(2-Hydroxyethyl)-α-D-Glucopyranoside-2′,3,4-trisphosphate: Synthesis of a Second Messenger Mimic Related to Adenophostin A

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A concise synthetic route from D-glucose to a chiral, biologically active, phosphorylated analogue of the highly potent Ca^{2+}-mobilising agonist adenophostin A has been developed, involving a regioselective dibenzylation of allyl α-D-glucopyranoside and a one-pot Lemieux-type allyl oxidation with subsequent reduction and neighbouring deketalisation, to provide the key intermediate for phosphorylation.

The second messenger D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] **1** (Fig. 1) mobilises intracellular Ca^{2+} as the prime response to the activation of phospholipase C by stimulation of an extracellular G-protein coupled receptor in many cell types.[1,2] Intensive biological interest has followed the discovery of the Ca^{2+} releasing activity of Ins(1,4,5)P_3 in 1983.[3] Additionally, there has been considerable chemical investigation aimed at the synthesis of inositol polyphosphates and understanding the structure-recognition parameters at the Ins(1,4,5)P_3 receptor and other binding proteins.[4–7] The synthesis of structurally-modified Ins(1,4,5)P_3 analogues offers the prospect of pharmacological intervention in this signalling pathway.

Structural modification of Ins(1,4,5)P_3 resulting in biologically active compounds has generally consisted of phosphate alteration (*e.g.* to phosphorothioates, phosphonates *etc.*) or hydroxyl group deletion, reorientation, alkylation, or replacement by isosteres and other groups in the cyclitol ring.[4–7] Much success has been achieved in understanding the structure activity profiles of Ins(1,4,5)P_3 analogues with respect to the Ins(1,4,5)P_3 receptor and metabolic enzymes. The recently reported naturally occurring adenophostins[8,9] A and B, **2a** and **2b** respectively, isolated from cultures of *Penicillium brevicompactum*, are full agonists with little apparent resemblance to Ins(1,4,5)P_3 and yet possess a Ca^{2+} mobilising potency some 100 times higher than Ins(1,4,5)P_3. While the broad basis for their Ins(1,4,5)P_3-like activity is clear, a structural rationalisation of their exceptional potency is presently lacking. The key feature for their recognition by the Ca^{2+} mobilizing receptor is probably the glucose 3,4-bisphosphate/2-hydroxy triad, analogous to the 4,5-bisphosphate/-6-hydroxy motif of Ins(1,4,5)P_3, with the pyranoside oxygen acting as a surrogate for the C-2 of Ins(1,4,5)P_3. The adenophostins are thus interesting targets for chemical modification. We report here the first step in this direction with the synthesis of the polyphosphorylated carbohydrate derivative (2-hydroxyethyl) α-D-glucopyranoside 2′,3,4-trisphosphate **3**.

Our route required preparation of the intermediate allyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-α-D-glucopyranoside **4**. We reasoned that it should be possible to obtain a 2,6-di-substituted α-anomeric derivative by a bis stannylene approach. Alcoholysis of D-glucose with allyl alcohol (Scheme 1) in the presence of a strong cation-exchange resin[10] provided a 7:3 α:β anomeric mixture estimated from the integral ratio of the anomeric protons (H-1α, δ 4.92, *J* 3.7; H-1β, δ 4.46, *J* 7.9) in the ^{1}H NMR spectrum of the product **5ab** in D_2O. When **5ab** was stannylated with 2.5 equiv. of dibutyltin oxide in toluene and the reaction mixture cooled, a precipitate formed which could not be redissolved (and therefore benzoylated) in toluene or dioxane. However, when 1.2 equiv. of dibutyltin oxide were used, followed by treatment of the cooled solution with 2.1 equiv. of benzoyl chloride, several minor products and a major product were produced, as observed by TLC. After standard work-up[11] the known allyl 2,6-di-*O*-benzoyl-α-D-glucopyranoside[12] **6** (mp 135–138 °C [lit.,[12] 136–137 °C]) was isolated in 34% yield by crystallisation from ethanol. Reaction of **6** with 2-methoxypropene provided fully protected **7** ($[\alpha]_D$ + 69.4°) as an oil. Benzoate hydrolysis with methanolic sodium hydroxide provided diol **8**($[\alpha]_D$ + 114.0°), which was smoothly benzylated under standard conditions to give syrupy **4**($[\alpha]_D$ + 27.8°).

Alternatively, allyl α-D-glucopyranoside[10] **5a** (Scheme 2), isolated from **5ab** by fractional crystallisation, was stannylated with 2.5 equiv. of dibutyltin oxide, followed by treatment of the product with neat benzyl bromide at 100–110 °C for two days to give a mixture of benzylated products by TLC. After standard work-up,[11] the required 2,6-dibenzyl derivative **9** (mp 74–77 °C; $[\alpha]_D$ + 76.4°) was isolated in 44% yield by crystallisation from diisopropyl ether. The structure of **9** was confirmed by preparation of its 3,4-dibenzoate ($[\alpha]_D$ −10.0°), the ^{1}H NMR spectrum of which displayed deshielded triplets at 5.52 and 5.98 ppm, corresponding to H-4 and H-3 respectively. While this work was in progress, similar selectivity on the corresponding methyl glycoside was reported,[13] confirming the general applicability of this method. Reaction of **9** with

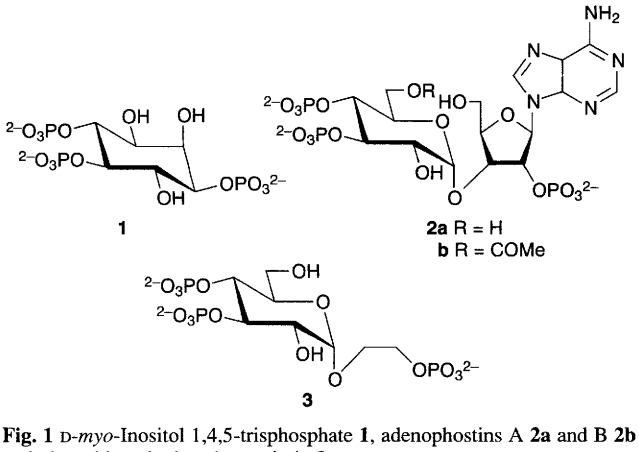
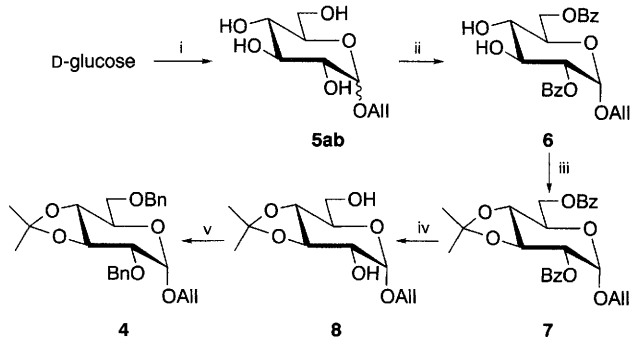


Fig. 1 D-*myo*-Inositol 1,4,5-trisphosphate **1**, adenophostins A **2a** and B **2b** and glucoside polyphosphate mimic **3**



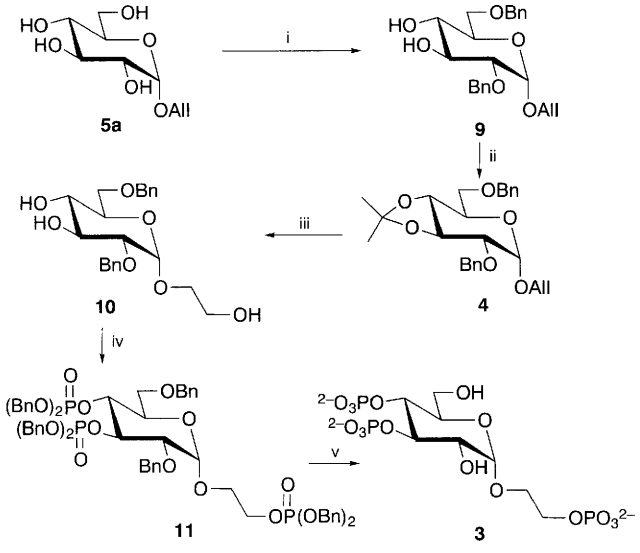
Scheme 1 Synthesis of allyl 2,6-di-*O*-benzyl 3,4-*O*-isopropylidene α-D-glucopyranoside. *Reagents and conditions*: i, Dowex-50 X-2, AllOH, reflux, 90 min; ii, (*a*) Bu_2SNO, toluene, reflux, $-H_2O$, 3 h (*b*) Et_3N, BzCl, room temp. 16 h, 34%; iii, 2-methoxypropene, acetone, -toluene-*p*-sulfonic acid, room temp., 5 min, 59%; iv, NaOH, MeOH, reflux, 1 h, 93%; v, NaH, BnBr, DMF, room temp., 76%. All = allyl.

2-methoxypropene provided **4**. Sequential treatment of **4** with OsO_4–$NaIO_4$ followed by excess $NaBH_4$[14] directly furnished (2-hydroxyethyl) 2,6-di-*O*-benzyl-α-D-glucopyranoside **10** ($[\alpha]_D$ + 48.2°) as an oil in 56% yield. ^1H and ^{13}C NMR spectral data of **10**† were consistent with those expected for an α-(2-hydroxyethyl) glucoside.[15]

Phosphitylation of **10** with bis(benzyloxy)(diisopropylamino)phosphine[16] followed by oxidation of phosphites with MCPBA gave the fully protected trisphosphate **11** ($[\alpha]_D$ + 12.5°). The intermediate vicinal 3,4-bisphosphite moiety exhibited the expected ^{31}P-^{31}P long range spin-spin coupling (^5J = 4.9 Hz).[17] The ^{31}P NMR spectrum of **11** showed a pseudo-septet at δ_P −0.89 (J_{HP} = 7.9 Hz), corresponding to the phosphorylated primary alcohol, and two pseudo-sextets at δ_P − 1.85 (J_{HP} 7.6 Hz) and −2.29 (J_{HP} 8.0 Hz) corresponding to the protected ring phosphates. Deprotection of **11** using sodium in liquid ammonia,[18] followed by ion-exchange chromatography of the crude product on Sepharose Q fast flow resin using buffers of triethylammonium bicarbonate gave the required trisphosphate **3**‡ ($[\alpha]_D$ + 90.5°, *c* 0.8 calc. for free acid, TEAB pH 8.6), eluting at *ca.* 470–550 mmol dm^{-3} buffer. Compound **3** was isolated as the triethylammonium salt and quantified by Briggs phosphate assay.

Trisphosphate **3** was examined for Ca^{2+} mobilising activity at the platelet Ins(1,4,5)P_3 receptor.[19] While **3** was found to release intracellular Ca^{2+}, its potency was not comparable to that reported for **2a**,[9] and was *ca.* 10 fold lower than Ins(1,4,5)P_3. Therefore, the adenosine motif appears to be an important requirement for the extreme potency of the adenophostins.

Thus, **3** represents a synthetic carbohydrate polyphosphate mimic of Ins(1,4,5)P_3 and, together with our recent demonstration that a cyclopentane-based analogue also exhibits activity,[20] contributes to the emerging class of structurally diverse Ins(1,4,5)P_3 mimics.



Scheme 2 Synthesis of (2-hydroxyethyl) α-D-glucopyranoside 2′,3,4-trisphosphate. *Reagents and conditions*: i, (*a*) Bu_2SnO, toluene, reflux, $-H_2O$, 4 h, (*b*) BnBr, 100–110 °C, N_2, 2 d, 44%; ii, 2-methoxypropene, acetone, toluene-*p*-sulfonic acid, room temp., 5 min, 71%; iii, (*a*) $NaIO_4$, OsO_4, H_2O–diethyl ether (1 : 1), room temp., 5 h, (*b*) $NaBH_4$, MeOH, 0 °C, 1 h, 56%; iv, (*a*) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, room temp. 30 min, (*b*) MCPBA, −78 °C to room temp. 10 min, 80%; v, Na/liquid NH_3.

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Footnotes

† *Spectroscopic data* for compound **10**: δ_H (400 MHz; $CDCl_3$) 3.35 (1 H, dd, *J* 3.4, 9.8, 2-H), 3.38–3.43 (1 H, m, C*H*HCH$_2$), 3.45 (1 H, t, *J* 9.3, 4-H), 3.58–3.69 (6 H, m, simplifies to 5 H, m, on D_2O exch, OH, 6-H, 6-H′, CH*H*CH$_2$), 3.74 (1 H, ddd, *J* 2.4, 4.9, 9.8, 5-H), 3.83 (2 H, brs, exch D_2O, 2 × OH), 3.97 (1 H, t, *J* 9.8, 3-H), 4.50, 4.55 (2 H, AB, J_{AB} 12.2, PhC*H*$_2$O), 4.56 (1 H, AB, J_{AB} 12.2, PhC*H*HO), 4.66 (1 H, d, *J* 3.9, 1-H), 4.73 (1 H, AB, J_{AB} 12.2, PhCH*H*O) and 7.23–7.36 (10 H, m, aromatic CH).

δ_C (100 MHz; $CDCl_3$) 61.47 (2′-C), 69.37 (6-C), 70.28 (1′-C), 70.50, 70.63, 72.88 (3-C, 4-C, 5-C), 73.55 (2 × PhCH_2O), 79.52 (2-C), 97.15 (1-C), 127.67, 127.71, 128.33, 128.38, 128.44, 128.66 (aromatic CH), 137.54 and 138.00 (2 × 1-C of benzyl rings). *m/z* (FAB$^-$) 403 (M − 1)$^-$, 50% and 557 (M + NBA)$^-$, 100%.

‡ *Spectroscopic data* for compound **3** δ_H (400 MHz; D_2O, pH *ca.* 4) 3.54–3.60 (1 H, m, 5-H), 3.55 (1 H, dd, *J* 3.9, 9.8, 2-H), 3.67–3.74 (4 H, m, OC*H*$_2$CH$_2$OPO$_3^{2-}$, 6-H, 6-H′), 3.82–3.91 (3 H, m, 3-H or 4-H, OCH$_2$-C*H*$_2$OPO$_3^{2-}$), 4.26 (1 H, ddd, *J* 8.8, 9.3, 3-H or 4-H) and 4.85 (1 H, d, *J* 3.9, 1-H). δ_P (36 MHz; D_2O, pH *ca.* 4) 0.32, 0.45 and 0.52 (3 s).

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