Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

NMR analysis of ^{15}N-labeled naphthyridine carbamate dimer (NCD) to contiguous CGG/CGG units in DNA

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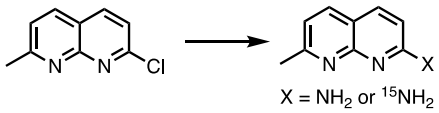
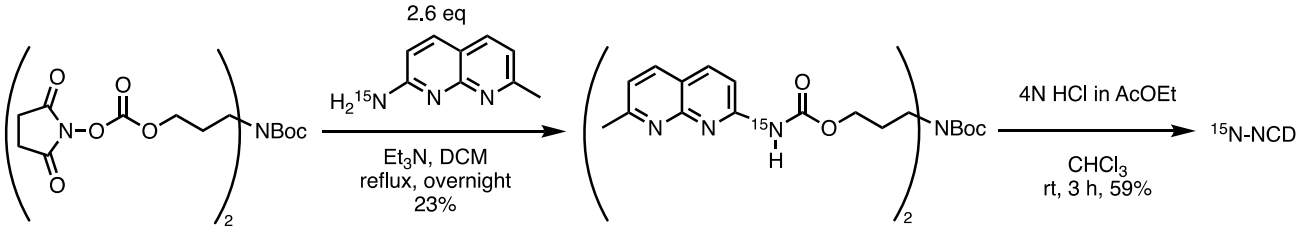
Supporting Figures

Table S1. The synthetic studies of the amination reaction on 2-chloro-7-methyl-1,8-naphthyridine. Entry 4 is used for the preparation of **2-[*15N*]amino-7-methyl-1, 8-naphthyridine**.



Entry	Amine source	X	Eq of X	additive	Time (h)	Yield (%)
1	22% NH_3aq	NH_2	100	-	12	ND
2	22% NH_3aq	NH_2	100	-	24	8
3	22% NH_3aq	NH_2	100	Cu (1 eq)	24	55
4	22% $^{15}NH_3$aq	$^{15}NH_2$	15	Cu (1 eq)	24	40



Scheme. S1 Synthesis of ^{15}N-NCD

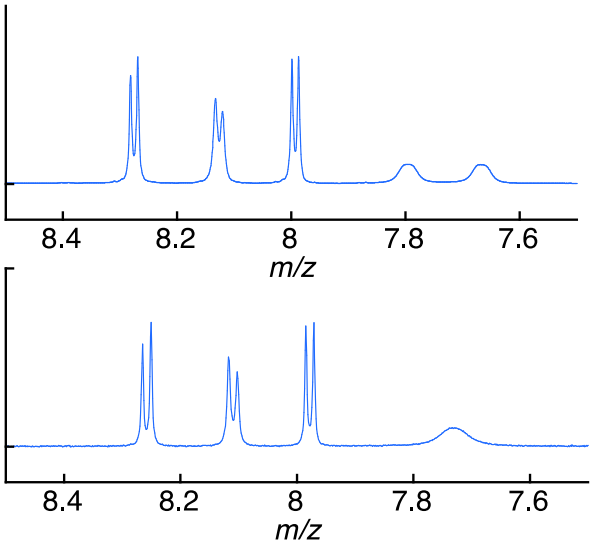
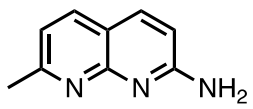


Fig. S1 The enlarged view of ^{1}H-NMR spectrum of (A) Boc-protected ^{15}N-NCD, and (B) Boc-protected NCD

Experimental section

General. Reagents and solvents were purchased from standard suppliers and used without further purification. Reactions were monitored with TLC plate silica gel 60 F_{254}. Spots on TLC were monitored with UV, phosphomolybdic acid, ninhydrin, or anisaldehyde. A C-200 Silica gel was used for silica gel flash chromatography. NMR spectra were measured with 600 MHz (JEOL) or 700 MHz (Bruker). NMR spectra were analyzed by Delta for Mac (JEOL, version 6.0) or Topspin for Mac (Bruker, version 4.1.4). The multiplicity was expressed as follows; s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. The chemical shifts are expressed in ppm relative to residual solvent as an internal standard, and coupling constants (*J* values) were represented in hertz.

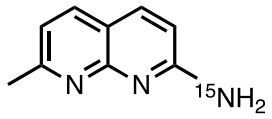
Preparation of 2-amino(^{15}N)-1,8-naphthyridine. To prepare ^{15}N-NCD, the synthesis method for 2-amino(^{15}N)-1,8-naphthyridine (**1**) was investigated. (Table S1) 2-Chloro-1,8-naphthyridine was selected as the precursor to compound **1**. Preliminary synthetic investigations were performed using unlabeled aqueous ammonia. When 2-chloro-1,8-naphthridine was treated with aqueous ammonia at 100 °C in a sealed tube for 12 h, 2-amino-1,8-naphthyridine was not detected. Even reaction time was extended to 24 h, the yield of 2-amino-1,8-naphthyridine was only an 8% yield. (Entry 2) Next, referring to the synthesis study demonstrating the copper (0) catalyzing nucleophilic substitution reaction of 2,6-dichloro-isoniconitic acid using ammonia,[1] 1.0 equivalent copper powder was incorporated into the reaction conditions. The yield was drastically improved to 55%. (Entry 3)



Chemical Formula: $C_9H_9N_3$
Exact Mass: 159.07965
Molecular Weight: 159.19200

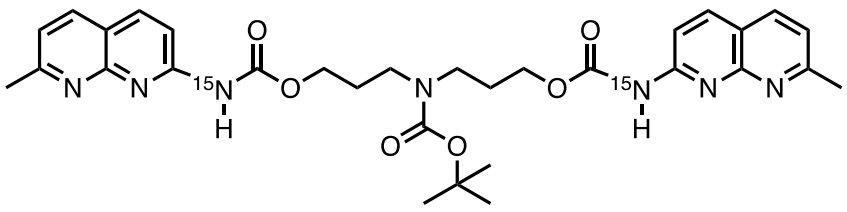
2-amino-7-methyl-1,8-naphthyridine. 2-Chloro-7-methyl-1,8-naphthyridine (100 mg, 0.560 mmol) and copper powder (35 mg, 0.550 mmol) were added to 6 M NH_3aq (22wt%, 60 mmol, 10 mL) in a sealed tube. Then the mixture was stirred for 24 h at 100 °C. The reaction mixture was filtered with celite. The celite was washed with 1 M NaOHaq. Then, all filtrate was recovered, and extracted with

$CHCl_3$. The organic layer was collected, dried with anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel eluted with $CHCl_3$/MeOH = 20/1 to give 2-amino-7-methyl-1,8-naphthyridine (49 mg, 0.307 mmol, 55%) as a white solid. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.83 (d, J = 3.0 Hz, 1H), 7.82 (d, J = 3.0 Hz, 1H), 7.08 (d, J = 8.5 Hz, 1H), 6.72 (d, J = 8.5 Hz, 1H), 5.10-4.90 (br, 2H), 2.69 (s, 3H).



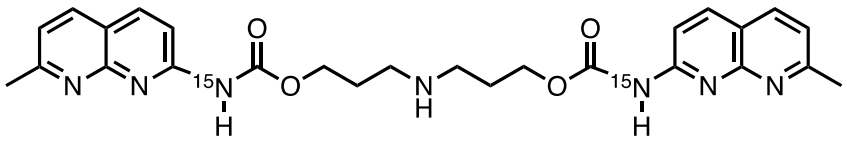
Chemical Formula: $C_9H_9N_2{}^{15}N$
Exact Mass: 160.07668
Molecular Weight: 160.18511

2-[*^{15}N*]amino-7-methyl-1, 8-naphthyridine. 2-Chloro-7-methyl-1,8-naphthyridine (101 mg, 0.565 mmol) and copper powder (38 mg, 0.598 mmol) were added to 6 M $^{15}NH_3$aq (22wt%, 9.0 mmol, 1.5 mL) in a sealed tube. Then, the mixture was stirred for 24 h at 100 °C. After cooling the mixture to room temperature, the reaction mixture was filtered with celite. The celite was washed with 1 M NaOHaq. All filtrate was recovered, and extracted with $CHCl_3$. The obtained organic layer was dried with anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluted with $CHCl_3$/MeOH = 20/1 to give 2-[^{15}N]amino-7-methyl-1, 8-naphthyridine (37 mg, 0.230 mmol, 40%) as white solid; ^{1}H-NMR (700 MHz, $CDCl_3$) δ 7.83 (d, J = 3.8 Hz, 1H), 7.82 (d, J = 3.8 Hz, 1H), 7.11 (d, J = 8.0 Hz, 1H), 6.74 (d, J = 8.0 Hz, 1H), 5.01 (d, J = 86.9 Hz, 2H), 2.69 (s, 3H); ^{13}C-NMR (175 MHz, $CDCl_3$) δ 162.2, 159.3, 159.2, 156.2, 138.0, 136.1, 118.9, 115.3, 111.3, 111.2, 25.4; ^{15}N-NMR (175 MHz, $CDCl_3$) δ 84.0; HR-ESI-MS *m/z* calcd for $C_9H_{10}N_2{}^{15}N$ $[M+H]^+$ 161.0840, found 161.0837.



Chemical Formula: $C_{31}H_{37}N_5{}^{15}N_2O_6$
Exact Mass: 605.27460
Molecular Weight: 605.66622

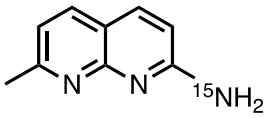
^{15}N-NCD-Boc. To the solution of tert-butyl bis(3-(succinimidyloxycarbonyloxy)-propyl)carbamate (96 mg, 0.18 mmol) in CH_2Cl_2 (10 mL) was added 2-[^{15}N]amino-7-methyl-1,8-naphthyridine (76 mg, 0.47 mmol) and triethylamine (1.6 mL, 11.5 mmol) at room temperature. The solution was stirred for 30 min at room temperature and then refluxed overnight. Then the solution was cooled to room temperature, diluted with $CHCl_3$, and washed with saturated $NaHCO_3$aq. The organic layer was collected, dried with anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel, eluted with AcOEt/MeOH = 100/0, 100/2, 100/4, 100/6, and then $CHCl_3$/MeOH = 100/2 to give ^{15}N-NCD-Boc (26 mg, 0.0430 mmol, 23%) as a white solid; ^{1}H-NMR (700 MHz, $CDCl_3$) δ 8.28 (d, J = 9.1 Hz, 2H), 8.13 (d, J = 9.1 Hz, 2H), 7.99 (d, J = 8.4 Hz, 2H), 7.73 (d, J = 88.6 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 4.27 (t, J = 6.2 Hz, 4H), 3.45-3.30 (br, 4H), 2.75 (s, 6H), 2.00-1.90 (br, 4H), 1.47 (s, 9H); ^{15}N-NMR (175 MHz, $CDCl_3$) δ 127.2; ^{13}C-NMR (175 MHz, $CDCl_3$) δ 163.2, 155.4, 154.7, 154.6, 153.3, 153.1, 153.0, 139.0, 136.4, 121.3, 118.0, 112.6, 79.9, 63.7, 63.5, 44.4, 44.2, 28.4, 28.2, 27.7, 25.6; HR-ESI-MS m/z calcd for $C_{31}H_{37}N_5{}^{15}N_2O_6[M+H]^+$ 606.2819, found 606.2818.

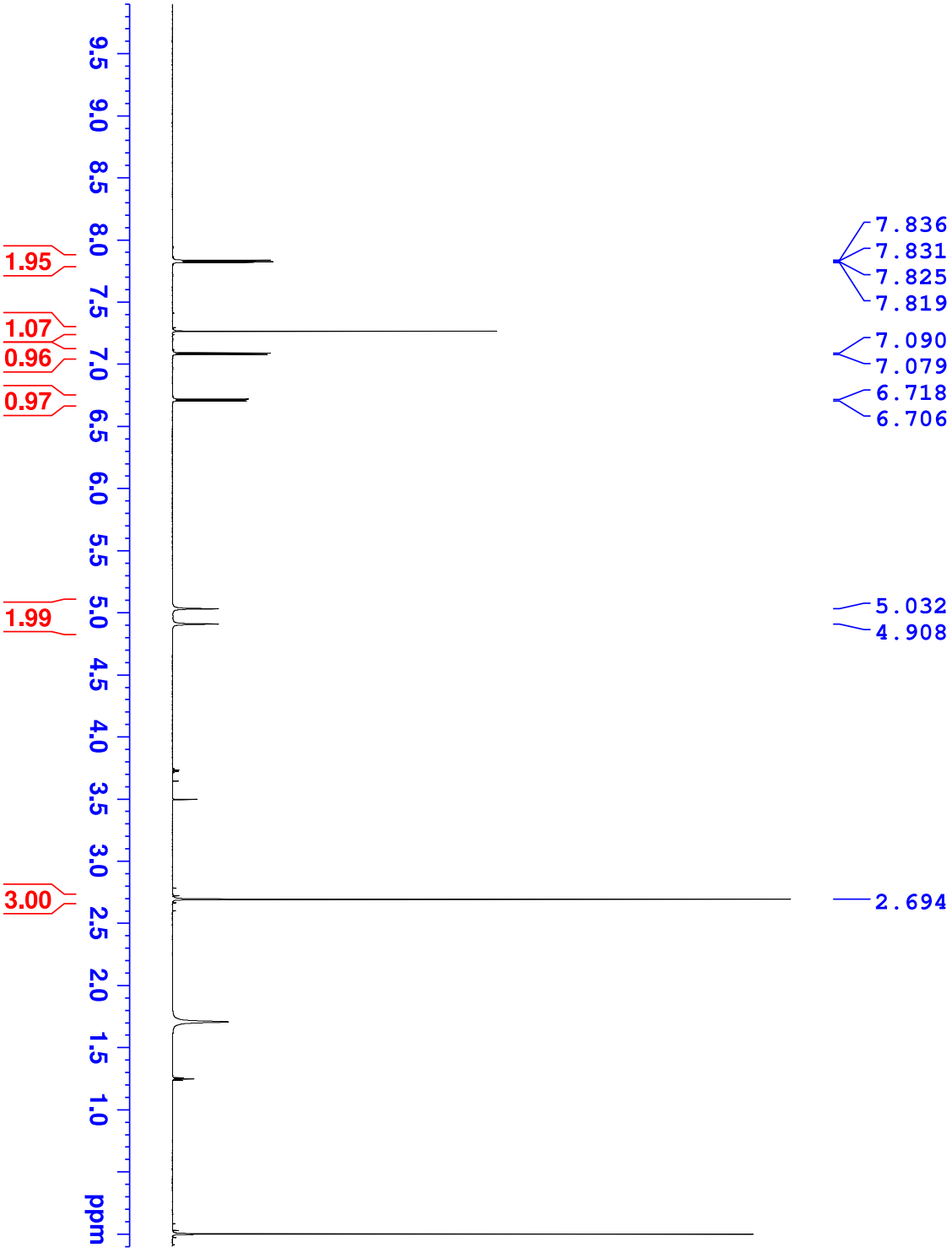


Chemical Formula: $C_{26}H_{29}N_5{}^{15}N_2O_4$
Exact Mass: 505.22217
Molecular Weight: 505.54922

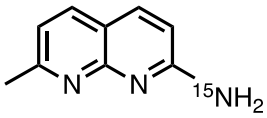
^{15}N-NCD. To the solution of ^{15}N-NCD-Boc (24.8 mg, 0.0411 mmol) in $CHCl_3$ (2 mL) was added 4 M HCl/AcOEt (10 mL) at 0 °C. The solution was stirred for 1 hour at room temperature. Then the reaction mixture of ^{15}N-NCD was further purified using RP-HPLC using 0.1% AcOH in H_2O and acetonitrile. To the solution of ^{15}N-NCD in H_2O (3 mL) was added 1 M HClaq dropwise. This process was three times for salt exchange, and freeze-dried to give ^{15}N-NCD +HCl (13.1 mg, 59%) ^{1}H-NMR (600 MHz, D_2O) δ 8.54 (d, J = 8.2 Hz, 2H), 8.25 (d, J = 8.9 Hz, 2H), 8.01 (d, J = 8.9 Hz, 2H), 7.53 (d, J = 8.2 Hz, 2H), 4.25 (t, J = 5.5 Hz, 4H), 3.15 (t, J = 7.2 Hz, 4H), 2.74 (s, 6H), 2.07-2.03 (m, 4H); ^{15}N-NMR (175 MHz, D_2O) δ 123.1; ^{13}C-NMR (175 MHz, D_2O) δ 159.5, 156.7, 156.6, 154.0, 153.8, 147.1, 147.1, 145.6, 140.0, 122.1, 119.1, 116.0, 116.0, 63.6, 45.3, 24.9, 20.2; HR-ESI-MS *m/z*: calcd for $C_{31}H_{37}N_5{}^{15}N_2O_6$ $[M+H]^+$ 506.2295, found 506.2331.

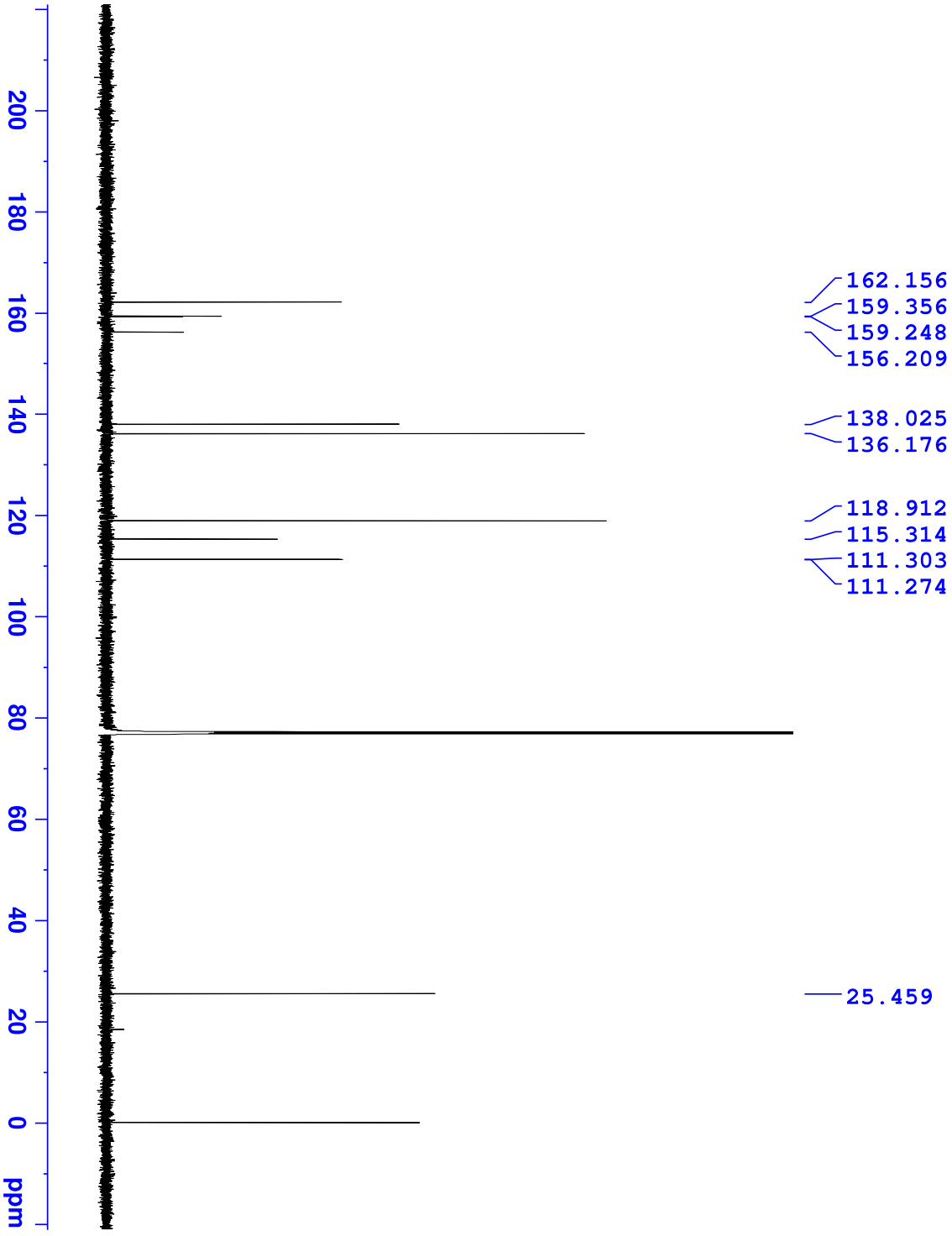
^{1}H-NMR of **2-[*^{15}N*]amino-7-methyl-1, 8-naphthyridine** in $CDCl_3$



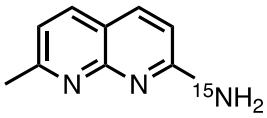


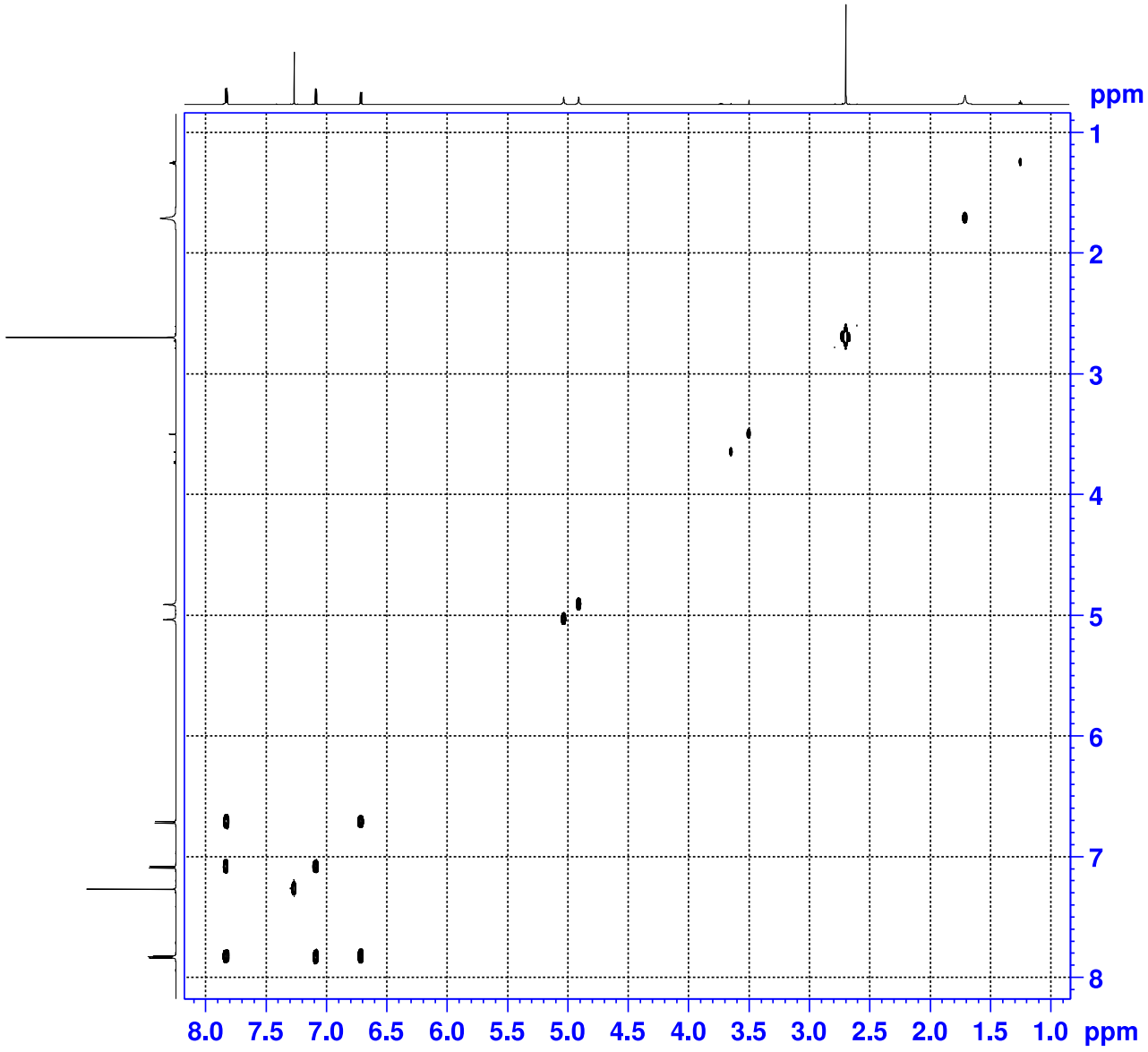
^{13}C-NMR of **2-[^{15}*N*]amino-7-methyl-1, 8-naphthyridine** in $CDCl_3$



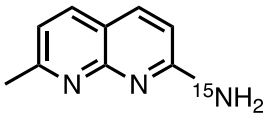


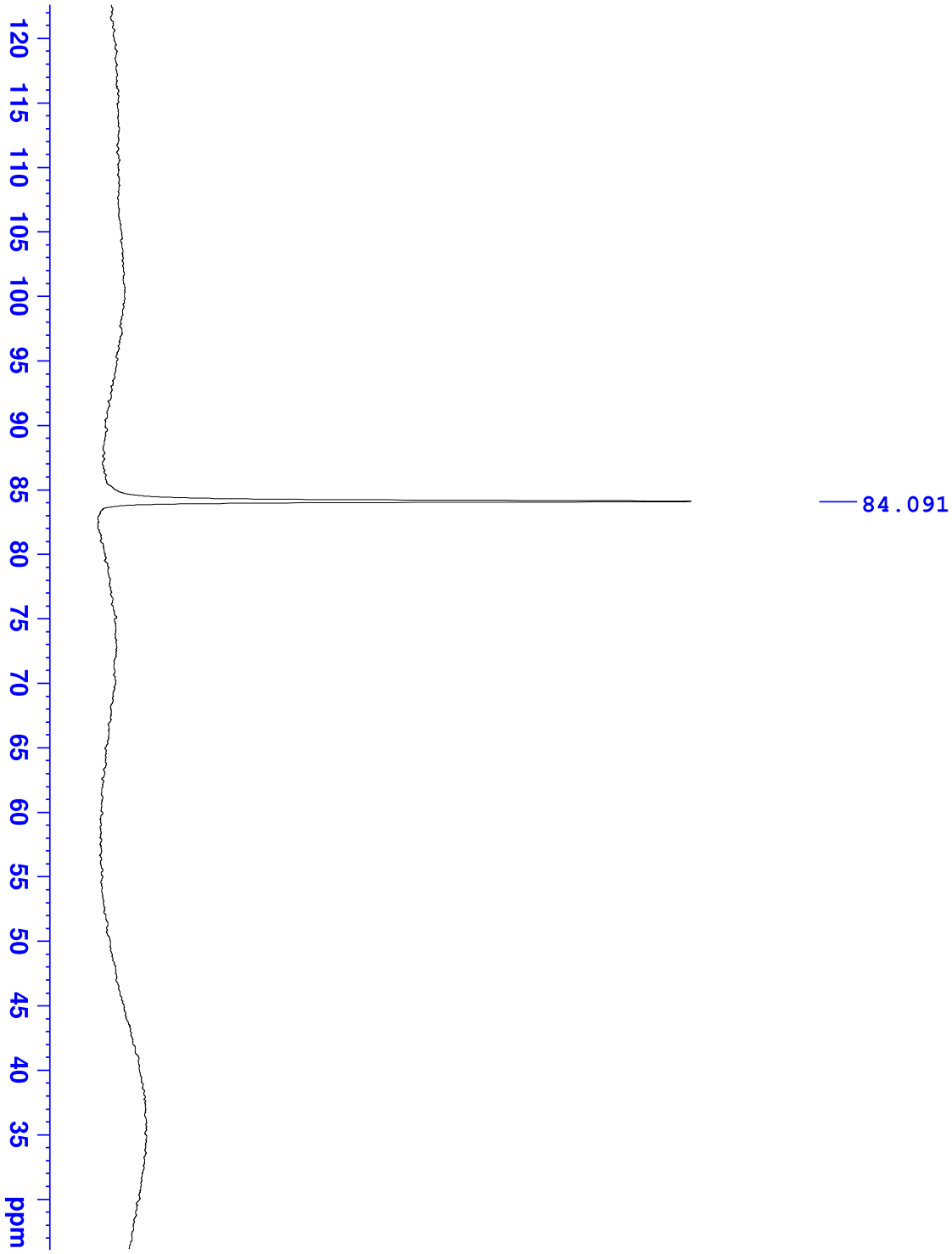
COSY of **2-[*15N*]amino-7-methyl-1, 8-naphthyridine** in $CDCl_3$



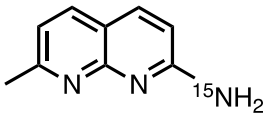


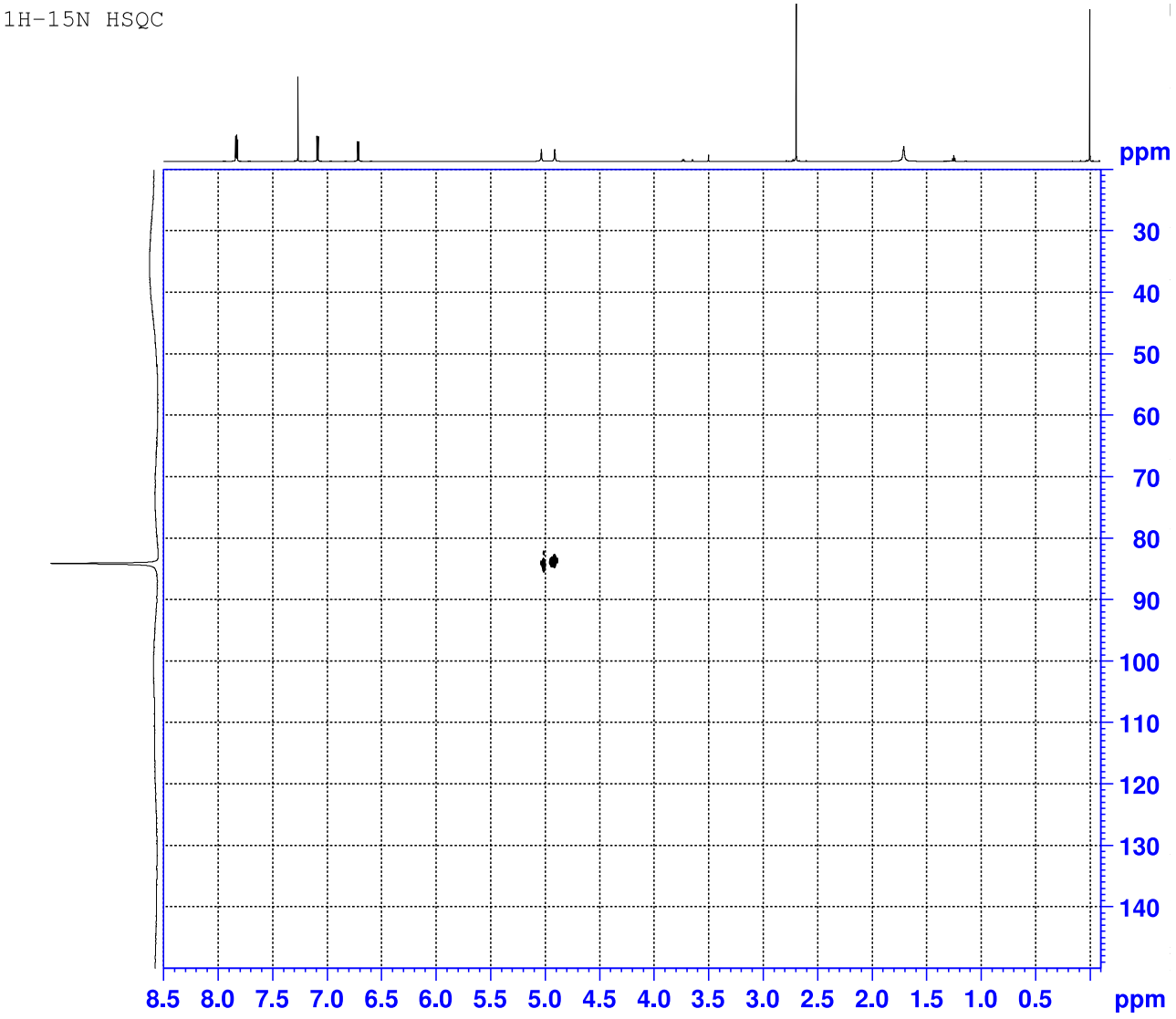
^{15}N-NMR of **2-[*^{15}N*]amino-7-methyl-1, 8-naphthyridine** in $CDCl_3$



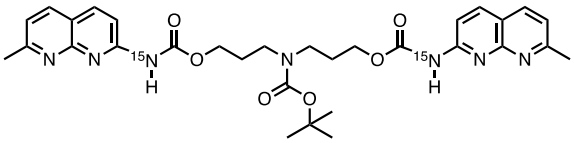


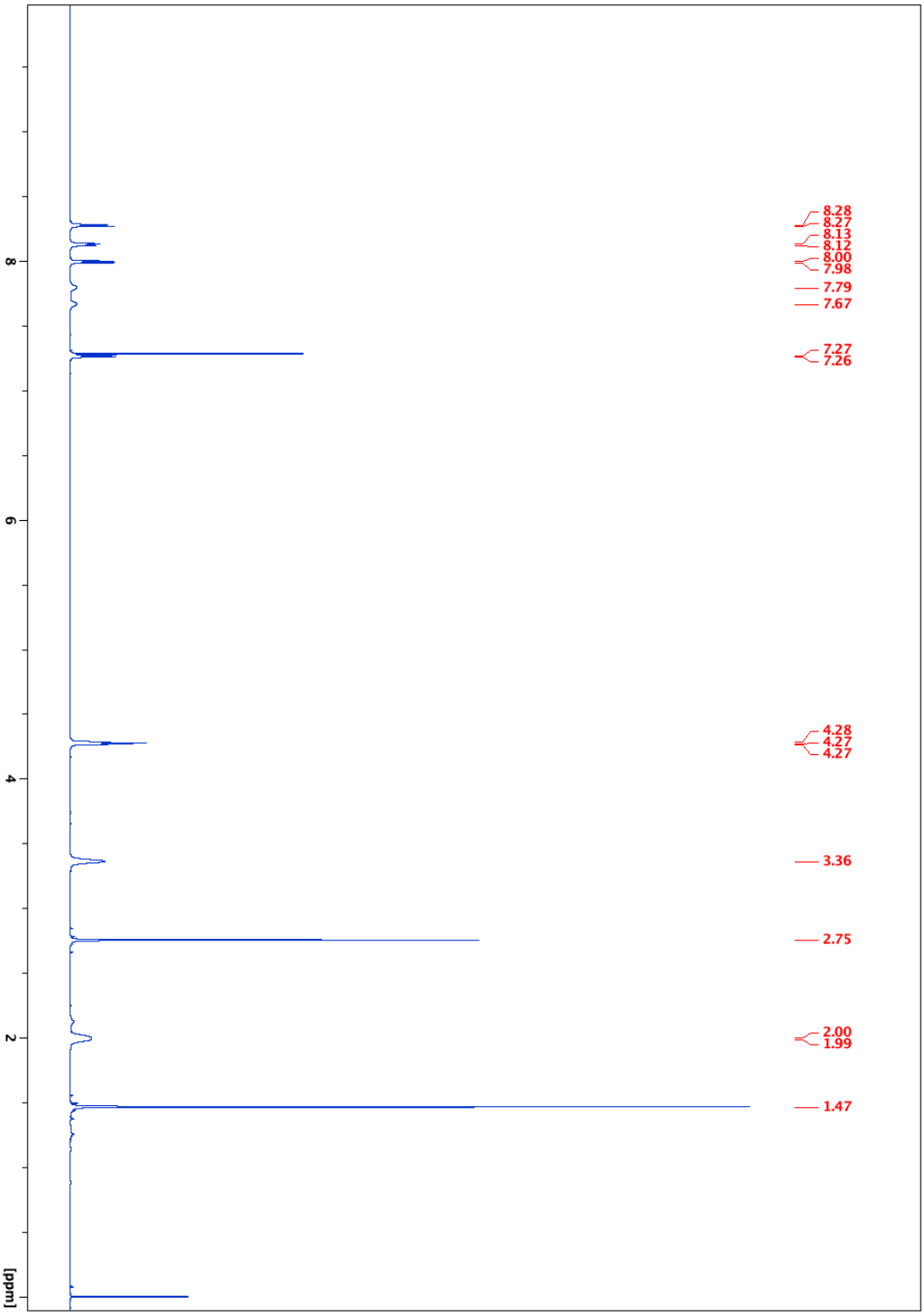
^{1}H-^{15}N HSQC of **2-[*^{15}N*]amino-7-methyl-1, 8-naphthyridine** in $CDCl_3$



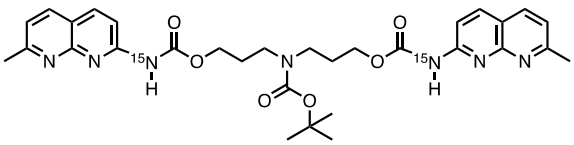


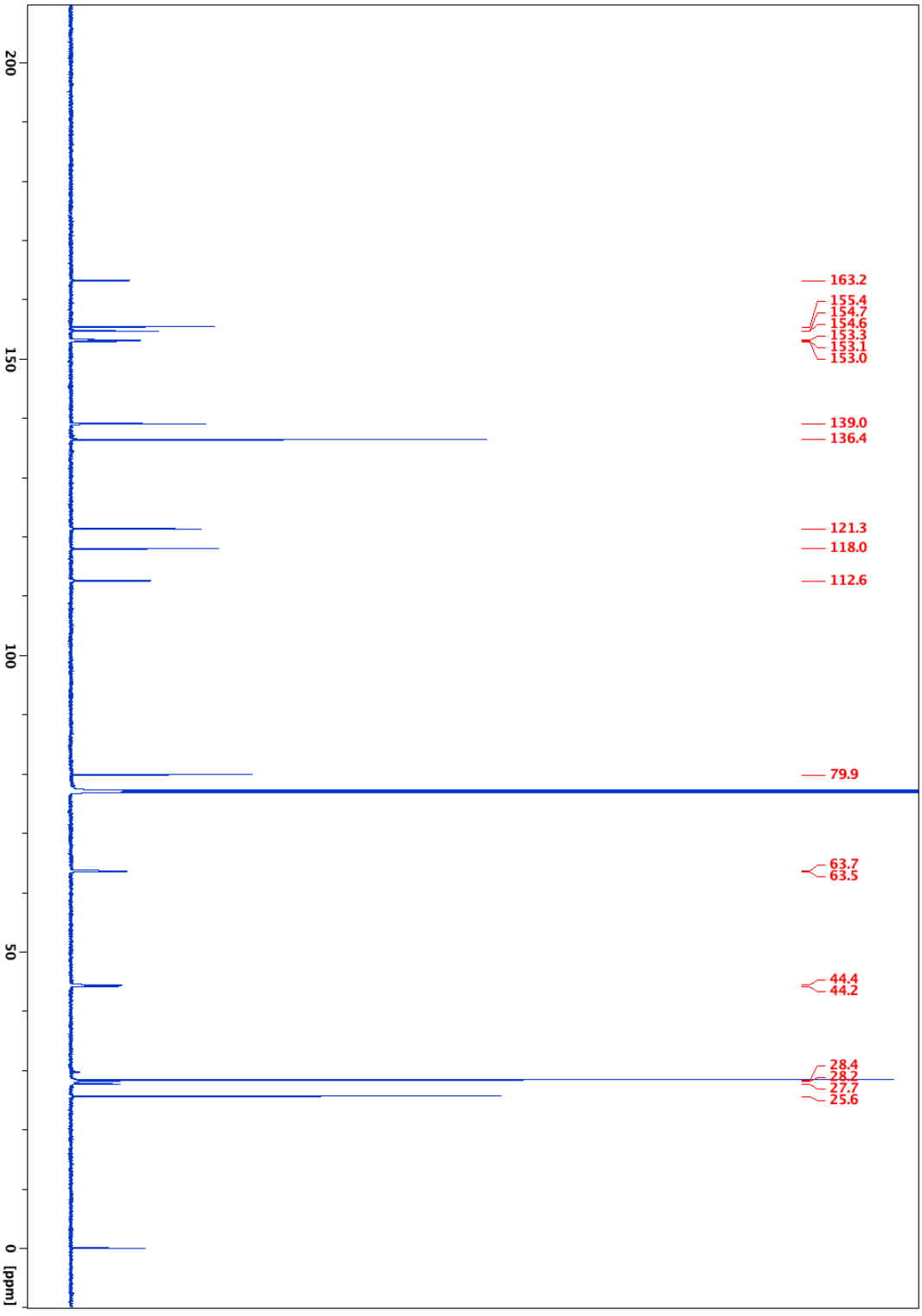
^{1}H-NMR spectrum of ^{15}**N-NCD-Boc** in $CDCl_3$



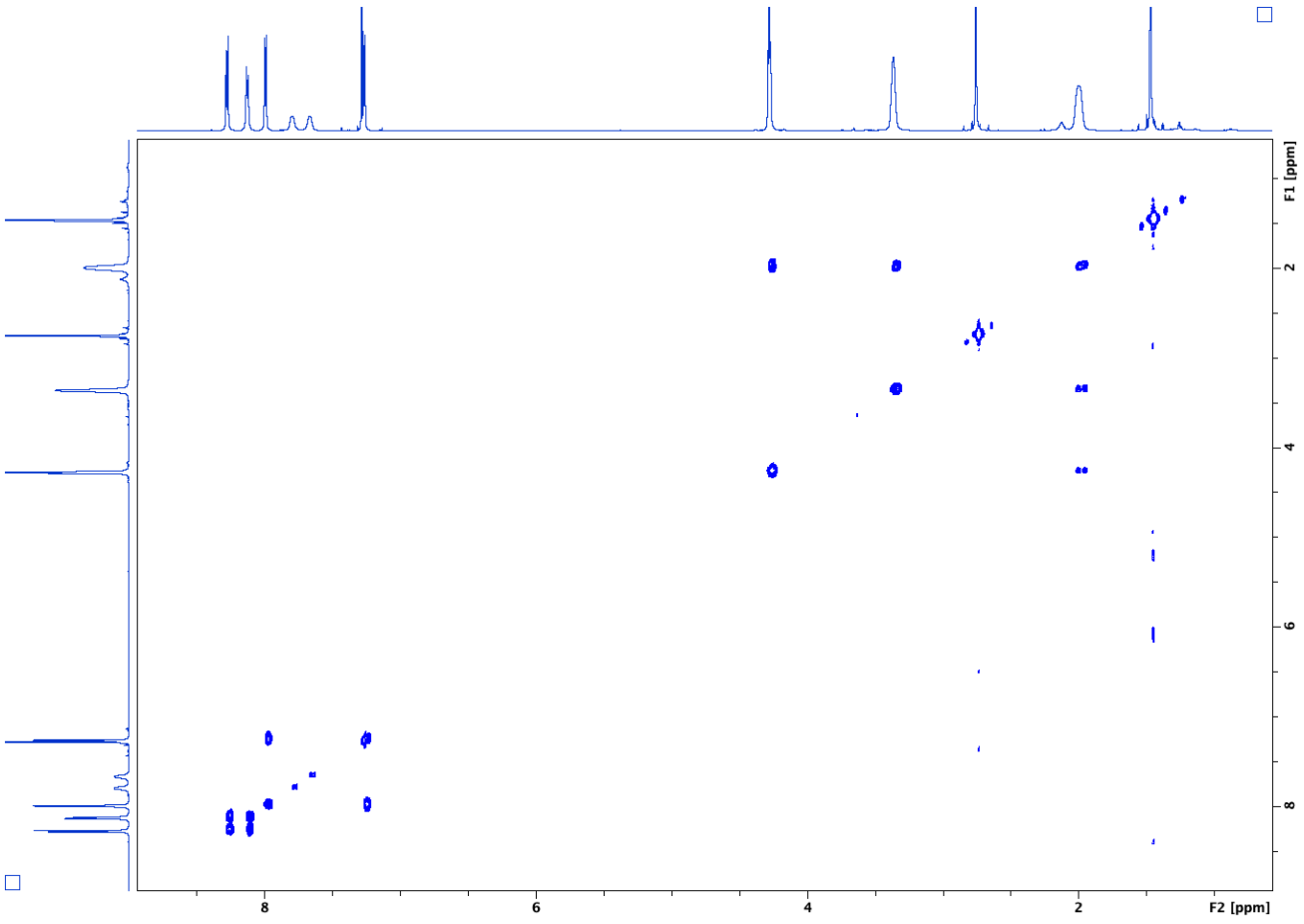


^{13}C-NMR spectrum of ^{15}**N-NCD-Boc** in $CDCl_3$

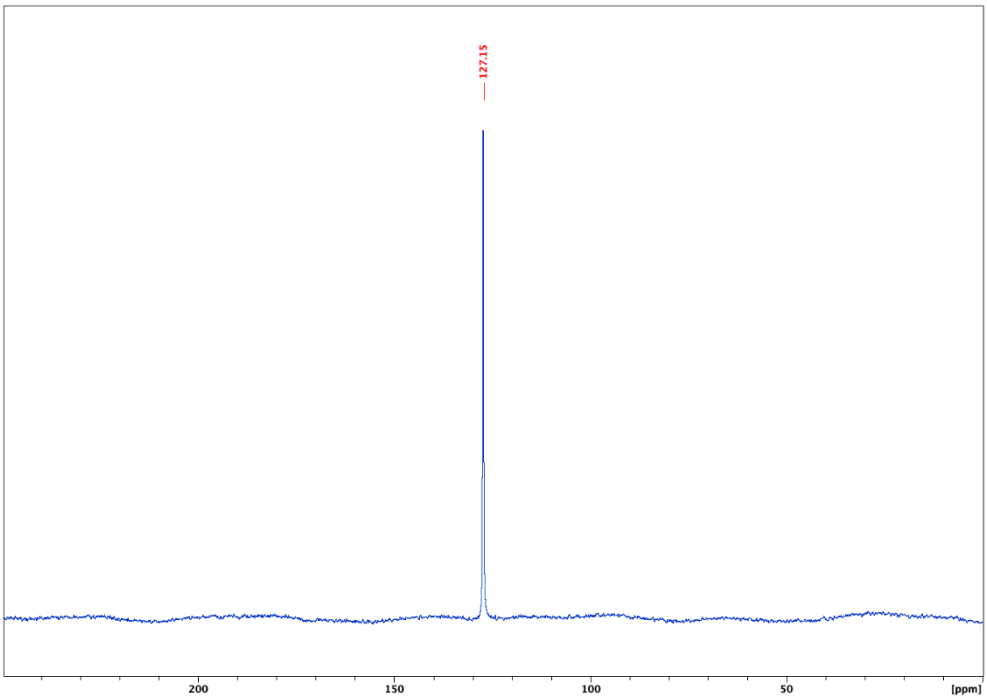


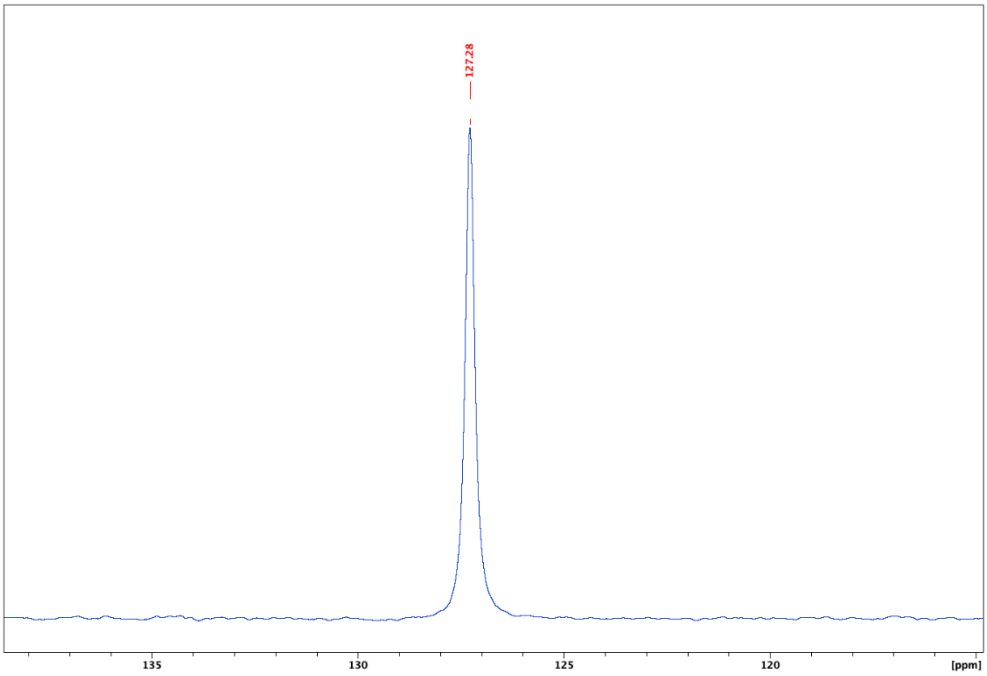


COSY spectrum of ^{15}**N-NCD-Boc** in $CDCl_3$

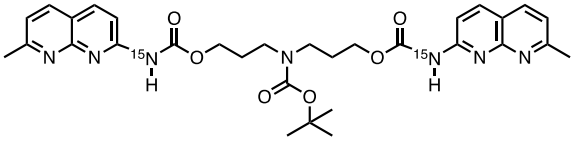


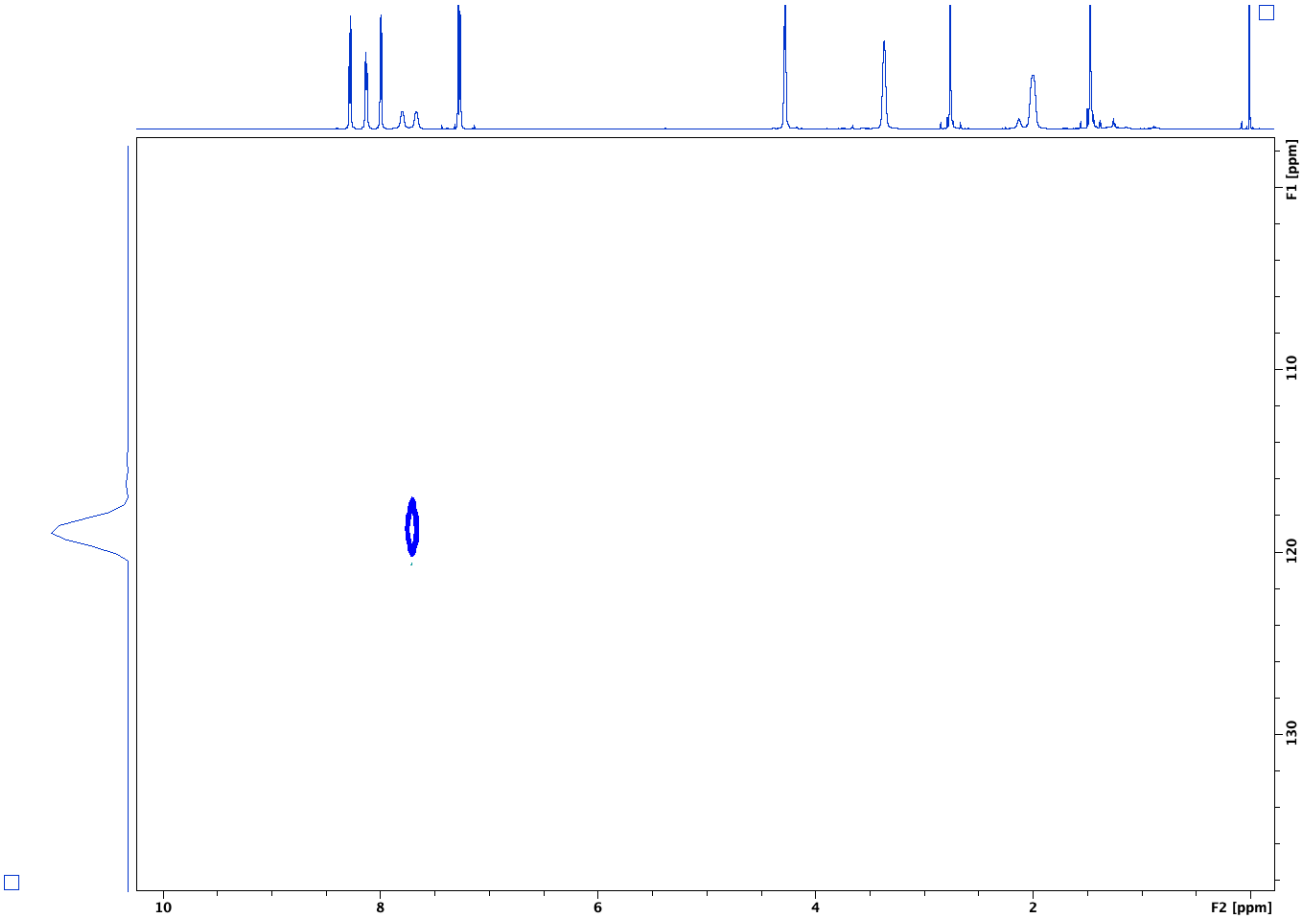
^{15}N-NMR spectrum of **^{15}N-NCD-Boc** in $CDCl_3$



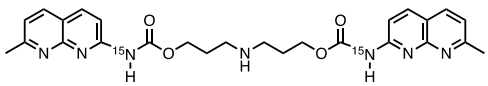


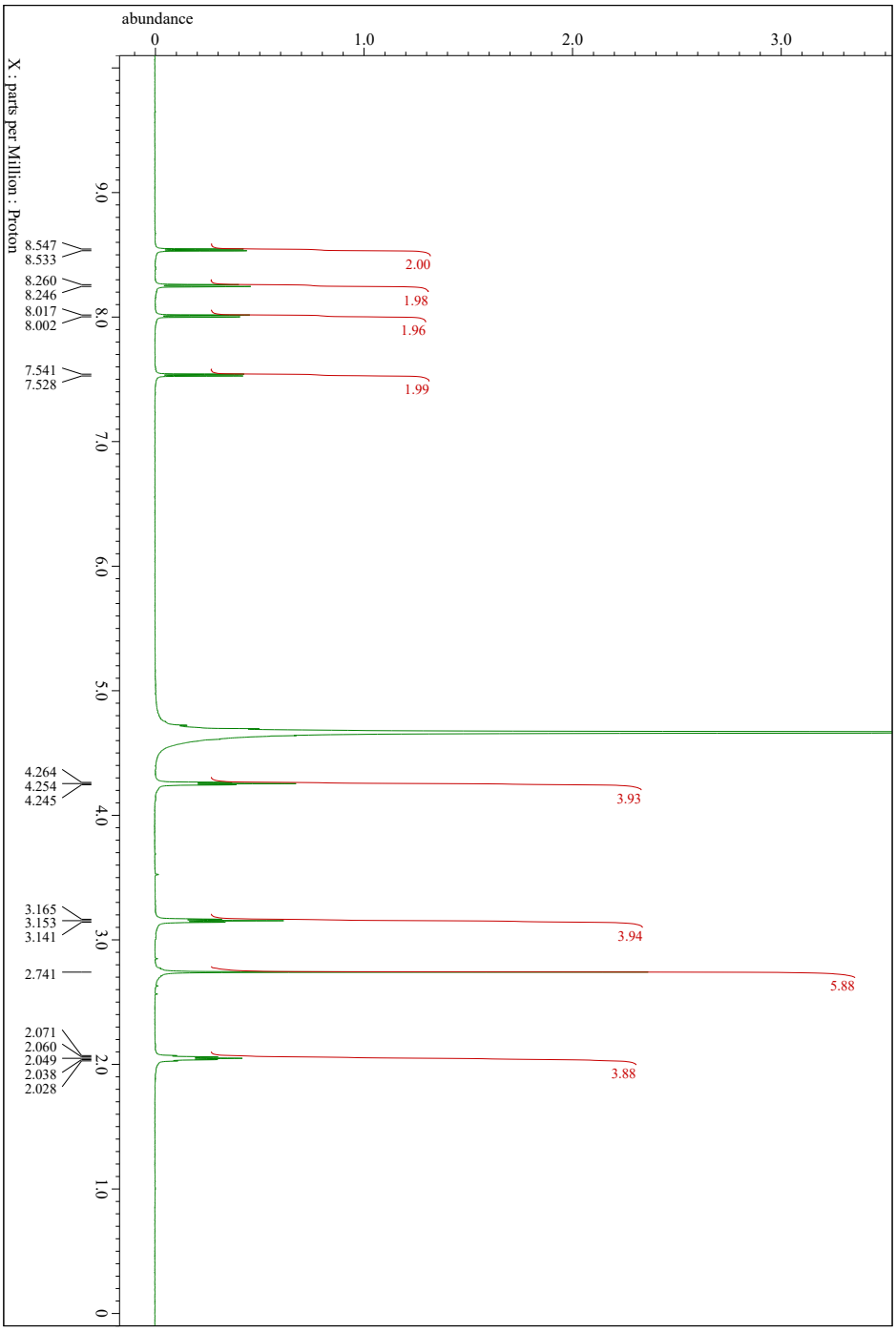
^{1}H-^{15}N-HSQC spectrum of ^{15}**N-NCD-Boc** in $CDCl_3$



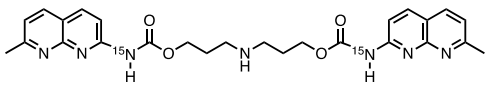


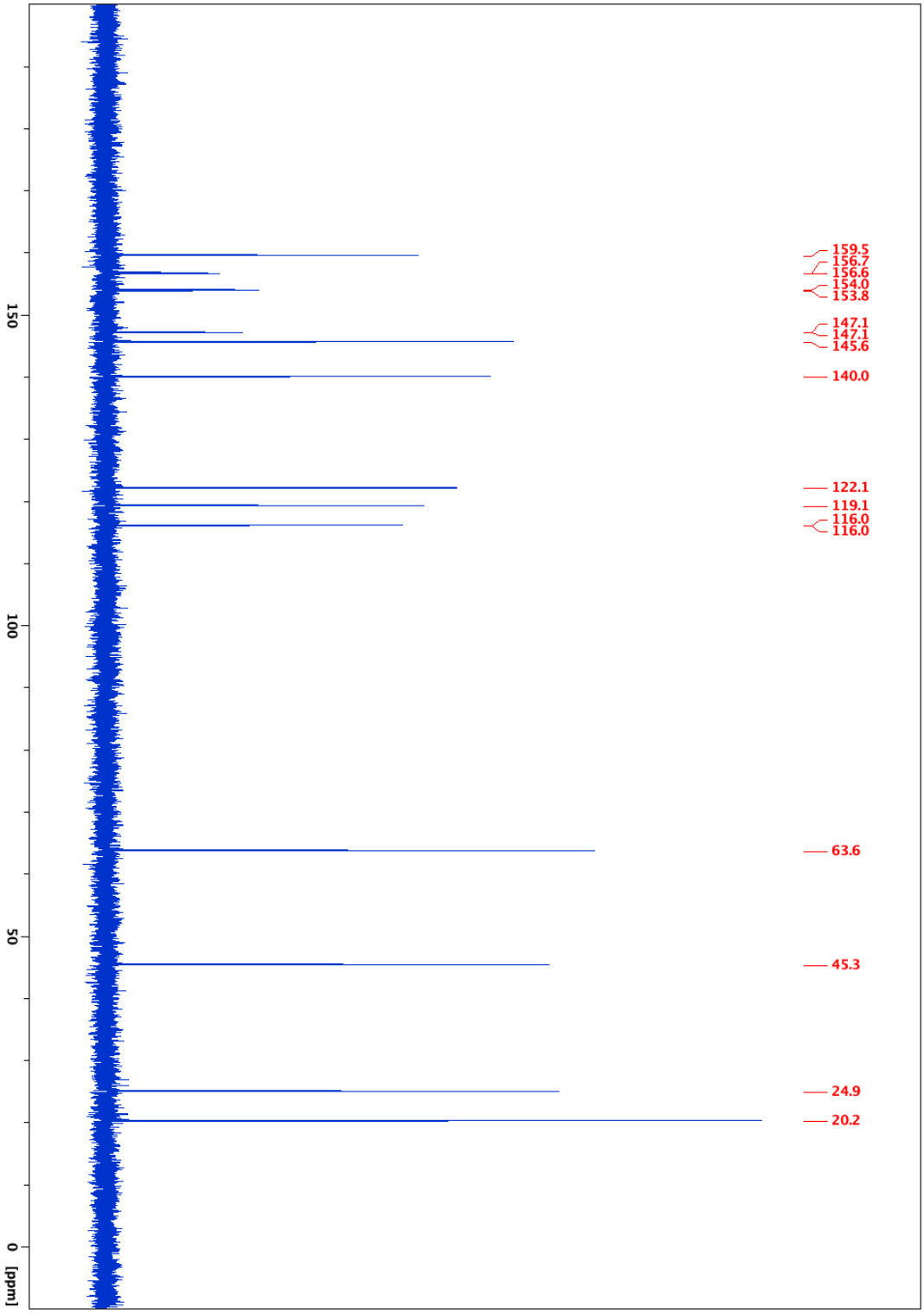
^{1}H-NMR spectrum of ^{15}**N-NCD** in D_2O



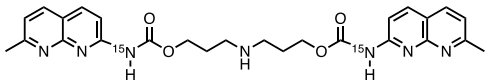


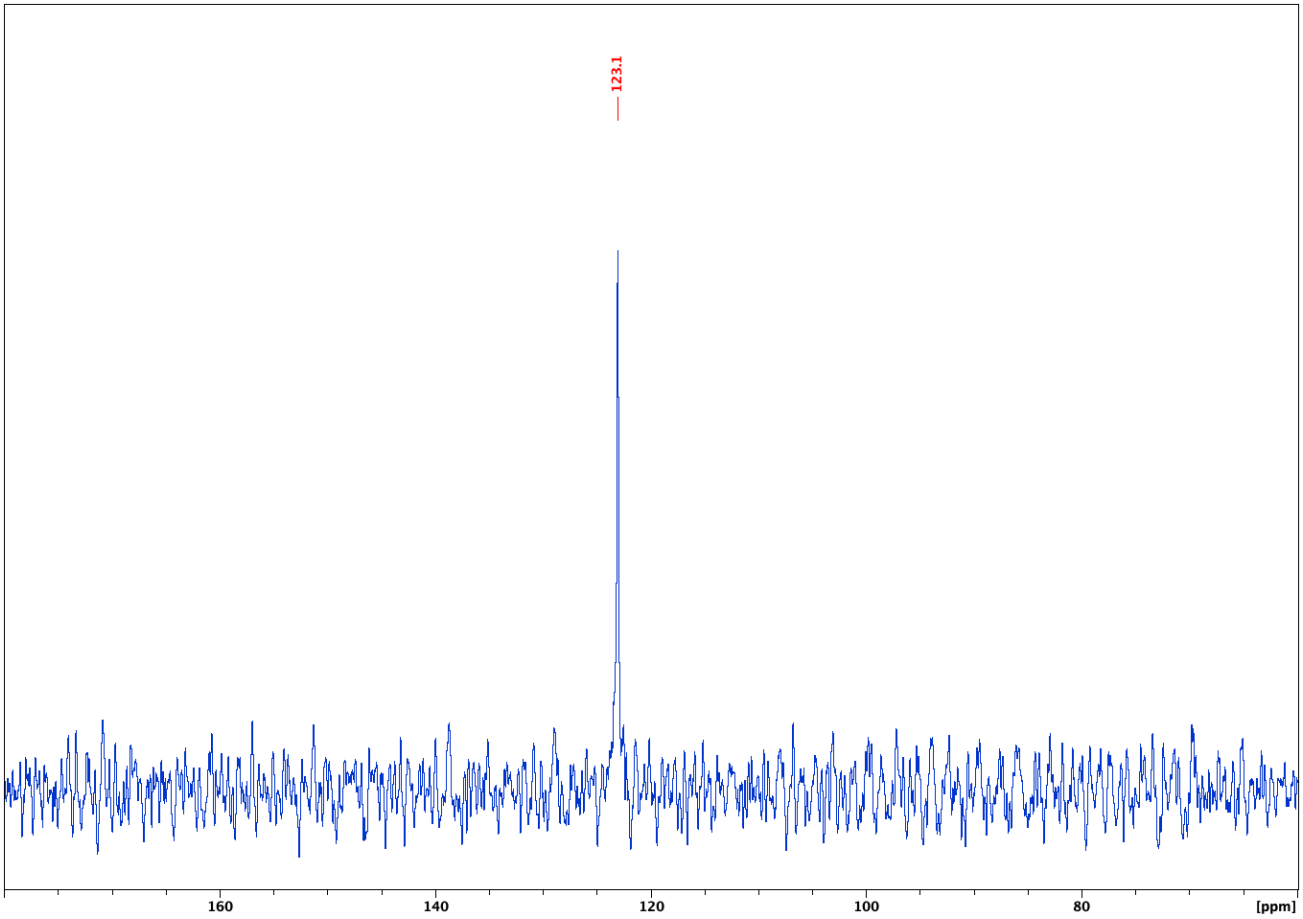
^{13}C-NMR spectrum of ^{15}**N-NCD** in D_2O



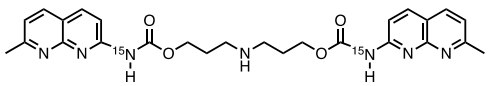


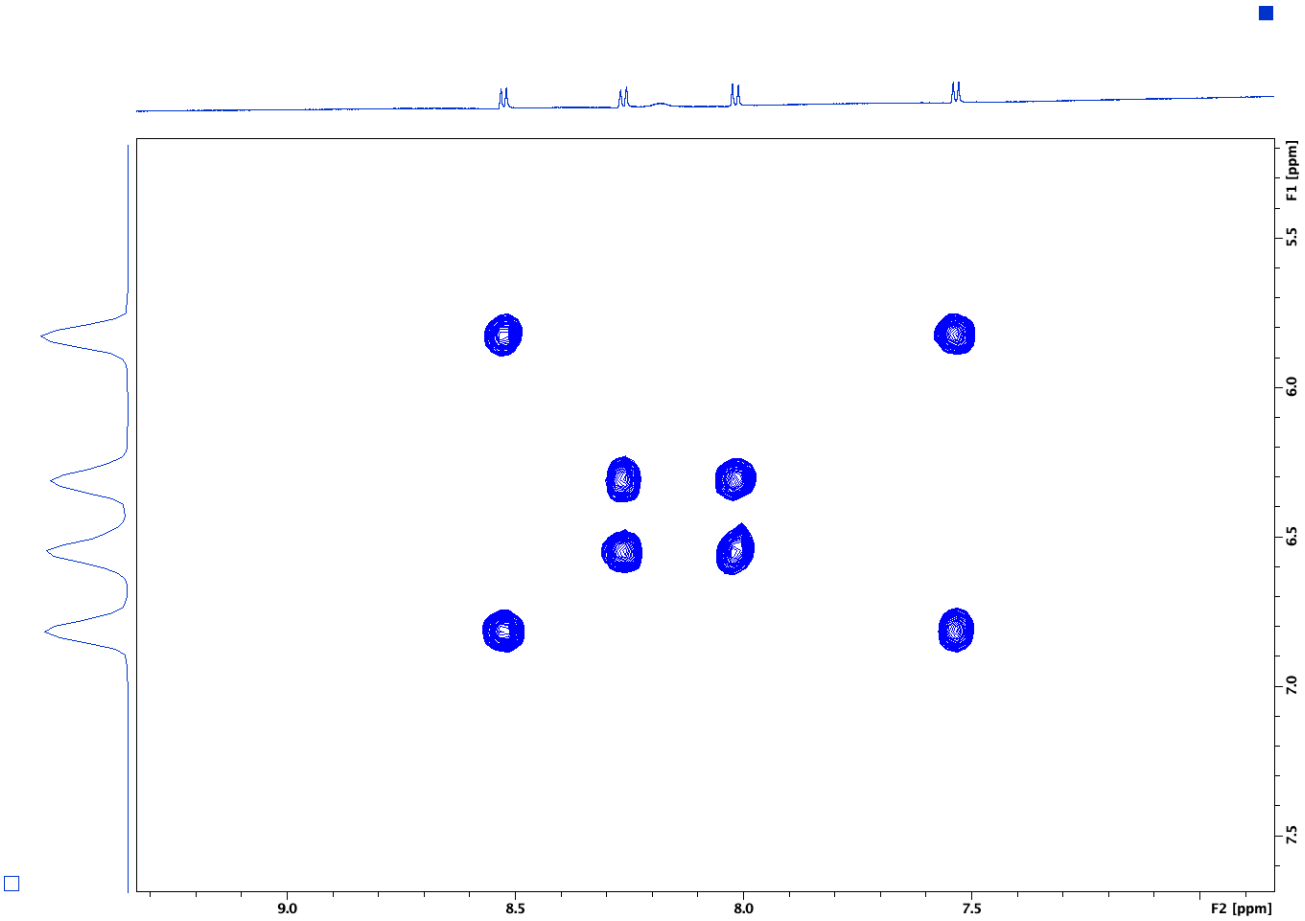
^{15}N-NMR spectrum of ^{15}**N-NCD** in D_2O





COSY spectrum of ^{15}**N-NCD** in D_2O (The aromatic region)





1 B. Brodbeck, B. Püllmann, S. E. Schmitt and M. Nettekoven, *Tetrahedron Lett.*, 2003, **44**, 1675–1678, 'Parallel iterative solution-phase synthesis of 5-amino-1-aryl-[1,2,4]triazolo[1,5-a]pyridine-7-carboxylic acid amide derivatives'.