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Enzyme Assisted Synthesis of D-*myo*-Inositol-1,2,6-trisphosphate

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Abstract: The title compound is prepared in enantiomerically pure form *via* a facile enzyme assisted route. Essential for the success of the described method were a) the highly enantioselective esterification of 4,6-*O*-dibenzoyl-*myo*-inositol **2**, b) the selective acylation of the axial hydroxyl function in **3** and c) the selective, base catalysed methanolysis of one benzoate group in **5**. The obtained, selectively protected 1,2,6-triol **6** was converted into the title compound **7** by phosphorylation using *N,N*-dimethyl dibenzyl phosphoamidite followed by deprotection.

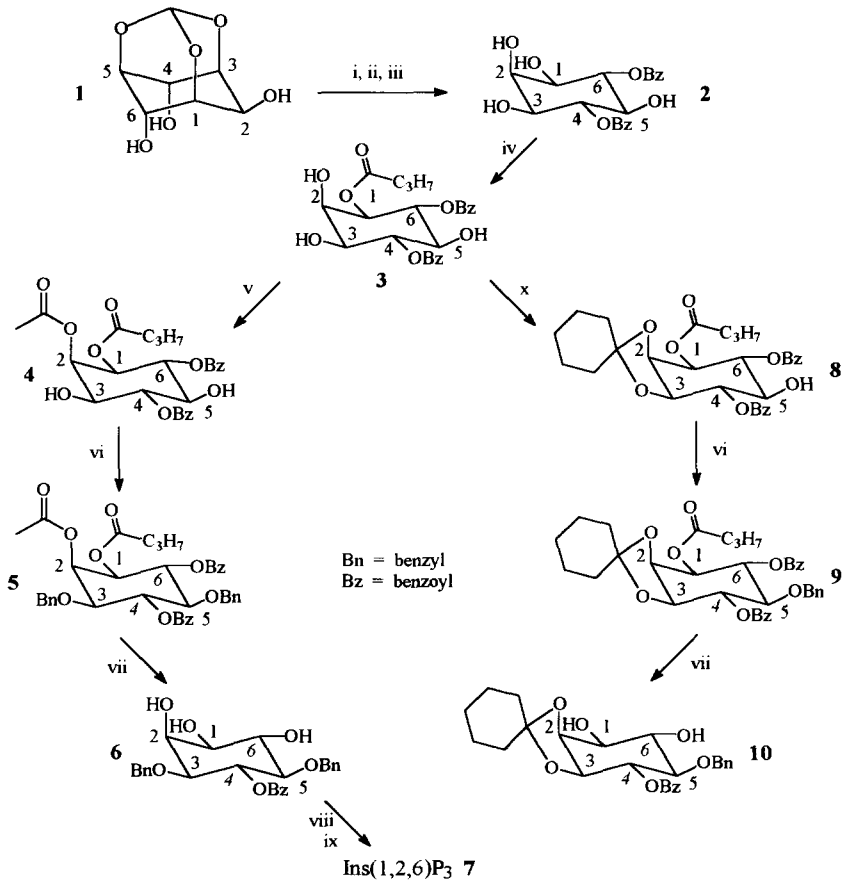
D-*myo*-inositol-1,2,6-trisphosphate (Ins(1,2,6)P_3; *PP56*; *α-Trinositol*) is a novel experimental drug, which displays a broad pharmacological profile in the treatment of numerous acute and chronic diseases. Ins(1,2,6)P_3 suppresses inflammatory processes e.g. caused by skin burns, arthritics and post-operative ileus[1], it prevents in experimental diabetes abnormal nerve functions causing secondary diabetic complications[1,2]. It constitutes the only known neuropeptide Y (NP-Y) antagonist[3,4] with a non peptide structure which does not bind at NP-Y receptors[5]. Ins(1,2,6)P_3 also influences the cholesterol transport[6] and reverses cadmium induced hypertension[7] in rats.

Ins(1,2,6)P_3 is presently mainly produced by partial degradation of phytic acid with a specific phytase from yeast. For the separation of Ins(1,2,6)P_3 from other inositol phosphates, proteins, buffer etc. the process involves the utilisation of ion exchange chromatography using gradient elution[1].

As an alternative we describe here a highly stereo- and enantioselective route to Ins(1,2,6)P_3 involving enzymatic and chemical reaction steps. (Scheme 1). Based on our previous published method[8] 1,3,5-*O*-methylidyne-*myo*-inositol **1** is converted *via* the symmetric 4,6-*O*-dibenzoyl-*myo*-inositol **2** into the enantiomerically pure 1D-1-*O*-butyryl-4,6-*O*-dibenzoyl-*myo*-inositol **3** (49 %) by enantioselective enzymatic esterification in presence of a lipoprotein lipase from *Pseudomonas species* (LPL)[9] using vinyl butyrate as acyl donor.

The axial 2-position of **3** was selectively protected using the "orthoester method"[10] resulting in the exclusive formation of the desired 1D-2-*O*-acetyl-1-*O*-butyryl-4,6-*O*-dibenzoyl-*myo*-inositol **4**. Benzylation of the remaining 3- and 5-positions in **4** using benzyl trichloroacetimidate/CF_3SO_3[11] led to the fully protected inositol derivative **5**. We were extremely pleased to find that the following removal of the ester functions was highly regioselective indeed, resulting in the rapid (1 h) formation of **6** with free hydroxy groups in the desired positions 1,2 and 6.

Scheme 1

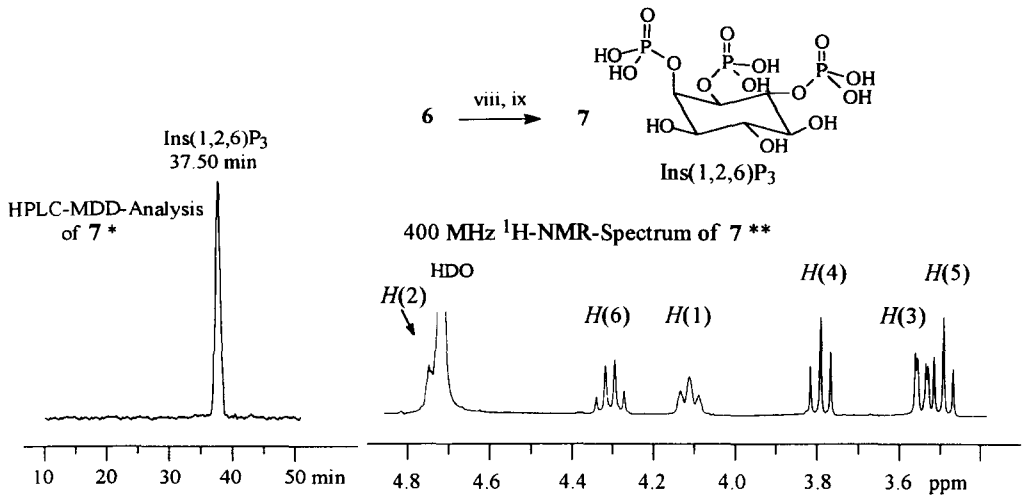


Reagents and conditions: i) THF, 3 eq. vinyl acetate, [LPL], 35°C, 5 d, quantitative; ii) Ar, CH_2Cl_2, pyridine, 2.1 eq. BzCl, [DMAP], 0 - 25°C, 12 h, 92 %; iii) MeOH/[HCl], reflux, 30 min, 68 %; iv) acetone, 3 eq. vinyl butyrate, [LPL], 35°C, 4 d, 80 %; v) THF, 2 eq. $CH_3(OEt)_3$, [*p*-TsOH], 25°C, 1.5 h; AcOH 80 %, 25°C, 1 h, 90 %; vi) Ar, cyclohexane/CH_2Cl_2 - 2:1, 1.5 - 3 eq. $Cl_3CCNHOBn$, [CF_3SO_3H], 25°C, 2 h, 71 % (**9**), 64 % (**5**); vii) MeOH/K_2CO_3, 25°C, 1 - 1.5 h, 72 % (**6**), 76 % (**10**); viii) Ar, CH_2Cl_2, 4 eq. Me_2N-P(OBn)$_2$, 4.1 eq. tetrazole, 25°C, 2 h; 4.5 eq. MCPBA, -40°C - 25°C, 2 h, 71 %; ix) 50 mbar H_2, ethanol 90 %, [Pd(OH)$_2$/C], 25°C, 12 h; H_2O - NaOH (pH 11 - 12), 25°C, 12 h; Amberlyst 15, 25°C, 15 min., 92 %; x) AcOEt, 4 eq. cyclohexanone, 4 eq. $CH_3(OEt)_3$, [*p*-TsOH], reflux, 1 h, 79 %.

While it is easily understandable that in the base catalysed methanolysis of **5** the acetate and butyrate functions are removed rapidly and faster than the more stable benzoate groups[12], it was somewhat surprising to find that in the progress of the reaction only one of the benzoate groups, exclusively the one in position 6, is removed selectively.

This observation can be rationalised by assuming that the formation of the tetrahedral intermediate required for the methanolysis of the ester function is more difficult in position 4 as compared to position 6 due to the sterically demanding groups in positions 3 and 5.

Scheme 2



*Mono Q-beads (5 µm, Pharmacia): column Ø = 10 mm, l = 100 mm; 25°C; buffer A: 50 mM Tris/HCl, pH 8.5, buffer B: 50 mM Tris/HCl, 400 mM KCl, pH 8.5; buffer C: 2 mM NH_4OAc, 30 µM YCl_3, 200 µM PAR, pH 5.0; flow: buffer A/B = 1.5 ml/min, gradient: % B (min) = 30 (0), 40 (2), 42 (16), 50 (20), 60 (38), 75 (48), 100 (50), buffer C *postcolumn* = 0.75 ml/min, detektor: 546 nm[16]. ** 400 MHz [D_2O, pH 6.0, 25°C] Na-salt[17].

This assumption is supported by similar observations made in the methanolysis of the structurally related 2,3-*O*-cyclohexylidene derivative **9** (Scheme 1). Again the benzoate function in the 6-position was preferentially removed during methanolysis and mainly **10** was formed. The 1,6-Diol **10** is also an important building block which could be useful e.g. for the preparation of glycosyl phosphatidylinositol protein anchors[13].

6 is highly stable under the conditions of methanolysis ($MeOH/K_2CO_3$, 25°C) and only traces of methyl benzoate can be detected after extended reaction times. **6** can be phosphorylated easily using *N,N*-dimethyl dibenzyl phosphoamidite, which is prepared in analogy to known procedures[14]. Deprotection of the resulting trisphosphate ester with H_2/Pd-C followed by saponification[15] (NaOH, pH 11 - 12) leads to Ins(1,2,6)P_3 **7** in nearly quantitative yield (Scheme 2). The observed chemical shifts and multiplicities of the ring proton resonances[17] in the ^{1}H-NMR-spectrum of **7** are due to the phosphorylation pattern (Scheme 2). The high isomeric purity of **7** was confirmed by ion exchange chromatography[16] (Scheme 2). All other data are in full agreement with recently published results[1b].

In summary, the above described method allows the conversion of *myo*-inositol *via* highly selective esterifications and regioselective deprotection steps into enantiomerically and isomerically pure **7**.

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[14] *N,N*-dimethyl dibenzyl phosphoamidite:
A mixture of 18.1 ml [100 mmol] **tris-(dimethylamino)-phosphine and 20.8 ml [200 mmol] benzyl alcohol is heated at 95 - 105°C under argon for 1 h. After this time the resulting products are fractionally distilled in vacuum over a vigreux column. See also ref. 14a.**
Yield: 20.3 - 23.2 g (70 - 80%) of a colourless liquid, bp: 132 - 134°C $_{0.05\ mbar}$
^{1}H-NMR (250.13 MHz, $CDCl_3$, δ [ppm], J [Hz]): δ = 3.47 2.64 (d, 6H, 2 CH_3, J_{PH} = 8.9), 4.74 - 4.89 (AB of ABX, 4H, 2 $C\underline{H}_2$-Ph, ν_a = 1201 Hz, ν_b = 1208 Hz, J_{AB} = 10.8, J_{PH} = J_{AX} = J_{BX} = 6.7), 7.41-7.43 (m, 10H, Ph); {^{1}H} ^{13}C-NMR (62.9 MHz, $CDCl_3$, δ [ppm], J [Hz]): δ = 34.50 (d, 2C, $\underline{C}H_3$, J_{CP} = 19.2); 65.20 (d, 4C, $\underline{C}H_2$-Ph, J_{CP} = 15.5); 127.09 (4C, *o*-Ph), 127.23 (2C, *p*-Ph), 128.09 (4C, *m*-Ph), 138.85 (d, 2C, *i*-Ph, J_{CP} = 5.9).
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[17] **7**(Na-salt): **^{1}H-NMR (400.13 MHz, D_2O, 25°C, pH 6.0, δ [ppm], J [Hz]): δ = 3.47 (dd as "t", *H*(5), ΣJ = 18.5), 3.53 (dd, *H*(3), $J_{2,3}$ = 2.2, $J_{3,4}$ = 10.0), 3.77 (dd as "t", *H*(4), ΣJ = 19.5), 4.08 (ddd as "t", *H*(1), ΣJ ≈18), 4.27 (ddd as "q", *H*(6), ΣJ = 27), 4.75 (*H*(2) hidden under HDO).**
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