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# Synthesis of analogues of *myo*-inositol 1,4,5-trisphosphate that contain sulfonamide, sulfate, methylphosphonate, and carboxymethyl groups

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ABSTRACT

The syntheses are described of four isosteric racemic *myo*-inositol 1,4,5-trisphosphate (**1**) analogues with the phosphate groups replaced by sulfonamide (**2**), sulfate (**3**), methylphosphonate (**4**), and carboxymethyl (**5**). None of these compounds had any affinity for the $IP_3$ receptor or induced platelet aggregation.

## INTRODUCTION

1D-*myo*-Inositol 1,4,5-trisphosphate (**1**, $IP_3$) exhibits its unique properties as a second messenger by reacting[1] with specific receptors on $Ca^{2+}$-sequestering organelles, thereby mobilising intracellular $Ca^{2+}$. $IP_3$ and its congeners are substrates for several kinases and phosphatases in the so-called phosphoinositide (PI) cycle[2]. The biological significance of the PI signalling system in general[3], and of $IP_3$ in particular, has stimulated much work on the chemical synthesis of phosphatidylinositol phosphates[4]. Moreover, biologically active $IP_3$ analogues that can permeate the cell membrane have potential in drug development[5]. In order to design $IP_3$ analogues that act as agonists or antagonists of the release of intracellular $Ca^{2+}$, a better understanding of the interaction of $IP_3$ with its receptors is essential and, in this respect, analogues can be used in structure–activity studies. In this context, we have reported the synthesis of analogues of *myo*-inositol 1,5-bis- and 1,4,5-trisphosphate that contain sulfate or sulfonamide moieties as phosphate-isosteric groups[6,7]. As part of an ongoing program[6–8], we now describe the synthesis of the racemic sulfonamide (**2**), sulfate (**3**), methylphosphonate (**4**), and carboxymethyl (**5**) analogues of *myo*-inositol 1,4,5-trisphosphate (**1**).

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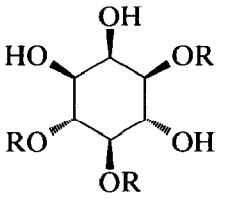
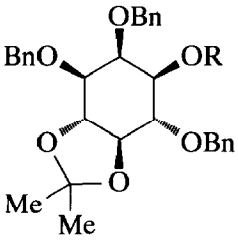
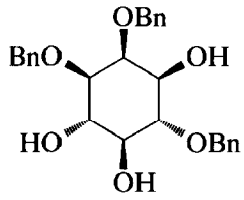
## RESULTS AND DISCUSSION

Treatment of 1-*O*-allyl-2,3,6-tri-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol[9] (**6**) with (1,5-cyclo-octadiene)bis(methyldiphenylphosphine)iridium hexafluorophosphate[10] afforded the *trans*-prop-1-enyl derivative **7**, acid hydrolysis (dioxane–methanol–0.1 M HCl) of which furnished the key-intermediate, 1,2,4-tri-*O*-benzyl-*myo*-inositol (**8**).




**1** R = $P(O)(OH)_2$
**2** R = $SO_2NH_2$
**3** R = $SO_2OH$
**4** R = $P(O)(CH_3)OH$
**5** R = $CH_2COOH$

**6** R = Allyl
**7** R = Prop-1-enyl

**8**

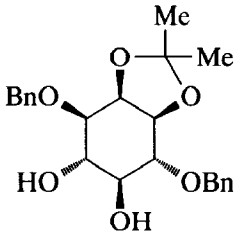
Reaction of **8** with sulfamoyl chloride and sodium hydride in *N*,*N*-dimethylformamide at 0° for 2 h gave, after chromatography, the sulfamoylated derivative, hydrogenolysis of which furnished the 1,4,5-trisulfonamide **2**. $^1H$ NMR spectroscopy revealed traces of under-sulfamoylated derivatives, which were removed by reversed-phase column chromatography to give the pure 1,4,5-trisulfonamide **2** (84% from **8**).

Treatment[11] of **8** for 16 h at 50° with the triethylamine–sulfur trioxide complex (15 equiv) in *N*,*N*-dimethylformamide gave the trisulfated derivative that was purified by chromatography on Sephadex LH-20 and then on silica gel. Hydrogenolysis then gave the 1,4,5-trisulfate **3** (86% from **8**).

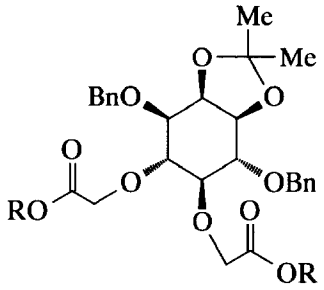
[1-(6-Trifluoromethyl)benzotriazolyl]methylphosphonate has been used[12] as a phosphonylating agent in the synthesis of an inositol monomethylphosphonate. However, bifunctional $P^V$ phosphonylating reagents, analogous to bifunctional $P^V$ phosphorylating reagents[8], are not suitable for the preparation of phosphonylated vicinal diols (as in **8**), because of the likely formation of cyclic phosphonates. Therefore, the utility of phosphinic acid $P^{III}$ derivatives[13] was explored. Thus, triethylammonium methylphosphinate[14] (6 equiv), obtained easily by hydrolysis of commercially available dichloro(methyl)phosphine, and pivaloyl chloride (6 equiv) were reacted with **8** for 1 h at 20° to give the 1,4,5-tris(methylphosphinate) derivative. Oxidation with iodine in aqueous pyridine, chromatography of the product on Sephadex LH-20, treatment with Dowex($Na^+$) resin, and then reversed-phase column chromatography gave the 1,4,5-tris(methylphosphonate)

derivative. Hydrogenolysis then gave the 1,4,5-tris(methylphosphonate) **4** (51% from **8**).

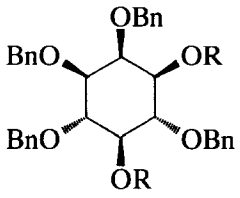
In seeking to prepare the 1,4,5-tri-*O*-carboxymethyl derivative **5**, conditions were sought that would permit efficient functionalisation of the 4,5-diol moiety. To this end, 3,6-di-*O*-benzyl-1,2-*O*-isopropylidene-*myo*-inositol[15] (**9**) was used in model studies. Application of the classical conditions for the introduction of carboxymethyl functions, namely, chloroacetic acid–sodium hydroxide, failed. Attention was then turned towards bromoacetates. However, none of the reactions attempted (methyl, ethyl, or *tert*-butyl bromoacetate in combination with butyllithium, sodium hydroxide, or sodium hydride) gave the desired 4,5-di-*O*-carboxymethyl derivative **10**.



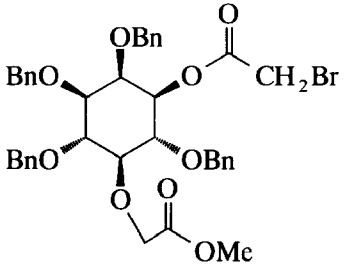

**9**




**10**



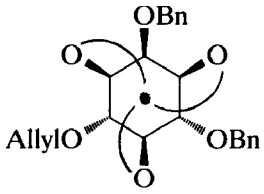

**11** R = H
**13** R = $CH_2C(O)O^tBu$



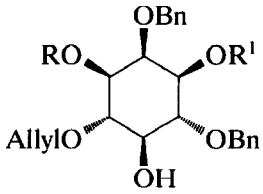

**12**

In further model studies, 1,2,4,6-tetra-*O*-benzyl-*myo*-inositol[6] (**11**) was treated with methyl bromoacetate in the presence of pulverised sodium hydroxide in dimethyl sulfoxide[16], butyl-lithium in tetrahydrofuran, or sodium hydride in *N*,*N*-dimethylformamide. However, the product (~ 30%) was the 3-*O*-bromoacetyl-5-*O*-methoxycarbonylmethyl derivative **12**. The different reactions at positions 3 and 5 in **11** may be be due to differences in nucleophilicity under the applied conditions. In order to avoid the trans-esterification reaction at position 3, a reagent containing a more-sterically hindered ester function was selected, namely, *tert*-butyl bromoacetate. Treatment of **11** with this *tert*-butyl ester in the presence of sodium hydride in *N*,*N*-dimethylformamide gave 91% of the 3,5-di-*O*-*tert*-butoxycarbonylmethyl derivative **13**.

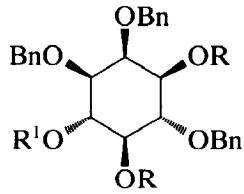
On the basis of these results, 4-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol (**16**) was synthesised, since the 4-*O*-allyl group might be convertible into a 4-*O*-carboxymethyl function after the introduction of the *tert*-butoxycarbonylmethyl groups at positions 1 and 5. Compound **16** was synthesised from 4-*O*-allyl-2,6-di-*O*-benzyl-*myo*-inositol 1,3,5-orthoformate[17] (**14**) by treatment with trifluoroacetic acid to afford the triol **15**, regioselective benzylation of which under phase-transfer conditions afforded **16** and 4-*O*-allyl-1,2,6-tri-*O*-benzyl-*myo*-inositol (**17**) in 42% yield from **14**. The structures of **16** and **17** were ascertained on the basis of $^1$H NMR (NOE) data, taking advantage of the interaction of the allyl $OCH_2$ and H-4 for the assignment of the resonance of H-4.




**14**




**15** R = R$^1$ = H
**16** R = Bn, R$^1$ = H
**17** R = H, R$^1$ = Bn




**18** R = $CH_2C(O)O^tBu$, R$^1$ = Allyl
**19** R = $CH_2C(O)O^tBu_2$, R$^1$ = $CH_2COOH$
**20** R = R$^1$ = $CH_2COOH$

Treatment of compound **16** with *tert*-butyl bromoacetate and sodium hydride in *N*,*N*-dimethylformamide gave the 1,5-di-*O*-*tert*-butoxycarbonylmethyl derivative **18** (84%). Oxidation of the 4-*O*-allyl group in **18** was studied using potassium permanganate, osmium tetraoxide, ozonolysis, and ruthenium tetraoxide. With the last-named procedure[18], 46% of the desired 1,2,4-tri-*O*-benzyl-3,5-di-*O*-*tert*-butoxycarbonylmethyl-6-*O*-carboxymethyl-*myo*-inositol (**19**) was obtained. Treatment of **19** with trifluoroacetic acid afforded **20**, hydrogenolysis of which furnished the desired 1,4,5-tri-*O*-carboxymethyl derivative **5**.

The identity and homogeneity of compounds **2**–**5** were each ascertained by $^1$H, $^{13}$C, and $^{31}$P NMR spectroscopy and FAB-mass spectrometry.

*Biological results.*—The biological properties of compounds **2**–**5** were examined using a permeabilised human-blood-platelet aggregation model and a radioligand binding assay; none of the compounds induced platelet aggregation or had any affinity for the $IP_3$ receptor. Probably, the specificity of the $IP_3$ receptor for its natural substrate is acquired via networks of hydrogen bonds and salt bridges, which are formed between the substrate and the protein. There are several studies described in the literature[19–21] explaining how particular proteins distinguish between phosphate, sulfate, and carboxylate groups by forming different patterns

of hydrogen bonds. We feel that the arguments in these studies may also be used to explain why none of our analogues is biologically active. In this respect, it is of interest that the replacement of one essential sulfate group in a synthetic heparin fragment by a phosphate group resulted in a complete loss of biological activity[22]. Up to now, *myo*-inositol 1,4,5-trisphosphorothioate[23] is the only isosteric $IP_3$ analogue, with all three phosphate groups modified, to retain affinity for the $IP_3$ receptor[24,25]. Clearly, the steric and electronic characteristics of $IP_3$ leave little or no space for modification of the phosphate groups without loss of affinity for the $IP_3$ receptor.

## EXPERIMENTAL

*General methods.*—TLC and HPTLC were performed on Kieselgel 60 F254 (Merck) with detection by charring with sulfuric acid or sulfuric acid–molybdate–phophoric acid. Column chromatography was performed on Kieselgel 60 (70–230 mesh, Merck) and reversed-phase column chromatography on LiChroprep RP-8 or RP-18 (40–63 $\mu$m, Merck). NMR spectra (internal $Me_4Si$ or $D_2O$) were recorded on a Bruker WM 360 spectrometer equipped with an ASPECT 3000 computer ($^1H$, 360 MHz; $^{13}C$, 90 MHz; $^{31}P$, 145 MHz). FAB-mass spectra were recorded with a Finnigan MAT 90 mass spectrometer equipped with a WATV Cs ion gun (Wagner Analysen Technik Vertriebs GmbH, Worpswede, Germany). The gun produced a beam of fast Cs ions (beam current $\sim 2$ $\mu$A at 22 kV). Glycerol or thioglycerol was used as the matrix.

The *myo*-inositol derivatives described below are racemic.

*1,2,4-Tri-O-benzyl-*myo*-inositol* (**8**).—To a solution of **6**[9] (2.0 g, 3.77 mmol) in THF (20 mL, freshly distilled from $LiAlH_4$) was added (1,5-cyclo-octadiene)bis-(methyldiphenylphosphine)iridium hexafluorophosphate (5 mg). The stirred solution was degassed, placed under $H_2$ for 2 min, degassed, and placed under $N_2$. After 4 h, the solvent was evaporated to give the 3-*O-trans*-prop-1-enyl derivative **7** ($\sim$ 100%). $^1H$ NMR data ($CDCl_3$): $\delta$ 1.50 (dd, 3 H, $CH_3CH$=CHO), 5.11 (dq, 1 H, $CH_3CH$=CHO), 6.19 (dq, 1 H, OCH=$CHCH_3$).

A solution of **7** (2.0 g, 3.77 mmol) in acetone–M HCl (55 mL, 10:1) was boiled under reflux for 10 min, then cooled to room temperature, diluted with $CH_2Cl_2$ (50 mL), washed with satd aq $NaHCO_3$ (25 mL) and brine (25 mL), dried ($MgSO_4$), and concentrated. Column chromatography (hexane–EtOAc, 2:1 → 1:1) of the residue gave **8** (1.63 g, 96%), $R_F$ 0.52 (1:1 hexane–EtOAc). NMR data ($CDCl_3$): $^1H$, $\delta$ 2.37 (d, 1 H, *J* 8 Hz, HO-1), 2.74 (d, 1 H, *J* 2 Hz, HO-4), 2.78 (d, 1 H, *J* 2 Hz, HO-5), 3.27 (dd, 1 H, *J* 9.5 and 2.0 Hz, H-3), 3.47 (dt, 1 H, *J* 9.5 and 2.0 Hz, H-5), 3.55 (ddd, 1 H, *J* 2.0, 9.5, and 8 Hz, H-1), 4.08 (t, 1 H, *J* 2.0 Hz, H-2), 4.53–4.96 (6 H, 3 PhC$H_2$), 7.27–7.39 (m, 15 H, 3 Ph); $^{13}C$, $\delta$ 73.44, 75.72, 76.03 (3 Ph$CH_2$), 73.65, 75.86, 77.45, 78.09, 78.72, 81.17, 82.67 (C-1/6).

myo-*Inositol 1,4,5-trisulfonamide* (**2**).—To a solution of **8** (106 mg, 0.236 mmol) in DMF (2 mL) was added NaH (34 mg, 1.42 mmol) at 0° under $N_2$. The mixture

was stirred for 5 min at 0°, sulfamoyl chloride (163 mg, 1.42 mmol) was added, stirring was continued for 2 h at 0°, and the mixture was diluted with $CH_2Cl_2$ (20 mL), washed with brine (10 mL) and water (10 mL), dried ($MgSO_4$), and concentrated. Column chromatography ($CH_2Cl_2$–MeOH, 99:1–95:5) of the residue afforded the protected sulfamoylated derivative (139 mg, 86%), $R_F$ 0.6 (95:5 $CH_2Cl_2$–MeOH).

A solution of this product in *tert*-butyl alcohol–water (20 mL, 3:1) was hydrogenolysed in the presence of 10% Pd/C (40 mg) for 16 h, then filtered, and concentrated. Reversed-phase column chromatography (RP-18; EtOH–$H_2O$, 1:3) of the residue afforded racemic **2** as a waxy solid (83 mg, 98%). Lyophilisation from *tert*-butyl alcohol–water (1:1) gave **2** as a white foam, $R_F$ 0.25 (RP-18; 3:1 EtOH–$H_2O$). NMR data ($D_2O$): $^1H$, $\delta$ 3.97 (dd, 1 H, $J$ 6.5 Hz, H-3), 4.16 (t, 1 H, $J$ 6.5 Hz, H-6), 4.47 (t, 1 H, $J$ 2.0 Hz, H-2), 4.55 (dd, 1 H, $J$ 2.0 and 6.5 Hz, H-1), 4.64 (t, 1 H, $J$ 6.5 Hz, H-5), 4.79 (t, 1 H, $J$ 6.5 Hz, H-4); $^{13}C$, $\delta$ 71.15 (2 C, C-2,3), 72.43 (C-6), 82.55 (C-1), 83.13 (C-4), 83.61 (C-5). FAB-mass spectrum: $m/z$ 418 $(M + H)^+$, 416 $(M - H)^-$.

myo-*Inositol 1,4,5-trisulfate* (**3**).—A solution of **8** (46 mg, 0.1 mmol) and $Et_3N \cdot SO_3$ (271 mg, 1.5 mmol) in DMF (2 mL) was stirred for 16 h at 50°, then cooled to 20°, and subjected to column chromatography on Sephadex LH-20, using DMF containing 0.5% of $Et_3N$. The appropriate fractions were collected, treated with aq $NaHCO_3$ (25.4 mg in 2 mL), and concentrated. Column chromatography ($CH_2Cl_2$–MeOH, 4:1) of the residue afforded the trisulfated derivative (66.8 mg, 88%), $R_F$ 0.33 (EtOAc–pyridine–AcOH–$H_2O$, 11:7:1.6:4). A solution of this derivative (66 mg, 0.086 mmol) in DMF–$H_2O$ (15 mL, 4:1) was treated in the presence of 10% Pd/C (35 mg) with $H_2$ for 16 h, then filtered, and concentrated. The residue was dissolved in water and lyophilised to afford **3** as a white solid (39 mg, 98%). NMR data ($D_2O$): $^1H$, $\delta$ 3.85 (dd, 1 H, $J$ 2.5 and 9.0 Hz, H-3), 3.96 (t, 1 H, $J$ 9.0 Hz, H-6), 4.25 (dd, 1 H, $J$ 2.5 and 9.0 Hz, H-1), 4.29 (t, 1 H, $J$ 9.0 Hz, H-5), 4.41 (t, 1 H, $J$ 2.0 Hz, H-2), 4.52 (t, 1 H, $J$ 9.0 Hz, H-4); $^{13}C$, $\delta$ 72.14 (C-2), 72.24 (C-3), 72.40 (C-4), 80.73 (C-1), 81.10 (C-4), 82.26 (C-5). FAB-mass spectrum: $m/z$ 463 $(M - Na)^-$.

*Triethylammonium methylphosphinate.*—To a mixture of MeCN–$H_2O$ (10 mL, 95:5) was added dichloro(methyl)phosphine (1 mL, 11.3 mmol) dropwise at 0°. The mixture was stirred for 15 min at 0°, then concentrated, and MeCN (3 × 10 mL) was evaporated from the residue. The final residue was redissolved in MeCN–$Et_3N$ (10 mL, 3:1), and the solvent was evaporated to give the title compound as a syrup that was used without further purification. NMR data ($CDCl_3$): $^{31}P$, $\delta$ 24.66; $^{13}C$, $\delta$ 8.53 (3 $CH_3CH_2$), 17.57 (d, $J$ 91 Hz, $PCH_3$), 45.48 (3 $CH_3CH_2$); $^1H$, $\delta$ 1.35 (m, 12 H, $PCH_3$ and 3 $CH_3CH_2$), 3.12 (q, 6 H, $J$ 7 Hz, 3 $CH_3CH_2$), 7.23 (dq, 1 H, $J$ 520 and 2 Hz, PH).

myo-*Inositol 1,4,5-tris(methylphosphonate)* (**4**).—To a solution of **8** (100 mg, 0.22 mmol) and triethylammonium methylphosphinate (240 mg, 1.33 mmol) in pyridine (2.5 mL) was added pivaloyl chloride (164 $\mu$L) at 20°. After 10 min, when $^{31}P$

NMR spectroscopy revealed the formation of the methylphosphinate ($\delta$ 31.6, broad), 0.5 M iodine in pyridine–water (4:1) was added. $^{31}$P NMR spectroscopy of the mixture showed the formation of one product within 5 min [$\delta$ 26.8 and 26.5 (double intensity)]. The mixture was concentrated to ~ 1 mL, applied to a column of Sephadex LH-20, and eluted with 1:1 $CH_2Cl_2$–MeOH. The appropriate fractions were combined and concentrated, and a solution of the residue in 2:1 MeOH–$H_2O$ (5 mL) was treated with Dowex($Na^+$) resin and concentrated. Reversed-phase (RP-8) column chromatography (2:8 MeCN–$H_2O$) of the residue gave the phosphonylated intermediate (88 mg, 53%), $R_F$ 0.63 (RP-8; 2:3 $CH_3CN$–$H_2O$).

Hydrogenolysis of this intermediate in DMF–$H_2O$ (15 mL, 3:1) in the presence of 10% Pd/C (30 mg) for 16 h afforded, after filtration, concentration, and lyophilisation ($H_2O$), **4** as a white solid (55 mg, 99%). NMR data ($D_2O$): $^{31}$P, $\delta$ 27.8, 28.6, 29.6; $^1$H, $\delta$ 1.32 (d, 3 H, *J* 17 Hz, P-1-$CH_3$), 1.34 (d, 3 H, *J* 14.5 Hz, P-4-$CH_3$), 1.37 (d, 3 H, *J* 14.5 Hz, P-5-$CH_3$), 3.74 (dd, 1 H, *J* 2.0 and 7.0 Hz, H-3), 3.87 (t, 1 H, *J* 7.0 Hz, H-6), 3.98 (q, 1 H, *J* 7.0 Hz, H-5), 4.01 (ddd, 1 H, *J* 2.0 and 7.0 Hz, H-1), 4.21 (t, 1 H, *J* 2.0 Hz, H-2), 4.24 (q, 1 H, *J* 7.0 Hz, H-4); $^{13}$C, $\delta$ 11.28 (d, *J* 137 Hz, P-1-$CH_3$), 11.81 (d, 2 P, *J* 138 Hz, P-4,5-$CH_3$), 72.95 (C-3), 73.38 (C-6), 73.79 (C-2), 77.02 (b, C-1), 77.67 (b, C-4), 79.12 (b, C-5). FAB-mass spectrum: *m/z* 481 $(M + H)^+$, 457 $(M - Na)^-$.

*1,2,4,6-Tetra*-O-*benzyl-3*-O-*bromoacetyl-5*-O-*methoxycarbonylmethyl*-myo-*inositol* (**12**).—To a vigorously stirred mixture of **11**[6] (250 mg, 0.5 mmol) and NaH (60 mg) in DMF (5 mL) was added methyl bromoacetate (5 mmol) dropwise at 20°. The mixture was stirred for 30 min at 20°, then diluted with $CH_2Cl_2$ (25 mL), washed with brine (2 × 15 mL), dried ($MgSO_4$), and concentrated. Column chromatography ($CH_2Cl_2$ → 96:4 $CH_2Cl_2$–acetone) of the residue gave **12** as an oil (110 mg, 30%), $R_F$ 0.56 (9:1 toluene–EtOAc). $^{13}$C NMR data ($CDCl_3$): $\delta$ 25.49 ($CH_2Br$), 52.61 ($OCH_3$), 71.16, 73.01, 74.38, 74.78, 75.85 (5 $OCH_2$), 74.53, 75.48, 78.60, 80.54, 81.00, 84.68 (C-1/6), 127.59–128.50, 137.56–138.17 (Ph), 166.58 ($COCH_2Br$), 169.32 (CO).

*1,2,4,6-Tetra*-O-*benzyl-3,5-di*-O-tert-*butoxycarbonylmethyl*-myo-*inositol* (**13**).—As described for **12**, treatment of **11** with *tert*-butyl bromoacetate afforded, after column chromatography (toluene–EtOAc, 9:1), **13** (349 mg, 91%), $R_F$ 0.42. NMR data ($CDCl_3$): $^{13}$C, $\delta$ 28.14 [$(CH_3)_3C$], 28.19 [$(CH_3)_3C$], 81.20, 81.34 [2 $C(CH_3)_3$], 69.61, 71.47, 72.34, 74.13, 74.22, 75.59, 75.63, 80.24, 80.76, 81.18, 81.39 (C-1/6 and 6 $OCH_2$); $^1$H, $\delta$ 1.43 (s, 9 H, $^t$Bu), 1.48 (s, 9 H, $^t$Bu), 3.23 (c, 3 H, H-1,3,5), 3.95 (t, 1 H, *J* 9.5 Hz, H-6), 4.07 (t, 1 H, *J* 9.5 Hz, H-4), 4.27 (t, 1 H, *J* 2.0 Hz, H-2).

*4*-O-*Allyl-2,6-di*-O-*benzyl*-myo-*inositol* (**15**).—A solution of **14**[17] (1.8 g, 4.39 mmol) in trifluoroacetic acid–water (20 mL, 5:1) was kept at 40° for 1 h, then concentrated, and toluene (2 × 25 mL) was evaporated from the residue. A solution of the residue in MeOH and concd ammonia (25 mL, 5:1) was kept for 30 min at 20°, then concentrated, and toluene (2 × 25 mL) was evaporated from the residue to give **15** (~ 100%), $R_F$ 0.25 (95:5 $CH_2Cl_2$–MeOH). $^{13}$C NMR data

($CDCl_3$): δ 73.99 ($OCH_2CH=CH_2$), 75.12, 75.42 (2 $PhCH_2$), 72.54, 74.94, 79.42, 81.84, 81.95 (C-1/6), 117.38 ($OCH_2CH=CH_2$), 135.09 ($OCH_2CH=CH_2$), 127.94–128.68, 138.57 (Ph).

*4-O-Allyl-2,3,6-tri-O-benzyl-*myo*-inositol* (**16**).—A mixture of **15** (1.6 g, 4 mmol), tetrabutylammonium bromide (360 mg), benzyl bromide (0.95 mL), and 2 M NaOH (15 mL) in $CH_2Cl_2$ (20 mL) was stirred vigorously for 16 h at 40°, then diluted with $CH_2Cl_2$ (50 mL), washed with $H_2O$ (3 × 25 mL), dried ($MgSO_4$), and concentrated. Column chromatography (9:1 $CH_2Cl_2$–EtOAc) of the residue gave **16** (431 mg, 22%), 4-*O*-allyl-1,2,6-tri-*O*-benzyl-*myo*-inositol (**17**, 20%), and **15** (43%).

Compound **16** had $R_F$ 0.55. NMR data ($CDCl_3$): $^1H$, δ 2.30 (d, 1 H, *J* 6 Hz, HO-1), 2.58 (d, 1 H, *J* 2.0 Hz, HO-5), 3.38 (dd, 1 H, *J* 2.0 and 9.5 Hz, H-3), 3.48 (c, 2 H, H-1,5), 3.67 (t, 1 H, *J* 9.5 Hz, H-6), 3.78 (t, 1 H, *J* 9.5 Hz, H-4), 4.03 (t, 1 H, *J* 2.0 Hz, H-2), 4.25–4.48 (m, 2 H, $OCH_2CH=CH_2$), 4.63–4.98 (c, 6 H, 3 $PhCH_2$), 5.25 (m, 2 H, $OCH_2CH=CH_2$), 5.96 (m, 1 H, $OCH_2CH=CH_2$), 7.13–7.41 (15 H, 3 Ph); $^{13}C$, δ 72.74, 74.34, 74.80, 75.00 (3 $PhCH_2$, $OCH_2CH=CH_2$), 72.21, 74.92, 77.24, 80.83, 80.95, 81.71 (C-1/6), 117.08 ($OCH_2CH=CH_2$), 135.25 ($OCH_2CH=CH_2$), 127.01–128.57, 137.30, 137.67, 138.43 (3 Ph).

Compound **17** had $R_F$ 0.43. NMR data ($CDCl_3$): $^1H$, δ 2.49 (d, 1 H, *J* 6 Hz, HO-3), 2.70 (d, 1 H, *J* 2.0 Hz, HO-5), 3.37–3.51 (c, 3 H, H-1,3,5), 3.58 (t, 1 H, *J* 9.5 Hz, H-6), 3.78 (t, 1 H, *J* 9.5 Hz, H-4), 4.03 (t, 1 H, *J* 2.0 Hz, H-2), 4.21–4.43 (m, 2 H, $OCH_2CH=CH_2$), 4.65–5.03 (m, 6 H, 3 $PhCH_2$), 5.10–5.32 (m, 2 H, $OCH_2CH=CH_2$), 5.84–6.00 (m, 1 H, $OCH_2CH=CH_2$), 7.21–7.43 (15 H, 3 Ph); $^{13}C$, δ 72.78, 73.94, 74.86, 75.56 (3 $PhCH_2$, $OCH_2CH=CH_2$), 72.24, 74.99, 77.19, 80.96, 81.35, 81.48 (C-1/6), 117.18 ($OCH_2CH=CH_2$), 135.35 ($OCH_2CH=CH_2$), 137.31, 137.71, 138.49 (3 quaternary C, Ph).

*4-O-Allyl-2,3,6-tri-O-benzyl-1,5-di-*O*-tert-butoxycarbonylmethyl-*myo*-inositol* (**18**).—As described for compound **13**, treatment of **16** (400 mg, 0.82 mmol) with NaH (4.0 mmol) and *tert*-butyl bromoacetate (8.0 mmol) afforded, after column chromatography (9:1 toluene–EtOAc), **18** (415 mg, 84%), $R_F$ 0.42. NMR data ($CDCl_3$): δ 1.43 (s, 9 H, $^t$Bu), 1.47 (s, 9 H, $^t$Bu), 3.16–3.31 (c, 3 H, H-1,3,5), 3.95 (t, 1 H, *J* 9.5 Hz, H-6), 4.06 (t, 1 H, *J* 9.5 Hz, H-4), 4.21 (t, 1 H, *J* 2.0 Hz, H-2), 4.26–4.97 (c, 12 H, 3 $PhCH_2$, $OCH_2CH=CH_2$, and 2 $CH_2CO$), 5.21 (m, 2 H, $OCH_2CH=CH_2$), 5.96 (m, 1 H, $OCH_2CH=CH_2$), 7.22–7.46 (15 H, 3 Ph); $^{13}C$, δ 28.14, 28.19 [2 $(CH_3)_3C$], 68.33, 69.57, 71.47, 72.31, 74.14, 75.59 (3 $PhCH_2$, $OCH_2CH=CH_2$, 2 $CH_2CO$), 74.22, 75.64, 80.27, 80.99, 81.23, 84.70 (C-1/6), 81.23, 81.39 [2 $(CH_3)_3C$], 116.53 ($OCH_2CH=CH_2$), 135.54 ($OCH_2CH=CH_2$), 127.25–128.39, 138.49, 138.83, 139.18 (Ph), 169.15, 169.94 (2 CO).

*1,2,4-Tri-O-benzyl-3,5-di-*O*-tert-butoxycarbonylmethyl-6-O-carboxymethyl-*myo*-inositol* (**19**).—To a solution of **18** (134 mg, 0.18 mmol) in $CCl_4$–MeCN–$H_2O$ (4 mL, 1:6:1) at 0° were added sodium periodate (163 mg) and ruthenium trichloride hydrate (2 mg). The mixture was stirred vigorously for 1 h at 20°, diluted with EtOAc (25 mL), washed with 0.1 M HCl (10 mL) and water (2 × 15 mL), dried ($MgSO_4$), and concentrated. Column chromatography ($CH_2Cl_2$–MeOH, 95:5 →

9:1) of the residue gave **19** (60 mg, 46%), $R_F$ 0.55 (9:1 $CH_2Cl_2$–MeOH). NMR data ($CDCl_3$): $^1H$, $\delta$ 1.42 (s, 9 H, $^t$Bu), 1.47 (s, 9 H, $^t$Bu), 3.21 (dd, 1 H, *J* 2.0 and 9.5 Hz, H-3), 3.34 (c, 2 H, H-1,5), 3.84 (t, 1 H, *J* 9.5 Hz, H-6), 4.04 (t, 1 H, *J* 9.5 Hz, H-4), 4.15–4.95 (c, 13 H, H-2, 3 PhC$H_2$ and 3 $CH_2CO$), 7.19–7.44 (15 H, Ph); $^{13}C$, $\delta$ 28.12 [2 ($CH_3$)$_3$C], 69.62, 69.80, 70.97, 71.05, 74.18, 74.68 (3 PhC$H_2$, and 3 $CH_2CO$), 74.21, 78.49, 80.51, 81.17, 81.41, 81.84 (double intensity), 82.32 [C-1/6, 2 ($CH_3$)$_3$C], 127.59–128.64, 136.41, 138.00, 138.54 (Ph), 169.40, 169.57, 172.11 (3 CO).

*1,2,4-Tri*-O-*benzyl-3,5,6-tri*-O-*carboxymethyl*-myo-*inositol* (**20**).—A solution of **19** (53 mg, 72 $\mu$mol) in $CH_2Cl_2$–trifluoroacetic acid (5 mL, 9:1) was stirred for 2 h at 20°, then diluted with toluene (10 mL), and concentrated, and toluene (2 × 10 mL) was evaporated from the residue. Reversed-phase (RP-18) chromatography (MeCN–$H_2O$, 1:1 → 7:3), combination of the appropriate fractions, treatment with Dowex($Na^+$) resin, and concentration gave **20** (23 mg, 50%). $^{13}C$ NMR data (MeOD): $\delta$ 176.14, 177.81, 177.94 (3 CO).

*1,4,5-Tri*-O-*carboxymethyl*-myo-*inositol* (**5**).—A solution of **20** (23 mg, 36 $\mu$mol) in *tert*-butyl alcohol–water (15 mL, 3:1) was hydrogenolysed in the presence of 10% Pd/C (20 mg) for 16 h, then filtered, and concentrated. The residue was lyophilised from water to give **5** as a white fluffy solid (12.1 mg, 80%). NMR data ($D_2O$): $^1H$, $\delta$ 3.35 (c, 1 H, H-5), 3.40 (dd, 1 H, *J* 2.5 and 9.5 Hz, H-1), 3.63 (c, 2 H, H-3,4), 3.88 (t, 1 H, *J* 9.5 Hz, H-6), 4.13 (s, 2 H, $CH_2CO$), 4.25 (t, 1 H, *J* 2.5 Hz, H-2), 4.26 (AB q, 2 H, $CH_2CO$), 4.30 (AB q, 2 H, $CH_2CO$); $^{13}C$, $\delta$ 67.54 (C-2), 67.89 (C-3), 69.49 (C-6), 70.42 (3 $CH_2CO$), 79.74 (C-1), 81.87 (C-4), 83.58 (C-5), 177.07, 177.51 (3 CO). FAB-mass spectrum: *m/z* 353 $(M - H)^-$, 375 $(M + Na - 2H)^-$, 397 $(M + Na - 3H)^-$.

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REFERENCES

1 H. Streb, R.F. Irvine, M.J. Berridge, and I. Schultz, *Nature (London)*, 306 (1983) 67–69; M.J. Berridge and R.F. Irvine, *ibid.*, 312 (1984) 315–321; R.H. Michell and J.W. Putney, *Inositol Lipids in Cellular Signalling*, Cold Spring Harbor, 1987; R.H. Michell, A.H. Drummond, and C.P. Downes, *Inositol Lipids in Cell Signalling*, Academic Press, London, 1989.

2 S.R. Nahorski and B.V.L. Potter, *Trends Pharmacol. Sci.*, 10 (1989) 139–144, and references therein.

3 R.F. Irvine, in S.R. Nahorski (Ed.), *Transmembrane Signalling, Intracellular Messengers and Implications for Drug Development*, Wiley, New York, 1990, pp. 169–183.

4 A.B. Reitz (Ed.), *ACS Symp. Ser.*, 463 (1991).

5 E.R. Chilvers, E.D. Kennedy, and B.V.L. Potter, *Drugs, News, Pers.*, 2 (1989) 342–346.

6 P. Westerduin, H.A.M. Willems, and C.A.A. van Boeckel, *Tetrahedron Lett.*, 47 (1990) 6915–6918.

7 P. Westerduin, H.A.M. Willems, and C.A.A. van Boeckel, *Tetrahedron Lett.*, 47 (1990) 6919–6922.
8 P. Westerduin and C.A.A. van Boeckel, *ACS Symp. Ser.*, 463 (1991) 111–121.
9 J. Gigg, R. Gigg, S. Payne, and R. Conant, *J. Chem. Soc., Perkin Trans. 1*, (1987) 1757–1762.
10 J.J. Oltvoort, C.A.A. van Boeckel, J.H. de Koning, and J.H. van Boom *Synthesis*, (1981) 305–308.
11 E.E. Gilbert, *Chem. Soc. Rev.*, 62 (1962) 549–589.
12 C.E. Dreef, R.J. Tuinman, A.W.M. Lefeber, C.E.E. Elie, G.A. van der Marel, and J.H. van Boom, *Tetrahedron*, 47 (1991) 4709–4722.
13 J. Stawinski, M. Thelin, E. Westman, and R. Zain, *J. Org. Chem.*, 55 (1990) 3503–3506.
14 H.A.M. Willems, G.H. Veeneman, and P. Westerduin, *Tetrahedron Lett.*, in press.
15 J. Gigg, R. Gigg, S. Payne, and R. Conant, *J. Chem. Soc., Perkin Trans. 1*, (1987) 423–429.
16 K. Kakinuma and H.-Y. Li, *Tetrahedron Lett.*, 30 (1989) 4157–4160.
17 D.C. Billington, *Chem. Soc. Rev.*, 18 (1989) 83–122.
18 P.H.J. Carlsen, T. Katsuki, V.S. Martin, and K.B. Sharpless, *J. Org. Chem.*, 46 (1981) 3936–3938.
19 Z.F. Kanyo and D.W. Christianson, *J. Biol. Chem.*, 266 (7) (1991) 4264–4268.
20 H. Luecke and F.A. Quiocho, *Nature (London)*, 347 (1990) 402–406.
21 J.A. Ippolito, R.S. Alexander, and D.W. Christianson, *J. Mol. Biol.*, 215 (1990) 457–471.
22 J.N. Vos, P. Westerduin, and C.A.A. van Boeckel, *Biorg. Med. Chem. Lett.*, 1 (1991) 143–146.
23 A.M. Cooke, R. Gigg and B.V.L. Potter, *J. Chem. Soc., Chem. Commun.*, (1987) 1525–1526.
24 A.L. Willcocks, B.V.L. Potter, A.M. Cooke, and S.R. Nahorski, *Eur. J. Pharmacol.*, 155 (1988) 181–183.
25 R.A.J. Challiss, S.M. Smith, B.V.L. Potter, and S.R. Nahorski, *FEBS Lett.*, 281 (1991) 101–104.