A

# APPENDIX A

## Report on NMR Analysis of Enoxaparin Oligosaccharides

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February 20, 2004

BY HAND DELIVERY

Dockets Management Branch (HFA-305)
Food and Drug Administration
Department of Health and Human Services
5630 Fishers Lane, Room 1061
Rockville, Maryland 20852

Re: **03P-0064/SUPP 1**

Ladies and Gentlemen:

On February 12, 2004, on behalf of Aventis Pharmaceuticals Inc. ("Aventis"), we filed a supplement (the "Supplement") to Aventis' Citizen Petition of February 19, 2003 (03P-0064/CP1). We understand that FDA filed the Supplement to that docket as 03P-0064/Supp 1.

The Supplement contains several reports of preclinical studies, included as Appendices A through I. Several pages of those reports bear "confidential" or "strictly confidential" stamps. Please be advised that these stamps should have been removed prior to submission of the Supplement, and are included on those documents in error. Aventis does not consider this material to be confidential at this time. Therefore, on behalf of Aventis, we request that you treat all of the materials accompanying the Supplement as non-confidential material.

Thank you for your attention to this matter. Please do not hesitate to contact me should you have any questions. Thank you.

Sincerely,

Peter O. Safir

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# Full NMR characterization of the Enoxaparin ATIII binding sites:

## NMR characterization of octasaccharides containing the ATIII binding pentasaccharide.

***Results of NMR analysis of pure octasaccharides.***

*(See figures of ANNEX 1)*

*Octasaccharide batch P-32444-19-1 (G4528).*

The proton spectra are shown in figures 1-3 and the signals integration agrees with an octasaccharide structure consisting of GlcN-IdoA-G-ΔU residues in 4:2:1:1 ratio. A weak signal at 5.40 ppm indicates the presence of another monosaccharide residue at the reducing end, as confirmed by the corresponding carbon chemical shift (95.7ppm) (fig.11). The proton chemical shift pattern of each residue was determined by COSY and TOCSY spectra (fig. 4-8) which indicate the presence of the following monosaccharide residues: ΔU, $GlcN_{NS,6S}$, $GlcN_{NS,6S}$red, $GlcN_{NS,3,6S}$, $GlcN_{NAc,6S}$, GlcA and $IdoA_{2S}$ (two residues). Moreover, the signals pattern of the weak anomeric signal at 5.40 ppm agrees with the presence of a reducing $ManN_{NS,6S}$ residue (fig. 8) (about 10%). The HSQC spectrum allowed both carbon chemical shifts measurement and resolution of the overlapping signals detected from homonuclear correlation spectra (fig. 9-11). All proton and carbon chemical shifts are reported in the table at page 16.

The interglycosidic correlations were determined by HMBC (fig. 14, 15) and NOESY (fig.12, 13) experiments, confirming ΔU-$GlcN_{NAc,6S}$-G-$GlcN_{NS,3,6S}$-$I_{2S}$-$GlcN_{NS,6S}$-$I_{2S}$-$GlcN_{NS,6S}$ as the main octasaccharide component. The NOE between H1 of $IdoA_{2S}$ and H4 of $ManN_{NS,6S}$ residues (fig. 13) confirms also the presence of a small amount of ΔU-$GlcN_{NAc,6S}$-G-$GlcN_{NS,3,6S}$-$I_{2S}$-$GlcN_{NS,6S}$-$I_{2S}$-$ManN_{NS,6S}$ octasaccharide. (fig. 17)

*Octasaccharide batch P-32077-80-3 (G4529).*

The proton spectra are shown in figures 18-20 and the signals integration agrees with the presence of the following residues: GlcN, IdoA, G, and ΔU, in 3:1:1:1 ratio. Moreover, four unknown anomeric signals at 5.57, 5.62, 5.36 and 5.34 ppm are present, which integration ratio is 0.53-0.38-0.53-0.38 respectively. A weak signal at 6.00 ppm indicates the presence of a ΔU residue bearing a sulfated group in position two (fig.20). The protons chemical shift pattern of each residue was determined by COSY and TOCSY spectra (fig. 21-24) which provide the presence of the following monosaccharide residues: ΔU, $GlcN_{NS,6S}$, $GlcN_{NS,3,6S}$, $GlcN_{NAc,6S}$, GlcA and three $IdoA_{2S}$. One of the $IdoA_{2S}$ residues corresponds to $IdoA_{2S}$ linked to $GlcN_{NS,6S}$, whereas the others two ($I_{2S}(1)$ and $I_{2S}(2)$) show unusual anomeric chemical shift values (5.36 and 5.34 ppm) (fig. 24). The chemical shifts pattern of the residues showing anomeric signals at 5.57-5.62 ppm agree with 1,6-anhydro-$GlcN_{NS,6S}$ and 1,6-anhydro-$ManN_{NS,6S}$ respectively, according to signal assignments described in the Aventis patent N° 2800074. The HSQC spectrum allowed carbon chemical shifts measurement of all residues including those of the 1,6-anhydro units (fig. 25-27). All proton and carbon chemical shifts are present in the table at page 29.

The interglycosidic correlations were determined by NOESY experiment (fig.28), confirming ΔU-$GlcN_{NAc,6S}$-G-$GlcN_{NS,3,6S}$-$I_{2S}$-$GlcN_{NS,6S}$-$I_{2S}$-(1,6-an-$GlcN_{NS,6S}$) and ΔU-$GlcN_{NAc,6S}$-G-$GlcN_{NS,3,6S}$-$I_{2S}$-$GlcN_{NS,6S}$-$I_{2S}$-(1,6-an-$ManN_{NS,6S}$) as the main octasaccharides present in the sample (58% and 42% respectively). Moreover, the NOESY experiment confirms that $IdoA_{2S}$ residues with anomeric signals at 5.357 and 5.343 ppm are linked to 1,6-an-$GlcN_{NS,6S}$ and 1,6-an-$ManN_{NS,6S}$, respectively.

*Octasaccharide batch P-32444-16-1 (G4530).*

The proton spectra are shown in figures 31-33 and the signals integration agrees with a octasaccharide structure consisting of GlcN, IdoA, G and ΔU in 4:2:1:1 ratio.

Protons chemical shift pattern was determined by COSY and TOCSY spectra (fig. 34-36) indicating the presence of the following monosaccharide residues: $ΔU_{2S}$, $GlcN_{NS,6S}$, $GlcN_{NS,6S}$red, $GlcN_{NS,3,6S}$, $GlcN_{NAc,6S}$, $IdoA_{2S}$, IdoA and GlcA. Moreover, a weak signal at

5.82 indicates the presence of a small amount of ΔU (less than 5%) (fig. 33). The HSQC spectrum allowed both carbon chemical shifts measurement and resolution of the overlapping signals detected from homonuclear correlation spectra (fig. 37-39). All proton and carbon chemical shifts are reported in the table at page 41.

The interglycosidic correlations were determined by NOESY experiment (fig.40), confirming $\Delta U_{2S}$-$GlcN_{NS,6S}$-I-$GlcN_{NAc,6S}$-G-$GlcN_{NS,3,6S}$-$I_{2S}$-$GlcN_{NS,6S}$ as predominant octasaccharide sequence (more than 95%). (fig. 42)

***Results of NMR analysis of octasaccharide mixtures.***

*Batches P31267-28-BIE10(G4532), P-32077-48-3 (G4531) and P-32077-48-4 (G4613).*

In order to define the different environment of 3-O-sulfated glucosamine residues (A*) inside the oligosaccharide chains, three octasaccharide mixtures were studied by NMR. In particular, an octasaccharide mixture (batch P31267-28-BIE10 - G4532), its AT-affine fraction (batch P32077-48-3 - G4531) and AT-non affine fraction (*P-32077-48-4 - G4613)* were studied. In figure 43 the proton spectra of the three mixtures are compared. The spectrum profile of the total mixture closely resembles the one of the non-affine fraction; only H4 signal of ΔU residue (5.82 ppm) is slightly stronger in the total mixture with respect to non-affine fraction. The profile of affine mixture significantly differs from the others: signals of H4 ΔU, H1 IdoA, H2 G and H2 A* in particular appear strongly enhanced if compared to the others two samples. Since these signals were originally present in the spectra of the total mixtures as minor signals, it can be concluded the total mixture to be mainly composed by non-affine structures. In order to detect other differences between total, affine and non-affine mixtures, the three samples were analysed by 2D-NMR spectroscopy (TOCSY and HSQC experiments). By comparison of the anomeric region of HSQC spectra (fig. 50, 56, 64) it could be observed that while total and non-affine fractions contain an $I_{2s}$red residue ($^{1}H$ 5.44 ppm, $^{13}C$ 95.57 ppm), the affine mixture does not contain this structure. Moreover, in HSQC spectra of total and non-affine mixtures (fig. 50 and 64) a signal at $^{1}H$ 4,77 ppm and $^{13}C$ 102.84 ppm can be spotted, which is totally absent in the spectrum of the affine fraction (fig. 56). By TOCSY assignment (fig. 61), this signal has been

attributed to a $G_{2S}$ residue, a quite unusual structure which NMR assignment has been previously reported by Sugahara (J. Biol. Chem. 1995, 270 (15), 8696-8705). Such residue was identified also in the hexasaccharide sample ZKA067 (described in a previous report). The closely resemblance between their chemical shifts suggests in both cases the occurrence of the structure -$A_{NS,6S}$-$G_{2S}$-. As reported in the cited article, even if G2S unit is located in the highly sulphated blocks in heparin, its structure-activity correlation has not been demonstrated. The almost exclusive presence of this residue in the non-affine fraction may suggest its poor contribution if any, to antithrombotic activity.

H1/H2 region of TOCSY spectrum of the total octasaccharide mixture (fig. 47) shows the presence of at least seven different signals belonging to A* residues. The chemical shift differences between each A* residues can be due to different neighbour residues or to the position of the pentasaccharide inside the octasaccharide chains. Since A* H1/H2 signals correlation are particularly resolved in comparison with the other signals of the pentasaccharide residues, they are used as probe to compare the different A* environment in the octasaccharide mixtures.

In the expansion of TOCSY spectrum (fig. 65) comparison between H1/H2 cross-peaks of A* residues of the total-mixture and the cross-peaks of the three pure octasaccharides (colored picture), is shown. In spite of the small differences in chemical shifts (probably due to small differences in ion strength or pH), the correlation peaks B and D correspond to internal (green and red) and reducing -$A_{NAc}$GA*IA (blue) sequences, respectively. In fact, octasaccharides G4528 and G4529, which differ only in the reducing end residue (four units distant from A*), show the same H1/H2 chemical shift values. The cross peaks A, C, E and F indicate the presence of different sequences containing the A* residue. Figure 66 shows the same region of TOCSY spectrum of the affine octasaccharide mixture: all signals observed in the spectra of the three pure octasaccharides described before are present. Signals not observed in the assigned octasaccharides sequences are also detected, such as signals A and G.

Finally, the same analysis performed on the non-affine mixture (G4613) permits to identify the environment of A* in non-affine sequences, by identification of further cross peaks not present in the affine fraction. Even if weak cross-peak A, present also in the affine-mixture, may be due to a non complete elimination of the affine structures, the intense cross-peaks C and the weaker

E and F peaks (fig. 67), not observed in the affine mixture, belong to A* residues of non affine sequences. The presence of no affinity sequences could be due to the cleavege of the pentasaccharide sequence or the lack of essential sulfate groups for the binding to AT.

In the following table the measured chemical shifts of the different A* residues (A-G) are shown. Comparison with A* chemical shifts values of pure octasaccharides is also indicated (coloured numbers).

| | H1 | H2 | H3 | H4 | H5 |
|---|---|---|---|---|---|
| **A* in AFFINE SEQUENCES** | | | | | |
| **G4531 residue A** | 5.485 | 3.475 | 4.392 | nd | nd |
| **G4531 residue B** | 5.523 (5.527-5.528) | 3.470 (5.459-5.455) | 4.395 (4.381-4.376) | 3.992 (3.978-3.975) | 4.168 (4.179-4.165) |
| **G4531 residue D** | 5.541 (5.546) | 3.470 (3.463) | 4.385 (4.371) | 3.986 (3.979) | 4.177 (4.179) |
| **G4531 residue G** | 5.506 | 3.444 | 4.421 | 3.972 | nd |
| **A* in NON-AFFINE SEQUENCES** | | | | | |
| **G4532/G4613 residue C** | 5.532/5.539 | 3.479/3.478 | 4.404/4.406 | 4.050/nd | 4.187/4.179 |
| **G4532/G4613 residue E** | 5.550/5.554 | 3.426/3.426 | 4.370/4.367 | 4.081/nd | nd |
| **G4532/G4613 residue F** | 5.532/5.539 | 3.398/3.399 | nd | nd | nd |

N.B. in green, red and blue are indicated chemical shifts of A* in G4528, G4529 and G4530 respectively.

G4531= affine fraction G4532= total mixture G4613= non-affine fraction

***NMR Methods***

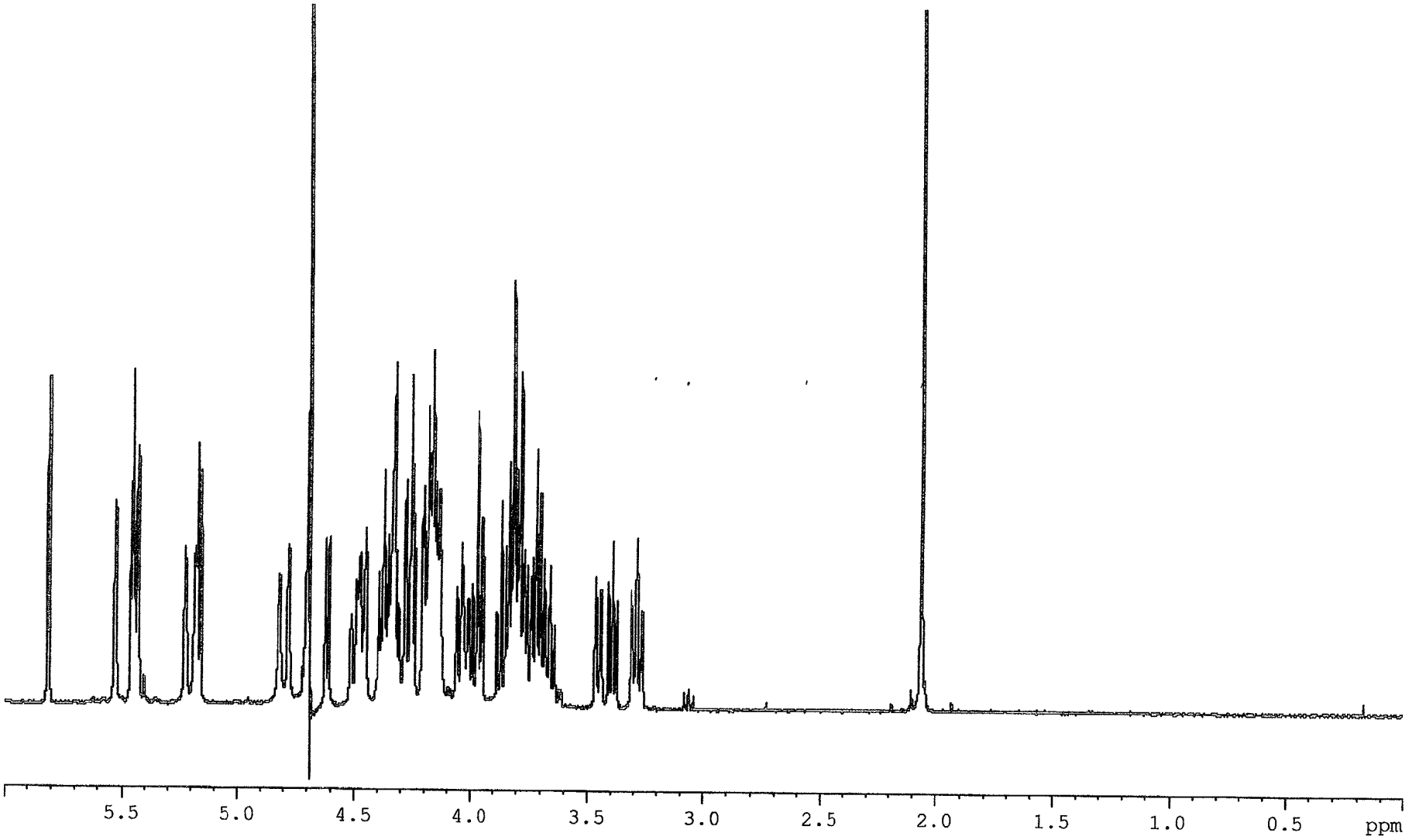
Monodimensional and bidimensional proton NMR spectra were recorded at 500MHz with a Bruker Avance 500 spectrometer equipped with a 5mm TXIz probe.

Samples were dissolved in $^2H_2O$ (99.9%) and freeze dried to remove residual water. After exchanging the samples three times, they were dissolved in 0.6 ml of $^2H_2O$ (99.996%). Chemical shifts are given in ppm downfield from sodium trimethylsilyl propionate as external standard (precision of ± 0.003ppm). All experiments were made at 25° C. Proton NMR spectra were recorded with presaturation of residual water signal, with a recicle delay of 12s and 128 scans. 2D homonuclear correlation spectra of pure octasaccharides were acquired with 32 scans for each of 512 free-induction decays. TOCSY spectra of the two octasaccharide mixtures were acquired with 1024 free-induction decay in order to increase $\omega 1$ resolution. 2D DQF-COSY and TOCSY were measured in phase-sensitive mode using TPPI, and Fourier transformed onto a data matrix of 4x2K with a phase shifted ($\pi/3$) square sine-bell function. The $^1H/^{13}C$ chemical shift correlation (HSQC) spectra were performed using z-gradients for coherence selection. They were obtained with carbon decoupling during acquisition period in phase sensitivity-enhanced pure absorption mode. The spectra were acquired with a nulling time of 2s, 1024 data points in F2, 512 increments in F1, 32 or 64 scans per increment. The final matrix size was zero-filled to 4Kx2K and multiplied with shifted ($\pi/3$) sine-bell-square prior Fourier transformation. NOESY experiments were recorded with 32 scans, 2k data points for $\omega 2$ and 512 increments in $\omega 1$. A mixing time of 300ms was used.

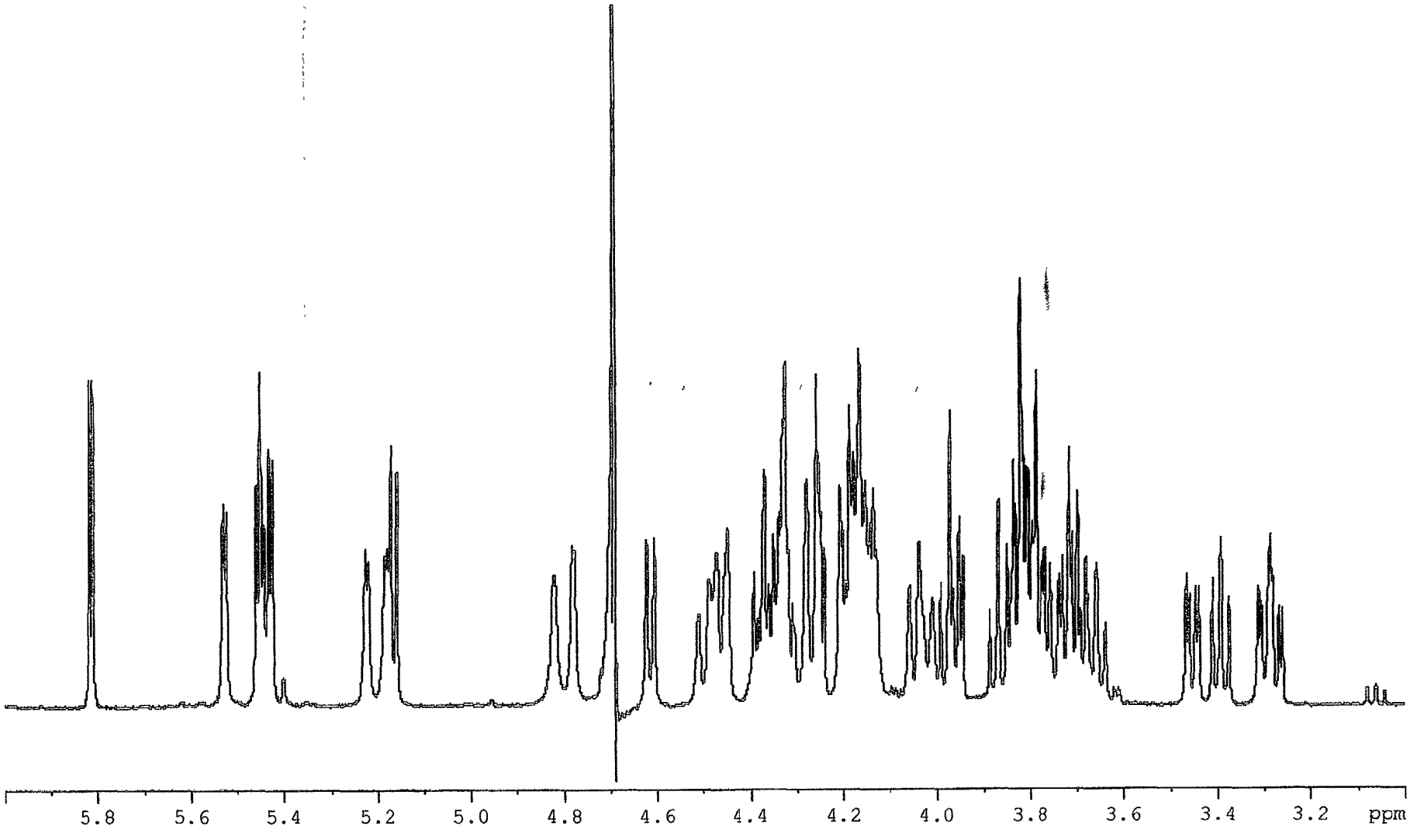
# ANNEX I

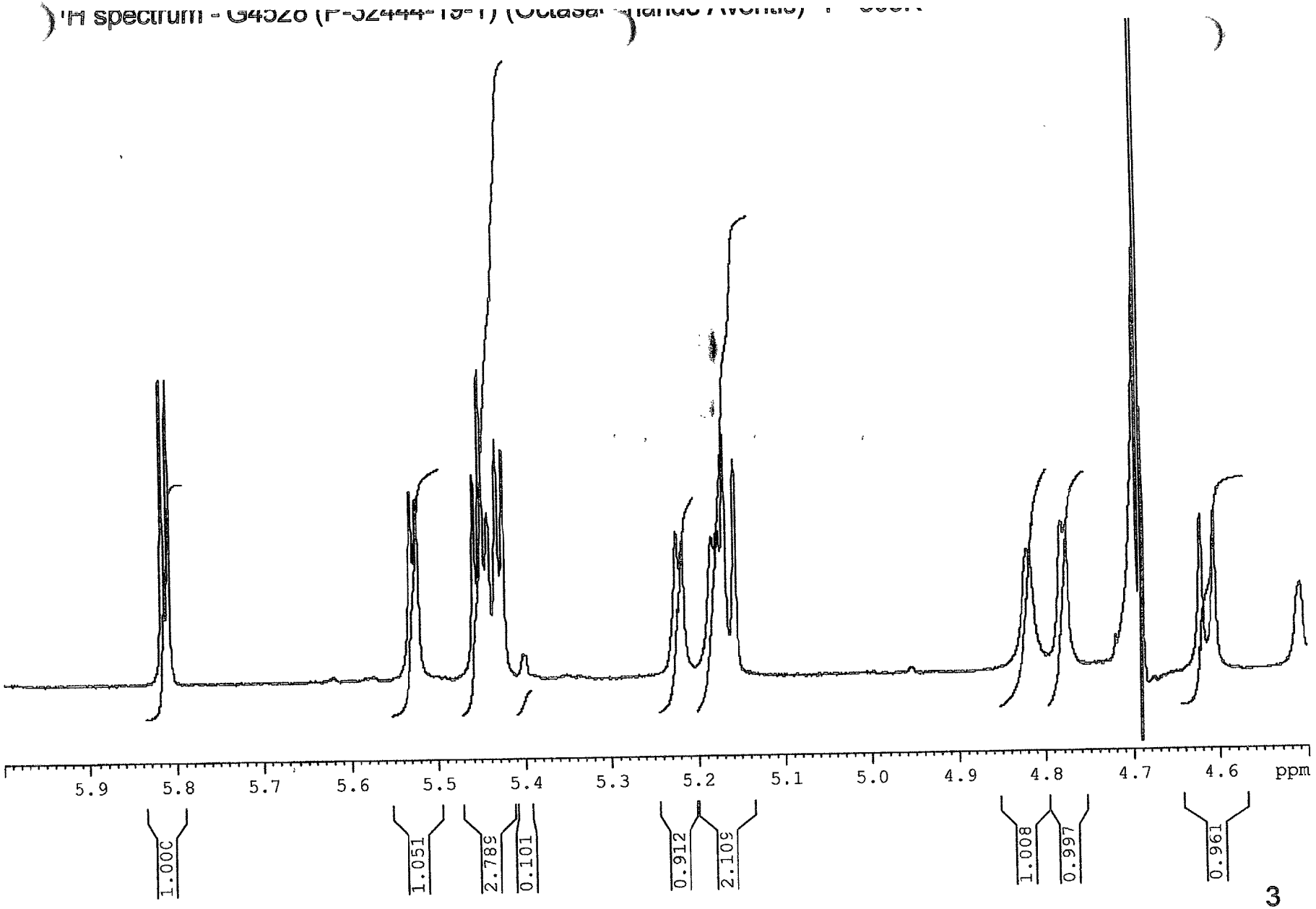
(FIGURES AND TABLES)

$^1$H spectrum - G4528 (P-32444-19-1) (Octasaccharide Aventis) T= 308K



$^1$H spectrum - G4528 (P-32444-19-1) (Octasaccharide Aventis) T= 308K

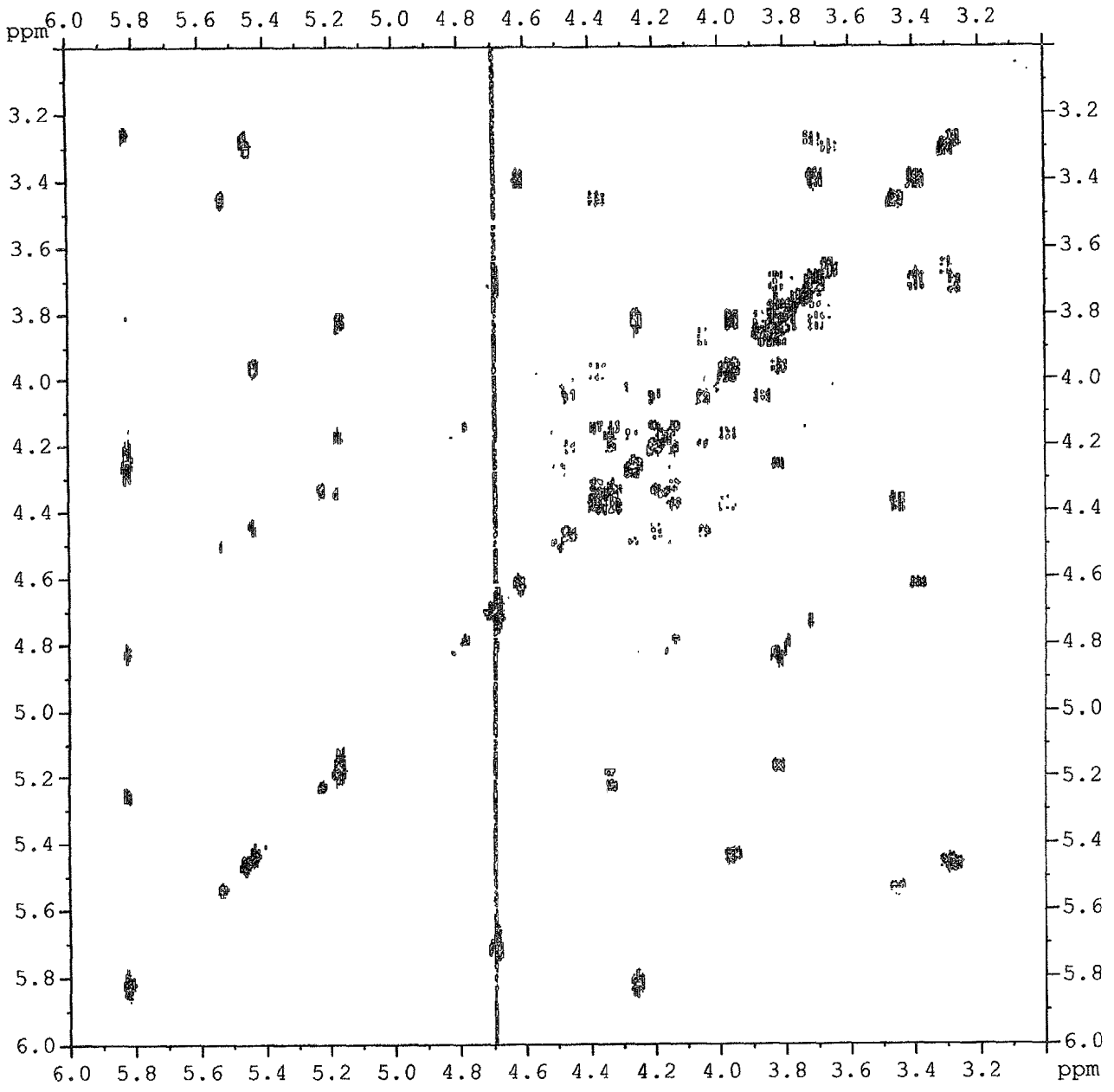




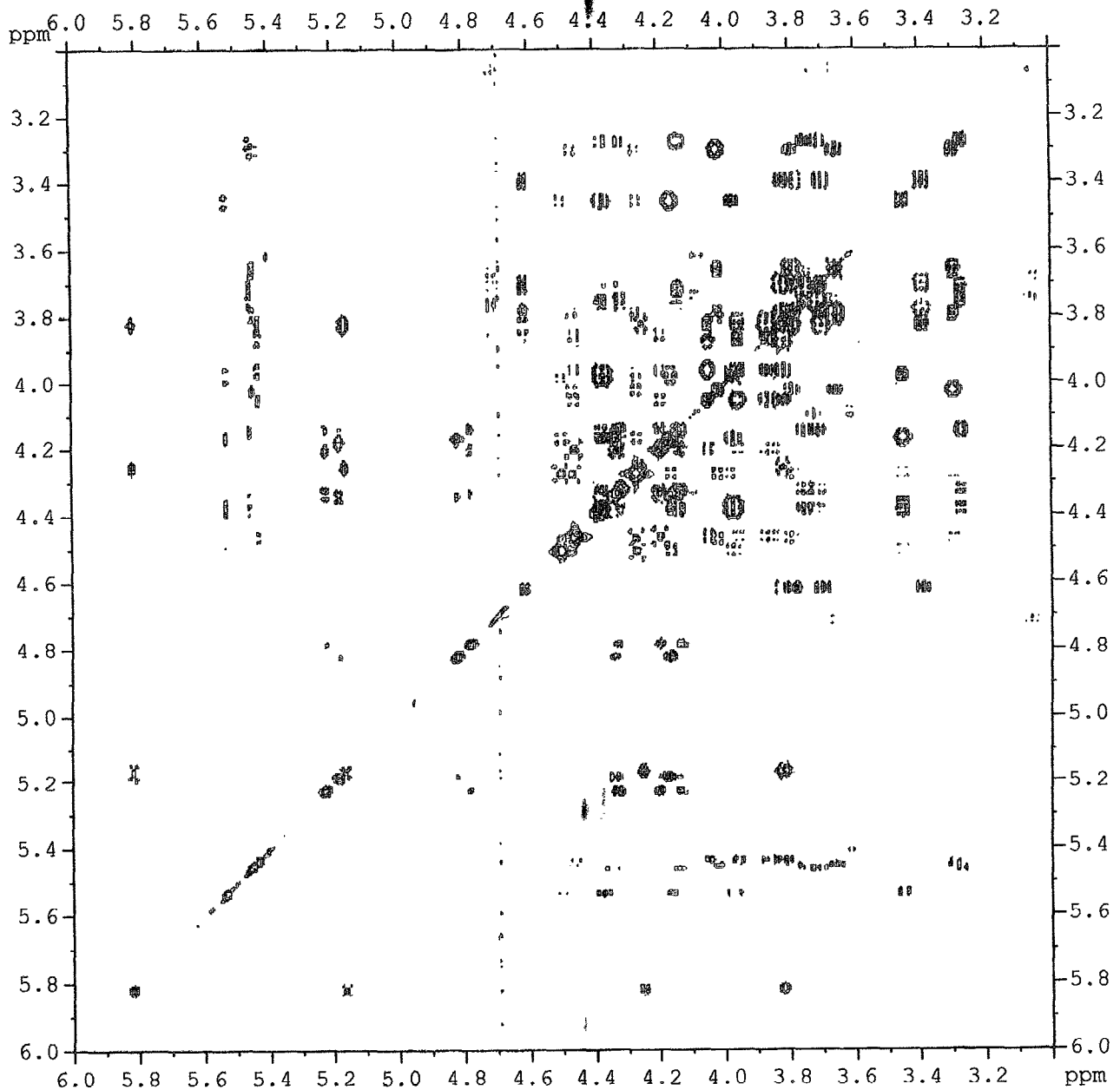
'H spectrum - G4528 (P-32444-19-1) (Octasa
5.9
5.8
5.7
5.6
5.5
5.4
5.3
5.2
5.1
5.0
4.9
4.8
4.7
4.6
ppm
1.000
1.051
2.789
0.101
0.912
2.109
1.008
0.997
0.961


3

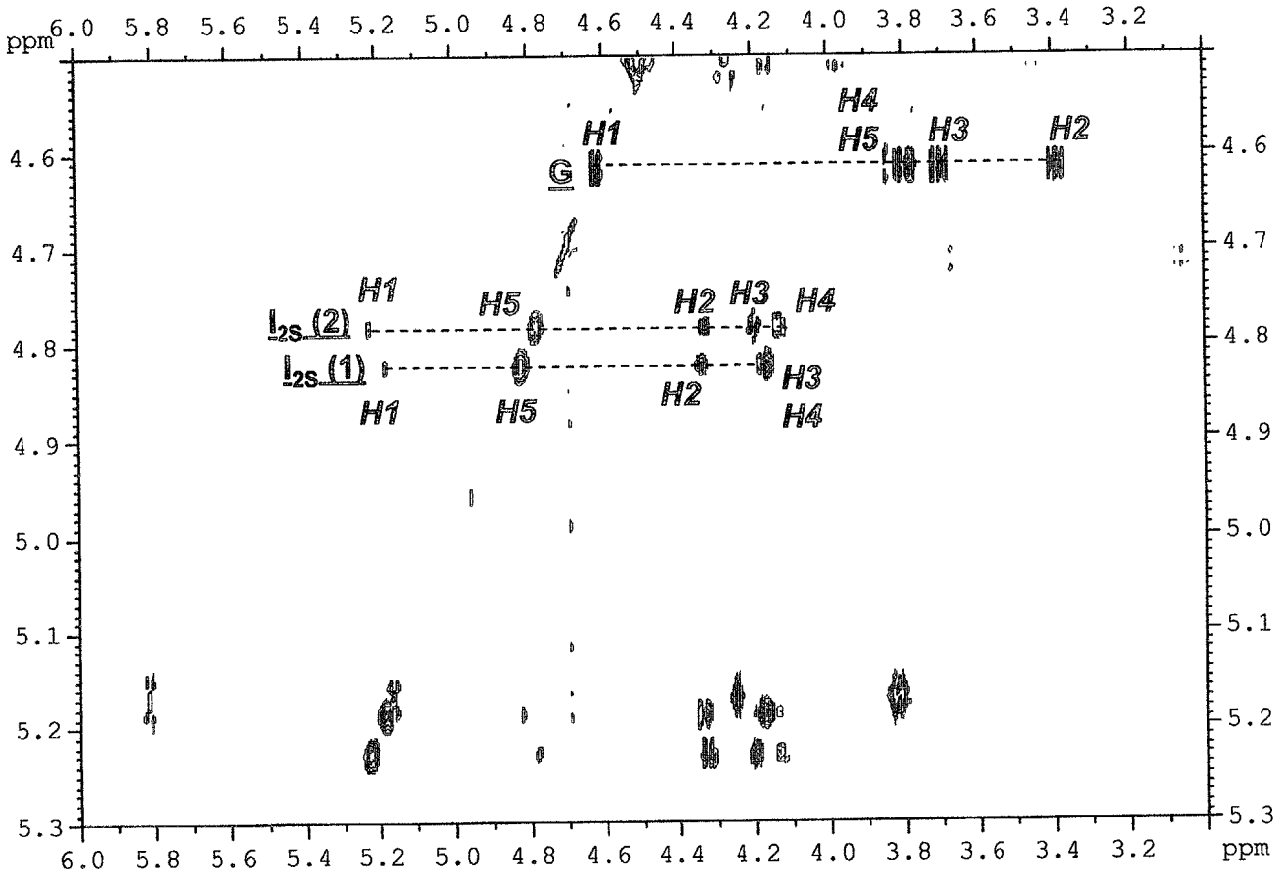
COSY spectrum - G4528 (P-3244 )19-1) (Octasaccharide Aventis) T= 308K



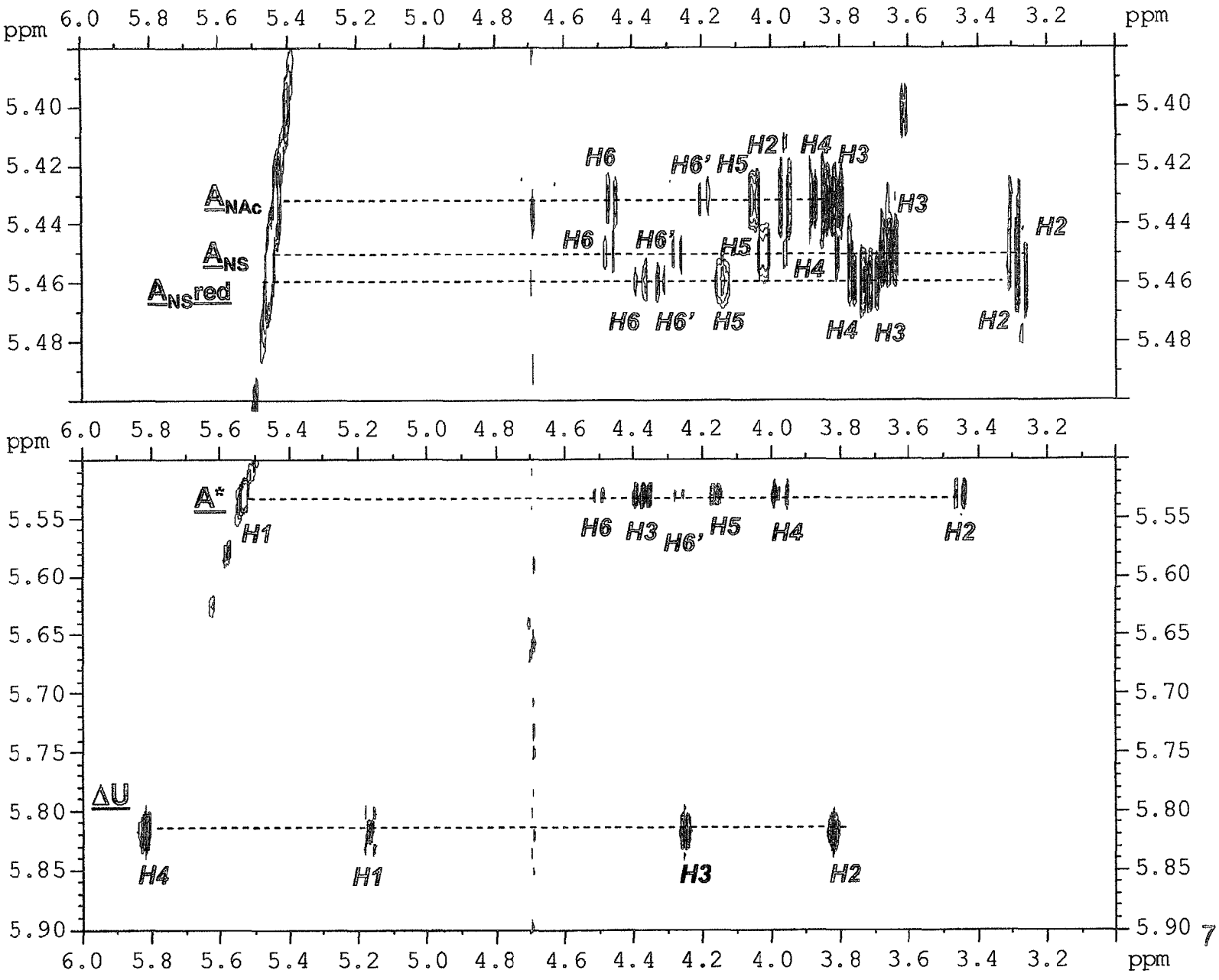
TOCSY spectrum - G4528 (P-32444-19-1) (C saccharide Aventis) T= 308K



TOCSY spectrum - G4528 (P-32444-19-1) (Octasaccharide Aventis) T= 308K

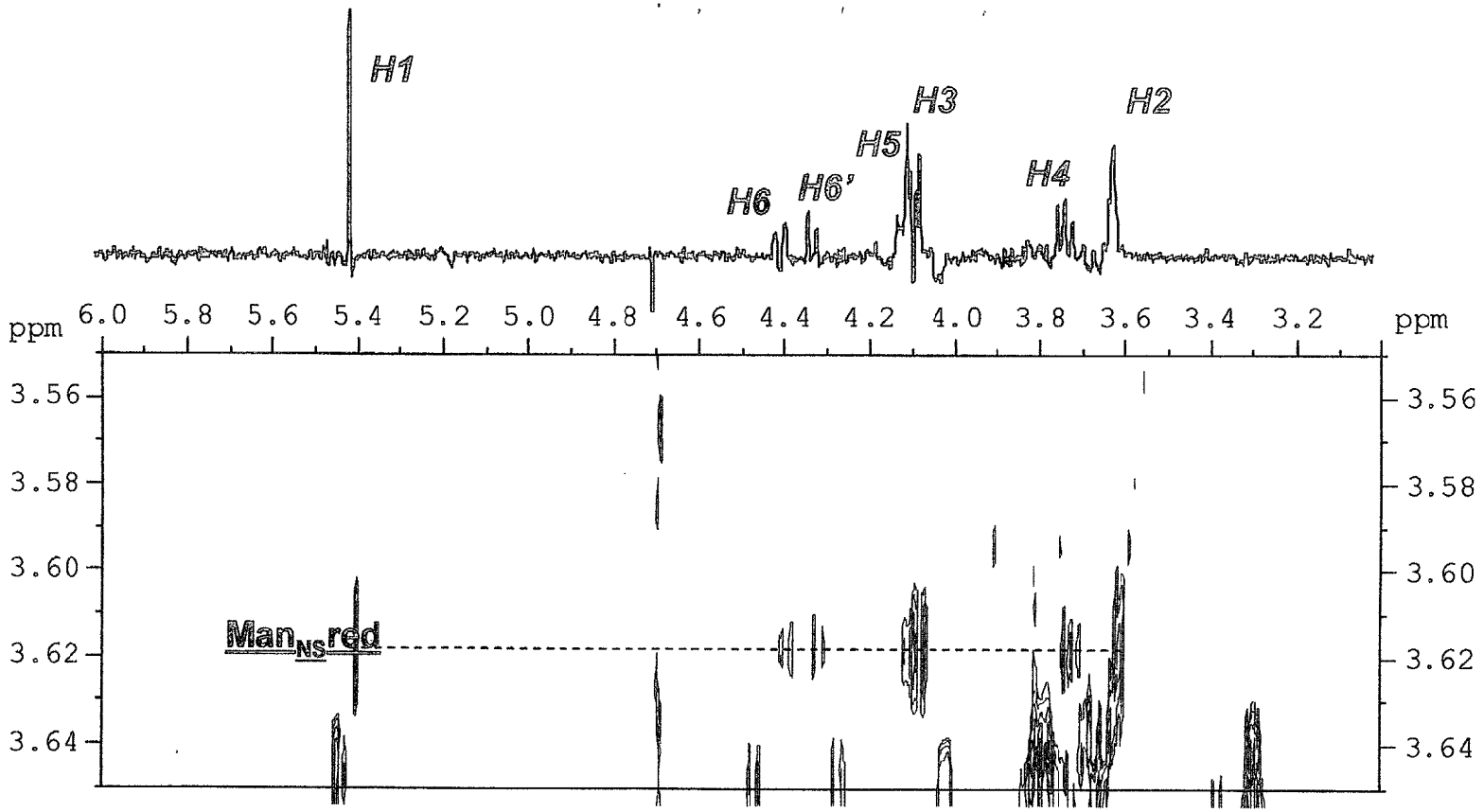


TOCSY spectrum - G4528 (P-3. 4-19-1) (Octasaccharide Aventis) T= 308K

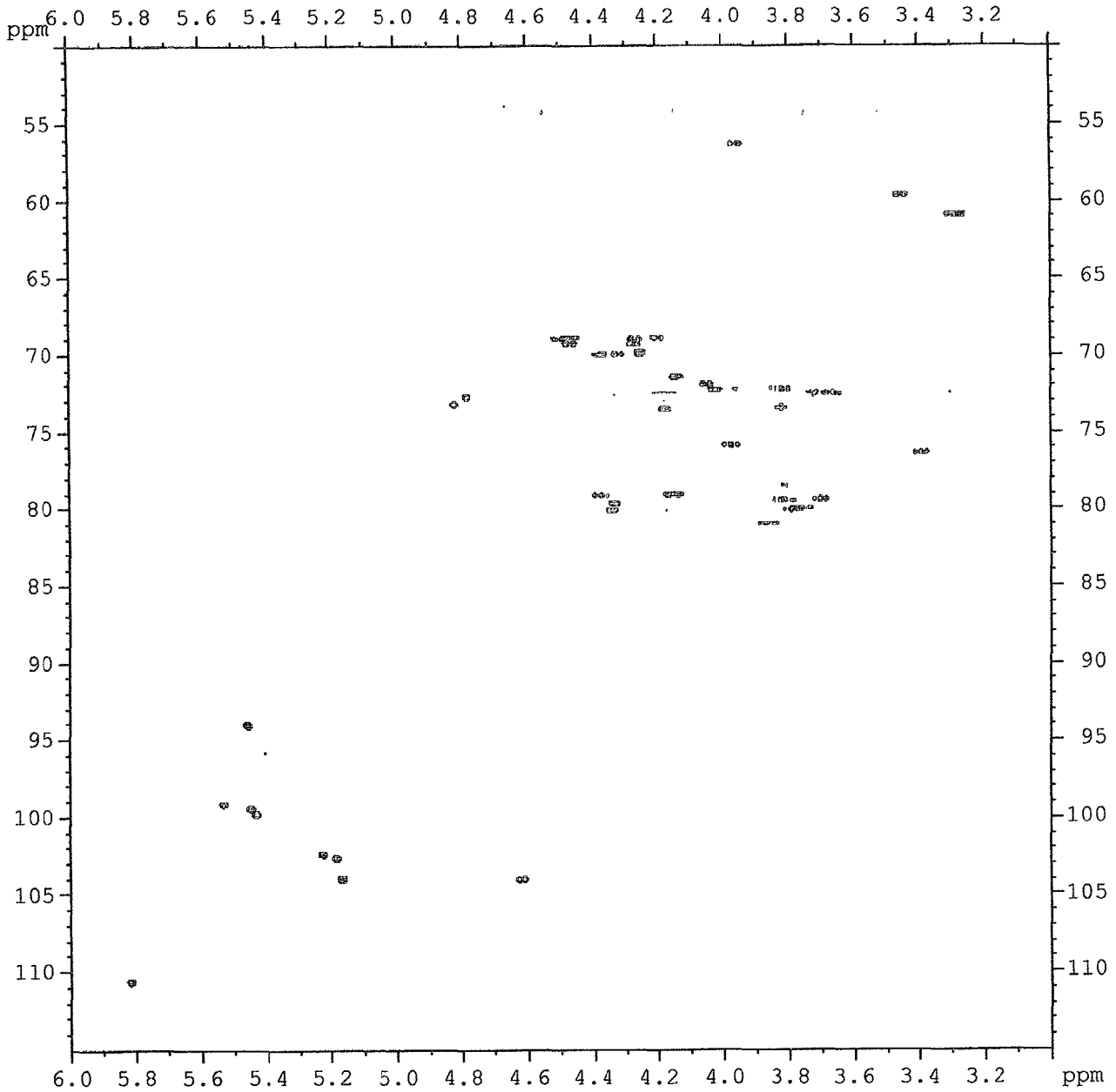



7

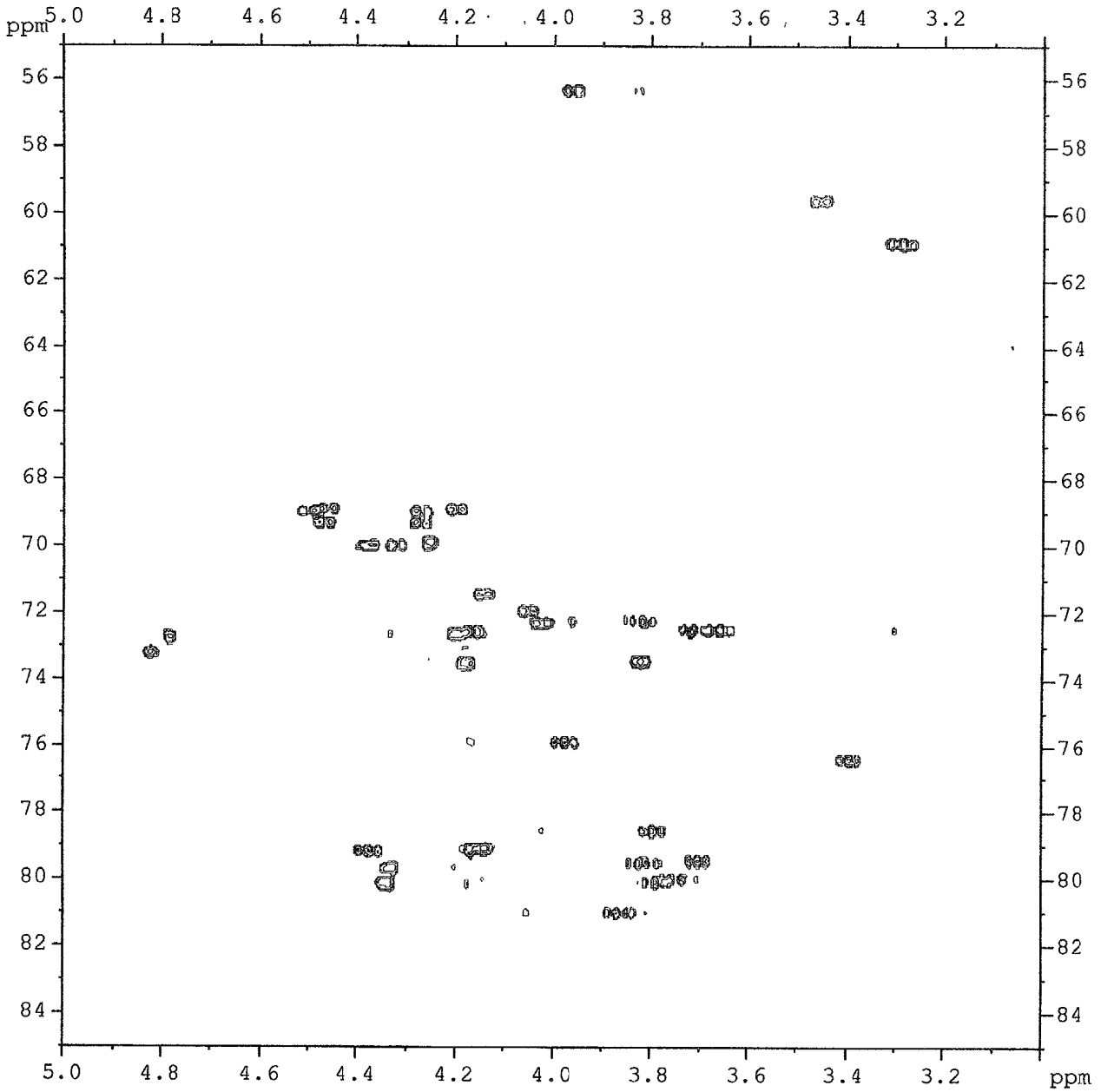
TOCSY spectrum - G4528 (P-32444-19-1) (Octasaccharide Aventis) T= 308K



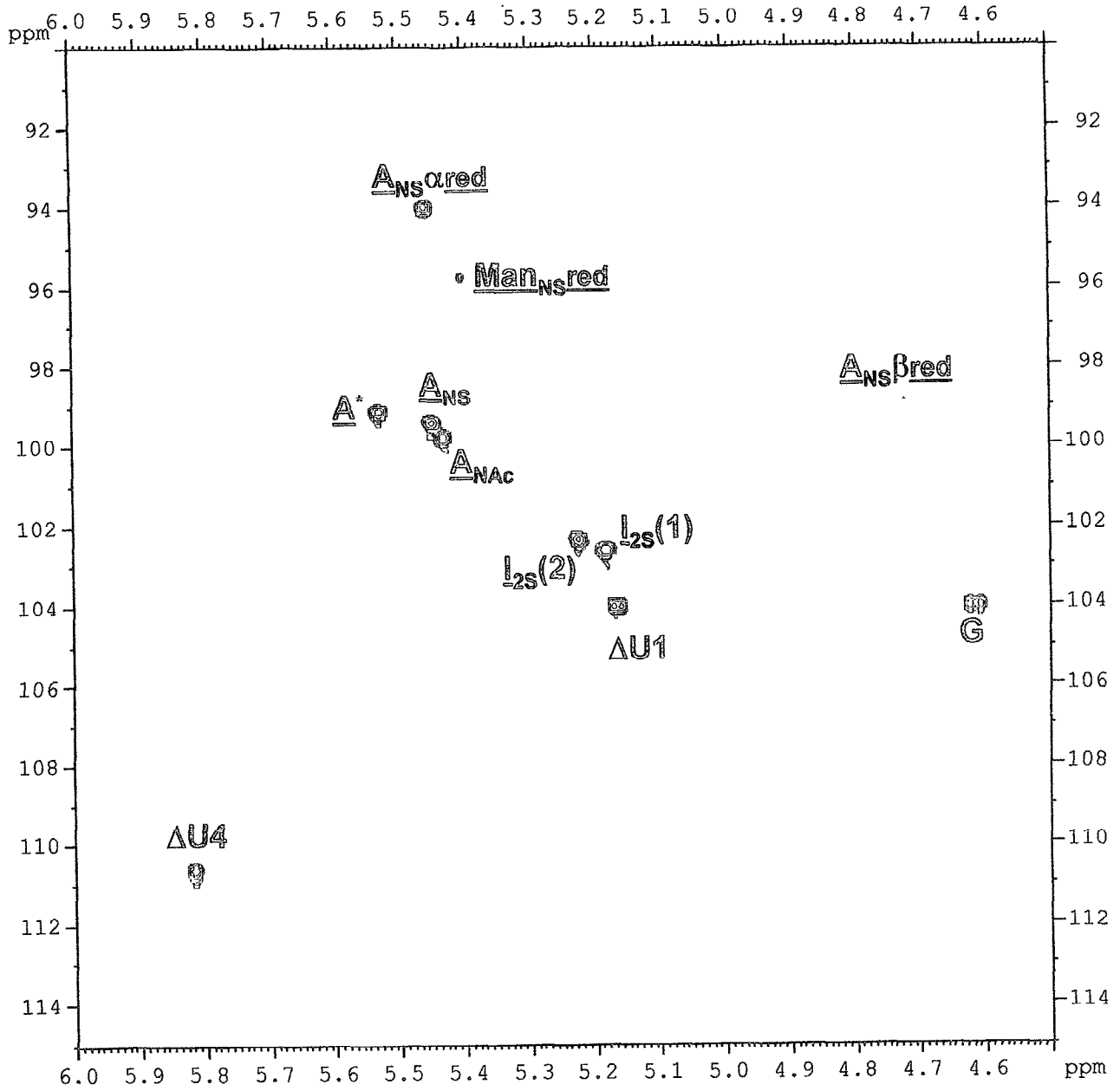
HSQC spectrum - G4528 (P-3_-44-19-1) (Octasaccharide Aventis) T= 308K



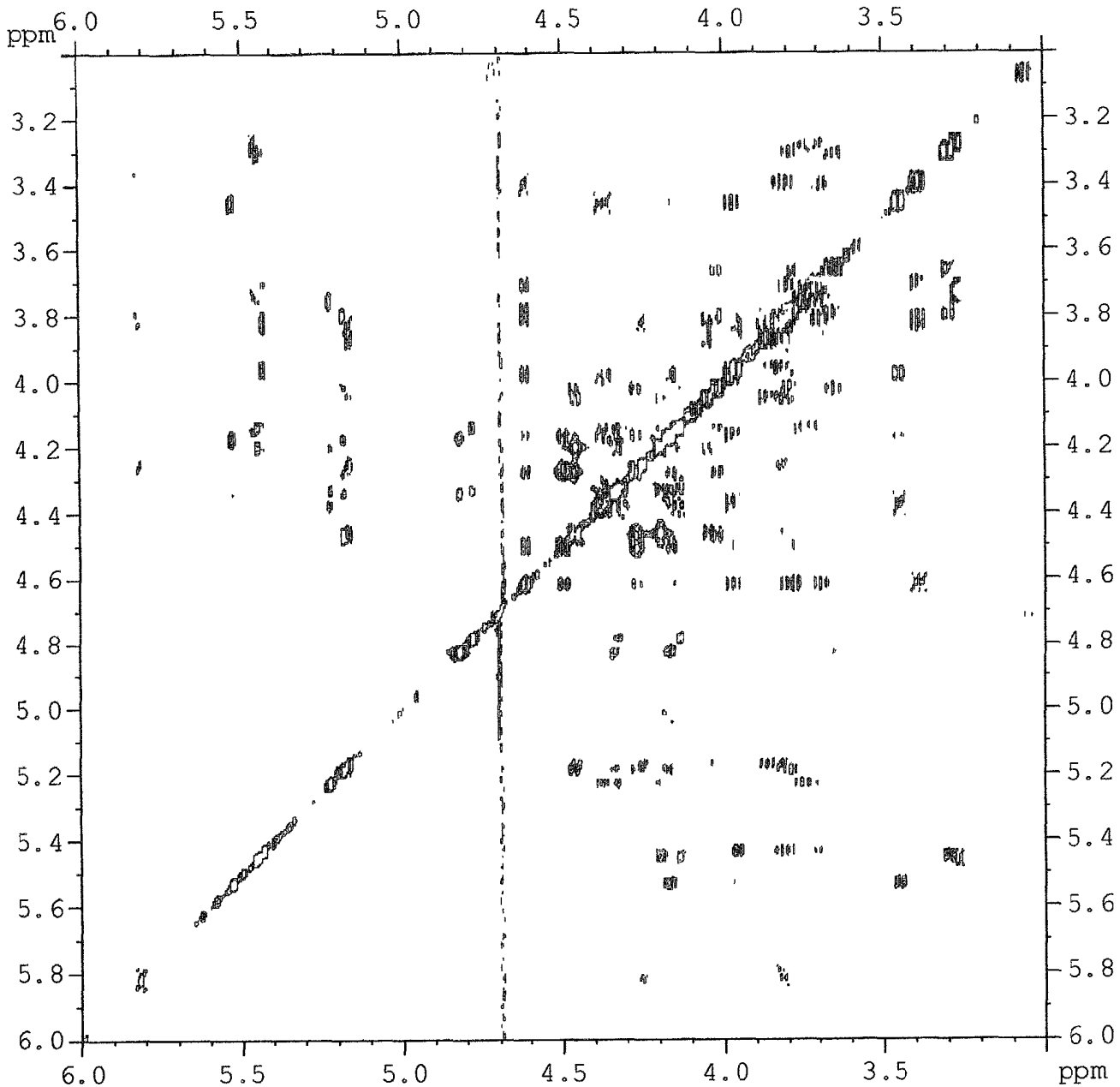
HSQC spectrum - G4528 (P-32444-19-1) (Octasaccharide Aventis) T= 308K



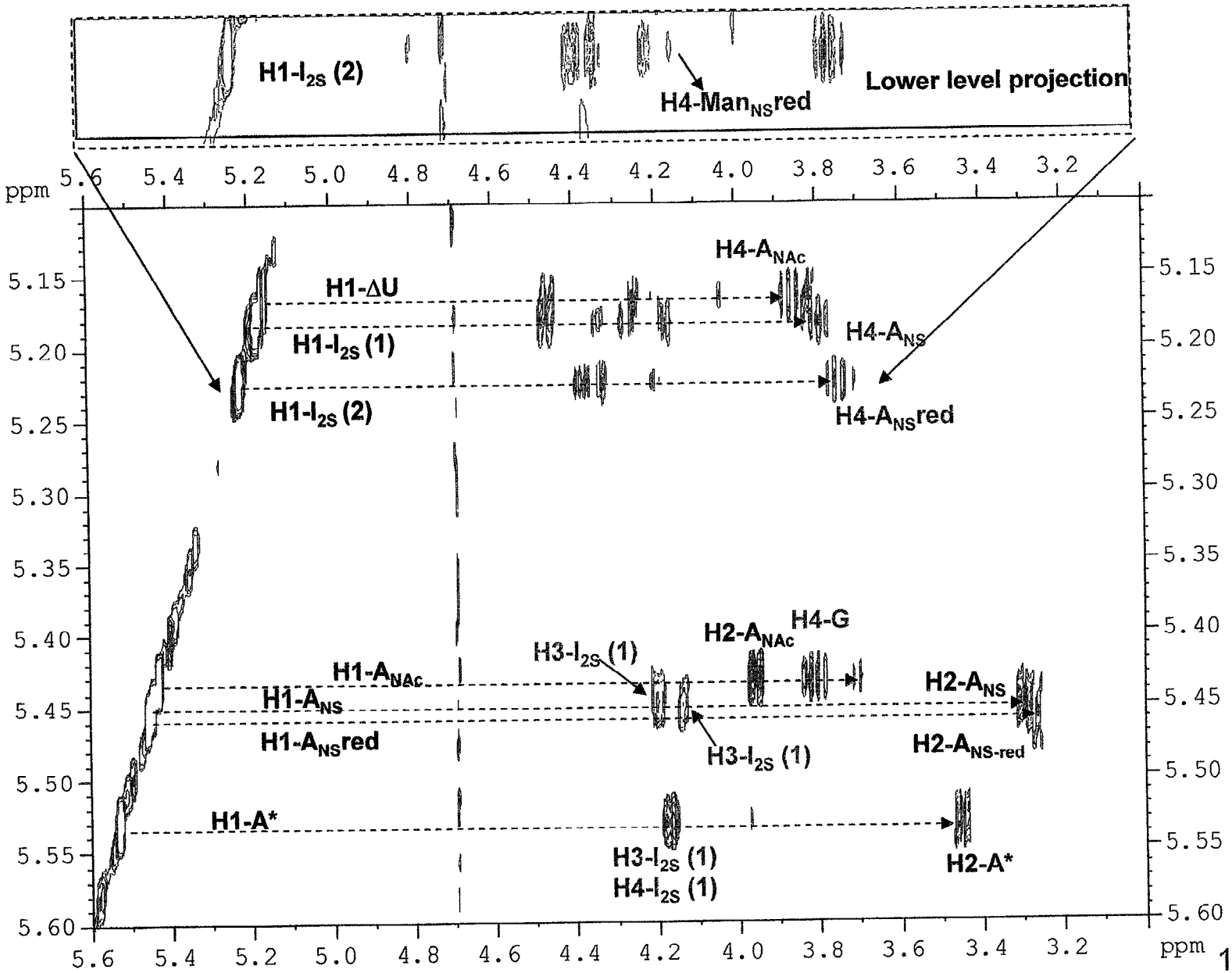
HSQC spectrum (anomeric region) - 4528 (P-32444-19-1) (Octasaccharide Aventis) T= 300K



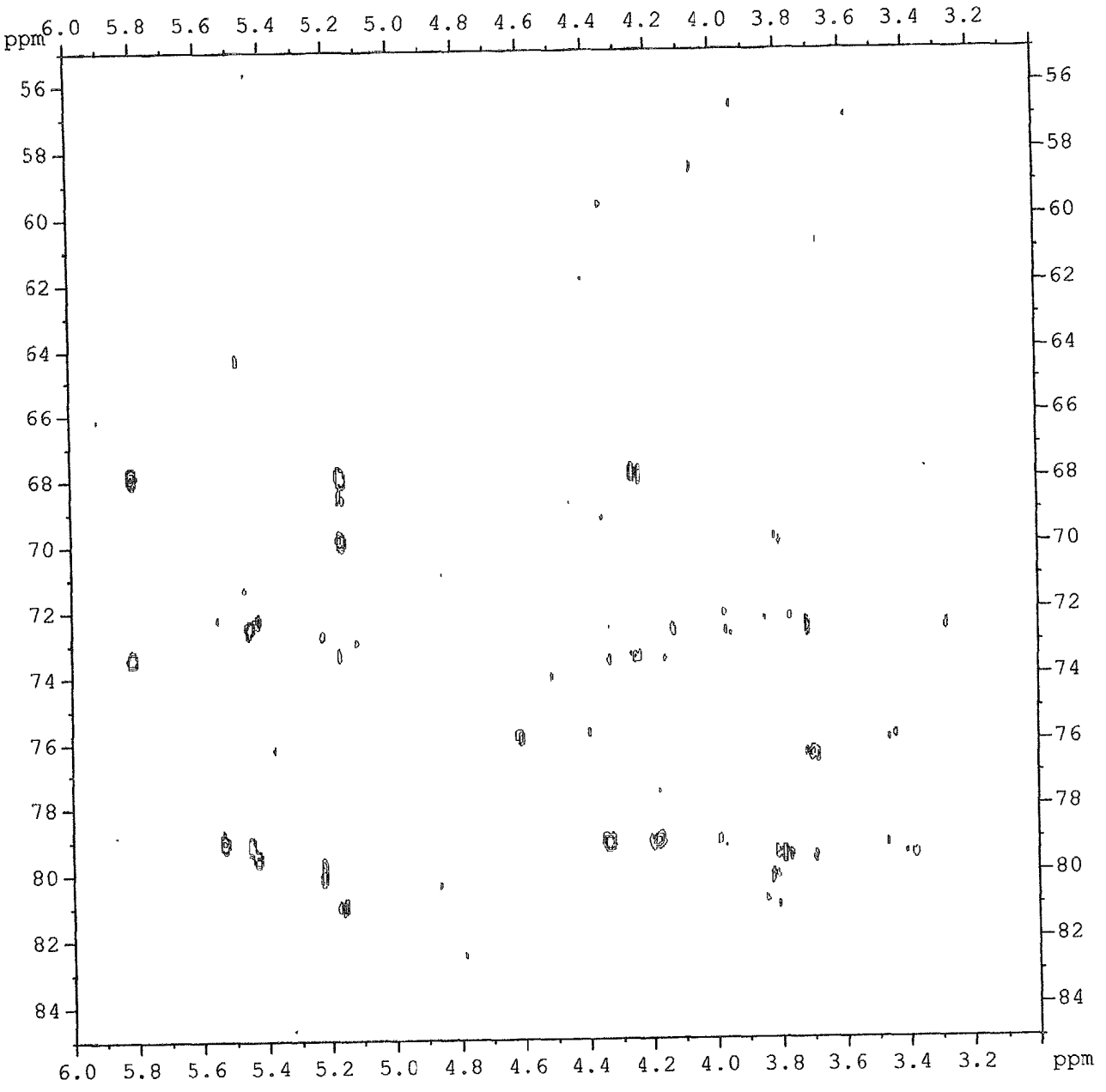
NOESY spectrum (mix. 300ms) - G4528 (P-32444-19-1) (Octasaccharide Aventis) T= 308K



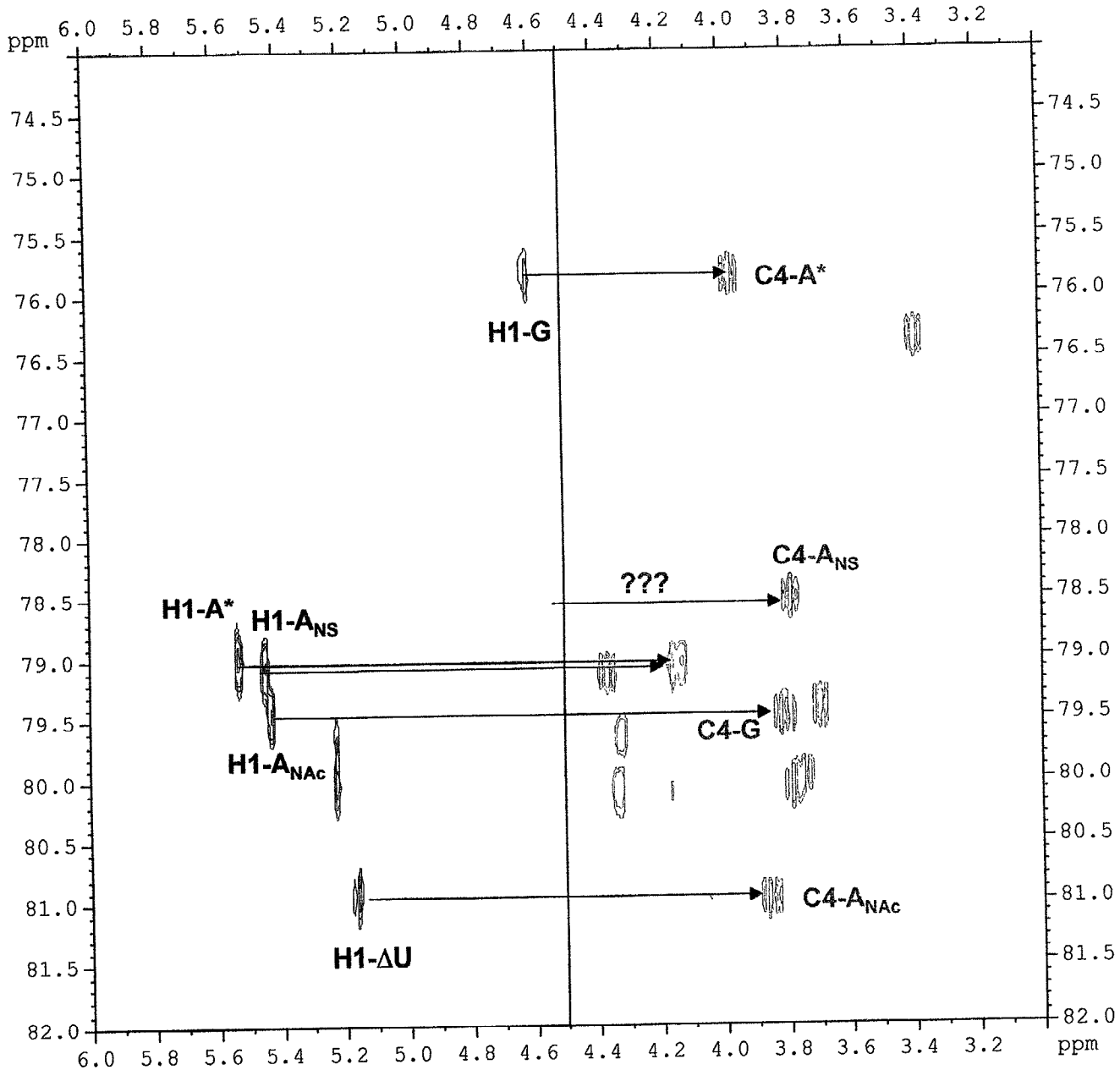
NOESY spectrum (mix. 300ms) - G4528 )-32444-19-1) (Octasaccharide Aventis) T= 308K



HMBC spectrum - G4528 (P- )444-19-1) (Octasaccharide Aventis) T= 308K



HSQC/HMBC - G4528 (P-32444-19-1) (Octasaccharide Aventis) T= 308K



$^{1}H$-$^{13}C$ chemical shifts G4528 (P-32 )4-19-1) (Octasaccharide Aventis) T= 308K

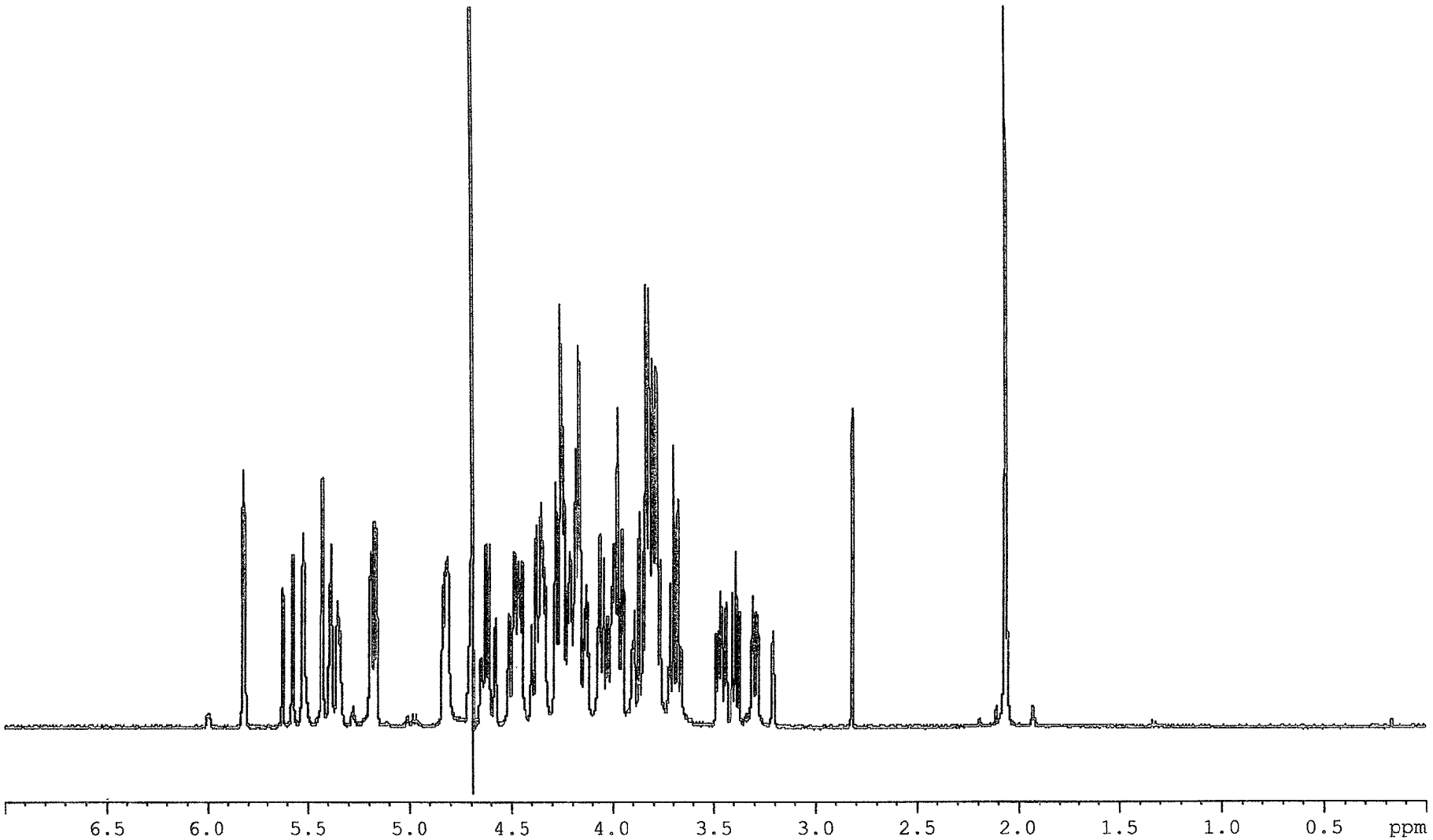
| | ΔU | $A_{NAc}$ | G | A* | $I_{2S}$ (1) | $A_{NS}$ | $I_{2S}$ (2) | $A_{NS}$αred ($A_{NS}$βred) | $Man_{NS}$red |
|---|---|---|---|---|---|---|---|---|---|
| H1/C1 | 5.164/ 103.96 | 5.430/ 99.69 | 4.615/ 103.92 | 5.528/ 99.05 | 5.181/ 102.53 | 5.447/ 99.32 | 5.222/ 102.28 | 5.456/ 93.94 (4.720/98.77) | 5.403/ 95.71 |
| H2/C2 | 3.820/ 73.35 | 3.960/ 56.26 | 3.394/ 76.30 | 3.455/ 59.96 | 4.340/ 79.99 | 3.298/ 60.83 | 4.332/ 79.56 | 3.276/ 60.83 (3.063/63.87) | 3.617/ 60.33 |
| H3/C3 | 4.252/ 72.41 | 3.829/ 72.13 | 3.705/ 79.33 | 4.376/ 79.06 | 4.178/ 73.43 | 3.659/ 72.38 | 4.200/ 72.54 | 3.712/ 72.38 | 4.081/ 70.12 |
| H4/C4 | 5.814/ 110.50 | 3.858/ 80.90 | 3.820/ 79.39 | 3.975/ 75.78 | 4.167/ 78.96 | 3.796/ 78.43 | 4.138/ 78.96 | 3.750/ 80.00 | 3.727/ nd |
| H5/C5 | | 4.051/ 71.84 | 3.777/ 79.90 | 4.165/ 72.47 | 4.822/ 73.13 | 4.022/ 72.20 | 4.782/ 72.65 | 4.144/ 71.33 | 4.108/ 71.95 |
| H6/C6 | | 4.461-4.198/ 68.79 | | 4.501-4.270/ 68.86 | | 4.469-4.271/ 69.20 | | 4.382-4.321/ 69.87 | 4.393-4.315/ 69.87 |

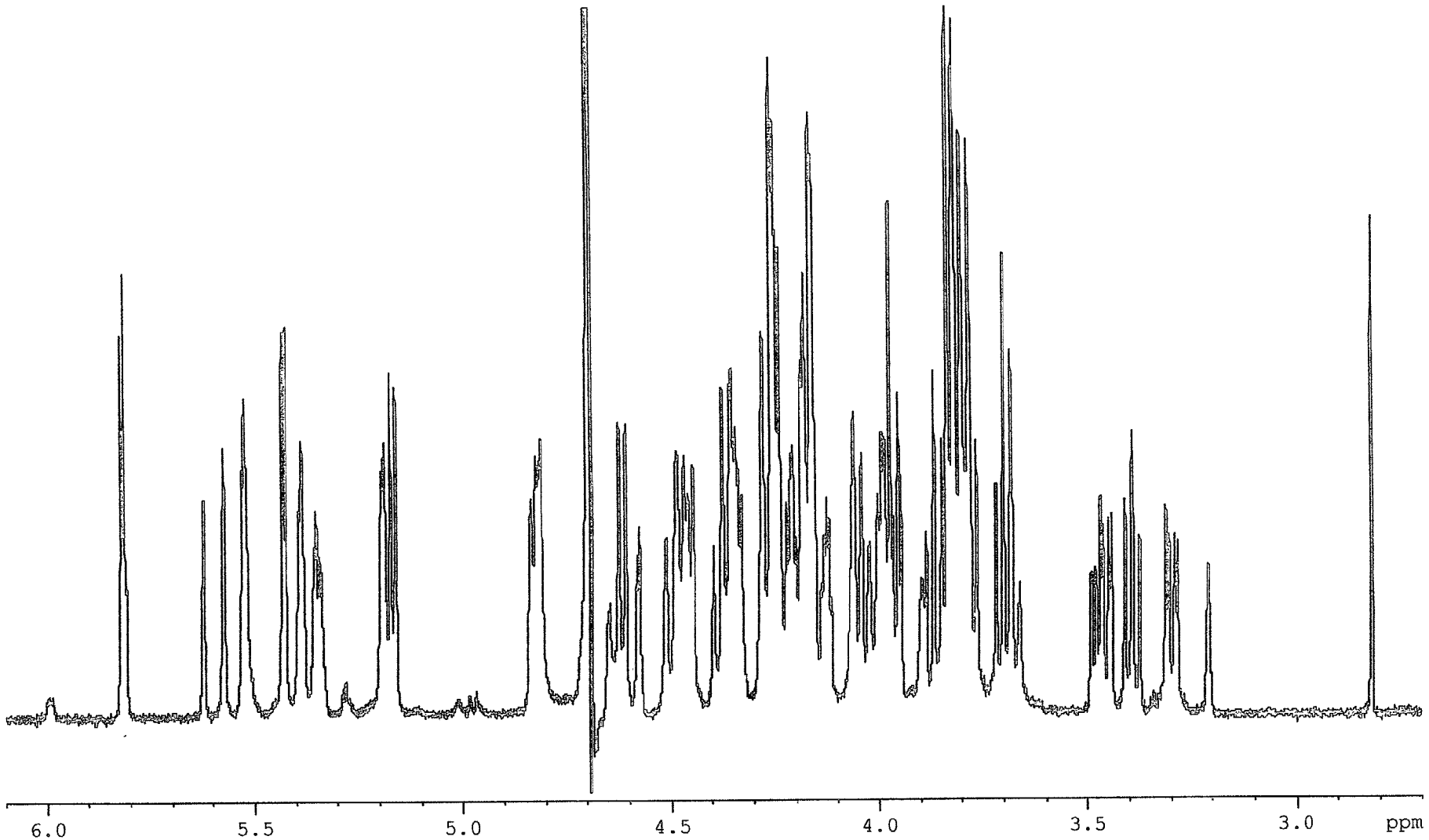
Octasaccharide sequences of sample batch- P-32444-19-1 (G4258) :

$\Delta U\text{-}A_{NAc,6S}\text{-}G\text{-}A_{NS,3,6S}\text{-}I_{2S}\text{-}A_{NS,6S}\text{-}I_{2S}\text{-}A_{NS,6S}$ $\cong 90\%$

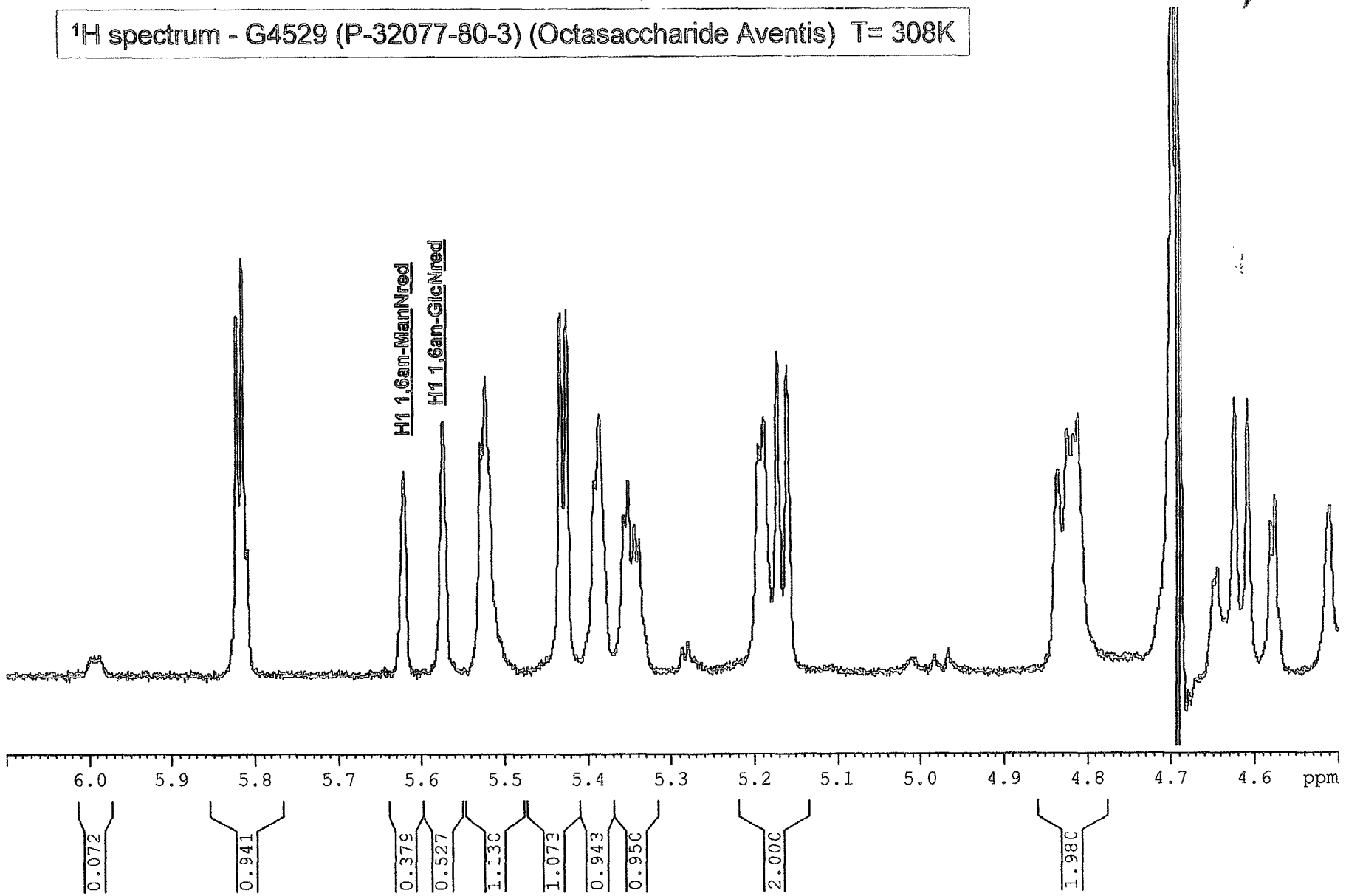
$\Delta U\text{-}A_{NAc,6S}\text{-}G\text{-}A_{NS,3,6S}\text{-}I_{2S}\text{-}A_{NS,6S}\text{-}I_{2S}\text{-}Man_{NS,6S}$ $\cong 10\%$

$^1$H spectrum - G4529 (P-32077-80-3) (Octas charide Aventis) T= 308K

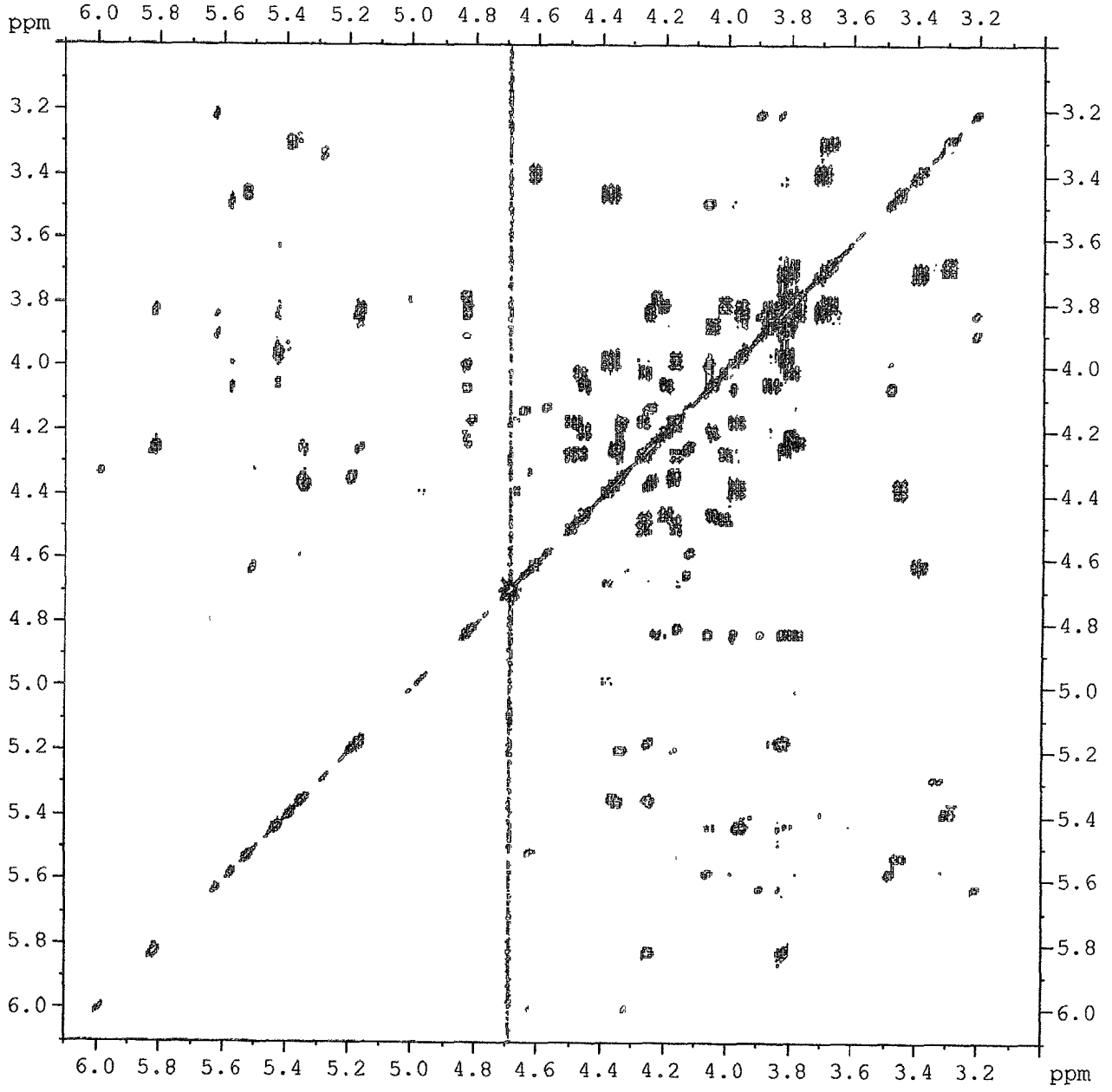



6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm

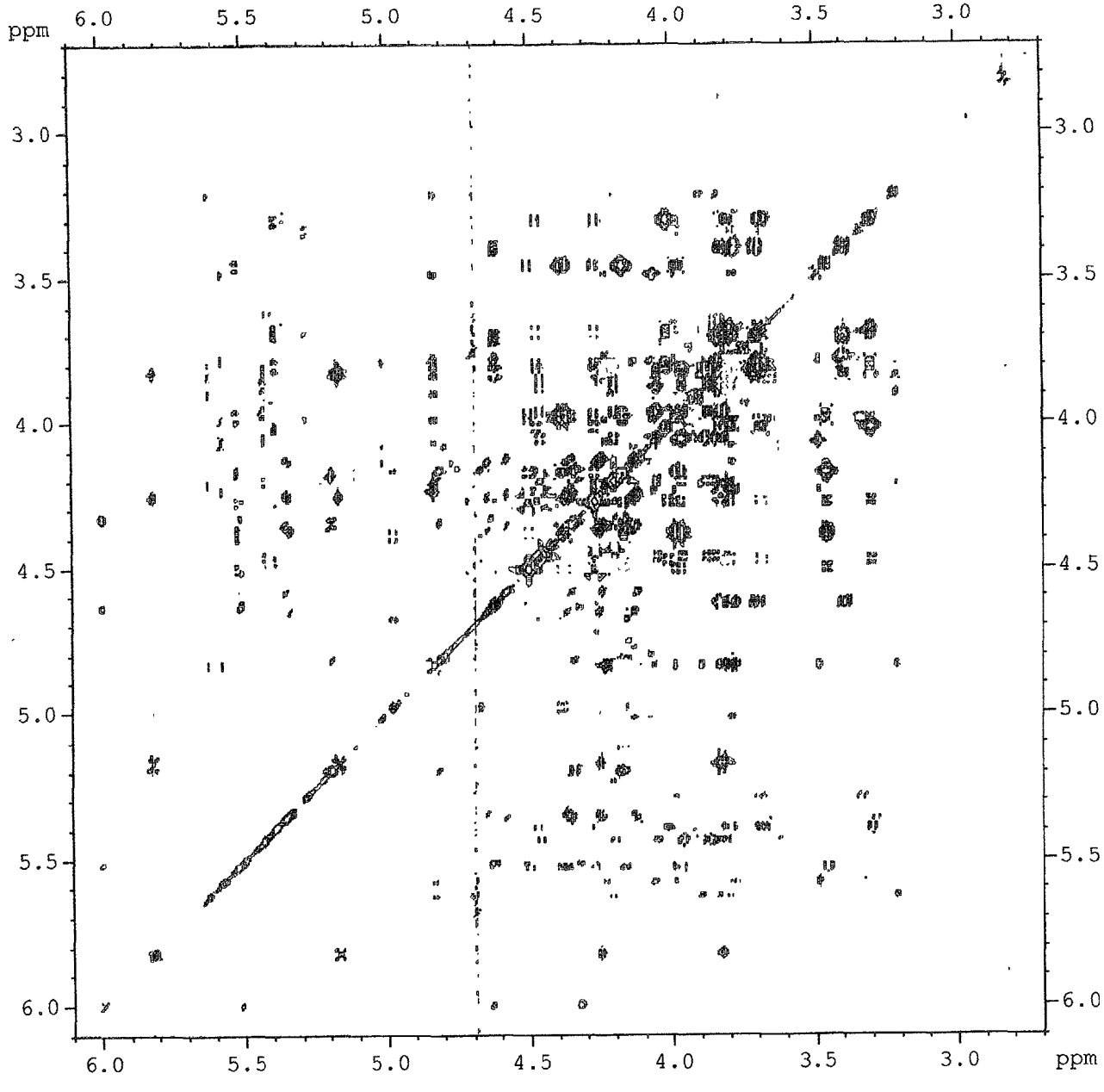
$^1$H spectrum - G4529 (P-32077-80-3) (Octasaccharide Aventis) T= 308K

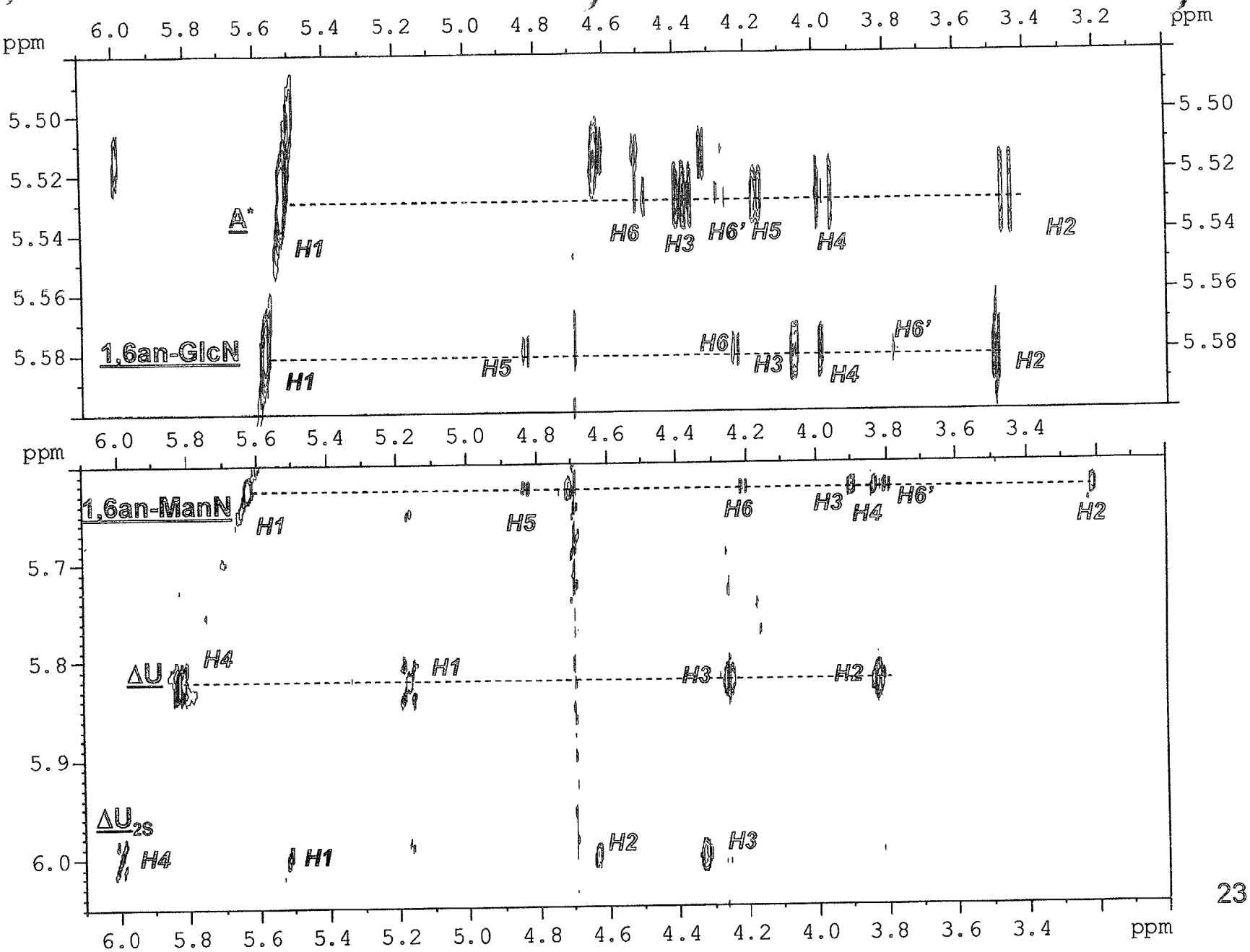


COSY spectrum - G4529 (P-32077-80-3) (Octasaccharide Aventis) T= 308K

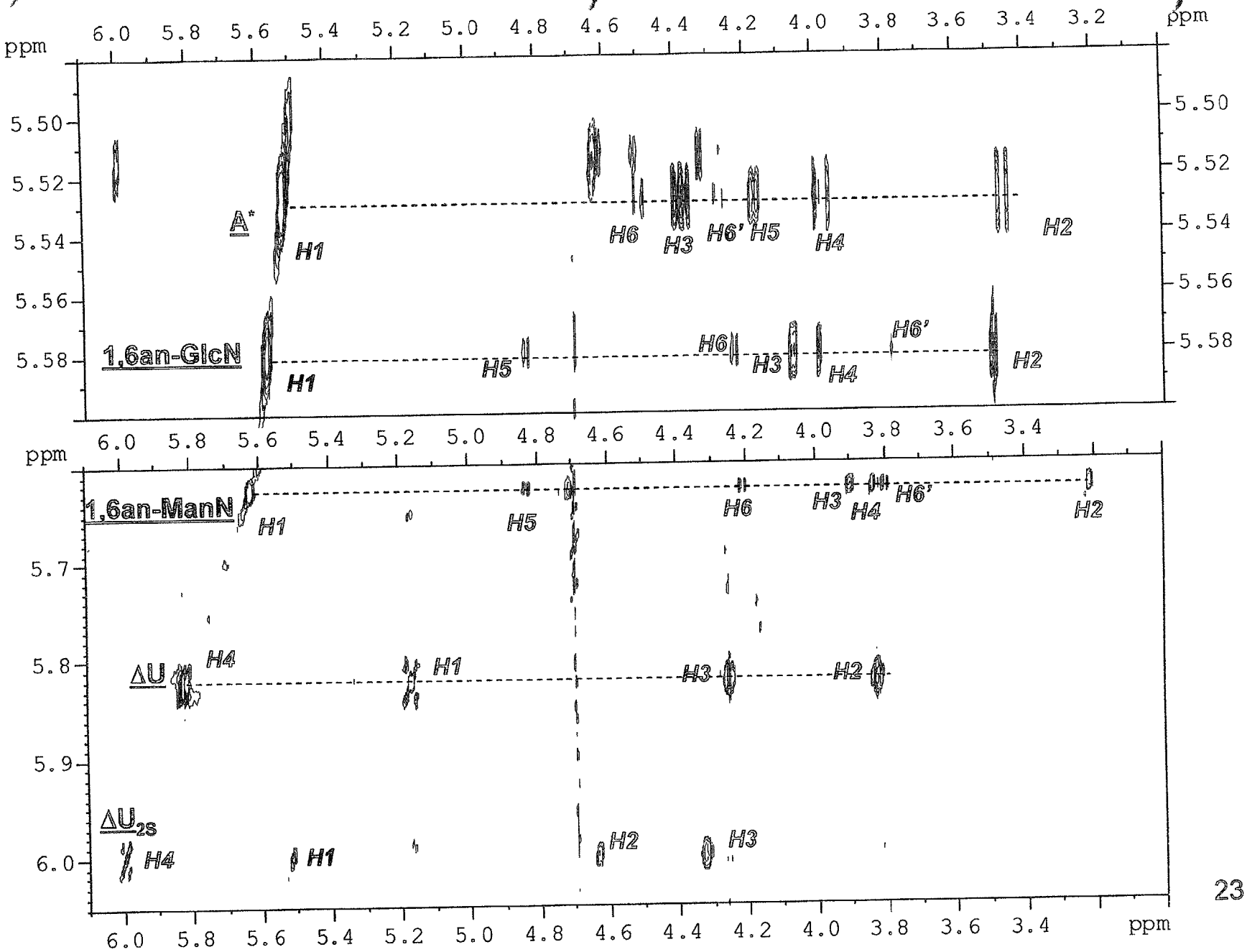


TOCSY spectrum - G4529 (P-32077-80-3) (Octasaccharide Aventis) T= 308K

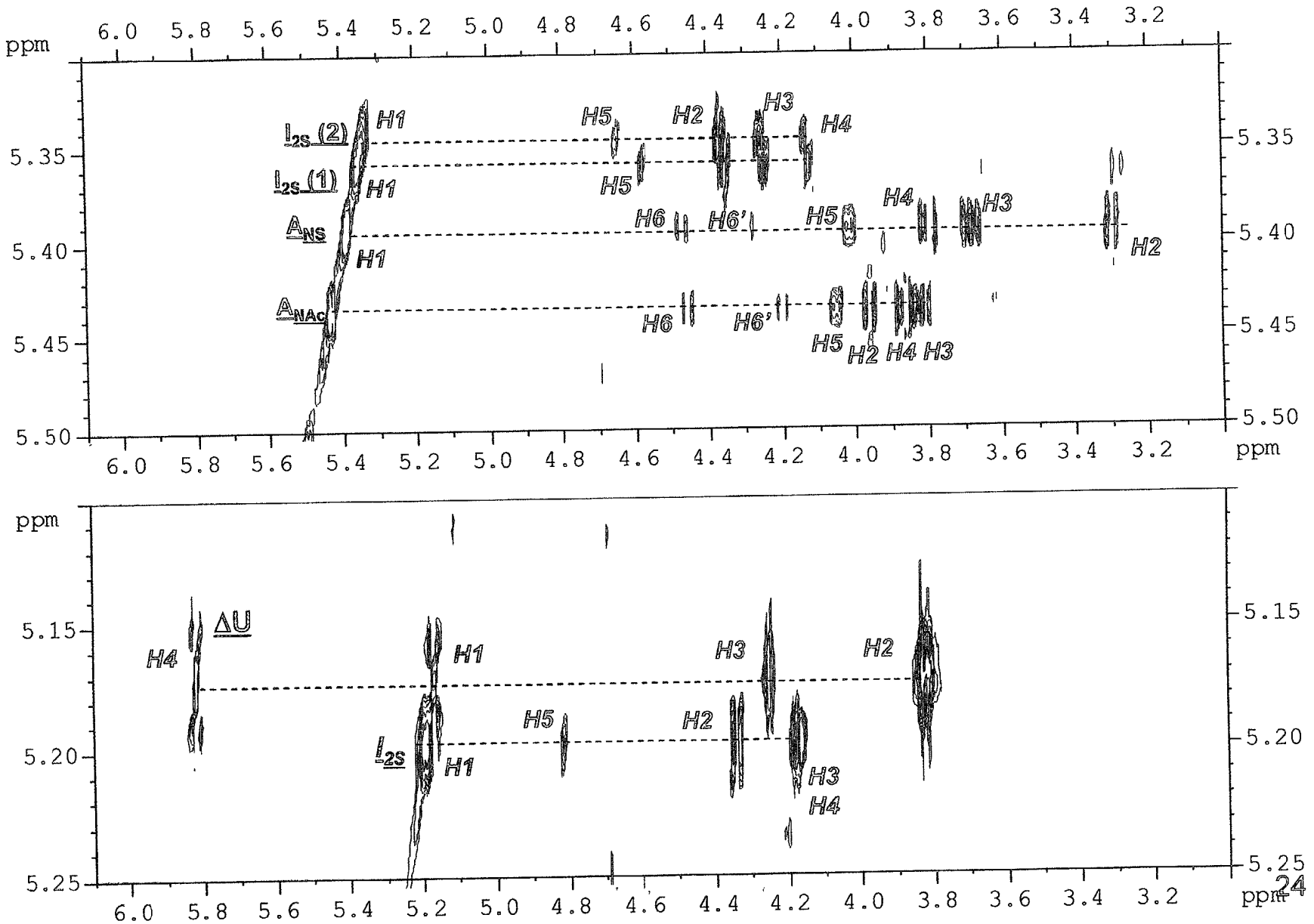


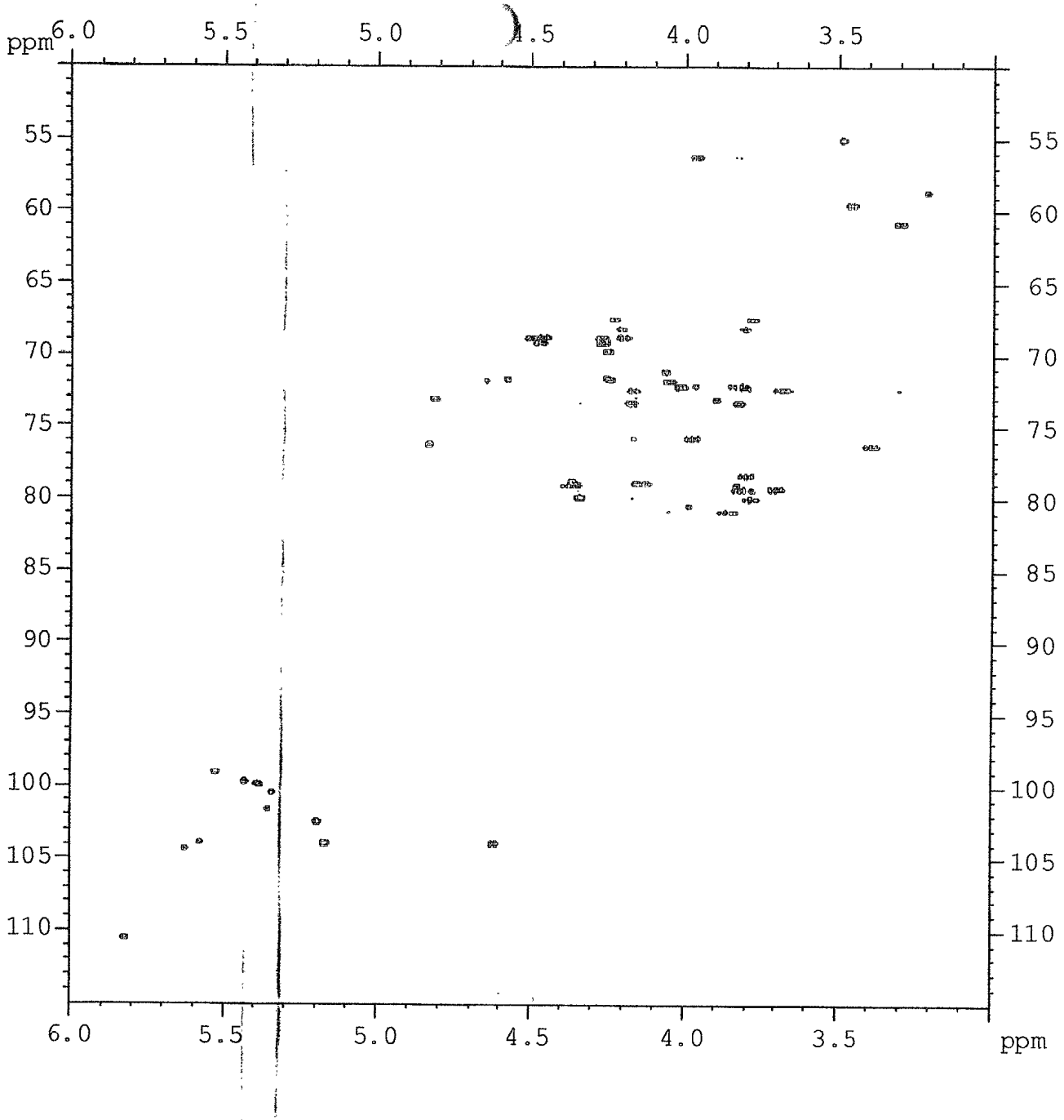

TOCSY spectrum - G4529 (P-3
(Octasaccharide Aventis)
ppm
6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2
5.50 5.52 5.54 5.56 5.58
A*
H1
H6
H3
H6'
H5
H4
H2
1,6an-GlcN
H1
H5
H6
H3
H4
H6'
H2
6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4
1,6an-ManN
H1
H5
H6
H3
H4
H6'
H2
5.7 5.8 5.9 6.0
ΔU
H4
H1
H3
H2
ΔU2S
H4
H1
H2
H3

TOCSY spectrum - G4529 (P-3[illegible]/7-80-5) (Octasaccharide Aventis) [illegible]

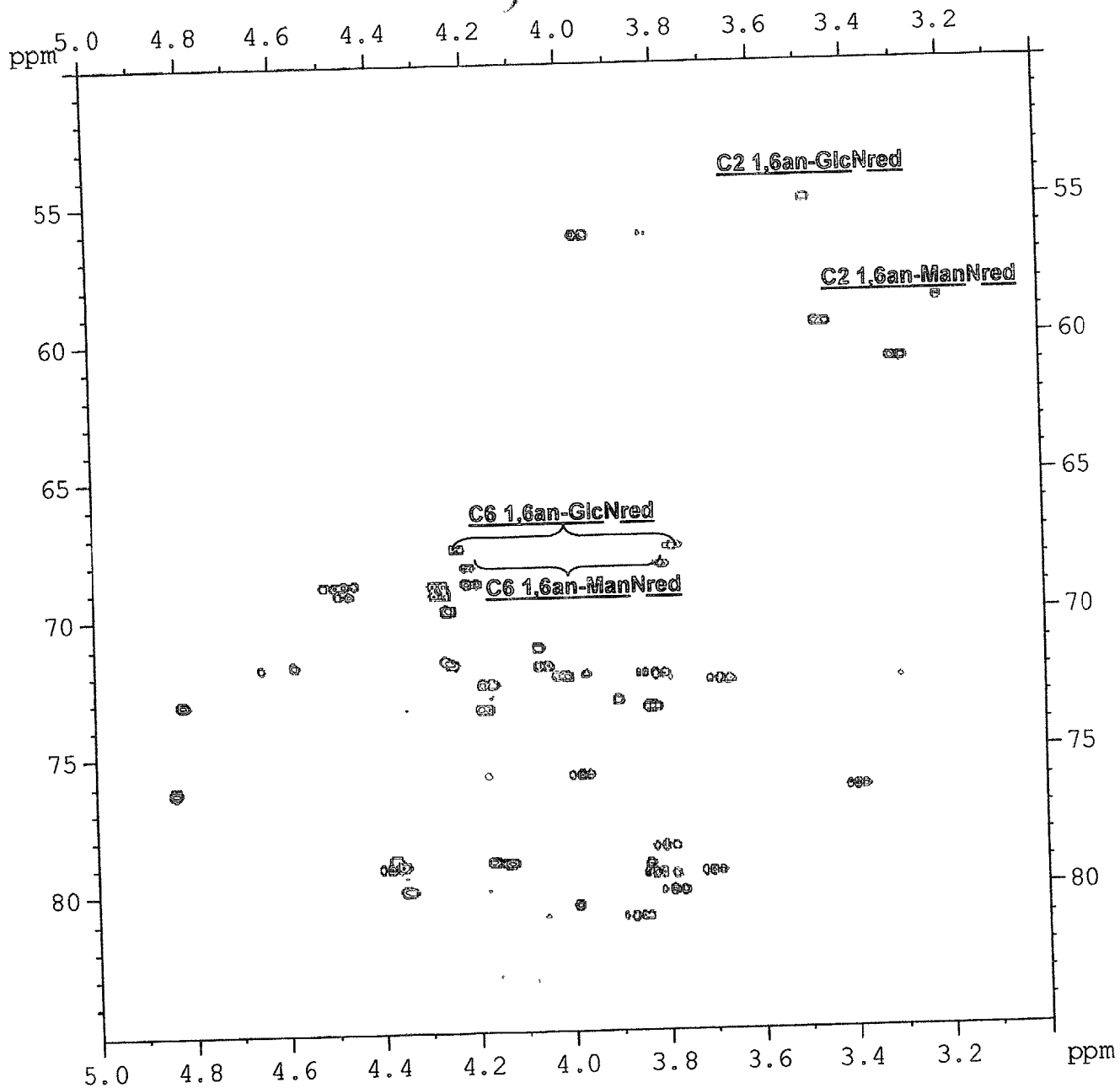


TOCSY spectrum - G4529 (P-32077-80-3) (Octasaccharide Aventis) T= 308K

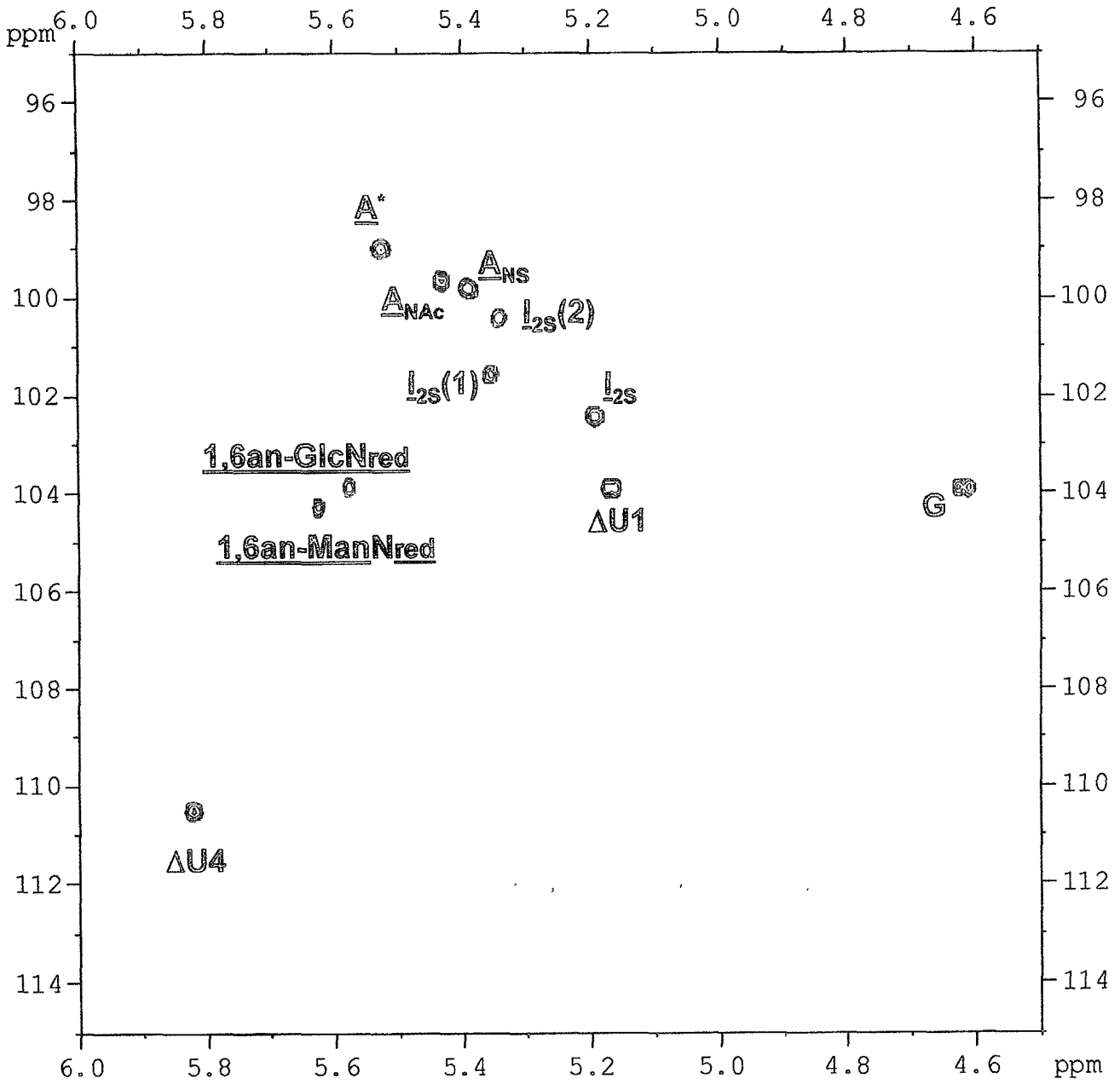



ppm
6.0
5.5
5.0
4.5
4.0
3.5
55
60
65
70
75
80
85
90
95
100
105
110
ppm

HSQC spectrum - G4529 (P-32077-80-3) (Oligosaccharide Aventis)

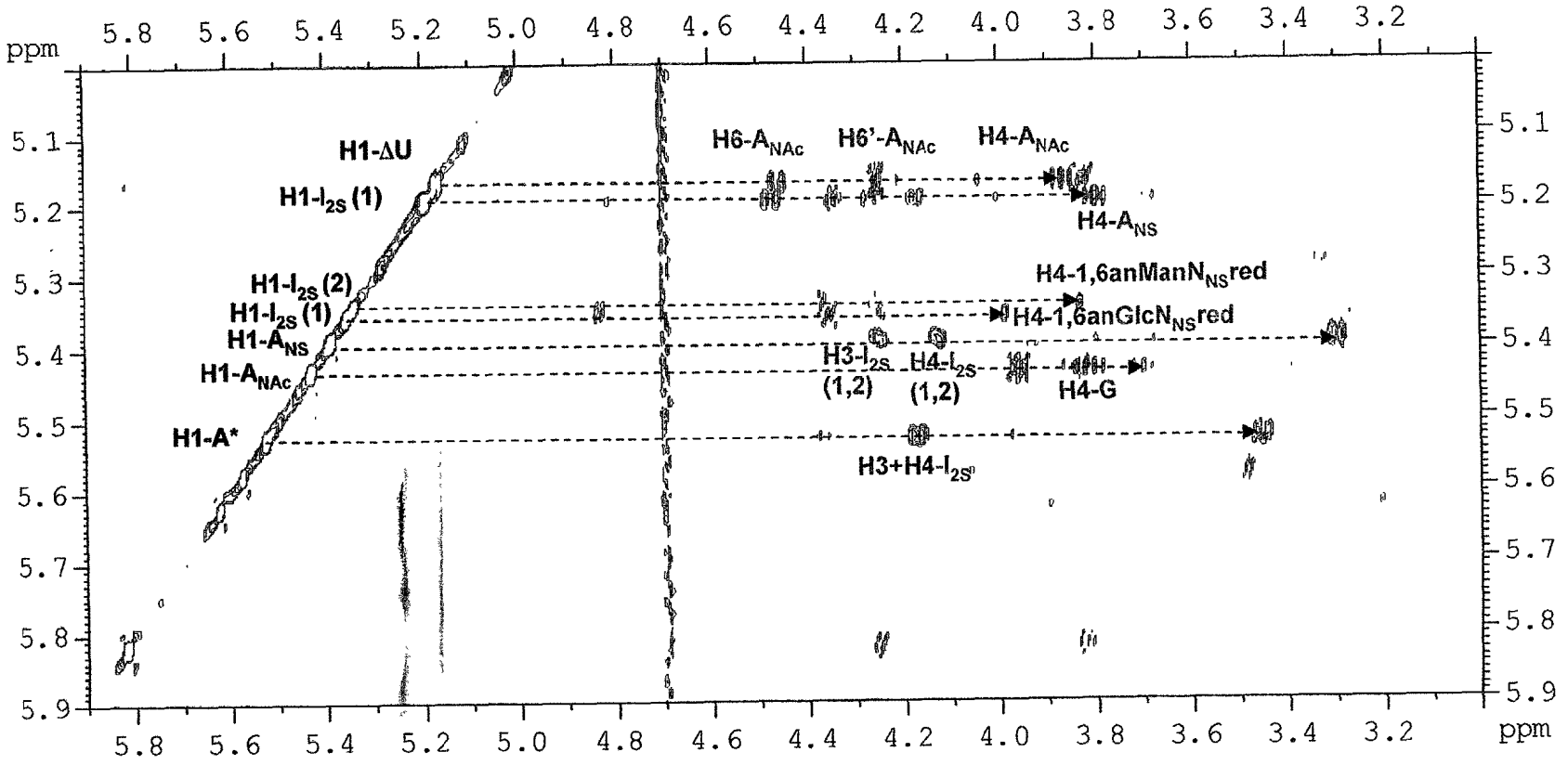


HSQC spectrum (anomeric region) - G4529 (P-32077-80-3) (Octasaccharide Aventis) T= 308K



NOESY spectrum (mix. 300ms) - G4529 (. 32077-80-3) (Octasaccharide Aventis) T= 308k

Red = inter-glycosidic linkage



H-C chemical shifts G4529 (F-32077-80-3) (Octasaccharide Aventis) T = 308K

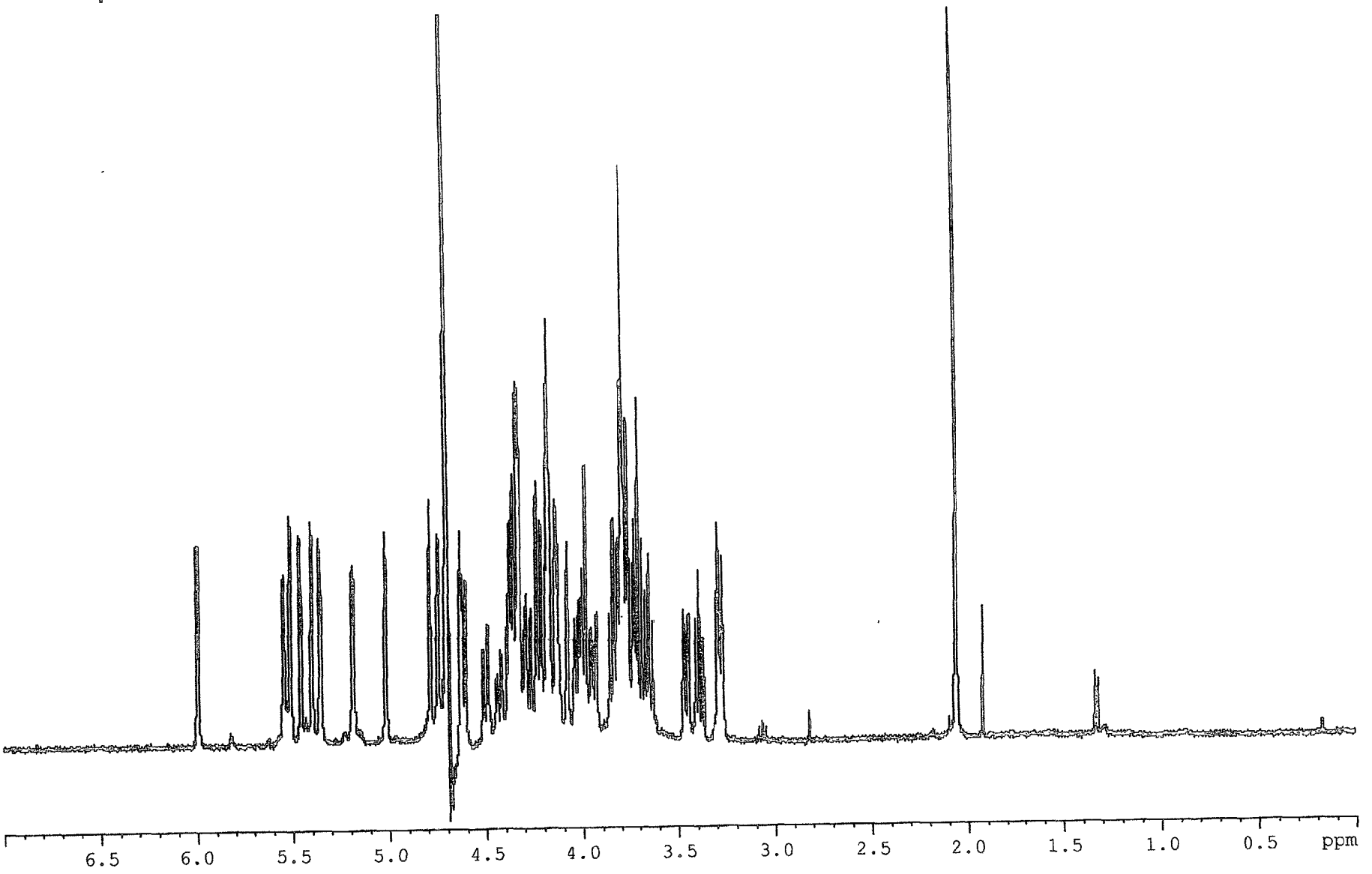
| | ΔU | $A_{NAc}$ | G | A* | $I_{2S}$ | $A_{NS}$ | $I_{2S}$ (1) | $I_{2S}$ (2) | 1,6an-ManN-red | 1,6an-GlcN-red |
|---|---|---|---|---|---|---|---|---|---|---|
| H1/C1 | 5.170/ 103.80 | 5.433/ 99.57 | 4.619/ 103.92 | 5.527/ 98.91 | 5.194/ 102.33 | 5.390/ 99.72 | 5.357/ 101.48 | 5.343/ 100.33 | 5.625/ 104.18 | 5.577/ 103.78 |
| H2/C2 | 3.827/ 73.23 | 3.964/ 56.16 | 3.396/ 76.20 | 3.459/ 59.43 | 4.345/ 79.82 | 3.302/ 60.74 | 4.37/ 78.7 | 4.37/ 78.7 | 3.213/ 58.59 | 3.488/ 54.96 |
| H3/C3 | 4.253/ 69.66 | 3.828/ 72.03 | 3.705/ 79.21 | 4.381/ 78.99 | 4.172/ 72.36 | 3.685/ 72.26 | 4.246/ 71.64 | 4.259/ 71.49 | 3.899/ 72.97 | 4.064/ 71.04 |
| H4/C4 | 5.821/ 110.4 | 3.870/ 80.82 | 3.817/ 79.26 | 3.978/ 75.68 | 4.166/ 78.79 | 3.804/ 78.29 | 4.129/ 78.85 | 4.129/ 78.85 | 3.835/ 78.93 | 3.990/ 80.38 |
| H5/C5 | | 4.018/ 72.09 | 3.789/ 79.89 | 4.179/ 73.23 | 4.815/ 72.95 | 4.055/ 71.72 | 4.581/ 71.60 | 4.649/ 71.67 | 4.83/ 76.1 | 4.83/ 76.1 |
| H6/C6 | | 4.461-4.203/ 68.69 | | 4.503-4.273/ 68.74 | | 4.474-4.269/ 69.09 | | | 4.210-3.807/ 68.08 | 4.234-3.780/ 67.43 |

Octasaccharide sequences of sample batch- P-32077-80-3 (G4259) :

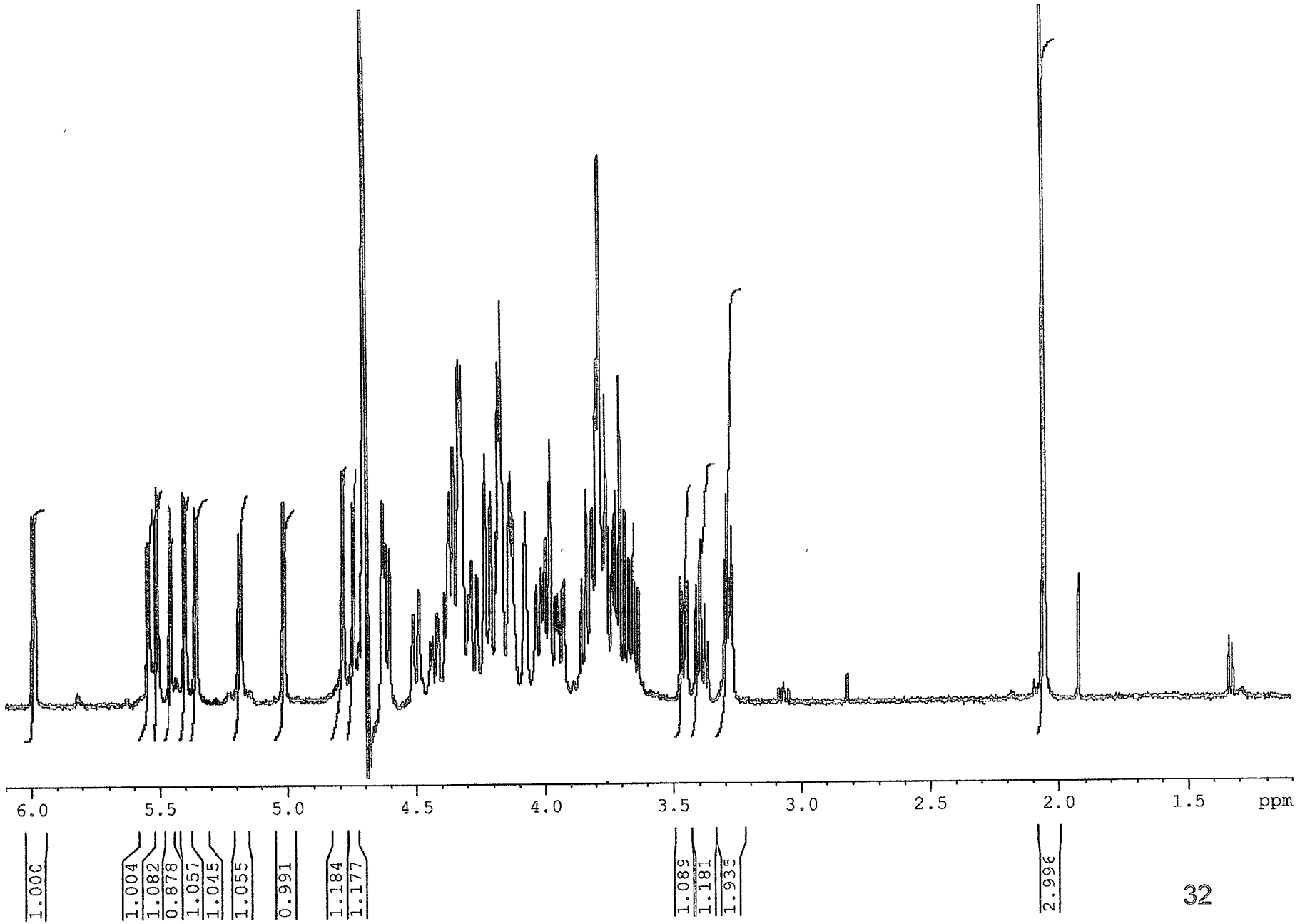
$\Delta U\text{-}A_{NAc,6S}\text{-}G\text{-}A_{NS,3,6S}\text{-}I_{2S}\text{-}A_{NS,6S}\text{-}I_{2S}\text{-}1,6\ an\text{-}GlcN_{NS,6S}$ (1) $\cong 58\%$

$\Delta U\text{-}A_{NAc,6S}\text{-}G\text{-}A_{NS,3,6S}\text{-}I_{2S}\text{-}A_{NS,6S}\text{-}I_{2S}\text{-}1,6an\text{-}ManN_{NS,6S}$ (2) $\cong 42\%$

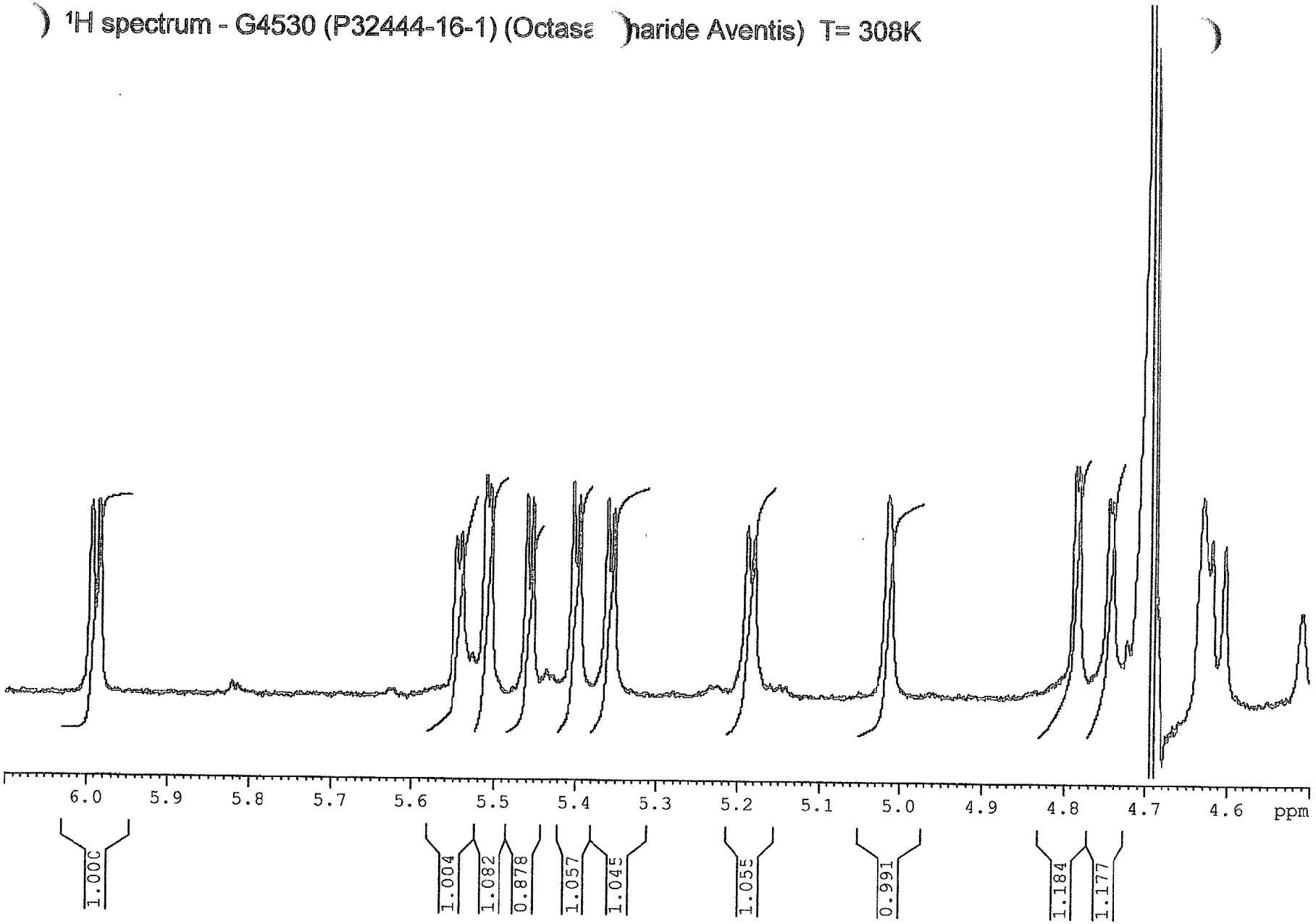
$^1$H spectrum - G4530 (P32444-16-1) (Octasaccharide Aventis) T= 308K



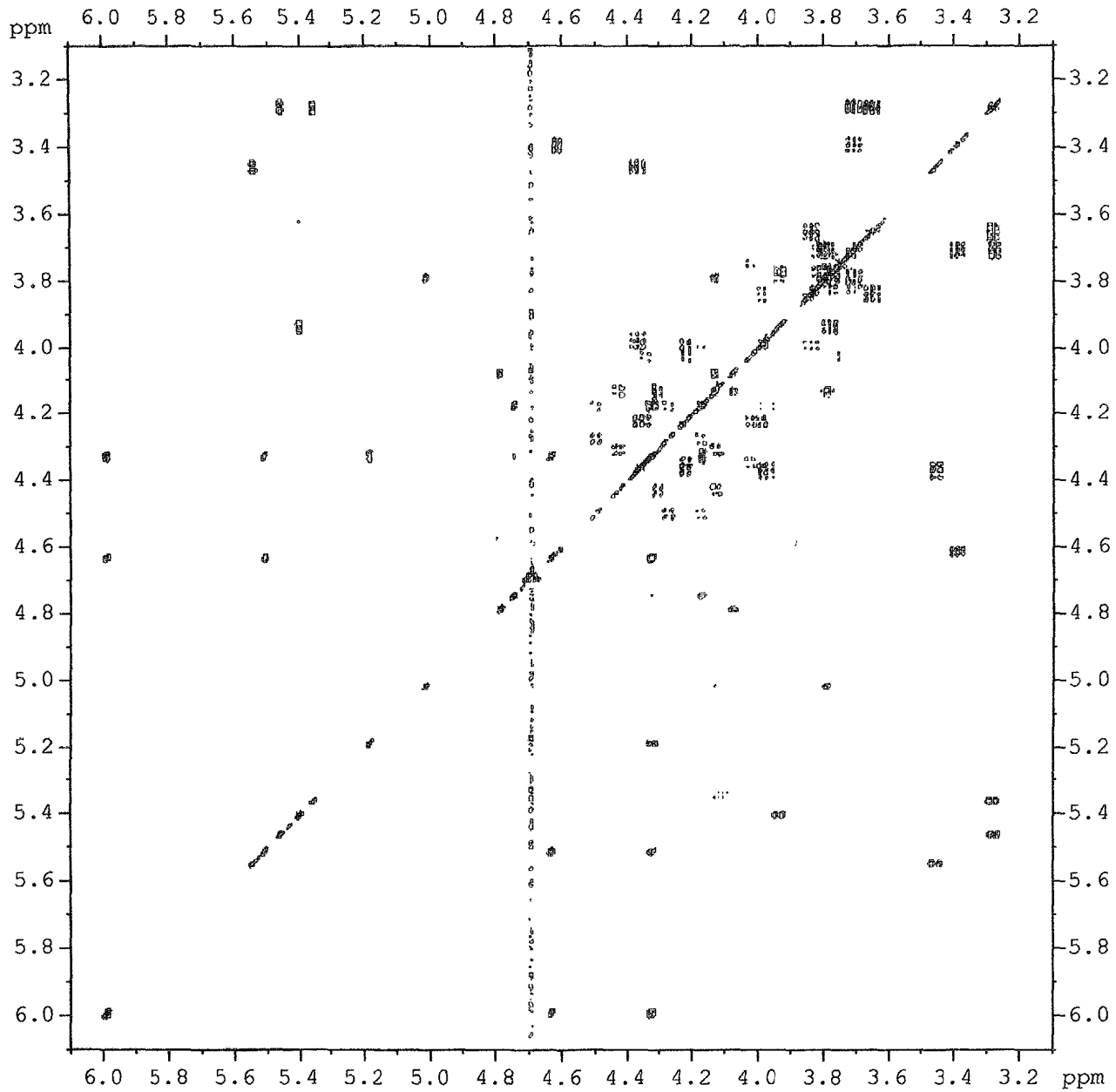
$^1$H spectrum - G4530 (P32444-16-1) (Octasa aride Aventis) T= 308K



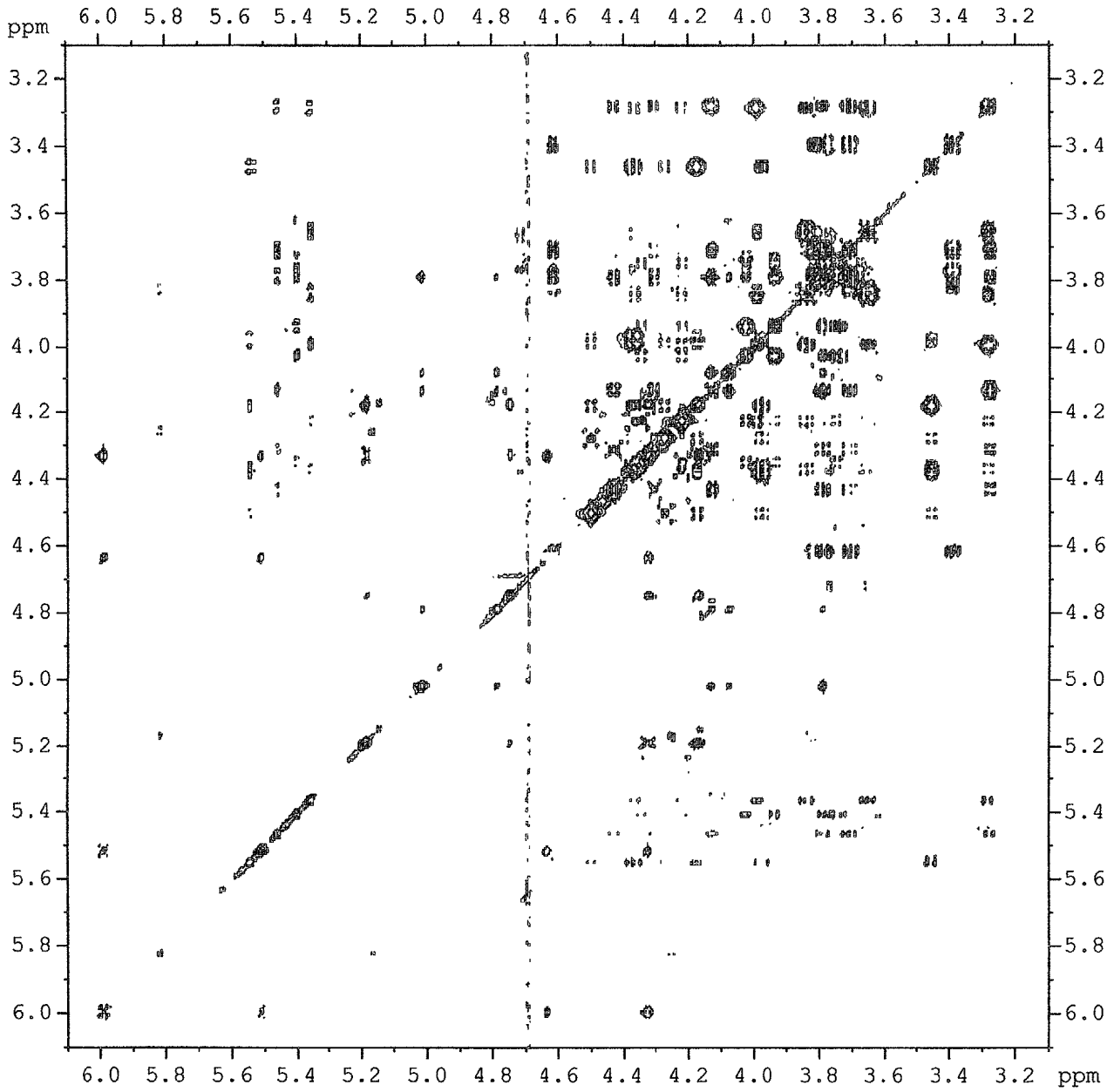
) $^1$H spectrum - G4530 (P32444-16-1) (Octasa haride Aventis) T= 308K



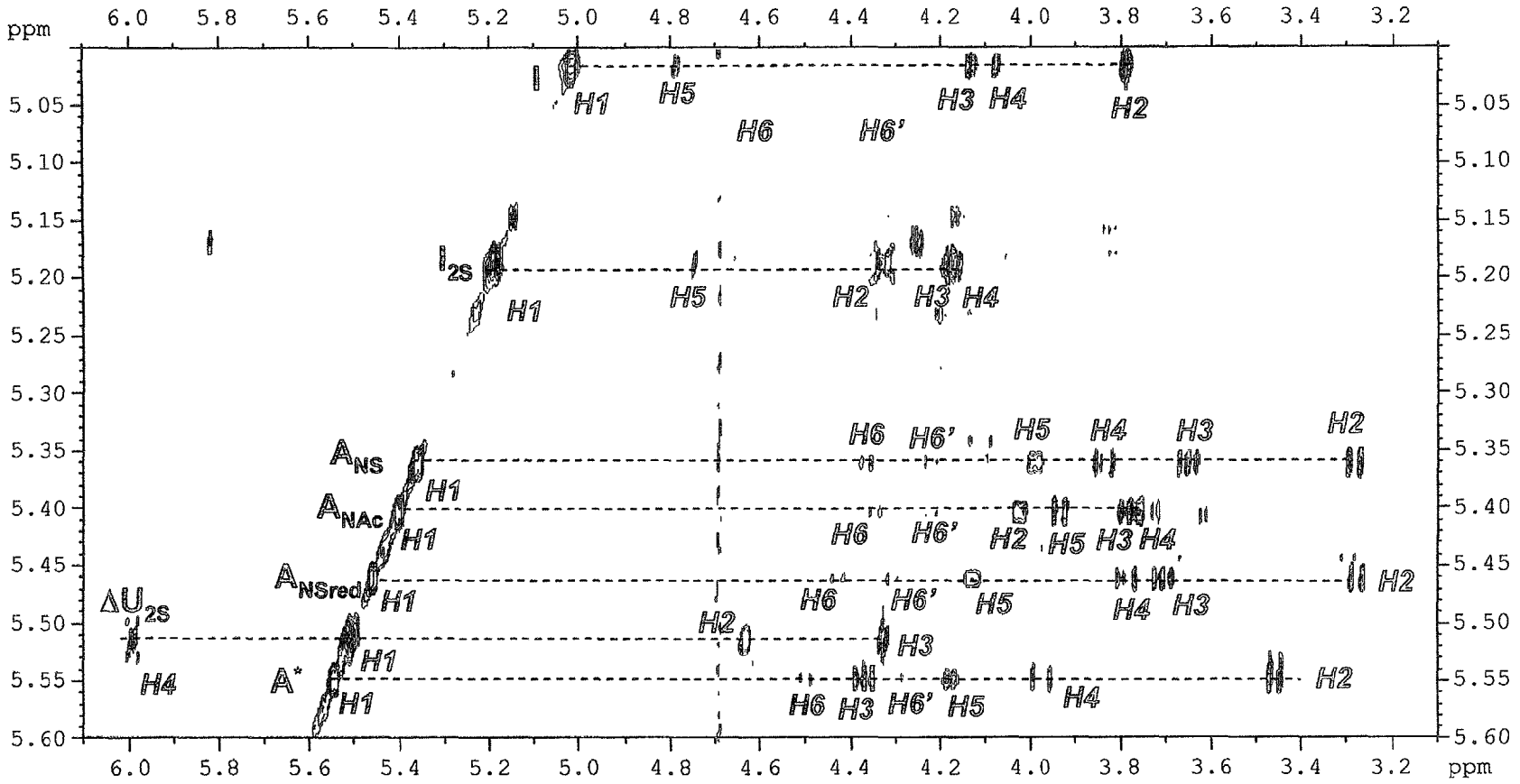
COSY spectrum - G4530 (P32444-16 ) (Octasaccharide Aventis) T= 308K



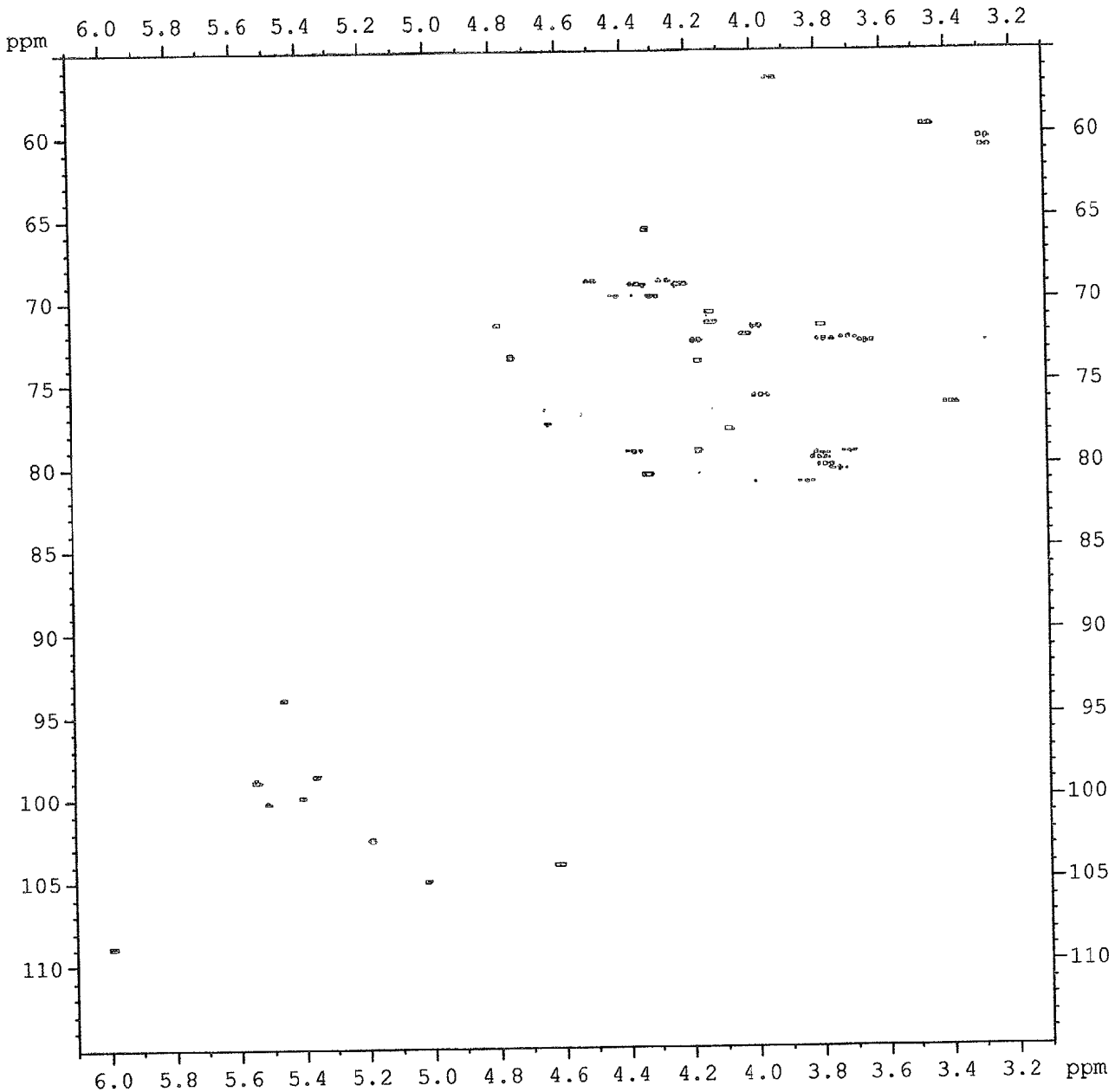
TOCSY spectrum - G4530 (P32444- 1) (Octasaccharide Aventis) T= 308K



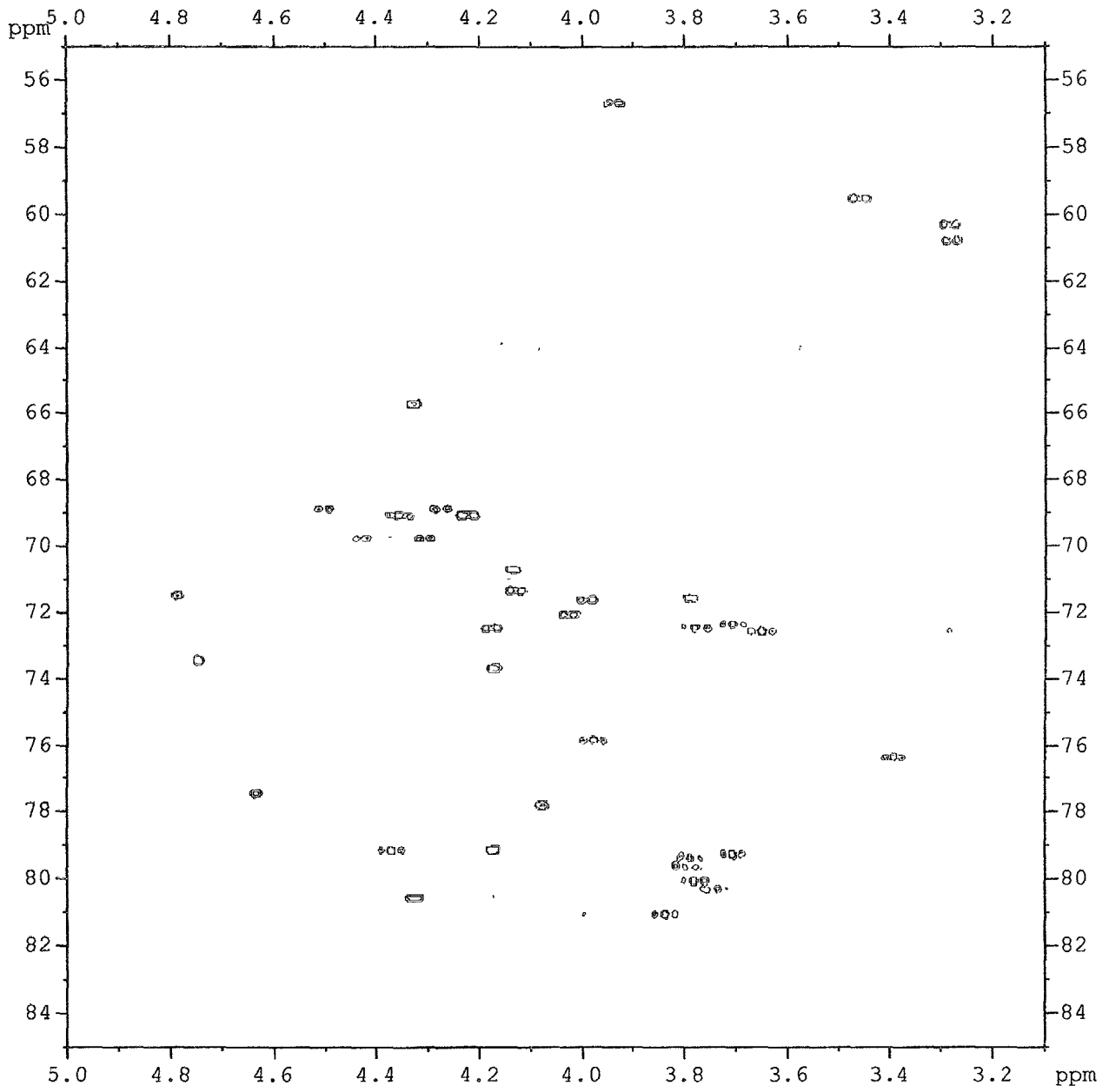
TOCSY spectrum - G4530 (P32444-16-1) (Octasaccharide Aventis) T= 308K



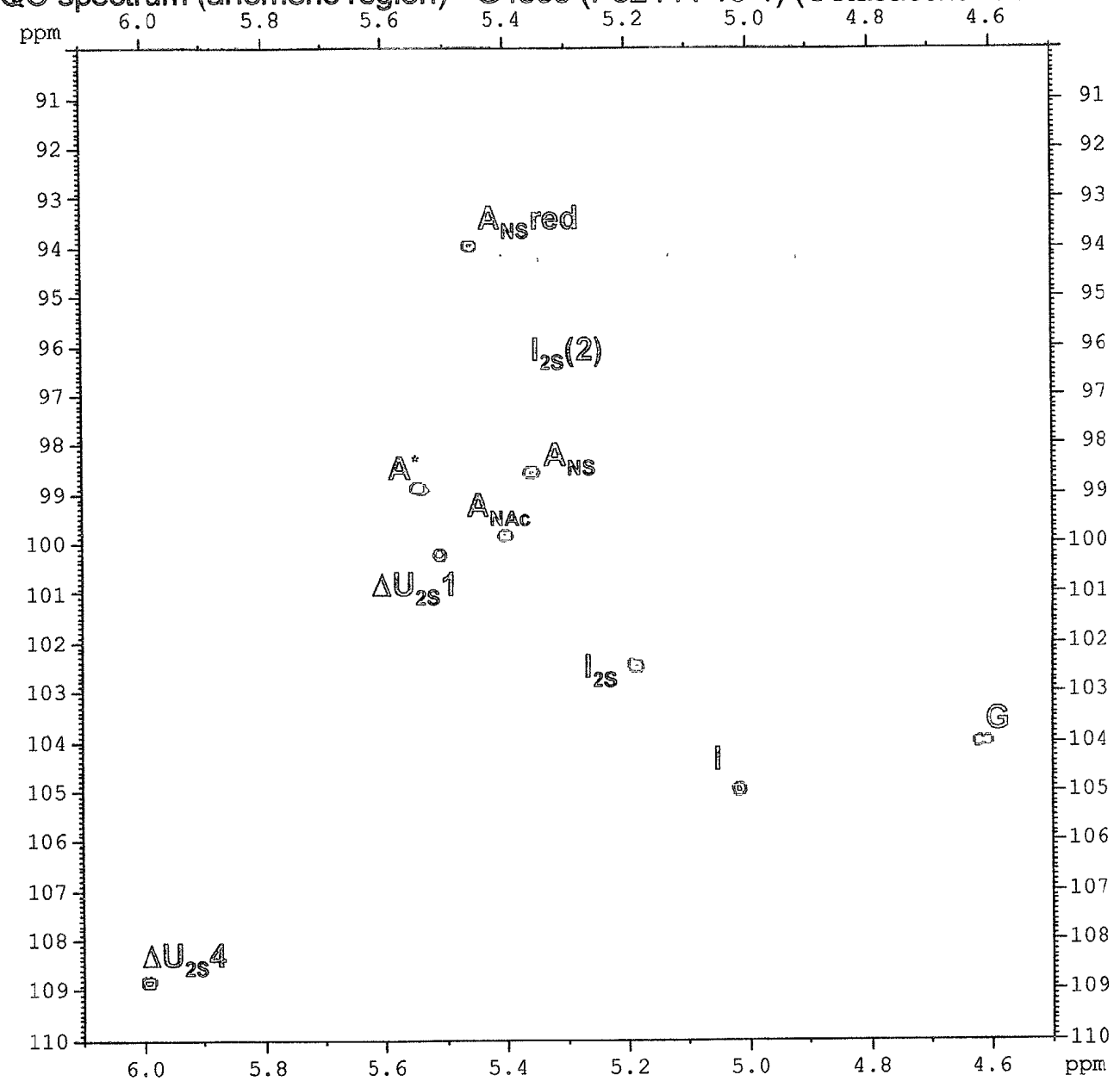
HSQC spectrum - G4530 (P32444 1) (Octasaccharide Aventis) T= 308K



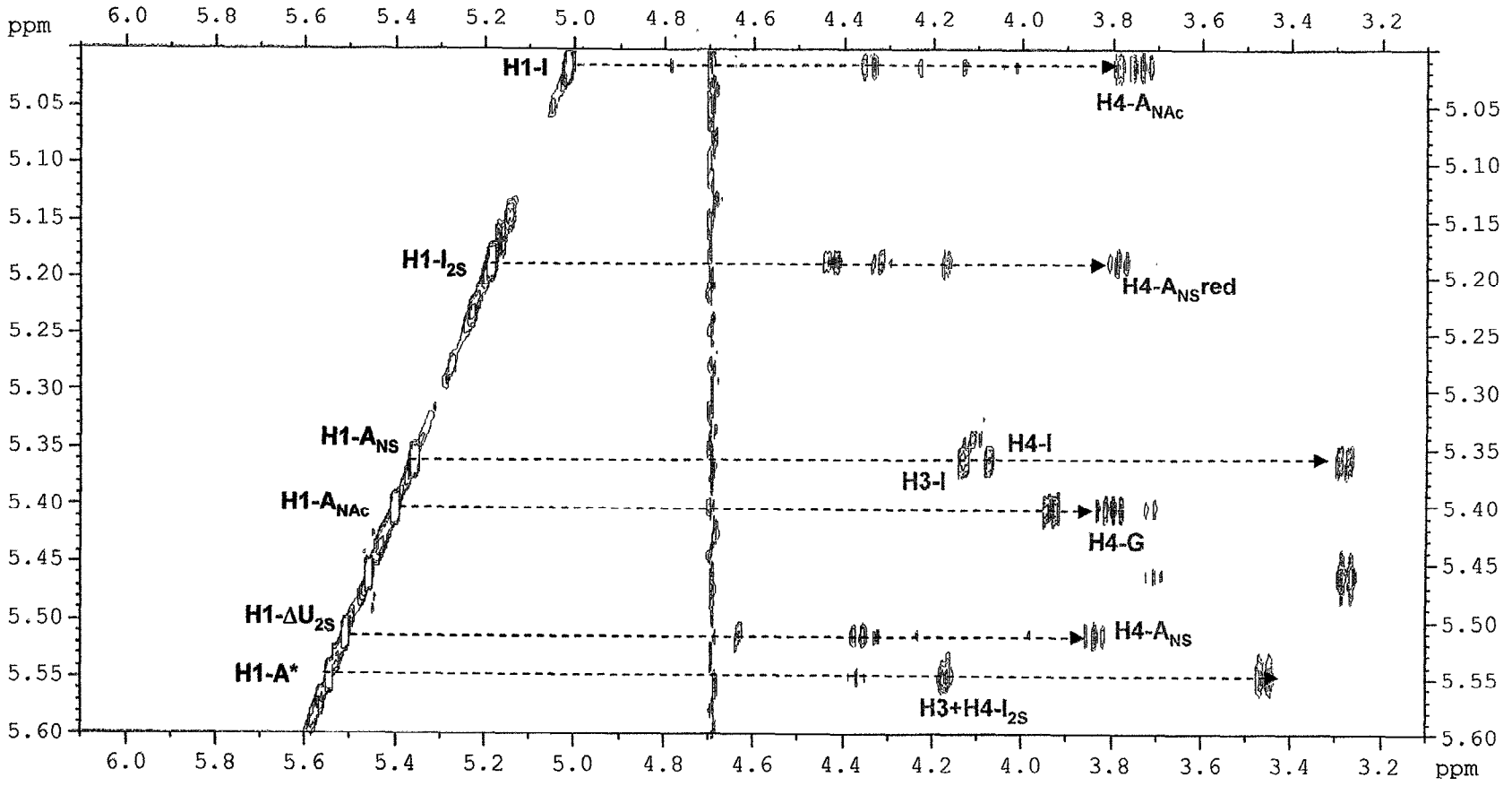
HSQC spectrum - G4530 (P32444-16-1) (O saccharide Aventis) T= 308K



HSQC spectrum (anomeric region) - G4530 (P32444-16-1) (Octasaccharide Aventis) T= 308K



NOESY spectrum (mix. 300ms) - G4530 (P32444-16-1) (Octasaccharide Aventis) T= 308K
Red = inter-glycosidic linkage

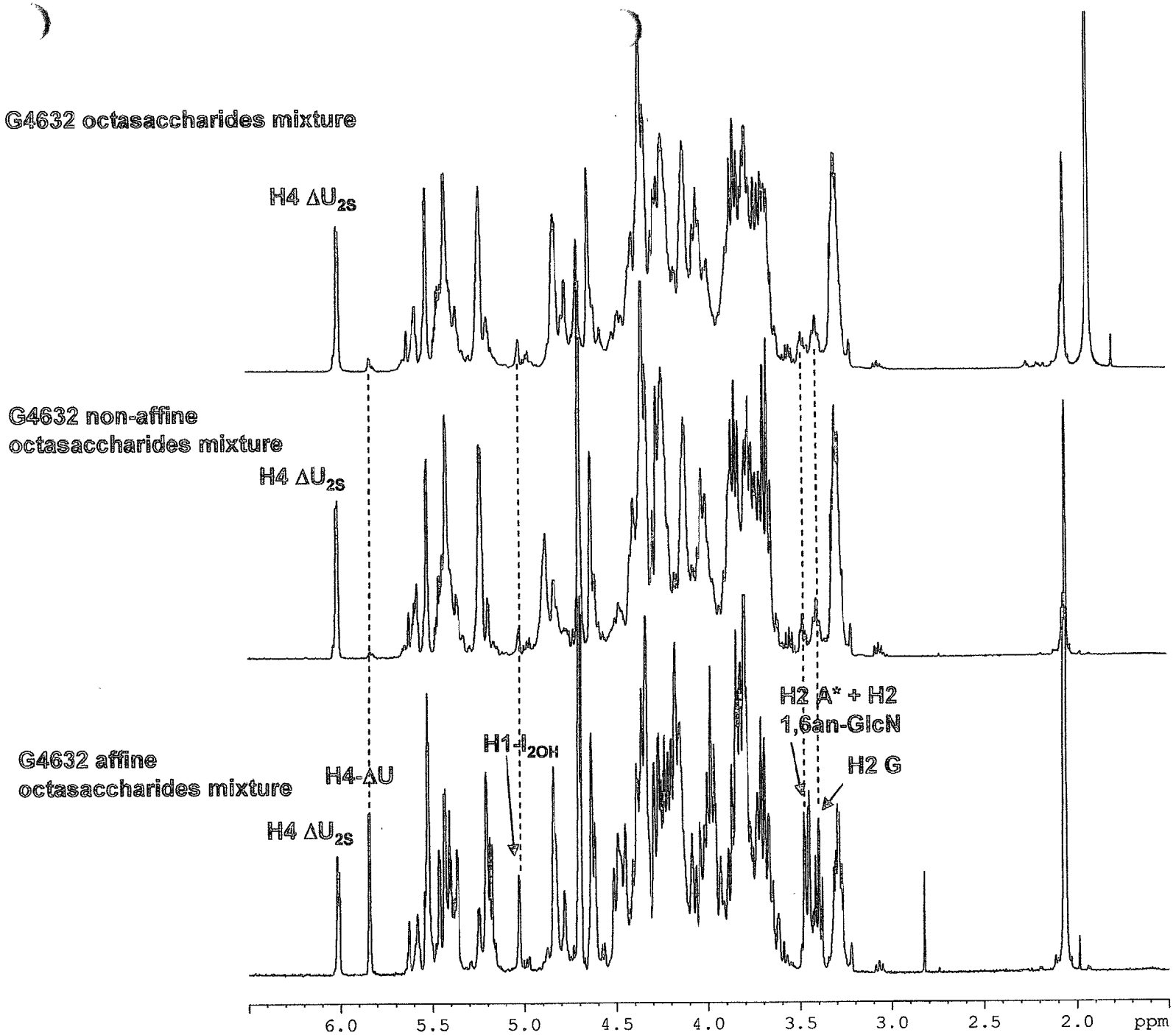


$^{1}H$-$^{13}C$ chemical shifts G4530 (P-32444-16-1) (Octasaccharide Aventis) T= 308K

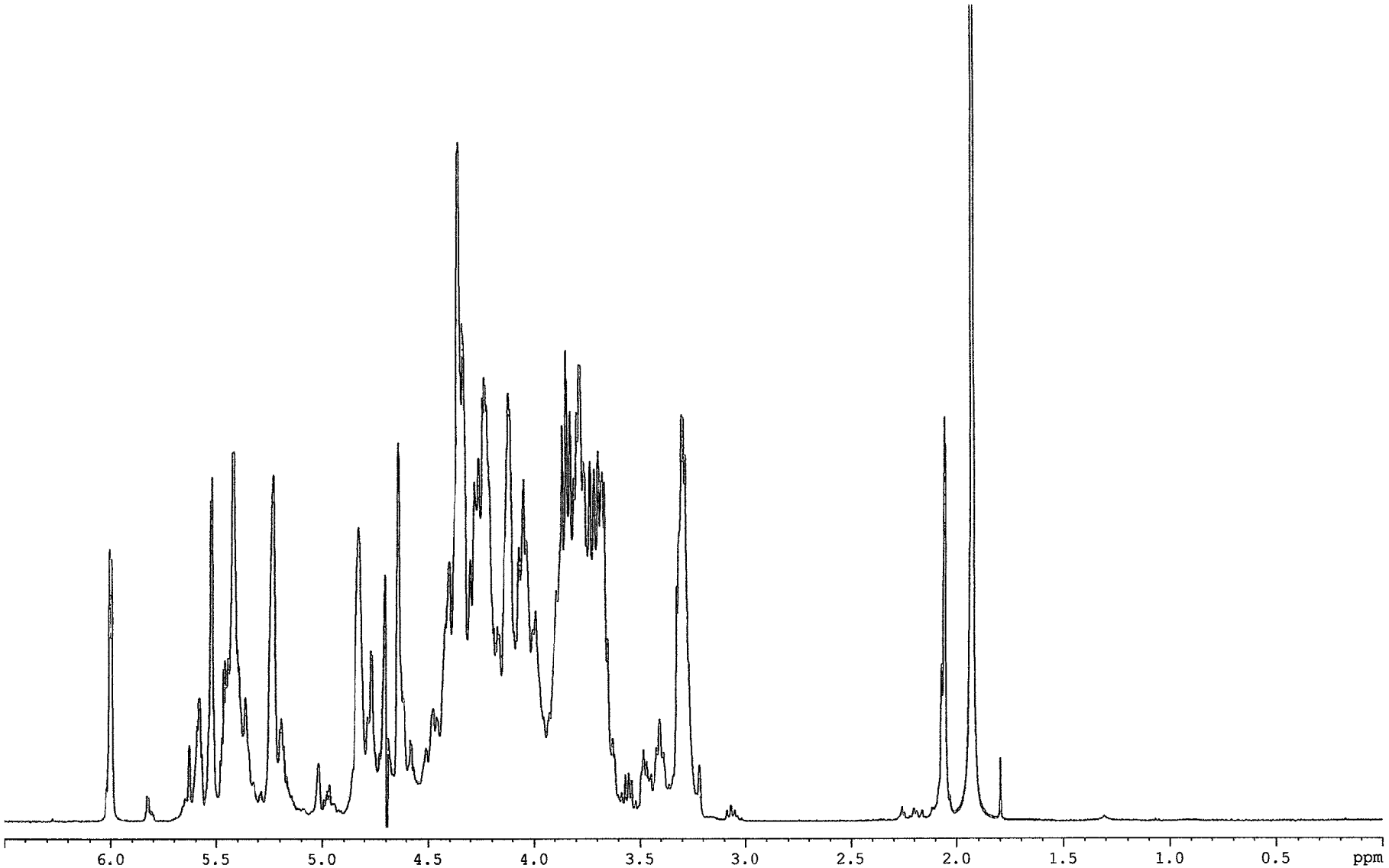
| | $\Delta U_{2S}$ | $A_{NS}$ | $I_{OH}$ | $A_{NAc}$ | G | $A^{*}$ | $I_{2S}$ | $A_{NS}red$ |
|---|---|---|---|---|---|---|---|---|
| H1/C1 | 5.511/ 100.13 | 5.360/ 98.47 | 5.018/ 104.84 | 5.402/ 99.74 | 4.616/ 103.89 | 5.546/ 98.78 | 5.188/ 102.38 | 5.459/ 93.87 |
| H2/C2 | 4.634/ 77.35 | 3.287/ 60.23 | 3.790/ 71.46 | 3.941/ 56.62 | 3.394/ 76.24 | 3.463/ 59.44 | 4.326/ 80.45 | 3.282/ 60.72 |
| H3/C3 | 4.327/ 65.60 | 3.654/ 72.45 | 4.134/ 70.62 | 3.783/ 72.36 | 3.708/ 79.12 | 4.371/ 79.02 | 4.173/ 73.57 | 3.709/ 72.25 |
| H4/C4 | 5.993/ 108.71 | 3.840/ 80.92 | 4.077/ 77.68 | 3.737/ 80.18 | 3.800/ 79.49 | 3.979/ 75.74 | 4.174/ 79.01 | 3.791/ 79.23 |
| H5/C5 | | 3.994/ 71.51 | 4.788/ 71.42 | 4.027/ 71.00 | 3.783/ 79.90 | 4.179/ 72.37 | 4.747/ 73.34 | 4.130/ 71.24 |
| H6/C6 | | 4.366-4.221/ 68.98 | | 4.348-4.221/ 68.98 | | 4.501-4.275/ 68.77 | | 4.429-4.307/ 69.65 |

Octasaccharide sequences of sample batch- P-32444-16-1 (G4530) :

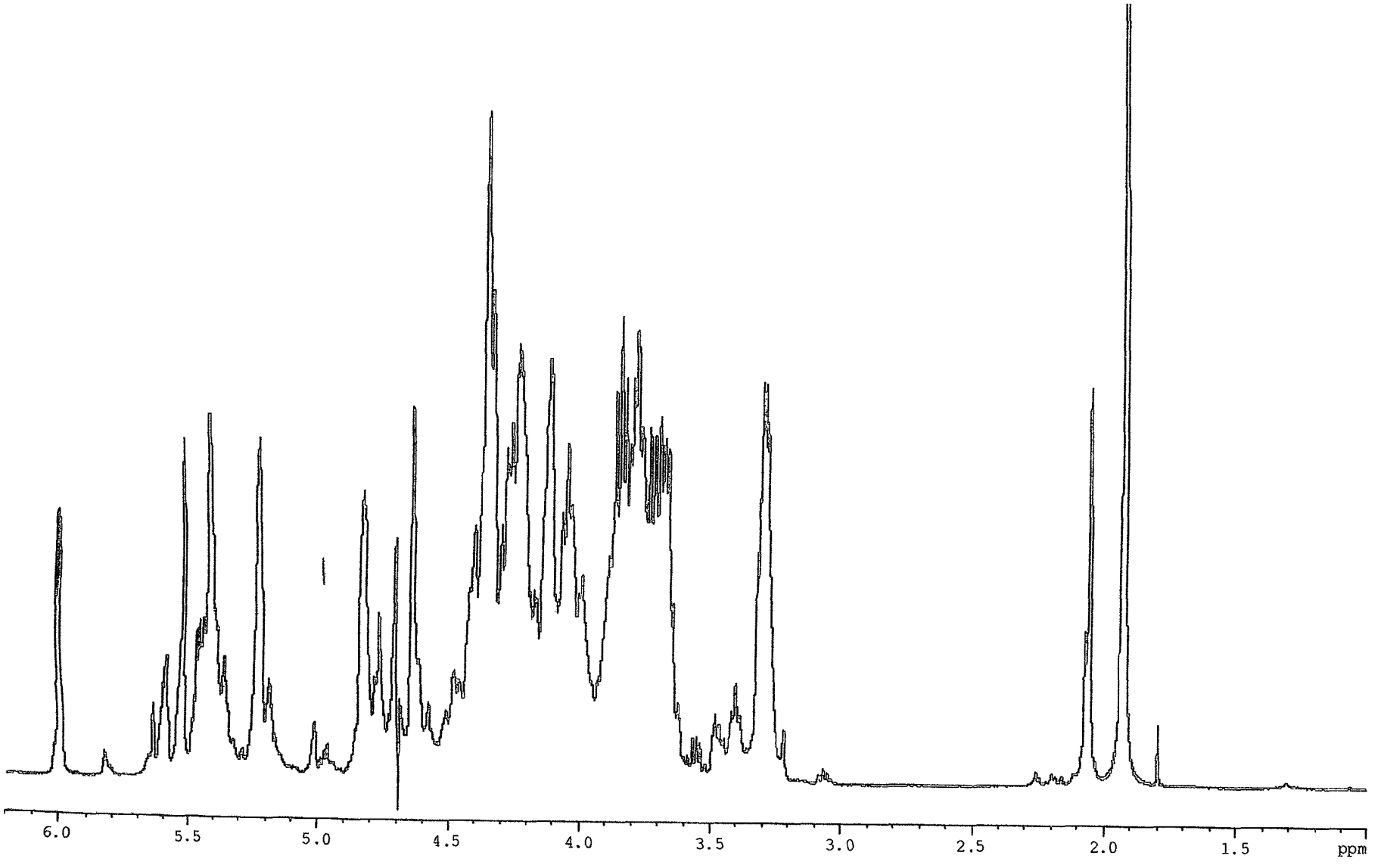
$$\Delta U_{2S}\text{-}A_{NS}\text{-}I\text{-}A_{NAc}\text{-}G\text{-}A_{NS,3,6S}\text{-}I_{2S}\text{-}A_{NS,6S} \quad \cong 100\%$$


G4632 octasaccharides mixture
H4 ΔU2S
G4632 non-affine octasaccharides mixture
H4 ΔU2S
G4632 affine octasaccharides mixture
H4-ΔU
H4 ΔU2S
H1-I2OH
H2 A* + H2 1,6an-GlcN
H2 G
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm

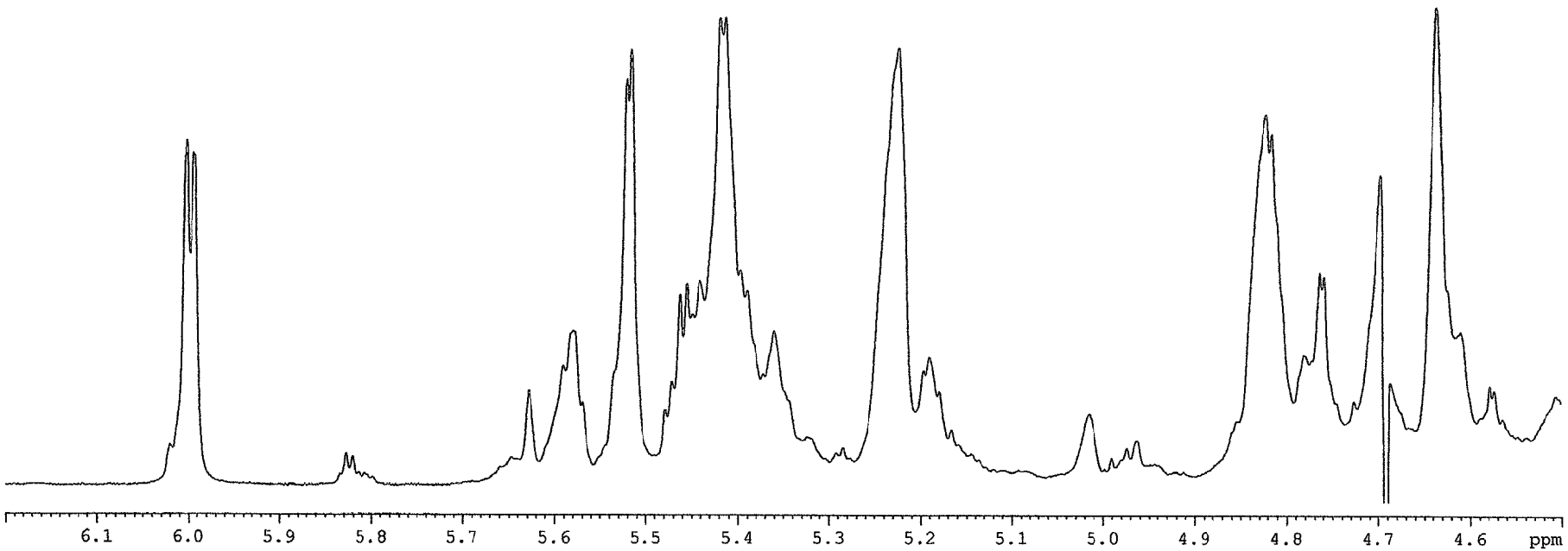
$^1$H spectrum - G4532 (P-31267-28-BIE10) (Octasaccharides mixture Aventis) T= 308K



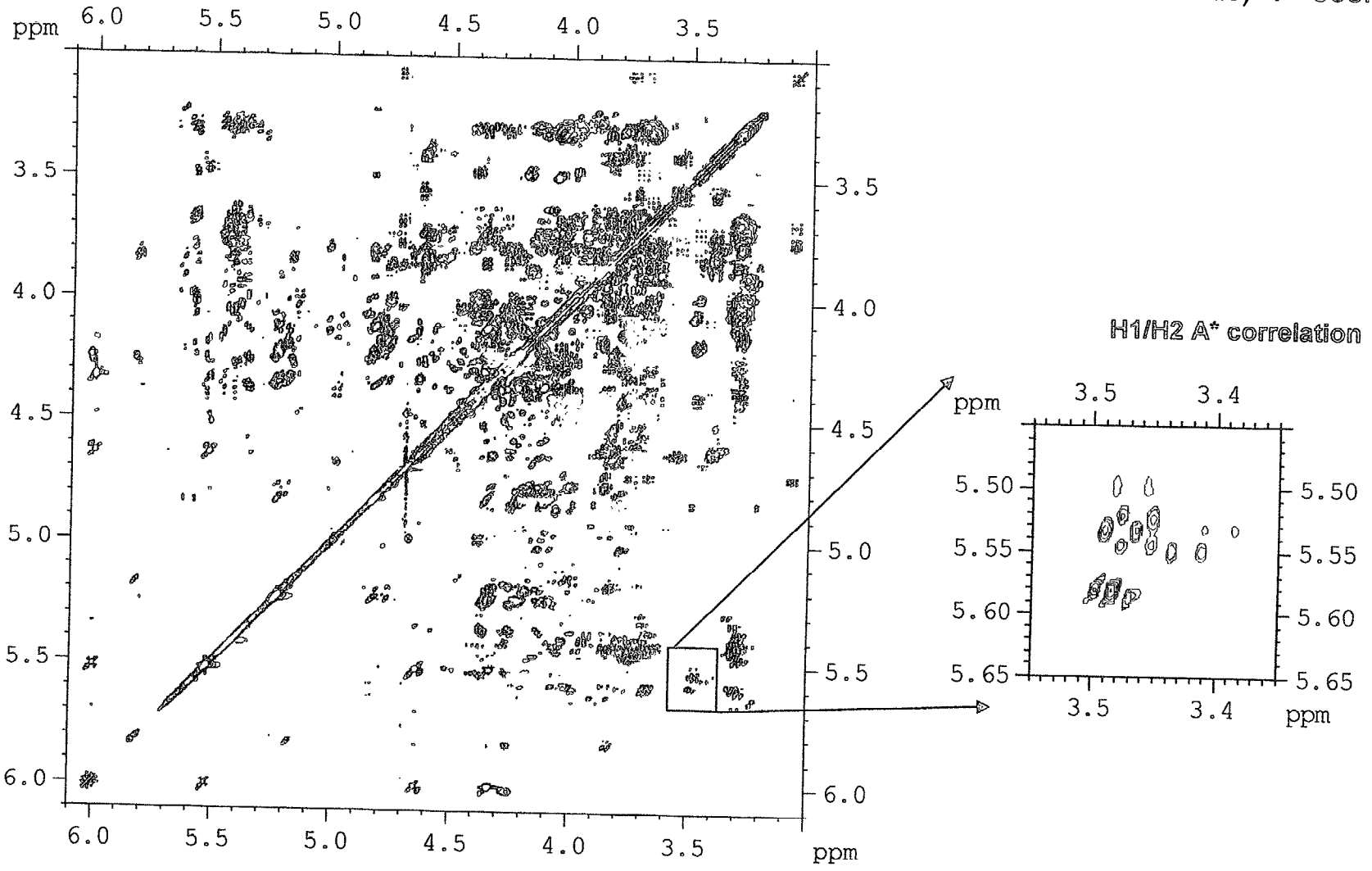
) $^1$H spectrum - G4532 (P-31267-28-BIE10) ( )tasaccharides mixture Aventis) T= 308K )

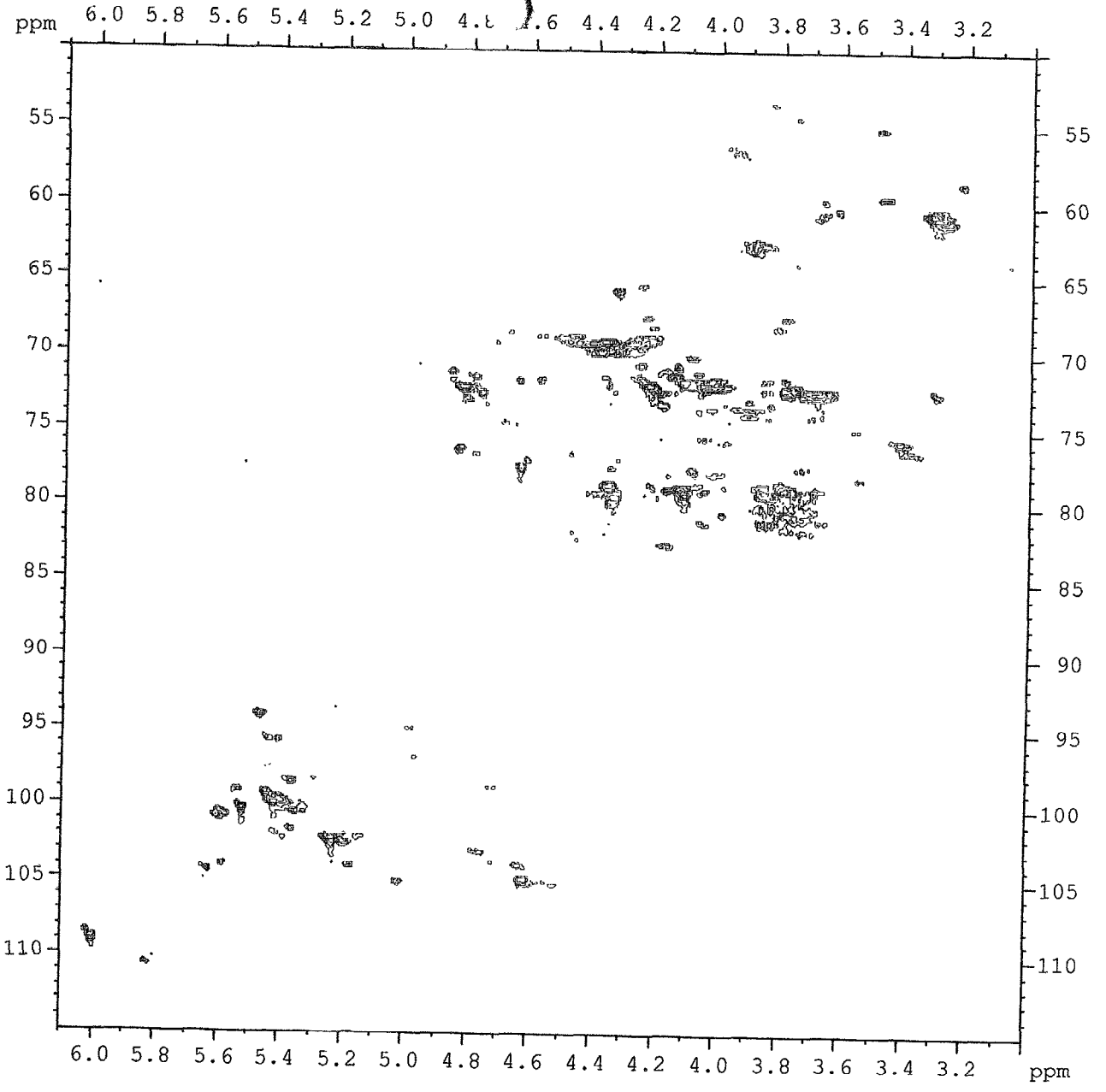


$^1$H spectrum - G4532 (P-31267-28-BIE10) (Octasaccharides mixture Aventis) T= 308K

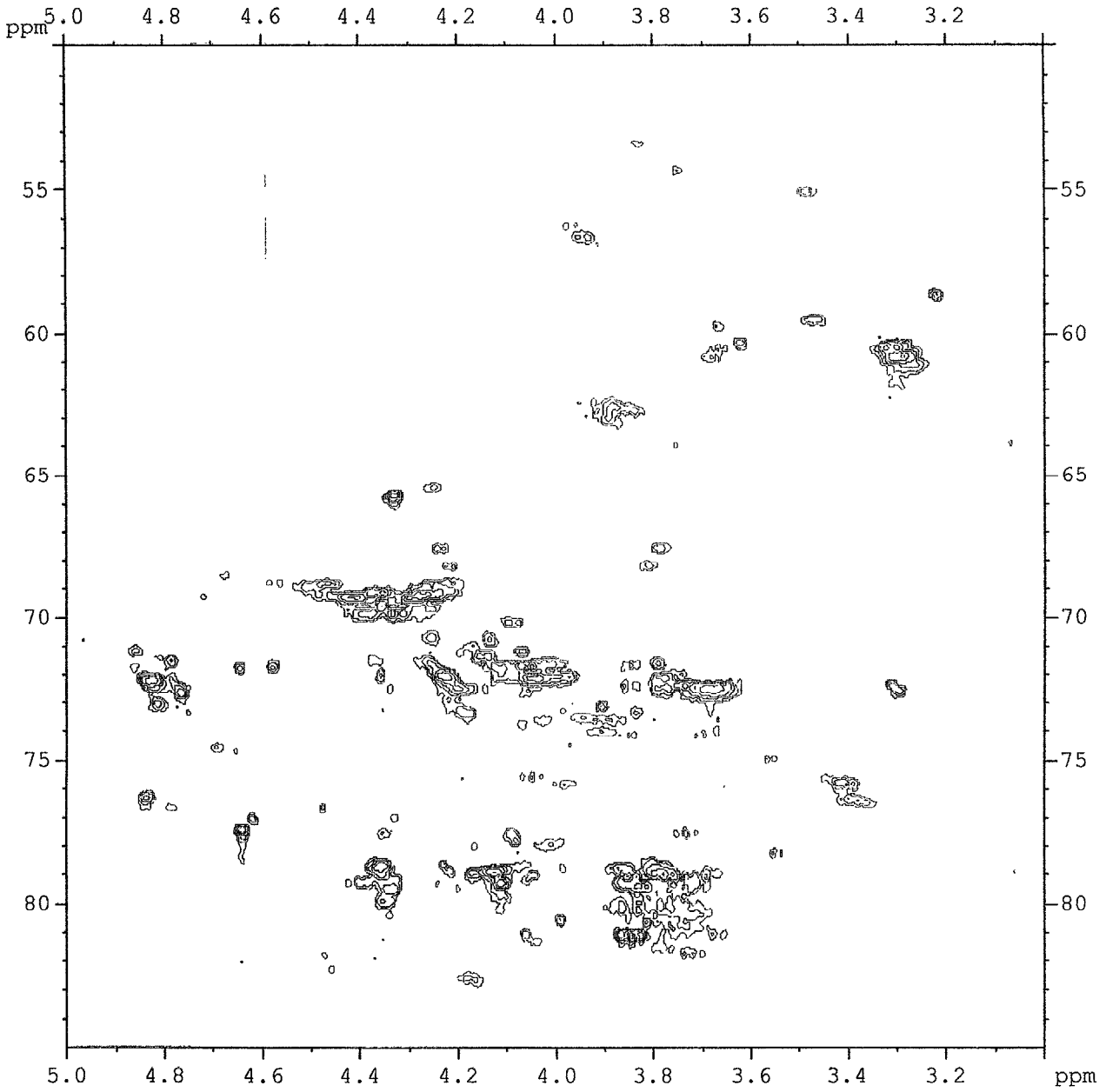


TOCSY spectrum - G4532 (P-31267-28-BIE10) (Octasaccharides mixture Aventis) T= 308K

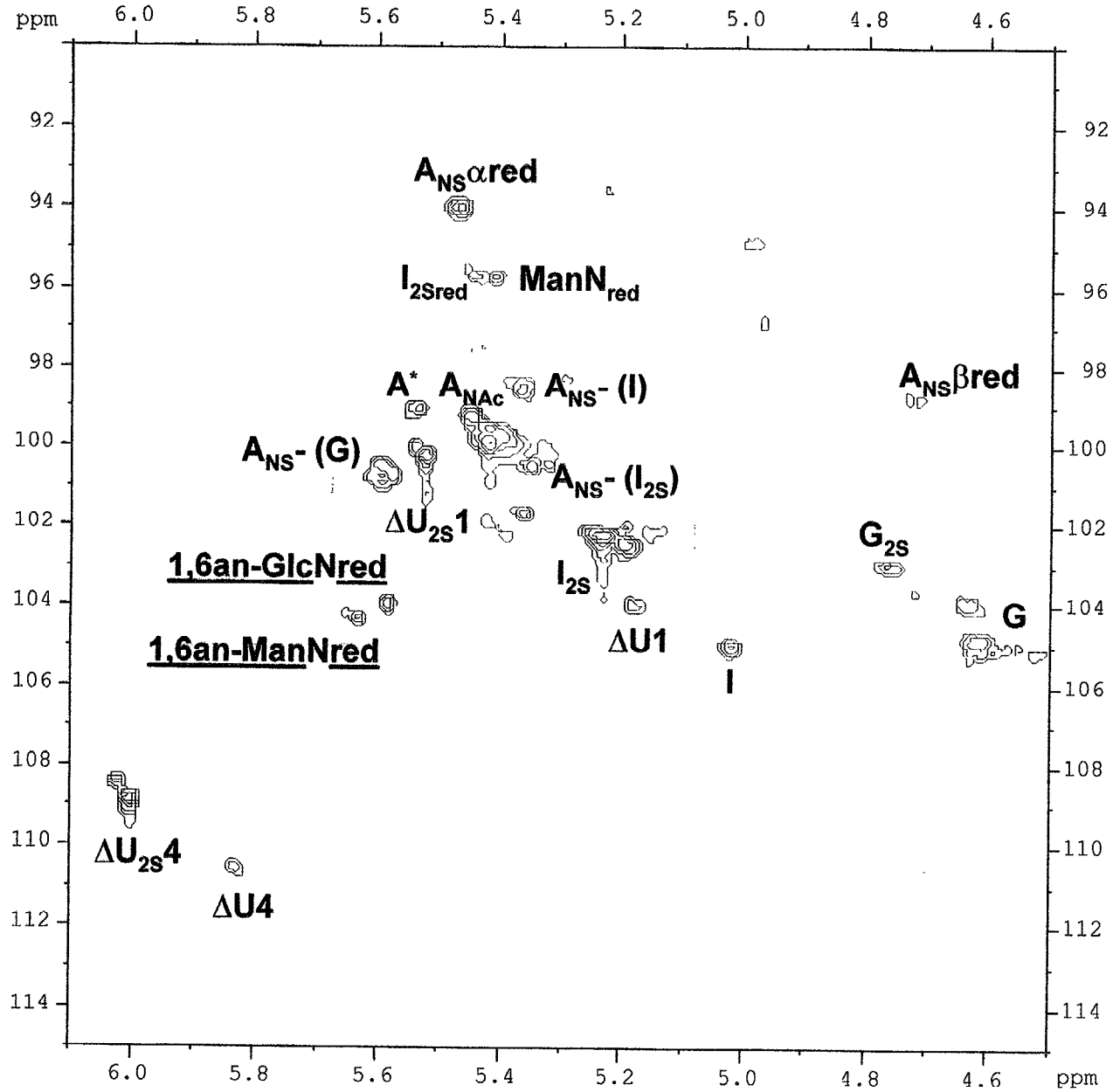



ppm
6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2
55 60 65 70 75 80 85 90 95 100 105 110
ppm

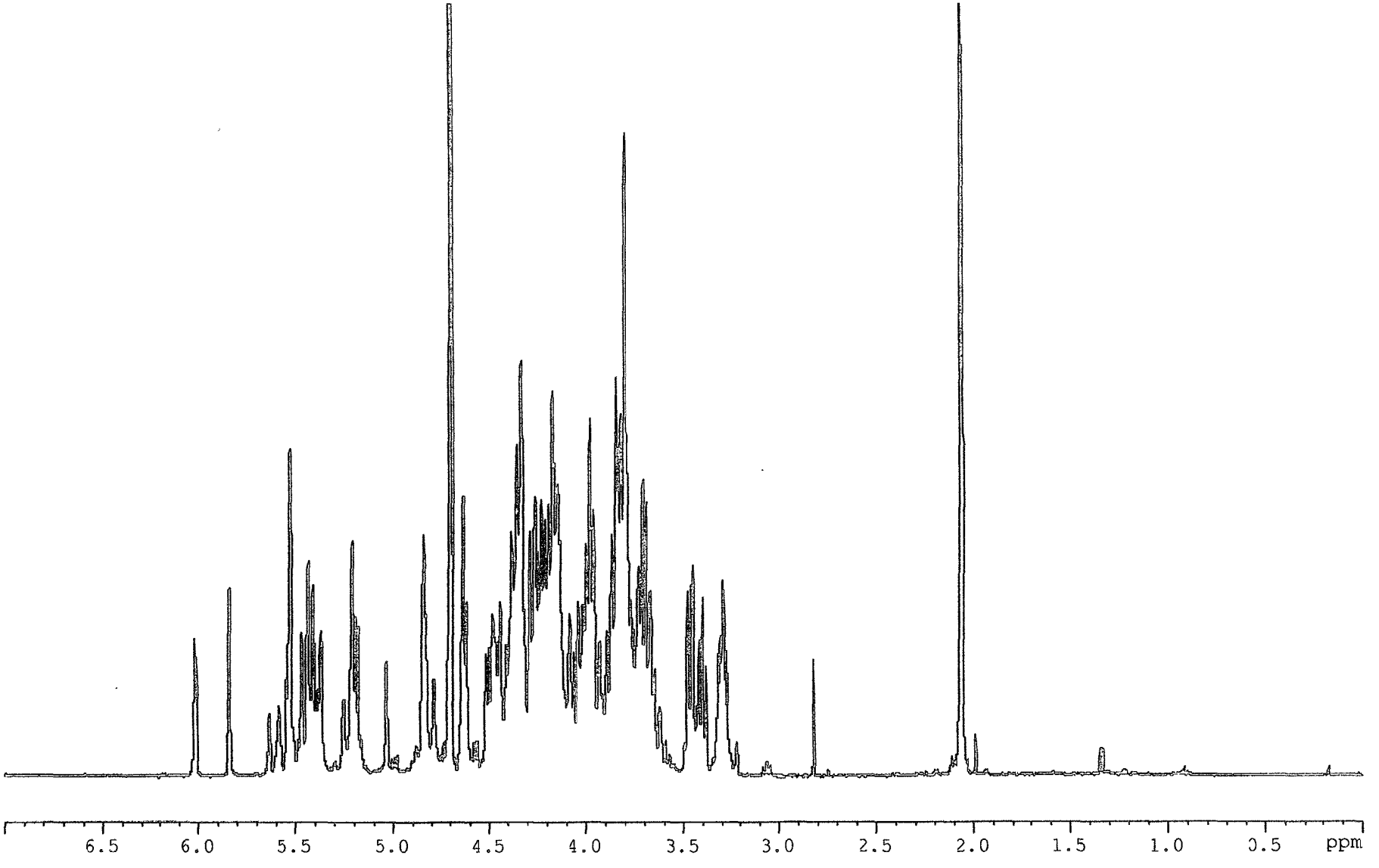
HSQC spectrum - G4532 (P-31267-28-BIE1 )(Octasaccharides mixture Aventis) T= 308K



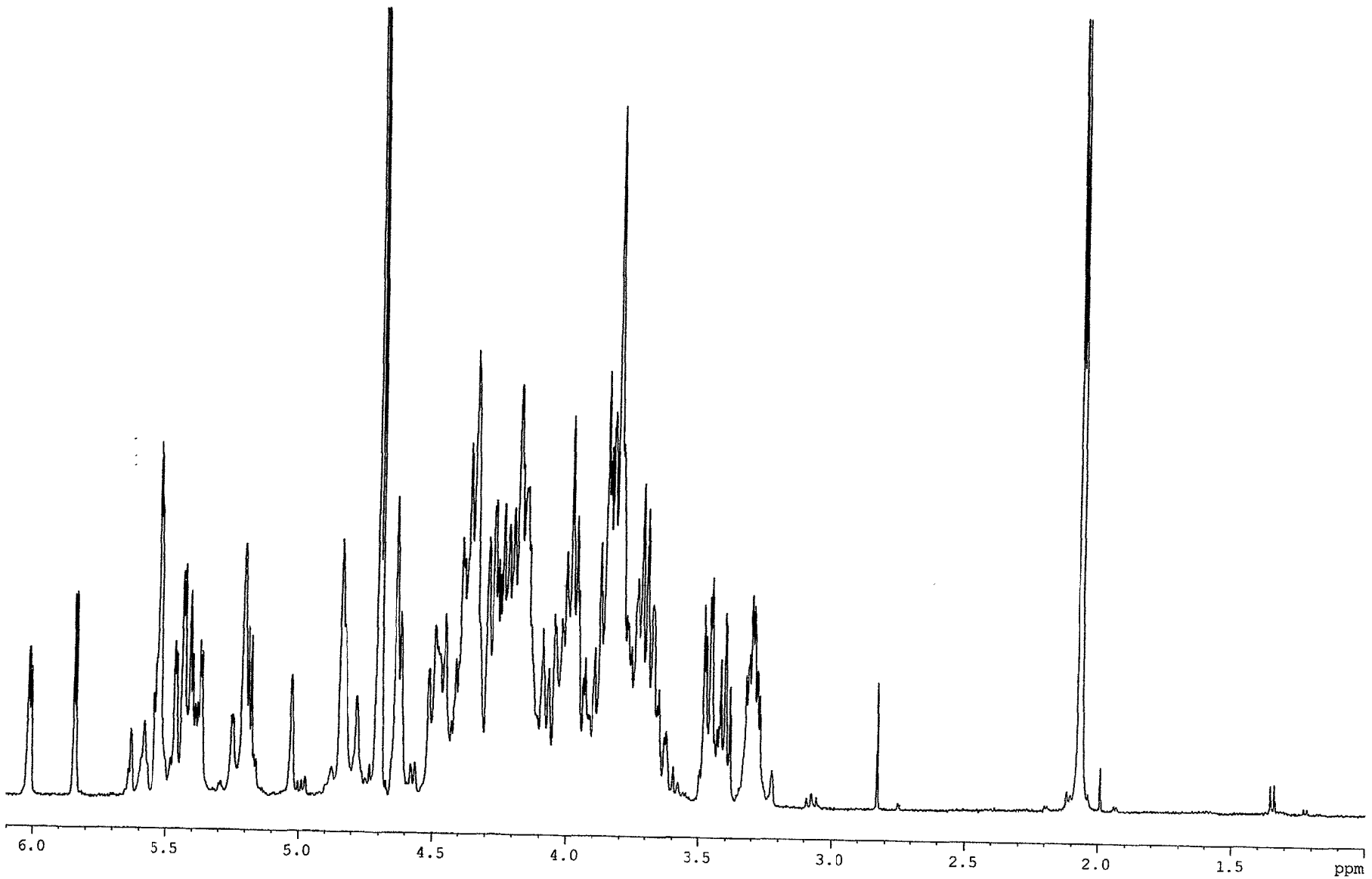
HSQC spectrum (anomeric region) - G4532 (P-31267-28-BIE10) (Octasaccharides mixture Aventis) T= 308K



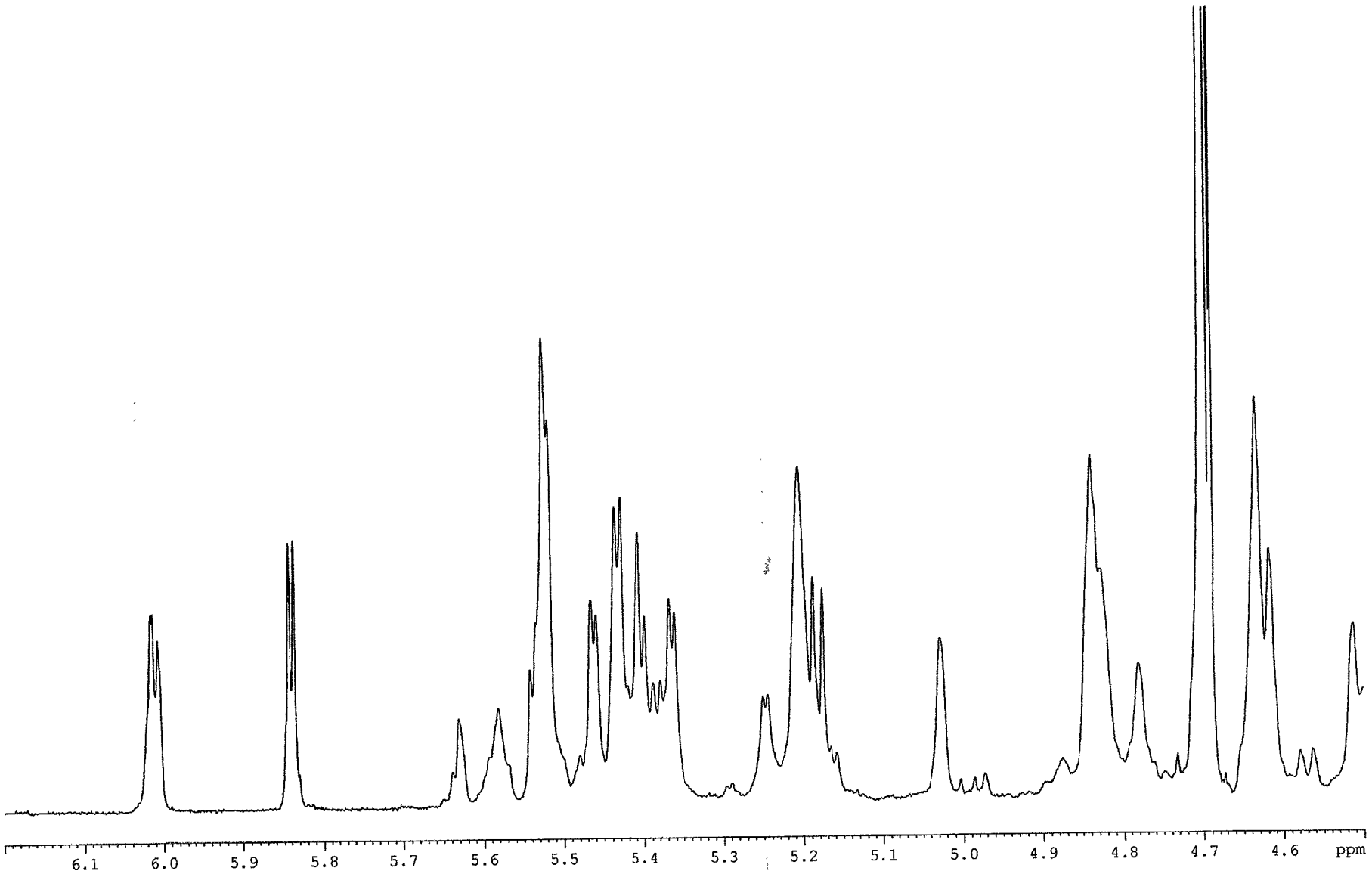
$^1$H spectrum - G4531 (P32077-48-3) (Octasaccharides mixture affine-fraction) T= 308K



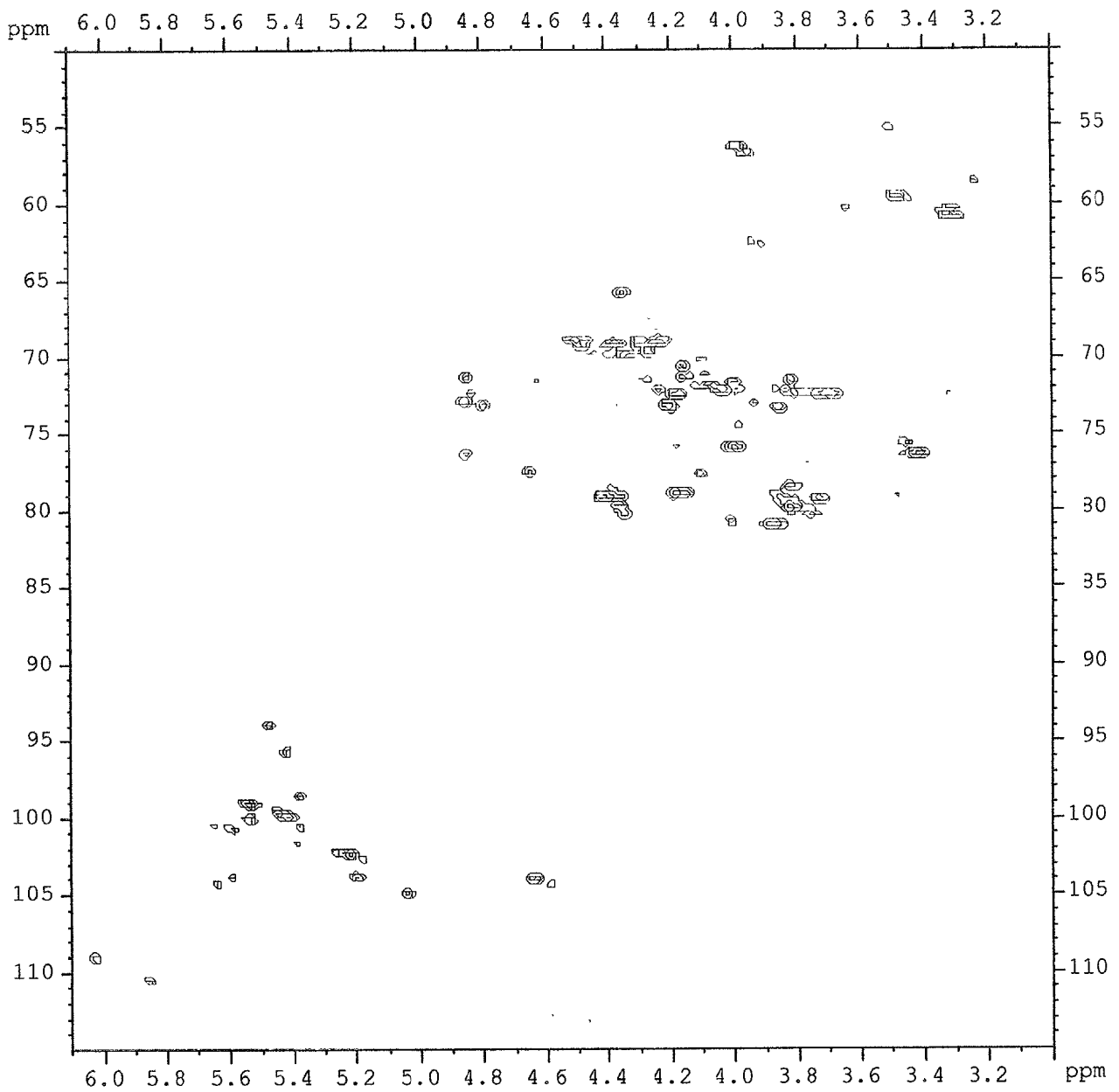
$^1$H spectrum - G4531 (P32077-48-3) (Octa. charides mixture affine-fraction) T= 308K



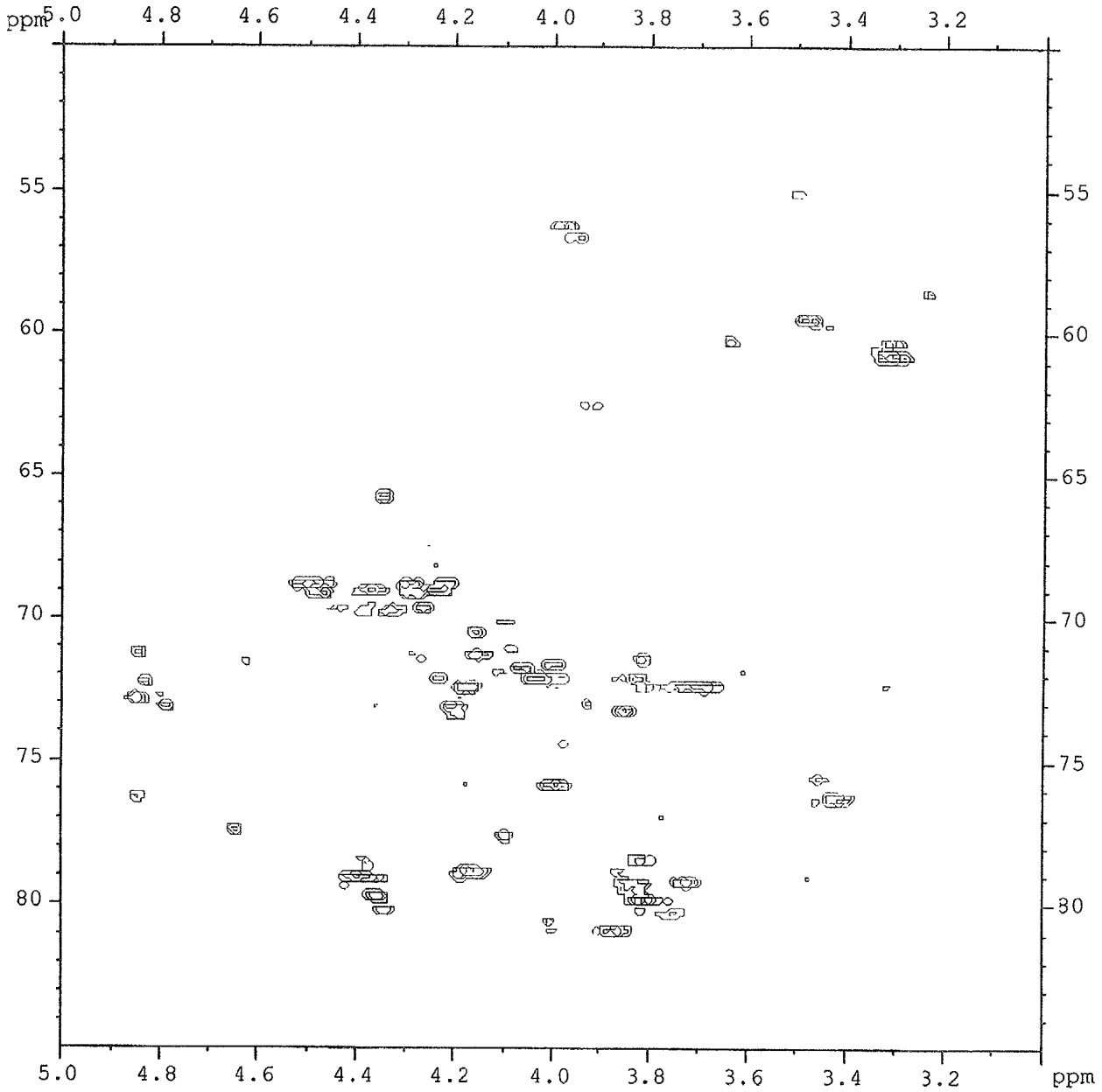
$^1$H spectrum - G4531 (P32077-48-3) (Octasaccharides mixture affine-fraction) T= 308K



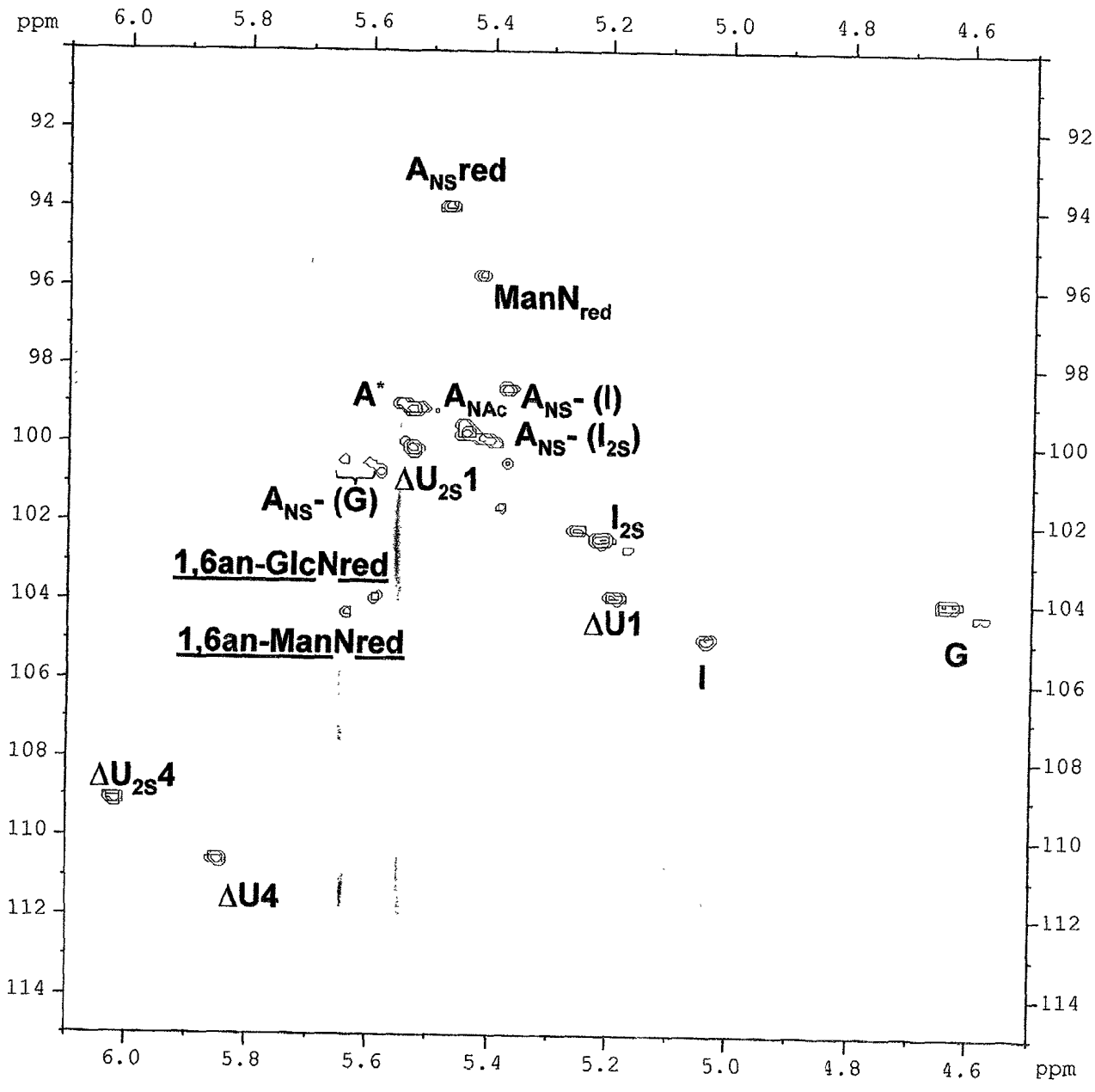
HSQC spectrum - G4531 (P32077-48-3) )ctasaccharides mixture affine-fraction) T= 308'



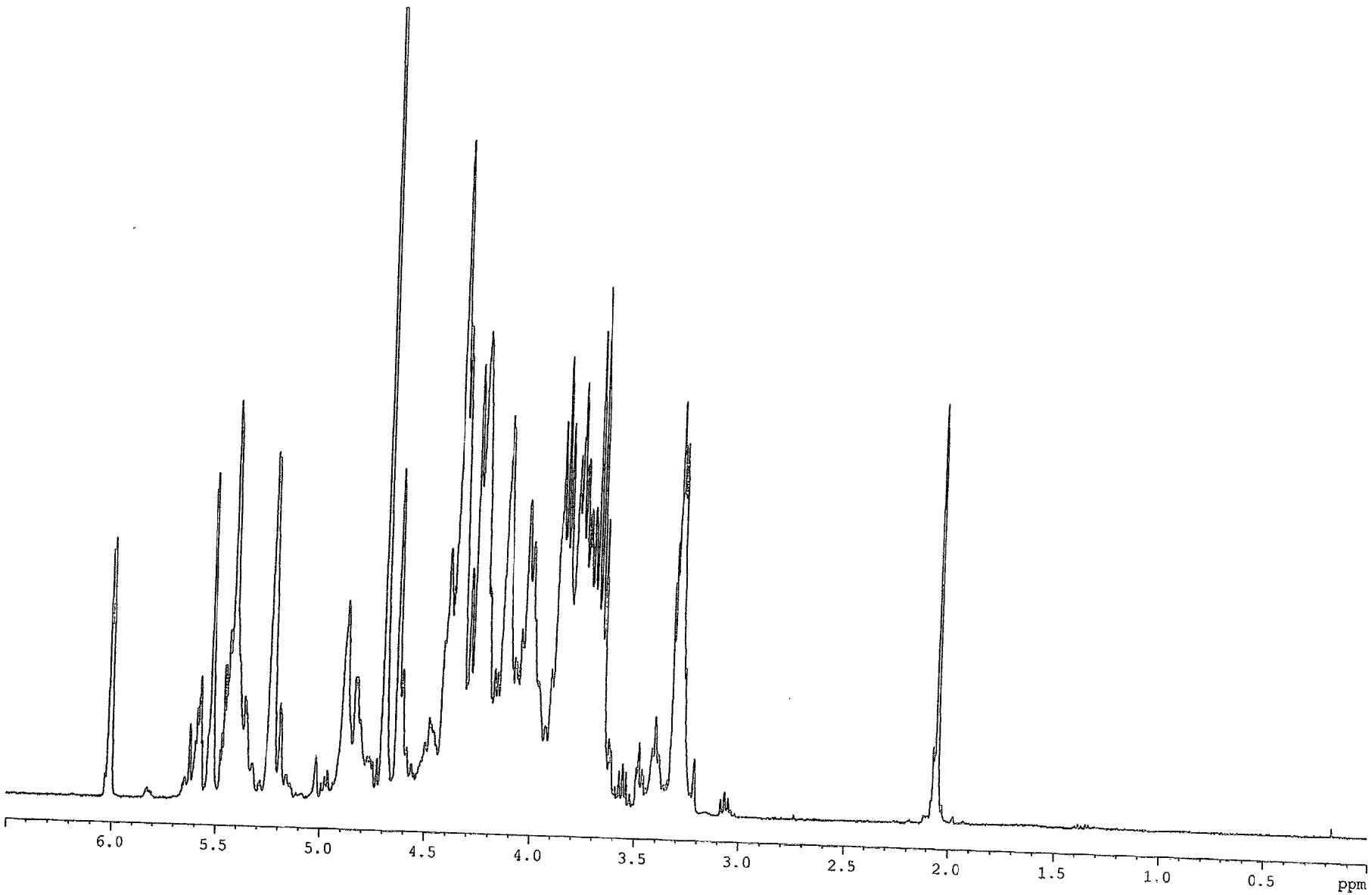
HSQC spectrum - G4531 (P32077-48-3) (Octasaccharides mixture affine-fraction) T= 308K



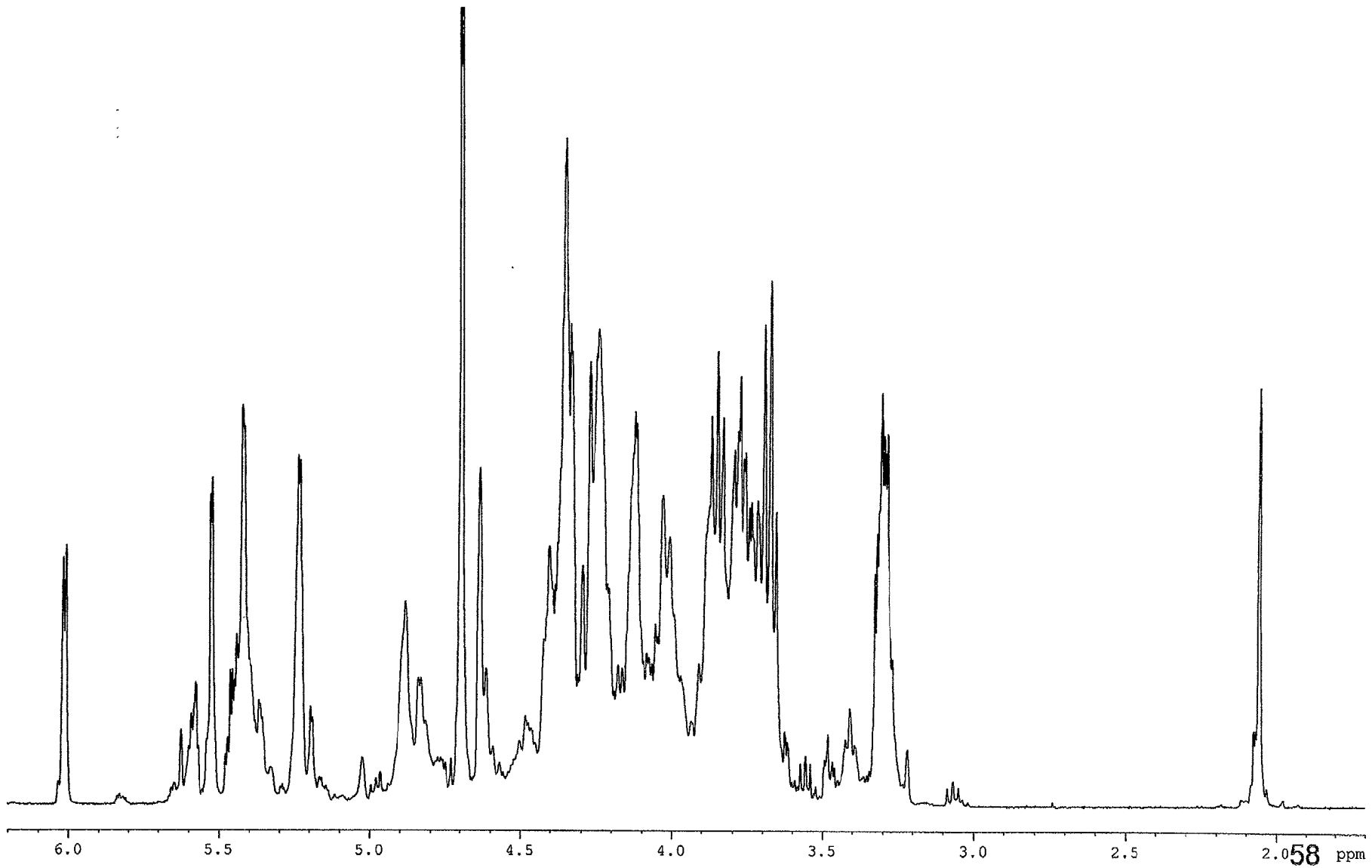
HSQC spectrum (anomeric region) - G4531 (P32077-48-3) (Octasaccharides mixture affine-fraction) T= 308K



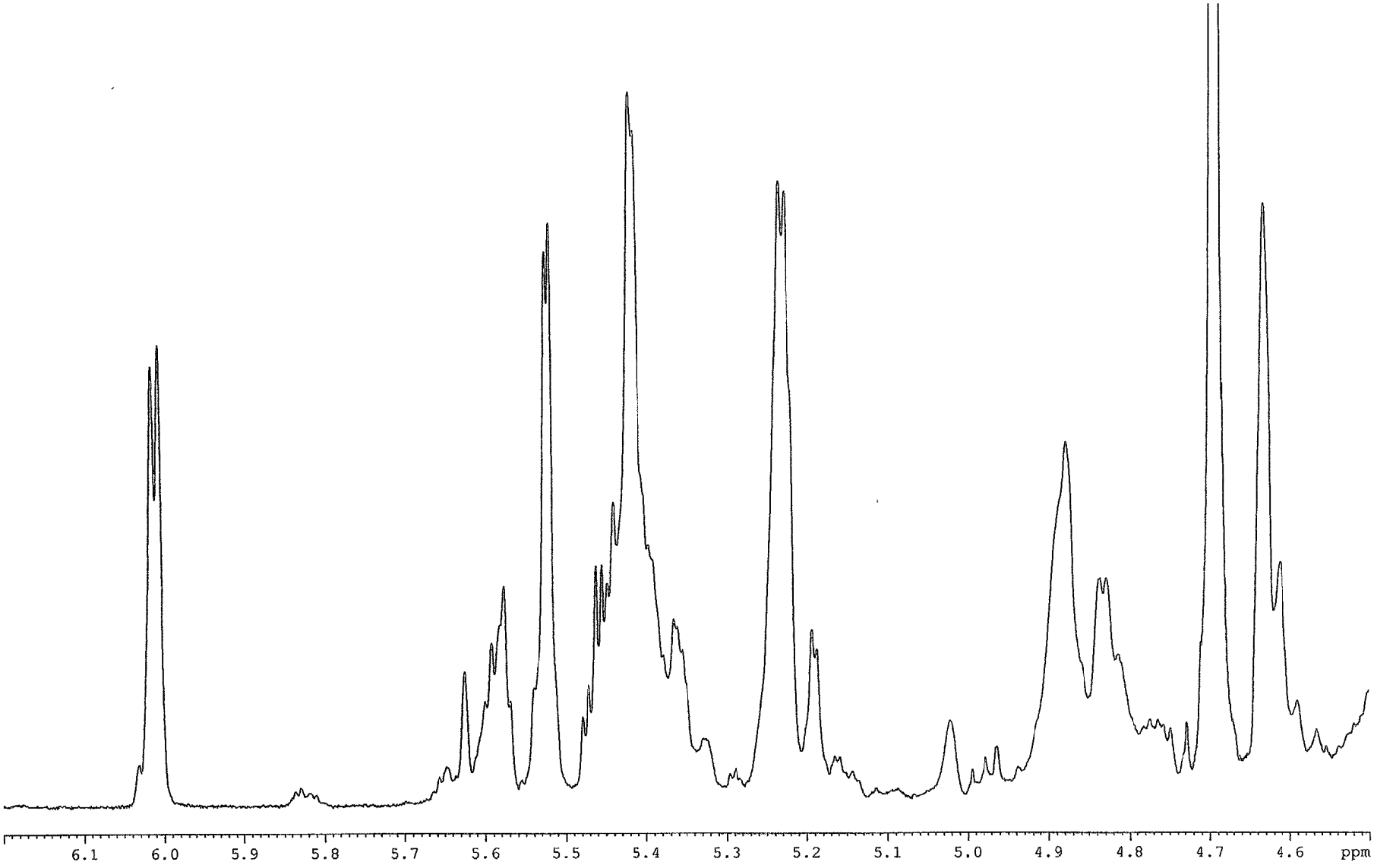
$^1$H spectrum - G4613 (P32077-48-4) (Octasaccharides mixture non-affine fraction ) T= 308K



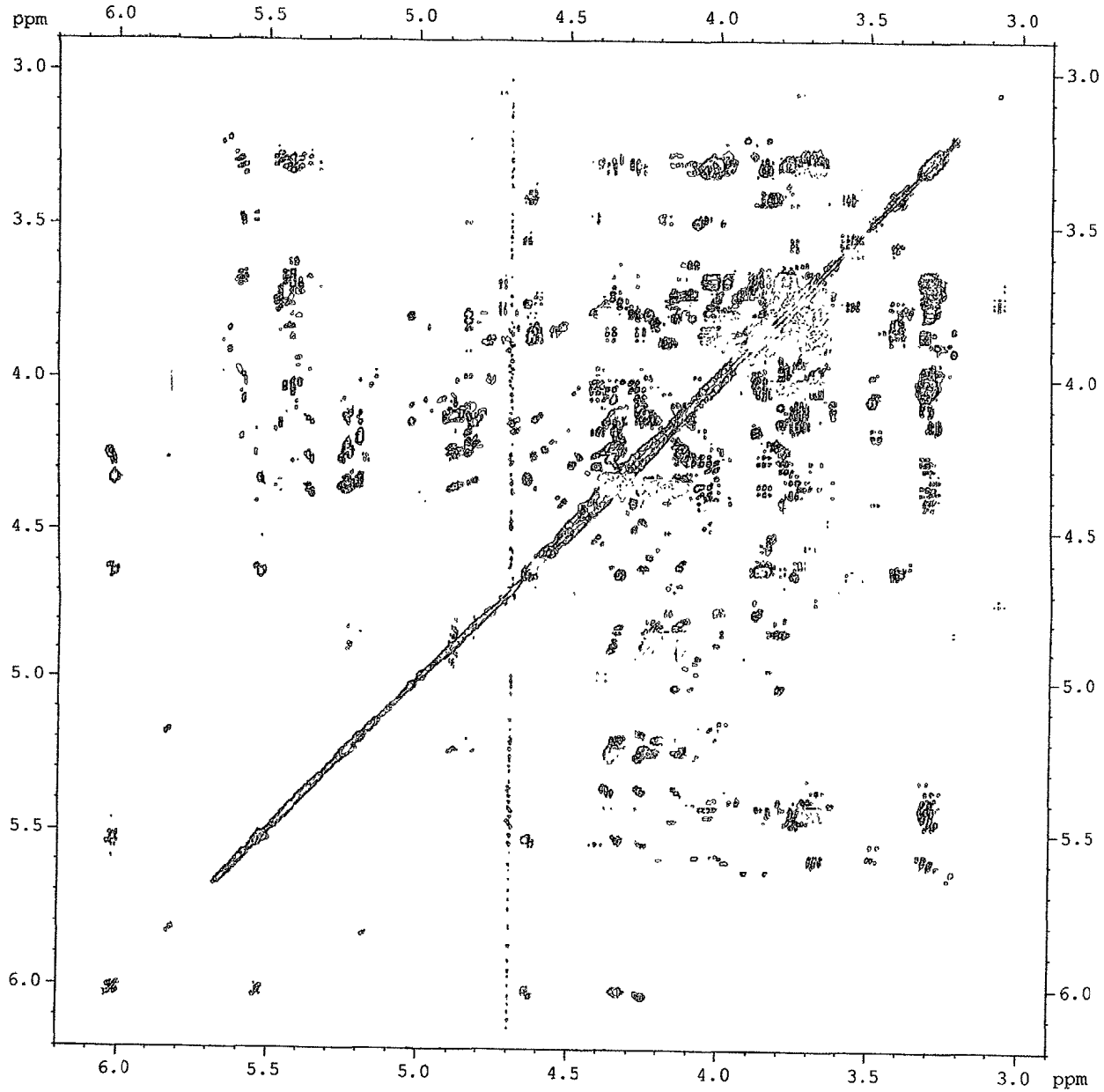
$^1$H spectrum - G4613 (P32077-48-4) (Octasaccharides mixture non-affine fraction ) T= 30oK

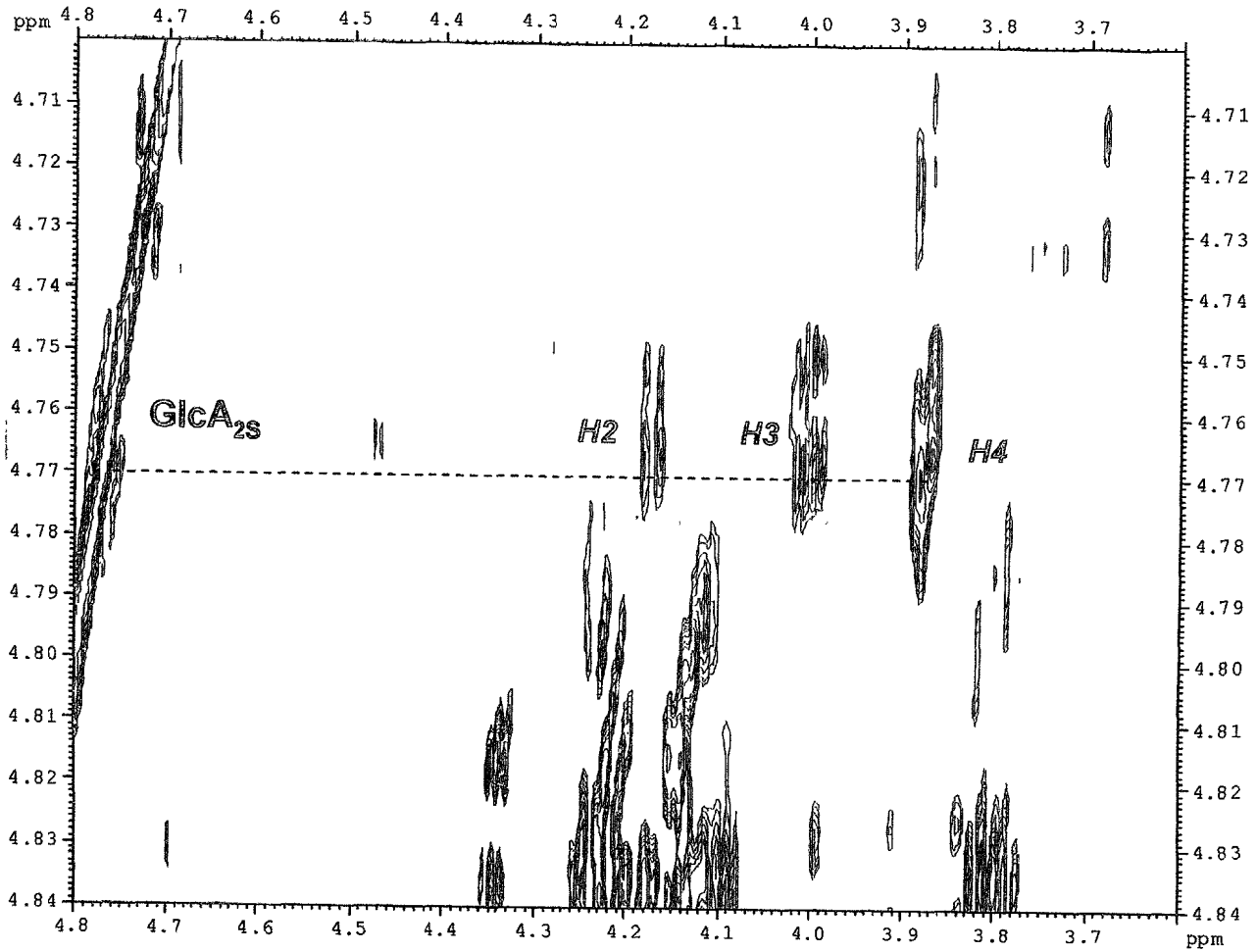


$^1$H spectrum - G4613 (P32077-48-4) (Octasaccharides mixture non-affine fraction ) T= 308K



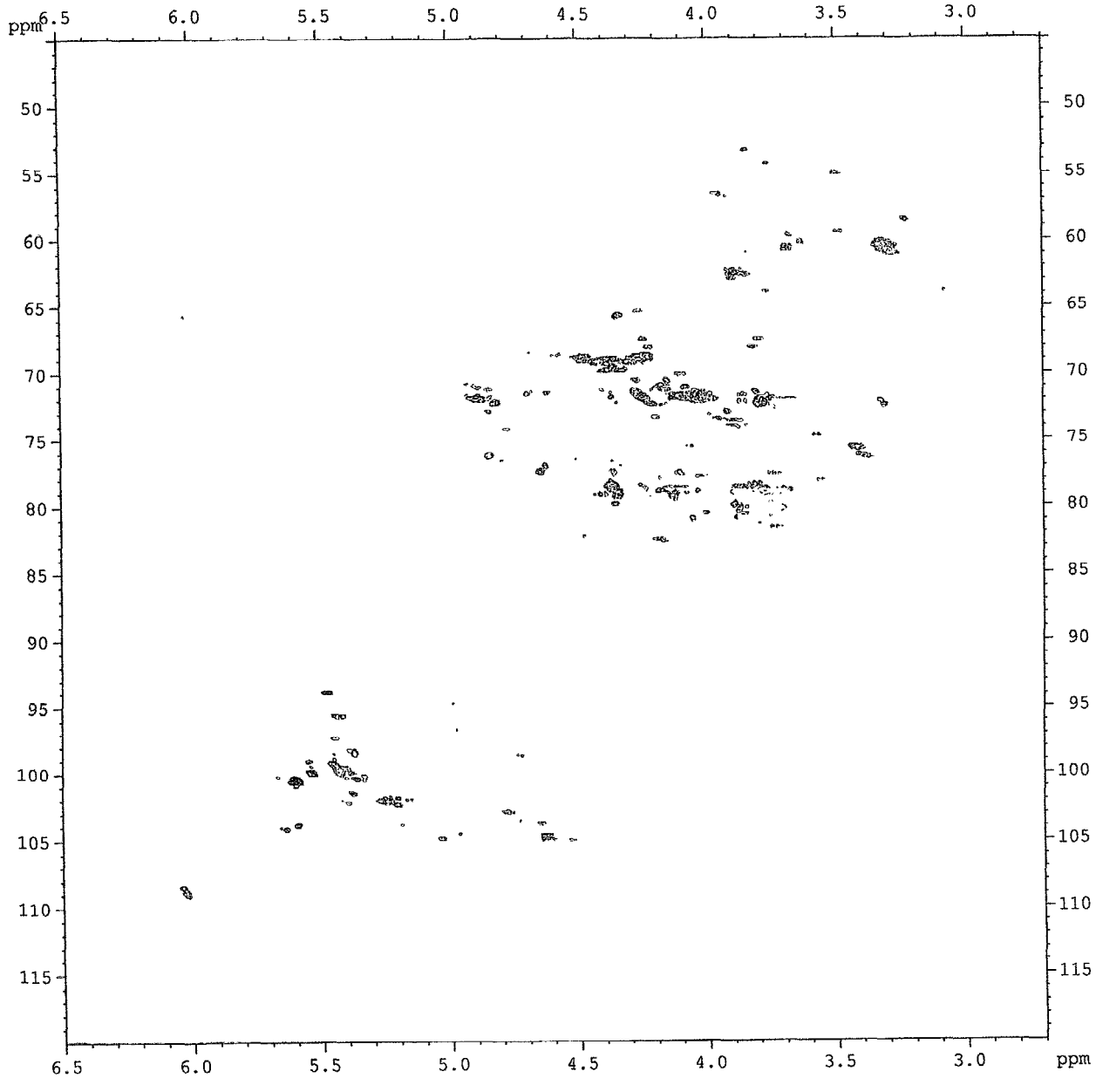
TOCSY spectrum - G4613 (P32077-48-4) (Octasaccharides mixture non-affine fraction ) T= 308K



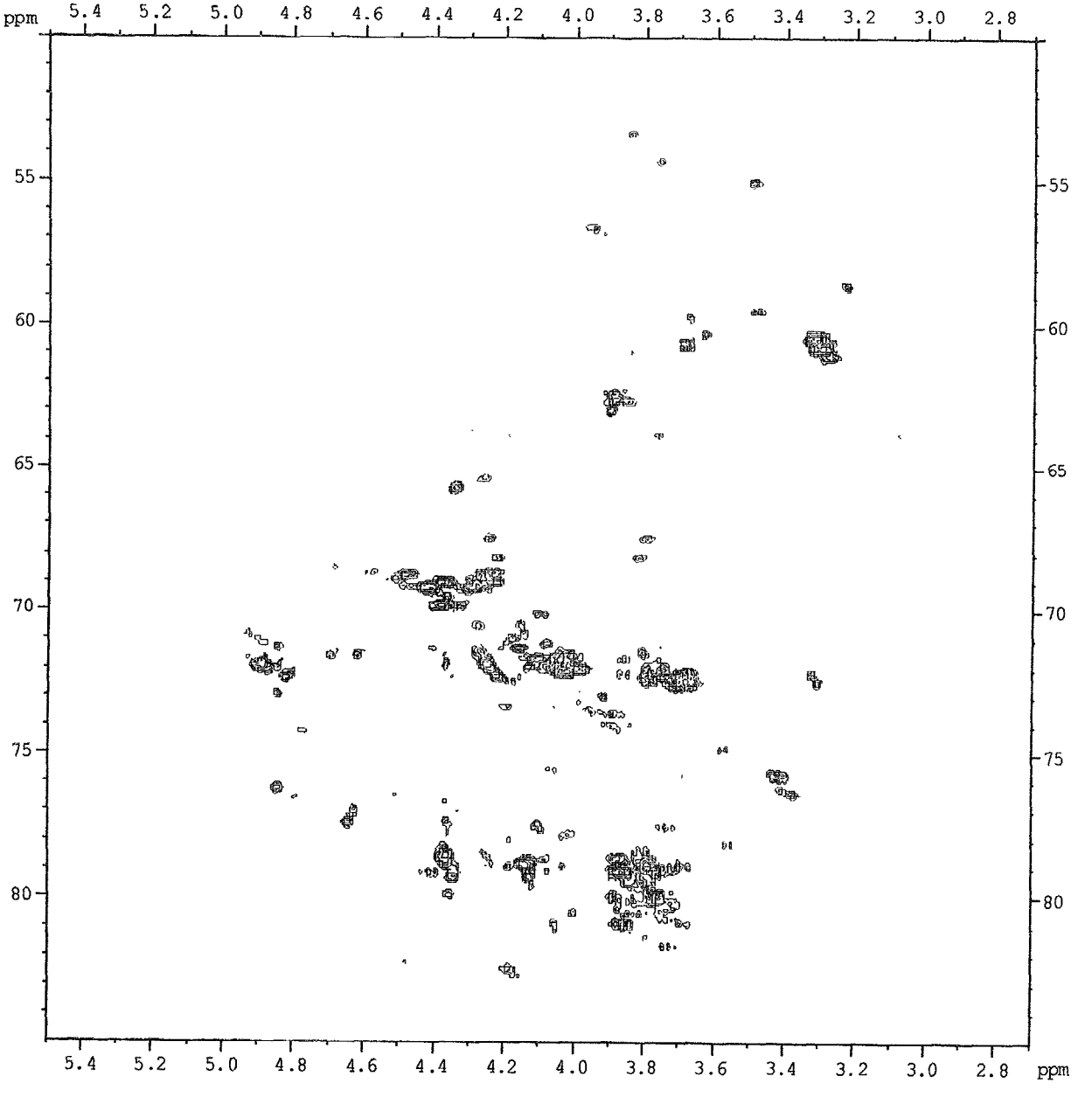



| H | $G_{2s}$ |
|---|---|
| 1 | 4.77/102.8 |
| 2 | 4.171/82.4 |
| 3 | 4.01 |
| 4 | 3.88 |
| 5 | nd |

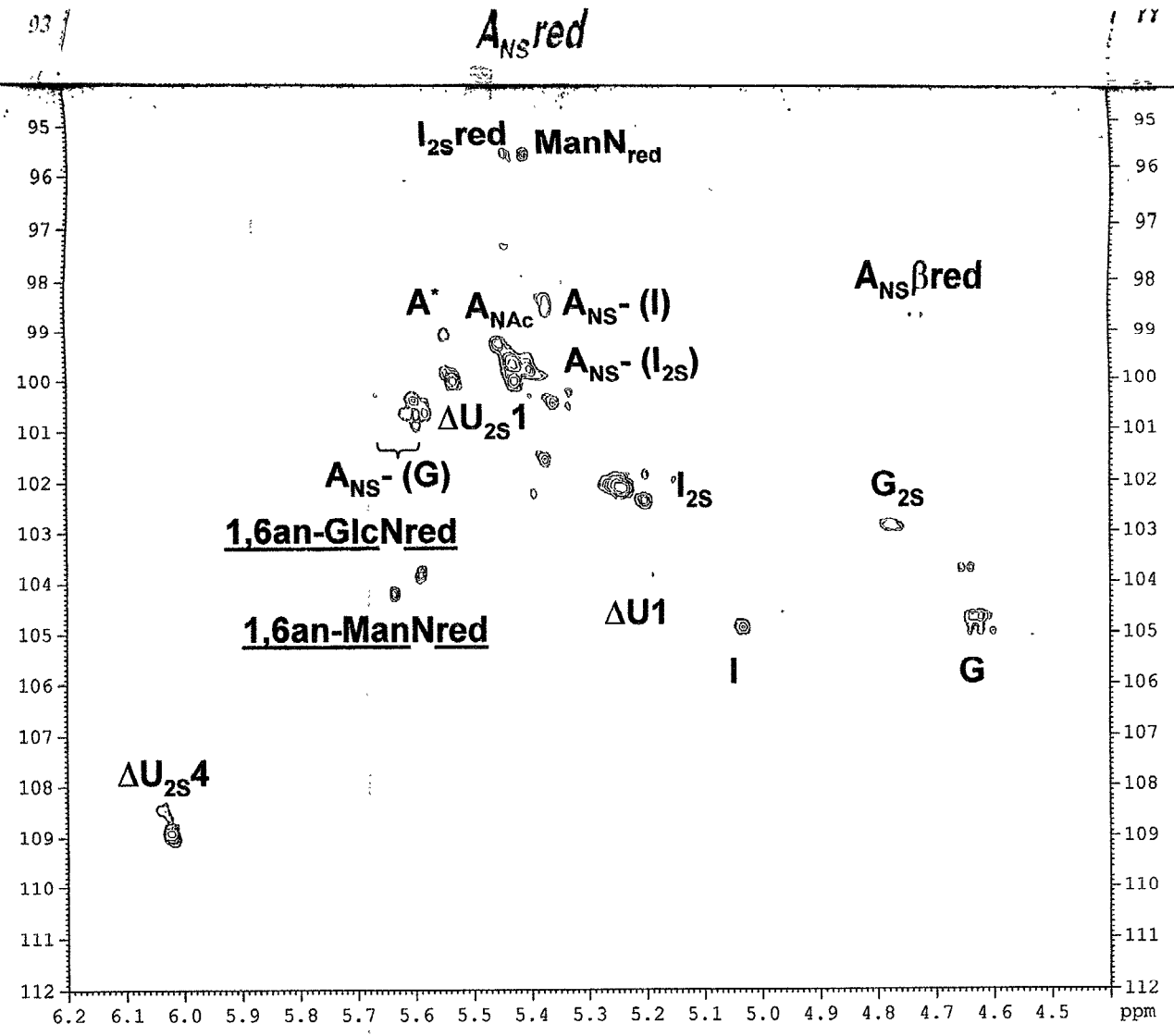
HSQC spectrum - G4613 (P32077-48-4) ctasaccharides mixture non-affine fraction ) T= 308K

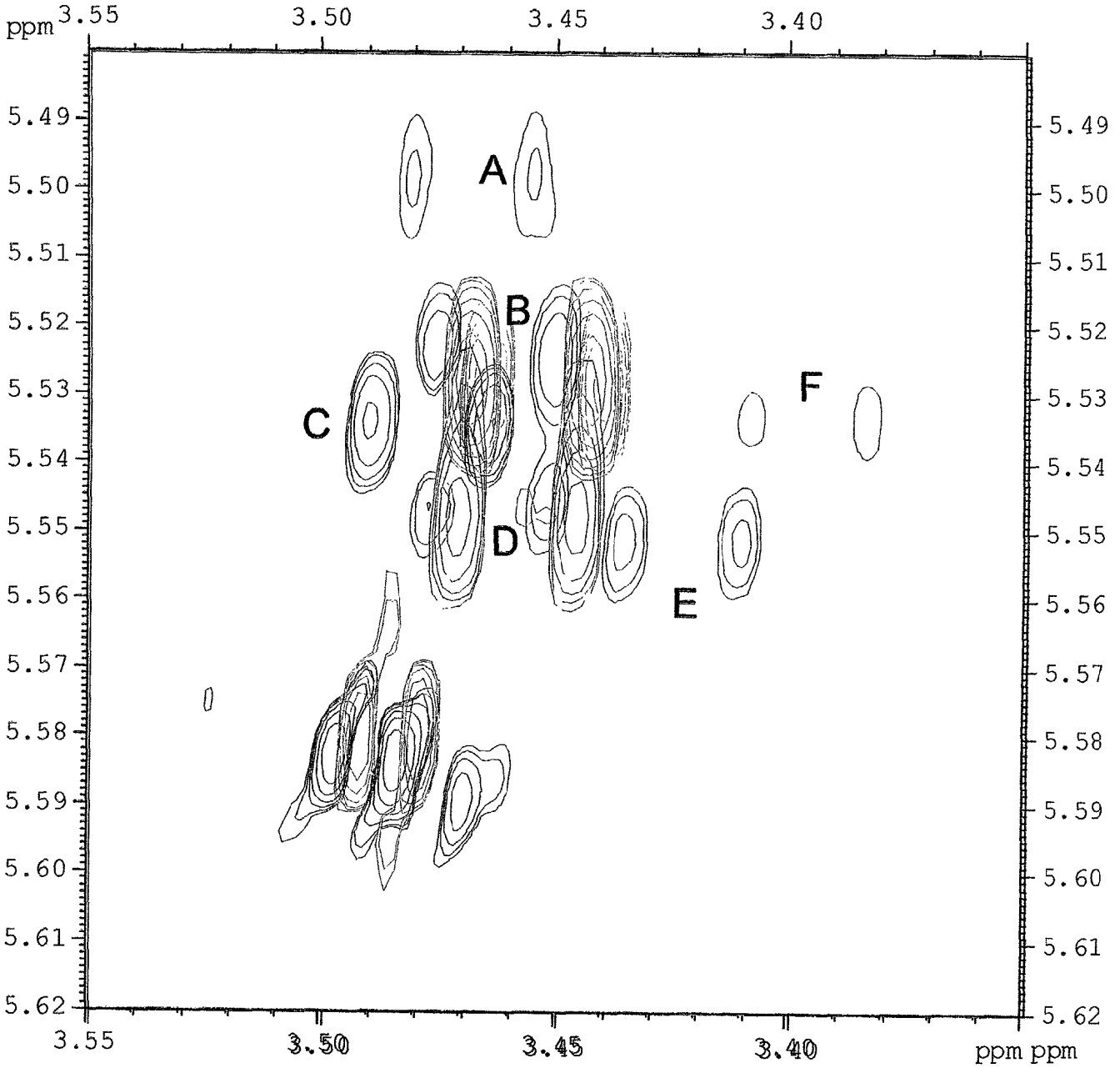


HSQC spectrum - G4613 (P32077-48-4) (Octasaccharides mixture non-affine fraction ) T= 308K



HSQC spectrum - G4613 (P32077-48-4 octasaccharides mixture non-affine fraction ) T= 308K






Green G4528

$UA_{NAc}GA^{*}IAIA$

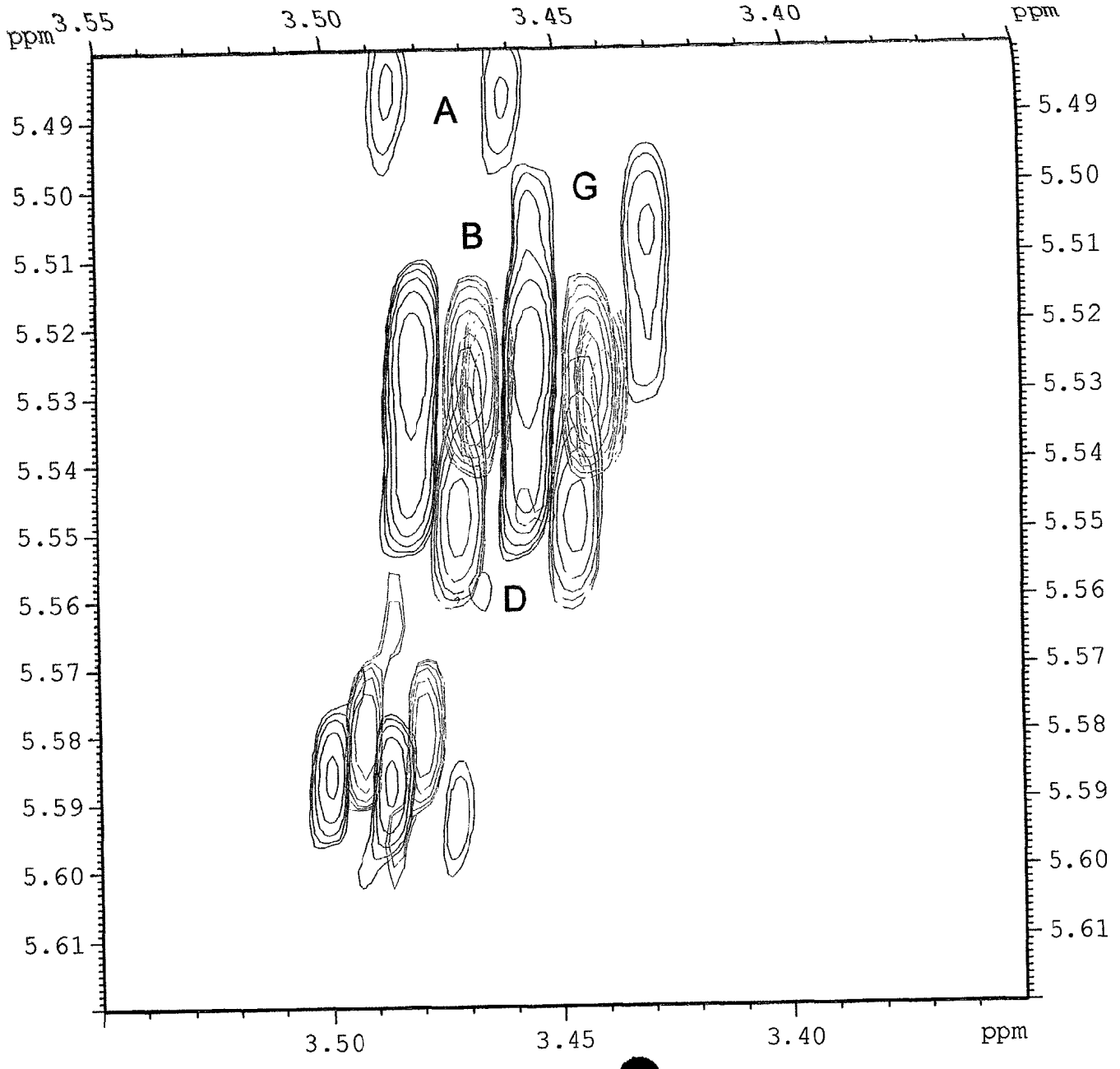
Red G4529

$UA_{NAc}GA^{*}IAI\text{-}Aan$

Blue G4530

$U_{2S}AI_{2OH}A_{NAc}GA^{*}IA$

**Black** total octasaccharides mixture




Green G4528

$UA_{NAc}GA^{*}IAIA$

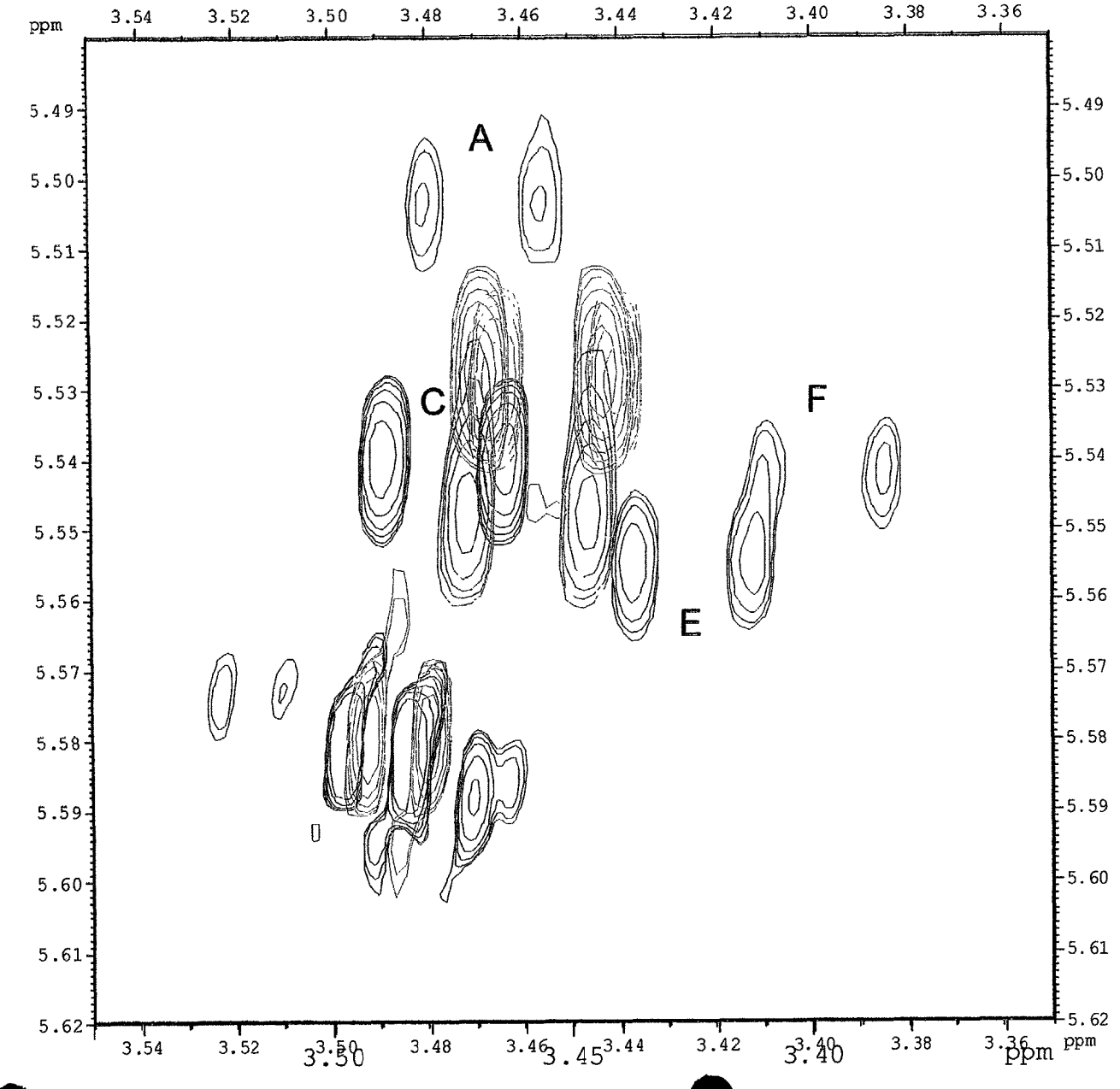
Red G4529

$UA_{NAc}GA^{*}IAI\text{-}Aan$

Blue G4530

$U_{2S}AI_{2OH}A_{NAc}GA^{*}IA$

**Black** "affine" octasaccharide mixtur




Green G4528

$UA_{NAc}GA^{*}IAIA$

Red G4529

$UA_{NAc}GA^{*}IAI\text{-}Aan$

Blue G4530

$U_{2S}AI_{2OH}A_{NAc}GA^{*}IA$

**Black** "non affine" octasaccharide mixture