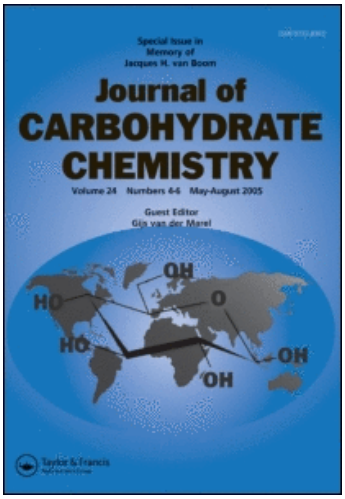

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A SEARCH FOR QUANTITATIVE ACYLATION OF α-TRINOSITOL (1D-*MYO*-INOSITOL 1,2,6-TRIS(DIHYDROGEN PHOSPHATE) PENTASODIUM SALT)

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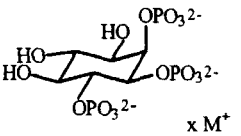
ABSTRACT

The acylation of α-trinositol is very sensitive to reaction conditions. Competing condensation reactions may give pyrophosphates and cyclic phosphates. Treatment of a *tert*-ammonium salt corresponding to α-trinositol with carboxylic acid anhydride and DMAP gives a good yield of the expected esters.

INTRODUCTION

α-Trinositol (1D-*myo*-inositol 1,2,6-tris(dihydrogen phosphate) pentasodium salt), **1**, can produce interesting physiological effects, such as acting as an antiinflammatory and analgesic compound.[1] This compound is prepared with high optical purity (ee > 98%) by fermentation of phytic acid (inositol hexakisphosphate) using baker's yeast and is one of few inositol phosphates available in kg quantities.[2]



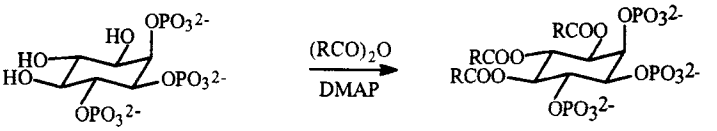
1, x M^+ = 5 Na^+, H^+

2, x M^+ = 3 EtN(*i*-Pr)$_2H^+$, 3 H^+

As part of a program dedicated to the synthesis of analogues and prodrugs of α-trinositol we became interested in 3,4,5-tri-*O*-carboxyl esters. It was found that some aliphatic esters are physiologically active *per se* and we therefore prepared a series of these esters (**3a-3g, 3n-3p**) for biological screening. The long-chain esters are amphiphilic and therefore membrane damaging. To avoid these effects but retain the steric influence of the substituent, some hydrophilic oxa-analogues were prepared (**3h, 3j**, and **3l**) by hydrogenolysis of the corresponding benzyl ethers (**3i, 3k**, and **3m**). To prepare derivatives with modified phosphate groups, e.g. acyloxymethyl derivatives via alkylation, it was deemed necessary to block the hydroxyl groups.[3] Nitropiperonylacetate **3q** provides adequate protection and is removed mildly by hydrogenation. Reduction of the nitro group results in spontaneous cyclization to a tetrahydroquinolinone and liberation of the trihydroxy moiety. The α-trinositol esters show increased resistance towards phosphatases and increased plasma protein binding, possibly indicating longer half-lives *in vivo*.

RESULTS AND DISCUSSION

The acylation of an alcohol is usually a rather trivial task and many good procedures are known.[4] The acylation of α-trinositol proved, however, to be very sensitive to reaction conditions. Being a sodium salt, α-trinositol is insoluble in most solvents except water. By exchanging sodium ions for *tert*-ammonium (from triethylamine or *N*-ethyldiisopropylamine) the inositol phosphate was made soluble in dichloromethane, chloroform, acetonitrile, pyridine, or DMF. The solubility in ethyl acetate, dioxane, tetrahydrofuran, or diethylene glycol diethyl ether remained low. Many unsatisfactory attempts were made to acylate various salts of α-trinositol. Techniques based on carboxylic acid anhydride and trifluoroacetic acid, carboxylic acid and trifluoroacetic acid anhydride, acetic acid anhydride and sodium acetate, pyridine, or tributylphosphine,[5] or carboxylic acid chloride and basic catalysts did not give a useful product. Finally, success was gained by dissolving a *tert*-ammonium salt corresponding to α-trinositol in dichloromethane, adding 4-(dimethylamino)pyridine (DMAP) and a carboxylic acid anhydride. The need for an excess of anhydride and DMAP was established. The amount of DMAP could be compensated for by a *tert*-amine only at the expense of a low reaction rate, whereas less than 6 equiv of anhydride gave partial acylation. By this method a variety of triesters were prepared successfully, e.g. esters of straight chain and substituted aliphatic acids. Even sterically demanding isobutyrate **3b** was prepared. In contrast to this, neither tri-(cyclohexylacetate) nor tri-(2-ethylbutyrate) could be prepared. Despite many attempts, benzoic acid anhydride persistently gave unsatisfying results.



2 $EtN(i\text{-}Pr)_2H^+$, H^+ 3a-3q Na^+

Compound, R	Na[a]	P[b]	imp[c]	DMAP[d]	H-4, H-5, H-3[e]
3a, CH_3CH_2	11.0	13.3	5	0.05	5.45, 5.23, 5.12
3b, $(CH_3)_2CH$	11.0	9.0	5	0.07	5.49, 5.26, 5.16
3c, $CH_3CH_2CH_2$	10.0	12.5	8	0.12	5.45, 5.23, 5.14
3d, $(CH_3)_2CHCH_2$	9.1	11.8	15	0.08	5.52, 5.29, 5.17
3e, $CH_3(CH_2)_3$	9.7	12.8	11	0.03	5.45, 5.25, 5.15
3f, $Me(CH_2)_5$	9.6	11.5	5	0.09	5.46, 5.23, 5.05
3g, $CH_3(CH_2)_{10}$	4.9	8.4	18	<0.1[f]	5.47, 5.23, 5.02
3h, $HOCH_2CH_2$	9.3	12.4	10	0.50	5.38, 5.14, 5.05
3i,[6] $BnOCH_2CH_2$	-	-	-[g]	0.80	5.46, 5.20, 5.00
3j, $HO(CH_2)_5$	7.6	10.0	7	<0.1[f]	5.50, 5.29, 5.19
3k,[7] $BnO(CH_2)_5$	-	-	8	<0.1[f]	5.40, 5.16, 4.95
3l, $HO(CH_2)_2O(CH_2)_2$	8.9	10.9	10	0.06	5.48, 5.27, 5.17
3m, $BnO(CH_2)_2O(CH_2)_2$	-	-	-[g]	<0.1[f]	5.45, 5.23, 5.05
3n, $PHCH_2$	9.9	10.4	13	0.53	5.37, 5.05, 4.86
3o,[8] p-$(AcNH)PhCH_2$	7.4	8.4	10	1.5	5.21, 5.01, 4.91
3p,[9] $PhCH_2CH_2$	9.2	9.6	8	<0.1[f]	5,18, 5.01, 4.82
3q,[10] 3-[2-NO_2-4,5-(OCH_2O)-Ph]CH_2CH_2	-	-	32[h]	-	5.57, 5.37, 5.17

a. sodium wt %, atomic absorption. b. Phosphorus wt %, according to *Svensk Standard 028226* and *028227*. c. Impurities mol %, partly acylated products estimated from NMR spectra. d. DMAP wt %, determined photometrically (λ=280 nm, pH6.0), may possess interfering physiological activity. e. Typical ^1H NMR shifts, further NMR characterization of final products included ^{13}C- and ^{31}P-spectra. f. Estimated from ^1H NMR. g. Indicative signals obscured. h. Procedure not optimized, is conveniently used as DMAP salt for further synthesis, see the following paper.

The ionic nature of α-trinositol esters and their limited stability in aqueous solution greatly restricted the choice of purification techniques and therefore a clean reaction was requisite. Typically, the acylation gave a reaction mixture with >95% triacylated material (^1H NMR). Work up, including precipitation of the crude product from diethyl ether, rapid ion exchange in aqueous solution and final precipitation of the product from ethanol, gave the triester as an amorphous sodium salt in circa 80% yield and 90% purity. Occasionally, substantial hydrolysis was observed during work-up, and

a modified procedure *via* precipitation as a barium salt and release in slightly acidic solution was developed. The acyl group in position three is hydrolysed preferentially but the rate is reduced in acidic media. Presumably the hydrolysis is assisted by the neighbouring phosphate. For lipophilic ester **3g**, a non-aqueous work-up was elaborated.

Successful acylation of a system with the structural features of α-trinositol is a delicate matter of finding precise reaction conditions favouring one reaction route over many other almost as likely ones. It is reasonable to assume initial acylation of the phosphate grouping followed by acylation of the hydroxyl groups.[11] Phosphate acylation gives a reactive mixed anhydride able to react with either a native neighbouring phosphate or a neighbouring hydroxyl group, thus forming a pyrophosphate or a cyclic phosphate respectively.[12] In support of an intermediary mixed anhydride, we recorded ^{31}P NMR of an acetylation of ammonium salt **2** and observed signals at -9.25, -9.33, and -9.65 ppm, typical of this functionality. By the addition of methanol-d_4 to the sample, the mixed anhydride was methanolyzed as indicated by a shift of ^{31}P NMR signals (1.39, 0.87, and 0.22 ppm), now corresponding to phosphate esters. By this mechanism small amounts of pyrophosphate derivatives occasionally occurring in the final product can be explained.

EXPERIMENTAL[13]

1D-*Myo*-inositol 1,2,6-tris(dihydrogen phosphate) *N*-ethyldiisopropylamine salt (2). A stock solution (0.5 M) of salt **2** was prepared from 1D-*myo*-inositol 1,2,6-tris(calcium phosphate) (300 g, water content 10%, 0.51 mol) by ion exchange (Dowex ® 50 W X 8, H^+), followed by neutralization with *N*-ethyldiisopropylamine (196 g, 1.52 mol) and adjusting to a final volume of 1 L. The dissolution of amine was facilitated by the addition of 1-propanol which gave a one-phase system. Removal of solvent gave a salt analysing for 2.3 ≤ nitrogen atoms/inositol ≤ 3.3. $[\alpha]_D^{22}$ -13.15° (*c* 0.2, aqueous buffer pH 7); ^1H NMR (D_2O) inositol signals δ 4.74 (d, 1 H, J = 8.9 Hz, H-2), 4.36 (q, 1 H, J = 9.2 Hz, H-6), 4.19 (t, 1 H, J = 9.7 Hz, H-1), 3.76 (H-4, 1 H, obscured by CHN), 3.63 (d, 1 H, J = 10.3 Hz, H-3), 3.52 (t, 1 H, J = 9.52 Hz, H-5), ammonium signals δ 3.73 (hept, 2 H, J = 6.6 Hz, CHN), 3.21 (q, 2 H, J = 7.5 Hz, CH_2N), 1.35 (m, 15 H, CH_3) .

Acylation of salt 2, general procedure. The solvent from a sample of the ammonium salt (**2**) stock solution (50 mL, 25 mmol) was removed and residual propanol and water were removed by co-evaporation with dry dichloromethane. The residue was dissolved in dry dichloromethane (300 mL) and DMAP (100 mmol, 4

equiv) was added (an immediate precipitate is formed) followed by the anhydride (250 mmol, 10 equiv). The mixture usually became homogeneous within a few minutes. A large excess of methanol was added after 2 d. After a few hours the solvent was removed and the residue was dissolved in methanol (30 mL) and added dropwise with stirring to dry diethyl ether (1.8 L). The precipitate was collected.

Isolation, procedure a: The precipitate was dissolved in a small volume of water (30 mL) and passed through a column of Dowex® (50 W X 8, Na^+, 100 equiv) with water as eluent (2.5 L). The water was removed at reduced pressure (foaming may be suppressed by addition of 2-propanol) and the residue, dissolved in a small volume of water (15 mL), was added with stirring into ethanol (200 mL). The precipitate was collected (centrifugation prior to decantation may be expedient) and dried.

Procedure b: The precipitate was dissolved in water (30 mL) and an aqueous solution of barium acetate (2.4 M, 35 mL) was added. The precipitate was collected after centrifugation, washed several times with water and suspended in acetic acid(*aq*) (1 M, 75 mL). A solution of sodium sulfate (0.5 M, 150 mL) was added and the mixture was stirred for 2 d. After centrifugation the solution was collected. Solvent was removed (2-propanol may be added to prevent foaming) and the residue was treated with ethanol as described above.

Procedure c, isolation of **3g**: After evaporation of the reaction solvent the residue was partitioned between heptane/methanol (1 L/ 500 mL). The methanol phase was extraced twice with heptane (500 mL), and the solvent was removed. The residue was refluxed in acetone (20 mL/g). The mixture was filtered at room temperature and the solid was suspended together with Dowex® (50 W X 8, Na^+, 50 equiv) in refluxing methanol for a few hours. The solution and the finely divided product were decanted. The solvent was removed and the product was collected.

The spectroscopic characteristics of the inositol part of esters **3a-3q** are all very similar. A typical example is given by 1D-3,4,5-tri-*O*-propanoyl-*myo*-inositol 1,2,6-trisphosphate sodium salt (**3a**): $[\alpha]_D^{23}$ +6.3° (*c* 0.7, aqueous buffer pH 7); ^{1}H NMR (D_2O) δ 5.29 (t, 1 H, J = 10.0 Hz, H-4), 5.07 (t, 1 H, J = 9.5 Hz, H-5), 4.99 (ddd, 1H, J = 10.4, 2.6, 1.1 Hz, H-3), 4.68 (dt, 1 H, J = 10.0, 2.6 Hz, H-2), 4.40 (q, 1 H, J = 9.3, H-6), 4.14 (tt, 1 H, J = 9.4, 2.8 Hz, H-1) 2.35-2.10 (m, 6 H, CH_2), 0.90-0.80 (m, 9 H, CH_3); ^{13}C-NMR (D_2O) δ 179.6, 179.5, 179.1, 77.1 (t, J = 5.8 Hz), 76.1 (d, J = 5.6 Hz) 75.9 (m), 74.9 (d, J = 2.2 Hz), 73.4 , 72.9 (d, J = 2.4 Hz), 30.3, 30.2, 30.1, 11.3, 11.2, 11.0; ^{31}P NMR (D_2O) δ 0 (set as zero), -1.1, -1.2.

6-Benzyloxy-4-oxahexanoic Acid. 1,4-Addition of benzyloxyethanol to acrylonitrile followed by acid-catalysed methanolysis and subsequent alkaline hydrolysis of the intermediary formed methyl ester gave, after acidification, 6-benzyloxy-4-oxahexanoic

acid as an oil (yield 65%, purity >95%).[6] ^{1}H NMR ($CDCl_3$) δ 10.95 (br s, 1 H), 7.39-7.23 (m, 5 H), 4.58 (s, 2 H), 3.78 (t, 2 H, J = 6.3 Hz) 3.69-3.65 (m, 2 H), 3.64-3.60 (m, 2 H), 2.67 (t, 2 H, J = 6.3).

Anhydrides, general procedure. The carboxylic acid was dissolved in dry dichloromethane. Dicyclohexylcarbodiimide (0.48 equiv), dissolved in the same solvent, was added. The reaction mixture was left overnight and filtered. The filtrate was used directly for acylation.

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13. Note well, characteristics of amorphous salts will inevitably be both compound and batchspecific, due to variations in the number of counter ions and water content. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on Varian Unity 300 by Reserca AB, Stockholm, Sweden.