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SYNTHESIS AND BINDING STUDIES OF AN OPTICALLY PURE HEXADEOXY-1,4,5-TRIS(METHYLENESULFONIC ACID) ANALOGUE OF IP_3

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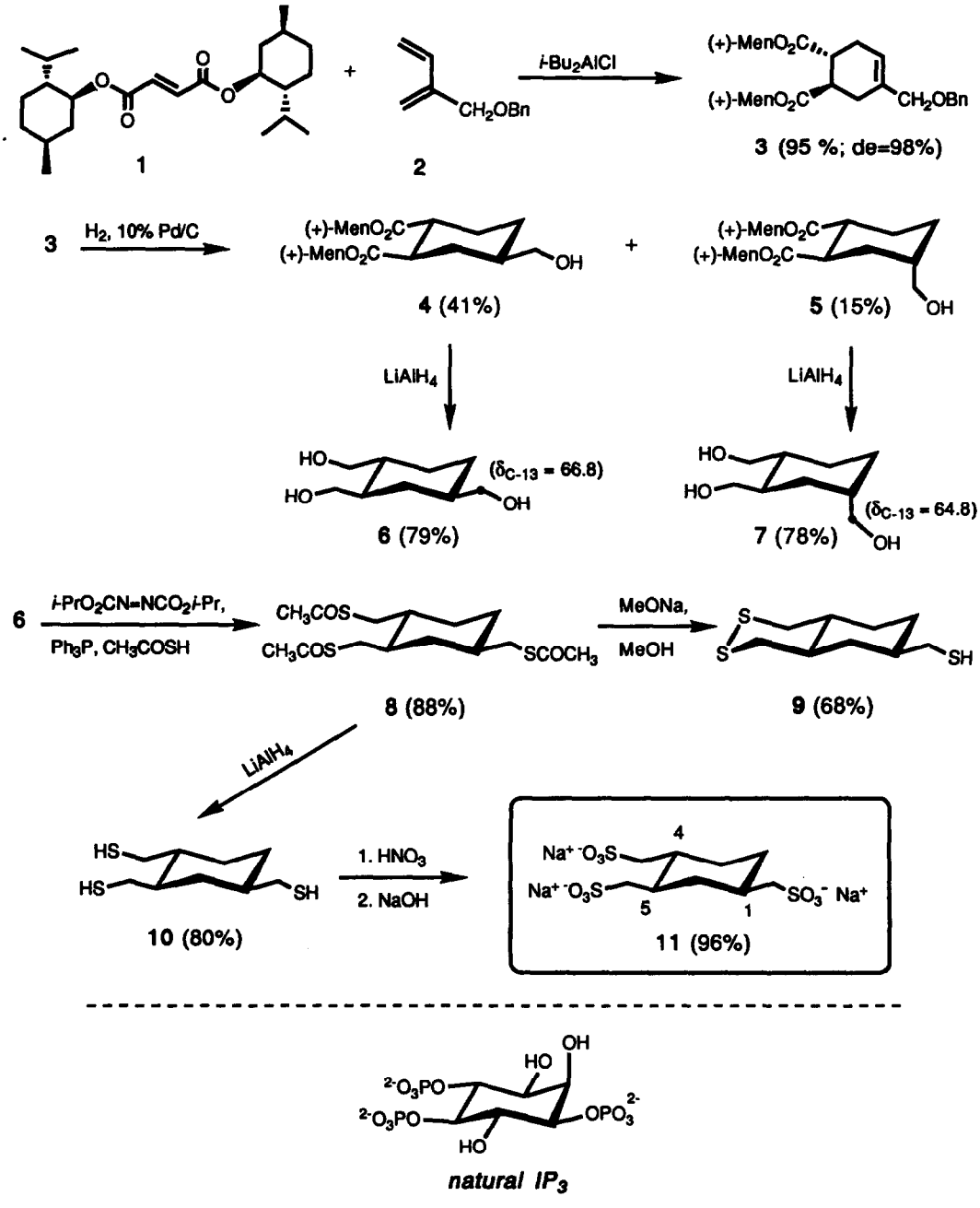
Abstract: The synthesis of the (-)-sodium salt of the hexadeoxy-1,4,5-tris(methylenesulfonic acid) analogue of IP_3 by use of an asymmetric Diels-Alder reaction is described together with the effects of this compound in binding to the IP_3 receptor.

The seminal idea that opened the area of *myo*-inositol signalling for intensive investigation came in 1975 with Michell's suggestion that the turnover of phosphoinositides coupled cell surface receptors to cellular Ca^{2+} mobilization.[1] Subsequently, Berridge was led to conclude that the water soluble product of $PI(4,5)P_2$ hydrolysis, namely $IP(1,4,5)_3$, was the likely intracellular candidate that released Ca^{2+} from intracellular stores and increased cytoplasmic free Ca^{2+} concentrations.[2] By a series of events that are still not fully understood, but probably involve a network of interacting protein kinases and phosphorylation of nuclear transcription factors,[3] the increase in intracellular Ca^{2+} together with the activation of PKC leads to transcriptional activation of genes responsible for a biological response.[4,5] In an effort to identify IP_3-like molecules that might find therapeutic application in the treatment of certain neurodegenerative disorders such as Alzheimer's disease,[6] a disease which may at least in part owe its etiology to improper calcium signalling, we have become interested in the creation of certain metabolically stable isosteres of IP_3. Herein we report the synthesis of a molecule which represents a simplified version of IP_3, namely the hexadeoxy analogue of IP_3 bearing methylenesulfonic acid groups in place of the phosphate groups of the parent structure. Although the 6-hydroxy group of IP_3 does play a more prominent role in Ca^{2+} release than either of the hydroxy groups at positions 2 or 3,[7,8] we were interested in constructing this minimally functionalized compound first in order to properly set the stage for further mapping the SAR requirements of the IP_3 recognition site.

Synthesis of the required sulfur analogue began by employing the di-(+)-menthyl ester of fumaric acid[9] 1 (Scheme 1) as the Diels-Alder dienophile in the cycloaddition reaction with 2-benzyloxymethyl-1,3-butadiene (2). This diene was prepared from isoprene by selenium dioxide oxidation[10] followed by *O*-benzylation. The cycloaddition reaction was carried out at -40 °C using diisobutylaluminum chloride as the homogeneous catalyst, the preferred catalyst in such

Scheme 1. Diastereofacially Selective Diels-Alder Route to an Analogue of IP_3.



asymmetric Diels-Alder reactions as elegantly described by Yamamoto and coworkers.[11] The cyclohexene **3** was formed in 95% yield which by HPLC determination was found to possess a diastereomeric purity of 98%. Next the cyclohexene **3** was hydrogenated over Pd/C to deliver the chromatographically separable compounds **4** and **5**, with the former isomer predominating. Reduction of these diesters individually with lithium aluminum hydride (LAH) then provided **6** and **7**. The assignment of stereochemistry to these cyclohexanes was made by examining the ^{13}C NMR chemical shift of the hydroxymethylene group at the C-1 postion. As a consequence of steric compression,[13] the axial hydroxymethylene group of **7** appears at a higher field strength than the equatorial C-1 hydroxymethylene group of **6** (see structures **6** and **7** for chemical shifts). Thus, the major product of the hydrogenation reaction was taken on to the synthesis of the target compound. After some experimentation, we found that sulfur was best introduced into this molecule by the Mitsunobu reaction involving use of thiolacetic acid.[14] The desired tris-thiolacetate **8** was obtained in 88% yield. Interestingly, when this compound was deprotected using catalytic amounts of sodium methoxide in methanol, the disulfide **9** could be isolated in 68% yield. Therefore, in order to avoid potential problems in the subsequent oxidation of this disulfide **9** to sulfonic acid, **8** was reduced directly to the tris-thiol **10** by LAH treatment, and **10** was then oxidized with concentrated nitric acid.[15] The desired IP_3 mimic **11** was isolated as its tris-sodium salt by treatment with sodium hydroxide. The sodium salt was obtained as a white powder which exhibited an optical rotation of -24.5° (*c* 1.0, H_2O).

To examine the ability of **11** to function as a mimic of IP_3, we investigated its ability to displace [^{3}H]IP_3 from rat cerebellar P_2 membrane fraction.[16] As is apparent from the graph presented in Figure 1, **11** is a rather poor mimic of natural IP_3, and exhibits a K_i of > 17 μM compared to a K_i of 10 nM for natural IP_3. Several factors are likely to contribute to the poor binding affinity of this compound, which may include the following: (a) the lack of the hydroxy groups at positions 2, 3, and 6; (b) substitution of one of the oxygen atoms present in each of the three phosphate groups of IP_3 by methylene; and (c) replacement of phosphate by sulfonic acid.[17] While the synthesis of additional IP_3 analogues will be required to sort out the contributions which each of the specific structural features delineated above contribute to binding, we note that deletion of the 6-hydroxy group from IP_3 results in about a 100-fold loss both in binding affinity and ability to release Ca^{2+} from rat parotid microsomal fraction.[7] Deletion of the 3-hydroxy group, however, has been shown to provide an IP_3 analogue of comparable Ca^{2+} releasing activity.[8] Furthermore, a recent article has revealed that *myo*-inositol 1,4,5-tris-sulphate is biologically inactive.[18]

In summation, while the IP_3 mimic disclosed herein is not useful from a biological standpoint, the chemical route developed may find application in the construction of improved compounds of this genre.

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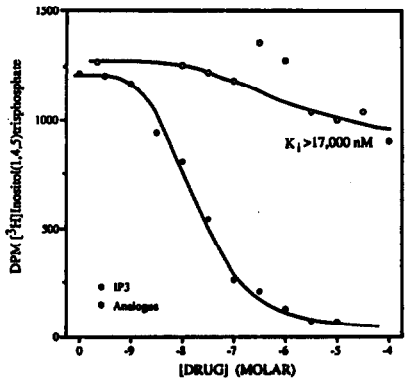

Figure 1. *Competition binding of [^{3}H]IP$_3$ with 11.* Each assay tube contained 12,000 dpm of [^{3}H]IP$_3$, 250 μg of rat cerebellar P$_2$ membrane fraction and 50 μL of IP$_3$ (0.06 to 2000 pmol) or **11** (0.6 to 10^6 pmol) in 100 mM Tris-HCl and 4 mM EDTA (pH = 7.8) in a final volume of 200 μL. Tubes were incubated for 30 min at 4 °C. Separation of bound and free ligand was achieved by rapid filtration. The K$_i$ was computed using computer assisted curve fitting (McPherson, G. A., BIOSOFT, Cambridge, U.K., 1985).

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