# Notes

## The Novel Ins(1,4,5)$P_3$ Analogue 3-Amino-3-deoxy-Ins(1,4,5)$P_3$: A pH-Dependent Ins(1,4,5)$P_3$ Receptor Partial Agonist in SH-SY5Y Neuroblastoma Cells

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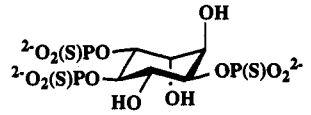
We have synthesized the first amino-substituted inositol 1,4,5-trisphosphate [Ins(1,4,5)$P_3$] analogue, D-3-amino-3-deoxy-*myo*-Ins(1,4,5)$P_3$ (**9**). Although **9** is a full agonist at the $Ca^{2+}$ mobilizing Ins(1,4,5)$P_3$ receptor at pH 7.2 and 7.6, it is apparently a high intrinsic activity partial agonist at pH 6.8, releasing only 80% of the Ins(1,4,5)$P_3$-sensitive $Ca^{2+}$ stores of SH-SY5Y cells. Additionally, **9** was able to fully displace [$^3$H]Ins(1,4,5)$P_3$ from binding sites in rat cerebellum membranes at both pH 6.8 and 7.6, indicating a full interaction with the Ins(1,4,5)$P_3$ receptor. The activity displayed by this amino analogue is unexpected and may be indicative of a pH-dependent conformational change in the amino acid residues comprising the Ins(1,4,5)$P_3$ binding site.

### Introduction

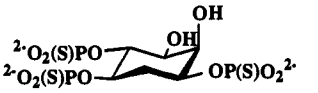
Many cell surface receptors activate phosphoinositidase C (PIC) via G-proteins. PIC catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to produce the second messengers, inositol 1,4,5-trisphosphate [Ins(1,4,5)$P_3$] and diacylglycerol.[1] Ins(1,4,5)$P_3$ interacts specifically with a family of tetrameric Ins(1,4,5)$P_3$ receptor-operated $Ca^{2+}$ channels to mobilize nonmitochondrial intracellular $Ca^{2+}$ stores.[2] Recently, several Ins(1,4,5)$P_3$ receptor ($IP_3R$) partial agonists have been detected. These include the high intrinsic activity Ins(1,3,4,6)$P_4$[3] and the low intrinsic activity analogues, L-*chiro*-inositol 2,3,5-trisphosphorothioate and D-6-deoxyinositol 1,4,5-trisphosphorothioate (Figure 1).[4] Herein we report the synthesis and biological characterization of the novel D-3-amino-3-deoxy-*myo*-Ins(1,4,5)$P_3$ (**9**). Under physiological ionic and pH conditions **9** probably exists largely in the 3-$NH_3^+$-Ins(1,4,5)$P_3$ form. Since the $NH_3^+$ moiety has approximately the same steric bulk as the native 3-hydroxyl group of Ins(1,4,5)$P_3$, **9** affords the opportunity to assess the biological effect of a positively charged substituent at the 3-position of Ins(1,4,5)$P_3$. By better understanding the structure–activity relationships that govern Ins(1,4,5)$P_3$'s calcium-releasing activity, it may be possible to discover new classes of Ins(1,4,5)$P_3$-based therapeutics for use in treating diseases like cancer that may be a consequence of abnormal intracellular calcium signalling.

### Chemistry

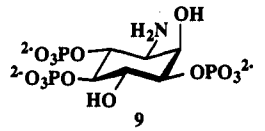
As shown in Scheme 1, the synthesis of D-3-amino-3-deoxy-*myo*-inositol 1,4,5-trisphosphate (**9**) employed D-3-azido-3-deoxy-*myo*-inositol (**1**)[5] as starting material. Thus, diacetonide formation from **1** with 2-methoxypropene and camphorsulfonic acid as catalyst gave a mixture of the diacetonides **2** and **3** in a 1.5:1 ratio.[16] The undesired diacetonide **3** could be recycled to the desired **2** via equilibration in acidic medium. Benzylation of the diacetonide **2** furnished **4** which could be selectively hydrolyzed to the monoacetonide **5**. Dibenzoylation, followed by hydrolysis of the *cis*-acetonide, gave the diol **6**. Differential benzoylation of the D-1 equatorial hydroxyl group followed by ethoxyethylation provided the fully protected diastereomeric derivatives **7** (the diastereoisomerism results from the ethoxyethyl group). Basic hydrolysis of **7** resulted in diastereomeric triols, the alkoxides of which were phosphorylated cleanly with tetrabenzyl pyrophosphate in DMF to give the protected trisphosphorylated derivatives **8**. Hydrogenolysis of the benzyl groups under 1 atm of pressure was accompanied by *in situ* hydrolysis of the ethoxyethyl function to provide the free acid which was titrated to pH = 8 with 1 N NaOH to yield **9** as a white amorphous hexasodium salt. Both the free acid and the salt were fully characterized by NMR analysis ($^1$H, $^{31}$P, and $^{13}$C), IR, and MS.




L-*chiro*-Inositol 2,3,5-trisphosphorothioate




D-6-Deoxy-inositol 1,4,5-trisphosphorothioate




**9**

**Figure 1.**

### Biological Testing

The ability of **9** to mobilize the Ins(1,4,5)$P_3$-sensitive intracellular $Ca^{2+}$ stores was assessed in $^{45}Ca^{2+}$-preloaded SH-SY5Y cells, permeabilized using saponin, as previously

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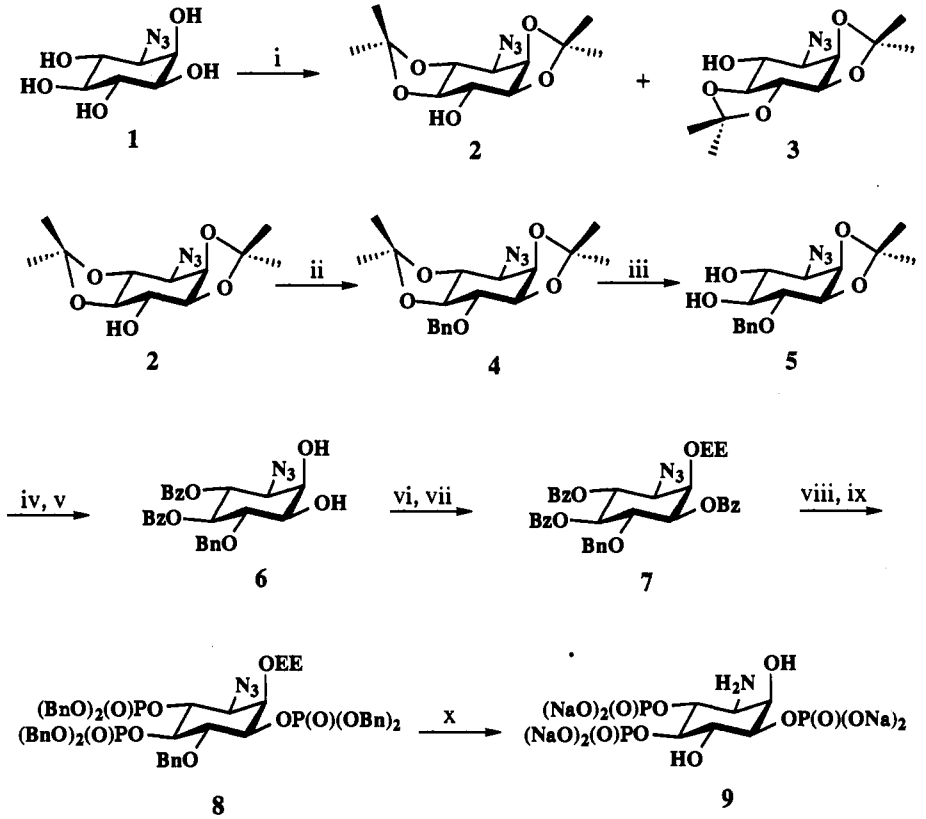
**Scheme I.**[a] Synthesis of D-3-Amino-3-deoxy-*myo*-inositol 1,4,5-Trisphosphate (9)




[a] (i) 2-Methoxypropene, camphorsulfonic acid, DMF, 65 °C (87%); (ii) NaH, BnBr, DMF, 0 °C (92%); (iii) AcCl (cat.), MeOH, dichloromethane (1:2 v/v), room temperature (81%); (iv) BzCl, pyridine, 0 °C to room temperature (v) conc. HCl, MeOH, room temperature (84% overall); (vi) BzCl, pyridine, room temperature (95%); (vii) ethyl vinyl ether, *p*-toluenesulfonic acid, dichloromethane, room temperature (viii) potassium carbonate, MeOH, room temperature (91% overall); (ix) tetrabenzyl pyrophosphate, NaH, DMF, 0 °C (73%); (x) $H_2$, 10% Pd–C, 90% aqueous AcOH, then titrate with 1 N NaOH (92%).

**Table 1.** The Effect of pH on Specific Ins(1,4,5)$P_3$ Receptor Binding and $^{45}Ca^{2+}$-Release Profiles of Ins(1,4,5)$P_3$ and 9

| buffer pH | Ins(1,4,5)$P_3$ | | 3-amino-3-deoxy-Ins(1,4,5)$P_3$ | |
|---|---|---|---|---|
| | $EC_{50}$ (nM)[a] | $IC_{50}$ (nM)[b] | $EC_{50}$ (nM)[a] | $IC_{50}$ (nM)[b] |
| 6.8 | 255 ± 49 | 78 ± 8 | 3676 ± 388 | 9114 ± 2282 |
| 7.2 | 52 ± 2 | | 1070 ± 130 | |
| 7.6 | 107 ± 15 | 61 ± 6 | 1491 ± 159 | 5652 ± 1779 |

[a] Ins(1,4,5)$P_3$ and 3-amino-3-deoxy-Ins(1,4,5)$P_3$ induced $^{45}Ca^{2+}$ mobilization in saponin permeabilized SH-SY5Y cells at pH 6.8, 7.2, and 7.6 and 20–22 °C. $EC_{50}$ values (nM) are shown as mean ± sem ($n$ = 4). [b] Displacement of specifically bound [$^3$H]Ins(1,4,5)$P_3$ from pig cerebellar membranes by Ins(1,4,5)$P_3$ and 3-amino-3-deoxy-Ins(1,4,5)$P_3$ at pH 6.8 and 7.6. $IC_{50}$ values (nM) are shown as mean ± sem ($n$ = 3).

described for 1321N1 cells.[6] The experiments were conducted in a cytosolic-like buffer (CLB)[7] with the pH adjusted to either 6.8, 7.2, or 7.6. All $^{45}Ca^{2+}$ release values were standardized (100% release) to the $^{45}Ca^{2+}$ release produced by a maximally effective concentration of Ins(1,4,5)$P_3$ (30 μM), included as an internal standard in all experiments ($n$ = 4). The binding affinity of the agents at the $IP_3R$ was assessed using pig cerebellar membranes, as described[8] using buffer adjusted to pH 6.8 or 7.6.

For both Ins(1,4,5)$P_3$ and 9 the optimum pH for $Ca^{2+}$ release was 7.2, and at all pH values Ins(1,4,5)$P_3$ was a significantly more potent agonist than 9 ($p < 0.01$). The $EC_{50}$ values of the concentration–response curves of both Ins(1,4,5)$P_3$ and 9 exhibited identical respective rightward shifts at pH 6.8 ($p < 0.05$) and also at pH 7.6 ($p < 0.05$) (Table 1). However, the concentration–response curve of 9 had a unique profile with maximal concentrations releasing only about 80% of the total intracellular Ins(1,4,5)$P_3$-sensitive $Ca^{2+}$ pool (Figure 2). Thus, 9 was a full agonist at pH 7.2 and 7.6, but apparently a partial agonist at pH 6.8. This partial agonism was unlikely to be an artifact arising from the relatively high $EC_{50}$ for $Ca^{2+}$ release exhibited by 9, since Ins(1,3,4,5)$P_4$ is a full agonist at pH 6.8, 7.2, and 7.6, despite the fact that it exhibits an even higher $EC_{50}$ value for $Ca^{2+}$ release at pH 6.8 in SH-SY5Y cells (5315 ± 419 nM, $n$ = 4).

## Discussion

Two of the other $IP_3R$ partial agonists, L-*chiro*-Ins(2,3,5)$PS_3$[4] and Ins(1,3,4,6)$P_4$[3], identified in SH-SY5Y cells can be visualized in binding conformations with the $IP_3R$ which mimic Ins(1,4,5)$P_3$ with the exception that their pseudo-3-position bears an axial hydroxyl group, as opposed to the native equatorial hydroxyl group.[9,10] Thus, the orientation and substituent status of the 3-position of Ins(1,4,5)$P_3$ may be an important regulator of the $IP_3R$. Ins(1,3,4,5)$P_4$ possesses all the critical structural motifs requisite for Ins(1,4,5)$P_3$ receptor interaction, plus an equatorially oriented phosphate group, but is a relatively weak agonist for $Ca^{2+}$ release.[7] In contrast, isosteric replacement of the native 3-OH of Ins(1,4,5)$P_3$ with fluorine, which has a similar electronegativity and size to a hydroxyl group,[11] produces 3-deoxy-3-fluoro-Ins(1,4,5)$P_3$, which shows only slightly weaker ligand and agonist activity than Ins(1,4,5)$P_3$.[6,10,12] Here we have demonstrated that the electronic charge as well as the size of the 3-position substituent appears to be influential since the positively charged $NH_3^+$ of 9 produces a significant

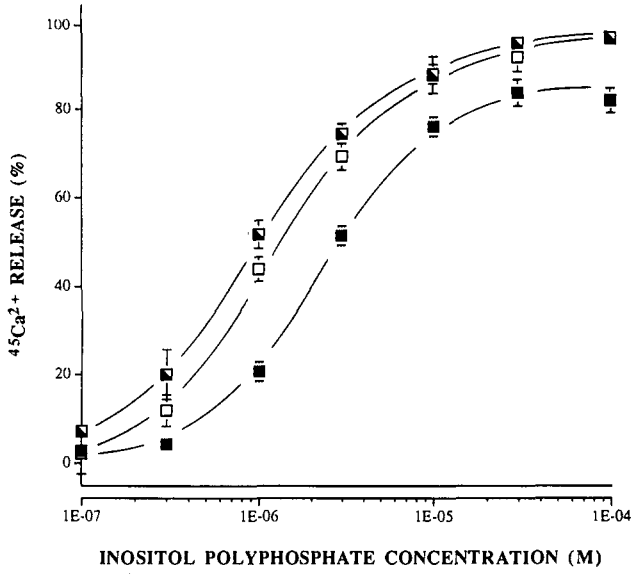



**Figure 2.** 3-Amino-3-deoxy-Ins(1,4,5)$P_3$ (**9**) induced $^{45}Ca^{2+}$ release from the Ins(1,4,5)$P_3$-sensitive calcium pool of saponin permeabilized SH-SY5Y cells at pH 6.8 (■), pH 7.2 (▣), and pH 7.6 (□). Note that at pH 6.8 the maximally effective concentration of **9** (30–100 μM) releases only 80% of the Ins(1,4,5)$P_3$-sensitive $Ca^{2+}$ pool (100% release).

reduction in agonist efficiency even though the amino group has steric bulk similar to both the fluoro and hydroxyl groups.

It is difficult to explain why **9** exhibited detectable partial agonism only at pH 6.8, since presumably $NH_3^+$ is the dominant form of the 3-position substituent at all pH's tested. Additionally, **9** was able to fully displace [$^3$H]-Ins(1,4,5)$P_3$ from binding sites in rat cerebellum membranes at both pH 6.8 and 7.6, indicating a full interaction with the $IP_3R$ (Table 1). Perhaps the partial agonism of **9** derives from subtle pH-dependent conformational modifications of the amino acid residues making up the binding site of the $IP_3R$, producing selectively a suboptimal interaction with **9**. We are currently developing other 3-position Ins(1,4,5)$P_3$ analogues to further probe the function of the Ins(1,4,5)$P_3$ receptor.

## Experimental Section

**General.** Nuclear magnetic resonance spectra ($^1$H, $^{13}$C, $^{31}$P) were measured with a Bruker WH-300 instrument ($^1$H frequency 300 MHz, $^{31}$P frequency 121.5 MHz) in the solvent noted. $^1$H chemical shifts are expressed in parts per million downfield from $Me_4Si$ used as internal standard. $^{31}$P chemical shifts were referenced to external aqueous 85% $H_3PO_4$. Infrared spectra were recorded on a 2020 Galaxy FT-IR spectrometer. Elemental analyses were performed by ONEIDA Research Services, Whitesboro, NY. Silica gel 60 (Merck, 230–400 mesh) was used for column chromatography. Thin-layer chromatography was performed on Merck silica gel F-254 plates (0.25 mm, precoated on glass). Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Tetrahydrofuran (THF) was distilled over sodium benzophenone ketyl, and *N,N*-dimethylformamide (DMF) and pyridine were distilled over $CaH_2$ at reduced and atmospheric pressure, respectively. Dichloromethane was distilled over $P_2O_5$. The phrase "standard workup" indicates extracting the aqueous phase three times with the noted organic solvent, washing the combined organic extracts sequentially with water and brine, drying with $MgSO_4$, and evaporating under aspirator pressure.

**D-3-Azido-3-deoxy-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol (2) and D-3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-*myo*-inositol (3).** A mixture of D-3-azido-3-deoxy-*myo*-inositol[5,13] (2.594 g, 12.6 mmol), camphorsulfonic acid (186 mg), and 2-methoxypropene (4.48 mL, 50.4 mmol) in 60 mL of dry DMF was heated under argon for 14 h at 46 °C. After cooling, triethylamine (5 mL) was added, and the DMF was distilled off *in vacuo*. Standard workup (ether) afforded a light yellow oil which was purified by chromatography (30% EtOAc/hexane) to give **2** (1.45 g, 47%; $R_f$ = 0.55) and **3** (1.68 g, 40%; $R_f$ = 0.35) as white solids. The regiochemistry of these diacetonides was confirmed by double resonance experiments conducted on D-3-azido-1,4,5-tri-*O*-benzoyl-6-*O*-benzyl-3-deoxy-*myo*-inositol (*vide infra*).

Physical and spectral data for **2**: mp 132–133 °C (hexane); $^1$H NMR ($CDCl_3$) δ 4.47 (1 H, t, *J* = 4.6 Hz), 4.06 (1 H, dd, *J* = 6.4, 5.0 Hz), 3.96 (1 H, dd, *J* = 10.7, 9.4 Hz), 3.90 (1 H, m), 3.73 (1 H, dd, *J* = 10.7, 5.0 Hz), 3.37 (1 H, dd, *J* = 10.4, 9.4 Hz), 2.71 (1 H, OH, d, *J* = 3.0 Hz), 1.54 (3 H, s), 1.49 (3 H, s), 1.46 (3 H, s), 1.39 (3 H, s); $^{13}$C NMR ($CDCl_3$) δ 113.06, 110.58, 81.77, 78.98, 76.69, 75.41, 74.41, 59.26, 28.03, 26.89, 26.83, 25.91; MS (EI) *m/z* 270 ($M^+ - CH_3$), 198, 113, 85; IR (thin film) 3460, 2991, 2106, 1377, 1052 cm$^{-1}$; $[\alpha]^{23}_D$ −8.6° (*c* 580 μg/mL, $CHCl_3$). Anal. ($C_{12}H_{19}N_3O_5$) C, H, N.

Physical and spectral data for **3**: mp 117–119 °C (hexane); $^1$H NMR ($CDCl_3$) δ 4.50 (1 H, t, *J* = 5.0 Hz), 4.26 (1 H, dd, *J* = 8.6, 5.0 Hz), 4.10 (1 H, ddd, *J* = 9.3, 7.6, 3.5 Hz), 3.71 (1 H, dd, *J* = 10.2, 8.6 Hz), 3.61 (1 H, dd, *J* = 7.6, 5.0 Hz), 3.38 (1 H, OH, d, *J* = 3.5 Hz), 3.34 (1 H, t, *J* = 9.8 Hz), 1.54 (3 H, s), 1.44 (3 H, s), 1.43 (3 H, s), 1.37 (3 H, s); $^{13}$C NMR ($CDCl_3$) δ 112.61, 110.77, 76.51, 78.23, 76.17, 76.01, 71.90, 64.17, 28.00, 26.83, 26.75, 25.74; MS (EI) *m/z* ($M^+ - CH_3$), 242, 198, 142, 113; IR (thin film) 3460, 2991, 2106, 1377, 1223, 1052 cm$^{-1}$; $[\alpha]^{23}_D$ +20° (*c* 250 μg/mL, $CHCl_3$). Anal. ($C_{12}H_{19}N_3O_5$) C, H, N.

**D-3-Azido-6-*O*-benzyl-3-deoxy-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol (4).** A solution of the diacetonide **2** (175 mg, 0.63 mmol) and benzyl bromide (298 μL, 2.5 mmol) in 5 mL of DMF was added via cannula to a stirred suspension of NaH (90 mg as 50% oil suspension, 1.88 mmol) in 3 mL of DMF at 0 °C under argon. The mixture was stirred at 0 °C for 2 h and then quenched by careful addition of a few drops of water, and DMF was removed *in vacuo*. Standard workup (ether) yielded a solid residue which on chromatography (10% EtOAc/hexane) furnished **4** (213 mg, 92%) as a white solid: mp 119–121 °C (hexane); $^1$H NMR ($CDCl_3$) δ 7.45–7.25 (5 H, m), 4.82 (2 H, s), 4.44 (1 H, t, *J* = 4.6 Hz), 4.18 (1 H, dd, *J* = 6.3, 5.1 Hz), 3.91 (1 H, dd, *J* = 10.7, 9.2 Hz), 3.73–3.65 (2 H, m), 3.45 (1 H, dd, *J* = 10.5, 9.2 Hz), 1.50 (3 H, s), 1.47 (3 H, s), 1.38 (3 H, s), 1.37 (3 H, s); $^{13}$C NMR ($CDCl_3$) δ 137.96, 128.23, 127.98, 127.59, 112.89, 110.27, 81.21, 79.76, 79.48, 76.75, 75.33, 72.09, 59.24, 27.72, 26.99, 26.91, 25.94; MS *m/z* 360 ($M^+ - CH_3$), 332, 275, 91; IR (thin film) 2988, 2104, 1373, 1224, 1085 cm$^{-1}$; $[\alpha]^{23}_D$ −56° (*c* 0.50 mg/mL, $CHCl_3$).

**D-3-Azido-4,5-di-*O*-benzoyl-6-*O*-benzyl-3-deoxy-*myo*-inositol (6).** The diacetonide **4** (213 mg, 0.58 mmol) was dissolved in 5 mL of $CH_2Cl_2$/MeOH (2:1 v/v) and stirred with 2 drops of AcCl at room temperature for 5 min. A few drops of triethylamine were added, and the volatiles were evaporated to leave a solid residue. Chromatography (40–50% EtOAc/hexane) yielded the diol **5** (137 mg, 72%) as a white solid. This material was dissolved in 5 mL of pyridine, benzoyl chloride (130 μL, 1.04 mmol) and 4-(*N,N*-dimethylamino)pyridine (DMAP; 10 mg) were added at 0 °C, and the mixture was stirred at this temperature for 2 h. Pyridine was distilled off in vacuo. Addition of EtOAc, filtration, and evaporation left a residue which was purified by chromatography (20% EtOAc/hexane) to give D-3-azido-4,5-di-*O*-benzoyl-6-*O*-benzyl-3-deoxy-1,2-*O*-isopropylidene-*myo*-inositol as a white solid. Methanol (14 mL), THF (4 mL), and concentrated HCl (50 μL) were added, and the resulting mixture was stirred at room temperature for 14 h. After rotary evaporation of the volatiles, the residue was directly chromatographed (50% EtOAc/hexane) to afford the diol **6** (170 mg, 61% over three steps) as a white solid: mp 52–53 °C; $^1$H NMR ($CDCl_3$) δ 8.00–7.91 (4 H, m), 7.55–7.45 (2 H, m), 7.42–7.30 (4 H, m), 7.30–7.15 (5 H, m), 5.94 (1 H, t, *J* = 10.1 Hz), 5.58 (1 H, t, *J* = 9.7 Hz), 4.71 (1 H, d, *J* = 11.3 Hz), 4.62 (1 H, dd, *J* = 11.3 Hz), 4.34 (1 H, br s), 4.08 (1 H, t, *J* = 9.6 Hz), 3.77 (1 H, m), 3.62 (1 H, dd, *J* = 10.1, 2.5 Hz); $^{13}$C NMR ($CDCl_3$) δ 165.68, 165.60, 137.49, 133.64, 133.24, 130.18, 129.79, 129.71, 129.20, 128.98, 128.54, 128.40, 128.32, 128.10, 79.03, 75.35, 73.65, 72.12, 71.10, 71.12, 61.74; MS (EI) *m/z* 336 ($M^+ - BzO - N_2 - H_2O$), 267, 207, 161, 105; IR (thin film)

3421, 3085, 2918, 2108, 1720, 1275, 1093 cm$^{-1}$; $[\alpha]^{23}_D$ −50° (c 1.4 mg/mL, $CHCl_3$).

**D-3-Azido-1,4,5-tri-*O*-benzoyl-6-*O*-benzyl-3-deoxy-*myo*-inositol.** The dibenzoate 6 (170 mg, 0.34 mmol) and DMAP (5 mg) were dissolved in 4 mL of dry pyridine, benzoyl chloride (49 μL) was added at 0 °C dropwise via syringe, and the reaction mixture was stirred at that temperature for 3 h. After two drops of water were added, the pyridine was evaporated under vacuum. The resulting residue was directly chromatographed (25% EtOAc/hexane) to furnish the 1,4,5-tribenzoate (194 mg, 95%) as a white solid. The structure of this compound (and, indirectly, of the diacetonides 2 and 3) was established on the basis of first-order analysis of the $^1$H NMR spectra decoupled at the C-1 and C-2 protons: mp 178–179 °C (EtOAc/hexane); $^1$H NMR ($CDCl_3$) δ 8.06 (2 H, d, $J$ = 7.3 Hz), 7.94 (2 H, d, $J$ = 7.4 Hz), 7.90 (2 H, d, $J$ = 7 Hz), 7.62 (1 H, d, $J$ = 7.4 Hz), 7.51 (2 H, d, $J$ = 7.9 Hz), 7.46 (2 H, d, $J$ = 7.9 Hz), 7.39–7.34 (4 H, m), 7.40–6.93 (5 H, m), 5.99 (t, $J$ = 10.2 Hz, 4-H), 5.67 (t, $J$ = 9.7 Hz, 5-H), 5.29 (dd, $J$ = 10, 2.6 Hz, 1-H), 4.67 (1 H, d, $J$ = 11.2 Hz), 4.60 (1 H, d, $J$ = 11.2 Hz), 4.58 (m, 2-H), 4.47 (t, $J$ = 9.7 Hz, 6-H), 3.84 (dd, $J$ = 10.5 Hz, 3-H); $^{13}$C NMR ($CDCl_3$) δ 165.65, 165.57, 165.48, 137.26, 133.56, 133.31, 133.14, 129.85, 129.73, 129.26, 128.93, 128.60, 128.37, 128.30, 128.15, 127.89, 127.64, 75.38, 74.15, 73.49, 71.00, 69.80, 61.94; MS (EI) $m/z$ 336 ($M^+$ − OBz − $N_2$ − $H_2O$), 267, 207, 161, 105; IR (thin film) 3500, 2106, 1728, 1269, 1097, 709 cm$^{-1}$; $[\alpha]^{23}_D$ −49.9° (c 2.7 mg/mL, $CHCl_3$). Anal. ($C_{34}H_{29}N_3O_8$) C, H, N.

**D-3-Azido-6-*O*-benzyl-3-deoxy-*myo*-inositol 1,4,5-Trisphosphoric Acid Hexabenzyl Ester.** To a solution of the tribenzoate, obtained above, and pyridinium $p$-toluenesulfonate (100 mg) in 4 mL of dry $CH_2Cl_2$ were added via syringe three 50-μL portions of ethyl vinyl ether at 1/2-h intervals. After stirring for a total of 3 h at room temperature, the reaction was quenched with saturated $NaHCO_3$ solution. The standard workup (ether) afforded 7 which was directly saponified at room temperature by reacting it overnight with anhydrous $K_2CO_3$ (200 mg) in 5 mL of MeOH. Filtration and evaporation left an oil which was purified by chromatography on silica gel with 80% EtOAc/hexane to give the 1,4,5-triol (105 mg, 91% overall). This material (53 mg, 144 μmol) and tetrabenzyl pyrophosphate (544 mg, 1.01 mmol) were dissolved in 3 mL of dry DMF and added, via cannula, to a stirred suspension of NaH (63 mg, 50% in oil) in 1 mL of DMF at 0 °C. After stirring for 3 h, a few drops of water were added, and the DMF was evaporated under high vacuum at room temperature. The solid residue thus obtained was directly loaded on a silica gel column. Elution with 40% EtOAc/hexane yielded a mixture of the diastereomers of 8 as an oil. To simplify analysis, the ethoxyethyl protecting group was removed by a procedure similar to the one described for compound 4 to give the free alcohol: $^1$H NMR ($CDCl_3$) δ 7.35–7.10 (33 H, m), 7.00–6.92 (2 H, m), 5.13–4.98 (5 H, m), 4.96–4.83 (7 H, m), 4.81 (1 H, d, $J$ = 9.3 Hz), 4.68 (1 H, d, $J$ = 9.3 Hz), 4.72–4.59 (2 H, m, benzylic H and 4-H), 4.50 (ddd, $J$ = 9.2 Hz, 5-H), 4.40 (m, 2-H), 4.18 (m, 1-H), 4.00 (t, $J$ = 9.5 Hz, 6-H), 3.62 (d, $J$ = 3.8 Hz, OH), 3.19 (dd, $J$ = 10.3, 2.0 Hz, 3-H); $^{31}$P NMR ($CDCl_3$) δ −0.92, −1.15, −1.46; IR (thin film) 3333, 3065, 3034, 2110, 1456, 1269, 1016 cm$^{-1}$; $[\alpha]^{23}_D$ −8.9° (c 37.5 mg/mL, $CHCl_3$). Anal. ($C_{55}H_{56}N_3O_{14}P_3$) C, H, N.

**D-3-Amino-3-deoxy-*myo*-inositol 1,4,5-Trisphosphate (9).** The diastereomeric mixture 8 (45 mg, 39.2 μmol) was dissolved in 9 mL of AcOH/$H_2O$ (9:1 v/v). Pd–C (10%, 90 mg) was added, and the resulting mixture was hydrogenolyzed at room temperature and atmospheric pressure during 12 h. After filtration over Celite, the volatiles were removed under high vacuum at room temperature. The residue, thus obtained, was dissolved in 4 mL of water and lyophilized to deliver the title $IP_3$ analogue as a fluffy solid. The free acid was converted to the sodium salt by neutralization with 1 N NaOH. The triethylammonium salt was also made by stirring an aqueous solution of the free acid with distilled triethylamine, followed by lyophilization: $^1$H NMR ($D_2O$, triethylammonium salt) δ 4.35 (t, $J$ = 2.9 Hz, 2-H), 4.26 (q, $J$ = 9.0 Hz, 4- or 5-H), 4.02 (q, $J$ = 9.0 Hz, 4- or 5-H), 3.95 (td, $J$ = 9, 2.9 Hz, 1-H), 3.85 (t, $J$ = 9 Hz, 6-H), 3.44 (dd, $J$ = 10.4, 2.9 Hz, 3-H); $^{13}$C NMR ($D_2O$, sodium salt) δ 81.06 (br s), 77.80 (d, $J$ = 5 Hz), 75.79 (br s), 72.90, 69.70, 55.36; $^{31}$P NMR ($D_2O$, triethylammonium salt, $^1$H-decoupled) δ 6.58, 5.78, 4.42; $[\alpha]^{25}_D$ −9.1° (c 6.25 mg/mL, $H_2O$); MS (plasma desorption, free acid) $m/z$ 279 ($M^+$ − $CHOCH_2OPO_3H_2$). Anal. ($C_{24}H_{69}N_4O_{18}P_3$, as triethylammonium salt, tetrahydrate) C, H, N.

**$^{45}Ca^{2+}$-Release Assay.** The ability to mobilize the Ins(1,4,5)$P_3$-sensitive intracellular $Ca^{2+}$ stores was assessed in $^{45}Ca^{2+}$-preloaded SH-SY5Y cells, permeabilized using saponin, as previously described for 1321N1 cells.[14] The experiments were conducted in a cytosolic-like buffer (CLB)[7] with the pH adjusted to either 6.8, 7.2, or 7.6. All $^{45}Ca^{2+}$-release values were standardized (100% release) to the $^{45}Ca^{2+}$ release produced by a maximally effective concentration of Ins(1,4,5)$P_3$ (30 μM), which was included as an internal control in all experiments.

**Preparation of Ins(1,4,5)$P_3$ Receptor-Rich Membranes from Pig Cerebellum.** Pig cerebella were obtained from a local abattoir and either used immediately or frozen in liquid nitrogen and stored at −70 °C. Pig cerebella "$P_2$" membrane preparations were prepared exactly as previously described for rat cerebellum.[15] The final membrane pellet was suspended in homogenization buffer (20 mM $NaHCO_3$ and 1 mM dithiothreitol) at 6–8 mg of protein/mL snap frozen in liquid nitrogen and stored at −79 °C until required.

**Ins(1,4,5)$P_3$ Receptor Binding Studies.** Increasing concentrations of Ins(1,4,5)$P_3$ or 3-amino-Ins(1,4,5)$P_3$ were incubated in a total assay volume of 120 μL with 1.5 nM [$^3$H]Ins(1,4,5)$P_3$ (17 Ci/mmol, Amersham International, U.K.) in a buffer containing 25 mM Tris/maleate, 5 mM $NaHCO_3$, 1 mM EDTA, and 0.25 mM DTT adjusted to the appropriate pH. The magnitude and affinity of Ins(1,4,5)$P_3$ receptor binding decreases with increasing acidity. Therefore incubations were initiated by addition of about 50 (at pH 7.6) or 70 (at pH 6.8) μg of cerebellar membrane protein and continued for 30 min at 4 °C. Bound and free ligand were separated by centrifugation (12000g, 4 min). Pellets were solubilized by addition of 100 μL of 2% sodium dodecyl sulfate before addition of 1 mL of scintillant. Residual bound radioactivity in the presence of 10 μM Ins(1,4,5)$P_3$ was defined as nonspecific binding.

**Data Analysis.** Agent concentrations producing 50% of the maximal response ($EC_{50}$ values) or 50% inhibition of the maximal response ($IC_{50}$ values) were calculated via computer-assisted curve fitting using GraphPad INPLOT VERSION 3.1 (GraphPad software, U.S.A.). Combined data from independent experiments were expressed as mean ± sem, where $n \geq 3$, and computer-assisted statistical analysis was performed in Excel (Microsoft Corp., WA).

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(16) The regiochemistry of compounds **2** and **3** was originally speculated on the basis of polarity differences exhibited by the corresponding fluoro diacetonide analogues.[12] However, the location of the hydroxyl group of the diacetonide **2** at C-6 was established definitively by analyzing detailed double resonance $^1H$ NMR experiments of a later intermediate, D-3-azido-1,4,5-tri-*O*-benzoyl-3-deoxy-*myo*-inositol (see the Experimental Section).