Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Supplementary Information for

Synthesis and Glycosidase Inhibitory Activity of Noeurostegine – a New and Potent Inhibitor of β-Glucoside Hydrolases

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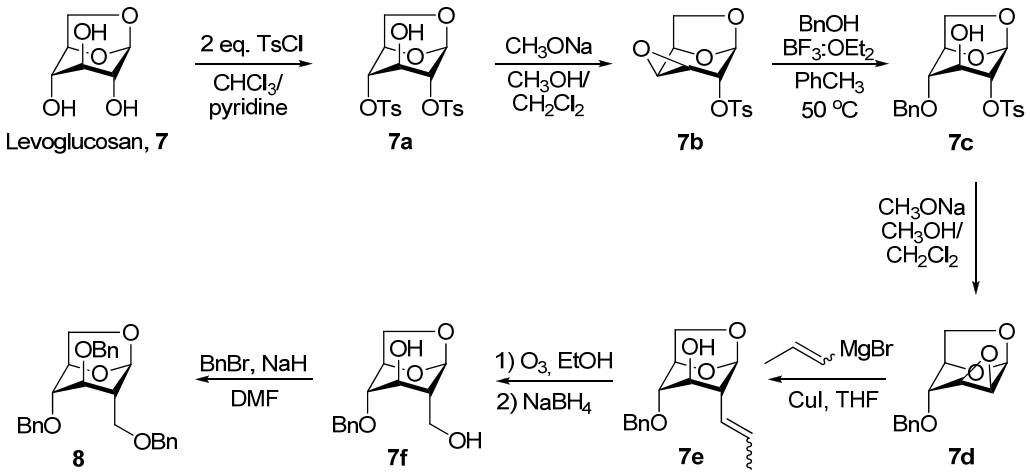
Streamlined synthesis of 8 from levoglucosan (7)

Levoglucosan (**7**) was treated with two equivalents of tosyl chloride[1,2] to predominantly give the di-tosylate **7a** along with a small proportion of the tri-tosylate which could be removed by crystallisation further down the synthetic sequence (Scheme A). The di-tosylate was then treated with methoxide to exclusively give the 3,4-epoxide (**7b**),[1] which subsequently was trans-diaxially opened under Lewis acidic conditions with benzyl alcohol to give alcohol **7c**.[1,3] This was converted into the 2,3-epoxide (**7d**) under alkaline conditions, which underwent copper(I) catalysed opening with a freshly prepared vinylogous Grignard reagent to give **7e**. This procedure proved far superior in terms of yield and cleanliness of reaction compared to the known procedure using commercial vinyl magnesiumbromide in refluxing THF.[4] Alkene ozonolysis followed by sodium borohydride reduction gave diol **7f**,[4] which subsequently underwent benzyl protection with benzyl bromide under basic conditions.[5] This gave tri-benzylated anhydrosugar **8** over eight steps in a yield of 28%. This series of reactions could be carried out on multigram scale without the need for intermediate chromatographic purification.



Scheme A. Synthesis of intermediate 8 from levoglucosan (7).

Experimental Section

1,6-Anhydro-2,4-di-*O*-*p*-tolylsulfonyl-β-D-glucopyranoside (7a)

To a cooled, stirred solution of levoglucosan (**7**) (14.95 g, 92.19 mmol) in dry pyridine (75 mL) was added a solution of *p*-toluenesulfonyl chloride (35.19 g, 184.55 mmol, 2.0 eq) in a mixture of dry pyridine (70 mL) and chloroform (100 mL) under an atmosphere of nitrogen.[2] The reaction mixture was stirred overnight during which it was allowed to reach rt. The reaction mixture was quenched with water (50 mL) and diluted with CH_2Cl_2 (200 mL). The organic phase was washed with 10 % aqueous H_2SO_4 (5 x 100 mL), dried over $MgSO_4$ and concentrated to give the product **7a** (45.64 g) as a colourless foam. NMR-analysis indicated >90% purity and the material was accordingly used directly for the next reaction. NMR data was in accordance with previously reported values.[6]

1,6:3,4-Dianhydro-2-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside (7b)

Sodium (2.73 g, 0.12 mol, 1.3 eq) was dissolved in dry MeOH (100 mL) and the resulting solution added to crude **7a** (43.37 g) in dry CH_2Cl_2 (150 mL) at 0 °C under an atmosphere of nitrogen. The reaction mixture was allowed to reach rt and then stirred for 1 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and the organic phase washed with water (3 x 150 mL), dried over $MgSO_4$ and concentrated. Crude **7b** (26.19 g) was isolated after workup

and used directly for the next reaction. Purity, as found by NMR analysis was found to be >90%. NMR data was in accordance with previously reported values.[6]

1,6-Anhydro-4-*O*-benzyl-2-*O*-*p*-tolylsulfonyl-β-D-glucopyranoside (7c)

To a solution of **7b** (26.19 g) in dry toluene (150 mL) was added benzyl alcohol (27.5 mL, 0.26 mol) and distilled boron trifluoride etherate (2.22 mL, 17.60 mmol, 0.2 eq) under an atmosphere of nitrogen. The reaction mixture was stirred at 50 °C overnight. The cooled solution was washed with a saturated aq. solution of $NaHCO_3$ (2 x 150 mL), water (2 x 150 mL), dried over $MgSO_4$ and concentrated. The solid crude product was washed with ether and filtered to remove un-reacted benzyl alcohol which gave the product **7c** as pure, small colourless crystals. These were used directly in the next reaction without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.82 (d, 2 H, J = 8.0 Hz, ArH), 7.38-7.28 (m, 7 H, ArH), 5.33 (s, 1 H, H1), 4.69 (d, 1 H, J_{gem} = 12.0 Hz, C$\underline{H}_2$Ph), 4.61 (d, 1 H, J_{gem} = 12.0 Hz, C$\underline{H}_2$Ph), 5.57 (m, 1 H, H5), 4.21 (d, 1 H, $J_{2,3}$ = 4.0 Hz, H2), 3.97 (t, 1 H, $J_{3,4;4,5}$ = 4.0 Hz, H4), 3.84 (d, 1 H, J_{gem} = 7.6 Hz, H6), 3.63 (dd, 1 H, $J_{5,6}$ = 5.6 Hz, J_{gem} = 7.6 Hz, H6´), 3.33 (d, 1H, $J_{3,4}$= 4.0 Hz, H3), 2.44 (s, 3 H, Ar-C$\underline{H}_3$), 1.60 (bs, 1 H, OH). NMR data was in accordance with previously reported data.[3]

1,6;2,3-Dianhydro-4-*O*-benzyl-β-D-mannopyranoside (7d)

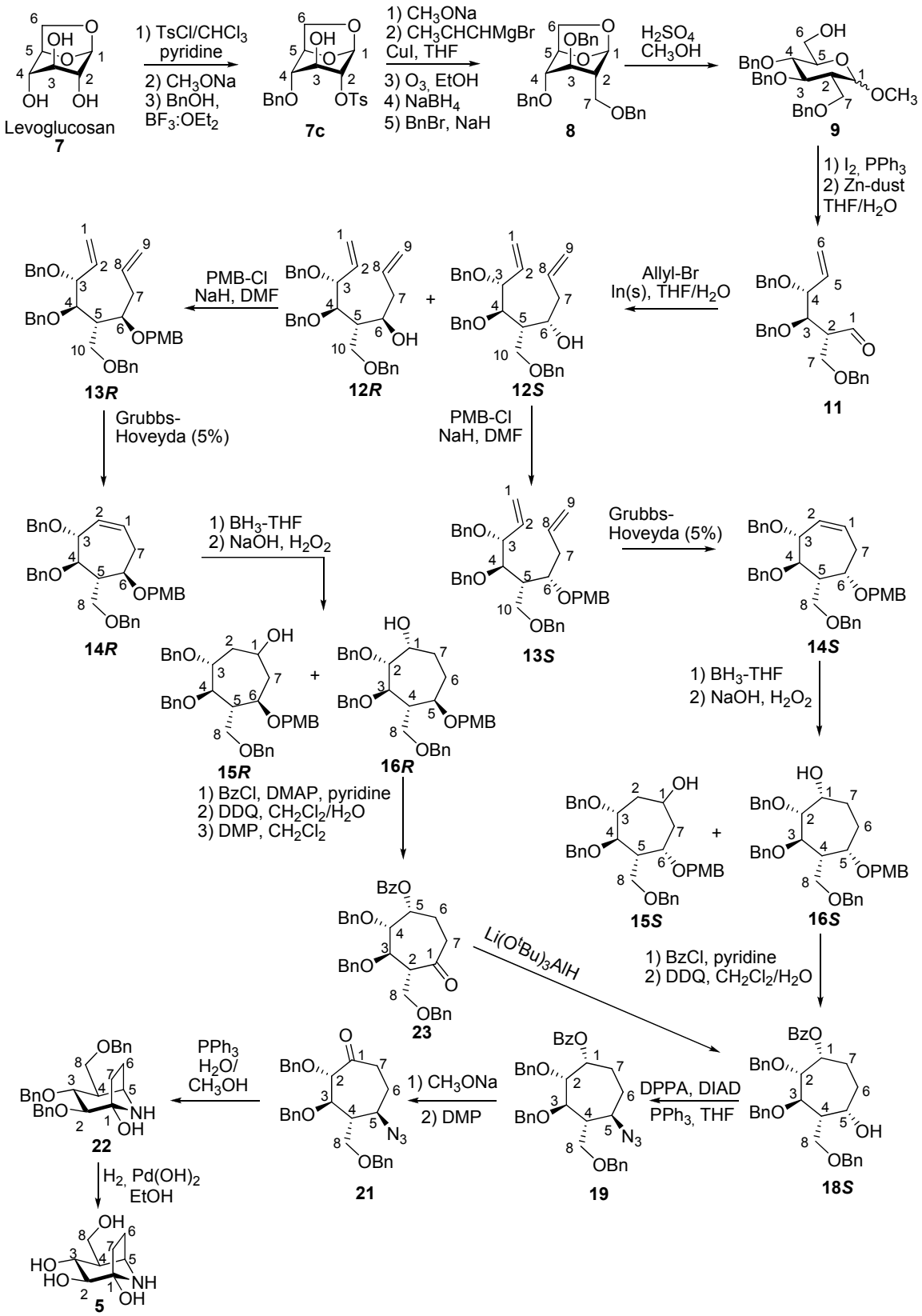
Sodium (1.46 g, 63.5 mmol, 1.3 eq) was dissolved in dry MeOH (60 mL) and the resulting solution added to **7c** (19.41 g) in dry CH_2Cl_2 (100 mL) at 0 °C under an atmosphere of nitrogen. The reaction mixture was allowed to reach rt and then stirred for 90 min. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and the organic phase was washed with water (3 x 150 mL), dried over $MgSO_4$ and concentrated. **7d** (11.59 g) was isolated after workup and used directly for the next reaction. Purity was found to be >90% by NMR analysis and spectral values in accordance with literature data.[7]

1,6-Anhydro-3,4-di-*O*-benzyl-2-*C*-benzyloxymethyl-2-deoxy-β-D-glucopyranoside (7e)

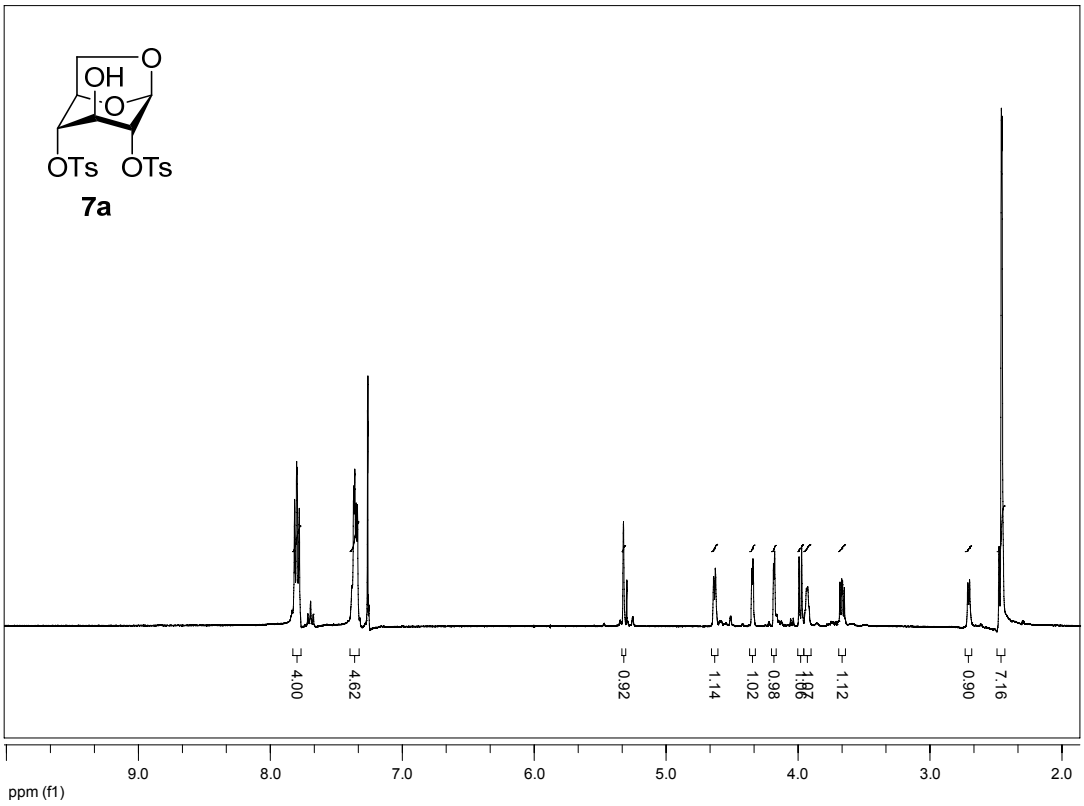
Freshly distilled THF (300 mL) and 1,2-dibromoethane (0.7 mL) was added to oven-dried Mg-turnings (17.49 g, 0.72 mol, 1.5 eq) in a three-necked round bottomed flask equipped

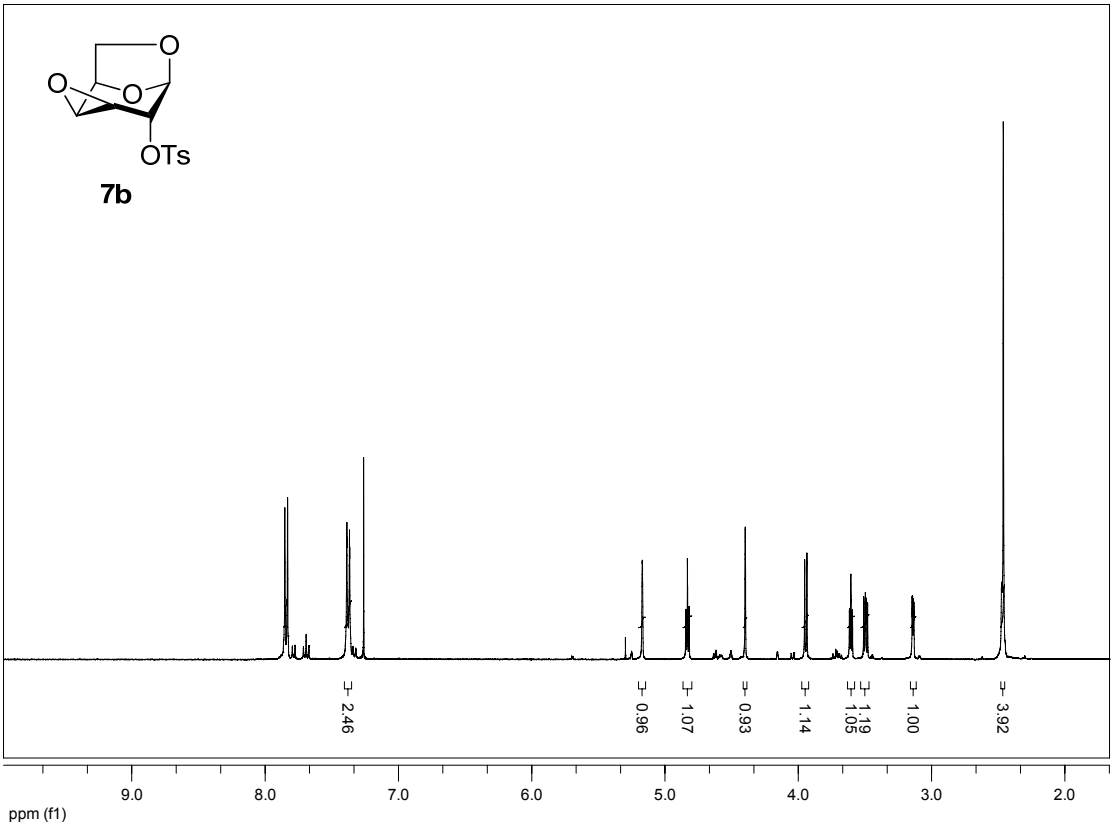
with a condenser. Localised heating (heat gun) was provided until the reaction mixture started to bubble (ethene formation). Then, cis/trans 1-bromo-1-propen (41.0 mL, 0.48 mol) was added at a rate to keep the reaction mixture at a gentle reflux. After complete addition the reaction mixture was refluxed for 90 min. during which the reaction was monitored by titration with menthol[8] to finally give a concentration of 1.24 M. The Grignard reagent was transferred by cannula into a dry round bottomed flask to make a stock solution. A solution of freshly the prepared Grignard reagent (350 mL, 0.43 mol, 9.0 eq) was then added to a flask containing CuI (0.943 g, 4.95 mmol, 0.1 eq) in dry ether (100 mL) at -78 °C. After 30 min, a solution of epoxide **7d** (11.59 g, 49.47 mmol) in dry ether was added to the stirred solution at -78 °C. The reaction mixture was allowed to reach rt and stirred overnight before quenched with a saturated aq. solution of NH_4Cl (200 mL). The layers were separated, and the aqueous phase extracted with ether (3 x 150 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The resulting product **7e** (13.70 g) was dissolved in ethanol (150 mL) and cooled to 0 °C. A stream of ozone was passed through this solution for 90 min., while keeping the temperature at 0 °C before a stream of O_2 and then nitrogen was passed through the solution. Sodium borohydride (15.05 g, 0.40 mol, 8.0 eq) was slowly added to the cooled and stirred solution of the ozonide. The reaction mixture was kept at 0 °C for 20 min, and then allowed to reach rt. After stirring for 3 h, Amberlite IR 120, H^+ (200 mL) was added and removed by filtration and rinsed with methanol after 30 min of stirring. The solution was concentrated leaving a colourless syrup of **7f**, which was used directly for the next reaction. Sodium hydride (60 %, 11.93 g, 0.30 mol, 6.0 eq) was added to a cooled (0 °C) and stirred solution of crude **7f** (13.20 g) in dry DMF (100 mL) under nitrogen atmosphere. Benzyl bromide (23.70 mL, 0.20 mol, 4.0 eq) was added to the mixture after 10 min and the reaction mixture was stirred overnight before quenched by addition of methanol (30 mL) and diluted with EtOAc (200 mL). The organic layer was washed with water (3 x 150 mL), dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash column chromatography (pentane/EtOAc 20:1→15:1→10:1) to give the product **8** (11.64 g, 28 % (8 steps, starting from levoglucosan)) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.34-7.26 (m, 15 H, ArH), 5.56 (s, 1 H, H1), 4.58-4.41 (m, 7 H, C$\underline{H}_2$Ph, H5), 4.12 (d, 1 H, J_{gem} = 7.2 Hz, H6), 3.72 (t, 1 H, $J_{gem;5,6'}$ = 6.4 Hz, H6), 3.62 (dd, 1 H, J_{gem} = 9.2 Hz, $J_{2,7}$ = 7.2 Hz, H7), 3.53-3.47 (m, 2 H, H3, H7'), 3.38 (bs, 1 H, H4), 2.24 (t, 1H, $J_{2,7;2,7'}$ = 7.2 Hz, H2). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 138.2, 138.1, 137.8 (ArC), 128.4 - 127.6 (m, ArC), 101.1, 76.9, 74.4, 73.4, 73.2, 71.2, 70.9, 69.1, 64.7, 43.6. NMR data was in accordance with previously reported data.[9]

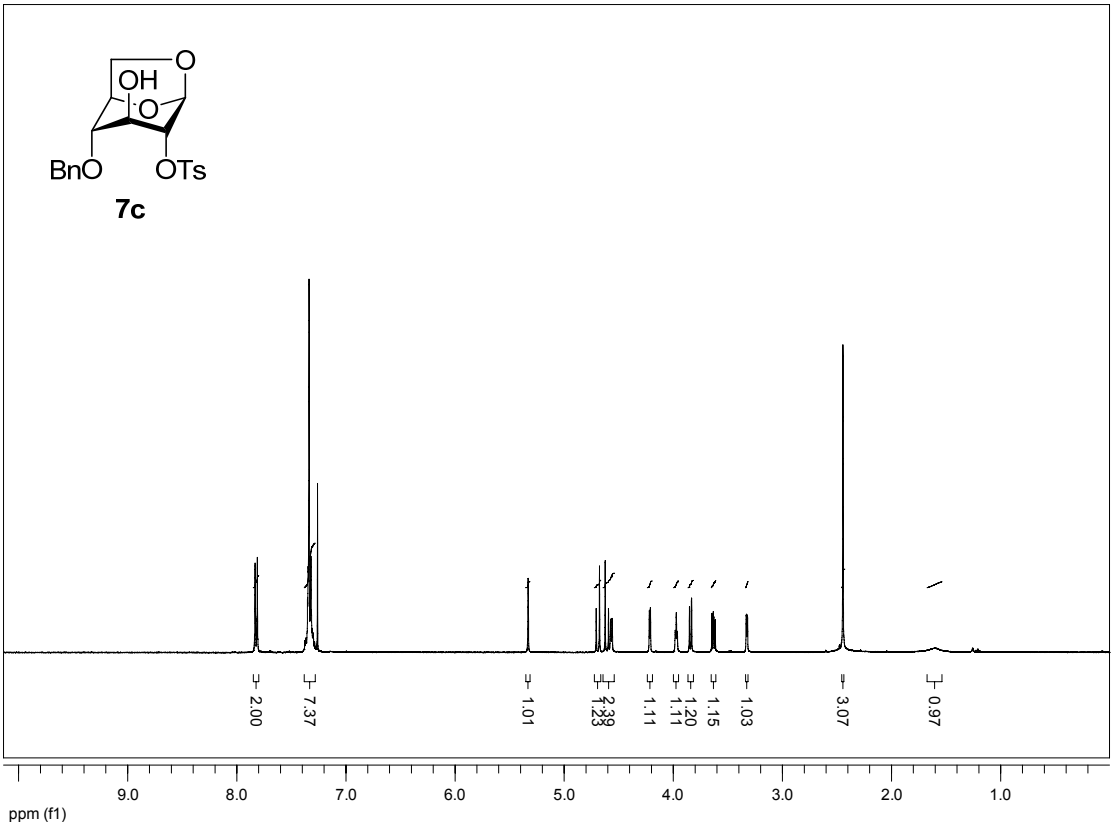
Compound numbering for NMR assignments.



NMR Spectra







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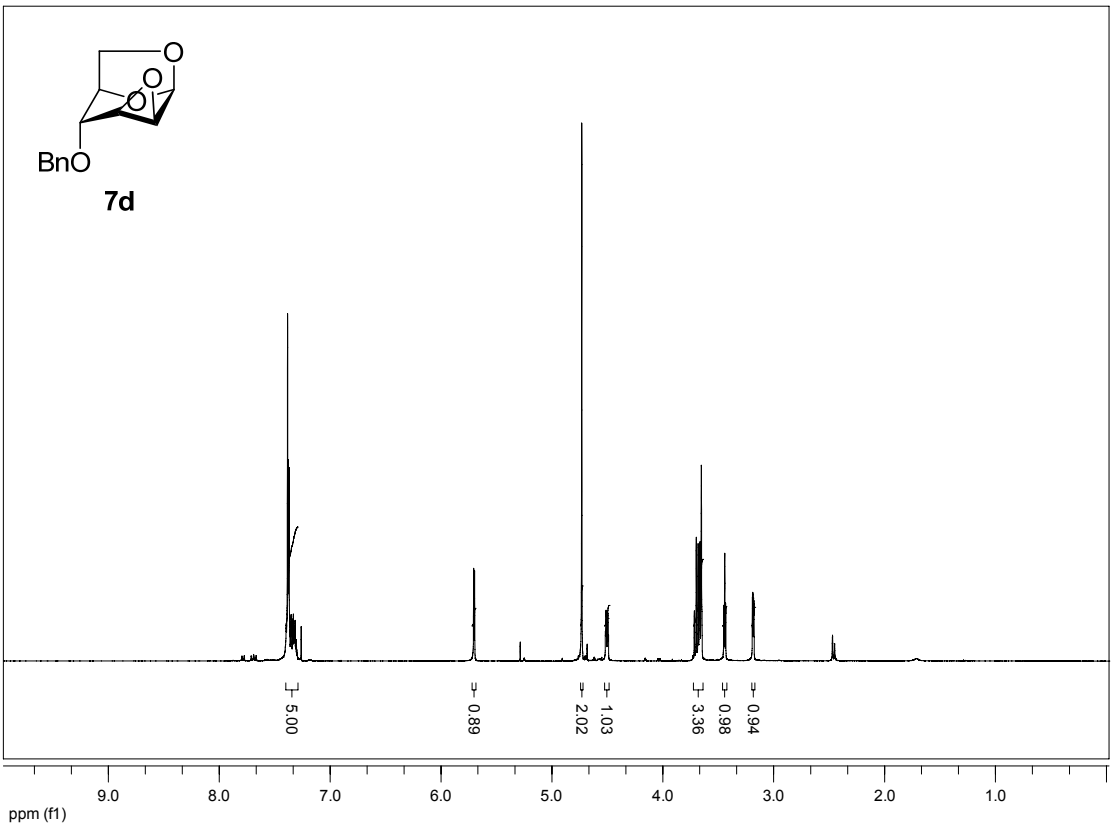
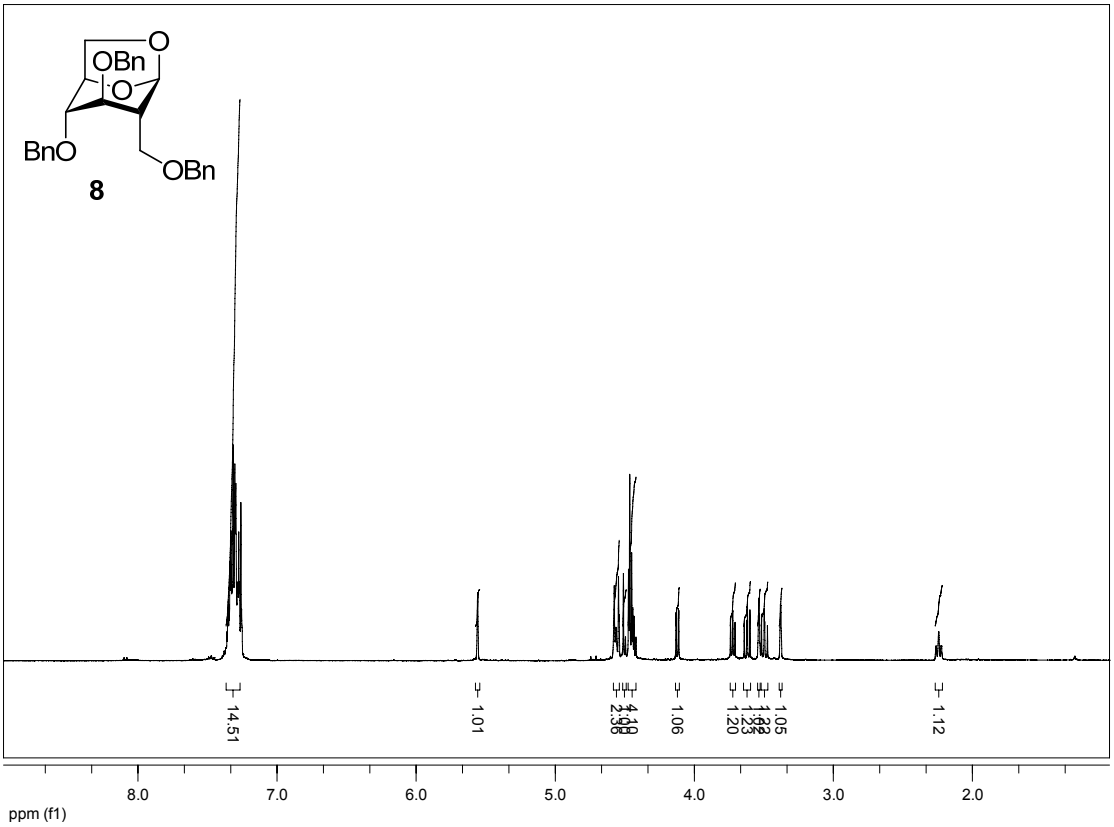
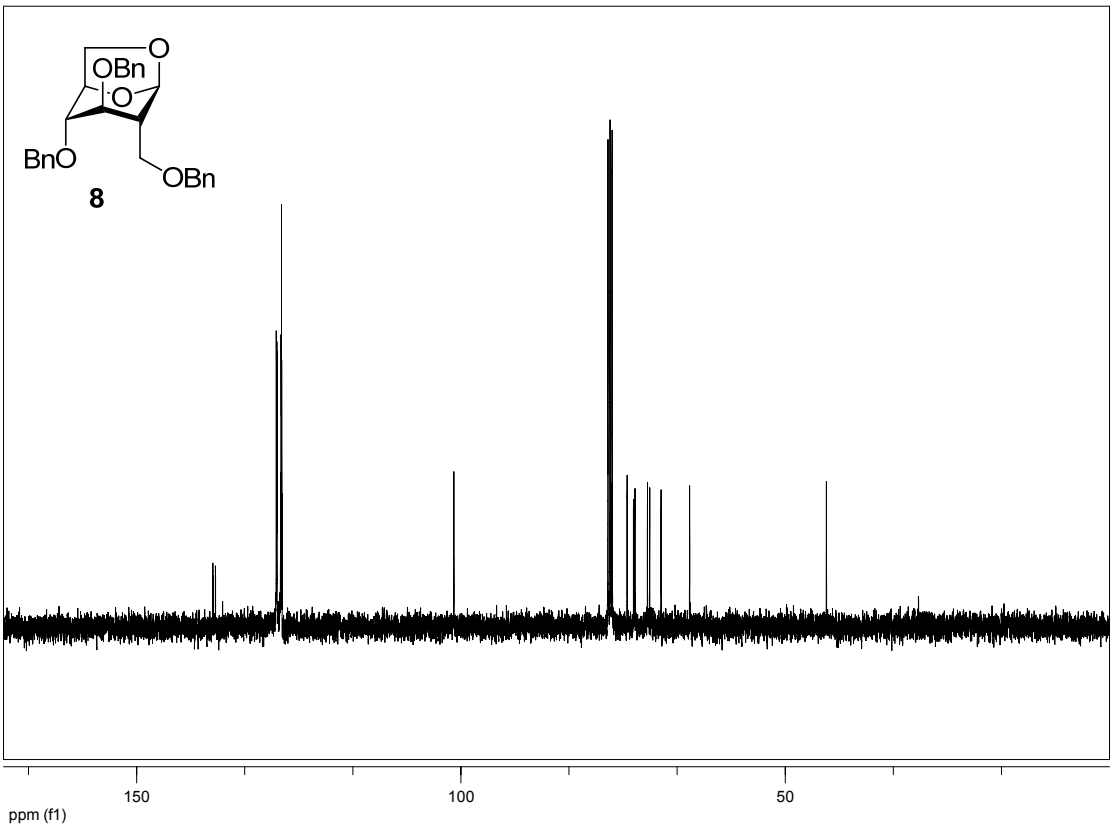
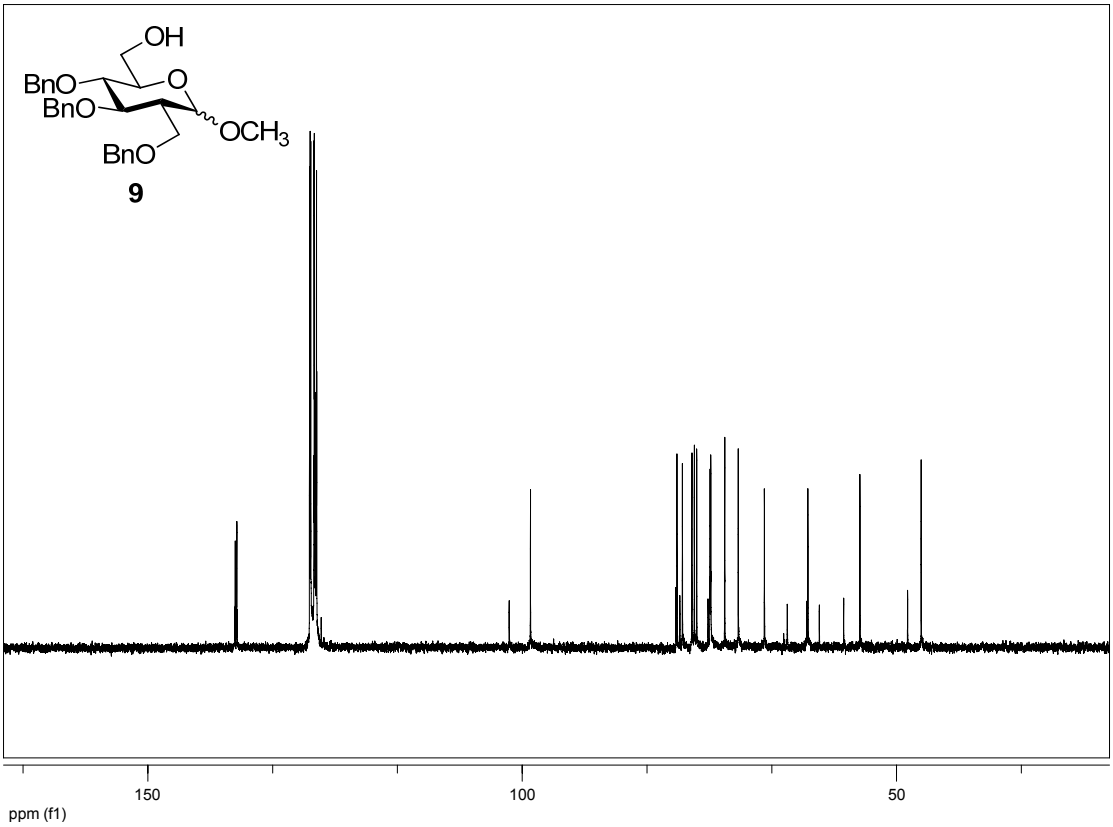
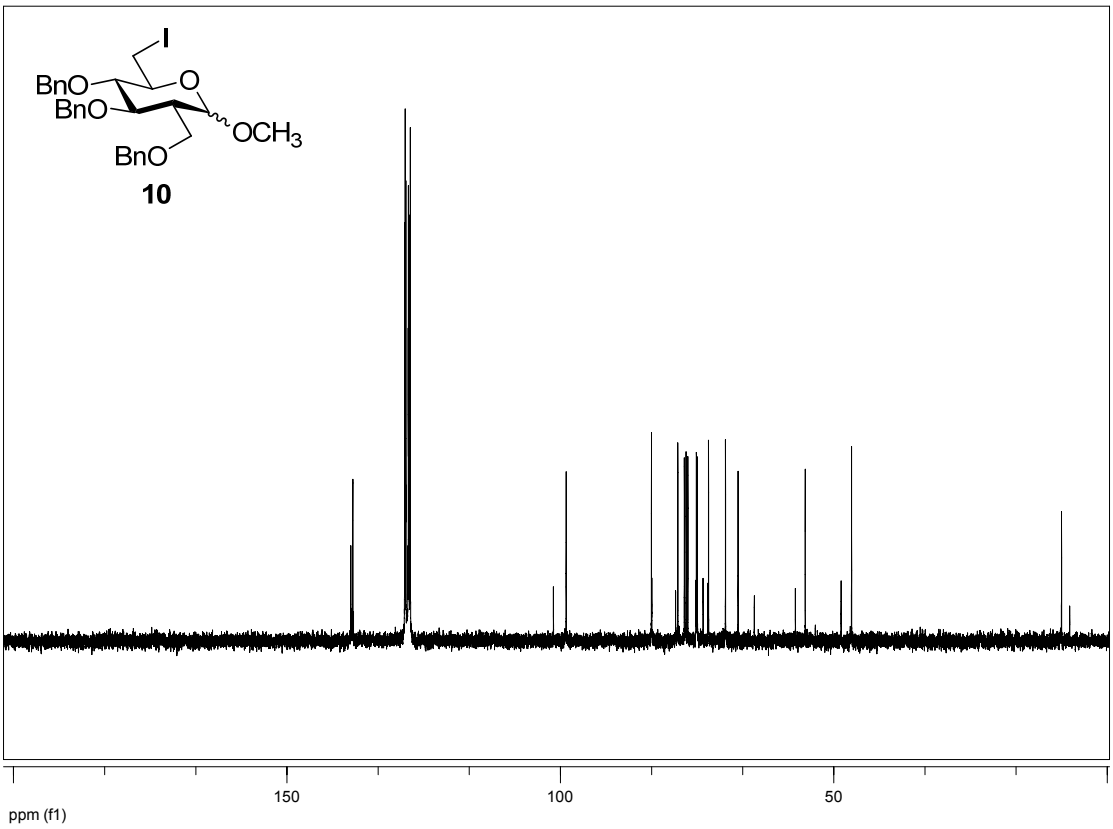


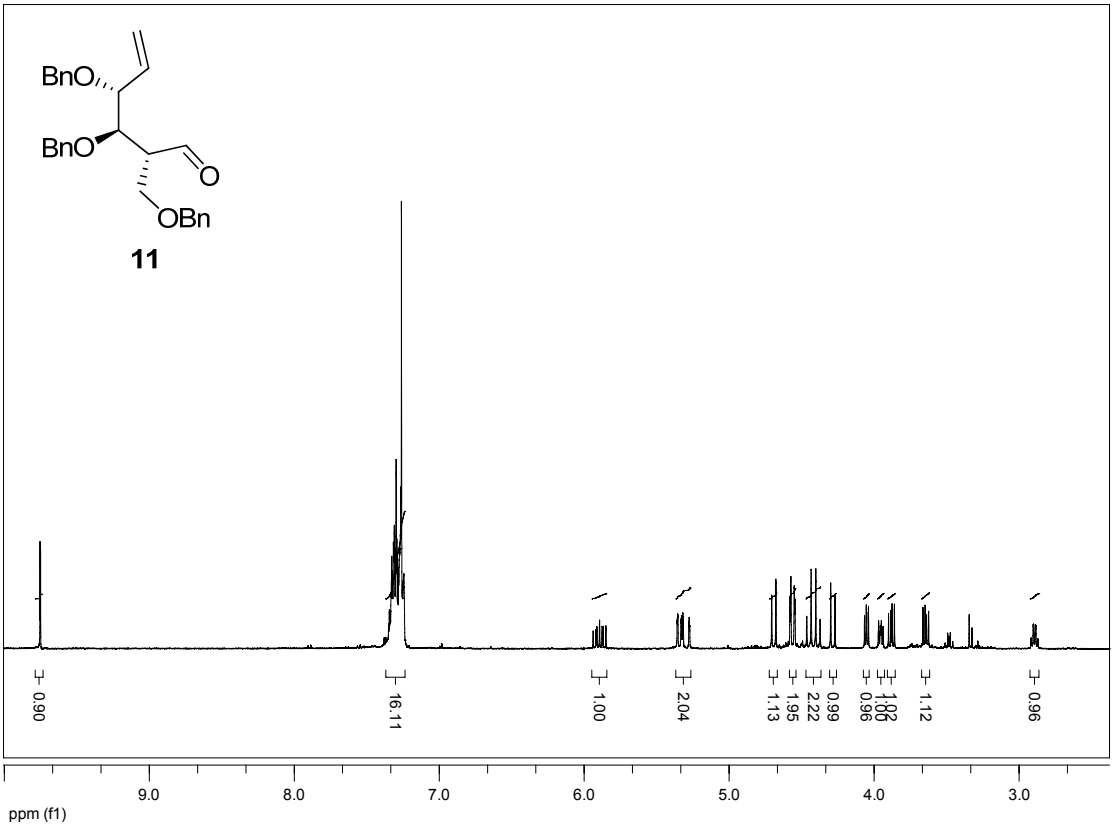
7d

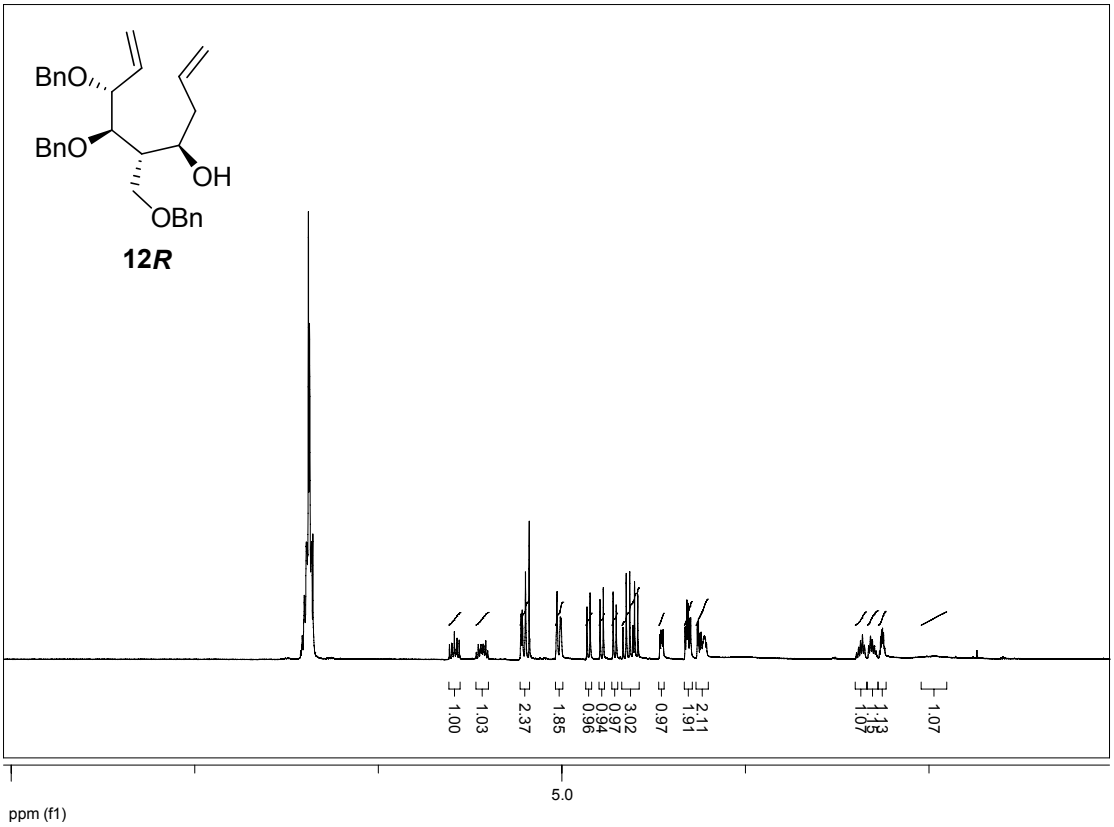


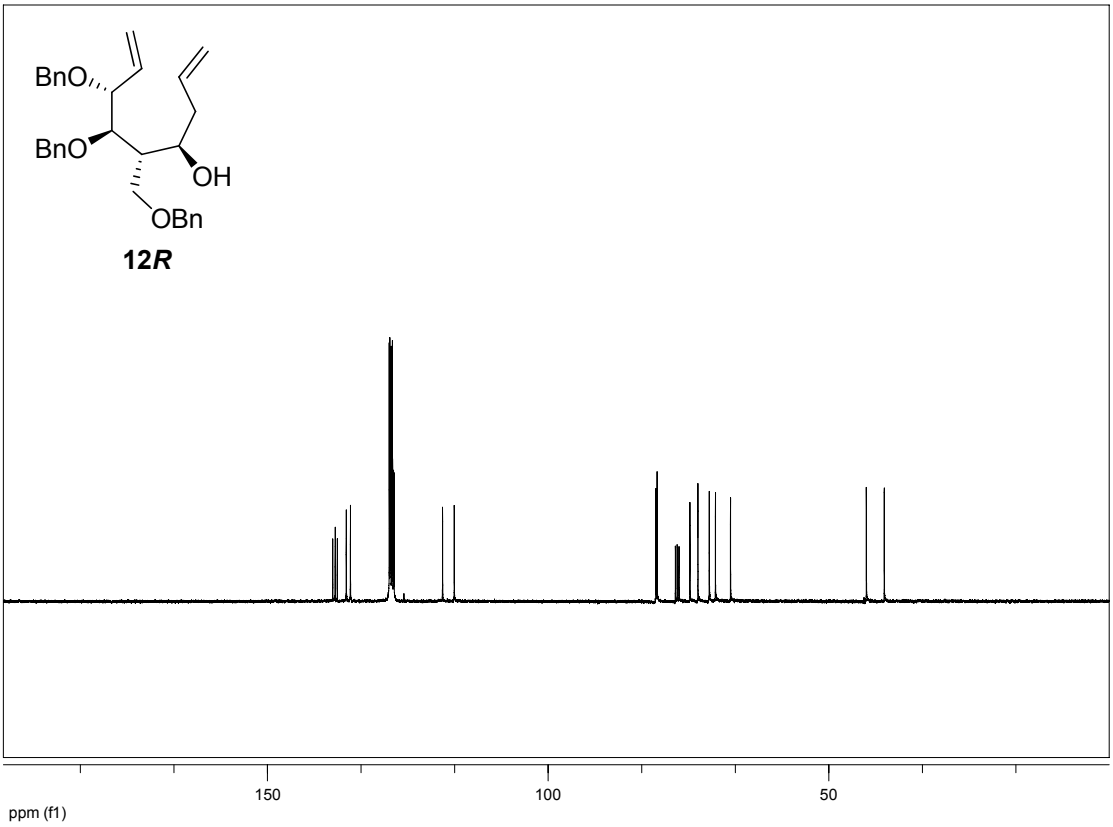


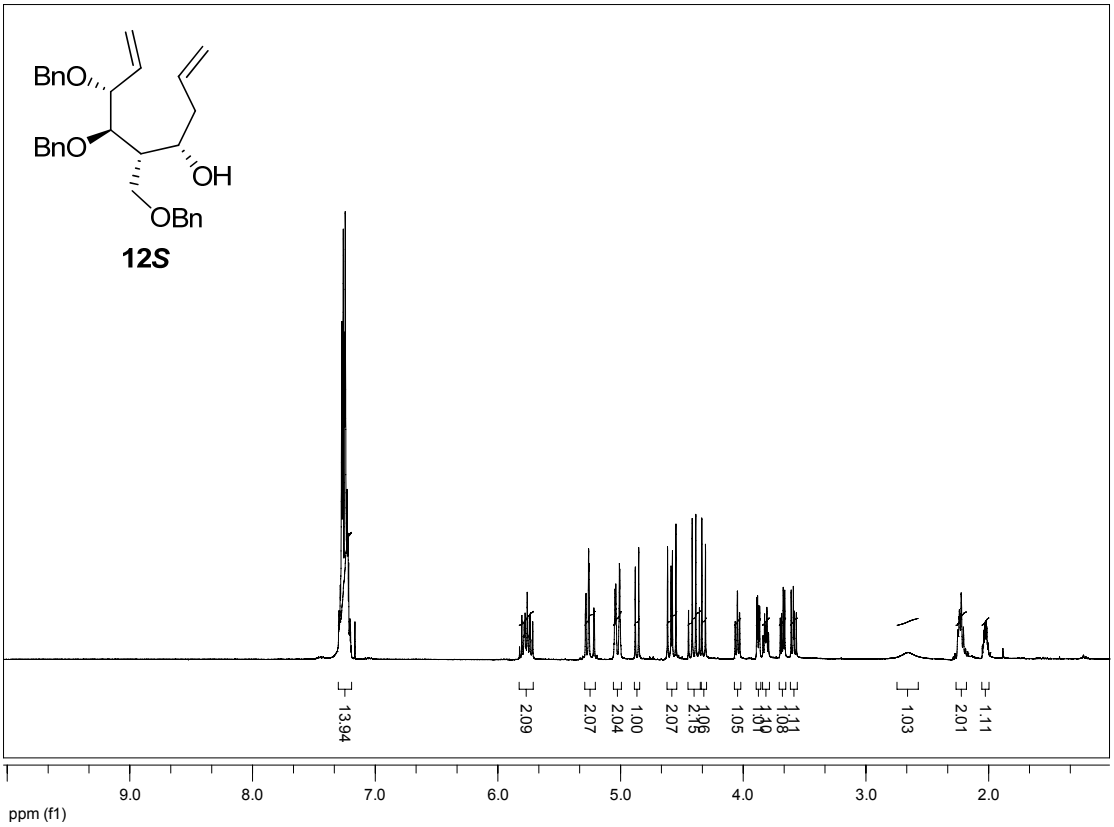


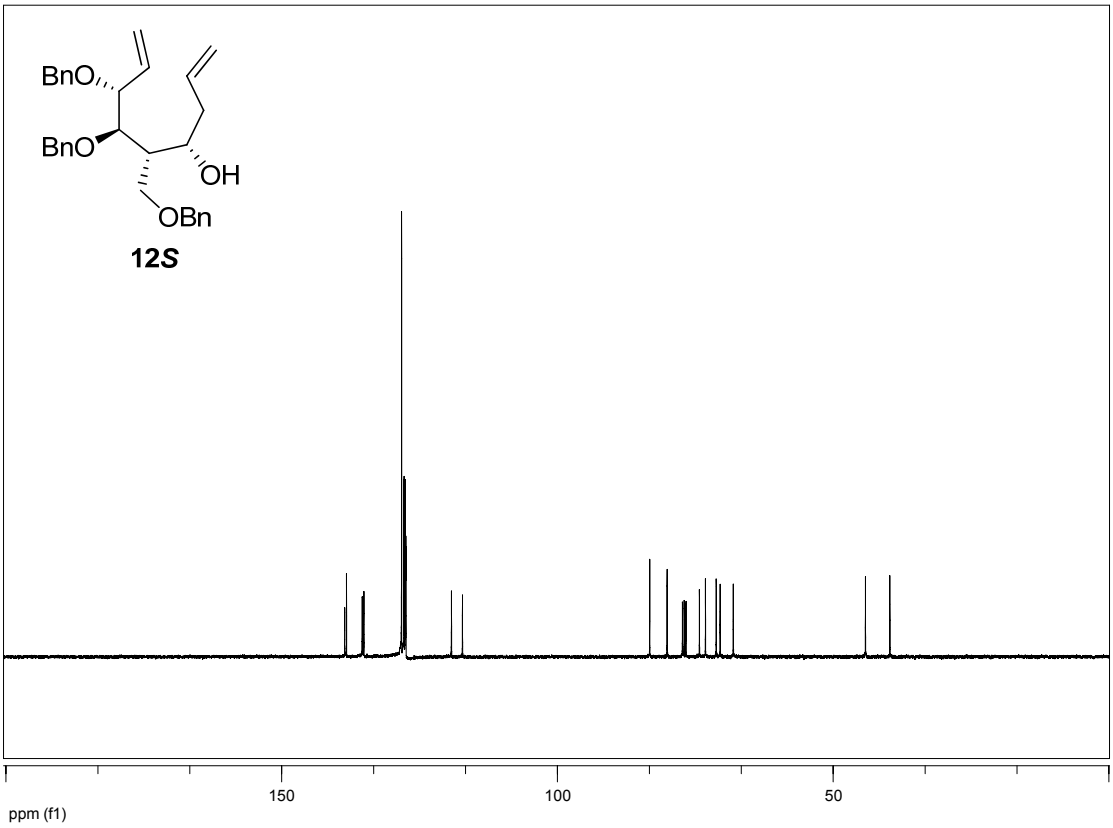


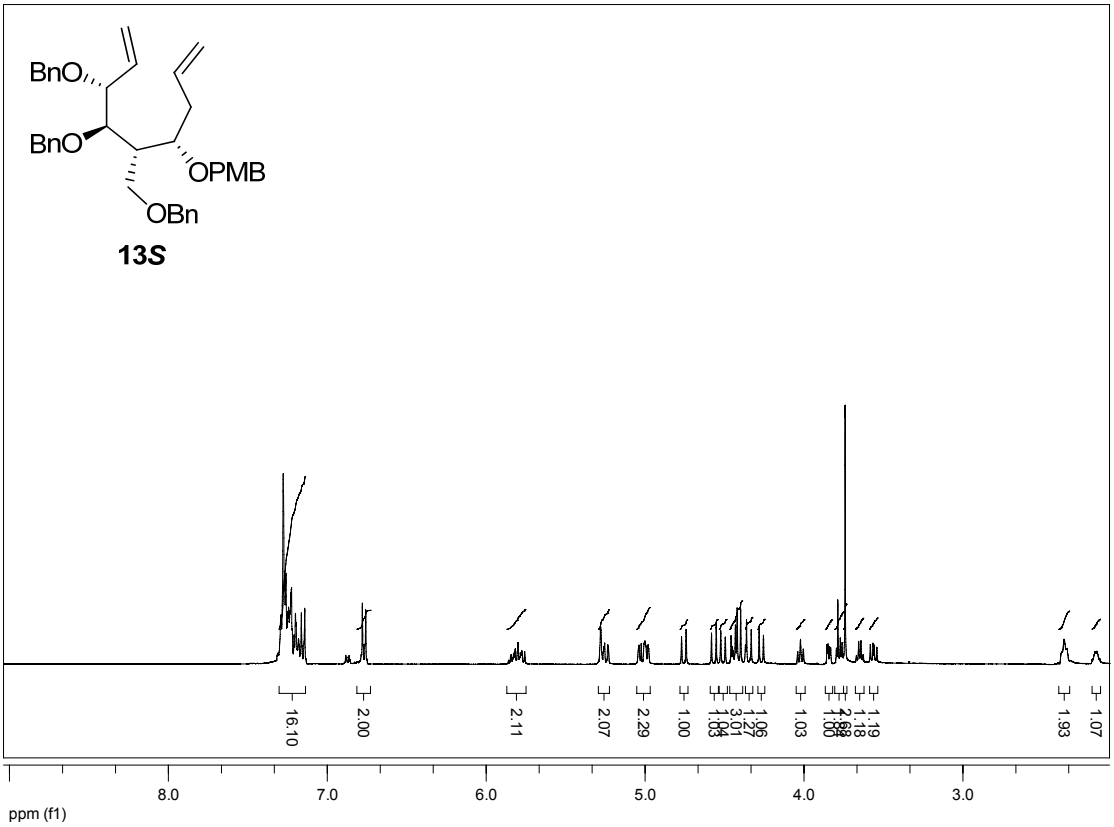




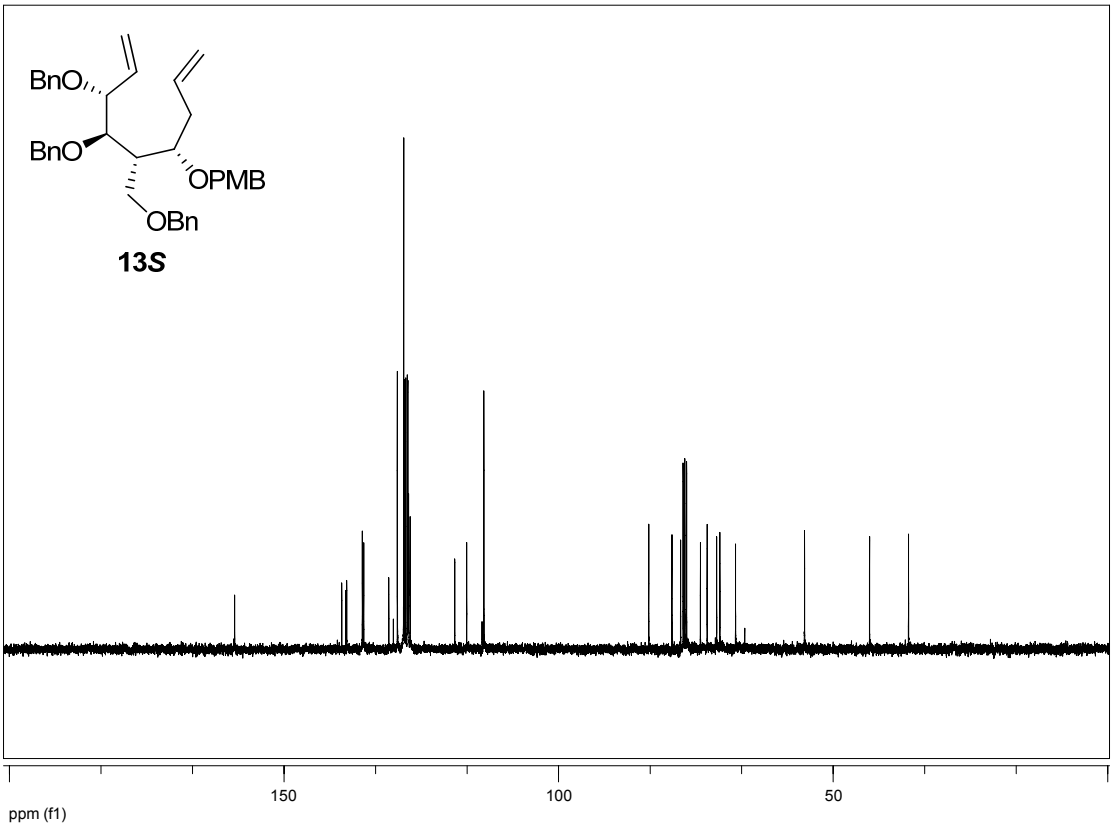








13*S*



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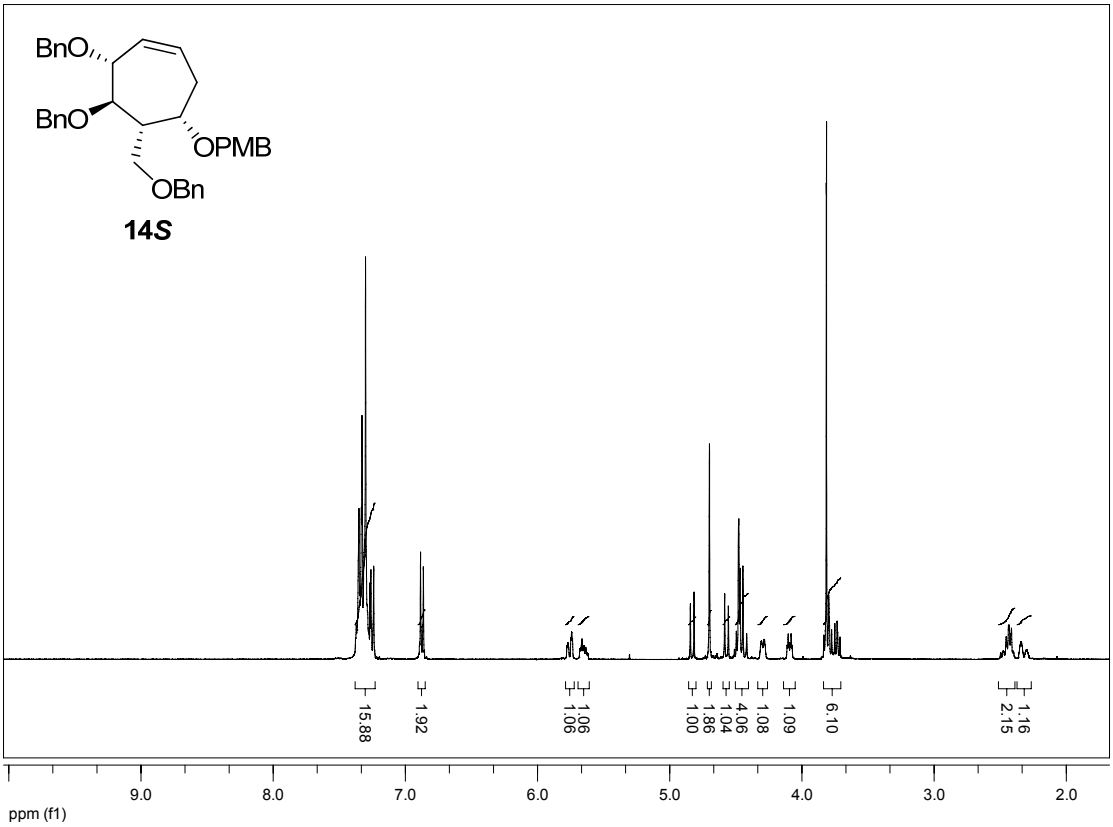
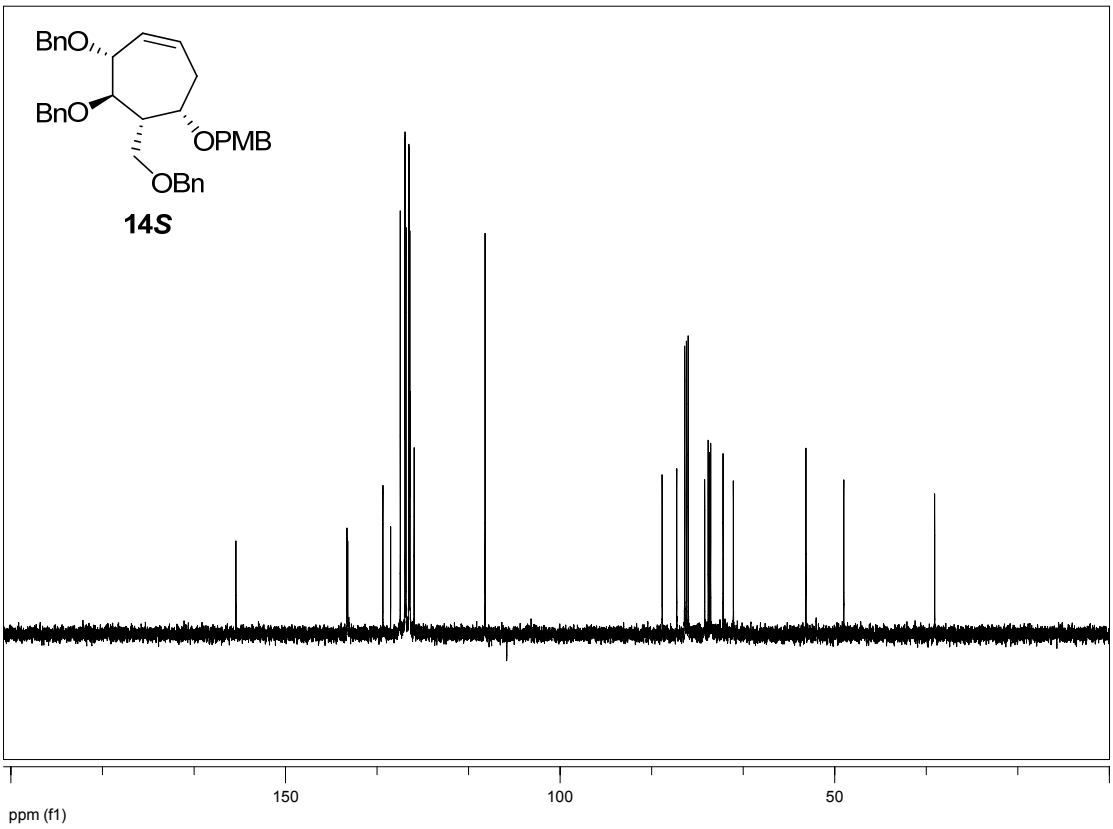
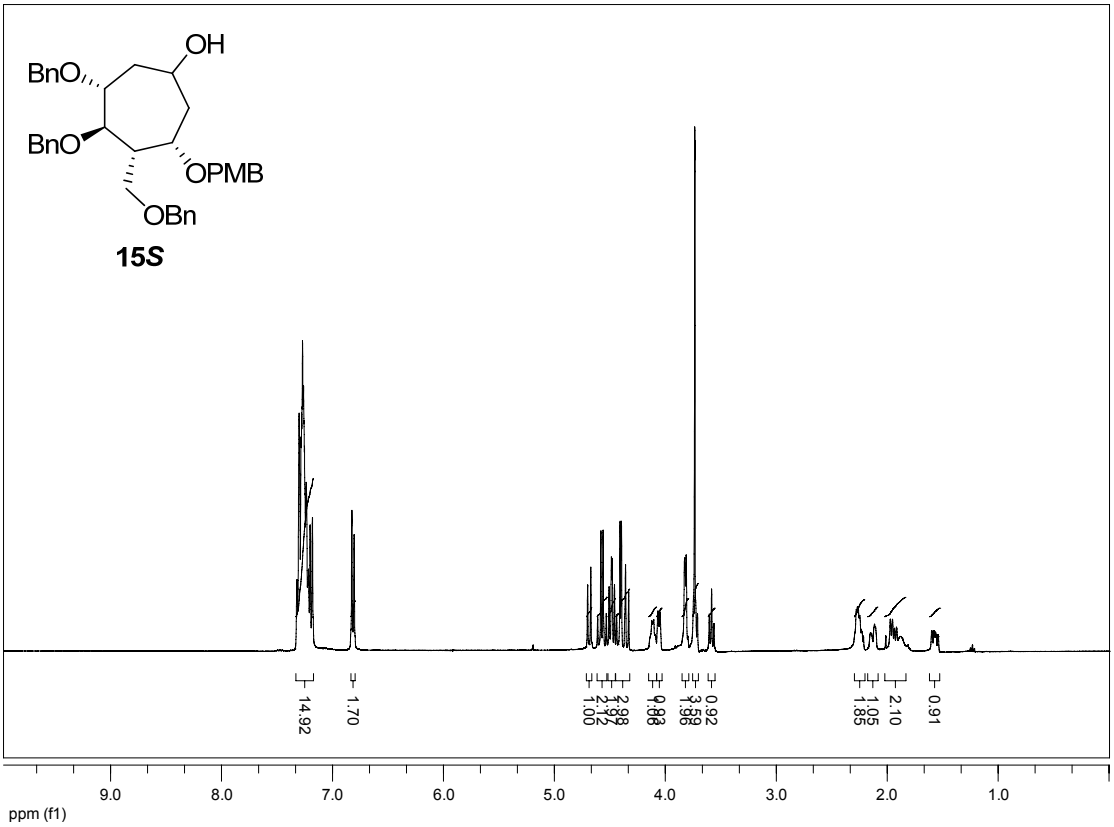
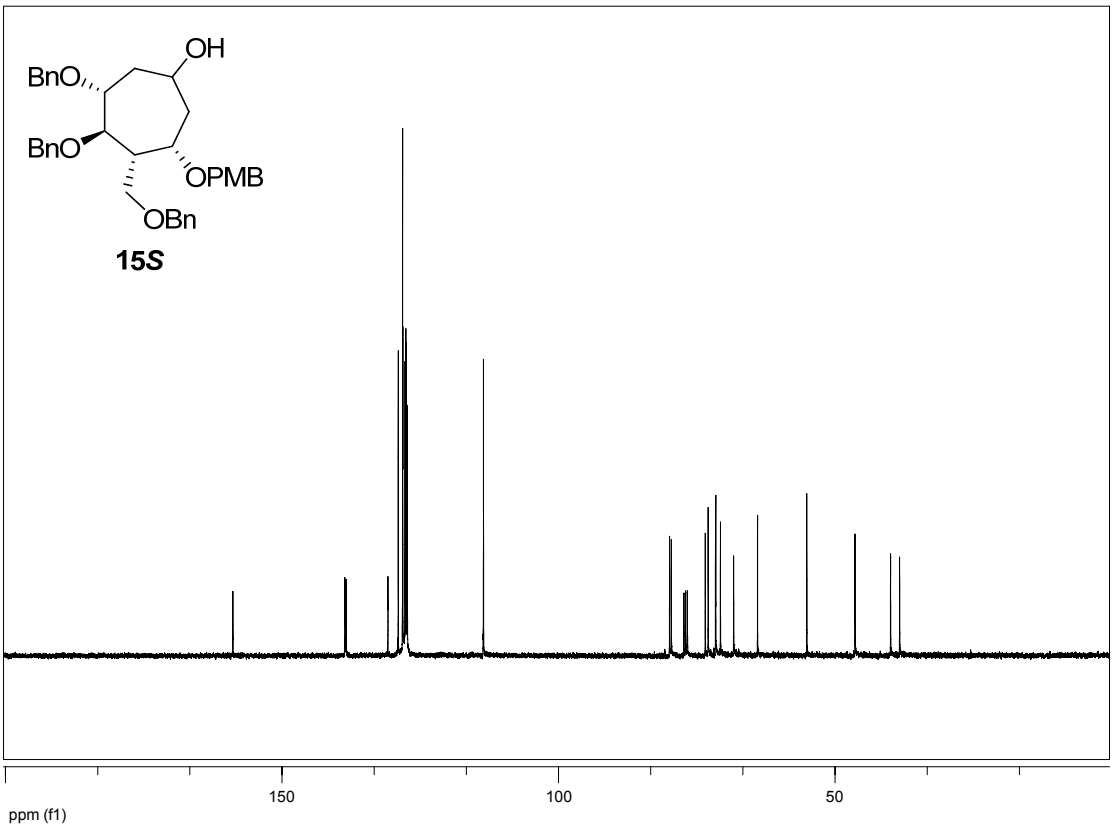
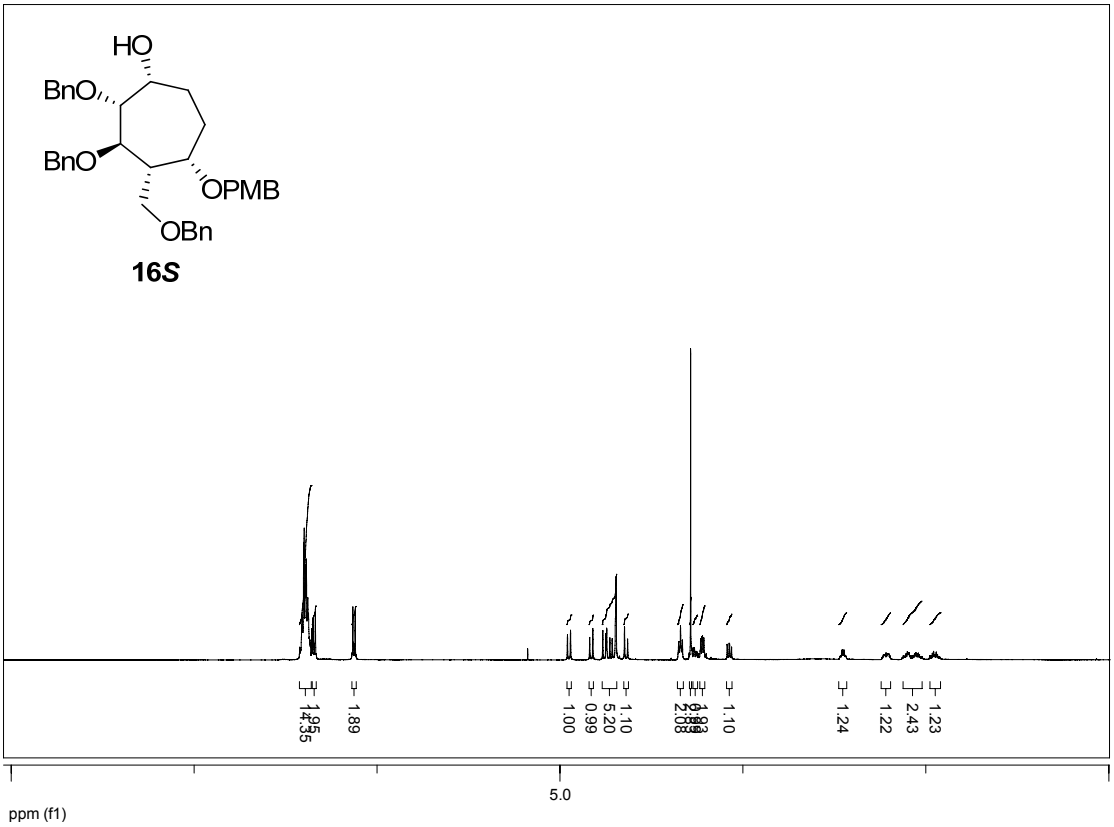
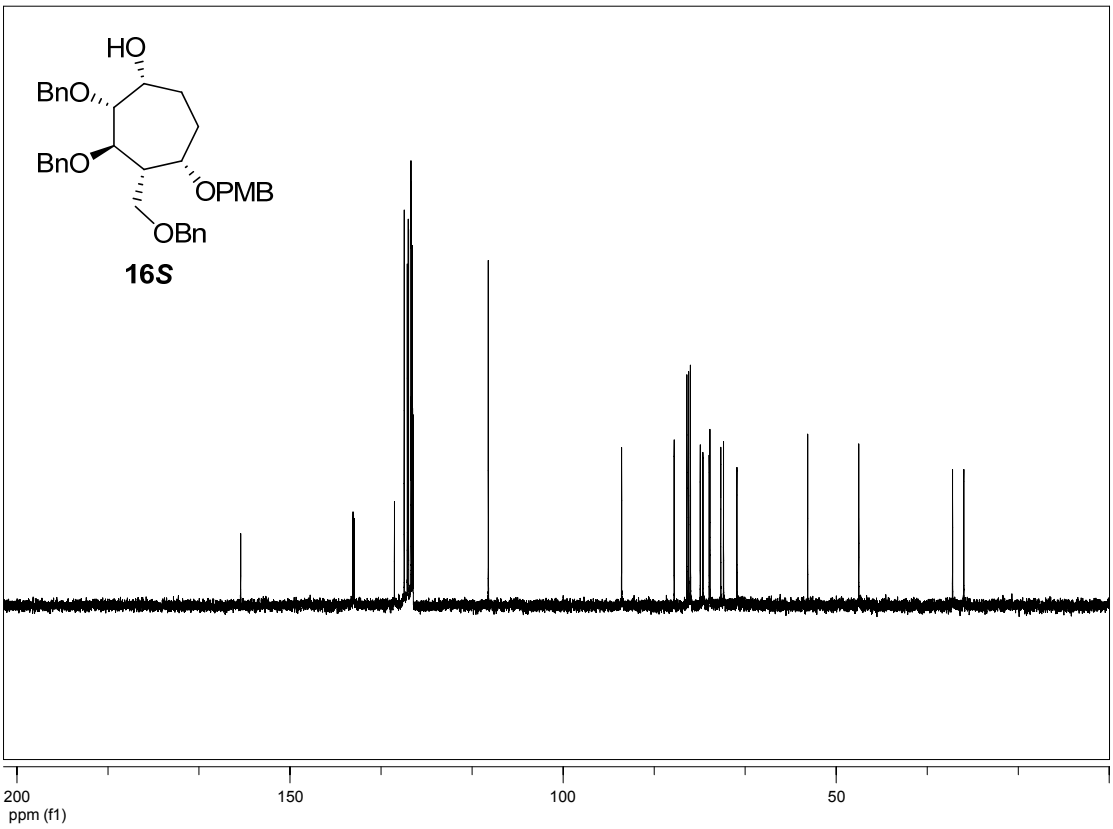


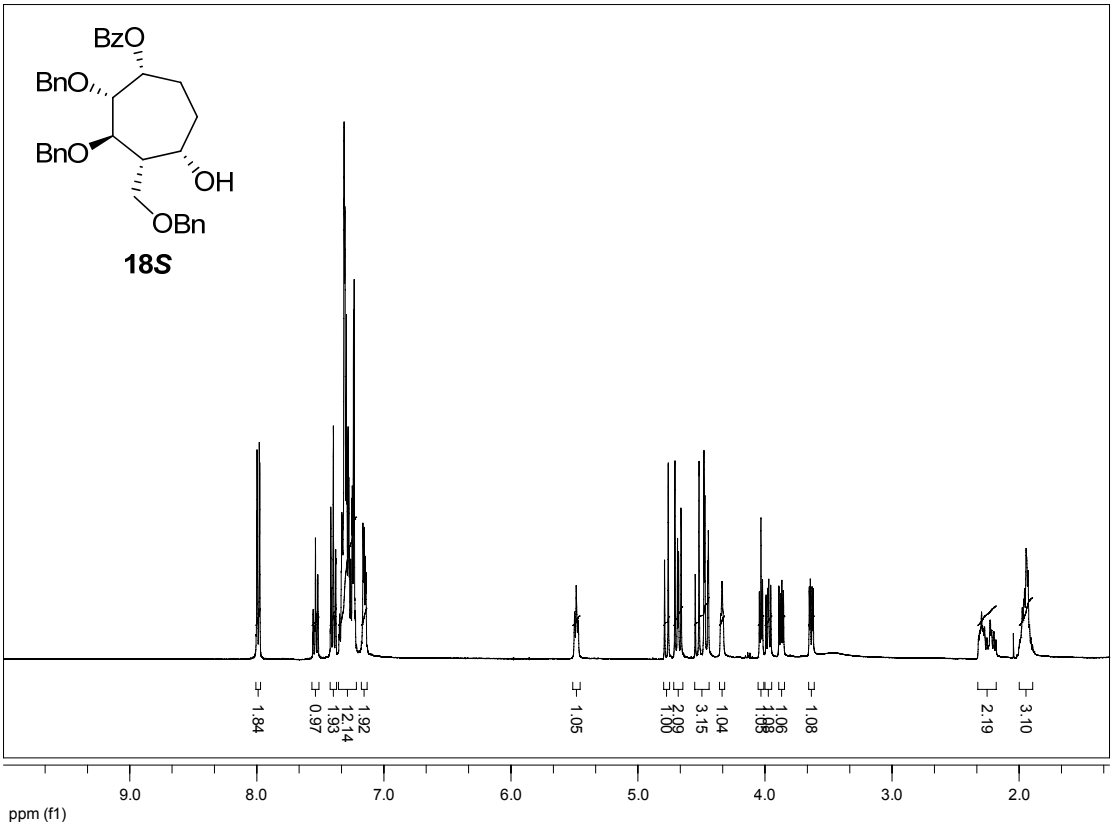


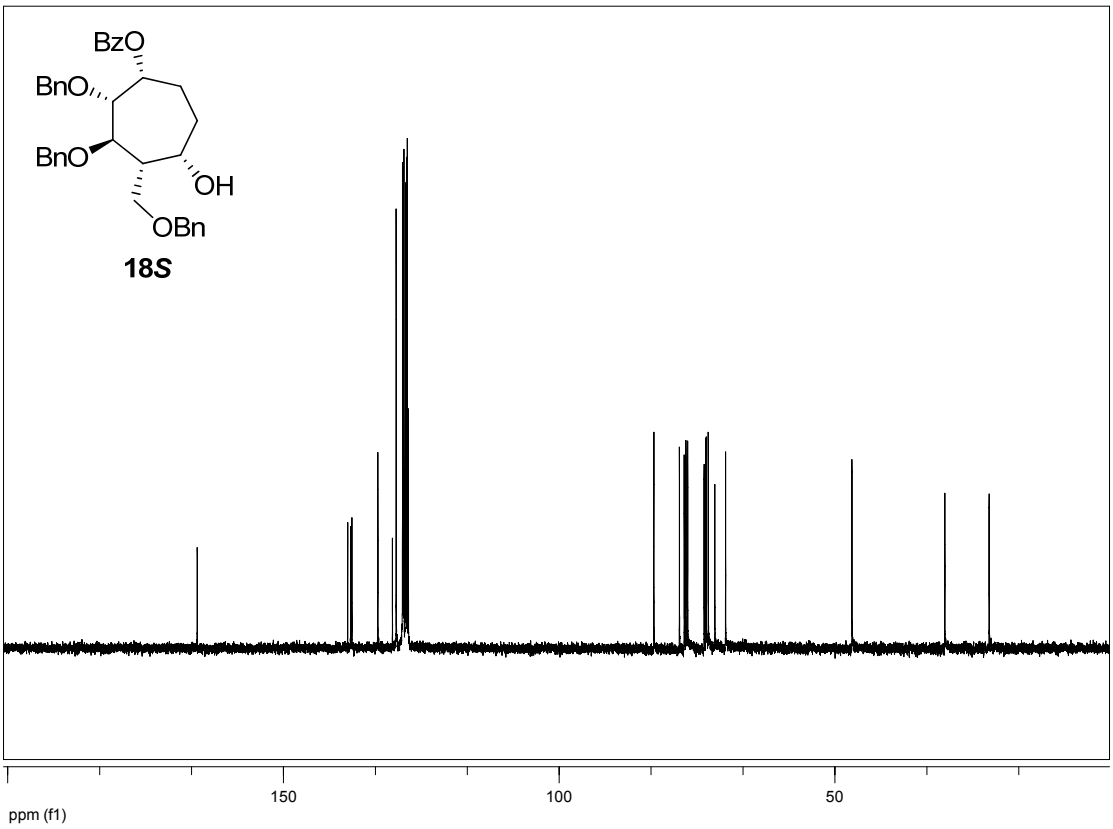


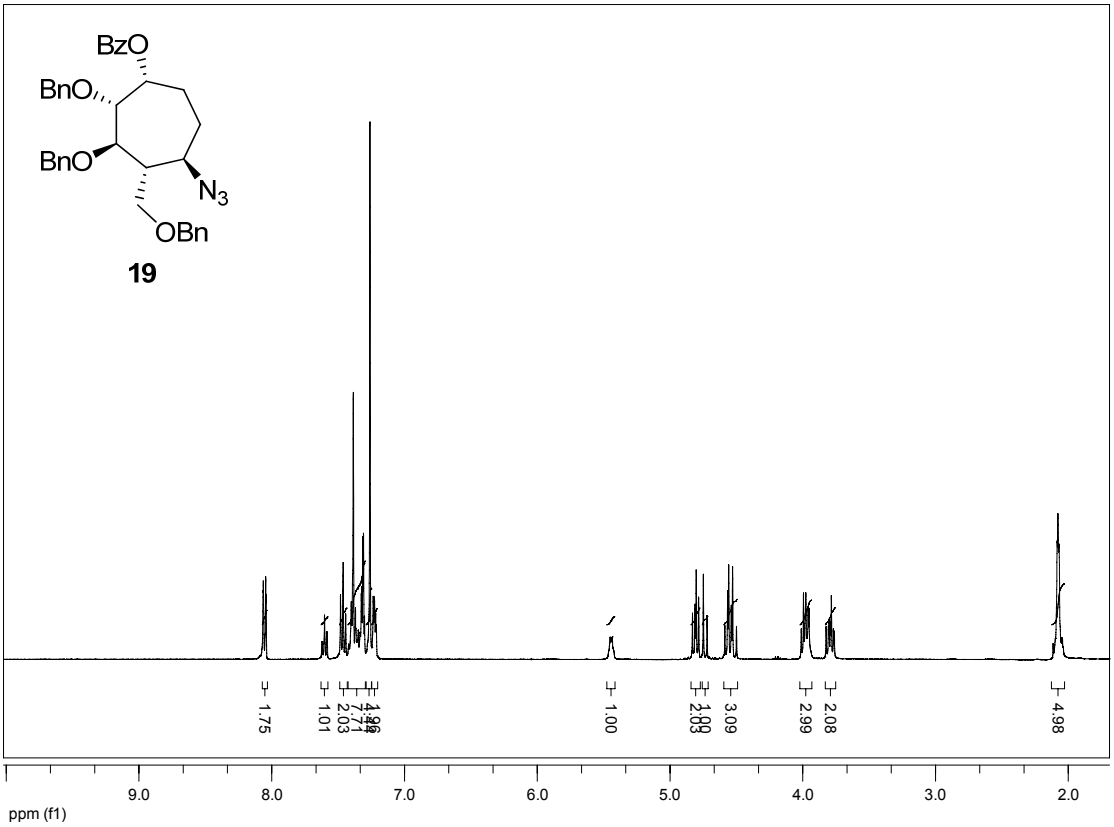


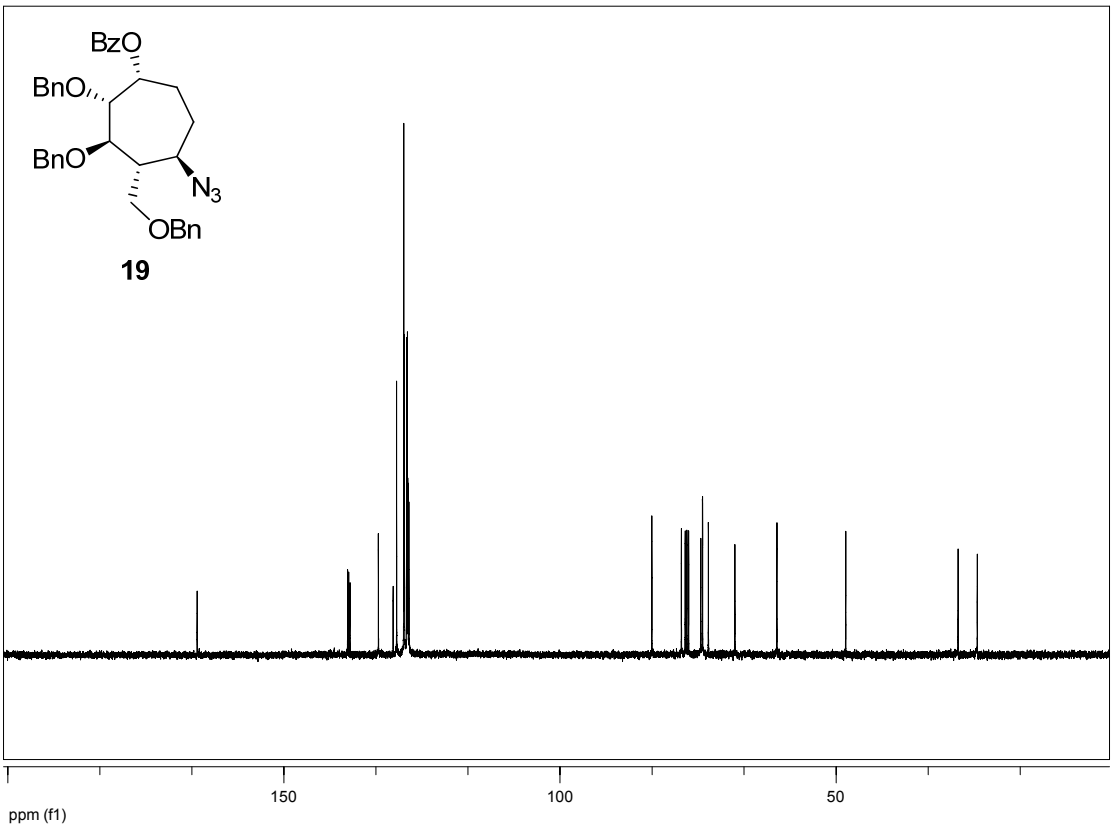


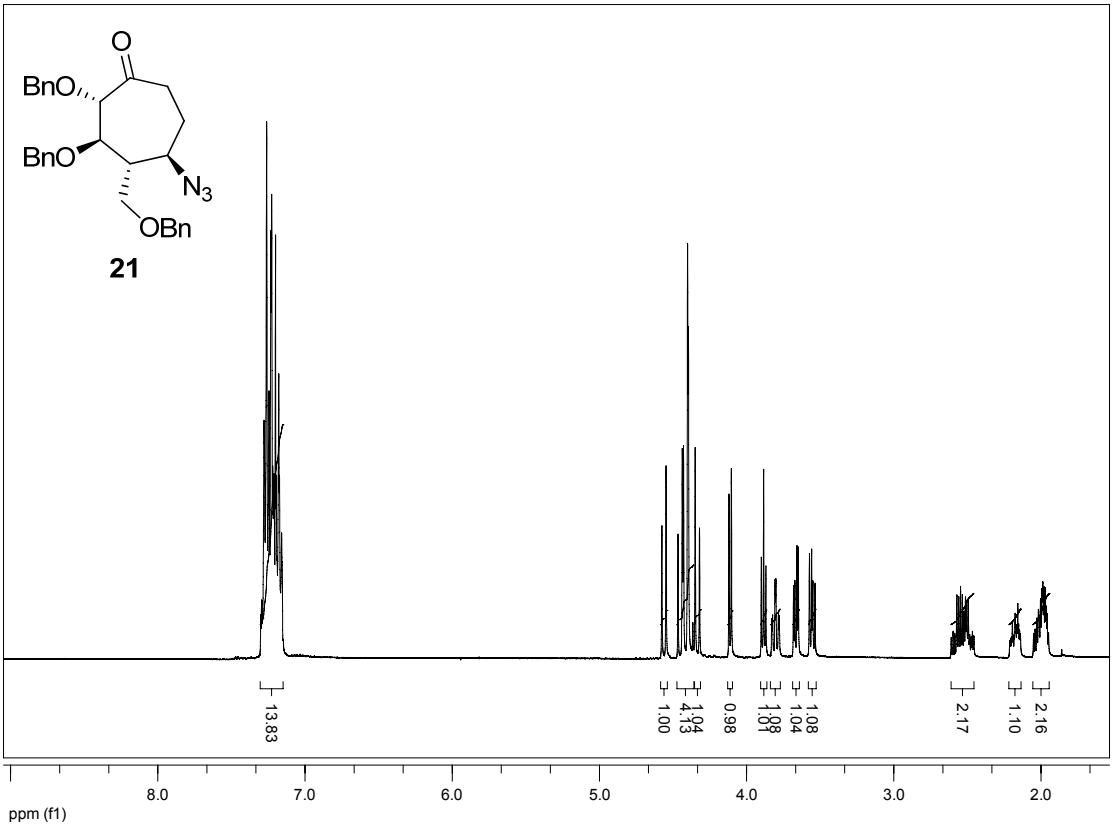


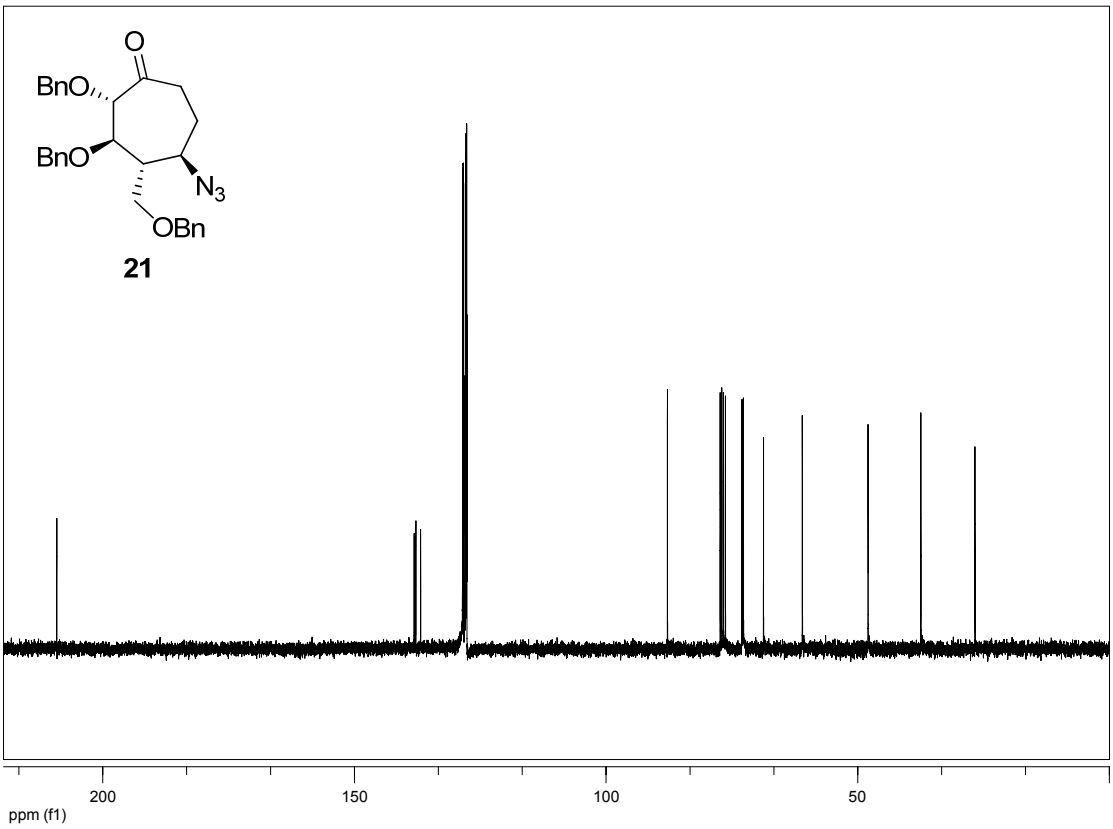


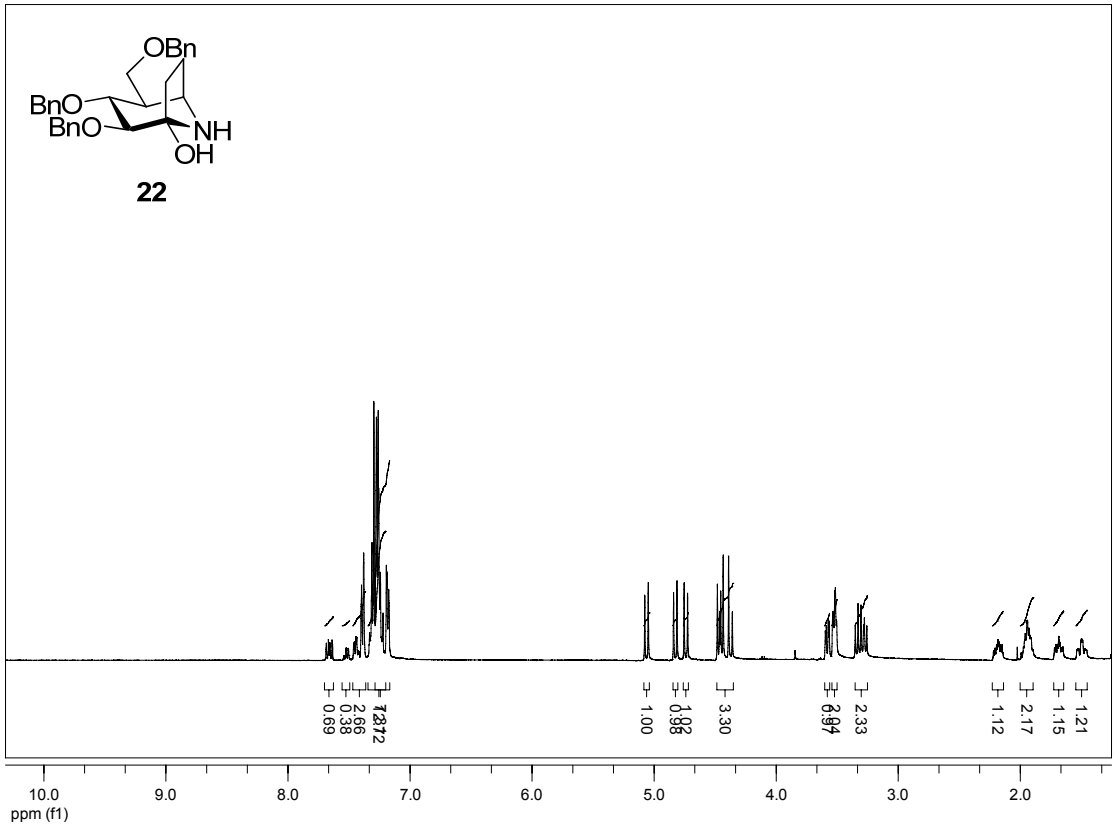


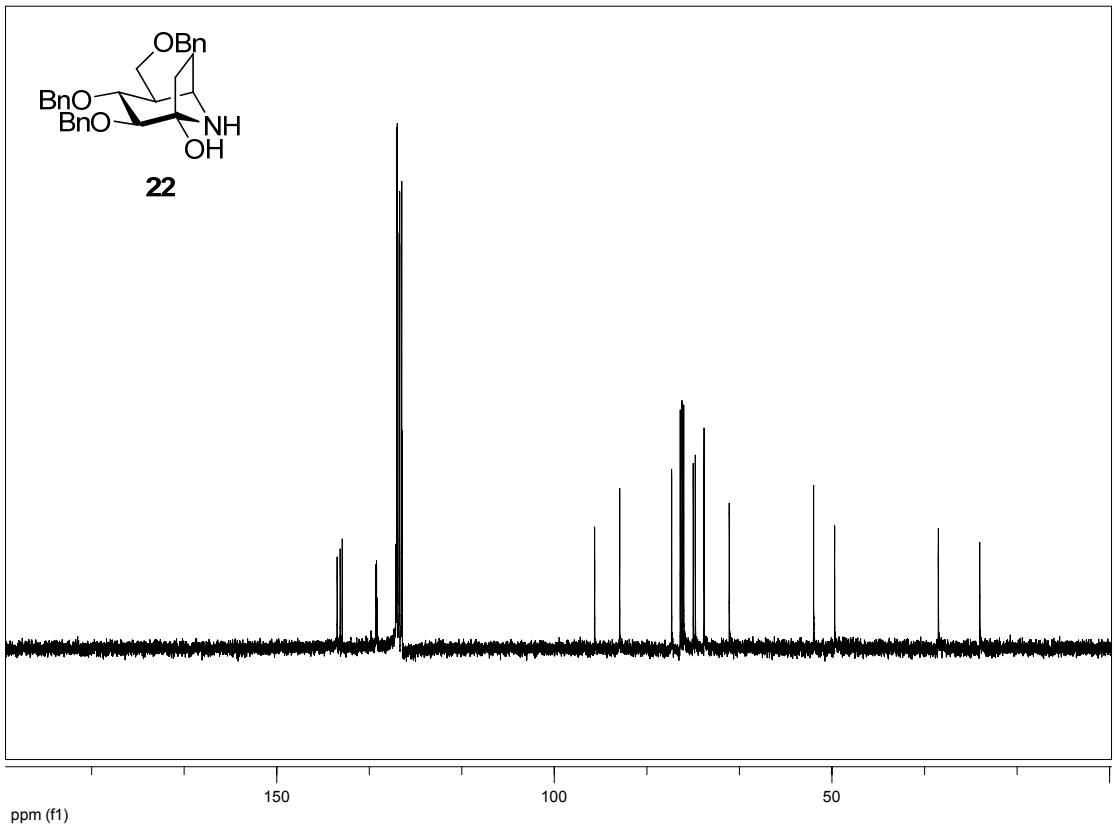


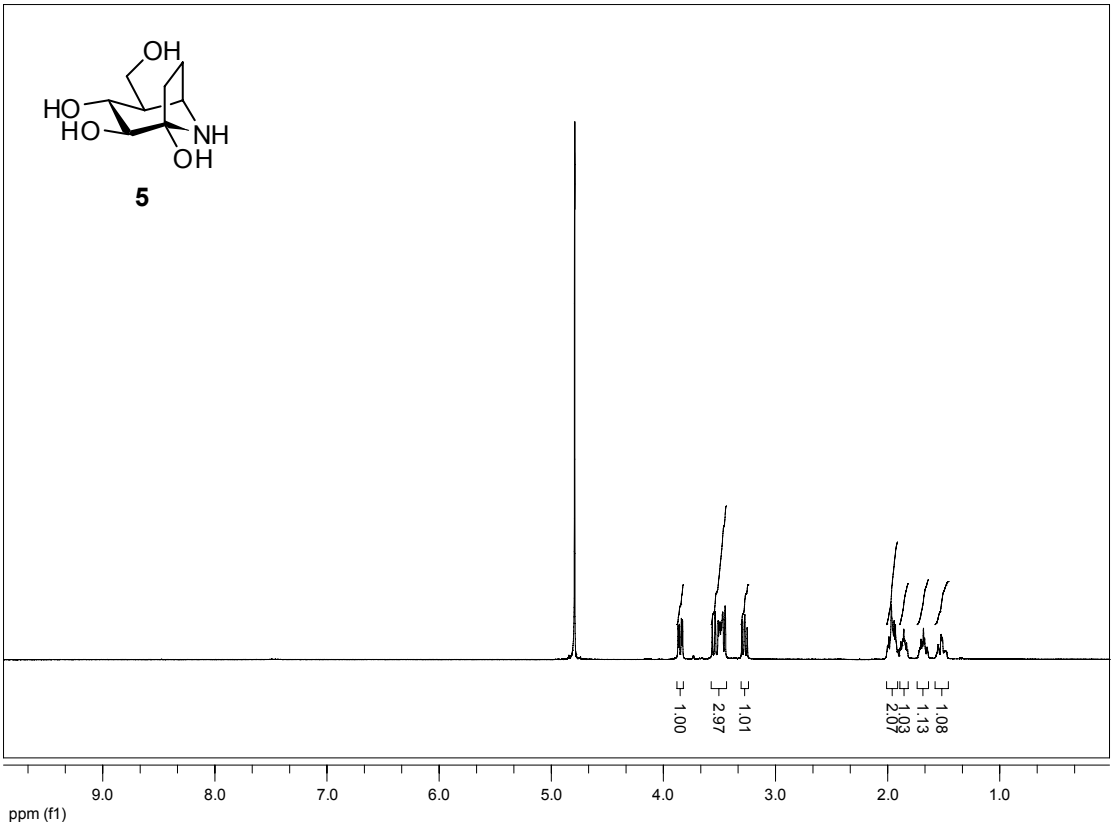


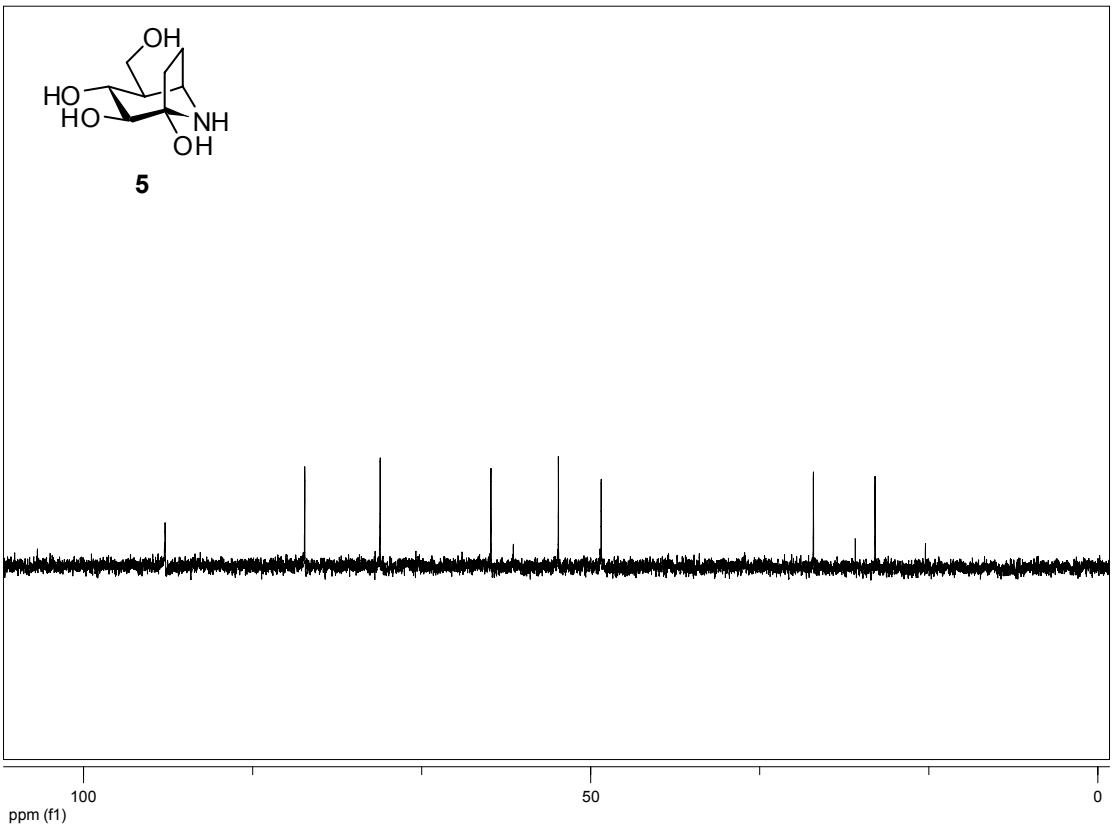


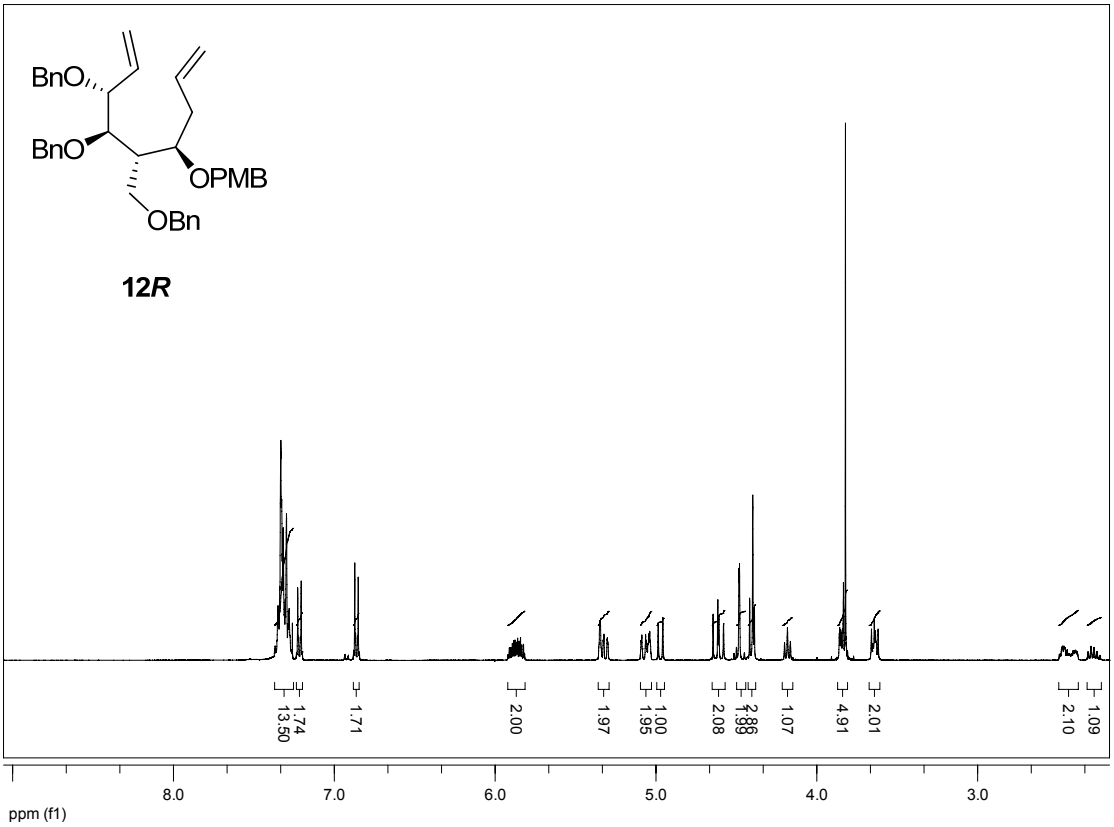


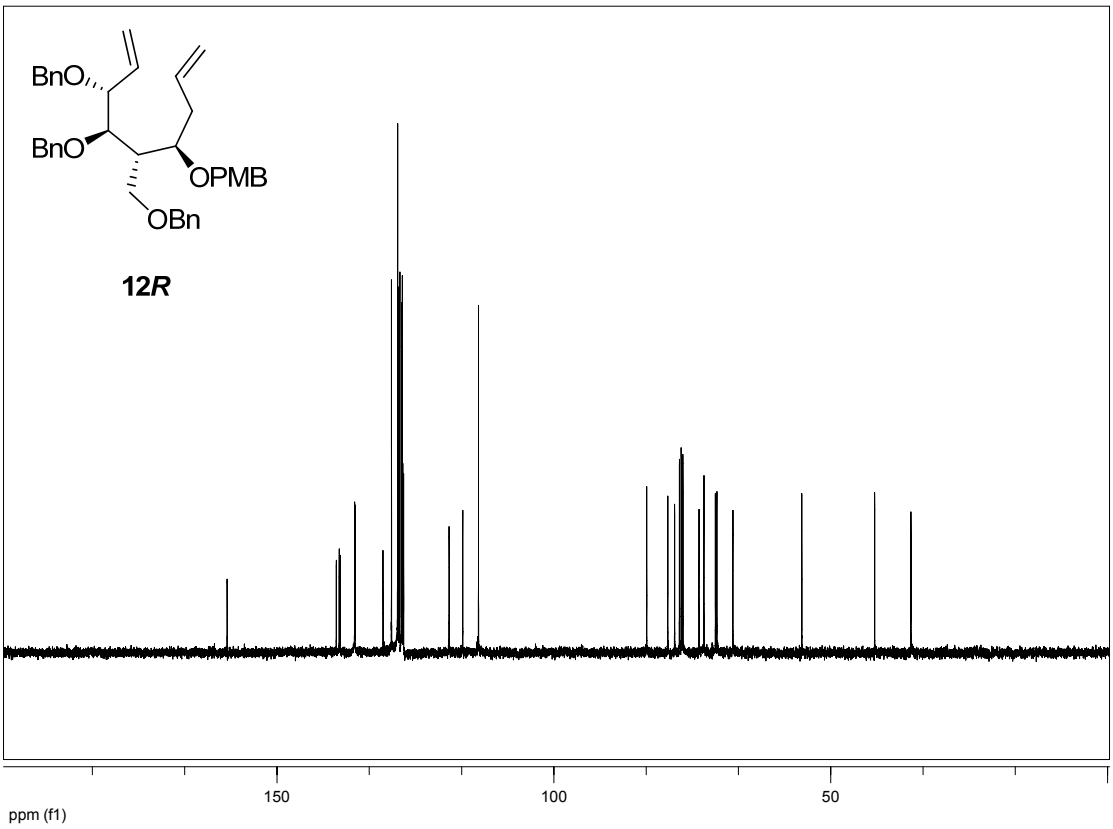


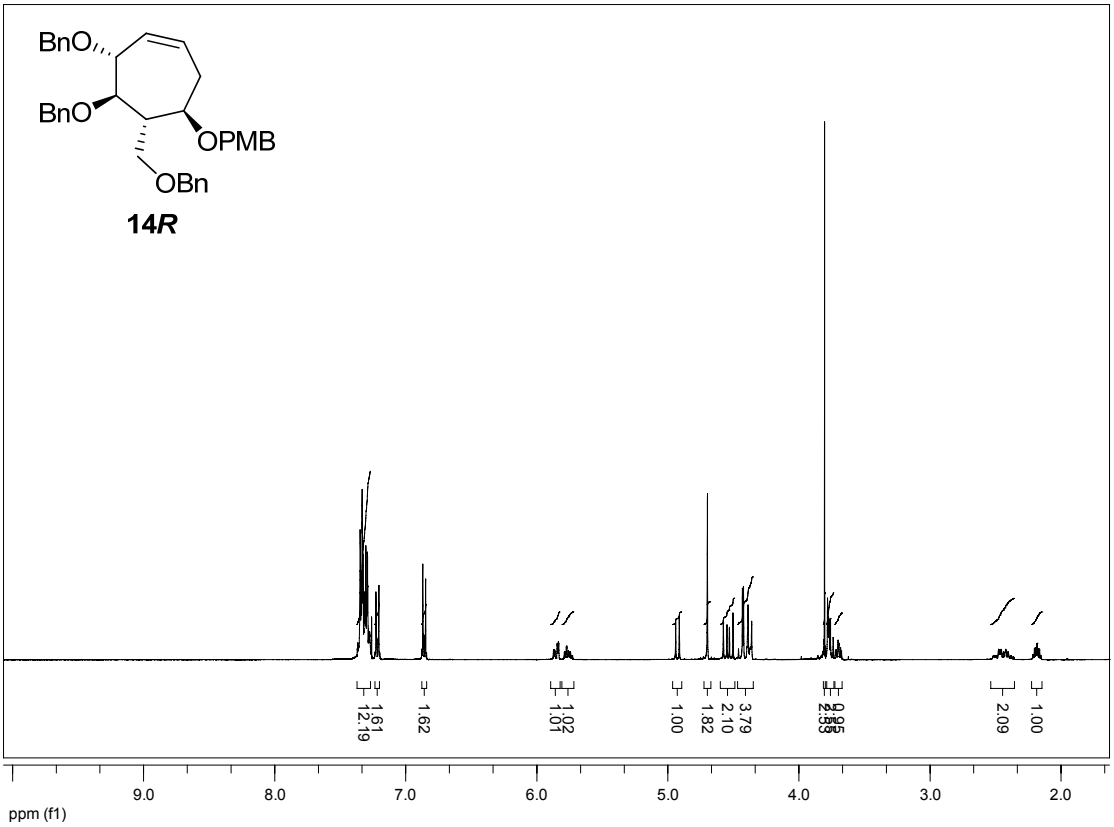


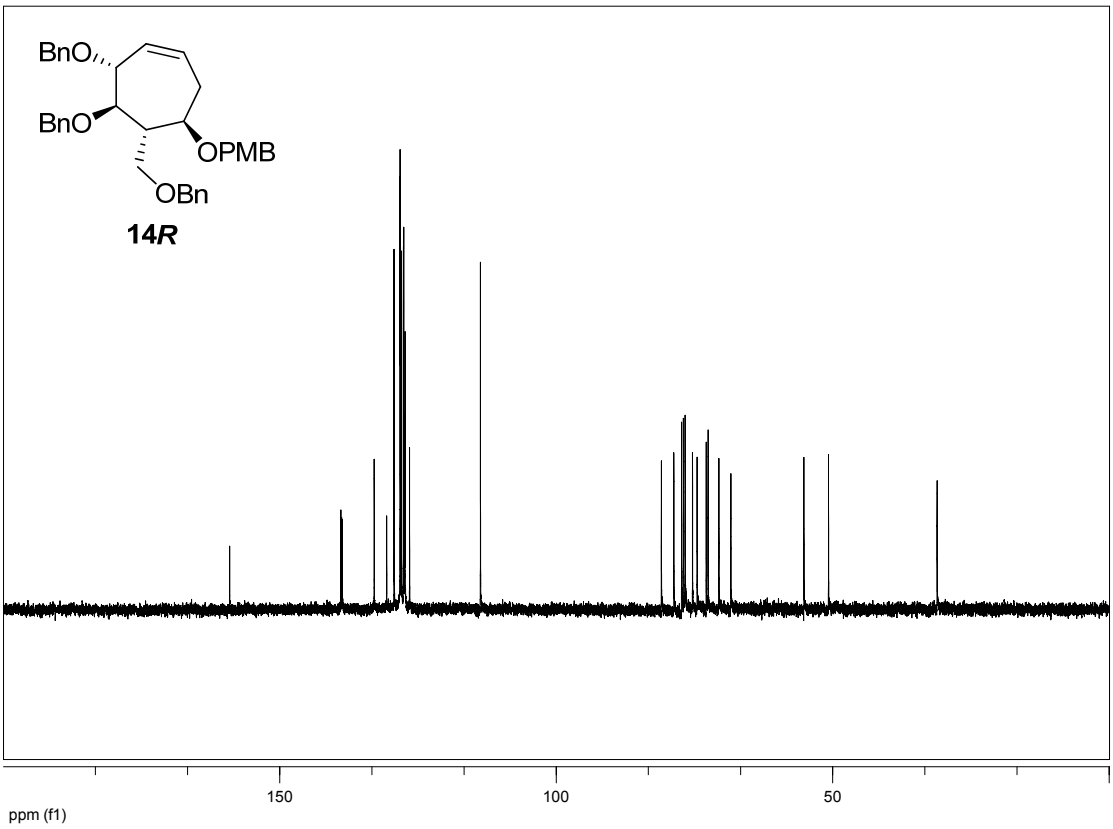


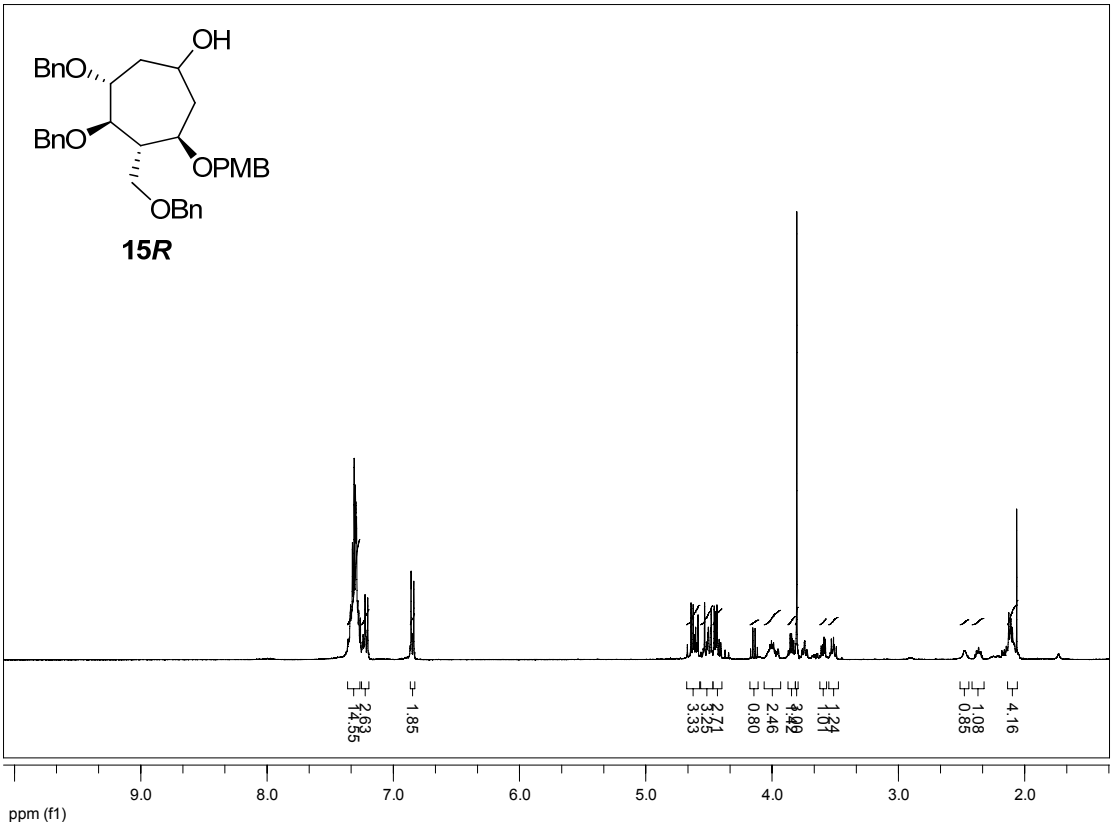


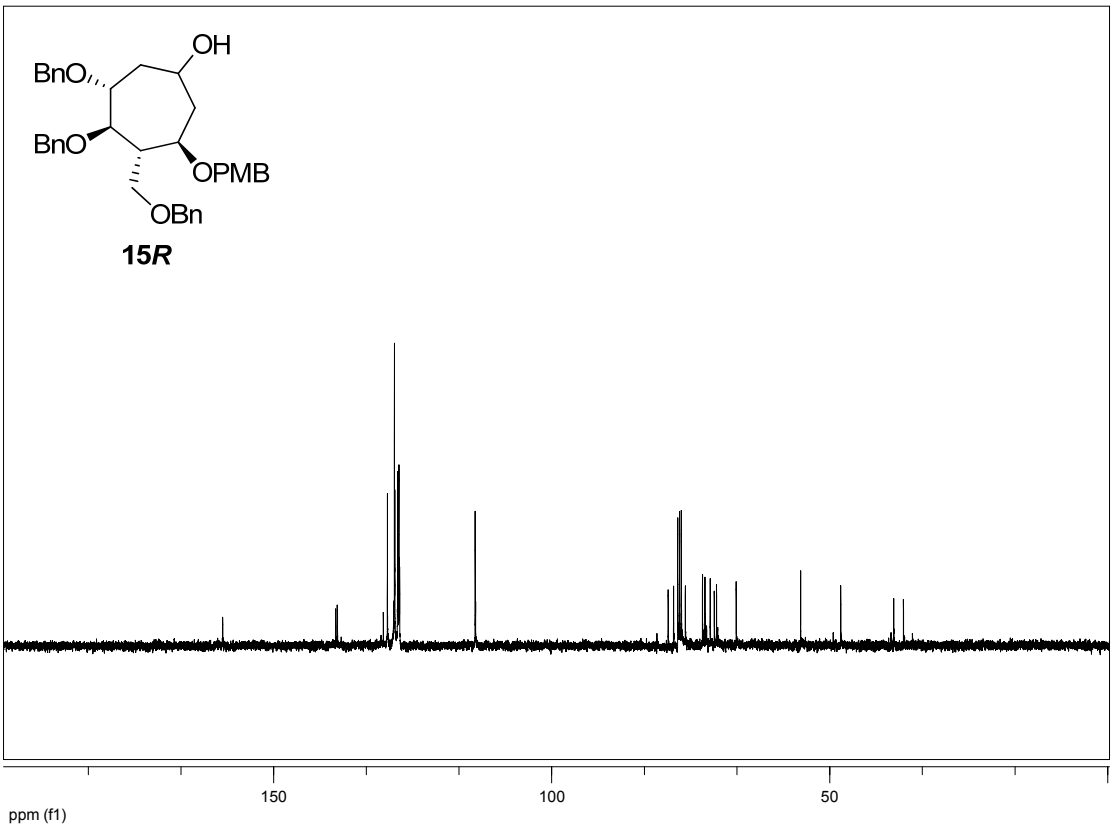


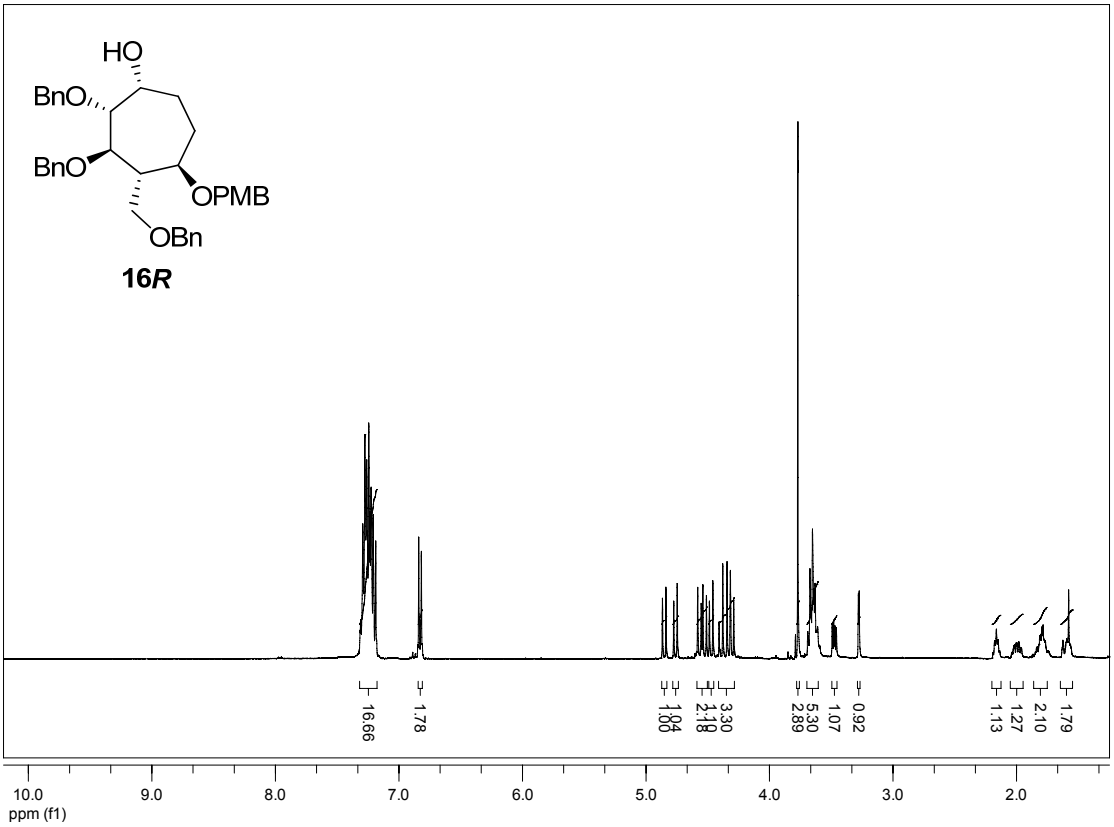




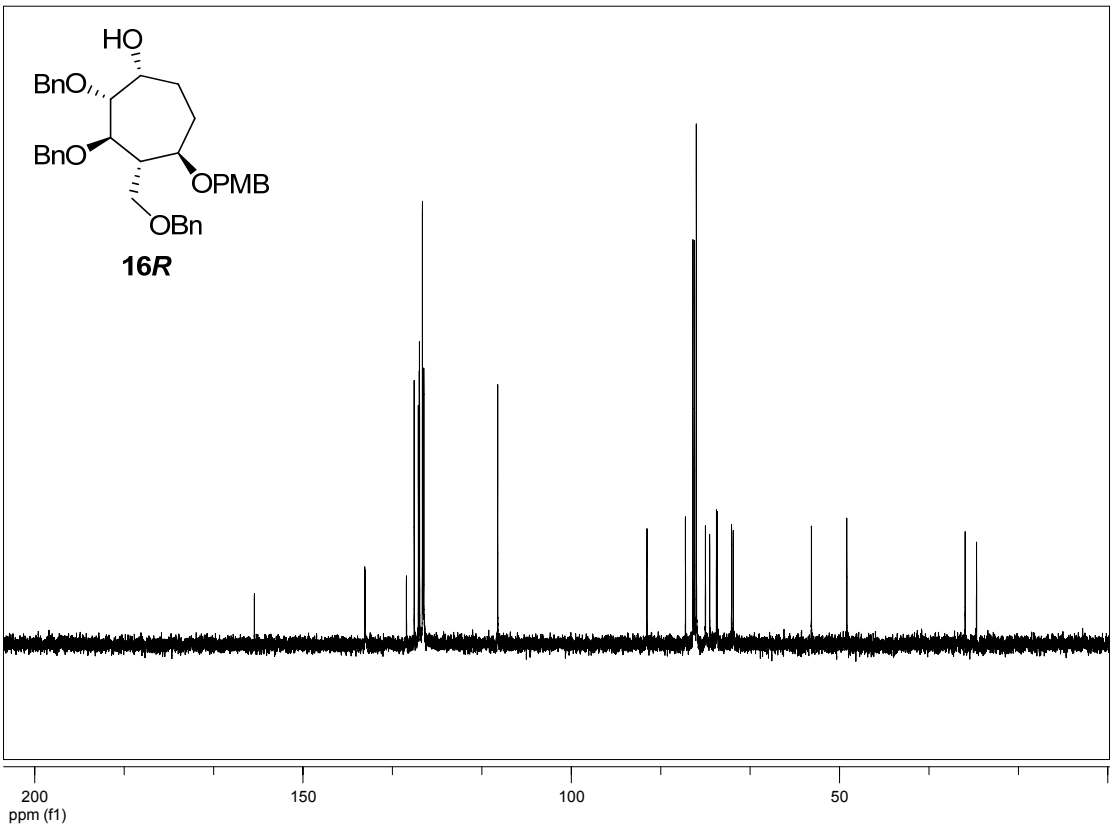




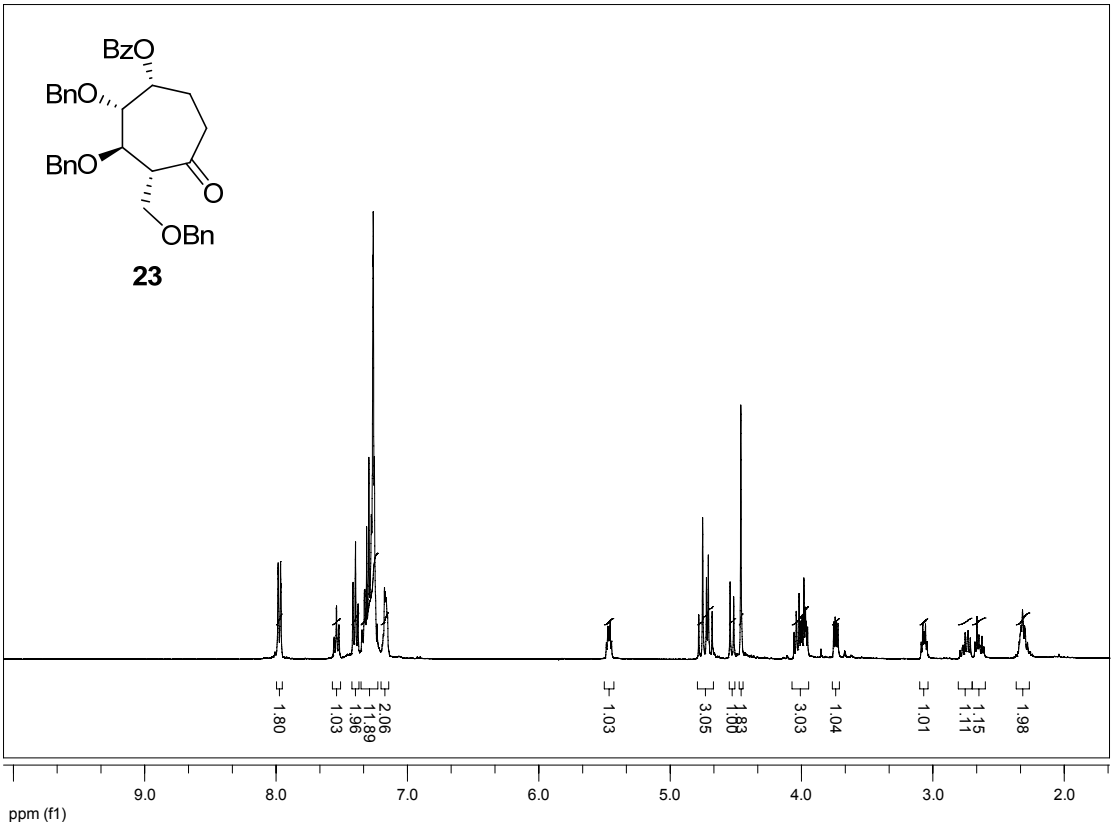


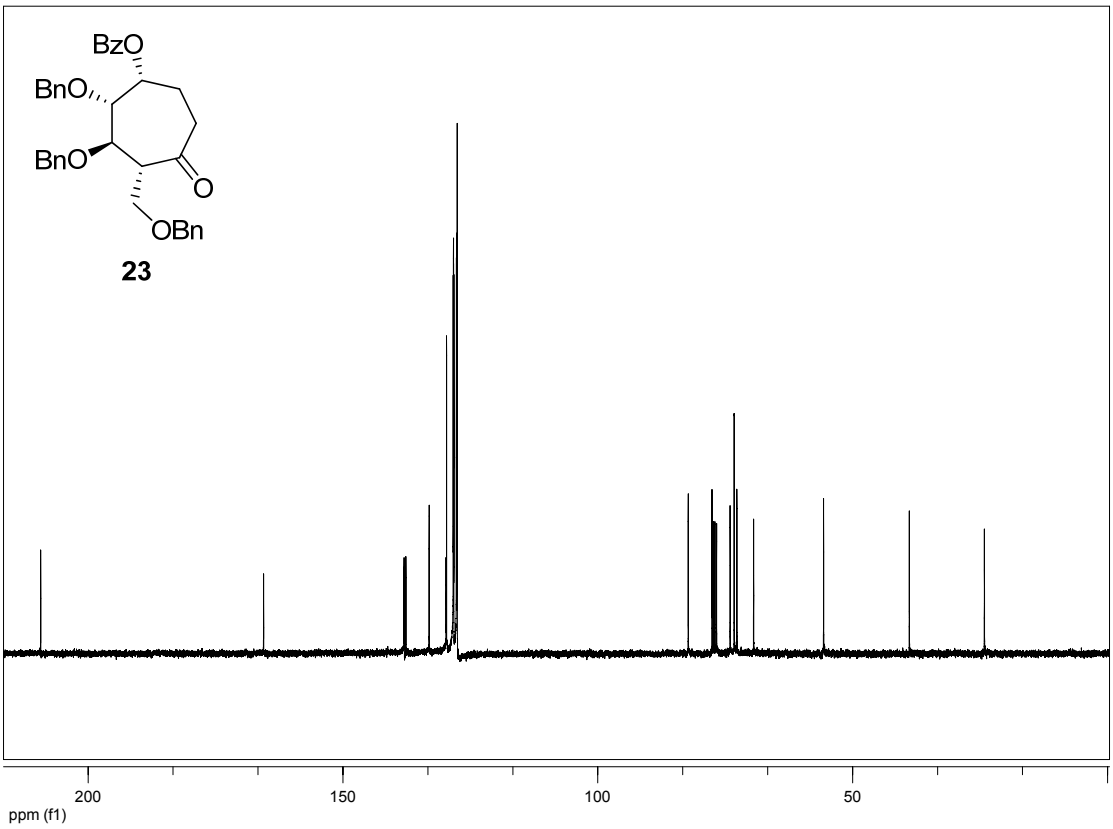


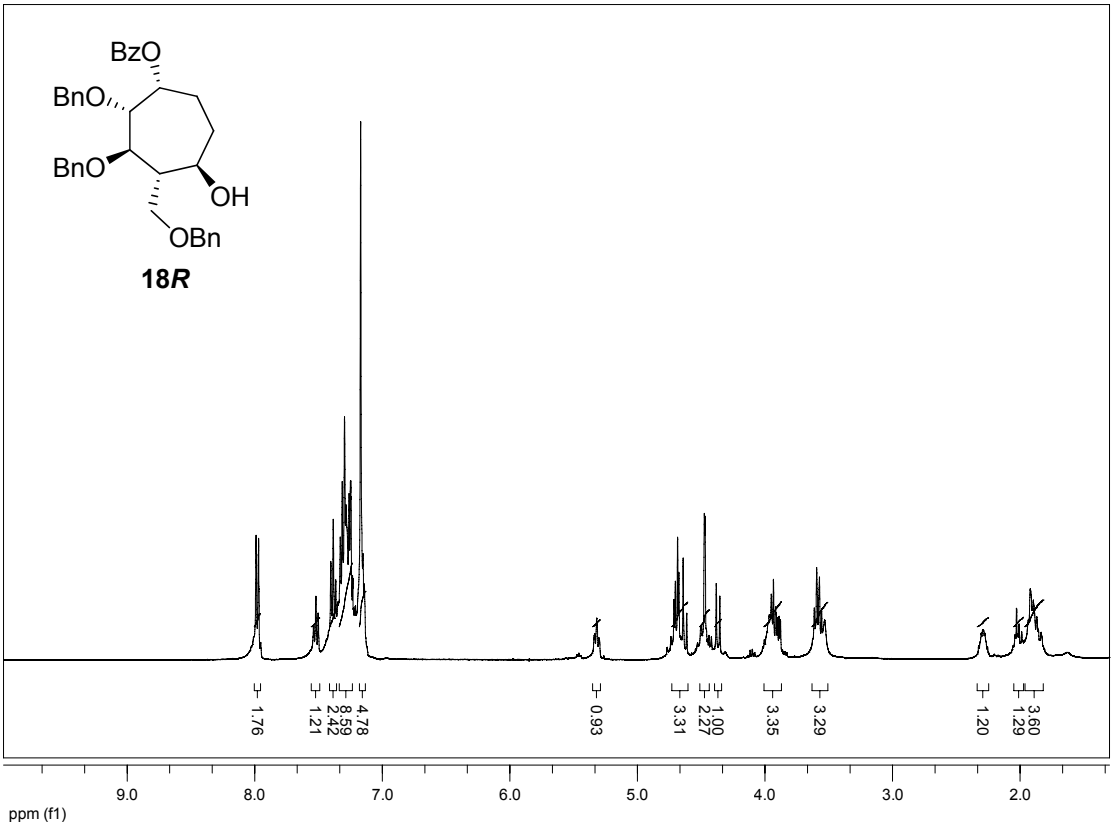
16*R*

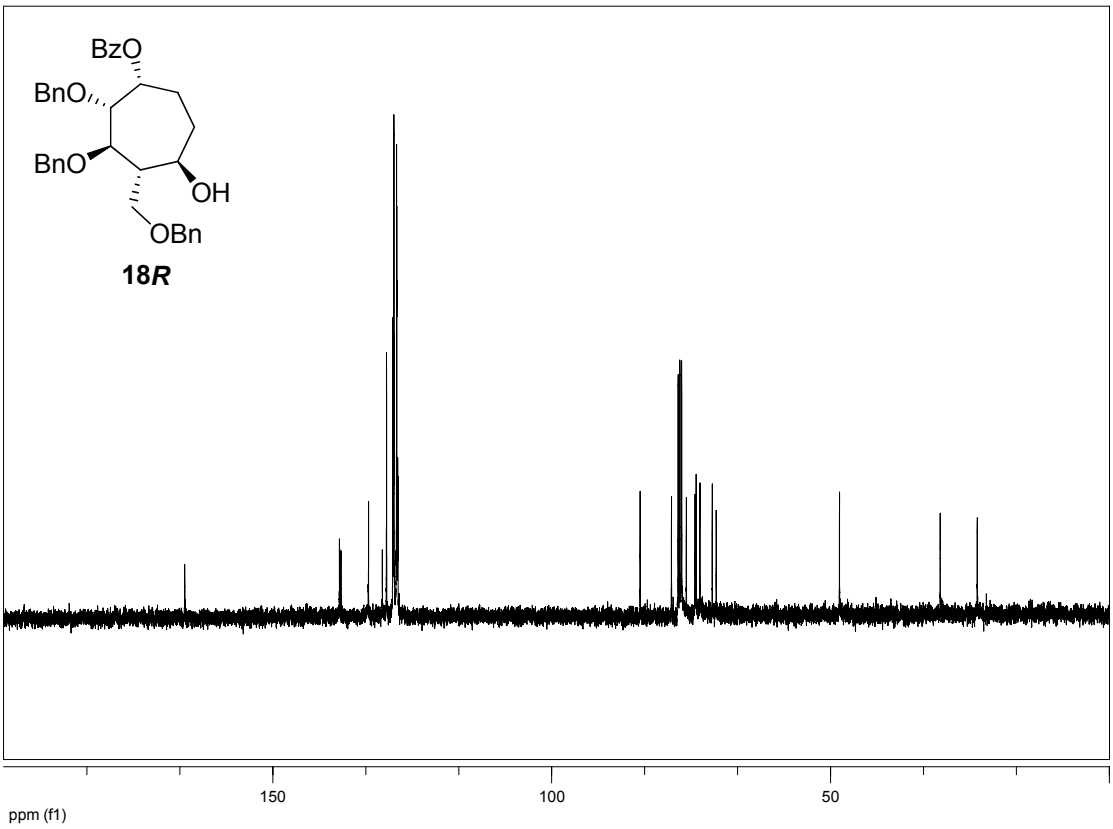


16*R*



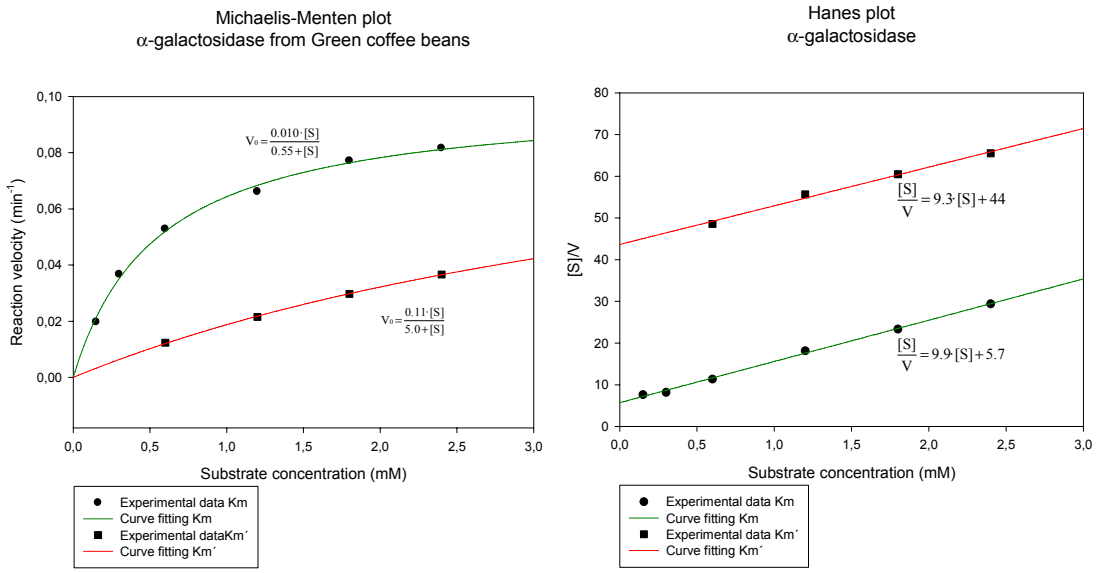




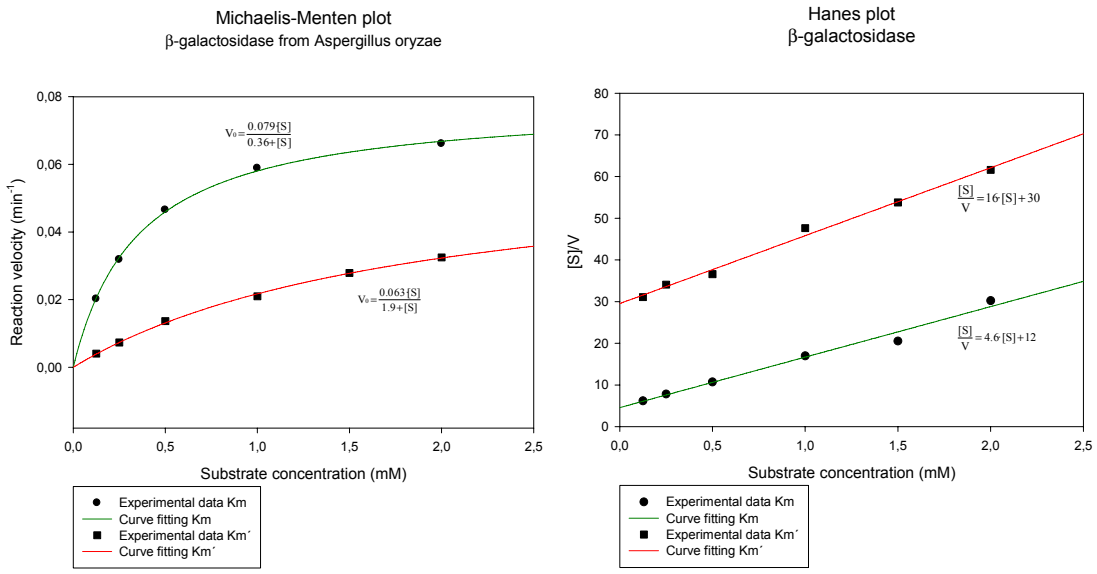


Michaelis-Menten and Hanes plots

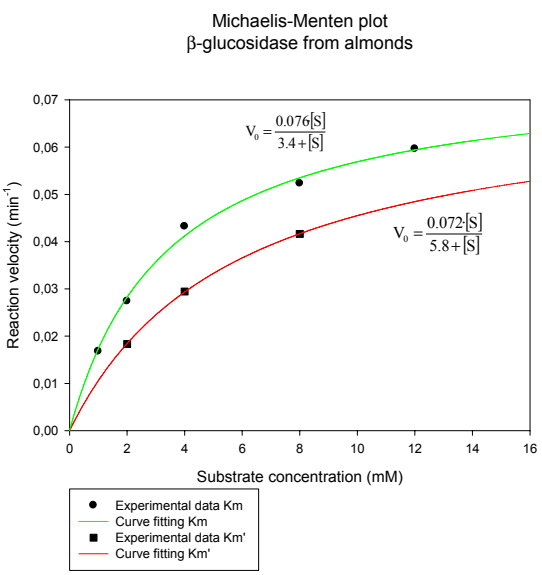
Michaelis-Menten and Hanes plots for inhibition of green coffee bean α-galactosidase, *Asp. oryzae* β-galactosidase, almonds β-glucosidase and *Thermotoga maritima* β-glucosidase. Inhibition of yeast α-glucosidase and *E. coli* β-galactosidase could not be detected at 1 mM.

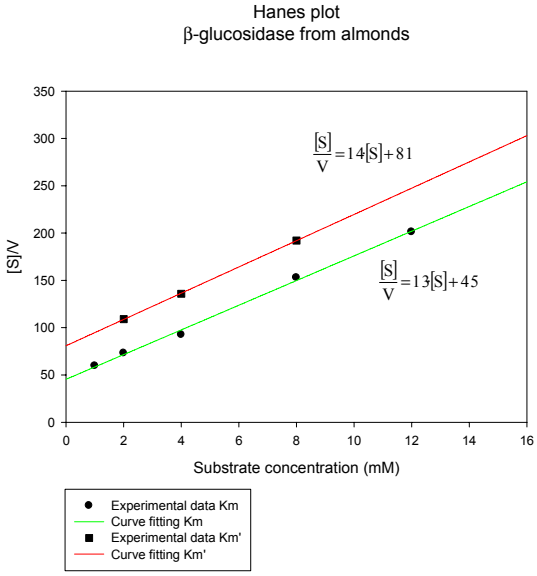


[I] = 20 μM, K_i = 2.5 μM

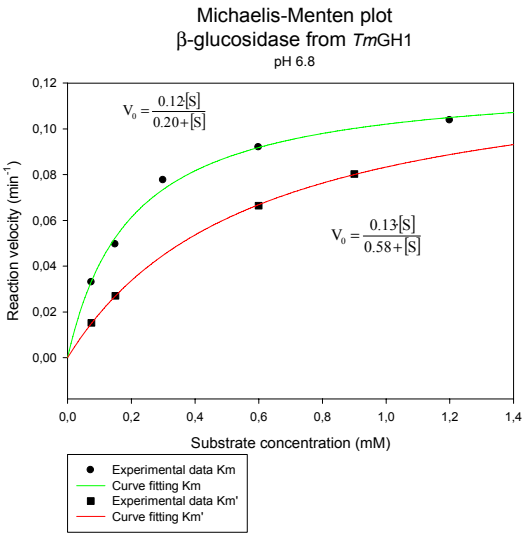


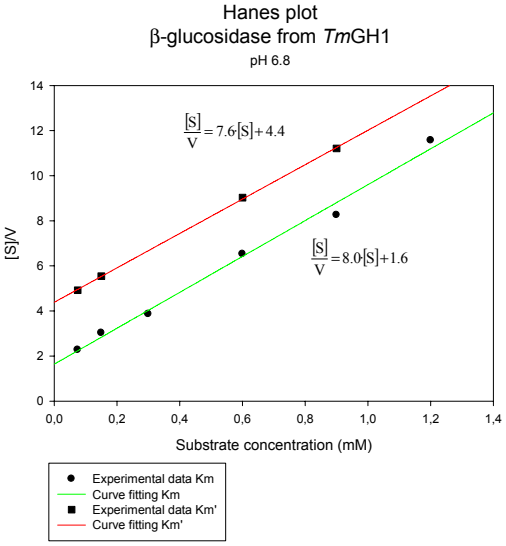
[I] = 100 μM, K_i = 23 μM



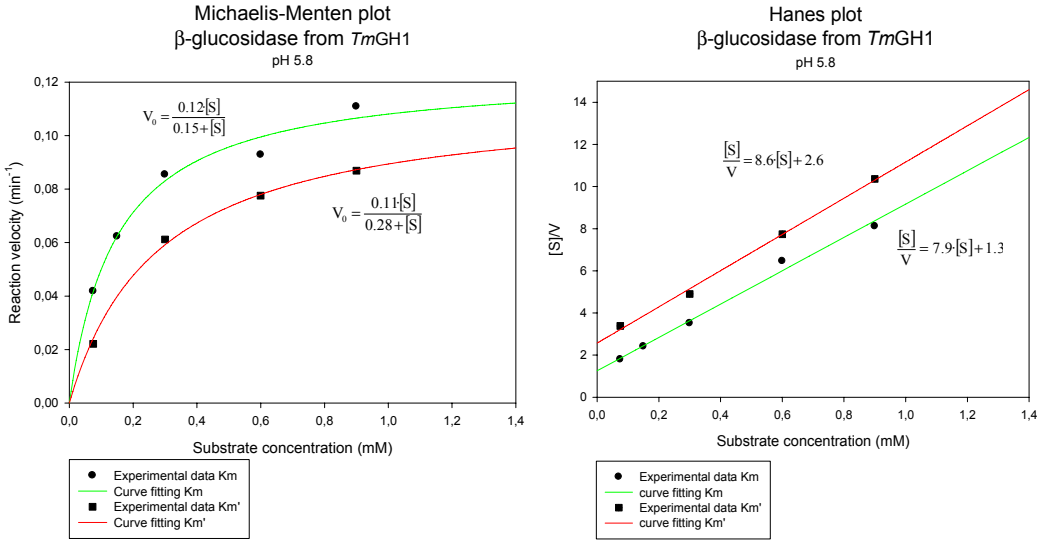


[I] = 1 μM, K_i = 1.5 μM





[I] = 250 nM, K_i = 140 nM



[I] = 1 μM, K_i = 1.1 μM

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