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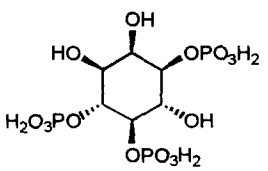
SYNTHESES OF *MYO*-INOSITOL-1,2,3,5- AND -2,4,5,6-TETRAKISPHOSPHATES, UNUSUAL INHIBITORS OF *MYO*-INOSITOL-1,4,5-TRISPHOSPHATE 3-KINASE

Sung-Kee Chung* and Young-Tae Chang

Department of Chemistry, Pohang University of Science & Technology, Pohang 790-784, Korea.

Abstract: D-*myo*-Inositol 1,4,5-trisphosphate [D-I(1,4,5)P_3], a calcium mobilizing second messenger, is converted to D-I(1,3,4,5)P_4 by D-I(1,4,5)P_3-3-kinase. Efficient syntheses of I(1,2,3,5)P_4 (**2**) and I(2,4,5,6)P_4 (**3**), novel 3-kinase inhibitors, are reported.© 1997 Elsevier Science Ltd.

Since the discovery that D-*myo*-inositol 1,4,5-trisphosphate [I(1,4,5)P_3, **1**] plays a pivotal role as a second messenger in the transmembrane signaling, thus mobilizing calcium ions from the intracellular storage, its interactions with the I(1,4,5)P_3 receptor and metabolic enzymes have been widely studied.[1] One of the major metabolic pathways involves a specific phosphorylation of I(1,4,5)P_3 to I(1,3,4,5)P_4, by I(1,4,5)P_3-3-kinase [IP3K].[2] It has been suggested that I(1,3,4,5)P_4 also acts as a second messenger mediating the entry of extracellular Ca^{2+} through plasma membrane ion channel,[3] although the detailed mechanistic understanding has not yet been achieved. The other major metabolic pathway involves Ins(1,4,5)P_3 5-phosphatase to yield Ins(1,4)P_2.[1] Thus, IP3K not only occupies a central position in regulating the availability of the two Ca^{2+} mobilizing second messangers but also provides an important branching point in the diverse pathways of the inositol polyphosphate metabolism. In the preceding paper,[4] we have reported the inhibitory activities of all possible regioisomers of IP_n on IP3K and proposed an active site model for the enzyme on the basis of the binding affinity data. However, we noted that Ins(1,2,3,5)P_4 (**2**) and Ins(2,4,5,6)P_4 (**3**) did show substantial inhibitory effects, although their structures do not contain the essential 1,4,5-trisphosphate motif of D-Ins(1,4,5)P_3. In order to help understand the structural characteristics of these substances in inhibiting IP3K, we sought efficient synthetic routes to these compounds. We report herein practical syntheses of compounds **2** and **3**.



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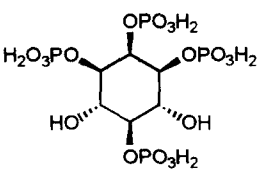

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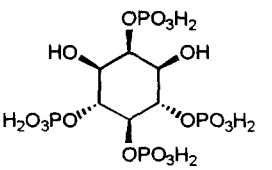

3

One of the key issues in the synthesis of inositol phosphates is to prepare suitable, selectively protected inositol intermediates. Inositol orthoformate, **4**, which was proven to be a useful intermediate for the synthesis of various inositol phosphates by us and others,[5] was selected as the key intermediates for the synthesis of **2** and **3**. The acetylation of compound **4**, prepared from *myo*-inositol,[6] under the usual conditions employing AcCl in pyridine showed an initial acetylation of the 2-OH selectively. A preferential alkylation at 4- and 6-OH of **4** was previously reported under the conditions employing an alkyl halide and a metal hydride base.[5c] Although the enzyme assisted selective acetylation[7] and TBDMS silylation[5d] of **4** at 2-OH are known, this is the first selective acetylation of 2-OH in **4** by a chemical method. Thus, successive treatments of **4** in pyridine with AcCl (1.6 eq., 1h) and then excess BzCl gave **5** as the major product together with a small amount of 2,4-diacetylated product (in ca. 3:1 ratio based on ^{1}H-NMR). A simple extractive work-up of the reation mixture was followed by an acid catalyzed hydrolysis to remove the acetyl and orthoester protecting groups. Pure 4,6-dibenzoated inositol, **6** was obtained by simple extraction in 56% yield over 3 steps from **4** and the by-product I(4)Bz was present exclusively in the water layer. Compound **6** was phosphorylated by successive treatments with diethyl chlorophosphite and diisopropylethylamine in DMF, and then 30% H_2O_2 to afford compound **7**[8] in 85% yield. In the final step, the protecting groups of **7** were removed by successive reactions with TMSBr and then LiOH. The target compound **2**[9] was obtained after ion exchange chromatography on Dowex 50x8-100 (H^+ form), pH adjustment to 10 with KOH, and lyophilization (Scheme 1).

I(2,4,5,6)P_4, **3** was also synthesized conveniently from the intermediate **4** (Scheme 2). An exhaustive benzylation of **4** using excess amounts of BnBr and NaH was followed by an acid-catalyzed hydroylsis to obtain **8** in 94%. A selective benzoylation of **8** afforded the 1,3-dibenzoated product, **9** in 64% yield. The enhanced nucleophilic reactivity of 1- and 3-OHs toward BzCl might be related to the through-space α-effect caused by the *cis*-related 2-oxygen,[10] although the exact origin of this selectivity is not clear. Removal of the benzyl protecting groups in **9** by hydrogenolysis gave I(1,3)Bz_2, **10**. Compound **10** was phosphorylated to give **11**[11], and the protecting groups of **11** were removed to give I(2,4,5,6)P_4, **3**[12] in good yield by the same procedures as described for I(1,2,3,5)P_4.

In conclusion, we successively prepared two novel IP3K inhibitors **2** and **3** in gram scales using inositol orthoformate as the key intermediate. These two routes represent the only synthetic pathways reported for I(1,2,3,5)P_4 and I(2,4,5,6)P_4 except the divergent total synthesis of all regioisomers of IP_4.[13]

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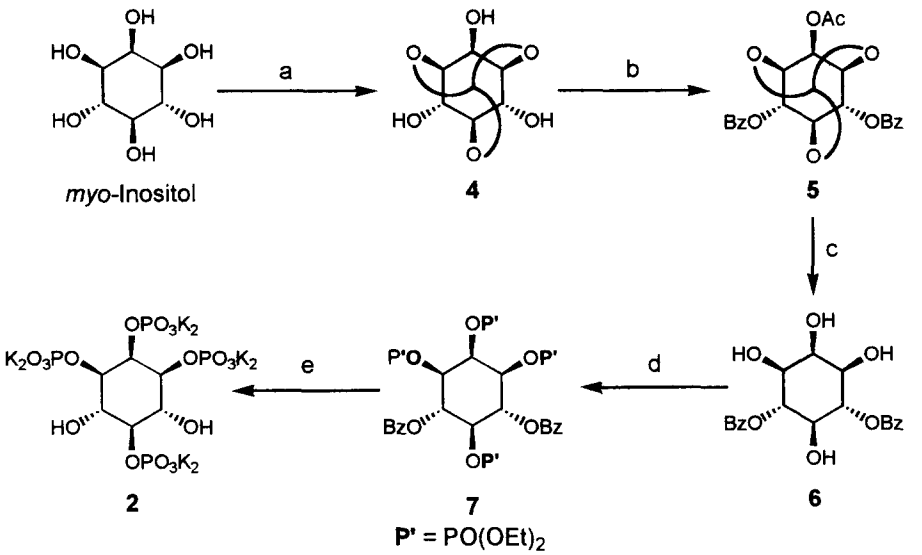
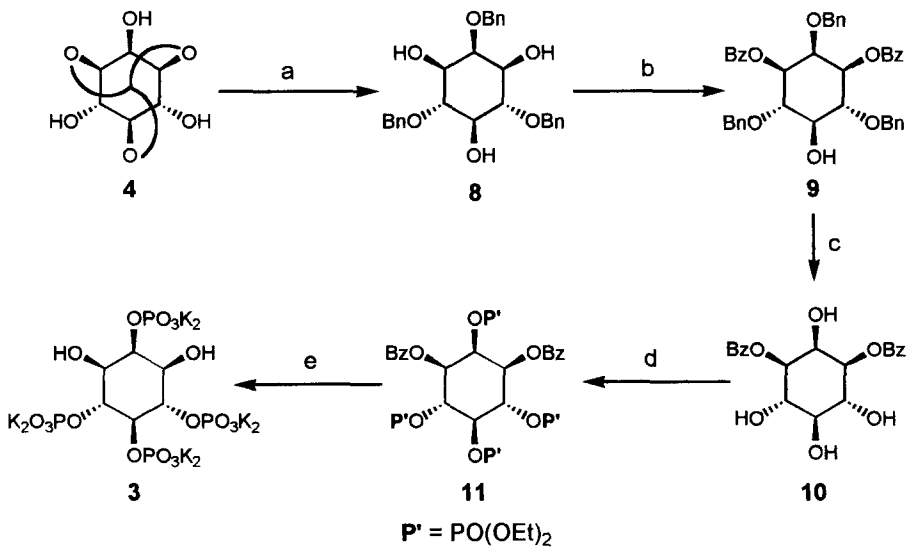


Scheme 1. a. $(EtO)_3CH$, pTSA, DMF, 89%. b.(i) AcCl (1.6 eq.), pyridine. (ii) BzCl (4 eq.). c. HCl-MeOH, 56% from **4**. d.(i) $(EtO)_2P$-Cl, iPr_2NEt, DMF. (ii) H_2O_2, 85%. e.(i) TMSBr, CH_2Cl_2. (ii) LiOH, Δ.(iii) H^+ ion-exchange. (iv) KOH, pH 10, quant.



Scheme 2. a.(i) BnBr, NaH. (ii) HCl-MeOH, 94%. b. BzCl (2.5 eq), pyridine, 67%. c. H_2 (1 atm)-$Pd(OH)_2$, quant. d.(i) $(EtO)_2P$-Cl, iPr_2NEt, DMF. (ii) H_2O_2, 82%. e.(i) TMSBr, CH_2Cl_2. (ii) LiOH, Δ.(iii) H^+ ion-exchange. (iv) KOH, pH 10, quant.

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8. **7**: mp 134-137 °C; ^{1}H-NMR ($CDCl_3$) δ 0.74-1.43 (m, 24H, $8CH_2C\mathbf{H}_3$), 3.49-4.29 (m, 16H, $8C\mathbf{H}_2CH_3$), 4.72 (app. tt, J = 1.9, 10.1 Hz, 2H, H-1 & H-3), 4.91 (app. q, J = 9.4 Hz, 1H, H-5), 5.27 (dt, J = 2.3, 9.1 Hz, 1H, H-2), 5.92 (app. t, J = 10.0 Hz, 2H, H-4 & H-6), 7.42-8.18 (m, 10H, 2Ph); ^{13}C-NMR ($CDCl_3$) δ 15.25-16.04 ($8CH_2\mathbf{C}H_3$), 63.82-64.50 ($8\mathbf{C}H_2CH_3$), [70.50(2C), 73.39(2C), 75.46, 76.57, inositol ring carbon], 128.36-133.29 (2Ph), 165.42 (2C, 2Ph**C**O); ^{31}P-NMR ($CDCl_3$) δ -1.97, -0.78, -0.59 (2P).
9. **2**: ^{1}H-NMR (D_2O, pH 10) δ 3.76 (q, J = 7.6 Hz, 1H, H-5), 3.81-3.90 (m, 4H, H-1, H-3, H-4, H-6), 4.52 (br d, J = 8.8 Hz, 1H, H-2); ^{13}C-NMR (D_2O, pH 10) δ 74.71 (2C), 76.56 (2C), 78.32, 81.69; ^{31}P-NMR (D_2O, pH 10) δ 3.41, 5.03, 5.75 (2P).
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11. **11**: Oil. ^{1}H-NMR ($CDCl_3$) δ 0.84-1.41 (m, 24H, $8CH_2C\mathbf{H}_3$), 3.64-4.31 (m, 16H, $8C\mathbf{H}_2CH_3$), 4.66 (app. q, J = 10.0 Hz, 1H, H-5), 5.09 (app. q, J = 9.3 Hz, 2H, H-4 & H-6), 5.24 (br d, J = 9.3 Hz, 1H, H-2), 5.34 (br d, J = 10.0 Hz, 2H, H-1 & H-3), 7.40-7.57 (m, 10H, 2Ph); ^{13}C-NMR ($CDCl_3$) δ 16.06-16.79 ($8CH_2\mathbf{C}H_3$), 64.54-65.19 ($8\mathbf{C}H_2CH_3$), [70.84 (2C), 74.17, 74.24, 75.87 (2C), inositol ring carbon], 128.93-134.10 (2Ph), 166.15 (2C, 2Ph**C**O); ^{31}P-NMR ($CDCl_3$) δ -1.36 (2P), -1.18, -0.65.
12. **3**: ^{1}H-NMR (D_2O, pH 10) δ 4.47 (m, 3H, H-4, H-5, H-6), 4.59 (br d, J = 10.6 Hz, H-2), 4.70 (br d, J = 11.9 Hz, 2H, H-1, H-3); ^{13}C-NMR (D_2O, pH 10) δ 70.26, 75.01 (3C), 76.69 (2C); ^{31}P-NMR (D_2O, pH 10) δ 3.82, 5.02 (3P).
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