CHEMBIOCHEM

Supporting Information

Cellular Internalisation of an Inositol Phosphate Visualised by Using Fluorescent $InsP_5$

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cbic_201300583_sm_miscellaneous_information.pdf

Supporting Information

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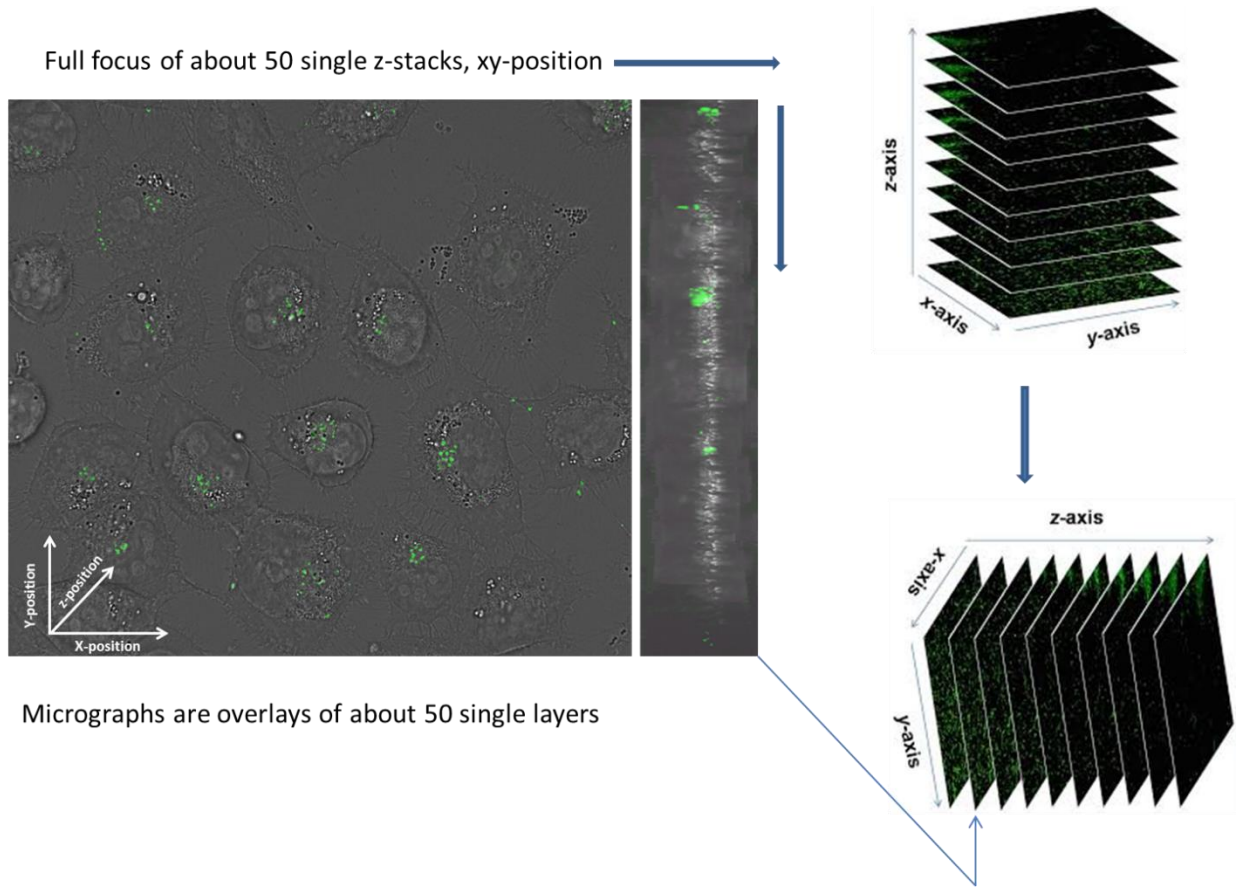


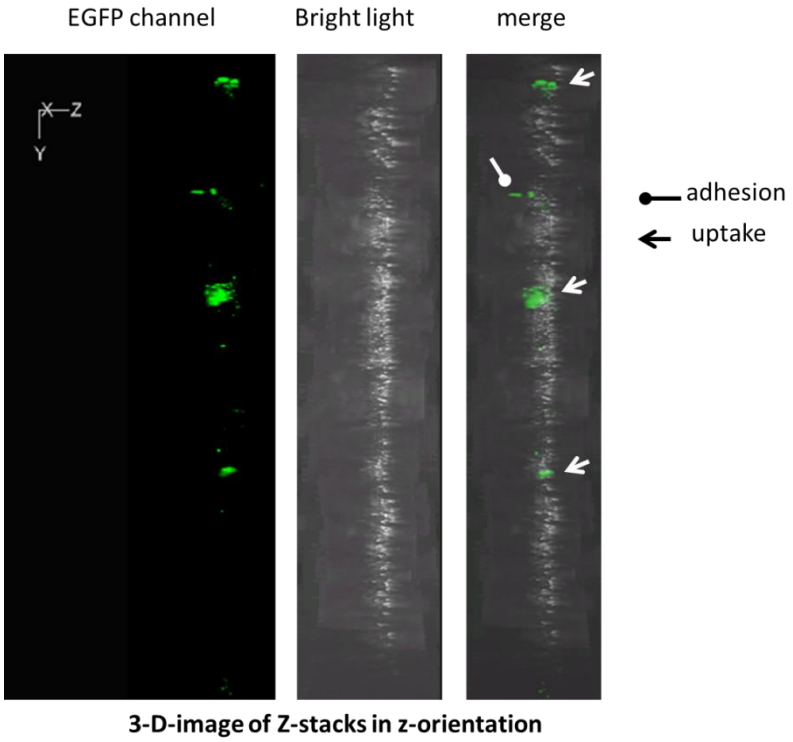


Figure S1. H1299 cells were treated with **5** (20 μM) for 16 h. Washed cells were fixed and analysed by fluorescence microscopy. Z-stacks and 3D-reconstituations of Z-stacks were performed and uptake of **5** was analysed in micrographs that were rotated in the Z-axis by 90°. Green dots at the outer layer were defined as adhesion (●—) and dots in the middle of the cell layer as uptake (←).

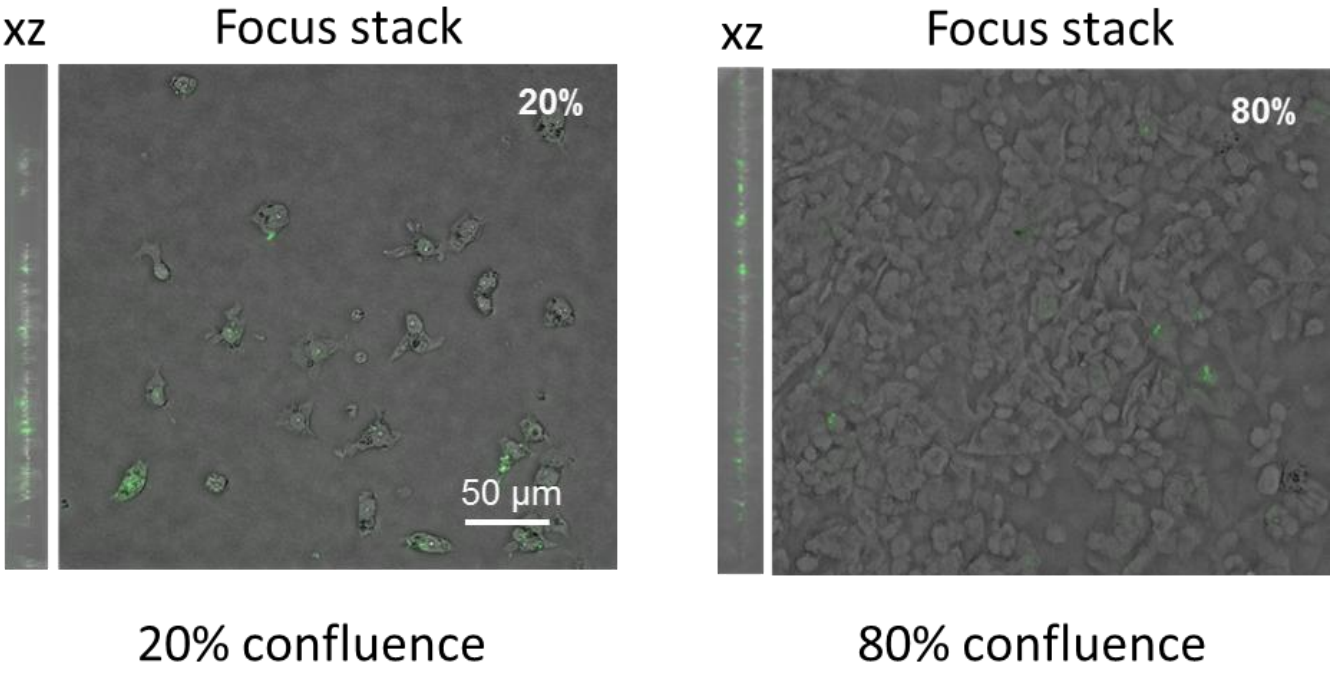


Figure S2. Uptake of FAM-$InsP_5$ (**5**) in H1299 cells. H1299 cells were grown to 20% or 80% confluence and treated with **5** (20 µM). After 16 h, washed cells were with paraformaldehyde and embedded in Fluoromount-G medium. Z-stacks of bright and fluorescence light micrographs were performed. Shown are the xz-layer and focus stackings of bright and fluorescence light overlays in a magnification of 10 x 20. Five micrographs per series (20% or 80% confluence) were performed and whole cell numbers and numbers of green fluorescent cells of the middle cell layers were counted to calculate % cellular uptake of **5**.

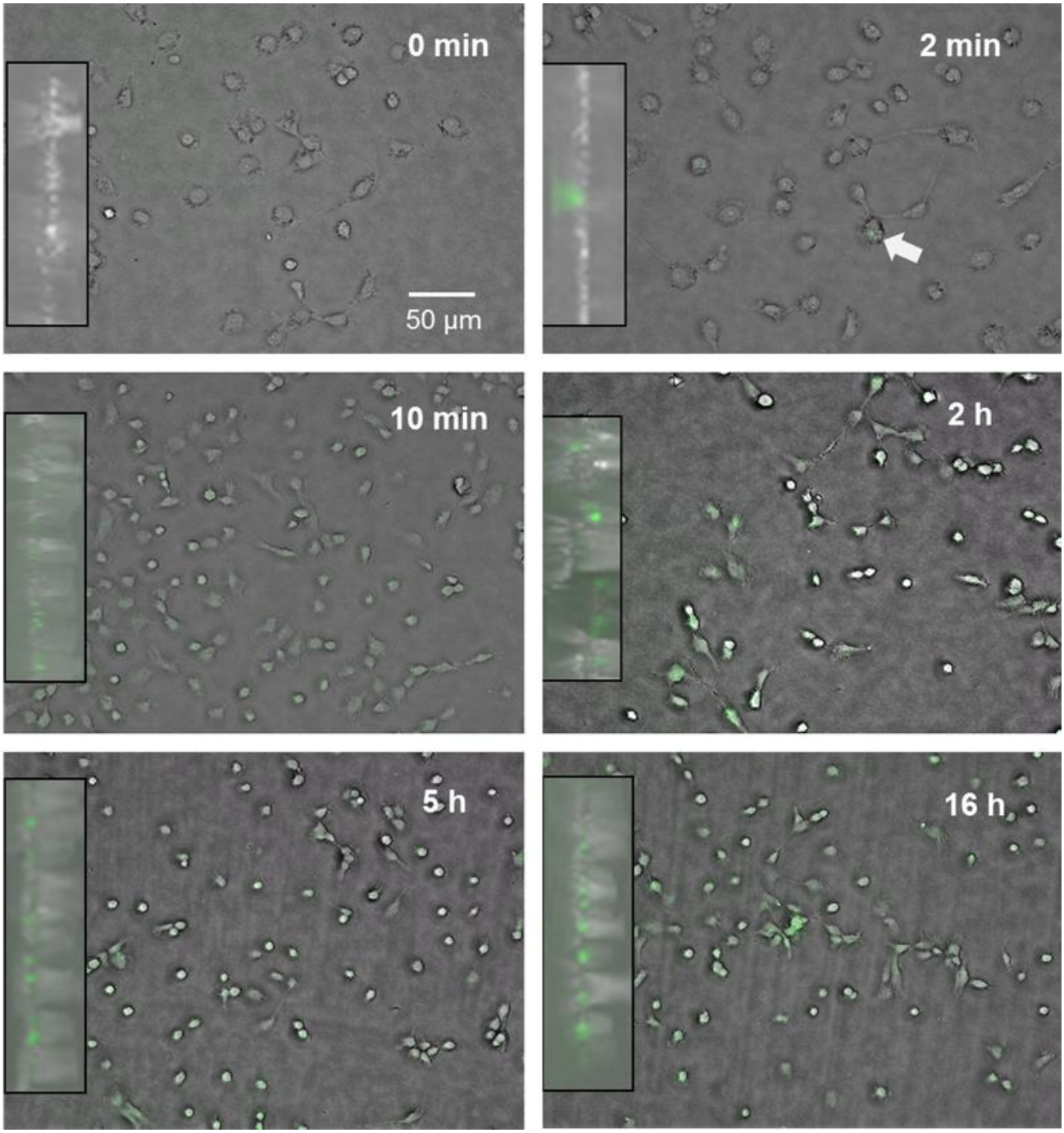
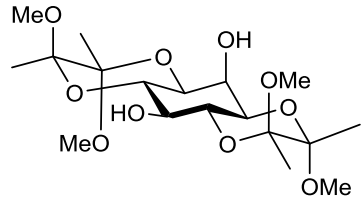
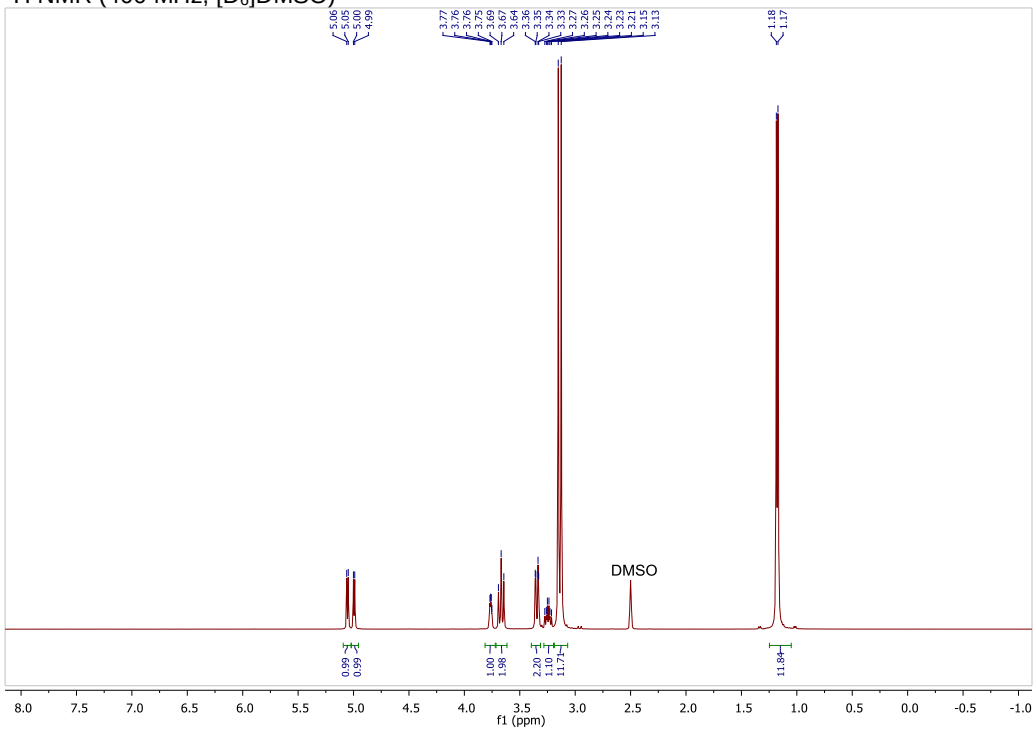


Figure S3. Time dependence of FAM-$InsP_5$ (**5**) uptake into H1299 cells. H1299 cells were grown to 50% confluence and treated with 20 µM **5** for the time points as indicated in the figure. Then, the washed cells were embedded in Fluoromount-G medium and uptake of **5** was determined as described above. To illustrate uptake over time, the xz-layers of representative micrographs are shown in higher magnification (left panels). After 2 min of incubation, only cell surface aggregates of **5** were visible (indicated by an arrow) and after 10 min, the first intracellular aggregates of **5** were detected. For each time point, at least five micrographs were evaluated, and % cellular uptake of **5** was calculated as before.

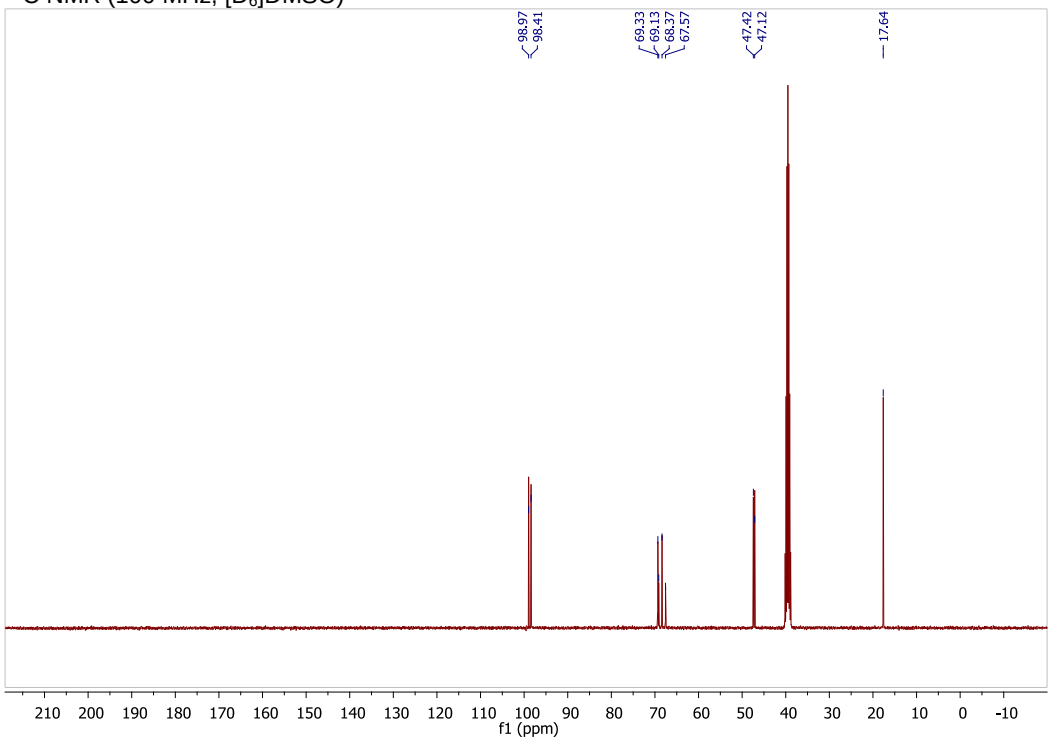
1,6:3,4-Bis-[*O*-(2,3-dimethoxybutane-2,3-diyl)]-*myo*-inositol (**6**)



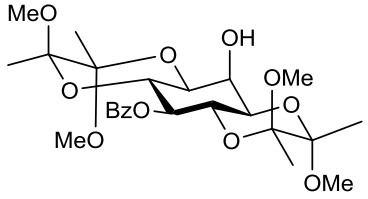
^{1}H NMR (400 MHz, [D$_6$]DMSO)



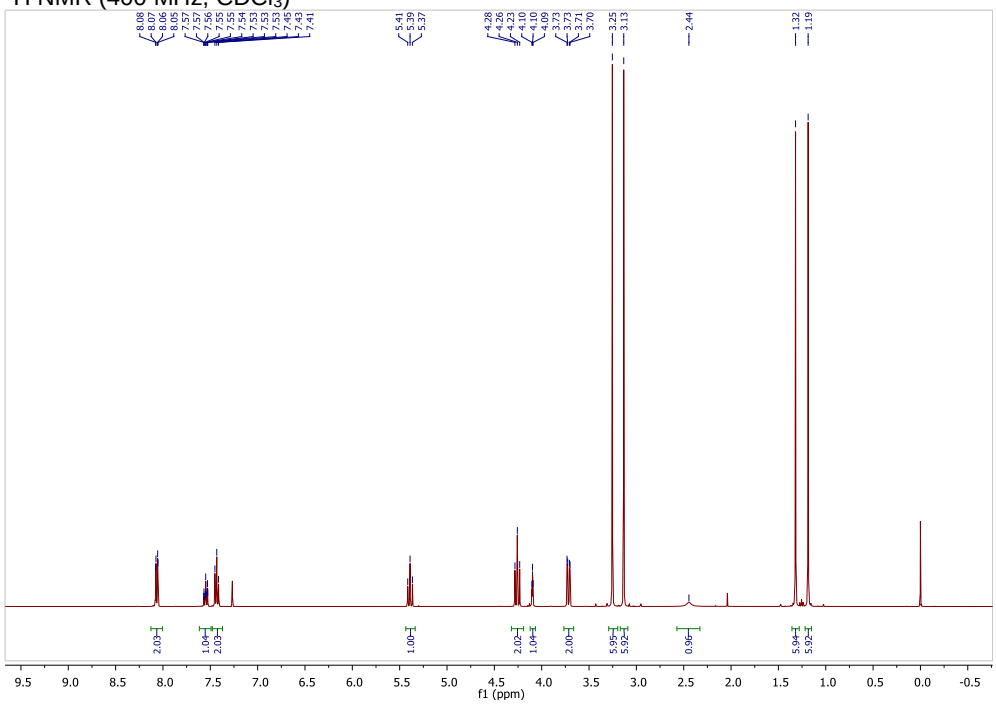
^{13}C NMR (100 MHz, [D$_6$]DMSO)



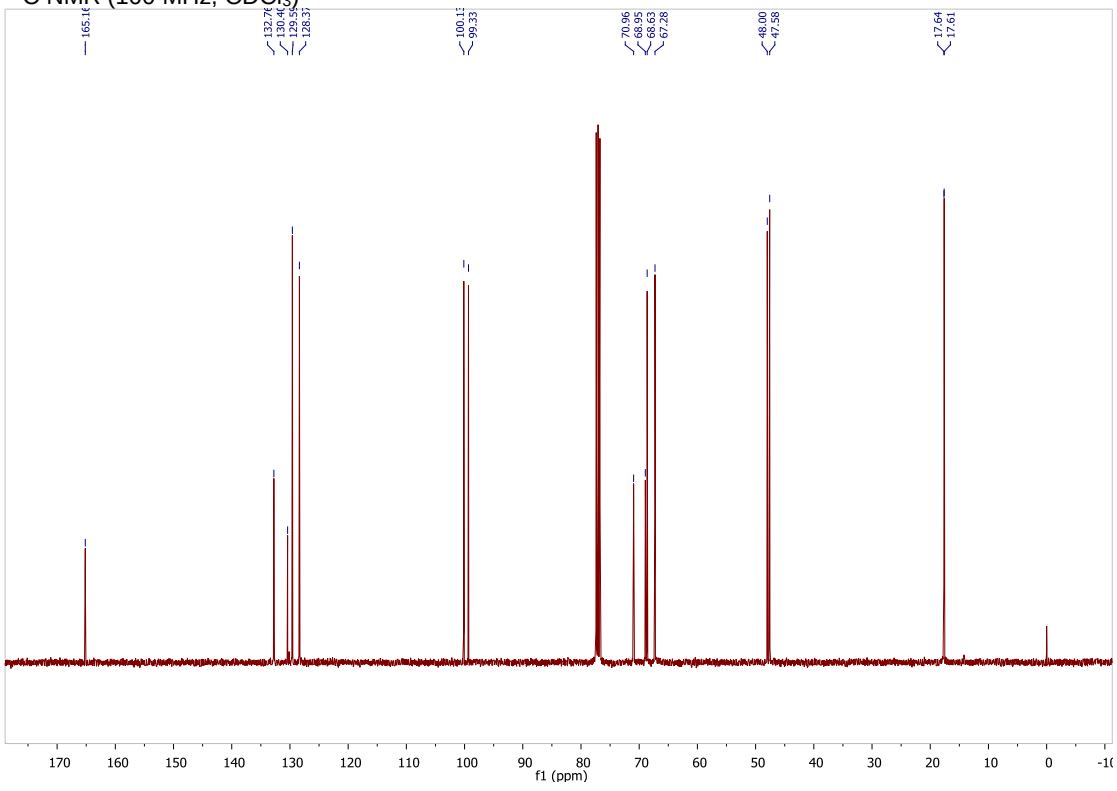
5-*O*-Benzoyl-1,6:3,4-bis-[*O*-(2,3-dimethoxybutane-2,3-diyl)]-*myo*-inositol (**7**)



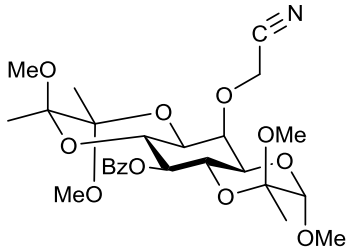
^{1}H NMR (400 MHz, $CDCl_3$)



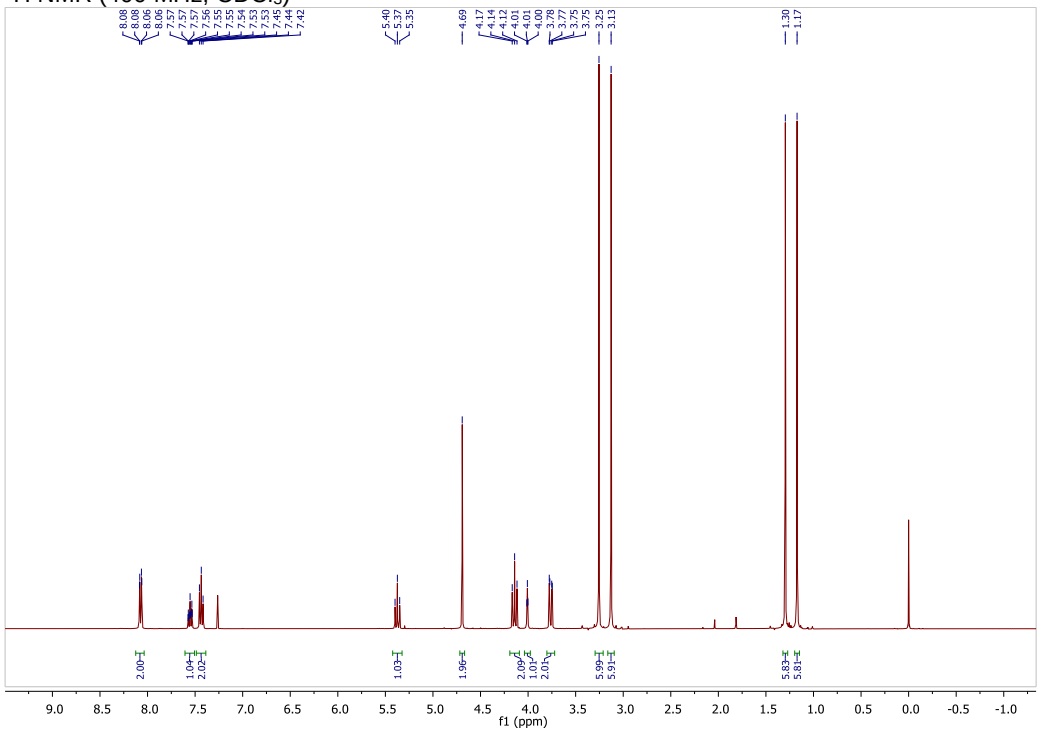
^{13}C NMR (100 MHz, $CDCl_3$)



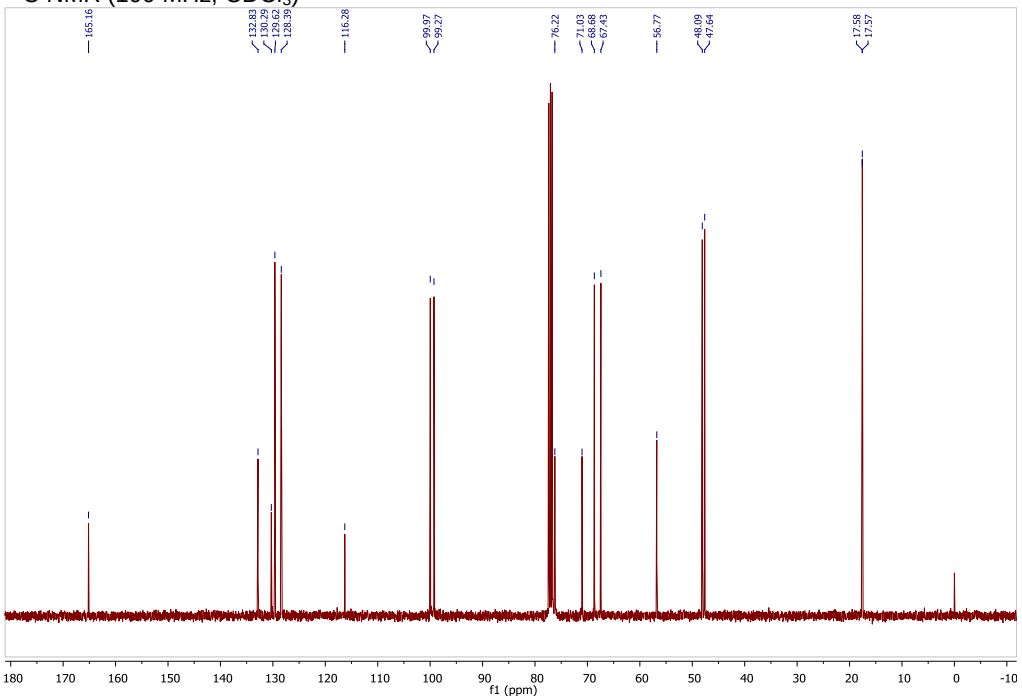
5-*O*-Benzoyl-2-*O*-cyanomethyl-1,6:3,4-bis-[*O*-(2,3-dimethoxybutane-2,3-diyl)]-*myo*-inositol (**8**)



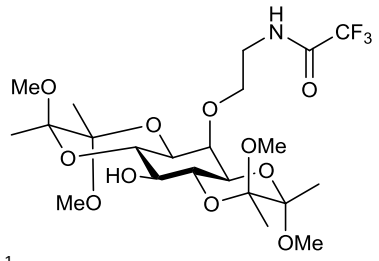
^{1}H NMR (400 MHz, $CDCl_3$)



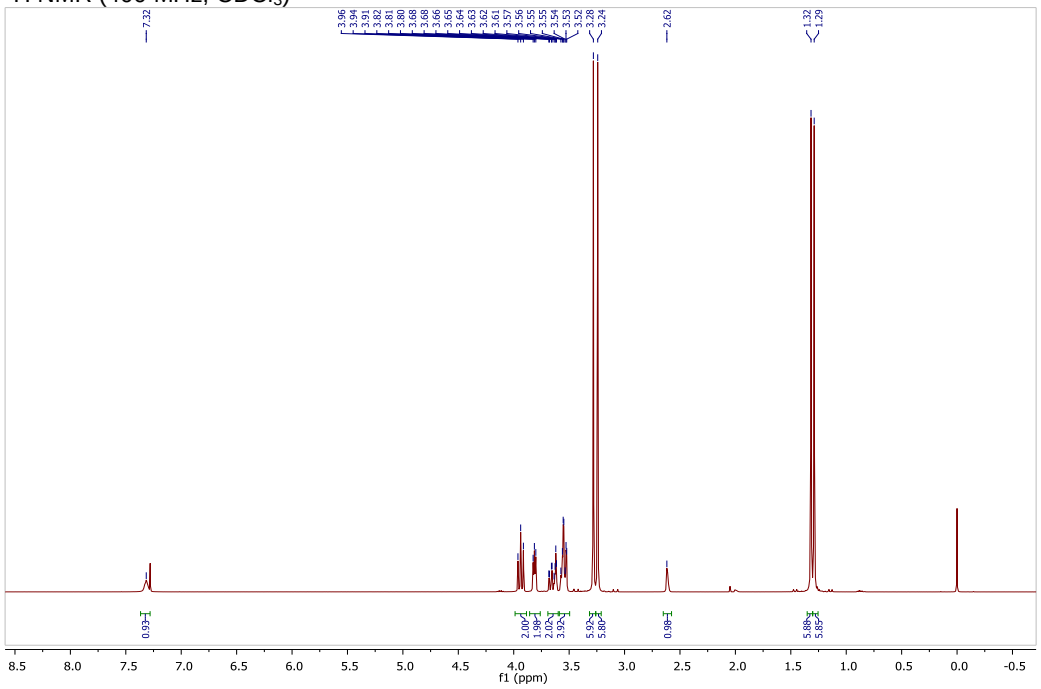
^{13}C NMR (100 MHz, $CDCl_3$)



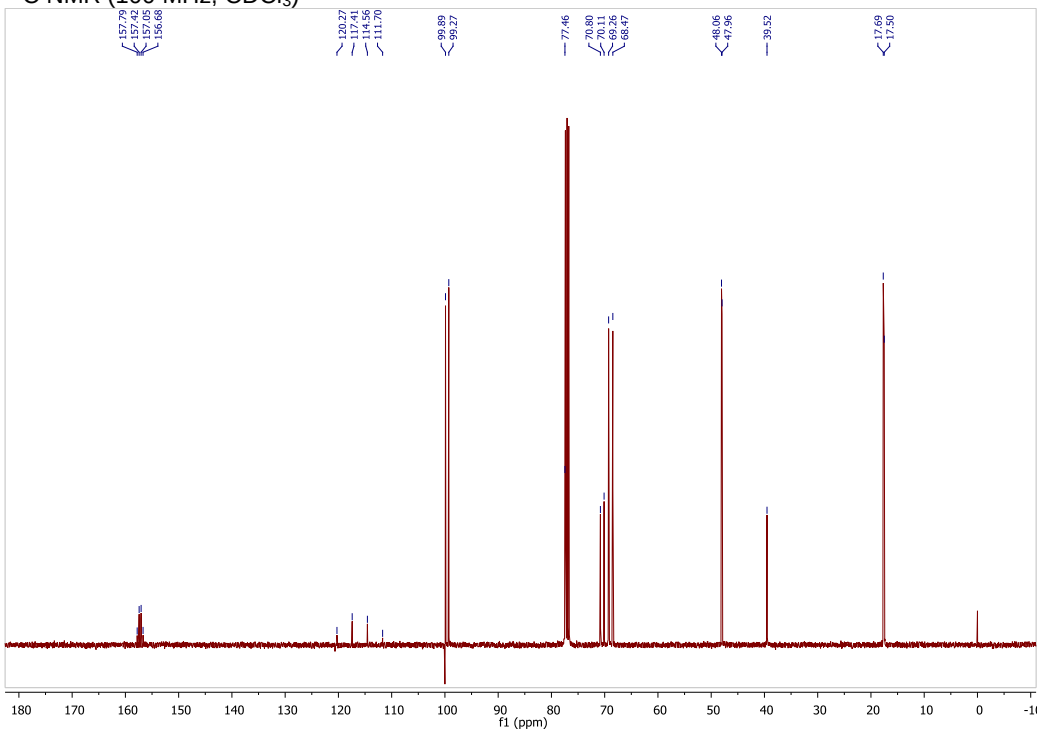
2-*O*-[2-(2,2,2-Trifluoroacetylamino)ethyl]-1,6:3,4-bis-[*O*-(2,3-dimethoxybutane-2,3-diyl)]-*myo*-inositol (**9**)



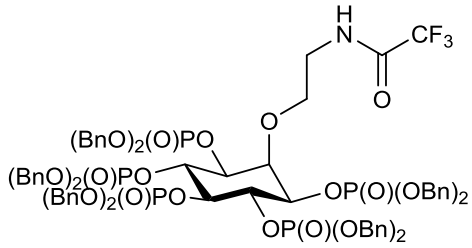
^{1}H NMR (400 MHz, $CDCl_3$)



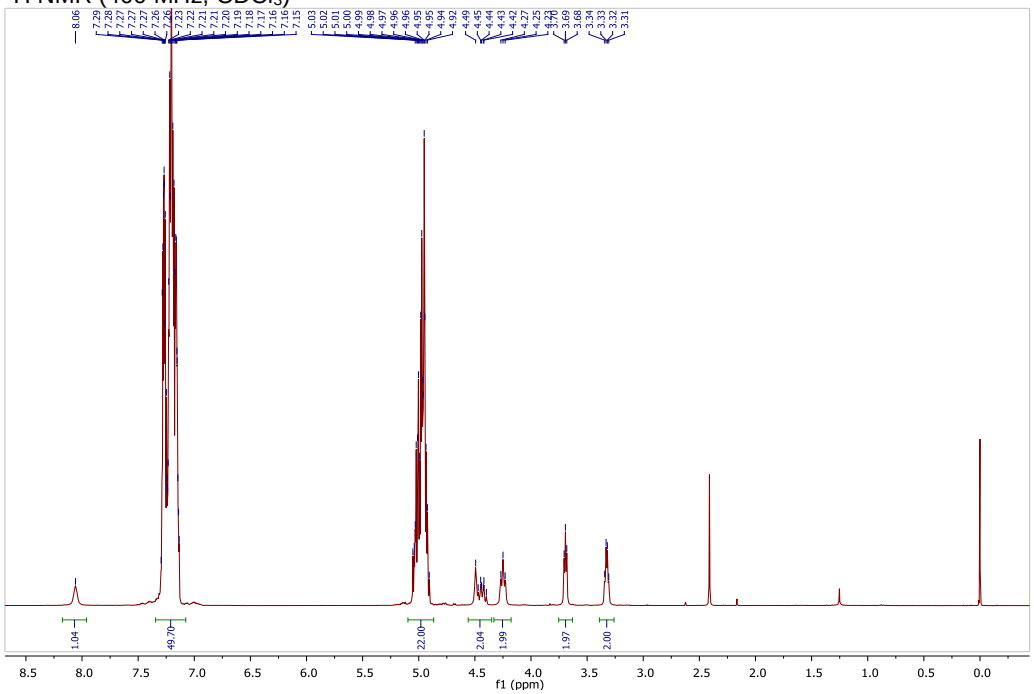
^{13}C NMR (100 MHz, $CDCl_3$)



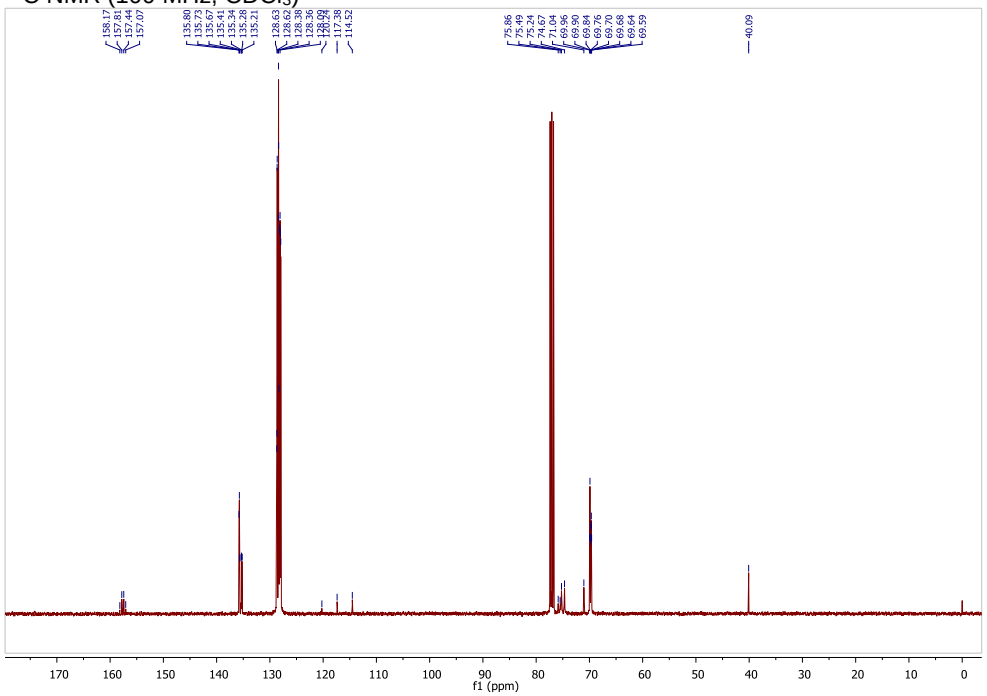
2-*O*-[2-(2,2,2-Trifluoroacetylamino)ethyl]-*myo*-inositol 1,3,4,5,6-pentakis (dibenzylphosphate) (**10**)



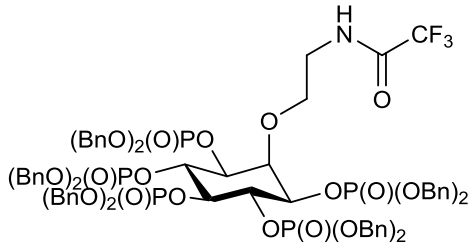
^{1}H NMR (400 MHz, $CDCl_3$)



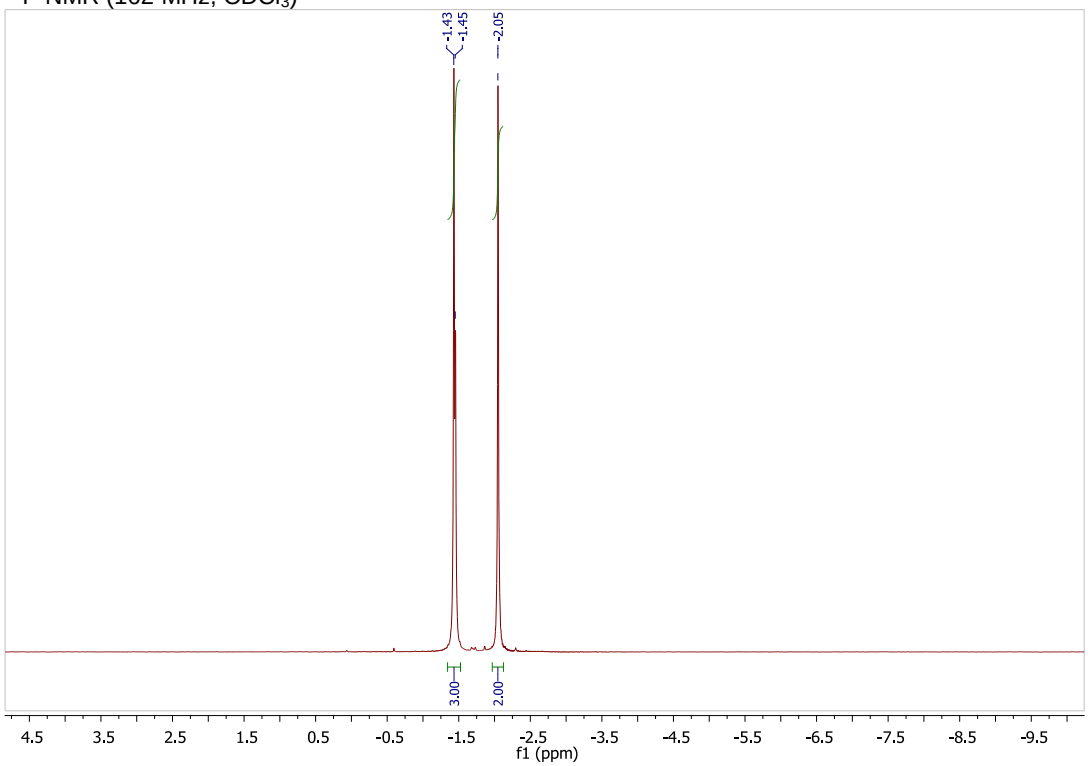
^{13}C NMR (100 MHz, $CDCl_3$)



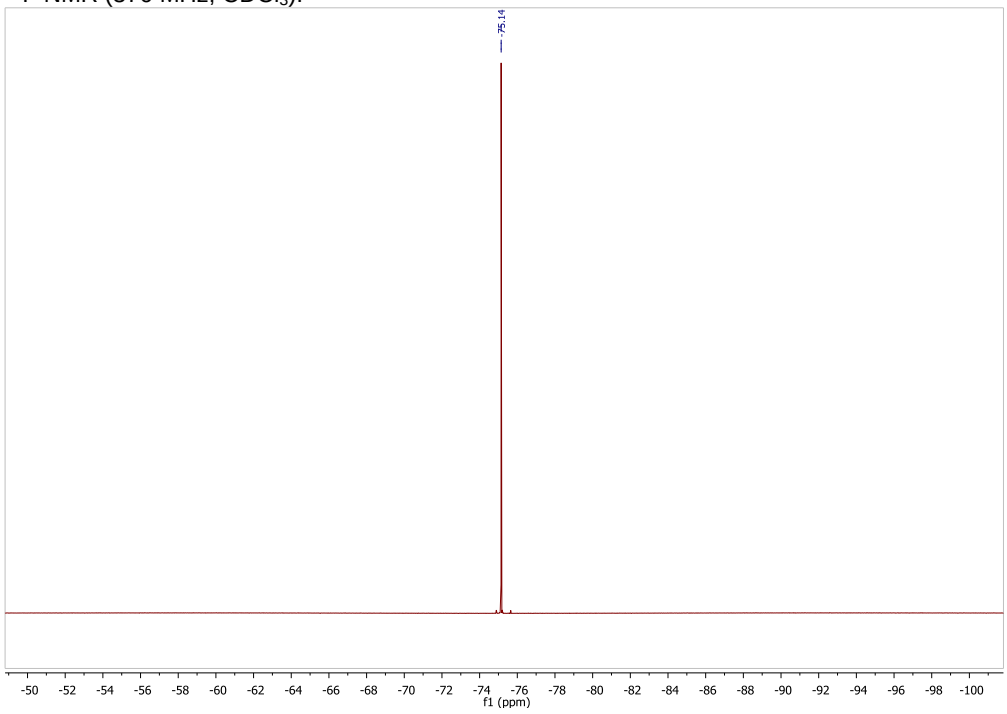
2-*O*-[2-(2,2,2-Trifluoroacetylamino)ethyl]-*myo*-inositol 1,3,4,5,6-pentakis (dibenzylphosphate) (**10**)



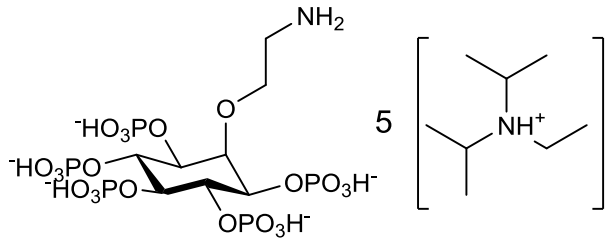
^{31}P NMR (162 MHz, $CDCl_3$)



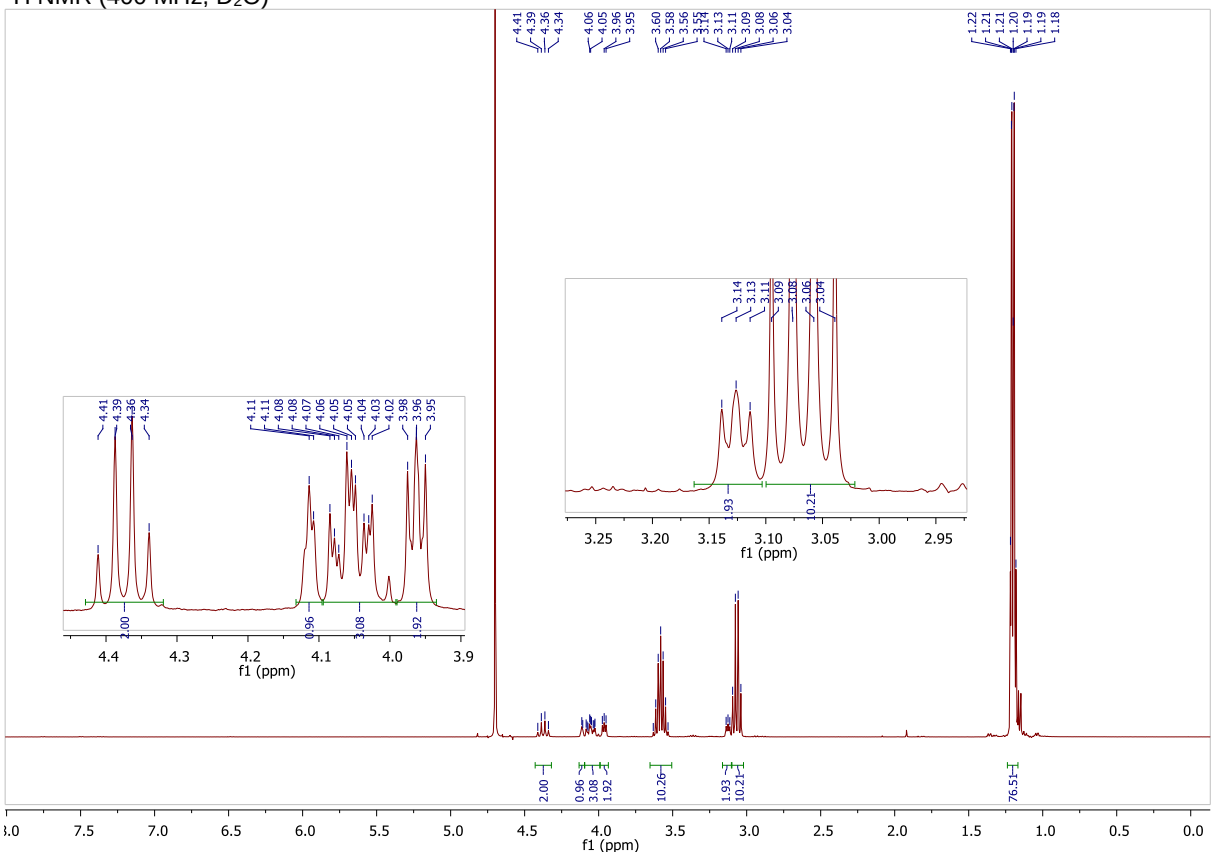
^{19}F NMR (376 MHz, $CDCl_3$):



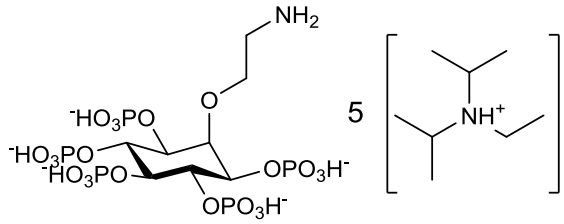
2-O-(2-Aminoethyl)-*myo*-inositol 1,3,4,5,6-pentakisphosphate (**4**)



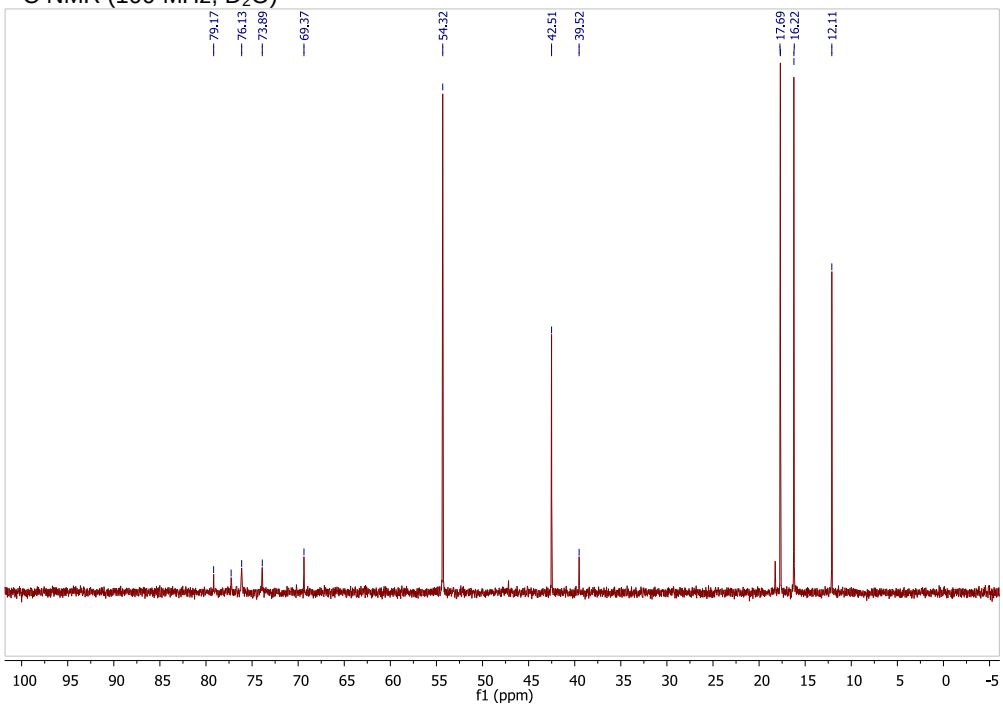
^{1}H NMR (400 MHz, D_2O)



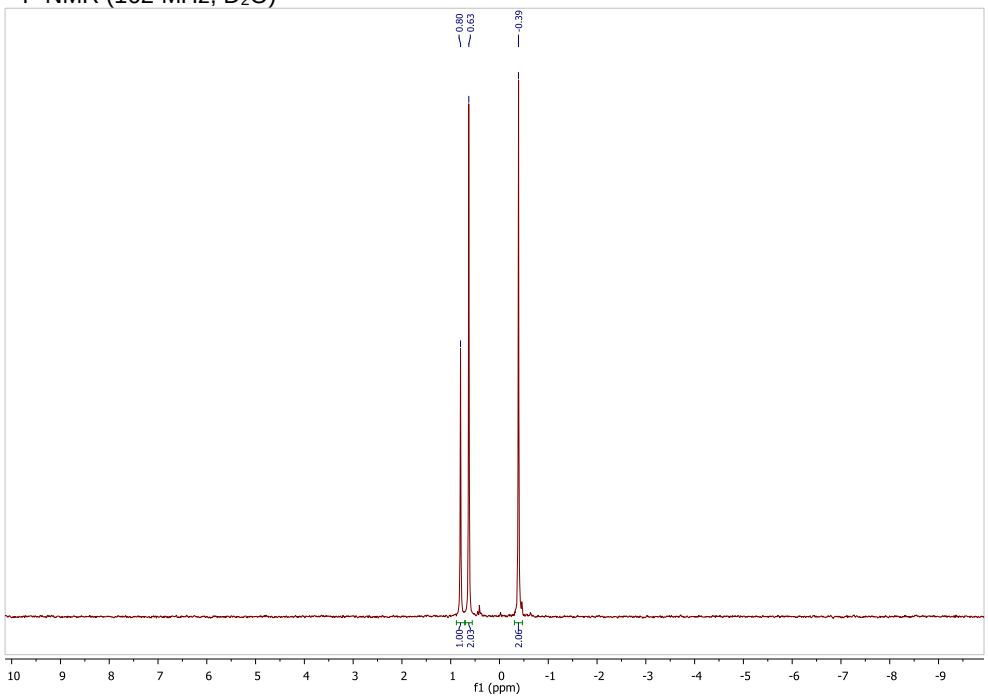
2-O-(2-Aminoethyl)-*myo*-inositol 1,3,4,5,6-pentakisphosphate (**4**)



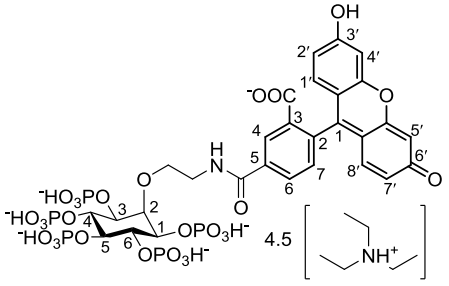
^{13}C NMR (100 MHz, D_2O)



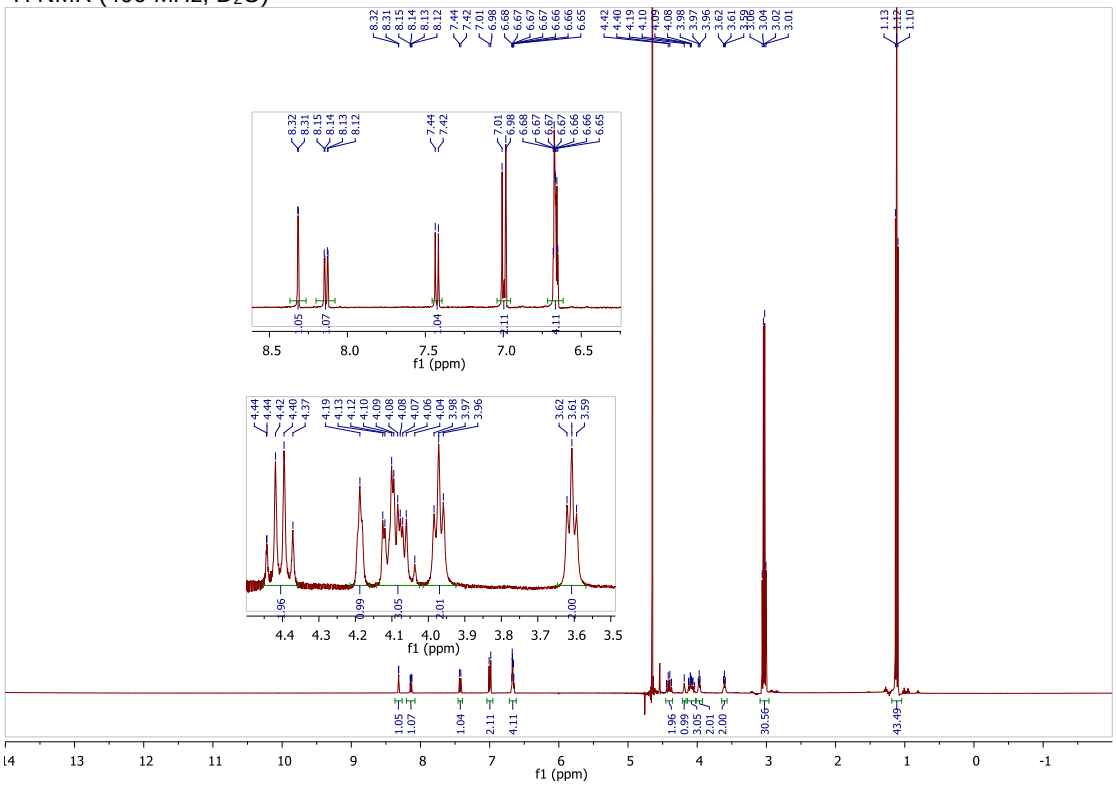
^{31}P NMR (162 MHz, D_2O)



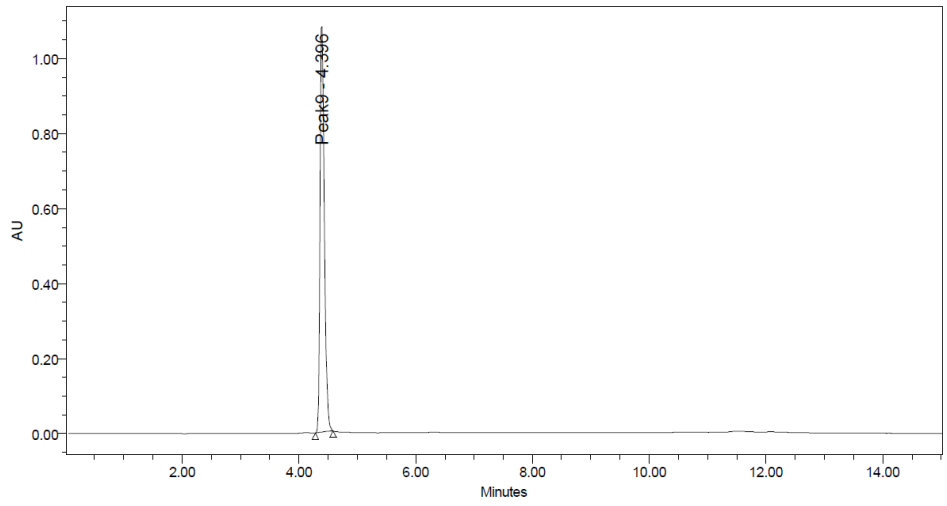
2-*O*-(2-(5-Fluoresceinylcarboxy)-aminoethyl)-*myo*-inositol 1,3,4,5,6-pentakisphosphate (**5**)



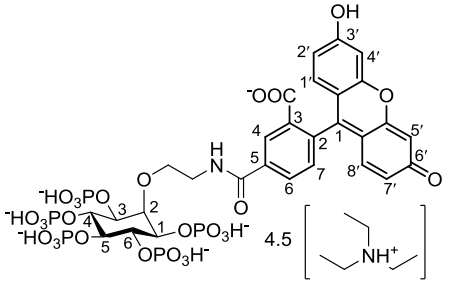
^{1}H NMR (400 MHz, D_2O)



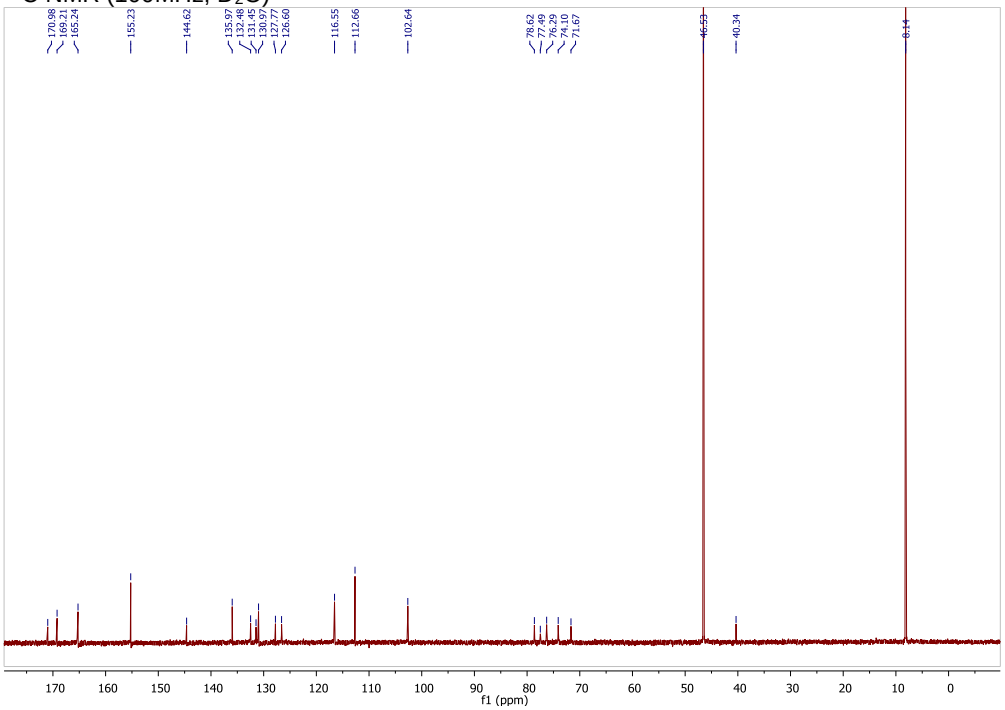
HPLC (5% to 70% CH_3CN in 0.1 m aqueous triethylammonium acetate, detection at 254 nm).



2-*O*-(2-(5-Fluoresceinylcarboxy)-aminoethyl)-*myo*-inositol 1,3,4,5,6-pentakisphosphate (**5**)



^{13}C NMR (100MHz, D_2O)



^{31}P NMR (109 MHz, CD_3OD)