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Note

Synthesis of (±) 3,6-di-*O*-benzyl-2-*myo*-inososse 1,4,5-tri-*O*-dibenzylphosphate as potential intermediate for the preparation of tritium-labelled *myo*-inositol 1,4,5-triphosphate

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D-*myo*-Inositol 1,4,5-triphosphate is well known as an intracellular second messenger in living cells of various tissues and, in particular, in brain membranes [1,2]. The potential therapeutic applications for the inositol derivatives appears multifarious since many receptor subtypes act with D-*myo*-inositol 1,4,5-triphosphate as messenger [3].

The elucidation of the complex mechanisms governing the inositol cascades requires the availability of labelled D-*myo*-inositol 1,4,5-triphosphate. Syntheses of this compound have been reported. They used either biosynthetic pathways [4] or organic syntheses [5]. The published preparations of labelled D-*myo*-inositol 1,4,5-triphosphate introduced the radioactive atom at a relatively early step of the synthesis [5]. Such approaches involve manipulating radioactive compounds along several steps and accumulate radioactive side products and polluted materials. Therefore, we decided to try to label D-*myo*-inositol 1,4,5-triphosphate with tritium as late as possible. We now report the synthesis of (±) 3,6-di-*O*-benzyl-2-*myo*-inososse 1,4,5-tri-*O*-dibenzylphosphate **11** which is the key intermediate in the preparation of [2-^{3}H]-*myo*-inositol 1,4,5-triphosphate **12**.

The starting material used was the commercially available *myo*-inositol **1** (Scheme 1). It was converted into (±) 3,6-di-*O*-benzyl-1,2-*O*-isopropylidene *myo*-inositol **2** in a five step well-described procedure [6]. The hydroxyls at C-4 and C-5 were protected as crotyl ethers [7] to give the totally protected inositol **3**. The crotyl protective group was preferred to the more classical allyl group since it enables more selective and milder deprotection [8]. The *cis*-isopropylidene protec-

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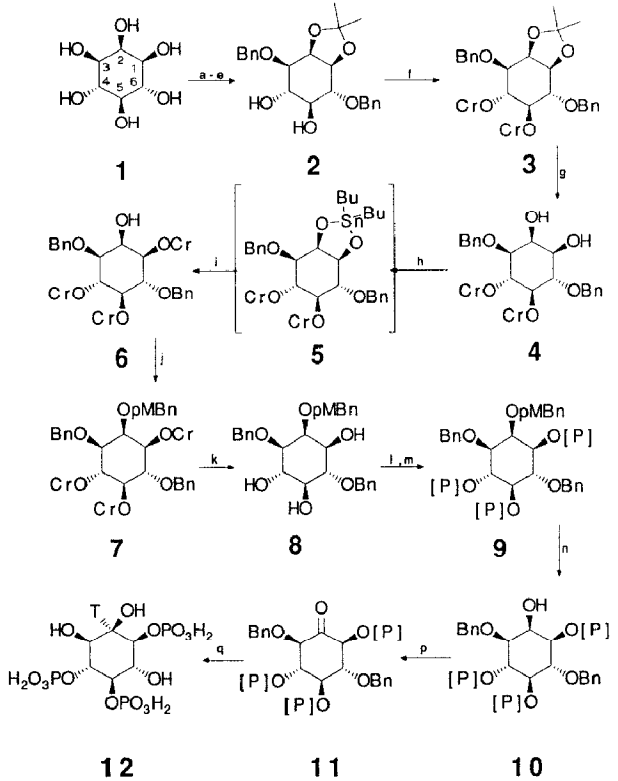

Scheme 1. Bn = $-CH_2Ph$; Cr = $-CH_2CH{=}CHCH_3$; *p*MBn = $pH_3COC_6H_4CH_2-$; [P] = $-PO(OCH_2Ph)2$. (a) 2,2-dimethoxypropane, DMF; (b) BzCl, Pyridine; (c) NaOH, MeOH; (d) BnBr, DMF, NaH; (e) acetone,H_2O; (f) Crotyl bromide, NaH, DMF; (g) HCl, acetone, MeOH; (h) Bu_2SnO, $Bu_4N^+Br^-$, H_3CCN; (i) CrBr, followed by $NaHCO_3$; (j) *p*MBnCl, DMF, NaH; (k) $tBuO^-K^+$, Me_2SO; (l) *N,N*-diethyl-dibenzylphosphoramidite, tetrazole, THF; (m) *m*CPBA, CH_2Cl_2; (n) cerium ammonium nitrate MeCN, H_2O; (p) Me_2SO, Ac_2O; (q) KB^3H_4, MeOH, followed by H_2, Pd/C.

tive group was then cleaved under acidic conditions to yield the diol **4**. Treatment of **4** with dibutyltin oxide resulted in a cyclic intermediate [9] **5** which was not isolated but directly reacted with crotyl bromide. After treatment with a potassium hydrogencarbonate solution in order to precipitate the tin byproducts, the crotyl ether **6** was the only product formed. The free hydroxyl group at C-2 was then protected as *p*-methoxybenzyl ether [10] **7**. This totally protected *myo*-inositol **7** was treated with potassium *tert*-butoxide which allowed the selective and mild deprotection of the three crotyl residues to yield the 1,4,5-triol **8**. This triol was phosphorylated in a 'one-pot' two-step procedure through a tri-*O*-dibenzylphosphite intermediate, prepared by means of *N,N*-diethyl-dibenzylphosphoramidate [11] which was oxidized in situ with *m*-chloroperoxybenzoic acid to give the triphosphate ester **9**. The next step was the selective removal of the C-2 *p*-methoxybenzyl protective group by means of cerium ammonium nitrate under mild

conditions [7]. The resulting alcohol group at C-2 in compound **10** was oxidized [12] with dimethylsulfoxide–acetic anhydride to the expected 2-inososе **11**.

Although the stereoselective reduction of 2-inososе is well described [13], the inososе derivative **11** seemed unfortunately not stable enough since its reduction did not afford the expected compound **12**. Noteworthy, numerous labelled (and also unlabelled) side products were formed after treatment with potassium[^{3}H]borohydride and deprotection, as can be seen from a HPLC profile of the crude reaction mixture.

1. Experimental

General methods.—Melting points were obtained on a Mettler PF 62 apparatus and are uncorrected. NMR spectra were recorded on a Bruker AC 200 spectrometer using the δ scale and $CHCl_3$ as internal reference. TLC were performed on Silica Gel precoated plates 60 F_{254} (E. Merck) with detection with phosphomolybdic acid, and UV. Column chromatography was carried out using Gerudan Si 60 (0.040–0.063 mm) Silica Gel (E. Merck).

(±) *3,6-Di-O-benzyl-4,5-di-O-crotyl-1,2-O-isopropylidene* myo-*inositol* (**3**).—(±) 3,6-Di-*O*-benzyl-1,2-*O*-isopropylidene *myo*-inositol [6] (**2**) (15 g, 3.74×10^{-2} mol) was dissolved in anhydrous DMF (250 mL). The solution was cooled at 0°C under argon atmosphere. NaH (3 equiv 5.0 g, 50% suspension) was added followed by crotyl bromide (3 equiv, 11.5 mL) under magnetic stirring for 2 h. The mixture was then stirred at room temperature for 72 h. The excess of NaH was neutralized by adding EtOH dropwise, the reaction mixture was poured into water and extracted with ether. The organic phase was dried over $MgSO_4$, filtered and the solvents were removed under reduced pressure (usual work up) to yield crude **3** which was used as such for the next step.

(±) *3,6-Di-O-benzyl-4,5-di-O-crotyl* myo-*inositol* (**4**).—The crude (±) 3,6-di-*O*-benzyl-4,5-di-*O*-crotyl-1,2-*O*-isopropylidene *myo*-inositol (**3**) was dissolved in a 7:3:1 MeOH–acetone–N HCl solution. The solution was refluxed for 30 min and, after cooling to room temperature, the medium was neutralized with NaOAc. The reaction mixture was evaporated to dryness and extracted by partition between water and ether. After the usual work up, the crude product crystallized from ether–petroleum ether (12.7 g, 73% starting from **2**); mp 88.7°C; ^{1}H NMR ($CDCl_3$): 7.4–7.3 (m, 10H, $(Ph)_2$), 5.8–5.6 (m, 4 H, $(-CH_2-CH{=}CH-CH_3)_2$), 4.84 (AB, J_{AB} 11.0, $\Delta\delta$ 0.26, 2 H, $-O-CH_2-Ph$), 4.71 (AB, J_{AB} 11.6, $\Delta\delta$ 0.05, $-O-CH_2-Ph$), 4.5–4.2 (m, 4 H,$(-CH_2-CH=)_2$), 4.16 (t, J 2.6, 1 H, H-2), 3.74 (t, J 9.5, 2H, H-4,5), 3.5–3.2 (m, 3 H, H-1,3,6), 2.54 (broad s, 2 H, $(OH)_2$), 1.9–1.6 (m, 6 H, $(=CH-CH_3)_2$). Anal. Calcd for $C_{28}H_{36}O_6$: C 71.77, H 7.74. Found: C 71.81, H 7.57.

(±) *3,6-Di-O-benzyl-1,4,5-tri-O-crotyl* myo-*inositol* (**6**).—Diol **4** (5.0 g, 1.06×10^{-2} mol), dibutyltin oxide (3.98 g, 1.5 equiv) and Bu_4NBr bromide (5.16 g, 1.5 equiv) were dissolved in MeCN (200 mL). Crotyl bromide (2.13 mL, 2.0 equiv) was

added and the reaction mixture was refluxed for 26 h; water was removed by azeotropic distillation over 4 Å molecular sieves. The reaction mixture was evaporated to dryness and the product extracted by partition between water and ether. The ether phase was treated with a saturated $NaHCO_3$ solution for 3 h to precipitate the tin derivatives. The mixture was passed through a Celite pad. Usual work-up yielded crude **6** (5.3 g, 95%) which was used as such for the next step.

(±) *3,6-Di-*O*-benzyl-1,4,5-tri-*O*-crotyl-2-*O*-*p*-methoxybenzyl* myo-*inositol* (**7**).—Alcohol **6** (5.28 g, 1.01×10^{-2} mol) was dissolved in DMF (250 mL) at 0°C. NaH (1.45 g of 50% suspension, 3 equiv) was added and the mixture stirred at 0°C. *p*-Methoxybenzyl chloride (1.51 mL, 1.1 equiv) was added and the reaction mixture was kept at room temperature for 13 h. The excess of NaH was neutralized with EtOH and the crude reaction mixture was poured into water and extracted with ether. The crude product obtained after the usual work up was purified by chromatography (1:1 ether–hexane), yielding **7** (5.90 g, 91%); ^{1}H NMR ($CDCl_3$): 7.4–6.8 (m, 14 H, $(Ph)_2$ and C_6H_4), 5.8–5.5 (m, 6 H, $(-CH{=}CH{-}CH_3)_3$), 4.9–4.6 (m, 6 H, $-CH_2Ph)_3$), 4.5–4.2 (m, 6 H, $(CH_2{-}CH{=})_3$), 4.0–3.8 (m, 3 H, H-2,4,5), 3.80 (s, 3 H, $-OCH_3$), 3.3–3.1 (m, 3 H, H-1,3,6), 1.7–1.6 (m, 9 H, $(CH{=}CH{-}CH_3)_3$). Anal. Calcd for $C_{40}H_{50}O_7$: C 74.74, H 7.84. Found: C 75.03, H 7.85.

(±) *3,6-Di-*O*-benzyl-2-*O*-p-methoxybenzyl* myo-*inositol* (**8**).—(±) 3,6-Di-*O*-benzyl-1,4,5-tri-*O*-crotyl-2-*O*-*p*-methoxybenzyl *myo*-inositol (**7**) (3.3 g, 5.13×10^{-3} mol) was dissolved in dry Me_2SO (200 mL). Potassium *tert*-butoxide (3.3 g) was added and the reaction vessel was kept at room temperature for 1 h and at 50°C for an additional 1 h. The vessel was then placed in crushed ice. The reaction mixture was neutralized with 0.1 M HCl and extracted with EtOAc. After the usual work up, the crude product was purified by chromatography (ether). The expected product was finally recrystallized from ether–hexane to give the triol **8** (1.03 g, 42%); mp 163.9°C; ^{1}H NMR ($CDCl_3$): 7.4–6.8 (m, 14 H, $(Ph)_2$ and $-C_6H_4$), 4.86 (s, 2 H, CH_2–Ph), 4.63 (AB, J_{AB} 11.6, $\Delta\delta$ 0.10, 4 H, $(-CH_2{-}Ph)_2$), 4.07 (t, 1 H, *J* 2.5, H-2), 4.00 (t, 1 H, *J* 9.4, H-6), 3.82 (s, 3 H,$-OCH_3$), 3.58 (AB of an ABXX′, J_{AB} 10.1, $\Delta\delta$ 0.20, J_{BX} 9.6, $J_{AX'}$ 9.4, 2 H, H-4,5), 3.51 (dd, *J* 16, *J* 2.6, 1 H, H-6), 3.28 (dd, 1 H, *J* 9.8, *J* 2.3, 1 H, H-1). Anal. Calcd for $C_{28}H_{32}O_7$: C:69.98, H 6.71. Found: C 69.69, H 6.58.

(±) *3,6-Di-*O*-benzyl-2-*O*-p-methoxybenzyl*-myo-*inositol 1,4,5-tri-*O*-dibenzylphosphate* (**9**).—(±) 3,6-Di-*O*-benzyl-2-*O*-*p*-methoxybenzyl *myo*-inositol (**8**) (500 mg, 1.04×10^{-3} mol) and tetrazole (1.0 g) were dried under high vacuum (5×10^{-2} Torr). THF (10 mL) was added followed by *N,N*-diethyl-dibenzylphosphoramidate (2.0 g, 6 equiv). The reaction mixture was stirred at room temperature for 4 h and then cooled at −40°C. At this temperature, *m*CPBA (1.82 g, 4 equiv) in CH_2Cl_2 (10 mL) was added. After 2 h at −40°C, the temperature was raised to room temperature and stirring was maintained for 15 h. The solvents were removed and the product extracted with CH_2Cl_2. The organic phase was washed with a 10% $Na_2S_2O_5$ sol, then with a 5% $NaHCO_3$ sol, dried over $MgSO_4$. After filtration and evaporation of the solvents, the crude crystals were recrystallized from ether–hexane (1.1 g, 94%); mp 117.8°C; ^{1}H NMR ($CDCl_3$): 7.5–6.7 (m, 44 H, $Ph_8 + p$-C_6H_4); 5.2–4.4 (m, 20 H, H-4,5, $(CH_2Ph)_8 + CH_2C_6H_4OCH_3$); 4.4–4.3 (m, 1 H,

H-2); 4.3–4.2 (m,1 H, H-1); 4.10 (t, 1 H, *J* 9.3, H-6); 3.74 (s, 3 H, OCH_3); 3.44 (dd, 1 H, *J* 10.2, *J* 1.2, H-3). Anal. Calcd for $C_{70}H_{71}O_{16}P_3$, $3H_2O$: C 63.91, H 5.90; P 7.06. Found: C 63.85, H 5.46, P 6.62.

(±) *3,6-Di-O-benzyl-1,4,5-tri-O-dibenzylphosphate* myo-*inositol* (**10**).—(±) 3,6-Di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-*myo*-inositol 1,4,5-tris(dibenzylphosphate) (**9**) (1.0 g, 0.794 mol), was dissolved in a 9:1 MeCN–water mixture and cooled to 0°C in an ice bath. Cerium ammonium nitrate (1.75 g, 4 equiv), dissolved in 10 mL of the same mixture was slowly added. The reaction vessel was maintained at 0°C for 1 h at room temp. The reaction mixture was poured into water and extracted with ether. After work up of the organic phase, crude crystals were obtained which were recrystallized from ether–hexane (0.70 g, 77%); mp 98.1°C; ^{1}H NMR ($CDCl_3$): 7.4–6.9 (m, 40 H, $(Ph)_8$), 5.1–4.4 (m, 18 H, $(-CH_2Ph)_8$ and, H-4,5), 4.36 (t, *J* 2.0, 1 H, H-2), 4.27 (t, *J* 2.0, 1 H, H-1), 4.08 (t, *J* 9.5, 1 H, H-6), 3.78 (dd, *J* 7.4, *J* 1.8, 1 H, H-3), 2.64 (broad s, 1 H, $-OH$). Anal. Calcd for $C_{62}H_{62}O_{15}P_3 \cdot H_2O$: C 64.24, H 5.65, P; 8.01. Found: C 64.34, H 5.52, P 8.16.

(±) *3,6-Di-O-benzyl-2-*myo-*inosose 1,4,5-tri-O-dibenzylphosphate* (**11**).—(±) 3,6-Di-*O*-benzyl-*myo*-inositol 1,4,5-tri-*O*-dibenzylphosphate (**10**) (500 mg, 4.38×10^{-4} mol) was dissolved in an anhydrous 3:2 Me_2SO–Ac_2O mixture. The mixture was kept at room temperature for 20 h and slowly poured into a saturated $NaHCO_3$ solution with stirring. Stirring was maintained for 2 h and the product was extracted with ether. After usual work up of the organic phase, the crude oil was purified by column chromatography (1:3 EtOAc–ether mixture) (35%). IR ($CHCl_3$): 1740 (C=O); 1600(Ar); ^{1}H NMR ($CDCl_3$): 7.4–7.0 (m, 40 H, $(CH_2Ph)_8$); 5.2–4.7 (m, 18 H, $(CH_2Ph)_8$, +H-4,5); 4.57 (d, 1 H *J* 10.8, H-1); 4.33 (d, 1 H, *J* 8.5, H-3); 3.85 (t, 1 H, *J* 8.0, H-6).

*Attempted preparation of [2-^{3}H]-*myo-*inositol 1,4,5-triphosphate* **12**.—Inosose **11** (1.47 mg, 1.30 μmol) was dissolved in MeOH (1 mL). This solution was directly introduced in a flask containing KB^3H_4 (500 mCi, 33 μmol). The mixture was stirred at room temp overnight and then, evaporated to dryness. The residue was taken up again in EtOH (1 mL) and evaporated to dryness to remove labile compounds. This last operation was repeated with additional 1 mL of EtOH. The residue was partitioned between water (2 mL) and CH_2Cl_2 (3×2 mL). CH_2Cl_2 was evaporated and the residue was dissolved in EtOH (2 mL). To this ethanolic solution which contained 4.9 mCi, Pd/C (6 mg) was added and the flask was placed under hydrogen (1 atm) overnight with stirring. After filtration (Millex SS) the crude material was analyzed by HPLC (Zorbax SAX column; eluant: KH_2PO_4 0.05M, pH 3.35; ^{3}H Detector Berthold). Only traces of the expected [2-^{3}H] IP_3 were dectected. Other assays, where we tried to isolate the reduced compound before final deprotection, also failed.

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