

Synthesis and Evaluation of 3-Modified 1D-*myo*-Inositols as Inhibitors and Substrates of Phosphatidylinositol Synthase and Inhibitors of *myo*-Inositol Uptake by Cells¹

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A number of 3-substituted 1D-*myo*-inositols were synthesized and evaluated as substrates for phosphatidylinositol synthase and uptake by intact cells. 1D-3-Amino-, -3-chloro-, and -3-(acetylthio)-3-deoxy-*myo*-inositols were all synthesized by nucleophilic displacement of the 6-*O*-(trifluoromethyl)sulfonyl group of 1L-1,2:3,4-di-*O*-cyclohexylidene-5-*O*-methyl-6-*O*-[(trifluoromethyl)sulfonyl]-*chiro*-inositol (which was prepared from *L*-quebrachitol), respectively, by reaction with LiN₃, followed by reduction of the azido function, and with LiCl and KSAc to give the *O*-protected compounds. *O*-Demethylation using BBr₃ and concomitant acetal hydrolysis furnished the free-hydroxy 3-amino- and 3-chloro-3-deoxy-1D-*myo*-inositols. The 3-mercapto analogue was obtained by removal of the acetal groups of the acetylthio analogue, followed by acetylation and purification of the peracetate, and subsequent *O*-demethylation and deacetylation. The 3-deoxy derivative was synthesized from the 6-*O*-(imidazol-1-ylthiocarbonyl) compound via Barton-McCombie deoxygenation. The 3-azido derivative was directly synthesized from 1L-1-*O*-tosyl-*chiro*-inositol via displacement with azide. The 3-keto analogue was prepared by Pt-catalyzed air oxidation of 1L-*chiro*-inositol. The compounds were all evaluated as substrates for phosphatidylinositol (PtdIns) synthase from mouse brain. The 3-NH₂, 3-F, 3-deoxy, and 3-keto analogues all showed activity as substrates, as measured by liberation of cytidine monophosphate. These compounds also showed inhibition of the reaction of *myo*-[³H]inositol with PtdIns synthase. These results taken together indicate that these compounds are likely to be incorporated into phospholipids. As a further indication that these compounds might be useful as probes for the PtdIns pathway, it was demonstrated that the 3-NH₂, 3-F, and 3-deoxy compounds are taken up by intact fibroblast cells as evidenced by their competing with *myo*-[³H]inositol uptake.

Research on the phosphatidylinositol (PtdIns) pathway² has in recent years assumed a position at the forefront of biochemical science, owing to the central role played by inositol lipids in cell physiology.³⁻⁶ In particular the phenomenon of transmembrane signaling, which has biomedical ramifications in a plethora of disease states, including arthritis, cancer, and diabetes, to name a few, has been the focus of extensive studies.⁷ This cell membrane receptor-effector function has among its key steps the hydrolysis of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P₂], mediated by a phosphatidylinositol-specific phospholipase C (PIC), to the well-recognized second messengers *sn*-1,2-diacylglycerol (Ac₂Gro) and 1D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P₃]. Studies on chemical intervention in this sequence of events have potential for developing pharmacological agents that could affect cell physiology and ultimately alter the course of various diseases. Thus, previous work involved the synthesis^{8,9} and evaluation^{10,11} of a number of modified cyclitols, of which the most promising was 5-deoxy-5-fluoro-*myo*-inositol. This compound was shown to be a substrate for PtdIns synthase (CDPdiacylglycerol:*myo*-inositol 3-phosphatidyltransferase, EC 2.7.8.11), the first enzyme of the PtdIns cycle, and it was found to be taken up by L1210 leukemia cells, where it was incorporated

into a phospholipid having all properties expected of a modified PtdIns 4-phosphate.^{10,11} Because of its lack of a hydroxyl function on C-5, such a species would cause an interruption of the cycle at the second phosphorylation step, mediated by 1-phosphatidylinositol 4-phosphate 5-kinase [PtdIns(4)P 5-kinase, EC 2.7.1.68].

A different approach to the exploitation of *myo*-inositol analogues was suggested by the discovery of 1-phosphatidylinositol 3-kinase (PtdIns 3-kinase, EC 2.7.1.137), which is responsible for the production of derivatives incorporating a 3-phosphate group, namely PtdIns(3)P, PtdIns(3,4)P₂, and PtdIns(3,4,5)P₃.¹²⁻¹⁴ These lipids have been found in many cells,⁷ especially in transformed fibroblasts.^{12,15-17} Clear evidence for the role of PtdIns 3-kinase in neoplastic transformation has been furnished by an experimental system involving the oncogenic mouse polyoma virus, where it has been shown that the ability of the virus both to transform cells in culture and to induce tumors in the animal depends on its ability to bind PtdIns 3-kinase through the middle T oncoprotein.^{18,19} Thus, considerable interest has been aroused in the possibility of blocking the synthesis of phosphoinositide 3-phosphates, perhaps with position 3-modified inositols.

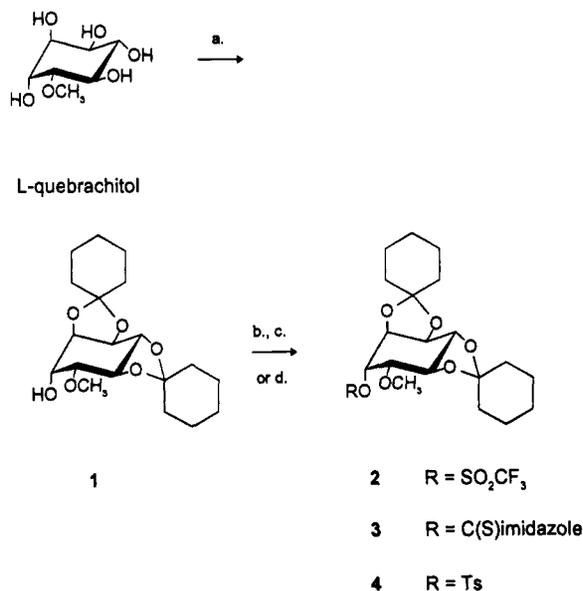
1D-*myo*-Inositols of this type have been reported by Kozikowski, Powis, and co-workers,²⁰⁻²² who examined their effects on cell growth, but to date little has been presented on the effects of these compounds on specific enzymes of the PtdIns pathway.²³ In the present report,²⁴ we describe the design, synthesis, and evaluation of

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Scheme I^a

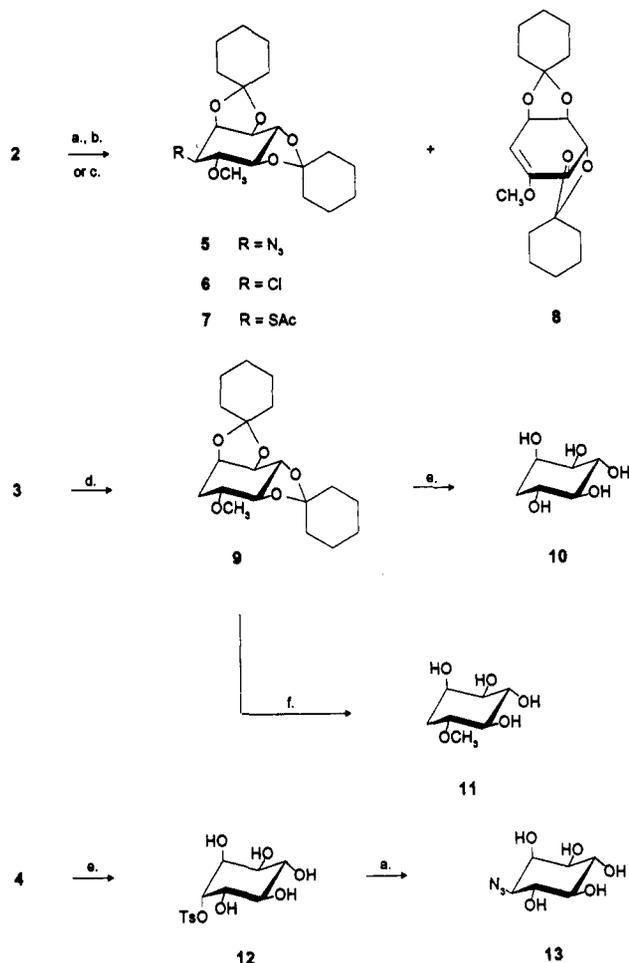
^aReagents: (a) 1-ethoxycyclohexene, DMF, *p*-TSA; (b) trifluoromethanesulfonic anhydride, pyridine, CH₂Cl₂; (c) 1,1'-thiocarbonyldiimidazole, DMF; (d) *p*-toluenesulfonyl chloride, pyridine.

3-modified 1D-*myo*-inositols as substrates or inhibitors for PtdIns synthase. Also described is the effect of some of these inositol analogues on the uptake of *myo*-inositol into intact cells.

Results and Discussion

Chemical Synthesis of the 3-Modified 1D-*myo*-Inositol Analogues. The requisite 3-substituted 1D-*myo*-inositol derivatives are best obtained from L-quebrachitol (1L-2-*O*-methyl-*chiro*-inositol) by inversion at the 1-position (1L-*chiro* numbering). Thus in Scheme I, L-quebrachitol is converted with 4 equiv of 1-ethoxycyclohexene²⁵ to 1L-1,2,3,4-di-*O*-cyclohexylidene-5-*O*-methyl-*chiro*-inositol (1).²⁶ Compound 1, with a free 6-OH group, is then activated for displacement or replacement via the three derivatives 2, 3, or 4 by reaction of 1 respectively with trifluoromethanesulfonic anhydride and pyridine in CH₂Cl₂,²⁷ 1,1'-thiocarbonyldiimidazole in DMF, or *p*-toluenesulfonyl chloride in pyridine to afford, respectively, the 6-*O*-(trifluoromethyl)sulfonyl (triflyl) derivative 2, the 6-*O*-(imidazol-1-ylthiocarbonyl) derivative 3, or the known²⁸ 6-*O*-tosyl derivative 4 (1L-*chiro* numbering). Both 2 and 3 were unstable, syrupy products that were identified by ¹H NMR spectroscopy (Table I) and used directly in the synthetic procedures; tosylate 4 was identical by melting point with the compound previously reported.²⁸ Both ¹H (Table I) and ¹³C NMR spectral data (Experimental Section) support the structure proposed for 4. All three derivatives showed in their ¹H NMR spectra H-3 (1D-*myo* numbering) as a "pseudo triplet" with $J_{2,3} \approx J_{3,4} \approx 4.8\text{--}6.6$ Hz (Table I).

1D-3-Azido-1,2:5,6-di-*O*-cyclohexylidene-3-deoxy-4-*O*-methyl-*myo*-inositol (5), 1D-3-chloro-1,2:5,6-di-*O*-cyclohexylidene-3-deoxy-4-*O*-methyl-*myo*-inositol (6), and 1D-3-(acetylthio)-1,2:5,6-di-*O*-cyclohexylidene-3-deoxy-4-*O*-methyl-*myo*-inositol (7) were prepared by nucleophilic displacement of the triflate group in 2 with lithium azide in DMF, lithium chloride in DMF, and potassium thioacetate in CH₃CN, respectively (Scheme II). Column chromatography furnished the pure displacement products 5, 6, and 7. These compounds showed $J_{2,3}$ in the range of

Scheme II^a

^aReagents: (a) LiN₃, DMF; (b) LiCl, imidazole, DMF; (c) KSAc, CH₃CN; (d) Bu₃SnH, AIBN, toluene; (e) BBr₃, CH₂Cl₂; CH₃OH; (f) 3:1 CF₃CO₂H-H₂O.

4.4–5.0 Hz and larger $J_{3,4}$ values of 8.6–9.8 Hz, reflecting the inversion at C-3 (1D-*myo* numbering). A byproduct, 2D-(2,4,5/3)-2,3:4,5-di-*O*-cyclohexylidene-1-*O*-methyl-1(6)-cyclohexene-1,2,3,4,5-pentol (8), the result of a competitive elimination reaction, was also isolated in yields ranging from 10 to 45%, depending upon the nucleophile and reaction conditions in general. Compound 8 was identified by its ¹H and ¹³C NMR spectra. Especially diagnostic was the ¹³C NMR resonance for the enolic C-4 (*myo*-inositol numbering) at δ 156.1 (see the Experimental Section) and the vinylic signal at δ 90.3, as well as the ¹H NMR data showing typical coupling expected for an allylic system (Table I). The compound gave an optical rotation in agreement with an earlier report.²⁸ Compounds 5–8 all gave acceptable elemental analyses.

Attempts to deprotect compound 5 with BBr₃ in CH₂Cl₂, followed by a workup in methanol, resulted in decomposition of the compound, as evidenced by a complex mixture that failed to show an azide stretch in the IR spectrum. Hydrogenation of 5 with palladium-on-charcoal (Pd/C) catalyst gave the protected 1D-3-amino-1,2:5,6-di-*O*-cyclohexylidene-3-deoxy-4-*O*-methyl-*myo*-inositol (14), which was then fully deprotected with BBr₃/CH₂Cl₂, with workup in methanol (conditions are acidic, as HBr is generated from the methanolysis of BBr₃), to give 1D-3-amino-3-deoxy-*myo*-inositol hydrobromide (15) in 66% yield (Scheme III). The structures of compounds 14 and 15 were confirmed by NMR spectroscopy (see Table I and

Table I. ¹H NMR Spectroscopic Data for Selected Cyclitol Derivatives^a

compd	solvent	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6}$)	H-6 ($J_{6,1}$)	other
2 ^b	CDCl ₃	3.56–3.69 (m)	4.41–4.47 (m) (5.7)	5.10 (ψt) (5.7)	4.41–4.47 (m)	3.56–3.69 (m)	3.84 (ψt) (5.2)	3.56 (s) OCH ₃ ; 1.41–1.65 (m) C ₆ H ₁₀
3 ^b	CDCl ₃	4.34 (dd) (6.6)	4.04 (dd) (6.6)	5.83 (ψt) (6.6)	3.62–3.76 (m)	3.62–3.76 (m)	4.51 (ψt) (6.6)	3.36 (s) OCH ₃ ; 1.21–1.90 (m) C ₆ H ₁₀
4 ^b	CDCl ₃	3.51–3.69 (m)	4.29–4.39 (m) (4.8)	4.98 (ψt) (4.8)	3.51–3.69 (m)	3.51–3.69 (m)	4.29–4.39 (m)	7.32–7.86 (dd) OTs; 3.30 (s) OCH ₃ ; 2.43 (s) ArCH ₃ ; 1.36–1.64 C ₆ H ₁₀
5	CDCl ₃	4.29 (dd) (6.5)	4.49 (dd) (4.4)	3.62–3.73 (m)	3.62–3.73 (m)	3.38 (dd) (8.2)	3.88 (dd) (10.5)	3.55 (s) OCH ₃ ; 1.19–1.77 (m) C ₆ H ₁₀
6	CDCl ₃	4.29 (dd) (6.0)	4.48 (dd) (4.6)	4.03–4.12 (m) (5.1)	3.81 (dd) (8.6)	3.38 (dd) (10.5)	4.03–4.12 (m)	3.56 (s) OCH ₃ ; 1.19–1.72 (m) C ₆ H ₁₀
7 ^c	CDCl ₃	4.23 (dd) (5.0)	4.39 (ψt) (5.0)	3.91 (dd) (8.2)	3.50–3.70 (m) (9.8)	3.43 (ψt) (9.8)	3.50–3.70 (m)	3.50–3.70 (m) OCH ₃ ; 2.25–2.37 (s) SC(O)CH ₃ ; 1.41–1.74 (m) C ₆ H ₁₀
8 ^d	CDCl ₃	4.35 (dd) (6.8)	4.92 (ddd) (3.4)	4.67 (dd) ^e		4.12 (dψt) (8.9)	3.80 (ψt) (9.6)	3.67 (s) OCH ₃ ; 1.25–1.87 (m) C ₆ H ₁₀
9	CDCl ₃	4.13 (dd) (8.5)	4.34 (dd) (4.3)	H-3 _a ^f 1.21–1.78 (m) H-3 _e 2.37 (ddd)	3.59 (m) (8.5)	3.28 (dd) (10.0)	3.49 (dd) (8.5)	3.39 (s) OCH ₃ ; 1.21–1.78 (m) C ₆ H ₁₀
10	D ₂ O	3.48–3.57 (m) (2.2)	4.06 (dd) ^h	H-3 _a ^f 1.54 (ddd) H-3 _e (2.09 (dψt))	3.75 (ddd) (9.0)	3.23 (ψt) (9.0)	3.48–3.57 (m)	
11	D ₂ O	3.42–3.49 (m) (2.8)	4.08 (dd) ⁱ	H-3 _a ^f 1.40 (ddd) H-3 _e 2.29 (dψt)	3.42–3.49 (m)	3.57 (ψt) (9.0)	3.32 (ψt) (9.0)	3.40 (s) OCH ₃
12	D ₂ O	3.46–3.95 (m)	3.46–3.95 (m)	4.74 (ψt) (4.7)	3.46–3.95 (m)	3.46–3.95 (m)	3.46–3.95 (m)	7.48–7.92 (dd) OTs; 2.48 (s) ArCH ₃
13 ⁱ	D ₂ O	3.89 (ψt) (4.5)	3.93 (ψt) (4.2)	3.74–3.81 (m)	3.34–3.51 (m)	3.34–3.51 (m)	3.34–3.51 (m)	
14	CDCl ₃	4.20 (dd) (5.1)	4.41 (ψt) (5.1)	2.94 (dd) (8.8)	3.28–3.32 (m)	3.28–3.32 (m) (9.2)	3.69 (ψt) (9.2)	3.61 (s) OCH ₃ ; 1.25–1.80 (m) C ₆ H ₁₀
15 ⁱ	D ₂ O	3.52–3.68 (m)	4.18 (ψt) (4.3)	3.32 (dd) (8.3)	3.52–3.68 (m)	3.41 (ψt) (10.2)	3.78 (ψt) (10.1)	
16 ⁱ	D ₂ O	3.44–3.61 (m)	4.12 (br s)	3.44–3.61 (m)	3.44–3.61 (m)	3.44–3.61 (m)	3.44–3.61 (m)	3.62 (s) OCH ₃
17 ^c	D ₂ O	3.21–3.46 (m)	3.69 (br s)	3.21–3.46 (m)	3.21–3.46 (m)	3.14 (m)	3.21–3.46 (m)	3.43 (s) OCH ₃
18	D ₂ O	3.55 (dd) (2.4)	4.14 (ψt) (2.4)	3.96 (dd) (10.0)	3.68 (ψt) (10.0)	3.64 (ψt) (10.0)	3.29 (ψt) (10.0)	
20	CDCl ₃	5.06 (dd) (2.6)	5.46 (ψt) (2.6)	3.86 (dd) (11.6)	3.54 (dd) (10.0)	5.18 (ψt) (10.0)	5.35 (ψt) (10.0)	3.42 (s) OCH ₃ ; 1.96, 2.05, 2.08, 2.16 (s/s) C(O)CH ₃ ; 2.34 (s) SC(O)CH ₃
21	D ₂ O	3.53–3.71 (m) (2.4)	4.05 (ψt) (2.4)	2.93 (dd) (10.9)	3.48 (dd) (9.0)	3.31 (ψt) (9.0)	3.53–3.71 (m)	

^a Spectra were recorded at 250 MHz unless otherwise indicated. Chemical shifts are reported in ppm, and first-order multiplicities (br, broad; d, doublet; dd, doublet of doublets; ddd, doublet of doubled doublets; m, multiplet; s, singlet; ψt, "pseudo triplet", i.e., a doublet of doublets with both couplings equivalent) immediately follow in parentheses. Spin-spin couplings (in hertz), where discernable, follow in parentheses. ^b Chemical shifts for all compounds are listed according to the 1D-*myo*-inositol numbering system. ^c At 200 MHz on a Nicolet NT-200 instrument. ^d At 360 MHz. ^e $J_{3,5} = 1.9$ Hz. ^f The methylene protons at C-3 are not chemically equivalent. Chemical shifts are reported separately for axial H-3_a and equatorial H-3_e. ^g $J_{3e,4} = 5.7$; $J_{gem} = 15.2$ Hz. ^h $J_{2,3a} = 3.0$; $J_{2,3e} = 4.2$ Hz. ⁱ $J_{3a,4} = 12.0$; $J_{3e,4} = 4.2$; $J_{gem} = 14.0$ Hz. ^j $J_{2,3a} = 2.2$ Hz. ^k $J_{3a,4} = 10.0$; $J_{3e,4} = 4.4$; $J_{gem} = 14.1$ Hz. ^l At 400 MHz.

the Experimental Section) and by elemental analyses. Surprisingly, **15** was found to be unstable to long-term storage as a second, so far uncharacterized, compound is found in samples of **15** that have been kept at room temperature for several months.

In order to obtain 1D-3-azido-3-deoxy-*myo*-inositol (**13**), the most robust of the three C-3 activated intermediates, the tosylate **4**, was demethylated with concomitant cleavage of the acetal groups using the BBr₃/CH₂Cl₂-MeOH procedure to furnish the known tosyl derivative **12**.²⁶ Compound **12** was subjected to displacement with lithium azide in DMF to give crude **13**. Purification of **13** was achieved via chromatography of the peracetylated compound, which, upon *O*-deacetylation with methanolic ammonia, gave **13** in 62% yield as a glassy solid. Compound **13** was difficult to purify for elemental analysis; however, HRMS gave an acceptable molecular ion peak, and the structures of **13** and its peracetate (data not

presented) were further established by ¹H NMR spectroscopy (Table I).

Using 9:1 CF₃CO₂H-water, the acetal groups of the protected azido derivative **5** were hydrolyzed, furnishing 1D-3-azido-3-deoxy-4-*O*-methyl-*myo*-inositol (**16**) in 66% yield. Reduction of the azido group with hydrogen and Pd/C catalyst gave 1D-3-amino-3-deoxy-4-*O*-methyl-*myo*-inositol (**17**) in 84% yield. The structures for both products were confirmed by ¹H NMR spectroscopy and elemental analyses. Good correspondence of chemical shifts and coupling constants was observed on comparing the spectrum of **15** with those of **16** and **17** (Table I).

By a straightforward application of the BBr₃/CH₂Cl₂-methanol method as for the synthesis of **15**, 1D-3-chloro-1,2:5,6-di-*O*-cyclohexylidene-3-deoxy-4-*O*-methyl-*myo*-inositol (**6**) was fully deprotected to give 1D-3-chloro-3-deoxy-*myo*-inositol (**18**) in 57% yield (Scheme III). The spin-spin coupling constants [$J_{2,3} = 2.4$, $J_{3,4} = 10.0$, and

Table III. *myo*-Inositol Analogues as Inhibitors of Phosphatidylinositol Synthase

analogue	% inhibn of PtdIns synthase ^a	analogue	% inhibn of PtdIns synthase ^a
10	9	18	30
11	28	21	40
13	17	22	37
15	19	23	47
16	10	24	22

^a Assay mixtures contained 0.5 mM *myo*-[³H]inositol and 5 mM analogue. The control rate was 3.6 nmol/min/mg protein. Data are averages of two or more determinations differing no more than 5%.

or smaller than the OH of the natural substrate are tolerated to a certain extent by the enzyme.

It is noteworthy that replacement of the 3-OH group by 3-F (compound 22) resulted in a 7% substrate activity, whereas the similarly substituted 5-F compound retained 25–30% substrate activity compared to *myo*-inositol^{10,11} (Table II). The activity of the 5-fluoro compound 24 has been attributed, at least in part, to the ability of the F atom to accept a hydrogen bond from the enzyme, perhaps in a fashion reminiscent of the natural substrate,¹¹ but this explanation seems less likely to apply to 22 in view of the finding that the deoxy compound 10 has about the same activity. As it is demonstrated that a simple halogen exchange of Cl for F in 18 vs 22, for example, is sufficient to virtually obliterate activity, steric bulk at C-3 appears to be the predominant factor in determining substrate activity. The 3-keto analogue 23 showed only 7% activity as a substrate for PtdIns, despite the fact that NMR studies (data not reported) reveal a preponderance of the *gem*-diol (i.e., the hydrated ketone, which would have an upwardly projecting OH group disposed in the 1D-*myo*-configuration) in aqueous solution. O-Substituents at C-4, such as in the methyl ethers 11 and 16, are not tolerated by the enzyme.

In a separate set of experiments, all of the analogues were tested against a reduced concentration of *myo*-inositol (analogue:inositol 10:1). All inhibited the incorporation of *myo*-inositol into PtdIns to varying degrees (9–47%, Table III). While little can be stated concerning the mode of inhibition by the 3-substituted analogues, their activity as inhibitors is important, as it indicates decreased activity of PtdIns synthase toward its natural substrate in the presence of an excess of analogue. The retention of measurable substrate activity by these compounds (see Table II), together with their inhibitory activity, may result in incorporation of the analogues themselves into phosphoinositides *in vivo*.

To further evaluate their usefulness as blocking agents for PtdIns 3-kinase *in vivo*, analogues that serve both as substrates and inhibitors of PtdIns synthase were tested for inhibition of the uptake of *myo*-inositol by rat embryo fibroblasts under conditions where the incorporated radiolabel is predominantly acid soluble. Inhibition of *myo*-inositol uptake by 3-modified cyclitols was measured as an indication of recognition by the inositol transport system. The results in Table IV demonstrate that those analogues that retain some substrate activity for PtdIns synthase also inhibit *myo*-inositol uptake into whole cells. Taken together, these results indicate that the 3-deoxy (10), 3-amino-3-deoxy (15), and the 3-deoxy-3-fluoro (22) analogues may be useful probes for studying the role of PtdIns 3-kinase in signal transduction and oncogenesis.

Table IV. Inhibition by Inositol Analogues of *myo*-Inositol Uptake in Normal Rat Fibroblasts

analogue	% inhibn of <i>myo</i> -[³ H]inositol uptake ^a
10	80
15	87
22	97

^a Analogues were tested at 5 mM and *myo*-[³H]inositol at 10 μM. Control without analogues incorporated 1.13 nmol of *myo*-inositol/mg of protein in 2 h. Data were from averages of four determinations differing by no more than 12%.

Experimental Section

Melting points were determined using a Thomas-Hoover "Unimelt" capillary melting point apparatus equipped with a Cole-Parmer Model 8520-50 Digi-Sense digital thermocouple combination that was calibrated with known standards. ¹H NMR spectra were recorded at 250, 360, or 400 MHz for solutions that were typically 1–2% (w/v) using respectively a Bruker AC 250, AM 360, or AMX 400 NMR spectrometer. Adsorption chromatography was carried out using E. Merck Silica Gel-60 products: (a) TLC on 0.2-mm aluminum-backed plates; (b) open column chromatography using either 63–212-μm (coarse) or 38–63-μm (fine) silica gel. TLC visualizations were carried out using the following techniques: (A) an anisaldehyde-sulfuric acid dip/heating;³⁶ (B) a spray with 1% aqueous NaIO₄, drying, then a spray with 1% aqueous KMnO₄, whereby the compound appeared as a yellow zone on a purple background; (C) a 0.4% solution of ninhydrin in EtOH containing 1.5% collidine/heat development.³⁷ Electron-impact (EI⁺) mass spectrometry was carried out on a VG ZAB-EQ (VG Analytical, Manchester, England) instrument at the Mass Spectrometry Center of the University of Tennessee. Optical rotations were measured in the indicated solvent and concentration at the sodium D line in a 1-dm cell at ambient temperature (21–27 °C), on a Perkin-Elmer Model 241 spectropolarimeter.

All solvents and reagents were of reagent grade and were used directly unless noted otherwise. *N,N*-Dimethylformamide (DMF) was distilled from calcium hydride at ca. 20 Torr, and methanol was distilled from magnesium turnings. Other solvents designated as "dry" or "anhydrous" were distilled from calcium hydride at atmospheric pressure and stored over molecular sieves (4A, except 3A in the case of DMF and methanol). Solvents, unless otherwise stated, were evaporated at ca. 40 °C/30 Torr on a Büchi Rotovapor. "Concentration under high vacuum" refers to rotary evaporation at pressures of ≤5 Torr. Elemental analyses were carried out by Atlantic Microlab, Inc., Norcross, GA. Solvents of inclusion or crystallization (except water) in the analyzed samples were confirmed by ¹H NMR spectroscopy. Analyses reported for a particular element are within ±0.3% of the theoretical.

Samples submitted for the enzymic assays were analyzed by GLC as their per-*O*-trimethylsilyl derivatives, prepared using Me₃Si-imidazole-CH₃CN according to the published procedure.³⁸ GLC conditions were as follows: Hewlett-Packard 5890 instrument; J&W Scientific DB-5 fused-silica capillary column, 0.03 mm × 30 m; column temperature, 200 °C, isothermal; helium carrier gas; flame-ionization detection. Per-*O*-(trimethylsilyl)-*myo*-inositol was used as the internal standard.

Preparation of 1L-1,2,3,4-Di-*O*-cyclohexylidene-5-*O*-methyl-*chiro*-inositol (1). Compound 1²⁶ was prepared from L-quebrachitol according to known methods.²⁵

1L-1,2,3,4-Di-*O*-cyclohexylidene-5-*O*-methyl-6-*O*-(trifluoromethylsulfonyl)-*chiro*-inositol (2). To a solution of 1²⁶ (1.59 g, 4.49 mmol) in dry CH₂Cl₂ (15 mL) at 0 °C was added dry pyridine (0.2 mL), followed by the dropwise addition of trifluoromethanesulfonic anhydride (0.90 mL, 4.76 mmol). The mixture was stirred at 0 °C for 2 h, at the end of which time TLC indicated one major product (*R*_f 0.61, CHCl₃). The mixture was concentrated, and the residual syrup was partitioned between EtOAc (10 mL) and H₂O (10 mL). The organic phase was washed with H₂O (10 mL) and dried (MgSO₄), and the solvent was evaporated to give 2.0 g (92%, crude) of a yellow syrup suitable for use directly in displacement reactions. For ¹H NMR data, see Table I.

1L-1,2,3,4-Di-O-cyclohexylidene-6-O-(imidazol-1-ylthiocarbonyl)-5-O-methyl-chiro-inositol (3). To a solution of **1**²⁸ (1.96 g, 5.53 mmol) in DMF (60 mL) was added dropwise 1,1'-thiocarbonyldiimidazole (2.2 g, 12.3 mmol). The mixture was stirred under a nitrogen atmosphere for 12 h at 90–100 °C, at the end of which time concentration under high vacuum yielded a yellow syrup. Purification by column chromatography (silica gel, 180 g, CHCl₃) gave 2.42 g (94%) of pure **3** as a white glass: *R*_f 0.15 (CHCl₃, A); for ¹H NMR data, see Table I; ¹³C NMR data (CDCl₃, 62.5 MHz) δ 23.4, 23.6, 23.8, 24.9, 34.5, 36.4, 36.5, 37.5, 58.5, 74.7, 75.5, 76.2, 76.5, 77.0, 77.2, 79.4, 111.8, 113.5, 118.1, 130.9, 137.0, 183.7 (C=S).

Preparation of 1L-1,2,3,4-Di-O-cyclohexylidene-5-O-methyl-6-O-(p-tolylsulfonyl)-chiro-inositol (4). Compound **4** was prepared from **1**²⁸ according to known methods:²⁸ mp 123–125 °C (lit.²⁸ mp 128.5–129 °C); [α]_D²⁵ -13.2° (c 0.90, CDCl₃) [lit.²⁸ [α]_D²⁵ -12° (c 0.733, CHCl₃)]; for ¹H NMR data, see Table I; ¹³C NMR data (CDCl₃, 62.5 MHz) δ 21.6, 23.4, 23.5, 23.6, 23.8, 24.8, 34.6, 36.3, 37.5, 58.1, 75.3, 75.9, 76.7, 78.7, 111.2, 112.7, 128.2, 129.6, 133.4, 144.9.

Preparation of 1D-3-Azido-1,2,5,6-di-O-cyclohexylidene-3-deoxy-4-O-methyl-myo-inositol (5). To a solution of **2** (ca. 2.0 g) in dry DMF (15 mL) at 0 °C was added lithium azide (0.38 g, 7.74 mmol). After stirring at 0 °C for 45 min, and then at ambient temperature for 9.75 h, the solvent was evaporated under high vacuum. The residual syrup was partitioned between CH₂Cl₂ (15 mL) and H₂O (15 mL). The organic phase was washed with H₂O (10 mL), dried (MgSO₄), and concentrated to give a mixture of products, including the elimination product **8**. Column chromatography (silica gel, 150 g, 2:98 EtOAc–hexanes) separated the elimination product **8** from the azide **5**. The azide was dried in vacuo at 50 °C for 19 h to give 0.755 g (48%) of **5** as a glass: *R*_f 0.76 (1:4 EtOAc–hexanes, A); [α]_D²⁵ +41.8° (c 1.0, CDCl₃) [lit.²⁹ [α]_D²⁵ +40° (c 1.00, CHCl₃)]; for ¹H NMR data, see Table I. Anal. (C₁₉H₂₉N₃O₆) C, H, N.

Physicochemical data for **8**: *R*_f 0.47 (CH₂Cl₂, A); for ¹H NMR data, see Table I; ¹³C NMR data (CDCl₃, 62.5 MHz) δ 23.3, 23.4, 23.6, 23.9, 24.9, 25.0, 34.5, 35.9, 36.3, 37.5, 55.4, 72.8, 74.3, 74.5, 80.6, 90.3 (C-3), 110.9, 114.1, 156.1 (C-4). Anal. (C₁₉H₂₈O₆) C, H.

1D-3-Chloro-1,2,5,6-di-O-cyclohexylidene-3-deoxy-4-O-methyl-myo-inositol (6). The crude triflate **2** (148 mg) in dry DMF (5 mL) was treated with imidazole (280 mg, 0.41 mmol), followed by the addition of lithium chloride (184 mg, 0.43 mmol). This mixture was stirred at ambient temperature for 12 h, at the end of which time TLC indicated two major products. The mixture was concentrated under high vacuum and purified by column chromatography (silica gel, 25 g, 1:30 EtOAc–hexanes) to provide 56.7 mg (36.4%) of **6** as a light-yellow syrup and 63 mg (44.8%) of the unsaturated compound **8**. Compound **8** was identical with the product obtained with **5**, above, by TLC and ¹H NMR spectroscopy.

Physicochemical data for **6**: *R*_f 0.30 (1:10 EtOAc–hexanes, A); for ¹H NMR data, see Table I; ¹³C NMR data (CDCl₃, 62.5 MHz) δ 23.5, 23.6, 23.9, 25.0, 25.1, 34.7, 36.5, 36.6, 58.6, 59.1, 75.2, 75.5, 76.3, 77.2, 83.1, 111.7, 113.0. Anal. (C₁₉H₂₉ClO₆) C, H, Cl.

1D-3-(Acetylthio)-1,2,5,6-di-O-cyclohexylidene-3-deoxy-4-O-methyl-myo-inositol (7). To a stirred solution of **2** (662 mg, 1.36 mmol) in CH₃CN (10 mL) was added potassium thioacetate (1.51 g, 13.2 mmol). The mixture was stirred at ambient temperature for 14 h, at the end of which time the solvent was evaporated, and the residue was partitioned between CHCl₃ (20 mL) and H₂O (20 mL). The organic phase was separated and dried (MgSO₄), and the product was purified by column chromatography (silica gel, 45 g, 1:15 EtOAc–hexanes) to provide 36.1 mg (8.6%) of **7** and 261 mg (51%) of **8** as colorless crystals after crystallization from hexanes. Compound **8** was identical with the product obtained with **5**, above, by TLC and ¹H NMR spectroscopy.

Physicochemical data for **7**: mp 115–117 °C; *R*_f 0.15 (1:15 EtOAc–hexanes, A); for ¹H NMR data, see Table I; ¹³C NMR data (CDCl₃, 62.5 MHz) δ 23.5, 23.7, 23.9, 25.0, 30.3, 34.9, 36.3, 36.5, 37.7, 45.5, 58.9, 76.1, 76.4, 78.9, 79.1, 80.0, 110.5, 112.3, 194.2. Anal. (C₂₁H₃₂O₆S) C, H, S.

1L-1,2,3,4-Di-O-cyclohexylidene-5-O-methyl-1,2,4/3,5-cyclohexanepentol (9). A solution of **3** (480 mg, 0.99 mmol) in

toluene (10 mL) was treated with a solution of tributyltin hydride (0.7 mL, 2.6 mmol) and 2,2'-azobis(2-methylpropanenitrile) (α,α'-azobisisobutyronitrile, AIBN, 0.25 g, 1.4 mmol). The mixture was refluxed under nitrogen for 3 h, at the end of which time the mixture was concentrated and purified by column chromatography (silica gel, 60 g, CHCl₃) to give 257 mg (74%) of pure **9** as a colorless syrup: *R*_f 0.30 (CHCl₃, A); for ¹H NMR data, see Table I; ¹³C NMR data (CDCl₃, 62.5 MHz) δ 22.6, 22.6, 22.7, 22.9, 24.0, 33.9, 35.5, 35.6, 37.1, 56.3, 72.7, 75.7, 75.9, 76.2, 78.5, 109.1, 111.0. Anal. (C₁₉H₃₀O₅·0.04CHCl₃) C, H.

Preparation of 1L-1,2,4/3,5-Cyclohexanepentol (10). To a solution of **9** (564 mg, 1.67 mmol) in CH₂Cl₂ (5 mL) was added dropwise a solution of boron tribromide (10 mL of a 1.0 M in CH₂Cl₂; 10 mmol). The solution was stirred at ambient temperature under a nitrogen atmosphere for 5.5 h, at the end of which time the mixture was concentrated to give a brown residue. The residue was successively dissolved in dry MeOH (4 × 10 mL), and the solvent was evaporated to eliminate boron byproducts. The residual syrup was dissolved in MeOH (10 mL) and treated with ethylene oxide to give a neutral pH, and the solvent was evaporated. The residual gum was partitioned between H₂O (20 mL) and CH₂Cl₂ (20 mL), and the aqueous phase was separated, filtered through Celite, and freeze-dried to give 228 mg (83%) of a light-yellow solid. Recrystallization from 95% EtOH gave 172 mg (63%) of **10** as a white solid: mp 179–181 °C (lit.³¹ mp 180–181 °C); [α]_D²⁵ -49.8° (c 0.5, H₂O) [lit.³¹ [α]_D²⁵ -50°]; for ¹H NMR data, see Table I. Anal. (C₆H₁₂O₅·0.3H₂O) C, H.

1L-5-O-Methyl-1,2,4/3,5-cyclohexanepentol (11). To **9** (78.5 mg, 0.23 mmol) was added 9:1 CF₃COOH–H₂O (8 mL), and the resulting solution was stirred at ambient temperature for 15 min. Concentration of the solution, followed by multiple coevaporations with absolute EtOH (4 × 10 mL), gave a residue that was partitioned between H₂O (10 mL) and CHCl₃ (10 mL). The aqueous phase was separated and freeze-dried to give 33 mg (81%) of **11** as a colorless solid: [α]_D²⁵ -69.1° (c 1.42, H₂O); for ¹H NMR data, see Table I. Anal. (C₇H₁₄O₅·0.07CHCl₃) C, H.

Preparation of 1L-1-O-(p-Tolylsulfonyl)-chiro-inositol (12). Compound **4** (1.56 g, 3.07 mmol) was reacted with BBr₃ in a manner similar to that described above for the preparation of compound **10** from **9**. Product **12** (1.15 g, 90%) was obtained as a white glass: mp 160–162 °C (lit.²⁶ mp 162.5–164 °C); [α]_D²⁵ -39.2° (c 0.95, D₂O) [lit.²⁶ [α]_D²⁵ -37.7° (c 0.45, MeOH)]; for ¹H NMR data, see Table I; ¹³C NMR data (D₂O, 90.5 MHz) δ 19.0, 66.2, 66.9, 68.1, 70.5, 74.8, 79.3, 126.1, 128.2.

Preparation of 1D-3-Azido-3-deoxy-myo-inositol (13). Compound **12** (836 mg, 2.50 mmol) was reacted in a manner similar to that described above for the preparation of compound **5** from **2**, except that the mixture was heated for 11 h at 60–65 °C. The crude reaction mixture was peracetylated using acetic anhydride in pyridine, and the product was purified by column chromatography (silica gel, 10 g, CH₂Cl₂), followed by deacetylation using a saturated methanolic ammonia solution at ambient temperature. Product **13** (318 mg, 62%) was obtained as a white glass: *R*_f 0.73 (4:1:3:2 PrOH–EtOAc–H₂O–HOAc; B); [α]_D²⁵ -2° (c 1.0, MeOH) [lit.²⁹ [α]_D²⁵ -6° (c 1.28, MeOH)]; IR 2100 (s) cm⁻¹; HRMS calcd for C₆H₁₁N₃O₅ 205.0695, found 205.0738; for ¹H NMR data, see Table I.

1D-3-Amino-1,2,5,6-di-O-cyclohexylidene-3-deoxy-4-O-methyl-myo-inositol (14). To a solution of **5** (241 mg, 0.64 mmol) in EtOAc (4 mL) was added a catalytic amount of 10% Pd/C in EtOAc (2 mL). This mixture was stirred under 55 psi H₂ for 20.5 h. Filtration, concentration of solvent, and drying the residue in vacuo at ambient temperature for 18.75 h gave 208 mg (92%) of a colorless syrup: *R*_f 0.15 (1:4 EtOAc–hexanes, A); [α]_D²⁵ -26.0° (c 0.6, CDCl₃); for ¹H NMR data, see Table I. Anal. (C₁₉H₃₁NO₅) C, H, N.

Preparation of 1D-3-Amino-3-deoxy-myo-inositol Hydrobromide (15). Compound **14** was treated in a manner similar to that described above for the preparation of compound **10** from **9**. Product **15** (300 mg, 66%) was obtained as a gray solid: *R*_f 0.26 (0.1 N NH₄Cl, C); [α]_D²⁵ +8.1° (c 1.1, H₂O) [lit.³⁹ [α]_D²⁵ +9.5° (c 6.2, H₂O)]; for ¹H NMR data, see Table I; ¹³C NMR data (D₂O, 90.5 MHz) δ 56.6, 71.4, 72.2, 74.4, 74.9, 77.5. Anal. (C₆H₁₃NO₅·0.92HBr) C, H, Br, N.

Preparation of 1D-3-Azido-3-deoxy-4-O-methyl-myo-inositol (16). Compound **5** was treated in a manner similar to that

described above for the preparation of compound 11 from 9. Product 16 (112 mg, 66%) was obtained as a white solid: mp 176–178 °C (lit.²⁹ mp 178–179 °C); $[\alpha]_D^{25}$ -25.5° (c 0.47, MeOH) [lit.²⁹ $[\alpha]_D$ -29° (c 1.04, MeOH)]; for ¹H NMR data, see Table I. Anal. (C₇H₁₃N₃O₅) C, H, N.

1D-3-Amino-3-deoxy-4-O-methyl-myo-inositol (17). Compound 16 was treated in a manner similar to that described above for the preparation of compound 14 from 5 with the substitution of EtOH as solvent. Product 17 (48.4 mg, 84%) was obtained as a mixture of the free base and the bicarbonate salt, a pale-yellow solid: mp 164–168 °C dec; *R*_f 0.56 (0.1 N NH₄Cl, C); for ¹H NMR data, see Table I. Anal. (C₇H₁₅NO₅·0.33 H₂CO₃) C, H, N.

1D-3-Chloro-3-deoxy-myo-inositol (18). Compound 6 was treated in a manner similar to that described above for the preparation of compound 10 from 9. Product 18 (34.5 mg, 57%) was obtained as a white solid: mp 235 °C dec; $[\alpha]_D^{25}$ +20.9° (c 0.54, H₂O); for ¹H NMR data, see Table I. Anal. (C₆H₁₁ClO₅·0.25H₂O) C, H.

1D-1,2,5,6-Tetra-O-acetyl-3-(acetylthio)-3-deoxy-4-O-methyl-myo-inositol (20). Compound 7 was treated in a manner similar to that described for the preparation of compound 11 from 9. Product 19 (410 mg, crude) was obtained as a light-yellow solid. Compound 19 was treated with acetic anhydride (4 mL) in pyridine (5 mL) and stirred at ambient temperature for 7 h, at which time the solvent was removed under vacuum. Column chromatography (silica gel, 25 g, 3:1 hexanes–EtOAc) afforded compound 20 (572 mg, 83% from 7) as colorless crystals: mp 103–105 °C; *R*_f 0.18 (1:3 EtOAc–hexanes, A); for ¹H NMR data, see Table I. Anal. (C₁₇H₂₄O₁₀S) C, H, S.

1D-3-Deoxy-3-mercapto-myo-inositol (21). Compound 20 was treated in a manner similar to that described for the preparation of compound 10 from 9. Product 21 (91 mg, 54%) was obtained as a white solid: $[\alpha]_D^{24}$ +40.9° (c 0.47, MeOH); for ¹H NMR data, see Table I. Anal. (C₆H₁₂O₅S·0.07CHCl₃) C, H, S.

PtdIns Synthase Assays. Mouse brain microsomes prepared as described by Benjamin and Agranoff³⁵ were the source of PtdIns synthase. Fresh brains from 12-day-old mice were homogenized in 6 volumes of 0.25 M sucrose, dialyzed 3 h against 70 mM potassium phosphate buffer, pH 8.1, and centrifuged at 900g for 10 min. The supernatant fraction was centrifuged at 12000g for 60 min. The resulting pellet was suspended in 0.25 M sucrose at a protein concentration of 20 mg/mL and stored at -80 °C. Protein was measured by the method of Bradford.⁴⁰ PtdIns synthase activity was measured essentially as described by Moyer et al.¹¹ The release of water-soluble CMP was monitored at 37 °C in the presence of 50 mM Tris-HCl buffer (pH 8.3), 48 mM MgCl₂, 0.5 mM Ptd-CMP (CDP-dipalmitin, Sigma), 0.25% Triton X-100, 5 mM inositol or analogue, and 0.5 mg of microsomal protein in a total volume of 0.2 mL. The reaction was stopped during the linear phase of the reaction by the addition of 3.8 mL of 2:1 CHCl₃-CH₃OH, and the phases were separated with 0.8 mL of water. CMP in the aqueous phase was quantitated from the absorbance at 280 nm using an extinction coefficient of 13 × 10⁶ mol⁻¹·cm². Alternatively, inhibition of PtdIns synthase was measured by monitoring the incorporation of 0.5 mM *myo*-[2-³H]inositol (12,000 cpm/nmol) (NEN-Dupont) at 37 °C into CHCl₃-soluble material in the presence of Tris-HCl (pH 8.3) 48 mM MgCl₂, 0.5 mM Ptd-CMP, 0.25% Triton X-100, 5 mM analogue, and 0.05 mg of microsomal protein in a total volume of 0.1 mL. The reaction was stopped after 20 min during the linear phase of the reaction by the addition of 0.5 mL of 0.1 N HCl in MeOH and 0.5 mL of CHCl₃, and the phases were separated with 0.5 mL of water. Radioactivity in the organic layer was quantitated in a scintillation counter.

Inhibition of *myo*-Inositol Uptake into F111 Cells. F111 rat fibroblasts (1.5 × 10⁶ cells/coverslip) were grown on 13-mm glass coverslips overnight in inositol-free Dulbecco's modified Eagle medium (DMEM) containing 10% dialyzed calf serum and 10 μM *myo*-inositol. Cells adhering to the coverslips were washed twice in Krebs-Ringer buffer (135 mM NaCl, 4.6 mM KCl, 1.2 mM CaCl₂, 1 mM Na phosphate, 1.3 mM MgSO₄, 5 mM HEPES pH 7.4), immersed in Krebs-Ringer buffer containing 10 μM *myo*-[³H]inositol (1000 cpm/pmol) and 5 mM analogue, and incubated in a 5% CO₂ incubator at 37 °C. After 120 min the cells were washed six times with 140 mM NaCl and lysed in

1% SDS. In a separate experiment, labeled cells were lysed with 10% trichloroacetic acid and separated by centrifugation into soluble and insoluble fractions. The cell lysates were assayed for radioactivity in a scintillation counter and for protein using Pierce Chemical Co.'s micro BCA protein assay reagents.

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References

- (1) Nomenclature used herein conforms to the IUPAC-IUB recommendations for cyclitols and phosphatidylinositols and related compounds published respectively in Nomenclature of Cyclitols. *Pure Appl. Chem.* 1974, 37, 285–297 and *Biochem. J.* 1978, 171, 1–19. Inasmuch as position numbers change with change in stereochemical designator, the numbering has been indicated as "1L-*chiro* numbering" or as "1D-*myo* numbering" where confusion may exist. The headings in Table I for the NMR data all refer to "1D-*myo* numbering" in order to permit necessary comparisons of chemical shifts (see *Biochem. J.* 1989, 258, 1–2 for rules for the use of "stereospecific numbering"). Abbreviations for the various compounds follow the recommendations in ref. 4, pp xxi–xxv, as currently modified and under consideration by IUPAC.
- (2) Vance, D. E. Complex Lipids. In *Biochemistry*, 2nd ed.; Zubay, G., Ed.; Macmillan: New York, 1988; pp 698–702.
- (3) *Inositol and Phosphoinositides*; Bleasdale, J. E., Eichberg, J., Hauser, G., Eds.; Humana: Clifton, NJ, 1985.
- (4) *Inositol Lipids in Cell Signaling*; Michell, R. H., Drummond, A. H., Downes, C. P., Eds.; Academic: San Diego, 1989.
- (5) Downes, C. P.; MacPhee, C. H. *myo*-Inositol Metabolites as Cellular Signals. *Eur. J. Biochem.* 1990, 193, 1–18.
- (6) Potter, B. V. L. Recent Advances in the Chemistry and Biochemistry of Inositol Phosphates of Biological Interest. *Nat. Prod. Rep.* 1990, 1–24.
- (7) Majerus, P. W.; Ross, T. S.; Cunningham, T. W.; Caldwell, K. K.; Jefferson, A. B.; Bansal, V. S. Recent Insights in Phosphatidylinositol Signaling. *Cell* 1990, 63, 459–465.
- (8) Jiang, C.; Moyer, J. D.; Baker, D. C. Synthesis of Deoxy and Deoxyhalogeno Analogues of *myo*-Inositol. *J. Carbohydr. Chem.* 1987, 6, 319–355.
- (9) Jiang, C.; Schedler, D. J. A.; Morris, P. E., Jr.; Zahed, A.-H. A.; Baker, D. C. Fluorinated cyclitols. An Improved Synthesis of 5-Deoxy-5-fluoro-*myo*-inositol, its Deuterium Labeling, and Synthesis of a 5,5-*gem*-Difluoro Analogue. *Carbohydr. Res.* 1990, 207, 277–285.
- (10) Moyer, J. D.; Reizes, O.; Ahir, S.; Jiang, C.; Malinowski, N.; Baker, D. C. Substrate Properties of Analogs of *myo*-Inositol. *Mol. Pharmacol.* 1988, 33, 683–689.
- (11) Moyer, J. D.; Reizes, O.; Malinowski, N.; Jiang, C.; Baker, D. C. Fluorinated Analogues of *myo*-Inositols as Biological Probes of Phosphatidylinositol Metabolism. In *Fluorinated Carbohydrates: Chemical and Biochemical Aspects*; Taylor, N. F., Ed.; ACS Symposium Series 374; American Chemical Society: Washington, DC, 1988; pp 43–58.
- (12) Whitman, M.; Downes, C. P.; Keeler, M.; Keller, T.; Cantley, L. Type I Phosphatidylinositol Kinase Makes a Novel Inositol Phospholipid, Phosphatidylinositol 3-Phosphate. *Nature* 1988, 332, 644–646.
- (13) Auger, K. R.; Serunian, L. A.; Soltoff, S. P.; Libby, P.; Cantley, L. C. PDGF-Dependent Tyrosine Phosphorylation Stimulates Production of Novel Polyphosphoinositides in Intact Cells. *Cell* 1989, 57, 167–175.
- (14) Schu, P. V.; Takegawa, K.; Fry, M. J.; Stack, J. H.; Waterfield, M. D.; Emr, S. D. Phosphatidylinositol 3-Kinase Encoded by Yeast VPS34 Gene Essential for Protein Sorting. *Science* 1993, 260, 88–91.
- (15) Cantley, L. C.; Auger, K. R.; Carpenter, C.; Duckworth, B.; Graziani, A.; Kapeller, R.; Soltoff, S. Oncogenes and Signal Transduction. *Cell* 1991, 64, 281–302.
- (16) Ulug, E. T.; Hawkins, P. T.; Hanley, M. R.; Courtneidge, S. A. Phosphatidylinositol Metabolism in Cells Transformed by Polyomavirus Middle T Antigen. *J. Virol.* 1990, 64, 3895–3904.
- (17) Lips, D. L.; Majerus, P. W.; Gorga, F. R.; Young, A. T.; Benjamin, T. L. Phosphatidylinositol 3-phosphate is Present in Normal and Transformed Fibroblasts and is Resistant to Hydrolysis by Bovine Brain Phospholipase C II. *J. Biol. Chem.* 1989, 264, 8759–8763.
- (18) Talmage, D. A.; Freund, R.; Young, A. T.; Dahl, J.; Dawe, C. J.; Benjamin, T. L. Phosphorylation of Middle T by pp60c-src: A Switch for Binding of Phosphatidylinositol 3-kinase and Optimal Tumorigenesis. *Cell* 1989, 59, 55–65.

- (19) Freund, R.; Dawe, C. J.; Benjamin, T. L. Changes in the Frequency, Morphology, and Behavior of Tumors Induced in Mice by a Polyomavirus Mutant with a Specifically Altered Oncogene. *Am. J. Pathol.* 1992, 141, 1409-1425.
- (20) Kozikowski, A. P.; Fauq, A. H.; Powis, G.; Melder, D. C. Efficient Synthetic Routes to Fluorinated Isosteres of Inositol and Their Effects on Cellular Growth. *J. Am. Chem. Soc.* 1990, 112, 4528-4531.
- (21) Powis, G.; Aksoy, I. A.; Melder, D. C.; Aksoy, S.; Eichinger, H.; Fauq, A. H.; Kozikowski, A. P. D-3-Deoxy-3-substituted myo-Inositol Analogues as Inhibitors of Cell Growth. *Cancer Chemother. Pharmacol.* 1991, 29, 95-104.
- (22) Kozikowski, A. P.; Fauq, A. H.; Powis, G.; Melder, D. C. D-3-Modified myo-Inositol Analogues: Synthesis and Growth Inhibitory Properties. *Med. Chem. Res.* 1991, 1, 277-282.
- (23) Offer, J.; Metcalfe, J. C.; Smith, G. A. The Uptake of ³H-Labelled Monodeoxyfluoro-myo-inositols into Thymocytes and their Incorporation into Phospholipid in Permeabilized Cells. *Biochem. J.* 1993, 291, 553-560.
- (24) For a preliminary report, see Shih, T.-L.; Johnson, S. C.; Schedler, D. J. A.; Baker, D. C.; Dahl, J.; Dower, K.; Benjamin, T. L.; Anderson, L. Synthesis of 3-Modified 1D-myo-Inositols as Potential Inhibitors of Phosphatidylinositol 3-Kinase. *Abstracts of Papers*; 201 National Meeting of the American Chemical Society, Atlanta, GA; American Chemical Society: Washington, DC, 1991; CARB 38.
- (25) Garegg, P. J.; Iverson, T.; Johansson, R.; Lindberg, B. Synthesis of Some Mono-O-benzyl and Penta-O-methyl-myo-inositols. *Carbohydr. Res.* 1984, 130, 322-326.
- (26) Gero, S. D. The preparation of 1-O-Tosyl(-)-Inositol from Quebrachitol. *Tetrahedron Lett.* 1966, 591-595.
- (27) Ambrose, M. G.; Binkley, R. W. Synthesis of Deoxyhalogeno Sugars. Reactions of Halide Ions with 1,2,3,4-Tetra-O-acetyl-6-O-[(trifluoromethyl)sulfonyl]-β-D-glucopyranose. *J. Org. Chem.* 1983, 48, 674-677.
- (28) Mercier, D.; Barnett, J. E. G.; Gero, S. D. Synthesis of Optically Active Derivatives of myo-Inositol. Preparation of 1L-myo-Inositol-1-phosphate. *Tetrahedron* 1969, 25, 5681-5687.
- (29) Olesker, A.; Mercier, D.; Gero, S. D.; Pearce, C. J.; Barnett, J. E. G. Conformation of the Structure of myo-Inosamine Components of Minosaminomycin. Synthesis of Derivatives of D-myo-Inosamine-1. *J. Antibiot.* 1975, 28, 490-491.
- (30) Barton, D. H. R.; McCombie, S. W. A New Method for the Deoxygenation of Secondary Alcohols. *J. Chem. Soc., Perkin Trans. 1* 1975, 1574-1585.
- (31) Anderson, L. The Cyclitols. In *The Carbohydrates*; Pigman, W.; Horton, D., Eds.; Academic: New York, 1972; Vol. 1A, p 558.
- (32) Kozikowski, A. P.; Ognyanov, V. I.; Fauq, A. H.; Nahorski, S. R.; Wilcox, R. A. Synthesis of 1D-3-Deoxy-, 1D-2,3-Dideoxy-, and 1D-2,3,6-Trideoxy-myo-inositol 1,4,5-Trisphosphate from Quebrachitol, Their Binding Affinities, and Calcium Release Activity. *J. Am. Chem. Soc.* 1993, 115, 4429-4434.
- (33) Post, G. G.; Anderson, L. Cyclitols and their Methyl Ethers. III. Catalytic Air Oxidation, the Hydrogenolysis of Inososes, and Some Pentol and Tetrol Methyl Ethers. *J. Am. Chem. Soc.* 1962, 84, 471-478.
- (34) Kurihara, N.; Hayashi, K.; Nakajima, M. Chemistry of Benzene-glycols Part XVIII. Synthesis of (-)-N-Methyl-2-deoxystreptamine and its Absolute Configuration. *Agric. Biol. Chem.* 1969, 33, 256-261.
- (35) Benjamin, J. A.; Agranoff, B. W. Distribution and Properties of CDP-diglyceride: Inositol Transferase from Brain. *J. Neurochem.* 1969, 16, 513-527.
- (36) Schaumberg, J. P.; Hokanson, G. C.; French, J. C.; Smal, E.; Baker, D. C. 2'-Chloropentostatin, a New Inhibitor of Adenosine Deaminase. *J. Org. Chem.* 1985, 50, 1651-1656.
- (37) Fieser, L. F. *Experiments in Organic Chemistry*, 3rd ed.; Heath: Boston, 1955; p 136.
- (38) Sweeley, C. C.; Bentley, R.; Makita, M.; Wells, W. W. Gas-Liquid Chromatography of Trimethylsilyl Derivatives of Sugars and Related Substances. *J. Am. Chem. Soc.* 1963, 85, 2497-2507.
- (39) Post, G. G. Ph.D. Dissertation, University of Wisconsin, 1959.
- (40) Bradford, M. M. A Rapid and Sensitive Method for Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* 1976, 72, 248-254.