

# 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

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

In the search for understanding the molecular basis for recognition of inositol polyphosphates, a number of analogues of 1,4,5-IP<sub>3</sub> have been recently synthesized.<sup>10-12</sup> It was concluded that the vicinal 4,5-phosphate groups of 1,4,5-IP<sub>3</sub> are of critical importance to receptor recognition and calcium mobilization,<sup>13</sup> while the 1-phosphate group plays an enhancing role in receptor binding.<sup>14</sup> Extending and consolidating previous findings regarding the importance of the individual hydroxyl groups of 1,4,5-IP<sub>3</sub>, 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.<sup>15</sup> Studies of the biological activity of 1,4,5-IP<sub>3</sub> analogues have shown that the 3-kinase is more specific in its binding of inositol phosphates than the relatively nonspecific 5-phosphatase.<sup>14,16-18</sup> On the other hand, 1,4,5-IP<sub>3</sub> analogues lacking a hydroxyl group at the 3-position could be crucial in establishing a role for 1,3,4,5-IP<sub>4</sub>, since such molecules would not be substrates for the 3-kinase and/or act as inhibitors of this enzyme. Therefore, in the course

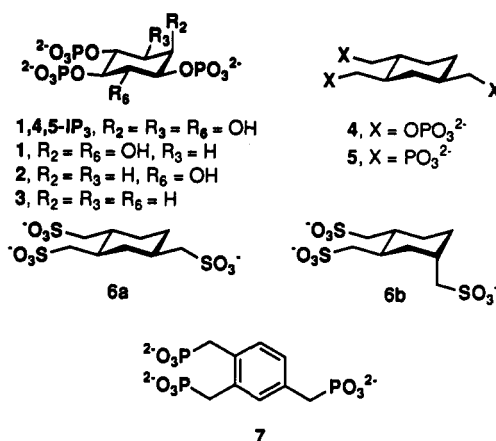


Figure 1. Deoxygenated 1,4,5-IP<sub>3</sub> analogues.

of our search for agonists and antagonists of the 1,4,5-IP<sub>3</sub> 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<sub>3</sub> (Figure 1). First we modified the parent molecule by stepwise deletion of the free hydroxyl groups as represented by 1*D*-3-deoxy-1,4,5-IP<sub>3</sub> (1), 1*D*-2,3-dideoxy-1,4,5-IP<sub>3</sub> (2), and 1*D*-2,3,6-trideoxy-1,4,5-IP<sub>3</sub> (3).

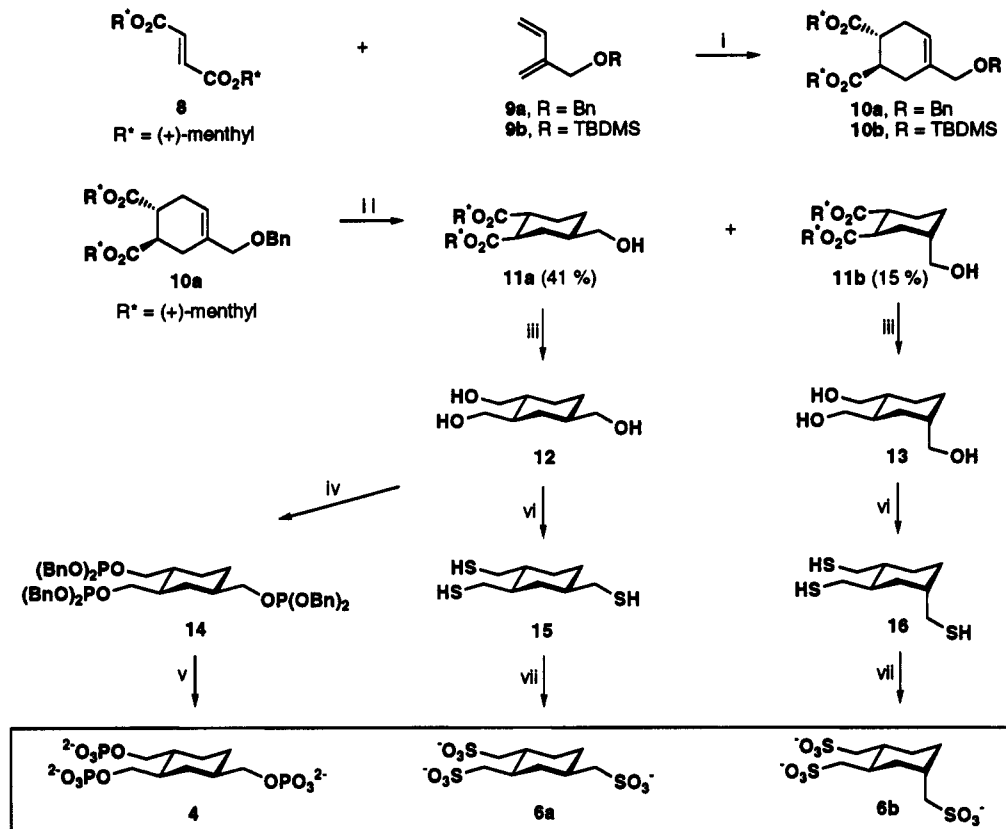
A second set of structurally simplified analogues was designed by more than one modification of 1,4,5-IP<sub>3</sub>, but by keeping the stereochemistry of the substituents at C-1, C-4, and C-5 intact. (1*R*,2*R*,4*R*)-Cyclohexane-1,2,4-tris(methylenephosphate) (4) was constructed by deletion of all of the free hydroxyl groups of 1,4,5-IP<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> analogues with the metabolic enzymes 5-phosphatase and 3-kinase, the bovine adrenal cortical 1,4,5-IP<sub>3</sub> receptor, and the Ca<sup>2+</sup> 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<sub>3</sub> analogues 1, 2, and 3 were synthesized from the naturally occurring cyclitol, L-quebrachitol, as recently described.<sup>15</sup>

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<sup>19</sup> employing an asymmetric Diels-Alder reaction<sup>20</sup> (Scheme I). Cycloaddition of the di-(+)-menthyl ester of fumaric acid (8)<sup>21</sup> with 2-[(benzyloxy)methyl]-1,3-butadiene (9a)<sup>19</sup> or 2-[[*tert*-butyldimethylsilyl]oxy]methyl]-1,3-butadiene (9b)<sup>22</sup> in the presence of diisobutylaluminum chloride gave the cyclohexenes 10a and 10b, respectively, with a diastereomeric purity of 98%. Hydrogenation of

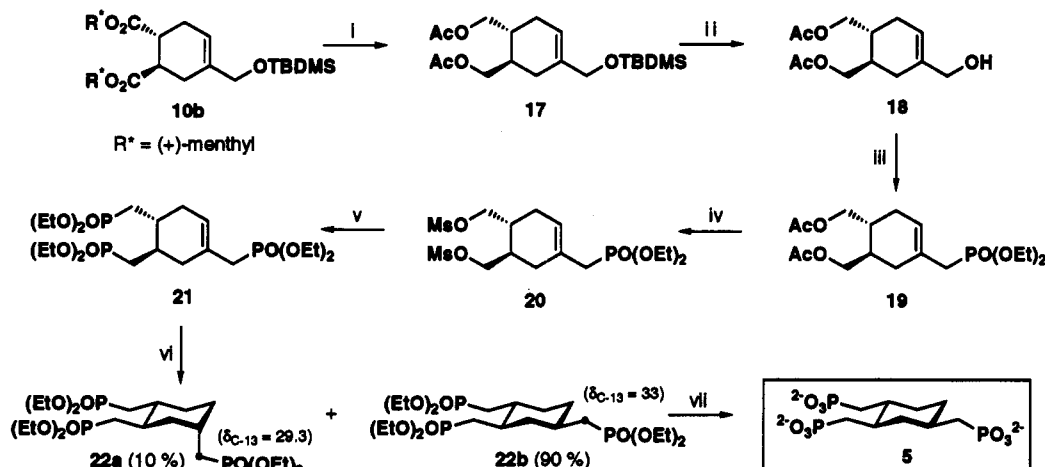
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### Scheme 1<sup>a</sup>



<sup>a</sup> (i)  $i\text{-Bu}_2\text{AlCl}$ , hexanes,  $-40^\circ\text{C}$ , 94%; (ii)  $\text{H}_2$ , 10% Pd/C, EtOH,  $25^\circ\text{C}$ ; (iii)  $\text{LiAlH}_4$ , THF,  $25^\circ\text{C}$ , 78%; (iv) NaH,  $[(\text{BuO})_2\text{PO}]_2\text{O}$ , DMF,  $0-25^\circ\text{C}$ , 67%; (v)  $\text{H}_2$  (70 psi), 10% Pd/C, EtOH,  $25^\circ\text{C}$ , then NaOH, 99%; (vi) 1.  $i\text{-PrO}_2\text{CN}=\text{NCO}_2i\text{-Pr}$ ,  $\text{Ph}_3\text{P}$ ,  $\text{CH}_3\text{COSH}$ , THF,  $0-25^\circ\text{C}$ , 88%; 2.  $\text{LiAlH}_4$ , THF,  $25^\circ\text{C}$ , 80%; (vii) concentrated  $\text{HNO}_3$ ,  $100^\circ\text{C}$ , then NaOH.

### Scheme II<sup>a</sup>

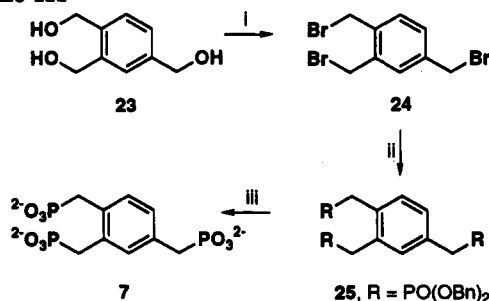


<sup>a</sup> (i) 1.  $\text{LiAlH}_4$ , THF, 25 °C, 96%; 2.  $\text{Ac}_2\text{O}$ , pyridine, THF, 25 °C, 92%; (ii)  $\text{TsOH}$ , THF,  $\text{H}_2\text{O}$ , 25 °C, 84%; (iii) 1.  $\text{SOCl}_2$ , pyridine, THF, 0–5 °C, 70%; 2.  $\text{P}(\text{OEt})_3$ , 130 °C, 81%; (iv) 1.  $\text{K}_2\text{CO}_3$ ,  $\text{EtOH}$ ,  $\text{H}_2\text{O}$ , 25 °C, 99%; 2.  $\text{MsCl}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , 25 °C, 86%; (v)  $\text{Na}$ ,  $\text{HPO}(\text{OEt})_2$ , toluene, 85 °C, 66%; (vi)  $\text{H}_2$  (70 psi), 10%  $\text{Pd/C}$ ,  $\text{EtOH}$ , 25 °C; (vii) concentrated  $\text{HCl}$ , reflux, then  $\text{NaOH}$ .

**10a** over Pd/C afforded the cyclohexanes **11a** and **11b**, which next were separated chromatographically and reduced with LiAlH<sub>4</sub> 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(methylene-phosphate) (**4**) as a hexasodium salt. Mitsunobu reaction of the triols **12** and **13** involving the use of thiolacetic acid<sup>23</sup>

gave the corresponding thiolacetates, which were reduced with  $\text{LiAlH}_4$  to the cyclohexane-1,2,4-tris(methylenethiols) **15** and **16**, respectively. Subsequent oxidation with nitric acid<sup>24</sup> and treatment with sodium hydroxide afforded the desired (1*R*,2*R*,4*R*)-cyclohexane-1,2,4-tris(methylene-sulfonate) (**6a**)<sup>19</sup> and (1*R*,2*R*,4*S*)-cyclohexane-1,2,4-tris(methylene-sulfonate) (**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<sup>a</sup>

<sup>a</sup> (i) PBr<sub>3</sub>, Et<sub>2</sub>O, 25 °C, 71%; (ii) HPO(OBn)<sub>2</sub>, KH, THF, 0–25 °C, 57%; (iii) H<sub>2</sub>, PtO<sub>2</sub>, EtOH, 25 °C, then NaOH, 93%.

**Table I.** In Vitro Biological Evaluation of the Deoxygenated 1,4,5-IP<sub>3</sub> Analogues<sup>a</sup>

1,4,5-IP <sub>3</sub> analogue	inhibition			
	5-phosphatase <sup>b</sup> K <sub>i</sub> (μM)	3-kinase <sup>c</sup> K <sub>i</sub> (μM)	binding <sup>d</sup> K <sub>i</sub> (nM)	Ca <sup>2+</sup> release <sup>e</sup> EC <sub>50</sub> (nM)
1,4,5-IP <sub>3</sub>	15 (S) <sup>f</sup>	0.85 (S)	2.2 ± 0.1 (K <sub>d</sub> )	52.1 ± 2.3
1	11.5 (S)	25 (r) <sup>g</sup>	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) <sup>h</sup>	>600 (R)	>10 <sup>5</sup>	>>10 <sup>5</sup>
5	535 (R)	>600 (R)	>10 <sup>5</sup>	>10 <sup>5</sup>
6a	3.9	>600 (R)	>10 <sup>5</sup>	>>10 <sup>5</sup>
6b	>10 <sup>3</sup> (R)	>600 (R)	>10 <sup>5</sup>	>>10 <sup>5</sup>
7	148 <sup>i</sup> (R)	>200 (R)	>10 <sup>5</sup>	>>10 <sup>5</sup>

<sup>a</sup> Results represent the average of at least four experiments.

<sup>b</sup> Assays performed using a 5-phosphatase preparation isolated from human erythrocyte membranes.<sup>28</sup> <sup>c</sup> Assays performed using a rat brain homogenate high in 3-kinase activity.<sup>29</sup> <sup>d</sup> Ability to displace [<sup>3</sup>H]-1,4,5-IP<sub>3</sub> (41 Ci/mmol, Amersham, UK) from the 1,4,5-IP<sub>3</sub> specific binding site of membranes prepared from bovine adrenal cortices.<sup>30</sup>

<sup>e</sup> Assessed in <sup>45</sup>Ca<sup>2+</sup>-preloaded SH-SY5Y cells, permeabilized using saponin, as previously described for 1321N1 cells.<sup>30</sup> (S) indicates that the analogue was a substrate of the enzyme. (r) indicates that resistance to 3-kinase was assumed. (R) indicates that the analogue was not a substrate and was metabolically resistant to the enzyme.

<sup>f</sup> Assays performed using only 10 μM 5-phosphatase and the K<sub>i</sub> value calculated from the IC<sub>50</sub> value using the Cheng–Prusoff equation.<sup>31</sup>

of the Michaelis–Arbusov or Michaelis–Becker reactions<sup>25</sup> 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<sup>25</sup> reaction to introduce the remaining two phosphonate functions. As shown in Scheme II, the ester groups of 10b were reduced with LiAlH<sub>4</sub>, 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<sup>26</sup> 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 <sup>13</sup>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)<sup>19</sup> and as a consequence of steric compression,<sup>27</sup> 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<sub>3</sub> 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<sub>3</sub> receptor, and to mobilize intracellular Ca<sup>2+</sup> stores (Table I).

While we have previously reported that 1*D*-3-deoxy-1,4,5-IP<sub>3</sub> (1) and 1*D*-2,3-dideoxy-1,4,5-IP<sub>3</sub> (2) possess relatively potent ligand and agonist activity, which was reduced dramatically for the analogue lacking the 6-hydroxyl group, i.e., 1*D*-2,3,6-trideoxy-1,4,5-IP<sub>3</sub> (3),<sup>15</sup> the rest of the deoxygenated analogues tested were essentially devoid of significant binding and Ca<sup>2+</sup> 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<sub>i</sub> 3.9 μM) exhibited potent inhibition of 5-phosphatase-catalyzed dephosphorylation of [<sup>3</sup>H]-1,4,5-IP<sub>3</sub>. 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<sup>32</sup> racemic *myo*-inositol 1,4,5-trissulfate, a 1,4,5-IP<sub>3</sub> 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.

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