Synthesis of 3-Position-Modified Analogues of *myo*-Inositol 1,4,5-Trisphosphate, Tools for Investigation of the Polyphosphoinositide Pathway of Cellular Signaling

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Methods for the synthesis of 3-*O*-(carboxymethyl)- and 3-*O*-alkylated *myo*-inositol 1,4,5-trisphosphates in racemic form from *myo*-inositol have been devised. For DL-3-*O*-(carboxymethyl)-*myo*-inositol 1,4,5-trisphosphate, an analogue of *myo*-inositol 1,3,4,5-tetrakisphosphate, DL-3-*O*-allyl-2,6-di-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-4,5-*O*-isopropylidene-*myo*-inositol (**14**) was prepared from *myo*-inositol in seven steps. The triol DL-3-*O*-allyl-2,6-di-*O*-benzyl-*myo*-inositol (**26**), which was obtained after treatment of **14** with acid, was phosphitylated and the product oxidized to give the fully protected trisphosphate **27**. The efficient oxidative cleavage of the 3-*O*-allyl ether of **27** in the presence of the cyanoethyl-protected phosphate triesters was achieved by treatment of **27** with $NaIO_4/RuCl_3$·hydrate to afford the fully protected 3-*O*-(carboxymethyl) trisphosphate **28**. After deblocking, DL-3-*O*-(carboxymethyl) trisphosphate **6** was obtained. For DL-3-*O*-alkylated *myo*-inositol 1,4,5-trisphosphate analogues, the fully protected **14** was isomerized to the *cis*-prop-1-enyl derivative **15**. The propenyl group was removed to give DL-2,6-di-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-4,5-isopropylidene-*myo*-inositol (**16**). The 3-*O*-methyl ether **17**, 3-*O*-ethyl ether **18**, and 3-*O*-*n*-propyl ether **19** derivatives were synthesized by treatment of the anion of **16** with methyl iodide, ethyl iodide, or *n*-propyl iodide, respectively. Removal of the isopropylidene and *p*-methoxybenzyl groups afforded 3-*O*-alkylated triols **20**, **21**, or **22**, which were phosphitylated and the products oxidized to give the respective fully protected 3-*O*-alkylated trisphosphates **23**−**25**. Deprotection furnished 3-*O*-methyl- (**3**), 3-*O*-ethyl- (**4**), or 3-*O*-*n*-propyl-*myo*-inositol 1,4,5-trisphosphate (**5**). These compounds will be useful pharmacological tools to explore the interaction of *myo*-inositol 1,4,5-trisphosphate with its receptor and metabolic enzymes.

Introduction

As a second messenger, D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3 (**1**)], which releases Ca^{2+} from an intracellular store[1,2] via an isolated,[3] cloned,[4] and sequenced[5] receptor, is now well established (Figure 1). Ins(1,4,5)P_3 is metabolized primarily via two pathways:[6] deactivation by a 5-phosphatase to Ins(1,4)P_2 or by phosphorylation by a 3-kinase to the tetrakisphosphate Ins(1,3,4,5)P_4 (**2**). The function of the latter remains controversial.[7] Recently, the identification of an Ins(1,3,4,5)P_3 binding protein[8] and the suggestion that it may be an Ins(1,3,4,5)P_3 receptor[9,10] have further spread interest in the potential cellular functions of this compound. Major challenges are now the elucidation of structure−activity relationships for the interaction of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 with their receptors and metabolic enzymes[11] and, additionally, the rational design of agonists, antagonists, and enzyme inhibitors. Recent progress in inositol phosphate chemistry has been reviewed.[11,12]

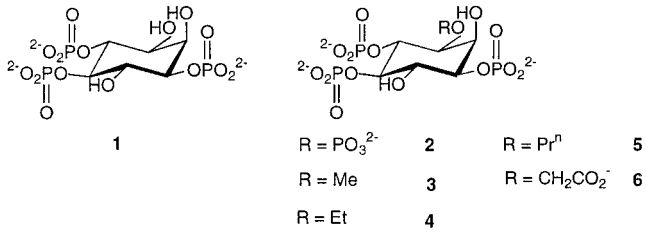

Figure 1. D-*myo*-Inositol 1,4,5-trisphosphate and 3-position modified analogues.

To investigate the role of the crucial hydroxyl group at the 3-position of Ins(1,4,5)P_3, the site of phosphorylation by Ins(1,4,5)P_3 3-kinase, a number of C-3-modified D-*myo*-inositol analogues have been synthesized and tested in previous work.[13−17] D-3-Deoxy-Ins(1,4,5)P_3,[18]

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(1) Berridge, M. J. *Annu. Rev. Biochem.* **1987**, *56*, 159−193.
(2) Berridge, M. J.; Irvine, R. F. *Nature* (*London*) **1989**, *341*, 197.
(3) Supattapone, S.; Worley, P. F.; Baraban, J. M.; Snyder, S. H. *J. Biol. Chem.* **1988**, *263*, 1530−1534.
(4) Furuichi, T.; Yoshikawa, S.; Miyawaki, A.; Waeda, A.; Mada, N.; Mikoshiba, K. *Nature* (*London*) **1989**, *342*, 32−38.
(5) Mignery, G. A.; Newton, C. L.; Archer, B. T., III; Südhof, T. C. *J. Biol. Chem.* **1990**, *265*, 12679−12685.
(6) Nahorski, S. R.; Potter, B. V. L. *Trends. Pharmacol. Soc.* **1989**, *10*, 139−144.
(7) Irvine, R. F. In *Advances in Second Messenger and Phosphoprotein Research*; Putney, J. W., Jr., Ed.; Raven Press: New York, 1992; pp 161−186.
(8) Cullen, P. J.; Hsuan, J. J.; Truong, O.; Letcher, A. J.; Jackson, T. R.; Dawson, A. P.; Irvine, R. F. *Nature* (*London*) **1995**, *376*, 527−530.
(9) Cullen, P. J.; Dawson, A. P.; Irvine, R. F. *Biochem. J.* **1995**, *305*, 139−143.
(10) Cullen, P. J.; Chung, S.-K.; Chang, Y.-T.; Dawson, A. P.; Irvine, R. F. *FEBS Lett.* **1995**, *358*, 240−242.
(11) Potter, B. V. L.; Nahorski, S. R. *Biochem. Soc. Trans.* **1992**, *20*, 434−442.
(12) Potter, B. V. L.; Lampe, D. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1933−1972.
(13) Kozikowski, A. P.; Fauq, A. H.; Rusnak, J. M. *Tetrahedron Lett.* **1989**, *30*, 3365−3368.
(14) Kozikowski, A. P.; Fauq, A. H.; Powis, G.; Melder, D. C. *Med. Chem. Res.* **1991**, *1*, 277−282.
(15) Powis, G.; Aksoy, I. A.; Melder, D. C.; Aksoy, S.; Eichinger, H.; Fauq, A. H.; Kozikowski, A. P. *Cancer Chemother. Pharmacol.* **1991**, *29*, 95−104.
(16) Kozikowski, A. P.; Powis, G.; Gallegos, A.; Tückmantel, W. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1323−1326.
(17) Kozikowski, A. P.; Fauq, A. H.; Malaska, M. J.; Tückmantel, W.; Ognyanov, V. I.; Powis, G. *Curr. Med. Chem.* **1994**, *1*, 1−12.

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D-3-C-(trifluoromethyl)-Ins(1,4,5)P_3,[19] 3-halo-Ins(1,4,5)-P_3,[17,20,21] and L-*chiro*-inositol 2,3,5-trisphosphate,[22−24] an Ins(1,4,5)P_3 analogue with an axial rather than an equatorial 3-hydroxy group, have also been synthesized and shown to have interesting properties. To explore further the steric constraints at the receptor and metabolic enzymes with respect to the 3-position, our first target was to obtain the DL-3-*O*-alkylated *myo*-inositol 1,4,5-trisphosphate analogues, 3-*O*-methyl- (**3**), 3-*O*-ethyl- (**4**), and 3-*O*-*n*-propyl-Ins(1,4,5)P_3 (**5**),[25] since analogues with substituents at C-3 of increasing steric bulk might display variable activity with respect to receptor binding and Ca^{2+} release and also toward metabolic enzymes.[26]

In the search for the structural requirements for effective Ca^{2+} release, several racemic analogues of Ins-(1,4,5)P_3, possessing multiple phosphate surrogate groups such as tris(sulfate), tris(sulfonamide), tris(carboxymethyl), and tris(methylphosphonate)[27,28] have been synthesized and did not have any Ca^{2+}-mobilizing or antagonistic properties. The 4,5-dimethylene phosphonate analogue of DL-Ins(4,5)P_2,[29] DL-inositol 1-phosphate 4,5-pyrophosphate,[30] and the D-hexadeoxy-1,4,5-tris(methylenesulfonic acid) analogue of Ins(1,4,5)P_3[31] were also found to be inactive. We believe that a more conservative approach, rather than that involving replacement with multiple phosphate surrogate groups, should be more successful. Consequently, we have synthesized initially, as an Ins(1,3,4,5)P_4 analogue, the first example of an inositol polyphosphate that possesses both phosphate groups and a nonphosphate group surrogate, *myo*-inositol 1,4,5-trisphosphate 3-*O*-methylenecarboxylate [3-CME Ins(1,4,5)P_3 (**6**)]. It is important to note that we do not include phosphorothioates in this definition, since they are well investigated (and several mixed phosphate/phosphorothioate ligands have been synthesized[12]) and generally well recognized by inositol polyphosphate binding proteins. Preliminary reports of the syntheses of the 3-*O*-alkylated Ins(1,4,5)P_3 analogues[25] and 3-CME-Ins-(1,4,5)P_3[32] have appeared already, and we now report here full details of this work.

Results and Discussion

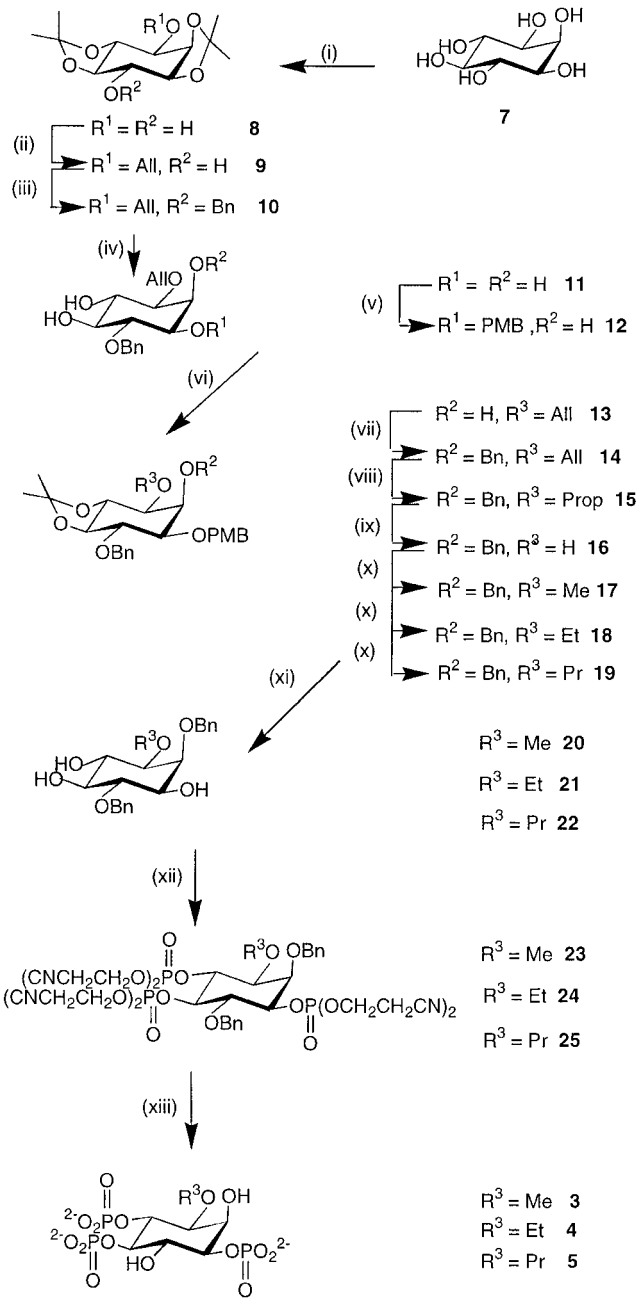
Synthesis of the target 3-modified compounds required initially the design of suitably protected inositol derivatives. Conversion of the racemic inositol 1,2:4,5 diketal[33] **8**, prepared from *myo*-inositol (**7**), to the corresponding 3-*O*-allyl **9**[34] and 3-*O*-allyl-6-*O*-benzyl **10** derivatives was achieved by treatment of **8** first with allyl bromide/barium oxide and barium hydroxide to give **9**, which was then treated with sodium hydride/benzyl bromide to give the fully protected **10** (Scheme 1). Removal of the isopropylidene groups afforded **11**, and regioselective introduction of a 1-*O*-*p*-methoxybenzyl ether in **11** was achieved by treatment first with dibutyltin oxide in refluxing toluene followed by reaction of the resulting stannylene with cesium fluoride/*p*-methoxybenzyl bromide to give **12**. After reintroduction of the 4,5-*O*-isopropylidene ketal giving **13**, the remaining 2-hydroxyl group was benzylated to produce **14**. The allyl group of **14** was isomerized to propenyl using the rhodium complex $[(Ph_3)P]_3RhCl$ in the presence of diazabicyclo[2.2.2]-octane to give **15**. Removal of the propenyl group by treatment of **15** with mercuric chloride and mercuric oxide in acetone/water afforded the key intermediate **16**. The fully protected 3-*O*-methyl ether **17**, 3-*O*-ethyl ether **18**, and 3-*O*-*n*-propyl ether **19** derivatives were synthesized by treatment of the anion of **16** with methyl iodide, ethyl iodide, or *n*-propyl iodide, respectively. The isopropylidene group and the 1-*O*-*p*-methoxybenzyl ether in each case were successively cleaved by treatment of **17**, **18**, or **19** with refluxing hydrochloric acid to produce the respective triols 3-*O*-methyl- (**20**), 3-*O*-ethyl- (**21**), or 3-*O*-*n*-propyl-2,6-di-*O*-benzyl-*myo*-inositol (**22**). Phosphitylation of **20**, **21**, or **22** was effected using bis(2-cyanoethyl) *N*,*N*-diisopropylphosphoramidite/tetrazole in dichloromethane[35] to afford the corresponding trisphosphites, which were smoothly oxidized with *t*-BuOOH to the fully protected trisphosphates **23**, **24**, or **25**, respectively. Treatment of **23**, **24**, or **25** each with sodium in liquid ammonia yielded the target compounds, racemic **3**, **4**, or **5**, respectively, which were purified by ion-exchange chromatography on Q-Sepharose, eluting with a gradient of triethylammonium bicarbonate buffer and quantified by the Briggs phosphate assay as their triethylammonium salts.

The synthesis of racemic *myo*-inositol 1,4,5-trisphosphate 3-*O*-methylenecarboxylate [3-CME Ins(1,4,5)P_3 (**6**)] is outlined in Scheme 2. Treatment of the fully protected compound **14** with refluxing hydrochloric acid produced the key triol 3-*O*-allyl-2,6-di-*O*-benzyl-*myo*-inositol (**26**). The fully protected corresponding trisphosphate **27** was obtained in a manner similar to that for **23** from **20**. The efficient oxidative cleavage of the 3-*O*-allyl ether of **27** in the presence of the cyanoethyl-protected phosphate triesters was achieved by treatment of **27** with $NaIO_4$/$RuCl_3$·hydrate[36] to afford **28**. The cyanoethyl and benzyl protecting groups of **28** were subsequently removed by treatment with sodium in liquid ammonia to provide crude **6**, which was subjected to ion-exchange chromatography on Q-Sepharose eluting with a gradient of triethylammonium bicarbonate buffer to give the pure Ins(1,3,4,5)P_4 analogue **6**.

The Ca^{2+} releasing properties of **3**−**5**, as well as their interaction with the metabolic enzymes Ins(1,4,5)P_3

(18) Seewald, M. J.; Aksoy, I. A.; Powis, G.; Fauq, A. H.; Kozikowski, A. P. *J. Chem. Soc., Chem. Commun.* **1990**, 1638−1639.
(19) Kozikowski, A. P.; Ognyanov, V. I.; Fauq, A. H.; Wilcox, R. A.; Nahorski, S. R. *J. Chem. Soc., Chem. Commun.* **1994**, 599−600.
(20) Kozikowski, A. P.; Fauq, A. H.; Aksoy, I. A.; Seewald, M. J.; Powis, G. *J. Am. Chem. Soc.* **1990**, *112*, 7403−7404.
(21) Fauq, A. H.; Kozikowski, A. P.; Ognyanov, V. I.; Wilcox, R. A.; Nahorski, S. R. *J. Chem. Soc., Chem. Commun.* **1994**, 1301−1302.
(22) Liu, C.; Nahorski, S. R.; Potter, B. V. L. *J. Chem. Soc., Chem. Commun.* **1991**, 1014−1016.
(23) Liu, C.; Nahorski, S. R.; Potter, B. V. L. *Carbohydr. Res.* **1992**, *234*, 107−115.
(24) Ward, S. G.; Mills, S. J.; Liu, C.; Westwick, J.; Potter, B. V. L. *J. Biol. Chem.* **1995**, *270*, 12075−12084.
(25) Liu, C.; Potter, B. V. L. *Tetrahedron Lett.* **1994**, *35*, 8457−8460.
(26) Hirata, M.; Watanabe, Y.; Kanematsu, T.; Ozaki, S.; Koga, T. *Biochim. Biophys. Acta* **1995**, *1244*, 404−410.
(27) Willems, H. A. M.; Veeneman, G. H.; Westerduin, P. *Tetrahedron Lett.* **1992**, *33*, 2075−2078.
(28) Westerduin, P.; Willems, H. A. M.; van Boeckel, C. A. A. *Carbohydr. Res.* **1992**, *234*, 131−140.
(29) Lyssikatos, J. P.; Bednarski, M. D. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 685−688.
(30) Noble, N. J.; Dubreuil, D.; Potter, B. V. L. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 471−476.
(31) Kozikowski, A. P.; Ognyanov, V. I.; Chen, C.; Kurian, P.; Crews, F. T. *Tetrahedron Lett.* **1993,** *34*, 219−222.
(32) Liu, C.; Thomas, N. F.; Potter, B. V. L. *J. Chem. Soc., Chem. Commun.* **1993**, 1687−1689.
(33) Gigg, J.; Gigg, R.; Payne, S.; Conant, R. *Carbohydr. Res.* **1985**, *142*, 132−134.
(34) Gigg, J.; Gigg, R.; Payne, S.; Conant, R. *J. Chem. Soc., Perkin Trans. 1* **1987**, 423−429.
(35) Cooke, A. M.; Potter, B. V. L.; Gigg, R. *Tetrahedron Lett.* **1987**, *28*, 2305−2308.
(36) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3937−3938.

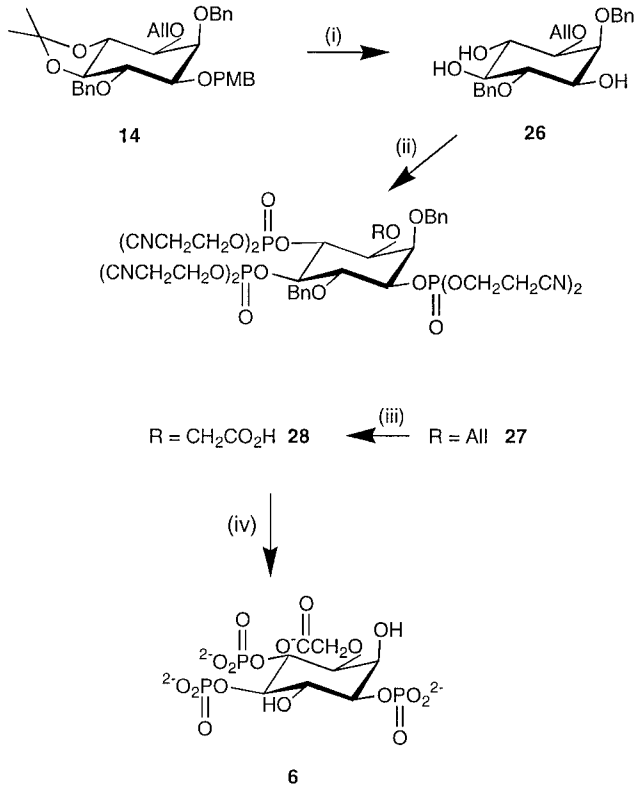
Scheme 1[a]



[a] Reagents and conditions: (i) (a) $CH_3C(OCH_3)_2CH_3$, PTSA, reflux, (b) BzCl, pyridine, (c) NaOH, reflux; (ii) allyl bromide, BaO, $Ba(OH)_2$, DMF; (iii) BnCl, NaH, DMF; (iv) PTSA, ethyl acetate/acetone/water; (v) (a) dibutyltin oxide, toluene, reflux, (b) CsF, (*p*-MeO)BnCl, DMF; (vi) 2-methoxypropene, PTSA, DMF; (vii) BnCl, NaH, DMF; (viii) diazabicyclo[2.2.2]octane, $[(Ph)_3P]_3RhCl$, ethanol/benzene/water; (ix) $HgCl_2$, HgO, acetone/water; (x) MeI, or EtI or Pr^nI, NaH, DMF; (xi) 1 M HCl MeOH, reflux; (xii) (a) $Pr^i_2NP(OCH_2CH_2CN)_2$, tetrazole, CH_2Cl_2, (b) 70% *t*-BuOOH; (xiii) (a) Na/liq NH_3, (b) H_2O. Bn = benzyl, PMB = *p*-methoxybenzyl, All = allyl, Prop = propenyl. All compounds are racemic.

5-phosphatase and 3-kinase, have been examined in permeabilized SH SY5Y cells relative to Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4. All three 3-*O*-alkylated analogues were evaluated as Ca^{2+}-mobilizing agonists, and compound **3** was found to be essentially equipotent to Ins(1,3,4,5)P_4, but relative EC_{50}'s increased markedly in the order of increasing 3-position chain length, i.e., R = Me < Et < Pr^n. They were all potent 5-phosphatase inhibitors with 3-*O*-methyl Ins(1,4,5)P_3 having a K_i some 5-fold lower

Scheme 2[a]



[a] Reagents and conditions: (i) 1 M HCl, MeOH, reflux; (ii) (a) $Pr^i_2NP(OCH_2CH_2CN)_2$, tetrazole, CH_2Cl_2, (b) 70% *t*-BuOOH; (iii) $NaIO_4$, $RuCl_3{\cdot}XH_2O$, CCl_4/acetonitrile/H_2O; (iv) (a) Na/liq NH_3, (b) H_2O. Bn = benzyl, PMB = *p*-methoxybenzyl, All = allyl. All compounds are racemic.

than the apparent K_i for Ins(1,4,5)P_3. Compound **3** also had a K_i for 3-kinase inhibition some 7-fold higher than the apparent K_i for Ins(1,3,4,5)P_4. Clearly, if the L-enantiomers of these mixtures are inactive, as expected,[6,37] then the true potencies of these compounds as receptor ligands and enzyme inhibitors are even more marked.

Racemic 3-CME-Ins(1,4,5)P_3 (**6**) was found to be a Ca^{2+}-mobilizing agonist in permeabilized neuroblastoma cells with a potency similar to that of Ins(1,3,4,5)P_4 [EC_{50} = 3.50 μM; cf 2.5 μM for D-Ins(1,3,4,5)P_4]. It had a 6-fold higher K_i for Ins(1,4,5)P_3 3-kinase than Ins(1,3,4,5)P_4 but was twice as potent at binding to Ins(1,4,5)P_3 5-phosphatase and was an inhibitor of this enzyme. Presumably, only the D-enantiomer is recognized by these proteins.

Analogues derived from inversion,[23] deletion,[38] and fluorination[20,39] of the crucial 3-hydroxy group have demonstrated little loss of Ca^{2+}-mobilizing activity relative to Ins(1,4,5)P_3, especially in the latter two cases. The present work is the first report to demonstrate that bulky substituents of a hydrophobic nature at the 3-position are reasonably well tolerated when R = Me, but not when R > Me. The situation is alleviated, however, in the case of substitution by an *O*-carboxymethyl[32] that, though

(37) Potter, B. V. L.; Nahorski, S. R. In *Drug Design for Neuroscience*; Kozikowski, A. P., Ed.; Raven Press: New York, 1993; pp 383−416.

(38) Kozikowski, A. P.; Ognyanov, V. I.; Chen, C.; Fauq, A. H.; Safrany, S. T.; Wilcox, R. A.; Nahorski, S. R. *J. Med. Chem.* **1993**, *36*, 3035−3038.

(39) Safrany, S. T.; Wilcox, R. A.; Liu, C.; Potter, B. V. L.; Nahorski, S. R. *Eur. J. Pharmacol.* **1992**, *226*, 265−272.

relatively bulky, will bear a negative charge at physiological pH. These data support the use of the carboxymethyl group as a phosphate group surrogate. Full biological results for new synthetic compounds will be reported elsewhere. These compounds should find applications as new tools for probe the polyphosphoinositide pathway of cellular signaling.

Experimental Section

Chemicals were purchased from Aldrich, Fluka, and Lancaster (U.K.). Dichloromethane, triethylamine, and dimethylformamide were dried over calcium hydride, distilled, and stored over 4A molecular sieves. TLC was performed on precoated plates (Merck TLC aluminum sheets silica $60F_{254}$ art. 5554). Products were visualized by spraying phosphomolybdic acid in methanol followed by heating. Flash chromatography was carried out using Sorbsil C60 silica gel. For ^{31}P NMR spectra chemical shifts were measured in ppm relative to external 85% H_3PO_4 and are positive when downfield from this reference. Melting points (uncorrected) were determined using a Kofler block. Ion-exchange chromatography was performed on a LKB-Pharmacia medium-pressure ion-exchange chromatograph using Q Sepharose Fast Flow with a gradient of triethylammonium bicarbonate (TEAB) as eluent. Column fractions containing inositol polyphosphate analogues were assayed for phosphate by a modification of the Briggs test[40] as described.[41]

DL-3-*O*-Allyl-1,2;4,5-di-*O*-isopropylidene-*myo*-inositol (9). To a suspension of 1,2;4,5-di-*O*-isopropylidene-*myo*-inositol[33] (**8**) (13.02 g, 50 mmol), barium oxide (15.35 g, 100 mmol), and barium hydroxide octahydrate (1.98 g, 6.25 mmol) in dry DMF (250 mL) was added allyl bromide (6.74 g, 4.82 mL, 55.7 mmol) dropwise at room temperature. The reaction mixture was stirred for 60 h at room temperature, and methanol (10 mL) was added. The resulting mixture was neutralized with AcOH/H_2O (1:1, v:v) and concentrated *in vacuo*. The residue was taken up in chloroform and washed successively with water, a saturated solution of sodium hydrogen carbonate, and again water. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The final product was obtained after flash column chromatography (ethyl acetate/petroleum ether, 3:7) and then crystallization to give compound **9** (9.47 g, 31.5 mmol, 63%): mp 128−130 °C (from petroleum ether bp 60−80 °C) (lit.[34] mp 127−129 °C); ^1H NMR ($CDCl_3$) δ 1.39, 1.45, 1.47, 1.55 (4s, 12H), 3.31 (dd, 1H, J = 9.5 Hz), 3.51 (br s, 1H), 3.81−4.05 (m, 4H), 4.25−4.33 (m, 2H), 4.48 (t, 1H, J = 4.4 Hz), 5.23 (d, 1H, J = 10.3 Hz), 5.32 (m, 1H), 5.98 (m, 1H); MS *m*/*z* (EI) 285 (6), 227 (1.4), 113 (100). Anal. Calcd for $C_{15}H_{24}O_6$: C, 60.00; H, 8.05. Found: C, 60.10; H, 8.15.

DL-3-*O*-Allyl-6-*O*-benzyl-1,2;4,5-di-*O*-isopropylidene-*myo*-inositol (10). To a solution of **9** (12.7 g, 42.33 mmol) in dry DMF (150 mL) was added sodium hydride (4.4 g, 4 equiv). After 15 min, benzyl chloride (9.77 mL, 84.76 mmol) was added, and the resulting mixture was stirred at room temperature for 2 h. Methanol was added to destroy the excess of sodium hydride, and the solution was partitioned between water (300 mL) and chloroform (400 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness. The crude product was crystallized from petroleum ether (40−60 °C) with a few drops of dichloromethane to give compound **10** (16.5 g, quantitative yield): mp 122−123 °C; ^1H NMR ($CDCl_3$) δ 1.36, 1.39, 1.45, 1.46 (4s, 12H), 3.38 (dd, 1H, J = 9.3 Hz, 10.5 Hz), 3.68 (dd, 1H, J = 6.5 Hz, 10.6 Hz), 3.79 (dd, 1H, J = 4.1 Hz, 10.1 Hz), 3.95 (dd, 1H, J = 9.4 Hz, 10.0 Hz), 4.13 (dd, 1H, J = 5.0 Hz, 6.5 Hz), 4.20−4.36 (m, 2H), 4.46 (dd, 1H, J = 4.5 Hz), 4.83 (s, 2H), 5.19−5.36 (m, 2H), 5.87−6.04 (m, 1H), 7.23−7.42 (m, 5H); MS *m*/*z* (EI) 391 (M, 81), 333 (49), 217 (16), 131 (46), 107 (16), 91 (100). Anal. Calcd for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 68.00; H, 7.86.

(40) Briggs, A. P. *J. Biol. Chem.* **1922**, *53*, 13.

(41) Lampe, D.; Liu, C.; Potter, B. V. L. *J. Med. Chem.* **1994**, *37*, 907−912.

DL-3-*O*-Allyl-6-*O*-benzyl-*myo*-inositol (11). A solution of **10** (9 g, 23.05 mmol) in ethyl acetate/acetone/water (80 mL:80 mL:8 mL, v:v:v) was stirred with *p*-toluenesulfonic acid (1.2 g, 6.3 mmol) at 40 °C for 5 h. The solvents were evaporated *in vacuo*, and the resulting solid was crystallized from ethyl acetate to give compound **11** (6.32 g, 20.4 mmol, 88.4%): mp 152−153 °C; ^1H NMR (DMSO) δ 2.99 (dd, 1H, J = 2.4 Hz, 9.7 Hz), 3.11 (ddd, 1H, J = 4.9 Hz, 9.0 Hz), 3.32 (ddd, 1H, J = 2.4 Hz, 7.9 Hz, 9.7 Hz), 3.41 (dd, 1H, J = 9.1 Hz), 3.53 (dd, 1H, J = 4.7 Hz, 9.3 Hz), 3.90 (ddd, 1H, J = 2.9 Hz, 4.2 Hz), 4.05−4.15 (m, 2H), 4.63 (d, 1H, J = 6.8 Hz), 4.68 (d, 1H, J = 8.0 Hz), 4.68 (s, 2H), 4.76 (d, 1H, J = 3.1 Hz), 4.79 (d, 1H, J = 4.9 Hz), 5.08−5.34 (m, 2H), 5.85−5.99 (m, 1H), 7.20−7.44 (m, 5H); MS *m*/*z* (EI) 310 (M, 9), 107 (13), 91 (100), 85 (40), 41 (54). Anal. Calcd for $C_{16}H_{22}O_6$: C, 61.92; H, 7.14. Found: C, 62.00; H, 7.18.

DL-3-*O*-Allyl-6-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (12). A mixture of **11** (9.5 g, 30.64 mmol) and dibutyltin oxide (11.44 g, 45.96 mmol) in dry toluene (200 mL) was refluxed for 3 h in a Dean−Stark apparatus to remove water, and the solvent was then removed *in vacuo*. To the residue was added cesium fluoride (9.2 g, 61.33 mmol), and the mixture was suspended in dry DMF (200 mL) followed by addition of *p*-methoxybenzyl chloride (4.3 mL, 36 mmol). The mixture was stirred at room temperature overnight. Water (300 mL) was added, the aqueous mixture was then extracted with chloroform (2 × 300 mL), and the organic layer was evaporated to a gum, which was crystallized from ethyl acetate/petroleum ether to give the compound **12** (9.7 g, 22.7 mmol, 74%): mp 132−133 °C; ^1H NMR ($CDCl_3$) δ 2.37 (b, 3H), 3.16 (dd, 1H, J = 2.7 Hz, 9.5 Hz), 3.41 (dd, 1H, J = 9.3 Hz), 3.41 (dd, 1H, J = 2.8 Hz, 9.5 Hz), 3.80 (dd, 1H, J = 9.5 Hz), 3.81 (s,3H), 3.92 (dd, 1H, J = 9.5 Hz), 4.07−4.24 (m, 2H), 4.23 (dd, 1H, J = 2.8 Hz), 4.65 (s, 2H), 4.77 and 4.95 (AB, 2H, J_{AB} = 11.2 Hz), 5.19−5.33 (m, 2H), 5.87−6.01 (m, 1H), 6.84−6.89 (m, 2H), 7.26−7.37 (m, 7H); MS *m*/*z* (EI) 429 (M, 4), 339 (89), 309 (47), 137 (16), 121 (100), 107 (11), 91 (16). Anal. Calcd for $C_{24}H_{30}O_7$: C, 66.98; H, 7.03. Found: C, 66.80; H, 7.14.

DL-3-*O*-Allyl-6-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-4,5-*O*-isopropylidene-*myo*-inositol (13). A solution of **12** (3.12 g, 7.25 mmol), *p*-toluenesulfonic acid (0.25 g), and 2-methoxypropene (2.12 mL, 22.5 mmol) in dry DMF (75 mL) was stirred at room-temperature overnight and then neutralized with ammonia. The DMF was evaporated *in vacuo* (<50 °C), and the residue was partitioned between water and chloroform (200 mL, each). The organic layer was dried ($MgSO_4$) and evaporated to dryness, and the crude product was obtained after flash column chromatography (ethyl acetate/petroleum ether 2:8) and then crystallization (ether/petroleum ether) to give the compound **13** (3.7 g, 6.5 mmol, 90%): mp 88.5 °C; ^1H NMR [$(CD_3)_2CO$] δ 1.37 (s, 6H), 3.42 (dd, 1H, J = 9.5 Hz), 3.42 (dd, 1H, J = 2.9 Hz, 8.8 Hz), 3.48 (dd, 1H, J = 2.7 Hz, 10.3 Hz), 3.76 (s, 3H), 3.78 (dd, 1H, J = 9.5 Hz), 3.85 (dd, 1H, J = 9.7 Hz), 4.00−4.14 (m, 2H), 4.25 (m, 1H), 4.47 and 4.62 (AB, 2H, J_{AB} = 11.3 Hz), 4.71 (s, 2H), 4.94 (d, 1H, J = 4.4 Hz), 5.11−5.32 (m, 2H), 5.83−5.97 (m, 1H), 6.81−6.88 (m, 2H), 7.27−7.33 (m, 7H); MS *m*/*z* (EI) 471 (M, 9), 379 (80), 349 (90), 137 (11), 121 (100), 107 (11), 91 (19). Anal. Calcd for $C_{27}H_{34}O_7$: C, 68.92; H, 7.28. Found: C, 68.90; H, 7.30.

DL-3-*O*-Allyl-2,6-di-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-4,5-*O*-isopropylidene-*myo*-inositol (14). To a solution of **13** (8.58 g, 18.22 mmol) in dry DMF (100 mL) was added sodium hydride (1.72 g, 44.8 mmol). After 15 min, benzyl chloride (4.16 mL, 36.03 mmol) was added, and the resulting mixture was stirred at room temperature for 2 h. Methanol was added to destroy the excess of sodium hydride, and the solution was partitioned between water (300 mL) and chloroform (500 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness. The crude product was purified after flash column chromatography (ethyl acetate/petroleum ether, 1:9) and crystallized from ethyl acetate/petroleum ether to give compound **14** (9.56 g, 17.1 mmol, 94%): mp 78 °C; ^1H NMR [$(CD_3)_2$-CO] δ 1.37, 1.38 (2s, 6H), 3.46 (dd, 1H, J = 9.5 Hz), 3.60 (dd, 1H, J = 2.8 Hz, 9.0 Hz), 3.65 (dd, 1H, J = 2.6 Hz, 10.3 Hz), 3.74 (s, 3H), 3.81 (dd, 1H, J = 9.5 Hz), 3.86 (dd, 1H, J = 10.3 Hz), 4.00−4.14 (m, 2H), 4.28 (dd, 1H, J = 2.7 Hz), 4.54 and

4.65 (AB, 2H, J_{AB} = 11.4 Hz), 4.71 and 4.75 (AB, 2H, J_{AB} = 14.8 Hz), 4.75 (s, 2H), 5.13−5.34 (m, 2H), 5.84−5.98 (m, 1H), 6.86−6.91 (m, 2H), 7.23−7.37 (m, 12H); MS *m/z* (EI) 561 (M, 7), 503 (5), 469 (82), 439 (96), 381 (12), 359 (15), 121 (100), 107 (15), 91 (35). Anal. Calcd for $C_{34}H_{40}O_7$: C, 72.83; H, 7.19. Found: C, 72.20; H, 7.22.

DL-3-*O*-(Prop-1-enyl)-1-*O*-(*p*-methoxybenzyl)-2,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol (15). To a solution of **14** (1.5 g, 2.68 mmol) in ethanol−benzene−water (100 mL, 7:3:1) was added diazabicyclo[2.2.2]octane (0.066 g, 0.6 mmol), and the mixture was heated to reflux followed by adding rhodium complex $[(Ph_3)P]_3RhCl$ (0.19 g, 0.21 mmol). After further reflux for 1 h, the cooled mixture was extracted with ether (2 × 100 mL), and the organic layer was dried ($MgSO_4$) and purified by flash column chromatography to give an oil that was crystallized from petroleum ether to give the compound **15** (1.4 g, 2.5 mmol, 93%): mp 96−98 °C; ^{1}H NMR ($CDCl_3$) δ (cis) 1.43, 1.46 (2s, 6H), 1.63 (dd, 3H, J = 1.7 Hz, 6.8 Hz), 3.40 (dd, 1H, J = 9.5 Hz), 3.41 (dd, 1H, J = 2.2 Hz, 9.7 Hz), 3.73 (dd, 1H, J = 2.7 Hz, 10.4 Hz), 3.80 (s, 3H), 4.02−4.08 (m, 2H), 4.14 (dd, 1H, J = 10.1 Hz), 4.49 (dq, 1H, J = 6.7 Hz), 4.57 and 4.63 (AB, 2H, J_{AB} = 11.5 Hz), 4.81 (s, 2H), 4.78 and 4.90 (AB, 2H, J_{AB} = 11.5 Hz), 6.12 (dd, 1H, J = 1.8 Hz, 6.4 Hz), 6.81−6.84 (m, 2H), 7.20−7.43 (m, 12H); MS *m/z* (EI) 561 (M, 2), 503 (2), 469 (13), 439 (6), 251 (10), 211 (25), 121 (100), 91 (65), 69 (33). Anal. Calcd for $C_{34}H_{40}O_7$: C, 72.83; H, 7.19. Found: C, 73.10; H, 7.30.

DL-1-*O*-(*p*-Methoxybenzyl)-2,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol (16). To a solution of mercuric chloride (0.63 g, 2.31 mmol) in acetone−water (7 mL, 10:1) was added dropwise with stirring a mixture of **15** (1.3 g, 2.32 mmol), mercuric oxide (0.63 g), and acetone−water (20 mL, 10:1) during 3 min. After a further 5 min, the mercuric oxide was removed by filtration through Celite, the acetone was evaporated *in vacuo*, and ether was added to the residue. The ether layer was washed with a semisaturated aqueous solution of potassium iodide (10 mL), dried ($MgSO_4$), and evaporated. The crude product was chromatographed on a flash column (light petroleum/ether 2:1), followed by crystallization from petroleum ether/ethyl acetate to give the compound **16** (0.9 g, 1.7 mmol, 75%); mp 99 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (2s, 6H), 2.35−2.45 (br s 1H), 3.38 (dd, 1H, J = 9.5 Hz), 3.49 (dd, 1H, J = 2.9 Hz, 9.5 Hz), 3.63 (dd, 1H, J = 2.7 Hz, 9.6 Hz), 3.73 (dd, 1H, J = 9.5 Hz), 3.79 (s, 3H), 3.91 (dd, 1H, J = 3.0 Hz), 4.00 (dd, 1H, J = 9.5 Hz), 4.58 and 5.06 (AB, 2H, J_{AB} = 11.2 Hz), 4.63 and 4.93 (AB, 2H, J_{AB} = 11.7 Hz), 4.75 and 4.79 (AB, 2H, J_{AB} = 11.5 Hz), 6.83−6.86 (m, 2H), 7.23−7.42 (m, 12H); MS *m/z* (EI) 521 (M, 2), 429 (6), 399 (8), 137 (20), 121 (100), 107 (33), 91 (32), 69 (14). Anal. Calcd for $C_{31}H_{36}O_7$: C, 71.52; H, 6.97. Found: C, 71.5; H, 6.95.

DL-3-*O*-Methyl-2,6-di-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-4,5-*O*-isopropylidene-*myo*-inositol (17). To a solution of **16** (0.09 g, 0.17 mmol) in dry DMF (10 mL) was added sodium hydride (0.24 g, 0.51 mmol). After 15 min, iodomethane (0.072 g, 0.51 mmol) was added, and the mixture was stirred at room temperature for 2 h. Methanol was added to destroy the excess of sodium hydride, and the solution was partitioned between water (10 mL) and chloroform (20 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness. The crude product was chromatographed on a flash column to give the compound **17** as an oil (0.09 g, 0.17 mmol, 98%): ^{1}H NMR ($CDCl_3$) δ 2.17 (2s, 6H), 2.94 (dd, 1H, J = 2.3 Hz, 9.5 Hz), 3.34 (s, 3H), 3.39 (dd, 1H, J = 2.4 Hz, 9.8 Hz), 3.44 (dd, 1H, J = 9.2 Hz), 3.81 (s, 3H), 3.90 (dd, 1H, J = 9.5 Hz), 3.96 (dd, 1H, J = 9.5 Hz), 4.11 (dd, 1H, J = 2.3 Hz), 4.61 (s, 2H), 4.76 and 4.98 (AB, 2H, J_{AB} = 11.0 Hz), 4.79 and 4.88 (AB, 2H, J_{AB} = 12.2 Hz), 6.86−6.88 (m, 2H), 7.26−7.41 (m, 12H); MS *m/z* (EI) 535 (M, 2%), 443 (9), 413 (14), 121 (100), 107 (20), 91 (32), 69 (14).

DL-3-*O*-Ethyl-2,6-di-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-4,5-*O*-isopropylidene-*myo*-inositol (18). The above compound was prepared in a fashion identical to that described for **17** in crystallized form, except EtI was used in place of MeI: mp 83 °C; ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3H, J = 7.0 Hz), 2.17 (s, 6H), 3.02 (dd, 1H, J = 2.4 Hz, 9.7 Hz), 3.38 (dd, 1H, J = 2.4 Hz, 9.5 Hz), 3.44 (dd, 1H, J = 9.2 Hz), 3.55 (q, 2H, J = 7.0 Hz), 3.81 (s, 3H), 3.90 (dd, 1H, J = 9.4 Hz), 3.96 (dd, 1H, J = 9.5 Hz), 4.07 (dd, 1H, J = 2.2 Hz), 4.59 (s, 2H), 4.78 and 4.96 (AB, 2H, J_{AB} = 11.2 Hz), 4.83 and 4.85 (AB, 2H, J_{AB} = 12.0 Hz), 6.82−6.89 (m, 2H), 7.22−7.41 (m, 12H); MS *m/z* (EI) 549 (M, 2), 457 (10), 427 (15), 121 (100), 91 (29). Anal. Calcd for $C_{33}H_{40}O_7$: C, 72.24; H, 7.35. Found: C, 72.2; H, 7.39.

DL-3-*O*-Propyl-2,6-di-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-4,5-*O*-isopropylidene-*myo*-inositol (19). The above compound was prepared in a fashion identical to that described for **17** as an oil, except that Pr^{n}I was used in place of MeI: ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H, J = 7.4 Hz), 1.43, 1.45 (2s, 6H), 1.63 (td, 2H, J = 7.2 Hz), 3.34−3.62 (m, 5H), 3.80 (s, 3H), 4.02−4.10 (m, 3H), 4.58 and 4.64 (AB, 2H, J_{AB} = 11.5 Hz), 4.78 and 4.90 (AB, 2H, J_{AB} = 11.5 Hz), 4.83 (s, 2H), 6.81−6.88 (m, 2H), 7.22−7.44 (m, 12H); MS *m/z* (EI) 563 (M, 2), 471 (10), 441 (18), 279 (55), 205 (75), 149 (66), 121 (100), 91 (30), 69 (19).

DL-3-*O*-Methyl-2,6-di-*O*-benzyl-*myo*-inositol (20). A solution of **17** (0.1 g, 0.18 mmol) in 1 M HCl−MeOH (2:8, v/v, 10 mL) was refluxed for 3 h, and the cooled solution was quenched with ammonia. The solvents were evaporated *in vacuo*, and the residue was partitioned between water and chloroform (30 mL, each). The organic layer was dried ($MgSO_4$) and evaporated to dryness, and the crude product was purified after flash column chromatography to give the compound **20** (0.064 g, 0.17 mmol, 95%): mp 116 °C; ^{1}H NMR ($CDCl_3$) δ 2.45 (br s, 1H), 3.00 (br, 2H), 3.01 (dd, 1H, J = 2.4 Hz, 9.7 Hz), 3.41 (s, 3H), 3.46 (dd, 1H, J = 9.0 Hz), 3.51 (dd, 1H, J = 2.8 Hz, 10.1 Hz), 3.68 (dd, 1H, J = 9.3 Hz), 3.93 (dd, 1H, J = 9.4 Hz), 4.11 (dd, 1H, J = 2.5 Hz), 4.71 and 4.91 (AB, 2H, J_{AB} = 11.5 Hz), 4.81 and 4.90 (AB, 2H, J_{AB} = 11.4 Hz), 7.26−7.77 (m, 10H); MS *m/z* (EI) 373 (M, 3), 283 (44), 195 (33), 181 (78), 159 (15), 107 (35), 91 (100). Anal. Calcd for $C_{21}H_{26}O_6$: C, 67.38; H, 6.97. Found: C, 67.4; H, 7.03.

DL-3-*O*-Ethyl-2,6-di-*O*-benzyl-*myo*-inositol (21). The above compound was prepared in a fashion similiar to that described for **20**: mp 115 °C; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, J = 7.1 Hz), 2.40 (br, 3H), 3.12 (dd, 1H, J = 2.3 Hz, 9.7 Hz), 3.48 (dd, 1H, J = 9.1 Hz), 3.50 (dd, 1H, J = 9.1 Hz), 3.53 (dd, 1H, J = 2.7 Hz, 9.0 Hz), 3.62−3.74 (m, 2H), 3.93 (dd, 1H, J = 9.4 Hz), 4.08 (dd, 1H, J = 2.6 Hz), 4.69 and 4.93 (AB, 2H, J_{AB} = 11.5 Hz), 4.87 and 4.88 (AB, 2H, J_{AB} = 11.4 Hz), 7.26−7.40 (m, 10H); MS *m/z* (EI) 387 (M, 3), 209 (36), 191 (20), 181 (73), 107 (38), 91 (100). Anal. Calcd for $C_{22}H_{28}O_6$: C, 68.00; H, 7.26. Found: C, 67.9; H, 7.29.

DL-3-*O*-*n*-Propyl-2,6-di-*O*-benzyl-*myo*-inositol (22). The above compound was prepared in a fashion similar to that described for **20**: mp 112 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, J = 7.4 Hz), 1.63 (td, 2H, J = 7.2 Hz), 2.35 (d, 1H, J = 6.8 Hz), 2.63 (br s, 1H), 2.65 (br s, 1H), 3.12 (dd, 1H, J = 2.2 Hz, 9.7 Hz), 3.40 (dd, 1H, J = 9.5 Hz), 3.49−3.60 (m, 3H), 3.67 (dd, 1H, J = 9.3 Hz), 3.95 (dd, 1H, J = 9.4 Hz), 4.09 (dd, 1H, J = 2.3 Hz), 4.68 and 4.94 (AB, 2H, J_{AB} = 11.5 Hz), 4.86 and 4.87 (AB, 2H, J_{AB} = 11.5 Hz), 7.26−7.40 (m, 10H); MS *m/z* (EI) 401 (M, 2), 311 (42), 223 (32), 205 (16), 181 (72), 107 (39), 91 (100). Anal. Calcd for $C_{23}H_{30}O_6$: C, 68.64; H, 7.5. Found: C, 68.7; H, 7.58.

DL-3-*O*-Methyl-2,6-di-*O*-benzyl-*myo*-inositol 1,4,5-Tris-[bis(2-cyanoethyl) phosphate] (23). To a mixture of **20** (0.05 g, 0.13 mmol) and 1*H*-tetrazole (0.17 g, 2.4 mmol) in dry CH_2Cl_2 (5 mL) was added bis(2-cyanoethyl) *N*,*N*-diisopropylphosphoramidite (0.5 g, 2.2 mL). The mixture was stirred at room temperature for 1 h, *t*-BuOOH (0.5 mL, 70% in H_2O) was added, and the resulting solution was stirred overnight and then washed with saturated aqueous $NaHCO_3$ (10 mL), dried ($MgSO_4$), and concentrated. Flash column chromatography of the residue gave compound **23** (0.085 g, 0.09 mmol, 71%) as an oil: ^{31}P NMR ($CDCl_3$) δ −3.43, −3.10; ^{1}H NMR ($CDCl_3$) δ 2.40−2.90 (m, 12H), 3.35 (dd, 1H, J = 2.1, 9.9 Hz), 3.45 (s, 3H), 4.03−4.59 (m, 16H), 4.76 (dd, 1H, J = 9.3 Hz), 4.81 and 4.92 (AB, 2H, J_{AB} = 11.5 Hz), 4.84 a nd 4.92 (AB, 2H, J_{AB} = 11.5 Hz), 7.09−7.65 (m, 10H); MS *m/z* (FAB) 933 [$(M + H)^+$, 15], 889 (2), 663 (2), 619 (3), 591 (4) 181 (10), 149 (13), 105 (10), 91 (100).

DL-3-*O*-Ethyl-2,6-di-*O*-benzyl-*myo*-inositol 1,4,5-Tris-[bis(2-cyanoethyl) phosphate] (24). The above compound was prepared in a fashion similar to that described for **23**: ^{31}P NMR ($CDCl_3$) δ −3.63, −3.37; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H,

J = 7.0 Hz), 2.42−2.90 (m, 12H), 3.44 (dd, 1H, J = 2.2, 9.9 Hz), 3.60−3.73 (m, 2H), 4.04−4.56 (m, 16H), 4.74 (dd, 1H, J = 9.4 Hz), 4.81 and 4.92 (AB, 2H, J_{AB} = 11.5 Hz), 4.84 and 4.92 (AB, 2H, J_{AB} = 11.5 Hz), 7.27−7.54 (m, 10H); MS *m/z* (FAB) 947 [$(M + H)^+$, 11], 934 (3), 889 (2), 227 (8), 149 (15), 135 (10), 91 (100).

DL-3-*O*-Propyl-2,6-di-*O*-benzyl-*myo*-inositol 1,4,5-Tris-[bis(2-cyanoethyl) phosphate] (25). The above compound was prepared in a fashion similar to that described for **23**: ^{31}P NMR ($CDCl_3$) δ −3.23, −3.09, −3.03; ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H, J = 7.4 Hz), 1.67 (td, 2H, J = 7.2 Hz), 2.40−2.90 (m, 12H), 3.49−3.60 (m, 3H), 4.00−4.50 (m, 16H), 4.74 (dd, 1H, J = 9.4 Hz), 4.81 and 4.92 (AB, 2H, J_{AB} = 11.5 Hz), 4.84 and 4.92 (AB, 2H, J_{AB} = 11.5 Hz), 7.01−7.64 (m, 10H); MS *m/z* (FAB) 962 [$(M + H)^+$, 10), 594 (1), 339 (10), 91 (100).

DL-3-*O*-Methyl-*myo*-inositol 1,4,5-trisphosphate (3). To liquid ammonia (40 mL) was added a solution of **23** (0.06 g, 0.064 mmol) in dry dioxane (1.8 mL) followed by sodium (0.1 g, 4.3 mmol) in small pieces. The solution was stirred for 5 min at room temperature and was quenched with ethanol. The ammonia was evaporated in a stream of nitrogen. Ion-exchange chromatography of the residue dissolved in water on Q Sepharose Fast Flow, using a gradient of H_2O to 1 M TEAB (pH 8.0), gave the compound **3** (0.047 g, 0.045 mmol, 70%); **3** was eluted at ca. 700 mM TEAB: ^{31}P NMR (D_2O) δ 0.67, 3.16, 3.57; ^{1}H NMR (D_2O) δ 3.32 (dd, 1H, J = 2.8 Hz, 9.9 Hz), 3.44 (s, 3H), 3.82 (dd, 1H, J = 9.4 Hz), 3.91 (ddd, 1H, J = 2.6 Hz, 7.7 Hz, 9.4 Hz), 3.97 (dd, 1H, J = 9.0 Hz), 4.27 (dd, 1H, J = 9.5 Hz), 4.45 (dd, 1H, J = 2.4 Hz); MS *m/z* (FAB) 433 [$(M - H)^-$, 100], 415 (5), 315 (8), 177 (5), 159 (6), 97 (9); HRMS (FAB) calcd for $C_7H_{17}O_{15}P_3$ 433.978, found 433.978.

DL-3-*O*-Ethyl-*myo*-inositol 1,4,5-Trisphosphate (4). The above compound was prepared in a fashion similar to that described for **3**: ^{31}P NMR (D_2O) δ 0.47, 3.23, 3.77; ^{1}H NMR (D_2O) δ 1.03 (t, 3H, J = 7.3 Hz), 3.38 (dd, 1H, J = 2.8 Hz, 9.8 Hz), 3.62−3.73 (m, 2H), 3.81−4.00 (m, 3H), 4.23 (m, 1H), 4.43 (dd, 1H, J = 2.4 Hz); MS *m/z* (FAB) 447 [$(M - H)^-$, 50] 287 (22), 217 (34), 134 (100), 97 (18), 81 (24); HRMS (FAB) calcd for $C_8H_{19}O_{15}P_3$ 446.985, found 446.984.

DL-3-*O*-Propyl-*myo*-inositol 1,4,5-Trisphosphate (5). The above compound was prepared in a fashion similar to that described for **3**: ^{31}P NMR (D_2O) δ −0.067, 2.89, 3.90; ^{1}H NMR (D_2O) δ 1.11 (t, 3H, J = 7.3 Hz), 1.43 (td, 2H, J = 7.3 Hz), 3.31 (dd, 1H, J = 2.8 Hz, 9.7 Hz), 3.43−3.91 (m, 2H), 3.72 (dd, 1H, J = 9.8 Hz), 3.77−3.91 (m, 2H), 4.19 (ddd, 1H, J = 9.8 Hz, 9.3 Hz), 4.27 (s, 1H); MS *m/z* (FAB) 461 [$(M - H)^-$, 100), 177 (8), 159 (9), 97 (13); HRMS (FAB) calcd for $C_9H_{21}O_{15}P_3$ 461.001, found 461.001.

DL-3-*O*-Allyl-2,6-di-*O*-benzyl-*myo*-inositol (26). DL-3-*O*-Allyl-2,6-di-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-4,5-*O*-isopropylidene-*myo*-inositol (**14**) (1.98 g, 3.53 mmol) was heated under reflux in 1 M hydrochloric acid/methanol (1:2, 40 mL) for 5 h whereupon the solvents were evaporated to dryness to yield a residue that was purified by flash column chromatography (petroleum ether/ethyl acetate 50−100%) to give the compound **26** in a crystallized form (petroleum ether 60−80 °C) (1.27 g, 3.18 mmol, 90%): mp 105−106 °C; ^{1}H NMR ($CDCl_3$) δ 2.37 (br s, 1H), 2.39 (br s, 1H), 2.75 (br s, 1H), 3.19 (dd, 1H, J = 2.4 Hz, 9.7 Hz), 3.47 (dd, 1H, J = 9.2 Hz), 3.53 (dd, 1H, J = 2.6 Hz, 9.4 Hz), 3.67 (dd, 1H, J = 9.4 Hz), 3.97 (dd, 1H, J = 9.6 Hz), 4.06 (dd, 1H, J = 2.6 Hz), 4.00−4.17 (m, 2H), 4.70 and 4.93 (AB, 2H, J_{AB} = 11.5 Hz), 4.85 and 4.88 (AB, 2H, J_{AB} = 11.4 Hz), 5.19−5.34 (m, 2H), 5.84−5.99 (m, 1H), 7.26−7.40 (m, 10H); MS *m/z* (EI) 399 (M, 3), 359 (2), 309 (41), 181 (51), 107 (40), 91 (100). Anal. Calcd for $C_{23}H_{28}O_6$: C, 68.98; H, 7.05. Found: C, 68.9; H, 7.00.

DL-3-*O*-Allyl-2,6-di-*O*-benzyl-*myo*-inositol 1,4,5-Tris-[bis(2-cyanoethyl) phosphate] (27). To a mixture of **26** (0.6 g, 1.5 mmol) and 1*H*-tetrazole (0.97 g, 13.7 mmol) in dry CH_2Cl_2 (15 mL) was added bis(2-cyanoethyl) *N*,*N*-diisopropylphosphoramidite (3.6 g, 15.8 mmol). The mixture was stirred at room temperature for 1 h, and *t*-BuOOH (5 mL, 70% in H_2O) was added. The resulting solution was stirred overnight and then washed with saturated aqueous $NaHCO_3$ (50 mL), dried ($MgSO_4$), and concentrated. Flash column chromatography of the residue gave compound **27** as an oil (1.15 g, 1.2 mmol, 80%): ^{31}P NMR ($CDCl_3$) δ 0.54, 1.48, 2.49; ^{1}H NMR ($CDCl_3$) δ 2.40−2.81 (m, 12H), 3.52 (dd, 1H, J = 2.4 Hz, 9.9 Hz), 3.96−4.55 (m, 19H), 4.79 and 4.91 (AB, 2H, J_{AB} = 11.4 Hz), 4.82 and 4.92 (AB, 2H, J_{AB} = 11.6 Hz), 5.23−5.41 (m, 2H), 5.85−5.97 (m, 1H), 7.29−7.44 (m, 10H); MS *m/z* (FAB) $C_{41}H_{50}O_{15}N_6P_3$ 959 [$(M + H)^+$, 7], 181 (8), 144 (5), 91 (100); HRMS (FAB) calcd for $C_{41}H_{50}O_{15}N_6P_3$ 959.255, found 959.255.

DL-3-*O*-(Carboxymethyl)-2,6-di-*O*-benzyl-*myo*-inositol 1,4,5-Tris[bis(2-cyanoethyl) phosphate] (28). To a mixture of 2 mL of carbon tetrachloride, 2 mL of acetonitrile, 3 mL of water and **27** (0.19 g, 0.20 mmol), and sodium periodate (0.175 g, 0.82 mmol) was added 1 mg of ruthenium trichloride hydrate, and the entire mixture was stirred vigorously for 2 h at room temperature. Then, 10 mL of CH_2Cl_2 was added, and the phases were separated. The upper aqueous phase was extracted three times with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$) and concentrated. The residue was diluted with 20 mL of ether, filtered through Celite, and concentrated. Flash column chromatography of the residue gave the compound **28** as an oil (0.13 g, 0.13 mmol, 64%): ^{31}P NMR ($CDCl_3$) δ −3.43, −3.16, −2.96; ^{1}H NMR ($CDCl_3$) δ 2.71−2.84 (m, 12H), 3.66 (dd, 1H, J = 2.8 Hz, 9.9 Hz), 4.02−4.50 (m, 18H), 4.52 (br s, 1H), 4.53−4.98 (m, 4H), 7.27−7.45 (m, 10H); MS *m/z* (FAB) 977 [$(M + H)^+$, 7], 338 (15), 149 (28), 91 (100); HRMS (FAB) calcd for $C_{40}H_{47}O_{17}N_6P_3$ 977.229, found 977.229.

DL-3-*O*-(Carboxymethyl)-*myo*-inositol 1,4,5-Trisphosphate (6). To liquid ammonia (40 mL) was added a solution of **28** (0.06 g, 0.06 mmol) in dry dioxane (1.8 mL) followed by sodium (0.1 g, 4.3 mmol) in small pieces. The solution was stirred for 5 min at room temperature and was quenched with ethanol. The ammonia was evaporated in a stream of nitrogen. Ion-exchange chromatography of the residue dissolved in water, on Q Sepharose Fast Flow, using a gradient from H_2O to 1 M TEAB (pH 8.0), gave the compound **6** (0.045 g, 0.041 mmol, 68%). **6** was eluted at ca. 800 mM TEAB: ^{31}P NMR (D_2O) δ 0.20, 0.30, 1.01; ^{1}H NMR (D_2O) δ 3.46 (dd, 1H, J = 9.6 Hz), 3.85−4.15 (m, 5H), 4.33 (dd, 1H, J = 9.2 Hz), 4.42 (br, 1H); MS *m/z* (FAB) 479 [$(M + H)^+$, 100), 177 (18), 159 (32), 97 (25); HRMS (FAB) calcd for $C_8H_{18}O_{17}P_3$ 478.976, found 478.976.

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