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# Synthesis of 2',3",4"-Trisphosphate-Containing Analogs of Adenophostin A

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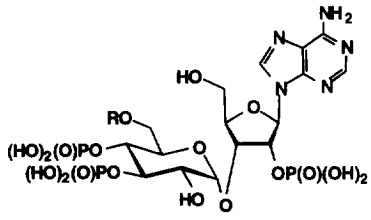
***Abstract:*** Adenophostin A analog **4** was prepared *via* trimethylsilyl trifluoromethanesulfonate (TMSOTf)-assisted glycosylation of (*S*)-6-*N*-diphenylacetyl-9-(2-*tert*-butyldiphenylsilyloxy-1-hydroxyprop-3-yl)-adenine (**11**) with trichloroacetimidate donor **12** to give dimer **13**. Protective group manipulations on **13** followed by phosphitylation with *N,N*-diisopropyl-bis-[2-(methylsulfonyl)ethyl] phosphoramidite (**17**) and *in situ* oxidation gave, after deprotection, (2*S*)-9-{1-(α-D-glucopyranosyloxy 3,4-bisphosphate)-2-monophosphate-prop-3-yl}-adenine (**4**). Condensation of phosphoryloxymethyl-adenosine **25** with D-arabinitol derivative **21** under the agency of TMSOTf afforded methylene acetal **26**. Protective group manipulations (→ **32**), phosphorylation, and deprotection yielded 3'-*O*-(D-arabinitol-4-*O*-methylene 2,3-bisphosphate)-adenosine 2'-monophosphate (**5**), a methylene acetal-containing analog of adenophostin A. © 1997 Elsevier Science Ltd.

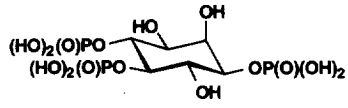
## Introduction

In 1993, Takahashi *et al.*[1] discovered that adenophostin A and B (*i.e.* **1** and **2** in Fig. 1), isolated from the fermentation broth of *Penicillium brevicompactum*, are full agonists for the D-*myo*-inositol receptor. It was also revealed that $Ca^{2+}$ mobilization and binding affinity of adenophostin A and B are approximately 10-100 times higher in comparison with the natural ligand D-*myo*-inositol 1,4,5-trisphosphate ($IP_3$, **3**). The exceptionally high biological activity of **1** and **2** is rather intriguing as they exhibit little structural resemblance with $IP_3$. However, it seems obvious that the vicinal 3,4-D-*threo* bisphosphate arrangement in the glucosyl and the 2'-phosphate in the ribosyl moiety are key elements for recognition of adenophostin A and B by the receptor. Indeed, adenophostin A derivatives lacking the 2'-ribosyl phosphate show a 1000-fold lower binding affinity.[1] On the other hand, the contribution of the adenine moiety to the biological activity is not fully understood. With the objective to get a deeper insight in the structure-activity profile of the $IP_3$ receptor, we[2] and other groups[3] embarked upon a program to prepare adenophostin A and analogs thereof.

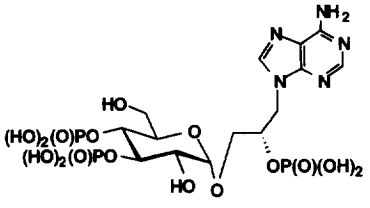
In this paper we present a convergent synthesis of adenophostin A analogs **4** and **5**, which both contain the 2',3",4"-trisphosphate function and the adenine moiety. The binding affinity and $Ca^{2+}$ mobilization of these analogs may be profoundly influenced by the enhanced conformational flexibility of the acyclic ribosyl and glucosyl unit in **4** and **5**, respectively.



1 R = H (adenophostin A)
2 R = Ac (adenophostin B)



3 ($IP_3$)



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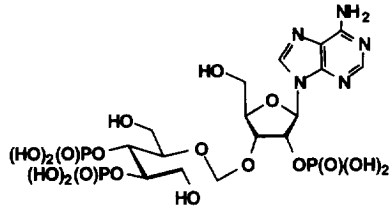

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**Figure 1**

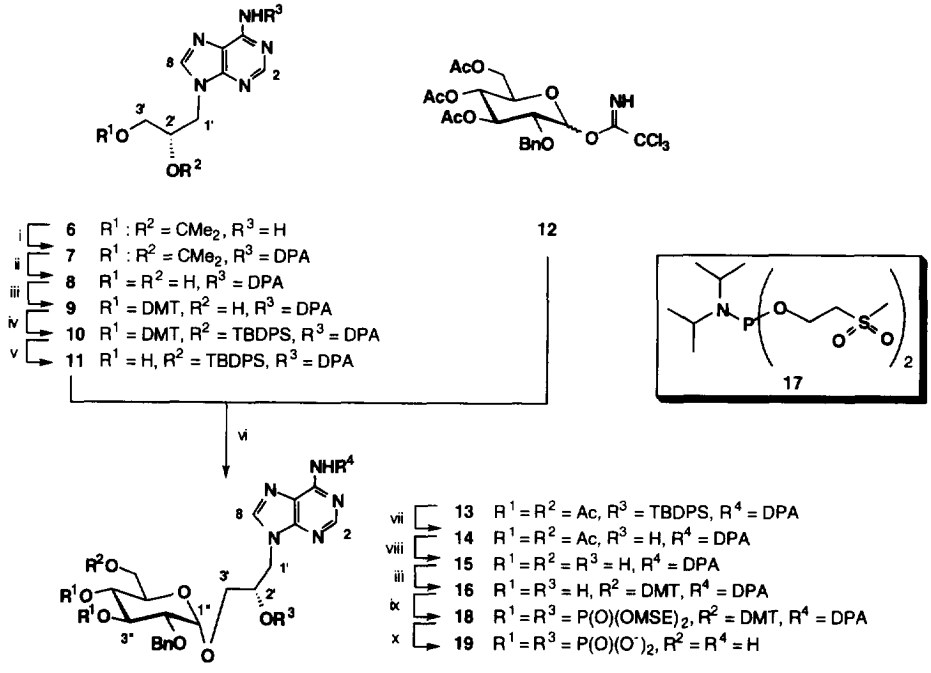
## Results and discussion

A key step in the convergent construction of the target adenophostin A analog (2*S*)-9-{1-(α-D-glucopyranosyloxy 3,4-bisphosphate)-2-monophosphate-prop-3-yl}-adenine (**4**) is the stereoselective glycosylation of a partially protected adenosyl acceptor with an appropriate glucosyl donor. In a similar fashion, 3'-*O*-(D-arabinityl-4"-*O*-methylene 2",3"-bisphosphate)-adenosine 2'-monophosphate (**5**) is accessible by condensation of a D-arabinitol acceptor with an appropriate 3'-*O*-methylene-containing adenosyl donor. Pioneering studies by Lichtenthaler *et al.*[4] showed that glycosylation of a partially protected nucleoside may be accompanied by the unwanted glycosylation of the nucleobase. In addition, preliminary studies[5] revealed that iodonium ion-mediated glycosylation of an adenosine derivative with an ethyl 1-thio-glucosyl donor led to iodination of the adenine moiety and Lewis acid-catalyzed depurination. Nevertheless, the successful introduction of a (3'→5') internucleosidic methylene acetal,[6] the preparation of 5-(β-D-glucopyranosyloxymethyl)-2'-deoxyuridine,[7] and the assembly of a shimofuridin analog[8] indicate that solely *O*-glycosylation of a nucleoside can be realized by judicious choice of a glycosyl donor and fine-tuning of the reaction conditions. Thus, it was expected that the synthesis of **4** is feasible by (see Scheme 1) (i) glycosylation of partially protected alkylated adenine **11** with glucosyl trichloroacetimidate **12** and (ii) introduction of the requisite 2',3",4"-phosphate monoesters. Similarly, methylene acetal analog **5** is accessible by (see Scheme 2) (i) reaction of 3'-phosphoryloxymethyladenosine **25** with D-arabinitol acceptor **21** followed by (ii) the installation of the 2',3",4"-phosphate monoesters.

The synthesis of adenophostin A analog **4** commences with the condensation of (*S*)-6-*N*-diphenylacetyl-9-(2-*tert*-butyldiphenylsilyloxy-1-hydroxyprop-3-yl)-adenine (**11**) with 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α/β-D-glucopyranosyl trichloroacetimidate (**12**). Suitably protected alkylated adeninyl acceptor **11** was readily

accessible from known[9] (*S*)-9-(1,2-isopropylidenedioxyprop-3-yl)-adenine (**6**). Thus, protection of the exocyclic amino function in **6** with a diphenylacetyl (DPA) group gave **7**. Deacetonation of **7** with aqueous acetic acid and regioselective protection of the primary hydroxyl in **8** with a 4,4'-dimethoxytrityl (DMT) group yielded adenine derivative **9**. Silylation of **9** with *tert*-butyldiphenylsilyl chloride (TBDPS-Cl) and pyrrole/trifluoroacetic acid-mediated removal of the DMT-group in **10** resulted in the isolation of acceptor **11** in a yield of 61% (based on **9**). Trichloroacetimidate donor **12** was prepared from 1,3,4,6-tetra-*O*-acetyl-2-*O*-benzyl-α/β–D-glucopyranose[10] by anomeric deacetylation and subsequent reaction with trichloroacetonitrile in the presence of potassium carbonate.

Having the requisite adeninyl acceptor **11** and glucopyranosyl donor **12** in hand, attention was focused on the purposive glycosylation. It was established that condensation of **11** with **12** under the controlled addition of excess trimethylsilyl trifluoromethanesulfonate (TMSOTf) proceeded smoothly to give exclusively α-linked (2*S*)-9-{1-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyloxy)-2-*tert*-butyldiphenylsilyloxy-prop-3-yl}-6-*N*-diphenylacetyl-adenine (**13**) in 80% yield, the anomeric configuration of which was firmly established by $^1$H NMR spectroscopy.



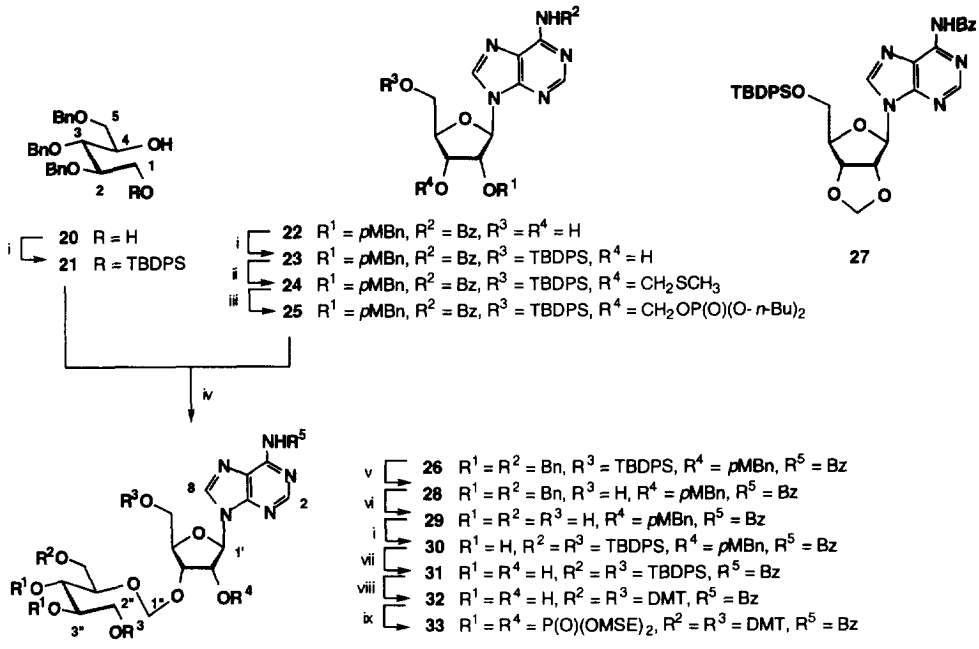

**Reagents and conditions:** (i) $(DPA)_2O$, pyr, 50 °C, 16 h, 82%; (ii) $AcOH/H_2O$, 4/1, v/v, 60 °C, 5 h; (iii) DMTCl, pyr, 2 h, **9**: 60% (based on **7**), **16**: 94%; (iv) TBDPSCl, imidazole, DMF, 60 °C, 16 h, 71%; (v) pyrrole, TFA, $CH_2Cl_2$, 0 °C, 10 min, 86%; (vi) TMSOTf, $CH_2Cl_2$, 10 min, 80% (α:β 1:0); (vii) TBAF (1 M in THF), pyr.HCl, THF, 50 °C, 2 h, 80%; (viii) KO*t*-Bu (1 M in MeOH)/1,4-dioxane, 2/1, v/v, 1 min, 84%; (ix) a. **17**, 1*H*-tetrazole, $CH_2Cl_2/CH_3CN$, 1/1, v/v, 15 min; b. *t*-BuOOH, 0 °C, 5 min; (x) 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.

**Scheme 1**

The next stage *en route* to adenophostin A analog **4** entails the introduction of the 2',3",4"-phosphate monoesters. To this end, dimer **13** was desilylated with fluoride ion followed by deacetylation of compound **14** to afford tetraol **15** in an overall yield of 67%. Protection of the 6"-OH with a DMT-group gave (2*S*)-9-{1-(2-*O*-benzyl-6-*O*-[4,4'-dimethoxytrityl]-α-D-glucopyranosyloxy)-2-hydroxyprop-3-yl}-6-*N*-diphenylacetyladenine (**16**). Phosphitylation of triol **16** with the reagent *N,N*-diisopropyl-bis-[2-(methylsulfonyl)ethyl] (MSE) phosphoramidite (**17**)[7] under the influence of 1*H*-tetrazole and subsequent *tert*-butyl hydroperoxide-mediated oxidation of the intermediate phosphite triesters to the corresponding phosphate triesters proceeded smoothly, as gauged by $^{31}$P NMR spectroscopy, to give the fully protected trisphosphate **18**. The protective groups were removed by the following sequence of reactions. Removal of the base-labile groups in **18** with NaOH, followed by aqueous acid-mediated removal of the DMT-group, and finally hydrogenolysis of **19** over Pd-black, led to the target analog **4**, the analytical data ($^1$H, $^{13}$C and $^{31}$P NMR spectroscopy, as well as ESI-mass spectrometry) of which were in complete accordance with the proposed structure.

The successful construction of **4** stimulated us to explore the assembly of adenophostin A analog **5**. The crucial step in the preparation of **5** comprises the installation of the methylene acetal in dimer **26** (Scheme 2) by condensation of 2,3,5-tri-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-D-arabinitol (**21**) and 6-*N*-benzoyl-3'-*O*-(di-*n*-butyloxy)phosphoryloxymethyl-2'-*O*-*p*-methoxybenzyl (*p*MBn)-5'-*O*-*tert*-butyldiphenylsilyladenosine (**25**) *via* the method developed by Quaedflieg *et al.*[6e] for the introduction of a dA(3'→5')dT internucleosidic methylene acetal. To this end, adenosine donor **25** was prepared from **22**[11] in three consecutive steps, as is vizualized in Scheme 2. Protection of the 5'-OH in **22** with a TBDPS-group and introduction of a methylthiomethyl function at the 3'-position by reaction of **23** with dimethyl sulfide and benzoyl peroxide (BPO) afforded **24**. Subsequent treatment of **24** with di-*n*-butylphosphate/*N*-iodosuccinimide (NIS) gave adenosyl donor **25** in 72%. Condensation of phosphoryloxymethyl donor **25** with arabinitol acceptor **21**, accessible by silylation of 2,3,5-tri-*O*-benzyl-D-arabinitol (**20**),[12] under the influence of TMSOTf led to the isolation of dimer **26** and the unwanted methylene acetal **27** in a ratio of 1:1. The formation of **27** may be explained by concomitant acid-mediated removal of the *p*-methoxybenzyl group.[13] Fortunately, dimer **26** could be isolated in an acceptable yield of 53% after TMSOTf-assisted condensation of **25** with excess **21**. Under these conditions, only trace amounts of the undesired derivative **27** were found.

Desilylation of **26** and hydrogenolysis of **28** over Pd-black gave 6-*N*-benzoyl-3'-*O*-(D-arabinityl-4"-*O*-methylene)-2'-*O*-*p*-methoxybenzyladenosine (**29**), indicating that the *p*MBn-group survives hydrogenation. The latter may be ascribed to stacking interactions of the *p*MBn-group with the adenine base.[3a,14] Transformation of **29** into 2',3",4"-triol **32** could be realized by the following four-step procedure: silylation of **29** with TBDPS-Cl and subsequent treatment of **30** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) gave **31**. Desilylation of **31** and regioselective protection of the three primary hydroxyls with DMT-groups afforded partially protected dimer **32**. The phosphate functions in 2',3",4"-triol **32** were installed following the same procedure as described earlier (*i.e.* **16** → **18**) using phosphitylating reagent **17**.[8] Removal of the base-labile Bz- and MSE-groups with NaOH and acidic deprotection of the primary hydroxyl functions yielded crude adenophostin A analog **5**. Purification and lyophilization gave homogeneous **5**, the identity of which was confirmed by $^1$H, $^{13}$C, and $^{31}$P NMR spectroscopy, as well as ESI-mass spectrometry.




**Reagents and conditions:** (i) TBDPSCl, pyr, 2 h, **21**: 99%, **23**: 88%, **30**: 72%; (ii) $(CH_3)_2S$, 2,6-lutidine, BPO, $CH_3CN$, 0 °C → rt, 1 h, 60%; (iii) $(n\text{-BuO})_2P(O)(OH)$, NIS, THF, 5 min, 72%; (iv) TMSOTf (0.5 eq + 3x 0.25 eq), 1,2-dichloroethane, 10 min, 53%; (v) TBAF (1 M in THF), THF, 50 °C, 2 h, 75%; (vi) Pd-black, $H_2$, *t*-BuOH/$H_2O$, 3/1, v/v, 48 h, quant.; (vii) DDQ, $CH_2Cl_2/H_2O$, 9/1, v/v, 16 h, 72%; (viii) a. TBAF (1 M in THF), 1,4-dioxane/THF, 1/1, v/v, 50 °C, 2 h; b. DMTCl, pyr, 6 h, 60% (2 steps); (ix) **17**, 1*H*-tetrazole, $CH_2Cl_2/CH_3CN$, 1/1, v/v, 15 min; b. *t*-BuOOH, 0 °C, 5 min.

**Scheme 2**

## Conclusion

In summary, the new 2',3",4"-trisphosphate monoesters-containing adenophostin A analogs **4** and **5** are readily accessible *via* a convergent approach. These compounds may contribute to a deeper insight in the structure-activity profile of the $IP_3$ receptor. The $Ca^{2+}$-mobilizing and receptor-binding properties of both analogs are currently under investigation and will be published in due course.

## 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, 2,6-lutidine, triethylamine and diethyl ether were refluxed for 2 h in the presence of $CaH_2$ (5 g $L^{-1}$), subsequently distilled and pyridine, 2,6-lutidine 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) was used as received. Eluents for column chromatography were of technical grade and distilled before use. All reactions were performed under anhydrous conditions at room temperature unless stated otherwise. Reactions were followed by TLC analysis conducted at Schleicher and Schüll DC Fertigfolien (F 1500 LS 254). The following eluents were used: diethyl ether (System A), methanol/dichloromethane, 2/98, v/v (System B), 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) at 20 °C unless stated otherwise. 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).

**(*S*)-6-*N*-Diphenylacetyl-9-(1,2-isopropylidenedioxyprop-3-yl)-adenine (7)** - To a solution of crystalline **6**[9] (10 g, 40 mmol) in pyridine (200 mL) was added diphenylacetic anhydride (49 g, 120 mmol) and the mixture was stirred at 50 °C. TLC analysis (System C) after 16 h showed almost complete conversion of starting material into a higher-running product. Methanol (50 mL) was added to destroy excess diphenylacetic anhydride and the reaction mixture was concentrated to a smaller volume. The residue was taken up in dichloromethane (200 mL), washed with aq. $NaHCO_3$ (10%, 100 mL) and water (100 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (eluent: dichloromethane/light petroleum, 1/1 to 1/0, v/v). Concentration of the appropriate fractions furnished **7** as a foam (15 g, 34 mmol, 82%); Rf 0.43; $^1$H NMR ($CDCl_3$): δ 9.22 (bs, 1H, NH), 8.71, 8.07 (2x s, 2H, H-2, H-8), 8.62, 8.60, 7.72-7.24 (m, 10H, H arom), 5.83 (s, 1H, H DPA), 4.48-4.42 (m, 2H, H-2', H-1a'), 4.28 (dd, 1H, H-1b', $J_{1b,2}$ 6.9 Hz, $J_{1a,1b}$ 14.7 Hz), 4.10 (dd, 1H, H-3a', $J_{2,3a}$ 6.4 Hz, $J_{3a,3b}$ 8.7 Hz), 3.67 (dd, 1H, H-3b', $J_{2,3b}$ 5.5 Hz), 1.36, 1.30 (2x s, 6H, 2x $CH_3$ isoprop); $^{13}$C{$^1$H} NMR ($CDCl_3$): δ 170.9 (C(O) DPA), 151.9, 143.8 (C-2, C-8), 151.6, 149.6 (C-4, C-6), 138.5 (2x Cq DPA), 129.2-127.2 (CH arom), 121.3 (C-5), 110.1 (Cq isoprop), 73.5 (C-2'), 66.3 (C-3'), 57.7 (CH DPA), 46.1 (C-1'), 26.7, 25.1 (2x $CH_3$ isoprop).

**(*S*)-9-(1,2-Dihydroxyprop-3-yl)-6-*N*-diphenylacetyladenine (8)** - Compound **7** (6.9 g, 16 mmol) was dissolved in a mixture of acetic acid and water (4/1, v/v, 75 mL) and stirred at 60 °C. TLC analysis (System C) after 5 h revealed almost complete disappearance of starting material into a more polar product and a small lower-running byproduct. The reaction mixture was diluted with toluene (50 mL) and concentrated. The residual oil was repeatedly diluted with toluene (5x 25 mL) and concentrated. Crude **8** was used without further purification; Rf 0.10; $^1$H NMR ($CDCl_3$/MeOD): δ 8.67, 8.12 (2x s, 2H, H-2, H-8), 7.45-7.23 (m, 10H, H arom), 5.49 (s, 1H, H DPA), 4.42 (dd, 1H, H-1a', $J_{1a,2}$ 0.8 Hz, $J_{1a,1b}$ 11.3 Hz), 4.27 (dd, 1H, H-1b', $J_{1b,2}$

6.7 Hz), 4.00-3.96 (m, 1H, H-2), 3.65-3.48 (2x dd, 2H, H-3a', H-3b', $J_{2,3a}$ 5.1 Hz, $J_{3a,3b}$ 13.1 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$/MeOD): δ 170.8 (C(O) DPA), 151.6, 144.9 (C-2, C-8), 151.6, 148.6 (C-4, C-6), 138.3 (2x Cq DPA), 128.5-126.7 (CH arom), 121.6 (C-5), 69.4 (C-2'), 63.2 (C-3'), 58.3 (CH DPA), 46.3 (C-1'); ESI-MS: $[M+Na]^+$ 426.

**(*S*)-9-(1-[4,4'-Dimethoxytrityloxy]-2-hydroxyprop-3-yl)-6-*N*-diphenylacetyladenine (9)** - To a solution of crude **8** (6.3 g, 16 mmol) in pyridine (75 mL) was added 4,4'-dimethoxytrityl chloride (6.9 g, 20 mmol). Stirring for 2 h indicated the reaction to be complete as gauged by TLC analysis (System C). Methanol (25 mL) was added and the reaction mixture was concentrated. Dilution of the residue with dichloromethane (75 mL), subsequent washing with aq. $NaHCO_3$ (10%, 25 mL) and water (25 mL), drying ($MgSO_4$), filtration and concentration gave crude **9**. Purification was achieved by silica gel column chromatography. Elution with dichloromethane/light petroleum (1/1 to 1/0, v/v) furnished pure **9** as a foam (6.6 g, 9.4 mmol, 60% over two steps); $[\alpha]_D$ -15.6° (*c* 1.0); Rf 0.37; $^1H$ NMR ($CDCl_3$): δ 9.48 (bs, 1H, NH), 8.98, 8.24 (2x s, 2H, H-2, H-8), 7.80-7.42 (m, 23H, H arom), 6.18 (s, 1H, H DPA), 4.80-4.42 (m, 3H, H-1a', H-1b', H-2), 4.45, 4.42 (2x s, 6H, 2x $OCH_3$), 3.46-3.42 (m, 2H, H-3a', H-3b'); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.7 (C(O) DPA), 158.1 (2x $COCH_3$), 151.7, 148.8 (C-2, C-8), 151.4, 148.6 (C-4, C-6), 144.2 (2x Cq DMT), 138.5 (2x Cq DPA), 135.3 (Cq DMT), 129.6-126.5 (CH arom), 112.8 (CH arom DMT), 121.2 (C-5), 86.0 (Cq DMT), 68.6 (C-2'), 64.6 (C-3'), 58.3 (CH DPA), 54.8 (2x $OCH_3$), 47.2 (C-1); ESI-MS: $[M+H]^+$: 706.

**(*S*)-9-(1-[4,4'-Dimethoxytrityloxy]-2-*tert*-butyldiphenylsilyloxyprop-3-yl)-6-*N*-diphenylacetyladenine (10)** - To a solution of **9** (5.9 g, 8.3 mmol) in DMF (50 mL) were subsequently added *tert*-butyldiphenylsilyl chloride (2.4 mL, 10.0 mmol) and imidazole (1.4 g, 21 mmol). The mixture was stirred at 60 °C for 16 h after which TLC analysis (System C) showed almost complete reaction. Excess *tert*-butyldiphenylsilyl chloride was destroyed by the addition of methanol (10 mL). The mixture was concentrated *in vacuo* and the residue was diluted with dichloromethane (75 mL). Successive washings with aq. $NaHCO_3$ (10%, 25 mL) and water (25 mL), drying of the combined organic layers ($MgSO_4$), filtration, and concentration yielded crude **10**. Purification by silica gel column chromatography (eluent: dichloromethane/light petroleum, 3/1 to 1/1, v/v) gave **10** as a white foam (5.6 g, 5.9 mmol, 71%); $[\alpha]_D$ +6.3° (*c* 1.0); Rf 0.80; $^1H$ NMR ($CDCl_3$): δ 8.86 (bs, 1H, NH), 8.56, 7.72 (2x s, 2H, H-2, H-8) 7.44-6.64 (m, 33H, H arom), 4.24 (m, 3H, H-1a', H-1b', H-2'), 3.78, 3.69 (2x s, 6H, 2x $OCH_3$), 3.19 (dd, 1H, H-3a', $J_{2,3a}$ 3.1 Hz, $J_{3a,3b}$ 12.3 Hz), 3.12 (dd, 1H, H-3b', $J_{2,3b}$ 0.8 Hz), 0.91 (s, 9H, $CH_3$ *t*-Bu); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 171.0 (C(O) DPA), 158.0 (2x $COCH_3$), 151.6, 143.7 (C-2, C-8), 151.4, 148.6 (C-4, C-6), 144.2 (2x Cq DMT), 138.7 (2x Cq DPA), 132.5, 131.8 (3x Cq Ph, DMT), 135.3-126.7 (CH arom), 112.7 (CH arom DMT), 121.3 (C-5), 86.1 (Cq DMT), 70.1 (C-2'), 64.4 (C-3'), 58.0 (CH DPA), 54.6 (2x $OCH_3$), 47.2 (C-1'), 26.4 ($CH_3$ *t*-Bu), 18.6 (Cq *t*-Bu); ESI-MS: $[M+H]^+$ 944.

**(*S*)-6-*N*-Diphenylacetyl-9-(2-*tert*-butyldiphenylsilyloxy-1-hydroxyprop-3-yl)-adenine (11)** - Fully protected adeninyl derivative **10** (5.6 g, 5.9 mmol) was dissolved in dichloromethane (25 mL) and cooled to 0 °C. Addition of pyrrole (2.5 mL, 17 mmol) and trifluoroacetic acid (0.88 mL, 11.4 mmol) followed by stirring for 10 min at ambient temperature led to complete removal of the 4,4'-dimethoxytrityl group as visualized by TLC analysis (System C). The reaction mixture was diluted with dichloromethane (25 mL) and

poured in ice cold aq. $NaHCO_3$ (10%, 25 mL). The layers were separated and the organic phase was washed with water (25 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was applied onto a column of silica gel. Elution was effected with dichloromethane/light petroleum (1/1, v/v). Subsequent concentration of the appropriate fractions furnished acceptor **11** as a white foam (3.3 g, 5.1 mmol, 86%); $[\alpha]_D$ +18.3° (*c* 1.0); Rf 0.33; $^1H$ NMR ($CDCl_3$): δ 9.24 (bs, 1H, NH), 8.63, 7.79 (2x s, 2H, H-2, H-8), 7.63-7.25 (m, 20H, H arom), 5.94 (bs, 1H, H DPA), 4.42 (dd, 1H, H-1a', $J_{1a,2}$ 2.8 Hz, $J_{1a,1b}$ 14.4 Hz), 4.18 (m, 1H, H-2'), 3.97 (dd, 1H, H-1b', $J_{1b,2}$ 4.3 Hz), 3.39 (m, 1H, H-3a'), 3.01 (m, 1H, H-3b'), 1.03 (s, 9H, $CH_3$ *t*-Bu); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 171.5 (C(O) DPA), 151.7, 144.4 (C-2, C-8), 151.7, 149.1 (C-4, C-6), 138.6 (2x Cq DPA), 132.5, 132.3 (2x Cq Ph), 135.3-127.0 (CH arom), 121.6 (C-5), 71.0 (C-2'), 61.8 (C-3'), 58.1 (CH DPA), 45.8 (C-1'), 26.7 ($CH_3$ *t*-Bu), 18.9 (Cq *t*-Bu); ESI-MS: $[M+H]^+$ 642; Anal. Calcd. for $C_{38}H_{39}N_5O_3Si$ (641.28): C, 71.11; H, 6.12; N, 10.91; Si, 4.38. Found: C, 71.14; H, 6.16; N, 10.89; Si, 4.36.

**3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α/β-D-glucopyranosyl trichloroacetimidate (12)** - To a cooled (0 °C) solution of known[11] 1,3,4,6-tetra-*O*-acetyl-2-*O*-benzyl-α/β–D–glucopyranose (4.6 g, 10.5 mmol) in DMF (88 mL) were subsequently added acetic acid (1.2 mL, 21 mmol) and hydrazine monohydrate (1.0 mL, 21 mmol). The reaction was stirred at ambient temperature for 30 min, after which TLC analysis (System A) revealed complete conversion of the starting compound into a lower-running product. The mixture was diluted with diethyl ether (50 mL) and poured into water (25 mL). The layers were separated and the organic phase was washed with aq. $NaHCO_3$ (10%, 25 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was applied onto a column of silica gel (eluent: ethyl acetate/light petroleum, 1/3 to 1/1, v/v) to give 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α/β–Δ–glucopyranose as an oil (3.5 g, 8.8 mmol, 84%); Rf 0.43; $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.5, 169.9, 169.6, 169.4 (C(O) Ac), 137.6, 137.1 (2x Cq Bn), 128.1-127.4 (CH arom), 96.9 (C-1β), 90.3 (C-1α), 76.6, 73.5, 71.4, 70.9, 68.2, 66.5 (C-2α, C-3α, C-4α, C-5α, C-2β, C-3β, C-4β, C-5β), 73.7, 72.3 (2x $CH_2$ Bn), 61.8 (C-6α/β), 20.4, 20.2 ($CH_3$ Ac). To a solution of 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α/β–D–glucopyranose (3.5 g, 8.8 mmol) in a mixture of dichloromethane/trichloroacetonitrile (15/1, v/v, 70 mL) was added $K_2CO_3$ (0.24 g, 1.8 mmol). The reaction was complete in 2 h as gauged by TLC analysis (System A). The mixture was filtered and concentrated *in vacuo* to afford crude imidate **12** which was used without further purification; Rf 0.56; $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 169.8, 169.1, 169.0 (C(O) Ac), 160.2 (C=NH), 137.1, 136.9 (2x Cq Bn), 127.9-127.1 (CH arom), 97.2 (C-1β), 92.8 (C-1α), 90.4 ($CCl_3$), 77.2, 75.4, 73.2, 71.8, 69.5, 67.7, 67.5 (C-2α, C-3α, C-4α, C-5α, C-2β, C-3β, C-4β, C-5β), 74.0, 72.3 (2x $CH_2$ Bn), 61.1 (C-6α/β), 20.1 ($CH_3$ Ac).

**(2*S*)-9-{1-(3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyloxy)-2-*tert*-butyldiphenylsilyloxyprop-3-yl}-6-*N*-diphenylacetyladenine (13)** - A mixture of trichlororacetimidate donor **12** (0.14 g, 0.25 mmol) and adeninyl acceptor **11** (0.15 g, 0.23 mmol) in dichloromethane (2 mL) was stirred for 30 min under a nitrogen atmosphere in the presence of activated molecular sieves (4Å). To this mixture TMSOTf (42 mg, 0.19 mmol) was added. TLC analysis (System C) after 5 min showed conversion of donor and acceptor in several intermediate products. Subsequent addition of an extra amount of TMSOTf (29 mg, 0.13 mmol) led to conversion of the intermediates into one main product as gauged by TLC. The reaction mixture was quenched with triethylamine (0.5 mL) and the mixture was diluted with dichloromethane (15 mL). Filtration and washing of the filtrate with aq. $NaHCO_3$ (10%, 10 mL), subsequent drying of the organic phase ($MgSO_4$),

filtration, and concentration *in vacuo* gave crude dimer **13**. Purification was accomplished by silica gel column chromatography (eluent: methanol/dichloromethane/light petroleum, 0/3/1 to 5/95/0, v/v/v). Further purification by Sephadex LH-20 gel-filtration (eluent: methanol/dichloromethane, 1/2, v/v) yielded α-linked **13** as the sole product (0.19 g, 0.18 mmol, 80%); Rf 0.50; $^1$H NMR ($CDCl_3$, 300 MHz, HH-COSY): δ 8.80 (bs, 1H, NH), 8.58, 7.84 (2x s, 2H, H-2, H-8), 7.52-7.22 (m, 25H, H arom), 5.95 (bs, 1H, H DPA), 5.35 (t, 1H, H-3", $J_{2,3}$, $J_{3,4}$ 9.6 Hz), 4.93 (d, 1H, H-1", $J_{1,2}$ 2.9 Hz), 4.92 (t, 1H, H-4", $J_{4,5}$ 9.9 Hz), 4.76-4.62 (AB, 2H, $CH_2$ Bn), 4.35 (bs, 3H, H-1a', H-1b', H-2'), 4.16 (dd, 1H, H-6a", $J_{5,6a}$ 4.2 Hz, $J_{6a,6b}$ 12.5 Hz), 3.82 (dd, 1H, H-6b", $J_{5,6b}$ 2.2 Hz), 3.66 (ddd, 1H, H-5"), 3.57 (dd, 1H, H-2"), 3.43 (dd, 1H, H-3a', $J_{2,3a}$ 3.4 Hz, $J_{3a,3b}$ 12.0 Hz), 3.32 (dd, 1H, H-3b', $J_{2,3b}$ 6.2 Hz), 2.00, 1.99, 1.98 (3x s, 9H, 3x $CH_3$ Ac), 0.99 (s, 9H, $CH_3$ *t*-Bu); $^{13}$C{$^1$H} NMR ($CDCl_3$, 75 MHz): δ 170.3, 170.0, 169.9, 169.6 (4x C(O) DPA, Ac), 151.9, 144.3 (C-2, C-8), 151.8, 148.7 (C-4, C-6), 138.7 (2x Cq DPA), 137.4 (Cq Bn), 132.4, 131.9 (2x Cq Ph), 135.5-127.1 (CH arom), 121.6 (C-5), 97.1 (C-1", $J_{C1",H1"}$ 171.8 Hz), 76.4, 71.7, 69.8, 68.2, 67.1 (C-2', C-2", C-3", C-4", C-5"), 72.8 ($CH_2$ Bn), 68.8 (C-3'), 61.6 (C-6"), 58.5 (CH DPA), 46.7 (C-1'), 26.7 ($CH_3$ *t*-Bu), 20.6, 20.5, 20.4 (3x $CH_3$ Ac), 18.9 (Cq *t*-Bu); ESI-MS: $[M+H]^+$ 1020; Anal. Calcd. for $C_{57}H_{61}N_5O_{11}Si$ (1019.41): C, 67.11; H, 6.03; N, 6.86; Si, 2.75. Found: C, 67.07; H, 6.06; N, 6.80; Si, 2.78.

**(2*S*)-9-{1-(3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyloxy)-2-hydroxyprop-3-yl}-6-*N*-diphenylacetyladenine (14)** - A mixture of dimer **13** (0.51 g, 0.50 mmol), pyridine.HCl (58 mg, 0.50 mmol), and TBAF (1 M in THF, 1.0 mL) in THF (2 mL) was stirred at 50 °C for 2 h, after which TLC analysis (System C) indicated the reaction to be complete. The mixture was diluted with ethyl acetate (15 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. Purification by silica gel column chromatography (eluent: dichloromethane) gave pure **14** as a white foam (0.30 g, 0.39 mmol, 80%); Rf 0.26; ESI-MS: $[M+H]^+$ 782; $^1$H NMR ($CDCl_3$): δ 9.22 (bs, 1H, NH), 8.67, 7.91 (2x s, 2H, H-2, H-8), 7.44-7.24 (m, 15H, H arom), 5.94 (bs, 1H, H DPA), 5.33 (t, 1H, H-3", $J_{2,3}$, $J_{3,4}$ 9.6 Hz), 4.93 (t, 1H, H-4", $J_{4,5}$ 9.8 Hz), 4.78 (d, 1H, H-1", $J_{1,2}$ 3.6 Hz), 4.70-4.53 (AB, 2H, $CH_2$ Bn), 4.42 (dd, 1H, H-1a', $J_{1a,2}$ 2.5 Hz, $J_{1a,1b}$ 11.9 Hz), 4.28 (dd, 1H, H-1b', $J_{1b,2}$ 5.0 Hz), 4.26-4.10 (m, 2H, H-2', H-6a"), 4.00 (dd, 1H, H-6b", $J_{5,6b}$ 1.9 Hz, $J_{6a,6b}$ 10.1 Hz), 3.92 (ddd, 1H, H-5"), 3.73 (dd, 1H, H-2"), 3.60 (dd, 1H, H-3a', $J_{2,3a}$ 2.9 Hz, $J_{3a,3b}$ 8.1 Hz), 3.44 (dd, 1H, H-3b', $J_{2,3b}$ 4.3 Hz), 2.04, 2.03, 1.97 (3x s, 9H, 3x $CH_3$ Ac); $^{13}$C{$^1$H} NMR ($CDCl_3$): δ 171.1, 170.2, 169.9, 169.4 (4x C(O) DPA, Ac), 151.6, 144.5 (C-2, C-8), 151.5, 148.7 (C-4, C-6), 138.5 (2x Cq DPA), 137.0 (Cq Bn), 128.7-126.9 (CH arom), 121.5 (C-5), 97.1 (C-1"), 76.5, 71.5, 68.2, 67.2 (C-2', C-2", C-3", C-4", C-5"), 72.9 ($CH_2$ Bn), 69.8 (C-3'), 61.6 (C-6"), 58.1 (CH DPA), 46.8 (C-1'), 20.5, 20.3, 20.2 (3x $CH_3$ Ac).

**(2*S*)-9-{1-(2-*O*-Benzyl-α-D-glucopyranosyloxy)-2-hydroxyprop-3-yl}-6-*N*-diphenylacetyladenine (15)** - To a stirred solution of dimer **14** (0.23 g, 0.30 mmol) in 1,4-dioxane (8 mL) was added potassium *tert*-butoxide (1 M in methanol, 16 mL). The mixture was stirred for 1 min, after which acetic acid (1.0 mL) was added. The reaction mixture was diluted with dichloromethane (10 mL), and poured in aq. $NaHCO_3$ (10%, 5 mL). The layers were separated and the organic phase was washed with water (5 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was applied onto a column of silica gel and elution was effected with methanol/dichloromethane (0/1 to 1/9, v/v). Concentration of the

appropriate fractions furnished tetraol **15** as a white foam (0.17 g, 0.25 mmol, 84%); Rf 0.05 (System D); $^1$H NMR ($CDCl_3$/MeOD): δ 8.62, 8.09 (2x s, 2H, H-2, H-8), 7.41-7.21 (m, 15H, H arom), 5.78 (bs, 1H, H DPA), 4.78-4.54 (AB, 2H $CH_2$ Bn), 4.61 (d, 1H, H-1", $J_{1,2}$ 3.1 Hz), 4.40-3.41 (m, 11H, H-1a', H-1b', H-2', H-3a', H-3b', H-2", H-3", H-4", H-5", H-6a", H-6b"); $^{13}$C{$^1$H} NMR ($CDCl_3$/MeOD): δ 170.3 (C(O) DPA), 151.6, 141.9 (C-2, C-8), 151.3, 148.7 (C-4, C-6), 138.2 (2x Cq DPA), 137.2 (Cq Bn), 128.5-126.9 (CH arom), 121.2 (C-5), 97.0 (C-1"), 79.1, 72.5, 71.7, 70.0, 68.0 (C-2', C-2", C-3", C-4", C-5"), 73.2 ($CH_2$ Bn), 69.2 (C-3'), 61.2 (C-6"), 58.3 (CH DPA), 46.5 (C-1'); ESI-MS: $[M+H]^+$ 656.

**(2*S*)-9-{1-(2-*O*-Benzyl-6-*O*-[4,4'-dimethoxytrityl]-α-D-glucopyranosyloxy)-2-hydroxyprop-3-yl}-6-*N*-diphenylacetyladenine (16)** - Regioselective introduction of the 4,4'-dimethoxytrityl groups in **15** (0.17 g, 0.25 mmol) was executed as described for compound **8**. Purification by silica gel column chromatography (eluent: dichloromethane/triethylamine, 98/2, v/v) afforded **16** as a yellowish foam (0.22 g, 0.23 mmol, 94%); Rf 0.49; $^1$H NMR ($CDCl_3$, 300 MHz, HH-COSY): δ 9.76 (bs, 1H, NH), 8.59, 7.99 (2x s, 2H, H-2, H-8), 7.41-6.74 (m, 28H, H arom), 6.11 (bs, 1H, H DPA), 4.65 (d, 1H, H-1", $J_{1,2}$ 3.6 Hz), 4.64-4.50 (AB, 2H, $CH_2$ Bn), 4.25 (dd, 1H, H-1a', $J_{1a,2}$ 3.2 Hz, $J_{1a,1b}$ 14.1 Hz), 4.17 (dd, 1H, H-1b', $J_{1b,2}$ 5.8 Hz), 4.06, 3.98 (2x bs, 2H, 2x OH), 3.82-3.46 (m, 2H, H-3a', H-4"), 3.39 (dd, 1H, H-3b', $J_{2,3b}$ 5.4 Hz, $J_{3a,3b}$ 11.5 Hz), 3.33 (dd, 1H, H-2", $J_{2,3}$ 9.7 Hz), 3.28 (dd, 1H, H-6a", $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 9.9 Hz), 3.22 (dd, 1H, H-6b", $J_{5,6b}$ 4.5 Hz); $^{13}$C{$^1$H} NMR ($CDCl_3$, 75 MHz): δ 172.3 (C(O) DPA), 158.2 (2x $COCH_3$), 151.8, 141.6 (C-2, C-8), 151.5, 148.8 (C-4, C-6), 144.6 (2x Cq DMT), 138.7 (2x Cq DPA), 137.4 (Cq Bn), 135.8 (2x Cq DMT), 129.9-126.6 (CH arom), 113.2 (CH arom DMT), 121.4 (C-5), 97.4 (C-1"), 85.9, (Cq DMT), 79.4, 73.0, 70.8, 70.7, 68.5 (C-2', C-2", C-3", C-4", C-5"), 73.7 ($CH_2$ Bn), 69.9 (C-3'), 63.1 (C-6"), 58.0 (CH DPA), 55.0 ($OCH_3$), 47.1 (C-1'); ESI-MS: $[M+H]^+$ 958.

**(2*S*)-9-{1-(α-D-Glucopyranosyl 3,4-bisphosphate)-2-monophosphate-prop-3-yl}-adenine (4)** - To a stirred mixture of triol **16** (0.22 g, 0.23 mmol) and phosphoramidite **17** (0.51 g, 1.4 mmol) in dichloromethane (4 mL) was added a solution of 1*H*-tetrazole (0.12 g, 1.7 mmol) in acetonitrile (4 mL). After stirring for 5 min $^{31}$P NMR ($CH_2Cl_2$) showed three major resonances at δ 141.1, 141.0 and 140.6. The mixture was cooled (0 °C) and *t*-BuOOH (2.0 mL) was added. Oxidation of the phosphite triesters to the corresponding phosphate triesters was complete in 15 min as gauged by $^{31}$P NMR ($CH_2Cl_2$: δ -2.3, -2.5, -2.6). The mixture was diluted with dichloromethane (15 mL) and washed with water (5 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure. Base-labile protective groups were removed by stirring the fully protected dimer in a mixture of 1,4-dioxane/methanol/NaOH (4 M) (14/5/1, v/v/v, 32 mL) for 16 h followed by neutralization (HOAc, 0.37 mL) and concentration. Removal of the 4,4'-dimethoxytrityl group was accomplished by stirring the crude material in acetic acid/water (4/1, v/v, 15 mL) for 1 h. Concentration of the product, subsequent evaporations with water (6x 10 mL), extraction with ethyl acetate (5 mL) and dichloromethane (5 mL), and concentration of the aqueous layer gave crude benzyl containing **19**. Finally, hydrogenation of purified **19** (obtained after HW-40 gel-filtration, eluent: TEAB (0.15 M) in a methanol/water mixture of 1/9, v/v) over Pd-black gave crude target adenophostin A analog **4**. Extensive purification was accomplished by gel-filtration (HW-40; eluent: TEAB (0.15 M) in a methanol/water mixture of 1/9, v/v) and ion-exchange chromatrography (Q-Sepharose; eluent: TEAB-gradient 0.05 M to 1.0 M). Ion-exchange with Dowex® 50Wx4 ($Na^+$-form) and lyophilization furnished homogeneous (2*S*)-9-{1-(α-D-

glucopyranosyl 3,4-bisphosphate)-2-monophosphate-prop-3-yl}-adenine (**4**) as a white fluffy solid (58 mg); $^{1}$H NMR ($D_2O$, 300 MHz, HH-COSY): δ 8.19 (s, 2H, H-2, H-8), 5.00 (d, 1H, H-1", $J_{1,2}$ 3.9 Hz), 4.63 (m, 1H, H-2'), 4.55 (d, 2H, H-1a', H-1b' $J_{1,2}$ 5.5 Hz), 4.45 (q, 1H, H-3", $J_{2,3}$, $J_{3,4}$, $^{3}J_{HP}$ 9.0 Hz), 3.99 (q, 1H, H-4", $J_{4,5}$, $^{3}J_{HP}$ 10.0 Hz), 3.86 (dd, 1H, H-6a", $J_{5,6a}$ 3.8 Hz, $J_{6a,6b}$ 13.1 Hz), 3.83 (dd, 1H, H-3a', $J_{2,3a}$ 3.4 Hz, $J_{3a,3b}$ 11.1 Hz), 3.72 (dd, 1H, H-2"), 3.68-3.65 (m, 2H, H-3b', H-6b"), 3.61 (ddd, 1H, H-5", $J_{5,6b}$ 1.8 Hz); $^{13}$C{$^{1}$H} NMR ($D_2O$, 75 MHz, CH-COSY): δ 149.7, 146.3 (C-2, C-8), 150.6, 144.7 (C-4, C-6), 118.8 (C-5), 99.3 (C-1"), 78.3 (C-3", $^{2}J_{CP}$ 2.9 Hz, $^{3}J_{CP}$ 6.0 Hz), 73.2 (C-4", $^{2}J_{CP}$ 5.9 Hz, $^{3}J_{CP}$ 8.6 Hz), 72.9 (C-2', $^{2}J_{CP}$ 5.3 Hz), 72.1 (C-2", $^{3}J_{CP}$ 3.3 Hz), 72.0 (C-5", $^{3}J_{CP}$ 7.5 Hz), 68.2 (C-3', $^{3}J_{CP}$ 2.9 Hz), 61.1 (C-6", $^{4}J_{CP}$ 4.7 Hz), 46.2 (C-1', $^{3}J_{CP}$ 3.6 Hz); $^{31}$P NMR ($D_2O$, 121 MHz, PH-COSY): δ 1.58 (C-4"-P), 1.50 (C-3"-P), 0.09 (C-2'-P); ESI-MS: $[M-H]^-$ 610; Anal. Calcd. for $C_{14}H_{24}N_5O_{16}P_3$ (611.29): C, 27.51; H, 3.96; N, 11.46; P, 15.20. Found: C, 7.46; H, 3.96; N, 11.49; P, 15.14.

**2,3,5-Tri-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-D-arabinitol (21)** - To a stirred solution of known[13] 2,3,5-tri-*O*-benzyl-D-arabinitol (**20**, 1.3 g, 3.0 mmol) in pyridine (15 mL) was added *tert*-butyldiphenylsilyl chloride (0.93 mL, 3.6 mmol) and a catalytic amount of 4-dimethylaminopyridine. TLC analysis (System A) after 2 h indicated the reaction to be complete. Excess *tert*-butyldiphenylsilyl chloride was destroyed with methanol (1 mL) and the mixture was concentrated *in vacuo*. The residue was diluted with diethyl ether (50 mL) and washed with aq. $NaHCO_3$ (10%, 25 mL) and water (25 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. Purification was accomplished by silica gel column chromatography (eluent: diethyl ether/light petroleum 1/3 to 1/0, v/v). Concentration of the appropriate fractions furnished pure **21** as a colorless oil (2.0 g, 3.0 mmol, 99%); Rf 0.71; $^{1}$H NMR ($CDCl_3$): δ 7.72-7.12 (m, 25H, H arom), 4.65-4.44 (AB, 2H, $CH_2$ Bn, 2x s, 4H, 2x $CH_2$ Bn), 4.10-3.62 (m, 7H, H-1a, H-1b, H-2, H-3, H-4, H-5a, H-5b), 1.05 (s, 9H, $CH_3$ *t*-Bu); $^{13}$C{$^{1}$H} NMR ($CDCl_3$): δ 138.5, 138.7 (3x Cq Bn), 133.5 (2x Cq Ph), 135.8-127.9 (CH arom), 79.6, 78.0, 70.5 (C-2, C-3, C-4), 74.1, 73.6, 73.5 (3x $CH_2$ Bn), 71.6 (C-5), 63.2 (C-1), 27.2 ($CH_3$ *t*-Bu), 19.4 (Cq *t*-Bu); ESI-MS: $[M+H]^+$ 661; Anal. Calcd. for $C_{42}H_{48}O_5Si$ (660.33): C, 76.33; H, 7.32; Si, 4.25. Found: C, 76.37; H, 7.34; Si, 4.28.

**6-*N*-Benzoyl-2'-*O*-*p*-methoxybenzyl-5'-*O*-*tert*-butyldiphenylsilyladenosine (23)** - Known[11] 6-*N*-benzoyl-2'-*O*-*p*-methoxybenzyladenosine (**22**, 5.6 g, 11.5 mmol) was silylated as described above for the preparation of **21**. Work-up in dichloromethane instead of diethyl ether and purification by silica gel column chromatography (eluent: methanol/dichloromethane/light petroleum 0/1/1 to 1/9/0, v/v/v) yielded pure **23** as a white foam (7.3 g, 10.1 mmol, 88%); Rf 0.45 (System C); $^{1}$H NMR ($CDCl_3$): δ 9.31 (bs, 1H, NH), 8.78, 8.18 (2x s, 2H, H-2, H-8), 8.07-7.27 (m, 15H, H arom Bz, Ph), 7.11, 7.07, 6.74, 6.69 (2x d, 4H, H arom *p*MBn), 6.21 (d, 1H, H-1', $J_{1,2}$ 4.6 Hz), 4.64 (s, 2H, $CH_2$ *p*MBn), 4.55 (t, 1H, H-2', $J_{2,3}$ 4.6 Hz), 4.52 (dd, 1H, H-3', $J_{3,4}$ 9.1 Hz), 4.21 (q, 1H, H-4', $J_{4,5a}$, $J_{4,5b}$ 3.3 Hz), 4.19 (dd, 1H, H-5a', $J_{5a,5b}$ 11.5 Hz), 3.89 (dd, 1H, H-5b), 3.74 (s, 3H, $OCH_3$), 2.76 (d, 1H, OH), 1.08 (s, 9H, $CH_3$ *t*-Bu); $^{13}$C{$^{1}$H} NMR ($CDCl_3$): δ 165.0 (C(O) Bz), 159.0 ($COCH_3$), 152.1, 148.9 (C-2, C-8), 150.6, 149.3 (C-4, C-6), 133.4, 132.3 (2x Cq Ph), 135.9-127.4 (CH arom), 122.3 (C-5), 113.2 (CH arom *p*MBn), 86.5 (C-1'), 84.9, 80.1, 68.8 (C-2', C-3', C-4'), 72.0 ($CH_2$ *p*MBn), 62.8 (C-5'), 54.7 ($OCH_3$), 26.5 ($CH_3$ *t*-Bu), 18.8 (Cq *t*-Bu).

**6-*N*-Benzoyl-2'-*O*-*p*-methoxybenzyl-3'-*O*-methylthiomethyl-5'-*O*-*tert*-butyldiphenylsilyl-adenosine (24)** - To a cooled (0 °C) solution of adenosyl derivative **23** (1.0 g, 1.3 mmol), dimethyl sulfide (0.97 mL, 13 mmol) and 2,6-lutidine (0.16 mL, 1.4 mmol) in acetonitrile (13 mL) was added benzoyl peroxide (1.3 g, 5.4 mmol) over a period of 30 min. After stirring for 30 min at room temperature, TLC analysis (System C) revealed conversion of starting material into a higher-running product. The reaction mixture was concentrated and diluted with dichloromethane (25 mL). Subsequent washings with water (10 mL) and aq. $NaHCO_3$ (10%, 10 mL), drying of the organic phase ($MgSO_4$), filtration, and concentration gave crude methylthiomethyl-containing adenosine **24**. Purification was accomplished by silica gel column chromatography. Elution with dichloromethane/light petroleum ether (3/1 to 1/0, v/v) afforded pure **24** (0.62 g, 0.78 mmol, 60%); Rf 0.52; $^1H$ NMR ($CDCl_3$/MeOD): δ 8.74, 8.21 (2x s, 2H, H-2, H-8), 7.89-6.67 (m, 19H, H arom), 6.22 (d, 1H, H-1', $J_{1,2}$ 4.1 Hz), 4.78-4.48 (2x AB, 4H, $CH_2$ *p*MBn, $OCH_2S$, m, 1H, H-2'), 4.41 (t, 1H, H-3', $J_{2,3}$, $J_{3,4}$ 3.8 Hz), 4.32 (m, 1H, H-4'), 4.24-4.01 (m, 2H, H-5a', H-5b'), 3.72 (s, 3H, $OCH_3$), 2.19 (s, 3H, $SCH_3$), 1.15 (s, 9H, $CH_3$ *t*-Bu); $^{13}C\{^1H\}$ NMR ($CDCl_3$/MeOD): δ 164.8 (C(O) Bz), 159.1 ($COCH_3$), 152.0, 141.5 (C-2, C-8), 151.0, 149.3 (C-4, C-6), 133.5, 132.9 (2x Cq Ph), 135.3-127.6 (CH arom), 123.3 (C-5), 113.4 (CH arom *p*MBn), 87.0 (C-1'), 83.3, 78.1, 72.5 (C-2', C-3', C-4'), 74.3 ($OCH_2S$), 71.8 ($CH_2$ *p*MBn), 62.8 (C-5'), 54.9 ($OCH_3$), 26.7 ($CH_3$ *t*-Bu), 18.9 (Cq *t*-Bu), 13.7 ($SCH_3$).

**6-*N*-Benzoyl-3'-*O*-(di-*n*-butyloxy)phosphoryloxymethyl-2'-*O*-*p*-methoxybenzyl-5'-*O*-*tert*-butyldiphenylsilyladenosine (25)** - A mixture of NIS (95 mg, 0.42 mmol) and di-*n*-butylphosphate (89 µL, 0.42 mmol) in THF (1 mL) was added to a stirred mixture of methylthiomethyl donor **24** (0.27 g, 0.35 mmol) and powdered molecular sieves (4Å) in 1,2-dichloroethane (1 mL). After stirring for 5 min, TLC analysis (System C) showed conversion of starting material into a lower-running product. The mixture was diluted with dichloromethane (10 mL) and the molecular sieves were removed by filtration. The filtrate was extracted with aq. $Na_2S_2O_3$ (10%, 5 mL) and aq. $NaHCO_3$ (10%, 5 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated under reduced pressure (maximum temperature of the water bath did not exceed 20 °C). The crude product was purified by silica gel column chromatography. Elution with methanol/dichloromethane (0/1 to 5/95, v/v) and concentration of the appropriate fractions gave **25** as an oil (0.24 g, 0.25 mmol, 72%); Rf 0.39; $^1H$ NMR ($CDCl_3$): δ 9.01 (bs, 1H, NH), 8.68, 8.04 (2x s, 2H, H-2, H-8), 7.68-6.62 (m, 19H, H arom), 6.14 (d, 1H, H-1', $J_{1,2}$ 6.2 Hz), 5.42-5.27 (dAB, 2H, $OCH_2O$, $^3J_{Ha,P}$ 10.0 Hz, $^3J_{Hb,P}$ 12.8 Hz), 4.77 (t, 1H, H-2', $J_{2,3}$ 6.2 Hz), 4.65-4.34 (AB, 2H, $CH_2$ *p*MBn, m, 2H, H-3', H-4'), 4.14-3.98 (q, 4H, $CH_2\alpha$ *n*-Bu, $^3J_{HP}$ 7.8 Hz, dd, 1H, H-5a', $J_{4,5a}$ 1.3 Hz, $J_{5a,5b}$ 11.0 Hz), 3.83 (dd, 1H, H-5b', $J_{4,5b}$ 3.5 Hz), 3.71 (s, 3H, $OCH_3$), 1.61 (m, 4H, $CH_2\beta$ *n*-Bu), 1.38 (m, 4H, $CH_2\gamma$ *n*-Bu), 1.08 (s, 9H, $CH_3$ *t*-Bu), 0.90 (t, 6H, $CH_3$ *n*-Bu); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 164.5 (C(O) Bz), 159.1 ($COCH_3$), 152.2, 141.1 (C-2, C-8), 149.3 (C-4, C-6), 133.5, 132.3 (2x Cq Ph), 135.3-127.6 (CH arom), 123.2 (C-5), 113.4 (CH arom *p*MBn), 91.6 ($OCH_2O$), 86.4 (C-1'), 83.7, 78.8, 75.5 (C-2', C-3', C-4'), 72.3 ($CH_2$ *p*MBn), 67.5 ($CH_2\alpha$ *n*-Bu, $^2J_{CP}$ 5.9 Hz), 63.0 (C-5'), 54.9 ($OCH_3$), 32.0 ($CH_2\beta$ *n*-Bu, $^3J_{CP}$ 5.9 Hz), 26.7 ($CH_3$ *t*-Bu), 19.0 (Cq *t*-Bu), 18.4 ($CH_2\gamma$ *n*-Bu), 13.3 ($CH_3$ *n*-Bu).

**6-*N*-Benzoyl-3'-*O*-(2",3",5"-tri-*O*-benzyl-1"-*O*-*tert*-butyldiphenylsilyl-D-arabinityl-4"-*O*-methylene)-2'-*O*-*p*-methoxybenzyl-5'-*O*-*tert*-butyldiphenylsilyladenosine (26)** - A mixture of phosphoryloxymethyl donor **25** (0.31 g, 0.32 mmol) and 2-arabinitol acceptor **21** (0.85 g, 1.3 mmol) in 1,2-

dichloroethane (1 mL) was stirred in the presence of activated molecular sieves (4Å) under a continuous stream of dry nitrogen. TMSOTf (33 mg, 0.15 mmol and 3 subsequent additions of 17 mg at 5 min intervals) was added. TLC analysis (System B) after stirring for 10 min showed almost complete consumption of donor. The reaction mixture was quenched with triethylamine (0.5 mL), diluted with dichloromethane (10 mL) and the molecular sieves were removed by filtration. The filtrate was washed with aq. $NaHCO_3$ (10%, 5 mL) and water (5 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. The residue was applied onto a column of silica gel. Elution was effected with methanol/dichloromethane/light petroleum (0/1/3 to 5/95/0, v/v/v). Concentration of the appropriate fractions and further purification by gel-filtration (LH-20, eluent: dichloromethane/methanol, 1/2, v/v) furnished dimer **26** (0.23 g, 0.17 mmol, 53%); Rf 0.21; $^1$H NMR ($CDCl_3$, 300 MHz, HH-COSY): δ 8.98 (bs, 1H, NH), 8.70, 7.96 (2x s, 2H, H-2, H-8), 8.04-6.58 (m, 44H, H arom), 6.13 (d, 1H, H-1', $J_{1,2}$ 6.7 Hz), 4.86-4.74 (AB, 2H, H-1a", H-1b"), 4.68 (dd, 1H, H-2', $J_{2,3}$ 4.5 Hz),4.63-4.24 (3x AB, 6H, 3x $CH_2$ (*p*M)Bn, s, 2H, $CH_2$ (*p*M)Bn, m, 2H, H-3', H-4'), 4.05 (m, 1H, H-5"), 3.96 (dd, 1H, H-4", $J_{3,4}$ 5.1 Hz, $J_{4,5}$ 3.3 Hz), 3.85-3.81 (dd, 1H, H-5a', $J_{4,5a}$ 4.1 Hz, $J_{5a,5b}$ 11.5 Hz, m, 3H, H-2a", H-2b", H-6a"), 3.76-3.71 (m, 1H, H-3", dd, 1H, H-5b', $J_{4,5b}$ 2.1 Hz), 3.67 (s, 3H, $OCH_3$), 3.66 (m, 1H, H-6b"), 1.05 (s, 18H, 2x $CH_3$ *t*-Bu); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 164.4 (C(O) Bz), 159.3 ($COCH_3$), 152.6, 141.6 (C-2, C-8), 151.5, 149.2 (C-4, C-6), 138.6, 138.4, 138.1 (3x Cq Bn), 133.9, 133.3, 133.2, 132.8, 132.7 (4x Cq Ph, Bz), 135.6-127.5 (CH arom), 123.2 (C-5), 113.6 (CH arom *p*MBn), 95.4 (C-1"), 86.6 (C-1'), 84.5, 79.8, 79.7, 78.2, 77.8, 75.2 (C-2', C-3', C-4', C-3", C-4", C-5"), 74.4, 73.1, 72.4 (4x $CH_2$ (*p*M)Bn), 70.1 (C-6"), 63.8, 63.4 (C-5', C-2"), 55.2 ($OCH_3$), 27.0, 26.9 (2x $CH_3$ *t*-Bu), 19.2, 19.1 (2x Cq *t*-Bu); ESI-MS: $[M+H]^+$ 1403; Anal. Calcd. for $C_{84}H_{91}N_5O_{11}Si_2$ (1401.63): C, 71.92; H, 6.54; N, 4.99; Si, 4.00. Found: C, 71.99; H, 6.57; N, 4.96; Si, 4.03.

**6-*N*-Benzoyl-3'-*O*-(2",3",5"-tri-*O*-benzyl-D-arabinityl-4"-*O*-methylene)-2'-*O*-*p*-methoxybenzyladenosine (28)** - Dimer **26** (0.67 g, 0.48 mmol) was desilylated as described for the desilylation of compound **13**. TLC analysis after stirring for 2 h at 50 °C (System C) indicated the reaction to be complete. The mixture was concentrated, diluted with dichloromethane (25 mL) and washed with aq. $NaHCO_3$ (10%, 10 mL) and water (10 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by silica gel column chromatography (eluent: methanol/dichloromethane, 0/1 to 5/95, v/v) to give pure **28** as a white foam (0.33 g, 0.36 mmol, 75%); Rf 0.32; $^1$H NMR ($CDCl_3$, 300 MHz, HH-COSY): δ 9.40 (bs, 1H, NH), 8.61, 7.80 (2x s, 2H, H-2, H-8), 8.07, 8.05, 7.62-7.34 (m, 5H, H arom Bz), 7.32-7.23 (m, 15H, H arom Bn), 6.81, 6.78, 6.43, 6.40 (2x d, 4H, H arom *p*MBn), 5.97 (bd, 1H, OH), 5.74 (d, 1H, H-1', $J_{1,2}$ 8.0 Hz), 5.01-4.85 (AB, 2H, H-1a", H-1b"), 4.81 (dd, 1H, H-2', $J_{2,3}$ 4.7 Hz), 4.74-4.07 (2x AB, 4H, 2x $CH_2$ (*p*M)Bn, 2x s, 4H, 2x $CH_2$ (*p*M)Bn), 4.53 (m, 1H, H-3'), 4.35 (m, 1H, H-4'), 4.14 (m, 1H, H-5"), 3.91 (t, 1H, H-4", $J_{3,4}$, $J_{4,5}$ 4.4 Hz), 3.83 (dd, 1H, H-6a", $J_{5,6a}$ 3.6 Hz, $J_{6a,6b}$ 10.4 Hz), 3.81-3.61 (m, 5H, H-5a', H-2a", H-2b", H-3", H-6b"), 3.59 (s, 3H, $OCH_3$), 3.57 (m, 1H, H-5b'); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 164.4 (C(O) Bz), 158.7 ($COCH_3$), 151.1, 142.3 (C-2, C-8), 149.7, 149.3 (C-4, C-6), 138.0, 137.9, 137.5 (3x Cq Bn), 133.4 (Cq Bz), 132.3-127.4 (CH arom), 123.5 (C-5), 112.7 (CH arom *p*MBn), 94.7 (C-1"), 88.7 (C-1'), 86.5, 79.4, 78.8, 77.5, 77.2, 74.8 (C-2', C-3', C-4', C-3", C-4", C-5"), 73.9, 73.0, 72.4, 72.0 (4x $CH_2$ (*p*M)Bn), 69.8 (C-6"), 62.5, 61.0 (C-5', C-2"), 54.7 ($OCH_3$); ESI-MS: $[M+H]^+$ 926.

**6-*N*-Benzoyl-3'-*O*-(D-arabinityl-4"-*O*-methylene)-2'-*O*-*p*-methoxybenzyladenosine (29)** - A mixture of dimer **28** (0.16 g, 0.18 mmol) and Pd-black (spatula) in *t*-butanol/water (3/1, v/v, 10 mL) was degassed and stirred under a hydrogen atmosphere for 48 h. The mixture was concentrated under reduced pressure. Crude **29** was used without further purification; Rf 0.05 (System C); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 164.0 (C(O) Bz), 159.6 ($COCH_3$), 151.8, 144.7 (C-2, C-8), 150.8, 150.3 (C-4, C-6), 134.1 (Cq Bz), 129.4 (Cq *p*MBn), 134.2-129.0 (CH arom), 124.2 (C-5), 113.8 (CH arom *p*MBn), 95.5 (C-1"), 88.9 (C-1'), 86.9, 79.7, 78.4, 75.5, 71.4, 70.3 (C-2', C-3', C-4', C-3", C-4", C-5"), 73.1 ($CH_2$ *p*MBn), 64.1, 63.2, 61.8 (C-5', C-2", C-5"), 55.7 ($OCH_3$).

**6-*N*-Benzoyl-2'-*O*-*p*-methoxybenzyl-3'-*O*-(1",5"-di-*O*-*tert*-butyldiphenylsilyl-D-arabinityl-4"-*O*-methylene)-5'-*O*-*tert*-butyldiphenylsilyladenosine (30)** - Silylation of all primary hydroxyl groups in 6-*N*-benzoyl-3'-*O*-(D-arabinitol-4-*O*-methylene)-2'-*O*-*p*-methoxybenzyl-adenosine (**29**, 0.18 mmol) was accomplished as described for the preparation of compound **21**. Work-up in dichloromethane instead of diethyl ether and purification by silica gel column chromatography (eluent: dichloromethane/light petroleum, 1/1 to 1/0, v/v) resulted in the isolation of diol **30** (0.17 g, 0.13 mmol, 72% over two steps); Rf 0.62 (System C); $^1H$ NMR ($CDCl_3$): δ 9.00 (bs, 1H, NH), 8.63, 7.91 (2x s, 2H, H-2, H-8), 7.69-7.18 (m, 35H, H arom), 6.87, 6.83, 6.54, 6.50 (2x d, 4H, H arom *p*MBn), 6.00 (d, 1H, H-1', $J_{1,2}$ 6.4 Hz), 4.94-4.89 (AB, 2H, H-1a", H-1b"), 4.90 (dd, 1H, H-2', $J_{2,3}$ 3.6 Hz), 4.88 (m, 1H, H-3'), 4.57-4.42 (AB, 2H, $CH_2$ *p*MBn), 4.44 (m, 1H, H-4'), 4.22-3.72 (m, 9H, H-5a', H-5b', H-2a", H-2b", H-3", H-4", H-5", H-6a", H-6b"), 3.65 (s, 3H, $OCH_3$), 3.27 (bd, 1H, OH), 2.95 (bd, 1H, OH), 1.05 (3x s, 27H, 3x $CH_3$ *t*-Bu); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 164.1 (C(O) Bz), 159.1 ($COCH_3$), 152.2, 141.8 (C-2, C-8), 151.0, 149.3 (C-4, C-6), 133.7-132.6 (6x Cq Ph, Bz), 128.5 (Cq *p*MBn), 135.4-127.7 (CH arom), 123.1 (C-5), 113.3 (CH arom *p*MBn), 94.8 (C-1"), 86.8 (C-1'), 83.7, 79.3, 78.7, 74.8, 70.6, 69.5 (C-2', C-3', C-4', C-3", C-4", C-5"), 72.5 ($CH_2$ *p*MBn), 66.9, 63.8, 63.1 (C-5', C-2", C-6"), 55.0 ($OCH_3$), 26.8, 26.7 ($CH_3$ *t*-Bu), 19.1 (Cq *t*-Bu); ESI-MS: $[M+H]^+$ 1370.

**6-*N*-Benzoyl-3'-*O*-(1",5"-di-*O*-*tert*-butyldiphenylsilyl-D-arabinityl-4"-*O*-methylene)-5'-*O*-*tert*-butyldiphenylsilyladenosine (31)** - To a solution of *p*-methoxybenzyl containing **30** (0.14 g, 0.10 mmol) in a mixture of dichloromethane/water (9/1 v/v, 4 mL) was added DDQ (50 mg, 0.22 mmol). The mixture was vigorously stirred for 16 h after which TLC analysis (System C) revealed almost complete conversion of starting material. The mixture was diluted with dichloromethane (15 mL) and poured into water (5 mL). The layers were separated and the organic phase was washed with aq. $NaHCO_3$ (10%, 5 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was applied onto a silica gel column (eluent: methanol/dichloromethane/light petroleum, 0/1/1 to 5/95/0, v/v/v). Concentration of the appropriate fractions gave pure **31** as a foam (90 mg, 72 μmol, 72%); Rf 0.44; $^1H$ NMR ($CDCl_3$, 300 MHz, HH-COSY): δ 9.10 (bs, 1H, NH), 8.70, 8.20 (2x s, 2H, H-2, H-8), 8.03-7.16 (m, 35H, H arom), 6.05 (d, 1H, H-1', $J_{1,2}$ 5.4 Hz), 4.98-4.81 (AB, 2H, H-1a", H-1b"), 4.86 (m, 1H, H-2'), 4.51 (t, 1H, H-3', $J_{2,3}$, $J_{3,4}$ 4.5 Hz), 4.24 (dd, 1H, H-4', $J_{4,5a}$ 3.8 Hz, $J_{4,5b}$ 7.0 Hz), 3.99-3.72 (m, 7H, H-2a", H-2b", H-3", H-4", H-5", H-6a", H-6b"), 3.92 (dd, 1H, H-5a', $J_{5a,5b}$ 11.5 Hz), 3.74 (dd, 1H, H-5b'), 3.58 (bs, 1H, OH), 3.32 (bs, 1H, OH), 1.91 (bs, 1H, OH), 1.05, 1.03, 1.02 (3x s, 27H, 3x $CH_3$ *t*-Bu); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 164.6 (C(O) Bz), 152.4, 141.6 (C-2, C-8), 151.3, 149.5 (C-4, C-6), 133.6, 132.8 (Cq Ph, Bz), 135.5-127.7 (CH arom), 123.2

(C-5), 94.8 (C-1"), 89.1 (C-1'), 83.7, 78.9, 77.8, 73.9, 70.7, 69.4 (C-2', C-3', C-4', C-3", C-4", C-5"), 67.9, 63.5, 63.1 (C-5', C-2", C-6"), 26.7 ($CH_3$ *t*-Bu), 19.1 (Cq *t*-Bu); ESI-MS: $[M+H]^+$ 1250.

**6-*N*-Benzoyl-3'-*O*-(1",5"-di-*O*-[4,4'dimethoxytrityl]-D-arabinityl-4"-*O*-methylene)-5'-[4,4'-dimethoxytrityl]-adenosine (32)** - Dimer **31** (90 mg, 72 µmol) was desilylated with TBAF (1 M in THF, 0.32 mL) in a mixture of 1,4-dioxane/THF (1/1, v/v, 2 mL) at 50 °C for 1 h. The mixture was diluted with pyridine (5 mL) and concentrated to a smaller volume. Repeated dilutions with pyridine (5x 5 mL) and subsequent concentrations gave crude desilylated dimer, which was taken up in pyridine (2 mL). 4,4'-Dimethoxytrityl chloride (95 mg, 0.28 mmol) was added and the mixture was stirred for 6 h. Methanol (1 mL) was added to destroy excess 4,4'-dimethoxytrityl chloride. The mixture was concentrated and diluted with dichloromethane (10 mL). Washings with aq. $NaHCO_3$ (10%, 5 mL) and water (5 mL), subsequent drying of the organic layer ($MgSO_4$), filtration, and concentration gave crude **32**. Purification was accomplished by silica gel column chromatography. Elution with methanol/dichloromethane/light petroleum (0/1/3 to 2/98/0 containing 2% triethylamine) furnished triol **32** as a yellowish foam (70 mg, 50 µmol, 60% over two steps); Rf 0.30 (System C); $^1H$ NMR ($CDCl_3$, 300 MHz, HH-COSY): δ 9.08 (bs, 1H, NH), 8.68, 8.20 (2x s, 2H, H-2, H-8), 8.04-6.74 (m, 44H, H arom), 6.06 (d, 1H, H-1', $J_{1,2}$ 5.2 Hz), 4.99-4.83 (AB, 2H, H-1a", H-1b", m, 1H, H-2'), 4.49 (t, 1H, H-3', $J_{2,3}$, $J_{3,4}$ 4.5 Hz), 4.31 (dd, 1H, H-4', $J_{4,5a}$ 3.8 Hz, $J_{4,5b}$ 5.7 Hz), 3.99-3.80 (m, 3H, H-3", H-4", H-5"), 3.75, 3.73, 3.71 (3x s, 18H, 6x $OCH_3$), 3.45 (dd, 1H, H-5a', $J_{5a,5b}$ 10.5 Hz), 3.34-3.27 (m, 4H, H-5b', H-2a", H-2b", H-6a"), 3.19 (dd, 1H, H-6b", $J_{5,6b}$ 5.0 Hz, $J_{6a,6b}$ 9.4 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 164.3 (C(O) Bz), 158.4 ($COCH_3$), 151.8, 141.8 (C-2, C-8), 151.4, 149.5 (C-4, C-6), 144.5, 144.3 (Cq DMT), 135.6, 135.5 (Cq DMT), 133.6 (Cq Bz), 132.7-126.8 (CH arom), 123.3 (C-5), 113.1 (CH arom DMT), 94.8 (C-1"), 89.2 (C-1'), 86.5, 86.4 (3x Cq DMT), 82.8, 78.4, 77.9, 73.9, 71.2, 68.8 (C-2', C-3', C-4', C-3", C-4", C-5"), 65.4, 63.1, 62.9 (C-5', C-2", C-6"), 55.1 ($OCH_3$); ESI-MS: $[M+H]^+$ 1444.

**3'-*O*-(D-Arabinityl-4"-*O*-methylene 2",3"-bisphosphate)-adenosine 2'-monophosphate (5)** - Phosphitylation of triol **32** (70 mg, 50 µmol) with phosphoramidite **17** (0.11 g, 0.3 mmol) was accomplished as described previously for the phosphitylation of **16**. $^{31}P$ NMR ($CH_2Cl_2$) showed three major resonances at δ 142.0, 141.4 and 140.0. $^{31}P$ NMR ($CH_2Cl_2$) after oxidation of the intermediate phosphite triesters to the corresponding phosphate triesters showed three major resonances at δ -1.3, -1.9, and -2.0. Removal of all base-labile protective groups and acid-labile groups as described before gave crude **5**. Purification by gel-filtration (HW-40, eluent: 0.15 M TEAB in water/methanol, 9/1, v/v) and concentration furnished trisphosphate **5** as its triethylammonium salt. Further purification was accomplished by Q-Sepharose ion-exchange chromatography in a TEAB-gradient (0.05 M to 0.5 M). Ion-exchange (Dowex® Wx50, $Na^+$-form) and lyophilization yielded 3'-*O*-(D-arabinitol-4-*O*-methylene 2,3-bisphosphate)-adenosine 2'-monophosphate (**5**) as a white fluffy solid (16 mg, 30 µmol, 60%); $^1H$ NMR ($D_2O$, 300 MHz, HH-COSY): δ 8.42, 8.30 (2x s, 2H, H-2, H-8), 6.22 (dd, 1H, H-1', $J_{1,2}$ 6.5 Hz), 5.32 (ddd, 1H, H-2', $J_{2,3}$ 5.3 Hz, $^3J_{HP}$ 11.7 Hz), 5.23-5.20 (AB, 2H, H-1a", H-1b"), 4.74 (dd, 1H, H-3', $J_{3,4}$ 2.8 Hz), 4.55 (m, 1H, H-4'), 4.40 (dddd, 1H, H-3", $J_{2a,3}$ 5.4 Hz, $J_{2b,3}$ 1.9 Hz, $J_{3,4}$ 5.4 Hz, $^3J_{HP}$ 10.2 Hz), 4.29 (ddd, H-4", $J_{4,5}$ 2.1 Hz, $^3J_{HP}$ 10.8 Hz), 4.05 (dd, 1H, H-6a", $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 13.2 Hz), 3.97 (m, 1H, H-5"), 3.95-3.90 (m, 4H, H-2a", H-2b", H-5a', H-5b'), 3.83 (dd, 1H, H-5b', $J_{4,5b}$ 8.5 Hz); $^{13}C\{^1H\}$ NMR ($D_2O$): δ 152.9, 149.1 (C-4, C-6), 152.6, 140.6 (C-2, C-8), 121.3 (C-5), 96.9 (C-

1"), 88.0 (C-1'), 85.7, 79.6, 76.4, 75.8, 72.4 (C-2', C-3', C-4', C-3", C-4", C-5"), 62.1, 62.0, 61.3 (C-5', C-2", C-6"); $^{31}$P NMR ($D_2O$): δ 5.2, 2.0, 1.2; ESI-MS: $[M-H]^-$ 670; Anal. Calcd. for $C_{16}H_{28}N_5O_{18}P_3$ (671.06): C, 28.63; H, 4.20; N, 10.43; P, 13.84. Found: C, 28.60; H, 4.24; N, 10.45; P, 13.89.

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