Electronic Supplementary Material (ESI) for Organic .

Supplementary Data for

Synthesis of High-Mannose 1-Thio Glycans and Their Conjugation to Protein

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Quantifying the degree of conjugation of compound 37: *N*-succinimidyl iodoacetate linker addition to ubiquitin.

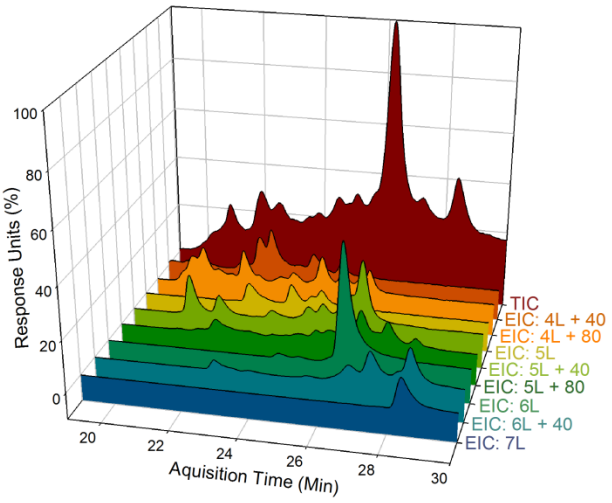


Figure S1. Total Ion Current (TIC) chromatogram and extracted-ion chromatograms (EICs) representing the differentially conjugated ubiquitin species of **37**. L=linkers, +40 signifies a mass increase of 40 MW, corresponding to an internal crosslink formed through reaction of the iodoacetyl moiety with a neighbouring amino acid functionality.

Table S1. Relative abundance of conjugate species of **37** as derived from peak integration from the PeakFit analysis of the UV chromatogram and identified by EIC correlation.

Peak	Identity	Area	Peak	Identity	Area
1	4L+80	0.12	16	4L+80	1.22
2	4L+80	0.27	17	5L+40	0.38
3	4L+80	0.24	18	5L+40	1.23
4	5L+40	0.42	19	4L+80	0.46
5	4L+80	0.18	20	5L+40	0.84
6	5L+40	0.21	21	5L+40	0.69
7	4L+80	0.31	22	6L	4.61
8	5L+80	0.35	23	5L+80	1.18
9	4L+40	0.10	24	6L+40	1.00
10	4L+40	0.21	25	5L+80	1.24
11	5L	0.47	26	6L+40	1.17
12	6L+40	0.19	27	5L+80	0.63
13	4L+40	0.14	28	6L+40	1.72
14	5L	0.13	29	7L	0.48
15	6L	0.27	Total Area:		20.46

Table S2. Summary of linker distribution of conjugate **37**.

37	Area	% Area
4 Linkers	3.11	15.20%
5 Linkers	7.91	38.66%
6 Linkers	8.96	43.79%
7 Linkers	0.48	2.35%
Total:	20.46	100.00%
Average # of linkers:		5.3

Note: L = linkers; +40 signifies a mass increase of 40 MW, corresponding to an internal crosslink formed through reaction of the iodoacetyl moiety with a neighbouring amino acid functionality.

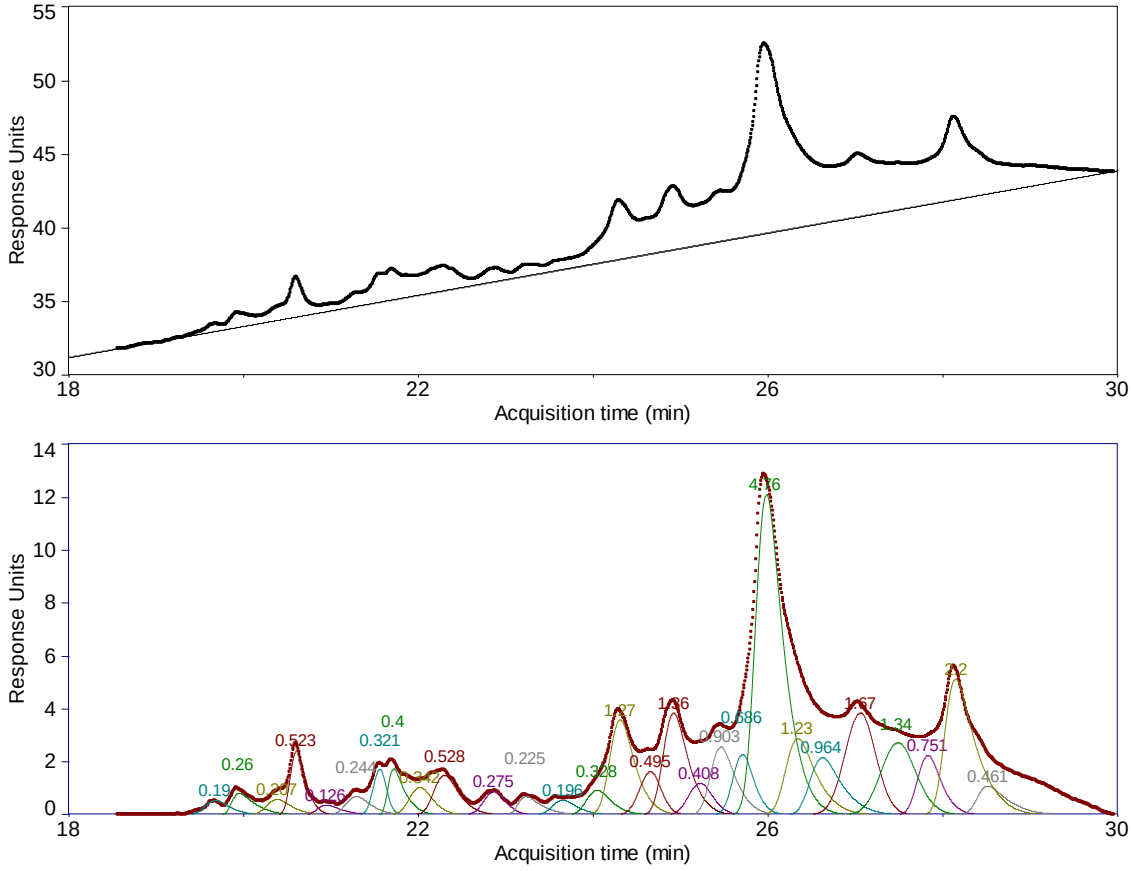


Figure S2. UV chromatogram (@210 nm) obtained from LC-UV-MS (above) and simulated chromatogram obtained by use of PeakFit software (below) for ubiquitin conjugate **37**.

Quantifying the degree of conjugation of compound 38: *N*-succinimidyl 4-pentynoate linker addition to ubiquitin.

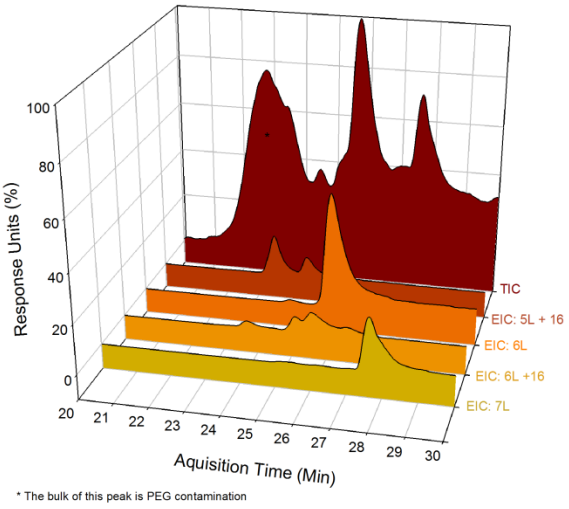


Figure S3. Total Ion Current (TIC) chromatogram and extracted-ion chromatograms (EICs) representing the differentially conjugated ubiquitin species of **38**. L = linkers, +16 signifies a mass increase of 16 MW corresponding to one oxidation.

Table S3. Relative abundance of conjugate species of **38** as derived from peak integration from the PeakFit analysis of the UV chromatogram and identified by EIC correlation.

Peak	Identity	Area
1	6L	0.49
2	5L+16	1.56
3	5L+16	1.15
4	6L+16	2.27
5	6L+16	0.66
6	6L	10.73
7	7L	4.54
8	7L	0.75
Total Area:		22.15

Note: L = linkers; +16 denotes a mass increase of 16 MW corresponding to one oxidation.

Table S4. Summary of linker distribution of conjugate **38**.

38	Area	% Area
5 Linkers	2.71	12.21%
6 Linkers	14.15	63.89%
7 Linkers	5.29	23.89%
Total:	22.15	100.00%
Average # of linkers:		6.1

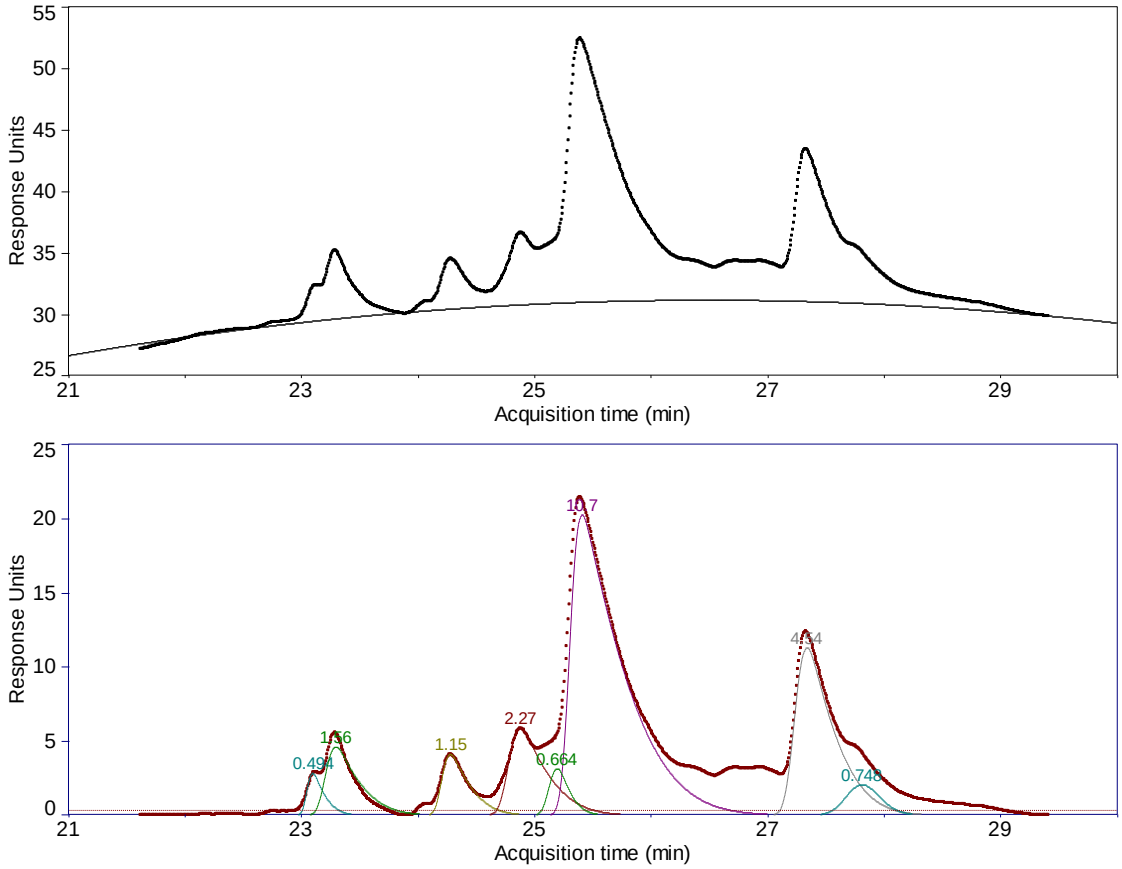


Figure S4. UV chromatogram (@210 nm) obtained from LC-UV-MS (above) and simulated chromatogram obtained by use of PeakFit software (below) for ubiquitin conjugate **38**. The PEG contamination observed in the TIC chromatogram does not interfere with protein quantification by UV peak integration as it does not absorb UV (@210 nm).

Quantifying the degree of conjugation of compound 39: glycoconjugation of 1 (Man_4) to 37.

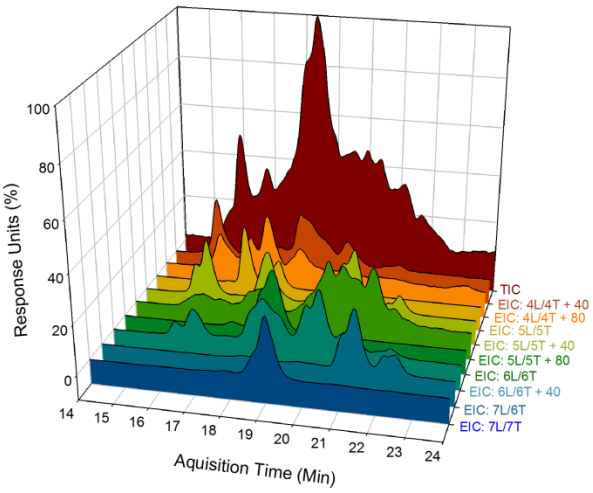


Figure S5. Total Ion Current (TIC) chromatogram and extracted-ion chromatograms (EICs) representing the differentially conjugated ubiquitin species of **39**. L = linkers, T = tetrasaccharides, +40 signifies a mass increase of 40 MW corresponding to an internal crosslink formed through reaction of the iodoacetyl moiety with a neighbouring amino acid functionality.

Table S5. Relative abundance of conjugate species of **39** as derived from peak integration from the PeakFit analysis of the UV chromatogram and identified by EIC correlation.

Peak	Identity	Area	Peak	Identity	Area
1	4L/4T+40	1.00	16	6L/6T+40	1.50
2	5L/5T+40	0.97	17	6L/6T+40	1.63
3	4L/4T+80	0.58	18	7L/7T	2.45
4	5L/5T+40	0.25	19	4L/4T+40	2.80
5	6L/6T+40	0.27	20	5L/5T+40	3.11
6	6L/6T+40	0.20	21	5L/5T+80	1.86
7	4L/4T+80	0.71	22	6L/6T+40	2.24
8	5L/5T	2.54	23	5L/5T+40	2.64
9	4L4T+80	1.29	24	5L/5T+80	1.66
10	5L/5T+40	1.61	25	5L/5T+80	0.48
11	5L/5T	3.74	26	5L/5T+40	2.27
12	4L/4T+40	1.81	27	7L/6T	1.36
13	6L/6T	6.08	28	7L/6T	0.48
14	4L/4T+80	1.98	29	7L/6T	0.26
15	6L/6T	10.40	Total Area:		58.17

Table S6. Summary of Man_4 distribution of conjugate **39**.

39	Area	% Area
4 Man_4	10.17	17.49
5 Man_4	21.12	36.32
6 Man_4	24.42	41.98
7 Man_4	2.45	4.21
Total:	58.17	100.00
Average # of Man_4:		5.3

Note: L = linkers; T = tetrasaccharides; +40 signifies a mass increase of 40 MW, corresponding to an internal crosslink formed through reaction of the iodoacetyl moiety with a neighbouring amino acid functionality.

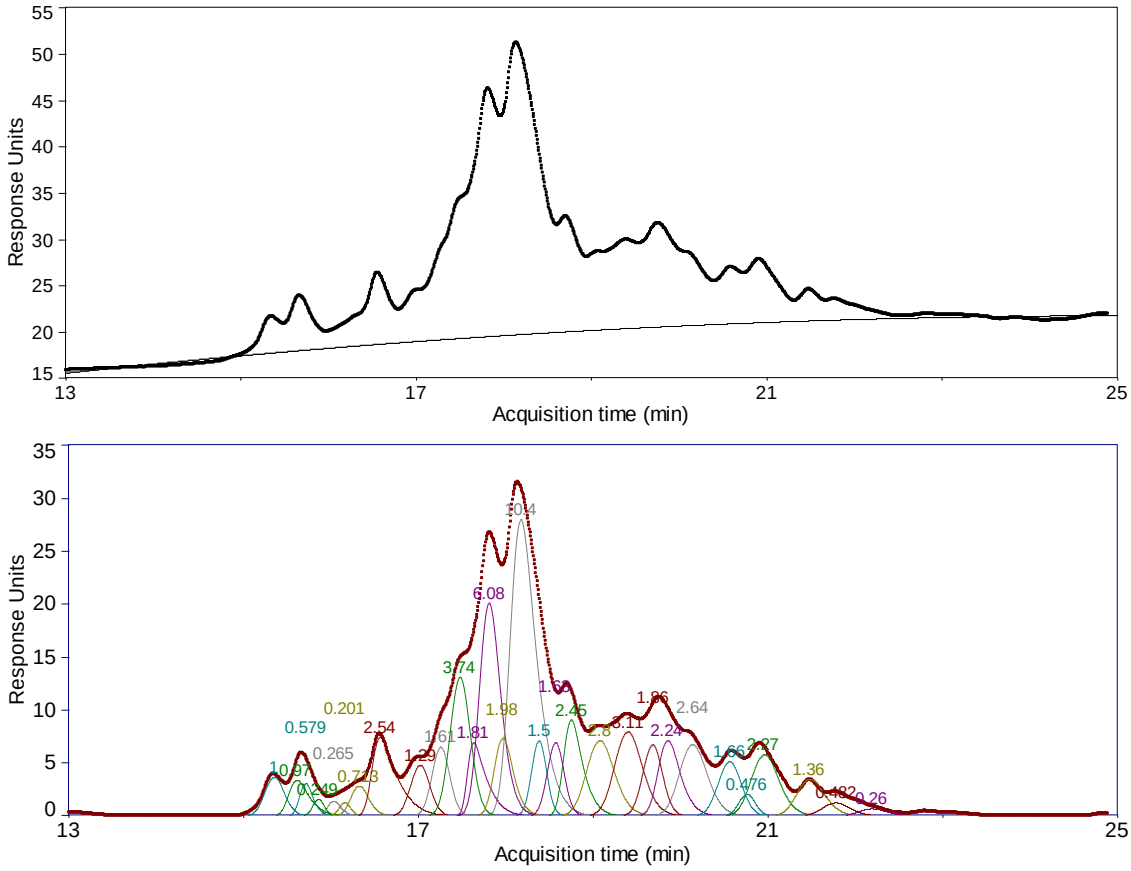


Figure S6. UV chromatogram (@210 nm) obtained from LC-UV-MS (above) and simulated chromatogram obtained by use of PeakFit software (below) for ubiquitin conjugate **39**.

Quantifying the degree of conjugation of compound 40: glycoconjugation of 2 (Man_5) to 37.

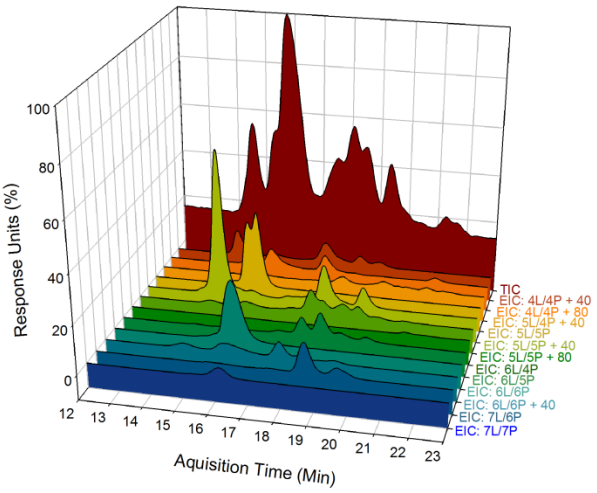


Figure S7. Total Ion Current (TIC) chromatogram and extracted-ion chromatograms (EICs) representing the differentially conjugated ubiquitin species of **40**. L = linkers, P = pentasaccharides, +40 signifies a mass increase of 40 MW corresponding to an internal crosslink formed through reaction of the iodoacetyl moiety with a neighbouring amino acid functionality.

Table S7. Relative abundance of conjugate species of **40** as derived from peak integration from the PeakFit analysis of the UV chromatogram and identified by EIC correlation.

Peak	Identity	Area	Peak	Identity	Area
1	4L/4P+40	0.41	17	6L/5P	0.66
2	5L/5P+40	0.63	18	7L/6P	0.60
3	5L/5P	1.96	19	5L/5P+80	0.22
4	5L/5P	0.61	20	6L/5P	0.18
5	6L/6P	7.29	21	4L/4P+40	0.16
6	6L/6P+40	0.83	22	5L/5P+40	0.62
7	7L/7P	0.24	23	5L/5P+80	0.36
8	4L/4P+40	0.32	24	7L/6P	0.18
9	5L/5P+40	0.26	25	5L/4P+40	0.24
10	4L/4P+40	0.70	26	6L/5P	0.20
11	4L/4P+80	1.22	27	5L4P+40	0.09
12	6L/6P+40	0.43	28	5L/5P+80	0.08
13	5L/5P+80	0.38	29	6L/4P	0.08
14	6L/5P	0.49	30	4L/4P+80	0.18
15	5L/5P+40	0.34	31	5L/4P+40	0.31
16	4L/4P+40	0.48	Total Area:		20.75

Table S8. Summary of Man_5 distribution of conjugate **40**.

40	Area	% Area
4 Man_5	4.20	20.23
5 Man_5	6.98	33.66
6 Man_5	9.33	44.97
7 Man_5	0.24	1.14
Total:	20.75	100.00
Average # of Man_5:		5.3

Note: L = linkers; P = Man_5; +40 signifies a mass increase of 40 MW, corresponding to an internal crosslink formed through reaction of the iodoacetyl moiety with a neighbouring amino acid functionality.

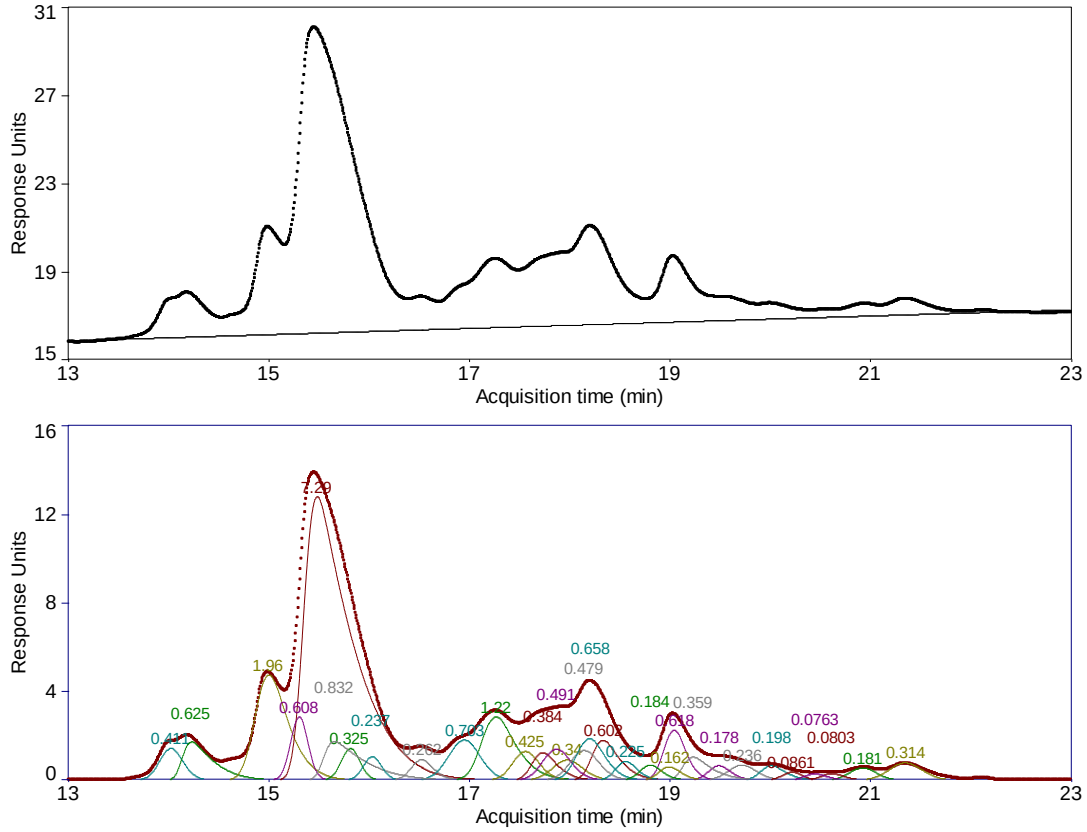


Figure S8. UV chromatogram (@210 nm) obtained from LC-UV-MS (above) and simulated chromatogram obtained by use of PeakFit software (below) for ubiquitin conjugate **40**.

Quantifying the degree of conjugation of compound 41: glycoconjugation of 33 (Man_4) to 38.

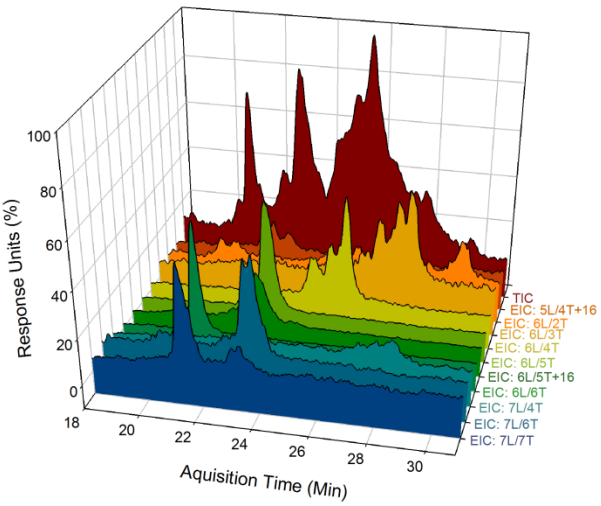


Figure S9. Total Ion Current (TIC) chromatogram and extracted-ion chromatograms (EICs) representing the differentially conjugated ubiquitin species of **41**. L = linkers, T = tetrasaccharides.

Table S9. Relative abundance of conjugate species of **41** as derived from peak integration from the PeakFit analysis of the UV chromatogram and identified by EIC correlation.

Peak	Identity	Area	Peak	Identity	Area
1	6L/5T+16	0.23	13	6L/4T	0.30
2	6L/6T	0.37	14	6L/2T	0.38
3	7L/7T	1.40	15	6L/4T	0.54
4	6L/5T	1.04	16	6L/2T	0.26
5	5L/4T+16	0.30	17	6L/3T	0.15
6	6L/5T	1.30	18	6L/3T	0.06
7	6L/5T+16	0.20	19	6L/3T	0.12
8	6L/5T	1.00	20	6L/2T	0.17
9	7L/6T	1.00	21	6L/2T	0.10
10	7L/6T	0.39	22	6L/2T	0.07
11	6L/4T	0.33	Total Area:		10.12
12	6L/4T	0.41			

Table S10. Summary of Man_4 distribution of conjugate **41**.

41	Area	% Area
2 Man_4	0.98	9.68
3 Man_4	0.33	3.26
4 Man_4	1.88	18.58
5 Man_4	3.77	37.25
6 Man_4	1.76	17.39
7 Man_4	1.40	13.83
Total:	10.12	100.00
Average # of Man_4:		4.9

Note: L = linkers; T = Man_4

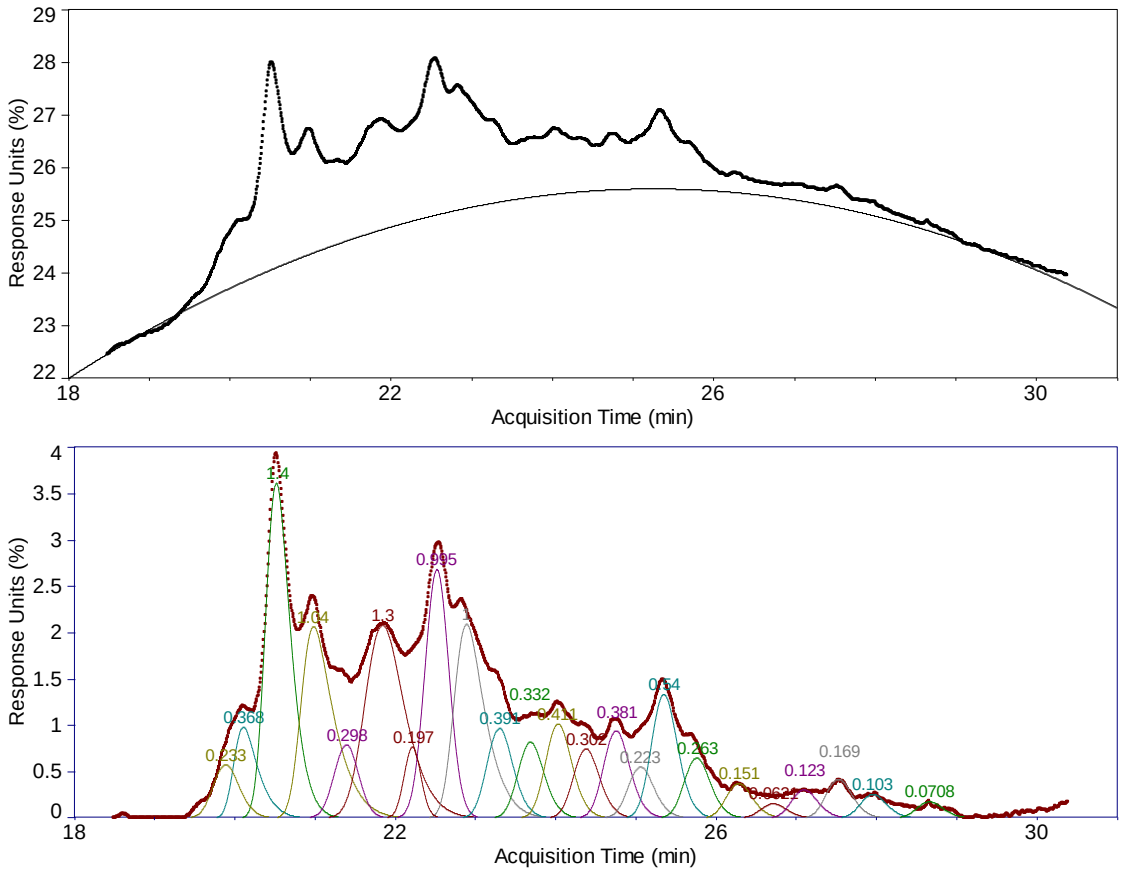


Figure S10. UV chromatogram (@210 nm) obtained from LC-UV-MS (above) and simulated chromatogram obtained by use of PeakFit software (below) for ubiquitin conjugate **41**.

Quantifying the degree of conjugation of compound 42: glycoconjugation of 34 (Man_5) to 38.

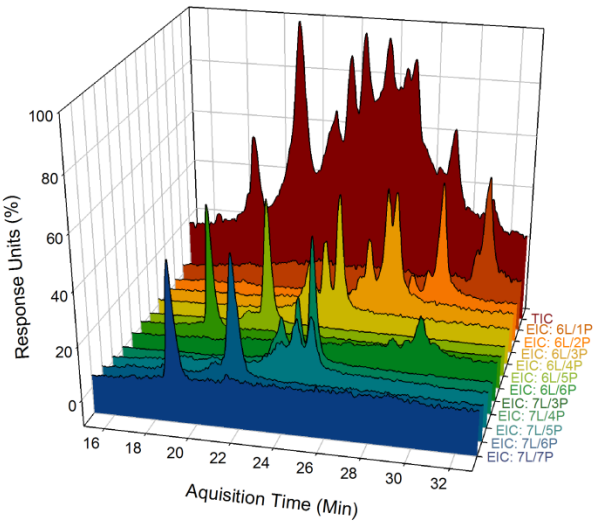


Figure S11. Total Ion Current (TIC) chromatogram and extracted-ion chromatograms (EICs) representing the differentially conjugated ubiquitin species of **42**. L = linkers, P = pentasaccharides. Oxidized products shared similar retention times to their non-oxidized counter parts are were ignored for simplicity.

Table S11. Relative abundance of conjugate species of **42** as derived from peak integration from the PeakFit analysis of the UV chromatogram and identified by EIC correlation.

Peak	Identity	Area	Peak	Identity	Area
1	6L/6P	0.89	13	6L/4P	1.51
2	7L/7P	3.03	14	7L/4P	0.97
3	6L/5P	0.51	15	7L/5P	0.42
4	7L/6P	0.62	16	6L/3P	1.10
5	6L/5P	2.14	17	6L/3P	1.82
6	7L/6P	5.20	18	6L/2P	1.06
7	6L/4P	1.10	19	6L/2P	0.90
8	7L/4P	0.95	20	7L/3P	0.29
9	7L/5P	1.25	21	6L/2P	0.23
10	6L/4P	1.20	22	7L/3P	0.28
11	7L/4P	0.99	23	6L/1P	0.08
12	7L/5P	0.46	Total Area:		26.98

Table S12. Summary of Man_5 distribution of conjugate **42**.

42	Area	% Area
1 Man_5	0.08	0.29
2 Man_5	2.18	8.09
3 Man_5	3.48	12.89
4 Man_5	6.72	24.92
5 Man_5	4.78	17.72
6 Man_5	6.71	24.88
7 Man_5	3.03	11.22
Average # of Man_5:		4.7

Note: L = linkers; P = Man_5

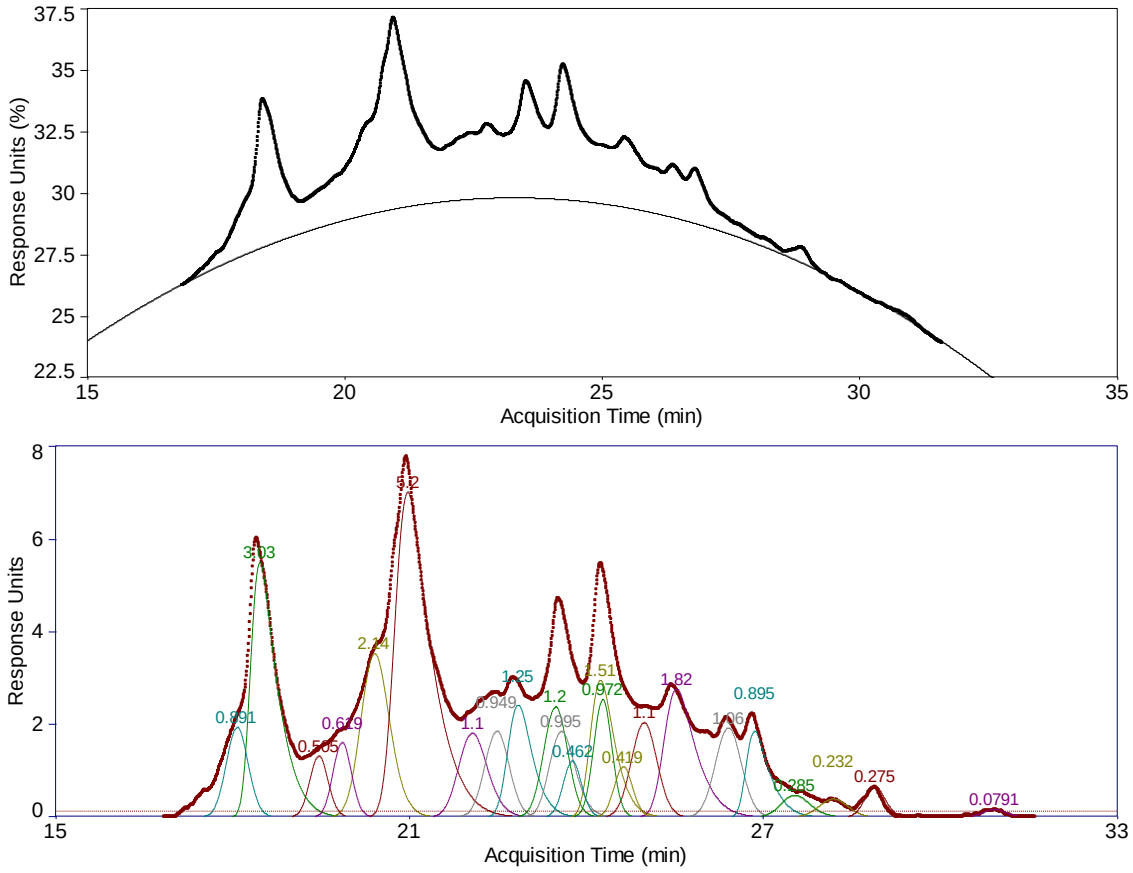
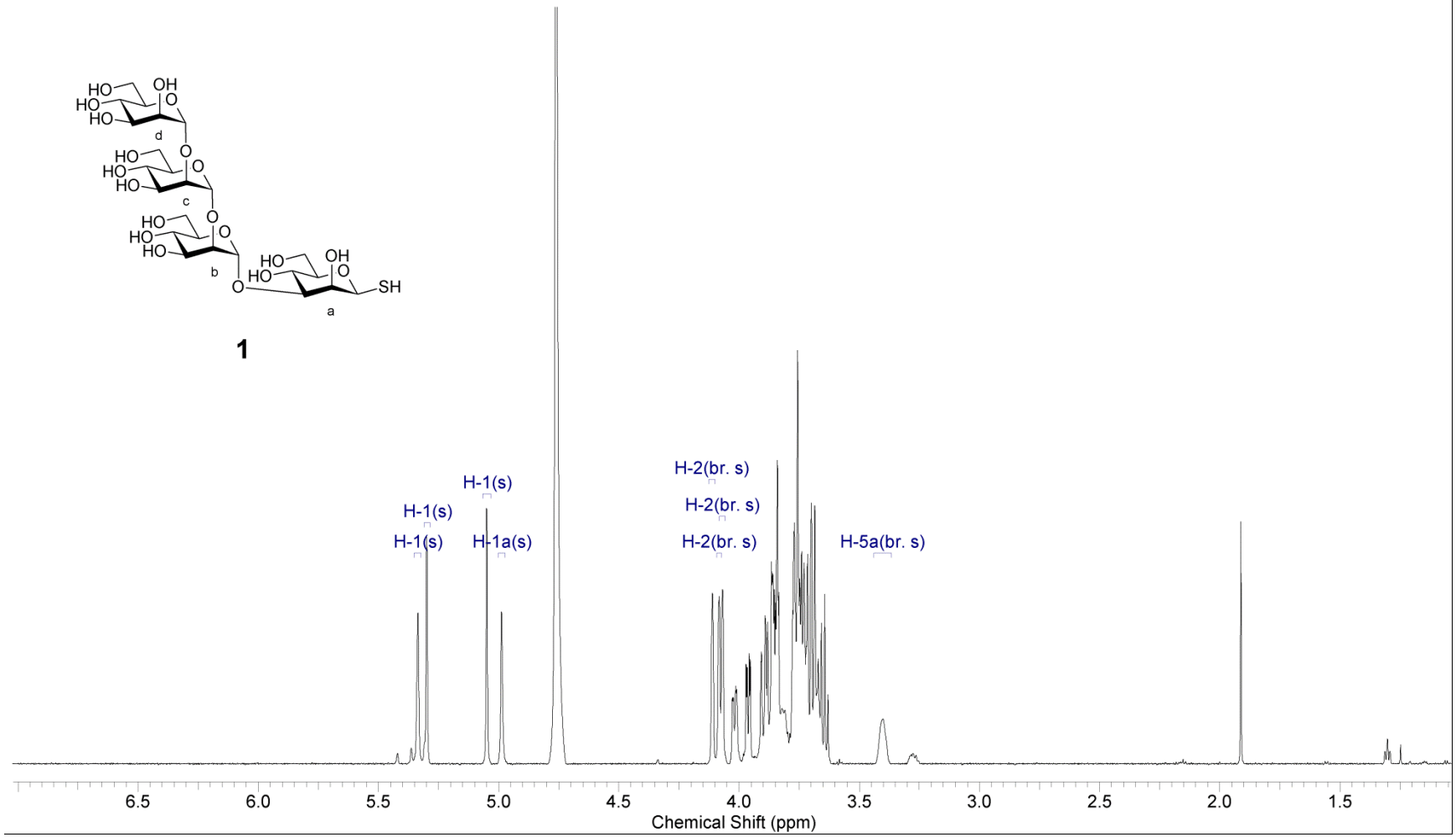
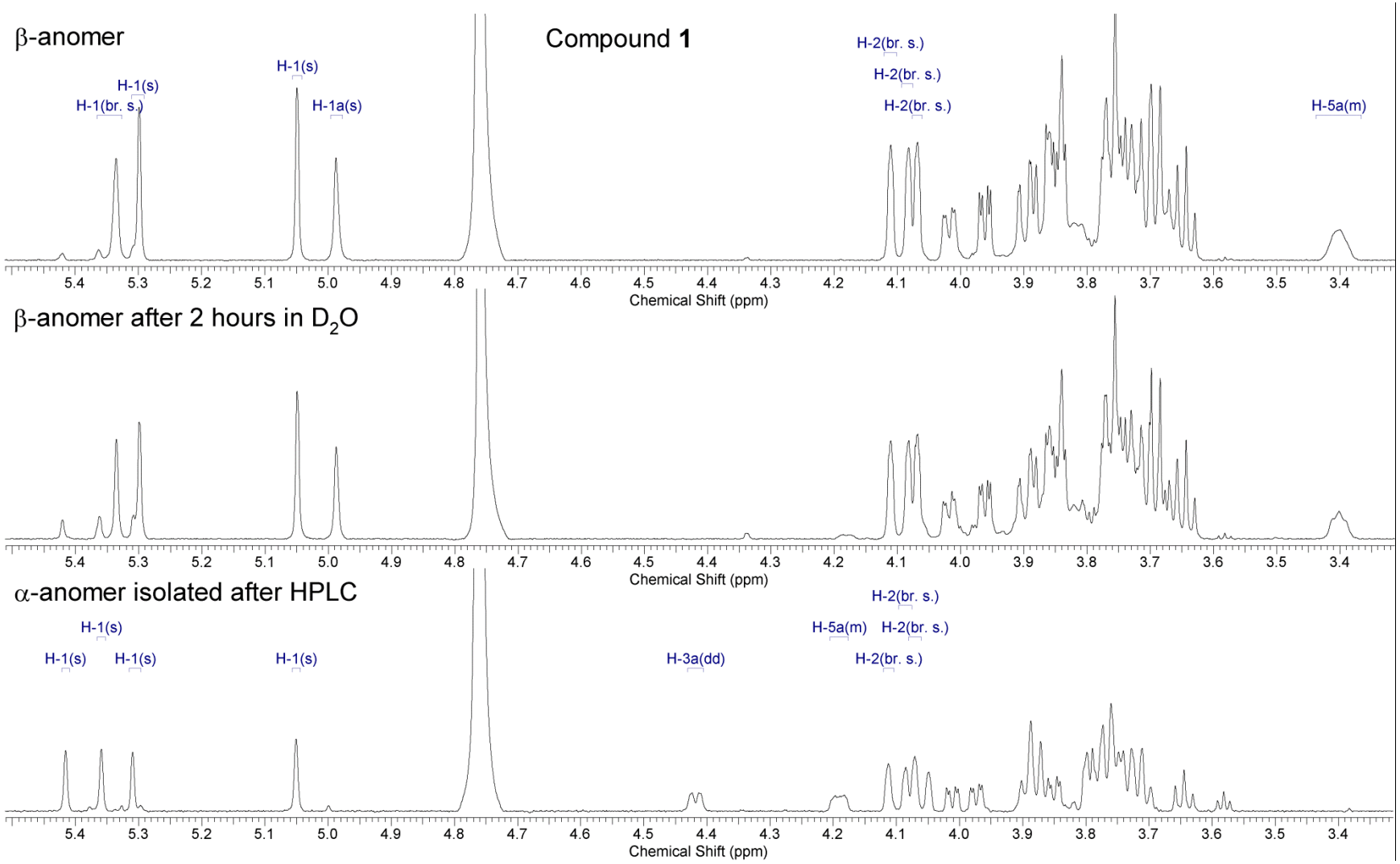


Figure S12. UV chromatogram (@210 nm) obtained from LC-UV-MS (above) and simulated chromatogram obtained by use of PeakFit software (below) for ubiquitin conjugate **42**.

JB-06-57 Tetra-SH 49m4 699.740 MHz H1 1D in d2o (ref. to external acetone @ 2.225 ppm), temp 27.5 C -> actual temp = 27.0 C, coldid probe

1

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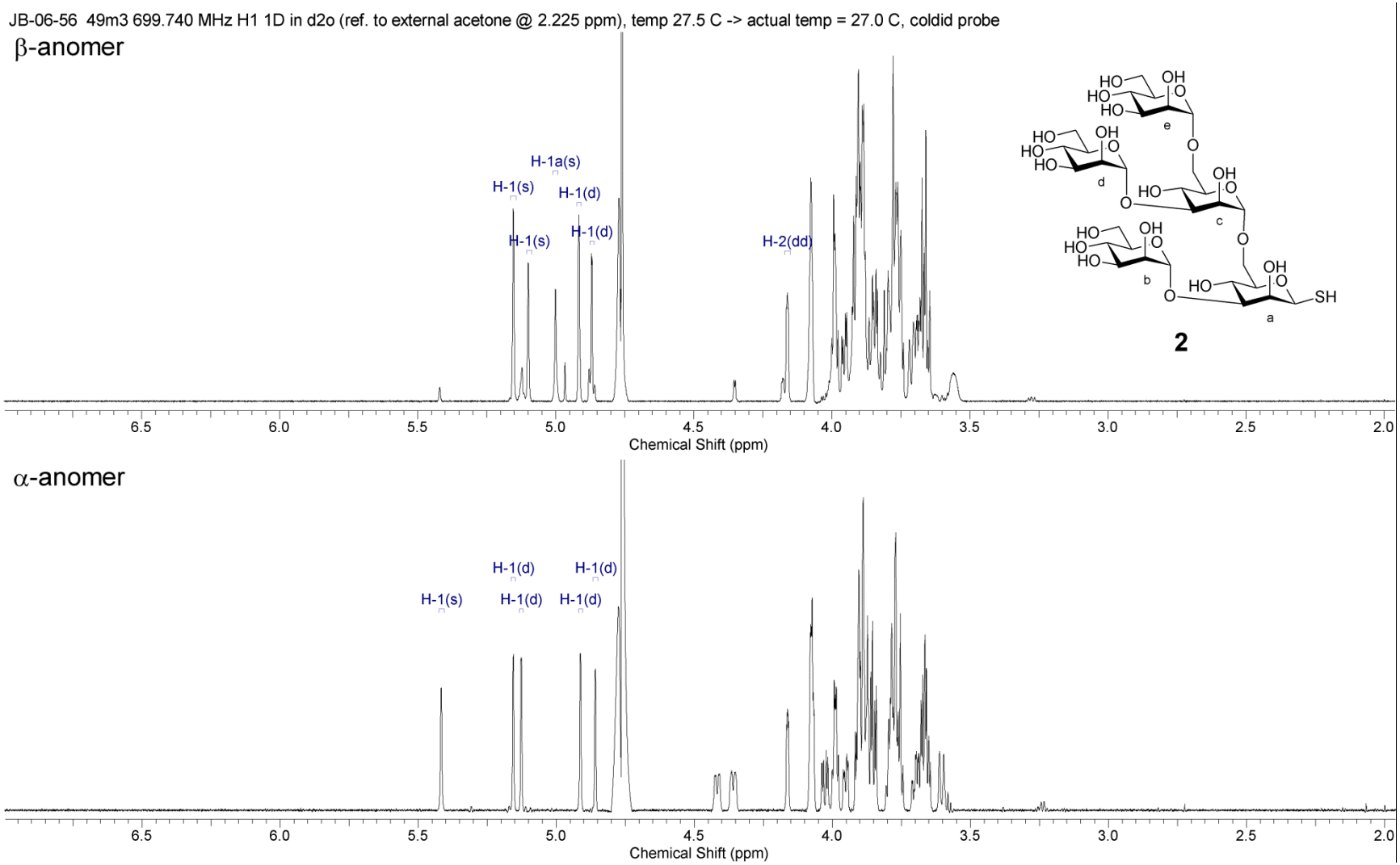
H-1(s)
H-1(s)
H-1(br. s.)
H-1a(s)
H-2(br. s.)
H-2(br. s.)
H-2(br. s.)
H-5a(m)
Chemical Shift (ppm)
β-anomer after 2 hours in D2O
Chemical Shift (ppm)
α-anomer isolated after HPLC
H-1(s)
H-1(s)
H-1(s)
H-1(s)
H-3a(dd)
H-2(br. s.)
H-5a(m)
H-2(br. s.)
H-2(br. s.)
Chemical Shift (ppm)


JB-06-56 49m3 699.740 MHz H1 1D in d2o (ref. to external acetone @ 2.225 ppm), temp 27.5 C -> actual temp = 27.0 C, coldid probe
β-anomer
H-1a(s)
H-1(s)
H-1(d)
H-1(d)
H-1(s)
H-2(dd)
HO
OH
O
SH
a
b
c
d
e
2
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Chemical Shift (ppm)
α-anomer
H-1(d)
H-1(d)
H-1(s)
H-1(d)
H-1(d)
6.5
6.0
5.5
5.0
4.5
4.0
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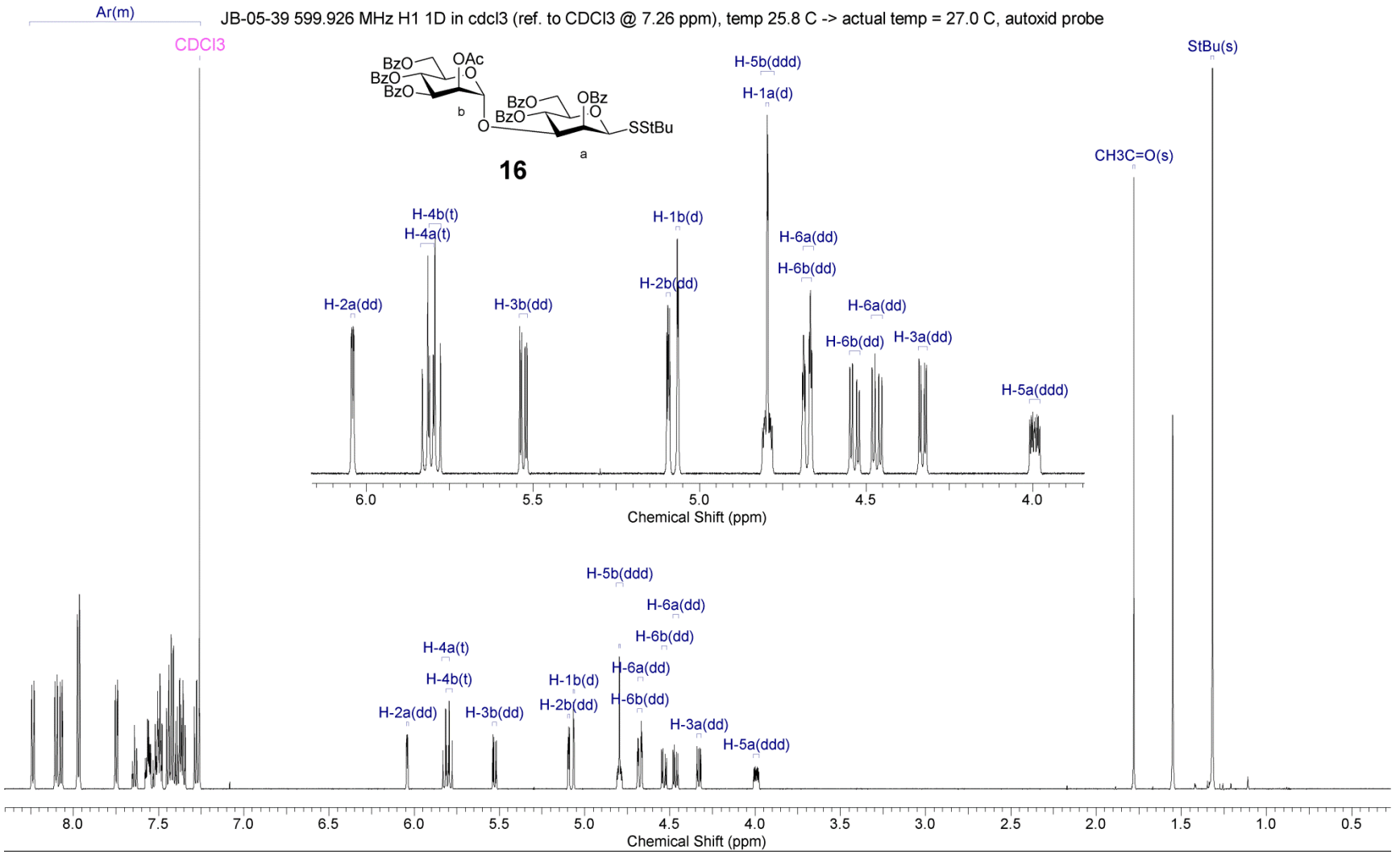
3.0
2.5
2.0
Chemical Shift (ppm)


Ar(m)
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BzO
OAc
BzO
BzO
O
b
BzO
OBz
BzO
O
O
SStBu
a
16
H-5b(ddd)
H-1a(d)
StBu(s)
CH3C=O(s)
H-4b(t)
H-4a(t)
H-1b(d)
H-6a(dd)
H-6b(dd)
H-2b(dd)
H-2a(dd)
H-3b(dd)
H-6a(dd)
H-6b(dd)
H-3a(dd)
H-5a(ddd)
6.0
5.5
5.0
4.5
4.0
Chemical Shift (ppm)
H-5b(ddd)
H-6a(dd)
H-6b(dd)
H-4a(t)
H-6a(dd)
H-4b(t)
H-1b(d)
H-6b(dd)
H-2a(dd)
H-3b(dd)
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7.5
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6.0
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1.5
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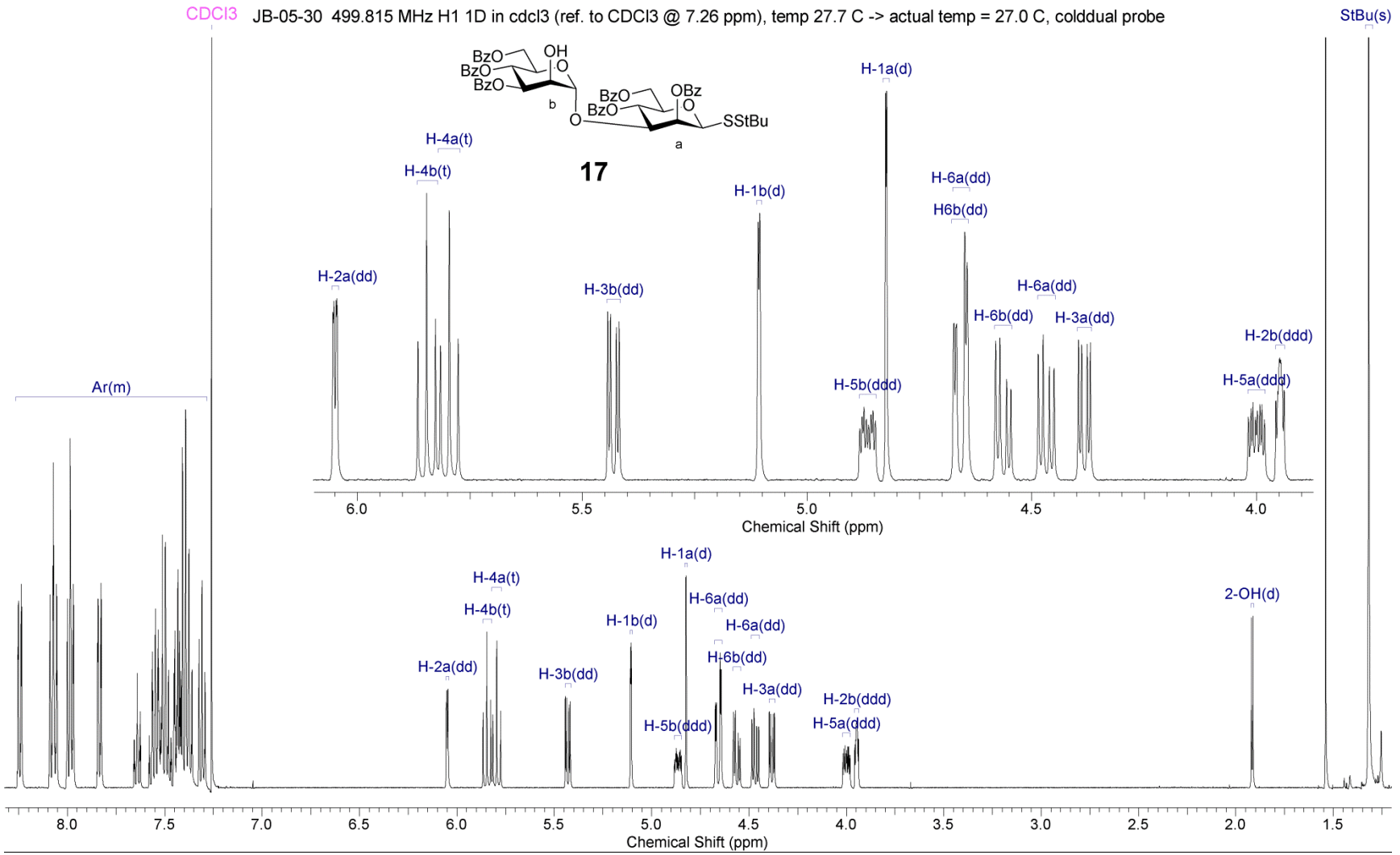
0.5
Chemical Shift (ppm)


CDCl3
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StBu(s)
17
H-1a(d)
H-4a(t)
H-4b(t)
H-1b(d)
H-6a(dd)
H6b(dd)
H-2a(dd)
H-3b(dd)
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H-6b(dd)
H-3a(dd)
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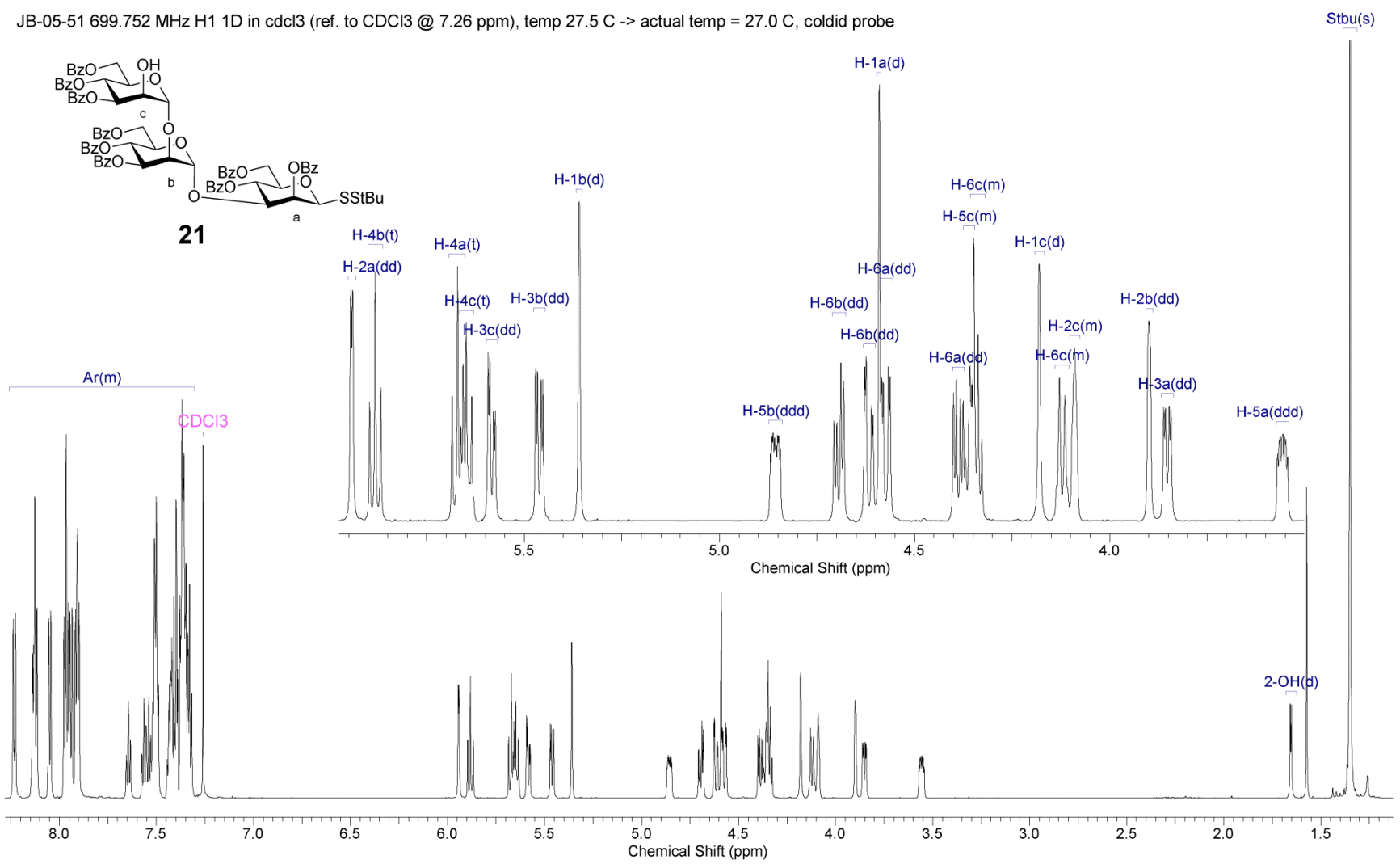
Ar(m)
2-OH(d)
Chemical Shift (ppm)
Chemical Shift (ppm)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21
Stbu(s)
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H-1b(d)
H-6c(m)
H-5c(m)
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H-4b(t)
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H-3a(dd)
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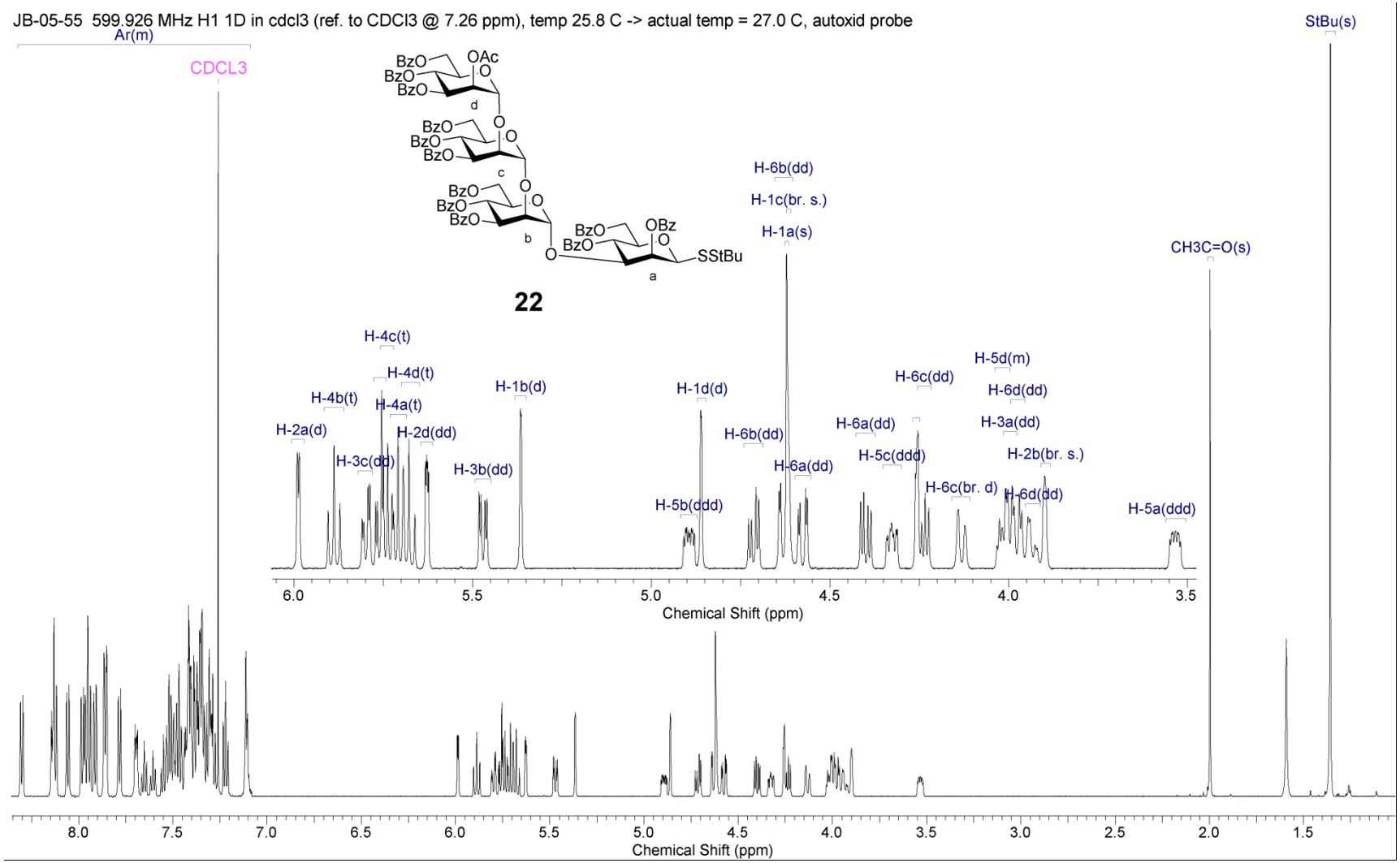
Ar(m)
CDCl3
2-OH(d)
Chemical Shift (ppm)
Chemical Shift (ppm)


JB-05-55 599.926 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 25.8 C -> actual temp = 27.0 C, autoxid probe
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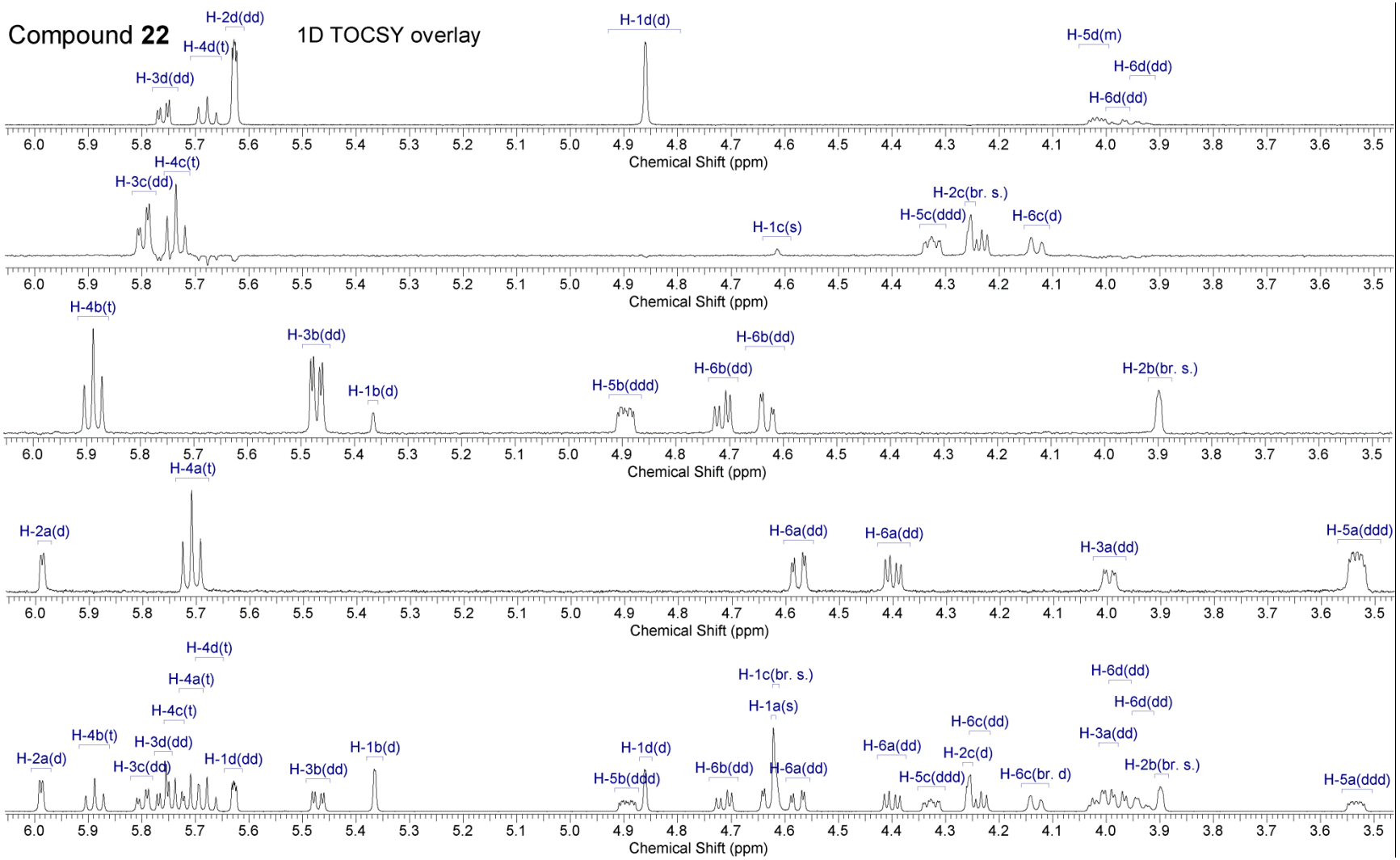
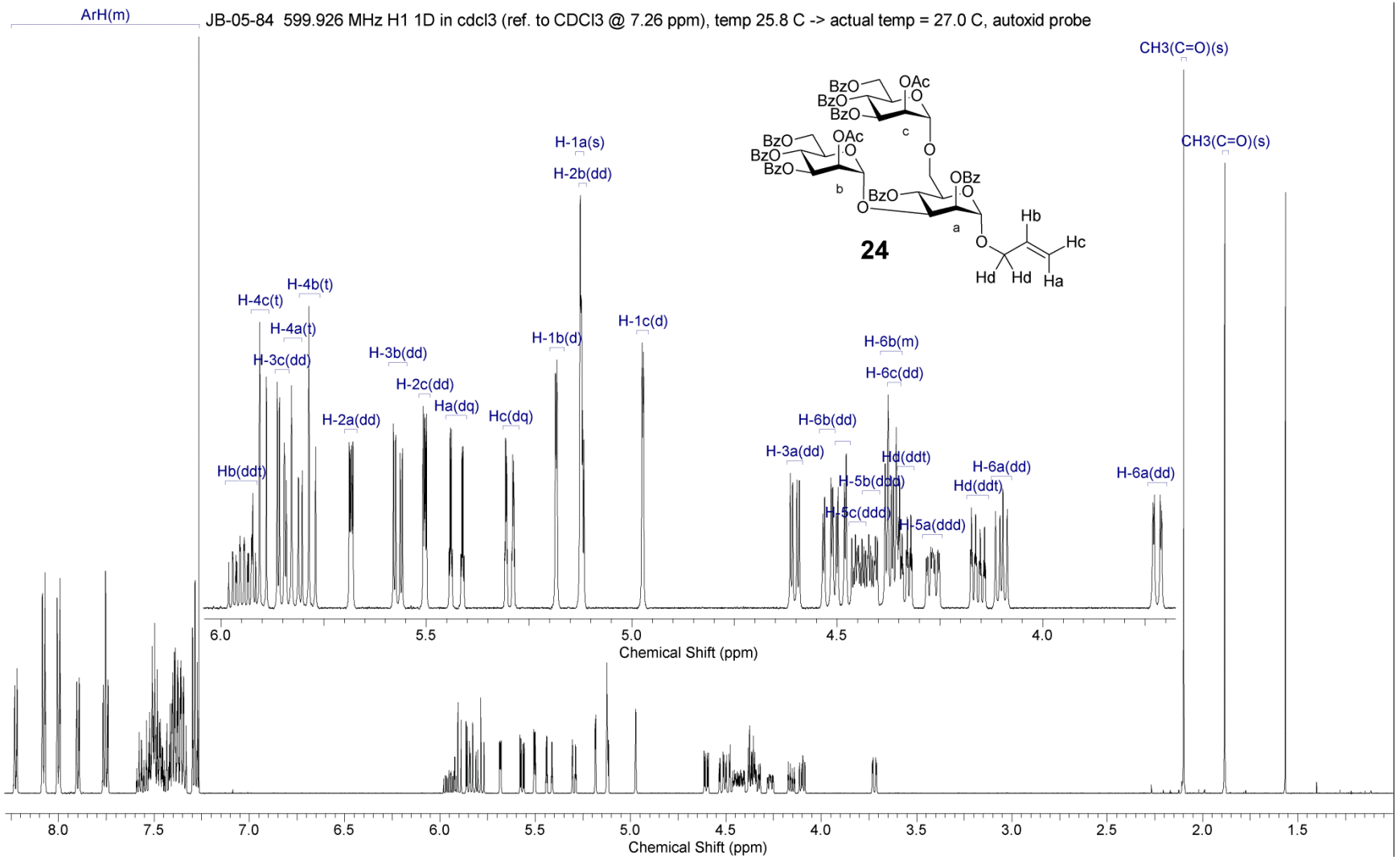
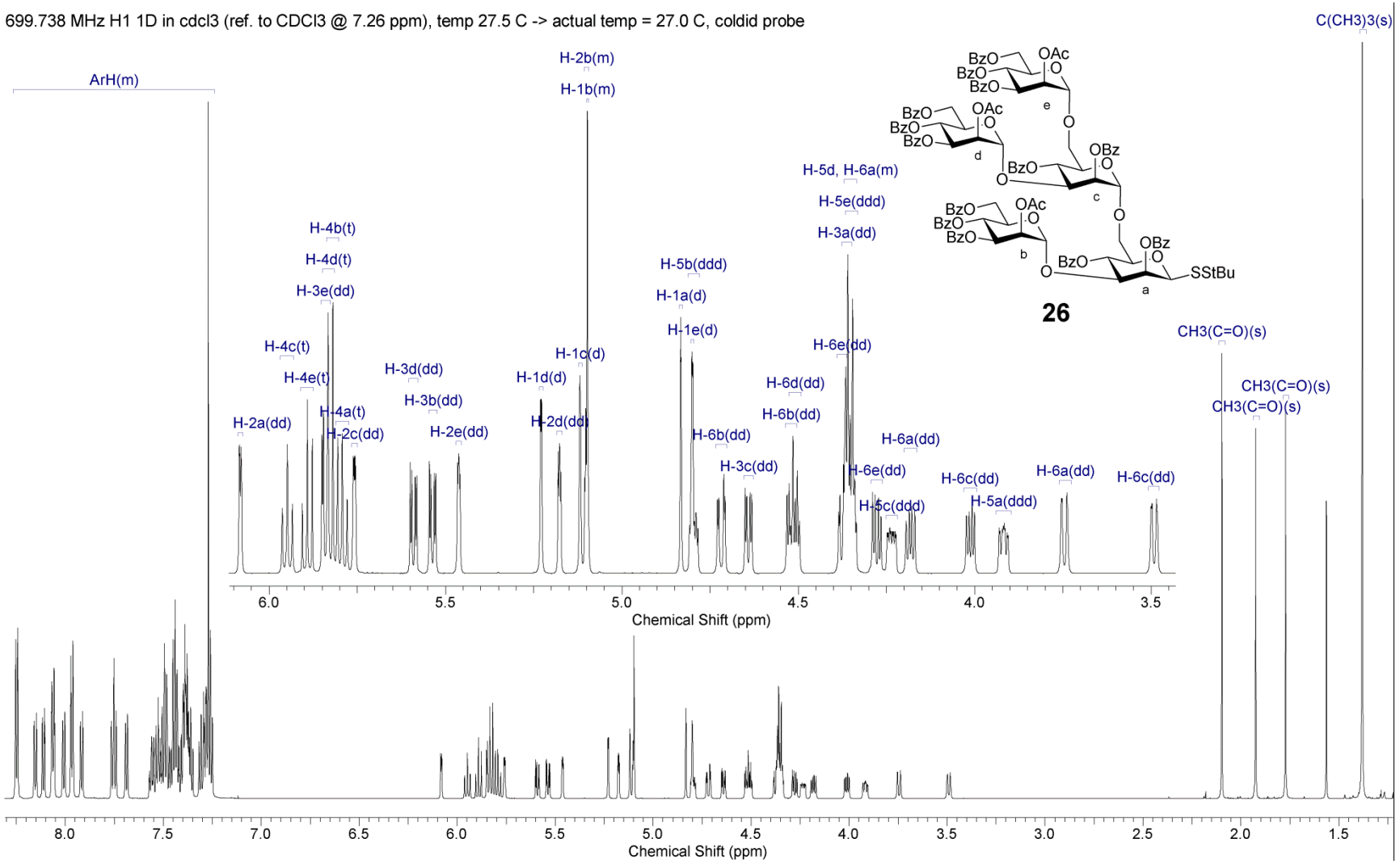
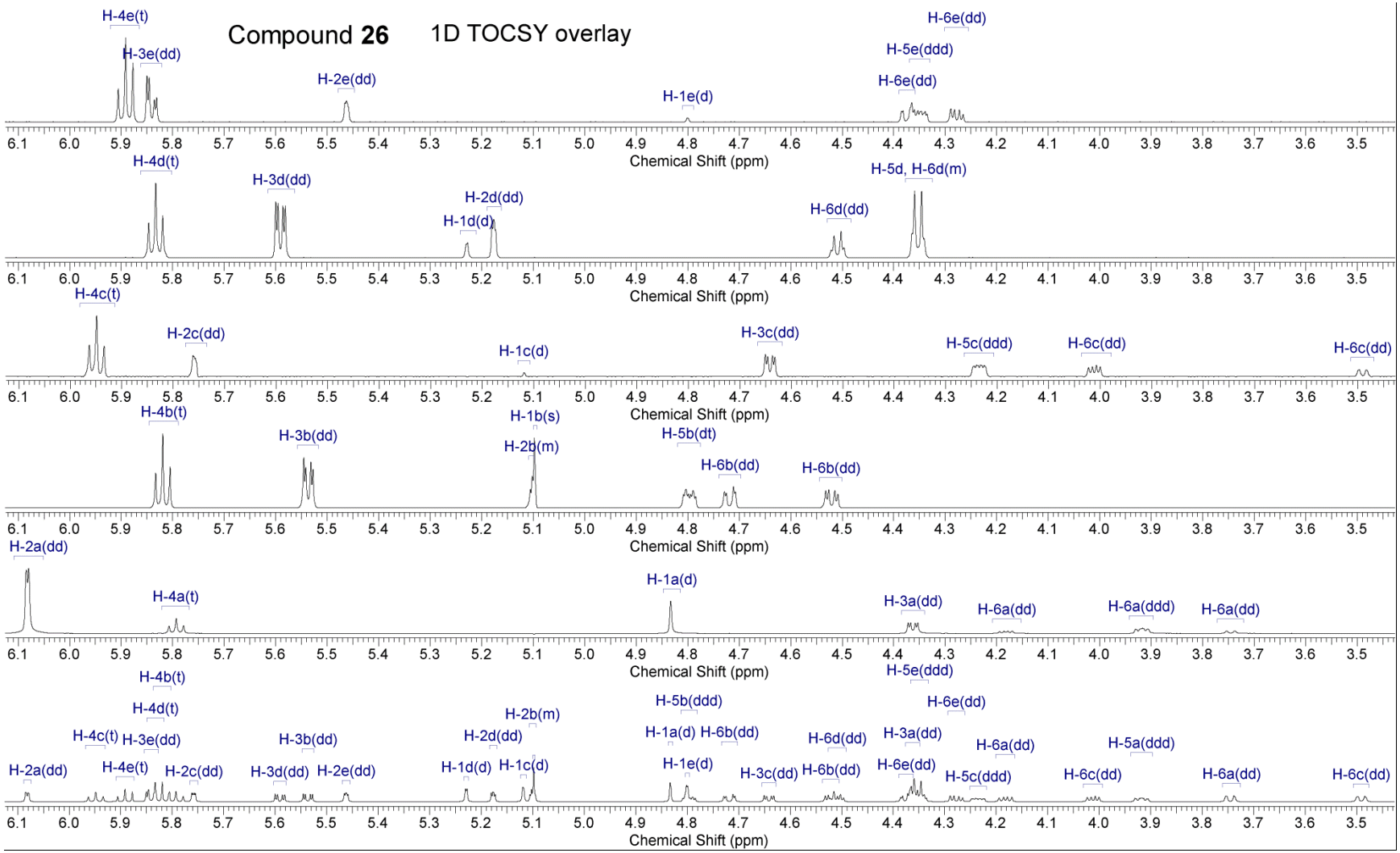
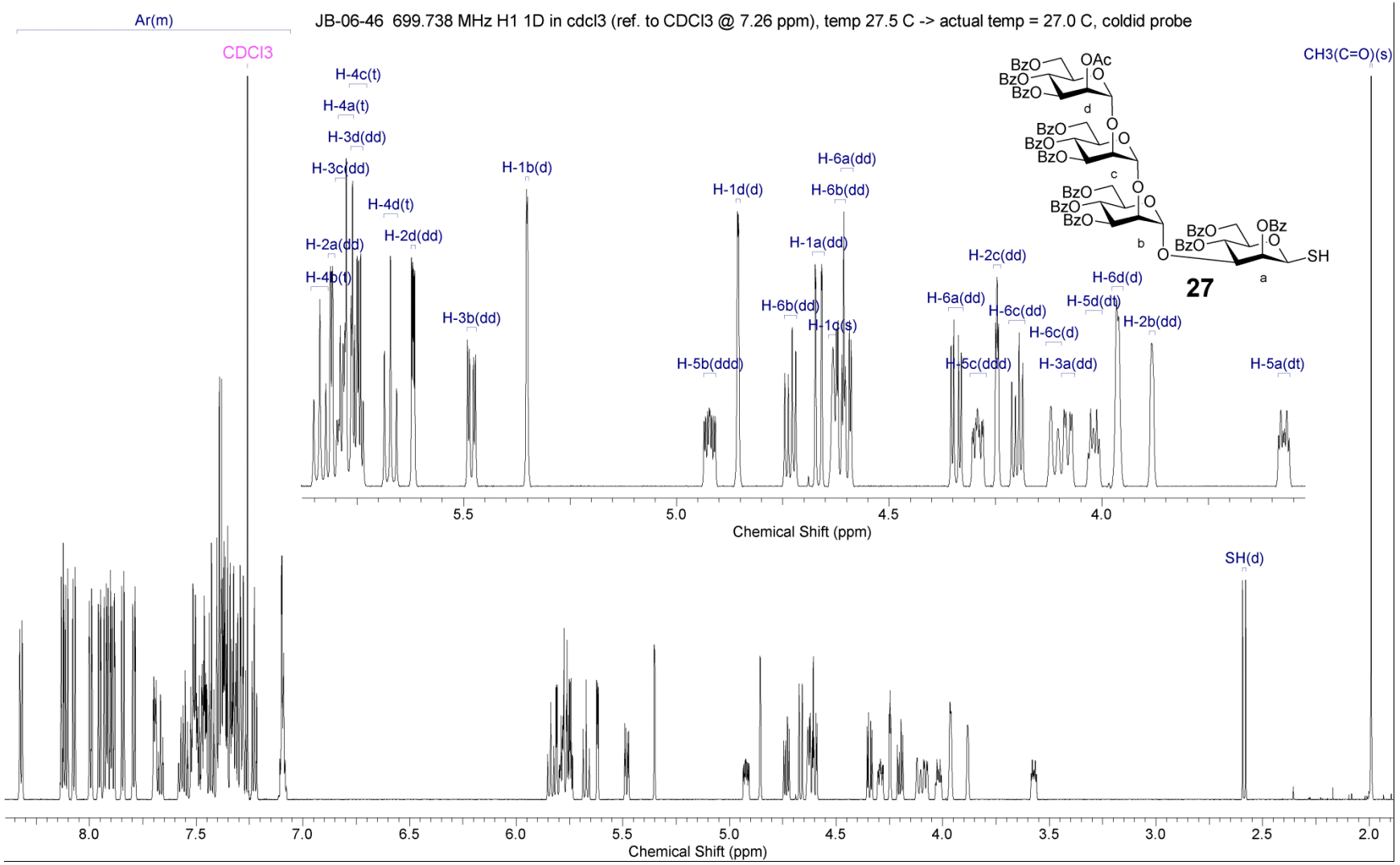
CDCL3
StBu(s)
CH3C=O(s)
22
H-6b(dd)
H-1c(br. s.)
H-1a(s)
H-4c(t)
H-4d(t)
H-4b(t)
H-4a(t)
H-2a(d)
H-2d(dd)
H-3c(dd)
H-3b(dd)
H-1b(d)
H-1d(d)
H-5b(ddd)
H-6b(dd)
H-6a(dd)
H-6a(dd)
H-5c(ddd)
H-6c(dd)
H-6c(br. d)
H-5d(m)
H-6d(dd)
H-3a(dd)
H-2b(br. s.)
H-6d(dd)
H-5a(ddd)
Chemical Shift (ppm)
Chemical Shift (ppm)

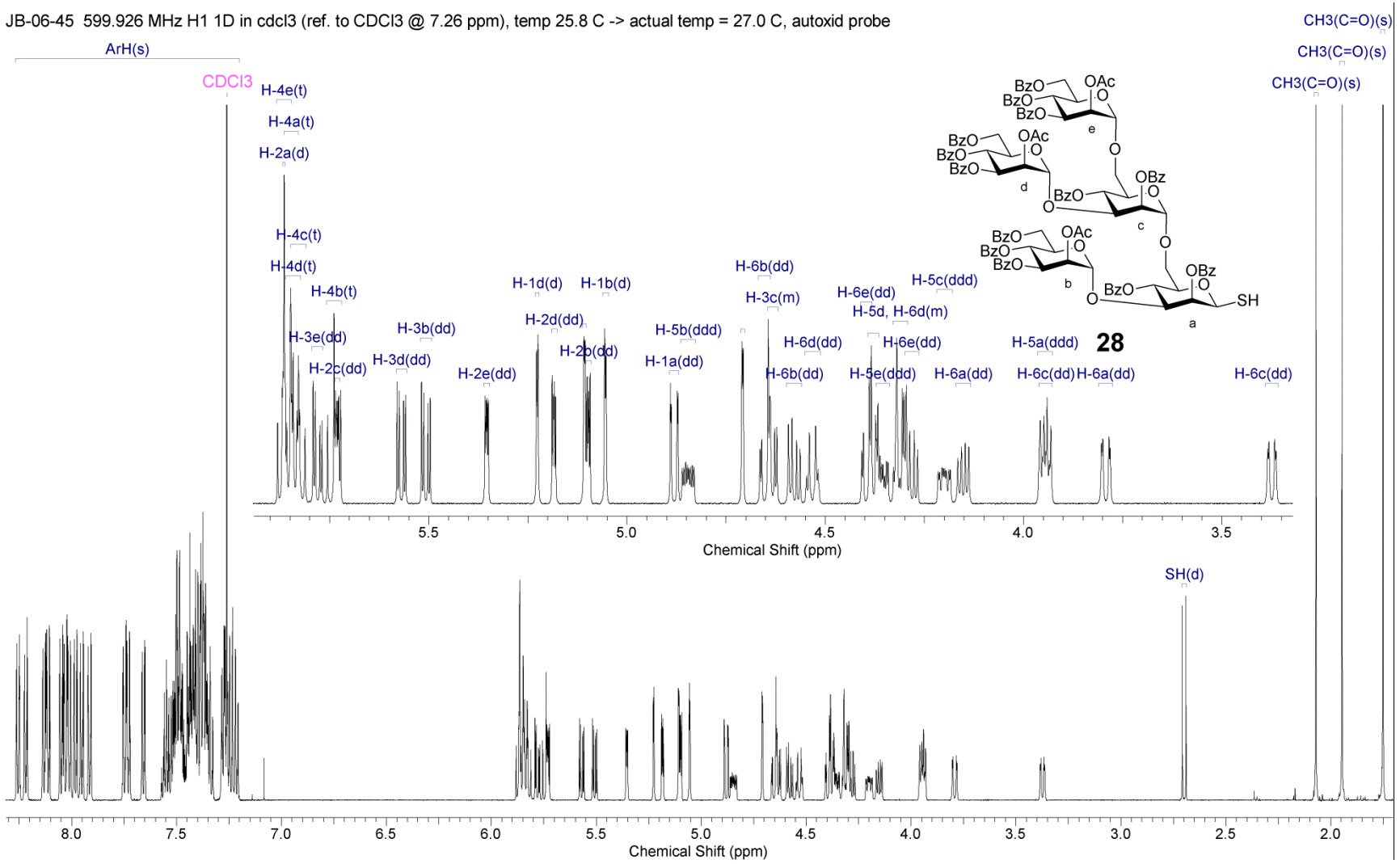

Compound 22
1D TOCSY overlay
H-2d(dd)
H-4d(t)
H-3d(dd)
H-1d(d)
H-5d(m)
H-6d(dd)
H-6d(dd)
H-4c(t)
H-3c(dd)
H-2c(br. s.)
H-5c(ddd)
H-6c(d)
H-1c(s)
H-4b(t)
H-3b(dd)
H-6b(dd)
H-6b(dd)
H-1b(d)
H-5b(ddd)
H-2b(br. s.)
H-4a(t)
H-2a(d)
H-6a(dd)
H-6a(dd)
H-3a(dd)
H-5a(ddd)
H-4d(t)
H-4a(t)
H-1c(br. s.)
H-6d(dd)
H-4c(t)
H-1a(s)
H-6d(dd)
H-6c(dd)
H-4b(t)
H-3d(dd)
H-3a(dd)
H-2a(d)
H-3c(dd)
H-1d(dd)
H-3b(dd)
H-1b(d)
H-1d(d)
H-6a(dd)
H-2c(d)
H-2b(br. s.)
H-5b(ddd)
H-6b(dd)
H-6a(dd)
H-5c(ddd)
H-6c(br. d)
H-5a(ddd)
6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5
Chemical Shift (ppm)

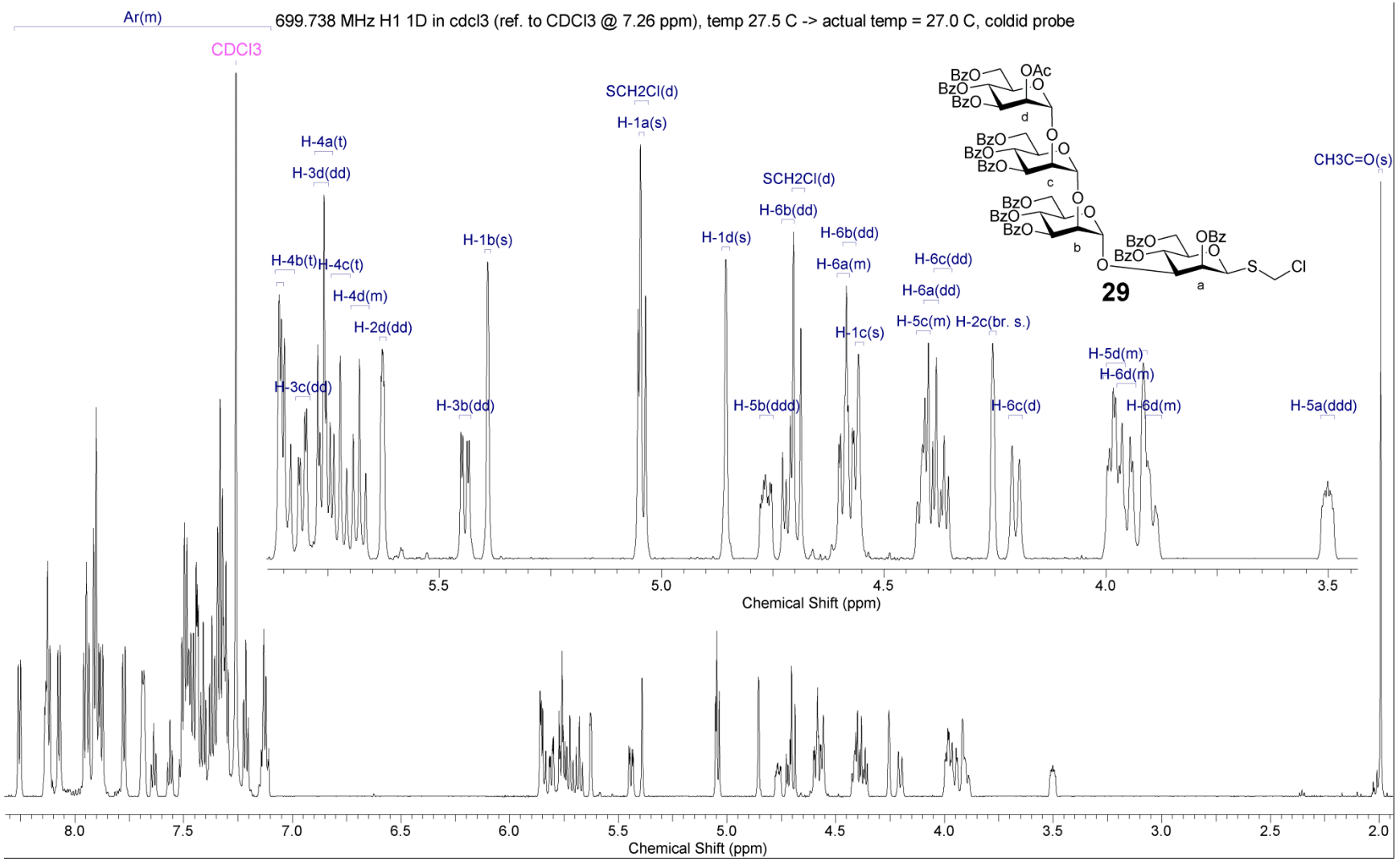

ArH(m)
JB-05-84 599.926 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 25.8 C -> actual temp = 27.0 C, autoxid probe
CH3(C=O)(s)
CH3(C=O)(s)
H-1a(s)
H-2b(dd)
H-4b(t)
H-4c(t)
H-4a(t)
H-3c(dd)
H-3b(dd)
H-2c(dd)
Ha(dq)
H-2a(dd)
Hc(dq)
H-1b(d)
H-1c(d)
H-6b(m)
H-6c(dd)
H-6b(dd)
H-3a(dd)
Hd(ddt)
H-6a(dd)
H-5b(ddd)
Hd(ddt)
H-5c(ddd)
H-5a(ddd)
H-6a(dd)
Hb(ddt)
BzO
OAc
OBz
Hb
Hc
Hd
Ha
24
Chemical Shift (ppm)
Chemical Shift (ppm)

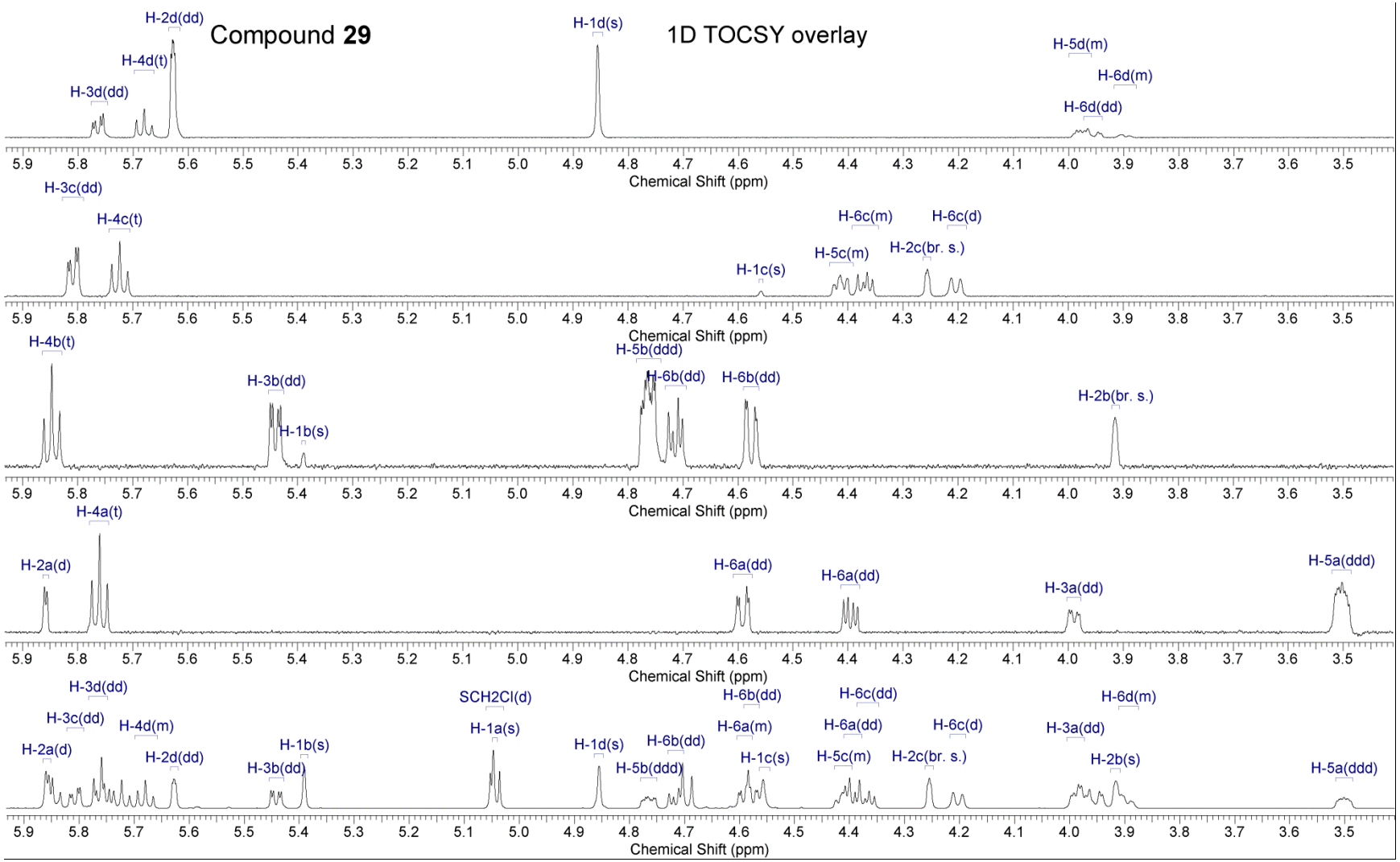

699.738 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 27.5 C -> actual temp = 27.0 C, coldid probe
ArH(m)
H-2b(m)
H-1b(m)
H-4b(t)
H-4d(t)
H-3e(dd)
H-4c(t)
H-4e(t)
H-4a(t)
H-2c(dd)
H-2a(dd)
H-3d(dd)
H-3b(dd)
H-2e(dd)
H-1d(d)
H-2d(dd)
H-1c(d)
H-5b(ddd)
H-1a(d)
H-1e(d)
H-6b(dd)
H-3c(dd)
H-5d, H-6a(m)
H-5e(ddd)
H-3a(dd)
H-6d(dd)
H-6b(dd)
H-6e(dd)
H-6a(dd)
H-6e(dd)
H-5c(ddd)
H-6c(dd)
H-5a(ddd)
H-6a(dd)
H-6c(dd)
CH3(C=O)(s)
CH3(C=O)(s)
CH3(C=O)(s)
C(CH3)3(s)
26
Chemical Shift (ppm)
Chemical Shift (ppm)

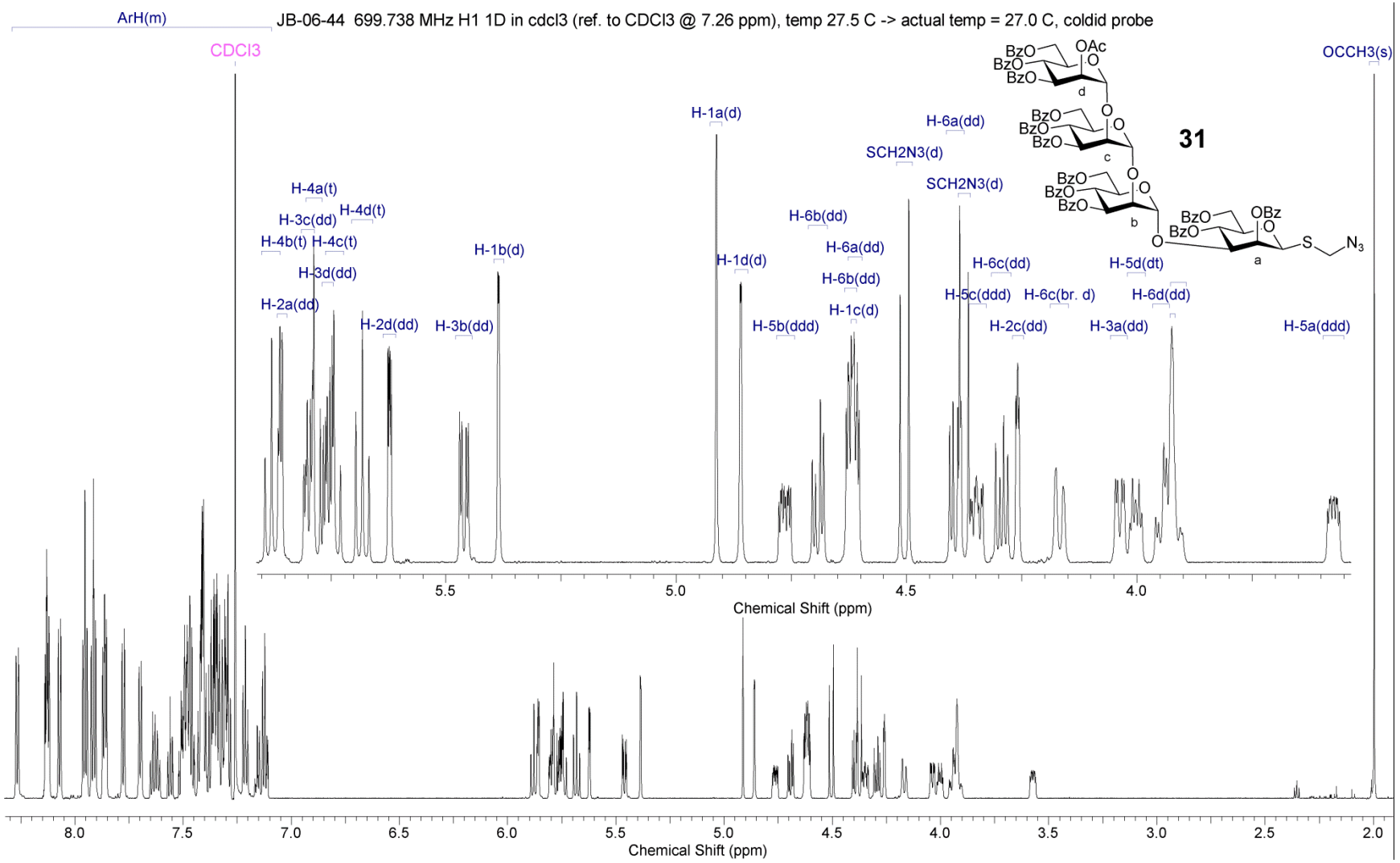

Compound 26
1D TOCSY overlay
H-4e(t)
H-3e(dd)
H-2e(dd)
H-1e(d)
H-6e(dd)
H-5e(ddd)
H-6e(dd)
H-4d(t)
H-3d(dd)
H-2d(dd)
H-1d(d)
H-6d(dd)
H-5d, H-6d(m)
H-4c(t)
H-2c(dd)
H-1c(d)
H-3c(dd)
H-5c(ddd)
H-6c(dd)
H-6c(dd)
H-4b(t)
H-3b(dd)
H-1b(s)
H-2b(m)
H-5b(dt)
H-6b(dd)
H-6b(dd)
H-2a(dd)
H-4a(t)
H-1a(d)
H-3a(dd)
H-6a(dd)
H-6a(ddd)
H-6a(dd)
H-4b(t)
H-4d(t)
H-4c(t)
H-3e(dd)
H-3b(dd)
H-2d(dd)
H-2b(m)
H-5b(ddd)
H-1a(d)
H-6b(dd)
H-6d(dd)
H-3a(dd)
H-5e(ddd)
H-6e(dd)
H-6a(dd)
H-5a(ddd)
H-2a(dd)
H-4e(t)
H-2c(dd)
H-3d(dd)
H-2e(dd)
H-1d(d)
H-1c(d)
H-1e(d)
H-3c(dd)
H-6b(dd)
H-6e(dd)
H-5c(ddd)
H-6c(dd)
H-6a(dd)
H-6c(dd)
6.1 6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5
Chemical Shift (ppm)

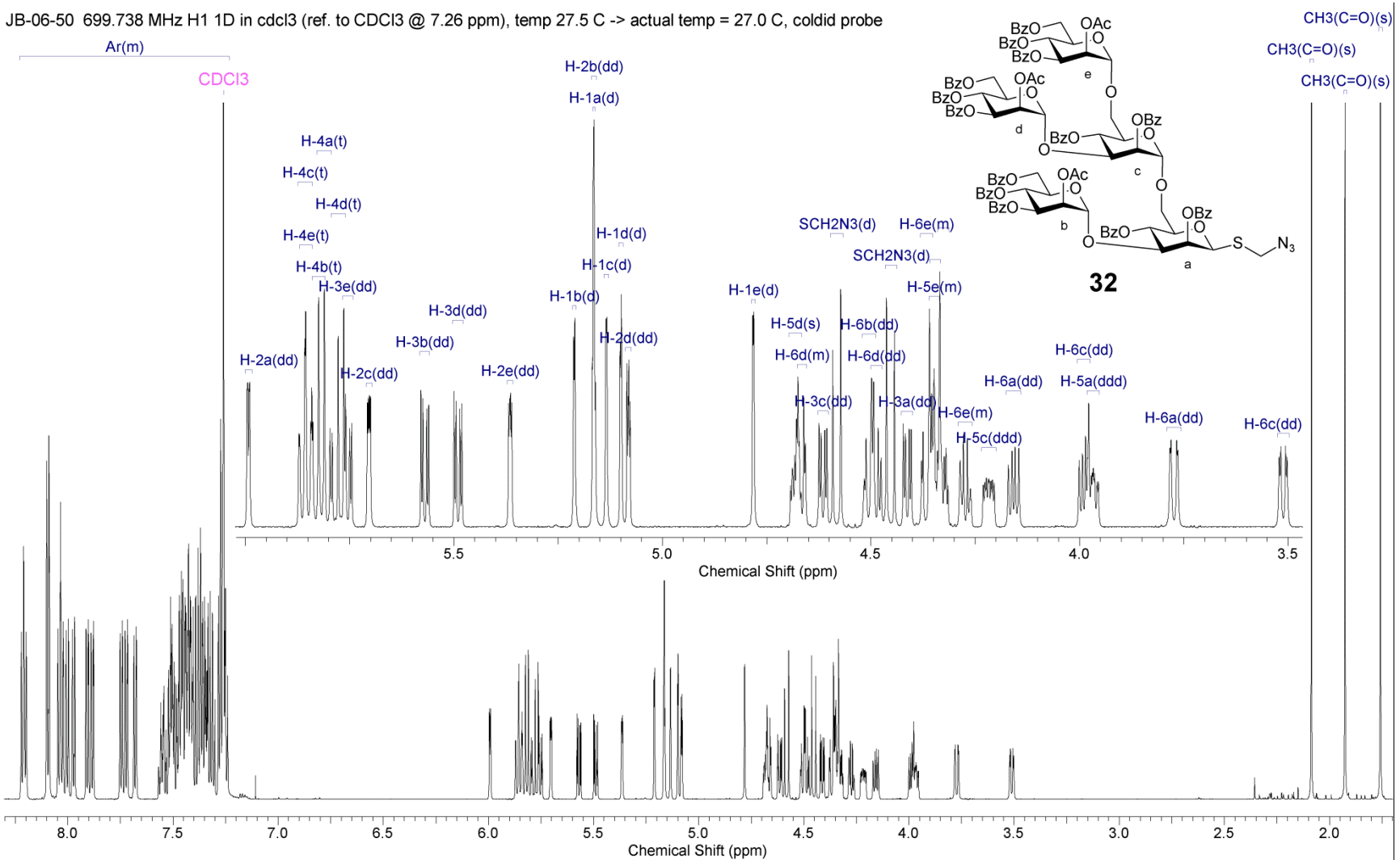

Ar(m)
JB-06-46 699.738 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 27.5 C -> actual temp = 27.0 C, coldid probe
CDCl3
CH3(C=O)(s)
H-4c(t)
H-4a(t)
H-3d(dd)
H-3c(dd)
H-4d(t)
H-2d(dd)
H-2a(dd)
H-4b(t)
H-3b(dd)
H-1b(d)
H-1d(d)
H-6a(dd)
H-6b(dd)
H-1a(dd)
H-6b(dd)
H-1c(s)
H-5b(ddd)
H-2c(dd)
H-6a(dd)
H-6c(dd)
H-6c(d)
H-5d(dt)
H-6d(d)
H-2b(dd)
H-5c(ddd)
H-3a(dd)
H-5a(dt)
SH(d)
27
Chemical Shift (ppm)
Chemical Shift (ppm)

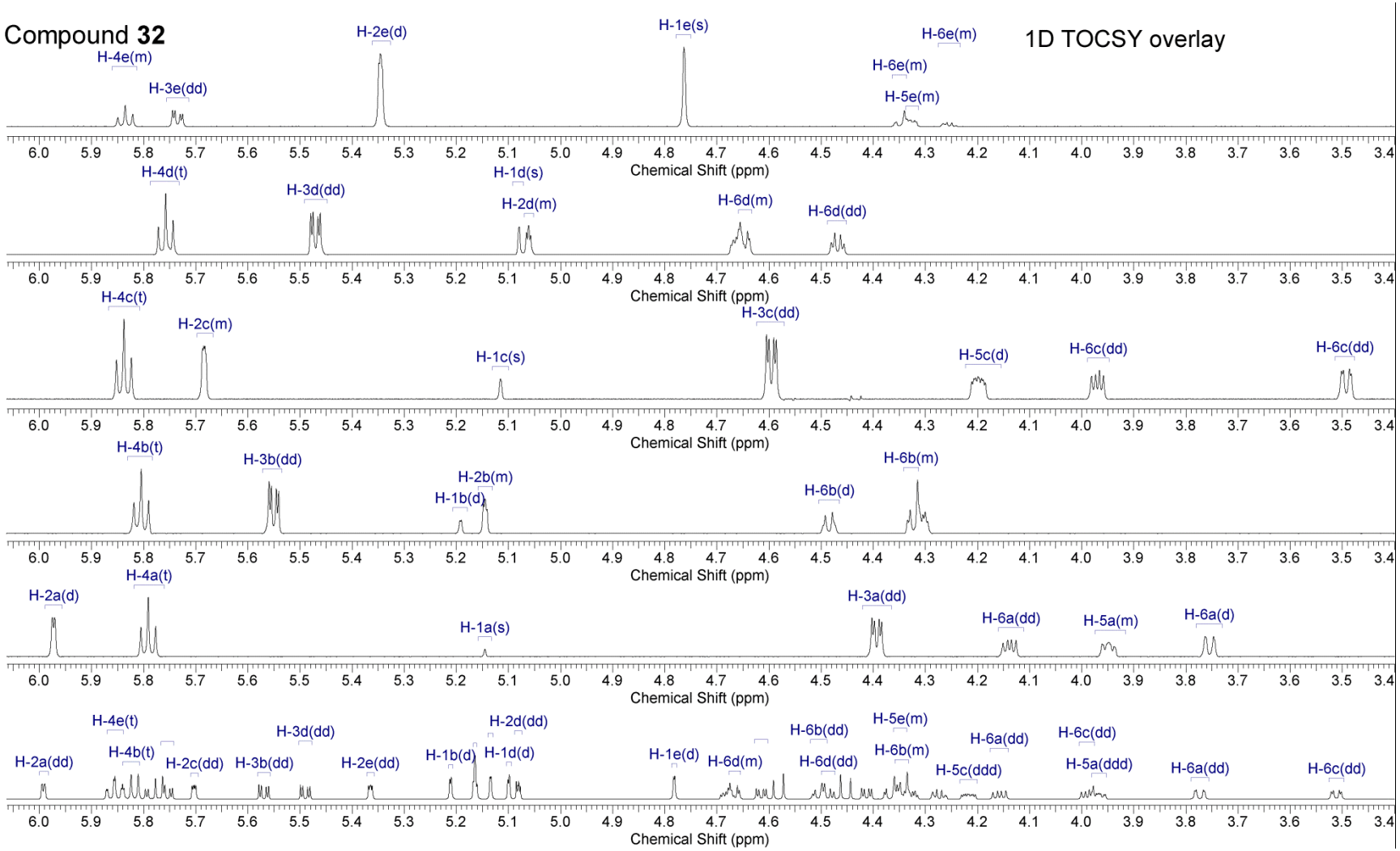

JB-06-45 599.926 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 25.8 C -> actual temp = 27.0 C, autoxid probe
ArH(s)
CDCl3
H-4e(t)
H-4a(t)
H-2a(d)
H-4c(t)
H-4d(t)
H-4b(t)
H-3e(dd)
H-2c(dd)
H-3b(dd)
H-3d(dd)
H-2e(dd)
H-1d(d)
H-1b(d)
H-2d(dd)
H-2b(dd)
H-5b(ddd)
H-1a(dd)
H-6b(dd)
H-3c(m)
H-6d(dd)
H-6b(dd)
H-6e(dd)
H-5d, H-6d(m)
H-5e(ddd)
H-5c(ddd)
H-6e(dd)
H-6a(dd)
H-5a(ddd)
H-6c(dd)
H-6a(dd)
H-6c(dd)
28
SH(d)
CH3(C=O)(s)
CH3(C=O)(s)
CH3(C=O)(s)
Chemical Shift (ppm)
Chemical Shift (ppm)

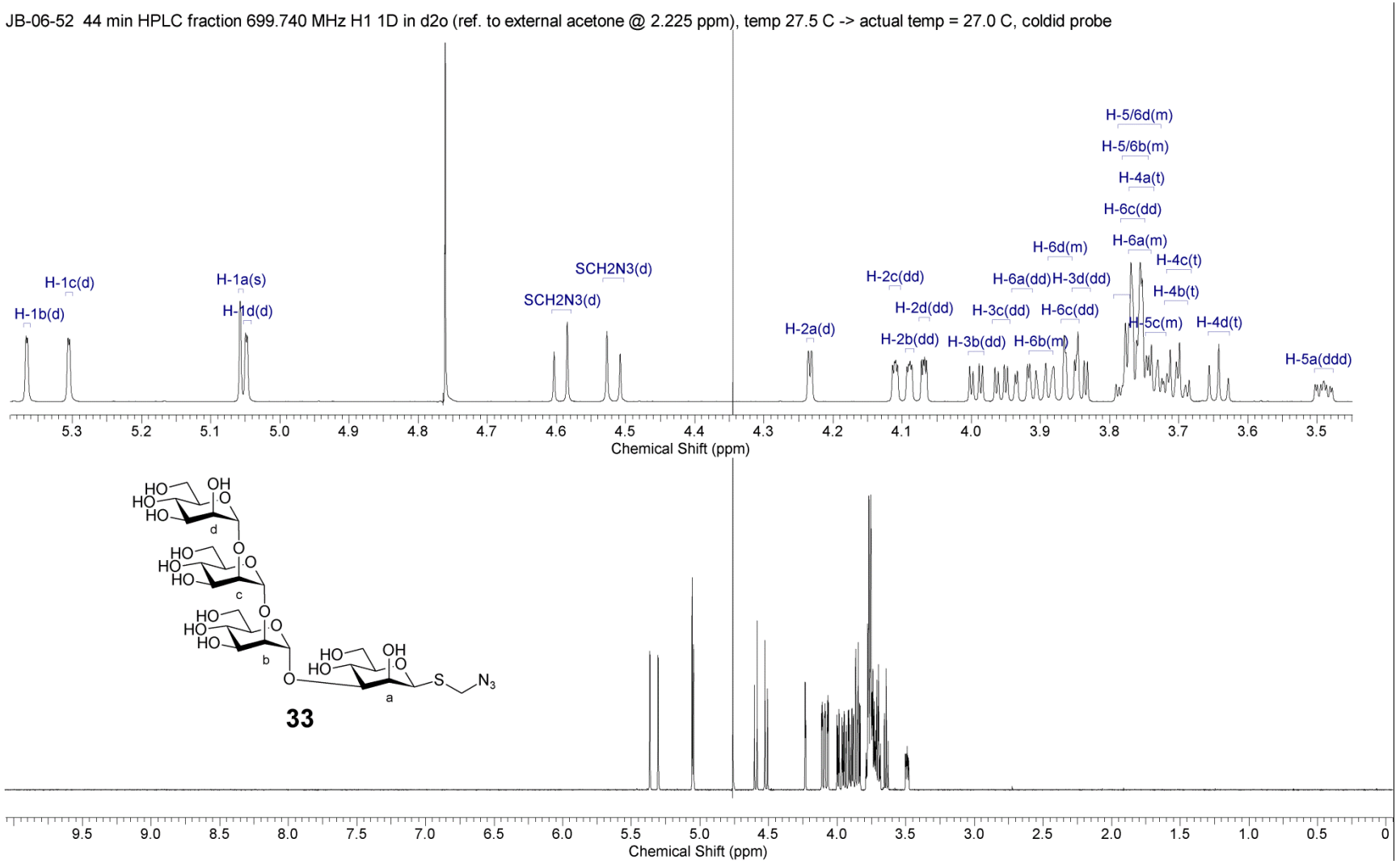

Ar(m)
699.738 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 27.5 C -> actual temp = 27.0 C, coldid probe
CDCl3
SCH2Cl(d)
H-1a(s)
H-4a(t)
H-3d(dd)
SCH2Cl(d)
H-6b(dd)
H-6b(dd)
H-1b(s)
H-1d(s)
H-4b(t)
H-4c(t)
H-6a(m)
H-6c(dd)
H-4d(m)
H-6a(dd)
H-2d(dd)
H-5c(m)
H-2c(br. s.)
H-1c(s)
H-5d(m)
H-6d(m)
H-3c(dd)
H-3b(dd)
H-5b(ddd)
H-6c(d)
H-6d(m)
H-5a(ddd)
CH3C=O(s)
29
Chemical Shift (ppm)
Chemical Shift (ppm)

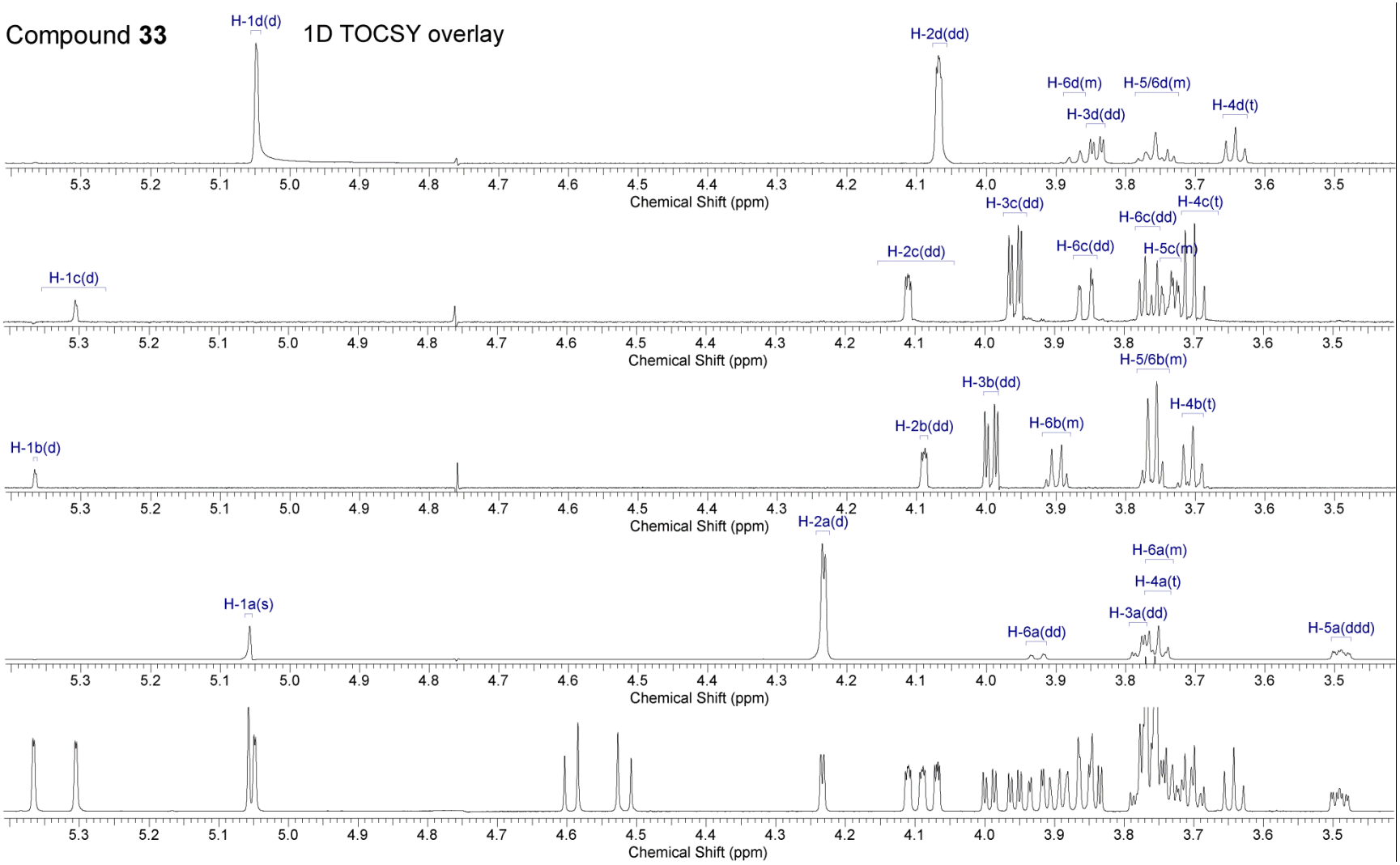

Compound 29
1D TOCSY overlay
H-2d(dd)
H-4d(t)
H-3d(dd)
H-1d(s)
H-5d(m)
H-6d(m)
H-6d(dd)
H-3c(dd)
H-4c(t)
H-6c(m)
H-6c(d)
H-5c(m)
H-2c(br. s.)
H-1c(s)
H-4b(t)
H-5b(ddd)
H-3b(dd)
H-6b(dd)
H-6b(dd)
H-2b(br. s.)
H-1b(s)
H-4a(t)
H-2a(d)
H-6a(dd)
H-6a(dd)
H-3a(dd)
H-5a(ddd)
SCH2Cl(d)
H-6b(dd)
H-6c(dd)
H-6d(m)
H-1a(s)
H-6a(m)
H-6a(dd)
H-6c(d)
H-1d(s)
H-6b(dd)
H-3a(dd)
H-4d(m)
H-1c(s)
H-5c(m)
H-2c(br. s.)
H-2b(s)
H-5b(ddd)
H-5a(ddd)
Chemical Shift (ppm)

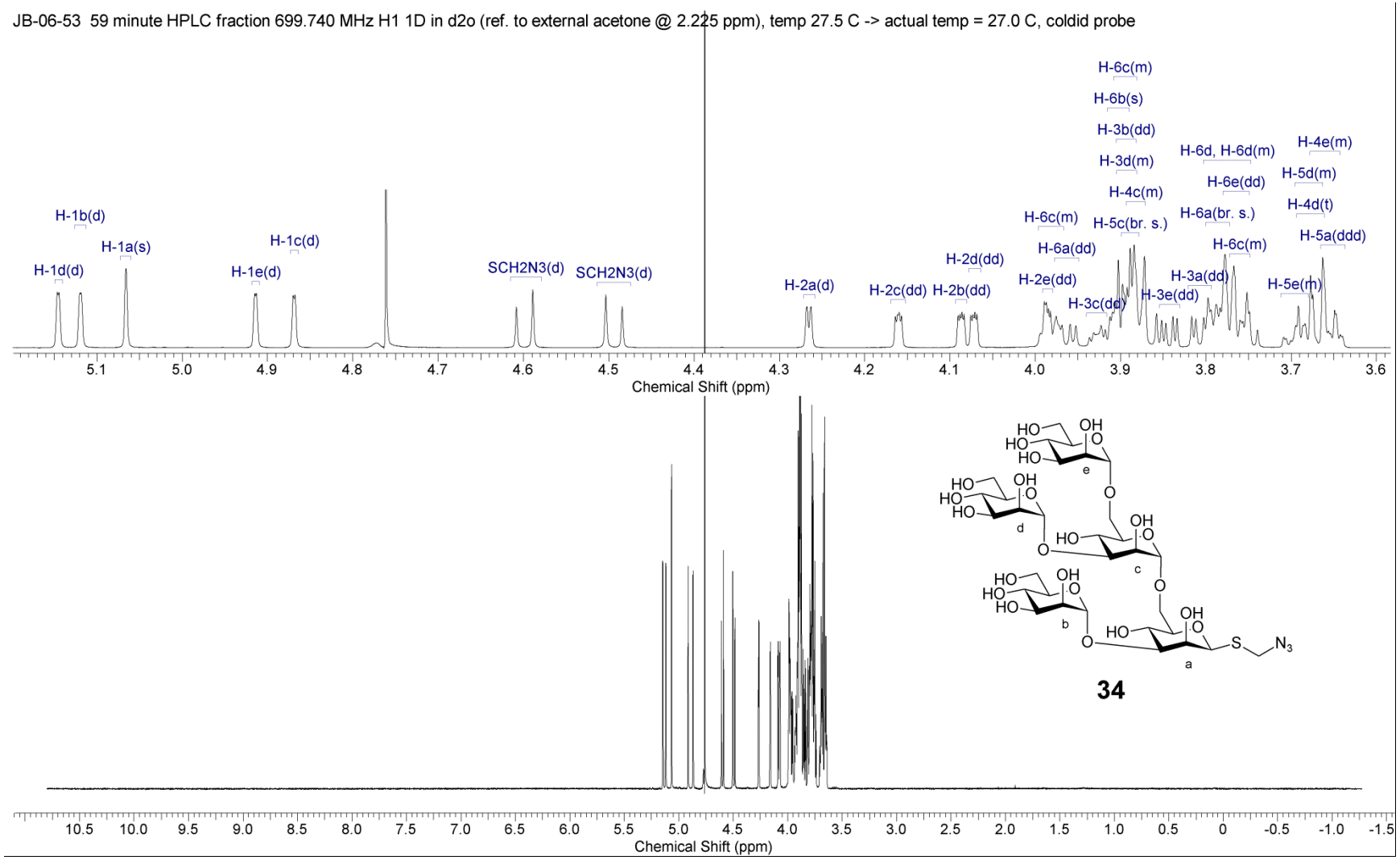

ArH(m)
JB-06-44 699.738 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 27.5 C -> actual temp = 27.0 C, coldid probe
CDCl3
OCCH3(s)
31
H-1a(d)
H-6a(dd)
SCH2N3(d)
SCH2N3(d)
H-4a(t)
H-3c(dd)
H-4d(t)
H-4b(t)
H-4c(t)
H-3d(dd)
H-2a(dd)
H-2d(dd)
H-3b(dd)
H-1b(d)
H-1d(d)
H-6b(dd)
H-6a(dd)
H-6b(dd)
H-1c(d)
H-5b(ddd)
H-6c(dd)
H-5c(ddd)
H-6c(br. d)
H-2c(dd)
H-5d(dt)
H-6d(dd)
H-3a(dd)
H-5a(ddd)
Chemical Shift (ppm)
Chemical Shift (ppm)

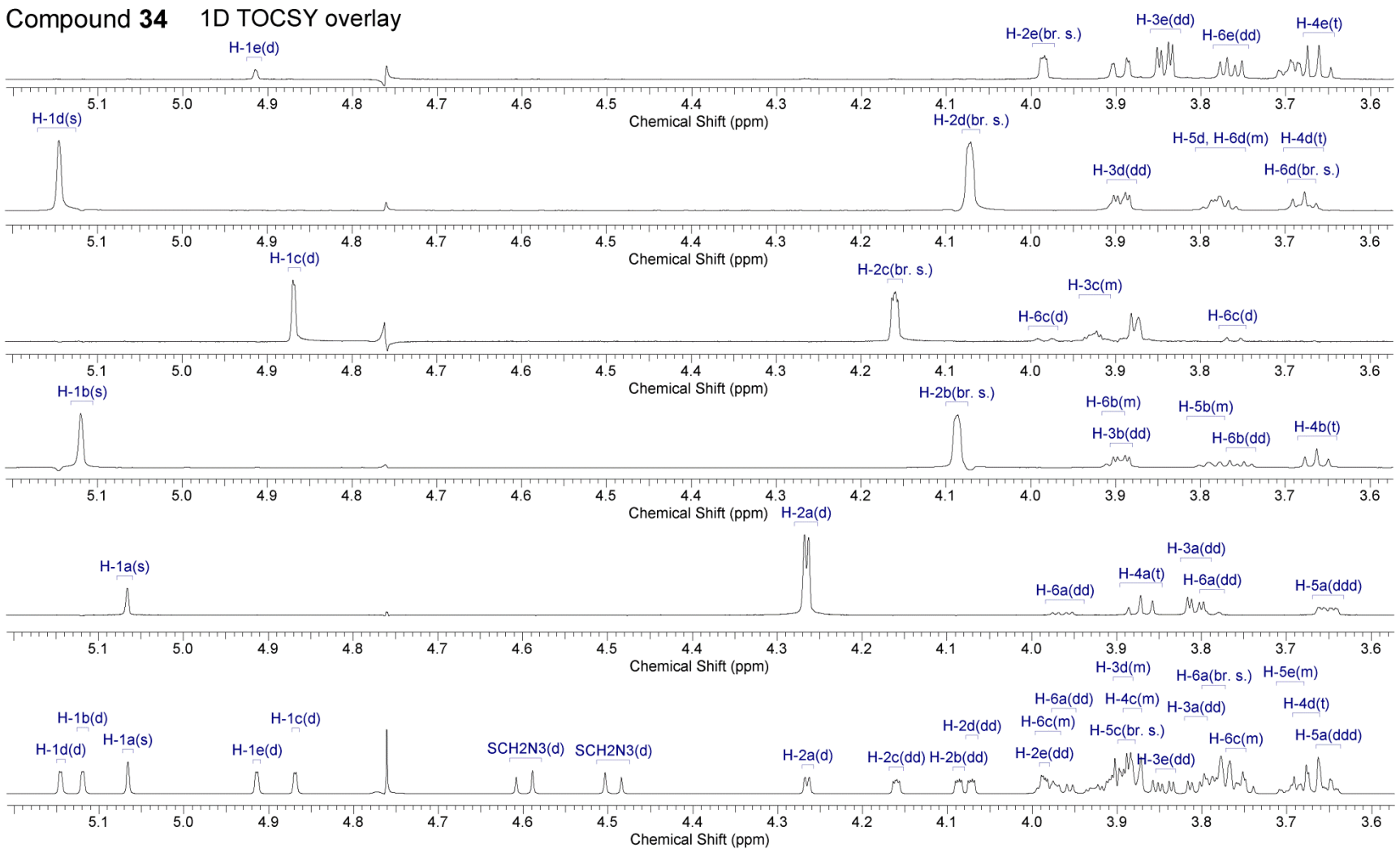

JB-06-50 699.738 MHz H1 1D in cdcl3 (ref. to CDCl3 @ 7.26 ppm), temp 27.5 C -> actual temp = 27.0 C, coldid probe
Ar(m)
CDCl3
H-2b(dd)
H-1a(d)
H-4a(t)
H-4c(t)
H-4d(t)
H-4e(t)
H-4b(t)
H-3e(dd)
H-1d(d)
H-1c(d)
H-1b(d)
H-3d(dd)
H-3b(dd)
H-2d(dd)
H-2a(dd)
H-2c(dd)
H-2e(dd)
SCH2N3(d)
H-6e(m)
SCH2N3(d)
H-1e(d)
H-5e(m)
H-5d(s)
H-6b(dd)
H-6d(m)
H-6d(dd)
H-6c(dd)
H-6a(dd)
H-5a(ddd)
H-3c(dd)
H-3a(dd)
H-6e(m)
H-6a(dd)
H-5c(ddd)
H-6c(dd)
CH3(C=O)(s)
CH3(C=O)(s)
CH3(C=O)(s)
32
Chemical Shift (ppm)
Chemical Shift (ppm)


Compound 32
1D TOCSY overlay
H-4e(m)
H-3e(dd)
H-2e(d)
H-1e(s)
H-6e(m)
H-6e(m)
H-5e(m)
H-4d(t)
H-3d(dd)
H-1d(s)
H-2d(m)
H-6d(m)
H-6d(dd)
H-4c(t)
H-2c(m)
H-1c(s)
H-3c(dd)
H-5c(d)
H-6c(dd)
H-6c(dd)
H-4b(t)
H-3b(dd)
H-2b(m)
H-1b(d)
H-6b(d)
H-6b(m)
H-2a(d)
H-4a(t)
H-1a(s)
H-3a(dd)
H-6a(dd)
H-5a(m)
H-6a(d)
H-4e(t)
H-2a(dd)
H-4b(t)
H-2c(dd)
H-3b(dd)
H-3d(dd)
H-2e(dd)
H-1b(d)
H-2d(dd)
H-1d(d)
H-1e(d)
H-6d(m)
H-6b(dd)
H-6d(dd)
H-5e(m)
H-6b(m)
H-5c(ddd)
H-6a(dd)
H-6c(dd)
H-5a(ddd)
H-6a(dd)
H-6c(dd)
6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4
Chemical Shift (ppm)


JB-06-52 44 min HPLC fraction 699.740 MHz H1 1D in d2o (ref. to external acetone @ 2.225 ppm), temp 27.5 C -> actual temp = 27.0 C, coldid probe
H-1b(d)
H-1c(d)
H-1a(s)
H-1d(d)
SCH2N3(d)
SCH2N3(d)
H-2a(d)
H-2c(dd)
H-2d(dd)
H-2b(dd)
H-3b(dd)
H-3c(dd)
H-6a(dd)
H-3d(dd)
H-6c(dd)
H-6b(m)
H-6d(m)
H-5/6d(m)
H-5/6b(m)
H-4a(t)
H-6c(dd)
H-6a(m)
H-4c(t)
H-4b(t)
H-5c(m)
H-4d(t)
H-5a(ddd)
5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5
Chemical Shift (ppm)
HO
OH
O
d
c
b
a
S
N3
33
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
Chemical Shift (ppm)


Compound 33
1D TOCSY overlay
H-1d(d)
H-2d(dd)
H-6d(m)
H-5/6d(m)
H-3d(dd)
H-4d(t)
H-3c(dd)
H-4c(t)
H-6c(dd)
H-6c(dd)
H-5c(m)
H-2c(dd)
H-1c(d)
H-5/6b(m)
H-3b(dd)
H-4b(t)
H-2b(dd)
H-6b(m)
H-1b(d)
H-2a(d)
H-6a(m)
H-4a(t)
H-1a(s)
H-3a(dd)
H-6a(dd)
H-5a(ddd)
5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5
Chemical Shift (ppm)


JB-06-53 59 minute HPLC fraction 699.740 MHz H1 1D in d2o (ref. to external acetone @ 2.225 ppm), temp 27.5 C -> actual temp = 27.0 C, coldid probe
H-1d(d)
H-1b(d)
H-1a(s)
H-1e(d)
H-1c(d)
SCH2N3(d)
SCH2N3(d)
H-2a(d)
H-2c(dd)
H-2b(dd)
H-2d(dd)
H-2e(dd)
H-6c(m)
H-6a(dd)
H-3c(dd)
H-6c(m)
H-6b(s)
H-3b(dd)
H-3d(m)
H-4c(m)
H-5c(br. s.)
H-3e(dd)
H-6d, H-6d(m)
H-6e(dd)
H-6a(br. s.)
H-3a(dd)
H-6c(m)
H-4e(m)
H-5d(m)
H-4d(t)
H-5a(ddd)
H-5e(m)
5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6
Chemical Shift (ppm)
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 -0.5 -1.0 -1.5
Chemical Shift (ppm)
34


Compound 34 1D TOCSY overlay
H-1e(d)
H-2e(br. s.)
H-3e(dd)
H-6e(dd)
H-4e(t)
H-1d(s)
H-2d(br. s.)
H-5d, H-6d(m)
H-4d(t)
H-3d(dd)
H-6d(br. s.)
H-1c(d)
H-2c(br. s.)
H-3c(m)
H-6c(d)
H-6c(d)
H-1b(s)
H-2b(br. s.)
H-6b(m)
H-5b(m)
H-3b(dd)
H-6b(dd)
H-4b(t)
H-2a(d)
H-1a(s)
H-3a(dd)
H-4a(t)
H-6a(dd)
H-6a(dd)
H-5a(ddd)
H-3d(m)
H-6a(br. s.)
H-5e(m)
H-6a(dd)
H-4c(m)
H-3a(dd)
H-4d(t)
H-1b(d)
H-1c(d)
H-2d(dd)
H-6c(m)
H-5c(br. s.)
H-6c(m)
H-5a(ddd)
H-1d(d)
H-1a(s)
H-1e(d)
SCH2N3(d)
SCH2N3(d)
H-2a(d)
H-2c(dd)
H-2b(dd)
H-2e(dd)
H-3e(dd)
5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6
Chemical Shift (ppm)