

SYNTHESIS AND BINDING STUDIES OF AN OPTICALLY PURE HEXADEOXY-1,4,5-TRIS(METHYLENESULFONIC ACID) ANALOGUE OF IP₃

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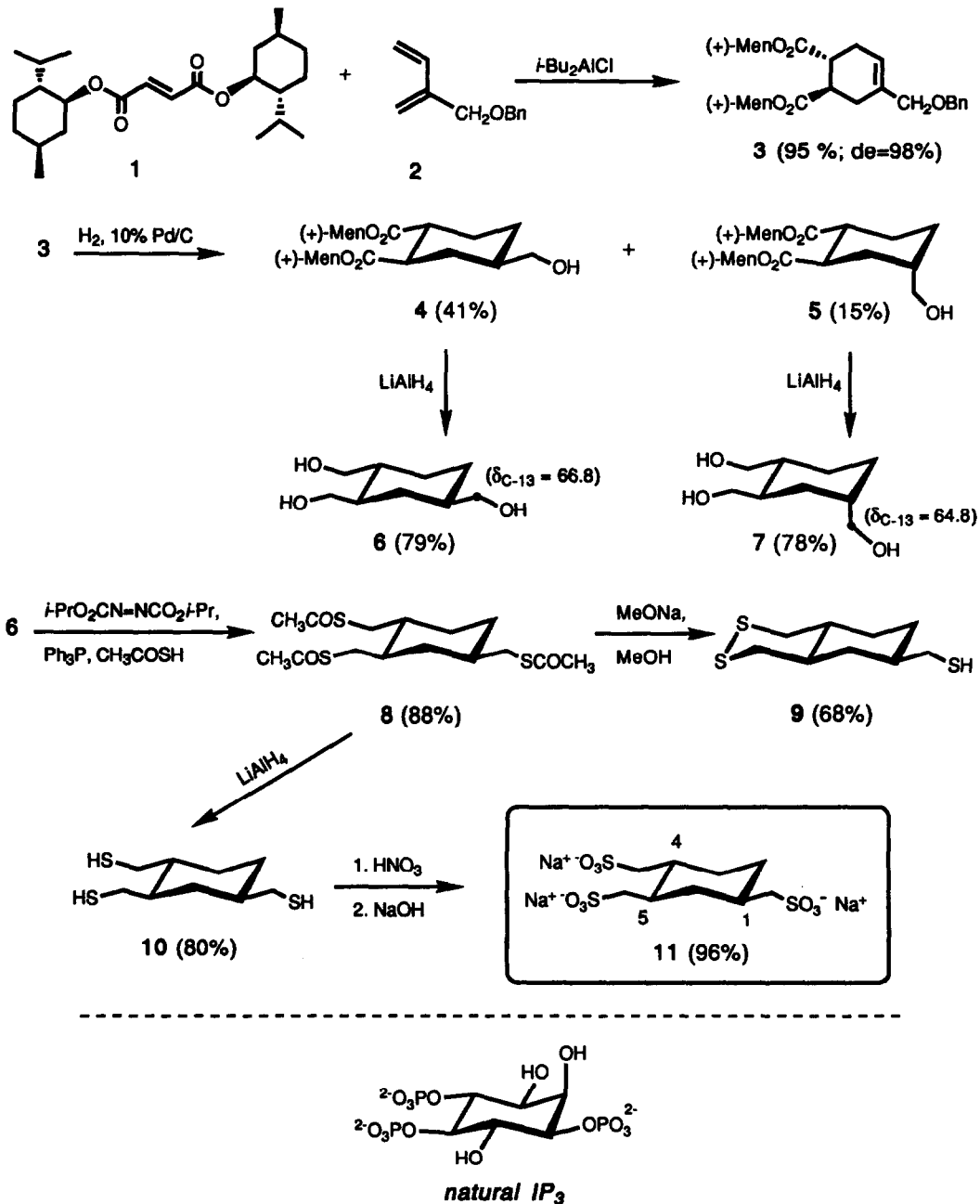
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Abstract: The synthesis of the (-)-sodium salt of the hexadeoxy-1,4,5-tris(methylene-sulfonic acid) analogue of IP₃ by use of an asymmetric Diels-Alder reaction is described together with the effects of this compound in binding to the IP₃ receptor.

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 Ca²⁺ mobilization.¹ Subsequently, Berridge was led to conclude that the water soluble product of PI(4,5)P₂ hydrolysis, namely IP(1,4,5)₃, was the likely intracellular candidate that released Ca²⁺ from intracellular stores and increased cytoplasmic free Ca²⁺ 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 Ca²⁺ together with the activation of PKC leads to transcriptional activation of genes responsible for a biological response.^{4,5} In an effort to identify IP₃-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 IP₃. Herein we report the synthesis of a molecule which represents a simplified version of IP₃, namely the hexadeoxy analogue of IP₃ bearing methylenesulfonic acid groups in place of the phosphate groups of the parent structure. Although the 6-hydroxy group of IP₃ does play a more prominent role in Ca²⁺ 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 IP₃ recognition site.

Synthesis of the required sulfur analogue began by employing the di-(+)-menthyl ester of fumaric acid⁹ 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 oxidation¹⁰ 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 IP₃.



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 ¹³C NMR chemical shift of the hydroxymethylene group at the C-1 position. 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 IP₃ 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, H₂O).

To examine the ability of **11** to function as a mimic of IP₃, we investigated its ability to displace [³H]IP₃ from rat cerebellar P₂ membrane fraction.¹⁶ As is apparent from the graph presented in Figure 1, **11** is a rather poor mimic of natural IP₃, and exhibits a K_i of > 17 μM compared to a K_i of 10 nM for natural IP₃. 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 IP₃ by methylene; and (c) replacement of phosphate by sulfonic acid.¹⁷ While the synthesis of additional IP₃ 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 IP₃ results in about a 100-fold loss both in binding affinity and ability to release Ca²⁺ from rat parotid microsomal fraction.⁷ Deletion of the 3-hydroxy group, however, has been shown to provide an IP₃ analogue of comparable Ca²⁺ releasing activity.⁸ Furthermore, a recent article has revealed that *myo*-inositol 1,4,5-trisulphate is biologically inactive.¹⁸

In summation, while the IP₃ 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.

Acknowledgements. We are indebted to the National Institute on Aging for their support of this work. We also wish to thank Dr. Roberto Pellicciari for valuable discussions.

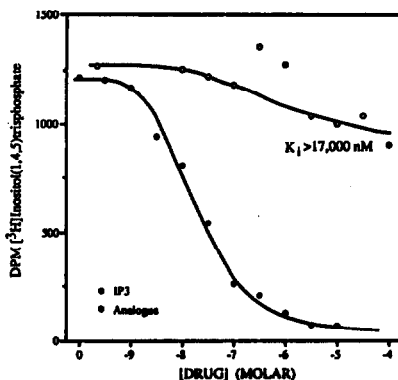


Figure 1. Competition binding of [^3H]IP $_3$ with 11. Each assay tube contained 12,000 dpm of [^3H]IP $_3$, 250 μg of rat cerebellar P $_2$ membrane fraction and 50 μL of IP $_3$ (0.06 to 2000 pmol) or 11 (0.6 to 10^6 pmol) in 100 mM Tris-HCl and 4 mM EDTA (pH = 7.8) in a final volume of 200 μL . Tubes were incubated for 30 min at 4 $^\circ\text{C}$. 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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13. Dalling, D. K.; Grant, D. M. *J. Am. Chem. Soc.* **1972**, *94*, 5318-5324. In addition, alcohols **4** and **5** were transformed to their corresponding aldehydes by Swern oxidation which proceeded without epimerization in each case. The ^1H NMR spectrum of the aldehyde derived from **5** revealed a signal for the proton at the aldehyde bearing carbon at $\delta = 2.52$. This signal appeared as a quintet with $J = 4.7$ Hz, thus reflecting its equatorial nature. No comparable signal was observed in the spectrum of the aldehyde derived from **4**, for this axially situated proton would be expected to resonate at higher field strengths and thus to overlap with other signals. The trans-diequatorial nature of the alkoxy carbonyl groups of **5** was also readily apparent from an examination of the appropriate ring protons centered at $\delta = 2.73$ (ABq, $J_{AB} = 9.7$ Hz, with both parts split into dd with $J = 9.2, 4.1$ and $9.2, 3.8$ Hz, respectively). The aldehydes derived from **4** and **5** were further shown to be equilibrated by DBU/ CD_3CN treatment to afford a 3:1 mixture in which the aldehyde derived originally from **4** predominated.
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(Received in USA 27 July 1992; accepted 8 October 1992)