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Laboratory note

Synthesis of α-trinositol related analogues. Structure–activity (analgesic and anti-inflammatory) relationships

S Ballereau[1], N Rehnberg[2], B Spiess[1], J Gigg[3], R Gigg[3], G Schlewer[1]*

[1]*Laboratoire de Pharmacochimie Moléculaire du CNRS, Faculté de Pharmacie, 74, route du Rhin, 67401 Illkirch, France;*
[2]*Perstorp Pharma, S-284 80 Perstorp, Sweden;*
[3]*Division of Lipid and General Chemistry, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK*

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Summary — α-Trinositol analogues, including methyl ethers, deoxy, oxa and aza derivatives were prepared. The parent compound possesses weak analgesic and anti-inflammatory properties. Removal of the non-phosphorylated hydroxyls generates a compound devoid of analgesic activity but which retains the anti-inflammatory property of the parent compound. The protection of these hydroxyls as methyl ethers leads to compounds which keep their anti-inflammatory activity, whereas the replacement of the cyclohexane carbone backbone by a tetrahydropyrane or a piperidine ring leads to compounds which increase the pain.

α-trinositol / inositol phosphate / analgesic activity / anti-inflammatory activity / oxa-inositol / aza-inositol

Introduction

α-Trinositol (D-*myo*-inositol-1,2,6-tris(phosphate) (Ins-(1,2,6)P_3)) [1, 2] **1** is a regioisomer of the well-known D-*myo*-inositol-1,4,5-tris(phosphate), Ins(1,4,5)P_3 **2**. It has been demonstrated that Ins(1,4,5)P_3 is an intracellular second messenger [3]. α-Trinositol does not bind to Ins(1,4,5)P_3 receptors, but possesses interesting anti-inflammatory and weak analgesic activities [1, 4–6].

Binding studies on rat membranes showed a marked influence of the pH, demonstrating that the ionization state of either the molecule or the receptor could play an important role in the strength of binding [7].

The behaviour of the protons present on the phosphate groups of inositol phosphates is largely influenced by the number and relative positions of the neighbouring functions and the cations present in the medium, particularly the alkali and alkali-earth cations [8–10]. The α-trinositol protons can be stabilized by two main factors: electrostatic interactions and hydrogen bonding.

As the hydroxyls can participate in this stabilization, it seemed of interest to prepare some analogues of α-trinositol in which the non-phosphorylated hydroxyls were modified, in order to compare the activity with that of the parent compound. The modifications included either removal of these hydroxyl groups, or transformation of one or all of them into methyl ethers. Other modifications concerned the replacement of the carbon ring backbone by an analogue containing an atom of oxygen or of nitrogen.

Herein we report the synthesis of (±)-(1α,2α,3β)-trihydroxy-cyclohexane-tris(phosphate) **3**, (±)-3,4,5-tri-*O*-methyl *myo*-inositol-1,2,6-tris(phosphate) **4**, and (±)-5-*O*-methyl *myo*-inositol-1,2,6-tris(phosphate) **5** (fig 1). These racemic compounds as well as 1,5-anhydro-D-arabinitol-2,3,4-tris(phosphate) **6** [11] and 1,5-dideoxy-1,5-imino-D-arabitol-2,3,4-tris(phosphate) **7** [12] were submitted to a preliminary screening for analgesic and anti-inflammatory properties. Their toxicity was also investigated.

Synthesis

The key intermediate for the synthesis of (±)-(1α,2α,3β)-trihydroxy-cyclohexane-tris(phosphate) **3** is (±)-(1α,2α,3β)trihydroxy-cyclohexane **8**. Among the reports published on the preparation of this triol [13–15], we selected a slightly modified procedure of

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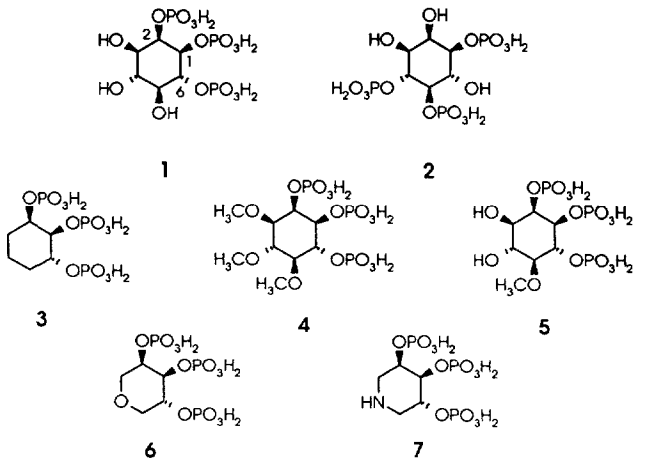


Fig 1. Inositol phosphate and analogues in their acid species.

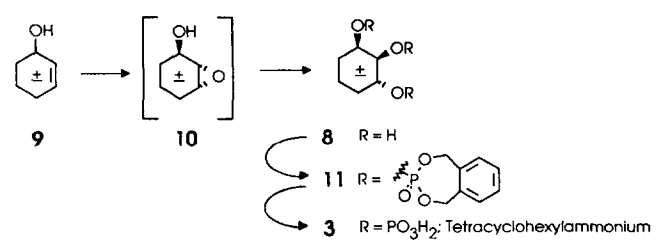
Fredericks and Guthrie [15]. Thus, treatment of 2-cyclohexenol **9**, dissolved in a mixture of water and tetrahydrofuran with *m*-CPBA furnished the epoxide **10**, which was slowly hydrolyzed in situ to give after extraction 45% of crystalline triol **8**. NMR studies confirmed the relative orientations of the three hydroxyl functions. The racemic triol was phosphorylated according to the method of Perich and Johns [16]. Thus, the triol was reacted with *N,N*-diethyl-*O*-xylylene phosphoramidite in the presence of tetrazole, yielding an intermediate tris(phosphite) which was oxidized to the tris(phosphate) **11** by means of *m*-CPBA. Hydrogenolysis with palladium on charcoal as catalyst removed the phosphate protective groups, giving the expected product **3**, which was stabilized as the cyclohexylammonium salt until used for pharmacological studies to avoid elimination and other side reactions (scheme 1).

The synthesis of (±)-3,4,5-tri-*O*-methyl *myo*-inositol-1,2,6-tris(phosphate) **4** starts with racemic 3,4-di-*O*-benzyl-1,2-*O*-isopropylidene *myo*-inositol **12**, obtained by saponification of the crystalline diacetate **13** [17]. The diol **12** was converted into the dimethyl ether **14** and the *O*-isopropylidene group was hydrolyzed to give the diol **15**, which yielded a crystalline diacetate **16**. Tin-mediated methylation of the equatorial hydroxyl group of **15** gave the trimethyl ether **17** and hydrogenolysis removed the benzyl ether groups, yielding the triol **19** which was characterized as the triacetate **20**. Phosphorylation was as described above; but using *N,N*-diisopropylamino dibenzyl phosphoramidite instead of *N,N*-diethyl-*O*-xylylene phosphoramidite led to the protected final product **21**. Hydrogenolysis in glacial acetic acid in the presence of three equivalents of sodium acetate gave the racemic 3,4,5-tri-*O*-methyl *myo*-inositol 1,2,6-tris(sodium hydrogen phosphate) **4** as a white powder (scheme 2).

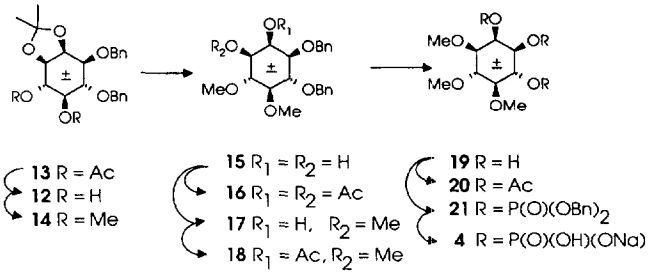
The (±)-5-*O*-methyl *myo*-inositol 1,2,6-tris(phosphate) **5** was prepared from the *p*-methoxybenzyl ether **22** [17]. The remaining free hydroxyl was transformed into methyl ether **23**. The *p*-methoxybenzyl group was removed from **23** by the action of dichlorodicyanobenzoquinone to give the alcohol **24**, which yielded a crystalline acetate **25**. Hydrolysis of the *O*-isopropylidene group from **24** gave the triol **26**, which was phosphorylated as described above, yielding **28**. Subsequent hydrogenolysis in glacial acetic acid in the presence of three equivalents of sodium acetate gave the expected racemic 5-*O*-methyl derivative **5** as a trisodium salt (scheme 3).

Pharmacological properties

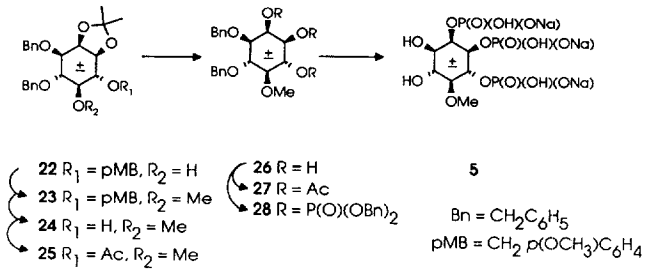
The compounds **1**, **3–7** were tested (**3–5** as racemates) for their acute toxicity according to Irwin's test [18]. The anti-inflammatory properties were tested after iv injection (64 mg/kg) in measuring the reduction of carrageenan-induced foot pad oedema [19]. The analgesic effects were tested against the acetic acid-induced writhing using ip injection (64 mg/kg) [20, 21].



Scheme 1. Synthesis of (±)-(1α,2α,3β)-cyclohexane-triol-tris(phosphate) **3**.



Scheme 2. Synthesis of 3,4,5-tri-*O*-methyl *myo*-inositol-1,2,6-tris(phosphate) **4**.



Scheme 3. Synthesis of 5-*O*-methyl *myo*-inositol-1,2,6-tris(phosphate) **5**.

Discussion

Toxicity appeared only at relatively high doses. All the compounds appeared atoxic at doses up to 256 mg/kg, and toxicity did not seem to be significantly influenced by the substituents (table I).

The anti-inflammatory activity evaluation of the compounds provided various results. The trideoxy derivative **3** significantly reduced the carrageenan-induced oedema. The compound seemed four-fold more potent than aspirin. Masking the non-phosphorylated hydroxyls as methyl ethers led to compounds **4** and **5**, showing relatively similar levels of anti-inflammatory activity to **1**. But replacing the cyclohexane backbone by a tetrahydropyrane or a piperidine ring seemed to suppress this property (compounds **6** and **7**). On the other hand, the latter compounds showed an aggravation in the acetic acid-induced writhing test. The methyl ethers **4** and **5** as well as the deoxy analogue **3** did not possess the analgesic property of the parent compound. However, the analgesic activity of the parent compound remained much lower than that of morphine.

For this preliminary screening we used racemates for compound **3–5**. It should be borne in mind that the two enantiomers may behave differently.

The parent compound **1** possesses both anti-inflammatory and weak analgesic properties. The structural variations around the vicinal tris(phosphate) separate the effects, and confer to the molecule only the anti-inflammatory effect (compound **3**).

Experimental protocols

Melting points were measured on a Mettler PF 62 apparatus and are uncorrected. NMR spectra were recorded either on a Bruker AC 200 spectrometer or on a Jeol FX90Q instrument using the δ scale. Coupling constants are given in Hz. Synthesis of 1,5-anhydro-D-arabinitol-2,3,4-tris(phosphate) **6** and 1,5-dideoxy-1,5-imino-D-arabitol-2,3,4-tris(phosphate) **7** have been described in [11] and [12] respectively. Male rats of NMRI strain, mean weight 22–26 g were used. Aspirin (Synthélabo) and morphine (Coopération Pharmaceutique Française) were dissolved in saline. The tests were carried out at 20–21 °C under artificial lighting between 7.00 and 19.00 h.

Table I. Pharmacological data for the tested compounds.

Compound	*Toxicity*		*Carrageenan-induced oedema (% antagonism)*[a]	*Writhing (% protection)*[a]
No	*256 mg/kg*	*512 mg/kg*		
1	Atox	3/3 < 1 min	32 ± 3	63 ± 5
3	Atox	3/3 < 1 min	50 ± 3	ns
4	Atox	3/3 < 1 min	26 ± 3	ns
5	Atox	2/3 < 1 min	25 ± 3	ns
6	Atox	2/3 < 1 min	ns	− 83 ± 9
7	Atox	3/3 < 1 min	ns	− 61 ± 5
Aspirin			54 ± 2[b]	
Morphine				99 ± 1[c]

Atox: atoxic; ns: not significant; [a]tested dose 64 mg/kg iv; [b]256 mg/kg; [c]4 mg/kg; [a,b, and c]groups of 10 mice; mean value ± standard deviation.

*(±)-1(*R*),2,3(*R*)-Trihydroxy-cyclohexane* ***8***
In a 50 mL flask, *m*-CPBA (0.75 g, 4.35 mmol) was dissolved in 5 mL of a water/THF mixture (5:1). The solution was heated at 40 °C and 2-cyclohexenol **9** (0.4 mL, 0.4 g, 4.07 mmol) was slowly added and heating maintained for 3 h. The flask was then kept overnight at 5 °C. The precipitate of *m*-chlorobenzoic acid was filtered off and the filtrate neutralized by means of a saturated solution of sodium hydrogen carbonate. The solid residue was dissolved in a mixture of ethanol/acetone (1:1). After filtration, the solvents were evaporated giving 0.39 g of a yellow oil, which was crystallized and recrystallized from ethyl acetate. Triol **8** (0.36 g, 37%) was obtained as white crystals, mp = 126.5 °C (lit 124 °C, Beilstein, E III, 6, 6250). ^{1}H-NMR ($(CD_3)_2CO$): 3.90 (dt, 1H, J = 3.1 Hz, J = 2.6 Hz, H_2), 3.69 (ddd, 1H, J = 9.0 Hz, J = 7.7 Hz, J = 4.3 Hz, H_3), 3.6–3.4 (m, 1H, O*H*), 3.30 (dd, 1H, J = 7.7 Hz, J = 3.1 Hz, H_2), 3.1–2.7 (m, 2H, $(OH)_2$), 1.0–1.9 (m, 6H, (CH_2) ring protons).

*(±)-Cyclohexane-1(*R*),2,3(*R*)-tri-*O*-(orthoxylylene)phosphate* ***11***
The triol **8** (84.1 mg, 0.5 mmol) and dried tetrazole (0.28 g, 4.0 mmol) were dissolved in anhydrous THF (10 mL). The flask was placed under an argon atmosphere and diethylamino-1,5-dihydro-2,4,3-benzo-dioxaphosphepine (*N,N*-diethyl-*O*-xylylene phosphoramidite) (0.6 g, 2.4 mmol) in 10 mL dried THF was added and the mixture stirred overnight at room temperature. The flask was cooled at 0 °C and *m*-CPBA (0.55 g, 3.2 mmol) in 10 mL CH_2Cl_2 was added. After 1 h of stirring at room temperature, the solvents were removed under vacuum and the residue redissolved in a mixture of ether/CH_2Cl_2 (2:1) and washed with a 10% solution of $Na_2S_2O_5$ (2 x 30 mL), washed again with a 5% $NaHCO_3$ (2 x 30 mL) and finally with a saturated solution of NaCl. After drying over Na_2SO_4, the solvents were removed under reduced pressure to give a brownish paste which was redissolved in a minimum of CH_2Cl_2 and precipitated in ether. The tris(phosphate) **11** was obtained as a white powder (158 mg, 47.3%) mp = 92–93 °C. ^{1}H-NMR ($CDCl_3$): 7.4–7.2 (m, 12H, $(C_6H_4)_3$), 5.5–5.0 (m, 15H, $(OCH)_3$ + $(OCH_2)_6$), 1.8–1.6 (m, 6H, CH_2 cycle).

*(±)-Cyclohexane-1(*R*),2,3(*R*)-tris(phosphate)* ***3*** *as its tetracyclohexylammonium salt*
The protected tris(phosphate) **11** (158 mg, 0.24 mmol) dissolved in 30 mL of a mixture of ethanol/CH_2Cl_2 (2:1) was hydrogenolyzed under atmospheric hydrogen pressure in the presence of 10% Pd/C (20 mg) for 12 h at 20 °C. The Pd/C was filtered off and the filtrate was evaporated to dryness and redissolved in water (1 mL). Cyclohexylamine (0.5 mL) was slowly added at 0 °C. The salt was precipitated by means of acetone. After decantation the supernatant was removed. The precipitate was washed twice with acetone and finally dried under vacuum. The final product was obtained as a white salt (173 mg, 95%). Anal $C_{30}H_{67}N_4O_{12}P_3$: (C, H, N, P). ^{1}H-NMR (D_2O): 4.6–4.5 (m, 1H, H_3), 4.4–4.3 (m, 1H, H_1), 4.0–4.1 (m, 1H, H_2), 3.7–3.3 (m, 4H, $[H_2$-C(H)$C_5H_{10}]_4$), 2.5–1.3 (m, 46H $[H_2N$-C(H)$C_5H_{10}]_4$, $(H_4)_2$, $(H_5)_2$, $(H_6)_2$).

*(±)-1,6-di-*O*-Benzyl-4,5-di-*O*-methyl* myo*-inositol* ***15*** *and the diacetate* ***16***
The diol **12** (3.0 g, 7.5 mmol), prepared by saponification of the crystalline diacetate **13** [17] was treated with an excess of sodium hydride and methyl iodide in DMF and the product isolated in the usual way. The crude product was purified by column chromatography on silica gel eluted with a 1:1 ether/light petroleum mixture, yielding **14** (2.34 g, 73%) as an oil. This was treated under reflux for 30 min with 1 M HCl/MeOH (1:9) (50 mL). An excess of $NaHCO_3$ was added to the cooled solution and the solvents were evaporated. The product was extracted from the residue with CH_2Cl_2 and recrystallization from 1:10 ethyl acetate/light petroleum gave the diol **15** (1.5 g, 71%) as crystals, mp = 77–79 °C. Anal $C_{22}H_{28}O_6$ (C, H).

A portion was treated with acetic anhydride-pyridine at 50 °C for 8 h and the product isolated in the usual way to give the diacetate **16**, which was recrystallized from 1:10 ethyl acetate/light petroleum, mp = 98–100 °C. Anal $C_{26}H_{32}O_8$: (C, H). ^{1}H-NMR ($CDCl_3$): 7.3–7.2 (m, 10H $(C_6H_5)_2$), 5.67 (t, J = 2.7 Hz, 1H, H_2), 4.9–4.3 (m, 5H, H_3 $(CH_2C_6H_5)_2$), 3.9–3.0 (m, 4H, H_1, H_4, H_5, H_6), 3.66 and 3.56 (2 s, each 3H, $(OCH_3)_2$), 2.15 and 2.06 (2 s, each 3H, $(H_3CCO)_2$).

*(±)-1,6-di-*O*-Benzyl-3,4,5-tri-*O*-methyl* myo*-inositol* ***17***
A mixture of the diol **15** (1.4 g, 3.6 mmol), dibutyl tin oxide (1.0 g, 4.0 mmol), tetrabutylammonium bromide (1.28 g, 4.0 mmol), acetonitrile (50 mL) and methyl iodide (10 mL, 160 mmol) was heated under reflux with a Soxhlet apparatus containing 3 Å molecular sieves for 24 h when TLC (ether) showed a major product (R_f 0.50). The solvents were evaporated from the cooled solution and the residue was stirred with ether (100 mL) and saturated $NaHCO_3$ (100 mL) for 2 h to precipitate the tin derivative. The mixture was filtered through a Celite pad and the ether layer was separated, dried (K_2CO_3) and concentrated. Column chromatography on silica gel eluted with ether gave the alcohol **17** (1.37 g, 94%), mp = 82–83 °C. Anal $C_{23}H_{30}O_6$: (C, H). ^{1}H-NMR ($CDCl_3$): 7.33 (s, 10H, $(C_6H_5)_2$), 4.84 and 4.72 (2 s, each 2H, $(CH_2C_6H_5)_2$), 4.24 (t, J = 2.5 Hz, 1H, H_2), 4.0–2.9 (m, 5H, H_1, H_3, H_4, H_5, H_6), 3.64, 3.62 and, 3.49 (3 s, each 3H, $(OCH_3)_3$).

A portion was acetylated with acetic anhydride-pyridine at 60 °C for 8 h to give the acetate **18** as a syrup. ^{1}H-NMR ($CDCl_3$): 7.4–7.3 (m, 10H $(C_6H_5)_2$), 5.77 (t, J = 2.5 Hz, 1H, H_2), 4.81 (AB, J_{AB} = 12.0 Hz, $\Delta\delta$ 0.14, 2H, $CH_2C_6H_5$), 4.62 (AB, J_{AB} = 12.0 Hz, $\Delta\delta$ 0.16 2H, $CH_2C_6H_5$), 3.8–3.0 (m, 5H, H_1, H_3, H_4, H_5, H_6), 3.66, 3.62 and 3.42 (3 s, each 3H $(OCH_3)_3$), 2.14 (s, 3H, H_3CCO).

*(±)-1,2,6-tri-*O*-Acetyl-3,4,5-tri-*O*-methyl* myo*-inositol* ***20***
A solution of the alcohol **17** (1.2 g, 2.98 mmol) in glacial acetic acid (20 mL) with 10% Pd/C (270 mg) was stirred under hydrogen at atmospheric pressure and room temperature for 36 h. The catalyst was removed by filtration, washed with acetic acid and the eluate was concentrated. Recrystallization of the crystalline product from EtOH (or EtOAc) gave the triol **19** (600 mg, 90%), mp = 172–174 °C. This was acetylated with acetic anhydride-pyridine at 60 °C for 9 h to give the triacetate **20**, mp = 106–108 °C. Anal $C_{15}H_{24}O_9$: (C, H). ^{1}H-NMR ($CDCl_3$): 5.63 (t, J = 2.7 Hz, 1H, H_2), 5.34 (t, J = 9.2 Hz, 1H, H_6), 4.82 (dd, J = 10.4 Hz, J = 2.4 Hz, 1H, H_1), 3.6–3.0 (m, 3H, H_3, H_4, H_5), 3.61, 3.55, 3.41 (3s, each 3H, $(OCH_3)_3$), 2.15, 2.08, 1.99 (3s, each 3H $(H_3CCO)_3$).

*(±)-3,4,5-tri-*O*-Methyl* myo*-inositol-1,2,6-tris(dibenzylphosphate)* ***21***
The triol **19** (460 mg, 2.07 mmol) was dissolved in warm CH_3CN (30 mL). Toluene (100 mL) was added and the solvents were evaporated to azeotrope any water from crystallization. The residual product was dissolved in CH_3CN (30 mL) and CH_2Cl_2 (30 mL) and tetrazole (870 mg, 12.4 mmol) and a solution of *N,N*-diisopropylamino-dibenzylphosphoramidite [22] (4.3 g, 12.4 mmol) in CH_2Cl_2 (70 mL) was added and the solution stirred at room temperature for 2 h, then cooled to 0 °C and a solution of technical *m*-chloroperbenzoic acid (4.9 g) in CH_2Cl_2 (40 mL) was added and the mixture stirred

for 1 h. The solution was concentrated to remove the CH_3CN and the residue diluted with CH_2Cl_2 (50 mL).

The solution was washed successively with aqueous sodium bisulfite solution and saturated $NaHCO_3$, dried ($MgSO_4$) and concentrated. Column chromatography on silica gel eluted with a 1:1 mixture of ether/EtOAc removed the non-polar byproducts, and further elution with EtOAc gave the tris-(dibenzylphosphate) derivative **21** (1.76 g, 85%) as a syrup. Anal $C_{51}H_{57}O_{15}P_3$: (C, H, P). ^{1}H-NMR ($CDCl_3$): 5.4–4.7 (m, 15H, (CH_2, $C_6H_5)_6$, and 3H) 4.30 (t, J = 8.3 Hz, 1H), 3.53, 3.51, 3.45 (3s, each 3H, ($OCH_3)_3$), 3.10 (t, J = 9.2 Hz, 2H). ^{31}P-NMR ($CDCl_3$): –2.42, –1.28, –0.87

*(±)-3,4,5-tri-*O*-Methyl* myo*-inositol-1,2,6-tris(sodium hydrogenphosphate) 4*

A solution of the tris(dibenzylphosphate) **21** (1.71 g, 1.7 mmol) in glacial acetic acid (25 mL) containing sodium acetate (420 mg, 5.12 mmol, 3 equiv) and 10% Pd/C (500 mg) was stirred at atmospheric hydrogen pressure and at room temperature for 20 h. The catalyst was removed by filtration through a Celite pad and the residue was washed with acetic acid and water. The filtrate was concentrated and toluene (3 x 50 mL) was evaporated from the residue to remove traces of acetic acid. Evaporation of EtOH from the residue then left the solid sodium salt of the expected compound **4** (900 mg, 100%). ^{31}P-NMR (D_2O): 0.0 (2P), 2.56 (1P).

*(±)-1,6-di-*O*-Benzyl-2,3-*O*-isopropylidene-4-*O*-*p*-methoxybenzyl-5-*O*-methyl* myo*-inositol 23*

A stored sample of the alcohol **22** (prepared as described in [17], where it was obtained as a syrup), had crystallized, and recrystallization from 1:20 EtOAc/light petroleum gave a crystalline product, mp = 68–70 °C. Anal $C_{31}H_{36}O_7$: (C, H). ^{1}H-NMR ($CDCl_3$): 7.4–6.8 (m, 14H, ($C_6H_5)_2$, and C_6H_4), 5.0–4.5 (m, 6H, ($CH_2C_6H_5)_2$ and $CH_2C_6H_4$), 4.4–3.0 (m, 2H, ring protons), 3.9–3.4 (m, 4H, ring protons), 3.79 (s, 3H, OCH_3), 2.61 (broad s, 1H, O*H*), 1.51, 1.35 (2s, each 3H, $C(CH_3)_2$). The alcohol **22** (1.0 g, 2.3 mmol) was treated with sodium hydride and methyl iodide in DMF, and the product was isolated in the usual way. Column chromatography on silica gel eluted with 1:2 ether/light petroleum mixture gave **23** (950 mg, 77%) as a syrup. Anal $C_{32}H_{38}O_7$: (C, H). ^{1}H-NMR ($CDCl_3$): 7.4–6.8 (m, 14H, ($C_6H_5)_2$ and C_6H_4), 4.9–4.6 (m, 6H, ($CH_2C_6H_5)_2$ and $CH_2C_6H_4$), 4.3–1.0 (m, 2H, ring protons), 3.7–3.6 (m, 3H, ring protons), 3.79, 3.58 (2s, each 3H, ($OCH_3)_2$), 3.13 (t, J = 8.5 Hz, 1H), 1.51, 1.34 (2s, each 3H, $C(CH_3)_2$).

*(±)-1,6-di-*O*-Benzyl-2,3-*O*-isopropylidene-5-*O*-methyl* myo*-inositol 24*

A mixture of the *p*-methoxybenzyl ether **23** (1.02 g, 1.9 mmol), dichlorodicyanoquinone (500 mg, 2.2 mmol), CH_2Cl_2 (30 mL) and water (2 mL) was stirred at room temperature for 1.5 h when TLC (1:1, ether/light petroleum) showed conversion of **23** (R_f 0.6) into a product (R_f 0.15) and *p*-methoxybenzaldehyde (R_f 0.65). The mixture was diluted with CH_2Cl_2 and the organic layer washed with saturated $NaHCO_3$, dried over K_2CO_3 and concentrated. Column chromatography on silica gel eluted with a 1:1 ether/light petroleum removed the aldehyde, and elution with ether gave the expected alcohol **24** (691 mg, 76%) as a syrup. Anal $C_{24}H_{30}O_6$: (C, H). ^{1}H-NMR ($CDCl_3$): 7.33 (s, 10H, ($C_6H_5)_2$), 4.75 (s, 2H, $CH_2C_6H_5$), 4.74 (AB, Δδ 0.16, J_{AB} = 10.2 Hz, 2H, $CH_2C_6H_5$), 4.3–4.2 (m, 1H, ring proton), 4.0–3.6 (m, 4H, ring protons), 3.58 (s, 3H, OCH_3), 2.96 (t, J = 8.5 Hz, 1H, ring proton), 2.69 (broad s, 1H, O*H*), 1.54, 1.34 (2s, each 3H, $C(CH_3)_2$). This gave a crystalline acetate **25**; mp = 77–79 °C (from light petroleum), ^{1}H-NMR ($CDCl_3$): 7.34 (s, 10H, ($C_6H_5)_2$), 5.23 (dd, J = 9.8 Hz, J = 7.3 Hz, 1H, H_4), 4.82 (s, 2H, $CH_2C_6H_5$), 4.79 (AB, Δδ 0.14, J_{AB} = 10.5 Hz, 2H, $CH_2C_6H_5$), 4.24 (t, J = 4.3 Hz, 1H, ring proton), 4.1–3.8 (m, 2H, ring protons), 3.65 (dd, J = 8.8 Hz, J = 3.9 Hz, 1H, ring proton), 3.50 (s, 3H, OCH_3), 3.08 (t, J = 8.5 Hz, 1H, ring proton), 2.09 (s, 3H, $OCCH_3$), 1.59, 1.33 (2s, each 3H, $C(CH_3)_2$).

*(±)-1,6-di-*O*-Benzyl-5-*O*-methyl* myo*-inositol 26 and the triacetate 27*

The alcohol **24** (620 mg, 1.5 mmol) was treated at reflux for 30 min with 1 M HCl/MeOH (1:9, 30 mL). An excess of $NaHCO_3$ was added, the mixture was concentrated and the product extracted with CH_2Cl_2. Column chromatography on silica gel eluted with EtOAc gave the triol **26** (520 mg, 91%) as crystals melting at 95–97 °C (from MeOH/water or EtOAc/light petroleum). Anal $C_{21}H_{26}O_6$, 0.25 H_2O: (C, H). A portion was acetylated as usual at 60 °C for 12 h and the product isolated in the usual way to give a crystalline triacetate **27**; mp = 99–101 °C (from EtOAc/light petroleum). Anal $C_{27}H_{32}O_9$: (C, H). ^{1}H-NMR ($CDCl_3$): 7.30 (broad s, 10H, ($C_6H_5)_2$), 5.70 (t, J = 2.4 Hz, 1H, H_2), 5.39 (t, J = 10.0 Hz, 1H, H_4), 4.9–4.4 (m, 5H, ($CH_2C_6H_5)_2$, and H_3), 3.6–3.5 (m, 1H, H_1), 3.56 (s, 3H, OCH_3), 3.24 (t, J = 9.2 Hz, 1H, H_5), 2.16, 2.08, 2.00 (3s, each 3H, ($OCCH_3)_2$).

*(±)-3,4-di-*O*-Benzyl-5-*O*-methyl* myo*-inositol-1,2,6-tris(dibenzylphosphate) 28*

The triol **26** (900 mg, 2.37 mmol) was taken up in CH_2Cl_2 (20 mL), toluene (100 mL) was added and the solution was concentrated to remove the water from crystallization present in the triol **26**. The residue was taken up into CH_2Cl_2 (50 mL) and *N,N*-diisopropylamino-dibenzylphosphoramidite (5.0 g, 14.5 mmol) and a solution of tetrazole (1.03 g, 14.7 mmol) in CH_3CN (25 mL) was added and after 1 h stirring at room temperature, TLC (EtOAc) showed conversion of the triol (R_f 0.25) into a product (R_f 0.95). The solution was cooled at 0 °C and *t*-butylhydroperoxide (2.0 mL) added. After 30 min, a 10% solution of sodium metabisulfite (10 mL) was added and the solution concentrated to remove the CH_3CN. The residue was diluted with CH_2Cl_2 (50 mL) and the solution washed with a 10% solution of sodium metabisulfite and a saturated solution of $NaHCO_3$, dried over $MgSO_4$ and concentrated. Column chromatography on silica gel eluted with 1:1 ether/light petroleum removed the apolar byproducts and elution with ether gave compound **28** (1.50 g, 55%) as a syrup. Anal $C_{63}H_{65}O_{15}P_3$: (C, H, P). ^{31}P-NMR ($CDCl_3$): –2.29, –1.28, –0.81.

*(±)-5-*O*-Methyl* myo*-inositol-1,2,6-tris(sodium hydrogenphosphate) 5*

A solution of the tris(dibenzylphosphate) **28** (1.46 g, 1.26 mmol) in glacial acetic acid (30 mL) containing sodium acetate (311 mg, 3.79 mmol, 3 equiv) and 10% Pd/C (600 mg) was stirred at atmospheric pressure at room temperature for 20 h. The product **5** (630 mg, 100%) was isolated as described for compound **4** and obtained as a white solid. ^{31}P-NMR (D_2O): –0.2 (1P), + 0.67 (2P).

Irwin's test [18]

Mice were administered the test substance (three animals per tested dose) and were observed in comparison with a control group (saline). Mortality, sedation, excitation, aggressiveness, Straub, writhes, convulsions, tremor exophthalmos, salivation, lacrimation, piloerection, defaecation, fear, traction, reactivity to touch, loss of righting reflex, sleep, motor uncoordination,

muscle tone, stereotypies, catalepsy, grasping, ptosis, difficulty in respiration, corneal reflex, analgaesia, gait, rectal temperature and pupil diameter were observed 15, 30, 60, 120, 180 min and 24 h after administration.

Carrageenan oedema test [19]
Mice (groups of 10 animals) were injected with a carrageenan solution into the lower surface of both hind-paws (0.75 mg per paw in 0.05 mL). The animals were sacrificed 3.5 h later by a blow to the cervical vertebrae and the hind-paws sectioned and weighed.

Acetic acid-induced writhing test [20, 21]
Mice (groups of ten animals) were injected ip with 0.5% acetic acid. The number of writhes were counted for 10 min beginning 3 min after acetic acid injection. The tested compounds were administered iv 5 min before acetic acid.

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