Total Synthesis of Nucleobase-Modified Adenophostin A Mimics

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Abstract: The adenophostins exhibit approximately 10–100 times higher receptor binding and Ca^{2+} mobilising potencies in comparison with the natural second messenger D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3]. Despite many synthetic attempts to determine the minimal structural requirement for this unusual behaviour of the adenophostins, few related simplified analogues displaying higher activity than that of Ins(1,4,5)P_3 have been reported. However, biological evaluation of such analogues has revealed that one of the key factors for the enhanced biological activity is the adenine moiety. To further understand the effect that the adenine base has upon the activity of the adenophostins, congeners in which this functionality is replaced by uracil, benzimidazole, 2-methoxynaphthalene, 4-methylanisole and 4-methylnaphthalene using the common intermediate 1,2-di-*O*-acetyl-5-*O*-benzyl-3-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranosyl)-ribofuranose have been synthesised using a base replacement strategy. The synthesis of the uracil and benzimidazole analogues was achieved using the Vorbrüggen condensation procedure. The 1′-*C*-glycosidic analogues were prepared using Friedel–Crafts type *C*-aryl glycosidation reactions. Phosphate groups were introduced using the phosphoramidite method with subsequent removal of all-benzyl protecting groups by catalytic hydrogenation or catalytic hydrogen transfer. Apart from one analogue with an α-glycosidic linkage all compounds were more potent than Ins(1,4,5)P_3 and most tended more towards adenophostin in activity. These analogues will be valuable tools to unravel the role that the adenine moiety plays in the potent activity of the adenophostins and demonstrate that this strategy is effective at producing highly potent ligands.

Keywords: adenophostin · C-glycosides · cyclitols · signal transduction

Introduction

The intracellular messenger 1-D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] (Figure 1) mobilises Ca^{2+} from intracellular stores upon binding to its specific receptor;[1] this results in an increase in cytosolic Ca^{2+} concentration that can regulate intracellular functions and control further release of Ca^{2+} from intracellular stores.[2] Because of its significant biological importance[3, 4] numerous analogues of Ins(1,4,5)P_3 have been extensively studied to develop specific ligands for the Ins(1,4,5)P_3 receptor to elucidate mechanistic aspects of Ins(1,4,5)P_3-mediated Ca^{2+} signalling pathways.[5] However, to date none of these inositol-based analogues has surpassed Ins(1,4,5)P_3 in binding affinity for the Ins(1,4,5)P_3 receptor or in Ca^{2+} mobilising ability.

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Recently, adenophostins A (**2**) and B (**3**) were isolated from *Pencillium brevicompactum*[6] and have been shown to be full agonists with affinities for Ins(1,4,5)P_3 receptors that are 10–100 fold greater than Ins(1,4,5)P_3.[6–10] Chemically, the adenophostins resemble Ins(1,4,5)P_3 in that the *trans* diequatorial bisphosphate arrangement flanked by a hydroxyl group (C-2″) which has been identified as a key point of the biological activity of Ins(1,4,5)P_3 is present (Figure 2).

Consequently all synthetic adenophostin analogues, to date, have this arrangement conserved. Attempts to determine which of the remaining structural features of the adenophostins are responsible for their high affinity interactions with Ins(1,4,5)P_3 receptors have resulted in the synthesis and biological evaluation of several related compounds[9–11] including **4**–**9** (Figure 1). To date, only one compound, possessing a ring-opened ribose surrogate "acyclophostin"[11h] has shown similar activity.[11f] From these studies (summarised in the SAR of Figure 2) the following observations regarding the structural requirements for adenophostin-like activity were apparent: 1) the α-D-glucopyranose structure is a good bioisostere of the *myo*-inositol backbone of Ins(1,4,5)P_3; 2) the three-dimensional arrangement of the three phosphate groups of adenophostin and its analogues is essential for biological activity; and 3) the adenine moiety enhances activity. To further understand the role that the adenine

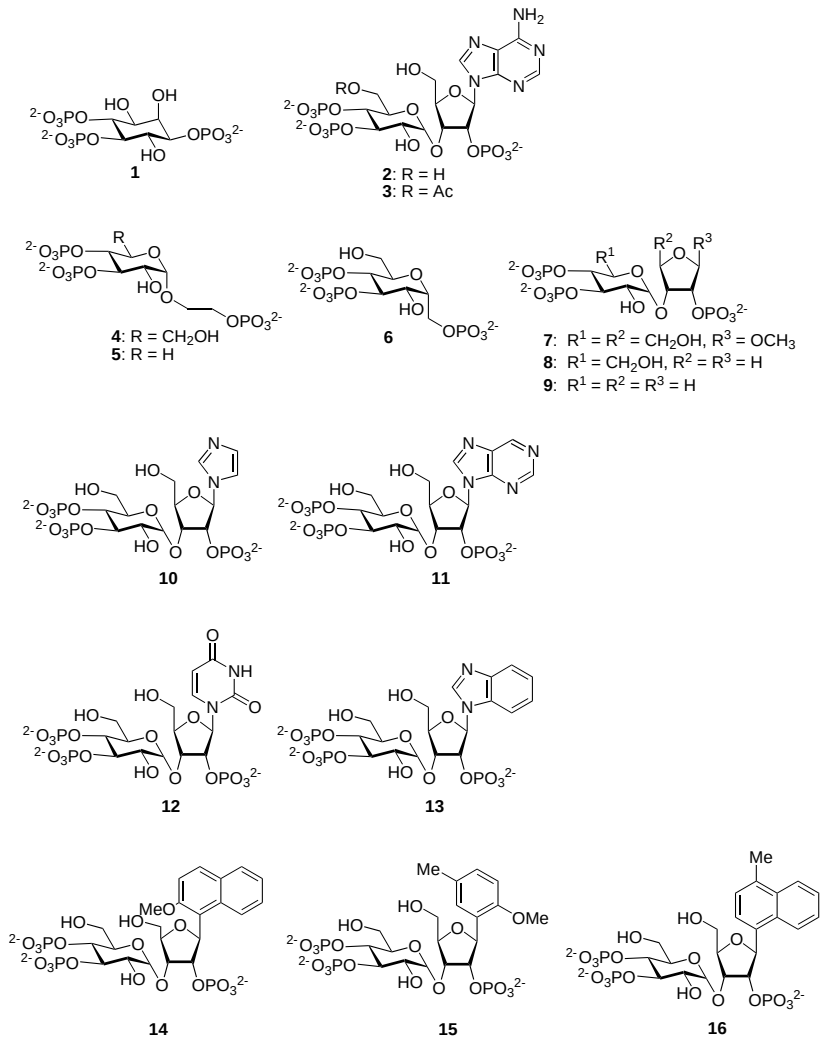

Figure 1. Ins(1,4,5)P_3 (**1**), the adenophostins (**2**, **3**) and analogues (**4**–**16**).

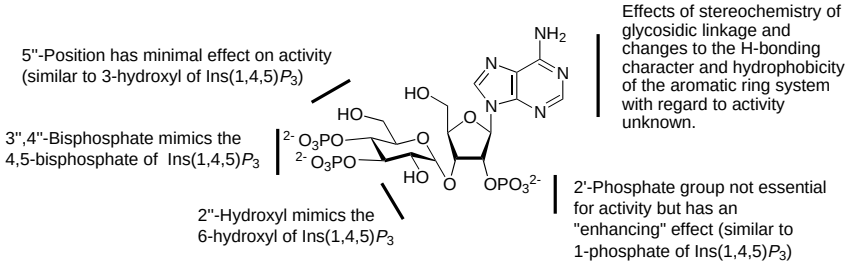

Figure 2. Key features of adenophostin A that contribute to its high-affinity binding at the Ins(1,4,5)P_3 receptor.

moiety plays in the high potency of the adenophostins (e.g. hydrogen bonding, hydrophobic interaction or a combination of the two) and what components of the adenine ring, if any, are instrumental for such activity, we recently reported the synthesis and evaluation of two adenophostin analogues in which the adenine moiety was replaced with an imidazole ring (**10**) and a purine ring (**11**).[12] The results of this study suggested that, although a purine ring (or equivalent) is necessary for high potency, the 6-amino substituent does not contribute greatly to the activity of adenophostin A and thus a hydrogen-bond interaction with the receptor via the N^6-amino group appears to be unlikely. However, adenophostin has three additional "potential" hydrogen-bonding sites (N^1, N^3 and N^7 in the adenine ring) which could help explain the high potency of interaction between adenophostin and the Ins(1,4,5)P_3 receptor that is observed. To investigate the potential of such hydrogen bonding between both the N^1, N^3 and N^7 in adenophostin with binding site residues in the Ins(1,4,5)P_3 receptor, and the extent to which such interactions and/or hydrophobic interactions might contribute to the binding efficiency and potency, adenophostin analogues in which the adenine ring has been replaced with nucleobase surrogates are required. We now report here further elaboration of our general methodology[12a] with the synthesis of adenophostin analogues in which the adenine ring has been replaced by uracil (**12**), benzimidazole (**13**), 2-methoxynaphthalene (**14**), 4-methylanisole (**15**) and 4-methylnaphthalene (**16**) (Figure 1). Some of these compounds have been reported in preliminary form.[12b]

Results and Discussion

The synthesis of adenophostin analogues **12**–**16** utilised the common disaccharide 1,2-di-*O*-acetyl-5-*O*-benzyl-3-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranosyl)-ribofuranose (**20**, Figure 3) which has previously been prepared in this laboratory and utilised in the synthesis of adenophostin analogues.[12]

This intermediate provides an excellent starting place for the synthesis of adenophostin analogues and similar compounds; the incorporation of acetates at positions 1 and 2 are a prerequisite for Vorbrüggen condensation and Friedel–Crafts type *C*-aryl glycosidation, with the additional incorporation of acetates at positions 3′ and 4′ allowing for one-step deprotection to yield the triols required for phosphorylation. Additionally, the orthogonal benzyl groups at positions 5, 2′ and 6′ would remain in place until the final deprotection step. In this paper we explore further the versatility of **20** and have also made some improvements en route to **20**. Although the disaccharide **20** is a versatile intermediate for synthesising

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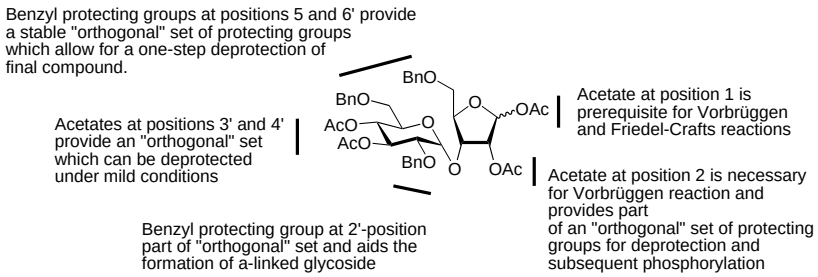
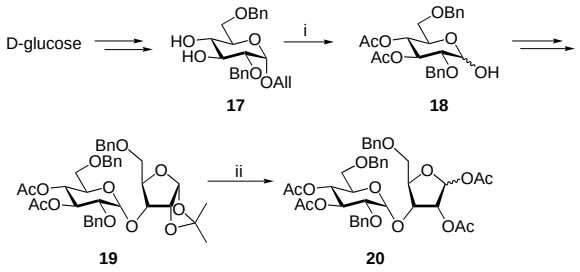


Figure 3. Versatility and synthetic utility of orthogonally protected disaccharidic donor **20**.

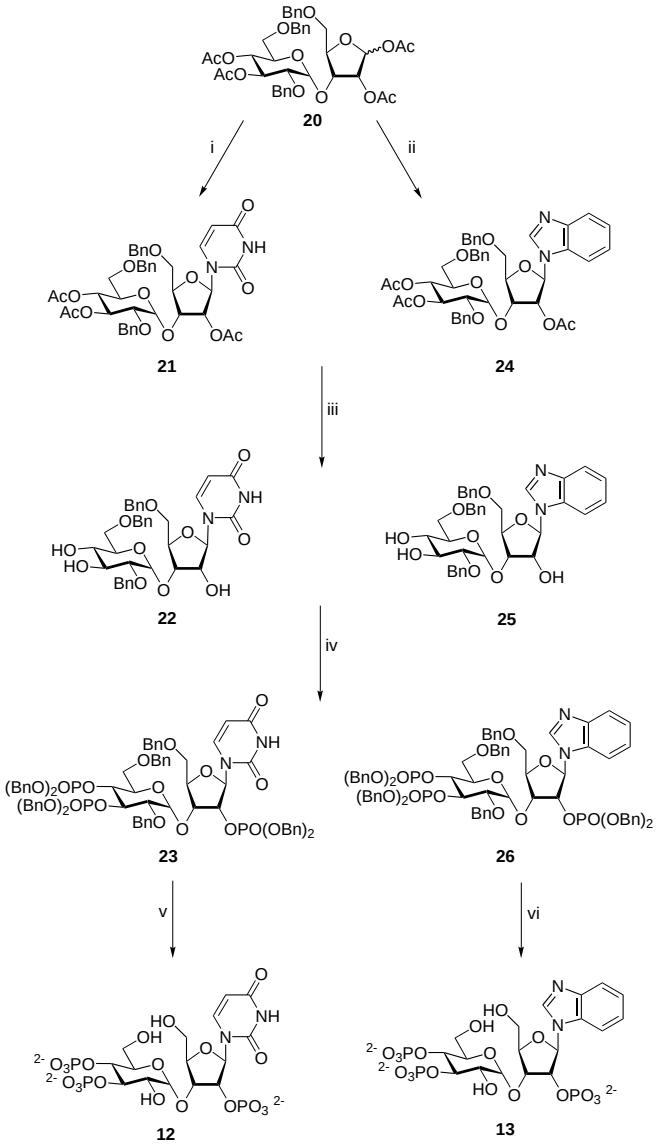
various adenophostin analogues, there were some drawbacks in the previously reported route to **20**. Consequently, technical improvements have been made, the results of which are shown in Scheme 1. These improvements primarily concern



Scheme 1. Details regarding further synthetic information can be found in the original paper[12a] i) a) Ac_2O, DMAP, CH_3CN, RT, 1 h; b) $PdCl_2$, MeOH, CH_2Cl_2, 10–20 °C, 18 h (**18**, 93 %); ii) a) 90 % aq TFA, RT, 5 min; b) Ac_2O, DMAP, CH_3CN, RT, 30 min (**20**, 80 %).

two steps from the original synthesis; the synthesis of 3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-D-glucopyranoside (**18**), and the synthesis of 1,2-di-*O*-acetyl-5-*O*-benzyl-3-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranosyl)-D-ribofuranose (**20**). For the original experimental details please refer to the previously reported synthesis.[12a] Glycosyl donor **18** was previously prepared by successive treatment of **17** with Ac_2O/pyridine and $PdCl_2$ in MeOH/dichloromethane in 78 % yield. However, it was found that this procedure was occasionally low yielding. By altering the original conditions for acetylation to three equivalents of Ac_2O and three equivalents of DMAP in acetonitrile, with subsequent removal of the anomeric *O*-allyl group we were able to obtain **18** reproducibly in high yield (>90 %). Further problems with the original synthesis of **20** occurred with the acidic hydrolysis of the *cis*-isopropylidene group of **19**. Previously, this step was carried out by refluxing an AcOH/H_2O solution of **19** in the presence of ethylene glycol. It was found that this reaction was difficult to reproduce in the reported yield and frequently yielded side-products. Furthermore, the purification was problematic due to the difficulty in separating the resulting diol from ethylene glycol. Consequently, a more convenient method for the synthesis of **20** was required. Treatment of **19** with 90 % aqueous TFA for five minutes at room temperature followed by evaporation of reaction solvent, yielded the corresponding *cis*-diol, which was acetylated without further purification using Ac_2O/DMAP in acetonitrile. This one-pot method yielded **20** in 80 % overall yield and was a more convenient procedure than the previously reported lower yielding preparation.

The preparation of the uracil- and benzimidazole-containing targets (**12** and **13**) followed the standard Vorbrüggen condensation procedure.[12] Treatment of **20** with silylated uracil (Scheme 2) and trimethylsilyl trifluoromethane sulfonate (TMSOTf) in acetonitrile at room temperature afforded uracil derivative (**21**) in 93 % yield.

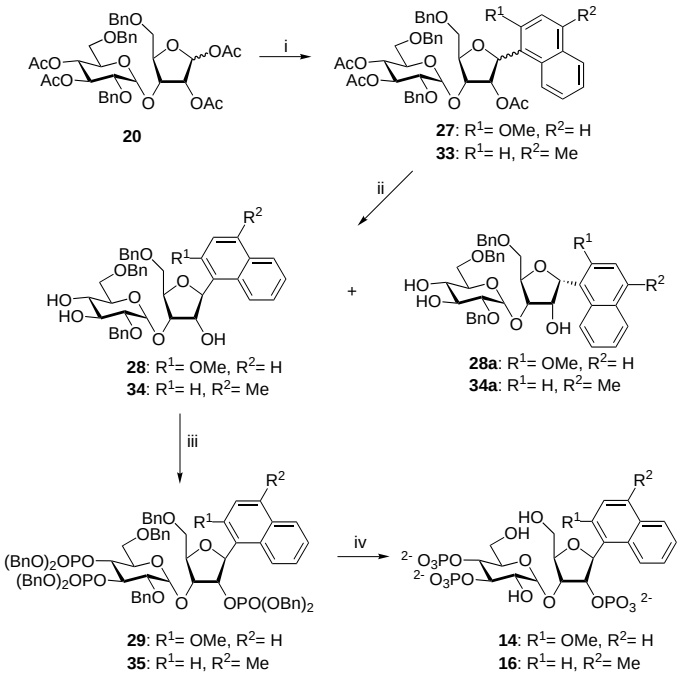


Scheme 2. i) Uracil, $(NH_4)_2SO_4$, *N,N,N′,N′*-hexamethyldisilazane, reflux, 3 h then residue dissolved in CH_3CN, TMSOTf, room temperature, 1 h (**21**, 93 %); ii) benzimidazole, $(NH_4)_2SO_4$, *N,N,N′,N′*-hexamethyldisilazane, reflux, 3 h then residue dissolved in acetonitrile, $SnCl_4$, room temperature, 1 h (**24**, 51 %); iii) NaOMe/MeOH, room temperature, 3 h (**22**, 88 %; **25**, 87 %); iv) 1*H*-tetrazole, dibenzyl(diisopropyl)phosphoramidite, CH_2Cl_2, RT, 3 h, then, *m*CPBA, −78 °C, (**23**, 85 %; **26**, 79 %); v) Pd/C, H_2, methanol, room temperature, 18 h (**12**, 98 %); vi) $Pd(OH)_2$, cyclohexene, methanol, reflux, 90 min (**13**, 74 %).

Similarly, reaction between silylated benzimidazole and TMSOTf in acetonitrile afforded the desired benzimidazole derivative **24** in only 44% yield. However, use of tin(IV)-chloride ($SnCl_4$) as promoter instead of TMSOTf improved the yield of the benzimidazole derivative by 7%. The acetyl groups of **21** and **24** were removed under Zemplén conditions[13] yielding triols **22** and **25**, respectively. Phosphate groups were introduced using the phosphoramidite method. Treatment of **22** and **25** with dibenzyldiisopropylphosphoramidite and 1*H*-tetrazole in dichloromethane followed by oxidation with *m*CPBA provided the corresponding tris(dibenzylphosphate) derivatives **23** and **26**, respectively. Oxidation of the intermediate trisphosphites was carried out at room temperature or at $-20\,^{\circ}C$. Simultaneous removal of all benzyl protecting groups was investigated by catalytic hydrogenation. When uridine derivative **23** in methanol was treated with Pd/C under atmospheric pressure of H_2 target compound **12** was obtained in high yield. However, similar treatment of the benzimidazole derivative **26** resulted in reduction of the aromatic aglycon moiety (analysis of reaction product by ^1H NMR). Removal of the benzyl protecting groups of **26** was achieved by catalytic hydrogen transfer[14] using palladium hydroxide [$Pd(OH)_2$] as catalyst and cyclohexene as transfer reagent providing **13** in 74% yield.

Attention was now turned to the construction of the 1′ *C*-aryl glycosides (**14**–**16**). O-Glycosides are usually prepared by reaction of an appropriate glycosyl donor, which is usually electrophilic and activated at the anomeric position, with a nucleophilic glycosyl acceptor in the presence of a promoter. The extension of this methodology to the synthesis of *C*-aryl glycosides can be considered to be an example of the Friedel–Crafts reaction.[15, 16] However, due to the weak nucleophilicity of the aryl ring, strong Lewis acids are often required as promoters.[17] This can be overcome by using an aromatic moiety which has been activated by electron-donating substituents, such as a hydroxyl or methoxy group or a fused second aromatic group, which require milder conditions for activation. Generally, carbohydrates bearing either acetyl or halogeno groups at the anomeric position are the donors used with the acetates requiring stronger activation than the halides. However, despite reduced reactivity, the anomeric acetates are generally the higher yielding. Another important factor in the success of reaction is the ring size of the glycosyl donor with pyranoses being more stereoselective than the furanoses with anomeric mixtures often resulting from use of the latter. When a pyranose sugar is used as the donor, unlike O-glycosylation, the presence of a participating group[18] at C-2 is not essential for controlling the stereoselectivity of the reaction; generally the more stable β-anomer, in which an aromatic ring at the anomeric position is in an equatorial orientation, will prevail. It has been proposed that under Lewis acidic conditions, these Friedel–Crafts *C*-aryl glycosidation products are in an equilibrium between the α- and β-anomers involving the pyranose ring-opened intermediate, giving rise to the thermodynamically favoured product.[15a] However, due to the similarity in stability of both anomers, when furanose sugars are used as the glycosyl donor, the reactions often result in yielding the anomeric mixture.

Recently, Kuribayashi and co-workers have found that $SnCl_4/AgCO_2CF_3$ is an efficient combination to promote β-selective Friedel–Crafts type *C*-aryl glycosidation reactions.[16f,g] A variety of *C*-aryl glycosides were prepared using this system from 1-*O*-acetyl sugars and aromatics substituted with a methoxy group. The yields for these conversions were 42–89% (α:β, 26:74 to 0:100); the tri-*O*-acetyl-β-anisoyl-ribofuranose was obtained as the major product in 62% yield (α:β, 26:74) with per-acetylated ribofuranose as the glycosyl donor. Application of this methodology to common intermediate **20** and 2-methoxynaphthalene using conditions similar to those of Kuribayashi (donor:acceptor:$SnCl_4$:$AgCO_2CF_3$ = 1:2:1.5:3, CH_2Cl_2, 0 °C) gave the desired C-glycoside in only 35% yield as an anomeric mixture (**27**, α:β = 1:8). Adaptation of the Kuribayashi conditions (donor:acceptor:$SnCl_4$:$AgCO_2CF_3$ = 1:20:1.5:2, $CHCl_3$, 20 °C, Scheme 3) resulted in a vast improvement in the overall yield



Scheme 3. i) 2-Methoxynaphthalene/1-methylnaphthalene, $AgCO_2CF_3$, $SnCl_4$, chloroform, room temperature, 45 min (**27**, 73%, α/β ratio 15:85 based on ^1H NMR analysis; **33**, 46%, %, α/β ratio 35:65 based on ^1H NMR analysis); ii) NaOMe/MeOH, room temperature, 3 h (**28**, 81%; **28a**, 5%; **34**, 57%; **34a**, 31%); iii) 1*H*-tetrazole, dibenzyl(diisopropyl)phosphoramidite, CH_2Cl_2, RT, 3 h, then, *m*CPBA, $-78\,^{\circ}C$, (**29**, 92%; **35**, 62%); iv) $Pd(OH)_2$, cyclohexene, methanol, reflux, 90 min (**14**, 64%; **16**, 67%).

(74%) but no improvement in the anomeric selectivity. Similar treatment of 4-methyl anisole and 1-methylnaphthalene with **20** in the presence of $SnCl_4/AgCO_2CF_3$ provided the corresponding *C*-aryl glycosides **30** (see Scheme 4) in 67% (α:β = 1:1), and **33** (see Scheme 3) in 46% yield (α:β = 1:2), respectively. Although these anomeric mixtures were inseparable at this stage and their regiochemistries were not confirmed, comparison with previous studies carried out on Friedel–Crafts type C-glycosidation reactions with 2-methoxynaphthalene,[16f] 4-methylanisole,[16f] and 1-methylnaphthalene[16c] suggested that C-glycosides had been formed at the

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1-position of 2-methoxynaphthalene, at the 2-position of 4-methylanisole, and at the 4-position of 1-methylnaphthalene. The acetyl groups of the anomeric mixtures **27**, **30**, and **33** were removed with catalytic sodium methoxide in methanol at room temperature yielding the corresponding triols, which were separated by silica gel chromatography providing **28** and **28a**, **31** and **31a**, and **34** and **34a**, respectively. The stereo- and regiochemistries of C-glycosides **28** and **31** were confirmed by NOESY spectroscopy (Figure 4). The ^{1}H NMR spectrum of

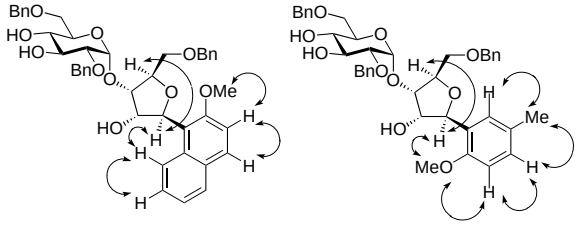


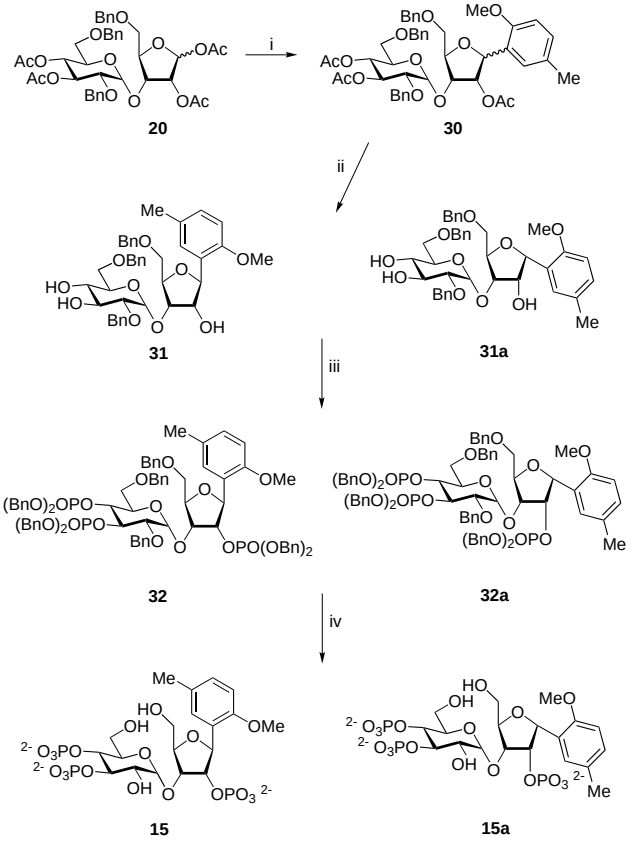
Figure 4. NOE correlations found for **28** and **31**.

28 revealed two doublets at $\delta = 8.34$ (H-8, $J_{7,8} = 8.3$ Hz) and 7.74 (H-5, $J_{5,6} = 8.3$ Hz) and a pair of doublets at 7.79 (H-4, $J_{3,4} = 8.8$ Hz) and 7.25 (H-3, $J_{3,4} = 8.8$ Hz), which also supported the conclusion that the glycosyl moiety had substituted at the 1-position of the naphthalene ring. $J_{1',2'}$ values for several *C*-aryl ribosides have been reported ($J_{1',2'}$ α-linked $< J_{1',2'}$ β-linked, typically 2–3 Hz for α-linked and 7–8 Hz for β-linked) and agreed well with those obtained for **28** and **28a** (3.4 Hz and 7.3 Hz, respectively), **31** and **31a** (3.7 Hz and 4.9 Hz, respectively), and **34** and **34a** (3.1 Hz and 3.5 Hz, respectively). Phosphate units were introduced by the phosphoramidite method as previously described for **22** and **25**. Removal of all benzyl protecting groups was attempted by catalytic hydrogenation but, as in the case for **26**, the aromatic moieties of **29**, **32** and **35**, appeared to be reduced (^{1}H NMR analysis). However, treatment with $Pd(OH)_2$ in the presence of cyclohexene in methanol at reflux yielded the corresponding trisphosphates **14**, **15** and **16**, respectively.

Additionally, an α-*C*-aryl glycoside analogue of adenophostin A was synthesised; the Friedel–Crafts type acylation of **20** with 4-methylanisole provided both anomers of **30** in approximately equal amounts which after separation of the triols **31** and **31a** provided sufficient material for the synthesis of the α-congener to be viable. Consequently, phosphitylation followed by oxidation of triol **31a** yielded fully protected **32a** which after deprotection yielded trisphosphate **15a** as the free acid (Scheme 4).

All trisphosphate targets were obtained and stored as the corresponding Na^+ salt and accurately quantified by total phosphate assay.

For all the modified compounds the EC_{50} of each was evaluated for Ca^{2+} mobilising ability in permeabilised hepatocytes relative to Ins(1,4,5)P_3 [145 nM] and our synthetic adenophostin A[19] [14.7 nM]. Uridophostin (**12**) was found to be the most active compound [34 nM], followed by the benzimidazole derivative **13** [48 nM], the β-4-methylanisolyl derivative **15** [48 nM], the β-4-methylnaphthalenyl derivative **16** [62 nM] and the β-2-methoxynaphthalenyl derivative **14**



Scheme 4. i) 4-Methylanisole, $AgCO_2CF_3$, $SnCl_4$, chloroform, RT, 45 min (**30**, 67%, α/β ratio 1:1 based on ^{1}H NMR analysis); ii) NaOMe/MeOH, RT, 3 h (**31**, 38%; **31a**, 36%); iii) 1*H*-tetrazole, dibenzyl(diisopropyl)phosphoramidite, CH_2Cl_2, RT, 3 h, then, *m*CPBA, $-78\,^\circ$C, (**32**, 96%; **32a**, 87%); iv) Pd/C, H_2, methanol, RT, 75 min (**15**, 80%, **15a**, 80%).

[99 nM]. Thus, all these compounds are more potent than Ins(1,4,5)P_3 and some have activity close to adenophostin A. The α-configured 4-methylanisolyl derivative **15a** showed a drastic decrease in potency [1372 nM] relative to the α-anomer [48 nM] and was even some 10 times weaker than Ins(1,4,5)P_3.

These results show that a base-modification approach represents a powerful strategy to develop high potency ligands. They also show that simple ring replacements of adenine can indeed produce highly potent compounds, more potent indeed than the single ring imidazole derivative we recently reported [108 nM].[12a] Additionally, we establish that naphthalene-based surrogates, completely unrelated to natural purine nucleobases, are relatively well tolerated and that a β-configuration is a prerequisite for potent adenophostin A-like activity. We also demonstrate that the traditional N-glycosidic linkage can be effectively replaced by a C-glycosidic one. These biological results and the pharmacological implications have been reported fully elsewhere.[19]

Thus, we further demonstrate that the nucleobase motif of adenophostin A can be replaced by both natural and unnatural surrogates and still maintain the high activity of the adenophostins. Our synthetic route to these modified compounds is versatile and should facilitate the synthesis of a range of further base-modified ligands, some with novel and finely tuned activity at the Ins(1,4,5)P_3 receptor.

The synthesis of the C-glycosidic analogue of adenophostin A and its uracil congener has recently been reported.[20] We have also recently reported modifications to the pyranose moiety of adenophostin A.[21]

Experimental Section

General methods: Chemicals were purchased from Aldrich, Fluka and Sigma. Dry toluene and dichloromethane were distilled from calcium hydride and stored over 4 Å molecular sieves. Acetonitrile was distilled from phosphorous pentoxide and stored over 3 Å molecular sieves. Pyridine was dried over potassium hydroxide pellets, distilled and then stored over potassium hydroxide pellets. Molecular sieves (3 and 4 Å) were pre-dried in an oven and activated for three hours under vacuum at 250 °C. Ether is diethyl ether. All aqueous (aq) solutions are saturated unless otherwise stated. Reactions were carried out at room temperature under a nitrogen atmosphere in pre-dried glassware unless otherwise stated. Analytical thin-layer chromatography (TLC) was performed on pre-coated plates (Merck TLC aluminium sheets, silica 60 F_{254}, Art. No. 5554): the products were visualised by UV radiation and staining with ethanolic phosphomolybdic acid followed by heating. Column chromatography was carried out under pressure on Sorbsil C60 silica gel. NMR spectra (^{31}P, ^{1}H, ^{13}C) were recorded on either a JEOL GX270 or EX 400 or a Varian Mercury 400 spectrometer with signals being assigned by 1D, DEPT, and 2D spectra (COSY, HETCOR, NOESY). Chemical shifts are quoted in parts per million (ppm) relative to tetramethylsilane (TMS), deuterium oxide (D_2O), [D]chloroform ($CDCl_3$), [D_4]methanol (CD_3OD). Coupling constants are quoted in Hz and refer to $^{3}J_{H,H}$ unless otherwise stated. The ^{31}P NMR shifts were measured in ppm relative to external 85 % phosphoric acid. Low-resolution mass spectra were recorded by the University of Bath Mass Spectrometry Service using fast atom bombardment (FAB, +ve and -ve) with 3-nitrobenzyl alcohol (NBA) as the matrix. High-resolution mass spectrometry (HRMS) was also carried out by the University of Bath Mass Spectrometry Service.

Free acids of final trisphosphates (**12**–**15**, **15a**) were converted into and stored as the corresponding Na^+ salt obtained by the following procedure: the free acid was dissolved in water and applied to a Diaion WK-20 resin column (Na^+ form) and developed with water. The eluent was evaporated in vacuo and the residue was co-evaporated with ethanol to give the corresponding sodium salt.

Total phosphate quantification was carried out using either a modified Briggs test[22] or the Ames assay.

Mass spectra of target trisphosphates (**12**–**15**, **15a**) were obtained as the corresponding triethylammonium salts prepared as follows: an aqueous solution of the sodium salt of **12**–**15**, **16a** was passed through a short column of Dowex 50 (H^+ form, developed with water) and the eluent was evaporated under reduced pressure. The resulting residue was dissolved in 0.5 M triethylammonium bicarbonate buffer, which was evaporated in vacuo to give the corresponding triethylammonium salts as solids.

3,4-Di-*O*-acetyl-2,6-di-*O*-benzyl-D-glucopyranose (18): A mixture of **17**[12a] (400 mg, 1 mmol), DMAP (366 mg, 3 mmol) and Ac_2O (300 μL, 3 mmol) in acetonitrile (15 mL) was stirred at room temperature for 1 h. Methanol was added and the solvent was evaporated under reduced pressure with the resulting residue being partitioned between chloroform and 0.5 N HCl. The organic layer was washed with brine, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by silica gel chromatography (hexane/ethyl acetate 10:1 then 5:1) to give allyl 3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranose as an oil (490 mg, quant.). The oil was then dissolved in methanol/dichloromethane (1:1, 6 mL) to which $PdCl_2$ (34 mg, 0.2 mmol) was added. The resulting mixture was stirred at 10–20 °C for 18 h after which the solution was filtered through Celite and the resulting filtrate was evaporated under reduced pressure. The resulting residue was then partitioned between chloroform and brine with the organic layer being dried ($MgSO_4$) and concentrated. The crude residue was purified by silica gel chromatography (hexane/ethyl acetate 5:1 then 3:1) yielding the title compound as white solids (**18**, 412 mg, 93 %). ^{1}H NMR spectrum was in accord with that of previously reported compound.[12a]

1,2-Di-*O*-acetyl-5-*O*-benzyl-3-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranosyl)-D-ribofuranose (20): A solution of **19** (141 mg, 0.2 mmol) in aqueous TFA (90 %, 3 mL) was stirred at room temperature for 5 min. The solution was diluted with water and the mixture was concentrated under reduced pressure. The resulting residue was partitioned between ethyl acetate and 0.5 N $NaHCO_3$ and the organic layer was washed with brine, dried ($MgSO_4$) and concentrated yielding the corresponding *cis*-diol as a non-isolated intermediate. Acetonitrile (3 mL) was added followed by Ac_2O (60 μL, 0.6 mmol) and DMAP (73 mg, 0.6 mmol) and the mixture was stirred at room temperature for 30 min. Methanol was then added and the solvent removed under reduced pressure. The resulting residue was partitioned between chloroform and 0.5 N HCl. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated. The resulting crude residue was purified by silica gel chromatography (hexane/ethyl acetate 5:1 then 3:1) yielding title compound as white solids (**20**, 119 mg, 80 %) of which ^{1}H NMR spectrum was in accord with the authentic compound.[12a]

2′-*O*-Acetyl-5′-*O*-benzyl-3′-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranosyl)uridine (21): A mixture of uracil (90 mg, 0.80 mmol) and $(NH_4)_2SO_4$ (4 mg) in *N*,*N*,*N*′,*N*′-hexamethyldisilazane (1.0 mL) was heated under reflux for 3 h. The solvent was evaporated under reduced pressure and the residue was co-evaporated with toluene to give silylated uracil as a non-purified residue. To a mixture of crude material and **20** (150 mg, 0.20 mmol) in acetonitrile (5 mL) was added TMSOTf (146 μL, 0.80 mmol) at 0 °C and the resulting mixture was stirred at room temperature for 1 h. The mixture was filtered through Celite with insoluble materials and Celite being washed with ethyl acetate. The filtrate and collected washings were combined, washed with $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated in vacuo. The crude residue was purified by silica gel chromatography (hexane/ethyl acetate 2:1 to 1:1) providing **21** (150 mg, 93 %). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.70$ (br s, 1H, NH), 7.74 (d, $J = 8.3$, 1H, H-6), 7.39–7.24 (m, 15H, ArH), 6.18 (d, $J = 4.9$, 1H, H-1′), 5.40 (dd, $J = 9.8$, 10.3, 1H, H-3″), 5.40 (dd, $J = 2.5$, 8.3, 1H, H-5), 5.19 (dd, $J = 4.9$, 5.4, 1H, H-2′), 5.01 (dd, $J = 9.5$, 9.8, 1H, H-3″), 4.99 (d, $J = 3.4$, 1H, H-1″), 4.64, 4.34 (AB, 2H, CH_2Ph), 4.53–4.40 (m, 6H, H-3′, H-4′, 2 × CH_2Ph), 3.92 (m, 1H, H-5″), 3.76 (dd, $J = 2.0$, 10.7, 1H, H-5′a), 3.65 (dd, $J = 1.5$, 10.7, 1H, H-5′b), 3.55 (dd, $J = 3.4$, 10.3, 1H, H-2″), 2.99 (m, 2H, H-6″), 1.96, 1.93, 1.89 (3 × s, 9H, 3 × OAc); HRMS-FAB: *m*/*z*: calcd for $C_{42}H_{47}N_2O_{14}$ 803.3027 $[M+H]^+$, found: 803.2969; UV (methanol): $\lambda_{max} = 255$ nm.

1-Benzimidazolyl-5-*O*-benzyl-3-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose (24): A mixture of benzimidazole (947 mg, 0.40 mmol) and $(NH_4)_2SO_4$ (1 mg) in *N*,*N*,*N*′,*N*′-hexamethyldisilazane (1.0 mL) was heated under reflux for 3 h. The solvent was evaporated in vacuo and the residue was co-evaporated with toluene to give silylated benzimidazole as a non-isolated residue. To a mixture of this residue and **20** (75 mg, 0.10 mmol) in acetonitrile (2 mL) was added $SnCl_4$ (1.0 M in CH_2Cl_2, 400 μL, 0.40 mmol) at 0 °C and the resulting mixture was stirred at room temperature for 1 h. The mixture was filtered through Celite and the insoluble materials and Celite were washed with ethyl acetate. The filtrate and washings were combined and washed with $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (hexane/ethyl acetate 5:4 to 1:1) to give **25** (41 mg, 51 %). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.19$ (s, 1H, H-2), 7.80 (d, $J = 7.8$, 1H, $H_{benzimidazole}$), 7.64 (d, $J = 8.3$, 1H, $H_{benzimidazole}$), 7.39–7.20 (m, 16H, $H_{benzimidazole}$, ArH), 7.16 (dd, $J = 7.3$, 7.8, 1H, $H_{benzimidazole}$), 6.15 (d, $J = 6.4$, 1H, H-1′), 5.47 (dd, $J = 9.8$, 10.2, 1H, H-3″), 5.38 (dd, $J = 5.9$, 6.4, 1H, H-2′), 5.02 (dd, $J = 9.8$, 9.8, 1H, H-4″), 4.91 (d, $J = 3.9$, 1H, H-1″), 4.65 (dd, $J = 3.4$, 5.9, 1H, H-3′), 4.63–4.36 (m, 7H, H-4′, 3 × CH_2Ph), 4.01 (m, 1H, H-5″), 3.69 (dd, $J = 2.4$, 10.7, 1H, H-5′a), 3.66 (dd, $J = 2.0$, 10.7, 1H, H-5′b), 3.55 (dd, $J = 3.9$, 10.2, 1H, H-2″), 3.99 (d, $J = 3.9$, 2H, H-6″), 2.00, 1.95, 1.85 (3 × s, 9H, 3 × OAc); HRMS-FAB: *m*/*z*: calcd for $C_{45}H_{49}N_2O_{12}$: 809.3286 $[M+H]^+$, found: 809.3321.

2-*O*-Acetyl-5-*O*-benzyl-1-(2-methoxynaphthalenyl)-3-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranosyl)-D-ribofuranose (27): $SnCl_4$ (94 μL, 0.80 mmol) was added at room temperature to a mixture of **20** (300 mg, 0.40 mmol), 2-methoxynaphthalene (1.27 g, 8.0 mmol) and $AgCO_2CF_3$ (132 mg, 0.60 mmol) in chloroform and the resulting mixture was stirred at room temperature for 45 min. $NaHCO_3$ was added and the mixture was stirred at room temperature for an additional 5 min. The mixture was filtered though Celite and the insoluble materials and Celite was washed with chloroform. The filtrate and washings were combined and washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The resulting residue was

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purified by silica gel chromatography (hexane/ethyl acetate 4:1 to 3:1) yielding title compound as a syrup (**27**, 233 mg, 73%, α/β ratio was 15:85 based on ^{1}H NMR analysis). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.63$ (d, $J =$ 8.5, 0.15 H, $H_{naphthalene}\alpha$), 8.32 (d, $J = 8.6$, 0.85 H, $H_{naphthalene}\beta$), 7.82 (d, $J = 8.9$, 0.85 H, $H_{naphthalene}\beta$), 7.78–7.68 (m, 1.15 H, $2 \times H_{naphthalene}\alpha$, $H_{naphthalene}\beta$), 7.53 (m, 1 H, $H_{naphthalene}\alpha$, $H_{naphthalene}\beta$), 7.34–7.18 (m, 17 H, ArH, $2 \times H_{naphthalene}\alpha$, $2 \times H_{naphthalene}\beta$), 6.21 (d, $J = 2.8$, 0.15 H, H-1′α), 5.94 (d, $J = 7.3$, 0.85 H, H-1′β), 5.81 (dd, $J = 2.8$, 4.1, 0.15 H, H-2′α), 5.64 (dd, $J = 7.0$, 7.3, 0.85 H, H-2′β), 5.51 (dd, $J = 9.5$, 10.0, 0.85 H, H-3″β), 5.44 (dd, $J = 9.8$, 9.8, 0.15 H, H-3″α), 5.15 (dd, $J = 4.3$, 8.9, 0.15 H, H-4″α), 5.11–5.03 (m, 1.7 H, H-1″β, H-4″β), 4.91 (d, $J = 4.3$, 0.15 H, H-1″α), 4.70–3.82 (m, 14 H, $3 \times CH_2$Ph, H-3′, H-4′, H-5′, H-5″, OCH_3), 3.60–3.31 (m, 3 H, H-2″, H-6″), 2.17, 1.97, 1.92, 1.88, 1.85 ($5 \times$ s, 9 H, $3 \times$ OAc); HRMS-FAB: m/z: calcd for $C_{49}H_{53}O_{13}$: 849.3486 $[M+H]^+$, found: 849.3462.

2-*O*-Acetyl-5-*O*-benzyl-1-(4-methylanisol-2-yl)-3-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranosyl)-D-ribofruanose (30): $SnCl_4$ (94 μL, 0.80 mmol) was added at room temperature to a mixture of **20** (300 mg, 0.40 mmol), 4-methylanisole (977 mg, 8.0 mmol) and $AgCO_2CF_3$ (132 mg, 0.60 mmol) in chloroform and the resulting mixture was stirred at room temperature for 45 min. $NaHCO_3$ was added and the mixture was stirred at room temperature for an additional 5 min. The mixture was filtered though Celite and the insoluble materials and Celite were washed with chloroform. The filtrate and washings were combined and washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (hexane/ethyl acetate 5:1 to 3:1) yielding title compound as a syrup (**30**, 217 mg, 67%, α/β 1:1 based on ^{1}H NMR analysis). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.39–7.16$ (m, 16 H, ArH, $H_{anisolyl}$), 7.05 (dd, $J = 1.8$, 8.2, 0.5 H, $H_{anisolyl}$), 6.99 (dd, $J = 1.8$, 8.2, 0.5 H, $H_{anisolyl}$), 6.77 (d, $J = 8.2$, 0.5 H, $H_{anisolyl}$), 6.67 (d, $J = 8.2$, 0.5 H, $H_{anisolyl}$), 5.84 (dd, $J = 3.4$, 3.7, 0.5 H, H-2′β), 5.48 (d, $J = 3.1$, 0.5 H, H-2′α), 5.43–5.36 (m, 1 H, H-1′α, H-3″β), 5.27 (d, $J = 3.1$, 0.5 H, H-3″α), 5.14 (d, $J = 3.7$, 0.5 H, H-1′β), 5.08–5.03 (m, 1 H, H-4″), 4.75–4.12 (m, 9 H, $3 \times CH_2$Ph, H-1″, H-3′, H-5″), 3.97–3.73 (m, 6 H, H-4′, H-5′, OCH_3), 3.58–3.51 (m, 1 H, H-2″), 3.35–3.12 (m, 2 H, H-6″), 2.27, 2.18, 2.04, 1.91, 1.88, 1.87, 1.86 ($7 \times$ s, 12 H, CH_3Ph, $3 \times$ OAc); HRMS-FAB: m/z: calcd for $C_{46}H_{53}O_{12}$: 813.3486 $[M+H]^+$, found: 813.3486.

2-*O*-Acetyl-5-*O*-benzyl-1-(4-methylnaphthalenyl)-3-*O*-(3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranosyl)-D-ribofruanose (33): $SnCl_4$ (94 μL, 0.80 mmol) was added at room temperature to a mixture of **20** (300 mg, 0.40 mmol), 1-methylnaphthalene (1.14 g, 8.0 mmol) and $AgCO_2CF_3$ (132 mg, 0.60 mmol) in chloroform and the resulting mixture was stirred at room temperature for 45 min. $NaHCO_3$ was added and the mixture was stirred at room temperature for an additional 5 min. The mixture was filtered though Celite and the insoluble materials and Celite was washed with chloroform. The filtrate and washings were combined and washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (hexane/ethyl acetate 4:1 to 3:1) yielding title compound as a syrup (**33**, 152 mg, 46%, α/β ratio was 35:65 based on ^{1}H NMR analysis). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.04–7.20$ (m, 21 H, ArH, $H_{naphthalenyl}$), 5.97–5.53 (m, 1 H, H-2′), 5.52–5.34 (m, 2 H, H-1′, H-3″), 5.15–4.87 (m, 3 H, H-3′, H-1″, H-4″), 4.67–4.22 (m, 8 H, $3 \times CH_2$Ph, H-4′, H-5″), 3.95–3.74 (m, 2 H, H-5′), 3.58–3.51 (m, 1 H, H-2″), 3.40–3.24 (m, 2 H, H-6″), 2.69, 2.67 ($2 \times$ s, 3 H, CH_3Ph), 1.98, 1.95, 1.92, 1.89 ($4 \times$ s, 9 H, OAc); HRMS-FAB: m/z: calcd for $C_{49}H_{53}O_{12}$: 833.3537 $[M+H]^+$, found: 833.3496.

5-*O*-Benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)uridine (22): NaOMe (cat) was added to a solution of **21** (71 mg, 0.9 mmol) in methanol (3 mL) and the solution was stirred at room temperature for 3 h. The mixture was neutralised with 1M AcOH (in THF) and concentrated in vacuo. The resulting residue was partitioned between chloroform and brine, separated, and the organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The crude residue was purified by silica gel chromatography (chloroform/methanol 30:1) yielding title compound (**22**, solids, 54 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.84$ (br s, 1 H, NH), 7.61 (d, $J = 7.8$, 1 H, H-6), 7.36–7.21 (m, 15 H, ArH), 6.10 (d, $J = 6.8$, 1 H, H-1′), 5.39 (d, $J = 7.8$, 1 H, H-5), 4.86 (d, $J = 3.4$, 1 H, H-1″), 4.75, 4.70 (AB, 2 H, CH_2Ph), 4.53–4.29 (m, 5-H, H-4′, $2 \times CH_2$Ph), 4.15 (m, 1 H, H-2′), 4.06–4.00 (m, 2 H, H-3′, H-3″), 3.93 (br s, 1 H, OH), 3.85 (m, 1 H, H-5″), 3.72 (d, $J = 8.8$, 1 H, H-6″a), 3.61–3.43 (m, 5 H, H-5′, H-2″, H-4″, H-6″b), 2.19 (br s, 2 H, $2 \times$ OH); HRMS-FAB: m/z: calcd for $C_{36}H_{41}N_2O_{11}$: 677.2710 $[M+H]^+$, found: 677.2726.

1-Benzimidazolyl-5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose (25): NaOMe (catalytic quantity) was added to a solution of **24** (65 mg, 0.4 mmol) in methanol (3 mL) and the solution was stirred at room temperature for 3 h. The mixture was neutralised with 1M AcOH (in THF) and concentrated in vacuo. The resulting residue was partitioned between chloroform and brine, separated, and the organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The crude residue was purified by silica gel chromatography (chloroform/methanol 60:1 to 40:1) yielding title compound as a yellow syrup (**25**, 48 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.08$ (s, 1 H, H-2), 7.75 (d, $J = 8.2$, 1 H, $H_{benzimidazole}$), 7.58 (d, $J = 8.2$, 1 H, $H_{benzimidazole}$), 7.36–7.21 (m, 16 H, $H_{benzimidazole}$, ArH), 7.09 (dd, $J = 7.6$, 7.6, 1 H, $H_{benzimidazole}$), 5.76 (d, $J = 6.1$, 1 H, H-1′), 4.87 (d, $J = 3.7$, 1 H, H-1″), 4.77, 4.67 (AB, 2 H, CH_2Ph), 4.55–4.45 (m, 5 H, H-2′, $2 \times CH_2$Ph), 4.30–4.26 (m, 2 H, H-3′, H-4′), 4.03 (dd, $J = 9.2$, 9.5, 1 H, H-3″), 3.85 (m, 1 H, H-5″), 3.71–3.52 (m, 5 H, H-5′, H-4″, H-6″), 3.47 (dd, $J = 3.7$, 9.5, 1 H, H-2″); HRMS-FAB: m/z: calcd for $C_{39}H_{43}N_2O_9$: 683.2969 $[M+H]^+$, found: 683.2967.

5-*O*-Benzyl-1-(2-methoxynaphthalenyl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose and **5-*O*-benzyl-1-(2-methoxynaphthalenyl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-α-D-ribofuranose (28** and **28a)**: NaOMe (catalytic quantity) was added to a solution of **27** (340 mg, 0.4 mmol) in methanol (3 mL) and the solution was stirred at room temperature for 3 h. The mixture was neutralised with 1M AcOH (in THF) and concentrated in vacuo. The resulting residue was partitioned between chloroform and brine, separated, and the organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The crude residue was purified by silica gel chromatography (chloroform/acetone 60:1 to 50:1) yielding 5-*O*-benzyl-1-(2-methoxynaphthalenyl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose as a yellow syrup (**28**, 235 mg, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.34$ (d, $J = 8.3$, 1 H, H-8), 7.79 (d, $J = 8.8$, 1 H, H-4), 7.74 (d, $J = 8.3$, 1 H, H-5), 7.34–7.23 (m, 16 H, H-6, ArH), 7.25 (d, $J = 8.8$, 1 H, H-3), 7.18 (dd, $J = 7.3$, 8.3, 1 H, H-7), 5.70 (d, $J = 7.3$, 1 H, H-1′), 4.94 (d, $J = 3.4$, 1 H, H-1″), 4.76–4.48 (m, 7 H, H-2′, CH_2Ph), 4.44 (dd, $J = 5.9$, 5.9, 1 H, H-3′), 4.18 (m, 1 H, H-4′), 4.03 (dd, $J = 9.3$, 9.3, 1 H, H-3″), 3.91–3.59 (m, 5 H, H-5′, H-5″, H-6″), 3.84 (s, 3 H, OMe), 3.56 (dd, $J = 9.3$, 9.3, 1 H, H-4″), 3.43 (dd, $J = 3.4$, 9.3, 1 H, H-2″); HRMS-FAB: m/z: calcd for $C_{43}H_{46}O_{10}$: 722.3091 $[M+H]^+$, found: 722.3097; and 5-*O*-benzyl-1-(2-methoxynaphthalenyl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-α-D-ribofuranose (**28a**, 15 mg, 5%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.57$ (d, $J =$ 8.8, 1 H, $H_{naphthalene}$), 7.81 (d, $J = 9.3$, 1 H, $H_{naphthalene}$), 7.73 (d, $J = 8.3$, 1 H, $H_{naphthalene}$), 7.42 (dd, $J = 8.3$, 8.8, 1 H, $H_{naphthalene}$), 7.34–7.29 (m, 17 H, $2 \times H_{naphthalene}$, $3 \times CH_2$Ph), 6.06 (d, $J = 3.4$, 1 H, H-1′), 4.93 (d, $J = 3.4$, 1 H, H-1″), 4.72–4.51 (m, 8 H, H-2′, H-3′, $3 \times CH_2$Ph), 3.98 (dd, $J = 9.3$, 9.3, 1 H, H-3″), 3.94 (s, 3 H, OMe), 3.87–3.56 (m, 7 H, H-4′, H-5′, H-4″, H-5″, H-6″), 3.38 (dd, $J = 3.4$, 9.3, 1 H, H-2″); HRMS-FAB: m/z: calcd for $C_{43}H_{46}O_{10}$: 722.3091 $[M+H]^+$, found: 722.3089.

5-*O*-Benzyl-1-(4-methylanisol-2-yl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose and **5-*O*-benzyl-1-(4-methylanisol-2-yl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-α-D-ribofuranose (31** and **31a)**: NaOMe (catalytic quantity) was added to a solution of **30** (230 mg, 0.25 mmol) in methanol (3 mL) and the solution was stirred at room temperature for 3 h. The mixture was neutralised with 1M AcOH (in THF) and concentrated in vacuo. The resulting residue was partitioned between chloroform and brine, separated, and the organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The crude residue was purified by silica gel chromatography (chloroform/acetone 30:1 to 20:1) yielding 5-*O*-benzyl-1-(4-methylanisol-2-yl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose as a yellow syrup (**31**, 66 mg, 38%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.37–7.27$ (m, 16 H, H-3, ArH), 7.03 (dd, $J = 2.1$, 8.2, 1 H, H-5), 6.76 (d, $J = 8.2$, 1 H, H-6), 5.18 (d, $J = 4.9$, 1 H, H-1′), 4.85 (d, $J = 3.7$, 1 H, H-1″), 4.77–4.43 (m, 6 H, $3 \times CH_2$Ph), 4.25–4.20 (m, 2 H, H-3′, H-4′), 4.12 (dd, $J =$ 4.9, 4.9, 1 H, H-2′), 3.98 (dd, $J = 9.2$, 9.2, 1 H, H-3″), 3.82–3.52 (m, 6 H, H-5′, H-4″, H-5″, H-6″), 3.77 (s, 3 H, OMe), 3.40 (dd, $J = 3.7$, 9.2, 1 H, H-2″), 2.18 (s, 3 H, Me); HRMS-FAB: m/z: calcd for $C_{40}H_{45}O_{10}$: 685.3013 $[M+H]^+$, found: 685.3017; and 5-*O*-benzyl-1-(4-methylanisol-2-yl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-α-D-ribofuranose (**31a**, 61 mg, 36%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.40$ (d, $J = 1.8$, 1 H, H-3), 7.36–7.27 (m, 15 H, ArH), 7.05 (dd, $J = 1.8$, 8.2, 1 H, 5-H), 6.76 (d, $J = 8.2$, 1 H, H-6), 5.36 (d, $J = 2.4$, 1 H, H-1′), 4.83 (d, $J = 3.7$, 1 H, H-1″), 4.71–4.46 (m, 7 H, H-2′, $3 \times CH_2$Ph), 4.36 (m, 1 H, H-3′), 3.90 (dd, $J = 9.1$, 9.5, 1 H, H-3″), 3.82 (s, 3 H, OMe), 3.78–3.51 (m, 7 H, H-4′, H-5′, H-4″, H-5″, H-6″), 3.35 (dd, $J = 3.7$,

9.5, 1H, H-2″), 3.05, 2.56, 2.54 (3 × br s, 3 × 1H, 3 × OH), 2.31 (s, 3H, Me); HRMS-FAB: *m/z*: calcd for $C_{40}H_{46}O_{10}$: 686.3091 $[M+H]^+$, found: 686.3093.

5-*O*-Benzyl-1-(4-methylnaphthalenyl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose and **5-*O*-benzyl-1-(4-methylnaphthalenyl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-α-D-ribofuranose (34** and **34a)**: NaOMe (catalytic quantity) was added to a solution of **33** (42 mg, 0.05 mmol) in methanol (3 mL) and the solution was stirred at room temperature for 3 h. The mixture was neutralised with 1M AcOH (in THF) and concentrated in vacuo. The resulting residue was partitioned between chloroform and brine, separated, and the organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The crude residue was purified by silica gel chromatography yielding 5-*O*-benzyl-1-(4-methylnaphthalenyl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose as a yellow syrup (**34**, 20 mg, 57%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.20$ (dd, $J =$ 1.8, 7.6, 1H, $H_{naphthalene}$), 8.04 (dd, $J = 1.8$, 7.4, 1H, $H_{naphthalene}$), 7.65 (d, $J = 7.4$, 1H, $H_{naphthalene}$), 7.57 – 7.49 (m, 2H, 2 × $H_{naphthalene}$), 7.37 – 7.23 (m, 16H, ArH, $H_{naphthalene}$), 5.57 (d, $J = 3.5$, 1H, H-1′), 4.74, 4.69 (AB, 2H, CH_2Ph), 4.71 (d, $J = 4.0$, 1H, H-1″), 4.64, 4.56 (AB, 2H, CH_2Ph), 4.54, 4.45 (AB, 2H, CH_2Ph), 4.35 – 4.32 (m, 1H, H-3′), 4.24 – 4.17 (m, 2H, H-2′, OH), 4.00 (t, $J = 9.4$, 1H, H-3″), 3.88 – 3.73 (m, 5H, H-4′, H-5′, H-5″, OH), 3.62 – 3.51 (m, 4H, H-4″, H-6″, OH), 3.4 (dd, $J = 3.5$, 9.7, 1H, H-2″), 2.52 (s, 3H, CH_3Ph); and 5-*O*-benzyl-1-(4-methylnaphthalenyl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-α-D-ribofuranose (**34a**, 11 mg, 31%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.02$ (m, 1H, $H_{naphthalene}$), 7.88 (m, 1H, $H_{naphthalene}$), 7.73 (d, $J =$ 7.4, 1H, $H_{naphthalene}$), 7.52 (m, 2H, 2 × $H_{naphthalene}$), 7.37 (d, $J = 7.4$, 1H, $H_{naphthalene}$), 7.36 – 7.16 (m, 15H, ArH), 5.88 (d, $J = 3.1$, 1H, H-1′), 4.78 (d, $J = 3.9$, 1H, H-1″), 4.61 – 4.28 (m, 9H, 3 × CH_2Ph, H-2′, H-3′, OH), 3.86 (t, $J = 9.0$, 1H, H-3″), 3.82 – 3.76 (m, 2H, H-5′), 3.70 – 3.53 (m, 6H, H-4′, H-4″, H-5″, H-6″, OH), 3.33 (dd, $J = 3.75$, 9.7, 1H, H-2″), 3.1 (br s, 1H, OH), 2.41 (s, 3H, CH_3Ph).

5-*O*-Benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)uridine 2′,3″,4″-tris(dibenzylphosphate) (23): Dibenzyl(diisopropyl)phosphoramidite (42 μL, 0.13 mmol) was added to a solution of 5-*O*-Benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)uridine (**22**, 19 mg, 0.028 mmol) and 1*H*-tetrazole (18 mg, 0.25 mmol) in dichloromethane (1 mL) and the mixture was stirred at room temperature for 3 h. To this mixture was then added *m*CPBA (38 mg, 0.13 mmol) at −78 °C and the resulting mixture was stirred at room temperature for an additional 2 h. The reaction mixture was diluted with ethyl acetate and washed with aq. 10% Na_2SO_3, 1N HCl, 0.5N $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated in vacuo. The resulting crude residue was purified by silica gel chromatography (chloroform/methanol 50:1) to give title compound (**23**, 33 mg, 85%, isolated as solid). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.12$ (br s, 1H, NH), 7.52 (d, $J = 8.3$, 1H, H-6), 7.38 – 7.06 (m, 45H, ArH), 6.35 (d, $J = 6.4$, 1H, H-1′), 5.31 (d, $J =$ 3.4, 1H, H-1″), 5.31 (d, $J = 8.3$, 1H, H-5), 5.04 – 4.69 (m, 15H, H-2′, H-3″, H-4″, 6 × CH_2PH), 4.49 – 4.21 (m, 8H, H-3′, H-4′, 3 × CH_2Ph), 3.80 – 3.47 (m, 6H, H-5′, H-2″, H-5″, H-6″); ^{31}P NMR (67.5 MHz, $CDCl_3$, H-decoupled): $\delta = -0.34$, −1.17, −1.29 (3 × s, 3 × P); HRMS-FAB: *m/z*: calcd for $C_{78}H_{80}N_2O_{20}P_3$: 1457.4517 $[M+H]^+$, found: 1457.4565; UV (methanol): $\lambda_{max} = 257$, 260 nm (sh).

1-Benzimidazolyl-5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-tris(dibenzylphosphate) (26): Dibenzyl(diisopropyl)phosphoramidite (42 μL, 0.13 mmol) was added to a solution of 1-benzimidazolyl-5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose (**25**, 48 mg, 0.07 mmol) and 1*H*-tetrazole (18 mg, 0.25 mmol) in dichloromethane and the mixture stirred at room temperature for 3 h. The solution was cooled to −78 °C and *m*CPBA (38 mg, 0.13 mmol) was added with stirring continuing for an additional 2 h at −20 °C. The mixture was diluted with ethyl acetate and was washed with 10% Na_2SO_3, 1N HCl, 0.5N $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated under reduced pressure. Crude residue was purified by silica gel chromatography (chloroform/acetone 200:1 to 100:1) yielding title compound as a syrup (**26**, 81 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 7.99 (s, 1H, H-2), 7.76 (d, $J = 8.2$, 1H, $H_{benzimidazole}$), 7.54 (d, $J = 8.3$, 1H, $H_{benzimidazole}$), 7.35 – 6.91 (m 47H, 2 × $H_{benzimidazole}$, ArH), 6.11 (d, $J = 6.8$, 1H, H-1′), 5.36 (d, $J = 3.4$, 1H, H-1″), 5.26 (m, 1H, H-2′), 5.05 – 4.89 (m, 8H, H-3″, H-4″, 3 × CH_2Ph), 4.80 – 4.70 (m, 2H, CH_2Ph), 4.55 – 4.29 (m, 11H, H-3′, 5 × CH_2Ph), 3.85 (m, 1H, H-4′), 3.74 – 3.53 (m, 6H, H-5′, H-2″, H-5″, H-6″); ^{31}P NMR (100 MHz, $CDCl_3$, H-decoupled): $\delta = -1.73$, −2.07, −2.18 (3 × s, 3 × P); HRMS-FAB: *m/z*: calcd for $C_{81}H_{82}N_2O_{18}P_3$: 1463.4776 $[M+H]^+$, found: 1463.4743.

1-(2-Methoxynaphthalenyl)-5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-tris(dibenzylphosphate) (29): Dibenzyl(diisopropyl)phosphoramidite (42 μL, 0.13 mmol) was added to a solution of 5-*O*-benzyl-1-(2-methoxynaphthalenyl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose (**28**, 181 mg, 0.025 mmol) and 1*H*-tetrazole (18 mg, 0.25 mmol) in dichloromethane and the mixture stirred at room temperature for 3 h. The solution was cooled to −78 °C and *m*CPBA (38 mg, 0.13 mmol) was added with stirring continuing for an additional 2 h at room temperature. The mixture was diluted with ethyl acetate and was washed with 10% Na_2SO_3, 1N HCl, 0.5N $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated under reduced pressure. The resulting crude residue was purified by silica gel chromatography (hexane/ethyl acetate 2:1 to 1:1) yielding title compound as a syrup (**29**, 345 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.32$ (d, $J = 8.8$, 1H, $H_{naphthalene}$), 7.77 (d, $J = 8.8$, 1H, $H_{naphthalene}$), 7.72 (d, $J = 7.3$, 1H, $H_{naphthalene}$), 7.43 – 6.65 (m, 48H, 3 × $H_{naphthalene}$, ArH), 6.10 (d, $J = 7.3$, 1H, H-1′), 5.65 (m, 1H, H-2′), 5.56 (d, $J = 3.4$, 1H, H-1″), 5.07 – 4.62 (m, 12H, 5 × CH_2Ph, H-3″, H-4″), 4.53 – 4.01 (m, 9H, 4 × CH_2Ph, H-3′), 3.90 – 3.56 (m, 7H, H-4′, H-5′, H-2″, H-5″, H-6″), 3.68 (s, 3H, OMe); ^{31}P NMR (100 MHz, $CDCl_3$, H-decoupled): $\delta = -1.49$, −2.28, −2.30 (3 × s, 3 × P); HRMS-FAB: *m/z*: calcd for $C_{85}H_{86}O_{19}P_3$: 1503.4976 $[M+H]^+$, found: 1503.5016.

1-(4-Methylanisol-2-yl)-5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-tris(dibenzylphosphate) (32): Dibenzyl(diisopropyl)phosphoramidite (42 μL, 0.13 mmol) was added to a solution of 5-*O*-benzyl-1-(4-methylanisol-2-yl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose (**31**, 38 mg, 0.055 mmol) and 1*H*-tetrazole (18 mg, 0.25 mmol) in dichloromethane and the mixture stirred at room temperature for 3 h. The solution was cooled to −78 °C and *m*CPBA (38 mg, 0.13 mmol) was added with stirring continuing for an additional 2 h at −20 °C. The mixture was diluted with ethyl acetate and was washed with 10% Na_2SO_3, 1N HCl, 0.5N $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated under reduced pressure. The crude residue was purified by silica gel chromatography (hexane/ethyl acetate 2:1 to 1:1) yielding title compound as a syrup (**32**, 78 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 7.39 – 7.05 (m, 46H, H-2, ArH), 7.01 (dd, $J = 2.0$, 8.3, 1H, H-5), 6.70 (d, $J =$ 8.3, 1H, H-6), 5.48 (d, $J = 5.4$, 1H, H-1′), 5.31 (d, $J = 3.4$, 1H, H-1″), 5.10 (m, 1H, H-2′), 5.04 – 4.88 (m, 8H, 3 × CH_2Ph, H-3″, H-4″), 4.79 – 4.57 (m, 6H, 3 × CH_2Ph), 4.51 – 4.24 (m, 7H, 3 × CH_2Ph, H-3′), 3.85 – 3.50 (m, 7H, H-4′, H-5′, H-2″, H-5″, H-6″), 3.63 (s, 3H, OMe), 2.11 (s, 3H, Me); ^{31}P NMR (67.5 MHz, $CDCl_3$, H-decoupled): $\delta = -1.10$, −1.23, −1.38 (3 × s, 3 × P); HRMS-FAB: *m/z*: calcd for $C_{82}H_{86}O_{19}P_3$: 1467.4976 $[M+H]^+$, found: 1467.5018.

1-(4-Methylanisol-2-yl)-5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-α-D-ribofuranose 2′,3″,4″-tris(dibenzylphosphate) (32a): Dibenzyl(diisopropyl)phosphoramidite (42 μL, 0.13 mmol) was added to a solution of 5-*O*-benzyl-1-(4-methylanisol-2-yl)-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-α-D-ribofuranose (**31a**, 58 mg, 0.085 mmol) and 1*H*-tetrazole (18 mg, 0.25 mmol) in dichloromethane and the mixture stirred at room temperature for 3 h. The solution was cooled to −78 °C and *m*CPBA (38 mg, 0.13 mmol) was added with stirring continuing for an additional 2 h at −20 °C. The mixture was diluted with ethyl acetate and was washed with 10% Na_2SO_3, 1N HCl, 0.5N $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated under reduced pressure. The crude residue was purified by silica gel chromatography yielding title compound as a syrup (**32a**, 108 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.42 – 6.92$ (m, 46H, H-5, ArH), 7.36 (d, $J = 2.0$, 1H, H-3), 6.58 (d, $J = 8.3$, 1H, H-6), 5.38 – 5.35 (m, 3H, H-1′, H-2′, H-1″), 5.06 – 4.15 (m, 21H, 9 × CH_2Ph, H-3′, H-3″, H-4″), 3.98 – 3.59 (m, 7H, H-4′, H-5′, H-2″, H-5″, H-6″), 3.72 (s, 3H, OMe), 2.21 (s, 3H, Me); ^{31}P NMR (67.5 MHz, $CDCl_3$, H-decoupled): $\delta = -1.07$, −1.29, −1.47 (3 × s, 3 × P); HRMS-FAB: *m/z*: calcd for $C_{82}H_{86}O_{19}P_3$: 1467.4976 $[M+H]^+$, found: 1467.4979.

1-(4-Methylnaphthalenyl)-5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-tris(dibenzylphosphate) (35): Dibenzyl(diisopropyl)phosphoramidite (21 μL, 0.07 mmol) was added to a solution of 1-(4-methylnaphthalenyl)-5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose (**34**, 12 mg, 0.017 mmol) and 1*H*-tetrazole (9 mg, 0.12 mmol) in dichloromethane (4 mL) and the reaction was stirred at room temperature for 2 h. The solution was cooled to −78 °C and *m*CPBA (19 mg, 0.07 mmol) was added with stirring continuing for an additional 2 h. The mixture was diluted with ethyl acetate and was washed with 10% Na_2SO_3, 1N HCl, 0.5N $NaHCO_3$, brine, dried ($MgSO_4$) and

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evaporated under reduced pressure. The crude residue was purified by silica gel chromatography yielding title compound as a syrup (**35**, 15 mg, 62%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.24$ (d, $J = 8.6$, 1H, $H_{naphthalene}$), 7.94 (d, $J = 8.6$, 1H, $H_{naphthalene}$), 7.68 (d, $J = 7.4$, 1H, $H_{naphthalene}$), 7.51–6.80 (m, 48H, $3 \times H_{naphthalene}$, ArH), 5.90 (d, $J = 7.0$, 1H, H-1′), 5.42 (d, $J = 3.5$, 1H, H-1″), 5.08–4.26 (m, 22H, $9 \times CH_2$Ph, H-2′, H-5′a, H-3″, H-4″), 3.89–3.55 (m, 7h, H-4′, H-5′b, H-2″, H-5″, H-6″), 2.21 (s, 3H, CH_3Ph); ^{31}P NMR (100 MHz, $CDCl_3$, H-decoupled): $\delta = -0.87, -1.06, -1.32$ ($3 \times$ s, $3 \times$ P).

3-*O*-(α-D-Glucopyranosyl)uridine 2′,3″,4″-trisphosphate (12): A mixture of 5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)uridine 2′,3″,4″-tris(dibenzylphosphate) (**23**, 131 mg, 0.90 mmol) and Pd/C (10%, 80 mg) in methanol (5 mL) was stirred under atmospheric pressure of H_2 at room temperature for 18 h. The catalyst was removed by filtration through Celite with the resulting filtrate being concentrated in vacuo. The residue was dissolved in water and applied to a Diaion WK-20 resin column (Na^+ form), which was developed by water. The eluent was evaporated under reduced pressure and the residues was co-evaporated with ethanol to give 3-*O*-(α-D-glucopyranosyl)uridine 2′,3″,4″-trisphosphate (**12**, 62 mg, 98%) as white solids. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.65$ (d, $J = 8.3$, 1H, H-6), 5.95 (d, $J = 4.4$, 1H, H-1′), 5.74 (d, $J = 8.3$, 1H, H-5), 5.15 (br s, 1H, H-1″), 4.74 (m, 1H, H-2′), 4.32–4.28 (m, 2H, H-3′, H-3″), 4.17 (m, 1H, H-4″), 3.90–3.50 (m, 7H, H-4′, H-5′, H-2″, H-5″, H-6″); ^{31}P NMR (100 MHz, D_2O, H-decoupled): $\delta = 1.78, 1.09, 0.23$ ($3 \times$ s, $3 \times$ P); HRMS-FAB (triethylammonium salts): *m/z*: calcd for $C_{15}H_{24}N_2O_{20}P_3$: 645.0135 $[M]^-$, found: 645.0130; UV (water): $\lambda_{max} = 260$ nm.

1-Benzimidazolyl-3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-trisphosphate (13): A mixture of 1-benzimidazolyl-5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-tris(dibenzylphosphate) (**26**, 48 mg, 0.033 mmol) and $Pd(OH)_2$ (10%, 120 mg) in a mixture of cyclohexene (3.0 mL) and methanol (6.0 mL) was heated under reflux for 90 min. The catalyst was removed by filtration through Celite with the filtrate being concentrated in vacuo. The residue was dissolved in water and applied to a Diaion WK-20 resin column (Na^+ form), which was developed with water. The eluent was evaporated under reduced pressure and the residue was co-evaporated with ethanol yielding 1-benzimidazolyl-3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-trisphosphate as the sodium salt (**13**, 19 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.38$ (s, 1H, H-2), 7.69 (d, $J = 8.2$, 1H, $H_{benzimidazole}$), 7.59 (d, $J = 7.3$, 1H, $H_{benzimidazole}$), 7.30–7.20 (m, 2H, $H_{benzimidazole}$), 6.21 (d, $J = 5.4$, 1H, H-1′), 5.17 (d, $J = 3.4$, 1H, H-1″), 4.96 (m, 1H, H-2′), 4.44 (dd, $J = 4.4, 4.8$, 1H, H-3′), 4.35 (m, 1H, H-3″), 4.27 (m, 1H, H-4′), 3.90 (m, 1H, H-5″), 3.78–3.58 (m, 6H, H-5′, H-2″, H-4″, H-6″); ^{31}P NMR (100 MHz, D_2O, H-decoupled): $\delta = 3.16, 2.76, 0.35$ ($3 \times$ s, $3 \times$ P); HRMS-FAB (triethylammonium salt): *m/z*: calcd for $C_{18}H_{26}O_{18}N_2P_3$: 651.0394 $[M+H]^+$, found: 651.0386.

1-(2-Methoxynaphthalenyl)-3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-trisphosphate (14): A mixture of 1-(2-methoxynaphthalenyl)-5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-tris(dibenzylphosphate) (**29**, 20 mg, 0.013mmol) and $Pd(OH)_2$ (10%, 120 mg) in a mixture of cyclohexene (3.0 mL) and methanol (6.0 mL) was heated under reflux for 90 min. The catalyst was removed by filtration through Celite with then resulting filtrate being concentrated in vacuo. The residue was then diluted with water and applied to a Diaion WK-20 resin column (Na^+ form), which was developed with water. The eluent was evaporated under reduced pressure and the residue co-evaporated with ethanol to yield the sodium salt of 1-(2-methoxynaphthalenyl)-3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-trisphosphate (**14**, 6 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.12$ (d, $J = 8.3$, 1H, $H_{naphthalene}$), 7.85 (d, $J = 8.8$, 1H, $H_{naphthalene}$), 7.76 (d, $J = 7.8$, 1H, $H_{naphthalene}$), 7.44 (t, $J = 7.3$, 1H, $H_{naphthalene}$), 7.29 (m, 2H, $2 \times H_{naphthalene}$), 5.81 (d, $J = 5.4$, 1H, H-1′), 5.19 (d, $J = 3.4$, 1H, H-1″), 5.17 (m, 1H, H-2′), 4.46–4.43 (m, 1H, H-3′), 4.36–4.32 (m, 1H, H-3″), 4.12 (m, 1H, H-4′), 3.90–3.78 (m, 8H, H-5′a, H-5″, H-6″, OCH_3, OH), 3.71–3.62 (m, 5H, H-5′b, H-2″, H-4″, $2 \times$ OH); ^{31}P NMR (100 MHz, $CDCl_3$, H-decoupled): $\delta = 2.58, 1.52, 0.27$ ($3 \times$ s, $3 \times$ P).

1-(4-Methylanisol-2-yl)-3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-trisphosphate (15): A mixture of 1-(4-methylanisol-2-yl)-5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-tris(dibenzylphosphate) (**32**, 40 mg, 0.27 mmol) and Pd/C (10%, 30 mg) in methanol was stirred under atmospheric pressure of H_2 at room temperature for 75 min. The catalyst was filtered off with Celite and the resulting filtrate concentrated in vacuo. The residue was dissolved in water and applied to a Diaion WK-20 resin column (Na^+ form), which was developed with water. The eluent was evaporated under reduced pressure and the residue was co-evaporated with ethanol to give the sodium salt of 1-(4-methylanisol-2-yl)-3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-trisphosphate (**15**, 19 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.16$ (s, 1H, H-3), 7.08 (d, $J = 8.2$, 1H, H-5), 6.84 (d, $J = 8.2$, 1H, H-6), 5.14 (d, $J = 3.7$, 1H, H-1′), 5.11 (d, $J = 6.1$, 1H, H-1″), 4.62 (m, 1H, H-2′), 4.31 (m, 1H, H-3″), 4.24 (dd, $J = 5.2, 5.2$, 1H, H-3′), 4.09 (m, 1H, H-4′), 3.89–3.58 (m, 7H, H-5′, H-2″, H-4″, H-5″, H-6″), 3.69 (s, 3H, OMe), 2.13 (s, 3H, Me); ^{31}P NMR (100 MHz, D_2O, H-decoupled): $\delta = -2.29, -1.35, -0.18$ ($3 \times$ s, $3 \times$ P); HRMS-FAB (triethylammonium salt): *m/z*: calcd for $C_{19}H_{30}O_{19}P_3$: 655.0594 $[M+H]^+$, found: 655.0592.

1-(4-Methylanisol-2-yl)-3-*O*-(α-D-glucopyranosyl)-α-D-ribofuranose 2′,3″,4″-trisphosphate (15a): A mixture of 1-(4-methylanisol-2-yl)-5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-α-D-ribofuranose 2′,3″,4″-tris(dibenzylphosphate) (**32a**, 44 mg, 0.30 mmol) and Pd/C (10%, 33 mg) in methanol was stirred under atmospheric pressure of H_2 at room temperature for 75 min. The catalyst was filtered off with Celite and the resulting filtrate concentrated in vacuo. The residue was dissolved in water and applied to a Diaion WK-20 resin column (Na^+ form), which was developed with water. The eluent was evaporated under reduced pressure and the residue was co-evaporated with ethanol to give the sodium salt of 1-(4-methylanisol-2-yl)-3-*O*-(α-D-glucopyranosyl)-α-D-ribofuranose 2′,3″,4″-trisphosphate (**15a**, 19 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.18$ (s, 1H, H-3), 7.03 (d, $J = 8.3$, 1H, H-5), 6.78 (d, $J = 8.3$, 1H, H-6), 5.31 (s, 1H, H-1′), 5.10 (d, $J = 3.4$, 1H, H-1″), 4.99 (m, 1H, H-2′), 4.45 (dd, $J = 3.9, 8.3$, 1H, H-3′), 4.35 (m, 1H, H-3″), 4.17 (m, 1H, H-4′), 4.03 (m, 1H, H-5″), 3.84 (d, $J = 10.7$, 1H, H-5′a), 3.71–3.60 (m, 5H, H-5′b, H-2″, H-4″, H-6″), 3.66 (s, 3H, OMe), 2.15 (s, 3H, Me); ^{31}P NMR (100 MHz, D_2O, H-decoupled): $\delta = 3.56, 2.65, 0.61$ ($3 \times$ s, $3 \times$ P); HRMS-FAB (triethylammonium salt): *m/z*: calcd for $C_{19}H_{30}O_{19}P_3$: 655.0594 $[M+H]^+$, found: 655.0588.

1-(4-Methylnaphthalenyl)-3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-trisphosphate (16): A mixture of 1-(4-methylnaphthalenyl)-5-*O*-benzyl-3-*O*-(2,6-di-*O*-benzyl-α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-tris(dibenzylphosphate) (**35**, 12 mg, 8 μmol) and $Pd(OH)_2$ (10%, 60 mg) in a mixture of cyclohexene (2.0 mL) and methanol (4.0 mL) was heated under reflux for 90 min. The catalyst was removed by filtration through Celite with the resulting filtrate being concentrated in vacuo. The residue was the diluted with water and applied to a Diaion WK-20 resin column (Na^+ form), which was developed with water. The eluent was evaporated under reduced pressure and the residue co-evaporated with ethanol to yield the sodium salt of 1-(4-methylnaphthalenyl)-3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranose 2′,3″,4″-trisphosphate (16, 4 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.19$ (m, 1H, $H_{naphthalene}$), 8.01 (m, 1H, $H_{naphthalene}$), 7.85 (m, 1H, $H_{naphthalene}$), 7.58–7.49 (m, 2H, $2 \times H_{naphthalene}$), 7.37–7.26 (m, 1H, $H_{naphthalene}$), 5.73 (d, $J = 4.1$, 1H, H-1′), 5.14 (d, $J = 3.1$, 1H, H-1″), 5.04 (m, 1H, H-2′), 4.43–4.28 (m, 2H, H-3′, H-3″), 4.16 (m, 1H, H-4′), 3.87–3.73 (m, 6H, H-5′, H-5″, H-6″, OH), 3.67–3.58 (m, 4H, H-2″, H-4″, $2 \times$ OH), 2.54 (s, 3H, CH_3Ph); ^{31}P NMR (100 MHz, $CDCl_3$, H-decoupled): $\delta = 2.52, 1.46, 0.72$ ($3 \times$ s, $3 \times$ P).

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[1] K. Mikoshiba, *Curr. Opin. Neurobiol.* **1997**, *7*, 339.
[2] M. J. Berridge, *J. Physiol.* **1997**, *499*, 291.
[3] M. J. Berridge, *Nature* **1993**, *361*, 315.
[4] B. V. L. Potter, S. R. Nahorski, in *Drug Design for Neuroscience* (Ed.: A. P. Kozikowski), Raven Press, New York, **1993**, pp. 383–416.
[5] B. V. L. Potter, D. Lampe, *Angew. Chem.* **1995**, *107*, 2085; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1933.

[6] M. Takahashi, T. Kagasaki, T. Hosoya, S. Takahashi, *J. Antiobiot.* **1993**, *46*, 1643.
[7] M. Takahashi, T. Kagasaki, S. Takahashi, *J. Biol. Chem.* **1994**, *269*, 369.
[8] J. Hirota, T. Michikawa, A. Miyakawa, M. Takahashi, K. Tanzawa, I. Okura, T. Furuichi, K. Mikoshiba, *FEBS Lett.* **1995**, *368*, 248.
[9] J. S. Marchant, M. D. Beecroft, A. M. Riley, D. J. Jenkins, R. D. Marwood, C. W. Taylor, B. V. L. Potter, *Biochemistry* **1997**, *36*, 12780.
[10] C. T. Murphy, A. M. Riley, C. J. Lindley, D. J. Jenkins, J. Westwick, B. V. L. Potter, *Mol. Pharmacol.* **1997**, *52*, 741.
[11] a) S. Shuto, K. Tatani, Y. Ueno, A. Matsuda, *J. Org. Chem.* **1998**, *63*, 8815; b) M. Kashiwayanagi, K. Tatani, S. Shuto, A. Matsuda, *Eur. J. Neurosci.* **2000**, *12*, 606; c) S. Shuto, Y. Yahiro, S. Ichikawa, A. Matsuda, *J. Org. Chem.* **2000**, *65*, 5547; d) D. J. Jenkins, B. V. L. Potter, *Carbohydr. Res.* **1996**, *287*, 169; e) R. D. Marwood, A. M. Riley, V. Correa, C. W. Taylor, B. V. L. Potter, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 453; f) M. D. Beecroft, J. S. Marchant, A. M. Riley, N. C. R. van Straten, A. G. van Marel, J. H. van Boom, B. V. L. Potter, C. W. Taylor, *Mol. Pharmacol.* **1999**, *55*, 109; g) R. A. Wilcox, C. Erneux, W. U. Primose, R. Gigg, S. Nahorski, *Mol. Pharmacol.* **1995**, *47*, 1204; h) N. C. R. van Straten, G. A. van der Marel, J. H. van Boom, *Tetrahedron* **1997**, *53*, 6523; i) N. C. R. van Straten, N. M. A. J. Kriek, Z. A. C. Cziria, G. A. van der Marel, J. H. van Boom, *Tetrahedron* **1997**, *53*, 6539; j) H. Hotoda, K. Murayama, S. Miyamoto, Y. Iwata, M. Takahashi, Y. Kawase, K. Tanzawa, M. Kaneko, *Biochemistry* **1999**, *38*, 9234; k) N. Motiessier, F. Chrétien, Y. Chapleur, C. Humeau, *Tetrahedron Lett.* **1995**, *44*, 8023; l) F. Roussel, M. Hilly, F. Chrétien, J.-P. Mauger, Y. Chapleur, *J. Carbohydr. Chem.* **1999**, *18*, 697; m) H. J. Rosenberg, A. M. Riley, V. Correa, C. W. Taylor, B. V. L. Potter, *Carbohydr. Res.* **2000**, *329*, 7; n) H. J. Rosenberg, A. M. Riley, R. D. Marwood, V. Correa, C. W. Taylor, B. V. L. Potter, *Carbohydr. Res.* **2001**, *332*, 53.
[12] a) R. D. Marwood, D. J. Jenkins, V. Correa, C. W. Taylor, B. V. L. Potter, *J. Med. Chem.* **2000**, *43*, 4278; b) R. D. Marwood, S. Shuto, D. J. Jenkins, B. V. L. Potter, *Chem. Commun.* **2000**, 219.
[13] A. Thomson, M. L. Wolfrom, *Methods Carbohydr. Chem.* **1963**, *2*, 427.
[14] a) G. M. Anantharamaiah, K. M. Sivanandaiah, *J. Chem. Soc. Perkin Trans. 1* **1977**, 490; b) S. Hanessian, T. J. Liak, B. Vanasse, *Synthesis* **1981**, 396.
[15] Reviewed in: a) M. H. D. Postema, *Tetrahedron* **1992**, *48*, 8545; b) C. Jaramillo, S. Knapp, *Synthesis* **1994**, 1; c) D. E. Levy, C. Tang, *The Chemistry of C-Glycosides*, Pergamon, **1995**; d) M. H. D. Hostema, *C-Glycoside Synthesis*, CRC, **1995**; e) Y. Du, R. J. Linhardt, *Tetrahedron* **1998**, *54*, 9913.
[16] For examples see: a) D. I. Kwok, G. D. Daves Jr., *J. Org. Chem.* **1989**, *54*, 4496; b) T. Matsumoto, M. Katsuki, K. Suzuki, *Tetrahedron Lett.* **1989**, *30*, 833; c) N. Hamamichi, T. Miyasaka, *J. Org. Chem.* **1991**, *56*, 3731; d) K. Toshima, G. Matsuo, K. Katsuta, *Tetrahedron Lett.* **1992**, *33*, 2175; e) J. C. Castro-Palomino, R. R. Schmidt, *Liebigs Ann.* **1996**, 1623; f) T. Kuribayashi, N. Ohkawa, S. Satoh, *Tetrahedron Lett.* **1998**, *39*, 4537; g) T. Kuribayashi, N. Ohkawa, S. Satoh, *Tetrahedron Lett.* **1998**, *39*, 4541.
[17] C. D. Hurd, W. A. Bonner, *J. Am. Chem. Soc.* **1945**, *67*, 1664.
[18] For reviews on O-glycosylation see: H. Paulsen, *Angew. Chem.* **1985**, *94*, 184; *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 155; K. Toshima, K. Tatsuta, *Chem. Rev.* **1993**, *93*, 1503; G. J. Boons, *Contemp. Org. Synth.* **1996**, *3*, 173; G. J. Boons, *Tetrahedron* **1996**, *52*, 1095; S. H. Kahn, R. A. O'Neill, in *Modern Methods in Carbohydrate Chemistry*, Harwood Academic, Amsterdam, **1996**; G. J. Boons in *Carbohydrate Chemistry*, Blackie Academic, London, **1998**; B. G. Davis *J. Chem. Soc. Perkin Trans. 1* **2000**, 2137.
[19] V. Correa, A. M. Riley, S. Shuto, G. Horne, E. P. Nerou, R. D. Marwood, B. V. L. Potter, C. W. Taylor, *Mol. Pharmacol.* **2001**, *59*, 1206.
[20] H. Abe, S. Shuto, A. Matsuda, *J. Org. Chem.* **2000**, *65*, 4315.
[21] R. D. Marwood, A. M. Riley, D. J. Jenkins, B. V. L. Potter, *J. Chem. Soc. Perkin Trans. 1* **2000**, *12*, 1935.
[22] D. Lampe, C. Liu, B. V. L. Potter, *J. Med. Chem.* **1994**, *37*, 907.

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