

Practical divergent synthesis of all possible regioisomers of *myo*-inositol trisphosphates

Sung-Kee Chung,* Young-Tae Chang and Kwang-Hoon Sohn

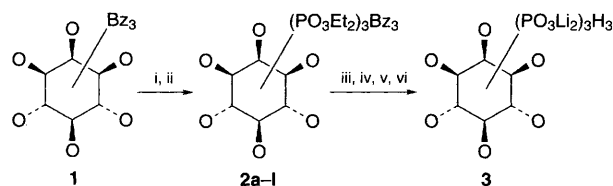
Department of Chemistry, Pohang University of Science and Technology, Pohang, 790-784, Korea

The synthesis of all possible 12 regioisomers of IP₃, some of which are implicated as second messengers in cellular signalling, is accomplished from *myo*-inositol via its tribenzoate derivatives (IBz₃) as the key intermediates.

D-*myo*-Inositol-1,4,5-trisphosphate [I(1,4,5)P₃] receptors are a homologous family of tetrameric ligand-gated Ca²⁺ channels which allow mobilization of intracellular Ca²⁺ stores in response to activation of cell-surface receptors linked to I(1,4,5)P₃ generation. Although the ligand recognition site of the IP₃ receptors appears to be in the *N*-terminal region, the exact nature of the molecular interactions is not understood. Several other IP₃s are also found in living systems, and studies to elucidate the receptor binding as well as the metabolism are in progress.^{1,2}

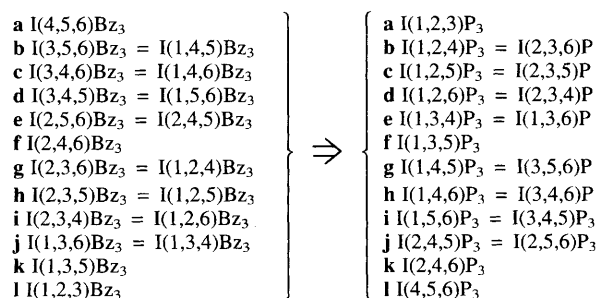
Systematic research on the relationship between the structure and biological function in these areas would be greatly facilitated by the ready availability of all IP₃ regioisomers. Until now nine out of the possible 12 (enantiomerically 20) IP₃ isomers have been synthesized by independent chemical routes: I(1,2,3)P₃,³ I(1,2,6)P₃,⁴ I(1,3,4)P₃,⁵ I(1,3,5)P₃,^{6,7} I(1,4,5)P₃,⁸ I(1,4,6)P₃,⁹ I(1,5,6)P₃,¹⁰ I(2,4,5)P₃¹¹ and I(2,4,6)P₃.⁷ Here we report a simple, divergent synthesis of all the possible 12 regioisomers of IP₃s via inositol tribenzoates (IBz₃).

One of the key problems in the synthesis of inositol phosphates is to prepare suitable, selectively protected inositol intermediates. We have previously reported syntheses of all the possible nine regioisomers of IP₄ through IBz₂¹² which were obtained by benzoyl group migration among the vicinal hydroxy groups of the *myo*-inositol structure.¹³ Based on the same synthetic strategy, the benzoyl migration technique was applied to the facile generation of all 12 regioisomers of *myo*-inositol tribenzoate (IBz₃) **1**, the key synthetic intermediates, which were then readily phosphorylated to provide the target IP₃ structures **2** (Scheme 1). Thus, the random, divergent benzoyl group migration in compound **1g**, prepared from *myo*-inositol,⁷ was successfully effected by treatment with 60% aq. pyridine at elevated temperatures. HPLC analysis conditions (RP18 Alltech, 250 × 4.6 mm, Mobile phase: MeCN: MeOH:H₂O (2:4:5), Flow rate: 1.5 ml min⁻¹) were found, which allowed separation of 10 out of the total 12 regioisomers (**1a–1**) present in the reaction mixture. The structures of the isolated regioisomers of **1** could be readily elucidated by



Scheme 1 Reagents and conditions: i, diethyl chlorophosphite (10 equiv.), diisopropylethylamine, DMF, -42 → 25 °C, 1 h; ii, hydrogen peroxide (35%), sodium phosphate buffer (1.0 mol dm⁻³, pH 7), 0 °C (70–99% overall yield); iii, Me₃SiBr, CH₂Cl₂, 25 °C, 1 d; iv, 1 mol dm⁻³ LiOH, 80 °C, 3 h; v, Dowex 50 × 8-100(H⁺). Benzoic acid produced was extracted with CH₂Cl₂; vi, pH adjusted to 10 (80–99% overall yield).

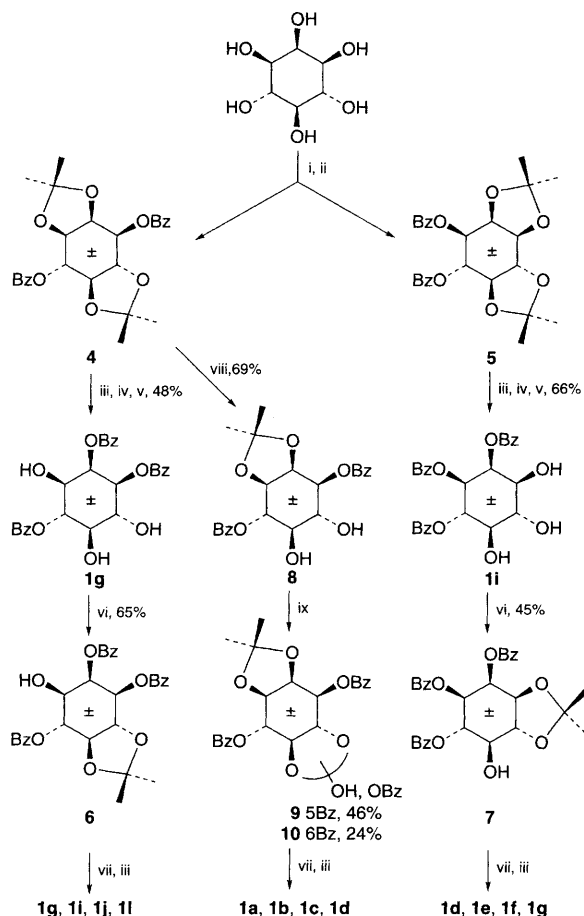
¹H NMR spectroscopy. The increasing order of the HPLC retention time of these regioisomers was found to be **1f**, **g**, **k**, **b**, **e**, **h**, **c**, **j**, **i**, (**a** + **l**) and **d**.



The separational difficulties associated with the mixture of 12 regioisomers could be substantially eased by carrying out the benzoyl group migrations in partially protected derivatives of IBz₃. In the event, compounds **4** and **5**, prepared from *myo*-inositol,¹⁴ were hydrolysed in acid and then selectively benzoylated on the axial 2-OH by way of the orthoester intermediates to give the two IBz₃, **1g** and **1i**.⁷ Each IBz₃ was monoacetalized under kinetic conditions to give the 4,5-acetal **6** and the 1,6-acetal **7**. Compound **8**, prepared from **4** by selective hydrolysis,¹² was monobenzoylelated to give a mixture of the 1,2-acetals **9** and **10** (Scheme 2). When compounds **6**, **7** and the mixture of compounds **9** and **10** were subjected to 60% aq. pyridine conditions and then 80% aq. acetic acid under reflux, three sets of four regioisomers providing a total of 10 different IBz₃ were obtained from the limited benzoyl group migrations. The kinetic behaviours of the benzoyl migration in each case was conveniently monitored by HPLC at various temperatures. More importantly, in these cases, the individual regioisomer was easily separated by silica gel chromatography at two stages, *i.e.* with or without the acetonide group. However, the remaining two IBz₃ intermediates **1f** and **1k** could not be obtained by this method and had to be separately prepared from *myo*-inositol orthoformate **11** (Scheme 3). Benzoylation of compound **11**, derived from *myo*-inositol,¹⁵ followed by acid-catalysed hydrolysis gave **1f**. Alternatively, benzoylation of **11**, followed by acid-catalysed hydrolysis, benzoylation and then hydrogenolysis provided **1k**. Each of the 12 regioisomers thus obtained was fully characterized by ¹H and ¹³C NMR including H–H COSY and FAB mass spectroscopy.

Each pure IBz₃ isomer **1** was separately phosphorylated by successive treatment with (i) diethyl chlorophosphite and *N,N*-diisopropylethylamine in DMF and (ii) 35% hydrogen peroxide¹² to yield all 12 regioisomers of compound **2**, which were thoroughly characterized by ¹H, ¹³C and ³¹P NMR including H–H COSY and FAB mass spectroscopy.† In the final steps, the protecting groups of **2** were removed by successive treatment with trimethylsilyl bromide and then LiOH. The product **3** was obtained after chromatography on Dowex 50 × 8-100 (H⁺ form), pH adjustment to 10 (LiOH) and then lyophilization.‡

It is now clear that all 39 regioisomers of the *myo*-inositol phosphates (IP_{*n*}) can be conveniently synthesized by acyl group migration as demonstrated here and previously.^{12,16} Fur-



thermore, the acyl group migration in conjunction with efficient separation techniques represents a method of generating molecular diversities in small molecules and in particular might prove to 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.

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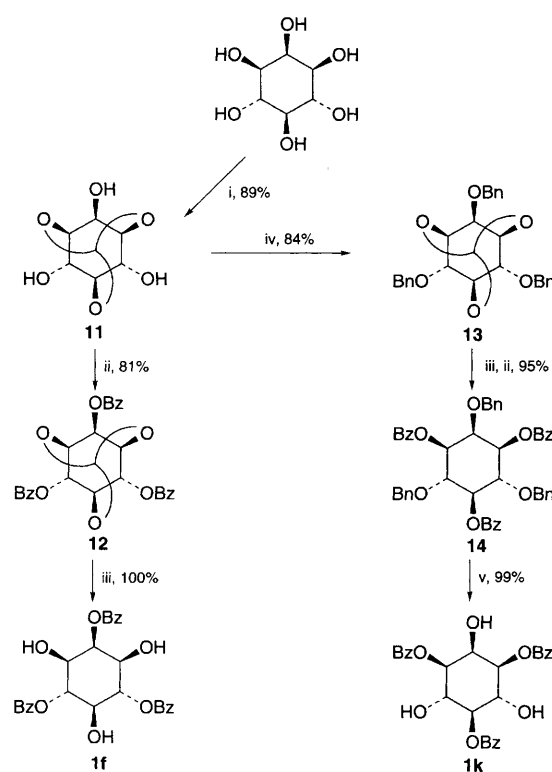
Footnotes

† ³¹P NMR data (121.5 MHz, CDCl₃, 25 °C, 85% H₃PO₄ as reference) for IBz₃ (PO₃Et₂)₃ **2**. For **2a**: δ_P -0.53(2 P) -1.76. For **2b**: δ_P -0.59, -0.73 and -1.42. For **2c**: δ_P -0.59, -0.78 and -1.54. For **2d**: δ_P -0.53, -1.26 and -1.59. For **2e**: δ_P -0.84(2 P) and -1.40. For **2f**: δ_P -0.59 and -0.81(2 P). **2g**: δ_P -0.89, -1.06 and -1.51. For **2h**: δ_P -0.75, -0.92 and -1.54. For **2i**: δ_P -0.98, -1.11 and -1.73. For **2j**: δ_P -0.64 and -1.28(2 P). For **2k**: δ_P -0.59 and -0.73(2 P). For **2l**: δ_P -0.98 and -1.45(2 P).

‡ ³¹P NMR data (121.5 MHz, D₂O, 25 °C, 85% H₃PO₄ as reference, pH 10) for IP₃ **3**. For **3a**: δ_P 5.81(2 P) and 4.27. For **3b**: δ_P 5.67, 5.53 and 5.44. For **3c**: δ_P 5.69 and 5.50(2 P). For **3d**: δ_P 5.39 and 4.88. For **3e**: δ_P 5.75, 5.11 and 4.30. For **3f**: δ_P 5.78 and 5.11(2 P). For **3g**: δ_P 5.58, 5.39 and 4.94. For **3h**: δ_P 6.08, 5.75 and 4.33. For **3i**: δ_P 5.86, 5.22 and 4.74. For **3j**: δ_P 5.92, 5.64 and 5.36. For **3k**: δ_P 5.94(3 P). For **3l**: δ_P 6.03(2 P) and 4.77.

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Scheme 3 Reagents and conditions: i, triethyl orthoformate, pTSA, DMF, 100 °C, 1 d; ii, BzCl, pyridine, room temp.; iii, pTSA, methanol, room temp., 1 h; iv, BnBr, NaH, DMF; v, H₂-Pd(OH)₂, EtOAc-MeOH

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