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Synthesis and Biological Evaluation of Cyclophostin: A 5′,6″-Tethered Analog of Adenophostin A

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Abstract—The synthesis, conformational analysis and biological evaluation of 5′-6″-tethered adenophostin A, so-called cyclophostin **14**, and its de-adeninylated analog **15** are described. They are prepared via ring-closing metathesis of diolefin **28**, consecutive coupling of the central building block **33** to 6-*N*-benzoyladenine or propargyl alcohol, respectively, followed by phosphorylation and deprotection. NMR spectroscopy and a molecular dynamics simulation indicated that the 5′-6″-tether induces a conformational change from 2′-*endo*/*syn* in **1** to 3′-*endo*/*anti* in **14**. The unexpected small loss of Ca^{2+}-releasing potency of cyclophostin **14**, which is reflected by the low EC_{50}/IC_{50} ratio in comparison with cycloribophostin **15**, suggests that the interaction of the adenine with IP_3R plays a decisive role in determining the high activity of adenophostin A (**1**).

Introduction

The gluconucleosides adenophostin A and B (**1** and **2**) exhibit ~10–100 times higher Ca^{2+}-mobilizing potencies in comparison with the natural second messenger D-*myo*-inositol 1,4,5-trisphosphate (IP_3, **4**).[1] Despite many synthetic efforts in determining the minimal structural requirements for the high activities of **1** and **2**, no simplified analogs displaying a higher activity than IP_3 (**4**) have been reported. The observed[1c] ~1000-fold reduced binding affinity of the 2′-dephosphorylated derivative **3** and the IP_3-like potencies found for the de-adeninylated analogs **5**–**7**[2] show that the extremely high potency of adenophostin A (**1**) is mainly governed by the adenine and the 2′-phosphate moieties. Additional studies have revealed that the Ca^{2+}-releasing activity of conformationally flexible analogs of **1** is significantly reduced. For instance, the hydroxyethyl glucosides **9**[3] and **10**[4] exhibit ~10-fold diminished potencies relative to **5**, whereas acyclophostin **11**[5] is a pH-dependent partial agonist. It has been proposed that the tripodal arrangement of the phosphates in adenophostin A (**1**) may stabilize a long-range interaction with the IP_3 receptor (IP_3R).[6] The latter is endorsed by molecular modeling[7,3b] studies, which showed that the 2′-phosphate in **1** occupies a more remote position from the *trans*-3″,4″-bisphosphate than the corresponding 1-phosphate in IP_3 (**4**). We recently reported[8] that the ~20-fold decreased activities of the rigid analogs spirophostin (3*R*)-**12** and (3*S*)-**13** relative to IP_3 (**4**) can be ascribed to an improper spatial orientation of the non-vicinal 3-phosphates. The aforementioned observations imply that a cooperative effect of the adenine moiety and the 2′-phosphate may be responsible for the enhanced activity of adenophostin A (**1**). It was envisaged that a non-de-adeninylated analog in which the conformational freedom of the isolated 2′-phosphate function is restricted would be of value in probing the possible existence of the proposed cooperative effect of the adenine.

In this paper, we describe the synthesis of the conformationally restricted adenophostin **14**, so-called cyclophostin, and its de-adeninylated analog **15**. In addition, the biological activity of both analogs in terms of conformational behavior is assessed by NMR spectroscopy and molecular modeling (Fig. 1).

Results and Discussion

Comparison of the biological activities of furanophostin **7**[9] and ribophostin **6**,[2] as well as those of xylose-derivative **10** and glucopyranose **9**,[3] shows that HO-5′ and HO-6″ do not contribute to the activity of **1**. Moreover, a recent molecular modeling study[7] of adenophostin A (**1**) revealed that both hydroxyls are in close proximity, inferring that the rotational freedom of **1** can be limited by anchoring either

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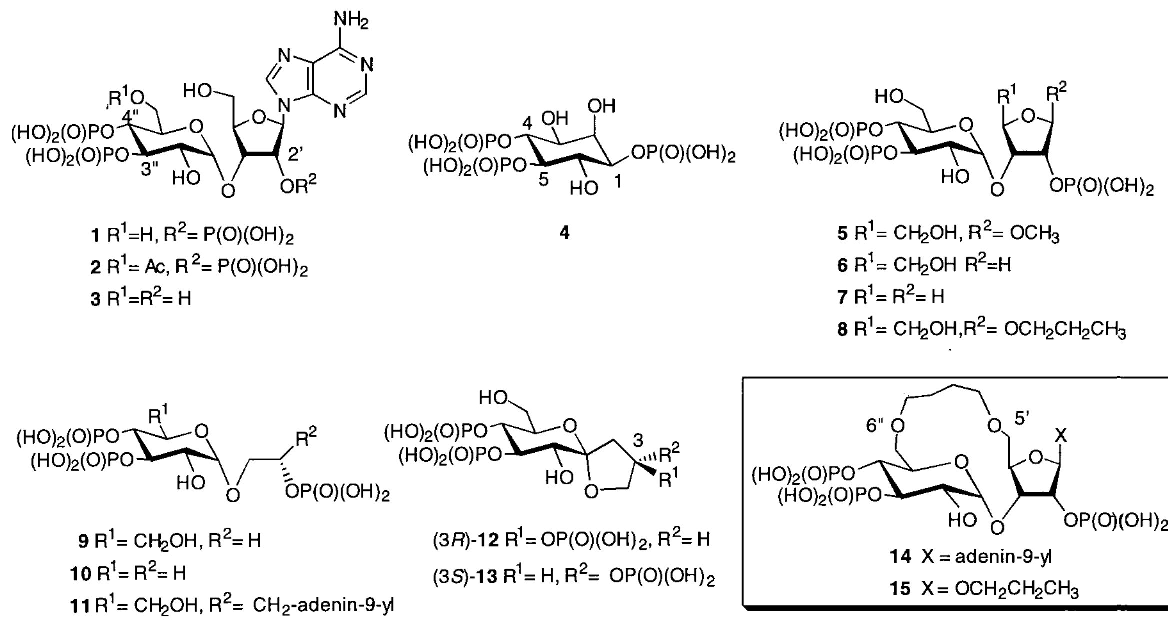
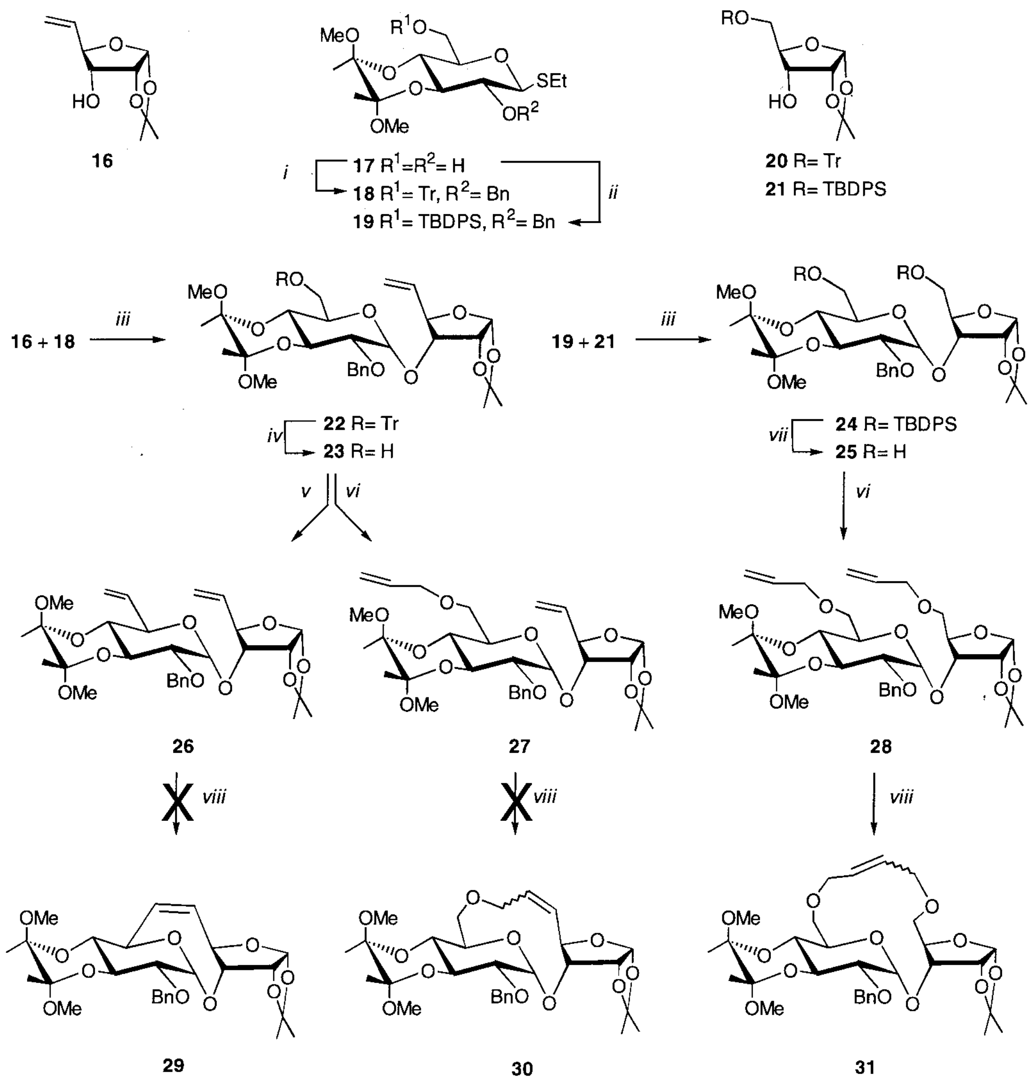


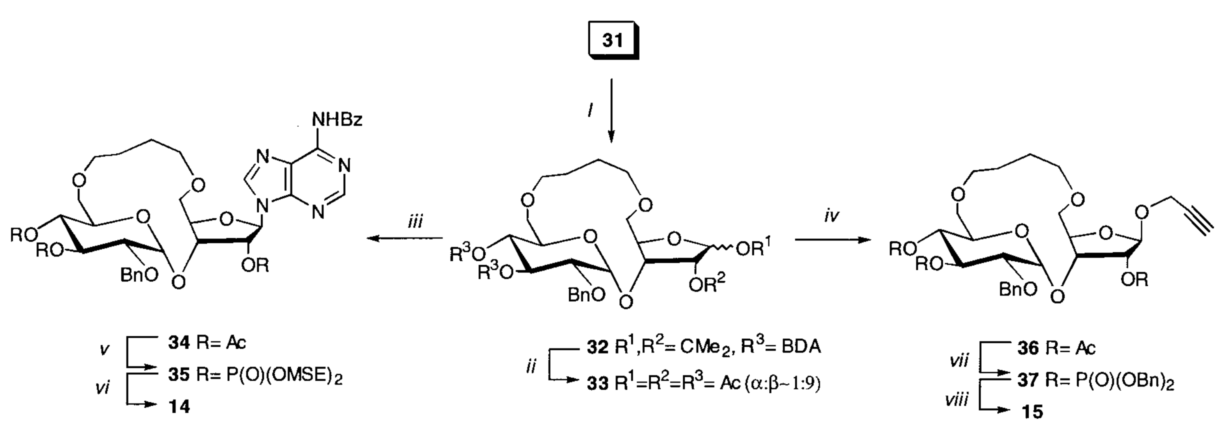
Figure 1. Structures of adenophostin A (**1**) and B (**2**), IP_3 (**4**), ribophostins **5–8**, acyclophostin **11**, spirophostins **12** and **13,** and cyclophostins **14** and **15**.

HO-5′ to HO-6″ or CH_2-5′ to CH_2-6″ with an appropriate alkyl spacer. To this end, attention was focused on the synthesis of disaccharides **26–28** (see Scheme 1) which, after ring-closing metathesis[10] (RCM), would lead to the respective eight-, eleven- and fourteen-membered ring derivatives **29–31**.

The synthesis of key dienes **26** and **27** commenced with *N*-iodosuccinimide (NIS)-mediated[11] coupling of known olefin **16**[12] with thioglucopyranoside **18**, prepared by tritylation and subsequent benzylation of known **17**,[16] to afford the α-linked disaccharide **22** in 86% yield. Detritylation of **22** under the influence of *p*-toluenesulfonic acid gave disaccharide **23**, which was readily converted to diene **26** by Swern oxidation of the primary hydroxyl group and subsequent Wittig olefination. Alternatively, allylation of **23** gave the 6′-*O*-allyl derivative **27**. Having the dienes **26** and **27** in hand, the formation of the eight- and eleven-membered rings **29** and **30** by RCM using the Grubbs' catalyst[14] $(PCy_3)_2Cl_2Ru{=}CHPh$ was undertaken. It was established that the bis-vinyl substrate **26** failed to give the eight-membered ring **29** under a variety of reaction conditions. On the other hand, RCM of the 6′-*O*-allyl-4-vinyl derivative **27** led to the exclusive formation of the unwanted 6′-tethered dimer in 69% yield. The outcome of these experiments may be explained by the steric congestion of the vinyl functions[15] and an indomitable ring strain.[16] In order to overcome the problems encountered in the above cyclizations, the sterically less demanding 5,6′-di-*O*-allyl derivative **28** was used for the construction of the fourteen-membered macrocycle **31** (see Scheme 1). Thus, coupling of 5-*O*-trityl-1,2-isopropylidene-α-D-ribofuranoside[26] (**20**) with donor **18** gave the α-linked dimer **24** (R=Tr). However, ensuing detritylation of **24** (R=Tr) under the influence of 1% *p*-TsOH in CH_2Cl_2/MeOH (1/1, v/v) was accompanied by concomitant cleavage of the glucosidic bond. The latter disadvantage could be circumvented as follows. Condensation of partially protected acceptor **21**[17] with donor **19**, prepared by silylation and benzylation of **17**, gave dimer **24** which, in turn, was readily transformed into diene **28** by desilylation (→**25**) and allylation in 72% yield over the last three steps. Gratifyingly, subjection of **28** to RCM proceeded uneventfully to give **31** as a mixture of *E*/*Z* isomers. Selective hydrogenation (Scheme 2) of the olefin bond in **31** with H_2/PtO_2 gave homogeneous **32**, the structure of which was fully ascertained by NMR spectroscopy and mass spectrometry. Acid-mediated deprotection[13] of **32** and subsequent acetylation afforded key building block **33**. Vorbrüggen-type condensation of **33** with silylated N^6-benzoyladenine in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave the tethered adenosine derivative **34** as a single isomer. The introduction of the three phosphate groups was performed by the following three-step sequence of reactions.[17] Selective deacetylation of **34** by short treatment with potassium *t*-butoxide in MeOH, followed by 1*H*-tetrazole-assisted phosphitylation of the alcohol functions with *N*,*N*-diisopropyl-bis-[2-(methylsulfonyl)-ethyl] (MSE) phosphoramidite,[18] and in situ oxidation of the intermediate phosphite triesters with *t*-butylhydroperoxide, gave trisphosphate **35**. Deprotection of the latter was affected in two consecutive steps involving removal of the base-labile groups with Tesser's base,[19] and hydrogenolysis of the 2′-*O*-benzyl ether to afford, after purification by HW-40 gel filtration and Dowex-ion exchange chromatography, homogeneous cyclophostin **14** (Na^+-salt). The de-adeninylated analog of **14**, i.e. cycloribophostin **15**, was prepared via deacetylation of propargyl glycoside **36**, obtained by glycosylation of propargyl alcohol with tetraacetate **33**, and phosphitylation of the intermediate triol with *N*,*N*-diisopropyl-dibenzyl phosphoramidite,[20] to furnish trisphosphate **37**. Debenzylation of **37** by hydrogenolysis, followed by purification as described earlier, gave homogeneous target compound **15**. The identity of **14** and **15** was fully ascertained by ^1H, ^{13}C and ^{31}P NMR spectroscopy, as well as by high resolution mass spectrometry.



Scheme 1. Reagents and conditions: (*i*) a. TrCl, pyridine, 16 h, 85%; b. NaH, BnBr, DMF, 2 h, 93%. (*ii*) a. TBDPSCl, pyridine, 16 h, 78%; b. NaH, BnBr, DMF, 2 h, 91%. (*iii*) NIS, TfOH, Ms 4 Å, Et_2O or toluene/1,4-dioxane, 1/3, v/v, 1 h, **22**: 86%, **24**: 85%. (*iv*) 1% *p*-TsOH, CH_2Cl_2/MeOH, 1/1, v/v, 4 h, 84%. (*v*) a. $(C(O)Cl)_2$, Et_3N, DMSO, CH_2Cl_2, −60°C, 2 h; b. $MePPh_3Br$, *n*-BuLi, THF, 0°C, 2 h, 71%, 2 steps. (*vi*) NaH, allylbromide, DMF, 3 h, **27**: 70%; **28**: 89%. (*vii*) 1 M TBAF, THF, 50°C, 16 h, 95%. (*viii*) 5 mol% $RuCl_2(PCy_3)_2CHPh$, 0.025 mM in toluene, 16 h, **29**: 0%, **30**: 0% (69% of 6′-*O*-allyl dimer formed), **31**: 75%.



Scheme 2. Reagents and conditions: (*i*) PtO_2, H_2, 2 h, quant. (*ii*) a. HOAc/H_2O/$(HOCH_2)_2$, 14/6/3, v/v/v, reflux, 90 min.; b. Ac_2O/pyridine, 4 h, 92%, 2 steps. (*iii*) a. HMDS, pyridine, 6-*N*-benzoyladenine, 7 h; b. TMSOTf, 1,2-dichloroethane, reflux, 16 h, 82%. (*iv*) propargyl alcohol, TMSOTf, $(CH_2Cl)_2$, 6 h, 82%. (*v*) a. *t*-BuOK (1 M in MeOH), 1 min; b. $(MSEO)_2PN(i\text{-}Pr)_2$, 1*H*-tetrazole, 30 min; c. *t*-BuOOH, 30 min. (*vi*) a. NaOH (4 M)/1,4-dioxane/MeOH, 1/14/5, v/v/v, 16 h; b) H_2, Pd-black, H_2O, 16 h, 56% (based on **34**). (*vii*) a. NaOMe, MeOH, 1 h; b. $(BnO)_2PN(i\text{-}Pr)_2$, 1*H*-tetrazole, 30 min; c. *t*-BuOOH 30 min, 60%. (*viii*) H_2, 10% Pd/C, 1,4-dioxane/*i*-PrOH/H_2O (4/2/1, v/v/v), 16 h, 84%.

Table 1. Coupling constants (in Hz) of adenophostin A (**1**), cyclophostins **14** and **15** and ribophostin **8**

Compound	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	Conformation	
				Ribose	Adenine
Adenophostin A (**1**)[a]	6.6	5.1	2.9	C-2′-*endo* (71%)[b]	*syn*
Cyclophostin (**14**)[c]	3.0	4.8	6.7	C-3′-*endo* (73%)[b]	*anti*
Ribophostin (**8**)[d]	~0	3.2	9.8	C-3′-*endo* (~100%)[e]	–
Cycloribophostin (**15**)[c]	~0	4.4	9.7	C-3′-*endo* (~100%)[e]	–

[a] See Ref. 7.
[b] Calculated with the conformational analysis program Pseurot v6.3.[30]
[c] Measured at 600 MHz in 50 mM phosphate buffer at p^2H 6.8.
[d] Measured at 300 MHz in D_2O.
[e] Based on the assumption that $10\times{}^2J_{1',2'}$ represents the percentage of 2′-*endo* (S-type) conformer.[21,22]

Conformational Analysis

In the first instance, the ^1H NMR spectroscopic data of cyclophostin **14** and its de-adeninylated analog **15** were compared with those of adenophostin A (**1**).[7] It turned out that the glucose moiety in **14** and **15** adopts, based on the coupling constants of the glucose ring protons (see Experimental section), a $^4C_1$ conformation as in **1**. In contrast, the coupling constants of the ribose protons (see Table 1) clearly show[21,22] that the C-3′-*endo* conformation in both **14** and **15** prevails over the C-2′-*endo* conformation of adenophostin A (**1**). The same holds, gauged by the absence of a vicinal coupling between H-1′ and H-2′ ($J_{1',2'}$~0), for ribophostin **8**.[2c,13]

As can be seen in Fig. 2, the NOEs between H-8 of the adenine and H-1′, H-2′ and H-3′ of the ribose ring clearly show that the nucleobase in cyclophostin **14** adopts an *anti* conformation. Moreover, the earlier assigned preference of **14** in adopting the C-3′-*endo* conformation is endorsed by the absence of a NOE signal between H-1′ and H-4′.

The effect of the 5′,6″-tether on the position of the 2′-phosphate (P-2′) and the conformational rigidity was investigated by a molecular dynamics simulation of cyclophostin **14**. The data of the global minimum conformation, presented in Fig. 4A and Table 2 (entry 3), clearly indicate that the distances between the *trans*-3″,4″-bisphosphate and P-2′ do not deviate substantially from those observed[7] in adenophostin A (**1**). The different values of τ_c and χ (see Table 2 and Fig. 3) of the global minimum 3′-*endo*/*anti* conformation of cyclophostin **14** are in accord with its NMR-spectroscopic data (see Table 1). Superimposition of cyclophostin **14** and adenophostin A (**1**), as illustrated in Fig. 4A, showed that the distance between the individual 2′-phosphates is 1.4 Å. Furthermore, it can be seen that the triangular arrangement of the phosphates in **14** is slightly smaller than the phosphorous triad in **1** and that the distances between C-5′ and C-6″ are the same (i.e. 4.4 Å, see entries 2 and 3 in Table 2). It can also be derived from the superimposition of the four lowest energy conformers of **14** (see Fig. 4B) that the conformation of the tethered disaccharide is constrained, whereas the adenine nucleobase adopts several preferred positions in the *anti* conformation. Apart from this, the presence of three conformers of cyclophostin **14** having a 2′-*endo* conformation similar to

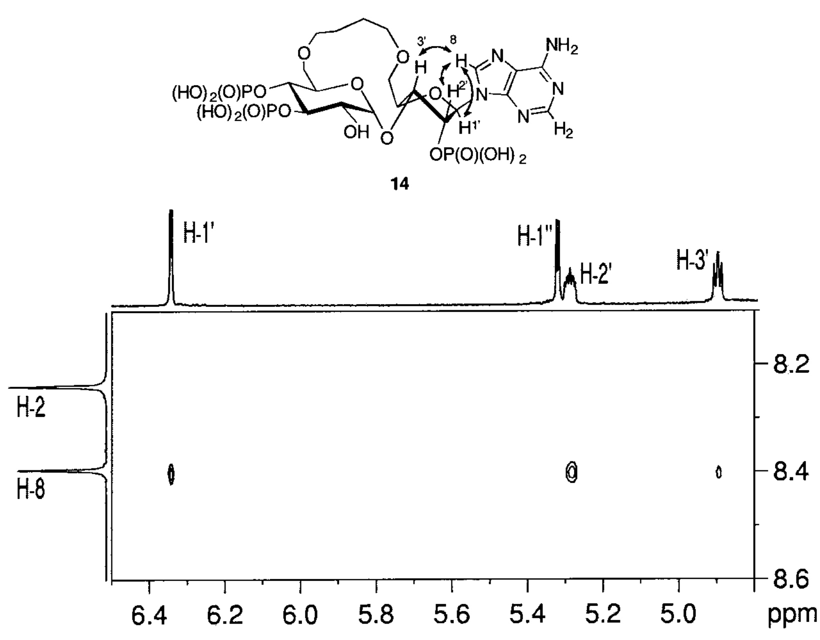


Figure 2. Part of the 600 MHz NOESY spectrum of cyclophostin **14** (10 mM) in 50 mM phosphate buffer in D_2O at p^2H 6.8.

Table 2. Conformational data for adenophostin A (**1**), IP_3 (**4**) and cyclophostin (**14**).[29]

Entry	Compound[a]	τ_a	τ_b	τ_c	τ_d	χ	Distance (Å)					
							ΔE[b]	O5′–O6″	C5′–C6″	P2′–P4″ (P1–P4)[c]	P2′–P3″ (P1–P5)[c]	P3″–P4″ (P4–P5)[c]
1	IP_3 (**4**)	–	–	–	–	–	–	–	–	8.1	6.9	4.1
2	Adenophostin A **1** (2′-*endo*/*syn*)	59°	100°	−23°	−127°	57°	–	4.9	4.4	9.6	8.2	4.1
3	Cyclophostin **14** (3′-*endo*/*anti*)	61°	85°	44°	−126°	178°	–	3.0	4.4	9.2	7.4	3.9
4	**14** (2′-*endo*/*syn*)	54°	87°	−17°	92°	−60°	17.6	3.0	3.1	8.9	7.1	3.9
5	**14** (2′-*endo*/*anti*)	90°	101°	−25°	−116°	−125°	18.0	3.3	3.5	8.9	7.0	3.8
6	**14** (2′-*endo*/*syn*)	87°	98°	−22°	−119°	57°	19.2	3.2	3.4	8.9	7.1	3.8

[a] Modeling of IP_3 and adenophostin A: see Ref. 7.
[b] Conformational energies relative to most stable conformer in entry 3 (kJ mol^{-1}).
[c] Numbering for IP_3.

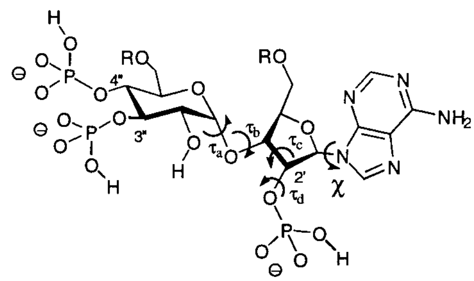


Figure 3. Torsion angles τ and χ assigned in molecular modeling of adenophostin A (**1**, R=H) and cyclophostin **14** [R,R=$(CH_2)_4$].

adenophostin A (**1**) was established (entries 4–6). However, the latter conformers are less stable (i.e. ~17 kJ mol^{-1}) than the energy-minimized 3′-*endo*-puckering structure (entry 3).

In summary, based on the molecular modeling results it may be concluded that the 3′-*O*-α-glucosyl ribose moiety in cyclophostin **14** closely resembles the geometry of the glucosidic bond in adenophostin A (**1**) and that the distance between the C-5′ and C-6″ positions is preserved. On the other hand, the adenosine moiety undergoes a conformational change from 2′-*endo*/*syn* in **1** to 3′-*endo*/*anti* in **14**.

Biological Evaluation

The IC_{50} values (see Table 3) of cyclophostin **14** and cycloribophostin **15**, as well as those of adenophostin A (**1**), IP_3 (**4**) and ribophostin **8**[2c,13] were determined in ^{3}H-IP_3 displacement binding experiments using bovine adrenal cortex membranes.[23] The relative displacing potencies of adenophostin A (**1**) and IP_3 (**4**) (entries 1 and 2) are consistent with earlier reported data while those of cyclophostin **14** (entry 3) and ribophostin **8** (entry 4) are similar to IP_3. The de-adeninylated analogs ribophostin **8** and cycloribophostin **15** (entry 5) both exhibit a ~20-fold decreased binding affinity to IP_3R in comparison with adenophostin A (**1**, entry 2) and cyclophostin **14** (entry 3), respectively. Moreover, the 5′,6″-tethered ligands **14** and **15** display a consistent ~15-fold diminished binding affinity in comparison with adenophostin A (**1**) and ribophostin **8**.

A functional response to cyclophostins **14** and **15** was studied by measuring the $^{45}Ca^{2+}$-release from intracellular stores upon binding to IP_3Rs in permeabilized SH-SY5Y neuroblastoma cells[24] in comparison with the activities of adenophostin A (**1**), IP_3 (**4**) and ribophostin **8** (see Table 4). Perusal of Table 4 reveals that the relative potencies and slope (*h*) of the concentration–response curves of all analogs are in agreement with the binding data presented in Table 3. Interestingly, the Ca^{2+}-mobilizing potency of cyclophostin **14** (entry 3) is now only ~5-fold decreased in comparison with adenophostin A (**1**), whereas the tethered ribophostin derivative **15** is ~13-fold less effective than the corresponding ribophostin **8**.

The respective binding and Ca^{2+}-release data in Tables 3 and 4 were compared by calculating the EC_{50}/IC_{50} ratios (see Table 4), which give an indication of the efficacy of each of the ligands. The relatively high value for IP_3 (**4**) (entry 1) can perhaps be ascribed, despite the short (30 s) incubation period, to partial metabolism of IP_3 in the $^{45}Ca^{2+}$-assay. The metabolically stable adenophostin A (**1**, entry 2) and its de-adeninylated analog **8** (entry 4) have similar EC_{50}/IC_{50} ratios. Despite the substantial loss of biological activity, the efficacy of the tethered cycloribophostin **15** (entry 5) is entirely predictable. In contrast, the EC_{50}/IC_{50} ratio of cyclophostin **14** (entry 3) is ~3-fold reduced, illustrating that the Ca^{2+}-releasing potency of **14** is less affected than the binding affinity for IP_3R.

In summary, the binding affinity of cyclophostin **14** and cycloribophostin **15** is in both cases ~15-fold diminished in comparison with adenophostin A (**1**) and ribophostin **8**, respectively. However, the Ca^{2+}-releasing activity of **14** is only ~5-fold lower than that observed for **1** (still ~5-fold higher than IP_3), which is in contrast to the ~13-fold reduced potency of **15**, relative to **8**.

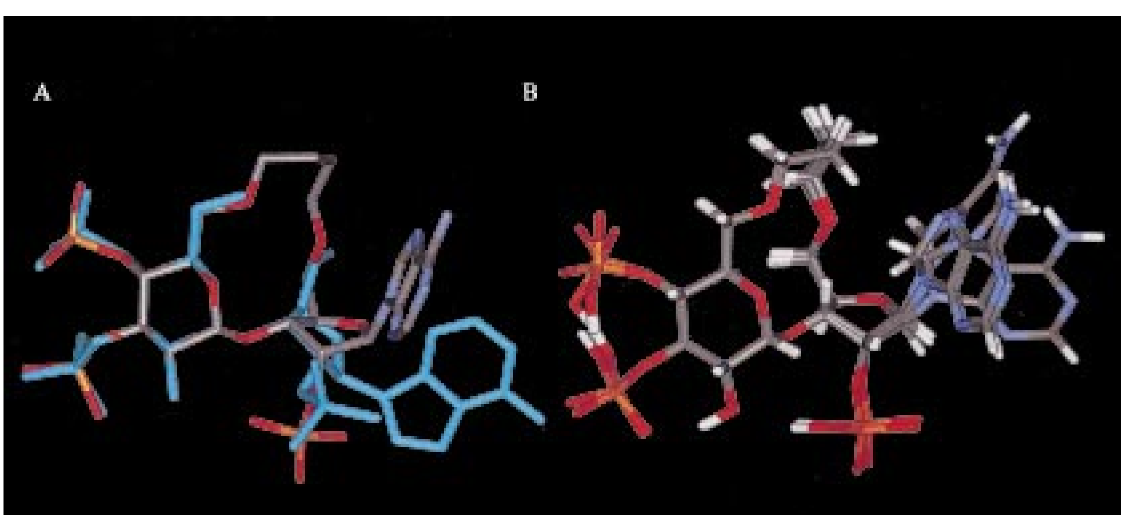


Figure 4. Graphical representation of the low-energy conformers with superimposition of the six-membered rings: A. global energy minimized conformations of cyclophostin **14** (blue) and adenophostin A (**1**); B. superposition of the four lowest-energy conformers of cyclophostin **14**.

Table 3. ^{3}H-IP_3-displacement/binding IC_{50} values of cyclophostins **14** and **15** in comparison with IP_3 (**4**), adenophostin A (**1**) and ribophostin **8** (values are shown as ±s.e. mean for the concentration which causes 50% of specific ^{3}H-IP_3 displacement (IC_{50}), with *h* as the slope of the concentration-response curve, for *n* experiments)

Entry	Compound	$-\log IC_{50}$	IC_{50} value (nM)	Ratio	*h*	n
1	IP_3 (**4**)	8.13±0.05	7.4	18	0.99±0.01	5
2	Adenophostin A (**1**)	9.39±0.07	0.41	1	1.51±0.13	4
3	Cyclophostin (**14**)	8.23±0.05	5.9	14	1.20±0.05	4
4	Ribophostin (**8**)	8.08±0.09	8.3	20	0.79±0.03	4
5	Cycloribophostin (**15**)	6.94±0.04	114	277	0.80±0.02	6

Table 4. $^{45}Ca^{2+}$-release EC_{50} values of cyclophostins **14** and **15** in comparison with IP_3 (**4**), adenophostin A (**1**) and ribophostin **8** (values are shown as ±s.e. mean for the concentration which causes 50% of maximal $^{45}Ca^{2+}$ release (EC_{50}), with *h* as the slope of the concentration-response curve, the % release is relative to ionomycin-induced Ca^{2+} release, for *n* experiments)

Entry	Compound	$-\log EC_{50}$	EC_{50} value (nM)	Ratio	Release (%)	*h*	EC_{50}/IC_{50} ratio	*n*
1	IP_3 (**4**)	6.73±0.09	188	27	75.7±5.8	0.98±0.05	25	5
2	Adenophostin A (**1**)	8.15±0.04	7.0	1	80.3±5.4	1.51±0.08	17	4
3	Cyclophostin (**14**)	7.42±0.08	38.2	5	79.9±4.7	1.44±0.10	**6.5**	4
4	Ribophostin (**8**)	6.84±0.09	145	21	76.6±3.9	1.17±0.07	18	4
5	Cycloribophostin (**15**)	5.73±0.13	1858	265	74.0±6.9	0.91±0.09	16	4

Conclusion

The synthesis of the fourteen-membered macrocycle cyclophostin **14**, as well as its de-adeninylated analog **15**, has been accomplished via ring-closing metathesis of carbohydrate diene **28**. NMR spectroscopy and molecular modeling studies of these conformationally restricted analogs of adenophostin A (**1**) revealed that the 5′,6″-*O*-*n*-butyl tether in **14** and **15** induces a C-3′-*endo*/*anti* conformation, rather than a C-2′-*endo*/*syn* conformation as in **1**. Although the mode of binding is at present not fully understood, the ~5-fold higher Ca^{2+}-releasing potency relative to IP_3 (**4**) indicates that cyclophostin **14**, despite the presence of a conformational restraint, closely resembles the bioactive conformation of adenophostin A (**1**). Moreover, the unexpected small loss of Ca^{2+}-mobilizing potency of cyclophostin **14**, which is reflected by the low EC_{50}/IC_{50} ratio in comparison with cycloribophostin **15**, shows that the loss of activity caused by the 5′,6″-tether can still be compensated by the adenine nucleobase. The latter implies that the adenine may have a more decisive influence on the enhanced activity of adenophostin A (**1**) than the orientation of the 2′-phosphate.[25] The cooperative effect of the adenine and the 2′-phosphate may be studied in more detail by varying the length of the 4C-tether in **14** or limiting the conformational freedom of the nucleobase by replacing the adenine with 8-bromoadenine.

Experimental

General methods and materials

CH_2Cl_2 and toluene were dried by distillation from P_2O_5 (5 g L^{-1}). Et_3N and pyridine were refluxed for 2 h in the presence of CaH_2 (5 g L^{-1}) and subsequently distilled. 1,2-dichloroethane (p.a. Rathburn), 1,4-dioxane (p.a. Baker), *i*-propanol (p.a. Baker), DMF (p.a. Baker), DMSO (p.a. Baker), THF (Acros) were stored over molecular sieves 4 Å. CH_3CN (p.a. Rathburn) and MeOH (HPLC-grade, Rathburn) were stored over molecular sieves 3 Å. Acetic acid (p.a. Baker) and acetic anhydride (p.a. Baker) were used as received. Allyl bromide, butane-2,3-dione, camphorsulfonic acid, ethylene glycol, trifluoromethane-sulfonic acid, oxalyl chloride, triphenylmethyl chloride, sodium hydride, *t*-butyldiphenylsilyl chloride, tetrabutylammonium fluoride and propargyl alcohol (Acros), Dowex® 50WX4, methyltriphenylphosphonium bromide and di-*t*-butyl peroxide (Fluka), *N*-iodosuccinimide, trimethylsilyl trifluoromethanesulfonate and 10% palladium on charcoal were purchased from Aldrich. Benzyl bromide, imidazole and *p*-toluenesulfonic acid (Merck) were used as received. *N*,*N*-Diisopropyl-bis-[2-(methylsulfonyl)ethyl] phosphoramidite[18] and *N*,*N*-diisopropyl-dibenzyl phosphoramidite[20] were prepared as described. All experiments 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). Compounds were visualized by UV light and by spraying with 20% sulfuric acid in MeOH followed by charring at 140°C. Column chromatography was performed on silica gel 60, 0.063–0.200 mm (Baker). NMR spectra were recorded with a Bruker WM-200 (^{1}H and ^{13}C at 200 and 50.1 MHz, respectively) and a Bruker WM-300 spectrometer (^{1}H at 300 MHz). ^{1}H- and ^{13}C-chemical shifts are given in ppm (δ) relative to tetramethylsilane as an internal standard. Mass spectra were recorded on a Finnigan MAT TSQ-70 or PE-SIEX API 165 mass spectrometer equipped with an electrospray inonization (ES) interface. HRMS (ES) spectra were measured with a Finnigan MAT 900 double focusing mass spectrometer equipped with an ES interface. The samples of the target compounds **14** and **15** were prepared in a mixture of isopropanol/H_2O (80/20, v/v) containing 1.0×10^{-4} M NaOAc, the clusters of which were used as internal standards in the negative ion detection mode.

(2′S,3′S) Ethyl 2-*O*-benzyl-3,4-di-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thio-6-*O*-trityl-β-D-glucopyranoside (18). Trityl chloride (5.19 g, 18.5 mmol) was added to a solution of **17**[13] (5.71 g, 16.8 mmol) in pyridine (25 mL) and the mixture was stirred overnight at 50°C. The reaction mixture was quenched with MeOH (1.0 mL) and concentrated. The residue was dissolved in EtOAc (100 mL) and washed with sat. aq. solution of $NaHCO_3$ (2×25 mL) and H_2O (25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. Traces of pyridine were removed by coevaporation with toluene (2×10 mL). The residue was applied onto a column of silica gel (Et_2O/light petroleum/Et_3N, 66/33/1, v/v/v) to yield the tritylated product as a white solid (6.69 g, 11.6 mmol). Mp 74°C. R_f 0.68 (Et_2O/light petroleum, 2/1, v/v). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 143.5 (Cq Ph), 128.4–125.0 (CH arom), 99.3, 99.0 (2×Cq BDA), 85.8 (C-1), 77.0, 73.7, 69.5, 65.2 (C-2, C-3, C-4, C-5), 61.6 (C-6), 47.6 (2×OCH_3 BDA), 23.5 (CH_2 SEt), 17.4, 17.2 (2×CH_3 BDA), 15.3 (CH_3 SEt). Sodium hydride (60% wt, 0.69 g, 17.3 mmol) was added to a cooled (0°C) solution of tritylated **17** (6.69 g, 11.6 mmol) in DMF (60 mL), followed by the addition of benzyl bromide (1.50 mL, 12.7 mmol). When TLC analysis showed complete conversion of starting material, excess sodium hydride was destroyed with MeOH (0.5 mL) and the reaction mixture was diluted with Et_2O (50 mL). H_2O (15 mL) was added and the DMF/H_2O layer was extracted with Et_2O. The organic layer was rinsed with H_2O (2×15 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. Compound **18** was obtained as a white solid by crystallization from Et_2O/light petroleum. Yield 7.42 g (11.0 mmol, 65% over two steps). Mp 69°C. R_f 0.85 (Et_2O/light petroleum, 1/1, v/v). ^1H NMR (300 MHz, H–H-COSY, $CDCl_3$): δ 7.52–7.18 (m, 20H, H arom), 4.87 (s, 2H, CH_2 Bn), 4.54 (d, 1H, H-1, $J_{1,2}$=9.5 Hz), 3.95 (t, 1H, H-2, $J_{2,3}$=9.5 Hz), 3.84 (t, 1H, H-3, $J_{3,4}$=8.8 Hz), 3.65–3.41 (m, 3H, H-4, H-5, H-6a), 3.28 (s, 3H, OCH_3 BDA), 3.13–3.08 (m, 1H, H-6b), 3.06 (s, 3H, OCH_3 BDA), 2.99–2.70 (m, 2H, CH_2 SEt), 1.42–1.27 (m, 3H, CH_3 SEt), 1.36, 1.16 (2×s, 6H, 2×CH_3 BDA); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 143.7 (Cq Tr), 138.0 (Cq Bn), 128.4–126.5 (CH arom), 99.2, 99.0 (2×Cq BDA), 85.9 (Cq Tr), 84.3 (C-1), 78.1, 76.7, 74.6, 65.3 (C-2, C-3, C-4, C-5), 75.0 (CH_2 Bn), 61.4 (C-6), 47.8, 47.4 (2×OCH_3 BDA), 23.8 (CH_2 SEt), 17.5, 17.1 (2×CH_3 BDA), 15.0 (CH_3 SEt). $C_{40}H_{46}O_7S$ (671): Calcd C 71.62, H 6.91; found C 71.73, H 6.96.

(2′*S*,3′*S*) Ethyl 2-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3,4-di-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thio-β-D-glucopyranoside (19). *t*-Butyldiphenylsilyl chloride (2.30 mL, 8.87 mmol) was added to a solution of **17**[13] (2.51 g, 7.39 mmol) in pyridine (15 mL) and the mixture was stirred for 2 h. MeOH (0.4 mL) was added and the mixture was concentrated. The residue was dissolved in EtOAc (50 mL) and washed with sat. aq. solution of $NaHCO_3$ (15 mL) and H_2O (15 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. Traces of pyridine were removed by coevaporation with dry toluene (2×10 mL). The crude product (R_f 0.67, Et_2O) was benzylated as described for **18**. Compound **19** was purified by column chromatography (EtOAc/light petroleum, 1/10→1/1, v/v) and obtained as a colorless oil. Yield 4.40 g (6.58 mmol, 89% over two steps). R_f 0.79 (Et_2O/light petroleum, 1/1, v/v). ^1H NMR (300 MHz, H–H-COSY, $CDCl_3$): δ 7.75–7.66 (m, 4H, H arom), 7.47–7.21 (m, 11H, H arom), 4.87 (AB, 2H, CH_2 Bn, J=−12.3 Hz), 4.47 (d, 1H, H-1, $J_{1,2}$=9.4 Hz), 4.16 (dd, 1H, H-3, $J_{2,3}$=8.9 Hz, $J_{2,3}$=9.9 Hz), 4.00 (dd, 1H, H-6, $J_{6a,6b}$=−11.4 Hz, $J_{5,6a}$=1.9 Hz), 3.83 (m, 2H, H-4, H-5), 3.36 (m, 2H, H-2, H-6b), 3.20, 3.18 (2×s, 6H, OCH_3 BDA), 2.74 (m, 2H, CH_2 SEt), 1.36, 1.28 (2×s, 6H, 2×CH_3 BDA), 1.33 (t, 3H, CH_3 SEt, J=−7.5 Hz), 1.04 (s, 9H, CH_3 *t*-Bu Si); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 138.1 (Cq Bn), 135.5, 135.3 (Cq TBDPS), 135.6, 129.5–126.8 (CH arom), 101.1, 101.0 (2×Cq BDA), 84.1 (C-1), 79.8, 79.0, 77.1, 69.0 (C-2, C-3, C-4, C-5), 74.5 (CH_2 Bn), 63.3 (C-6), 47.8 (2×OCH_3 BDA), 26.6 (*t*-Bu Si), 24.5 (CH_2 SEt), 19.2 (Cq *t*-Bu Si), 17.8, 17.6 (2×CH_3 BDA), 15.1 (CH_3 SEt). ES-MS: 689 $[M+Na]^+$. HRMS (ES): Calcd $C_{37}H_{51}O_7SSi$ $[M+H]^+$ 667.3135, found 667.3139 (± 0.0023).

1,2-*O*-Isopropylidene-5-*O*-trityl-α-D-ribofuranoside (20). 1,2-*O*-Isopropylidene-α-D-ribofuranoside[26] (4.8 g, 25.0 mmol) was tritylated as described for the synthesis of **18**. Purification of the product was accomplished by column chromatography (Et_2O/light petroleum/Et_3N, 14/85/1, v/v/v) to give **18** as a white solid (9.13 g, 21.3 mmol, 86%). R_f 0.94 (Et_2O). Mp 101°C. ^1H NMR ($CDCl_3$): δ 7.58–7.18 (m, 15H, H arom), 5.89 (d, 1H, H-1, $J_{1,2}$=4.4 Hz), 4.59 (t, 1H, H-2, $J_{2,3}$=3.7 Hz), 4.17–4.07 (m, 1H, H-4), 4.04–3.87 (m, 1H, H-3), 3.63–3.24 (m, 2H, H-5a, H-5b), 2.30 (d, 1H, OH), 1.57, 1.38 (2×s, 6H, 2×CH_3 isoprop). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 143.5 (Cq Ph), 128.4–126.7 (CH arom), 112.1 (Cq isoprop), 103.8 (C-1), 86.3 (Cq Tr), 79.4, 78.3, 71.8 (C-2, C-3, C-4), 62.7 (C-5), 26.2 (2×CH_3 isoprop). $C_{27}H_{28}O_5$ (432): Calcd C 74.98, H 6.52; found C 75.14, H 6.59.

General glycosylation procedure

A mixture of acceptor **16**, **20** or **21** (1.00 mmol) and donor **18** or **19** (1.00–1.25 mmol) was dried by coevaporation with 1,4-dioxane (3×5 mL) and was stirred for 15 min in the appropriate solvent mixture (10 mL) with powdered molecular sieves (4 Å) under an atmosphere of argon. NIS (0.27 g, 1.20 mmol) and a catalytic amount of TfOH (9 μL, 0.11 mmol) were subsequently added. After completion of the reaction (±30 min) as judged by TLC analysis, the reaction mixture was filtered and diluted with EtOAc (15 mL). The filtrate was washed with aq. $Na_2S_2O_3$ (1 M, 5 mL) and sat. aq. $NaHCO_3$ (5 mL), dried over $MgSO_4$ and concentrated in vacuo to afford the crude product.

(2″*S*,3″*S*) 3-*O*-(2′-*O*-Benzyl-3′,4′-di-*O*-(2″,3″-dimethoxybutane-2″,3″-diyl)-6-*O*-trityl-α-D-glucopyranosyl)-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*allo*-hex-5-enofuranoside (22). Glycosylation of 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*allo*-hex-5-enofuranoside (**16**, 0.93 g, 5.0 mmol) with thioglucoside **17** (4.01 g, 6.0 mmol) was carried out in a mixture of Et_2O/CH_2Cl_2 (50 mL, 10/1, v/v) as described in the general procedure. The product was purified by column chromatography (Et_2O/light petroleum, 1/4→1/1, v/v) to give dimer **22** as a white foam. Yield 3.28 g (4.3 mmol, 86%). R_f 0.31 (Et_2O/light petroleum, 1/1, v/v). ^1H NMR ($CDCl_3$): δ 7.47–7.18 (m, 20H, H arom), 6.01–5.87 (m, 1H, H-5), 5.83 (d, 1H, H-1, $J_{1,2}$=3.7 Hz), 5.47 (d, 1H, H-6a, $J_{5,6a}$=16.8 Hz), 5.29 (d, 1H, H-1′, $J_{1',2'}$=3.7 Hz), 5.23 (d, 1H, H-6b, $J_{5,6b}$=10.2 Hz), 4.82 (s, 2H, CH_2 Bn), 4.76 (t, 1H, H-2, $J_{2,3}$=3.9 Hz), 4.62 (m, 1H, H-3), 4.12 (t, 1H, H-3′, $J_{2'3'}$=$J_{3'4'}$=9.4 Hz), 3.93–3.67 (m, 4H, H-4, H-2′, H-4′, H-5′), 3.32 (m, 1H, H-6a′), 3.29 (s, 3H, OCH_3 BDA), 3.04 (m, 1H, H-6b′), 3.01 (s, 3H, OCH_3 BDA), 1.57, 1.39, 1.32, 1.16 (4×s, 12H, 4×CH_3 isoprop, BDA); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 143.5 (Cq Tr), 138.7 (Cq Bn), 134.3 (C-5), 128.3–126.6 (CH arom), 117.5 (C-6), 112.4 (Cq isoprop), 103.7 (C-1), 99.0 (2×Cq BDA), 95.2 (C-1′), 86.0 (Cq Tr), 78.4, 77.9, 76.4, 76.0, 69.3, 69.1, 66.2 (C-2, C-3, C-4, C-2′, C-3′, C-4′, C-5′), 71.5 (CH_2 Bn), 61.5 (C-6′), 47.6 (2×OCH_3 BDA), 27.3 (2×CH_3 isoprop), 17.5, 17.2 (2×CH_3 BDA). ES-MS: 818 $[M+Na]^+$. $C_{47}H_{54}O_{11}$ (795): Calcd C 71.01, H 6.85; found C 70.93, H 6.79.

(2″*S*,3″*S*) 3-*O*-(2′-*O*-Benzyl-3′,4′-di-*O*-(2″,3″-dimethoxybutane-2″,3″-diyl)-α-D-glucopyranosyl)-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*allo*-hex-5-enofuranoside (23). Dimer **22** (0.30 g, 0.38 mmol) was dissolved in a solution of 1% *p*-TsOH in MeOH/CH_2Cl_2 (7.5 mL, 1/1, v/v) and stirred for 4 h. The mixture was neutralized with Et_3N and concentrated. Purification of the crude product by column chromatography (light petroleum/EtOAc, 1/1, v/v) gave alcohol **23** (0.18 g, 0.32 mmol, 84%) as a white foam. R_f 0.15 (Et_2O/light petroleum, 2/3, v/v); ^1H NMR ($CDCl_3$): δ 7.43–7.26 (m, 5H, H arom), 5.86–5.78 (m, 1H, H-5), 5.81 (d, 1H, H-1, $J_{1,2}$=3.7 Hz), 5.51–5.25 (m, 2H, H-6a, H-6b), 5.13 (d, 1H, H-1′, $J_{1',2'}$=3.7 Hz), 4.78 (AB, 2H, CH_2 Bn, J=−12.4 Hz), 4.69 (t, 1H, H-2, $J_{2,3}$=4.0 Hz), 4.56 (m, 1H, H-3), 4.15 (t, 1H, H-3′, $J_{2'3'}$=$J_{3'4'}$=9.6 Hz), 3.77–3.57 (m, 6H, H-4, H-2′, H-4′, H-5′, H-6a′, H-6b′), 3.32, 3.27 (2×s, 6H, 2×OCH_3 BDA), 2.05 (s, 1H, OH), 1.55, 1.35, 1.32 (3×s, 12H, 4×CH_3 isoprop, BDA); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 138.9 (Cq Ph), 134.3 (C-5), 128.5–127.1 (CH arom), 118.7 (C-6), 112.9 (Cq isoprop), 103.8 (C-1), 99.6, 99.4 (2×Cq BDA), 95.9 (C-1′), 78.9, 78.2, 76.5, 76.2, 69.7, 69.0, 66.1 (C-2, C-3, C-4, C-2′, C-3′, C-4′, C-5′), 71.9 (CH_2 Bn), 60.9 (C-6′), 48.1, 47.8 (2×OCH_3 BDA), 26.6 (2×CH_3 isoprop), 17.8, 17.6 (2×CH_3 BDA).

(2″*S*,3″*S*) 3-*O*-(2′-*O*-Benzyl-3′,4′-di-*O*-(2″,3″-dimethoxybutane-2″,3″-diyl)-6′-*O*-trityl-α-D-glucopyranosyl)-1,2-*O*-isopropylidene-5-*O*-trityl-α-D-ribofuranoside (24 *R*=Tr). Condensation of trityl derivative **20** (0.76 g, 1.1 mmol) with **18** (0.47 g, 1.1 mmol) was performed in a mixture of Et_2O/CH_2Cl_2 (10 mL, 10/1, v/v) as described in the general glycosylation procedure. The disaccharide was purified by column chromatography (Et_2O/light petroleum, 1/4, v/v) to give the title compound as a white foam. Yield 0.85 g (0.82 mmol, 75%). R_f 0.68 (Et_2O/light petroleum, 1/1, v/v); ^1H NMR ($CDCl_3$): δ 7.41–7.05 (H arom), 6.04 (d, 1H, H-1, $J_{1,2}$=3.7 Hz), 5.31 (d, 1H, H-1′, $J_{1',2'}$=3.7 Hz), 4.85 (t, 1H, H-2, $J_{2,3}$=3.8 Hz), 4.76 (s, 2H, CH_2 Bn), 4.36 (dd, 1H, H-3, $J_{3,4}$=9.5 Hz), 4.27 (d, 1H, H-4), 3.87–3.70 (m, 4H, H-2′, H-3′, H-4′, H-5′), 3.57, 3.39 (m, 2H, H-5), 3.27 (m, 1H, H-6a′), 3.15, 3.18 (2×s, 6H, 2×OCH_3 BDA), 2.84 (m, 1H, H-6b′), 1.56, 1.43, 1.30, 1.11 (4×s, 4×CH_3 isoprop, BDA); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 143.5 (Cq Tr), 138.7 (Cq Bn), 112.5 (Cq isoprop), 104.2 (C-1), 99.0 (2×Cq BDA), 94.6 (C-1′), 85.8, 85.7 (2×Cq Tr), 77.4, 76.2, 69.5, 68.4, 59.8 (C-2, C-3, C-4, C-2′, C-3′, C-4′, C-5′), 71.7 (CH_2 Bn), 65.5, 59.8 (C-5, C-6′), 47.9, 47.6 (2×OCH_3 BDA), 26.5 (2×CH_3 isoprop), 17.8, 17.3 (2×CH_3 BDA). ES-MS: 1042 $[M+H]^+$, 1064 $[M+Na]^+$. HRMS (ES): Calcd $C_{65}H_{69}O_{12}$ $[M+H]^+$ 1041.4799, found 1041.4794 (± 0.0029).

(2″*S*,3″*S*) 3-*O*-(2′-*O*-Benzyl-6-*O*-*t*-butyldiphenylsilyl-3′,4′-di-*O*-(2″,3″-dimethoxybutane-2″,3″-diyl)-α-D-glucopyranosyl)-1,2-*O*-isopropylidene-5-*O*-*t*-butyldiphenylsilyl-α-D-ribofuranoside (24). Condensation of compound **21** (0.43 g, 1.00 mmol) with thioglucoside **19** (0.76 g, 1.15 mmol) was accomplished in a mixture of toluene and 1,4-dioxane (10 mL, 3/1, v/v) according to the general glycosylation procedure. Purification was performed by column chromatography (light petroleum/EtOAc, 6/1→1/1, v/v) to afford bis-silylated dimer **24** in 74% yield (0.76 g, 0.74 mmol), as a white foam. R_f 0.57 (light petroleum/EtOAc, 5/1, v/v). ^1H NMR (300 MHz, H–H-COSY, $CDCl_3$): δ 7.76–7.53 (m, 8H, H-arom Ph-Si), 7.43–7.18 (m, 17H, arom Bn, Ph-Si), 5.76 (d, 1H, H-1, $J_{1,2}$=3.9 Hz), 5.24 (d, 1H, H-1′, $J_{1',2'}$=3.9 Hz), 4.80 (s, 2H, CH_2 Bn), 4.64 (t, 1H, H-2, $J_{2,3}$=4.0 Hz), 4.24 (ddd, 1H, H-4, $J_{3,4}$=4.2 Hz, $J_{4,5a}$=2.5 Hz, $J_{4,5b}$=9.2 Hz), 4.15 (t, 1H, H-3′, $J_{3',4'}$=$J_{2',3'}$=9.9 Hz), 4.13 (dd, 1H, H-3), 3.92 (dd, H-5a, $J_{5a,5b}$=−9.8 Hz), 3.88 (t, 1H, H-4′, $J_{4',5'}$=9.8 Hz), 3.82–3.66 (m, 4H, H-5b, H-5′, H-6′), 3.68 (dd, 1H, H-2′), 3.29, 3.26 (2×s, 6H, 2×OCH_3 BDA), 1.52, 1.39, 1.38, 1.33 (4×s, 4×CH_3 isoprop, BDA), 0.99, 0.91 (2×s, 18H, CH_3 *t*-Bu Si); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 139.1 (Cq Bn), 135.9, 135.7, 135.5, 135.4 (CH arom Ph-Si), 133.8, 133.4, 133.0 (Cq Ph-Si), 129.6–127.2 (CH arom Bn, Ph-Si), 112.9 (Cq isoprop), 104.3 (C-1), 99.5 (2×Cq BDA), 95.3 (C-1′), 79.5, 77.2, 76.6, 72.7, 70.4, 69.5, 65.5 (C-2, C-3, C-4, C-2′, C-3′, C-4′, C-5′), 72.0 (CH_2 Bn), 62.0, 61.5 (C-5, C-6′), 48.1, 48.0 (2×OCH_3 BDA), 26.9, 26.8 (2×CH_3 isoprop, CH_3 *t*-Bu Si), 19.3, 19.2 (2×Cq *t*-Bu Si), 18.1, 17.8 (2×CH_3 BDA). ES-MS: 1056 $[M+Na]^+$. $[\alpha]_D^{20}$=+91.0° (*c* 1.0 $CHCl_3$). $C_{59}H_{76}O_{12}Si_2$ (1033): Calcd C 68.57, H 7.41; found C 68.75, H 7.49.

(2″*S*,3″*S*) 3-*O*-(2′-*O*-Benzyl-6,7-dideoxy-3′,4′-di-*O*-(2″,3″-dimethoxybutane-2″,3″-diyl)-α-D-*gluco*-hept-6-enopyranosyl)-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*allo*-hex-5-enofuranoside (26). To a cooled (−60°C) solution of oxalyl chloride (0.38 mL, 4.3 mmol) in CH_2Cl_2 (6.7 mL) under a N_2 atmosphere was added dropwise a solution of DMSO (0.61 mL, 8.3 mmol) in CH_2Cl_2 (3.4 mL). After stirring for 2 min, compound **23** (0.88 g, 1.60 mmol) in CH_2Cl_2 (5 mL) was added dropwise and the reaction mixture was stirred at −60°C for 30 min. Et_3N (2.6 mL, 18.4 mmol) was added and after 10 min the solution was allowed to warm to RT. The mixture was diluted with CH_2Cl_2 (30 mL) and washed with sat. aq. NaCl (2×10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude aldehyde was immediately used without further purification. To a solution of methyltriphenylphosphonium bromide (0.86 g, 2.4 mmol) in THF (18 mL) under a nitrogen atmosphere was added *n*-butyllithium (1.5 mL, 1.6 M in hexanes). After stirring for 1 h, a solution of the aldehyde (1.6 mmol) in CH_2Cl_2 (3.5 mL) was added dropwise at 0°C to the yellow suspension. When TLC analysis showed complete conversion into a more lipophilic product the reaction mixture was filtered over silica gel, concentrated and purified by column chromatography (Et_2O/light petroleum, 1/4, v/v) to afford disaccharide **26** as a colorless oil. Yield 0.62 g (1.12 mmol, 70%). R_f 0.63 (light petroleum/EtOAc, 3/1, v/v); ^1H NMR ($CDCl_3$): δ 7.44–7.22 (m, 5H, H arom), 5.96–5.76 (m, 2H, H-5, H-6′), 5.80 (d, 1H, H-1, $J_{1,2}$=3.7 Hz), 5.52–5.22 (m, 4H, H-6, H-7′), 5.14 (d, 1H, H-1′, $J_{1',2'}$=3.7 Hz), 4.79 (AB, 2H, CH_2 Bn, J=−12.4 Hz), 4.71 (dd, 1H, H-2, $J_{2,3}$=4.4 Hz), 4.57 (dd, 1H, H-3, $J_{3,4}$=9.5 Hz), 4.14 (m, 2H, H-4′, H-3′), 3.72 (dd, 1H, H-5′, $J_{5',6'}$=4.4 Hz, $J_{4',5'}$=9.5 Hz), 3.62 (dd, 1H, H-2′, $J_{2',3'}$=9.3 Hz), 3.42 (t, 1H, H-4′, $J_{3',4'}$=9.5 Hz), 3.32, 3.21 (2×s, 6H, 2×OCH_3 BDA), 1.56, 1.36, 1.35, 1.31 (4×s, 12H, 4×CH_3 isoprop, BDA); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 138.9 (Cq Ph), 134.3 (C-5), 133.6 (C-6′), 128.0–127.2 (CH arom), 118.6 (C-7′), 118.0 (C-6), 112.9 (Cq isoprop), 103.8 (C-1), 99.6, 99.3 (2×Cq BDA), 95.7 (C-1′), 78.8, 78.2, 76.6, 76.2, 70.4, 69.8, 69.2 (C-2, C-3, C-4, C-2′, C-3′, C-4′, C-5′), 71.5 (CH_2 Bn), 48.1, 47.7 (2×OCH_3 BDA), 26.6 (2×CH_3 isoprop), 17.8, 17.6 (2×CH_3 BDA); ES-MS: 566 $[M+NH_4]^+$, 571 $[M+Na]^+$, 587 $[M+K]^+$. HRMS (ES): Calcd $C_{29}H_{41}O_{10}$ $[M+H]^+$ 549.2710, found 549.2714 (± 0.0019).

(2″S,3″S) 3-*O*-(6′-*O*-Allyl-2′-*O*-benzyl-3′,4′-di-*O*-(2″,3″-dimethoxybutane-2″,3″-diyl)-α-D-glucopyranosyl)-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*allo*-hex-5-enofuranoside (27). To a cooled solution (0°C) of dimer **23** (0.20 g, 0.36 mmol) in DMF (3 mL) was added sodium hydride (60% wt, 22 mg, 0.54 mmol). After stirring for 15 min, allyl bromide (34 μL, 0.40 mmol) was added and the reaction mixture allowed to stir for 3 h after which it was quenched with MeOH (0.3 mL). The mixture was diluted with Et_2O (5 mL) H_2O (15 mL) was added and the DMF/H_2O layer was extracted with Et_2O. The organic layer was rinsed with H_2O (2×5 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. After purification by column chromatography (Et_2O/light petroleum, 1/3, v/v) compound **27** was obtained as a white foam. Yield 0.15 g (0.25 mmol, 70%). R_f 0.41 (Et_2O/light petroleum, 1/1, v/v); ^1H NMR ($CDCl_3$): δ 7.43–7.24 (m, 5H, H arom), 5.95–5.73 (m, 2H, H-5, CH allyl), 5.79 (d, 1H, H-1, $J_{1,2}$=3.7 Hz), 5.51–5.18 (m, 4H, H-6, CH_2=CH allyl), 5.14 (d, 1H, H-1′, $J_{1′,2′}$=4.4 Hz), 4.78 (AB, 2H, CH_2 Bn, J=−12.4 Hz), 4.70 (t, 1H, H-2, $J_{2,3}$=3.9 Hz), 4.55 (m, 1H, H-3), 4.17–3.97 (m, 3H, H-4, H-3′, OCH_2 allyl), 3.80–3.60 (m, 6H, H-2′, H-4′, H-5′, H-6′, OCH_2 allyl), 3.31, 3.25 (2×s, 6H, 2×OCH_3 BDA), 1.55, 1.35, 1.33, 1.31 (4×s, 12H, 4×CH_3 isoprop, BDA); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 138.9 (Cq Bn), 134.4, 134.3 (C-5, OCH_2 allyl), 128.0, 127.2 (CH arom Bn), 118.5 (C-6), 116.9 (CH_2=CH allyl), 112.8 (Cq isoprop), 103.8 (C-1), 99.5, 99.3 (2×Cq BDA), 95.8 (C-1′), 78.7, 78.2, 76.5, 76.0, 69.2, 69.0, 65.8 (C-2, C-3, C-4, C-2′, C-3′, C-4′, C-5′), 72.4, 71.9 (CH_2 Bn, CH allyl), 67.5 (C-6′), 48.0, 47.8 (2×OCH_3 BDA), 26.6 (2×CH_3 isoprop), 17.8, 17.6 (2×CH_3 BDA); ES-MS: 594 $[M+H]^+$, 516 $[M+Na]^+$. HRMS (ES): Calcd $C_{31}H_{45}O_{11}$ $[M+H]^+$ 593.2972, found 593.2969 (± 0.0017).

(2″S,3″S) 5-*O*-Allyl-3-*O*-(6′-*O*-allyl-2′-*O*-benzyl-3′,4′-di-*O*-(2″,3″-dimethoxybutane-2″,3″-diyl)-α-D-glucopyranosyl)-1,2-*O*-isopropylidene-α-D-ribofuranoside (28). Compound **24** (1.14 g, 1.10 mmol) was dissolved in a mixture of 1,4-dioxane (15 mL) and a 1.0 M solution of TBAF in THF (3.29 mL). After stirring overnight at 50°C the mixture was concentrated and the oily residue was dissolved in EtOAc (25 mL). The solution was rinsed with sat. aq. NaCl (3×10 mL) and H_2O (10 mL). The organic layer was dried ($MgSO_4$) and concentrated. Purification was effected by column chromatography (EtOAc/MeOH, 1/0→98/2, v/v) to give diol **25** as a white foam. Yield 0.59 g (1.04 mmol, 95%). R_f 0.18 (EtOAc). Allylation of compound **25** (0.57 g, 1.02 mmol) was executed as described for the preparation of **27**. The crude product was purified by column chromatography (Et_2O/light petroleum, 1/6, v/v), to afford **28** as a colorless oil. Yield 0.59 g (0.93 mmol, 89%). R_f 0.90 (EtOAc); ^1H NMR (300 MHz, H–H-COSY, $CDCl_3$): δ 7.42–7.24 (H arom), 5.97–5.83 (m, 2H, 2×CH allyl), 5.80 (d, 1H, H-1, $J_{1,2}$=3.7 Hz), 5.31–5.13 (m, 4H, 2×CH_2CH allyl), 5.18 (d, 1H, H-1′, $J_{1′,2′}$=4.4 Hz), 4.77 (AB, 2H, CH_2 Bn, J=−12.4 Hz), 4.70 (t, 1H, H-2, $J_{2,3}$=4.0 Hz), 4.29 (ddd, H-4, $J_{3,4}$=9.3 Hz, $J_{4,5a}$=1.9 Hz, $J_{4,5b}$=4.0 Hz), 4.18–3.92 (m, 8H, H-3, H-3′, H-4′, H-5′, 2×OCH_2 allyl), 3.81 (m, 1H, H-6a′), 3.75 (dd, 1H, H-5a, $J_{5a,5b}$=−11.2 Hz), 3.65–3.51 (m, 3H, H-2′, H-6b′, H-5b), 3.32, 3.25 (2×s, 6H, 2×OCH_3 BDA), 1.53, 1.35, 1.31 (3×s, 12H, 4×CH_3 isoprop, BDA). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 138.7 (Cq Bn), 134.4, 134.2 (2×CH allyl), 127.8, 127.1 (CH arom Bn), 116.9 (2×CH_2=CH allyl), 112.7 (Cq isoprop), 104.0 (C-1), 99.4, 99.3 (2×Cq BDA), 95.6 (C-1′), 77.4, 76.3, 75.9, 73.1, 69.2, 68.9, 65.5 (C-2, C-3, C-4, C-2′, C-3′, C-4′, C-5′), 72.3, 71.8 (CH_2 Bn, OCH_2 allyl), 67.8, 67.2 (C-6′, C-5), 48.0, 47.7 (2×OCH_3 BDA), 26.5 (2×CH_3 isoprop), 17.5, 17.5 (2×CH_3 BDA); ES-MS: 659 $[M+Na]^+$. $[\alpha]_D^{20}$=+135.4° (c 2.0 $CHCl_3$). HRMS (ES): Calcd $C_{33}H_{49}O_{12}$ $[M+H]^+$ 637.3223, found 637.3219 (± 0.0027). $C_{33}H_{48}O_{12}$ (636): Calcd C 62.25, H 7.60; found C 62.45, H 7.64.

General procedure for ring-closing metathesis

Residual H_2O was removed from the diene (1.0 mmol) by coevaporation with dry toluene (3×5 mL), after which it was dissolved in dry toluene (40 mL). The solution was degassed by bubbling through with argon for 20 min. Catalyst **29** (41 mg, 5 mol%) was added and degassing was continued for 20 min, after which the solution was stirred overnight under an argon atmosphere. When TLC analysis indicated termination of the reaction, the reaction mixture was concentrated and the product was purified by column chromatography (light petroleum/Et_2O, 8/1→1/1, v/v).

Dimer of 27. Attempted ring-closing metathesis of diene **27** (100 mg, 0.17 mmol) according to the general procedure yielded a single isomer of dimer **30** as a brownish oil. Yield 66 mg (0.12 mmol, 69%). R_f 0.83 (Et_2O); ^1H NMR ($CDCl_3$): δ 7.43–7.27 (m, 10H, H arom Bn), 5.89–5.73 (m, 4H, H-5, CH allyl), 5.80 (d, 2H, H-1, $J_{1,2}$=3.7 Hz), 5.50–5.23 (m, 4H, H-6a, H-6b), 5.14 (d, 2H, H-1′, $J_{1′,2′}$=3.7 Hz), 4.77 (AB, 4H, CH_2 Bn, J=−12.4 Hz), 4.69 (t, 2H, H-2, $J_{2,3}$=3.9 Hz), 4.57 (dd, 2H, H-3), 4.09 (t, 2H, H-3′, $J_{3′,4′}$=$J_{2′,3′}$=9.7 Hz), 4.00–3.98 (m, 4H, H-4, OCH_2 allyl), 3.78–3.43 (m, 12H, H-2′, H-4′, H-5′, H-6a′, H-6b′, OCH_2 allyl), 3.31, 3.23 (2×s, 12H, 2×OCH_3 BDA), 1.55, 1.35, 1.34, 1.30 (4×s, 24H, CH_3 isoprop, BDA); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 138.9 (Cq Ph), 134.4 (C-5, CH allyl), 128.0, 127.6, 127.2 (CH arom Bn), 118.6 (C-6), 112.9 (Cq isoprop), 103.8 (C-1), 99.6, 99.3 (2×Cq BDA), 95.9 (C-1′), 79.6, 78.7, 76.5, 76.0, 69.2, 69.0, 65.8 (C-2, C-3, C-4, C-2′, C-3′, C-4′, C-5′), 72.4 (OCH_2 allyl), 71.3 (CH_2 Bn), 67.6 (C-6′), 48.1, 47.8 (2×OCH_3 BDA), 26.6 (2×CH_3 isoprop), 17.8, 17.7 (2×CH_3 BDA). ES-MS: 1158 $[M+H]^+$, 1170 $[M+Na]^+$. HRMS (ES): Calcd $C_{60}H_{85}O_{22}$ $[M+H]^+$ 1157.5542, found 1157.5549 (± 0.0028).

(*E*/*Z*, 2″S,3″S) 5,6′-Di-*O*-but-2-en-1,4-diyl-3-*O*-(2′-*O*-benzyl-3′,4′-di-O-(2″,3″-dimethoxybutane-2″,3″-diyl)-α-D-glucopyranosyl)-1,2-*O*-isopropylidene-α-D-ribofuranoside (31). Ring-closing metathesis of diene **28** (0.30 g, 0.47 mmol) was effected according to the general procedure to give a 2:1 mixture of *E*/*Z*-isomers of 14-membered macrocycle **31**. The light brownish oily product was used in the next reaction without purification. Combined yields 0.21 g (0.32 mmol, 75%). R_f 0.53 and 0.42 (Et_2O). The higher-running product was the major isomer: ^1H NMR (600 MHz, H–H-COSY, $CDCl_3$): δ 7.44–7.23 (m, 5H, H arom Bn), 5.89 (m, 2H, H-b, H-c), 5.78 (d, 1H, H-1, $J_{1,2}$=3.7 Hz), 5.12 (d, 1H, H-1′, $J_{1',2'}$=4.2 Hz), 5.78 (AB, 2H, CH_2 Bn, J=−12.1 Hz), 4.69 (t, 1H, H-2′, $J_{2,3}$=4.0 Hz), 4.52 (dd, 1H, H-3, $J_{3,4}$=9.1 Hz), 4.27 (m, 2H, H-4, H-a), 4.17 (m, 1H, H-d), 4.14 (t, 1H, H-3′, $J_{2',3'}$=$J_{3',4'}$=9.8 Hz), 4.06 (dt, 1H, H-5′, $J_{4',5'}$=$J_{5',6b'}$=9.9 Hz, $J_{5',6a'}$ 1.5 Hz), 3.95 (dd, 1H, H-d′, $J_{c,d'}$=5.3 Hz, $J_{d,d'}$=−10.2 Hz), 3.89 (dd, 1H, H-a′, $J_{a',b}$=5.3 Hz, $J_{a,a'}$=−9.9 Hz), 3.83 (d, 1H, H-5a, $J_{5a,5b}$=−11.2 Hz), 3.75 (dd, 1H, H-6a′, $J_{6a,6b}$=−12.0 Hz), 3.67 (dd, 1H, H-5b, $J_{4,5b}$=1.8 Hz), 3.64 (dd, 1H, H-2′), 3.59 (dd, 1H, H-6b′), 3.42 (t, 1H, H-4′), 3.33, 3.21 (2×s, 6H, 2×OCH_3 BDA), 1.50, 1.35, 1.34, 1.32 (4×s, 12H, 4×CH_3 isoprop, BDA); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 139.0 (Cq Ph), 130.5, 129.9 (C-b, C-c), 128.0–127.0 (CH arom), 112.8 (Cq isoprop), 104.2 (C-1), 99.7, 99.4 (2×Cq BDA), 95.4 (C-1′), 77.6, 76.2, 71.9, 69.5, 67.7, 67.4 (C-2, C-3, C-4, C-2′, C-3′, C-4′, C-5′), 71.8, 69.4, 67.2, 66.4, 66.0 (C-a, C-d, C-5, C-6′, CH_2 Bn), 48.3, 47.8 (2×OCH_3 BDA), 26.8, 26.6 (2×CH_3 isoprop), 17.9, 17.7 (2×CH_3 BDA); ES-MS: 632 $[M+Na]^+$. $C_{31}H_{44}O_{12}$ (609): Calcd C 61.17, H 7.29; found C 61.11, H 7.35.

(2″*S*,3″*S*) 3-*O*-(2′-*O*-Benzyl-3′,4′-di-*O*-(2″,3″-dimethoxybutane-2″,3″-diyl)-α-D-glucopyranosyl)-5,6′-di-*O*-(butane-1,4-diyl)-1,2-*O*-isopropylidene-α-D-ribofuranoside (32). A mixture of *E*/*Z* isomers of **31** (0.21 g, 0.32 mmol) was dissolved in EtOAc (10 mL) and the solution was degassed and placed under a blanket of N_2. A catalytic amount of PtO_2 (7 mg, 10 mol%) was added, the mixture was degassed once more and stirred under a H_2-atmosphere. TLC analysis revealed complete conversion of **31** after 25 min into a single higher-running product. The reaction mixture was filtered over Glass Fiber (GF/2A, Whatman®) and concentrated in vacuo to give **32** in quantitative yield (0.21 g, 0.32 mmol). R_f 0.64 (Et_2O) as a brownish oil. ^1H NMR ($CDCl_3$, 300 MHz, H–H-COSY): δ 7.43–7.22 (m, 5H, H arom), 5.83 (d, 1H, H-1, $J_{1,2}$=3.8 Hz), 5.17 (d, 1H, H-1′, $J_{1',2'}$=4.2 Hz), 4.79 (AB, 2H, CH_2 Bn, J=−12.4 Hz), 4.74 (dd, 1H, H-2, $J_{2,3}$=4.5 Hz), 4.52 (dd, 1H, H-3, $J_{3,4}$=9.1 Hz), 4.25 (dd, 1H, H-4, $J_{4,5a}$=1.4 Hz), 4.15–4.04 (m, 3H, H-3′, H-5′), 3.86 (dd, 1H, H-5a, $J_{5a,5b}$=−11.6 Hz), 3.74 (dd, 1H, H-6a′, $J_{6a',6b'}$=−11.7 Hz, $J_{5',6b'}$=1.6 Hz), 3.65 (dd, 1H, H-2′, $J_{2',3'}$=10.1 Hz), 3.61–3.41 (m, 7H, H-5b, H-4′, H-6b′, OCH_2 *n*-Bu), 3.33, 3.21 (2×s, 6H, 2×OCH_3 BDA), 1.88, 1.63 (m, 4H, CH_2 *n*-Bu), 1.49, 1.35, 1.32 (4×s, 12H, 4×CH_3 isoprop, BDA); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 138.8 (Cq Ph), 127.9–126.9 (CH arom), 112.7 (Cq isoprop), 104.0 (C-1), 99.5, 99.3 (2×Cq BDA), 95.2 (C-1′), 78.0, 76.2, 71.9, 69.6, 67.3 (C-2, C-3, C-4, C-2′, C-3′, C-4′, C-5′), 71.8, 70.0, 69.1, 68.7, 67.0 (2×OCH_2 *n*-Bu, C-5, C-6′, CH_2 Bn), 48.1, 47.7 (2×OCH_3 BDA), 26.6 (2×CH_3 isoprop), 25.8, 25.5 (2×CH_2 *n*-Bu), 17.7, 17.5 (2×CH_3 BDA). $[\alpha]_D^{20}$=+138.6° (*c* 1.0 $CHCl_3$). ES-MS: 634 $[M+Na]^+$. HRMS (ES): Calcd $C_{31}H_{47}O_{12}$ $[M+H]^+$ 611.3067, found 611.3072 (± 0.0019). Calcd C 57.69, H 6.45; found C 57.82, H 6.53.

1,2-Di-*O*-acetyl-3-*O*-(3′,4′-di-*O*-acetyl-2′-*O*-benzyl-β-D-glucopyranosyl)-5,6′-di-*O*-(butane-1,4-diyl)-D-ribofuranoside (33). Compound **32** (0.15 g, 0.24 mmol) was dissolved in a solution of $AcOH/H_2O/(CH_2OH)_2$, 14/6/3, v/v/v (7 mL) and was refluxed for 90 min. TLC analysis (MeOH/EtOAc, 8/92, v/v) indicated the appearance of one product (R_f 0.42). The reaction mixture was concentrated under reduced pressure, coevaporated with toluene (3×5 mL) and stirred in a mixture of in pyridine/acetic anhydride (8 mL, 2/1, v/v). The mixture was concentrated, coevaporated with toluene (3×5 mL) and subjected to column chromatography (light petroleum/EtOAc, 1/0→1/1, v/v) to yield **33** (138 mg, 0.22 mmol, 92%, α:β∼1:9) as a white foam. R_f 0.15 (EtOAc/light petroleum, 1/1, v/v). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 169.9 (C=O Ac), 138.5 (Cq Bn), 128.2, 127.7, 127.4 (CH arom Bn), 97.7 (C-1), 94.9 (C-1′), 80.7, 76.7, 71.6, 70.9, 70.3, 69.6, 68.7 (C-2, C-3, C-4, C-2′, C-3′, C-4′, C-5′), 77.6, 76.4, 72.3, 69.9, 76.6 (2×OCH_2 *n*-Bu, C-5, C-6′, CH_2 Bn), 26.3 (2×CH_2 *n*-Bu), 20.6 (4×CH_3 Ac). ES-MS: 565 $[M-OAc]^+$, 647 $[M+Na]^+$, 1272 $[2M-Na]^+$. HRMS (ES): Calcd $C_{30}H_{41}O_{14}$ $[M+H]^+$ 625.2506, found 625.2508 (± 0.0016).

2′-*O*-Acetyl-6-*N*-benzoyl-5′,6″-di-*O*-(*n*-butane-1,4-diyl)-3′-*O*-(3″,4″-di-*O*-acetyl-2″-*O*-benzyl-α-d-glucopyranosyl)-adenosine (34). A suspension of 6-*N*-benzoyladenine (0.14 g, 0.60 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (1.1 mL) and pyridine (0.5 mL) was refluxed for 7 h under an Ar atmosphere. The reaction mixture was cooled, diluted with toluene (5 mL) and concentrated in vacuo under careful exclusion of H_2O. The residual oil was diluted with toluene (3×5 mL) and concentrated in vacuo to remove excess 1,1,1,3,3,3-hexamethyldisilazane. Tetraacetate **33** (0.13 g, 0.20 mmol) in $(CH_2Cl)_2$ (5 mL) and a catalytic amount of TMSOTf (8 μL, **25** mol%) were added to the silylated 6-*N*-benzoyladenine. After stirring for 16 h at reflux temperature TLC analysis showed conversion of **33** into a lower-running product. The reaction mixture was quenched with Et_3N (0.25 mL), diluted with CH_2Cl_2 (10 mL) and poured into sat. aq. $NaHCO_3$ (5 mL). The organic phase was washed with H_2O (5 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2/MeOH, 1/0 to 95/5, v/v). Concentration of the appropiate fractions yielded **34** as a yellowish foam. Yield 0.13 g (0.16 mmol, 82%); R_f 0.65 (EtOAc/MeOH, 95/5, v/v); ^1H NMR ($CDCl_3$, 600 MHz, H–H-COSY): δ 9.05 (s, 1H, NH), 8.81 (s, 1H, H-2), 8.63 (s, 1H, H-8), 8.02, (d, 2H, arom Bz, J=7.4 Hz), 7.60 (t, 1H, arom Bz, J=7.4 Hz), 7.52 (t, 2H, arom Bz, J=7.8 Hz), 7.38–7.28 (m, 5H, H arom Bn), 6.27 (d, 1H, H-1′, $J_{1',2'}$=1.8 Hz), 5.88 (dd, 1H, H-2′, $J_{2',3'}$=4.7 Hz), 5.44 (t, 1H, H-3″, $J_{2'',3''}$=$J_{3'',4''}$=9.6 Hz), 5.07 (d, 1H, H-1″, $J_{1'',2''}$=3.7 Hz), 4.86 (dd, 1H, H-3′, $J_{3',4'}$=7.9 Hz), 4.71 (t, 1H, H-4″, $J_{4'',5''}$=9.8 Hz), 4.60 (AB, 2H, CH_2 Bn, J=−11.9 Hz), 4.48 (m, 1H, H-4′), 4.01 (dd, 1H, H-5a′, $J_{5a',5b'}$=−11.2 Hz, $J_{4',5a'}$=2.7 Hz), 3.97 (dt, 1H, H-5″, $J_{5'',6''}$=2.0 Hz), 3.71 (dd, 1H, H-5b′, $J_{4',5b'}$=2.7 Hz), 3.69–3.67, 3.64–3.60 (2×m, 2H, H-a/d), 3.55 (1H, dd, H-2″, $J_{2'',3''}$=10.0 Hz), 3.50–3.42 (m, 2H, H-6a″, H-a/d), 3.39 (1H, dd, H-6b″, $J_{6a'',6b''}$=−11.0 Hz), 2.04, 1.92, 1.87 (3×s, 9H, CH_3 Ac), 1.94–1.82, 1.74–1.69 (2×m, 4H, H-b, H-c); $^{13}C\{^1H\}$ NMR (150 MHz, C–H-COSY, $CDCl_3$): δ 170.1, 170.0, 168.8 (3×C=O Ac), 164.6 (C=O Bz), 152.3, 151.1, 149.4, 141.8 (C-4, C-6, C-2, C-8), 137.6 (Cq Bn), 133.7 (Cq Bz), 132.7 (CH arom Bz), 128.3, 128.2, 127.9, 127.6 (CH arom), 123.5 (C-5), 95.9 (C-1″), 87.8 (C-1′), 81.3 (C-4′), 76.9 (C-2″), 73.8 (CH_2 Bn), 72.8 (C-2′), 71.7 (C-3″), 71.4 (C-3′), 71.1, 70.7 (OCH_2 *n*-Bu), 70.0 (C-6″), 69.9 (C-5″), 69.8 (C-4″), 68.0 (C-5′), 26.7, 26.3 (CH_2 *n*-Bu), 20.8, 20.7, 20.4 (3×CH_3 Ac); ES-MS: 827 $[M+Na]^+$, 805 $[M+H]^+$, 565 $[M-A^{Bz}]$+, 240 $[A^{Bz}+H]^+$. $[\alpha]_D^{20}=+43.8°$ (*c* 1.0 $CHCl_3$). HRMS (ES): Calcd $C_{40}H_{46}N_5O_{13}$ $[M+H]^+$ 804.3091, found 804.3082 (± 0.0029). Calcd C 59.77, H 5.64; found C 59.85, H 7.58.

5′,6″-Di-*O*-(*n*-butane-1,4-diyl)-3′-*O*-(α-D-glucopyranosyl)-adenosine 2′,3″,4″-trisphosphate—Cyclophostin (14). To a solution of glucopyranosyl adenosine **34** (83 mg, 0.10 mmol) in 1,4-dioxane (3 mL) was added a solution of KO-*t*Bu in MeOH (1 M, 4 mL). After stirring vigorously for 1 min, the reaction mixture was neutralized by the addition of AcOH (0.23 mL, 4.0 mmol). The solution was poured into sat. aq. $NaHCO_3$ (5 mL) and the resulting mixture which was extracted with CH_2Cl_2 (2×10 mL). The organic phase was washed with H_2O (3 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. The crude deacetylated dimer was used without purification. R_f 0.43 (EtOAc/MeOH, 9/1, v/v). ES-MS: 679 $[M+H]^+$, 701 $[M+Na]^+$. A mixture of the triol (70 mg, 0.10 mmol) and *N*,*N*-diisopropylamino-bis-[2-(methylsulfonyl)ethyl]-phosphine (0.23 g, 0.62 mmol) was coevaporated with 1,4-dioxane (2×5 mL). The mixture was dissolved in CH_2Cl_2 (3 mL) and a solution of 1*H*-tetrazole (56 mg, 0.80 mmol) in CH_3CN (3 mL) was added. TLC analysis indicated the formation of a single product (R_f 0.43, CH_2Cl_2/MeOH, 9/1, v/v) which was complete after 30 min. Oxidation of the intermediate phosphite triesters was effected by the addition of *t*-BuOOH (0.80 mL) at 0°C. After 30 min the mixture was diluted with CH_2Cl_2 (10 mL), washed with H_2O (5 mL) and dried ($MgSO_4$). Concentration of the organic phase gave trisphosphate **35**. R_f 0.14 (CH_2Cl_2/MeOH, 9/1, v/v). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ −2.00, −2.39, −2.45. Crude **35** 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 (0.06 mL), concentrated and the product was purified by gel-filtration over a Fractogel HW-40 column (elution: 0.15 M triethyl ammonium carbonate). Concentration, coevaporation with MeOH/H_2O (3×5 mL, 4/1, v/v) and lyophilization of the appropriate fractions afforded the 2′-*O*-benzyl derivative of cyclophostin in pure form. Yield 82 mg (56% over the three steps from **34**). Debenzylation was accomplished by dissolving the compound in H_2O (10 mL) and stirring the solution in the presence of Pd-black (50 mg) and AcOH (2 drops) under a H_2 atmosphere. After 16 h the catalyst was removed by filtration over Glass Fiber (GF/2A, Whatman®). The filtrate was concentrated and lyophilized to give cyclophostin **14** which was converted into the Na^+-form by ion-exchange with Dowex® 50Wx4 (Na^+-form) and imino diacetate resin (Chelex®, Na^+ form). Lyophilization gave **14** as a white fluffy foam. Yield 45 mg (48 μmol). ^1H NMR (D_2O, 600 MHz, H–H-COSY): δ 8.39 (H-2), 8.25 (H-8), 6.44 (d, 1H, H-1′, $J_{1',2'}$=2.3 Hz), 5.32 (d, 1H, H-1″, $J_{1'',2''}$=4.1 Hz), 5.29 (ddd, 1H, H-2′, $J_{2',3'}$=5.0 Hz, ^3J_P=7.9 Hz), 4.95 (dd, 1H, H-3′, $J_{3',4'}$=5.9 Hz), 4.47 (m, 1H, H-4′), 4.28 (q, 1H, H-3″, $J_{2'',3''}=J_{3'',4''}={}^3J_P$=9.2 Hz), 4.11 (d, 1H, H-6a″, J=−11.7 Hz), 4.00 (t, 1H, H-5″, $J_{4'',5''}=J_{5'',6b''}$=9.8 Hz), 3.86–3.81 (m, 2H, H-4″, H-2″), 3.77 (dd, 1H, H-5a′, $J_{4',5a'}$=3.9 Hz, $J_{5a',5b'}$=−11.7 Hz), 3.69–3.61 (m, 3H, OCH_2 *n*-Bu, H-6b″, H-5b″), 3.57–3.51, 3.47–3.44 (2×m, 3H, OCH_2 *n*-Bu), 1.68–1.63, 1.56–1.52, 1.50–1.42 (3×m, 4H, 2×CH_2 *n*-Bu); $^{13}C\{^1H\}$ NMR (D_2O): δ 148.1 (C-4/C-6), 153.6 (C-2), 120.1 (C-5), 96.4 (C-1″), 88.2 (C-1′), 81.9 (C-4′), 77.3, 74.0, 73.3, 72.8, 71.1 (C-2′, C-3′, C-2″, C-3″, C-4″, C-5″), 70.7, 70.3, 70.0, 68.4, 69.5 (2×OCH_2 *n*-Bu, C-5′, C-6″), 26.3, 25.9 (2×CH_2 *n*-Bu); ^{31}P NMR (D_2O, 242 MHz, P–H-COSY): δ 4.44 (P-3″, P-2′), 2.92 (P-4″); ES-MS: 722 $[M-H]^-$, 360.5 $[M-2H]^{2-}$, 691 $[M-3H+2Na]^-$, 713 $[M-4H+3Na]^-$. HRMS (ES): Calcd $C_{20}H_{31}N_5O_{18}P_3$ $[M-H]^-$722.0877, found 722.0865 (± 0.0025).

Propargyl 2′-*O*-acetyl-3′-*O*-(3″,4″-di-*O*-acetyl-2″-*O*-benzyl-α-D-glucopyranosyl)-5′,6″-di-*O*-(butane-1,4-diyl)-β-D-ribofuranoside (36). Compound **33** (0.13 g, 0.21 mmol) was coevaporated with $(CH_2Cl)_2$ and dissolved in $(CH_2Cl)_2$ (3 mL). Propargyl alcohol (18 μL, 0.31 mmol) and activated molecular sieves (4 Å) were added and the mixture was stirred for 15 min under a blanket of N_2. TMSOTf (8 μL, 25 mol%) was added and after 6 h the reaction mixture was filtered over Hyflo®, diluted with Et_2O (10 mL) and washed with sat. aq. $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The crude residue was purified by column chromatography (light petroleum/EtOAc, 3/1→0/1, v/v) to afford acetylene derivative **34** as a colorless oil (70 mg, 0.11 mmol, 54%). R_f 0.38 (EtOAc/light petroleum, 1/1, v/v). ^1H NMR ($CDCl_3$): δ 7.33–7.29 (m, 5H, H-arom Bn), 5.39 (t, 1H, H-3″, $J_{2'',3''}=J_{3'',4''}$=9.5 Hz), 5.36 (d, 1H, H-2′, $J_{2',3'}$=4.4 Hz), 5.16 (s, 1H, H-1′), 5.02 (d, 1H, H-1″, $J_{1'',2''}$=3.6 Hz), 4.76–4.69 (m, 2H, H-4″, H-5″), 4.59 (AB, 2H, CH_2 Bn, J=−11.4 Hz), 4.32–4.22 (m, 3H, H-3′, H-1 propargyl), 3.97 (ddd, 1H, H-4′, $J_{4',5'}$=8.6 Hz, $J_{4',5b'}$=1.1 Hz), 3.77 (dd, 1H, H-5a′, $J_{5a',5b'}$=−11.7 Hz, $J_{4',5a'}$=2.9 Hz), 3.69–3.30 (m, 7H, H-5b′, 2×OCH_2 *n*-Bu, H-6″), 2.03, 1.92, 1.88 (3×s, 9H, CH_3 Ac). 1.95–1.51 (m, 4H, 2×CH_2 *n*-Bu); $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.0 (3×C(O) Ac), 137.5 (Cq Ph), 128.2–127.5 (CH arom), 102.2 (C-1′), 95.5 (C-1″), 80.3, 76.7, 73.2, 71.6, 69.8, 69.2 (C-2′, C-3′, C-4′, C-2″, C-3″, C-4″, C-5″, CH propargyl), 73.1, 70.8, 70.6, 70.1, 68.6 (2×OCH_2 *n*-Bu, C-5′, C-6″, CH_2 Bn, Cq propargyl), 53.3 (CH_2 propargyl), 26.5, 25.9 (2×CH_2 *n*-Bu), 20.6 (3×CH_3 Ac). $[\alpha]_D^{20}=+61.3°$ (*c* 1.0 $CHCl_3$). HRMS (ES): Calcd $C_{31}H_{41}O_{13}$ $[M+H]^+$ 621.2547, found 621.2543 (± 0.0021). Calcd C 59.99, H 6.50; found C 60.08, H 6.57.

***n*-Propyl 5′,6″-di-*O*-(*n*-butane-1,4-diyl)-3′-*O*-(α-D-glucopyranosyl)-β-D-ribofuranoside 2′,3″,4″-trisphosphate (15).** Triacetate **36** (70 mg, 0.11 mmol) was dissolved in a 0.1 M solution of NaOMe in MeOH. After 1 h the mixture was neutralized with Dowex® H^+ which was subsequently removed by filtration. The filtrate was concentrated and the residual oil was dried by coevaparation with 1,4-dioxane (3×5 mL). The thus obtained triol (56 mg, 0.11 mmol) and dibenzyl *N*,*N*-diisopropyl phosphoramidite (0.14 mL, 0.68 mmol) were dissolved in $(CH_2Cl)_2$ (4 mL) and a solution of 1*H*-tetrazole (65 mg, 0.96 mmol) in CH_3CN (2 mL) was added under N_2. After stirring for 30 min TLC analysis

(Et_2O/light petroleum, 1/1, v/v) showed complete conversion of starting material into a higher-running product (R_f 0.56, Et_2O/light petroleum 1/1, v/v). The reaction mixture was cooled (0°C), *t*-butyl hydroperoxide (0.26 mL) was added and stirring was continued for 30 min after which TLC analysis revealed complete disappearance of the phosphite triester intermediate into a lower-running product (R_f 0.24, Et_2O). The reaction mixture was diluted with EtOAc (10 mL), washed with H_2O, dried over $MgSO_4$, filtered and concentrated in vacuo. Compound **37** was obtained as a colorless oil after purification by column chromatography (light petroleum/EtOAc, 3/1→0/1, v/v) in a 60% yield (86 mg, 68 μmol). $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ −3.2, −3.7, −3.9; ES-MS: 1275 $[M+H]^+$, 1297 $[M+Na]^+$; $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 137.3 (Cq Bn-2″), 136.4, 135.8, 135.7, 135.4 (6×Cq Bn), 128.7–127.6 (CH Bn), 102.3 (C-1′), 94.5 (C-1″), 80.4, 80.2, 79.1, 78.2, 77.1, 74.9, 72.7, 72.6 (C-2″, C-3″, C-4″, C-5″, C-2′, C-3′, C-4′, C-3 propargyl), 74.5, 71.9, 70.6 (C-2 propargyl, 2×OCH_2 *n*-Bu), 69.9, 69.7, 69.6, 69.2, 69.1, 68.9 (7×CH_2 Bn), 65.7, 65.4 (C-6″, C-5′), 53.1 (C-1 propargyl), 26.9, 25.7 (2×CH_2 *n*-Bu). Trisphosphate **37** (86 mg, 68 μmol) was dissolved in a mixture of 1,4-dioxane (5 mL), *i*-propanol (2.5 mL) and H_2O (1.25 mL) containing NaOAc (66 mg, 0.80 mmol). The mixture was degassed and 10% Pd/C (50 mg), was added and stirred under an atmosphere of H_2 for 16 h. The catalyst was removed by filtration over Glass Fiber (GF/2A, Whatman®) and the filtrate was concentrated. Purification was performed as described for the synthesis of cyclophostin **14** to give derivative **15** as a white fluffy foam. Yield 52 mg (57 μmol, 84%, Na^+-form). ^1H NMR (D_2O, 600 MHz, H–H-COSY): δ 5.15 (s, 1H, H-1′), 5.11 (d, 1H, H-1″, $J_{1'',2''}$=3.9 Hz), 4.55 (dd, 1H, H-2′, $J_{2',3'}$=4.4 Hz, ^3J_P=8.5 Hz), 4.46 (dd, 1H, H-3′, $J_{3',4'}$=9.7 Hz), 4.34 (q, 1H, H-3″, $J_{2'',3''}$=$J_{3'',4''}$=^3J_P=9.7 Hz), 4.27 (m, 1H, H-4′), 4.02 (t, 1H, H-5″, $J_{4'',5''}$=$J_{5'',6b''}$=9.2 Hz), 3.91 (d, 1H, H-6a″, J=−11.8 Hz), 3.89 (d, 1H, H-5a′, $J_{5a',5b'}$=−11.6 Hz), 3.80 (q, 1H, H-4″ ^3J_P=9.6 Hz), 3.69 (dd, 1H, H-2″), 3.67–3.56 (m, 6H, H-6b″, 2×OCH_2 *n*-Bu, H-1a *n*-Pr), 3.51 (dd, 1H, H-5b′, $J_{4',5b'}$=4.5 Hz), 3.51 (m, 1H, H-1b *n*-Pr), 1.79–1.74; 1.64–1.58 (2×m, 4H, 2×CH_2 *n*-Bu), 1.55 (m, 2H, H-2 *n*-Pr), 0.86 (t, 3H, H-3 *n*-Pr, J=7.7 Hz); $^{13}C\{^1H\}$ NMR (D_2O): δ 106.7 (C-1′), 98.5 (C-1″), 80.5 (C-4′), 77.7, 75.8, 75.2, 71.8 (C-2′, C-3′, C-2″, C-3″, C-4″, C-5″), 71.0, 70.9, 70.3, 69.9, 69.5 (2×OCH_2 *n*-Bu, C-1 *n*-Pr, C-5′, C-6″), 26.2, 25.3 (CH_2 *n*-Bu), 22.9 (C-2 *n*-Pr), 22.9 (C-3 *n*-Pr); ^{31}P NMR (D_2O, 242 MHz, P–H-COSY): δ 2.35 (P-3″), 1.35 (P-2′), 1.11 (P-4″); ES-MS: 647 $[M-H]^-$, 669 $[M-2H+Na]^-$, 691 $[M-3H+2Na]^-$, 713 $[M-4H+3Na]^-$. HRMS (ES): Calcd $C_{19}H_{38}O_{19}P_3$ $[M-H]^-$ 663.1219, found 663.1208 (± 0.0023).

Biological Evaluation

^{3}H-IP$_3$ Displacement binding experiments

A 'P_2' fraction of bovine adrenal cortex was prepared as described previously.[27] Increasing concentrations of adenophostin A (**1**),[28] IP_3 (**4**), cyclophostin **14**, ribophostin **8**[2c,13] and cycloribophostin **15** were incubated with a constant amount of ^3H-IP_3 (approx. 9000 d.p.m. per assay; stock: 21 Ci $mmol^{-1}$; NEN) and adrenal cortex membranes; incubations were stopped after 30 min at 4°C by rapid vacuum filtration.[23] Non-specific binding was defined in the presence of 10 μM IP_3. The displacement isotherm of each of the ligands (not shown) was used to obtain an estimate of the IC_{50} values (see Table 3) using GraphPad Prism and are given as $-\log IC_{50}$ values (±s.e. mean).

$^{45}Ca^{2+}$-Release experiments

Assays were performed using SH-SY5Y human neuroblastoma cells (passage 20–30) essentially as described previously[24] with certain modifications. Confluent monolayers of SH-SY5Y cells were washed and harvested using 10 mM HEPES, 0.9% NaCl, 0.02% EDTA, pH 7.4 and recovered by centrifugation (400×*g*, 3 min). Cells were resuspended in an 'intracellular-like' buffer (ICB: 20 mM HEPES, 135 mM KCl, 2.5 mM $MgCl_2$, 2 mM ATP, 20 μM $CaCl_2$, pH 7.1; the free $[Ca^{2+}]$ was buffered to 100–150 nM by addition of EGTA) and centrifuged (400×*g*, 3 min), this latter step was repeated and the final cell pellet was gently resuspended in ICB supplemented with an ATP regenerating system (10 mM phosphocreatine, 10 U mL^{-1} creatine phosphokinase) and permeabilization was achieved by addition of 50 μg mL^{-1} α-escin. After 2 min 1 μCi mL^{-1} $^{45}Ca^{2+}$ (1000 Ci $mmol^{-1}$, Amersham, Little Chalfont, UK) was added and the permeabilized cell suspension was added to ICB containing different concentrations of adenophostin A (**1**), IP_3 (**4**), cyclophostin **14**, ribophostin **8** or cycloribophostin **15**. Incubations were continued for 2 min (IP_3, 30 s) and samples were then centrifuged (13,000×*g*, 3 min). A silicone oil mixture (300 μL of Dow-Corning 556/550, 1:1, v/v) was then added to each tube and the samples were recentrifuged (13,000×*g*, 3 min). The ICB and oil were then aspirated, tubes inverted and allowed to drain for ≥60 min before addition of 1.1 mL FloScint IV scintillation cocktail (Packard Bioscience BV, Groningen, the Netherlands). Samples were stored overnight in the dark before scintillation counting. The total releaseable $^{45}Ca^{2+}$-pool was defined as that released by addition of 10 μM ionomycin. Each release isotherm was used to obtain estimates of the EC_{50} value, the slope factor (*h*) and the maximum obtainable release (expressed as a % of the total ionomycin-releaseable pool) using GraphPad Prism.

Molecular modeling

All calculations were run on IRIS workstations according to Hotoda et al.[7] Full details on the molecular dynamics simulation will be published elsewhere.[29]

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