Electronic Supplementary Material (ESI) for RSC Advances.
This journal is © The Royal Society of Chemistry 2015

SUPPORTING INFORMATION

Plasmodium *falciparum* Subtilisin-like Protease 1: Discovery of Potent Difluorostatone-based Inhibitors

S. Giovani,[a,b,c,†] M. Penzo,[d,†] S. Butini,[a,b,c] M. Brindisi,[a,b,c] S. Gemma,[a,b,c,*] E. Novellino,[a,e] G. Campiani,[a,b,c,*], M. J. Blackman,[d] S. Brogi[a,b,c]

[a]*European Research Centre for Drug Discovery and Development (NatSynDrug), University of Siena, via Aldo Moro 2, 53100, Siena, Italy.*

[b]*Dept. Biotechnology, Chemistry, and Pharmacy, University of Siena, via Aldo Moro 2, 53100, Siena, Italy.*

[c]*Centro Interuniversitario di Ricerche sulla Malaria (CIRM), University of Perugia, Perugia, Italy.*

[d]*Division of Parasitology, MRC National Institute for Medical Research, Mill Hill, London, NW7 1AA, UK*

[e]*Dip. di Farmacia, University of Naples Federico II, Via D. Montesano 49, 80131 Naples, Italy*

Table of Contents

Figure S1 S2

Figure S2 S2

Figure S3 S3

Figure S4 S3

Figure S5 S4

^{1}H NMR spectra S5

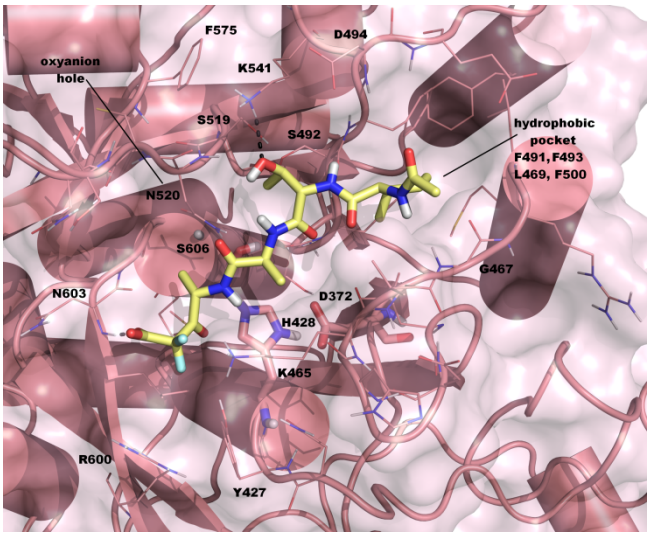


Figure S1. Docked pose of **3** (light yellow sticks) in the cavity of the PfSUB1 (PDB ID: 4LVN). H-bonds are indicated by black dotted lines. The catalytic triad is represented by salmon sticks while the other residues in the binding site were represented by lines. Molecular graphics were generated by PyMOL (The PyMOL Molecular Graphics System, v1.6-alpha; Schrodinger LLC: New York, 2013). Nonpolar hydrogens were omitted for clarity**.**

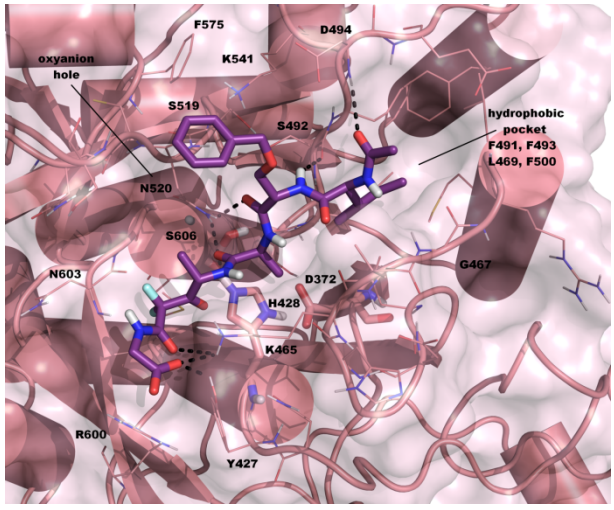


Figure S2. Docked pose of **9** (light yellow sticks) in the cavity of the PfSUB1 (PDB ID: 4LVN). H-bonds are indicated by black dotted lines. Catalytic triad is represented by salmon sticks while the other residues in the binding site were represented by lines. Molecular graphics were generated by PyMOL (The PyMOL Molecular Graphics System, v1.6-alpha; Schrodinger LLC: New York, 2013). Nonpolar hydrogens were omitted for clarity.

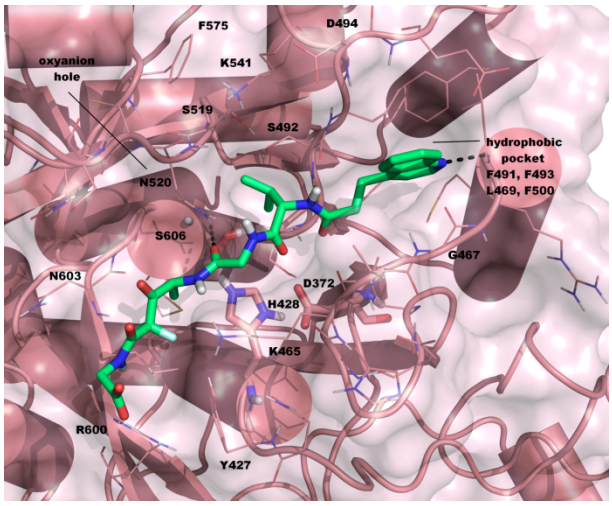


Figure S3. Docked pose of **17** (light green sticks) in its neutral state, in the cavity of the PfSUB1 (PDB ID: 4LVN). H-bonds are indicated by black dotted lines. Catalytic triad is represented by salmon sticks while the other residues in the binding site were represented by lines. Molecular graphics were generated by PyMOL (The PyMOL Molecular Graphics System, v1.6-alpha; Schrodinger LLC: New York, 2013). Nonpolar hydrogens were omitted for clarity.

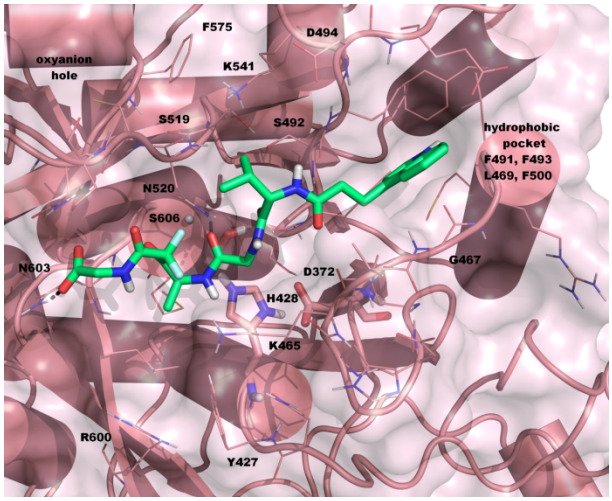


Figure S4. Docked pose of **17** (light green sticks) in its protonated state, in the cavity of the PfSUB1 (PDB ID: 4LVN). H-bonds are indicated by black dotted lines. Catalytic triad is represented by salmon sticks while the other residues in the binding site were represented by lines. Molecular graphics were generated by PyMOL (The PyMOL Molecular Graphics System, v1.6-alpha; Schrodinger LLC: New York, 2013). Nonpolar hydrogens were omitted for clarity.

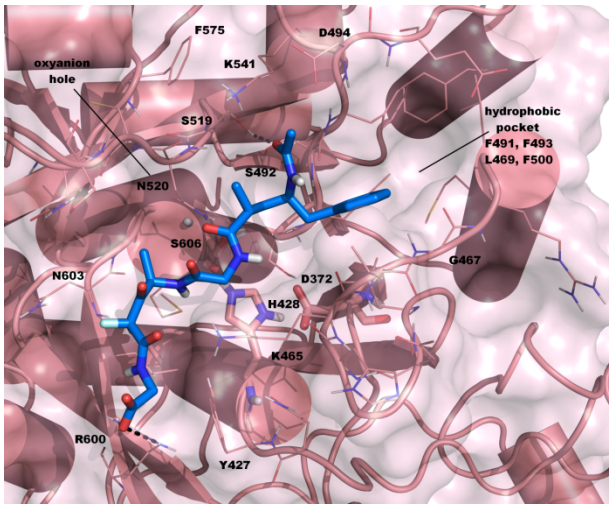
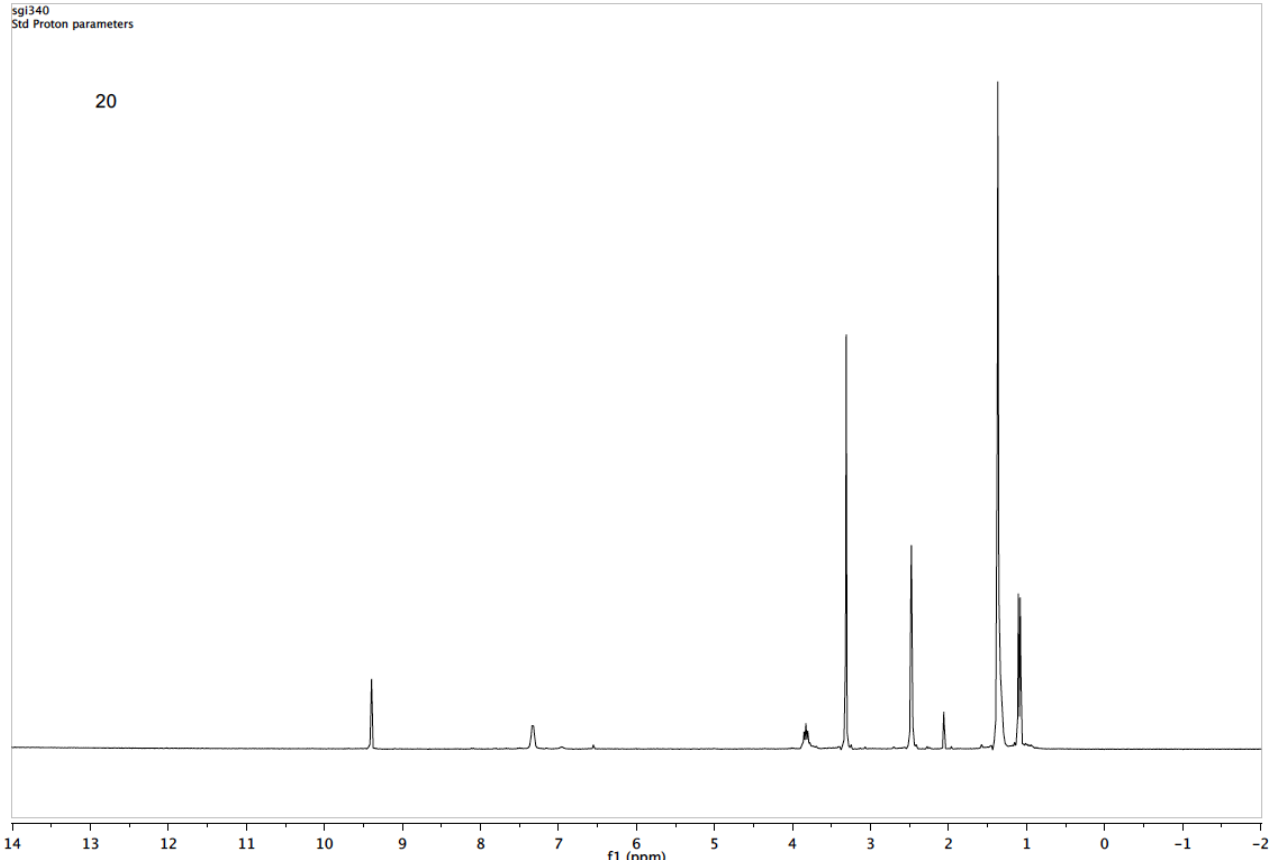
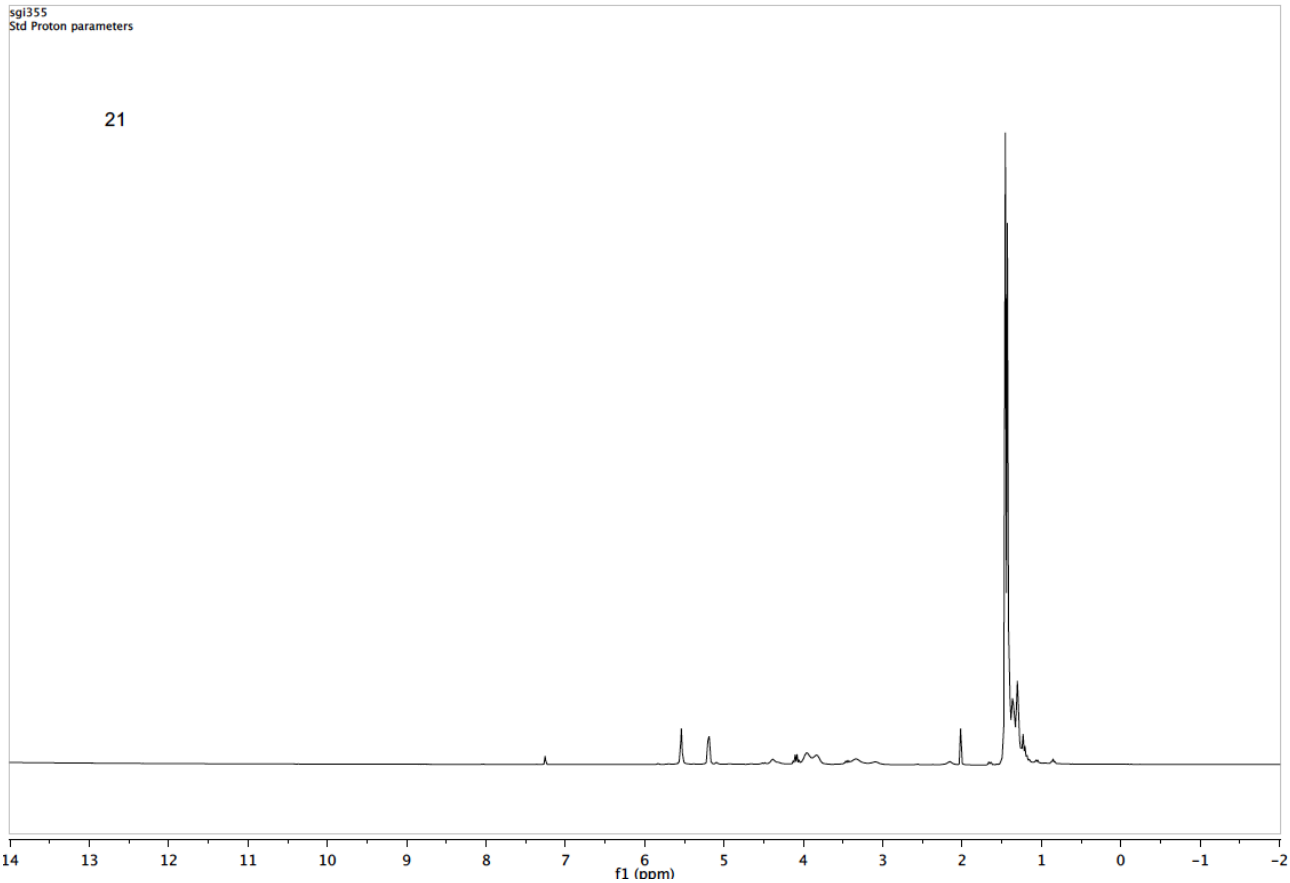
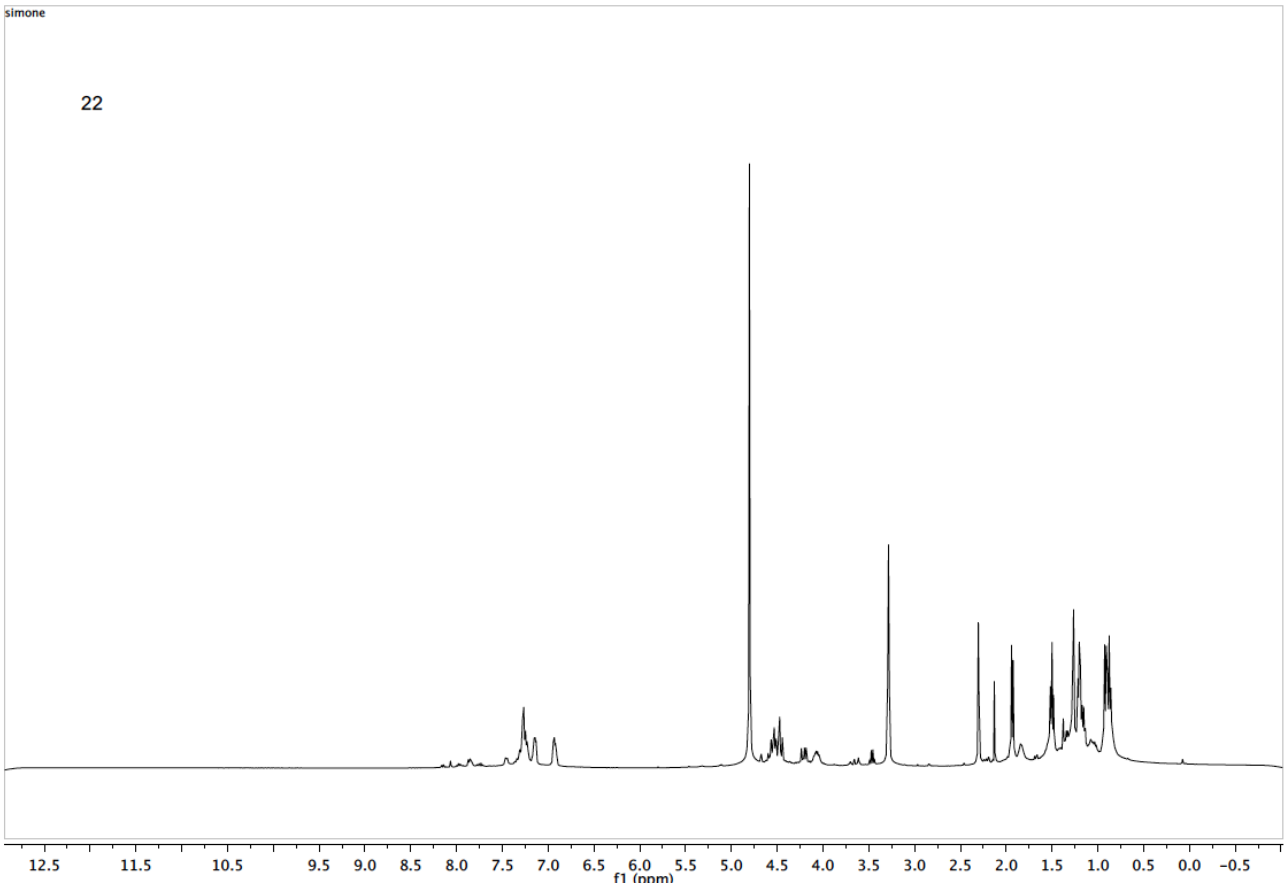


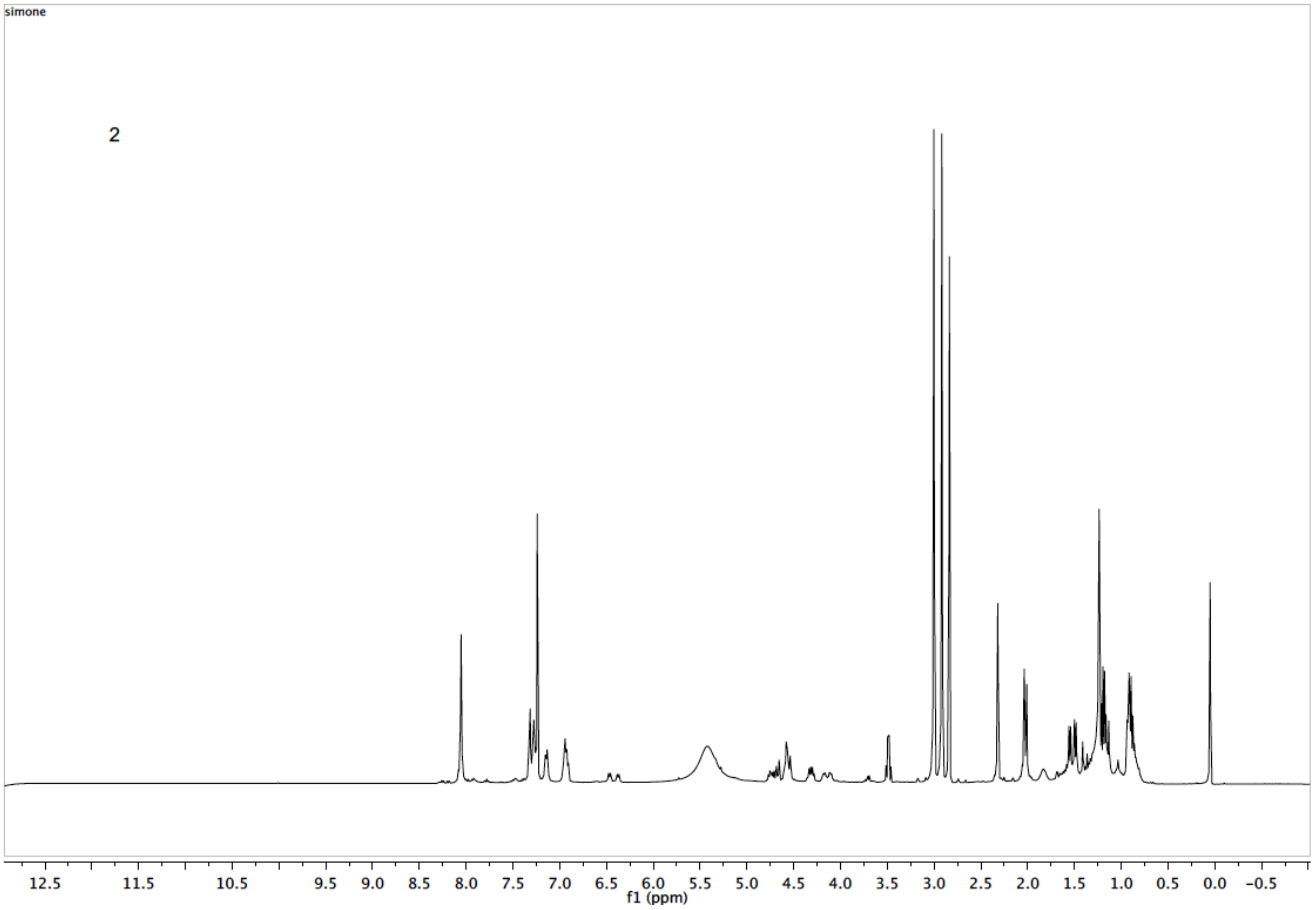
Figure S5. Docked pose of **18** (blue sticks) in the cavity of the PfSUB1 (PDB ID: 4LVN). H-bonds are indicated by black dotted lines. Catalytic triad is represented by salmon sticks while the other residues in the binding site were represented by lines. Accordingly, the Goldscore value is 67.34, while the ΔG_{bind} is -71.95 kcal/mol. Molecular graphics were generated by PyMOL (The PyMOL Molecular Graphics System, v1.6-alpha; Schrodinger LLC: New York, 2013). Nonpolar hydrogens were omitted for clarity.

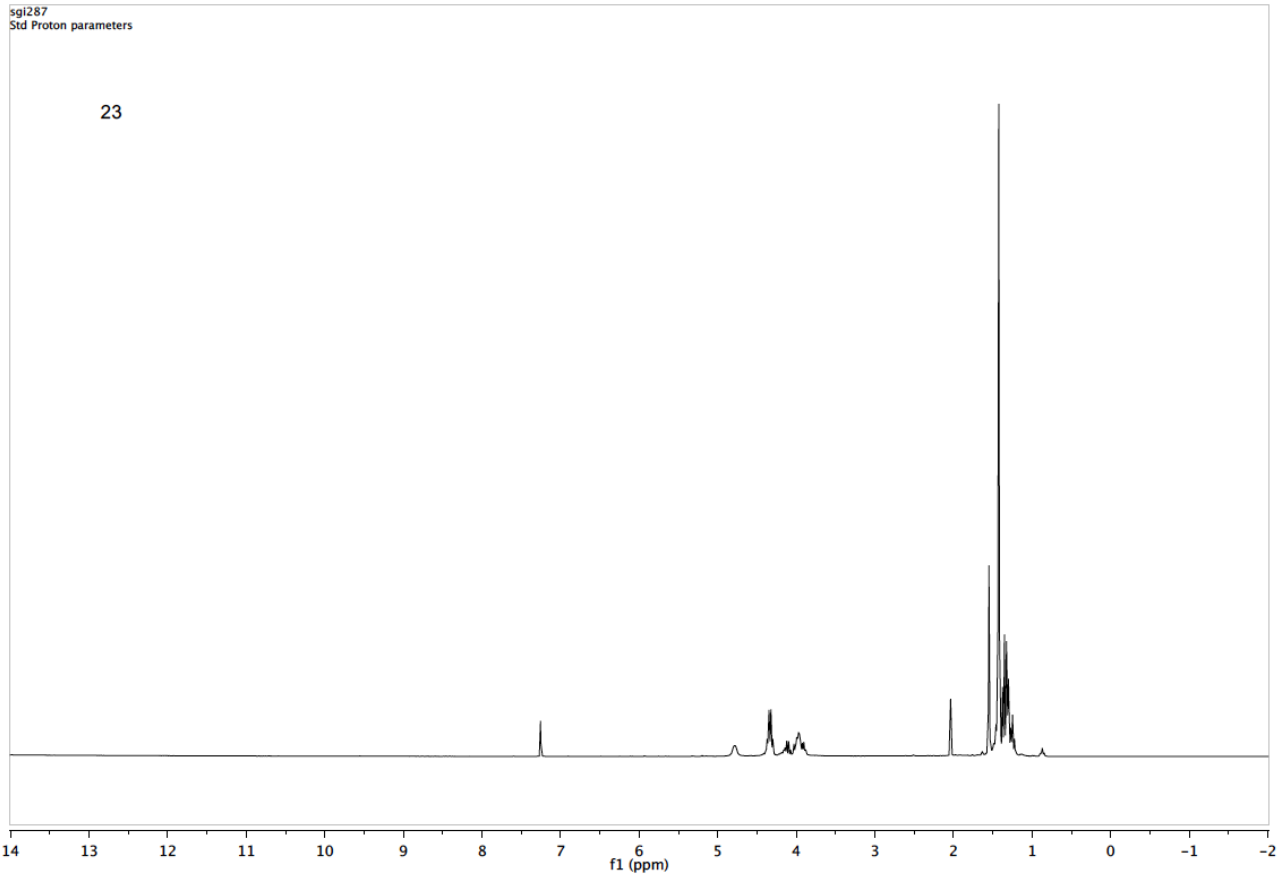
^{1}H NMR spectra

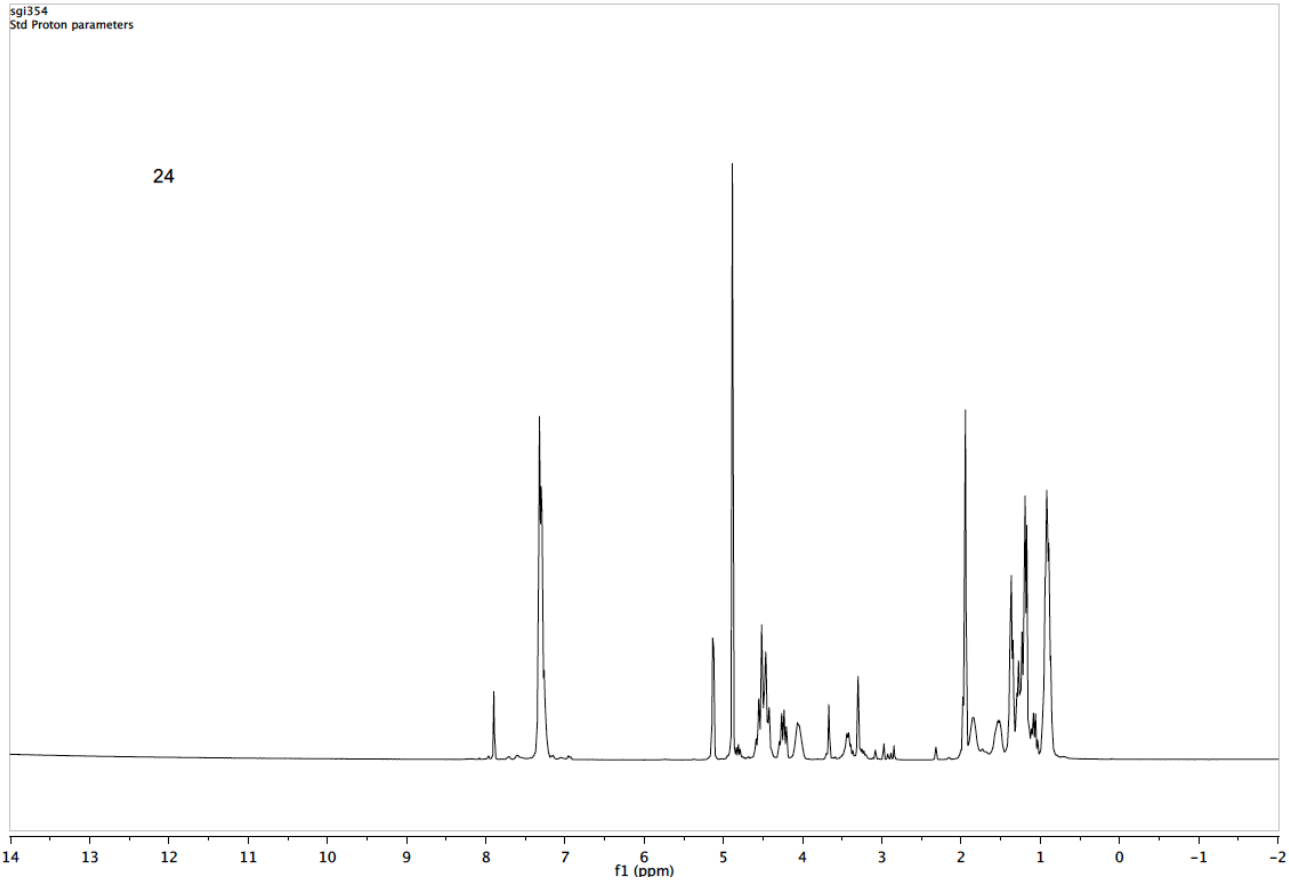


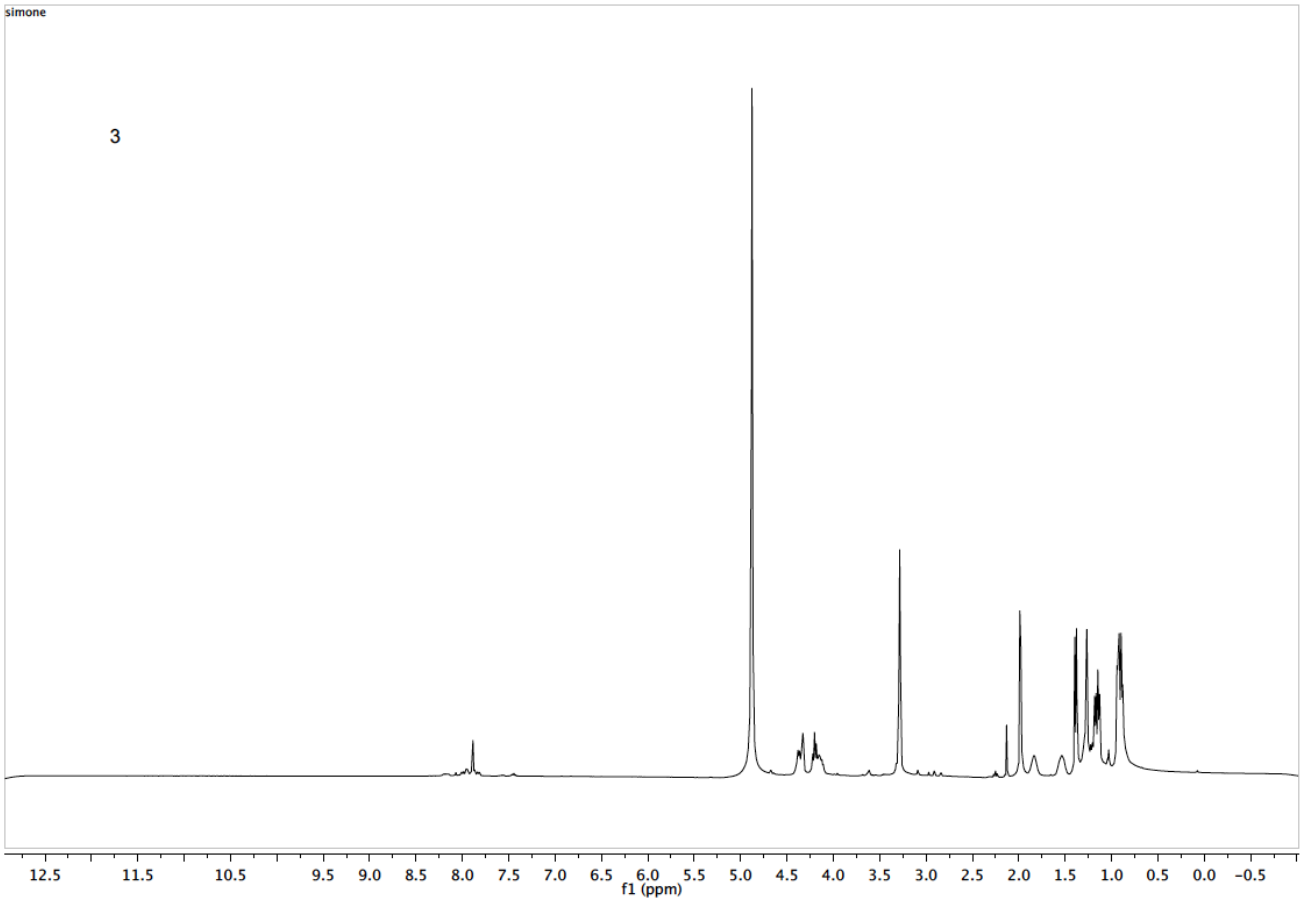


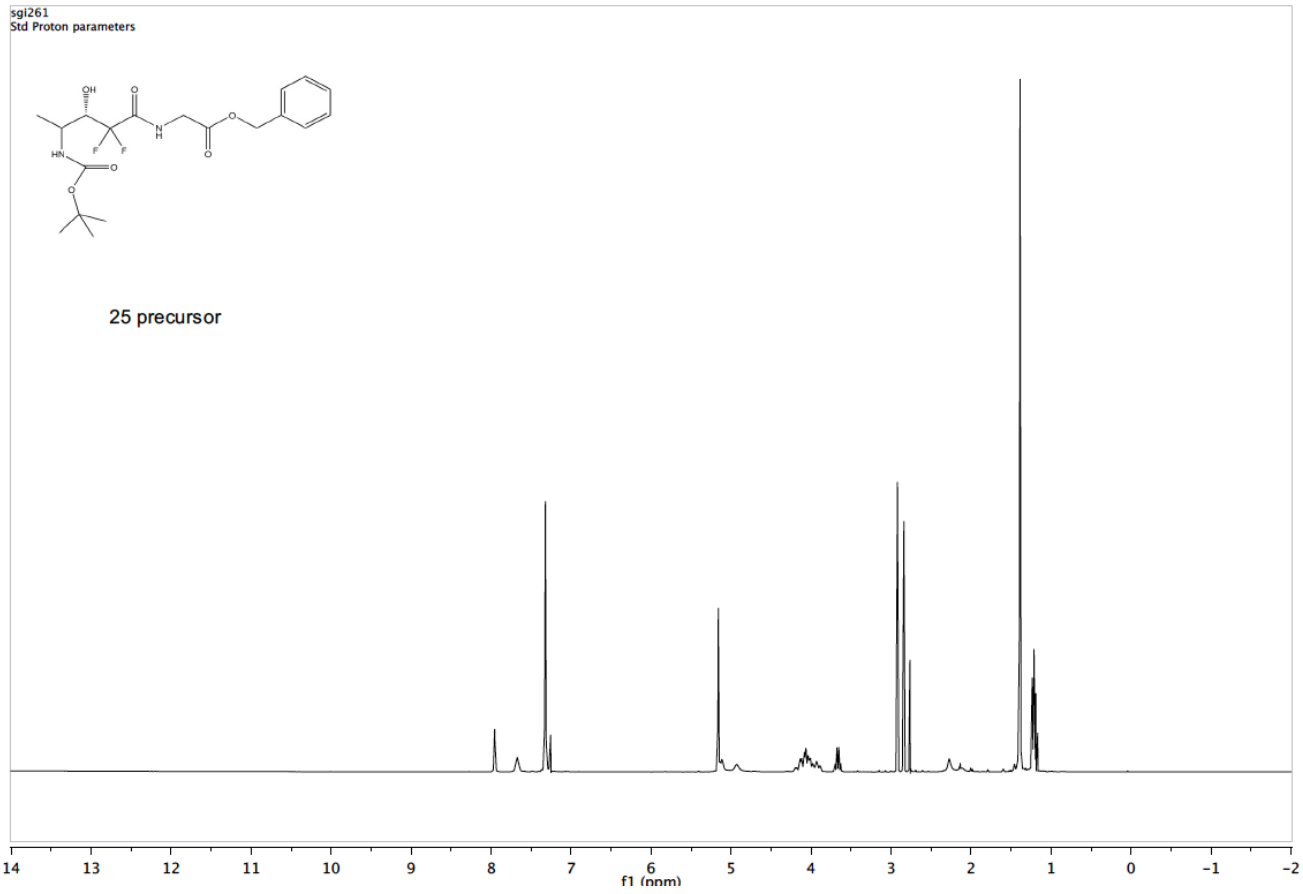

simone
22
12.5 11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

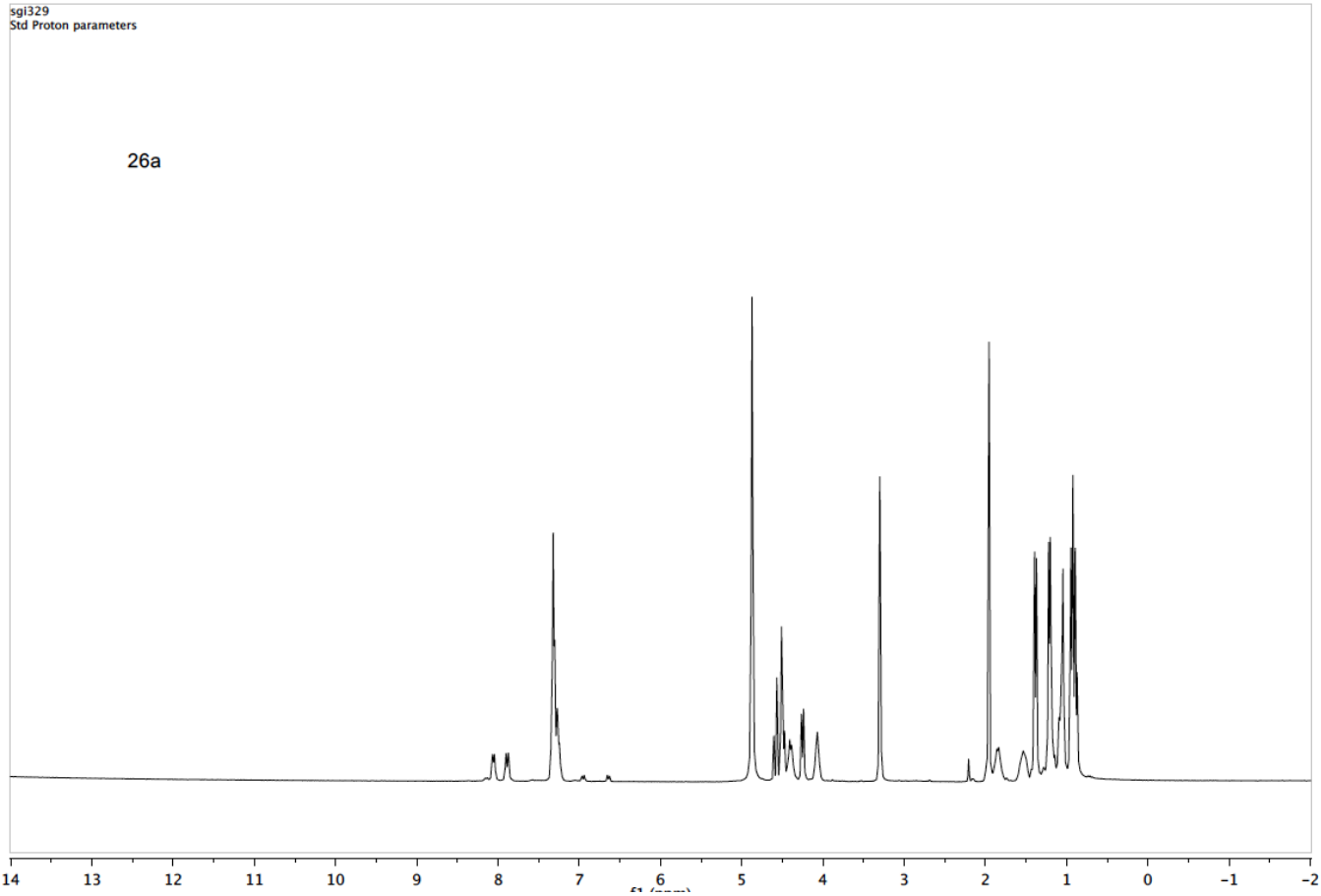

simone
2
12.5 11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

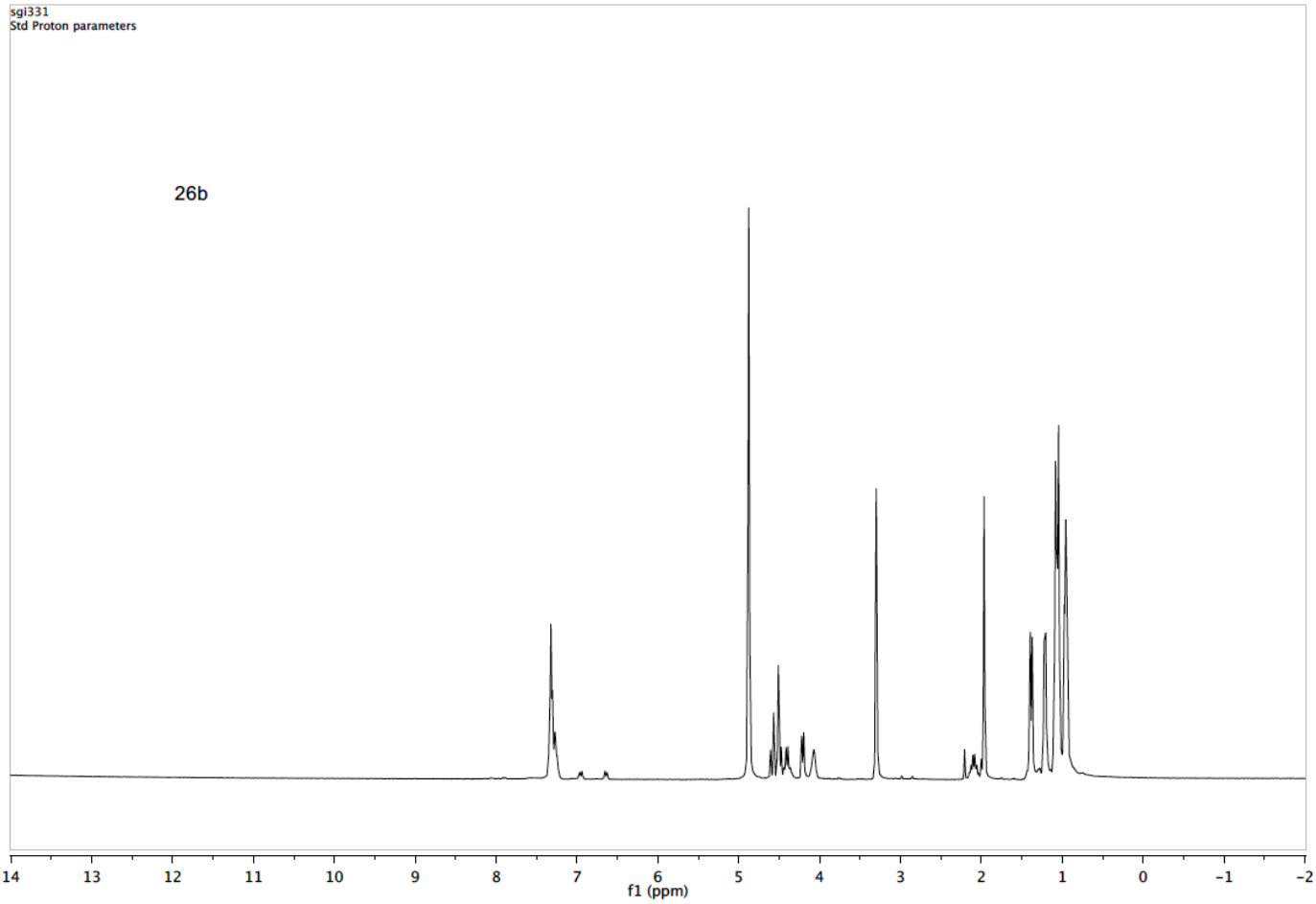

sgi287
Std Proton parameters
23
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

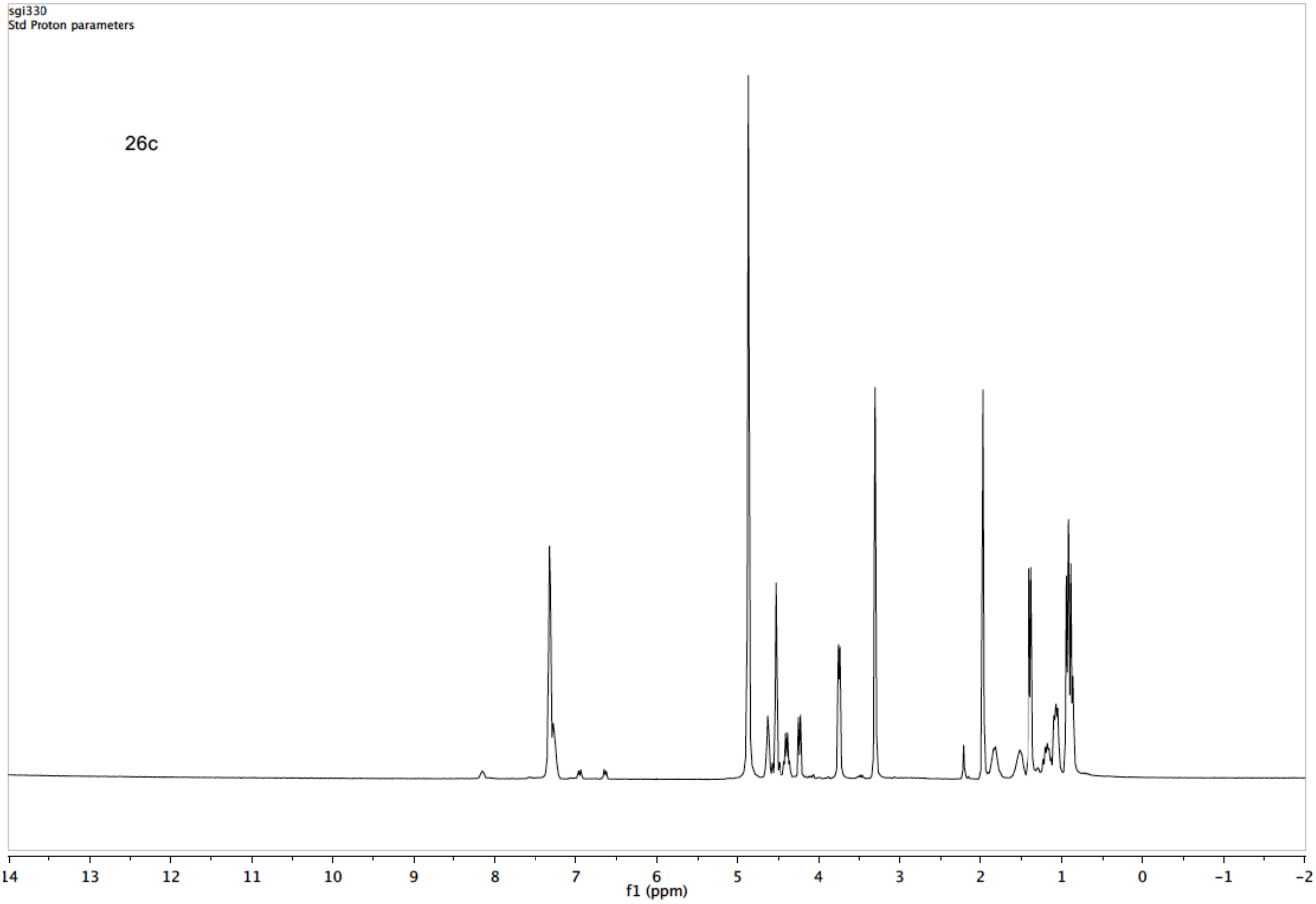

sgi354
Std Proton parameters
24
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

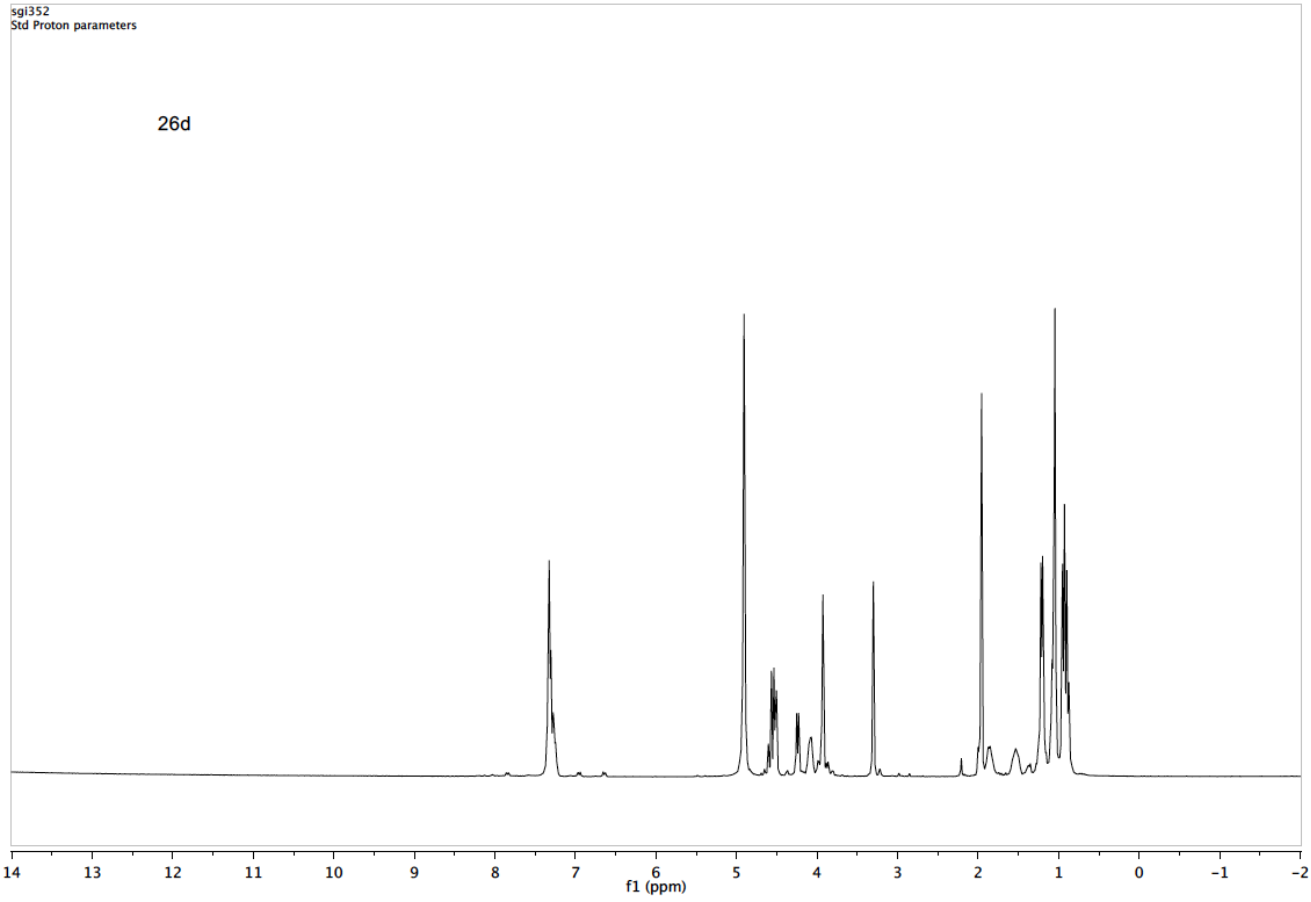

simone
3
12.5 11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

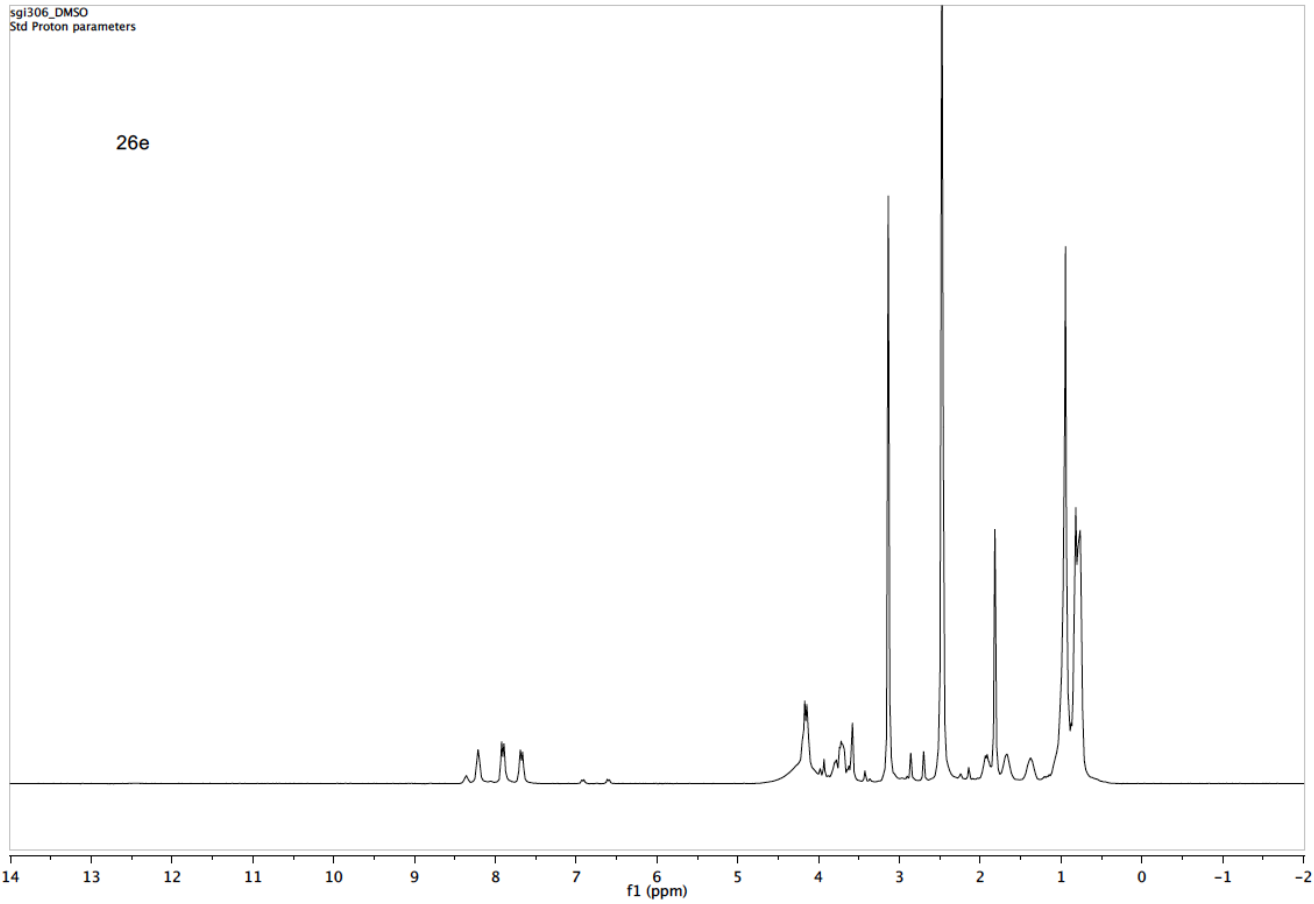

sgi261
Std Proton parameters
25 precursor
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

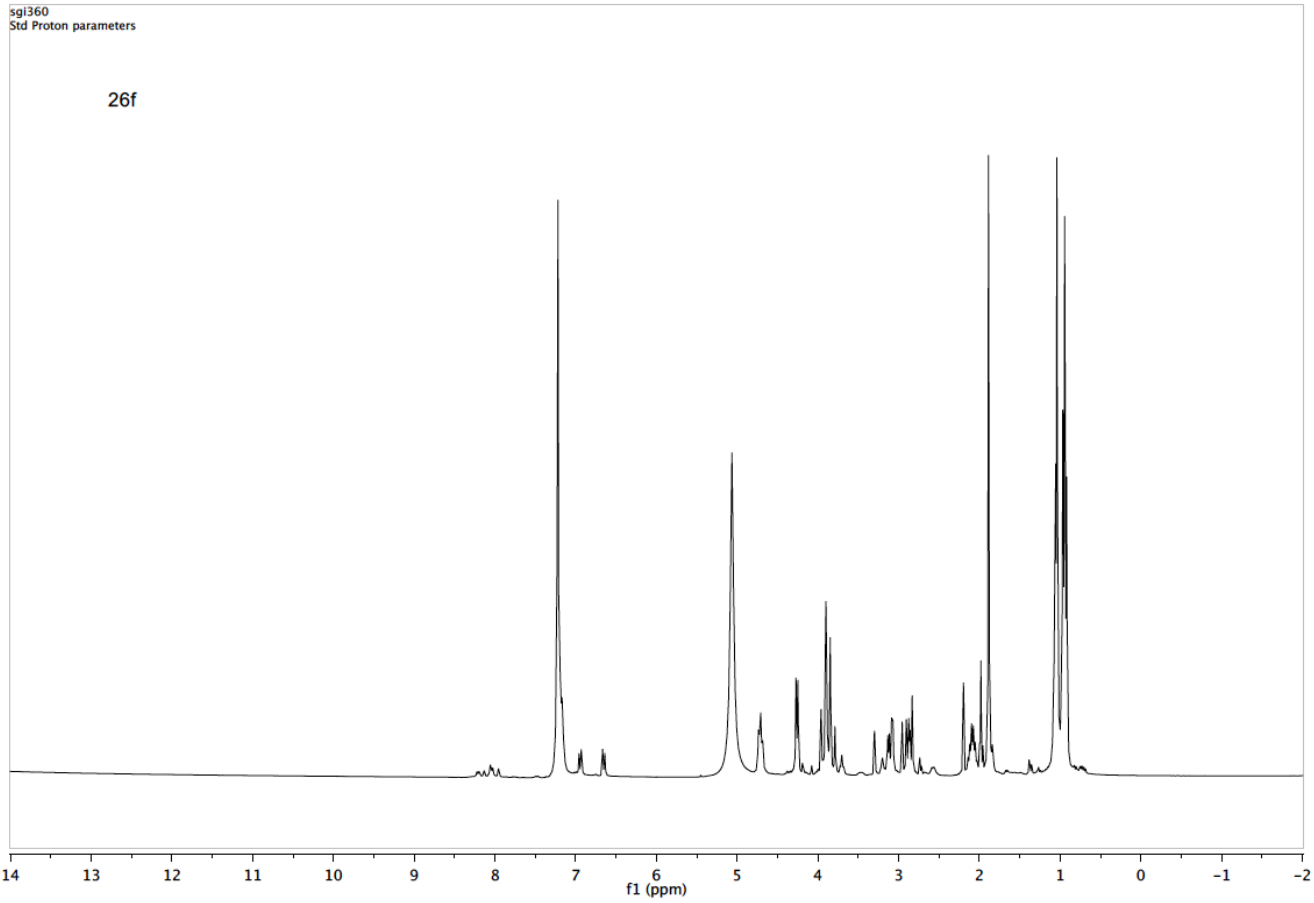

sgi329
Std Proton parameters
26a
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2

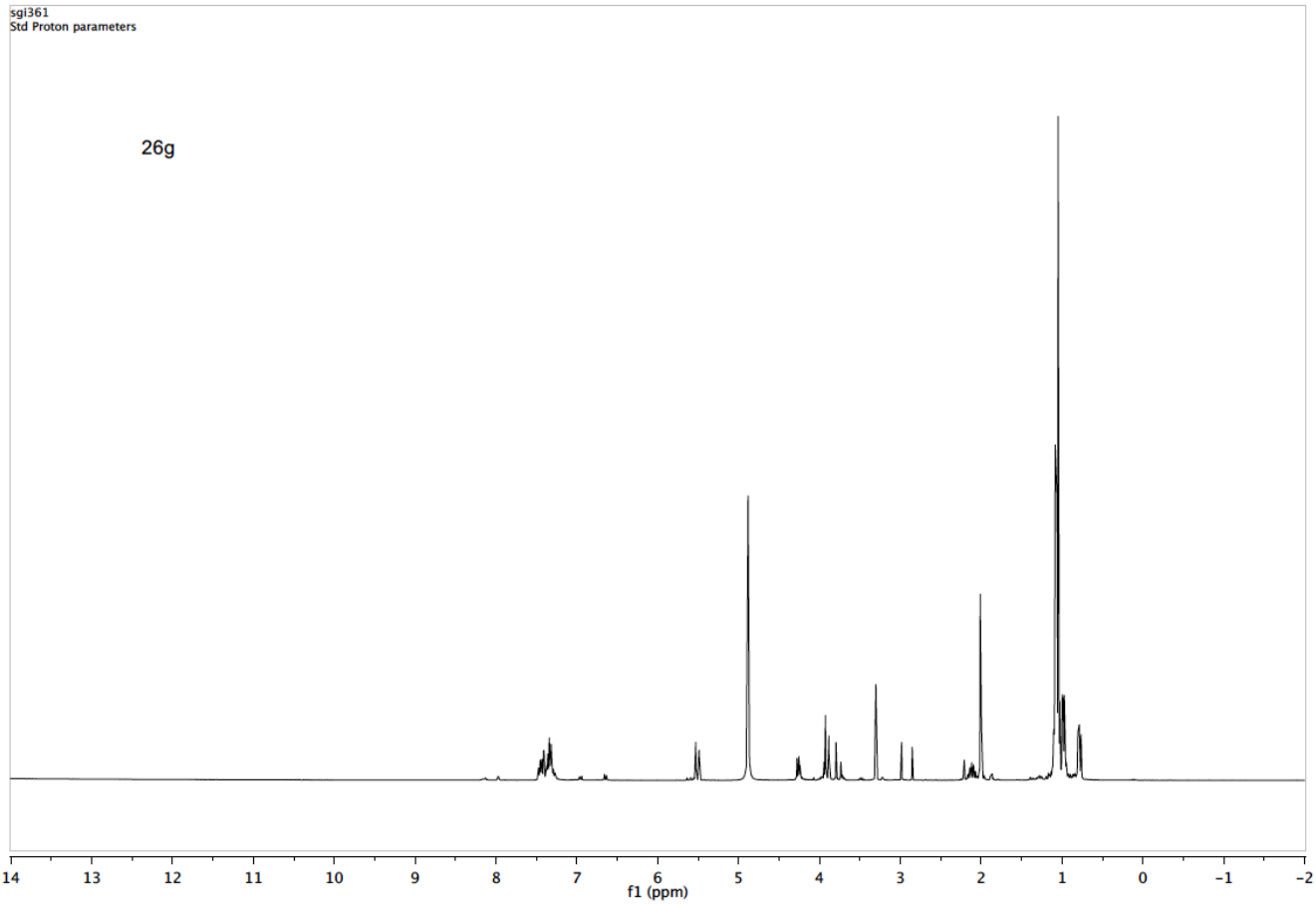

sgi331
Std Proton parameters
26b
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

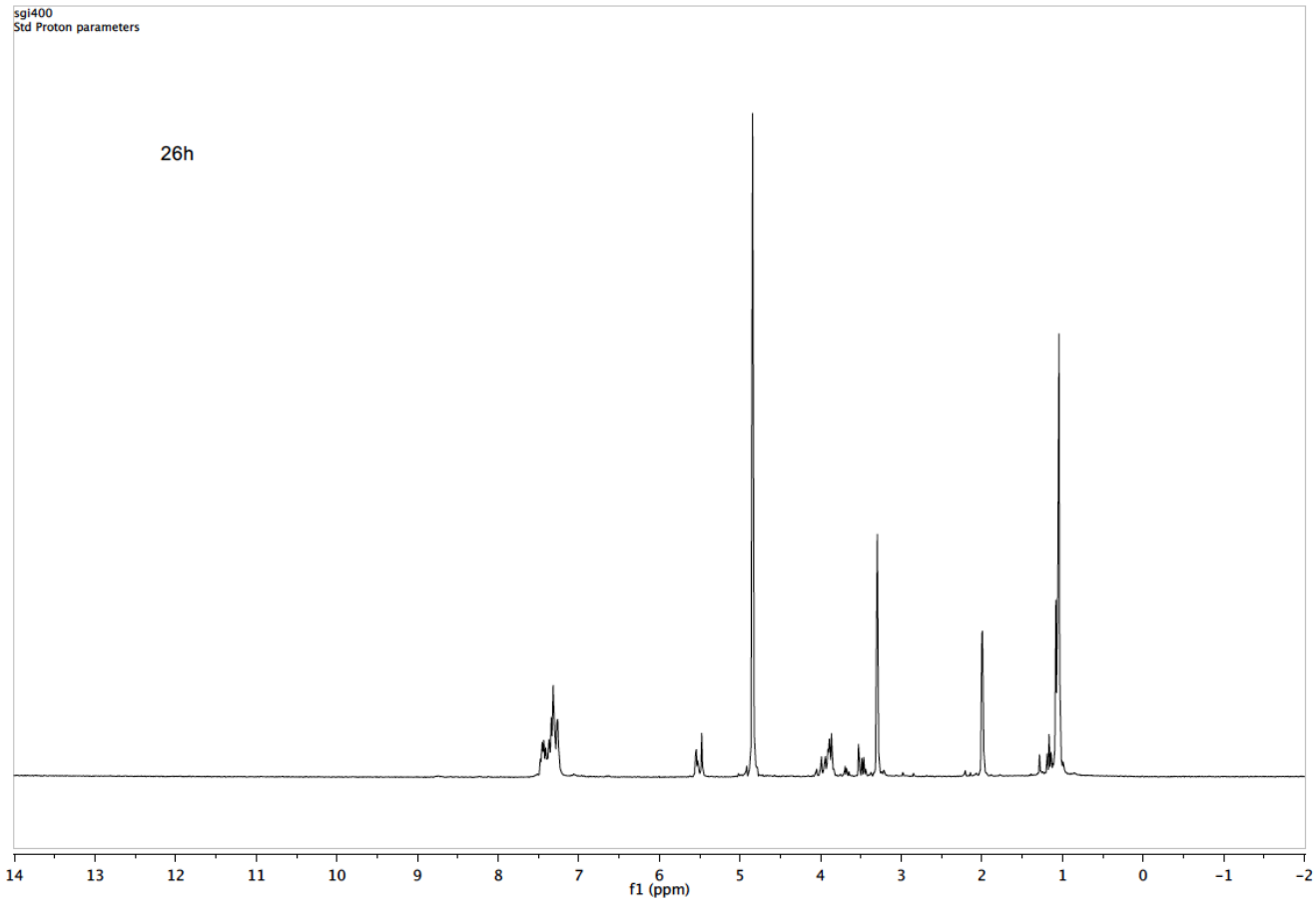

sgi330
Std Proton parameters
26c
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

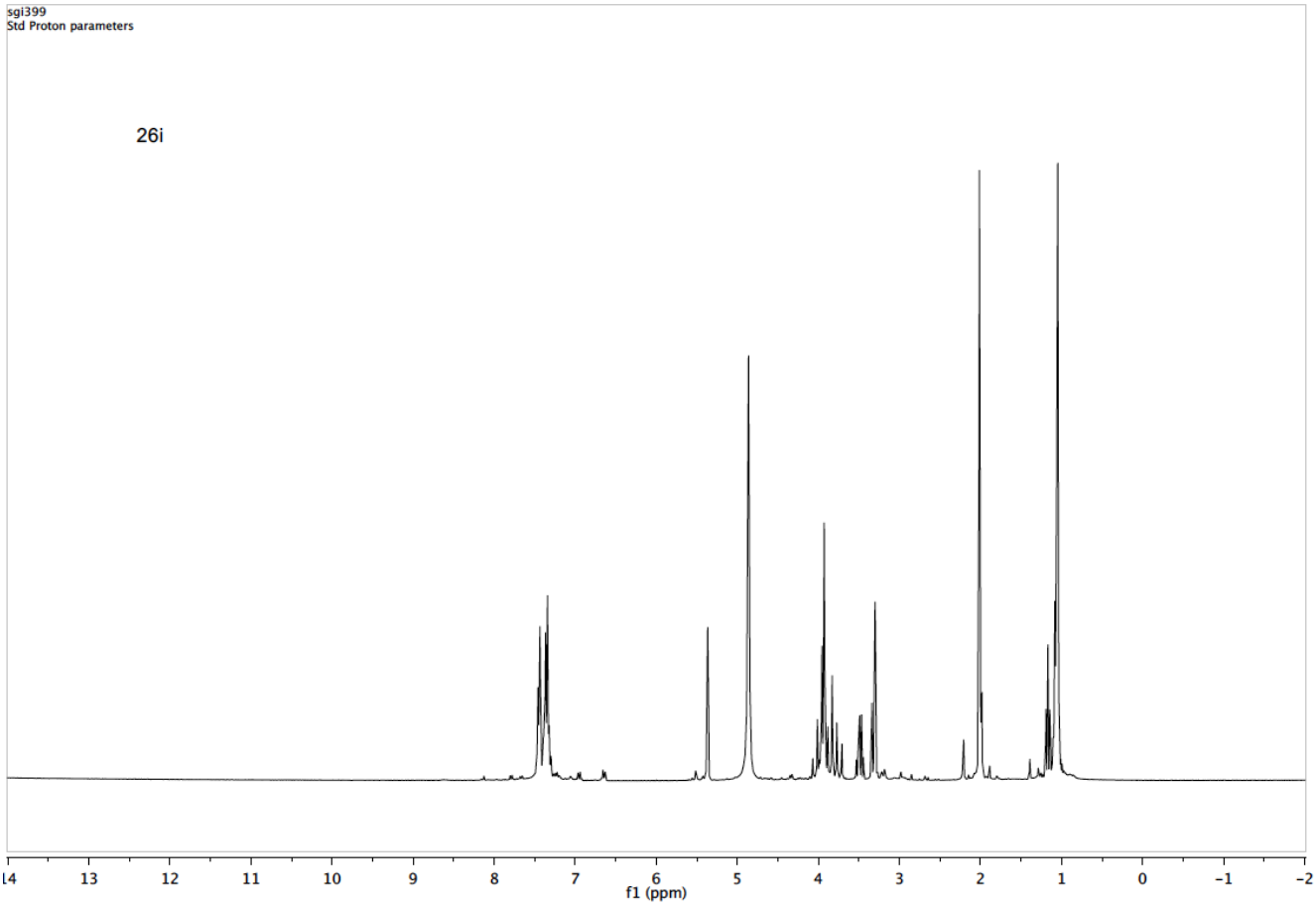

sgi352
Std Proton parameters
26d
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

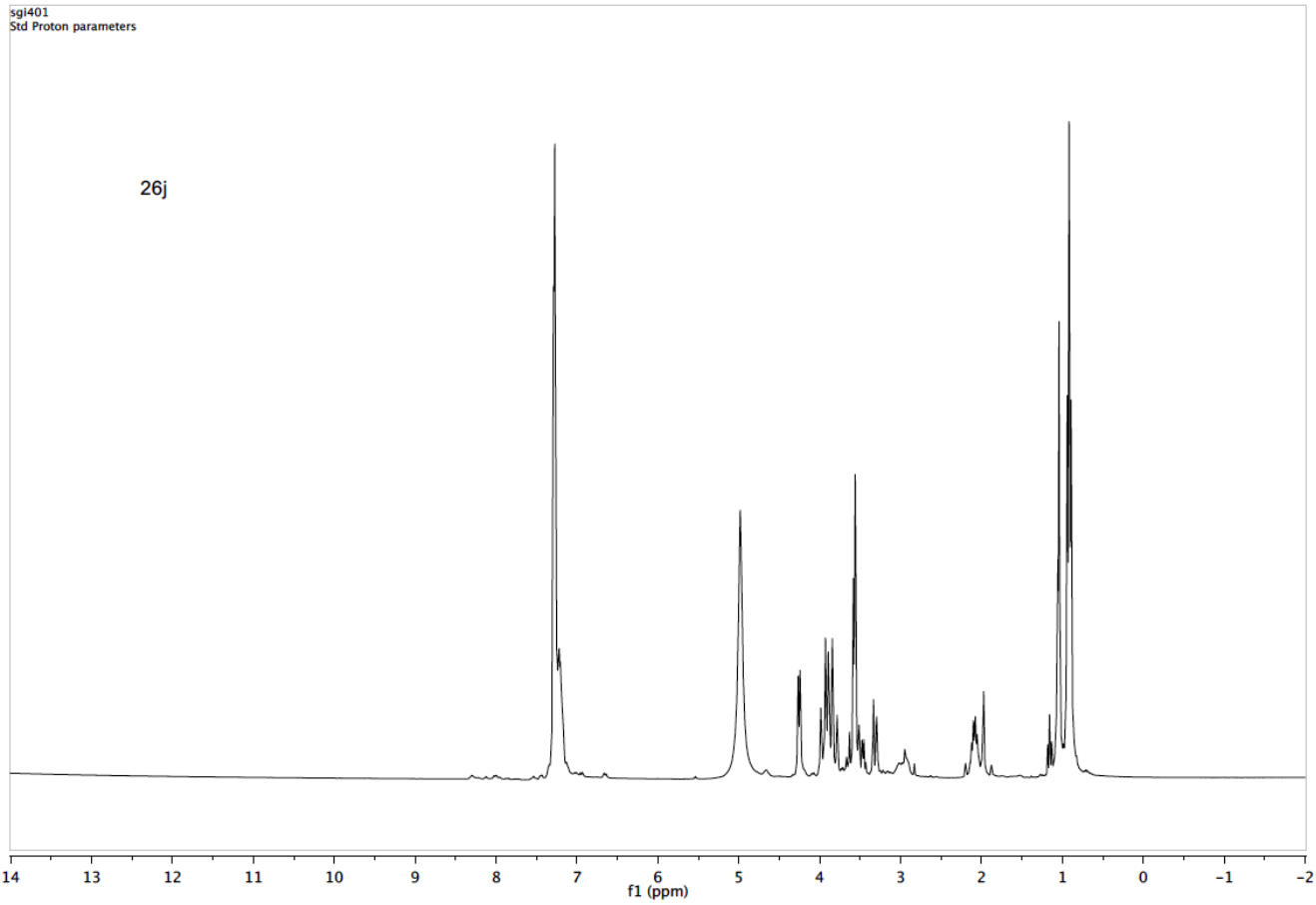

sgi306_DMSO
Std Proton parameters
26e
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

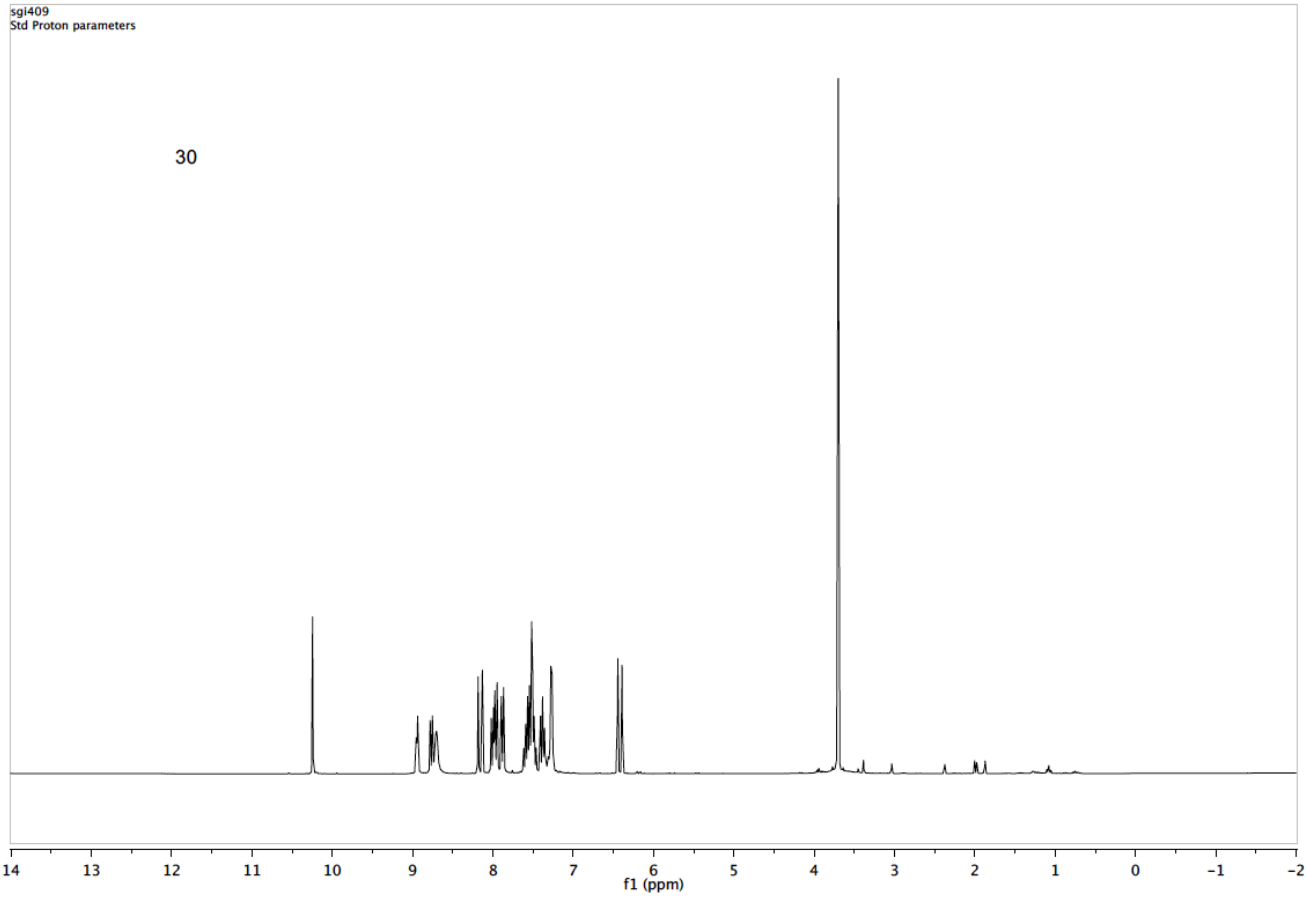

sgi360
Std Proton parameters
26f
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

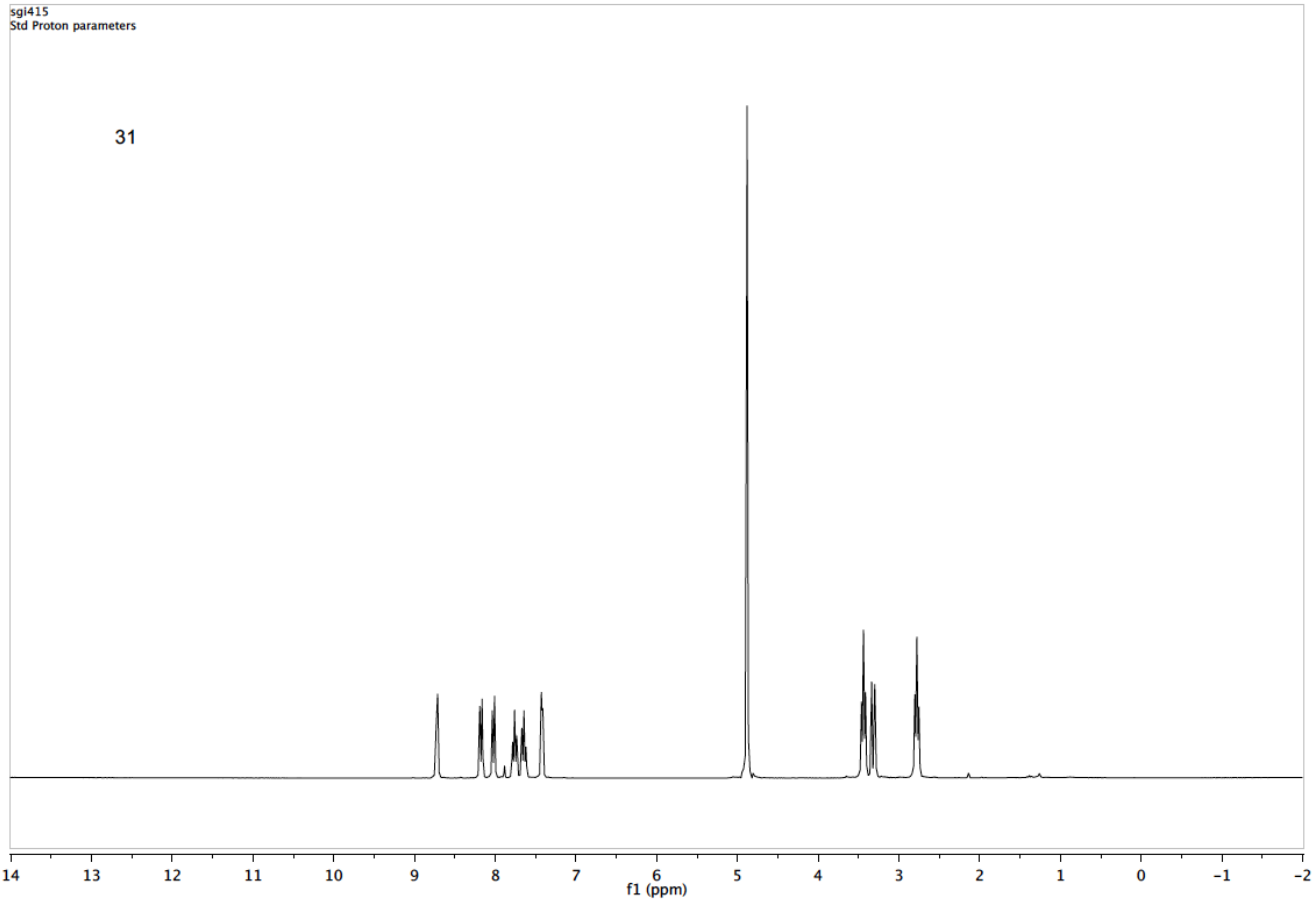

sgi361
Std Proton parameters
26g
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

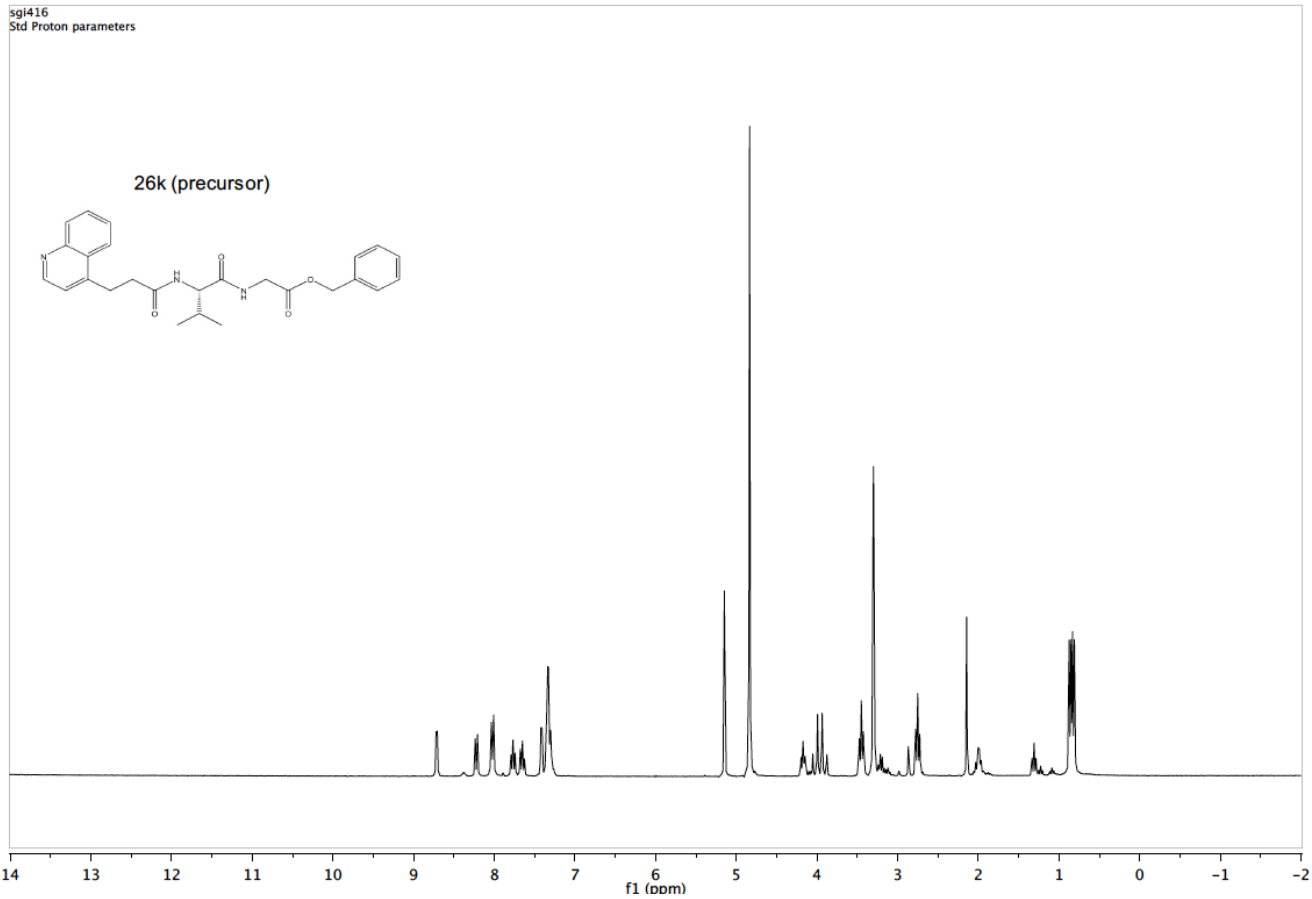

sgi400
Std Proton parameters
26h
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

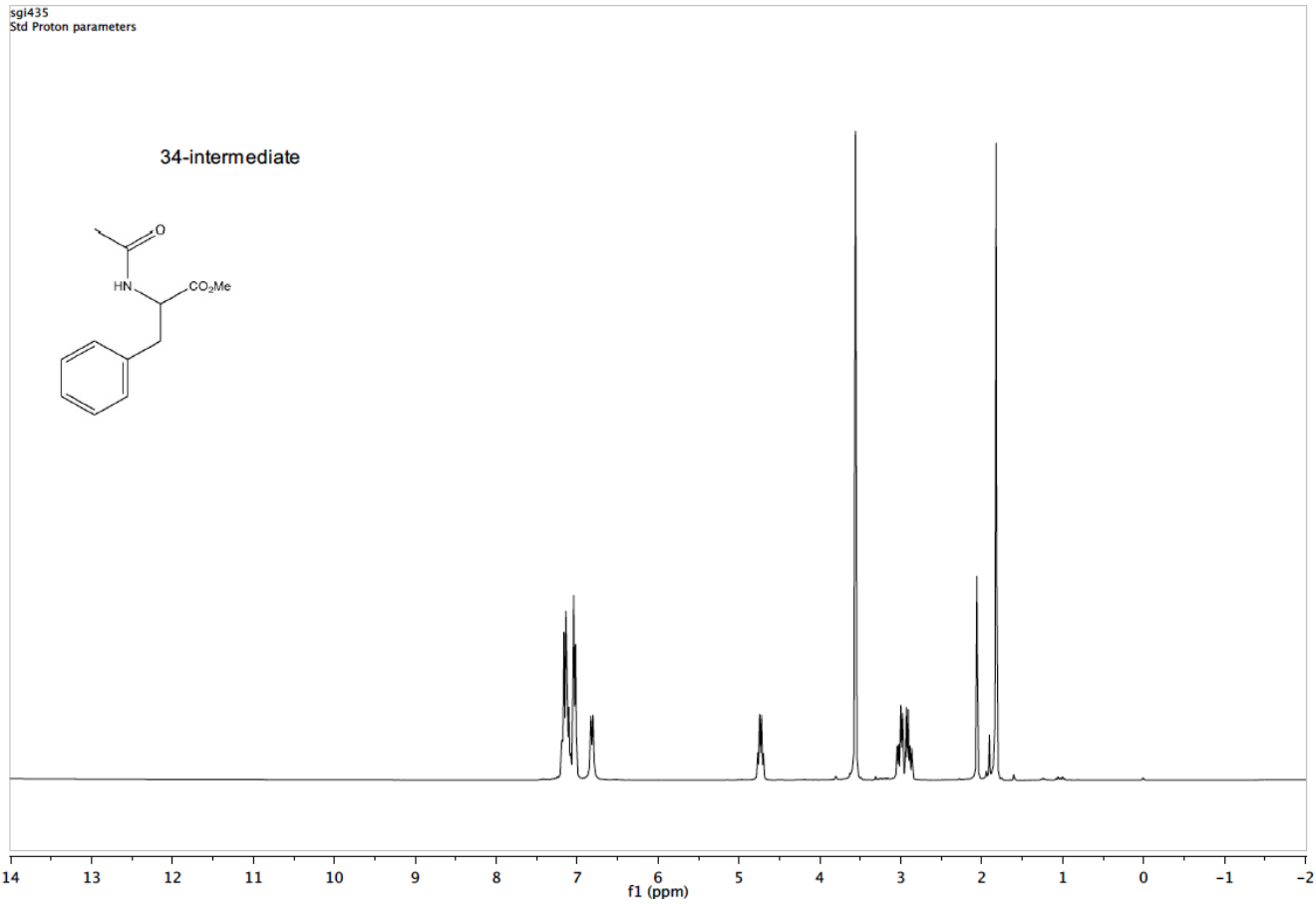

sgi399
Std Proton parameters
26i
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

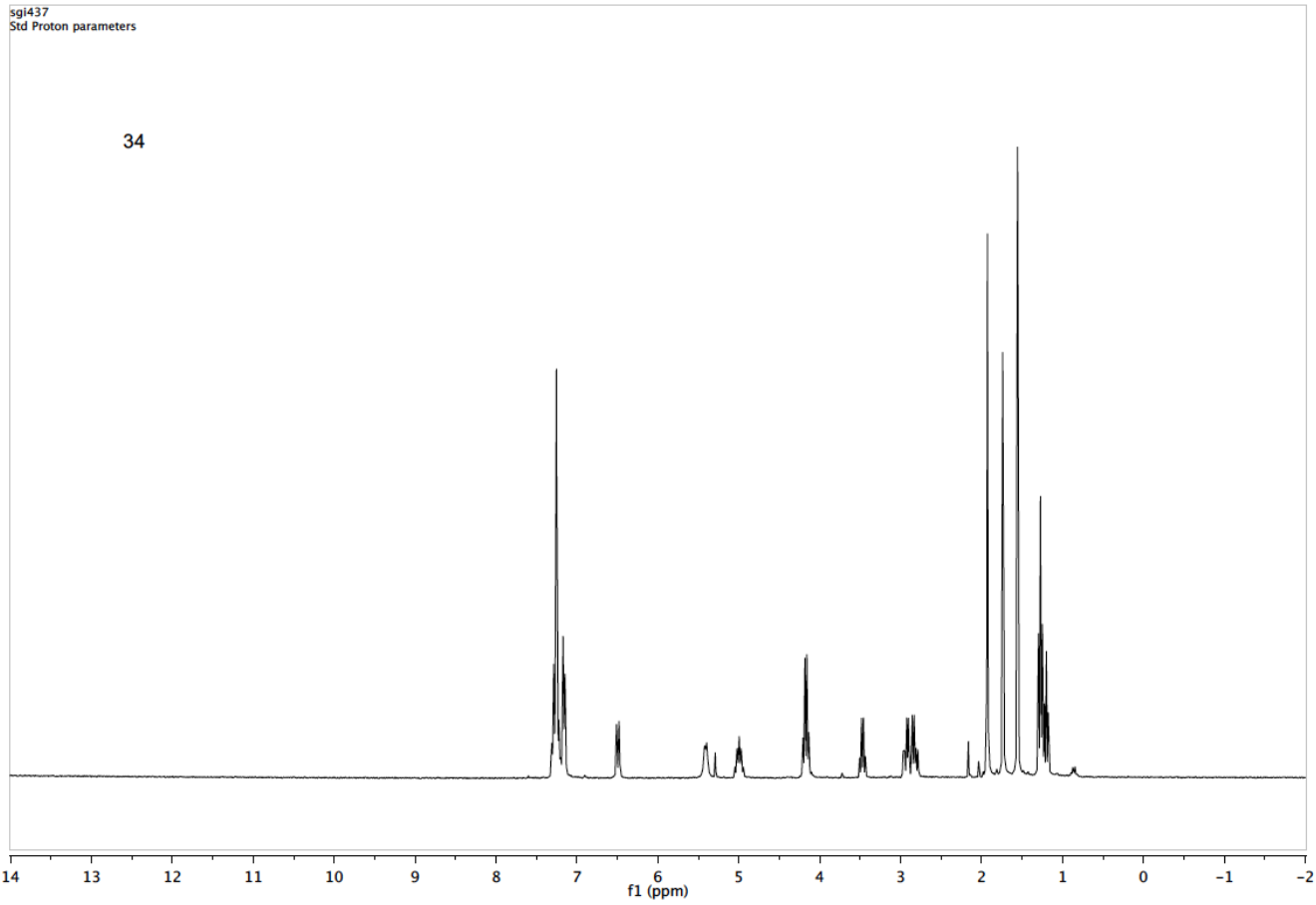

sgi401
Std Proton parameters
26j
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

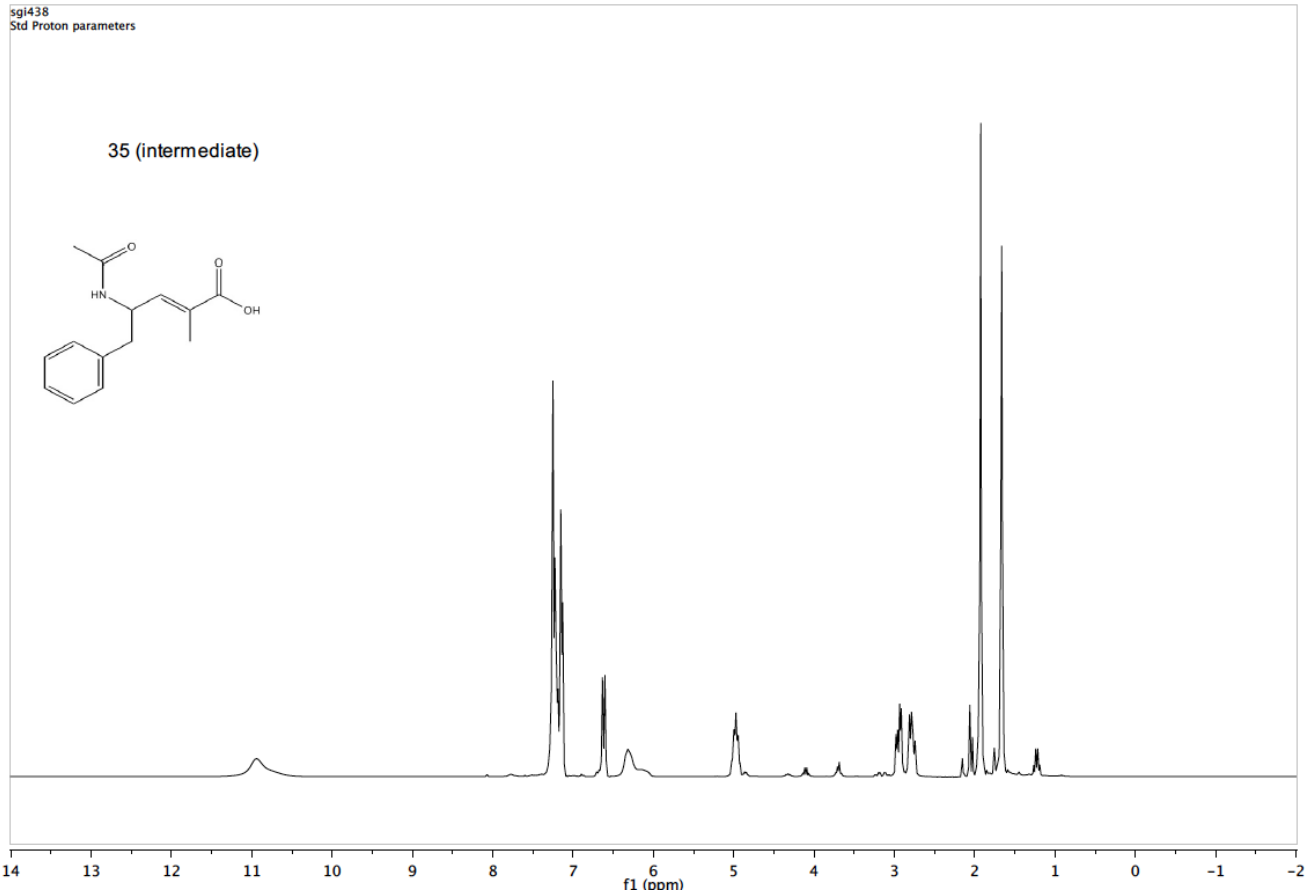

sgi409
Std Proton parameters
30
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

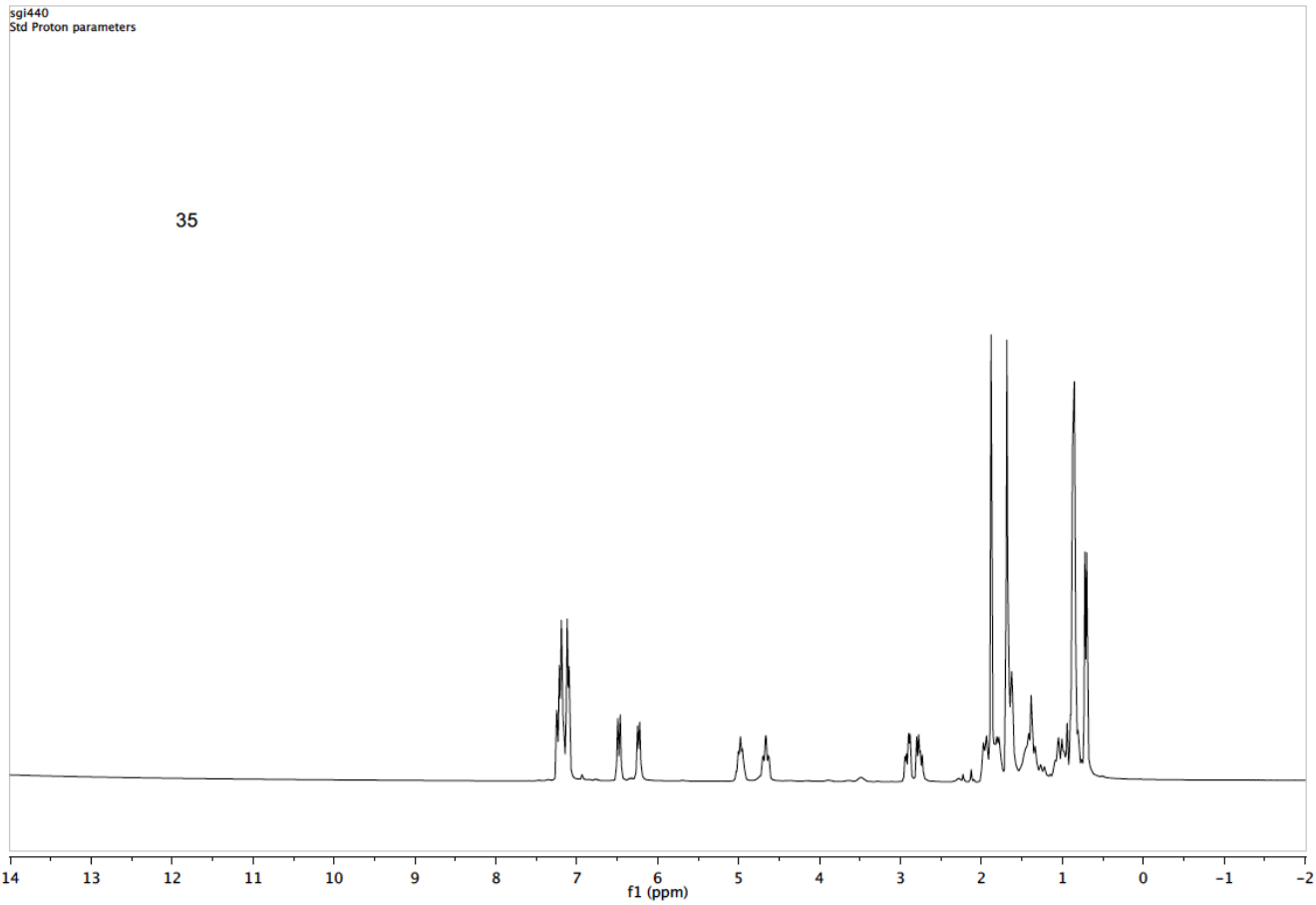

sgi415
Std Proton parameters
31
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

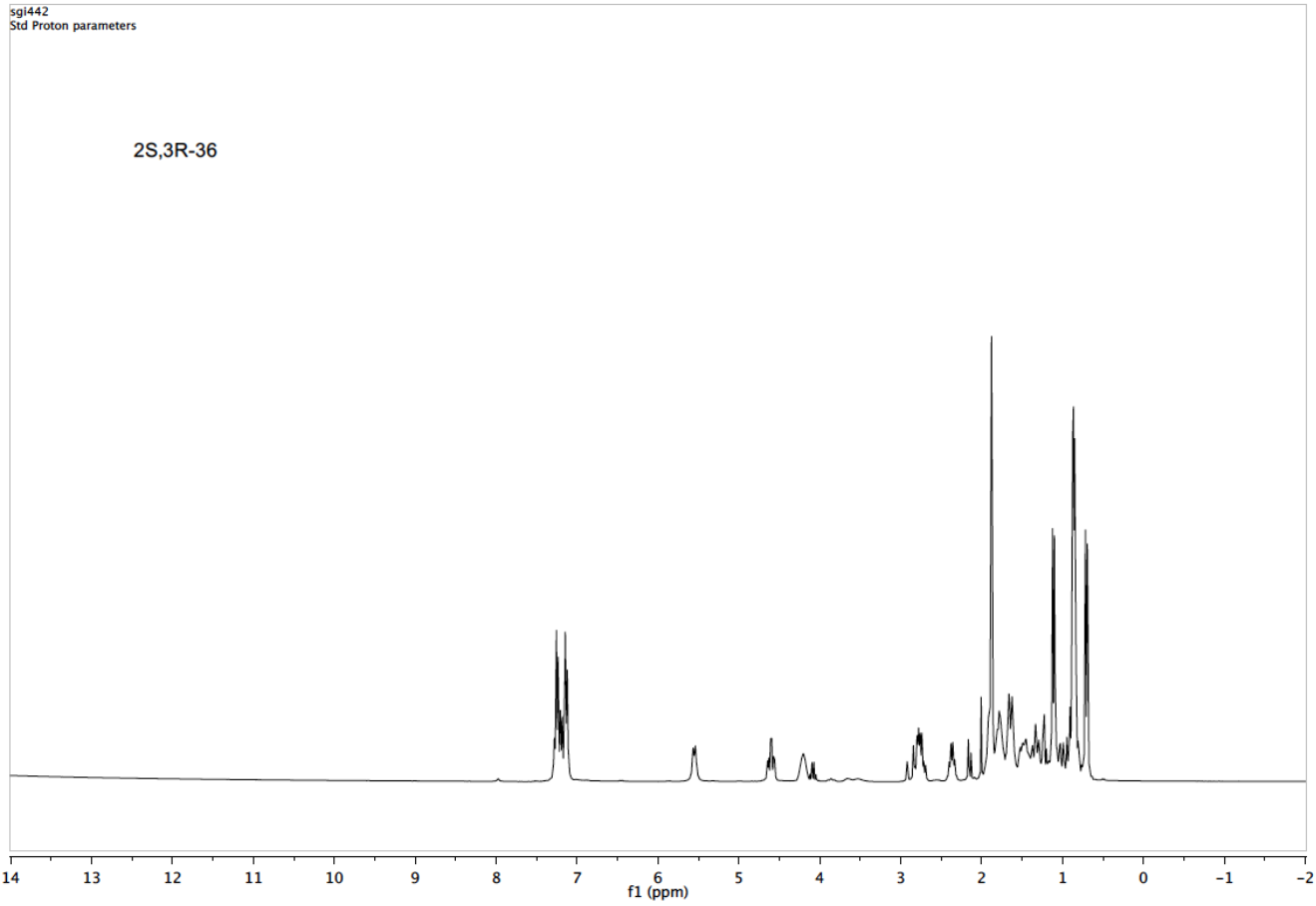

sgi416
Std Proton parameters
26k (precursor)
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

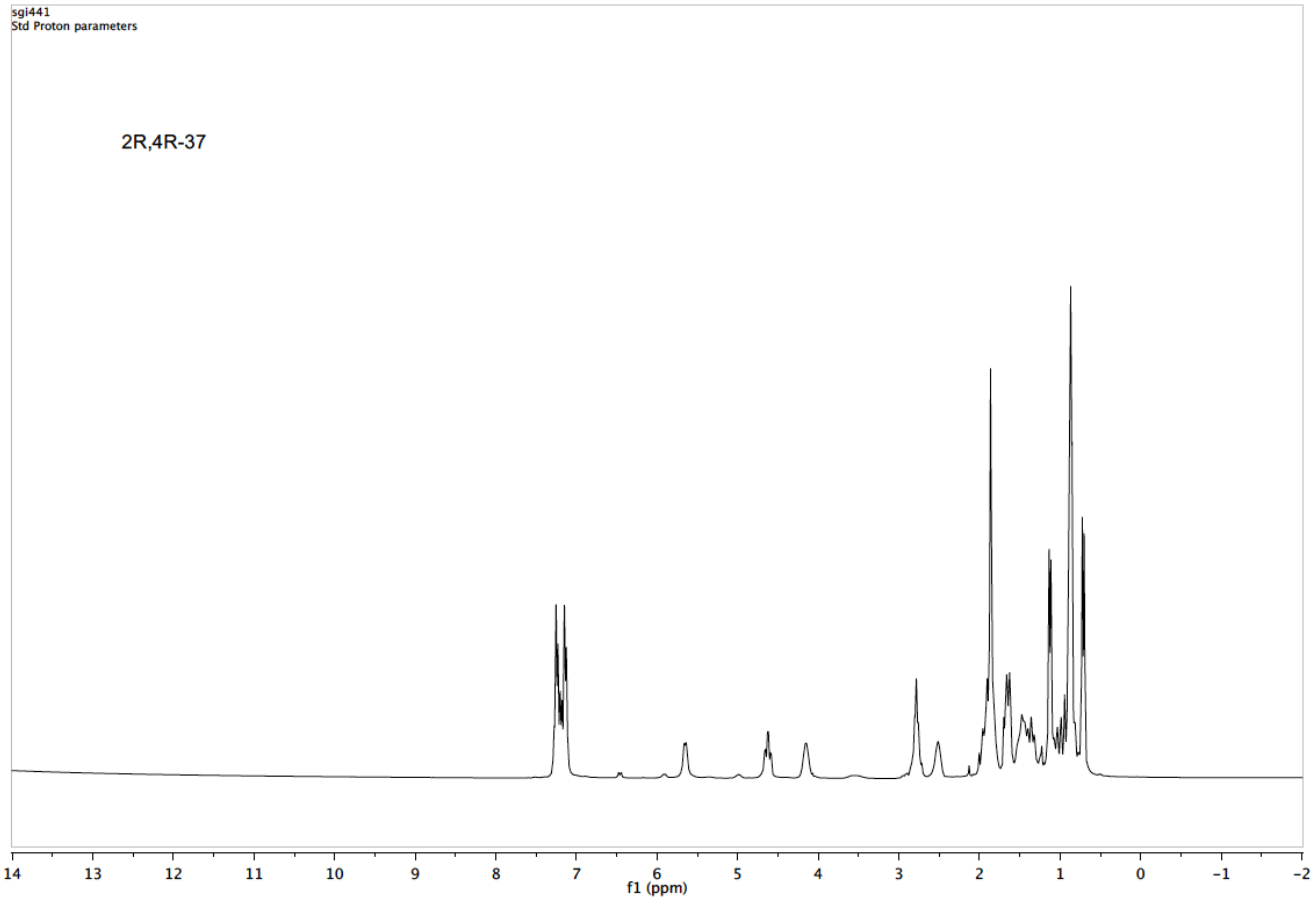

sgi435
Std Proton parameters
34-intermediate
HN
CO2Me
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

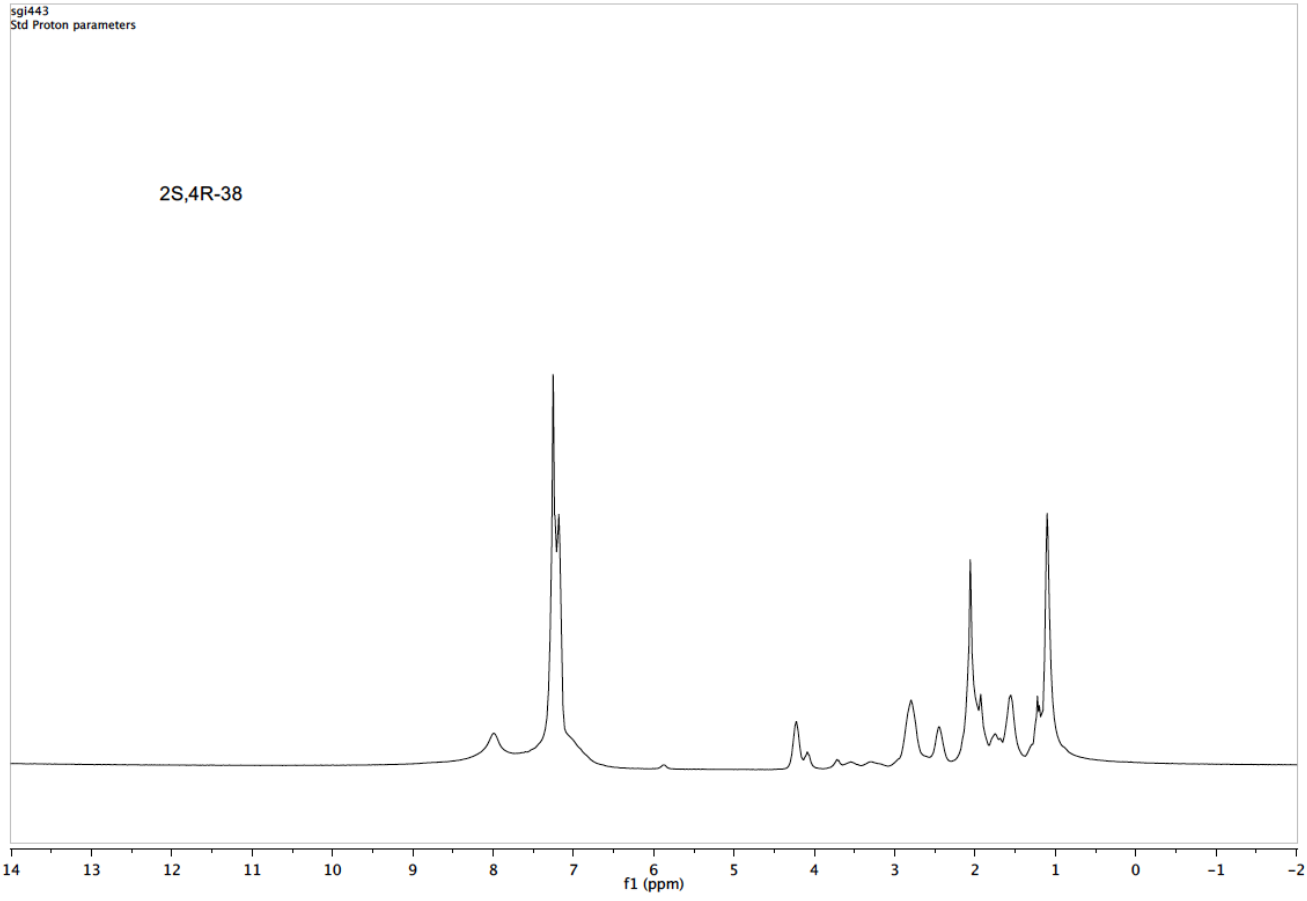

sgi437
Std Proton parameters
34
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

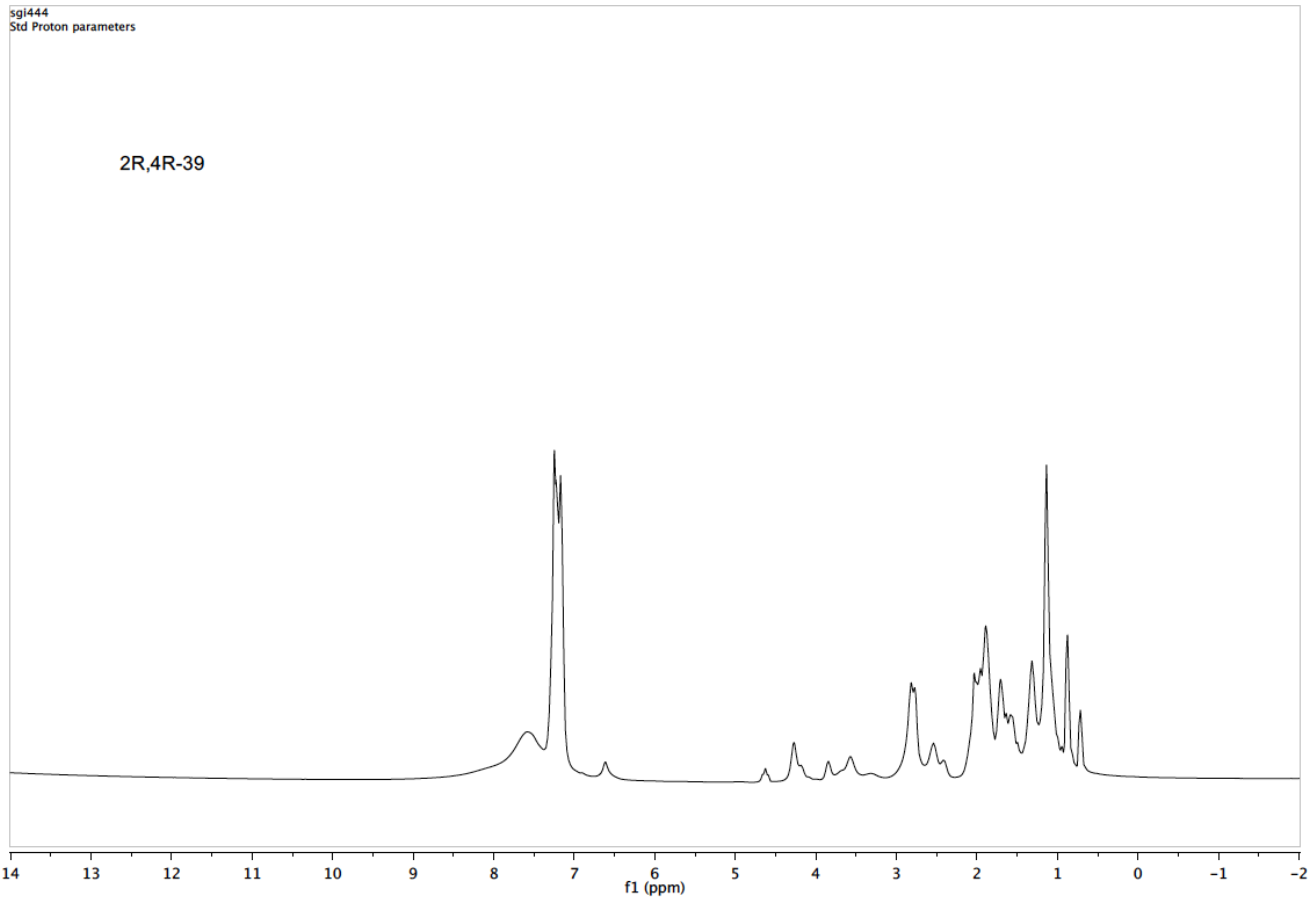

sgi438
Std Proton parameters
35 (intermediate)
HN
O
O
OH
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

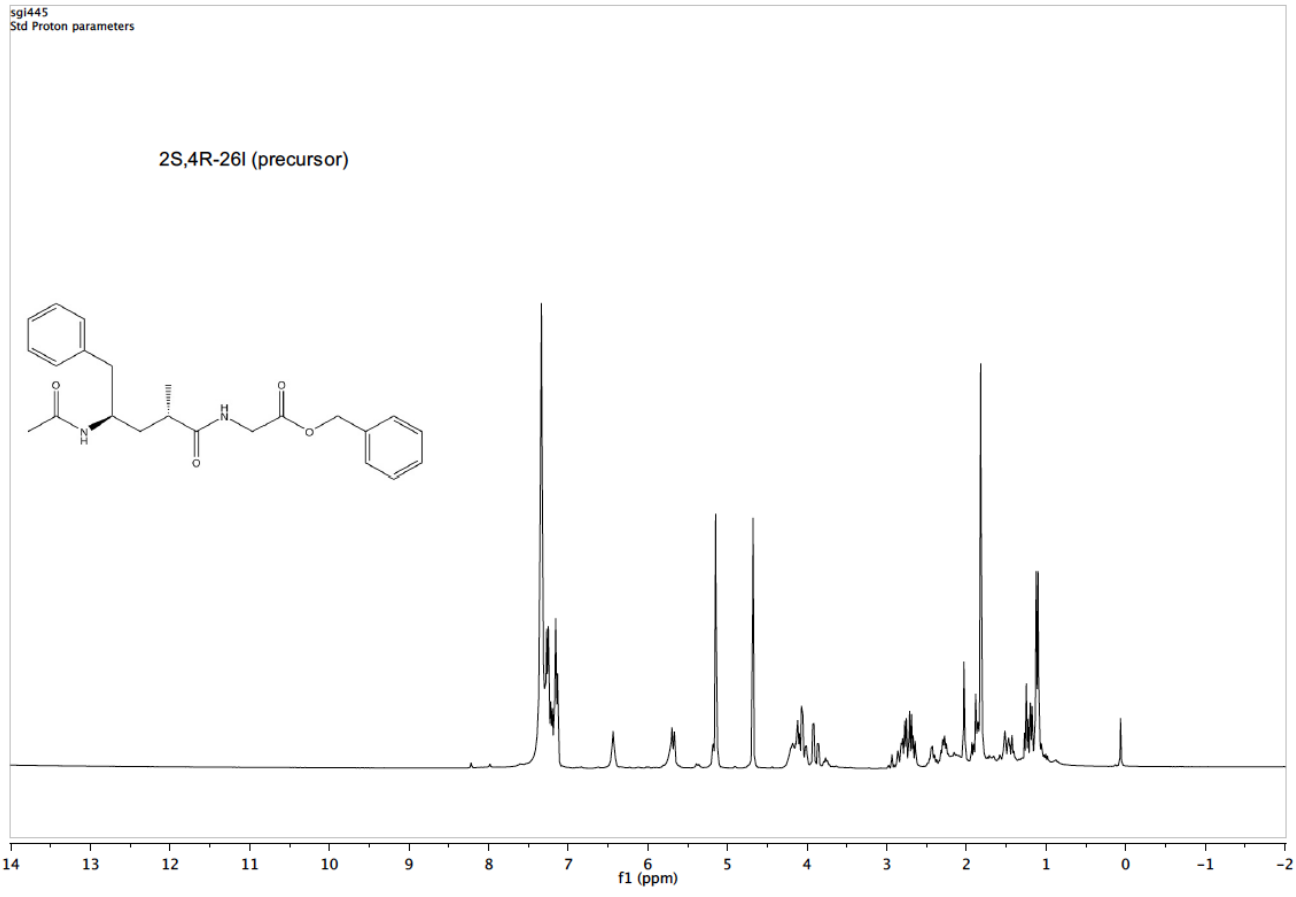

sgi440
Std Proton parameters
35
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

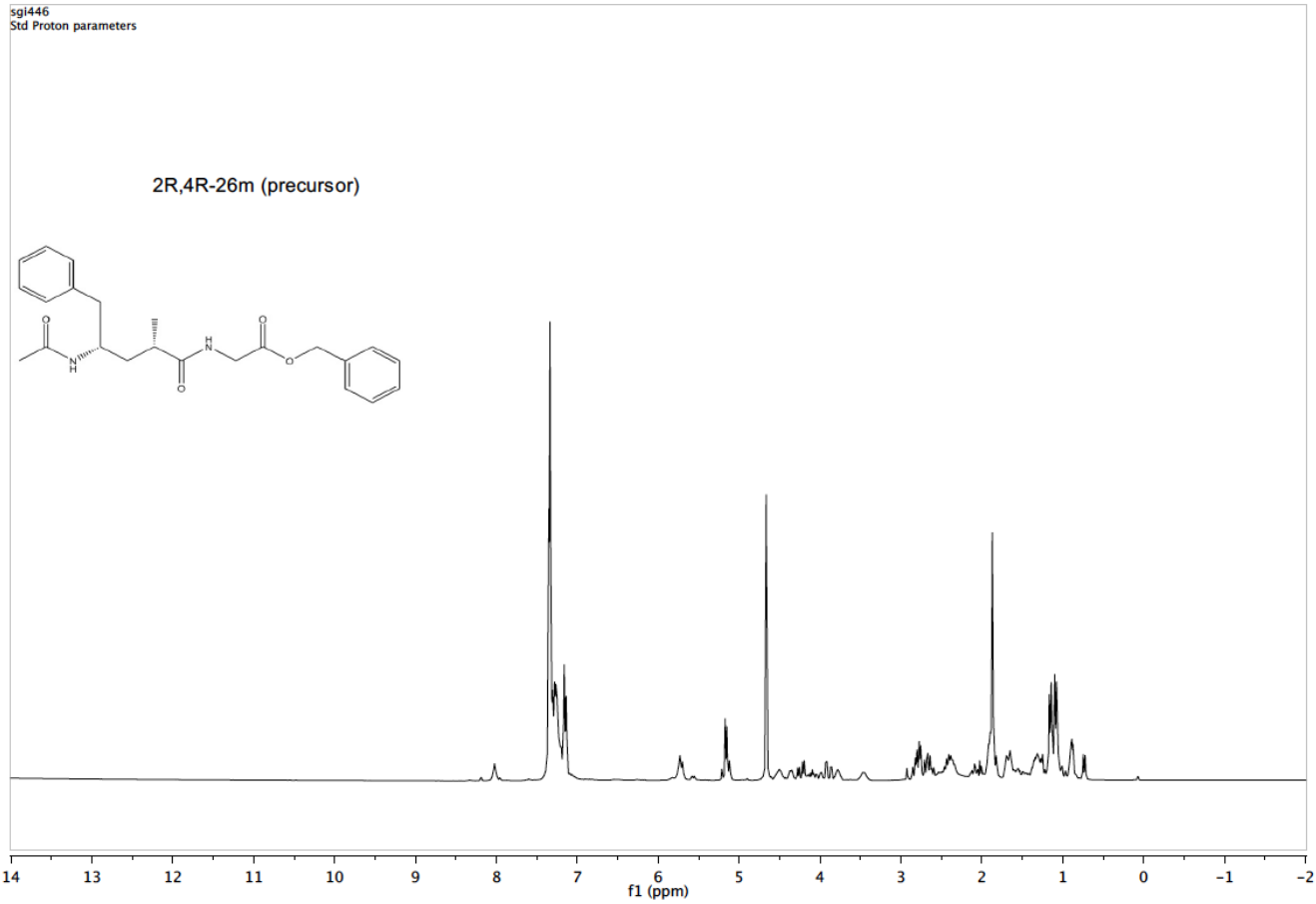

sgi442
Std Proton parameters
2S,3R-36
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

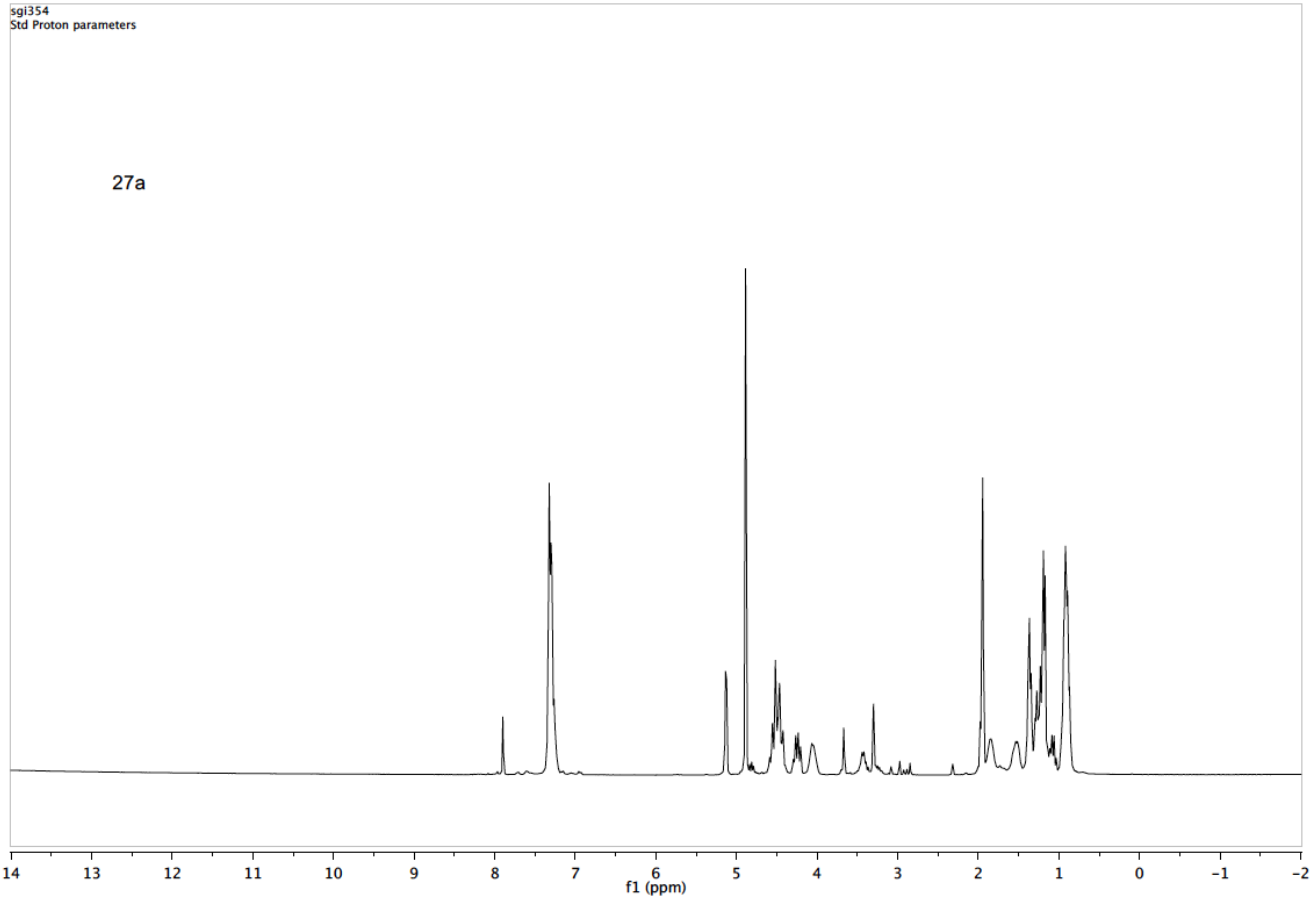

sgi441
Std Proton parameters
2R,4R-37
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

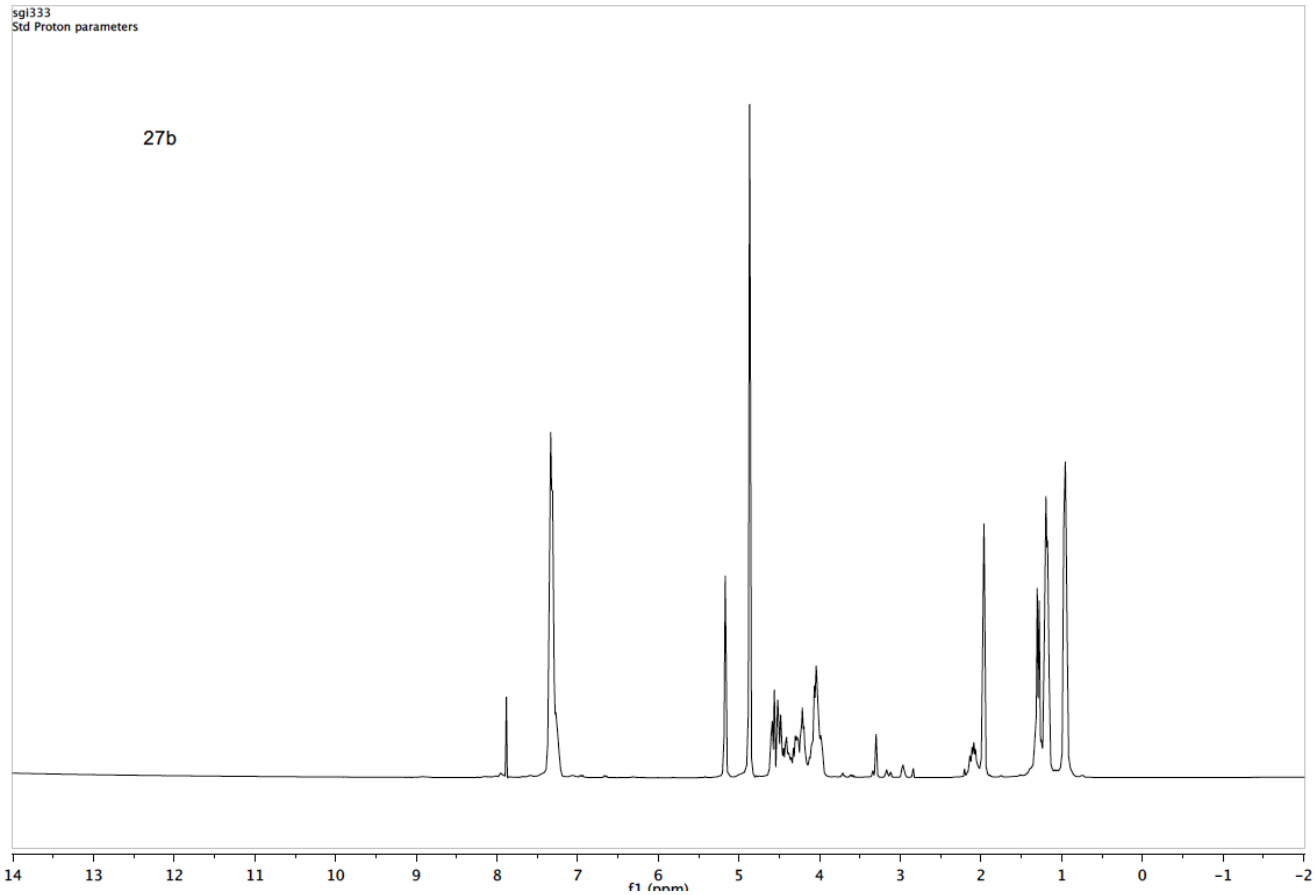

sgi443
Std Proton parameters
2S,4R-38
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

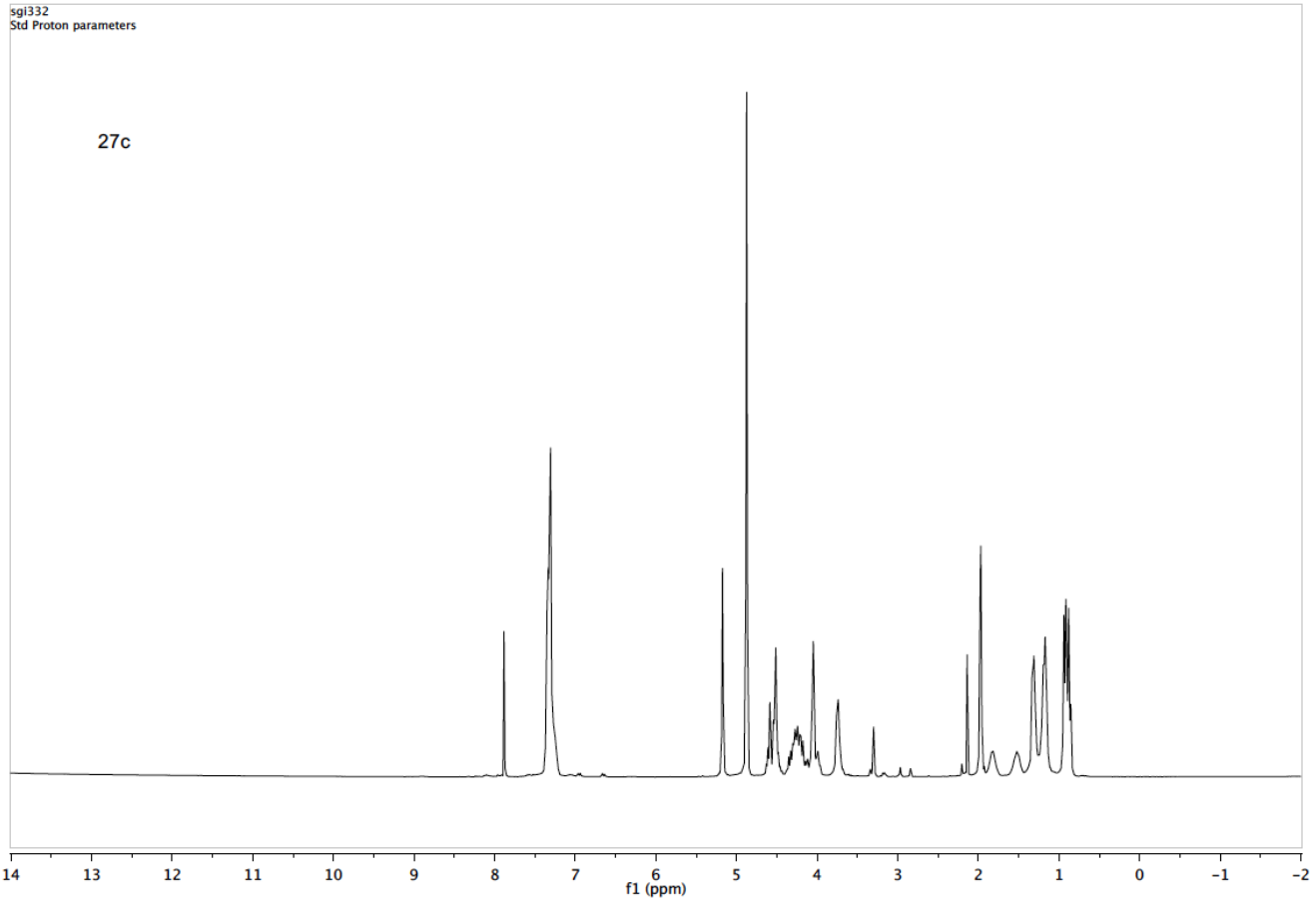

sgi444
Std Proton parameters
2R,4R-39
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

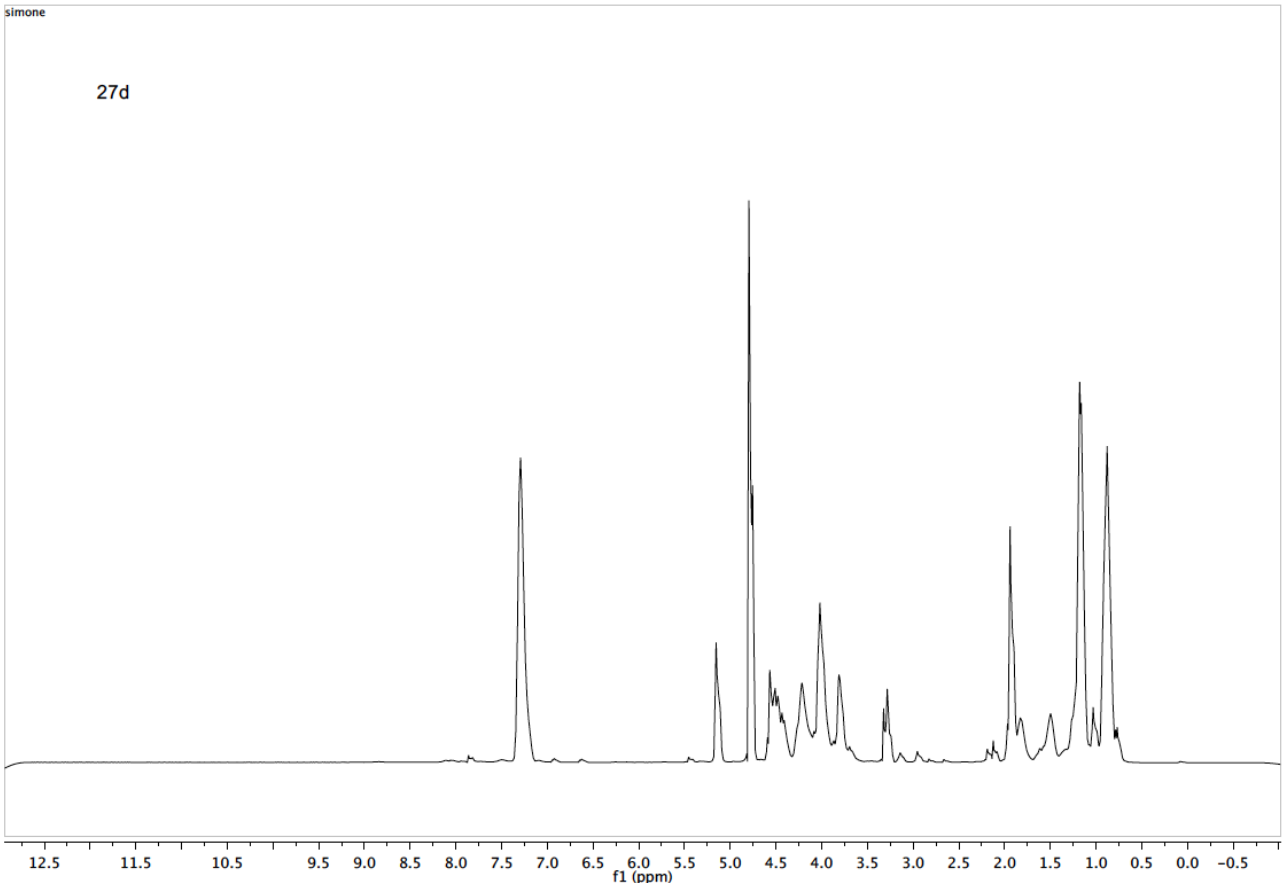

sgi445
Std Proton parameters
2S,4R-26l (precursor)
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

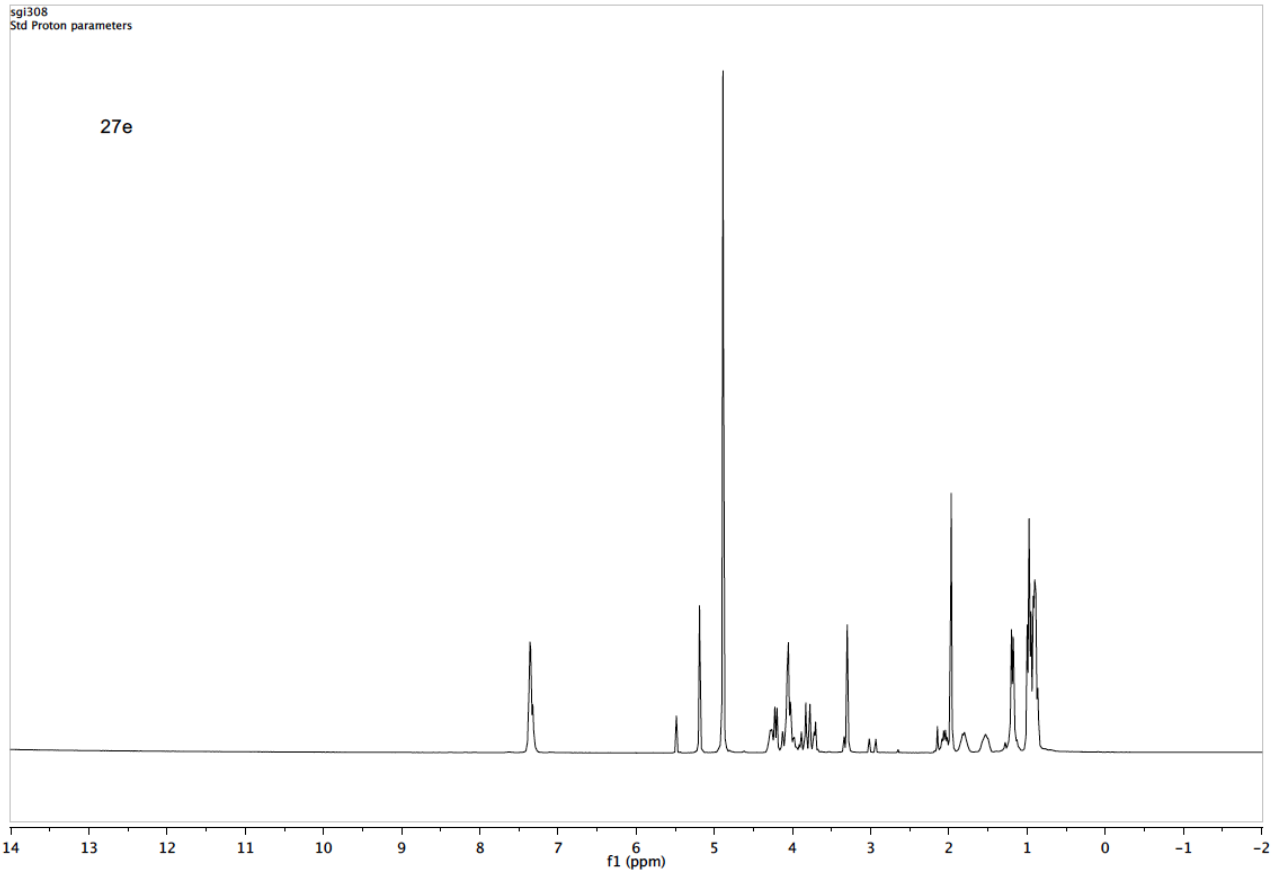

sgi446
Std Proton parameters
2R,4R-26m (precursor)
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

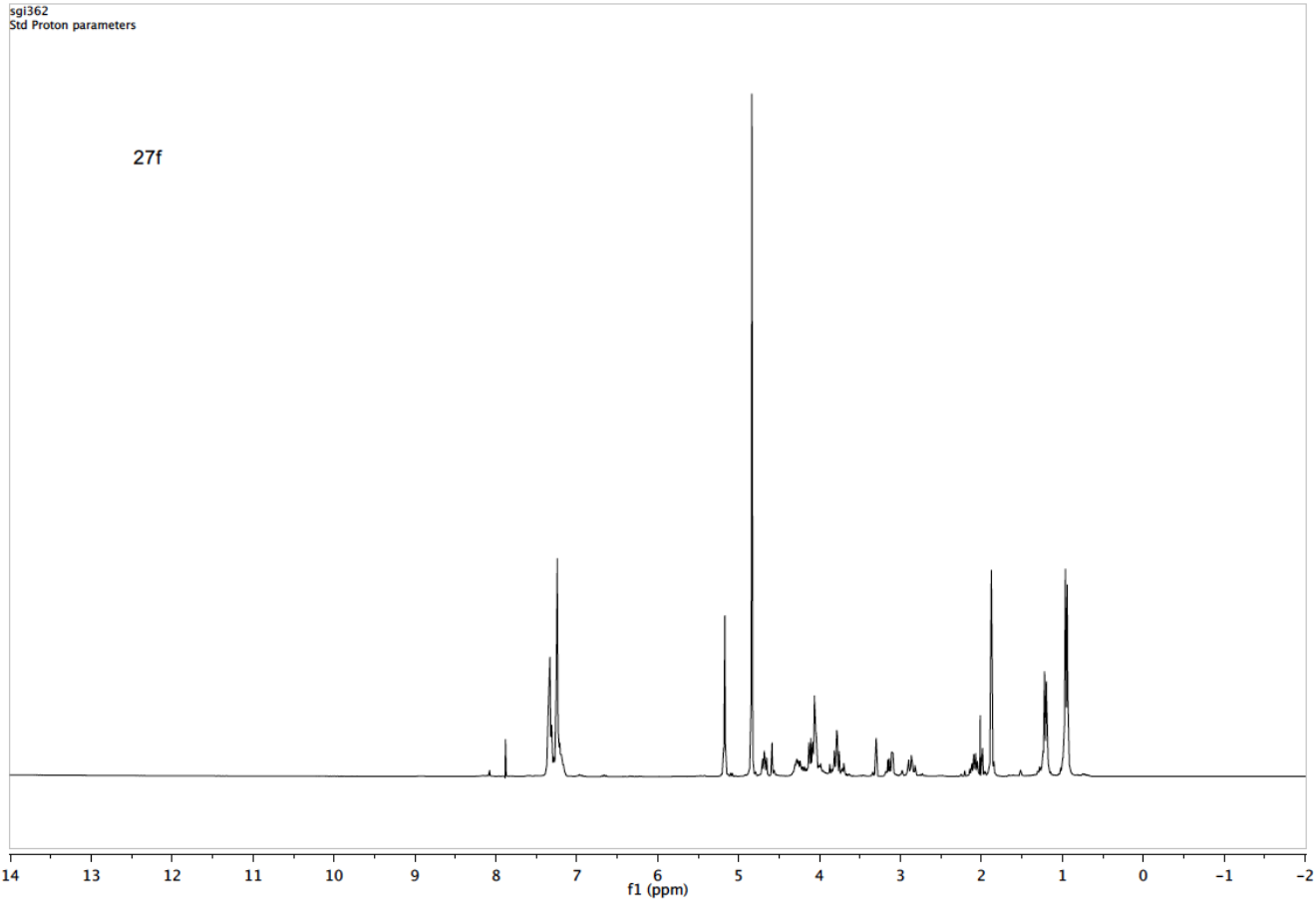

sgi354
Std Proton parameters
27a
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

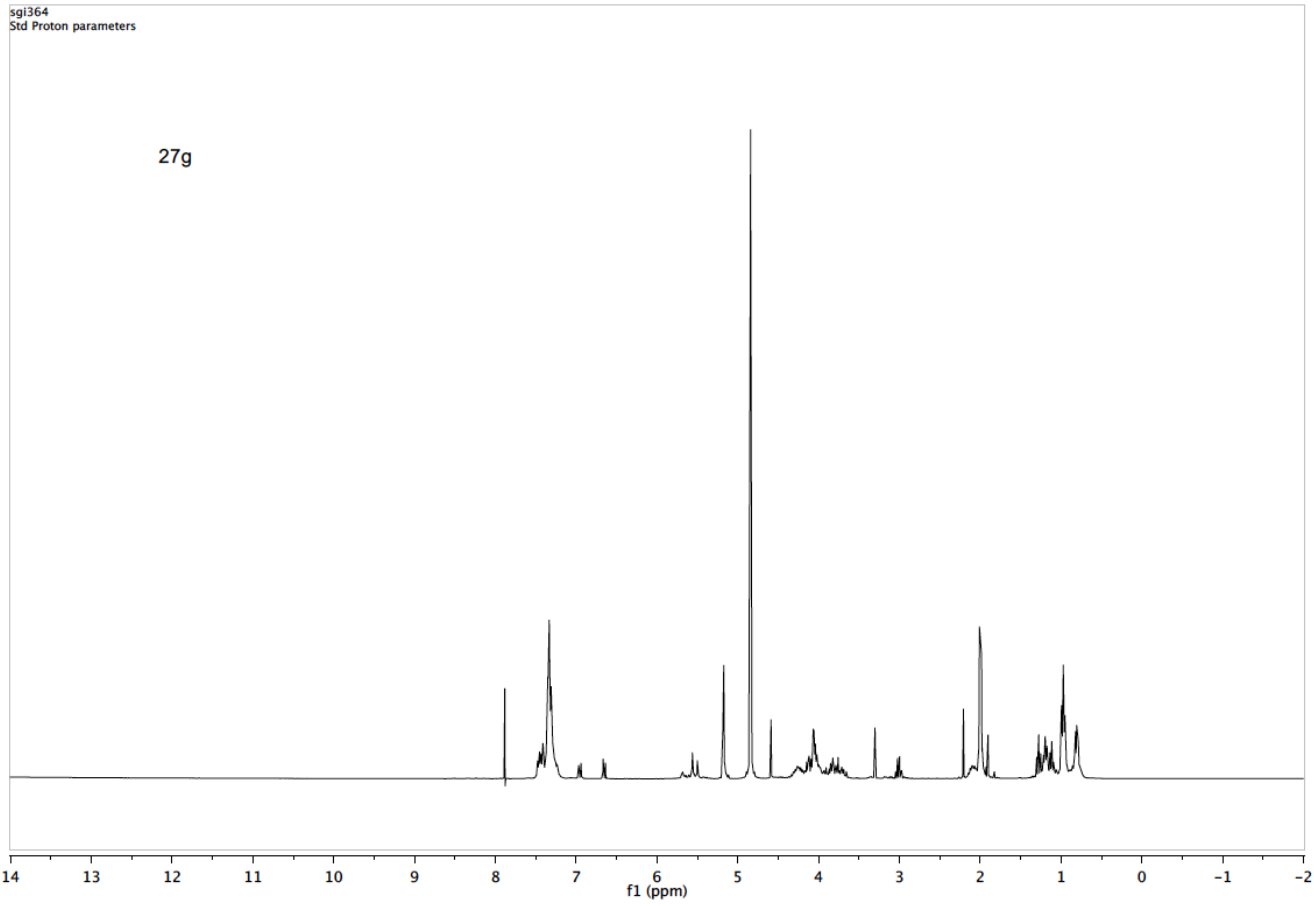

sgi333
Std Proton parameters
27b
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

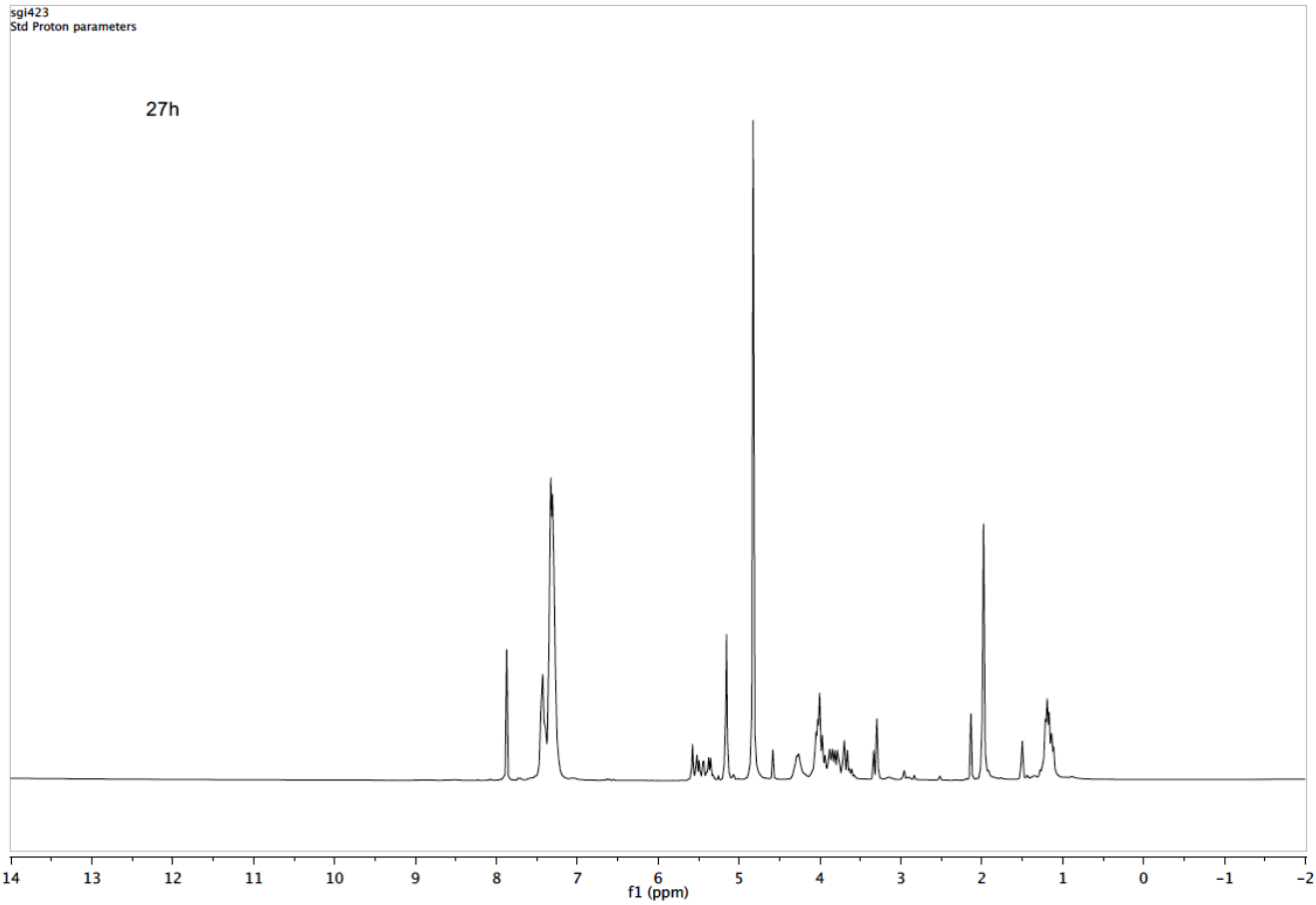

sgi332
Std Proton parameters
27c
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

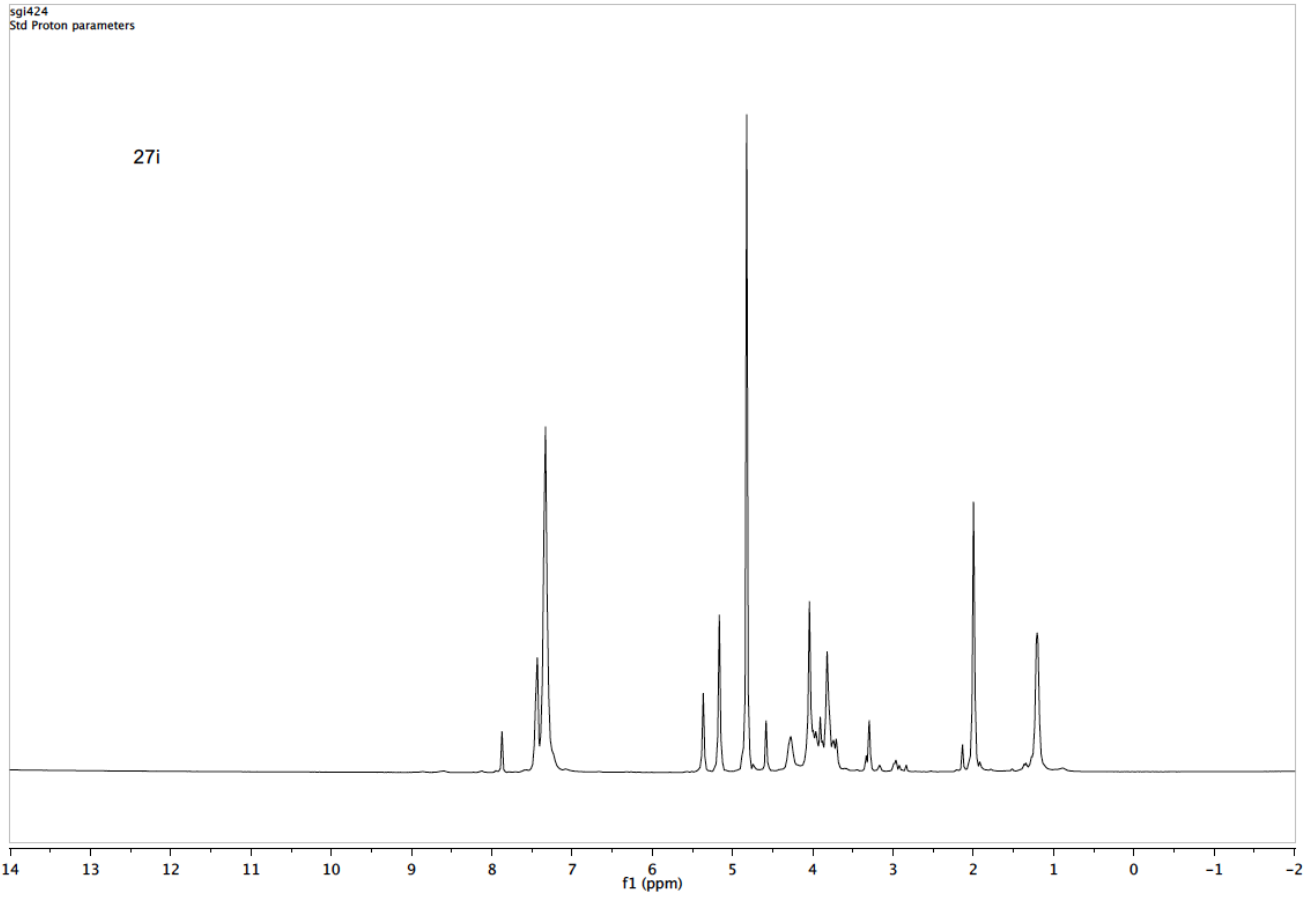

simone
27d
12.5 11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

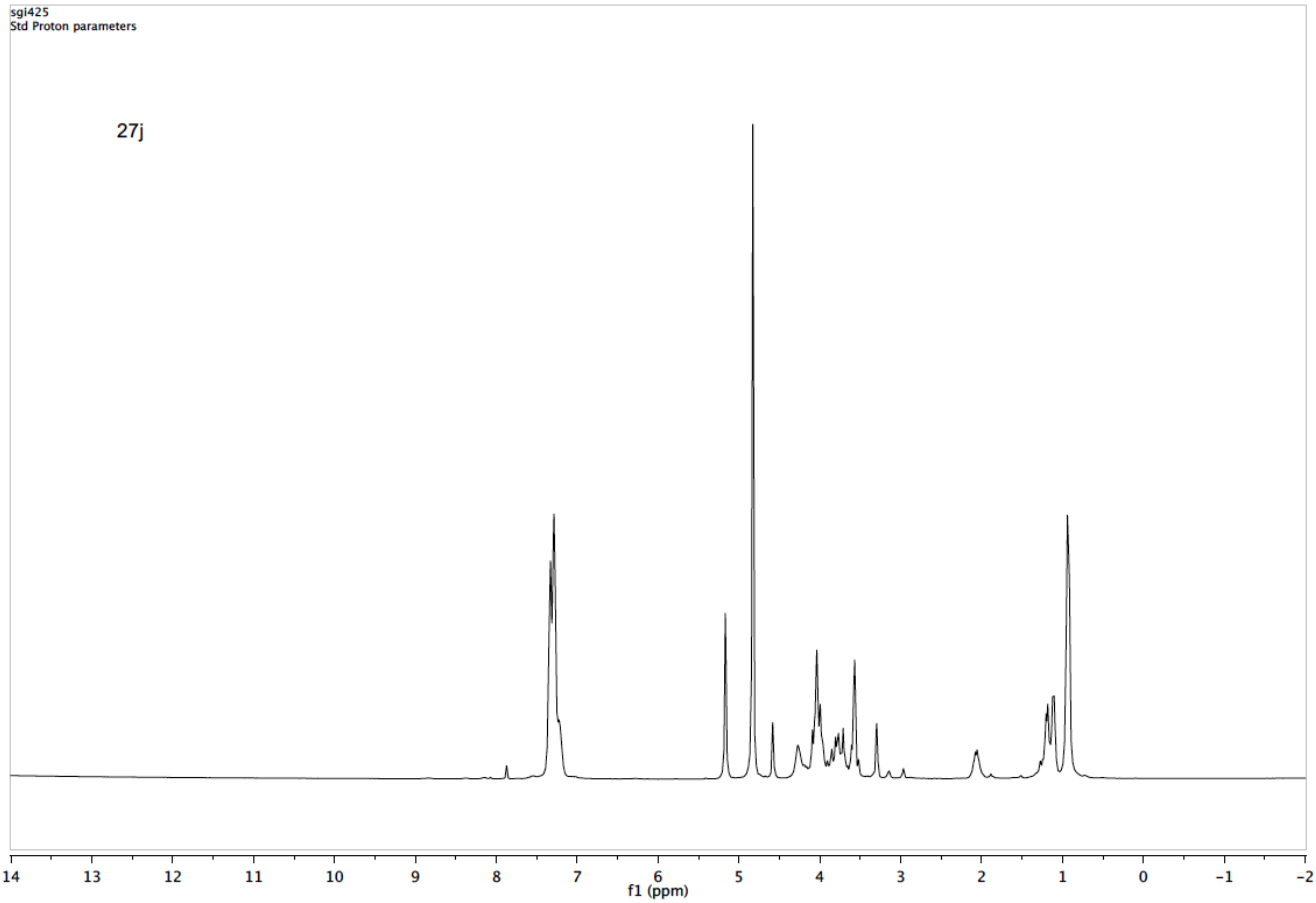

sgi308
Std Proton parameters
27e
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

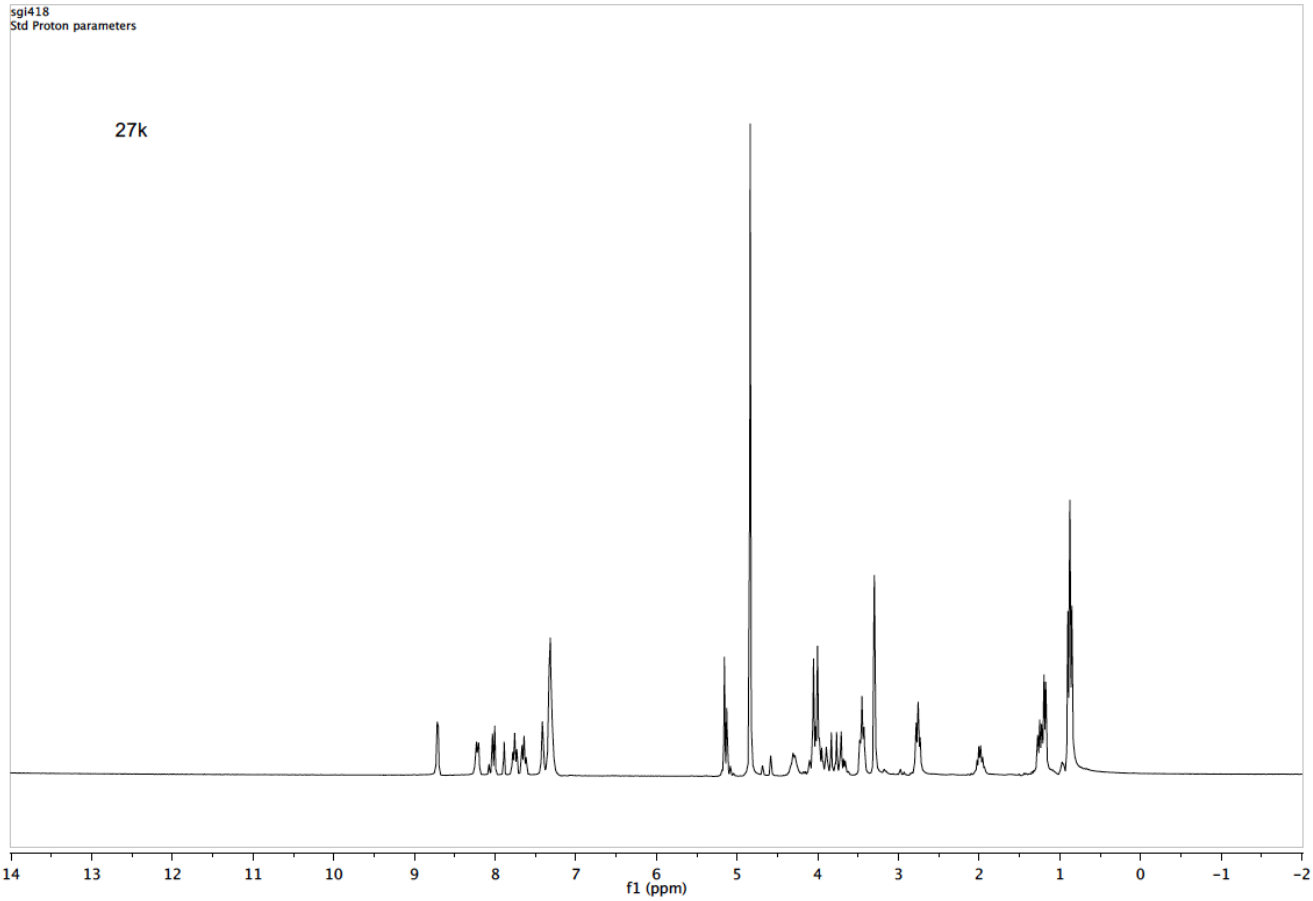

sgi362
Std Proton parameters
27f
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

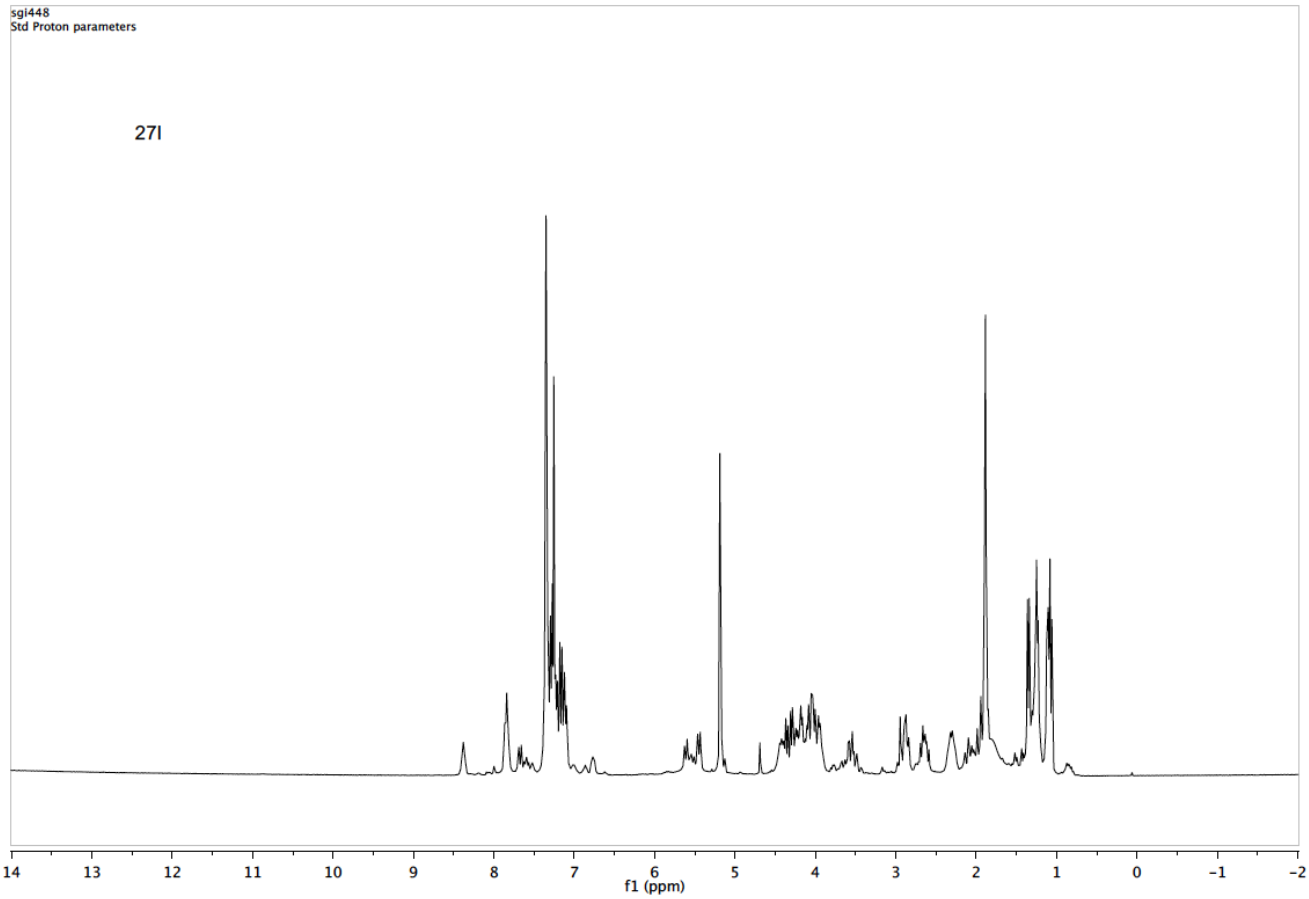

sgi364
Std Proton parameters
27g
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

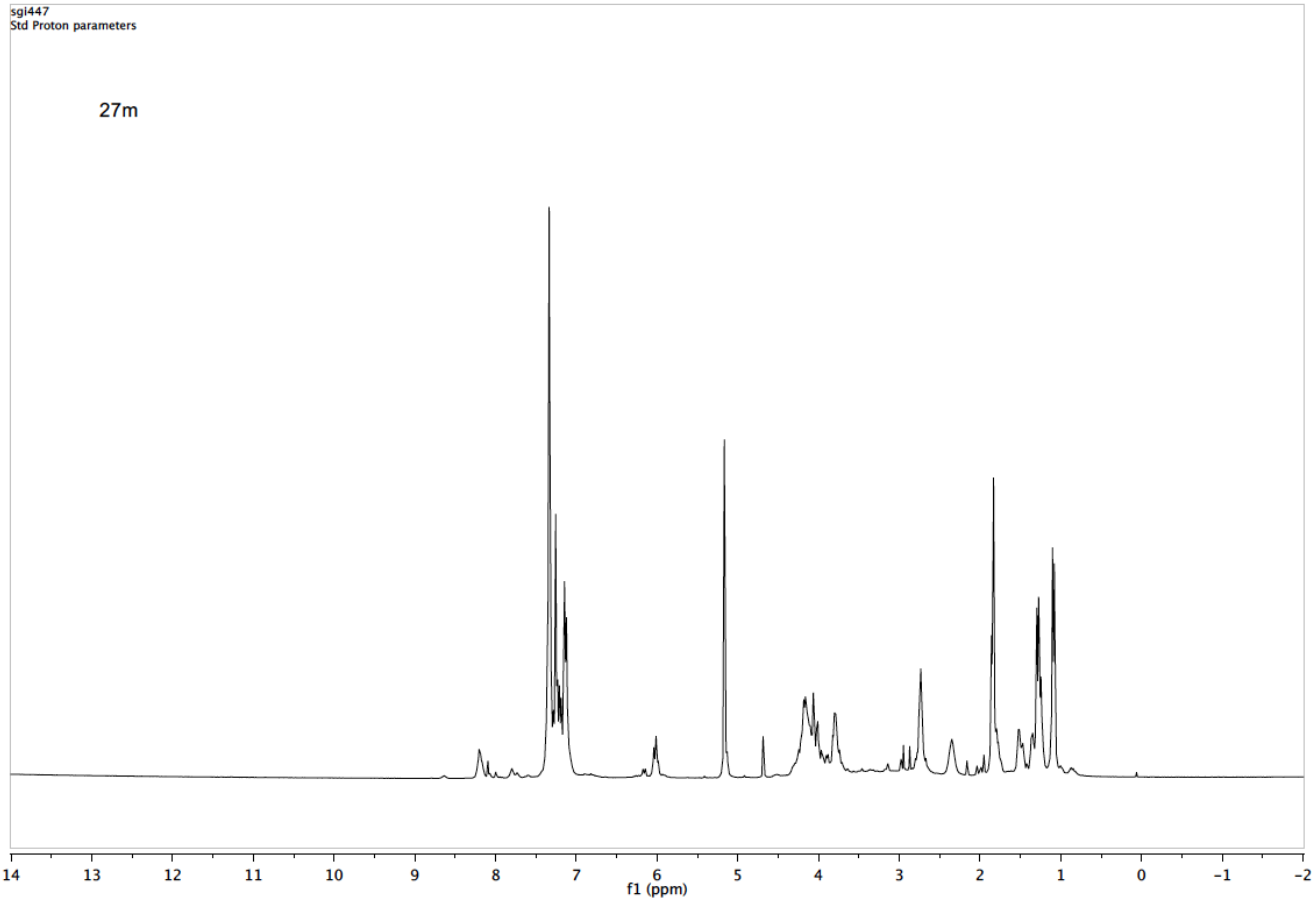

sgi423
Std Proton parameters
27h
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

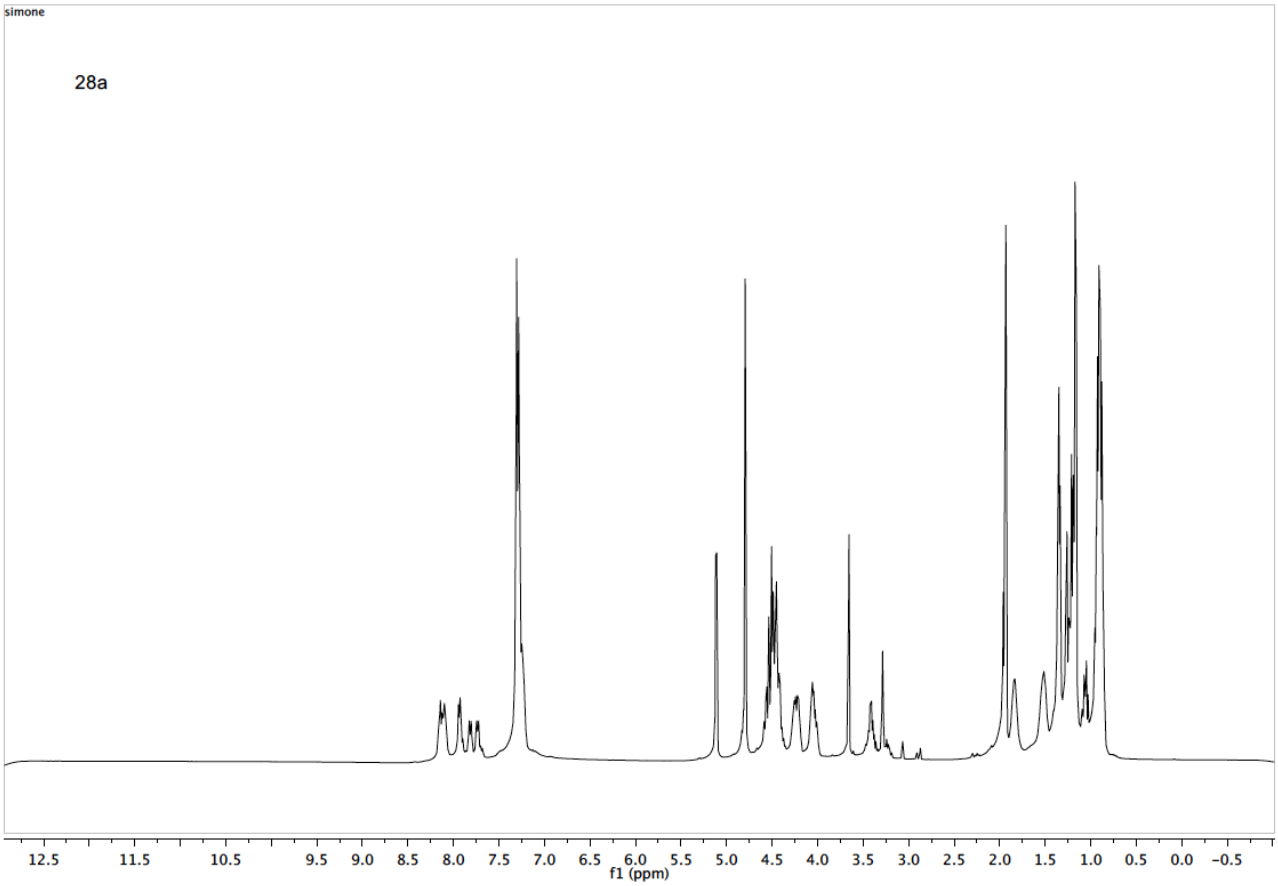

sgi424
Std Proton parameters
27i
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

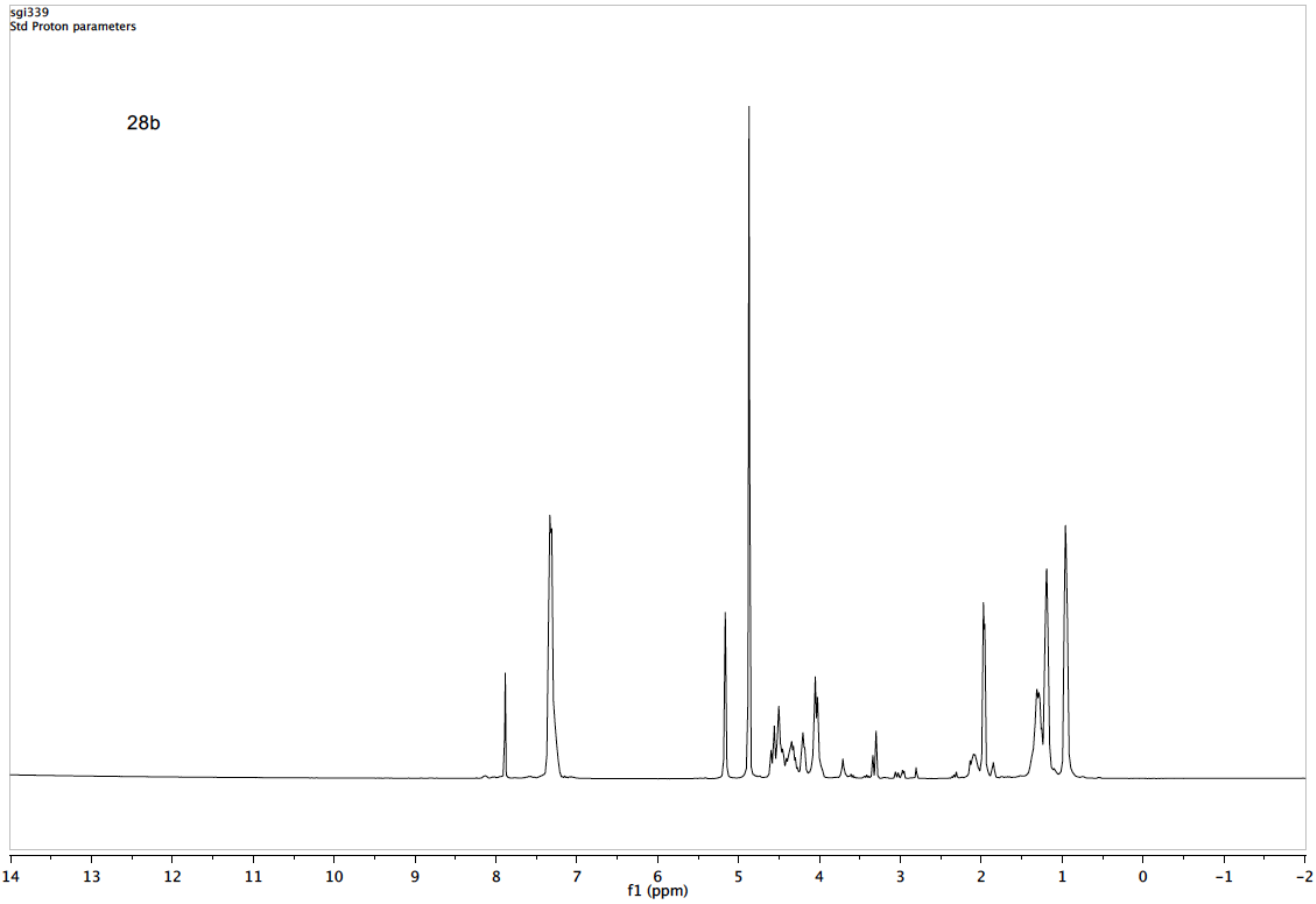

sgi425
Std Proton parameters
27j
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

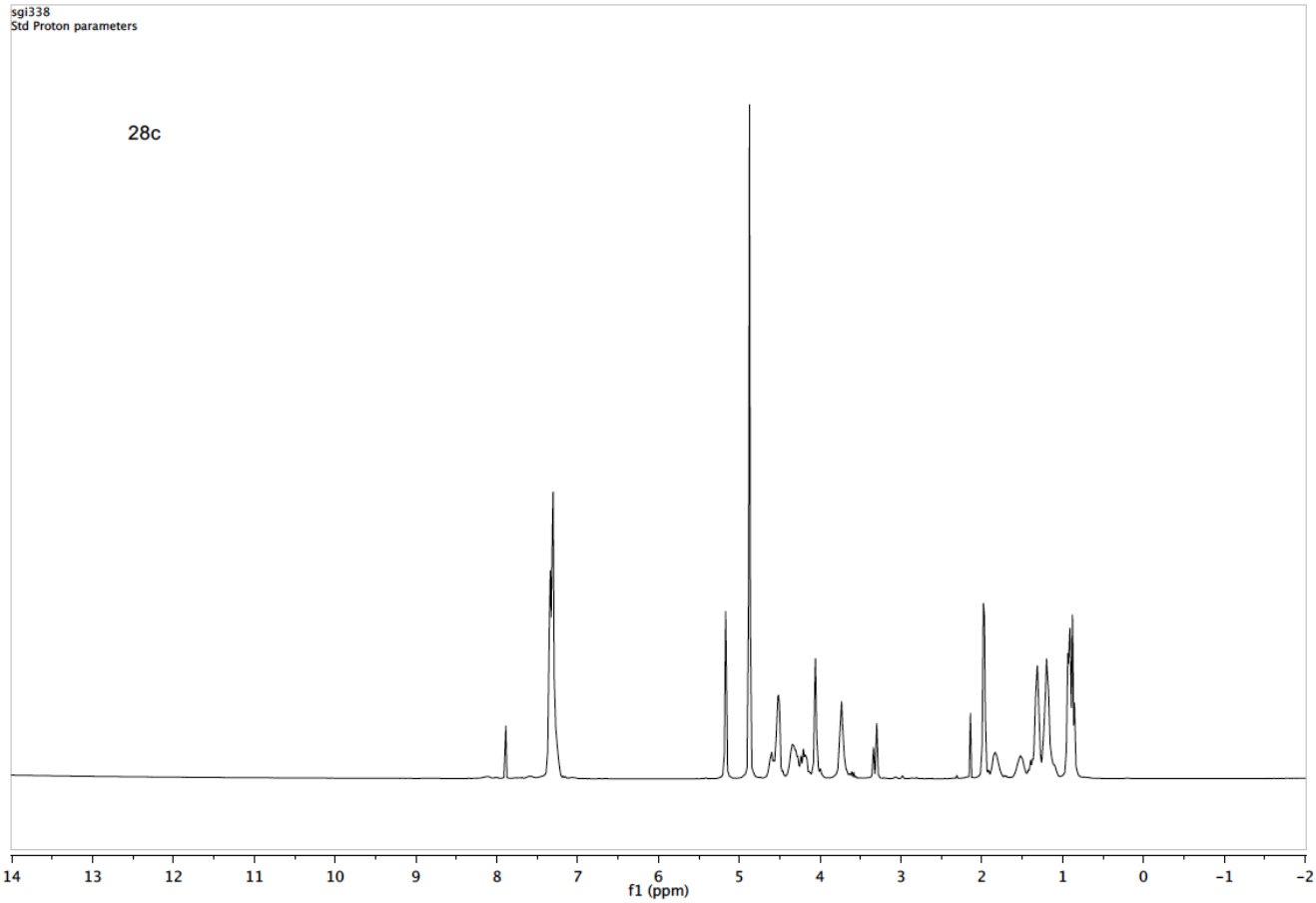

sgi418
Std Proton parameters
27k
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

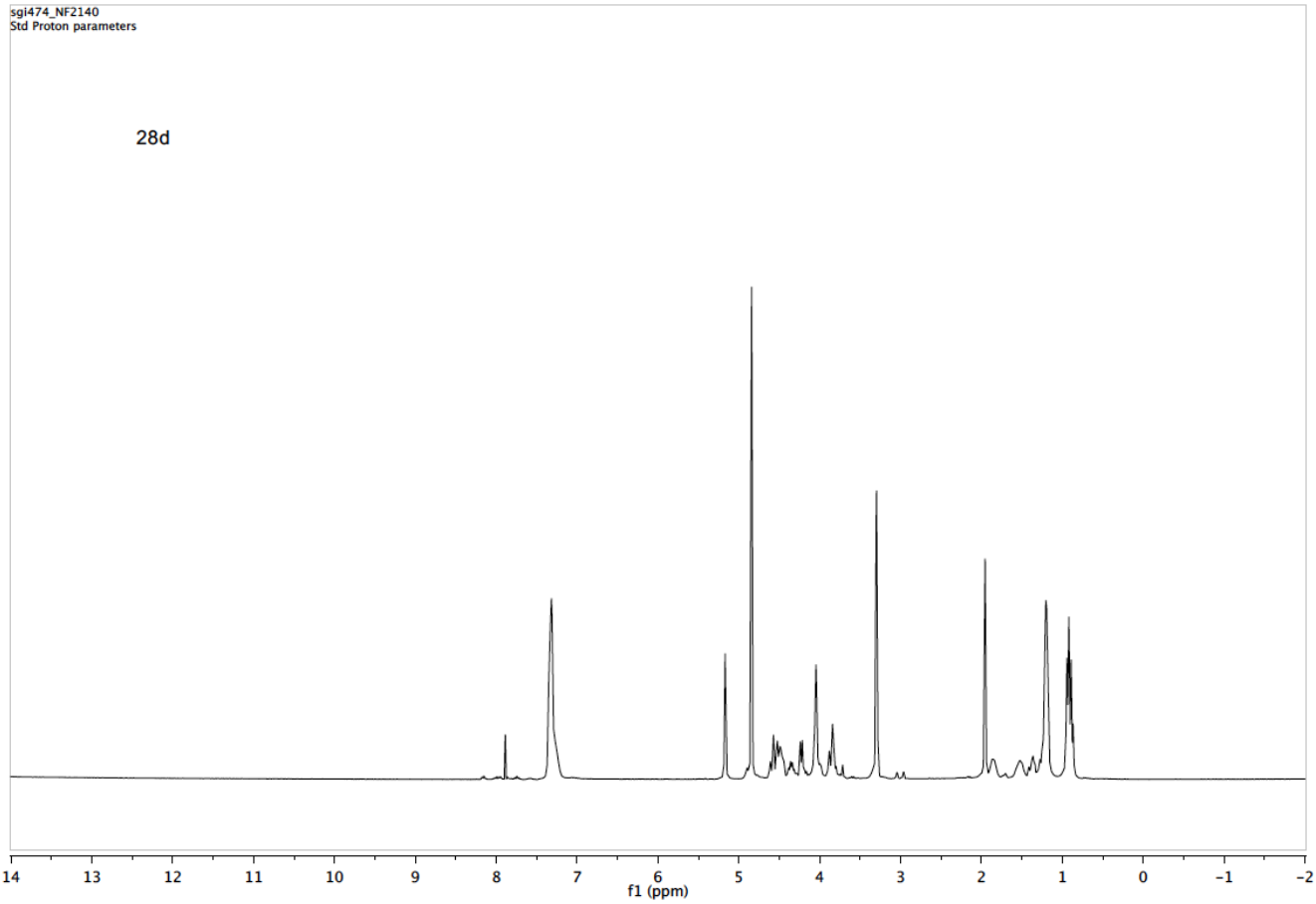

sgi448
Std Proton parameters
27l
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

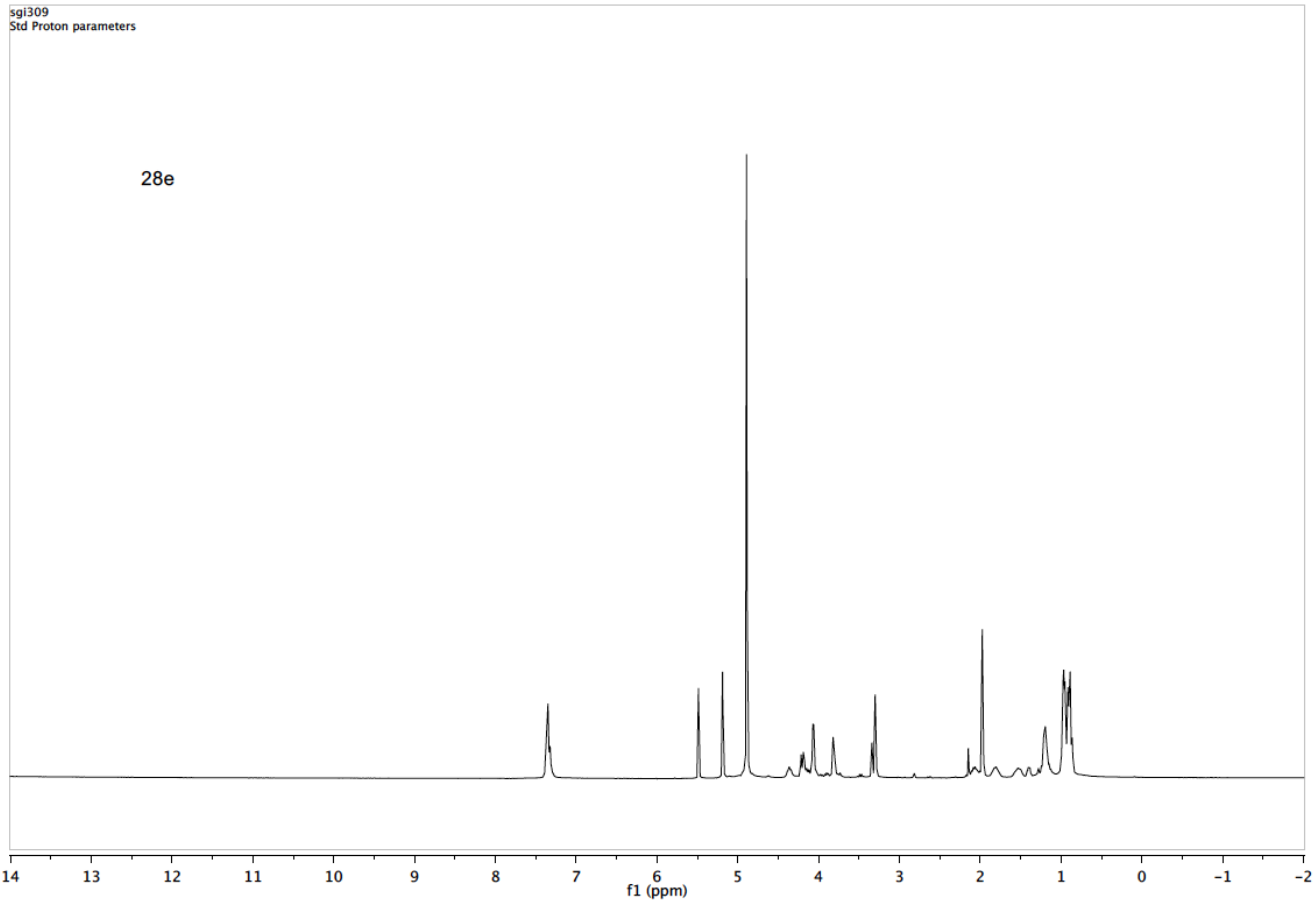

sgi447
Std Proton parameters
27m
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

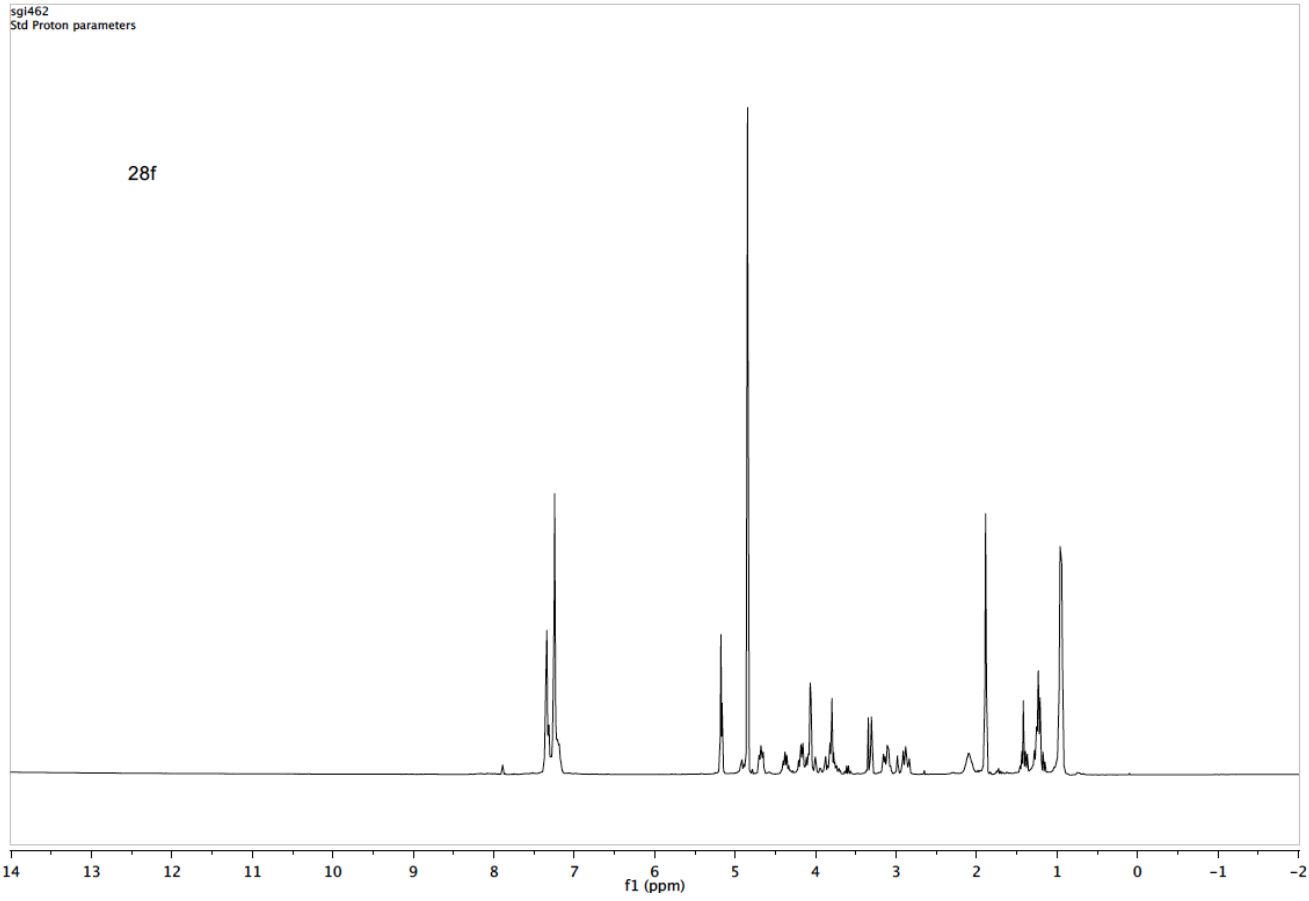

simone
28a
12.5 11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

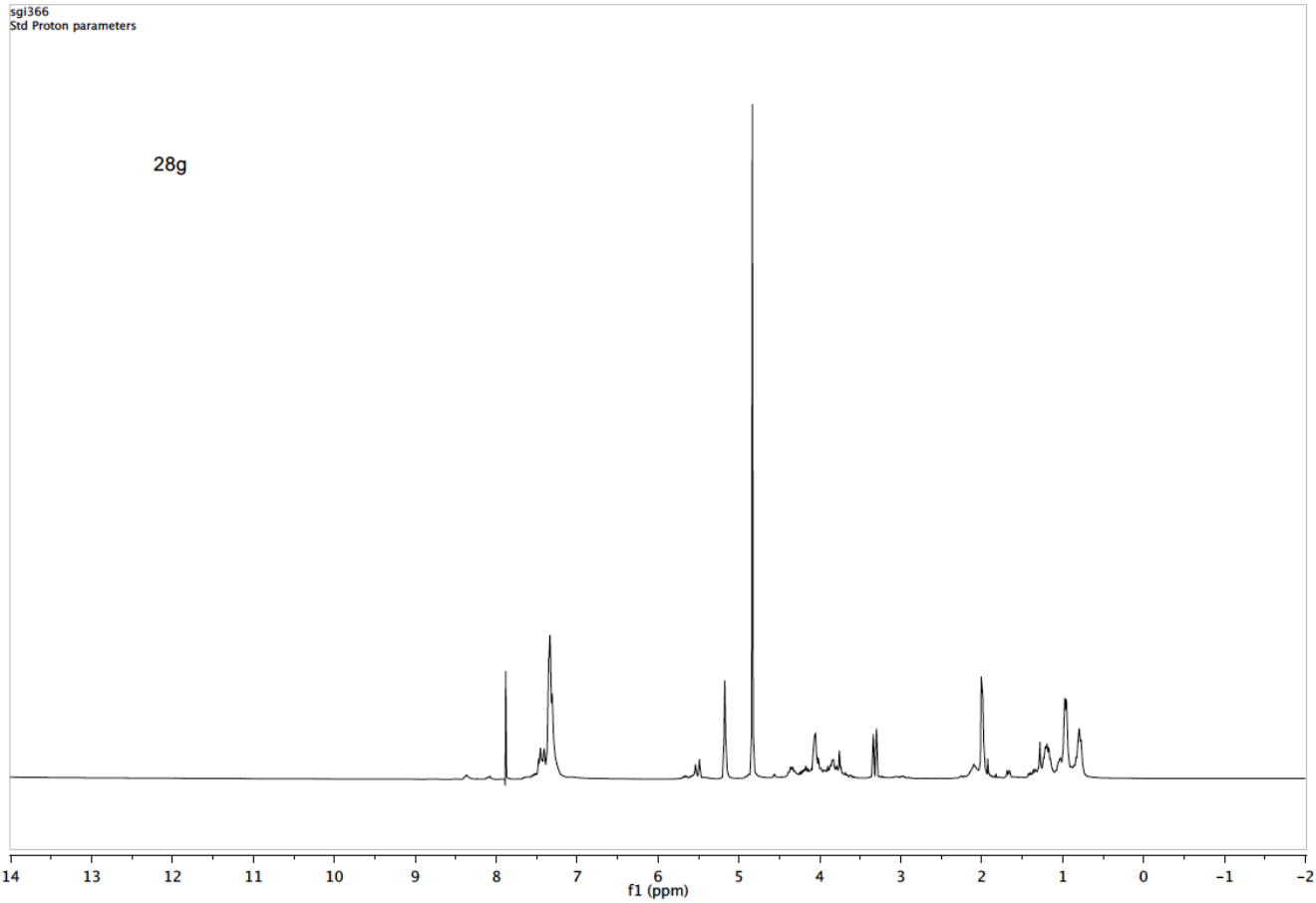

sgi339
Std Proton parameters
28b
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

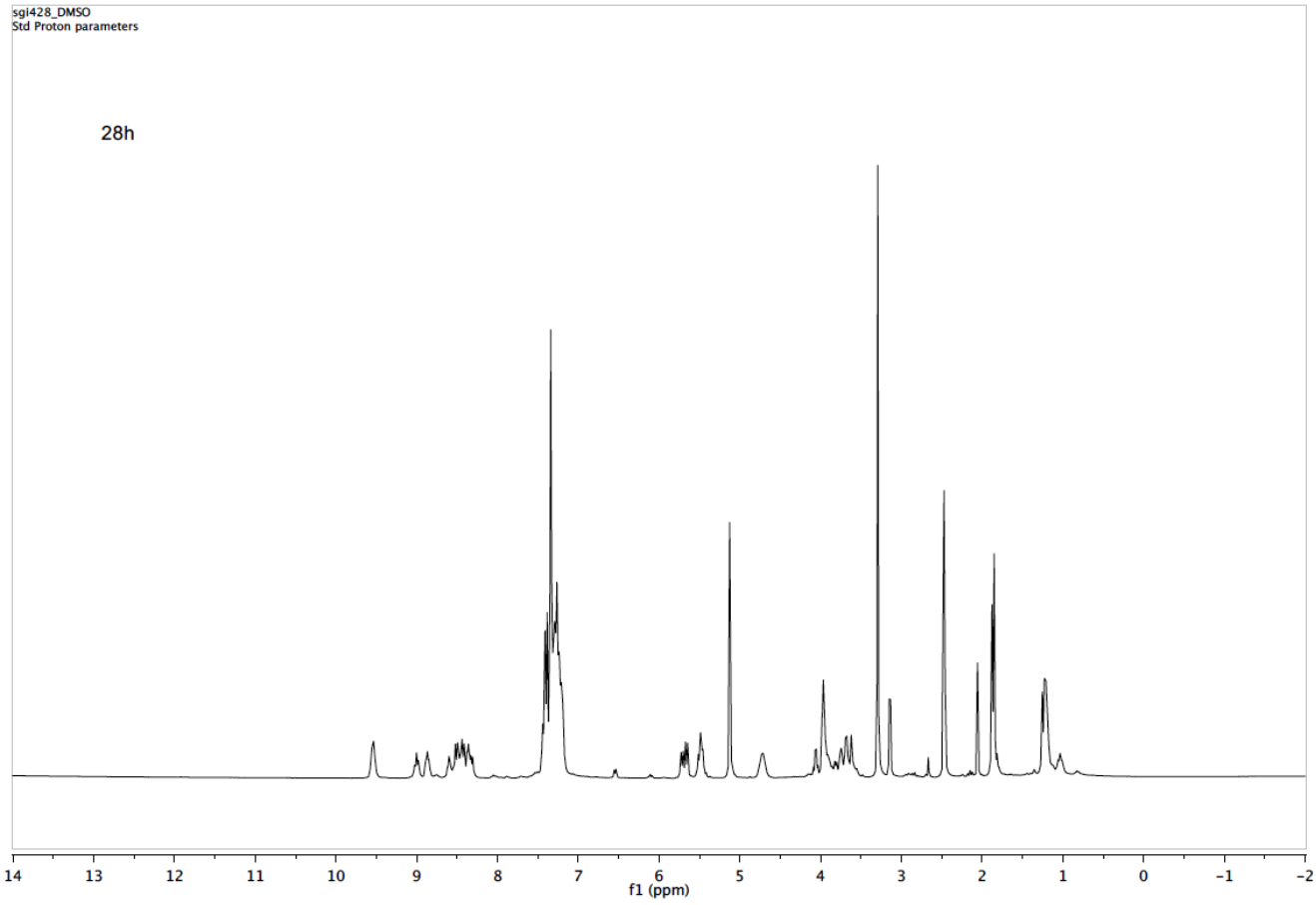

sgi338
Std Proton parameters
28c
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

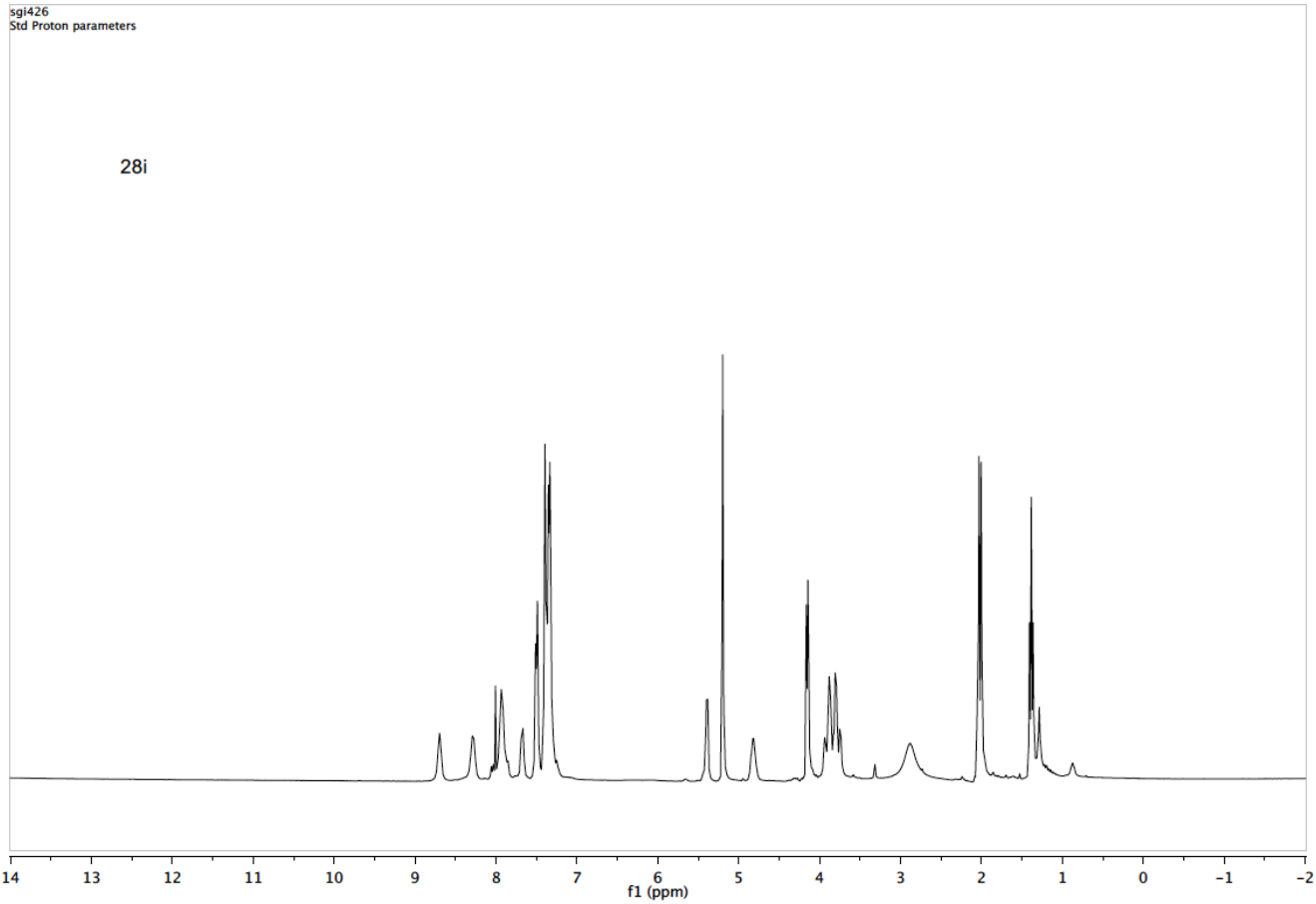

sgi474_NF2140
Std Proton parameters
28d
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

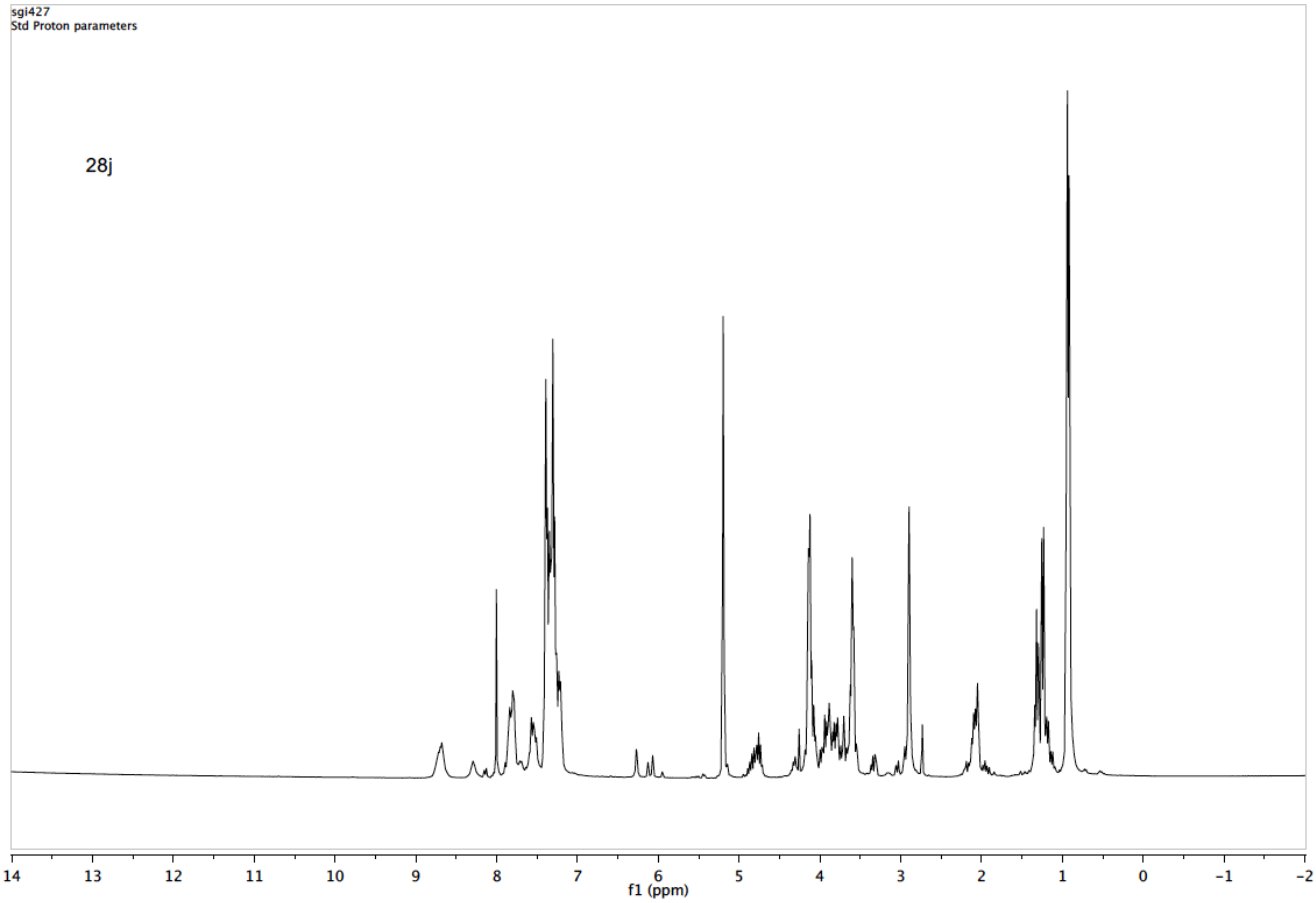

sgi309
Std Proton parameters
28e
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

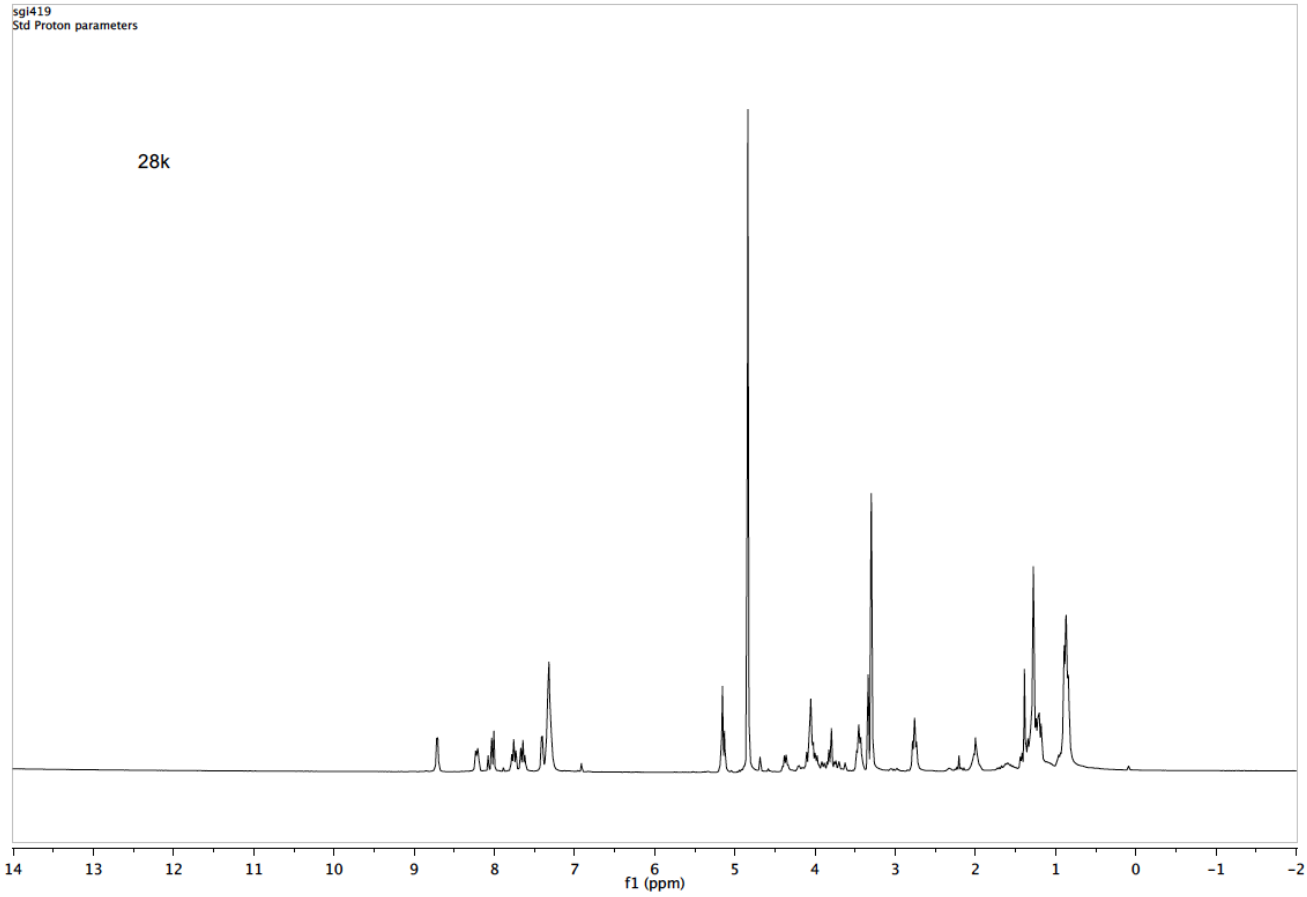

sgi462
Std Proton parameters
28f
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

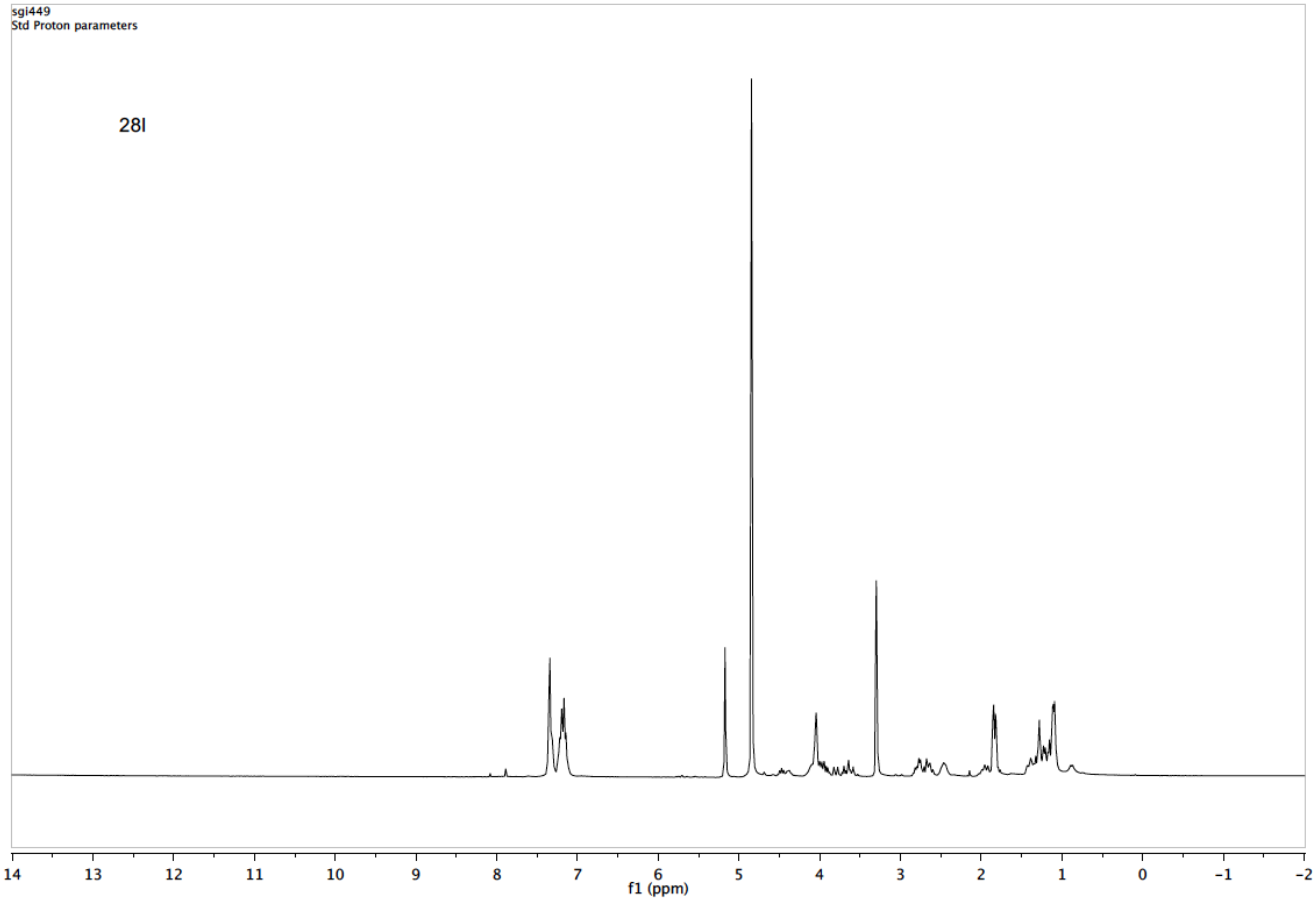

sgi366
Std Proton parameters
28g
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

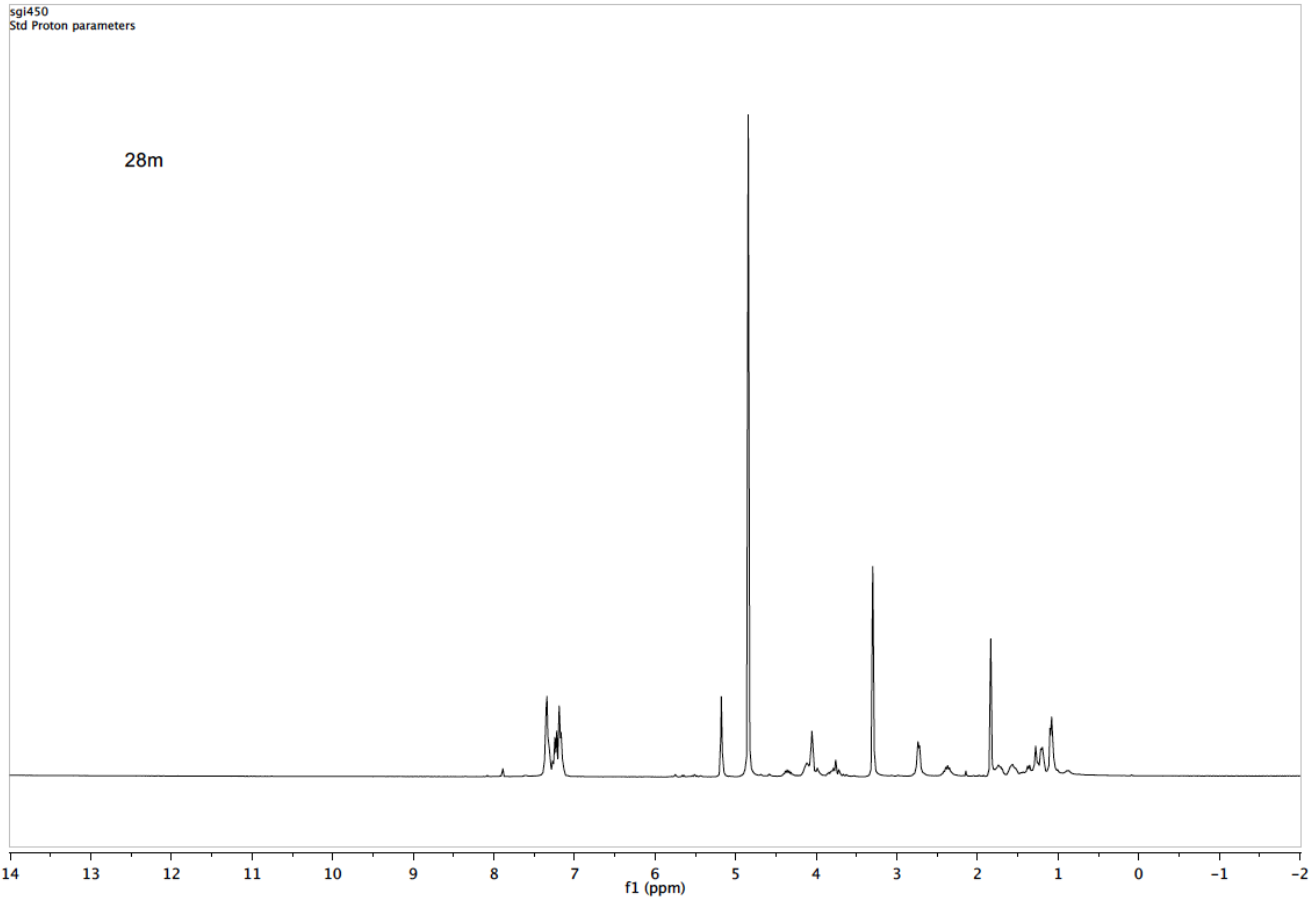

sgi428_DMSO
Std Proton parameters
28h
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

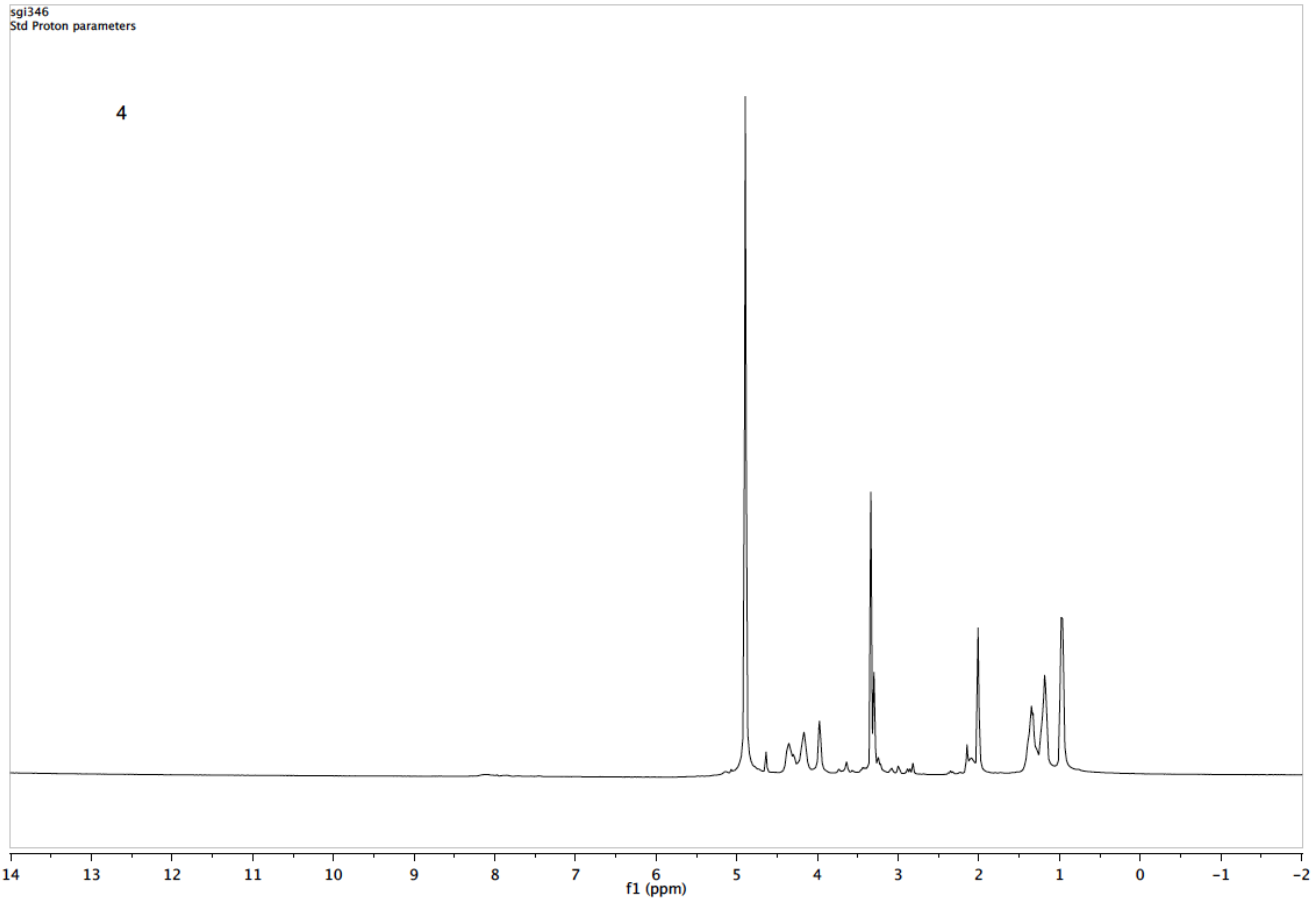

sgi426
Std Proton parameters
28i
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

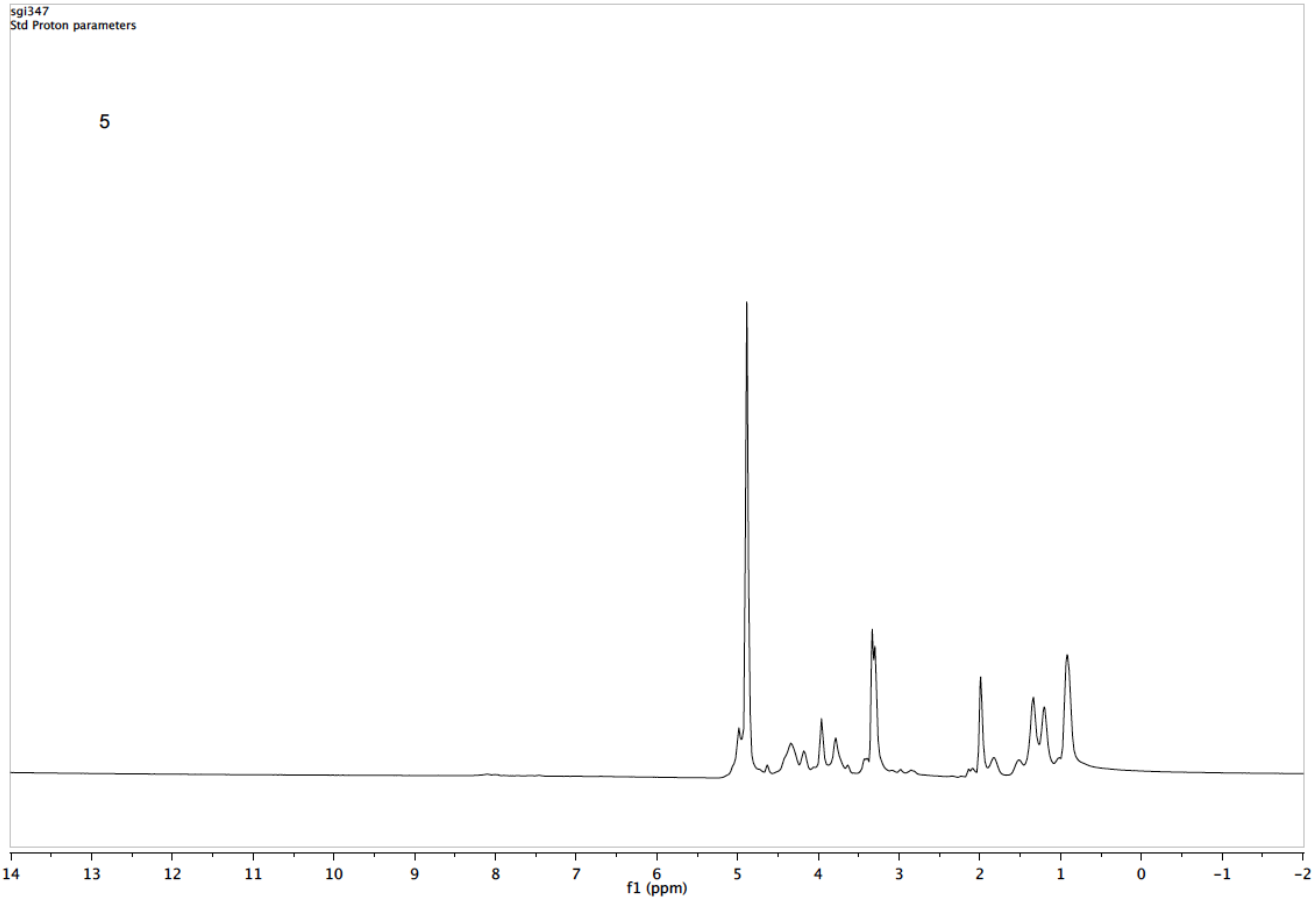

sgi427
Std Proton parameters
28j
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

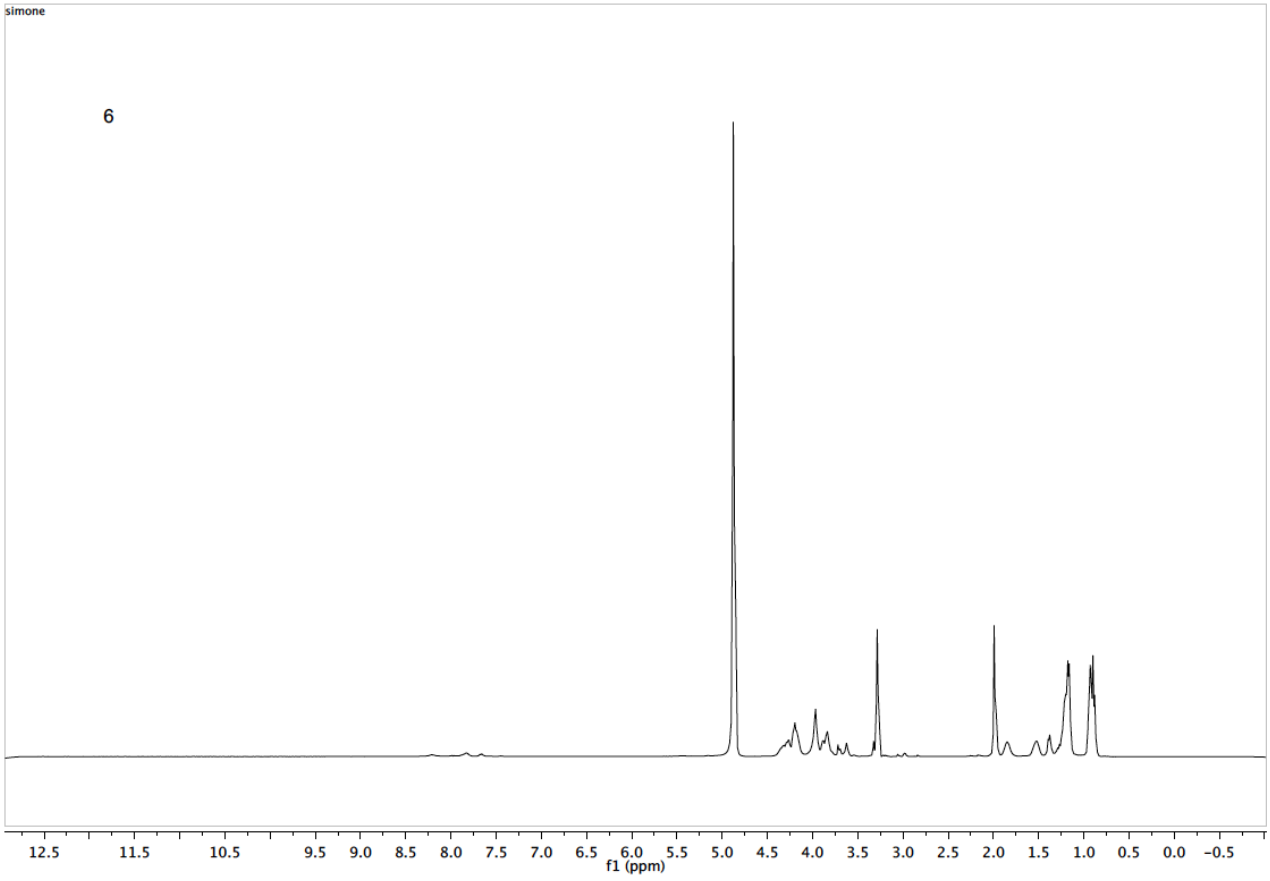

sgl419
Std Proton parameters
28k
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

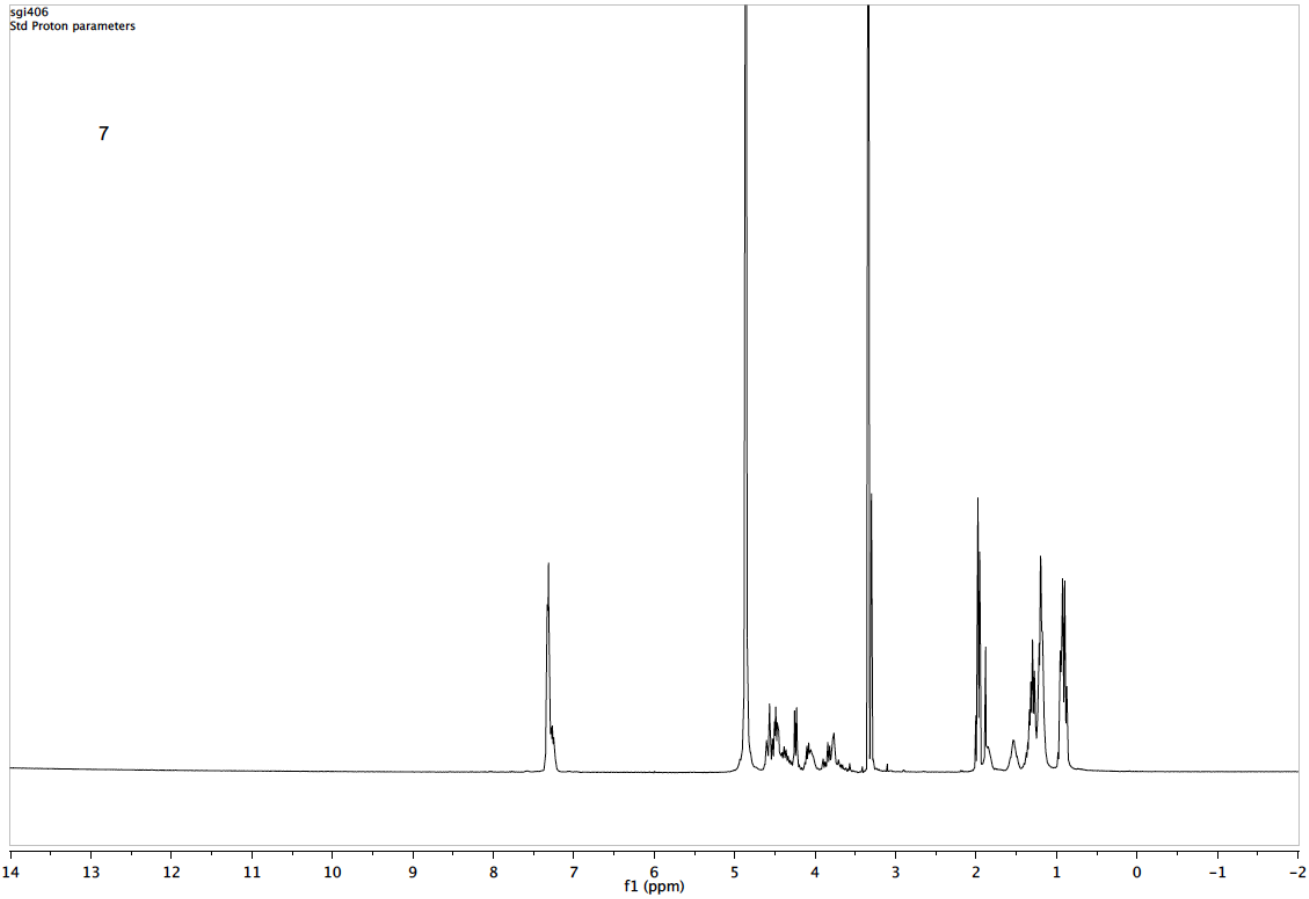

sgl449
Std Proton parameters
28l
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

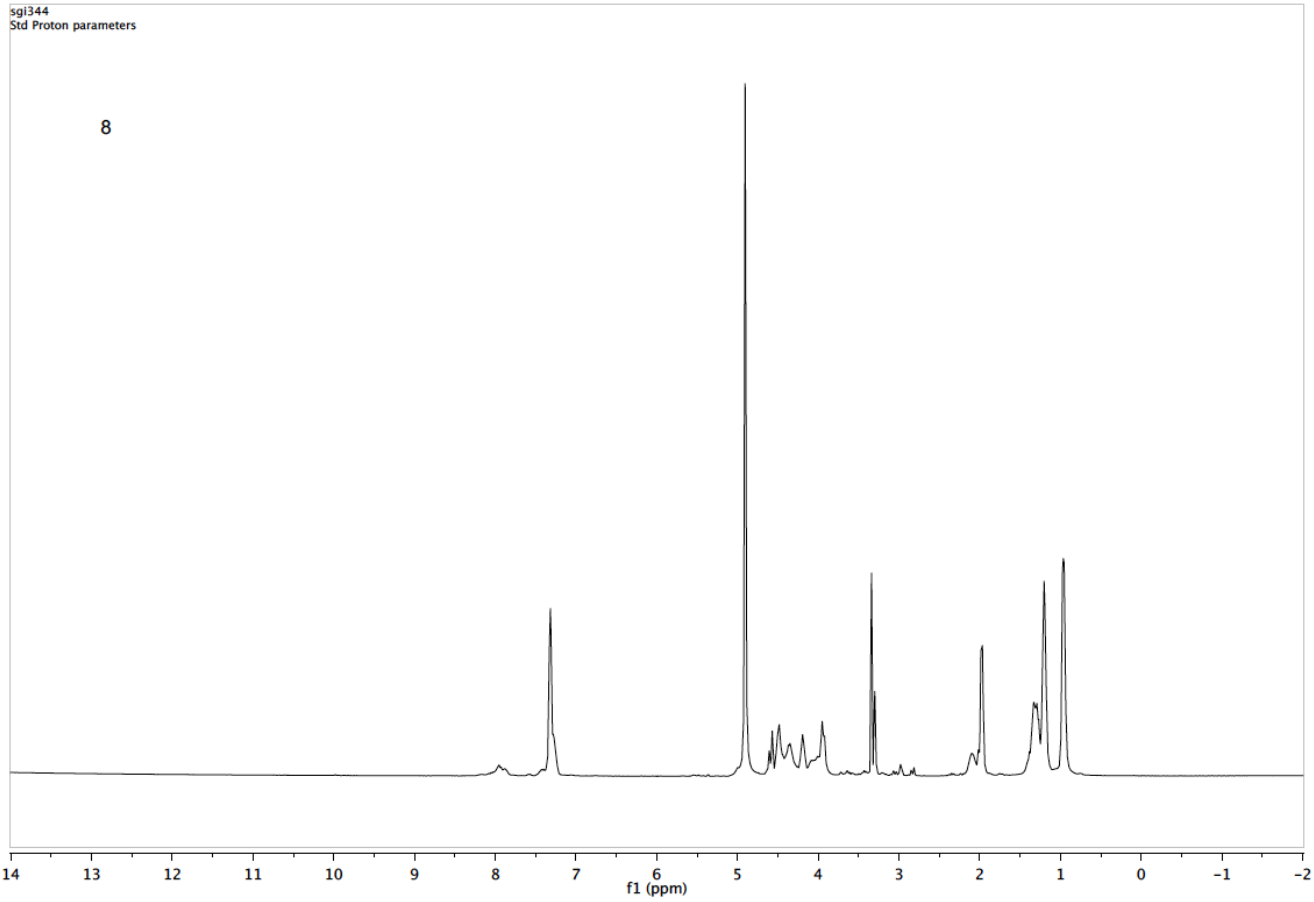

sgi450
Std Proton parameters
28m
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

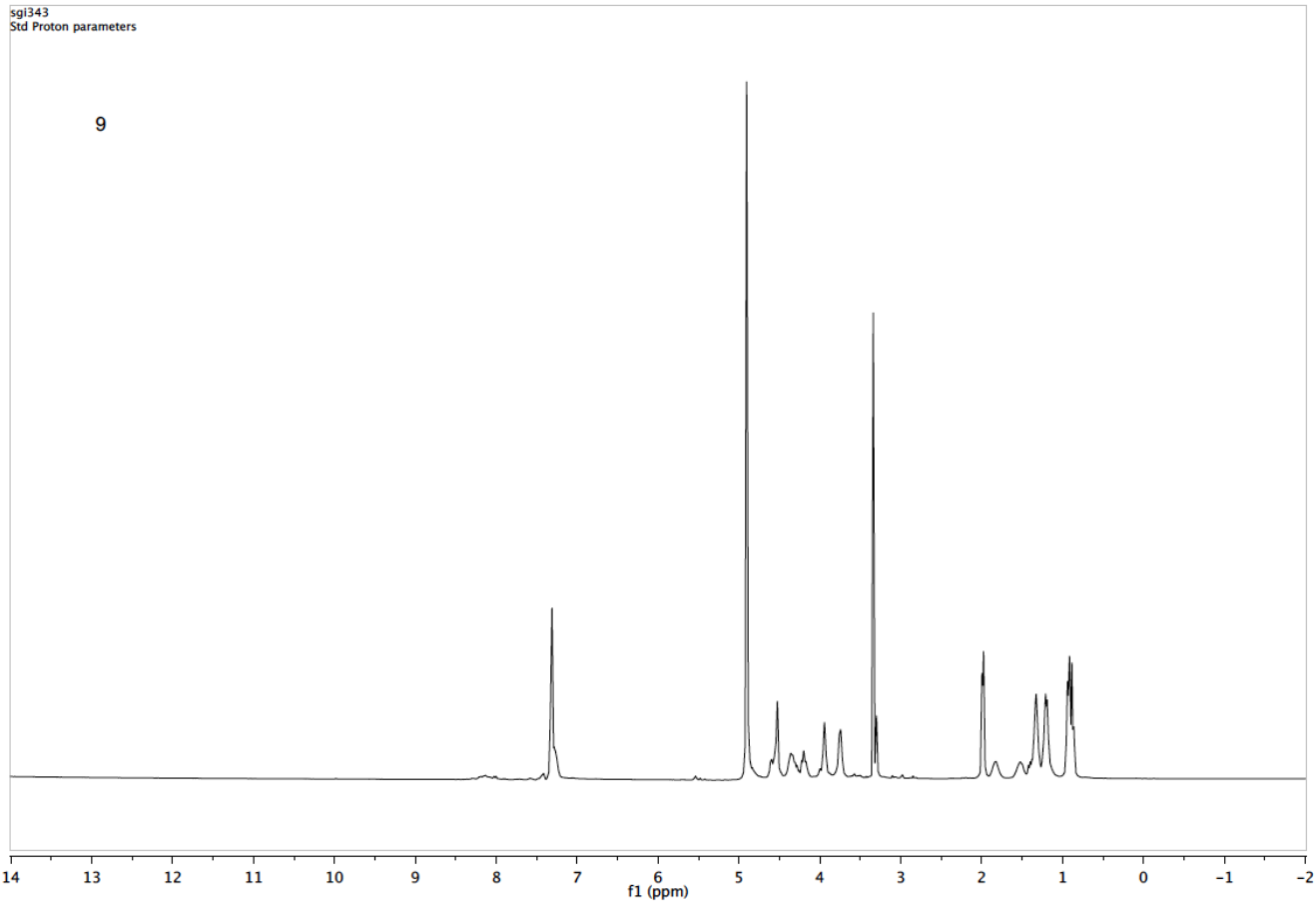

sgi346
Std Proton parameters
4
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

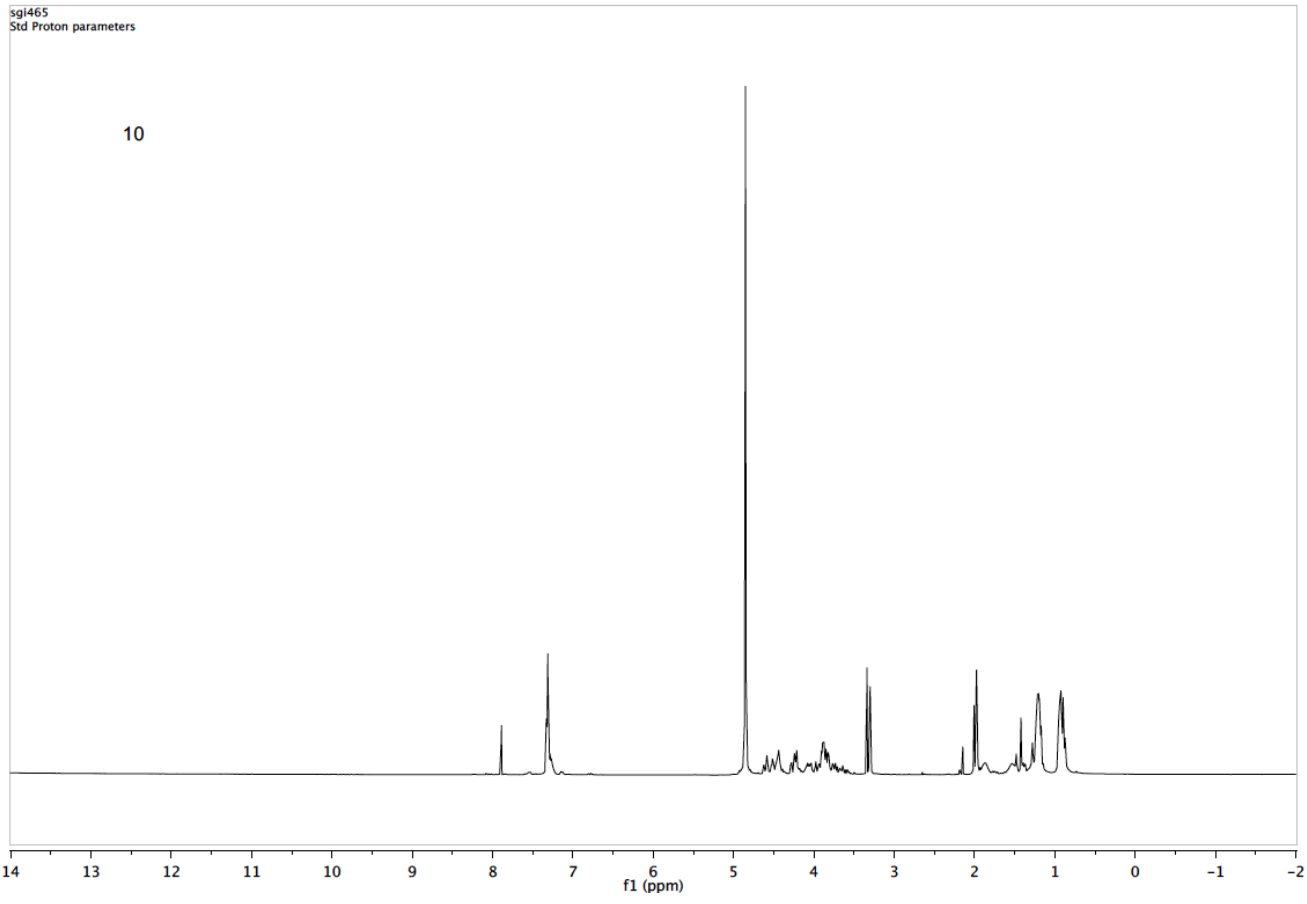

sgi347
Std Proton parameters
5
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

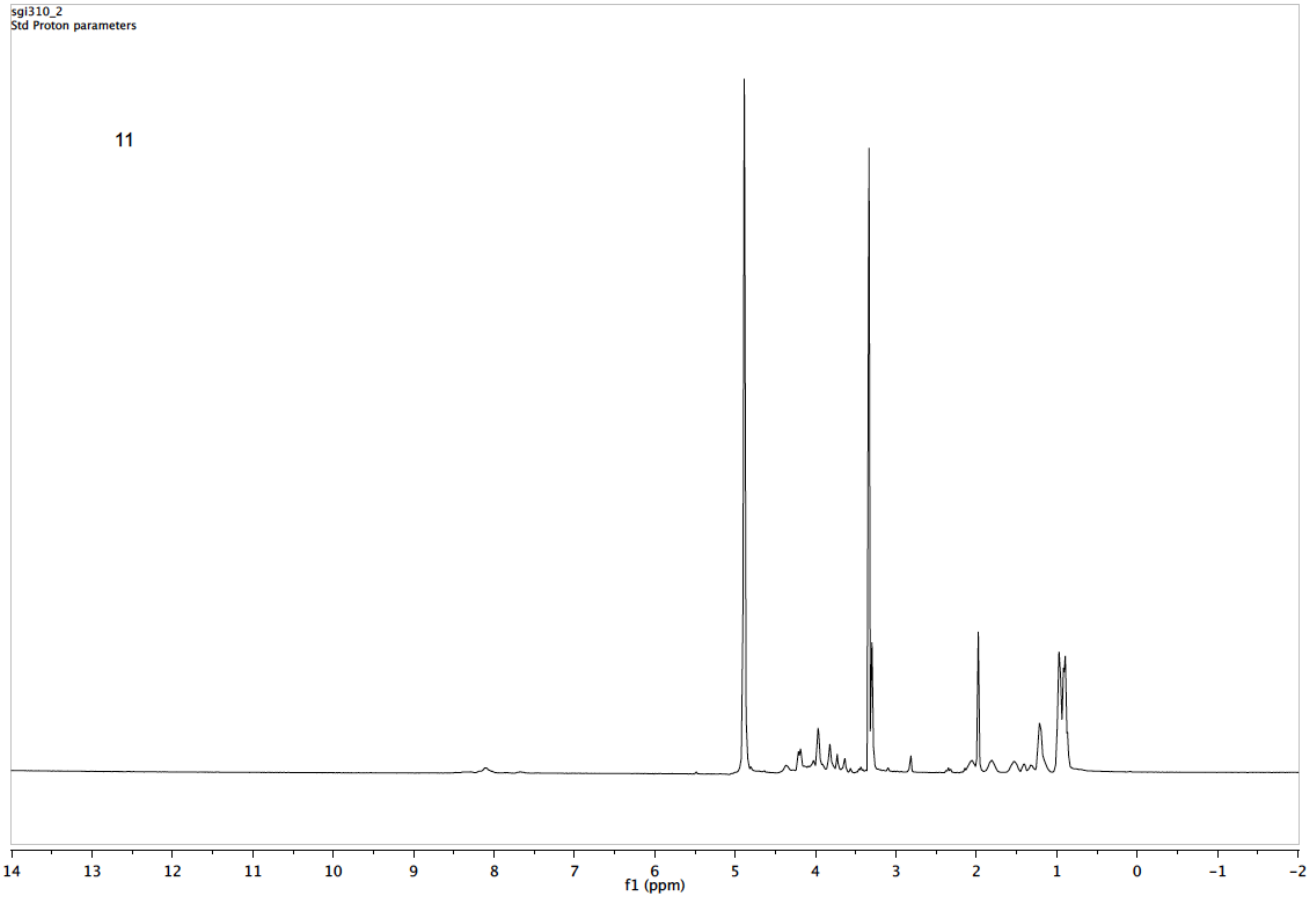

simone
6
12.5 11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 −0.5
f1 (ppm)

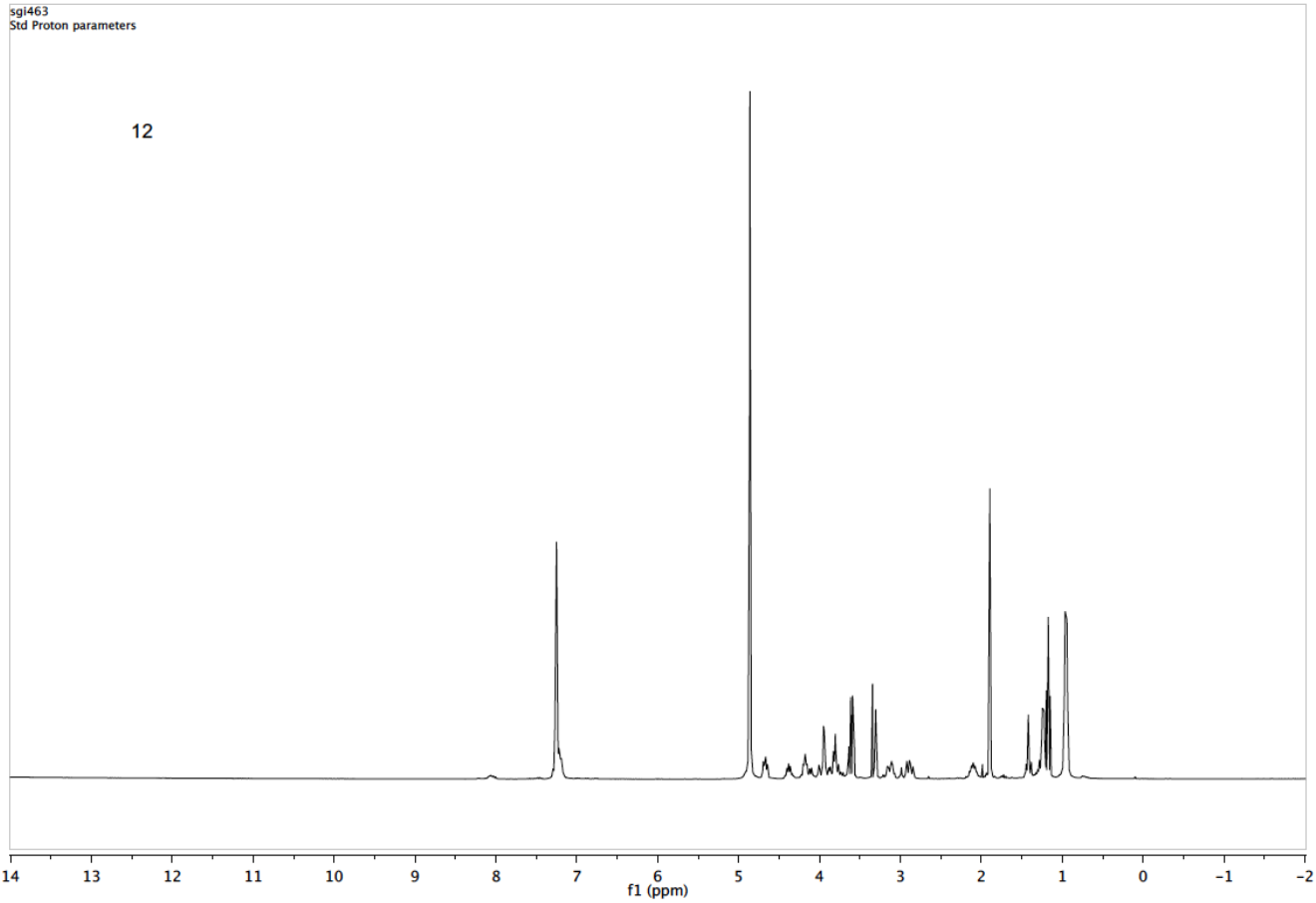

sgi406
Std Proton parameters
7
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

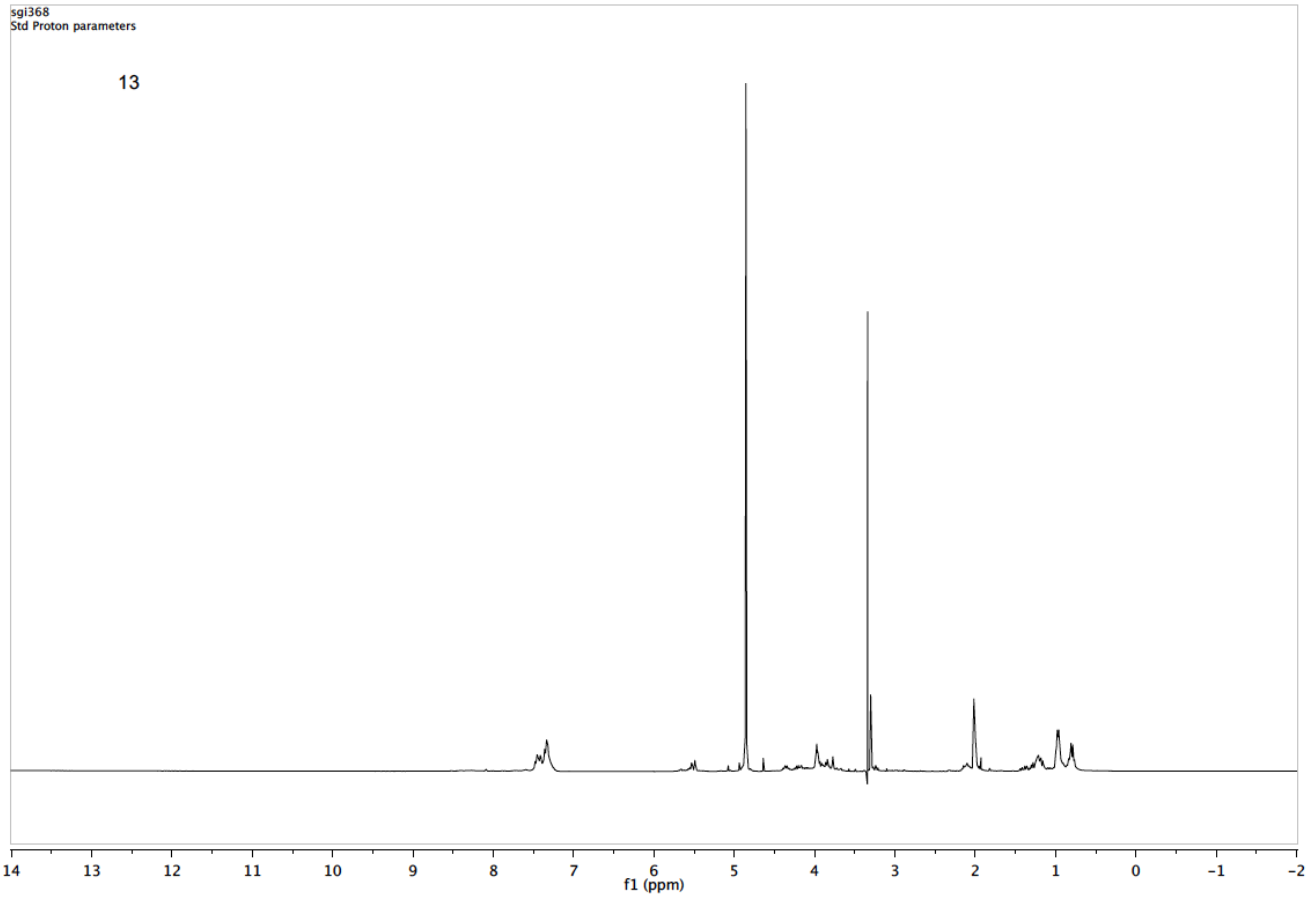

sgi344
Std Proton parameters
8
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

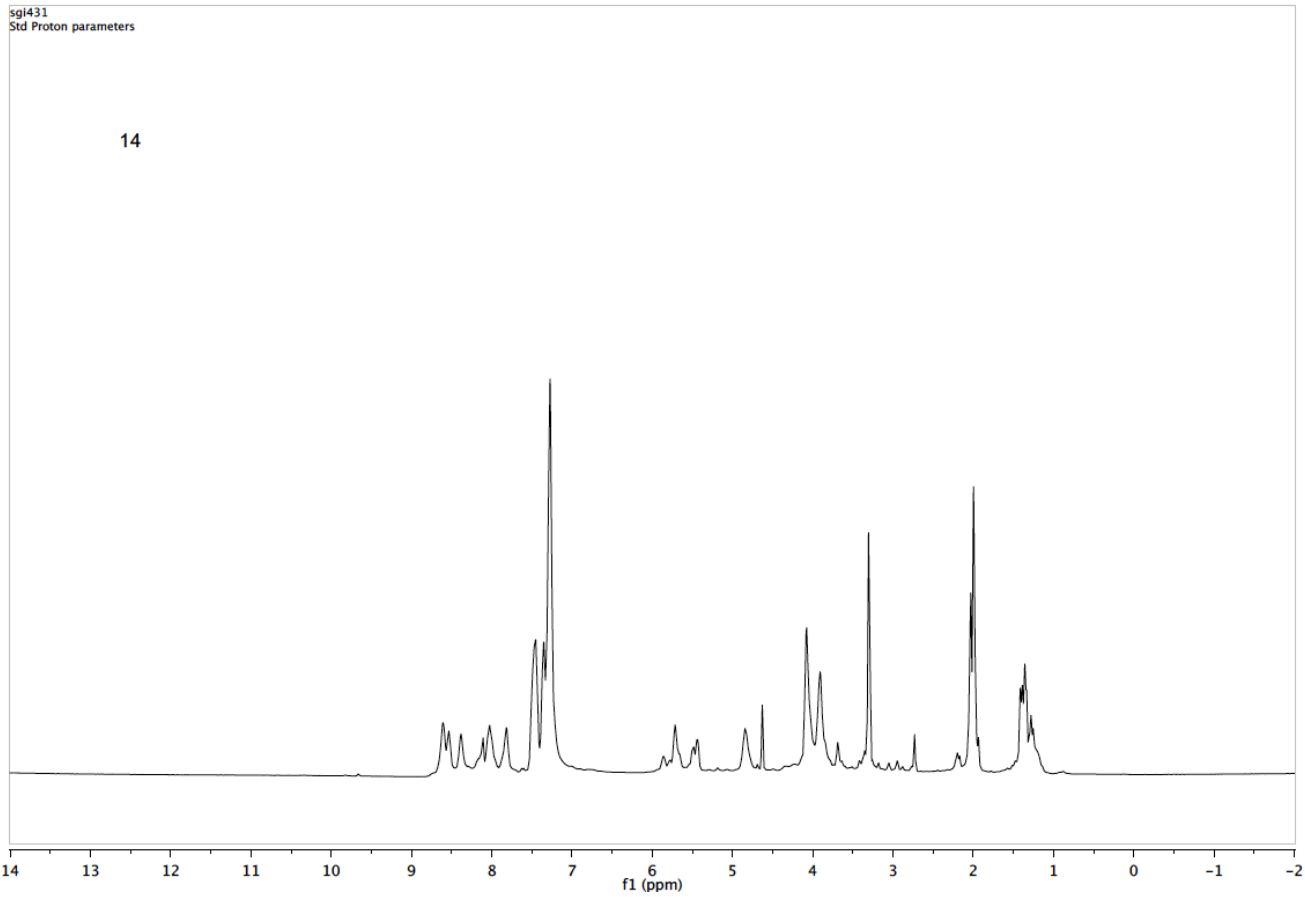

sgi343
Std Proton parameters
9
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

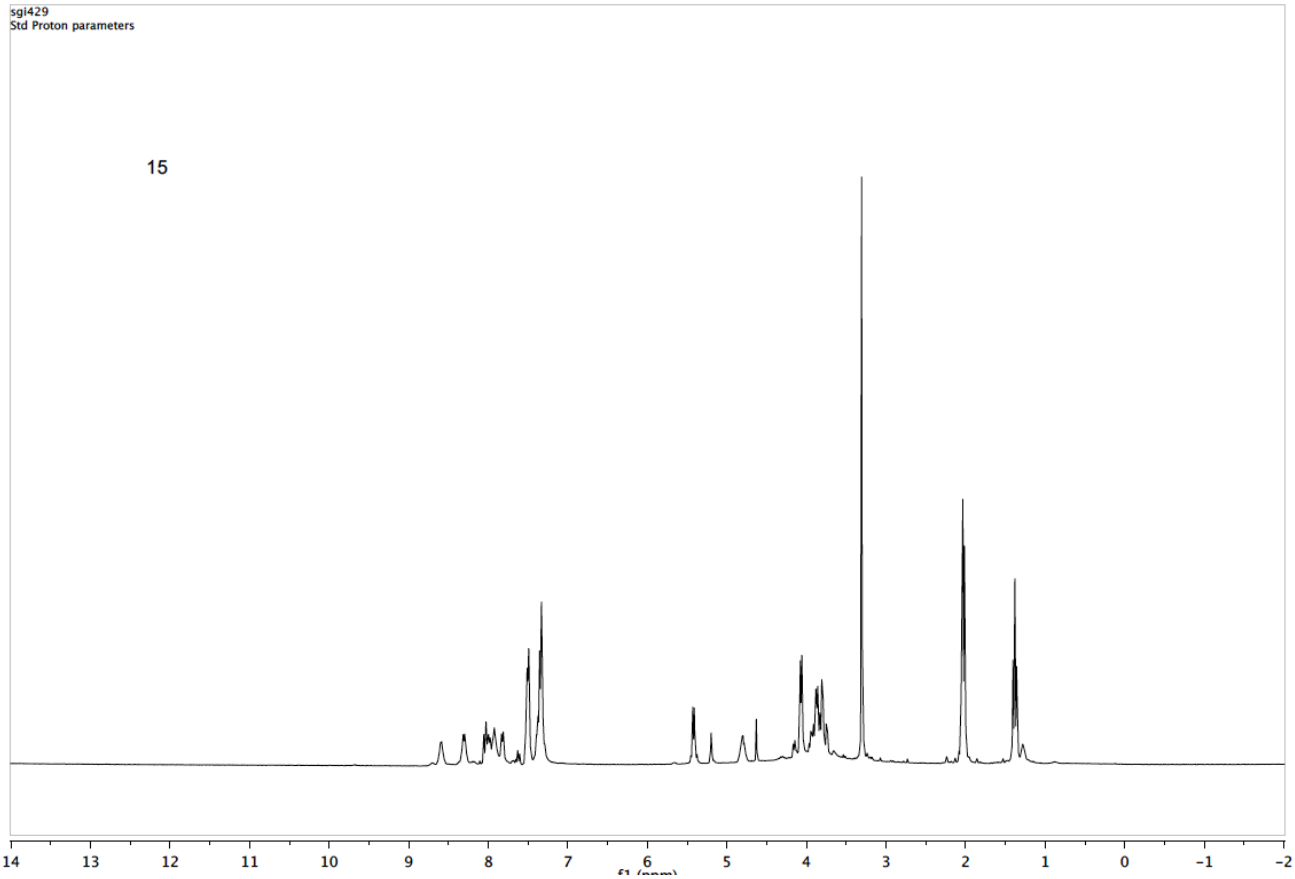

sgi465
Std Proton parameters
10
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

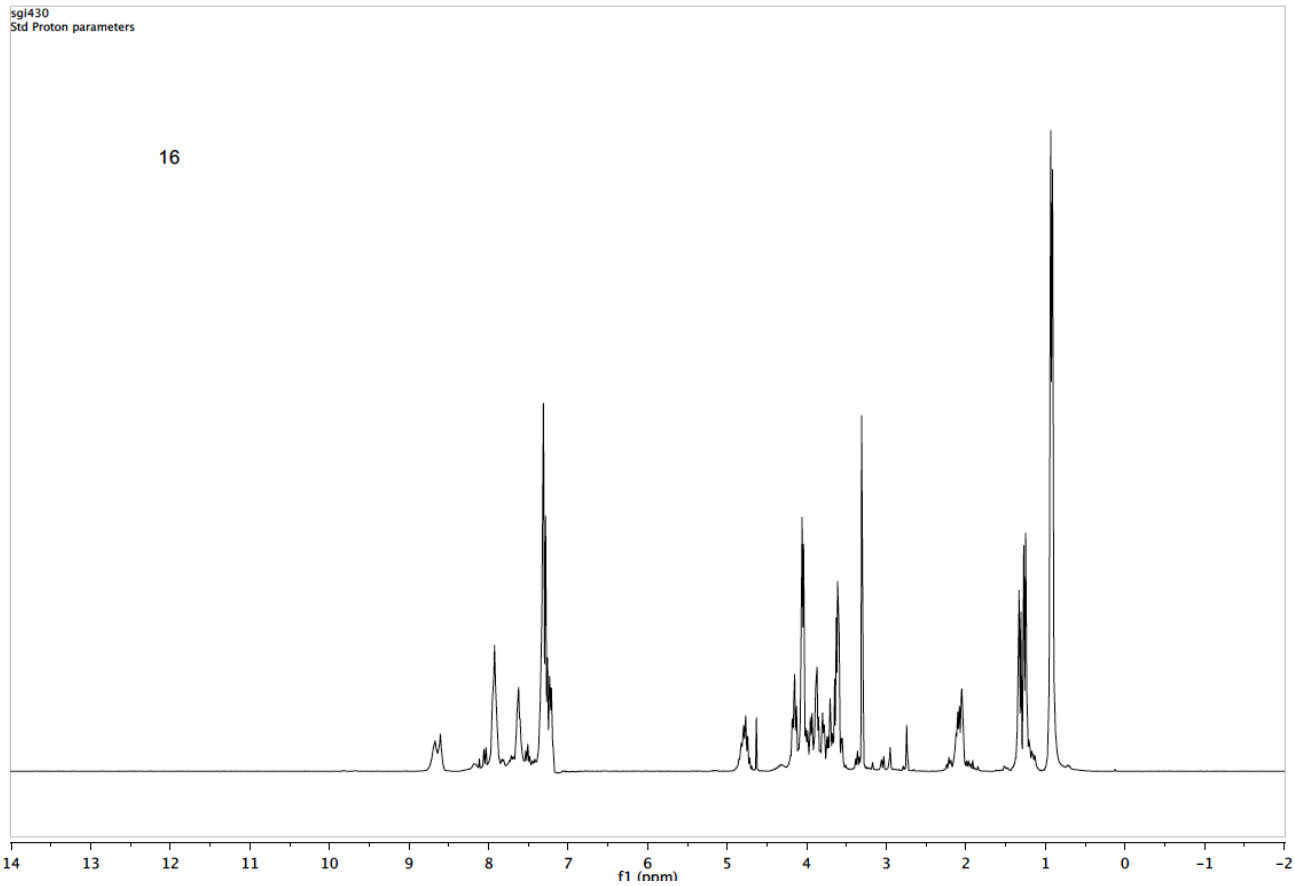

sgi310_2
Std Proton parameters
11
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

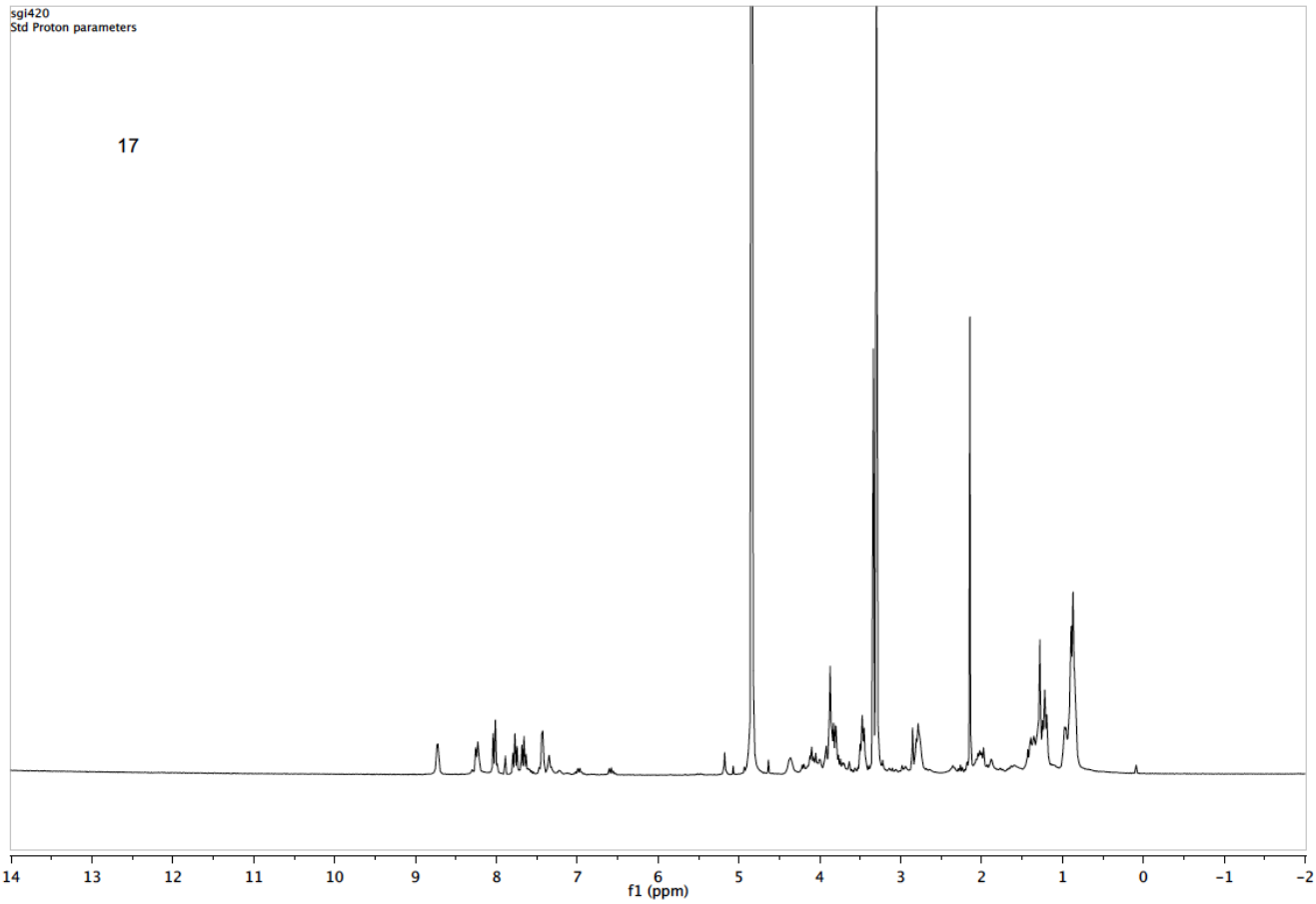

sgi463
Std Proton parameters
12
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f1 (ppm)

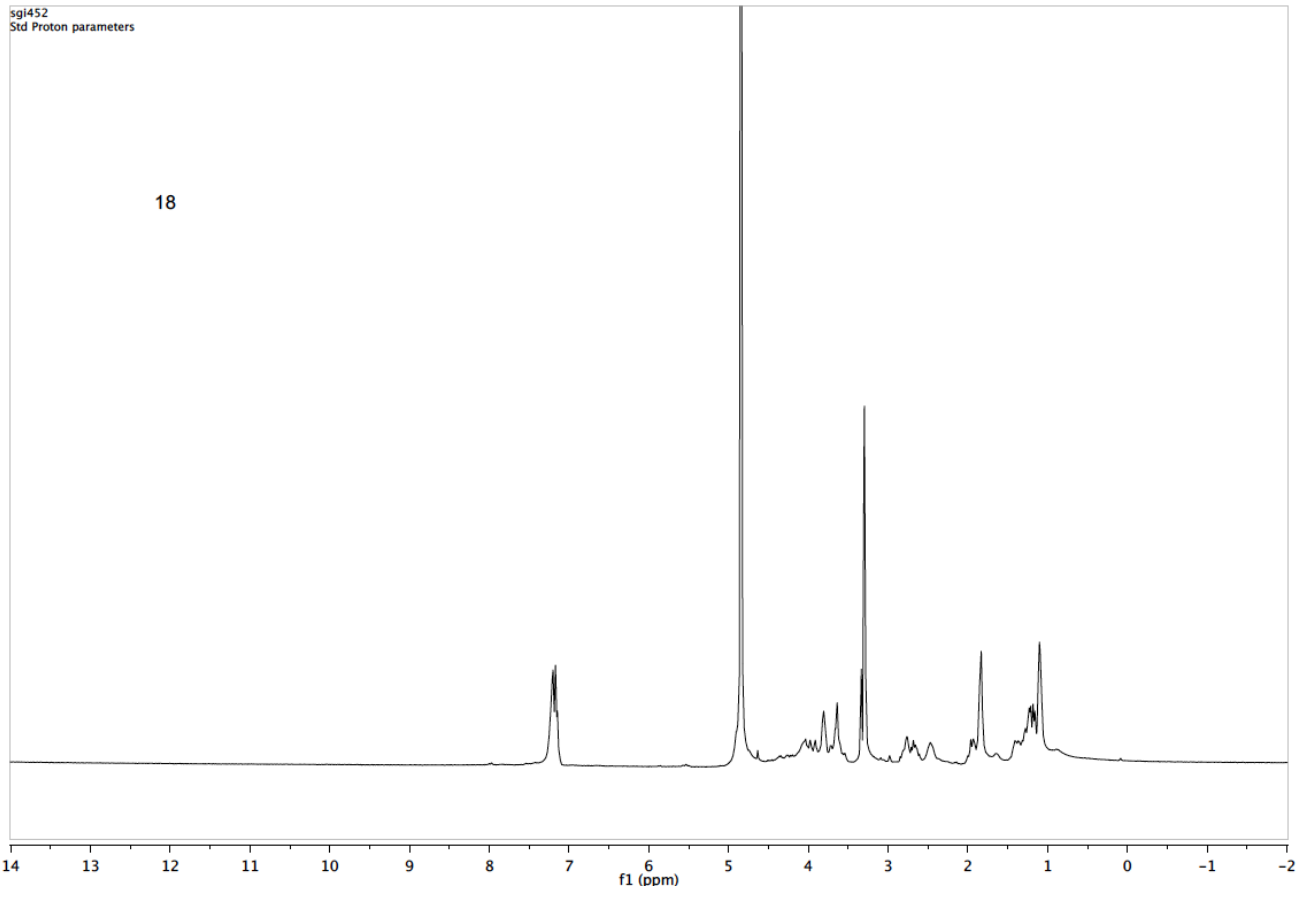

sgi368
Std Proton parameters
13
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)

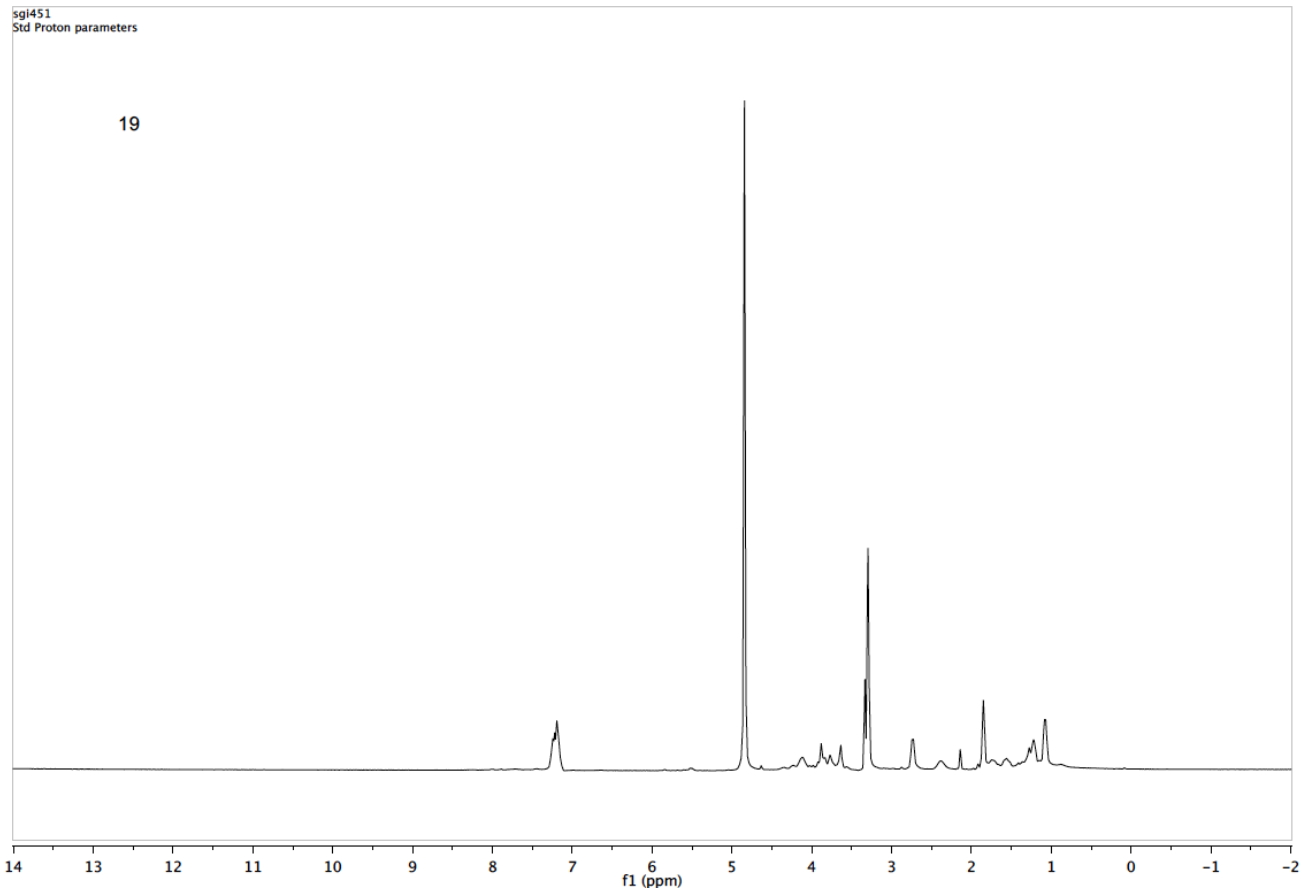

sgi431
Std Proton parameters
14
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)


sgi429
Std Proton parameters
15
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2


sgi430
Std Proton parameters
16
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)


sgi420
Std Proton parameters
17
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)


sgi452
Std Proton parameters
18
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2
f1 (ppm)


sgi451
Std Proton parameters
19
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
−2
f1 (ppm)