Synthesis of Glycopolymers for Microarray Applications via Ligation of Reducing Sugars to a Poly(acryloyl hydrazide) Scaffold

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Supporting Information

Materials and General Procedures:

All chemicals, unless stated otherwise, were purchased from Aldrich Chemicals. Lectins were purchased from Vector laboratories and labeled with amine-reactive Cy5 fluorophore (GE Healthcare) according to published protocol.[1] Solvents were purified on a Glass Contour solvent purification system. Column chromatography was performed on Biotage SP1 flash chromatography system. Distillations were performed on Kugelrohr short-path distillation apparatus (AC input 115 V) purchased from Aldrich. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Biospin Advance II 500 MHz High Performance NMR spectrometer with multinuclear CP-MAS probe. Spectra were recorded in $CDCl_3$ or D_2O solutions at 293K and referenced to residual solvent peaks Size exclusion chromatography (SEC) was performed on Viscotek TDA 302 SEC system with triple detector array. For measurements in DMF (0.2% LiBr) the instrument was equipped with 2 in-series Mixed Bed GMHHR-M columns, separation range 100-4M (30 cm x 7.8 mm i.d.) at 70 °C. Aqueous SEC analysis (100 mM sodium phosphate, 150 mM NaCl, pH = 7.2, 0.02% NaN_3) was performed on Shodex SB-804 HQ column,

separation range 100-1M (300 mm x 8 mm i.d.) at 35 °C. All glycopolymers were isolated by gel filtration on Sephadex G-25 columns (PD-10, GE Healthcare). Anionic glycopolymers 5n, p and r were purified on monovalent avidin agarose resin column (Thermo scientific) prior to gel filtration. Microarrays were fabricated on SuperStreptavidin glass substrates (Arrayit) using UCSF DeRisi linear servomotor microarrayer equipped with 75-µm silicon PETC pins from Parallel Synthesis Technologies, Inc. Microarrays stained with fluorescent lectins were analyzed on Axon GenePix 4000B scanner. MS analysis was performed in positive mode on Thermo LTQ Mass Spectrometer. ATR analyses were performed using Pike Technologies MIRacle^{TM} single refraction ATR accessory (ZnSe crystal). UV-VIS spectra were collected on a Perkin Elmer Lambda 35 UV/VIS spectrometer.

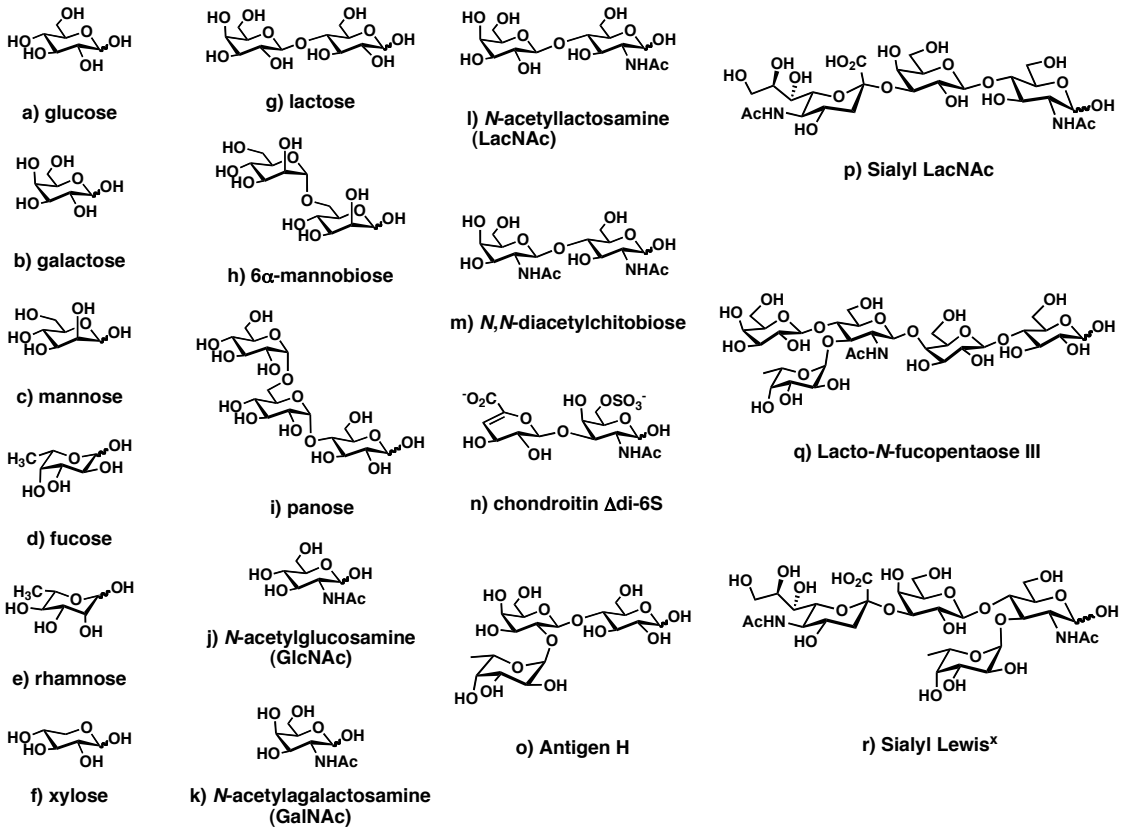
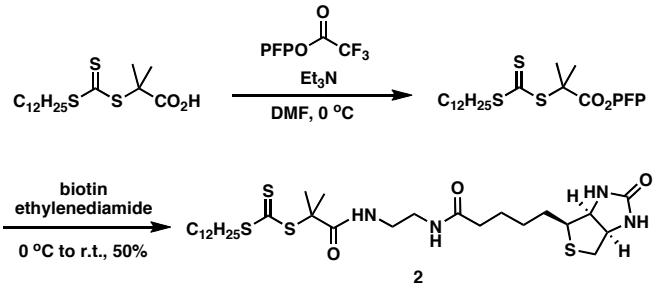


Chart SI 1. Molecular structures of glycan epitopes.

I. Glycopolymer synthesis.



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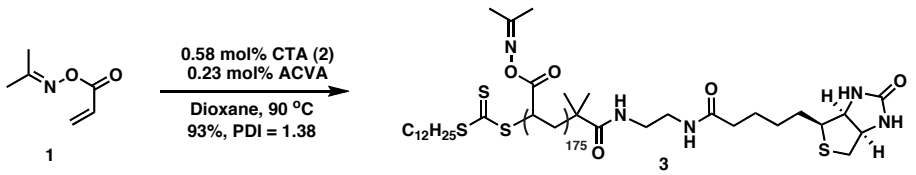
Chain transfer agent (2). A flame-dried Schlenk flask (25 mL) equipped with a magnetic stirring bar was charged with *S*-dodecyl-*S'*-(α,α-dimethylacetic acid)trithiocarbonate[2] (50 mg, 0.14 mmol) under a stream of nitrogen. Dry DMF (1 mL) was added. The flask was equipped with a rubber septum and the yellow solution was cooled to 0 °C. Triethylamine (48 μL, 0.34 mmol, 2.5 equiv.) was added followed by pentafluorophenyl trifluoroacetate (26 μL, 0.15 mmol, 1.1 equiv.). The reaction was stirred at 0 °C. After 1 hr, the acid was fully consumed and biotin ethylenediamide[3] (43.2 mg, 0.15 mmol, 1.1 equiv.) was added in one portion. The reaction was allowed to warm to r. t. and stirred for 6 h. After this time, the solvent was removed, the residue was washed with ether, chromatographed (methanol/dichloromethane 1→10%) and crystallized from ethyl acetate to give 45.4 mg of yellow crystals (50%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 7.13 (bs, 1H), 6.99 (bs, 1H), 6.93 (s, 1H), 5.94 (s, 1H), 4.53 (m, 1H), 4.33 (m, 1H), 3.33 (bm, 4H), 3.26 (t, J = 7.4 Hz, 2H), 3.13 (bm, 1H), 2.91 (dd, J_1 = 12.8 Hz, J_2 = 4.8 Hz, 1H), 2.76 (d, J = 12.8 Hz, 1H), 2.19 (m, 2H), 1.80-1.60 (m, 12H), 1.48-1.20 (m, 20H), 0.87 (t, J = 7.0 Hz, 3H).

^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 221.2, 174.2, 173.6, 61.7, 60.3, 57.1, 55.8, 40.7, 40.6, 39.2, 37.2, 36.0, 31.9, 29.6, 29.6, 29.5, 29.3, 29.1, 29.0, 28.2, 28.1, 27.8, 25.8, 25.8, 25.7, 22.7, 14.1.

MS (ITMS$^+$ c NSI) *m/z*: calcd: 633.30, $(M+H)^+$, found: 633.34 $(M+H)^+$; MS2 (633.34): 431.08 $(M\text{-}H\text{-}SC_{12}H_{25})^+$, 347.04 $(COC(CH_3)_2SCS_2C_{12}H_{25})^+$.

ATR (ZnSe) ν (cm^{-1}): 3294.2, 3221.0, 3100.4, 2919.9, 2850.3, 1706.2, 1644.2, 1628.9, 1462.1, 1267.6, 1242.6, 1070.7, 811.3, 760.0.

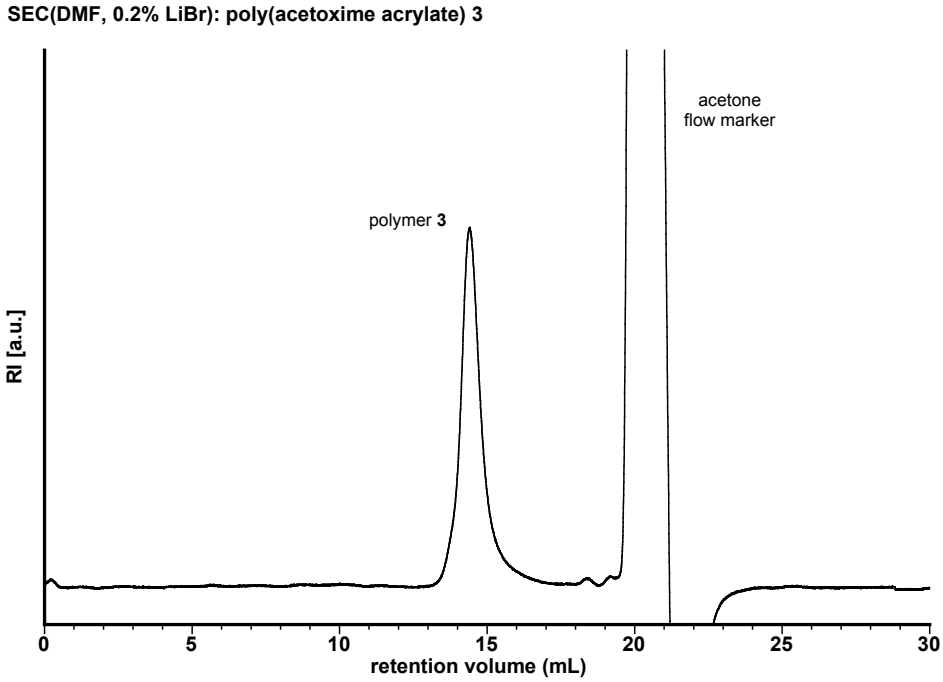


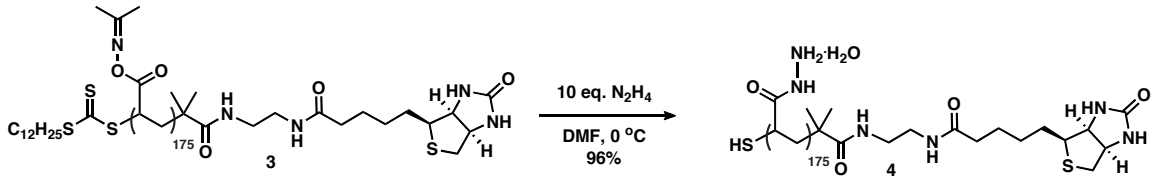
RAFT polymerization of acetoneoxime acrylate[4] monomer (1) in the presence of chain transfer agent (2). A flame-dried Schlenk flask (10 mL) equipped with a magnetic stirring bar was charged with **2** (11.9 mg, 0.019 mmol, 0.58 mol%), ACVA (4,4'-azobis(4-cyanovaleric acid), 2.2 mg, 0.008 mmol, 0.23 mol%), monomer **1** (411.7 mg, 3.240 mmol, filtered through basic alumina) and anhydrous dioxane (489 mg). The flask was equipped with a rubber septum and attached to a Schlenk line. The yellow solution was thoroughly degassed by three freeze-pump-thaw cycles. After the final cycle, the flask was allowed to warm to r. t. and then immersed into an oil bath preheated to 90 °C. After 2 h, the mixture became viscose. A small sample was analyzed by ^{1}H NMR spectroscopy. The monomer conversion was estimated at 90%. The reaction mixture was diluted with CH_2Cl_2 and precipitated into hexanes. The residue was re-dissolved in a minimal quantity of CH_2Cl_2 and precipitated again into hexanes with vigorous stirring. This was repeated twice more. The yellow polymer was concentrated from CH_2Cl_2 three times to remove residual hexanes and dried under high vacuum overnight to give polymer **3** as a pale yellow solid (382 mg, 93%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): see attached spectrum.

ATR (ZnSe) ν (cm^{-1}): 2955.7, 2918.0, 2849.0, 1756.5, 1649.4, 1442.0, 1372.4, 1273.1, 1239.7, 1130.8, 1065.3, 862.2, 752.3.

SEC (DMF, 0.2% LiBr): M_w = 22.75 kDa, M_n = 16.52 kDa, DP = 174, PDI = 1.38.





Synthesis of poly(acryloyl hydrazide hydrate) (4). Poly(acetoneoxime acrylate) **3** (100 mg, 0.79 mmol) was dissolved in DMF (2 mL). The yellow solution was cooled to 0 °C and hydrazine was added (246 μL, 7.90 mmol, 10 equiv.). Within 10 min, the yellow color disappeared and white solid precipitated from the solution. The reaction was stirred at 0 °C for additional 1 hr. After this time, the solvent was decanted and the solid was washed with ether and methanol. The residue was dialyzed against DI water and lyophilized to obtain 65.2 mg hydrate of polymer **4** as white solid (96%).

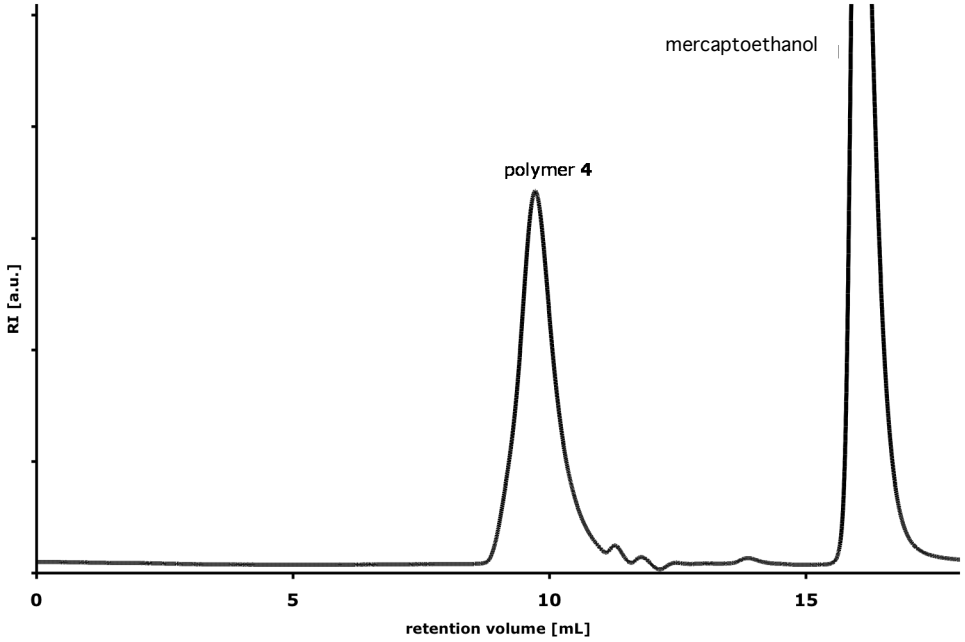
^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): see attached spectrum

^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): see attached spectrum

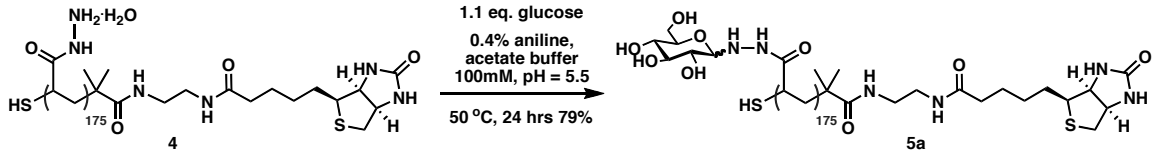
ATR (ZnSe) ν (cm^{-1}): 3267.4, 1645.9, 1541.3, 1448.6, 1387.1, 1303.0, 1272.1, 990.6.

Elemental Analysis: calcd: C, 34.61; H, 7.75; N, 26.91; O, 30.74; found: C, 36.51; H, 7.96; N, 26.98; O, 28.55.

SEC (100mM PBS): M_w = 20.30 kDa, M_n = 13.96 kDa, DP = 191, PDI = 1.45.



A representative synthesis of glycopolymers 5.



Glucose glycopolymer 5a. An Eppendorf tube (250 μL) was charged with glucose (0.44 mg, 0.002 mmol, 1.1 equiv.). A solution of poly(acryloyl hydrazide) (**4**) (8.9 μL, c = 0.25

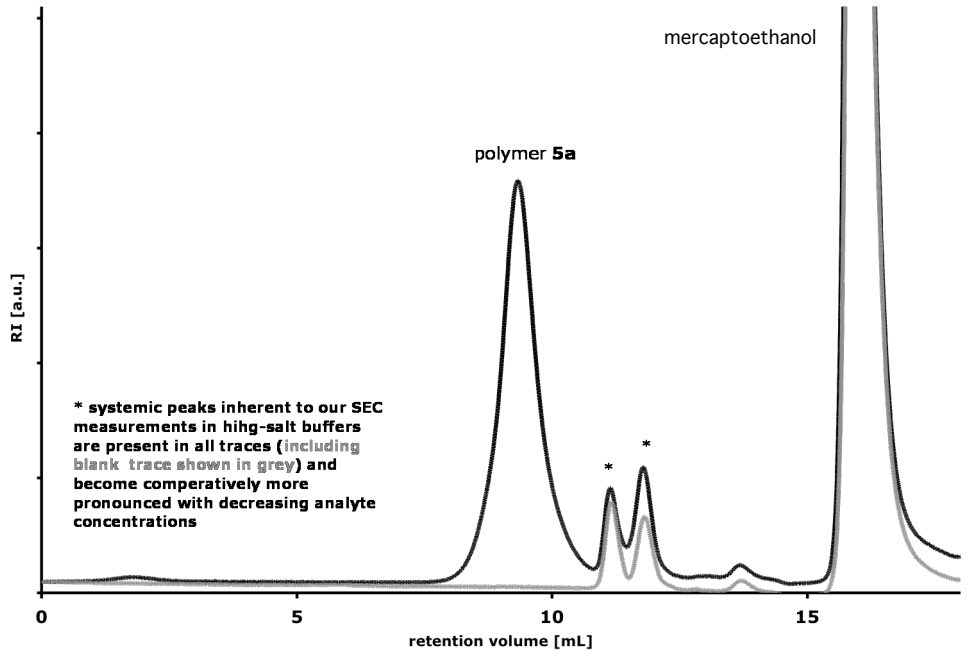
M) in acetate buffer (100 mM, pH = 5.5) containing aniline (1 mM) was added. The tube was closed, vortexed until all glucose dissolved and heated at 50 °C for 24 h. After this time, the solvent was removed under reduced pressure and the residue was purified on Sephadex G-25 column (eluting with DI water adjusted to pH = 8 with NH_4OH) and lyophilized to give 0.48 mg of white solid (98%, 79% glycosylation efficiency, α/β = 7 : 93).

^{1}H NMR (D_2O, 500 MHz) δ (ppm): see attached spectrum

ATR (ZnSe) ν (cm^{-1}): 3269.5, 2922.8, 1658.5, 1445.2, 1365.5, 1079.6, 1023.2.

SEC (100 mM PBS buffer, pH = 7.2): M_w = 38.22 kDa, M_n = 27.42 kDa, PDI = 1.39.

M_w(^{1}H NMR) = 38.32 kDa.



All polymers **5** were synthesized according to the above procedure. Charged glycopolymers **5n**, **p**, and **r** were first purified on Sephadex G-25 columns with 60 mM guanidinium chloride in PBS and then desalted on a Sephadex G-25 with aqueous NH_4OH elution. The yields and isomeric compositions of polymers **5a-r** are

summarized in Table 1 and Figure 2A in the body of this article. Molecular weights of polymers **5a-r** and polydispersities determined by SEC together with those calculated based on ^{1}H NMR analysis of glycosylation efficiencies are organized in **Table SI 1**.* Representative examples of SEC traces and all ^{1}H NMR data corresponding to compounds **1-5** are attached below.

		1.1 equivalent of glycan				2.0 equivalents of glycan			
#	*glycan*	*Mw (NMR)*	*Mw (SEC)*	*Mn (SEC)*	*PDI (SEC)*	*Mw (NMR)*	*Mw (SEC)*	*Mn (SEC)*	*PDI (SEC)*
5a	Glucose	38,316	38,223	27,416	1.39	39,821	38,792	28,518	1.36
5b	Galactose	39,570	37,167	26,870	1.38	41,828	37,551	27,214	1.38
5c	Mannose	37,814	40,738	29,064	1.40	41,075	40,635	29,070	1.40
5d	Fucose	30,542	36,752	24,947	1.47	30,988	37,312	25,777	1.45
5e	Rhamnose	35,894	39,592	27,584	1.44	37,678	39,848	28,025	1.42
5f	Xylose	36,174	41,235	28,350	1.45	36,770	39,476	27,737	1.42
5g	Lactose	59,538	47,009	33,112	1.42	69,131	57,684	36,258	1.59
5h	6α-mannobiose	58,472	51,478	35,631	1.44	63,802	51,981	36,120	1.44
5i	Panose	70,661	53,213	37,367	1.42	73,106	53,547	38,162	1.40
5j	*N*-acetylglucosamine	38,801	36,334	25,403	1.43	40,090	37,457	24,293	1.54
5k	*N*-acetylgalactosamine	38,479	40,905	28,163	1.45	40,090	42,591	29,416	1.45
5l	*N*-acetyllactosamine	35,425	35,734	24,366	1.47	37,843	43,275	29,664	1.46
5m	*N,N*-diacetylchitobiose	43,505	41,136	28,232	1.46	41,478	47,318	32,716	1.45
5n	Chondroitin Δdi-6S	50,211	75,393	51,262	1.47	54,276	75,241	49,952	1.51

		0.6 equivalents of glycan			
#	*glycan*	Mw (NMR)	Mw (SEC)	Mn (SEC)	PDI (SEC)
5o	Antigen H	46,841	39,866	28,052	1.42
5p	Sialyl LacNAc	31,834	42,326	28,398	1.49
5q	Lacto-*N*-fucopentaose III	76,842	65,893	44,568	1.48
5r	Sialyl Lewis X	32,154	44,925	30,963	1.45

Table SI 1. Molecular weights and polydispersity indexes (PDIs) of glycopolymers 5a-r determined by size exclusion chromatography (SEC) and from 1H NMR analysis.

NOTE: We observed reasonable match between Mw's determined by SEC and by ^{1}H NMR analysis for most monosaccharide glycopolymers **5**. In the case of di- and oligosaccharide polymers **5g-i**, **o**, and **q**, SEC gave significantly lower M_w's. On the other hand, charged glycopolymers **5n**, **p**, and **r** eluted typically at shorter retention times (See **Chart SI 2**). Although it is still to be determined how and why exactly the glycopolymer's structure affects its residence time on the SEC column, we speculate that it is a result of non-covalent interactions between the glycans, unsubstituted hydrazide

groups, and/or charges in those glycopolymers and the column sorbent. The broadening of the SEC signals and high apparent PDIs of the glycopolymers further support this hypothesis.

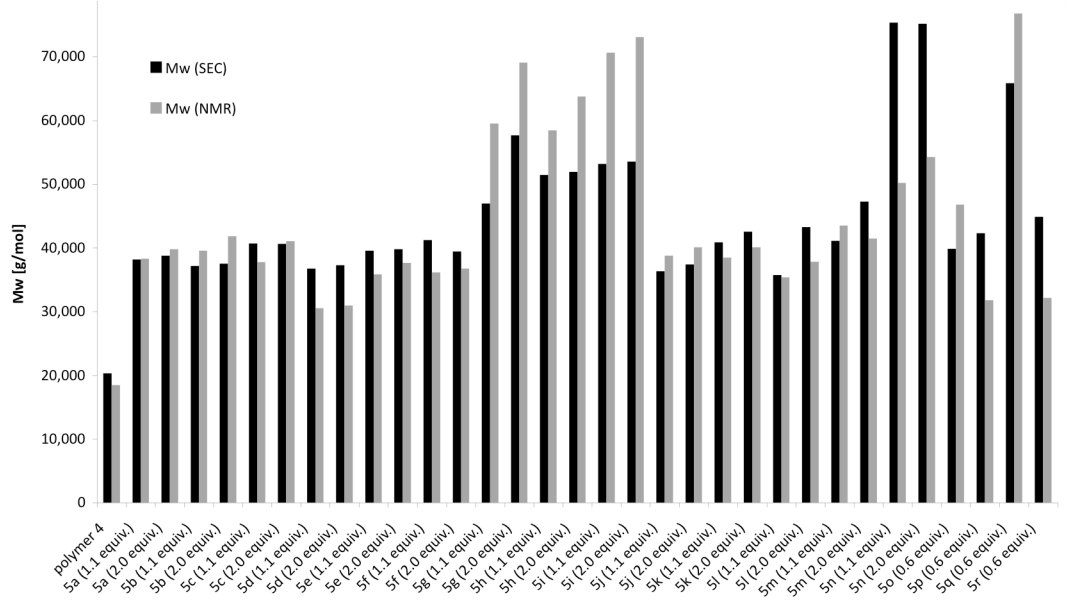
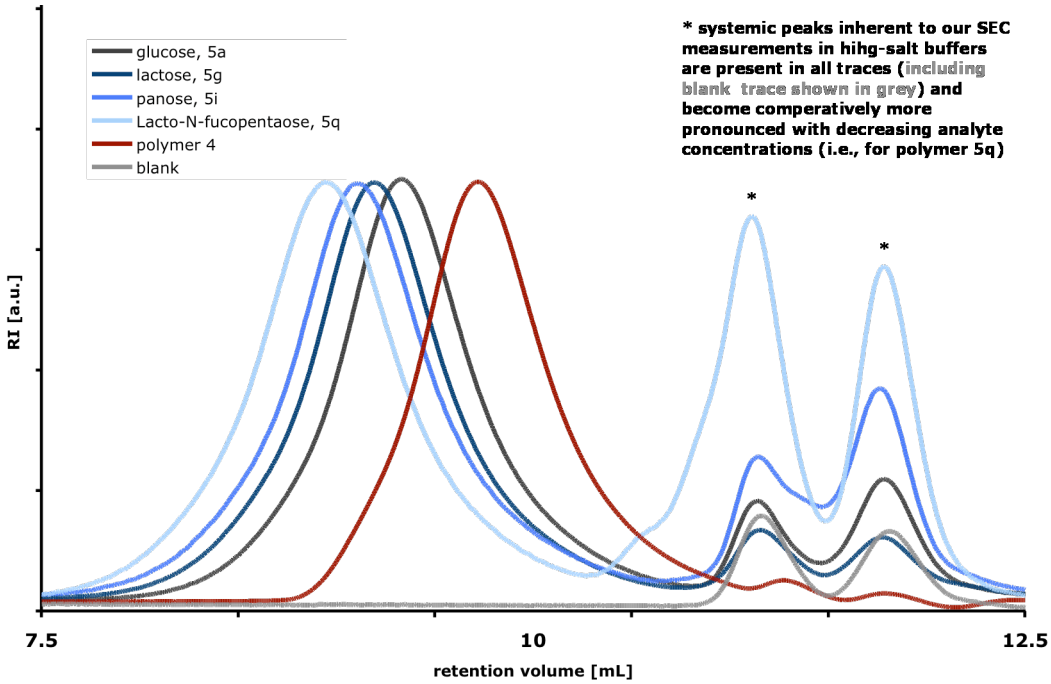


Chart SI 2. Plot of Mw of polymers **4** and **5a-r** as listed in **Table SI 1**.

SEC (100 mM PBS buffer, pH = 7.2) of polymers **4**, **5a**, **g**, **i**, and **q**.



II. Microarray construction and lectin binding

Solutions of glycopolymers **5a-r** in 3 x SSC buffer ([glycan] = 250 μM) were printed on streptavidin-coated glass at relative humidity (RH) = 80%). After printing, the slides were allowed to hydrate at RH = 100% for 1-2 h (*caution*: prolonged hydration may cause spot-walking). The slides were allowed to dry in air and all unbound streptavidin was blocked with biotin solution (2mM in 100 mM PBS, pH = 7.2, containing 1% BSA)) for 30 min. The slides were washed thoroughly with DI water and dried.

The resulting microarrays were incubated separately for 1 hr with the following lectin solutions:

1) ConA (0.1 μg/mL, 10 mM HEPES, 150 mM NaCl, 0.1 mM $CaCl_2$, 0.1% Tween 20, pH = 7.5, dye/protein = 1.43 per tetramer)
2) RCA I (0.1 μg/mL, 10 mM sodium phosphate, 150 mM NaCl, 0.1% Tween 20, pH = 7.8, dye/protein = 1.53 per aggregate)
3) HPA (0.1 μg/mL, 10 mM TRIS, 150 mM NaCl, 0.1% Tween 20, pH = 8.0, dye/protein = 3.83 per hexamer)
4) AAL (1.0 μg/mL, 10 mM HEPES, 150 mM NaCl, 0.1% Tween 20, pH = 8.5, dye/protein = 0.49 per dimer)

After incubation, the slides were washed with PBS buffer (100 mM, pH = 7.2, 3 x 10 min), rinsed with DI water, dried, and scanned at λ = 649 nm.

Ligation efficiencies were determined from ^{1}H NMR spectra of polymers **5a-r** as described here for glucose polymer **5a**:

All carbohydrate protons in **5a** were added (0.13 + 1.00 + 39.33 = 40.46) and divided by the total number of C-H signals in glucose (40.46 / 7 = 5.78). Multiplication of this number by 3 backbone C-Hs gives the number of backbone protons corresponding to units ligated with glucose (5.78 * 3 = 17.34).

Ligation efficiency is defined as the percentage of polymer units that are glycosylated:

l.g. = 17.34 / 20.87 * 100% = 83%.

Since generally the β-anomeric proton signal could not be resolved, we calculated the **percent isomeric ratio** as follows: α-anomeric and open form hydrazide protons were added and multiplied by 7 to give the total proton signals corresponding to these two isomers ((0.13 + 1.00) * 7 = 7.91). β-anomeric proton signal was calculated by subtracting α-anomeric and open form protons from the total glucose proton signal and dividing the resulting number by 7 for each C-H of glucose ((40.46 – 7.91) / 7 = 4.65).

The **isomeric ratios** are then defined as:

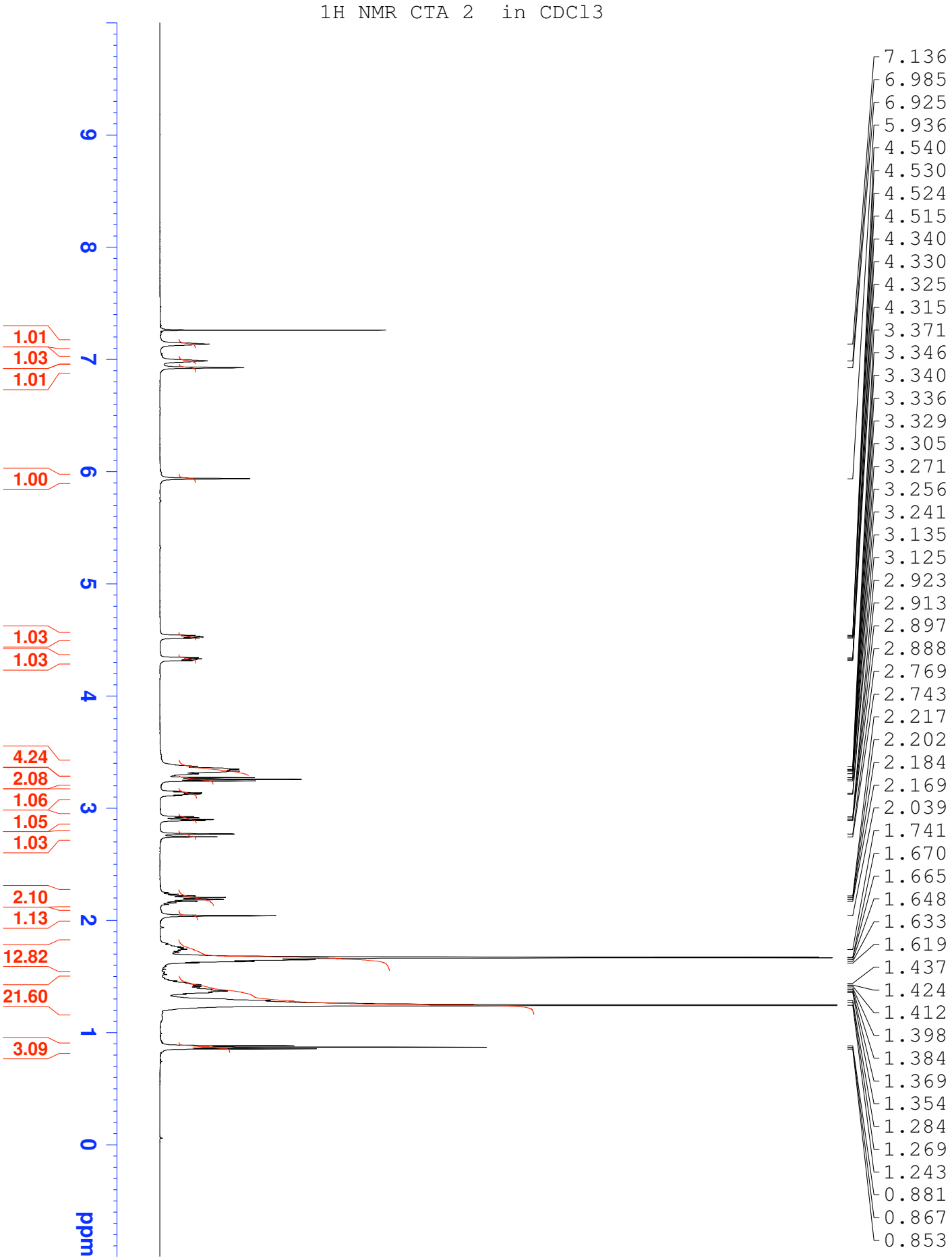
% β form = 4.65 / (0.13 + 1.00 + 4.65) * 100% = 80%

% α form = 1.00 / (0.13 + 1.00 + 4.65) * 100% = 18%

% open form = 0.13 / (0.13 + 1.00 + 4.65) * 100% = 2%

We were not able to clearly resolve α-anomeric proton signals for polymers **5f**, **k**, **m**, and **n** and therefore could not assign β / α ratio for these polymers and, therefore, we only reported open / (β+α) ratio.

III. Spectral data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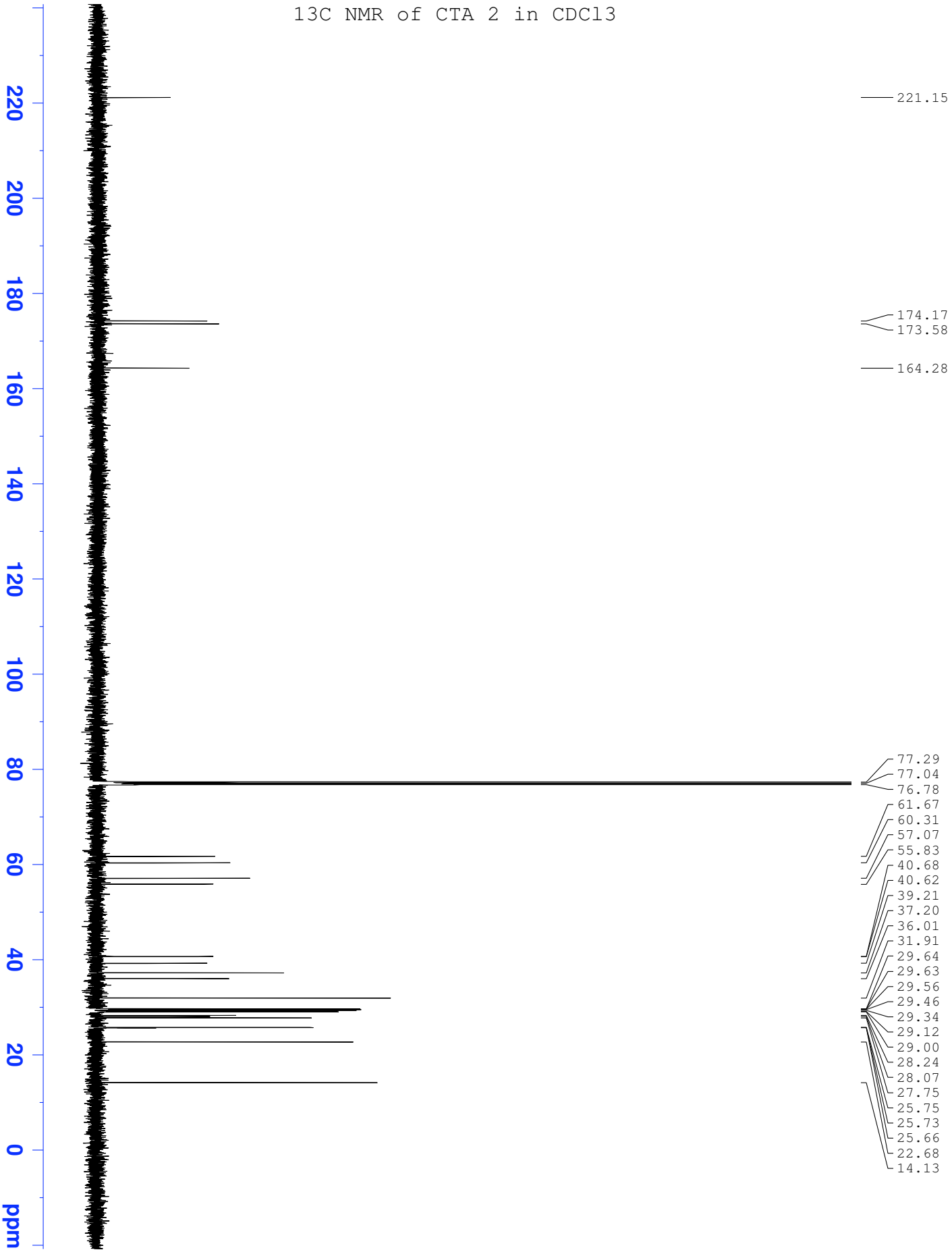
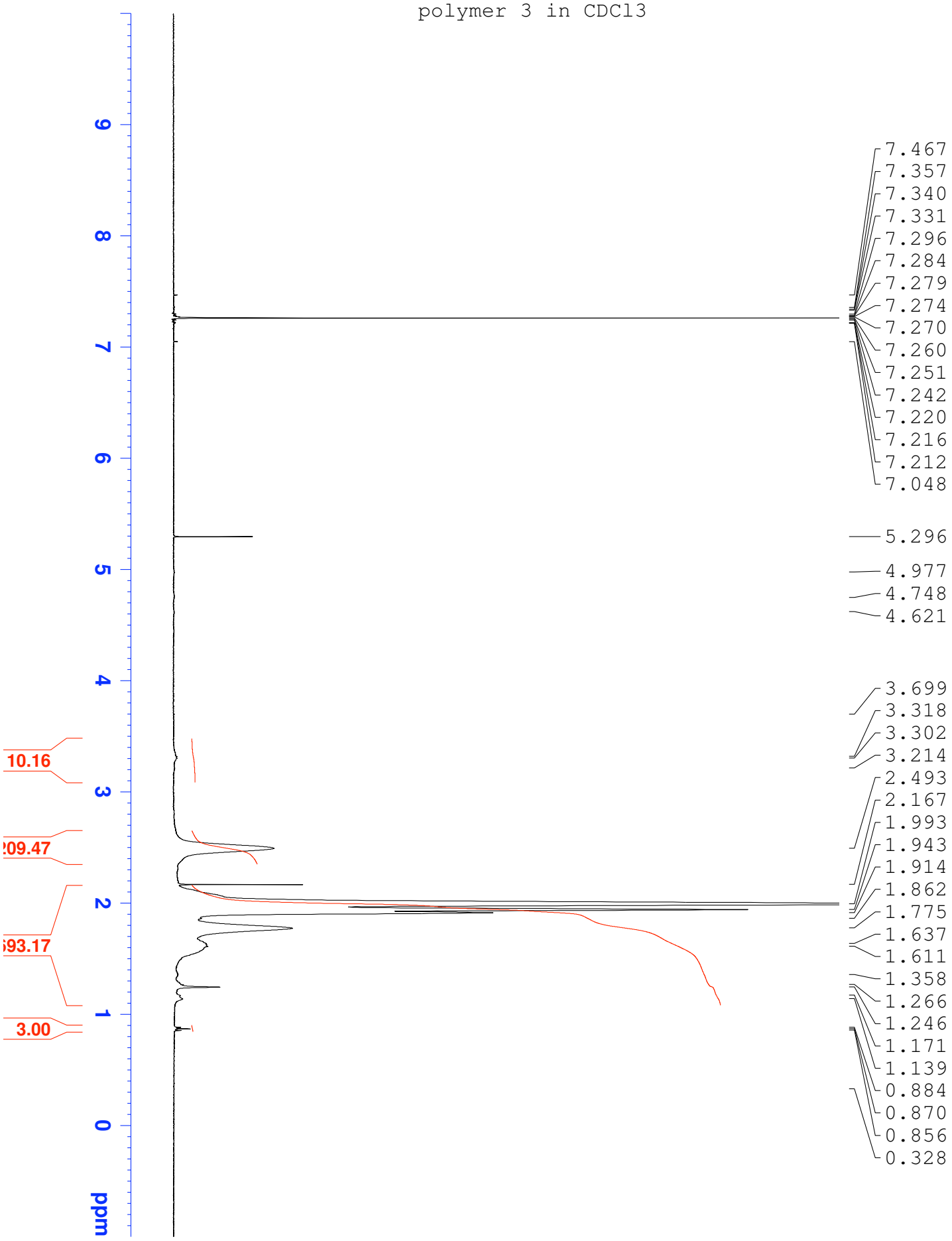
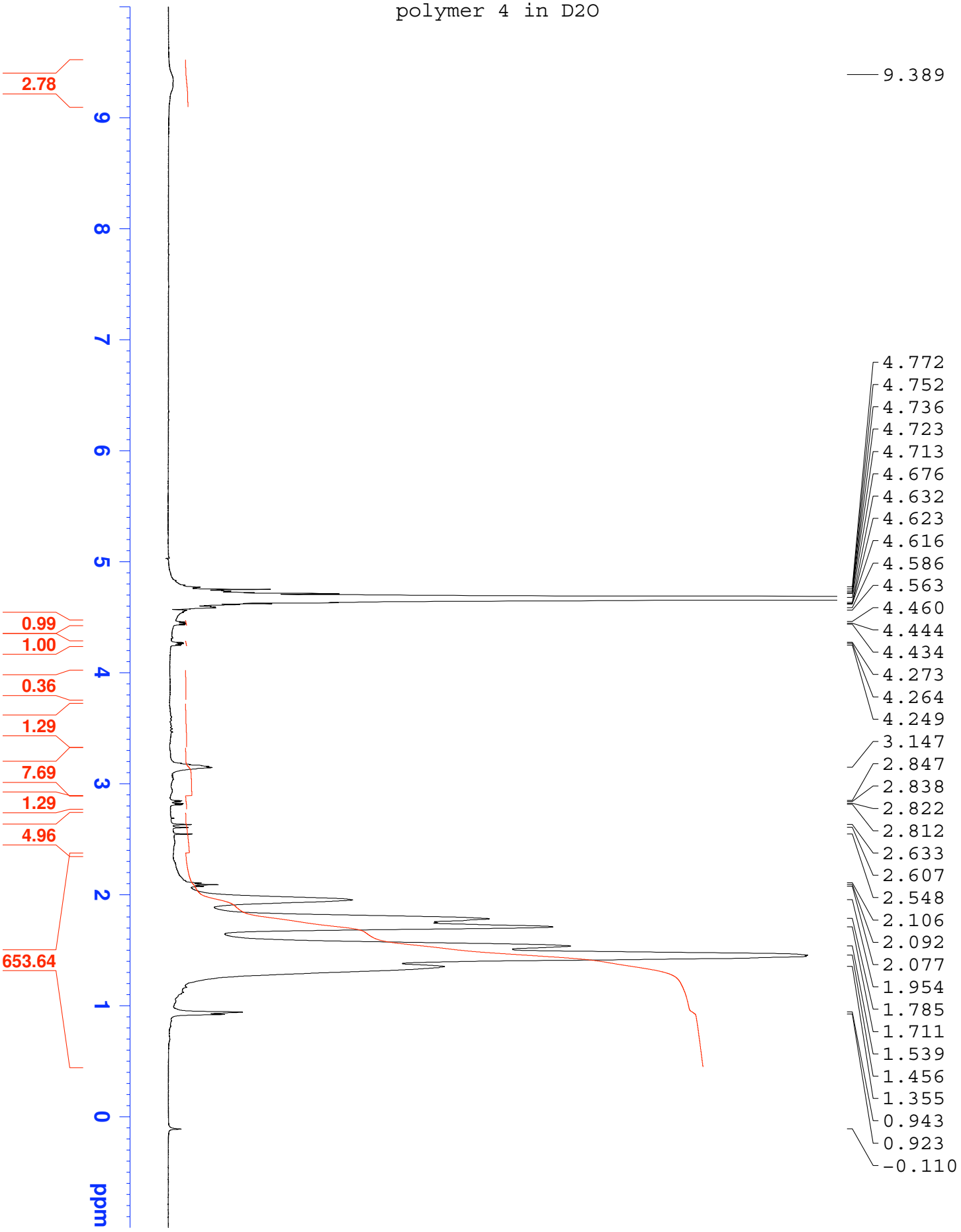
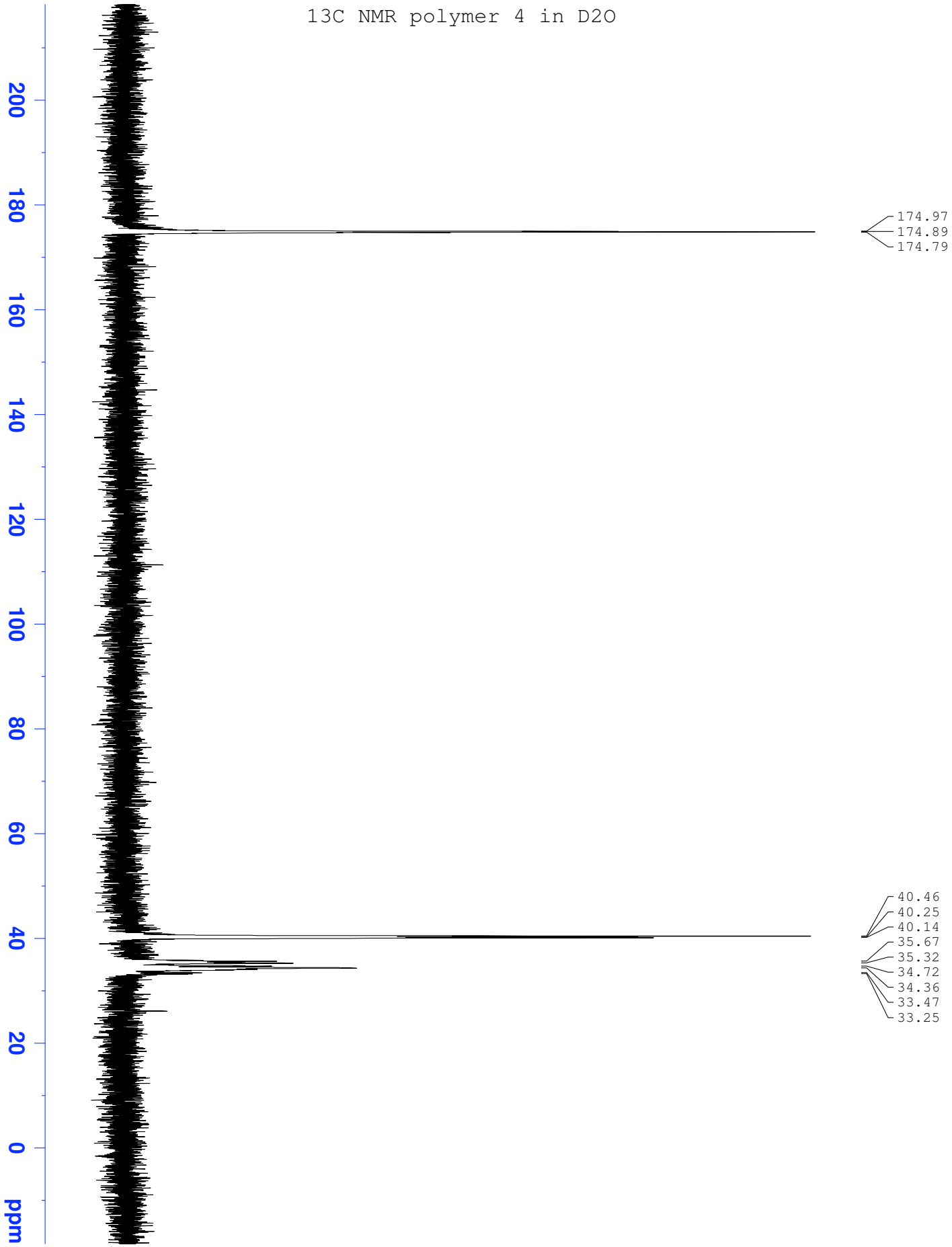
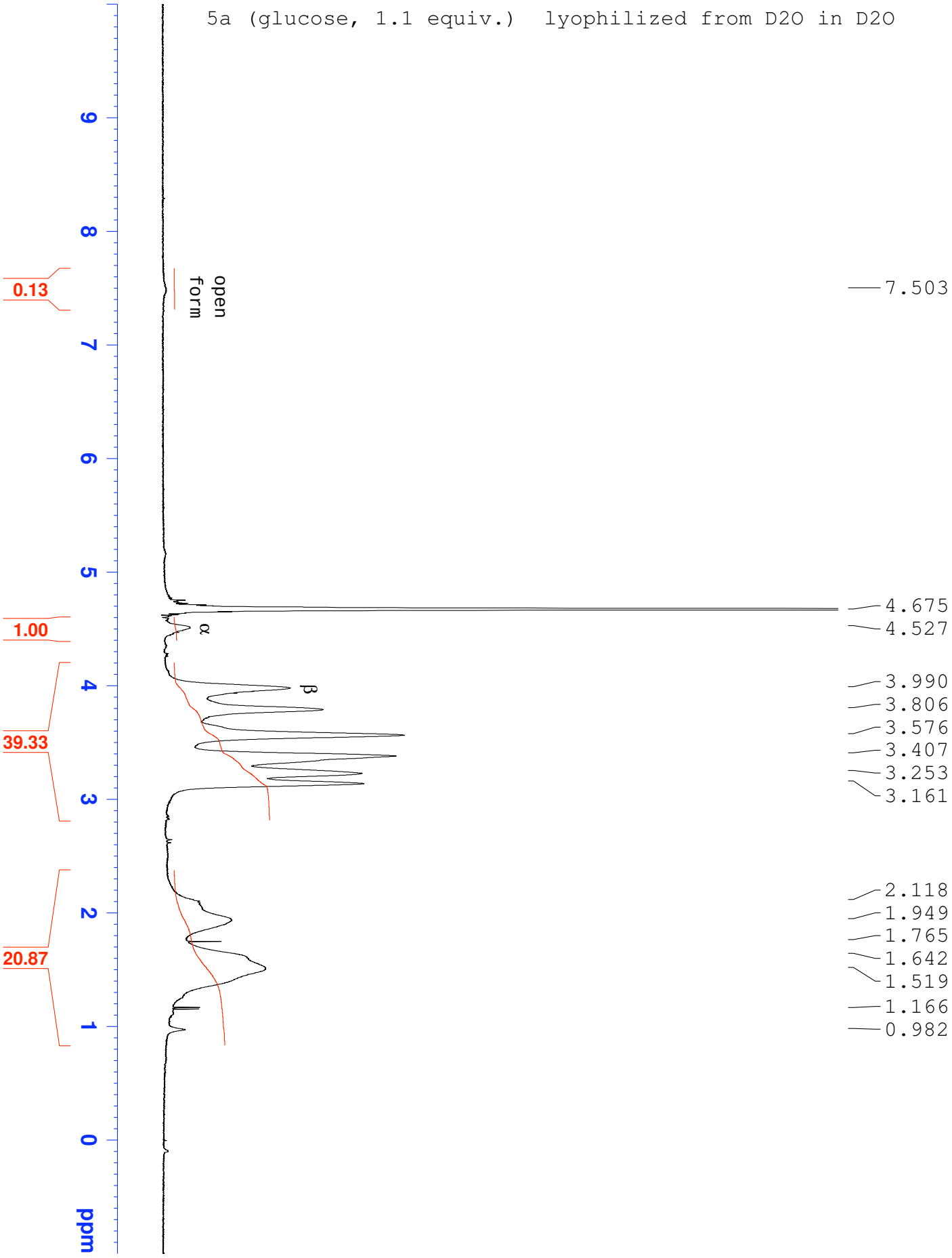
7.136
6.985
6.925
5.936
4.540
4.530
4.524
4.515
4.340
4.330
4.325
4.315
3.371
3.346
3.340
3.336
3.329
3.305
3.271
3.256
3.241
3.135
3.125
2.923
2.913
2.897
2.888
2.769
2.743
2.217
2.202
2.184
2.169
2.039
1.741
1.670
1.665
1.648
1.633
1.619
1.437
1.424
1.412
1.398
1.384
1.369
1.354
1.284
1.269
1.243
0.881
0.867
0.853
1.01
1.03
1.01
1.00
1.03
1.03
4.24
2.08
1.06
1.05
1.03
2.10
1.13
12.82
21.60
3.09
ppm

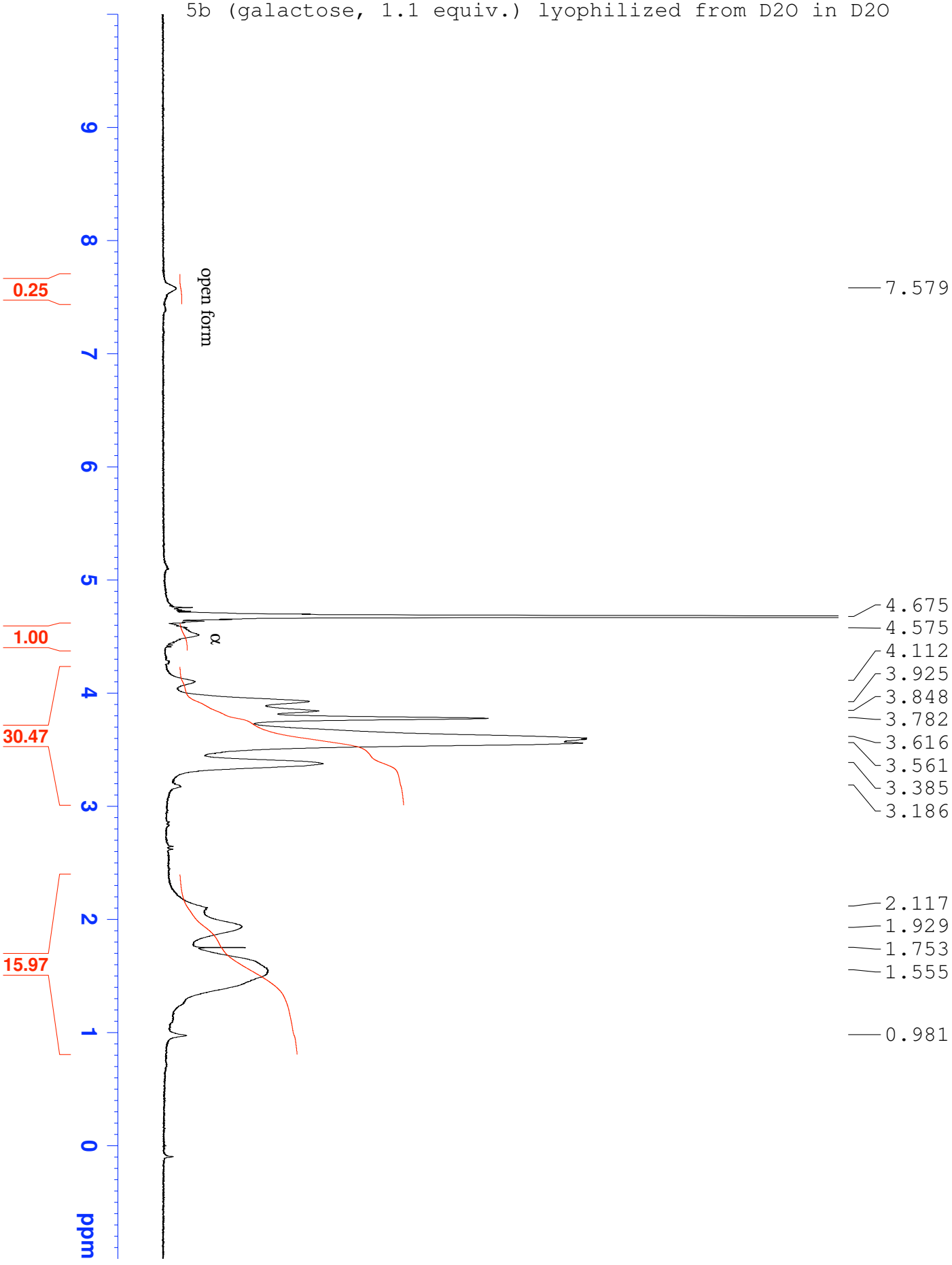

13C NMR of CTA 2 in CDCl3
221.15
174.17
173.58
164.28
77.29
77.04
76.78
61.67
60.31
57.07
55.83
40.68
40.62
39.21
37.20
36.01
31.91
29.64
29.63
29.56
29.46
29.34
29.12
29.00
28.24
28.07
27.75
25.75
25.73
25.66
22.68
14.13
220
200
180
160
140
120
100
80
60
40
20
0
ppm

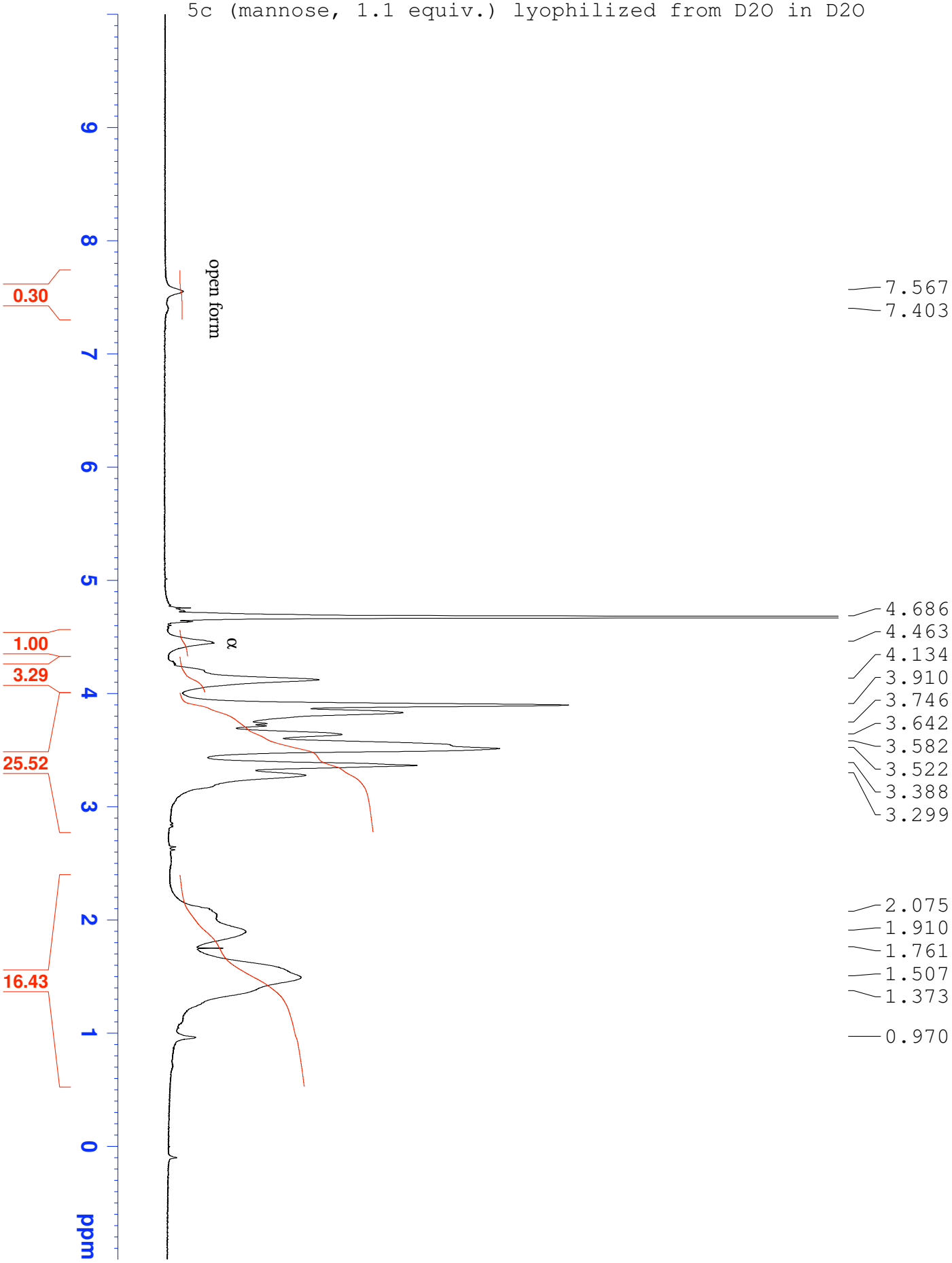

polymer 3 in CDCl3
7.467
7.357
7.340
7.331
7.296
7.284
7.279
7.274
7.270
7.260
7.251
7.242
7.220
7.216
7.212
7.048
5.296
4.977
4.748
4.621
3.699
3.318
3.302
3.214
2.493
2.167
1.993
1.943
1.914
1.862
1.775
1.637
1.611
1.358
1.266
1.246
1.171
1.139
0.884
0.870
0.856
0.328
10.16
09.47
93.17
3.00
ppm

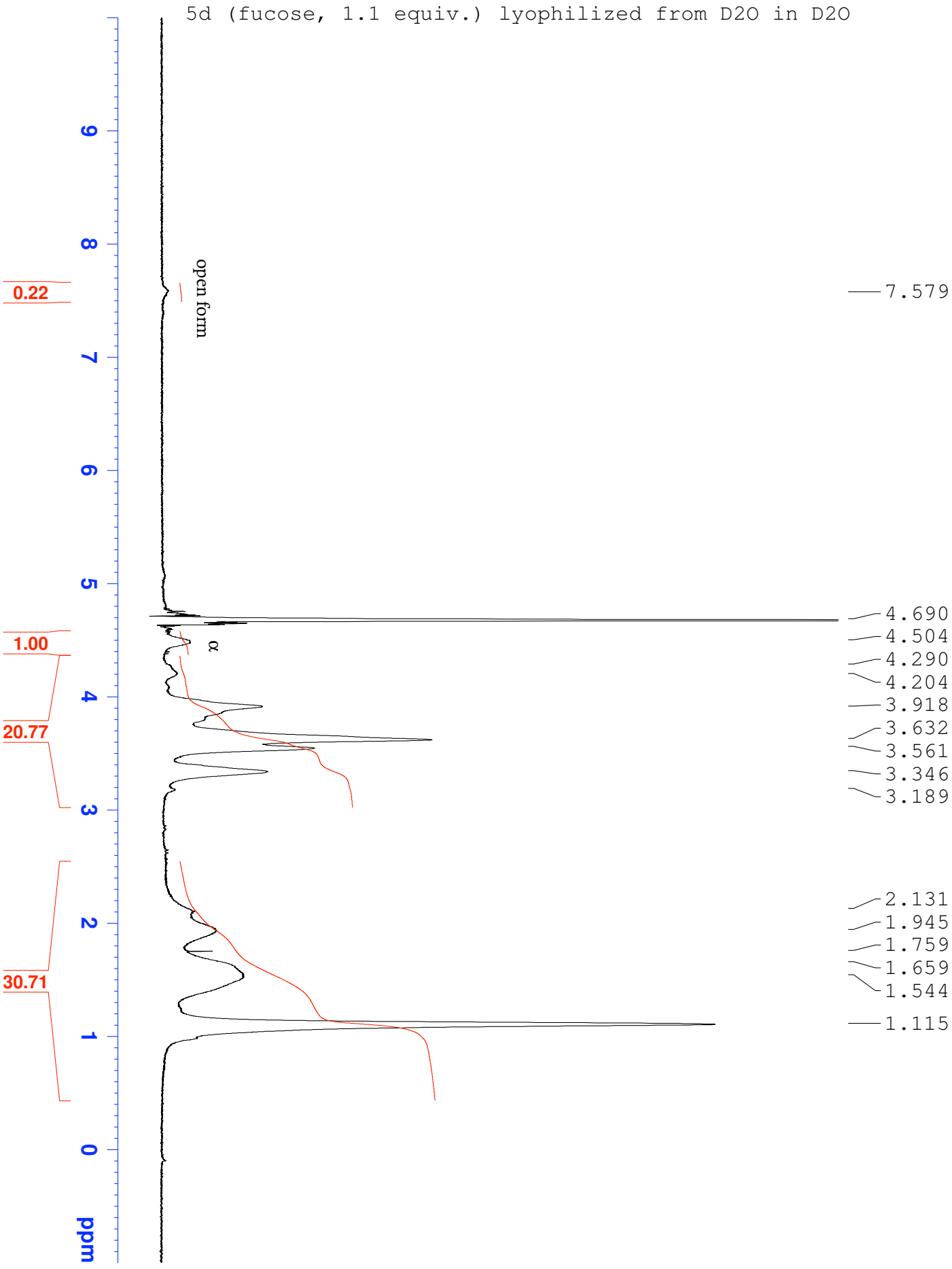

polymer 4 in D2O
2.78
0.99
1.00
0.36
1.29
7.69
1.29
4.96
653.64
9.389
4.772
4.752
4.736
4.723
4.713
4.676
4.632
4.623
4.616
4.586
4.563
4.460
4.444
4.434
4.273
4.264
4.249
3.147
2.847
2.838
2.822
2.812
2.633
2.607
2.548
2.106
2.092
2.077
1.954
1.785
1.711
1.539
1.456
1.355
0.943
0.923
-0.110
ppm

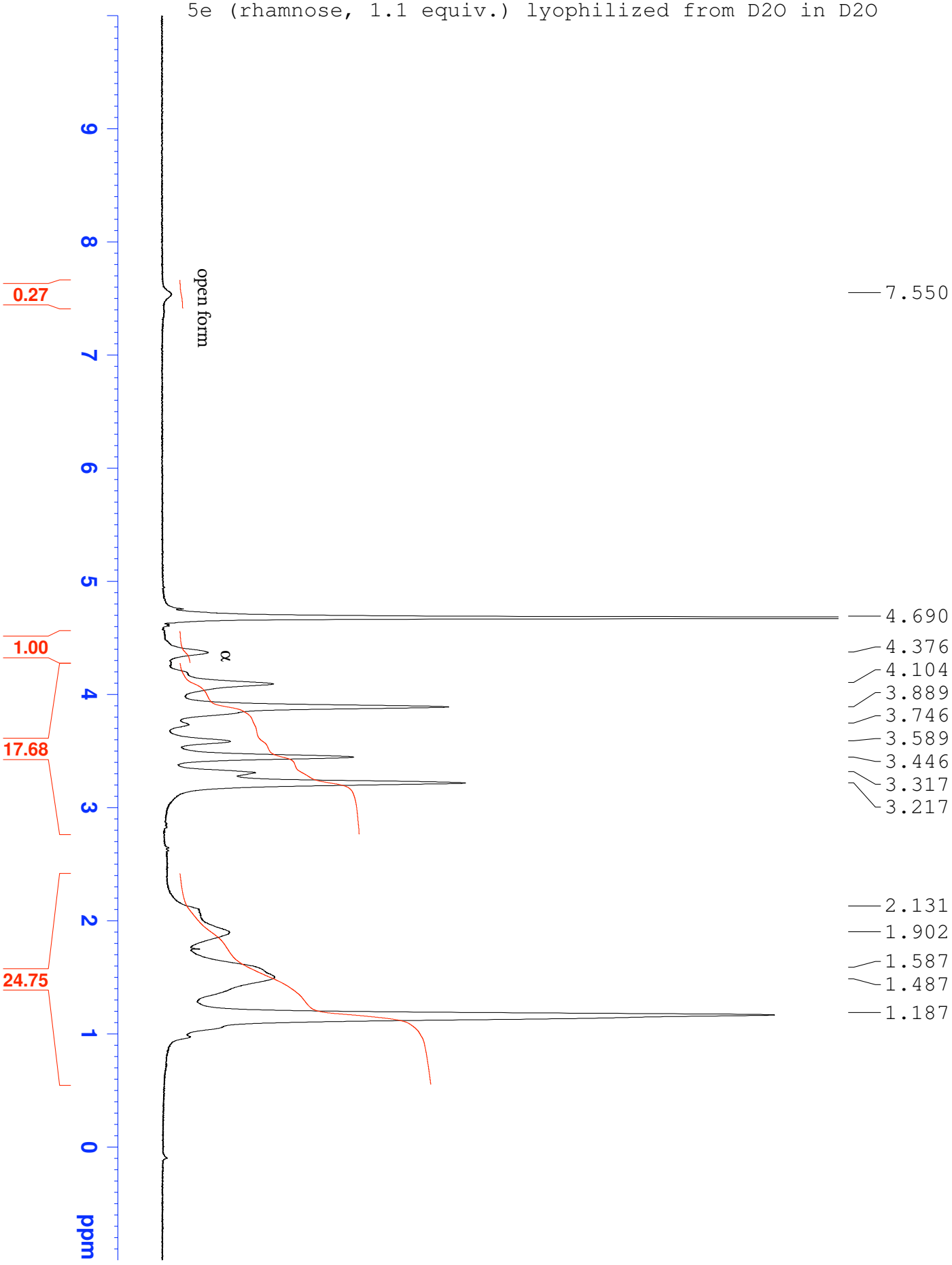

13C NMR polymer 4 in D2O
200
180
160
140
120
100
80
60
40
20
0
ppm
174.97
174.89
174.79
40.46
40.25
40.14
35.67
35.32
34.72
34.36
33.47
33.25

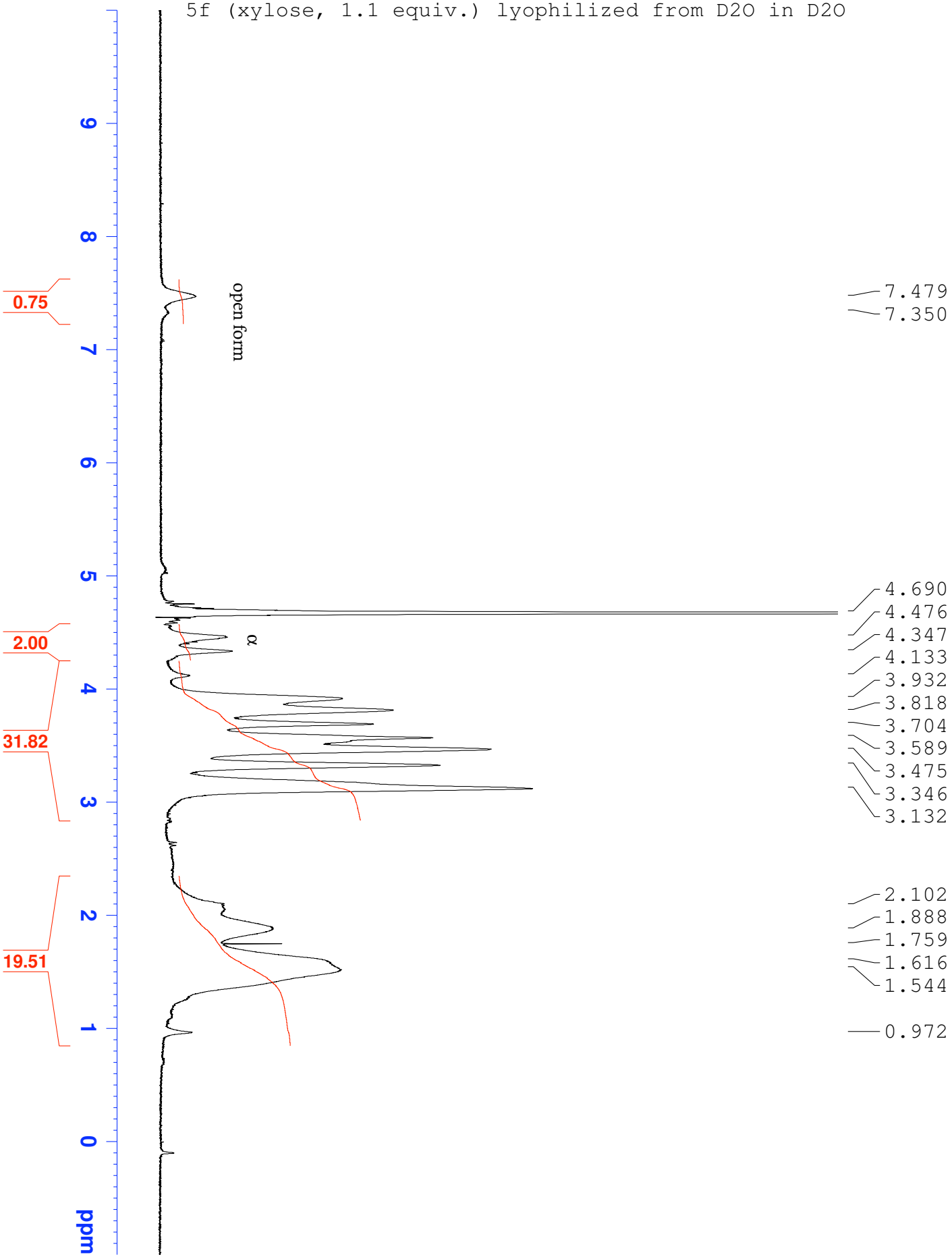

5a (glucose, 1.1 equiv.) lyophilized from D2O in D2O
open form
α
β
0.13
1.00
39.33
20.87
9
8
7
6
5
4
3
2
1
0
ppm
7.503
4.675
4.527
3.990
3.806
3.576
3.407
3.253
3.161
2.118
1.949
1.765
1.642
1.519
1.166
0.982


5b (galactose, 1.1 equiv.) lyophilized from D2O in D2O
open form
α
0.25
1.00
30.47
15.97
7.579
4.675
4.575
4.112
3.925
3.848
3.782
3.616
3.561
3.385
3.186
2.117
1.929
1.753
1.555
0.981
ppm

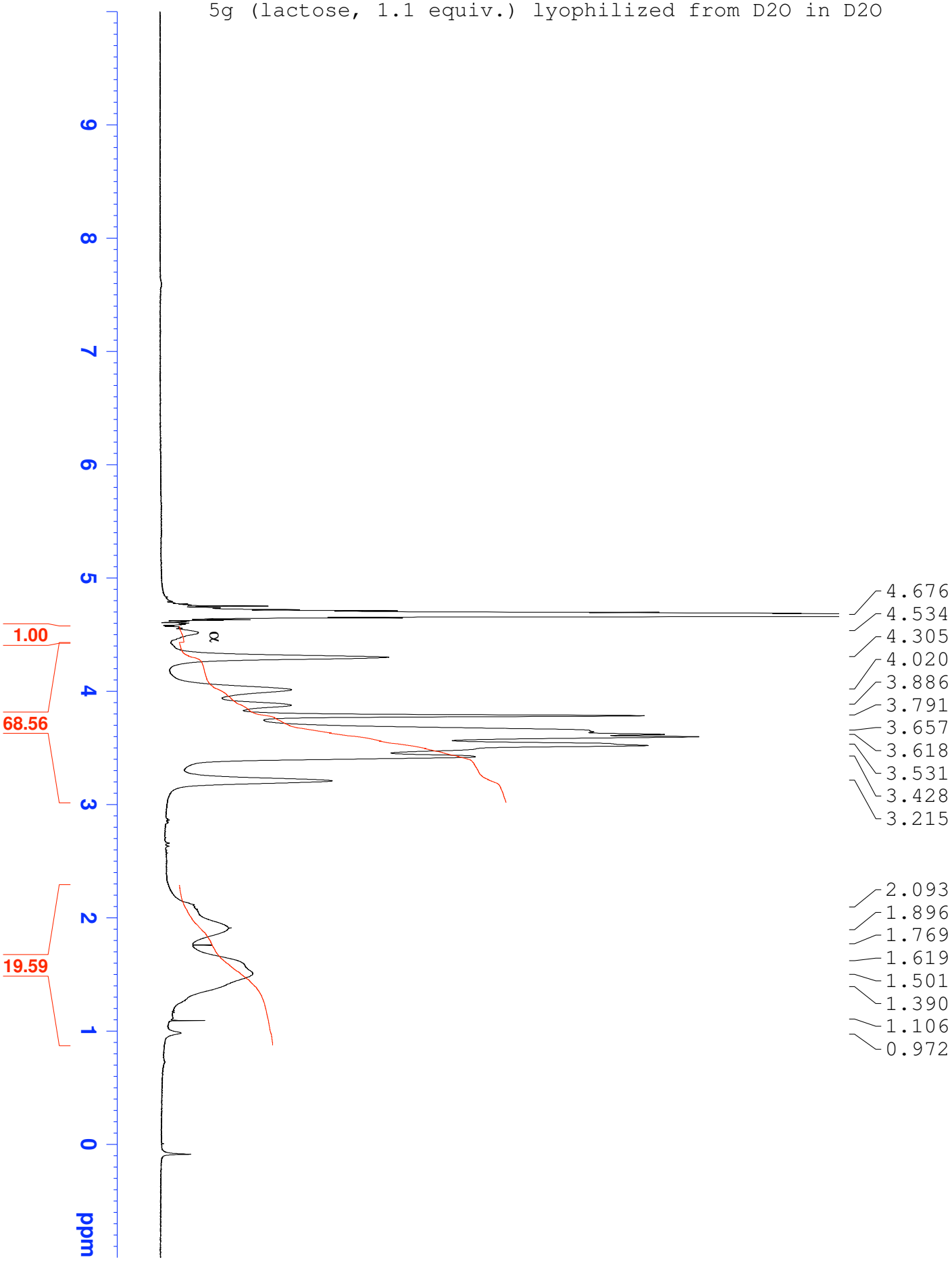

5c (mannose, 1.1 equiv.) lyophilized from D2O in D2O
open form
α
7.567
7.403
4.686
4.463
4.134
3.910
3.746
3.642
3.582
3.522
3.388
3.299
2.075
1.910
1.761
1.507
1.373
0.970
0.30
1.00
3.29
25.52
16.43
ppm

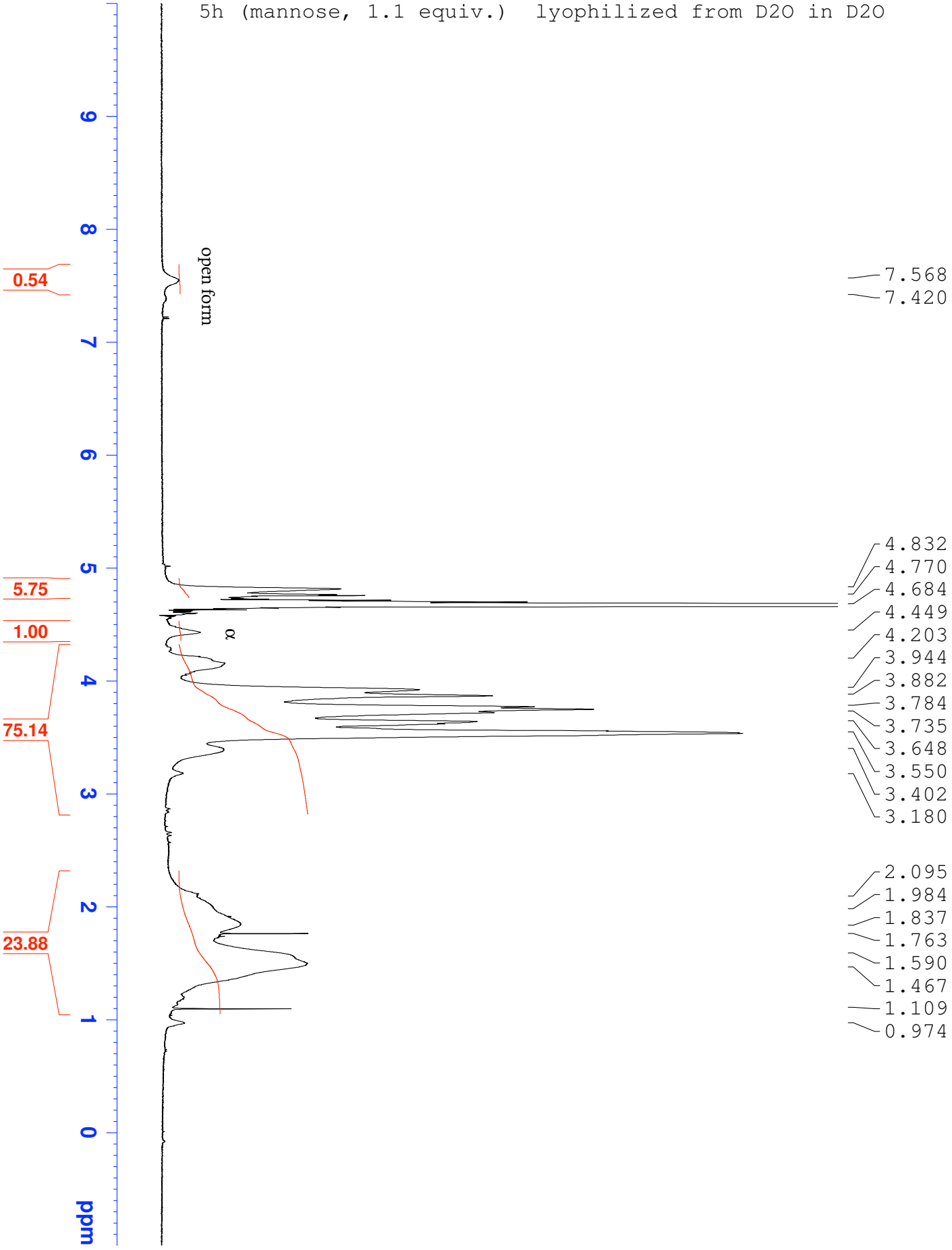

5d (fucose, 1.1 equiv.) lyophilized from D2O in D2O
open form
α
0.22
1.00
20.77
30.71
7.579
4.690
4.504
4.290
4.204
3.918
3.632
3.561
3.346
3.189
2.131
1.945
1.759
1.659
1.544
1.115
ppm

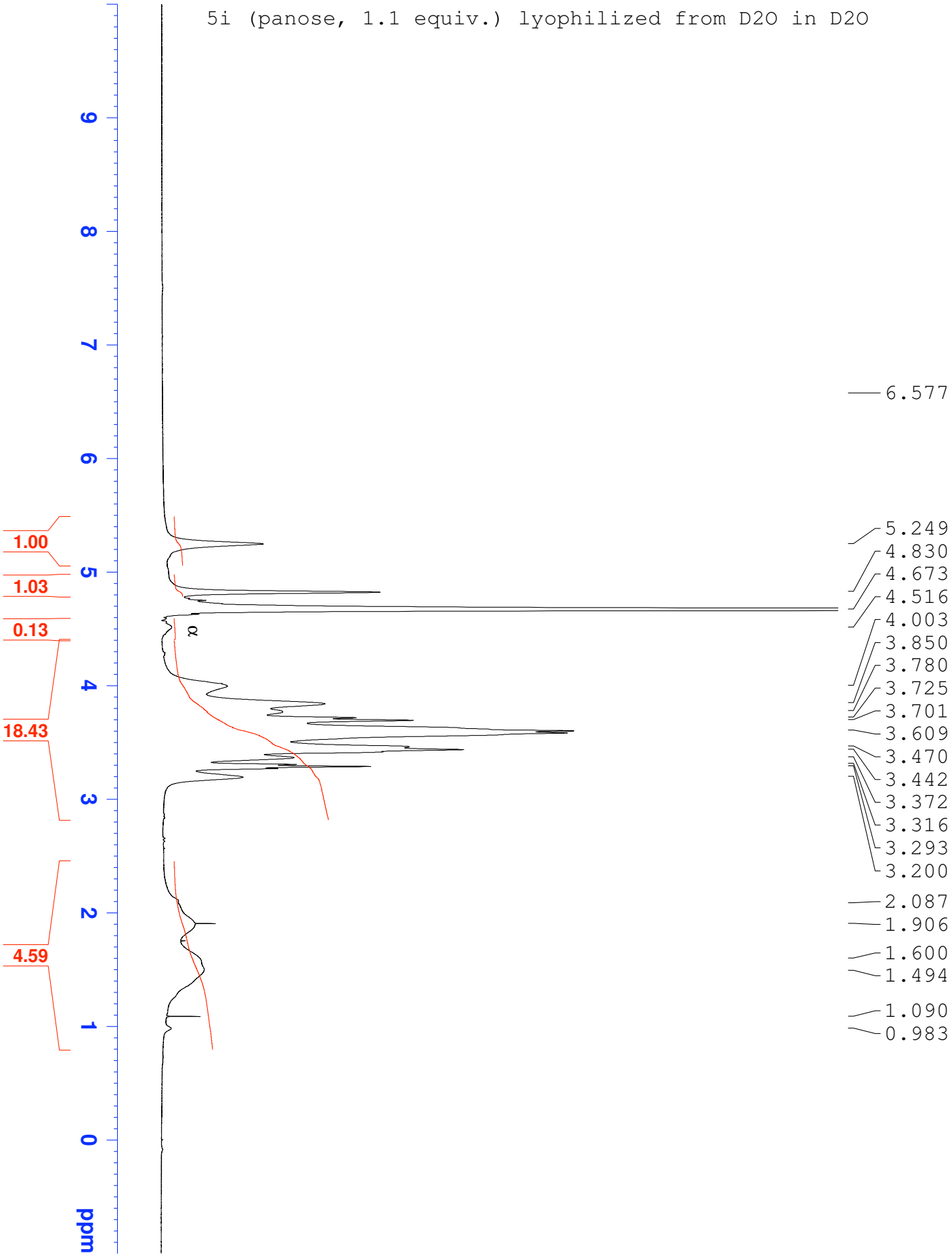

5e (rhamnose, 1.1 equiv.) lyophilized from D2O in D2O
open form
α
0.27
1.00
17.68
24.75
9
8
7
6
5
4
3
2
1
0
ppm
7.550
4.690
4.376
4.104
3.889
3.746
3.589
3.446
3.317
3.217
2.131
1.902
1.587
1.487
1.187

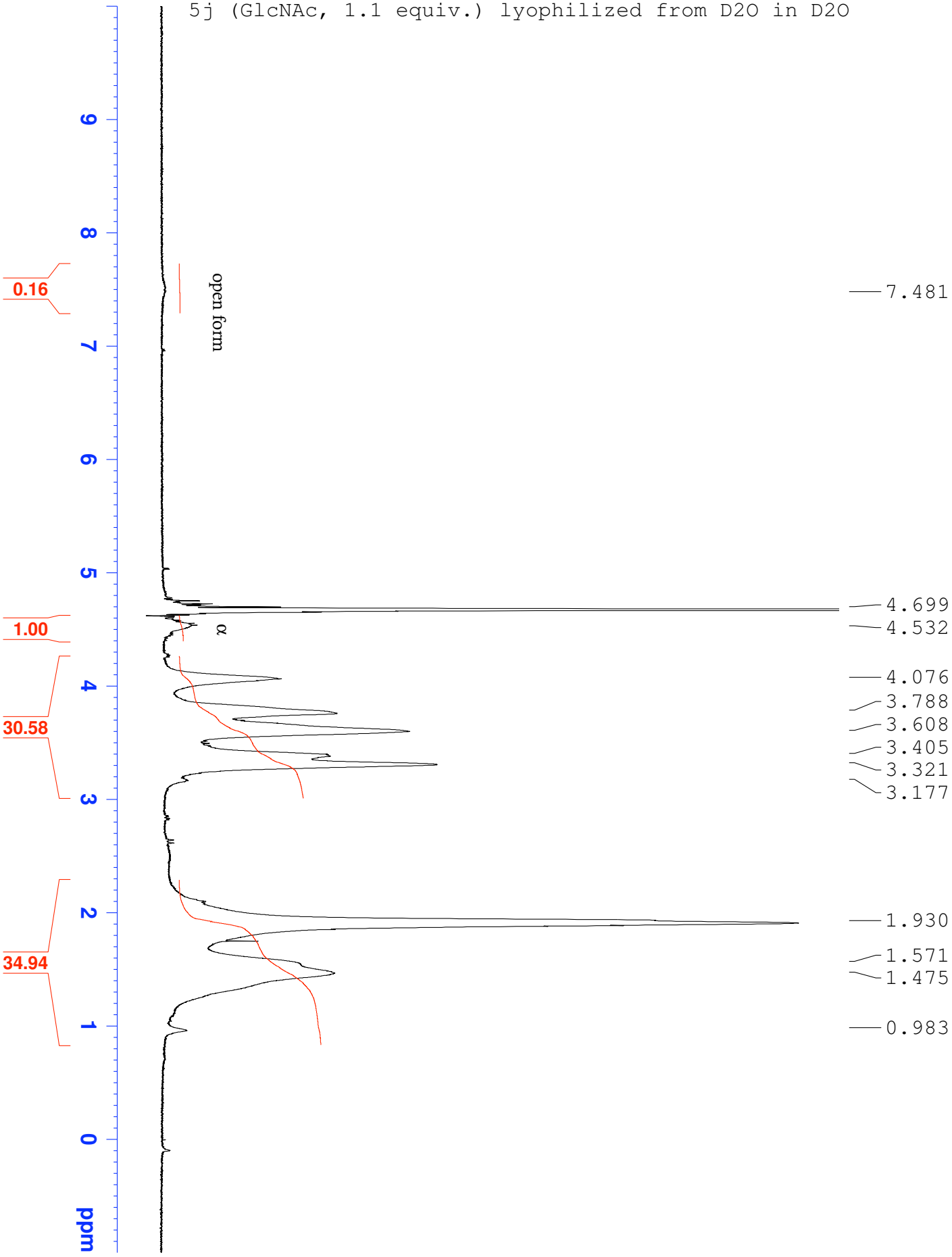

5f (xylose, 1.1 equiv.) lyophilized from D2O in D2O
open form
α
0.75
2.00
31.82
19.51
ppm
7.479
7.350
4.690
4.476
4.347
4.133
3.932
3.818
3.704
3.589
3.475
3.346
3.132
2.102
1.888
1.759
1.616
1.544
0.972

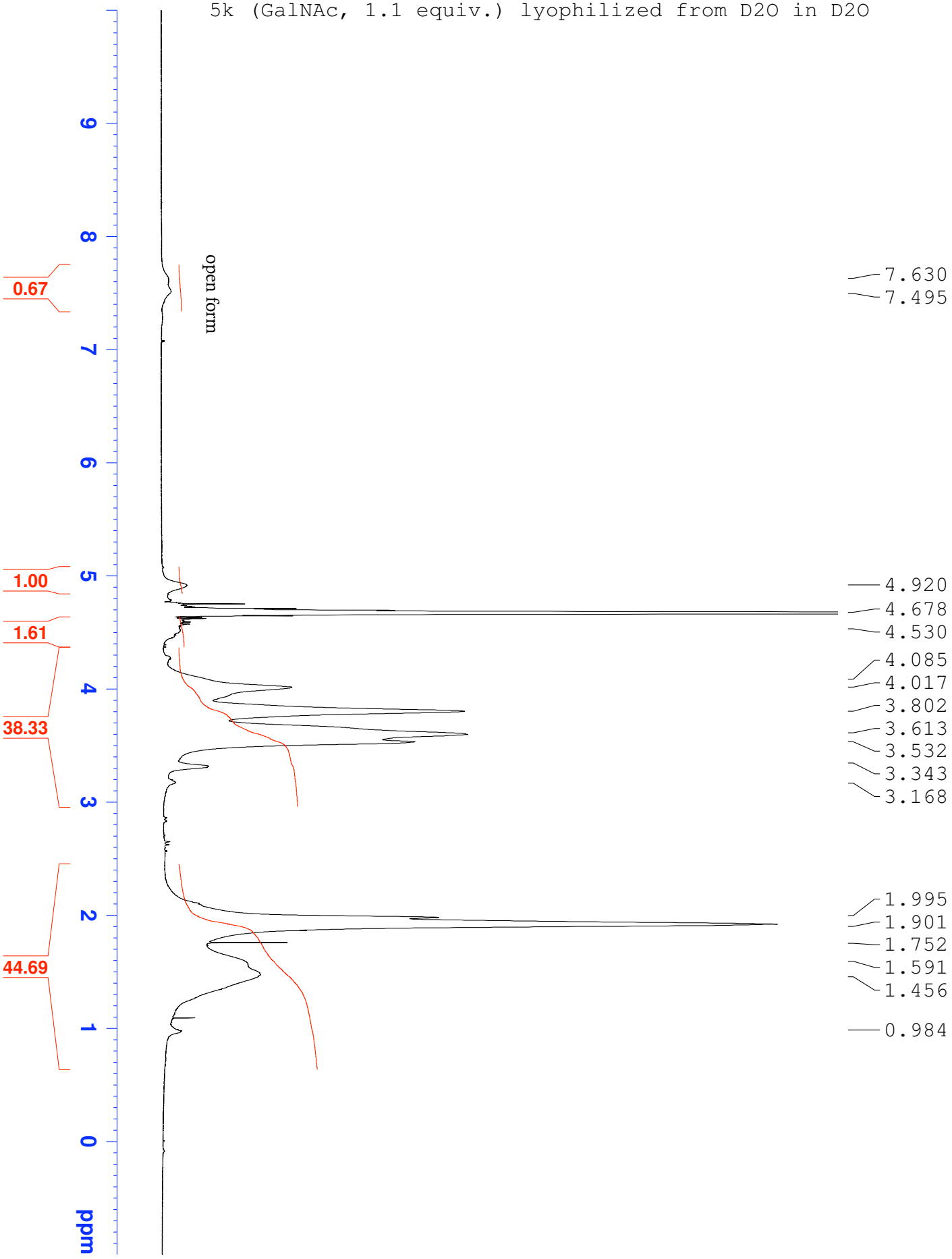
5g (lactose, 1.1 equiv.) lyophilized from D2O in D2O

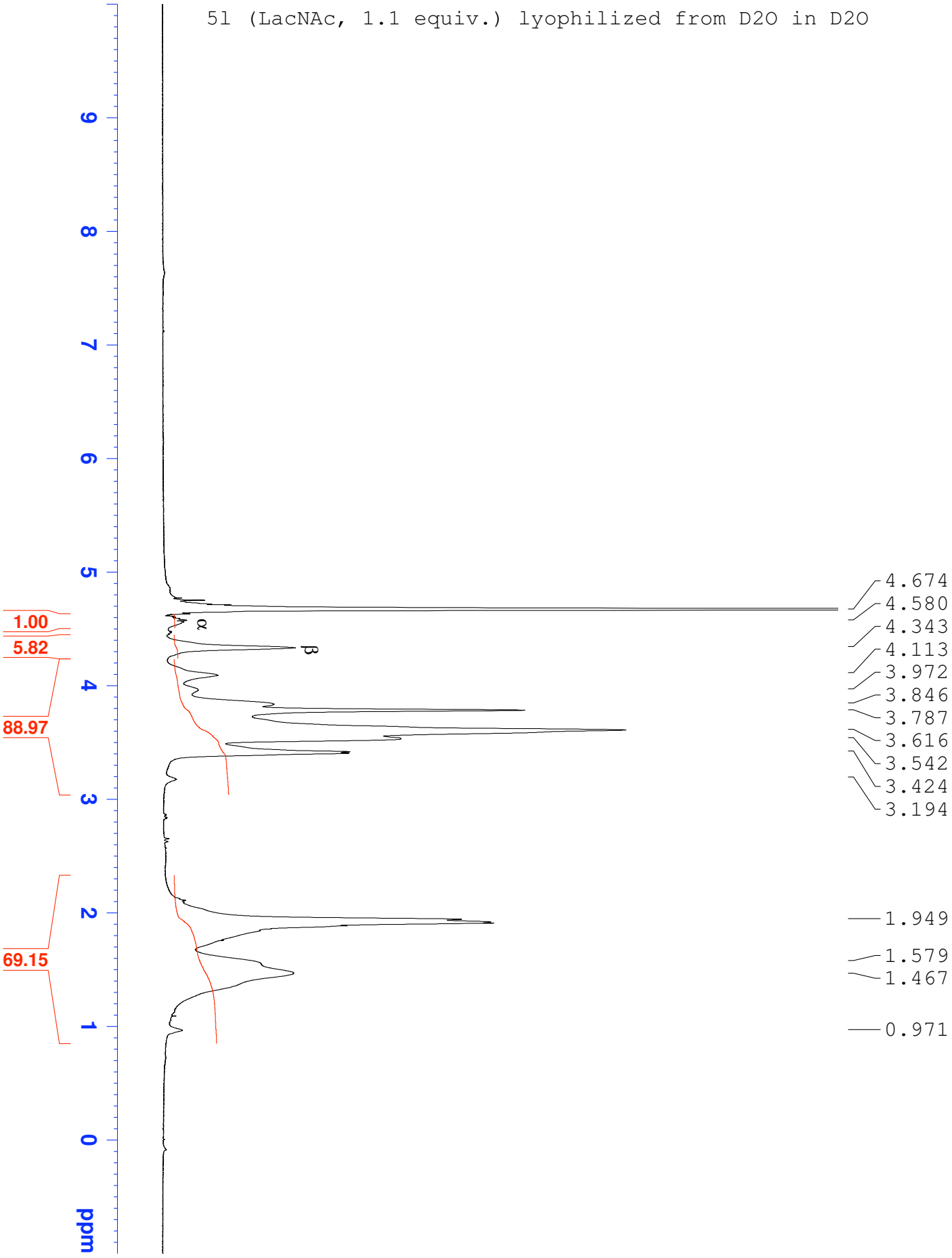


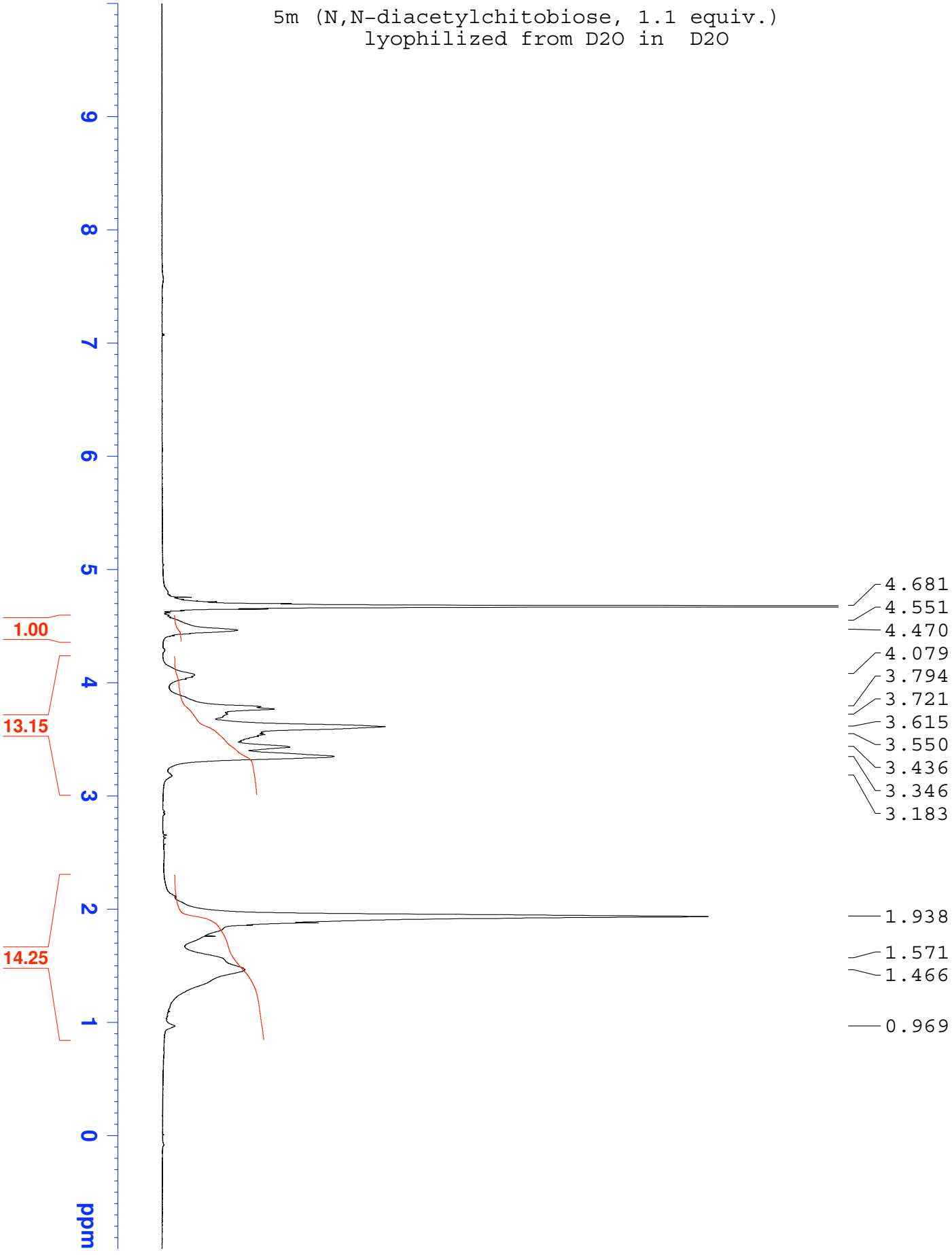

5h (mannose, 1.1 equiv.) lyophilized from D2O in D2O
open form
α
0.54
5.75
1.00
75.14
23.88
ppm
9
8
7
6
5
4
3
2
1
0
7.568
7.420
4.832
4.770
4.684
4.449
4.203
3.944
3.882
3.784
3.735
3.648
3.550
3.402
3.180
2.095
1.984
1.837
1.763
1.590
1.467
1.109
0.974

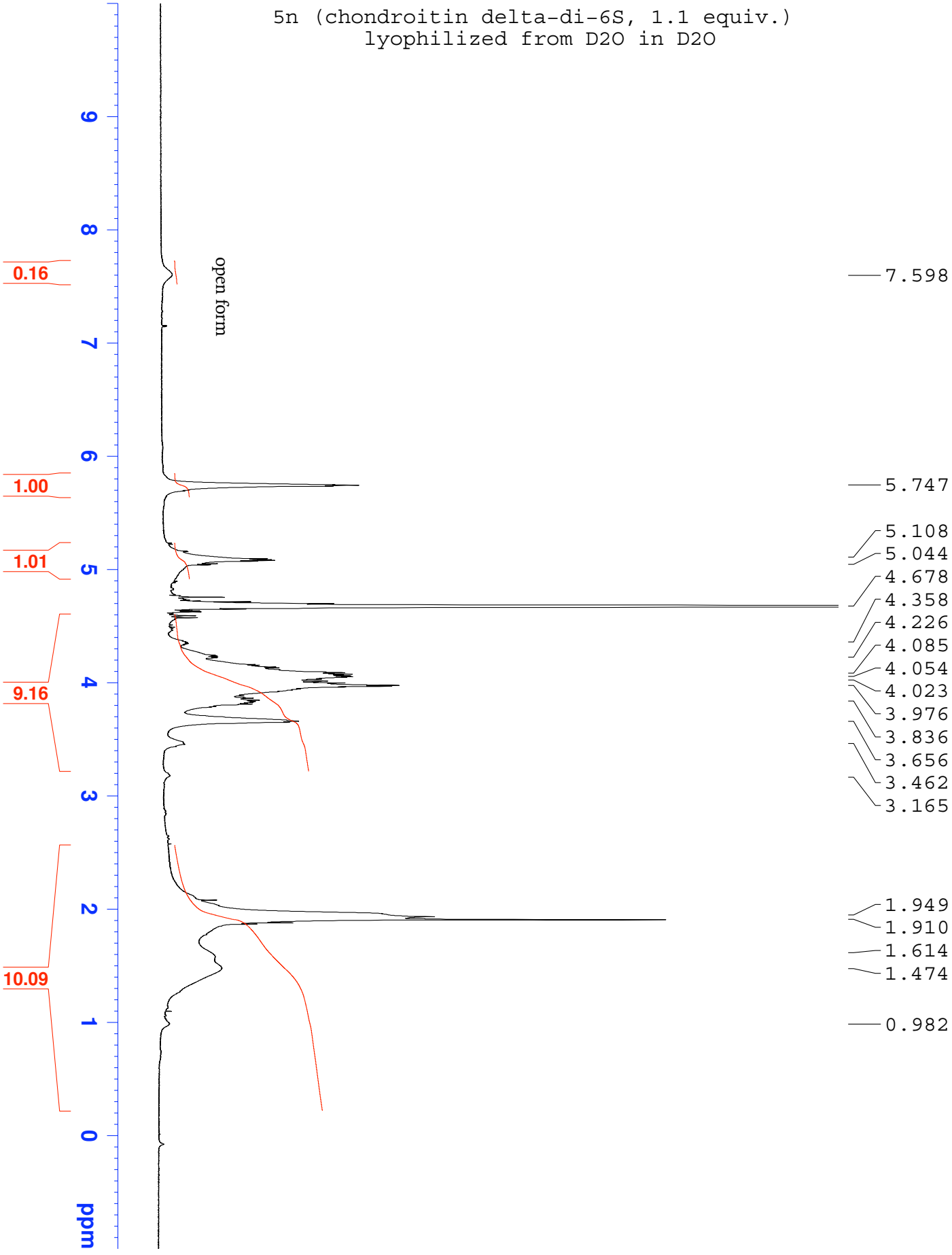

5i (panose, 1.1 equiv.) lyophilized from D2O in D2O
6.577
5.249
4.830
4.673
4.516
4.003
3.850
3.780
3.725
3.701
3.609
3.470
3.442
3.372
3.316
3.293
3.200
2.087
1.906
1.600
1.494
1.090
0.983
1.00
1.03
0.13
18.43
4.59
ppm

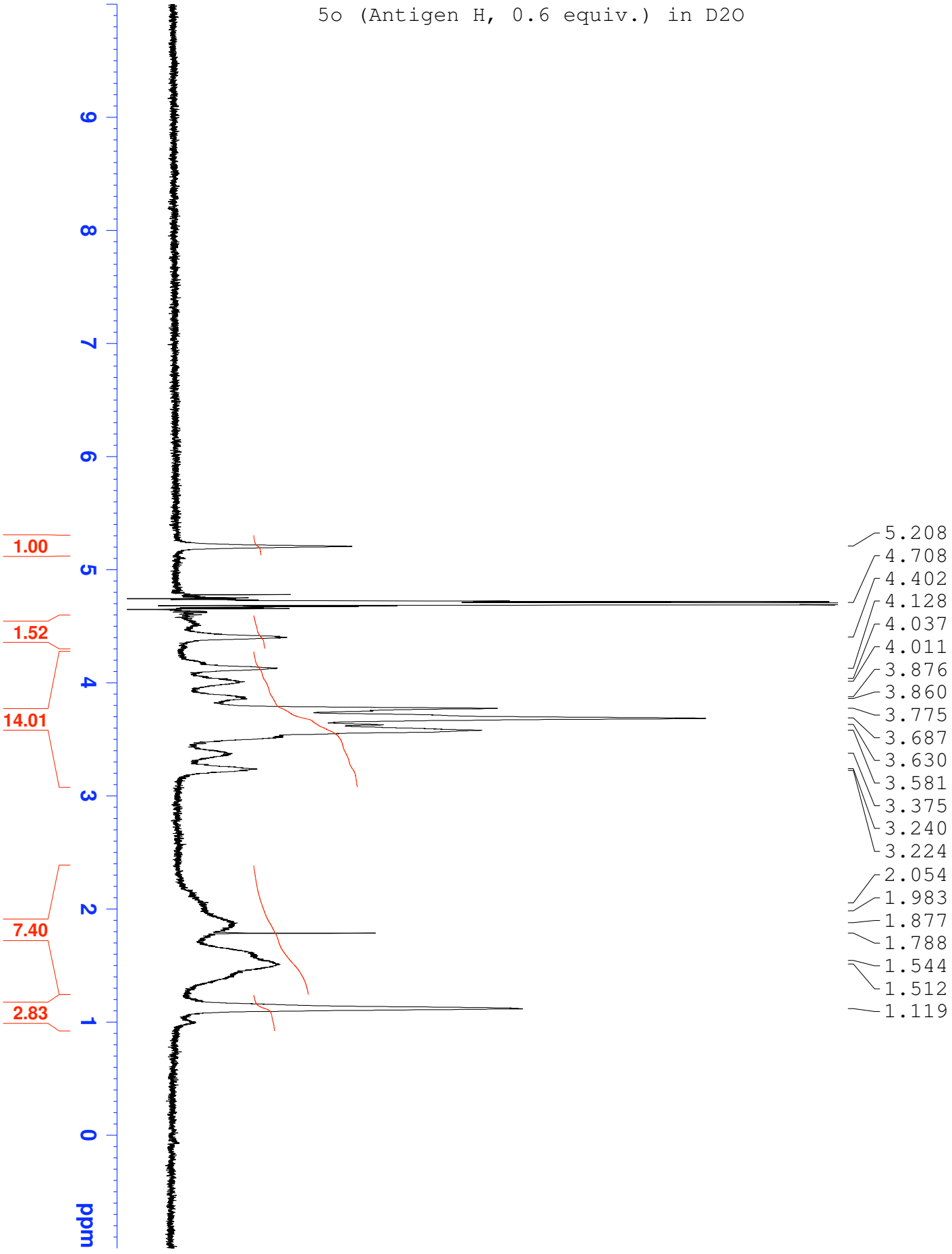

5j (GlcNAc, 1.1 equiv.) lyophilized from D2O in D2O
open form
α
7.481
4.699
4.532
4.076
3.788
3.608
3.405
3.321
3.177
1.930
1.571
1.475
0.983
0.16
1.00
30.58
34.94
ppm

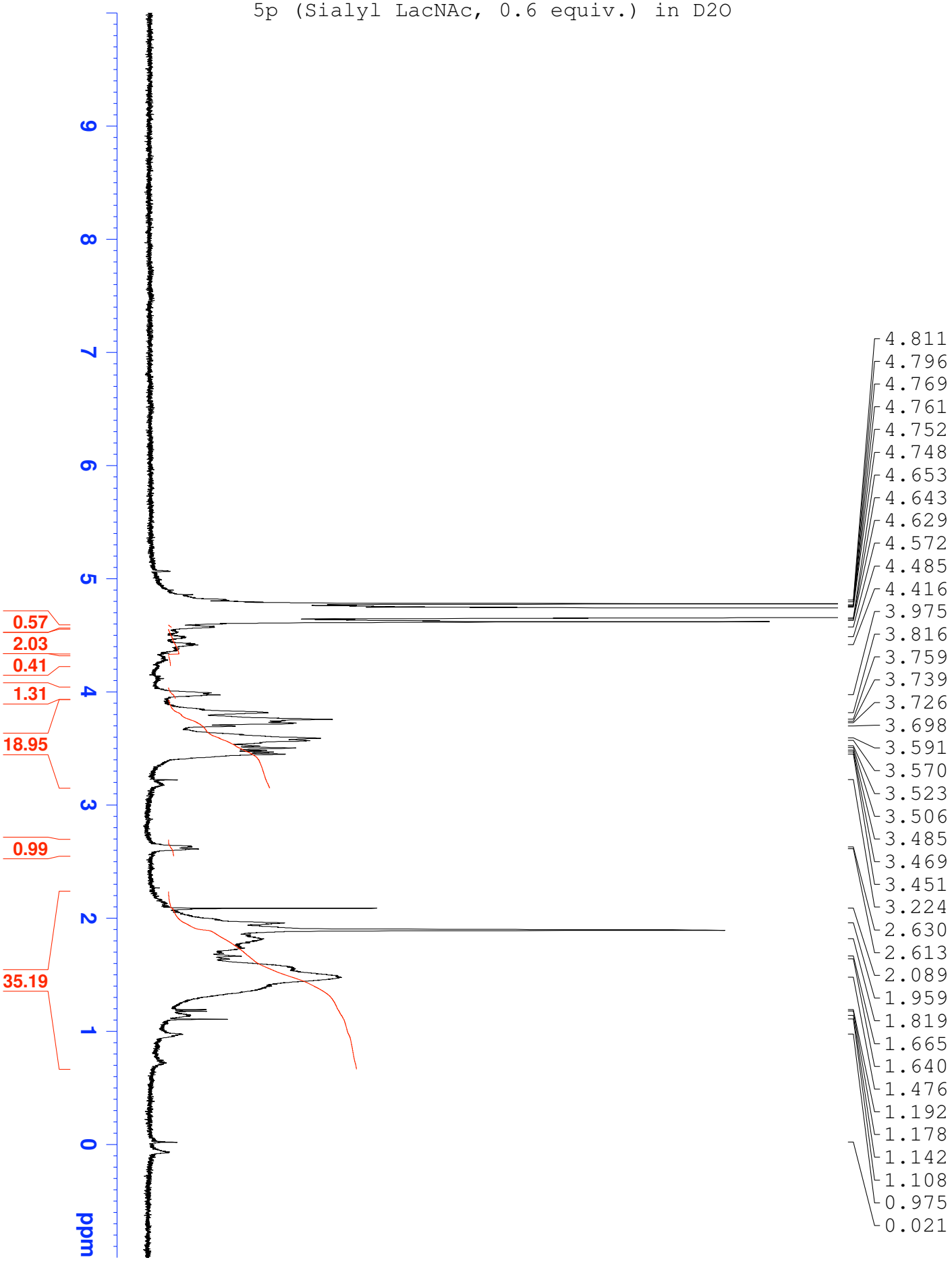

5k (GalNAc, 1.1 equiv.) lyophilized from D2O in D2O
open form
0.67
1.00
1.61
38.33
44.69
9
8
7
6
5
4
3
2
1
0
ppm
7.630
7.495
4.920
4.678
4.530
4.085
4.017
3.802
3.613
3.532
3.343
3.168
1.995
1.901
1.752
1.591
1.456
0.984


5l (LacNAc, 1.1 equiv.) lyophilized from D2O in D2O
9
8
7
6
5
4
3
2
1
0
ppm
1.00
5.82
88.97
69.15
α
β
4.674
4.580
4.343
4.113
3.972
3.846
3.787
3.616
3.542
3.424
3.194
1.949
1.579
1.467
0.971

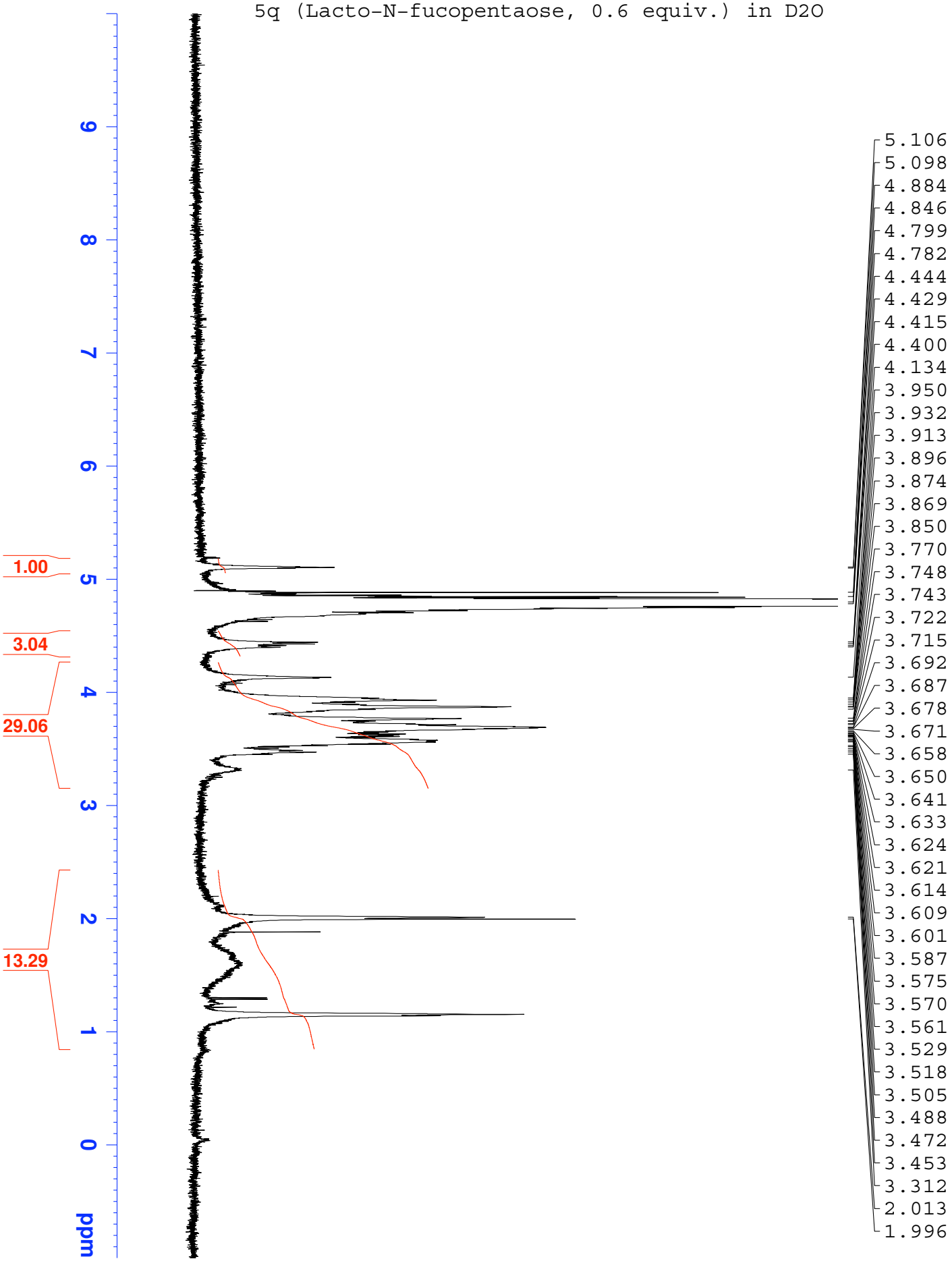

5m (N,N-diacetylchitobiose, 1.1 equiv.)
lyophilized from D2O in D2O
ppm
0
1
2
3
4
5
6
7
8
9
1.00
13.15
14.25
4.681
4.551
4.470
4.079
3.794
3.721
3.615
3.550
3.436
3.346
3.183
1.938
1.571
1.466
0.969


5n (chondroitin delta-di-6S, 1.1 equiv.)
lyophilized from D2O in D2O
open form
0.16
1.00
1.01
9.16
10.09
7.598
5.747
5.108
5.044
4.678
4.358
4.226
4.085
4.054
4.023
3.976
3.836
3.656
3.462
3.165
1.949
1.910
1.614
1.474
0.982
9
8
7
6
5
4
3
2
1
0
ppm

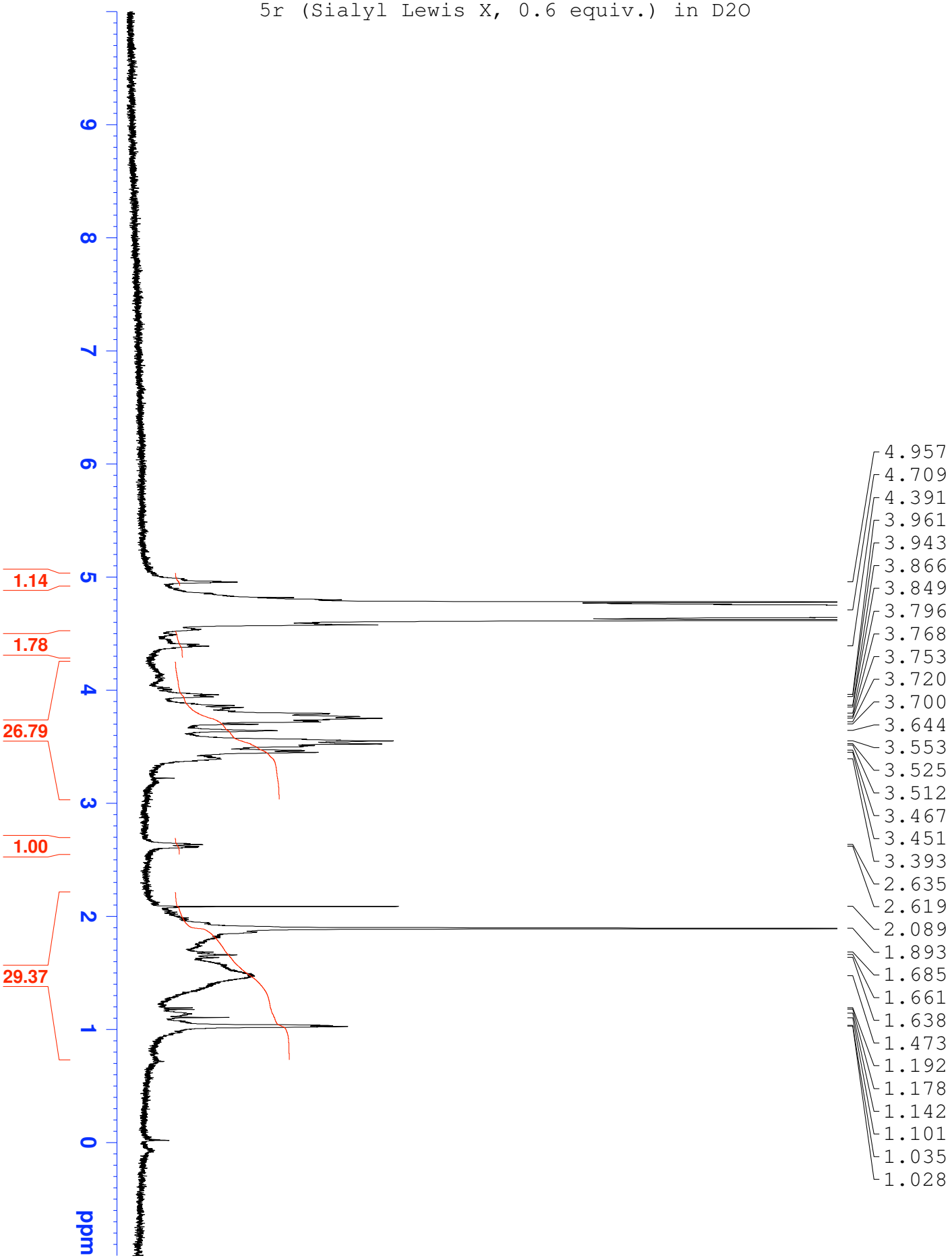

5o (Antigen H, 0.6 equiv.) in D2O
1.00
1.52
14.01
7.40
2.83
ppm
5.208
4.708
4.402
4.128
4.037
4.011
3.876
3.860
3.775
3.687
3.630
3.581
3.375
3.240
3.224
2.054
1.983
1.877
1.788
1.544
1.512
1.119


5p (Sialyl LacNAc, 0.6 equiv.) in D2O
9
8
7
6
5
4
3
2
1
0
ppm
0.57
2.03
0.41
1.31
18.95
0.99
35.19
4.811
4.796
4.769
4.761
4.752
4.748
4.653
4.643
4.629
4.572
4.485
4.416
3.975
3.816
3.759
3.739
3.726
3.698
3.591
3.570
3.523
3.506
3.485
3.469
3.451
3.224
2.630
2.613
2.089
1.959
1.819
1.665
1.640
1.476
1.192
1.178
1.142
1.108
0.975
0.021

5q (Lacto-N-fucopentaose, 0.6 equiv.) in D2O



5r (Sialyl Lewis X, 0.6 equiv.) in D2O



IV. References:

(1)Park, S.; Lee, M.-R., Shin, I. *Nature Protocols* **2007**, *2*, 2747-2758.
(2) Lai, J. T.; Filla, D.; Shea, R. *Macromolecules* **2002**, *35*, 6754-6756.
(3) Bottari, P.; Aebersold, R.; Turecek, F.; Gelb, M. H. *Bioconjugate Chem.* **2004**, *15*, 380-388.
(4) Metz, N.; Theato, P. *Eur. Pol. J.* **2007**, *43*, 1202-1209.