Synthesis of Potent Agonists of the D-*myo*-Inositol 1,4,5-Trisphosphate Receptor Based on Clustered Disaccharide Polyphosphate Analogues of Adenophostin A

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Clustered disaccharide analogues of adenophostin A (**2**), i.e. mono-, di-, and tetravalent derivatives **6**−**8**, respectively, were synthesized and evaluated as novel ligands for the tetrameric D-*myo*-inositol 1,4,5-trisphosphate receptor (IP_3R). The synthesis was accomplished via Sonogashira coupling of propargyl 2-*O*-acetyl-5-*O*-benzyl-3-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranosyl)-*β*-D-ribofuranoside (**16**) with iodobenzene **18**, **22**, or **25**, followed by deacetylation, phosphorylation, and deprotection. The abilities of the target compounds **6**−**8**, as well as ribophostin **4**, propylphostin **5**, and IP_3 (**1**), to evoke Ca^{2+} release from permeabilized hepatocytes or displacement of [^{3}H]IP_3 from its receptor in hepatic membranes were compared. Although the binding affinities of **4**−**8** were similar, there were modest though significant differences in their potencies in Ca^{2+} release assays: tetraphostin **8** > IP_3 ∼ diphostin **7** > phenylphostin **6** > ribophostin **4** ∼ propylphostin **5**.

Introduction

The intracellular messenger D-*myo*-inositol 1,4,5-trisphosphate (IP_3, **1**) is released after activation of receptors that stimulate phospholipase C activity[1,2] and then binds to IP_3 receptors (IP_3R) within intracellular stores, causing the large conductance channel of the IP_3R to open.[3] Ca^{2+} flowing through the open channel causes a local increase in cytosolic [Ca^{2+}] that can both regulate intracellular events[4] and control further release of intracellular Ca^{2+} stores to produce more widespread increases in cytosolic [Ca^{2+}].[5]

In mammals, the three closely related subtypes of IP_3R (types 1−3)[6] assemble to form tetrameric Ca^{2+} channels which may be either homo- or heteromeric[6] and which cryo-electron microscopy studies have revealed as large particles with 4-fold symmetry and a width of about 12 nm.[7] For all three receptor subtypes, the Ca^{2+} channel is formed by membrane-spanning regions close to the C-terminal, while the IP_3-binding domain lies within the N-terminal.[8,9]

More detailed studies of type 1 IP_3Rs indicate that the pore of the channel is formed by the fifth and sixth (final) transmembrane regions[10] and the intervening loop,[10] while the IP_3-binding site is formed by two distinct domains within the N-terminus.[11] Although the different IP_3R subtypes are differentially expressed in different tissues[12] and differ in some aspects of their regulation,[6] the physiological significance of the receptor diversity is unclear.[13,14]

Activation, inhibition, and inactivation of IP_3R are complex processes regulated by both IP_3 and Ca^{2+}. Channel opening appears to require binding of IP_3 to at least three, and possibly all four, subunits of the IP_3R[15−17] followed by binding of Ca^{2+} to at least one site on either the receptor or an associated protein.[15,17,18] Inhibition of IP_3Rs by cytosolic Ca^{2+} is regulated by IP_3, with IP_3 binding controlling whether stimulatory or inhibitory Ca^{2+}-binding sites are accessible.[19] Finally, IP_3 binding also causes a slower partial inactivation of the receptor.[20] It is important to resolve whether IP_3 binding to a single subunit within the tetrameric receptor is sufficient to cause inactivation and whether IP_3 binding regulates the Ca^{2+}-binding sites of only the subunit to which IP_3 is bound or Ca^{2+} binding to each subunit of the tetramer.[15,19]

It occurred to us that these IP_3-binding events to the tetrameric receptor could be studied in more detail by using multivalent ligands. The latter class of molecules has been used to explore both carbohydrate−protein interactions[21] and the interactions between cyclic GMP and cation channels[22] and can give rise to enhanced binding affinity[22,23] relative to their monovalent counterparts. However, it might be expected that the functionalization of IP_3 (**1**) necessary for coupling to a central scaffold would result in loss of potency. In this respect, the metabolically resistant adenophostins A and B (**2** and **3**, Figure 1)[24] as highly potent,[25] full agonists of IP_3R provide attractive alternatives. More importantly, analogues[26−28] lacking the adeninyl moiety (e.g. ribophostin **4**)[29] are still equipotent to IP_3.[30] The latter implies that analogues of adenophostin A (**2**) in which the adenine is replaced by an appropriate spacer might be used as an acceptable substitute for a corresponding IP_3 derivative.

We report here in full detail[31] the synthesis and biological evaluation of the novel mono-, di-, and tetravalent adenophostin A analogues: 'propylphostin' **5**, 'phenylphostin' **6**, 'diphostin' **7**, and 'tetraphostin' **8**.

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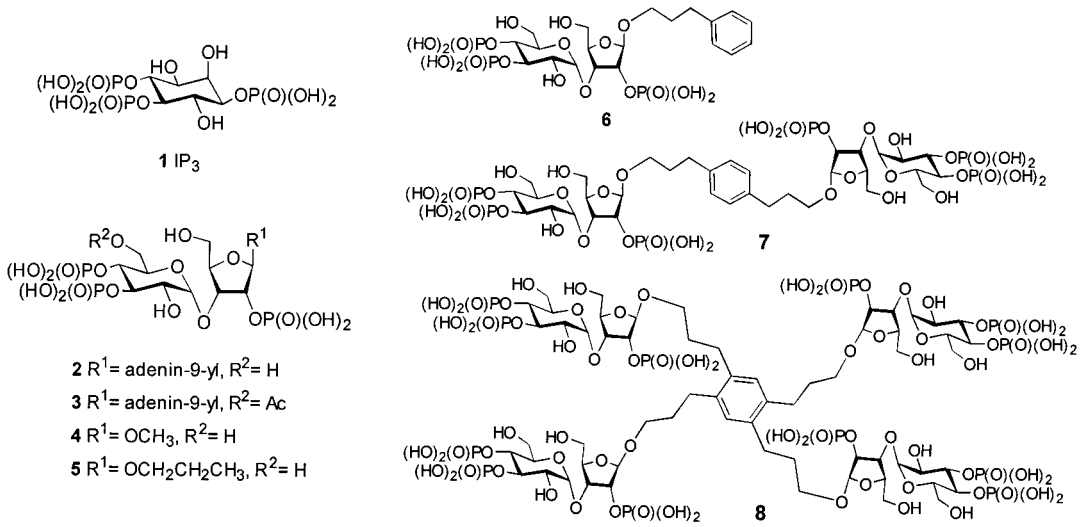
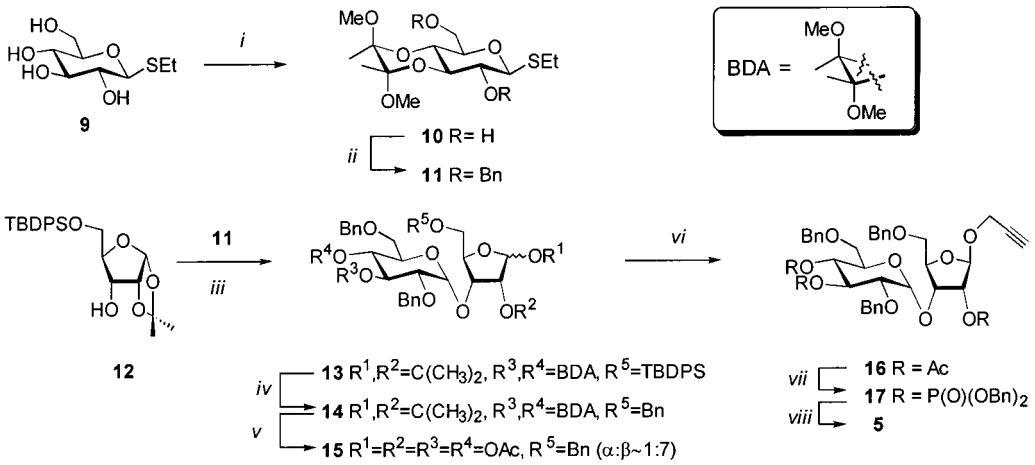


Figure 1. Structures of D-*myo*-inositol 1,4,5-trisphosphate (IP_3, **1**), adenophostin A (**2**) and B (**3**), ribophostin **4**, propylphostin **5**, phenylphostin **6**, diphostin **7**, and tetraphostin **8**.

Scheme 1. Construction of Acetylene Derivative **16** and Synthesis of Propylphostin **5**[a]



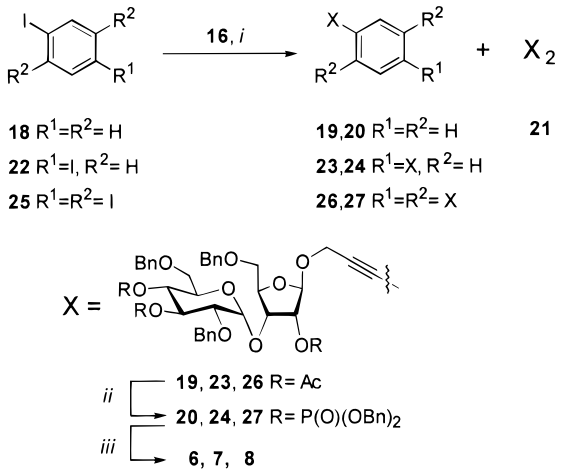
[a] Reagents and conditions: (i) butane-2,3-dione (1.1 equiv), $CH(OCH_3)_3$, cat. CSA, CH_3OH, reflux, 3 h, 86% (2 regioisomers); (ii) BnBr, NaH, DMF, 1 h, 98%; (iii) NIS/cat. TfOH, Et_2O, 45 min, 83%; (iv) a. TBAF (1 M in THF)/1,4-dioxane, 1/4, v/v, 50 °C, 8 h, b. BnBr, NaH, DMF, 92%; (v) a. $HOAc/H_2O/(HOCH_2)_2$, 14/6/3, v/v/v, reflux, 75 min, b. Ac_2O, pyr, 16 h, 86%; (vi) propargyl alcohol (2 equiv), TMSOTf, $(CH_2Cl)_2$, 30 min, 81%; (vii) a. NaOMe, MeOH, then Dowex H^+, b. dibenzyl (*N*,*N*-diisopropyl)phosphoramidite, 1*H*-tetrazole, $(CH_2Cl)_2$/CH_3CN, 3/1, v/v, 30 min, then *t*-BuOOH, 0 °C, 30 min, 80%; (viii) Pd/C, H_2 (1 atm), NaOAc, 1,4-dioxane/*i*-PrOH/H_2O, 4/2/1, v/v/v, 16 h, 85%.

Results and Discussion

Synthesis. Target compounds **6**−**8** (Figure 1) are composed of a phenyl core that is mono-, di-, or tetrasubstituted with phosphorylated *β*-*O*-propyl-(3-*O*-α-glucosyl)ribosyl units, respectively. Retrosynthetic analysis indicates that these molecules can be assembled via Sonogashira coupling[32,33] of iodobenzene **(18)**, 1,4-diiodobenzene **(22)**, or 1,2,4,5-tetraiodobenzene **(25)** with the common building block propargyl 2-*O*-acetyl-3-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranosyl)-5-*O*-benzyl-*β*-D-ribofuranoside (**16**). The α-glucosidic linkage in key disaccharide **16** can in principle be introduced by condensing, as reported[34] for the synthesis of adenophostin A, the ribose unit **12** (see Scheme 1) with ethyl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-1-thio-*β*-D-glucopyranoside. In our route, the latter glucosyl donor is replaced by the more easily accessible thioglucoside **11**, which is prepared by the following two-step procedure (see Scheme 1). Protection of known[35] ethyl 1-thio-*β*-D-glucopyranoside (**9**) with trimethyl orthoformate and butane-2,3-dione[36,37] in the presence of a catalytic amount of camphorsulfonic acid (CSA) gave, after separation of two regioisomers, 3,4-*O*-butane-2,3-diacetal (BDA) derivative **10**. Benzylation of **10** with benzyl bromide (BnBr) and sodium hydride (NaH) yielded fully protected glucosyl donor **11**.

Glycosylation of known[34] ribose acceptor **12** with **11** in the presence of the promotor *N*-iodosuccinimide (NIS) and a catalytic amount of trifluoromethanesulfonic acid (TfOH) proceeded in a stereoselective fashion to afford α-linked disaccharide **13** in 83% yield. Conversion of **13** into the more acid-stable derivative **14** was effected by desilylation of **13** with *n*-tetrabutylammonium fluoride (TBAF) and subsequent benzylation of the resulting primary hydroxyl function. Concomitant removal of the BDA and isopropylidene groups in **14** under mild acidic conditions[34] followed by acetylation of the resulting hydroxyl functions afforded the fully protected dimer

Scheme 2. Synthesis of Mono-, Di-, and Tetravalent Analogues **6**−**8** by Sonogashira Coupling of **16** with Mono-, Di-, or Tetraiodobenzene (**18**, **22**, or **25**), respectively, followed by Phosphorylation and Deprotection[a]



[a] Reagents and conditions: (i) 5 mol % $PdCl_2(PPh_3)_2$, 10 mol % CuI, Et_3N/DMF, 1/20, v/v, 16 h, **19**: 74%; **23**: 100%; **26**: 52%; (ii) a. NaOMe, MeOH, then Dowex H^+, b. dibenzyl (*N*,*N*-diisopropyl)-phosphoramidite, 1*H*-tetrazole, $(CH_2Cl)_2/CH_3CN$, 3/1, v/v, 30 min, then *t*-BuOOH, 0 °C, 1 h; **20**: 88%; **24**: 88%; **27**: 65%; (iii) Pd/C, H_2 (1 atm), NaOAc, 1,4-dioxane/*i*-PrOH/H_2O, 4/2/1, v/v/v, 16 h, **6**: 80%; **7**: 42%; **8**: 42%.

15 as a mixture of anomers. Glycosidation of **15** with propargyl alcohol under the agency of a catalytic amount of trimethylsilyl triflate (TMSOTf) gave building block **16** in 53% yield based on **12**. Deacetylation of **16** and subsequent phosphitylation with dibenzyl *N*,*N*-diisopropylphosphoramidite[38] followed by in situ oxidation of the resulting phosphite triesters afforded trisphosphate **17** in 80% over the three steps. Debenzylation and concomitant reduction of the acetylene moiety was effected by hydrogenation to give, after purification by HW-40 gel filtration, propylphostin **5** (Na^+ salt).

The assembly of phenyl derivatives **6**−**8** as presented in Scheme 2 commences with Sonogashira coupling[9] of terminal acetylene **16** with monoiodobenzene (**18**) under the influence of $PdCl_2(PPh_3)$/CuI/Et_3N to give the expected phenylacetylene derivative **19** and the homocoupled byproduct **21** in a 2:1 ratio. Formation of the latter compound could be suppressed by slow addition (∼1 h) of acetylene **16** to the iodobenzene/catalyst solution. In a similar way, the di- and tetravalent derivatives **23** and **26** were readily available starting from 1,4-diiodobenzene (**22**) and 1,2,4,5-tetraiodobenzene[39] (**25**), respectively. Phosphorylation and deprotection of **19**, **23**, and **26**, as described for the preparation of compound **5**, furnished phenylphostin **6**, diphostin **7**, and tetraphostin **8**, the identity and homogeneity of which were fully ascertained by ^1H, ^{13}C, and ^{31}P NMR spectroscopy as well as ES mass spectrometry.

Biological Evaluation. Preliminary evaluation of the mobilizing ability of the novel compounds was made using a sea urchin homogenate assay system[40] coupled with fluorescence techniques. All of the compounds were found to release Ca^{2+} with a roughly similar potency when compared to IP_3 (data not shown). With this in hand, the accurate binding affinity and Ca^{2+}-releasing ability of phenylphostin **6**, diphostin **7,** and tetraphostin **8** at rat liver IP_3Rs were determined using equilibrium competition binding experiments with $[^3H]IP_3$, and $^{45}Ca^{2+}$-flux measurements. The results of these experiments were compared with those obtained for IP_3 (**1**), ribophostin **4**,[29,30] and propylphostin **5**. As can be seen in Table 1, a maximally effective concentration of either IP_3 (**1**) or the analogues (**4**−**8**) releases about the same fraction of the intracellular Ca^{2+} stores (∼35%) and that combined applications of 10 μM IP_3 with 10 μM of any of the analogues releases no more Ca^{2+} than a maximal concentration of either agonist alone (Table 1, penultimate column). Each analogue (**4**−**8**) binds with similar affinity to liver IP_3Rs (∼2-fold less than the affinity of IP_3), and none are more than 2-fold less potent than IP_3 in stimulating Ca^{2+} release. The steep Hill coefficients ($h > 2$) in the functional analysis of each agonist (**1**, **4**−**8**) and the unitary Hill slope for IP_3 binding are consistent with previous suggestions that at least three of the four IP_3 receptor subunits must independently bind IP_3 before the Ca^{2+} channel opens.[15,16] The unitary Hill coefficients of the binding curves for the monomeric ligands **4**−**6** are as expected, but the similar unitary coefficients for diphostin **7** and tetraphostin **8** suggest that it is unlikely that more than one of their possible ligands binds simultaneously. It is, however, noteworthy that the potency of the ligands increases with the number of phosphorylated disaccharide within the cluster: tetraphostin **8** (which is actually slightly more potent than IP_3) > diphostin **7** > monovalent ligands **4**−**6**. Because it is difficult to define the number of disaccharide units available for binding within the clusters (binding of one unit may restrict binding of another within the cluster), it is not yet clear whether the greater potency of polyvalent ligands simply reflects an increase in effective concentration of the agonist. However, closer inspection of Table 1 suggests that diphostin **7** and tetraphostin **8** achieve greater potency (lower EC_{50}) without an increase in their apparent affinity (K_d): the EC_{50}/K_d ratio is significantly lower for these ligands than for the monovalent ligands. This observation is difficult to reconcile with the increased potency of the polyvalent ligands resulting solely from an increase in the effective concentration of the agonist and instead suggests that they may be more efficacious than their monovalent counterparts (i.e. more effectively activate the IP_3 receptor once they have bound).

The structural basis for the superpotent activity of the adenophostins is still unclear. It has recently been demonstrated that the phosphate charge distribution on adenophostin A (**2**) is very similar to that observed in IP3 (**1**),[41] and it now seems that a specific interaction of the adenine base moiety with the receptor (and an associated interplay with the conformationally mobile disaccharide unit) is most likely the prime factor underlying the unusual potency.[42−45] It is thus of interest to evaluate analogues with modified bases and their surrogates.[42,46] In this context, it is useful to compare the relative potencies of the series ribophostin **4**, propylphostin **5**, and phenylphostin **6**. Ribophostin **4** and propylphostin **5** are essentially identical in activity, and the extra length of the hydrophobic chain of **5** over **4** apparently confers no relative advantage, nor adenophostin-like potency. In particular **6**, which possesses both a flexible chain and a flat aromatic hydrophobic motif, exhibits only a relatively marginal improvement

Table 1. $^{45}Ca^{2+}$ Release EC_{50} Values and K_d Values of Ribophostin **4**, Propylphostin **5**, Phenylphostin **6**, Diphostin **7**, and Tetraphostin **8** Obtained by Equilibrium Competition Binding with $[^3H]IP_3$

	equilibrium binding[a]		Ca^{2+} release[b]				
compd	K_d (nM)	h	EC_{50} (nM)[a]	h	max response (% Ca^{2+} stores)	release with 10 μM IP_3 (% IP_3-sensitive Ca^{2+} stores)[c]	EC_{50}/K_d
IP_3, **1**	1.7 ± 0.3	0.88 ± 0.15	201 ± 20	2.9 ± 0.5	32 ± 4		120 ± 28
ribophostin **4**	4.4 ± 0.8	0.99 ± 0.05	484 ± 20	3.4 ± 1.4	33 ± 3	89 ± 6	110 ± 20
propylphostin **5**	4.7 ± 1.2	0.97 ± 0.17	491 ± 36	2.1 ± 0.3	42 ± 6	87 ± 7	104 ± 28
phenylphostin **6**	3.1 ± 0.8	1.16 ± 0.08	381 ± 10	3.9 ± 1.7	34 ± 7	87 ± 6	123 ± 32
diphostin **7**	3.6 ± 1.1	0.95 ± 0.14	215 ± 15	2.2 ± 0.3	31 ± 2	92 ± 8	60 ± 19
tetraphostin **8**	4.3 ± 0.7	1.04 ± 0.18	161 ± 8	2.1 ± 0.3	42 ± 5	101 ± 5	37 ± 6

[a] Results are means ± SEM; $n = 3$ (7 for **1**). [b] Results are means ± SEM; $n = 3$ (5 for **1**). [c] Fraction of the IP_3-sensitive Ca^{2+} stores released by 10 μM ligand in combination with 10 μM IP_3.

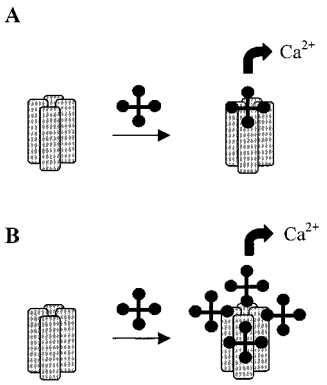


Figure 2. Schematic representation for IP_3R activation with tetraphostin **8**: A, cooperative mode of binding of tetraphostin **8**; B, noncooperative binding of four tetraphostin **8** molecules.

in potency. We conclude, therefore, that the phenylpropoxy group of **6** is unable to access the presumed hydrophobic receptor motif or is too flexible to allow subsequent correct positioning of the 2′-phosphate group for adenophostin-like activity. In this regard, it would clearly be worthwhile to evaluate biologically the less flexible corresponding ribofuranosyl 1′-*O*-benzyl or phenyl derivatives, since adenophostin analogues with surrogate bases are still able to exhibit potent activity.[46]

Conclusions

The results indicate that the mono-, di-, and tetravalent adenophostin A analogues **6**−**8** do not exhibit cooperative binding to the tetravalent IP_3R, excluding the (rather unlikely) possibility that activation of IP_3R occurs after binding of a single tetravalent ligand as illustrated in Figure 2A. Instead, we suggest that the four IP_3-binding sites of the receptor are capable of accommodating four molecules of tetraphostin **8** (Figure 2B), which is, of course, much larger than the natural ligand, IP_3 (**1**), implying that the IP_3-binding sites are either exposed at the surface of the receptor or at least accessible to bulky negatively charged ligands.

In summary, the mono-, di-, and tetravalent adenophostin A analogues **6**−**8** present a novel class of ligands for IP_3R, with the polyvalent compounds not showing the cooperative binding we had anticipated but rather an apparent increase in their efficacy. When the location of the IP_3-binding sites on the receptor becomes established, it may be possible to exploit methods similar to those reported herein to synthesize polyvalent adenophostin analogues with the size and geometry of the central scaffold more precisely matched to the dimensions of the binding sites.

Experimental Section

General Methods. ^1H, ^{13}C, 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 mass spectrometer equipped with a custom-made electrospray interface (ES). HRMS (ES) spectra were measured in the negative mode with a MAT 900 double-focusing mass spectrometer equipped with an electrospray interface. The samples were prepared in a mixture of 2-propanol/H_2O (80/20, v/v) containing 1.0×10^{-4} M NaOAc, the clusters of which were used as internal standards. Optical rotations were determined with a Propol automatic polarimeter. Melting points were determined with a Büchi (Flawil, Switzerland) melting point apparatus. Elemental analysis was performed with a Perkin-Elmer Series II CHNS/O analyzer 2400. Toluene, CH_2Cl_2 and pyridine were boiled under reflux for 3 h with P_2O_5 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 charring with 20% H_2SO_4 in EtOH or ammonium molybdate (25 g·L^{-1}) and ceric ammonium sulfate (10 g·L^{-1}) in 10% aqueous 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 water were removed by coevaporation with toluene or pyridine. Butanedione, propargyl alcohol, *N*-iodosuccinimide, Dowex 50XW4, bis(triphenylphosphine)palladium(II) chloride, camphor sulfonic acid, copper(I) iodide, trifluoromethanesulfonic acid, trimethyl orthoformate, tetrabutylammonium fluoride (1.0 M in THF) (Acros), benzyl bromide, ethylene glycol, sodium hydride (60%), *t*-BuOOH (80% in di-*tert*-butyl peroxide) (Merck), trimethylsilyl triflate, iodobenzene, 1,4-diiodobenzene, 1*H*-tetrazole (Aldrich) and acetic anhydride (Baker) were all used as received. Imino diacetate resin (Chelex; Na^+ form) 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 mL·min^{-1}). The collected product was analyzed with strong anion-exchange HPLC on a Pharmacia MonoQ column by elution with a mixture of buffers (pH 12) A: 0.01 N NaOH and B: 0.01 N NaOH in 1.2 N NaCl (gradient 0 → 50% B).

$^{45}Ca^{2+}$ Release. For the preliminary sea urchin homogenate assay *L. Pictus* egg homogenate (2.5% v/v, prepared as previously described[40]) was incubated at 17 °C in intracellular-like medium (IM) containing an ATP-regenerating system, mitochondrial inhibitors and Fluo-3 (3 μM), and extra-microsomal Ca^{2+} was measured by monitoring Fluo-3 fluoresence (excitation 490 nm and emission 535 nm) using a Perkin-Elmer LS50-B luminescence spectrophotometer controlled by FL-

Winlab. Homogenate (0.5 mL) was placed in a cuvette and additions of test compound (dissolved in IM with 10 μM EGTA) were made. Estimates of the amount of Ca^{2+} released were based upon calibrations with known amounts of Ca^{2+} added to the assay homogenate as described previously.[40] For the quantitative work, hepatocytes (10^6 cells·mL^{-1}) permeabilized with saponin (10 μg·mL^{-1}) were loaded to steady state with $^{45}Ca^{2+}$ (20 μCi·mL^{-1}) at 37 °C in a cytosol-like medium (CLM) with a free [Ca^{2+}] of 200 nM.[30] After 5 min, cells were diluted 5-fold into similar medium at 37 °C but without ATP and supplemented with thapsigargin (1.25 μM) to inhibit the Ca^{2+} pumps of the intracellular Ca^{2+} stores. After 15 s, appropriate concentrations of the ligand were added, and after a further 60 s, the $^{45}Ca^{2+}$ contents of the intracellular stores were determined after rapid filtration using a Brandel receptor-binding harvester.[30] CLM had the following composition: 140 mM KCl, 20 mM NaCl, 2 mM $MgCl_2$, 1 mM ethylene glycol bis(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid (EGTA), 300 μM $CaCl_2$ (free [Ca^{2+}] = 200 nM), 10 μM carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP), 7.5 mM ATP, 20 mM 1,4-piperazinediethanesulfonic acid (PIPES), pH 7.0 at 37 °C.

[^{3}H]IP$_3$ Binding. Hepatic membranes were prepared by a method modified from that described by Prpic et al.[47] The livers of two male Wistar rats (250−300 g) were perfused through the portal vein with cold saline (50 mL: 118 mM NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4$, 0.96 mM NaH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose, 1 mM EGTA, pH 7.4 at 4 °C); the livers were then removed and homogenized in cold buffered sucrose (50 mL: 250 mM sucrose, 1 mM EGTA, 5 mM Hepes, pH 7.4 at 4 °C) in a Dounce homogenizer with 10 passes of a loose-fitting plunger and 5 passes of a tight plunger. The homogenate from both livers (in 100 mL of buffered sucrose) was filtered through gauze and centrifuged (10 min, 2500*g*), and the pellet resuspended in buffered sucrose (96 mL) supplemented with Percoll (12.9 mL, 1.13 g·mL^{-1}). After centrifugation (30 min, 35000*g*) membranes were harvested as a discrete layer near the top of the tube, resuspended in cold hypoosmotic buffer (100 mL: 1 mM EGTA, 5 mM *N*-(2-hydroxyethyl)piperazine-*N′*-(2-ethanesulfonic acid) (Hepes), pH 7.4), and recentrifuged (10 min, 48000*g*). The pellet was resuspended in binding medium (5 mL: 50 mM Tris, 1 mM EGTA, pH 8.3) to give a final protein concentration of about 3 mg protein·mL^{-1} and then frozen in liquid nitrogen before storage at −80 °C. For equilibrium competition binding assays, membranes in binding buffer (500 μL containing 0.1 mg membrane protein) were incubated for 5 min at 4 °C with [^{3}H]IP$_3$ (typically, 45 nCi, 40 Ci/mmol) and appropriate concentrations of competing ligands. Incubations were terminated by centrifugation (5 min, 20000*g*, 4 °C), and the pellets resuspended for liquid scintillation counting.

Data Analysis. Ca^{2+} release: Concentration−response relationships were fitted to logistic equations using Kaleidegraph software (Synergy Software, PA) from which the maximal response, half-maximally effective agonist concentration (EC_{50}), and Hill slope (h) were determined. All results are expressed as means ± SEM. Binding parameters were determined by curve-fitting to logistic equations as previously described.[30] Nonspecific binding was typically 10−15% of total binding. All experiments were performed in triplicate ($n = 3$; $n = 5 - 7$ for IP$_3$), and the combined results are expressed as means ± SEM.

(2′*S*,3′*S*)-Ethyl 3,4-Di-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thio-β-D-glucopyranoside (10) and (2′*R*,3′*R*)-Ethyl 2,3-Di-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thio-β-D-glucopyranoside. Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (33.2 g, 84.7 mmol) was deacylated with NaOMe (0.22 g, 4.20 mmol) in MeOH (300 mL). When TLC analysis showed complete conversion of starting material the reaction mixture was neutralized with Dowex 50WX4 H^+ and filtered. Trimethyl orthoformate (27.2 mL, 280 mmol), butane-2,3-dione (7.37 mL, 93.2 mmol) and camphorsulfonic acid (1.93 g, 9.3 mmol) were added and the mixture was heated until reflux. After 3 h triethylamine (1.42 mL, 10.0 mmol) was added and the reaction mixture was concentrated. The crude oil was purified by column chromatography (Et_2O/light petroleum, 1/1 → 1/0, v/v) which afforded **10** and its regioisomer as white foams in a 1:1 ratio. Combined yield: 24.6 g (72.8 mmol, 86%). **3,4-BDA isomer**: R_f 0.59 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 4.41 (d, 1 H, H-1, $J_{1,2}$ 9.5 Hz), 3.88 (ddd, 1 H, H-5, $J_{5,6a}$ 12.1 Hz, $J_{5,6b}$ 2.7 Hz, $J_{4,5}$ 5.6 Hz), 3.80−3.52 (m, 5 H, H-2, H-3, H-4, H-6), 3.32, 3.26 (2× s, 6 H, 2× OCH_3), 2.75 (q, 2 H, CH_2 of SEt, J 7.4 Hz), 2.53 (d, 1 H, OH-2, J 1.8 Hz), 2.04 (dd, 1 H, OH-6, J 7.4 Hz, J 5.6 Hz), 1.35, 1.31 (2× s, 6 H, 2× CH_3), 1.29 (t, 3 H, CH_3 of SEt); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 99.5, 99.2 (2× Cq, BDA), 86.2 (C-1), 77.8, 73.2, 69.6, 65.3 (C-4, C-5, C-3, C-2), 60.9 (C-6), 47.7 (2× CH_3 OMe), 24.2 (CH_2 of SEt), 17.4, 17.3 (2× CH_3, BDA), 15.0 (CH_3 of SEt). **2,3-BDA isomer**: R_f 0.44 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 4.63 (d, 1 H, H-1, $J_{1,2}$ 9.8 Hz), 3.88−3.82 (m, 4 H, H-3, H-4, H-5, H-2), 3.53−3.46 (m, 2 H, H-6), 3.30, 3.29 (2× s, 6 H, 2× OCH_3), 2.72 (q, 2 H, CH_2 of SEt, J 7.5 Hz), 1.34, 1.33 (2× s, 6 H, 2× CH_3), 1.31 (t, 3 H, CH_3 of SEt); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 99.5, 99.1 (2× Cq, BDA), 82.4 (C-1), 79.7, 73.8, 68.5, 66.6 (C-3, C-5, C-2, C-4), 61.5 (C-6), 47.6 (2× CH_3 OMe), 24.2 (CH_2 of SEt), 17.2 (2× CH_3, BDA), 14.6 (CH_3 of SEt). Anal. ($C_{14}H_{26}O_7S$) C, H.

(2′*S*,3′*S*)-Ethyl 2,6-Di-*O*-benzyl-3,4-di-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thio-β-D-glucopyranoside (11). Sodium hydride (60%, 1.74 g, 50.8 mmol) was added to a cooled (0 °C) solution of **10** (4.9 g, 14.5 mmol) in DMF (75 mL). After strirring for 15 min, benzyl bromide (3.77 mL, 31.9 mmol) was added. When TLC analysis showed complete conversion of starting material into a more lipophilic product, the excess of sodium hydride was destroyed with MeOH and the reaction mixture was diluted with Et_2O (100 mL) and H_2O (20 mL). The layers were separated and the DMF/H_2O mixture was extracted with Et_2O (2 × 50 mL). The combined organic layers were washed with H_2O, dried ($MgSO_4$) and concentrated in vacuo. Purification by column chromatography (Et_2O/light petroleum, 3/1 → 1/1, v/v) gave **11** as a white solid: yield 7.24 g (14.2 mmol, 98%); R_f 0.91(Et_2O/light petroleum, 2/1, v/v); ^{1}H NMR ($CDCl_3$) δ 7.45−7.23 (m, 10H, CH arom), 4.83 (AB, 2 H, CH_2 Bn, J -12.1 Hz), 4.58 (AB, 2 H, CH_2 Bn, J -12.3 Hz), 4.46 (d, 1 H, H-1, $J_{1,2}$ 9.3 Hz), 3.86 (t, 1 H, H-3, $J_{2,3} = J_{3,4}$ 9.4 Hz), 3.80−3.57 (m, 4 H, H-4, H-5, H-6), 3.48 (t, 1 H, H-2), 3.29, 3.20 (2× s, 6 H, 2× OCH_3), 2.82−2.64 (m, 2 H, CH_2 of SEt), 1.35, 1.29 (2× s, 6 H, 2× CH_3), 1.30 (t, 3 H, CH_3 of SEt, J 7.5 Hz); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 138.4 (2× Cq Bn), 128.8, 127.6, 127.4 (CH Bn), 99.5 (2× Cq, BDA), 85.0 (C-1), 78.3, 77.5, 74.8, 65.9 (C-2, C-3, C-4, C-5), 75.2, 73.3 (2× CH_2 Bn), 68.5 (C-6), 47.8, 47.7 (2× CH_3 OMe), 24.4 (CH_2 of SEt), 17.8, 17.7 (2× CH_3, BDA), 15.3 (CH_3 of SEt); mp 78−80 °C. Anal. ($C_{28}H_{38}O_7$) C, H.

(2″*S*,3″*S*)-3-*O*-[2′,6′-Di-*O*-benzyl-3′,4′-di-*O*-(2″,3″-dimethoxybutane-2″,3″-diyl)-α-D-glucopyranosyl]-1,2-*O*-isopropylidene-5-*O*-*tert*-butyldiphenylsilyl-α-D-ribofuranoside (13). 1,2-*O*-Isopropylidene-5-*O*-*tert*-butyldiphenylsilyl-α-D-ribofuranose (**12**) (1.71 g, 3.99 mmol) and compound **11** (2.27 g, 4.38 mmol) were dried by coevaporation with 1,2-dichloroethane. The remaining oil was dissolved in Et_2O (20 mL) and stirred with powdered molecular sieves (4 Å) under an inert atmosphere. After 5 min *N*-iodosuccinimide (0.99 g, 4.38 mmol) and trifluoromethanesulfonic acid (60 μL, 0.44 mmol) were added. The resulting mixture was allowed to stir at room temperature for 45 min after which TLC analysis revealed complete disappearance of glucosyl donor and acceptor. The molecular sieves were filtered off over Hyflo and rinsed with Et_2O. The filtrate was washed with aqueous $Na_2S_2O_3$ (10%), aqueous $NaHCO_3$ (10%), H_2O and dried ($MgSO_4$). The crude product was purified by column chromatography (Et_2O/light petroleum, 1/6 → 1/3, v/v) to afford dimer **13** in a yield of 2.93 g (3.31 mmol, 83%) as a colorless oil: R_f 0.60 (Et_2O/light petroleum, 2/1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$, HH-COSY) δ 7.70−7.62 (m, 4 H, CH arom Ph), 7.41−7.21 (m, 16 H, CH arom), 5.79 (d, 1 H, H-1, $J_{1,2}$ 3.7 Hz), 5.25 (d, 1 H, H-1′, $J_{1',2'}$ 4.0 Hz), 4.78−4.73 (m, 3 H, H-2, CH_2 6′-*O*-Bn), 4.47 (AB, 2 H, CH_2 Bn, J -12.1 Hz), 4.32 (d, 1 H, H-3, $J_{2,3}$ 4.3 Hz, $J_{3,4}$ 8.8 Hz,), 4.23−4.12 (m, 2 H, H-4, H-3′), 3.98 (dd, 1 H, H-5a, $J_{5a,5b}$ −11.9 Hz, $J_{4,5a}$ 1.2 Hz), 3.87−3.81 (m, 3 H, H-4′, H-5′, H-5b),

3.67−3.60 (m, 2 H, H-2′, H-6a′), 3.51 (dd, 1 H, H-6b′, $J_{6a',6b'}$ −11.0 Hz, $J_{5',6b'}$ 1.9 Hz), 3.29, 3.17 (2× s, 6 H, 2× OCH_3), 1.52 (s, 3 H, CH_3 isoprop), 1.35 (2× s, 6 H, CH_3, BDA, CH_3 isoprop), 1.30 (s, 3 H, CH_3, BDA), 1.01 (s, 9 H, *t*-Bu TBDPS); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 138.8, 138.7, 135.5, 135.3 (4× Cq arom), 129.4−127.0 (CH arom), 112.7 (Cq isoprop), 104.1 (C-1), 99.4, 99.3 (2× Cq, BDA), 95.8 (C-1′), 79.2, 76.9, 75.9, 72.7, 69.3, 65.5 (C-2′, C-3′, C-4′, C-5′, C-2, C-3, C-4), 73.4, 71.8 (2× CH_2 Bn), 67.4 (C-6′), 61.5 (C-5), 47.8, 47.7 (2× CH_3 OMe), 26.7, 26.6 (CH_3 *t*-Bu TBDPS, isoprop), 19.1 (Cq *t*-Bu TBDPS), 17.8, 17.6 (2× CH_3, BDA), $J_{H-1',C-1'}$ 171.4 Hz: α-glucoside; $J_{H-1,C-1}$ 181.7 Hz; ES-MS *m*/*z* 908 $[Na]^+$, 924 $[M + K]^+$. Anal. ($C_{50}H_{64}O_{12}Si$) C, H.

(2″*S*,3″*S*)-5-*O*-Benzyl-3-*O*-[2′,6′-di-*O*-benzyl-3′,4′-di-*O*-(2″,3″-dimethoxybutane-2″,3″-diyl)-α-D-glucopyranosyl]-1,2-*O*-isopropylidene-α-D-ribofuranoside (14). Compound **13** (3.51 g, 3.97 mmol) was stirred at 50 °C in a mixture of dioxane (26 mL) and TBAF (1.0 M in THF, 6.13 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 (100 mL), washed with brine (2 × 20 mL), H_2O (10 mL) and dried ($MgSO_4$). Purification by column chromatography (Et_2O/light petroleum, 1/1 → 1/0, v/v) gave the desilylated disaccharide: yield of 2.37 g; R_f 0.47 (Et_2O); ^1H NMR ($CDCl_3$) δ 7.42−7.26 (m, 10 H, CH arom), 5.76 (d, 1 H, H-1, $J_{1,2}$ 3.6 Hz), 5.15 (d, 1 H, H-1′, $J_{1',2'}$ 3.8 Hz), 4.77 (AB, 2 H, CH_2 Bn, *J* -11.9 Hz), 4.68 (dd, 1 H, H-2, $J_{2,3}$ 3.9 Hz), 4.56 (AB, 2 H, CH_2 Bn, *J* -12.2 Hz), 4.13 (m, 3 H, H-3, H-4, H-3′), 3.86 (m, 2 H, H-4′, H-5′), 3.65 (m, 5 H, H-2′, H-5, H-6′), 3.30, 3.17 (2× s, 6 H, 2× OCH_3), 1.53 (s, 3 H, CH_3 isoprop), 1.34 (2× s, 6 H, CH_3, BDA, CH_3 isoprop), 1.28 (s, 3 H, CH_3, BDA); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 138.7, 138.5 (2× Cq Bn), 128.1−127.1 (CH arom), 112.8 (Cq isoprop), 104.0 (C-1), 99.5, 99.3 (2× Cq, BDA), 95.6 (C-1′), 78.4, 76.5, 75.9, 72.3, 69.2, 69.1, 66.0 (C-2′, C-3′, C-4′, C-5′, C-2, C-3, C-4), 73.3, 71.8 (2× CH_2 Bn), 67.8 (C-6′), 59.8 (C-5), 48.0, 47.7 (2× CH_3 OMe), 26.6, 26.5 (2× CH_3 isoprop), 17.7, 17.5 (2× CH_3, BDA).

Benzylation was performed as described earlier for **11**. Purification was effected by column chromatography (Et_2O/light petroleum, 1/5 → 1/2, v/v) to give fully protected **14** as a white solid: yield 2.66 g (92%, 2 steps); R_f 0.65 (Et_2O/light petroleum, 2/1, v/v); ^1H NMR (300 MHz, $CDCl_3$, HH-COSY) δ 7.42−7.23 (m, 15 H, CH arom), 5.79 (d, 1 H, H-1, $J_{1,2}$ 3.7 Hz), 5.19 (d, 1 H, H-1′, $J_{1',2'}$ 4.0 Hz), 4.76 (AB, 2 H, CH_2 Bn, *J* -12.4 Hz), 4.70 (dd, 1 H, H-2, $J_{2,3}$ 3.8 Hz), 4.56 (AB, 2 H, CH_2 Bn, *J* -12.1 Hz), 4.50 (AB, 2 H, CH_2 Bn, *J* -12.1 Hz), 4.32 (ddd, 1 H, H-4, $J_{3,4}$ 9.3 Hz, $J_{4,5a}$ 1.8 Hz, $J_{4,5b}$ 3.9 Hz), 4.14−4.07 (m, 2 H, H-3, H-3′), 3.82−3.76 (m, 3 H, H-5b, H-2′, H-4′), 3.56 (dd, 1 H, H-6b′, $J_{6a',6b'}$ -10.2 Hz, $J_{5',6b'}$ 1.5 Hz), 3.30, 3.18 (2× s, 6 H, 2× OCH_3), 1.53 (s, 3 H, CH_3 isoprop), 1.35 (2× s, 6 H, CH_3, BDA, CH_3 isoprop), 1.31 (s, 3 H, CH_3, BDA); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 138.8, 138.1, 137.9 (3× Cq Bn), 128.2−127.2 (CH arom), 112.8 (Cq isoprop), 104.1 (C-1), 99.5, 99.4 (2× Cq, BDA), 95.7 (C-1′), 77.8, 76.5, 76.0, 69.4, 69.2, 65.9 (C-2′, C-3′, C-4′, C-5′, C-2, C-3, C-4), 73.4, 71.9 (3× CH_2 Bn), 68.1, 67.7 (C-6′, C-5), 48.0, 47.9 (2× CH_3 OMe), 26.7, 26.6 (2× CH_3 isoprop), 17.9, 17.6 (2× CH_3, BDA); $[\alpha]_D^{20}$ +150.8 (*c* 1.0, $CHCl_3$); mp 93−94 °C; ES-MS *m*/*z* 754 $[M + NH_4]^+$, 759 $[M + Na]^+$, 775 $[M + K]^+$. Anal. ($C_{41}H_{52}O_{12}$) C, H.

1,2-Di-*O*-acetyl-3-*O*-(3′,4′-di-*O*-acetyl-2′,6′-di-*O*-benzyl-α-D-glucopyranosyl)-5-*O*-benzyl-D-ribofuranoside (15). Compound **14** (2.66 g, 3.61 mmol) was heated until reflux in a mixture of acetic acid/H_2O/ethylene glycol (75 mL, 14/6/3, v/v/v). After 75 min the reaction mixture was cooled (0 °C) and quenched with aqueous $NaHCO_3$ (10%, 125 mL). The resulting suspension was extracted with EtOAc (3 × 100 mL). The organic layer was washed with H_2O (3 × 50 mL), dried ($MgSO_4$) and concentrated. The crude product (R_f 0.53, MeOH/EtOAc, 8/92, v/v) was dissolved in a mixture of acetic anhydride/pyridine (50 mL, 3/7, v/v) and stirred for 16 h at room temperature. The mixture was diluted with toluene and concentrated under reduced pressure (3×). The oily product was applied onto a column of silica gel which was eluted with Et_2O/light petroleum (1/9 → 1/1, v/v). Concentration of the appropriate fractions afforded tetraacetate **15** as a white foam: yield 2.33 g (86%, 2 steps, α:β = 1:7); R_f 0.56 (EtOAc/light petroleum, 1/1, v/v); ^1H NMR ($CDCl_3$) δ 7.29−7.26 (m, 15 H, CH arom), 6.43 (d, 0.13 H, H-1α, $J_{1,2}$ 4.4 Hz), 6.12 (s, 0.87 H, H-1β), 5.42−5.30 (m, 6 H, 2× CH_2 Bn, H-1′, H-3′), 5.10−5.00 (m, 3 H, CH_2 Bn, H-4′), 4.68−4.10 (m, 4 H, H-2, H-3, H-4, H-5′), 3.71−3.30 (m, 5 H, H-5, H-6, H-2′), 1.96, 1.93, 1.87, 1.86 (4 × s, 4 × CH_3 Ac); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 170.0, 169.9, 169.4, 169.0 (4 × C(O) Ac), 137.9, 137.6, 137.3 (3× Cq Bn), 128.2−127.1 (CH arom), 98.3 (C-1), 96.2 (C-1′), 81.0, 76.4, 73.7, 73.4, 71.7, 68.7, 68.8 (C-2′, C-3′, C-4′, C-5′, C-2, C-3, C-4), 73.2, 73.0 (3× CH_2 Bn), 69.1, 67.4 (C-6′, C-5), 20.8, 20.7, 20.5, 20.3 (4 × CH_3 Ac); ES-MS *m*/*z* 691 $[M - OAc]^+$, 773 $[M + Na]^+$, 790 $[M + K]^+$. Anal. ($C_{40}H_{46}O_{14}$) C, H.

[2′-*O*-Acetyl-3′-*O*-(3″,4″-di-*O*-acetyl-2″,6″-di-*O*-benzyl-α-D-glucopyranosyl)-5′-*O*-benzyl-β-D-ribofuranosyloxy]prop-3-yne (16). A solution of compound **15** (0.75 g, 1.00 mmol) in dichloroethane (10 mL) was stirred with powdered molecular sieves 4 Å (0.5 g) under a nitrogen atmosphere. Propargyl alcohol (0.15 mL, 2.50 mmol) was added via syringe, followed by the addition of TMSOTf (0.05 mL, 0.25 mmol). The mixture was stirred for 30 min at room temperature, after which TLC analysis showed the appearance of a higher-running product. The reaction was quenched by the addition of triethylamine (0.10 mL, 0.70 mmol), filtered over Hyflo, and the solvents were removed in vacuo. Purification was performed by column chromatography (EtOAc/light petroleum, 1/4 → 1/1, v/v) to give acetylene derivative **16** as a colorless oil: yield 0.25 g (81%) + 0.09 g (12%) of recovered α-**15**; R_f 0.33 (acetone/CH_2Cl_2, 2/98, v/v); ^1H NMR (600 MHz, $CDCl_3$, HH-COSY) δ 7.43−7.23 (m, 15 H, CH arom), 5.38 (t, 1 H, H-3″, $J_{2''3''} = J_{3''4''}$ 9.8 Hz), 5.31 (d, 1 H, H-2′, $J_{2',3'}$ 4.6 Hz), 5.19 (s, 1 H, H-1′), 5.03 (t, 1 H, H-4″, $J_{4'',5''}$ 9.8 Hz), 5.00 (d, 1 H, H-1″, $J_{1'',2''}$ 3.5 Hz), 4.61 (AB, 2 H, CH_2 Bn, *J* -12.2 Hz), 4.55 (dd, 1 H, H-3′, $J_{3',4'}$ 7.4 Hz), 4.50 (AB, 2 H, CH_2 Bn, *J* -12.0 Hz), 4.39 (AB, 2 H, CH_2 Bn, *J* -12.1 Hz), 4.37 (m, 1 H, H-4′), 4.20 (ABX, 2 H, H-1, $^4J_{1,3}$ 2.4 Hz, $J_{1a,1b}$ -15.3 Hz), 3.85 (m, 1 H, H-5"), 3.69 (dd, 1 H, H-5a′, $J_{5a',5b'}$ -10.9 Hz), 3.57 (dd, 1 H, H-5b′, $J_{4',5b'}$ 5.1 Hz), 3.54 (dd, 1 H, H-2"), 3.33 (dd, 1 H, H-6a", $J_{6a'',6b''}$ -10.8 Hz, $J_{5'',6a''}$ 2.6 Hz), 3.27 (dd, 1 H, H-6b", $J_{5'',6b''}$ 3.9 Hz), 2.43 (t, 1 H, H-3), 1.92, 1.87, 1.86 (3 × s, 9H, 3 × CH_3 Ac); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 170.2, 170.1, 169.6, (3 × C(O) Ac), 138.0, 137.7, 137.6 (3× Cq Bn), 128.3−127.4 (CH arom), 103.2 (C-1′), 96.3 (C-1"), 80.4, 76.6, 74.7, 73.5, 71.9, 69.0, 68.8 (C-2″, C-3″, C-4″, C-5″, C-2′, C-3′, C-4′, C-3), 74.2 (C-2), 72.4, 72.3 72.1 (3× CH_2 Bn), 69.4, 66.7 (C-6″, C-5′), 53.0 (C-1), 19.8, 19.6, 19.5 (3 × CH_3 Ac); $[\alpha]_D^{20}$ +74.4 (*c* 2.0, $CHCl_3$); ES-MS *m*/*z* 769 $[M + Na]^+$. Anal. ($C_{41}H_{46}O_{13}$) C, H.

[5′-*O*-benzyl-3′-*O*-(2″,6″-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranosyloxy]prop-3-yne 2′,3″,4″-Tris(dibenzyl phosphate) (17). Triacetate **16** (63 mg, 84 μmol) was dissolved in a mixture of sodium methoxide (3 mg, 50 μmol) in dry MeOH (5 mL) and stirred for 1.5 h. The solution was neutralized with Dowex H^+, filtered and repeatedly concentrated under reduced pressure. The residue was concentrated with 1,2-dichloroethane (2 × 5 mL), dissolved in CH_2Cl_2 (5 mL) and subsequently dibenzyl (*N*,*N*-diisopropyl)phosphoramidite[38] (0.10 mL, 0.29 mmol) and a solution of 1*H*-tetrazole (52 mg, 0.70 mmol) in CH_3CN (2 mL) were added. The solution was stirred under an inert atmosphere for 30 min after which TLC analysis (toluene/EtOAc, 9/1, v/v) revealed complete conversion into a higher running product (R_f 0.82). The mixture was cooled (0 °C) and *t*-BuOOH (0.15 mL, 85% solution in di-*tert*-butyl peroxide, 1.3 mmol) was added. After 30 min at room temperature 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, 1/4 → 1/0, v/v) to give **17** as a colorless oil: yield 93 mg (80%, 2 steps); R_f 0.68 (toluene/EtOAc/MeOH, 180/50/5, v/v/v); ^1H NMR ($CDCl_3$) δ 7.42−7.01 (m, 45 H, CH arom), 5.33 (s, 1 H, H-1′), 5.08 (d, 1 H, H-1", $J_{1'',2''}$ 3.4 Hz), 5.01−4.82 (m, 14 H, H-2′, H-3″, 6× CH_2 Bn), 4.69 (AB, 2 H, CH_2 Bn, *J* −12.1 Hz), 4.54−4.45 (m, 5 H, CH_2 Bn, H-3′, H-4″, H-4′), 4.35 (AB, 2 H, CH_2 Bn, *J* −11.6 Hz), 4.13 (d, 2 H, H-1, $^4J_{1,3}$ 2.3 Hz), 3.81 (m,

1 H, H-5″), 3.67−3.45 (m, 5 H, H-5′, H-2″, H-6″), 2.30 (t, 1 H, H-3); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 138.1, 137.9, 137.6 (3× Cq Bn), 136.2, 136.0, 135.9, 135.8, 135.6, 135.5 (6× Cq Bn), 128.7−127.4 (CH Bn), 102.9 (C-1′), 95.1 (C-1″), 80.0, 78.1, 77.3, 77.2, 76.9, 74.3, 73.8, 73.6 (C-2“, C-3”, C-4“, C-5”, C-2′, C-3′, C-4′, C-3), 74.8, 73.1, 72.0 (3× CH_2 Bn), 70.1, 69.7, 69.6, 69.5, 69.3, 69.2, 69.0, 68.9, 67.9 (6× CH_2 Bn, C-6″, C-5′, C-2), 54.3 (C-1); ^{31}P{^{1}H} NMR ($CDCl_3$) δ −0.74, −1.34, −1.69; $[\alpha]_D^{20}$ +21.2 (*c* 0.5, $CHCl_3$); ES-MS *m/z* 1401 $[M + H]^+$, 1423 $[M + Na]^+$. Anal. ($C_{77}H_{79}O_{19}P_3$) C, H.

[2′-*O*-Acetyl-5′-*O*-benzyl-3′-*O*-(3″,4″-di-*O*-acetyl-2″,6″-di-*O*-benzyl-α-D-glucopyranosyl)-*β*-D-ribofuranosyloxy]prop-3-ynylbenzene (19). A solution of iodobenzene (**18**; 48 μL, 0.43 mmol) in DMF (4 mL) was degassed and stirred under an inert atmosphere. Et_3N (0.5 mL), $Pd(PPh_3)_2Cl_2$ (15 mg, 5 mol %) and CuI (8 mg, 10 mol %) were added and the mixture was degassed again. Acetylene derivative **16** (0.32 g, 0.43 mmol) in DMF (6 mL) was added via syringe during a 1 h period. The reaction mixture was stirred until, after 16 h, complete conversion of the acetylene (R_f 0.68) into a higher-running product was observed (16 h) with TLC analysis. The reaction mixture was diluted with Et_2O, washed with brine, aqueous $NaHCO_3$ (10%) and H_2O. The organic layer was dried with $MgSO_4$ and concentrated. The brown residue was subjected to column chromatography (EtOAc/light petroleum, 1/3 → 2/3, v/v) to give phenyl acetylene derivative **19** as a brownish foam: yield 0.26 g (74%); R_f 0.78 (toluene/EtOAc/MeOH, 90/25/2.5, v/v/v); ^{1}H NMR (HH-COSY, 300 MHz, $CDCl_3$) δ 7.62−7.26 (m, 20 H, CH arom), 5.39 (t, 1 H, H-3″, $J_{2'',3''} = J_{3'',4''}$ 9.6 Hz), 5.35 (d, 1 H, H-2′, $J_{2',3'}$ 4.5 Hz), 5.27 (s, 1 H, H-1′), 5.03 (t, 1 H, H-4″, $J_{4'',5''}$ 9.7 Hz), 4.99 (d, 1 H, H-1″, $J_{1'',2''}$ 3.3 Hz), 4.56 (dd, 1 H, H-3′, $J_{3',4'}$ 7.9 Hz), 4.54 (AB, 2 H, CH_2 Bn, *J* -12.1 Hz), 4.45 (m, 4 H, CH_2 Bn, H-1), 4.38 (m, 3 H, CH_2 Bn, H-4′), 3.86 (dt, 1 H, H-5″, $J_{5'',6''}$ 3.0 Hz, $J_{4'',5''}$ 7.4 Hz), 3.72 (dd, 1 H, H-5a′, $J_{5a',5b'}$ −10.5 Hz, $J_{4',5a'}$ 3.4 Hz), 3.55 (dd, 1 H, H-5b′, $J_{4',5b'}$ 4.9 Hz), 3.54 (dd, 1 H, H-2″), 3.31 (ABX, 2 H, H-6″, $J_{6a'',6b''}$ −10.9 Hz), 1.92, 1.87, 1.86 (3× s, 9 H, CH_3 Ac); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 170.0, 169.9, 169.4, (3 × C(O) Ac), 137.9, 137.6, 137.4 (3× Cq Bn), 131.6, 128.3−127.4 (CH arom), 122.2 (Cq Ph), 103.1 (C-1′), 96.1 (C-1″), 86.4, 83.8 (C-2, C-3 alkyne), 80.2, 76.5, 74.6, 73.4, 71.8, 68.9, 68.6 (C-2″, C-3″, C-4″, C-5″, C-2′, C-3′, C-4′), 73.3, 73.2 73.0 (3× CH_2 Bn), 70.4, 67.5 (C-6″, C-5′), 54.7 (C-1), 20.7, 20.6, 20.4 (3 × CH_3 Ac); $[\alpha]_D^{20}$ +54.8 (*c* 1.0, $CHCl_3$); ES-MS *m/z* 840 $[M + NH_4]^+$, 845 $[M + Na]^+$, 861 $[M + K]^+$. Anal. ($C_{47}H_{50}O_{13}$) C, H.

[5′-*O*-Benzyl-3′-*O*-(2″,6″-di-*O*-benzyl-α-D-glucopyranosyl)-*β*-D-ribofuranosyloxy]prop-3-ynylbenzene 2′,3″,4″-Tris(dibenzyl phosphate) (20). Deacetylation and phosphorylation of phenylacetylene derivative **19** were performed as described for the synthesis of **17**. The product was purified by column chromatography (EtOAc/light petroleum, 1/3 → 1/1, v/v) to give **20** as a colorless oil: yield 0.33 g (88%, 2 steps); R_f 0.75 (EtOAc/light petroleum, 1/1, v/v); ^{1}H NMR ($CDCl_3$) δ 7.52−7.08 (m, 50 H, CH arom), 5.46 (s, 1 H, H-1′), 5.13 (d, 1 H, H-1″, $J_{1'',2''}$ 2.9 Hz), 5.08−4.85 (m, 14 H, H-2′, H-3″, 6× CH_2 Bn), 4.73 (AB, 2 H, CH_2 Bn, *J* −12.2 Hz), 4.59−4.47 (m, 5 H, CH_2 Bn, H-3′, H-4″, H-4′), 4.39 (m, 4 H, CH_2 Bn, H-1), 3.89 (m, 1 H, H-5″), 3.72−3.48 (m, 5 H, H-5′, H-2″, H-6″); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 137.9, 137.4 (3× Cq Bn), 136.0, 135.9, 135.7, 135.6, 135.4, 135.3 (6× Cq Bn), 131.6 (CH Ph), 129.7−126.8 (CH Bn), 122.2 (Cq Ph), 103.0 (C-1′), 95.0 (C-1″), 86.4, 83.9 (C-2, C-3 alkyne), 79.9, 78.0, 77.2, 76.8, 76.8, 74.1, 73.6, 73.6 (C-2″, C-3″, C-4″, C-5″, C-2′, C-3′, C-4′), 74.1, 73.6, 73.0, 71.9, 70.0, 69.6, 69.2, 68.9, 67.8 (9× CH_2 Bn, C-6″, C-5′), 55.1 (C-1); ^{31}P{^{1}H} NMR ($CDCl_3$) δ −0.74, −1.36, −1.70. Anal. ($C_{83}H_{83}O_{19}P_3$) C, H.

1,6-Bis[2′-*O*-acetyl-5′-*O*-benzyl-3′-*O*-(3″,4″-di-*O*-acetyl-2″,6″-di-*O*-benzyl-α-D-glucopyranosyl)-*β*-D-ribofuranosyloxy]-2,4-hexadiyne (21): colorless oil; R_f 0.41 (toluene/EtOAc/MeOH, 90/15/2, v/v/v); ^{1}H NMR (300 MHz, $CDCl_3$, HH-COSY) δ 7.38−7.17 (m, 30H, CH arom), 5.37 (t, 2 H, H-3″, $J_{2'',3''} = J_{3'',4''}$ 9.7 Hz), 5.30 (d, 2 H, H-2′, $J_{2',3'}$ 4.6 Hz), 5.15 (s, 2 H, H-1′), 5.02 (t, 2H, H-4″, $J_{4'',5''}$ 9.7 Hz), 5.00 (d, 2 H, H-1″, $J_{1'',2''}$ 3.1 Hz), 4.68−4.44 (m, 20 H, H-3′, 6× CH_2 Bn, H-1, H-6, H-4′), 3.85 (dt, 2 H, H-5″, $J_{5'',6''}$ 3.1 Hz, $J_{4'',5''}$ 9.9 Hz), 3.68 (dd, 2 H, H-5a′, $J_{5a',5b'}$ −10.8 Hz, $J_{4',5a'}$ 3.4 Hz), 3.56 (dd, 2 H, H-5b′, $J_{4',5b'}$ 4.7 Hz), 3.53 (dd, 2 H, H-2″), 3.29 (ABX, 4 H, H-6″, $J_{6a'',6b''}$ −10.8 Hz), 1.92, 1.88, 1.86 (3× s, 18 H, CH_3 Ac); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 170.1, 170.0, 169.5, (3× C(O) Ac), 137.9, 137.7, 137.5 (3× Cq Bn), 128.3−127.4 (CH Bn), 103.2 (C-1′), 96.2 (C-1″), 80.4, 76.5, 74.5, 73.4, 71.9, 69.0, 68.7 (C-2″, C-3″, C-4″, C-5″, C-2′, C-3′, C-4′), 74.4 (C-2), 73.4, 73.3 73.1 (3× CH_2 Bn), 70.5 (C-3), 70.3, 67.6 (C-6″, C-5′), 54.3 (C-1), 20.8, 20.6, 20.5 (3× CH_3 Ac); ES-MS *m/z* 1508 $[M + NH_4]^+$, 1513 $[M + Na]^+$, 1529 $[M + K]^+$. Anal. ($C_{82}H_{90}O_{26}$) C, H.

1,4-Bis{[2′-*O*-acetyl-5′-*O*-benzyl-3′-*O*-(3″,4″-di-*O*-acetyl-2″,6″-di-*O*-benzyl-α-D-glucopyranosyl)-*β*-D-ribofuranosyloxy]prop-3-ynyl}benzene (23). 1,4-Diiodobenzene (**22**; 60 mg, 0.19 mmol) was treated with acetylene **16** (0.35 g, 0.47 mmol) as described for for the preparation of **19**. TLC analysis revealed complete conversion into a lower-running product after 2 h. Column chromatography (EtOAc/light petroleum, 2/3, v/v) afforded disubstituted benzene derivative **23** as a brownish foam: R_f 0.24 (toluene/EtOAc/MeOH, 180/25/2.5, v/v/v); yield 0.29 g (100%); ^{1}H NMR (HH-COSY, 300 MHz, $CDCl_3$) δ 7.36 (s, 4 H, CH-arom Ph), 7.34−7.26 (m, 30 H, CH-arom Bn), 5.38 (t, 2 H, H-3″, $J_{2'',3''} = J_{3'',4''}$ 9.7 Hz), 5.36 (d, 2 H, H-2′, $J_{2',3'}$ 4.8 Hz), 5.27 (s, 2 H, H-1′), 5.03 (t, 2 H, H-4″, $J_{4'',5''}$ 9.6 Hz), 5.01 (d, 2 H, H-1″, $J_{1'',2''}$ 3.1 Hz), 4.58 (dd, 2 H, H-3′, $J_{3',4'}$ 8.2 Hz), 4.51 (AB, 4 H, CH_2 Bn, *J* -12.2 Hz), 4.48 (m, 8 H, CH_2 Bn, H-1), 4.38 (AB, 4 H, CH_2 Bn, *J* −12.1 Hz), 4.36 (m, 2 H, H-4′), 3.85 (dt, 2 H, H-5″, $J_{5'',6''}$ 2.9 Hz, $J_{4'',5''}$ 7.3 Hz), 3.70 (dd, 2 H, H-5a′, $J_{5a',5b'}$ −10.7 Hz, $J_{4',5a'}$ 3.4 Hz), 3.57 (dd, 2 H, H-5b′, $J_{4',5b'}$ 4.8 Hz), 3.54 (dd, 2 H, H-2″), 3.28 (ABX, 4 H, H-6″, $J_{6a'',6b''}$ −10.6 Hz), 1.92, 1.87, 1.86 (3× s, 18 H, CH_3 Ac); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 170.1, 169.5, (3 × C(O) Ac), 137.6, 137.5, 137.4 (3× Cq Bn), 133.1 (CH Ph), 128.2−127.3 (CH arom), 122.4 (Cq Ph), 103.2 (C-1′), 96.1 (C-1″), 85.8 (C-2, C-3 alkyne), 80.3, 76.5, 74.6, 73.4, 71.8, 68.9, 68.6 (C-2″, C-3″, C-4″, C-5″, C-2′, C-3′, C-4′), 73.2, 73.1 73.0 (3× CH_2 Bn), 70.4, 67.5 (C-6″, C-5′), 54.7 (C-1), 20.6, 20.4 (3 × CH_3 Ac); $[\alpha]_D^{20}$ +56.5 (*c* 0.87, $CHCl_3$); ES-MS *m/z* 1584 $[M + NH_4]^+$, 1589 $[M + Na]^+$, 1605 $[M + K]^+$. Anal. ($C_{88}H_{94}O_{26}$) C, H.

1,4-Bis{[5′-*O*-benzyl-3′-*O*-(2″,6″-di-*O*-benzyl-α-D-glucopyranosyl)-*β*-D-ribofuranosyloxy]prop-3-ynyl 2′,3″,4″-tris(dibenzyl phosphate)}benzene (24). Deacetylation and phosphorylation of **23** were performed as described for the synthesis of **17**. Purification was established by column chromatography (EtOAc/light petroleum, 1/3 → 1/0, v/v) to give **24** as a colorless oil: yield 0.15 g (88%, 2 steps); R_f 0.50 (EtOAc/light petroleum, 2/1, v/v); ^{1}H NMR ($CDCl_3$) δ 7.41−7.02 (m, 94 H, CH arom), 5.38 (s, 2 H, H-1′), 5.08 (d, 2 H, H-1″, $J_{1'',2''}$ 3.7 Hz), 5.03−4.81 (m, 28 H, H-2′, H-3″, 12× CH_2 Bn), 4.69 (AB, 4 H, CH_2 Bn, *J* −11.8 Hz), 4.57−4.26 (m, 18 H, 2× CH_2 Bn, H-3′, H-4″, H-4′, H-1), 3.83 (m, 2 H, H-5″), 3.71−3.48 (m, 10H, H-5′, H-2″, H-6″); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 138.0, 137.5, 135.8, 135.6, 135.5, 135.4 (9× Cq Bn), 131.6 (CH Ph), 128.4−127.4 (CH Bn), 122.4 (Cq Ph), 103.2 (C-1′), 95.1 (C-1″), 85.9 (C-2, C-3 alkyne), 80.0, 78.1, 77.4, 76.9, 74.3, 73.7, 73.6 (C-2″, C-3″, C-4″, C-5″, C-2′, C-3′, C-4′), 73.1, 72.0, 70.1, 69.7, 69.3, 67.9 (9× CH_2 Bn, C-6", C-5′), 55.2 (C-1); ^{31}P{^{1}H} NMR ($CDCl_3$) δ −0.75, −1.42, −1.74. Anal. ($C_{160}H_{160}O_{38}P_6$) C, H.

1,2,4,5-Tetrakis{[2′-*O*-acetyl-5′-*O*-benzyl-3′-*O*-(3″,4″-di-*O*-acetyl-2″,6″-di-*O*-benzyl-α-D-glucopyranosyl)-*β*-D-ribofuranosyloxy]prop-3-ynyl}benzene (26). 1,2,4,5-Tetraiodobenzene[39] (**25**; 53 mg, 91 μmol) was treated with acetylene **16** (0.30 g, 0.40 mmol) as described for the preparation of **19**. After 4 h TLC analysis (toluene/EtOAc/MeOH, 90/15/2, v/v/v) revealed complete conversion of **16** (R_f 0.54) into a lower-running product. Column chromatography (EtOAc/light petroleum, 2/3 → 3/2, v/v) afforded tetramer **26** as a brown foam: R_f 0.26; yield 0.14 g (52%); ^{1}H NMR (300 MHz, $CDCl_3$, HH-COSY) δ 7.48 (s, 2 H, H-2 and H-5 Ph) 7.34−7.19 (m, 60 H, CH arom), 5.37 (t, 4 H, H-3″, $J_{2'',3''} = J_{3'',4''}$ 9.7 Hz), 5.35 (d, 4 H, H-2′, $J_{2',3'}$ 4.7 Hz), 5.26 (s, 4 H, H-1′), 5.03 (t, 4 H, H-4″, $J_{4'',5''}$ 9.7 Hz), 5.03 (d, 4 H, H-1″, $J_{1'',2''}$ 3.0 Hz), 4.58 (dd, 4 H, H-3′, $J_{3',4'}$ 8.2 Hz), 4.51 (AB, 8 H, CH_2 Bn, *J* −12.2 Hz), 4.44 (m, 16 H, CH_2 Bn, H-1), 4.38 (AB, 8 H, CH_2 Bn, *J* -11.9 Hz), 4.37 (m, 4 H,

H-4′), 3.85 (dt, 4 H, H-5″, $J_{5″,6″}$ 3.0 Hz, $J_{4″,5″}$ 7.4 Hz), 3.69 (dd, 4 H, H-5a′, $J_{5a′,5b′}$ −10.9 Hz, $J_{4′,5a′}$ 3.3 Hz), 3.57 (dd, 4 H, H-5b′, $J_{4′,5b′}$ 4.9 Hz), 3.56 (dd, 4 H, H-2″), 3.28 (ABX, 8 H, H-6″, $J_{6a″,6b″}$ −10.8 Hz), 1.91, 1.85 (2 × s, 36 H, 3 × CH_3 Ac); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 170.2, 170.0, 169.6, (3 × C(O) Ac), 138.0, 137.8, 137.6 (3× Cq Bn), 135.4 (CH Ph), 128.3−127.4 (CH Bn), 124.9 (Cq Ph), 103.0 (C-1′), 96.1 (C-1″), 90.2, 83.9 (C-2, C-3 alkyne), 80.3, 76.6, 74.5, 73.5, 72.0, 69.1, 68.7 (C-2″, C-3″, C-4″, C-5″, C-2′, C-3′, C-4′), 73.4, 73.3 73.1 (3× CH_2 Bn), 70.4, 67.6 (C-6″, C-5′), 54.5 (C-1), 20.8, 20.6, 20.5 (3 × CH_3 Ac); $[\alpha]_D^{20}$ +52.0 (*c* 0.4, $CHCl_3$). Anal. ($C_{170}H_{182}O_{52}$) C, H.

1,2,4,5-Tetrakis{[5′-*O*-benzyl-3′-*O*-(2″,6″-di-*O*-benzyl-α-D-glucopyranosyl)-*β*-D-ribofuranosyloxy]prop-3-ynyl-2′,3″,4″-tris(dibenzyl phosphate)}benzene (27). Deacetylation and phosphorylation of **26** were performed as described for the synthesis of **17**. Purification was established by column chromatography (EtOAc/light petroleum, 1/2 → 1/0, v/v) to afford **27** as a slightly yellowish oil: yield 77 mg (65%, 2 steps); R_f 0.54 (toluene/EtOAc/MeOH, 25/10/1, v/v/v); ^1H NMR ($CDCl_3$) δ 7.39−6.99 (m, 182 H, CH arom), 5.58 (s, 4 H, H-1′), 5.08 (d, 2 H, H-1″, $J_{1″,2″}$ 3.7 Hz), 5.03−4.81 (m, 28 H, H-2′, H-3″, 12× CH_2 Bn), 4.69 (AB, 4 H, CH_2 Bn, *J* −11.8 Hz), 4.57−4.26 (m, 18 H, 2× CH_2 Bn, H-3′, H-4″, H-4′, H-1), 3.83 (m, 2 H, H-5″), 3.71−3.48 (m, 10 H, H-5′, H-2″, H-6″); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 138.0, 137.4 (3× Cq Bn), 136.0, 135.8, 135.7, 135.6, 135.5, 135.3 (6× Cq Bn), 135.4 (CH Ph), 128.5−127.4 (CH Bn), 124.9 (CH Ph), 103.0 (C-1′), 95.1 (C-1″), 90.4 (C-2), 83.5 (C-3), 79.9, 78.3, 77.4, 76.9, 74.3, 73.8, 73.7 (C-2″, C-3″, C-4″, C-5″, C-2′, C-3′, C-4′), 74.6, 73.2, 72.1 (3× CH_2 Bn), 69.8, 69.7, 69.6, 69.5, 69.3, 69.2, 69.1, 67.8 (6× CH_2 Bn, C-6″, C-5′), 55.0 (C-1); $^{31}P\{^1H\}$ NMR ($CDCl_3$) δ −0.74, −1.53, −1.83; $[\alpha]_D^{20}$ +8.4 (*c* 0.5, $CHCl_3$). Anal. ($C_{314}H_{314}O_{76}P_{12}$) C, H.

Propyl 3′-*O*-(α-D-Glucopyranosyl)-*β*-D-ribofuranoside 2′,3″,4″-Trisphosphate (Na^+ Salt), 'Propylphostin' (5). Compound **17** (64 mg, 46 μmol) and NaOAc (45 mg, 4 equiv/phosphate) were dissolved in a mixture of dioxane/2-propanol/H_2O (7 mL, 4/2/1, v/v/v) and the solution was degassed. Palladium on carbon (10%, 50 mg) was added and the reaction mixture was stirred under an atmosphere of hydrogen gas. After 16 h the catalyst was removed by filtration over glass Fiber (GF/2A, Whatman). The filtrate was concentrated under reduced pressure and the product was purified by gel-filtration over a Fractogel HW-40 column by elution with a triethylammonium bicarbonate buffer (0.15 M). Concentration and repeated concentration (MeOH/H_2O, 4/1, v/v, 3 × 15 mL) of the appropriate fractions (at *t* = 85 min), followed by lyophilization gave trisphosphate **5** in pure form. The product was converted to the Na^+ form by ion-exchange with Dowex 50WX4 (Na^+-form) and imino diacetate resin (Chelex, Na^+ form), followed by lyophylization: yield 28 mg (85%, 39 μmol); anion-exchange HPLC elution at 24% buffer B; ^1H NMR (300 MHz, D_2O, HH-COSY) δ 5.32 (s, 1 H, H-1′), 5.11 (d, 1 H, H-1″, $J_{1″,2″}$ 3.9 Hz), 4.48 (dd, 1 H, H-2′, $J_{2′,3′}$ 3.2 Hz, $^3J_{2′,P}$ 6.8 Hz), 4.24−4.19 (m, 3 H, H-3″,H-3′, H-4′), 3.97 (dd, 1 H, H-5a′, $J_{5a′,5b′}$ -10.1 Hz, $J_{4′,5a′}$ 3.0 Hz), 3.91 (q, 1 H, H-4″, $J_{3″,4″} = {}^3J_{4″,P}$ 9.0 Hz), 3.81−3.73 (m, 2 H, H-5″, H-5b′), 3.71−3.55 (m, 4 H, H-6″, H-2″, H-1a), 3.48 (m, 1 H, H-1b), 1.55 (m, 2 H, H-2), 0.86 (t, 3 H, H-3, *J* 7.4 Hz); $^{13}C\{^1H\}$ NMR (D_2O, CH-COSY, 75 MHz) δ 107.1 (C-1′), 96.4 (C-1″), 81.6 (C-4′), 76.6 (C-3″), 74.2 (C-2′), 73.4 (C-3′), 73.3 (C-5″), 73.1 (C-2″), 73.0 (C-4″), 70.6 (C-1), 63.9 (C-6″), 60.9 (C-5′), 22.7 (C-2), 10.5 (C-3); $^{31}P\{^1H\}$ NMR (PH-COSY, D_2O, 121 MHz) δ 5.1 (P-3″), 4.5 (P-4″), 4.2 (P-2′).; ES-MS *m*/*z* 593 $[M - H]^-$, 615 $[M - 2H + Na]^-$, 637 $[M - 3H + 2Na]^-$; HR-MS $C_{14}H_{28}O_{19}P_3$ $[M - H]^-$ calcd 593.0437, found 593.0434 (±0.0028).

[3′-*O*-(α-D-Glucopyranosyl)-*β*-D-ribofuranosyloxy]-prop-3-ylbenzene 2′,3″,4″-Trisphosphate (Na^+ Salt), 'Phenylphostin' (6). Deprotection of **20** and purification as described for the synthesis of **5** gave phenyl derivative **6** as a white fluffy solid. Release from HW40 fractogel column at *t* = 110 min: yield 80% (49 mg, 76 μmol); anion-exchange HPLC elution at 26% buffer B; ^1H NMR (300 MHz, D_2O, HH-COSY) δ 7.37−7.20 (m, 5 H, CH Ph), 5.20 (s, 1 H, H-1′), 5.15 (d, 1 H, H-1″, $J_{1″,2″}$ 3.9 Hz), 4.53 (dd, 1 H, H-2′, $J_{2′,3′}$ 4.1 Hz, $^3J_{2′,P}$ 8.4 Hz), 4.37 (q, 1 H, H-3″, $J_{2″,3″} = J_{3″,4″} = {}^3J_{3″,P}$ 9.2 Hz), 4.25 (dd, 1 H, H-3′, $J_{3′,4′}$ 7.6 Hz), 4.20 (m, 1 H, H-4′), 3.97 (q, 1 H, H-4″, $J_{4″,5″} = {}^3J_{4″,P}$ 9.6 Hz), 3.89 (dd, 1 H, H-6a″, $J_{6a″,6b″}$ -9.6 Hz, $J_{5″,6a″}$ 3.7 Hz), 3.83−3.61 (m, 5 H, H-5a′, H-6b″, H-5″, H-2″, H-1a), 3.58−3.47 (m, 2 H, H-5b′, H-1b), 2.68 (t, 2 H, H-3, *J* 7.5 Hz), 1.88 (m, 2 H, H-2); $^{13}C\{^1H\}$ NMR (D_2O, CH-COSY, 75 MHz) δ 143.1 (Cq Ph), 129.5, 129.4, 126.8 (CH Ph), 107.2 (C-1′), 97.7 (C-1″), 81.6 (C-4′), 77.9 (C-3″), 74.7 (C-2′, C-3′), 72.7 (C-4″, C-2″), 71.7 (C-5″), 68.3 (C-1), 64.0 (C-5′), 61.0 (C-6″), 32.3 (C-3), 31.2 (C-2); $^{31}P\{^1H\}$ NMR (PH-COSY, 121 MHz, D_2O) δ 3.37 (P-4″), 3.13 (P-2′), 2.17 (P-3″); ES-MS *m*/*z* 669 $[M - H]^-$, 691 $[M - 2H + Na]^-$, 713 $[M - 3H + 2Na]^-$; HR-MS $C_{20}H_{32}O_{19}P_3$ $[M - H]^-$calcd 669.0751, found 669.0747 (±0.0033).

1,4-Bis{[3′-*O*-(α-D-glucopyranosyl)-*β*-D-ribofuranosyloxy]-prop-3-yl 2′,3″,4″-trisphosphate}benzene (Na^+ Salt), 'Diphostin' (7). Deprotection of **24** and purification as described for the synthesis of **5** afforded dimer **7** as a white hygroscopic powder. Release from HW40 fractogel column at *t* = 69 min: yield 42% (18 mg, 27 μmol); anion-exchange HPLC elution at 30% buffer B; ^1H NMR (300 MHz, D_2O, HH-COSY) δ 7.24 (s, 4 H, CH Ph), 5.34 (s, 2 H, H-1′), 5.15 (d, 2 H, H-1″, $J_{1″,2″}$ 3.9 Hz), 4.52 (dd, 2 H, H-2′, $J_{2′,3′}$ 3.4 Hz, $^3J_{2′,P}$ 6.9 Hz), 4.33−4.22 (m, 6 H, H-3″,H-3′, H-4′), 3.99 (q, 2 H, H-4″, $J_{4″,5″} = {}^3J_{4″,P}$ 9.4 Hz), 3.93 (dd, 2 H, H-6a″, $J_{6a″,6b″}$ −10.3 Hz, $J_{5″,6a″}$ 3.6 Hz), 3.87−3.71 (m, 10 H, H-5a′, H-6b″, H-5″, H-2″, H-1a), 3.68−3.52 (m, 4 H, H-5b′, H-1b), 2.65 (t, 4 H, H-3, *J* 7.7 Hz), 1.88 (m, 4 H, H-2); $^{13}C\{^1H\}$ NMR (D_2O) δ 140.3 (Cq Ph), 130.4 (CH Ph), 107.3 (C-1′), 97.4 (C-1″), 81.8 (C-4′), 77.5 (C-3″), 74.5, 74.2 (C-2′, C-3′), 73.3, 72.8 (C-4″, C-2″), 71.9 (C-5″), 68.7 (C-1), 64.1 (C-5′), 61.2 (C-6″), 32.8 (C-3), 31.0 (C-2); $^{31}P\{^1H\}$ NMR (PH-COSY, 121 MHz, D_2O) δ 4.17 (broad, P-4″, P-2′, P-3″); ES-MS *m*/*z* 630 $[M - 2H]^{2-}$, 641 $[M - 3H + Na]^{2-}$; HR-MS $C_{34}H_{58}O_{38}P_6$ $[M - 2H]^{2-}$ calcd 630.0515, found 630.0508 (±0.0025).

1,2,4,5-Tetrakis{[3′-*O*-(α-D-glucopyranosyl)-*β*-D-ribofuranosyloxy]prop-3-yl 2′,3″,4″-trisphosphate}benzene (Na^+ Salt), 'Tetraphostin' (8). Deprotection of **26** and purification were performed as described for the synthesis of **5** to give tetramer **8** as a highly hygroscopic solid. Release from HW40 fractogel column at *t* = 65 min: yield 42% (15 mg, 6.1 μmol); anion-exchange HPLC elution at 36% buffer B; ^1H NMR (600 MHz, D_2O, HH-COSY) δ 7.10 (s, 2 H, CH Ph), 5.32 (s, 4 H, H-1′), 5.13 (d, 4 H, H-1″, $J_{1″,2″}$ 4.0 Hz), 4.51 (dd, 4 H, H-2′, $J_{2′,3′}$ 4.5 Hz, $^3J_{2′,P}$ 7.3 Hz), 4.25 (m, 12 H, H-3″, H-4′, H-3′), 3.96 (m, 8 H, H-4″, H-6a″), 3.78 (m, 12 H, H-5a′, H-2″, H-1a), 3.70 (dd, 4 H, H-6b″, $J_{6a″,6b″}$ −13.3 Hz, $J_{5″,6b″}$ 2.1 Hz), 3.66−3.57 (m, 12 H, H-5b′, H-5″, H-1b), 2.61 (t, 8 H, H-3, *J* 8.0 Hz), 1.84 (m, 8 H, H-2); $^{13}C\{^1H\}$ NMR (D_2O, CH-COSY, 75 MHz) δ 138.8 (Cq Ph), 131.1 (CH Ph), 107.5 (C-1′), 96.9 (C-1″), 81.9 (C-4′), 77.0 (C-3″), 74.4 (C-2′), 73.8 (C-3′), 72.9 (C-5″, C-2″), 72.0 (C-4″), 69.2 (C-1), 64.2 (C-5′), 61.1 (C-6″), 31.3 (C-3), 28.8 (C-2); $^{31}P\{^1H\}$ NMR (PH-COSY, 121 MHz, D_2O) δ 4.57 (P-3″), 4.34 (P-4″), 4.25 (P-2′); ES-MS *m*/*z* 611 $[M - 4H]^{4-}$, 616 $[M - 5H + Na]^{4-}$, 622 $[M - 6H + 2Na]^{4-}$, 627 $[M - 7H + 3Na]^{4-}$, 633 $[M - 8H + 4Na]^{4-}$, 822 $[M - 4H + Na]^{3-}$, 830 $[M - 5H + 2Na]^{3-}$, 837 $[M - 6H + 3Na]^{3-}$, 845 $[M - 7H + 4Na]^{3-}$, 852 $[M - 8H + 5Na]^{3-}$, 859 $[M - 9H + 6Na]^{3-}$; HR-MS $C_{62}H_{110}O_{76}P_{12}$ $[M - 4H]^{4-}$ calcd 610.5398, found 610.5405 (±0.0021).

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References

(1) Berridge, M. J. Inositol trisphosphate and calcium signaling. *Nature* **1993**, *361*, 315−325.
(2) Potter, B. V. L.; Lampe, D. Chemistry of inositol lipid mediated cellular signaling. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1933−1972.
(3) Taylor, C. W. Inositol trisphosphate receptors: Ca^{2+}-modulated intracellular Ca^{2+} channels. *Biochim. Biophys. Acta* **1998**, *1426*, 19−33.
(4) Berridge, M. J. The AM and FM of calcium signaling. *Nature* **1997**, *386*, 759−760.
(5) Berridge, M. J. Elementary and global aspects of calcium signaling. *J. Physiol.* **1997**, *499*, 291−306.
(6) Patel, S.; Joseph, S. K.; Thomas, A. P. Molecular properties of inositol 1,4,5-trisphosphate receptors. *Cell Calcium* **1999**, *25*, 247−264.
(7) Katayama, E.; Funashashi, H.; Michikawa, T.; Shiraishi, T.; Ikemoto, T.; Iino, M.; Hirosawa, K.; Mikoshiba, K. Native structure and arrangement of inositol-1,4,5-trisphosphate receptor molecules in bovine cerebellar Purkinje cells as studied by quick-freeze deep-etch electron microscopy. *EMBO J.* **1996**, *15*, 4844−4851.
(8) Mignery, G. A.; Newton, C. L.; Archer, B. T., III; Südhof, T. C. Structure and expression of the rat inositol 1,4,5-trisphosphate receptor. *J. Biol. Chem.* **1990**, *265*, 12679−12685.
(9) Miyawaki, A.; Furuichi, T.; Ryou, Y.; Yoshikawa, S.; Nakagawa, T.; Saitoh, T.; Mikoshiba, K. Structure−function relationships of the mouse inositol 1,4,5-trisphosphate receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4911−4915.
(10) Ramos-Franco, J.; Galvan, D.; Mignery, G. A.; Fill, M. Location of the permeation pathway in the recombinant type-1 inositol 1,4,5-trisphosphate receptor. *J. Gen. Physiol.* **1999**, *114*, 243−250.
(11) Yoshikawa, F.; Iwasaki, H.; Michikawa, T.; Furuichi, T.; Mikoshiba, K. Cooperative formation of the ligand-binding site of the inositol 1,4,5-trisphosphate receptor by two separable domains. *J. Biol. Chem.* **1999**, *274*, 328−334.
(12) Taylor, C. W.; Genazzani, A.; Morris, S. A. Expression of inositol trisphosphate receptors. *Cell Calcium* **1999**, *26*, 237−251.
(13) DeLisle, S.; Blondel, O.; Longo, F. J.; Schnabel, W. E.; Bell, G. I.; Welsh, M. J. Expression of inositol 1,4,5-trisphosphate receptors changes the Ca2+ signal of Xenopus oocytes. *Am. J. Physiol.* **1996**, *270*, C1255−C1261.
(14) Miyakawa, T.; Maeda, A.; Yamazawa, T.; Hirose, K.; Kurosaki, T.; Iino, M. Encoding of Ca^{2+} signals by differential expression of IP_3 receptor subtypes. *EMBO J.* **1999**, *18*, 1303−1308.
(15) Marchant, J. S.; Taylor, C. W. Cooperative activation of IP_3 receptors by sequential binding of IP_3 and Ca^{2+} safeguards against spontaneous activity. *Curr. Biol.* **1997**, *7*, 510−518.
(16) Meyer, T.; Wensel, T.; Stryer, L. Kinetics of calcium channel opening by inositol 1,4,5-trisphosphate. *Biochemistry* **1990**, *29*, 32−37.
(17) Parker, I.; Yao, Y.; Ilyin, V. Fast kinetics of calcium liberation induced in *Xenopus* oocytes by photoreleased inositol trisphosphate. *Biophys. J.* **1996**, *70*, 222−237.
(18) Finch, E. A.; Turner, T. J.; Goldin, S. M. Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release. *Science* **1991**, *252*, 443−445.
(19) Adkins, C. E.; Taylor, C. W. Lateral inhibition of inositol 1,4,5-trisphosphate receptors by cytosolic Ca^{2+}. *Curr. Biol.* **1999**, *9*, 1115−1118.
(20) Marchant, J. S.; Taylor, C. W. Rapid activation and partial inactivation of inositol trisphosphate receptors by inositol trisphosphate. *Biochemistry* **1998**, *37*, 11525−11533.
(21) Mammen, M.; Choi, S.-K.; Whitesides, G. M. Polyvalent interactions in biological systems: implications for design and use of multivalent ligands and inhibitors. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754−2794.
(22) Kramer, R. H.; Karpen, J. W. Spanning binding sites on allosteric proteins with polymer-linked ligand dimers. *Nature* **1998**, *395*, 710−713.
(23) Valentijn, A. R. P. M.; van der Marel, G. A.; van Boom, J. H. Solid-phase synthesis of lysine-based cluster galactosides with high affinity for the asialoglycoprotein receptor. *Tetrahedron* **1997**, *53*, 759−770.
(24) Takahashi, M.; Tanzawa, K.; Takahashi, S. Adenophostins, newly discovered metabolites of *Penicillium Brevicompactum*, act as potent agonist of the inositol 1,4,5-trisphosphate receptor. *J. Biol. Chem.* **1994**, *269*, 369−372.
(25) Hirota, J.; Michikawa, T.; Miyawaki, A.; Takahashi, M.; Tanzawa, K.; Okura, I.; Mikoshiba, K. Adenophostin-mediated quantal Ca^{2+}-release in the purified and reconstituted inositol 1,4,5-trisphosphate receptor-type-1. *FEBS Lett.* **1995**, *368*, 248−251.
(26) Hotoda, H.; Murayama, K.; Miyamoto, S.; Iwata, Y.; Takahashi, M.; Kawase, Y.; Tanzawa, K.; Kaneko, M. Molecular recognition of adenophostin, a very potent Ca^{2+} inducer, at the D-*myo*-inositol 1,4,5-trisphosphate receptor. *Biochemistry* **1999**, *38*, 9234−9241.
(27) Marwood, R. D.; Riley, A. M.; Correa, V.; Taylor, C. W.; Potter, B. V. L. Simplification of adenophostin A defines a minimal structure for potent glucopyranoside-based mimics of D-*myo*-inositol 1,4,5-trisphosphate. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 453−458.
(28) Shuto, S.; Tatani, K.; Ueno, Y.; Matsuda, A. Synthesis of adenophostin analogues lacking the adenine moiety as novel IP_3 receptor ligands: some structural requirements for the significant activity of adenophostin A. *J. Org. Chem.* **1998**, *63*, 8815−8824.
(29) Jenkins, D. J.; Marwood, R. D.; Potter, B. V. L. A disaccharide polyphosphate mimic of D-*myo*-inositol 1,4,5-trisphosphate. *Chem. Commun.* **1997**, 449−450.
(30) Marchant, J. S.; Beecroft, M. D.; Riley, A. M.; Jenkins, D. J.; Marwood, R. D.; Taylor, C. W.; Potter, B. V. L. Disaccharide polyphosphates based upon adenophostin A activate hepatic D-*myo*-inositol 1,4,5-trisphosphate receptors. *Biochemistry* **1997**, *36*, 12780−12790.
(31) de Kort, M.; Valentijn, A. R. P. M.; van der Marel, G. A.; van Boom, J. H. Synthesis of clustered disaccharide polyphosphate analogues of adenophostin A. *Tetrahedron Lett.* **1997**, *38*, 7629−7632.
(32) Sonogashira, K.; Tohda, Y.; Hagihara, N. A convenient synthesis of acetylenes: catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes, and bromopyridines. *Tetrahedron Lett.* **1975**, 4467−4471.
(33) Rossi, R.; Carpita, A.; Bellina, F. Palladium-and/or copper-mediated cross-coupling reactions between 1-alkynes and vinyl, aryl, 1-alkynyl, 1,2-propadienyl, propargyl and allylic halides or related compounds. A review. *Org. Prep. Proc.* **1995**, *27*, 129−160.
(34) Van Straten, N. C. R.; van der Marel, G. A.; van Boom, J. H. An expeditious route to the synthesis of adenophostin A. *Tetrahedron* **1997**, *53*, 6509−6522.
(35) Lemieux, R. U. Mercaptolysis of glucose and galactose pentaacetates. *Can. J. Chem.* **1951**, *29*, 1079−1084.
(36) Montchamp, J.-L.; Tian, F.; Hart, M. E.; Frost, J. W. Butane 2,3-bisacetal protection of vicinal diequatorial diols. *J. Org. Chem.* **1996**, *61*, 3897−3899.
(37) Douglas, N. L.; Ley, S. V.; Osborn, H. M. I.; Owen, D. R.; Priepke, H. W. M.; Warriner, S. L. Direct protection of 1,2-diols from alpha-diketones. *Synlett* **1996**, 793−794.
(38) Bannwarth, W.; Trzeciak, A. A simple and effective chemical phosphorylation procedure for biomolecules. *Helv. Chim. Acta* **1987**, *70*, 175−186.
(39) Mattern, D. L. Periodonation of benzene with periodate/iodide. *J. Org. Chem.* **1983**, *48*, 4773−4774.
(40) Clapper, D. L.; Walseth, T. H.; Dargie, P. J.; Lee, H.-C. Pyridine nucleotide metabolites stimulate calcium release from sea urchin egg microsomes desensitised to inositol trisphosphate. *J. Biol. Chem.* **1987**, *262*, 9561−9568.
(41) Felemez, M.; Marwood, R. D.; Potter, B. V. L.; Spiess, B. Inframolecular studies of the protonation of adenophostin A: comparison with D-*myo*-inositol 1,4,5-trisphosphate. *Biochem. Biophys. Res. Commun.* **1999**, *286*, 334−340.
(42) Marwood, R. D.; Shuto, S.; Jenkins, D. J.; Potter, B. V. L. Convergent synthesis of adenophostin A analogues via a base replacement strategy. *Chem. Commun.* **2000**, 219−220.
(43) Marwood, R. D.; Correa, V.; Taylor, C. W.; Potter, B. V. L. Synthesis of adenophostin A. *Tetrahedron Asymmetry* **2000**, *11*, 397−403.
(44) de Kort, M.; Regenbogen, A. D.; Valentijn, A. R. P. M.; Challiss, R. A. J.; Iwata, Y.; Miyamoto, S.; van der Marel, G. A.; van Boom, J. H. Spirophostins: conformationally restricted analogues of adenophostin A. *Chem. Eur. J.* **2000**, in press.
(45) de Kort, M.; Regenbogen, A. D.; Overkleeft, H. S.; Challiss, R. A. J.; Iwata, Y.; Miyamoto, S.; van der Marel, G. A.; van Boom, J. H. Synthesis and biological evaluation of cyclophostin: a 5′,6″-tethered analog of adenophostin A. *Tetrahedron* **2000**, in press.
(46) Correa, V.; Marwood, R. D.; Shuto, S.; Riley, A. M.; Jenkins, D. J.; Potter, B. V. L.; Taylor, C. W. Potent analogues of adenophostin A that release the intracellular Ca^{2+}stores of hepatocytes. *Br. J. Pharmacol.* **2000**, in press (Abstract).
(47) Prpic, V.; Green, K. C.; Blackmore, P. F.; Exton, J. H. Vasopressin-, angiotensin II-, and α_1-adrenergic-induced inhibition of Ca^{2+} transport by rat liver plasma membrane vesicles. *J. Biol. Chem.* **1984**, *259*, 1382−1385.

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