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An Expeditious Route to the Synthesis of Adenophostin A

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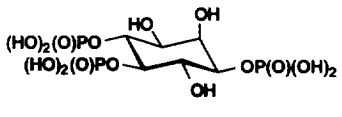
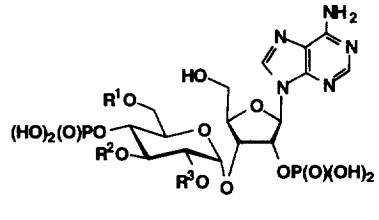
Abstract: Glycosylation of 1,2-*O*-isopropylidene-5-*O*-*tert*-butyldiphenylsilyl-α-D-ribofuranose (**8**) with ethyl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-1-thio-α/β-D-glucopyranoside (**7**) under the agency of *N*-iodosuccinimide and trifluoromethanesulfonic acid afforded α-linked dimer **10** in 95% yield. Acetylation of **13**, obtained after hydrogenation of **10** followed by pivaloylation of **11** (→ **12**) and deacetonation, yielded penta-acetate **14**. Vorbrüggen-type condensation of **14** with bis-trimethylsilyl 6-*N*-benzoyladenine (**9**) gave adenosyl glucoside **17**. Deacetylation of **17** resulted in migration of the pivaloyl group from the 2"-OH to the 3"-OH of the glucosyl moiety (→ **18**), giving access, after phosphorylation and deprotection, to adenophostin A analog **4** containing two (2"-4")-*cis* oriented phosphate groups. Vorbrüggen-type condensation of **9** with **16**, obtained by deacetonation of **10** and subsequent acetylation, gave adenosyl glucoside **22**. Protective group manipulations followed by phosphorylation furnished, after deprotection, homogeneous adenophostin A (**2**) in a high overall yield. © 1997 Elsevier Science Ltd.

Introduction

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-trisphosphate[1] (IP_3, **1**, Fig. 1). Growing evidence indicates that IP_3 is essential in various cellular functions such as smooth muscle contractility, neuronal excitability, activation of inflammatory cells, and cell proliferation. In 1993, adenophostin 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 10-100 times higher[3] receptor-binding affinity and Ca^{2+}-mobilizing activity in comparison 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)$_2$

Figure 1

Recently, Hotoda *et al.*[4] reported for the first time an eight-step approach to the synthesis of adenophostin A using 2-*O*-benzyl-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide (**5**, Fig. 2) and 6-di-*N*-benzoyl-2'-*O*-*p*-methoxybenzyl-5'-*O*-[4-monomethoxy-trityl]-adenosine (**6**) as building units. In this particular case, 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.

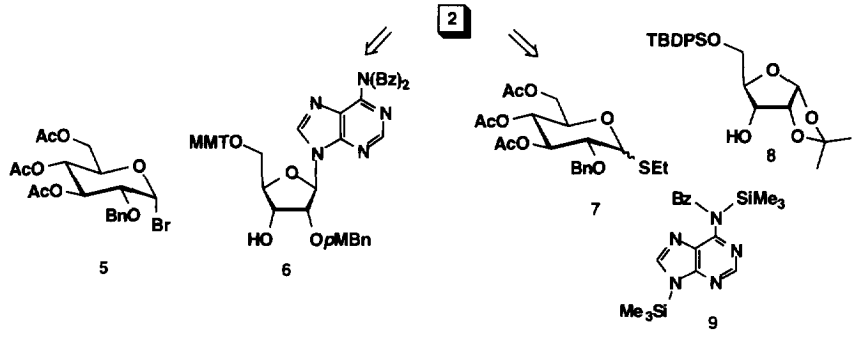
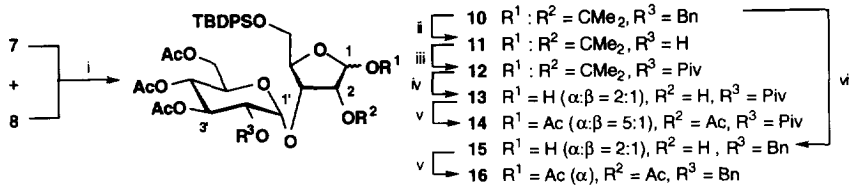


Figure 2

With the objective to get a better insight into the structure-activity relationship of this new type of IP_3 agonists, we here report[5] a versatile approach to the preparation of adenophostin A (**2**) starting from the properly protected ethyl 1-thio-α/β-D-glucopyranose, D-ribofuranose and adenine derivatives **7**, **8** and **9**, respectively. Moreover, adenophostin A analog **4** having two (2"-4")-*cis* oriented phosphate groups in the glucosyl moiety could be attained following a slightly different protective group strategy.

Results and discussion

The route of synthesis to adenophostin A (**2**) commences with the introduction of the requisite 1,2-*cis* glycosidic linkage between the glucopyranosyl donor **7** and the ribofuranose acceptor **8** (Scheme 1). It was anticipated[6] that glycosylation of **8** with **7**, readily available by treatment of 1,3,4,6-tetra-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranose[7] with (ethylthio)trimethylsilane in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf)[8], would proceed with a high degree of α-stereoselectivity. Indeed, iodonium ion (NIS)/catalytic triflic acid (TfOH)-mediated condensation of **7** with **8**, prepared in three consecutive steps by regioselective silylation of 1,2-*O*-isopropylidene-α-D-xylofuranose[9] with *tert*-butyldiphenylsilyl chloride followed by oxidation of the resulting product with $DMSO/Ac_2O$ and reduction of the ulose derivative with $NaBH_4$, led to the exclusive formation of the α-linked dimer **10**.



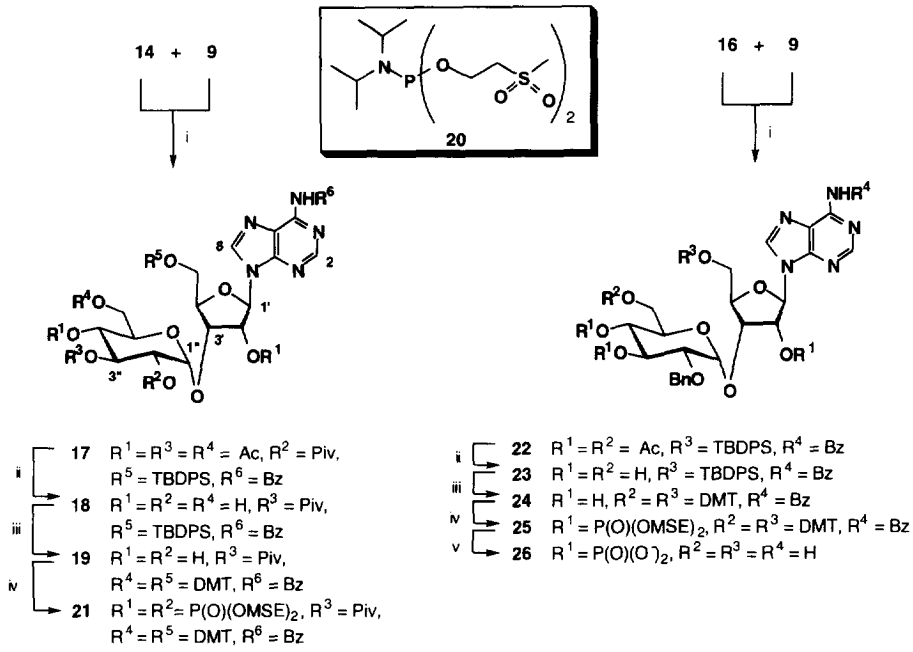
Reagents and conditions: (i) NIS/(*cat.*) TfOH, $(CH_2Cl)_2/Et_2O$, 1/1, v/v, 5 min, 95%; (ii) Pd,C (10%), H_2 (35 psi), *i*-PrOH/HOAc, 16 h, quant.; (iii) PivCl, 1,4-dioxane/pyr, 20/1, v/v, 16 h, 76%; (iv) $HOAc/H_2O$, 7/3, v/v, reflux, 30 min, 60%; (v) Ac_2O, pyr, 4 h, **14**: 91%, **16**: 94%; (vi) $HOAc/H_2O/(HOCH_2)_2$, 14/6/3, v/v/v, 30 min, 84%.

Scheme 1

However, subsequent deacetonation of **10** with acetic acid-water at elevated temperature was accompanied by an unacceptable degree of interglycosidic bond cleavage as well as partial removal of the *tert*-butyldiphenylsilyl (TBDPS) group. With the objective to eliminate these unwanted side reactions, we first examined whether the replacement of the 2-*O*-benzyl in **10** by the rather base-stabile 2-*O*-pivaloyl would be a viable alternative. To this end, the benzyl group in **10** was removed by hydrogenolysis to give, after pivaloylation of **11**, the corresponding 2-*O*-pivaloyl derivative **12**. Subjection of the latter compound to the same deacetonation conditions led to the isolation of the 1,2-diol derivative **13** in an acceptable yield of 60%. The successful synthesis of **13** urged us to establish whether the pivaloyl group would be compatible with the removal of the acetyl groups in the glucosyl adenosine **17**. The latter dimer was readily accessible by Vorbrüggen condensation[10] of fully acetylated dimer **14**, obtained by acetylation of **13**, with bis-silylated 6-*N*-benzoyladenine (**9**). Thus, TMSOTf-mediated coupling of **14** with **9** (see Scheme 2) gave homogeneous **17** in 80% yield. Unfortunately, short treatment of **17** with potassium *tert*-butoxide in methanol led, as evidenced by ^{1}H-NMR spectroscopy, to a near quantitative migration of the Piv-group to the neighbouring 3"-position (→ **18**). Removal of the TBDPS-group in **18** with fluoride ion, and subsequent regioselective protection of the two primary hydroxyl functions with 4,4'-dimethoxytrityl (DMT) groups, led to the isolation of the homogeneous 2',2",4"-triol-derivative **19**, which proved to be (see later) a suitable precursor for the synthesis of adenophostin A analog **4**.

The unexpected migration of the Piv-group in **17** was an incentive to study in more detail the acidolysis of the isopropylidene function in **10**. Attempts to remove the 1,2-acetonide function by reaction with $CuCl_2.2H_2O$[11] or $PdCl_2(CH_3CN)_2$[12] yielded only traces of diol **15**. In addition, treatment of **10** with trifluoroacetic acid[13] to give **15** or acetolysis with acetic anhydride-trifluoroacetic acid (→ **16**) were both abortive. On the other hand, a high-yielding and smooth transformation of **10** into **15** occurred by removing the 1,2-*O*-isopropylidene group with acetic acid-water containing ethylene glycol. Vorbrüggen-type condensation[10] of **16**, obtained after acetylation of **15**, with **9** (see Scheme 2) led to the isolation of fully protected 2'-*O*-acetyl-3'-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-6-*N*-benzoyl-5'-*O*-*tert*-butyldiphenylsilyladenosine (**22**). Dimer **22** was now subjected to the same sequence of protective group manipulations as described for the conversion of **17** into **19**. Thus, saponification of the acetyl protective groups in **22** by short treatment with potassium *tert*-butoxide in methanol gave, after removal of the TBDPS-group in **23** with tetrabutylammonium

fluoride and subsequent regioselective protection of the two primary hydroxyl functions with 4,4'-dimethoxytrityl groups, triol derivative **24** in 64% yield (based on **22**).



Reagents and conditions: (i) TMSOTf, $(ClCH_2)_2$, reflux, 16 h, **17**: 80%, **22**: 71%; (ii) KO*t*-Bu (1 M in MeOH)/1,4-dioxane, 2/1, v/v, 1 min, **18**: 76%; **23**: 99%; (iii) a. TBAF (1 M in THF)/1,4-dioxane, 1/4, v/v, 50 °C, 2 h; b. DMTCl, pyr, 16 h, **19**: 78%, **24**: 65% (2 steps); (iv) a. **20**, 1*H*-tetrazole, CH_2Cl_2/CH_3CN, 1/1, v/v, 15 min; b. *t*-BuOOH, 0 °C, 30 min; (v) a. NaOH (4 M)/1,4-dioxane/MeOH, 1/14/5, v/v/v, 16 h; b. HOAc/H_2O, 4/1, v/v, 1 h, 86% (based on **24**).

Scheme 2

Having the 2',2",4"- and 2',3",4"-triols (*i.e.* **19** and **24**) in hand, attention was now focused on the introduction of the three phosphate monoesters. Phosphorylation of **19** and **24** was readily accomplished using the in our laboratory recently developed[14] reagent *N,N*-diisopropyl-bis-[2-(methylsulfonyl)ethyl] (MSE) phosphoramidite (**20**). Thus, 1*H*-tetrazole-assisted phosphitylation of **19** and **24** with **20**, followed by *in situ* oxidation of the intermediate phosphite triesters with *tert*-butyl hydroperoxide gave the fully protected phosphate triesters **21** and **25**, respectively. A one-pot sequential removal of the base-labile and acid-labile protective groups from **21** afforded, after HW-40 gel-filtration, homogeneous adenophostin A analog **4** (30%, Na^+-salt), the ^1H, ^{13}C and ^{31}P-NMR and ESI-mass analytical data of which were in complete accordance with the proposed structure. In an analogous way, fully protected adenophostin A (**25**) was transformed into the mono-benzyl protected derivative **26** in 86% yield (based on **24**). Finally, hydrogenolysis of **26** over Pd-black (1 atm. H_2) resulted, after purification by gel-filtration, in the isolation of homogeneous **2**[15] (65%, Na^+-salt), the

analytical data - ^{1}H, ^{13}C as well as ^{31}P-NMR spectroscopy and ESI-mass spectrometry - of which were in full accord with those reported for naturally occurring[2] and synthetic[4] adenophostin A.

Conclusion

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 **16**. In addition, the unexpected migration of the pivaloyl moiety in **17** gave access to the interesting adenophostin A analog **4**. The ready availability of dimers **10, 15** and **16** may open the way to the preparation of biologically important base-modified analogs of adenophostin A.

Experimental

General methods and materials

Dichloromethane and toluene were dried by distillation from P_2O_5 (5 g L^{-1}) and stored over molecular sieves 4Å (Acros). Pyridine, diethyl ether and triethylamine were heated under reflux for 2 h in the presence of CaH_2 (5 g L^{-1}) and subsequently distilled. Pyridine and diethyl ether were stored over molecular sieves 4Å. *N,N*-Dimethylformamide (p.a. Baker), 1,2-dichloroethane (p.a. Rathburn), 1,4-dioxane (p.a. Baker), *iso*-propanol (p.a. Baker), and acetonitrile (p.a. Rathburn) were stored over molecular sieves 4Å. Methanol (HPLC-grade, Rathburn) was stored over molecular sieves 3Å and all solvents were used without further purification. Acetic acid (p.a. Baker) and acetic anhydride (p.a. Baker) were used as received. Eluents for column chromatography were of technical grade and distilled before use. Reactions were followed by TLC analysis conducted at Schleicher and Schüll DC Fertigfolien (F 1500 LS 254). The following eluents were used: ethyl acetate/light petroleum, 1/3, v/v (System A), and 1/1, v/v (System B), methanol/dichloromethane, 5/95, v/v (System C), and 1/10, v/v (System D). Compounds were visualized by UV light and by spraying with 20% sulfuric acid in methanol followed by charring at 140°C. Column chromatography was performed on silica gel 60, 0.063-0.200 mm (Baker). Gel-filtration was performed on Sephadex LH-20 (Pharmacia). Optical rotations were measured with a Propol polarimeter for solutions in chloroform (p.a. Baker) unless stated otherwise (20 °C). NMR spectra were recorded with a Jeol JNM-FX-200 (^{1}H, ^{13}C, and ^{31}P at 200, 50.1, and 80.7 MHz, respectively), a Bruker WM-300 spectrometer equipped with an Aspect 2000 computer (^{1}H, ^{13}C, and ^{31}P at 300, 75, and 121 MHz respectively), and a Bruker 600-DMX spectrometer (^{1}H, ^{13}C, and ^{31}P at 600, 150, and 242 MHz, respectively). ^{1}H and ^{13}C-Chemical shifts are given in ppm (δ) relative to tetramethylsilane as an internal standard and ^{31}P-chemical shifts are given relative to 85% H_3PO_4 as an external standard. Mass spectra were recorded on a Finnigan MAT TSQ-70 equipped with a custom-made Electrospray Interface (ESI).

Ethyl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-1-thio-α/β-D-glucopyranoside (7)

To a cooled (0 °C) solution of known[7] 1,3,4,6-tetra-*O*-acetyl-2-*O*-benzyl-α/β-D-glucopyranoside (3.8 g, 8.7 mmol) and (ethylthio)trimethylsilane (3.2 mL, 19.5 mmol) in dichloromethane (45 mL) was added a catalytic

amount of TMSOTf (0.17 mL, 0.87 mmol). After stirring for 1 h at ambient temperature an additional amount of TMSOTf (0.17 mL, 0.87 mmol) was added and the mixture was stirred for 16 h, after which TLC analysis (System B) showed almost complete disappearance of starting material. The reaction mixture was diluted with dichloromethane (50 mL), washed with aq. $NaHCO_3$ (10%, 50 mL) and water (50 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (eluent: ethyl acetate/light petroleum 1/3, v/v) to yield pure glucopyranoside **7** as an α/β (2/1) mixture (2.4 g, 5.4 mmol, 62%); Rf 0.68; ^{1}H NMR ($CDCl_3$, α anomer): δ 7.36-7.29 (m, 5H, H arom), 5.40 (d, 1H, H-1, $J_{1,2}$ 5.8 Hz), 5.32 (t, 1H, H-3, $J_{2,3}$, $J_{3,4}$ 9.6 Hz), 4.95 (t, 1H, H-4, $J_{4,5}$ 9.6 Hz), 4.61 (AB, 2H, CH_2 Bn), 4.43 (ddd, 1H, H-5), 4.31 (dd, 1H, H-6a, $J_{5,6a}$ 4.6 Hz, $J_{6a,6b}$ 12.1 Hz), 4.02 (dd, 1H, H-6b, $J_{5,6b}$ 1.9 Hz), 3.82 (dd, 1H, H-2), 2.58-2.48 (m, 2H, CH_2 SEt), 2.07, 2.02, 2.00 (3x s, 9H, 3x CH_3 Ac), 1.29 (t, 3H, CH_3 SEt); $^{13}C\{^1H\}$ NMR ($CDCl_3$, α/β anomers): δ 169.6, 169.4, 169.0 (C(O) Ac), 136.8 (Cq Bn), 128.1-127.1 (CH arom), 84.4 (C-1β), 82.0 (C-1α), 78.4, 75.5, 74.8, 74.7, 71.6, 68.2, 66.8 (C-2α, C-3α, C-4α, C-5α, C-2β, C-3β, C-4β, C-5β), 71.3 (CH_2 Bn), 61.4 (C-6α/β), 24.4 (CH_2 SEtβ), 22.9 (CH_2 SEtα), 19.9, 19.8, 19.6 (3x CH_3 Ac), 14.4 (CH_3 SEtβ), 14.0 (CH_3 SEtα); ESI-MS: $[M+H]^+$ 441; Anal. Calcd. for $C_{21}H_{28}O_8S$ (440.15): C, 57.26; H, 6.41; S, 7.28. Found: C, 57.34; H, 6.45, S, 7.30.

1,2-*O*-Isopropylidene-5-*O*-*tert*-butyldiphenylsilyl-α-D-ribofuranose (8)

To a solution of known[9] 1,2-*O*-isopropylidene-α-D-xylofuranose (12.8 g, 67.0 mmol) in pyridine (300 mL) was added *tert*-butyldiphenylsilyl chloride (21.0 mL, 80.4 mmol). After stirring for 3 h, TLC analysis (System A) showed complete conversion of starting material into a more lipophilic product. The reaction mixture was quenched with methanol (25 mL) and concentrated. The residue was taken up in diethyl ether (200 mL), and washed with aq. $NaHCO_3$ (10%, 100 mL) and water (100 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification was achieved by silica gel column chromatography (eluent: diethyl ether/light petroleum, 1/3 to 1/1, v/v) to give pure 1,2-*O*-isopropylidene-5-*O*-*tert*-butyldiphenylsilyl-α-D-xylofuranose as an oil (27.2 g, 63.7 mmol, 95%); Rf 0.60; $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 132.3 (Cq Ph), 136.0-127.6 (CH arom), 104.9 (C-1), 85.4, 78.9, 76.1 (C-2, C-3, C-4), 62.4 (C-5), 26.7 (CH_3 *t*-Bu, isoprop), 19.0 (Cq *t*-Bu).

1,2-*O*-Isopropylidene-5-*O*-*tert*-butyldiphenylsilyl-α-D-xylofuranose (27.2 g, 63.7 mmol) was stirred in a mixture of dimethyl sulfoxide and acetic anhydride (4/1, v/v, 250 mL). After 16 h, TLC analysis (System A) showed complete conversion of the starting material into a higher-running product. The mixture was poured into ice water (200 mL) and the solution was extracted with diethyl ether (3x 100 mL). The combined organic layers were washed with aq. $NaHCO_3$ (10%, 100 mL) and water (100 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude ulose was reduced without purification: to the vigorously stirred and cooled (0 °C) solution of the ulose in methanol/dichloromethane (1/1, v/v, 500 mL) was added $NaBH_4$ (12.0 g, in 10 portions). Five minutes after the last addition TLC analysis (System A) showed disappearance of the ulose and a product at the same height as the starting xylose derivative. The reaction mixture was poured into water (200 mL), washed, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification of the residue was accomplished using a silica gel column (eluent: diethyl ether/light petroleum, 1/2, v/v) followed by crystallization from ethanol (300 mL) to obtain ribofuranose **8** as a white solid (17.2 g, 40.1 mmol, 63% over two steps). $[\alpha]_D$ +28.0° (*c* 1.0); Rf 0.53; ^{1}H NMR ($CDCl_3$): δ 7.71-7.40 (m, 10H, H arom), 5.84 (d, 1H, H-1, $J_{1,2}$ 3.9 Hz), 4.57 (t, 1H, H-3, $J_{2,3}$ 4.0 Hz), 4.18 (m, 1H, H-4), 4.06-3.88 (m, 3H, H-3, H-5a, H-5b), 2.32 (d, 1H, OH), 1.56, 1.38

(2x s, 6H, 2x CH_3 isoprop), 1.05 (s, 9H, CH_3 *t*-Bu); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 133.2 (Cq Ph), 135.5-127.6 (CH arom), 112.4 (Cq isoprop), 104.1 (C-1), 81.2, 78.7, 71.2 (C-2, C-3, C-4), 62.3 (C-5), 26.7, 26.5 (3x CH_3 *t*-Bu, isoprop), 19.2 (Cq *t*-Bu); Anal. Calcd. for $C_{24}H_{32}O_5Si$ (428.60): C, 67.26; H, 7.53; Si, 6.55. Found: C, 67.21; H, 7.56, Si, 6.56.

3-*O*-(3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-1,2-*O*-iso-propylidene-5-*O*-*tert*-butyldiphenylsilyl-α-D-ribofuranose (10)

A mixture of ethyl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-1-thio-α/β-D-glucopyranoside (**7**, 2.8 g, 6.3 mmol), 1,2-*O*-isopropylidene-5-*O*-*tert*-butyldiphenylsilyl-α-D-ribofuranose (**8**, 2.1 g, 5.0 mmol) and activated molecular sieves (4Å) in 1,2-dichloroethane/diethyl ether (1/1, v/v, 25 mL) was stirred under a blanket of argon. The mixture was cooled (0 °C) and NIS (1.4 g, 6.3 mmol) and a catalytic amount of TfOH (95 mg, 0.63 mmol) were subsequently added. After stirring for 5 min TLC analysis (System A) showed complete disappearance of acceptor **8** and the presence of a product with an Rf-value equal to that of donor **7**. The reaction mixture was quenched with triethylamine (1 mL) and filtered. The filtrate was diluted with dichloromethane (25 mL), and successively washed with aq. $Na_2S_2O_3$ (20%, 25 mL), aq. $NaHCO_3$ (10%, 25 mL) and water (25 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude coupling product was applied onto a column of silica gel and elution was effected with diethyl ether/light petroleum (1/9 to 1/3, v/v). Further purification was achieved by Sephadex LH-20 gel-filtration (eluent: methanol/dichloromethane, 1/2, v/v) to remove excess donor. Concentration of the appropriate fractions yielded solely α-linked disaccharide **10** as a colorless foam (3.8 g, 4.8 mmol, 95%); $[\alpha]_D$ +91.0° (*c* 1.0); Rf 0.34; ^1H NMR ($CDCl_3$, 300 MHz, HH-COSY): δ 7.71-7.25 (m, 15H, H arom), 5.87 (d, 1H, H-1, $J_{1,2}$ 3.7 Hz), 5.48 (t, 1H, H-3', $J_{2,3}$ $J_{3,4}$ 9.7 Hz), 5.32 (d, 1H, H-1', $J_{1,2}$ 3.7 Hz), 5.03 (t, 1H, H-4', $J_{4,5}$ 9.8 Hz), 4.78 (t, 1H, H-2, $J_{2,3}$ 4.0 Hz), 4.76-4.60 (AB, 2H, CH_2 Bn), 4.33 (dd, H-3, $J_{3,4}$ 8.9 Hz), 4.23 (bd, 1H, H-4), 4.15 (dd, 1H, H-6a', $J_{5,6a}$ 3.6 Hz, $J_{6a,6b}$ 12.3 Hz), 4.10-3.96 (m, 3H, H-5', H-5a, H-6b'), 3.92 (dd, 1H, H-5b, $J_{4,5b}$ 2.2 Hz, $J_{5a,5b}$ 12.3 Hz), 3.63 (dd, 1H, H-2'), 2.04, 1.99, 1.98 (3x s, 9H, 3x CH_3 Ac), 1.52, 1.37 (2x s, 6H, 2x CH_3 isoprop), 1.05 (s, 9H, CH_3 *t*-Bu); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.1, 169.7, 169.5 (3x C(O) Ac), 137.5 (Cq Bn), 133.1, 132.5 (2x Cq Ph), 133.1-127.4 (CH arom), 112.8 (Cq isoprop), 104.2 (C-1), 94.3 (C-1', $J_{C\text{-}1',H\text{-}1'}$ 171.5 Hz), 78.8, 76.4, 75.4, 72.4, 71.5, 68.1, 67.5 (C-2, C-3, C-4, C-2', C-3', C-4', C-5'), 71.2 (CH_2 Bn), 61.3, 61.1 (C-5, C-6'), 26.6 (CH_3 *t*-Bu, isoprop), 20.6, 20.5, 20.4 (3x CH_3 Ac), 19.1 (Cq *t*-Bu); ESI-MS $[M+H]^+$ 807; Anal. Calcd. for $C_{43}H_{54}O_{13}Si$ (806.33): C, 64.00; H, 6.74; Si, 3.48. Found: C, 63.98; H, 6.74, Si, 3.46.

3-*O*-(3,4,6-Tri-*O*-acetyl-α-D-glucopyranosyl)-1,2-*O*-isopropylidene-5-*O*-*tert*-butyldiphenylsilyl-α-D-ribofuranose (11)

To a solution of dimer **10** (1.6 g, 2.0 mmol) in *iso*-propanol (15 mL) and acetic acid (2 drops) was added palladium on charcoal (10%). The mixture was degassed and shaken under hydrogen pressure (35 psi) for 16 h. The reaction mixture was filtered over a bed of Hyflo and the filtrate was concentrated to give debenzylated disaccharide **11**, which was used without further purification (quant. yield); Rf 0.13 (System A); ^{13}C NMR ($CDCl_3$): δ 170.1-168.9 (3x C(O) Ac), 132.7, 132.3 (2x Cq Ph), 135.1-127.3 (CH arom), 112.6 (Cq isoprop), 104.1 (C-1), 97.8 (C-1'), 78.8, 76.2, 75.2, 72.7, 69.8, 67.9, 67.3 (C-2, C-3, C-4, C-2', C-3', C-4', C-5'), 61.6, 61.1 (C-5, C-6'), 26.3, 26.1 (CH_3 *t*-Bu, isoprop), 20.4, 20.1 (3x CH_3 Ac), 18.8 (Cq *t*-Bu).

3-*O*-(3,4,6-Tri-*O*-acetyl-2-*O*-pivaloyl-α-D-glucopyranosyl)-1,2-*O*-isopropylidene-5-*O*-*tert*-butyldiphenyl-silyl-α-D-ribofuranose (12)

Disaccha-ride **11** (0.86 g, 1.2 mmol) was dissolved in 1,4-dioxane (8 mL). Pyridine (0.44 mL) and pivaloyl chloride (0.23 mL, 1.8 mmol) were added and the reaction mixture was stirred for 16 h, after which TLC analysis (System A) revealed complete conversion of **11** into a more lipophilic product. Excess pivaloyl chloride was destroyed by the addition of water (5 mL) and the mixture was concentrated *in vacuo*. The oily residue was dissolved in ethyl acetate (25 mL) and washed with aq. $NaHCO_3$ (10%, 10 mL) and water (10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The thus obtained oil was purified by silica gel column chromatography (eluent: ethyl acetate/light petroleum, 1/9 to 1/3, v/v). Concentration of the appropriate fractions afforded fully protected **12** as an oil (0.73 g, 0.91 mmol, 76%); $[\alpha]_D$ +100.6° (*c* 2.6); Rf 0.53; ^{1}H NMR ($CDCl_3$): δ 7.70–7.27 (m, 10H, H arom), 5.79 (d, 1H, H-1, $J_{1,2}$ 3.4 Hz), 5.56 (t, 1H, H-3', $J_{2,3}$, $J_{3,4}$ 9.8 Hz), 5.32 (d, 1H, H-1', $J_{1,2}$ 3.9 Hz), 4.91 (t, 1H, H-4', $J_{4,5}$ 9.9 Hz), 4.88 (dd, 1H, H-2'), 4.66 (t, 1H, H-2, $J_{2,3}$ 3.4 Hz), 4.24 (dd, 1H, H-3, $J_{3,4}$ 9.0 Hz), 4.12-3.81 (m, 6H, H-4, H-5', H-5a, H-5b, H-6a', H-6b'), 1.98 (3x s, 9H, 3x CH_3 Ac), 1.44, 1.28 (2x s, 6H, 2x CH_3 isoprop), 1.16 (s, 9H, CH_3 Piv), 1.00 (s, 9H, CH_3 TBDPS); ^{13}C{^{1}H} NMR ($CDCl_3$): δ 176.7 (C(O) Piv), 169.6, 169.2, 168.7 (3x C(O) Ac), 132.9, 132.2 (2x Cq Ph), 135.2-127.4 (CH arom), 112.4 (Cq isoprop), 103.1 (C-1), 93.5 (C-1'), 78.1, 75.9, 72.7, 69.7, 69.4, 67.6, 67.4 (C-2, C-3, C-4, C-2', C-3', C-4', C-5'), 61.1 (C-5, C-6'), 38.1 (Cq Piv), 26.4, 26.1 (CH_3 TBDPS, Piv, isoprop), 20.1 (3x CH_3 Ac), 18.8 (Cq *t*-Bu); ESI-MS: $[M+Na]^+$ 823; Anal. Calcd. for $C_{41}H_{56}O_{14}Si$ (800.34): C, 61.48; H, 7.05; Si, 3.51. Found: C, 61.43; H, 7.07, Si, 3.54.

3-*O*-(3,4,6-Tri-*O*-acetyl-2-*O*-pivaloyl-α-D-glucopyranosyl)-5-*O*-*tert*-butyldiphenylsilyl-α/β-D-ribofuranose (13)

The acetonide function in compound **12** (0.56 g, 0.70 mmol) was removed by refluxing in a mixture of acetic acid/water (7/3, v/v, 10 mL) for 30 min. The mixture was cooled, diluted with toluene (10 mL) and concentrated to a smaller volume. The residue was repeatedly diluted with toluene (5x 10 mL) and concentrated *in vacuo*. Application of the residue onto a column of silica gel (eluent: ethyl acetate/light petroleum, 1/3 to 1/1, v/v) and concentration of the appropriate fractions yielded diol **13** (0.32 g, 0.42 mmol, 60%) as a mixture of anomers (α:β = 2:1); Rf 0.30 (System B); ^{13}C{^{1}H} NMR ($CDCl_3$): δ 177.5 (C(O) Piv), 170.6, 170.1, 169.5 (3x C(O) Ac), 132.7, 132.4 (2x Cq Ph), 135.3-127.6 (CH arom), 101.4 (C-1β), 96.5, 96.1 (C-1α, C-1'), 82.2, 81.3, 77.7, 76.7, 74.2, 71.2, 70.1, 69.7, 68.1, 67.7 (C-2, C-3, C-4, C-2', C-3', C-4', C-5'), 63.6, 61.7 (C-5, C-6'), 38.6 (Cq Piv), 26.6 (CH_3 TBDPS, Piv), 20.3 (3x CH_3 Ac), 18.9 (Cq *t*-Bu); ESI-MS: $[M+Na]^+$ 783.

1,2-di-*O*-Acetyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-pivaloyl-α-D-glucopyranosyl)-5-*O*-*tert*-butyl-diphenylsilyl-α/β-D-ribofuranose (14)

Diol **13** (0.32 g, 0.42 mmol) was dissolved in a mixture of pyridine/acetic anhydride (2/1, v/v, 10 mL). After stirring for 4 h TLC analysis (System B) revealed complete conversion of starting material into a somewhat higher-running product. The reaction mixture was diluted with toluene (25 mL) and concentrated to a smaller volume. The residual oil was repeatedly diluted with toluene (6x 10 mL) and concentrated again. Crude **14** was applied onto a column of silica gel. Elution with ethyl acetate/light petroleum (1/3, v/v) and concentration of the appropriate fractions furnished diacetate **14** (α:β = 5:1, 0.31 g, 0.38 mmol, 91%); Rf 0.72; ^{13}C{^{1}H} NMR

($CDCl_3$): δ 177.4 (C(O) Piv), 170.2, 169.6, 169.3 (5x C(O) Ac), 132.5 (2x Cq Ph), 135.2-127.7 (CH arom), 97.8 (C-1), 95.9 (C-1'), 84.1, 83.4, 75.6, 74.8, 74.0, 71.8, 70.5, 70.3, 69.3, 68.4, 68.2, 68.0 (C-2, C-3, C-4, C-2', C-3', C-4', C-5'), 62.6, 61.6 (C-5, C-6'), 38.6 (Cq Piv), 26.7, 26.6 (CH_3 TBDPS, Piv), 20.8, 20.5 (5x CH_3 Ac), 19.1 (Cq *t*-Bu); ESI-MS: $[M+Na]^+$ 867; Anal. Calcd. for $C_{42}H_{56}O_{16}Si$ (844.98): C, 59.70; H, 6.68; Si, 3.32. Found: C, 59.75; H, 6.70, Si, 3.33.

3-*O*-(3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-5-*O*-*tert*-butyldiphenylsilyl-α/β-D-ribofuranose (15)

Disaccharide **10** (0.18 g, 0.22 mmol) was dissolved in acetic acid/water/ethylene glycol (14/6/3, v/v/v, 5 mL). After stirring at reflux temperature for 30 min the reaction mixture was cooled and poured into aq. $NaHCO_3$ (10%, 10 mL). The product was extracted with ethyl acetate (3x 10 mL). The combined organic phases were washed with water (10 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was repeatedly diluted with toluene (4x 10 mL) and concentrated. Purification was performed by silica gel column chromatography (eluent: ethyl acetate/light petroleum, 1/1, v/v). Concentration of the appropriate fractions afforded diol **15** as a mixture of anomers (α:β = 2:1, 0.14 g, 0.18 mmol, 84%); Rf 0.23 (System B); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.3-169.6 (C(O) Ac), 136.5 (Cq Bn), 132.7, 132.2 (2x Cq Ph), 135.3-127.6 (CH arom), 101.6 (C-1β), 97.7, 97.4 (C-1'), 96.6 (C-1α), 81.9, 77.6, 76.1, 74.2, 71.7, 71.5, 68.0, 67.7 (C-2, C-3, C-4, C-2', C-3', C-4', C-5'), 73.9, 73.7 (CH_2 Bn), 63.4, 61.6 (C-5, C-6'), 26.5 (CH_3 *t*-Bu), 20.6, 20.5, 20.3 (3x CH_3 Ac), 19.0 (Cq *t*-Bu); ESI-MS: $[M+Na]^+$ 789.

1,2-di-*O*-Acetyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-5-*O*-*tert*-butyldiphenylsilyl-α-D-ribofuranose (16)

Diol **15** (1.4 g, 1.8 mmol) was acetylated as described previously for the synthesis of **14**. Crude **16** was applied onto a column of silica gel. Elution with ethyl acetate/light petroleum (1/3, v/v) and concentration of the appropriate fractions furnished diacetate **12** (α:β = 1:0, 1.4 g, 1.7 mmol, 94%); Rf 0.63 (System B); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.2, 169.7, 169.4 (3x C(O) Ac), 137.6 (Cq Bn), 132.8 (2x Cq Ph), 135.5-127.7 (CH arom), 98.5 (C-1'), 97.1 (C-1), 83.1, 76.6, 74.6, 73.9, 71.5, 68.3, 67.9 (C-2, C-3, C-4, C-2', C-3', C-4', C-5'), 73.4 (CH_2 Bn), 63.0, 61.6 (C-5, C-6'), 26.8 (CH_3 *t*-Bu), 20.9, 20.6, 20.5 (3x CH_3 Ac) 19.2 (Cq *t*-Bu); ESI-MS: $[M+Na]^+$ 873; Anal. Calcd. for $C_{44}H_{54}O_{15}Si$ (850.32): C, 62.10; H, 6.40; Si, 3.30. Found: C, 62.04; H, 6.36, Si, 3.29.

2'-*O*-Acetyl-3'-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-pivaloyl-α-D-glucopyranosyl)-6-*N*-benzoyl-5'-*O*-*tert*-butyldiphenylsilyladenosine (17)

A suspension of 6-*N*-benzoyladenine (**9**, 0.22 g, 0.93 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (1.7 mL) and pyridine (0.60 mL) was refluxed for 7 h. The reaction mixture was cooled, diluted with toluene (5 mL) and concentrated. The residual oil was repeatedly diluted with toluene (5x 5 mL) and concentrated *in vacuo* to remove excess 1,1,1,3,3,3-hexamethyldisilazane. Disaccharide **14** (0.32 g, 0.38 mmol) in 1,2-dichloroethane (5 mL) and a catalytic amount of TMSOTf (9 mg, 0.04 mmol) were added to the silylated 6-*N*-benzoyladenine. After stirring for 16 h at reflux temperature TLC analysis (System C) showed conversion of the starting disaccharide into one major lower-running product. The reaction mixture was quenched with triethylamine (0.5 mL), diluted with dichloromethane (10 mL) and poured into aq. $NaHCO_3$ (10%, 5 mL). The organic phase was

washed with water (5 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (eluent: dichloromethane/light petroleum, 3/1 to 1/0, v/v). Concentration of the appropriate fractions afforded **17** as a yellowish foam (0.31 g, 0.30 mmol, 80%); $[\alpha]_D$ +56.1 (*c* 0.8); Rf 0.47; ^{1}H NMR ($CDCl_3$): δ 9.19 (bs, 1H, NH), 8.66, 8.24 (2x s, 2H, H-2, H-8), 8.04-7.28 (m, 15H, H arom), 6.37 (d, 1H, H-1', $J_{1,2}$ 8.0 Hz), 6.04 (dd, 1H, H-2', $J_{2,3}$ 4.9 Hz), 5.60 (t, 1H, H-3", $J_{2,3}$, $J_{3,4}$ 9.9 Hz), 5.32 (d, 1H, H-1", $J_{1,2}$ 3.6 Hz), 5.11 (t, 1H, H-4", $J_{4,5}$ 9.8 Hz), 4.83 (dd, 1H, H-2"), 4.71 (d, 1H, H-3'), 4.42 (bs, 1H, H-4'), 4.21 (dd, 1H, H-6a", $J_{5,6a}$ 3.5 Hz, $J_{6a,6b}$ 11.0 Hz), 4.12 (dd, 1H, H-5a', $J_{4,5a}$ 2.9 Hz, $J_{5a,5b}$ 10.0 Hz), 4.00-3.68 (m, 3H, H-5", H-5b', H-6b"), 2.12 (4x s, 12H, 4x CH_3 Ac), 1.22 (s, 9H, CH_3 Piv), 1.16 (s, 9H, CH_3 TBDPS); ^{13}C{^{1}H} NMR ($CDCl_3$): δ 177.6 (C(O) Piv), 170.4, 169.7, 169.6, 169.4 (4x C(O) Ac), 164.8 (C(O) Bz), 152.5, 141.1 (C-2, C-8), 152.1, 149.6 (C-4, C-6), 133.4, 132.0 (2x Cq Ph), 135.-127.9 (CH arom), 123.2 (C-5), 95.1 (C-1"), 84.2 (C-1'), 74.2, 73.7, 70.4, 69.2, 68.0 (C-2', C-3', C-4', C-2", C-3", C-4", C-5"), 63.5, 61.5 (C-5', C-6"), 38.7 (Cq Piv), 27.0, 26.8 (CH_3 TBDPS, Piv), 20.5, 20.3 (4x CH_3 Ac), 19.1 (Cq *t*-Bu); ESI-MS: $[M+H]^+$ 1025; Anal. Calcd. for $C_{52}H_{61}N_5O_{15}Si$ (1023.39): C, 60.98; H, 6.00; N, 6.84; Si, 2.74. Found: C, 61.03; H, 6.04; N, 6.83; Si, 2.76.

6-*N*-Benzoyl-3'-*O*-(3-*O*-pivaloyl-α-D-glucopyranosyl)-5'-*O*-*tert*-butyldiphenylsilyladenosine (18)

To a solution of glucopyranosyl adenosine **17** (0.27 g, 0.26 mmol) in 1,4-dioxane (8 mL) was added a solution of potassium *tert*-butoxide in methanol (1 M, 16 mL). After stirring for 1 min, the reaction mixture was neutralized upon the addition of acetic acid (0.93 mL). The solution was poured into aq. $NaHCO_3$ (10%, 10 mL), and the solution was extracted with dichloromethane (2x 10 mL). The organic phase was washed with water (10 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude deacetylated dimer was purified by silica gel column chromatography. Elution was effected with methanol/dichloromethane (0/1 to 5/95, v/v). Concentration of the appropriate fractions yielded **18** as a white foam (0.17 g, 0.20 mmol, 76%); $[\alpha]_D$ +44.7° (*c* 1.3); Rf 0.27 (System C); ESI-MS: $[M+H]^+$ 856; ^{1}H NMR ($CDCl_3$): δ 9.37 (bs, 1H, NH), 8.69-8.25 (2x s, 2H, H-2, H-8), 8.03-7.27 (m, 15H, H arom), 6.13 (d, 1H, H-1', $J_{1,2}$ 7.8 Hz), 5.16 (t, 1H, H-3", $J_{2,3}$, $J_{3,4}$ 9.4 Hz), 5.10 (d, 1H, H-1", $J_{1,2}$ 3.8 Hz), 4.81 (t, 1H, H-2', $J_{2,3}$ 5.6 Hz), 4.46 (bs, 1H, H-4'), 4.40 (dd, 1H, H-3', $J_{3,4}$ 2.6 Hz), 3.91-3.40 (m, 7H, H-5a', H-5b', H-2", H-4", H-5", H-6a", H-6b"), 1.23 (s, 9H, CH_3 Piv), 1.02 (s, 9H, CH_3 TBDPS); ^{13}C NMR ($CDCl_3$): δ 180.5 (C(O) Piv), 165.1 (C(O) Bz), 152.3, 141.5 (C-2, C-8), 151.3, 151.2 (C-4, C-6), 133.2, 132.4 (2x Cq Ph), 135.4-127.8 (CH arom), 122.4 (C-5), 100.6 (C-1"), 88.0 (C-1'), 84.2, 78.1, 76.3, 74.8, 72.7, 70.5, 68.7 (C-2', C-3', C-4', C-2", C-3", C-4", C-5"), 63.4, 61.4 (C-5', C-6"), 39.0 (Cq Piv), 27.0, 26.8 (CH_3 TBDPS, Piv), 19.1 (Cq *t*-Bu).

6-*N*-Benzoyl-3'-*O*-(6-*O*-[4,4'-dimethoxytrityl]-3-*O*-pivaloyl-α-D-glucopyranosyl)-5'-*O*-[4,4'-dimethoxytrityl]-adenosine (19)

Tetrabutylammonium fluoride (0.23 mL, 1 M in THF) was added to a solution of dimer **18** (0.13 g, 0.15 mmol) in 1,4-dioxane (1 mL). After stirring for 2 h at 50 °C TLC analysis (System C) showed conversion of starting material into a lower-running product (Rf 0.05). Pyridine (5 mL) was added and the reaction mixture was concentrated to a smaller volume. Crude desilylated product was dried by repeated evaporation with pyridine (3x 5 mL) and subsequently dissolved in pyridine (1 mL). To this stirred solution 4,4'-dimethoxytrityl

chloride (0.31 g, 0.36 mmol) was added and the mixture was stirred for 16 h. Excess 4,4'-dimethoxytrityl chloride was destroyed with methanol (2 mL) and the solution was concentrated under reduced pressure. The residue was taken up in dichloromethane (10 mL) and washed with aq. $NaHCO_3$ (10%, 5 mL) and water (5 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The residual oil was applied onto a silica gel column (eluent: dichloromethane/light petroleum/triethylamine, 49/49/2 to 98/0/2, v/v/v) to afford dimer **19** as a foam (0.14 g, 0.12 mmol, 78%); $[\alpha]_D$ +38.7° (*c* 0.7); Rf 0.36 (System C); ^{1}H NMR ($CDCl_3$): δ 9.09 (bs, 1H, NH), 8.71, 8.26 (2x s, 2H, H-2, H-8), 8.04-6.64 (m, 31H, H arom), 6.12 (d, 1H, H-1', $J_{1,2}$ 6.5 Hz), 5.18 (t, 1H, H-3", $J_{2,3}$, $J_{3,4}$ 9.5 Hz), 5.14 (d, 1H, H-1", $J_{1,2}$ 4.0 Hz), 4.99 (t, 1H, H-2', $J_{2,3}$ 5.9 Hz), 4.54 (bs, 1H, H-4'), 4.44 (dd, 1H, H-3', $J_{3,4}$ 2.1 Hz), 3.86 (m, 1H, H-5"), 3.79 (dd, 1H, H-2", $J_{2,3}$ 9.8 Hz), 3.71 (2x s, 12H, 4x OCH_3), 3.56 (t, 1H, H-4", $J_{4,5}$ 9.5 Hz), 3.46 (dd, 1H, H-5a', $J_{4,5a}$ 3.3 Hz, $J_{5a,5b}$ 10.7 Hz), 3.39 (dd, 1H, H-6a", $J_{5,6a}$ 3.2 Hz, $J_{6a,6b}$ 10.2 Hz), 3.30 (dd, 1H, H-5b', $J_{4,5b}$ 3.1 Hz), 3.25 (dd, 1H, H-6b", $J_{5,6b}$ 5.3 Hz), 2.70 (bs, 1H, OH), 1.80 (bs, 2H, 2x OH), 1.22 (s, 9H, CH_3 Piv); ^{13}C{^{1}H} NMR ($CDCl_3$): δ 180.3 (C(O) Piv), 164.7 (C(O) Bz), 158.4, 158.3 (2x $COCH_3$), 152.2, 141.7 (C-2, C-8), 151.4, 149.6 (C-4, C-6), 144.6, 144.2 (2x Cq DMT), 135.7, 135.1 (Cq DMT, Bz), 135.5-126.7 (CH arom), 123.0 (C-5), 113.1-113.0 (CH arom DMT), 100.9 (C-1"), 88.7 (C-1'), 86.6, 86.1 (2x Cq DMT), 83.6, 80.1, 77.2, 74.9, 71.9, 70.4, 70.0 (C-2', C-3', C-4', C-2", C-3", C-4", C-5"), 63.5, 63.3 (C-5', C-6"), 55.0 (2x OCH_3), 39.0 (Cq Piv), 27.0 (CH_3 Piv); ESI-MS: $[M+H]^+$ 1222.

3-*O*-(α-D-Glucopyranosyl 2,4-bisphosphate)-adenosine 2'-monophosphate (4)

To a mixture of triol **19** (81 mg, 66 μmol) and *N,N*-diisopropyl-bis-[2-(methylsulfonyl)ethyl] phosphoramidite (**20**, 0.15 g, 0.40 mmol) in dichloromethane (2 mL) was added a solution of 1*H*-tetrazole (36 mg, 0.53 mmol) in acetonitrile (2 mL). After stirring for 15 min TLC analysis (System D) showed complete conversion of starting triol in a higher-running product (Rf 0.71). ^{31}P NMR (CH_2Cl_2) showed the presence of three major resonances (δ 140.6, 140.5 and 140.4). The reaction mixture was cooled (0 °C), *tert*-butyl hydroperoxide (0.5 mL) was added and stirring was continued for 30 min after which TLC analysis (System D) revealed complete disappearance of the intermediate phosphite triesters into a lower-running product (Rf 0.53, ^{31}P NMR (CH_2Cl_2): δ -1.7, -2.1, -2.4). The reaction mixture was diluted with dichloromethane (10 mL), washed with water (5 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Fully protected adenophostin A analog **21** was dissolved in a mixture of NaOH (4 M)/1,4-dioxane/methanol (1/14/5, v/v/v, 10 mL). After stirring for 16 h the reaction mixture was neutralized with acetic acid (0.12 mL) and concentrated. The residue was dissolved in acetic acid/water (4/1, v/v, 5 mL) and stirred for 1 h. The mixture was concentrated and acid was removed by repeated evaporations with water (5x 10 mL). The remaining solid was dissolved in water (10 mL) and apolair compounds were extracted with ethyl acetate and dichloromethane (2x 5 mL). The aqueous layer was concentrated under reduced pressure. Extensive purification of crude deprotected **4** was accomplished by gel-filtration over Fractogel HW-40 (S, Omnilabo). The column was eluted with a solution of TEAB (0.15 M) in methanol/water (1/9, v/v). Further purification by Q-Sepharose ion-exchange (eluent: 0.05 M TEAB → 1.0 M TEAB) and ion-exchange by Dowex® 50Wx4 (Na^+-form) gave after lyophilization pure **4** (13 mg, 20 μmol, 30%); ^{1}H NMR (D_2O, 600 MHz, HH-COSY): δ 8.31, 8.21 (H-2, H-8), 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), 4.66 (dd, 1H, H-3', $J_{3,4}$ 5.7 Hz), 4.46 (dd, 1H, H-4', $J_{4,5}$ 6.4 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), 3.88 (dd, 1H, H-5a', $J_{4,5a}$ 2.7 Hz, $J_{5a,5b}$ 13.0 Hz), 3.85 (dd, 1H, H-

6a", $J_{5,6}$ 4.8 Hz, $J_{6a,6b}$ 12.9 Hz), 3.82 (dd, 1H, H-5b', $J_{4,5b}$ 3.5 Hz), 3.80 (dd, 1H, H-6b", $J_{5,6b}$ 3.5 Hz), 3.71 (ddd, 1H, H-5"); ^{13}C NMR (D_2O, 150 MHz, CH-COSY): δ 149.6, 142.2 (C-4, C-6), 120.2 (C-5), 98.4 (C-1"), 88.8 (C-1', $^3J_{CP}$ 4.5 Hz), 85.1 (C-4'), 75.6 (C-3'), 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), 72.3 (C-5"), 62.2 (C-5'), 61.3 (C-6"); ^{31}P NMR (D_2O, 242 MHz, PH-COSY): δ 3.64 (C-4"-P), 1.61 (C-2'-P), 1.20 (C-2"-P); ESI-MS: $[M-H]^-$ 667; Anal. Calcd. for $C_{16}H_{26}N_5O_{18}P_3$ (669.05): C, 28.71; H, 3.92; N, 10.46; P, 13.88. Found: C, 28.70; H, 3.96; N, 10.49; P, 13.94.

2'-*O*-Acetyl-3'-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-6-*N*-benzoyl-5'-*O*-*tert*-butyldiphenylsilyladenosine (22)

Vorbrüggen-type condensation of 3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl)-1,2-di-*O*-acetyl-5-*O*-*tert*-butyldiphenylsilyl-α-D-ribofuranose (**16**, 1.0 g, 1.2 mmol) with commercially available 6-*N*-benzoyladenine (**9**) was accomplished as described for the preparation of **17**. Extensive purification of crude **22** by silica gel column chromatography (eluent: methanol/dichloromethane/light petroleum, 0/3/1 to 2/98/0, v/v/v) furnished glucosyl adenosine **22** as a yellowish foam (0.88 g, 0.85 mmol, 71%); $[\alpha]_D$ +31.5° (*c* 1.8); Rf 0.68 (System C); ^{1}H NMR ($CDCl_3$, 300 MHz, HH-COSY): δ 9.11 (bs, 1H, NH), 8.72, 8.20 (2x s, 2H, H-2, H-8), 8.04-7.25 (m, 20H, H arom), 6.36 (d, 1H, H-1', $J_{1,2}$ 6.7 Hz), 5.86 (t, 1H, H-2', $J_{2,3}$ 6.2 Hz), 5.47 (t, 1H, H-3", $J_{2,3}$, $J_{3,4}$ 9.8 Hz), 4.97 (t, 1H, H-4", $J_{4,5}$ 9.8 Hz), 4.90 (d, 1H, H-1", $J_{1,2}$ 3.6 Hz), 4.80 (dd, 1H, H-3', $J_{3,4}$ 3.1 Hz), 4.63-4.57 (AB, 2H, CH_2 Bn), 4.42 (m, 1H, H-4'), 4.15 (dd, 1H, H-6a", $J_{5,6a}$ 4.8 Hz, $J_{6a,6b}$ 12.1 Hz), 4.09-4.02 (dd, 1H, H-5a', $J_{4,5a}$ 3.5 Hz, m, 1H, H-5"), 3.91 (dd, 1H, H-6b", $J_{5,6b}$ 2.0 Hz), 3.86 (dd, 1H, H-5b', $J_{4,5b}$ 3.5 Hz), 2.04-1.91 (4x s, 12H, 4x CH_3 Ac), 1.09 (s, 9H, CH_3 *t*-Bu); ^{13}C{^{1}H} NMR ($CDCl_3$): δ 170.3, 170.1, 169.9, 169.6 (4x C(O) Ac), 164.7 (C(O) Bz), 152.7, 141.5 (C-2, C-8), 151.8, 149.6 (C-4, C-6), 137.3 (Cq Bn), 133.4, 132.3 (2x Cq Ph), 135.5-127.3 (CH arom), 123.4 (C-5), 98.6 (C-1"), 85.8 (C-1'), 84.2, 77.2, 76.5, 73.7, 71.3, 68.4, 68.2 (C-2', C-3', C-4', C-2", C-3", C-4", C-5"), 73.1 (CH_2 Bn), 63.2, 61.9 (C-5', C-6"), 26.8, (CH_3 *t*-Bu), 20.7, 20.5, 20.2 (4x CH_3 Ac), 19.1 (Cq *t*-Bu); ESI-MS: $[M+H]^+$ 1030; Anal. Calcd. for $C_{54}H_{59}N_5O_{14}Si$ (1029.38): C, 62.96; H, 5.77; N, 6.80; Si, 2.73. Found: C, 62.99; H, 5.79; N, 6.83; Si, 2.71.

6-*N*-Benzoyl-3'-*O*-(2-*O*-benzyl-α-D-glucopyranosyl)-5'-*O*-*tert*-butyldiphenylsilyladenosine (23)

Deacetylation of **22** (0.88 g, 0.85 mmol) was performed as described for dimer **17** (→ **18**). The oily residue obtained after work-up was applied onto a column of silica gel. Elution with methanol/dichloromethane (0/1 to 5/95, v/v) afforded pure **23** as a white foam (0.73 g, 0.84 mmol, 99%); $[\alpha]_D$ +23.9° (*c* 1.5); Rf 0.18 (System C); ^{1}H NMR ($CDCl_3$/MeOD): δ 8.68, 8.23 (2x s, 2H, H-2, H-8), 8.06-7.27 (m, 20H, H arom), 6.60 (d, 1H, H-1', $J_{1,2}$ 6.8 Hz), 4.88 (m, 1H, H-2'), 4.80-4.66 (AB, 2H, CH_2 Bn, d, 1H, H-1", $J_{1,2}$ 3.6 Hz), 4.46 (bd, 1H, H-4'), 4.26 (dd, 1H, H-3', $J_{2,3}$ 5.3 Hz, $J_{3,4}$ 2.1 Hz), 4.08-3.26 (m, 8H, H-5a', H-5b', H-2", H-3", H-4", H-5", H-6a", H-6b"), 1.04 (s, 9H, CH_3 *t*-Bu); ^{13}C {^{1}H} NMR ($CDCl_3$/CD_3OD): δ 165.5 (C(O) Bz), 151.9, 141.4 (C-2, C-8), 151.4, 149.2 (C-4, C-6), 136.8 (Cq Bn), 133.0, 131.9 (2x Cq Ph), 134.9-126.9 (CH arom), 122.3 (C-5), 98.5 (C-1"), 87.9 (C-1'), 83.2, 78.5, 77.1, 73.8, 72.5, 72.2, 69.3 (C-2', C-3', C-4', C-2", C-3", C-4", C-5"), 73.4 (CH_2 Bn), 62.9, 60.5 (C-5', C-6"), 26.3 (CH_3 *t*-Bu), 18.6 (Cq *t*-Bu); ESI-MS: $[M+Na]^+$ 880.

6-*N*-Benzoyl-3'-*O*-(2-*O*-benzyl-6-*O*-[4,4'-dimethoxytrityl]-α-D-glucopyranosyl)-5'-*O*-[4,4'-dimethoxytrityl]-adenosine (24)

Desilylation of **23** (0.73 g, 0.84 mmol) and subsequent protection of the primary hydroxyl functions with a 4,4'-dimethoxytrityl group was accomplished *via* the same procedure as described for **18** (→ **19**). The crude product was purified by silica gel column chromatography (eluent: dichloromethane/light petroleum/triethylamine, 49/49/2 to 98/0/2, v/v/v) to furnish dimer **24** as a yellowish solid (0.67 g, 0.55 mmol, 65%); $[\alpha]_D$ +9.4° (*c* 1.3); Rf 0.31 (System C); ^{1}H NMR ($CDCl_3$, 300 MHz, HH-COSY): δ 9.13 (s, 1H, NH), 8.58, 8.17 (2x s, 2H, H-2, H-8), 8.04-6.67 (m, 36H, H arom), 6.13 (d, 1H, H-1', $J_{1,2}$ 6.7 Hz), 4.90 (t, 1H, H-2'), 4.86 (d, 1H, H-1", $J_{1,2}$ 3.6 Hz), 4.71 (AB, 2H, CH_2 Bn), 4.49 (bd, 1H, H-4'), 4.26 (dd, 1H, H-3', $J_{2,3}$ 5.1 Hz, $J_{3,4}$ 1.7 Hz), 4.05 (t, 1H, H-3", $J_{2,3}$, $J_{3,4}$ 9.3 Hz), 3.81 (m, 1H, H-5"), 3.69, 3.68 (2x s, 12H, 4x OCH_3), 3.54 (t, 1H, H-4", $J_{4,5}$ 9.3 Hz), 3.45 (dd, 1H, H-2", dd, 1H, H-5a'), 3.22 (dd, 1H, H-5b', $J_{4,5b}$ 5.3 Hz, $J_{5a,5b}$ 10.1 Hz), 3.28 (dd, 1H, H-6a", $J_{5,6a}$ 3.3 Hz, $J_{6a,6b}$ 10.7 Hz), 3.36 (dd, 1H, H-6b", $J_{5,6b}$ 2.8 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$): δ 164.7 (C(O) Bz), 158.2 ($COCH_3$ DMT), 152.2, 141.6 (C-2, C-8), 151.5, 149.4 (C-4, C-6), 144.7, 144.2 (Cq DMT), 137.2 (Cq Bn), 135.6-135.0 (Cq DMT, Bz), 129.8-127.6 (CH arom), 113.3 (CH arom DMT), 123.1 (C-5), 99.6 (C-1"), 88.2 (C-1'), 86.5, 85.8 (2x Cq DMT), 83.1, 80.0, 79.0, 74.6, 73.1, 71.9, 70.6 (C-2', C-3', C-4', C-2", C-3", C-4", C-5"), 73.8 (CH_2 Bn), 63.2 (C-5', C-6"), 54.8 (2x OCH_3); ESI-MS: $[M+H]^+$ 1229.

3-*O*-(α-D-Glucopyranosyl 3,4-bisphosphate)-adenosine 2'-monophosphate (adenophostin A, 2)

Phosphorylation and deprotection of triol **24** (0.24 g, 0.20 mmol) was accomplished as described for the synthesis of adenophostin A analog **4**. ^{31}P NMR (CH_2Cl_2) after phosphitylation showed the presence of two major resonances (δ 141.1, 140.5). ^{31}P NMR (CH_2Cl_2) after oxidation of the intermediate phosphite triesters to the corresponding phosphate triesters showed three major resonances (δ -2.1, -2.3, -2.6). Deprotection of the base- and acid-labile protective groups in compound **25** was readily accomplished as described for **21**. Crude benzyl-containing adenophostin A (**26**) was purified by gel-filtration over Fractogel HW-40 (S Omnilabo). The column was eluted with a solution of TEAB (0.15 M) in methanol/water (1/9, v/v). Compound **26** was dissolved in water (10 mL) and acetic acid (2 drops) was added. Pd-black (spatula) was added and the mixture was degassed and stirred under a H_2-atmosphere for 6 h. The mixture was filtered and concentrated. Purification by gel-filtration over Fractogel HW-40 (S, Omnilabo), eluent TEAB (0.15 M) in methanol/water (1/9, v/v) followed by ion-exchange by Dowex® 50Wx4 (Na^+ form) and lyophilization furnished adenophostin A (**2**, 87 mg, 0.13 mmol, 65%); ^{1}H NMR (D_2O, 600 MHz, HH-COSY): δ 8.28, 8.10 (2x s, 2H, H-2, H-8), 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), 4.62 (dd, 1H, H-3', $J_{3,4}$ 5.1 Hz), 4.48-4.44 (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), 3.87 (dd, 1H, H-5a', $J_{4,5a}$ 2.6 Hz, $J_{5a,5b}$ 13.0 Hz, dd, 1H, H-6a", $J_{5,6a}$ 4.5 Hz, $J_{6a,6b}$ 13.0 Hz), 3.77 (m, 2H, H-5", H-6b"), 3.74 (dd, 1H, H-2", $J_{2,3}$ 9.7 Hz); ^{13}C{^{1}H}NMR (D_2O, 150 MHz, CH-COSY): δ 152.5, 142.0 (C-2, C-8), 149.0 (C-4, C-6), 119.7 (C-5), 96.7 (C-1"), 88.2 (C-1', $^3J_{CP}$ 5.4 Hz), 85.3 (C-4'), 77.7 (C-3", $^2J_{CP}$ 8.2 Hz), 75.7 (C-2', $^2J_{CP}$ 4.3 Hz), 74.4 (C-3', $^3J_{CP}$ 3.6 Hz), 72.8 (C-5"), 72.6 (C-4", $^2J_{CP}$ 3.3 Hz), 71.7 (C-2", $^3J_{CP}$ 3.1 Hz), 62.1 (C-5'), 60.9 (C-6"); ^{31}P NMR (D_2O, 242 MHz, PH-COSY): δ 2.44 (C-4"-P), 1.91 (C-3"-P), 0.79 (C-2'-P); ESI-MS:$[M-H]^-$ 668; Anal. Calcd. for

$C_{16}H_{26}N_5O_{18}P_3$ (669.05): C, 28.71; H, 3.92; N, 10.46; P, 13.88. Found: C, 28.68; H, 3.94; N, 10.47; P, 13.83.

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