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

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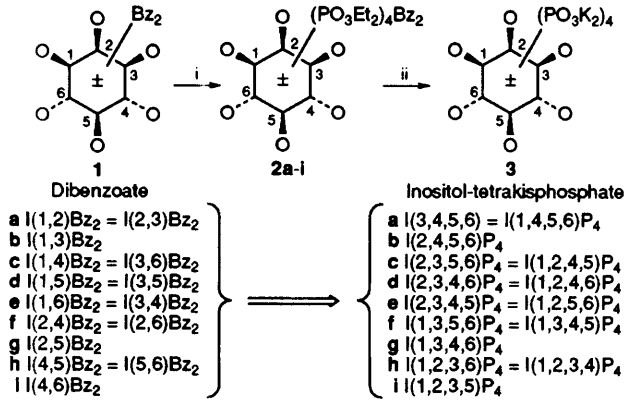
Synthesis of all possible nine regioisomers of IP_4, some of which are implicated as second messengers in the cellular signalling, was accomplished from *myo*-inositol *via* its dibenzoate derivatives (IBz_2) as the key intermediates; base-catalysed isomerization of readily available I(1,4)Bz_2 and its derivatives, followed by suitable separation procedures efficiently provided all nine regioisomers of IBz_2.

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 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 have been widely studied.[1–8] 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 extracellular Ca^{2+} through plasma membrane ion channels.[9] Several other IP_4s were also found in living systems, and studies to elucidate their functions including binding of specific proteins,[10,11] are in progress. Until now only four out of a possible nine (enantiomerically 15) IP_4 isomers, I(1,2,4,5)P_4,[12,13] I(1,3,4,5)P_4,[7,12,14–23] I(1,3,4,6)P_4,[7,24] I(1,4,5,6)P_4[12,25] have been synthesized by independent chemical routes. Systematic research on the structure and biological function of IP_4 has been hampered by the limited availability of IP_4 regioisomers. Here we report the total synthesis of all possible nine regioisomers of IP_4s using inositol dibenzoates (IBz_2) as the key intermediates.

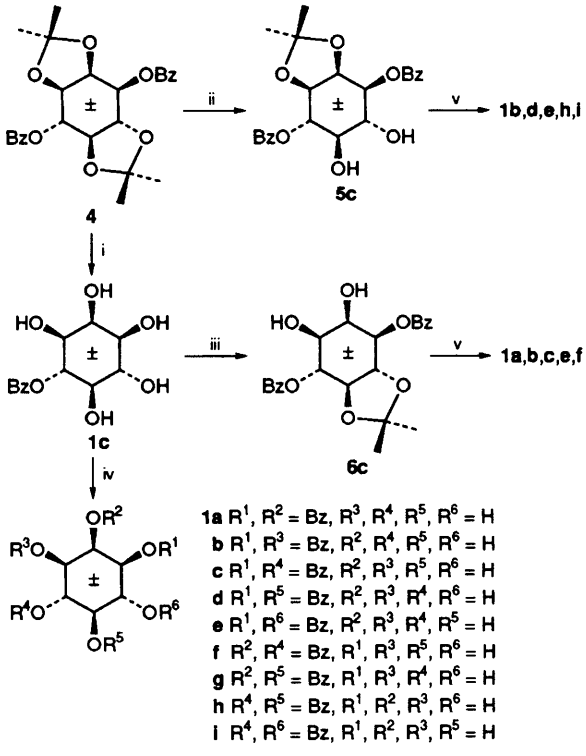
One of the key problems in the syntheses of inositol phosphates is to prepare suitable, selectively protected inositol intermediates. Our synthetic strategy is based on the facile generation of all nine regioisomers of *myo*-inositol dibenzoate (IBz_2) as the key intermediates, which are expected to be readily amenable to phosphorylation to provide the target structures (Scheme 1). Since inositol acetates and benzoates are known to isomerize upon base treatment,[12] we have examined this method as a quick way of generating IBz_2 isomers. Thus, compound **4**, prepared from *myo*-inositol,[26] was hydrolysed in 80% aqueous acetic acid at reflux to give I(1,4)Bz_2 (**1c**). The desired benzoyl migration in **1c** was successfully effected upon treatment with 60% aq. pyridine at elevated temperatures, but without much selectivity. However, the HPLC analysis conditions[27] were found, which allowed a complete separation of the nine isomers (**1a–i**) present in the reaction mixture. In practice the nine isomers of Ibz_2 (**1**) were separated in pure forms by combination of silica gel column chromatography, fractional crystallisation and preparative HPLC, and they were fully characterised by ^1H and ^{13}C NMR spectroscopy including H–H COSY.† It has been determined that the increasing order of the HPLC retention time of these isomers is 1,4(**1c**), 2,4(**1f**), 2,5(**1g**), 1,5(**1d**), 1,2(**1a**), 4,6(**1i**), 1,3(**1b**), 4,5(**1h**) 1,6(**1e**), and the increasing order of R_f values on SiO_2 is **1a**, **1h**, **1e**, **1f**, **1b**, **1g**, **1i**, **1d**, **1c**. There is no obvious correlation between these two sequences.

The separational difficulties could be substantially ameliorated by carrying out the benzoyl group migration in partially protected derivatives of I(1,4)Bz_2. Thus, compound **5c** was prepared from **4** by selective hydrolysis, and compound **6c** from **1c** by monoacetalisation (Scheme 2). When **5c** and **6c** were subjected to 60% aqueous pyridine conditions and then 80% aqueous acetic acid at reflux, two sets of five isomers of IBz_2 were obtained from the limited benzoyl group migrations. The kinetic behaviours of the benzoyl migration in **1c**, **5c** and **6c** at various temperatures were monitored by HPLC (see following paper).[27]

Each IBz_2 isomer was separately phosphorylated by successive treatment with diethylchlorophosphite and *N*,*N*-diiso-



Scheme 1 *Reagents and conditions*: i, (*a*) Diethylchlorophosphite (10 equiv.), diisopropylethylamine, DMF, −42 °C → 25 °C (*b*) hydrogen peroxide (30%), sodium phosphate buffer (1.0 mol dm^{-3}, pH 7), at 0 °C (70–95% overall yield); ii, (*a*) bromotrimethylsilane, $CDCl_3$, 25 °C, (*b*) 1 mol dm^{-3} KOH, 80 °C, 1 h, (*c*) Dowex 50 × 8-100(H^+). Benzoic acid produced was extracted out with dichloromethane, (*d*) pH adjusted to 10 (70–90% overall yield).



Scheme 2 *Reagents and conditions*: preparation of IBz_2(**4**). i, 80% aq. acetic acid, reflux, 15 min, quantitative; ii, cat. TSA (toluene-*p*-sulfonic acid), methanol–dichloromethane (1:3), 25 °C, 1.5 h, followed by chromatography on silica gel (**5c** 69%, **1c** 22% and **4** 5%); iii, cat. TSA, 2-methoxypropene (2 equiv.) in DMF (dimethylformamide), 10 °C, 2 h, followed by recrystallisation (**6c** 40%); iv, pyridine–water (6:4), at elevated temperature; v, (*a*) pyridine–water(6:4), at elevated temperature, (*b*) 80% aq. acetic acid, reflux, 15 min

propylethylamine in DMF, and then 30% hydrogen peroxide to yield *all nine* isomers of compound **2**, which were thoroughly characterised by ^{1}H, ^{13}C and ^{31}P NMR.‡ In the final steps, the protecting groups of **2** were removed by successive reactions with trimethylsilyl bromide and then KOH. Cleavage of the ethyl phosphate esters was monitored by ^{31}P NMR, which clearly showed upfield chemical shift changes of 10–20 ppm due to the conversion of the ethyl ester to the silyl ester.[28] The product **3** was obtained after chromatography on Dowex 50 × 8-100 (H^+ form), pH adjustment to 10 with KOH, and lyophilization.§ Biological studies on the IP_4 isomers are currently in progress.

It is suggested that the group migration method in conjunction with some efficient separational techniques as delineated above might be a very useful and general synthetic strategy to generate a diverse molecular array of carbohydrate isomers, which would be necessary for the determination of structural specificities in their reactions with biological macromolecules such as receptors, enzymes and antibodies. We are currently pursuing syntheses of all twelve regioisomers of IP_3 and the optically active versions of IP_4 and IP_3 isomers by the group migration method.

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Footnotes

† ^{1}H NMR data (CD_3OD) for the ring protons in IBz_2(**1**) are as follows. **1a**: δ 3.45 (dd, *J* = 8.8, 9.8 Hz, 1H, H-5), 3.84 (dd, *J* = 2.6, 9.9 Hz, 1H, H-3), 3.97 (dd, *J* = 8.8, 9.9 Hz, 1H, H-4), 4.04 (dd, *J* = 9.8, 9.8 Hz, 1H, H-6), 5.13 (dd, *J* = 2.6, 9.8 Hz, 1H, H-1), 5.87 (dd, *J* = 2.6, 2.6 Hz, 1H, H-2). **1b**: δ 3.46 (t, *J* = 9.3 Hz, 1H, H-5), 4.10 (dd, *J* = 9.3, 10.2, 2H, H-4 and H-6), 4.46 (t, *J* = 2.5 Hz, 1H, H-2), 5.03 (dd, *J* = 2.5, 10.2 Hz, 2H, H-1 and H-3). **1c**: δ 3.62 (dd, *J* = 9.4, 9.4 Hz, 1H, H-5), 3.84 (dd, *J* = 2.5, 10.1 Hz, 1H,H-3), 4.14 (dd, *J* = 9.4, 10.0 Hz, 1H, H-6), 4.25 (dd, *J* = 2.5, 2.5 Hz, 1H, H-2), 4.96 (dd, *J* = 2.5, 10.0 Hz, 1H, H-1), 5.50 (dd, *J* = 9.4, 10.1 Hz, 1H, H-4). **1d**: δ 3.65 (dd, *J* = 2.5, 9.9 Hz, 1H, H-3), 3.98 (dd, *J* = 9.8, 9.9 Hz, 1H, H-4), 4.26 (dd, *J* = 2.5, 2.5 Hz, 1H, H-2), 4.30 (dd, *J* = 9.6, 10.2 Hz, 1H, H-6), 5.03 (dd, *J* = 2.5, 10.2 Hz, 1H, H-1), 5.17 (dd, *J* = 9.6, 9.8 Hz, 1H, H-5). **1e**: δ 3.67 (dd, *J* = 2.6, 9.6 Hz, 1H, H-3), 3.72 (dd, *J* = 9.5, 9.5 Hz, 1H, H-5), 3.92 (dd, *J* = 9.5, 9.6 Hz, 1H, H-4), 4.35 (dd, *J* = 2.6, 2.6 Hz, 1H, H-2), 5.25 (dd, *J* = 2.6, 10.4 Hz, 1H, H-1), 5.87 (dd, *J* = 9.5, 10.4 Hz, 1H, H-6). **1f**: δ 3.62 (dd, *J* = 9.5, 10.0 Hz, 1H, H-5), 3.75 (dd, *J* = 2.7, 10.1 Hz, 1H, H-1), 3.88 (dd, *J* = 9.5, 10.1 Hz, 1H, H-6), 4.02 (dd, *J* = 2.7, 9.8 Hz, 1H, H-3), 5.55 (dd, *J* = 9.8, 10.0 Hz, 1H, H-4), 5.76 (dd, *J* = 2.7, 2.7 Hz, 1H, H-2). **1g**: δ 3.81 (dd, *J* = 2.9, 9.9 Hz, 2H, H-1 and H-3), 4.01 (d, *J* = 9.6, 9.9 Hz, 2H, H-4 and H-6), 5.14 (t, *J* = 9.6 Hz, 1H, H-5), 5.77 (t, *J* = 2.9 Hz, 1H, H-2). **1h**: δ 3.66 (dd, *J* = 2.7, 9.7 Hz, 1H, H-1), 3.92 (dd, *J* = 2.7, 9.9 Hz, 1H, H-3), 4.10 (dd, *J* = 9.7, 9.7 Hz, 1H, H-6), 4.15 (dd, *J* = 2.7, 2.7 Hz, 1H, H-2), 5.36 (dd, *J* = 9.7, 9.8 Hz, 1H, H-5), 5.74 (dd, *J* = 9.8, 9.9 Hz, 1H, H-4), **1i**: δ 3.83 (dd, *J* = 2.7, 9.8 Hz, 2H, H-1 and H-3), 3.86 (t, *J* = 9.8 Hz, 1H, H-5), 4.12 (t, *J* = 2.7 Hz, 1H, H-2), 5.59 (dd, *J* = 9.8, 9.8 Hz, 2H, H-4 and H-6).

‡ ^{31}P NMR data ($CDCl_3$) for IBz_2 $(PO_3Et_2)_4$(**2**) are as follows (85% H_3PO_4 as reference). **2a**: δ −1.64, −1.61, −1.08, −0.96. **2b**: δ −1.36(2P), −1.18, −0.65. **2c**: δ −1.54, −1.22, −1.17, −0.58. **2d**: δ −1.70, −1.31, −0.70, −0.55. **2e**: δ −1.61(2P), −1.23, −0.64. **2f**: δ −1.58, −0.98, −0.92, −0.72. **2g**: δ −1.29(2P), −0.89(2P). **2h**: δ −1.87, −1.30, −0.56, −0.39. **2i**: δ −1.97, −0.78, −0.59(2P).

§ ^{31}P NMR data (D_2O, pH 10) for IP_4(**3**) are as follows (85% H_3PO_4 as reference). **3a**: δ 4.14, 4.43, 4.60, 5.18. **3b**: δ 3.82, 5.02(3P). **3c**: δ 4.52, 5.06, 5.28, 5.37. **3d**: δ 4.82, 4.94, 5.22, 5.97. **3e**: δ 5.14, 5.17, 5.42, 5.55. **3f**: δ 3.96, 4.46, 4.63, 5.39. **3g**: δ 4.57(2P), 5.49(2P). **3h**: δ 3.82, 5.46, 6.36, 6.48. **3i**: δ 3.41, 5.03, 5.75(2P).

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