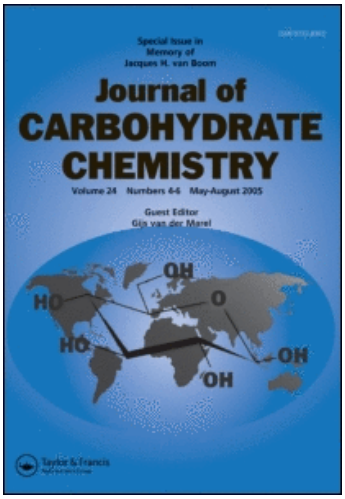


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# SYNTHESIS OF AN ACYLOXYMETHYL PRODRUG OF THE INOSITOL PHOSPHATE α-TRINOSITOL

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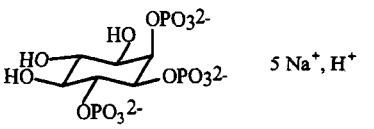
## ABSTRACT

Acyloxymethylation of an acylated silver salt of α-trinositol gives, after deprotection, membrane permeable 1D-*myo*-inositol 1,2,6-tris(ethoxycarbonyloxymethyl sodium phosphate). The acyl groups, 3-(4,5-methylenedioxy-2-nitrophenyl)propanoyl, are cleaved by hydrogenolysis.

## INTRODUCTION

α-Trinositol (1D-*myo*-inositol 1,2,6-tris(dihydrogen phosphate) pentasodium salt, **1**) can produce antiinflammatory and analgesic properties of pharmaceutical interest[1] and is prepared with high optically purity (ee >98 %) in large quantities by fermentation of phytic acid (inositol hexakisphosphate) using baker's yeast.[2]




**1**

The ionic nature of α-trinositol makes it membrane impermeable and limits clinical applications as oral administration is ineffective. Consequently, a program designed to increase the oral bioavailability was initiated and partly dedicated to the synthesis of prodrugs.[3] This letter presents and discusses the synthesis of the acyloxymethyl prodrug 1D-*myo*-inositol 1,2,6-tris-(ethoxycarbonyloxymethyl sodium phosphate) (7).

## RESULTS AND DISCUSSION

We have observed that tris(monoalkyl) esters of α-trinositol, although negatively charged, pass through intestinal membranes. Simple inositol alkyl phosphates may, however, be too stable to ensure a reasonable rate of drug recovery. A plausible alternative is to structurally modify α-trinositol to methylene double esters, acyloxymethyl phosphates, prepared by alkylation of inositol phosphate silver salts with an acyloxymethyl halide.[4]

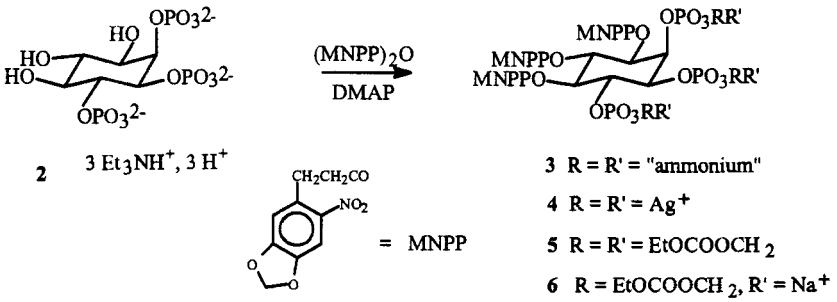


Alkylation of the phosphates of α-trinositol requires protection of the hydroxyl functionalities to suppress side reactions, e.g., phosphate migration. The susceptibility of the double esters to nucleophilic attack necessitates careful selection of the protective groups. Several obvious protective groups were tested without appreciable success. Frequently, the protective group reagent, e.g., benzyl chloride, bromide, and trichloroacetimidate, alkylsilyl chlorides, benzyl and *t*-butyl pyrocarbonates and chloroformates, 2,4-dinitrophenylsulphenyl chloride, and butansulphonyl chloride, activated the phosphate grouping giving rise to *inter alia* pyro- and cyclic phosphates as was evident from $^{31}$P NMR spectra.

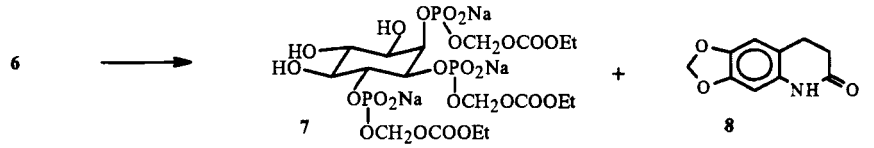
Acylation of α-trinositol is a quite facile reaction[5] but unsubstituted alkanoates will not fulfil the selectivity requirement in the final deprotection (see below). Proper substitution may, however, improve selectivity. Esters of 4-pentenoic acid have successfully been used to protect the anomeric position of carbohydrates.[6] Deprotection is initiated by mild electrophiles, e.g., iodine or *N*-bromosuccinimide. Application of this technique to the protection of α-trinositol gave unsatisfactory results. Esterification with 4-pentenoic acid anhydride of α-trinositol gave the expected product. Attempted deprotection with mild electrophiles (see above) gave halohydrins that only slowly lactonized with concomitant recovery of the hydroxyl functionalities. The rate of lactonization was increased by methyl substitution as was evident from studies of the model compound methyl 3,3-dimethyl-4-pentenoic acid. The corresponding α-trinositol triester could, however not be prepared due to steric reasons.[5]

Finally, success was gained by using a nitrophenylalkanoate. A few were considered but rejected, e.g., 3-(*o*-nitrophenyl)propanoate, 3-(2,4-dinitrophenyl)propanoate, and 2-

(*o*-nitrophenyl)acetate, on grounds of expected problems of isomeric purity and stability. 3-(4,5-Methylenedioxy-2-nitrophenyl)propanoate (MNPP) did, however, fulfil our requirements. MNPP anhydride was synthesized from commercially available 3-(3,4-methylenedioxyphenyl)-2-propenoic acid by hydrogenation to the corresponding propanoic acid followed by nitration and condensation with dicyclohexylcarbodiimide. Acylation of the α-trinositol triethylammonium salt **2** gave ester **3**. This was converted *in situ* to silver salt **4**.[7] Alkylation with chloromethyl ethyl carbonate gave non-ionic ester **5**. Sodium iodide in refluxing acetone gave salt **6**.




Hydrogenation (Pd(C)/HOAc) of nitroderivative **6** gave an *o*-aminophenylpropanoate that spontaneously cyclized to give the final product **7** and the insoluble quinolinone **8**. Prodrug **7** was isolated and used as such after centrifugation, removal of solvents and precipitation from ethanol.




After intraduodenal administration to minipigs,[8] ester **7** was absorbed as was evident after acid hydrolysis and analysis of plasma samples. Before hydrolysis only low concentrations of α-trinositol could be detected. Conclusively, the prodrug did pass the intestinal membranes but no appreciable reconversion to the parent drug took place. Since the pharmacokinetic of the prodrug is unknown it is not possible to acurately calculate the fraction of absorbed ester **7**.

By analogy with the enzymatic hydrolysis of diethyl succinate to monoethyl succinate, an anionic product that is not further degraded, we hypothesise that the faliure of **7** to undergo deprotection is due to the presence of negative charges in the proximity of the ethoxycarbonyloxy groups.[4b,9]

## EXPERIMENTAL[10]

**3-(4,5-Methylenedioxy-2-nitrophenyl)propanoic acid.** 3-(3,4-Methylenedioxyphenyl)propanoic acid[11] (60 g, 0.31 mol) in acetic acid (200 mL) was added with stirring during 1 h to nitric acid (6 M, 360 mL) at 30 °C. The heterogenous reaction mixture was added to ice and the precipitate was filtered off and washed free from nitric acid with water to give the title product (59 g, 80%): mp 157-160 °C (EtOAc); $^1$H NMR (DMSO-$d_6$) δ 12.2 (s, 1 H, OH), 7.50 and 7.02 (2 s, 2 H, ArH), 6.17 (s, 2 H, $OCH_2O$), 2.98 (t, 2 H, J = 7.6 Hz, $ArCH_2$), 2.54 (t, 2 H, J = 7.6 Hz, $CH_2CO$); $^{13}$C NMR (DMSO-$d_6$) δ 173.3, 151.4, 146.2, 142.3, 132.5, 110.2, 104.9, 103.1, 34.2, 27.9.

Anal. Calcd for $C_{10}H_9NO_6$: C, 50.2; H, 3.8; N, 5.9. Found: C, 50.4; H, 3.6; N, 5.8.

**3-(4,5-Methylenedioxy-2-nitrophenyl)propanoic acid anhydride (MNPP anhydride).** 3-(4,5-Methylenedioxy-2-nitrophenyl)propanoic acid (59 g, 0.25 mol) and dicyclohexylcarbodiimide (26.2 g, 0.127 mol) in dichloromethane/ethyl acetate (800 mL 1:1) was refluxed overnight. Solvents were removed and acetone (490 mL) was added. The mixture was heated to reflux and *N,N'*-dicyclohexylurea was filtered off. The filtrate was cooled to 5 °C. The precipitate was filtered off and washed with cool acetone to give the title compound (33 g, 58%): mp 126-128 °C; $^1$H NMR (DMSO-$d_6$) δ 7.54 (s, 1 H, ArH), 7.07 (s, 1 H, ArH), 6.17 (s, 2 H, $OCH_2O$), 3.13 (t, 2 H, J = 7.6 Hz, $ArCH_2$), 2.88 (t, 2 H, J = 7.6 Hz, $CH_2CO$); $^{13}$C NMR (DMSO-$d_6$) δ 168.3, 151.5, 146.4, 142.4, 131.5, 110.4, 105.1, 103.2, 35.0, 27.2.

Anal. Calcd for $C_{20}H_{16}N_2O_5$: C, 52.2; H, 3.5; N, 6.1. Found: C, 52.4; H, 3.4, 6.0.

**1D-*myo*-Inositol 1,2,6-tris(dihydrogen phosphate) triethylamine salt (2).** α-Trinositol (22.1 g, water content 10 %, 37.5 mmol) was dissolved in water and passed through a column of Dowex® (50 W x 8, $H^+$, 100 eq) with water as eluent. Triethylamine (15.2 g, 150 mmol, 4 eq) was added and the volume adjusted with water to 1 L. Removal of solvent gives a salt with an average N/P-ratio of 1.

**1D-3,4,5-Tri-*O*-[3-(4,5-methylenedioxy-2-nitrophenyl)propanoyl]-*myo*-inositol 1,2,6-tris(dihydrogen phosphate) 4-(dimethylamino)pyridine triethylamine salt (3).** Water was removed from a sample of the ammonium salt (2) stock solution (400 mL, 15 mmol) and residual moisture was removed by repeated co-evaporation with DMF. DMAP (7.33 g, 60 mmol), dichloromethane (700 mL) and MNPP anhydride (48.3 g, 105 mmol) was added. By stirring for 20 h the mixture slowly became homogenous. Methanol (100 mL) was added to decompose anhydrides. Solvents were removed, the residue was dissolved in methanol (250 mL), and added dropwise with stirring to dry diethyl ether (2.5 L). The ether solution was decanted and the dried residue was dissolved in water/methanol (250 mL, 3:2). The solution was used in the next step. The $^1$H NMR

spectrum of **3** was poorly resolved but established the cations to be derived from DMAP and triethylamine in a ratio of 1.9 and 0.3 per inositol, respectively. $^1$H NMR spectroscopy of the corresponding sodium salt gave a spectrum of improved resolution: $^1$H NMR ($CD_3COOD$) δ 7.50, 7.48, 7.45, 6.14, 6.12, and 6.10 (6 s, 6 H, ArH), 6.96, 6.94, and 6.92 (3 s, 6 H, $OCH_2O$), 5.60 (t, 1 H, J = 10.1 Hz, H-4), 5.42 (t, 1 H, J = 9.5 Hz, H-5), 5.20 (d, 1H, J = 10.9 Hz, H-3), 5.10 (d, 1 H, J = 9.3 Hz, H-2), 4.76 (q, 1 H, J = 10.3 Hz, H-6), 4.56 (t, 1 H, J = 9.9 Hz, H-1), 3.12 (m, 6 H, $ArCH_2$), 2.73 (m, 6 H, $CH_2CO$).

**1D-3,4,5-Tri-*O*-[3-(4,5-methylenedioxy-2-nitrophenyl)propanoyl]-myo-inositol 1,2,6-tris[bis(ethoxycarbonyloxymethyl) phosphate] (5).** The above solution of ester **3** (ca. 15 mmol) was passed trough a column of Dowex® (50 W x 8, $H^+$, 60 eq) with water/methanol (3:2) as eluent and immediately treated with silver carbonate (20.7 g, 75 mmol). The slurry was stirred for 1 h, solvent was removed, residual water and methanol were removed by repeated co-evaporations with dry acetonitrile, and the residue dried *in vacuo*. The silver salt was suspended in dry acetonitrile (500 mL) and chloromethyl ethyl carbonate[12] (20.3 g, 150 mmol) was added. The mixture was refluxed for 2 d in the dark and the solution was decanted. Acetonitrile was evaporated and the crude product was chromatographed on silica (1.4 L, 15-40 μm) with heptane/ethyl acetate (3:7) as eluent to give **5** (14.3 g, 56 %, from **2**). $^1$H NMR ($CDCl_3$) δ 7.46 (br s, 2 H, ArH), 7.43 (s, 1 H, ArH), 6.91, 6.87, and 6.85 (3 s, 3 H, ArH), 6.07 (br s, 4 H, $OCH_2O$), 6.05 (s, 2 H, $OCH_2O$), 5.82-5.58 (m, 12 H, $POCH_2O$), 5.48 (t, 1 H, J = 10.2 Hz, H-4), 5.27 (t, 1 H, J = 10.1 Hz, H-5), 5.25 (br d, 1 H, J = 10 Hz, H-3), 5.08 (br d, 1H, J = 10.5 Hz, H-2), 4.86 (q, 1 H, J = 9.5 Hz, H-6), 4.67 (br t, 1 H, J = 9.2 Hz, H-1), 4.31-4.17 (m, 12 H, $CH_3CH_2$), 3.24-3.01 (m, 6 H, $ArCH_2$) 2.89-2.57 (m, 6 H, $CH_2CO$), 1.40-1.22 (m, 18 H, $CH_3$).

**1D-3,4,5-Tri-*O*-[3-(4,5-methylenedioxy-2-nitrophenyl)propanoyl]-*myo*-inositol 1,2,6-tris(ethoxycarbonyloxymethyl sodium phosphate) (6).** **5** (14.2 g, 8.4 mmol) and sodium iodide (4.5 g, 30 mmol) was dissolved in refluxing acetone (500 mL). After 17 h the reacton mixture was allowed to cool and the volume was reduced to 0.2 L. By dropwise addition of the acetone solution to ethanol (1 L) the product was precipitated and isolated by filtration to give, after washing with ethanol and drying *in vacuo*, **6** (11.4 g, 92 %). $^1$H NMR ($CD_3COOD$) δ 7.49, 7.48, 7.44, 7.02, 6.97, and 6.96 (6 s, 6 H, ArH), 6.14, 6.13, and 6.11 (3 s, 6 H, $OCH_2O$), 5.85-5.52 (m, 7 H, $POCH_2O$ and H-4), 5.42 (t, 1 H, J = 9.5 Hz, H-5), 5.30 (br d, 1 H, J = 10.2 Hz, H-3), 5.20 (br d, 1 H, J = 9.1 Hz, H-2) 4.86 (q, 1 H, J = 9.7 Hz, H-6), 4.62 (br t, 1 H, J = 9.5 Hz, H-1), 4.33-4.19 (m, 6 H, $CH_3CH_2$), 3.31-3.00 (m, 6 H, $ArCH_2$), 2.98-2.57 (m, 6 H, $CH_2CO$), 1.36-1.26 (m, 9 H, $CH_3$).

**1D-*myo*-Inositol 1,2,6-tris(ethoxycarbonyloxymethyl sodium phosphate) (7).** **6** was dissolved in acetic acid (250 mL) and hydrogenated (1 atm, 20 °C) over Pd(C) (8 g, 5 %). The reaction was complete within 20 h. Insoluble quinolinone was removed by centrifugation and acetic acid evaporated. The residue was suspended in water and insoluble material was removed by centrifugation. The water solution was extracted twice with dichloromethane before evaporation. The residue was dissolved in water (10 mL) and added dropwise to ethanol (350 mL). The precipitate was filtered off, washed with ethanol and dried to give **7** (5.10 g, 82 %): $[\alpha]_D^{21}$ -27.4° (*c* 0.6, $H_2O$); IR 3400 (OH), 1750 (C=O), 1269 (C-O) $cm^{-1}$; $^1H$ NMR ($D_2O$) δ 5.69-5.53 (m, 6 H, $OCH_2O$), 4.81 (dt, 1 H, J = 8.9, 2.1 Hz, H-2), 4.37 (q, 1 H, J = 9.1 Hz, H-6), 4.31, 4.31, 4.31 (3 q, 2 H each, J = 7.2 Hz, $CH_3CH_2$), 4.14 (td, 1 H, J = 9.9, 2.1 Hz, H-1), 3.78 (dd, 1 H, J = 9.9, 9.4, H-4), 3.63 (dd, 1 H, J = 10.0, 2.2 Hz, H-3), 3.54 (t, 1 H, J = 9.1 Hz, H-5), 1.34, 1.34, 1.33 (3 t, 3 H each, J = 7.2 Hz, $CH_3CH_2$); $^{13}C$ NMR ($D_2O$) δ 157.6, 157.5, 157.5, 88.9 (m), 79.9 (d), 79.6 (t), 76.7, 76.0 (d), 75.3, 73.2, 68.3, 16.2; $^{31}P$ NMR ($D_2O$) δ -0.51 (P-6), -0.97 (P-2), -1.58 (P-1).

Anal. Calcd for $C_{18}H_{30.3}Na_{2.7}O_{24}P_3$: Na, 7.9; P, 11.8. Found: Na, 7.7; P, 11.4.

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