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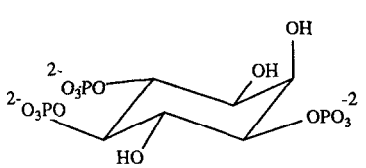
SYNTHESIS OF TETHERED PHYTIC ACID

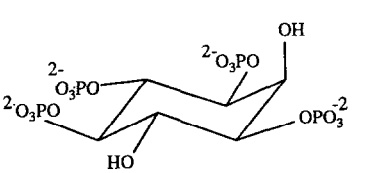
James F. Marecek and Glenn D. Prestwich*
Department of Chemistry
State University of New York
Stony Brook, New York 11794-3400

Summary: The synthesis of 2-*O*-(3-aminopropyl) and 2-*O*-(6-aminohexyl)inositol hexakisphosphate provides two tetherable probes for isolation and characterization of IP_6 and IP_5 receptors.

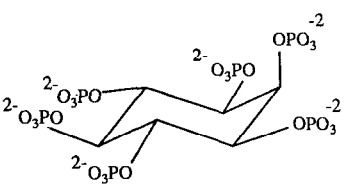
Since the discovery that D-*myo*-inositol 1,4,5-trisphosphate (IP_3 or Ins(1,4,5)P_3), one of the products of a specific phospholipase C-catalyzed cleavage of phosphatidylinositol 4,5-bisphosphate, acts as a second messenger[1], considerable effort has been expended on biological studies of this molecule and its metabolites.[2] To facilitate these studies, numerous syntheses have been reported for the preparation of these species.[3] The most abundant inositol polyphosphate, Ins(1,2,3,4,5,6)P_6 (phytic acid, IP_6), is found in large quantities in the seeds of many plants and in small amounts in most mammalian cells; however, much less is known about its origin and function. It has been postulated that it serves as a phosphorus and/or inositol reserve in plants, but this does not explain its widespread occurrence in animal cells. One suggestion is that it may have an extracellular function as a neurotransmitter.[4] High specific activity binding sites for IP_6 have been identified in cerebellar membranes and in the anterior pituitary.[5]



D-*myo*-Ins(1,4,5)P_3



D-*myo*-Ins(1,3,4,5)P_4

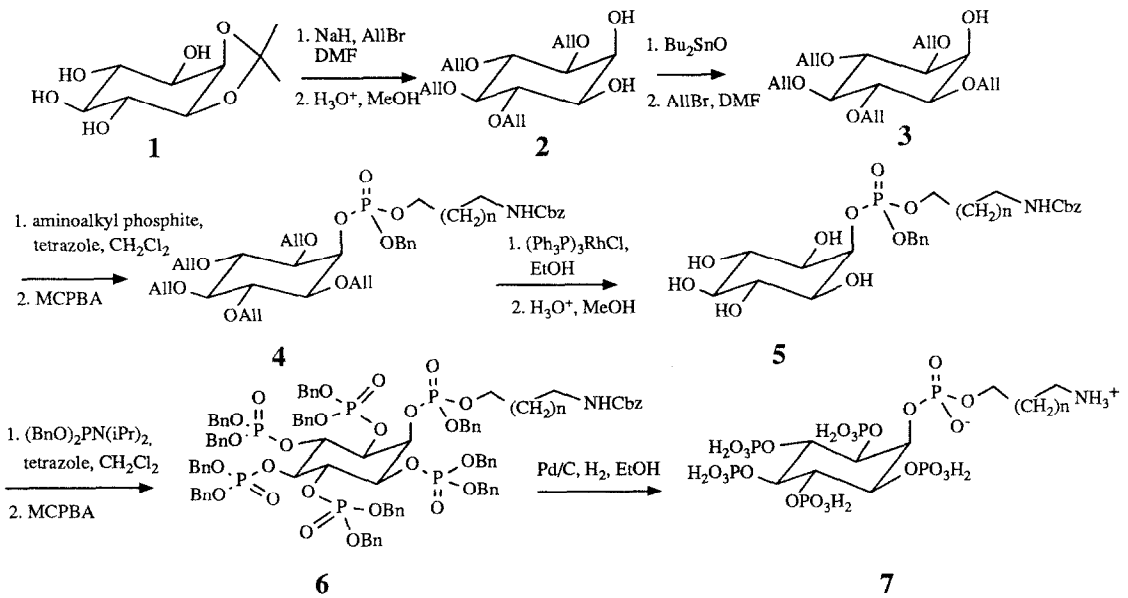


D-*myo*-Ins(1,2,3,4,5,6)P_6

We recently reported the preparation of P-1 tethered ω-aminoalkyl phosphodiester derivatives and their use in purification and affinity labeling of IP_3 and IP_4 receptors.[6] We now extend this work to include a convenient synthesis of tetherable IP_6 derivatives for use as affinity probes for the IP_6 receptors.

The common intermediate for these syntheses is 1,3,4,5,6-penta-*O*-allyl-*myo*-inositol (**3**), which was prepared from 1,2-*O*-isopropylidene *myo*-inositol (**1**) as shown in Scheme 1. Thus, allylation of ketal **1** followed by acidic hydrolysis of the ketal protecting group afforded 1,4,5,6-tetra-*O*-allyl-*myo*-inositol (**2**). The stannylene derivative of diol **2** was formed and allylated in the equatorial position[7] to give the pentaallyl inositol **3** in 68% overall yield from **1**.[8]

* Address correspondence to this author.



Scheme I. Synthesis of 2-*O*-(ω-aminoalkyl) phosphates of IP_6 (**a**, n = 1; **b**, n = 4).

Attempts to phosphorylate the hindered 2-hydroxyl group of **3** by standard phosphorylating procedures (e.g., mesitylene sulfonyl tetrazole[9], mesitylene sulfonyl 3-nitro-1,2,4-triazole[10], alkyl cyclic enediol phosphates[11]) were unsuccessful. Therefore, we turned to phosphite chemistry[12] as shown. A recent report on the use of bis(allyloxy)(di-*iso*-propylamino)phosphine as a phosphitylating reagent[13] suggested that oxidation of the intermediate phosphite to the corresponding phosphate should be possible without affecting the five allyl groups. Thus, *N*-Cbz-protected aminoalkyl benzyl (*N,N*-diisopropylamino) phosphites were prepared from benzyloxy (*N,N*-diisopropylamino)chlorophosphine and either *N*-Cbz-3-aminopropanol or *N*-Cbz-6-aminohexanol.[6a] These reagents were used to phosphitylate the pentaallyl intermediate **3**. Clean oxidation of the intermediate phosphite to the phosphate was accomplished in the presence of the five allyl groups by using a slight excess of *m*-chloroperbenzoic acid at -40 °C (3 min) and then at 0 °C (12 min); remaining oxidizing reagent was destroyed by addition of aqueous sodium sulfite to the cold solution. The benzyl (1,3,4,5,6-penta-*O*-allyl-*myo*-inosityl) 2-*O*-(*N*-Cbz-ω-aminoalkyl)phosphates **4a** (n = 1) and **4b** (n = 4) were obtained in 70% yield.[14]

The allyl groups were removed by a two-step procedure. Isomerization to the 1-propenyl ethers using Wilkinson's catalyst in refluxing ethanol[15], rapid silica chromatography, and then a brief treatment with warm acidic methanol provided the desired pentahydroxy 2-*O*-aminoalkyl phosphates **5a** and **5b**. Since the phosphotriester group can readily migrate to the 1-hydroxyl (same as the 3 position in this *meso* compound) once the protecting groups are removed[16], the solvent was quickly removed at low temperature and the crude pentaols **5** were immediately phosphitylated with dibenzyl

(*N,N*-diisopropylamino)phosphine.[17] The five phosphite groups were oxidized to phosphates (*m*-CPBA), and the perbenzyl hexaphosphate **6** was obtained in 57% yield from pentaallyl intermediate **4** after silica gel chromatography.

If the hexaphosphate were to exist in the simple chair form with an axial 2-hydroxyl group and five equatorial hydroxyl groups, the ^{31}P NMR should show only four peaks in a 1 : 2 : 2 : 1 ratio, since the molecule contains a *meso* plane. However, the ^{31}P-NMR spectrum of the perbenzyl hexaphosphate **6b** showed six peaks of equal intensity[18]; apparently, the cyclohexyl ring is twisted as a result of severe steric crowding. In fact, IP_6 itself has been shown to have the expected 2-axial phosphoryl group and five equatorial phosphoryl groups only at low pH when in the completely protonated form. At high pH, with the phosphates fully ionized, the 2-phosphoryl becomes equatorial and the remaining five phosphoryl groups adopt axial conformations as a result of charge-charge repulsion.[19]

The twelve benzyl protecting groups were simultaneously removed by catalytic hydrogenolysis to give the aminoalkyl hexaphosphates **7a** and **7b**. The tethered phytic acids were converted to their ammonium salts before further workup, and were then converted to the sodium salt with concomitant removal of divalent metal ions by passage through a Chelex 100 column. Since the hexaphosphate can complex strongly to polyvalent metal ions[20], the Chelex treatment to remove paramagnetic impurities is required to obtain sharp NMR signals. The ^{31}P-NMR (D_2O at pH 9.02) showed the expected four peaks in a 1 : 2 : 1 : 2 ratio[21], suggesting that the deprotected polyphosphate adopts the normal chair conformation with a *meso* plane. The 2-*O*-(ω-aminoalkyl)IP_6 derivatives **7a** and **7b** were allowed to react with an *N*-hydroxysuccinimide (NHS)-activated resin (Affigel-10) in sodium bicarbonate buffer at pH 8.5 to give the affinity matrix and with the NHS ester of 4-azidosalicylic acid to produce a photoaffinity label. The biological data on these compounds will be reported elsewhere.

Acknowledgements

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8. Compound **3**: ^{1}H-NMR ($CDCl_3$) δ 3.12 (dd, 2H, *J* = 2.8, 9.7 Hz), 3.60 (t, 2H, *J* = 9.5 Hz), 4.0-4.4 (m, 12H), 5.0-5.3 (m, 10H), 5.8-6.0 (m, 5H); ^{13}C-NMR ($CDCl_3$) δ 67.86, 71.52, 74.14, 74.20, 79.01, 80.35, 82.39, 116.05, 116.77, 134.56, 135.17.

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14. Compound **4b**: ^{31}P-NMR ($CDCl_3$) δ -2.6; ^{1}H-NMR ($CDCl_3$) δ 1.2-1.7 (m, 8H), 3.05-3.2 (m, 4H), 3.53 (td, 2H, *J* = 2.4, 9.5 Hz), 3.9-4.4 (m, 11H), 5.03 (s, 2H), 5.03-5.30 (m, 15H), 5.75-6.0 (m, 5H), 7.22-7.32 (m, 10H); ^{13}C-NMR ($CDCl_3$) δ 24.77, 25.88, 29.51, 29.71 (d, *J* = 7.5 Hz), 40.56, 66.06, 67.41 (d, *J* = 6.0 Hz), 68.51 (d, *J* = 5.2 Hz), 70.92, 71.02, 73.72 (d, *J* = 6.1 Hz), 74.14, 74.39, 77.86, 80.11 (d, *J* = 5.8 Hz), 82.11, 116.17, 116.36, 116.68, 116.85, 127.17, 127.65, 127.70, 127.74, 128.04, 128.11, 134.22, 134.30, 134.97, 135.10, 136.14 (d, *J* = 8 Hz), 136.50, 156.12; MS (FAB) *m/z* 784 (M+1).

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18. Compound **6b**: ^{31}P-NMR ($CDCl_3$) δ -3.56, -2.97, -2.73, -2.16, -2.12, -2.07; MS (FAB) *m/z* 1884 $(M+1)^+$.

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21. Compound **7b**: ^{31}P-NMR (D_2O, pH 9.02) δ 0.43, 2.72, 2.93, 4.66; ^{13}C-NMR δ 24.09, 24.66, 26.38, 29.00 (d, *J* = 5.3 Hz), 39.21, 66.49 (d, *J* = 3.8 Hz), 72.52 (broad), 75.52 (very broad), 77.66 (very broad); ^{1}H-NMR δ 1.23-1.44 (m, 4H), 1.44-1.70 (m, 4H), 2.87 (t, 2H), 3.82-4.15 (m, 6H), 4.20-4.45 (m, 2H).

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