Asymmetric Syntheses of L,L- and L,D-Di-*myo*-inositol-1,1'-phosphate and their Behavior as Stabilizers of I-Monophosphatase Activity at Extreme Temperatures

Christina M. Longo,[‡] Yang Wei,[§] Mary F. Roberts[§] and Scott J. Miller[*‡]

[‡]*Department of Chemistry, Yale University, 225 Prospect Street, New Haven, Connecticut 06520-4900, and*
[§]*Department of Chemistry, Merkert Chemistry Center, Boston College, Chestnut Hill, Massachusetts 02467*

Supporting Information

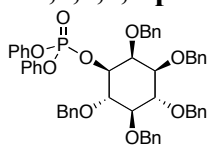
General Procedures. Proton NMR spectra were recorded on Bruker 400 or 500 MHz spectrometers. Proton chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane (TMS, δ 0.0) or with the solvent reference relative to TMS employed as the internal standard ($CDCl_3$, δ 7.26; D_2O, δ 4.79). Data are reported as follows: chemical shift (multiplicity [singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), doublet of triplets (dt), doublet of doublet of doublets (ddd), triplet (t), triplet of doublets (td), quartet (q), multiplet (m)], integration, coupling constants [Hz]). Carbon NMR spectra were recorded on Bruker 400 (100 MHz) or 500 (125 MHz) spectrometers with complete proton decoupling. Carbon chemical shifts are reported in ppm (δ) relative to TMS with the respective solvent reference as the internal standard ($CDCl_3$, δ 77.0), or with CH_3OH (δ 49.50) as an internal standard. Phosphorus NMR spectra were recorded on Bruker 400 (162 MHz) or 500 (202 MHz) spectrometers with complete proton decoupling. Phosphorus chemical shifts are reported in ppm (δ) relative to an 85% H_3PO_4 external standard. NMR data were recorded at 25 ˚C, unless otherwise indicated. Infrared spectra were obtained on a ThermoNicolet Avatar 210 Series spectrometer, υ_{max} (cm^{-1}) and are partially reported. Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 Å F-254 precoated plates (0.25 mm thickness). TLC R*f* values are reported. Visualization was accomplished by irradiation with a UV lamp and ceric ammonium molybdate (CAM) solutions. Flash column chromatography was performed using Silica Gel 60 Å (32-56 μm). Optical rotations were recorded on a Perkin Elmer 341 Series polarimeter at the sodium D line (path length 50 mm). High resolution mass spectra were obtained at the Mass Spectrometry Facility at the University of Illinois Urbana-Champaign. The method of ionization is given in parentheses.

Analytical normal-phase HPLC was performed on a Hewlett-Packard 1100 Series chromatograph equipped with a diode array detector (214 nm and 254 nm). All reactions were performed under an argon or nitrogen atmosphere employing oven- or flame-dried glassware. All solvents were purified according to standard procedures or using a Solvent Purification System. 2,4,6-tri-*O*-benzyl-*myo*-inositol was prepared according to the method of Billington and coworkers.[1] L-2,4,6-tri-*O*-benzyl-*myo*-inositol-diphenyl-1-phosphate (**2**) was prepared according to the method of Miller and coworkers.[2] 2-benzyloxy-1-methylpyridinium triflate was prepared according to the method of Dudley and coworkers.[3] Peptide catalysts C-W[4] were prepared on solid phase according to standard procedures.[5] Peptides were purified by reverse phase flash chromatography on

C18 silica eluting with a 55-85% methanol in water gradient on a Biotage SP4 system. All other chemicals were purchased commercially and used as received.

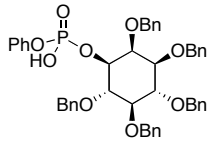
The amino acid abbreviations beyond the most standard proteinogenic amino acids are as follows: Abu – 2-aminobutyric acid, Aib – α-aminoisobutyric acid, Hfe – homophenylalanine, Hyp – 4-hydroxyproline, Phg – phenylglycine, Pip – pipecolic acid, Pmh – π-methylhistidine, Sp5 – 1-aminocyclopentane-1-carboxylic acid.

L-2,3,4,5,6-pentabenzyl-*myo*-inositol-diphenyl-1-phosphate



L-2,4,6-tri-*O*-benzyl-*myo*-inositol-diphenyl-1-phosphate (1.53 g, 2.24 mmol) was dissolved in benzotrifluoride (25 mL), and magnesium oxide (904 mg, 22.4 mmol) was added, followed by 2-benzyloxy-1-methylpyridinium triflate (7.83 g, 22.4 mmol). The white suspension was stirred vigorously and heated to reflux for three days and then cooled to room temperature, filtered through Celite, and concentrated. The yellow oil was purified by silica gel flash chromatography (gradient elution 10% - 20% ethyl acetate in hexanes containing 0.05% methanol) to afford 1.01 g (73% yield) of a colorless oil. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.34 (d, J = 4.4 Hz, 1H), 7.32-7.11 (m, 34H), 4.93-4.74 (m, 6H), 4.68-4.63 (m, 2H), 4.60-4.51 (m, 2H), 4.30 (t, J = 2.2 Hz, 1H), 4.20-4.05 (m, 2H), 4.02-3.86 (m, 1H), 3.60-3.45 (m, 2H); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ 150.4, 138.6, 138.6, 138.4, 138.3, 138.0, 129.8, 129.7, 128.9, 128.6, 128.4, 128.3, 128.2, 128.1, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4, 127.4, 127.0, 125.4, 125.2, 120.2, 120.1, 120.0, 119.9, 83.1, 81.2, 80.5, 79.8, 76.0, 75.9, 75.5, 75.0, 73.0, 65.3, 60.4; **^{31}P NMR** ($CDCl_3$, 162 MHz) δ -12.4; **IR** (film, cm^{-1}) 3061, 3027, 2923, 2858, 1589, 1489, 1452, 1357, 1288, 1217, 1188, 1160, 1122, 1073, 1025, 954, 773, 751, 733 696; **TLC** R_f 0.7 (50% ethyl acetate/ hexanes); **$[\alpha]_D^{20}$** = + 5.4 (c 1.0, $CHCl_3$, at 99% ee); **HRMS** calcd for $[C_{53}H_{51}O_9PNa]^+$ requires m/z 885.3168; found 885.3136 (ESI+).

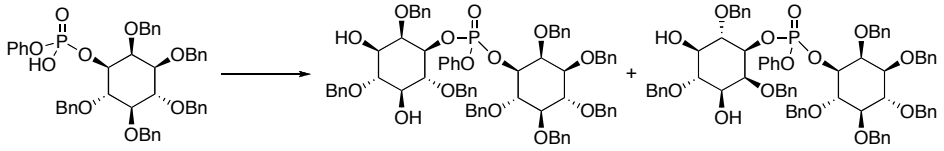
L-2,3,4,5,6-pentabenzyl-*myo*-inositol-phenyl-1-phosphate (7)



L-2,3,4,5,6-pentabenzyl-*myo*-inositol-diphenyl-1-phosphate (2.01 g, 2.3 mmol) was dissolved in THF (10 mL) and lithium hydroxide monohydrate (980 mg, 23.3 mmol) and water (10 mL) were added respectively, and the reaction was stirred at reflux for 12 hours. The reaction was cooled to room temperature, silica gel was added (10 g), and the mixture was concentrated to dryness. The crude product was purified by silica gel flash chromatography (1% - 10% methanol in methylene chloride) to afford the lithium salt as a white solid. The solid was dissolved in a minimum amount of 60% methylene chloride in methanol and eluted on DOWEX 50X2-200 resin with 60% methylene chloride in methanol to afford 1.79 g (98% yield) of a thick colorless oil. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 12.8 (br s, 1H), 7.40-6.95 (m, 30H), 4.85 (t, J = 10.8 Hz, 1H), 4.81-4.73 (m, 4H), 4.68 (d, J = 6.8 Hz, 1H), 4.60 (d, J = 6.8 Hz, 1H), 4.55 (s, 1H), 4.32 (td, J = 2.4 Hz, J =

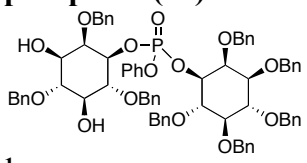
8.92 Hz, 1H), 4.23 (t, *J* = 2.2 Hz, 1H), 4.08-3.98 (m, 2H), 3.94-3.82 (m, 2H), 3.43 (t, *J* = 9.2 Hz, 1H), 3.36 (dd, *J* = 2.1 Hz, *J* = 9.8 Hz, 1H); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 150.4, 150.3, 138.7, 138.6, 138.5, 138.1, 137.9, 129.7, 128.9, 128.8, 128.8, 128.7, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.4, 125.3, 120.2, 120.1; **^{31}P NMR** ($CDCl_3$, 202 MHz) δ -4.4; **IR** (film, cm^{-1}) 3060, 3027, 2917, 2864, 1589, 1486, 1450, 1356, 1209, 1160, 1123, 1066, 1045, 1025, 955, 731, 690; **TLC** R_f 0.7 (20% methanol/ methylene chloride); **$[\alpha]_D^{20}$** = + 5.9 (*c* 1.0, $CHCl_3$, at 99% ee); **HRMS** calcd for $[C_{47}H_{47}O_9PH]^+$ requires m/z 787.3030; found 787.3050 (ESI+).

Optimized chlorination and coupling



L-2,3,4,5,6-pentabenzyl-*myo*-inositol-phenyl-1-phosphate (464 mg, 0.59 mmol) was dissolved in methylene chloride (4 mL), and oxalyl chloride (103 μL, 1.18 mmol) was added, followed by DMF (46 μL, 0.59 mmol), resulting in a colorless effervescing solution. The solution was allowed to stir for 2 minutes, and was transferred via cannula into a solution containing tribenzylinositol (532 mg, 1.18 mmol), triethylamine (329 μL, 2.36 mmol), and DMAP (73 mg, 0.59 mmol) in methylene chloride (6 mL). The solution immediately turned orange and was stirred for 12 hours. The reaction was quenched with water and concentrated to yield an orange foam. Purification by silica gel flash chromatography (gradient elution 10% - 50% ethyl acetate in hexanes) yielded 410 mg (57% yield) of a white foam as a 3:1 mixture of diastereomers **8a**:**9a**. The diastereomers were separated using a Chromatotron (Harrison Research) on a 2 mm rotor (30% ethyl acetate in hexanes containing 0.1% methanol). Reaction can be done with DMAP (20 mol %) to afford 39% yield as a 3:1 mixture of diastereomers **8a**:**9a**.
Procedure repeated with catalyst **10** (20 mol %) to afford 64% yield of a white foam as a 13:1 mixture of diastereomers **8a**:**9a**.
Procedure repeated with catalyst **11** (20 mol %) to afford 33% yield of a white foam as a 1:2.5 mixture of diastereomers **8a**:**9a**.
See Table 1 for additional catalyst screens.

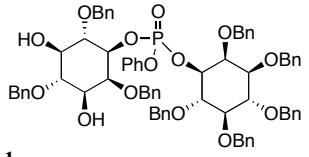
Phenyl-L-2,3,4,5,6-pentabenzyl-*myo*-inositol-D-2',4',6'-tribenzyl-*myo*-inositol-1,1'-phosphate (8a)



^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38-7.15 (m, 45H), 4.85 (dd, *J* = 11.4, *J* = 2.4, 4H), 4.78 (t, *J* = 10.4 Hz, 2H), 4.72 (m, 4H), 4.64 (m, 6H), 4.48 (dd, *J* = 6.8 Hz, *J* = 4.8 Hz, 1H), 4.40-4.30 (m, 2H), 4.26 (dt, *J* = 10.7 Hz, *J* = 2.4 Hz, 1H), 4.01-3.92 (m, 2H), 3.77 (t, *J* = 9.4 Hz, 1H), 3.60 (t, *J* = 9.4 Hz, 1H), 3.36-3.27 (m, 2H), 3.11 (t, *J* = 9.3 Hz, 1H), 3.02

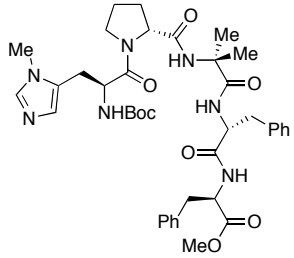
(td, J = 9.2 Hz, J = 2.3 Hz, 1H), 2.25 (d, J = 2.3 Hz, 1H), 2.14 (d, J = 5.1, 1H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 150.5, 150.4, 138.9, 138.8, 138.7, 138.6, 138.5, 138.3, 138.1, 137.9, 129.8, 128.6, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 127.4, 127.3, 127.3, 127.2, 125.4, 120.2, 120.1, 82.8, 81.1, 80.6, 80.3, 80.0, 80.0, 79.7, 79.6, 79.3, 79.2, 79.2, 79.1, 78.7, 76.5, 75.9, 75.5, 75.3, 75.0, 74.9, 74.8, 74.5, 74.4, 72.8, 72.0, 71.7; **^{31}P NMR** ($CDCl_3$, 202 MHz) δ -6.7; **IR** (film, cm^{-1}) 3566, 3427, 3088, 3062, 3030, 2923, 2868, 1496, 1454, 1395, 1358, 1259, 1207, 1120, 1070, 1027, 958, 731, 697; **TLC** R_f 0.9 (50% ethyl acetate/ hexanes); **$[\alpha]_D^{20}$** = + 2.1 (c 1.0, $CHCl_3$); **HRMS** calcd for $[C_{74}H_{76}O_{14}P]^+$ requires m/z 1219.4973; found 1219.5012 (ESI+).

Phenyl-L-2,3,4,5,6-pentabenzyl-*myo*-inositol-L-2',4',6'-tribenzyl-*myo*-inositol-1,1'-phosphate (9a)



^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37-7.06 (m, 45H), 4.91-4.64 (m, 14H), 4.54-4.42 (m, 4H), 4.38 (t, J = 2.4 Hz, 1H), 4.08 (t, J = 2.4 Hz, 1H), 4.05 (t, J = 9.6 Hz, 1H), 3.95 (m, 1H), 3.85 (m, 1H), 3.56 (t, J = 9.4 Hz, 1H), 3.37 (dd, J = 9.0 Hz, J = 2.0 Hz, 1H), 3.32 (t, J = 9.3 Hz, 1H), 3.04 (dd, J = 9.8 Hz, J = 2.0 Hz, 1H), 2.88 (m, 1H), 2.37 (d, J = 2.0 Hz, 1H), 1.99 (d, J = 5.8 Hz, 1H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 160.1, 150.6, 150.5, 138.9, 138.7, 138.6, 138.5, 138.4, 138.4, 138.3, 138.3, 138.2, 138.1, 138.0, 129.7, 128.9, 128.8, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 127.3, 127.2, 125.2, 120.1, 120.0, 82.7, 81.3, 81.0, 80.3, 80.1, 80.0, 79.8, 79.7, 79.4, 79.2, 79.1, 78.8, 78.4, 78.3, 77.5, 76.0, 75.8, 75.5, 75.4, 75.3, 75.2, 75.1, 75.0, 74.9, 74.8, 74.6, 74.4, 73.4, 72.6, 72.0, 71.4; **^{31}P NMR** ($CDCl_3$, 162 MHz) δ -8.4; **IR** (film, cm^{-1}) 3423, 3087, 3062, 3026, 2924, 2865, 1493, 1453, 1357, 1261, 1208, 1122, 1068, 1026, 955, 734, 696; **TLC** R_f 0.9 (50% ethyl acetate/ hexanes); **$[\alpha]_D^{20}$** = + 7.7 (c 1.0, $CHCl_3$); **HRMS** calcd for $[C_{74}H_{76}O_{14}P]^+$ requires *m/z* 1219.4973; found 1219.5010 (ESI+).

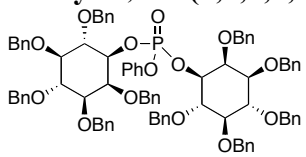
Peptide 11



Peptide **11** was prepared using standard solution phase techniques. The residue was purified by reverse phase flash chromatography on C18 silica eluting with a 55-85% methanol in water gradient on a Biotage SP4 system. **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.40 (s, 1H), 7.26-7.14 (m, 9H), 6.83 (s, 1H), 6.71 (s, 1H), 5.57 (d, J = 7.0 Hz, 1H), 4.77 (q, J = 8.0 Hz, J = 6.2 Hz, 1H), 4.52-4.47 (m, 1H), 4.41 (q, J = 7.3 Hz, J = 7.2 Hz, 1H), 4.06 (t, J = 6.2 Hz, 1H), 3.72-3.68 (m, 1H), 3.66 (s, 1H), 3.58 (s, 1H), 3.31 (dd, J = 14.3 Hz, J = 4.6 Hz, 1H), 3.24-3.16 (m, 2H), 3.12-2.93 (m, 4H), 2.10-1.93 (m, 3H), 1.84-1.79

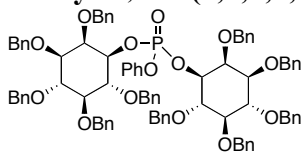
(m, 4H), 1.44 (s, 3H), 1.41 (s, 9H), 1.25 (s, 3H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 173.7, 172.1, 171.3, 171.2, 170.8, 138.3, 138.2, 137.0, 129.3, 129.2, 128.3, 128.3, 128.1, 126.9, 126.6, 126.4, 129.2, 128.3, 128.3, 128.1, 126.9, 126.6, 126.4, 80.7, 61.9, 57.2, 55.2, 53.8, 52.5, 52.2, 47.8, 37.6, 36.5, 31.4, 28.9, 28.3, 26.5, 25.8, 25.0, 24.9; **IR** (film, cm^{-1}) 3366, 3313, 3276, 3027, 2974, 2917, 1744, 1662, 1654, 1638, 1527, 1503, 1446, 1429, 1384, 1364, 1282, 1249, 1213, 1168, 1106, 1021, 923; **TLC** R_f 0.4 (10% methanol/ methylene chloride); **HRMS** calcd for $[C_{40}H_{54}O_8N_7]^+$ requires *m/z* 760.4034; found 760.4020 (ESI+).

Phenyl-L,L-di(2,3,4,5,6-pentabenzyl-*myo*-inositol)-1,1'-phosphate (9b)



Phenyl-L-2,3,4,5,6-pentabenzyl-*myo*-inositol-L-2',4',6'-tribenzyl-*myo*-inositol-1,1'-phosphate (160 mg, 0.13 mmol) was dissolved in benzotrifluoride (3 mL), and 2-benzyloxy-1-methylpyridinium triflate (917 mg, 2.6 mmol) was added, followed by magnesium oxide (106 mg, 2.6 mmol). The reaction was heated in a sealed tube at 90 ˚C for three days, and subsequently cooled to room temperature, filtered through Celite with toluene, and concentrated. The residue was purified by silica gel flash chromatography (gradient elution 10% - 20% ethyl acetate in hexanes containing 0.5% methanol) to yield a colorless residue (49 mg, 27% yield). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.32-7.06 (m, 55H), 4.88-4.84 (m, 4H), 4.80-4.71 (m, 10H), 4.53-4.43 (m, 6H), 4.34 (t, *J* = 2.3 Hz, 1H), 4.30 (t, *J* = 2.3 Hz, 1H), 4.09-3.86 (m, 6H), 3.38 (t, *J* = 9.3 Hz, 1H), 3.30 (t, *J* = 9.3 Hz, 1H), 3.12 (dd, *J* = 12.0 Hz, *J* = 2.2 Hz, 1H), 3.03 (dd, *J* = 12.0 Hz, *J* = 2.2 Hz, 1H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 150.5, 138.9, 138.8, 138.7, 138.5, 138.4, 138.2, 138.1, 129.6, 128.9, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4, 127.2, 127.1, 126.8, 126.0, 125.2, 120.0, 82.9, 82.6, 81.2, 80.4, 80.2, 79.7, 79.3, 78.6, 76.5, 76.0, 76.0, 75.9, 75.6, 75.5, 75.3, 75.0, 74.8, 72.7, 72.5; **^{31}P NMR** ($CDCl_3$, 202 MHz) δ -8.3; **IR** (film, cm^{-1}) 3685-3178 br, 3088, 3063, 3028, 2918, 2849, 1717, 1594, 1495, 1451, 1360, 1290, 1260, 1210, 1161, 1123, 1068, 1026, 952, 732, 694; **TLC** R_f 0.5 (30% ethyl acetate/ hexanes); **$[\alpha]_D^{20}$** = +1.2 (*c* 1.0, $CHCl_3$); **HRMS** calcd for $[C_{88}H_{87}O_{14}PNa]^+$ requires m/z 1421.5731; found 1421.5704 (ESI+).

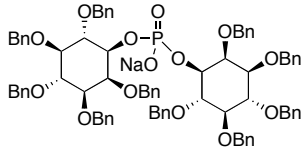
Phenyl-L,D-di(2,3,4,5,6-pentabenzyl-*myo*-inositol)-1,1'-phosphate (8b)



Phenyl-L-2,3,4,5,6-pentabenzyl-*myo*-inositol-D-2',4',6'-tribenzyl-*myo*-inositol-1,1'-phosphate (45 mg, 0.037 mmol) was dissolved in benzotrifluoride (1 mL), and 2-benzyloxy-1-methylpyridinium triflate (258 mg, 0.74 mmol) was added, followed by magnesium oxide (30 mg, 0.74 mmol). The reaction was heated in a sealed tube at 90 ˚C for three days, and subsequently cooled to room temperature, filtered through Celite with toluene, and concentrated. The residue was purified by silica gel flash chromatography (gradient elution 10% - 20% ethyl acetate in hexanes containing 0.5% methanol) to yield

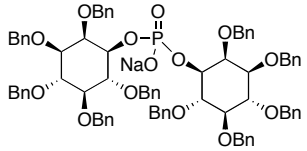
a colorless residue (18 mg, 35% yield). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.35-7.00 (m, 55H), 4.88-4.82 (m, 4H), 4.78 (t, *J* = 10.4 Hz, 4H), 4.66-4.52 (m, 14H), 4.34 (td, *J* = 2.5 Hz, *J* = 8.6 Hz, 2H), 4.31 (t, *J* = 2.2 Hz, 1H), 3.97-3.90 (m, 4H), 3.24 (dd, *J* = 2.0 Hz, *J* = 9.8 Hz, 1H), 3.02 (t, *J* = 9.2 Hz, 1H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 150.5, 139.0, 138.9, 138.7, 138.4, 138.2, 129.8, 128.9, 128.9, 128.7, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.6, 127.5, 127.5, 127.3, 127.0, 125.3, 120.2, 120.2, 82.8, 81.1, 80.3, 79.9, 79.8, 79.3, 79.2, 76.3, 75.8, 75.4, 74.9, 72.8; **^{31}P NMR** ($CDCl_3$, 202 MHz) δ -7.0; **IR** (film, cm^{-1}) 3088, 3063, 3030, 2923, 2856, 1717, 1590, 1493, 1452, 1355, 1277, 1255, 1207, 1161, 1122, 1069, 1025, 955, 734, 697; **TLC** R_f 0.5 (30% ethyl acetate/ hexanes); **$[\alpha]_D^{20}$** = 0 (*c* 1.0, $CHCl_3$); **HRMS** calcd for $[C_{88}H_{87}O_{14}PNa]^+$ requires *m/z* 1421.5731; found 1421.5721 (ESI+).

Sodium-L,L-di(2,3,4,5,6-pentabenzyl-*myo*-inositol)-1,1'-phosphate (9c)



Phenyl-L,L-di(2,3,4,5,6-pentabenzyl-*myo*-inositol)-1,1'-phosphate (18 mg, 0.013 mmol) was dissolved in THF (50 μL) and lithium hydroxide monohydrate (6 mg, 0.13 mmol) was added followed by water (50 μL). The reaction was heated to 70 ºC for 12 hours in a heavy-walled conical vial under pressure. Upon cooling to room temperature, the mixture was concentrated and purified by silica gel flash chromatography (2% methanol in methylene chloride) to afford the lithium salt as a colorless residue. The lithium salt was processed on Chelex 100 Na form resin with ethyl acetate to afford a colorless residue as the sodium salt (8 mg, 47% yield). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.28-6.96 (m, 50H), 4.92-4.81 (m, 6H), 4.70 (d, *J* = 10.8 Hz, 2H), 4.58-4.36 (m, 10H), 4.03-3.79 (m, 11H), 3.31 (d, *J* = 8.6 Hz, 2H), 2.93 (br t, 1H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 129.2, 128.9, 128.6, 128.3, 128.2, 128.1, 127.6, 127.4, 127.3; **^{31}P NMR** ($CDCl_3$, 202 MHz) δ -1.2; **IR** (film, cm^{-1}) 3063, 3030, 2957, 2922, 2851, 1724, 1671, 1604, 1492, 1453, 1359, 1257, 1208, 1128, 1070, 1025, 872, 799, 730, 696; **TLC** R_f 0.5 (10% methanol/ methylene chloride); **$[\alpha]_D^{20}$** = -7.2 (*c* 0.5, $CHCl_3$); **HRMS** calcd for $[C_{82}H_{84}O_{14}P]^+$ requires *m/z* 1323.5599; found 1323.5596 (ESI+).

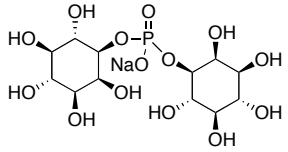
Sodium-L,D-di(2,3,4,5,6-pentabenzyl-*myo*-inositol)-1,1'-phosphate (8c)



Phenyl-L,D-di(2,3,4,5,6-pentabenzyl-*myo*-inositol)-1,1'-phosphate (18 mg, 0.013 mmol) was dissolved in THF (50 μL) and lithium hydroxide monohydrate (6 mg, 0.13 mmol) was added followed by water (50 μL), and the reaction heated to 70 ºC for 12 hours in a heavy-walled conical vial under pressure. Upon cooling to room temperature, the mixture was concentrated and purified by silica gel flash chromatography (2% methanol in methylene chloride) to afford the lithium salt as a colorless residue. The lithium salt was processed on Chelex 100 Na form resin with ethyl acetate to afford a colorless residue as

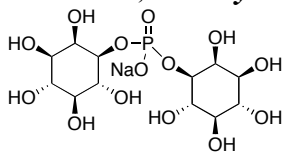
the sodium salt (12 mg, 69% yield). **^{1}H NMR** ($CDCl_3$, 500 MHz) δ 7.32-6.82 (m, 50H), 4.91-4.83 (m, 6H), 4.73-4.67 (m, 6H), 4.45-4.38 (m, 5H), 4.20 (br, 2H), 4.03-3.92 (m, 4H), 3.80 (t, *J* = 13.3 Hz, 1H), 3.32-2.88 (m, 8H); **^{13}C NMR** ($CDCl_3$, 126 MHz) δ 139.1, 138.4, 128.9, 128.6, 128.4, 128.2, 128.0, 127.8, 127.7, 127.3, 127.1, 82.8, 81.5, 80.8, 76.2, 75.7, 74.9, 72.4; **^{31}P NMR** ($CDCl_3$, 162 MHz) δ -1.5; **IR** (film, cm^{-1}) 3063, 3027, 2959, 2923, 2851, 1497, 1452, 1360, 1260, 1209, 1127, 1069, 1024, 731, 696; **TLC** R_f 0.5 (10% methanol/ methylene chloride); **$[\alpha]_D^{20}$** = 0 (*c* 0.25, $CHCl_3$); **HRMS** calcd for $[C_{82}H_{82}O_{14}P]^-$ requires *m/z* 1321.5442; found 1321.5366 (ESI-).

Sodium-L,L-di-*myo*-inositol-1,1'-phosphate



Sodium-L,L-di(2,3,4,5,6-pentabenzyl-*myo*-inositol)-1,1'-phosphate (10.4 mg, 0.008 mmol) was dissolved in ethyl acetate (150 μL) and methanol (150 μL) was added. Palladium (II) hydroxide (100 mg) was added, and the system was purged and flushed with hydrogen gas five times and then stirred for 24 hours under a hydrogen balloon (1 atm). The mixture was filtered through Celite, washing with water, and then lyophilized as the sodium salt (2.8 mg, 82% yield). **^{1}H NMR** (D_2O, 500 MHz) δ 4.28 (t, *J* = 2.7 Hz, 2H), 4.03 (ddd, *J* = 8.7 Hz, *J* = 7.0 Hz, *J* = 2.7 Hz, 2H), 3.74 (t, *J* = 9.7 Hz, 2H), 3.63 (t, *J* = 9.5 Hz, 2H), 3.54 (dd, *J* = 10.0 Hz, *J* = 2.9 Hz, 2H), 3.30 (t, *J* = 9.4 Hz, 2H); **^{13}C NMR** (D_2O, 126 MHz) δ 77.7, 77.7, 75.5, 73.7, 73.0, 73.0, 72.8, 72.2; **^{31}P NMR** (D_2O, 202 MHz) δ -1.2; **$[\alpha]_D^{20}$** = + 1.2 (*c* 0.4, D_2O; pH = 8); **HRMS** calcd for $[C_{12}H_{23}O_{14}PNa]^+$ requires m/z 445.0723; found 445.0718 (ESI+).

Sodium-L,D-di-*myo*-inositol-1,1'-phosphate



Sodium-L,D-di(2,3,4,5,6-pentabenzyl-*myo*-inositol)-1,1'-phosphate (18.9 mg, 0.014 mmol) was dissolved in ethyl acetate (200 μL) and methanol (200 μL) was added. Palladium (II) hydroxide (150 mg) was added, and the system was purged and flushed with hydrogen gas five times and then stirred for 24 hours under a hydrogen balloon (1 atm). The mixture was filtered through Celite, washing with water, and then lyophilized as the sodium salt (6.0 mg, 96% yield). **^{1}H NMR** (D_2O, 500 MHz) δ 4.30 (t, *J* = 2.7, 2H), 4.05 (ddd, *J* = 8.8 Hz, *J* = 6.7 Hz, *J* = 2.8 Hz, 2H), 3.79 (t, *J* = 9.7 Hz, 2H), 3.66 (t, *J* = 9.4 Hz, 2H), 3.57 (dd, *J* = 10.0 Hz, *J* = 2.8 Hz, 2H), 3.34 (t, *J* = 9.4 Hz, 2H); **^{13}C NMR** (D_2O, 126 MHz) δ 78.0, 78.0, 75.4, 73.7, 72.9, 72.9, 72.7, 72.1; **^{31}P NMR** (D_2O, 202 MHz) δ -1.3; **$[\alpha]_D^{20}$** = 0 (*c* 0.4, D_2O; pH = 8); **HRMS** calcd for $[C_{12}H_{23}O_{14}PNa]^+$ requires m/z 445.0723; found 445.0719 (ESI+).

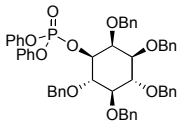
Phosphatase Assays. Recombinant *A. fulgidus* IMPase, expressed and purified as described previously[6], in 50 mM Tris HCl, pH 8.0, was placed in 200 μl PCR tubes to

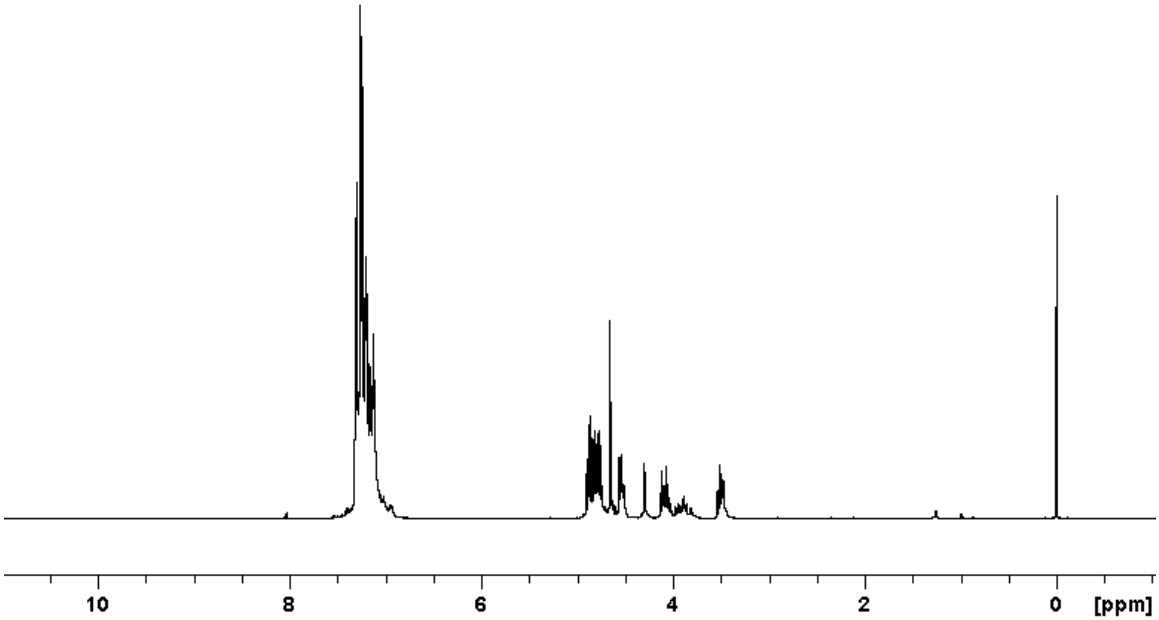
which different compatible solutes (DIP, KCl, NaCl, K^+-glutamate or Na^+-glutamate) were added. The total volume was 50 µl, and the final concentration of protein was 52 µg/ml (determined by Lowry Assay). Each solution was heated at 95°C in the PCR machine (GeneAmp PCR System 9700, PE Applied Biosystems). After 15 min, the samples were removed and kept on ice prior to measuring residual catalytic activity.

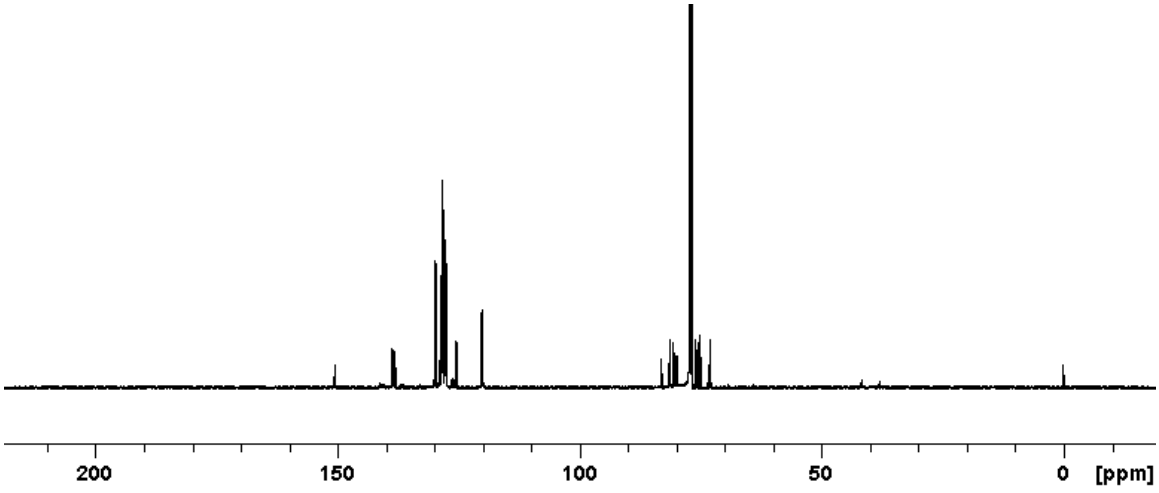
A. fulgidus IMPase specific activity was measured towards D-I-1-P (0.5 mM) at 85°C with a colorimetric phosphate assay[7]. Assay mixtures contained the substrate in 50 mM Tris-HCl, pH 8.0, with 1 mM EDTA and 5 mM Mg^{2+} in a total volume of 50 µl. The amount of enzyme added was adjusted to give 5-20% conversion of substrate to inorganic phosphate (Pi) during the 5 min incubation at 85°C (for enzyme not subjected to heating, 0.31µg was added). After incubation, the reaction mixtures were placed on ice and 1 mL of Malachite green reagent added to quench the reaction. The absorbance at 660 nm was measured and compared with the standard Pi samples to determine the reaction rate. The residual activity was calculated by comparing the specific activity from each assay to what was observed after heating at 95°C for 15 min in the absence of any thermoprotective agents. Errors in specific activities were usually <20%.

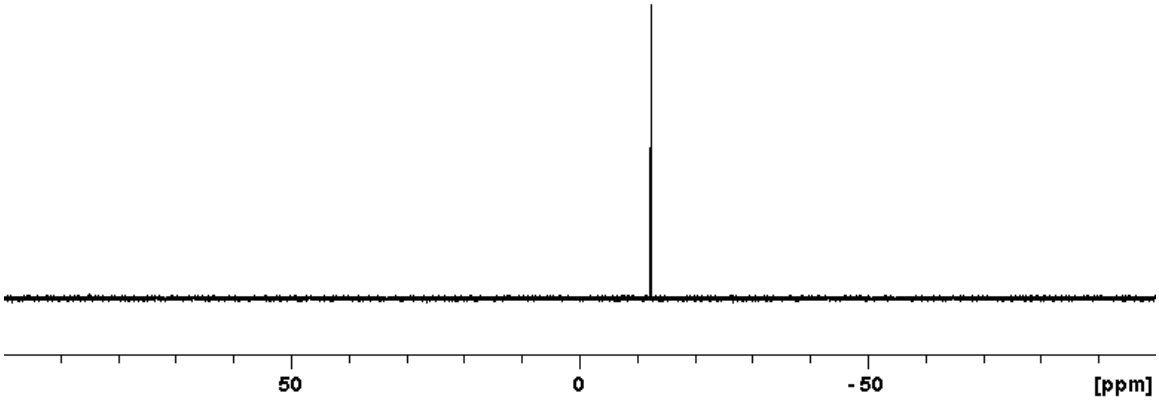
Table. Ability of solutes (100 mM) to protect *A. fulgidus* IMPase from thermal inactivation after heating at 95°C for 15 min.

Solute	Residual Activity
–	0.030±0.003
Na^+ L,L-DIP	0.17±0.04
Na^+ L,D-DIP	0.11±0.01
NaCl	0.035±0.005
Na^+-glutamate	0.035±0.005
Na^+-dimethylphosphate (DMP)	0.043±0.003
KCl	0.18±0.01
K^+-glutamate	0.12±0.01
myo-inositol	0.051±0.006

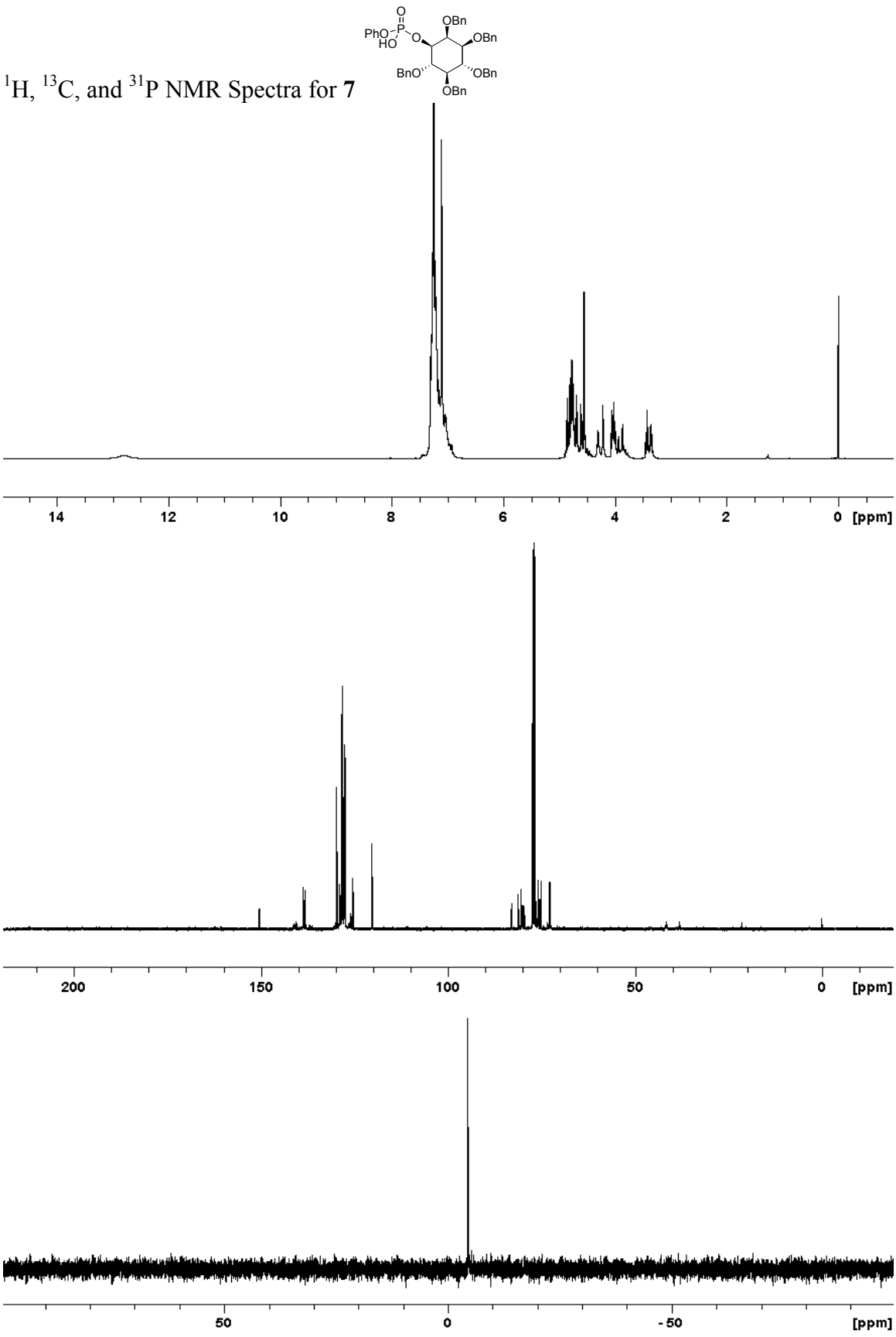
^{1}H, ^{13}C, and ^{31}P NMR Spectra for 



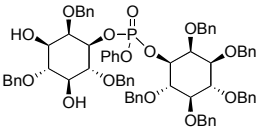


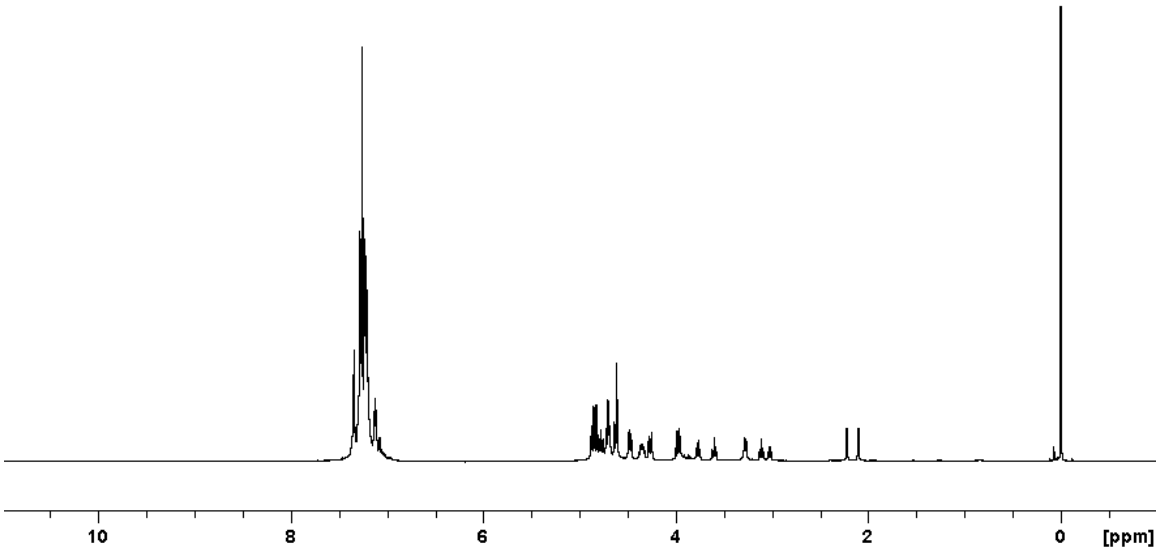


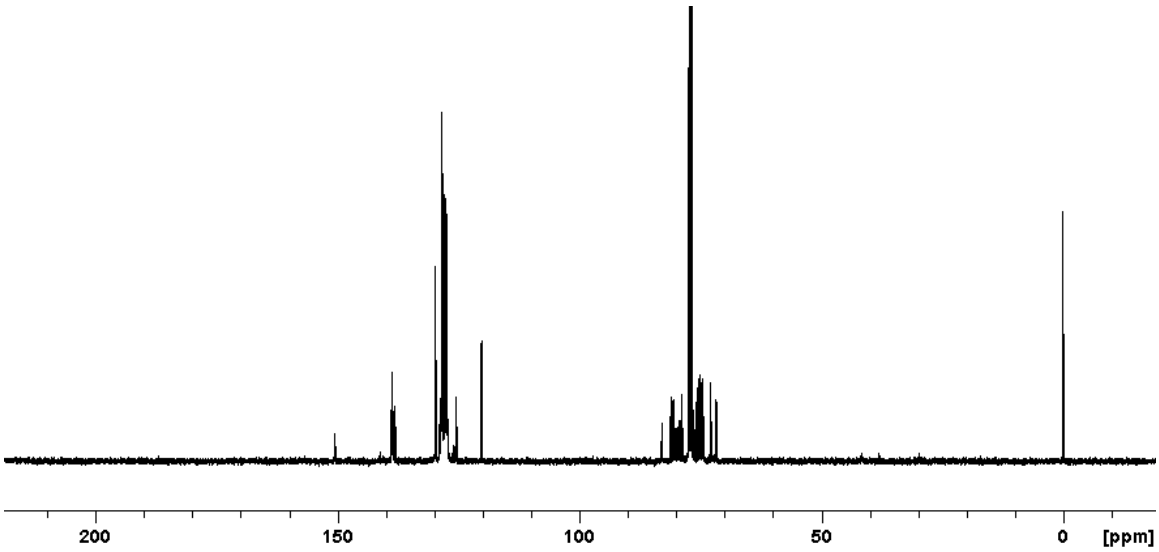
^{1}H, ^{13}C, and ^{31}P NMR Spectra for **7**

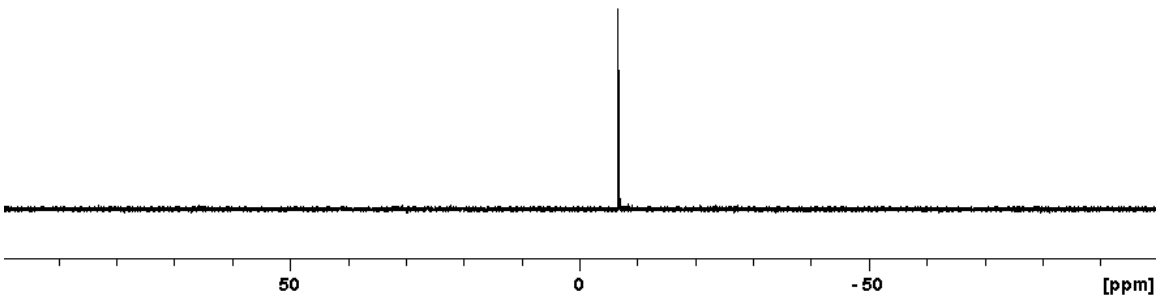


^{1}H, ^{13}C, and ^{31}P NMR Spectra for **8a**

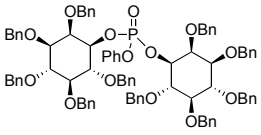


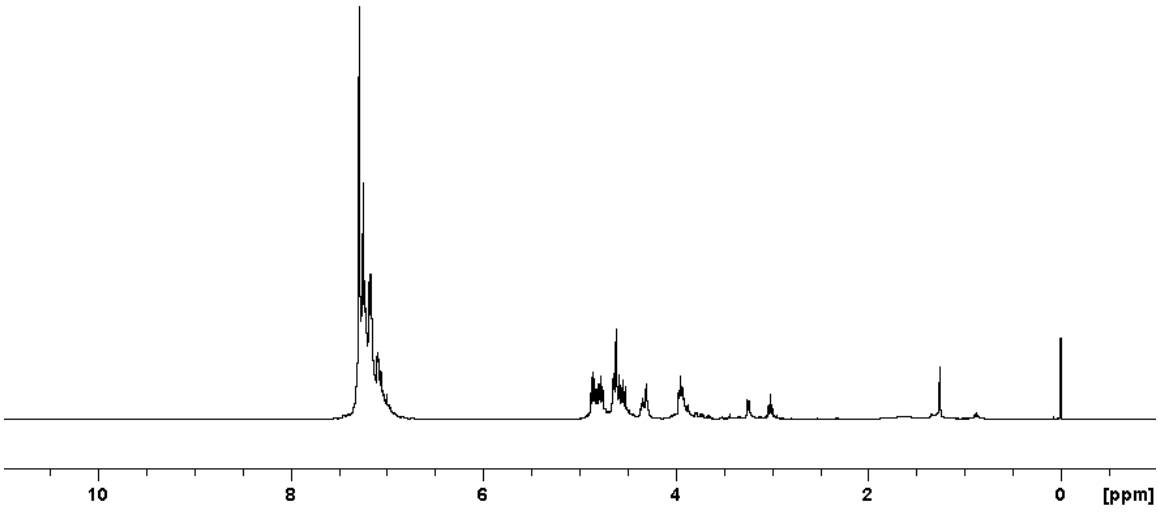


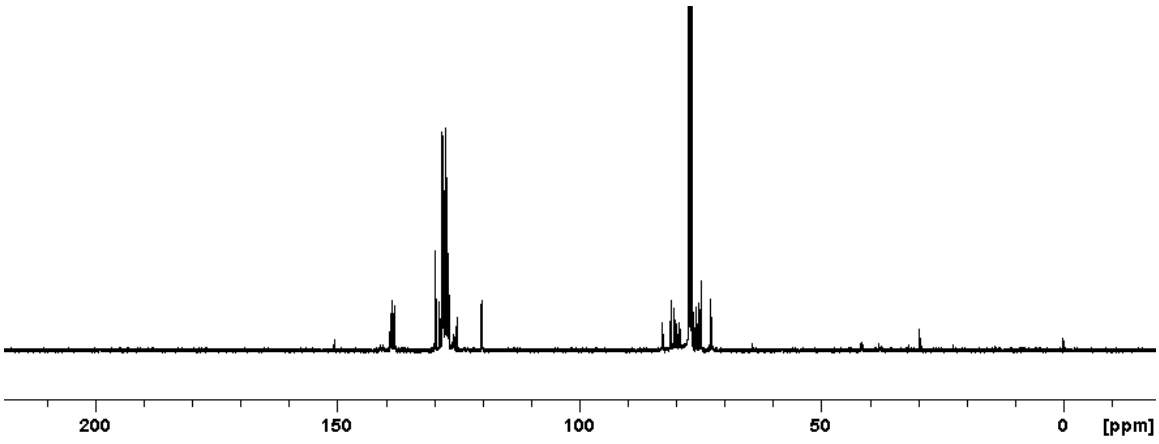


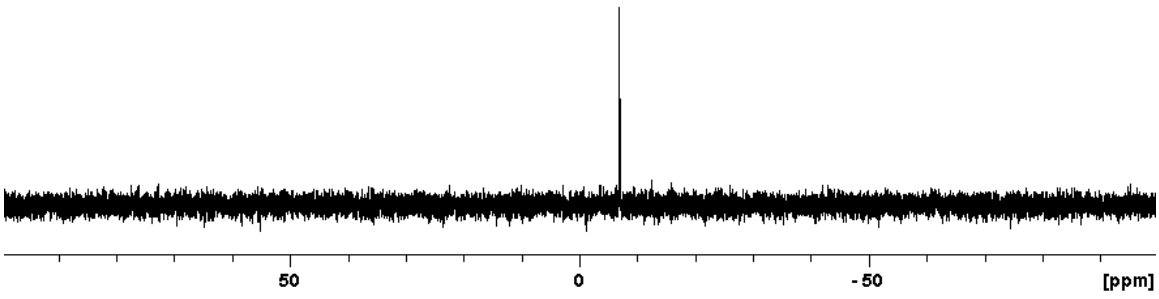


^{1}H, ^{13}C, and ^{31}P NMR Spectra for **8b**

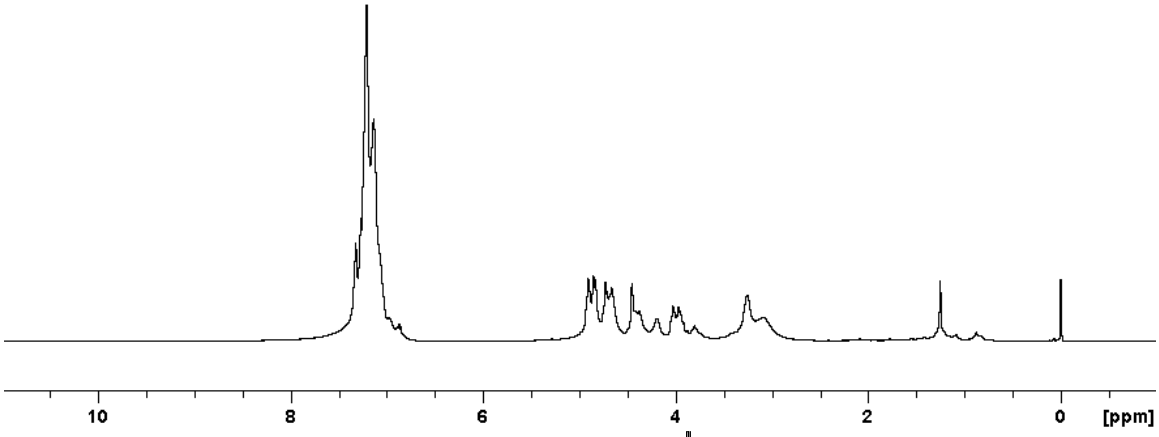


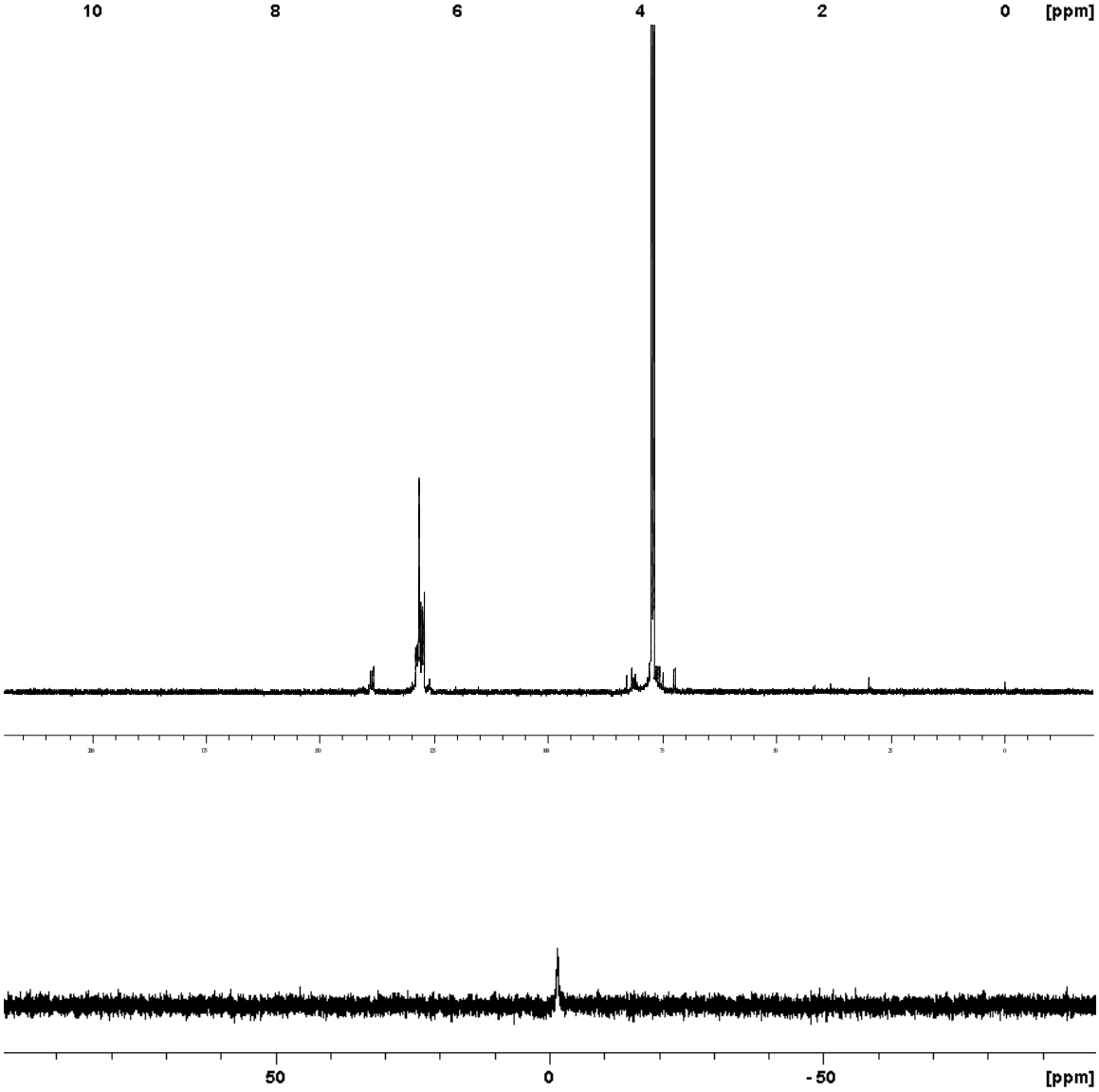


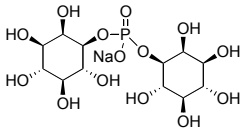


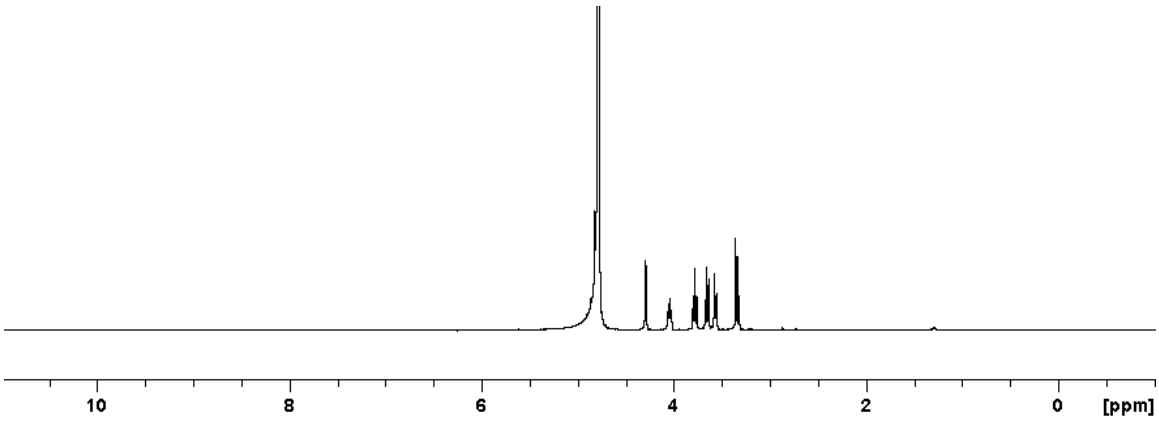


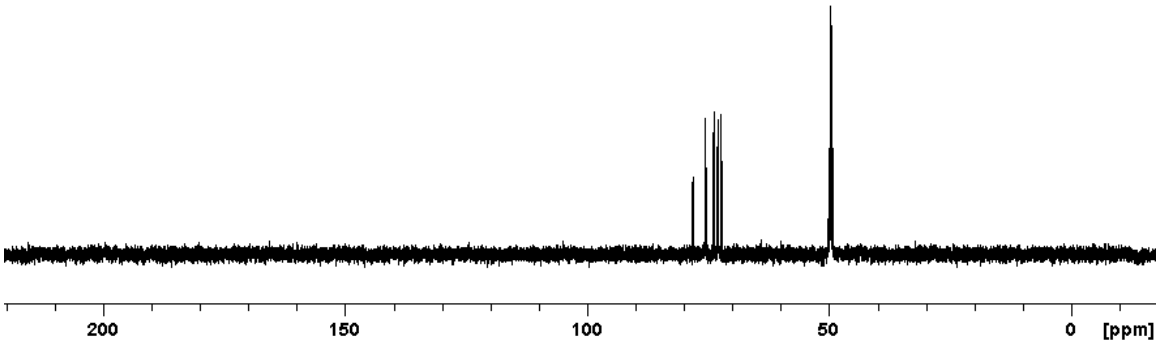
^{1}H, ^{13}C, and ^{31}P NMR Spectra for **8c**

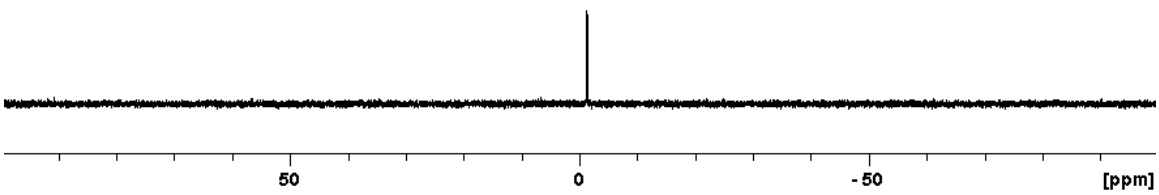




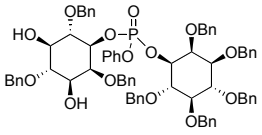
^{1}H, ^{13}C, and ^{31}P NMR Spectra for 

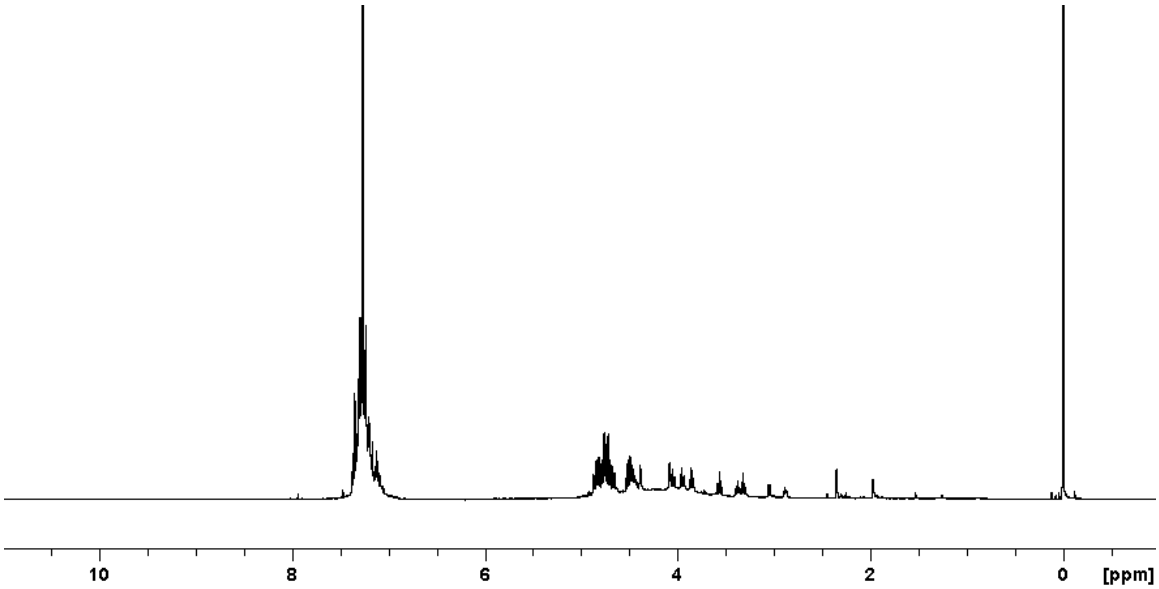


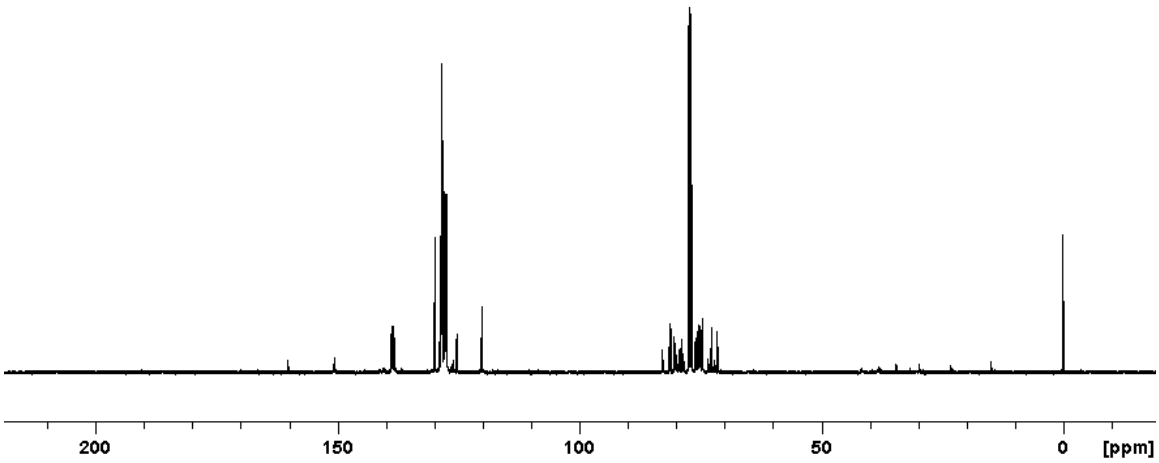


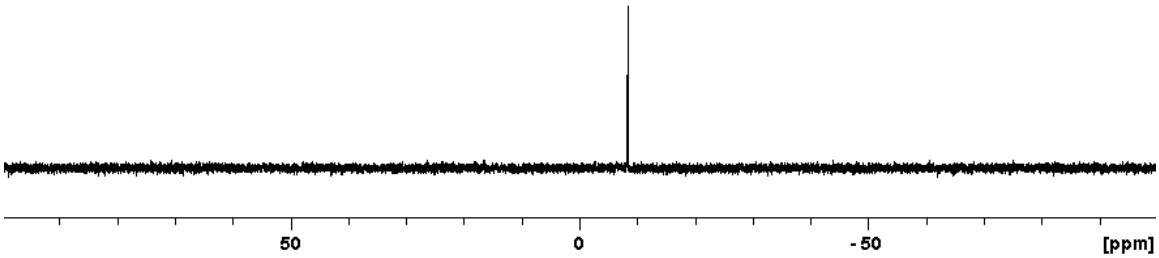


^{1}H, ^{13}C, and ^{31}P NMR Spectra for **9a**

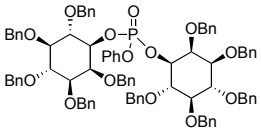


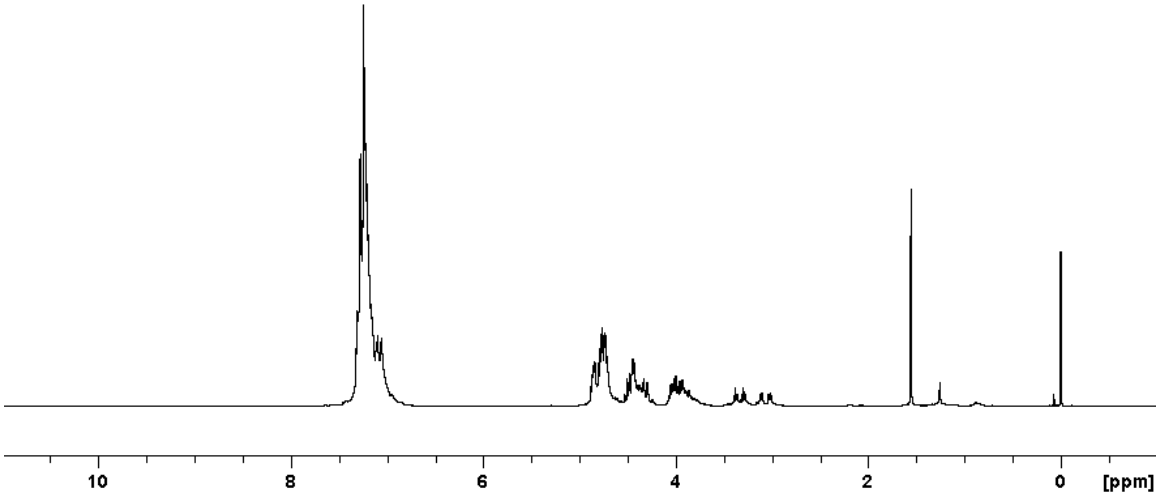


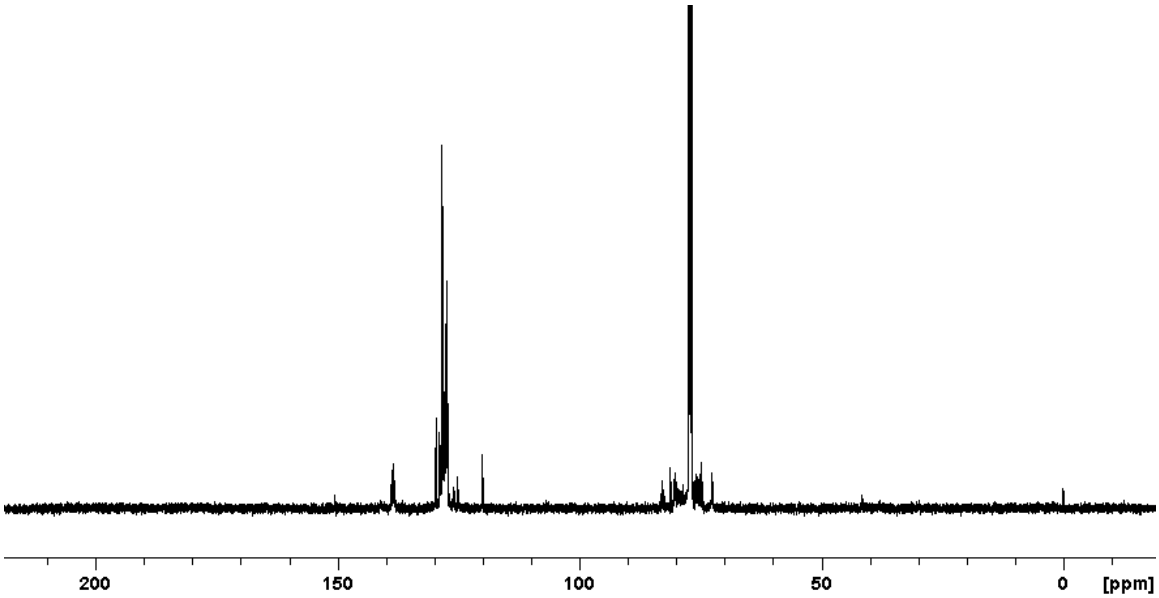


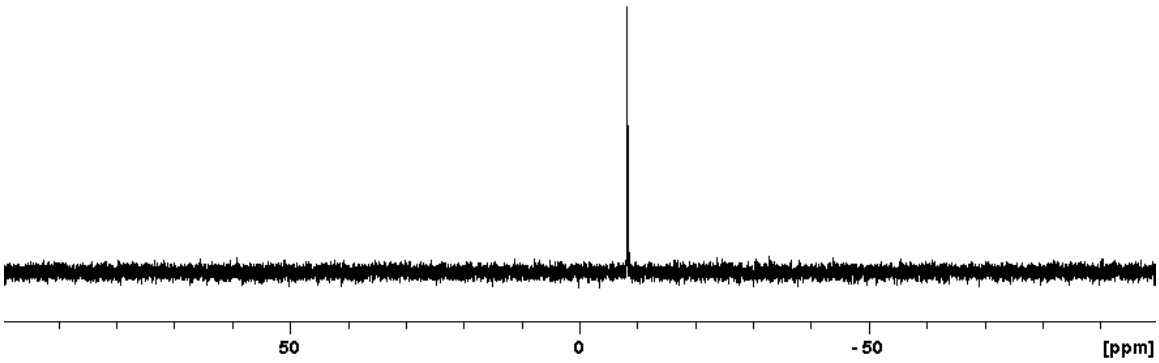


^{1}H, ^{13}C, and ^{31}P NMR Spectra for **9b**

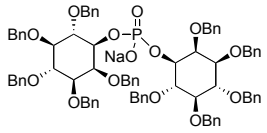


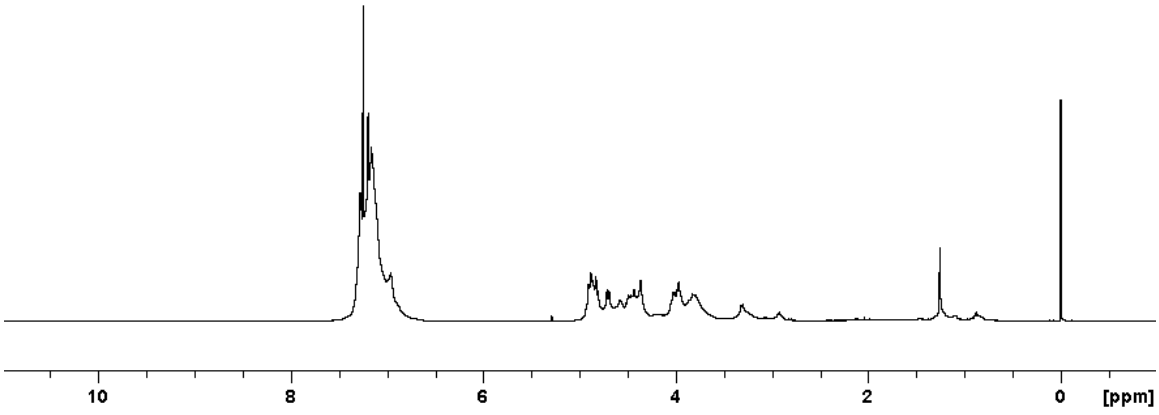


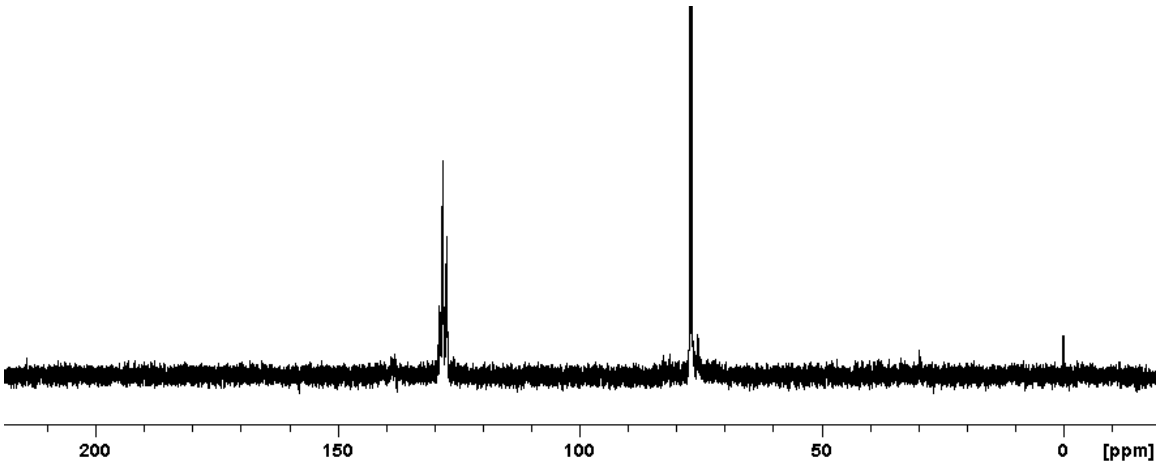


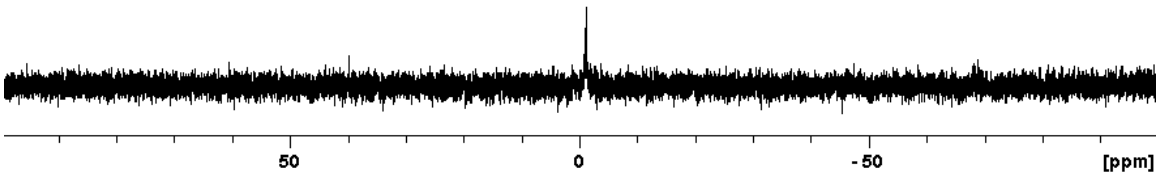


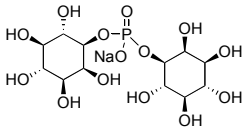
^{1}H, ^{13}C, and ^{31}P NMR Spectra for **9c**

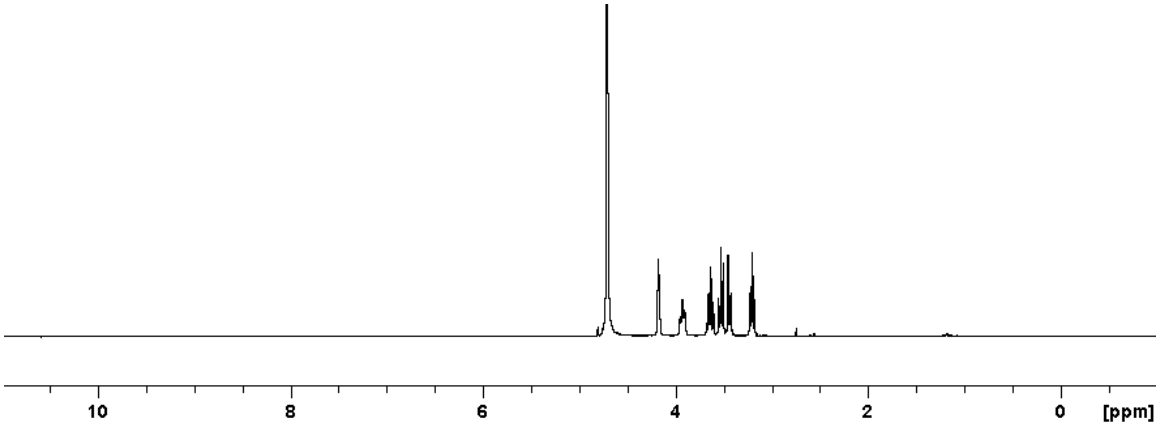


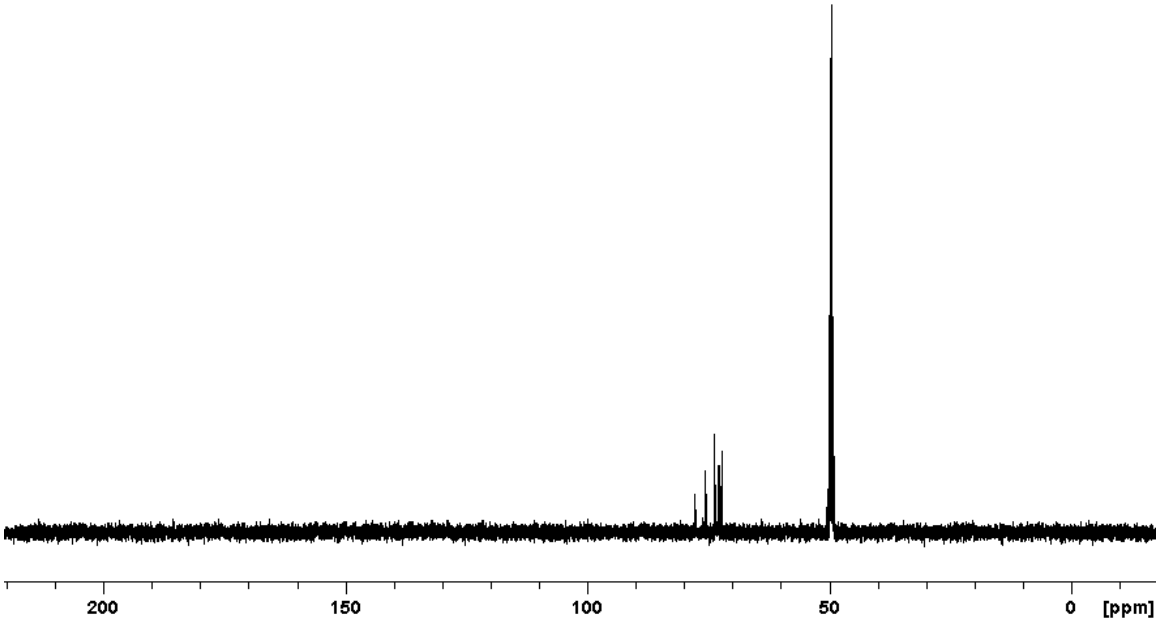


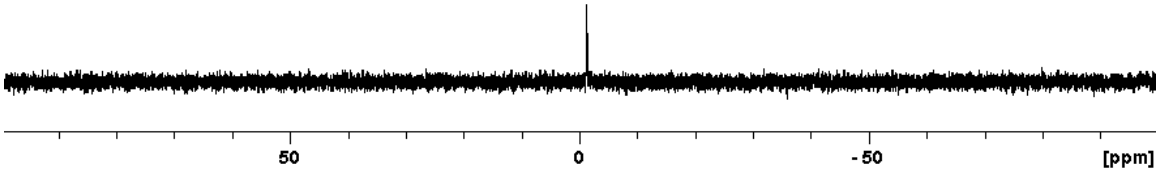


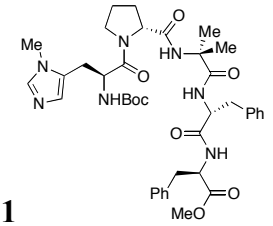


^{1}H, ^{13}C, and ^{31}P NMR Spectra for 

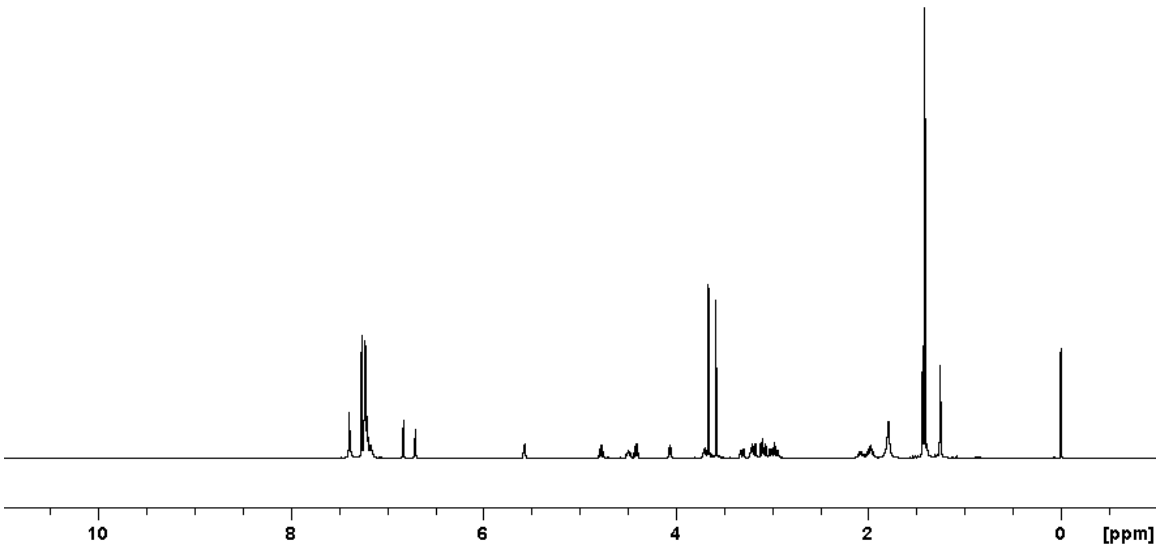








^{1}H, and ^{13}C NMR Spectra for peptide **11**



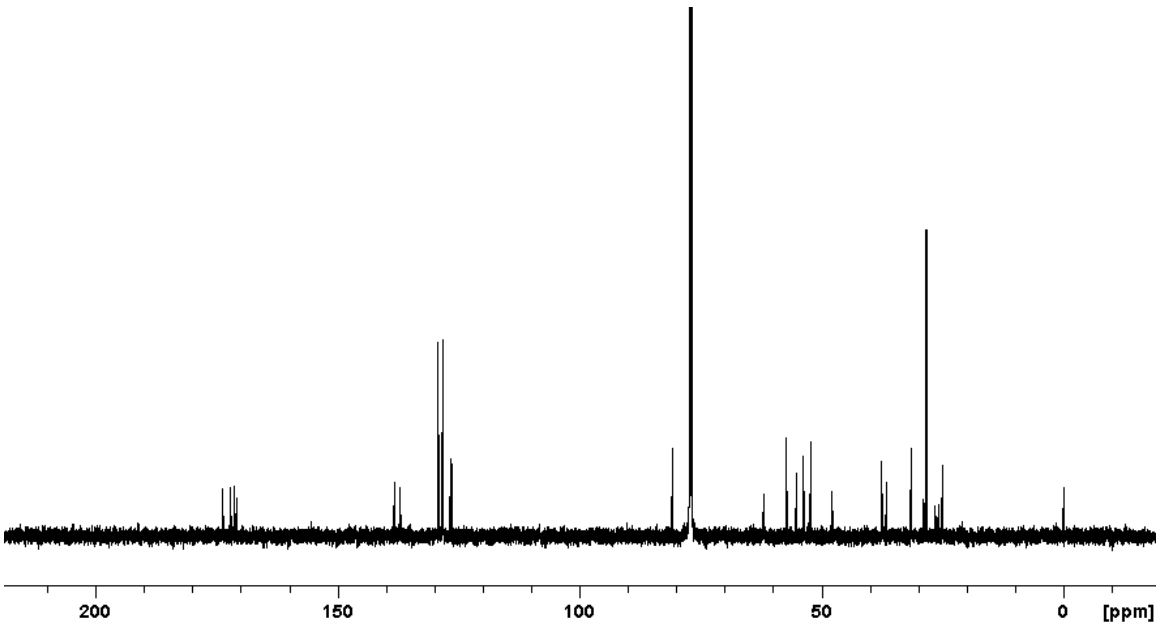


Table 1.

Catalyst	Sequence	8a	9a
A	DMAP	3	1
B	NMI	4	1
C	Boc-Pmh-OMe	3	1
D (**4**)	Boc-Pmh-Hyp(*t*Bu)-Sp5-Tyr(*t*Bu)-Phe-OMe	3.6	1
E (**5**)	Boc -Pmh-Asn(trt)-His(*t*Bu)-Asp(O*t*Bu)-Ala-OMe	1	1.25
F (**10**)	Boc -D-Pmh-Pro-Aib-Trp(Boc)-Phe-OMe	13	1
G	Boc -Pmh-D-Pro-Aib-D-Trp(Boc)-D-Phe-OMe	1	1.2
H	Boc -Pmh-D-Pro-Abu-Phe-D-Phe-OMe	1.4	1
I	Boc -Pmh-D-Pro-His(Bzl)-Phe-D-Phe-OMe	3.6	1
J	Boc -Pmh-D-Pro-D-Val-Phe-D-Phe-OMe	2.5	1
K	Boc -Pmh-D-Pip-Aib-Trp(Boc)-D-Phe-OMe	5	1
L	Boc -Pmh-D-Pip-Leu-Phe-D-Phe-OMe	1	1
M	Boc -Pmh-Asn(Trt)-Aib-Ser(*t*Bu)-D-Tyr(*t*Bu)-Ala-OMe	1	1
N	Boc -Pmh-His(Trt)-Aib-Ser(*t*Bu)-D-Tyr(*t*Bu)-Phg-OMe	1	1
O	Boc -Pmh-Asn(Trt)-Ala-Ser(*t*Bu)-D-Phe-Phe-OMe	1.8	1
P	Boc -Pmh-D-Pro-D-Asp(O*t*Bu)-Tyr(OBn)-D-Phe-OMe	2	1
Q	Boc -Pmh-Thr(*t*Bu)-D-Val-His(trt)-D-Phe-D-Val-Thr(*t*Bu)-Ile-OMe	1	1
R	Boc -Pmh-D-Pro-Asp(O*t*Bu)-Phe-D-Phe-OMe	2	1
S	Boc -Pmh-D-Pro-D-Hfe-Phe-D-Phe-OMe	3	1
T (**11**)	Boc -Pmh-D-Pro-Aib-D-Phe-D-Phe-OMe	1	2.5

Product ratios determined by ^{31}P NMR.

[1] Billington, D. C.; Baker, R.; Kulagowski, J. J.; Mawer, I. M.; Vacca, J. P.; deSolms, S. J.; Huff, J. R. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1423.

[2] Sculimbrene, B. R.; Morgan, A. J.; Miller, S. J. *J. Am. Chem. Soc*. **2002**, *124*, 11653.

[3] Poon, K. W. C.; Dudley, G. B. *J. Org. Chem*. **2006**, *71*, 3923.

[4] Imbriglio, J.; Vasbinder, M. M.; Miller, S. J. *Org. Lett*. **2003,** *5*, 3741. Sculimbrene, B. R.; Morgan, A. J.; Miller, S. J. *J. Am. Chem. Soc*. **2002**, *124*, 11653. Sculimbene, B. R.; Miller, S. J. *J. Am. Chem. Soc*. **2001**, *123*, 10125. Lewis, C. A.; Merkel, J.; Miller, S. J. *Bioorg. Med. Chem. Lett*. **2008**, ASAP. doi:10.1016/j.bmcl.2008.09.019.

[5] Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: Berlin, 1993.

[6] Stieglitz, K.A.; Johnson, K.A.; Yang, H.; Roberts, M.F.; Seaton, B.A.; Head, J.F.; Stec, B. *J. Biol. Chem*. **2002**, *277*, 22863.

[7] Itaya, K.; Ui, M. *Clin. Chim. Acta* **1966**, *14*, 361.