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

Stephen C. Johnson,† Jean Dahl,‡ Tzenge-Lien Shih,† David J. A. Schedler,† Laurens Anderson,§ Thomas L. Benjamin,*,‡ and David C. Baker*,†

Department of Chemistry, The University of Tennessee, Knoxville, Tennessee 37996-1600, Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115, and Department of Biochemistry, The University of Wisconsin—Madison, Madison, Wisconsin 53706

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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 LiN_3, followed by reduction of the azido function, and with LiCl and KSAc to give the *O*-protected compounds. *O*-Demethylation using BBr_3 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-NH_2, 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*-[^{3}H]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-NH_2, 3-F, and 3-deoxy compounds are taken up by intact fibroblast cells as evidenced by their competing with *myo*-[^{3}H]inositol uptake.

Research on the phosphatidylinositol (PtdIns) pathway[2] 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)P_2], mediated by a phosphatidylinositol-specific phospholipase C (PIC), to the well-recognized second messengers *sn*-1,2-diacylglycerol (Ac_2Gro) and 1D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3]. 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)P_2, and PtdIns(3,4,5)P_3.[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

† The University of Tennessee.
‡ Harvard University.
§ The University of Wisconsin—Madison.
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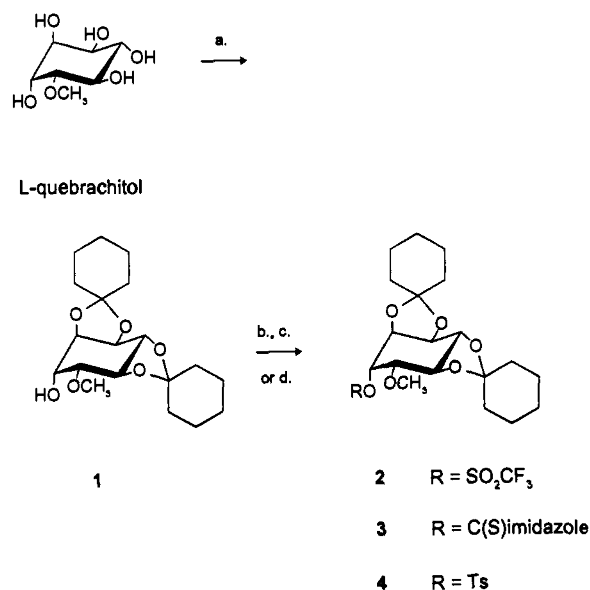
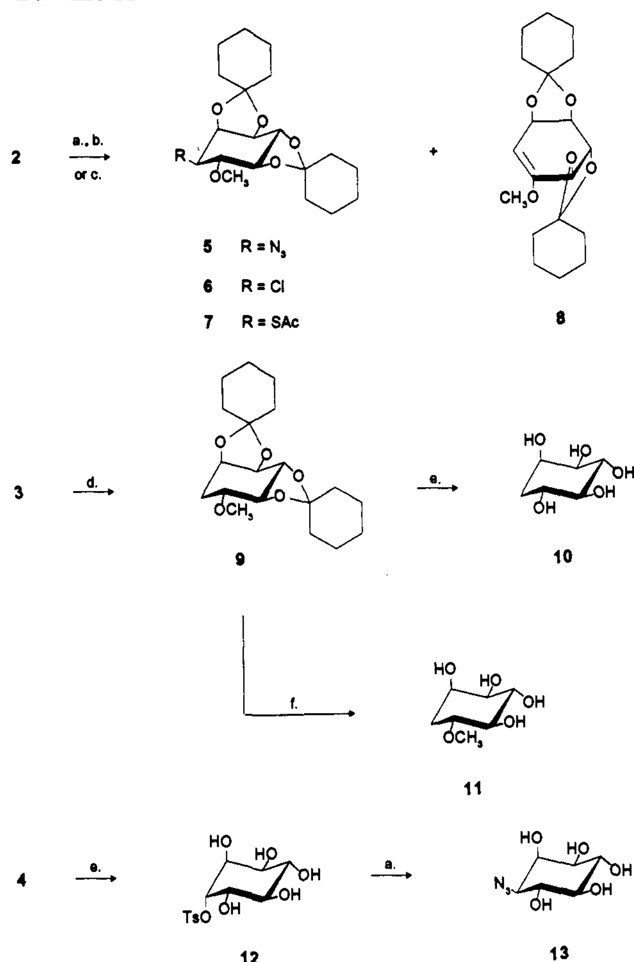
Scheme I[a]



[a]Reagents: (a) 1-ethoxycyclohexene, DMF, *p*-TSA; (b) trifluoromethanesulfonic anhydride, pyridine, CH_2Cl_2; (c) 1,1′-thiocarbonyldiimidazole, DMF; (d) *p*-toluenesulfonyl chloride, pyridine.

Scheme II[a]



[a]Reagents: (a) LiN_3, DMF; (b) LiCl, imidazole, DMF; (c) KSAc, CH_3CN; (d) Bu_3SnH, AIBN, toluene; (e) BBr_3, CH_2Cl_2; CH_3OH; (f) 3:1 CF_3CO_2H–H_2O.

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-ethoxycyclohexene[25] 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 CH_2Cl_2,[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 known[28] 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 ^{13}C 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 $J_{2,3} \approx J_{3,4} \approx 4.8$–$6.6$ 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 CH_3CN, respectively (Scheme II). Column chromatography furnished the pure displacement products 5, 6, and 7. These compounds showed $J_{2,3}$ in the range of 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 ^{13}C NMR spectra. Especially diagnostic was the ^{13}C 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 BBr_3 in CH_2Cl_2, followed by a workup in methanol, resulted in decompositon 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 BBr_3/CH_2Cl_2, with workup in methanol (conditions are acidic, as HBr is generated from the methanolysis of BBr_3), 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. ^{1}H 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_6H_{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_6H_{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_6H_{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_6H_{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_6H_{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_6H_{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_6H_{10}
9	$CDCl_3$	4.13 (dd) (8.5)	4.34 (dd) (4.3)	H-3$_a$[f,g] 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_6H_{10}
10	D_2O	3.48–3.57 (m) (2.2)	4.06 (dd)[h]	H-3$_a$[f,i] 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_2O	3.42–3.49 (m) (2.8)	4.08 (dd)[j]	H-3$_a$[f,k] 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_2O	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[l]	D_2O	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_6H_{10}
15[l]	D_2O	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[l]	D_2O	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_2O	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_2O	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_2O	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-3a and equatorial H-3e. [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.

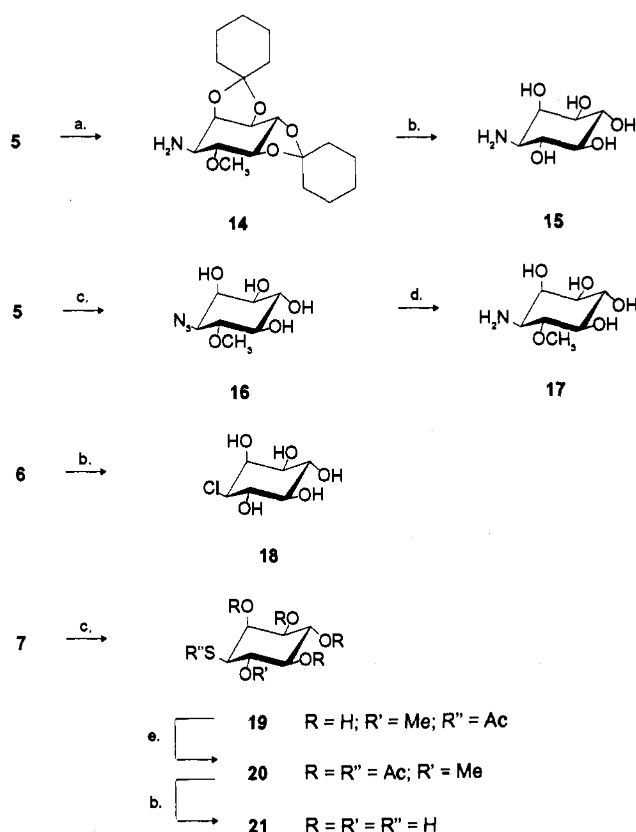
the Experimental Section) and by elemental analyses. Surprisingly, 15 was found to be unstable to long-term storage as a second, so far uncharacterized, compound is found in samples of 15 that have been kept at room temperature for several months.

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 BBr_3/CH_2Cl_2–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 ^{1}H NMR spectroscopy (Table I).

Using 9:1 CF_3CO_2H–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 ^{1}H NMR spectroscopy and elemental analyses. Good correspondence of chemical shifts and coupling constants was observed on camparing the spectrum of 15 with those of 16 and 17 (Table I).

By a straightforward application of the BBr_3/CH_2Cl_2–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

Scheme III[a]



[a]Reagents: (a) H_2, 10% Pd–C, EtOAc; (b) BBr_3, CH_2Cl_2; CH_3OH; (c) 9:1 CF_3CO_2H–H_2O; (d) H_2, 10% Pd–C, EtOH; (e) Ac_2O, pyridine.

$J_{4,5}$ = 10.0 Hz (Table I)] confirm the inversion of **6** to give the 3-substituted 1D-*myo* derivative. Compound **18** also gave an acceptable elemental analysis.

Barton–McCombie[30] free-radical deoxygenation of 1L-1,2:3,4-di-*O*-cyclohexylidene-6-*O*-(imidazol-1-ylthiocarbonyl)-5-*O*-methyl-*chiro*-inositol (**3**) at C-6 with AIBN–Bu_3SnH gave 1L-1,2:3,4-di-*O*-cyclohexylidene-5-*O*-methyl-1,2,4/3,5-cyclohexanepentol (**9**) in 74% yield. Evidence of the deoxygenation was provided by the appearance of two complex multiplets for H-3_a and H-3_b (1D-*myo* numbering) upfield in the δ 1.2–2.4 region (Table I). Deprotection of **9** with a boron tribromide solution in CH_2Cl_2, followed by hydrolytic workup in methanol, gave 1L-1,2,4/3,5-cyclohexanepentol (**10**) in 63% yield (Scheme II), which had a melting point and optical rotation identical with the values reported for (–)-viburnitol.[31] This approach to **10** (which is very similar to a procedure just published[32]) is superior to previously reported routes that involve tedious product separations and are low yielding.[33,34]

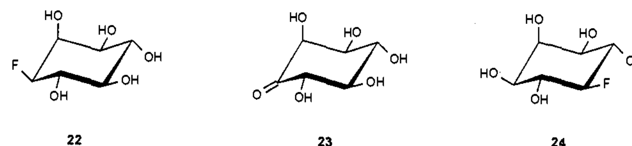
For the preparation of 1D-3-deoxy-3-mercapto-*myo*-inositol (**21**), 1D-3-(acetylthio)-1,2:5,6-di-*O*-cyclohexylidene-3-deoxy-4-*O*-methyl-*myo*-inositol (**7**) was subjected to acidic hydrolysis in 9:1 CF_3CO_2H–water to give crude **19**. This product was peracetylated, and the peracetate **20** was purified by column chromatography and characterized by ^1H NMR spectroscopy and elemental analysis. *O,S*-Deacetylation of **20** occurred along with *O*-demethylation upon treatment with BBr_3/CH_2Cl_2–MeOH, giving the mercapto analogue **21** in 54% yield. The structure was confirmed by ^1H NMR spectroscopy and elemental analysis.

Two additional analogues desired for study, 1D-3-deoxy-3-fluoro-*myo*-inositol (**22**) and "1D-*myo*-inosose-3" (**23**), were synthesized by routes not involving activated derivatives **2**, **3**, or **4**. The 3-fluoro analogue **22** was prepared

Table II. *myo*-Inositol Analogues as Substrates of Phosphatidylinositol Synthase

analogue	CMP formation[a] (%, compared with *myo*-inositol)	analogue	CMP formation[a] (%, compared with *myo*-inositol)
myo-inositol	100	18	1
10	6	21	1.5
11	0	22	7
13	1	23	7
15	9	24	28
16	0		

[a] Analogues and *myo*-inositol were tested at 5 mM. The apparent K_m of *myo*-inositol in this assay was ~1.5 mM (see ref 34 and confirmed in the present experiments). The control rate was 11.4 nmol/min/mg protein. Averages of two or more determinations differing no more than 5% are presented.



directly from L-quebrachitol by the DAST-mediated fluorination method of Kozikowski and co-workers,[20] while compound **23** was obtained by the platinum-catalyzed air oxidation of 1L-*chiro*-inositol. All attempts to accomplish the oxidation using platinum deposited on Norit as the catalyst were complete failures, but platinum on Darco G-60 proved effective as described by Post and Anderson.[33] The isolation and purification of **23** was carried out as described by these authors. 5-Deoxy-5-fluoro-*myo*-inositol (**24**) was prepared by the improved procedure as recently described.[9]

3-Modified 1D-*myo*-Inositol Analogues as Substrates or Inhibitors of Phosphatidylinositol Synthase. The 3-modified 1D-*myo*-inositol analogues (Table II) were evaluated as substrates or inhibitors of PtdIns synthase from mouse brain (for details, see the Experimental Section). Substrate activity was determined by the release of cytidine 5′-monophosphate (CMP) from cytidine diphosphodiacylglycerol (PtdCMP), as described in previous studies.[10,11,35] Our data confirm that PtdIns synthase has a marked preference for the natural substrate, *myo*-inositol. Modification of *myo*-inositol at the 3-position reduced its efficacy as a substrate by 90% or greater. Of the compounds tested, 1D-3-amino-3-deoxy-*myo*-inositol (**15**) showed the highest substrate activity, namely 9% that of *myo*-inositol at the highest concentration tested. Next highest was 1D-3-deoxy-3-fluoro-*myo*-inositol (**22**),[20] followed by the 3-deoxy analogue, 1L-1,2,4/3,5-cyclohexanepentol (**10**), showing respectively 7% and 6% substrate activity relative to *myo*-inositol. Similar results have been reported for the incorporation of 1D-3-deoxy-3-fluoro-*myo*-[2-^3H]inositol into phospholipid by permeabilized cells.[23] The other analogues, namely the 1D-3-azido-3-deoxy- (**13**), the 1D-3-chloro-3-deoxy- (**18**), and the 1D-3-deoxy-3-mercapto-*myo*-inositol (**21**), all showed negligible activity as substrates for the enzyme. This result is in line with the trends observed earlier for 5-modified *myo*-inositols wherein the substrate activities were shown to diminish with increasing steric bulk of the substituent.[10,11] Thus it is apparent from the data in Table II that substituents (NH_2, F, and H) that are either approximately equal to

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*-[^{3}H]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 *myo*-[^{3}H]inositol uptake[a]
10	80
15	87
22	97

[a] Analogues were tested at 5 mM and *myo*-[^{3}H]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. ^{1}H 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 $NaIO_4$, drying, then a spray with 1% aqueous $KMnO_4$, 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 ^{1}H 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 Me_3Si–imidazole–CH_3CN 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 **1**[26] 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 **1**[26] (1.59 g, 4.49 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C was added dry pyridine (0.2 mL), followed by the dropwise addition of trifluoromethanesulfonic anhydride (0.80 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, $CHCl_3$). The mixture was concentrated, and the residual syrup was partitioned between EtOAc (10 mL) and H_2O (10 mL). The organic phase was washed with H_2O (10 mL) and dried ($MgSO_4$), and the solvent was evaporated to give 2.0 g (92%, crude) of a yellow syrup suitable for use directly in displacement reactions. For ^{1}H 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[26] (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, $CHCl_3$) gave 2.42 g (94%) of pure 3 as a white glass: R_f 0.15 ($CHCl_3$, A); for ^1H NMR data, see Table I; ^{13}C NMR data ($CDCl_3$, 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[26] according to known methods:[28] mp 123–125 °C (lit.[28] mp 128.5–129 °C); $[\alpha]^{26}_D$ −13.2° (c 0.90, $CDCl_3$) [lit.[28] $[\alpha]^{29}_D$ −12° (c 0.733, $CHCl_3$)]; for ^1H NMR data, see Table I; ^{13}C NMR data ($CDCl_3$, 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 CH_2Cl_2 (15 mL) and H_2O (15 mL). The organic phase was washed with H_2O (10 mL), dried ($MgSO_4$), 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); $[\alpha]^{22}_D$ +41.8° (c 1.0, $CDCl_3$) [lit.[29] $[\alpha]_D$ +40° (c 1.00, $CHCl_3$)]; for ^1H NMR data, see Table I. Anal. ($C_{19}H_{29}N_3O_5$) C, H, N.

Physicochemical data for 8: R_f 0.47 (CH_2Cl_2, A); for ^1H NMR data, see Table I; ^{13}C NMR data ($CDCl_3$, 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. ($C_{19}H_{28}O_5$) 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; ^{13}C NMR data ($CDCl_3$, 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. ($C_{19}H_{29}ClO_5$) 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 CH_3CN (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 $CHCl_3$ (20 mL) and H_2O (20 mL). The organic phase was separated and dried ($MgSO_4$), and the product was purified by column chromatography (silica gel, 45 g, 1:15 EtOAc–hexanes) to provide 36.1 mg (8.6%) of 8 and 261 mg (51%) of 7 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; ^{13}C NMR data ($CDCl_3$, 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. ($C_{21}H_{32}O_6S$) 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, $CHCl_3$) to give 257 mg (74%) of pure 9 as a colorless syrup: R_f 0.30 ($CHCl_3$, A); for ^1H NMR data, see Table I; ^{13}C NMR data ($CDCl_3$, 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. ($C_{19}H_{30}O_5$·0.04$CHCl_3$) C, H.

Preparation of 1L-1,2,4/3,5-Cyclohexanepentol (10). To a solution of 9 (564 mg, 1.67 mmol) in CH_2Cl_2 (5 mL) was added dropwise a solution of boron tribromide (10 mL of a 1.0 M in CH_2Cl_2; 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 H_2O (20 mL) and CH_2Cl_2 (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); $[\alpha]^{25}_D$ −49.8° (c 0.5, H_2O) [lit.[31] $[\alpha]_D$ −50°]; for ^1H NMR data, see Table I. Anal. ($C_6H_{12}O_5$·0.3H_2O) 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 CF_3COOH–H_2O (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 H_2O (10 mL) and $CHCl_3$ (10 mL). The aqueous phase was separated and freeze-dried to give 33 mg (81%) of 11 as a colorless solid: $[\alpha]^{25}_D$ −69.1° (c 1.42, H_2O); for ^1H NMR data, see Table I. Anal. ($C_7H_{14}O_5$·0.07$CHCl_3$) C, H.

Preparation of 1L-1-O-(p-Tolylsulfonyl)-*chiro*-inositol (12). Compound 4 (1.56 g, 3.07 mmol) was reacted with BBr_3 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); $[\alpha]^{23}_D$ −39.2° (c 0.95, D_2O) [lit.[26] $[\alpha]^{30}_D$ −37.7° (c 0.45, MeOH)]; for ^1H NMR data, see Table I; ^{13}C NMR data (D_2O, 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, CH_2Cl_2), 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–H_2O–HOAc; B); $[\alpha]^{22}_D$ −2° (c 1.0, MeOH) [lit.[29] $[\alpha]_D$ −6° (c 1.28, MeOH)]; IR 2100 (s) cm^{-1}; HRMS calcd for $C_6H_{11}N_3O_5$ 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 H_2 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); $[\alpha]^{22}_D$ −26.0° (c 0.6, $CDCl_3$); for ^1H NMR data, see Table I. Anal. ($C_{19}H_{31}NO_5$) 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 NH_4Cl, C); $[\alpha]^{28}_D$ +8.1° (c 1.1, H_2O) [lit.[39] $[\alpha]_D$ +9.5° (c 6.2, H_2O)]; for ^1H NMR data, see Table I; ^{13}C NMR data (D_2O, 90.5 MHz) δ 56.6, 71.4, 72.2, 74.4, 74.9, 77.5. Anal. ($C_6H_{13}NO_5$·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); $[\alpha]^{23}_D$ −25.5° (c 0.47, MeOH) [lit.[29] $[\alpha]_D$ −29° (c 1.04, MeOH)]; for ^{1}H NMR data, see Table I. Anal. ($C_7H_{13}N_3O_5$) 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 NH_4Cl, C); for ^{1}H NMR data, see Table I. Anal. ($C_7H_{15}NO_5 \cdot 0.33\ H_2CO_3$) 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; $[\alpha]^{25}_D$ +20.9° (c 0.54, H_2O); for ^{1}H NMR data, see Table I. Anal. ($C_6H_{11}ClO_5 \cdot 0.25H_2O$) 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 ^{1}H NMR data, see Table I. Anal. ($C_{17}H_{24}O_{10}S$) 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: $[\alpha]^{24}_D$ +40.9° (c 0.47, MeOH); for ^{1}H NMR data, see Table I. Anal. ($C_6H_{12}O_5S \cdot 0.07CHCl_3$) C, H, S.

PtdIns Synthase Assays. Mouse brain microsomes prepared as described by Benjamin and Agranoff[35] 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 900*g* for 10 min. The supernatant fraction was centrifuged at 12000*g* 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 $MgCl_2$, 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 $CHCl_3$–CH_3OH, 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 × 10^6 $mol^{-1} \cdot cm^2$. Alternatively, inhibition of PtdIns synthase was measured by monitoring the incorporation of 0.5 mM *myo*-[2-^{3}H]inositol (12,000 cpm/nmol) (NEN-Dupont) at 37 °C into $CHCl_3$-soluble material in the presence of Tris-HCl (pH 8.3) 48 mM $MgCl_2$, 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 $CHCl_3$, 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 × 10^5 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 $CaCl_2$, 1 mM Na phosphate, 1.3 mM $MgSO_4$, 5 mM HEPES pH 7.4), immersed in Krebs-Ringer buffer containing 10 μM *myo*-[^{3}H]inositol (1000 cpm/pmol) and 5 mM analogue, and incubated in a 5% CO_2 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.
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