Concurrent Display of Both α- and β-Turns in a Model Peptide

Deekonda Srinivas[†], *Kuruppanthara N. Vijayadas,* [†] *Rajesh Gonnade*[‡], *Usha D. Phalgune*[§], *P. R. Rajamohanan**[§], *and Gangadhar J. Sanjayan**[†]

† Division of Organic Synthesis, ‡ Center for Materials Characterization, § Central NMR Facility, National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411 008, India;

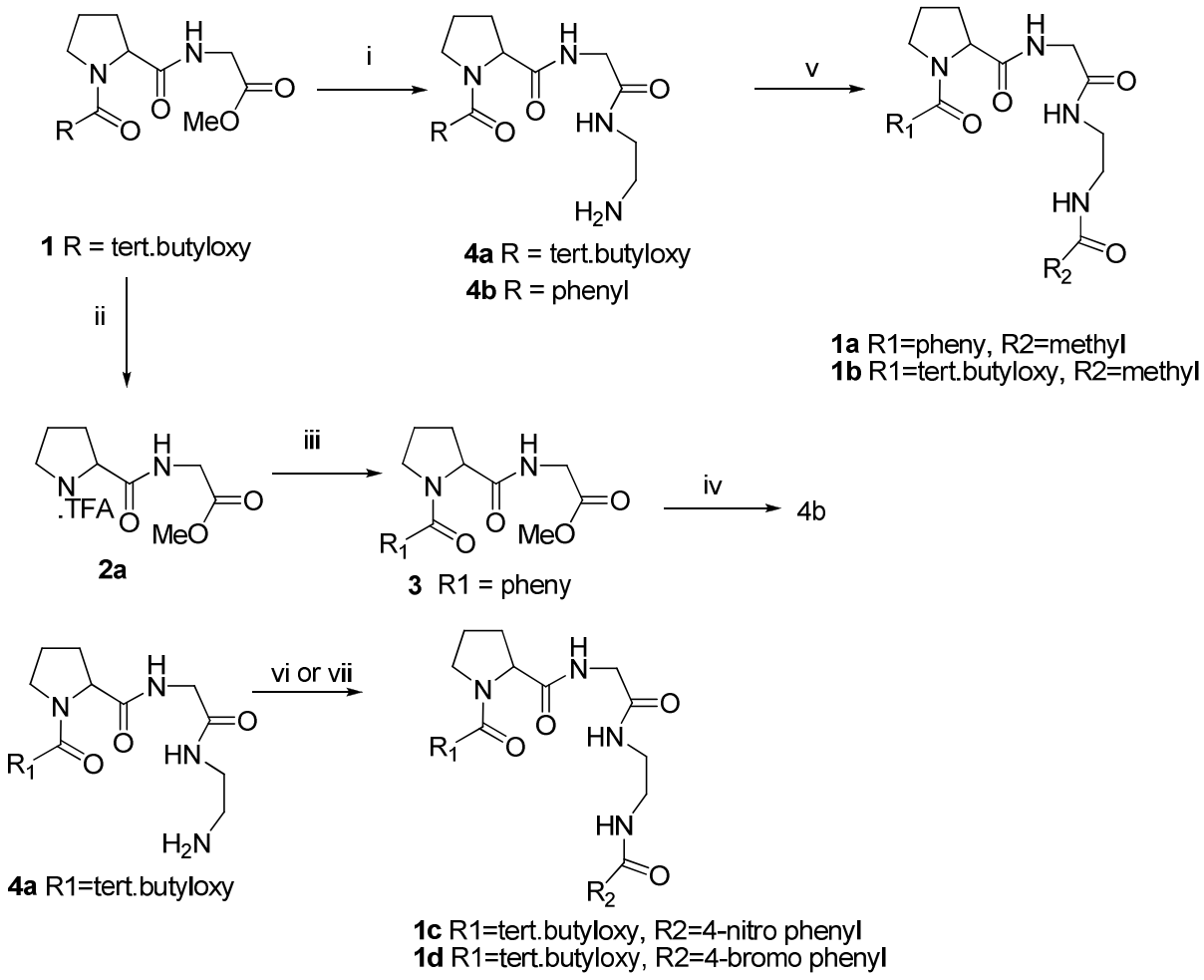
Contents | Pages

General Methods

Unless otherwise stated, all the chemicals and reagents were obtained commercially. Acetonitrile was dried by distilling over calcium hydride and kept it over 4 Å mol sieves, prior to use. Chromatography was done on pre-coated silica gel plates. Column chromatographic purifications were done with 100-200 Mesh Silica gel. NMR spectra were recorded in $CDCl_3$ on Ac 200 MHz or DRX-500 MHz NMR spectrometers. All chemical shifts are reported in δ ppm downfield to TMS and peak multiplicities are reported as singlet (s), doublet (d), quartet (q), broad (br), broad singlet (bs) and multiplet (m). IR spectra were recorded in nujol or $CHCl_3$. Single crystal X-ray data were collected with graphite monochromatized (Mo K_α = 0.71073Å) radiation at room temperature. All the data were corrected for Lorentzian, polarization and absorption effects using SAINT and SADABS programs. SHELX-97 was used for structure solution with full matrix least squares refinement on F^2. Hydrogen atoms were included in the refinement as per the refinement model.

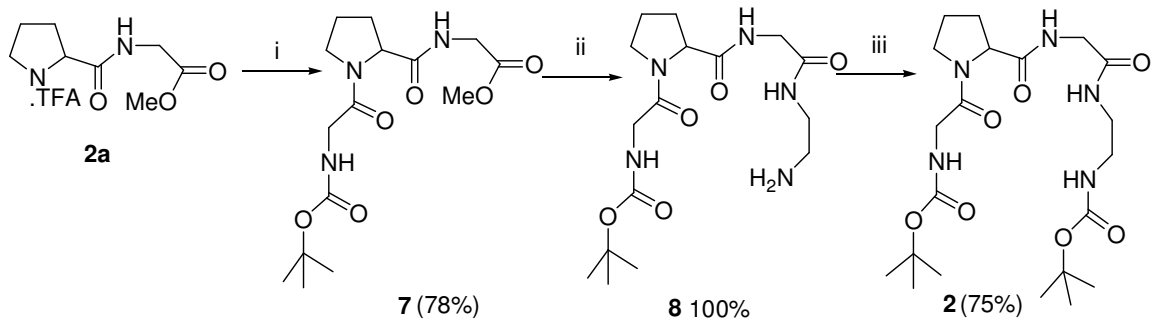
Synthetic Scheme:

Scheme 1



Reagents and conditions: (i) ethylenediamine, methanol, rt, 2h; (ii) trifluoro acetic acid, dichloromethane, rt, 2h; (iii) benzoyl chloride, Et_3N, dry dichloromethane, rt, 5h; (iv) ethylenediamine, methanol, rt, 1h; (v) acetic anhydride, pyridine, rt, 6h; (vi) 4-nitrobenzoic acid, TBTU, DIPEA, dry acetonitrile, rt, 6h; (vii) 4-bromo benzoic acid, TBTU, DIPEA, dry acetonitrile, rt, 6h.

Scheme 2



Reagents and conditions:(i) Boc-Gly-OH, EDCI, triethylamine, dry dichloromethane, rt, 6h; (ii) ethylenediamine, methanol, rt, 2h; (iii) Boc-anhydride, THF, rt, 3h.

Experimental Procedures:

Crystallographic data of 1a: ($C_{18}H_{24}N_4O_4$): M = 360.41, Crystal dimensions 0.76 x 0.27 x 0.18 mm^3, monoclinic, space group $P\ 2_1$, a = 4.7957(14), b = 19.605(6), c = 9.913(3) Å, β = 100.076(4)°, V = 917.7(5) $Å^3$, Z = 2, ρ_{calcd} = 1.304 gcm^{-3}, μ (Mo-K_α) = 0.094 mm^{-1}, *F(000)* = 384, $2\theta_{max}$ = 50.00°, T = 297(2) K, 10080 reflections collected, 4092 unique, 3811 observed ($I > 2\sigma(I)$) reflections, 248 refined parameters, R value 0.0505, $wR2$ = 0.1348 (all data R = 0.0537, $wR2$ = 0.1377), S = 1.070, minimum and maximum transmission 0.970 and 0.983; maximum and minimum residual electron densities +0.269 and –0.195 e $Å^{-3}$.

Crystallographic data of 2: ($C_{21}H_{39}N_5O_8 .H_2O$): M = 489.57, Crystal dimensions 0.54 x 0.38 x 0.07 mm^3, orthorhombic, space group $P\ 2_12_12_1$, a = 9.0917(8), b = 9.8531(9), c = 29.228(2) Å, V = 2618.3(4) $Å^3$, Z = 4, ρ_{calcd} = 1.242 gcm^{-3}, μ (Mo-K_α) = 0.095 mm^{-1}, *F(000)* = 1056, $2\theta_{max}$ = 50.00°, T = 297(2) K, 13265 reflections collected, 4607 unique, 3636 observed ($I > 2\sigma(I)$) reflections, 321 refined parameters, R value 0.0641, $wR2$ = 0.1324 (all data R = 0.0855, $wR2$ = 0.1411), S = 1.116, minimum and maximum transmission 0.9501 and 0.9938; maximum and minimum residual electron densities +0.221 and –0.142 e $Å^{-3}$.

Tert-butyl 2-((methoxycarbonyl)methylcarbamoyl)pyrrolidine-1-carboxylate 1: To an ice-cold stirred solution of the Boc-Pro-OH (1.2 g, 5.58 mmol, 1 equiv.) in dry dichloromethane (15 mL) was added Et_3N (1.94 mL, 13.9 mmol, 2.5 equiv.) and isobutyl chloroformate (0.51 mL, 3.95 mmol, 1 equiv.). The resulting mixture was stirred vigorously for 5 min, and glycine methyl ester (0.62 g, 5.02 mmol, 0.9 equiv.) was added**.** The resulting reaction mixture was stirred for 5 h. The reaction mixture was

diluted with dichloromethane and washed sequentially with potassium hydrogen sulphate solution, saturated sodium bicarbonate, and water. Drying and concentration of the dichloromethane extract under reduced pressure gave the crude product which on column chromatography (40% EtOAc/Hexane) afforded the desired known product **1** (1.2 g, 75%).

[(Pyrrolidine-2-carbonyl)-amino]-acetic acid methyl ester. trifluoro acetate 2a. To an ice-cold stirred solution of **1** (1.0 g, 3.49 mmol) in dichloromethane (10 mL) was added, 50% trifluoro acetic acid-dichloromethane mixture (6 mL). The resulting reaction mixture was stirred at room temperature for 2h. The solvent was stripped off under reduced pressure, and the resultant residue was dissolved in methanol. Methanol was removed under reduced pressure; the process was repeated for two times to remove the excess trifluoro acetic acid from the reaction mixture. The residue was dried under vacuum to yield the desired product **2a** as a white gummy liquid (1.0 g, quantitative), which was used for the next reaction, without further purification.

Methyl 2-(1-benzoyl-pyrrolidine-2-carboxamido)acetate 3. To an ice-cold stirred solution of **2a** (2.0 g, 6.68 mmol, 1 equiv.) in dry dichloromethane (15 mL) was added Et_3N (2.78 mL, 20.0 mmol, 3 equiv.) followed by benzoyl chloride (1.1 mL, 8.0 mmol, 1.2 equiv.). The resulting reaction mixture was stirred at room temperature for 6 h. The reaction mixture was diluted with dichloromethane (80 mL) and washed sequentially with saturated sodium bicarbonate solution and water. Drying and concentration of the dichloromethane extract under reduced pressure gave the crude product which on column chromatography (60% EtOAc/Hexane) afforded the desired pure product **3** (1.1 g, 56%), $[\alpha]^{26}_{D}$ −9.5 (c=0.2, chloroform); IR ($CHCl_3$) ν (cm^{-1}): 3018, 1749, 1679, 1602, 1215,

759; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60-7.30 (m, 5H), 4.90-4.70 (m, 1H), 4.10-3.95 (m, 2H), 3.72 (s, 3H), 3.65-3.30 (m, 2H), 2.55-1.70 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 170.9, 170.0, 135.9, 130.1, 128.2, 127.0, 59.5, 52.0, 50.2, 41.0, 27.4, 25.1; ESI Mass: 313.3 (M+Na); Anal. Calcd. for $C_{15}H_{18}N_2O_4$: C, 62.06; H, 6.25; N, 9.65. Found: C, 62.15; H, 6.19; N, 9.59.

Tert-butyl 2-((2-aminoethylcarbamoyl)methylcarbamoyl)pyrrolidine-1-carboxylate 4a: To an ice-cold stirred solution of **1** (1.0 g, 3.49 mmol, 1 equiv.) in methanol (20 mL) was added ethylenediamine (3 mL). The resulting reaction mixture was stirred at 0°C for 30 min, and continued at room temperature for 1 hr. The solvent was stripped off under reduced pressure, the resultant residue was taken in toluene, and toluene was stripped off under reduced pressure. The process was repeated for two times to remove excess ethylenediamine. The residue was dried under vacuum to yield the desired product **4a** as a thick liquid (1.09 g, 100%) which was used for the next reaction, without further purification.

N-((2-aminoethylcarbamoyl)methyl)-1-benzoyl-pyrrolidine-2-carboxamide 4b: To an ice-cold stirred solution of **3** (0.5 g, 1.72 mmol, 1 equiv.) in methanol (10 mL) was added ethylenediamine (1.5 mL). The resulting reaction mixture was stirred at 0°C for 30 min, and continued at room temperature for 30 min. The solvent was stripped off under reduced pressure, the resultant residue was taken in toluene, and toluene was stripped off under reduced pressure, the process was repeated two times to remove excess ethylenediamine. The residue was dried under vacuum to yield the desired product **4b** as

thick liquid (0.54 g, 100%) which was used for the next reaction, without further purification.

1-Benzoyl-pyrrolidine-2-carboxylic acid [(2-acetylamino-ethylcarbamoyl)-methyl]-amide 1a: To a solution of **4b** (0.5 g, 1.57 mmol, 1 equiv.) in dry pyridine (5 mL) was added acetic anhydride (0.44 mL, 4.71 mmol, 3 equiv.). The resulting reaction mixture was stirred at room temperature for 5h. The solvent was stripped off under reduced pressure to get the crude product which on column chromatography (100% EtOAc) afforded the desired pure product **1a** (0.39 g, 69%); mp 157-160^{0}C; $[\alpha]^{26}_D$ −11.5 (c=0.2, chloroform); IR ($CHCl_3$) ν (cm^{-1}): 3336, 3018, 1662, 1612, 1217, 771; ^{1}H NMR (400 MHz, CDCl3): δ 8.24 (bs, 1H), 8.07 (bs, 1H), 8.05-7.95 (m, 2H), 7.88 (bs, 1H), 7.85-7.70 (m, 3H), 4.95-4.75 (m, 1H), 4.75-4.55 (m, 1H), 4.30-3.60 (m, 7H), 2.70-1.90 (m, 4H), 2.15 (s, 3H), 3; ^{13}C NMR (100MHz, $CDCl_3$): δ 172.5, 170.8, 170.0, 135.5, 130.6, 128.5, 126.9, 61.6, 50.7, 43.3, 39.6, 39.1, 29.4, 25.6, 22.7; ESI Mass: 383.17 (M+Na); Anal. Calcd. for $C_{18}H_{24}N_4O_4$: C, 59.99; H, 6.71; N, 15.55. Found: C, 59.82; H, 6.69; N, 15.40.

2-{[(2-Acetylamino-ethylcarbamoyl)-methyl]-carbamoyl}-pyrrolidine-1-carboxylic acid tert-butyl ester 1b: To a solution of **4a** (3.0 g, 9.55 mmol, 1 equiv.) in dry pyridine (15 mL) was added acetic anhydride (2.71 mL, 28.66 mmol, 3 equiv.). The resulting reaction mixture was stirred at room temperature for 5h. The solvent was stripped off under reduced pressure to get the crude product which on column chromatography (80% EtOAc: Pet. ether) afforded the desired pure product **1b** as thick liquid (2.5 g, 73%) $[\alpha]^{26}_D$ −10.7 (c=0.2, chloroform); IR ($CHCl_3$) ν (cm^{-1}): 3323, 3016, 1668, 1533, 1411, 1215, 756; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (bs, 1H), 7.49 (bs, 1H), 7.10 (bs, 1H),

4.17 (bs, 1H), 4.00-3.75 (m, 2H), 3.50-3.0 (m, 6H), 2.20-1.80 (m, 4H), 1.94 (s, 3H), 1.42 (s, 9H) ; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6, 171.1, 170.3, 155.5, 80.7, 60.8, 47.3, 43.2, 39.2, 29.8, 28.4, 24.6, 23.0; ESI Mass: 395.3 (M+K); Anal. Calcd. for $C_{16}H_{28}N_4O_5$: C, 53.92; H, 7.92; N, 15.72. Found: C, 53.65; H, 7.70; N, 15.63.

2-({[2-(4-Nitro-benzoylamino)-ethylcarbamoyl]-methyl}-carbamoyl)-pyrrolidine-1-carboxylicacid tert-butyl ester 1c : To an ice-cold stirred solution of 4-nitro- benzoic acid (0.5 g, 3.04 mmol, 1.2 equiv.) and amine **4a** (0.8 g, 2.54 mmol, 1 equiv.) in dry acetonitrile (15mL) was added DIPEA (1.09 mL, 6.09 mmol, 2.4 equiv.) followed by TBTU (1.14 g, 3.55 mmol, 1.4 equiv.) The resulting reaction mixture was stirred for overnight at room temperature. The solvent was stripped off under reduced pressure; the resultant residue was dissolved in dichloromethane (100 mL) and washed sequentially with potassium hydrogen sulphate solution, saturated sodium bicarbonate and water. Drying and concentration in vacuo yielded the crude product which on column chromatography (70% ethyl acetate/pet-ether) afforded **1c** (0.75 g, 64%); mp 173-176^{0}C; $[\alpha]^{26}_D$ −9.0 (c=0.2, chloroform); IR ($CHCl_3$) ν (cm^{-1}): 3325, 3018, 1666, 1527, 1215, 756; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30-8.15 (d, 2H), 8.10-7.95 (d, 2H), 7.90 (bs, 1H), 7.79 (bs, 1H), 6.87 (bs, 1H), 4.20-3.85 (m, 3H), 3.65-3.30 (m, 6H), 2.30-1.75 (m, 4H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6, 170.9, 165.6, 155.7, 149.3, 139.7, 128.4, 123.4, 80.9, 60.9, 47.3, 43.2, 40.8, 39.0, 29.8, 28.3, 24.5 ; ESI Mass: 486.20 (M+Na); Anal. Calcd. for $C_{21}H_{29}N_5O_7$: C, 54.42; H, 6.31; N, 15.11. Found: C, 54.27; H, 6.19; N, 14.99.

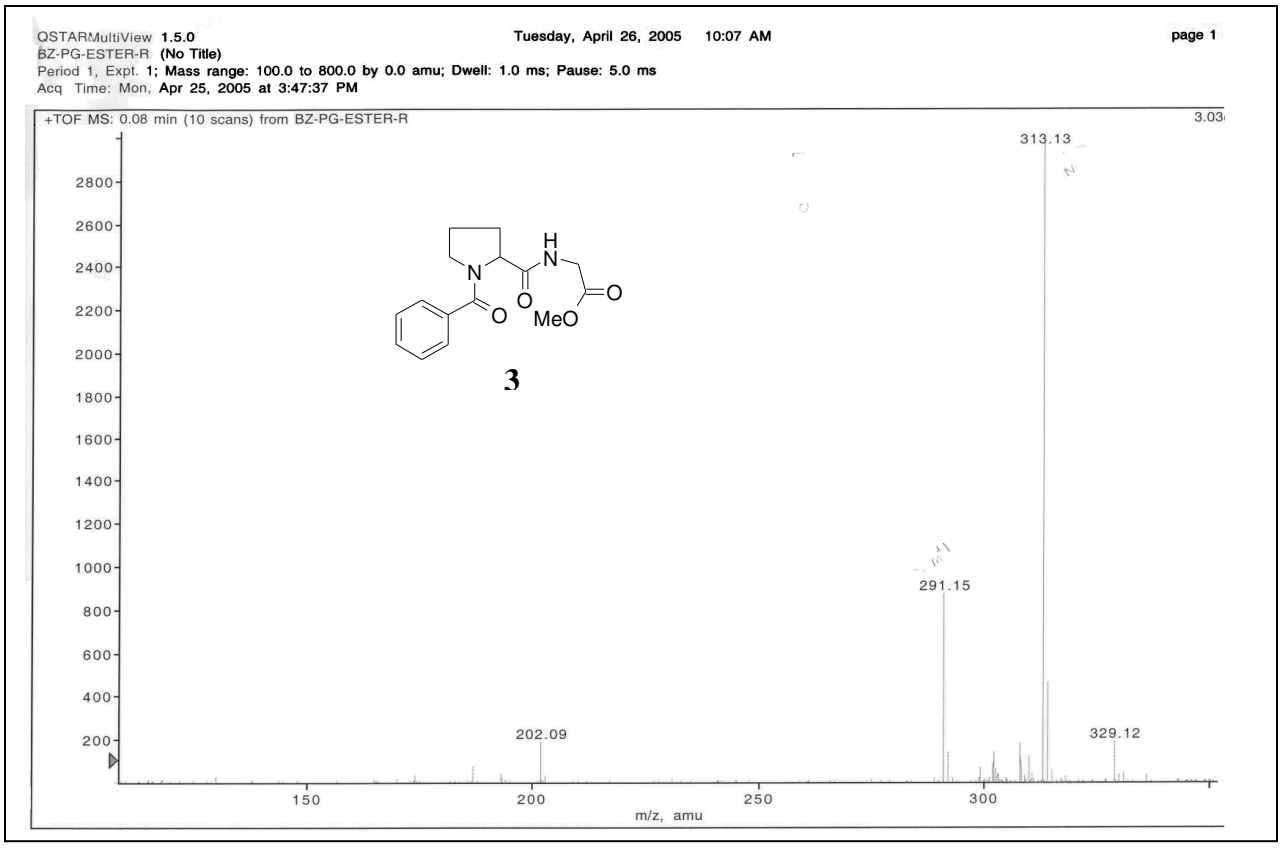
2-({[2-(4-Bromo-benzoylamino)-ethylcarbamoyl]-methyl}-carbamoyl)-pyrrolidine-1-carboxylicacid tert-butyl ester 1d: To an ice-cold stirred solution of 4-bromo-

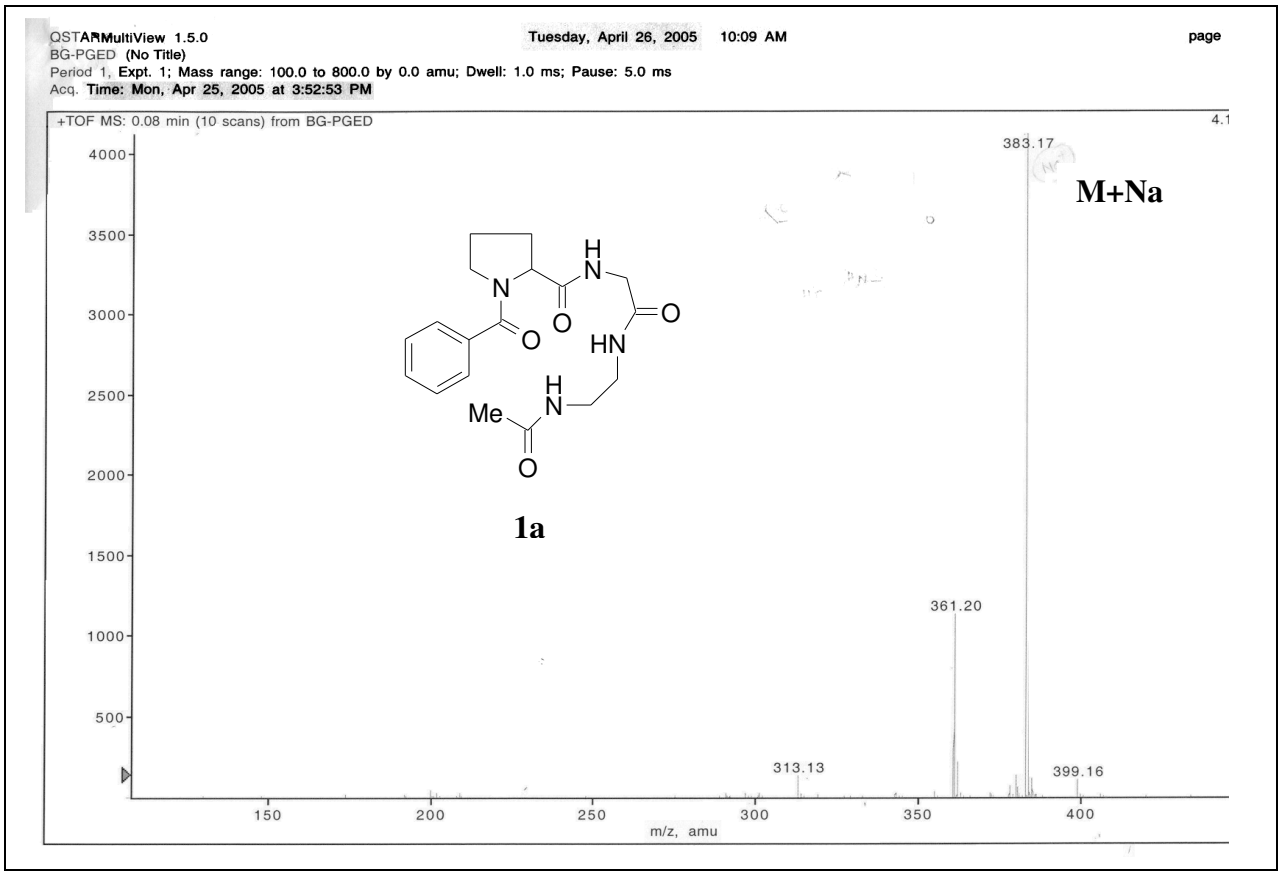
benzoic acid (0.5 g, 3.04 mmol, 1.2 equiv.) and amine **4a** (0.8 g, 2.54 mmol, 1 equiv.) in dry acetonitrile (15 mL) was added DIPEA (1.09 mL, 6.09 mmol, 2.4 equiv.) followed by the addition of TBTU (1.14 g, 3.55 mmol, 1.4 equiv.) The resulting reaction mixture was stirred for overnight at room temperature. The solvent was stripped off under reduced pressure; the resultant residue was dissolved in dichloromethane (100 mL) and washed sequentially with potassium hydrogen sulphate solution, saturated sodium bicarbonate and water. Drying and concentration in vacuo yielded the crude product which on column chromatography (90% ethyl acetate/pet-ether) afforded **1d** (0.79 g, 68%); mp 180-182^{0}C; $[\alpha]^{26}_D$ −5.0 (c=0.2, chloroform); IR ($CHCl_3$) ν (cm^{-1}): 3334, 3018, 1662, 1411, 1215, 756; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85-7.40 (m, 6H), 7.02 (bs, 1H), 4.20-3.75 (m, 3H), 3.65-3.25 (m, 6H), 2.25-1.70 (m, 4H), 1.40 (s, 9H) ; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 170.7, 166.7, 155.5, 132.9, 131.4, 128.8, 125.8, 80.7, 60.8, 47.2, 43.1, 40.6, 39.1, 29.7, 28.2, 24.5 ; ESI Mass: 520.25 (M+Na); Anal. Calcd. for $C_{21}H_{29}N_4O_5Br$: C, 50.71; H, 5.88; N, 11.26. Found: C, 50.65; H, 5.80; N, 11.19.

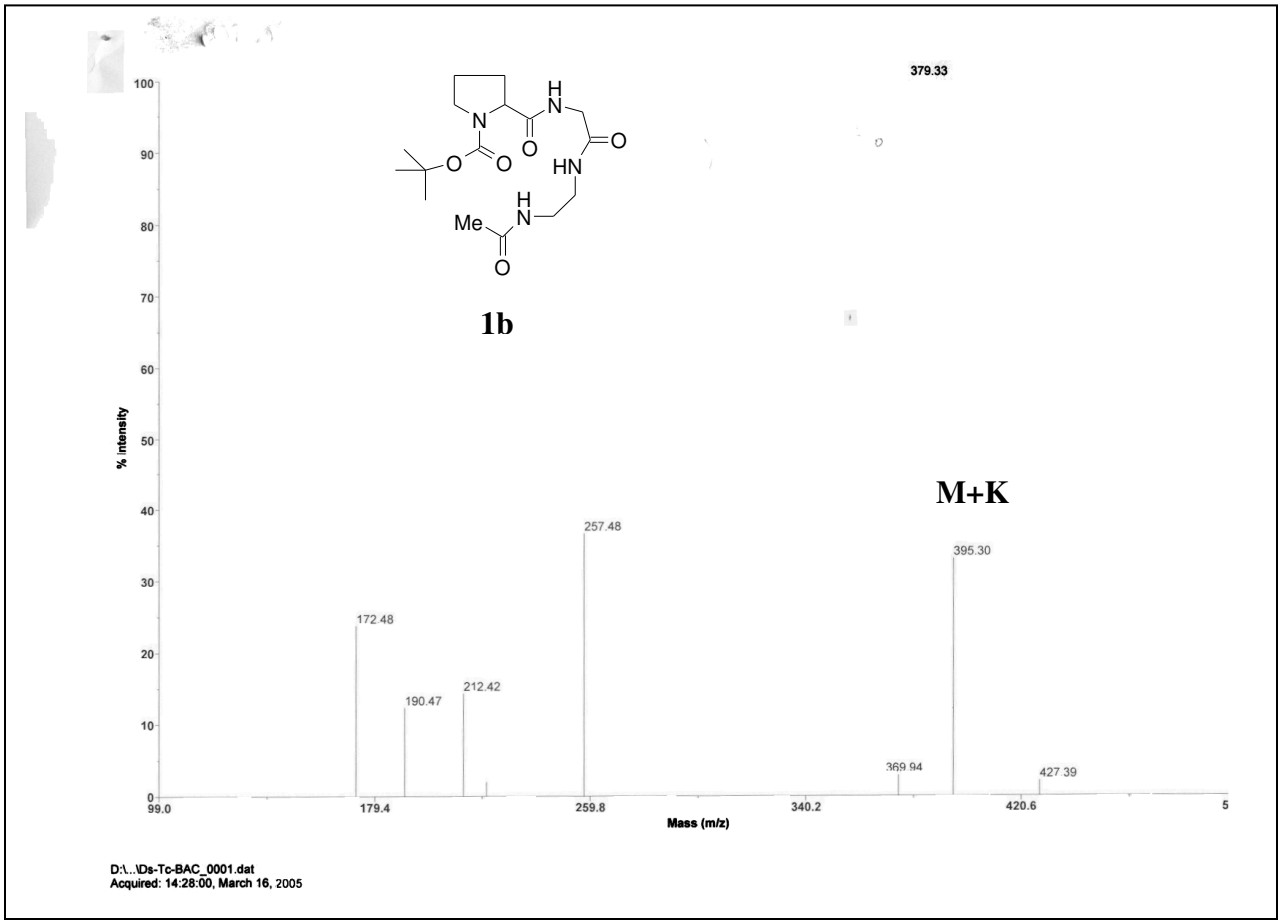
{[1-(2-tert-Butoxycarbonylamino-acetyl)-pyrrolidine-2-carbonyl]-amino}-acetic acid methyl ester 7: To an ice-cold stirred solution **2a** (0.5 g, 1.67 mmol, 1 equiv.) and Boc-Gly-OH (0.29 g, 1.67 mmol, 1 equiv.) in dry dichloromethane (10 mL) was added DIPEA (1.19 mL, 6.68 mmol, 4 equiv.) followed by EDCI (0.44 g, 2.33 mmol, 1.4 equiv.) The resulting reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with dichloromethane (80mL) and washed sequentially with potassium hydrogen sulphate solution, saturated sodium bicarbonate and water. Drying and concentration in vacuo yielded the crude product **7** (0.45 g, 78%), which was used for the next step without further purification.

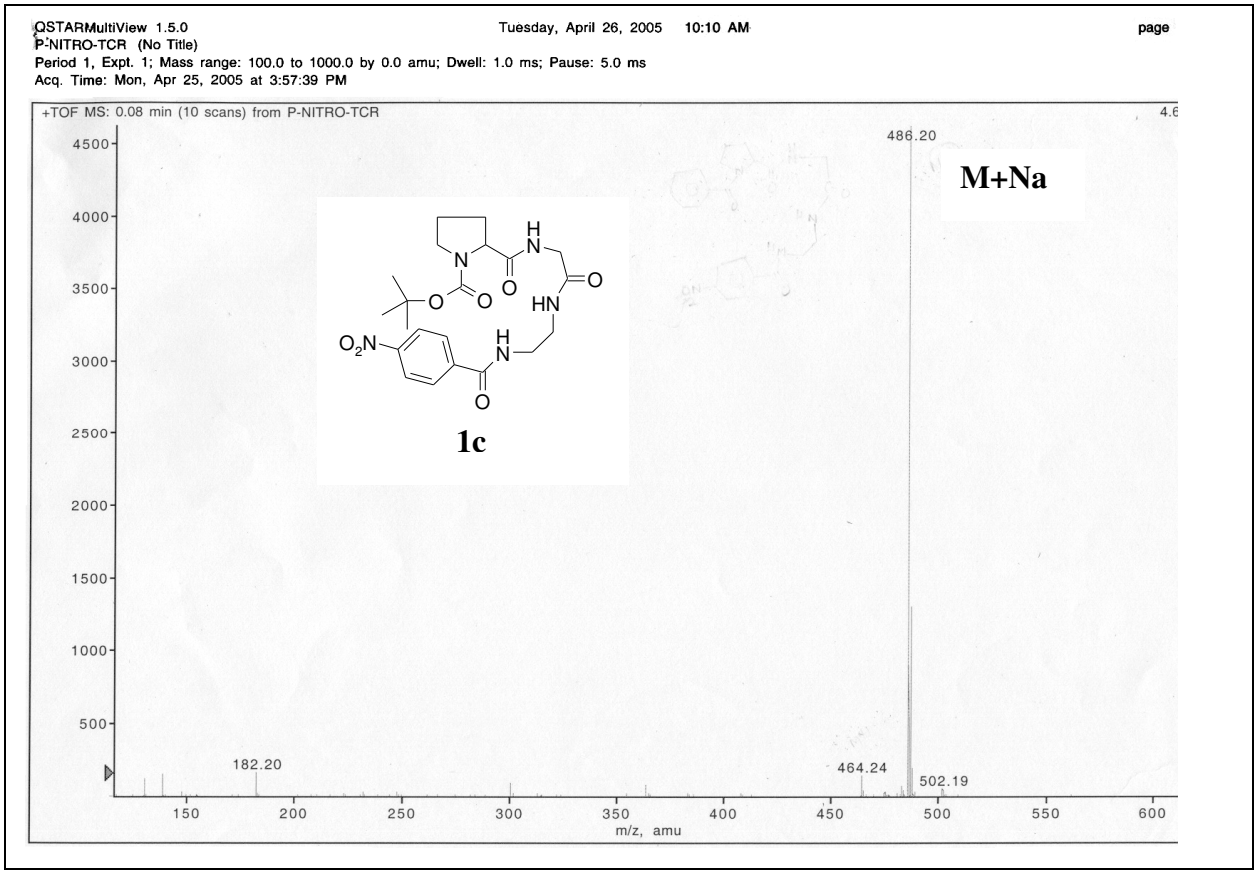
[2-(2-{[(2-Amino-ethylcarbamoyl)-methyl]-carbamoyl}-pyrrolidin-1-yl)-oxo-ethyl]-carbamic acid tert-butyl ester 8: To an ice-cold stirred solution of **7** (1.0 g, 3.49 mmol, 1 equiv.) in methanol (10 mL) was added ethylenediamine (2 mL). The resulting reaction mixture was stirred at 0^0C for 30 min, and continued at room temperature for 30 min. The solvent was stripped off under reduced pressure, and then the residue was taken in toluene, and again stripped off the solvent under reduced pressure. The residue was dried under vacuum to yield the desired product **8** as a thick liquid (1.09 g, 100%) which was used for the next reaction, without further purification.

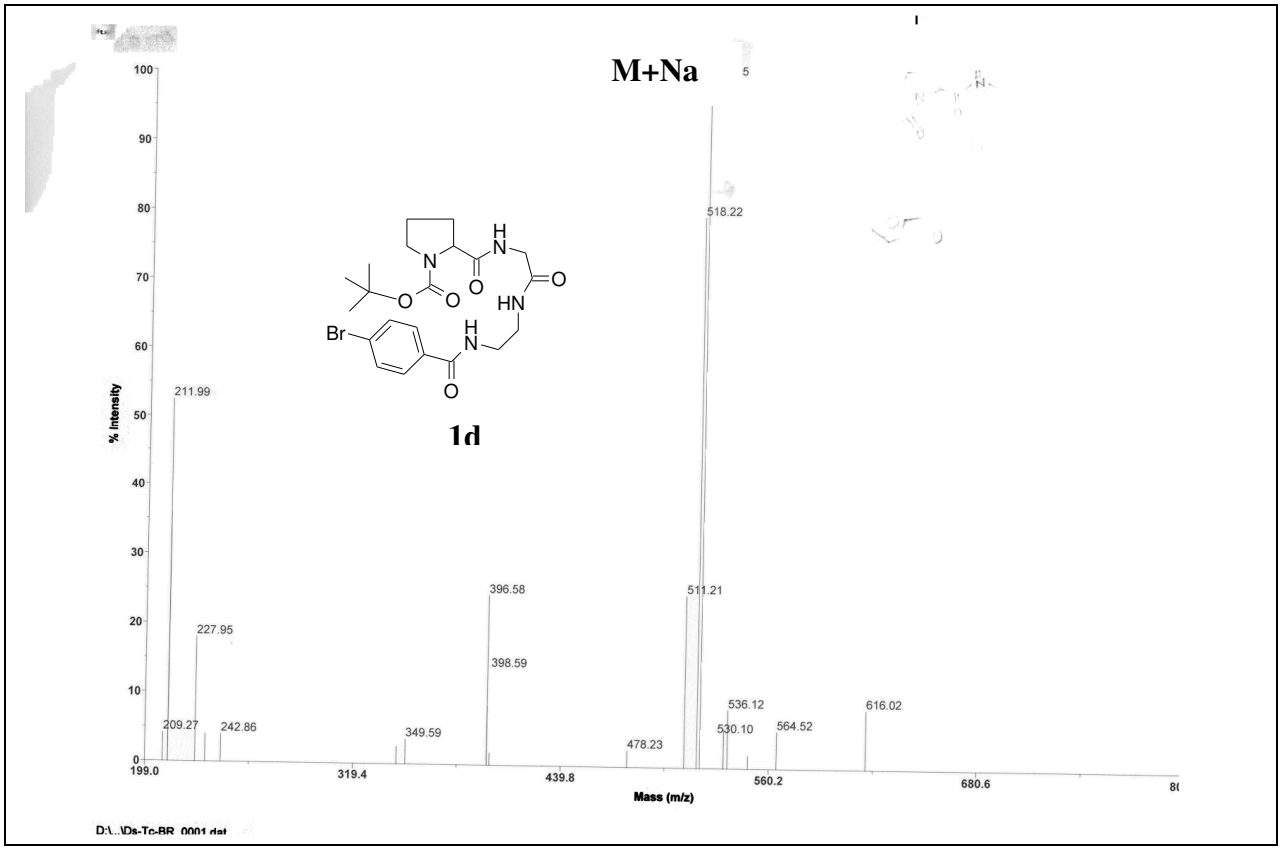
[2-(2-{[(2-tert-Butoxycarbonylamino-ethylcarbamoyl]-carbamoyl}-pyrrolidin-1-yl)-2-oxo-ethyl]-carbamic acid tert-butyl ester 2: To an ice cold solution of the compound **8** (0.54 g, 1.45 mmol, 1 equiv.) in tetrahydrofuran (10 mL), Boc anhydride (0.63 g, 2.91 mmol, 2 equiv.) was added and the resulting reaction mixture was stirred at room temperature for one hour. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water and saturated sodium chloride solution. Drying and concentration of the ethyl acetate extract under reduced pressure gave the crude product which on column chromatography (100% EtOAc) afforded the desired pure product **2** (0.55 g, 75%); mp 195-198^{0}C; $[\alpha]^{26}_D$ −11.2 (c=0.2, chloroform); IR ($CHCl_3$) ν (cm^{-1}): 3325, 3018, 1666, 1612, 1215, 756; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30-8.15 (d, 2H), 8.10-7.95 (d, 2H), 7.90 (bs, 1H), 7.79 (bs, 1H), 6.87 (bs, 1H), 4.20-3.85 (m, 3H), 3.65-3.30 (m, 6H), 2.30-1.75 (m, 4H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 169.8, 169.6, 157.5, 156.6, 156.0, 79.8, 79.5, 61.3, 47.0, 43.0, 40.1, 29.0, 28.2, 24.9 ; ESI Mass: 494.20 (M+Na); Anal. Calcd. for $C_{21}H_{37}N_5O_7$: C, 53.49; H, 7.91; N, 14.85 Found: C, 53.15; H, 7.75; N, 14.67.

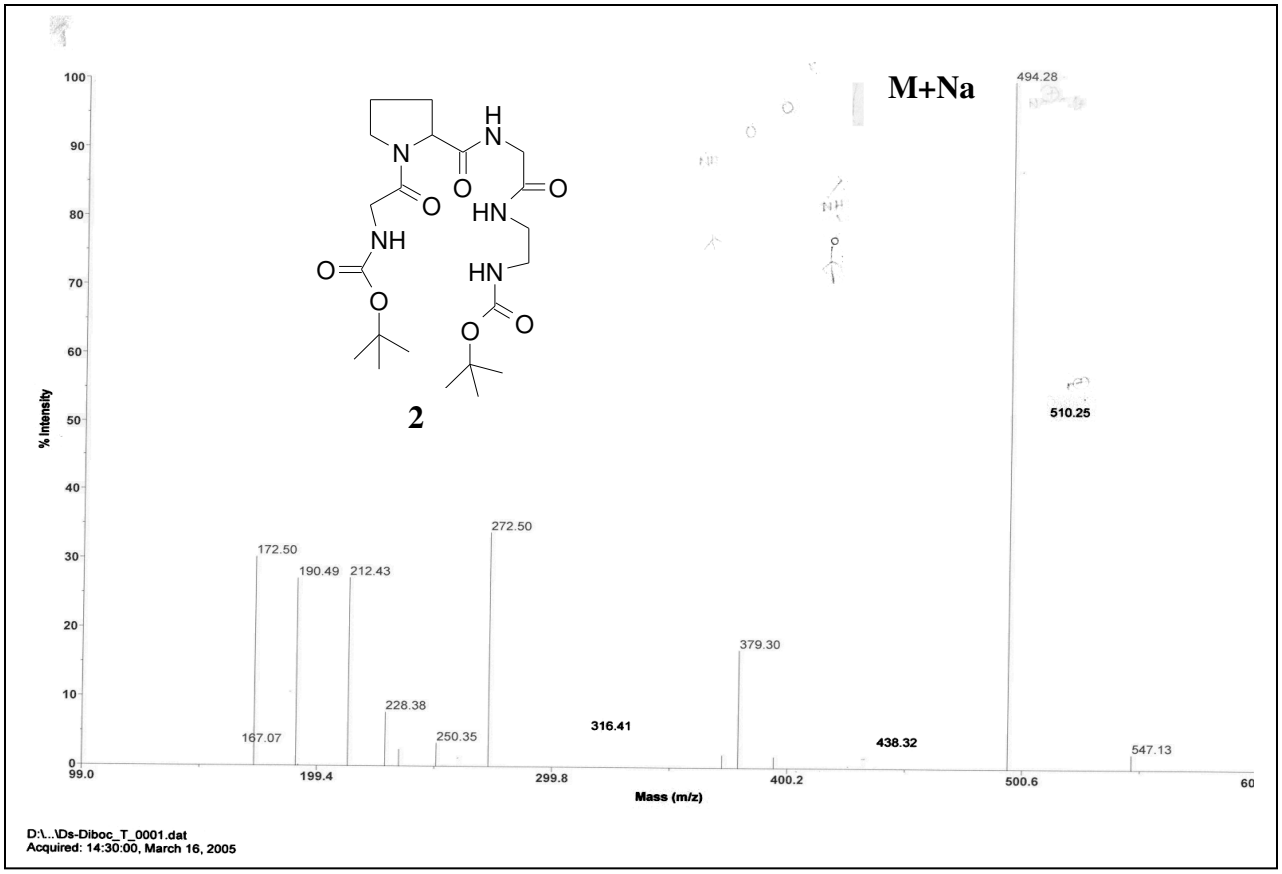


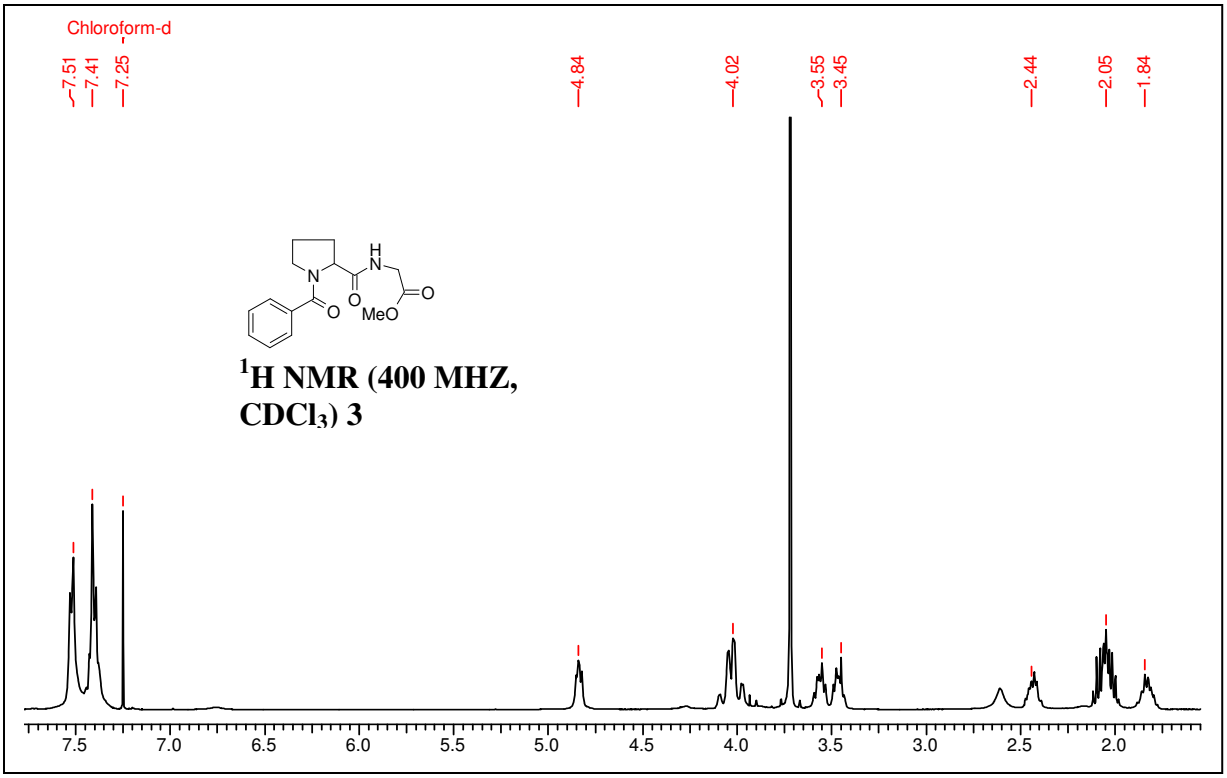




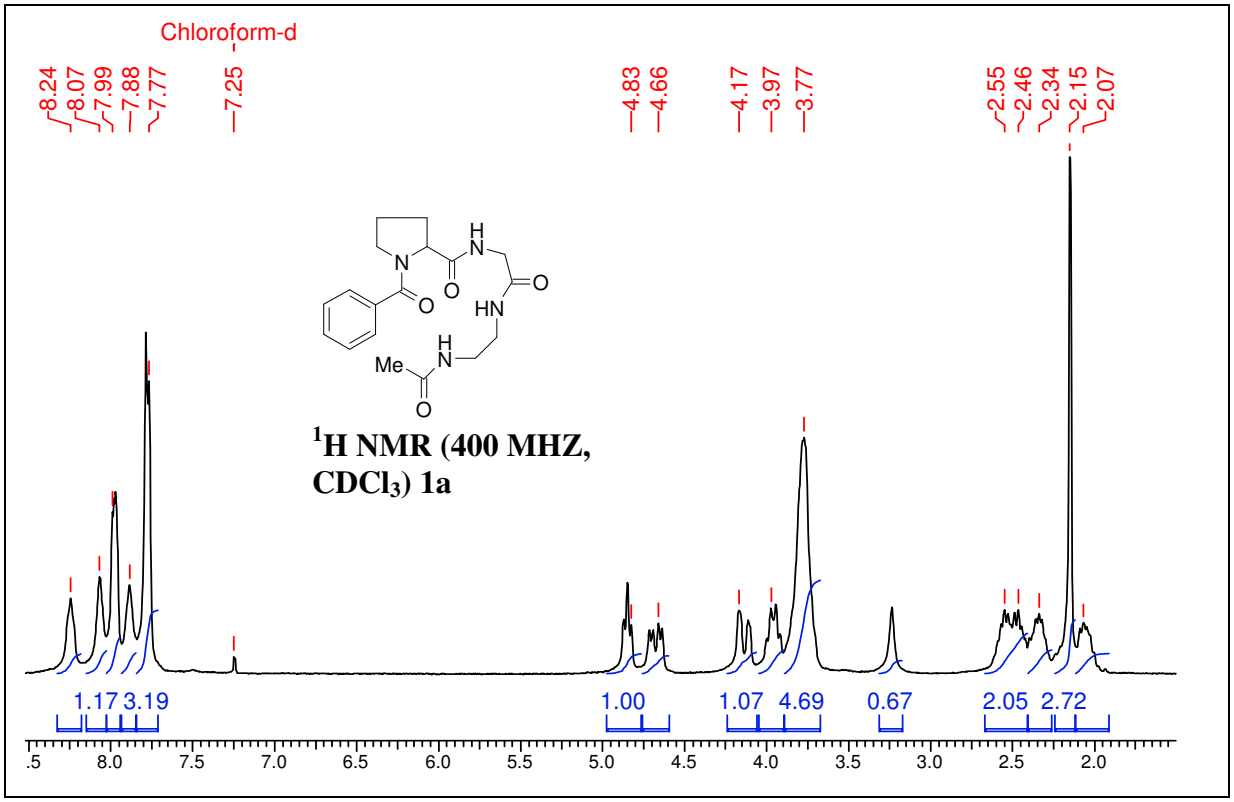




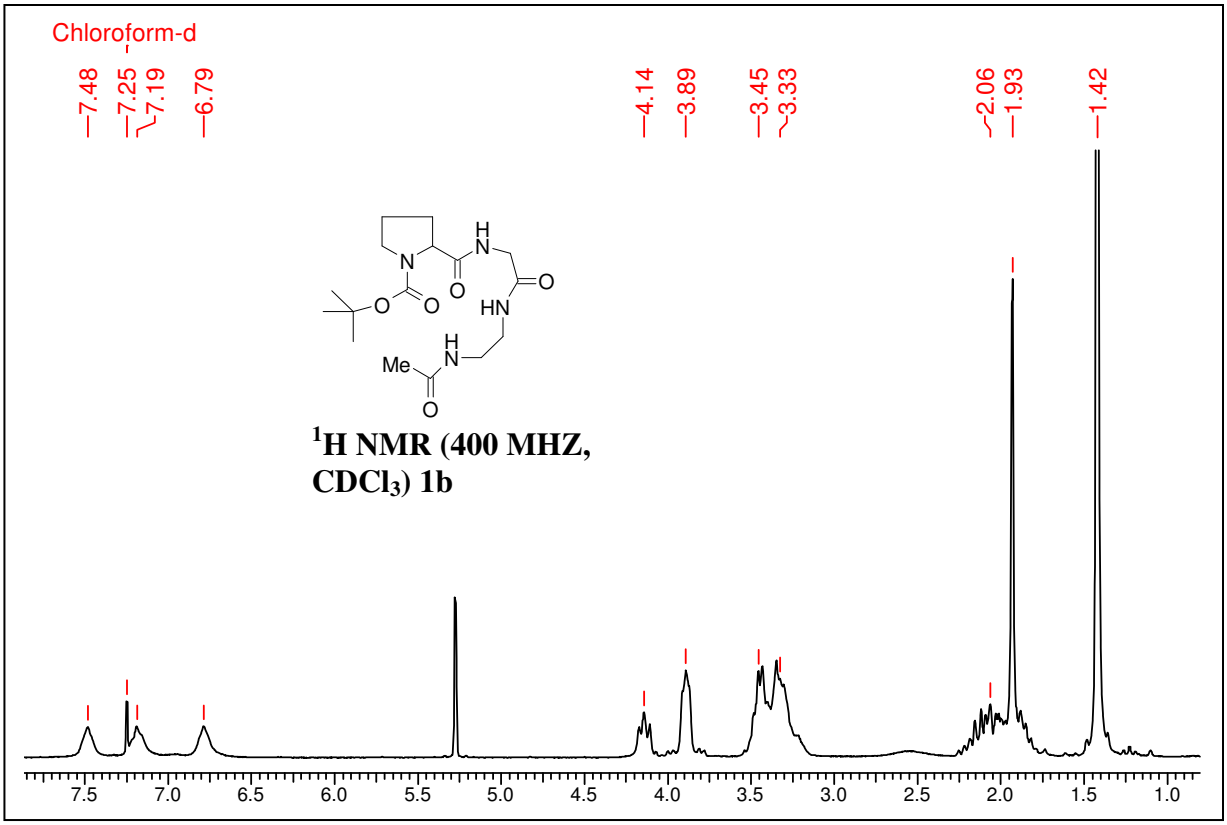




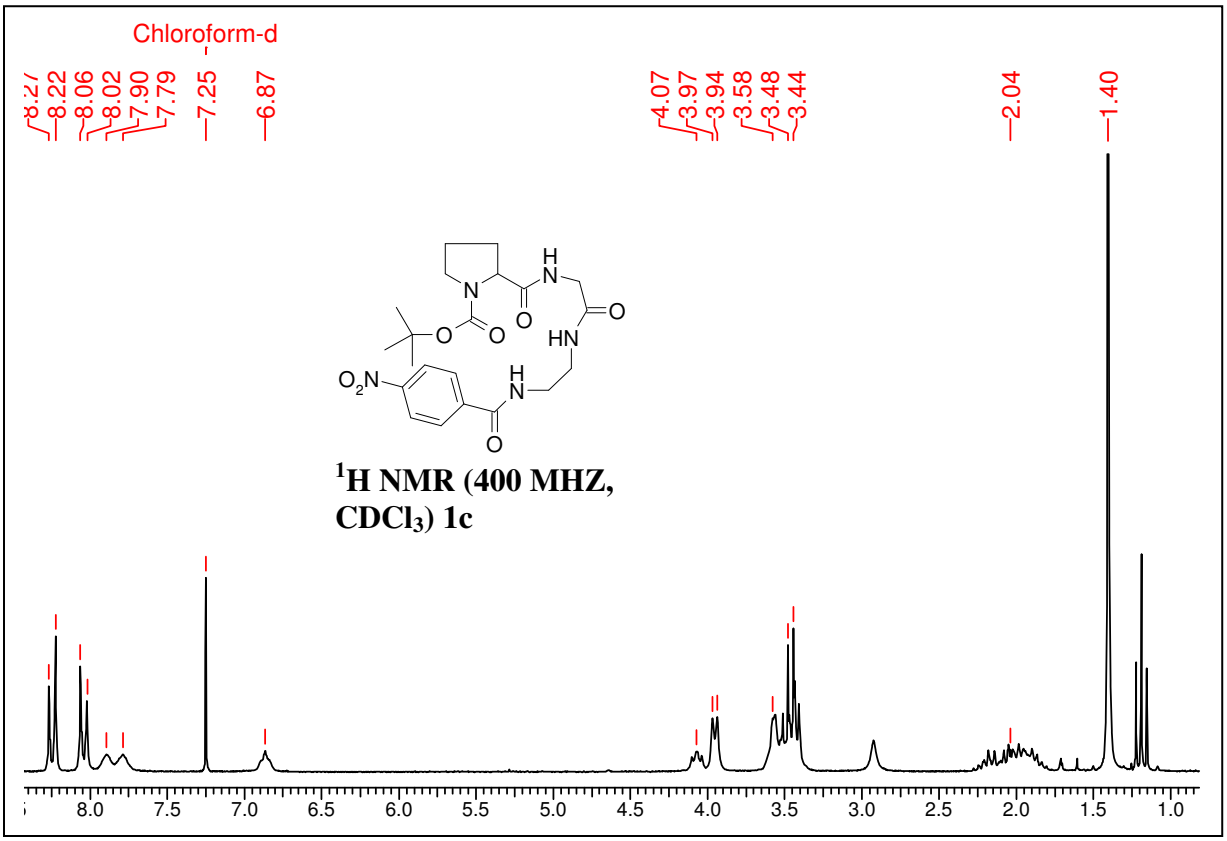
^{1}H NMR (400 MHZ, $CDCl_3$) 3



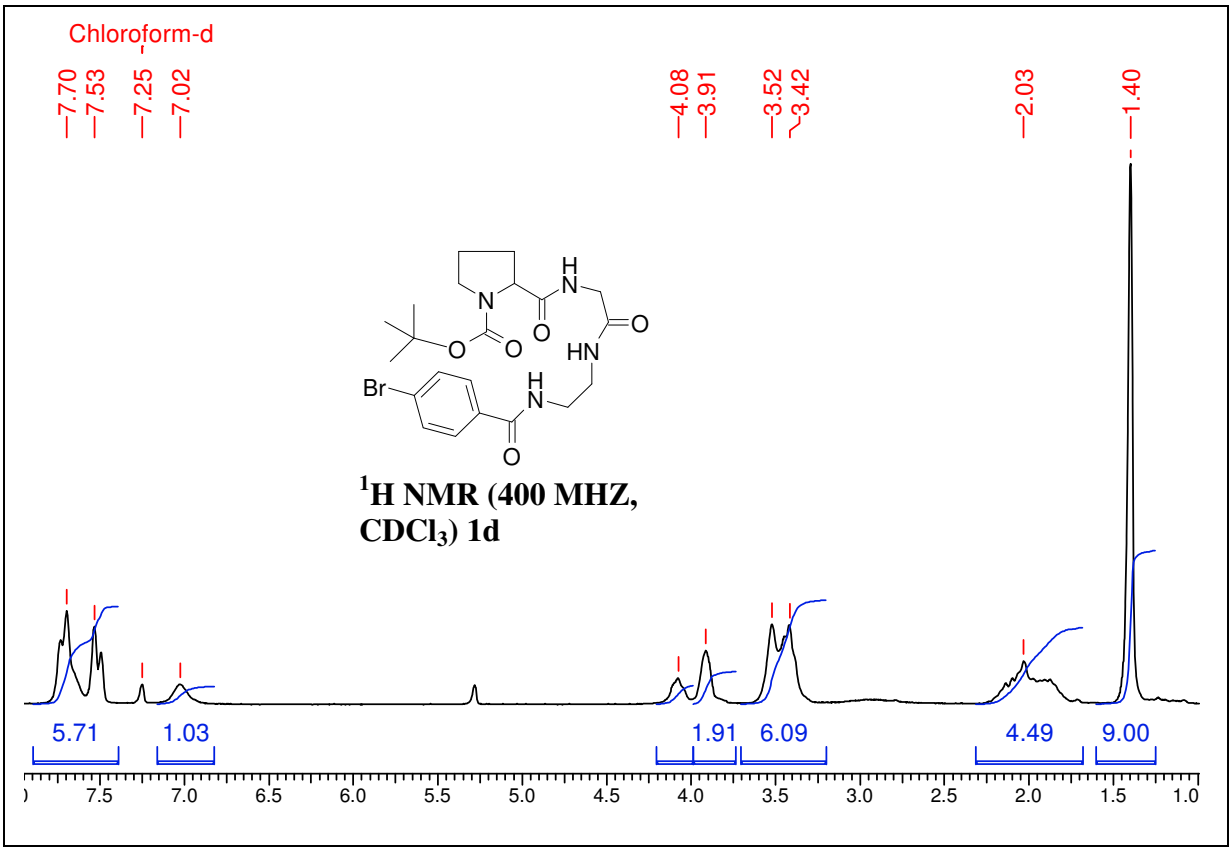
^{1}H NMR (400 MHZ, $CDCl_3$) 1a



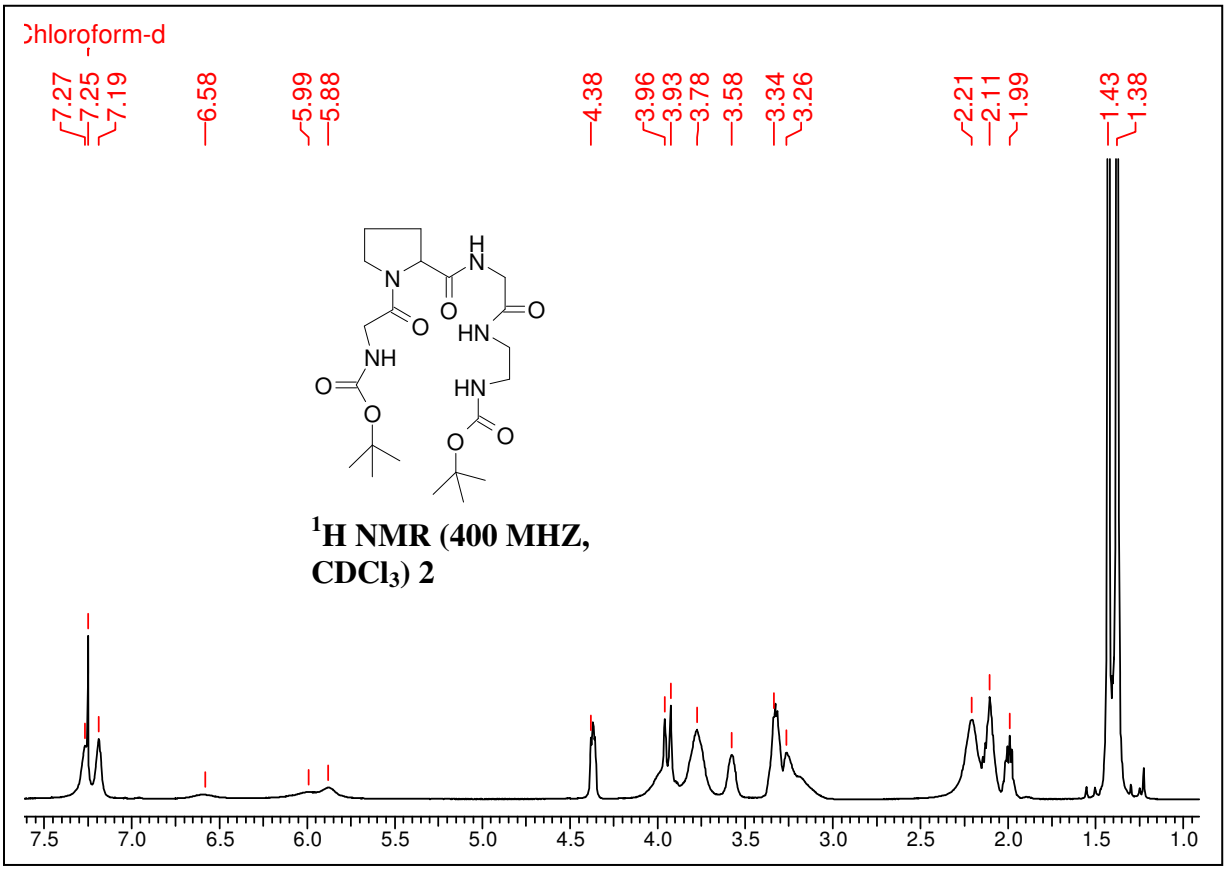
^{1}H NMR (400 MHZ, $CDCl_3$) 1b



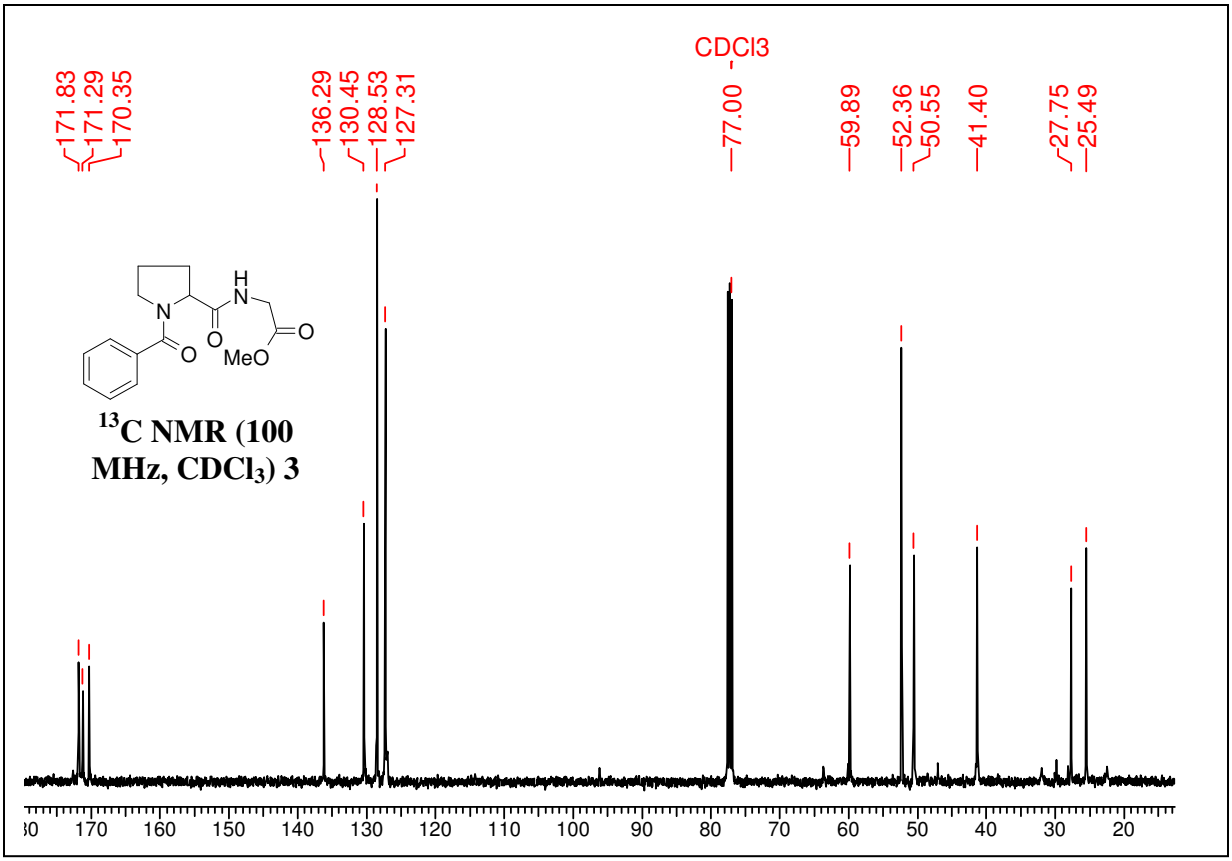
^{1}H NMR (400 MHZ, $CDCl_3$) 1c



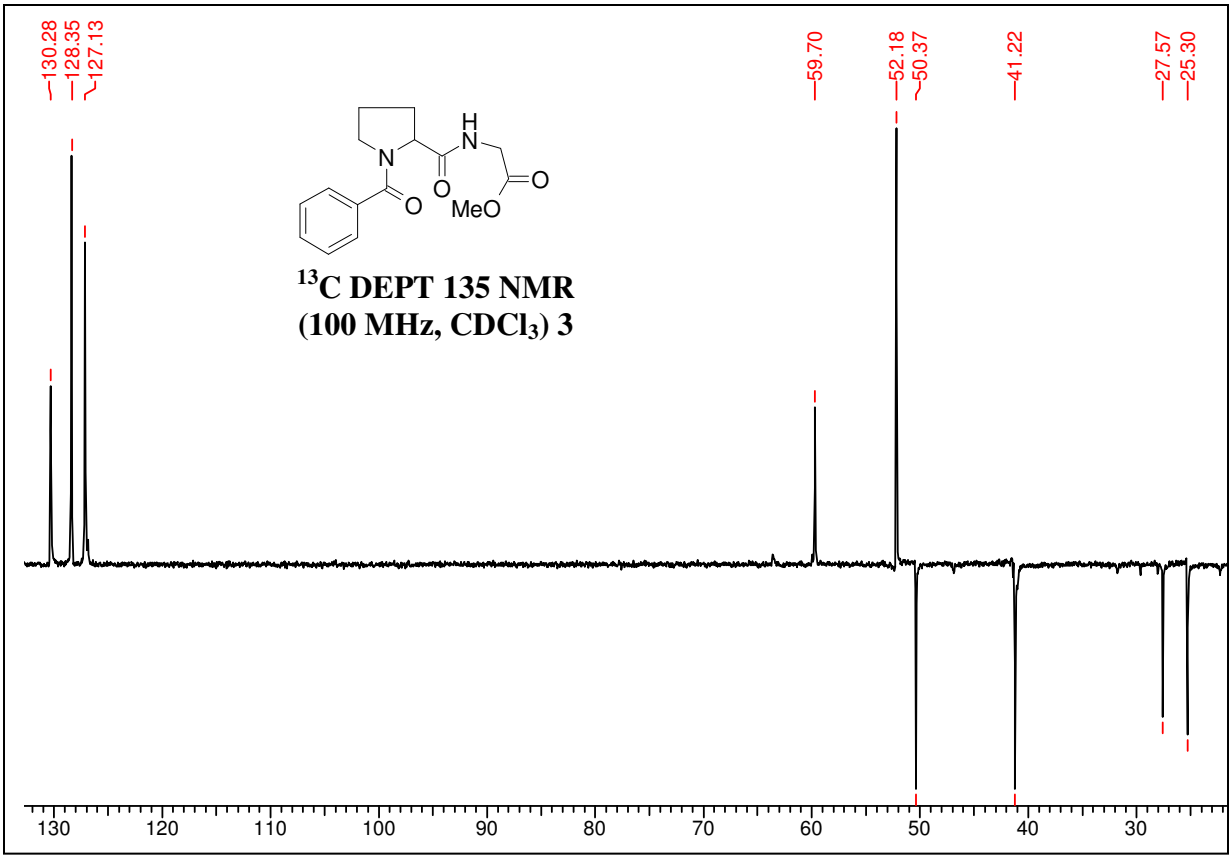
^{1}H NMR (400 MHZ, $CDCl_3$) 1d



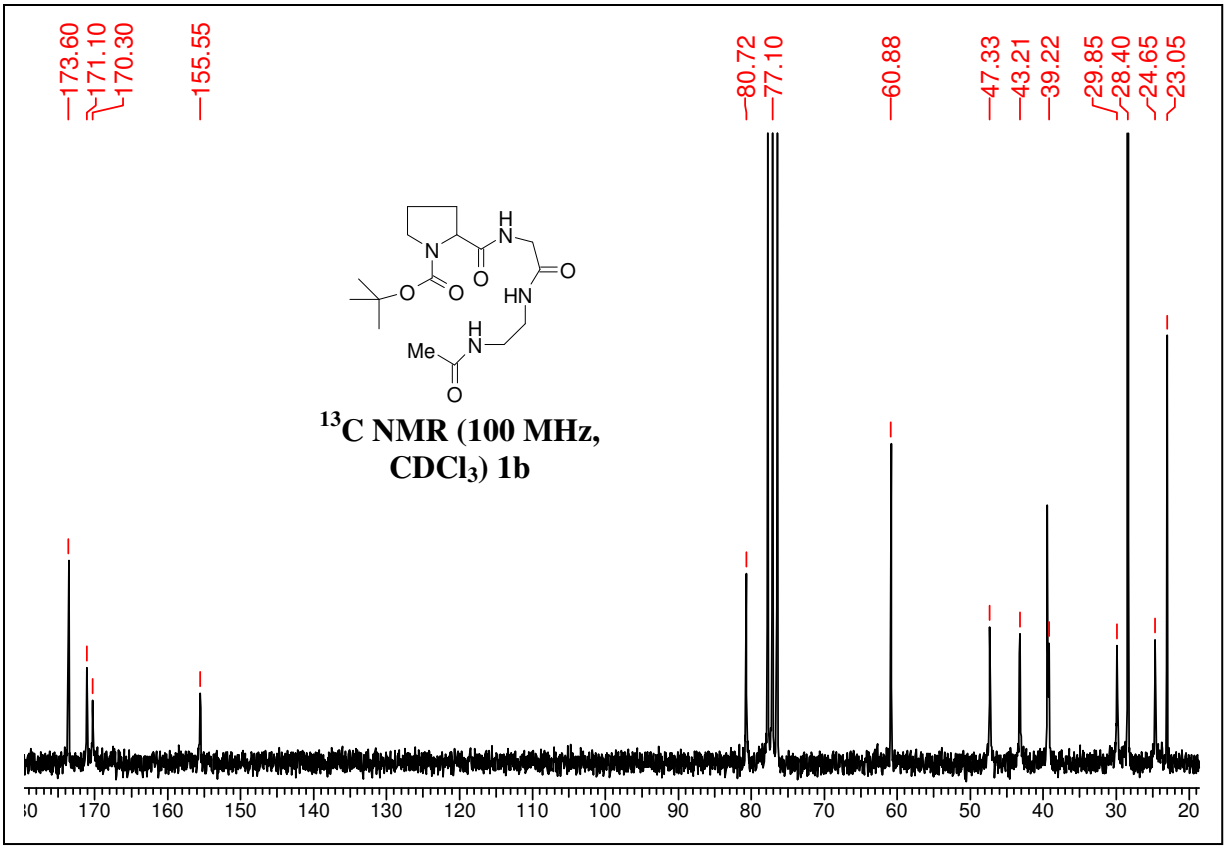
^{1}H NMR (400 MHZ, $CDCl_3$) 2



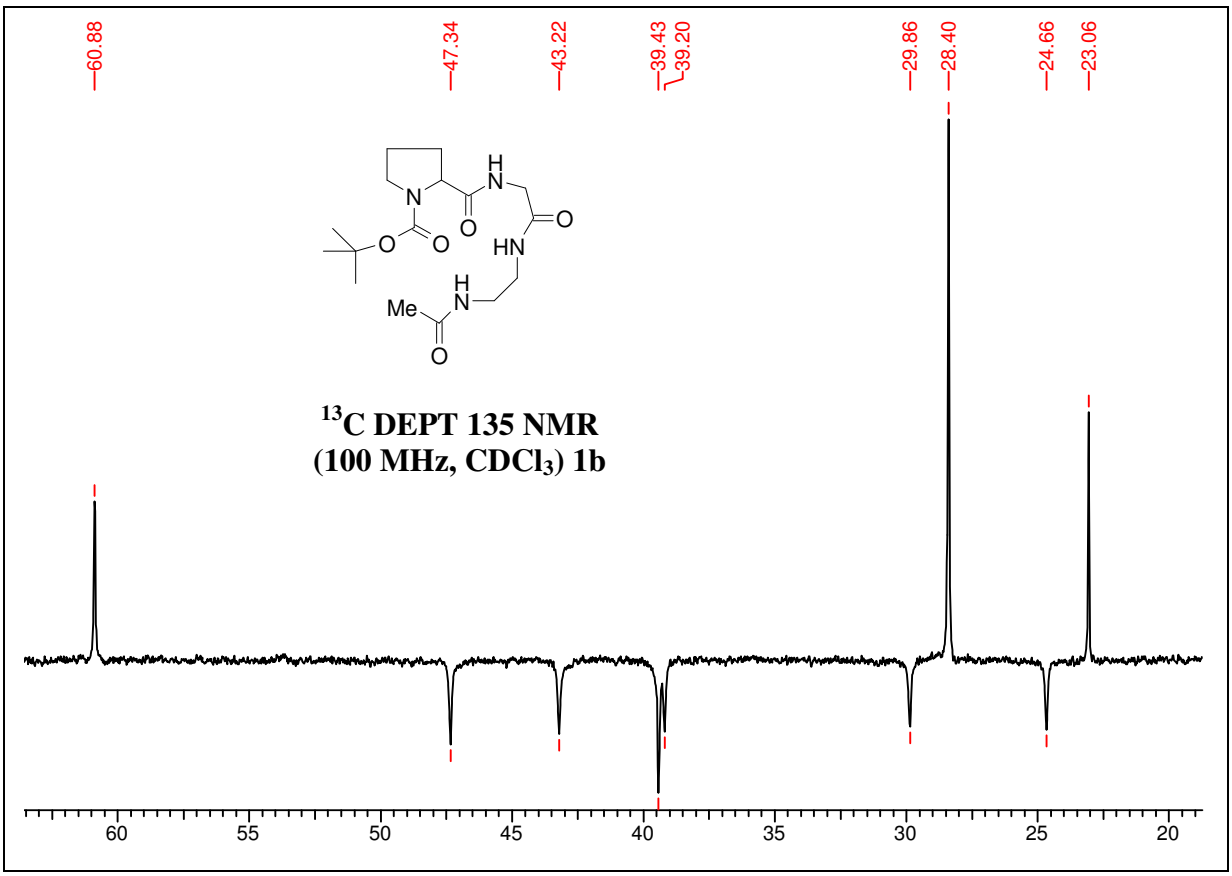
^{13}C NMR (100 MHz, $CDCl_3$) 3



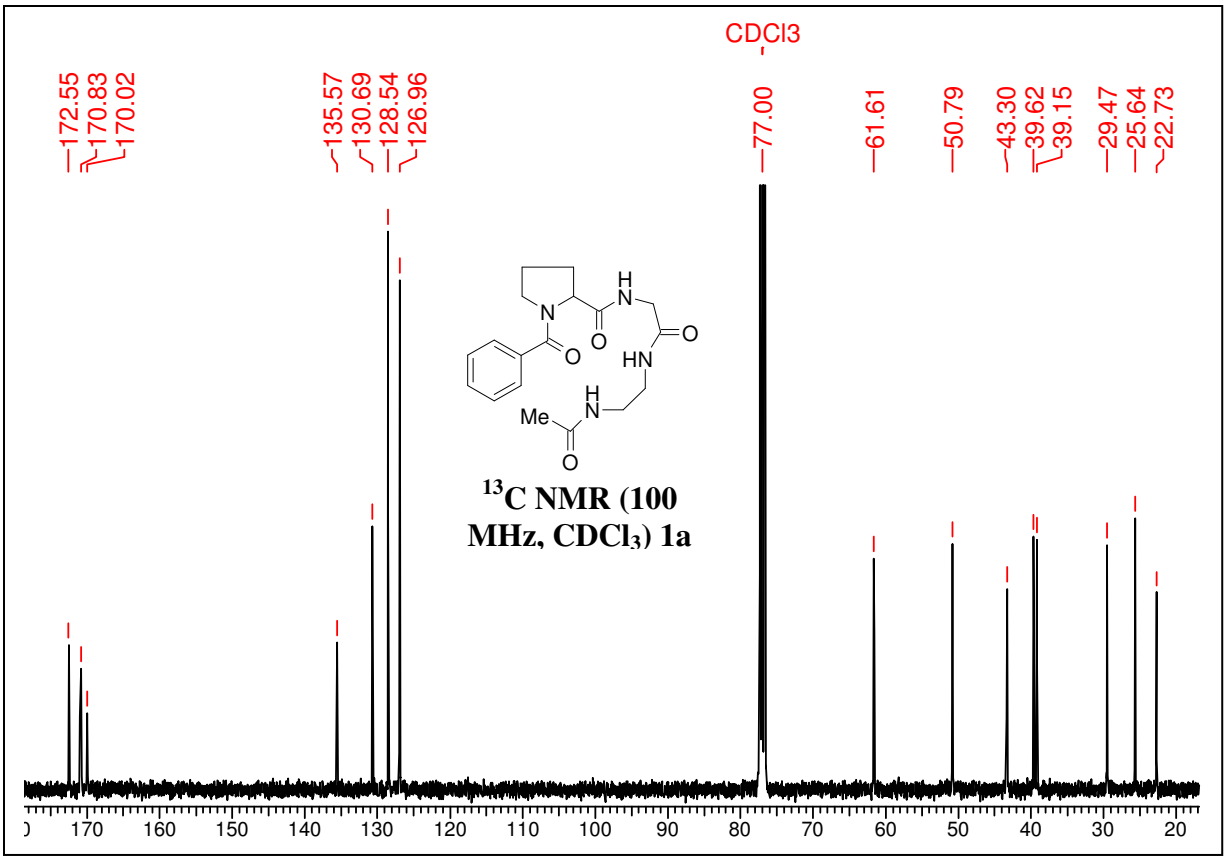
^{13}C DEPT 135 NMR (100 MHz, $CDCl_3$) 3

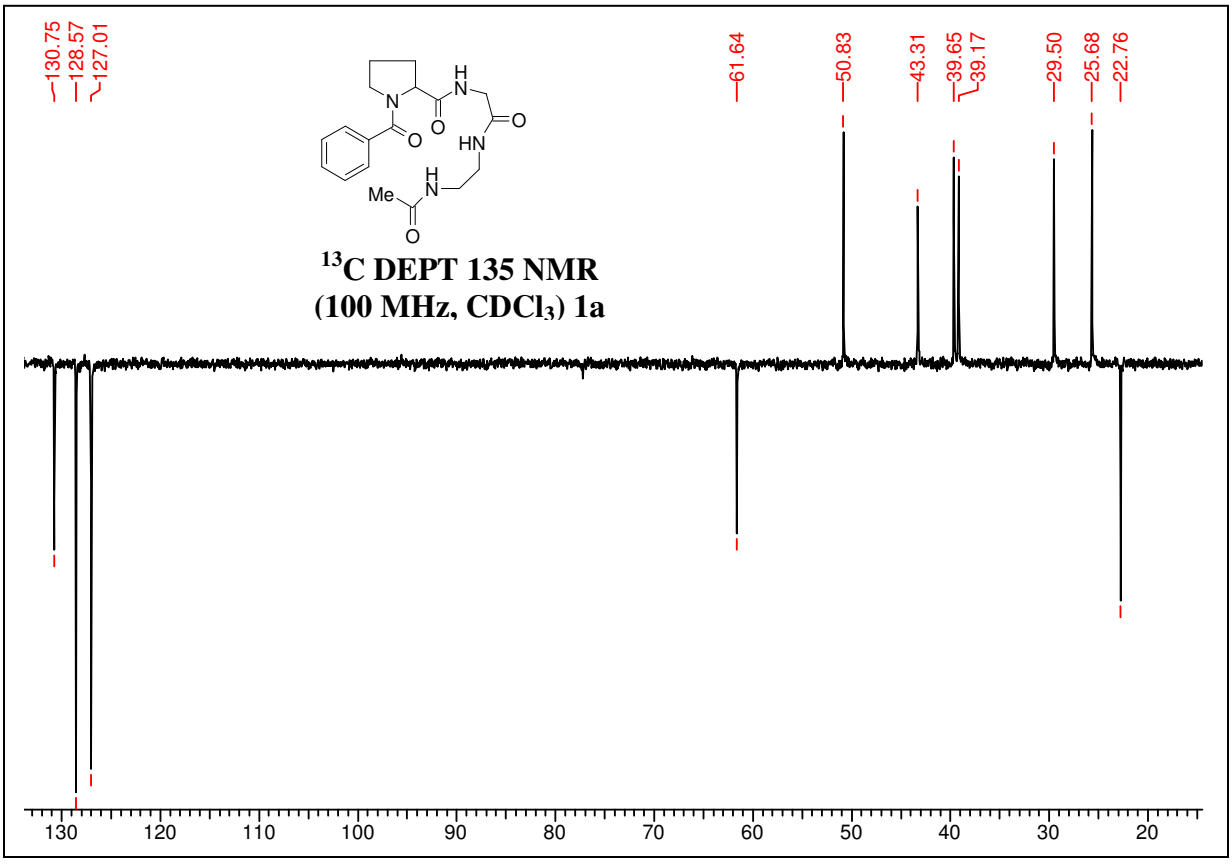


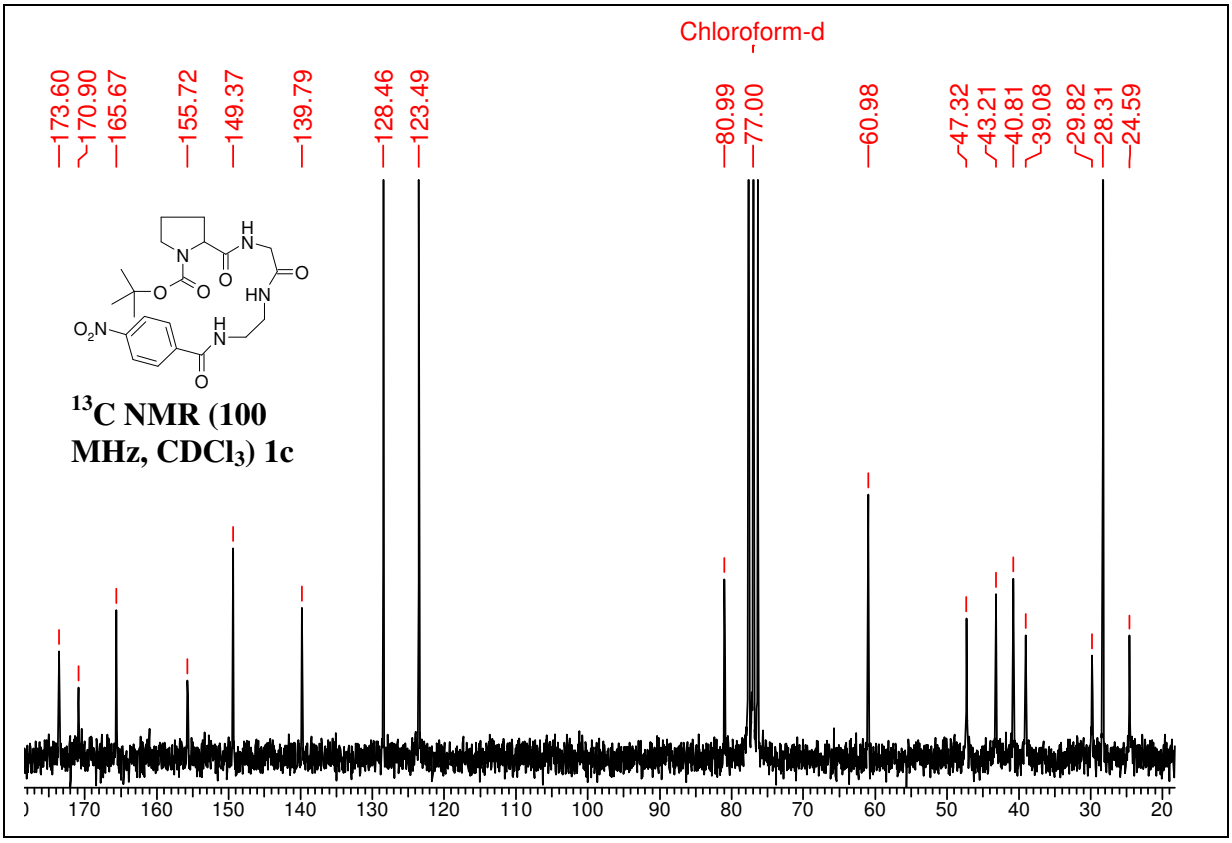
^{13}C NMR (100 MHz, $CDCl_3$) 1b

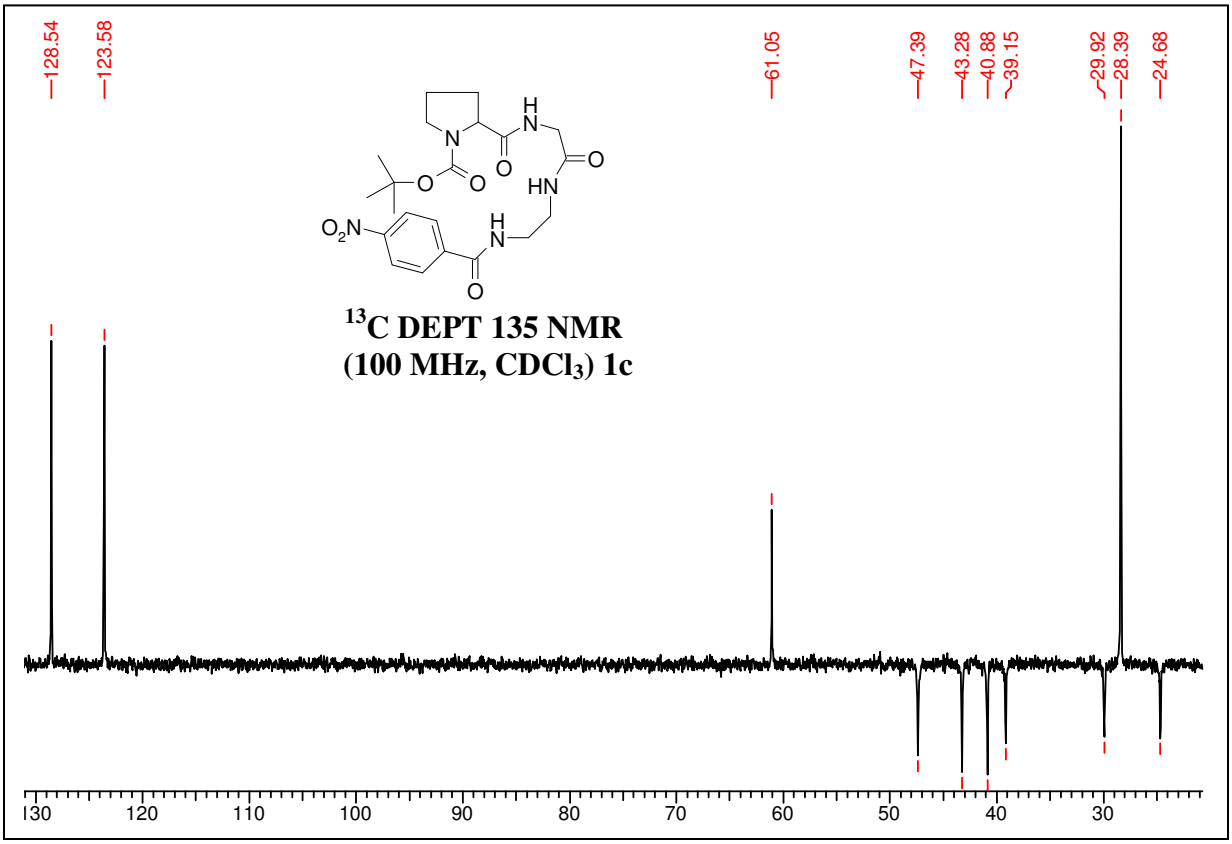


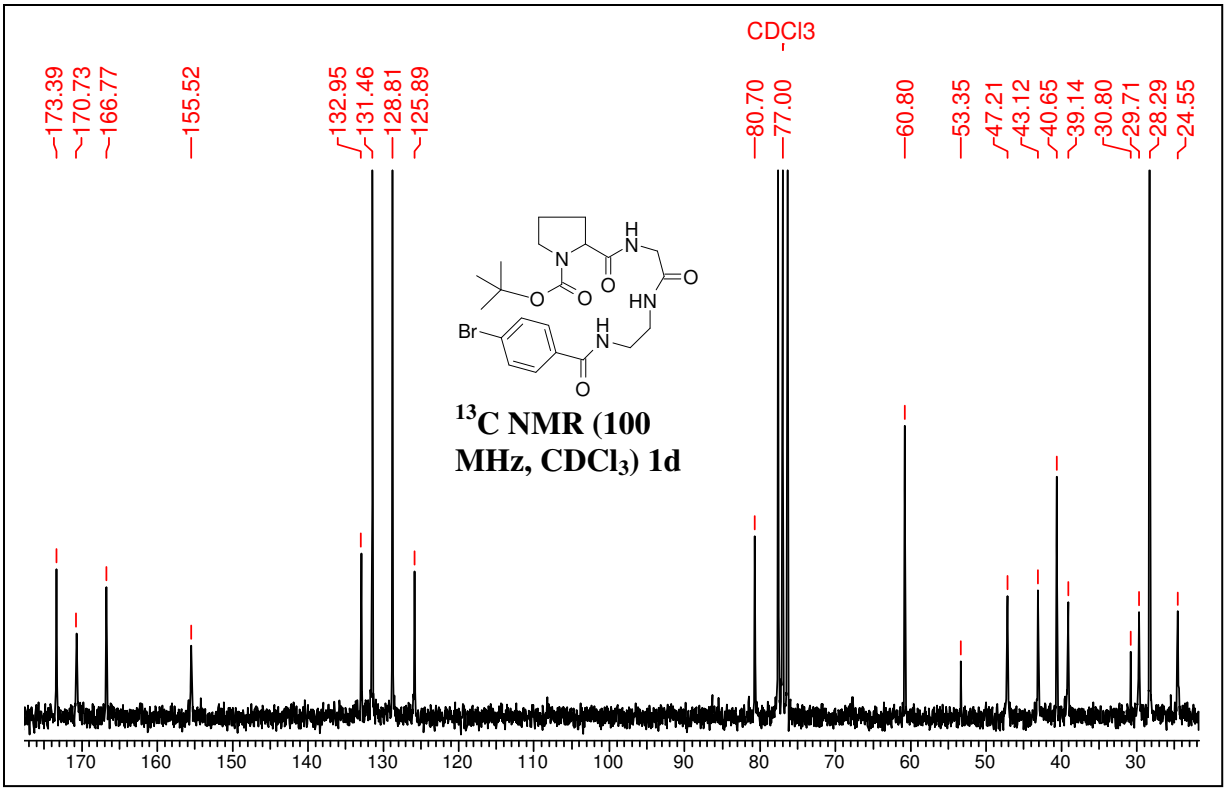
^{13}C DEPT 135 NMR (100 MHz, $CDCl_3$) 1b

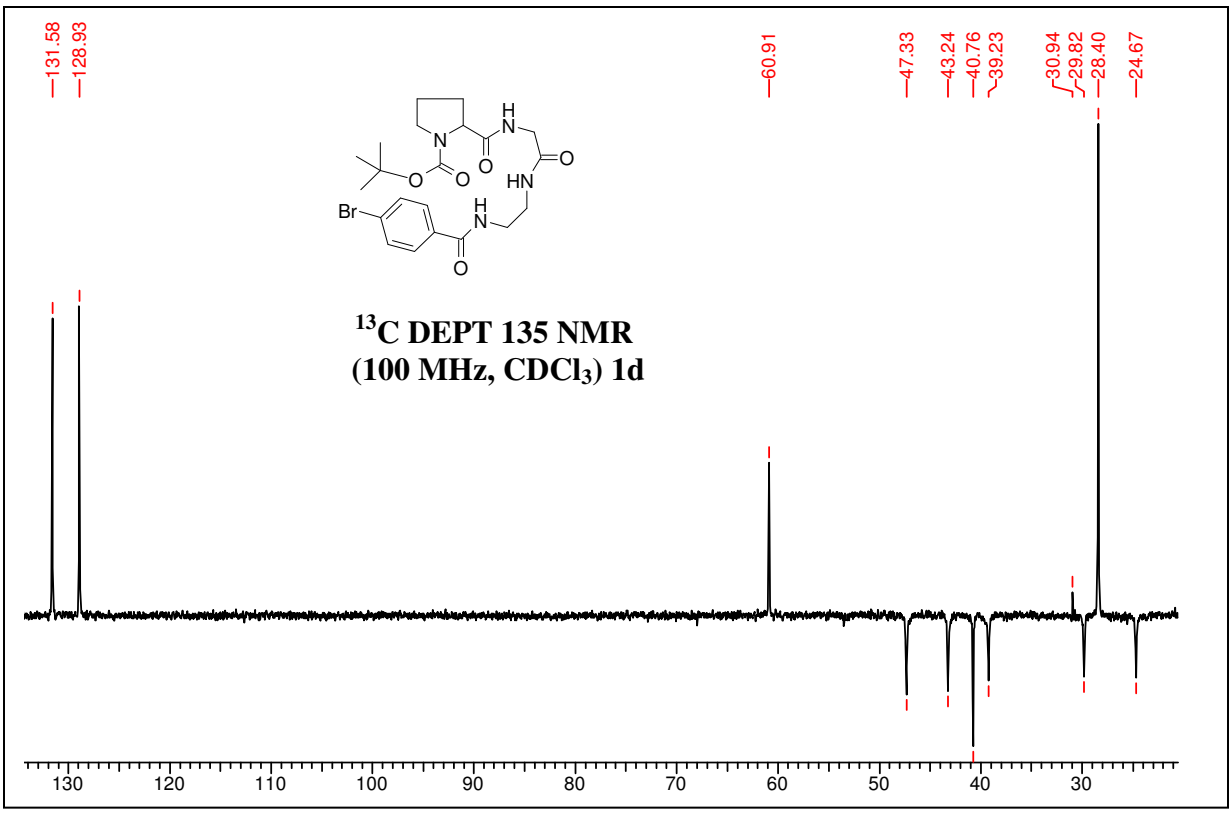


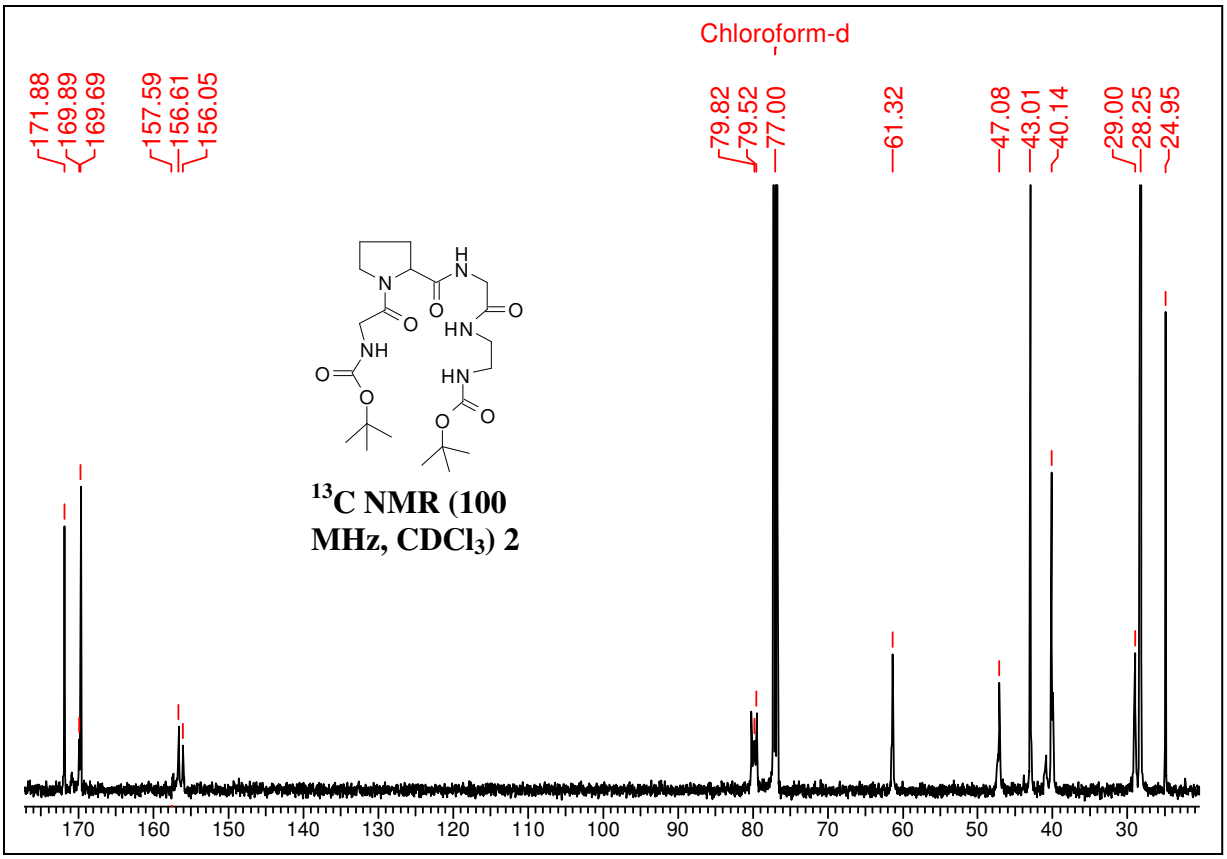




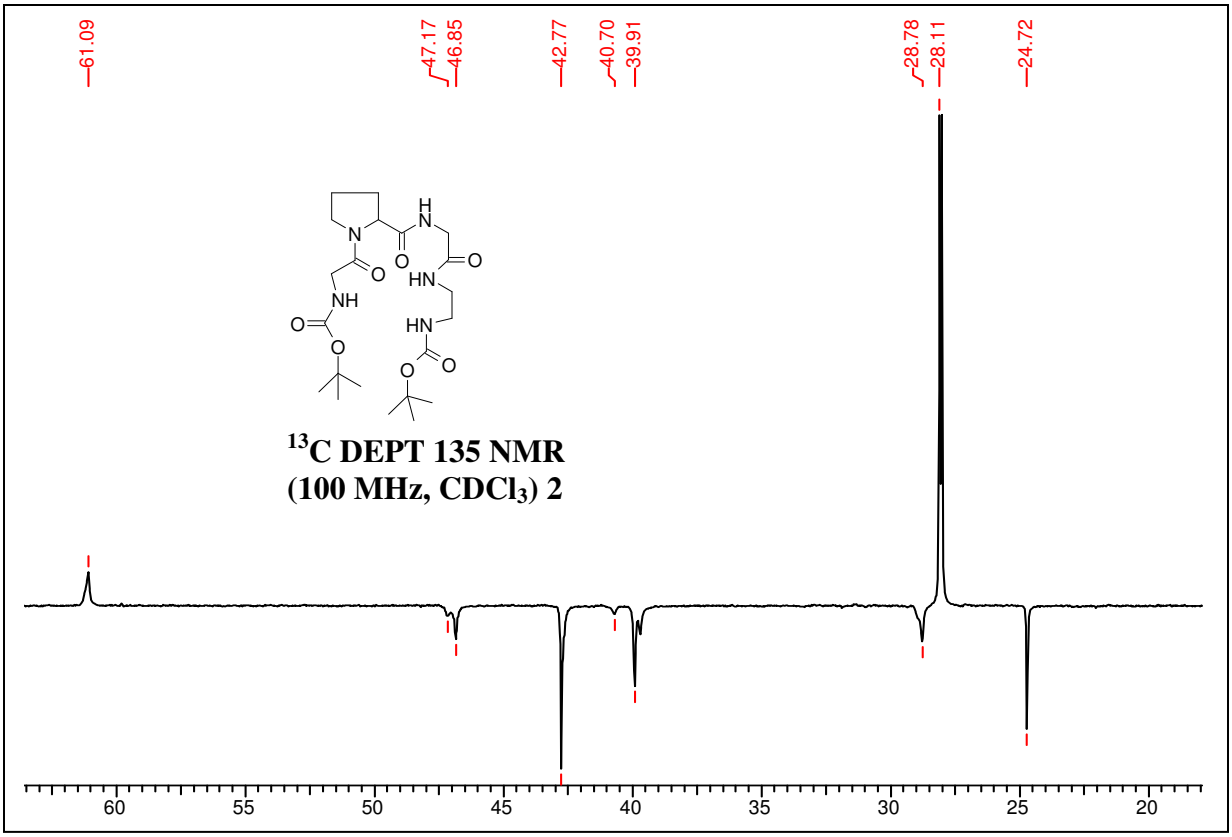



CDCl3
173.39
170.73
166.77
155.52
132.95
131.46
128.81
125.89
80.70
77.00
60.80
53.35
47.21
43.12
40.65
39.14
30.80
29.71
28.29
24.55
Br
HN
^{13}C NMR (100 MHz, CDCl$_3$) 1d

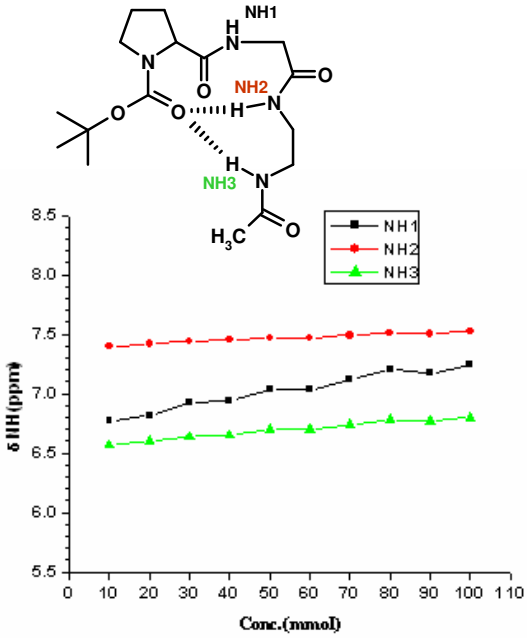

131.58
128.93
60.91
47.33
43.24
40.76
39.23
30.94
29.82
28.40
24.67
Br
HN
^{13}C DEPT 135 NMR (100 MHz, CDCl$_3$) 1d



^{13}C NMR (100 MHz, $CDCl_3$) 2



^{13}C DEPT 135 NMR (100 MHz, $CDCl_3$) 2

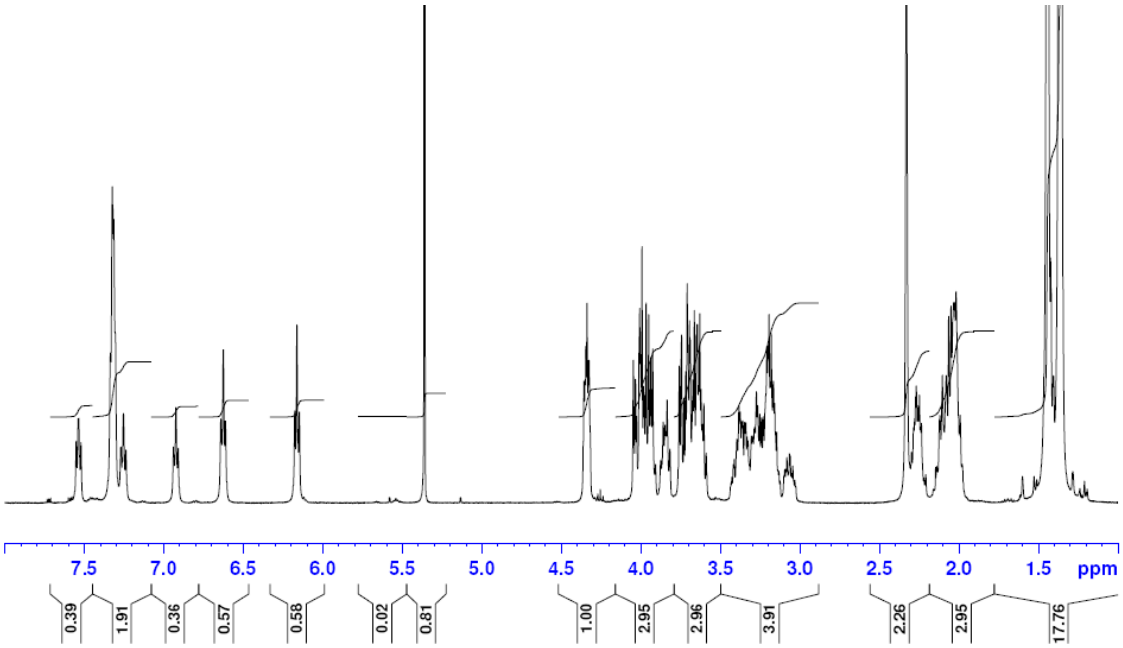


S.No	Conc. (mmol)	NH1	NH2	NH3
1	10	6.77	7.4	6.57
2	20	6.82	7.42	6.6
3	30	6.92	7.44	6.64
4	40	6.94	7.45	6.65
5	50	7.03	7.47	6.7
6	60	7.03	7.47	6.7
7	70	7.12	7.49	6.74
8	80	7.2	7.51	6.78
9	90	7.17	7.5	6.77
10	100	7.24	7.52	6.8

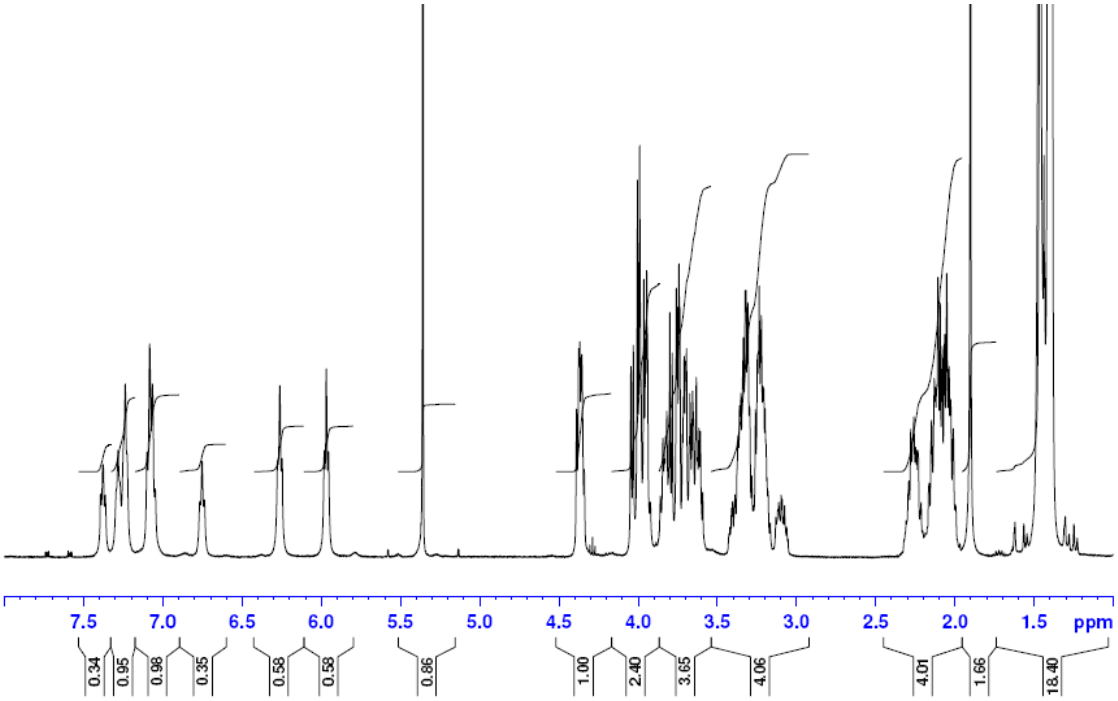
Figure 1: Dilution experiment of 1b (400 MHz, CDCl3). Concentration varies 10 mmol to 100 mmol

Table 1: Dilution data for 1b

^{1}H spectrum of 2 in CD_2Cl_2 (20 mg/ml) at 243K

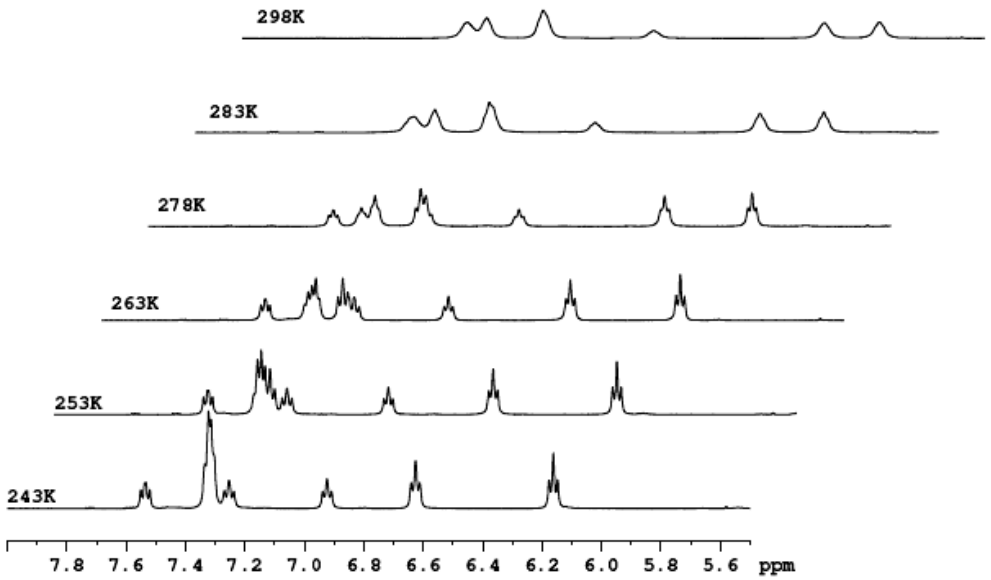


^{1}H spectrum of 2 in CD_2Cl_2 (20 mg/ml) at 278K



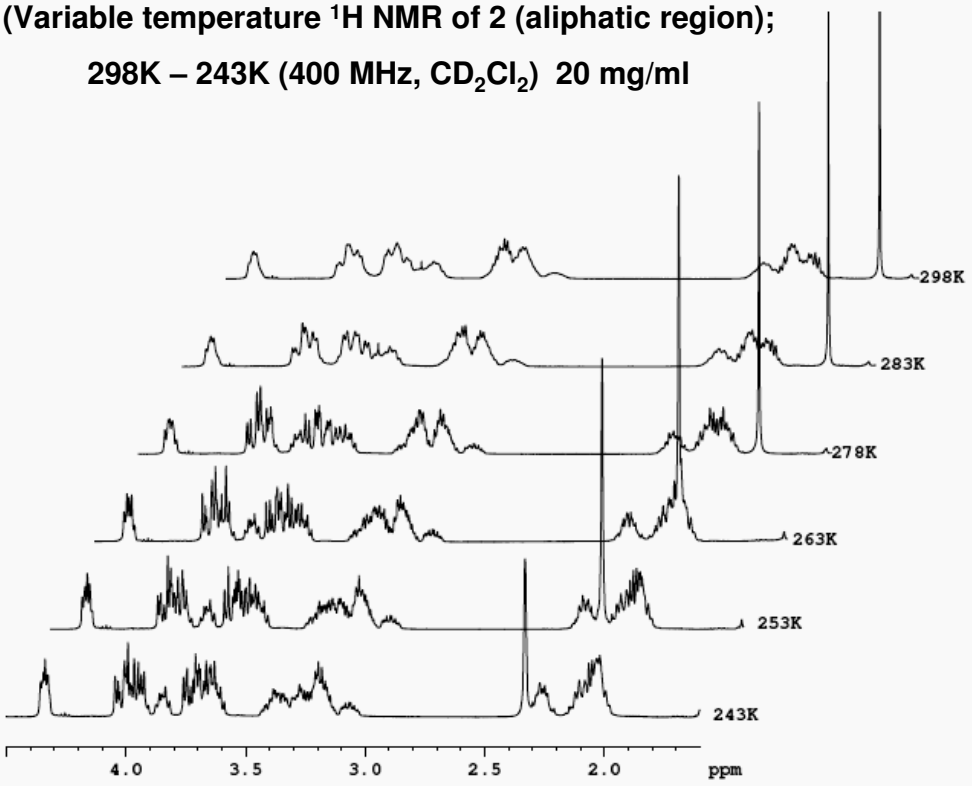
(Variable temperature ^{1}H NMR spectra of 2 (NH region);

298K – 243K (400 MHz, CD_2Cl_2) 20 mg/ml

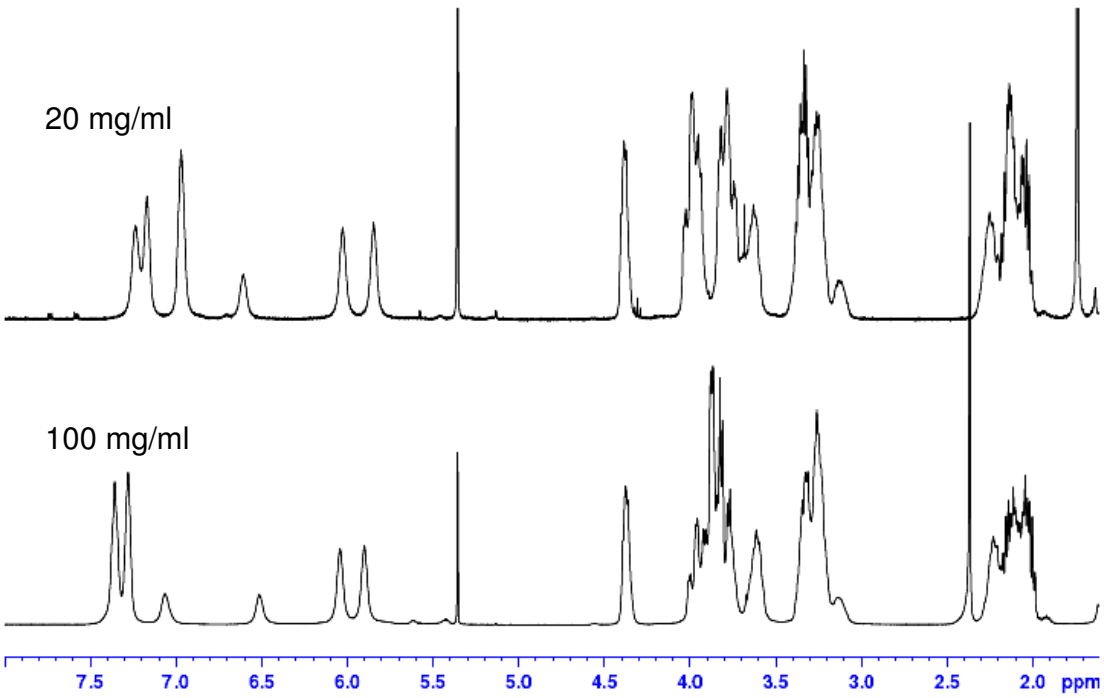


(Variable temperature ^{1}H NMR of 2 (aliphatic region);

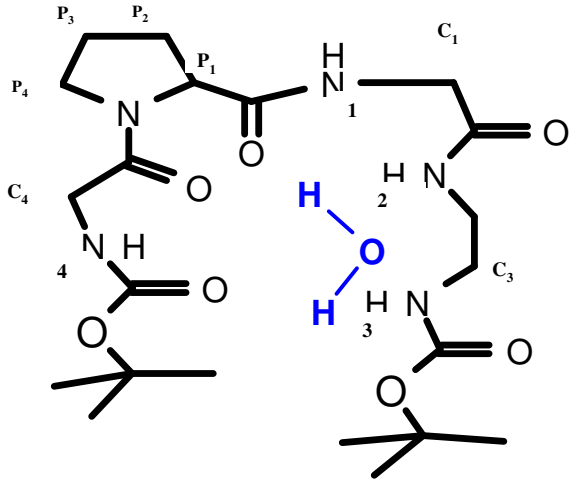
298K – 243K (400 MHz, CD_2Cl_2) 20 mg/ml



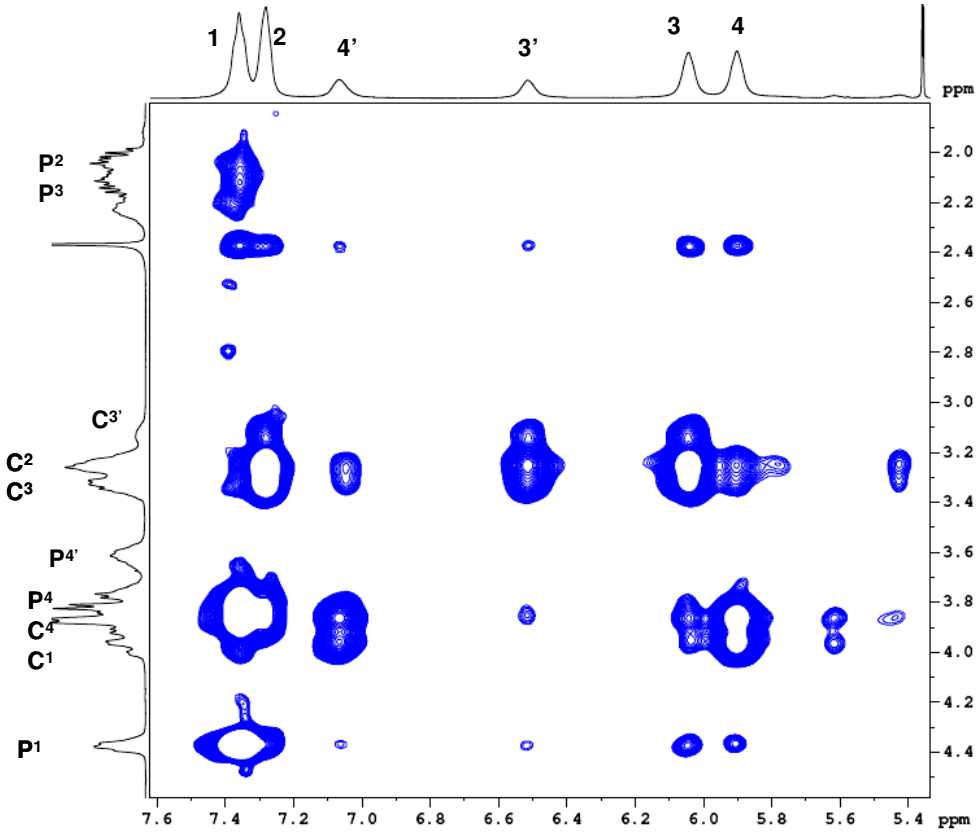
^{1}H NMR spectra of 2 in CD_2Cl_2



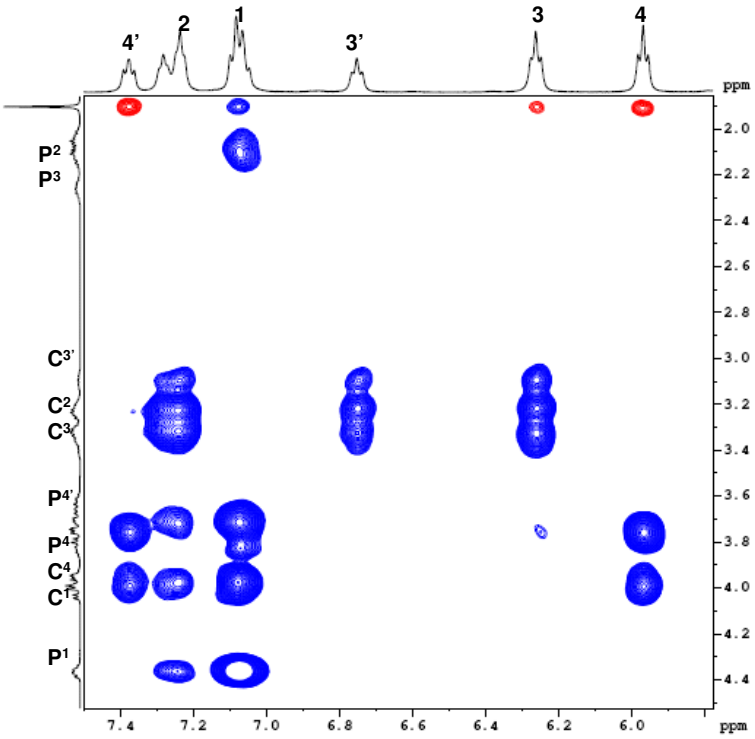
Molecular Structure of 2 with selected atom labeling



2D NOESY of 2 in CD_2Cl_2 (100 mg/ml) with 1.25 s Mixing time at 298K



2D NOESY of 2 in CD_2Cl_2 (20 mg/ml) with 1.25 s Mixing time at 278K



DOSY spectra of 2 at different concentrations

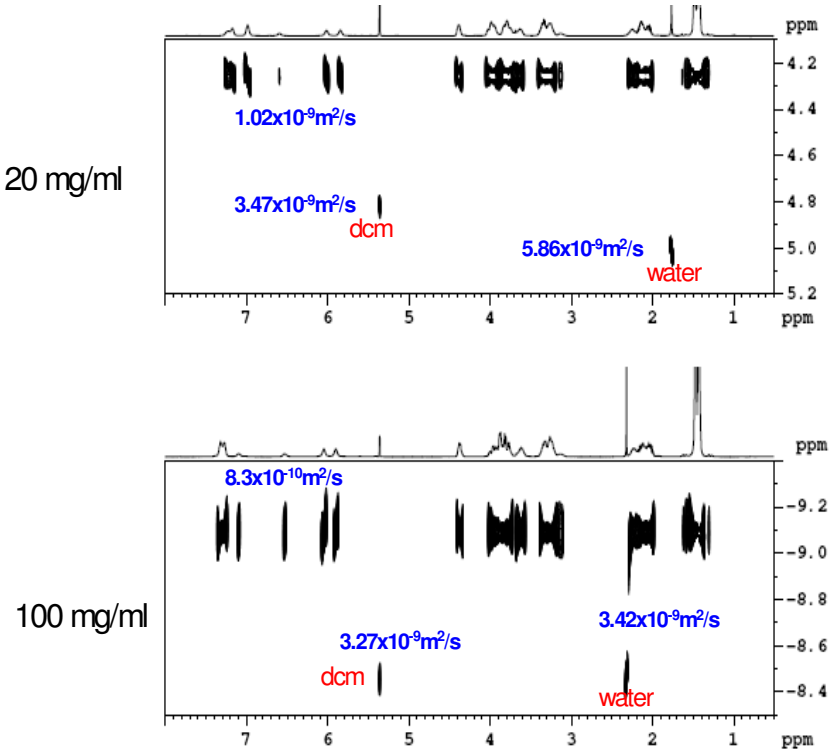


Table I: Self diffusion coefficient table for 2 in DCM

Conc. mmol	Self diffusion coefficient ($10^{-9}m^2$ sec^{-1})		
	Substrate	Water	DCM
--	--	6.58	3.57
53	1.02	5.86	3.47
265	0.82	3.42	3.27

PyMOL-rendered crystal structure of **1a** showing molecular self-assembly in the solid-state. Hydrogens other than at the hydrogen bonding sites have been deleted for clarity.