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Carbohydrate Research 341 (2006) 897–902
Carbohydrate RESEARCH

Note

Synthesis of *myo*-inositol 1,2,3-tris- and 1,2,3,5-tetrakis(dihydrogen phosphate)s as a tool for the inhibition of iron-gall-ink corrosion

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Received 11 November 2005; received in revised form 16 February 2006; accepted 23 February 2006
Available online 13 March 2006

Abstract—Two *myo*-inositol phosphates, *myo*-inositol 1,2,3-tris(dihydrogen phosphate) and *myo*-inositol 1,2,3,5-tetrakis(dihydrogen phosphate), have been synthesised in several steps from *myo*-inositol (in *Chem. Abstr.*: D-*myo*-inositol) in the form of their sodium salts. They were shown to prevent iron-gall-ink decay in cellulose items at the same level as phytic acid dodecasodium salt.

Keywords: *myo*-Inositol phosphates; Synthesis; Antioxidant activity; Iron-gall-ink corrosion; Paper stabilisation

1. Introduction

One of the most important inks in the history of the western world is called iron gall ink; it is named after the two colour-forming ingredients: gallic acid from the tannins and iron ions. Unfortunately, these components induce severe damage or even the complete destruction of numerous historical artifacts, and significant efforts are devoted to the development of processes that would increase their stability.[1,2] The two main reasons for iron-gall-ink corrosion have been identified as acid hydrolysis and oxidation, catalysed by ferrous ions. Acid hydrolysis of cellulose is prevented by deacidification, whereas oxidative decay is usually inhibited by the addition of antioxidants. The only antioxidant that has been used for the prevention of ink decay is the iron chelating agent *myo*-inositol hexakis(dihydrogen phosphate) (phytic acid),[3] a naturally occurring antioxidant,[4–6] which theoretically doubles the lifetime of iron-containing paper, though its mode of action is still not completely clear.[7] Its anion (phytate) apparently occupies all the available coordination sites, thus preventing the reaction of ferrous ions with hydroperoxides. Since it represents only a modest health hazard, it has been recently proposed as a preventive antioxidant for iron-gall-ink-containing cellulose.[8]

Unfortunately, due to the poor solubility of phytate in non-aqueous media, it cannot be used for water-sensitive items, such as bound books. We have therefore decided to investigate other *myo*-inositol derivatives that might exhibit the same effect on the stabilisation of the paper items, but contain free hydroxy groups, which might be easily derivatised to give less polar compounds, which would consequently be soluble in non-aqueous solvents. Previously it was shown that a 1,2,3-grouping of *myo*-inositol phosphates inhibited the production of hydroxy radicals (HO$^{\bullet}$) in the iron-catalysed Haber–Weiss reaction.[9] It also prevented the iron-catalysed oxidation of ascorbic acid and the peroxidation of arachidonic acid.[10] On this basis it was concluded that such pattern might be essential for the properties of phytate.[11] To establish whether they might stabilise cellulose containing iron gall ink and consequently to confirm the above conclusion, we have decided to compare the effects of sodium salts of *myo*-inositol 1,2,3-tris(dihydrogen phosphate) **7** (Scheme 1), 1,2,3,5-tetrakis(dihydrogen phosphate) **10** and phytic acid on the stabilisation of paper items. Compound **7** has been previously synthesised

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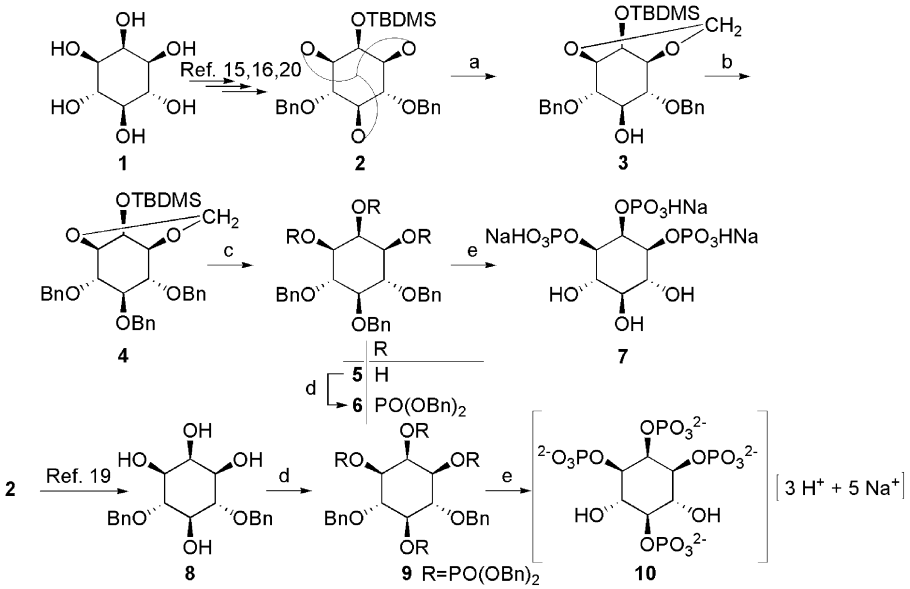
doi:10.1016/j.carres.2006.02.029



Scheme 1. Reagents and conditions: (a) DIBALH, −20 °C, CH_2Cl_2 (76% yield); (b) BnBr, NaH, Bu_4NI, DMF (70%); (c) HCl, MeOH, Δ (93%); (d) 1. $(BnO)_2PN(i\text{-}Pr)_2$, 1*H*-tetrazole, CH_2Cl_2; 2. *m*-CPBA (for **6** 90%, for **9** 93%); (e) H_2/1 bar, Pd/C (10%), MeOH/H_2O (19:1), then ion-exchange (quantitatively for **7** and **10**).

from *myo*-inositol in several steps in the form of monosodium tetra(cyclohexylammonium) *myo*-inositol-1,2,3-trisphosphate,[9] and derivative **10** as an octapotassium salt of *myo*-inositol-1,2,3,5-tetrakisphosphate.[12]

2. Results and discussion

Our idea was to start from a single intermediate, which would enable (by the application of selective protecting groups)[13,14] the synthesis of both products in the form of sodium salts in order to exclude the effect of the cation on accelerated ageing tests (phytic acid is also commercially available as dodecasodium salt). *myo*-Inositol 1,3,5-orthoformate and its derivatives have been widely used as precursors for the synthesis of variously substituted inositol phosphates.[12,15–23] The selective protection or deprotection of the hydroxy groups of inositol orthoformate was achieved either by the enzymatic[16,18] or chemical methods.[12,15,17–20,23] We decided to start from the previously known 2-*O*-*tert*-butyldimethylsilyl derivative **2**,[15] which can be prepared in several steps from *myo*-inositol (**1**).[15,16,20] Compound **2** contains three different orthogonal sets[14] of protecting groups and, thus, allows us to transform it selectively into phosphates **7** and **10**.

Treatment of **2** with an excess (5 equiv) of diisobutylaluminium hydride (DIBALH) in dichloromethane (CH_2Cl_2)[21,22] at −20 °C, gave selectively derivative **3** in a 76% yield. At −60 °C the reaction did not take place at all, but at 0 °C or at room temperature the cleavage was not selective and the TBDMS group was also partially eliminated. The same situation occurred when lower quantities of DIBALH and, as a consequence, longer reaction times were employed. Moreover, lowering the quantity of the solvent also resulted in a lower yield. The compound **3** was benzylated with benzyl bromide in the presence of sodium hydride as a base, and tetrabutylammonium iodide as a catalyst at the remaining free hydroxy group to give **4**, which was further hydrolysed under acidic conditions to obtain previously described[22] tribenzyl derivative **5**. The latter was phosphorylated by a modified method from the literature,[24] employing treatment with dibenzyl *N*,*N*-diisopropylphosphoramidite in the presence of 1*H*-tetrazole, followed by an oxidation with *m*-chloroperbenzoic acid (*m*-CPBA) in CH_2Cl_2 yielding tris(dibenzyl phosphate) **6** (90% yield). The complete removal of all benzyl groups was accomplished by hydrogenolysis in the presence of Pd/C (10%) in a mixture MeOH/H_2O 19:1, then the deprotected **6** was run through an ion-exchange column (50X-8-100 Na^+ form). Finally, fractions containing the product were combined and lyophilised to give trisodium salt of trisphosphate **7** as a white powder. The overall yield for the eight steps from the starting *myo*-inositol (**1**) was 25% (in the calculation the best yields from the literature[15,16,20] were taken for the synthesis of **2**). The obtained yield is much higher than that obtained for the synthesis of the previously mentioned sodium tetra(cyclohexylammonium) salt of *myo*-inositol-1,2,3-trisphosphate, which was synthesised from **1** in seven steps, but with a very low overall yield (below 4%).[9,25]

The synthesis of the *myo*-inositol 1,2,3,5-tetrakisphosphate **10** from **2**, via the previously known deprotected derivative **8**,[19] which was treated in a similar way as

compound **5** with dibenzyl *N*,*N*-diisopropylphosphoramidite in the presence of 1*H*-tetrazole in CH_2Cl_2, was followed by the addition of *m*-CPBA to give 1,2,3,5-tetrakis(dibenzyl phosphate) **9**; further hydrogenation and ion-exchange resulted in pentasodium salt **10** with an overall yield of 44% for six steps (in comparison with the previous synthesis of the octapotassium salt of *myo*-inositol 1,2,3,5-tetrakisphosphate **10** from *myo*-inositol in six steps with a 42% overall yield).[12] To establish the potential stabilising effect during the iron-gall-ink-induced decay of paper, the samples containing iron gall ink were treated with aqueous solutions of sodium salts of either of the two *myo*-inositol derivatives, **7** and **10**, or phytate.[26] Sodium salts were used instead of the usually employed calcium and ammonium salts,[3] due to the poorly defined composition of the latter. After accelerated ageing, the degree of polymerisation (DP) was determined using viscometry. The rate constant was obtained, according to Ekenstam,[27] as the slope of $(1/DP_t - 1/DP_0)$ over time t, where DP_t and DP_0 are the degrees of polymerisation at time t and 0, respectively. The results presented in Figure 1 demonstrate a significant improvement in the cellulose stability after the treatment with phytate or with compounds **7** and **10**. While the paper treated with phytate was 13 ± 4 times more stable than the deacidified control, the effect obtained with the treatments using compounds **7** and **10** was 6 ± 1 and 15 ± 7 times, respectively.

In conclusion, we have presented a new and efficient synthesis of two *myo*-inositol derivatives (**7** and **10**) that inhibit cellulose degradation in the range similar to that of phytate. Due to the presence of hydroxy groups, which might be transformed to form derivatives soluble in non-aqueous solvents, compounds **7** and **10** in their suitably derivatised forms might have a strong potential for the stabilisation of water-sensitive items that are endangered by corrosive iron gall inks.

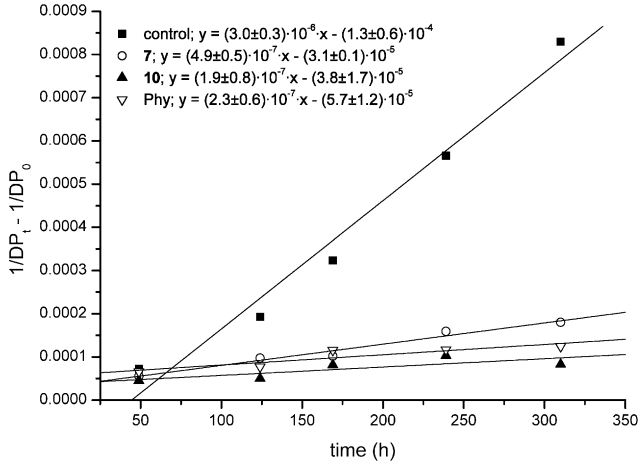


Figure 1. Graph showing the changes of $1/DP_t - 1/DP_0$ over time during accelerated ageing at 80 °C and 65% relative humidity. All the samples were deacidified with $Ca(HCO_3)_2$ and treated with **7**, **10** or phytate; the control was only deacidified.

3. Experimental

Melting points were determined on a Kofler micro-hot-stage and are uncorrected. ^1H (300 MHz), ^{31}P (121 MHz) and ^{13}C NMR (75 MHz) spectra were recorded with a Bruker Avance DPX 300 spectrometer. In all the ^1H NMR spectra TMS was used as an internal reference, in the ^{13}C NMR spectra the residual solvent signal was used as an internal reference ($CDCl_3$, triplet at 77.0 ppm) unless otherwise stated. All ^{31}P spectra were referenced to 85% phosphoric acid. All ^{31}P and ^{13}C NMR spectra were ^1H decoupled unless otherwise stated. The coupling constants (J) are given in hertz. IR spectra were obtained with a Perkin–Elmer Spectrum 1000 spectrophotometer. Mass spectra were recorded with a VG-Analytical AutoSpec Q instrument. Elemental analyses (C, H, N) were performed with a Perkin–Elmer 2400 CHN Analyser. Column chromatography was performed with Silica Gel 60 (E. Merck, 60–63 mm). Thin-layer chromatography was carried out on Fluka silica-gel TLC cards. The starting compound **2** was prepared as described in the literature.[15,16,20] CH_2Cl_2 was dried over P_4O_{10} and distilled before use. All other reagents were used as received from commercial suppliers. For the paper-stabilisation studies, purified cotton linters cellulose sheets (Whatman filter paper no. 1; 86.0 g m^{-2}, degree of polymerisation (DP): 2630, RSD 0.74%) were used. Model ink containing tannin (puro, Carlo Erba) (49.2 g L^{-1}), gum arabic (31.4 g L^{-1}) and $FeSO_4 \times 7H_2O$ (42 g L^{-1}) in deionised water was applied as 1 cm broad lines to model paper using a Roland plotter DXY 990 with a 0.6 mm pen at a speed of 5 cm s^{-1}. The samples were immersed in aqueous solutions of $Ca(HCO_3)_2$ (0.01 mol L^{-1}, 2×15 min). Apart from one sample, which was used as a control, the dry papers were immersed in 0.01 mol L^{-1} solutions of chelator (phytate or **7** or **10**, 2×15 min). All samples were aged according to ISO Standard 5630/3 conditions (80 °C and 65% RH) in a Vötsch VC 0020 ageing oven. Viscometric determinations of the degree of polymerisation (DP) were performed according to the standard procedure (SCAN-CM 15:88), using 1 mol L^{-1} cupriethylenediamine solvent (Carlo Erba), with the typical RSD below 0.8%. DP was calculated from the intrinsic viscosity using the equation $DP^{0.85} = 1.1 \times [\eta]$.[28]

3.1. 4,6-Di-*O*-benzyl-2-*O*-*tert*-butyldimethylsilyl-1,3-*O*-methylene-*myo*-inositol (3)

To a cooled (−20 °C) solution of **2** (1.00 g, 2.1 mmol) in anhydrous CH_2Cl_2 (25 mL) DIBALH (10 mL of 1 M

solution in CH_2Cl_2, 10 mmol) was added under an Ar atmosphere. After 1 h of stirring the mixture was poured into a cooled (−20 °C) solution of potassium tartrate (60 g in 100 mL of water) and saturated aqueous NH_4Cl (80 mL). Ethyl acetate (200 mL) was added and the mixture was stirred for 1 h at room temperature. Layers were separated and the aqueous layer was additionally extracted with ethyl acetate (2 × 80 mL), combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with CH_2Cl_2/ethyl acetate (15:1) to give **3** as a bright-yellow oil (760 mg, 76%). IR (neat): *ν* 3567, 2953, 2928, 2857, 1497, 1463, 1455 cm^{-1}; ^1H NMR ($CDCl_3$): *δ* 7.28 (m, 10H, 2 × Ph), 5.63 (d, 1H, *J* 4.6 Hz, $H_AH_BCO_2$), 4.69 (d, 2H, *J* 12.0 Hz, $PhCH_AH_B$), 4.64 (d, 1H, *J* 4.6 Hz, $H_AH_BCO_2$), 4.63 (t, 1H, *J* 1.6 Hz, 2-H), 4.59 (d, 2H, *J* 12.0 Hz, $PhCH_AH_B$), 4.24 (m, 2H, 1-H, 3-H), 4.00–3.94 (m, 3H, 4-H, 5-H, 6-H), 3.00 (d, 1H, *J* 10.6 Hz, 5-OH), 0.90 (s, 9H, *t*-Bu), 0.10 (s, 6H, 2 × Me); ^{13}C ($CDCl_3$): *δ* 138.0, 128.3, 127.5, 127.3, 85.4, 81.2, 75.4, 71.9, 68.8, 64.0, 25.7, 17.9, −4.8; EI-MS: *m*/*z* 485 [$(M-H)^+$, 1], 181 (100), 92 (78); HRMS (EI) calcd for $C_{27}H_{38}O_6Si$ (M^+) 486.2438, found 486.2446.

3.2. 4,5,6-Tri-*O*-benzyl-2-*O*-*tert*-butyldimethylsilyl-1,3-*O*-methylene-*myo*-inositol (4)

To a stirred solution of **3** (1.00 g, 2.1 mmol) in DMF (8 mL), 60% sodium hydride (140 mg, 3.5 mmol) and tetrabutylammonium iodide (20 mg, 0.07 mmol) were added at room temperature. After 5 min of stirring benzyl bromide (650 μL, 5 mmol) was added dropwise and the mixture was stirred for 1 h at room temperature. The reaction was quenched with water (150 mg, 8 mmol) and evaporated, then water (10 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 20 mL), combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with petroleum benzine (boiling point 40–60 °C)/ethyl acetate (15:1) to give **4** as a bright-yellow oil (830 mg, 70%). IR (neat): *ν* 3342 br, 2953, 2928, 2857, 1725, 1497, 1454 cm^{-1}; ^1H NMR ($CDCl_3$): *δ* 7.29 (m, 15H, 3 × Ph), 5.33 (d, 1H, *J* 4.7 Hz, $H_AH_BCO_2$), 4.77 (d, 1H, *J* 4.7 Hz, $H_AH_BCO_2$), 4.61 (s, 2H, 5-CH_2Ph), 4.60 (d, 2H, *J* 12.0 Hz, 4- and 6-CH_AH_BPh), 4.53 (d, 2H, *J* 12.0 Hz, 4- and 6-CH_AH_BPh), 4.24 (t, 1H, *J* 1.8 Hz, 2-H), 4.08 (dd, 2H, *J* 1.8 and 1.8 Hz, 1-H, 3-H), 3.93 (dd, 2H, *J* 1.8 and 4.6 Hz, 4-H, 6-H), 3.64 (t, 1H, *J* 4.6 Hz, 5-H), 0.93 (s, 9H, *t*-Bu), 0.12 (s, 6H, 2 × Me); ^{13}C ($CDCl_3$): *δ* 138.4, 137.9, 128.4, 128.3, 127.9, 127.7, 127.6, 85.4, 82.1, 79.2, 74.5, 72.9, 71.7, 64.4, 25.8, 18.1, −4.7 (one signal is hidden); EI-MS: *m*/*z* 575 [$(M-H)^+$, 0.2], 181 (42), 91 (100); HRMS (EI) calcd for $C_{34}H_{44}O_6Si$ (M^+) 576.2907, found 576.2926.

3.3. Preparation of 4,5,6-tri-*O*-benzyl-*myo*-inositol (5)[22]

A solution of **4** (1.00 g, 1.7 mmol) in methanol (30 mL) and concentrated aqueous HCl (4 mL) was refluxed for 3 h, then it was evaporated, water (10 mL) was added to the residue and the mixture was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The product was obtained as a white solid (722 mg, 93%): mp (from MeOH/H_2O) 118–120 °C; IR (KBr): *ν* 3379 br, 2922, 1497, 1454, 1360 cm^{-1}; ^1H NMR ($CDCl_3$): *δ* 7.33 (m, 15H, 3 × Ph), 4.95 (d, 2H, *J* 11.3 Hz, 4- and 6-CH_AH_BPh), 4.92 (s, 2H, 5-CH_2Ph), 4.76 (d, 2H, *J* 11.3 Hz, 4- and 6-CH_AH_BPh), 4.17 (dt, 1H, *J* 1.8 and 2.8 Hz, 2-H), 3.80 (dd, 2H, *J* 9.4 and 9.5 Hz, 4-H, 6-H), 3.54 (ddd, 2H, *J* 2.8, 4.4 and 9.5 Hz, 1-H, 3-H), 3.49 (t, 1H, *J* 9.4 Hz, 5-H), 3.55 (d, 1H, *J* 1.8 Hz, 2-OH), 3.40 (d, 2H, *J* 4.4 Hz, 1-OH, 3-OH); ^{13}C ($CDCl_3$): *δ* 138.4, 138.3, 128.5, 128.3, 127.8, 127.75, 127.70, 127.6, 83.2, 81.7, 75.5, 75.4, 71.9, 71.3; EI-MS: *m*/*z* 359 [$(M-CH_2Ph)^+$, 23], 107 (22), 92 (39), 91 (100). Anal. Calcd for $C_{27}H_{30}O_6$: C, 71.98; H, 6.71. Found: C, 71.83; H, 6.92.

3.4. 4,5,6-Tri-*O*-benzyl-*myo*-inositol 1,2,3-tris(dibenzyl phosphate) (6)

To a mixture of triol **5** (200 mg, 0.44 mmol) and 1-*H*-tetrazole (360 mg, 5.1 mmol) in MeCN (30 mL) *N*,*N*-diisopropyl dibenzyl phosphoramidite (720 mg, 2.28 mmol) in CH_2Cl_2 (10 mL) was added. After 240 min of stirring at room temperature the reaction mixture was cooled to −40 °C and *m*-CPBA (80–85%, 600 mg, 2.8 mmol) in CH_2Cl_2 (10 mL) was added. The resulting solution was stirred at 0 °C for 240 min, then the reaction mixture was diluted with CH_2Cl_2 (60 mL), washed with Na_2SO_3 (10%, 2 × 40 mL), saturated $NaHCO_3$ (2 × 30 mL), water (30 mL) and saturated aqueous NaCl solution (30 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product thus obtained was purified by column chromatography on silica gel with petroleum benzine (boiling point 40–60 °C)/CH_2Cl_2/ethyl acetate (3:10:3) to give **6** (492 mg, 90%) as a bright-yellow oil. IR (neat): *ν* 3466 br, 3066, 3033, 2951, 2894, 1497, 1456 cm^{-1}; ^1H NMR ($CDCl_3$): *δ* 7.35–7.10 (m, 45H, 9 × Ph), 5.40 (td, 1H, *J* 2.3 and 8.4 Hz, 2-H), 5.13–4.74 (m, 18H, 9 × $PhCH_2$), 4.41 (dddd, 2H, *J* 2.2, 2.3, 9.6 and 9.6 Hz, 1-H, 3-H), 3.92 (dd, 2H, *J* 9.4 and 9.6 Hz, 4-H, 6-H), 3.52 (t, 1H, *J* 9.4 Hz, 5-H); ^{13}C ($CDCl_3$): *δ* 138.0, 137.9, 135.9 (d, *J* 7 Hz), 135.8 (d, *J* 7 Hz), 135.7 (d, *J* 7 Hz), 128.44, 128.43, 128.39, 128.34, 128.32, 128.28, 128.23, 128.22, 128.0, 127.92, 127.88, 127.7, 127.6 (two s), 127.5, 82.3 (m), 79.4 (d, *J* 5 Hz), 77.7 (m), 76.1, 75.9 (dd, *J* 3 and 6 Hz), 75.5, 69.6 (d, *J* 5 Hz), 69.40 (d, *J* 5 Hz), 69.38 (d, *J* 6 Hz); ^{31}P NMR (121 MHz, $CDCl_3$): *δ* 2.75 (2P),

1.93 (1P); FAB$^+$-MS: *m*/*z* 1231.5 [(M+H)$^+$], 1141.3, 181; HRMS (ESI) calcd for $C_{69}H_{70}O_{15}P_3$ (M+H)$^+$ 1231.3928, found 1231.3930.

3.5. Trisodium salt of *myo*-inositol 1,2,3-tris(dihydrogen phosphate) (7)

A mixture of **6** (492 mg, 0.4 mmol) in methanol/water (19:1, 20 mL), and Pd/C (10%, 100 mg) was stirred under a H_2 atmosphere for 12 h at room temperature. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure at room temperature. The residue was dissolved in water (1 mL) and applied to a column of cation-exchange resin (Dowex 50-X8, mesh 20–50, Na^+ form) that was eluted with water. Fractions containing the product were combined and lyophilised to give **7** quantitatively as a white solid: mp >300 °C; IR (neat): *v* 3423 br, 1656 br, 1198 br cm^{-1}; ^{1}H NMR (D_2O, referenced to DMSO at 2.71 ppm): δ 4.94 (td, 1H, *J* 2.5 and 9.9 Hz, 2-H), 4.06 (m, 2H, 1-H, 3-H), 3.84 (dd, $J_1 \sim J_2 \sim 9.5$ Hz, 4-H, 6-H), 3.44 (t, *J* 9.4 Hz, 5-H); ^{13}C (D_2O, referenced to DMSO at 39.39 ppm): δ 76.4 (m), 75.9 (m), 74.8, 72.8 (m); ^{31}P NMR (121 MHz, D_2O): δ 4.06 (2P), 3.79 (1P); FAB$^-$MS: *m*/*z* 485 [$(C_6H_{11}Na_3O_{15}P_3)^-$; free acid+3Na$^+$−4H$^+$], 463 [$(C_6H_{12}Na_2O_{15}P_3)^-$; free acid+2Na$^+$−3H$^+$], 441 [$(C_6H_{13}NaO_{15}P_3)^-$; free acid+Na$^+$−2H$^+$], 419 [$(C_6H_{14}O_{15}P_3)^-$; free acid−H$^+$], 181. Anal. Calcd for $C_6H_{12}Na_3O_{15}P_3 \times 3H_2O$: C, 13.34; H, 3.36. Found: C, 13.71; H, 3.62.

3.6. 4,6-Di-*O*-benzyl-*myo*-inositol 1,2,3,5-tetrakis-(dibenzyl phosphate) (9)

To a mixture of tetrol **8** (200 mg, 0.54 mmol) and 1-*H*-tetrazole (470 mg, 6.7 mmol) in MeCN (10 mL), *N*,*N*-diisopropyl dibenzyl phosphoramidite (1.20 g, 3.46 mmol) in CH_2Cl_2 (10 mL) was added. After 240 min of stirring at room temperature the reaction mixture was cooled to −40 °C, and *m*-CPBA (80–85%, 1.00 g, 4.64 mmol) in CH_2Cl_2 (10 mL) was added. The resulting solution was stirred at 0 °C for 240 min, then the reaction mixture was diluted with CH_2Cl_2 (60 mL), washed with Na_2SO_3 (10%, 2 × 40 mL), saturated $NaHCO_3$ (2 × 30 mL), water (30 mL) and saturated aqueous NaCl solution (30 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product thus obtained was purified by column chromatography on silica gel with petroleum benzine (boiling point 40–60 °C)/CH_2Cl_2/ethyl acetate (5:5:3) to give **9** (620 mg, 93%) as a bright-yellow oil. IR (neat): *v* 3426 br, 3064, 3033, 2951, 2893, 1497, 1455 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.36–6.95 (m, 50H, 10 × Ph), 5.49 (td, 1H, *J* 2.2 and 8.4 Hz, 2-H), 5.14–4.60 (m, 20H, 10 × PhCH_2), 4.52 (dt, 1H, *J* 9.4 and 9.4 Hz, 5-H), 4.44 (dddd, 2H, *J* 2.0, 2.2, 9.6 and 9.6 Hz, 1-H, 3-H), 4.00 (dd, 2H, *J* 9.4 and 9.6 Hz, 4-H, 6-H); ^{13}C ($CDCl_3$): δ 138.3, 136.4 (d, *J* 8 Hz), 136.3 (d, *J* 7 Hz), 136.2 (d, *J* 7 Hz) 136.0 (d, *J* 7 Hz), 128.93, 128.91, 128.87, 128.82, 128.80, 128.77, 128.75, 128.6, 128.5, 128.47, 128.45, 128.13, 128.10, 127.9, 127.8, 74.5, 69.6 (d, *J* 5 Hz), 69.4 (d, *J* 5 Hz, two C), 69.2 (d, *J* 5 Hz), 79.3 (m), 77.6 (m, two C), 77.1 (m), 75.2 (m, two C); ^{31}P NMR (121 MHz, $CDCl_3$): δ 2.61 (2P), 2.31 (1P), 2.10 (1P); FAB$^+$-MS *m*/*z* 1401.9 [(M+H)$^+$, 23], 307.1, 181; HRMS (ESI) calcd for $C_{76}H_{77}O_{18}P_4$ (M+H)$^+$ 1401.4060, found 1401.4070. Anal. Calcd for $C_{76}H_{76}O_{18}P_4$: C, 65.14; H, 5.47. Found: C, 65.12; H, 5.69.

3.7. Pentasodium salt of *myo*-inositol 1,2,3,5-tetrakis-(dihydrogen phosphate) (10)

A mixture of **9** (620 mg, 0.4 mmol) in methanol/water (19:1, 20 mL), Pd/C (10%, 150 mg) was stirred under a H_2 atmosphere for 12 h at room temperature. The catalyst was removed by filtration and the filtrate concentrated under reduced pressure at room temperature. The residue was dissolved in water (1 mL) and applied to a column of cation-exchange resin (Dowex 50-X8, mesh 20–50, Na^+ form) that was eluted with water. Fractions containing the product were combined and lyophilised to give **10** quantitatively as a white solid: mp >300 °C; IR (neat): *v* 3424 br, 1647 br, 1198 br, 1050 br cm^{-1}; ^{1}H NMR (D_2O, referenced to DMSO at 2.71 ppm): δ 4.75 (td, 1H, *J* 2.6 and 9.9 Hz, 2-H), 3.93 (m, 2H, 1-H, 3-H), 3.87–3.71 (m, 3H, 4-H, 5-H, 6-H); ^{13}C (75 MHz, D_2O, referenced to DMSO at 39.5 ppm): δ 80.2 (m), 76.1 (m), 75.6 (m), 72.3 (m); ^{31}P NMR (121 MHz, D_2O): δ 6.28 (1P), 4.85 (1P), 4.59 (2P); FAB$^-$MS: *m*/*z* 609 [$(C_6H_{10}Na_5O_{18}P_4)^-$; free acid+5Na$^+$−6H$^+$], 587 [$(C_6H_{11}Na_4O_{18}P_4)^-$; free acid+4Na$^+$−5H$^+$], 565 [$(C_6H_{12}Na_3O_{18}P_4)^-$; free acid+3Na$^+$−4H$^+$], 543 [$(C_6H_{13}Na_2O_{18}P_4)^-$; free acid+2Na$^+$−3H$^+$], 521 [$(C_6H_{14}NaO_{18}P_4)^-$; free acid+Na$^+$−2H$^+$], 499 [$(C_6H_{15}O_{18}P_4)^-$; free acid−H$^+$]. Anal. Calcd for $C_6H_{11}Na_5O_{18}P_4 \times 3H_2O$: C, 10.85; H, 2.58. Found: C, 11.06; H, 2.91.

Acknowledgements

The authors gratefully acknowledge the support of the European Commission, the Fifth Framework Programme (EVK4-CT-2001-0049) and the support of the Ministry of Higher Education, Science and Technology of the Republic of Slovenia, as well as Slovenian Research Agency (P1-0230 and P1-0153). Dr. B. Kralj and Dr. D. Žigon (Center for Mass Spectroscopy, 'Jožef Stefan' Institute, Ljubljana, Slovenia) are gratefully acknowledged for mass spectroscopy measurements.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.02.029.

References

1. Daniels, V. D. *Chem. Soc. Rev.* **1996**, *25*, 179–186.
2. Jones, N. *New Sci.* **2002**, *175*, 38–42.
3. Neevel, J. G. *Restaurator* **1995**, *16*, 143–160.
4. Raboy, V. *Phytochemistry* **2003**, *64*, 1033–1043.
5. Graf, E. *J. Am. Oil Chem. Soc.* **1983**, *60*, 1861–1867.
6. Graf, E.; Empson, K. L.; Eaton, J. W. *J. Biol. Chem.* **1987**, *262*, 11647–11650.
7. Strlič, M.; Kolar, J.; Pihlar, B. *Polym. Degrad. Stab.* **2001**, *73*, 535–539.
8. Reißland, B.; de Groot, S. *Iada Preprints*; The Royal Academy of Fine Arts: Copenhagen, 1999; pp 121–129.
9. Spiers, I. D.; Barker, C. J.; Chung, S.-K.; Chang, Y.-T.; Freeman, S.; Gardiner, J. M.; Hirst, P. H.; Lambert, P. A.; Michell, R. H.; Poyner, D. R.; Schwalbe, C. H.; Smith, A. W.; Solomons, K. R. H. *Carbohydr. Res.* **1996**, *282*, 81–99.
10. Phillippy, B. Q.; Graf, E. *Free Radical Biol. Med.* **1997**, *22*, 939–946.
11. Hawkins, P. T.; Poyner, D. R.; Jackson, T. R.; Letcher, A. J.; Lander, D. A.; Irvine, R. F. *Biochem. J.* **1993**, *294*, 929–934.
12. Chung, S.-K.; Chang, Y.-T. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2715–2718.
13. Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 3rd ed.; J. Wiley & Sons: New York, 1999.
14. Kocieñski, P. J. *Protecting Groups*, corrected ed.; Georg Thieme: Stuttgart, 2000.
15. Lee, H. W.; Kishi, Y. *J. Org. Chem.* **1985**, *50*, 4402–4404.
16. Baudin, G.; Glänzer, B. I.; Swaminathan, K. S.; Vasella, A. *Helv. Chim. Acta* **1988**, *71*, 1367–1378.
17. Billington, D. C.; Baker, R.; Kulagowski, J. J.; Mawer, I. M.; Vacca, J. P.; deSolms, S. J.; Huff, J. R. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1423–1429.
18. Andersch, P.; Schneider, M. P. *Tetrahedron: Asymmetry* **1993**, *4*, 2135–2138.
19. Laumen, K.; Ghisalba, O. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 2046–2049.
20. Angyal, S. J. *Carbohydr. Res.* **2000**, *325*, 313–320.
21. Gilbert, I. H.; Holmes, A. B.; Young, R. C. *Tetrahedron Lett.* **1990**, *31*, 2633–2634.
22. Gilbert, I. H.; Holmes, A. B.; Pestchanker, M. J.; Young, R. C. *Carbohydr. Res.* **1992**, *234*, 117–130.
23. Sato, K.-i.; Akai, S.; Sugita, N.; Ohsawa, T.; Kogure, T.; Shoji, H.; Yoshimura, J. *J. Org. Chem.* **2005**, *70*, 7496–7504.
24. Yu, K.-L.; Fraser-Reid, B. *Tetrahedron Lett.* **1988**, *29*, 979–982.
25. Baker, G. R.; Billington, D. C.; Gani, D. *Tetrahedron* **1991**, *47*, 3895–3908.
26. Anders, M.; Lichtblau, D. A.; Kolar, J.; Malešič, J.; Strlič, M.; Šala, M.; Kočevar, M. Antioxidant for paper. PCT Int. Appl., 2005, 32 pp.
27. Emsley, A. M.; Stevens, G. C. *Cellulose* **1994**, *1*, 26–56.
28. Evans, R.; Wallis, A. F. A. *4th Int. Symp. Wood Chem.*, 1987; pp 201–205.