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

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

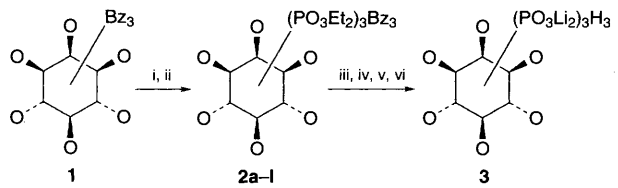
Department of Chemistry, Pohang University of Science and Technology, Pohang, 790-784, Korea

The synthesis of all possible 12 regioisomers of IP_3, some of which are implicated as second messengers in cellular signalling, is accomplished from *myo*-inositol *via* its tribenzoate derivatives(IBz_3) as the key intermediates.

D-*myo*-Inositol-1,4,5-trisphosphate [I(1,4,5)P_3] receptors are a homologous family of tetrameric ligand-gated Ca^{2+} channels which allow mobilization of intracellular Ca^{2+} stores in response to activation of cell-surface receptors linked to I(1,4,5)P_3 generation. Although the ligand recognition site of the IP_3 receptors appears to be in the *N*-terminal region, the exact nature of the molecular interactions is not understood. Several other IP_3s 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 IP_3 regioisomers. Until now nine out of the possible 12 (enantiomerically 20) IP_3 isomers have been synthesized by independent chemical routes: I(1,2,3)P_3,[3] I(1,2,6)P_3,[4] I(1,3,4)P_3,[5] I(1,3,5)P_3,[6,7] I(1,4,5)P_3,[8] I(1,4,6)P_3,[9] I(1,5,6)P_3,[10] I(2,4,5)P_3[11] and I(2,4,6)P_3.[7] Here we report a simple, divergent synthesis of all the possible 12 regioisomers of IP_3s *via* inositol tribenzoates (IBz_3).

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 IP_4 through IBz_2[12] 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(IBz_3) **1**, the key synthetic intermediates, which were then readily phosphorylated to provide the target IP_3 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:H_2O (2:4:5), Flow rate: 1.5 ml min^{-1}) were found, which allowed separation of 10 out of the total 12 regioisomers (**1a–l**) 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, Me_3SiBr, CH_2Cl_2, 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 CH_2Cl_2; vi, pH adjusted to 10 (80–99% overall yield).

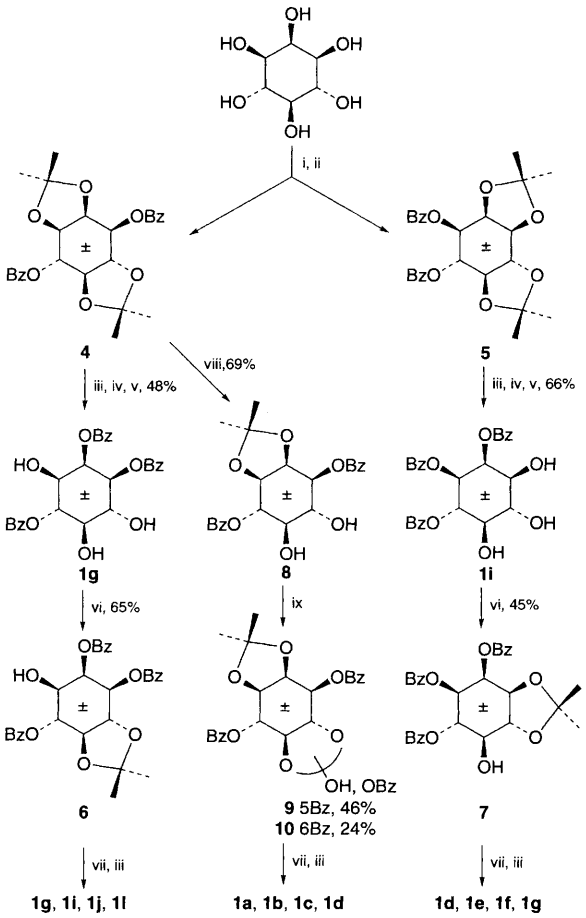
^1H NMR spectroscopy. The increasing order of the HPLC retention time of these regioisomers was found to be **1f, g, k, b, e, h, c, j, i, (a + l)** and **d**.

a I(4,5,6)Bz_3		**a** I(1,2,3)P_3
b I(3,5,6)Bz_3 = I(1,4,5)Bz_3		**b** I(1,2,4)P_3 = I(2,3,6)P
c I(3,4,6)Bz_3 = I(1,4,6)Bz_3		**c** I(1,2,5)P_3 = I(2,3,5)P
d I(3,4,5)Bz_3 = I(1,5,6)Bz_3		**d** I(1,2,6)P_3 = I(2,3,4)P
e I(2,5,6)Bz_3 = I(2,4,5)Bz_3		**e** I(1,3,4)P_3 = I(1,3,6)P
f I(2,4,6)Bz_3	⇒	**f** I(1,3,5)P_3
g I(2,3,6)Bz_3 = I(1,2,4)Bz_3		**g** I(1,4,5)P_3 = I(3,5,6)P
h I(2,3,5)Bz_3 = I(1,2,5)Bz_3		**h** I(1,4,6)P_3 = I(3,4,6)P
i I(2,3,4)Bz_3 = I(1,2,6)Bz_3		**i** I(1,5,6)P_3 = I(3,4,5)P_3
j I(1,3,6)Bz_3 = I(1,3,4)Bz_3		**j** I(2,4,5)P_3 = I(2,5,6)P_3
k I(1,3,5)Bz_3		**k** I(2,4,6)P_3
l I(1,2,3)Bz_3		**l** I(4,5,6)P_3

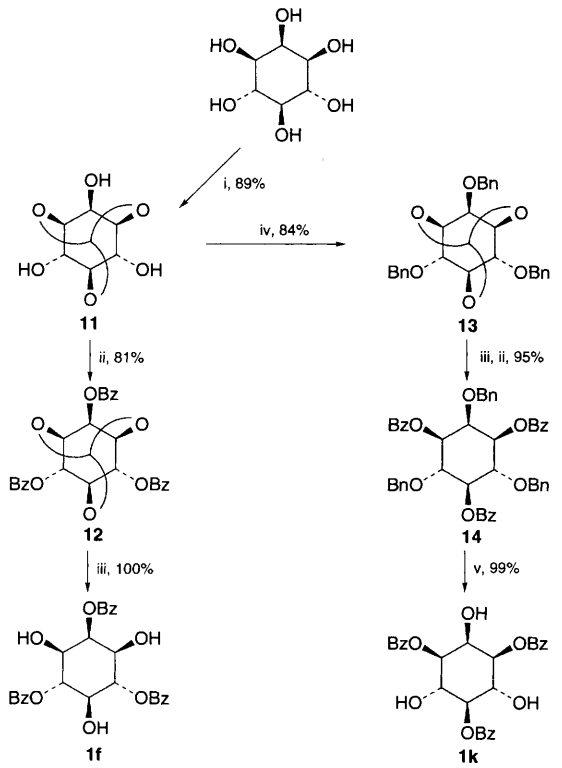
The separational difficulties associated with the mixture of 12 regioisomers could be substantially eased by carrying out the benzoyl group migrations in partially protected derivatives of IBz_3. In the event, compounds **4** and **5**, prepared from *myo*-inositol,[14] were hydrolysed in acid and then selectively benzoylated on the axial 2-OH by way of the orthoester intermediates to give the two IBz_3, **1g** and **1i**.[7] Each IBz_3 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 monobenzoylated 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 IBz_3 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 IBz_3 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, benzylation of **11**, followed by acid-catalysed hydrolysis, benzoylation and then hydrogenolysis provided **1k**. Each of the 12 regioisomers thus obtained was fully characterised by ^1H and ^{13}C NMR including H–H COSY and FAB mass spectroscopy.

Each pure IBz_3 isomer **1** was separately phosphorylated by successive treatment with (i) diethyl chlorophosphite and *N*,*N*-diisopropylethylamine in DMF and (ii) 35% hydrogen peroxide[12] to yield all 12 regioisomers of compound **2**, which were thoroughly characterized by ^1H, ^{13}C and ^{31}P 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-



Scheme 2 *Reagents and conditions*: i, 2,2-dimethoxypropane, pTSA, DMF, 100 °C, 3 h; ii, BzCl, pyridine, room temp. fractional crystallization; iii, 80% aq. AcOH, reflux, 30 min; iv, trimethyl orthobenzoate, pTSA, THF, room temp.; v, drops of water; vi, 2-methoxypropene, pTSA, THF, room temp.; vii, pyridine–water (6:4), 100 °C; viii, cat. pTSA, MeOH–CH_2Cl_2 (1:3), room temp., 1.5 h; ix, BzCl (1.2 equiv.), pyridine



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, H_2–$Pd(OH)_2$, EtOAc–MeOH

thermore, the acyl group migration in conjuction 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.

This work was supported by the Korea Science & Engineering Foundation (Centre for Biofunctional Molecules) and the Ministry of Education (Basic Science Research Fund).

Footnotes

† ^{31}P NMR data (121.5 MHz, $CDCl_3$, 25 °C, 85% H_3PO_4 as reference) for $IBz_3(PO_3Et_2)_3$ **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).

‡ ^{31}P NMR data (121.5 MHz, D_2O, 25 °C, 85% H_3PO_4 as reference, pH 10) for IP_3 **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_P78, 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.

References

1 B. V. L. Potter, *Nat. Prod. Rep.*, 1990, 1; M. J. Berridge, *Nature (London)*, 1993, **361**, 315; D. C. Billington, *The inositol phosphates, Chemical synthesis and Biological significance*, VCH, Weinheim, 1993.
2 *Inositol Phosphates and Derivatives*, ed. A. B. Reitz, *ACS Symposium Series 463*, ACS, Washington DC, 1991.
3 I. D. Spiers, S. Freeman, D. R. Poyner and C. H. Schwalbe, *Tetrahedron Lett.*, 1995, **36**, 2125.
4 T. Desai, A. Fernandez-Mayoralas, J. Gigg, R. Gigg, C. Jaramillo, S. Payne, S. Penades and N. Schnetz, ref. 2, p. 86.
5 S. Ozaki, M. Kohno and H. Nakahira, *Chem. Lett.*, 1988, 77; C. E. Ballou and W. Tegge, ref. 2, p. 33; D. M. Gou and C. S. Chen, *Tetrahedron Lett.*, 1992, **33**, 721; M. F. Boehm and G. D. Prestwich, *Tetrahedron Lett.*, 1988, **29**, 5217; A. M. Riley, R. Payne and B. V. L. Potter, *J. Med. Chem.*, 1994, **37**, 3918.
6 J. P. Vacca, S. J. de Solms, S. D. Young, J. R. Huff, D. C. Billington, R. Baker, J. J. Kulagowski and I. M. Mawer, ref. 2, p. 66.
7 S.-K. Chung, Y.-T. Chang and K.-H. Sohn, *Kor. J. Med. Chem.*, 1994, **4**, 57.
8 S. Ozaki, Y. Watanabe, T. Ogasawara, Y. Kondo, N. Shiotani, H. Nishii and T. Matsuki, *Tetrahedron Lett.*, 1986, **27**, 3157; C. B. Reese and J. G. Ward, *Tetrahedron Lett.*, 1987, **28**, 2309; J. P. Vacca, S. J. de Solms and J. R. Huff, *J. Am. Chem. Soc.*, 1987, **109**, 3478; A. M. Cooke, B. V. L. Potter and R. Gigg, *Tetrahedron Lett.*, 1987, **28**, 2305; S.-K. Chung, Y. Ryu, Y.-T. Chang and S.-H. Moon, *Kor. J. Med. Chem.*, 1992, **2**, 33.
9 Y. Watanabe, T. Ogasawara, S. Ozaki and M. Hirata, *Carbohydr. Res.*, 1994, **258**, 87.
10 J. R. Falck and A. Abdali, ref. 2, p. 145.
11 W. Tegge, G. V. Denis and C. E. Ballou, *Carbohydr. Res.*, 1991, **217**, 107; Y. Watanabe, T. Ogawara, N. Shiotani and S. Ozaki, *Tetrahedron Lett.*, 1987, **28**, 2607.
12 S.-K. Chung and Y.-T. Chang, *J. Chem. Soc., Chem. Commun.*, 1995, 11.
13 S.-K. Chung and Y.-T. Chang, *J. Chem. Soc., Chem. Commun.*, 1995, 13.
14 S.-K. Chung and Y. Ryu, *Carbohydr. Res.*, 1994, **258**, 145.
15 H. W. Lee and Y. Kishi, *J. Org. Chem.*, 1985, **50**, 4402.
16 P. J. Cullen, S.-K. Chung, Y.-T. Chang, A. P. Dawson and R. F. Irvine, *FEBS Lett.*, 1995, **358**, 240.

Received, 11th August 1995; Com. 5/05405B