Supplementary material

Characterization of Danaparoid complex extractive drug by an orthogonal analytical approach

Cristina Gardini [1], Elena Urso [2], Marco Guerrini [2], René van Herpen [3], Pauline de Wit [3] and Annamaria Naggi [2,*]

[1] Centro Alta Tecnologia Istituto di Ricerche Chimiche e Biochimiche G. Ronzoni S.r.l., via G. Colombo 81, Milan, 20133, Italy; gardini@cat-ronzoni.it (C.G.)
[2] Istituto di Ricerche Chimiche e Biochimiche G. Ronzoni S.r.l., via G. Colombo 81, Milan, 20133, Italy; urso@ronzoni.it (E.U.); guerrini@ronzoni.it (M.G.); naggi@ronzoni.it (A.N.)
[3] Aspen Oss B.V., Kloosterstraat 6, 5349 AB Oss, Netherlands; rvanherpen@nl.aspenpharma.com (R.v.H.); pdewit@nl.aspenpharma.com (P.d.W.)
* Correspondence: naggi@ronzoni.it; Tel.: +39 02 70641626

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Table S1. Abbreviations of assigned fragments.

Residue	Abbreviation
H2/C2 N-sulfated glucosamine	ANS
H2/C2 3-O,N-sulfated glucosamine	A2*
H2/C2 N-acetyl glucosamine	ANAc
H6/C6 6-O-unsulfated glucosamine	A6OH
H6/C6 6-O-sulfated glucosamine	A6S
H1/C1 N-sulfated glucosamine *linked to* β-D-glucuronic acid	ANS-(G)
H1/C1 3-O,N-sulfated glucosamine	A1*
H1/C1 N-sulfated glucosamine *linked to* 2-O-sulfo α-L-iduronic acid+ H1/C1 N-acetyl glucosamine *linked to* β-D-glucuronic acid	Atot
H1/C1 N-sulfated glucosamine *linked to* α-L-iduronic acid	ANS-(I)
H1/C1 N-acetyl glucosamine *linked to* α-L-iduronic acid	ANAc-(Ix)
H1/C1 reducing N-sulfated α-D-glucosamine + H1/C1α-D-glucosamine	ANSαred+ANH_2
H1/C1 reducing N-acetyl α-D- glucosamine	ANAcαred
H1/C1 reducing N-sulfated β-D-glucosamine	Aβred
H1/C1 2-O-sulfo α-L-iduronic acid	I2S
H1/C1 α-L-iduronic acid *linked to* H6/C6 N sulfate /acetylated 6-O-sulfated α-D-glucosamine	I-(A6S)
H1/C1 α-L-iduronic acid *linked to* H6/C6 of N sulfate /acetylated α-D-glucosamine	I-(A6OH)
H1/C1 β-D-glucuronic acid *linked to* N-sulfated α-D-glucosamine	G-(ANS)
H1/C1 β-D-glucuronic acid *linked to* N-acetyl α-D-glucosamine	G-(ANAc)
Oxidized N-acetylglucosamine	ANAc-ox
H1/C1 β-D-glucuronic acid *linked to* 4-O-sulfo-N-acetylgalactosamine	G-(GalNAc,4S)
H1/C1 β-D-glucuronic acid *linked to* 6-O-sulfo-N-acetylgalactosamine	G-(GalNAc,6S)
H1/C1 α-L-iduronic acid *linked to* β-N-acetylgalactosamine	I_DS
H1/C1 2-O-sulfo α-L-iduronic acid *linked to* β-N-acetylgalactosamine	I2S_DS
H2/C2 β-N-acetylgalactosamine (DS+CS)	GalNAc
H1/C1 α-L-iduronic acid *linked to* Oxidized N-acetylglucosamine	I-(ANAcox)
Oxidized N-acetyl galactosamine	GalNAc-ox
Oxidized HS ox1	ox1
Oxidizex HS ox2	ox2
Non reducing N- acetyl galactosamine	GalNAc_NR

Table S2. Results summary table regarding CS/DS fractions: assignment of main signals.

Peak N.	Experimental m/z (z)	MW	Assigned composition
1	903.7(-2)	1809.4	-
	634.6(-2)	1271.2	A6,2,3(Ra)
2	991.7(-2)	1985.4	-
3	910.7(-2)	1823.4	A7,4,4(T1)
4	968.7(-2)	1939.4	A8,4,4(Ra)
5	1056.7(-2)	2115.4	-
	1285.8(-2)	2573.6	-
6	1204.8(-2)	2411.6	A9,5,5(T1)
7	1262.8(-2)	2527.6	A10,5,5(Ra)
8	1415.4(-2)	2832.8	-
9	1563.5(-2)	3129.0	A11,6,6(T1)
10	951.5(-3)	2857.5	-
11	1195.0(-3)	3588.0	A13,7,7(T1)
12	1233.7(-3)	3704.1	A14,7,7(Ra)
13	1304.9(-3)	3917.7	-
14	1429.7(-3)	4292.1	A16,8,8(Ra)
15	1587.1(-3)	4764.3	A17,9,9(T1)
16	1625.8(-3)	4880.4	A18,9,9(Ra)
17	1783.2(-3)	5352.6	A19,10,10(T1)
18	1821.9(-3)	5468.7	A20,10,10(Ra)
19	1451.9(-4)	5811.6	A21,11,11(T1)
20	1448.7(-4)	5798.8	A22,11,11(Ra)
21	1598.9(-4)	6399.6	A23,12,12(T1)
22	1628.2(-4)	6516.8	A24,12,12(Ra)
23	1746.5(-4)	6990	A25,13,13(T1)
24	1775.3(-4)	7105.2	A26,13,13(Ra)
25	1893.6(-4)	7578.4	A27,14,14(T1)
26	1922.6(-4)	7694.4	A28,14,14(Ra)
27	2069.6(-4)	8282.4	A30,15,15(Ra)

Table S3. Comparison of the main mass signals between Danaparoid CAT272 and its HS fraction.

HS-Danaparoid			CAT272		
Peak N.	Experimental m/z	Assigned composition	Peak N.	Experimental m/z	Assigned composition
1	568.6062	A5,2,3*	-		
2	647.6176	ΔU6,2,3*	-		
3	648.5645	A5,4,3*	-		
4	695.5908	ΔU6,3,3(T1)*	a	625.5269	A5,5,0
5	727.5733	ΔU6,4,3*	-		
6	713.6211	ΔU7,2,3(Ra)*	b	505.0112	U4,4,0
7	614.5498	A5,4,1(T1)	c	614.5497	A5,4,1(T1)
8	702.5671	U6,4,1(T1)	d	702.5654	U6,4,1(T1)
9	654.529	A5,5,1(T1)	e	654.5277	A5,5,1(T1)
10	742.5434	U6,5,1(T1)	f	742.5419	U6,5,1(T1)
11	967.6087	A7,7,1(T1)	g	967.6096	A7,7,1(T1)
12	1055.6233	U8,7,1(T1)	h	1055.627	U8,7,1(T1)
13	1072.1597	A7,8,1(T1)	i	1072.1602	A7,8,1(T1)
14	1160.1763	U8,8,1(T1)	l	1160.1752	U8,8,1(T1)
15	1176.7109	A7,9,1(T1)	m	1176.7131	A7,9,1(T1)

* These components could be CS/DS fragments not completely separated from the HS fraction.

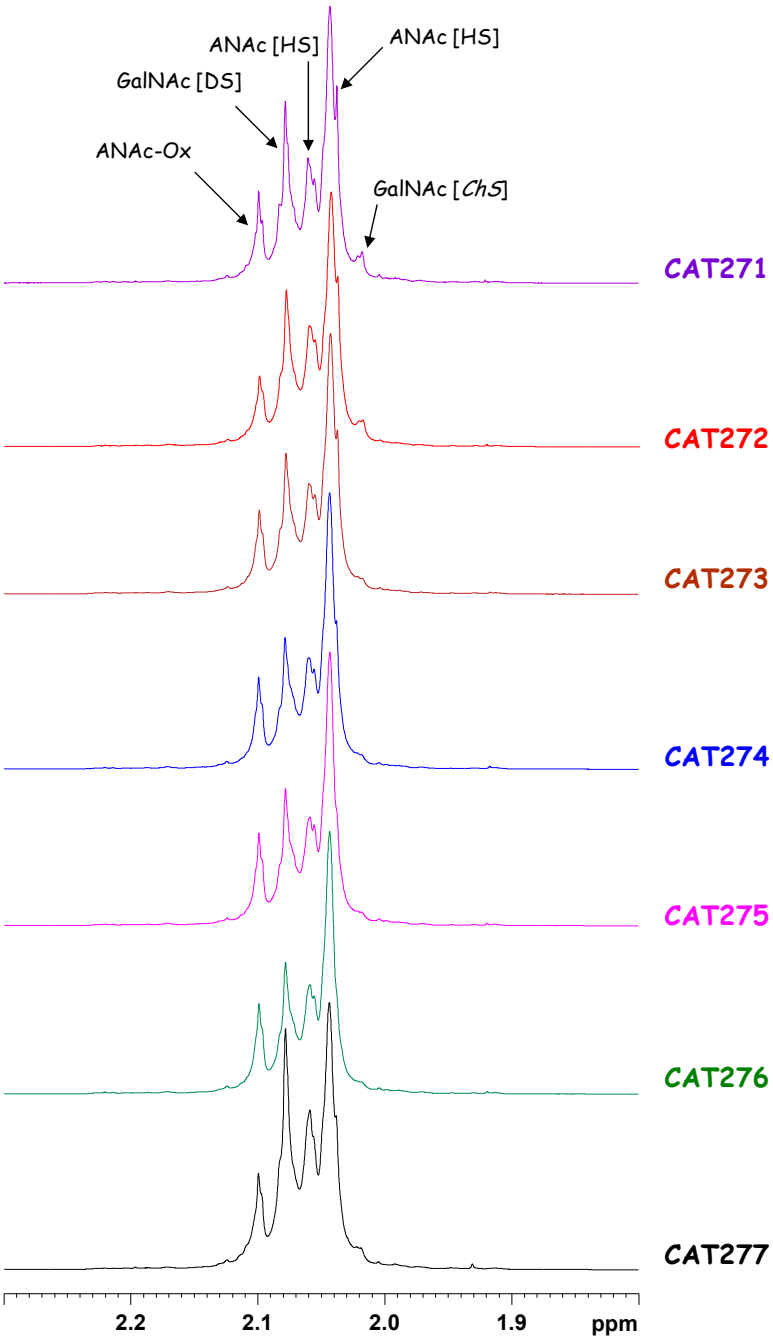


Figure S1. ^{1}H NMR spectrum of a Danaparoid samples: expansion of the acetyl region.

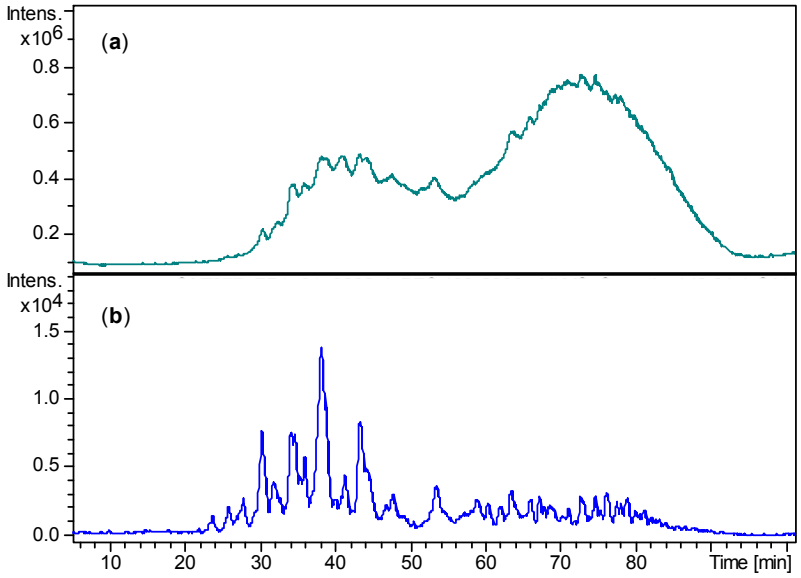


Figure S2. LC-MS chromatograms of a Danaparoid sample CAT277, the LC method was composed by an initial isocratic step followed by linear gradient: (a) Total Ion Chromatogram (TIC); (**b**) Base Peak Chromatogram (BPC).

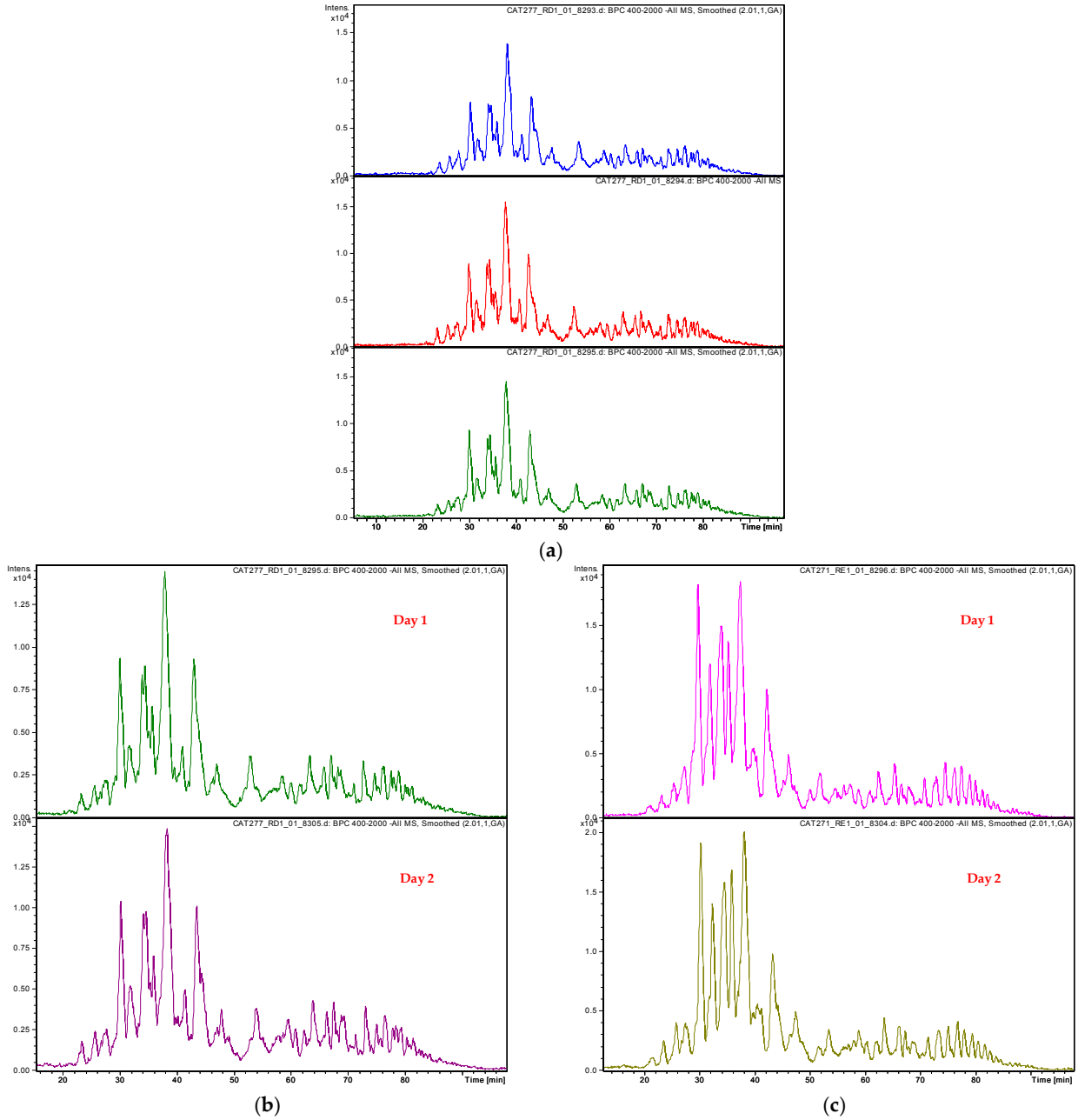


Figure S3. LC-MS chromatograms: (a) repeatability of CAT277; (b) interday precision of CAT277; (c) interday precision of CAT271.

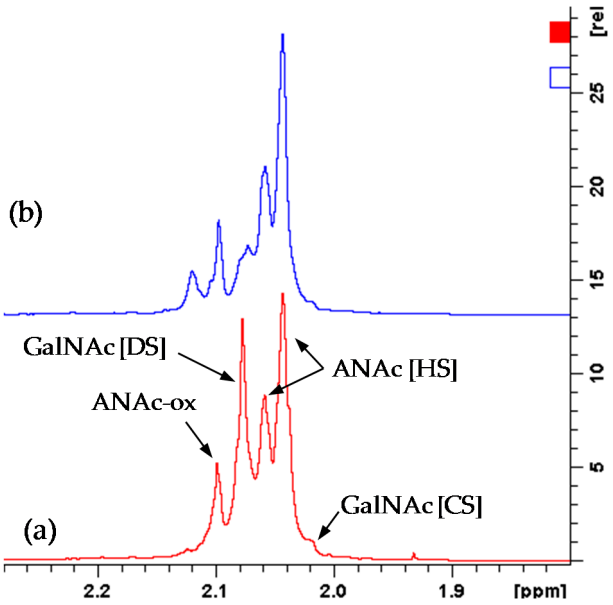
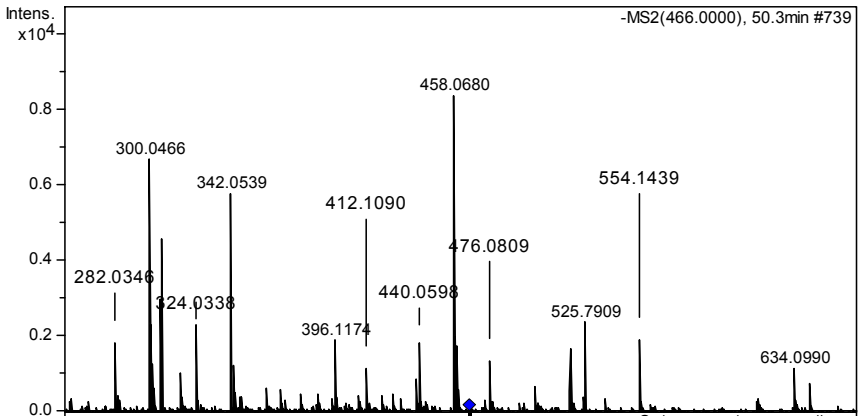


Figure S4. Acetyl region of proton spectra: comparison between (**a**) one Danaparoid sample CAT277; (**b**) its ChABC digestion product CAT469.



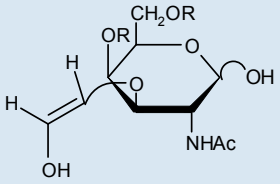
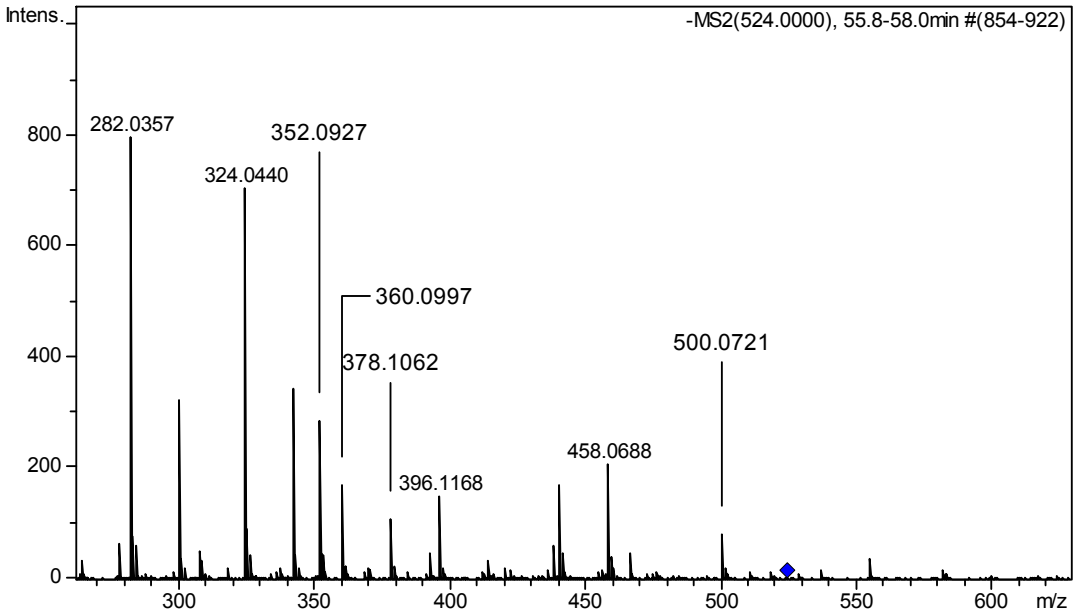
m/z (-z)	M	Structure hypothesis
466.0 (-2)	**934**	**ΔU4,2,2+O**
458.1 (-1)	459	ΔU2,1,1
440.0 (-1)	441	ΔU2,1,1(– H_2O)
412.1 (-1)	413	**U-A_{NAc} +O**
396.1 (-1)	397	U-A_{NAc}
342.1 (-1)	343	 where R = SO_3, H
316.0 (-1)	317	**$A_{NAc, S}$ +O**
300.0 (-1)	301	$A_{NAc, S}$
282.0 (-1)	283	$A_{NAc, S}$ (– H_2O)
554.1 (-1)	555	ΔU-A_{NAc}-U
634.1 (-1)	635	ΔU-A_{NAc}-U (+SO_3)

Figure S5. MS/MS spectrum of mass signal at m/z 466.0 (z -2) attributed to ΔU4,2,2(T1) at the Collision Energy (CE) of 25 eV and the assignment of fragments.



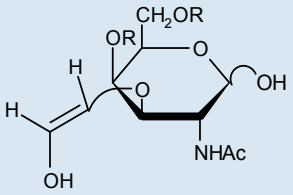
m/z (-z)	M	Structure hypothesis
524.0 (-2)	**1050**	**ΔU4,2,2(+$C_4H_4O_5$)**
500.1 (-1)	501	-
458.1 (-1)	459	ΔU2,1,1
440.0 (-1)	441	ΔU2,1,1(-H_2O)
396.1 (-1)	397	U-A_{NAc}
378.1 (-1)	379	ΔU-A_{NAc}
360.1 (-1)	361	ΔU-A_{NAc} (-H_2O)
352.1 (-1)	353	A_{NAc} (+$C_4H_4O_5$)
342.1 (-1)	343	 where R = SO_3, H
324.1 (-1)	325	-
536.1 (-1)	537	ΔU-A_{NAc}-U(-H_2O)
554.1 (-1)	555	ΔU-A_{NAc}-U

Figure S6. MS/MS spectrum of mass signal at m/z 524.0 (z -2) attributed to ΔU5,2,2(Ra) at the Collision Energy (CE) of 25 eV and the assignment of fragments.

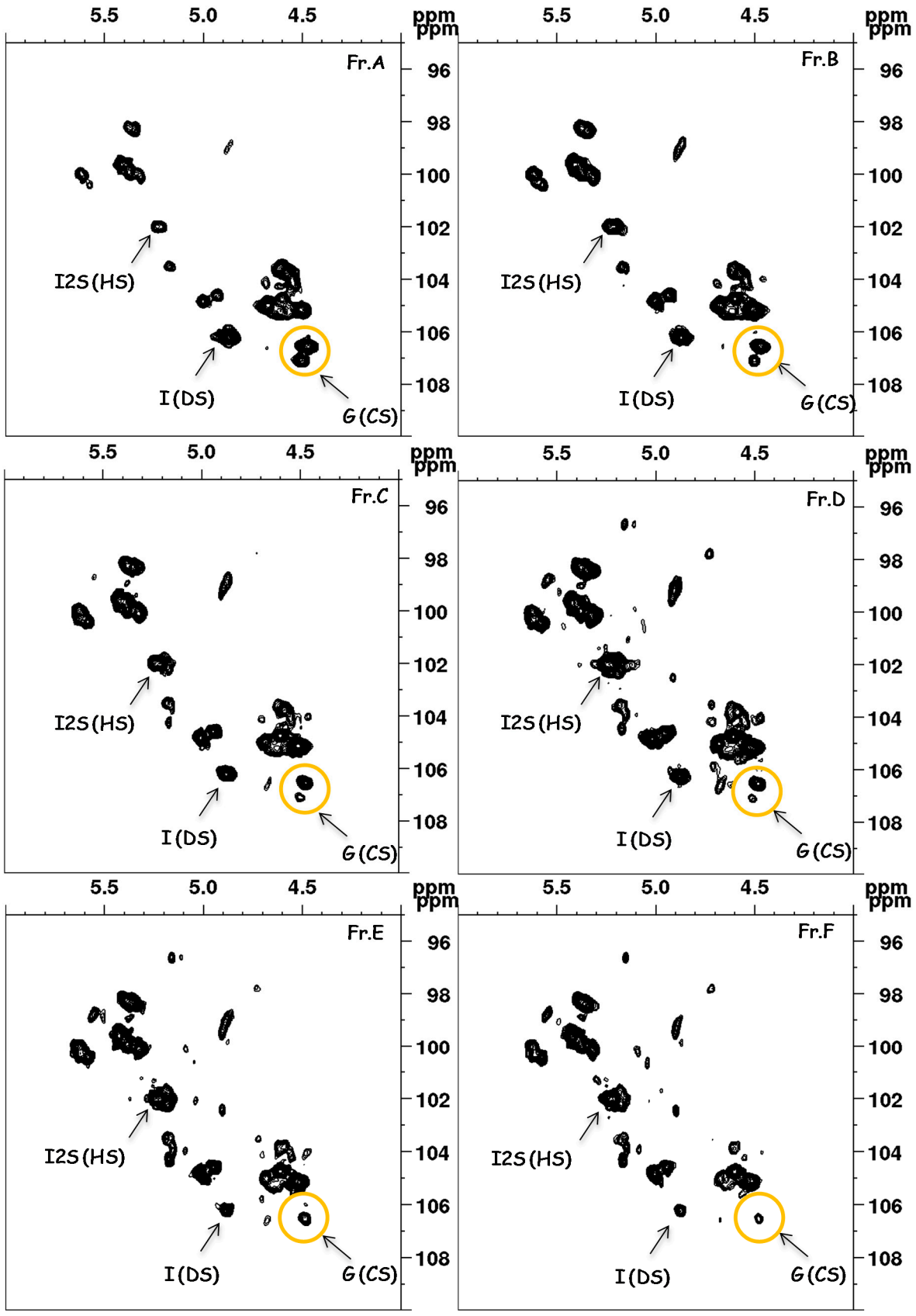


Figure S7(a). Anomeric region of SEC fractions A-F of one Danaparoid sample (CAT272).

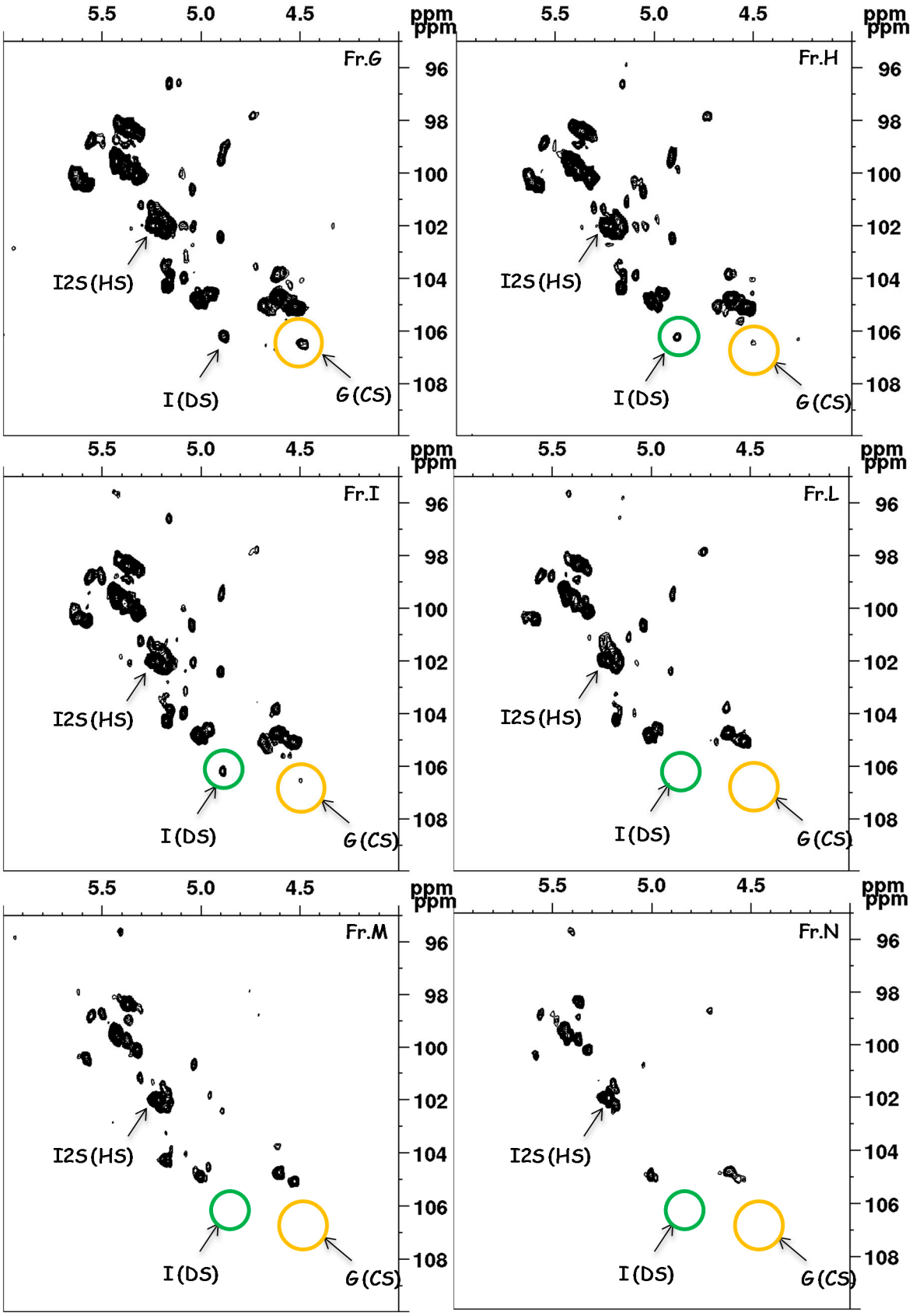


Figure S7(b). Anomeric region of SEC fractions G-N of one Danaparoid sample (CAT272).