New (green) methodology for efficient hydrazine cleavage

Lenka Kubovičová[a], Kristýna Bürglová[b] and Jan Hlaváč [* a,b]

[a]Department of Organic Chemistry, Faculty of Science, Palacký University, 771 46 Olomouc, Czech Republic

[b]Institute of Molecular and Translation Medicine, Faculty of Medicine and Dentistry, Hněvotínská 5, 779 00 Olomouc, Czech Republic

1. General considerations

LC/MS analyses were performed using UHPLC/MS with an UHPLC chromatograph Acquity with PDA detector and a single quadrupole mass spectrometer (Waters) with an X-Select C18 column at 30 °C and a flow rate of 600 µl/min. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with linearly programmed gradient over the course of 2.5 min and then maintains this concentration for 1.5 min. Two methods with various solvent gradients were used for the measurements (change of % A): method 1 (from 80 to 20), method 2 (from 100 to 50). The column was re-equilibrated at 10% B for 1 min. The APCI ionization operated at a discharge current of 5 µA, vaporizer temperature of 350 °C and capillary temperature of 200 °C.

Purity of compounds was determed as ratio of appropriate peak area to sum of areas of all peaks of the mixture. Areas were determined by integration of the peaks from PDA detector response.

Purification was performed using semipreparative HPLC with a Waters 1500 series HPLC equipped with an Autosampler 2707, a Binary HPLC pump 1525, a Waters Photodiode Array Detector 2998 and a Waters Fraction Collector III with a YMC C18 reverse phase column, 20 x 100 mm, with 5 µm particles. The mobile phase consisted of acetonitrile and a 10 mM aqueous ammonium acetate gradient over 6 min.

NMR spectra were measured in DMSO-d_6, water-d_2 DMF-d_7 using JEOL ECX-500 (500 MHz) spectrometer. Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (*J*) are reported in Hertz (Hz). Acetate salts exhibited singlet at 1.7 – 1.9 ppm in the ^{1}H NMR spectrum and two resonances at 173 and 23 ppm in ^{13}C spectrum.

Solvents and chemicals were purchased from Sigma-Aldrich (Milwaukee, IL, www.sigmaaldrich.com) or Aapptec (USA, http://www.aapptec.com).

Following abbreviations were used: AAL (amino acid linker), DCM (dichloromethane), DIC (*N,N'*-diisopropylcarbodiimide), DIEA (*N,N*-diisopropylethylamine), DMF (*N,N*-dimethylformamide), HOBt (1-hydroxybenzotriazole hydrate), RT (room temperature), TFA (trifluoroacetic acid), THF (tetrahydrofuran), TMSOK (potassium trimethylsilanolate).

Deprotected resin means that resin was treated in mixture of 50% of piperidine in DMF for 20 min at RT to removed Fmoc group.

For the analysis of the product immobilized on the resin following procedure was used: analytical sample of resin (~ 5 mg) was treated with cleavage cocktail 50% TFA in DCM for 15 min at RT. The cleavage cocktail was evaporated by a stream of nitrogen and resin was extracted into 1 mL of 50% MeOH/water and analyzed by LC/MS.

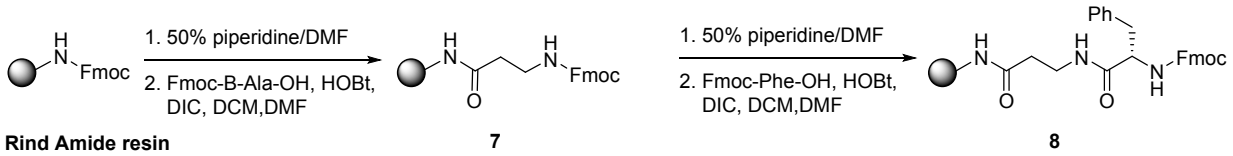
Analysis of preparative sample for full characterization of the compound was done by the same procedure with use of 10 ml of TFA/DCM per 1g of the resin and extraction of the residue to 10 ml of 50% MeOH/water. All compounds after semipreparative HPLC were obtained with purity ≥98% according to the PDA detector.

NMR quantification was determined directly using ^{1}H NMR by comparison with the residual solvent signal[1].

2. Reactions on solid support

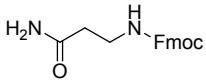
2.1. Synthesis of β-Ala-Phe spacer – resins 7 and 8

Resin 7 and **resin 8** were prepared according to the following scheme:



Resin 7: To the deprotected Rink Amide resin (1 g; 100-200 mash; loading 0.6 mmol/g) solution of an Fmoc-β-Ala (1.24 g; 4 mmol), DIC (0.62 mL; 4 mmol) and HOBt (0.61 g; 4 mmol) in mixture of DMF and DCM (50% vw; 12 mL) was added. Slurry was stirred 1 h at RT. Resin was washed with 3×12 mL DMF and 3×12 mL DCM and used in following steps.

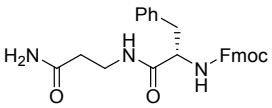
Analysis of the cleaved compound from the resin **7.**



Crude product: LC/MS analysis after cleavage form the resin: MS (ESI) exact mass calcd. for $C_{18}H_{19}N_2O_3$ $[M+H]^+$: 311.13; found: 311.27, t_R = 2.42 min (method 1), purity: 99%.

Resin 8: Attachment of the second amino acid followed the same procedure with use of resin **7** and Fmoc-Phe as the aminoacid. The Fmoc groups were deprotected and the resin was used in following steps.

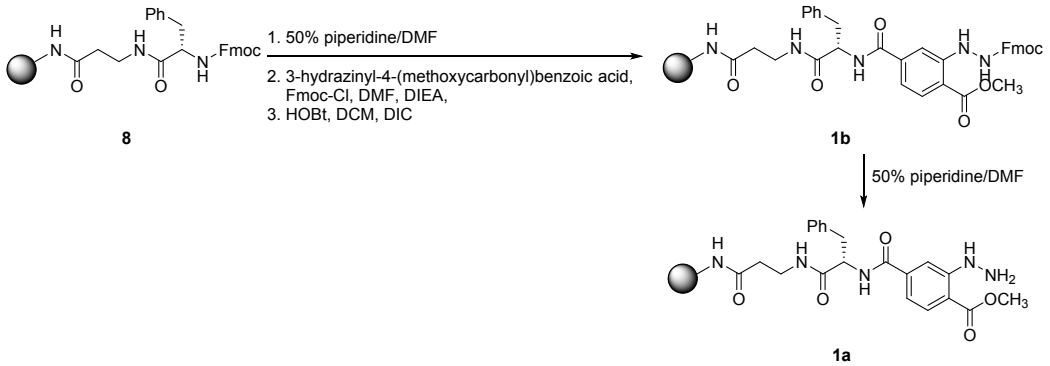
Analysis of the cleaved compound from the resin **8.**



Crude product: LC/MS analysis after cleavage form the resin: MS (ESI) exact mass calcd. for $C_{27}H_{28}N_3O_4$ $[M+H]^+$: 458.20; found: 458.40, t_R = 2.81 min (method 1), purity: 99%.

2.2. Preparation of monosubstituted and Fmocylated hydrazines 1a, 1b, 1e, 1f, 1i, 1j.

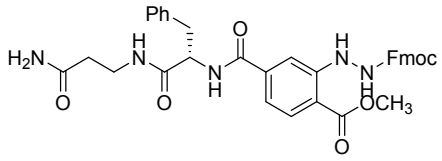
Resin **1a** and resin **1b** were prepared according to the following scheme:



Resin 1b: 3-hydrazinyl-4-(methoxycarbonyl)benzoic acid **5d**[1] (0.55 g; 2.6 mmol), DIEA (1 mL; 2.6 mmol) and Fmoc chloride (0.59 g; 2.3 mmol) were dissolved in DMF (5 mL). After 20 min, solution of HOBt (0.49 g; 3.6 mmol) and DIC (0.52 mL; 4 mmol) in DCM (5 mL) was added. Meanwhile, immobilized peptide **8** (1 g) was deprotected. The resin was washed with 3×12 mL DMF and 3×12 mL

DCM followed by the addition of the prepared solution of the hydrazine. The mixture was stirred for 1 h at RT. Finally, the resin was washed with 3×12 mL DCM and 3×12 mL DMF.

Analysis of the cleaved compound from the resin **1b.**

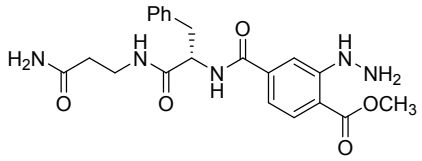


Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{36}H_{36}N_5O_7$ $[M+H]^+$: 650.25; found: 650.27, t_R = 2.88 min, (method 1), purity: 88%.

Pure product: Yield: 41 mg, 48%. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.78 (bs, 1H), 8.62 (d, J = 8.1 Hz, 1H), 8.12 (t, J = 5.6 Hz, 1H), 7.85 (bs, 2H), 7.71 (bs, 2H), 7.43 – 7.26 (m, 8H), 7.19 (t, J = 6.5 Hz, 3H), 7.09 (t, J = 7.1 Hz, 1H), 6.79 (s, 1H), 4.64 – 4.55 (m, 1H), 4.45 – 4.11 (m, 3H), 3.82 (s, 3H), 3.36 – 3.16 (m, 4H), 3.07 – 2.88 (m, 2H), 2.19 (td, J = 7.2, 1.9 Hz, 2H).

Resin 1a: The resin **1b** was deprotected and washed with 3×12 mL DMF and 3×12 mL DCM.

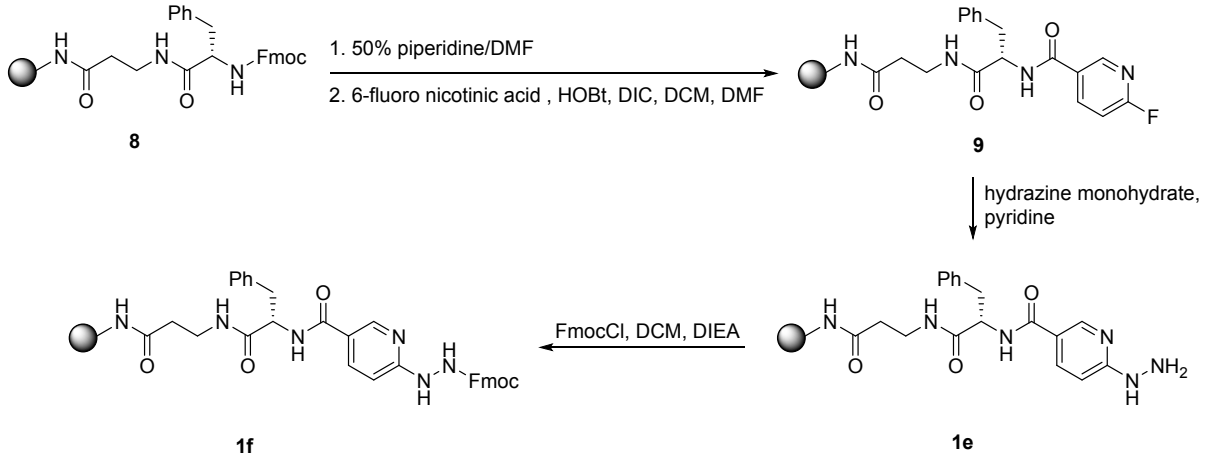
Analysis of the cleaved compound from the resin **1a.**



Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{21}H_{26}N_5O_5$ $[M+H]^+$: 428.19; found: 428.40, t_R = 1.71 min (method 1), purity: 57%.

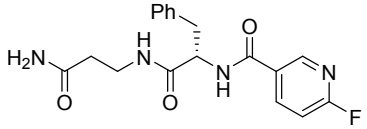
The derivative **1a** was very unstable during purification, therefore its structure was confirmed only by MS analysis and used directly for next reaction.

Resin **1e** and resin **1f** were prepared according to the following scheme:



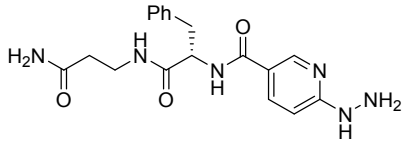
Resin 9: The resin **8** (1 g) was deprotected and washed with 3×12 mL DMF and 3×12 mL DCM. Then, solution of 6-fluoro nicotinic acid (0.43 g; 3 mmol), HOBt (0.46 g; 3.4 mmol) and DIC (0.47 mL; 3.7 mmol) in DMF and DCM (50% vv; 12 mL) was added. The mixture was stirred for 1 h at room temperature. Finally, resin was washed with 3×12 mL DMF, 3×12 mL DCM and used for next step.

Analysis of the cleaved compound from the resin **9.**



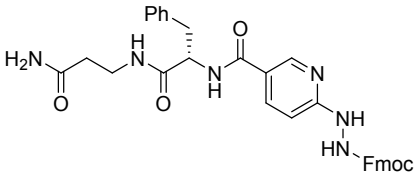
Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{18}H_{20}FN_4O_3$ $[M+H]^+$: 359.14; found: 359.45, t_R = 1.41 min (method 1), purity: 99%.

Resin 1e: The resin **9** (1 g) was stirred in solution of hydrazine monohydrate (0.29 mL; 1 mmol) in pyridine (12 mL) for 48 h at RT. Finally, the resin was washed with 3×12 mL DMF and 3×12 mL DCM.
Analysis of the cleaved compound from the resin **1e.**



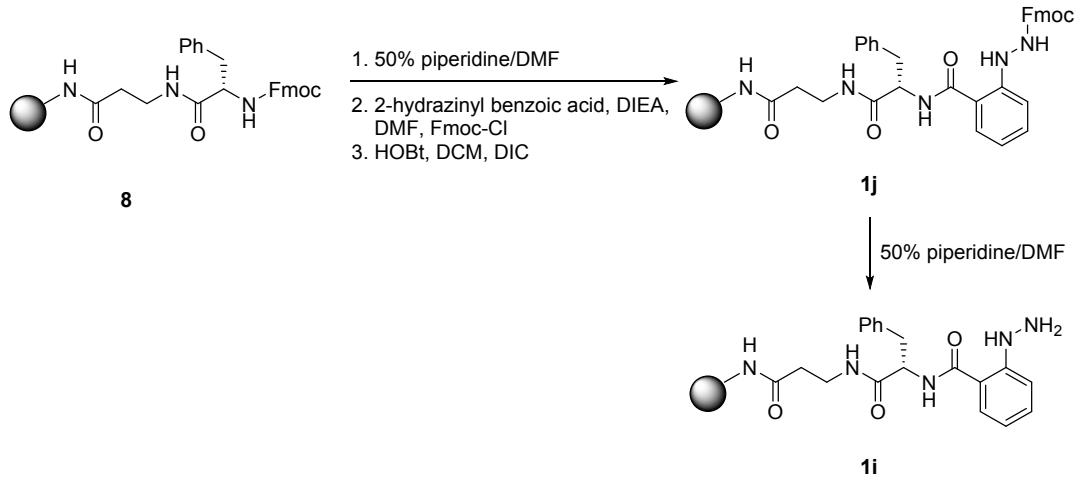
Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{18}H_{23}N_6O_3$ $[M+H]^+$: 371.18; found: 371.40, t_R = 0.98 min (method 1), purity: 61%.
The derivative **1e** was very unstable during purification, therefore its structure was confirmed only by MS analysis and used directly for next reaction.

Resin 1f : The resin **1e** (1 g) was stirred in solution of Fmoc chloride (1.48 g; 5.7 mmol) and DIEA (1.04 mL; 6 mmol) in DCM (12 mL) for 30 min at RT. Then, the resin was washed with 5×12 mL DCM.
Analysis of the cleaved compound from the resin **1f.**



Crude product: LC/MS analysis MS (ESI) exact mass calcd. for $C_{33}H_{33}N_6O_5$ $[M+H]^+$: 593.24; found: 593.22, t_R = 2.55 min, (method 1), purity: 98%.
Pure product: Yield: 59 mg, 44%. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.38 (s, 1H), 8.50 (s, 1H), 8.41 (d, J = 8.2 Hz, 1H), 8.10 (d, J = 5.0 Hz, 1H), 7.91 (t, J = 9.4 Hz, 3H), 7.75 (d, J = 7.1 Hz, 2H), 7.45 (t, J = 7.2 Hz, 2H), 7.34 (dd, J = 22.2, 5.4 Hz, 6H), 7.24 (t, J = 7.6 Hz, 3H), 7.15 (d, J = 7.4 Hz, 1H), 6.84 (s, 1H), 4.67 – 4.58 (m, 1H), 4.45 – 4.26 (m, 3H), 3.30 – 3.24 (m, 2H), 3.08 (dd, J = 13.6, 4.1 Hz, 1H), 2.97 – 2.91 (m, 1H), 2.23 (td, J = 9, 6.9 Hz, 2H). ^{13}C NMR (101 MHz) δ 173, 172, 165, 157, 144, 141, 139, 130, 129, 128 (2C), 127 (3C), 126, 121 (2C), 66, 55, 47, 38, 36, 35.

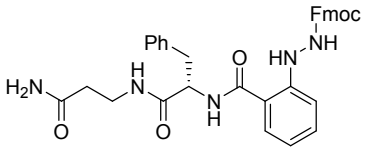
Resin 1i and **resin 1j** were prepared according to the following scheme:



8 **1j** **1i**

Resin 1j: 2-hydrazinyl benzoic acid **5a**[1] (0.4 g; 2.6 mmol), DIEA (1 mL) and Fmoc Chloride (0.59 g; 2.3 mmol) were dissolved in DMF (5 mL). After 20 min, solution of HOBt (0.49 g; 3.6 mmol) and DIC (0.52 mL; 4 mmol) in DCM (5 mL) was added. Meanwhile, immobilized peptide **8** (1 g) was deprotected. The resin was washed with 3×12 mL DMF and 3×12 mL DCM followed by the addition of the prepared solution of the 2-hydrazinylbenzoic acid. The mixture was stirred for 1 h at RT. Finally, the resin was washed with 3×12 mL DCM and 3×12 mL DMF.

Analysis of the cleaved compound from the resin **1j**

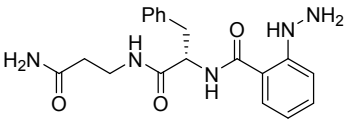


Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{34}H_{34}N_5O_5$ $[M+H]^+$: 592.25; found: 592.22, t_R = 3.03 min (method 1), purity: 99%.

Pure product: Yield: 52 mg, 39%. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.31 (s, 1H), 8.82 (s, 1H), 8.47 (d, *J* = 7.1 Hz, 1H), 8.13 (s, 1H), 7.90 (d, *J* = 6.2 Hz, 2H), 7.72 (d, *J* = 6.1 Hz, 2H), 7.60 (d, *J* = 6.3 Hz, 1H), 7.43 (d, *J* = 7.0 Hz, 2H), 7.33 (d, *J* = 7.0 Hz, 4H), 7.26 (d, *J* = 7.0 Hz, 2H), 7.16 (d, *J* = 5.9 Hz, 1H), 6.83 (s, 1H), 6.72 (dd, *J* = 38.3, 6.7 Hz, 2H), 4.62 (d, *J* = 1.5 Hz, 1H), 4.48 – 4.18 (m, 3H), 3.30 – 3.24 (m, 2H), 3.06 (dd, *J* = 13.7, 4.3 Hz, 1H), 3.01 – 2.93 (m, 1H), 2.24 (t, *J* = 7.5 Hz, 2H).

Resin 1i: Prepared by defmocation of resin **1j**.

Analysis of the cleaved compound from the resin **1i.**



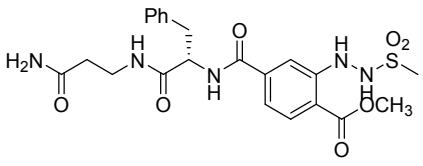
LC/MS analysis: MS (ESI) exact mass calcd. for $C_{19}H_{24}N_5O_3$ $[M+H]^+$: 370.18; found: 370.40, t_R = 1.35 min (method 1), purity: 70%.

The derivative **1i** was very unstable during purification, therefore its structure was confirmed only by MS analysis and used directly for next reaction.

2.3. General Procedure for Preparation of Mesyl Derivatives 1c, 1g and 1k.

Immobilized hydrazine derivatives **1b, 1f, 1j** (1 g) were deprotected, washed with 3×12 mL DMF and 3×12 mL DCM, and then 12 mL of solution of mesyl chloride (0.52 mL; 6 mmol) and pyridine (0.49 mL; 6 mmol) in DCM (12 mL) was added. Slurry was stirred for 3 h at RT. Resin was then washed with 3×12 mL DMF, 3×12 mL DCM.

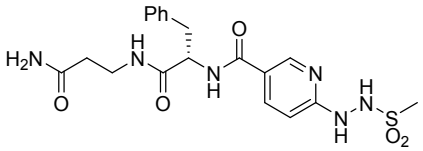
Analysis of the cleaved compound from the resin **1c.**



Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{22}H_{28}N_5O_7S$ $[M+H]^+$: 506.16; found: 506.31, t_R = 1.83 min (method 1), purity: 80%.

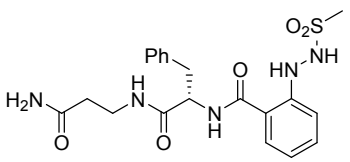
Pure product: Yield: 38 mg, 37%. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.15 (s, 1H), 9.03 (s, 1H), 8.64 (d, *J* = 8.5 Hz, 1H), 8.15 (t, *J* = 5.7 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.69 (d, *J* = 1.6 Hz, 1H), 7.34 – 7.30 (m, 3H), 7.24 (dd, *J* = 14.1, 6.3 Hz, 3H), 7.15 (t, *J* = 7.3 Hz, 1H), 6.83 (s, 1H), 4.67 – 4.60 (m, 1H), 3.86 (s, 3H), 3.29 – 3.24 (m, 2H), 3.06 (dd, *J* = 13.7, 4.2 Hz, 1H), 3.01 (s, 3H), 2.97 (dd, *J* = 13.6, 10.5 Hz, 1H), 2.23 (t, *J* = 7.4 Hz, 2H).

Analysis of the cleaved compound from the resin **1g.**



Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{19}H_{25}N_6O_5S$ $[M+H]^+$: 449.15; found: 449.28, t_R = 1.27 min (method 1), purity: 11%. The compound was not purified and was not used for any other reaction.

Analysis of the cleaved compound from the resin **1k.**



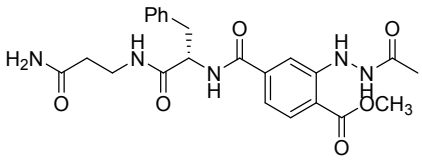
Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{20}H_{26}N_5O_5S$ $[M+H]^+$: 448.16; found: 448.19, t_R = 2.47 min (method 1), purity: 90%.

Pure product: Yield: 35 mg, 39%. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.13 (s, 1H), 8.55 (d, J = 8.3 Hz, 1H), 8.15 (t, J = 5.6 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.33 (dd, J = 15.0, 8.0 Hz, 3H), 7.24 (dd, J = 15.9, 8.1 Hz, 3H), 7.16 (t, J = 7.4 Hz, 1H), 6.83 (s, 1H), 6.78 (t, J = 7.3 Hz, 1H), 4.73 – 4.53 (m, 1H), 3.30 – 3.20 (m, 2H), 3.08 (dd, J = 13.7, 4.2 Hz, 1H), 3.02 – 2.94 (m, 1H), 2.94 (d, J = 9.0 Hz, 3H), 2.24 (t, J = 7.2 Hz, 2H). ^{13}C NMR (126 MHz) δ 173, 171, 168, 149, 138, 132, 129, 128, 126, 118, 116, 114, 55, 37 (2C), 35 (2C).

2.4. General Procedure for Preparation of Acetyl derivatives 1d, 1h and 1l.

After the deprotection of Fmoc group from the resine **1b, 1f, 1j** (1 g) the slurry was stirred in solution of acetanhydride (0.57 mL; 6 mmol) and DIEA (1.04 mL; 6 mmol) in DMF (12 mL) for 0.5 h at RT. Then, resin was washed with 3×12 mL DMF, 3×12 mL DCM.

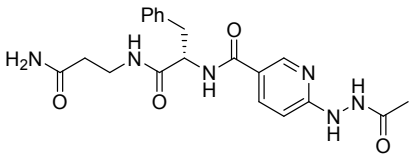
Analysis of the cleaved compound from the resin **1d.**



Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{23}H_{28}N_5O_6$ $[M+H]^+$: 470.20; found: 470.15, t_R = 1.59 min (method 1), purity: 68%.

Pure product: Yield: 42 mg, 42%. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.98 (d, J = 2.1 Hz, 1H), 8.87 (d, J = 1.9 Hz, 1H), 8.66 (d, J = 8.4 Hz, 1H), 8.15 (t, J = 5.6 Hz, 1H), 7.89 – 7.85 (m, 1H), 7.32 (d, J = 7.4 Hz, 3H), 7.28 – 7.23 (m, 3H), 7.19 – 7.13 (m, 2H), 6.84 (s, 1H), 4.63 – 4.56 (m, 1H), 3.85 (s, 3H), 3.31 – 3.23 (m, 2H), 3.06 (dd, J = 13.6, 4.2 Hz, 1H), 2.96 (dd, J = 13.5, 10.7 Hz, 1H), 2.23 (t, J = 7.2 Hz, 2H), 1.96 (s, 3H). ^{13}C NMR (101 MHz) δ 173, 171, 169, 167, 166, 151, 140, 139, 131, 129, 128, 126, 116, 112 (2C), 55, 52, 37, 35 (2C), 21.

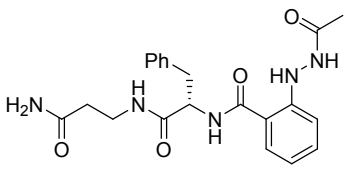
Analysis of the cleaved compound from the resin **1h.**



Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{20}H_{25}N_6O_4$ $[M+H]^+$: 413.13; found: 413.19, t_R = 0.84 min (method 1), purity: 83%.

Pure product: Yield: 39 mg, 44%. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.83 (s, 1H), 8.78 (s, 1H), 8.51 (d, J = 3.0 Hz, 1H), 8.43 (d, J = 8.4 Hz, 1H), 8.13 (t, J = 5.6 Hz, 1H), 7.89 (dd, J = 8.8, 2.4 Hz, 1H), 7.38 (s, 1H), 7.33 – 7.30 (m, 2H), 7.25 (d, J = 7.3 Hz, 2H), 7.18 – 7.13 (m, 1H), 6.90 (s, 1H), 6.54 (d, J = 8.4 Hz, 1H), 4.67 – 4.59 (m, 1H), 3.29 (dt, J = 11.2, 7.1 Hz, 2H), 3.09 (dd, J = 13.6, 4.1 Hz, 1H), 2.94 (dd, J = 13.5, 10.8 Hz, 1H), 2.25 (t, J = 7.4 Hz, 2H), 1.92 (s, 3H). ^{13}C NMR (101 MHz) δ 173, 172, 170, 166, 162, 149, 139, 137, 130, 129, 127, 121, 106, 55, 38, 36, 35, 21.

Analysis of the cleaved compound from the resin **1l.**



Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{21}H_{26}N_5O_4$ $[M+H]^+$: 412.13; found: 412.37, t_R = 1.53 min, (method 1), purity: 88%.

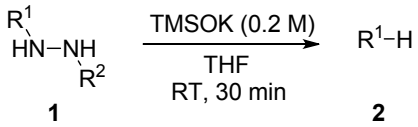
Pure product: Yield: 53 mg, 34%. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.79 (d, J = 2.7 Hz, 1H), 8.91 (d, J = 2.5 Hz, 1H), 8.52 (d, J = 8.3 Hz, 1H), 8.17 (t, J = 5.7 Hz, 1H), 7.57 (dd, J = 7.9, 1.2 Hz, 1H), 7.34 (d, J = 7.1 Hz, 3H), 7.27 (dd, J = 13.8, 6.4 Hz, 3H), 7.16 (t, J = 7.3 Hz, 1H), 6.78 (dt, J = 15.0, 12.4 Hz, 3H), 4.67 – 4.58 (m, 1H), 3.32 – 3.25 (m, 2H), 3.07 (dd, J = 13.7, 4.3 Hz, 1H), 2.96 (dd, J = 13.7, 10.7 Hz, 1H), 2.24 (t, J = 7.1 Hz, 2H), 1.89

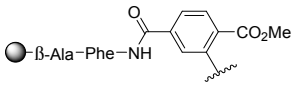
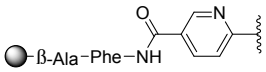
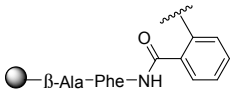
(s, 3H). ^{13}C NMR (101 MHz) δ 173, 172, 169, 168, 150, 139, 132, 129, 128, 126, 117, 116, 113, 55, 37, 35 (2C), 21.

2.5. General procedure for hydrazine cleavage on solid phase

Solution of 0.2 M TMSOK (0.3 g, 2 mmol) in THF (10 mL) was added to the immobilized hydrazine **1a-l** (1 g) which had been swelled in DCM. Slurry was stirred for 0.5 h at RT. Resin was then washed with 3×12 mL DMF, 3×12 mL DCM. Yields are summarized in the following table:

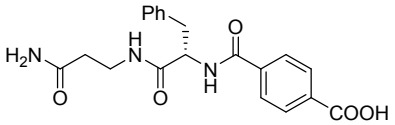
Table 1: Synthesis of derivative **2** via dehydrazination of compounds **1**.



1	R^1	R^2	**2**	Yield of **2** (%)*
1a		-H	**2a**	20**
1b		-Fmoc	**2a**	***
1c		-Ms	**2a**	16**
1d		-COMe	**2a**	19**
1e		-H	**2e**	53
1f		-Fmoc	**2e**	***
1g		-Ms	**2e**	****
1h		-COMe	**2e**	54
1i		-H	**2i**	58
1j		-Fmoc	**2i**	***
1k		-Ms	**2i**	17
1l		-COMe	**2i**	57

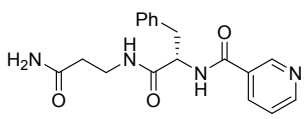
*Yield determined by ^{1}H NMR spectroscopy after HPLC purification.
**Product was isolated as carboxylic acid.
***Product was not observed.
****Reaction was not studied because the preparation of product **1g** proceeded with very low purity.

Analysis of the cleaved compound from the resin **2a.**



Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{20}H_{22}N_3O_5$ $[M+H]^+$: 384.15; found: 384.34, t_R = 2.47 min (method 2). Pure product: ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.65 (d, J = 8.4 Hz, 1H), 8.18 (t, J = 5.6 Hz, 1H), 7.91 (d, J = 8.1 Hz, 2H), 7.79 (d, J = 8.3 Hz, 2H), 7.37 – 7.31 (m, 3H), 7.24 (t, J = 7.6 Hz, 2H), 7.15 (tt, J = 10, 2 Hz, 1H), 6.83 (s, 1H), 4.68 – 4.63 (m, 1H), 3.30 – 3.24 (m, 2H), 3.08 (dd, J = 13.7, 4.3 Hz, 1H), 2.98 (dd, J = 13.6, 10.6 Hz, 1H), 2.54 (s, 1H), 2.24 (J = 7.4 Hz, 2H).^{13}C NMR (126 MHz) δ 173, 172, 171, 166, 139, 136, 129 (2C) , 128, 127, 126, 55, 35 (2C), 21.

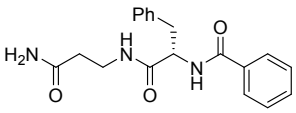
Analysis of the cleaved compound from the resin **2e.**



Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{18}H_{21}N_4O_3$ $[M+H]^+$: 341.15; found: 341.34, t_R = 2.47 min (method 1).

Pure product: ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.92 (d, J = 2.2 Hz, 1H), 8.81 (d, J = 8.5 Hz, 1H), 8.68 (dd, J = 4.8, 1.7 Hz, 1H), 8.16 (t, J = 5.7 Hz, 1H), 8.10 (dt, J = 4, 2 Hz, 1H), 7.49 – 7.45 (m, 1H), 7.33 (d, J = 7 Hz, 3H), 7.25 (t, J = 10.4 Hz, 2H), 7.18 – 7.13 (m, 1H), 6.84 (s, 1H), 4.70 – 4.63 (m, 1H), 3.31 – 3.24 (m, 2H), 3.11 (dd, J = 13.7, 4.2 Hz, 1H), 2.94 (dd, J = 13.7, 10.8 Hz, 1H), 2.24 (td, J = 7.3, 1.4 Hz, 2H).^{13}C NMR (126 MHz) δ 173, 171, 165, 152, 149, 138, 135, 130, 129, 128, 126, 123, 55, 37, 35(2C).

Analysis of the cleaved compound from the resin **2i.**



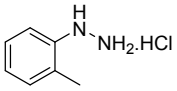
Crude product: LC/MS analysis: MS (ESI) exact mass calcd. for $C_{19}H_{22}N_3O_3$ $[M+H]^+$: 340.13; found: 340.34, t_R = 2.47 min (method 1).
Pure product: ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.53 (d, J = 8.4 Hz, 1H), 8.11 (t, J = 5.7 Hz, 1H), 7.78 (d, J = 7.0 Hz, 2H), 7.50 (t, J = 7.9 Hz, 1H), 7.43 (t, J = 7.5 Hz, 2H), 7.32 (d, J = 7.2 Hz, 3H), 7.24 (t, J = 7.6 Hz, 2H), 7.15 (t, J = 7.3 Hz, 1H), 6.84 (s, 1H), 4.68 – 4.62 (m, 1H), 3.30 – 3.24 (m, 2H), 3.08 (dd, J = 13.7, 4.2 Hz, 1H), 2.97 (dd, J = 13.7, 10.7 Hz, 1H), 2.23 (td, J = 7.3, 1.4 Hz, 2H).^{13}C NMR (126 MHz) δ 172, 171, 166, 138, 134, 131, 129, 128 (2C), 127, 126, 55, 37, 35 (2C).

3. Reactions in solution

3.1. Preparation of hydrazines in solution

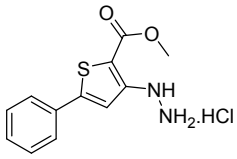
Hydrazines **3a-3c** and **3e – 3i** are commercially available.

2-tolyl hydrazine hydrochloride 3d



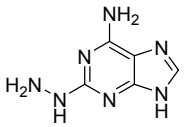
The compound was prepared according to the published procedure[2].
Yield: 1.0 g; 63% of light brown powder. LC/MS analysis: MS (ESI) exact mass calcd. for $C_7H_{11}N_2$ $[M+H]^+$: 123.06; found: 123.36, t_R = 1.53 min (method 1), purity: 99%.

Methyl-3-hydrazinyl-5-phenylthiophene-2-carboxylate hydrochloride 3j



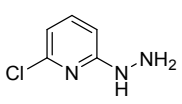
The compound was prepared according to the published procedure[2].
Yield: 0.71 g; 58% of white powder. LC/MS analysis: MS (ESI) exact mass calcd. for $C_{12}H_{13}N_2O_2S$ $[M+H]^+$: 249.06; found: 249.26, t_R = 2.93 min (method 1), purity: 86%.

2-hydrazinyl-9*H*-purin 6-amine 3k



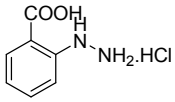
The compound was prepared according to the published procedure[3].
Yield: 1.27 g; 65% of white powder. LC/MS analysis: MS (ESI) exact mass calcd. for $C_5H_8N_7$ $[M+H]^+$: 166.08; found: 166.18, t_R = 0.38 min (method 1), purity: 99%.

2-chloro-6-hydrazinyl pyridine 3l



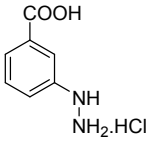
The compound was prepared according to the published procedure[4].
Yield: 1.57 g; 80% of white powder. LC/MS analysis: MS (ESI) exact mass calcd. for $C_5H_7ClN_3$ $[M+H]^+$: 144.03; found: 144.57, t_R = 1.28 min (method 1), purity: 95%.

2-hydrazinyl benzoic acid 5a



The compound was prepared according to the published procedure[2]. Yield: 1.39 g; 90% of white powder. LC/MS analysis: MS (ESI) exact mass calcd. for $C_7H_9N_2O_2$ $[M+H]^+$: 153.06; found: 153.36, t_R = 0.41 min (method 2), purity: 99%.

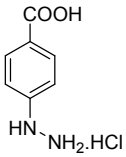
3-hydrazinyl benzoic acid 5b



The compound was prepared according to the published procedure[2].

Yield: 1.7 g; 90% of white powder. LC/MS analysis: MS (ESI) exact mass calcd. for $C_7H_9N_2O_2$ $[M+H]^+$: 153.06; found: 153.16, t_R = 0.57 min (method 2), purity: 95%.

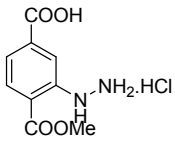
4-hydrazinyl benzoic acid 5c



The compound was prepared according to the published procedure[2].

Yield: 1.8 g; 92% of light yellow powder. LC/MS analysis: MS (ESI) exact mass calcd. for $C_7H_9N_2O_2$ $[M+H]^+$: 153.06; found: 153.16, t_R = 0.57 min (method 2), purity: 99%.

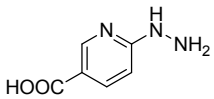
3-hydrazinyl-4-(methoxycarbonyl)benzoic acid 5d



The compound was prepared according to the published procedure[2].

Yield: 2.1 g; 84% of light yellow powder. LC/MS analysis: MS (ESI) exact mass calcd. for $C_9H_{11}N_2O_4$ $[M+H]^+$: 210.06; found: 211.20, t_R = 0.94 min (method 2), purity: 99%.

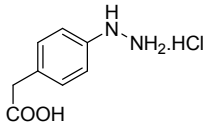
6-hydrazinyl nicotinic acid 5e



The compound was prepared according to the published procedure[5].

Yield: 1.25 g; 82% of light grey powder. LC/MS analysis: MS (ESI) exact mass calcd. for $C_6H_8N_3O_2$ $[M+H]^+$: 154.05; found: 154.16 t_R = 0.51 min (method 2), purity: 91%.

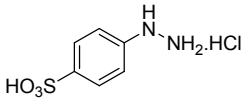
4-hydrazinyl phenylacetic acid 5f



The compound was prepared according to the published procedure[2].

Yield: 1.38 g; 89% of light brown powder. LC/MS analysis: MS (ESI) exact mass calcd. for $C_8H_{11}N_2O_2$ $[M+H]^+$: 167.08; found: 167.30, t_R = 0.36 min (method 2), purity: 98%.

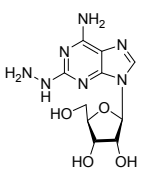
4-hydrazinyl sulphanilic acid 5g



The compound was prepared according to the published procedure[2].

Yield: 1.19 g; 92% of light yellow powder. LC/MS analysis: MS (ESI) exact mass calcd. for $C_6H_9N_2O_3S$ $[M+H]^+$: 189.03; found:189.05, t_R = 0.47 min (method 2) purity: 91%.

2-hydrazinyl adenosine 5h



The compound was prepared according to the published procedure[6].

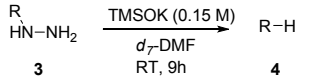
Yield: 1.58 g; 80% of light yellow powder. LC/MS analysis: MS (ESI) exact mass calcd. for $C_{10}H_{16}N_7O_4$ $[M+H]^+$: 298.12; found:298.24, t_R = 2.49 min (method 2) purity: 98%.

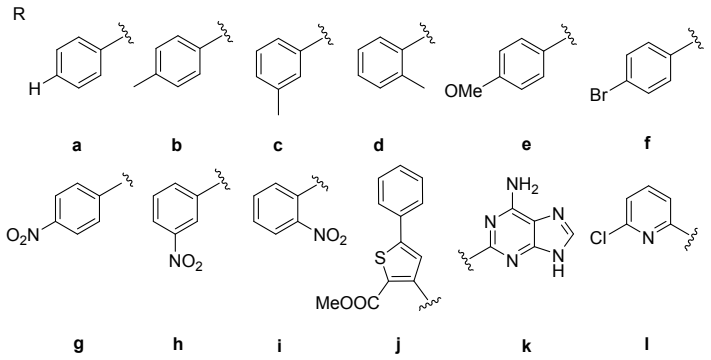
3.2. General procedure for hydrazines cleavage in solution

A/ Cleavage of compounds insoluble in water

Solution of 0.15 M TMSOK (0.02 g, 0.15 mmol) in d_7-DMF (1 mL) was added to the hydrazines **3a-l** (0.02 g). Slurry was stirred for 9 h at RT. Final solution was then analyzed by ^{1}H NMR. The NMR spectra of crude compounds are placed in chapter 9. The yields are summarized in the following table:

Table 2: Hydrazine cleavage in solution with corresponding yields in d_7-DMF.





Compound **3**	Product **4**	Yield of **4** (%)*	Yield of **4** at 70 °C for 48 h (%)*
3a	**4a**	99	-
3b	**4b**	95	-
3c	**4b**	35	70
3d	**4b**	SM	51
3e	**4e**	89	-
3f	**4f**	81	-
3g	**4g**	55	-
3h	**4g**	85	-
3i	**4g**	90	-
3j	**4j**	69	-
3k	**4k**	insoluble	50
3l	**4l**	42	97

*Yield determined by ^{1}H NMR spectroscopy.

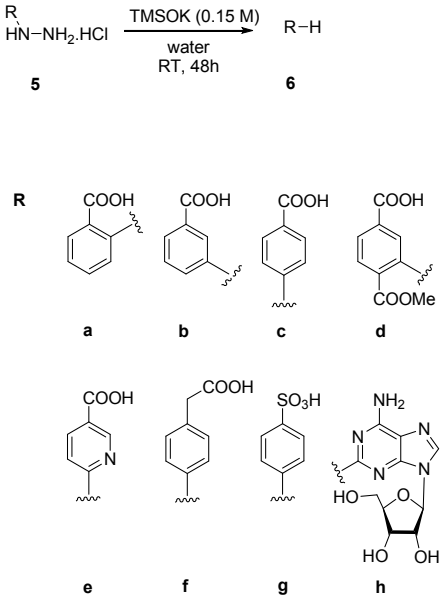
^{1}H NMR data of all final products (**4a**[7], **4b**[8], **4e**[9], **4f**[10], **4g**[11], **4j**[12], **4k**[13],**4l**[14]) are in accordance with published data.

B/ Cleavage of compounds soluble in water

Solution of 0.15 M TMSOK (0.02 g, 0.15 mmol) in water (1 mL) was added to the hydrazine **5a-h** and **3k** (0.02 g). Slurry was stirred for 48 h at RT. Final solution was lyophilized to dryness, residue was dissolved in deuterium oxide and analyzed by ^{1}H NMR. The NMR spectra of crude compounds are placed in chapter 9.

^{1}H NMR data of all final products (**6a**[15]**, 6d**[16]**, 6e**[17], **6f**[18]**, 6g**[19]**,6h**[20]**, 4k**[13]) are in accordance with published data

Table 3: Hydrazine cleavage in solution with corresponding yields in D_2O.



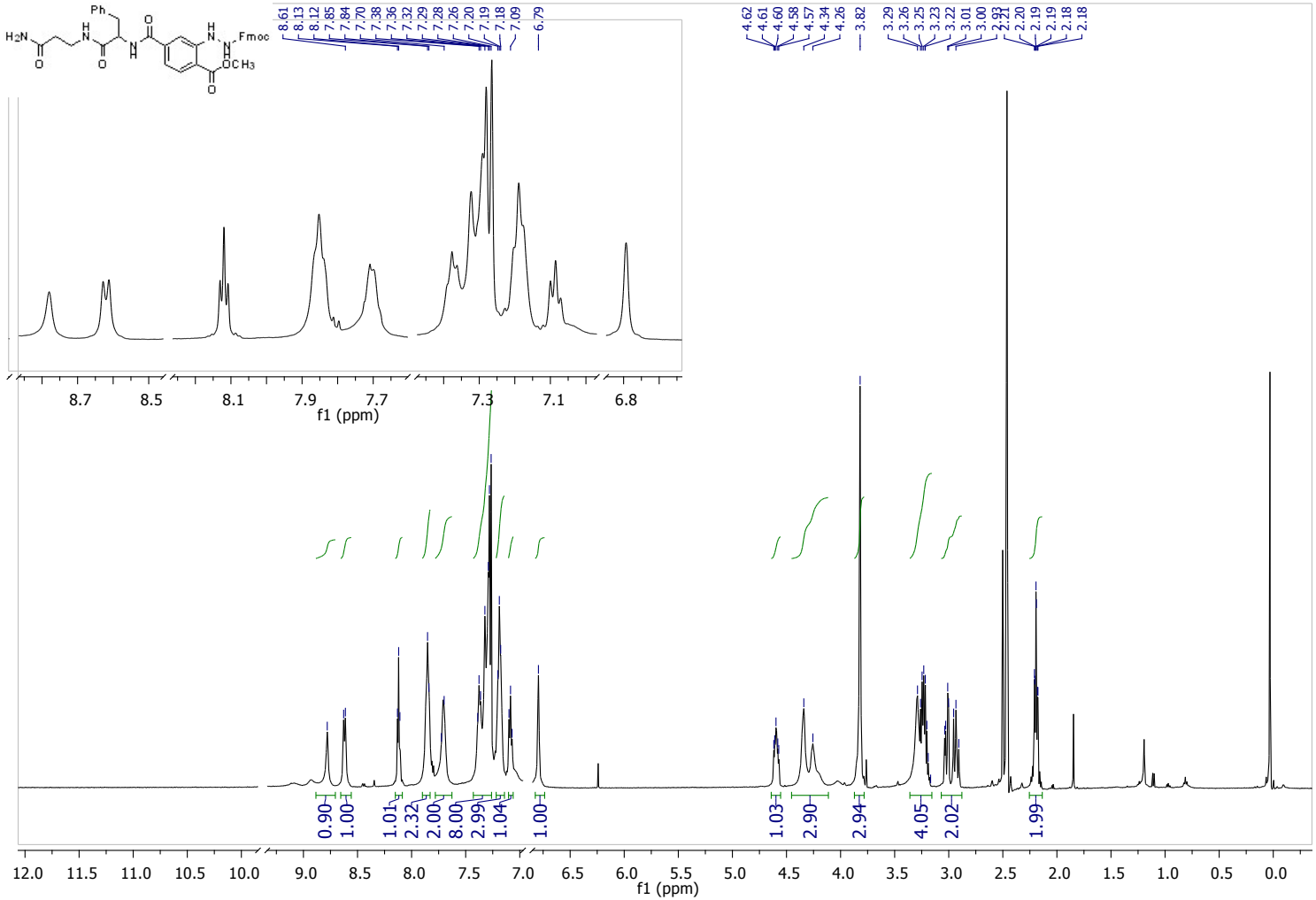
Entry	Compound **5**	Product **6**	Yield of **6a-h** (%)*	Yield of **6(R)** at 70 °C for 48 h (%)*
1	**5a**	**6a**	99	-
2	**5b**	**6a**	64	-
3	**5c**	**6a**	77	-
4	**5d**	**6d**	81	-
5	**5e**	**6e**	71	-
6	**5f**	**6f**	90	-
7	**5g**	**6g**	98	-
8	**5h**	**6h**	40	89
9	**3k**	**4k**	49	45

*Yield determined by ^{1}H NMR spectroscopy.

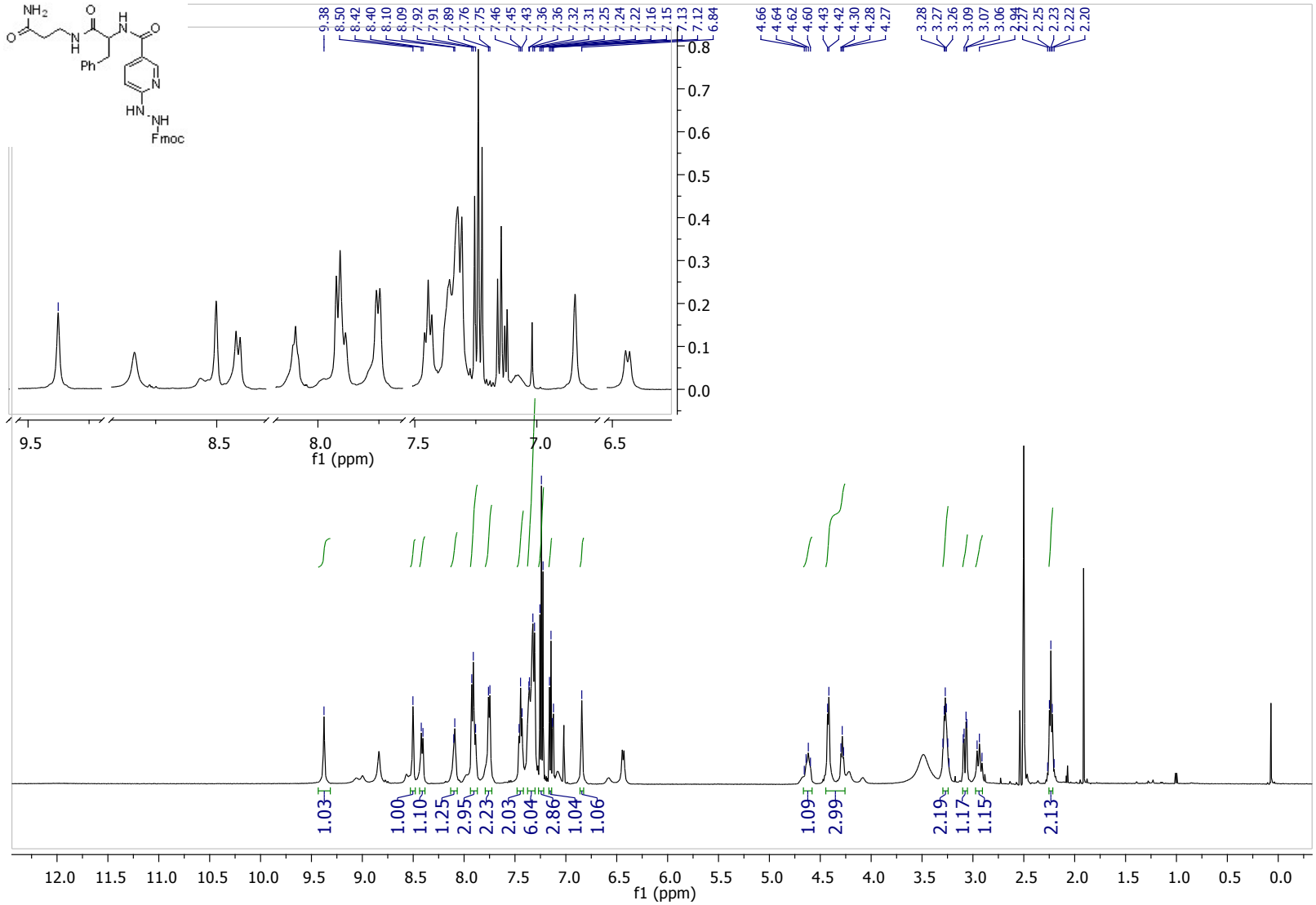
4. NMR spectra

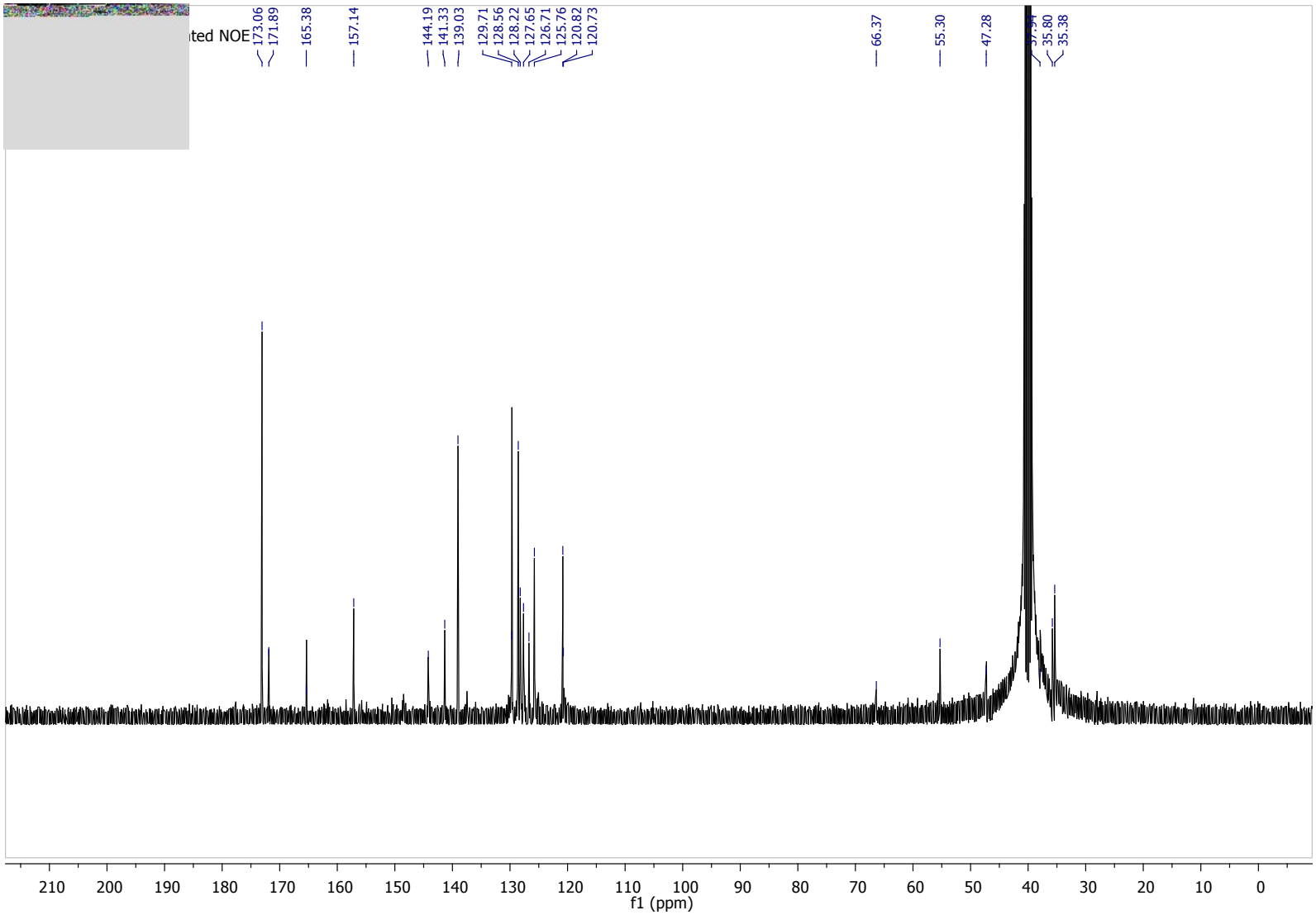
4.1. NMR spectra of products from solid-phase synthesis isolated by HPLC

Product released from the resin 1b

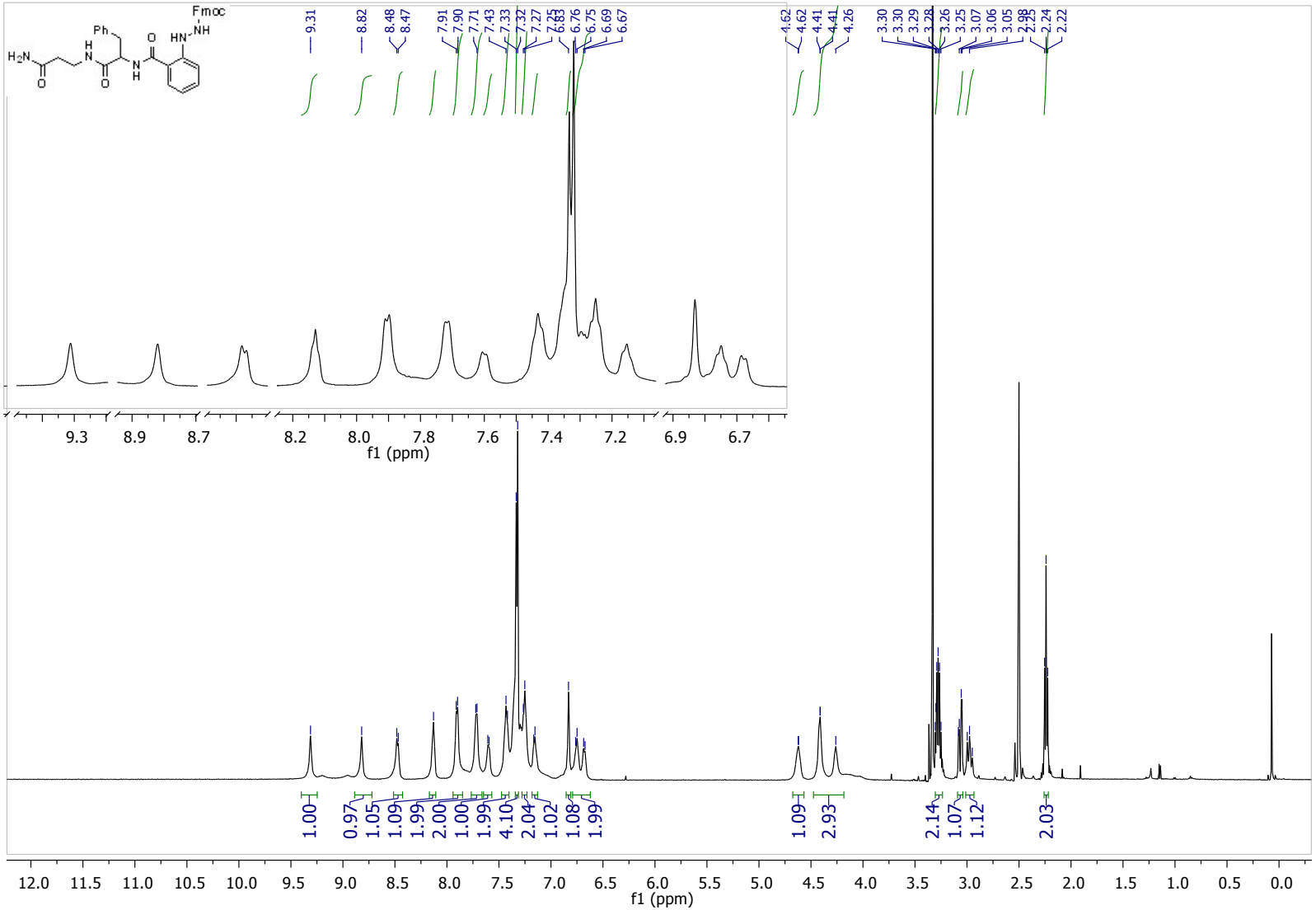


Product released from the resin 1f

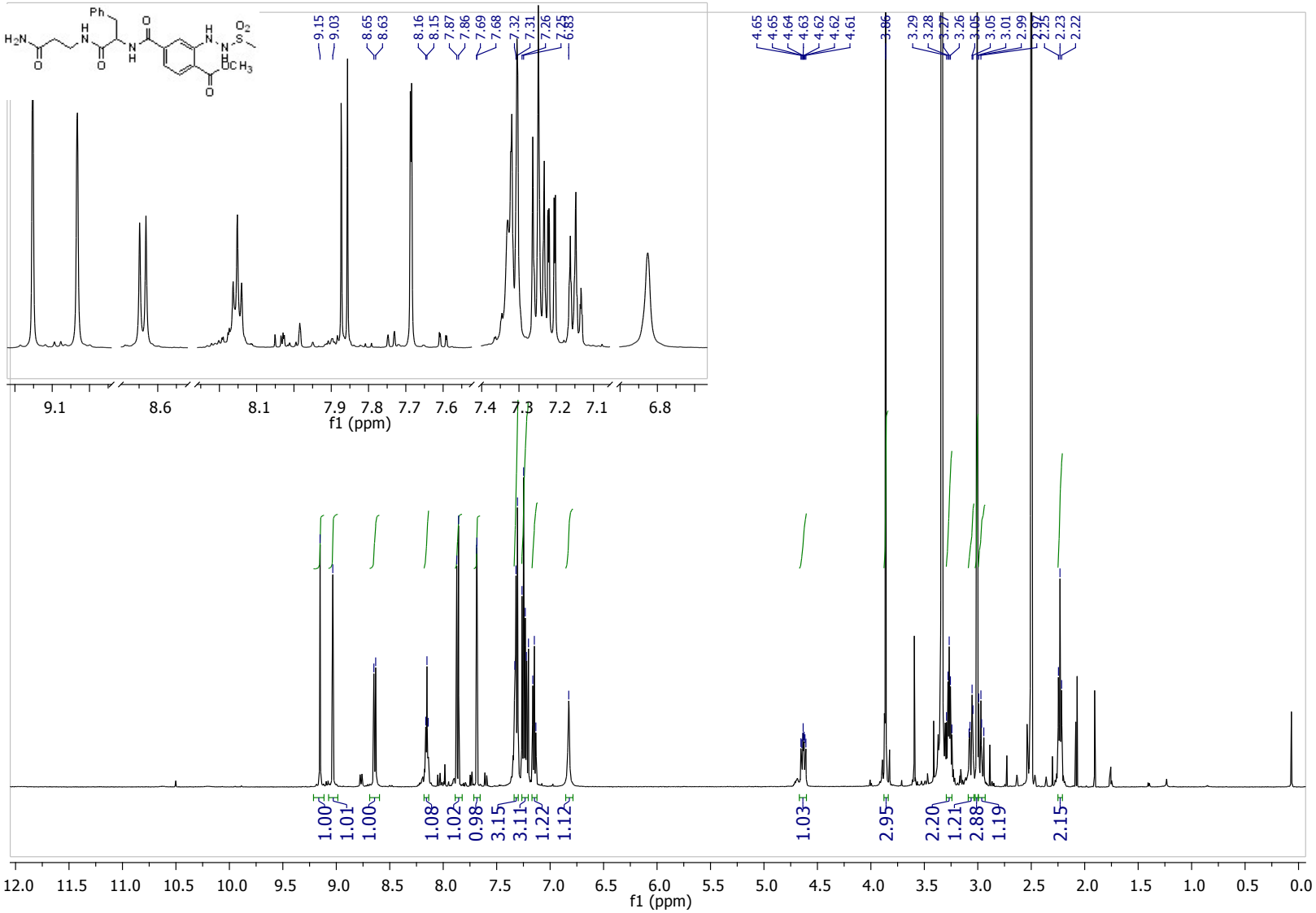


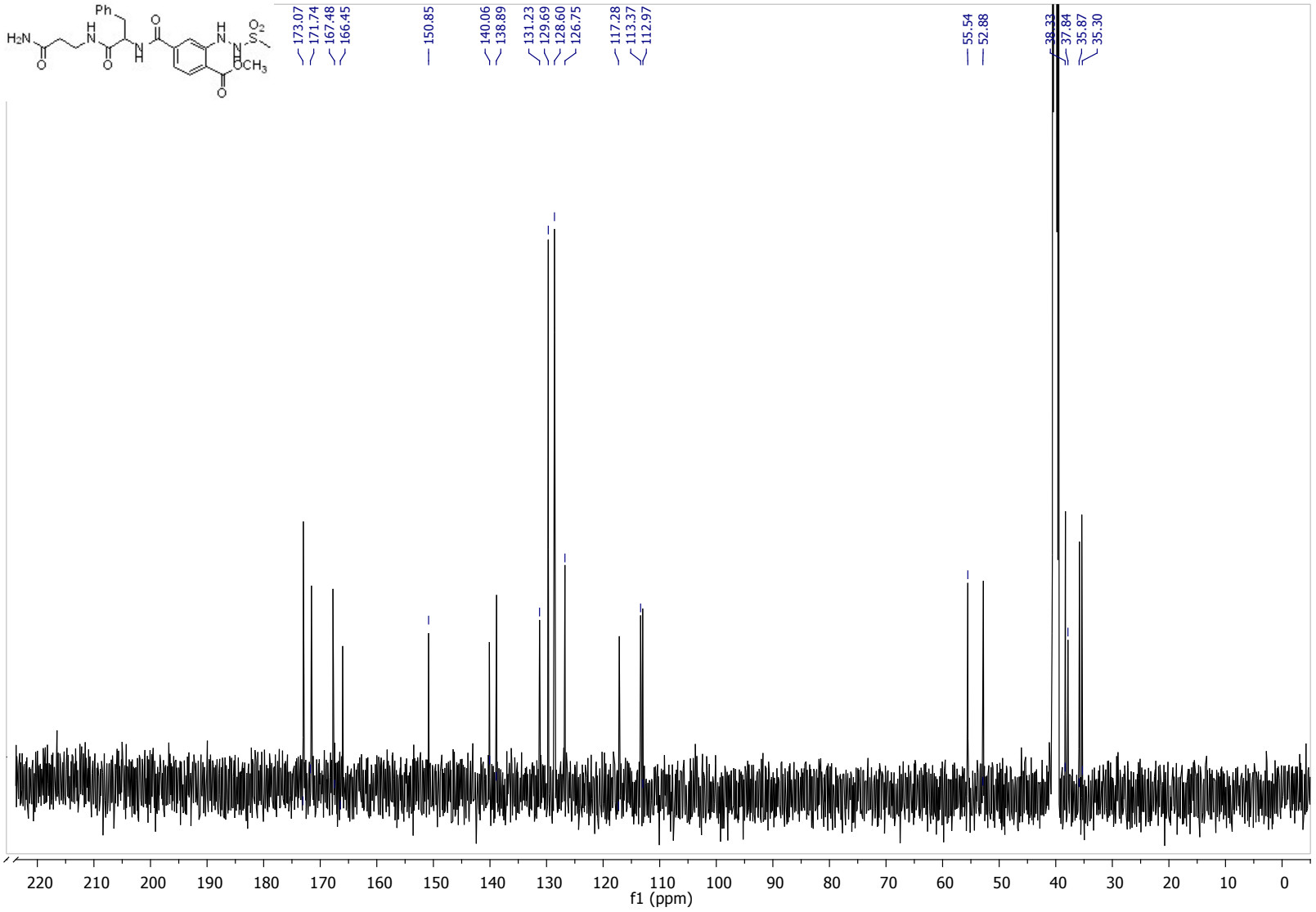

ted NOE
173.06
171.89
165.38
157.14
144.19
141.33
139.03
129.71
128.56
128.22
127.65
126.71
125.76
120.82
120.73
66.37
55.30
47.28
37.94
35.80
35.38
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

Product released from the resin 1j

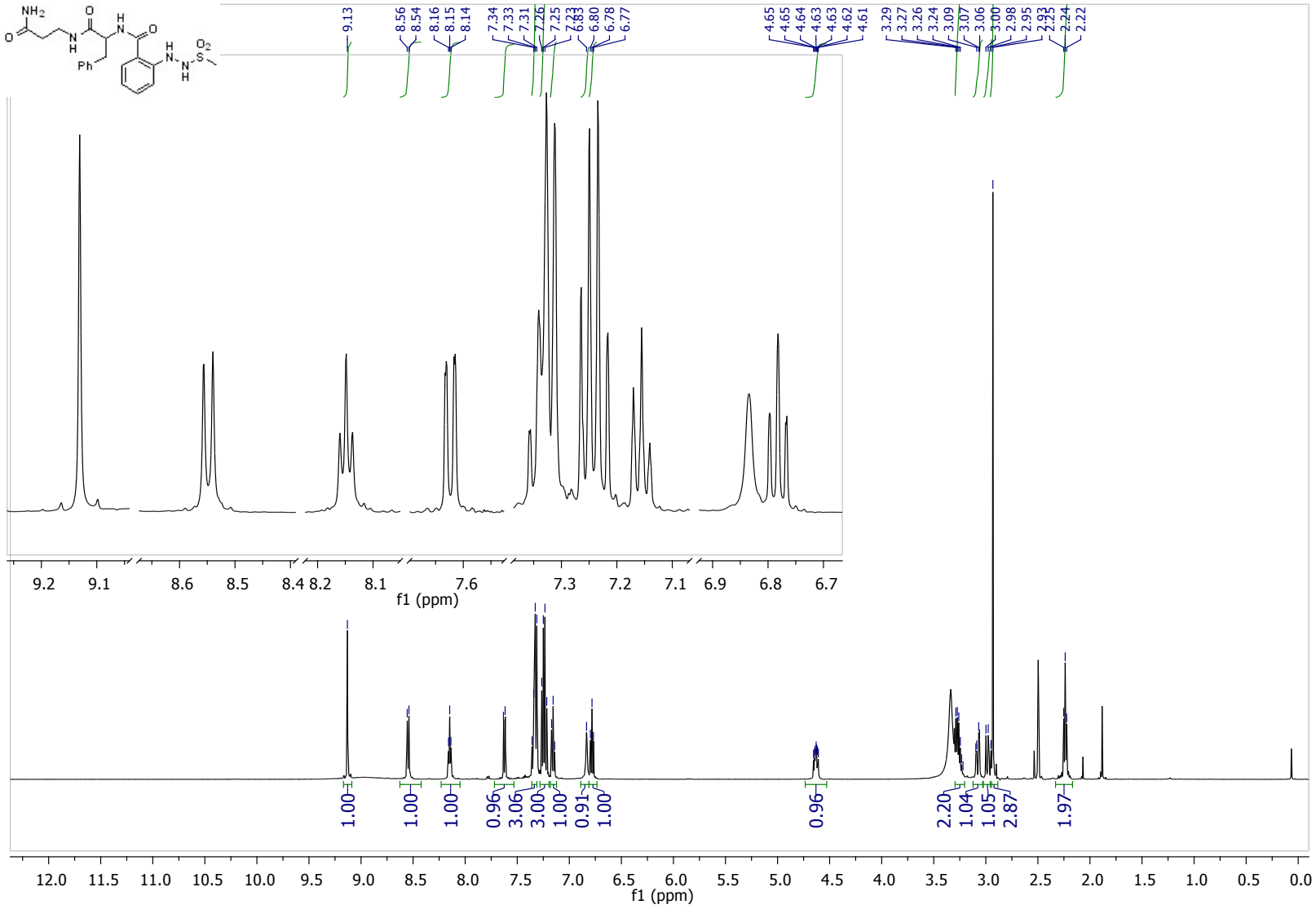


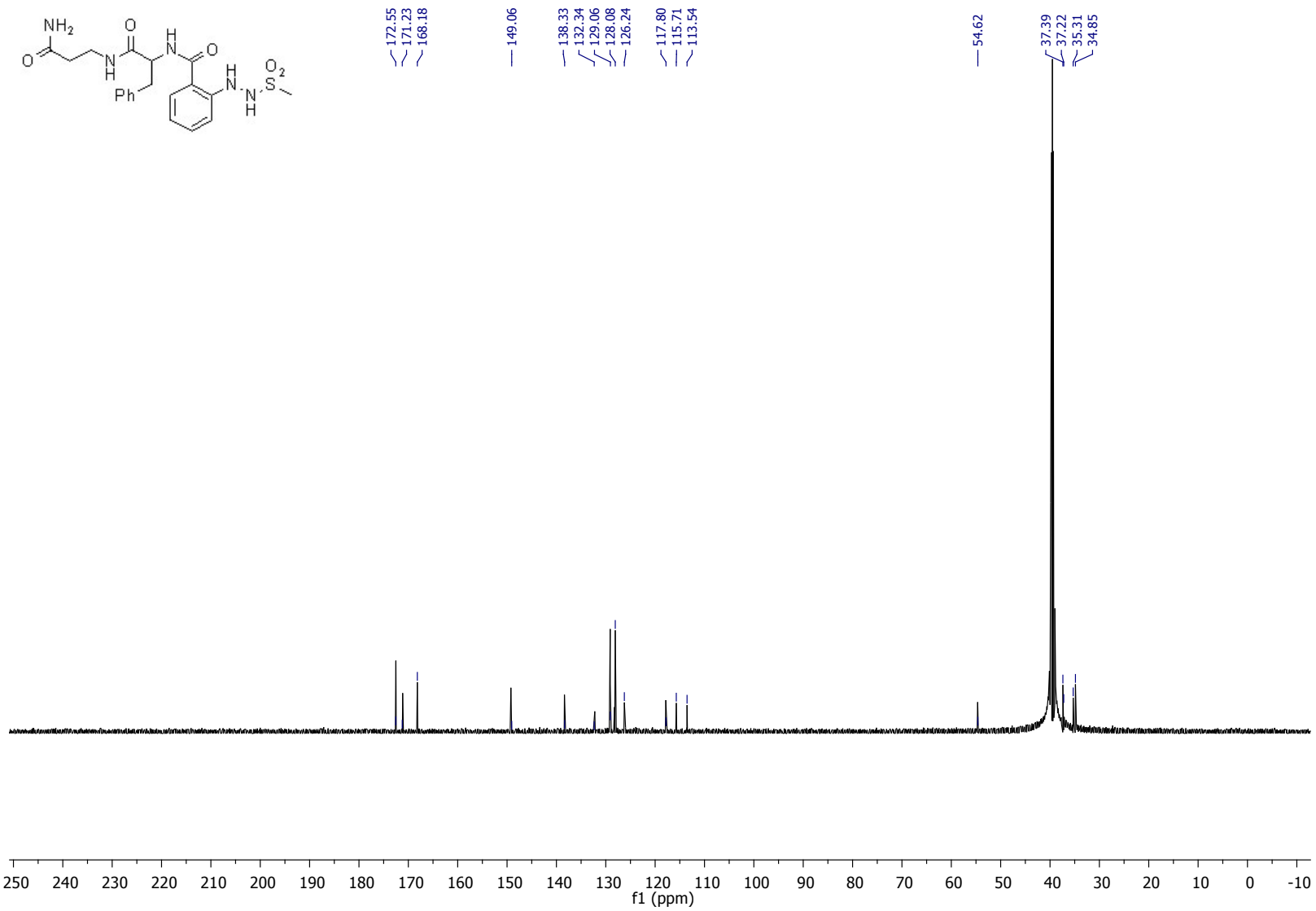
Product released from the resin 1c



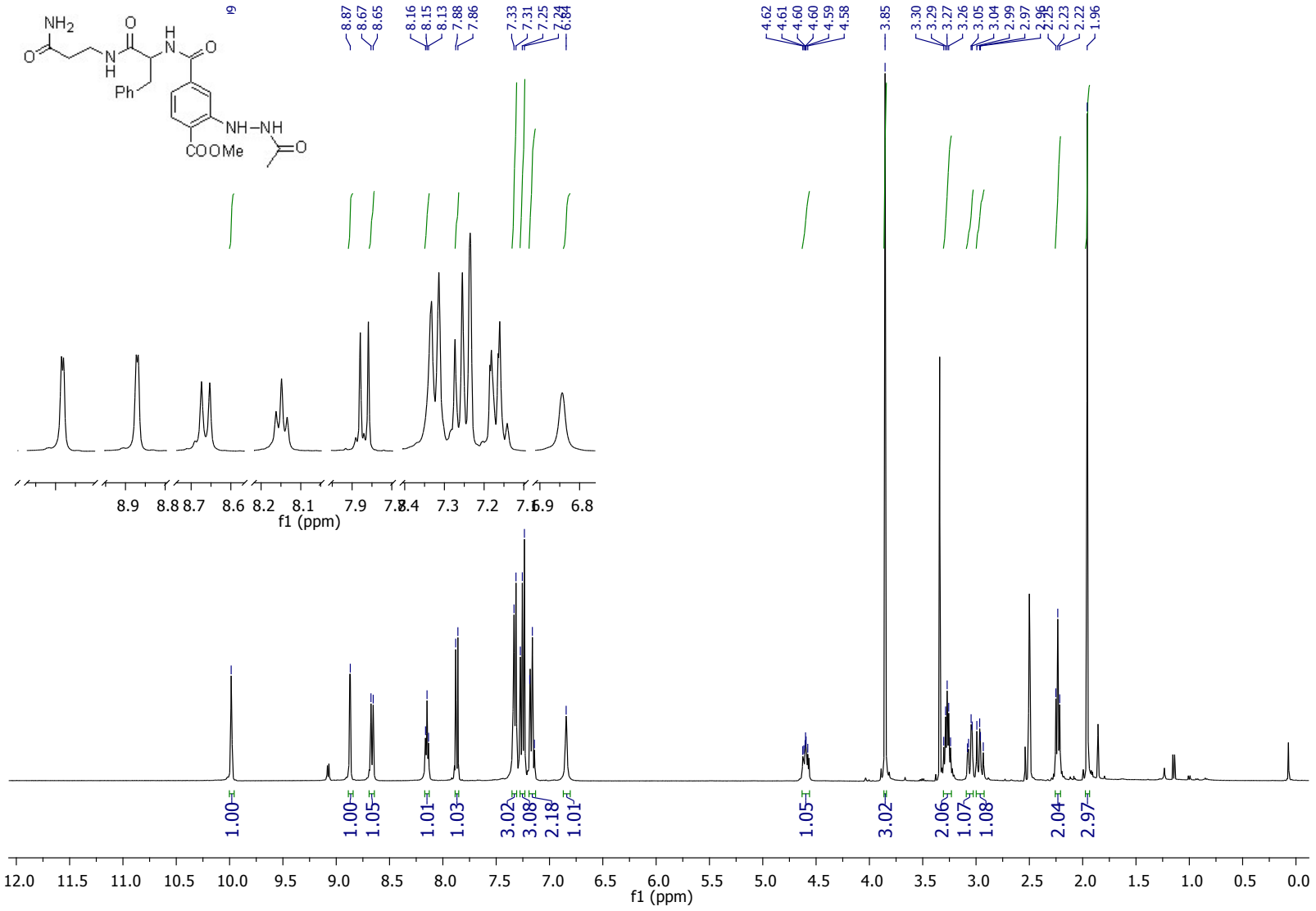

173.07
171.74
167.48
166.45
150.85
140.06
138.89
131.23
129.69
128.60
126.75
117.28
113.37
112.97
55.54
52.88
38.33
37.84
35.87
35.30
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

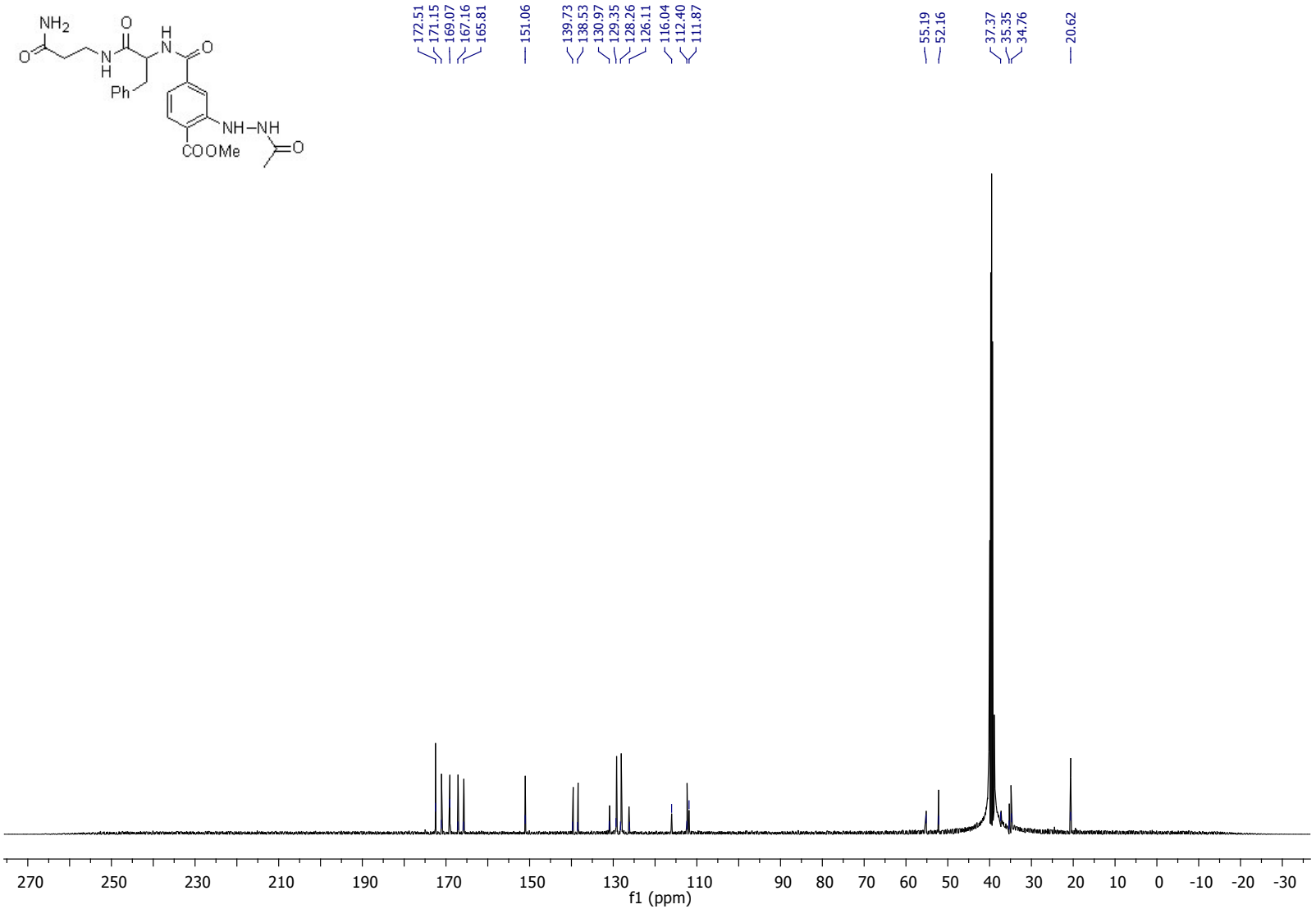
Product released from the resin 1k



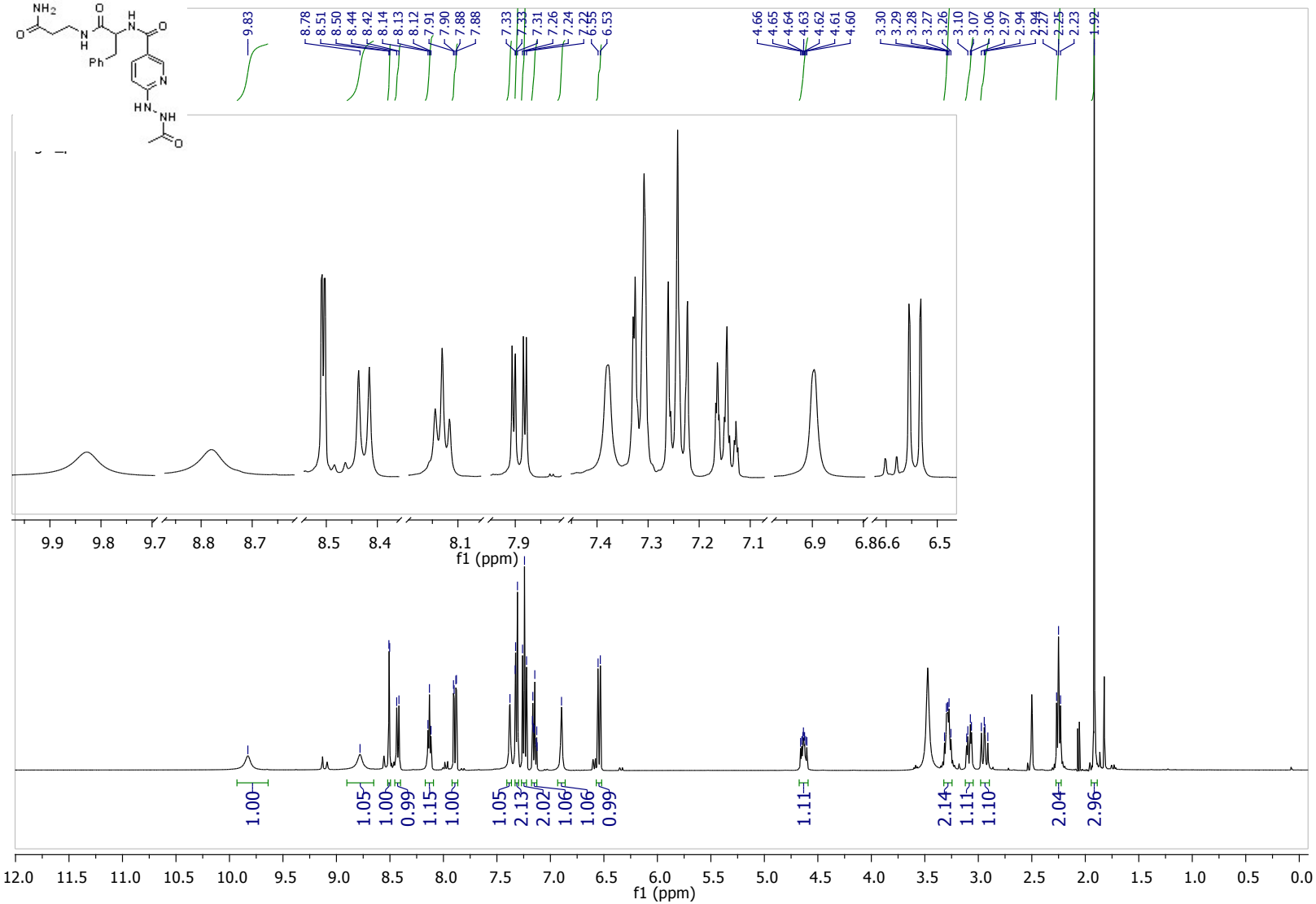

172.55
171.23
168.18
149.06
138.33
132.34
129.06
128.08
126.24
117.80
115.71
113.54
54.62
37.39
37.22
35.31
34.85
250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

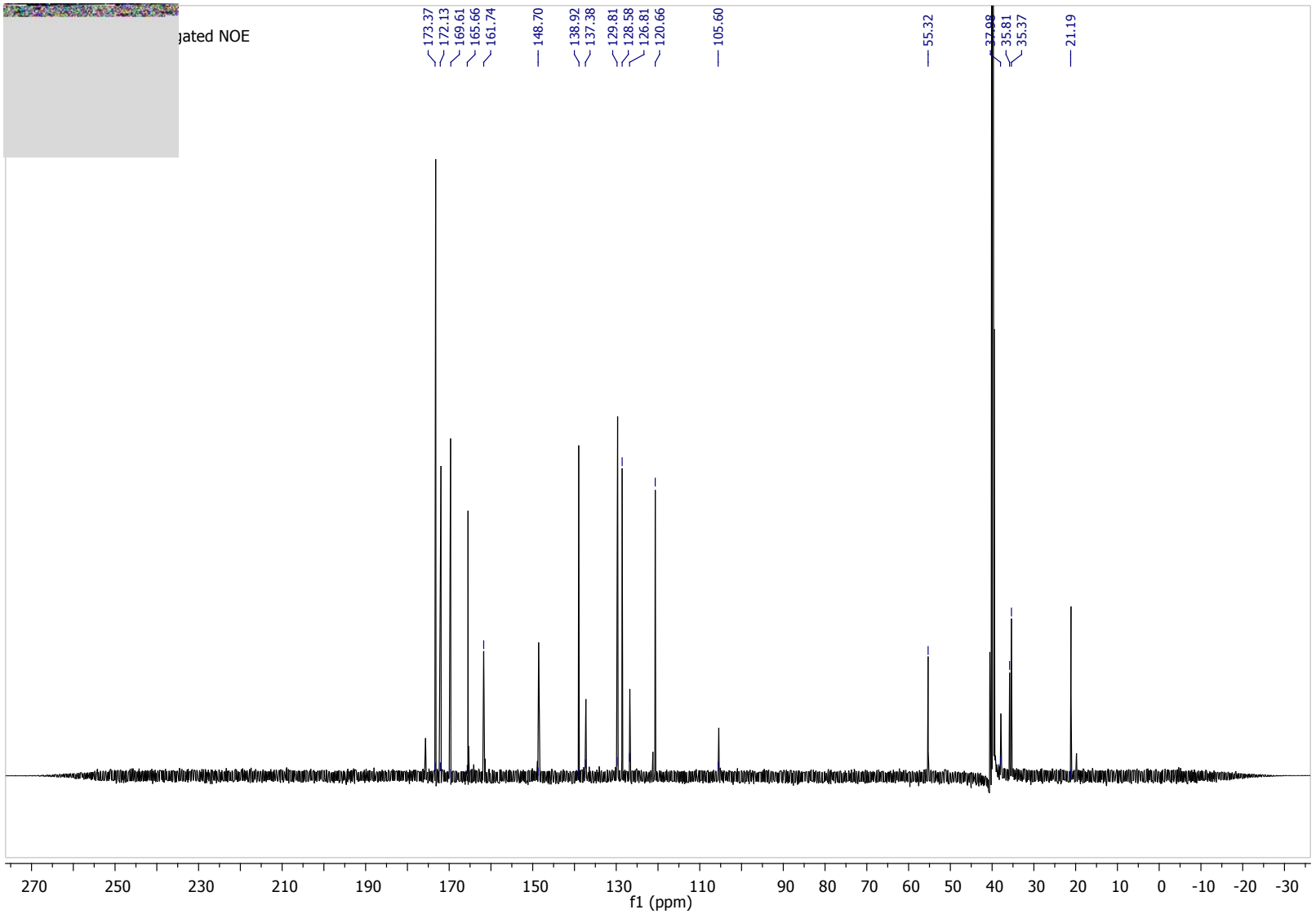
Product released from the resin 1d



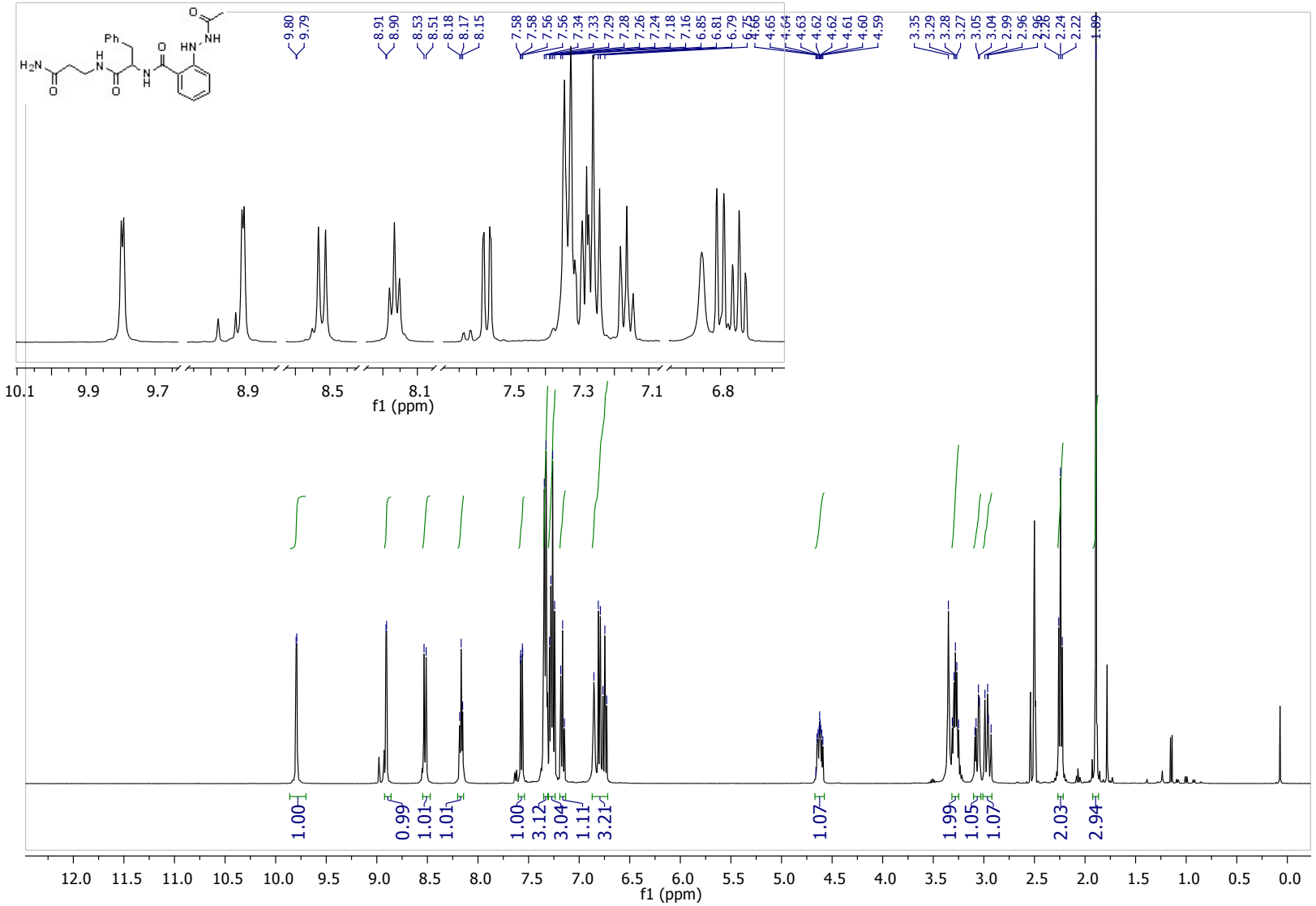

NH2
O
O
N
H
H
N
O
Ph
NH–NH
COOMe
O
172.51
171.15
169.07
167.16
165.81
151.06
139.73
138.53
130.97
129.35
128.26
126.11
116.04
112.40
111.87
55.19
52.16
37.37
35.35
34.76
20.62
270
250
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
f1 (ppm)

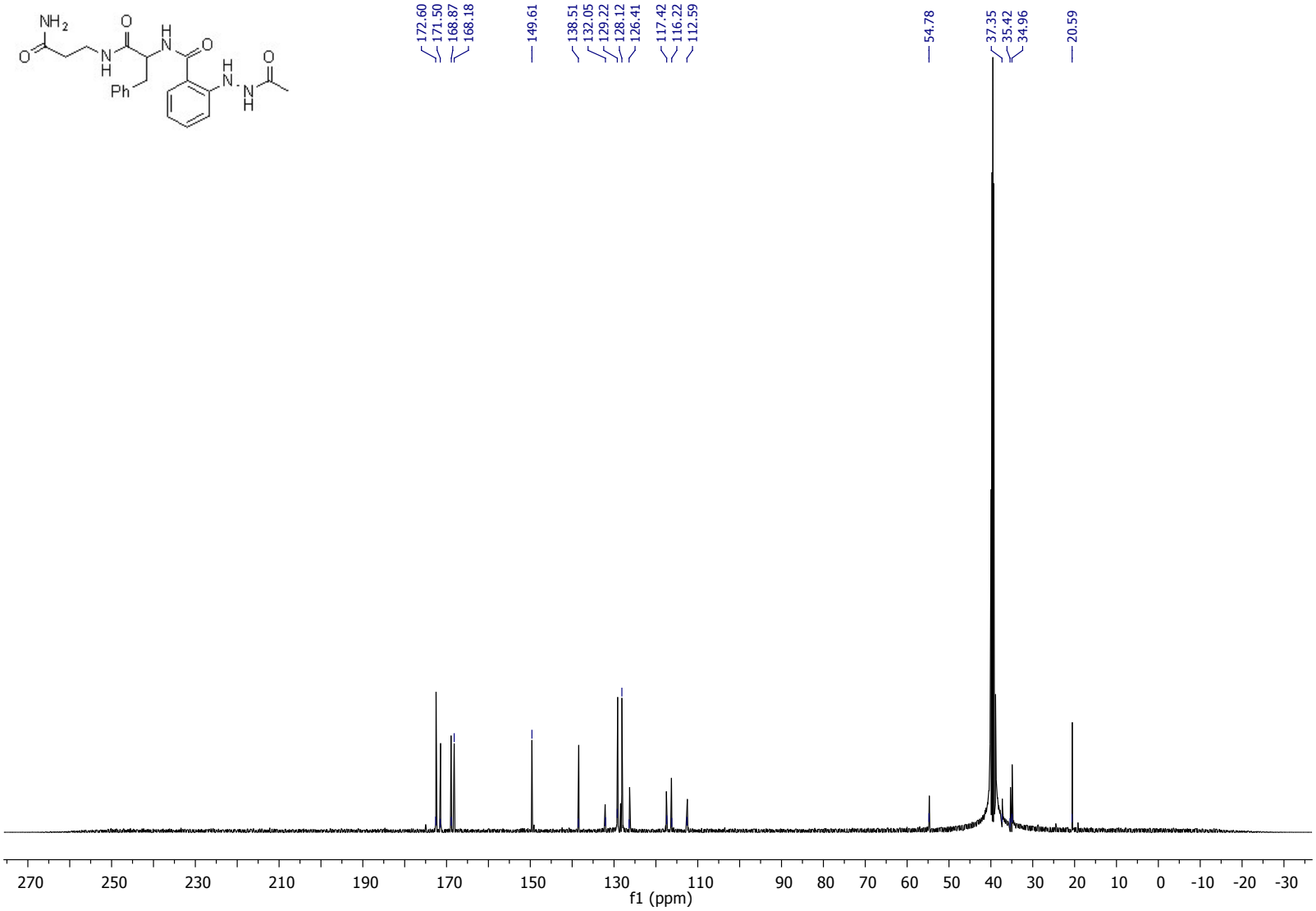
Product released from the resin 1h



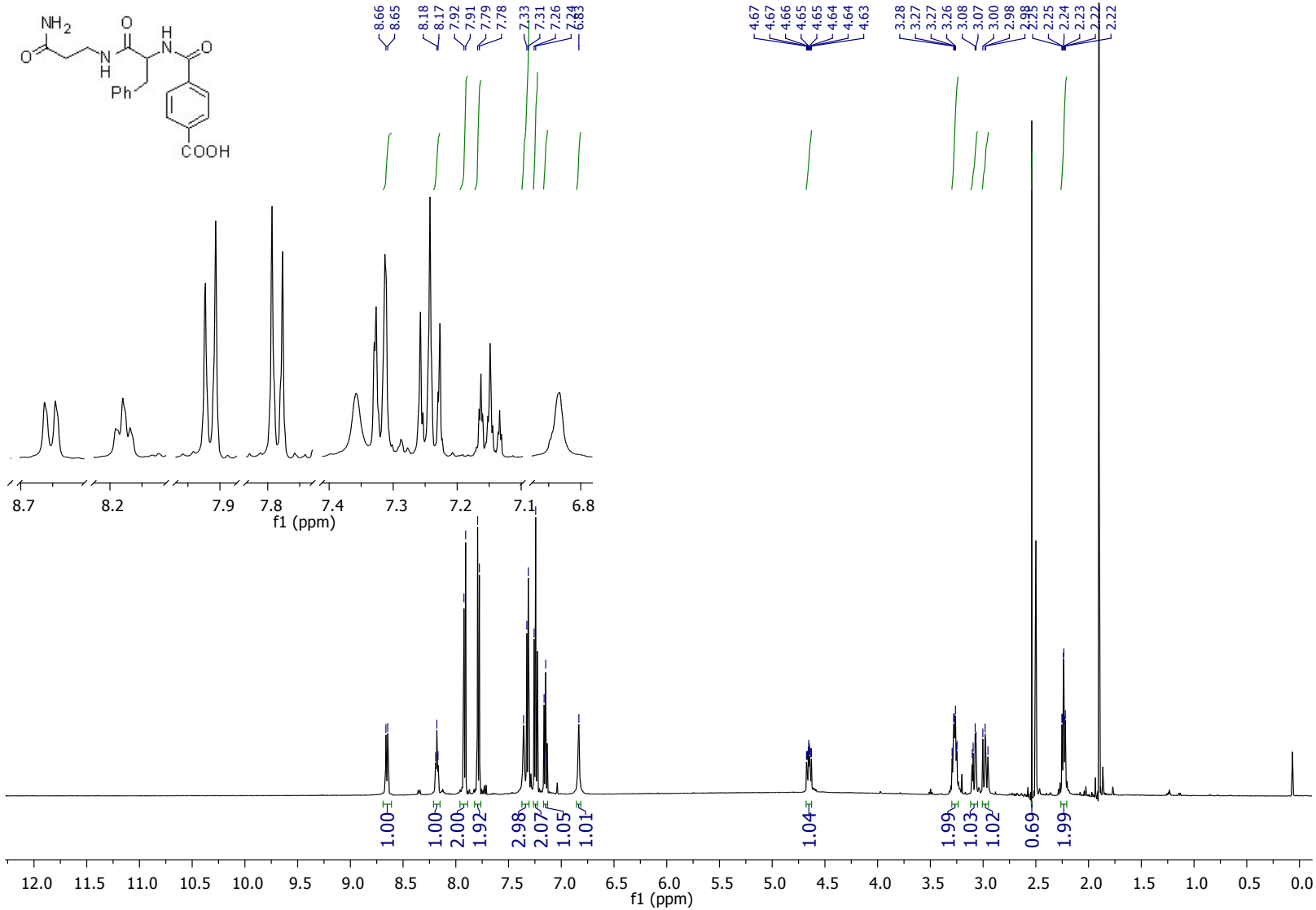

gated NOE
173.37
172.13
169.61
165.66
161.74
148.70
138.92
137.38
129.81
128.58
126.81
120.66
105.60
55.32
37.08
35.81
35.37
21.19
270
250
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
f1 (ppm)

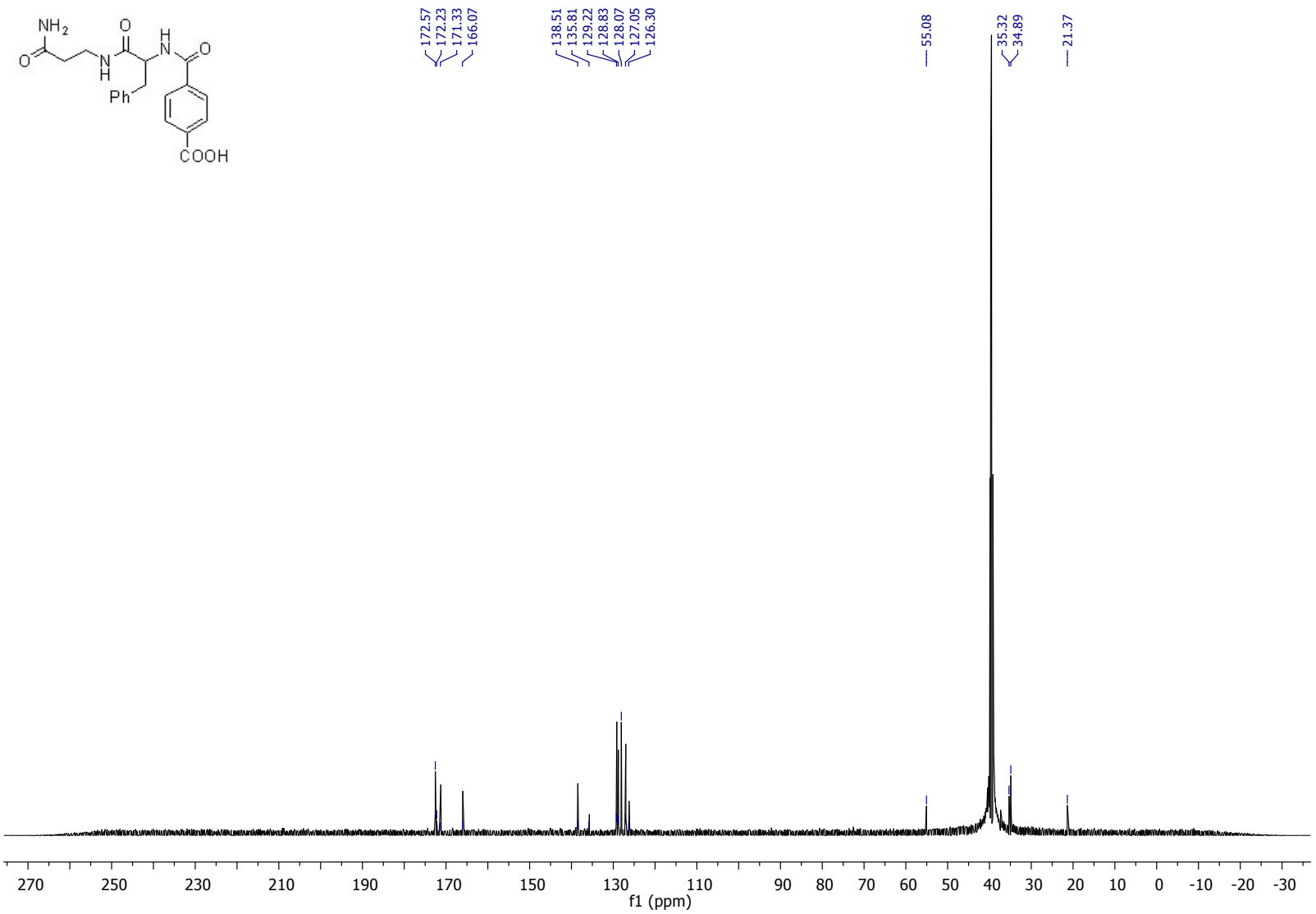
Product released from the resin 1l



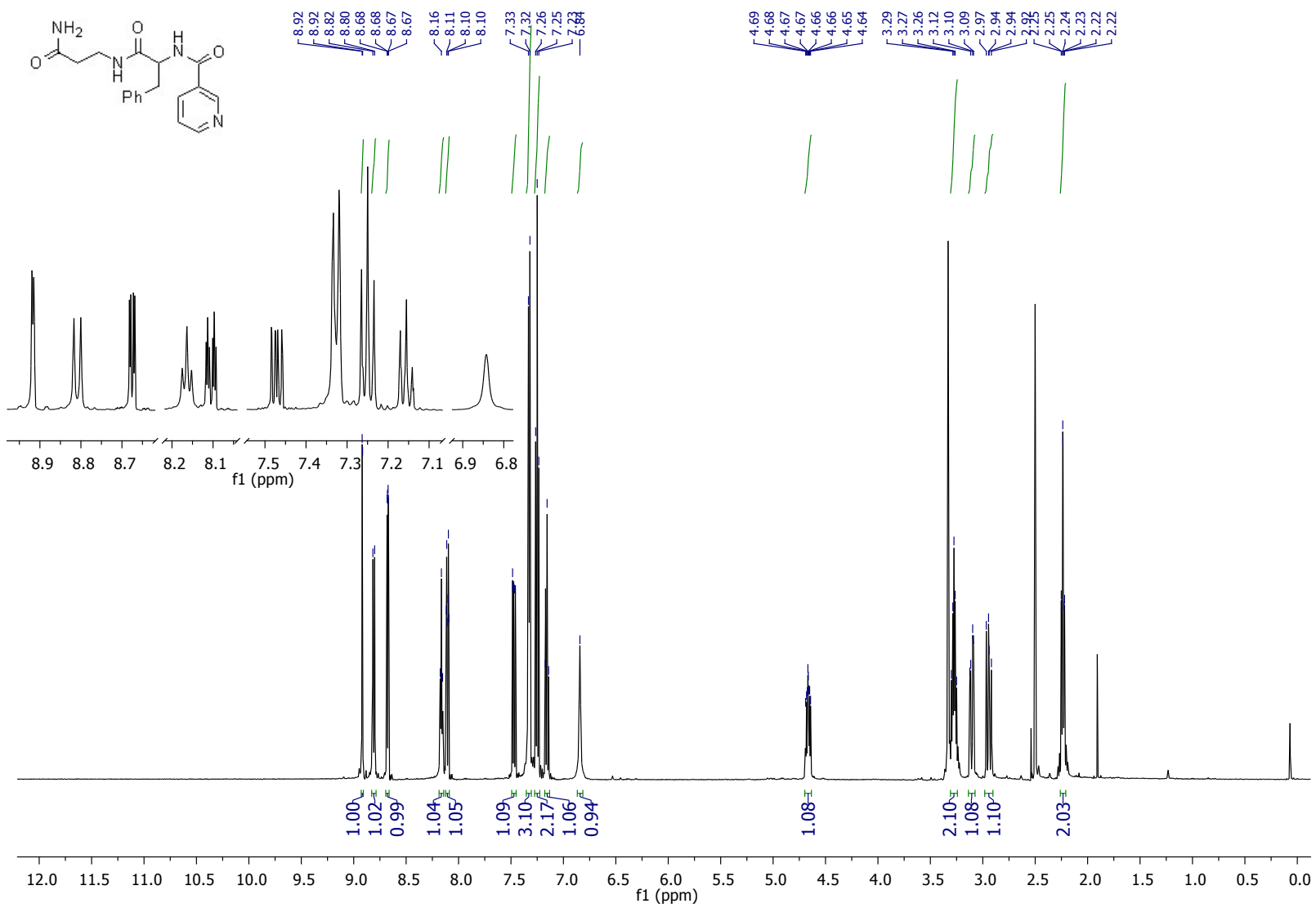

NH2
O
N
H
O
H
N
O
Ph
H
N
N
H
O
172.60
171.50
168.87
168.18
149.61
138.51
132.05
129.22
128.12
126.41
117.42
116.22
112.59
54.78
37.35
35.42
34.96
20.59
270
250
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
f1 (ppm)

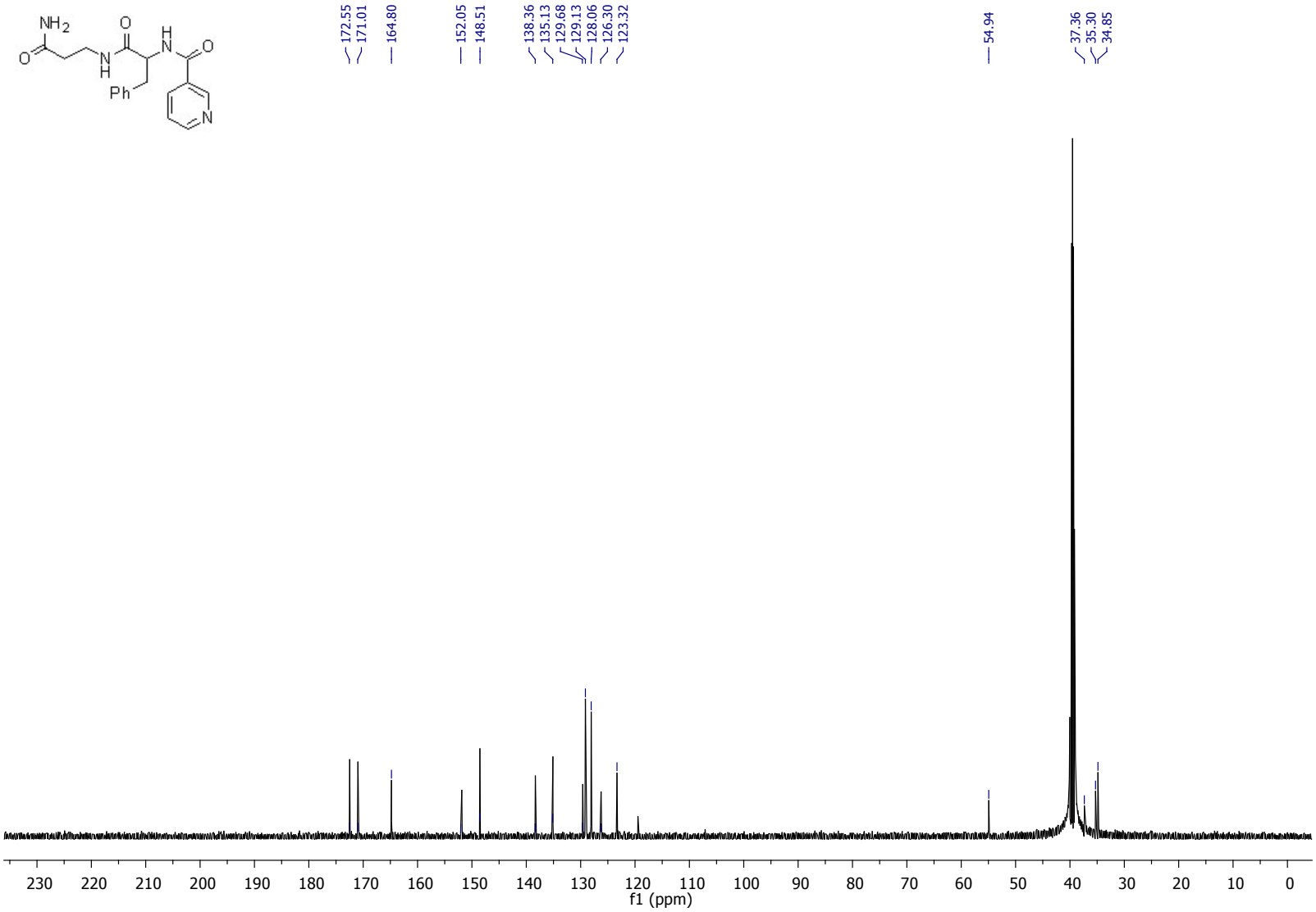
Product released from the resin 2a



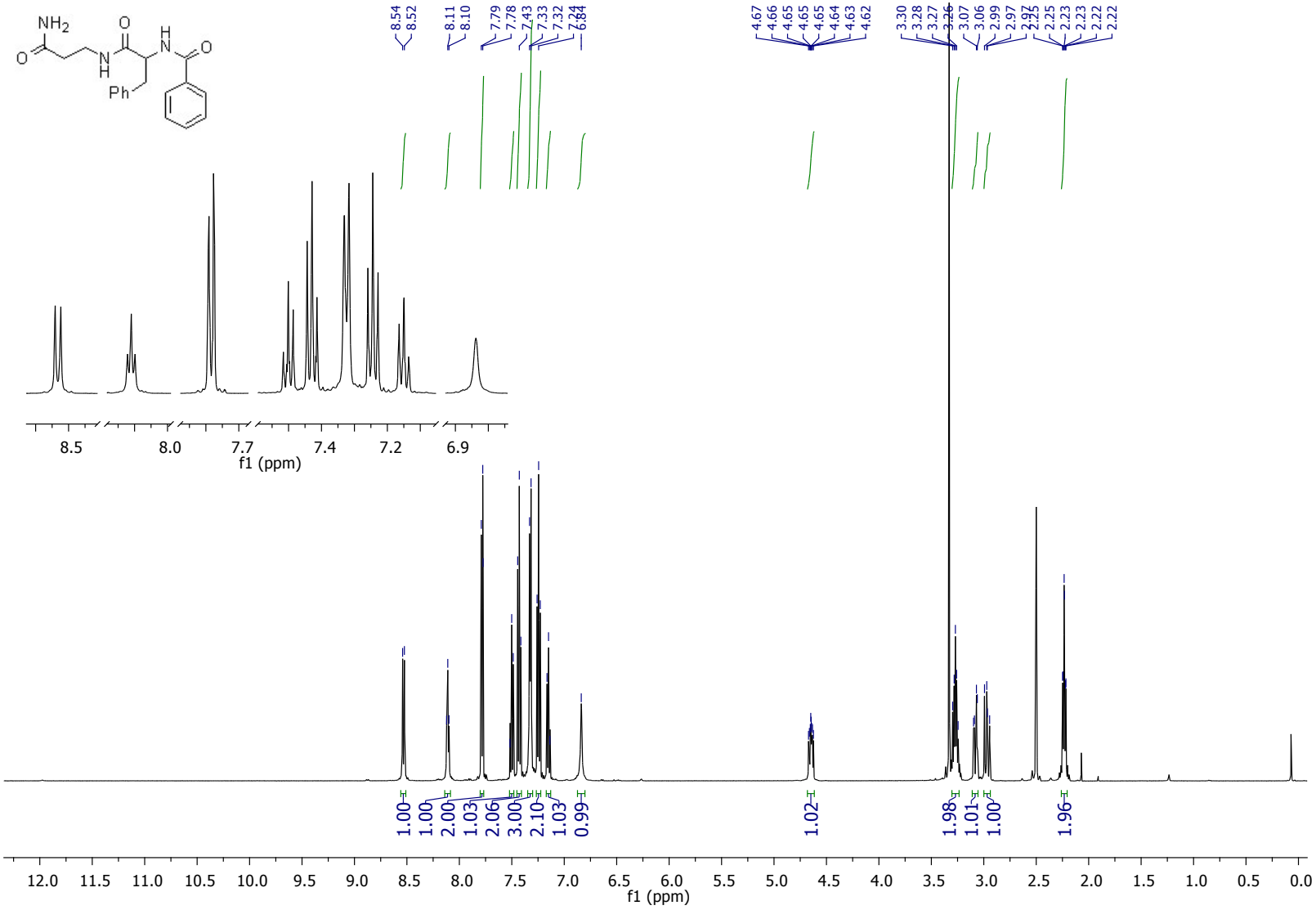

NH2
O
O
N
H
H
N
O
Ph
COOH
172.57
172.23
171.33
166.07
138.51
135.81
129.22
128.83
128.07
127.05
126.30
55.08
35.32
34.89
21.37
270 250 230 210 190 170 150 130 110 90 80 70 60 50 40 30 20 10 0 -10 -20 -30
f1 (ppm)

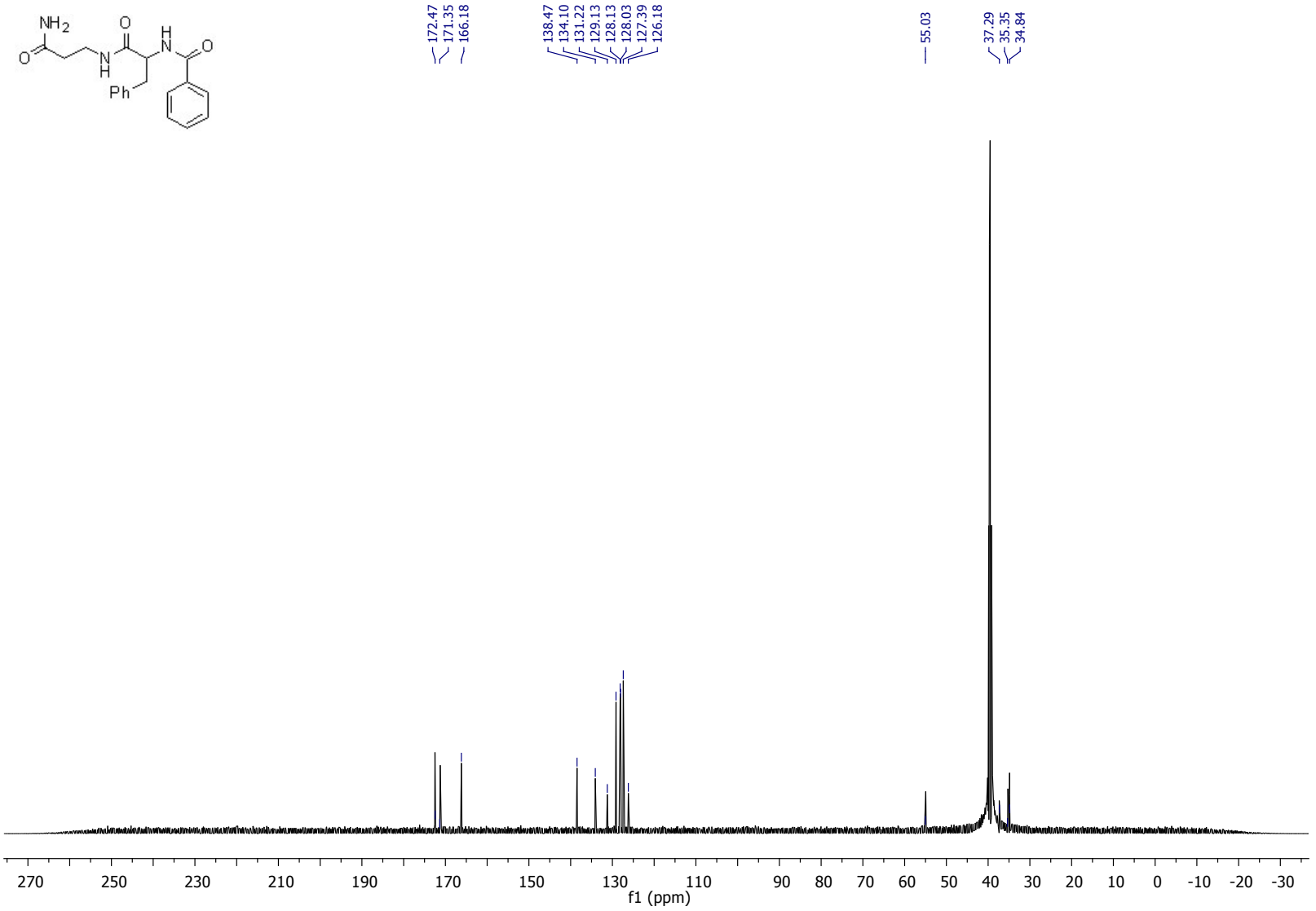
Product released from the resin 2b




NH2
O
N
H
O
N
H
O
Ph
N
172.55
171.01
164.80
152.05
148.51
138.36
135.13
129.68
129.13
128.06
126.30
123.32
54.94
37.36
35.30
34.85
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

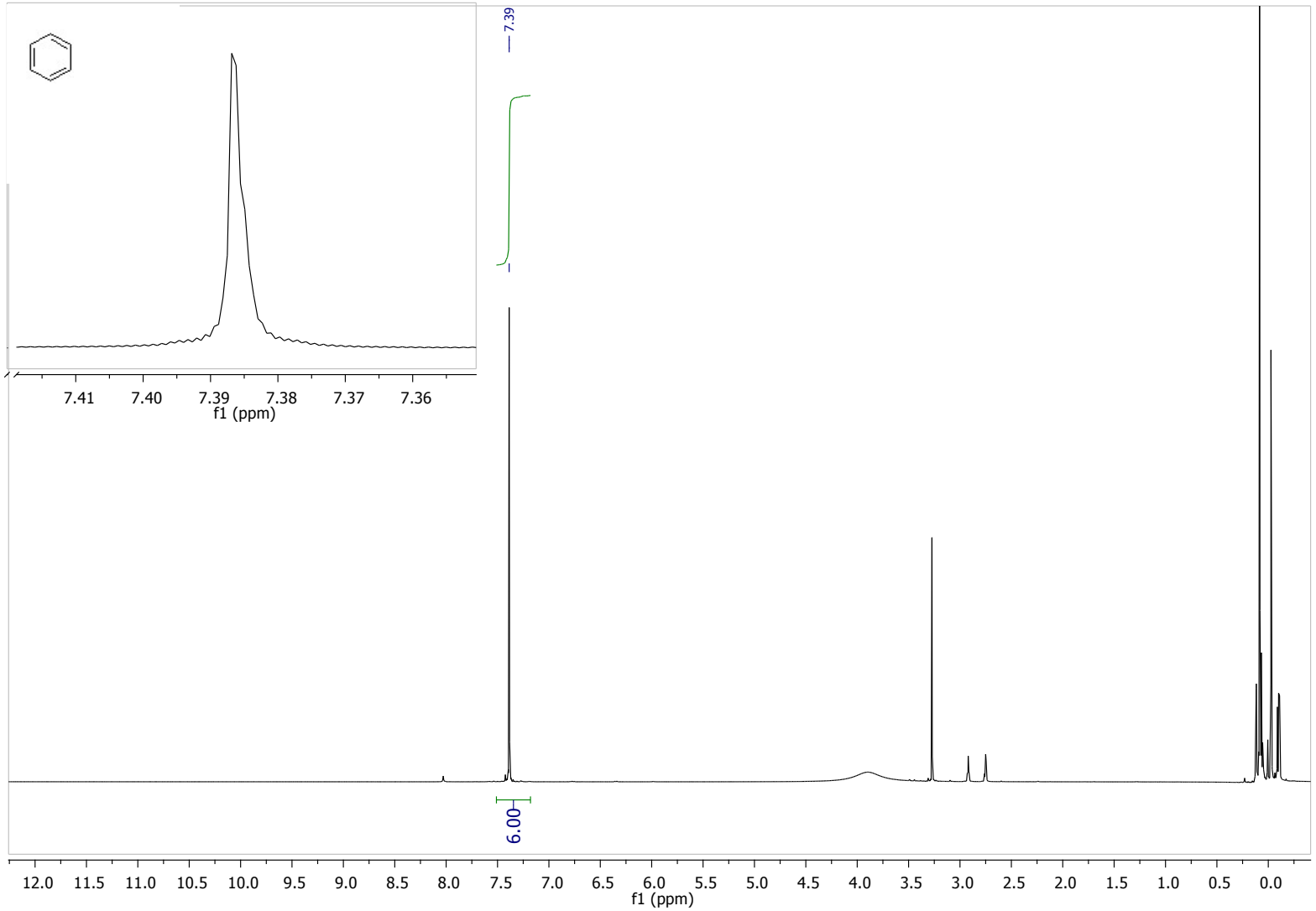
Product released from the resin 2c



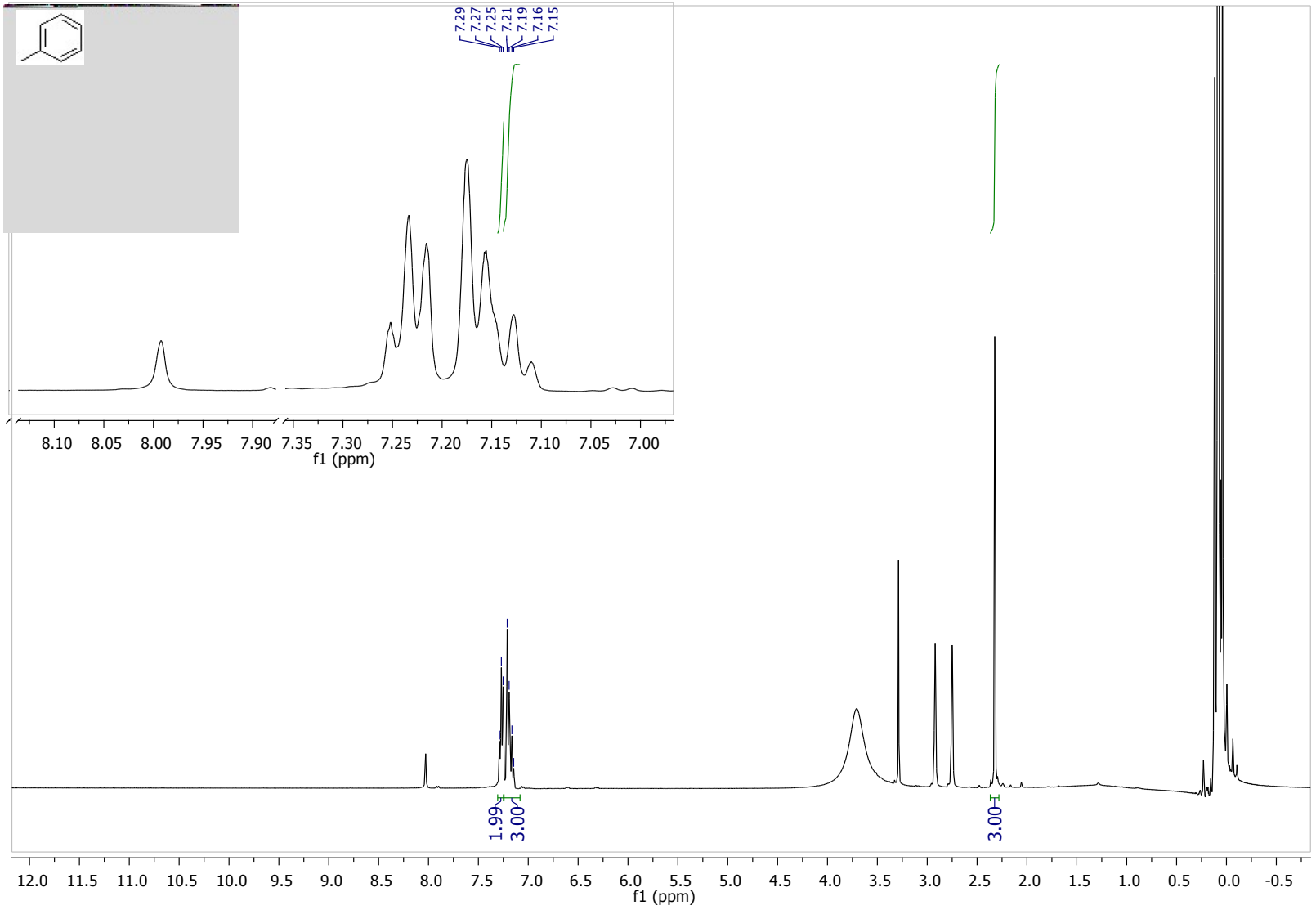

NH2
O
O
N
H
H
N
O
Ph
172.47
171.35
166.18
138.47
134.10
131.22
129.13
128.13
128.03
127.39
126.18
55.03
37.29
35.35
34.84
270
250
230
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
f1 (ppm)

4.2. NMR spectra of crude products of hydrazine cleavage in solution

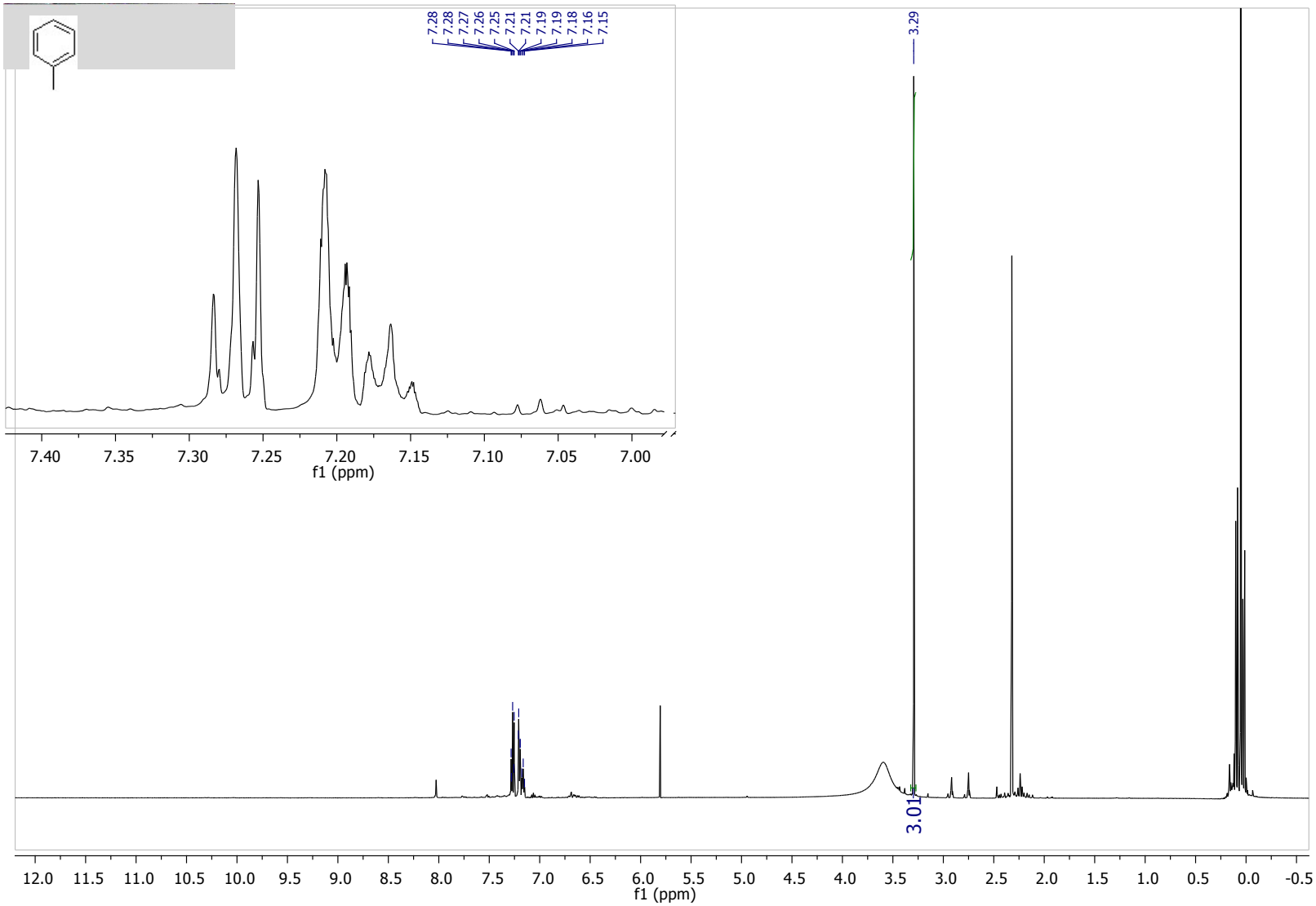
4a



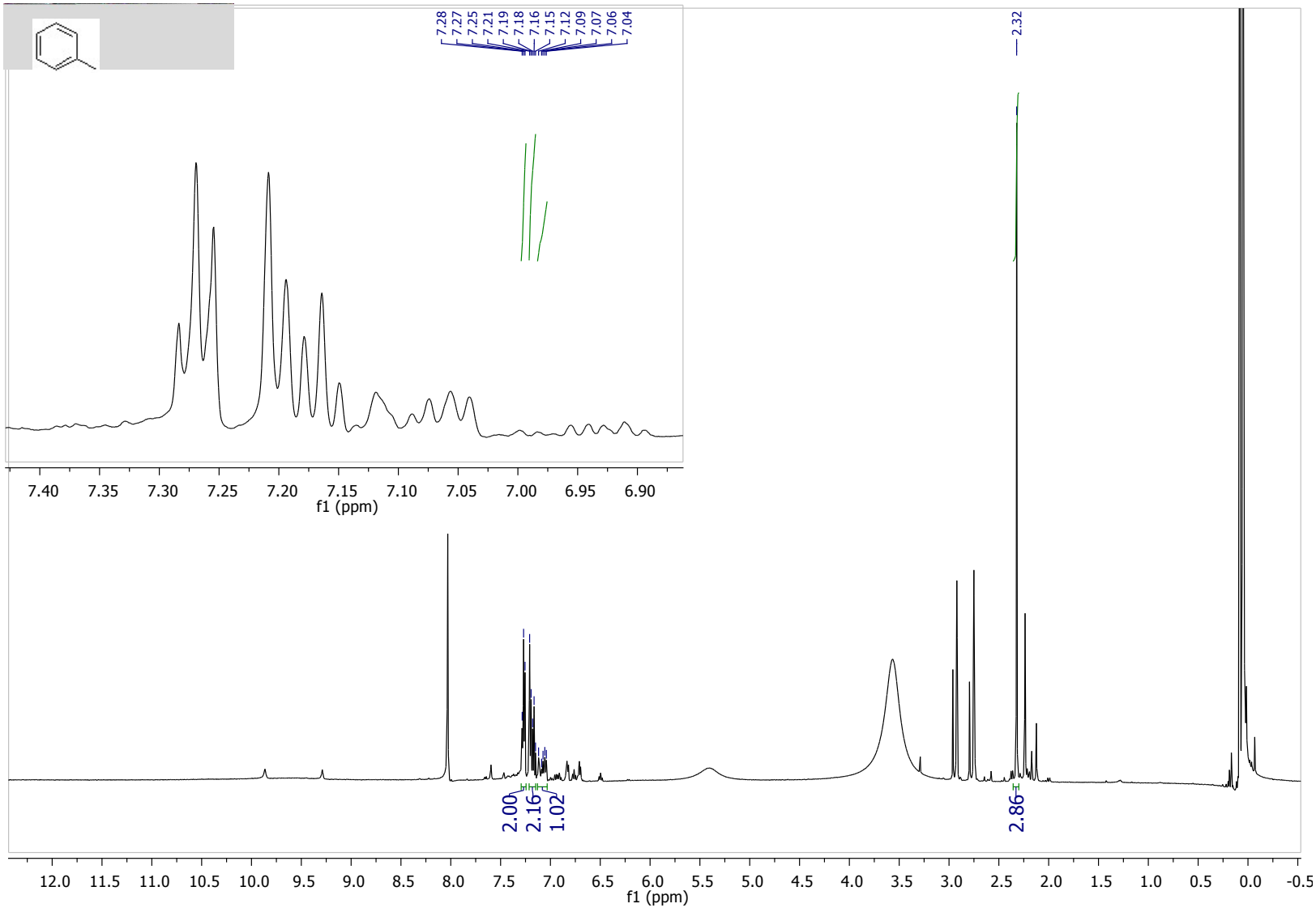
4b prepared from from 3b



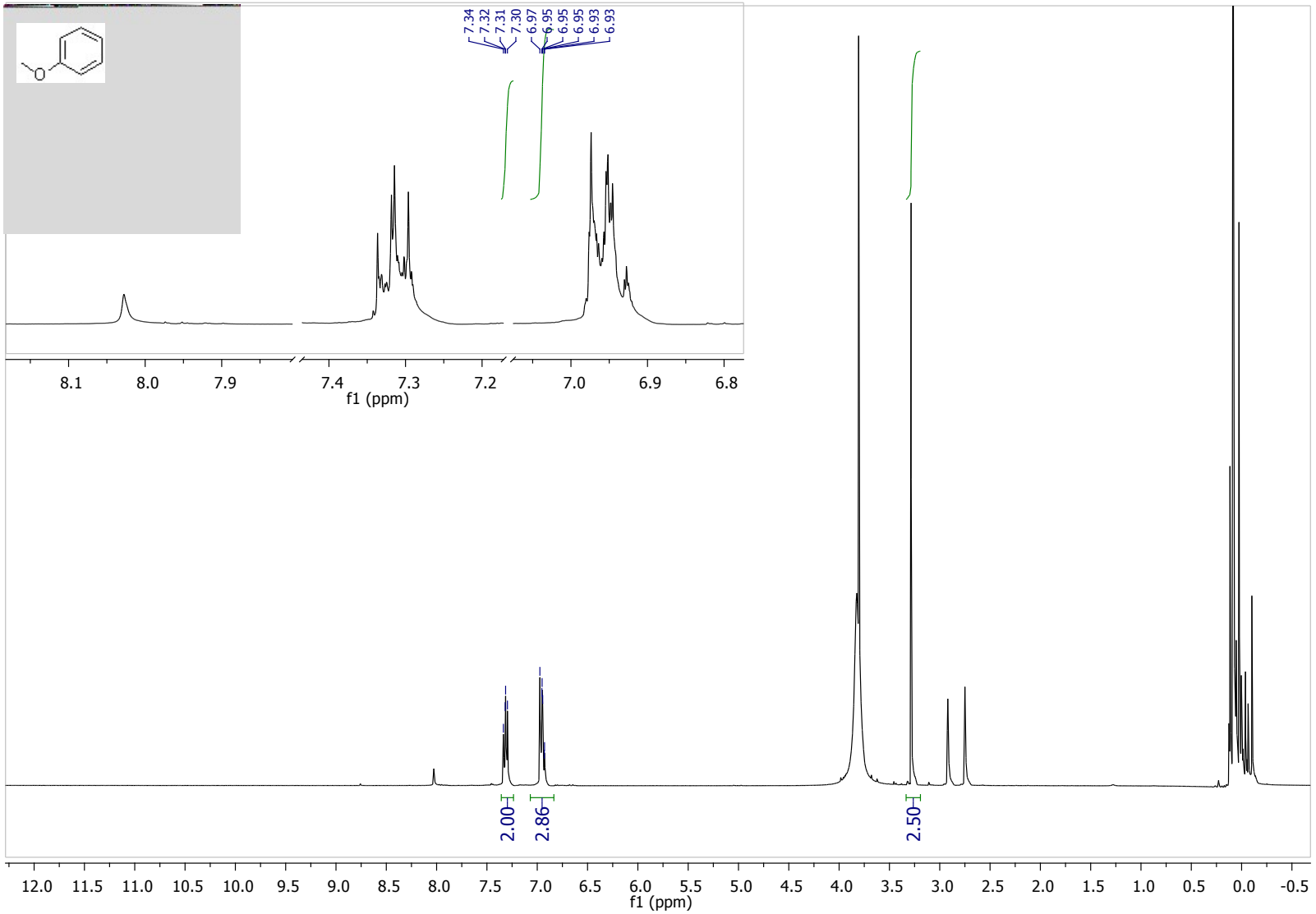
4b prepared from 3c under heating



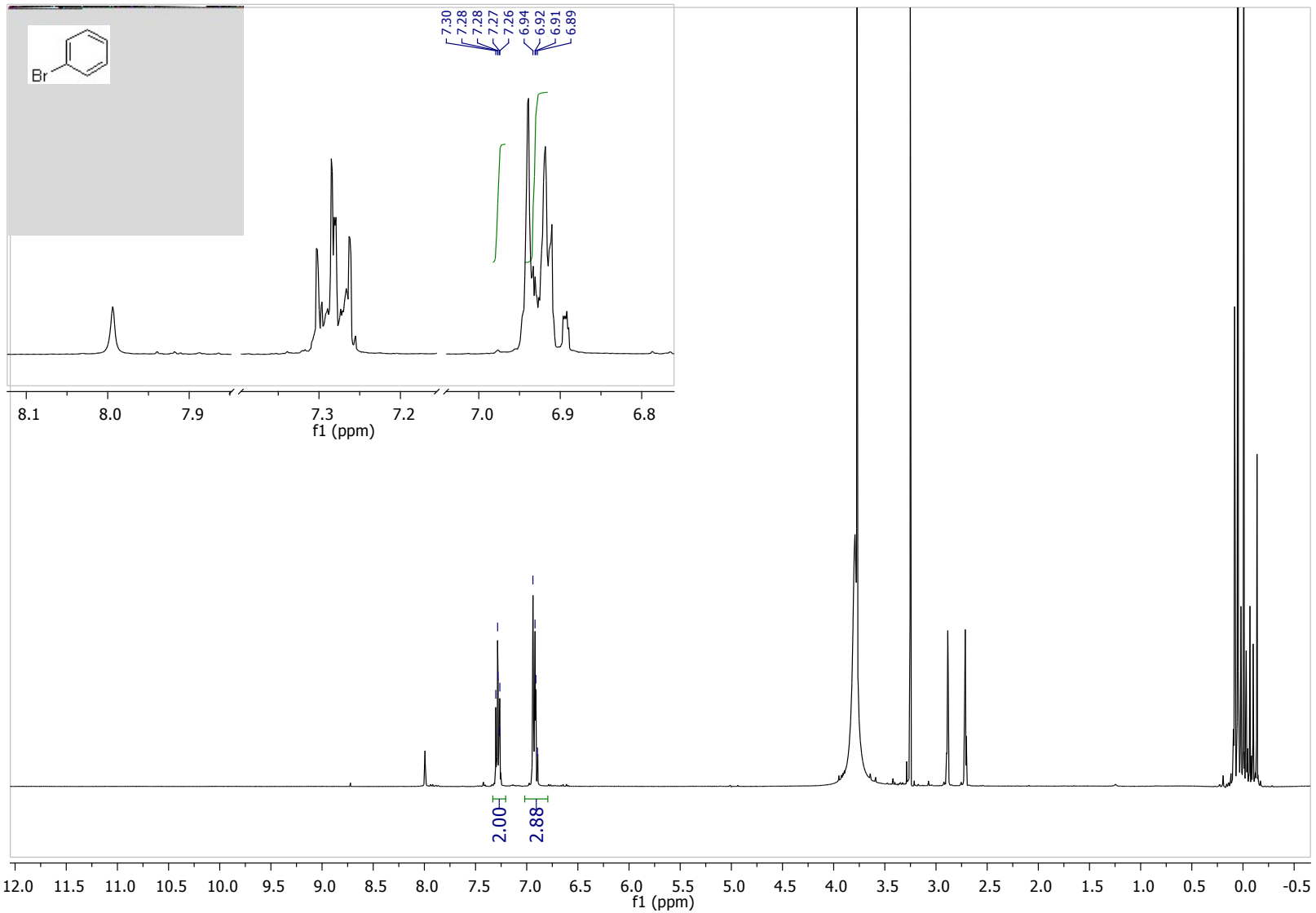
4b prepared from 3d under heating



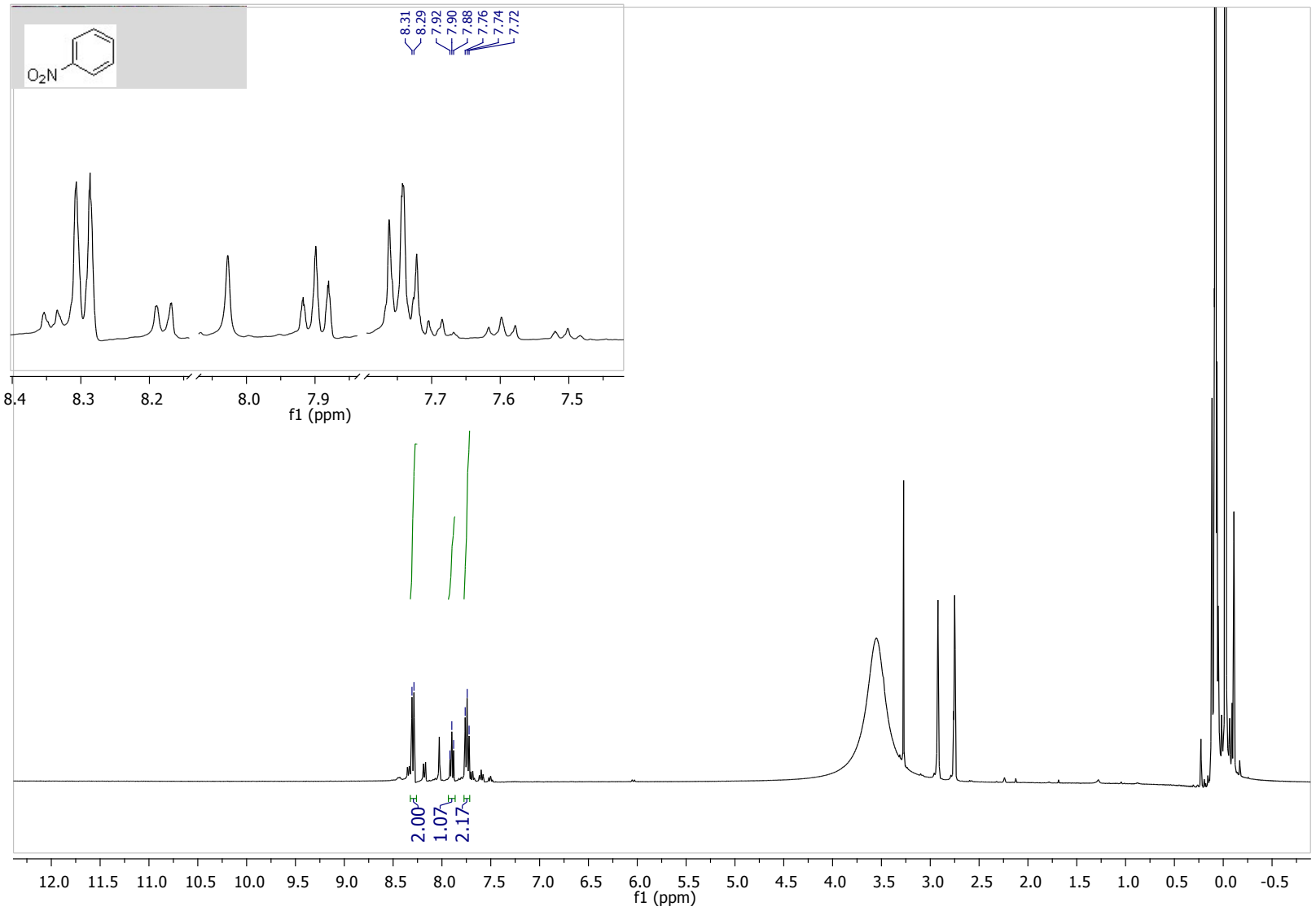
4e


7.34
7.32
7.31
7.30
6.97
6.95
6.95
6.95
6.93
6.93
8.1
8.0
7.9
7.4
7.3
7.2
7.0
6.9
6.8
f1 (ppm)
2.00
2.86
2.50
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

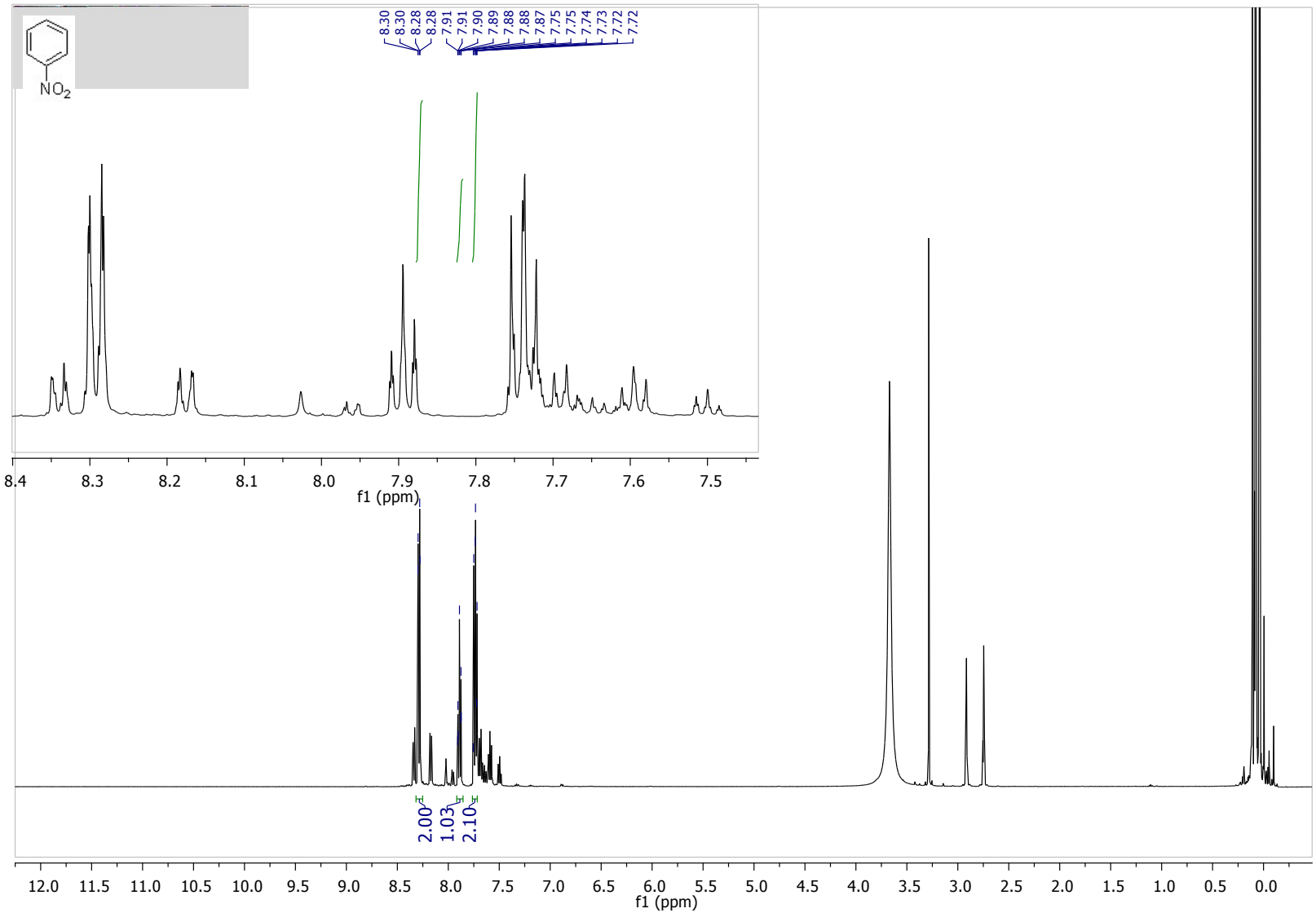
4f


Br
7.30
7.28
7.28
7.27
7.26
6.94
6.92
6.91
6.89
8.1
8.0
7.9
7.3
7.2
7.0
6.9
6.8
f1 (ppm)
2.00
2.88
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

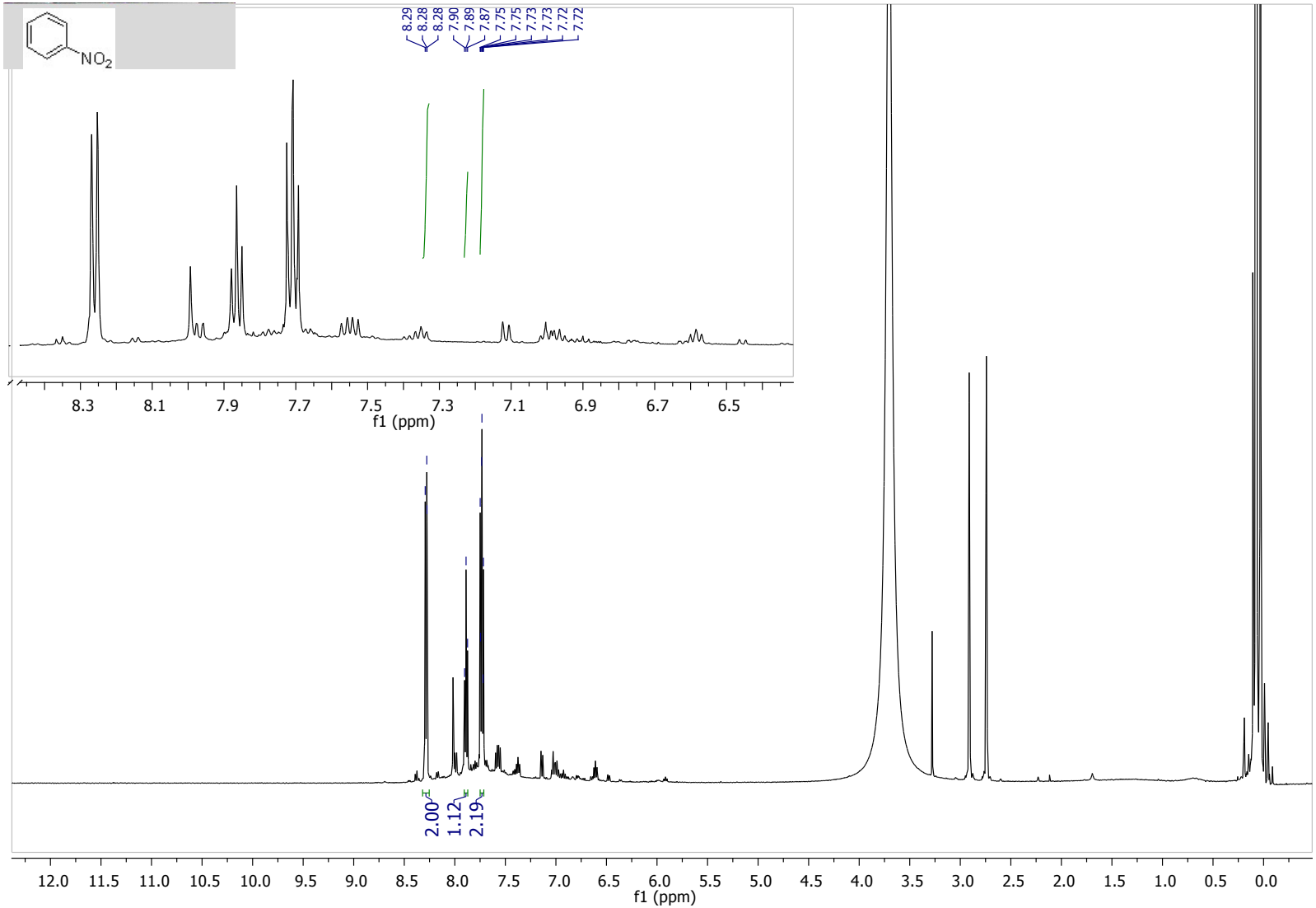
4g prepared from 3g



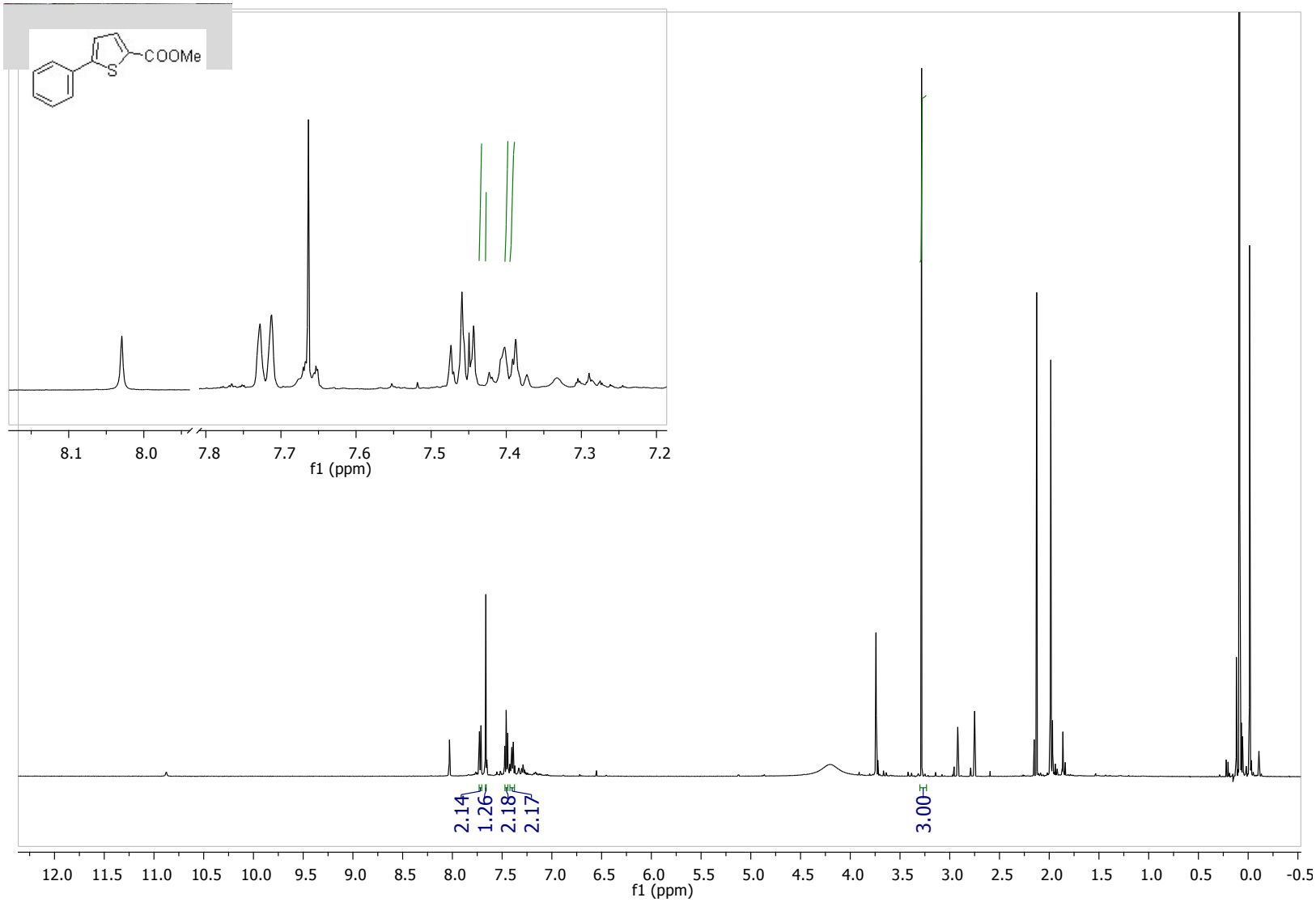
4g prepared from 3h



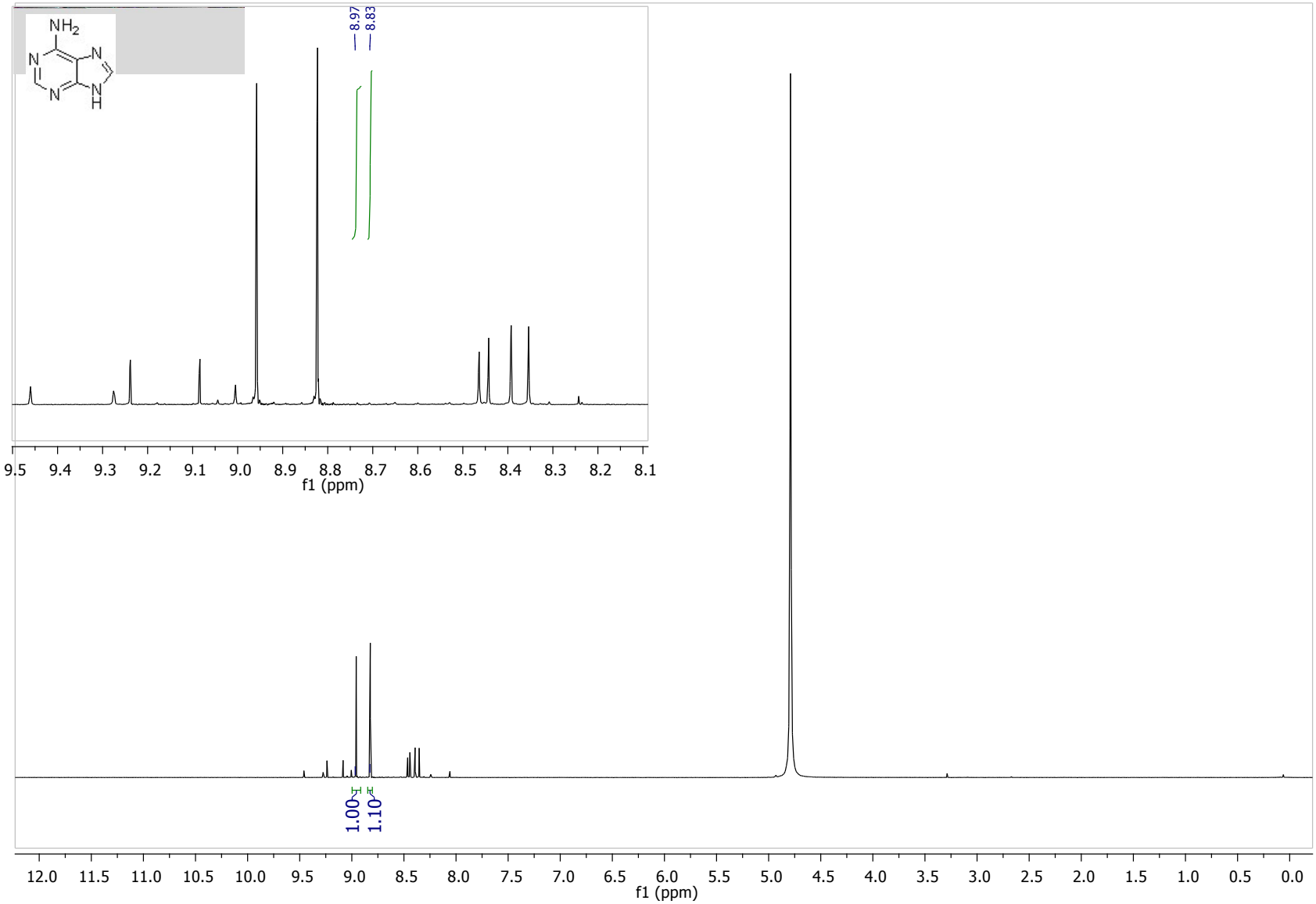
4g prepared from 3i



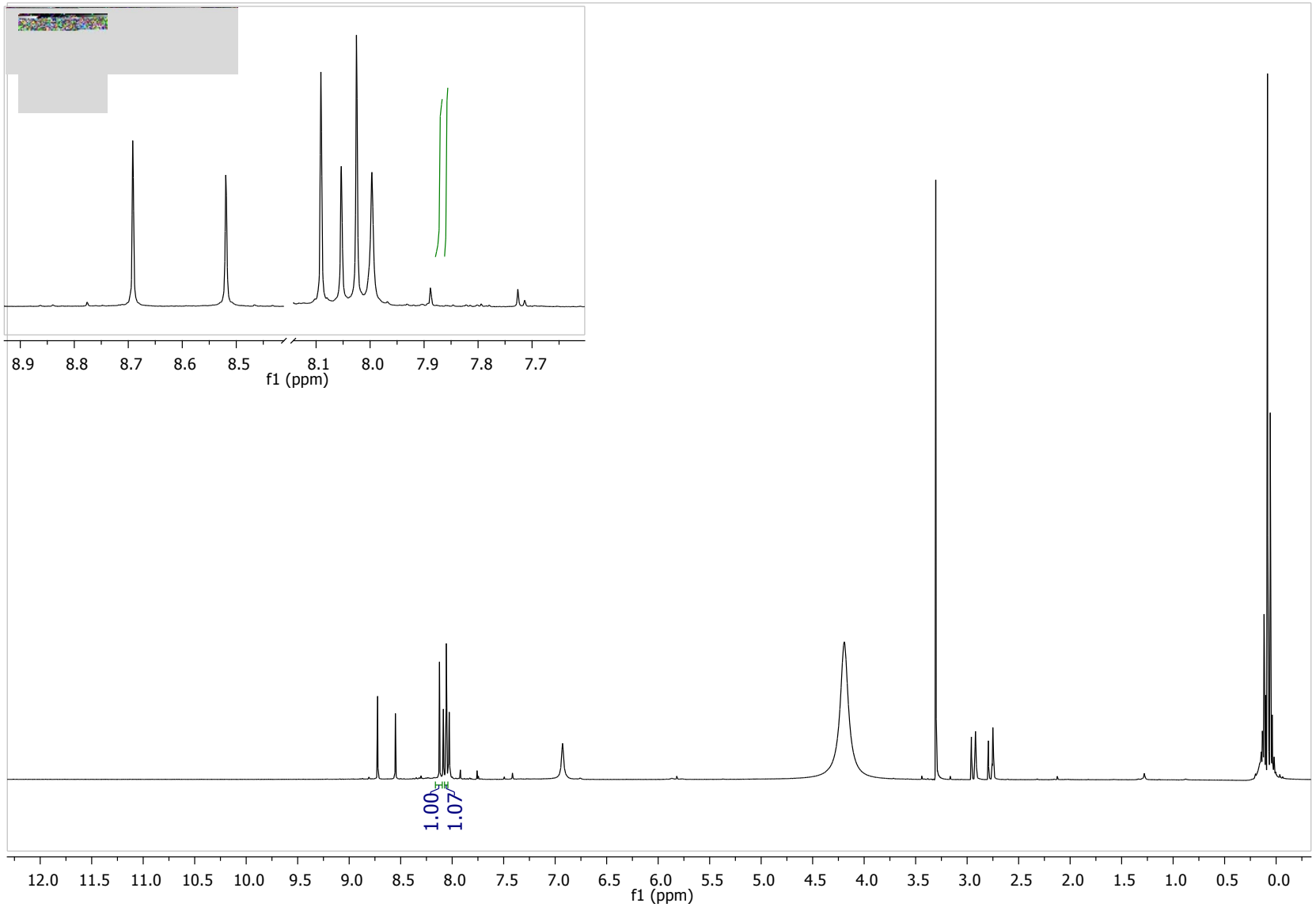
4j


COOMe
S
8.1
8.0
7.8
7.7
7.6
7.5
7.4
7.3
7.2
f1 (ppm)
2.14
1.26
2.18
2.17
3.00
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

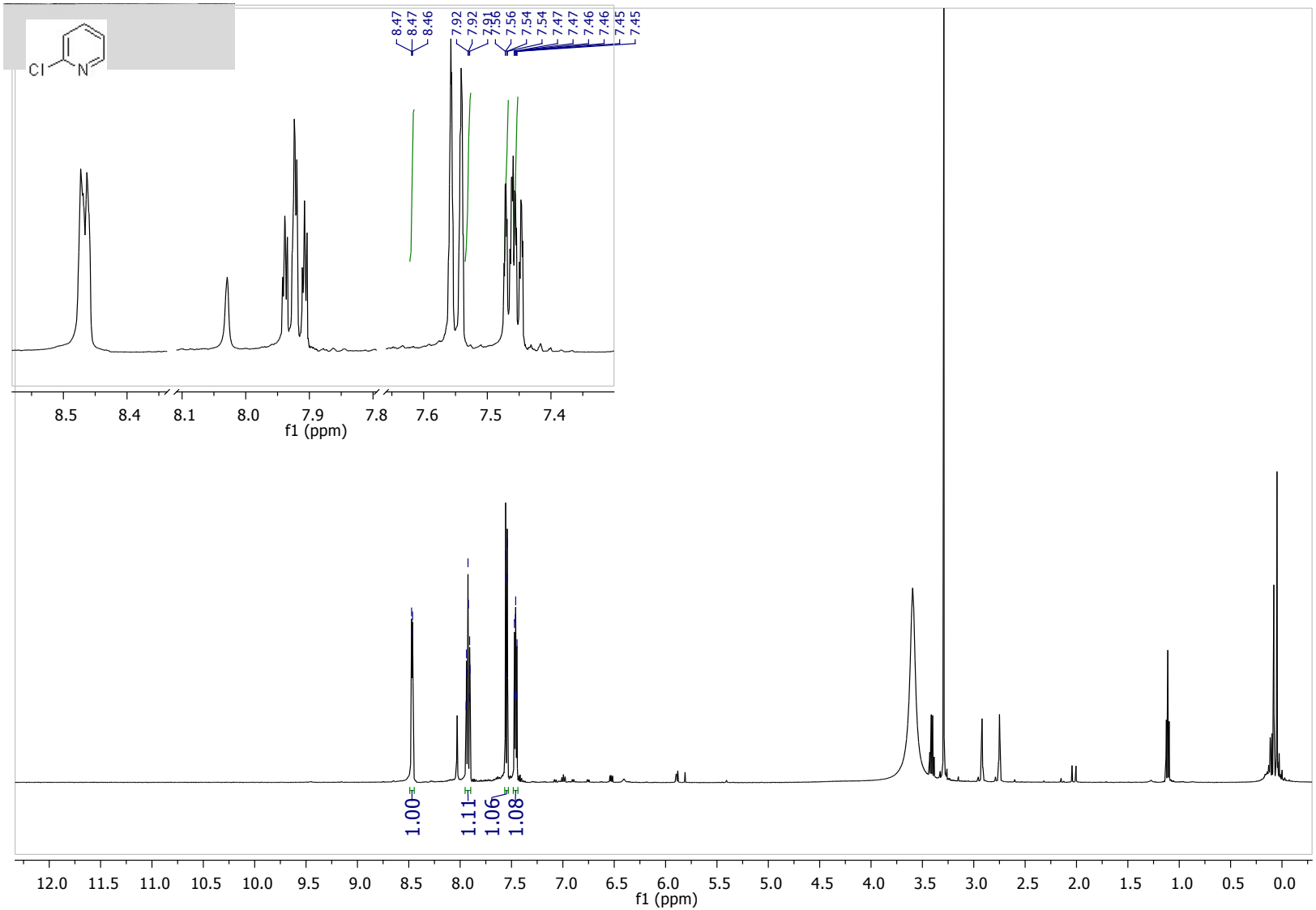
4k prepared from 3k in D_2O under room temperature



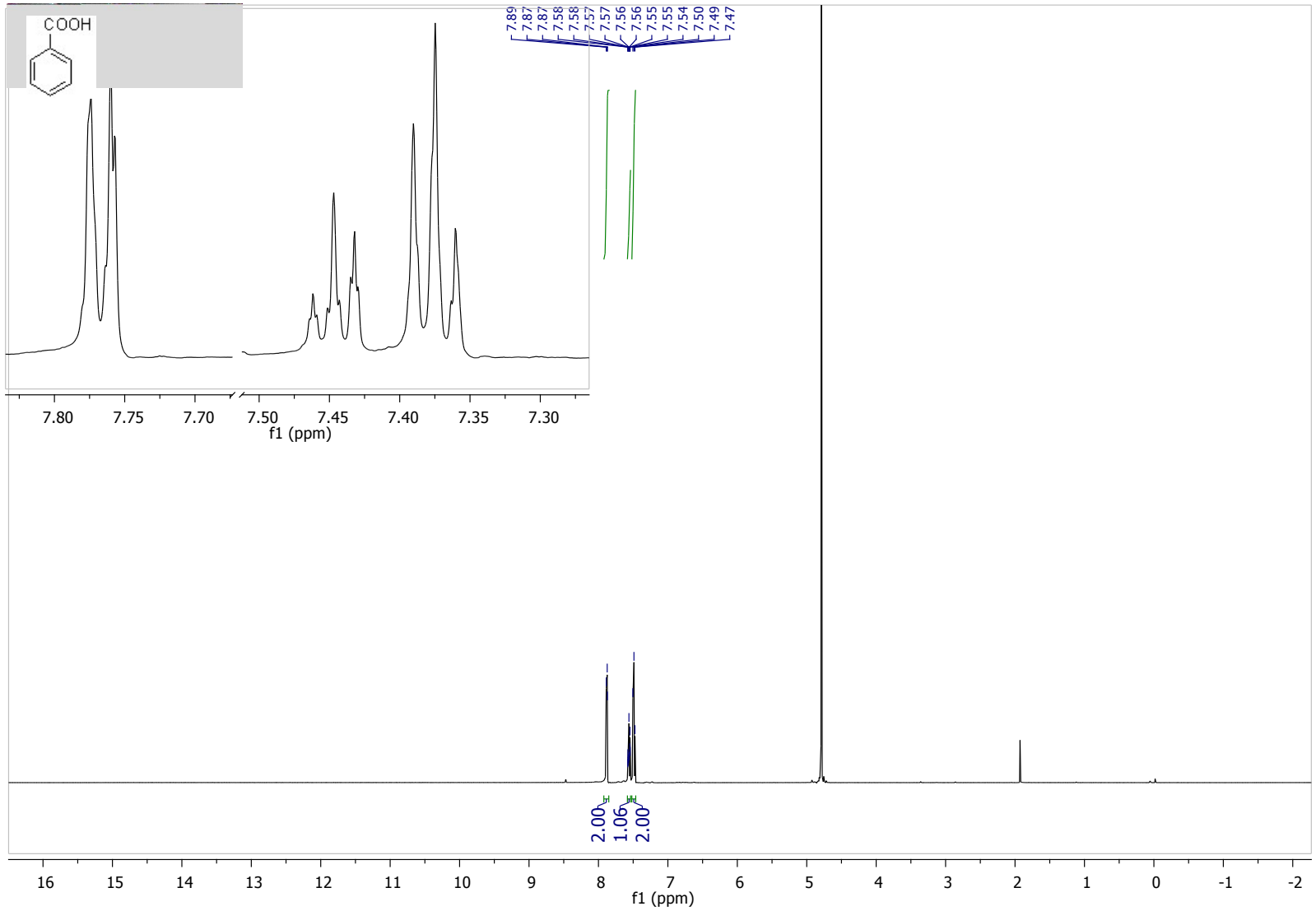
4k prepared from 3k in *d7*-DMF under heating



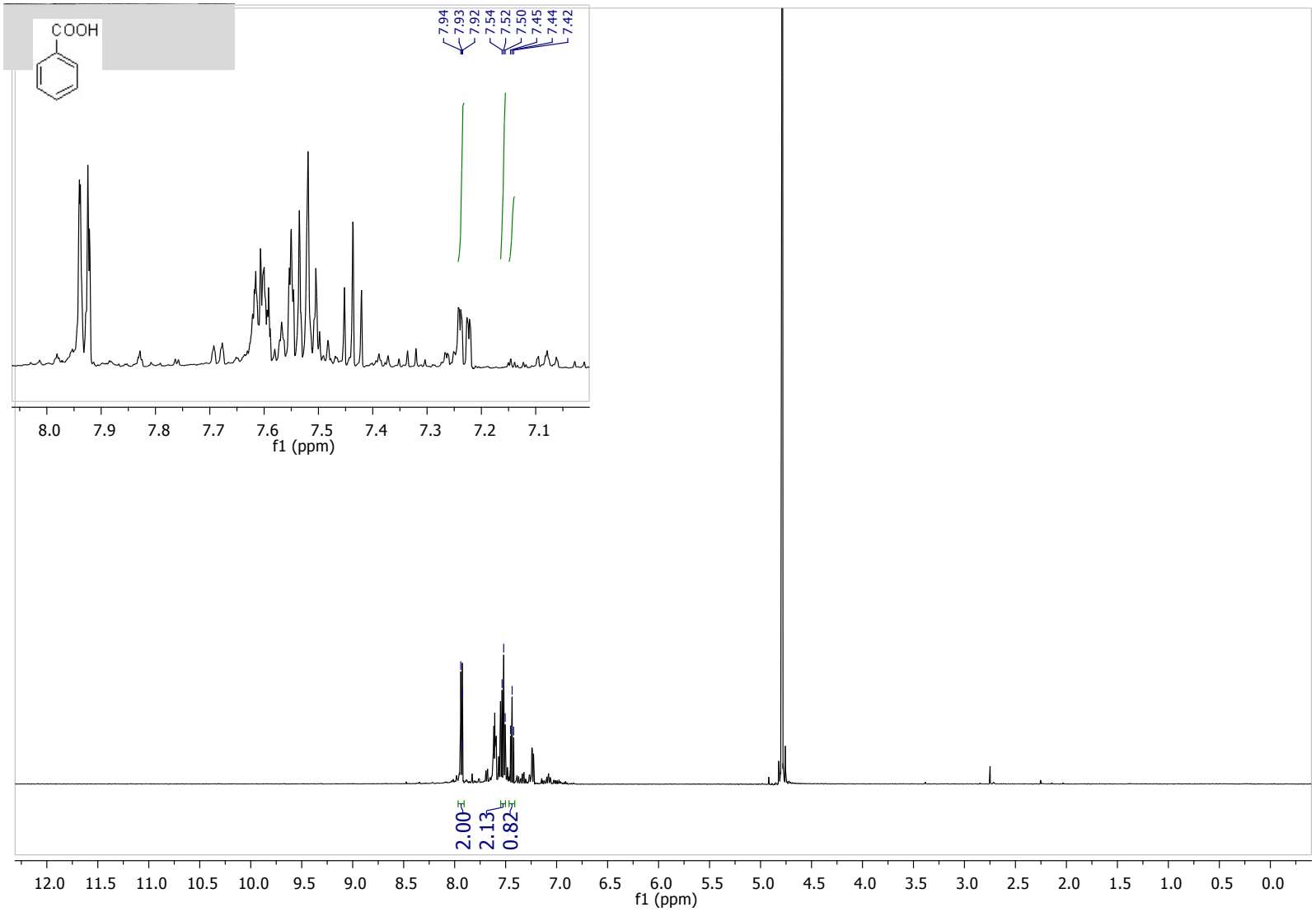
4l prepared from 3l under heating



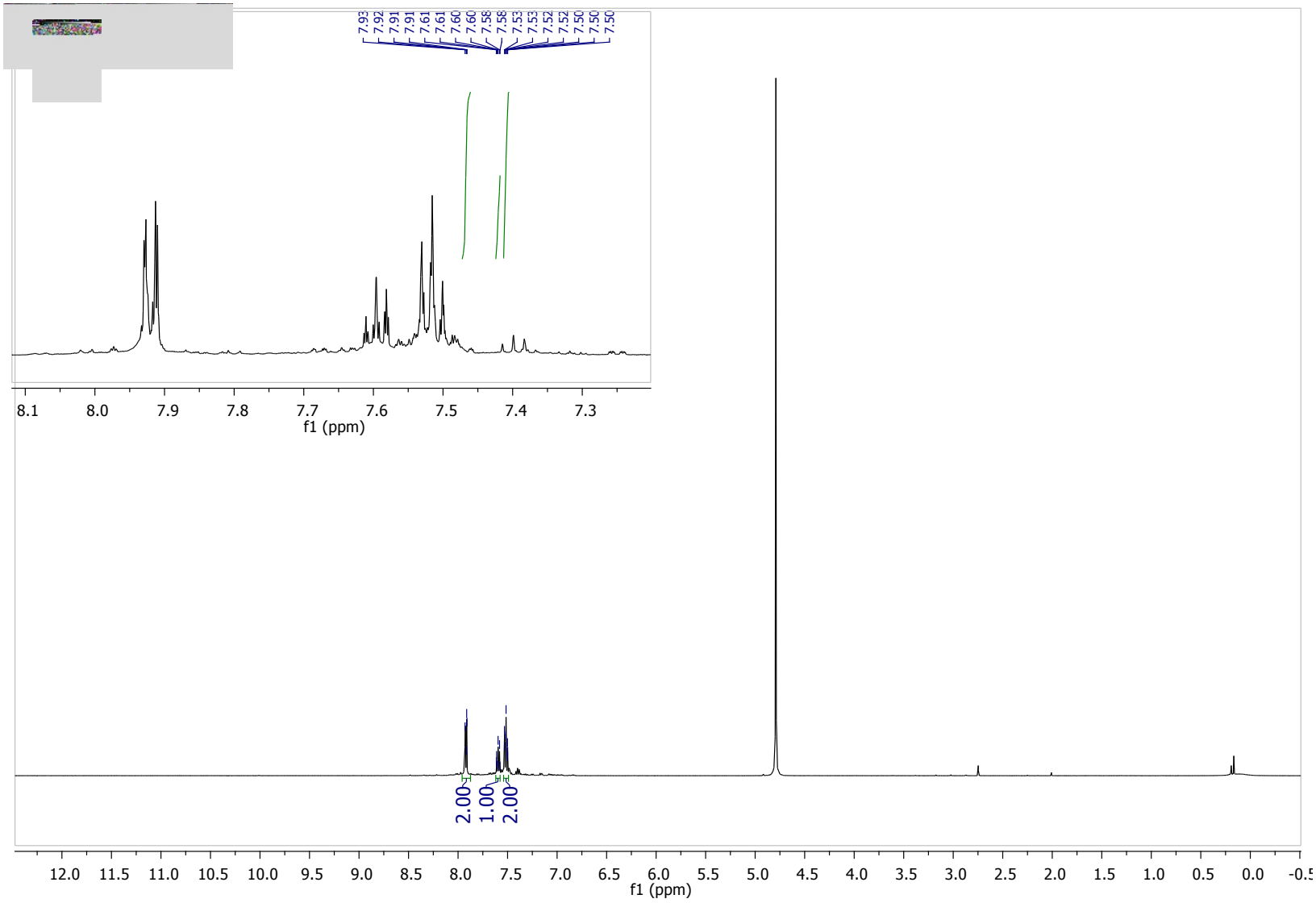
6a prepared from 5a



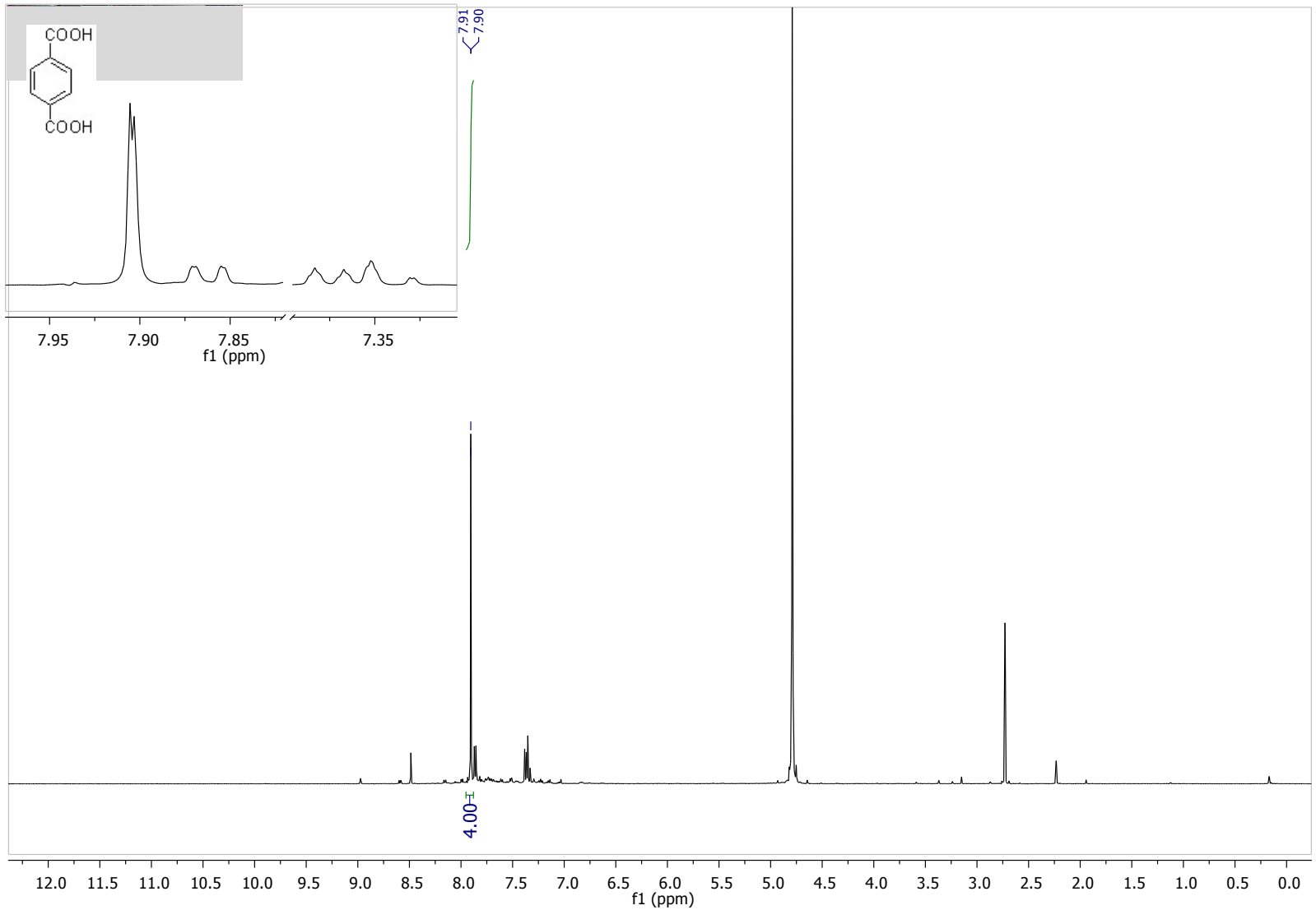
6a prepared from 5b



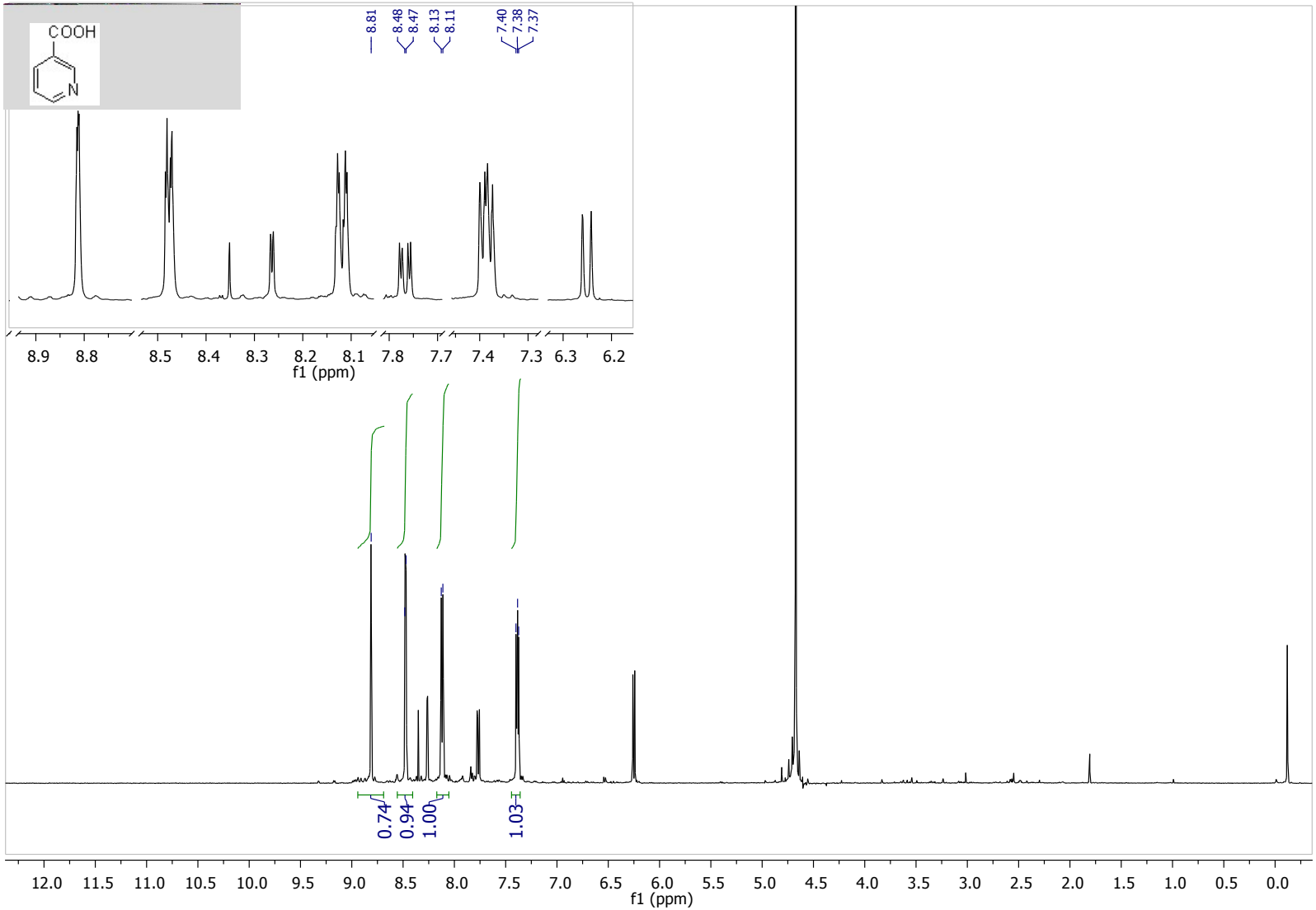
6a prepared from 5c



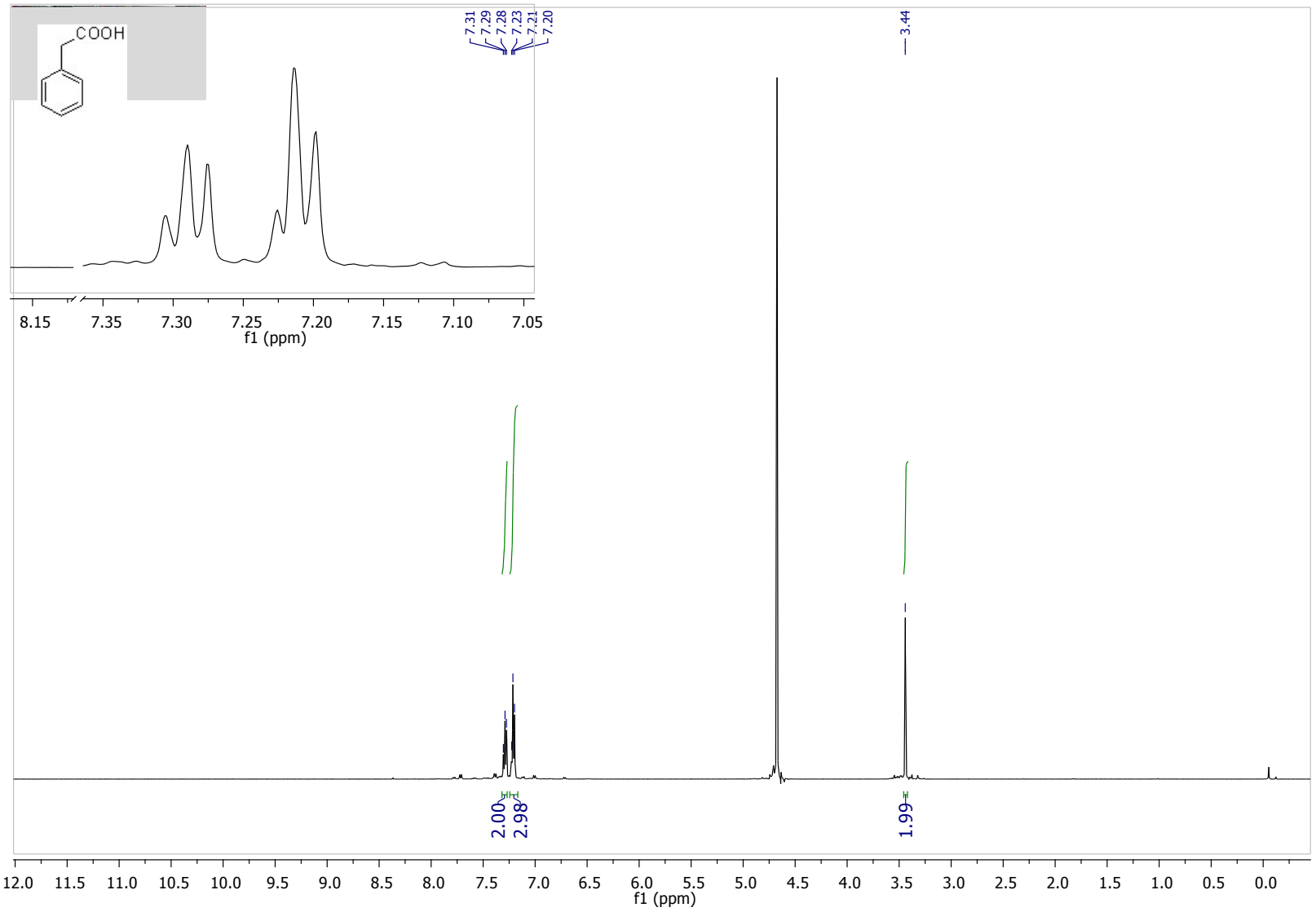
6d


COOH
COOH
7.91
7.90
7.95
7.90
7.85
7.35
f1 (ppm)
4.00
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

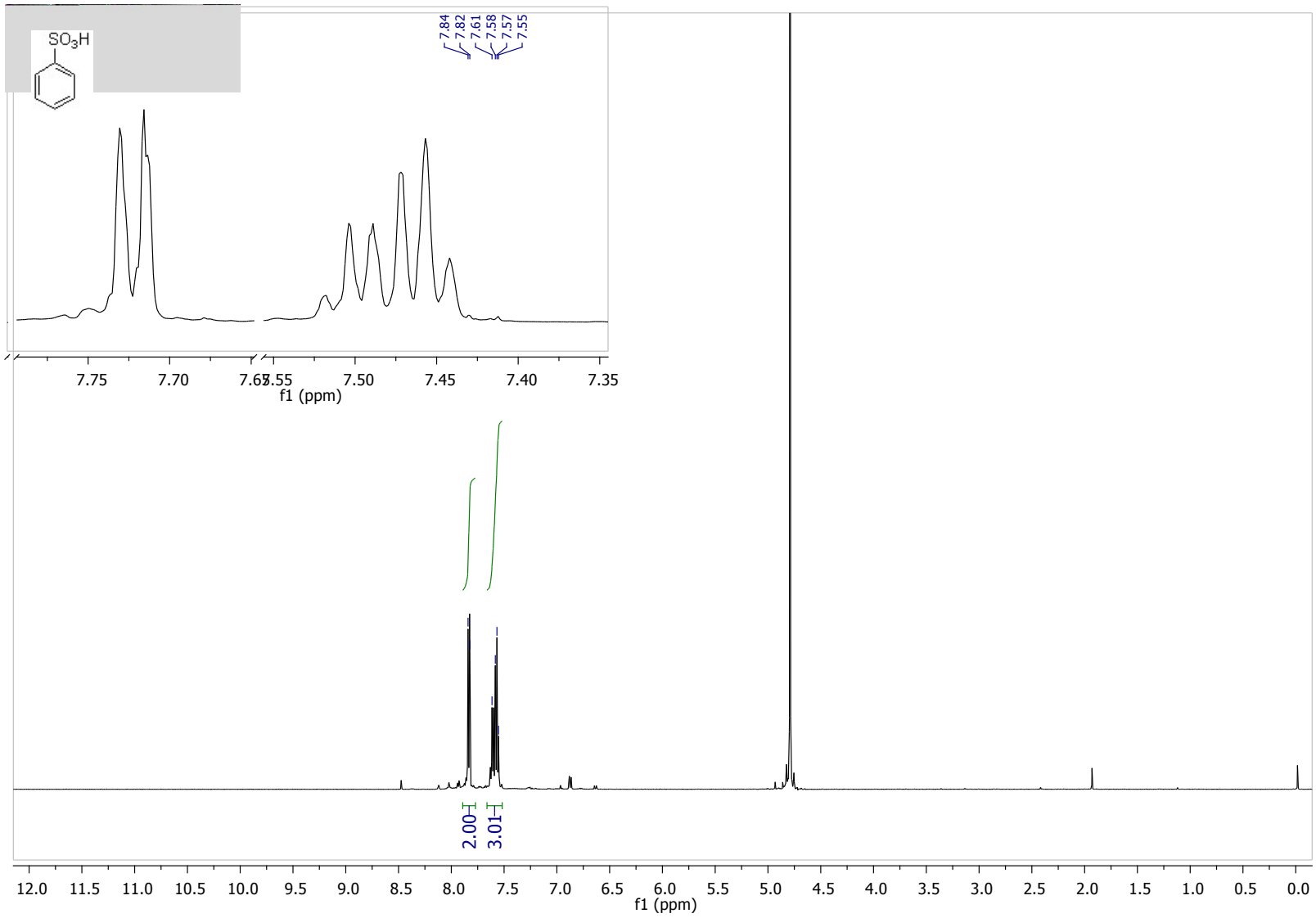
6e


COOH
N
8.81
8.48
8.47
8.13
8.11
7.40
7.38
7.37
f1 (ppm)
0.74
0.94
1.00
1.03
f1 (ppm)

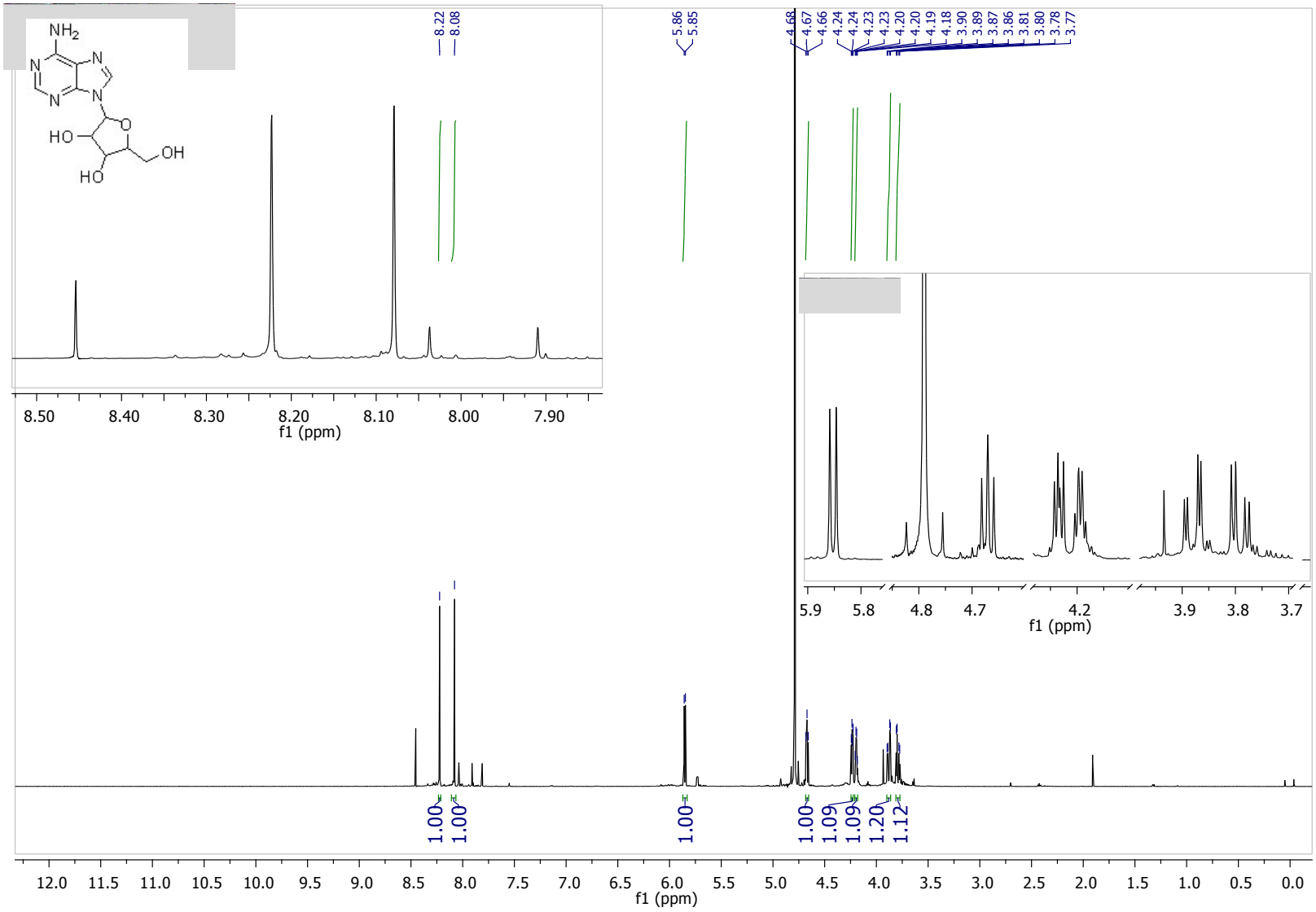
6f


COOH
7.31
7.29
7.28
7.23
7.21
7.20
3.44
8.15
7.35
7.30
7.25
7.20
7.15
7.10
7.05
f1 (ppm)
2.00
2.98
1.99
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

6g


SO3H
7.84
7.82
7.61
7.58
7.57
7.55
7.75
7.70
7.65
7.55
7.50
7.45
7.40
7.35
f1 (ppm)
2.00
3.01
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

6h prepared from 5h under heating



5. References

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