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An Expeditious Route to the Synthesis of Adenophostin A

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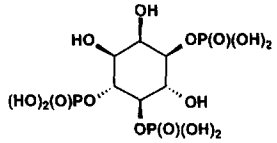
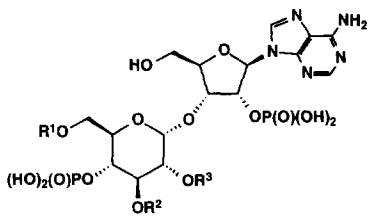
Abstract: Glycosylation of 1,2-*O*-isopropylidene-5-*O*-*tert*-butyldiphenylsilyl-α-D-ribofuranose (**8**) with ethyl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-1-thio-α/β-D-glucopyranoside (**7**) under the agency of *N*-iodosuccinimide and trifluoromethanesulfonic acid (*cat.*) afforded α-linked dimer **10** in 95% yield. Vorbrüggen-type condensation of **12**, obtained by deacetonation of **10** and subsequent acetylation, with bis-trimethylsilyl N^6-benzoyl adenine gave adenosyl glucoside **13**. Protective group manipulations followed by phosphorylation furnished, after deprotection, homogeneous **2** in high overall yield. Copyright © 1996 Elsevier Science Ltd

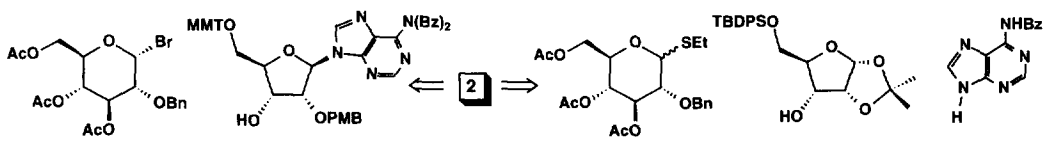
Stimulation of an extracellular G-protein coupled receptor induces in many cell types intracellular Ca^{2+} mobilization *via* the second messenger D-*myo*-inositol 1,4,5-triphosphate[1] (IP_3, **1**). Growing evidence indicates that IP_3 may be an essential element in various cellular functions, *i.e.* smooth muscle contractility, secretion, neuronal excitability, the activation of inflammatory cells and cell proliferation. Recently, adenophostins A (**2**) and B (**3**), isolated from the fermentation broth of *Penicillium brevicompactum* SANK 11991 and SANK 12177, were discovered as potent IP_3 receptor agonists[2], with a 100 times higher IP_3 receptor-binding affinity and Ca^{2+}-mobilizing activity in comparison[3] with the natural ligand IP_3.



1 IP_3



2 R^1 = H, R^2 = P(O)(OH)$_2$, R^3 = H (Adenophostin A)
3 R^1 = Ac, R^2 = P(O)(OH)$_2$, R^3 = H (Adenophostin B)
4 R^1 = H, R^2 = H, R^3 = P(O)(OH)$_2$



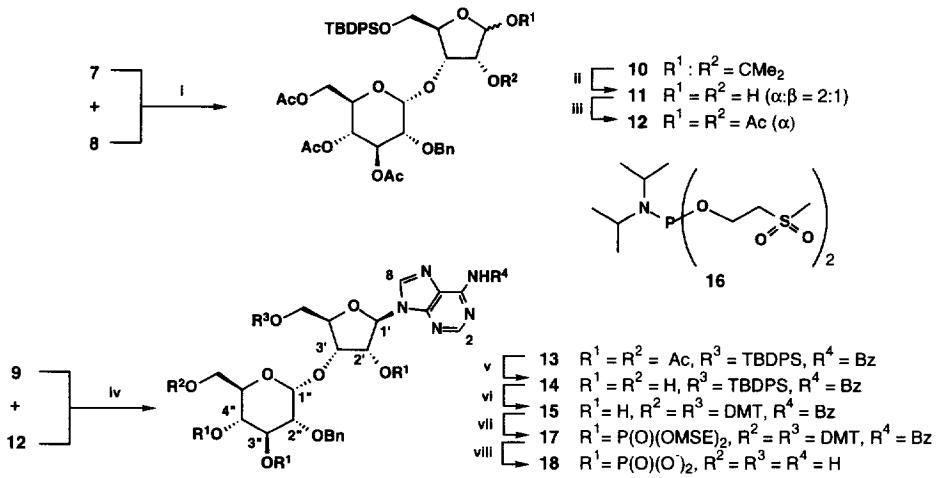
5 **6** **7** **8** **9**

Recently, Hotoda *et al.*[4] reported for the first time an eight-step approach to the synthesis of adenophostin A (**2**) using 2-*O*-benzyl-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide (**5**) and di-N^6-benzoyl-2'-*O*-*p*-methoxybenzyl-5'-*O*-monomethoxytrityl adenosine (**6**) as the building units. Glycosylation of **6** with **5** under the influence of $AgClO_4$ followed by protective group manipulations of the resulting α-linked dimer and subsequent phosphorylation gave, after deprotection, compound **2** in 22% overall yield.

With the objective to get an insight into the structure activity relationship of this new type of second messengers, we here report a versatile approach to the preparation of adenophostin A based on the properly protected units ethyl 1-thio-α/β-D-glucopyranoside **7**, the ribofuranoside **8** and the adenine derivative **9**.

The route of synthesis is depicted in Scheme 1 and commences with the introduction of the requisite 1,2-*cis* linkage between the glucopyranosyl donor **7** and the ribofuranoside acceptor **8**. It was anticipated[5] that glycosylation of **8** with **7**, readily available by treatment of 1,3,4,6-tetra-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranose with (ethylthio)trimethylsilane in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf)[6], would proceed with a high degree of α-stereoselectivity. Indeed, iodonium ion (NIS)-catalytic triflic acid (TfOH) mediated condensation of **7** with **8**, prepared from known[7] 1,2-*O*-isopropylidene-α-D-xylofuranose in three steps[8], led to the exclusive formation of the α-linked dimer **10**[9]. Unexpectedly, the purposive deacetonation of **10** in a mixture of acetic acid-water under reflux conditions was accompanied by cleavage of the interglycosidic linkage and partial removal of the *tert*-butyldiphenylsilyl (TBDPS) group. However, a high yielding and smooth transformation of **10** into **11**[9] was attained by unleashing the 1,2-*O*-isopropylidene function with HOAc-H_2O containing ethylene glycol. Acetylation of the 1,2-diol function in **11** gave key intermediate **12**[9] in 75% yield over the three steps.

Scheme 1



Reagents and conditions: (i) NIS/*cat.* TfOH, $(CH_2Cl)_2/Et_2O$ (1:1, v/v), 5 min, 95% (α:β = 1:0); (ii) HOAc/H_2O/$(HOCH_2)_2$ (6:14:3, v/v/v), reflux, 30 min, 84%; (iii) Ac_2O, pyr, 4 h, 94%; (iv) $(Me_3Si)_2A^{Bz}$, TMSOTf, $(CH_2Cl)_2$, reflux, 16 h, 71%; (v) KO*t*-Bu/MeOH (1M)/dioxane (2:1, v/v), 1 min, 96%; (vi) a. TBAF/THF (1M)/dioxane (1:3, v/v), 40 °C, 2 h; b. DMTCl, pyr, 16 h, 65% (two steps); (vii) a. **16**, 1*H*-tetrazole, CH_2Cl_2, 15 min; b. *t*-BuOOH, 0 °C, 5 min; (viii) a. NaOH (4N)/dioxane/MeOH, 1/14/5, v/v/v, 16 h; b. HOAc/H_2O, 8/2, v/v, 1 h, 86% (based on **15**).

Vorbrüggen condensation[10] of **12** with the bis-trimethylsilyl derivative of **9** led to the isolation of the fully protected adenosyl glucoside **13**. In order to introduce the requisite phosphate groups, compound **13** was now subjected to the following protective group manipulations. Deacetylation of **13** to yield **14** was effected by short treatment with KO*t*-Bu in methanol. Removal of the TBDPS group in **14** with fluoride ions, and subsequent regioselective masking of the two primary hydroxyl functions with 4,4'-dimethoxytrityl (DMT) groups, led to the isolation of the triol-derivative **15** in 62% yield (based on **13**). Phosphorylation of **15** was readily accomplished using the recently by us developed[11] reagent *N,N*-diisopropyl-bis [2-(methylsulfonyl)ethyl] (MSE) phosphoramidite (**16**). Thus, 1*H*-tetrazole assisted phosphitylation of **15** with **16**, followed by *in situ* oxidation (*t*-BuOOH) of the intermediate phosphite triesters, gave the fully protected derivative **17**[12]. A one-pot sequential removal of the base labile benzoyl and 2-(methylsulfonyl)ethyl and the acid labile 4,4'-dimethoxytrityl groups from **17** gave, after purification by HW-40 gel filtration, the mono-benzyl protected derivative **18** in 86% yield (based on **15**). Finally, hydrogenation of **18** with Pd-black-H_2O (1 atm. H_2) gave, after gel filtration (HW-40), homogeneous **2**[13] (65% yield, Na^+-salt), the spectroscopic data - ^1H as well as ^{13}C-NMR spectroscopy - of which were in full accord with those reported for naturally occurring[2] and synthetic[4] adenophostin A. In addition, the two-dimensional ^1H-^{31}P correlated NMR-spectrum of **2** (see Fig. 1) firmly established the presence of two (3"-4")-*trans* oriented phosphate groups in the glucopyranosyl moiety. The spatial arrangement of the latter two phosphates was also independently confirmed by comparison (see Fig. 1) of the ^1H-^{31}P correlated spectrum of **2** with that of synthetically prepared[14] adenophostin A analogue **4** containing (2"-4")-*cis* oriented phosphate groups.

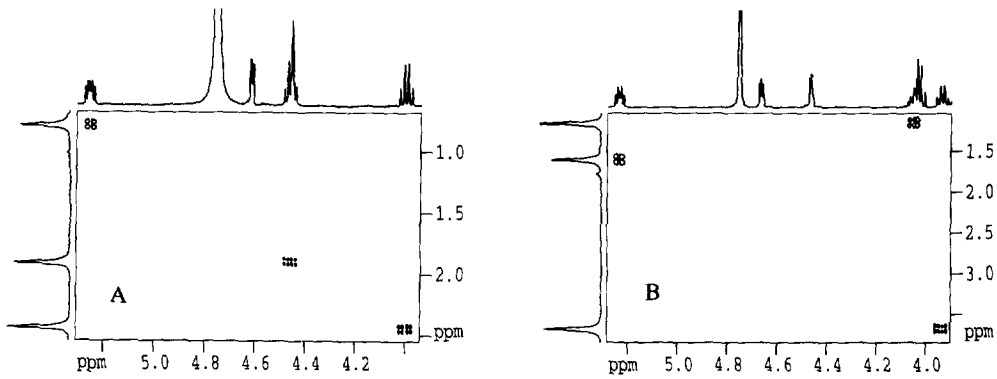


Figure 1. ^1H-^{31}P Correlated NMR spectra of compounds **2** (A) and **4** (B).

The successful assembly of adenophostin A (28% yield over the nine steps) is mainly due to the high yielding and stereoselective synthesis of dimer **10** and its smooth conversion into the functionalized dimer **12**. The synthesis of other biologically interesting analogues of adenophostin A starting from the now readily available dimers **10-12** will be reported in due course.

References and Notes

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8. 1,2-*O*-Isopropylidene-α-D-xylofuranose was regioselectively silylated with *tert*-butyldiphenylsilyl chloride. Oxidation of the resulting product with DMSO/Ac_2O and reduction of the ulose derivative with $NaBH_4$ afforded crystalline (ethanol) **8** in 60% yield.
9 Relevant spectroscopic data ($CDCl_3$): (**10**): ^{1}H-NMR: δ 5.87 (d, 1H, H-1, $J_{1,2}$ = 3.7 Hz), 5.32 (d, 1H, H-1', $J_{1',2'}$ = 3.7 Hz); ^{13}C-NMR: δ 104.2 (C-1), 94.3 (C-1', $J_{C\text{-}1,\ H\text{-}1}$ = 171.5 Hz); ESI-MS $[M+H]^+$ 807; (**11**): ^{13}C-NMR: δ 101.6 (C-1 β), 97.7 (C-1'), 96.6 (C-1 α); ESI-MS $[M+Na]^+$ 789; (**12**): ^{13}C-NMR: δ 98.5 (C-1 α), 97.1 (C'-1); ESI-MS $[M+Na]^+$ 873.
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12. ^{31}P-NMR (CH_2Cl_2): δ -2.01, -2.33, -2.63.
13. Relevant spectroscopic data of target compound **2**: ^{1}H-NMR (D_2O): δ 8.28 (s, 1H, H-8), 8.10 (s, 1H, H-2), 6.24 (d, 1H, H-1', $J_{1',2'}$ = 6.7 Hz), 5.33 (d, 1H, H-1", $J_{1",2"}$ = 3.8 Hz), 5.26 (ddd, 1H, H-2', $J_{2',3'}$ = 2.7 Hz, $^3J_{HP}$ = 9.8 Hz), 4.46 (m, 2H, H-4', H-3"), 4.00 (q, 1H, H-4", $J_{3",4"} = J_{4",5"}$ = 9.9 Hz, $^3J_{HP}$ = 9.9 Hz); ^{13}C-NMR (D_2O): δ 155.7, 149.0 (C-4, C-6), 152.2, 142.0 (C-2, C-8), 119.7 (C-5), 98.7 (C-1"), 88.2 (C-1'), 77.7 (C-3", $^2J_{CP}$ = 8.2 Hz), 75.7 (C-2', $^2J_{CP}$ = 4.3 Hz), 72.6 (C-4", $^2J_{CP}$ = 3.3 Hz); ^{31}P-NMR (D_2O): δ 2.44 (C-4" P), 1.91 (C-3" P), 0.79 (C-2' P); ESI-MS $[M-H]^-$ 668.
14. The synthesis of **4** was accomplished by subjecting **10** to the following sequence of reactions: a. H_2, Pd(C); b. pivaloyl chloride (76% over the two steps); c. HOAc-H_2O-$(HOCH_2)_2$; d. Ac_2O; e. $(Me_3Si)_2A^{Bz}$, TMSOTf; f. KO*t*-Bu, methanol; g. TBAF; h. DMT-Cl; i. **16**, 1*H*-tetrazole; j. *t*-BuOOH; k. NaOH; l. HOAc-H_2O. The conditions and yields of the individual steps c-l did not deviate substantially from the steps ii-viii mentioned in Scheme 1. Interestingly, step f led to a near quantitative migration of the pivaloyl group to the 3"-position in the glucosyl moiety. Relevant spectroscopic data for **4**: ^{1}H-NMR (D_2O): δ 8.31 (s, 1H, H-8), 8.21 (s, 1H, H-2), 6.28 (d, 1H, H-1', $J_{1',2'}$ = 6.0 Hz), 5.39 (d, 1H, H-1", $J_{1",2"}$ = 3.3 Hz), 5.23 (ddd, 1H, H-2', $J_{2',3'}$ = 3.7 Hz, $^3J_{HP}$ = 9.0 Hz), 4.22 (m, 1H, H-2"), 4.02 (t, 1H, H-3", $J_{2",3"} = J_{3",4"}$ = 9.8 Hz), 3.94 (q, 1H, H-4", $J_{4",5"}$ = 9.7 Hz, $^3J_{HP}$ = 9.2 Hz); ^{13}C-NMR (D_2O): δ 149.6, 142.2 (C-4, C-6), 120.2 (C-5), 98.4 (C-1"), 88.8 (C-1'), 75.2 (C-3"), 75.1 (C-2', $^2J_{CP}$ = 4.8 Hz), 73.4 (C-4", $^2J_{CP}$ = 4.7 Hz), 72.6 (C-2", $^2J_{CP}$ = 5.6 Hz); ^{31}P-NMR (D_2O): δ 3.64 (C-4" P), 1.61 (C-2' P), 1.20 (C-2" P).

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