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TETRAHEDRON: *ASYMMETRY*

Synthesis of adenophostin A

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Abstract

The natural product and potent agonist of the D-*myo*-inositol 1,4,5-trisphosphate receptor, adenophostin A, was synthesised from adenosine and D-glucose using efficient methodology. The synthetic material was equipotent with naturally occurring adenophostin A in evoking Ca^{2+} release from the intracellular stores of permeabilised cells.

The second messenger role of D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] in mediating the release of Ca^{2+} from intracellular stores on binding to its own ligand-gated receptor is well established.[1] In conjunction with pharmacological investigations the synthesis of many Ins(1,4,5)P_3 analogues has provided insights into the structural motifs required for binding and Ca^{2+} release at the Ins(1,4,5)P_3 receptor,[2] although synthetic analogues with potency exceeding that of Ins(1,4,5)P_3 have yet to be synthesised.

The discovery in 1993 of adenophostins A and B (Fig. 1; **2** and **3**),[3] isolated from a culture broth of *Penicillium brevicompactum*, brought with it a new perspective on the pharmacophore required for activity at Ins(1,4,5)P_3 receptors. Both molecules were found to be 10- to 100-fold more potent than Ins(1,4,5)P_3 in releasing intracellular Ca^{2+} from permeabilised cells and in competitive receptor-binding assays.[4,5] This was the first time that derivatives of a core structure other than inositol had exhibited Ins(1,4,5)P_3-like activity. Although the adenophostins seem structurally quite different to Ins(1,4,5)P_3, they do possess certain direct similarities, i. e. the 3′′,4′′-(bisphosphate)/2′′-hydroxyl triad and the 4,5-(bisphosphate)/6-hydroxyl motifs, respectively, together with another phosphate group (these crucial features for activity are highlighted green in Fig. 1, although the suspected importance of the 2′′-hydroxyl group of **2** has yet to be formally established). Syntheses of chiral inositol phosphates and their derivatives have, in the main, been by means of long multistep routes and resolution of intermediates is normally required.[2] Because they offer a template for the design of new classes of the chiral Ins(1,4,5)P_3 receptor ligand based upon carbohydrates rather than cyclitols, the adenophostins are of considerable interest as

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starting points for novel synthetic potential signal transduction modulators. Adenophostin A itself is also now finding widespread use as a pharmacological tool to investigate cell signalling mechanisms.[6]

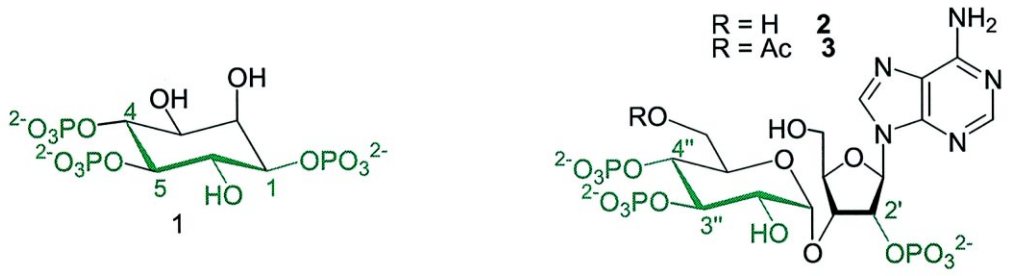
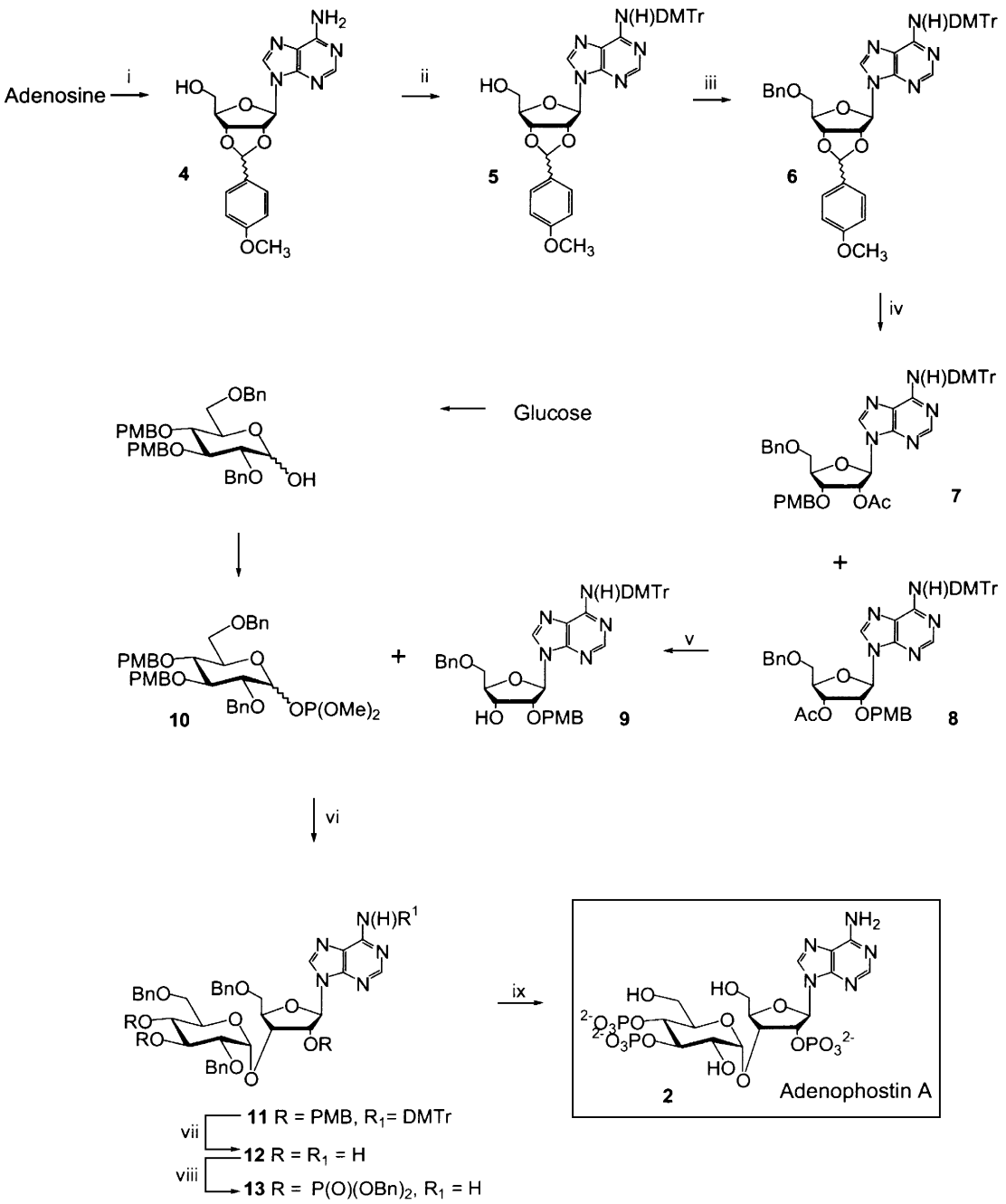


Fig. 1. Structures of Ins(1,4,5)P_3 **1**, adenophostin A **2** and adenophostin B **3**

In an effort to elucidate the structural motifs responsible for the extraordinary activity of the adenophostins we and others have synthesised several analogues of adenophostin A.[5,7–12] There have also been two syntheses of adenophostin A itself.[13,14] As part of a more general synthetic programme we also required an efficient route to the natural molecule and we now report here a total synthesis of adenophostin A.

A synthetic strategy† involving the glycosidation of a regioselectively protected adenosine-based acceptor (**9**) with a regioselectively protected glucosyl phosphite donor (**10**) was adopted (Scheme 1). First, a novel route to a suitably protected adenosine-based acceptor was devised. The 2′,3′-diol of adenosine was protected by treatment of adenosine with freshly distilled *p*-methoxybenzaldehyde in the presence of triethyl orthoformate and dry *p*TSA[15] at 35−40°C to give the 2′,3′-*O*-*p*-methoxybenzylidene acetal (**4**) as a ca. 3:2 diastereoisomeric mixture as judged by ^{1}H NMR spectroscopy. Selective protection of the N^6-position with a dimethoxytrityl (DMTr) group in the presence of the free 5′-hydroxyl group was accomplished by a one-pot reaction. Thus, the free 5′-hydroxyl of **4** was transiently protected as a trimethylsilyl (TMS) ether using chlorotrimethysilane in pyridine;[16,17] subsequent addition of dimethoxytrityl (DMTr) chloride resulted in the introduction of a DMTr group at the N^6-position of the adenine moiety. The transient TMS ether was then cleaved by addition of concentrated aqueous ammonia solution to give **5** in 97% yield after column chromatography. Initial attempts to benzylate the 5′-hydroxyl of **5** with sodium hydride and benzyl bromide in DMF only resulted in a poor yield; therefore, an alternative method was sought. Benzylation of the 5′-hydroxyl was finally achieved in high yield by heating a solution of **5** in a mixture of dioxane and benzene, in the presence of benzyl chloride and potassium hydroxide.[18] Finally, the *p*-methoxybenzylidene acetal of the resulting **6** was reductively cleaved using DIBAL-H in dichloromethane to give the required **9** and its more polar regioisomer, in a ca. 3:2 diastereoisomeric mixture as judged by ^{1}H NMR spectroscopy. The crude products from the treatment of **6** with DIBAL-H were converted to their 3′- and 2′-*O*-acetates, respectively, using standard conditions to enable their separation by careful column chromatography and identification, since in both ^{1}H NMR spectra of **8** and **7** there were deshielded protons corresponding to the respective acylated positions. Once

† All new compounds exhibited satisfactory spectroscopic and analytical data in accord with their structure.



Scheme 1. Synthetic route to adenophostin A. Reagents and conditions: (i) *p*-methoxybenzaldehyde, triethyl orthoformate, *p*TSA, 35–40°C; 90%; (ii) (a) TMSCl, pyridine; (b) DMTrCl; (c) NH_4OH; 97% yield; (iii) BnCl, KOH, benzene, dioxane, reflux, 95%; (iv) (a) DIBAL-H, CH_2Cl_2, −78°C; (b) Ac_2O, pyridine; **8** (55% yield), **7** (38% yield); (v) NH_4OH, MeOH, 100%; (vi) $AgClO_4$, $ZnCl_2$, dioxane, toluene, 4 Å sieves, 53%; (vii) CF_3COOH, CH_2Cl_2, 81%; (viii) (a) $(BnO)_2PNPr^i_2$, imidazolium triflate; (b) MCPBA, −78°C; 70% yield over two steps; (ix) wet $Pd(OH)_2/C$, cyclohexene:MeOH:H_2O, 14:7:1, reflux, 92%. DMTr=dimethoxytrityl, PMB=*p*-methoxybenzyl, Bn=benzyl

identified, the 3′-*O*-acetyl derivative was then treated with methanolic ammonia to furnish the desired glycosyl acceptor in quantitative yield.

The desired glucopyranosyl phosphite donor was synthesised essentially using a method that we reported recently.[12b] This glycosyl donor **10** and acceptor **9** were coupled together in a mixture of dioxane and toluene[19] with dry zinc chloride and silver perchlorate as promoters in the presence of 4 Å molecular sieves.[20] The α-coupled **11** was the sole isolated product, with the formation of the required 1,2-*cis* linkage being confirmed by the H-1′′ resonance in the ^{1}H NMR spectrum of **11**, which appeared as a doublet with a small axial–equatorial coupling constant (δ_H 5.22, J 3.4 Hz). Deprotection of all the acid-sensitive groups with TFA in dichloromethane led to the required triol **12**. Phosphitylation of **12** was accomplished by a recently reported method which does not necessitate prior protection of the adenine N^6-position; if a stoichiometric amount of imidazolium triflate is employed as phosphoramidite activator, selective hydroxyl phosphitylation can be achieved.[21] Thus, **12** was phosphitylated with a mixture of bis(benzyloxy)(diisopropylamino)phosphine and imidazolium triflate in dichloromethane. As may be expected, monitoring the reaction by TLC indicated that phosphitylation with imidazolium triflate instead of tetrazole was more sluggish. On complete conversion of starting material into the trisphosphite the reaction mixture was cooled to −78°C before oxidation to the trisphosphate with MCPBA.

Deprotection of the fully protected adenophostin precursor **13** proved difficult, with the benzyl ethers being particularly stable. Initial attempts with sodium and liquid ammonia were unsuccessful. Catalytic hydrogenation was also less than satisfactory. Compound **13** was treated separately with 10% palladium on carbon, palladium black and 20% palladium hydroxide and, while use of the former two catalysts was unsuccessful, the latter was very slow, with complete deprotection taking 5 days, and this was accompanied by a low yield. Complete deprotection in high yield was finally achieved via catalytic transfer hydrogenation.[22] A solution of **13** was refluxed in a mixture of methanol, water and cyclohexene with 20% palladium hydroxide for 2.5 h. Following removal of the catalyst, the crude product was purified on an MP1 AG ion exchange resin column, being eluted with a gradient of 0–100% 150 mM TFA. Adenophostin A was isolated as the free acid {$[\alpha]_D^{18}$=+41.6, *c* 0.5 in H_2O} and subsequently converted to the hexakis–sodium salt {$[\alpha]_D^{25}$=+28.8, *c* 0.7 in H_2O[13]}. Quantification was accomplished by UV assay. Both the ^{1}H and ^{31}P NMR spectra and the negative ion FAB mass spectrum of **2** were in agreement with those reported previously for both natural[3] and synthetic adenophostin A.[13,14] Synthetic adenophostin A had the same retention time as a sample of authentic natural material as investigated by reverse phase HPLC on an ODS column (using as eluent a gradient of 10–30% acetonitrile and 0.05 M phosphate buffer, containing 0.1% w/v of tetrabutylammonium hydrogen sulphate, acting as an ion-pair reagent).

This approach to the assembly of the glyconucleoside component of adenosphostin A is more similar to that used by Hotoda et al.[13] than that of van Straten et al.,[14] which involved a very different convergent approach whereby the adenine base was introduced onto a disaccharide intermediate using Vorbrüggen condensation methodology. The present strategy, however, offers several advantages over that of Hotoda et al.[13] The glycosyl donor is prepared in final form and no further unnecessary protecting group manipulation is required after coupling. The phosphite donor is easy to synthesise in high yield, giving complete α-selectivity on coupling. For the glycosyl acceptor 'permanent' 5′-protection is clearly highly desirable using e.g. a benzyl group; this is, however, not compatible with the N^6-benzyl protection as used previously[13] and also widely in nucleoside chemistry, since concomitant N^6-alkylation is an undesirable consequence. N^6-Dimethoxytrityl protection has not been used widely before, but offers here the clear advantage that the protecting group can be removed in one step under acidic conditions and in high yield simultaneously with the three *p*-methoxybenzyl groups, without the disadvantage of other protecting groups being affected.[13] This provides a highly attractive strategy in concert with the latest methodology

for phosphitylation without amino group protection[21] and our present route stands as one of the first examples of the application of this useful advance. Finally, our strategy permits the fully protected trisphosphate to be deprotected in one step to give adenophostin A.

The effects of synthetic adenophostin A, natural adenophostin A and Ins(1,4,5)P_3 on unidirectional $^{45}Ca^{2+}$ efflux from the intracellular stores of permeabilised rat hepatocytes[5] were determined (Fig. 2) using the following method: permeabilised hepatocytes were loaded to steady state (5 min at 37°C) with $^{45}Ca^{2+}$ in a cytosol-like medium (140 mM KCl, 20 mM NaCl, 2 mM $MgCl_2$, 1 mM EGTA, 300 μM $CaCl_2$, 20 mM PIPES, pH 7.0) containing ATP (1.5 mM), creatine phosphate (5 mM) creatine phosphokinase (5 units/ml) and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (10 μM). After 5 min, thapsigargin (1.25 μM) was added to the cells to inhibit further Ca^{2+} uptake; 30 s later the cells were added to appropriate concentrations of the agonists and after a further 60 s the $^{45}Ca^{2+}$ content of the stores was determined by rapid filtration. Concentration–response relationships were fitted to a four parameter logistic equation using Kaleidegraph software from which the maximal response, half-maximally effective agonist concentration (EC_{50}) and Hill slope (*h*) were determined, as shown in Table 1. Clearly, synthetic adenophostin A from this route is equipotent with the natural material and is approximately an order of magnitude more potent than Ins(1,4,5)P_3.

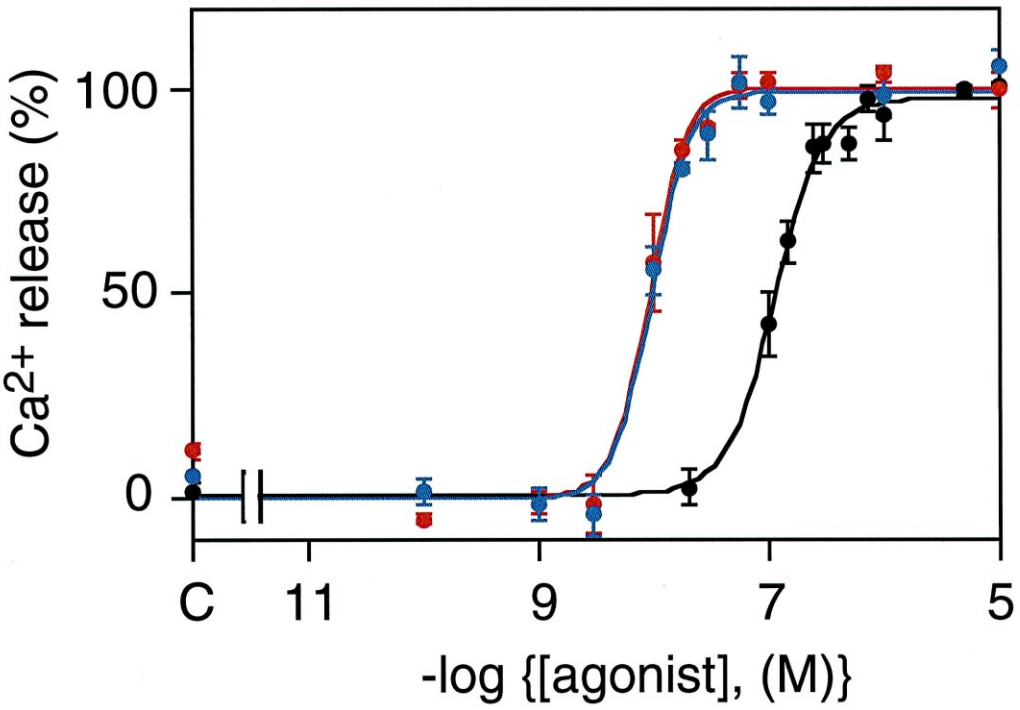


Fig. 2. Ca^{2+} mobilisation evoked by natural and synthetic adenophostin A, and Ins(1,4,5)P_3. Results (mean±SEM, *n*=3 or 4) show the concentration-dependent effects of Ins(1,4,5)P_3 (black), natural (blue) or synthetic (red) adenophostin A on Ca^{2+} release from the Ins(1,4,5)P_3-sensitive intracellular Ca^{2+} stores of permeabilised rat hepatocytes

We have modelled[23] adenophostin A and compared its conformation to that of Ins(1,4,5)P_3 in complex with an Ins(1,4,5)P_3 binding protein, obtained from a crystal structure analysis.[24] The results are shown in Fig. 3 and illustrate how the three phosphate groups of **1** can mimic those of **2**. The positioning of the

Table 1
Data for agonist-induced Ca^{2+} release from permeabilised rat hepatocytes: results are expressed as mean±SEM; percentage release is expressed as a % of the total Ca^{2+} pool; *n*=number of experiments

Agonist	*EC_{50} (nM)*	*h*	*% Release*	*n*
Ins(1,4,5)P_3	115±14	2.05±0.38	59±6	4
Natural adenophostin A	9.2±1.7	2.99±0.32	49±3	3
Synthetic Adenophostin A	9.6±1.0	2.46±0.19	50±1	3

2′-phosphate group is clearly of crucial importance. It seems likely that hydrophobic interactions of the adenine base with the receptor are important in underlying the unusual activity of adenophostin A.

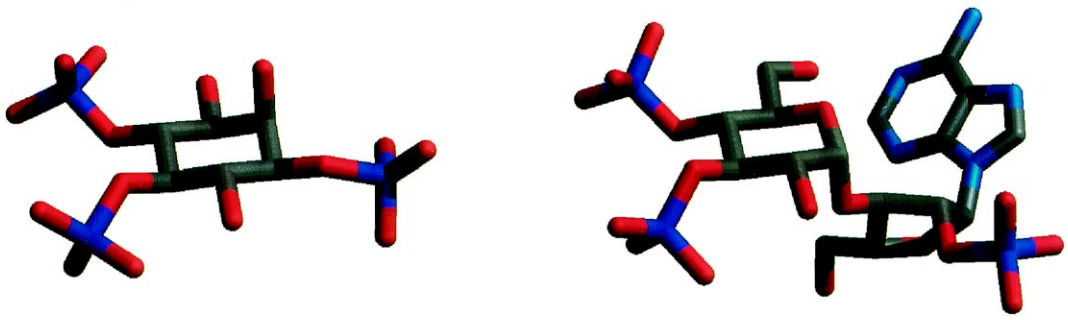


Fig. 3. Comparison of conformations of **1** and **2**. Left: structure of Ins(1,4,5)P_3 taken from the X-ray crystal structure of the phospholipase C-δ_1 pleckstrin homology domain complex with Ins(1,4,5)P_3.[24] Right: representative energy-minimised conformer of adenophostin A obtained from molecular dynamics simulations at 300 K[23]

In summary, we have designed an efficient synthesis of adenophostin A and have demonstrated the authenticity of the product. This route should facilitate the preparation of structurally modified analogues to explore the utility of such compounds for pharmacological intervention in the polyphosphoinositide pathway of cellular signalling.

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References

1. Berridge, M. J. *Nature (London)* **1993**, *361*, 315–325.
2. Potter, B. V. L.; Lampe, D. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1933–1972.
3. (a) Takahashi, M.; Kagasaki, T.; Hosoya, T.; Takahashi, S. *J. Antibiot*. **1993**, *46*, 1643–1647. (b) Takahashi, S.; Kinoshita, T.; Takahashi, M. *J. Antibiot.* **1994**, *47*, 95–100.
4. (a) Takahashi, M.; Tanzawa, K.; Takahashi, S. *J. Biol. Chem.* **1994**, *269*, 369–372; (b) Hirota, J.; Michikawa, T.; Miyawaki, A.; Takahashi, M.; Tanzawa, K.; Okura, I.; Furuichi, T.; Mikoshiba, K. *FEBS Lett.* **1995**, *368*, 248–252.
5. Marchant, J. S.; Beecroft, M. D.; Riley, A. M.; Jenkins, D. J.; Marwood, R. D.; Taylor, C. W.; Potter, B. V. L. *Biochemistry* **1997**, *36*, 12780–12790.
6. (a) Marchant, J. S.; Parker, I. *Biochem. J.* **1998**, *334*, 505–509; (b) Hartzell, H. C.; Machaca, K.; Hirayama, Y. *Mol. Pharmacol.* **1997**, *51*, 683–692; (c) Huang, Y.; Takahashi, M.; Tanzawa, K.; Putney Jr., J. W. *J. Biol. Chem.* **1998**, *273*, 31815–31821; (d) Machaca, K.; Hartzell, H. C. *J. Biol. Chem.* **1999**, *274*, 4824–4831; (e) DeLisle, S.; Marksberry, E. W.; Bonnett, C.; Jenkins, D. J.; Potter, B. V. L.; Takahashi, M.; Tanzawa, K. *J. Biol. Chem.* **1997**, *272*, 9956–9961; (f) Gregory, R. B.; Wilcox, R. A.; Berven, L. A.; van Straten, N. C. R.; van der Marel, G. A.; van Boom, J. H.; Barritt, G. J. *Biochem. J.* **1999**, *341*, 401–408; (g) Bird, G. S.; Takahashi, M.; Tanzawa, K.; Putney Jr., J. W. *J. Biol. Chem.* **1999**, *274*, 20643–20649; (h) Sato, Y.; Miyazaki, S.; Shikano, T.; Mitsuhashi, N.; Takeuchi, H.; Mikoshiba, K.; Kuwabara, Y. *Biology of Reproduction* **1999**, *58*, 867–873; (i) Broad, L. M.; Armstrong, D. L.; Putney, J. W., Jr. *J. Biol. Chem.* **1999**, *274*, 32881–32888.
7. Jenkins, D. J.; Potter, B. V. L. *Carbohydr. Res.* **1996**, *287*, 169–182.
8. Wilcox, R. A.; Erneux, C.; Primrose, W. U.; Gigg, R.; Nahorski, S. R. *Mol. Pharmacol.* **1995**, *47*, 1204–1211.
9. Moitessier, N.; Christian, A. L.; Chapleur, Y.; Humeau, C. *Tetrahedron Lett.* **1995**, *46*, 8023–8026.
10. Murphy, C. T.; Riley, A. M.; Lindley, C. J.; Jenkins, D. J.; Westwick, J.; Potter, B. V. L. *Mol. Pharmacol.* **1997**, *52*, 741–748.
11. Jenkins, D. J.; Marwood, R. D.; Potter, B. V. L. *Chem. Commun.* **1997**, 449–450.
12. (a) Shuto, S.; Tatani, K.; Ueno, Y.; Matsuda, A. *J. Org. Chem*. **1998,** *63*, 8815–8824; (b) Marwood, R. D.; Riley, A. M.; Correa, V.; Taylor, C. W.; Potter, B. V. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 453–458.
13. Hotoda, H.; Takahashi, M.; Tanzawa, K.; Takahashi, S.; Kaneko, M. *Tetrahedron Lett.* **1995**, *36*, 5037–5040.
14. van Straten, N. C. R.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1997**, *53*, 6509–6522.
15. Morley, C. G. D.; Hogencamp, H. P. C. *Arch. Biochem. Biophys.* **1968**, *123*, 207–208.
16. McLaughlin, L. W.; Piel, N.; Hellmann, T. *Synth. Commun.* **1985**, 322–323.
17. Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316–1319.
18. Pankiewicz, K. W.; Krzeminski, J.; Ciszewski, L. A.; Ren, W.-Y.; Watanabe, K. A. *J. Org. Chem.* **1992**, *57*, 553–559.
19. Demchenko, A.; Stauch, T.; Boons, G.-J. *Synlett* **1997**, 818–820.
20. Watanabe, Y.; Nakamoto, C.; Yamamoto, T.; Shoichiro, O. *Tetrahedron* **1994**, *50*, 6523–6536.
21. Hayakawa, Y.; Kataoka, M. *J. Am. Chem. Soc.* **1998**, *120*, 12395–12401.
22. Hanessian, S.; Liak, T. J.; Vanasse, B. *Synthesis* **1981**, 396–397.
23. Modelling was carried out using the Sybyl software package (v6.3, Tripos Associates) on a Silicon Graphics workstation. The TRIPOS force field was used, with added parameters for carbohydrates (Pérez, S.; Meyer, C.; Imberty, A. In *Modelling of Biomolecular Structures and Mechanisms*; Pullman, A; Jortner, J.; Pullman, B., Eds.; Kluwer: Dordrecht, 1995; pp. 425–454).
24. Ferguson, K. M.; Lemmon, M. A.; Schlessinger, J.; Sigler, P. B. *Cell* **1995**, *83*, 1037–1046.