Convergent synthesis of adenophostin A analogues *via* a base replacement strategy

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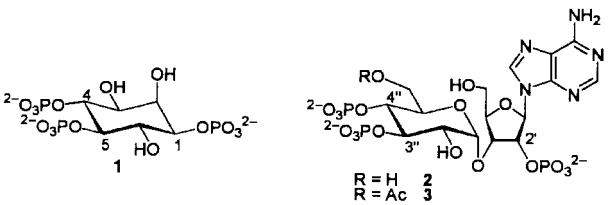
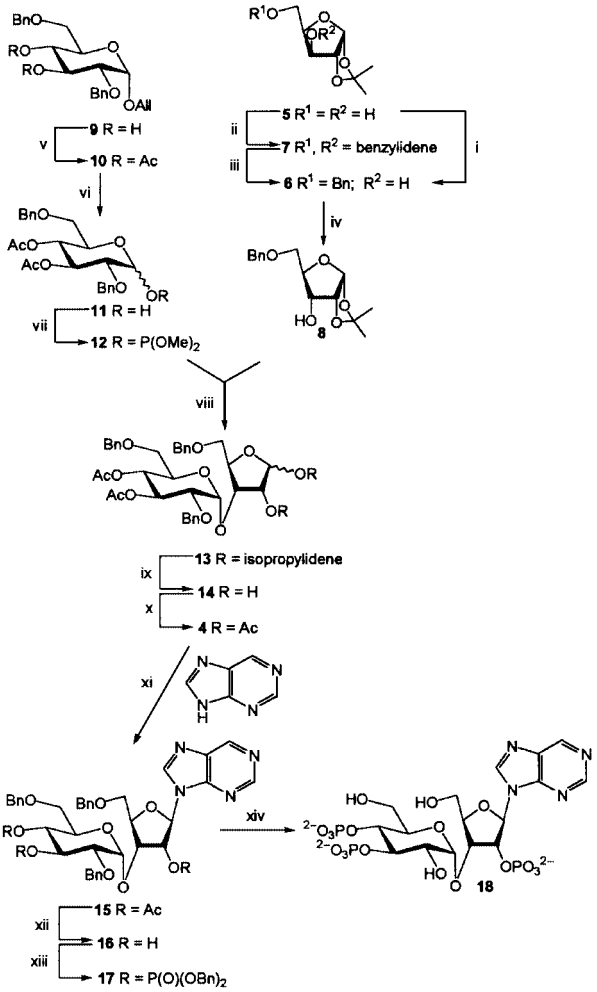
The first totally synthetic base-modified analogues of the natural product and potent D-*myo*-inositol 1,4,5-trisphosphate receptor agonist adenophostin A were efficiently synthesised from D-xylose and D-glucose using methodology employing base and surrogate base addition to a common disaccharide intermediate.

D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] is a second messenger which mediates the release of intracellular Ca^{2+} on binding to its own ligand-gated receptor.[1] The synthesis of Ins(1,4,5)P_3 analogues has provided insights into the structural requirements for binding and Ca^{2+} release at the Ins(1,4,5)P_3 receptor,[2] although inositol-based mimics with a potency exceeding that of Ins(1,4,5)P_3 have yet to be synthesised.



Adenophostins A and B (**2** and **3**)[3] were isolated from broths of *Penicillium brevicompactum*, and were found to be 10- to 100-fold more potent than Ins(1,4,5)P_3 in releasing Ca^{2+} and in receptor binding assays.[4,5] Design of chiral ligands based upon carbohydrates rather than cyclitols could provide novel synthetic signal transduction modulators. The biological activity of minimal structural analogues synthesised so far has not exceeded that of Ins(1,4,5)P_3[5–11] and it is likely that the adenine base is crucial for potency. Adenophostin A is finding widespread use as a tool to investigate cell signalling mechanisms[12] and there have been three syntheses of the molecule.[13–15] We required base-modified analogues of **2** and report here an efficient convergent route to five such compounds *via* a common disaccharide intermediate.

The disaccharide required for Vorbrüggen condensations, 1,2,3′,4′-tetra-*O*-acetyl-2′,5,6′-tri-*O*-benzyl-3-*O*-α-D-glucopyranosyl-D-ribofuranose (**4**), was prepared as follows. Species **5**[16] (Scheme 1) was selectively 5-*O*-benzylated by two different methods. Regioselective ring opening of a 3,5-*O*-dibutylstannylene derivative with BnBr yielded the 5-*O*-benzyl ether **6** (mp 45–47 °C; $[\alpha]_D$ +2.9) in one step. A 78% yield of benzylated products was isolated containing 90% of the desired regioisomer **6**. This method has since been reported by another group.[17] Alternatively, acid-catalysed treatment of **5** with benzaldehyde dimethyl acetal gave the crystalline benzylidene acetal **7** (mp 125 °C; $[\alpha]_D$ +3.8) as a single diastereoisomer in 89% yield; regiospecific cleavage of this acetal with $NaCNBH_3$–HCl also gave **6** in 68% yield. Inversion of the 3-hydroxy group to give the acceptor **8** (mp 83–85 °C; lit.[18] 81–83 °C) was achieved by oxidation with Ac_2O and DMSO and reduction of the corresponding 3-ulose with $NaBH_4$.[17] Acetylation of **9**, available in two steps from D-glucose,[6] gave diacetate **10** quantitatively and cleavage of the allyl glycoside was achieved using $PdCl_2$[19] to give **11** (mp 71–74 °C; $[\alpha]_D$ +83.0). Phosphitylation[15] gave the phosphite donor **12** as an oil in a 1:1 anomeric mixture as judged by ^1H and ^{31}P NMR spectroscopy.



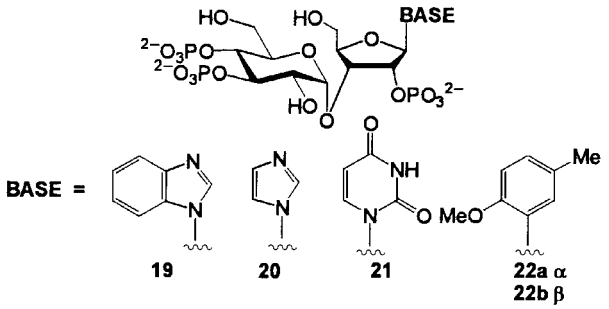
Scheme 1 *Reagents and conditions*: i, Bu_2SnO, BnBr, Bu_4NBr, MeCN, 4 Å sieves, Soxhlet, reflux, 24 h, 78%; ii, $PhCH(OMe)_2$, TsOH, DMF, 70 °C, –MeOH, 2 h, 89%; iii, $NaCNBH_3$–HCl, THF–Et_2O, 3 Å sieves, room temp., 5 min, 68%; iv, (*a*) Ac_2O–DMSO, 18 h, room temp., (*b*) $NaBH_4$, EtOH–H_2O (5:4), room temp., 1 h, 50% over two steps; v, Ac_2O, pyridine, room temp., 20 h, 100%; vi, $PdCl_2$, MeOH–CH_2Cl_2 (1:1), room temp., 3 h, 78%; vii, $(MeO)_2PNEt_2$, 1*H*-tetrazole, CH_2Cl_2, room temp., 30 min; viii, dioxane–toluene (3:1), 4 Å sieves, room temp., 2 h then $ZnCl_2$, $AgClO_4$, dark, room temp., 20 h, 81% (based on **8**); ix, AcOH–H_2O–$(CH_2OH)_2$ (14:6:3), reflux, 15 min, 75%; x, Ac_2O, pyridine, room temp., 20 h, 82%; xi, (*a*) purine, $(Me_3Si)_2NH$–Me_3SiCl (2:1), reflux, 20 h, (*b*) TMSOTf, $(CH_2Cl)_2$, reflux, 7 h, 61%; xii, conc. aq. NH_3–MeOH (1:5), sealed vessel, room temp., 20 h, 97%; xiii, $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2, room temp., 30 min, then MCPBA, −78 °C, 5 min, 80%; xiv, wet $Pd(OH)_2$/C, MeOH–cyclohexene–H_2O (11:5:1), reflux, 17 h, 56%.

Donor **12** and acceptor **8** were coupled together in a similar fashion to that described[11*b*] to give the desired disaccharide **13**

(mp 125–127 °C; $[\alpha]_D$ +101.6) in 81% yield; the reaction time had to be extended because of the deactivating effects[20] of the donor 3,4-di-*O*-acetate protecting groups. Note the high regiospecificity of this glycosylation, the α-coupled anomer being the sole isolated product in high yield. Cleavage of the isopropylidene acetal was accomplished by heating **13** at reflux in aqueous AcOH containing ethylene glycol[14] to give **14** (mp 126–128 °C; $[\alpha]_D$ +116.5) which was acetylated to give **4**.†

To exemplify our route the condensation of **4** with purine is described, using the method of Vorbrüggen *et al.*[21] with TMSOTf as catalyst. The major product was the 9-β-D-ribofuranosidopurine nucleoside (nebularine) derivative **15** ($[\alpha]_D$ +73.6) which exhibited a deshielded doublet at δ_H 6.44 (*J* 4.9 Hz) corresponding to H-1′ of a β-substituted product; purine signals in the ^{13}C NMR spectrum also corresponded closely to those of the known[22] 2′,3′,5′-tri-*O*-acetyl nebularine. Stirring **15** in a mixture of concentrated aqueous ammonia and methanol gave triol **16** ($[\alpha]_D$ +23.5) required for phosphorylation. Triol **16** was phosphitylated and the resulting trisphosphite was oxidised to trisphosphate **17** ($[\alpha]_D$ +17.9).[15] Deprotection of **17** to the purine analogue of **2** ('purinophostin', **18**) was achieved with catalytic transfer hydrogenation. The free acid was eluted from MP1 AG ion exchange resin with a gradient of aqueous TFA and converted into the sodium salt.

Other adenine-related base surrogates used were benzimidazole and imidazole, condensation being achieved similarly to above to give **19** and **20**. The imidazole analogue of adenophostin A ('imidophostin') **20**, in which the adenine six-membered



ring had been effectively deleted, was readily accessible by condensation of **4** with *N*-trimethylsilylimidazole.[23] A small amount of bis-glycosylated material was also formed. The desired product was deprotected, phosphorylated and deprotected as previously. We also synthesised an analogue ('uridophostin', **21**†) possessing the natural nucleic acid base, uracil. Condensation of **4** with 4-methylanisole gave both α- and β-substituted aryl C-glycosides in a *ca.* 1:1 ratio from ^1H NMR spectroscopy; these products were used to prepare the α- and β-4-methyl anisole analogues of adenophostin A, **22a** and **22b** respectively, by the coupling of 4-methylanisole and **4** in the presence of $AgCO_2CF_3$ and $SnCl_4$. This demonstrates the utility of our route also for the preparation of C-nucleoside analogues.

Convergent construction of **2** was described by van Straten *et al.*[14] This elegant approach was somewhat hampered, however, by a large number of protection/deprotection steps. Our present route allows efficient access to a central disaccharide **4**, whilst the choice of protecting groups requires minimal manipulation between Vorbrüggen condensation and target trisphosphates.

In summary, we report here an efficient route to the first synthetic base-modified analogues of adenophostin A.

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Notes and references

† *Selected data* for **4**: mp 105–107 °C (EtOAc–hexane); R_f 0.29 (EtOAc–hexane 3:7); $[\alpha]_D^{20}$ +98.2 (*c* 0.2, $CHCl_3$) (Found: C, 64.00; H, 6.15. Calc. for $C_{40}H_{46}O_{14}$: C, 63.98; H, 6.18%); δ_H(400 MHz; $CDCl_3$) 1.86, 1.87, 1.93, 1.96 (12H, 4s, CH_3CO), 3.29, 3.36 (2H, ABX, $^2J_{AB}$ 10.7, $^3J_{AX}$ 3.9, $^3J_{BX}$ 2.4, H-6′$_A$, H-6′$_B$), 3.56 (1H, *J* 9.8, 3.4, H-2′), 3.63, 3.72 (2H, ABX, $^2J_{AB}$ 11.2, $^3J_{AX}$ 3.9, $^3J_{BX}$ 2.9, H-5$_A$, H-5$_B$), 3.88 (1H, ddd, *J* 10.3, 2.9, H-5′), 4.29 (1H, AB, J_{AB} 12.2, OCH*H*Ar), 4.37–4.40 (1H, m, H-4), 4.47–4.57 (4H, m, 2 × OC*H*$_2$Ar), 4.63–4.64 (2H, m, H-3, OCH*H*Ar), 5.03–5.08 (2H, m, H-1′, H-4′), 5.33 (1H, d, *J* 4.9, H-2), 5.38 (1H, dd, *J* 9.3, H-3′), 6.12 (1H, s, H-1), 7.23–7.34 (15H, m, ArCH); δ_C (100.4 MHz; $CDCl_3$) 20.45, 20.56, 20.63, 20.74, 20.83, 20.94, 21.05, 21.16 (8q, 4 × *C*H$_3$CO$_{\alpha \text{ and } \beta}$), 67.52 (t, C-6′), 68.85 (d, C-5′), 69.00, 69.28 (2t, C-5$_{\alpha \text{ and } \beta}$), 71.89, 72.02 (2d, C-3′$_{\alpha \text{ and } \beta}$), 73.02 (d, C-2), 73.17, 73.26, 73.35, 73.41, 73.48, 73.55 (4t and 2d, C-3$_{\alpha \text{ and } \beta}$, OCH$_2Ar_{\alpha \text{ and } \beta}$), 76.59, 76.76 (2d, C-2′$_{\alpha \text{ and } \beta}$), 81.27 (d, C-4), 96.28, 96.47 (2d, C-1′$_{\alpha \text{ and } \beta}$), 98.46, 98.50 (d, C-1$_{\alpha \text{ and } \beta}$), 127.32, 127.61, 127.74, 127.91, 128.02, 128.36, 128.46 (7d, ArCH), 137.51, 137.76, 138.11 (3s, 3 × C-1 of Bn rings), 169.35, 169.69, 170.17, 170.28 (4s, 4 × CH_3CO); α and β subscripts denote signals arising from α and β-anomers respectively; *m/z* (FAB$^+$) 750 (M^+, 1%), 91 (100). For uridophostin: δ_H (D_2O) 3.90–3.50 (7H, m, H-4′, H-5′, H-2″, H-5″, and H-6″), 4.17 (1H, m, H-4″), 4.32–4.28 (2H, m, H-3′ and H-3″), 4.74 (1H, m, H-2′), 5.15 (1H, br s, H-1″), 5.74 (1H, d, *J* 8.3, H-5), 5.95 (1H, d, *J* 4.4, H-1′), 7.65 (1H, d, *J* 8.3, H-6); δ_P(100 MHz, D_2O, ^1H-decoupled) 0.23, 1.09, 1.78; HRMS (triethylammonium salt, FAB) calc. for $C_{15}H_{24}N_2O_{20}P_3$ 645.0135, found 645.0130 (100%, M^-); $\lambda_{max}(H_2O)$/nm 260.

1 M. J. Berridge, *Nature (London)*, 1993, **361**, 315.
2 B. V. L. Potter and D. Lampe, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1933.
3 S. Takahashi, T. Kinoshita and M. Takahashi, *J. Antibiot.*, 1994, **47**, 95.
4 M. Takahashi, K. Tanzawa and S. Takahashi, *J. Biol. Chem.*, 1994, **269**, 369; J. Hirota, T. Michikawa, A. Miyawaki, M. Takahashi, K. Tanzawa, I. Okura, T. Furuichi and K. Mikoshiba, *FEBS Lett.*, 1995, **368**, 248.
5 J. S. Marchant, M. D. Beecroft, A. M. Riley, D. J. Jenkins, R. D. Marwood, C. W. Taylor and B. V. L. Potter, *Biochemistry*, 1997, **36**, 12 780 and references therein.
6 D. J. Jenkins and B. V. L. Potter, *Carbohydr. Res.*, 1996, **287**, 169.
7 R. A. Wilcox, C. Erneux, W. U. Primrose, R. Gigg and S. R. Nahorski, *Mol. Pharmacol.*, 1995, **47**, 1204.
8 N. Moitessier, F. Chrétien, Y. Chapleur and C. Humeau, *Tetrahedron Lett.*, 1995, **46**, 8023.
9 C. T. Murphy, A. M. Riley, C. J. Lindley, D. J. Jenkins, J. Westwick and B. V. L. Potter, *Mol. Pharmacol.*, 1997, **52**, 741.
10 D. J. Jenkins, R. D. Marwood and B. V. L. Potter, *Chem. Commun.*, 1997, 449; Corrigendum, 805.
11 (*a*) S. Shuto, K. Tatani, Y. Ueno and A. Matsuda, *J. Org. Chem.*, 1998, **63**, 8815; (*b*) R. D. Marwood, A. M. Riley, V. Correa, C. W. Taylor and B. V. L. Potter, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 453.
12 Y. Huang, M. Takahashi, K. Tanzawa and J. W. Putney Jr., *J. Biol. Chem.*, 1998, **273**, 31 815; G. S. Bird, M. Takahashi, K. Tanzawa and J. W. Putney Jr., *J. Biol. Chem.*, 1999, **274**, 20 643; L. M. Broad, D. L. Armstrong and J. W. Putney Jr., *J. Biol. Chem.*, 1999, **274**, 32 881 and references therein.
13 H. Hotoda, M. Takahashi, K. Tanzawa, S. Takahashi and M. Kaneko, *Tetrahedron Lett.*, 1995, **36**, 5037.
14 N. C. R. van Straten, G. A. van der Marel and J. H. van Boom, *Tetrahedron*, 1997, **53**, 6509.
15 R. D. Marwood, V. Correa, C. W. Taylor and B. V. L. Potter, *Tetrahedron: Asymmetry*, 2000, in press.
16 J. Moravcova, J. Capkova and J. Stanek, *Carbohydr. Res.*, 1994, **263**, 61.
17 T. Q. Chen and M. M. Greenberg, *Tetrahedron Lett.*, 1998, **39**, 1103; P. B. Alper, M. Hendrix, P. Sears and C.-H. Wong, *J. Am. Chem. Soc.*, 1998, **120**, 1965.
18 T. Desai, J. Gigg and R. Gigg, *Carbohydr. Res.*, 1996, **280**, 209.
19 W. Liao and D. Lu, *Carbohydr. Res.*, 1996, **296**, 171.
20 N. L. Douglas, S. V. Ley, U. Lücking and S. L. Warriner, *J. Chem. Soc., Perkin Trans. 1*, 1998, 51.
21 H. Vorbrüggen, K. Krolikiewicz and B. Bennua, *Chem. Ber.*, 1981, **114**, 1234.
22 V. Nair and S. G. Richardson, *J. Org. Chem.*, 1980, **45**, 3969.
23 A. Al Mourabit, M. Beckmann, A. Poupat, A. Ahond and P. Potier, *Tetrahedron: Asymmetry*, 1996, **7**, 3455.

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