Deoxygenated Inositol 1,4,5-Trisphosphate Analogues and Their Interaction with Metabolic Enzymes. (1*R*,2*R*,4*R*)-Cyclohexane-1,2,4-tris(methylenesulfonate): A Potent Selective 5-Phosphatase Inhibitor

Alan P. Kozikowski,* Vassil I. Ognyanov, Chinpiao Chen, Abdul H. Fauq, Stephen T. Safrany,† Robert A. Wilcox,† and Stefan R. Nahorski†

Neurochemistry Research, Mayo Foundation for Medical Education and Research, 4500 San Pablo Road, Jacksonville, Florida 32224, and Department of Pharmacology and Therapeutics, University of Leicester, P.O. Box 138, MSB, University Road, Leicester LE1 9HN, UK

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Introduction. 1D-*myo*-Inositol 1,4,5-trisphosphate (1,4,5-IP_3) plays an important role in the signal transduction processes as an intracellular second messenger.[1] Extracellular agonist stimulation generates 1,4,5-IP_3 via phospholipase C catalyzed cleavage of the minor membrane lipid phosphatidylinositol 4,5-bisphosphate. 1,4,5-IP_3 mediates the release of Ca^{2+} from a store in the endoplasmic reticulum by an interaction with a specific receptor, which has been purified,[2,3] cloned, and sequenced.[4,5] The 1,4,5-IP_3 calcium mobilizing signal is terminated by the metabolism of 1,4,5-IP_3 involving two main pathways. Dephosphorylation of the 5-phosphate group catalyzed by the enzyme 5-phosphatase results in the formation of 1D-*myo*-inositol 1,4-bisphosphate (1,4-IP_2), which is not active as a calcium mobilizing agent and is subsequently degraded to free inositol by other phosphatases.[6] The second metabolic route involves phosphorylation of the 3-hydroxyl group of 1,4,5-IP_3 by an ATP-dependent 3-kinase yielding 1D-*myo*-inositol 1,3,4,5-tetrakisphosphate (1,3,4,5-IP_4), whose putative role of stimulating the entry of extracellular calcium by acting on a different receptor is still controversial.[7-9]

In the search for understanding the molecular basis for recognition of inositol polyphosphates, a number of analogues of 1,4,5-IP_3 have been recently synthesized.[10-12] It was concluded that the vicinal 4,5-phosphate groups of 1,4,5-IP_3 are of critical importance to receptor recognition and calcium mobilization,[13] while the 1-phosphate group plays an enhancing role in receptor binding.[14] Extending and consolidating previous findings regarding the importance of the individual hydroxyl groups of 1,4,5-IP_3, we recently gave a solid proof for the minor role of the 2- and 3-hydroxyl groups in receptor binding, and the critical role for the 6-hydroxyl group, which may be involved in fixing the solution conformation by intramolecular hydrogen bonds to the neighboring phosphate group.[15] Studies of the biological activity of 1,4,5-IP_3 analogues have shown that the 3-kinase is more specific in its binding of inositol phosphates than the relatively nonspecific 5-phosphatase.[14,16-18] On the other hand, 1,4,5-IP_3 analogues lacking a hydroxyl group at the 3-position could be crucial in establishing a role for 1,3,4,5-IP_4, since such molecules would not be substrates for the 3-kinase and/or act as inhibitors of this enzyme. Therefore, in the course

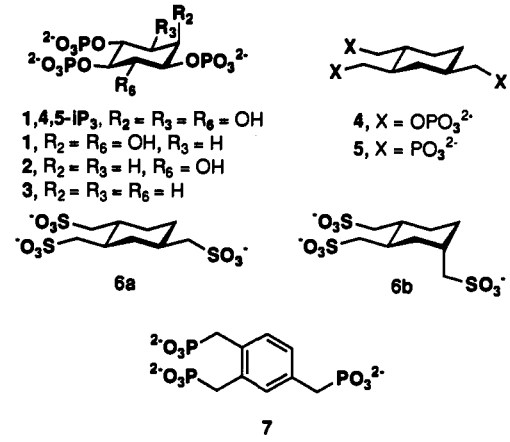

Figure 1. Deoxygenated 1,4,5-IP_3 analogues.

of our search for agonists and antagonists of the 1,4,5-IP_3 receptor and for 5-phosphatase and 3-kinase enzyme inhibitors, we designed and synthesized several different types of structurally diverse deoxygenated analogues of 1,4,5-IP_3 (Figure 1). First we modified the parent molecule by stepwise deletion of the free hydroxyl groups as represented by 1D-3-deoxy-1,4,5-IP_3 (**1**), 1D-2,3-dideoxy-1,4,5-IP_3 (**2**), and 1D-2,3,6-trideoxy-1,4,5-IP_3 (**3**).

A second set of structurally simplified analogues was designed by more than one modification of 1,4,5-IP_3, but by keeping the stereochemistry of the substituents at C-1, C-4, and C-5 intact. (1*R*,2*R*,4*R*)-Cyclohexane-1,2,4-tris(methylenephosphate) (**4**) was constructed by deletion of all of the free hydroxyl groups of 1,4,5-IP_3 and insertion of methylene groups between the phosphate groups and the cyclohexane ring. Next, substitution of the phosphate groups with phosphonic and sulfonic acid groups led to (1*R*,2*R*,4*R*)-cyclohexane-1,2,4-tris(methylenephosphonate) (**5**) and -tris(methylenesulfonate) (**6a**), respectively. In addition, inversion of the configuration of the substituent at C-4 gave (1*R*,2*R*,4*S*)-cyclohexane-1,2,4-tris(methylenesulfonate) (**6b**). Finally, a more drastic structural simplification was achieved by the substitution of the cyclohexane moiety of the parent structure with a benzene ring as represented by benzene-1,2,4-tris(methylenephosphonate) (**7**), since excellent overlap of the phosphate groups of 1,4,5-IP_3 with the phosphonate groups of **7** was observed in molecular modeling studies. Herein we report the synthesis and the interaction of the deoxygenated 1,4,5-IP_3 analogues with the metabolic enzymes 5-phosphatase and 3-kinase, the bovine adrenal cortical 1,4,5-IP_3 receptor, and the Ca^{2+} mobilizing receptor of SH-SY5Y human neuroblastoma cells. The novel (1*R*,2*R*,4*R*)-cyclohexane-1,2,4-tris(methylenesulfonate) (**6a**) appeared as a highly potent and selective inhibitor of the 5-phosphatase.

Chemistry. The deoxy-1,4,5-IP_3 analogues **1**, **2**, and **3** were synthesized from the naturally occurring cyclitol, L-quebrachitol, as recently described.[15]

The phosphate, phosphonate, and sulfonate analogues **4**, **5**, **6a**, and **6b** were obtained from a common precursor constructed via a synthetic route recently described by us[19] employing an asymmetric Diels–Alder reaction[20] (Scheme I). Cycloaddition of the di-(+)-menthyl ester of fumaric acid (**8**)[21] with 2-[(benzyloxy)methyl]-1,3-butadiene (**9a**)[19] or 2-[[(*tert*-butyldimethylsilyl)oxy]methyl]-1,3-butadiene (**9b**)[22] in the presence of diisobutylaluminum chloride gave the cyclohexenes **10a** and **10b**, respectively, with a diastereomeric purity of 98%. Hydrogenation of

† University of Leicester.

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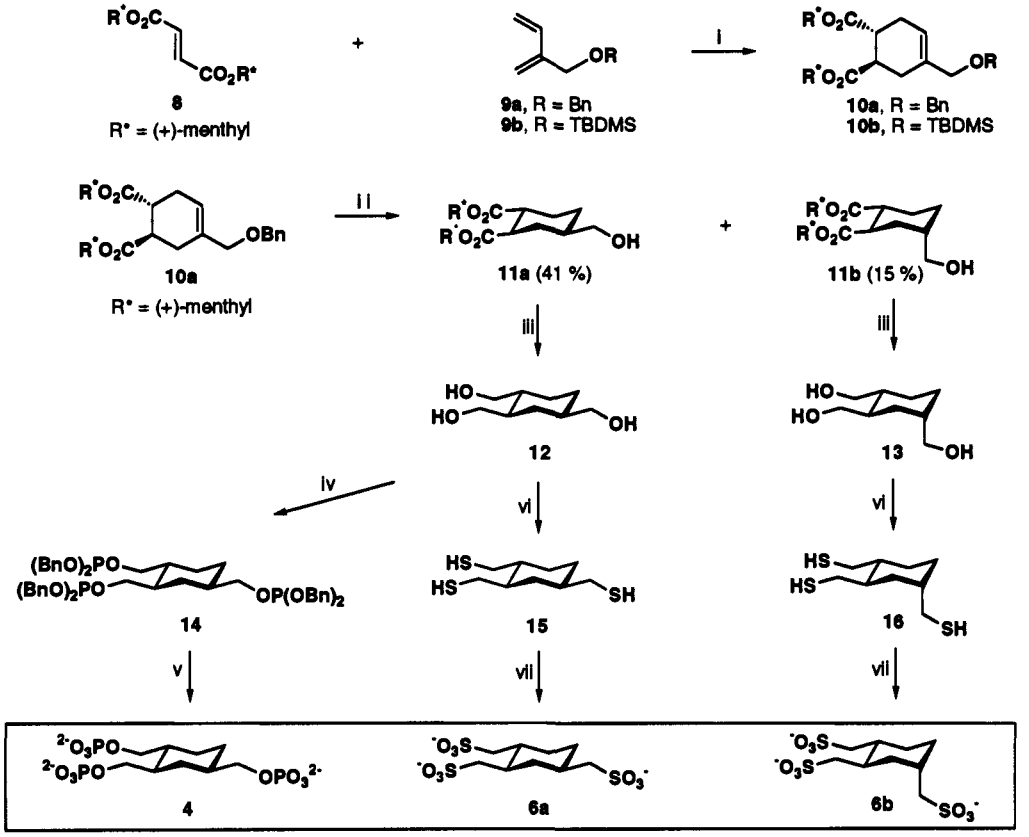
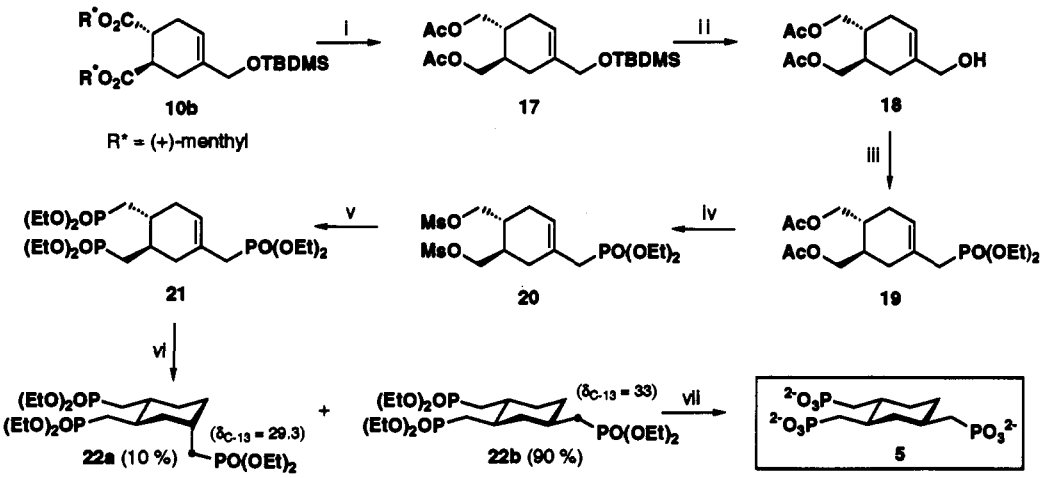
Scheme I[a]



[a] (i) *i*-Bu_2AlCl, hexanes, −40 °C, 94%; (ii) H_2, 10% Pd/C, EtOH, 25 °C; (iii) $LiAlH_4$, THF, 25 °C, 78%; (iv) NaH, $[(BnO)_2PO]_2O$, DMF, 0–25 °C, 67%; (v) H_2 (70 psi), 10% Pd/C, EtOH, 25 °C, then NaOH, 99%; (vi) 1. *i*-$PrO_2CN{=}NCO_2$*i*-Pr, Ph_3P, CH_3COSH, THF, 0–25 °C, 88%; 2. $LiAlH_4$, THF, 25 °C, 80%; (vii) concentrated HNO_3, 100 °C, then NaOH.

Scheme II[a]

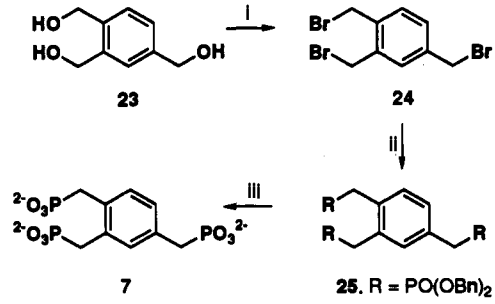


[a] (i) 1. $LiAlH_4$, THF, 25 °C, 96%; 2. Ac_2O, pyridine, THF, 25 °C, 92%; (ii) TsOH, THF, H_2O, 25 °C, 84%; (iii) 1. $SOCl_2$, pyridine, THF, 0–5 °C, 70%; 2. $P(OEt)_3$, 130 °C, 81%; (iv) 1. K_2CO_3, EtOH, H_2O, 25 °C, 99%; 2. MsCl, Et_3N, CH_2Cl_2, 25 °C, 86%; (v) Na, $HPO(OEt)_2$, toluene, 85 °C, 66%; (vi) H_2 (70 psi), 10% Pd/C, EtOH, 25 °C; (vii) concentrated HCl, reflux, then NaOH.

10a over Pd/C afforded the cyclohexanes **11a** and **11b**, which next were separated chromatographically and reduced with $LiAlH_4$ to give the enantiomerically pure cyclohexane-1,2,4-trismethanols **12** and **13**. Treatment of (1*R*,2*R*,4*R*)-cyclohexane-1,2,4-trismethanol (**12**) with sodium hydride and dibenzyl pyrophosphate led to the protected phosphate **14**, which after hydrogenation over Pd/C and treatment with sodium hydroxide gave the target analogue (1*R*,2*R*,4*R*)-cyclohexane-1,2,4-tris(methylenephosphate) (**4**) as a hexasodium salt. Mitsunobu reaction of the triols **12** and **13** involving the use of thiolacetic acid[23] gave the corresponding thiolacetates, which were reduced with $LiAlH_4$ to the cyclohexane-1,2,4-tris(methylenethiols) **15** and **16**, respectively. Subsequent oxidation with nitric acid[24] and treatment with sodium hydroxide afforded the desired (1*R*,2*R*,4*R*)-cyclohexane-1,2,4-tris(methylenesulfonate) (**6a**)[19] and (1*R*,2*R*,4*S*)-cyclohexane-1,2,4-trismethylenesulfonate (**6b**) as its trissodium salt.

Initial attempts to prepare the analogue (1*R*,2*R*,4*R*)-cyclohexane-1,2,4-tris(methylenephosphonate) (**5**) from the triol **12** via nucleophilic displacement of the corresponding triiodide with phosphorus under the conditions

Scheme III[a]



[a] (i) PBr_3, Et_2O, 25 °C, 71%; (ii) $HPO(OBn)_2$, KH, THF, 0–25 °C, 57%; (iii) H_2, PtO_2, EtOH, 25 °C, then NaOH, 93%.

Table I. In Vitro Biological Evaluation of the Deoxygenated 1,4,5-IP_3 Analogues[a]

	inhibition			
1,4,5-IP_3 analogue	5-phosphatase[b] K_i (μM)	3-kinase[c] K_i (μM)	binding[d] K_i (nM)	Ca^{2+} release[e] EC_{50} (nM)
1,4,5-IP_3	15 (S)[f]	0.85 (S)	2.2 ± 0.1 (K_d)	52.1 ± 2.3
1	11.5 (S)	25 (r)[g]	23.4 ± 1.5	155.7 ± 20.1
2	16.5 (S)	19 (r)	39.6 ± 1.0	185.7 ± 6.9
3	81.4 (S)	36 (r)	3948 ± 376	>10000
4	144 (R)[h]	>600 (R)	$>10^5$	$\gg 10^5$
5	535 (R)	>600 (R)	$>10^5$	$>10^5$
6a	3.9	>600 (R)	$>10^5$	$\gg 10^5$
6b	$>10^3$ (R)	>600 (R)	$>10^5$	$\gg 10^5$
7	148[i] (R)	>200 (R)	$>10^5$	$\gg 10^5$

[a] Results represent the average of at least four experiments. [b] Assays performed using a 5-phosphatase preparation isolated from human erythrocyte membranes.[28] [c] Assays performed using a rat brain homogenate high in 3-kinase activity.[29] [d] Ability to displace [^{3}H]-1,4,5-IP_3 (41 Ci/mmol, Amersham, UK) from the 1,4,5-IP_3 specific binding site of membranes prepared from bovine adrenal cortices.[30] [e] Assessed in $^{45}Ca^{2+}$-preloaded SH-SY5Y cells, permeabilized using saponin, as previously described for 1321N1 cells.[30] [f] (S) indicates that the analogue was a substrate of the enzyme. [g] (r) indicates that resistance to 3-kinase was assumed. [h] (R) indicates that the analogue was not a substrate and was metabolically resistant to the enzyme. [i] Assays performed using only 10 μM 5-phosphatase and the K_i value calculated from the IC_{50} value using the Cheng–Prusoff equation.[31]

of the Michaelis–Arbusov or Michaelis–Becker reactions[25] were unsuccessful. Therefore, we decided first to employ the more reactive allylic function of the starting adduct **10b** to install a phosphonate group by the Michaelis–Arbusov reaction and then to use the Michaelis–Becker[25] reaction to introduce the remaining two phosphonate functions. As shown in Scheme II, the ester groups of **10b** were reduced with $LiAlH_4$, and the resulting diol was protected as the diacetate **17**. Cleavage of the TBDMS group with *p*-toluenesulfonic acid gave the allylic alcohol **18**, which after conversion to the corresponding chloride, followed by treatment with triethyl phosphite, led to the phosphonate **19**. Saponification of the acetate groups in **19** followed by mesylation afforded **20**, which was reacted with sodium diethyl phosphite[26] to give the trisphosphonate **21**. Finally, hydrogenation of **21** over Pd/C led to the formation of the chromatographically separable cyclohexanes **22a** and **22b**, with the latter isomer predominating. The assignment of stereochemistry to **22a** and **22b** was made by examining the ^{13}C NMR chemical shifts of the methylene groups at the C-4 position. Similarly to the isomeric cyclohexane-1,2,4-trismethanols **12** and **13** (Scheme I)[19] and as a consequence of steric compression,[27] the equatorial hydroxymethylene group at C-4 of **22b** appears at lower field than the axial hydroxymethylene group of **22a** (see structures **22a** and **22b** for chemical shifts). Thus the major isomer of the hydrogenation reaction was assigned as **22b** and was further hydrolyzed with concentrated hydrochloric acid and treated with sodium hydroxide to give the required analogue (1*R*,2*R*,4*R*)-cyclohexane-1,2,4-tris(methylenephosphonate) (**5**) as its hexasodium salt.

The analogue **7** was prepared in a few steps from benzene-1,2,4-trismethanol (**23**) as described in Scheme III. The triol **23** was converted to the corresponding tribromide **24**, which was reacted with dibenzyl phosphite under the conditions of the Michaelis–Becker reaction to give the tris(dibenzyl phosphonate) **25**. Subsequent hydrolysis and treatment with sodium hydroxide gave the desired analogue benzene-1,2,4-tris(methylenephosphonate) (**7**) as its hexasodium salt.

Biology. All of the deoxygenated 1,4,5-IP_3 analogues were examined for their ability to interact with the metabolic enzymes 3-kinase and 5-phosphatase, to bind to the 1,4,5-IP_3 receptor, and to mobilize intracellular Ca^{2+} stores (Table I).

While we have previously reported that 1D-3-deoxy-1,4,5-IP_3 (**1**) and 1D-2,3-dideoxy-1,4,5-IP_3 (**2**) possess relatively potent ligand and agonist activity, which was reduced dramatically for the analogue lacking the 6-hydroxyl group, i.e., 1D-2,3,6-trideoxy-1,4,5-IP_3 (**3**),[15] the rest of the deoxygenated analogues tested were essentially devoid of significant binding and Ca^{2+} mobilizing activity.

Several of the analogues were relatively active inhibitors of the enzyme preparations. In particular (1*R*,2*R*,4*R*)-cyclohexane-1,2,4-tris(methylenesulfonate) (**6a**) (K_i 3.9 μM) exhibited potent inhibition of 5-phosphatase-catalyzed dephosphorylation of [^{3}H]-1,4,5-IP_3. In contrast, inversion of the configuration at C-4 of **6a**, as represented by the isomeric analogue (1*R*,2*R*,4*S*)-cyclohexane-1,2,4-tris(methylenesulfonate) (**6b**) led to the loss of the inhibitory activity against 5-phosphatase. In this respect, we should note that the recently described[32] racemic *myo*-inositol 1,4,5-trissulfate, a 1,4,5-IP_3 analogue with oxygen atoms in place of the methylene groups of **6a**, was reported to be inactive in calcium release studies. Unfortunately, no data on the interaction of this analogue with the metabolic enzymes were provided.

In conclusion, the novel methylenesulfonate **6a** may be a valuable tool for studies requiring specific 5-phosphatase inhibition, since it fails to exhibit significant ligand or agonist activity and does not react with the 3-kinase. We are currently investigating the ability of cell-permeable derivatives and analogues of (1*R*,2*R*,4*R*)-cyclohexane-1,2,4-tris(methylenesulfonate) (**6a**) to inhibit 5-phosphatase activity in intact cells.

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Supplementary Material Available: Analytical and spectral data for compounds **4**, **5**, **6a,b**, **7**, **9a,b**, **10a,b**, **11a,b**, **12–21**, **22a,b**, **24**, and **25** (8 pages). Ordering information is given on any current masthead page.

References

(1) Berridge, M. J.; Irvine, R. F. Inositol Phosphates and Cell Signalling. *Nature (London)* **1989**, *341*, 197–202.
(2) Supattapone, S.; Worley, P. F.; Baraban, J. M.; Snyder, S. H. Solubilization, Purification, and Characterization of an Inositol Trisphosphate Receptor. *J. Biol. Chem.* **1988**, *263*, 1530–1534.
(3) Maeda, N. M.; Niinobe, M.; Mikoshiba, K. A. Cerebellar Purkinje Cell Marker P_{400} Protein is an Inositol 1,4,5-Trisphosphate Receptor Protein. Purification and Characterization of $InsP_3$ Receptor Complex. *EMBO J.* **1990**, *9*, 61–67.

(4) Furuichi, T.; Yoshikawa, S.; Miyawaki, A.; Wada, A.; Maeda, N.; Mikoshiba, K. Primary Structure and Functional Expression of the Inositol 1,4,5-Trisphosphate-Binding Protein P_{400}. *Nature (London)* **1989**, *342*, 32–38.
(5) Mignery, G. A.; Newton, C. L.; Archer, B. T.; Sudhof, T. C. Structure and Expression of the Rat Inositol 1,4,5-Trisphosphate Receptor. *J. Biol. Chem.* **1990**, *265*, 12679–12685.
(6) Shears, S. B. Metabolism of Inositol Phosphates. In *Advances in Second Messenger Research*; Putney, J. W. J., Ed.; Raven Press Ltd.: New York, 1992; Vol. 26, pp 63–92.
(7) Challiss, R. A. J.; Willcocks, A. L.; Mulloy, B.; Potter, B. V. L.; Nahorski, S. R. Characterization of Inositol 1,4,5-Trisphosphate- and Inositol 1,3,4,5-Tetrakisphosphate-Binding Sites in Rat Cerebellum. *Biochem. J.* **1991**, *274*, 861–867.
(8) Wilcox, R. A.; Challiss, R. A. J.; Willcocks, A. L.; Baudin, G.; Vasella, A.; Potter, B. V. L.; Nahorski, S. R. Stereoselectivity of Ins(1,3,4,5)-P_4 Recognition Sites: Implications for the Mechanism of the Ins-(1,3,4,5)P_4 Induced Ca^{2+} Mobilization. *Biochem. J.* **1993**, *294*, 191–194.
(9) Irvine, R. F. Inositol Tetrakisphosphate as a Second Messenger: Confusions, Contradictions, and a Potential Resolution. *BioEssays* **1991**, *13*, 419–428.
(10) Billington, D. C. *The Inositol Phosphates – Chemical Synthesis and Biological Significance*; VCH: Weinheim; New York, 1993.
(11) Potter, B. V. L. Recent Advances in the Chemistry and Biochemistry of Inositol Phosphates of Biological Interest. *Nat. Prod. Rep.* **1990**, *7*, 1–24.
(12) Potter, B. V. L.; Nahorski, S. R. Synthesis and Biology of Inositol Polyphosphate Analogues. *Biochem. Soc. Trans.* **1992**, *20*, 434–442.
(13) Gawler, D. J.; Potter, B. V. L.; Gigg, R.; Nahorski, S. R. Interactions between Inositol-Tris- and Tetrakis-Phosphates. *Biochem. J.* **1991**, *276*, 163–167.
(14) Nahorski, S. R.; Potter, B. V. L. Molecular Recognition of Inositol Polyphosphates by Intracellular Receptors and Metabolic Enzymes. *Trends Pharmacol. Sci.* **1989**, *10*, 139–144.
(15) 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.
(16) Safrany, S. T.; Wilcox, R. A.; Liu, C.; Potter, B. V. L.; Nahorski, S. R. 3-Position Modification of *myo*-Inositol 1,4,5-Trisphosphate: Consequences for Intracellular Ca^{2+} Mobilization and Enzyme Recognition. *Eur. J. Pharmacol. (Mol. Pharmacol. Sect.)* **1992**, *226*, 265–272.
(17) Polokoff, M. A.; Bencen, G. H.; Vacca, S. J.; deSolms, S. J.; Young, S. D.; Huff, J. R. Metabolism of Synthetic Inositol Trisphosphate Analogs. *J. Biol. Chem.* **1988**, *263*, 11922–11926.
(18) Hirata, M.; Watanabe, Y.; Ishimatsu, T.; Ikebe, T.; Kimura, Y.; Yamaguchi, K.; Ozaki, S.; Koga, T. Synthetic Inositol Trisphosphate Analogs and Their Effects on Phosphatase, Kinase, and the Release of Ca^{2+}. *J. Biol. Chem.* **1989**, *264*, 20303–20308.
(19) Kozikowski, A. P.; Ognyanov, V. I.; Chen, C.; Kurian, P.; Crews, F. T. Synthesis and Binding Studies of an Optically Pure Hexadeoxy-1,4,5-Tris(Methylenesulfonic Acid) Analogue of IP_3. *Tetrahedron Lett.* **1993**, *34*, 219–222.
(20) Furuta, K.; Iwanaga, K.; Yamamoto, H. Asymmetric Diels-Alder Reaction. Cooperative Blocking Effect in Organic Synthesis. *Tetrahedron Lett.* **1986**, *27*, 4507–4510.
(21) Heathcock, C. H.; Davis, B. R.; Hadley, C. R. Synthesis and Biological Evaluation of a Monocyclic, Fully Functional Analogue of Compactin. *J. Med. Chem.* **1989**, *32*, 197–202.
(22) The diene **9b** was prepared in 68% yield by treatment of 2-(hydroxymethyl)-1,3-butadiene[33] with TBDMSCl in the presence of imidazole and DMAP in acetonitrile.
(23) Volante, R. P. A New, Highly Efficient Method for the Conversion of Alcohols to Thiolesters and Thiols. *Tetrahedron Lett.* **1981**, *22*, 3119–3122.
(24) Bauer, L.; Gardella, L. A. Addition of Thiourea to 2- and 4-Vinylpyridines. *J. Org. Chem.* **1961**, *26*, 82–85.
(25) Engel, R. *Synthesis of Carbon-Phosphorus Bonds*; CRC Press, Inc.: Boca Raton, FL, 1988.
(26) Baraldi, P. G.; Guarneri, M.; Moroder, F.; Pollini, G. P.; Simoni, D. Synthesis of 1-Phthalimidoalkenephosphonates. *Synthesis* **1982**, 653–655.
(27) Dalling, D. K.; Grant, D. M. Carbon-13 Magnetic Resonance. XXI. Steric Interactions in the Methylcyclohexanes. *J. Am. Chem. Soc.* **1972**, *94*, 5318–5324.
(28) Safrany, S. T.; Sawyer, D. A.; Nahorski, S. R.; Potter, B. V. L. Synthetic D- and L-Enantiomeric Fluoro-Analogues of *myo*-Inositol 1,4,5-Trisphosphate Interact Differently with *myo*-Inositol 1,4,5-Trisphosphate, 3-Kinase and 5-Phosphatase: Identification of the First Potent Small Molecule 3-Kinase Inhibitor. *Chirality* **1992**, *4*, 415–422.
(29) Safrany, S. T.; Wojcikiewicz, R. J. H.; Strupish, J.; McBain, J.; Cooke, A. M.; Potter, B. V. L.; Nahorski, S. R. Synthetic Phosphorothioate-Containing Analogues of Inositol 1,4,5-Trisphosphate Mobilize Intracellular Ca^{2+} Stores and Interact Differentially with Inositol 1,4,5-Trisphosphate, 5-Phosphatase and 3-Kinase. *Mol. Pharmacol.* **1991**, *39*, 754–761.
(30) Wilcox, R. A.; Sawyer, D. A.; Liu, C.; Nahorski, S. R.; Potter, B. V. L. The Role of the 2- and 3-Hydroxyl Groups of 1D-*myo*-Inositol 1,4,5-Trisphosphate in Calcium Mobilization from Permeabilized Human 1321N1 Astrocytoma Cells. *Carbohydr. Res.* **1992**, *234*, 237–246.
(31) Cheng, Y.; Prusoff, W. H. Relationship Between the Inhibition Constant (K_i) and the Concentration of Inhibitor which Causes 50 Percent Inhibition (I_{50}) of an Enzyme Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
(32) Willems, H. A. M.; Veeneman, G. H.; Westerduin, P. Synthesis of Racemic *myo*-Inositol 1,4,5-Trismethylphosphonate, *myo*-Inositol 4,5-Bismethylphosphonate and *myo*-Inositol 5-Methylphosphonate via a Phosphinate Approach. *Tetrahedron Lett.* **1992**, *33*, 2075–2078.
(33) Tanaka, J.; Suzuki, T.; Takabe, K.; Katagiri, T. A. Selenium Dioxide Oxidation of Isoprene. *Nippon Kagaku Kaishi* **1973**, *2*, 292–295.