Synthesis of Photoaffinity Derivatives of Adenophostin A

Martin de Kort,[a] Jaco Luijendijk,[a] Gijs A. van der Marel,[a] and Jacques H. van Boom*[a]

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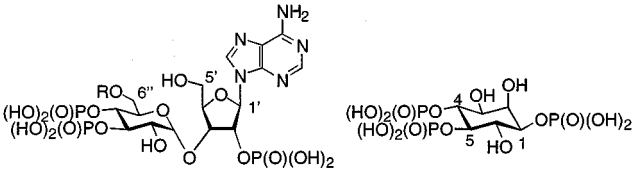
Photoaffinity derivatives **7** and **8** of adenophostin A, modified at the 5′- and 1′-positions, were prepared by a chemoselective reaction of the aminophostins **15** and **30** with *N*-succinimidyl *p*-benzoyl-2,3-dihydrocinnamate (*p*-benzoyldihydrocinnamoyl-*N*-hydroxysuccinimide; BZDC-NHS, **21**). The latter compound was prepared by Heck coupling of 4-iodobenzophenone (**16**) with methyl acrylate.

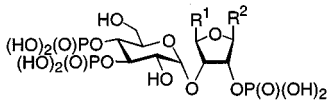
Introduction

Adenophostin A (**1**) and B (**2**) (see Scheme 1) have attracted considerable interest as full agonists of the D-*myo*-inositol 1,4,5-trisphosphate receptor (IP_3R), exhibiting binding affinities and Ca^{2+}-mobilizing potencies 10−100 times higher than those of the natural ligand IP_3 (**3**).[1] Initially, it was proposed that **1** and **2** interact with a regulatory ATP binding site of IP_3R. However, recent findings[2] confirmed that the binding site of both compounds is the same as for IP_3 (**3**). Furthermore, the Ca^{2+} signaling induced by the metabolically resistant adenophostins is, in contrast with that of IP_3, prolonged and spatially restricted.[3] These favorable properties render both compounds useful pharmacological tools[4] in studying in more detail the activation of IP_3Rs at specific locations in the cell.[5] The latter aspect was nicely illustrated by the recent discovery[6] of a small sub-region in the endoplasmic reticulum of hepatocytes that may be responsible for IP_3R-induced Ca^{2+} entry.[7] It was envisaged that the availability of photoaffinity derivatives of adenophostin A (**1**) would be of general value in unraveling the complex Ca^{2+} signaling pathways. In order to attain this goal, it is essential that the presence of a photolabel does not impair the binding affinity for the receptor. It is well established that the high activity of adenophostin A (**1**) is mainly dictated by the phosphate triad,[1a,8] the adenine residue,[9] and[10] HO-2′′, while the contributions of HO-5′ (cf. **4**)[9a,9b] and HO-6" (cf. **2**) are less important.[11,12] In addition, analogs of **1** in which the adenine is replaced[13] by an alkyl group, as in ribophostin **5**,[14] still exert IP_3-like potency. The same holds for the corresponding phenyl derivative **6**,[14] which exhibits a slightly higher potency than **5**. It is also of interest to note that adenophostin analogs with surrogate bases[15] are more active than IP_3 (**3**). This scrutinizing of the structure-activity profile for **1** suggests a strategy of introducing a photoaffinity label at either the 5′, the 6′′, or the 1′-position without substantially impairing the biological activity.

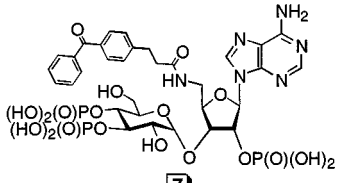


1 R= H
2 R= Ac

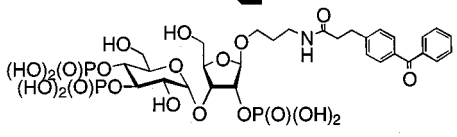
3 IP_3



4 $R^1 = R^2 = H$
5 $R^1 = CH_2OH$, $R^2 = OCH_2CH_2CH_3$
6 $R^1 = CH_2OH$, $R^2 = O(CH_2)_3Ph$



7



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We report here the synthesis of the two photoaffinity derivatives **7** and **8** (see Scheme 1) based on adenophostin A (**1**). In compound **7**, the chemically stable *p*-benzoyldihydrocinnamoyl (BZDC) photoprobe[16] is joined directly by an amide bond to the 5′-position, while in **8** it is tethered to an aminopropyl spacer at the anomeric center of the ribosyl moiety.

Results and Discussion

In the first instance, attention was focused on the synthesis of a photoaffinity derivative in which either the 5′-

[a] Leiden Institute of Chemistry, Gorlaeus Laboratories, University of Leiden,
P. O. Box 9502, 2300 RA Leiden, The Netherlands
Fax: (internat.) + 31-71/527-4307
E-mail: j.boom@chem.leidenuniv.nl

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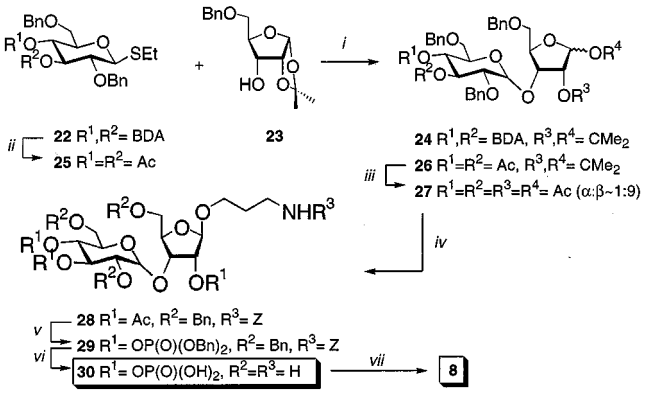
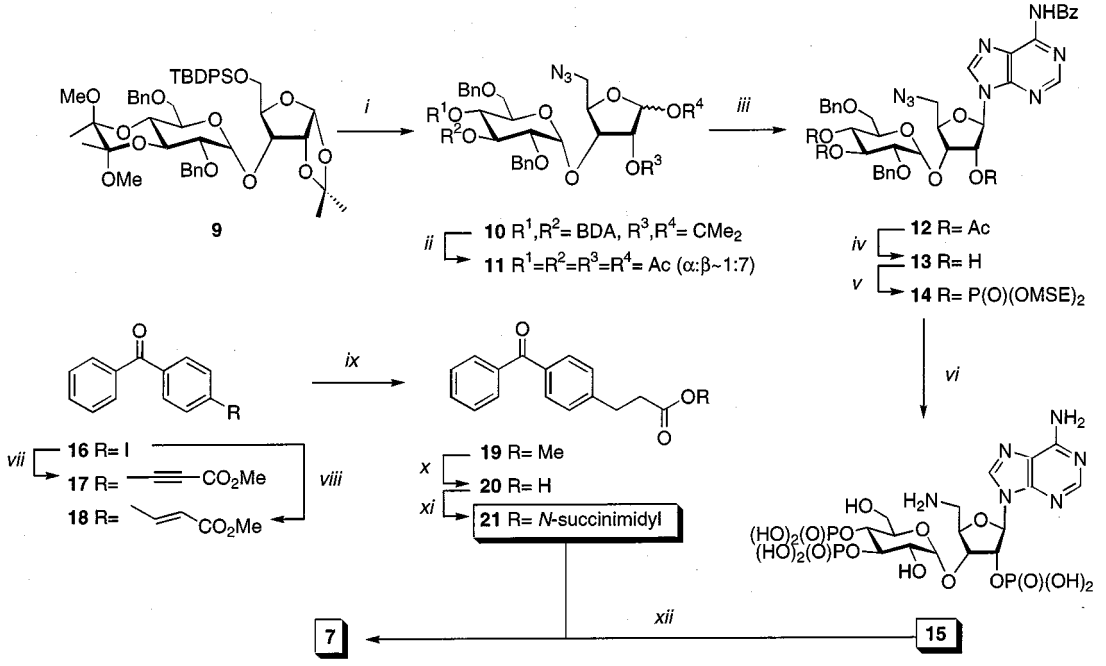
or the 6′′-position was replaced by a BZDC moiety. Previous work from our laboratory[14,17] had revealed that the silyl-protected disaccharide **9** (see Scheme 2) is a valuable building block in the construction of adenophostin analogs. Retrosynthetic analysis shows that the same dimer is also an ideal starting compound for the synthesis of key 5′-aminophostin **15**, which can readily be converted into the 5′-BZDC derivative **7** by reaction with *N*-hydroxysuccinimide **21**. Desilylation of **9** with *n*-tetrabutylammonium fluoride (TBAF) and subjection of the resulting primary hydroxy function to diphenylphosphoryl azide and diethyl azodicarboxylate[18] gave the 5-azido derivative **10**. Subsequently, concomitant removal of the 3,4-butane diacetal (BDA) and isopropylidene protecting groups in **10**, followed by acetylation, yielded tetraacetate **11**. Vorbrüggen-type condensation of **11** with silylated N^6-benzoyladenine in the presence of catalytic TMSOTf gave the expected[19] glucosyl adenosine derivative **12**. The introduction of the three phosphate groups was effected by the following well-established[19] three-step sequence of reactions. Selective deacetylation of **12** by short treatment with potassium *tert*-butoxide in MeOH resulted in **13**. Phosphitylation of the alcohol functions in **13** with *N*,*N*-diisopropylbis[2-(methylsulfonyl)ethyl] (MSE) phosphoramidite, assisted by 1*H*-tetrazole,[20] and in situ oxidation of the intermediate phosphite triesters with *tert*-butyl hydroperoxide, afforded trisphosphate **14**. Deprotection of **14** was accomplished by removal of the base-labile groups with Tesser's base,[21] followed by hydrogenolysis of the remaining benzyl ethers to give, after purification by HW-40 gel filtration and Dowex Na^+ ion-exchange chromatography, the homogeneous 5′-amino derivative of adenophostin A (**15**, Na^+ salt). The observed low yield (27%) of intermediate **14** may be due to the occurrence of an undesired Staudinger reaction[22] of the 5′-azide functionality with the phosphoramidite reagent. The low recovery of **14** could in principle be overcome by prior conversion of the azide into a protected amine function (cf. phosphorylation of **28** in Scheme 3).



Scheme 3. Reagents and conditions: (*i*) see Table 1, 15 min; (*ii*) a. 80% HOAc, reflux, 1 h; b. Ac_2O/pyridine, 30 min., 92%; (*iii*) a. HOAc/H_2O/$(HOCH_2)_2$, 14:6:3, v/v/v, reflux, 1 h; b. Ac_2O/pyridine, 4 h, 94% (2 steps); (*iv*) $SnCl_4$, $HO(CH_2)_3NHZ$, $(CH_2Cl)_2$, mol. sieves 4 Å, 16 h, 92%; (*v*) a. NaOMe, MeOH, then Dowex H^+; b. $(BnO)_2PN(iPr)_2$, 1*H*-tetrazole, $(CH_2Cl)_2$/CH_3CN, 3:1, v/v, 30 min, then *t*BuOOH, 0°C, 1 h, 77%; (*vi*) Pd/C, H_2 (1 atm), NaOAc, 1,4-dioxane/*i*PrOH/H_2O, 4:2:1, v/v/v, 16 h, 82%; (*vii*) **21**, DMF, 0.2 M, Et_3NHCO_3, pH = 8.5, 16 h, 95%



Scheme 2. Reagents and conditions: (*i*) a. TBAF (1.0 M in THF)/1,4-dioxane, 1:4, v/v, 50°C, 8 h; b. Ph_3P, DEAD, $(PhO)_2P(O)N_3$, 16 h, 60%, (2 steps); (*ii*) a. HOAc/H_2O/$(HOCH_2)_2$, 14:6:3, v/v/v, reflux, 1 h; b. Ac_2O/pyridine, 4 h, 79% (2 steps); (*iii*) TMS-$A^{Bz,TMS}$, TMSOTf, $(CH_2Cl)_2$, reflux, 16 h, 68%; (*iv*) *t*BuOK (1 M in MeOH), 1 min, 88%; (*v*) $(MSEO)_2PN(iPr)_2$, 1*H*-tetrazole, 30 min, then *t*BuOOH, 0 °C, 30 min, 27%; (*vi*) a. NaOH (4 M)/1,4-dioxane/MeOH, 1:14:5, v/v/v, 16 h; b. H_2, Pd black, 16 h, 62% (2 steps); (*vii*) methyl propiolate, $PdCl_2(PPh_3)_2$, CuI, K_2CO_3, THF, 65 °C, 6 h, 56%; (*viii*) methyl acrylate, $Pd(OAc)_2$, Bu_4NCl, $NaHCO_3$, DMF, 3 h, 85%; (*ix*) H_2, PtO_2, EtOAc, 5 min, 95%; (*x*) 0.5 M NaOH, 1,4-dioxane/H_2O (2:1, v/v), 2 h, quant.; (*xi*) NHS, DCC, CH_2Cl_2; (*xii*) DMF, 0.2 M, Et_3NHCO_3 H, pH = 8.5, 20 h, 95%, see ref.[28]

The BZDC succinimyl reagent **21**, required for the introduction of the photoaffinity probe, was prepared according to the sequence of reactions presented in Scheme 2. Sonogashira coupling[23] of 4-iodobenzophenone (**16**)[24] with methyl propiolate gave the acetylene derivative **17** in a moderate yield. Alternatively, Heck coupling[25] of iodide **16** with methyl acrylate[26] proceeded smoothly to provide methyl (*E*)-*p*-benzoylcinnamate (**18**). Short treatment[27] (5 min) of either **17** or **18** with H_2/PtO_2 furnished the dihydrocinnamoyl derivative **19**, which after saponification of the methyl ester afforded the free acid **20**. Condensation of **20** with *N*-hydroxysuccinimide (NHS) assisted by *N*,*N*′-dicyclohexylcarbodiimide (DCC) led to the activated ester **21**, which was in all aspects identical to a sample prepared previously.[28] LC-MS analysis showed that the condensation of amino derivative **15** with **21** proceeded, as expected,[29] in a chemoselective fashion to give **7**. Purification of the crude product by ion-exchange chromatography provided the homogeneous 5′-BZDC photoaffinity probe **7** (Na^+ salt) in 78% yield. The identity of **7** was unambiguously corroborated by mass spectrometry, ^{31}P and ^1H NMR spectroscopy.

It is evident that the production of photoaffinity analog **8**, containing the BZDC group tethered via an aminopropyl spacer to the 1′-position, could also be effected starting from the disaccharide **9** mentioned earlier. Nonetheless, it was decided to replace the 5′-*O*-*tert*-butyldiphenylsilyl group in **9** by a benzyl group, as in the disaccharide **24**. This change in protecting group strategy would facilitate the deprotection in a later stage of the synthesis (vide infra). The construction of target compound **8** is presented in Scheme 3 and commences with the preparation of dimer **24**. Condensation of the readily accessible[14,17] BDA-protected donor **22** with the known[30] acceptor 5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribose (**23**), mediated by iodonium ion (NIS) and catalytic amounts of triflic acid (TfOH), gave dimer **24** as a mixture of anomers (see Entry 1, Table 1). The low stereoselectivity is in contrast with the exclusive formation of the α anomer in the coupling of **22** with the corresponding 5-*O*-*tert*-butyldiphenylsilyl-1,2-*O*-isopropylidene-α-D-ribofuranoside.[17] Executing the glycosylation of **23** with **22** in a mixture of 1,4-dioxane and toluene (3:1, v/v)[31] led to a slight improvement in the α/β ratio and yield (Entry 2). A similar result was obtained using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the catalyst (Entry 3). Interestingly, the coupling of **23** with the less reactive 3,4-di-*O*-acetylated donor **25**, prepared by demasking of the BDA function in **22** and acetylation of the resulting diol, proceeded with an acceptable degree of α selectivity, to afford dimer **26** in good yield (Entry 4). Removal of the acetonide group in α anomer **26**, followed by acetylation of the hydroxy functions, afforded the valuable[15,17,32] tetraacetate **27**. Glycosidation of **27** with 3-(benzyloxycarbonylamino)-1-propanol[33] under the influence of $SnCl_4$ led to the exclusive formation of the β-aminopropyl derivative **28**. Deacetylation of **28** and subsequent phosphitylation of the triol with dibenzyloxy(*N*,*N*-diisopropylamino)phosphane,[34] followed by in situ oxidation of the phosphite triesters with *tert*-butyl hydroperoxide, gave triphosphate **29** in 77% yield. One-step removal of the benzyloxycarbonyl (*Z*) and benzyl groups in **29** by hydrogenolysis afforded derivative **30**, containing the amino spacer. Treatment of **30** with BZDC-NHS (**21**) as described for the preparation of **7** gave, after purification by ion-exchange chromatography, the 1′-BZDC-aminopropyl derivative **8** (Na^+ salt) in 95% yield. The identity of **8** was unambiguously corroborated by mass spectrometry and ^{31}P, ^{13}C, and ^1H NMR spectroscopy.

Table 1. NIS-mediated glucosylation of **23** with **22** and **25**

Entry	Donor	Product	Conditions[a]	α/β ratio[b]	Yield (%)
1	**22**	**24**	A	2.2:1	62
2	**22**	**24**	B	3.0:1	72
3	**22**	**24**	C	3.5:1	75
4	**25**	**26**	C	11.8:1	89

[a] Conditions: mol. sieves 4 Å, room temp.; A: NIS, TfOH, Et_2O; B: NIS, TfOH, toluene/1,4-dioxane, 1:3, v/v; C: NIS, TMSOTf, toluene/1,4-dioxane, 1:3, v/v. – [b] Determined by integration of the 1′-H signals in the ^1H NMR spectra of the crude products.

Conclusion

This paper describes the first synthesis of photoaffinity derivatives of adenophostin A (**1**) – i.e. the benzophenone ligands **7** and **8** – by chemoselective functionalization of the respective aminophostins **15** and **30**. Preliminary biological assay indicated that both ligands **7** and **8** are full agonists of IP_3R. A full account on the binding affinity, Ca^{2+}-releasing properties, and the potential of these novel ligands to induce IP_3R-mediated Ca^{2+} entry is in progress.

Experimental Section

General Procedures: ^1H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded with a Jeol JNM-FX-200 (200/50.1/80.7 MHz), a Bruker WM-300 (300/75.1/121 MHz), or a Bruker DMX-600 spectrometer (600/150/242 MHz). ^1H and ^{13}C chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard, and ^{31}P chemical shifts relative to 85% H_3PO_4 as external standard. – Mass spectra were recorded with a Finnigan MAT TSQ70 triple quadrupole or a PE-SCIEX API 165 mass spectrometer equipped with a custom-made electrospray (ES) interface. – Optical rotations were determined with a Propol automatic polarimeter. – Melting points were determined with a Büchi (Flawil, Switzerland) melting point apparatus. – Toluene, CH_2Cl_2, and pyridine were boiled under reflux for 3 h with P_2O_5, distilled, and stored over molecular sieves (4 Å). Et_2O was freshly distilled from $LiAlH_4$. 1,2-Dichloroethane (Biosolve, HPLC grade), 2-propanol, DMF, and 1,4-dioxane (Baker, p.a.) were stored over molecular sieves (4 Å). MeOH (Rathburn, HPLC grade) was stored over molecular sieves (3 Å). Column chromatography was performed on Baker silica gel (0.063–0.200 mm) and TLC analysis on "DC-Fertigfolien" (Schleicher & Schüll F1500, LS 254) with detection by UV absorption (254 nm) and spraying with 20% H_2SO_4 in EtOH, or ammonium molybdate (25 gL^{-1}) and ceric ammonium sulfate (10 gL^{-1}) in 10%

H_2SO_4, followed by charring at 140 °C. Reactions were carried out at ambient temperature, unless otherwise stated. Prior to reactions that required anhydrous conditions, traces of H_2O were removed by repeated concentration with toluene, pyridine, or 1,4-dioxane. – Propargyl alcohol, diethyl azodicarboxylate, trifluoromethanesulfonic acid, trimethylsilyl trifluoromethanesulfonate, $PdCl_2(PPh_3)_2$, methyl propiolate, copper(I) iodide, tetrabutylammonium chloride (Acros), ethylene glycol, *t*BuOK, tin(IV) chloride, methyl acrylate, K_2CO_3, *N*-hydroxysuccinimide (NHS), dicyclohexylcarbodiimide (DCC), *t*BuOOH (80% in di-*tert*-butyl peroxide) (Merck), platinum(IV) oxide, 1*H*-tetrazole, palladium(II) acetate (Aldrich), and acetic anhydride (Baker) were all used as received. Imino diacetate resin (Chelex®, Na^+ salt) was purchased from Sigma. Purification of the target compounds was performed by gel filtration with a Fractogel column [HW 40(s), 26/60] with triethylammonium bicarbonate buffer (0.15 M) as eluent (1.5 mLmin^{-1}) or FPLC chromatography (Pharmacia) with ammonium bicarbonate (0.15 M) as eluent. Analytical anion exchange HPLC was performed on a Mono Q HR 5/5 column (Pharmacia), flow rate 2.0 mLmin^{-1}. Elution was effected at pH = 12.0 with a mixture of buffer A (0.01 N NaOH) and buffer B (0.01 N NaOH + 1.2 N NaCl) with the following gradient: t = 0 to t = 2.5 min 0% B, t = 2.5 to t = 12.5 min 0–40% B.

5-Azido-5-deoxy-3-*O*-{2,6-di-*O*-benzyl-3,4-di-*O*-[(2′*S*,3′*S*)-2′,3′-dimethoxybutane-2′,3′-diyl]-α-D-glucopyranosyl}-1,2-*O*-isopropylidene-α-D-ribofuranoside (10): Compound **9**[17] (3.00 g, 3.40 mmol) was stirred at 50 °C in a mixture of 1,4-dioxane (20 mL) and TBAF (1.0 M in THF, 5.24 mL). After 8 h, TLC analysis revealed the reaction to be complete and the mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (75 mL), washed with brine (2 × 15 mL) and H_2O (10 mL), and dried ($MgSO_4$). Purification by column chromatography (Et_2O/light petroleum ether, 1:1→1:0, v/v) gave the desilylated disaccharide in a yield of 2.02 g. R_f = 0.47 (Et_2O). The alcohol (1.98 g, 3.06 mmol) was stirred in a mixture containing triphenylphosphane (1.06 g, 4.10 mmol) and diethyl azodicarboxylate (0.31 g, 4.10 mmol) in dry THF (15 mL). Diphenylphosphoryl azide (0.88 mL, 4.10 mmol) was added over a period of 15 min. The reaction mixture was stirred for 16 h, after which TLC analysis showed conversion of the starting alcohol into a faster running product. The solvent was removed under reduced pressure and the product was purified by column chromatography (eluent: EtOAc/light petroleum ether, 0:1→1:9, v/v) to afford azide **10**. Yield 1.22 g (1.82 mmol, 60%, 2 steps). – R_f = 0.81 (EtOAc/light petroleum ether, 1:9, v/v). – ^{1}H NMR ($CDCl_3$): δ = 7.42–7.15 (m, 10 H, CH arom), 5.78 (d, 1 H, 1′-H, $J_{1',2'}$ = 3.6 Hz), 5.14 (d, 1 H, 1′′-H, $J_{1'',2''}$ = 4.0 Hz), 4.76 (AB, 2 H, CH_2 Bn, J = –11.9 Hz), 4.70 (t, 1 H, 2′-H), 4.56 (AB, CH_2 Bn, J = –11.8 Hz), 4.32 (m, 1 H, 4′-H), 4.10 (t, 1 H, 3′′-H, $J_{2'',3''}$ = $J_{3'',4''}$ = 9.6 Hz), 4.04 (m, 1 H, 3′-H), 3.87 (m, 1 H, 5′′-H), 3.78 (t, 1 H, 4′′-H), 3.73–3.66 (m, 3 H, 6′′-H, 5a′-H), 3.64 (dd, 1 H, 2′′-H), 3.33 (dd, 1 H, 5b′-H, $J_{5a',5b'}$ = –9.7 Hz, $J_{4',5a'}$ 5.8 Hz), 3.31, 3.19 (2 s, 6 H, CH_3 OMe), 1,54, 1.35 (2 s, 6 H, CH_3 BDA), 1.35, 1.30 (2 s, 6 H, CH_3 isoprop). – ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 138.4, 137.6 (2 C_q Bn), 127.9–126.9 (CH arom), 112.7 (C_q isoprop), 103.6 (C-1′′), 99.2, 99.1 (2 C_q BDA), 95.5 (C-1′), 76.7, 76.2, 75.6, 73.7, 69.0, 65.7 (C-2′, C-3′, C-4′, C-2′′, C-3′′, C-4′′, C-5′′), 73.0, 71.7 (2 CH_2 Bn), 67.6 (C-6′′), 50.1 (C-5′), 47.7, 47.5 (2 CH_3 OMe), 26.3, 26.2 (2 CH_3 BDA), 17.5, 17.3 (2 CH_3 isoprop). – $C_{34}H_{45}N_3O_{11}$ (671.7 *((AUTHOR: We added mol. masses, please check them all!))*): calcd. C 60.79, H 6.75, N 6.26; found C 60.60, H 6.84, N 6.21. – ES-MS; *m/z*: 694 [M + Na]$^+$.

1,2-Di-*O*-acetyl-3-*O*-(3′,4′-di-*O*-acetyl-2′,6′-di-*O*-benzyl-α-D-glucopyranosyl)-5-azido-5-deoxy-α-D-ribofuranoside (11): Compound **10** (1.17 g, 1.75 mmol) was heated to reflux in a mixture of AcOH/H_2O/$(CH_2OH)_2$ (20 mL, 14:6:3, v/v/v). After 1 h, the reaction mixture was cooled (0°C) and quenched with sat. aq. $NaHCO_3$ (30 mL). The resulting suspension was extracted with EtOAc (3 × 25 mL). The organic layer was washed with H_2O (3 × 10 mL), dried ($MgSO_4$), and concentrated. The intermediate tetraol (R_f = 0.29, MeOH/EtOAc, 5:95, v/v) was dissolved in a mixture of acetic anhydride/pyridine (25 mL, 3:7, v/v) and stirred for 4 h. The mixture was diluted with toluene (10 mL) and concentrated under reduced pressure (3 ×). The oily product was subjected to column chromatography by elution with Et_2O/light petroleum ether (1:9→1:1, v/v). Concentration of the appropriate fractions afforded tetraacetate **11** as a white foam. Yield 0.95 g (1.38 mmol, 79%, 2 steps); α/β ≈ 1:7. – ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 169.9, 169.6, 169.4, 168.7 (4 C=O Ac), 137.6, 137,4 (2 C_q Bn), 128.2–127.5 (CH arom), 97.9 (C-1′′), 96.1 (C-1′), 80.4, 76.4, 73.0, 72.7, 71.5, 69.0, 68.8 (C-2′, C-3′, C-4′, C-2′′, C-3′′, C-4′′, C-5′′), 73.3 (2 CH_2 Bn), 67.8 (C-6′′), 50.8 (C-5′), 20.7, 20.5, 20.4, 20.1 (4 CH_3 Ac). – ES-MS; *m/z*: 687 [M + H]$^+$.

2′-*O*-Acetyl-3′-*O*-(3′′,4′′-di-*O*-acetyl-2′′,6′′-di-*O*-benzyl-α-D-glucopyranosyl)-5′-azido-*N*^{6}-benzoyl-5′-deoxyadenosine (12): A suspension of 6-*N*-benzoyladenine (0.91 g, 3.79 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (6.8 mL) and pyridine (2.4 mL) was refluxed for 7 h. The reaction mixture was cooled, diluted with toluene (5 mL), and concentrated in vacuo. The residual oil was repeatedly diluted with toluene (3 × 5 mL) and concentrated in vacuo to remove excess 1,1,1,3,3,3-hexamethyldisilazane. Disaccharide **11** (0.95 g, 1.38 mmol) in 1,2-dichloroethane (18 mL), and a catalytic amount of TMSOTf (61 μL, 0.36 mmol) were added to the silylated *N*^{6}-benzoyladenine. After stirring for 16 h at reflux temperature, TLC analysis showed conversion of the starting tetraacetate into a slower running product. The reaction mixture was quenched with Et_3N (1.5 mL), diluted with CH_2Cl_2 (30 mL), and poured into sat. aq. $NaHCO_3$ (15 mL). The organic phase was washed with H_2O (10 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2/MeOH, 1:0 →95:5, v/v) to give **12** as a yellowish foam. Yield 0.80 g (0.93 mmol, 68%). – $[\alpha]_D$ = +74.0 (c =1.0 $CHCl_3$). – ^{1}H NMR ($CDCl_3$): δ = 9.15 (s, 1 H, NH), 8.81 (s, 1 H, 2-H), 8.23 (s, 1 H, 8-H), 8.02 (d, 2 H, CH arom Bz), 7.63–7.44 (m, 3 H, CH arom Bz), 7.37–7.23 (m, 10 H, CH arom Bn), 6.25 (d, 1 H, 1′-H, $J_{1',2'}$ = 5.1 Hz), 5.86 (t, 1 H, 2′-H, $J_{2',3'}$ = 5.2 Hz), 5.44 (t, 1 H, 3′′-H, $J_{3'',4''}$ = 9.6 Hz), 5.00 (t, 1 H, 4′′-H, $J_{4'',5''}$ = 9.8 Hz), 4.98 (d, 1 H, 1′′-H, $J_{1'',2''}$ = 3.6 Hz), 4.73 (t, 1 H, 3′-H, $J_{3',4'}$ = 5.2 Hz), 4.64–4.43 (m, 4 H, 2 CH_2 Bn), 4.45 (m, 1 H, 4′-H), 3.98 (m, 1 H, 5′′-H), 3.61 (AB, 2 H, 5′-H, $J_{4',5'}$ 6.3 Hz, $J_{5a',5b'}$ = –10.2 Hz), 3.57 (dd, 1 H, 2′′-H, $J_{2'',3''}$ 10.1 Hz), 3.51–3.49 (m, 2 H, 6′′-H), 1.98, 1.95, 1.90 (3 s, 9 H, 3 CH_3 Ac). – ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 169.7, 169.6 (3 C=O Ac), 164.8 (C=O Bz), 152.3 (C-2), 151.2 (C-4), 149.5 (C-6), 140.3 (C-8), 137.1, 137.0 (2 C_q Bn), 133.0 (C_q Bz), 132.2 (CH Bz), 128.2–127.3 (CH arom), 123.5 (C-5), 97.8 (C-1′′), 86.4 (C-1′), 81.3, 76.1, 75.7, 72.7, 71.1, 69.1, 68.7 (C-2′, C-3′, C-4′, C-2′′, C-3′′, C-4′′, C-5′′), 73.2, 72.9 (2 CH_2 Bn), 67.8 (C-6′′), 50.9 (C-5′), 20.4, 20.1, 19.8 (3 CH_3 Ac). – $C_{43}H_{44}N_8O_{12}$ (864.9): calcd. C 59.72, H 5.13, N 12.96; found C 59.89, H 5.19, N 13.05. – ES-MS; *m/z*: 866 [M + H]$^+$, 889 [M + Na]$^+$.

5′-Azido-*N*^{6}-benzoyl-3′-*O*-(2′′,6′′-di-*O*-benzyl-α-D-glucopyranosyl)-5′-deoxyadenosine (13): To a vigorously stirred solution of glucopyranosyl adenosine **12** (0.73 g, 0.85 mmol) in 1,4-dioxane (25 mL)

was added a solution of *t*BuOK in MeOH (1.0 M, 35 mL). After stirring for 1 min, the reaction mixture was neutralized upon the addition of AcOH (2.0 mL, 35 mmol). The solution was poured into sat. aq. $NaHCO_3$ (25 mL), and the solution was extracted with CH_2Cl_2 (2 × 25 mL). The organic phase was washed with H_2O (15 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by column chromatography (eluent: CH_2Cl_2/MeOH, 1:0 to 95:5, v/v) afforded triol **13** as a white foam. Yield 0.55 g (0.75 mmol, 88%). $[\alpha]_D$ = +57.8 (*c* =1.0 $CHCl_3$). − R_f = 0.45 (MeOH/EtOAc, 5:95, v/v). − ^{1}H NMR ($CDCl_3$): δ = 9.41 (s, 1 H, NH), 8.73 (s, 1 H, 2-H), 8.20 (s, 1 H, 8-H), 8.02 (d, 2 H, CH Bz), 7.61−7.46 (m, 3 H, CH arom Bz), 7.36−7.16 (m, 10 H, CH arom Bn), 6.07 (d, 1 H, 1′-H, $J_{1',2'}$ = 5.3 Hz), 4.89 (d, 1 H, 1′′-H, $J_{1'',2''}$ = 3.6 Hz), 4.72 (m, 1 H, 2′-H), 4.71 (AB, 2 H, CH_2 Bn, *J* = −11.7 Hz), 4.53 (AB, 2 H, CH_2 Bn, *J* = −12.2 Hz), 4.28−4.24 (m, 2 H, 3′-H, 4′-H), 4.03 (t, 1 H, 3′′-H, $J_{3'',4''} = J_{2'',3''}$ = 9.5 Hz), 3.84 (m, 1 H, 5′′-H), 3.77 (dd, 1 H, 6a′′-H, $J_{5'',6a''}$ = 6.9 Hz), 3.68−3.62 (m, 2 H, 6b′′-H, 5a′-H), 3.61−3.51 (m, 2 H, 4′′-H, 5b′-H), 3.44 (dd, 1 H, 2′′-H). − ^{13}C{^{1}H} NMR (MeOD): δ = 167.6 (C=O Bz), 153.1 (C-2), 152.9 (C-4), 150.8 (C-6), 144.2 (C-8), 139.3, 139.0 (2 C_q Bn), 134.6 (C_q Bz), 133.7 (CH Bz), 129.5−128.5 (CH arom), 124.8 (C-5), 98.8 (C-1′′), 90.0 (C-1′), 82.9, 80.4, 77.8, 73.2, 71.7 (C-2′, C-3′, C-4′, C-2′′, C-3′′, C-4′′, C-5′′), 74.2, 74.1 (2 CH_2 Bn), 70.8 (C-6′′), 52.8 (C-5′). − ES-MS: 740 $[M + H]^+$, 762 $[M + Na]^+$.

5′-Amino-5′-deoxy-3′*O*-α-D-glucopyranosyl-3′′,4′′-bisphosphate)-adenosine 2′-Monophosphate 15, Na$^+$ Salt: To a mixture of triol **13** (0.11 g, 0.14 mmol) and *N*,*N*-diisopropylbis[2-(methylsulfonyl)-ethyl] phosphoramidite[19,20] (0.30 g, 0.81 mmol) in CH_2Cl_2 (4 mL) was added a solution of 1*H*-tetrazole (75 mg, 1.1 mmol) in CH_3CN (4 mL). After stirring for 30 min, TLC analysis (MeOH/CH_2Cl_2, 15:85, v/v) showed complete conversion of starting triol into several faster running products (major product: R_f = 0.57). The reaction mixture was cooled, *t*BuOOH (0.17 mL) was added, and stirring was continued for 30 min, after which TLC analysis revealed complete conversion of the intermediate phosphite triester into slower running products. The phosphate triester **14** was obtained after purification by column chromatography (eluent: CH_2Cl_2/MeOH, 1:0 to 9:2, v/v). Yield 55 mg (38 μmol, 27%). − R_f = 0.41 (CH_2Cl_2/MeOH, 90:8, v/v). − ^{31}P NMR ($CDCl_3$): δ = −2.0, −1.9, −1.8. − Compound **14** (33 mg, 23 μmol) was dissolved in a mixture of NaOH (4 M)/1,4-dioxane/MeOH (1:14:5, v/v/v, 10 mL) and stirred for 16 h. The mixture was neutralized with AcOH (0.12 mL) and concentrated. Purification of the trisphosphate was accomplished by gel filtration on an HW-40 column eluted with triethyl ammonium bicarbonate buffer (0.15 M). Complete deprotection of the purified 2′′,6′′-di-*O*-benzyl-5′-azidophostin intermediate was effected by hydrogenation in MeOH/H_2O and AcOH (3 drops) in the presence of Pd black. After 16 h, the mixture was neutralized with Et_3N and filtered through glass fiber (Whatman GF/A) and concentrated under reduced pressure. Ion-exchange chromatography by Dowex® 50Wx4 (Na^+ salt) and treatment with imino diacetate resin (Chelex®, Na^+ salt), followed by lyophilization, gave pure 5′-aminophostin **15**. Yield 9.3 mg (14 μmol, 62%). − ^{1}H NMR (D_2O, 600 MHz, HH-COSY): δ = 8.22 (s, 1 H, 2-H), 8.18 (s, 1 H, 8-H), 6.27 (d, 1 H, 1′-H, $J_{1',2'}$ = 5.1 Hz), 5.37 (d, 1 H, 1′′-H, $J_{1'',2''}$ = 3.9 Hz), 5.08 (ddd, 1 H, 2′-H, $J_{2',3'}$ = 2.9 Hz, $J_{2',P}$ = 8.4 Hz), 4.65 (dd, 1 H, 3′-H, $J_{3',4'}$ = 4.8 Hz), 4.52 (q, 1 H, 3′′-H, $J_{3'',4''} = J_{2'',3''} = J_{3'',P}$ = 8.4 Hz), 4.32 (m, 1 H, 4′-H), 3.91 (m, 2 H, 4′′-H, 6a′′-H), 3.73 (m, 3 H, 5′′-H, 6b′′-H, 2′′-H), 3.32 (dAB, 2 H, 5′-H, $J_{4',5'}$ = 2.9 Hz, $J_{5a',5b'}$ = −10.7 Hz). − ^{31}P NMR (D_2O): δ = 4.25, 3.59, 3.08. − HR-MS: $C_{16}H_{26}N_6O_{17}P_3$ $[M - H]^-$ calcd. 667.0566; found 667.0573. − ES-MS; *m*/*z*: 669 $[M + H]^+$.

Methyl *p*-Benzoyl-2,3-didehydrocinnamate (17): 4-Iodobenzophenone (**16**, 0.32 g, 1.04 mmol) and methyl propiolate (0.35 g, 0.37 mL, 4.16 mmol) were dissolved in THF (5 mL) and the solution was degassed. $PdCl_2(PPh_3)_2$ (15 mg, 0.02 mmol), copper(I) iodide (8 mg, 0.05 mmol), and K_2CO_3 (0.29 g, 2.1 mmol) were added and the mixture was refluxed for 6 h under argon. The dark brown solution was concentrated and dissolved in Et_2O (50 mL). The organic phase was washed with sat. aq. $NaHCO_3$ (25 mL) and H_2O (25 mL), dried ($MgSO_4$), and concentrated. The dark brown residue was applied to a column of silica gel, which was eluted with EtOAc/light petroleum ether (1:9, v/v) to give **17**. Yield 0.15 g (0.58 mmol, 56%). − R_f = 0.36 (EtOAc/light petroleum ether, 1:9, v/v). − ^{1}H NMR ($CDCl_3$): δ = 7.85−7.75, 7.72−7.57, 7.54−7.44 (m, 9 H, H arom), 3.87 (s, 3 H, OMe). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 200.7 (C=O benzophenone), 153.9 (C=O), 137.8, 135.3, 123.2 (Cq arom), 132.7, 132.6, 129.8, 128.3 (CH arom), 86.9 (C-2), 52.8 (OMe). − ES-MS; *m*/*z*: 265 $[M + H]^+$.

(*E*)-Methyl *p*-Benzoylcinnamate (18): 4-Iodobenzophenone (**16**, 0.31 g, 1.00 mmol) was dissolved in DMF (4 mL) and the solution was degassed. $Pd(OAc)_2$ (11 mg, 0.05 mmol), methyl acrylate (0.17 g, 0.18 mL, 2.0 mmol), tetrabutylammonium chloride (0.28 g, 1.0 mmol), and $NaHCO_3$ (0.21 g, 0.25 mmol) were added, and the solution was degassed again. The reaction mixture was stirred for 3 h under argon, after which it was concentrated to 1 mL, diluted with Et_2O (20 mL), and rinsed with H_2O (10 mL), sat. aq. $NaHCO_3$ (10 mL), and H_2O (10 mL). Purification was effected by column chromatography (EtOAc/light petroleum ether, 0:1→2:8, v/v) to give cinnamic acid derivative **18** in pure form. Yield 0.23 g (0.85 mmol, 85%). − R_f = 0.31 (EtOAc/light petroleum ether, 1:9, v/v). − ^{1}H NMR ($CDCl_3$): δ = 7.85−7.44 (m, 10 H, H arom, 3-H), 6.54 (d, 1 H, 2-H, J_{trans} = 16 Hz), 3.84 (s, 3 H, OMe). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 195.6 (C=O benzophenone), 166.8 (C=O), 143.2 (C-3), 138.6, 137.9, 137.1 (C_q arom), 132.5, 131.3, 130.4, 129.8, 128.2, 127.7 (CH arom, C-3), 120.0 (C-2), 51.7 (OMe). − $C_{17}H_{14}O_3$: calcd. C 76.68, H 5.30; found C 76.61, H 5.25. − ES-MS; *m*/*z*: 267 $[M + H]^+$, 289 $[M + Na]^+$, 555 $[2 M + Na]^+$.

Methyl *p*-Benzoyl-2,3-dihydrocinnamate (19): Cinnamic ester derivative **17** (0.13 g, 0.50 mmol) or **18** (0.13 g, 0.50 mmol) was dissolved in EtOAc (5 mL). Platinum(IV) oxide (7 mg, 0.03 mmol) was added and the solution was degassed. The reaction mixture was vigorously stirred under H_2. After 5 min, the solution was degassed, immediately filtered through glass fiber (GF/2A, Whatman®) and concentrated. Purification was effected by column chromatography (EtOAc/light petroleum ether, 1:9→3:7, v/v) to give compound **19** in pure form. Yield 0.12 g (0.45 mmol, 95%). − R_f = 0.30 (EtOAc/light petroleum ether, 2:8, v/v). − ^{1}H NMR ($CDCl_3$): δ = 7.84−7.20 (m, 9 H, H arom), 3.69 (s, 3 H, CH_3), 3.04 (t, 2 H, 3-H, $J_{2,3}$ = 7.3 Hz), 2.69 (t, 2 H, CH_2). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 178.2 (C=O), 144.8, 137.9, 135.1 (C_q arom), 132.0, 130.6, 129.1, 128.4 (CH arom), 34.1, 29.2 (C-2, C-3). − $C_{17}H_{16}O_3$ (268.3): calcd. C 76.10, H 6.01; found C 75.99, H 6.03. − ES-MS; *m*/*z*: 269 $[M + H]^+$, 291 $[M + Na]^+$.

***N*-Succinimidyl *p*-Benzoyl-2,3-dihydrocinnamate (21):** Compound **19** (0.10 g, 0.37 mmol) was dissolved in a 0.5 M solution of NaOH in 1,4-dioxane/H_2O (2:1, v/v, 5 mL). After stirring for 2 h, the mixture was acidified with AcOH (pH = 5). The solution was diluted with EtOAc (15 mL), washed with H_2O (2 × 5 mL), dried ($MgSO_4$), and concentrated. The product **20** was used in the next reaction without purification. Yield 95 mg (quant.). − R_f = 0.23 (EtOAc/light petroleum ether 1:1, v/v). − ^{1}H NMR ($CDCl_3$): δ = 7.85−7.25 (m, 9 H, H arom), 3.05 (t, 2 H, 3-H, $J_{2,3}$ = 7.3 Hz), 2.74 (t, 2 H, 2-H, CH_2). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 178.4 (C=O),

145.2, 137.6, 135.7 (C_q arom), 132.3, 130.5, 129.9, 128.2 (CH arom), 35.0, 30.4 (C-2, C-3). − ES-MS; *m/z*: 255 $[M + H]^+$, 277 $[M + Na]^+$, 531 $[2\,M + Na]^+$. − Dihydrocinnamic acid **20** was converted into *N*-succinimidyl cinnamate **21** as described by Prestwich et al.[28]

5′-(*p*-Benzoyl-2,3-dihydrocinnamido)-5′-deoxy-3′-*O*-(α-D-glucopyranosyl 3′′,4′′-bisphosphate)-adenosine 2′-Monophosphate (7), Na^+ Salt: Aminophostin **15** (Na^+ salt, 5.0 mg, 7.5 μmol) was subjected to the activated ester derivative **21** (8.0 mg, 23 μmol) as described by Prestwich et al.[29] The reaction was monitored by LC/MS analysis, which indicated complete conversion of the starting material after 20 h. No side products arising from reaction with the adenine base were detected. The product was purified by Q-Sepharose column chromatography (Pharmacia). After lyophilization of the appropriate fractions, photoaffinity derivative **7** was converted into the Na^+ salt by sequential treatment with Dowex® 50Wx4 (Na^+ salt) and imino diacetate resin (Chelex®, Na^+ salt), followed by lyophilization. Yield 5.1 mg (5.5 μmol, 78%). − ^{1}H NMR (D_2O, 600 MHz, HH-COSY): δ = 8.24 (s, 1 H, 2-H), 8.13 (s, 1 H, 8-H), 7.82 (t, 1 H, *J* = 7.5 Hz), 7.65 (m, 4 H), 7.51 (d, 2 H, *J* = 7.9 Hz), 7.28 (d, 2 H, *J* = 8.2 Hz), 6.28 (d, 1 H, 1′-H, $J_{1',2'}$ = 1.1 Hz), 5.33 (d, 1 H, 1′′-H, $J_{1'',2''}$ = 3.4 Hz), 4.52 (dd, 1 H, 2′-H, $J_{2',P}$ = 7.3 Hz), 4.27 (q, 1 H, 3′′-H, *J* = 8.1 Hz), 4.22 (m, 2 H, 3′-H, 4′-H), 3.86 (dd, 1 H, 6a′′-H, $J_{6a'',6b''}$ = −9.6 Hz, $J_{5'',6a''}$ = 3.4 Hz), 3.81 (m, 2 H, 5′′-H, 4′′-H), 3.68 (m, 2 H, 2′′-H, 6b′′-H), 3.10−3.01 (m, 2 H, 5′-H), 2.75 (m, 1 H, CH_2 BZDC), 2.57 (m, 3 H, CH_2 BZDC). − ^{31}P NMR (D_2O, 242 MHz): δ = 2.86 (P-4′), 2.27 (P-3′′), 1.43 (P-2′). − HR-MS: $C_{32}H_{38}N_6O_{19}P_3$ $[M - H]^-$ calcd. 903.1403; found 903.1398. − ES-MS; *m/z*: 905 $[M + H]^+$.

5-*O*-Benzyl-3-*O*-{2′,6′-di-*O*-benzyl-3′,4′-di-*O*-[(2′′*S*,3′′*S*)-2′′,3′′-dimethoxybutane-2′′,3′′-diyl]-α-D-glucopyranosyl}-1,2-*O*-isopropylidene-α-D-ribofuranoside (24): Acceptor **23** (0.97 g, 3.49 mmol) and donor **22** (1.98 g, 3.83 mmol) were repeatedly concentrated with toluene (3 × 5 mL) and dissolved in a mixture of toluene and 1,4-dioxane (1:3, v/v, 30 mL). Activated molecular sieves (4 Å) were added and the mixture was stirred under a stream of N_2. NIS (0.86 g, 3.83 mmol) and a catalytic amount of TMSOTf (0.07 mL, 0.43 mmol) were subsequently added. After stirring for 10 min, TLC analysis showed complete disappearance of **23**. The reaction mixture was filtered through Hyflo® and the filtrate was diluted with EtOAc (25 mL), washed with aq. $Na_2S_2O_3$ (15 mL) and aq. $NaHCO_3$ (1 M, 15 mL), dried with $MgSO_4$ and concentrated in vacuo. The α and β anomers were separated by column chromatography (eluent: toluene/EtOAc, 19:1→8:2, v/v), to give disaccharide **24** in pure form. Yield 2.09 g (2.62 mmol, 75%) The analytical data are in all aspects identical to a previously prepared sample.[14,17]

Ethyl 3,4-Di-*O*-acetyl-2,6-di-*O*-benzyl-1-thio-β-D-glucopyranoside (25): Compound **22** (7.96 g, 15.6 mmol) was dissolved in 80% aq. AcOH (75 mL) and heated to reflux. After stirring for 1 h at that temperature, the reaction mixture was concentrated in vacuo. The oily residue was repeatedly concentrated with pyridine (3 × 10 mL) and dissolved in a mixture of pyridine/acetic anhydride (25 mL, 2:1, v/v). After 30 min, the mixture was concentrated and the residue was concentrated with toluene (3 × 20 mL). The product **25** was isolated as white needles after crystallization from Et_2O/light petroleum ether (5:2, v/v). Yield 7.00 g (14.4 mmol, 92%). − R_f = 0.81 (EtOAc). − M.p. 108 °C. − ^{1}H NMR ($CDCl_3$): δ = 7.30−7.26 (m, 10 H, H arom), 5.19 (t, 1 H, 3-H, $J_{3,4}$ = 9.5 Hz), 4.90 (t, 1 H, 4-H, $J_{4,5}$ = 9.5 Hz), 4.71 (AB, 2 H, CH_2 Bn, *J* = −12.1 Hz), 4.52 (m, 3 H, 1-H, CH_2 Bn), 3.67−3.54 (m, 3 H, 5-H, 6a-H, 6b-H), 3.47 (t, 1 H, 2-H, $J_{2,3}$ = 9.5 Hz), 2.83−2.72 (m, 2 H, CH_2 SEt), 1.33 (t, 3 H, CH_3 SEt, *J* = 7.4 Hz). − ES-MS; *m/z*: 489 $[M + H]^+$.

3-*O*-(3′,4′-Di-*O*-acetyl-2′,6′-di-*O*-benzyl-α-D-glucopyranosyl)-5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranoside (26): Acceptor **23** (0.54 g, 1.94 mmol) and donor **25** (1.04 g, 2.13 mmol) were dissolved in a mixture of toluene and 1,4-dioxane (1:3, v/v, 20 mL) and were coupled as described for **24**. The α and β anomers were separated by column chromatography (eluent: toluene/EtOAc 9:1→8:2, v/v), to give disaccharide **26** in pure form. Yield 1.31 g (1.86 mmol, 89%). − R_f = 0.64 (toluene/EtOAc/MeOH, 90:25:2.5, v/v/v). − $[\alpha]_D^{20}$ = +103.5 (*c* = 2.0 $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 7.36−7.22 (m, 15 H, H arom), 5.83 (d, 1 H, 1-H, $J_{1,2}$ = 4.4 Hz), 5.38 (t, 1 H, 3′-H, $J_{2',3'}$, $J_{3,4}$ = 9.5 Hz), 5.21 (d, 1 H, 1′-H, $J_{1',2'}$ = 3.7 Hz), 5.09 (t, 1 H, 4′-H, $J_{4',5'}$ = 9.5 Hz), 4.72−4.47 (m, 7 H, 2 CH_2 Bn, 2-H, 3-H, 4-H), 4.30 (d, 2 H, CH_2 Bn), 4.16 (dd, 1 H, 5′-H, $J_{5',6'}$ = 4.4 Hz), 3.81 (dd, 1 H, 5a-H, $J_{4,5a}$ = 2.2 Hz, $J_{5a,5b}$ = 11.7 Hz), 3.71 (dd, 1 H, 5b-H, $J_{4,5b}$ = 3.0 Hz), 3.62 (dd, 1 H, 2′-H), 3.32 (m, 2 H, 6-H), 2.03, 1.88 (s, 6 H, 2 CH_3 Ac), 1.53, 1.37 (s, 6 H, 2 CH_3 isoprop). − ^{13}C{^{1}H} NMR ($CDCl_3$): δ = 168.8, 169.3 (2 C=O Ac), 137.6, 137.3 (3 C_q Bn), 128.0−127.2 (CH arom), 112.6 (C_q isoprop), 104.0 (C-1), 94.0 (C-1′), 77.3, 75.9, 75.5, 72.5, 71.7, 68.6, 68.2 (C-2, C-3, C-4, C-2′, C-3′, C-4′, C-5′), 73.2, 73.0 (3 CH_2 Bn), 67.6, 67.1 (C-5, C-6′), 26.4 (2 CH_3 Ac), 20.5, 20.3 (2 CH_3 isoprop). − $C_{39}H_{46}N_8O_{12}$ (818.8): calcd. C 66.28, H 6.56; found C 66.37, H 6.53. − ES-MS; *m/z*: 706 $[M + H]^+$.

1,2-Di-*O*-acetyl-3-*O*-(3′,4′-di-*O*-acetyl-2′,6′-di-*O*-benzyl-α-D-glucopyranosyl)-5-*O*-benzyl-α-D-ribofuranoside (27): Compound **26** (2.26 g, 3.20 mmol) was heated to reflux in a mixture of AcOH/H_2O/$(CH_2OH)_2$ (35 mL, 14:6:3, v/v/v). After 1 h, the reaction mixture was cooled (0 °C) and quenched with sat. aq. $NaHCO_3$ (60 mL). The resulting suspension was extracted with EtOAc (3 × 50 mL). The organic layer was washed with H_2O (3 × 20 mL), dried ($MgSO_4$), and concentrated. The intermediate diol (R_f = 0.42, MeOH/EtOAc, 4:96, v/v) was dissolved in a mixture of acetic anhydride/pyridine (40 mL, 3:7, v/v) and stirred for 4 h. The mixture was diluted with toluene (3 × 10 mL) and concentrated under reduced pressure. The oily product was subjected to column chromatography, eluting with Et_2O/light petroleum ether (1:9→1:1, v/v). Concentration of the appropriate fractions afforded tetraacetate **27** as a white foam. Yield 2.33 g (94%, 3.10 mmol, 2 steps). − R_f = 0.56 (EtOAc/light petroleum ether, 1:1, v/v). − α/β ≈ 1:7. The analytical data are in all aspects identical to a previously prepared sample.[14,15a,17]

1-((3-Benzyloxycarbonylamino)-propyl)-2-*O*-acetyl-5-*O*-benzyl-3-*O*-(3′,4′-di-*O*-acetyl-2′,6′-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranoside (28): Tetraacetate **27** (0.97 g, 1.29 mmol) and 3-(benzyloxycarbonylamino)-1-propanol[33] (0.24 g, 1.94 mmol) were dissolved in 1,2-dichloroethane (20 mL). The solution was stirred with powdered molecular sieves (4 Å) under a stream of argon. $SnCl_4$ (76 μL, 0.65 mmol) was added and the mixture was stirred overnight. The mixture was neutralized with Et_3N (0.2 mL), filtered through Hyflo,® and diluted with Et_2O (20 mL). The solution was washed with H_2O (10 mL) and sat. aq. $NaHCO_3$ (10 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/light petroleum ether, 1:6⟶1:1, v/v) to give **28** as a single isomer. Yield 1.07 g (1.19 mmol, 92%). − R_f = 0.86 (toluene/EtOAc/MeOH, 90:25:2.5, v/v/v). − ^{1}H NMR (300 MHz, HH COSY, $CDCl_3$): δ = 7.36−7.21 (m, 20 H, H arom), 5.38 (t, 1 H, 3′′-H, $J_{2'',3''}$= $J_{3'',4''}$ = 9.7 Hz), 5.19 (dd, 1 H, 2′-H, $J_{1',2'}$ = 1.1 Hz, $J_{2',3'}$ = 4.7 Hz), 5.07 (AB, 2 H, CH_2 Bn, *J* = −12.0 Hz), 5.03 (t, 1 H, 4′′-H, $J_{4'',5''}$ = 9.5 Hz), 4.96 (m, 2 H, 1′-H, 1′′-H), 4.51−4.44 (m, 5 H, 2 CH_2 Bn, 3′-H),

4.32 (m, 1 H, 4′-H), 3.86 (ddd, 1 H, 5′′-H, $J_{4'',5''}$ = 10.7 Hz, $J_{5'',6''}$ = 4.5 Hz), 3.75 (m, 1 H, 1a-H), 3.66 (dd, 1 H, 5a′-H, $J_{4',5'}$ = 5.8 Hz), 3.56−3.50 (m, 2 H, 5b′-H, 2′′-H), 3.45 (m, 1 H, 1b′-H), 3.37 (dd, 1 H, 6a′′-H, $J_{6a'',6b''}$ = −11.0 Hz, $J_{5'',6''}$ = 2.6 Hz), 3.27 (dd, 1 H, 6b′′-H, $J_{5'',6''}$ = 3.9 Hz), 3.19 (m, 2 H, 2-H), 1.93, 1.87, 1.82 (s, 3 CH_3 Ac), 1.70 (m, 2 H, 3-H). − $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 170.3, 169.3 (3 C=O Ac), 156.3 (C=O Z), 137.9, 137.7, 137.5 (3 C_q Bn), 136.6 (C_q Z), 128.8−127.5 (CH arom), 105.2 (C-1′′), 96.5 (C-1′), 80.1, 76.5, 75.0, 73.8, 71.9, 69.0, 68.8 (C-2′, C-3′, C-4, C-2′′, C-3′′, C-4′′, C-5′′), 73.4, 73.2 (2 CH_2 Bn), 70.3, 67.6, 66.5, 65.5 (CH_2 Bn, C-5′, C-6′′, C-1), 38.2 (C-3), 29.3 (C-2), 20.8, 20.6 (CH_3 Ac). − $C_{49}H_{57}NO_{15}$ (899.9): calcd. C 65.39, H 6.38, N 1.56; found C 65.51, H 6.42, N 1.59. − ES-MS; *m/z*: 923 $[M + Na]^+$.

1-(3-Aminopropyl)-3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranoside 2,3′,4′-Trisphosphate (30) (Et_3NH^+ Salt): Triacetate **28** (0.39 g, 0.43 mmol) was dissolved in dry MeOH (10 mL) containing sodium methoxide (7 mg, 0.12 mmol) and was stirred for 1.5 h. When TLC analysis (toluene/EtOAc/MeOH, 90:25:2.5, v/v/v) showed complete conversion of the starting material into a slower running product (R_f = 0.59), the solution was neutralized with Dowex H^+, filtered, and concentrated under reduced pressure. − ES-MS; *m/z*: 774 $[M + H]^+$, 797 $[M + Na]^+$. − The triol (0.12 g, 0.15 mmol) was repeatedly concentrated with CH_3CN (2 × 5 mL) and dissolved in CH_2Cl_2 (5 mL). Dibenzyloxy(*N*,*N*-diisopropylamino)-phosphane (0.20 mL, 0.60 mmol) and a solution of 1*H*-tetrazole (84 mg, 1.20 mmol) in CH_3CN (2 mL) were subsequently added. The mixture was stirred under nitrogen for 30 min, after which TLC analysis (toluene/EtOAc, 9:1, v/v) revealed complete conversion into a faster running product (R_f = 0.72). The mixture was cooled (0 °C) and *t*BuOOH (0.25 mL, 2.2 mmol) was added. After 1 h, the solution was diluted with EtOAc (20 mL), washed with H_2O, dried ($MgSO_4$) and concentrated. The product was purified by column chromatography (EtOAc/light petroleum ether, 1:4⟶1:0, v/v) to give **29** as a colorless oil. Yield 0.18 g (0.16 mmol, 77%, 2 steps). − ^{31}P NMR ($CDCl_3$): δ = 0.86, 1.39, 2.00. − Compound **29** (0.14 g, 89 μmol) and NaOAc (87 mg, 0.11 mmol) were dissolved in a mixture of dioxane/2-propanol/H_2O (15 mL, 4:2:1, v/v/v) and the solution was degassed. Palladium on carbon (10%, 75 mg) was added and the reaction mixture was stirred under H_2. After 16 h, the catalyst was removed by filtration through glass fiber (GF/2A, Whatman). The filtrate was concentrated under reduced pressure and the product was purified by gel filtration on a Fractogel HW-40 column as described for **15**, to give aminopropyl derivative **30**. Yield 0.11 g (Et_3NH^+ salt, 73 μmol, 82%). An analytical sample was prepared by conversion of the trisphosphate into the Na^+ salt as described for compound **15**. − ^1H NMR (300 MHz, HH-COSY, D_2O): δ = 5.22 (s, 1 H, 1′-H), 5.17 (d, 1 H, 1′′-H, $J_{1'',2''}$ = 3.8 Hz), 4.55 (dd, 1 H, 2′-H, $J_{2',3'}$ = 4.2 Hz, $J_{2',P}$ = 8.3 Hz), 4.32 (q, 1 H, 3′′-H, $J_{3'',4''}$ = $J_{3'',4''}$ = $J_{3'',P}$ = 9.5 Hz), 4.29−4.16 (m, 2 H, 3′-H, 4′-H), 3.93−3.87 (m, 2 H, 5′′-H, 1a-H), 3.82 (dd, 1 H, 5a′-H, $J_{4',5a'}$ = 3.0 Hz, $J_{5a',b'}$ = −12.4 Hz), 3.75−3.60 (m, 7 H, 2′′-H, 4′′-H, 1b-H, 5b′-H, 6′′-H), 3.11 (dt, 2 H, 3-H, *J* = 1.9 Hz, *J* = 6.7 Hz), 1.95 (m, 2 H, 2-H). − $^{13}C\{^1H\}$ NMR (D_2O): δ = 106.9 (C-1′′), 97.8 (C-1′), 82.0, 77.8, 75.5, 74.0, 72.6, 71.7 (C-2′, C-3′, C-4′, C-2′′, C-3′′, C-4′′, C-5′′), 66.9 (C-1), 62.8, 60.9 (C-5′, C-6′′), 38.7 (C-3), 27.2 (C-2). − HR-MS: $C_{14}H_{30}NO_{19}P_3$ $[M - H]^-$ calcd. 608.0546; found 608.0541. − ES-MS; *m/z*: 608 $[M - H]^-$.

1-(3-(*p*-Benzoyldihydrocinnamido)propyl)-3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranoside 2,3′,4′-Trisphosphate (8) (Na^+ Salt): Compound **30** (Et_3NH^+ salt, 17 mg, 15 μmol) was coupled with the activated ester derivative **21** (16 mg, 45 μmol) as described by Prestwich et al.[29] Purification was effected as described for compound **7**, to give compound **8** as the Na^+ salt. Yield 14 mg (Na^+ salt, 14 μmol, 95%). − ^1H NMR (600 MHz, HH-COSY, D_2O): δ = 7.76−7.72 (m, 4 H, H arom), 7.69 (t, 1 H, H arom, *J* = 7.4 Hz), 7.55 (t, 2 H, H arom, *J* = 7.9 Hz), 7.37 (d, 2 H, H arom, *J* = 8.1 Hz), 5.28 (s, 1 H, 1′-H), 5.15 (d, 1 H, 1′′-H, $J_{1'',2''}$ = 3.8 Hz), 4.47 (dd, 1 H, 2′-H, $J_{2',3'}$ = 4.5 Hz, $J_{2',P}$ = 7.8 Hz), 4.37 (q, 1 H, 3′′-H, *J* = 7.5 Hz), 4.18 (dd, 1 H, 3′-H, $J_{3',4'}$ = 7.4 Hz), 4.09 (m, 1 H, 4′-H), 3.97 (q, 1 H, 4′′-H, *J* = 8.1 Hz), 3.78 (dd, 1 H, 6a′′-H), 3.68−3.63−3.58 (m, 4 H, 5′′-H, 5b′-H, 6b′′-H, 1a-H), 3.28 (dt, 1 H, 1b-H, $J_{1,2}$ = 6.2 Hz, $J_{1a,b}$ = 10.0 Hz), 3.18 (dt, 2 H, 3-H, *J* = 6.5 Hz, *J* = 13.5 Hz), 3.06 (t, 2 H, 6-H, *J* = 6.7 Hz), 2.63 (t, 2 H, 7-H, *J* = 7.2 Hz), 1.63 (m, 2 H, 2-H). − $^{13}C\{^1H\}$ NMR (D_2O): δ = 134.2, 131.6, 131.0, 129.5, 129.3 (CH arom), 106.6 (C-1′′), 96.4 (C-1′), 81.7, 77.9, 75.7, 75.5, 73.0, 72.5, 71.7 (C-2′, C-3′, C-4′, C-2′′, C-3′′, C-4′′, C-5′′), 66.1 (C-1), 63.3, 60.9 (C-5′, C-6′′), 37.8, 36.8 (C-6, C-7), 32.3 (C-3), 28.9 (C-2). − ^{31}P NMR (D_2O): 1.89, 0.61. − HR-MS: $C_{30}H_{42}NO_{21}P_3$ $[M - H]^-$ calcd. 844.1383; found 844.1390. − ES-MS; *m/z*: 846 $[M + H]^+$, 905 $[M + Na]^+$.

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