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Synthesis of All Possible Regioisomers of *myo*-Inositol Pentakisphosphate

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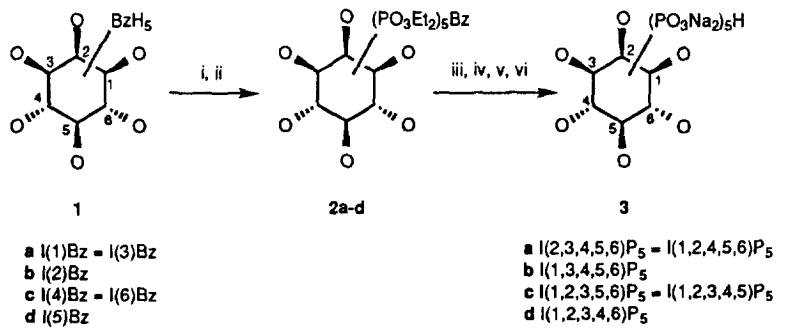
Abstract: Synthesis of all possible 4 regioisomers of IP_5s, naturally occurring metabolites of IP_3 and IP_4, was accomplished from *myo*-inositol *via* its monobenzoate derivatives (IBz_1) as the key intermediates; base-catalyzed isomerization of readily available IBz_1 derivatives, followed by suitable separation procedures efficiently provided the requisite regioisomers of IBz_1.

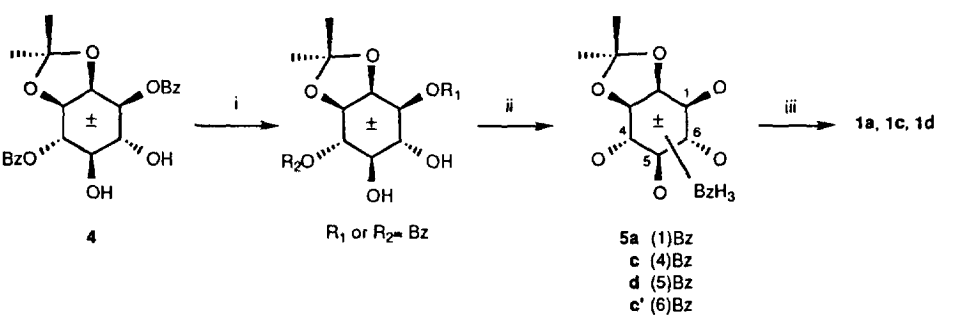
Since the discovery that D-*myo*-inositol-1,4,5-trisphosphate[I(1,4,5)P_3] plays a pivotal role as a second messenger in the transmembrane signalling, thus mobilizing calcium ions from the intracellular storage, its interaction with I(1,4,5)P_3 receptors and the metabolism of IP_3 were widely studied.[1] 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, and it has been suggested that I(1,3,4,5)P_4 also acts as a second messenger mediating the entry of extracelluar Ca^{2+} through plasma membrane ion channel.[2] Although IP_5s have been recognized for some time as naturally occurring metabolites of IP_3 and IP_4, studies on their metabolism and possible functional roles are currently in progress. For example, I(1,3,4,5,6)P_5 was shown to have a potent inhibitory activity toward I(1,3,4,5)P_4 3-phosphatase, which is a key enzyme in the regeneration of the second messenger I(1,4,5)P_3 from I(1,3,4,5)P_4.[3] I(1,3,4,5,6)P_5 was also found to inhibit the I(1,3,4,5)P_4 binding to the purified putative I(1,3,4,5)P_4 binding protein with an IC_{50} close to that of I(1,3,4,5)P_4 itself.[4] There exist four possible IP_5 regioisomers: two meso compounds, and two pairs of enantiomers. Surprisingly, efficient synthetic procedures for all IP_5s have not yet been worked out. The syntheses of I(1,2,4,5,6)P_5 and I(1,2,3,4,5)P_5 based on the old phosphorylation method were reported by Angyal and Russel.[5] More recently, I(1,3,4,5,6)P_5 was prepared by Ozaki, et al.[6] Systematic understanding on the relationship between the structure of IP_5 and the biolgical function of the metabolic enzymes would be greatly facilitated by the ready availability of all IP_5 regioisomers. Here we report the total synthesis of all possible 4 regioisomers of IP_5s using inositol monobenzoates (IBz_1) as the key intermediates.

One of the key problems in the synthesis of inositol phosphates is to prepare suitable, selectively protected inositol intermediates. We have previously reported synthesis of all possible regioisomers of IP_4 and IP_3 through IBz_2 and IBz_3,[7] which were obtained by the benzoyl group migration among the vicinal hydroxyl groups of the *myo*-inositol structure.[8] Based on the same synthetic strategy, now the benzoyl migration technique was applied to generate the 4 regioisomers of *myo*-inositol monobenzoate (IBz_1), **1**, which were expected to be phosphorylated to provide the 4 regioisomers of the target IP_5 structure, **2** (Scheme 1).



Scheme 1. i) diethyl chlorophosphite(30 eq.), diisopropylethylamine, DMF, - 42 °C ⟶ 25 °C, 3day; ii) hydrogen peroxide(30 %), sodium phosphate buffer(1.0 M, pH 7), 0 °C (40-60 % overall yield from **1**); iii) bromotrimethylsilane, dichloromethane, 25 °C, 3 day; iv) 1M LiOH, 80 °C, 3h; v) Dowex 50x8-100(H^+). Benzoic acid produced was extracted out with dichloromethane; vi) pH adjusted to 10 (80-99 % overall yield from **2**).

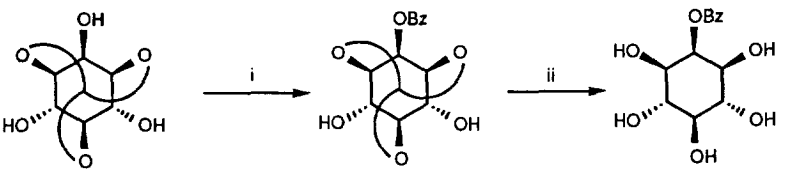
Thus, compound **4**, prepared from *myo*-inositol,[7] was partially hydrolyzed with NaOMe in MeOH-acetone to give a mixture of the monobenzoate derivatives (**5a** and **5c**). Without separation, this mixture was subjected to the migration conditions (60 % aqueous pyridine, 100 °C, 1h) and then each regioisomer (**5a**, **5c**, **5d**, **5c'**) was easily separated by silica gel column chromatography (eluted with ethyl acetate-hexane gradients).[9] The hydrolysis of the monoacetal protecting group of **5** in 80 % aqueous acetic acid at reflux gave three (**1a**, **1c**, **1d**) of four IBz_1 regioisomers (Scheme 2).



Scheme 2. i) NaOMe, MeOH-acetone(1:10), 15 min, IBz_1 mixture(72%), S.M.(12%), tetraol(15%); ii) pyridine-water(6:4), 100 °C, 1h (**5a** 26%, **5c** 23%, **5d** 31%, **5c'** 20%), followed by silica-gel column chromatography; iii) 80% aq. AcOH, reflux, 30 min, 100%.

The remaining regioisomer (**1b**) of IBz_1 could not be obtained this way and had to be independently prepared from *myo*-inositol orthoformate **6** (Scheme 3). A selective monobenzoylation at the 2-OH group of

compound **6**, derived from *myo*-inositol,[10] was effected under the usual conditions employing BzCl in pyridine to give **7**,[6] while a preferential alkylation at 4- or 6-OH has been reported under the conditions using an alkyl halide and a metal hydride base.[11] Acid-catalysed hydrolysis of **7** gave **1b** in quantitative yield. Each of the 4 regioisomers of IBz_1 thus obtained was fully characterised by ^1H, ^{13}C NMR including H-H COSY, and mass (FAB) spectrometry.[12]



6 **7** **1b**

Scheme 3. i) BzCl(1 eq.), pyridine, r.t., 60%; ii) HCl, MeOH, r.t., 100%

Each IBz_1 isomer was separately phosphorylated by successive treatments with diethyl chlorophosphite and *N,N*-diisopropylethylamine in DMF, and then 30 % hydrogen peroxide to yield all 4 regioisomers of compound **2** (Scheme 1), which were thoroughly characterized by ^1H, ^{13}C and ^{31}P NMR.[13] However, it is to be noted that the phosphorylation yields (40-60 %) were generally not as good as in the cases for IP_3 and IP_4 (80-99 %)[7] even with a large excess amount of phophorylating agent and a longer reaction time (3 days), perhaps due to the unfavorable steric hindrances between the vicinal phosphate groups. Therefore, each protected IP_5 isomer **2** had to be purified by column chromatography. In the final steps, the protecting groups of **2** were removed by successive reactions with trimethylsilyl bromide and then LiOH. Cleavage of the ethyl phosphate esters was monitored by ^{31}P-NMR, which clearly showed upfield chemical shift changes of 10-20 ppm upon the conversion of the ethyl ester to the silyl ester.[14] Each regioisomers of the product (**3**) was obtained after ion exchange chromatography on Dowex 50x8-100 (H^+ form), pH adjustment to 10 with NaOH, and lyophilization.[15] Biological studies on the IP_5 isomers are currently in progress.

It is to be stressed that the group migration method in conjuction with some efficient separational techniques as delineated here as well as in the previous reports[7] has proven to be a very useful and general synthetic strategy to generate a diverse molecular array of the inositol and carbohydrate isomers, which would be necessary for the determination of structural specificities in their reactions with biological macromolecules such as receptors, enzymes and antibodies. The syntheses of the optically active versions of IP_n isomers by the group migration method are also in progress.

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REFERENCES AND NOTES

1. a) Shears, S. B. *Biochem. J.* **1989**, *260*, 313-324; b) Berridge, M. J. *Nature*(London) **1993**, *361*, 315-325.
2. Irvine, R. F.; Cullen, P. J. *Current Biology* **1993**, *3*, 540-543.
3. Hughes, P. J.; Shears, S. B. *J. Biol. Chem.* **1990**, *265*, 9869-9875.
4. Donie, F.; Reiser, G. *Biochem. J.*, **1991**, *275*, 453-457.
5. Angyal, S. J.; Russell, A. F. *Aust. J. Chem.* **1968**, *21*, 391-404.
6. Ozaki, S.; Koga, Y.; Ling, L.; Watanabe, Y.; Kimura, Y.; Hirata, M. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1058-1063.
7. a) Chung, S. K.; Chang, Y. T. *J. Chem. Soc., Chem. Commun.* **1995**, 11-12; b) Chung, S. K.; Chang, Y. T.; Sohn, K. H. *J. Chem. Soc., Chem. Commun.* **1996**, 163-164.
8. Chung, S. K.; Chang, Y. T. *J. Chem. Soc., Chem. Commun.* **1995**, 13-14.
9. The increasing order of R_f values on SiO_2 TLC (ethyl acetate-hexane 2:1, three times) is **5c** (0.10), **5a** (0.15), **5d** (0.25), **5c'** (0.3). All isomers are crystalline solids and their melting points are **5c** (183-184 ℃), **5a** (209-211 °C), **5d** (205-208 ℃), **5c'** (158-161 ℃).
10. Andersch, P; Schneider, M. P. *Tetrahedron: Asymmetry* **1993**, *4*, 2135-2138.
11. Billington, D. C.; Baker, R.; Kulagowski, J. J.; Mawer, I.; Vacca, J. P.; deSolms, S. J.; Huff, J. R. *J. Chem. Soc., Perkin Trans. 1*, **1989**, 1423-1429.
12. The melting points and ^{1}H-NMR data (CD_3OD) for the ring protons in IBz (**1**) are as follows. **1a**. 219-220 ℃; δ 3.26 (t, *J* = 9.1 Hz, 1H, H-5), 3.41 (dd, *J* = 2.7, 9.7 Hz, 1H, H-3), 3.60 (dd, *J* = 9.1, 9.7 Hz, 1H, H-4), 3.91 (dd, *J* = 9.1, 10.2 Hz, 1H, H-6), 4.10 (t, *J* = 2.7 Hz, 1H, H-2), 4.80 (dd, *J* = 2.7, 10.2 Hz, 1H, H-1). **1b**. 240-242 °C; δ 3.30 (t, *J* = 8.9 Hz, 1H, H-5), 3.66 (dd, *J* = 2.6, 9.7 Hz, 2H, H-1 & H-3), 3.73 (dd, *J* = 8.9, 9.7 Hz, 2H, H-4 & H-6), 5.69 (t, *J* = 2.6 Hz, 1H, H-2). **1c**. 208-210 °C; δ 3.46 (dd, *J* = 2.7, 9.7 Hz, 1H, H-1), 3.49 (t, *J* = 9.5 Hz, 1H, H-5), 3.71 (dd, *J* = 2.7, 10.0 Hz, 1H, H-3), 3.78 (dd, *J* = 9.5, 9.7 Hz, 1H, H-6), 4.04 (t, *J* = 2.7 Hz, 1H, H-2), 5.45 (app t, *J* = 9.8 Hz, 1H, H-4). **1d**. 238-240 °C; δ 3.52 (dd, *J* = 2.8, 9.8 Hz, 2H, H-1 & H-3), 3.91 (app t, *J* = 9.7 Hz, 2H, H-4 & H-6), 4.05 (t, *J* = 2.8 Hz, 1H, H-2), 5.04 (t, *J* = 9.6 Hz, 1H, H-5).
13. ^{31}P-NMR data ($CDCl_3$) for $IBz_1(PO_3Et_2)_5$ (**2**) are as follows(85 % H_3PO_4 as the reference standard). **2a**. δ 0.26 (2P), 0.46, 0.82, 1.06. **2b**. δ 0.46 (2P), 0.61 (3P). **2c**. δ -0.11, 0.08, 0.67, 1.32 (2P). **2d**. δ -0.18, 0.61 (2P), 1.45 (2P).
14. McKenna, C. E.; Higa, M. T.; Cheung, N. H.; McKenna, M.-C. *Tetraheron Lett.* **1977**, *18*, 155-158.
15. ^{31}P-NMR data (D_2O, pH 10) for IP_5 (**3**) are as follows (85 % H_3PO_4 as the reference standard). **3a**. δ 6.07, 6.20, 6.54, 6.77, 7.22. **3b**. δ 5.98 (2P), 6.35, 6.83 (2P). **3c**. δ 6.05 (2P), 6.75, 7.13, 7.27. **3d**. δ 6.00, 6.92 (2P), 7.90 (2P).

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