidazol-1-yl or pyrid-3-yl substituted side chain in the 2-position were synthesized and tested for antihypertensive activity in spontaneously hypertensive rats and for inhibition of TXA_2 synthetase in rabbit platelets, in vitro. 1,4-Dihydro-2-(1*H*-imidazol-1-ylmethyl)-6-methyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid 3-ethyl 5-methyl diester (1) was shown to be similar in potency to nitrendipine as an antihypertensive agent. Compound 1 inhibited TXA_2 synthetase in rabbit and human platelets in vitro and reduced plasma TXB_2 levels in rats at antihypertensive dose levels. The reductions in thromboxane production observed in vivo and in vitro were accompanied by enhanced levels of 6-KPGF$_{1\alpha}$, reflecting diversion of the arachidonic acid cascade toward prostacyclin synthesis.

Hypertension is an important risk factor for a variety of cardiovascular disorders. With the availability of effective antihypertensive agents, therapy for marked and moderate hypertension is now routine. Although effective antihypertensive therapy reduces the incidence of cardiovascular related morbidity and mortality in previously hypertensive patients, the level of risk is not entirely reduced to that present in normotensive subjects. For example, extensive trials of antihypertensive drug therapy in hypertensive patients have shown a significant reduction in the incidence of stroke, congestive heart failure, and renal damage but little or no reduction in coronary artery disease (CAD) and associated myocardial infarction or sudden death.[1] Given the present availability of safe and effective antihypertensive agents with relatively few side-effects, further advance may require the development of agents having additional properties capable of reducing the incidence of CAD related events. One such approach would be to design a combined antithrombotic–antihypertensive agent.

CAD is characterized by the presence of atherosclerotic plaque on the coronary blood vessel wall. Acute myocardial infarction is believed to be initiated by plaque disruption and exposure of underlying collagen which stimulates platelet aggregation leading to thrombus formation and vasospasm due to release of vasoactive substances from the aggregating platelets.[2] Recent studies have highlighted the role of prostaglandins in CAD.[3,4] Thromboxane A_2 (TXA_2) is synthesized in platelets by the action of TXA_2 synthetase on prostaglandin endoperoxides and is one of the products released during platelet aggregation.[5] TXA_2 has been shown to be a potent vasoconstrictor and platelet-aggregating agent and these pro-

Chart I



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