Bicyclic Amino acid-Carbohydrate-Conjugates as Conformationally Restricted Hydroxyethylamine (HEA) Transition-State Isosteres

Sangram S. Kale,[a] Sanjay T. Chavan,[b] Sushma G. Sabharwal,[b] Vedavati G. Puranik[c] and Gangadhar J. Sanjayan,*[a]

[a]Division of Organic Chemistry, [b]Biochemistry Division, University of Pune, Pune-411007, [c]Center for Materials Characterization, National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411 008, India. E-mail: gj.sanjayan@ncl.res.in

General Methods.

Unless otherwise stated, all the chemicals and reagents were obtained commercially. Dry solvents were prepared by the standard procedures. Analytical Thin Layer Chromatography was done on precoated silica gel plates (Kieselgel $60F_{254}$, Merck). Unless otherwise stated Column Chromatographic purifications were done with 100-200 Mesh Silica gel. NMR spectra were recorded in $CDCl_3$ on AV 200 MHz, or AV 400 MHz spectrometers. All chemical shifts are reported in δ ppm downfield to TMS and peak multiplicities as singlet (s), doublet (d), quartet (q), broad singlet (bs), and multiplet (m). IR spectra were recorded in $CHCl_3$.

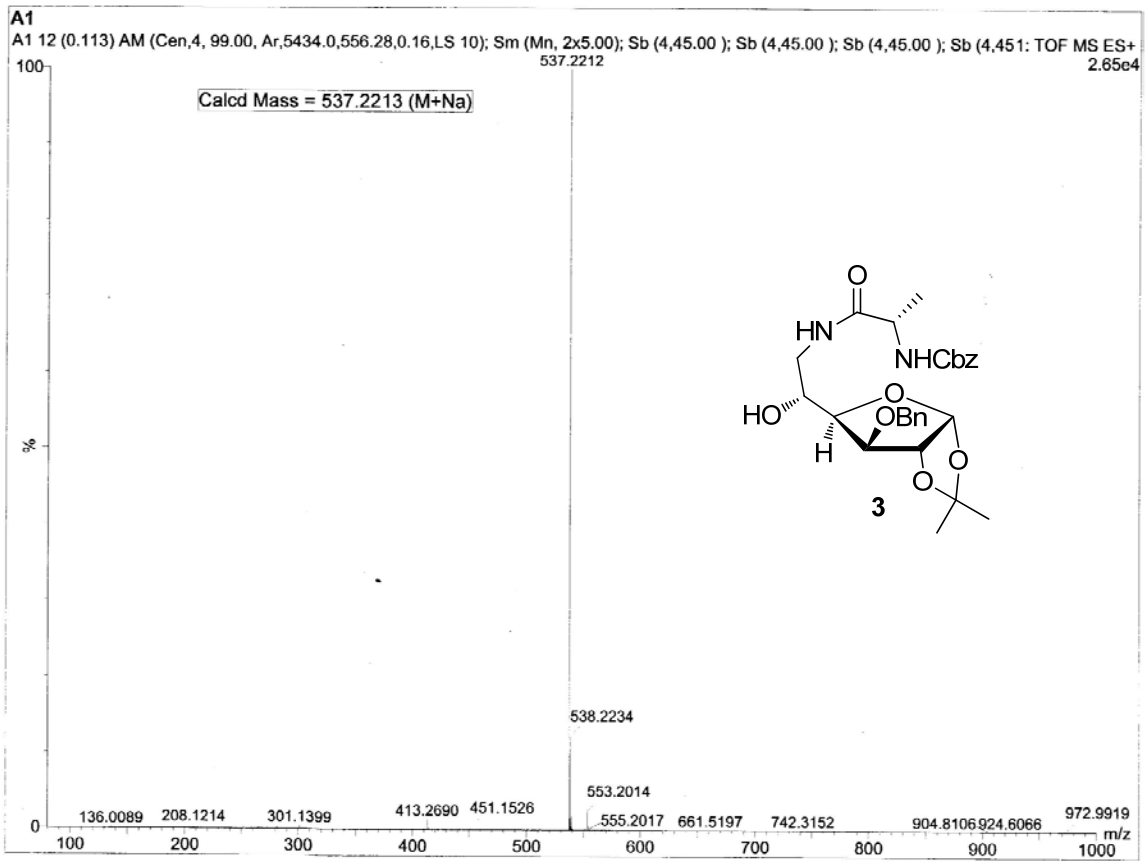
Experimental Procedures:

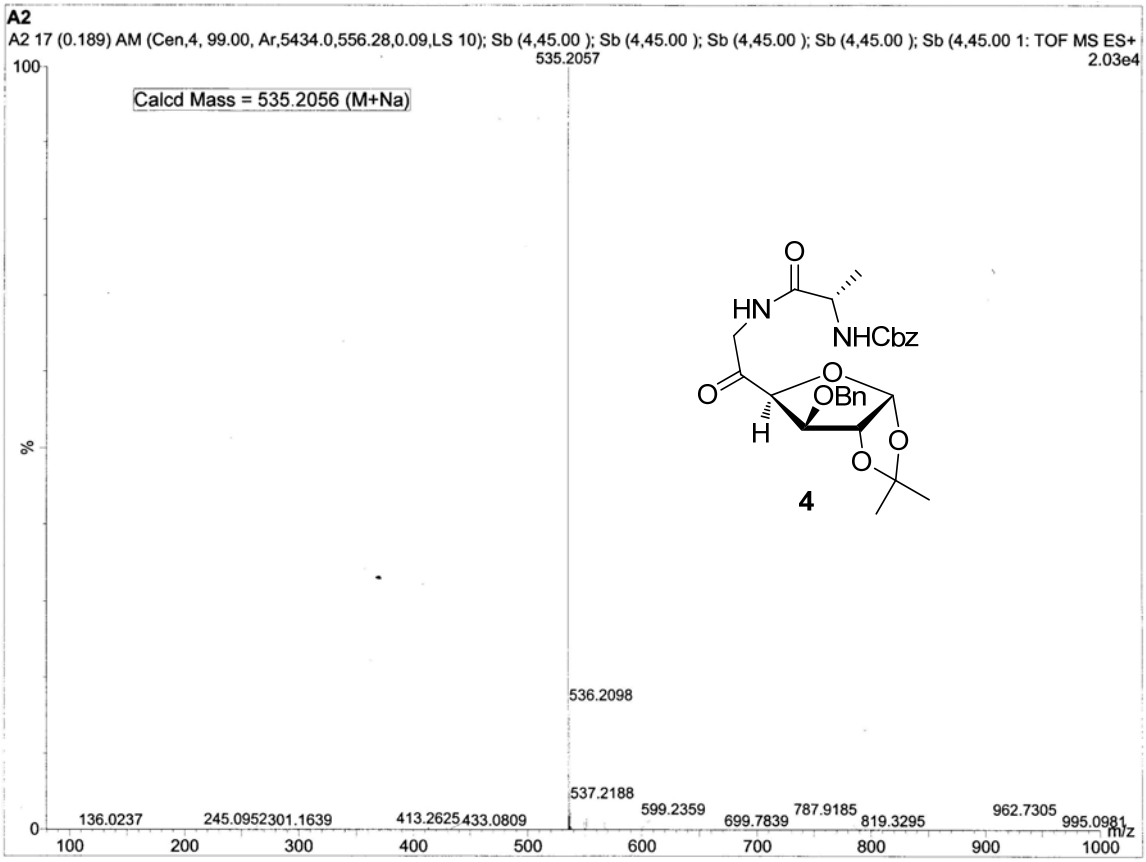
Benzyl((2S)-1-(((2R)-2-((3aR,5R,6aR)-6-(benzyloxy)-2,2dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-2-hydroxyethyl)amino)-1-oxopropan-2-yl)carbamate 3. PPh_3 (0.46 g, 1.79 mmol) was added to a solution of **2**[16] (0.4 g, 1.19 mmol) in THF:H_2O [15 mL, 4:1 (v/v)] and was stirred for 3h at room temperature. THF was evaporated completely and the residue was extracted with ethyl acetate, dried over Na_2SO_4 and concentrated. The residue containing the free amine (not purified) was dissolved in dry acetonitrile (20 mL) at 0^oC, and to this mixture was added sequentially Cbz-Ala-OH (0.21 g, 0.97 mmol), HOBT (0.18 g, 0.87 mmol), EDC.HCl (0.22 g, 0.11 mmol) and DMAP (0.14 g, 0.15 mmol). The reaction mixture was allowed to stir at r.t. for 12 h. The reaction mixture was diluted with ethyl acetate (15 mL) and washed with $NaHCO_3$, water, and the organic layer was separated and dried over Na_2SO_4, concentrated and purified by column chromatography to furnish **3** (0.45 g, 73%); *Rf* 0.35 (pet. ether/ethyl acetate = 40/60); mp: 91.5-93.2 ^oC; $[\alpha]^{25}_D$= -11.91 (c 4.7, $CHCl_3$); IR (ν) $CHCl_3$, (cm^{-1}) 3431, 3018, 1697, 1665, 1215, 1074; ^1H NMR (200 MHz, $CDCl_3$) δ: 7.38- 7.28 (m, 10H), 6.87-6.81 (m, 1H), 5.90-5.86 (d, *J* = 4.0 Hz, 1H), 5.56-5.53 (d, *J* = 6.0 Hz, 1H), 5.14-5.00 (dd, *J* = 16.0, 12.0 Hz, 2H), 4.72- 4.57 (m, 3H), 4.29-4.17 (m, 1H), 4.09- 4.03 (m, 3H), 3.74- 3.65 (m, 2H), 3.36-3.26 (m, 1H), 1.45 (s, 3H), 1.38-1.35 (d, *J* = 6.0 Hz, 3H), 1.29 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 174.3, 155.8, 137.3, 136.0, 128.3, 127.9, 127.8, 127.6, 111.5, 104.5, 82.1, 81.5, 80.7, 72.2, 68.0, 60.8, 26.5, 26.0,18.7. HRMS calcd. for $C_{27}H_{34}N_2O_8Na$, 537.2213; Found, 537.2212. Elemental analysis calcd. for $C_{27}H_{34}N_2O_8$: Anal. C, 63.02; H, 6.66; N, 5.44; Found: C, 62.63; H, 6.92; N, 5.66.

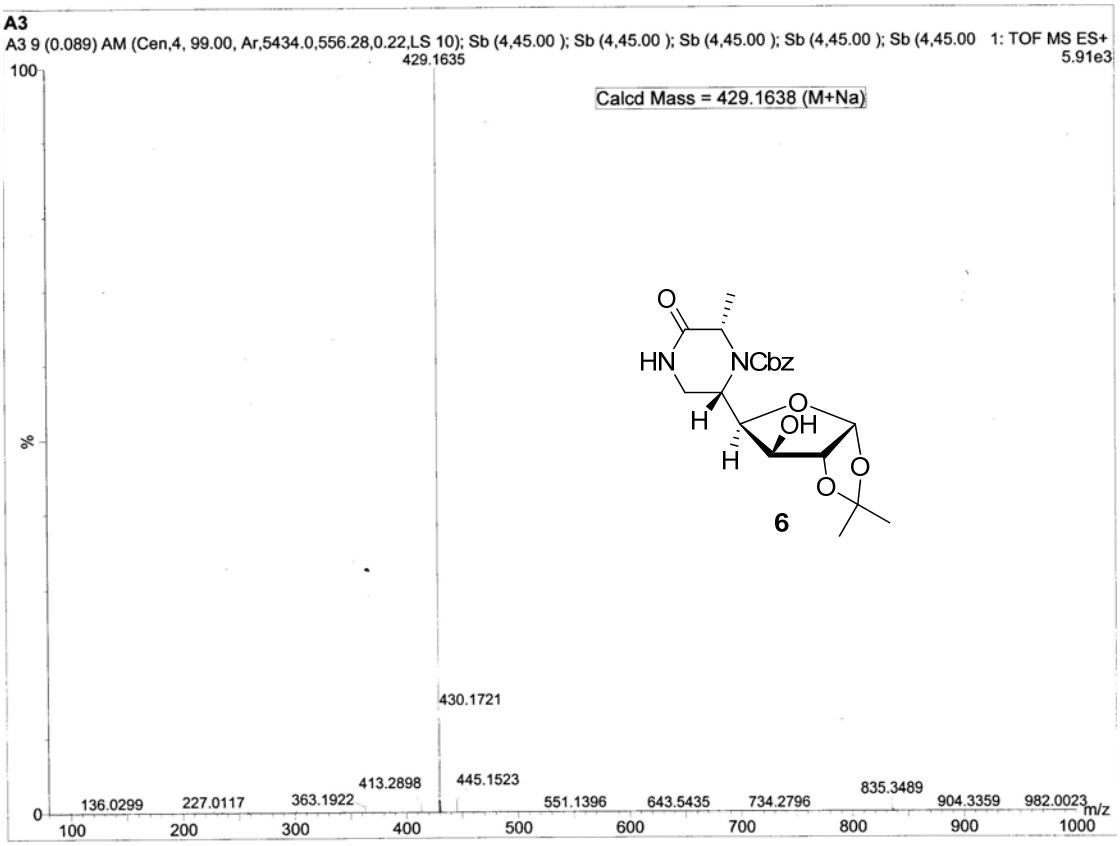
benzyl((2S)-1-((2-((3aR,5S,6aR)-6-(benzyloxy)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)-2-oxoethyl)amino)-1-oxopropan-2-yl)carbamate 4. To a stirred solution of **3** (5.8 g, 11.3 mmol) in dry DCM (30 mL) containing 4Å molecular sieves (6g) was added pyridinium chlorochromate (3.65 g, 16.9 mmol) at 0°C followed by NaOAc (0.46 g, 5.65 mmol). After the addition, the ice bath was removed and the reaction mixture was stirred at r.t. for 5 h. DCM was evaporated and the residue dissolved in ethyl acetate (50 mL), filtered through celite, the organic layer was concentrated, and the residue thus obtained was purified by column chromatography to yield **4** (4.2 g, 72%); *Rf* 0.40 (pet ether /ethyl acetate = 40/60); $[\alpha]^{25}_{D}$= -50.78 (c 1.03, $CHCl_3$); IR (ν) $CHCl_3$, (cm^{-1}) 3018, 1720, 1685, 1676, 1217, 1074; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.37-7.33 (m, 6H), 7.31-7.28 (m, 2H), 7.19- 7.14 (m, 2H), 6.55 (m, 1H), 6.08-6.07 (d, *J* = 3.4 Hz, 1H), 5.39-5.36 (m, 1H), 5.12 (s, 2H), 4.76-4.74 (d, *J* = 3.8 Hz, 1H), 4.60- 4.55 (m, 2H), 4.47-4.41 (m, 1H), 4.40-4.34 (m, 2H), 4.29-4.26 (2H), 1.47 (s, 3H), 1.41-1.38 (d, *J* = 7.0 Hz, 3H), 1.32 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 203.4, 172.2, 155.8, 136.5, 136.1, 128.5, 128.1, 128.0, 112.6, 106.0, 84.6, 83.5, 81.6, 72.4, 66.9, 50.4, 48.6, 26.9, 26.2, 18.9; HRMS calcd. for $C_{27}H_{32}N_2O_8Na$, 535.2056; Found, 535.2057. Elemental analysis calcd. for $C_{27}H_{32}N_2O_8$: Anal. C, 63.27; H, 6.29; N, 5.47; Found: C, 62.94; H, 6.09; N. 5.88.

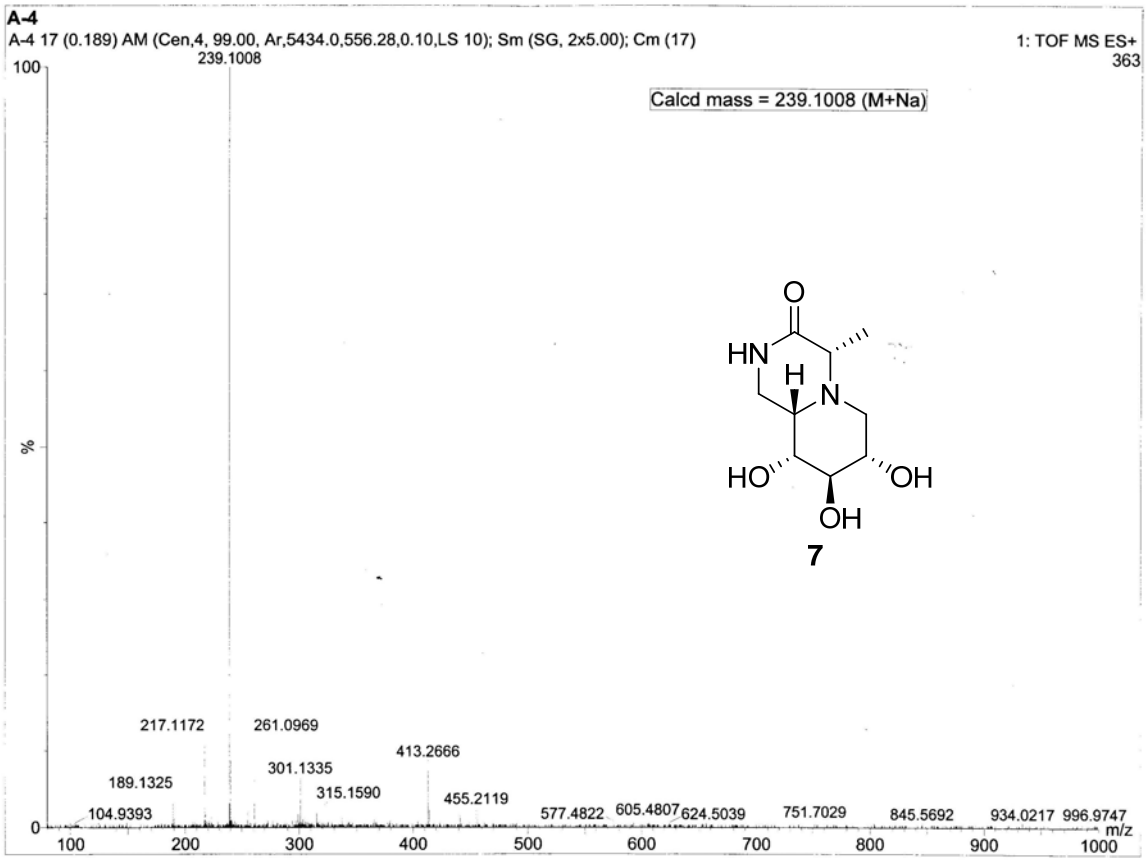
(2R,6S)-benzyl-6-((3aR,5R,6aR)-6-hydroxy-2,2-dimethyltetrahydrofuro[2,3d][1,3]dioxol-5-yl)-2-methyl-3-oxopiperazine-1-carboxylate 6. To a solution of **4** (0.5 g, 0.97 mmol) in methanol (15 mL) was added 20% $Pd(OH)_2$ (0.12 g). The reaction mixture was stirred at 120 psi for 24 h. The catalyst was filtered through celite and the filtrate was evaporated to give a thick oil of **5**, which was carried forward to the next reaction without

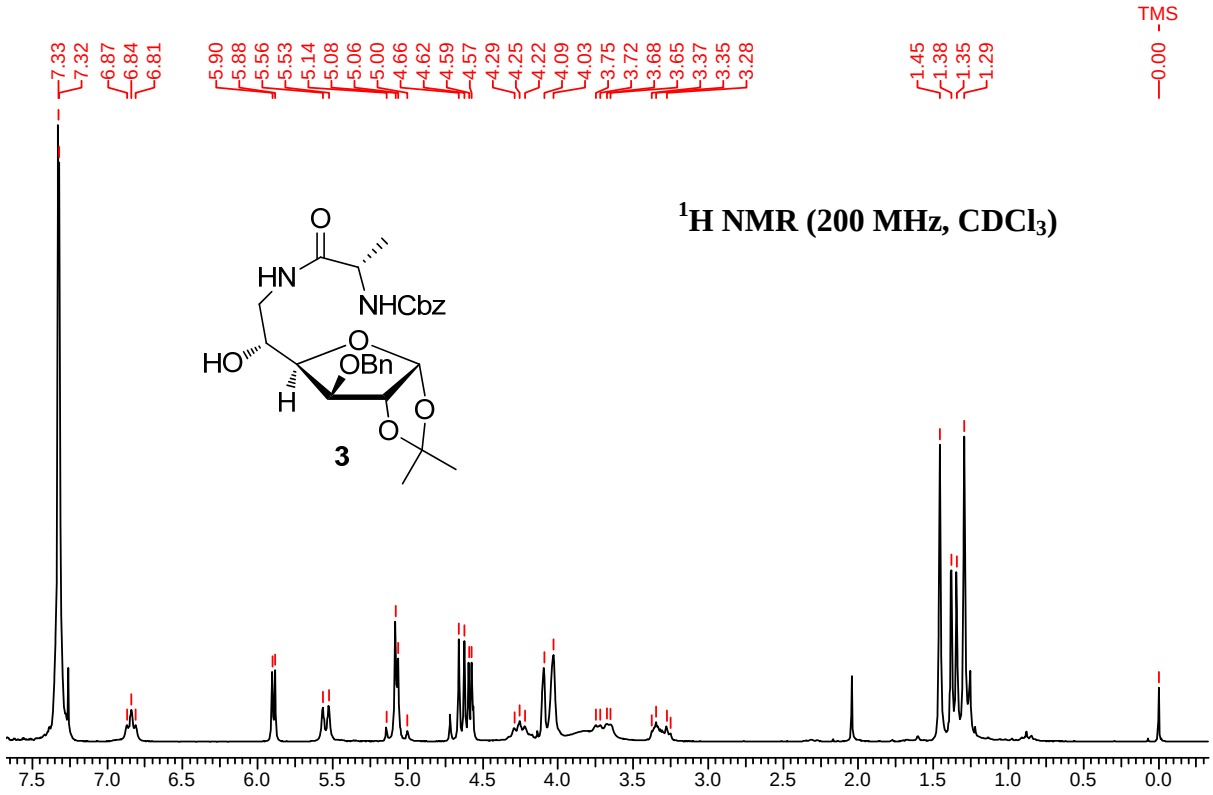
further purification. To a solution of **5** (0.4 g, 1.47 mmol), in methanol-water (40 mL, 9:1) was added sodium bicarbonate (0.37 g, 4.41 mmol) and benzylchloroformate (0.38 g, 2.2 mmol) at 0 °C and the reaction mixture was stirred for 3 h. The reaction mixture was stripped off the solvent under reduced pressure and the residue was extracted with DCM (2 X 20 mL). The organic layer was dried with Na_2SO_4, concentrated, and purified by column chromatography to yield **6** (0.24 g, 60%); *Rf* 0.3 (pet ether /ethyl acetate = 5/95);); mp: 117.1-119.7 ^oC; $[\alpha]^{22}_D$= -18.18 (c 1.1, $CHCl_3$); IR (ν) $CHCl_3$, (cm^{-1}) 3406, 3325, 1694, 1674, 1418; ^1H NMR (400 MHz, $CDCl_3$) δ: 7.34 (s, 5H), 6.49 (bs, 1H), 5.89 (d, *J* = 3.3 Hz, 1H), 5.19-5.12 (dd, *J* = 18.0, 12.0 Hz, 2H), 4.83-4.82 (m, 1H), 4.59-4.54 (m, 2H), 4.36-4.33 (d, *J* = 10.0 Hz, 1H), 4.12-4.08 (m, 2H), 3.58-3.55 (m, 1H), 3.35-3.31 (m, 1H), 1.57-1.55 (d, *J* = 7.0 Hz, 3H), 1.43 (s, 3H), 1.27 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 170.6, 155.2, 135.8, 128.5, 128.2, 127.7, 111.5, 104.5, 85.5, 77.5, 74.5, 67.8, 57.8, 47.2, 42.4, 26.6, 26.0, 19.0; HRMS calcd. for $C_{20}H_{26}N_2O_7Na$, 429.1638; Found, 429.1635. Elemental analysis calcd. for $C_{20}H_{26}N_2O_7$: Anal. C, 59.10; H, 6.45; N, 6.89; Found: C, 59.41, H, 6.65, N, 6.55.



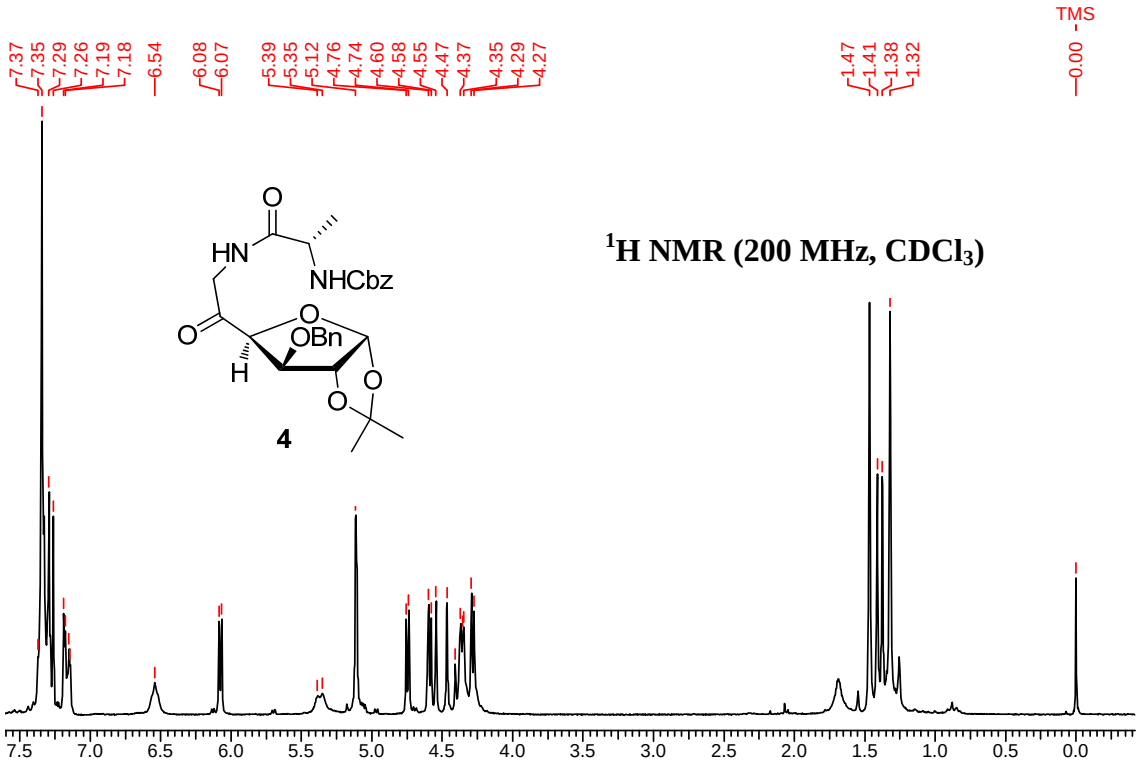




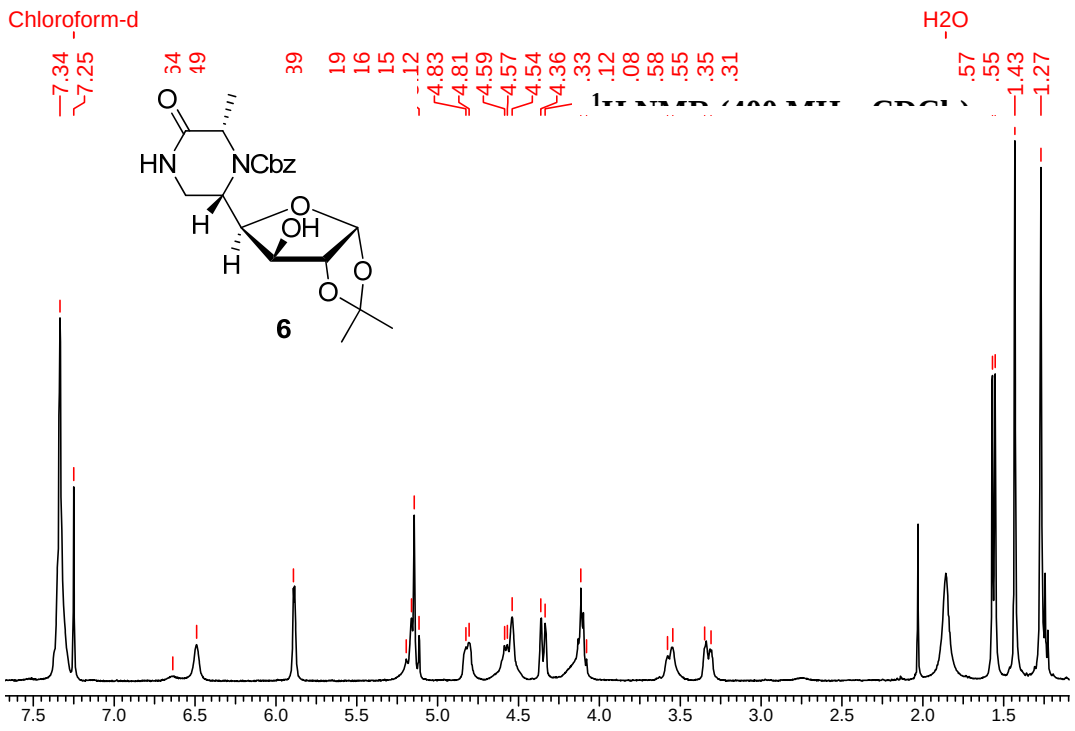


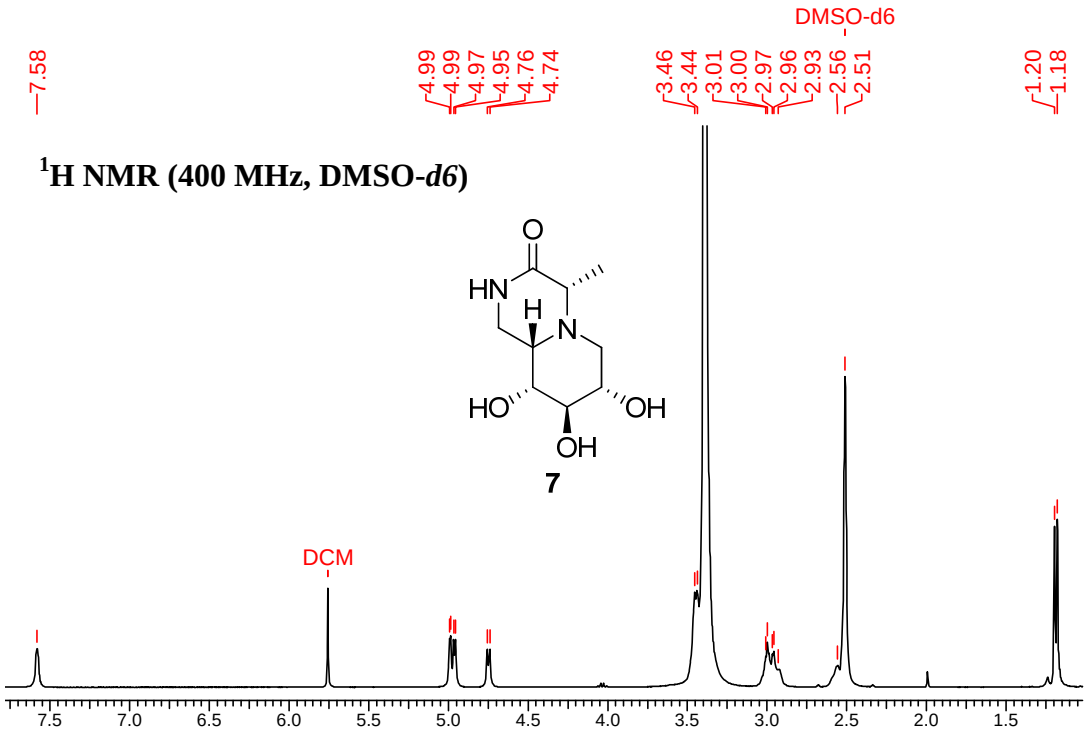


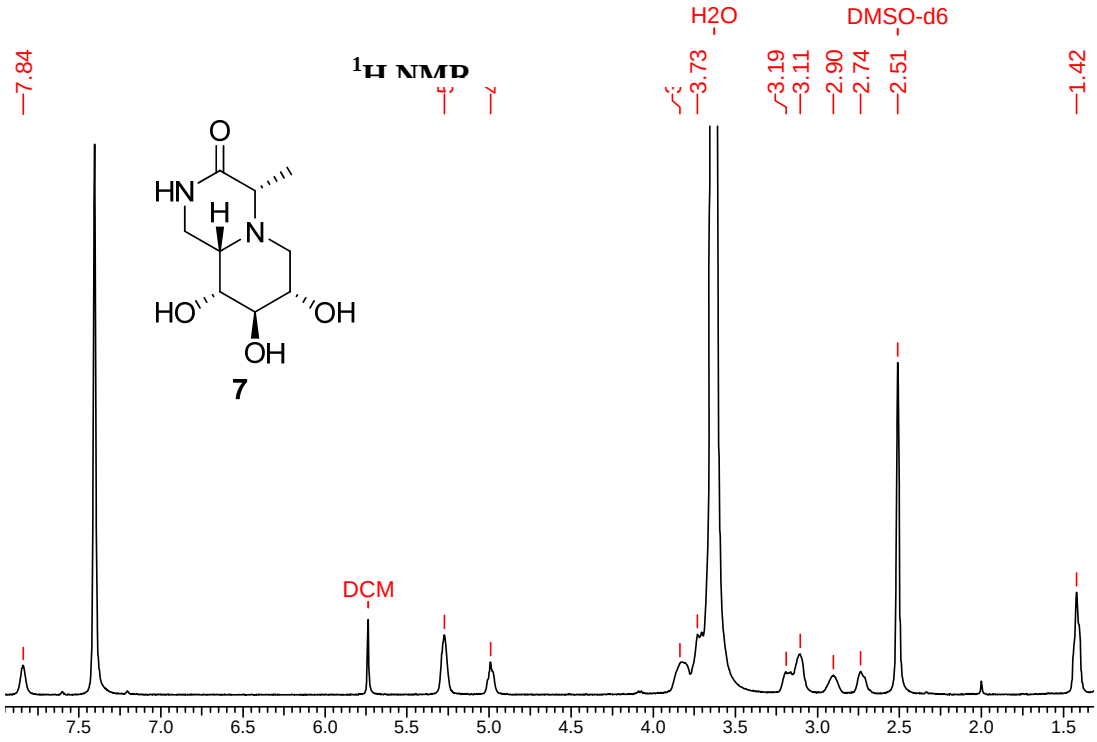
^{1}H NMR (200 MHz, $CDCl_3$)

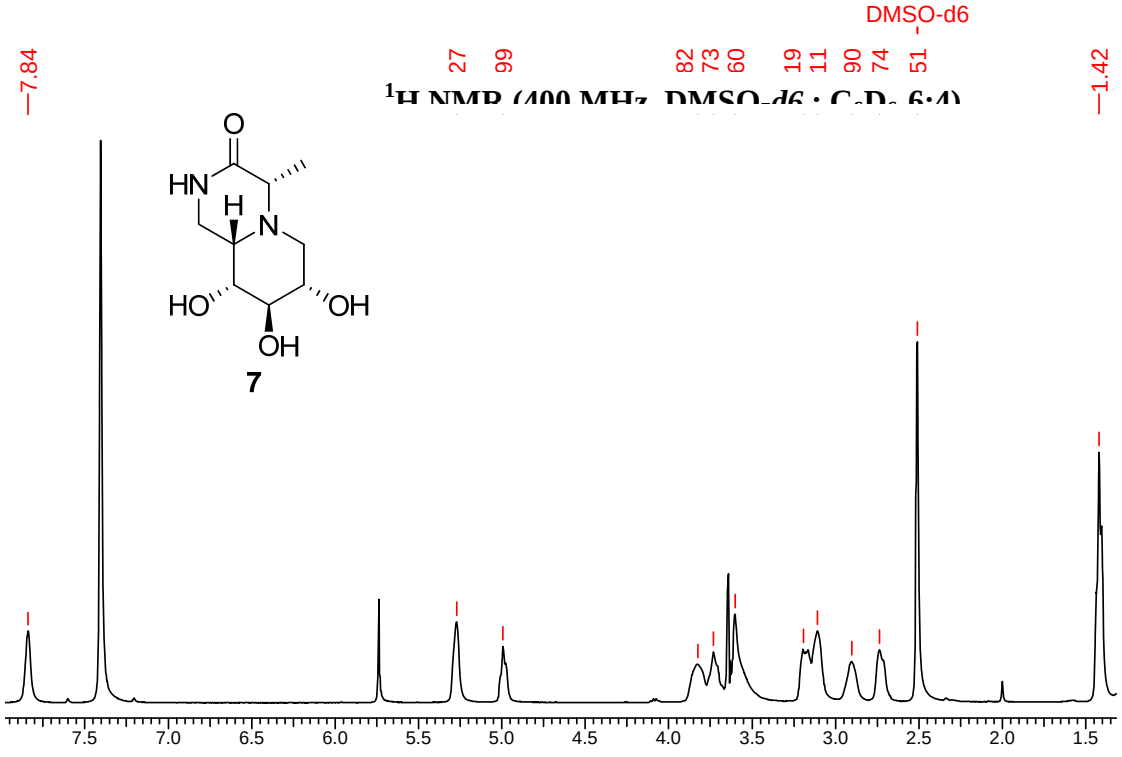


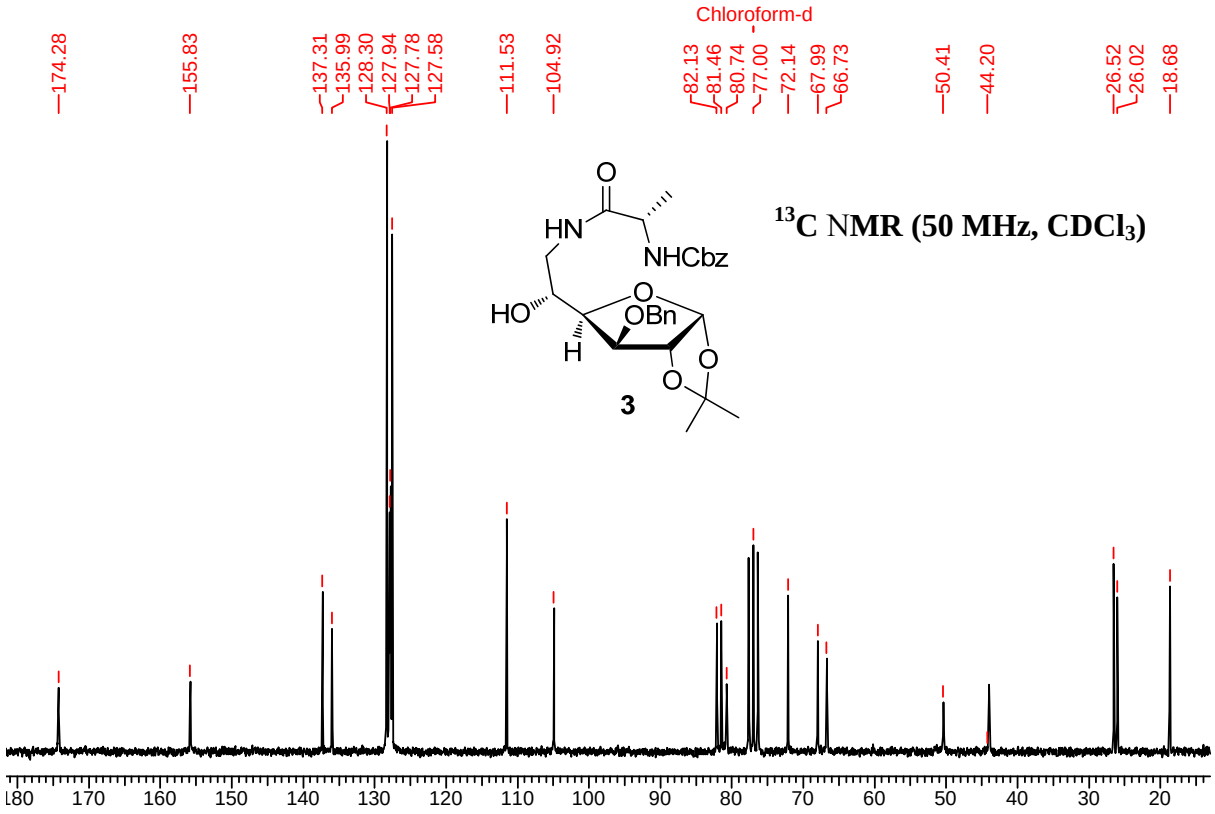
^{1}H NMR (200 MHz, $CDCl_3$)



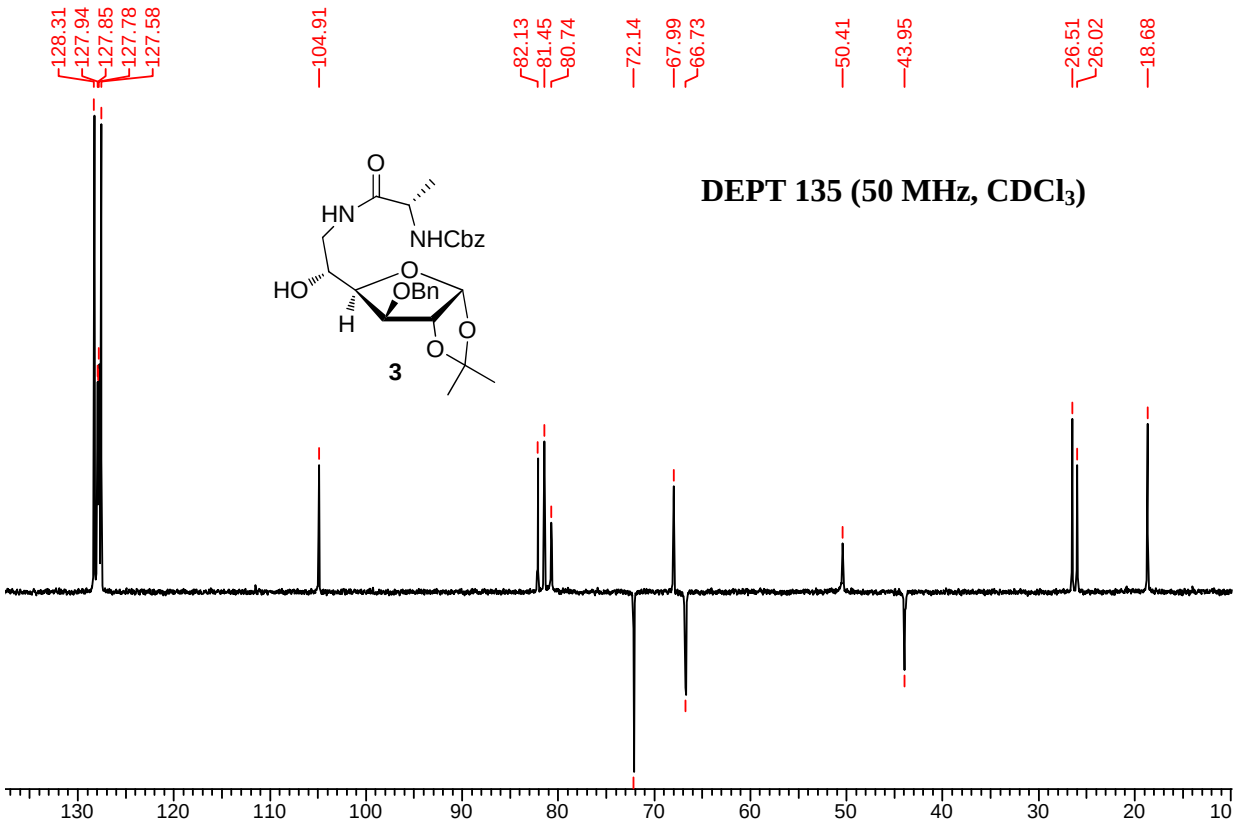




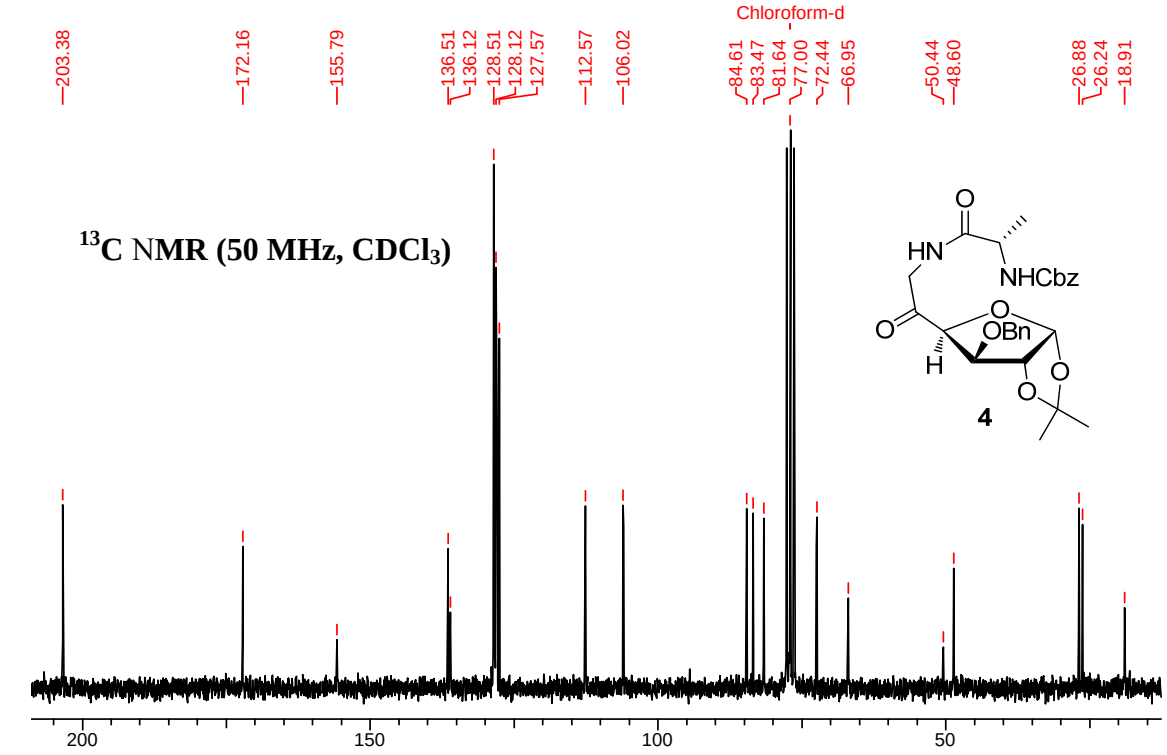




^{13}C NMR (50 MHz, $CDCl_3$)

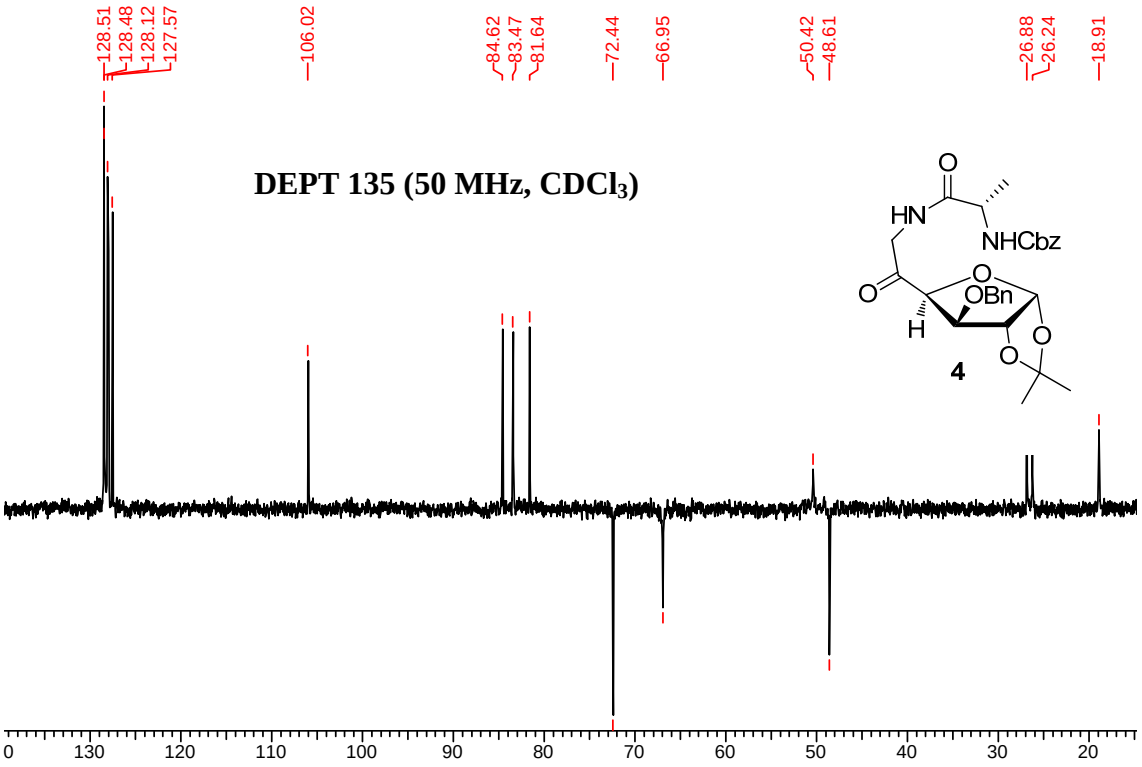


DEPT 135 (50 MHz, $CDCl_3$)



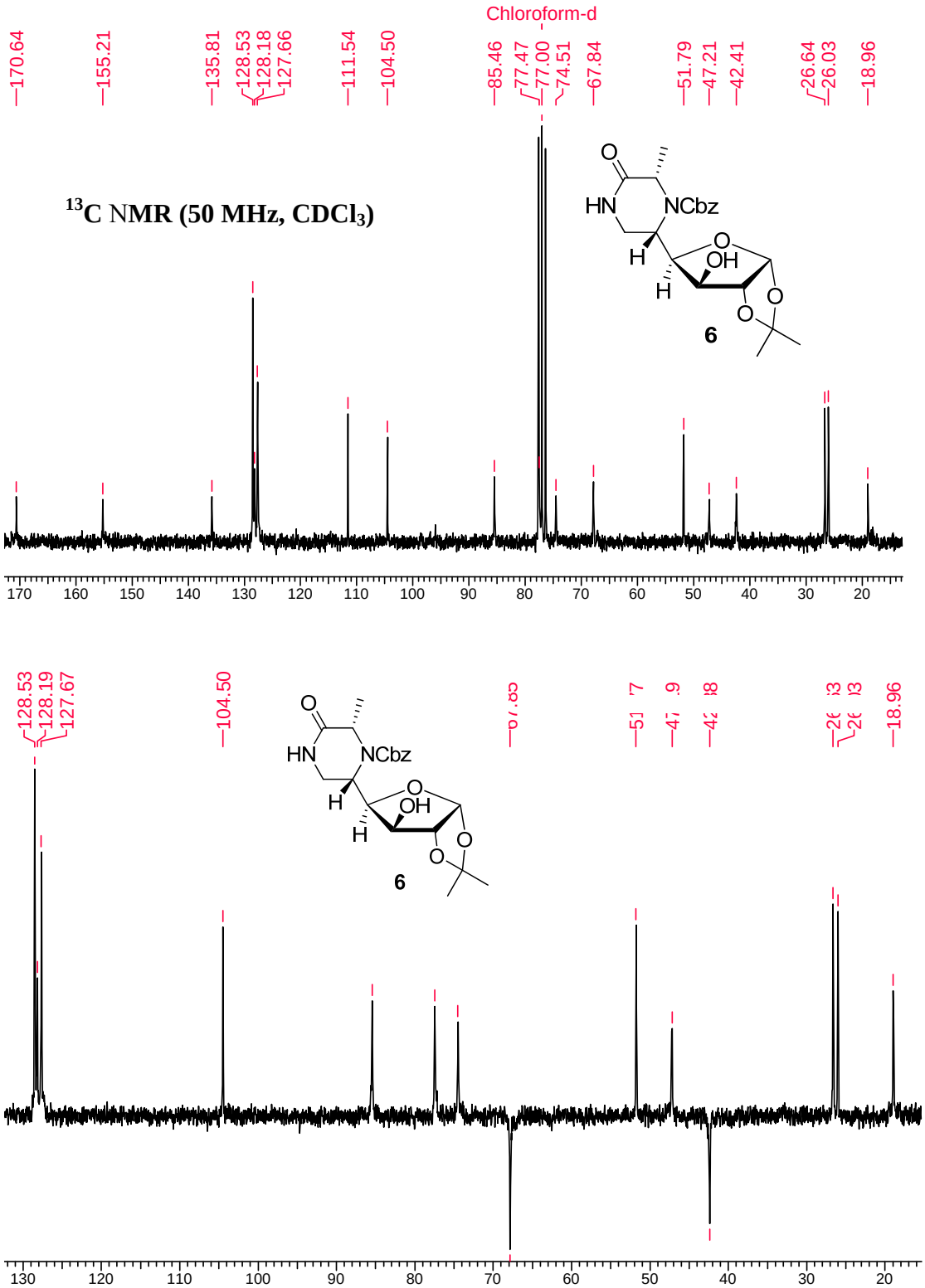
^{13}C NMR (50 MHz, $CDCl_3$)

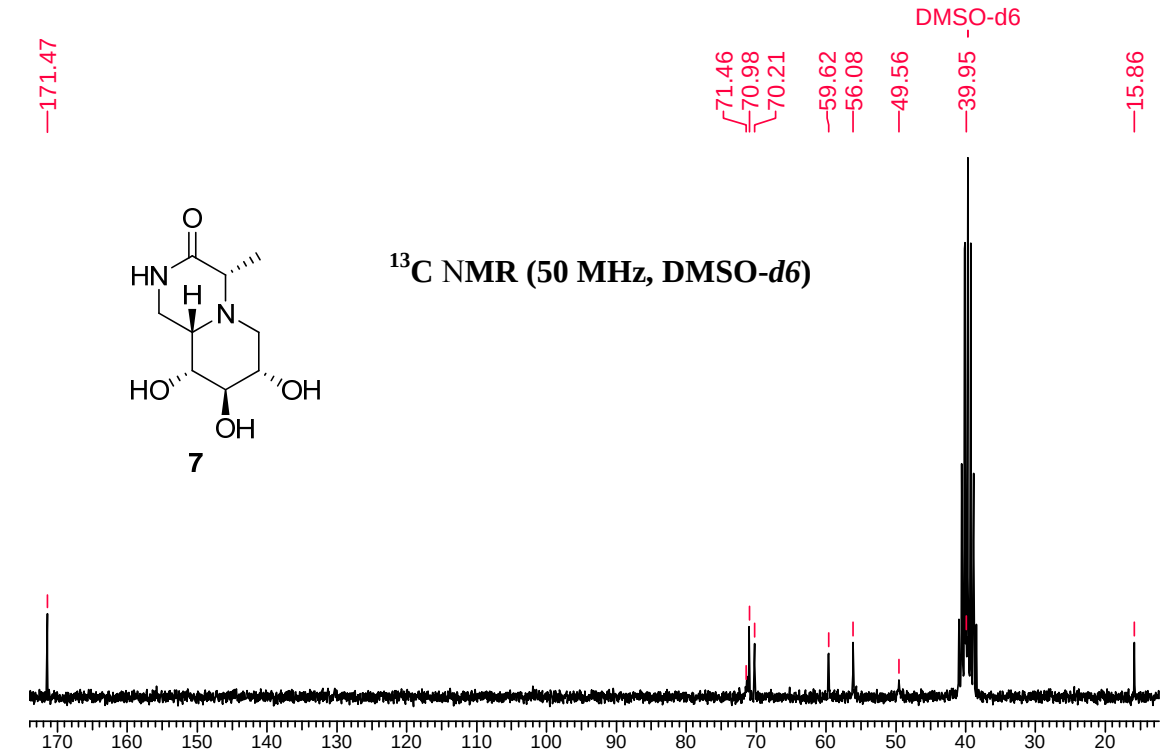
4



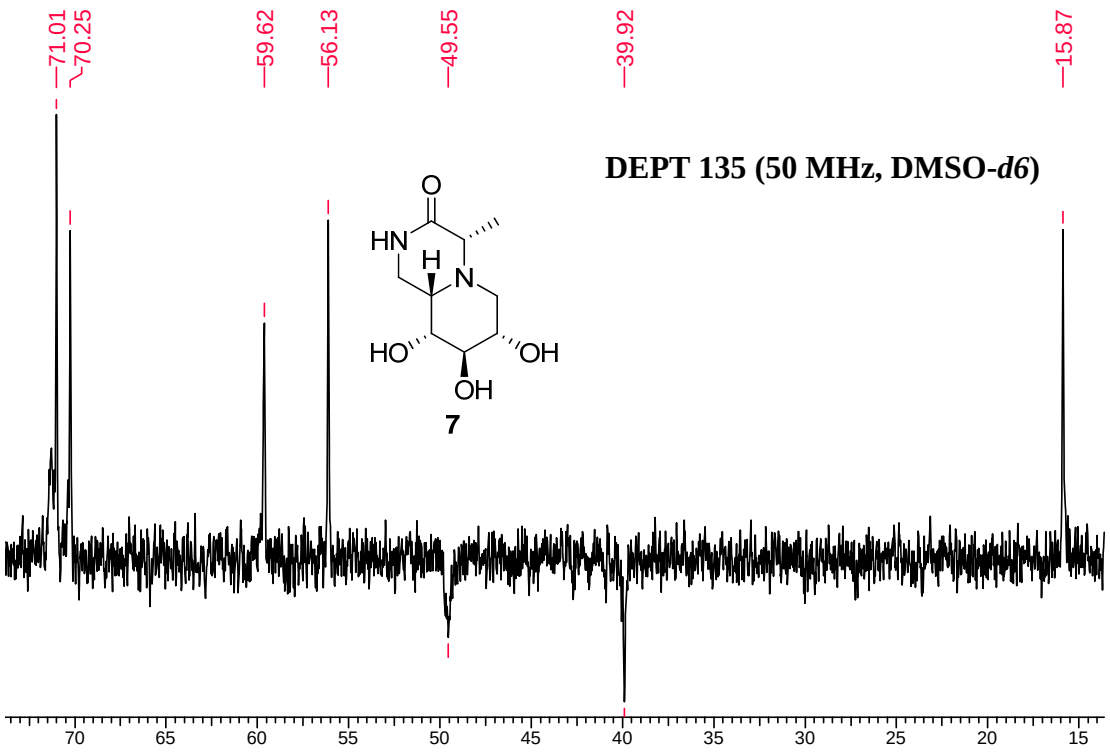
DEPT 135 (50 MHz, $CDCl_3$)

4





^{13}C NMR (50 MHz, DMSO-*d6*)



DEPT 135 (50 MHz, DMSO-*d6*)

General Procedure for the Inhibition Assay:

The substrates: *p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenyl-α-D-galactopyranoside, *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-α-D-mannopyranoside and *p*-nitrophenyl *N*-acetyl-β-D-glucosaminidase were procured from sigma chemicals. The inhibition assay with compound **7** was performed by measuring the residual hydrolytic activities of the glycosidases with 2 mM concentration of *p*-nitrophenyl- glycopyranoside prepared in citrate buffer (0.025 M, pH 4.0) and used for assay. The test compound was pre-incubated with the enzyme, buffered at its optimal pH, for 1 h at 37 ºC (for α-galactosidase at 60 ºC). The enzyme reaction was initiated by the addition of 100 µL of substrate. Reaction was terminated with the addition of 0.05 M borate buffer (pH 9.8) and absorbance of the liberated *p*-nitrophenol was measured at 405 nm with a UV-visible Spectrophotometer. Controls were run simultaneously in the absence of test compound. One unit of glycosidase activity is defined as the amount of enzyme that hydrolyzed 1 µmol of *p*-nitrophenol per minute under assay condition.

Inhibitory potencies of compound **7**.

Enzymes(Source)	Compound % inhibition
α-Galactosidase (Geobacillus toebii BK 1)	NI
β-Galactosidase (Almond)	NI
α-Glucosidase (Baker's yeast)	NI
α-Glucosidase (Rice)	NI
β-Glucosidase (Almond)	NI
α-Manosidase (Jack bean)	NI
N-Acetyl-b-D-glucosaminidase (Jack bean)	NI

NI = No inhibition (data is average of three sets of assay performed)

Inhibitory activity evaluation:

The inhibitory activity of **7** was studied against the following glycosidases, procured from Sigma chemical company: β-Galactosidase and β-glucosidase (isolated from almond seeds), α-galactosidase (isolated from g*eobacillus toebii* BK1), α-galactosidase (isolated from rice), α-mannosidase and N-acetyl-β-D-glucosaminidase (isolated from jack bean seeds) and α-glucosidase (bakers yeast). As seen in the above table, the compound **7** did not show any inhibitory activity against any of the glycosidases tested, under the assay conditions.

Trypsin/Chymotrypsin Inhibitory Studies.

General Procedure for Trypsin/Chymotrypsin Inhibitory Assay[1,2]

Trypsin/chymotrypsin inhibitory activity was determined by the pre-incubation of the inhibitor with the trypsin/chymotrypsin (2.0 mg/ml in 0.01N HCl) in phosphate buffer (pH 7.4, 50 mM) for 60 minutes at 37^0C in a total volume of 2 ml. Trypsin/chymotrypsin reaction was initiated by the addition of casein (2%) and the mixture was incubated at 37^0C for 15 minutes. The enzyme action was arrested by adding 3 ml trichloro acetic acid (TCA) (10% w/v). TCA soluble peptides were collected by centrifugation and estimated by reading the absorbance at 280 nm on UV-visible spectrophotometer and by Lowry's method. One unit of inhibitory activity is defined as the amount of the inhibitor that reduces the trypsin activity by one unit. The enzymes trypsin and chymotrypsin were procured from Sigma Chemicals. The compound **7** showed 15% trypsin inhibitory activity, while no inhibition was observed against chymotrypsin.

References.

1. Robert A. McRorie, William L. Williams. Biochemistry of Mammalian Fertilization. *Annu. Rev. Biochem*. 1974, **43**, 777-802.
2. M.L. Kakade, Simons N, Liener I.E. *Cereal Chem*, 1969, **46**, 518-526.