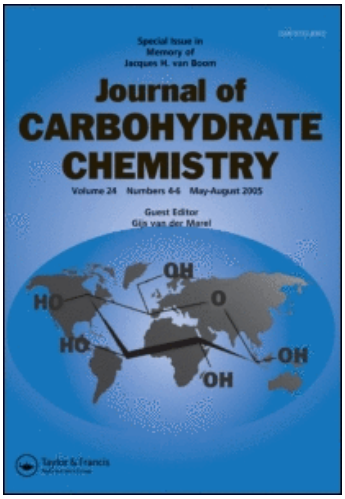

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New Sugar-Based Permeant Analogs of D-*Myo*-Inositol 1,4,5-Trisphosphate Mimicking the Effect of Vasopressin: Synthesis and Biologic Evaluation

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On the basis of the xylose-inositol analogy, a series of permeant analogs of D-myo-inositol 1,4,5-trisphosphate ($InsP_3$) have been synthesized by various esterifications of the phosphate groups. Their ability to cross the cell membrane has been tested on vasopressin cells. Very fast liberation of calcium occurs when active analogs are introduced in the extracellular medium on intact cells. Membrane crossing as well as hydrolysis of the phosphate is very rapid using acyloxymethyl esterification of all the phosphate groups. The free compounds behave in the cell like $InsP_3$. One of the analogs

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Dedicated to the memory of Jacques H. van Boom and his outstanding contribution to the field.
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prepared this way behaves like vasopressin for rat hepatocyte cells expressing vasopressin receptor.

Keywords Inositol 1,4,5-trisphosphate, Permeant analogs, Carbohydrate, Vasopressin, Cell, Calcium liberation

INTRODUCTION

The increasing knowledge of cellular mechanisms provides new opportunities for the development of molecular tools for the studies of biologic processes. Cellular response to an external stimulus is often the result of a cascade of events. Most of primary messengers bind to a specific G protein-coupled receptor (GPCR). It is then followed by a second series of signalling events such as calcium liberation. The discovery of this calcium signalling pathway has been a major event in cellular biology.[1–3] Even more interesting was the identification of the second messengers derived from the phosphoinositide pathway, in particular inositol 1,4,5-trisphosphate ($InsP_3$) **1**.[4–6] This small molecule is formed by hydrolysis of a membrane-anchored phosphoinositide (PIP_3) by a phospholipase C, which is activated following ligand binding to a GPCR. This hydrolysis produces diacylglycerol and the second messenger inositol 1,4,5-triphosphate ($InsP_3$). Binding of the latter to its specific receptor ($InsP_3R$), located on the endoplasmic reticulum, triggers calcium liberation and cellular response via calmodulin and other calcium-sensitive proteins. Complex cascades of events degrade $InsP_3$ to inositol and then recycle inositol to PIP3.

$InsP_3$ is a ubiquitous second messenger, which prompted much interest in the scientific community in the last 20 years. On the one hand, the possibility of blocking its binding to $InsP_3R$ was explored, and the search for antagonists of this receptor has been a long-standing interest.[7] Only a few antagonists of low potency have been found. On the other hand, $InsP_3R$ agonists, that is, analogs of $InsP_3$, have been developed to study $InsP_3R$, the signalling pathway, or to trigger at will biologic events such as secretion, cell proliferation, or cell contraction. This task was easier, and a number of synthetic analogs of $InsP_3$ have been proposed.[8–10]

In our group, we studied heterocyclic structures designed by analogy with $InsP_3$. These sugar-based analogs of $InsP_3$ are potent ligands of $InsP_3R$.[11–15] Moreover, while our studies were in progress, adenophostins **2**, the most potent agonists of $InsP_3R$, have been isolated[16] and supported our idea of replacing the cyclohexane ring of inositol by sugar templates. These molecules provide good lead compounds for the design of potent agonists. The chemistry of adenophostin[17] and congeners[18] has been thoroughly explored, in particular in J. van Boom group. The biologic activity of all these analogs has been explored on isolated receptor $InsP_3$ or on permeabilized cells and confirmed

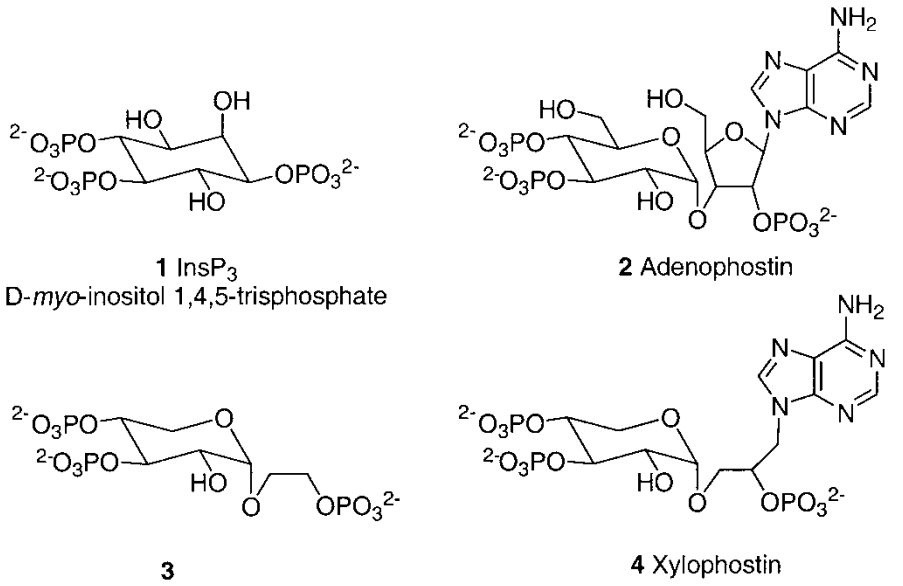


Figure 1: Sugars based analogs of $InsP_3$.

the potency of these ligands to trigger calcium release from endoplasmic reticulum stores.[19] We were also able to construct simplified analogs of adenophostins, which we named xylophostins and which are very potent agonists of $InsP_3R$ (Fig. 1).[15]

As above mentioned, one interest of these agonists is their possible use as biochemical tools. Indeed, it would be of interest to trigger calcium release using the second messenger itself without external stimulus by the normal primary messenger and to trigger biologic events in intact cells. This would be particularly useful on abnormal cells not expressing GPCR.

However, the intrinsic high polarity of these water-soluble polyphosphates precludes their use on intact cells. Analogs of $InsP_3$ that could be transported across cell membranes and could undergo subsequent degradation to the free phosphate derivatives, the so-called permeant derivatives, could provide a solution to this problem. Various permeant inositol phosphates have already been proposed,[20] and here we report the results of our investigations along these lines using our xylose-based analogs. We also report the details of the synthetic studies and some preliminary biologic results providing the clues to cell external activation via $InsP_3$ analogs.

RESULTS AND DISCUSSION

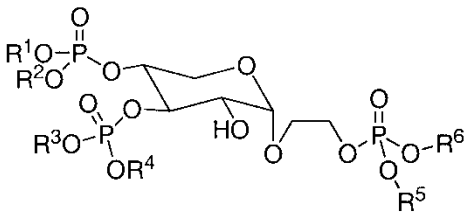
Among the $InsP_3$ analogs synthesized in our group, compound **3** was found very active[9] and would be a good candidate to test several strategies for making

this compound permeant. Different approaches were tested, and six target compounds depicted in Figure 2 were devised and prepared.

In the first series of experiments, three possibilities were explored. The simplest way was the phosphorylation of the appropriate triol with a protected alkylphosphate, assuming that the recovery of the phosphate group should be carried out via a phospholipase hydrolysis of the alkylphosphate groups. The synthesis of **5** bearing dialkylphosphate was envisioned. The chain length of the alkyl group of the dialkylphosphate should play a crucial role either in the transport or in the phosphate liberation inside the cell and would need to be carefully chosen. We chose to limit our investigation to the medium-sized octyl group. One drawback of this approach is the very low solubility in water of this type of fully protected compound. To overcome this drawback, a possibility was to decrease the number of protected phosphates Protection of each of the three phosphates with single alkyl group as in **6** was thus considered. Finally, the construction of **7** and **8**, with only one of the three phosphates protected with two alkyl groups, was also envisioned. It is of note that such monoprotected trisphosphates should act as a surfactant and interact with cell membranes in several ways.

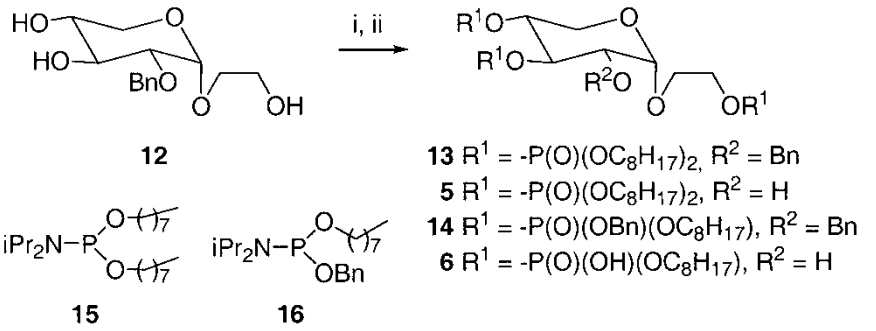
The synthesis of **5** and **6** started from the previously described triol **12** obtained in five steps from allyl-α-D-xylopyranoside via the 2-*O*-benzyl derivative **17**.[11] Reaction of **12** with the phosphoramidite **15** followed by *in situ* oxidation with tBuOOH gave the protected derivative **13** in 42% yield. Hydrogenolysis of this compound gave the free hydroxyl group of **5** in good yield.

The synthesis of **6** proceeded along the same lines by changing the phosphitylating agent to **16**. The protected trisphosphate **14** was obtained in 52% yield



5 $R^1 = R^3 = R^5 = R^2 = R^4 = R^6 = C_8H_{17}$
6 $R^1 = R^3 = R^5 = H$, $R^2 = R^4 = R^6 = C_8H_{17}$
7 $R^1 = R^2 = R^3 = R^4 = H$, $R^5 = R^6 = C_8H_{17}$
8 $R^1 = R^2 = R^3 = R^4 = R^5 = H$, $R^6 = C_8H_{17}$
9 $R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = CH_2\text{-}O\text{-}COCH_3$
10 $R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = CH_2\text{-}O\text{-}CO(CH_2)_2CH_3$
11 $R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = CH_2\text{-}O\text{-}CO(CH_2)_6CH_3$

Figure 2: Structure of target compounds.



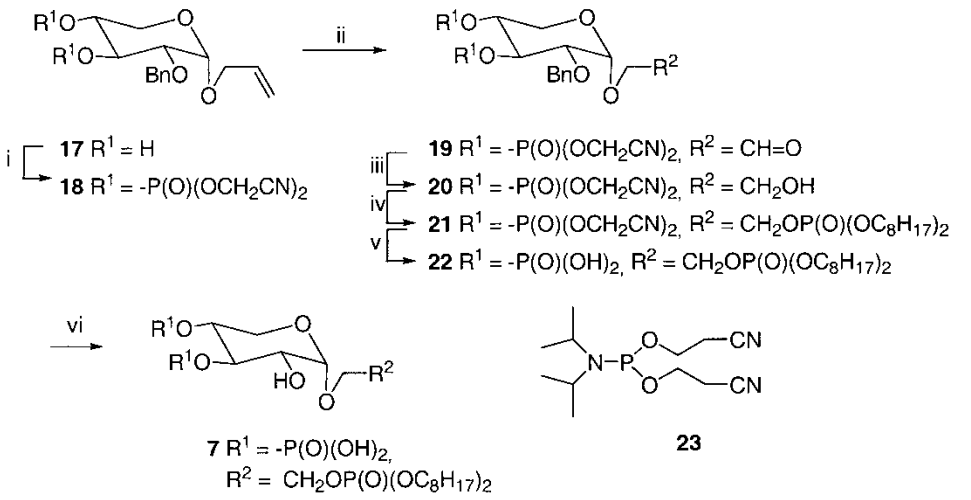
Scheme 1: Reagents i: **15**, 1H-tetrazole, CH_2Cl_2, rt, then 0°C, tBuOOH; ii: Pd/C 10% H_2, 20 bars, MeOH.

for the two steps. Deprotection of the four benzyl groups by hydrogenolysis at 20 bars in methanol gave the free derivative **6** isolated as the sodium salt in quantitative yield (Sch. 1).

It was known from the previous studies that the two vicinal phosphates at position 4 and 5 of the inositol ring are crucial for recognition by the $InsP_3R$. Thus, it could be useful to modify only the phosphate of the aglycon that mimics the phosphate at position 1 of $InsP_3$. The synthetic strategy toward the triol **12** allowed the construction of such unsymmetrical trisphosphates. We started from the protected allyl xyloside **17** that was phosphorylated at position 3 and 4 (carbohydrate numbering) using the phosphoramidite **23**,[21] followed by oxidation with *t*BuOOH to give the protected bisphosphate **18** in 70% yield. The double bond was cleaved using OsO_4 and $NaIO_4$ in a dioxane--water mixture to yield the corresponding aldehyde **19** in 98% yield. Reduction of the carbonyl group with sodium borohydride in MeOH for 3 min gave the expected alcohol **20** in 57% yield. Phosphorylation of this alcohol by the phosphoramidite method using **15** gave the protected trisphosphate **21** in 42% yield after oxidation. Deprotection of the cyano-ethyl groups of **21** gave the semi-protected derivative **22**. Deprotection of the cyano-ethyl groups and the benzyl group of **21** was achieved by treatment with sodium in liquid ammonia and gave quantitatively the free bisphosphate **7** (Sch. 2).

The same alcohol **20** was engaged in a phosphorylation reaction using the *H*-phosphonate **25**[22] in the presence of pivaloyl chloride[23] followed by oxidation with iodine in a 98 : 2 pyridine/water mixture[24,25] to provide the protected phosphate **24**. Removal of the cyanoethyl and benzyl groups by treatment with sodium in liquid ammonia afforded the free derivative **8** in 98% yield (Sch. 3).

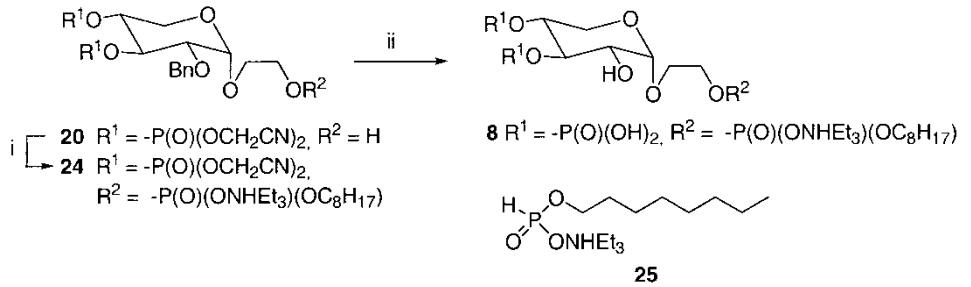
Preliminary studies of the properties of the four phosphates **5–8** were rather disappointing. The biologic testings (vide infra) consisted of measuring calcium liberation from intact cells under physiologic conditions. None of



Scheme 2: Reagents i: **23**, 1H-tetrazole, CH_2Cl_2, rt, then 0°C, tBuOOH; ii: OsO_4, $NaIO_4$, dioxane/H_2O, rt; iii: $NaBH_4$, MeOH; iv: **15**, 1H-tetrazole, CH_2Cl_2, rt, then 0°C, tBuOOH; v: KOH, MeOH 0.5 M, 40°C, vi: from **21** Na, NH_3 liq., dioxane.

these compounds showed significant activity, which means that either these compounds do not cross the cell membrane or if they do, are not released significantly to the free phosphates. Only compound **6** showed an ionophoric effect.

To improve the permeability and the hydrolysis to the free phosphate we turned our attention to more labile phosphate protection.[26] Acyloxy derivatives have been considered to produce prodrugs of charged or polar bioactive compounds.[27] Acetoxymethyl groups have been introduced in the phosphoinositol field to prepare permeant derivatives of $InsP_3$.[20,28] These groups are easily introduced on the free phosphates under smooth reaction conditions. Three different acyloxymethyl groups, octyl, butyl, and ethyl, which differed by the chain length, have been used in this study. The strategy consists of

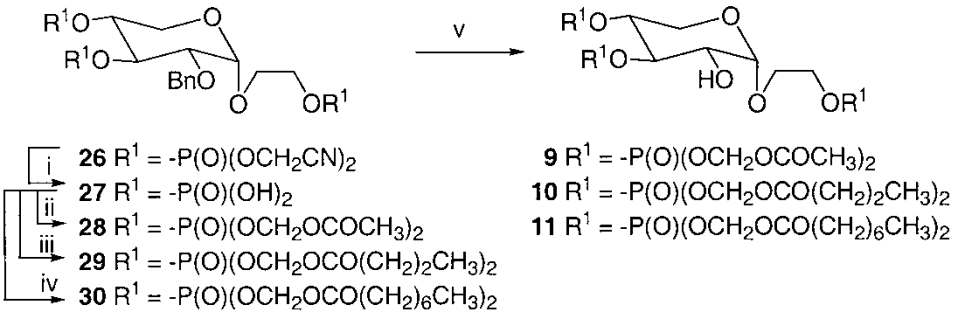


Scheme 3: Reagents i: **BF**, pivaloyl chloride, pyridine, − 20°C, 2 hr then I_2, pyridine/H_2O (98/2 v : v), − 20°C, then rt, 40 min; ii: Na, NH_3 liq., dioxane.

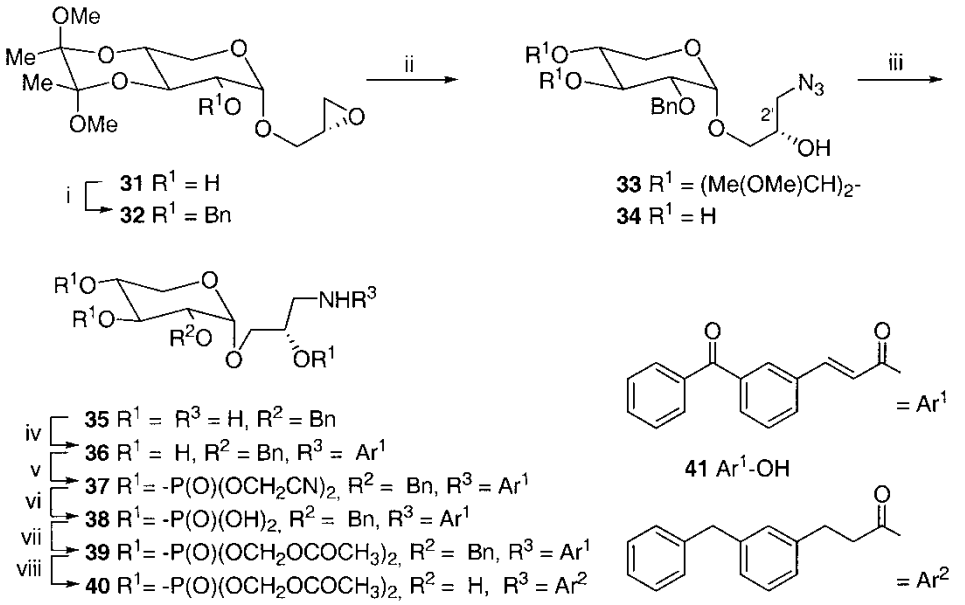
the formation of the free xylose-derived trisphosphate **27** that could be alkylated and then deprotected to give the expected permeant derivatives. Phosphorylation of **12** using the phosphoramidite **23** followed by *t*BuOOH oxidation gave the protected trisphosphate **26** in 58% yield. Removal of the phosphate groups by treatment with potassium hydroxide in methanol gave the free trisphosphate **27** in excellent yield (Sch. 4).

This key intermediate was then treated with iodomethyl octanoate prepared by a three-step sequence from octanoic acid using modification of literature procedures.[29] The fully protected derivative **30** was obtained in modest yield. The same procedure was applied to **27** using iodomethylbutanoate prepared from the known bromoderivative[30] and bromomethyl acetate[31] to provide the protected derivatives **29** and **28**, respectively, in about 20% yield. Final deprotection of the 2-*O*-benzyl group was cleanly achieved by hydrogenolysis (20 bars H_2) in ethylacetate in the presence of palladium on charcoal as the catalyst to provide the permeant derivatives **9, 10**, and **11**.

Given the easy access to the enantiomerically pure xylose trisphosphate **3** and given its high potency, it seemed interesting to use it to construct more complex molecular tools. Photoaffinity labelling is a key method to localize receptors in cells for example. This has been used in the inositol field and developed recently by Prestwich.[32] In most of these studies a photoaffinity ligand is carried by the 1-phosphate group of the inositol derivative. We have developed xylose-based analogs of $InsP_3$, which are equipped with an aglycon bearing the phosphate group and an anchoring point. This has been used for linking the adenine residue in the synthesis of xylophostin. We explored the same approach to construct an affinity labelling ligand of $InsP_3R$, in which the three phosphates would be present in a protected form to make this compound permeant. A derivative of cinnamic acid developed by Prestwich[33] and acetoxy methyl groups were chosen as the photosensitive and protecting groups, respectively (Sch. 5).



Scheme 4: Reagents i: KOH, MeOH 0.5 M, 40°C; ii: bromomethyl acetate, DIEA, CH_3CN, rt; iii: iodomethylbutanoate, DIEA, CH_3CN, rt; iv: iodomethyl octanoate, DIEA, CH_3CN, rt; v: Pd/C 10%, H_2, 20 bars, AcOEt.



Scheme 5: Reagents i: NaH, BnBr, DMF; ii: 1) NaN_3, $MeOCH_2CH_2OH$, H_2O, NH_4Cl, 2) TFA/H_2O; (95/5 v : v), CH_2Cl_2; iii: Pd/C 10%, H_2, MeOH; iv: **41**, BOP, NEt_3, DMF; v: **23**, 1H-tetrazole, CH_2Cl_2, rt, then 0°C, tBuOOH; vi: KOH, MeOH 0.5 M, 40°C; vii: bromomethyl acetate, DIEA, CH_3CN, rt; viii: Pd/C 10%, H_2, 20 bars, AcOEt.

Benzylation of the 2-OH group of known epoxide **31**[34] gave **32** in 91% yield. Treatment of this compound with sodium azide in methoxyethanol in the presence of ammonium chloride gave **33** in 80% yield. Attempts to carry out the phosphorylation steps in the presence of the azide group did not lead to the expected compounds in satisfactory yield and purity. Thus, the azide function was reduced to the corresponding amine by hydrogenolysis using 10% Pd/C in methanol for 4 hr, providing **35** in 97% yield. Coupling of the free amine with the *E* acid **41** in the presence of BOP reagent in DMF gave the corresponding amide **36** in 72% yield. A set of two NMR signals was observed for this compound, which is likely due to two conformational isomers in the p-benzoylcinnamyl system. Phosphitylation of the free OH groups of **36** with **23** and oxidation of the phosphites to the phosphates gave **37** in 42% overall yield. Removal of the phosphate-protecting groups in basic medium gave the free trisphosphate **38** in excellent yield. After ion exchange the diisopropylammonium salts were reacted with 10 equivalents of acetoxymethyl bromide in acetonitrile to provide the protected derivative **39** in 20% yield. All these compounds were also mixtures of conformational isomers as shown by ^1H and ^{13}C NMR. The last step was removal of 2-*O*-benzyl group by hydrogenolysis, which was accompanied by reduction of the cinnamoyl double bond. Treatment of **39** under 15 bars of hydrogen atmosphere for

10 hr in the presence of 10% Pd/C in ethyl acetate was needed to achieve the benzyl group hydrogenolysis. The end product of the reaction **40** was also no longer a mixture of conformers but was also no longer a benzophenone system. Indeed, reduction of the carbonyl group occurred under such drastic hydrogenolysis conditions. This type of reduction has some literature precedents.[35] Despite this unexpected loss of the carbonyl group, this compound has an interest as a permeant analog of $InsP_3$ bearing a lipophilic group near the 1′-*O*-phosphate. It could be used to test a hypothesis about the potent activity of adenophostin, which should be due to lipophilic or stacking interactions between adenosine and an appropriate pocket on $InsP_3R$.[36]

BIOLOGICAL ACTIVITIES

The binding efficiency of the compounds **3**, **8**, and **7** was assayed in competition binding experiments of [^{3}H]$InsP_3$ to rat cerebellar membranes. The analogs inhibited specific [^{3}H]$InsP_3$ binding in the following order of potency, $InsP_3 > \mathbf{3} > \mathbf{8} > \mathbf{7}$ (Fig. 3). This indicates that the protection of the 2′-O-phosphate with one or two alkyl groups is not detrimental to binding to the receptor, confirming the minor role of this phosphate group (corresponding to the 1-*O*-phosphate in $InsP_3$) in calcium liberation. It also indicates that the phosphate esters are not cleaved under the experimental conditions to produce **3**.

Analog-evoked Ca^{2+} Responses in Isolated Hepatocytes

Analogs **6**, **9**, or **40** were added to quin2-loaded rat hepatocyte suspension and calcium responses were studied using spectrofluorimetry. All three analogs induced an increase in quin2 fluorescence, indicating an increase of the

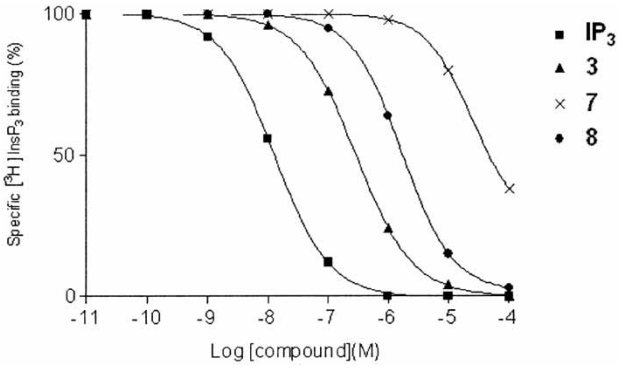


Figure 3: Inhibition of specific (^{3}H)$InsP_3$ binding to rat cerebellar cells $InsP_3R$ by **3**, **7**, and **8**.

cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$). Maximal responses were obtained in the presence of 100 μM of **6**, 100 μM of **9**, and 10 μM of **40**. In the presence of extracellular Ca^{2+} this increase of $[Ca^{2+}]_c$ is sustained, and the following addition of 10 nM of the Ca^{2+} mobilizing agonist vasopressin did not induce further ($[Ca^{2+}]_c$ increase, most likely because quin2 was saturated (Fig. 4). The $[Ca^{2+}]_c$ increase developed within 1 to 2 min after the addition of **9** or **40**. This delay is most likely due to limiting steps like the entry of the analog through the plasma membrane and to the hydrolysis of the ester function by the cell esterases. The Ca^{2+} increase developed much more rapidly after the addition of **6**; this suggests that it induced a nonspecific Ca^{2+} entry through the plasma membrane.

In the absence of extracellular Ca^{2+}, maximal concentrations of **6** (100 μM), **9** (100 μM), or **40** (10 μM) induced a transient $[Ca^{2+}]_c$ increase, which developed within 1 to 2 min after the addition of the analog (Fig. 5). Since there was no Ca^{2+} entry from the extracellular medium, these increases are due to the mobilization of Ca^{2+} accumulated in the intracellular Ca^{2+} store. The addition of 10 nM vasopressin evoked a calcium release through the production of $InsP_3$.[37] This response was reduced when vasopressin was added after **6** or suppressed when added after **9** or **40**. This suggests that the analogs induced the release of Ca^{2+} from the same intracellular store as the one, which is sensitive to $InsP_3$, namely, the endoplasmic reticulum. Accordingly, the previous stimulation of the hepatocytes by vasopressin induced a transient increase of $[Ca^{2+}]_c$, which emptied the $InsP_3$-sensitive Ca^{2+} store consequently, and the following addition of the analogs did not induce further Ca^{2+} release.

We have studied in more detail the effects of the analog **40** on the Ca^{2+} mobilization of quin2-loaded hepatocytes. Figure 5c indicates that the addition of 0.1 μM of **40** to cells incubated in a Ca^{2+}-free medium did not evoke Ca^{2+} release, and the following addition of vasopressin was not precluded. The addition of 1 μM or 10 μM **40** induced progressive increases of

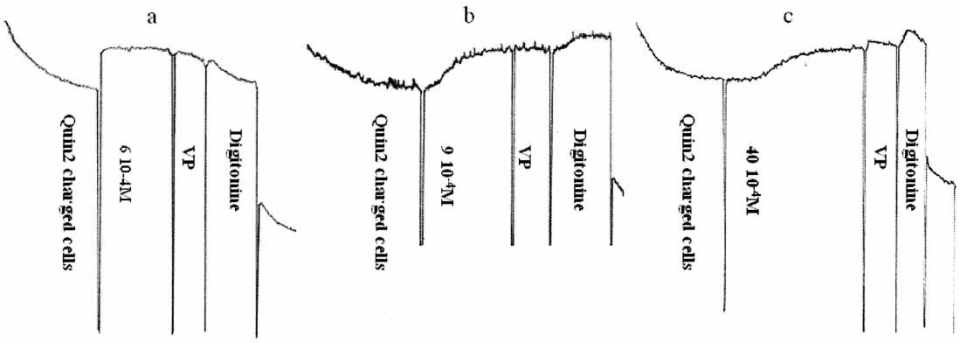


Figure 4: Calcium mobilization by **6**, **9**, and **40** in intact rat hepatocytes in the presence of extracellular calcium (1.8 nM).

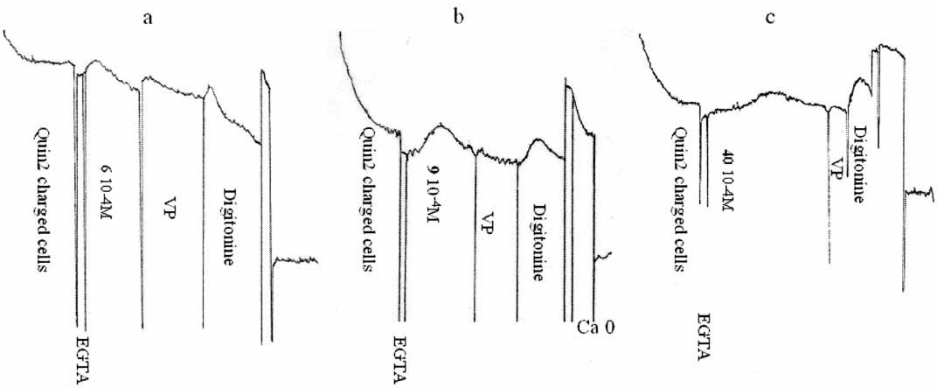


Figure 5: Calcium mobilization by **6**, **9**, and **40** in intact rat hepatocytes in the absence of extracellular calcium.

$[Ca^{2+}]_c$, which led to progressive decreases of the response to vasopressin. This confirms that **40** and vasopressin mobilized Ca^{2+} from the same intracellular Ca^{2+} store. The addition of 6 μM of thapsigargin, an inhibitor of the Ca^{2+} pump that actively accumulates Ca^{2+} in the lumen of the endoplasmic reticulum, evoked transient increases of $[Ca^{2+}]_c$ and precluded the effect of **40** (Fig. 6b). This confirms that **40** mobilized Ca^{2+} from the endoplasmic reticulum. Accordingly, the addition of **40** before the addition of thapsigargin precluded the Ca^{2+} release by the Ca^{2+} pump inhibitor (Fig. 6a). These data clearly indicate that **40** mimics the effect of $InsP_3$ in the hepatocytes.

In the presence of extracellular Ca^{2+}, increasing concentrations of **40** from 0.1 μM to 10 μM induced progressive increase of the $[Ca^{2+}]_c$ (Fig. 4c). This increase was maintained during the presence of the analog, and the further addition of vasopressin induced a reduced increase of $[Ca^{2+}]_c$. The sustained

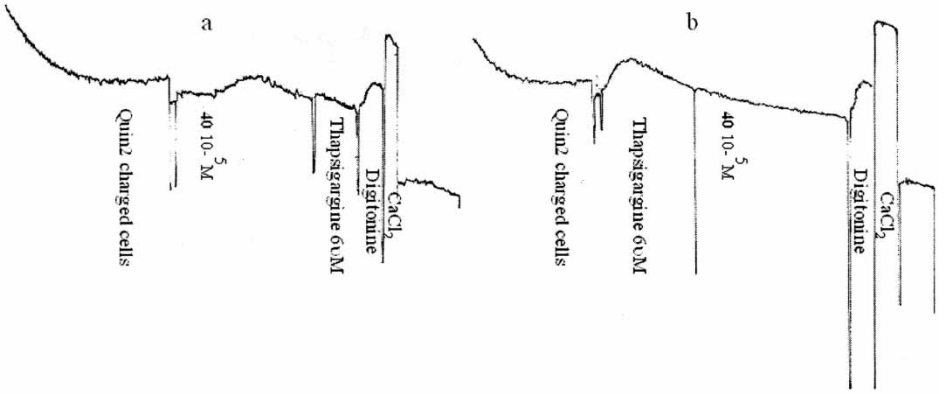


Figure 6: Effect of thapsigargine on calcium mobilization by **40** on intact rat hepatocytes.

response observed in the presence of **40** suggests that the analog stimulated the Ca^{2+} entry through the plasma membrane. This is in agreement with the capacitative hypothesis that assumes that the stimulation of Ca^{2+} influx directly depends on the Ca^{2+} concentration in the lumen of the endoplasmic reticulum of stimulated cells.[38]

CONCLUSION

Different protected analogs of our xylose-based full agonist of $InsP_3$ **3** have been prepared and tested for their ability to bind the $InsP_3$ R or to mobilize calcium from its internal stores. Fully protected analogs of **3** on all phosphates did not mobilize calcium in intact cells. Partially protected derivatives on 2′-O-phosphate do not cross cell membranes but bind to $InsP_3R$, although less potently than **3**. The most efficient compound was the acetoxymethyl derivative **9**, which crossed cell membranes and is able to mobilize calcium, other acyl oxy derivatives being less interesting. Interestingly, compound **40**, primarily designed as a photoaffinity ligand, is also a good permeant agonist of $InsP_3R$. This should be in favor of the existence of a hydrophobic pocket on the receptor that can accommodate a rather large aromatic group like adenosine or the phenyl benzyl group of **40**. All these analogs can serve as biologic tools for further investigation of the calcium-signalling pathway.

EXPERIMENTAL SECTION

Optical rotations were determined at 20°C with a Perkin Elmer model 141 automatic polarimeter. NMR spectra were recorded at 25°C with a Bruker AC250 spectrometer. Chemical shift (δ) are given in ppm relative to the signal for internal tetrametylsilane for ^1H NMR and indirectly to the central line of $CDCl_3$, δ 77.03 for ^{13}C NMR spectra; those in deuterium oxide are reported relative to external 2,3 dimethyl-2-silapentane-5 sulfate (DSS). Infrared spectra were recorded on a Perkin-Elmer Spectrum 1000 FTIR spectrometer. All reactions were monitored by thin layer chromatography on Kieselgel $60F_{254}$ (Merck) with detection by UV light and/or by charring with 15% sulfuric acid in ethanol. Elemental analyses and mass spectra were performed by the Service Central d'Analyses du CNRS at Vernaison (France).

General procedure for ozonolysis followed by a reductive cleavage (Procedure A): A solution of allyl xyloside (1 mmol) in a dichloromethane-methanol mixture (10 mL, 1 : 1 v/v) was cooled to −70°C and ozone was bubbled through the solution (flow of 0.4 L/min) until the disappearance of the starting material. The solution was degassed using a stream of argon for 15 min. Sodium borohydride (4 eq.) was added and the reaction mixture was allowed to warm to rt. After completion of the reaction (TLC monitoring) the

solvents were evaporated under reduced pressure and the residue was diluted with ethyl acetate. The organic layer was washed with 3N hydrochloric acid and water, dried over magnesium sulfate, and concentrated to provide the corresponding 2-hydroxyethyl derivative.

General procedure for phosphorylation reactions (Procedure B): To a solution of the alcohol derivative in dichloromethane (12 mL/mmol) were added under argon 1*H*-tetrazole (4 eq./OH) and the required phosphoramidite (2.5 eq./OH). The reaction mixture was stirred at rt until TLC and ^{31}P NMR spectroscopy analysis showed complete conversion of starting material into the corresponding phosphite derivative. The solution was cooled to 0°C, an excess of *t*BuOOH was added, and the reaction mixture was warmed to rt. After completion of the reaction (TLC monitoring), the medium was diluted with dichloromethane; successively washed with water, saturated aqueous $NaHCO_3$ solution, and water; dried over magnesium sulfate; and concentrated in vacuo. The residue was purified by column chromatography.

General procedure for hydrogenolysis reactions (Procedure C): To a mixture of the phosphorylated derivative in methanol (40 mL/mmol) was added the same amount of 10% Pd/C. The mixture was placed under hydrogen atmosphere at 20 bars. After 14 hr, the catalyst was removed by filtration on a pad of Celite and the solvent evaporated in vacuo. In the case of the presence of benzylic phosphodiester groups, the residue was dissolved in water and aqueous NH_3 was added to reach pH 8.0, and the solution was applied to a column of Bio-Rad Chelex 100 resin (Na^+ form). The compound was eluted with water; fractions containing the desired compound were combined and freeze dried.

General procedure for debenzylation using sodium in liquid ammonia (Procedure D): Liquid ammonia was condensed into a two-necked flask at −78°C and sodium was added. A solution of a benzylated compound in dry dioxane was added and the mixture was stirred for 20 min. The reaction was quenched with ethanol and the medium was warmed to rt. The solution was degassed with nitrogen for 15 min, neutralized to pH 7.00 with DOWEX 50W (H^+) cation exchange resin, and filtered, and the resin was washed with deionized water. After evaporation of the solvents, the residue was applied to a column of Bio-Rad Chelex 100 resin (Na^+ form). The compounds were eluted with water; fractions containing the desired compound were combined and freeze dried.

General procedure for methanolic cleavage of cyanoethyl phosphate (Procedure E): The protected compound was dissolved in a 0.5M potassium hydroxide methanolic solution (2.3 OH equivalent/phosphate group). The solution was heated at 35°C until all the cyanoethyl groups were removed (^{31}P NMR monitoring) and then allowed to cool to rt. The solution was neutralized to pH 7.00 with DOWEX 50W (H^+) cation exchange resin and filtered, and

the resin was washed with deionized water. After evaporation of the solvents the residue was applied to a column of Bio-Rad Chelex 100 resin (Na^+ form). The column was eluted with water; fractions containing the desired compound were combined and freeze dried.

(2-Hydroxyethyl) 2-*O*-benzyl-α-D-xylopyranoside (12). The allyl xyloside **17** (2.22 g, 7.92 mmol) was converted into 2-hydroxyethyl derivative according to procedure A to provide **12** (1.84 g, 6.5 mmol, 82%) as a syrup. R_f 0.65 (CH_2Cl_2/MeOH,9:1); $[\alpha]_D + 65.0$ (c 2.8, $CHCl_3$); IR 3374 (OH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.23–7.36 (m, 5H, Ar); 4.66 (AB, 1H, J_{AB} 11.5 Hz, C*H*$_2$Ph); 4.56 (d, 1H, $J_{1\text{-}2}$ 3.5 Hz, *H*-1); 4.52 (AB, 1H, C*H*$_2$Ph); 4.49 (m, 1H, O*H*); 4.28 (m, 2H, O*H*); 3.83 (dd, 1H, $J_{2\text{-}3}$ 9.6, $J_{3\text{-}4}$ 8.5 Hz, *H*-3); 3.56–3.68 (m, 3H, *H*-1′a, *H*-1′b, *H*-5ax); 3.44–3.49 (m, 1H, *H*-2′a); 3.38–3.42 (m, 1H, *H*-2′b); 3.33–3.37 (m, 1H, *H*-4); 3.29 (dd, 1H, $J_{4\text{-}5eq}$ 4.5, $J_{5eq\text{-}5ax}$ 11.5 Hz, *H*-5eq); 3.23 (dd, 1H, *H*-2); ^{13}C NMR ($CDCl_3$) δ 137.8 (*C* ipso); 128.3; 127.9; 127.8; (5C, *C* Ar); 96.7 (*C*-1); 79.5 (*C*-3); 72.9 (*C*-2); 73.4 (*C*H$_2$Ph); 69.9 (*C*-4); 69.5 (*C*-1′); 61.3 (2C, *C*-5, *C*-2′). Anal. Calcd for $C_{14}H_{20}O_6$: C, 59.10; H, 7.10. Found: C, 59.26; H, 7.31.

Bis octyloxy (diisopropylamino)phosphine (15). A solution of dichloro-(*N*,*N*-diisopropylamino)-phosphine (28.08 g, 40 mmol) in anhydrous diethyl ether (6 mL) was added dropwise under argon to a solution of octyl alcohol (2 eq., 12.59 mL, 80 mmol) and triethylamine (2 eq., 11.15 mL, 80 mmol) in Et_2O (26 mL) at 0°C. The mixture was stirred at rt until ^{31}P NMR indicated complete consumption of the dichlorophosphine (170 ppm) to give the corresponding phosphoramidite (150 ppm) (6 hr). After filtration over Celite and washing with Et_2O (100 mL), the solvent was evaporated to afford **15** (95%, 14.79 g, 38 mmol) as an oil. ^1H NMR ($CDCl_3$) δ 3.53–3.71 (m, 6H, 4C*H*(CH_3)$_2$, *H*-1, *H*-1′); 1.56–1.70 (m, 4H, *H*-2, *H*-2′); 1.25–1.42 (m, 20H, 2(*H*-3, *H*-3′, *H*-4, *H*-4′, *H*-5, *H*-5′, *H*-6, *H*-6′, *H*-7, *H*-7′)); 1.19 (d, 12H, $J_{H\text{-}H}$ 6.8 Hz, 2 CH(C*H*$_3$)$_2$); 0.89 (t, 6H, 6*H*-8); ^{13}C NMR ($CDCl_3$) δ 63.4 (2C, $^2J_{C\text{-}P}$ 16.7 Hz, 2*C*-1); 42.7 (2C, $^2J_{C\text{-}P}$ 10.8 Hz, 2*C*H(CH_3)$_2$); 31.8 (2C, 2*C*-6); 31.3 (2C, $^3J_{C\text{-}P}$ 6.9 Hz, 2*C*-2); 29.3 (4C, 2*C*-4, 2*C*-5); 26.0 (2C, 2*C*-3); 24.6 (4C, $^3J_{C\text{-}P}$ 6.9 Hz, 2CH(*C*H$_3$)$_2$); 22.6 (2C, 2*C*-7); 14.0 (2C, 2*C*-8); ^{31}P NMR ($CDCl_3$) δ 150.0 (s).

[(2-Dioctylphosphonoxy)ethyl] 2-*O*-benzyl-3,4-bis(dioctylphosphate)-α-D-xylopyranoside (13). The alcohol derivative **12** was phosphorylated according to procedure B with the phosphoramidite **15** to give **13** (42%) as a gum. R_f 0.5 (Hex/EtOAc, 3:7); $[\alpha]_D + 17.7$ (c 1.66, $CHCl_3$); IR 1232 (P=O); 1025 (P–O). cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.22–7.41 (m, 5H, Ar); 4.65–4.75 (m, 3H, *H*-1, *H*-3, C*H*$_2$Ph); 4.58 (AB, 1H, J_{AB} 12.1 Hz, C*H*$_2$Ph); 4.26 (m, 1H, *H*-4); 4.13–4.19 (m, 2H, *H*-2′a, *H*-2′b); 3.86–4.12 (m, 13H, 6*H*-1″a, 6*H*-1″b, *H*-5eq); 3.75 (m, 1H, *H*-1′a); 3.54–3.66 (m, 2H, *H*-5ax, *H*-1′b); 3.44 (dd, 1H, $J_{1\text{-}2}$ 3.3, $J_{2\text{-}3}$ 9.3 Hz, *H*-2); 1.58–1.70 (m, 12H, 6*H*-2″a, 6*H*-2″b); 1.15-1.41 (m, 60H, 6(*H*-3″a, *H*-3″b, *H*-4″a, *H*-4″b, *H*-5″a, *H*-5″b,

H-6″a, *H*-6″b, *H*-7″a, *H*-7″b); 0.80–0.92 (m, 18H, 6C*H*$_3$); ^{13}C NMR ($CDCl_3$) δ: 137.5 (*C* ipso); 128.0; 127.5 (5C, *C* Ar); 96.6 (*C*-1); 77.4 (*C*-2); 77.1 (dd, $^2J_{C\text{-}P}$ 6.1, $^3J_{C\text{-}P}$ 7.3 Hz, *C*-3); 73.2 (dd, $^2J_{C\text{-}P}$ 3.8, $^3J_{C\text{-}P}$ 4.8 Hz, *C*-4); 72.4 (*C*H$_2$Ph); 67.5–68.5 (6d, $^2J_{C\text{-}P}$ 6.2 Hz, 6*C*-1″); 66.6 (d, $^3J_{C\text{-}P}$ 7.1 Hz, *C*-1′); 65.5 (d, $^2J_{C\text{-}P}$ 5.7 Hz, *C*-2′); 59.2 (*C*-5); 31.9 (6*C*-6″); 29.9 (d, $^3J_{C\text{-}P}$ 6.7 Hz, 6*C*-2″); 28.8 (6*C*-4″, 6*C*-5″); 25.1 (6*C*-3″); 22.2 (6*C*-7″); 13.7 (6*C*-8″); ^{31}P NMR ($CDCl_3$) δ: 0.55 (s, C-2′O*P*); −0.27 (s, 2P, C-3O*P*, C-4O*P*). Anal. Calcd for $C_{62}H_{119}O_{15}P_3$: C, 62.18; H, 10.02; P, 7.76. Found: C, 62.32; H, 10.21; P, 7.62.

[(2-Dioctylphosphonoxy)ethyl]-3,4-bis(dioctylphosphate)-α-D-xylopyranoside (5). Tris(dioctyl)phosphate **13** (150 mg, 0.125 mmol) was hydrogenated according to procedure C to yield **5** (99%, 137 mg, 0.124 mmol) as a gum. R_f 0.59 (Hex/EtOAc,1:9); $[\alpha]_D + 24.0$ (c 1.6, $CHCl_3$); IR 1232 (P=O); 1026(P-O). cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.87 (d, 1H, $J_{1\text{-}2}$ 3.6 Hz, *H*-1); 4.51 (m, 1H, $J_{2\text{-}3}$ 9.4, $J_{3\text{-}4}$ 9.3, $^3J_{H\text{-}P}$ 8.1 Hz, *H*-3); 3.82–4.40 (m, 18H, *H*-4, *H*-5eq, *H*-1′a, *H*-1′b, *H*-2′a, *H*-2′b, 6*H*-1″a, 6*H*-1″b); 3.54–3.78 (m, 2H, *H*-2, *H*-5ax); 1.53–1.83 (m, 12H, 6*H*-2″a, 6*H*-2″b); 1.17–1.47 (m, 60H, 6(*H*-3″a, *H*-3″b, *H*-4″a, *H*-4″b, *H*-5″a, *H*-5″b, *H*-6″a, *H*-6″b, *H*-7″a, *H*-7″b)); 0.77–0.98 (m, 18H, 6C*H*$_3$); ^{13}C NMR ($CDCl_3$) δ 99.0 (*C*-1); 79.0 (dd, $^2J_{C\text{-}P}$ 5.7, $^3J_{C\text{-}P}$ 7.6 Hz, *C*-3); 73.0 (dd, $^2J_{C\text{-}P}$ 5.2, $^3J_{C\text{-}P}$ 5.7 Hz, *C*-4); 71.4 (*C*-2); 67.5–68.5 (6d, 6C, $^2J_{C\text{-}P}$ 6.2 Hz, 6*C*-1″); 67.5 (d, $^3J_{C\text{-}P}$ 6.2 Hz, *C*-1′); 66.1 (d, 1C, $^2J_{C\text{-}P}$ 5.7 Hz, *C*-2′); 59.9 (*C*-5); 31.9 (s, 6C, 6*C*-6″); 30.5 (d, $^3J_{C\text{-}P}$ 5.9 Hz, 6*C*-2″); 29.3 (s, 12C, 6*C*-4″, 6*C*-5″); 25.6 (s, 6C, 6*C*-3″); 22.8 (s, 6C, 6*C*-7″); 14.2 (s, 6C, 6*C*-8″); ^{31}P NMR ($CDCl_3$) δ: 1.09; 0.57; −0.21 (3s, 3P); ESI-MS (positive mode): calcd for $C_{55}H_{113}O_{15}P_3$ *m/z*: 1108 $[M + H]^+$; Anal. Calcd for $C_{55}H_{113}O_{15}P_3$: C, 59.64; H, 10.20; P, 8.38. Found: C, 59.44; H, 10.19; P, 8.36.

(Benzyloxy)(octyloxy)(diisopropylamino)phosphine (16). Benzyloxy (diisopropylamino)phosphine (17.34 g, 51.23 mmol) and 1*H*-tetrazole (1 eq., 3.58 g, 51.23 mmol) were dissolved in dichloromethane (80 mL) under argon. After stirring for 30 min, a solution of 1-octanol (0.95 eq., 7.66 mL, 51.2 mmol) in CH_2Cl_2 (55 mL) was added dropwise over 45 min. The mixture was stirred for 2 hr, when ^{31}P NMR indicated complete consumption of the starting phosphine (126.7 ppm) to give a product with a signal at 147.5 ppm. The medium was diluted with CH_2Cl_2 (600 mL) and washed with saturated aqueous $NaHCO_3$ solution (3 × 50 mL) and water. The organic layer was dried over Na_2SO_4 and concentrated in vacuo, and the residue was purified by column chromatography on silica gel deactivated with 5% of triethylamine to afford **16** (61%, 11.48 g, 31.25 mmol) as a gum. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.18–7.44 (m, 5H, *H* Ar); 4.58–4.95 (2dd, 2H, J_{AB} 12.6, $^3J_{H\text{-}P}$ 8.8 Hz, C*H*$_2$Ph); 3.54–3.90 (m, 4H, 2C*H*(CH$_3$)$_2$, *H*-1, *H*-1′); 1.55–1.71 (m, 2H, *H*-2, *H*-2′); 1.27–1.50 (m, 10H, *H*-3, *H*-3′, *H*-4, *H*-4′, *H*-5, *H*-5′, *H*-6, *H*-6′, *H*-7, *H*-7′); 1.23 (d, 6H, $J_{H\text{-}H}$ 1.5 Hz, CH(C*H*$_3$)$_2$); 1.20 (d, 6H, $J_{H\text{-}H}$ 2.2 Hz, CH(C*H*$_3$)$_2$); 0.92 (t, 3H, *H*-8, *H*-8′, *H*-8″); ^{13}CNMR ($CDCl_3$) δ 136.8 (*C*ipso); 127.8; 126.8; 126.6

(3s, 5C, *C*H Ar); 64.8 (d, $^{2}J_{C\text{-}P}$ 18.3 Hz, *C*H_2Ph or *C*-1); 63.4 (d, $^{2}J_{C\text{-}P}$ 17.1 Hz, *C*H_2Ph or *C*-1); 42.6 (d, $^{2}J_{C\text{-}P}$ 13.4 Hz, *C*H(CH_3)$_2$); 31.5 (*C*-6); 31.0 (d, $^{3}J_{C\text{-}P}$ 7.3 Hz, *C*-2); 30.9 (2C, *C*-4, *C*-5); 25.6 (*C*-3); 24.2 (d, 4C, $^{3}J_{C\text{-}P}$ 6.1 Hz, 2CH(*C*H_3)$_2$); 22.3 (*C*-7); 13.8 (*C*-8); ^{31}PNMR δ 147.5 (s).

[(2-Benzyloctylphosphonoxy)ethyl] 2-*O*-benzyl-3,4-bis(benzyl-octylphosphate) α-D-xylopyranoside (14). Compound **12** was phosphorylated according to procedure B with the phosphoramidite **16** to give the corresponding compound **14** (52%) as a gum after purification by column chromatography (Hex/EtOAc 6 : 4 → EtOAc). R_f 0.79 (Hex/EtOAc,2 : 8); $[\alpha]_D$ + 18.8 (c 0.85, $CHCl_3$); IR 1272 (P═O); 1019 (P-O) cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.13–7.43 (m, 5H, *H* Ar); 4.88–5.19 (m, 6H, 3POC*H*$_2$Ph); 4.47–4.87 (m, 4H, *H*-1, *H*-3, C*H*$_2$Ph); 4.33 (m, *H*-4); 4.06–4.21 (m, 2H, *H*-2′a, *H*-2′b); 3.81–4.05 (m, 7H, 6*H*-1″, *H*-5a); 3.73 (ddd, 1H, *H*-1′a); 3.50–3.67 (m, 2H, *H*-5b, *H*-1′b); 3.44 (dd, 1H, $J_{1\text{-}2}$ 2.9, $J_{2\text{-}3}$ 9.5 Hz, *H*-2); 1.48–1.71 (m, 6H, 3(2*H*-2″)); 0.85–1.30 (m, 30H, 3(2*H*-3″, 2*H*-4″, 2*H*-5″, 2*H*-6″, 2*H*-7″)); 0.79–0.99 (m, 9H, 3(2*H*-8″)); ^{13}CNMR ($CDCl_3$) δ 137.5 (*C*ipso); 135.7; 135.6 (2s, 3C, *C*ipso); 128.2; 128.1; 127.8; 127.6; 127.4; 127.2 (20C, *C*H Ar); 96.5 (*C*-1); 77.4 (*C*-2); 77.0 (dd, $^{3}J_{C\text{-}P}$ 6.1, $^{4}J_{C\text{-}P}$ 1.1 Hz, *C*-3); 73.4 (m, *C*-4); 72.5 (*C*H$_2$Ph); 69.3 (d, 1C, $^{2}J_{C\text{-}P}$ 4.9 Hz, PO*C*H$_2$Ph); 68.6–69.0 (2d, 2C, 2PO*C*H$_2$Ph); 68.1 (d, 1C, $^{2}J_{C\text{-}P}$ 6.1 Hz, C-1″); 67.7–68.0 (2d, 2C, 2C-1″); 66.6 (d, 1C, $^{3}J_{C\text{-}P}$ 7.3 Hz, *C*-1′); 65.6 (d, 1C, $^{2}J_{C\text{-}P}$ 6.1 Hz, *C*-2′); 59.2 (*C*-5); 31.4 (*C*-6″); 29.9 (d, $^{3}J_{C\text{-}P}$ 6.1 Hz, *C*-2″); 28.8 (2C, *C*-4″, *C*-5″); 25.0 (*C*-3″); 22.3 (*C*-7″); 13.7 (*C*-8″); ^{31}PNMR ($CDCl_3$) δ 0.48 (s, 1P, $C_{2'}$O*P*); 0.28 (s, 2P, C_3O*P*, C_4O*P*); FAB-MS (positive mode): calcd for $C_{59}H_{89}O_{15}P_3$ *m*/*z*: 1132 $[M + H]^+$. Anal. Calcd for $C_{59}H_{89}O_{15}P_3$: C, 62.68; H, 7.87; P, 8.21. Found: C, 62.74; H, 7.84; P, 8.25.

[(2-Benzyloctylphosphonoxy)ethyl] 3,4-bis(benzyl-octylphosphate) α-D-xylopyranoside (6). The title compound was obtained as a white powder in 99% yield by hydrogenolysis of **14** according to procedure C. $[\alpha]_D$ +12.0 (c 0.5, H_2O); IR 1230(P═O); 1105(P-O) cm^{-1}; ^{1}H NMR (250 MHz, D_20) δ4.97 (d, 1H, $J_{1\text{-}2}$ 3.0 Hz, *H*-1); 4.27 (dd, 1H, $J_{2\text{-}3}$ 9.5, $J_{3\text{-}4}$ 9.3, $^{3}J_{H\text{-}P}$ 8.0 Hz, *H*-3); 3.57–4.18 (m, 14H, *H*-2, *H*-4, *H*-5a, *H*-5b, *H*-1′a, *H*-1′b, *H*-2′a, *H*-2′b, 6*H*-1″); 1.57–1.78 (m, 6H, 3(2*H*-2″)); 1.23–1.39 (m, 30H, 3(2*H*-3″, 2*H*-4″, 2*H*-5″, 2*H*-6″, 2*H*-7″)); 0.83–1.06 (m, 9H, 3(3*H*-8″)); ^{13}CNMR (D_2O) δ 100.5 (*C*-1); 78.5 (dd, *C*-3); 73.8 (*C*-2); 73.5 (dd, *C*-4); 70.1 (d, 1C, $^{3}J_{C\text{-}P}$ 7.6 Hz, *C*-1′); 69.1 (d, 1C, $^{2}J_{C\text{-}P}$ 4.8 Hz, C-1″); 68.6-68.7 (2d, 2C, 2C-1″); 66.7 (d, 1C, $^{2}J_{C\text{-}P}$ 4.3 Hz, *C*-2′); 63.2 (*C*-5); 34.4 (3C, *C*-6″); 33.0 (d, 3C, $^{3}J_{C\text{-}P}$ 6.0 Hz, *C*-2″); 31.9 (6C, *C*-4″, *C*-5″); 28.1 (3C, *C*-3″); 25.1 (3C, *C*-7″); 16.3 (3C, *C*-8″); ^{31}PNMR (D_2O) δ 4.34; 4.29; 3.79 (3s, 3P). ESI-MS (positive mode): Calcd for $C_{31}H_{62}O_{15}P_3Na_3$ *m*/*z*: 859 $[M + Na]^+$; 837 $[M + H]^+$; 815 $[M\text{-}Na + 2H]^+$; 793 $[(M\text{-}2Na + 3H]^+$.

Allyl 2-*O*-benzyl-3,4-bis[(2-cyanoethyl) phosphate] α-D-xylopyranoside (18). Compound **17** was phosphorylated with **23** according to the procedure B

to afford **18** (70%) as a gum. R_f 0.38 (EtOAc/MeOH,97:3); $[\alpha]_D + 31.1$ (c 0.8, CH_2Cl_2); IR 2254 (CN); 1256 (P=O); 1040 (P-O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30–7.45 (m, 5H, *H* Ar); 5.93 (m, 1H, *H*-7); 5.38 (dd, 1H, $J_{7\text{-}8'}$ 17.1, $J_{8\text{-}8'}$ 1.5 Hz, *H*-8′); 5.27 (dd, 1H, $J_{7\text{-}8}$ 11.5 Hz, *H*-8); 4.92 (d, 1H, $J_{1\text{-}2}$ 3.5 Hz, *H*-1); 4.78 (m, 1H, $J_{3\text{-}4}$ 9.3, $^3J_{P\text{-}H}$ 8.8 Hz, *H*-3); 4.64 (AB, 2H, J_{AB} 11.4 Hz, CH_2Ph); 4.03–4.50 (m, 10H, *H*-4, *H*-6, 4CH_2CH_2CN); 3.92–4.02 (m, 2H, *H*-5, *H*-6′); 3.72 (dd, 1H, $J_{4\text{-}5}$ 10.1, $J_{5\text{-}5'}$ 10.8 Hz, *H*-5′); 3.59 (dd, 1H, $J_{2\text{-}3}$ 9.8 Hz, *H*-2); 2.79–2.88 (m, 4H, CH_2CH_2CN); 2.70–2.77 (m, 2H, CH_2CH_2CN); 2.47–2.57 (m, 1H, CH_2CH_2CN); 2.33–2.42 (m, 1H, CH_2CH_2CN); ^{13}CNMR ($CDCl_3$) δ 137.2 (s, *C*ipso); 133.1 (*C*-7); 128.6; 128.3; 128.2 (3s, 5C, *C*H Ar); 118.6 (*C*-8); 117.0; 116.8; 116.7 (3s, *C*N); 94.8 (*C*-1); 78.2 (m, *C*-3); 77.5 (*C*-2); 74.4 (m, *C*-4); 72.5 (*C*H_2Ph); 68.6 (*C*-6); 62.1–63.3 (4d, 4C, $^2J_{C\text{-}P}$ 4.9 Hz, 4*C*H_2*C*H_2CN); 59.0 (*C*-5); 19.3–19.5 (4d, 4C, 4CH_2*C*H_2CN); ^{31}PNMR ($CDCl_3$) δ −1.86; −1.94 (2s, 2P). Anal. Calcd for $C_{27}H_{34}O_{11}N_4P_2$: C, 49.72; H, 5.21; N, 8.58; P, 9.49. Found: C, 49.79; H, 5.24; N, 8.43; P, 9.54.

(Oxoethanyl) 2-*O*-benzyl-3,4-bis[(2-cyanoethyl) phosphate] α-D-xylopyranoside (19). Compound **18** (1.86 g, 2.85 mmol) was dissolved in dioxane (25 mL) and water was added (25 mL). To this solution were added dropwise 2.65 mL of osmium tetroxide (2.5% in *tert*-butanol) and sodium periodate (5 eq., 3.05 g, 14.2 mmol). After stirring for 2 hr 30 min, TLC analysis showed complete consumption of the starting material and the solution was filtered over Celite. The mixture was diluted with CH_2Cl_2 (300 mL) and the organic layer was washed twice with water (50 mL), dried over $MgSO_4$, and concentrated in vacuo to afford **19** (99%, 1.86 g, 2.84 mmol) as a yellow gum. R_f 0.45 (EtOAc/MeOH,9 : 1); $[\alpha]_D + 32.2$ (c 1.3, $CHCl_3$); IR 2255 (CN); 1734 (C=O); 1280(P=O); 1039(P-O) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 9.70 (s, 1H, CO*H*); 7.22–7.48 (m, 5H, *H* Ar); 4.92 (d, 1H, $J_{1\text{-}2}$ 3.9 Hz, *H*-1); 4.60–4.85 (m, 2H, CH_2Ph, *H*-3); 4.64 (AB, 1H, J_{AB} 10.7 Hz, CH_2Ph); 3.95–4.50 (m, 11H, *H*-4, *H*-5a, *H*-1′a, 4CH_2CH_2CN); 3.45–3.84 (m, 3H, *H*-2, *H*-5b, *H*-1′b); 2.31–2.89 (m, 8H, 4CH_2CH_2CN), ^{13}C NMR ($CDCl_3$) δ: 198.3 (*C*=O); 137.2 (Cipso); 128.7; 128.5 (2s, 5C, *C*H Ar); 116.9 (s, 4C, *C*N); 96.2 (*C*-1); 77.6 (m, *C*-3); 76.6 (*C*-2); 74.1 (dd, $^2J_{C\text{-}P}$ 5.7, $^3J_{C\text{-}P}$ 2.8 Hz, *C*-4); 72.9 (*C*H_2Ph); 72.3 (*C*-1′); 62.3–63.4 (4d, 4C, $^2J_{C\text{-}P}$ 5.2 Hz, 4*C*H_2*C*H_2CN); 59.4 (*C*-5); 19.5–19.8 (4d, 4C, 4CH_2*C*H_2CN); ^{31}P NMR ($CDCl_3$) δ −1.96 (s, 2P). Anal. Calcd for $C_{26}H_{32}O_{12}N_4P_2$: C, 47.74; H, 4.89; N, 8.56; P, 9.46. Found: C, 47.70; H, 4.94; N, 8.53; P, 9.47.

(2-Hydroxyethyl) 2-*O*-benzyl-3,4-bis[(2-cyanoethyl) phosphate] α-D-xylopyranoside (20). To a solution of **19** (1.86 g, 2.79 mmol) in methanol (88 mL) was added $NaBH_4$ (2 eq., 224 mg, 5.58 mmol). After stirring for 3 min, the solution was neutralized with 3 N HCl, the solvent was evaporated, and the residue was diluted in CH_2Cl_2 (30 mL). The organic layer was washed with water (2 × 40 mL), dried over $MgSO_4$, and concentrated in vacuo. The

crude mixture was purified by preparative HPLC (EtOAc/MeOH 96.5 : 3.5) to give **20** (57%, 1.07 g, 1.60 mmol) as a gum. R_f 0.36 (EtOAc/MeOH,9 : 1); $[\alpha]_D + 30.1$ (c 1.8, $CHCl_3$); IR 2255 (CN); 1276 (P═O); 1037 (P-O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.32–7.47 (m, 5H, *H* Ar); 4.80–4.89 (m, 2H, *H*-1, *H*-3); 4.68 (s, 2H, CH_2Ph); 4.14–4.50 (m, 9H, *H*-4, 4CH_2CH_2CN); 4.00 (dd, 1H, $J_{4\text{-}5eq}$ 6.0, $J_{5eq\text{-}5ax}$ 11.1 Hz, *H*-5eq); 3.73–3.86 (m, 4H, *H*-5ax, *H*-1′a, *H*-2′a, *H*-2′b); 3.59 (dd, 1H, $J_{1\text{-}2}$ 3.3, $J_{2\text{-}3}$ 9.8 Hz, *H*-2); 3.49 (m, 1H, *H*-1′b); 2.78–2.91 (m, 4H, 2CH_2CH_2CN); 2.67–2.74 (m, 2H, CH_2CH_2CN); 2.48–2.62 (m, 2H, CH_2CH_2CN); 1.90 (s, 1H, O*H*), ^{13}C NMR ($CDCl_3$) δ: 137.1 (s, *C*ipso); 128.6; 128.5; 128.3 (3s, 5C, *C*H Ar); 116.8 (s, 4C, *C*N); 96.3 (*C*-1); 78.1 (m, *C*-3); 77.7 (*C*-2); 74.4 (dd, $^2J_{C\text{-}P}$ 5.7, $^3J_{C\text{-}P}$ 2.8 Hz, *C*-4); 72.8 (*C*H_2Ph); 70.1 (*C*-1′); 62.2–63.3 (4d, 4C, $^2J_{C\text{-}P}$ 5.2 Hz, 4*C*H_2CH_2CN); 61.2 (*C*-2′); 59.0 (*C*-5); 19.3–19.5 (4d, 4C, 4CH_2*C*H_2CN)); ^{31}P NMR ($CDCl_3$) δ −3.31; −3.09 (2s, 2P). Anal. Calcd for $C_{26}H_{34}O_{12}N_4P_2$: C, 47.59; H, 5.18; N, 8.53; P, 9.44. Found: C, 47.52; H, 5.24; N, 8.62; P, 9.45.

[(2-Dioctylphosphonoxy)ethyl] 2-*O*-benzyl-3,4-bis[(2-cyanoethyl)phosphate] α-D-xylopyranoside (21). The alcohol **20** was phosphorylated with the phosphoramidite **15** according to the procedure B. The residue was purified by column chromatography (EtOAc/MeOH 98 : 2 → 96 : 4) to give **21** (60%) as a gum. R_f 0.5 (EtOAc/MeOH,95 : 5); $[\alpha]_D + 29.8$ (c 1.56, CH_2Cl_2); IR 2255 (CN); 1277(P═O); 1037 (P-O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.22–7.42 (m, 5H, *H* Ar); 4.92 (d, 1H, $J_{1\text{-}2}$ 3.6 Hz, *H*-1); 4.63–4.80 (m, 2H, CH_2Ph, *H*-3); 4.56 (AB, 1H, J_{AB} 11 Hz, CH_2Ph); 3.89–4.48 (m, 16H, 4*H*-1″, *H*-4, 4CH_2CH_2CN, *H*-5a, *H*-2′a, *H*-2′b); 3.82 (m, 1H, *H*-1′a); 3.61–3.76 (m, 4H, *H*-5ax, *H*-1′b); 3.55 (dd, 1H, $J_{2\text{-}3}$ 9.8 Hz, *H*-2); 2.64–2.86 (m, 6H, CH_2CH_2CN); 2.24–2.55 (m, 2H, CH_2CH_2CN); 1.51–1.72 (m, 4H, 2*H*-2″a, 2*H*-2″b); 1.12–1.42 (m, 20H, 2(*H*-3″a, *H*-3″b, *H*-4″a, *H*-4″b, *H*-5″a, *H*-5″b, *H*-6″a, *H*-6″b, *H*-7″a, *H*-7″b)); 0.77–0.93 (m, 6H, 6*H*-8″); ^{13}C NMR ($CDCl_3$) δ 137.4 (*C*ipso); 128.8; 128.4; (2s, 5C, *C*H Ar); 116.9; 116.8 (2s, 4C, *C*N); 96.4 (*C*-1); 78.1 (m, *C*-3); 77.7 (*C*-2); 74.4 (m, *C*-4); 72.5 (*C*H_2Ph); 68.2 (m, 2C, 2*C*-1″); 67.2 (d, $^3J_{C\text{-}P}$ 7.3 Hz, *C*-1′); 66.2 (d, 1C, $^2J_{C\text{-}P}$ 5.7 Hz, *C*-2′); 63.2 (d, 1C, $^2J_{C\text{-}P}$ 6.2 Hz, *C*H_2CH_2CN); 63.0 (m, 2C, 2*C*H_2CH_2CN); 62.5 (d, 1C, $^2J_{C\text{-}P}$ 4.9 Hz, *C*H_2CH_2CN); 59.3 (*C*-5); 31.9 (s, 2C, 2*C*-6″); 30.5 (d, 2C, $^3J_{C\text{-}P}$ 7.3 Hz, 2*C*-2″); 30.4; 29.3 (2s, 4C, 2*C*-4″, 2*C*-5″); 25.6 (s, 2*C*-3″); 22.8 (s, 2*C*-7″); 19.4-19.9 (4d, 4C, 4CH_2*C*H_2CN); 14.3 (s, 6*C*-8″); ^{31}P NMR ($CDCl_3$) δ: 0.49; −1.92; −1.95 (3s, 3P). Anal. Calcd for $C_{42}H_{67}O_{15}N_4P_3$: C, 52.49; H, 6.97; N, 5.82; P, 9.66. Found: C, 52.20; H, 7.07; N, 5.77; P, 9.63.

[(2-Dioctylphosphonoxy)ethyl] 3,4-bis[(2-cyanoethyl)phosphate] α-D-xylopyranoside (22). Compound **21** (280 mg, 0.291 mmol) was dissolved in a solution of KOH (0.5 M in methanol, 4.85 mL) and heated at 40°C for 3 hr. The reaction mixture was then cooled to rt and neutralized to pH 7 with Dowex 50W (H^+) cation-exchange resin. The resin was filtered off and

washed twice with methanol (10 mL). The filtrate was concentrated to dryness; the residue was dissolved in deionized water (3 mL) and applied to a column of Bio-Rad Chelex 100 resin (Na^+ form). The compound was eluted with deionized water; fractions containing compound **22** were combined and freeze dried to give **22** (99%, 241 mg, 0.288 mmol) as a white powder. $[\alpha]_D + 4.5$ (c 1.60, H_2O); IR (KBr) 1263 (P═O); 1037 (P-O) cm^{-1}; ^1H NMR (D_2O; 250 MHz) δ 7.17–7.53 (m, 5H, *H* Ar); 4.92 (AB, 1H, J_{AB} 12 Hz, C*H*$_2$Ph); 4.73 (d, 1H, $J_{1\text{-}2}$ 3.4 Hz, *H*-1); 4.67 (AB, 1H, C*H*$_2$Ph); 4.42 (ddd, 1H, $J_{2\text{-}3}$ 9.5, $J_{3\text{-}4}$ 9.6, $^3J_{H\text{-}P}$ 7.3 Hz, *H*-3); 3.75–4.33 (m, 9H, 4*H*-1″, *H*-4, *H*-5a, *H*-1′a, *H*-2′a, *H*-2′b); 3.44–3.71 (m, 3H, *H*-2, *H*-5b, *H*-1′b); 1.50–1.73 (m, 4H, 2*H*-2″a, 2*H*-2″b); 1.19–1.47 (m, 20H, 2(*H*-3″a, *H*-3″b, *H*-4″a, *H*-4″b, *H*-5″a, *H*-5″b, *H*-6″a, *H*-6″b, *H*-7″a, *H*-7″b)); 0.81–1.06 (m, 6H, 6*H*-8″); ^{13}C NMR (D_2O) δ 139.3 (*C*ipso); 128.9; 128.8 (2s, 5C, *C*H Ar); 99.9 (*C*-1); 80.7 (*C*-2); 78.4 (dd, *C*-3); 75.8 (*C*H$_2$Ph); 74.1 (dd, *C*-4); 70.3–71.3 (2d, 2C, $^2J_{C\text{-}P}$ 6.7 Hz, 2*C*-1″); 69.1 (d, 1C, $^2J_{C\text{-}P}$ 5.7 Hz, *C*-2′); 68.8 (d, $^3J_{C\text{-}P}$ 7.6 Hz, *C*-1′); 63.4 (*C*-5); 34.3 (s, 2C, 2*C*-6″); 32.2–32.6 (m, 2C, 2*C*-2″); 31.8; 31.6 (2s, 4C, 2*C*-4″, 2*C*-5″); 28.0 (s, 2*C*-3″); 25.1 (s, 2*C*-7″); 16.2 (s, 6*C*-8″); ^{31}P NMR (D_2O) δ 6.58; 4.13 (2s, 2P, 2O*P*O$_3$); −3.36 (s, 1P, C2′O*P*). ESI-MS (negative mode): Calcd for $C_{30}H_{55}O_{15}P_3$ *m*/*z*: 747 [M-H]$^-$; 769 [M-2H + Na]$^-$.

[(2-Dioctylphosphonoxy)ethyl] 3,4-bis(dihydrogen-phosphate) α-D-xylopyranoside tetrasodium salt (7). Compound **7** was obtained in 99% yield as a white powder by treatment of **21** according to procedure D. $[\alpha]_D + 3.3$ (c 0.45, H_2O); ^1H NMR (400 MHz, D_20) δ4.84 (d, 1H, $J_{1\text{-}2}$ 3.5 Hz, *H*-1); 4.15–4.24 (m, 2H, *H*-2′b, *H*-2′a); 4.01–4.14 (m, 5H, *H*-3, 4*H*-1″); 3.84–3.95 (m, 3H, *H*-4, *H*-5 eq, *H*-1′a); 3.62–3.78 (m, 3H, *H*-2, *H*-5ax, *H*-1′b); 1.53–1.71 (m, 4H, 2*H*-2″a, 2*H*-2″b); 1.07–1.43 (m, 20H, 2(*H*-3″a, *H*-3″b, *H*-4″a, *H*-4″b, *H*-5″a, *H*-5″b, *H*-6″a, *H*-6″b, *H*-7″a, *H*-7″b)); 0.71–0.89 (m, 6H, 6*H*-8″); ^{13}CNMR (D_20) δ 101.3 (*C*-1); 76.0 (m, *C*-3); 73.8 (*C*-2); 74.1 (m, *C*-4); 71.3 (d, 2C, $^2J_{C\text{-}P}$ 6.2 Hz, 2*C*-1″); 68.9–69.5 (m, 2C, *C*-1′, *C*-2′); 64.6 (*C*-5); 34.4 (s, 2C, 2*C*-6″); 32.7 (d, 2C, $^3J_{C\text{-}P}$ 6.7 Hz, 2*C*-2″); 31.8; 31.7 (4C, 2*C*-4″, 2*C*-5″); 28.1 (2C, 2*C*-3″); 25.3 (2C, 2*C*-7″); 16.6 (2C, 2*C*-8″); ^{31}PNMR (D_20) δ 7.96; 7.31 (2s, 2P, 2O*P*O$_3$Na$_2$); -3.18 (s, 1P, C2′O*P*); ESI-MS (negative mode): Calcd for $C_{23}H_{49}O_{15}P_3$ *m*/*z*: 657 [M-H]$^-$; 679 [M-2H + Na]$^-$; 701 [M-3H + 2Na]$^-$.

Octyl phosphonate triethylamonium salt (25). To a solution of 1-octanol (651 mg, 5 mmol) and NEt_3 (1.39 mL, 10 mmol) in dioxane (7 mL) was added under argon a dioxane solution (5 mL) of 2-chloro-1,3,2-benzodioxaphosphorin-4-one (1.01 g, 5 mmol). After 30 min at rt, ^{31}P NMR indicated complete consumption of the starting chlorophosphite (149.6 ppm) to give a product with a signal at 125.6 ppm; water (1 mL) was then added to the medium. The solvents were evaporated to dryness and the residue was coevaporated twice with toluene. Compound **25** (812 mg, 2.73 mmol) was as a white solid in 55% yield after purification by column chromatography (AcOEt → AcOEt/MeOH

85 : 15). ^{1}H NMR (250 MHz, $CDCl_3$): δ 6.78 (d, 1H, $J_{P\text{-}H}$ 628 Hz, P-*H*); 3.85 (m, 2H, 2*H*-1); 3.03 (q, 6H, J 7.1 Hz, $N(CH_2CH_3)_3$); 1.53–1.67 (m, 2H, 2*H*-2); 1.13–1.40 (m, 19H, 2(*H*-3, *H*-3′, *H*-4, *H*-4′, *H*-5, *H*-5′, *H*-6, *H*-6′, *H*-7, *H*-7′), $N(CH_2CH_3)_3$)); 0.83 (t, 3H, $J_{H\text{-}H}$ 5.8 Hz, 3*H*-8). ^{13}C NMR ($CDCl_3$): δ 63.8 (d, $^{2}J_{C\text{-}P}$ 409 Hz, *C*-1); 4504 (s, 3C, $N(CH_2CH_3)_3$; 3104 (s, *C*-6); 3003 (d, $^{3}J_{C\text{-}P}$ 703 Hz, *C*-2); 2808 (s, 2C, *C*-4, *C*-5); 25.4 (s, *C*-3); 22.2 (s, *C*-7); 13.8 (s, *C*-8); 8.2 (s, 3C, $N(CH_2CH_3)_3$); ^{31}P NMR ($CDCl_3$): δ 3.51 (s, 1P).

[(2-Octylphosphonoxy)ethyl] 2-*O*-benzyl-3,4-bis[(2-cyanoethyl)phosphate] α-D-xylopyranoside (24). To a solution of xyloside **20** (250 mg, 0.37 mmol) and H-phosphonate monoester **25** (1 eq., 110 mg, 0.37 mmol) in pyridine (5 mL) was added dropwise a solution of pivaloyl chloride (3 eq., 0.13 mL, 1.11 mmol) in pyridine (1 mL) at -20°C for 25 min under argon. After being stirred for 2 hr, a solution of iodine (1.25 eq., 118 mg, 0.46 mmol) in a pyridine/water mixture (0.9 mL, 98 : 2, v/v) was added. The medium was warmed to rt and after 45 min, a solution of sodium sulfite in water (1%) was added until complete discoloration of the medium. The solvents were evaporated in vacuo, the residue was coevaporated three times with toluene and the crude product was purified by column chromatography on silicic acid (EtOAc/MeOH 98 : 2 → 80 : 20) and then on LH20 Sephadex gel ($CHCl_3$/MeOH 1 : 1) to give **24** (40%, 140 mg, 0.15 mmol) as a gum. $[\alpha]_D$ + 23.0 (c 0.98, $CHCl_3$); IR 2251 (CN); 1280 (P═O); 1038 (P-O) cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$/MeOD, 1 : 1) δ 7.07–7.29 (m, 5H, *H* Ar); 4.73 (d, 1H, $J_{1\text{-}2}$ 2.9 Hz, *H*-1); 4.37–4.60 (m, 3H, *H*-3, CH_2Ph); 3.47–4.34 (m, 17H, *H*-4, *H*-5, *H*-5′, *H*-1′a, *H*-1′b, *H*-2′a, *H*-2′b, 2*H*-1″, $4CH_2CH_2CN$); 3.41 (dd, 1H, $J_{2\text{-}3}$ 9.5 Hz, *H*-2); 3.14 (q, 6H, J 7.1 Hz, $N(CH_2CH_3)_3$); 2.50–2.75 (m, 6H, $3CH_2CH_2CN$); 2.17–2.49 (m, 2H, CH_2CH_2CN); 1.32–1.61 (m, 2H, 2*H*-2″); 0.85–1.30 (m, 19H, 2(*H*-3″a, *H*-3″b, *H*-4″a, *H*-4″b, *H*-5″a, *H*-5″b, *H*-6″a, *H*-6″b, *H*-7″a, *H*-7″b), $N(CH_2CH_3)_3$)); 0.56–0.77 (t, 3H, $J_{H\text{-}H}$ 5.8 Hz, 3*H*-8″); ^{13}CNMR($CDCl_3$/MeOD, 1 : 1) δ 136.8 (*C*ipso); 128.0; 127.9; 127.6 (5C, *C*H Ar); 117.6 (s, *C*N); 95.8 (*C*-1); 77.9 (m, *C*-3); 77.1 (*C*-2); 74.1 (m, *C*-4); 72.1 (CH_2Ph); 67.2 (m, *C*-1′); 62.2–63.3 (m, 6C, *C*-1″, *C*-2′, $4CH_2CH_2CN$); 58.6 (*C*-5); 45.8 (s, 3C, $N(CH_2CH_3)_3$); 31.4 (*C*-6″); 30.0 (d, $^{3}J_{C\text{-}P}$ 7.2 Hz, *C*-2″); 29.2; 28.9 (2C, *C*-4″, *C*-5″); 25.4 (*C*-3″); 22.2 (*C*-7″); 13.4 (*C*-8″); 8.0 (3C, $N(CH_2CH_3)_3$); ^{31}PNMR ($CDCl_3$/MeOD, 1 : 1)δ −2.43; −1.78 (2s, $2OPO(OCH_2CH_2CN)_2$); 0.40 (s, $OPORO_2^-$); ESI-MS (negative mode): Calcd for $C_{34}H_{51}O_{15}N_4P_3$ *m*/*z*: 847 $[M\text{-}H]^-$.

[(2-Octylphosphonoxy)ethyl] 3,4-bis(dihydrogen-phosphate) α-D- xylopyranoside pentatriethylamonium salt (8). The title compound was obtained as a white powder from xyloside **24** in 98% yield according to procedure D. $[\alpha]_D$ + 16.1 (c 1.05, H_2O); IR 1232 (P═O); 1100 (P-O) cm^{-1}; ^{1}H NMR (250 MHz, D_2O) δ 4.96 (d, 1H, $J_{1\text{-}2}$ 3 Hz, *H*-1); 4.23 (ddd, 1H, $J_{2\text{-}3}$ 9.4, $J_{3\text{-}4}$ 9.5, $^{3}J_{H\text{-}P}$ 8.0 Hz, *H*-3); 3.62–4.14 (m, 10H, *H*-2, *H*-4, *H*-5a, *H*-5b, *H*-1′a, *H*-1′b, *H*-2′a, *H*-2′b, 2*H*-1″); 1.52–1.75 (m, 2H, 2*H*-2″); 1.18–1.48 (m, 10H,

2(*H*-3″a, *H*-3″b, *H*-4″a, *H*-4″b, *H*-5″a, *H*-5″b, *H*-6″a, *H*-6″b, *H*-7″a, *H*-7″b); 0.80–0.98 (t, 3H, $J_{H\text{-}H}$ 5.8 Hz, 3*H*-8″); ^{13}CNMR (D_2O) δ 99.2 (*C*-1); 75.4 (m, *C*-3); 72.5 (*C*-2); 71.5 (dd, $^2J_{C\text{-}P}$ 5.4 Hz, *C*-4); 68.5 (d, $^3J_{C\text{-}P}$ 7.1 Hz, *C*-1′); 67.6 (d, $^2J_{C\text{-}P}$ 6.2 Hz, *C*-1″); 65.6 (d, $^2J_{C\text{-}P}$ 5.7 Hz, *C*-2′); 63.0 (*C*-5); 32.1 (*C*-6″); 30.9 (d, $^3J_{C\text{-}P}$ 7.2 Hz, *C*-2″); 29.4 (2C, *C*-4″, *C*-5″); 26.1 (*C*-3″); 23.1 (*C*-7″); 14.5 (*C*-8″); ^{31}PNMR (D_20) δ 7.80; 7.20 (2s, 2O*P*O$_3^{2-}$); 4.60 (s, O*P*ORO$_2^-$); ESI-MS (negative mode): Calcd for $C_{15}H_{33}O_{15}P_3$ *m/z*: 545 [M-H]$^-$.

[(2-Dicyanoethylphosphonoxy)ethyl] 2-*O*-benzyl-3,4-bis[(2-cyanoethyl)-phosphate] α-D-xylopyranoside (26). Compound **12** was phosphorylated according to procedure B with the phosphoramidite **23** to give the compound **26** in 58% as a gum after purification by column chromatography (AcOEt/MeOH 98 : 2 → 88 : 12). R_f 0.36 (AcOEt/MeOH,9 : 1); $[\alpha]_D + 26.1$ (c 0.72, $CHCl_3$); IR 2255 (CN); 1279 (P═O); 1036 (P-O) cm^{-1}. ^{1}H NMR (250 MHz $CDCl_3$): δ 7.28–7.43 (m, 5H, *H* Ar); 4.83 (d, 1H, $J_{1\text{-}2}$ 3.6 Hz, *H*-1); 4.76 (ddd, 1H, $J_{2\text{-}3}$ 9.5, $J_{3\text{-}4}$ 9.2, $^3J_{H\text{-}P}$ 7.9 Hz, *H*-3); 4.66 (s, 2H, C*H*$_2$Ph); 4.22–4.52 (m, 15H, *H*-4, *H*-2′a, *H*-2′b, 6 C*H*$_2$CH$_2$CN); 4.01 (dd, 1H, $J_{4\text{-}5eq}$ 6.0, $J_{5eq\text{-}5ax}$ 11.7 Hz, *H*-5eq); 3.89 (m, 1H, *H*-1′a); 3.75 (dd, 1H, $J_{4\text{-}5ax}$ 9.7 Hz, *H*-5ax); 3.53–3.68 (m, 2H, *H*-2, *H*-1′b); 2.33–2.92 (m, 12H, 6 CH$_2$C*H*$_2$CN); ^{13}C NMR ($CDCl_3$): δ 137.0 (s, C_{ipso}); 128.3; 128.0; 127.8; (3s, 5C, *C*H Ar); 116.8; 116.7; 116.6 (3s, 3*C*N); 95.8 (s, *C*-1); 77.6 (dd, $^2J_{C\text{-}P}$ 7.8, $^3J_{C\text{-}P}$ 5.9 Hz, *C*-3); 77.2 (s, *C*-2); 73.7 (dd, $^2J_{C\text{-}P}$ 5.2, $^3J_{C\text{-}P}$ 3.3 Hz, *C*-4); 72.3 (s, *C*H$_2$Ph); 66.7 (d, $^2J_{C\text{-}P}$ 5.7 Hz, *C*-2′); 66.7 (d, $^3J_{C\text{-}P}$ 6.7 Hz, *C*-1′); 62.3-63.3 (6d, 6C, $^2J_{C\text{-}P}$ 5.7 Hz, 6 *C*H$_2$CH$_2$CN); 58.7 (s, *C*-5); 19.0-19.3 (6d, 6C, 6 CH$_2$*C*H$_2$CN); ^{31}P NMR ($CDCl_3$): δ −1.38; −1.48 (2s); −1.92 (s, C-2′O*P*). Anal. Calcd for $C_{32}H_{41}O_{15}N_6P_3$: C, 45.61; H, 4.90; N, 9.97; P, 11.03. Found: C, 45.37; H, 4.85; N, 10.15; P, 10.92.

[2-(Phosphonoxy)ethyl] 2-*O*-benzyl-3,4-bis(dihydrogen-phosphate) α-D-xylopyranoside hexasodium salt (27). Compound **27** was obtained as a white solid by treatment of **26** according to procedure E. $[\alpha]_D + 26.0$ (c 1.5, H_2O); IR 1232 (P═O), 1100 (P-O) cm^{-1}. ^{1}H NMR (250 MHz D_2O): δ 7.37–7.62 (m, 5H, *H* Ar); 4.95 (AB, 1H, J_{AB} 11.9 Hz, C*H*$_2$Ph); 4.70–4.87 (m, 2H, *H*-1, C*H*$_2$Ph); 4.46 (ddd, 1H, $J_{2\text{-}3}$ 9.2, $J_{3\text{-}4}$ 9.0, $^3J_{H\text{-}P}$ 7.9 Hz, *H*-3); 4.09 (dddd, 1H, *H*-4); 3.90–4.02 (m, 2H, *H*-2′a, *H*-2′b); 3.74–3.89 (m, 2H, *H*-5eq, *H*-1′a); 3.57–3.73 (m, 3H, *H*-2, *H*-5ax, *H*-1′b); ^{13}C NMR (D_2O): δ 138.2 (s, C_{ipso}); 129.4; 129.0 (2s, 5C, *C*H Ar); 99.4 (s, *C*-1); 79.9 (d, $^3J_{C\text{-}P}$ 3.8 Hz, *C*-2); 79.1 (dd, $^2J_{C\text{-}P}$ 5.9, $^3J_{C\text{-}P}$ 4.7 Hz, *C*-3); 76.0 (s, *C*H$_2$Ph); 74.5 (dd, $^2J_{C\text{-}P}$ 4.8 Hz, *C*-4); 70.0 (d, $^3J_{C\text{-}P}$ 7.6 Hz, *C*-1′); 65.9 (d, $^2J_{C\text{-}P}$ 4.8 Hz, *C*-2′); 59.1 (s, *C*-5). ^{31}P NMR (D_2O): δ 7.66; 6.84 (2s, 2O*P*O$_3^{2-}$); 4.25 (s, C-2′O*P*); ESI-MS (Negative mode): Calcd for $C_{14}H_{23}O_{15}P_3$ *m/z*: 611 [M-5H + 4Na]$^-$; 589 [M-4H + 3Na]$^-$; 567 [M-3H + 2Na]$^-$; 545 [M-2H + Na]$^-$; 523 [M-H]$^-$.

[(2-(Diacetoxymethylphosphonoxy)ethyl] 2-O-benzyl-3,4 bis(diacethoxy-methyl)phosphate α-D-xylopyranoside (28). Compound **27** (139 mg,

0.21 mmol) was dissolved in water (2 mL) and applied to column of DOWEX 50W H^+ resin. The column was eluted with deionized water; fractions containing the free phosphate compound (114 mg, 0.21 mmol) were combined and freeze dried. The resulting powder was suspended in acetonitrile (1.45 mL) containing *N*,*N*-diisopropylethylamine (0.3 mL). The suspension was sonicated and concentrated. This operation was repeated twice or more to get an oily residue, which was dissolved in acetonitrile (1.45 mL), and then DIEA (0.73 mL) and acetoxymethyl bromide (1.95 g, 12.78 mmol) were added. The solution was stirred 28 hr (^{31}P RMN monitoring) at rt and the solvent was concentrated. Compound **28** (80 mg, 0.08 mmol, 39%) was obtained as a gum after purification by column chromatography (AcOEt). R_f 0.24 (AcOEt/MeOH, 98:2); $[\alpha]_D + 22.8$ (c 0.7, $CHCl_3$); IR: 1766 (C=O); 1216 (P=O) cm^{-1}; ^{1}H NMR (250 MHz $CDCl_3$): δ 7.21–7.39 (m, 5H, *H* Ar); 5.42–5.73 (m, 12H, 6O-C*H*$_2$-O); 4.62–4.78 (m, 3H, *H*-1, *H*-3, C*H*$_2$Ph); 4.55 (AB, 1H, J_{AB} 11.7 Hz, C*H*$_2$Ph); 4.37 (dddd, 1H, *H*-4); 4.18–4.30 (m, 2H, *H*-2′a, *H*-2′b); 3.88 (ddd, 1H, $J_{4\text{-}5eq}$ 5.8, $J_{5eq\text{-}5ax}$ 11. $^4J_{H\text{-}P}$ 2,9 Hz, *H*-5eq); 3.76 (dddd, 1H, *H*-1′a); 3.51–3.70 (m, 1H, *H*-5ax, *H*-1′b); 3.44 (dd, 1H, $J_{1\text{-}2}$ 3.6, $J_{2\text{-}3}$ 9.5 Hz, *H*-2); 2.12; 2.10; 2.07 (3s, 18H, 6C*H*$_3$C=O); ^{13}C NMR ($CDCl_3$): δ 168.9 (s, 6C, 6*C*=O); 137.1 (s, C_{ipso}); 128.2; 127.8 (2s, 5C, *C*H Ar); 96.4 (s, *C*-1); 81.5–82.5 (6d, 6C, 6O*C*H$_2$C=O); 77.7 (dd, $^2J_{C\text{-}P}$ 7.3 Hz, *C*-3); 77.0 (s, *C*-2); 73.6 (dd, $^2J_{C\text{-}P}$ 4.9 Hz, *C*-4); 72.8 (s, *C*H$_2$Ph); 66.5 (m, 2C, *C*-1′, *C*-2′); 58.7 (s, *C*-5); 20.3 (s, 6C, 6*C*H$_3$C=O); ^{31}P NMR ($CDCl_3$; 100 MHz): δ −2.98; −3.31 (2s); −3.95 (s, C-2′O*P*). ESI-MS (positive mode): Calcd for $C_{32}H_{47}O_{27}P_3$ m/z: 956 $[M]^+$.

[(2-(Dibutyryloxymethylphosphonoxy)ethyl] 2-O-benzyl-3,4 bis(dibutyryloxymethyl phosphate) α-D-xylopyranoside (29). Compound **29** was obtained as a gum in 22% yield after purification by column chromatography (AcOEt/Hex 65:35 → 75:25) by treatment of **27** with butyryloxymethyl iodide using the procedure described for the preparation of **28**. R_f 0.3 (Hex/AcOEt, 3:7), $[\alpha]_D + 20.0$ (c 0.6, $CHCl_3$); IR 1766 (C=O); 1218 (P=O) cm^{-1}; ^{1}H NMR (250 MHz $CDCl_3$): δ 7.22–7.42 (m, 5H, *H* aromatics); 5.46–5.77 (m, 12H, 6O-C*H*$_2$-O); 4.65–4.82 (m, 3H, *H*-1, *H*-3, C*H*$_2$Ph); 4.55 (AB, 1H, J_{AB} 11.7 Hz, C*H*$_2$Ph); 4.36 (dddd, 1H, *H*-4); 4.17–4.32 (m, 2H, *H*-2′a, *H*-2′b); 3.89 (ddd, 1H, $J_{4\text{-}5eq}$ 5.8, $J_{5eq\text{-}5ax}$ 11. $^4J_{H\text{-}P}$ 2.9 Hz, *H*-5eq); 3.77 (dddd, 1H, *H*-1′a); 3.50–3.69 (m, 2H, *H*-5ax, *H*-1′b); 3.44 (dd, 1H, $J_{1\text{-}2}$ 2.9, $J_{2\text{-}3}$ 9.5 Hz, *H*-2); 2.23–2.43 (m, 12H, 6*H*-1″a, 6*H*-1″b); 1.56–1.75 (m, 12H, 6*H*-2″a, 6*H*-2″b); 0.88–1.04 (m, 18H, 6C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 171.5 (s, 6C, 6*C*=O); 137.1 (s, C_{ipso}); 128.2; 127.8 (2s, 5C, *C*H Ar); 96.4 (s, *C*-1); 82.0–82.8 (6d, 6C, 6O*C*H$_2$C=O); 77.7 (dd, $^2J_{C\text{-}P}$ 6.1, $^3J_{C\text{-}P}$ 7.3 Hz, *C*-3); 77.0 (s, *C*-2); 73.6 (dd, $J_{C\text{-}P}$ 4.8 Hz, *C*-4); 72.7 (s, *C*H$_2$Ph); 66.4 (2d, 2C, *C*-1′, *C*-2′); 58.7 (s, *C*-5); 35.3 (s, 6*C*, 6*C*-1″); 17.6 (s, 6*C*, 6*C*-2″); 13.2 (s, 6*C*, 6*C*H$_3$); ^{31}P NMR ($CDCl_3$; 100 MHz): δ −3.07; −3.37 (2s); −3.99 (s, C-2′O*P*); Anal. Calcd for $C_{44}H_{71}O_{27}P_3$; C, 46.98; H, 6.36; P, 8.26. Found: C, 46.87; H, 6.48; P, 8.13.

[(2-(Dioctanoyloxymethylphosphonoxy)ethyl] 2-O-benzyl-3,4-bis(dioctanoyloxymethylphosphate) α-D-xylopyranoside (30). Compound **30** was obtained as a gum in 35% yield after purification by column chromatography (AcOEt/Hex 3 : 7 → 45 : 55) by treatment of **27** with octanoyloxymethyl iodide using the procedure described for the preparation of **28.** R_f 0.43 (Hex/AcOEt, 5 : 5); $[\alpha]_D$ + 12.7 (c 1.14, $CHCl_3$); IR 1766 (C═O); 1284 (P═O) cm^{-1}; ^1H NMR (250 MHz $CDCl_3$): δ 7.27–7.42 (m, 5H, *H* Ar); 5.45–5.75 (m, 12H, 6O-C*H*$_2$-O); 4.65–4.82 (m, 3H, *H*-1, *H*-3, C*H*$_2$Ph); 4.57 (AB, 1H, J_{AB} 11.7 Hz, C*H*$_2$Ph); 4.38 (dddd, 1H, *H*-4); 4.17–4.32 (m, 2H, *H*-2′a, *H*-2′b); 3.89 (ddd, 1H, $J_{4\text{-}5eq}$ 5.8, $J_{5eq\text{-}5ax}$ 11. $^4J_{H\text{-}P}$ 2.9 Hz, *H*-5eq); 3.78 (dddd, 1H, *H*-1′a); 3.51–3.70 (m, 2H, *H*-5ax, *H*-1′b); 3.45 (dd, 1H, $J_{1\text{-}2}$ 3.6, $J_{2\text{-}3}$ 9.5 Hz, *H*-2); 2.27–2.45 (m, 12H, 6*H*-1″a, 6*H*-1″b); 1.50–1.73 (m, 12H, 6*H*-2″a, 6*H*-2″b); 1.18–1.43 (m, 48H, 6(*H*-3″a, *H*-3″b, *H*-4″a, *H*-4″b, *H*-5″a, *H*-5″b, *H*-6″a, *H*-6″b); 0.79–0.99 (m, 18H, 6C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 171.6; 171.5 (2s, 6C, 6*C*═O); 137.1 (s, C_{ipso}); 128.1; 127.7 (2s. 5C, *C*H Ar); 96.3 (s, *C*-1); 82.0–82.7 (6d, 6C, 6O*C*H$_2$C═O); 77.7 (dd, $^2J_{C\text{-}P}$ 6.1, $^3J_{C\text{-}P}$ 7.3 Hz, *C*-3); 77.0 (s, *C*-2); 73.6 (dd, $J_{C\text{-}P}$ 4.8 Hz, *C*-4); 72.7 (s, *C*H$_2$Ph); 66.5 (d, $^2J_{C\text{-}P}$ 4.9 Hz, *C*-2′); 66.3 (d, 1C, $^3J_{C\text{-}P}$ 7.3 Hz, *C*-1′); 58.7 (s, *C*-5); 33.4 (s, 6*C*, C); 31.2 (s, 6*C*, C) 28.5 (s, 6*C*, C); 24.4 (s, 6*C*, C); 24.0 (s, 6*C*, C); 22.5 (s, 6*C*, C); 13.6 (s, 6*C*, 6C7″); ^{31}P NMR ($CDCl_3$; 100 MHz): δ −3.18; −3.46 (2s); −4.12 (s, C-2′O*P*). Anal. Calcd for $C_{68}H_{119}O_{27}P_3$: C, 55.88; H, 8.21; P, 6.36. Found: C, 55.99; H, 8.14; P, 6.68.

[(2-(Diacetoxymethylphosphonoxy)ethyl] 3,4-bis(diacethoxymethyl)-phosphate α-D-xylopyranoside (9). To a solution of **28** (20 mg, 0.020 mmol) in ethyl acetate (10 mL) was added Pd/C 10% (20 mg). The medium was hydrogenated under 20 bars. After 12 hr, the catalyst was removed on a pad of Celite and the solvent evaporated in vacuo to give compound **9** (17.8 mg, 0.020 mmol) as a gum. R_f = 0.37 (AcOEt/MeOH, 93 : 7); $[\alpha]_D$ = +33.0 (c 1.54, $CHCl_3$); IR 1767 (C═O); 1216 (P═O) cm^{-1}; ^1H NMR (400 MHz $CDCl_3$): δ 5.58–5.78 (m, 12H, 6O-C*H*$_2$-O); 4.92 (d, 1H, $J_{1\text{-}2}$ 3.7 Hz, *H*-1); 4.63 (ddd, 1H, $J_{2\text{-}3}$ 9.3, $J_{3\text{-}4}$ 9.3, $^3J_{H\text{-}P}$ 8.0 Hz, *H*-3); 4.41 (dddd, 1H, *H*-4); 4.19–4.36 (m, 2H, *H*-2′a, *H*-2′b); 3.89–4.01 (m, 2H, *H*-5eq, *H*-1′a); 3.59–3.75 (m, 3H, *H*-1′b, *H*-5ax, *H*-2); 2.14; 2.11; 2.09 (3s, 18H, 6C*H*$_3$C═O); ^{13}C NMR ($CDCl_3$): δ 168.9 (s, 6C, 6*C*═O); 98.5 (s, *C*-1); 82.4–82.8 (6d, 6C, 6O*C*H$_2$C═O); 79.6 (dd, $^2J_{C\text{-}P}$ 6.1 Hz, *C*-3); 73.3 (dd, $^2J_{C\text{-}P}$ 4.9 Hz, *C*-4); 70.7 (s, *C*-2); 67.1 (d, $^3J_{C\text{-}P}$ 6.1 Hz, *C*-1′); 66.8 (d, $^2J_{C\text{-}P}$ 3.7 Hz, *C*-2′); 59.1 (s, *C*-5); 20.3 (s, 6C, 6*C*H$_3$C═O); ^{31}P NMR ($CDCl_3$): δ −2.90; −3.08 (2s); −3.83 (s, C-2′O*P*); LSIMS-MS (positive mode): Calcd for $C_{25}H_{41}O_{27}P_3$ *m*/*z*: 867 $[M + H]^+$.

[(2-(Dibutyryloxymethylphosphonoxy)ethyl] 3,4-bis(dibutyryloxymethyl) phosphate α-D-xylopyranoside (10). Compound **10** was quantitatively obtained as a gum by treatment of **29** using the procedure described for the preparation of **9.** R_f 0.55(Hex/AcOEt, 4 : 6); $[\alpha]_D$ + 35.2 (c 0.68, $CHCl_3$); IR 1767 (C═O); 1211 (P═O) cm^{-1}; ^1H NMR (250 MHz $CDCl_3$): δ 5.53–5.79 (m, 12H, 6O-C*H*$_2$-O);

4.89 (d, 1H, $J_{1\text{-}2}$ 3.6 Hz, *H*-1); 4.60 (ddd, 1H, $J_{2\text{-}3}$ 9.3, $J_{3\text{-}4}$ 9.3, $^{3}J_{H\text{-}P}$ 8.0 Hz, *H*-3); 4.37 (dddd, 1H, *H*-4); 4.21–4.33 (m, 2H, *H*-2′a, *H*-2′b); 3.84–4.00 (m, 2H, *H*-5eq, *H*-1′a); 3.53–3.76 (m, 3H, *H*-1′b, *H*-5ax, *H*-2); 2.28–2.43 (m 12H, 6*H*-1″a, 6*H*-1″b); 1.55–1.77 (m, 12H, 6*H*-2″a, 6*H*-2″b); 0.88–1.07 (m, 18H, 6CH_3); ^{13}C NMR ($CDCl_3$): δ 172.2; 172.1 (2s, 6C, 6*C*=O); 99.2 (s, *C*-1); 82.7–83.3 (6d, 6C, 6O*C*H_2C=O); 80.2 (dd, $^{2}J_{C\text{-}P}$ 6.1, $^{3}J_{C\text{-}P}$ 7.3 Hz, *C*-3); 74.0 (dd, $J_{C\text{-}P}$ 6.0 Hz, *C*-4); 71.4 (s, *C*-2); 67.5 (2d, 2C, *C*-1′, *C*-2′); 59.8 (s, *C*-5); 36.0 (s, 6*C*, 6*C*-1″); 18.3 (s, 6*C*, 6*C*-2″); 13.8 (s, 6*C*, 6*C*H_3); ^{31}P NMR ($CDCl_3$; 100 MHz): −2.83; −3.11 (2s); −3.90 (s, C-2′O*P*); ESI-MS (positive mode): Calcd for $C_{37}H_{65}O_{27}P_3$ *m*/*z*: 1057 $[M\text{-}H + Na]^+$; 1035 $[M]^+$.

[(2-(Dioctanoylphosphonoxy)ethyl] 3,4-bis(dioctanoyloxymethyl)phosphate α-D-xylopyranoside (11). Compound **11** was quantitatively obtained as a gum by treatment of **29** using the procedure described for the preparation of **9.** R_f 0.55 (Hex/AcOEt, 4 : 6); $[\alpha]_D + 23.3$ (c 1.24, $CHCl_3$), IR 1770 (C=O); 1277 (P=O) cm^{-1}; ^{1}H NMR (250 MHz $CDCl_3$): δ 5.52–5.77 (m, 12H, 6O-CH_2-O); 4.88 (d, 1H, $J_{1\text{-}2}$ 3.7 Hz, *H*-1); 4.60 (ddd, 1H, $J_{2\text{-}3}$ 9.3, $J_{3\text{-}4}$ 9.3, $^{3}J_{H\text{-}P}$ 8.1 Hz, *H*-3); 4.37 (dddd, 1H, *H*-4); 4.17–4.33 (m, 2H, *H*-2′a, *H*-2′b); 3.82–3.99 (m, 2H, *H*-5eq, *H*-1′a); 3.53–3.75 (m, 3H, *H*-1′b, *H*-5ax, *H*-2); 2.24–2.47 (6s, 12H, 6*H*-1″a, 6*H*-1″b); 1.50–1.73 (m, 12H, 6*H*-2″a, 6*H*-2″b); 1.18–1.43 (m, 48H, 6(*H*-3″a, *H*-3″b, *H*-4″a, *H*-4″b, *H*-5″a, *H*-5″b, *H*-6″a, *H*-6″b); 0.78–0.98 (m, 18H, 6CH_3); ^{13}C NMR ($CDCl_3$): δ 178.1; 171.5 (2s, 6C, 6*C*=O); 98.5 (s, *C*-1); 82.0–82.8 (6d, 6C, 6O*C*H_2C=O); 79.5 (dd, $J_{C\text{-}P}$ 6.1 Hz, *C*-3); 73.3 (dd, $^{2}J_{C\text{-}P}$ 4.9 Hz, *C*-4); 70.7 (s, *C*-2); 66.8 (d, $^{3}J_{C\text{-}P}$ 6.1 Hz, *C*-1′); 66.7 (d, $^{2}J_{C\text{-}P}$ 3.7 Hz, *C*-2′); 59.0 (s, *C*-5); 33.4 (s, 6*C*, C); 31.2 (s, 6*C*, C); 28.5 (s, 6*C*, C); 24.4 (s, 6*C*, C); 24.0 (s, 6*C*, C); 22.2 (s, 6*C*, C); 13.6 (s, 6*C*, 6C-7″); ^{31}P NMR ($CDCl_3$; 100 MHz): δ −2.90; −3.29 (2s); −4.05 (s, C-2′O*P*); Anal. Calcd for $C_{61}H_{113}O_{27}P_3$ C, 53.40; H, 8.31; P, 6.78. Found: C, 53.72; H, 8.36; P, 6.65; ESI-MS (positive mode): Calcd for $C_{61}H_{113}O_{27}P_3$ *m*/*z*: 1371 $[M]^+$.

(2(*S*)-Hydroxy-3-azidopropyl) 2-*O*-benzyl-3,4-*O*-[(2S,3S) (2,3-dimethoxybutane-2,3-diyl)]-α-D-xylopyranoside (33). To a solution of **32** (1.53 g, 3.73 mmol) in 2-methoxyethanol (40 mL) were added NH_4Cl (490 mg, 9.32 mmol), water (13 mL), and NaN_3 (970 mg, 14.9 mmol). The mixture was heated under reflux for 1 hr and concentrated under reduced pressure, and the residue was diluted in water (20 mL). The aqueous layer was extracted with ethyl acetate. The organic layers were combined and dried over $MgSO_4$ and concentrated in vacuo. Compound **33** (1.350 g, 2.98 mmol, 80%) was obtained as a gum after purification by chromatography (Hex/AcOEt,1 : 1). R_f 0.26 (Hex/AcOEt,7 : 3); $[\alpha]_D + 157.3$ (c 0.96, $CHCl_3$); IR 3445 (OH); 2101 (N_3) $cm^{-1.}$ ^{1}H NMR (250 MHz $CDCl_3$): δ 7.22–7.40 (m, 5H, *H* Ar); 4.90 (AB, 1H, J_{AB} 11.7 Hz, CH_2Ph); 4.73 (d, 1H, $J_{1\text{-}2}$ 3.7 Hz, *H*-1); 4,64 (AB, 1H, CH_2Ph); 4.09 (dd, 1H, $J_{2\text{-}3}$ 9.6, $J_{3\text{-}4}$ 9.4 Hz, *H*-3); 3.87 (m, 1H, $J_{2'\text{-}3'a}$ 4.4, $J_{2'\text{-}3'b}$ 4.9 Hz, *H*-2′); 3.68–3.78 (m, 3H, *H*-4, *H*-5a, *H*-1′a); 3.46–3.62 (m, 3H, *H*-2, *H*-5b, *H*-1′b); 3.21–3.41 (m, 8H, 2OCH_3, *H*-3′a, *H*-3′b); 1.82 (s, 1H, O*H*); 1.35; 1.31

(2s, 6H, 2CH_3). ^{13}C NMR ($CDCl_3$): δ 137.8 (s, C_{ipso}); 128.0; 127.4 (2s, 5C, *C*H Ar); 99.3; 99.1; (2s, 2CH_3*C*OCH_3); 98.6 (s, *C*-1); 76.3 (s, *C*-2); 73.4 (s, *C*H_2Ph); 70.0 (s, 1C, *C*-3); 69.8 (s, *C*-1′); 69.0 (s, *C*-2′); 66.0 (s, 1C, *C*-4); 59.3 (s, *C*-5); 53.0 (s, *C*-3′); 47.5 (s, 2C, 2O*C*H_3); 17.5; 17.2 (2s, 2*C*H_3); Anal. Calcd for $C_{21}H_{31}N_3O_8$: C, 55.60; H, 6.89; N, 9.27. Found: C, 55.45; H, 6.72; N, 9.14.

(2(*S*)-Hydroxy-3-azidopropyl) 2-*O*-benzyl-α-D-xylopyranoside (34). To a solution of **33** (1.017 g, 3 mmol) in CH_2Cl_2 (20 mL) was added 95% aqueous TFA solution (15 mL). The mixture was stirred at rt for 10 min and concentrated under reduced pressure. The residue was diluted with ethyl acetate and the solution was neutralized by adding solid $NaHCO_3$. Compound **34** (814 mg, 2.49 mmol) was obtained as a gum in 83% yield after purification by column chromatography (Hex/AcOEt,1 : 9). R_f 0.46 (AcOEt); $[\alpha]_D$. + 74.0 (c 0.36, $CHCl_3$), IR 3390 (OH); 2101 (N_3) cm^{-1}; ^{1}H NMR (250 MHz $CDCl_3$): δ 7.23–7.40 (m, 5H, *H* Ar); 4.72 (AB, 1H, J_{AB} 11.6 Hz, C*H*$_2$Ph); 4.65 (d, 1H, $J_{1\text{-}2}$ 3.6 Hz, *H*-1); 4.60 (AB, 1H, C*H*$_2$Ph); 3.77–4.08 (m, 2H, *H*-3, *H*-2′); 3.43–3.71 (m, 4H, *H*-4, *H*-5a, *H*-5b, *H*-1′a); 3.18–3.42 (m, 4H, *H*-2, *H*-1′b, *H*-3′a, *H*-3′b); 1.72 (s, 3H, 3O*H*); ^{13}C NMR ($CDCl_3$): δ 137.0 (s, C_{ipso}); 128.4; 128.1 (2s, 5C, *C*H Ar); 96.8 (s, *C*-1); 79.3 (s, *C*-2); 73.3 (s, *C*H_2Ph); 72.8 (s, *C*-3); 69.5 (s, *C*-4); 69.4 (s, *C*-1′); 69.1 (s, *C*-2′); 61.2 (s, *C*-5); 52.9 (s, *C*-3′); Anal. Calcd for $C_{15}H_{21}N_3O_6$: C, 53.09; H, 6.24; N, 12.38. Found: C, 53.25; H, 6.17; N, 13.54.

(2(*S*)-Hydroxy-3-aminopropyl) 2-*O*-benzyl-α-D-xylopyranoside (35). To a solution of **34** (228 mg, 0.67 mmol) in methanol (4.4 mL) was added Pd/C 10% (34 mg) and the solution hydrogenated for 4 hr. The catalyst was removed by filtration through a pad of Celite and the solvent was concentrated to afford compound **35** (203 mg, 0.065 mmol; 97%) as a white solid. R_f 0.10 (CH_2Cl_2/MeOH/H_2O (65 : 25 : 4)); $[\alpha]_D$ + 72.7 (c 1.02, MeOH); IR 3368 (OH, NH_2) cm^{-1}; ^{1}H NMR (250 MHz CD_3OD): δ 7.13–7.43 (m, 5H, *H* Ar); 4.66–4.76 (m, 2H, C*H*$_2$Ph, *H*-1); 4.58 (AB, 1H, J_{AB} 11.7 Hz, C*H*$_2$Ph); 3.54–3.80 (m, 3H, *H*-3, *H*-2′, *H*-1′a); 3.35–3.53 (m, 3H, *H*-4, *H*-5a, *H*-5b); 3.17–3.34 (m, 2H, *H*-2, *H*-1′b); 2.52–2.83 (m, 2H, *H*-3′a, *H*-3′b); ^{13}C NMR (CD_3OD): δ 140.2 (s, C_{ipso}); 130.1; 130.0 (2s, 5C, *C*H Ar); 99.3 (s, *C*-1); 81.7 (s, 2C, *C*-2, *C*-2′); 75.0 (s, *C*-3); 74.9 (s, *C*H_2Ph); 72.1 (s, 2C, *C*-4, *C*-1′); 63.5 (s, *C*-5); 45.9 (s, *C*-3′), Anal. Calcd for $C_{15}H_{23}NO_6$: C, 57.48; H, 7.40; N, 4.47. Found: C, 57.64; H, 7.49; N, 4.35.

[3-(*p*-Benzoylcinnamido)-2(*S*)-hydroxy-propyl] 2-*O*-benzyl-α-D-xylopyranoside (36). To a solution of **41** (257 mg, 1 mmol) in DMF (3 mL) were added NEt_3 (0.15 mL, 1.12 mmol) and BOP (495 mg, 1.12 mmol). The mixture was stirred at rt for 15 min and then a solution of **35** (319 mg, 1 mmol) in DMF (7 mL) was added. After 40 min, the solvent was removed in vacuo and the residue was diluted with ethyl acetate. The organic layer was washed with 3N HCl and water, dried over Na_2SO_4, and concentrated. Compound **36**

(398 mg, 0.73 mmol) was obtained as a gum in 72% yield after purification by column chromatography (CH_2Cl_2/MeOH 97 : 3 → 94 : 6). R_f 0.50 (CH_2Cl_2/MeOH 9 : 1); $[\alpha]_D + 33.4$ (c 1.0, $CHCl_3$); IR 3351 (OH, NH); 1658; 1651; 1645 (PhC(O)Ph-, OC(O)N, -Ph-C=C-) cm^{-1}; ^1H NMR (250 MHz $CDCl_3$): δ 7.65–7.78 (m, 2H, *H* Ar); 7.39–7.63 (m, 8H, -C=C*H*Ph, *H* Ar); 7.19–7.35 (m, 5H, *H* Ar); 7.11 (t, 1H, J, N*H*); 6.56 (d, 1H, J_{trans} 15.4 Hz, -C=C*H*C(O)); 4.48–4.77 (m, 3H, CH_2Ph, *H*-1); 3.82–4.00 (m, 3H, *H*-3, *H*-2′, O*H*); 3.23–3.70 (m, 8H, *H*-4, *H*-5a, *H*-5b, *H*-2, *H*-1′a, *H*-1′b, *H*-3′a, *H*-3′b); 2.93 (s, 2H, O*H*); ^{13}C NMR ($CDCl_3$): 196.1 (*C*=O); 166.6 (C(O)NH); 139.3 (C=*C*-Ph); 138.3; 137.7; 137.1; 136.8 (4s, 4C, C_{ipso}); 132.7; 130.2; 129.6; 128.3; 128.0; 127.3 (6s, 14C, *C*H Ar); 122.8 (C=*C*-C(O)); 96.8 (s, *C*-1); 79.4 (s, *C*-2); 73.3 (s, *C*H_2Ph); 72.8 (s, *C*-3 or *C*-2′); 69.9 (s, *C*-3 or *C*-2′); 69.8 (s, *C*-1′); 68.9 (s, *C*-4); 61.3 (s, *C*-5); 42.7 (s, *C*-3′); Anal. Calcd for $C_{31}H_{33}NO_8$: C, 67.99; H, 6.07; N, 2.56. Found: C, 68.12; H, 6.19; N, 2.45.

[3-(*p*-Benzoylcinnamido)-2(*S*)-(2-dicyanoethylphosphonoxy)-propyl] 2-*O*-benzyl-3,4-bis[(2-dicyanoethyl) phosphate]-α-D-xylopyranoside (37). The triol derivative **36** was phosphorylated with the phosphoramidite **23** according to procedure B. The residue was purified by column chromatography (CH_2Cl_2/MeOH 98 : 2 → 9 : 1) to give **37** (42%) as a gum. R_f 0.12 (CH_2Cl_2/MeOH 9 : 1); $[\alpha]_D + 24.6$ (c 0.8, $CHCl_3$); IR 3320 (NH); 2251 (CN); 1658; 1651; 1646 (PhC(O)Ph-, OC(O)N, –Ph-C=C–; 1279 (P=O); 1040 (P-O) cm^{-1}; ^1H NMR (250 MHz $CDCl_3$): δ 7.66–7.78 (m, 2H, *H* Ar); 7.38–7.64 (m, 8H, –C=C*H*Ph, *H* Ar); 7.22–7.37 (m, 5H, *H* Ar); 6.90 (t, 1H, J, N*H*); 6.62 (d, 1H, J_{trans} 15.2 Hz, –C=C*H*C(O)); 4.92 (d, 1H, $J_{1\text{-}2}$ 2.9 Hz, *H*-1); 4.58–4.84 (m, 4H, CH_2Ph, *H*-3, *H*-2′); 3.94–4.52 (m, 16H, *H*-4, *H*-5eq, *H*-1′a, *H*-1′b, 6CH_2CH_2CN); 3.88 (dd, 1H, $J_{4\text{-}5ax}$ 6.6 Hz, *H*-5ax); 3.50–3.79 (m, 3H, *H*-2, *H*-3′a, *H*-3′b); ^{13}C NMR ($CDCl_3$): δ 195.7 (*C*=O); 165.8 (C(O)NH); 139.7 (C=*C*-Ph); 138.3; 137.9; 136.9 (3s, 4C, C_{ipso}); 132.4; 130.2; 129.6; 128.4; 127.8; 127.5 (6s, 14C, *C*H Ar); 122.5 (C=*C*–C(O)); 116.9; 116.7; 116.6 (3s, 3*C*N); 96.1 (s, *C*-1); 77.6 (m, *C*-3); 77.1 (s, *C*-2); 76.6 (m, *C*-2′); 73.8 (s, *C*-4); 72.4 (s, *C*H_2Ph); 67.7 (m, *C*-1′); 58.8 (s, *C*-5); 40.6 (s, *C*-3′); 21.2 (s, 6C, 6CH_2*C*H_2CN); ^{31}P NMR ($CDCl_3$; 100 MHz): δ −1.47; −1.61; −1.98 (s, 3P); Anal. Calcd for $C_{49}H_{54}N_7O_{17}P_3$: C, 53.22; H, 4.92; N, 8.87; P, 8.40. Found: C, 53.05; H, 4.75; N, 8.98; P, 8.23.

[3-(*p*-Benzoylcinnamido)-2(*S*)-(dihydrogen phosphate)-propyl] 2-*O*-benzyl-3,4-bis-dihydrogen phosphate α-D-xylopyranoside hexasodium salt (38). Compound **38** was obtained as a white solid by treatment of **37** according to procedure E. $[\alpha]_D + 23.8$ (c 1.2, H_2O), IR 3317 (NH); 1658; 1652; 1646 (PhC(O)Ph-, OC(O)N, -Ph-C=C-C(O)); 1278(P=O) cm^{-1}; ^1H NMR (250 MHz D_2O): 7.03–7.65 (m, 15H, *H* Ar, –C=C*H*Ph); 6.66 (d, 1H, J_{trans} 15.0 Hz, –C=C*H*C(O)); 4.59–4.94 (m, 3H, *H*-1, CH_2Ph); 4.40–4.57 (m, 2H, *H*-3, *H*-2′); 4.28 (m, 1H, *H*-4); 3.85–4.11 (m, 4H, *H*-5ax, *H*-5eq, *H*-1′a, *H*-1′b); 3.79 (m, 1H, *H*-2); 3.42–3.65 (m, 2H, *H*-3′a, *H*-3′b); ^{13}C NMR (D_2O): δ 199.4

(*C*═O); 168.0 (C(O)NH); 139.4 (C═*C*-Ph); 138.0; 137.1; 136.4 (3s, 4C, C_{ipso}); 133.6; 130.2; 128.1; 127.9; 127.7 (5s, 14C, *C*H Ar); 123.2 (C═*C*-C(O)); 99.6 (s, *C*-1); 76.4 (s, *C*-2); 73.1 (s, CH_2Ph); 70.5 (s, 2C, *C*-3 or *C*-4 or *C*-2′); 70.0 (m, *C*-1′); 69.4 (m, 1C, *C*-3 or *C*-4 or *C*-2′); 65.8 (s, *C*-5); 42.6 (s, *C*-3′); ^{31}P NMR (D_2O; 100 MHz): δ 7.65; 7.50; 7.26 (3s, 3P); Anal. Calcd for $C_{31}H_{30}N_1O_{17}P_3Na_6$: C, 40.50; H, 3.29; N, 1.52; P, 10.11. Found: C, 40.26; H, 3.51; N, 1.36; P, 9.97.

[3-(*p*-Benzoylcinnamido)-2(*S*)-(diacetoxymethylphosphonoxy) propyl] 2-*O*-benzyl-3,4-bis (diacetoxymethyl)phosphate-α-D-xylopyranoside (39). Compound **39** was obtained as a gum in 20% yield after purification by column chromatography (AcOEt/MeOH 98 : 2 → 95 : 5) by treatment of **38** using the procedure described for the preparation of **28**. $[\alpha]_D + 27.5$ (c 0.5, $CHCl_3$); IR 3315 (NH); 1769 (OC(O)CH_3); 1662; 1660; 1656 (PhC(O)Ph-, OC(O)N, -Ph-C═C-C(O)); 1217 (P═O) cm^{-1}; ^{1}H NMR (250 MHz $CDCl_3$): δ 7.72–7.83 (m, 2H, *H* Ar); 7.53–7.70 (m, 2H, -C═C*H*Ph, *H* Ar); 7.21–7.52 (m, 13H, *H* Ar); 6.91 (t, 1H, $J_{NH\text{-}H3'a} = J_{NH\text{-}H3'b}$ 5.8 Hz, N*H*); 6.62 (d, 1H, J_{trans} 15.3 Hz, -C═C*H*C(O)); 5.44–5.75 (m, 12H, O-CH_2-O); 4.57–4.83 (m, 5H, *H*-1, CH_2Ph, *H*-3, *H*-2′); 4.36 (m, 1H, *H*-4); 3.53–3.99 (m, 6H, *H*-5eq, *H*-5ax, *H*-1′a, *H*-1′b, *H*-3′a, *H*-3′b); 3.50 (dd, 1H, $J_{1\text{-}2}$ 3.5, $J_{2\text{-}3}$ 9.5 Hz, *H*-2). ^{13}C NMR ($CDCl_3$): δ 196.0 (*C*═O); 169.2 (s, 6C, 6O*C*═OCH_3); 165.9 (C(O)NH); 139.7 (C═*C*-Ph); 138.4; 136.8 (2s, 4C, C_{ipso}); 132.2; 130.2; 129.6; 128.3; 127.9 (5s, 14C, *C*H Ar); 122.5 (C═*C*-C(O)); 96.7 (s, *C*-1); 81.5–82.55 (6d, 6C, 6O*C*H_2C═O); 77.7 (m, 2C, *C*-2 or *C*-3 or *C*-2′); 77.0 (m, 1C, *C*-2 or *C*-3 or *C*-2′); 73.5 (m, *C*-4); 72.8 (s, *C*H_2Ph); 68.0 (m, *C*-1′); 58.8 (s, *C*-5); 40.9 (m, *C*-3′); 20.4 (s, 6C, 6*C*H_3C═O). ^{31}P NMR ($CDCl_3$; 100 MHz): δ −3.37; −3.61; −4.00 (3s, 3P); Anal. Calcd for $C_{49}H_{60}N_1O_{29}P_3$: C, 48.24; H, 4.96; N, 1.15; P, 7.62. Found: C, 48.09; H, 5.12; N, 1.02; P, 7.43.

[3-Biphenyldihydrocinnamido)2(*S*) (diacetoxymethylphosphonoxy) propyl] 3,4-bis(diacetoxymethyl)phosphate α-D-xylopyranoside (40). Compound **40** was quantitatively obtained as a gum by treatment of **39** using the procedure described for the preparation of **9.** $[\alpha]_D + 27.0$ (c 0.5, $CHCl_3$); IR 3315 (NH); 1768 (OC(O)CH_3); 1668 (OC(O)N); 1216(P═O); 1151(P-O) cm^{-1}; ^{1}H NMR (250 MHz $CDCl_3$): δ 7.23–7.33 (m, 2H, *H* Ar); 7.12–7.21 (m, 7H, *H* Ar); 6.48 (t, 1H, $J_{NH\text{-}H3'a} = J_{NH\text{-}H3'b}$ 5.9 Hz, N*H*); 5.50–5.76 (m, 12H, O-CH_2-O); 4.81 (d, 1H, $J_{1\text{-}2}$ 3.6 Hz, *H*-1); 4.53–4.68 (m, 2H, *H*-3, *H*-2′); 4.37 (m, 1H, *H*-4); 3.87–3.99 (m, 3H, *H*-5a, Ph-CH_2-Ph-); 3.74 (dd, 1H, $J_{1'a\text{-}1'b'}$ 11.0, $J_{1'a\text{-}2'}$ 6.6 Hz, *H*-1′a); 3.40–3.68 (m, 5H, *H*-2, *H*-5b, *H*-1′b, *H*-3′a, *H*-3′b); 2.91 (m, 2H, CH_2Ph-); 2.51 (m, 2H, CH_2C(O)); ^{13}C NMR ($CDCl_3$): δ 172.6 (C(O)NH); 169.1; 168.9 (2s, 6C, 6O*C*═OCH_3); 140.8; 138.8; 138.1 (3s, 3C, C_{ipso}); 128.7; 128.5; 128.1 (3s, 5C, *C*H Ar); 98.5 (s, *C*-1); 81.6–82.8 (6d, 6C, 6O*C*H_2C═O); 79.6 (dd, $^2J_{C\text{-}P}$ 5.2, $^3J_{C\text{-}P}$ 7.1 Hz, *C*-3); 77.0 (m, 1C, *C*-2′); 73.4 (dd, $^2J_{C\text{-}P}$ 4.8, $^3J_{C\text{-}P}$ 5.2 Hz, *C*-4); 70.7 (s, *C*-2); 67.1 (d, $^3J_{C\text{-}P}$ 4.3 Hz, *C*-1′); 59.1 (s, *C*-5); 41.2 (s, -Ph*C*H_2Ph); 39.9 (m, *C*-3′); 37.6 (s, *C*H_2C(O)); 30.7

(s, CH_2Ph-); 20.3 (s, 6C, 6CH_3C=O); ^{31}P NMR ($CDCl_3$; 100 MHz): δ −3.36; −3.83; −3.91 (3s, 3P); Anal. Calcd for $C_{42}H_{58}N_1O_{28}P_3$: C, 45.13; H, 5.23; N, 1.25; P, 8.31. Found: C, 45.31; H, 5.11; N, 1.07; P, 8.19.

Biological Evaluation

The preparation of rat cerebellar microsomes was achieved according to ref. 15.

Rat hepatocytes were isolated by the two-step collagenase perfusion technique,[39] modified as described in ref. 40. Cell viability, as assessed by trypan blue exclusion, was >96%. After isolation, cells were maintained in suspension in Eagle's medium supplemented with 15 g/L gelatin and gassed with O2/CO2 (19 : 1) at rt, during the following 8 hr, during which [Ca^{2+}]c determination was performed.

Equilibrium [^{3}H]InsP_3-binding studies were achieved according to the procedure described in ref 15.

Spectrofluorimetry studies: Before experiment, cells were loaded with the Ca^{2+}-sensitive indicator quin2-acetoxymethyl (AM) ester. Briefly, an aliquot corresponding to 10^6 cells was incubated at 37°C in 0.5 mL incubation medium containing 50 μM quin2-AM for 180 seconds, centrifuged, washed, and resuspended in 2 mL of Dulbecco's modified Eagle medium. The cell suspension was then transferred to the oxygenated spectrofluorimeter cuvette (under O2/CO2 (19 : 1)) and stirred magnetically at 37°C (JY3D fluorometer, Jobin & Yvon). Calcium movements were measured from the observed changes in the fluorescence of quin2 (excitation wavelength, 340 nm; emission wavelength, 492 nm). At the end of each experiment, the fluorescence signal was calibrated by making the cell membrane permeable with 5 μg/mL digitonin (quin2 saturation by external Ca^{2+} gave maximal fluorescence) and then by adding 20 mM EGTA to the suspension of lyzed cells to obtain the minimal fluorescence.

Experiments in Ca^{2+}-free medium were performed to evaluate the mobilization of intracellular Ca^{2+} during hormone or analog stimulation. The Ca^{2+} influx was suppressed by adding EGTA (4 mM) to the incubation medium. The hormone or analogs were then added to the medium before the addition of the hormone or analog.

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