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Virtual Coupling of Pyran Protons in the ^{1}H NMR Spectra of *C*- and *N*-Glucoronides: Dependence on Substitution and Solvent

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ABSTRACT We have observed that certain *C*- and *N*-glucuronides prepared as intermediates for breast cancer preventives demonstrate non-first order ^{1}H NMR spectra that are not the result of impurities or degradation but are instead due to virtual coupling in the pyran proton network. This virtual coupling shows the expected dependence on solvent and field strength and, more importantly, on the nature of the C-1 substitution. Although the hybridization of the atom bonded to C-1 may play a role, it appears that steric and/or electronic factors, which have the effect of increasing $\Delta\nu/J$ for H-3 and H-4, are critical for eliminating the spectral complexity. These observations, which appear to be fairly general, suggest that this phenomenon should be considered when addressing the purity of pharmaceutical agents containing these types of structural units.

Key Words: ^{1}H NMR, glucuronides, breast cancer, chemoprevention, virtual coupling

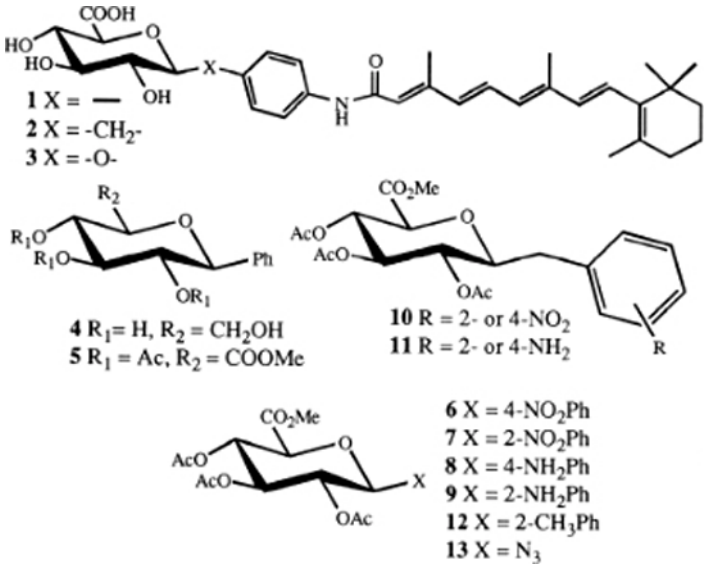

Figure 1. *C*- and *N*-linked glucuronides investigated

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INTRODUCTION

The *O*-glucuronide metabolites of retinoic acid and certain of its natural and synthetic analogues have been suggested to be biologically active forms of the parent molecule (1). As a class, these retinoids regulate epithelial tissue differentiation and show utility in treating dermatological diseases as well as promise for the treatment and prevention of cancer (2). Because of the relative chemical and metabolic instability of these glucuronides, we have been synthesizing *C*- and *N*-glucuronosyl analogues of some of these metabolites in an effort to improve the activity of these compounds and/or to determine whether these metabolites are active themselves or are hydrolyzed to the active parent retinoid (3). Thus, we have prepared *C*-glucuronosyl analogues 1 and 2 (Figure 1) of the *O*-glucuronide 3 of the semisynthetic retinoid *N*-(4-hydroxyphenyl) retinamide. Our results suggest these compounds show promise as mammary tumor chemopreventive agents (4,5).

In the course of synthesizing 1, selective PtO_2-mediated oxidation (6, 7) of the 6-hydroxymethyl group of glucosylbenzene (4) followed by esterification and acetylation produced a product 5 that showed unusual complexity in the ^{1}H NMR spectrum in the region of the pyran ring protons. This was true for all resonances except that assigned for the H-1 proton. Since the Adams' catalyst that promoted oxidation had not to our knowledge been previously employed for the oxidation of *C*-glycosyl compounds into their glucuronide analogues, and given that this aryl-*C*-glycoside contains a tertiary carbon and benzylic ether unit (carbohydrate position 1), both of which may be prone to oxidation, we were concerned that other products might have been produced during the reaction that would compromise the purity of the materials and hence the validity of bioactivity assays performed with them.

After careful chromatographic purification and recrystallization of 5 to apparent homogeneity, while its ^{1}H NMR spectrum remained unchanged, other available spectroscopic evidence (^{13}C NMR, IR, and MS) was consistent with a single compound assigned the structure 5. The possibility that the complexity of the ^{1}H NMR spectrum resulted from long-range virtual ^{1}H-^{1}H coupling was thus considered (8). Spin simulation of the spectrum using PANIC (Parameter Adjustment in NMR by Iterative Calculation) appeared to confirm this explanation.

Prompted by the report of Saito et al (9) on their observation of virtual ^{1}H-^{1}H coupling in glucuronosyl moieties within *O*-disaccharides and their conjugates, we wish to report our interesting observations of similar phenomena in *C*- and *N*-glucuronosyl compounds, which appears to depend on the structure of the pyran C-1 substituent and the solvent employed in NMR measurements. This observation of deceptively complex spectra appears to be surprisingly general and should be considered when evaluating the purity, including the stereochemical purity, of potential pharmaceutical agents containing these structural units.

MATERIALS AND METHODS

Fourier-transformed ^{1}H NMR spectra were obtained on sample solutions in glass 175 x 5 mm sample tubes (Wilmad; Buena, NJ). Spectra were collected for 20 mg/mL solutions at 250, 400, 600, and 800 MHz on AC250 or DPX250, DRX400, DMX600, and DMX800 instruments, respectively (Bruker Instruments; Billerica, MA). Samples were dissolved in $CDCl_3$, CD_2Cl_2, acetone-d_6, benzene-d_6, DMSO-d_6, CD_3OD, pyridine-d_5, and tetrahydrofuraN-d_8 as appropriate (Cambridge Isotope Laboratories; Andover, MA) and spectra referenced to the residual protio solvent (relative to TMS) in the deuterated solvents. Spectra were collected at ambient temperature using 90° pulse widths and transformed after exponential multiplication (LB = 0.2 Hz). Spectral simulation (see Table 1) was performed using PANIC version 840419 implemented on an ASPECT 3000 computer (Bruker Instruments).

The compounds studied were prepared as previously published (3, 10, 11). Entries 2 and 9 (Table 2) were prepared by methods identical to those used for entries 1 and 10 using the appropriate Grignard reagents, while entries 17 and 18 were prepared by methods identical to those used in entry 19 using acetyl and benzoyl chloride respectively.

Table 1. Chemical Shifts and Coupling Constants Simulated for H-1 to H-5 of 5

Proton	Chemical Shift (ppm)	Coupling Constant (Hz)
H-1	4.40	$J_{1,2}$ = 9.895 $J_{1,3}$ =-0.172 $J_{1,4}$ = 0.0 $J_{1,5}$ = 0.0
H-2	5.15	$J_{2,3}$ = 9.294 $J_{2,4}$ = 0.0 $J_{2,5}$ = 0.0
H-3	5.34	$J_{3,4}$ = 9.800 $J_{3,5}$ = 0.012 H-4 5.37 J4,5 = 9.485 H-5 4.16

Table 2. Virtual Coupling Dependence on C-1 Substituent

Entry No.	C-1 B Substituent	Virtual Coupling[a]
1	Ph-(5)	YES[b]
2	1-Naphthyl-	NO
3	4-NO_2Ph-(6)	YES
4	2-NO_2Ph-(7)	NO
5	4-NH_2Ph-(8)	YES
6	2-NH_2Ph-(9)	NO
7	4-CH_3Ph-	YES
8	2-CH_3Ph-(12)	YES[c]
9	CH_3-	YES
10	$PhCH_2$-	NO
11	4-NO_2PhCH_2-(10a)	NO
12	2-NO_2PhCH_2-(10b)	NO
13	4-NH_2PhCH_2-(11a)	NO
14	2-NH_2PhCH_2-(11b)	NO
15	N_3-(13)	YES[d]
16	H_2N-	NO
17	CH_3CONH-	NO
18	PhCONH-	NO
19	Retinoyl NH-	NO
20	CH_3COO-	NO

[a]In $CDCl_3$ at 250 MHz; [b]Eliminated in acetone-d_6 and at 800 MHz (see Appendix); [c]Weakly present; [d]Eliminated in acetone-d_6, benzene-d_6, pyridine-d_5, tetrahydrofuran-d_8, CD_2Cl_2, CD_3OD, and DMSO-d_6 and at 400 MHz (see Appendix).

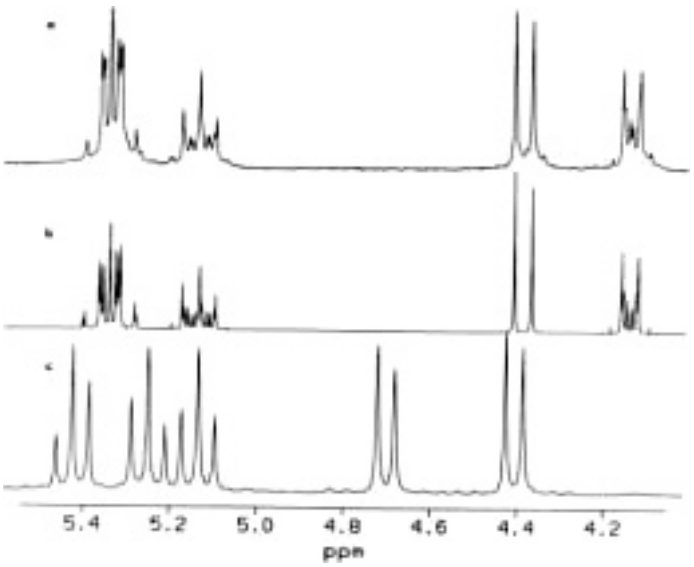

Figure 2. Partial 250 MHz ^{1}H NMR of 5 in a) $CDCl_3$, b) simulated, and c) CD_3CO

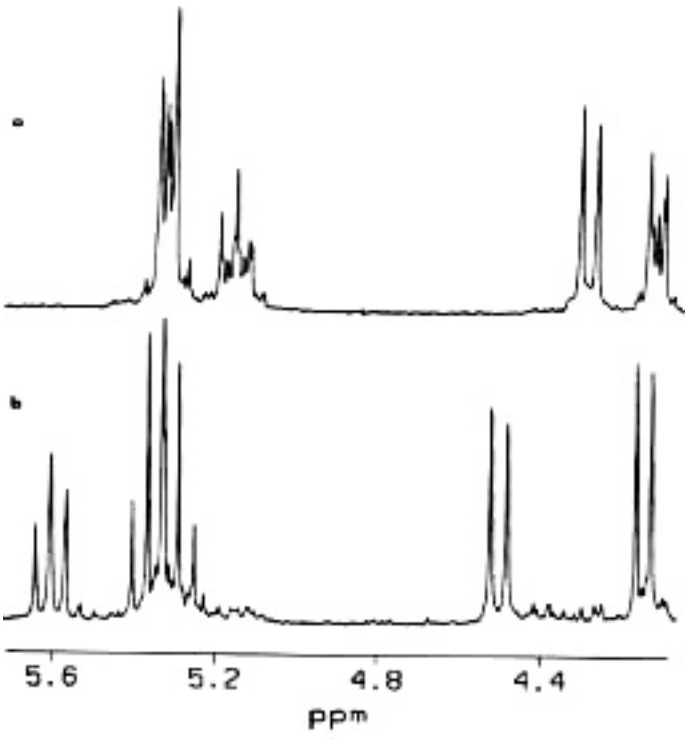

Figure 3. Partial 250 MHz ^{1}H NMR $CDCl_3$ spectrum of a) 8 and b) 9

RESULTS AND DISCUSSION

The **5** used in this study was prepared as previously described (3). The 250 MHz ^{1}H NMR spectrum of this compound in $CDCl_3$, in the region of the pyran protons, is shown in Figure 2. The surprising complexity of this spectrum, which is still present at 400 MHz (but is reduced at 600 MHz and and eliminated at 800 MHz), and the possibility that it arose from virtual coupling between H-2 and H-5, led us to simulate the spectrum using PANIC, as is also shown in Figure 2. The chemical shifts and calculated coupling constants derived from simulating the spectrum of 5 are shown in Table 1. For this simulation, the apparent couplings constants $J_{1,4,}$, $J_{1,5,}$ $J_{2,4,}$ and $J_{2,5}$ are sufficiently small that they can be set to zero and a satisfactory simulation can be obtained. Nonetheless, the H-2 and H-5 nuclei appear to show the observed complexity by virtue of being coupled as X parts of ABX spectra to H-3 and H-4, which themselves form a strongly coupled AB system with $\Delta\nu/J = 0.82$ at 250 MHz. As might be expected, this phenomenon can be eliminated by recording the ^{1}H NMR spectrum of 5 in different solvents. As also shown in Figure 2, the spectrum of 5 in acetone-d_6 can be analyzed as first order,with $\Delta\nu$ /J for H-3 and H-4 now being 2.96.

Interestingly, our chemistry to further elaborate 5 to 1 produced intermediates that show virtual coupling that depends on both the nature and site of aromatic ring substitution. Nitration of 5 produced a 3:2 mixture of isomers 6 and 7, which were difficult to separate (3). In one instance, small quantities of pure 6 and 7 were obtained by preparative TLC. Their 250 MHz ^{1}H NMR spectrum in $CDCl_3$ showed virtual coupling comparable to that of 5 for 6 but not to that of 5 for 7 (Data not shown). Reduction of the nitroaromatic isomer mixture produced the readily separable *O*- and *p*-anilines 8 and 9 (3). In this instance, the *para* substituted aniline 8 also shows strong virtual coupling that was not simulated but appears likely to result from the even smaller $\Delta\nu/J_{3,4}$ ratio (Figure 3). For the *ortho* regioisomer 9, this virtual coupling observed for 5 and 8 is also absent. Homonuclear decoupling and NOE difference spectra established that H-2 in 9 has moved substantially downfield to 5.61 ppm. More importantly, the chemical shift of H-3 and H-4 has reversed relative to 5 (5.37 and 5.29 ppm respectively) and $\Delta\nu/J_{3,4}$ has increased to 1.91, which

appears to be sufficient to eliminate this coupling phenomenon.

Because both the *O*-nitrophenyl and *O*-aminophenyl isomers 7 and 9 fail to show the virtual coupling present in 5, 6, and 8, which bear a C_2-symmetric substituent at C-1, it seems plausible that this lack of virtual coupling results from steric interactions of the *O*-substituent with the axial H-1 or H-2 protons. This results in a different favored rotamer about the C-1-Ar bond and/or causes subtle changes in the conformation of the pyran ring, changes that have the effect of increasing $\Delta\nu/J_{3,4}$. In support of this concept, none of the *ortho* nitro or amino *C*-benzyl analogues 10 or 11 (3) (which we required for the preparation of 2) that have an interposed methylene unit show evidence of virtual coupling in the 250 MHz ^{1}H NMR spectra in $CDCl_3$ (see Table 2 for a summary of the compounds we investigated to determine whether the phenomenon is observed). That other more subtle influences such as electronics may also play a role is suggested by inspection of the spectrum of the *O*-tolyl analog 12, which we prepared serendipitously during efforts to synthesize 2 (10). In the $CDCl_3$ ^{1}H NMR spectrum of 12, the H-2, H-3, and H-4 resonances overlap extensively, unlike any of the other compounds reported here. However, the H-5 resonance at 4.16 ppm shows some evidence of much less extensive virtual coupling than for 5, implying that the impact of the *O*-methyl substituent is insufficient to change $\Delta\nu/J_{3,4}$ enough to eliminate virtual coupling under these spectroscopic conditions. Furthermore, we observed that the 1-ß-azido glucuronide 13 we previously prepared (11) demonstrated virtual coupling in the ^{1}H NMR spectrum in $CDCl_3$, which is nearly identical to that of 5. This coupling is absent at 400 MHz and in the 250 MHz acetone-d_6, benzene-d_6, CD_2Cl_2, CD_3OD, pyridine-d_5, and tetrahydrofuraN-d_8 DMSO-d_6 spectra of 13 and also in the $CDCl_3$ spectrum of the amine prepared by reduction of 13 as well as its acylated derivatives (11). Once again, linear, symmetrical azide substitution results in virtual coupling while reduction products do not show this property, suggesting, perhaps, that the hybridization of the C-1 attached atom may play a role in causing this phenomenon. However, as shown in entry 9 of Table 2, the spherically symmetrical, sterically undemanding methyl substituted compound also demonstrates this virtual coupling. Thus, with the limited set of examples explored here, while those with atoms with sp^2-like character bonded to C-1 demonstrate this coupling, steric and electronic effects from the C-1 substituent are likely to be more important contributors to the complexity of the observed spectra than is hybridization.

It might be expected that homonuclear decoupling experiments would allow elimination of this observed virtual coupling in many instances. In the present case, this is only a partially successful strategy because the phenomenon is driven by the small value of $\Delta\nu/J_{3,4}$ and thus selective irradiation of H-3 or H-4 is not possible. As shown for compound 13 in the Appendix, irradiation of H-5 and H-2 (4.1 and 4.95 spm respectively) still leaves some significant evidence of a noN-first order spectrum. More successful in this case is the impact of raising the temperature on spectral appearance (also see Appendix). Interestingly, we have observed this virtual coupling for *C*- and *N*-glucuronides only when samples are dissolved in $CDCl_3$. Thus, it appears that in this solvent a unique pyran ring conformation and fortuitous ^{1}H chemical shifts create the observed phenomenon. Given the high volatility of $CDCl_3$, limits are placed on routine use of elevated temperature experiments. Nonetheless, raising the temperature for 13 in $CDCl_3$ by 20°C above ambient clearly alters spectral appearance in a manner consistent with movement toward a first order spectrum.

CONCLUSIONS

Thus, as in some ß-D-glucopyranosuronate systems (9), certain C- and N-glucuronides can show surprisingly complex ^{1}H NMR spectra. These appear to be the result of long-range virtual coupling and are not caused by the presence of isomer mixtures at C-1 or in substitution of the aromatic ring in *C*-aryl glucuronides. The phenomenon shows sensitivity to substituents at the *O*-position of *C*-aryl glucuronides, but this is observed strongly only when the *O*-positions are unsubstituted. Both solvent and field strength dependences are observed. Changing the solvent from $CDCl_3$ to other solvents causes a greater chemical shift dispersion, thereby removing virtual coupling effects in these ^{1}H NMR spectra. By increasing the spectrometer magnetic field, the value of $\Delta\nu/J$ becomes sufficiently large to no longer exhibit virtual coupling effects. The relatively high

frequency with which this spectral phenomenon is observed in these types of structural units suggest it should be considered when the purity of potential pharmaceutical agents containing these structural units is in doubt based on ^{1}H NMR analysis.

ACKNOWLEDGEMENTS

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REFERENCES

1. Mehta RG, Barua AB, Olson JA, Moon RC. Effects of retinoid glucuronides on mammary gland development in organ culture. *Oncology.* 1991;48:505-509.

2. Hill DL, Grubbs CJ. Retinoids and cancer prevention. *Annu Rev Nutr*. 1992;12:161-181.

3. Panigot MJ, Humphries KA, Curley RW, Jr. Preparation of 4-retinamidophenyl- and 4-retinamidobenzyl-C-glycosyl and C-glucuronosyl analogues of the glucuronide of 4-hydroxyphenylretinamide as potential stable cancer chemopreventive agents. *J Carbohydr Chem*. 1994;13:303-321.

4. Curley RW, Jr, Abou-Issa H, Panigot MJ, Repa JJ, Clagett-Dame M, Ashafie G. Chemopreventive activities of C-glucuronide/glycoside analogs of retinoid-O-glucuronides against breast cancer development and growth. *Anticancer Res.* 1996;16:757-764.

5. Abou-Issa HM, Alshafie GA, Wong MF, Clagett-Dame M, Repa JJ, Sikri V, Curley RW, Jr. Chemopreventive activity of a C-glucuronide analog of N-(4-hydroxyphenyl)retinamide-O-glucuronide against mammary tumor growth and development. *Anticancer Res*. 1999;19:999-1004.

6. Heyns K, Paulsen H. Selective catalytic oxidation of carbohydrates, employing platinum catalysts. *Adv Carbohydr Chem*. 1962;17:169-211.

7. Wong MF, Weiss KL, Curley RW, Jr. Recent improvements towards the synthesis of the C-glucuronosyl cancer chemopreventive (B-D-glucopyranosyluronate)-4-retinamidophenylmethane. *J Carbohydr Chem*. 1996;15:763-768.

8. Musher JI, Corey EJ. Virtual long-range spiN-spin couplings in nuclear magnetic resonance (N.M.R.): The linear 3-spin system and qualitative implications of higher systems. *Tetrahedron*. 1962;18:791-809.

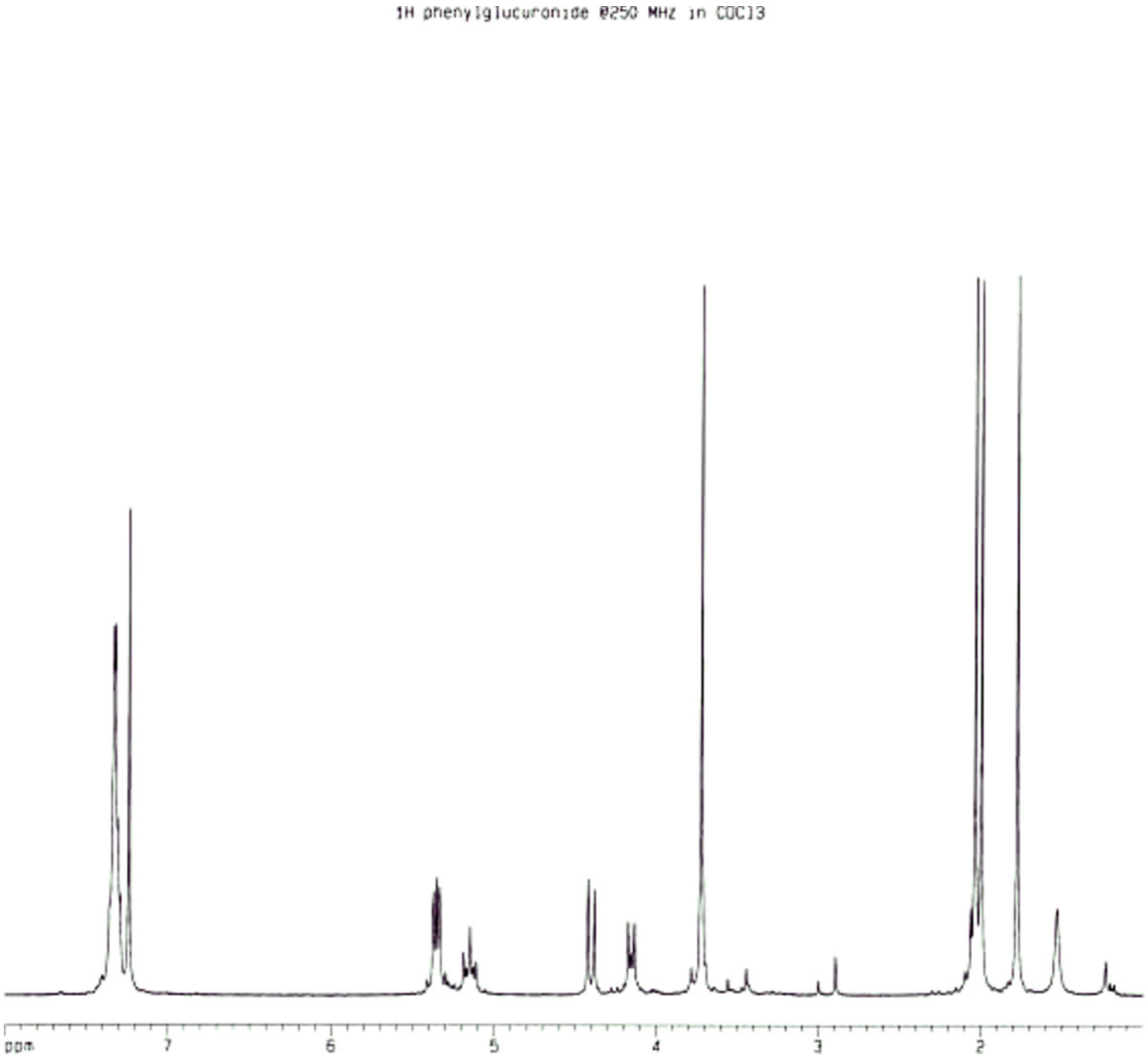
9. Saito S, Sasaki Y, Furomoto T, Sumita S, Hinomoto T. Virtual ^{1}H-^{1}H spiN-spin coupling in a linear five-spin system on the pyramose rings of some glucuronides. *Carbohydr Res*. 1994; 258:59-75.

10. Panigot MJ, Curley RW, Jr. Reaction of glycosyl halide with benzyl Grignard reagents: unexpected O-tolyl alkylation of tetra-O-acetylglucopyranosyl bromide and direct synthesis of (B-glycosyl)phenylmethanes. *J Carbohydr Chem*. 1994;13:293-302.

11. Robarge MJ, Repa JJ, Hanson KK, Seth S, Clagett-Dame M, Abou-Issa H, Curley RW, Jr. N-Linked analogs of retinoid O-glucuronides: potential cancer chemopreventive/chemotherapeutic agents. *Bioorg Med Chem Lett*. 1994;4:2117-2122.

Appendix

Additional Spectra for Table 2, Entry 1 (5) and Entry 15 (13)



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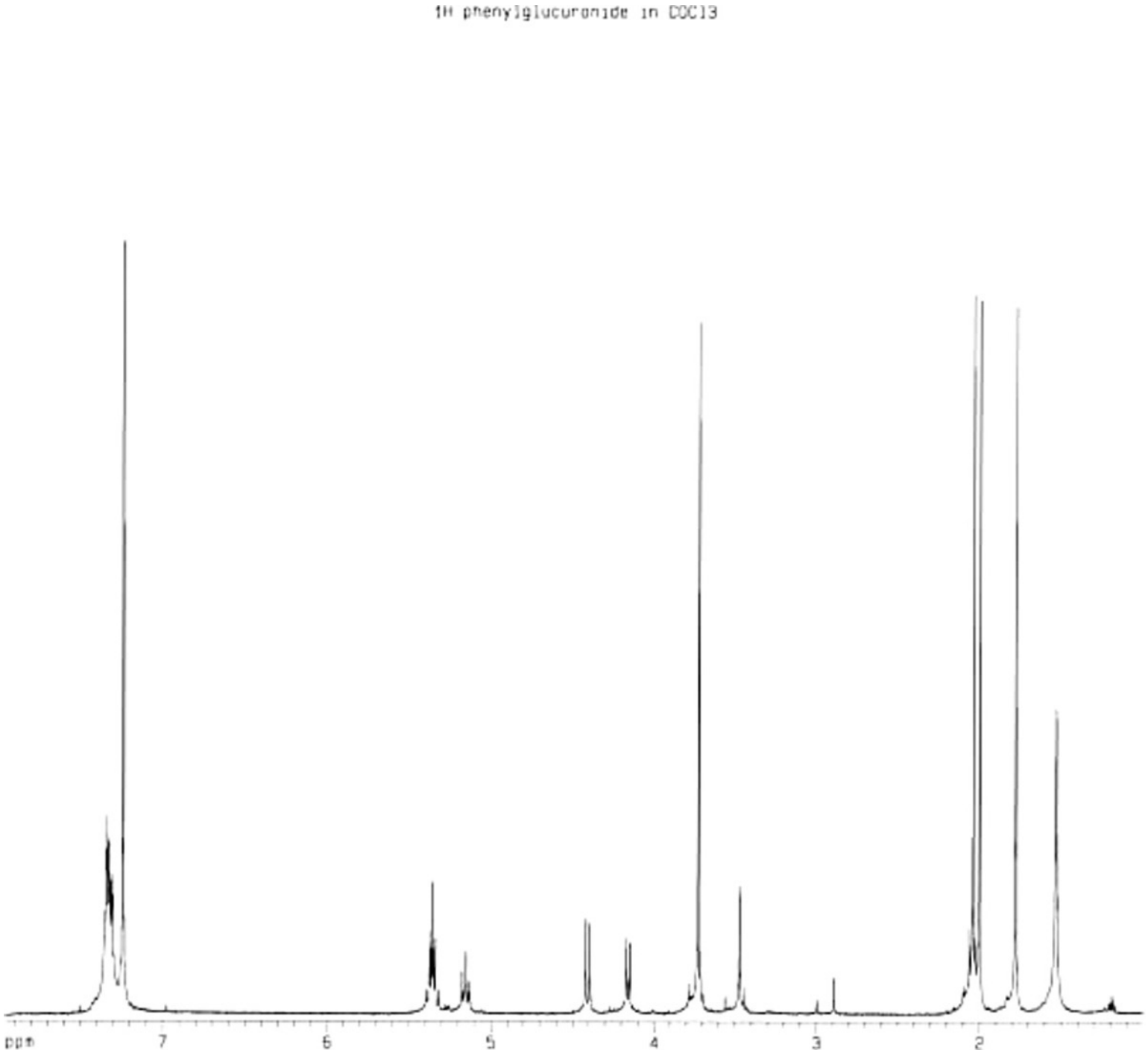

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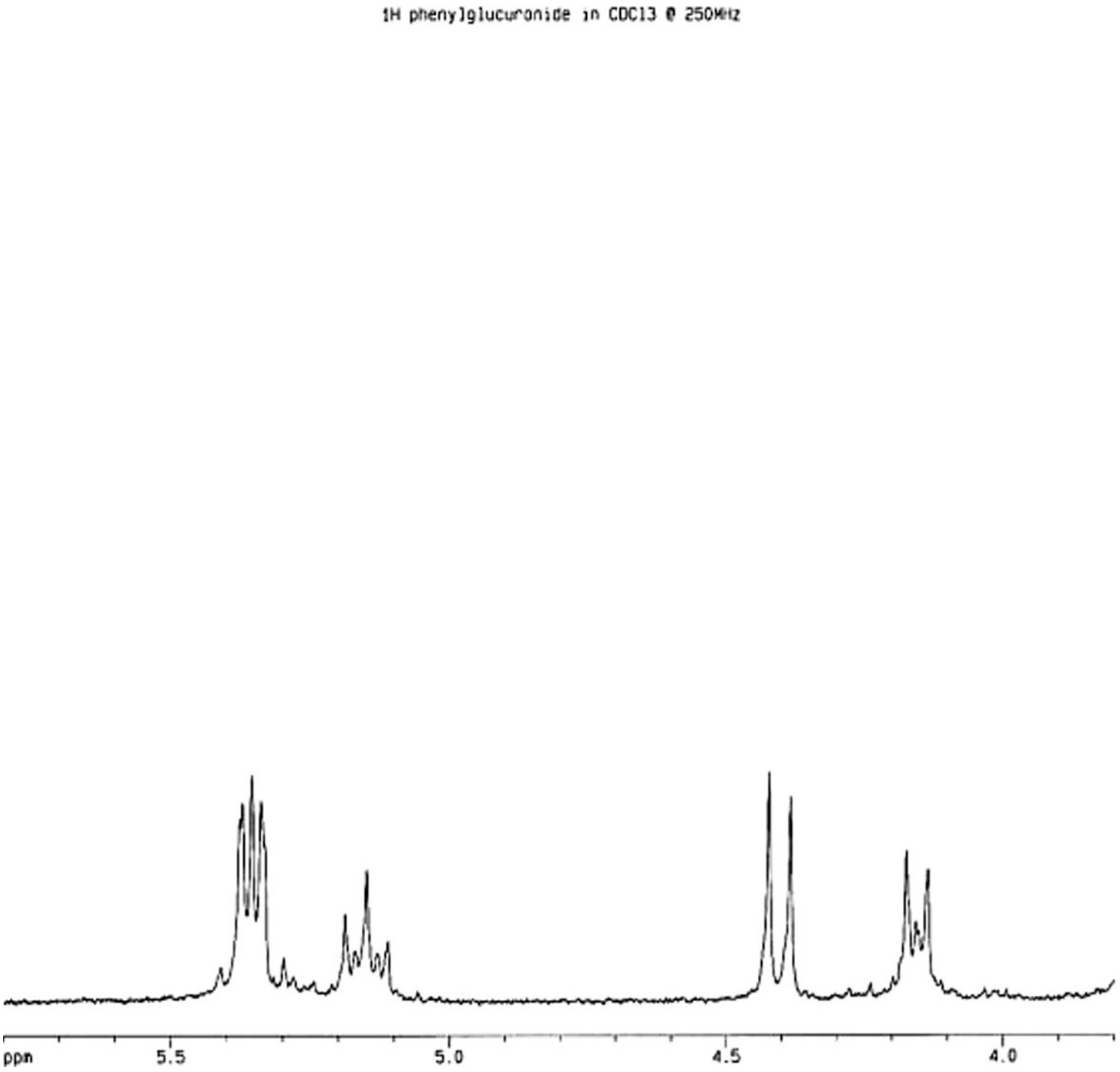

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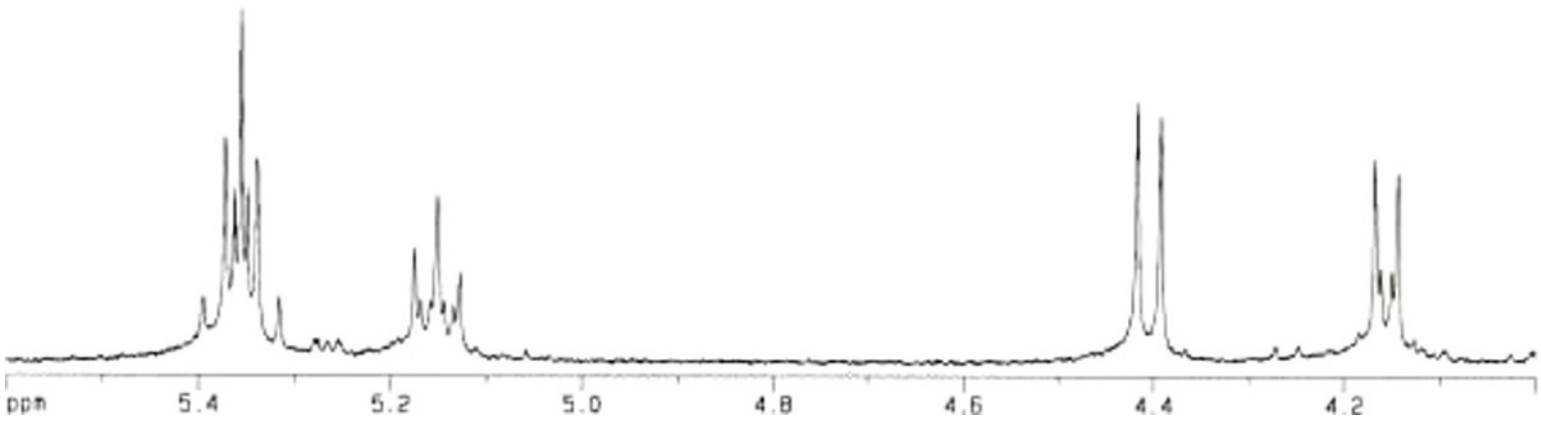

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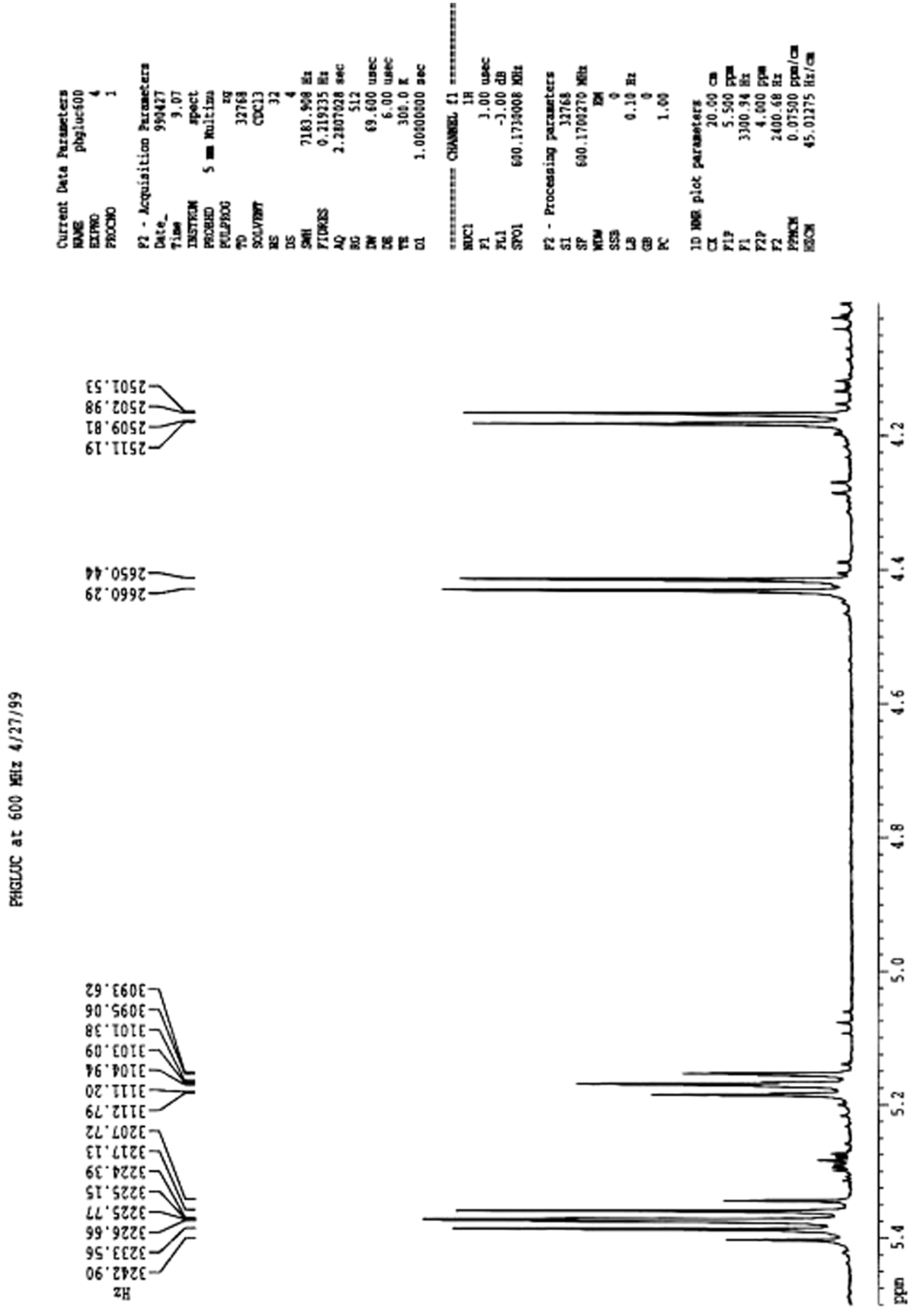
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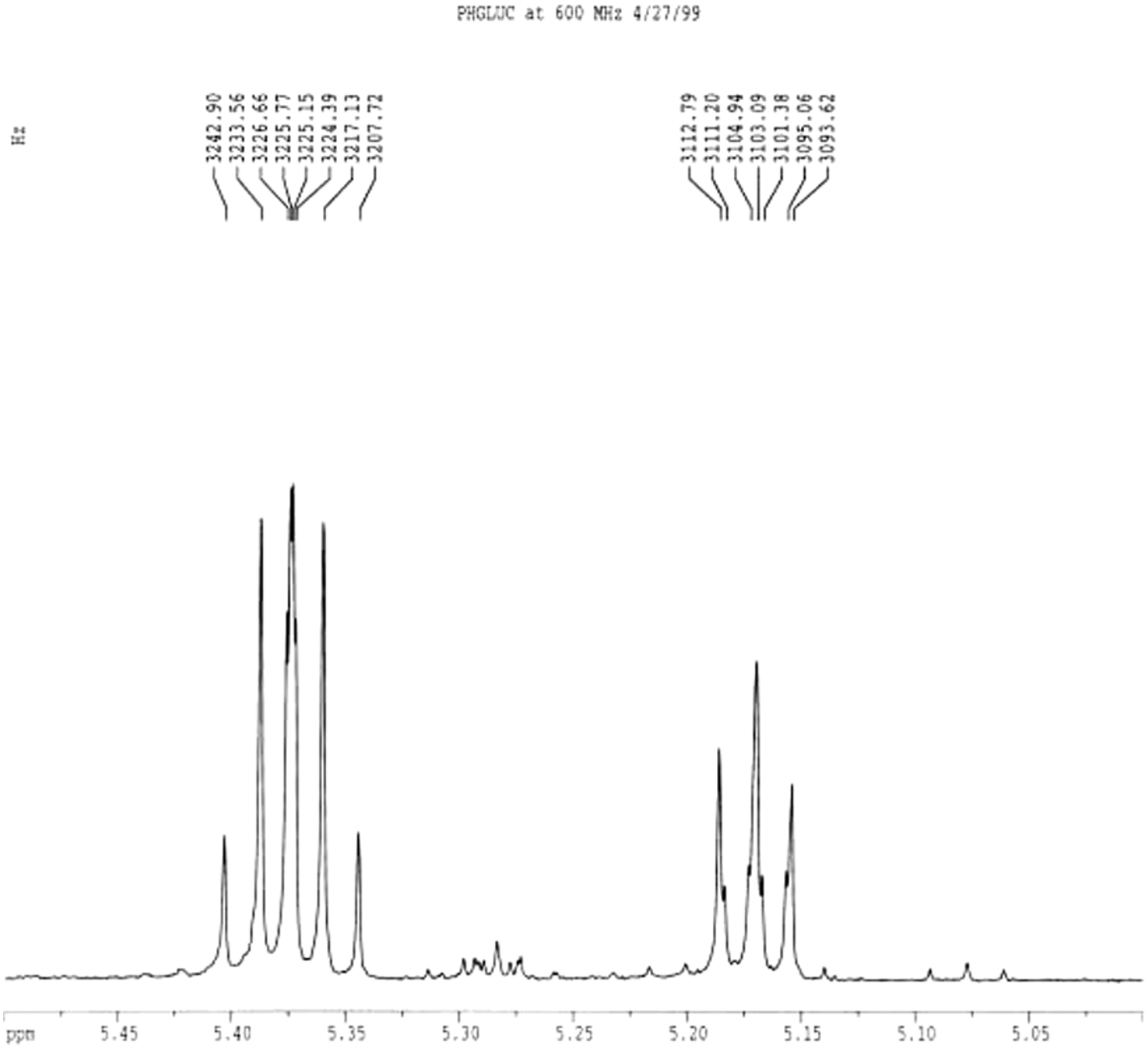

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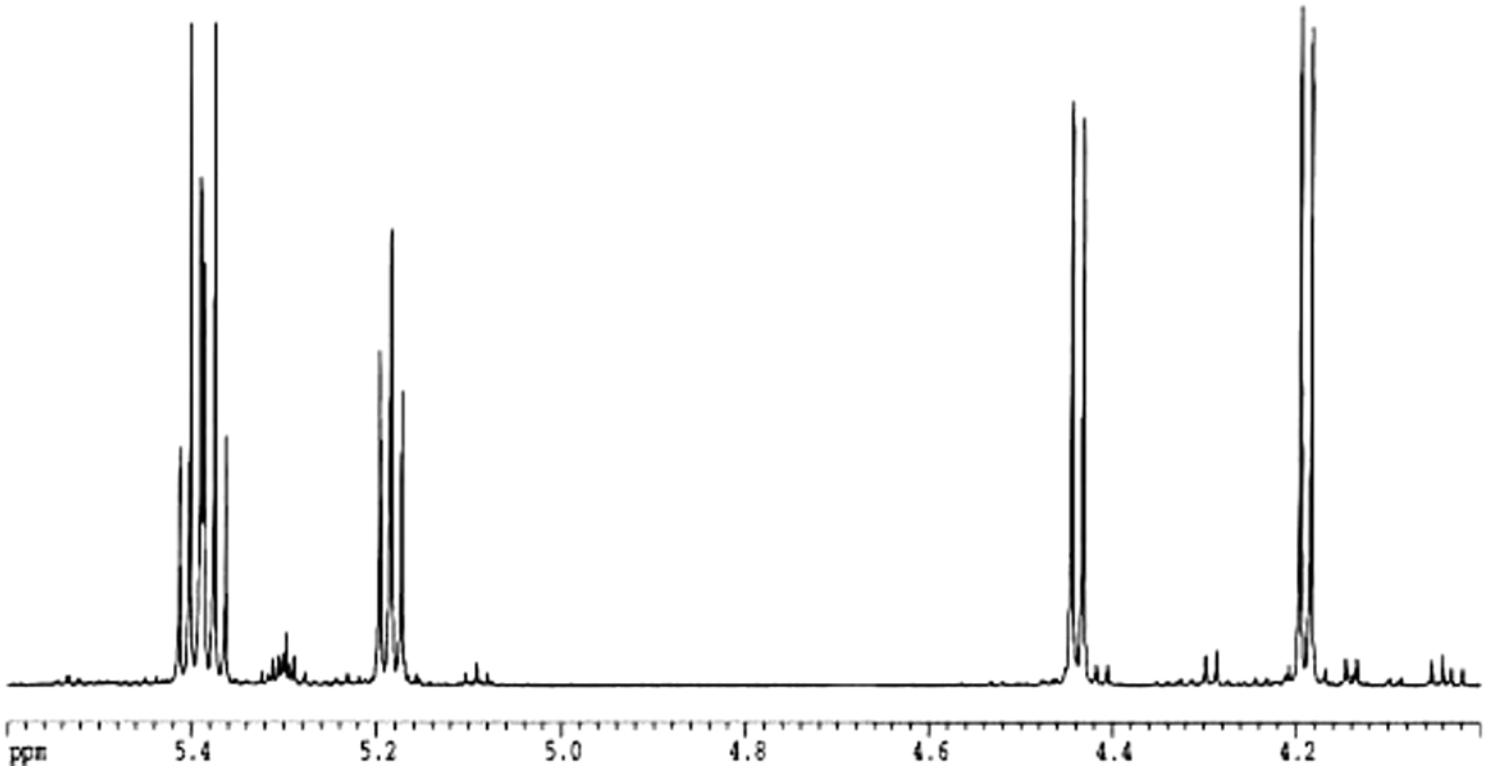


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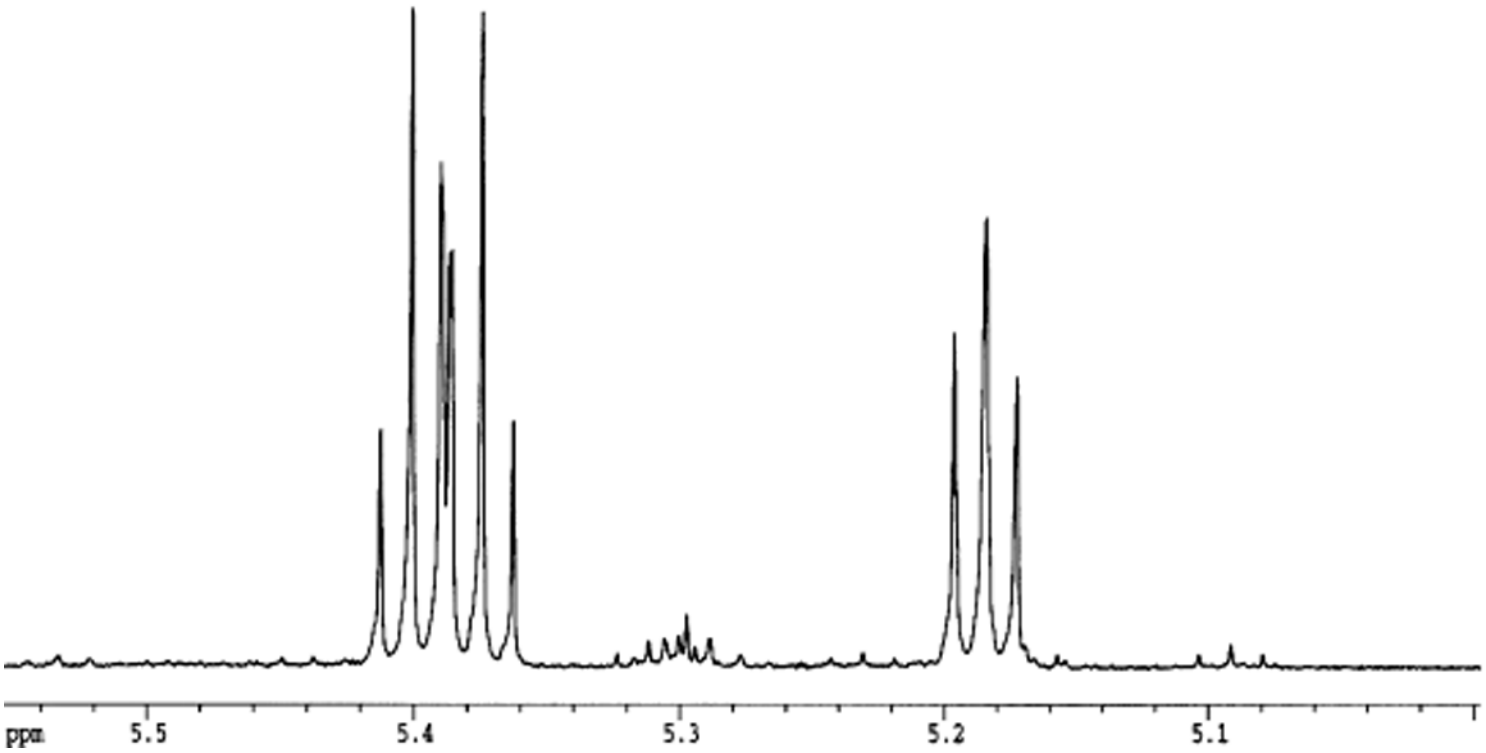
FIDRES            0.158946 Hz
AQ            3.1457779 sec
RG                  256
DW                48.000 usec
DE                 6.00 usec
TE                323.0 K
D1            1.20000005 sec

============ CHANNEL f1 ============
NUC1                1H
P1                4.00 usec
PL1              -3.00 dB
SFO1        800.1337464 MHz

F2 - Processing parameters
SI                65536
SF          800.1300000 MHz
WDW                 EM
SSB                 0
LB                0.10 Hz
GB                  0
PC                1.00

1D NMR plot parameters
CX               20.00 cm
F1P               5.600 ppm
F1              4480.73 Hz
F2P               4.000 ppm
F2              3200.52 Hz
PPMCM            0.08000 ppm/cm
HZCM            64.01040 Hz/cm
```

PHGLUC 800 MHz




```
Current Data Parameters
NAME                pbgluc800
EXPNO                       4
PROCNO                      1

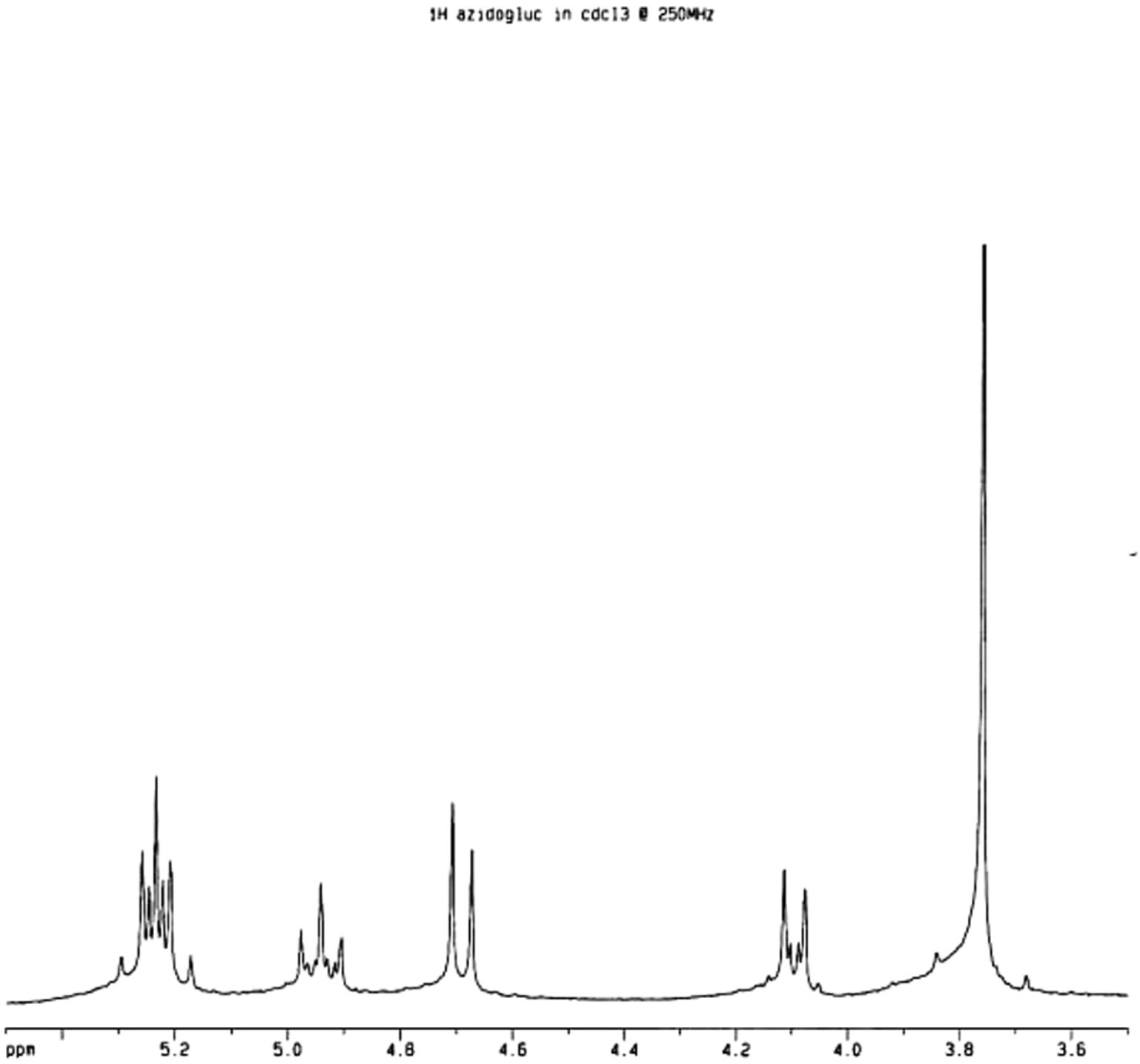
F2 - Acquisition Parameters
Date_                  990503
Time                    15.05
INSTRUM                 spect
PROBHD       5 mm QXI noI
PULPROG                    zg
TD                      65536
SOLVENT                 CDCl3
NS                         16
DS                          4
SWH                10416.667 Hz
FIDRES              0.158946 Hz
AQ                  3.1457779 sec
RG                        256
DW                   48.000 usec
DE                    6.00 usec
TE                    323.0 K
D1                 1.20000005 sec

============ CHANNEL f1 ============
NUC1                       1H
P1                    4.00 usec
PL1                  -3.00 dB
SFO1              800.1337464 MHz

F2 - Processing parameters
SI                      65536
SF                800.1300000 MHz
WDW                        EM
SSB                         0
LB                    0.10 Hz
GB                          0
PC                       1.00

1D NMR plot parameters
CX                   20.00 cm
F1P                   5.554 ppm
F1                  4443.97 Hz
F2P                   4.997 ppm
F2                  3998.42 Hz
PPMCM                0.02784 ppm/cm
HZCM                22.27728 Hz/cm
```

AAPS PharmSci 2001; 3 (1) Article 4 (http://www.pharmsci.org/)



Current Data Parameters

NAME	azidoglcccdcl3
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

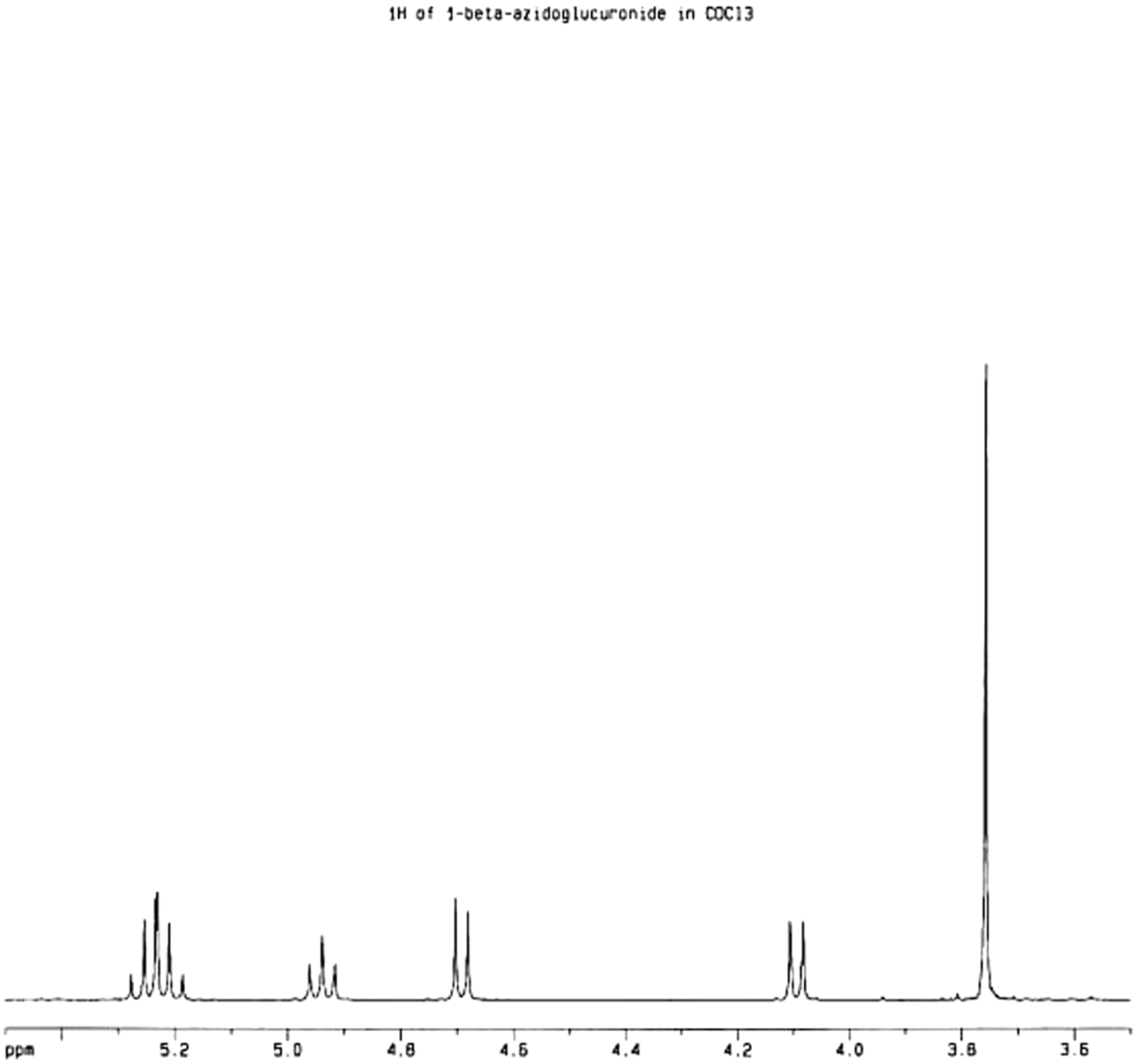
Date_	990203
Time	9.03
INSTRUM	spect
PROBHD	5 mm QNP 1H
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	32
DS	2
SWH	2003.205 Hz
FIDRES	0.061133 Hz
AQ	8.1789427 sec
RG	912.3
DW	249.600 usec
DE	6.00 usec
TE	300.0 K
D1	2.00000000 sec
P1	8.00 usec
SFO1	250.1310005 MHz
NUC1	1H
PL1	-3.00 dB

F2 - Processing parameters

SI	16384
SF	250.1300130 MHz
WDW	EM
SSB	0
LB	0.20 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	20.00 cm
F1P	5.500 ppm
F1	1375.72 Hz
F2P	3.500 ppm
F2	875.46 Hz
PPMCM	0.10000 ppm/cm
HZCM	25.01300 Hz/cm



Current Data Parameters

NAME	azgluc
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

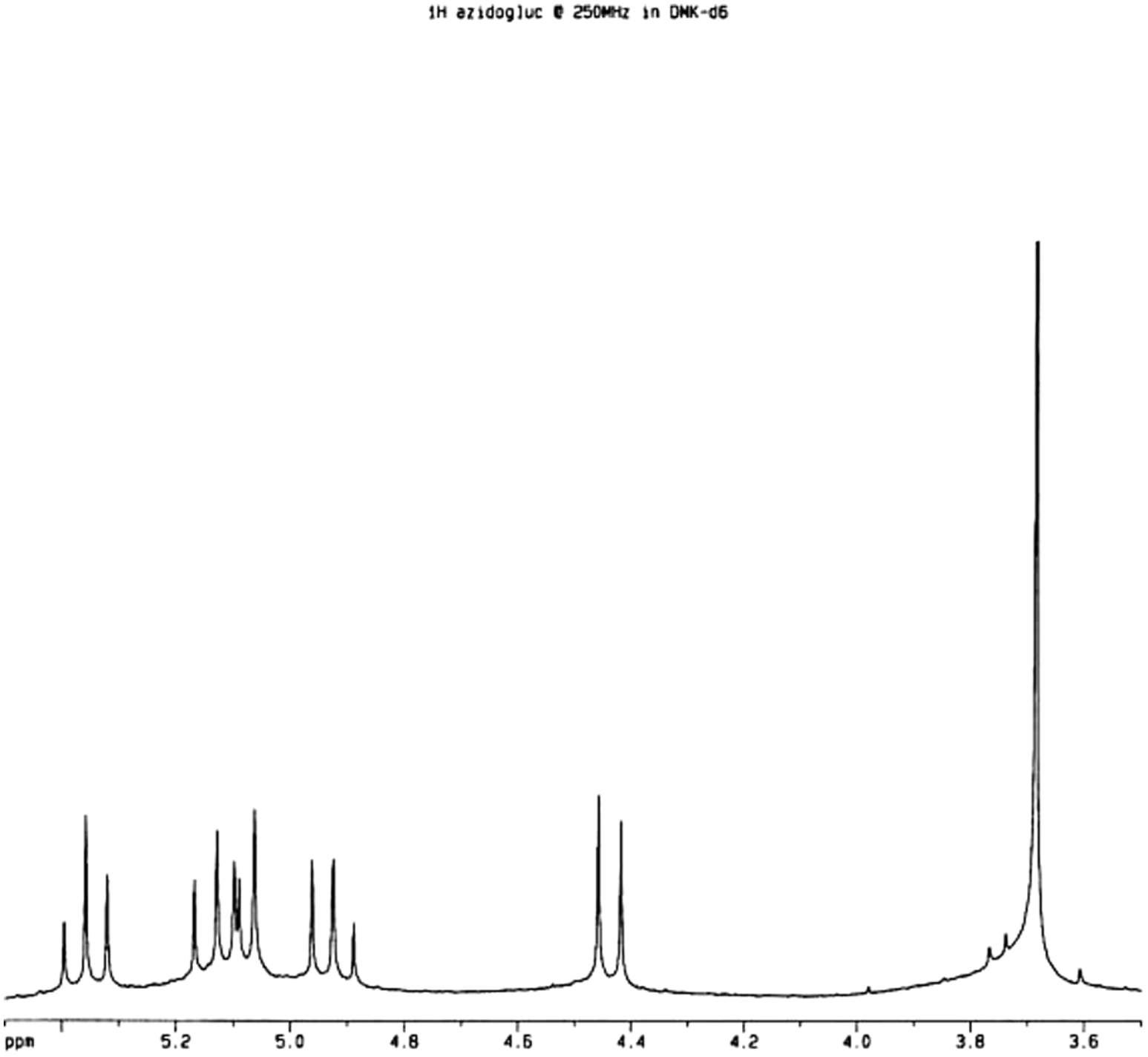
Date_	980928
Time	14.05
INSTRUM	spect
PROBHD	5 mm TBO
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	8
DS	2
SWH	3205.128 Hz
FIDRES	0.097813 Hz
AQ	5.1118579 sec
RG	256
DW	156.000 usec
DE	6.00 usec
TE	300.0 K
D1	2.00000000 sec
P1	10.00 usec
SFO1	400.1316005 MHz
NUC1	1H
PL1	-6.00 dB

F2 - Processing parameters

SI	16384
SF	400.1300170 MHz
WDW	EM
SSB	0
LB	0.30 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	20.00 cm
F1P	5.500 ppm
F1	2200.72 Hz
F2P	3.500 ppm
F2	1400.46 Hz
PPMCM	0.10000 ppm/cm
HZCM	40.01300 Hz/cm



Current Data Parameters

NAME	azidoglucdmk
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

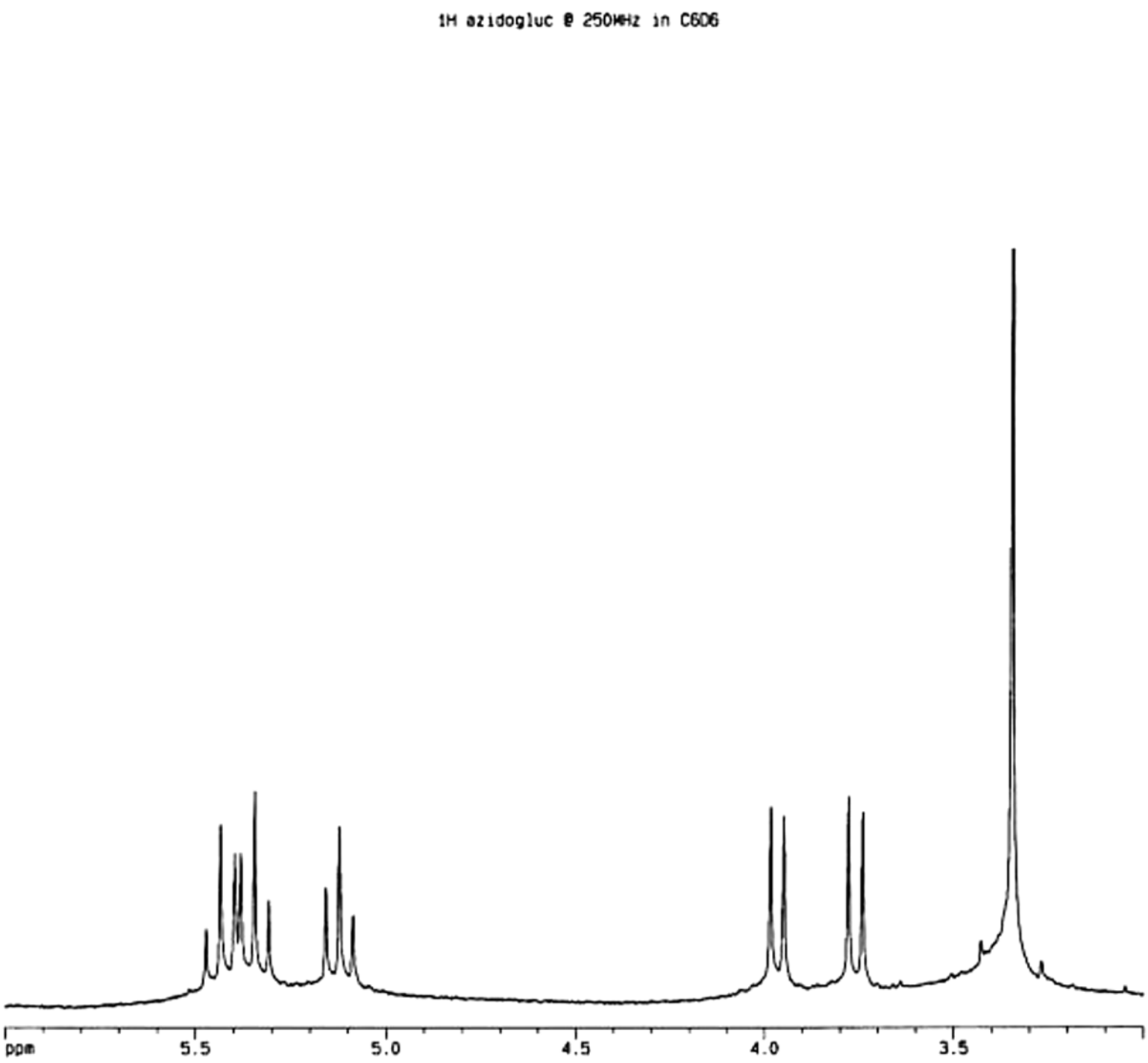
Date_	990203
Time	11.41
INSTRUM	spect
PROBHD	5 mm QNP 1H
PULPROG	zg
TD	32768
SOLVENT	Aceton
NS	32
DS	2
SWH	2003.205 Hz
FIDRES	0.061133 Hz
AQ	8.1789427 sec
RG	645.1
DW	249.600 usec
DE	6.00 usec
TE	300.0 K
D1	2.00000000 sec
P1	8.00 usec
SFO1	250.1310005 MHz
NUC1	1H
PL1	-3.00 dB

F2 - Processing parameters

SI	16384
SF	250.1300130 MHz
WDW	EM
SSB	0
LB	0.20 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	20.00 cm
F1P	5.500 ppm
F1	1375.72 Hz
F2P	3.500 ppm
F2	875.46 Hz
PPMCM	0.10000 ppm/cm
HZCM	25.01300 Hz/cm



Current Data Parameters

NAME	azidoglucbenz
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

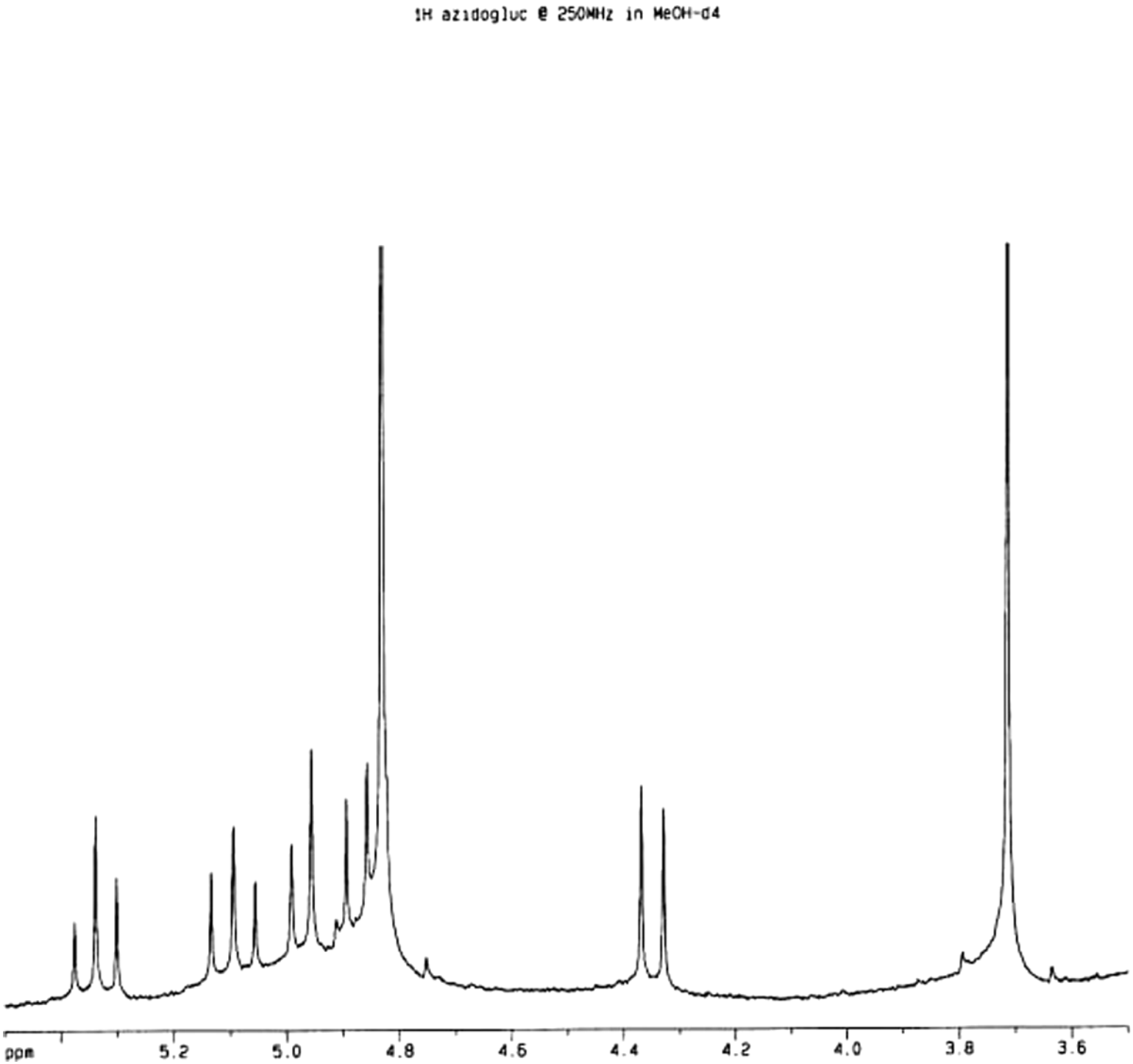
Date_	990203
Time	17.23
INSTRUM	spect
PROBHD	5 mm GNP 1H
PULPROG	zg
TD	32768
SOLVENT	C6D6
NS	32
DS	2
SWH	2003.205 Hz
FIDRES	0.061133 Hz
AQ	8.1789427 sec
RG	1024
DW	249.600 usec
DE	6.00 usec
TE	300.0 K
D1	2.00000000 sec
P1	8.00 usec
SFO1	250.1310005 MHz
NUC1	1H
PL1	-3.00 dB

F2 - Processing parameters

SI	16384
SF	250.1300130 MHz
WDW	EM
SSB	0
LB	0.20 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	20.00 cm
F1P	6.000 ppm
F1	1500.78 Hz
F2P	3.000 ppm
F2	750.39 Hz
PPMCM	0.15000 ppm/cm
HZCM	37.51950 Hz/cm



Current Data Parameters

NAME	azidoglucmeoh
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	990203
Time	15.02
INSTRUM	spect
PROBHD	5 mm DMP 1H
PULPROG	zg
TD	32768
SOLVENT	MeOH
NS	32
DS	2
SWH	2003.205 Hz
FIDRES	0.061133 Hz
AQ	8.1789427 sec
RG	1024
DW	249.600 usec
DE	6.00 usec
TE	300.0 K
D1	2.00000000 sec
P1	8.00 usec
SFO1	250.1310005 MHz
NUC1	1H
PL1	-3.00 dB

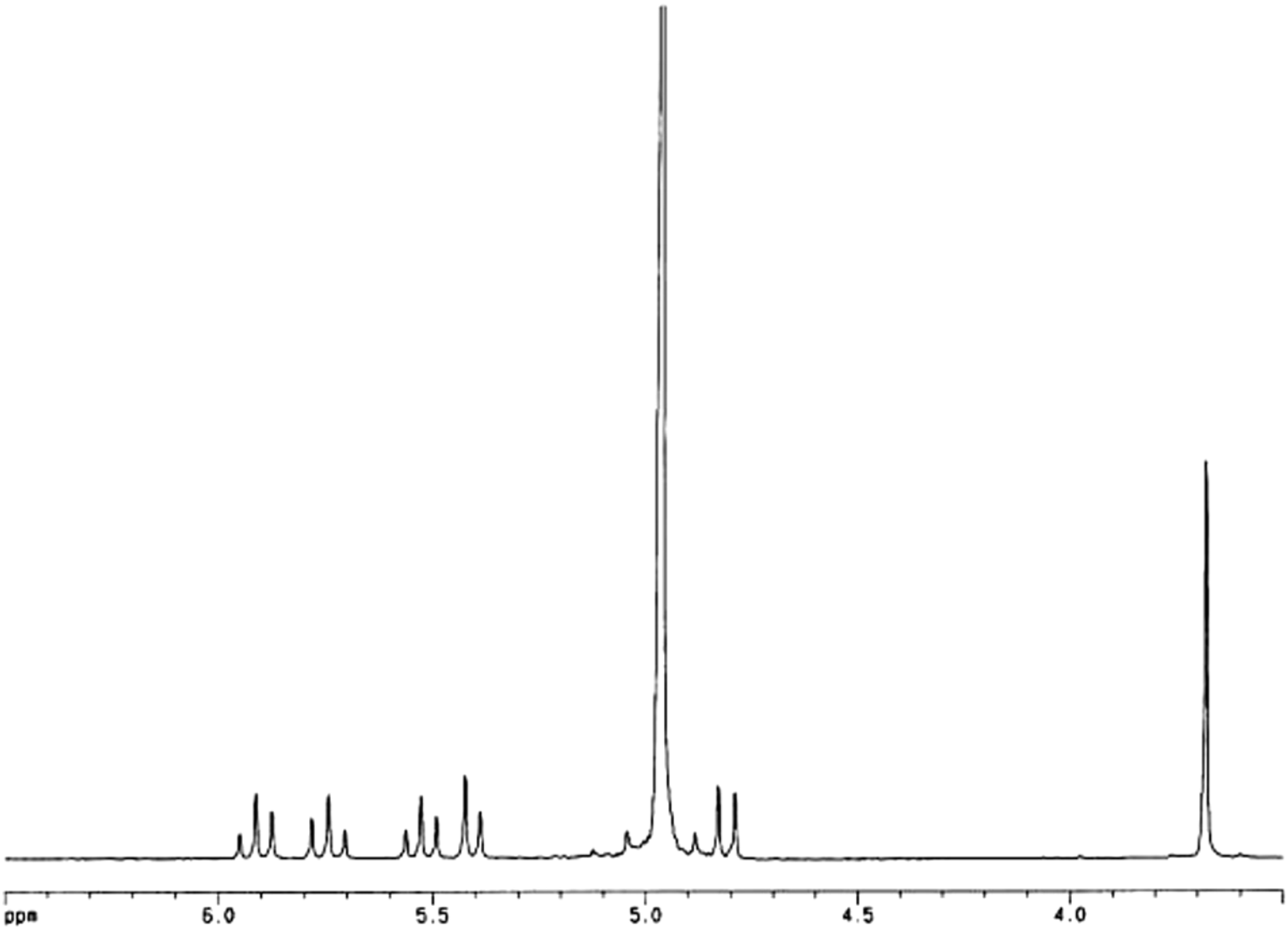
F2 - Processing parameters

SI	16384
SF	250.1300130 MHz
WDW	EM
SSB	0
LB	0.20 Hz
GB	0
PC	1.00

1D NMR plot parameters

CX	20.00 cm
F1P	5.500 ppm
F1	1375.72 Hz
F2P	3.500 ppm
F2	875.46 Hz
PPMCM	0.10000 ppm/cm
HZCM	25.01300 Hz/cm

1H azidogluc @ 250MHz in pyr-d5

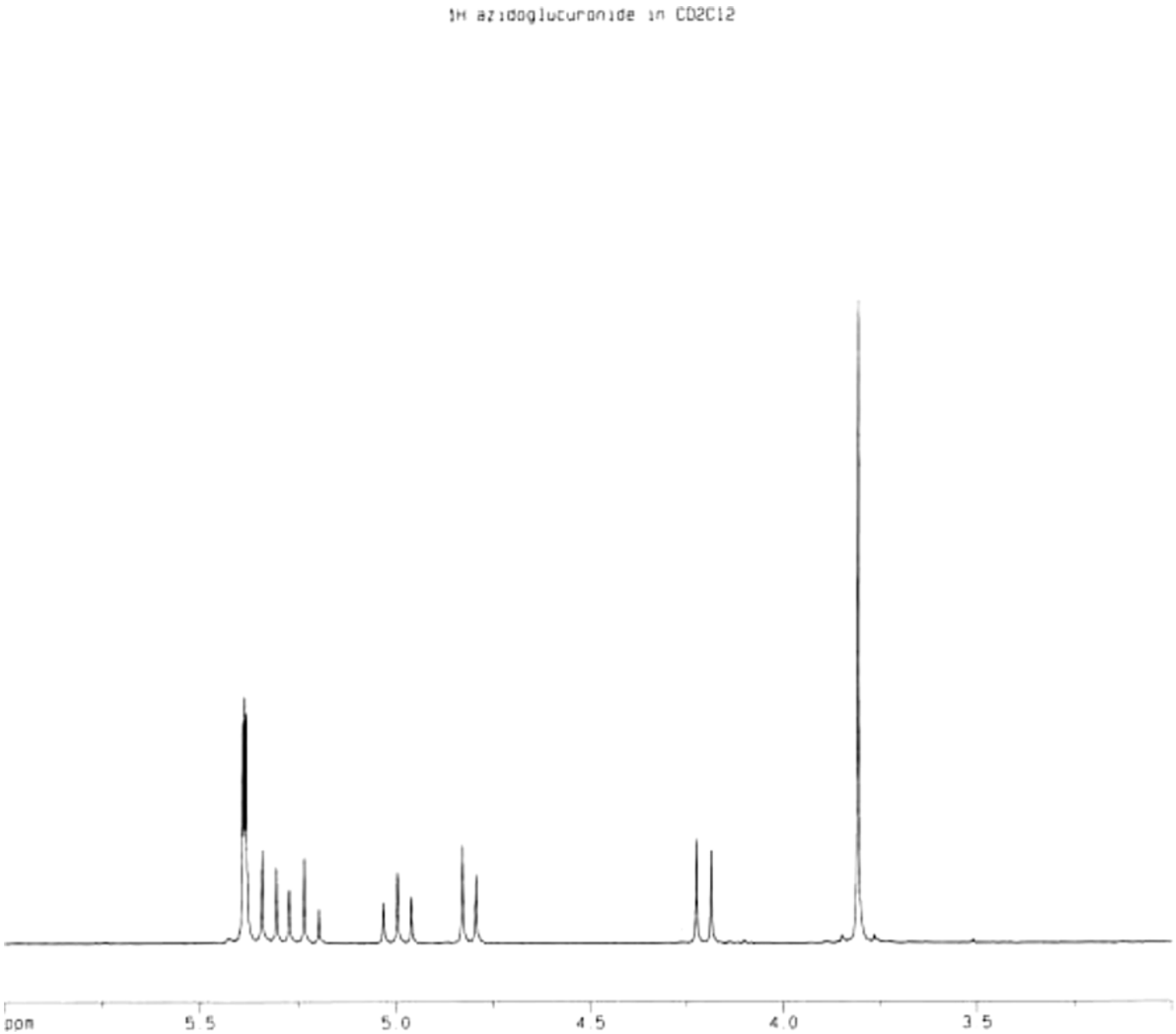


Current Data Parameters	
NAME	azidoglucpyr
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters		
Date_	990204	
Time	9.06	
INSTRUM	spect	
PROBHD	5 mm GNP 1H	
PULPROG	zg	
TD	32768	
SOLVENT	Aceton	
NS	32	
DS	2	
SWH	2495.010	Hz
FIDRES	0.076142	Hz
AQ	6.5667572	sec
RG	400	
DW	200.400	usec
DE	6.00	usec
TE	300.0	K
D1	2.00000000	sec
P1	8.00	usec
SFO1	250.1312505	MHz
NUC1	1H	
PL1	-3.00	dB

F2 - Processing parameters		
SI	16384	
SF	250.1303781	MHz
WDW	EM	
SSB	0	
LB	0.20	Hz
GB	0	
PC	1.00	

1D NMR plot parameters		
CX	20.00	cm
F1P	6.500	ppm
F1	1625.85	Hz
F2P	3.500	ppm
F2	875.46	Hz
PPMCM	0.15000	ppm/cm
HZCM	37.51955	Hz/cm



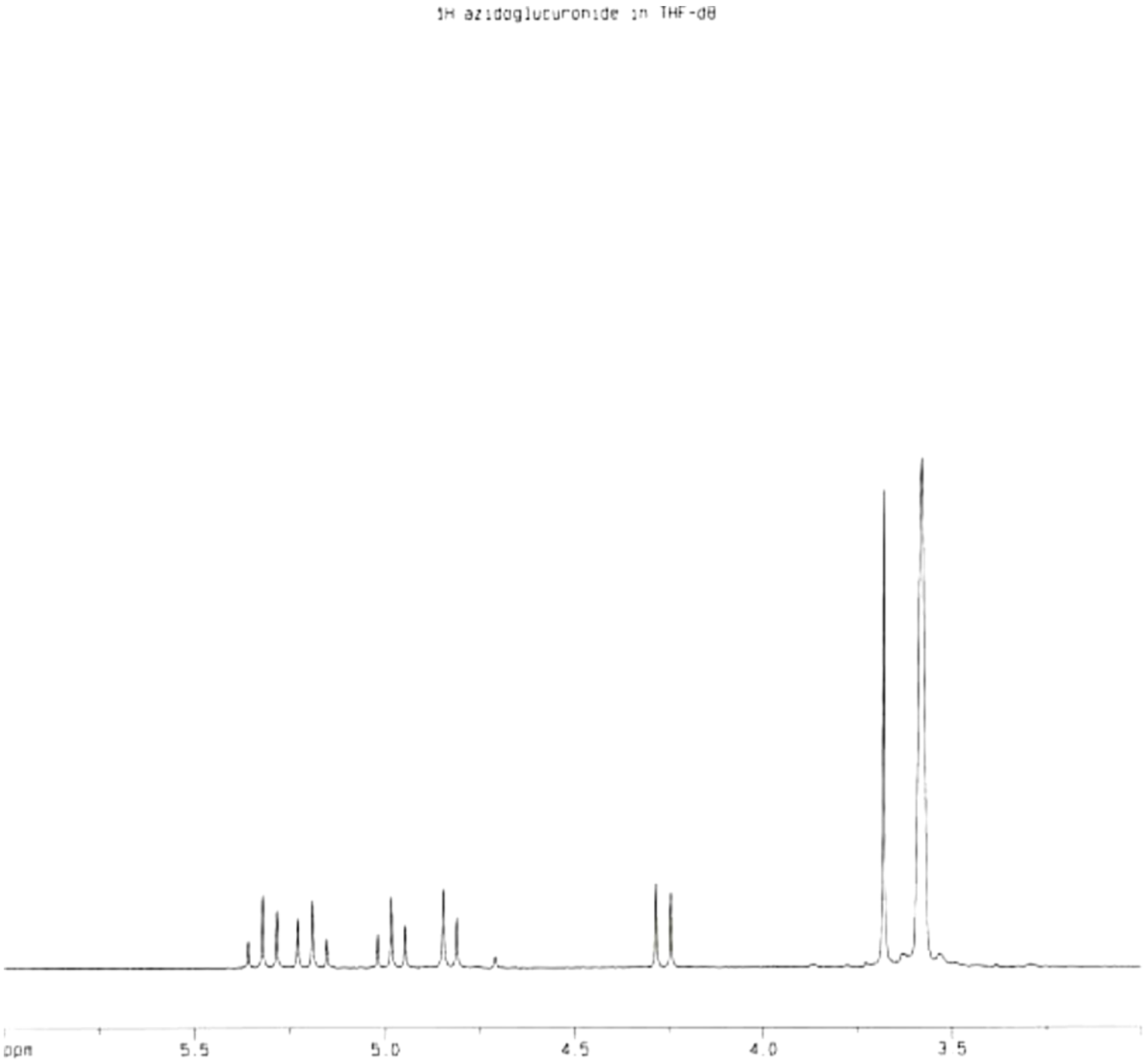

```
Current Data Parameters
NAME        azidogluc13c
EXPNO                   6
PROCNO                  1

F2 - Acquisition Parameters
Date_            20001201
Time                11.07
INSTRUM             spect
PROBHD     5 mm QNP  1H
PULPROG                zg
TD                  32768
SOLVENT            CD2Cl2
NS                      8
DS                      2
SWH              1755.618 Hz
FIDRES           0.053577 Hz
AQ               9.3323765 sec
RG                  912.3
DW                284.800 usec
DE                  6.00 usec
TE                 300.0 K
D1              2.50000000 sec

------------ CHANNEL f1 --------
NUC1                    1H
P1                  11.00 usec
PL1                 -3.00 dB
SFO1         250.1311256 MHz

F2 - Processing parameters
SI                  32768
SF           250.1299942 MHz
WDW                    EM
SSB                     0
LB                  0.20 Hz
GB                      0
PC                  1.40

1D NMR plot parameters
CX                 20.00 cm
F1P                6.000 ppm
F1                1500.78 Hz
F2P                3.000 ppm
F2                 750.39 Hz
PPMCM             0.15000 ppm/cm
HZCM             37.51950 Hz/cm
```



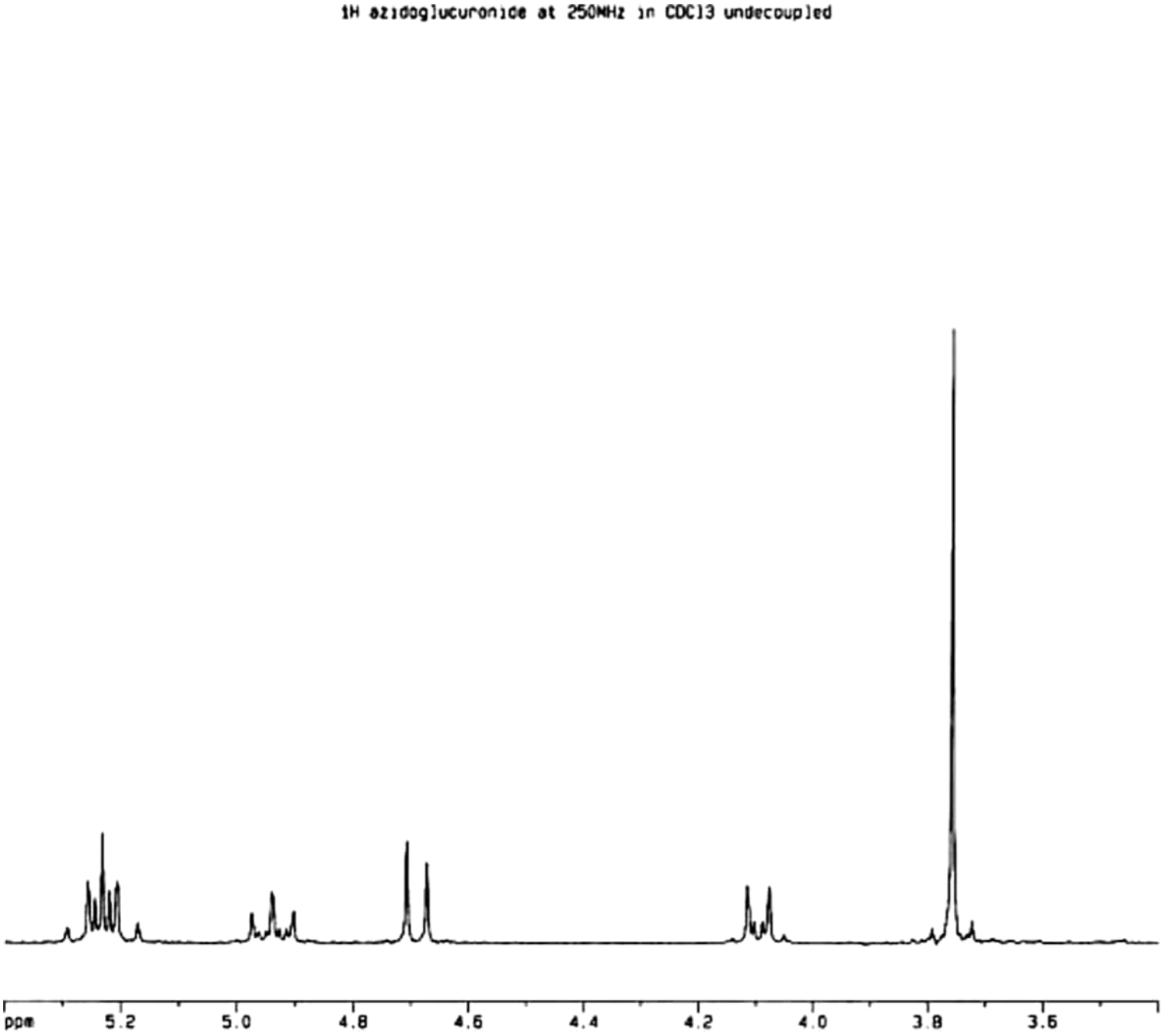
Current Data Parameters
NAME azidogluc13c
EXPNO 7
PROCNO 1

F2 - Acquisition Parameters
Date_ 20001201
Time 11 18
INSTRUM spect
PROBHD 5 mm QNP 1H
PULPROG zg
TD 32768
SOLVENT Aceton
NS 8
DS 2
SWH 1755.618 Hz
FIDRES 0.053577 Hz
AQ 9.3323765 sec
RG 812.7
DW 284.800 usec
DE 6.00 usec
TE 300.0 K
D1 2.50000000 sec

========== CHANNEL f1 ========
NUC1 1H
P1 11.00 usec
PL1 -3.00 dB
SFO1 250.1311256 MHz

F2 - Processing parameters
SI 32768
SF 250.1300102 MHz
WDW EM
SSB 0
LB 0.20 Hz
GB 0
PC 1.40

1D NMR plot parameters
CX 20.00 cm
F1P 6.000 ppm
F1 1500.78 Hz
F2P 3.000 ppm
F2 750.39 Hz
PPMCM 0.15000 ppm/cm
HZCM 37.51950 Hz/cm



Current Data Parameters

NAME	azidogluc
EXPNO	9
PROCNO	1

F2 - Acquisition Parameters

Date_	20001031
Time	9.14
INSTRUM	spect
PROBHD	5 mm QNP 1H
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	1
DS	0
SWH	1745.810 Hz
FIDRES	0.053278 Hz
AQ	9.3848047 sec
RG	645.1
DW	286.400 usec
DE	6.00 usec
TE	300.0 K
D1	2.50000000 sec

---------- CHANNEL f1 ---------

NUC1	1H
P1	11.00 usec
PL1	-3.00 dB
SFO1	250.1311256 MHz

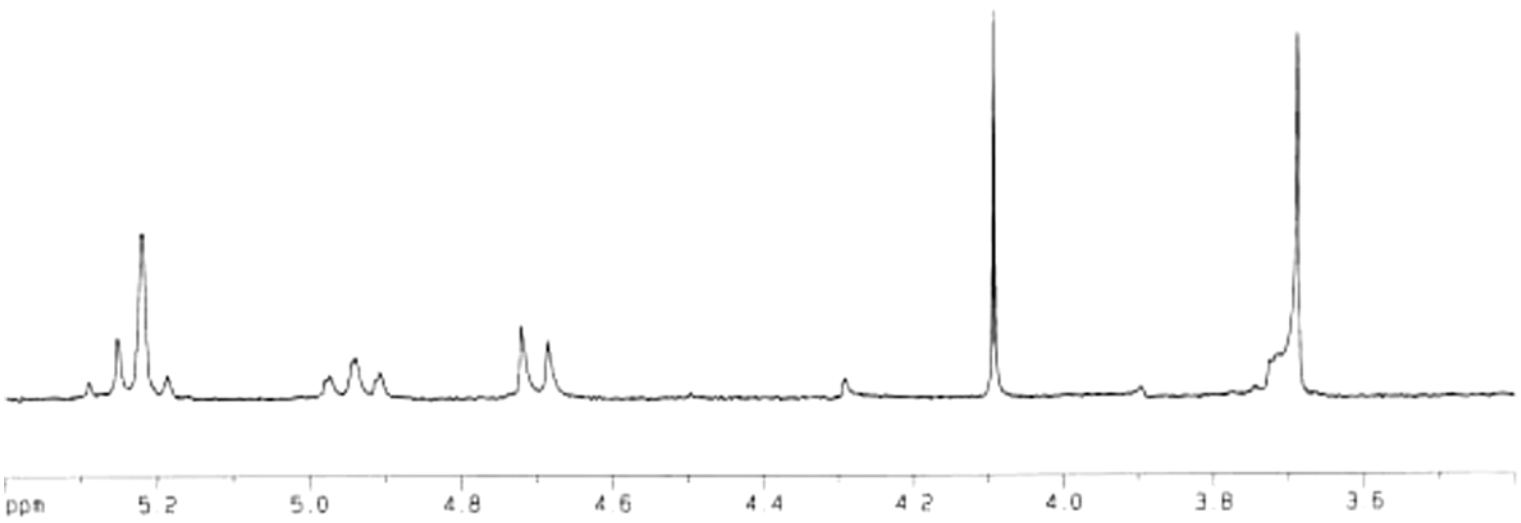
F2 - Processing parameters

SI	32768
SF	250.1300126 MHz
WDW	EM
SSB	0
LB	0.20 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	5.400 ppm
F1	1350.70 Hz
F2P	3.400 ppm
F2	850.44 Hz
PPMCM	0.10000 ppm/cm
HZCM	25.01300 Hz/cm

1H azidoglucuronide at 250MHz in CDCl3 decoupled at 4.1ppm




```
Current Data Parameters
NAME                 azidogluc
EXPNO                       10
PROCNO                       1

F2 - Acquisition Parameters
Date_               20001031
Time                    9.37
INSTRUM                 spect
PROBHD     5 mm QNP  1H
PULPROG                 zghd
TD                      32768
SOLVENT                 CDCl3
NS                         32
DS                          2
SWH                 1745.810 Hz
FIDRES              0.053278 Hz
AQ                  9.3848047 sec
RG                      645.1
DW                    286.400 usec
DE                      6.00 usec
TE                     300.0 K
D1                  2.00000000 sec
d12                 0.00002000 sec

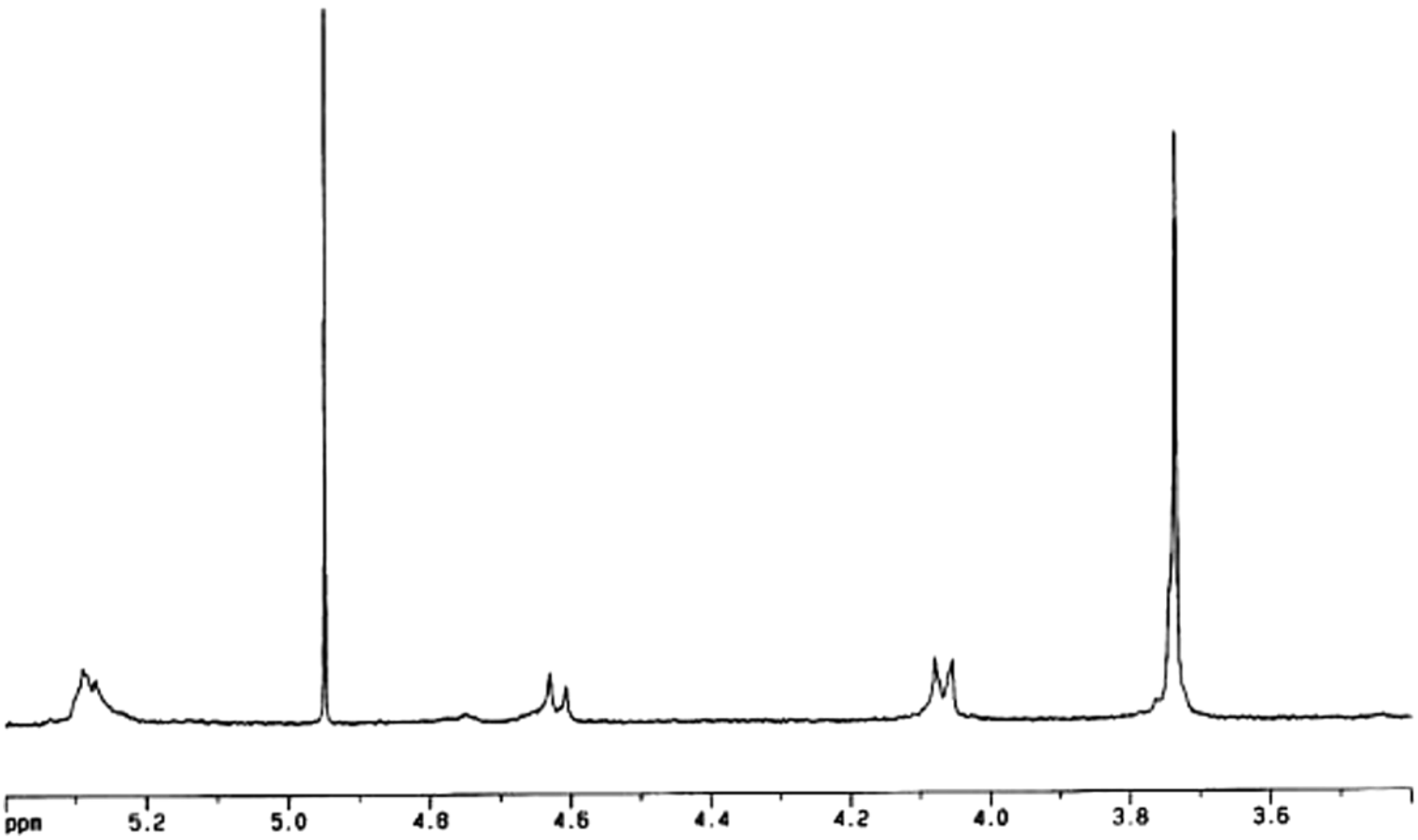
====== CHANNEL f1 ========
NUC1                       1H
P1                      11.00 usec
PL1                     -3.00 dB
SFO1              250.1311256 MHz

====== CHANNEL f2 ========
NUC2                       1H
PL2                      0.00 dB
PL24                    40.00 dB
SFO2              250.1310410 MHz

F2 - Processing parameters
SI                      32768
SF                250.1300172 MHz
WDW                        EM
SSB                         0
LB                      0.20 Hz
GB                          0
PC                      1.40

1D NMR plot parameters
CX                     20.00 cm
F1P                     5.400 ppm
F1                   1350.70 Hz
F2P                     3.400 ppm
F2                    850.44 Hz
PPMCM                0.10000 ppm/cm
HZCM                25.01300 Hz/cm
```

1H azidoglucuronide at 250MHz in CDCl3 decoupled at 4.9ppm



Current Data Parameters

NAME	azidogluc
EXPNO	11
PROCNO	1

F2 - Acquisition Parameters

Date_	20001031
Time	9.50
INSTRUM	spect
PROBHD	5 mm QNP 1H
PULPROG	zghd
TD	32768
SOLVENT	Aceton
NS	32
DS	2
SWH	1745.810 Hz
FIDRES	0.053278 Hz
AQ	9.3848047 sec
RG	645.1
DW	286.400 usec
DE	6.00 usec
TE	300.0 K
D1	2.50000000 sec
d12	0.00002000 sec

---------- CHANNEL f1 ---------

NUC1	1H
P1	11.00 usec
PL1	-3.00 dB
SFO1	250.1311256 MHz

---------- CHANNEL f2 ---------

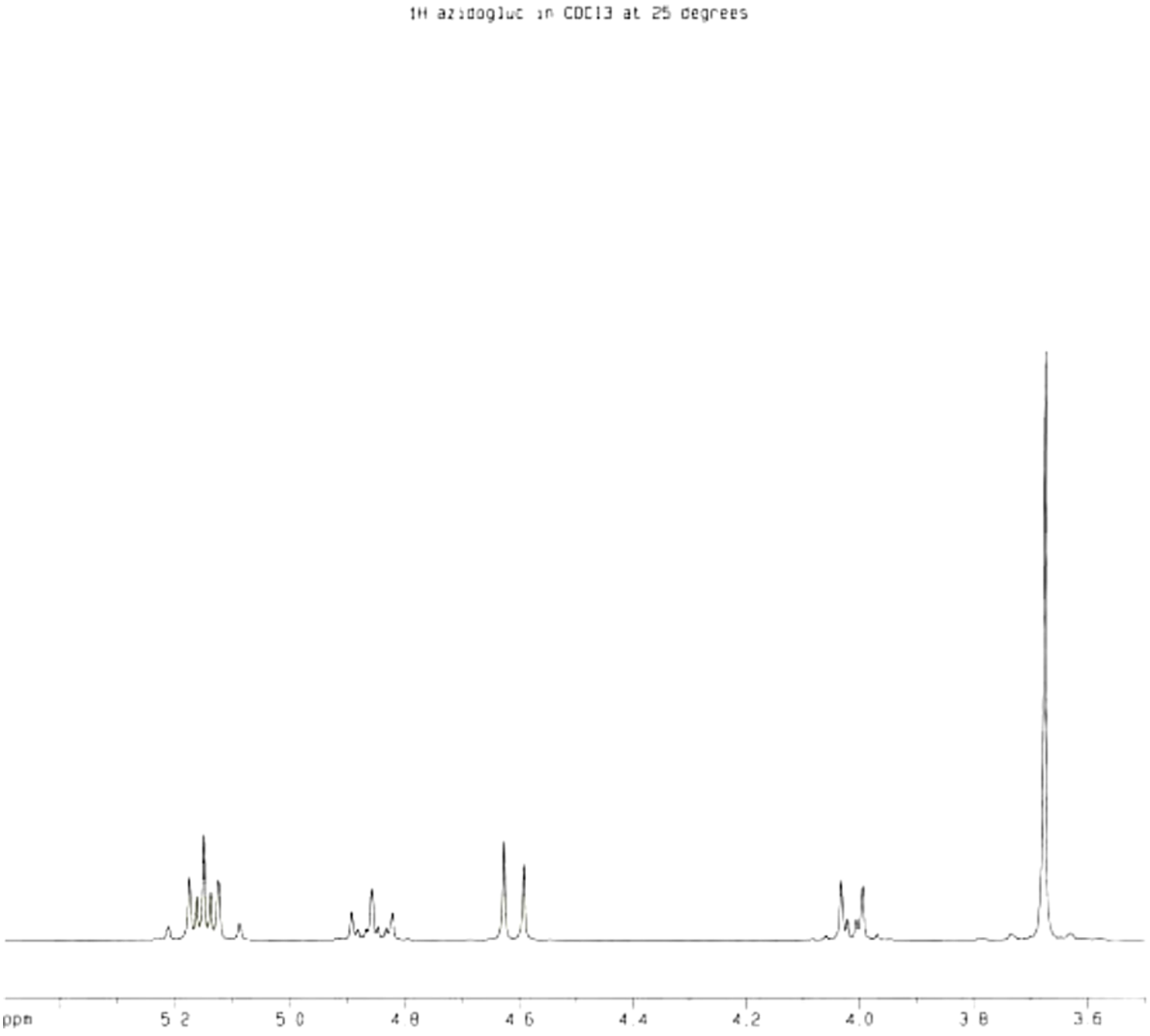
NUC2	1H
PL2	0.00 dB
PL24	40.00 dB
SFO2	250.1312520 MHz

F2 - Processing parameters

SI	32768
SF	250.1300143 MHz
WDW	EM
SSB	0
LB	0.20 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	5.400 ppm
F1	1350.70 Hz
F2P	3.400 ppm
F2	850.44 Hz
PPMCM	0.10000 ppm/cm
HZCM	25.01300 Hz/cm




```
Current Data Parameters
NAME                 azidoglu
EXPNO                       5
PROCNO                      1

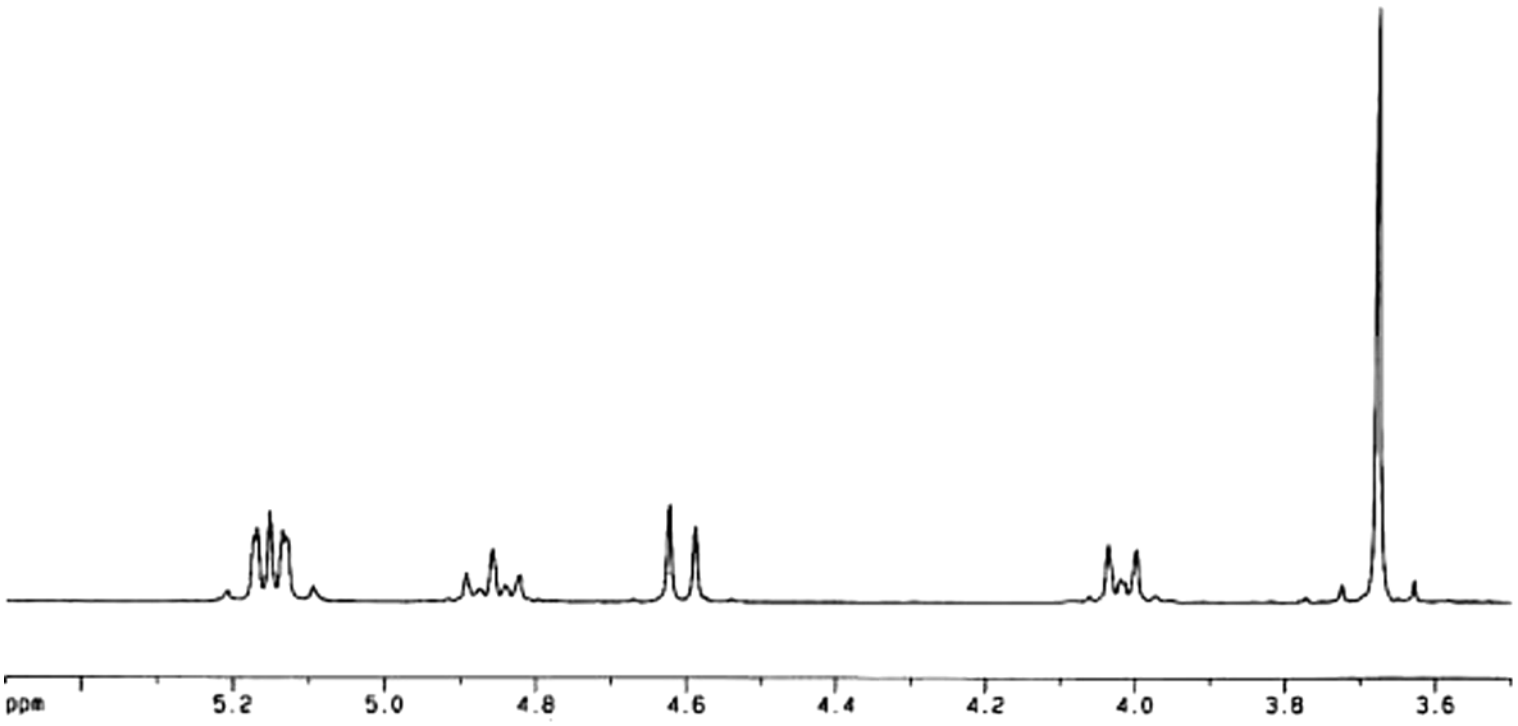
F2 - Acquisition Parameters
Date_               20001012
Time                   13.55
INSTRUM                spect
PROBHD     5 mm DNP  1H
PULPROG                 zg
TD                     32768
SOLVENT                CDCl3
NS                         8
DS                         2
SWH                1755.618 Hz
FIDRES             0.053577 Hz
AQ                 9.3323765 sec
RG                       512
DW                   284.800 usec
DE                     6.00 usec
TE                     300.0 K
D1                 2.50000000 sec

============ CHANNEL f1 ========
NUC1                       1H
P1                     11.00 usec
PL1                    -3.00 dB
SFO1               250.1311256 MHz

F2 - Processing parameters
SI                     32768
SF                 250.1300328 MHz
WDW                        EM
SSB                         0
LB                      0.20 Hz
GB                          0
PC                      1.40

1D NMR plot parameters
CX                     20.00 cm
F1P                    5.500 ppm
F1                   1375.71 Hz
F2P                    3.500 ppm
F2                    875.46 Hz
PPMCM                0.10000 ppm/cm
HZCM                25.01300 Hz/cm
```

AAPS PharmSci 2001; 3 (1) Article 4 (http://www.pharmsci.org/)



Current Data Parameters

NAME	azidogluc
EXPNO	6
PROCNO	1

F2 - Acquisition Parameters

Date_	20001012
Time	13.59
INSTRUM	spect
PROBHD	5 mm DMP 1H
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	8
DS	2
SWH	1755.618 Hz
FIDRES	0.053577 Hz
AQ	9.3323765 sec
RG	512
DW	284.800 usec
DE	6.00 usec
TE	300.0 K
D1	2.50000000 sec

********** CHANNEL f1 ********

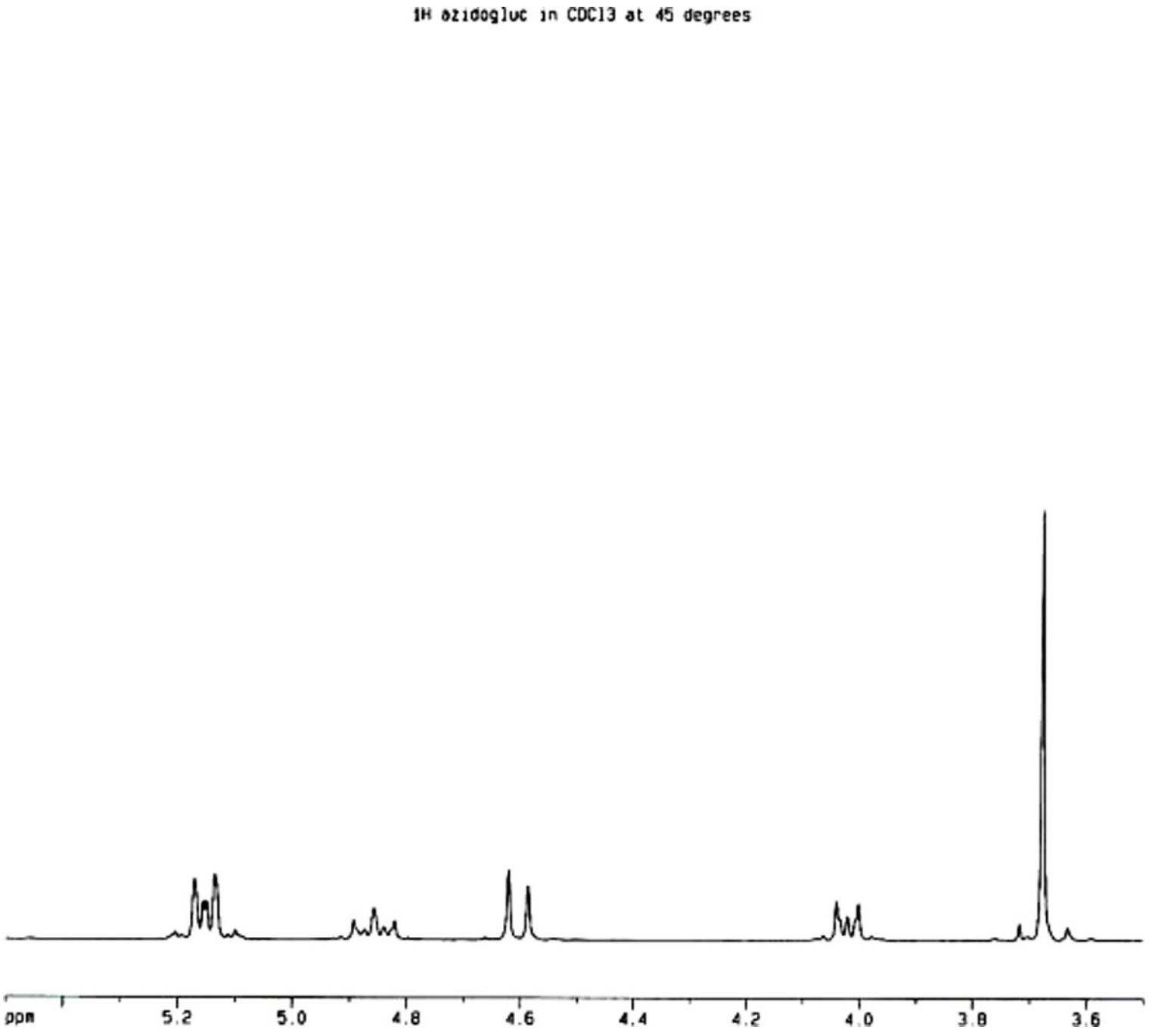
NUC1	1H
P1	11.00 usec
PL1	-3.00 dB
SFO1	250.1311256 MHz

F2 - Processing parameters

SI	32768
SF	250.1300328 MHz
WDW	EM
SSB	0
LB	0.20 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	5.500 ppm
F1	1375.72 Hz
F2P	3.500 ppm
F2	875.46 Hz
PPMCM	0.10000 ppm/cm
HZCM	25.01300 Hz/cm



Current Data Parameters

NAME	azidogluc
EXPNO	7
PROCNO	1

F2 - Acquisition Parameters

Date_	20001012
Time	14.03
INSTRUM	spect
PROBHD	5 mm QNP 1H
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	8
DS	2
SWH	1755.618 Hz
FIDRES	0.053577 Hz
AQ	9.3323765 sec
RG	512
DW	284.800 usec
DE	6.00 usec
TE	300.0 K
D1	2.50000000 sec

********** CHANNEL f1 ********

NUC1	1H
P1	11.00 usec
PL1	-3.00 dB
SFO1	250.1311256 MHz

F2 - Processing parameters

SI	32768
SF	250.1300328 MHz
WDW	EM
SSB	0
LB	0.20 Hz
GB	0
PC	1.40

1D NMR plot parameters

CX	20.00 cm
F1P	5.500 ppm
F1	1375.72 Hz
F2P	3.500 ppm
F2	875.46 Hz
PPMCM	0.10000 ppm/cm
HZCM	25.01300 Hz/cm