# Asymmetric Phosphorylation through Catalytic P(III) Phosphoramidite Transfer: Enantioselective Total Synthesis of *myo*-Inositol-6-Phosphate

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**Contents**



**General Procedures.** Proton NMR spectra were collected on Bruker 400 or 500 MHz spectrometers at 25 °C. Proton chemical shifts are reported in ppm ($\delta$) relative to internal tetramethylsilane (TMS, $\delta$ 0.0 ppm) or with the solvent reference relative to TMS employed as the internal standard ($CDCl_3$, $\delta$ 7.26 ppm; $CD_3OD$, $\delta$ 3.31 ppm; $D_2O$, $\delta$ 4.79 ppm). Data are reported as follows: chemical shift (multiplicity [singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m)], coupling constants [Hz], integration). Broad peaks are denoted by (br) before the chemical shift multiplicity. Carbon NMR spectra were collected on Bruker 400 (101 MHz) or 500 (126 MHz) NMR spectrometers with complete proton decoupling. Carbon chemical shifts are reported in ppm ($\delta$) relative to TMS with respective solvent resonances as the internal standard ($CDCl_3$, $\delta$ 77.16). A drop of methanol was added as an internal standard ($\delta$ 49.5) for carbon NMR experiments performed in $D_2O$. Where indicated, carbon-phosphorous couplings are reported as: chemical shift, multiplicity, and coupling constant. Phosphorous NMR spectra were recorded on Bruker 400 (162 MHz) and 500 (202 MHz) NMR spectrometers with complete proton and carbon decoupling. Phosphorous chemical shifts are reported in ppm ($\delta$) relative to 85% $H_3PO_4$ as an external standard. Infrared spectra were obtained using a Thermo Electron Corporation Nicolet 6700 FT-IR instrument. Thin-layer chromatography (TLC) was performed using silica gel 60 Å F254 precoated plates (0.25 mm thickness) and TLC $R_f$ values are reported herein. Analytical TLC plates were developed utilizing cerium ammonium molybdate (CAM) or by UV absorbance. Preparatory TLC was performed using 20 x 20 cm Silica Gel 60 Å F254 precoated plates (0.25 mm thickness) and visualized by UV absorbance. Flash chromatography was performed using Silica Gel 60Å (32-63 μM). Specific rotations were determined with a Perkin Elmer Polarimeter 341 at 20 °C on the sodium D line (path length 10.0 cm). High resolution mass spectra were obtained from institutional providers and the method of ionization reported herein.

Phosphitylations were monitored by $^{31}P$ NMR using the following procedure: 100-200 μL of reaction solvent (including molecular sieves) were added to 600 μL of $CDCl_3$. Chemical shifts representing the phosphoramidite (147.8 ppm) and the corresponding inositol phosphite (monophosphite 142.4 ppm, bisphosphite 142.5 ppm) were integrated to determine reaction progress.

Analytical reverse phase high-pressure liquid chromatography (HPLC) was performed employing a single-wavelength UV detector equipped with a Waters Sunfire RP C-18 (4.6x150 mm) column on a gradient of 0-100% acetonitrile in water with 0.1% TFA over 36 min. Preparative reverse phase peptide purifications were performed on a BioTage SP4 instrument (C18HS 25+3, 0-100% MeOH in water with 0.1% TFA). Measurements of enantiomeric excess were carried out via analytical normal phase HPLC equipped with a diode array detector and employing *Chiracel*® columns. Specific methods are described below.

Reactions were performed under inert atmosphere ($N_2$ or argon) employing flame- or oven-dried glassware. All solvents were either distilled or taken from a solvent purification system. Chloroform (Aldrich, >98.8%, ACS grade) was dried according to a previously reported procedure as follows: Chloroform stabilized with 0.5-1% ethanol was washed 2 times with equal volumes of deionized water, dried with anhydrous $K_2CO_3$, refluxed over $P_2O_5$, collected, and stored for up to 5 days wrapped in aluminum foil.[1] Molecular sieves (10 Å, UOP type, powder) were used as provided by Fluka without further preparation. Dibenzyl *N*,*N*-diethylphosphoramidite (90% pure) was purchased from Alfa Aesar and used without further purification.

**General observations regarding the use of 10 Å molecular sieves.**

During the course of reaction optimization, we made several observations regarding the use of 10 Å molecular sieves for this chemistry. We observed a nearly linear rate dependence upon the quantities of molecular sieves used. While reaction rates were clearly affected by molecular sieve loading, they did not appear to influence selectivities up to 333 mg/mL. By controlling the reaction rate with molecular sieve loading we were able to lower catalyst loading from 20 mol % to 5 mol % for the desymmetrization step, and 5 mol % for the kinetic resolution.

When mixing 10 Å molecular sieves and chloroform we frequently observed the evolution of a significant amount of heat as well as a strongly acidic vapor. Early large-scale reactions (500 mg substrate) frequently gave erratic yields and selectivities. A number of reports in the literature have documented a highly exothermic dechlorination reaction that occurs for a variety of chlorocarbons when they are mixed with 10 Å molecular sieves.[2 3 4] In general, the dechlorination of chloroform is thought to occur upon initial mixing whereupon a significant amount of HCl is generated, but upon further mixing very little HCl evolves. Indeed, this was consistent with our observation; after 30 min HCl evolution appeared to cease and the solution was not acidic as indicated by pH paper.

Our early efforts to control the evolution of HCl involved cooling the chloroform to 0 °C before adding the molecular sieves. The reaction would then be warmed to room temperature prior to the addition of other reagents. Although it did not compromise selectivity, the initial cooling appeared to lower reaction rates. Again, this was consistent with previous reports in which lower temperature mixing resulted in less HCl generation. Based upon these results, we surmised that the rate dependence upon molecular sieve loading was largely due to the activation of the sieves by HCl gas, which was controlled by both the temperature during addition and the quantity of sieves used. In light of this, we found that reproducible results could be obtained by simply adding the molecular sieves to stirring chloroform at room temperature instead of adding the chloroform to the sieves, which allowed the bulk solvent to quench the heat generated. Additionally, by letting the heat and HCl dissipate for 30 min before adding the other components of the reaction we were able to achieve highly reproducible results on larger scales.

**2,4,6-Tri-*O*-allyl-*myo*-inositol.**
2,4,6-Tri-*O*-allyl-*myo*-inositol was prepared from *myo*-inositol using a previously reported procedure. Compound characterization was consistent with what was previously reported.[5]

**2,4,6-Tri-*O*-allyl-1,3,5-tri-*O*-PMB-*myo*-inositol.**
To a flame-dried 500 mL flask was added 350 mL dry DMF. Sodium hydride (3.3 g, 138 mmol) was added under a stream of nitrogen. 2,4,6-Triallyl inositol was then added in small portions over the course of 30 min. The slow addition was necessary to prevent the vigorous evolution of gas. After stirring for an additional 30 min, the reaction was cooled to 0 °C and 4-methoxybenzyl chloride (17.1 mL, 126 mmol) was added *via* syringe. The reaction was stirred at room temperature for 20 h, then checked by TLC (50% ethyl acetate in hexanes), which showed the starting material had been consumed. The reaction was quenched with 150 mL water (added slowly) and concentrated *in vacuo*. The resulting residue was dissolved in 500 mL ethyl acetate and washed with 300 mL water and 150 mL brine. The combined aqueous layers were then back-extracted with 100 mL ethyl acetate. The combined organics were dried with $Na_2SO_4$, filtered, and concentrated *in vacuo*. The resulting residue was purified by flash chromatography (20% ethyl acetate in hexanes) to yield a white fluffy solid (12.234 g, 81% yield). **$^1$H NMR** (500 MHz, $CDCl_3$) $\delta$ 7.32 – 7.24 (m, 6H), 6.90 – 6.84 (m, 6H), 6.04 – 5.94 (m, 2H), 5.94 – 5.86 (m, 1H), 5.31 – 5.20 (m, 3H), 5.18 – 5.10 (m, 3H), 4.74 (s, 2H), 4.58 (d, $J$ = 11.4, 2H), 4.55 (d, $J$ = 11.4, 2H), 4.36 (m, 2H), 4.29 (m, 2H), 4.25 (m, 2H), 3.85 (m, 1H), 3.81 (s, 6H), 3.80 (s, 3H), 3.78 (t, $J$ = 9.5, 2H), 3.29 (t, $J$ = 9.1, 1H), 3.20 (m, 2H). **$^{13}$C NMR** (126 MHz, $CDCl_3$) $\delta$ 159.3, 159.3, 136.0, 135.7, 131.3, 130.7, 129.9, 129.3, 116.7, 116.5, 113.9, 83.5, 81.5, 80.4, 77.4, 77.2, 76.9, 75.8, 74.6, 74.1, 73.3, 72.5, 55.4; **IR** (film, cm$^{-1}$) 2926, 2866, 2837, 1644, 1614, 1586, 1516, 1456, 1440, 1410, 1360, 1302, 1251, 1173, 1141, 1078, 1059, 1035; **TLC** *Rf* = 0.25 (20 % ethyl acetate in hexanes); **Exact mass** calc'd for $[C_{39}H_{48}O_9]Na^+$ requires *m/z* 683.3191, found 683.3169 (ESI+).

**1,3,5-Tri-*O*-PMB-*myo*-inositol (4).**
To a stirred suspension of 2,4,6-tri-*O*-allyl-1,3,5-tri-*O*-PMB-*myo*-inositol (5 g, 7.60 mmol) in a 9:1 mixture of EtOH:water (211.25 mL) was added $RhCl(PPh_3)_3$ (0.350 g, 0.38 mmol) followed by 1,4-diazabicyclo[2.2.2]octane (DABCO) (0.307 g, 2.74 mmol). The reaction was refluxed for 19 h, after which time the reaction was cooled to room temperature, filtered, and concentrated *in vacuo*. The residue was dissolved in MeOH (120 mL) and 2 M aqueous HCl (3.25 mL) was added. The reaction was stirred at room temperature for 1.5 h after which time it was deemed complete by TLC. The reaction was basified with concentrated aqueous $NH_4OH$ to pH 8 and concentrated. The residue was dissolved in ethyl acetate (EtOAc, 100 mL) and dried with $Na_2SO_4$, filtered, and concentrated *in vacuo*. The resulting residue was then purified by column chromatography (70% EtOAc in hexanes) to afford pure **4** as a white solid (3.092 g, 75% yield). **$^1$H NMR** (400 MHz, $CDCl_3$) $\delta$ 7.34 – 7.24 (m, 6H), 6.93 – 6.84 (m, 6H), 4.79 (s, 2H), 4.66 (d, $J$ = 11.4, 2H), 4.61 (d, 4.19 $J$ = 11.5, 2H), (t, $J$ = 2.6, 1H), 3.99 (td, $J$ = 9.3, 1.3, 2H), 3.81 (s, 6H), 3.79 (s, 3H), 3.24 – 3.16 (m, 3H), 2.49 (d, $J$ = 1.3, 2H), 2.38 (s, 1H); **$^{13}$C NMR** (101 MHz, $CDCl_3$) $\delta$ 159.7, 159.5, 131.0, 130.0, 129.9, 129.8, 114.2, 114.1, 82.2, 79.1, 74.5, 72.2, 72.0, 66.5, 55.5, 55.5; **IR** (film, $cm^{-1}$) 2494, 3416, 3259, 2945, 2906, 2874, 2835, 1610, 1512, 1243; **TLC** $Rf$ = 0.28 (60% EtOAc in hexanes); **Exact mass** calcd for $[C_{30}H_{36}O_9]Na^+$ requires $m/z$ 563.2252, found 563.2242 ($ESI^+$).

**D-*myo*-Inositol-6-phosphate (11).**
A flame-dried two-neck flask fitted with a dry ice/acetone cold finger was cooled to –78 °C in a dry ice/acetone bath. The apparatus was flushed with argon and in it 12 mL anhydrous ammonia was condensed. In a separate flask, 6-dibenzylphosphate-1,3,5-tri-*O*-PMB-myo-inositol **5** (187 mg, 0.26 mmol) was dissolved in a minimal amount of tetrahydrofuran (1 – 2 mL). A small piece of sodium metal (~ 6 mg, 0.25 mmol) was added to the ammonia, which was stirred until it dissolved. The solution of **5** was then added dropwise until the deep blue color of the solution dissipated, after which another piece of sodium metal (~6 mg) was added. Upon complete addition of **5**, the solution was allowed to stir for 5 min before being quenched with pieces of ice until the blue color disappeared. The flask was removed from the ammonia solution and the cold finger. Over a heavy stream of nitrogen, the flask was slowly brought to room temperature and all of the ammonia removed. The resulting white solid was then dissolved in a minimal amount of water and passed through a column of DOWEX 50WX8-400 ion exchange resin (10 mL of resin) eluting with water. The acidic fractions were combined and added to a separatory funnel with cyclohexylamine (172 μL, 1.5 mmol). The solution was vigorously swirled and the excess cyclohexyl-amine was extracted with three 30 mL volumes of diethyl ether. Acetonitrile was added to the aqueous layer to make a solution of ~20% aqueous acetonitrile. The solution was frozen and lyophilized overnight giving a white fluffy solid (102 mg, 95% yield). **$^1$H NMR** (400 MHz, $D_2O$) $\delta$ 4.00 (dt, $J_{P\text{-}H}$ = 7.8, $J_{H\text{-}H}$ = 9.1, 1H), 3.95 (t, $J$ = 2.7, 1H), 3.60 (t, $J$ = 9.7, 1H), 3.51 (dd, $J$ = 9.6, 2.7, 1H), 3.44 (dd, $J$ = 10.0, 2.8, 1H), 3.30 (t, $J$ = 9.2, 1H), 3.05 (m, 2H), 1.87 (m, 4H), 1.70 (m, 4H), 1.54 (m, $J$ = 12.7, 2H), 1.23 (m, 8H), 1.07 (m, 2H); **$^{13}$C NMR** (126 MHz, $D_2O$) $\delta$ 76.7 (d, $J_{C\text{-}P}$ = 5.5), 74.8 (d, $J_{C\text{-}P}$ = 3.2), 73.0, 72.3, 72.0 (d, $J_{C\text{-}P}$ = 2.8), 71.7, 50.9, 49.5, 31.0, 24.9, 24.4; **$^{31}$P NMR** (162 MHz, $D_2O$) $\delta$ 4.59; **IR** (powder, $cm^{-1}$) 2937, 2881, 2859, 2833, 2786, 2677, 2622, 2575, 2518, 2206, 2063, 1616, 1587, 1503, 1457, 1447, 1408, 1390, 1378, 1355, 1339, 1327, 1048. **Exact mass** calc'd for $[C_{18}H_{39}N_2O_9P]H^+$ requires $m/z$ 459.247, found 459.247; **$[\alpha]_D$** = –0.3 ($c$ = 2.0, $H_2O$, pH 8 - 9).

**6-Dibenzylphosphate-*myo*-inositol-1,2,3,4,5-pentaacetate (14).**
The tetrabutyl ammonium salt **12** was formed by passing **11** though DOWEX 50WX8-400 ion exchange resin and treating the acidic fractions with 2 equiv of aqueous 1.0 M tetrabutyl ammonium hydroxide, and lyophilizing the solution to give **12** as a white solid. $CDCl_3$ (0.5 mL) and 0.5 mL pyridine was added to a flask containing **11** (12.4 mg, 0.017 mmol). To this stirring solution was added acetic anhydride (0.157 mL, 1.7 mmol). The solution was stirred under nitrogen and reaction progress was checked by $^{31}$P and $^1$H NMR. After 2 days, six inositol proton resonances were found from 5.22 to 4.6 ppm, indicative of the formation of a single product. Similarly, in the phosphorous field a single resonance was observed at -10 ppm. The reaction was poured into 10 mL of water and extracted two times with 100 mL diethyl ether. The aqueous layer was concentrated *in vacuo* and the remaining water as an azeotrope with two 25 mL volumes of toluene. The resulting residue was dissolved in 1 mL $CDCl_3$, dried with anhydrous potassium carbonate, transferred to a 5 mL flask, and concentrated *in vacuo*. To **13** was added 0.5 mL ACN-$d_3$, benzyl bromide (0.44 mL, 3.7 mmol), tetrabutyl ammonium iodide (12.3 mg, 0.033 mmol) and 100 mg

anhydrous $K_2CO_3$. The reaction was stirred overnight and checked by $^{31}P$ and $^{1}H$ NMR. Six distinct inositol proton resonances were observed between 6.3 and 5.6 ppm, and a single phosphorous resonance was observed at -1.14 ppm. The reaction mixture was poured into a separatory funnel containing 25 mL each of diethyl ether and water. The ether layer was dried with anhydrous sodium sulfate and concentrated. The resulting residue was dissolved in 50 mL acetonitrile and washed eight times with 50 mL of hexanes to remove the benzyl bromide and tetrabutyl ammonium salts. The acetonitrile layer was then concentrated and **14** was purified by preparatory TLC (40% ethyl acetate in hexanes), to give a clear oil (4.5 mg, 41% yield, 95.8% e.e.). **$^{1}$H NMR** (400 MHz, $CDCl_3$) $\delta$ 7.41 – 7.28 (m, 10H), 5.59 (t, $J$ = 2.8, 1H), 5.46 (t, $J$ = 10.2, 1H), 5.27 (t, $J$ = 9.7, 1H), 5.17 (dd, $J$ = 10.1, 2.8, 1H), 5.09 (dd, $J$ = 10.5, 2.9, 1H), 5.01 – 4.87 (m, 5H), 2.19 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.89 (s, 3H), 1.83 (s, 3H); **$^{13}$C NMR** (101 MHz, $CDCl_3$) $\delta$ 170.0, 169.8, 169.7, 169.6, 169.5, 135.4, 135.3, 135.2, 128.8, 128.8, 128.7, 128.7, 128.1, 127.9, 75.5 (d, $J_{C-P}$ = 5.5), 71.2 (d, $J_{C-P}$ = 3.3), 69.8 (d, $J_{C-P}$ = 5.9), 69.6 (d, $J_{C-P}$ = 5.7), 69.4, 68.9 (d, $J_{C-P}$ = 3.6), 68.6, 68.3, 20.9, 20.7, 20.6, 20.5; **$^{31}$P NMR** (162 MHz, $CDCl_3$) $\delta$ -2.07; **IR** (film, $cm^{-1}$) 2937, 2857, 1750, 1457, 1431, 1367, 1270, 1211, 1110, 1082, 1035, 1013, 959, 933, 906; TLC *Rf* 0.22 (50% ethyl acetate in hexanes). **Exact mass** calc'd for $[C_{30}H_{35}O_{14}P]H^+$ requires *m/z* 651.184, found 651.183; **HPLC** $t_R$ 40.0 min employing a *Chiracel*® AD column eluting with 35% ethanol in hexanes at a flow rate of 0.5 mL/min.; $\mathbf{[\alpha]_D}$ = +15.7 (*c* = 0.5, $CHCl_3$). **Note:** *The above procedure was performed on a sample of **11** derived from 6-dibenzylphosphate-1,3,5-tri-O-benzyl-myo-inositol (94.7% ee). This same procedure was performed with a sample of **11** derived from **5** (>98 % ee) which yielded **14** in 98.1% ee. In each case, the erosion of enantiomeric excess was negligible.*

**(+/-)-4/6-Dibenzylphosphate-1,3,5-tri-*O*-PMB-myo-inositol (5).**
To a flame dried flask was added 20 mL chloroform, followed by 3.22 g of 10 Å molecular sieves. The solution was allowed to stir for 30 min before adding 5-(*p*-nitrophenyl)-1H-tetrazole (22 mg, 0.114 mmol), 1,3,5-tri-*O*-PMB-*myo*-inositol (310 mg, 0.57 mmol) and dibenzyl *N*,*N*-diethylphosphoramidite (259 µL, 0.71 mmol). The solution was allowed to stir 21 h before being filtered through a celite pad, cooled to 0 °C, and oxidized with 30% aqueous $H_2O_2$ (682 µL, 6.68 mmol) for 1 h. The reaction was then poured over ice in a separatory funnel and the remaining hydrogen peroxide was quenched with saturated $Na_2SO_3$. The aqueous layer was extracted three times with 50 mL dichloromethane. The organic layers were combined, dried with anhydrous $Na_2SO_4$, and concentrated. The resulting residue was then purified by flash chromatography (2% – 4% ethanol in diethyl ether) to yield a colorless oil (136.6 mg, 30% yield). See synthesis of optically pure **5** for full characterization.

**(+/-)-4/6-Dibenzylphosphorothioate-1,3,5-trisPMB-myo-inositol (9).**
To a flame dried flask was added 8 mL chloroform, followed by 1.33 g of 10 Å molecular sieves. The solution was allowed to stir for 30 min before adding 5-(*p*-nitrophenyl)-1H-tetrazole (8.4 mg, 0.044 mmol), 1,3,5-*O*-triPMB-*myo*-inositol (310 mg, 0.22 mmol) and dibenzyl *N*,*N*-diethylphosphoramidite (100 µL, 0.289 mmol). The solution was allowed to stir 21 h then oxidized with the addition of Beaucage's reagent (89 mg, 0.444 mmol) and stirred for 1 h. The reaction was then poured into a separatory funnel with saturated aqueous $NaHCO_3$. The aqueous layer was extracted three times with 50 mL dichloromethane. The organic layer was combined, dried with anhydrous $Na_2SO_3$, and concentrated. The resulting residue was then purified by flash chromatography (20 – 60% ethyl acetate in hexanes) followed by a second flash column to remove the product functionalized in the 2-position (0.5% EtOH, 55% $Et_2O$ in hexanes) to yield a colorless oil (41 mg, 22.6 % yield). **$^{1}$H NMR** (400 MHz, $CDCl_3$) $\delta$ 7.29 – 7.03 (m, 16H), 6.82 – 6.75 (m, 2H), 6.75 – 6.67 (m, 4H), 4.99 – 4.75 (m, 5H), 4.70 (d, $J$ = 10.7, 1H), 4.60 (d, $J$ = 10.7, 1H), 4.53 (d, $J$ = 11.5, 1H), 4.48 (d, $J$ = 4.8, 1H), 4.45 (d, $J$ = 4.8, 1H), 4.41 (d, $J$ = 11.7, 1H), 4.05 – 3.94 (m, 2H), 3.71 (s, 3H), 3.69 (s, 3H), 3.66 (s, 3H), 3.31 – 3.23 (m, 2H), 3.07 (dd, $J$ = 9.6, 2.5, 1H), 2.40 (d, $J$ = 2.1, 1H), 2.37 (s, 1H); **$^{13}$C NMR** (101 MHz, $CDCl_3$) $\delta$ 159.5, 159.4, 159.1, 136.3, 136.2, 136.1, 130.7, 129.8, 129.6, 129.5, 129.4, 128.4, 128.3, 128.1, 128.1, 127.8, 127.8, 114.0, 113.9, 113.8, 81.0 (d, $J_{C-P}$ = 3.65) 79.5 (d, $J_{C-P}$ = 7.0), 78.2, 77.8 (d, $J_{C-P}$ = 2.6), 73.8, 71.9, 71.9, 71.8, 69.7 (d, $J_{C-P}$ = 4.2), 69.7 (d, $J_{C-P}$ = 4.4), 66.5, 55.3, 55.3, 55.3; **$^{31}$P NMR** (162 MHz, $CDCl_3$) $\delta$ 69.13; **IR** (film, $cm^{-1}$) 3446, 3063, 3032, 3000, 2934, 2885, 2835, 1611, 1585, 1511, 1455, 1369, 1301, 1243, 1174, 1064, 991; **Exact mass** calc'd for $[C_{44}H_{49}O_{11}PS]H^+$ requires *m/z* 817.281, found 817.280; **HPLC** $t_R$ 24.6 min (early enantiomer) and $t_R$ 33.2 min (late enantiomer) employing a *Chiracel*® AD column eluting with 37% ethanol in hexanes at a flow rate of 0.5 mL/min.

**Synthesis of Tetrazolyl Alanine.**
Tetrazolyl alanine was prepared according to a previous report. Compound characterization was consistent with what was previously reported. [6] While it was reported that the product, tetrazolyl alanine, could be used without purification, we found it necessary to purify by flash chromatography (77.54 : 23.23 : 2.23 DCM/MeOH/ $H_2O$).

## Experiments to determine $k_{rel}$ of peptide 3

**Kinetic Resolution of Racemic Monophosphate.**
To a flame-dried 25 mL round bottom flask was added 7.9 mL of freshly distilled chloroform followed by 10 Å molecular sieves (1.8 g). In a separate vial, 200 mg of racemic **5** (0.25 mmol) was dissolved in 1.5 mL chloroform. After 30 min of stirring the chloroform/molecular sieves mixture under a heavy stream of nitrogen, racemic **5** was added and the vial containing **5** was washed with another 1.5 mL of chloroform, which was added to the reaction. Peptide **3** was added (11.6 mg, 0.013 mmol) and the reaction was cooled to 0 °C. To the reaction was added dibenzyl *N*,*N*-diethylphosphoramidite (43.5 μL, 0.125 mmol). The reaction was then moved to a 4 °C cold room. The reaction was monitored for the disappearance of the phosphoramidite (usually 12-24 h) by $^{31}$P NMR (see General Procedures). Upon complete consumption of phosphoramidite, the reaction was vacuum filtered through a celite pad and the celite was washed with DCM. The filtrate was then cooled to 0 °C and 500 μL of 30% aqueous hydrogen peroxide was added. After an hour, the reaction was poured into a separatory funnel 1/3 full with ice. Excess peroxide was quenched with saturated aqueous $Na_2SO_3$. The organic layer was collected and the aqueous layer was extracted twice with DCM. The combined organics were dried with sodium sulfate and concentrated. The crude oil was used for determination of the precise conversion by $^{31}$P and $^{1}$H NMR. A portion of the remaining crude material was passed through a small silica flash column to remove peptide and phosphoramidite byproducts (40% ethyl acetate in hexanes to remove byproducts, 80% ethyl acetate in hexanes to elute mono and bis). The resulting purified mixture of monophosphate and bisphosphate was concentrated and the enantiomeric excess was determined by chiral HPLC (see characterization of **5** above).

**Kinetic Resolution of Racemic Monosphosphorothioate.**
To a flame dried 5 mL flask was added freshly distilled chloroform followed by 664 mg 10 Å molecular sieves, and then stirred slowly. The solution was stirred for 30 additional min under a heavy stream of nitrogen. The suspension was transferred, *via* syringe, to a flask containing racemic phosphorothioate **9** (73 mg, 0.089 mmol). Peptide **3** was added (4 mg, 0.0045 mmol) and the solution was cooled to 0 °C in an ice bath. Dibenzyl *N*,*N*-diethylphosphoramidite (14.3 μL, 0.045 mmol) was added and the reaction moved to a 4 °C cold room. The reaction was monitored by $^{1}$ and $^{31}$P NMR for the consumption of phosphoramidite (see General Procedures). Upon completion, Beaucage's reagent (35.6 mg, 0.178) was added to the solution, which was stirred for 30 min and then brought to room temperature. The reaction was filtered through a pad of celite. The filtrate was diluted with DCM (50 mL) and washed with saturated aqueous sodium bicarbonate. The organic phase was removed and the aqueous layer was washed two times with 50 mL DCM. The combined organics were dried with anhydrous sodium sulfate and concentrated. $^{1}$H and $^{31}$P NMR were used to determine conversion to the bis product. The monophosphorothioate was purified by flash chromatography (20 – 45% ethyl acetate in hexanes) and the enantiomeric excess was determined by chiral HPLC employing a *Chiracel*® AD column eluting with 37% ethanol/hexanes at a flow rate of 0.5 mL/min. HPLC $t_R$ 24.6 min and $t_R$ 33.2 min.

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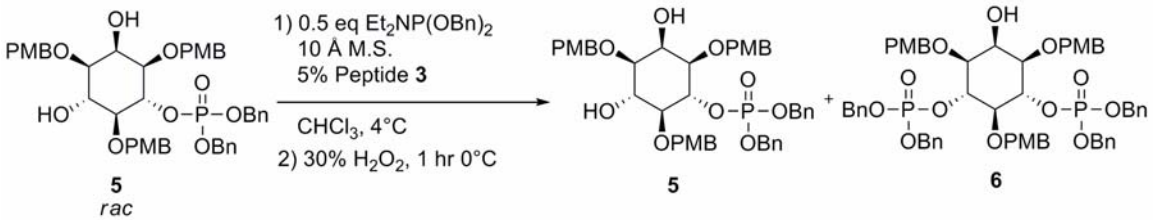
[5] Mills SJ, Backers K, Erneux C, Potter BVL (2003) Synthesis of D- and L-*myo*-inositol 1,2,4,6-tetrakisphosphate, regioisomers of *myo*-inositol 1,3,4,5 tetrakisphosphate: activity against Ins(1,4,5)$P_3$ binding proteins, *Org. Biomol. Chem.* 1: 3546–3556.
[6] Vlasuk GP, Webb TR, Abelman MM, Pearson DA, Miller TA U.S. Patent 5492895, September 21, (1999)**.**

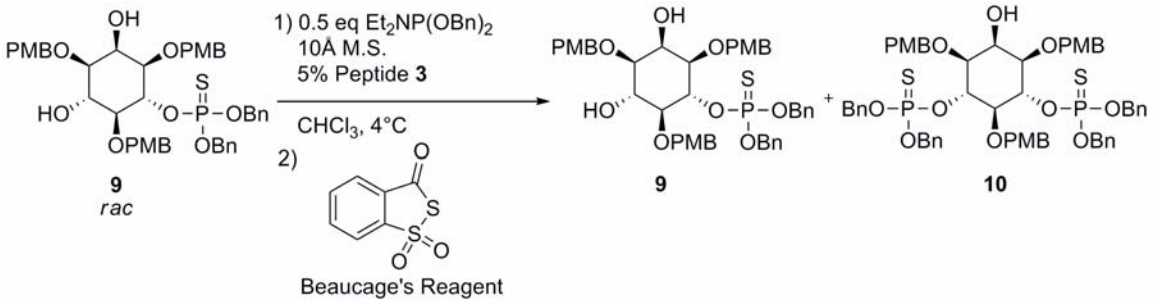
Supplemental Table 1: Kinetic Resolution of Racemic Monophosphate **5**




| | Conversion to **6** | | | | $k_{rel}$ based upon conversion to **6** | | |
|---|---|---|---|---|---|---|---|
| Trial | H-Shifts[α] | P Shifts[β] | Average | % *ee* | H Shifts[γ] | P Shifts[δ] | Average |
| 1 | 0.44 | 0.46 | 0.45 | 71.4 | 37.6 | 26.5 | 31.1 |
| 2 | 0.43 | 0.42 | 0.42 | 65.1 | 28.3 | 46.4 | 35.1 |
| 3 | 0.54 | 0.53 | 0.54 | 93.4 | 27.9 | 38.8 | 32.5 |
| 4 | 0.51 | 0.48 | 0.50 | 85.6 | 28.5 | 70.5 | 40.7 |

Conversions were calculated using $^{31}$P and $^{1}$H NMR on a Bruker 500 MHz NMR spectrometer. [α]Conversions based upon proton resonances were calculated by averaging the normalized chemical shift ratios for three distinct proton chemical shifts for both starting monophosphate **5** and bisphosphate **6**. [β]Phosphorous conversions were calculated using the normalized chemical shift ratio for the monophosphate (-1.49 ppm) and bisphosphate (-1.63 ppm). Values for $k_{rel}$ were calculated utilizing the method described by Kagan et al. ($k_{rel}$ = ln[(1 - c)(1 - ee)]/ln[(1 - c)(1 + ee)]).[i] Conversions based upon proton[γ] and phosphorous[δ] signals were used to calculate values for $k_{rel}$. An average $k_{rel}$ for each trial was calculated for each run giving 34.9 ± 3.7 as an average of 4 trials.

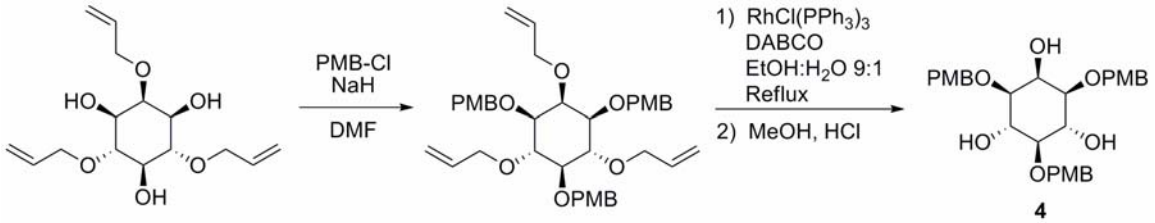
Supplemental Table 2: Kinetic Resolution of Phosphorothioate **9**



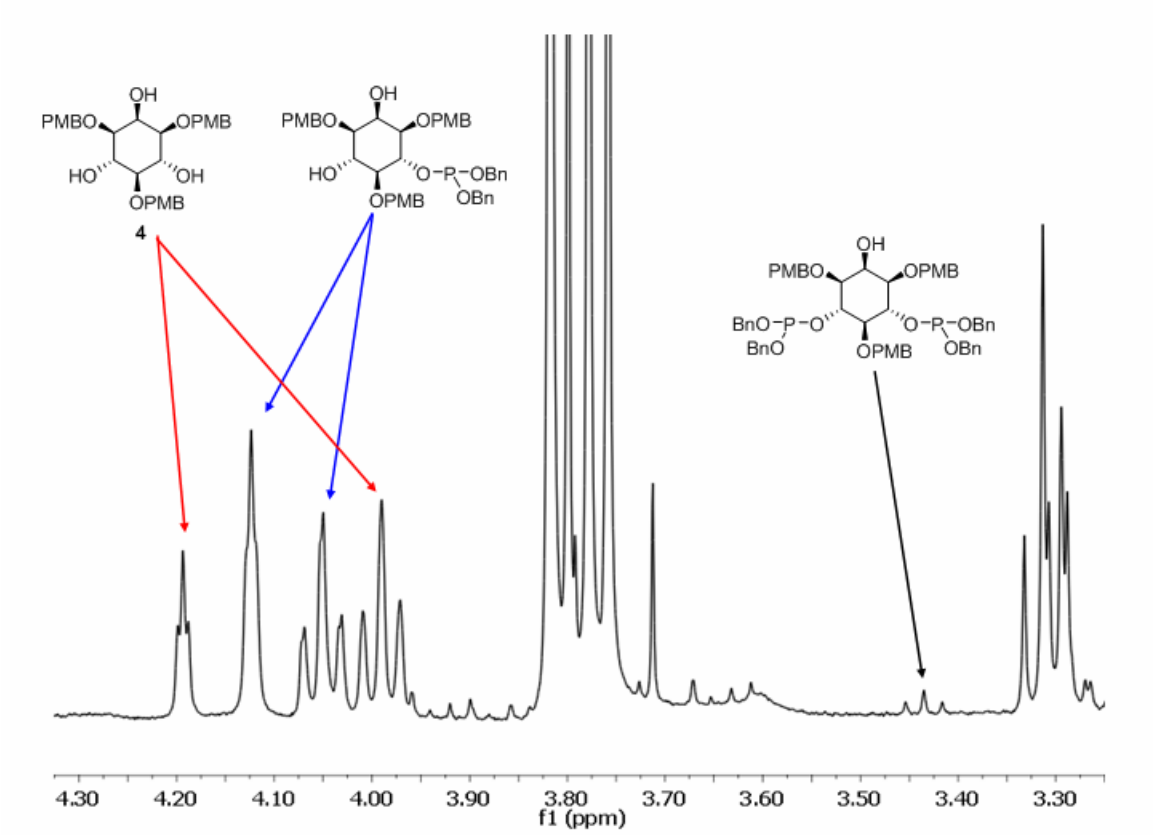

| | Conversion to **10** | | | | $k_{rel}$ based upon conversion to **10** | | |
|---|---|---|---|---|---|---|---|
| Trial | H Shifts[α] | P Shifts[β] | Average | % *ee* | H Shifts[γ] | P Shifts[δ] | Average |
| 1 | 0.36 | 0.35 | o.36 | 30.4 | 4.4 | 4.6 | 4.5 |
| 2 | 0.56 | 0.58 | 0.57 | 83.5 | 12.3 | 10.1 | 11.1 |
| 3 | 0.51 | 0.51 | 0.51 | 67.6 | 9.6 | 9.6 | 9.6 |

Conversions were calculated using $^{31}$P and $^{1}$H NMR on a Bruker 500 MHz NMR spectrometer. [α]Conversions based upon proton resonances were calculated by averaging the normalized chemical shift ratios for three distinct proton chemical shifts for both starting monophosphorothioate and bisphosphorothioate. [β]Phosphorous conversions were calculated using the normalized chemical shift ratio for the monophosphorothioate (69.13 ppm) and bisphosphorothioate (69.00 ppm). Values for $k_{rel}$ were calculated utilizing the method described by Kagan et. al.[1] ($k_{rel}$ = ln[(1 - c)(1 - ee)]/ln[(1 - c)(1 + ee)]).[4] Conversions based upon proton[γ] and phosphorous[δ] signals were used to calculate values for $k_{rel}$. An average $k_{rel}$ for each trial was calculated for each run giving 8.4 ± 2.8 as an average of 3 trials.

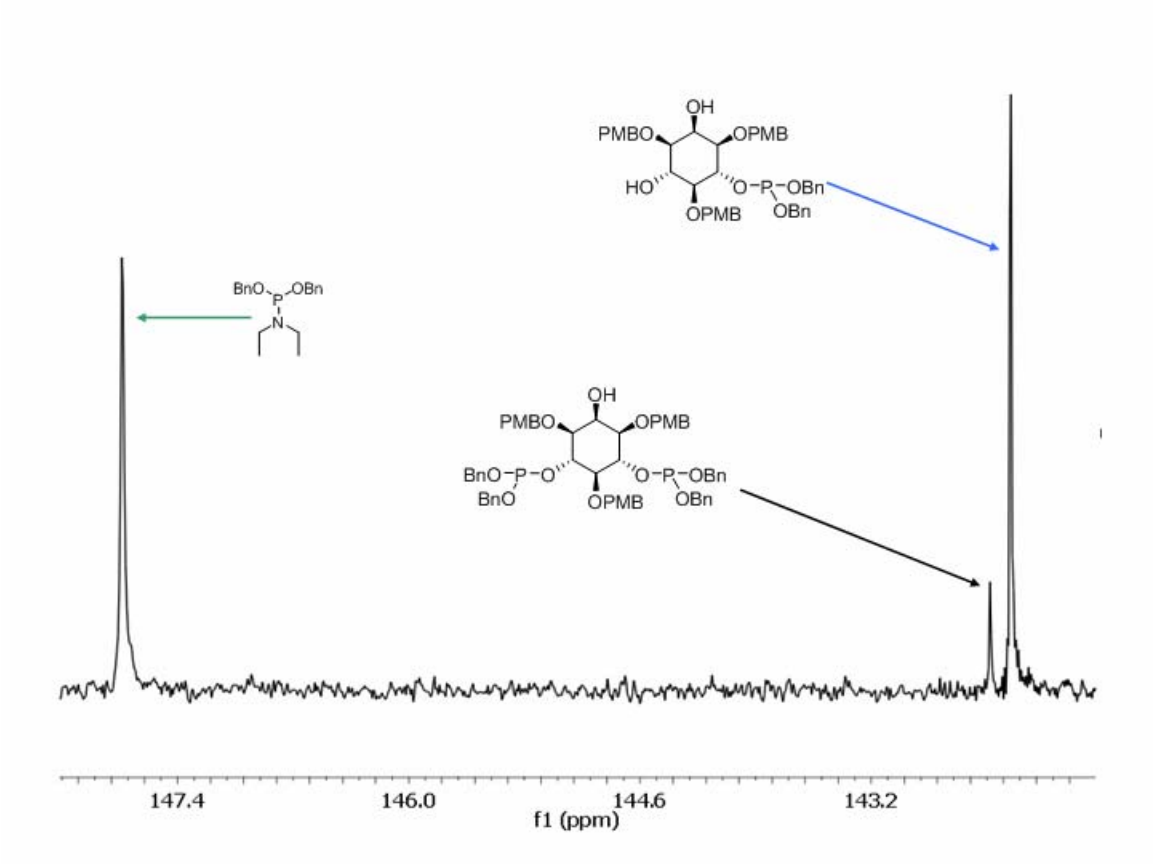
[1] Kagan HB, Fiaud JC (**1985)** Kinetic resolution, *Top. Stereochem.* 18: 249–330.



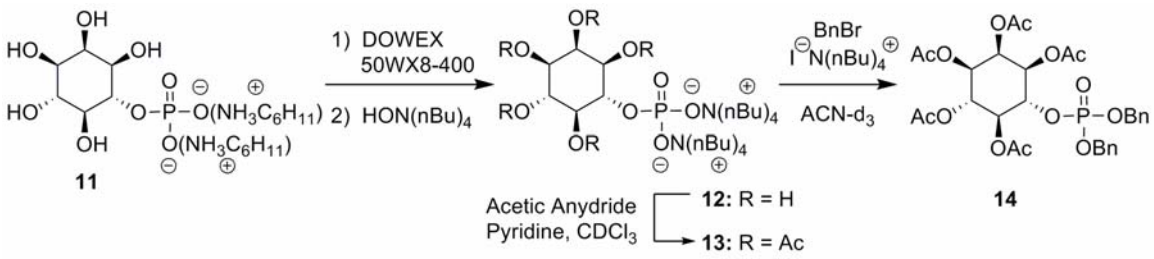

Supplemental Scheme 1: Synthesis of 1,3,5-Tri-*O*-PMB-*myo*-inositol **(4)**




Supplemental Figure 1: Characteristic $^{1}$H-NMR chemical shifts corresponding to 1,3,5-Tri-*O*-PMB-*myo*-inositol (**4)** and intermediate inositol monophosphite and inositol bisphosphite.




Supplemental Figure 2: Characteristic $^{31}$P-NMR chemical shifts corresponding to dibenzyl *N*,*N*-diethylphosphoramidite, intermediate inositol monophosphite, and inositol bisphosphite.



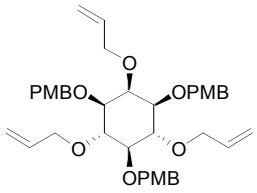

Supplemental Scheme 2: Synthetic scheme for the preparation of protected inositol **14** for chiral HPLC analysis. Procedure is included in supporting text.

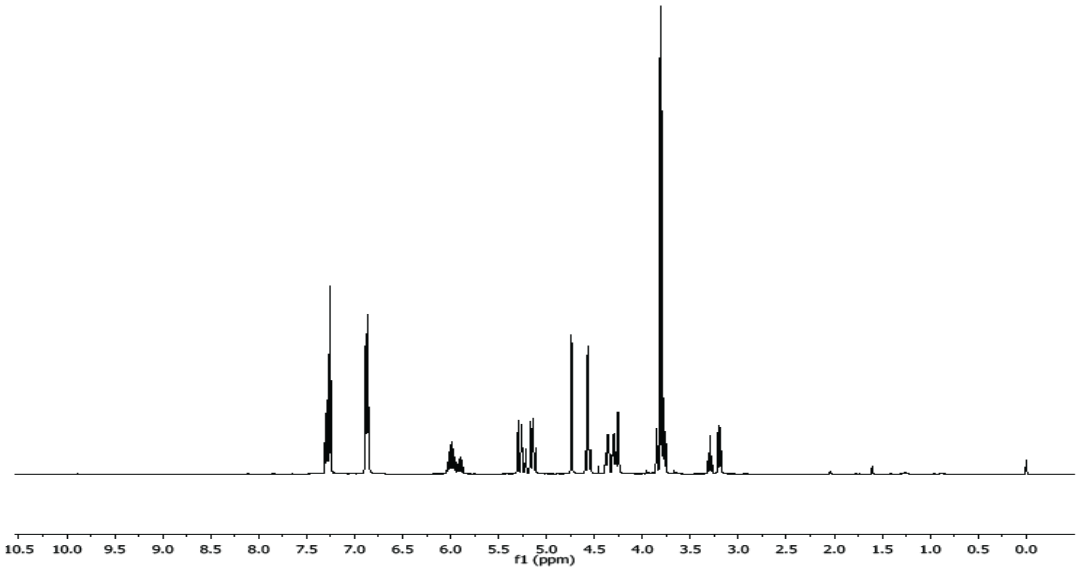
**Asymmetric Phosphorylation through Catalytic P(III) Phosphoramidite Transfer: Enantioselective Total Synthesis of *myo*-Inositol-6-Phosphate**

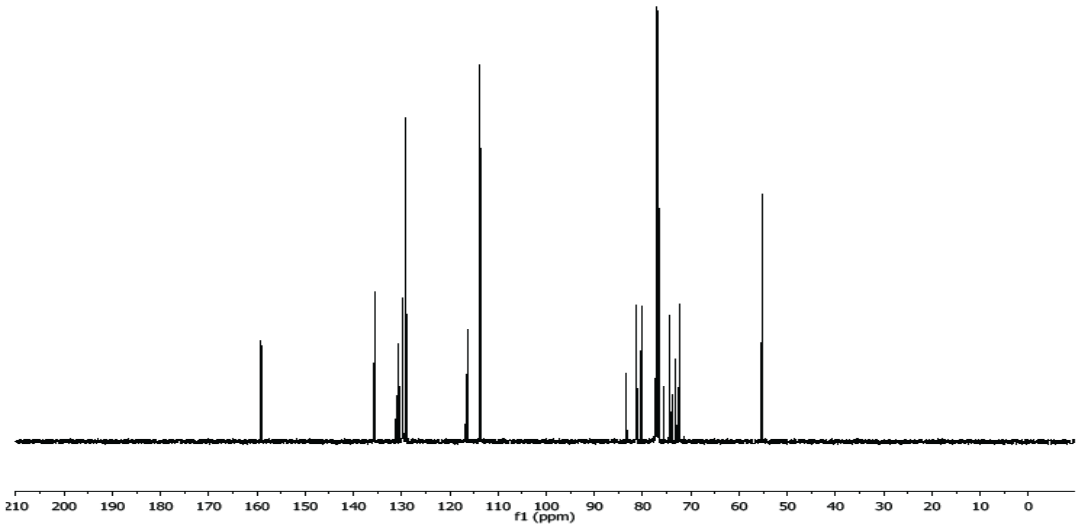
Peter A. Jordan, Katherine J. Kayser-Bricker and Scott J. Miller*

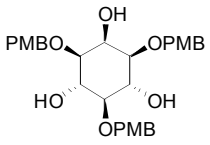
*[*]Department of Chemistry, Yale University, New Haven, Connecticut, 06520*

$^{1}$H NMR, $^{13}$C NMR and $^{31}$P NMR for Characterized Compounds

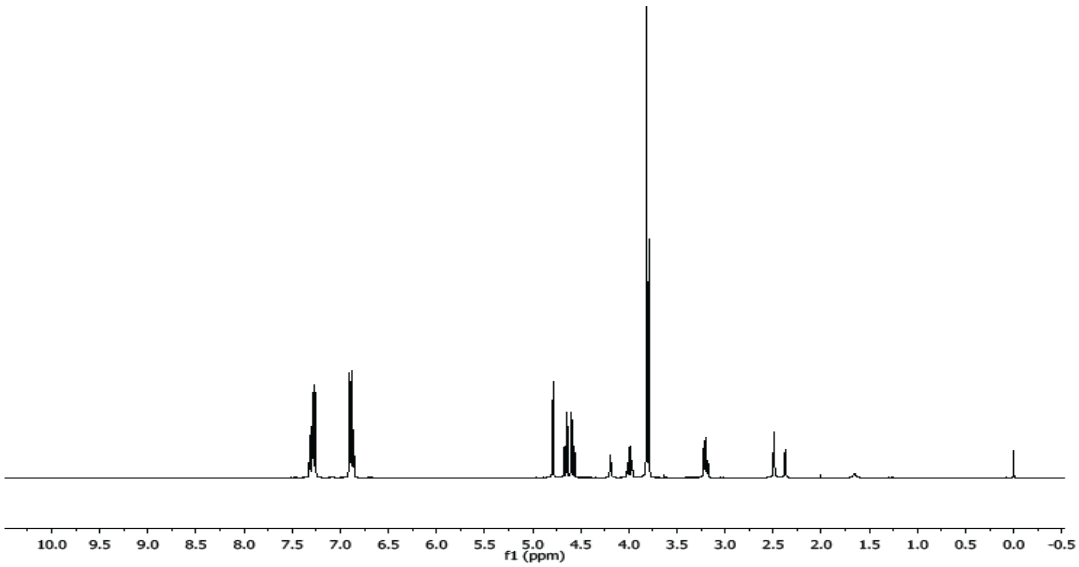


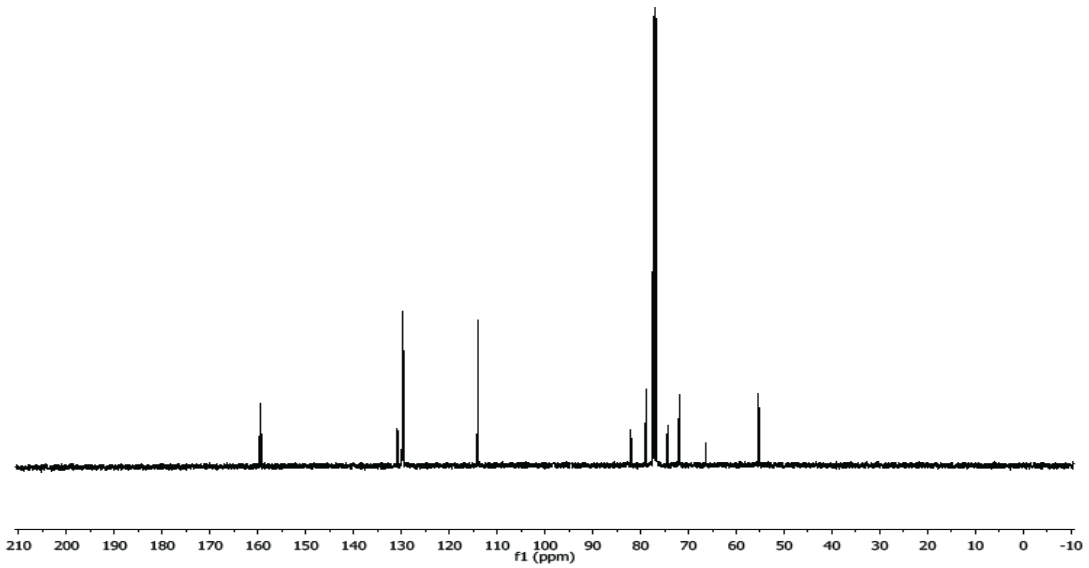




OH
PMBO
OPMB
HO
OH
OPMB

**4**


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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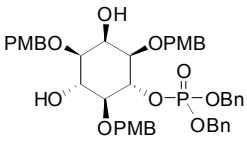
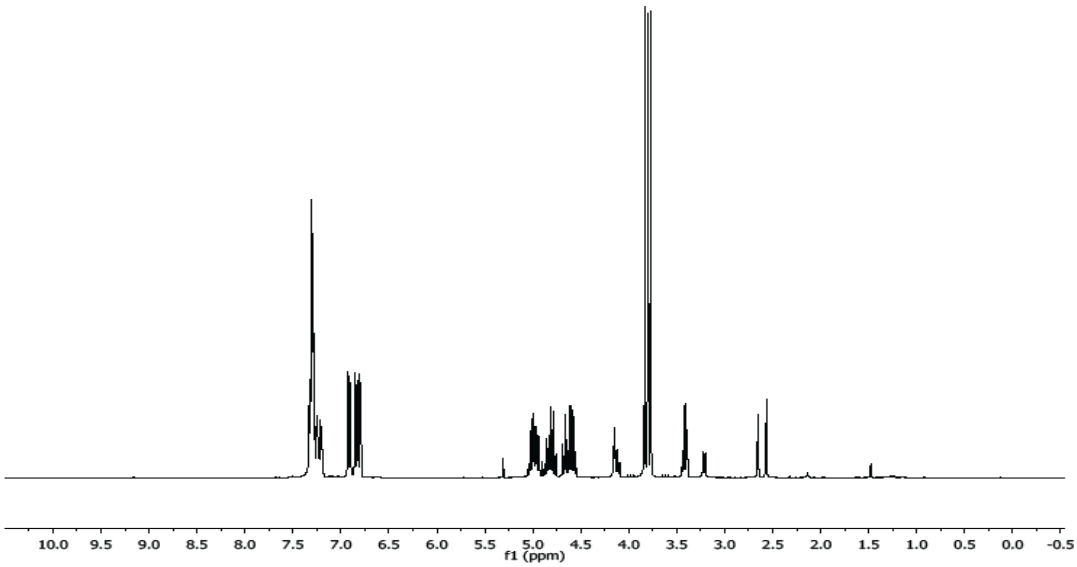
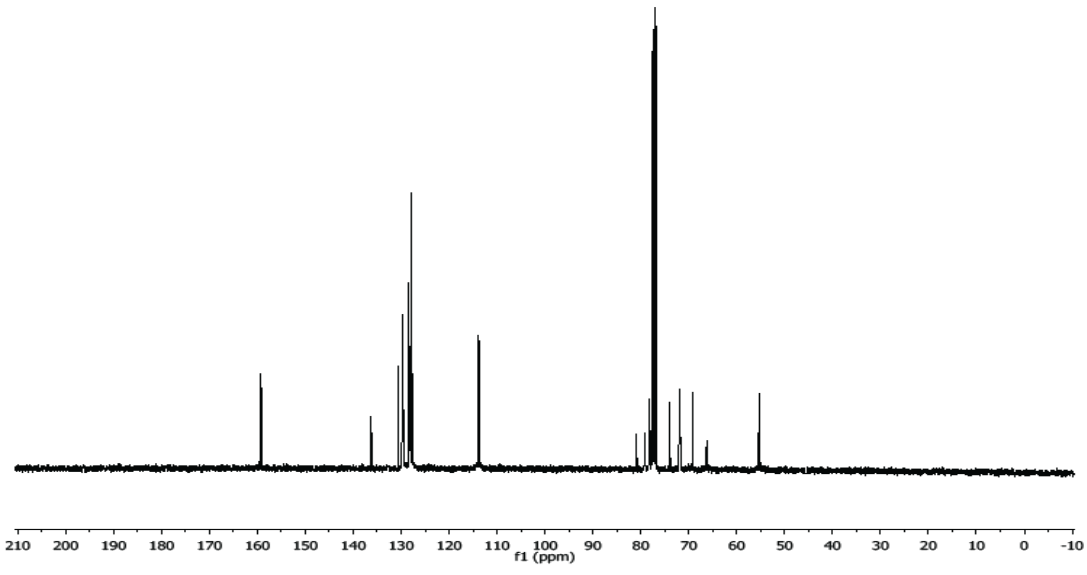
f1 (ppm)


OH
PMBO
OPMB
O
HO
O–P–OBn
OPMB
OBn

**5**


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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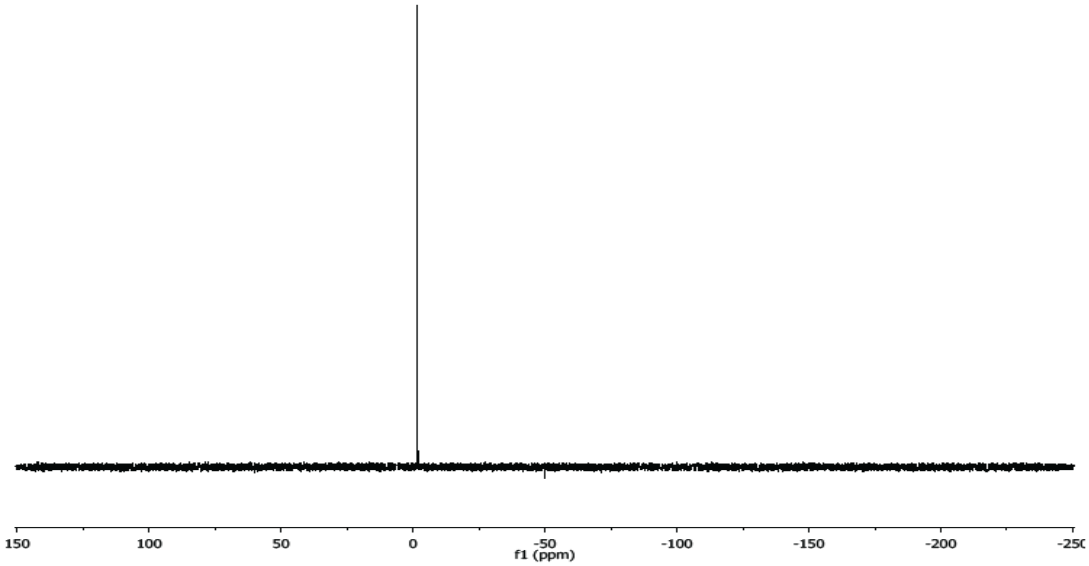
f1 (ppm)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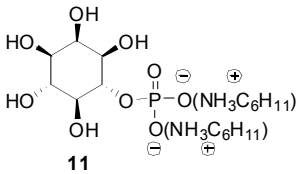
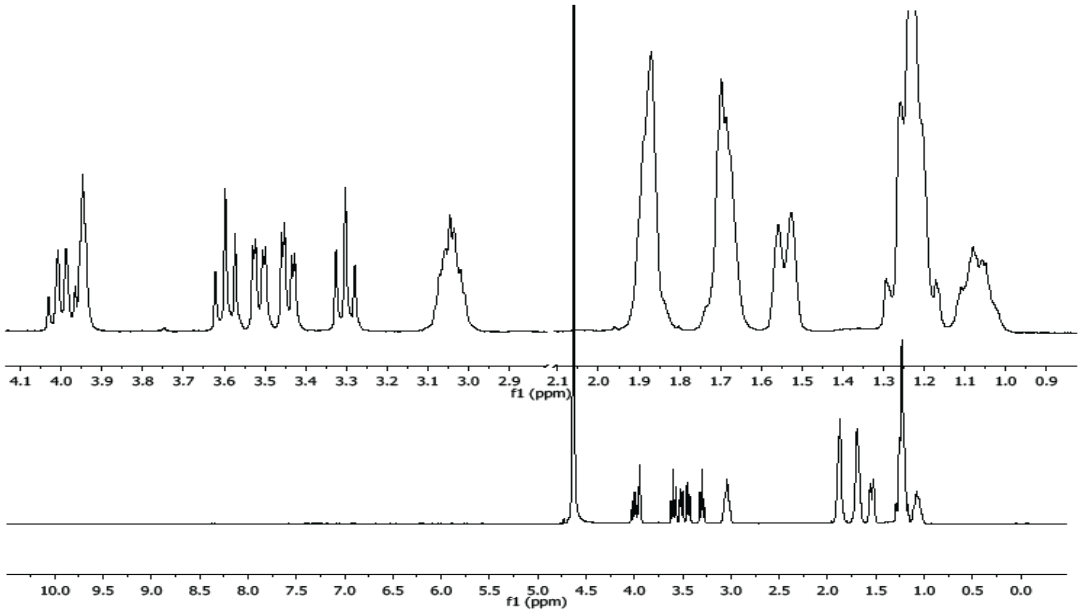
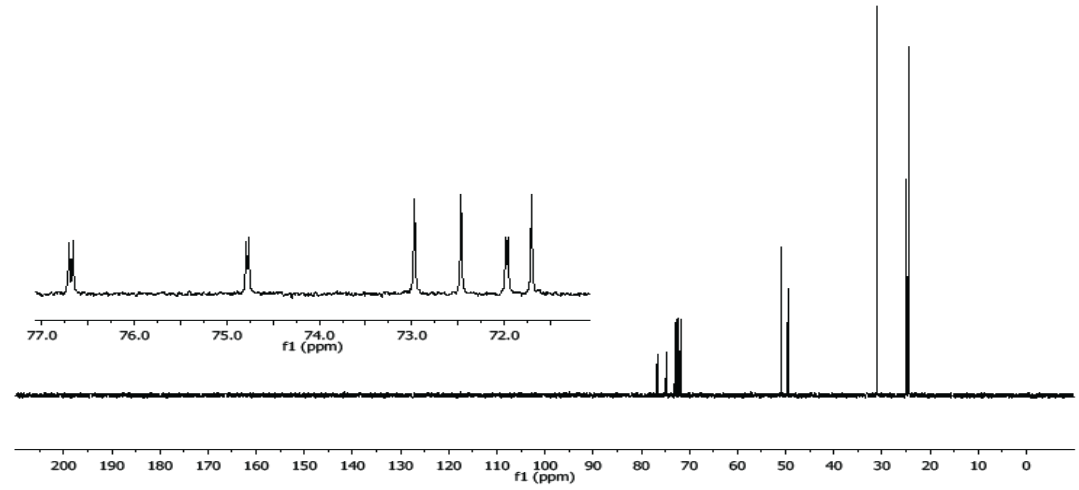
100
50
0
-50
-100
-150
-200
f1 (ppm)


OH
HO
OH
HO
O
P
O(NH3C6H11)
O(NH3C6H11)
OH

**11**


4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9
f1 (ppm)
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


77.0
76.0
75.0
74.0
73.0
72.0
f1 (ppm)
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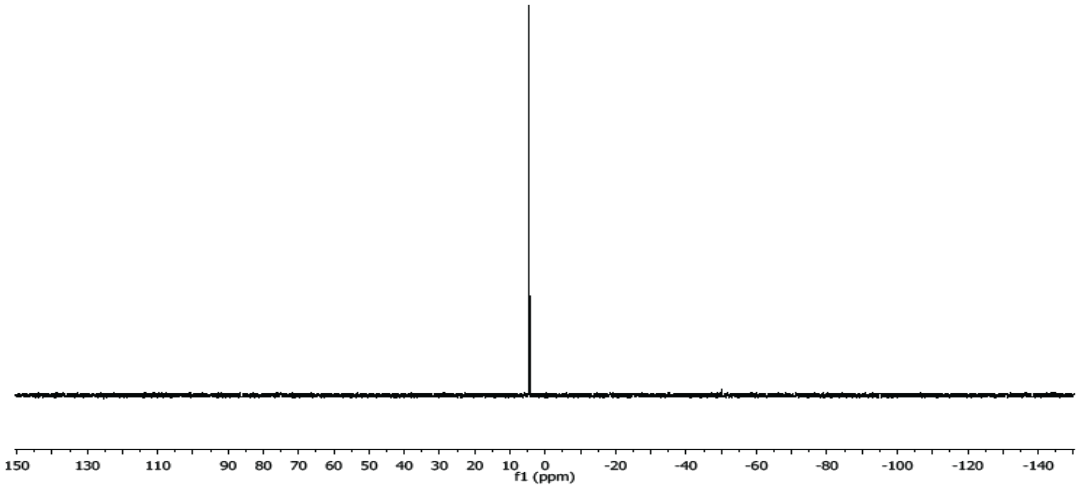
f1 (ppm)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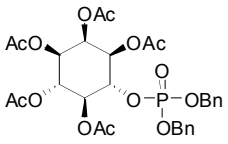
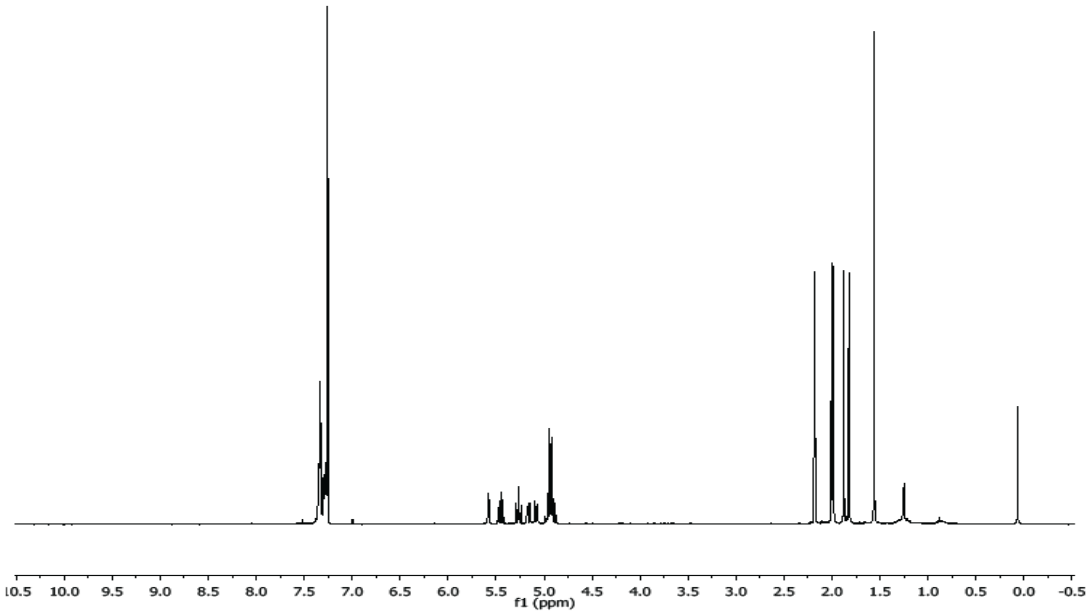
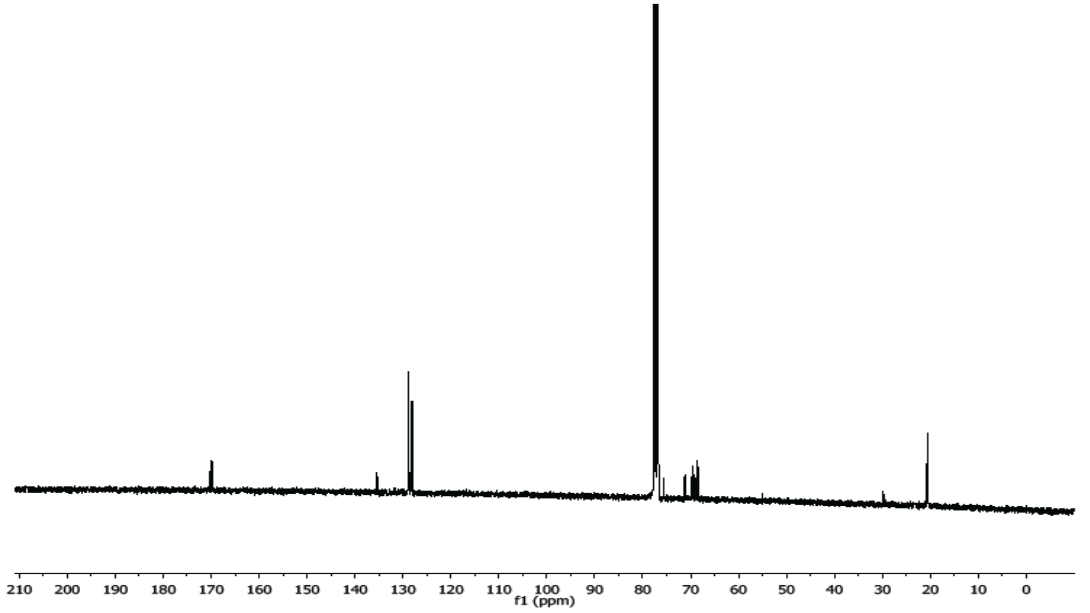
f1 (ppm)


OAc
AcO
OAc
O
AcO
O–P–OBn
OAc
OBn

**14**


10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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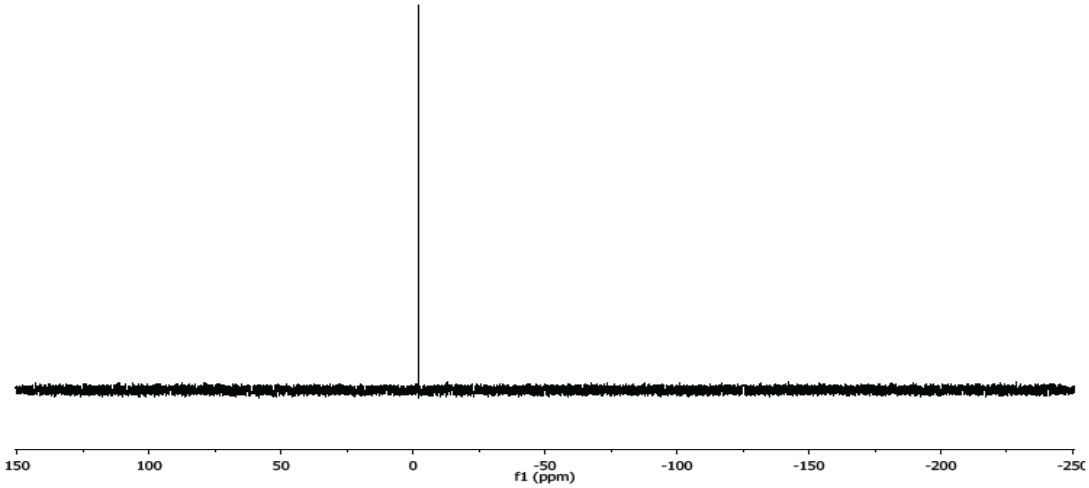
f1 (ppm)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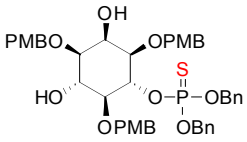
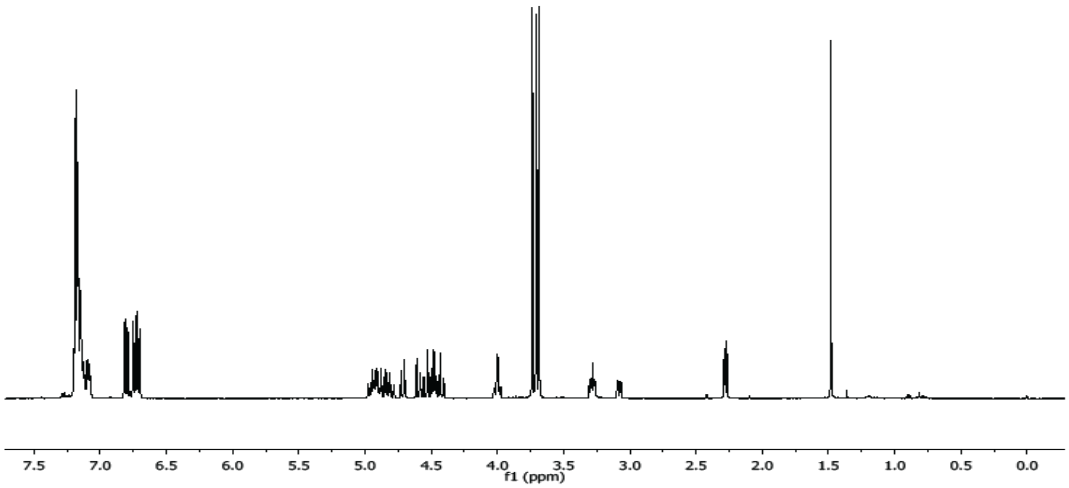
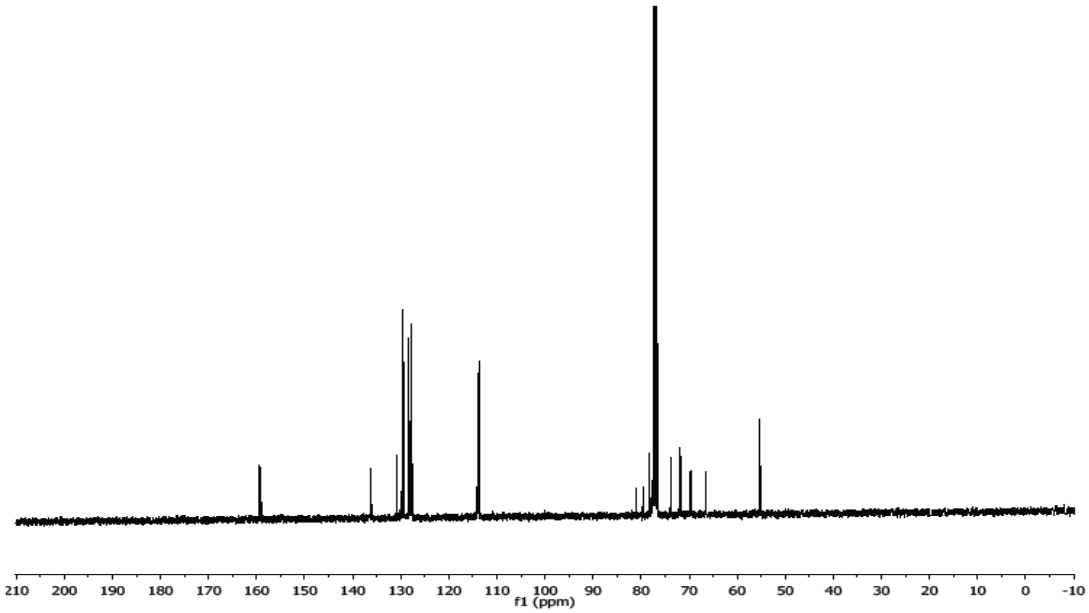
100
50
0
-50
-100
-150
-200
f1 (ppm)


OH
PMBO
OPMB
S
HO
O–P–OBn
OPMB
OBn

**9**


7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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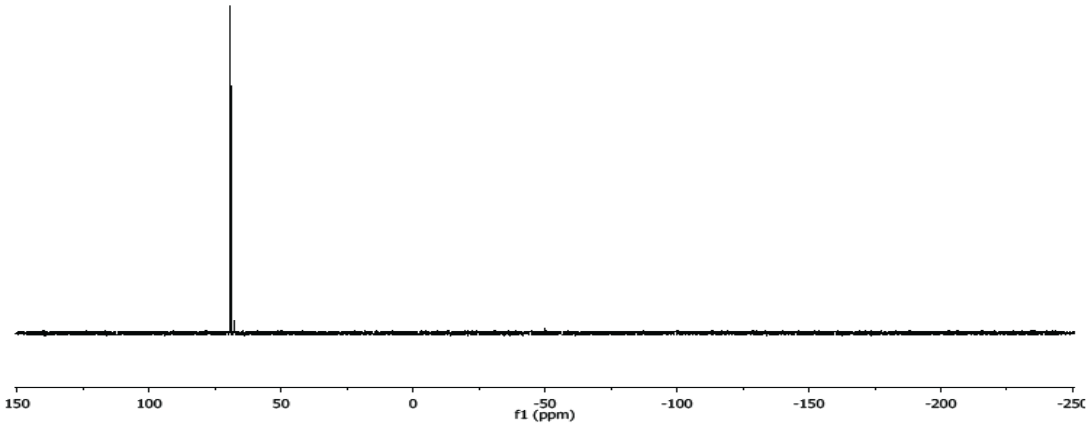
f1 (ppm)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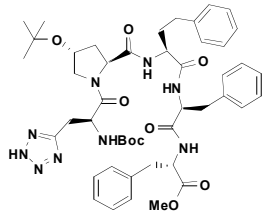
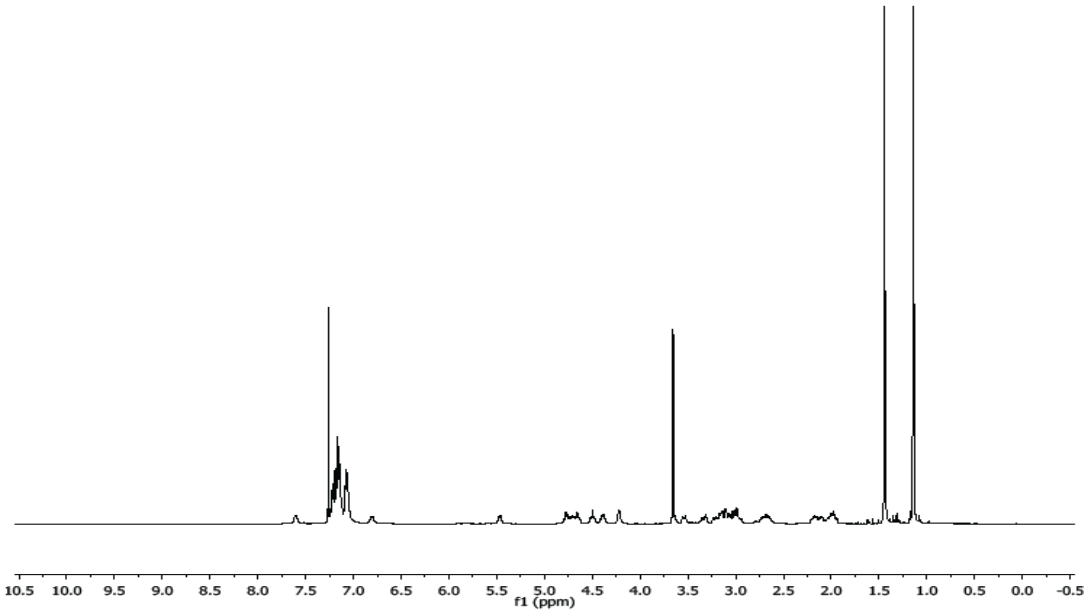
f1 (ppm)


O
N
H
O
HN
N
O
NHBoc
O
NH
HN
N
N
O
MeO

Peptide **3**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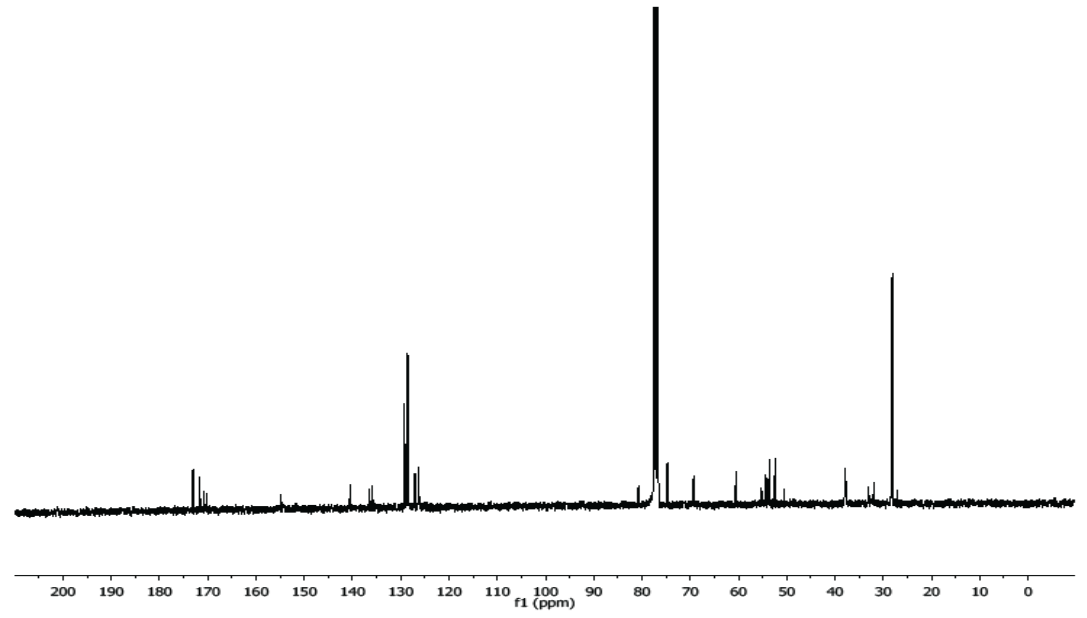
f1 (ppm)


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)