Brief Articles

Synthesis of (±)-2-*O*-[4′-(*N*-9″-Purinyl)butyl] *myo*-Inositol 1,4,5-Tris(phosphate), a Potent Full Agonist at the D-*myo*-Inositol 1,4,5-Tris(phosphate) Receptor

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Racemic 2-*O*-[4′(9″-*N*-purinyl)butyl] *myo*-inositol 1,4,5-tris(phosphate) **8** was synthesized starting from *myo*-inositol. Substitution of position 2 by an alkyl side chain was rendered possible by inversion of the chair conformation of the inositol ring by means of an orthoester. The final compound is a full agonist with the same order of potency as D-*myo*-inositol 1,4,5-tris(phosphate).

Introduction

The second messenger functions of D-*myo*-inositol 1,4,5-tris(phosphate) (Ins(1,4,5)P_3) **1** (Figure 1) are now well established.[1−3] The activation of GPCRs linked to G_q/G_{16} result in the hydrolysis of phosphatidyl *myo*-inositol 4,5-bis(phosphate) and liberation of this intracellular second messenger. By occupying its receptors, Ins(1,4,5)P_3 provokes the mobilization of the intracellular calcium stores.[4]

The structure activity relationship undertaken around Ins(1,4,5)P_3[5,6] allowed Kozikowski to propose a pharmacophore model[7,8] (Figure 2). But, to date, none of the analogues directly derived from the Ins(1,4,5)P_3 skeleton attained either the affinity or the activity of the parent compound.

Ten years ago, the structures and activities of adenophostins A and B, **2** and **3**, respectively, were published (Figure 1). These disaccharide tris(phosphates) are 10−100 times more potent as agonists than the endogenous product.[9−12] Molecular modeling studies have readily shown that adenophostins contain all of the important structural functions of Ins(1,4,5)P_3. Moreover, the adenyl base seemed to play an important role in the increase of activity. This was confirmed by the structure activity relationship around these molecules. A functional simplification of the adenophostins revealed that, as soon as the adenyl part was modified or removed, the activity decreased (Figure 3, compounds **4**−**6**).[11] If the adenophostin structure was reduced to the elements strictly necessary to mimic Ins(1,4,5)P_3 (Figure 3, compound **7**), there was a loss of activity compared to the endogenous compound.

The goal of our project was to synthesize an adenophostin mimic based on the Ins(1,4,5)P_3 backbone.

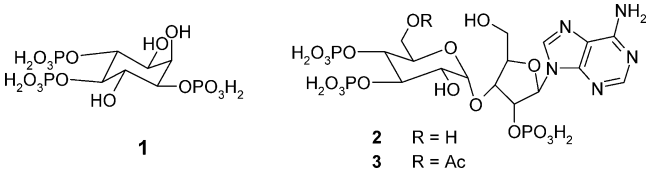

Figure 1. Structures of Ins(1,4,5)P_3 (**1**) and adenophostins A and B (**2**) and (**3**), respectively.

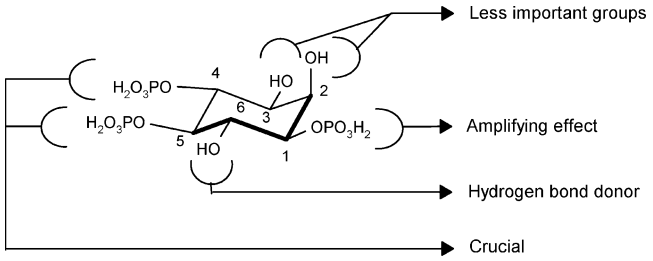

Figure 2. Pharmacophore model proposed by Kozikowski.[7,8]

Following Kozikowski's conclusions, it seemed possible to use position 2 of the inositol ring to introduce a spacer ω substituted by an adenyl-mimic moiety. Using simple Dreiding stereomodels, a four methylenic unit spacer engrafted in position 2 generated a chimeric adenophostin molecule based on the Ins(1,4,5)P_3 structure (Figure 4, compound **8**).

This molecule does not contain the adenyl NH_2. In a first approach, this seemed not of major importance as the deaminated analogue of adenophostin A (purinophostin, compound **4**) displays the same order of magnitude in affinity and activity.[11]

We report here the synthesis and biological properties of compound **8**.

During this synthesis the XR structure of the type 1 receptor binding site cocrystallized with the endogenous ligand was published.[13] Preliminary docking studies where the binding site was considered rigid showed that compound **8** fit well in the binding site of Ins(1,4,5)P_3.

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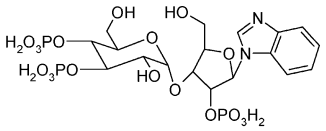

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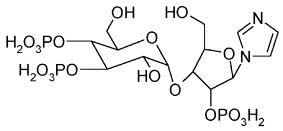

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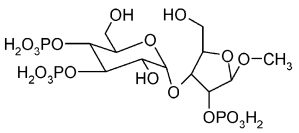

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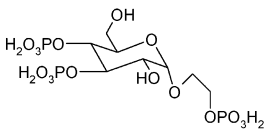

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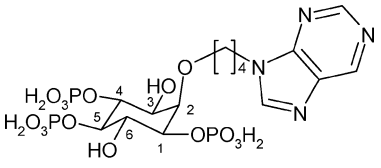
Compound	Relative Activity	Relative Affinity
1	1	1
2	10	6
4	8	**4**
5	1.3	1.4
6	0.4	0.7
7	0.08	-

Figure 3. Simplified analogues of adenophostin A.[11]



8

Figure 4. Structure of an adenophostin mimic molecule **8** based on the Ins(1,4,5)P_3 skeleton.

The side chain seemed able to establish additional interactions with one side of the binding site[13] (not shown).

Synthesis

The major difficulty of this synthesis was the poor reactivity toward nonactivated alkylating agents of the position 2 of *myo*-inositol, which is usually in an axial conformation. In our case, the O-alkylation of the axial hydroxyl of a suitably protected inositol failed even if drastic conditions for the formation of the oxyanion or chelation of the counterion of this oxyanion in different solvents were used. Alkylation of position 2 succeeded if activated alkyl halides such as benzyl halide, allyl halide, well-known protective groups,[5−7] or, more recently, bromoacetonitrile[14] were used.

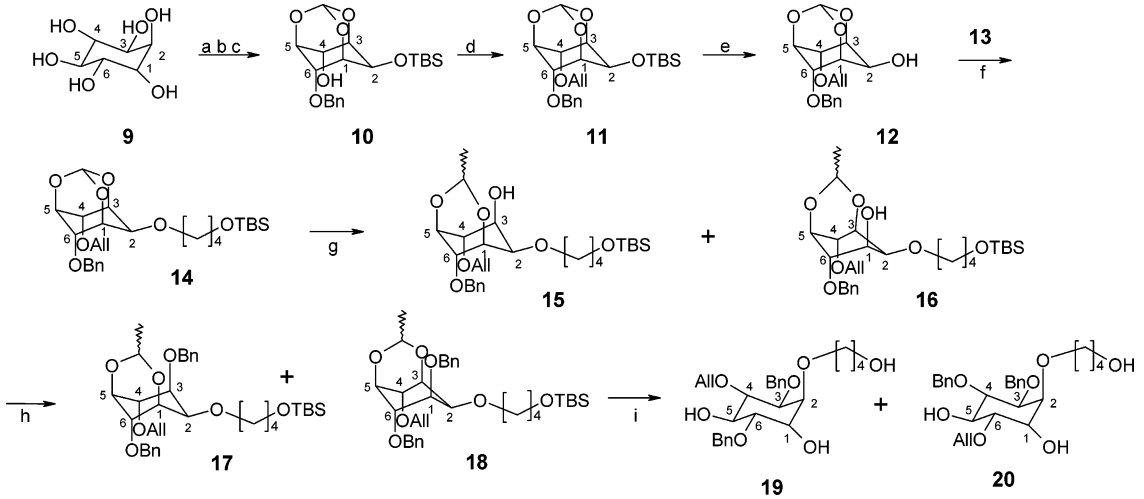
To promote the reactivity of position 2, it was helpful to invert the inositol chair by means of the orthoester **10**. Such an inversion drastically modified the behavior of the different hydroxyls and permitted specific reactions.[15] Using such an inversion the expected product was prepared according to the following sequences (Schemes 1 and 2).

myo-Inositol **9** was converted to the racemate orthoester **10** by well-known methods.[5,6,16] Allylation of the last free hydroxyl yielded quantitatively the totally protected compound **11**. Position 2 was selectively and classically deprotected using tetrabutylammonium fluoride in THF,[16] giving the alcohol **12** in quantitative yield. This equatorial alcohol **12** was then easily substituted even when non particularly activated alkylating reagents were used. Thus, the alcohol **12** was treated with sodium hydride to form the alcoholate, which was reacted with 1-*O*-tosyl-4-*O*-TBS butane-1,4-diol[17,18] **13**, to yield the totally protected inositol **14**. The orthoester was then selectively opened using trimethylaluminum.[19]

However, if the selectivity prevented the deprotection of position 5, the reaction failed in the selectivity between positions 1 and 3, leading to a mixture of two alcohols, **15** and **16**, which are both of interest. The free alcohol of compounds **15** and **16** was protected as benzyl ether by the classical reaction of the alcoholate with benzyl bromide giving two new totally protected inositols **17** and **18**. Simultaneous hydrolysis of ethylidene acetal and the silyl ether led to the triols **19** and **20**.

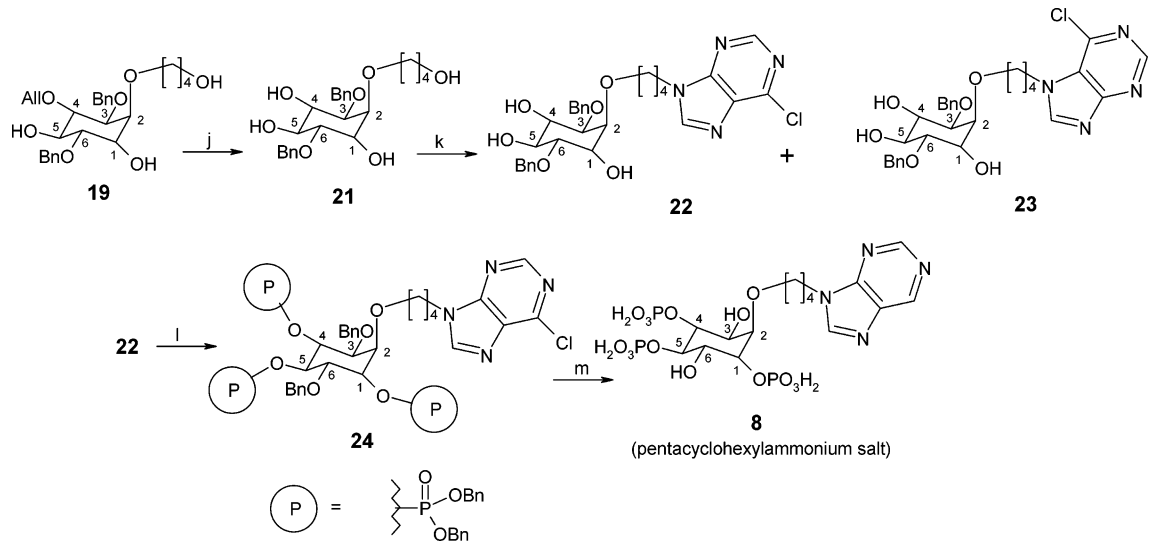
The allyl protective group of compound **19** was removed,[20] leading to tetraol **21**. A selective Mitsunobu reaction on the primary alcohol permitted the introduction of a chloropurine in the ω position of the spacer. In addition to the expected N-9 purinyl derivative **22**, a second product identified as the N-7 purinyl analogue **23** was also formed. These two isomers were separated. The 1,4,5-triol **22** was phosphorylated by means of tetrabenzyl pyrophosphate[21] to give the protected final product **24**. All the protective groups and the chlorine were simultaneously removed by treatment with cyclo-

Scheme 1. Synthesis of Analogue **8**: Part 1[a]



[a] (a) $HC(OEt)_3$, DMF, pTsOH, Ar, 70% yield; (b) TBSCl, imidazole, DMF, Ar, 62% yield; (c) NaH, BnBr, DMF, Ar, 90% yield; (d) NaH, AllBr, DMF, Ar, 97% yield; (e) TBAF, THF, 99% yield; (f) NaH, **13**, Ar, 67% yield; (g) $AlMe_3$, CH_2Cl_2, 0 °C, 70% yield; (h) NaH, BnBr, DMF; (i) MeOH, pTsOH, reflux, **19** 23% yield, **20** 22% yield.

Scheme 2. Synthesis of Analogue **8**: Part 2[a]



[a] (j) $Rh(P(C_6H_5)_3)_3Cl$, DABCO, EtOH−H_2O 9:1, reflux, 62% yield; (k) DEAD, $P(C_6H_5)_3$, 6-chloropurine, THF, Ar, **22** 40% yield; **23** 7% yield; (l) NaH, tetrabenzyl pyrophosphate, THF, Ar, 39% yield; (m) MeOH, cyclohexene, $Pd(OH)_2$, μW, 120 °C, 5 min, cyclohexylamine.

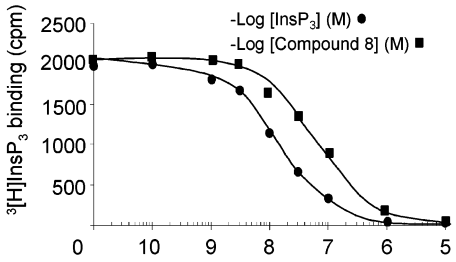


Figure 5. Compound **8** inhibits in a dose-dependent fashion the binding of [^{3}H]Ins(1,4,5)P_3 to bovine adrenal cortex microsomes (n = 6).

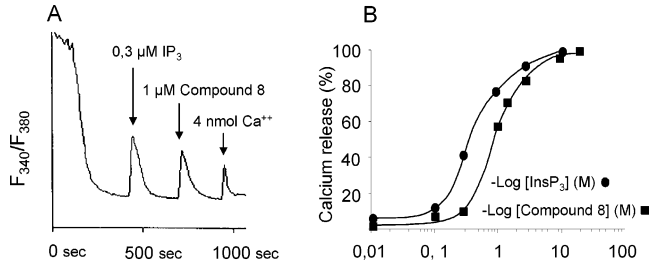


Figure 6. (A) Typical fura 2 fluorescence trace showing that the Ca^{2+} stored within the endoplasmic reticulum of permeabilized RINm5F cells is readily releasable by Ins(1,4,5)P_3 and by compound **8**. (B) Dose−response curves for compound **8** release and Ins(1,4,5)P_3-induced Ca^{2+} release from permeabilized RINm5F cells (n = 6).

hexene in the presence of palladium dihydroxide in a microwave oven. The final product **8**, obtained as racemate, was stabilized as a pentacyclohexylammonium salt.

Biochemical Properties and Discussion

The binding properties of compound **8** were measured on bovine adrenal cortex microsomes as shown in Figure 5. Increasing concentrations of compound **8** diminished the binding of [^{3}H]Ins(1,4,5)P_3 in a dose-dependent manner. The IC_{50} value (concentration inhibiting 50% of tracer binding) was 40 nM, which is equipotent to that of Ins(1,4,5)P_3 **1** if we take into account that compound **8** was obtained as a racemate (see below).

The functional properties of compound **8** were evaluated for its ability to release Ca^{2+} from permeabilized pancreatic cells (RINm5F).[23] Figure 6 (panel A) shows that, as well as Ins(1,4,5)P_3, compound **8** could efficiently release stored Ca^{2+} that was thereafter slowly recaptured within the endoplasmic reticulum of permeabilized cells.

As previously explained,[22] this slow recapture is indicative of an efficient degradation of Ins(1,4,5)P_3 and likely of compound **8** by endogenous phosphatases. Compound **8** behaved as a full agonist, releasing the same maximal amount of Ca^{2+} as Ins(1,4,5)P_3 (Figure 6, panel B). Its potency to mobilize Ca^{2+} was about one-third (EC_{50} of 0.8 μM) of that of Ins(1,4,5)P_3 (EC_{50} of 0.3 μM). In this series Ins(1,4,5)P_3 is 3 orders of magnitude more active than its enantiomer Ins(3,5,6)P_3. Again, if we consider that compound **8** is a racemate, this suggests that the active enantiomer could be considered as equipotent to Ins(1,4,5)P_3.

The synthesized compound behaves as a good Ins(1,4,5)P_3 mimic. The side chain introduced in position 2 does not restrain significantly the docking of the molecule in the Ins(1,4,5)P_3 receptor. These results support those of previous studies indicating that this position 2 tolerates some substitution leading to Kozikowski's pharmacophore model. The results obtained for compound **8** could suggest that the receptor essentially recognizes the Ins(1,4,5)P_3 part of the product. The 4 methylenic unit side chain length does not allow the additional interactions supposed to be due to the adenyl part of adenophostins. Either the area occupied by the purine of our analogue does not correspond to that of the base of the adenophostins or the chain length is inappropriate to establish additional interactions. It is now of first importance to resolve the racemate to confirm what is generally observed for Ins(1,4,5)P_3 analogues; that is to say that the 2-substituted Ins(1,4,5)P_3 could be much more active than its enantiomer (2-substituted Ins(3,5,6)P_3). A second aim will be the crystallization of the more active enantiomer in the binding site to see how the side chain in position 2

behaves in the binding pocket. More work is in progress to optimize the length and character of the side chain.

Experimental Section

General. If not specified, NMR spectra were recorded on a Bruker Avance 300 spectrometer using the δ scale. The abbreviations s, d, t, q, qu, m are related to singlet, doublet, triplet, quadruplet, quintuplet, and multiplet, respectively; coupling constants are given in Hz.

(±)-4-*O*-Allyl-6-*O*-benzyl-2-*O*-(4′-*O*-*tert*-butyldimethylsilyl-butyl) *myo*-Inositol 1,3,5-*O*-Orthoformate (14). Compound **12** (1.91 g, 5.96 mmol) was dissolved in dry DMF (50 mL) and kept under argon. NaH (1.3 equiv, 3.75 mmol, 310 mg) was added at 0 °C with stirring for 5 min. The reaction mixture was then warmed to room temperature and stirred for 15 min. The tosylate **13** (1.25 equiv, 7.45 mmol, 2.45 g)[17,18] was added dropwise, and stirring was maintained overnight. The reaction mixture was quenched by ice cooled water (5 mL), diluted with NaCl saturated water (100 mL), and extracted with ether (2 times 250 mL) and CH_2Cl_2 (2 times 250 mL). The combined organic layers were dried over $MgSO_4$. The crude product obtained after solvent evaporation was purified by column chromatography on silica gel eluted with a gradient of heptane and ether. The products were not completely separated. The expected 2-O-substituted analogue **14** (2.02 g, 67% yield): R_f 0.46 (heptane−ether 1/1); ^{1}H NMR (600 MHz, $CDCl_3$, COSY, HSQC) 7.4−7.3 (m, 5H, C_6H_5), 5.90 (ddt, J = 17.2, J = 10.4, J = 5.7, 1H, $OCH_2-CH{=}CH_2$), 5.51 (s, 1H, $HC(OCH)_3$), 5.29 (dq, J = 17.2, J = 1.6, 1H, $OCH_2-CH{=}CH_{trans}H$), 5.22 (dq, J = 10.4, J = 1.4, 1H, $OCH_2CH{=}CH_{cis}H$), 4.64 (AB, $\Delta\delta$ = 0.12, J_{AB} =11.9, 2H, $CH_2-C_6H_5$), 4.43 (td, J = 2.3, J = 1,6, 1H, C*H*-5), 4.35−4.33 (m, 1H, C*H*-4), 4.32 (tq, J = 3.8, J = 1.7, 1H, C*H*-1), 4.30−4.28 (m, 1H, C*H*-6), 4.28−4.26 (m, 1H, C*H*-3); 4.11 (AB part of an ABXYY′, $\Delta\delta$ = 0.20, J_{AB} = 12.7, J_{AX} = 5.8, J_{BX} = 5.5, J_{AY} = $J_{AY'}$ = 1.4, J_{BY} = $J_{BY'}$ = 1.2, 2H, $OCH_2-CH{=}CH_2$), 3.85 (t, J = 1.5, 1H, C*H*-2), 3.64 (t, J = 6.4, 2H, C*H*$_2$-4′), 3.58 (t, J = 6.6, 2H, C*H*$_2$-1′), 1.71 (qu, J = 7.1, 2H, C*H*$_2$-2′), 1.60 (qu, J = 6,9, 2H, C*H*$_2$-3′), 0.89 (s, 9H, *t*Bu); 0.05 (s, 6H, Me_2); ^{13}C NMR (150 MHz, $CDCl_3$) 134.1 *C*H = allyl, 128.4 2C, *CH* = arom ortho, 127.9, *C*H = arom para, 127.6 2C, *C*H = arom meta, 117.6 CH_2 = allyl, 103.2 *C*H orthoester, 73.9 *C*-4, 73.8 *C*-6, 71.7 CH_2 benzyl, 70.8 $-CH_2-$ allyl, 70.5 *C*-1, 70.4 *C*-3, 69.5 *C*-1′, 68.0, 2C, *C*-2 and *C*-5, 62.9 *C*-4′, 29.4 *C*-3′; 26.4 *C*-2′, 25.9, 3C, Me_3, 18.3 *C*(Me)$_3$, −5.3, 2C, Me_2.

2-O-Tosylated derivative (0.12 g, 4% yield): R_f 0.21 (heptane−ether 1/1); ^{1}H NMR (200 MHz, $CDCl_3$) 7.62 ((AB)$_2$, $\Delta\delta$ = 0.44, J_{AB} = 7.6, 4H, $OC_6H_4CH_3$), 7.4−7.3 (m, 5H, C_6H_5), 5.81 (ddt, J = 17.4, J = 10.5, J = 5.3, 1H, $OCH_2-CH{=}CH_2$), 5.49 (broad d, J = 1.2, 1H, $HC(OCH)_3$), 5.22 (dq, J = 12.2, J = 1.5, 1H, $OCH_2-CH{=}CH_{trans}H$), 5.16 (dq, J = 4.9, J = 1.7, 1H, $OCH_2-CH{=}CH_{cis}H$), 5.02(q, J = 1.6, 1H, *H*-2), 4.52 (AB $\Delta\delta$ = 0.12, J_{AB} = 11.8, 2H, $CH_2-C_6H_5$), 4.45−4.15 (3m, 5H, *H*-1, *H*-3, *H*-4, *H*-5, *H*-6), 4.02 (AB part of an ABMXX′, $\Delta\delta$ = 0.16, J_{AB} = 12.7, J_{AX} = 5.6, J_{BX} = 5.4, J_{AY} = $J_{AY'}$ = J_{BY} = $J_{BY'}$ = 1.5, 2H, $OCH_2-CH{=}CH_2$), 2.41 (s, 3H, $OC_6H_4CH_3$); a fraction containing a mixture of the expected compound **14** and the 2-O-tosylated compound (0.42 g) and starting material **12** (0.40 g, 21% yield) were also obtained.

(±)-1,4-Di-*O*-benzyl-2-*O*-[4′(9″-*N*-(6″-Cl-purinyl))butyl] *myo*-Inositol (22) and (±)-1,4-Di-*O*-benzyl-2-*O*-[4′(7″-*N*-(6″-Cl-purinyl))butyl] *myo*-Inositol (23). The tetraol **21** (280 mg, 647 μmol) was dissolved in dry THF (5 mL) and injected at room temperature into a vessel kept under argon containing 6-chloropurine (1 equiv, 100 mg), PPh_3 (1 equiv, 170 mg), and DEAD (1 equiv, 120 μL) dissolved in dry THF (30 mL). After 24 h of stirring, the reaction mixture was evaporated to dryness. After crystallization of DEAD side product in ether, the filtrate was evaporated and purified by silica gel column chromatography eluted with ethyl acetate. Unreacted tetraol **21** (85 mg) was recovered. The expected chloropurine derivative **22** (149 mg, 40% yield) was obtained as a beige paste: R_f 0.54 in (DCM−MeOH 9/1); ^{1}H NMR ($CDCl_3$, COSY) 8.76 and 8.08 (2s, 2H, *H*-2″ and *H*-8″), 7.6−7.4 (m, 10H (C_6H_5)$_2$), 4.88 (AB, $\Delta\delta$ = 0.11, J_{AB} = 11.4, 2H, $CH_2-C_6H_5$), 4.70 (AB, $\Delta\delta$ = 0.04, J_{AB} = 11.8, 2H, $CH_2-C_6H_5$), 4.29 (AB part of an ABX$_2$, $\Delta\delta$ = 0.03, J_{AB} = 7.1, J_{AX} = J_{BX} = 3.6, 2H, C*H*$_2$-4′), 4.0−3.7 (m, 4H, containing at 3.94 (t, J = 2.3, 1H, *H*-2), at 3.93 (t, J = 9.3, 1H, *H*-6), and at 3.38 (AB part of an ABX$_2$, $\Delta\delta$ = 0.13, J_{AB} = 9.3, J_{BX} = 6.0, J_{AX} = 5.6, 2H, C*H*$_2$-1′)), 3.65 (t, J = 9.2, 1H, *H*-4), 3.53 (dd, partially masked, 1H, *H*-3), 3.51 (t, J = 8.9, 1H, *H*-5), 3.20 (dd, J = 9.8, J = 2.3, 1H, *H*-1), 2.79 (broad s, 2H, exchangeable with CD_3OD, (O*H*)$_2$), 2.69 (s, 1H, exchangeable with CD_3OD, O*H*), 2.02 (qu, J = 7.2, 2H, C*H*$_2$-3′), 1.62 (qu, J = 7.2, 2H, C*H*$_2$-2′); ^{13}C NMR (75.43 MHz, $CDCl_3$, DEPT) 152.01 *C*″-2, 145.53 *C*″-8, 132.3−128.0 (*C*H Ar)$_2$, 81,43 *C*-2, 8.032 *C*-6, 77.60 *C*-3, 75.22 *C*-4, 74.91 $CH_2C_6H_5$, 72.98 *C*-5, 72.89 $CH_2C_6H_5$, 72.54 *C*′1, 44.23 *C*′-4, 27.13, and 27.02 *C*′-2, *C*′-3. A more polar product, R_f 0.08 (DCM−MeOH 9/1) (7% yield), was identified as the isomer **23**: ^{1}H NMR ($CDCl_3$, COSY) 8.86 and 8.22 (2s, 2H, *H*-2″ and *H*-8″), 7.45−7.25 (m, 10H (C_6H_5)$_2$), 4.89 (AB, $\Delta\delta$ = 0.16, J_{AB} = 11.4, 2H, $CH_2-C_6H_5$), 4.69 (s, 2H, $CH_2-C_6H_5$), 4.40 (AB part of an ABX$_2$, $\Delta\delta$ = 0.03, J_{AB} = 8.2, J_{AX} = J_{BX} = 7.2, 2H, C*H*$_2$-4′), 3.94 (t, J = 2.2, 1H, *H*-2), 3.91 (t, J = 9.4, 1H, *H*-6), 3.81 (AB part of an ABX$_2$, $\Delta\delta$ = 0.02, J_{AB} = 6.0, J_{AX} = J_{BX} = 4.1, C*H*$_2$-1′), 3.63 (t, J = 9.2, 1H, *H*-4), 3.52 (t, J = 9.1, 1H, *H*-5), 3.50 (dd, J = 9.1, J = 4.5, 1H, *H*-3), 3.31 (dd, J = 9.7, J = 2.3, 1H, *H*-1), 2.60 (broad s, 1H, exchangeable with CD_3OD, O*H*), 2.00 (qu, J = 7.4, 2H, C*H*$_2$-3′), 1.68 (qu, J = 6.5, 2H, C*H*$_2$-2′); ^{13}C NMR (50.29 MHz, $CDCl_3$) 162.08, 152.66, 149.98, 143.40, 138.91, 138.01, 122.69, 81.54, 80.53, 75.55, 75.26, 73.34, 72.87, 72.30, 47.22, 29.03, 26.96.

(±)3,6-Di-*O*-benzyl-2-*O*-[4′(9″-*N*-(6″-Cl-purinyl))butyl] *myo*-Inositol 1,4,5-Tris-*O*-dibenzyl(phosphate) (24). The triol **22** (217 mg, 381 μmol) was dissolved in dry THF (40 mL), kept under argon, and cooled at 0 °C. NaH suspension (6 equiv, 2.29 mmol, 91 mg) and crown ether [15-5] (100 μL) were added with stirring for 3 min, then heated at 50 °C for 30 min, and cooled again to 0 °C. Tetrabenzylpyrophosphate (6 equiv, 1.23 g) was added, and stirring was maintained overnight. The mixture was then warmed to room temperature. The reaction mixture was treated with $NaHCO_3$ (100 mL of saturated aqueous solution) and extracted with CH_2Cl_2 (twice 100 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. Purification on a silica gel column eluted with a mixture of ether−MeOH 19/1gave the expected tris(phosphate) **24** (201 mg, 39% yield): R_f 0.45 in ether−EtOH 19/1; ^{1}H NMR ($CDCl_3$, COSY) 8.71 and 8.16 (2s, 2H, *H*-2″ and *H*-8″), 7.4−6.9 (m, 40H, (C_6H_5)$_8$), 5.1−4.4 (m, 18H, ($CH_2C_6H_5$)$_8$ and *H*-4 at 4.97 and *H*-5 at 4.56; complexity of the m is simplified by {^{31}P}), 4.23 (ddd, J = 9.8, J = 7.4, J = 1.4, 1H, *H*-1, became dd J = 9.8, J = 1.9 after {^{31}P}), 4.15 (broad t, J = 7.4, 3H, C*H*$_2$-1′ + *H*-2), 3.68 (broad t, J = 4.5, 2H, C*H*$_2$-4′), 3.41 (dd, J = 9.5, J = 1.7, 1H, *H*-3), 1.94 (qu, J = 7.4, 2H, C*H*$_2$-3′), 1.53 (qu, J = 6.3, 2H, C*H*$_2$-2′); {^{1}H}-^{31}P NMR (121.49 MHz, $CDCl_3$) −0.94, −1.07, −1.19.

(±)-2-*O*-[4′(9″-*N*-Purinyl)butyl] *myo*-Inositol 1,4,5-Tris-(phosphate)-pentacyclohexylammonium (8). Protected inositol **24** (140 mg, 103 μmol) was dissolved in MeOH (5 mL) containing cyclohexene (0.9 mL) and $Pd(OH)_2$ (10 mg) in a sealed tube placed in a microwave oven (120 °C) for 5 min. The reaction mixture was filtered through a Millipore Millex GV 0.22 μM membrane which was rinsed with MeOH (30 mL) and water (10 mL). The dark solution was treated with black charcoal and filtered again. The solvents were evaporated and the residue redissolved in pure water (5 mL) and treated with a large excess of cyclohexylamine (5 mL). After 1 min of stirring, the mixture was evaporated to dryness again and dissolved in pure water (0.5 mL), which was added dropwise to dry acetone (100 mL). After overnight at 4 °C, crystals of the final product **8** were obtained: ^{1}H NMR (200 MHz, D_2O) 9.08, 8.90, 8.57 (3s, 3H, *H*-2″, *H*-6″ and *H*-8″), 4.4−3.6 (m, 10H, C*H*$_2$-1′, C*H*$_2$-4′ and *H*-1, *H*-2, *H*-3 *H*-4, *H*-5, *H*-6), 3.2−3.0 (m, 5H, (NC*H*)$_5$), 2.1−1.2 (m, 54H, (C*H*$_2$)$_{27}$); {^{1}H}-^{31}P NMR (121.49 MHz, $CDCl_3$) 4.34, 3.31, 2.92.

An analytical sample was passed through an ion exchanger column (IRA 120 H^+) to generate the free phosphate. MS ($C_{15}H_{25}N_4P_3O_{15}$ 594): (M − 1)/1 = 593, (M − 2)/2 = 296. HRMS: (M − 2)/2 calcd 296.0106, found 296.0095. {^{31}P}-^{1}H NMR (D_2O, COSY): 9.35, 9.24, 8.93 (3s, 3H, *H*-2″, *H*-6″ and *H*-8″), 4.47 (t, *J* = 7.2, 2H, C*H*$_2$-1′), 4.25−3.55 (m, 8H, containing at 4.23 *H*-4, 3.95 *H*-5, 3.90 *H*-2, 3.86 *H*-1, 3.75 *H*-6, 3.78 C*H*$_2$-4′, 3.67 *H*-3), 2.13 (qu, *J* = 7.2, 2H, C*H*$_2$-3′), 1.61 (qu, *J* = 8.0, 2H, C*H*$_2$-2′). {^{1}H}-^{31}P NMR (121.49 MHz, $CDCl_3$): 1.26, 0.87, 0.04.

The final product, as its pentacyclohexylammonium salt, was kept at −28 °C until tested.

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Supporting Information Available: Complementary experimental section. This material is available free of charge via the Internet at http://pubs.acs.org.

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