Bioorganic & Medicinal Chemistry Letters, Vol.3, No.4, pp. 685-688, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

SYNTHESIS OF THE 4,5 DIPHOSPHONATE ANALOG OF D,L-*myo*-INOSITOL 4,5-DIPHOSPHATE

Joseph P. Lyssikatos and Mark D. Bednarski*

Department of Chemistry, University of California at Berkeley, Berkeley, California 94720, USA

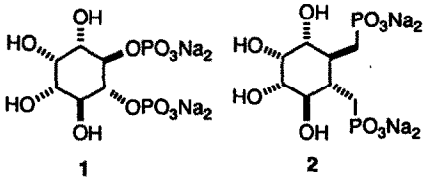
(*Received in USA* 8 *September* 1992)

Abstract

The first synthesis of the 4,5 diphosphonate analog (4,5 IPn_2; compound **2**) of D,L-4,5 *myo*-inositol 4,5 diphosphate **1** (4,5 IP_2) is described. Key steps in the synthesis include the introduction of the diphosphonate into dimesylate **5** using sodium diethyl phosphite in DME to give **6**. The 3,6 trans hydroxyl groups were introduced into **6** using the Bäckvall trans-diacetoxylation reaction. The use of the C_2 symmetry in the synthesis was utilized to introduce the final two stereocenters by osmylation of **14** to give compound **2** in an overall yield of 4.5% in only nine steps starting form readily available phthalic acid. Alternatively, the key intermediated **6** can be constructed by introducing the diphosphonate into compound **8** followed by conversion to **6**. This results in an overall yield of 7% for the synthesis of 4,5 IPn_2 (**2**) from butadiene and dimethyl fumarate. Compound **2** did not mobilize calcium in rat basophilic leukemia cells but is stable *in vivo* and can potentially be used for the production of antibodies.

The inositol phosphate cycle regulates secretion, contraction, T-lymphocyte activation, and a myriad of other biological pathways.[1-4] The key component of this cycle is the 1,4,5 inositol triphosphate (IP_3) which acts as a secondary messenger for Ca^{+2} mobilization. Derivatives of IP_3 such as 4,5 IP_2, compound **1**, also have been found to mobilize calcium in permeabilized Swiss-3T3 cells[5] and guinea pig hepatocytes.[6] Stable analogs of inositol phosphates and its derivatives can potentially act as a long-lived agonists or antagonists to study Ca^{+2} mobilization, and as materials that can be conjugated to carrier proteins for antibody production. Therefore, these molecules may have important applications in helping to decipher the IP_3 pathway. Current research in this area has led to the synthesis of various phosphothiolate,[7,8] sulfate,[9] and the 5-methylene phosphonate[10] analogs of IP_3. In this letter, we report the first synthesis of the 4,5 diphosphonate analog **2** (4,5 IPn_2) of D,L-*myo*-inositol 4,5-diphosphate **1** (4,5 IP_2).

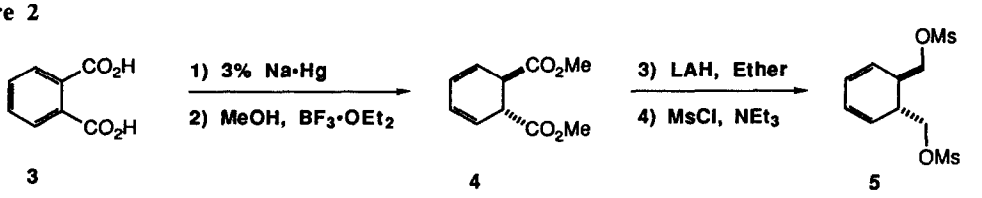
Figure 1



Our first approach to the synthesis of **2** starts with the reduction of phthalic acid, compound **3**, using 3% sodium amalgum in an aqueous solution buffered with sodium acetate-acetic acid to give the trans-1,2-dicarboxylate-3,5-cyclohexadiene[11] which was directly converted into its dimethyl ester **4** using boron trifluoride etherate (BF_3•OEt_2) in refluxing methanol.[12] The diester **3** was then reduced with lithium aluminum hydride

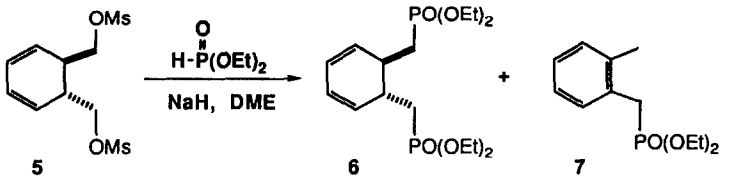
(LAH)[13] and the resulting diol converted to the dimesylate **5** using methanesulfonyl chloride (MsCl) and triethylamine (Et_3N) in an overall yield of 50% from phthalic acid.

Figure 2

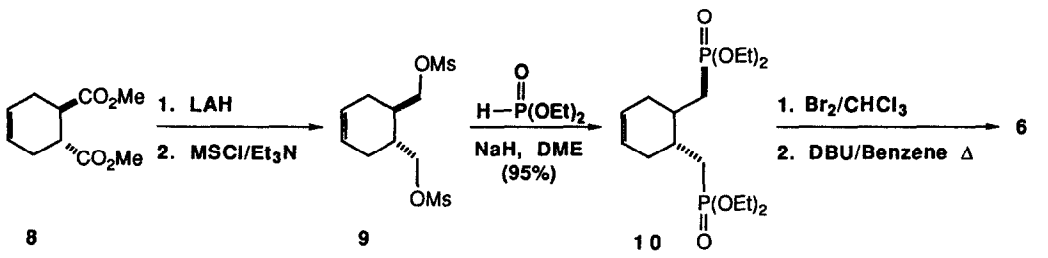


The diphosphonate moiety was introduced by the reaction of **4** with diethyl phosphite using sodium hydride as a base in refluxing DME to give a 48% yield of **6**.[16] The other product was the aromatic phosphonate **7**.

Figure 3

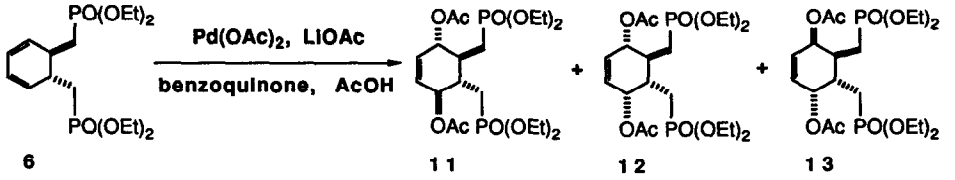


In order to avoid the formation of the major by-product **7**, the diphosphonate could be introduced instead into dimesylate **9** using the same conditions as those used in the conversion of compound **5** to **6** (sodium diethylphosphite/DME). Dimesylate **9** is readily available from compound **8** by reduction and mesylation. Compound **8** is the Diels-Alder adduct derived from dimethyl fumarate and butadiene using boron trifluoride-etherate as a catalyst. The absence of the diene prevents the elimination and aromatization during the phosphonate reaction that results in the formation of **7**. Compound **9** can then be converted into the key intermediate **6** by bromination and elimination under standard conditions ($Br_2/CHCl_3$; DBU).



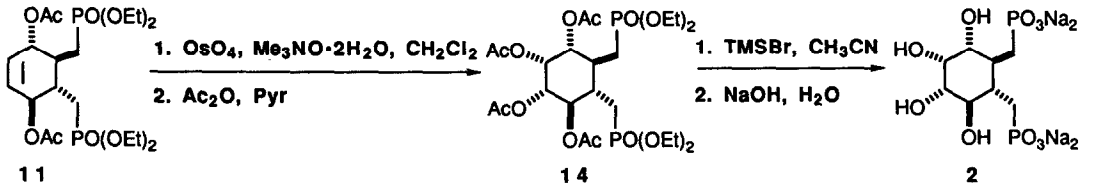
Compound **6** was then subjected to the Bäckvall transdiacetoxylation reaction[14] to give a 3:1:1 mixture of compounds **11**, **12** and **13** in an overall yield of 75%. The mixture of compounds **11-13** was not separable using silica gel chromatography and was, therefore, directly osmylated[15] and the resulting diols acetylated using

Figure 4



acetic anhyride in pyridine. At this point the major isomer (compound **14**) was isolated and purified.[16] Compound **14** was then deprotected using trimethylsilyl bromide (TMSBr) in acetonitrile to remove the ethyl groups[17] and then treated with an aqueous solution of sodium hydroxide to remove the acetates to give the target molecule **2**.

Figure 5



Compound **2** was tested for calcium mobilization using rat basophilic leukemia cells[18] and showed no activity even at concentrations as high as 5mM. DL-4,5-IP_2 was reported to have an Ec50 of 70μM in this cell line.[19] Compound **2** also was also not a competitive inhibitor for IP_3-mediated calcium release. We conclude, therefore, that disubstitution of phosphonate groups for the 4,5-phosphates greatly reduces the efficacy of inositol phosphate mediated calcium release. We plan to use compound **2** to test the hypothesis that antibodies toward **2** should still cross react with **1** despite the fact that **2** is not effective at Ca^{+2} mobilization.

Acknowledgement

This research was supported by the Director, Office of Energy Research, Office of Basic Sciences, Division of Materials Sciences, by the Division of Energy Biosciences of the U.S. Department of Energy (DE-AC03-76SF0098), and the National Institutes of Health Award #R29 GM43037-02. Mark Bednarski thanks the American Cancer Society for a Junior Faculty Award and Eli Lilly for the 1991 Eli Lilly Grantee Award. Joseph Lyssikatos thanks the National Institutes of Health for a predoctoral fellowship.

References and Notes

1. Sambrook, J. F. *Cell* **1990**, *61*, 197.
2. Berridge, M. *J. Ann. Rev. Biochem.* **1987**, *56*, 159.
3. Gardner, P. *Cell* **1989**, *59*, 15.

4. Inositol Phosphates and Derivatives: Synthesis, Biochemistry, and Therapeutic Potential; Reitz, A. B. (ed.), American Chemical Society Symposium Series 463, American Chemical Society, Washington, D.C., 1990, 235 p.
5. Irvine, R. F.; Brown, K. D.; Berridge, M. J. *Biochem. J.* **1984**, *221*, 269.
6. Burgess, G. M.; Irvine, R. F.; Berridge, M. J.; McKinney, J. S.; Putney, Jr. J. W. *Biochem. J.* **1984**, *224*, 741.
7. Nahorski, S. R.; Potter, B. V. L. *Trends Pharmacol. Sci.* **1989**, *10*, 139.
8. Cooke, A. M.; Gigg, R.; Potter, B. V. L. *J. Chem. Soc., Chem. Commun.* **1987**, 1525.
9. Westerduin, P.; Willems, H. A. M.; Van Boeckel, C. A. A. *Tetrahedron Lett.* **1990**, *47*, 6919.
10. Falck, J. R.; Abdali, A.; Wittenberger, S. J. *J. Chem. Soc., Chem. Commun.* **1990**, 953.
11. McDonald, R. N.; Reineke, C. E. *Org. Syn.* **1970**, *50*, 50.
12. Cerefice, S. A.; Fields, E. K. *J. Org. Chem.* **1979**, *39*, 971.
13. Askani, R.; Scheider, W. *Chem. Ber.* **1983**, *116*, 2355.
14. Bäckvall, J. E.; Nordberg, R. F. *J. Am. Chem. Soc.* **1981**, *103*, 4959.
15. Poli, G. *Tetrahedron Lett.* **1989**, *30*, 7385.
16. All compounds gave spectra that are consistant with their assigned structures. The spectral data for compounds **2**, **6** and **11** are given as follows: Compound **2**: ^{1}H NMR (500 MHz, D_2O) δ 3.78 (appt. J=2.6 Hz, 1H), 3.42 (appt, J=10Hz, 1H) 3.33 (dd, J=2.6, 10.0 Hz, 2H), 1.56 → 1.72 (m, 3H), 1.23 → 1.35 (m, 3H) ^{31}P NMR d 18.38, 18.29. FAB H^+ 337. Compound **6**: IR (neat) 3498, 3041, 2982, 2931, 2906, 1751, 1721, 1652; ^{1}H NMR (400MHz, $CDCl_3$) δ 5.84-5.92 (m, 4H), 4.01-4.18 (m, 8H), 2.55-2.62 (m, 2H), 1.73-1.96 (m, 4H), 1.33 (t, J=7.0 Hz, 6H); ^{13}C NMR (100MHz, $CDCl_3$) δ 128.17, 128.13, 61.52, 61.45, 61.41, 33.49, 33.38, 29.60, 28.22, 16.45, 16.38; HRMS (MH^+) calcd for $C_{16}H_{31}O_6P_2$ 381.1596 found 381.1590. Compound **11**: ^{1}H NMR (500MHz, $CDCl_3$) δ 5.56 (appt, J=2.6 Hz, 1H), 5.36 (appt, J=10.4 Hz, 1H), 4.98 (dd, J=2.8, 10.0 Hz, 1H), 4.92 (dd, J=2.5 Hz, 11.6 Hz, 1H), 4.03-4.19 (m, 8H), 2.55-2.67 (m, 1H), 2.00-2.40 (m, 5H), 2.15 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 1.30-1.38 (m, 12H); ^{13}C NMR (125MHz, $CDCl_3$) δ 170.15, 169.91, 169.70, 169.60, 71.73, 71.69, 71.60, 71.57, 71.01, 69.21, 61.77, 61.70, 61.68, 61.64, 61.58, 37.13, 37.09, 34.49, 34.35, 24.92, 24.48, 23.52, 23.09, 20.81, 20.76, 20.63, 20.53, 16.46, 16.38, 16.30, 16.24; HRMS (MH^+) calcd for $C_{24}H_{43}O_{14}P_2$ 617.2128 found 617.2127. Anal. calcd. for $C_{24}H_{42}O_{14}P_2 \cdot H_2O$: C, 45.43; H, 6.99; Found: C, 45.48; H, 6.82.
17. McKenna, C. E.; Schmidhauser, J. *J. Chem. Soc. Chem. Commun.* **1979**, 739.
18. We would like to thank Lubert Stryer and Alan Kindman for assaying compound **2**.
19. Tegge, W.; Denis, G. V.; Ballou, C. E. *Carbohydr. Res.* **1991**, *217*, 107.