Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Ring-opening reactions for the solid-phase synthesis of nisin A lipopeptide analogues

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General methods for solid-phase peptide synthesis

Reaction vessel for manual peptide synthesis

For this procedure, a specially designed fritted reaction column was used. The column has a 10 - 100 mL volume with a 24/40 or 14/20 female ground glass joint for pushing solvent through with argon from the top of the vessel to aid filtration. The bottom of the vessel is a frit, after which the glass is tapered to a stopcock with a 3-way valve connected to an argon inlet for bubbling solvents and output tap for draining solvents. All solid-phase reactions were done in such vessels using the below general procedures, unless stated otherwise.

2-Chlorotrityl-chloride resin loading

A vial is first flame dried, then filled with dry dichloromethane (10 mL/g of resin). 2-Chlorotritylchloride resin (0.3 – 1.2 meq/g, 200 – 400 mesh, styrene-2% divinylbenzene) is added, and to the suspension an N⍰-Fmoc-amino acid with acid-labile side chain protected (2 mol eq/meq of resin) and DIPEA (5 mol eq/ meq resin) are added. The vial is sealed over argon and shaken for 3 h.

Reduced loading

To reduce the loading of the resin to 0.1 – 0.15 meq/g of resin, standard procedures below are followed to deprotect the Fmoc group, then the next amino acid is loaded in reduced quantity. To do this, in a separate vial is added the Fmoc-AA-OH (0.1 – 0.15 meq/g of resin), DMF (10 mL/g of resin), and DIPEA (2 mmol/mmol of amino acid), which is shaken and left to sit for 5 m. The solution is added to the resin and bubbled together with argon for 3 h, after which point the resin is washed with DMF (10 mL/g of resin for 3 × 30 s), then dichloromethane (10 mL/g of resin for 3 × 30 s).

Resin swelling

The resin is swollen by adding DMF (10 mL/g of resin) and for 15 m, bubbling argon through the suspension. After this time, the DMF is pushed through the reaction vessel with argon.

Deprotection of Fmoc

To the reaction vessel, 20% piperidine (10 mL/g of resin) is added, then bubbled with argon (3 × 5 m). Between each addition of 20% piperidine, the DMF (10 mL/g of resin) is flushed through the vessel without

bubbling (× 3). After the final 5 m period, DMF (10 mL/g of resin) is added and bubbled with argon (3 × 20 s).

Addition of amino acid to the growing peptide chain

In a vial, HATU (4.95 eq. relative to resin loading) and the Fmoc-AA-OH (5 eq. relative to resin loading are combined. To the same vial, DMF (10 mL/g of resin) is added, followed by DIPEA (10 eq. relative to resin loading). The vial is then shaken or sonicated until the solids are fully dissolved, then left to react in the vial for 5 – 10 m. After this time, the activated-ester-containing solution is added to the reaction vessel containing the resin. The reaction solution is then bubbled with argon for 1 h. After this time, the resin is rinsed with DMF (3 × 10 mL/g of resin) by bubbling each addition of DMF with argon for 20 s, then filtering it off before the next addition. At this point, either the "deprotection of Fmoc" step is then repeated, otherwise the peptide is removed from the resin (small scale test cleavage or complete deprotection) following the "cleavage of the peptide from the resin chlorotrityl" step. *Note that for specially synthesized amino acids such as orthogonally protected lanthionine (**18**/**19**), methyl lanthionine (**20**/**21**) and unsaturated residues (**6**/**7**) the ratio of amino acid:HATU:DIPEA were decreased to 2:1.99:4 relative to resin loading and the reaction time is extended to 3 h; repeated couplings were sometimes necessary.

Cleavage of the peptide from 2-chlorotrityl chloride resin

Before cleavage, the resin is bubbled with dry dichloromethane (10 mL/g of resin) for 3 × 20 s, filtering dichloromethane away after each 20 s interval. Then either procedure 1 or 2 can be followed depending on the goal of the cleavage.

1. <u>For a small scale test cleavage between amino acid additions</u> (used to ensure completion of the addition): a small amount of mostly dry resin (30 – 100 beads by estimate) is added to a small reaction vessel using a spatula, and to it 95:2.5:2.5 TFA:TIPS:H_2O (100 – 200 μL) is added. The vessel is then shaken for 5 m, after which time the solution is filtered through a pipette-cotton plug into an Eppendorf tube. The solvent of the resulting solution is evaporated using a gentle stream of argon. To the resulting oil, ice cold ether is added and the precipitate is collected on the side of the Eppendorf tube through centrifugation (30 s). The contaminated ether is either poured or pipetted carefully out of the Eppendorf tube. The resulting solid is then dissolved in 30% acetonitrile in H_2O with 0.1% TFA, and analyzed by LCMS (using vial inserts for small tests) or MALDI-TOF MS. Due to the small reaction time required for test cleavages some side-chain protecting groups (Boc, *t*Bu, Pbf) occasionally remain on the peptide, which should be considered when analyzing the MS data.

2. For the complete cleavage (and isolation) of the final peptide, the reaction vessel is flushed with argon for 15 m or until the resin becomes free flowing. Alternatively, the resin is placed under high-vacuum until it becomes a dry clumpy solid. After drying, the resin is weighed to obtain an accurate yield for the cleavage. In a vial, the peptide is cleaved by shaking the 2-chlorotrityl resin for 2 h with either 95:2.5:2.5 TFA:TIPS:H_2O (10 mL/g of resin) to remove all protecting groups or using 20% HFIP in dry dichloromethane (10 mL/g of resin) to maintain Boc side chain protection. The resulting solution is then filtered into a large vial using a series of cotton-pipette filters, avoiding taking up any of the resin (which leads to clogging), then rinsing the filters with dry dichloromethane. The resulting solution is then placed under reduced pressure to remove volatile components. If the resulting oil is too viscous to be transferred, a small amount of dichloromethane is added. To as many Eppendorf tubes as necessary, -78 ºC ether (1 mL) is added, then the concentrated dichloromethane-peptide solution is added dropwise to each, leading to peptide precipitation. The peptide is collected on the side of the Eppendorf tube through centrifugation (2 m). The contaminated ether is decanted or carefully pipetted off, and the peptide is washed further with -78 ºC ether (3×) by shaking/sonicating then centrifuging. The peptide is then dissolved in choice solvent (30% acetonitrile in H_2O with 0.1% TFA), then analyzed by LCMS or MALDI, and purified using HPLC.

Storing resin

When peptide coupling must cease for the day, the resin is bubbled with dry dichloromethane (10 mL/g of resin) for 3 × 20 s, filtering dichloromethane away after each 20 s interval. The resin-containing vessel is then flushed with argon for 15 m or placed on high vacuum and filled with argon after dryness is obtained. The peptide vessel is sealed with a septum, then placed in 4 ºC, away from volatile compounds

Removal of allyl/alloc protecting groups from resin-bound peptides

In a vial, a suspension of resin in DMF (10 mL/g of resin) is bubbled with argon. To the vial, $Pd(PPh_3)_4$ (2.0 eq.) and $PhSiH_3$ (10 eq.) is added. The reaction vessel is then sealed under argon, covered in aluminum foil, and shaken at room temperature for 2 h. The resin is then washed with dry dichloromethane (5 × 10 mL/g of resin for 20 s), DMF (5 × 10 mL/g of resin for 20 s), then 0.5% sodium diethyldithiocarbamate in DMF (3 × 10 mL/g of resin for 20 s), and then again with dichloromethane (3 × 10 mL/g of resin for 20 s) and DMF (3 × 10 mL/g of resin for 20 s).

Note: occasionally, after the washing step with 0.5% sodium diethyldithiocarbamate for the allyl/alloc deprotection, the reaction vessel frit would become partially clogged. Allowing the vessel to sit overnight in a kiln at 500 ºC, followed by an HF soak for 1 h was effective at unclogging the frit.

Macrocyclization of resin-bound peptides

The Fmoc group is first removed using 20% piperidine as previously described. The peptide is then cyclized by adding a solution of PyAOP (5 eq.), HOBt (5 eq.) and DIPEA (10 eq.) in DMF (10 mL/g of resin) and bubbled with argon for 3 h. The resin is then washed with DMF (3 × 10 mL/g of resin for 20 s), dichloromethane (3 × 10 mL/g of resin for 20 s) and the cyclization checked by MALDI. Deprotection and cyclization are repeated as necessary until completion.

On-resin conversion of Cys/MeCys (SSMe or SStBu) to Dha/Dhb

In a small SPPS reaction vessel, the resin is suspended in 9:1, THF:H_2O (30 mL/g resin) and through the solution argon is bubbled for 15 m. After this time, thiophenol is added (50 eq.), followed by tributylphosphine (1.0 M in THF) (20 eq.). The reaction vessel is sealed under argon and shaken for 18 h, after which time the reducing solution is pushed out of the reaction vessel with argon and the resin washed with DMF (3 × 10 mL/g of resin for 20 s), then dichloromethane (3 × 10 mL/g of resin for 20 s). A test cleavage is then done to determine completion of the reaction. To the vessel, 50 mM ascorbic acid in DMF (minimal, but enough to allow resin stay swollen during shaking) is added and argon is bubble through for 15 m. After this time, 2,5-dibromovalerate (4 eq.) and DIPEA (5 eq.) are added, the reaction vessel is sealed under argon and subsequently shaken for 96 h. The resin is then washed with DMF (3 × 10 mL/g of resin for 20 s), then dichloromethane (3 × 10 mL/g of resin for 20 s), and a test cleavage is done to determine completion of the reaction.

Synthetic SPPS lipopeptide synthesis general procedure (1, 2 and 3)

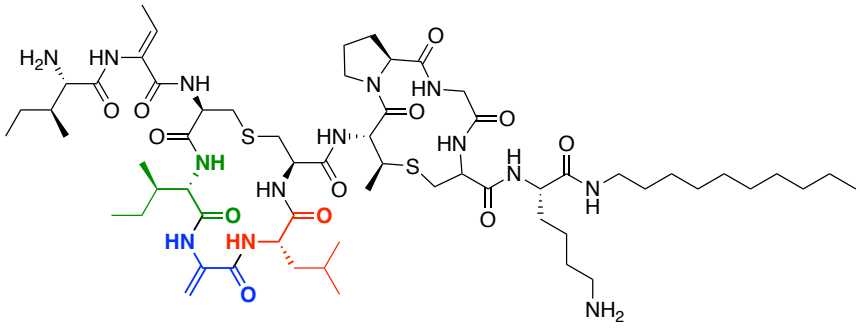
The peptides were synthesized on solid phase according to the general procedures listed above. The macrocycles were installed by coupling orthogonally protected methyllanthionine **20**/**21** and lanthionine **18**/**19** at position 11 and 7 respectively, and after coupling the subsequent amino acids in each cycle, deprotection and cyclization were done according to the general procedures. After the completion of bicyclic chain, on-resin conversion of Cys/MeCys (SSMe or SS*t*Bu) to Dha/Dhb was performed as per the general procedures. The resulting resin was cleaved with 20% HFIP in dichloromethane for 2 h and worked-up according to the general procedures to obtain a crude white solid that was purified by HPLC.

The protected peptides (prior to coupling of the lipid tail) were purified on a C8 column (Vydac 208TP1010) with a flow rate of 5 or 10 mL/min. Detection was done at 220 and 280 nm. The gradient began with 50% MeCN in H_2O (0.1% TFA) for the first 5 m. The gradient then ramped up to 75% MeCN over 35 m. The final ramp up to 90% MeCN occurred over 0.2 m, where it was for 5 m. The gradient ramped down to 50% MeCN over 1 m and was held there for 5 m.

The peptides were transformed into lipopeptides **1**, **2** and **3** using a procedure modified from the work of Nathaniel Martin and coworkers.[4] The protected peptide was dissolved in DMF (10 µL/ mg of peptide) in a small glass reaction vessel with a conical bottom, and to it PyBOP (1 eq.) and DIPEA (2 eq.) were added. After 15 m, decylamine (1.1 eq.) was added and the reaction was left stationary for 3 h. After this time 10% AcOH in H_2O (100 µL/ mg of peptide) was added and the mixture was extracted with chloroform (4 × 100 µL/ mg of peptide). The combined organic fractions were subjected to reduced pressure evaporation to provide a crude oil or solid that was dissolved in pure TFA (10 µL/ mg of peptide) for 30 m before evaporation of TFA.

The lipopeptide analogues **1**, **2** and **3** were purified on a C8 column (Vydac 208TP1010) with a flow rate of 5 or 10 mL/min. Detection was done at 220 and 280 nm. The gradient began with 40% MeCN in H_2O (0.1% TFA) for the first 5 m. The gradient then ramped up to 90% MeCN over 40 m, where it was for 5 m. The gradient ramped down to 40% MeCN over 1 m and was held there for 5 m.

Nisin lipopeptide 1



1

The *C*-terminal amino acid peptide was synthesized using the methods described in the section on peptide synthesis and purified using the method described in the section on purification (R_t = 33.9 m, 90% based on resin loading); **HRMS** (MALDI-TOF) Calc'd for $C_{61}H_{99}N_{13}O_{17}S_2$ $[M+H]^+$ 1350.6796; found 1350.6815. The peptide **1** was synthesized using the methods described in the section on peptide synthesis and purified using the method described in the section on purification. (R_t = 33.9 m, 85%). **HRMS** (MALDI-TOF) Calc'd for $C_{61}H_{104}N_{14}O_{12}S_2$ $[M+2H]^{+2}$ 645.3773, found 645.3704.

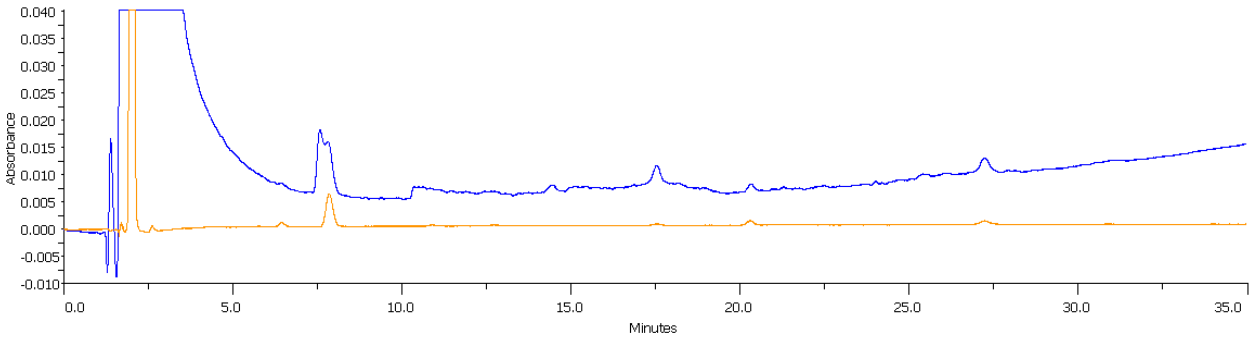


Figure 1 – HPLC trace for lipopeptide **1**

Table 1 – Synthetic lipopeptide (**1**) ^{1}H-NMR assigned with the aid of TOCSY

	Chemical shifts / ppm					
Residue	**NH**	**Hα**	**Hβ**	**Hγ**	**Hδ**	**Other**
Ile[1]	-	4.03	2.04	1.24, 1.60	1.0	-
Dhb[2]	9.87	-	6.62	1.78		-
Lan1[3*]	7.63	4.51	3.30, 2.86	-	-	-
Ile[4]	8.14	4.15	1.95	1.40	1.10, 0.83	-
Dha[5]	8.51	-	6.20, 5.40	-	-	-
Leu[6]	8.63	3.98	1.72	1.59	0.89	-
Lan1[7*]	7.83	4.70	2.74, 3.10	–	-	-
MeLan1[8*]	8.31	4.94	3.48	1.22	-	-
Pro[9]	-	4.26	2.30	1.80	2.00	-
Gly[10]	8.43	3.59, 4.23	-	-	-	-
MeLan1[11*]	7.96	3.79	3.03, 3.50	-	-	-
Lys[12]	7.72	4.24	1.80	1.38	1.60	2.90 (Hε)
Lipid-tail	7.14	3.10	1.43	1.25 $(CH_2)_n$	-	0.73 (CH_3)

Values given for the protons of each amino acid are given in ppm

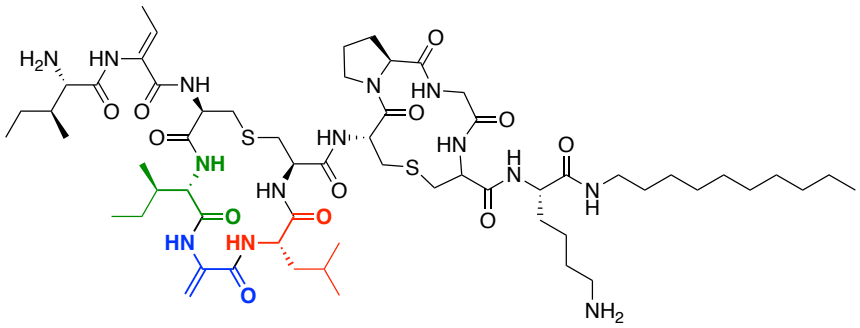
*Lan1 and MeLan1 denote two halves of lanthionine and methyllanthionine residues

Table 2 – Synthetic lipopeptide (**1**) ROESY Data

Amino acid	Amide NH of AA / ppm	ROE detected
Ile^{1}	4.03 (Hα)*	1.00 (Ile^{1} Hδ), 2.04 (Ile^{1} Hβ), 3.09 (Lan1^{7} Hβ)
Dhb^{2}	9.87	4.04 (Ile$_{1}$), 7.63 (Lan1^{3} NH)
Lan1^{3*}	7.63	2.84 (Lan1^{3} Hβ), 4.47 (Lan1^{3} Hα), **6.60 (Dhb^{2} Hβ)**
Ile^{4}	8.14	1.10 (Ile^{4} Hδ), 1.41 (Ile^{4} Hγ), 2.91 (Lan^{3} Hβ)
Dha^{5}	8.51	8.14 (Ile^{4} NH), 4.11 (Ile^{4} Hα), 1.96 (Ile^{4} Hβ)
Leu^{6}	8.63	3.98 (Leu^{6} Hβ), 5.4 (Dha^{5} Hβ)
Lan1^{7*}	7.83	3.98 (Leu^{6} Hβ), 4.70 (Lan^{7} Hα)
MeLan1^{8*}	8.31	1.24 (MeLan1^{8} Hγ), 3.50 (MeLan1^{11} Hβ), 4.68 (Lan1^{7} Hα)
Pro^{9}	4.26 (Hα)*	1.73 (Leu^{6} Hβ), 3.56 (Gly^{10} Hα)
Gly^{10}	8.43	3.54 (Gly^{10} Hα), 4.24 (Pro^{9} Hα)
MeLan1^{11*}	7.96	3.08 (Lan1^{7} Hβ), 3.47 (MeLan1^{11} Hβ), 3.81 (MeLan1^{11} Hα, 4.16 (Gly^{10} Hα, 4.30 (Lys^{12} Hα)
Lys^{12}	7.72	1.33 (Lys^{12} Hγ), 1.61 (Lys^{12} Hδ), 3.79 (MeLan1^{11} Hα), 4.24 (Lys^{12} Hα)
Lipid-tail	7.14	1.40 (Lipid-tail Hβ), 4.24 (Lys^{12} Hα)

Hα was used for Ile^{1} and Pro^{9} as there is no NH signal on the spectra or they have no NH, respectively. Bold indicates ROE used to determine *Z*-Dhb stereochemistry and is identical to that reported in previous literature.[5]

Nisin lipopeptide 2



2

The *C*-terminal carboxylic acid peptide was synthesized using the methods described in the section on peptide synthesis and purified using the method described in the section on purification. HRMS (MALDI-TOF) Calc'd for $C_{60}H_{97}N_{13}O_{17}S_2$ $[M+H]^+$ 1335.6567, found 1335.6565. The lipopeptide 2 was synthesized using the methods described in the section on peptide synthesis and purified using the method described in the section on purification. (R_t = 32.5 m, 90%). HRMS (MALDI-TOF) Calc'd for $C_{60}H_{102}N_{14}O_{12}S_2$ $[M+2H]^{+2}$ 638.3694, found 638.3691.

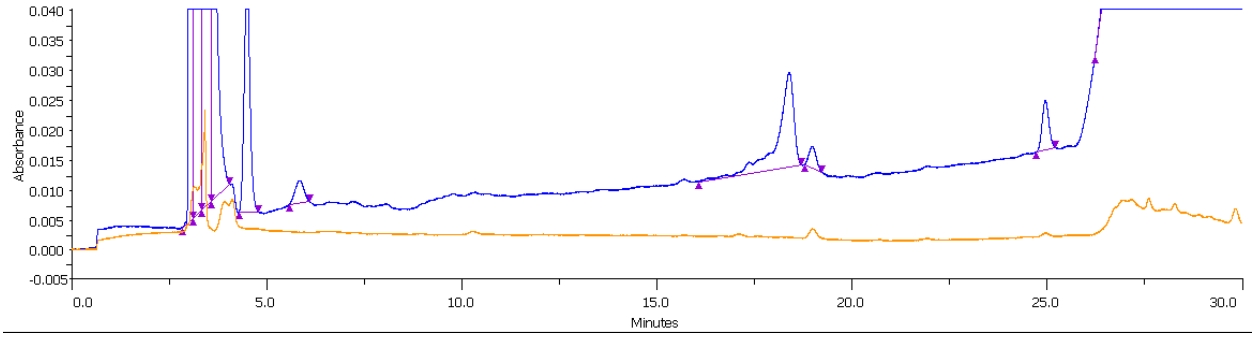
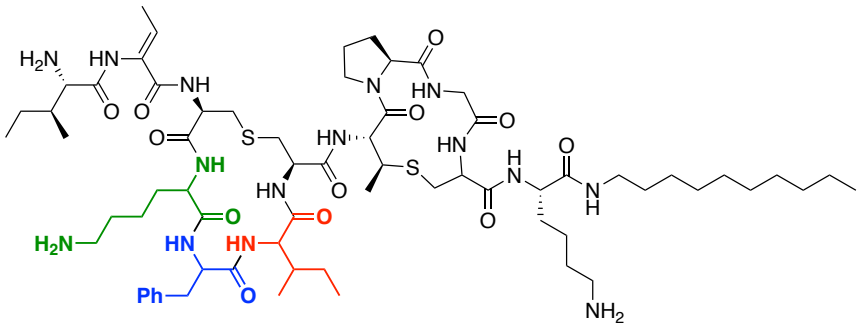


Figure 2 – HPLC trace for lipopeptide **2**

Nisin lipopeptide 3



3

The C-terminal carboxylic acid peptide was synthesized using the methods described in the section on peptide synthesis and purified using the method described in the section on purification. HRMS (MALDI-TOF) Calc'd for $C_{72}H_{114}N_{14}O_{19}S_2$ $[M+H]^+$ 1542.7826, found 1542.7831. The lipopeptide 3 was synthesized using the methods described in the section on peptide synthesis and purified using the method described in the section on purification. (R_t = 23.4 m, 87%). HRMS (MALDI-TOF) Calc'd for $C_{132}H_{189}N_{35}O_{38}S_4$ $[M+Na]^+$ 1404.7870, found 1404.7882.

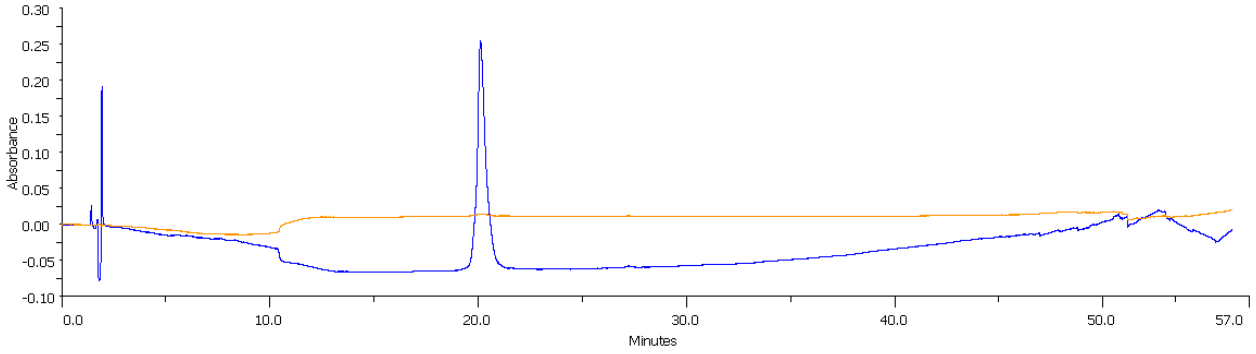


Figure 3 – HPLC trace for lipopeptide **3**

Semi-synthetic lipopeptide synthesis (1*)

The procedure for isolation of nisin, cleavage of nisin, and purification of the A and B rings was done using a method developed by Slootweg *et al*.[6] The semi-synthetic nisin A and B ring lipopeptide **1*** was synthesized using a method based on the work of Nathaniel Martin and coworkers.[4]

Isolation of nisin: A commercially available crude nisin extract (20 g) was dissolved in H_2O (500 mL) and was stirred vigorously for 15 m at which point the solution was separated into 20 conical tubes (~ 20 mL each). To each tube, dichloromethane (15 mL) was added; the tubes were vortexed for 15 s; then they were centrifuged (15 m at 1000 × g). Both the H_2O and dichloromethane were decanted to leave a brown pellet that was redissolved in H_2O (100 mL), filtered through celite, which was further rinsed with H_2O (100 mL). The H_2O was lyophilized to provide a crude off-white powder that was purified by preparative RP-HPLC (C18, R_t = 21.4 m). Pure nisin was obtained as a fluffy white powder (14 mg, 4.18 mmol, 7%).

Cleavage and purification of the A and B rings: Pure nisin (10 mg) was dissolved in Tris buffer (4 mL, 25 mM NaOAc, 5 mM Tris–HCl, 5 mM $CaCl_2$, pH 6), and to it trypsin (1 mg) was added. The reaction was shaken at 30 ºC for 12 h, at which point more trypsin was added (2 mg). The reaction was shaken for a further 16 h at which point no more starting material was observed by MALDI-TOF-MS. The material was frozen and lyophilized to provide a white powder that was purified by semi-preparative RP-HPLC (C8, R_t = 25 m). The A and B rings (amino acids 1 – 12 of nisin) were isolated as a white powder (3.0 mg, 2.07 mmol, 69%).

Coupling of the lipid tail: The A and B rings (3.0 mg) were dissolved in DMF (100 μL/mg), and to them a solution of PyBOP (1.1 eq.), DIPEA (2 eq.), and decylamine (50 eq.) was added. The reaction sat at room temperature for 20 m before being diluted with 30% acetonitrile in H_2O with 0.1% TFA to 500 μL total. The reaction was centrifuged and injected onto a semi-preparative HPLC.

Purification: The lipopeptide analogue **1*** was purified on a C8 column (Vydac 208TP1010) with a flow rate of 5 or 10 mL/min. Detection was done at 220 and 280 nm. The gradient began with 40% MeCN in H_2O (0.1% TFA) for the first 5 m. The gradient then ramped up to 90% MeCN over 40 m, where it was for 5 m. The gradient ramped down to 40% MeCN over 1 m and was held there for 5 m.

Antimicrobial activity assays

Growth of bacterial strains

Bacillus subtilis JH642 and *Staphylococcus aureus* ATCC 6538 were grown in Tryptic Soy Broth (TSB) at 37 °C, and *Lactococcus lactis* IL1403 was grown in All Purpose Tween 80 (APT) broth at 30 °C.

Spot-on-lawn assays

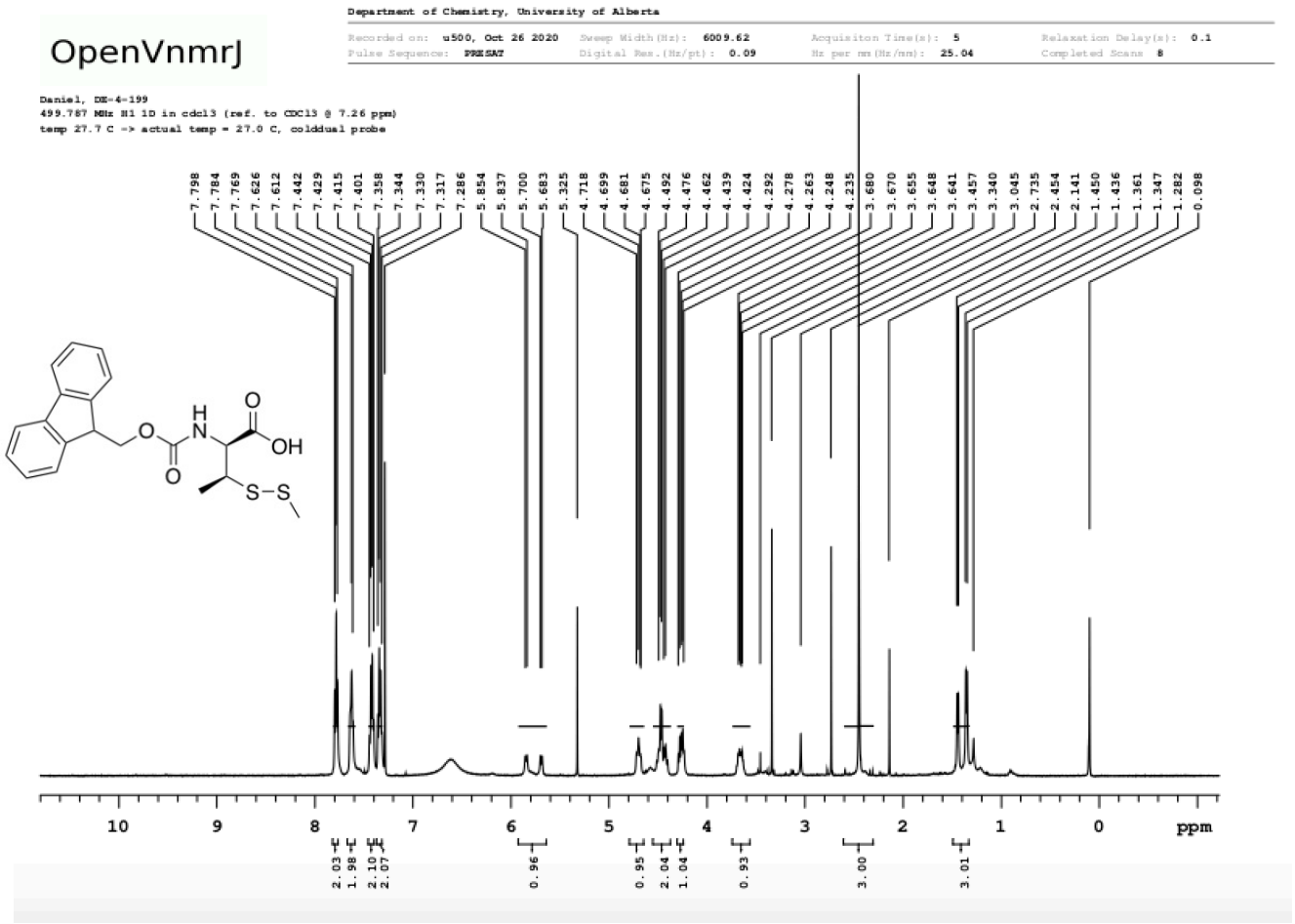
Spot-on-lawn assays were performed to determine the antibacterial activity of the various lipopeptide analogs. Overnight cultures of bacterial indicator strains were used to inoculate 5 mL of soft agar (0.75% agar) containing the appropriate medium and poured onto hard agar media (1.5% agar) plates. Compounds to be tested for inhibitory activity were dissolved in MilliQ H_2O with DMSO (1%) and various concentrations were made by series of two-fold dilution of the peptide stock solutions in MilliQ H_2O with DMSO (1%). An aliquot of 10 µL from each concentration was spotted onto the plates and after drying, the plates were incubated overnight at the appropriate temperature. Minimum inhibitory concentration (MIC) was determined from the duplicate experiments based on the growth inhibition observed after 12 h at the locations where the compound solutions were spotted.

Microdilution assays

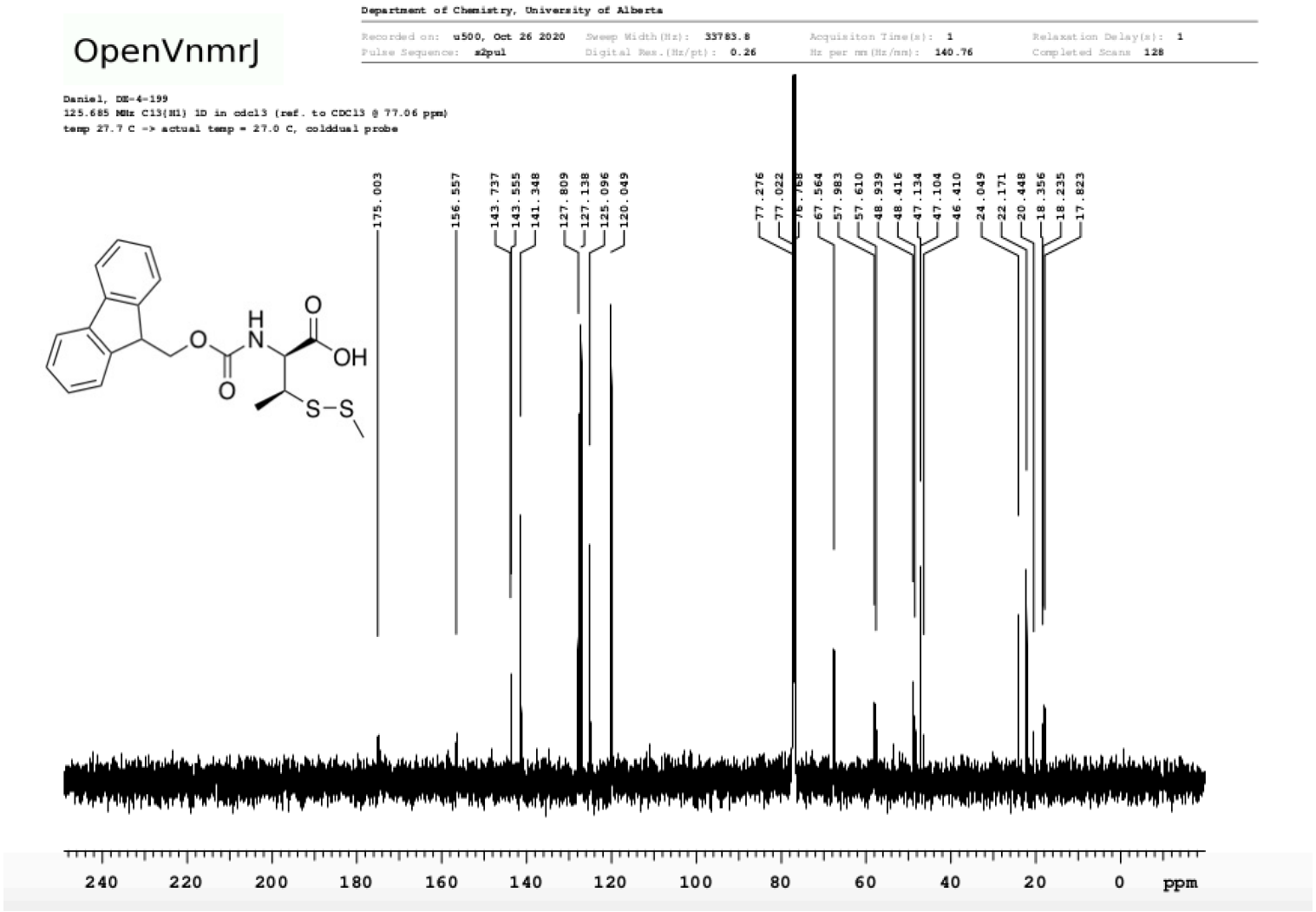
MICs of the lipopeptide compounds were also determined using a broth microdilution assay. The lipopeptides were dissolved in 10% DMSO in MilliQ H_2O, then two-fold dilution series of these peptides were made in MilliQ H_2O. *L. lactis* was grown in APT broth overnight and then diluted 1000-fold in APT to achieve a concentration of approximately 2×10^5 cells/90 µL of APT broth. The wells of a 96-well polypropylene microtiter plate were filled with 90 µL of the inoculated broth solutions, followed by 10 µL of the appropriate lipopeptide dilution at decreasing concentrations across the plate. Nisin Z was used as a positive control in all experiments. As a negative control, broth with 10 µL of MilliQ H_2O was used. The plate was incubated 20 h at 30 ºC. During incubation absorbance readings (600 nm) were taken every 10 min. on a SpectraMax i3x Plate Reader (Molecular Devices, Sunnyvale, CA). The concentration of compound was deemed effective if it completely inhibited growth in the well for 20 h and MICs were recorded as the lowest concentration of peptide to do so.

Compound Spectra

7 – ^{1}H-NMR



7 – ^{13}C-NMR


OpenVnmrJ
Department of Chemistry, University of Alberta
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57.983
57.610
48.939
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47.134
47.104
46.410
24.049
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18.235
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O
H
N
O
OH
O
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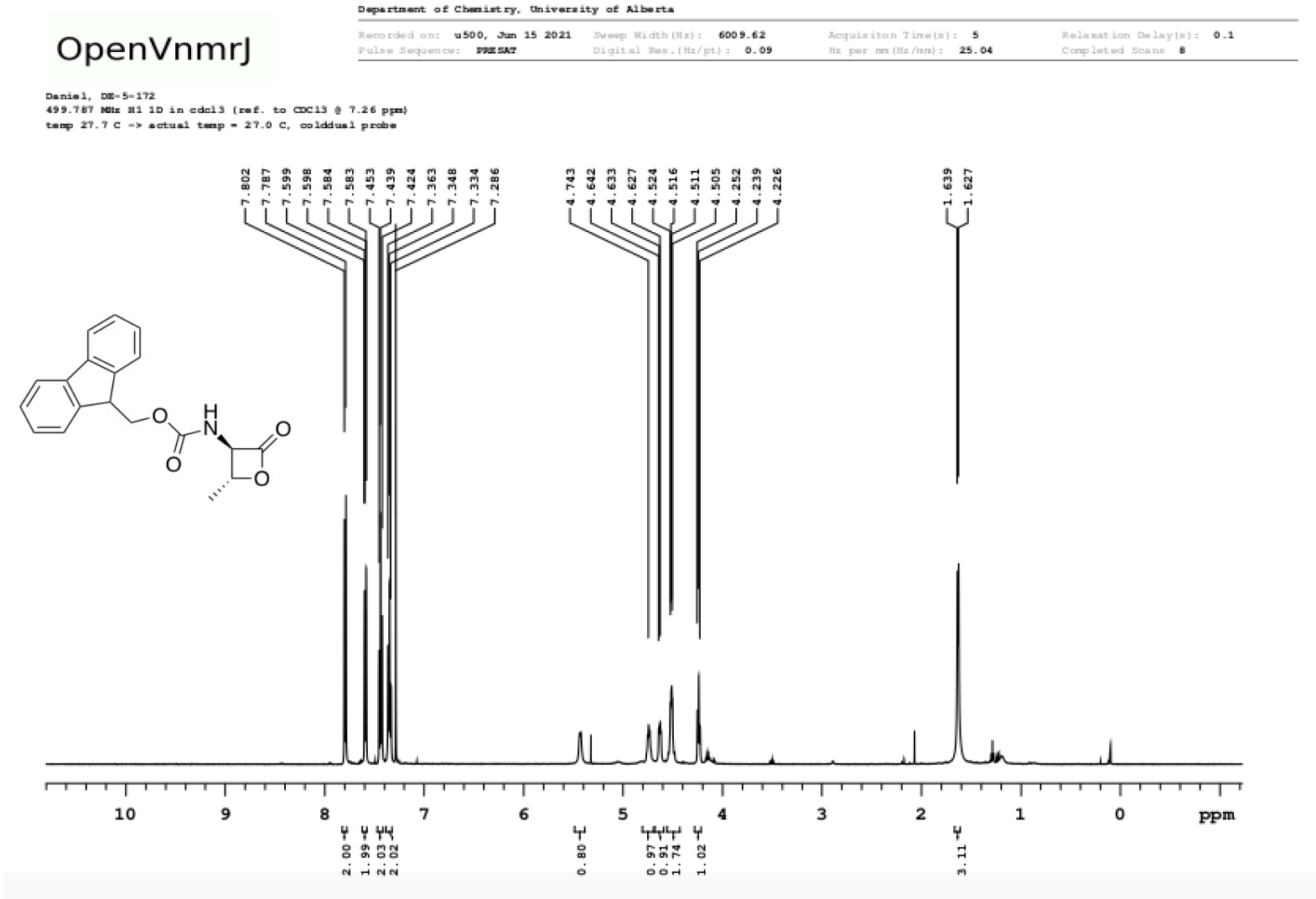
ppm

8 – ^{1}H-NMR


OpenVnmrJ
Department of Chemistry, University of Alberta
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Daniel, DE-5-172
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7.787
7.599
7.598
7.584
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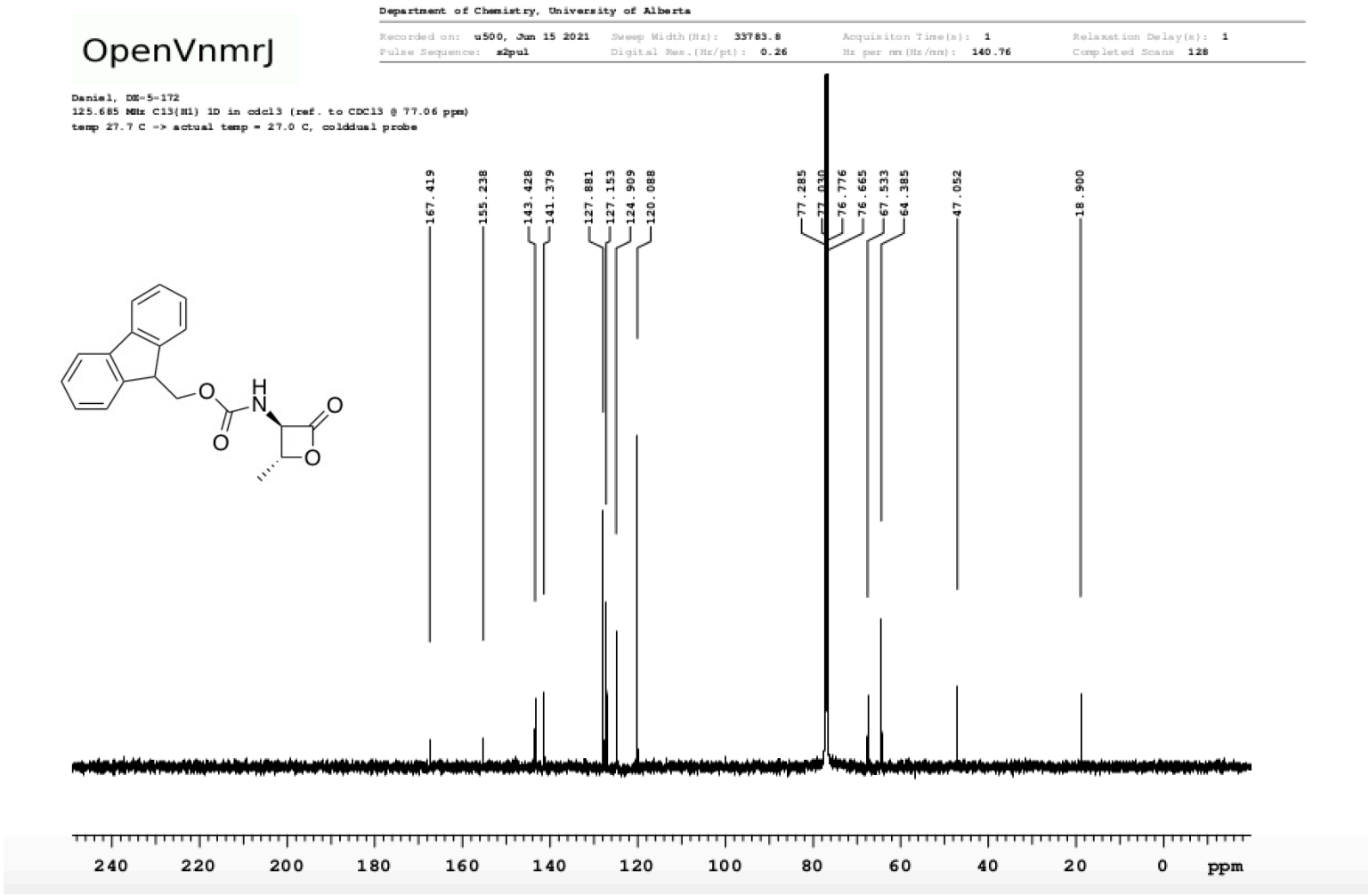
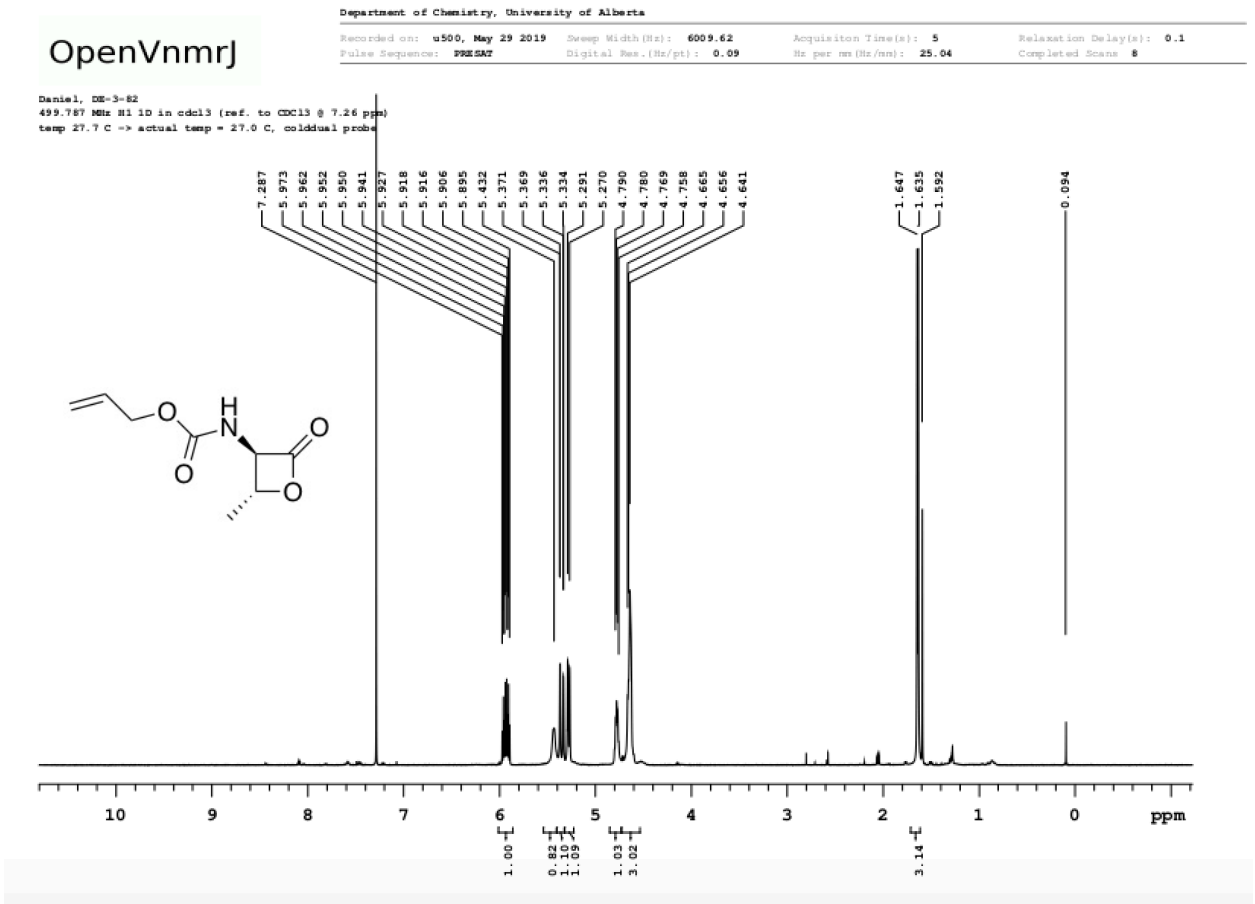
4.511
4.505
4.252
4.239
4.226
1.639
1.627
10
9
8
7
6
5
4
3
2
1
0
ppm
2.00
1.99
2.03
2.02
0.80
0.97
0.91
1.74
1.02
3.11

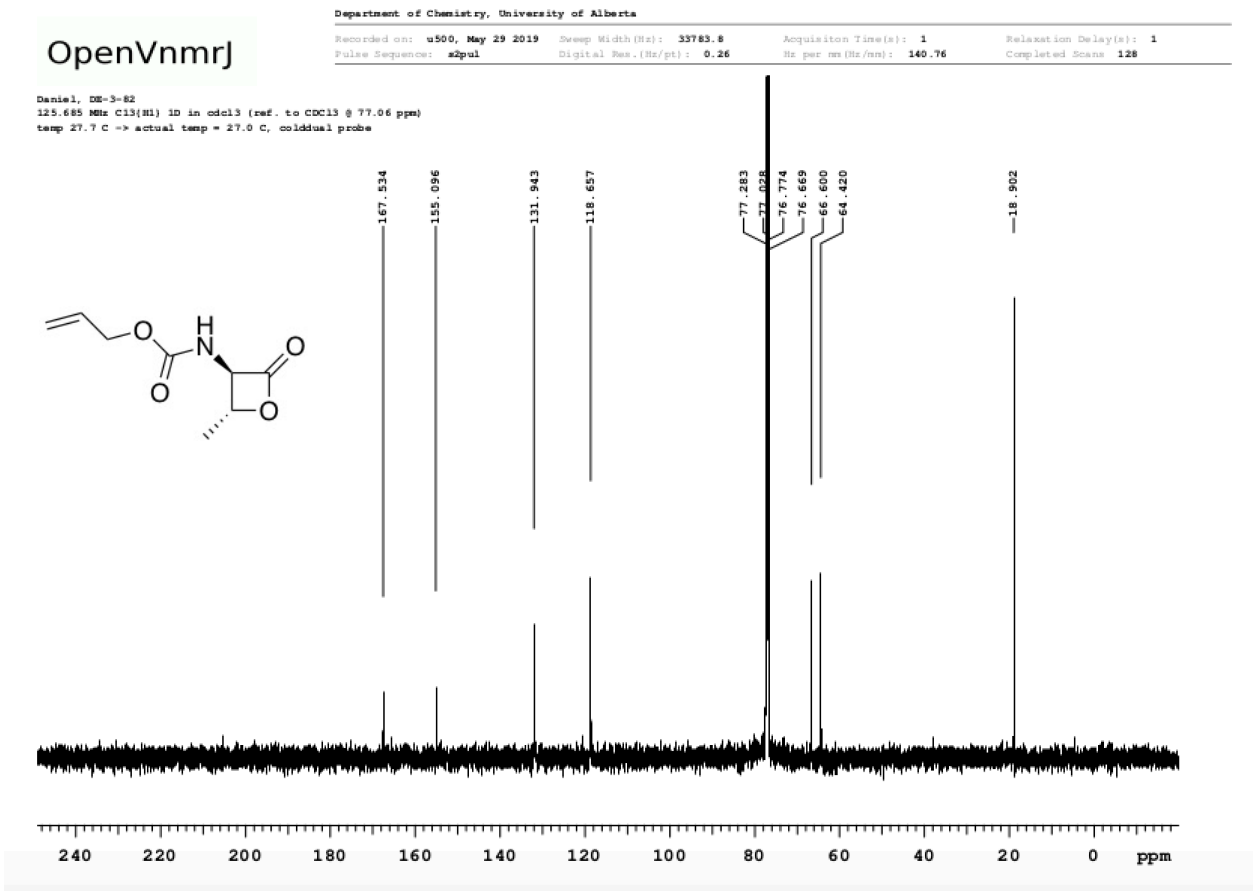
8 – ^{13}C-NMR


Department of Chemistry, University of Alberta
OpenVnmrJ
Recorded on: u500, Jun 15 2021
Sweep Width(Hz): 33783.8
Acquisiton Time(s): 1
Relaxation Delay(s): 1
Pulse Sequence: s2pul
Digital Res.(Hz/pt): 0.26
Hz per mm(Hz/mm): 140.76
Completed Scans 128
Daniel, DE-5-172
125.685 MHz C13{H1} 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 27.7 C -> actual temp = 27.0 C, colddual probe
167.419
155.238
143.428
141.379
127.881
127.153
124.909
120.088
77.285
77.030
76.776
76.665
67.533
64.385
47.052
18.900
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

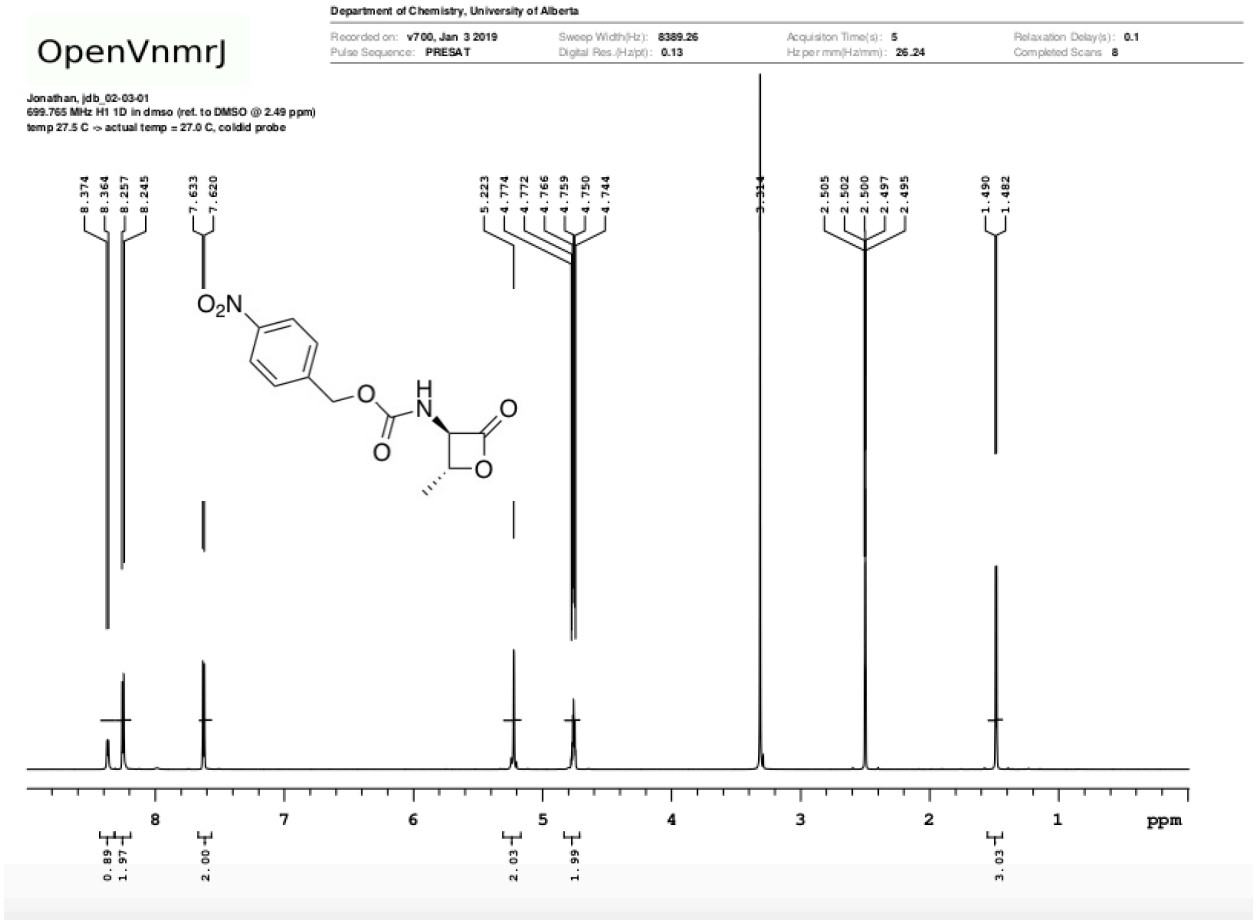
9 – ^{1}H-NMR


Department of Chemistry, University of Alberta
OpenVnmrJ
Recorded on: u500, May 29 2019
Pulse Sequence: PRESAT
Sweep Width(Hz): 6009.62
Digital Res.(Hz/pt): 0.09
Acquisiton Time(s): 5
Hz per mm(Hz/mm): 25.04
Relaxation Delay(s): 0.1
Completed Scans 8
Daniel, DE-3-82
499.787 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 27.7 C => actual temp = 27.0 C, colddual probe
7.287
5.973
5.962
5.952
5.950
5.941
5.927
5.918
5.916
5.906
5.895
5.432
5.371
5.369
5.336
5.334
5.291
5.270
4.790
4.780
4.769
4.758
4.665
4.656
4.641
1.647
1.635
1.592
0.094
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
0.82
1.10
1.09
1.03
3.02
3.14

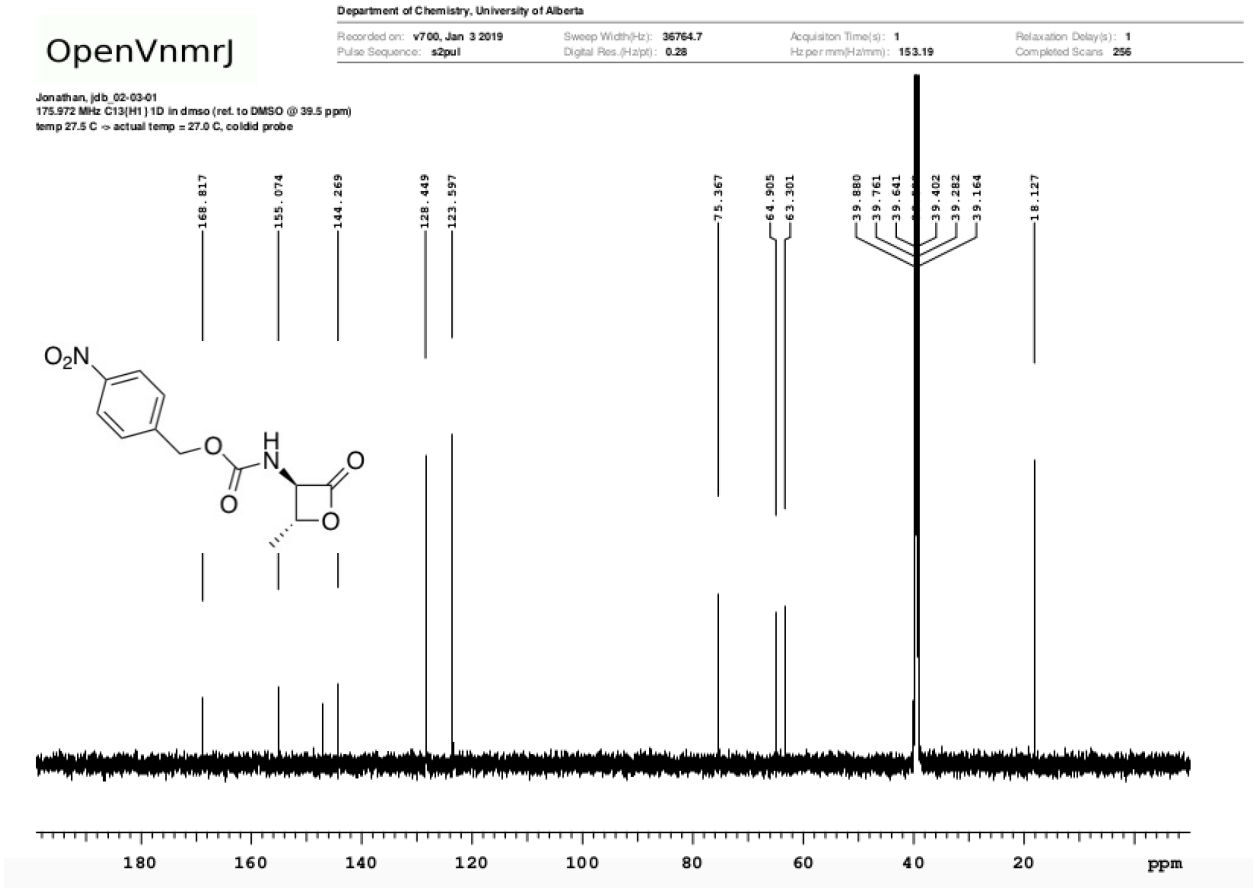
9 – ^{13}C-NMR


OpenVnmrJ
Department of Chemistry, University of Alberta
Recorded on: u500, May 29 2019
Pulse Sequence: s2pul
Sweep Width(Hz): 33783.8
Digital Res.(Hz/pt): 0.26
Acquisiton Time(s): 1
Hz per mm(Hz/mm): 140.76
Relaxation Delay(s): 1
Completed Scans 128
Daniel, DE-3-82
125.685 MHz C13{H1} 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 27.7 C => actual temp = 27.0 C, colddual probe
167.534
155.096
131.943
118.657
77.283
77.028
76.774
76.669
66.600
64.420
18.902
O
H
N
O
O
O
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

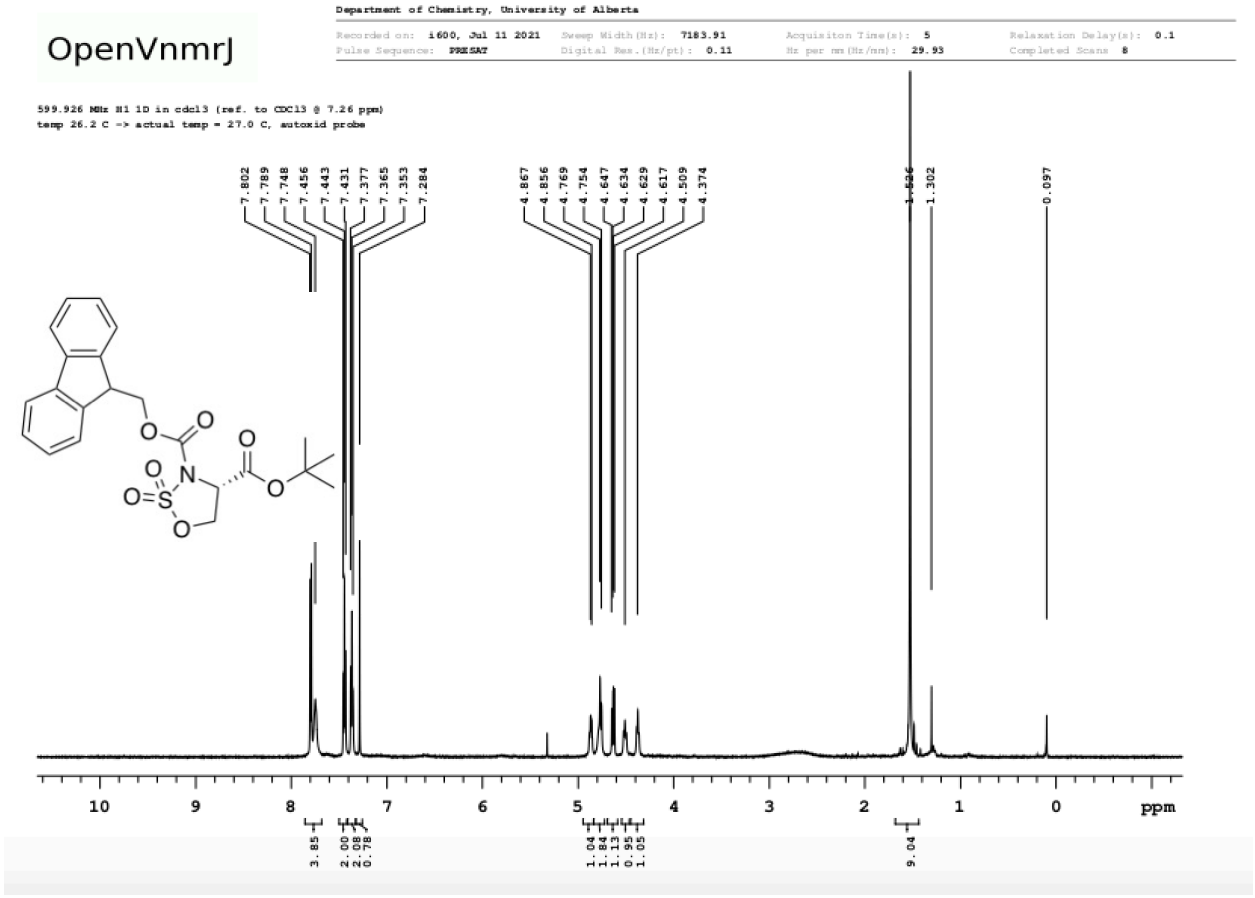
10 – ^{1}H-NMR


Department of Chemistry, University of Alberta
OpenVnmrJ
Recorded on: v700, Jan 3 2019
Pulse Sequence: PRESAT
Sweep Width(Hz): 8389.26
Digital Res.(Hz/pt): 0.13
Acquisiton Time(s): 5
Hz per mm(Hz/mm): 26.24
Relaxation Delay(s): 0.1
Completed Scans 8
Jonathan, jdb_02-03-01
699.765 MHz H1 1D in dmso (ref. to DMSO @ 2.49 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
8.374
8.364
8.257
8.245
7.633
7.620
5.223
4.774
4.772
4.766
4.759
4.750
4.744
3.314
2.505
2.502
2.500
2.497
2.495
1.490
1.482
O2N
O
H
N
O
O
O
8
7
6
5
4
3
2
1
ppm
0.89
1.97
2.00
2.03
1.99
3.03

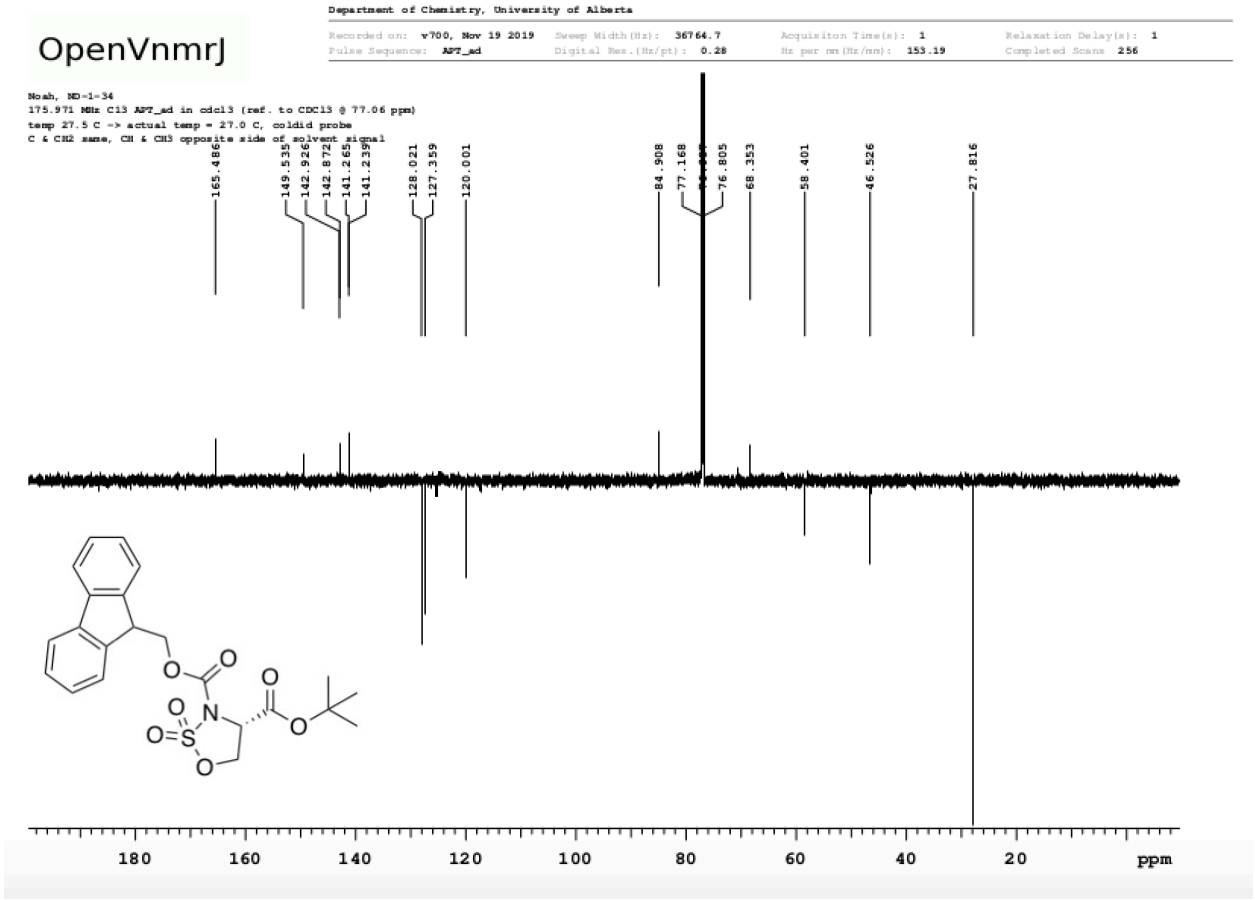
10 – ^{13}C-NMR


OpenVnmrJ
Department of Chemistry, University of Alberta
Recorded on: v700, Jan 3 2019
Pulse Sequence: s2pul
Sweep Width(Hz): 36764.7
Digital Res.(Hz/pt): 0.28
Acquisiton Time(s): 1
Hz per mm(Hz/mm): 153.19
Relaxation Delay(s): 1
Completed Scans 256
Jonathan, jdb_02-03-01
175.972 MHz C13{H1} 1D in dmso (ref. to DMSO @ 39.5 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
168.817
155.074
144.269
128.449
123.597
75.367
64.905
63.301
39.880
39.761
39.641
39.402
39.282
39.164
18.127
O2N
O
H
N
O
O
O
180
160
140
120
100
80
60
40
20
ppm

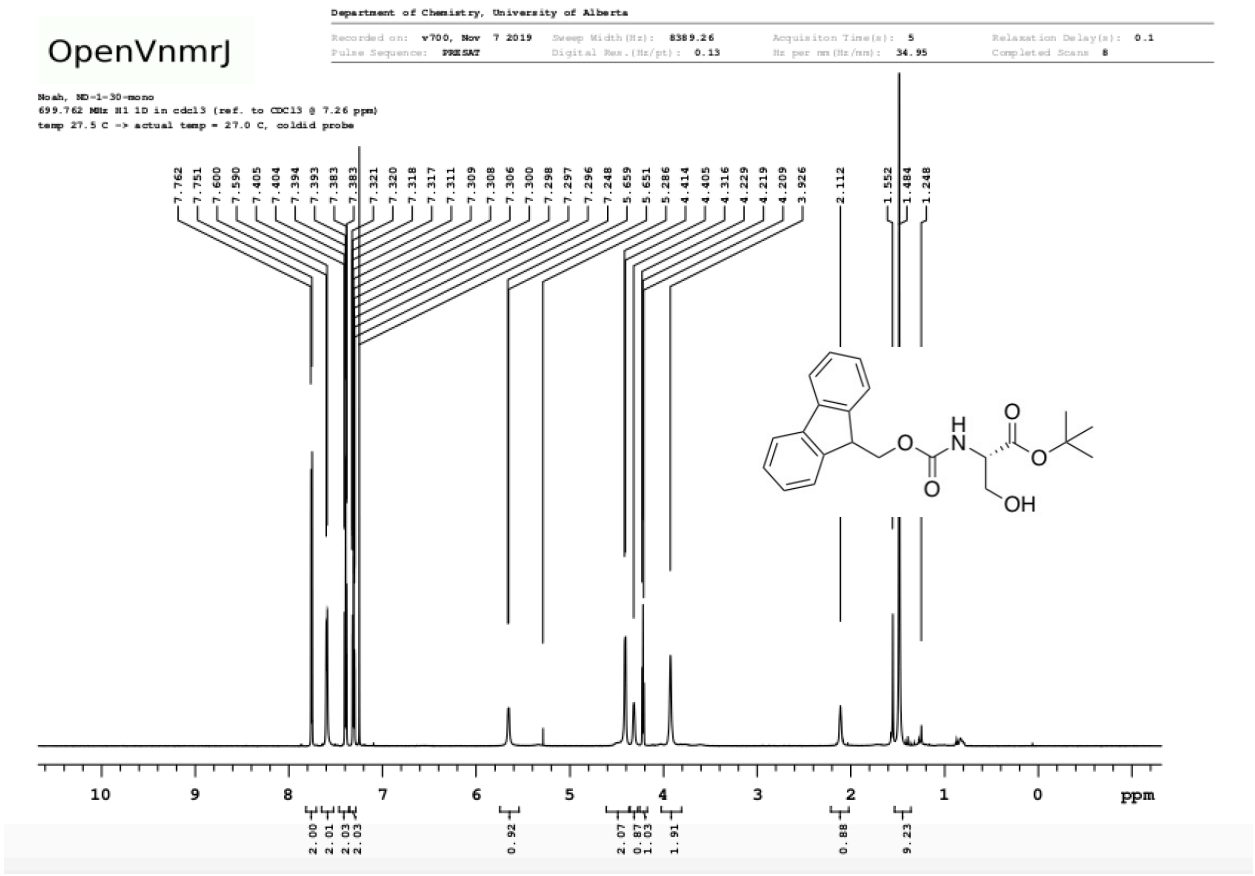
14 – ^{1}H-NMR


OpenVnmrJ
Department of Chemistry, University of Alberta
599.926 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.2 C -> actual temp = 27.0 C, autoxid probe
7.802
7.789
7.748
7.456
7.443
7.431
7.377
7.365
7.353
7.284
4.867
4.856
4.769
4.754
4.647
4.634
4.629
4.617
4.509
4.374
1.536
1.302
0.097
ppm

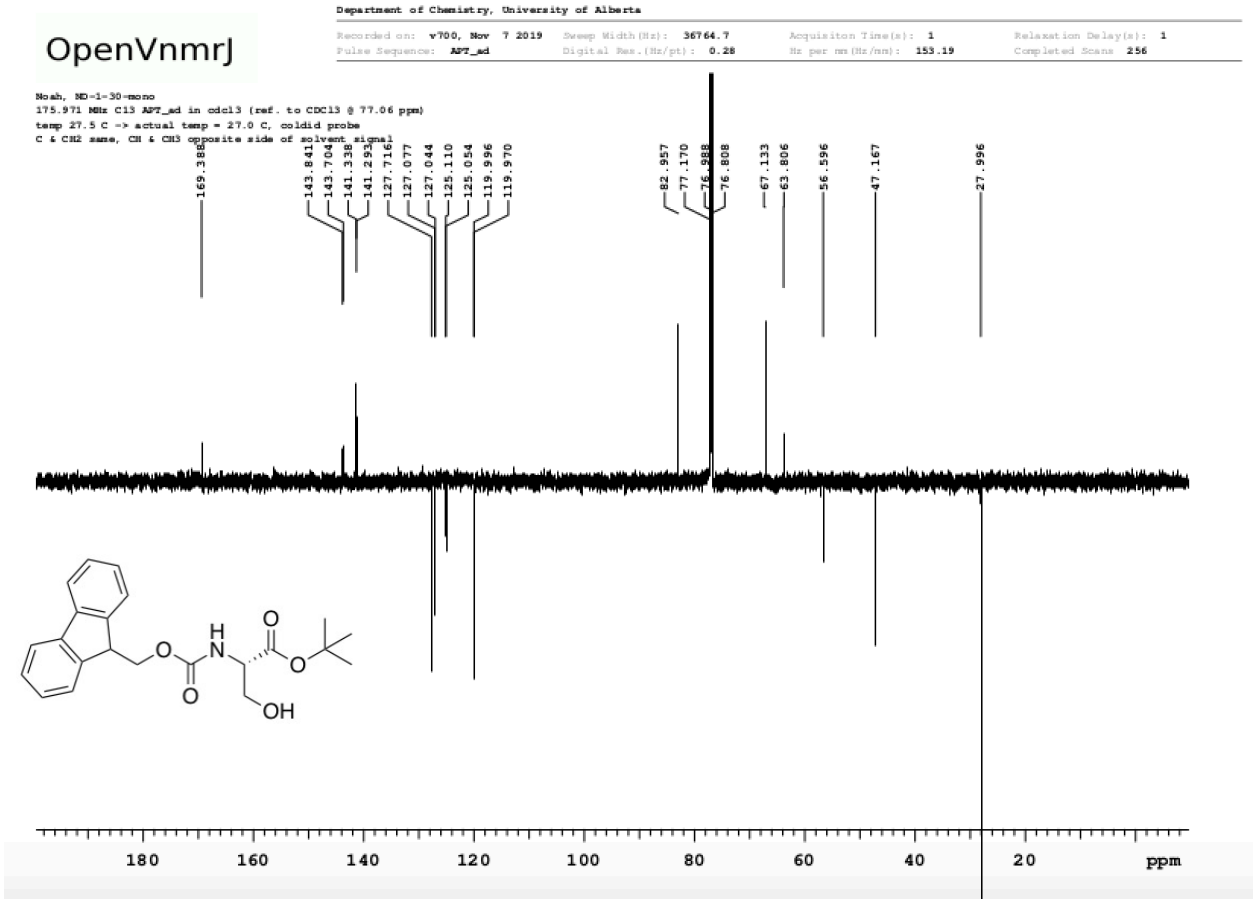
14 – ^{13}C-DEPT NMR



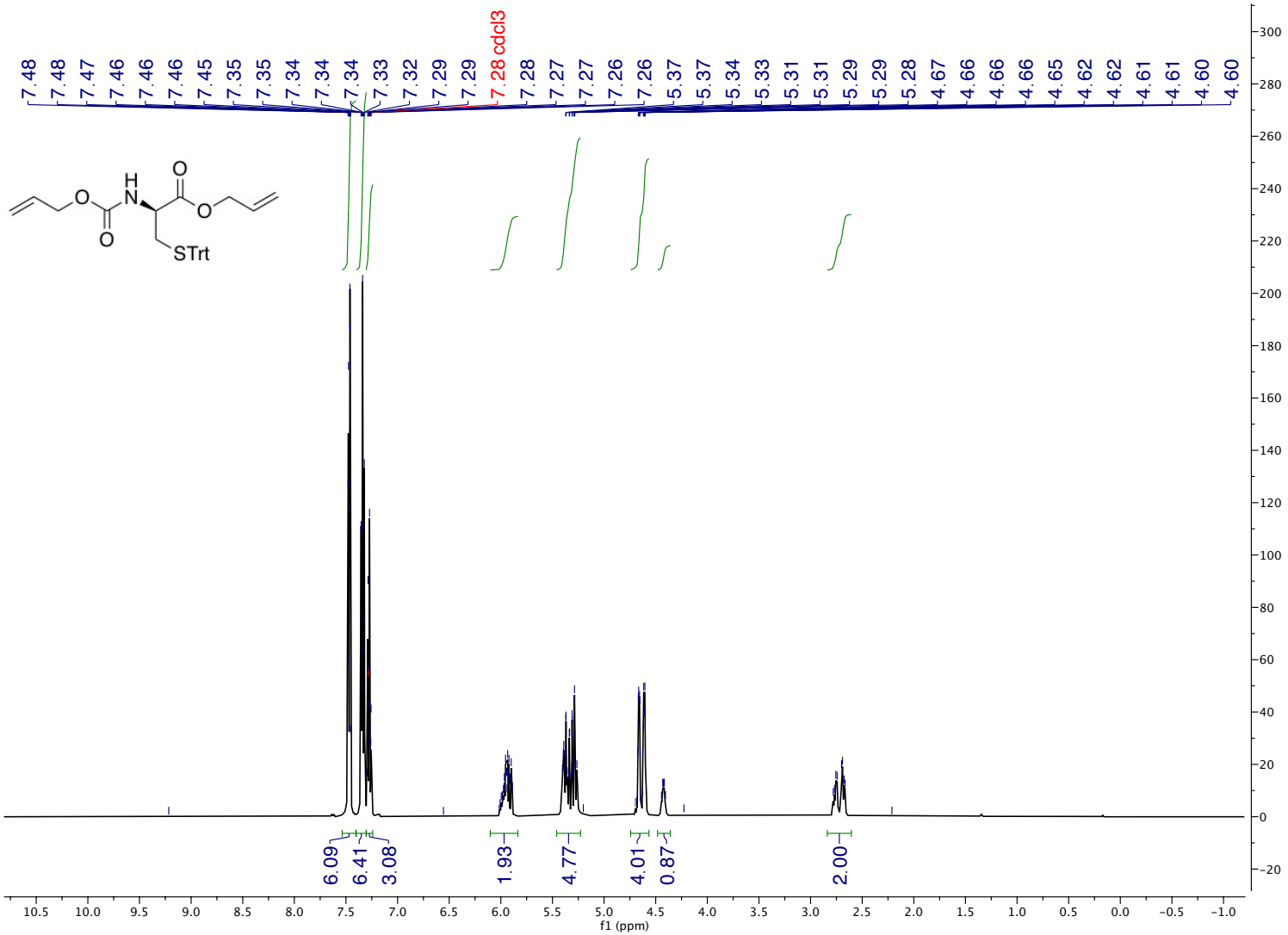
15 – ^{1}H-NMR


Department of Chemistry, University of Alberta
OpenVnmrJ
Recorded on: v700, Nov 7 2019
Pulse Sequence: PRESAT
Sweep Width(Hz): 8389.26
Digital Res.(Hz/pt): 0.13
Acquisiton Time(s): 5
Hz per mm(Hz/mm): 34.95
Relaxation Delay(s): 0.1
Completed Scans 8
Noah, ND-1-30-mono
699.762 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
7.762
7.751
7.600
7.590
7.405
7.404
7.394
7.393
7.383
7.383
7.321
7.320
7.318
7.317
7.311
7.309
7.308
7.306
7.300
7.298
7.297
7.296
7.248
5.659
5.651
5.286
4.414
4.405
4.316
4.229
4.219
4.209
3.926
2.112
1.552
1.484
1.248
10
9
8
7
6
5
4
3
2
1
0
ppm
2.00
2.01
2.03
2.03
0.92
2.07
0.87
1.03
1.91
0.88
9.23
O
H
N
O
O
O
O
OH

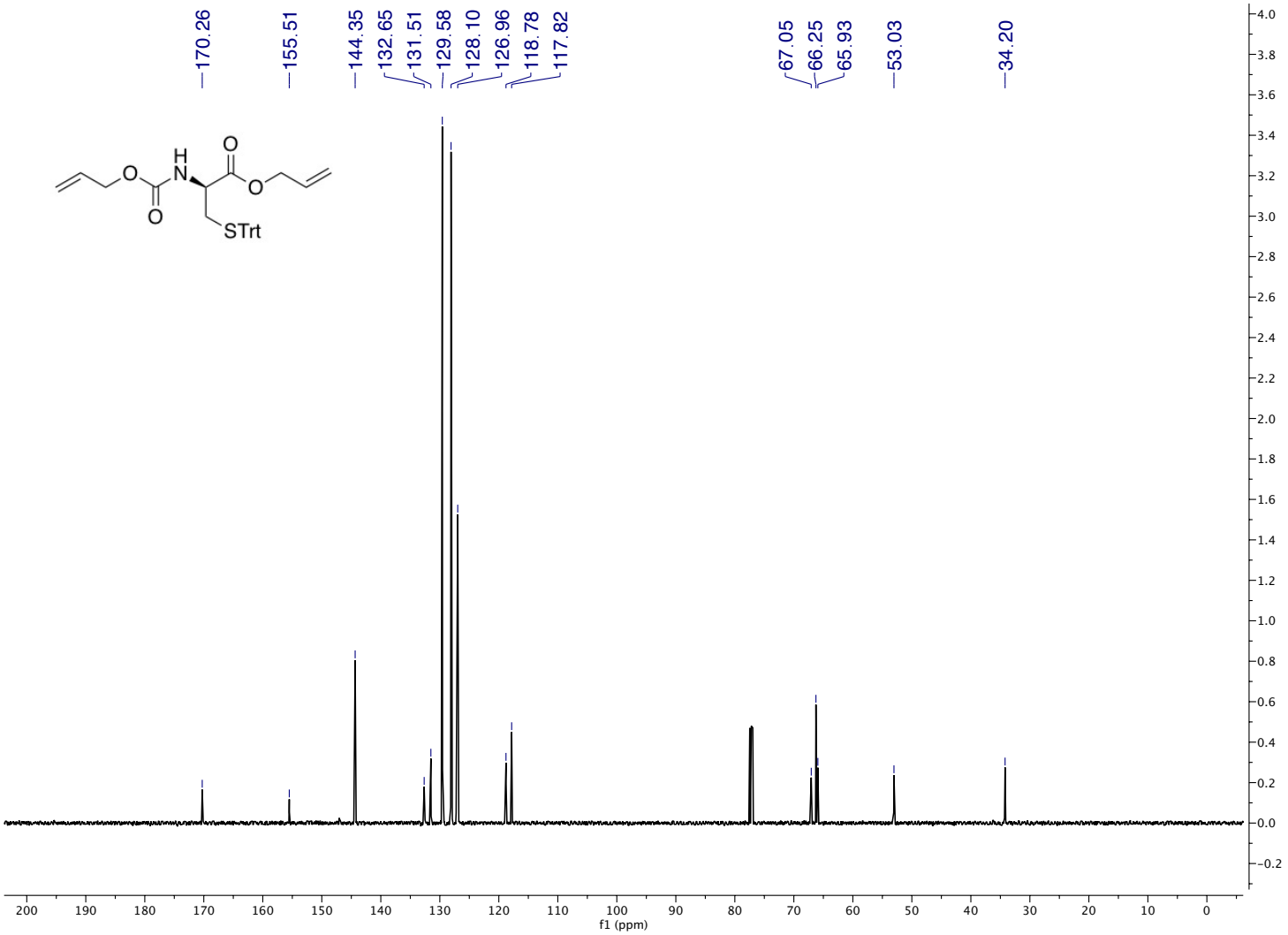
15 – ^{13}C DEPT NMR



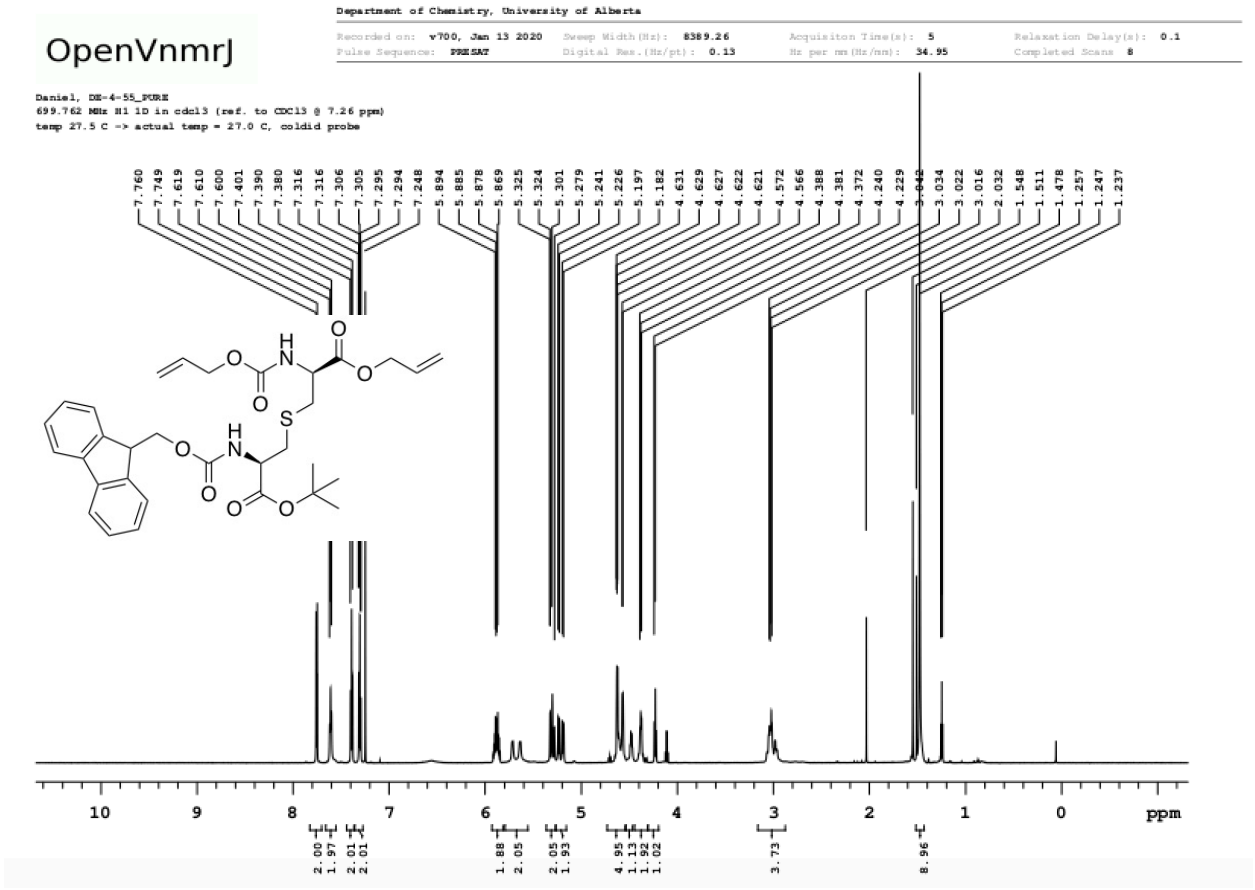
16 – ^{1}H-NMR


7.48 7.48 7.47 7.46 7.46 7.46 7.45 7.35 7.35 7.34 7.34 7.34 7.33 7.32 7.29 7.29 7.28 cdcl3 7.28 7.27 7.27 7.26 7.26 5.37 5.37 5.34 5.33 5.31 5.31 5.29 5.29 5.28 4.67 4.66 4.66 4.66 4.65 4.62 4.62 4.61 4.61 4.60 4.60
O
H
N
O
O
O
STrt
6.09
6.41
3.08
1.93
4.77
4.01
0.87
2.00
f1 (ppm)

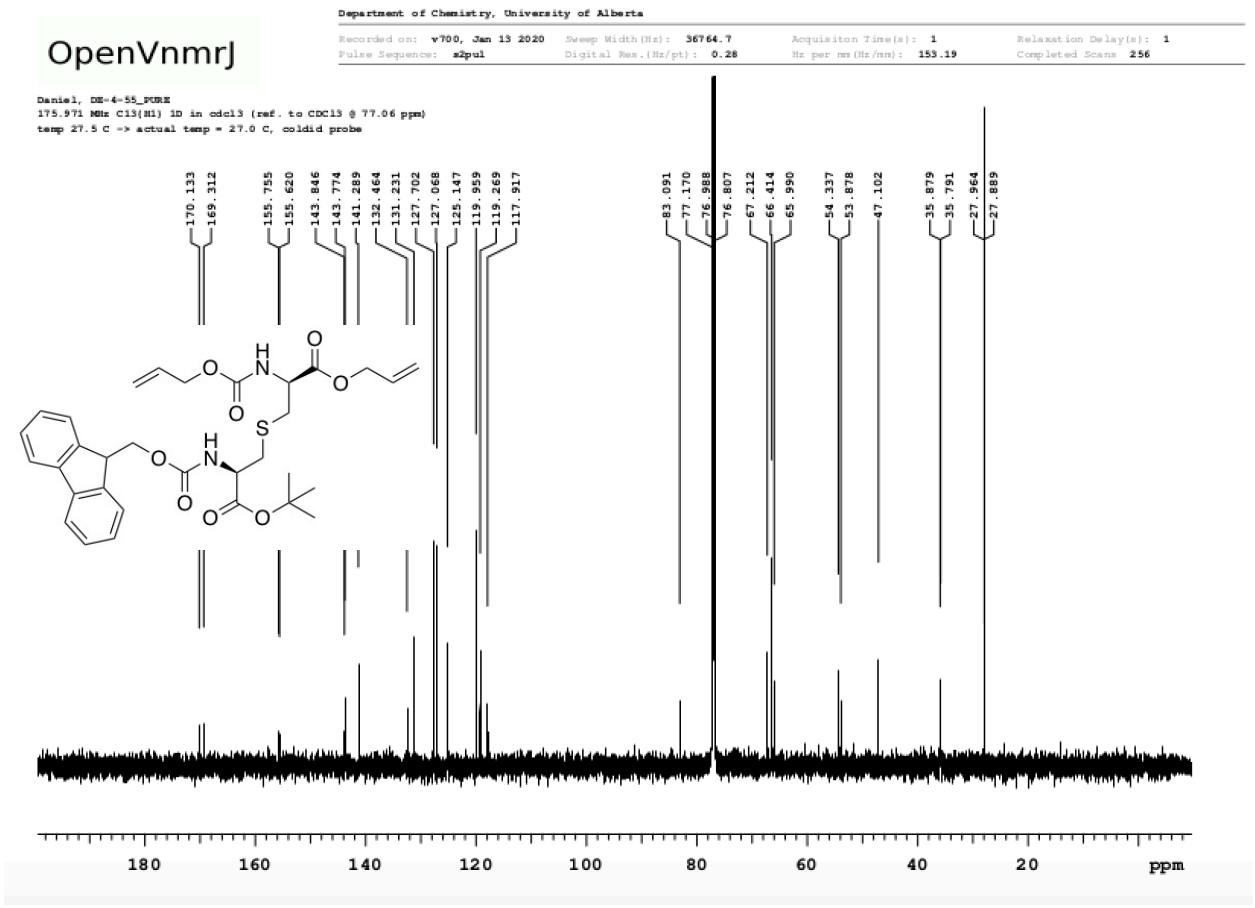
16 – ^{13}C-NMR


170.26
155.51
144.35
132.65
131.51
129.58
128.10
126.96
118.78
117.82
67.05
66.25
65.93
53.03
34.20
STrt
f1 (ppm)

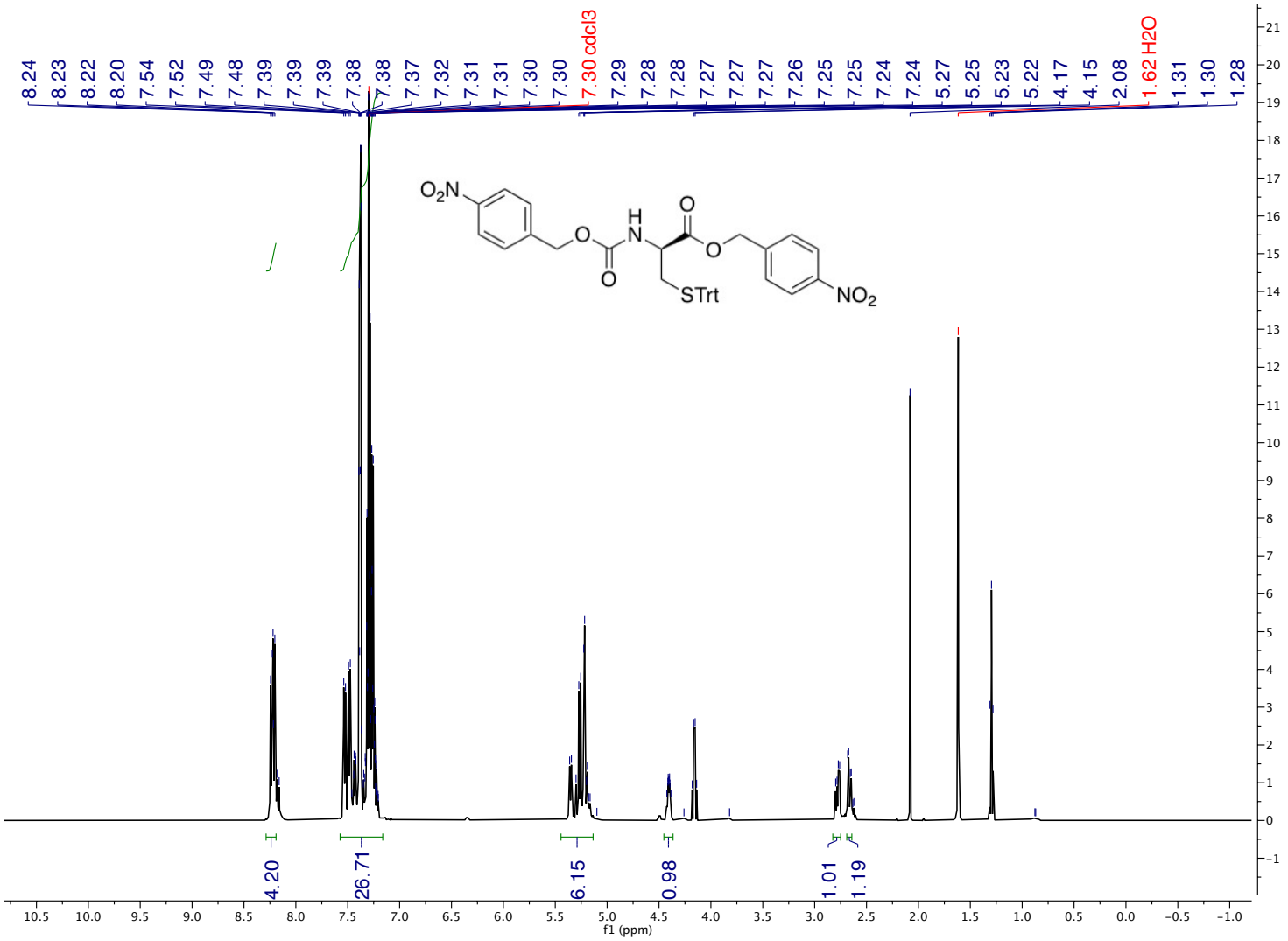
S1 – ^{1}H-NMR


Department of Chemistry, University of Alberta
OpenVnmrJ
Recorded on: v700, Jan 13 2020 Sweep Width(Hz): 8389.26 Acquisiton Time(s): 5 Relaxation Delay(s): 0.1
Pulse Sequence: PRESAT Digital Res.(Hz/pt): 0.13 Hz per mm(Hz/mm): 34.95 Completed Scans 8
Daniel, DE-4-55_PURE
699.762 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
7.760 7.749 7.619 7.610 7.600 7.401 7.390 7.380 7.316 7.316 7.306 7.305 7.295 7.294 7.248
5.894 5.885 5.878 5.869 5.325 5.324 5.301 5.279 5.241 5.226 5.197 5.182 4.631 4.629 4.627 4.622 4.621 4.572 4.566 4.388 4.381 4.372 4.240 4.229
3.042 3.034 3.022 3.016 2.032 1.548 1.511 1.478 1.257 1.247 1.237
10 9 8 7 6 5 4 3 2 1 0 ppm
2.00 1.97 2.01 2.01 1.88 2.05 2.05 1.93 4.95 1.13 1.92 1.02 3.73 8.96

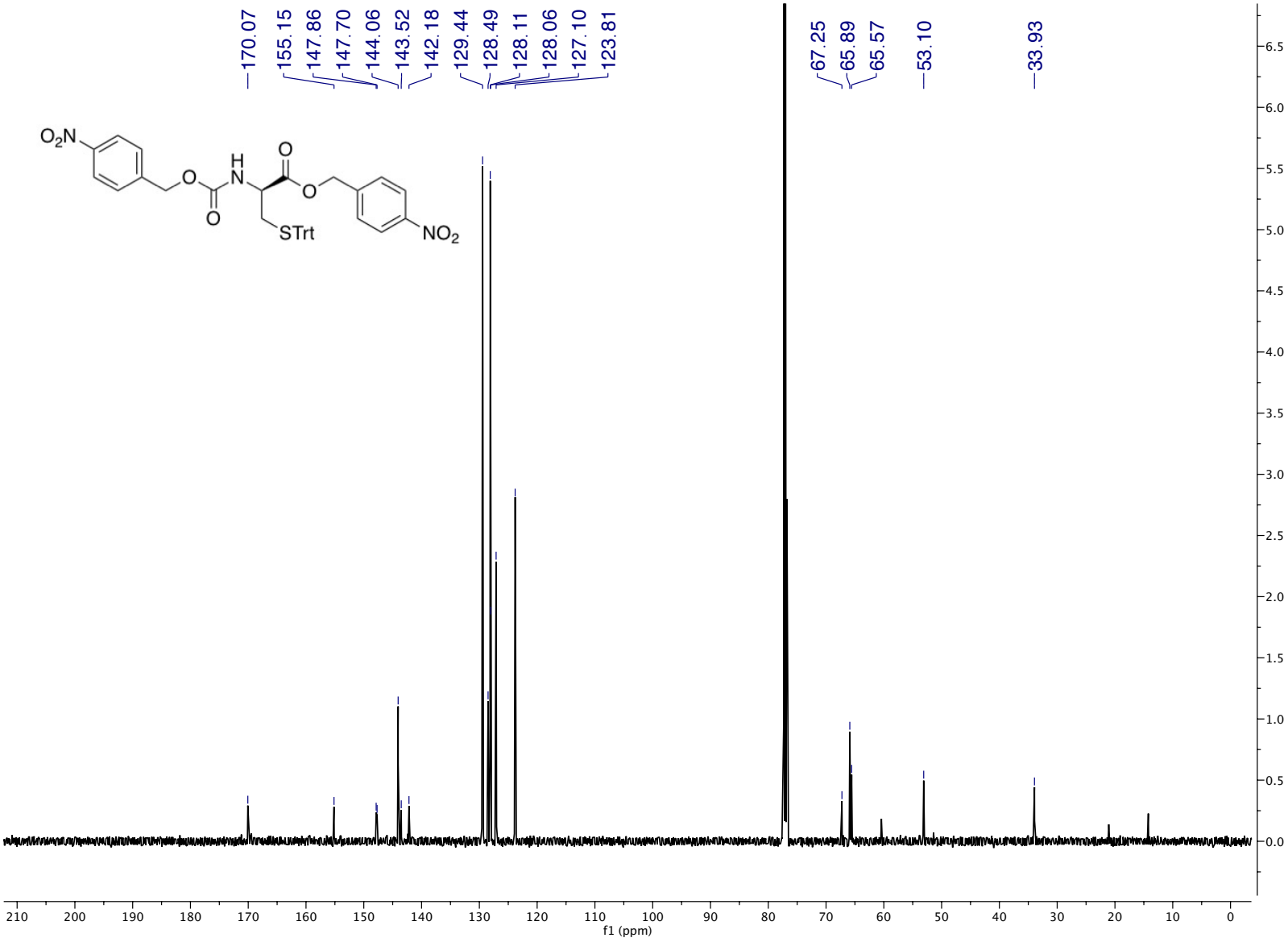
S1 – ^{13}C-NMR


OpenVnmrJ
Department of Chemistry, University of Alberta
Recorded on: v700, Jan 13 2020 Sweep Width(Hz): 36764.7 Acquisiton Time(s): 1 Relaxation Delay(s): 1
Pulse Sequence: s2pul Digital Res.(Hz/pt): 0.28 Hz per mm(Hz/mm): 153.19 Completed Scans 256
Daniel, DE-4-55_PURE
175.971 MHz C13{H1} 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 27.5 C => actual temp = 27.0 C, coldid probe
170.133
169.312
155.755
155.620
143.846
143.774
141.289
132.464
131.231
127.702
127.068
125.147
119.959
119.269
117.917
83.091
77.170
76.988
76.807
67.212
66.414
65.990
54.337
53.878
47.102
35.879
35.791
27.964
27.889
180
160
140
120
100
80
60
40
20
ppm

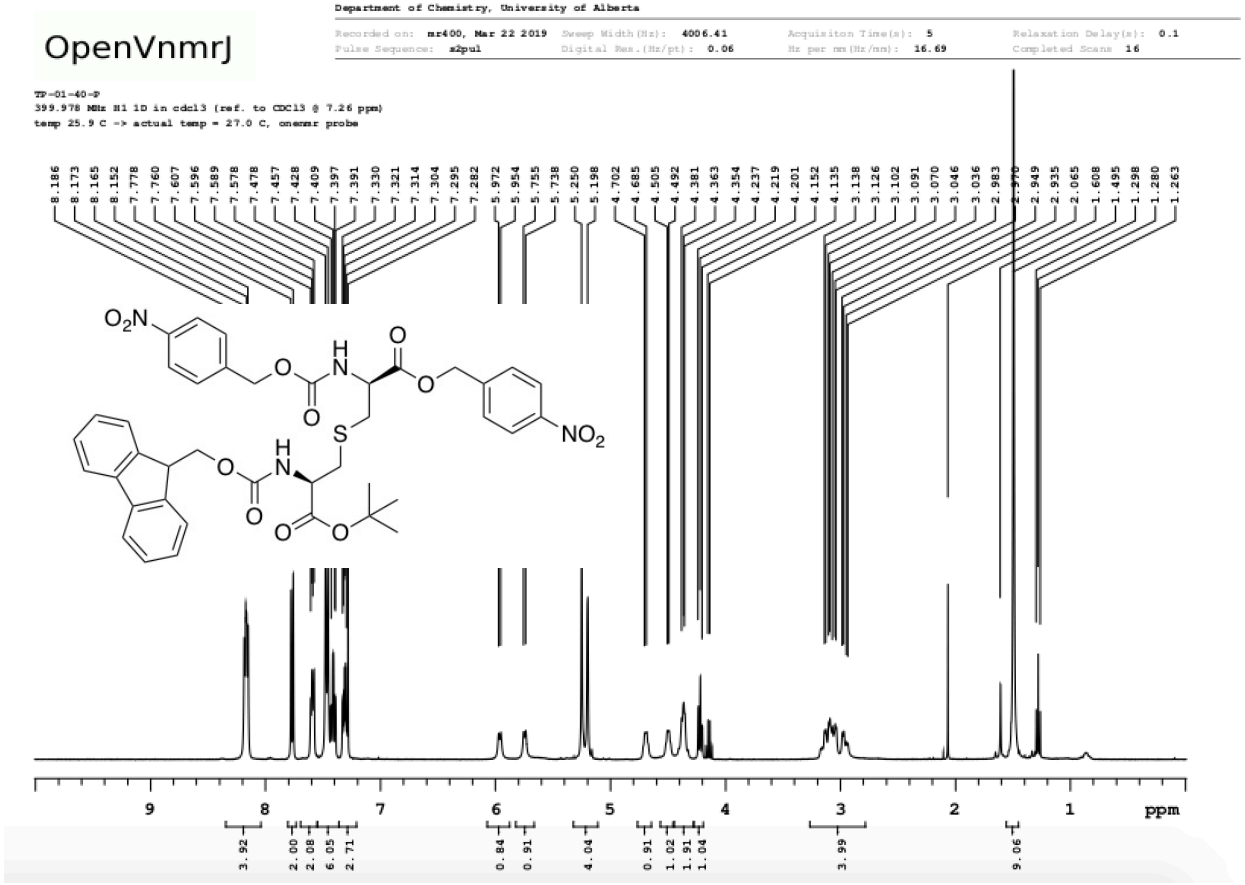
17 – ^{1}H-NMR


8.24
8.23
8.22
8.20
7.54
7.52
7.49
7.48
7.39
7.39
7.39
7.38
7.38
7.37
7.32
7.31
7.31
7.30
7.30
7.30 cdcl3
7.29
7.28
7.28
7.27
7.27
7.27
7.26
7.25
7.25
7.24
7.24
5.27
5.25
5.23
5.22
4.17
4.15
2.08
1.62 H2O
1.31
1.30
1.28
O2N
O
H
N
O
O
O
O
STrt
NO2
4.20
26.71
6.15
0.98
1.01
1.19
f1 (ppm)

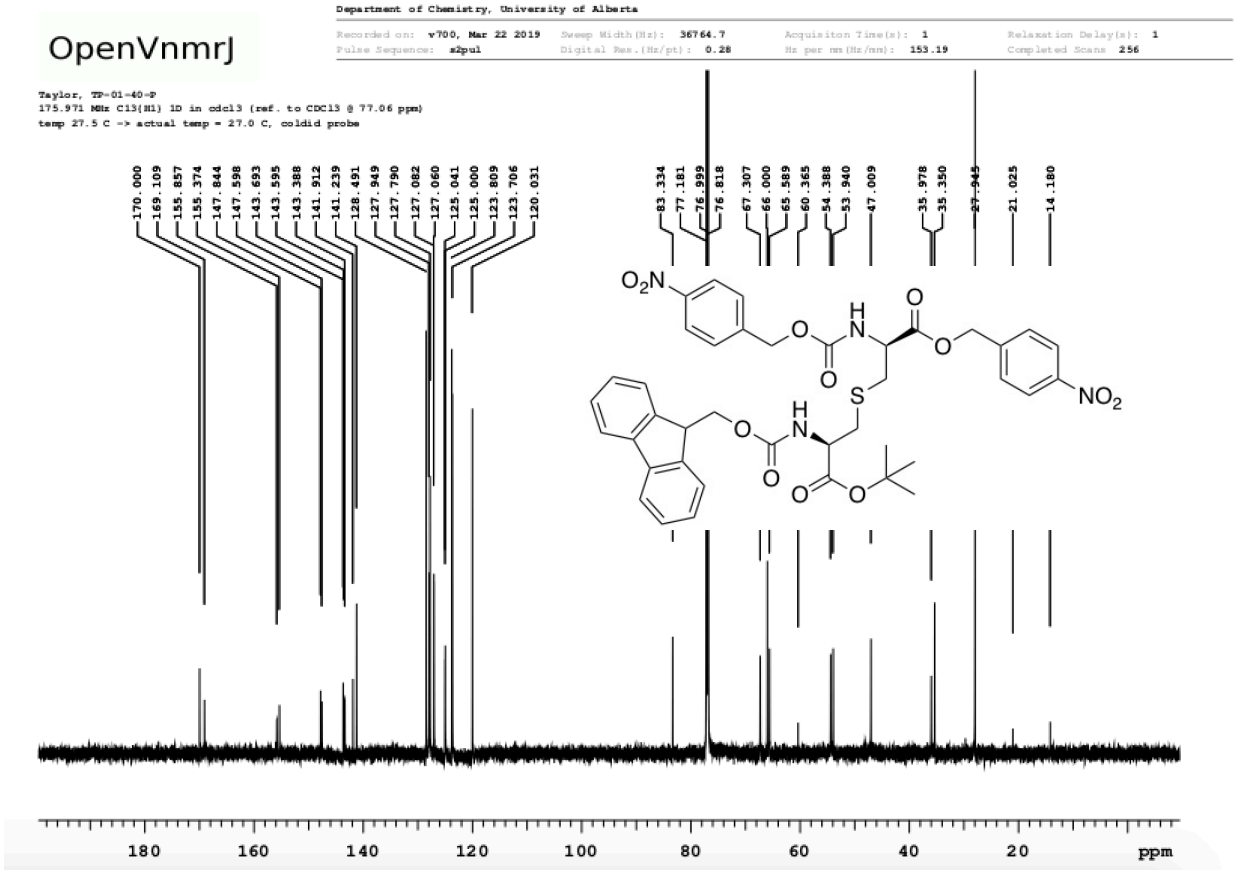
17 – ^{13}C-NMR


170.07
155.15
147.86
147.70
144.06
143.52
142.18
129.44
128.49
128.11
128.06
127.10
123.81
67.25
65.89
65.57
53.10
33.93
O2N
NO2
STrt
f1 (ppm)

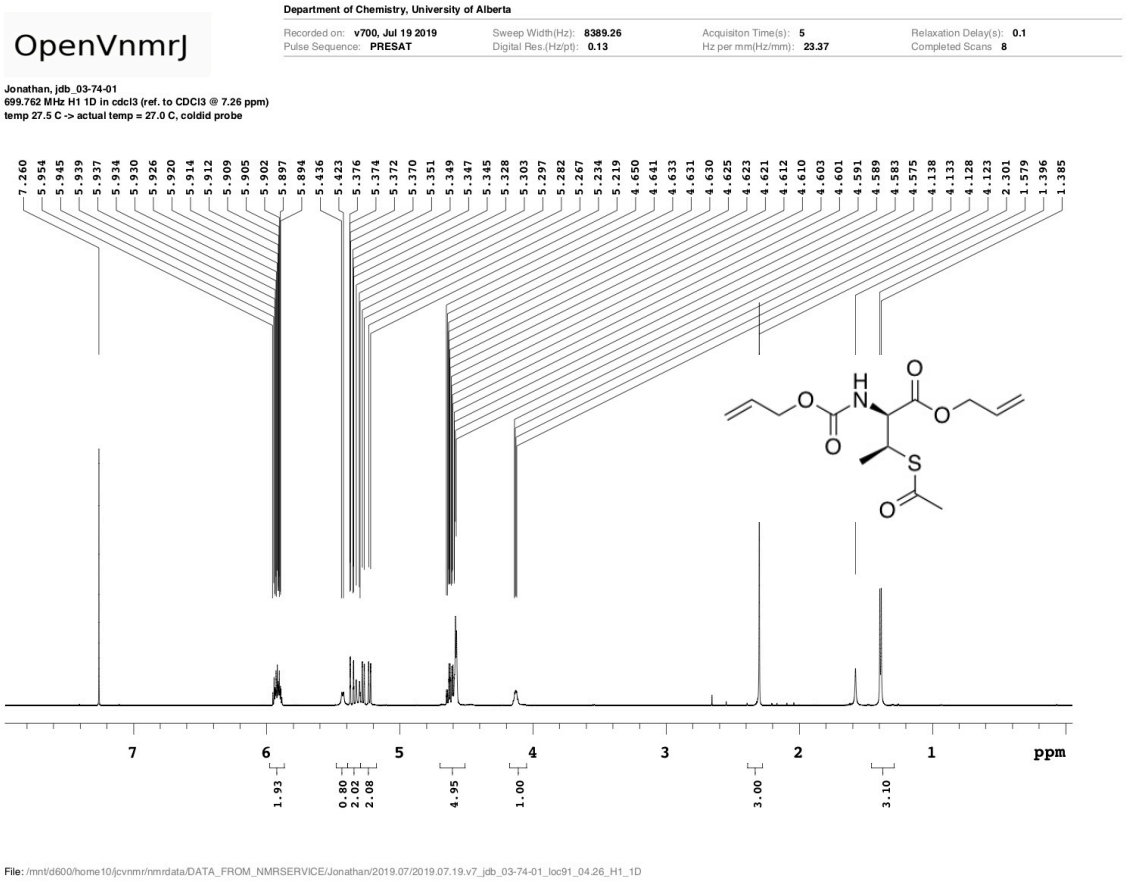
S2 -^{1}H-NMR


Department of Chemistry, University of Alberta
OpenVnmrJ
TP-01-40-P
399.978 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 25.9 C -> actual temp = 27.0 C, onenmr probe
8.186 8.173 8.165 8.152 7.778 7.760 7.607 7.596 7.589 7.578 7.478 7.457 7.428 7.409 7.397 7.391 7.330 7.321 7.314 7.304 7.295 7.282 5.972 5.954 5.755 5.738 5.250 5.198 4.702 4.685 4.505 4.492 4.381 4.363 4.354 4.237 4.219 4.201 4.152 4.135 3.138 3.126 3.102 3.091 3.070 3.046 3.036 2.983 2.970 2.949 2.935 2.065 1.608 1.495 1.298 1.280 1.263
O2N
NO2
9 8 7 6 5 4 3 2 1 ppm
3.92 2.00 2.08 6.05 2.71 0.84 0.91 4.04 0.91 1.02 1.91 1.04 3.99 9.06

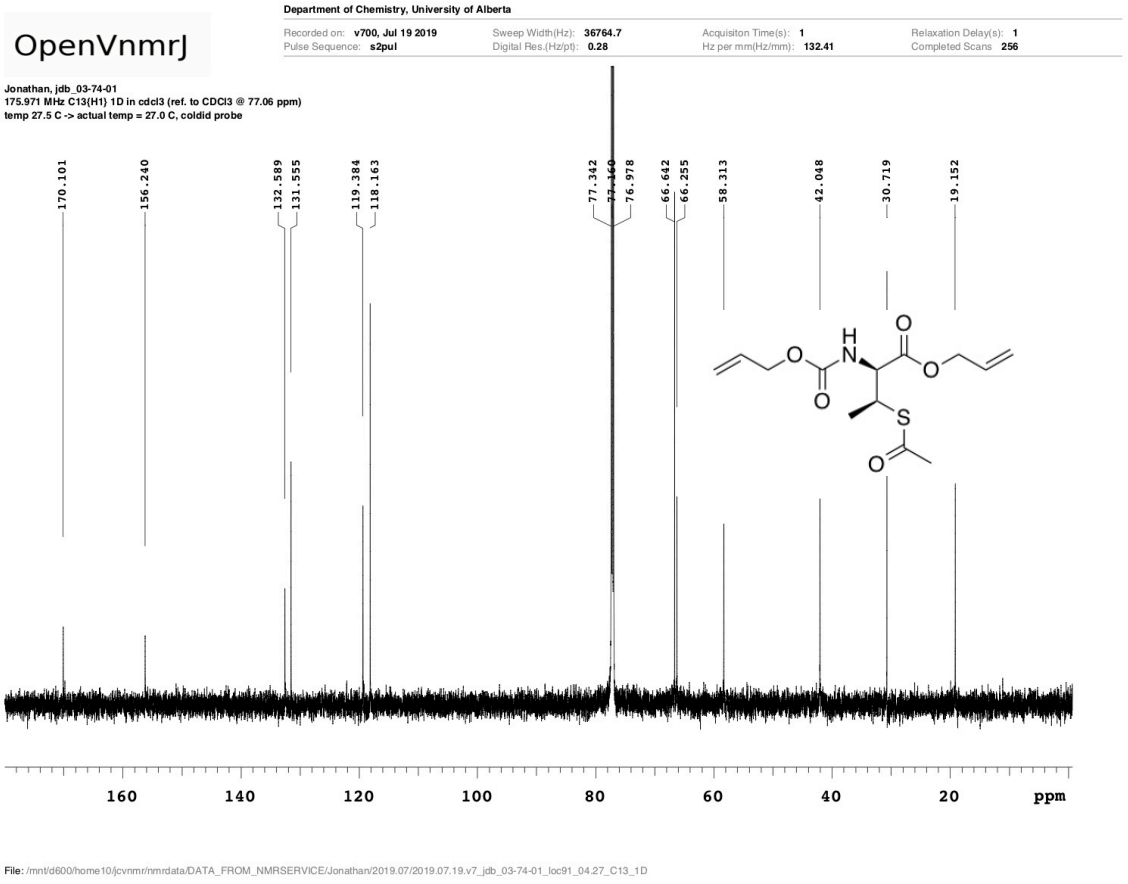
S2 – 13C-NMR


Department of Chemistry, University of Alberta
OpenVnmrJ
Recorded on: v700, Mar 22 2019
Pulse Sequence: s2pul
Sweep Width(Hz): 36764.7
Digital Res.(Hz/pt): 0.28
Acquisiton Time(s): 1
Hz per mm(Hz/mm): 153.19
Relaxation Delay(s): 1
Completed Scans 256
Taylor, TP-01-40-P
175.971 MHz C13{H1} 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
170.000
169.109
155.857
155.374
147.844
147.598
143.693
143.595
143.388
141.912
141.239
128.491
127.949
127.790
127.082
127.060
125.041
125.000
123.809
123.706
120.031
83.334
77.181
76.999
76.818
67.307
66.000
65.589
60.365
54.388
53.940
47.009
35.978
35.350
27.945
21.025
14.180
180
160
140
120
100
80
60
40
20
ppm

12 – ^{1}H-NMR

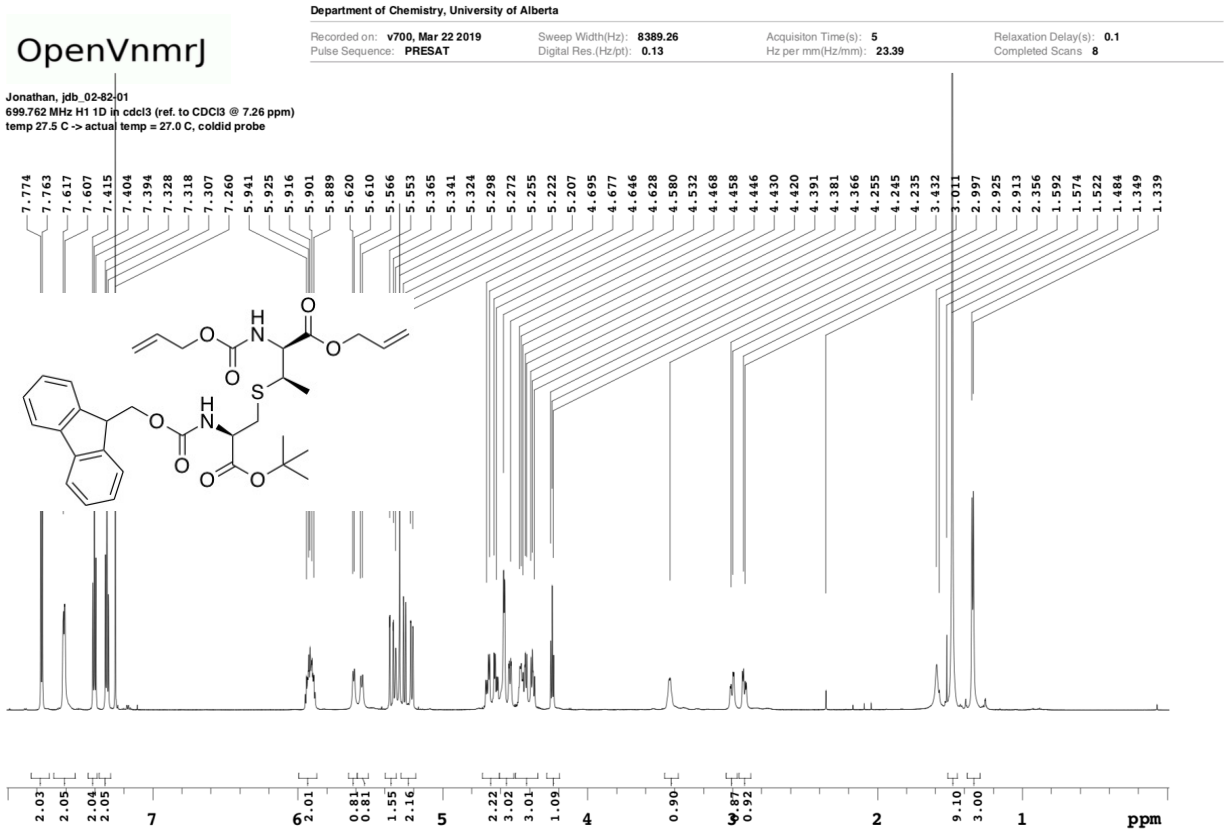

Department of Chemistry, University of Alberta
OpenVnmrJ
Recorded on: v700, Jul 19 2019
Pulse Sequence: PRESAT
Sweep Width(Hz): 8389.26
Digital Res.(Hz/pt): 0.13
Acquisiton Time(s): 5
Hz per mm(Hz/mm): 23.37
Relaxation Delay(s): 0.1
Completed Scans 8
Jonathan, jdb_03-74-01
699.762 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
7.260
5.954
5.945
5.939
5.937
5.934
5.930
5.926
5.920
5.914
5.912
5.909
5.905
5.902
5.897
5.894
5.436
5.423
5.376
5.374
5.372
5.370
5.351
5.349
5.347
5.345
5.328
5.303
5.297
5.282
5.267
5.234
5.219
4.650
4.641
4.633
4.631
4.630
4.625
4.623
4.621
4.612
4.610
4.603
4.601
4.591
4.589
4.583
4.575
4.138
4.133
4.128
4.123
2.301
1.579
1.396
1.385
7
6
5
4
3
2
1
ppm
1.93
0.80
2.02
2.08
4.95
1.00
3.00
3.10
File: /mnt/d600/home10/jcvnmr/nmrdata/DATA_FROM_NMRSERVICE/Jonathan/2019.07/2019.07.19.v7_jdb_03-74-01_loc91_04.26_H1_1D

12 – ^{13}C-NMR

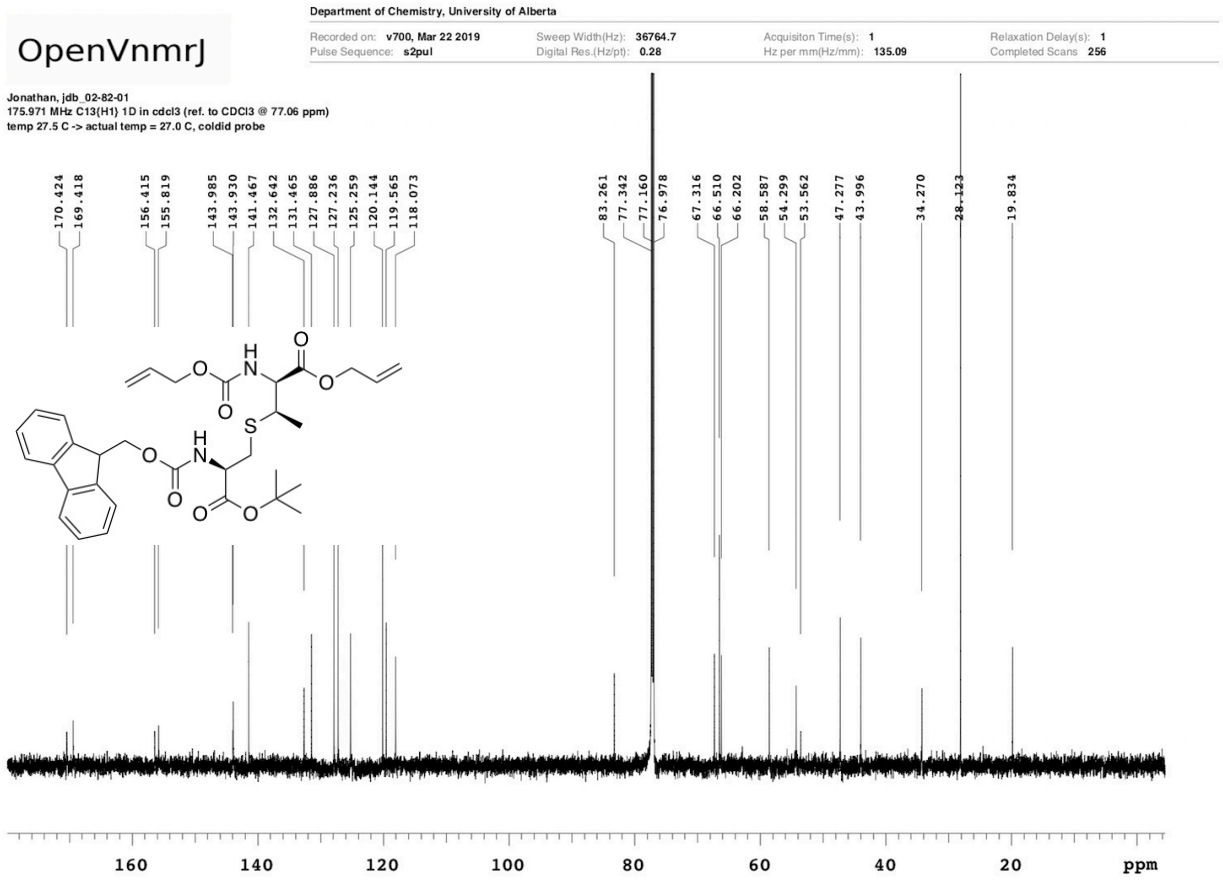


File: /mnt/d600/home10/jcvnmr/nmrdata/DATA_FROM_NMRSERVICE/Jonathan/2019.07/2019.07.19.v7_jdb_03-74-01_loc91_04.27_C13_1D

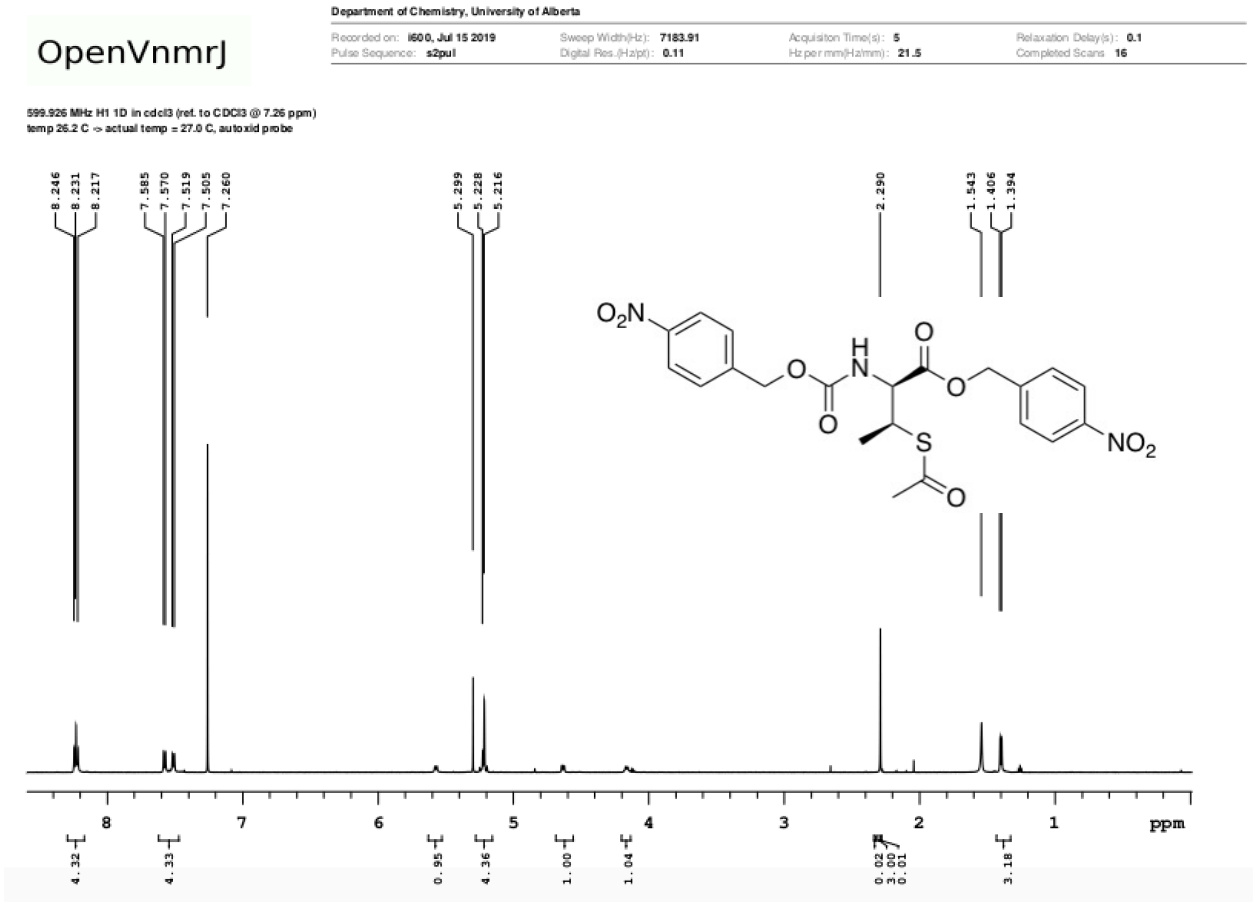
S3 – ^{1}H-NMR


Department of Chemistry, University of Alberta
OpenVnmrJ
Recorded on: v700, Mar 22 2019
Pulse Sequence: PRESAT
Sweep Width(Hz): 8389.26
Digital Res.(Hz/pt): 0.13
Acquisiton Time(s): 5
Hz per mm(Hz/mm): 23.39
Relaxation Delay(s): 0.1
Completed Scans 8
Jonathan, jdb_02-82-01
699.762 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
7.774 7.763 7.617 7.607 7.415 7.404 7.394 7.328 7.318 7.307 7.260 5.941 5.925 5.916 5.901 5.889 5.620 5.610 5.566 5.553 5.365 5.341 5.324 5.298 5.272 5.255 5.222 5.207 4.695 4.677 4.646 4.628 4.580 4.532 4.468 4.458 4.446 4.430 4.420 4.391 4.381 4.366 4.255 4.245 4.235 3.432 3.011 2.997 2.925 2.913 2.356 1.592 1.574 1.522 1.484 1.349 1.339
2.03 2.05 2.04 2.05 2.01 0.81 0.81 1.55 2.16 2.22 3.02 3.01 1.09 0.90 3.87 0.92 9.10 3.00
7 6 5 4 3 2 1 ppm

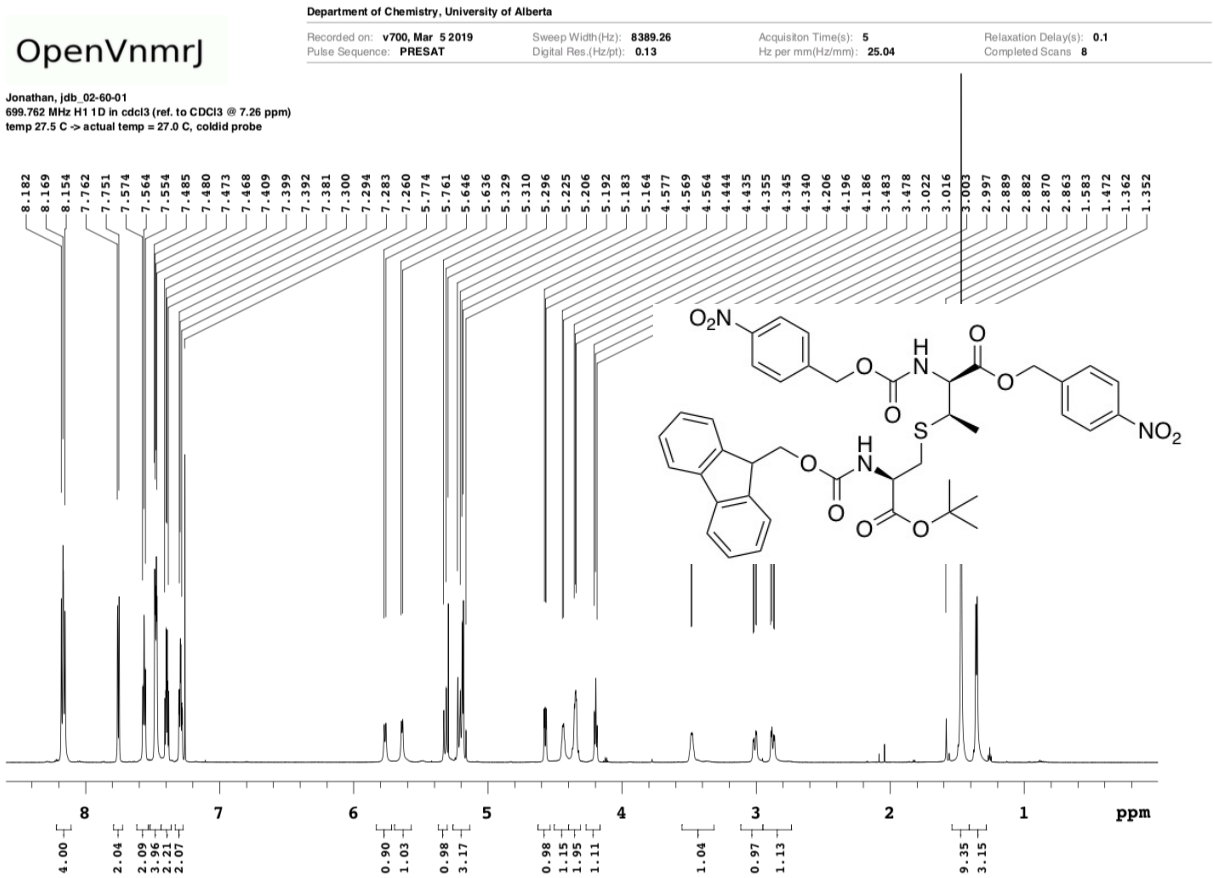
S3 – ^{13}C-NMR


OpenVnmrJ
Department of Chemistry, University of Alberta
Recorded on: v700, Mar 22 2019
Pulse Sequence: s2pul
Sweep Width(Hz): 36764.7
Digital Res.(Hz/pt): 0.28
Acquisiton Time(s): 1
Hz per mm(Hz/mm): 135.09
Relaxation Delay(s): 1
Completed Scans 256
Jonathan, jdb_02-82-01
175.971 MHz C13(H1) 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
170.424
169.418
156.415
155.819
143.985
143.930
141.467
132.642
131.465
127.886
127.236
125.259
120.144
119.565
118.073
83.261
77.342
77.160
76.978
67.316
66.510
66.202
58.587
54.299
53.562
47.277
43.996
34.270
28.123
19.834
160
140
120
100
80
60
40
20
ppm

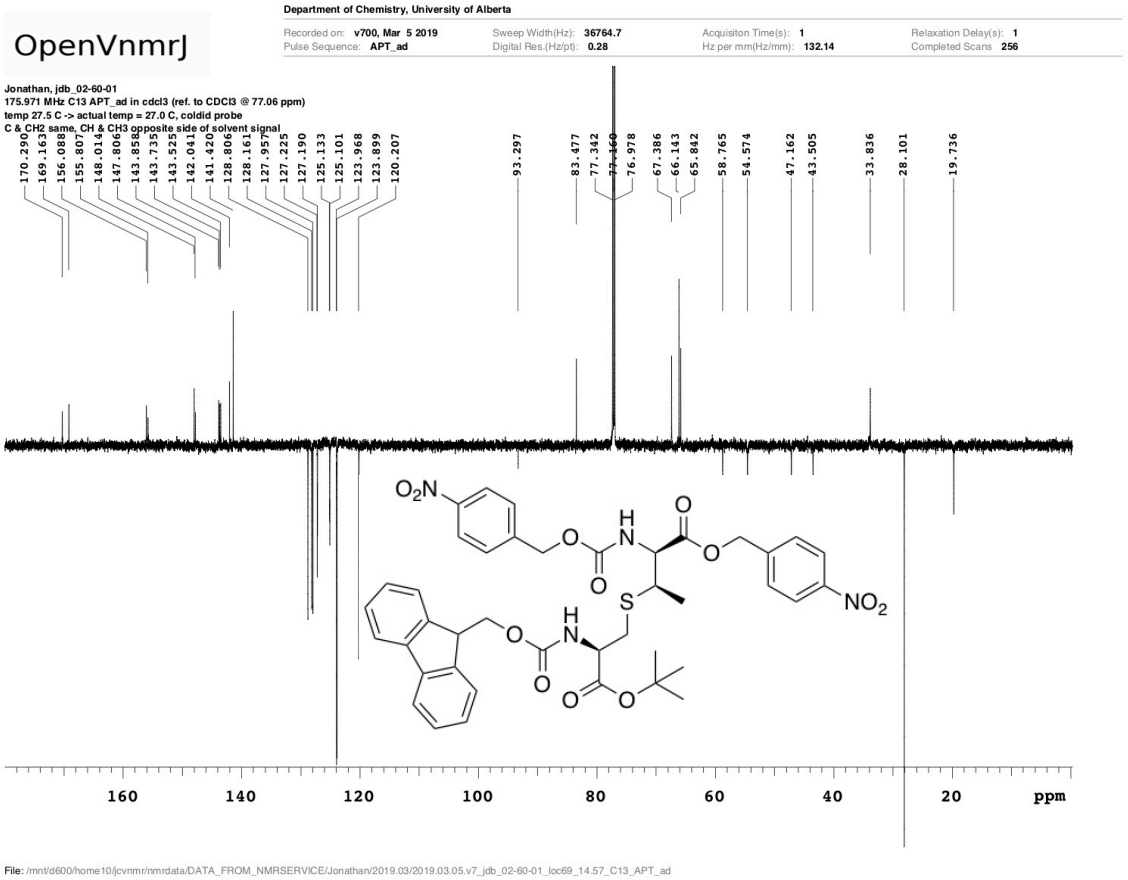
13 – ^{1}H-NMR


OpenVnmrJ
Department of Chemistry, University of Alberta
Recorded on: i600, Jul 15 2019
Pulse Sequence: s2pul
Sweep Width(Hz): 7183.91
Digital Res.(Hz/pt): 0.11
Acquisiton Time(s): 5
Hz per mm(Hz/mm): 21.5
Relaxation Delay(s): 0.1
Completed Scans 16
599.926 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 26.2 C -> actual temp = 27.0 C, autoxid probe
8.246
8.231
8.217
7.585
7.570
7.519
7.505
7.260
5.299
5.228
5.216
2.290
1.543
1.406
1.394
O2N
NO2
ppm
4.32
4.33
0.95
4.36
1.00
1.04
0.02
3.00
0.01
3.18

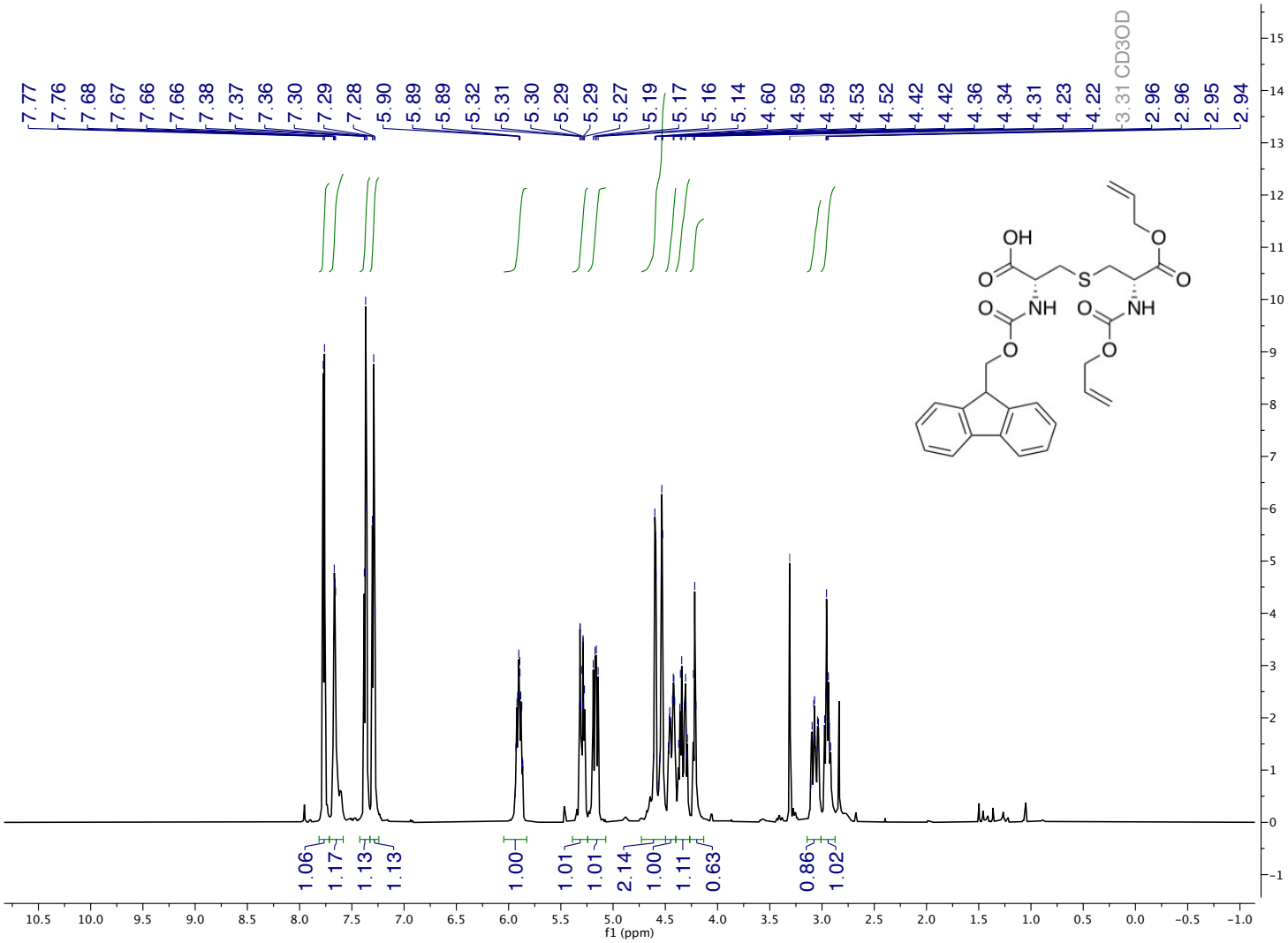
S4 – ^{1}H-NMR


Department of Chemistry, University of Alberta
OpenVnmrJ
Recorded on: v700, Mar 5 2019
Pulse Sequence: PRESAT
Sweep Width(Hz): 8389.26
Digital Res.(Hz/pt): 0.13
Acquisiton Time(s): 5
Hz per mm(Hz/mm): 25.04
Relaxation Delay(s): 0.1
Completed Scans 8
Jonathan, jdb_02-60-01
699.762 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
8.182 8.169 8.154 7.762 7.751 7.574 7.564 7.554 7.485 7.480 7.473 7.468 7.409 7.399 7.392 7.381 7.300 7.294 7.283 7.260 5.774 5.761 5.646 5.636 5.329 5.310 5.296 5.225 5.206 5.192 5.183 5.164 4.577 4.569 4.564 4.444 4.435 4.355 4.345 4.340 4.206 4.196 4.186 3.483 3.478 3.022 3.016 3.003 2.997 2.889 2.882 2.870 2.863 1.583 1.472 1.362 1.352
O2N
NO2
8 7 6 5 4 3 2 1 ppm
4.00 2.04 2.09 3.96 2.21 2.07 0.90 1.03 0.98 3.17 0.98 1.15 1.95 1.11 1.04 0.97 1.13 9.35 3.15

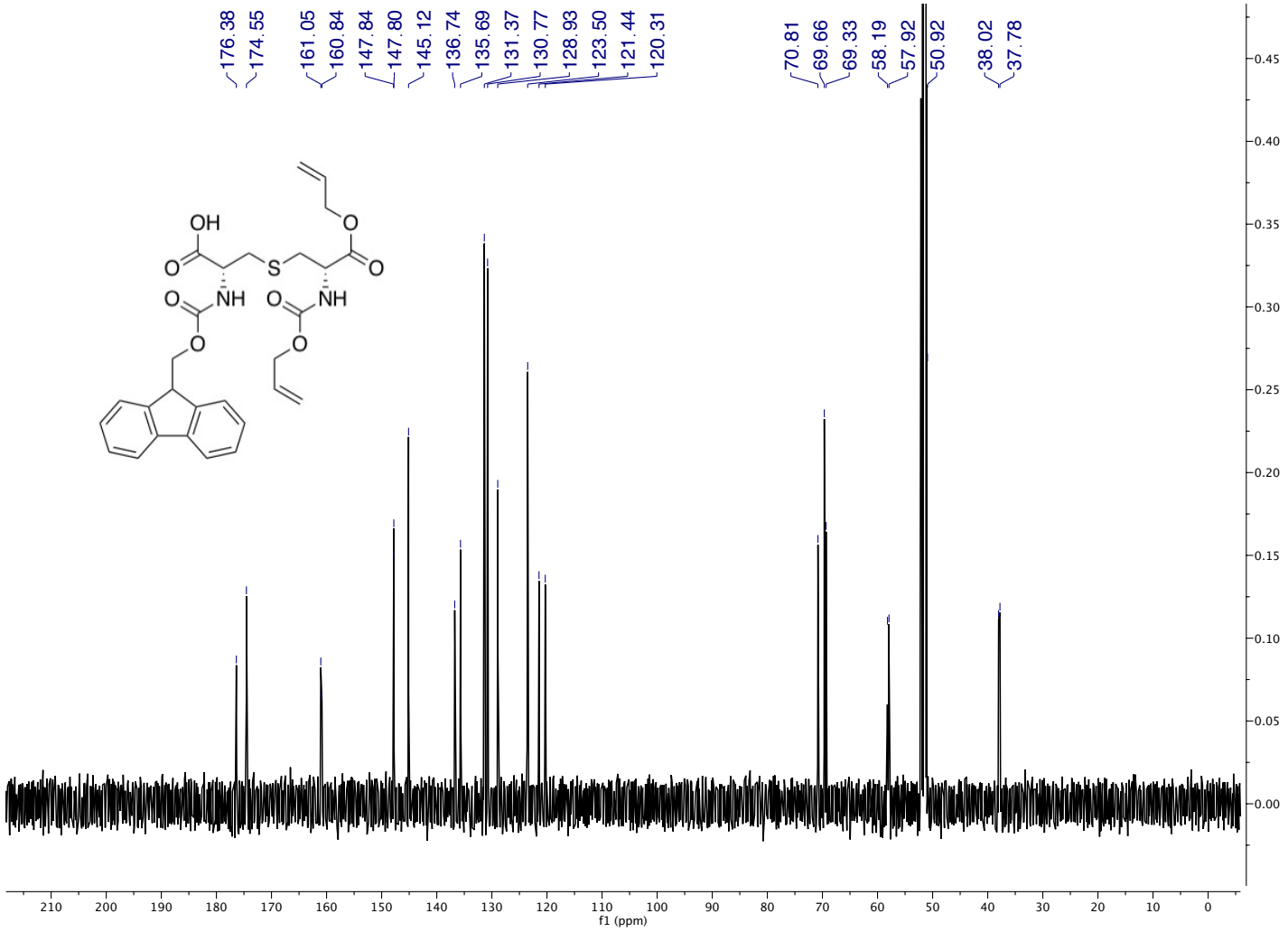
S4 – ^{13}C-DEPT NMR



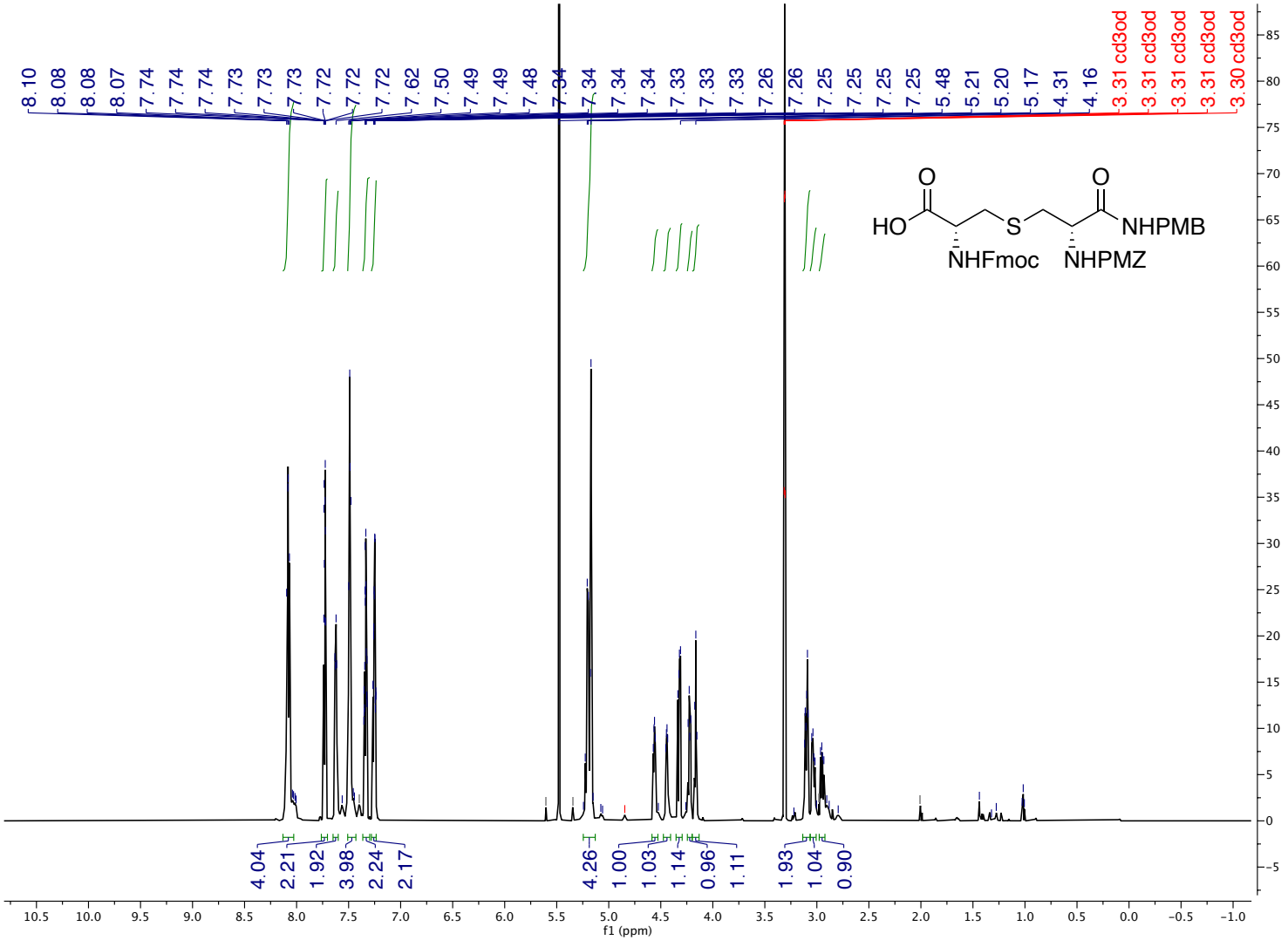
18 – ^{1}H-NMR


7.77
7.76
7.68
7.67
7.66
7.66
7.38
7.37
7.36
7.30
7.29
7.28
5.90
5.89
5.89
5.32
5.31
5.30
5.29
5.29
5.27
5.19
5.17
5.16
5.14
4.60
4.59
4.59
4.53
4.52
4.42
4.42
4.36
4.34
4.31
4.23
4.22
3.31 CD3OD
2.96
2.96
2.95
2.94
OH
O
O
S
O
O
NH
NH
O
O
O
O
1.06
1.17
1.13
1.13
1.00
1.01
1.01
2.14
1.00
1.11
0.63
0.86
1.02
f1 (ppm)

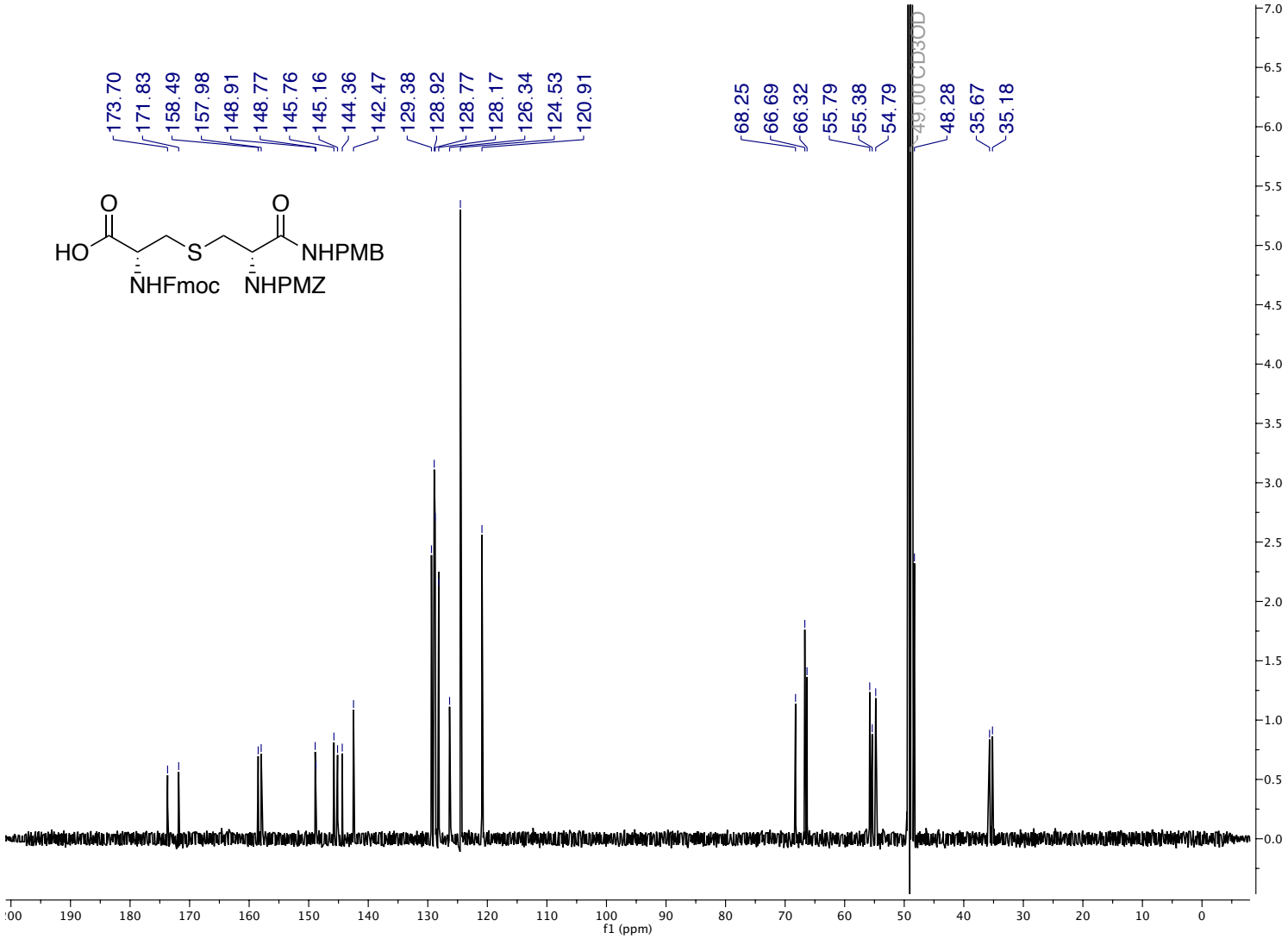
18 – ^{13}C-NMR


176.38
174.55
161.05
160.84
147.84
147.80
145.12
136.74
135.69
131.37
130.77
128.93
123.50
121.44
120.31
70.81
69.66
69.33
58.19
57.92
50.92
38.02
37.78
OH
O
S
NH
NH
f1 (ppm)

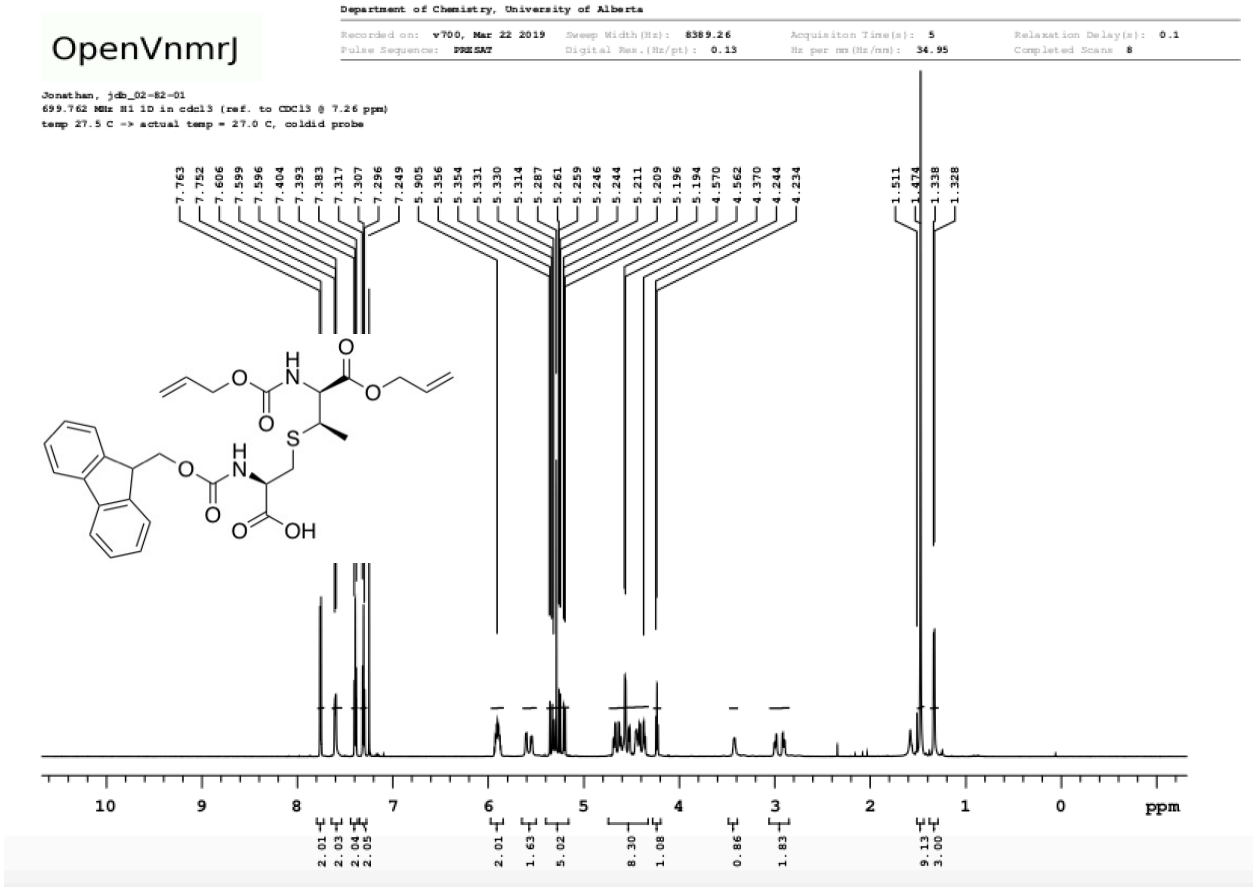
19 – ^{1}H-NMR


8.10 8.08 8.08 8.07 7.74 7.74 7.74 7.73 7.73 7.73 7.72 7.72 7.72 7.62 7.50 7.49 7.49 7.48 7.34 7.34 7.34 7.34 7.33 7.33 7.33 7.26 7.26 7.25 7.25 7.25 7.25 5.48 5.21 5.20 5.17 4.31 4.16 3.31 cd3od 3.31 cd3od 3.31 cd3od 3.31 cd3od 3.30 cd3od
HO
O
S
O
NHPMB
NHFmoc
NHPMZ
4.04 2.21 1.92 3.98 2.24 2.17 4.26 1.00 1.03 1.14 0.96 1.11 1.93 1.04 0.90
f1 (ppm)

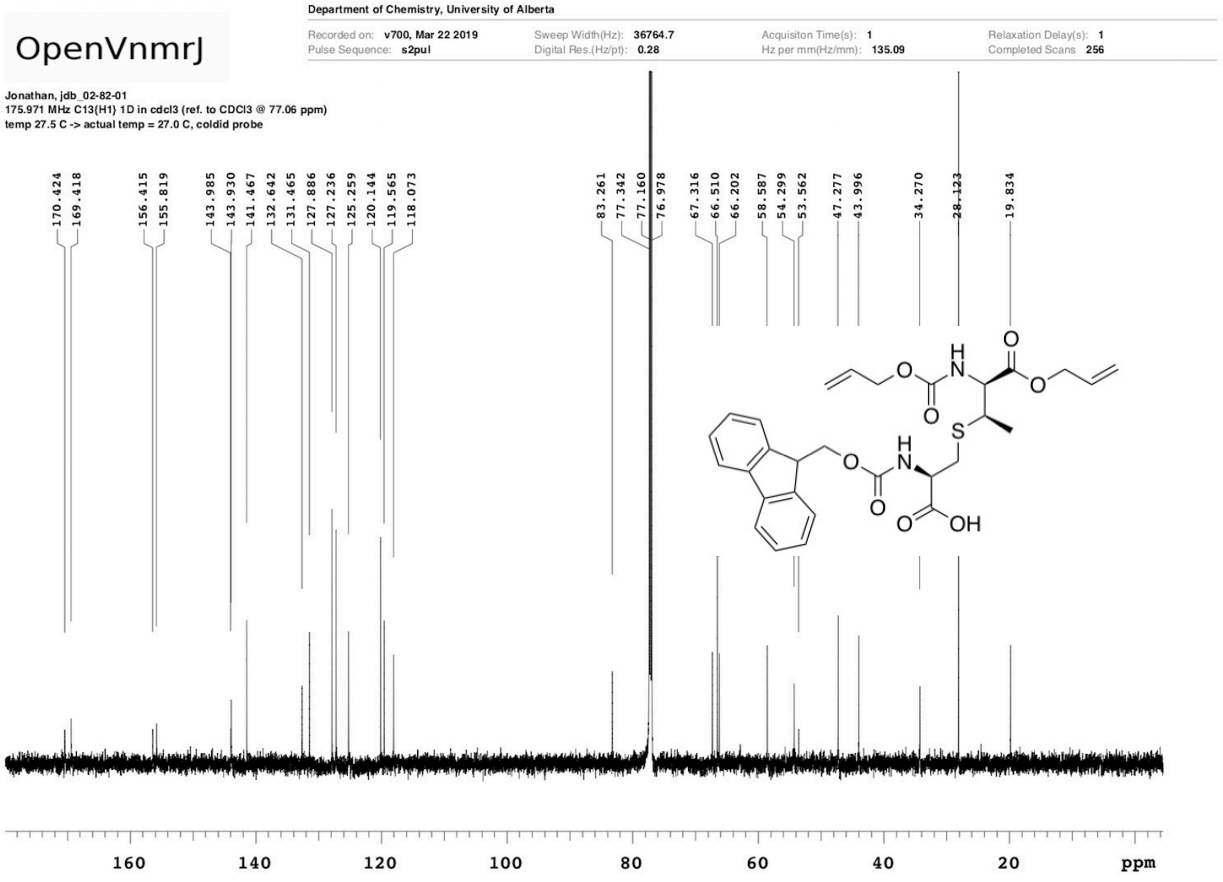
19 – ^{13}C-NMR


173.70
171.83
158.49
157.98
148.91
148.77
145.76
145.16
144.36
142.47
129.38
128.92
128.77
128.17
126.34
124.53
120.91
68.25
66.69
66.32
55.79
55.38
54.79
49.00 CD3OD
48.28
35.67
35.18
O
HO
S
O
NHPMB
NHFmoc
NHPMZ
f1 (ppm)

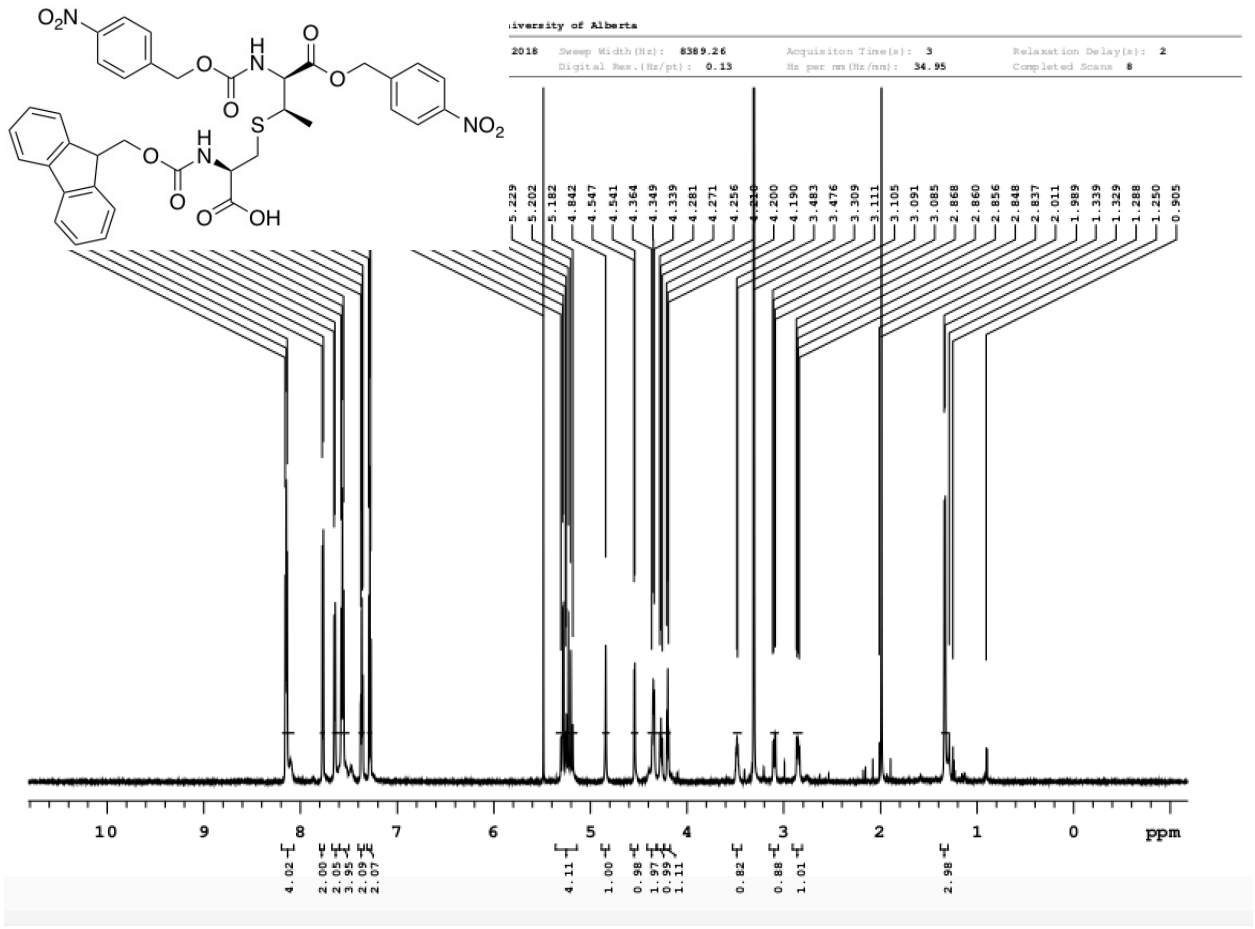
20 – ^{1}H-NMR


Department of Chemistry, University of Alberta
OpenVnmrJ
Recorded on: v700, Mar 22 2019 Sweep Width(Hz): 8389.26 Acquisiton Time(s): 5 Relaxation Delay(s): 0.1
Pulse Sequence: PRESAT Digital Res.(Hz/pt): 0.13 Hz per mm(Hz/mm): 34.95 Completed Scans 8
Jonathan, jdb_02-82-01
699.762 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm)
temp 27.5 C => actual temp = 27.0 C, coldid probe
7.763 7.752 7.606 7.599 7.596 7.404 7.393 7.383 7.317 7.307 7.296 7.249 5.905 5.356 5.354 5.331 5.330 5.314 5.287 5.261 5.259 5.246 5.244 5.211 5.209 5.196 5.194 4.570 4.562 4.370 4.244 4.234 1.511 1.474 1.338 1.328
10 9 8 7 6 5 4 3 2 1 0 ppm
2.01 2.03 2.04 2.05 2.01 1.63 5.02 8.30 1.08 0.86 1.83 9.13 3.00

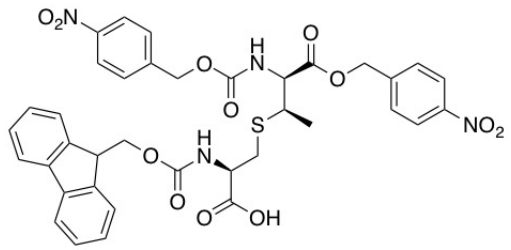
20 – ^{13}C-NMR

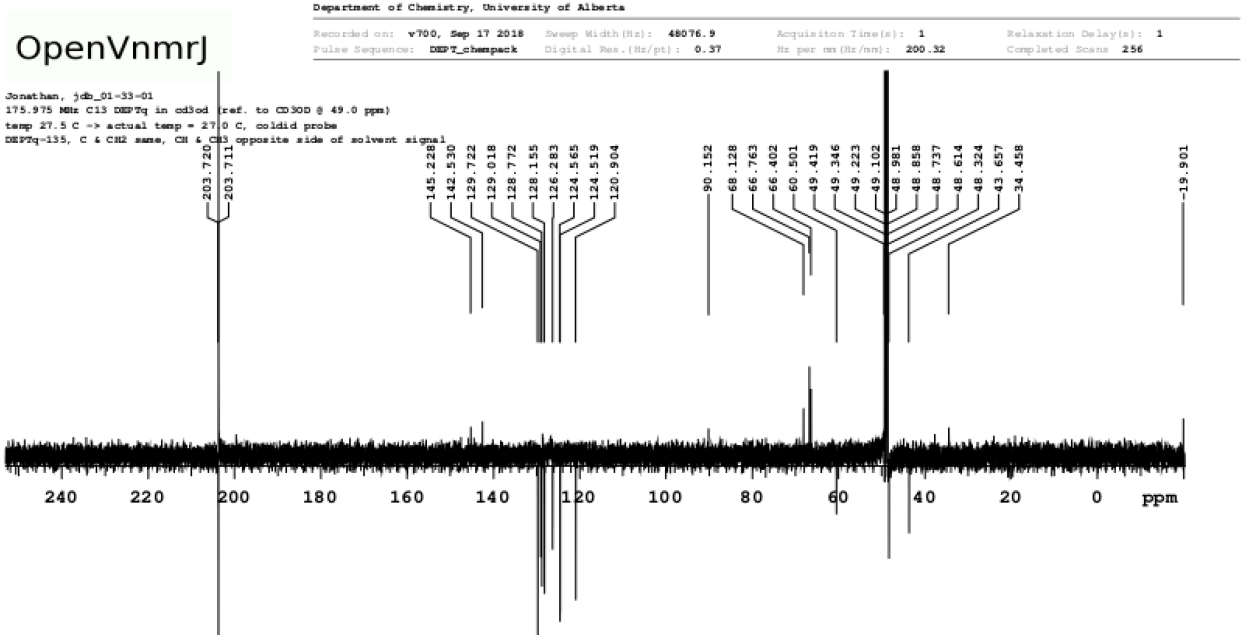

Department of Chemistry, University of Alberta
OpenVnmrJ
Recorded on: v700, Mar 22 2019
Pulse Sequence: s2pul
Sweep Width(Hz): 36764.7
Digital Res.(Hz/pt): 0.28
Acquisiton Time(s): 1
Hz per mm(Hz/mm): 135.09
Relaxation Delay(s): 1
Completed Scans 256
Jonathan, jdb_02-82-01
175.971 MHz C13{H1} 1D in cdcl3 (ref. to CDCl3 @ 77.06 ppm)
temp 27.5 C -> actual temp = 27.0 C, coldid probe
170.424
169.418
156.415
155.819
143.985
143.930
141.467
132.642
131.465
127.886
127.236
125.259
120.144
119.565
118.073
83.261
77.342
77.160
76.978
67.316
66.510
66.202
58.587
54.299
53.562
47.277
43.996
34.270
28.123
19.834
160
140
120
100
80
60
40
20
ppm

21 – ^{1}H-NMR

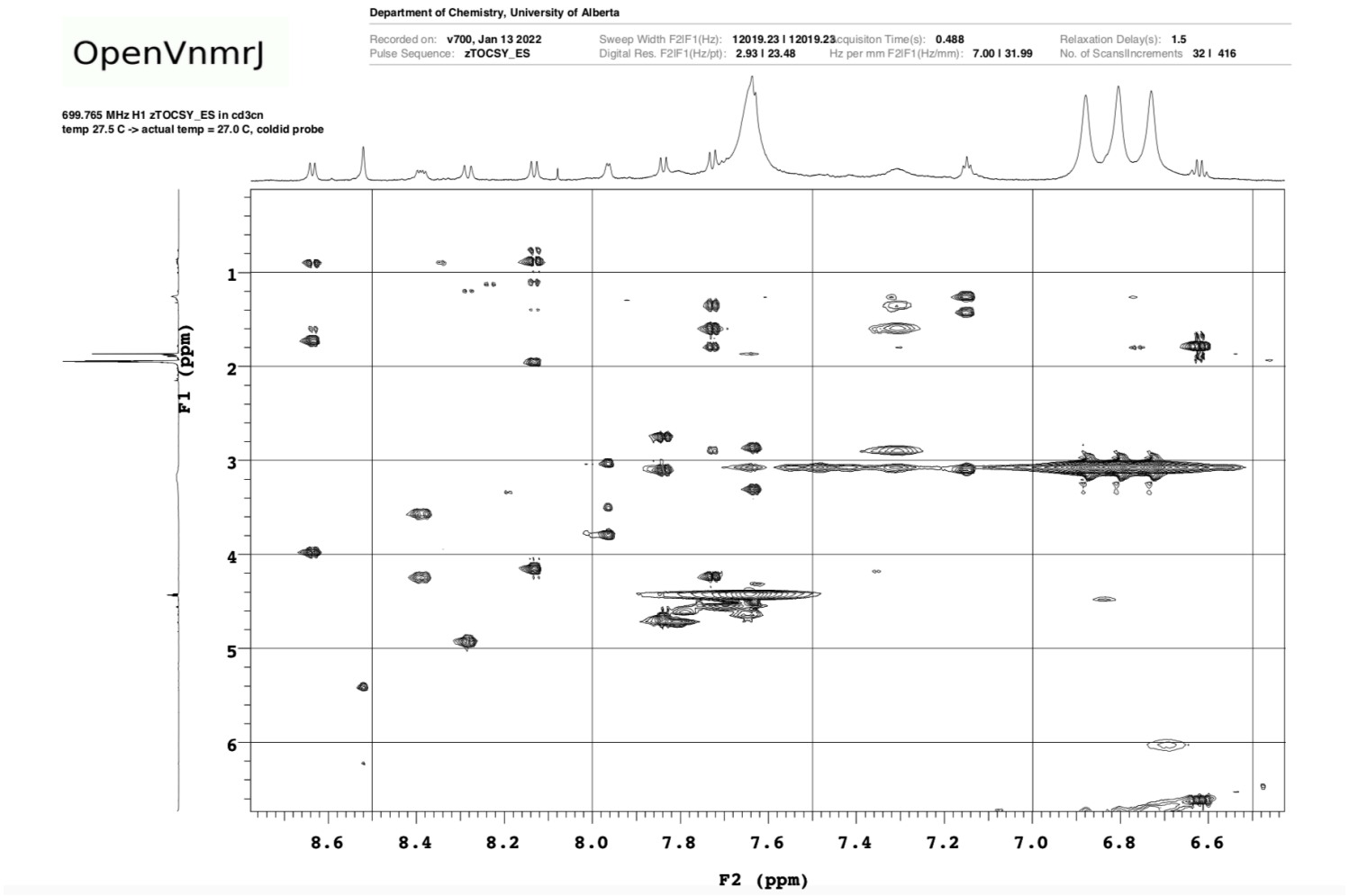

iversity of Alberta
2018 Sweep Width(Hz): 8389.26 Acquisition Time(s): 3 Relaxation Delay(s): 2
Digital Res.(Hz/pt): 0.13 Hz per mm(Hz/mm): 34.95 Completed Scans 8
5.229 5.202 5.182 4.842 4.547 4.541 4.364 4.349 4.339 4.281 4.271 4.256 4.210 4.200 4.190 3.483 3.476 3.309 3.111 3.105 3.091 3.085 2.868 2.860 2.856 2.848 2.837 2.011 1.989 1.339 1.329 1.288 1.250 0.905
10 9 8 7 6 5 4 3 2 1 0 ppm
4.02 2.00 2.05 2.05 2.09 2.07 4.11 1.00 0.98 1.97 0.99 1.11 0.82 0.88 1.01 2.98

21 – ^{13}C DEPT NMR

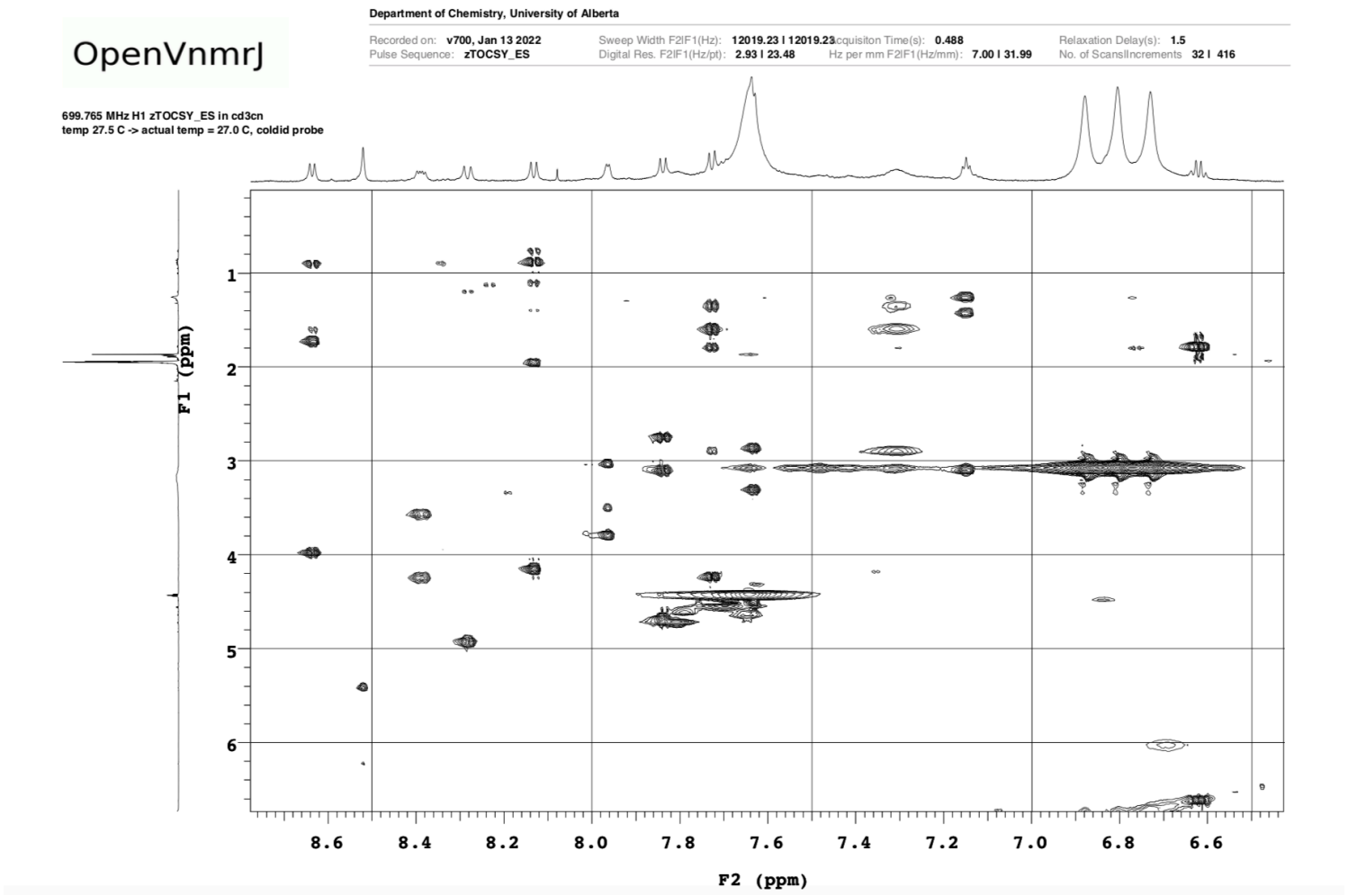




Synthetic Lipopeptide **1** - TOCSY Data



Synthetic Lipopeptide **1** - ROESY Data



Synthetic (1) and semi-synthetic (1*) lipopeptide TOCSY NMR comparison

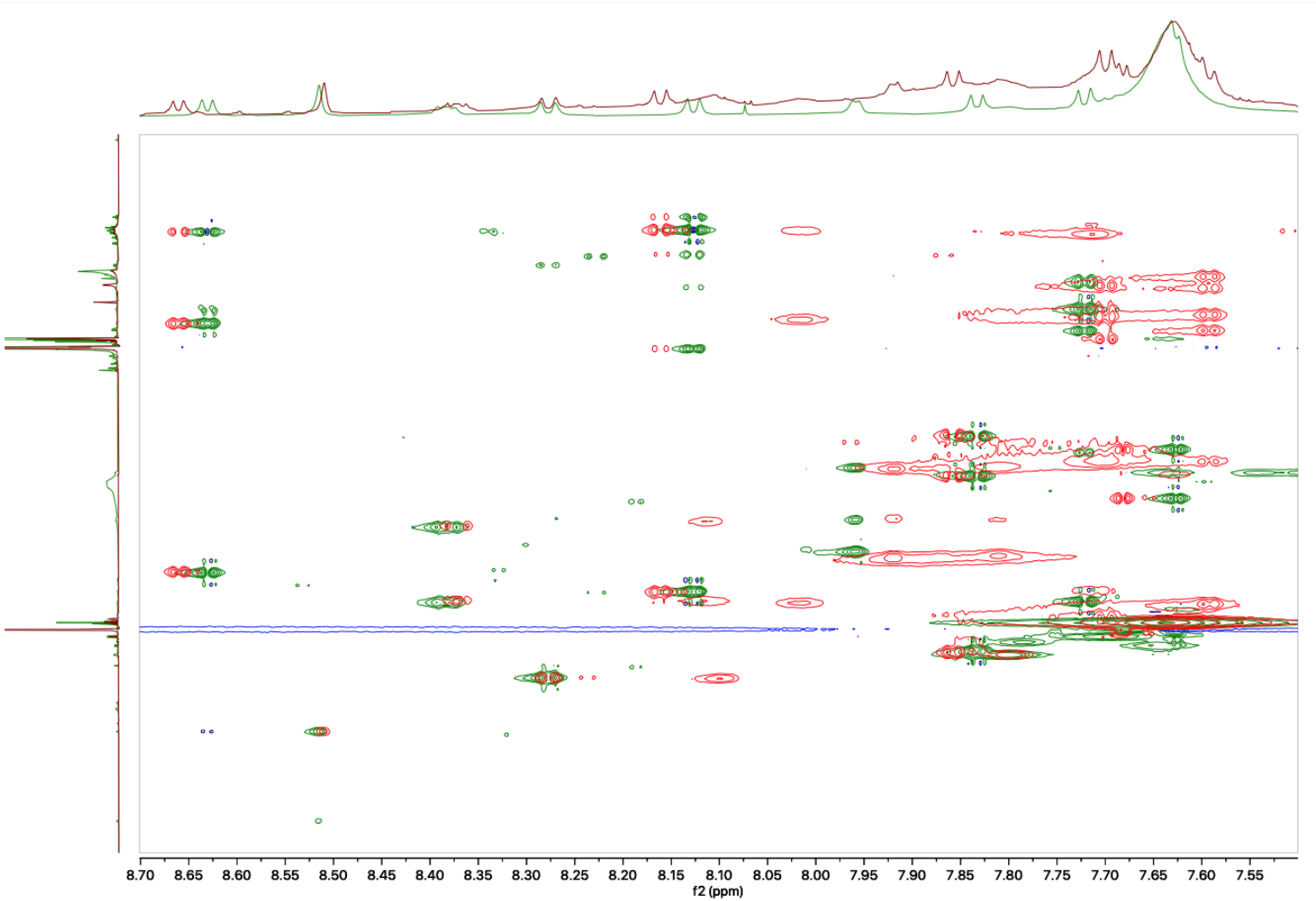


Figure 4 – Overlay of the ^{1}H-NMR and TOCSY NMR spectra of the synthetic lipopeptide (**1**) in red and the semi-synthetic lipopeptide (**1***) in green showing small discrepancies in the chemical shifts presumably due to slight differences in pH and/or concentration.

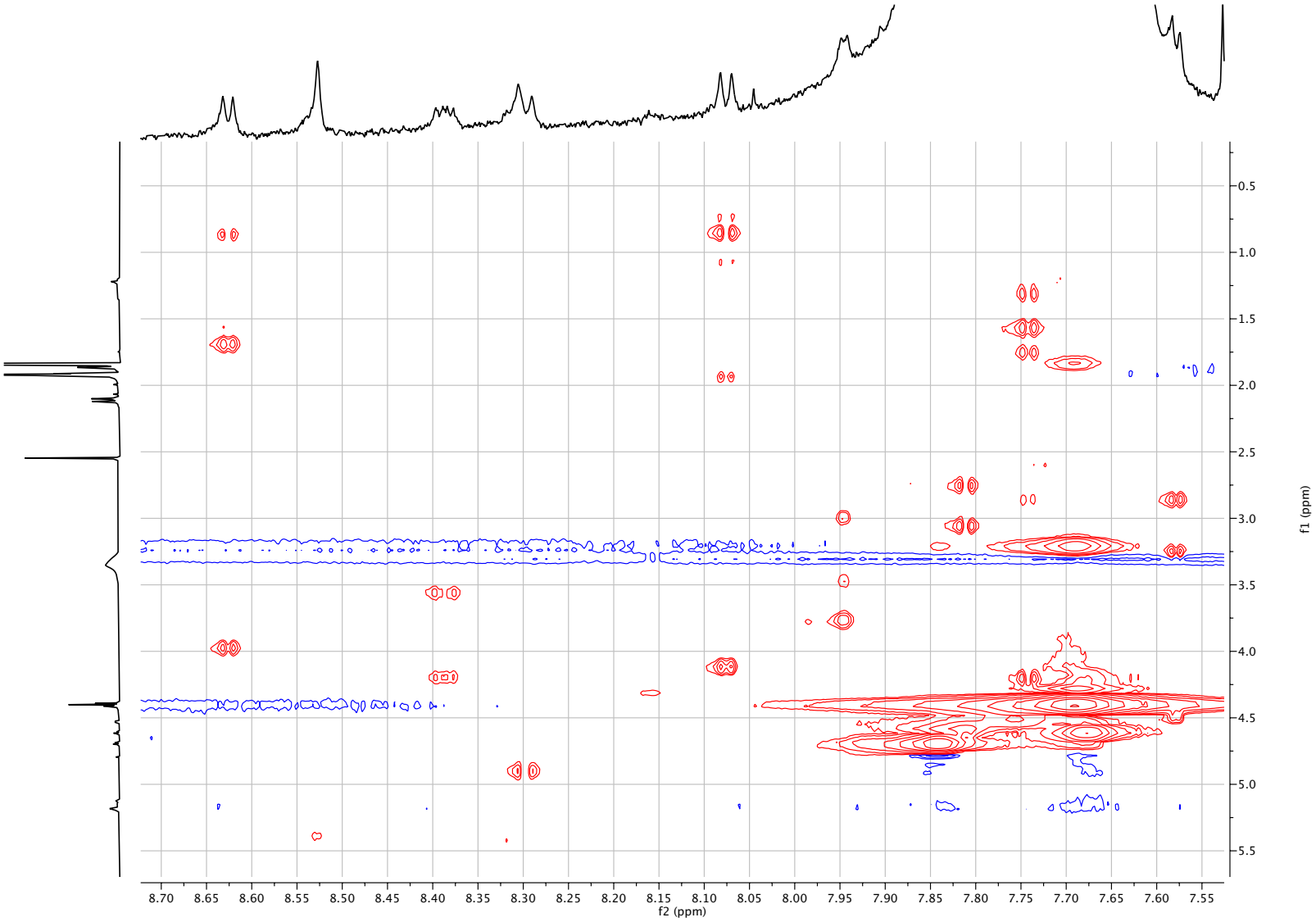


Figure 5 – Mixed sample ^{1}H-NMR and TOCSY NMR experiment showing a 1:2 mixture of synthetic lipopeptide (**1**) and semi-synthetic lipopeptide (**1***) confirming that the two compounds are chemically identical.

References

1 S. Xiong, N. Wang, C. Liu, H. Shen, Z. Qu, L. Zhu, X. Bai, H.-G. Hu, W. Cong and L. Zhao, *Bioorganic Med. Chem. Lett.*, 2021, **37**, 127831.

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