Synthesis, Acid−Base Behavior, and Binding Properties of 6-Modified *myo*-Inositol 1,4,5-Tris(phosphate)s

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myo-Inositol 1,4,5-tris(phosphate) was modified at position 6. The analogues synthesized are reported in this publication are 6-deoxy-*myo*-inositol 1,4,5-tris(phosphate), 6-fluoro-6-deoxy-*myo*-inositol 1,4,5-tris(phosphate), *epi*-inositol 1,4,5-tris(phosphate), and 6-amino-6-deoxy-*myo*-inositol 1,4,5-tris(phosphate). These derivatives showed poor affinity for the Ins(1,4,5)P_3 receptors. The inframolecular acid−base behavior and the cooperative effects between the phosphate groups could help explain the loss of affinity of these 6-modified analogues.

Introduction

The fact that *myo*-inositol 1,4,5-tris(phosphate) (Ins(1,4,5,)P_3, **1**) (Chart 1) acts as an intracellular second messenger is now well-established. The binding of **1** to its endoplasmic reticulum receptors (ERR) induces the release of calcium from intracellular stores.[1−3]

Several research groups have developed structure−activity relationships (SAR) around this compound, especially toward its binding to the ERR.[4−6] Compilation of the SAR results allowed Kozikowski to propose a pharmacophore model showing the crucial role of the vicinal bis(phosphate) in positions 4 and 5, the amplifying effect of the third phosphate in position 1, the slight importance of the two hydroxyls in positions 2 and 3, and the major role played by the hydroxyl group in position 6. Specifically, the hydroxyl group in position 6 seems to be involved in a hydrogen bond as the hydrogen bond donor (Figure 1).[7,8]

In the classical approach, a pharmacophore model is formed by considering the variation of activty as a direct consequence of the chemical variation of a given functional group. The activity is indicative of its ability to reach its counterpart of the binding site. Since inositol phosphates contain multiple phosphate and hydroxyl moieties, intramolecular interactions should be considered. In fact, a chemical modification at a given position on the inositol ring could induce changes in the other functional groups in the ring (i.e. by changing charge distributions, intramolecular hydrogen bond, conformations, etc.). Thus, the hydroxyl group in position 6 could be involved in intramolecular interactions as well as in a probable supramolecular hydrogen bond with the receptor site. Therefore, the polyfunctionality of inositol tris(phosphates) ($InsP_3$) led us to investigate some potentiometric and ^{31}P NMR physicochemical studies to see if intramolecular interfunctional cooperation could help in the explanation of the SAR results.

Potentiometric analyses give a global view of the acid−base behavior of the molecule. According to the

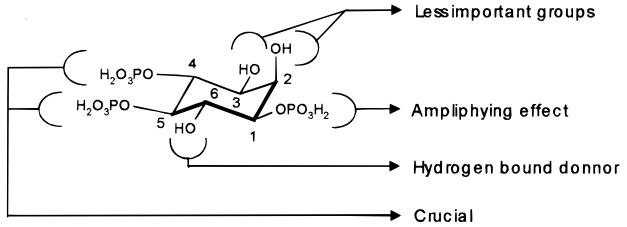

Figure 1. Pharmacophore model proposed by Kozikowski.[7]

pH variations, a series of equilibria have to be considered. The behavior can be described by the general equation associated with the corresponding stepwise equilibrium constants K_y:

$$H^+ + H_{y-1}InsP_3^{(6-y+1)-} \overset{K_y}{\rightleftharpoons} H_yInsP_3^{(6-y)-}$$

$$K_y = \frac{[H_yInsP_3^{(6-y)-}]}{[H^+][H_{y-1}InsP_3^{(6-y+1)-}]}$$

For polyfunctional compounds such as $InsP_3$, the protonation−deprotonation process of each phosphate cannot simply be described using these macroscopic constants or their co-logarithm (p*K*). One must consider a more detailed ionization scheme, function by function, defining, in that manner, microspecies. This approach will be titled as the inframolecular approach hereafter.

For $InsP_3$, 8 microspecies have to be considered in the studied pH range. These 8 microspecies are connected through 12 microequilibria (Figure 2).[9] In this case, ^{31}P NMR is very useful to resolve such a complex system.

Because the phosphate groups can freely rotate around the σ bond[10] and no conformational changes have been observed on the cyclohexyl ring as a function of the pH,[11] one can hypothesize that the chemical shift observed (δ_i^{obs}) for a given phosphate versus the pH is essentially related to its ionization state. It should be noted that for each phosphate, only one acidic proton is concerned in the pH range of this study ($2.5 < pH < 10$). It is reasonable to deduce that, at a given pH, δ_i^{obs} is a weighted average of the chemical shifts of the

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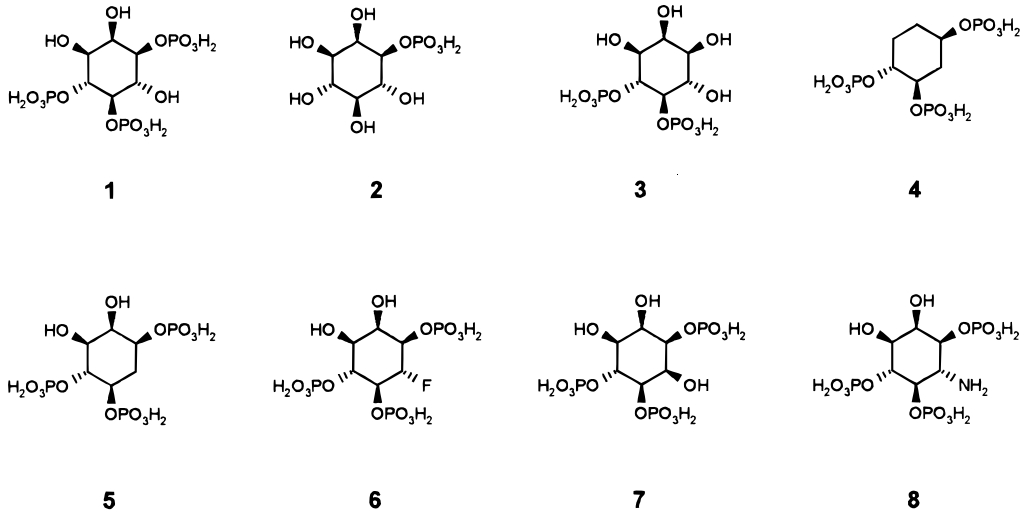
Chart 1



monoprotonated phosphate (δ_{ip}) and of the totally deprotonated form (δ_{id}).[12] Consequently, by following the phosphorus chemical shift variations of each phosphate over a large pH range, it is possible to describe the behavior, and particularly the ionization state, of the molecule at an inframolecular level.

Finally, mathematical treatment of data obtained by potentiometry and ^{31}P NMR titrations by the programs MICROPOT,[13] EQUIPOT,[14] SUPERQUAD,[15] HYPNMR,[16] and EASYPLOT[17] yield equilibrium constants (macroscopic and microscopic) for the protonation state of the molecule.

For an inositol monophosphate, such as Ins(1)P$_1$ (**2**), the curve of the chemical shift variation versus the pH looks like a classical titration of an acidic proton.[12] For a vicinal bis(phosphate) such as Ins(4,5)P$_2$ (**3**), the curves become more complicated. Each curve concerns only one acidic proton but appears biphasic. These aspects demonstrate the cooperative effect, which exists between the two vicinal phosphates,[12] a phenomenon whose interactivity parameters have been evaluated.[18]

The situation becomes very complex in the case of Ins(1,4,5)P$_3$ (**1**) (Figure 3). The curves show cooperative effects between all three phosphates. Particularly, the phosphate in position 1 seems to be dependent on the behavior of the vicinal phosphate in positions 4 and 5. This effect probably involves the neighboring hydroxyl groups. This was verified by preparing and analyzing the 2,3,6-trideoxy derivative **4**.[19,20] The compound displayed a 100-fold lower affinity than the parent compound **1**. The ^{31}P NMR clearly showed that the three phosphates of compound **4** were divided into two independent groups (Figure 4). The phosphate in position 1 became an isolated phosphate and showed a classical monophasic curve similar to that observed for Ins(1)P$_1$, while the phosphates in positions 4 and 5 showed curves which mimic those of the Ins(4,5)P$_2$ (**3**).[19]

According to Kozikowski's pharmacophore model, the hydroxyls in positions 2 and 3 do not seem important for affinity; thus, we focused on the synthesis, acid–base properties, and binding evaluation of 6-modified Ins(1,4,5)P$_3$. In this manuscript we report our studies on (±)-6-deoxy-*myo*-inositol 1,4,5-tris(phosphate) (**5**), (±)-6-deoxy-6-fluoro-*myo*-inositol 1,4,5-tris(phosphate) (**6**), (±)-*epi*-inositol 1,4,5-tris(phosphate) (**7**), and (±)-6-deoxy-6-amino-*myo*-inositol 1,4,5-tris(phosphate) (**8**).[19]

Synthesis

The physicochemical analyses do not require optically active compounds; therefore the synthesis were devel-

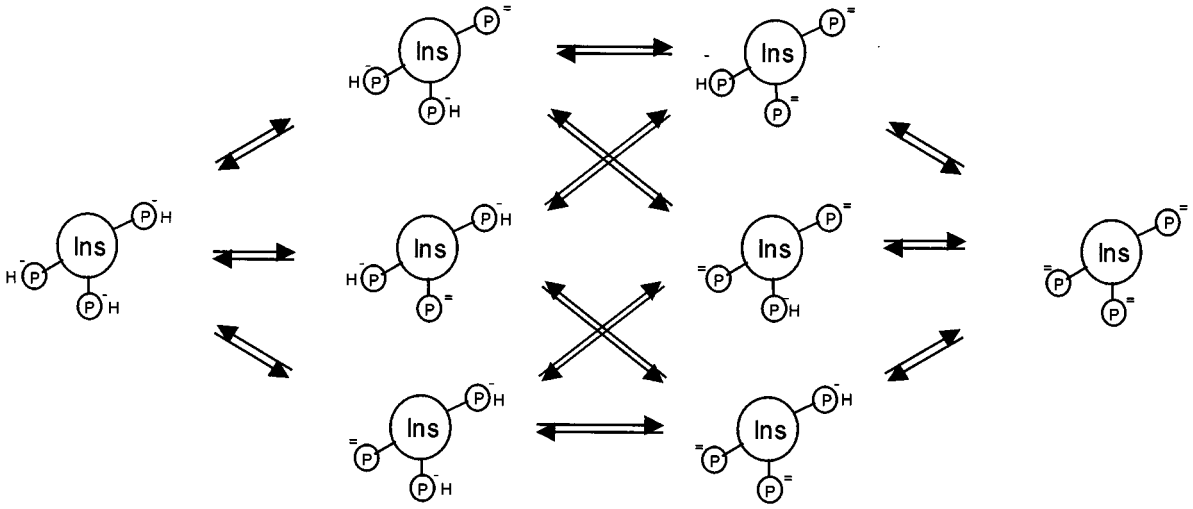
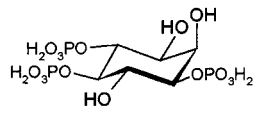


Figure 2. Microspecies and microequilibria for an IP$_3$.



Ins(1,4,5)P_3

1

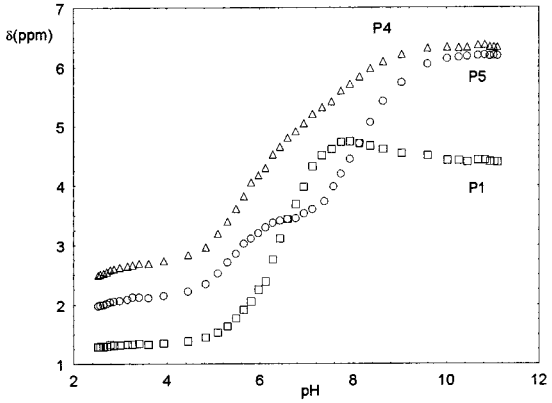


Figure 3. ^{31}P NMR titration curves for *myo*-inositol 1,4,5-tris(phosphate) (**1**).

2,3,6-tri-deoxy-Ins(1,4,5)P_3

4

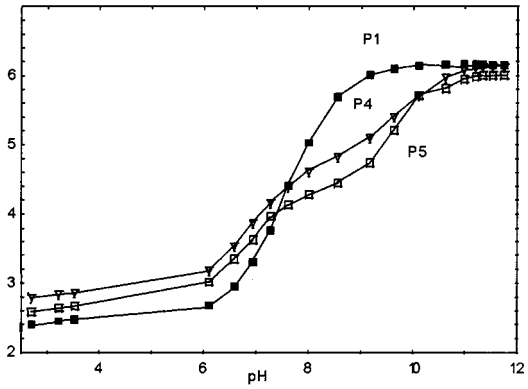
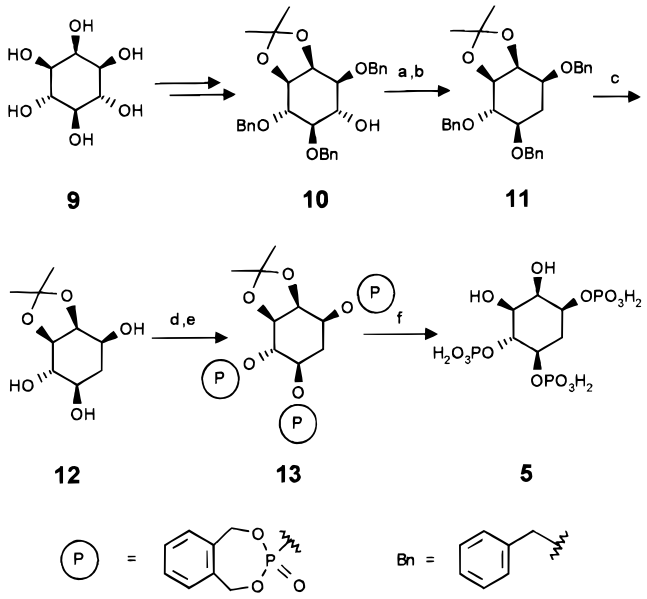


Figure 4. ^{31}P NMR titration curves for 2,3,6-trideoxy-*myo*-inositol 1,4,5-tris(phosphate) (**4**).

oped in the racemate series. If the binding property had justified it, resolution of the racemates would have been possible, as was done by the preparation of camphanic,[21,22] menthoxy acetic,[23] or orthoesters[24] diastereomers.

Synthesis of (±)-6-Deoxy-*myo*-inositol 1,4,5-Tris(phosphate) (5). The synthesis of this compound will give us some information on the influence of the OH in position 6 on the ionization state of the phosphate groups and its possible influence on the cooperation among the phosphates. Its synthesis has been previously reported starting from benzene[25] or from galactose,[26,27] while our starting material was *myo*-inositol (**9**) (Scheme 1). The *myo*-inositol was converted into 1,4,5-tri-*O*-benzyl-2,3-*O*-isopropylidene-*myo*-inositol (**10**) using the following conditions. Massy's procedure[28] was used to form the isopropylidene moiety. Subsequent treatment with benzyl bromide in the presence of dibutyltin oxide using the procedure of Gigg and Co.[29,30]

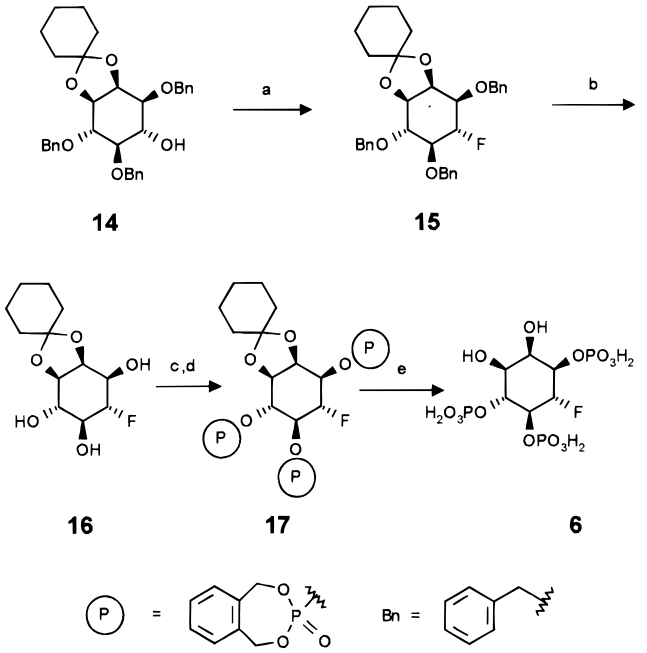
Scheme 1. Synthesis of 6-Deoxy-*myo*-inositol 1,4,5-Tris(phosphate) (**5**)[a]



9 **10** **11** **12** **13** **5**

[a] (a) NaH, CS_2, MeI; (b) Bu_3SnH, AIBN; (c) H_2, 10% Pd/C; (d) *o*-$C_6H_4(CH_2O)_2PN(C_2H_5)_2$, *1H*-tetrazole; (e) *m*-CPBA; (f) H_2, 10% Pd/C, H^+.

Scheme 2. Synthesis of 6-Deoxy-6-fluoro-*myo*-inositol 1,4,5-Tris(phosphate) (**6**)[a]

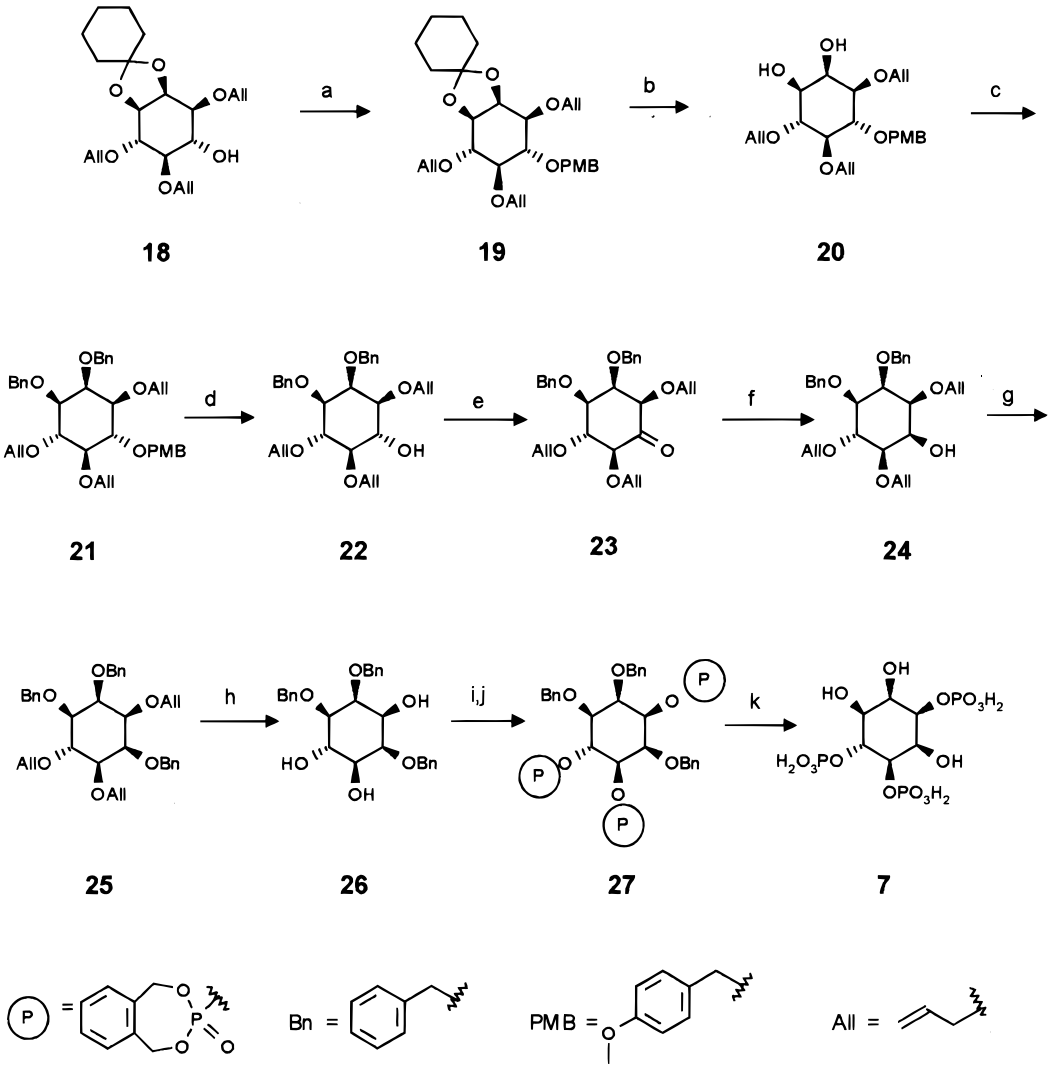


14 **15** **16** **17** **6**

[a] (a) DAST; (b) H_2, 10% Pd/C; (c) *o*-$C_6H_4(CH_2O)_2PN(C_2H_5)_2$, *1H*-tetrazole; (d) *m*-CPBA; (e) H_2, 10% Pd/C, H^+.

formed the derivative **10**. The hydroxyl in position 6 of compound **10** was removed, using the method proposed by Barton-McCombie.[31] Thus, treatment of the alcohol **10** with sodium hydride followed by carbone disulfide and finally methyl iodide formed the *S*-methylxanthate, which was reduced by tributyltin hydride to yield the deoxy derivative **11**. Neutral hydrogenolysis in the presence of palladium on charcoal (10%) gave the 1,4,5-trihydroxy derivative **12**, which was phosphorylated by the phosphite method[32] to form the protected tris(phosphate) **13**. The final product **5** was obtained by hydrogenolysis using palladium on charcoal (10%), but

Scheme 3. Synthesis of *epi*-Inositol 1,4,5-Tris(phosphate) (**7**)[a]



[a] (a) NaH, PMBCl; (b) THF, MeOH, H_2O, TFA; (c) NaH, BnBr; (d) THF, MeOH, HCl; (e) DMSO, $(COCl)_2$, Et_3N; (f) EtOH, $NaBH_4$; (g) NaH, benzyl bromide; (h) DABCO, $RhCl(PC_6H_5)_3$, then $Hg(OAc)_2$; (i) *o*-$C_6H_4(CH_2O)_2PN(C_2H_5)_2$, 1*H*-tetrazole; (j) *m*-CPBA; (k) H_2, 10% Pd/C.

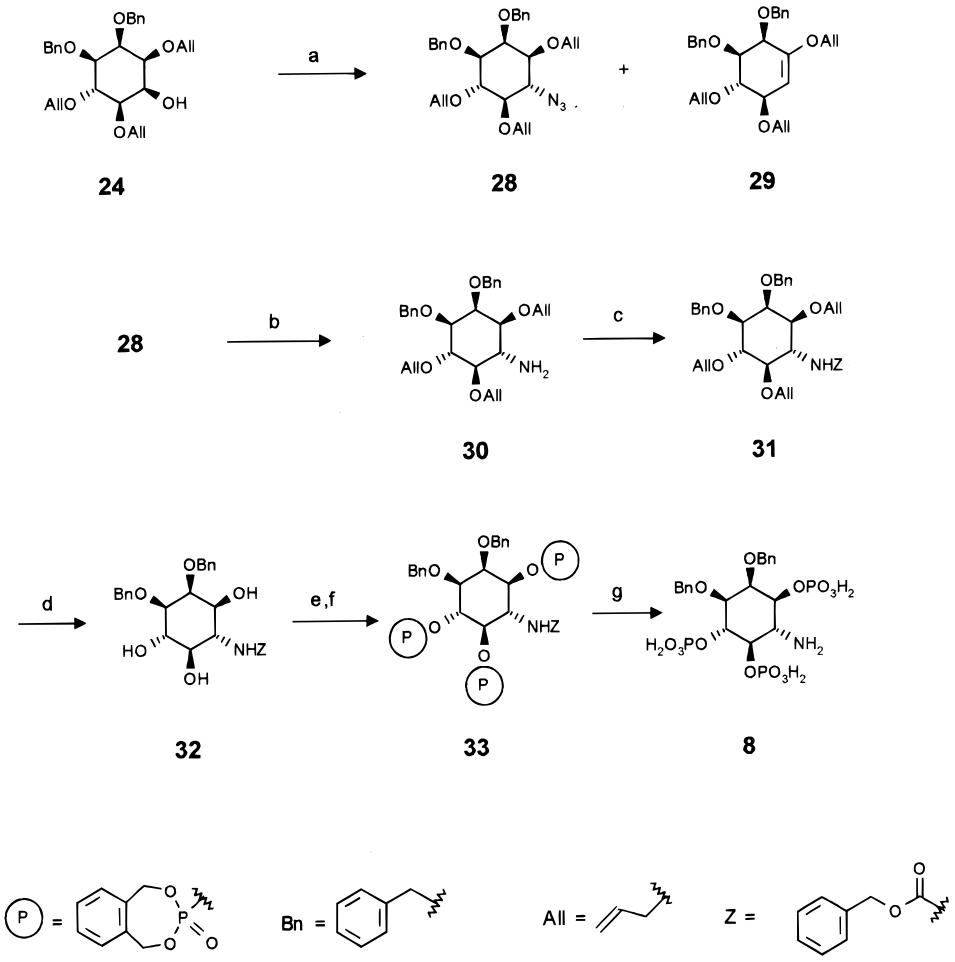
this time in an acidic medium, leading to the simultaneous deprotection of the benzyl-like groups as well as the isopropylidene moiety. The product **5** was stored as its cyclohexylammonium salt until used for physicochemical or binding investigations.

Synthesis of (±)-6-Deoxy-6-fluoro-*myo*-inositol 1,4,5-Tris(phosphate) (6). The isosteric replacement of the hydroxyl group by a fluorine atom could give some information about an intramolecular receiving or donating hydrogen bond. The synthesis of this fluoro derivative was previously described by Ley and et al.[25] in a synthetic scheme requiring 12 steps. We have developed a short, 6-step synthesis of this analogue. According to the same procedure described above for the deoxy derivative **5**, *myo*-inositol (**9**) was transformed into 2,3-*O*-cyclohexylidene-1,4,5-tri-*O*-benzyl-*myo*-inositol (**14**)[28,29,30] (Scheme 2). The use of DAST led to an unusual retention of the *myo* configuration giving the fluorinated derivative **15**. Such retentions have been previously reported[33,34] and involved anchimeric assistance of neighboring groups. In our case, this assistance could be obtained from the benzyl ethers through the formation of benzyloxonium. The formation of the oxomium and the introduction of the fluorine probably act in a concerted mechanism to form the fluoro derivative **15** with retention of configuration.[35] As for the deoxy derivative **5** the final steps of the synthesis were the neutral hydrogenolysis of the benzyl ethers to give the triol **16** followed by their phosphorylation to give compound **17**. Total deprotection yielded the expected compound **6**, stored as its cyclohexylammonium salt.[36]

Synthesis of (±)-*epi*-Inositol 1,4,5-Tris(phosphate) (7). The orientation of the hydroxyl group could influence the ionization state or/and the binding property and modify the ability to establish a hydrogen bond. To probe the influence of the orientation, we prepared an analogue where the carbon in position 6 was inverted. As was done for the two previous molecules, we started the synthesis with *myo*-inositol (**9**), which was converted into the 2,3-*O*-cyclohexylidene-1,4,5-tri-*O*-allyl derivative **18** using a similar procedure to the one reported above for compounds **10** and **14** (Scheme 3).

As long as a cyclohexylidene group was maintained to protect positions 2 and 3 the reactivity of position 6 remained very low.[37] For example, it was not possible to invert the orientation of this OH by a Mitsunobu

Scheme 4. Synthesis of 6-Amino-6-deoxy-*myo*-inositol 1,4,5-Tris(phosphate) (**8**)[a]



[a] (a) Triflic anhydride, NaN_3; (b) $SnCl_2$, C_6H_5SH, Et_3N; (c) NaOH, $C_6H_5CH_2OCOCl$; (d) $ZnCl_2$, $Pd(P(C_6H_5)_3)_4$; (e) *o*-$C_6H_4(CH_2O)_2PN(C_2H_5)_2$, *1H*-tetrazole; (f) *m*-CPBA; (g) H_2, 10% Pd/C.

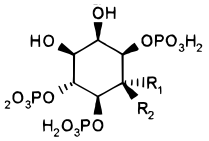
reaction. It was therefore necessary to use an alternative protecting group. Thus, the last hydroxyl of compound **18** was protected as a *p*-methoxybenzyl ether, **19**. The cyclohexylidene moiety was subsequently removed by hydrolysis to form **20**. Reprotection of the two hydroxyls as benzyl ethers yielded the derivative **21**. The free hydroxyl in position 6 was recovered by acidic hydrolysis to give the alcohol **22**. To obtain the inverted alcohol, it was necessary to proceed through a Swern oxidation to give the ketone **23**. Stereoselective reduction of this ketone by sodium borohydride[38] yielded mainly the alcohol **24**, in the *epi* configuration. This hydroxyl was protected as a benzyl ether to obtain the fully protected derivative **25.** The allyl groups were then removed by successive treatments with tris(triphenylphosphine)rhodium chloride followed by mercuric acetate to yield the triol **26**. The triol was phosphorylated (compound **27**) and deprotected as seen above to supply the expected *epi*-inositol **7**.

Synthesis of (±)-6-Deoxy-6-amino-*myo*-inositol 1,4,5-Tris(phosphate) (8). At physiological pH the 6-amino function should be protonated. The presence of a positive charge between the phosphates in positions 1 and 6 could induce Coulombic interactions. For this synthesis, we used the same intermediate **24** reported for the synthesis of the *epi*-inositol 1,4,5-tris(phosphate) (**7**). The free alcohol of compound **24** was activated as a trifluoromethanesulfonate and then substituted by sodium azide to give the azide **28** possessing, again, the *myo* configuration accompanied by a significant amount of the elimination product **29** (Scheme 4). The azido function was reduced to the primary amine **30** according to Barta et al. by treatment with stannyl chloride and thiophenol in the presence of triethylamine.[39] The amino group was protected as a benzyloxycarbamate to give the totally protected derivative **31**. Treatment of the allyl ethers with tributyltin hydride in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium and zinc chloride[40,41] yielded the free 1,4,5- triol **32** which was phosphorylated (compound **33**) and finally deprotected to yield the expected compound **8** and converted to the cyclohexylammonium salt as described above.

Results

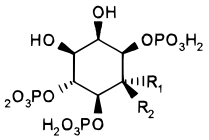
Binding Properties. The affinity of the prepared compounds for the Ins(1,4,5)P_3 receptor was measured by binding on bovine adrenal cortex microsomes.[36,42] The microsomes (1 mg of protein) were incubated in a buffer solution containing 25 mM Tris/HCl, pH 8.5, 100 mM KCl, 20 mM NaCl, 5 mM KH_2PO_4, 1 mM EDTA, and 0.1% bovine serum albumin. Incubations were performed for 60 min at 0 °C, in a final volume of 500 μL, with [^{3}H]InsP_3 (15 000 cpm) and concentrations of compounds ranging from 0.1 nM to 10 μM. Nonspecific binding was determined in the presence of 1 μM InsP_3.

Table 1. Binding Properties



compd	R_1	R_2	IC_{50} (μM)
5	H	H	1.0
6	F	H	2.0
7	H	OH	3.0
8	NH_2	H	5.0
1	OH	H	14 nM
4			3.9

Table 2. Stepwise Macroscopic Equilibrium Constants



			log K_y			
compd	R_1	R_2	$y = 1$	$y = 2$	$y = 3$	$y = 4$
5	H	H	8.30	6.65	5.53	
6	F	H	8.76	6.64	5.56	
7	H	OH	8.01	6.39	5.40	
8	NH_2	H	10.69	7.35	5.58	4.62
1	OH	H	7.85	6.40	5.31	

Incubations were terminated by centrifugation at 15000*g* for 15 min. The supernatant, which contained the free ligand, was removed, and the microsomal pellet containing the receptor bound radioactivity was analyzed by liquid scintillation counting. The experimental results were analyzed with the KELL program (distributed by Biosoft, Cambridge, UK) which uses a weighted nonlinear curve-fitting routine with the Marquardt–Levenberg modification of the Gauss–Newton technique.[43] The results are reported as IC_{50} values (concentration of compound inhibiting 50% of tracer binding) in Table 1.

All the modifications made on the position 6 led to compounds with dramatically lower affinities toward the ERR. All analogues were generally 2 orders of magnitude less active than the parent compound **1**.

Acid–Base Studies. Acid–base analyses were run at 37 °C, in a 0.2 M KCl medium which roughly mimics the temperature and the ionic strength of the cell.

Macroscopic Constants. Mathemathical treatments[13–15] of the data obtained by potentiometric analyses led to the stepwise equilibrium constants reported in Table 2.

Inframolecular Results. Figures 5–8 report the inframolecular ^{31}P NMR titration curves obtained for the derivatives **5**–**8**, respectively.

Discussion

The hydrogen bond donor character and the orientation of the hydroxyl in this position both seem important for the affinity. To explain the decrease in affinity for the ERR by 2 orders of magnitude, it may be necessary to add an additional factor to the classical formation of a hydrogen bond between position 6 and the receptor sites.

6-deoxy-Ins(1,4,5)P_3

5

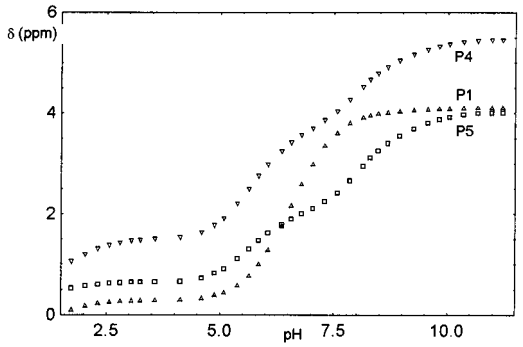
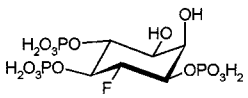


Figure 5. ^{31}P NMR titration curves for 6-deoxy-*myo*-inositol 1,4,5-tris(phosphate) (**5**).



6-F-Ins(1,4,5)P_3

6

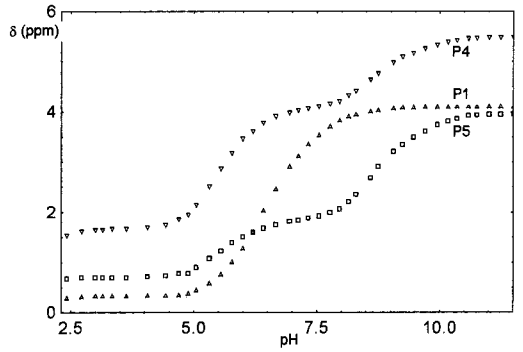
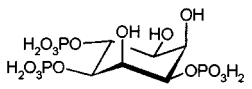


Figure 6. ^{31}P NMR titration curves for 6-deoxy-6-fluoro *myo*-inositol 1,4,5-tris(phosphate) (**6**).

Macroscopic Approach. In terms of the macroscopic protonation constants K_1, the synthesized compounds and the parent compound **1** can be classified according to their decreasing basicity: 6-fluoro derivative **6** > 6-deoxy derivative **5** > *epi* derivative **7** > Ins(145)P_3 (**1**). As the positions of the phosphate groups always remain the same for all compounds, such a variation involves, a fortiori, the neighboring hydroxyls.

The hydroxyls can contribute in differents ways to diminish the basicity of the phosphate. They can modify the solvation shell of the phosphate. They can also form an intramolecular hydrogen bond with the phosphate group or proceed by an attractor inducing effect through the bond between the hydroxyls and the phosphate groups.[10] This last contribution can be discarded due to the induction effect of the fluorine atom. Because this effect is stronger than that of the hydroxyl, it should lead to a decrease in basicity of the fluoro derivative **6** in comparison to the parent compound **1**. In fact, the fluoro derivative **6** is the most basic compound of this series.



epi-Ins(1,4,5)P_3

7

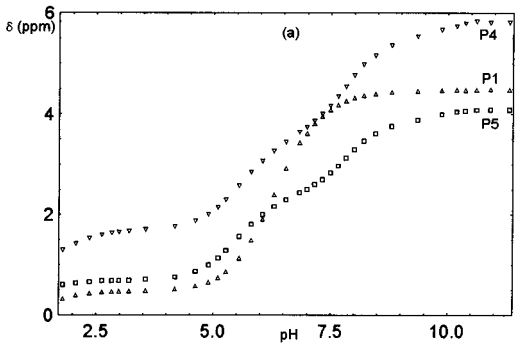
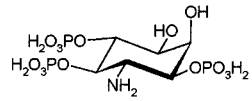


Figure 7. ^{31}P NMR titration curves for *epi*-inositol 1,4,5-tris(phosphate) (**7**).



6-NH_2-Ins(1,4,5)P_3

8

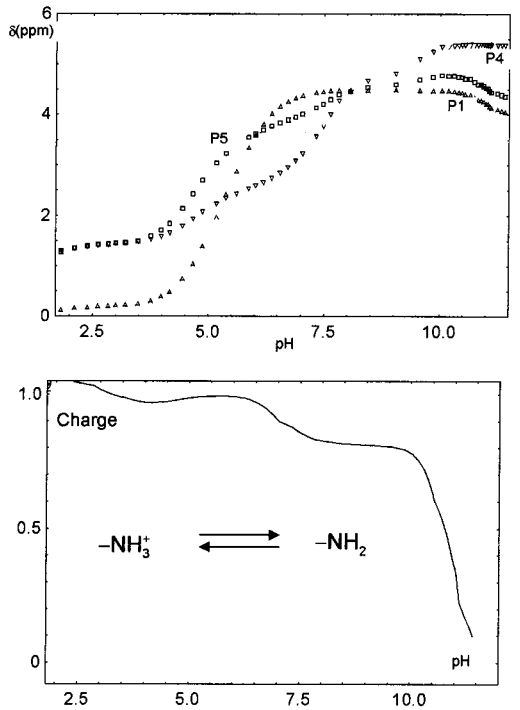


Figure 8. ^{31}P NMR titration curves and ammonium−amine equilibria for 6-amino-6-deoxy-*myo*-inositol 1,4,5-tris(phosphate) (**8**).

If we take into account the π factor which gives a measure of the hydrophobicity of the different substituents of position 6, the classification will be as follows: fluoro derivative **6** (+0.14) > deoxy derivative **5** (0.00) > hydroxy derivative **1** (−0.67). Consequently, one could presume that the observed increase in basicity going from the epimerized hydroxyl to the 6-deoxy derivative to the fluorinated analogue is due to the reduction of the local dielectric constant of the medium, as these modifications diminish the hydrophilicity of the local zone.

Inframolecular Approach. The shapes of the inframolecular titration curves obtained by ^{31}P NMR are relatively similar for the deoxy **5**, fluoro **6**, and epi **7** analogues (Figures 5−7). In all cases, the complex pattern of the parent compound **1** is notably simplified. The contribution of intramolecular cooperation looks reduced, and the aspect of the curves is reduced to the superimposition of two independent systems. One is constituted by the phosphate in position 1 which exhibits a classical monophasic evolution as observed for an independent phosphate. The other is constituted by the two vicinal phosphates in positions 4 and 5 showing a biphasic behavior comparable to that which we have observed for a simple vicinal bis(phosphate). Even if modest, the substituent variations we have made on position 6 lead to dissociate these two groups. It is intriguing to observe that this dissociation in the cooperation effects seems consistent with a dramatic loss of affinity. If we compare the different analogues, shifts are also observed for the limit values of the chemical shift of both the monoprotonated species (δ_p) and the totally deprotonated species (δ_d). These magnetic shieldings are due to local fields linked to local dielectric constants or to local lipophilic factors (such as those mentioned for the macroscopic approach). A detailed analysis of these shieldings will be published in a future manuscript.

For the aminated derivative **8**, the presence of the amine, an additional protonable function, gives a more complex problem. In addition to the titration of the different phosphate groups, the potentiometric analysis combined with the ^{31}P NMR results permits the reconstitution of the titration of the amine in position 6 (Figure 8). It is interesting to note that, even at relatively high pH values, a partial positive charge remains on this ammonium. Such a charge could induce Coulombic interactions with the neighboring phosphate leading to an unsuitable charge distribution into the space. In addition, the phosphate may be forced into conformations inadequate for binding to the receptor sites. One can also consider that such a partial charge on the ammonium is incompatible with the corresponding area of the receptor sites.

Even if tenuous, the modifications we made in position 6 led to a dramatic loss of affinity and induced large changes in the behavior of all three phosphate groups. Particularly, the cooperative effects between the phosphate groups were greatly modified. In addition to the interactions between Ins(1,4,5)P_3 (**1**) and the ERR, it seems possible that some intramolecular interactions on Ins(1,4,5)P_3 (**1**) could generate the right conformation with the right charge distributions for suitable dynamic interactions with the receptors.

More work is in progress to probe the preservation or the re-establishment of the cooperative effects between the two groups of phosphates observed for Ins(1,4,5)P_3 (**1**), and to examine if the cooperative effects could be associated with the affinity, or even to the activity, of this important second messenger.

Experimental Section

General Methods. Melting points were measured on a Mettler PF62 apparatus and are uncorrected. Microanalyses were performed at the Service Central d'Analyses du CNRS in Vernaison, France. Mass spectra were recorded at the Faculté de Chimie de Strasbourg, France. NMR spectra were run on a Bruker Avance DPX 300-MHz spectrometer using the δ scale with residual $CHCl_3$, 85% H_3PO_4, and $CFCl_3$ as references for proton, phosphorus, and fluorine, respectively. Coupling constants are given in Hz; s, d, t, q, m, and ls means singlet, doublet, triplet, quadruplet, multiplet, and large singlet, respectively. THF, ether, and toluene were dried over sodium; CH_2Cl_2 was distilled over CaH_2; triethylamine and pyridine were dried over KOH; acetonitrile and benzene were kept over 3 Å molecular sieves. Column chromatographies were performed with silca gel 60 (Merck).

(±)-1,4,5-Tri-*O*-benzyl-6-deoxy-2,3-*O*-isopropylidene-*myo*-inositol (11). Dry (±)-1,4,5-tri−*O*-benzyl-2,3−*O*−isopropylidene-*myo*−inositol (**10**)[28−30,44] (800 mg; 1.63 mmol) was dissolved in dried THF (8 mL). The reaction mixture was cooled to 0 °C and sodium hydride (5 equiv; 326 mg; 60% dispersion; 8.15 mmol) was added. The mixture was warmed again to room temperature and stirred for 20 min. Carbone disulfide (15 equiv; 1.50 mL; 24.4 mmol) was slowly added and then the mixture was refluxed for 1 h. After cooling to room temperature, methyl iodide (5 equiv; 220 μL; 3.60 mmol) was added and stirring at room temperature was maintained for 16 h. The mixture was diluted with ethanol (2 mL), water (4 mL), and ethyl acetate (50 mL). The organic layer was washed with an aqueous saturated NH_4Cl solution (50 mL) then brine (50 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. The crude product was dissolved in anhydrous toluene (15 mL) and Bu_3SnH (4 equiv; 1.75 mL; 6.5 mmol) and AIBN (30 mg) were added. The reaction mixture was refluxed in an argon atmosphere for 30 min then concentrated and the crude product was purified by silica gel column chromatography (ether−hexane, 1:2) giving 630 mg (82%) of compound **11** as a colorless oil. R_f = 0.57 (ether−hexane, 1:1). Anal. ($C_{30}H_{34}O_5$) C, H. ^{1}H NMR ($CDCl_3$): 7.5−7.1 (m, 15H, -(C_6H_5)$_3$); 4.85 (AB, J_{AB} = 11.5, $\Delta\delta$ = 0.09, 2H, −O−CH_2−C_6H_5); 4.68 (AB, J_{AB} = 12.3, $\Delta\delta$ = 0.05, 2H, −O−CH_2−C_6H_5); 4.87 (AB, J_{AB} = 11.7, $\Delta\delta$ = 0.02, 2H, −O−CH_2−C_6H_5); 4.35 (t, *J* = 4.0, 1H, *H-2*); 4.07 (dd, *J* = 6.8, *J* = 5.2, 1H, *H-3*); 3.66 (dd, *J* = 9.3, *J* = 6.8, 1H, *H-4*); 3.61 (dt, *J* = 12.1, *J* = 4.0, 1H, *H-1*); 3.33 (ddd, *J* = 11.5, *J* = 9.3, *J* = 4.0, 1H, *H-5*); 2.21 (dt, *J* = 12.2, *J* = 3.8, 1H, *H-6$_{eq}$*); 1.96 (q, *J* = 12.1, 1H, *H-6$_{ax}$*); 1.54 (s, 3H, CH_3); 1.42 (s, 3H, CH_3).

(±)-6-Deoxy-2,3-*O*-isopropylidene-*myo*-inositol (12). The tribenzylated derivative **11** (100 mg; 0.22 mmol) was dissolved in 15 mL of ether−EtOH−H_2O (2:2:1) mixture. 10% Pd/C (50 mg) was added to the reaction mixture which was hydrogenized (5 atm) for 16 h. The reaction mixture was filtered and evaporated to dryness. Pure triol **12** was obtained as an oil (44 mg, 98%). R_f = 0.38 (CH_2Cl_2−MeOH, 9:1). Anal. ($C_9H_{16}O_5$) C, H. ^{1}H NMR (DMSO-d_6): 4.81 (d, *J* = 4.2, 1H, exchangeable, O*H-1*); 4.80 (d, *J* = 6.0, 1H, exchangeable, O*H-4*); 4.60 (d, *J* = 4.2, 1H, exchangeable, O*H-5*); 4.10 (t, *J* = 4.0, 1H, *H-2*); 3.9−3.7 (m, 2H, containing at 4.72 (dd, *J* = 6.9, *J* = 5.1, 1H, *H-3*) and *H-1*); 3.3−3.0 (m, 2H, *H-4* and *H-5* giving after exchange with D_2O at 3.19 (dd, *J* = 9.5, *J* = 6.9, 1H, *H-4*) and at 3.12 (td, *J* = 9.9, *J* = 3.8, 1H, *H-5*)); 1.72 (dt, *J* = 11.9 and *J* = 4.2, 1H, *H-6$_{eq}$*); 1.55 (q, *J* = 11.7, 1H, *H-6$_{ax}$*); 1.42 (s, 3H, CH_3); 1.26 (s, 3H, CH_3).

(±)-6-Deoxy-2,3-*O*-isopropylidene-*myo*-inositol 1,4,5-Tri-*O*-(orthoxylylene)phosphate (13). Dry triol **12** (50 mg; 0.25 mmol) and 1*H*-tetrazole (12 equiv; 210 mg; 3.0 mmol) were dissolved in 5 mL of anhydrous THF. The solution was cooled at −78 °C and *N*,*N*-diethyl *O*-xylylene phosphoramidite (6 equiv; 360 mg; 1.5 mmol) was added. After warming up again to room temperature for 8 h the reaction mixture was cooled again at −78 °C, and *m*CPBA (12 equiv; 740 mg; 3.0 mmol) dissolved in 4 mL of CH_2Cl_2 was added. After warming up again to room temperature and stirring at this temperature for 30 min, CH_2Cl_2 (30 mL) was added and the mixture was washed with a 10% aqueous solution of Na_2SO_3 (2 × 50 mL) and a saturated aqueous solution of $NaHCO_3$ (2 × 50 mL). The organic layer was dried over Na_2SO_4, evaporated to dryness, and purified by silica gel column chromatography (CH_2Cl_2−MeOH, 97:3). The tris(phosphorylated) derivative **13** was obtained as a white powder (133 mg, 70%). R_f = 0.21 (CH_2Cl_2−MeOH, 97:3). Anal. ($C_{33}H_{37}O_{14}P_3$) C, H. ^{1}H NMR{^{31}P} ($CDCl_3$): 7.5−7.1 (m, 12H, −(C_6H_4)$_3$); 5.6−5.0 (m, 12H, −(O−CH_2−C_6H_4−CH_2−O)-$_3$); 4.90 (dt, *J* = 11.9, *J* = 4.0, 1H, *H-1*); 4.78 (dd, *J* = 9.3, *J* = 7.5, 1H, *H-4*); 4.62 (t, *J* = 4.2, 1H, *H-2*); 4.55 (td, *J* = 10.6, *J* = 4.2, 1H, *H-5*); 4.21 (dd, *J* = 7.1, *J* = 4.7, 1H, *H-3*); 2.76 (dt, *J* = 12.4, *J* = 4.2, 1H, *H-6$_{eq}$*); 2.43 (q, *J* = 11.9, 1H, *H-6$_{ax}$*); 1.67 (s, 3H, CH_3); 1.46 (s, 3H, CH_3). ^{31}P NMR{^{1}H} ($CDCl_3$): −0.11 (s, 1P); −0.90 (s, 1P); −2.11 (s, 1P). MS (FAB$^+$) M 750.1: *m*/*z* 751.0 [M + H].

(±)-6-Deoxy-*myo*-inositol 1,4,5-Tris(phosphate) (5). Phosphotriester **13** (100 mg; 0.13 mmol) dissolved in 16 mL of CH_2Cl_2−MeOH−H_2O (1: 2: 1) mixture was hydrogenized (5 atm) in the presence of 10% Pd/C (0.1 g) and acetic acid (0.5 mL) for 12 h at 20 °C. Pd/C was filtered and the filtrate was evaporated to dryness. The crude product was dissolved in 2 mL of bidistilled water then cooled to 0 °C, and cyclohexylamine (1 mL) was added. The reaction mixture was evaporated to dryness and redissolved in 0.5 mL of bidistilled water and the cyclohexylammonium salts were precipitated by addition of 80 mL of acetone. The salts were filtered and precipitated again by 80 mL of acetone. After filtration 54 mg of (±)-6-deoxy-*myo*-inositol 1,4,5-tris(phosphate) cyclohexylammonium (**5**) were obtained[41] (68%). ^{1}H NMR{^{31}P} (D_2O): 4.5−4.1 (m, 4H, *H-1*, *H-2*, *H-4*, *H-5*); 3.78 (dd, *J* = 9.2, *J* = 4.2, 1H, *H-3*); 2.39 (dt, *J* = 11.9, *J* = 4.2, 1H, *H-6$_{eq}$*); 2.14 (q, *J* = 11.9, 1H, *H-6$_{ax}$*). ^{31}P NMR{^{1}H} (pH = 11.2) (D_2O): 5.45 (s, 1P, *P-4*); 4.11 (s, 1P, *P-1*); 4.01 (s, 1P, *P-5*). MS (FAB$^-$) M 404.1: *m*/*z* 403.1 [M − H].

(±)-1,4,5-Tri-*O*-benzyl-2,3-*O*-cyclohexylidene-6-deoxy-6-fluoro-*myo*-inositol (15). DMAP (2.2 equiv; 269 mg; 2.2 mmol) was added to a solution of (±)-1,4,5-tri-*O*-benzyl-2,3-*O*-cyclohexylidene-*myo*-inositol (**14**)[28,30,45] (550 mg; 1.04 mmol) in 10 mL of anhydrous toluene. The solution was cooled to −30 °C and DAST (1.9 equiv; 260 μL; 2 mmol) was added. The reaction mixture was then heated at 60 °C for 3 h. The excess of DAST was neutralized by adding 10 mL of a saturated aqueous $NaHCO_3$ solution. The reaction mixture was extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography (ether−hexane, 1:2). The fluoro derivative **15** was obtained (443 mg; 80.2%) as a yellowish oil. R_f = 0.67 (ether−hexane, 1:2). Anal. ($C_{33}H_{37}FO_5$) C, H, F. ^{1}H NMR ($CDCl_3$−C_6D_6, 1:3): 7.5−7.2 (m, 15H, -($CH_2C_6H_5$)$_3$); 5.00 (dt, J_{HF} = 51.2, *J* = 8.1, 1H, *H-6*); 4.9−4.6 (m, 6H, -($CH_2C_6H_5$)$_3$); 4.18 (dt, *J* = 5.6, *J* = J_{H2F} = 4.0, 1H, *H-2*); 3.97 (t, *J* = 6.2, 1H, *H-3*); 3.77 (dd, *J* = 8.6, *J* = 6.4, 1H, *H-4*); 3.62 (ddd, J_{HFcis} = 11.3, *J* = 8.7, *J* = 3.8, 1H, *H-1*); 3.46 (dt, J_{HFcis} = 16.2, *J* = 8.1, 1H, *H-5*); 1.8−0.7 (m, 10H, C_6H_{10}). ^{19}F NMR ($CDCl_3$): −195.7 (dddd, J_{HF} = 51.2, J_{HFcis} = 16.2, J_{HFcis} = 11.3, J_{H2F} = 4.0, 1F, *F-6*).

(±)-2,3-*O*-Cyclohexylidene-6-deoxy-6-fluoro-*myo*-inositol (16). (±)-1,4,5-Tri−*O*-benzyl-2,3−*O*-cyclohexylidene-6-deoxy-6-fluoro−*myo*−inositol (**15**) (240 mg; 0.45 mmol) was dissolved in 20 mL of ether−EtOH (1:3) mixture. 10% Pd/C (100 mg) was added to the reaction mixture which was hydrogenized (5 atm) for 16 h. The reaction mixture was filtered and evaporated to dryness. Triol **16** (115 mg; 97%) was obtained as an oil. R_f = 0.25 (AcOEt). ^{1}H NMR (D_2O−(CD_3)$_2$-CO, 1:1): 4.40 (dt, *J* = 8.8, J_{HF} = 52.6, 1H, *H-6*); 4.33 (dd, *J* = 8.8, *J* = 4.4, 1H); 4.0−3.8 (2H); 3.6−3.3 (m, 2H); 1.7−1.2 (m, 10H, C_6H_{10}). ^{19}F −NMR (D_2O): −194.8 (ddd, J_{HF} = 55.5, J_{HFcis} = 15.0, J_{HFcis} = 4.5, 1F, *F-6*).

(±)-2,3-*O*-Cyclohexylidene-6-deoxy-6-fluoro-*myo*-inositol 1,4,5-Tri-*O*-(orthoxylylene)phosphate (17). (±)-2,3−*O*-Cyclohexylidene-6-deoxy-6-fluoro−*myo*−inositol (**16**) (80 mg; 0.31 mmol) and 1*H*-tetrazole (10 equiv; 215 mg; 3.05 mmol) were dissolved in 10 mL of anhydrous THF. The solution was cooled to −78 °C and *N*,*N*-diethyl *O*-xylylene phosphoramidite

(5 equiv; 365 mg; 1.53 mmol) was added. After warming up to room temperature the mixture was stirred for 8 h and then cooled again to −78 °C and *m*CPBA (3.3 equiv; 500 mg; 2 mmol) dissolved in 8 mL of CH_2Cl_2 was added. The reaction mixture was then kept at room temperature for 30 min. After evaporation to dryness, the residue was redissolved in 30 mL of CH_2Cl_2 and washed with a 10% Na_2SO_3 aqueous solution (2 × 50 mL) then with a 5% $NaHCO_3$ aqueous solution (2 × 50 mL). The organic layer was dried over Na_2SO_4, evaporated to dryness, and chromatographed on a silica gel column (AcOEt + 0.1% Et_3N). The tris(phosphorylated) compound **17** (160 mg, 65%) was obtained as a white powder. R_f = 0.25 (AcOEt + 0.1% Et_3N). ^{1}H NMR ($CDCl_3$): 7.5−7.2 (m, 12H, (−CH_2−C_6H_4−CH_2-)$_3$); 5.2−4.6 (m, 13H, (−CH_2−C_6H_4−CH_2-)$_3$ and *H-6*); 4.35 (dd, J = 6.4, J = 3.7, 1H, *H-2*); 4.16 (t, J = 6.2, 1H, *H-4*); 3.9−3.7 (m, 2H, *H-1* and *H-3*) 3.57 (dt, J = 8.8, J_{HFcis} = 8.0, 1H, *H-5*); 1.8−1.3 (m, 1H, C_6H_{10}). ^{19}F NMR{1H} ($CDCl_3$): −196.0 (s, 1F, *F-6*). ^{31}P NMR{^{1}H} ($CDCl_3$): −1.00 (s, 1P); −1.32 (s, 1P); −1.84 (s, 1P). MS (FAB$^+$) M 808: *m/z* 809 [M + H].

(±)-6-Deoxy-6-fluoro-*myo*-inositol 1,4,5-Tris(phosphate) (6). (±)-2,3−*O*-Cyclohexylidene-6-deoxy-6-fluoro−*myo*-inositol 1,4,5-tri−*O*−(orthoxylylene)phosphate (**17**) (50 mg; 0.062 mmol) was dissolved in a EtOH−H_2O−CH_2Cl_2 (1:1:1) mixture (25 mL). 10% Pd/C (100 mg) and AcOH (0.5 mL) were added and the reaction mixture was hydrogenolized (5 atm H_2) for 16 h at room temperature. After filtration and evaporation to dryness the crude product was dissolved in 5 mL of bidistilled water and cooled to 0 °C; cyclohexylamine (1 mL) was added. The reaction mixture was evaporated to dryness and redissolved in 1 mL of bidistilled water. The cyclohexylammonium salts were precipitated by addition of 80 mL of acetone. After filtration the salts were again dissolved in 1 mL of bidistilled water and precipitated again by 80 mL of acetone. After filtration 43 mg of cyclohexylammonium salts of (±)-6-deoxy-6-fluoro-*myo*-inositol 1,4,5-tris(phosphate) (**6**) were obtained. ^{1}H NMR (D_2O, pH = 11.0, 37 °C): 4.72 (dt, J = 8.4, J_{HF} = 53.5, 1H, *H-6*) 4.3−4.4 (m, 1H, *H-2*); 4.3−4.2 (m, 3H, *H-1*, *H-4* and *H-5*); 3.83 (dd, J = 9.2, J = 2.6, 1H, *H-3*). ^{19}F NMR (D_2O, pH = 11.0): −194.4 (d, J_{HF} = 53.5, *F-6*). ^{31}P NMR (D_2O, pH = 11.0, 37 °C): 5.48 (d, J_{HP} = 6.1, *P-4*); 4.11 (d, J_{HP} = 8.5, *P-1*); 3.96 (d, J_{HP} = 7.9, *P-5*).

(±)-1,4,5-Tri-*O*-allyl-2,3-*O*-cyclohexylidene-6-*O*-*p*-methoxybenzyl-*myo*-inositol (19). (±)-1,4,5-Tri−*O*-allyl-2,3−*O*-cyclohexylidene−*myo*−inositol (**18**)[28−30] (2.88 g; 7.57 mmol) was dissolved in anhydrous DMF (40 mL). The solution was cooled to 0 °C and sodium hydride (2.5 equiv; 756 mg; 60% dispersion, 19 mmol) and *p*-methoxybenzyl chloride (2 equiv; 2.05 mL; 15 mmol) were added. The reaction mixture was warmed again to room temperature and stirred overnight. The mixture was poured in cooled water and extracted with ethyl acetate (2 × 40 mL). The organic layer was washed with water (3 × 25 mL), dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by silica gel column chromatography (ether−hexane, 1:4). *p*-Methoxybenzyl derivative **19** was obtained in quantitative yield (3.8 g). R_f = 0.59 (ether−hexane, 1:1). Anal. ($C_{29}H_{40}O_7$) C, H. ^{1}H NMR ($CDCl_3$): 7.32 and 6.89 (2d, J = 8.6, 4H, −C_6H_4−OCH_3); 6.1−5.9 (m, 3H, -(O−CH_2−C*H*=CH_2)$_3$); 5.4−5.0 (m, 6H, -(O−CH_2−CH=CH_2)$_3$); 4.74 (AB, J_{AB} = 10.1, Δδ = 0.03, 2H, −O−CH_2−C_6H_4−OCH_3); 4.4−4.1 (m, 7H, containing at 4.38 (t, J = 4.7, 1H, *H-2*) and -(O−CH_2−CH=CH_2)$_3$); 4.03 (dd, J = 6.9, J = 5.5, 1H, *H-3*); 3.82 (s, 3H, −OCH_3); 3.81 (t, J = 8.6, 1H, *H-6*); 3.62 (dd, J = 9.7, J = 7.1, 1H, *H-4*); 3.57 (dd, J = 8.8, J = 3.8, 1H, *H-1*); 3.22 (dd, J = 9.5, J = 9.0, 1H, *H-5*); 1.9−1.1 (m, 10H, C_6H_{10}).

(±)-1,4,5-Tri-*O*-allyl-6-*O*-*p*-methoxybenzyl-*myo*-inositol (20). (±)-1,4,5-Tri-*O*-allyl-2,3−*O*-cyclohexylidene-6−*O*−*p*-methoxybenzyl−*myo*−inositol (**19**) (3.87 g; 7.57 mmol) was dissolved in THF (2.5 mL) and EtOH (95%; 25 mL). Water (5 mL) and TFA (1.5 mL) were added and the mixture was refluxed for 2 h. The solution was evaporated to dryness and the crude product was redissolved in H_2O−AcOEt. The aqueous phase was reextracted with AcOEt. The organic layer was washed with water, dried over Na_2SO_4, filtered, and evaporated. Column chromatography on silica gel (ether−hexane, 2:1) gave 2.64 g of diol **20** (84%). R_f = 0.17 (ether−hexane, 2:1). Anal. ($C_{23}H_{32}O_7$) C, H. ^{1}H NMR ($CDCl_3$): 7.30 and 6.88 (2d, J = 8.6, 4H, −C_6H_4−OCH_3); 6.1−5.8 (m, 3H, -(O−CH_2−C*H*=CH_2)$_3$); 5.4−4.9 (m, 6H, -(O−CH_2−CH=CH_2)$_3$); 4.74 (AB, J_{AB} = 10.2, Δδ = 0.04, 2H, −O−CH_2−C_6H_4−OCH_3); 4.5−3.9 (m, 7H, *H-2* and -(O−CH_2−CH=CH_2)$_3$); 3.81 (t, J = 8.6, 1H, *H-6*); 3.80 (s, 3H, −OCH_3); 3.65 (t, J = 9.5, 1H, *H-4*); 3.42 (ddd, J = 9.5, J_{H3OH} = 4.2, J = 3.1, 1H, *H-3*); 3.30 (dd, J = 9.5, J = 2.8, 1H, *H-1*); 3.24 (t, J = 9.4, 1H, *H-5*); 2.78 (d, J = 4.2, 1H, exchangeable, O*H-3*); 2.77 (s, 1H, exchangeable, O*H-2*).

(±)-1,4,5-Tri-*O*-allyl-2,3-di-*O*-benzyl-6-*O*-*p*-methoxybenzyl-*myo*-inositol (21). (±)-1,4,5-Tri-*O*-allyl-6-*O*-*p*-methoxybenzyl-*myo*-inositol (**20**) (2.45 g; 5.83 mmol) was dissolved in anhydrous DMF (40 mL). The solution was placed at 0 °C and sodium hydride (6 equiv; 1.68 g; 60% dispersion; 35.0 mmol) and benzyl bromide (5 equiv; 3.5 mL; 30 mmol) were added. The reaction mixture was warmed again and left at room temperature overnight. The mixture was poured in water and extracted with AcOEt (2 × 40 mL). The organic layer was washed with water (3 × 25 mL), dried over Na_2SO_4, filtered, and evaporated. The crude product was chromatographed on a silica gel column (ether−hexane, 1:4). Compound **21** was obtained in 96% yield (3.35 g) R_f = 0.58 (ether−hexane, 1:1). Anal. ($C_{37}H_{44}O_7$) C, H. ^{1}H NMR (C_6D_6): 7.6−6.8 (m, 14H, containing at 7.32 and 6.88 (2d, J = 8.6, 4H, −C_6H_4−OCH_3) and -(C_6H_5)$_2$); 6.2−5.8 (m, 3H, -(O−CH_2−C*H*=CH_2)$_3$); 5.5−5.1 (m, 6H, -(O−CH_2−CH=CH_2)$_3$); 4.96 (s, 2H, −O−CH_2−C_6H_5); 4.90 (AB, J_{AB} = 10.6, Δδ = 0.04, 2H, −O−CH_2−C_6H_4−OCH_3); 4.7−4.3 (m, 6H, containing at 4.59 (AB, J_{AB} = 11.9, Δδ = 0.10, 2H, −O−CH_2−C_6H_5) and -(O−CH_2−CH=CH_2)$_2$); 4.2−3.9 (m, 5H, containing at 4.14 (t, J = 9.5, 1H, *H-6*), at 4.10 (t, J = 9.7, 1H, *H-4*), at 3.98 (s, 1H, *H-2*) and −O−CH_2−CH=CH_2); 3.49 (s, 3H, −OCH_3); 3.37 (t, J = 9.3, 1H, *H-5*); 3.23 (dd, J = 9.7, J = 1.8, 1H, *H-3*); 3.15 (dd, J = 9.7, J = 2,0. 1H, *H-1*).

(±)-1,4,5-Tri-*O*-allyl-2,3-di-*O*-benzyl-*myo*-inositol (22). (±)-1,4,5-Tri-*O*-allyl-2,3-di−*O*-benzyl-6−*O*−*p*-methoxybenzyl−*myo*−inositol (**21**) (3.25 g; 5.41 mmol) was dissolved in THF (30 mL) and methanol (100 mL). 3 N HCl (50 mL) was added and the mixture was refluxed for 2.5 h. The solvents were evaporated and the crude product was purified by silica gel column chromatography (ether−hexane, 1:1). (±)-1,4,5-Tri−*O*-allyl-2,3-di−*O*-benzyl−*myo*−inositol (**22**) was obtained in 96% yield (2.5 g). R_f = 0.41 (ether−hexane, 1:1). Anal. ($C_{29}H_{36}O_6$) C, H. ^{1}H NMR ($CDCl_3$): 7.5−7.1 (m, 10H, −(C_6H_5)$_2$); 6.2−5.8 (m, 3H, -(O−CH_2−C*H*=CH_2)$_3$); 5.5−5.1 (m, 6H, -(O−CH_2−CH=CH_2)$_3$); 4.84 (AB, J_{AB} = 12.1, Δδ = 0.04, 2H, −O−CH_2−C_6H_5); 4.68 (AB, J_{AB} = 11.9, Δδ = 0.08, 2H, −O−CH_2−C_6H_5); 4.5−4.3 (m, 4H, -(O−CH_2−CH=CH_2)$_2$); 4.2−3.9 (m, 4H, *H-6*, *H-2* and −O−CH_2−CH=CH_2); 3.88 (t, J = 9.5, 1H, *H-4*); 3.33 (dd, J = 9.9, J = 2.2, 1H, *H-3*); 3.22 (t, J = 9.2, 1H, *H-5*); 3.11 (dd, J = 9.9, J = 2.0, 1H, *H-1*); 2.60 (s, 1H, exchangeable, O*H-6*).

(±)-1,4,5-Tri-*O*-allyl-2,3-di-*O*-benzyl-6-*myo*-inosose (23). DMSO (6.3 equiv; 1.4 mL; 20 mmol) was dissolved in CH_2Cl_2 (10 mL) and the solution was cooled to − 78 °C. Oxalyl chloride (5 equiv; 1.4 mL; 16 mmol) was slowly added. The mixture was kept at room temperature for 10 min and then cooled to −78 °C. The alcohol **22** (1.5 g; 3.12 mmol) dissolved in CH_2Cl_2 (2 × 5 mL) was slowly added. After 40 min, triethylamine (20 equiv; 8.7 mL; 62.4 mmol) was added to the mixture which was warmed again to room temperature for 20 min. The mixture was diluted with CH_2Cl_2 (40 mL) and washed with water (2 × 50 mL) and brine (2 × 50 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated. The crude product **23** (2.1 g) was used as such for the next step. An aliquot was purified by chromatography on a silica gel column (ether−hexane, 1:2). R_f = 0.46 (ether−hexane, 1:1). ^{1}H NMR ($CDCl_3$): 7.5−7.2 (m, 10H, -(C_6H_5)$_2$); 6.1−5.8 (m, 3H, -(O−CH_2−C*H*=CH_2)$_3$); 5.4−5.1 (m, 6H, -(O−CH_2−CH=CH_2)$_3$); 4.83 (AB, J_{AB} = 12.1, Δδ = 0.07, 2H, −O−CH_2−C_6H_5); 4.67 (AB, J_{AB} = 11.9, Δδ = 0.05, 2H, −O−CH_2−C_6H_5); 4.5−4.2 (m, 4H, -(O−CH_2−CH=CH_2)$_2$); 4.2−3.8 (m, 6H, containing at 4.14 (t, J = 2.2, 1H. *H-2*), at 4.03 (t, J = 9.3, 1H, *H-4*), at 3.88 (d, J =

1.6, 1H, *H-1*), at 3.90 (d, J = 9.6, 1H, *H-5*) and −O−CH_2−CH=CH_2); 3.72 (dd, J = 9.3, J = 2.2, 1H, *H-3*).

(±)-1,4,5-Tri-*O*-allyl-2,3-di-*O*-benzyl-*epi*-inositol (24). The crude **23** obtained above (2.1 g) was dissolved in absolute ethanol (50 mL) and the temperature was adjusted to 0 °C. $NaBH_4$ (5 equiv; 590 mg; 15.6 mmol) was added and the mixture was stirred at room temperature for 1 h. Water (30 mL) was added and the solvents were evaporated. The residue was redissolved in AcOEt (100 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness.The crude product was chromatographed on a silica gel column (ether−hexane, 1:2). (±)-1,4,5-Tri-*O*-allyl-2,3-di-*O*-benzyl-*epi*-inositol (**24**) was obtained (1.09 g, 73% from **22**). R_f = 0.34 (ether−hexane, 1:1). Anal. ($C_{29}H_{36}O_6$) C, H. ^{1}H NMR ($CDCl_3$): 7.5−7.2 (m, 10H, -(C_6H_5)$_2$); 6.2−5.8 (m, 3H, -(O−CH_2−CH=CH_2)$_3$); 5.4−5.1 (m, 6H, -(O−CH_2−CH=CH_2)$_3$); 4.86 (AB, J_{AB} = 11.2, $\Delta\delta$ = 0.05, 2H, −O−CH_2−C_6H_5); 4.70 (AB, J_{AB} = 11.9, $\Delta\delta$ = 0.12, 2H, −O−CH_2−C_6H_5); 4.5−4.3 (m, 2H, -(O−CH_2−CH=CH_2)$_2$); 4.3−3.9 (m, 8H, containing at 4.04 (t, J = 9.7, 1H, *H-4*), at 4.02 (s, 1H, exchangeable, O*H-6*), *H-6*, *H-2* and -(O−CH_2−CH=CH_2)$_2$); 3.30 (dd, J = 9.7, J = 2.8, 1H, *H-3*); 3.20 (t, J = 2.8, 1H, *H-1*); 3.16 (dd, J = 9.7, J = 3.1, 1H, *H-5*). Alcohol **22** (68 mg) with the *myo* configuration was also obtained.

(±)-1,4,5-Tri-*O*-allyl-2,3,6-tri-*O*-benzyl-*epi*-inositol (25). (±)-1,4,5-Tri-*O*-allyl-2,3-di−*O*-benzyl−*epi*-inositol (**24**) (250 mg; 0.52 mmol) was dissolved in anhydrous DMF (4 mL). The solution was cooled to 0 °C and sodium hydride (1.3 mmol; 2.5 equiv; 52 mg; 60% dispersion) was added. Benzyl bromide (1.04 mmol; 2 equiv; 124 μL) was also added. The mixture was warmed again overnight to room temperature. The mixture was poured in water and extracted with AcOEt (2 × 20 mL). The organic layer was washed with water (3 × 20 mL), dried over Na_2SO_4, filtered, and evaporated. The crude product was purified on a silica gel column (ether−hexane, 1:2). The benzylated compound **25** was obtained in 92% yield (274 mg). R_f = 0.54 (ether−hexane, 1:1). Anal. ($C_{36}H_{42}O_6$) C, H. ^{1}H NMR ($CDCl_3$): 7.5−7.2 (m, 15H, -(C_6H_5)$_3$); 6.2−5.8 (m, 3H, -(O−CH_2−CH=CH_2)$_3$); 5.4−5.1 (m, 6H, -(O−CH_2−CH=CH_2)$_3$); 4.93 (AB, J_{AB} = 12.2, $\Delta\delta$ = 0.06, 2H, −O−CH_2−C_6H_5); 4.92 (AB, J_{AB} = 12.1, $\Delta\delta$ = 0.06, 2H, −O−CH_2−C_6H_5); 4.69 (AB, J_{AB} = 12.1, $\Delta\delta$ = 0.06, 2H, −O−CH_2−C_6H_5); 4.40 (dt, J = 5.7, J = 2.9, 2H, −O−CH_2−CH=CH_2); 4.3−4.0 (m, 5H, containing at 4.22 (t, J = 9.7, 1H, *H-4*), at 4.12 (t, J = 2.6, 1H, *H-2*), *H-6* and −O−CH_2−CH=CH_2); 3.93 (dt, J = 5.1, 4J = 2.9, 2H, −O−CH_2−CH=CH_2); 3.27 (dd, J = 9.7, J = 2.9, 1H, *H-3*); 3.19 (dd, J = 9.9, J = 3.1, 1H, *H-5*); 3.14 (t, J = 2.7, 1H, *H-1*).

(±)-2,3,6-Tri-*O*-benzyl-*epi*-inositol (26). (±)-1,4,5-Tri-*O*-allyl-2,3,6-tri−*O*-benzyl−*epi*-inositol (**25**) (713 mg; 1.25 mmol) and DABCO (0.6 equiv; 277 mg; 0.75 mmol) were dissolved in ethanol−water (9:1, 22 mL). $RhCl(PPh_3)_3$ (0.3 equiv; 280 mg; 0.30 mmol) was added and the mixture was refluxed for 12 h. The reaction mixture was poured in water (100 mL) and extracted with AcOEt (3 × 60 mL). The organic layers were dried over Na_2SO_4, filtered, and evaporated. The crude product was dissolved in a mixture of THF (4.5 mL) and water (3 mL). Under vigorous stirring a solution of mercuric acetate(II) (3.7 equiv; 1.46 g; 4.6 mmol) in water (3.5 mL) was rapidly added to the mixture. After 20 min, the mixture was diluted with AcOEt (40 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and evaporated to dryness. Pure triol **26** was obtained by chromatography on a silica gel column (ether−hexane, 2:1) (460 mg, 80%). R_f = 0.48 (AcOEt). Anal. ($C_{27}H_{30}O_6$) C, H. ^{1}H NMR ($CDCl_3$): 7.5−7.2 (m, 15H, -(C_6H_5)$_3$); 4.94 (AB, J_{AB} = 11.5, $\Delta\delta$ = 0.47, 2H, −O−CH_2−C_6H_5); 4.83 (AB, J_{AB} = 11.9, $\Delta\delta$ = 0.21, 2H, −O−CH_2−C_6H_5); 4.76 (AB, J_{AB} = 11.9, $\Delta\delta$ = 0.10, 2H, −O−CH_2−C_6H_5); 4.26 (t, J = 9.3, 1H, *H-4*); 4.03 (t, J = 3.1, 1H, *H-2*); 3.95 (t, J = 3.5, 1H, *H-6*); 3.72 (dt, J_{H1OH} = 10.8, J = 3.7, 1H, *H-1*); 3.56 (ddd, J = 9.3, J_{H5OH} = 6.4, J = 3.7, 1H, *H-5*); 3.33 (dd, J = 9.1, J = 2.8, 1H, *H-3*); 3.02 (d, J = 10.8, 1H, exchangeable, O*H-1*); 2.63 (d, J = 6.4, 1H, exchangeable, O*H-5*); 2.47 (s, 1H, exchangeable, O*H-4*).

(±)-2,3,6-Tri-*O*-benzyl-*epi*-inositol 1,4,5-Tri-*O*-(*o*-xylylene)phosphate (27). Triol **26** (120 mg; 0.27 mmol) and 1*H*-tetrazole (9 equiv; 168 mg; 2.39 mmol) were dissolved in 8 mL of anhydrous THF. The solution was cooled to −78 °C and *N*,*N*-diethyl *O*-xylylene phosphoramidite (6 equiv; 382 mg; 1.6 mmol) was added. The mixture was warmed again to room temperature. Stirring was maintained for 8 h. The reaction mixture was cooled again to −78 °C, and *m*CPBA (10 equiv; 657 mg; 2.7 mmol) dissolved in CH_2Cl_2 (4 mL) was added. After warming up and stirring at room temperature for 30 min, the mixture was evaporated to dryness, redissolved in 30 mL of CH_2Cl_2, and washed with a 10% Na_2SO_3 aqueous solution (2 × 50 mL) and a $NaHCO_3$ saturated aqueous solution (2 × 50 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness, and the crude residue was chromatographed on a silica gel column (CH_2Cl_2−MeOH, 97:3). The tris(phosphorylated) compound **27** was obtained as a white powder (238 mg; 88%). R_f = 0.44 (CH_2Cl_2−MeOH, 95:5). Anal. ($C_{51}H_{51}O_{15}P_3$) C, H, P. ^{1}H NMR{^{31}P} (DMSO-d_6): 7.6−7.0 (m, 27H, -(C_6H_4)$_3$ and -(C_6H_5)$_3$); 5.6−4.6 (m, 21H, -(O−CH_2−C_6H_4−CH_2−O)$_3$, -(O−CH_2−C_6H_5)$_3$ and 3H cycle); 4.6−4.2 (m, 2H cycle); 4.1−3.9 (m, 1H cycle). ^{31}P NMR{^{1}H} (DMSO-d_6): −0.16 (s, 1P); −2.45 (s, 1P); −3.24 (s, 1P). MS (FAB$^+$) M 996.2: m/z 997.1 [M + H].

(±)-*epi*-Inositol 1,4,5-Tris(phosphate) (7). The phosphotriester **27** (238 mg; 0.24 mmol) dissolved in a CH_2Cl_2−MeOH−H_2O (1: 2: 1) mixture (6 mL) was hydrogenized in the presence of 10% Pd/C (0.2 g) under a hydrogen atmosphere (5 atm) for 12 h at 20 °C. Pd/C was filtered over Celite and the filtrate was evaporated to dryness. The crude product was dissolved in 2 mL of bidistilled water and cooled at 0 °C. Cyclohexylamine (1 mL) was added. The reaction mixture was evaporated to dryness and redissolved in 0.5 mL of bidistilled water. The cyclohexylammonium salts were precipitated by adding 80 mL of acetone. The salts were filtered and precipitated again in 80 mL of acetone. After filtration 178 mg (81%) of (±)-*epi*-inositol 1,4,5-tris(phosphate) (**7**) cyclohexylammonium salt were obtained ^{1}H NMR{^{31}P} (D_2O, pH = 11.3): 4.53 (t, J = 8.7, 1H, *H-4*); 4.44 (s, 1H, *H-6*); 4.31 (s, 1H, *H-2*); 4.20 (s, 1H, *H-1*); 4.16 (t, J = 9.3, 1H, *H-5*); 3.76 (dd, J = 9.3, J = 2.4, 1H, *H-3*). ^{31}P NMR{^{1}H} (D_2O, pH = 11.3): 5.82 (s, 1P, *P-4*); 4.47 (s, 1P, *P-1*); 4.08 (s, 1P, *P-5*). MS (FAB$^-$) M 420.1: m/z 419.1 [M − H, 100%], 509.2 [M − H + C_7H_7, 60%].

(±)-1,4,5-Tri-*O*-allyl-6-azido-2,3-di-*O*-benzyl-6-deoxy-*myo*-inositol (28). (±)-1,4,5-Tri-*O*-allyl-2,3-di-*O*-benzyl-*epi*-inositol (**24**) (500 mg; 1.04 mmol) was dissolved in CH_2Cl_2 (5 mL) and the solution was cooled to 0 °C. Pyridine (0.3 mL) and triflic anhydride (2.2 equiv; 630 μL; 2.3 mmol) were slowly added. After 2 h at 0 °C, the mixture was diluted with CH_2Cl_2 (20 mL) and washed with H_2O at 0 °C (20 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and evaporated to dryness. The crude product was dissolved in DMF (6 mL) and at 0 °C, NaN_3 (5 equiv; 340 mg; 5.20 mmol) was added. After warming up again to room temperature the mixture was stirred overnight, poured in water, and extracted with AcOEt (2 × 20 mL). The organic layer was washed with water, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by silica gel column chromatography (ether−hexane, 1:4). The expected compound **28** was obtained with 59% yield (309 mg). R_f = 0.41 (ether−hexane, 1:4). ^{1}H NMR ($CDCl_3$): 7.5−7.2 (m, 10H, -(C_6H_5)$_2$); 6.1−5.8 (m, 3H, -(O−CH_2−CH=CH_2)$_3$); 5.4−5.1 (m, 6H, -(O−CH_2−CH=CH_2)$_3$); 4.85 (s, 2H, −O−CH_2−C_6H_5); 4.64 (AB, J_{AB} = 11.9, $\Delta\delta$ = 0.08, 2H, −O−CH_2−C_6H_5); 4.5−4.2 (m, 4H, -(O−CH_2−CH=CH_2)$_2$); 4.1−4.0 (m, 2H, −O−CH_2−CH=CH_2); 3.98 (t, J = 2.0, 1H, *H-2*); 3.90 (t, J = 10.0, 1H, *H-6*); 3.88 (t, J = 9.3, 1H, *H-4*); 3.22 (dd, J = 9.9, J = 2.0, 1H, *H-3*); 3.08 (t, J = 9.5, 1H, *H-5*); 3.03 (dd, J = 10.0, J = 2.0, 1H, *H-1*). IR ($CHCl_3$): 2111.3 cm^{-1} (N_3).

Elimination product **29** (130 mg, 27%) was also obtained. R_f = 0.24 (ether−hexane, 1:4). ^{1}H NMR ($CDCl_3$): 7.5−7.2 (m, 10H, -(C_6H_5)$_2$); 6.2−5.9 (m, 3H, -(O−CH_2−CH=CH_2)$_3$); 5.4−5.2 (m, 6H, -(O−CH_2−CH=CH_2)$_3$); 4.85 (AB, J_{AB} = 12.3, $\Delta\delta$ = 0.02, 2H, −O−CH_2−C_6H_5); 4.8−4.6 (m, 3H, containing at 4.71 (AB, J_{AB} = 11.9, $\Delta\delta$ = 0.05, 2H, −O−CH_2−C_6H_5) and *H-6*); 4.44 (*AB* part of an $ABMX_2$, J_{AB} = 12.5, J_{AM} = J_{BM} = 5.7, J_{AX}

$= J_{BX} = 1.3$, $\Delta\delta = 0.12$, 2H, $-O-CH_2-CH=CH_2$); 4.3−4.0 (m, 7H, containing at 4.12 (dd, $J = 6.8$, $J = 3.3$, 1H, *H-5*), at 4.09 (dd, $J = 3.5$, $J = 1.1$, 1H, *H-2*), at 4.09 (dd, $J = 9.5$, $J = 6.9$, 1H, *H-4*) and -$(O-CH_2-CH=CH_2)_2$); 3.53 (dd, $J = 9.9$, $J = 3.5$, 1H, *H-3*).

(±)-1,4,5-Tri-*O*-allyl-6-amino-2,3-di-*O*-benzyl-6-deoxy-*myo*-inositol (30). Dried (5×10^{-3} mbar; 50 °C; 12 h) tin chloride (2 equiv; 276 mg; 1.46 mmol) was dissolved in anhydrous acetonitrile (7 mL). Thiophenol (8 equiv; 600 μL; 5.84 mmol) and triethylamine (6 equiv; 610 μL; 4.38 mmol) were added to the solution. (±)-1,4,5-Tri−*O*-allyl-6-azido-2,3-di−*O*-benzyl-6-deoxy−*myo*−inositol (**28**) dissolved in anhydrous acetonitrile (2 × 2 mL) was injected into the mixture. After 1 h at TA, the solvents were removed and the residue was dissolved in CH_2Cl_2 (20 mL) and 2 N NaOH (20 mL). The aqueous phase was washed with CH_2Cl_2 (2 × 10 mL). The organic layers were dried over Na_2SO_4, filtered, and evaporated to dryness. The crude product was used as such for the next step. An aliquot was purified by column chromatography (ether). $R_f = 0.13$ (ether). Anal. ($C_{29}H_{37}O_5N$) C, H, N. ^{1}H NMR ($CDCl_3$): 7.5−7.2 (m, 10H, -$(C_6H_5)_2$); 6.1−5.8 (m, 3H, -$(O-CH_2-CH=CH_2)_3$); 5.4−5.1 (m, 6H, -$(O-CH_2-CH=CH_2)_3$); 4.83 (AB, $J_{AB} = 12.1$, $\Delta\delta = 0.05$, 2H, $-O-CH_2-C_6H_5$); 4.68 (AB, $J_{AB} = 11.7$, $\Delta\delta = 0.07$, 2H, $-O-CH_2-C_6H_5$); 4.5−4.1 (m, 4H, -$(O-CH_2-CH=CH_2)_2$); 4.1−3.8 (m, 4H, containing at 4.03 (t, $J = 2.4$, 1H, *H-2*), at 3.89 (t, $J = 9.3$, 1H, *H-4*) and $-O-CH_2-CH=CH_2$); 3.39 (t, $J = 9.9$, 1H, *H-6*); 3.31 (dd, $J = 9.7$, $J = 2.4$, 1H, *H-3*); 3.08 (t, $J = 9.5$, 1H, *H-5*); 3.00 (dd, $J = 10.2$, $J = 2.2$, 1H, *H-1*); 1.83 (s, 2H, exchangeable, $-NH_2$).

(±)-1,4,5-Tri-*O*-allyl-6-amino-2,3-di-*O*-benzyl-6-*N*-benzyloxycarbonyl-6-deoxy-*myo*-inositol (31). Crude (±)-1,4,5-tri-*O*-allyl-6-amino-2,3-di-*O*-benzyl-6-deoxy-*myo*-inositol (**30**) was dissolved in dioxane (14 mL) and 2 N NaOH (4 equiv; 1.5 mL; 2.92 mmol). At 0 °C benzyl chloroformate (4 equiv; 420 μL; 2.92 mmol) was slowly added. After 2 h at room temperature, the solvents were evaporated and the crude product was purified via silica gel column chromatography (ether−hexane, 1:2). The expected compound **31** was obtained with 88% yield (from azide **28**) (390 mg). $R_f = 0.33$ (ether−hexane, 1:1). Anal. ($C_{37}H_{43}O_7N$) C, H, N. ^{1}H NMR (DMSO-d_6): 7.5−7.1 (m, 15H, -$(C_6H_5)_3$); 6.1−5.8 (m, 3H, -$(O-CH_2-CH=CH_2)_3$); 5.4−5.1 (m, 8H, -$(O-CH_2-CH=CH_2)_3$ and $-O-CH_2-C_6H_5$); 4.75 (s, 2H, $-O-CH_2-C_6H_5$); 4.64 (AB, $J_{AB} = 12.1$, $\Delta\delta = 0.07$, 2H, $-O-CH_2-C_6H_5$); 4.3−3.9 (m, 7H, -$(O-CH_2-CH=CH_2)_3$ and *H-2*); 3.77 (q, $J = J_{H6NH} = 10.2$, 1H, *H-6*); 3.59 (t, $J = 9.3$, 1H, *H-4*); 3.4−3.2 (m, 3H, *H-3*, *H-1*, N*H*); 3.19 (t, $J = 9.5$, 1H, *H-5*). MS (FAB^+) M 613.3: *m/z* 614.2 [M + H].

(±)-6-Amino-2,3-di-*O*-benzyl-6-*N*-benzyloxycarbonyl-6-deoxy-*myo*-inositol (32). (±)-1,4,5-Tri-*O*-allyl-6-amino-2,3-di−*O*-benzyl-6−*N*-benzyloxycarbonyl-6-deoxy−*myo*−inositol (**31**) (270 mg; 0.451 mmol) was dissolved in anhydrous THF (12 mL); dried (5×10^{-3} mbar, 100 °C, 12 h) $ZnCl_2$ (8.1 equiv; 500 mg; 3.65 mmol) was added. After 15 min stirring at room temperature, $Pd(PPh_3)_4$ (0.66 equiv; 345 mg; 0.3 mmol) was added and stirring was maintained for 10 min. Bu_3SnH (12 equiv; 1.45 mL; 5.42 mmol) was slowly added. After 1 h, the reaction mixture was diluted with AcOEt (30 mL) and H_2O (6 mL) and acidified. The aqueous phase was extracted with AcOEt (3 × 30 mL). The organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was dissolved in acetonitrile (30 mL) and hexane (60 mL). The mixture was stirred for 30 min. The acetonitrile phase was recovered and evaporated to dryness. Silica gel column chromatography (CH_2Cl_2−MeOH, 97:3) furnished 187 mg (86%) of triol **32**. $R_f = 0.41$ (CH_2Cl_2−MeOH 97:3). Anal. ($C_{28}H_{31}O_7N$) C, H, N. ^{1}H NMR ($CDCl_3$): 7.5−7.2 (m, 15H, -$(C_6H_5)_3$); 5.12 (s, 2H, $-O-CH_2-C_6H_5$); 4.87 (AB, $J_{AB} = 11.5$, $\Delta\delta = 0.30$, 2H, $-O-CH_2-C_6H_5$); 4.69 (AB, $J_{AB} = 11.7$, $\Delta\delta = 0.07$, 2H, $-O-CH_2-C_6H_5$); 4.07 (t, $J = 2.6$, 1H, *H-2*); 4.03 (td, $J = 9.3$, $J = 1.8$, 1H, *H-4*); 3.82 (td, $J = 10.3$, $J = 6.6$, 1H, *H-6*); 3.5−3.3 (m, 3H, *H-1*, *H-5* and N*H*); 3.28 (dd, $J = 9.7$, $J = 2.0$, 1H, *H-3*); 3.0−2.8 (m, 1H, exchangeable. O*H-5*); 2.76 (d, $J = 2.0$, 1H, exchangeable, O*H-4*). MS (FAB^+) M 493.2: *m/z* 494.1 [M + H].

(±)-6-Amino-2,3-di-*O*-benzyl-6-*N*-benzyloxycarbonyl-6-deoxy-*myo*-inositol 1,4,5-Tri-*O*-(*o*-xylylene)phosphate (33). (±)-6-Amino-2,3-di-*O*-benzyl-6−*N*-benzyloxycarbonyl-6-deoxy−*myo*−inositol (**32**) (158 mg; 0.32 mmol) and 1*H*-tetrazole (9 equiv; 210 mg; 3.00 mmol) were dissolved in 15 mL of anhydrous THF. The solution was cooled to −78 °C and *N*,*N*-diethyl *O*-xylylene phosphoramidite (6 equiv; 475 mg; 2 mmol) was added. After warming up the solution was stirred at room temperature for 8 h. The reaction mixture was cooled again to −78 °C, and *m*CPBA (10 equiv; 816 mg; 3.3 mmol) dissolved in 5 mL of CH_2Cl_2 was added. The reaction mixture was then kept at room temperature for 30 min. The solvents were removed by evaporation and the residue was dissolved in 30 mL of CH_2Cl_2 and washed with 10% Na_2SO_3 aqueous solution (2 × 50 mL) and saturated $NaHCO_3$ aqueous solution (2 × 50 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness, and the residue was chromatographed on a silica gel column (CH_2Cl_2−MeOH, 97:3). 6-Amino-2,3-di-*O*-benzyl-6-*N*-benzyloxycarbonyl-6-deoxy-*myo*-inositol 1,4,5-tri-*O*-(orthoxylylene)phosphate (**33**) was obtained as a white powder (295 mg; 88%). $R_f = 0.42$ (CH_2Cl_2−MeOH, 97:3). ^{1}H NMR{^{31}P} ($CDCl_3$): 7.5−7.0 (m, 27H, -$(C_6H_5)_3$ and -(C_6H_4)-$_3$); 5.7−4.5 (m, 24H, containing at 5.35 (AB, $J_{AB} = 13.9$, $\Delta\delta = 0.47$, 2H, $-O-CH_2-C_6H_4$-), at 5.28 (AB, $J_{AB} = 13.7$, $\Delta\delta = 0.34$, 2H, $-O-CH_2-C_6H_4$-), at 5.20 (t, $J = 9.3$, 1H, *H-4*), at 4.93 (AB, $J_{AB} = 13.7$, $\Delta\delta = 0.64$, 2H $-O-CH_2-C_6H_4$-), at 4.51 (s, 1H, *H-2*), -$(O-CH_2-C_6H_5)_3$, -$(O-CH_2-C_6H_4)$-$_3$, *H-1*, *H-5*, *H-6* and N*H*); 4.16 (AB, $J_{AB} = 11.3$, $\Delta\delta = 0.10$, 2H, $-O-CH_2-C_6H_5$); 3.75 (dd, $J = 9.7$, $J = 2.0$, 1H, *H-3*). ^{31}P NMR{1H} ($CDCl_3$): −0.12 (s, 1P); −2.47 (s, 1P); −2.59 (s, 1P). MS (FAB^+) M 1039.2: *m/z* 1040.1 [M + H].

(±)-6-Amino-6-deoxy-*myo*-inositol 1,4,5-Tris(phosphate) (8). (±)-6-amino-2,3-di-*O*-benzyl-6−*N*-benzyloxycarbonyl-6-deoxy−*myo*-inositol 1,4,5-tri−*O*−(orthoxylylene)phosphate (**33**) (100 mg; 0.096 mmol) dissolved in a CH_2Cl_2−MeOH−H_2O (1:2:1) mixture (16 mL) was hydrogenized (5 atm) in the presence of 10% Pd/C (50 mg) for 12 h at 20 °C. The Pd/C was filtered through a Celite pad and the filtrate was evaporated to dryness. The residue was dissolved in 1 mL bidistilled water and then cooled to 0 °C; cyclohexylamine (1 mL) was added. The mixture was evaporated to dryness and dissolved again in 0.5 mL of bidistilled water. The cyclohexylammonium salts were precipitated by adding 40 mL of acetone. The salts were filtered and precipitated again by 40 mL of acetone. After filtration and drying 53 mg (89%) of cyclohexylammonium salts of (±)-6-amino-6-deoxy-*myo*-inositol 1,4,5-tris(phosphate) (**8**) were obtained. ^{1}H NMR{^{31}P} (D_2O, pH = 11.4): 4.39 (t, $J = 2.6$, 1H, *H-2*); 4.25 (t, $J = 9.4$, 1H, *H-4*); 3.94 (dd, $J = 9.7$, $J = 2.6$, 1H, *H-1*); 3.93 (t, $J = 9.1$, 1H, *H-5*); 3.79 (dd, $J = 9.4$, $J = 2.6$, 1H, *H-3*); 3.25 (t, $J = 9.9$, 1H, *H-6*). ^{31}P NMR{1H} (D_2O, pH = 11.4): 5.38 (s, 1P, *P-4*); 4.36 (s, 1P, *P-5*); 4.05 (s, 1P, *P-1*). MS (FAB^-) M 419.1: *m/z* 418.1 [M − H].

Ins(1,4,5)P_3 Derivatives Binding Assay. Bovine adrenal cortex microsomes were prepared as previously described.[46] Bovine adrenal microsomes were incubated for 30 min at 0 °C in a medium containing 25 mM Tris/HCl buffered at pH 8.5, 100 mM KCl, 20 mM NaCl, 5 mM KH_2PO_4, and 1 mM EDTA in a final volume of 500 μL with the appropriate concentration of [^{3}H]Ins(1,4,5)P_3 (0.9 nM), Ins(1,4,5)P_3, and Ins(1,4,5)P_3 derivatives. Nonspecific binding was determined in the presence of 1 μM Ins(1,4,5)P_3. Incubations were terminated by centrifugation at 15000*g* for 5 min at 0 °C. The receptor-bound radioactivity was analyzed by liquid scintillation spectrometry.

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References

(1) Streb, H.; Irvine, R. F.; Berridge, M. J.; Schultz, I. Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. *Nature* **1983**, *312*, 375−376.

(2) Berridge, M. J.; Irvine, R. F. Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* **1984**, *312*, 315−321.
(3) Berridge, M. J. Inositol trisphosphate and calcium signaling. *Nature* **1993**, *361*, 315−325.
(4) Billington, D. C. Recent developments in the synthesis of *myo*-inositol phosphates. *Chem. Soc. Rev.* **1989**, *18*, 83−122.
(5) Billington, D. C. *The inositol phosphates: chemical synthesis and biological significance*; VCH Publishers: Weinheim, 1993.
(6) Potter, B. V. L.; Lampe, D. Chemistry of inositol lipid mediated cellular signaling. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1933−1972.
(7) Kozikowski, A. P.; Ognyanov, V. I.; Fauq, A. H.; Nahorski, S. R.; Wilcox, R. A. Synthesis of 1D-3-deoxy-, 1D-2,3-dideoxy-, and 1D-2,3,6-trideoxy- *myo*-inositol 1,4,5-trisphosphate from quebrachitol, their binding affinities, and calcium release activity. *J. Am. Chem. Soc.* **1993**, *115*, 4429−4434.
(8) Bonis, D.; Sezan, A.; Mauduit, P.; Cleophax, J.; Gero, S. D.; Rossignol, B. Comparative studies of D-*myo*-inositol 1,4,5-trisphosphate and synthetic 6-deoxy D-*myo*-inositol 1, 4, 5 trisphosphate: binding and calcium release activity in rat parotid microsomes. *Biochem. Biophys. Res. Commun.* **1991**; *175*; 894−900.
(9) Mernissi Arifi, K.; Schmitt, L.; Schlewer, G.; Spiess, B. Complete resolution of the microscopic: Protonation equilibria of D-*myo*-inositol 1,2,6-tris(phosphate) and related compounds by ^{31}P NMR and potentiometry. *Anal. Chem.* **1995**; *67*; 2567−2574.
(10) Cozzone, P. J. Jardetzky, O. Phosphorus-31 Fourier transform nuclear magnetic resonance study of mononucleotides and dinucleotides. 1. Chemical shifts. *Biochemistry* **1976**; *15*, 4853−4859.
(11) Barrientos, L. G. Murthy, P. P. N. Conformational studies of myo-inositol phosphates. *Carbohydr. Res.* **1996**; *296*; 39−54.
(12) Schmitt, L.; Bortmann, P.; Schlewer, G.; Spiess, B. *Myo*-inositol 1,4,5-triphosphate and related compounds' protonation sequence: potentiometric and ^{31}P NMR studies. *J. Chem. Soc., Perkin Trans. 2* **1993**; 2257−2263.
(13) Jost, P. Heulin, B.; Réalisation d'un dosage contrôlé par micro-ordinateur. *Actual. Chim.* **1984**, *4*, 34−37.
(14) Jost, P.; Igot, E.; Claisse, T.; Delfan, O. *Log. Chim.* **1986**.
(15) Gans, P.; Sabatini, A.; Vacca, A. Superquad: An improved general program for computation of formation constants from potentiometric data. *J. Chem. Soc., Dalton Trans.* **1985**, 1195−1200.
(16) Frassineti, C.; Ghelli, S.; Gans, P.; Sabatini, A.; Moruzzi, M. S.; Vacca, A.; Nuclear magnetic resonance as a tool for determining protonation constants of natural polyprotic bases in solution. *Anal. Biochem.* **1995**; *231*; 374−382.
(17) Karon, S. *Scientific plotting and data analysis*; 1995.
(18) Mernissi Arifi, K. Caractères macro et micro acido-basiques d′inositol-phosphates, propriétés complexantes vis-à-vis de cations de transition, d'Al3+ et de polyamines. 1994.
(19) Numbering of Ins(1,4,5)P_3 was used for all derivatives to facilitate the comparison, even if this does not strictly follow the IUPAC rules.
(20) Schmitt, L.; Spiess, B.; Schlewer, G. Synthesis of (±) cyclohexane-1(R),2(R),4(R)-triol-triphosphate, a deoxygenated analogue of *myo*-inositol-1,4,5-triphosphate. *Tetrahedron Lett.* **1992**, *33*, 2013−2016.
(21) Vacca, J. P.; deSolms, S. J.; Huff, J. R. Total synthesis of D- and L-*myo*-inositol 1,4,5-trisphosphate. *J. Am. Chem. Soc.* **1987**, *109*, 3478−3479.
(22) Gigg, J.; Gigg, R.; Payne, S.; Conant, R. The allyl group for protection in carbohydrate chemistry. Part 20. Synthesis of 1L-1-*O*-methyl-*myo*-inositol [(+)-bornesitol] by resolution of (±)-1,2,4-tri-*O*-benzyl-*myo*-inositol. *J. Chem. Soc., Perkin Trans. 2* **1987**, 1757−1762.
(23) Ozaki, S.; Watanabe, Y.; Ogasawara, T.; Kondo, Y.; Shiotani, N.; Nishii, H.; Matsuki, T. Total synthesis of optically active *myo*-inositol 1,4,5- tris(phosphate). *Tetrahedron Lett.* **1986**, *27*, 3157−3160.
(24) Stepanov, A. E.; Runova, O. B.; Schlewer, G.; Spiess, B.; Shvets, V. I. Total synthesis of *sn-myo*-inositol-1,4,5-triphosphate and its enantiomer. *Tetrahedron Lett.* **1989**, *30*, 5125−5128.
(25) Ley, S. V.; Parra, M.; Redgrave, J. L.; Sternfeld, F. Microbial oxidation in synthesis: preparation of *myo*-inositol phosphates and related cyclitol derivatives from benzene. *Tetrahedron* **1990**, *46*, 4995−5026.
(26) Cleophax, J.; Dubreuil, D.; Gero, S. D.; Loupy, A.; Dealmeida, M. V.; Dasilva, A. D.; Vass, G.; Bischoff, E.; Perzborn, E.; Hecker, G.; Lockhoff, O. Enantioselective synthesis of inositols as intermediates for the preparation of deoxy-inositol phosphates from D-galactose. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 831−834.
(27) Dubreuil, D.; Cleophax, J.; Dealmeida, M. V.; Verresebrie, C.; Liaigre, J.; Vass, G.; Gero, S. D. Stereoselective synthesis of 6-deoxy and 3,6-dideoxy-d-myo-inositol precursors of deoxy-myo-inositol phosphate analogues from d-galactose. *Tetrahedron* **1997**, *53*, 16747−16766.
(28) Massy, D. J. Wyss, P. The synthesis of DL-1-(hexadecanoyloxy)-methyl- and 1-*O*-hexadecanoyl-inositols as potential inhibitors of phospholipase C. *Helv. Chim. Acta* **1990**, *73*, 1037−1040.
(29) Desai, T.; Gigg, J.; Gigg, R.; Payne, S.; Penades, S.; Rogers, H. J. The allylation of dibutylstannylene derivatives of *myo*-inositol. *Carbohydr. Res.* **1991**, *216*, 197−209.
(30) Gigg, J.; Gigg, R.; Payne, S.; Conant, R. The allyl group for protection in carbohydrate chemistry. Part 18.1 Allyl and benzyl ethers of *myo*-inositol. Intermediates for the synthesis of *myo*-inositol trisphosphates. *J. Chem. Soc., Perkin Trans. 1* **1987**, 423−429.
(31) Barton, D. H. R.; McCombie, S. W. A new method for the deoxygenation of secondary alcohols. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574−1585.
(32) Watanabe, Y.; Komoda, Y.; Ebisuya, K.; Ozaki, S. An efficient phosphorylation method using a new phosphitylating agent, 2-diethyl-1,3,2-benzodioxaphosphepane. *Tetrahedron Lett.* **1990**, *31*, 255−256.
(33) Yang, S. S.; Chiang, Y. C. P.; Beattie, T. R. Synthesis of DL-1-deoxy-1-fluoro-6-*O*-methyl-*chiro*-inositol − Confirmation of a structural-DAST fluorination correlation. *Carbohydr. Res.* **1993**, *249*, 259−263.
(34) Jiang, C.; Moyer, J. D.; Baker, D. C. Synthesis od deoxy deoxyhalogeno analogues of *myo*-inositol. *J. Carbohydr. Chem.* **1987**, *6*, 319−355.
(35) Ballereau, S.; Guédat, P.; Spiess, B.; Rehnberg, N.; Schlewer, G. Synthesis of (±)-4-deoxy-4-fluoro-*myo*-inositol. *Tetrahedron Lett.* **1995**, *36*, 7449−7450.
(36) Guédat, P.; Poitras, M.; Spiess, B.; Guillemette, G.; Schlewer, G. Rapid synthesis and properties of (±)-6-deoxy-6-fluoro-myo-inositol-1,4,5-tris(phosphate) an analogue of myo-inositol-1,4,5-tris(phosphate). *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1175−1178.
(37) Bruzik, K. S.; Tsai, M.−D. Efficient and systematic syntheses of enantiomerically pure and regiospecifically protected *myo*-inositol. *J. Am. Chem. Soc.* **1992**, *114*, 6361−6374.
(38) Gigg, J.; Gigg, R. Reactions of the ketone derived from (±)-3,4,5-tri-o-benzyl-1,2-o-isopropylidene-myo-inositol − preparation of racemic derivatives of epi-inositol and of 4-c-methyl-epi-[(±)-isolaminitol] and 4-c-methyl-myo-inositol [(±)-laminitol]. *Carbohydr. Res.* **1997**; *299*; 77−83.
(39) Barta, M.; Romea, P.; Urpi, F.; Vilarrasa, J. A fast procedure for the reduction of azides and nitro compounds based on the reducing ability of $Sn(SR)_3^-$ species. *Tetrahedron* **1990**, *46*, 587−594.
(40) Four, P.; Guibé, F. Palladium-catalysed conjugate reduction of α,*β*-unsaturated carbonyl compound with tributyltin hydride. The promoting influence of the presence of protonic or lewis acid. *Tetrahedron Lett.* **1982**, *23*, 1825−1828.
(41) Guibé, F.; Dangles, O.; Balavoine, G. Palladium-catalysed reaction of tributyltin hydride. Selective and very mild deprotection of allyl and allyoxycarbonyl derivatives of amino acids. *Tetrahedron Lett.* **1986**, *27*, 2365−2368.
(42) Poitras, M.; Bernier, S.; Richard, D.; Servant, M.; Guillemette, G. The high affinity state of inositol 1,4,5-trisphosphate receptor is a functional state. *J. Biol. Chem.* **1993**, *268*, 24078−24082.
(43) Rodbard, D. Lessons from the computerization of radioimmunoassays: An introduction to basic principles of modeling. *Computers in Endocrinology*; Raven Press: New York, 1984.
(44) Desai, T.; Gigg, J.; Gigg, R.; Martin-Zamora, E. The preparation of racemic and enantiomerically pure *myo*-inositol derivatives for the synthesis of phosphatidylinositol 3-,3,4-bis-, and 3,4,5-tris-phosphates and for the synthesis of analogues of 1d-*myo*-inositol 1,3,4,5-tetrakisphosphate. *Carbohydr. Res.* **1996**, *296*, 97−133.
(45) Marecek, J. F.; Prestwich, G. D. Fluorinated analogues of Ins-(1,4,5)P_3. *Tetrahedron Lett.* **1989**, *30*, 5401−5404.
(46) Poitras, M.; Ribeiro-Do-Valle, R. M.; Poirier, S. N.; Guillemette, G. Bidirectional activity of the endoplasmic reticulum Ca2+-ATPase of bovine adrenal cortex. *Biochemistry* **1995**, *34*, 9755−9761.

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