A disaccharide polyphosphate mimic of 1d-*myo*-inositol 1,4,5-trisphosphate

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A concise route from D-glucose and D-ribose to a potent sugar polyphosphate second messenger mimic related to adenophostin A is described; a role for the adenine base of the adenophostins is suggested.

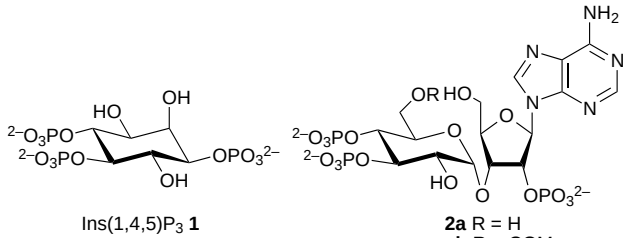
1d-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] is a second messenger responsible for increasing the intracellular Ca^{2+} concentration in stimulated cells.[1] Structure–activity studies now allow a good understanding of the relationship between **1** and its receptor.[2] A specific vicinal d-*threo* bisphosphate is essential for activity, while the third phosphate and 6-hydroxy group help to enhance binding affinity.

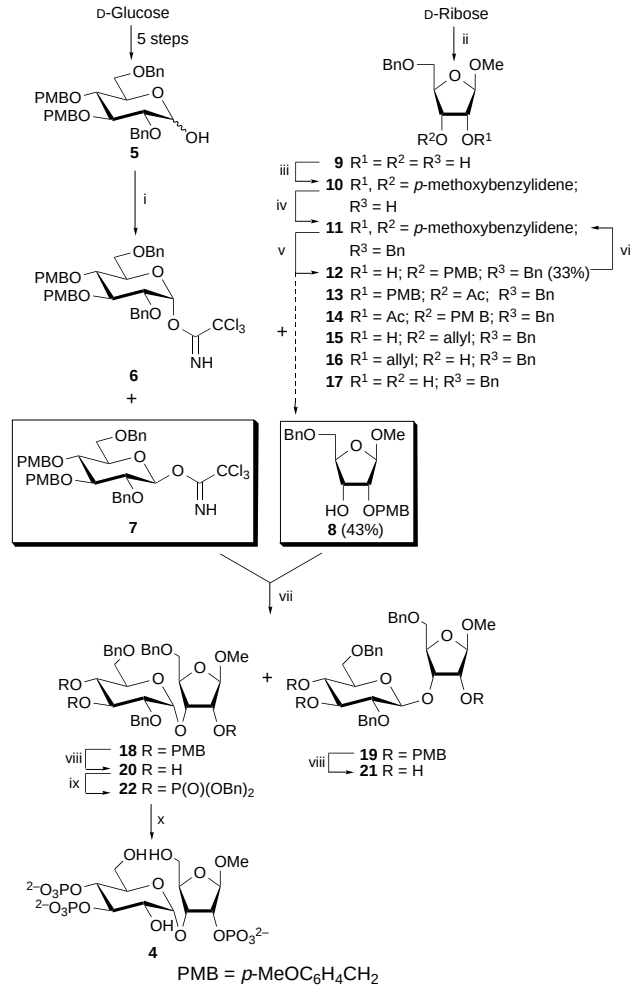
Most active inositol polyphosphates and related compounds tested at the Ins(1,4,5)P_3 receptor exhibit potencies comparable to or less than that of **1**.[2] However, in 1993 a Japanese group reported the isolation of two potently agonistic trisphosphate glyconucleotides from culture broths of *Penicillium brevicompactum*,[3] named adenophostins A and B and identified as **2a** and **2b**, respectively.[4] The structure of **2a** has now been confirmed by total synthesis.[5] Both adenophostins are effective at concentrations 10–100-fold lower than for **1**; these relative potencies are consistent with binding data.[6,7] Thus, **2a** and **2b** are by far the most potent agonists yet described at the Ins(1,4,5)P_3 receptor, despite their structural dissimilarity to **1**, and are attractive leads to carbohydrate-based Ins(1,4,5)P_3 mimics and receptor modulators.

To elucidate the structural motifs of the adenophostins responsible for their activity we[8] and others[9] prepared the minimal structure **3**, which exhibited a potency 10-fold lower than **1**, demonstrating that while the glucose bisphosphate contained the pharmacophore responsible for Ca^{2+} release, at least part of the adenosine moiety was responsible for the high activity of the adenophostins. Molecular modelling studies on **3** established[9] that the staggered conformation of its bimethylene chain did not allow the 2′-phosphate to mimic the corresponding phosphate of either **1** or **2a**. We report here the synthesis of methyl 3-*O*-(α-d-glucopyranosyl)-β-d-ribofuranoside 2,3′,4′-trisphosphate **4**, in which the adenine ring of **2a** has effectively been deleted, but the third phosphate is held similarly to **2a**, and which should clarify the relative importance of conformational restriction of this phosphate for the potency of **2a**.

2,6-Di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-d-glucopyranose **5** was prepared in five steps from d-glucose.[8b] Reaction of **5** with Cl_3CCN in the presence of K_2CO_3[10] gave the α-trichloroacetimidate **6** ($[\alpha]_D$ +13.6) and the crystalline β-anomer **7** (mp 80–81 °C; $[\alpha]_D$ +16.5), separated by flash chromatography, in the ratio of 1 : 2.5, based on isolated products.

With a suitable glycosyl donor in hand, we required **8** as an acceptor. d-Ribose was converted into known[11] **9**. Reaction of **9** with 1.05 equiv. of *p*-methoxybenzaldehyde dimethyl





Scheme 1 *Reagents and conditions*: i, Cl_3CCN, K_2CO_3, CH_2Cl_2, N_2, room temp. 2 h, 65%; ii, MeOH, H_2SO_4, room temp., 20 h (β-anomer obtained by crystallisation); iii, *p*-$MeOC_6H_4CH(OMe)_2$, PTSA, DMF, 70 °C, −MeOH, 4 h, 93%; iv, NaH, BnBr, DMF, 3 h, 82%; v, DIBAL-H, CH_2Cl_2, room temp., 3 h, 76%; vi, DDQ, CH_2Cl_2, 3 Å sieves, room temp., 3 h, 71%; vii, $Me_3SiOSO_2CF_3$, Et_2O, 3 Å sieves, room temp., 10 min; viii, DDQ, CH_2Cl_2–H_2O (10 : 1), room temp., 1 h, 56%; ix, $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, room temp., 30 min, then MCPBA, −78 °C to room temp., 10 min, 82%; x, H_2, Pd–C, 40 psi, MeOH–H_2O 4:1, room temp., 18 h, 70%

acetal,[12] with continuous removal of the liberated MeOH *via* an air condenser,[13] gave the 2,3-*O*-(*p*-methoxybenzylidene) derivative **10** ($[\alpha]_D$ −43.0) as a *ca.* 3 : 2 diastereoisomeric mixture, as judged by NMR. Benzylation of **10** gave **11** ($[\alpha]_D$ −27.2). Cleavage of the acetal with $LiAlH_4$–$AlCl_3$ in refluxing THF,[14] $NaCNBH_3$–Me_3SiCl in MeCN[12] or DIBAL-H in CH_2Cl_2[15] all gave the required **8** (mp 42–43 °C; $[\alpha]_D$ +34.6), and the more polar **12** ($[\alpha]_D$ −28.8), in approximately equal proportions, the latter reagent giving by far the best yield. The structures of **8** and **12** were confirmed by preparation of acetates **13** ($[\alpha]_D$ +17.5) and **14** ($[\alpha]_D$ +21.5), the ^{1}H NMR spectra of which respectively revealed a triplet at δ 5.13 (*J* 5.3 Hz) corresponding to H-3, and a doublet at δ 5.18 (*J* 4.4 Hz; H-1 presented as a singlet) corresponding to H-2. Although the regioselectivity of acetal cleavage was disappointing, isomer **12** was easily reoxidised to **11** (as a 92 : 8 diastereoisomeric mixture, $[\alpha]_D$ −26.4; diastereoisomers not assigned) using DDQ in dry CH_2Cl_2.[16]

A preparation of the related allyl-protected ribosides **15** and **16** by a different route has recently been reported.[17] Anomerisation of methyl 5-*O*-benzyl-2,3-*O*-isopropylidene-β-d-ribofuranoside on acidic hydrolysis, to give a *ca.* 1 : 4 α : β-anomeric mixture of products was described. We found that the more labile *p*-methoxybenzylidene acetal of **11** could be removed without anomerisation by treatment with 80% (*v*/*v*) aqueous acetic acid at 60 °C for 25 min, to give **17** ($[\alpha]_D$ −42.0; lit.[17] −47.7). Therefore, **10** may be a more suitable intermediate than methyl 2,3-*O*-isopropylidene-β-d-ribofuranoside to prepare derivatives of methyl β-d-ribofuranoside substituted at position 5.

Coupling of **7** and **8** was achieved using $Me_3SiOSO_2CF_3$ as promoter.[10] The ^{1}H NMR spectrum indicated that although the α-glucopyranosyl compound **18** was the major product (H-1′, δ 5.09, *J* 3.4 Hz), the anomer **19** was present as a *ca.* 20% contaminant which could not be removed at this stage. However, on treatment with DDQ, the required triol **20**‡ (mp 103–105 °C; $[\alpha]_D$ +28.3) could be separated from **21** by column chromatography. Phosphitylation of **20** followed by oxidation gave the trisphosphate **22** ($[\alpha]_D$ +34.7). Compound **22** was hydrogenolysed to give the required trisphosphate **4**§ which was purified on Q Sepharose resin eluting with a 0–1 mol dm^{-3} gradient of triethylammonium hydrogen carbonate, pH 7.5. The triethylammonium salt of **4** eluted at *ca.* 800–850 mmol dm^{-3} buffer. As this form was surprisingly poorly soluble in water, it was converted to the freely soluble hexapotassium salt ($[\alpha]_D$ +67.3 calc. for free acid, H_2O, pH 8.5) before quantification by total phosphate assay[18] and biological evaluation.

Preliminary biological evaluation of 'ribophostin' **4** using permeabilised hepatocytes[19] revealed a Ca^{2+}-mobilising potency 10-fold better than **3** and very close to that of Ins(1,4,5)P_3. Full biological characterisation will be reported elsewhere. Noting that 2′-dephosphorylation of **2a** reduces its binding affinity 1000-fold,[4] this suggests that conformational restriction of the 2′-phosphate alone can engender Ins(1,4,5)P_3-like, but not adenophostin-like, potency. Therefore the adenine base of adenophostin plays an important, but as yet undefined, role in enhancing activity. We believe that it probably contributes to the positioning of the ribose phosphate such that it can mimic the 1-phosphate of Ins(1,4,5)P_3 in a unique way. Further clarification must now await the synthesis of suitably conformationally restricted compounds for accurate positioning of the 2′-phosphate group.

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Footnotes

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‡ *Spectroscopic data* for compound **20**: δ_H (400 MHz; $CDCl_3$; *J*/Hz) 1.69 (1 H, br s, exch. D_2O, OH), 2.74, 2.87 (2 H, 2 br s, exch. D_2O, 2 × OH), 3.32 (3 H, s, OCH_3), 3.38 (1 H, dd, *J* 3.4, 9.8, 2′-H), 3.45–3.57 (5 H, m, 4′-H, 5-Ha, 5-Hb, 6′-Ha, 6′-Hb), 3.74 (1 H, dt, *J* 3.9, 9.8, 5′-H), 3.92 (1 H, t, *J* 9.3, 3′-H), 4.01 (1 H, m, 3-H), 4.22 (2 H m, 2-H, 4-H), 4.44, 4.52 (2 H, AB, J_{AB} 12.2, CH_2Ph), 4.51 (2 H, s, CH_2Ph), 4.69, 4.74 (2 H, AB, J_{AB} 11.7, CH_2Ph), 4.69 (1 H, d, *J* 3.4, 1′-H), 4.88 (1 H, s, 1-H), 7.23–7.36 (15 H, m, 3 × Ph); δ_C(100.4 MHz; $CDCl_3$) 55.03 (q, OCH_3), 69.00 (t, C-5 or C-6′), 70.74 (d, C-4′), 70.84 (d, C-5′), 71.69 (t, C-5 or C-6′), 73.24 (d, C-3′), 73.28 (d, C-3), 73.32, 73.55, 74.14 (3 t, CH_2Ph), 78.35 (d, C-2′), 79.11 (d, C-2 or C-4), 80.27 (d, C-2 or C-4), 97.79 (d, C-1′), 108.34 (d, C-1), 127.57, 127.63, 127.69, 127.74, 128.33, 128.42, 128.51, 128.75 (8 d, Ph), 137.18, 137.86, 138.06 (3 s, 3 × C-1 of phenyl ring); *m*/*z* (FAB^+) 597 [$(M + 1)^+$, 12%], 565 [$(M - OCH_3)^+$, 48], 343 [$(M - C_{13}H_{18}O_5)^+$, 3], 255 [$(M - C_{20}H_{24}O_5)^+$, 2]; *m*/*z* (FAB^-) 595 [$(M - 1)^-$, 28%], 505 [$(M - C_7H_7)^-$, 15], 253 [$(M - C_{20}H_{24}O_5)^-$, 25].

§ *Spectroscopic data* for compound **4** (triethylammonium salt): δ_H(400 MHz; CD_3OD; *J*/Hz) 3.35 (3 H, s, OCH_3), 3.54 (1 H, ABX, $^2J_{AB}$ 11.9, ^3J 6.4, 5-Ha), 3.62 (1 H, dd, *J* 3.8, 9.6, 2′-H). 3.66 (1 H, br s, OH), 3.69–3.73 (3 H, m, 5-Hb, 5′-H, 6′-Ha), 3.93 (1 H, ABX, $^2J_{AB}$ 13.0, ^3J 3.5, 6′-Hb), 4.04–4.11 (2 H, m, 4-H, 4′-H), 4.44 (1 H, dd, *J* 4.3, 7.3, 3-H), 4.45 (1 H, q, $J_{3\text{-H, 2-H}} = J_{3\text{-H, 4-H}} = J_{HP} = 9$, 3′-H), 4.58 (1 H, dd, *J* 4.3, J_{HP} 9.5, 2-H), 4.94 (1 H, s, 1-H), 5.03 (2 H, br s, 2 × OH), 5.13 (1 H, d, *J* 3.7, 1′-H); δ_C(100.4 MHz; CD_3OD) 55.18 (q, OCH_3), 61.98, 64.87 (2 t, C-5, C-6′), 73.32, 73.52, 73.78, 76.29, 76.46 (with C–P coupling), 78.83 (6 d, C-2, C-3, C-2′–C-5′), 82.71 (d, C-4), 98.93 (d, C-1′), 108.96 (dd, $^3J_{CP}$ 3.7, C-1); δ_P(161.7 MHz; CD_3OD) −0.38, 1.05, 1.10 (3s); *m*/*z* (FAB^-) 565 [M^-, 100%] (Found: M^-, 565.012. $C_{12}H_{24}O_{19}P_3$ requires, 565.012).

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