Supporting Information

Exploration of the active site of β4GalT7: Modifications of the aglycon of aromatic xylosides

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Mass analysis

Table S1. HRMS for all galactosylated products.

Acceptor substrate	**Galactosylation product** Chemical formula	**HRMS** calcd for $[M+H]^+$	**HRMS** found
1	$C_{21}H_{26}O_{10}$	439.1599	439.1598
2	$C_{12}H_{22}O_{10}$	327.1286	327.1287
3	$C_{17}H_{24}O_{10}$	389.1442	389.1441
4	$C_{21}H_{26}O_{10}$	439.1599	439.1598
5	$C_{23}H_{28}O_{10}$	465.1755	465.1755
6	$C_{26}H_{30}O_{10}$	520.2177[a]	520.2179
7	$C_{21}H_{26}O_9S$	455.1370	455.1370
8	$C_{21}H_{26}O_{11}S$	487.1269	487.1270
9	$C_{22}H_{28}O_9$	437.1806	437.1804
12	$C_{22}H_{28}O_{10}$	453.1755	453.1755
13	$C_{23}H_{30}O_{11}$	483.1861	483.1858
14	$C_{25}H_{34}O_{12}$	527.2123	527.2123
15	$C_{27}H_{38}O_{13}$	571.2385	571.2388
16	$C_{22}H_{28}O_{11}$	486.1973[a]	486.1972
17	$C_{21}H_{26}O_{11}$	455.1548	455.1549
18	$C_{23}H_{28}O_{12}$	497.1654	497.1655
19	$C_{22}H_{25}NO_{10}$	464.1551	464.1550

[a] HRMS for $[M+NH_4]^+$

Cloning, expression and purification of β4GALT7

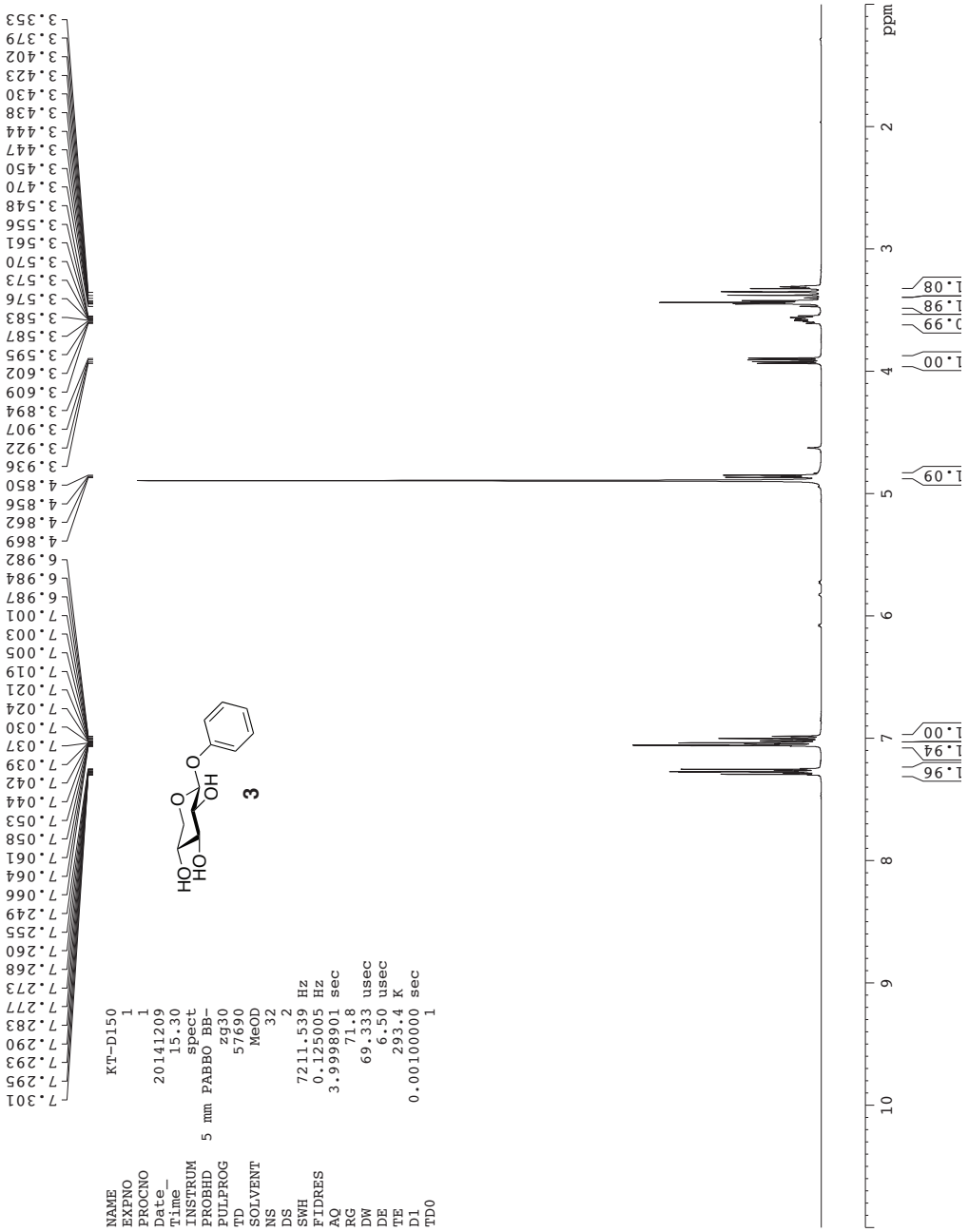
The part of the b4GALT7 open reading frame (sequence harmonized for expression in *E. Coli*, Genewiz) corresponding to aa 82 to aa 327 was subcloned into the NcoI and NotI sites of a pET-41a(+) (Merck Millipore) expression vector using the following primers (Sigma): Forward (NcoI restriction site in bold letters)

aa 82: 5'-GCATCTC**CCATGG**AGCATTGGGAGGAAGATGCCAGTT-3'

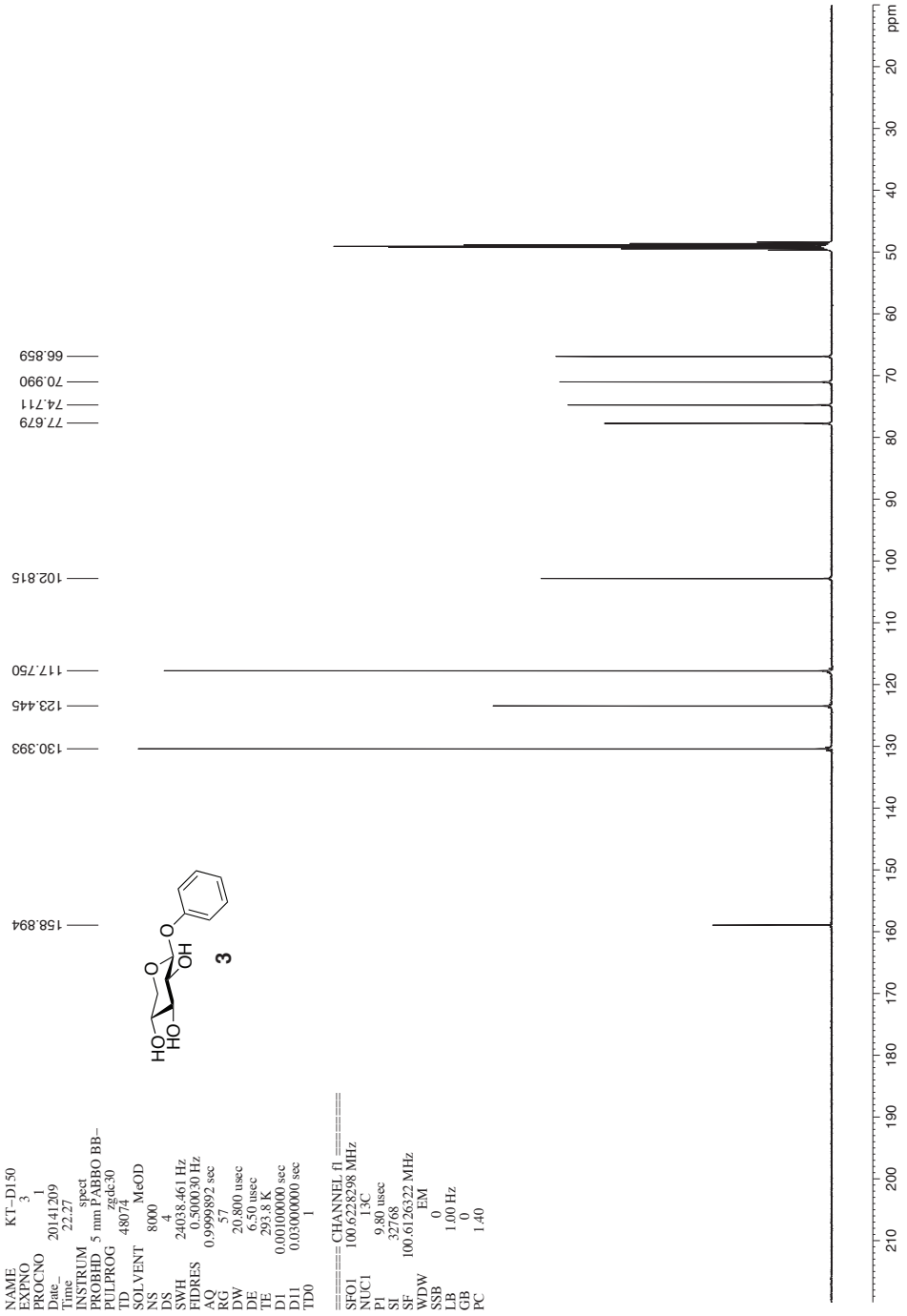
Reverse (NotI restriction site in bold letters) aa 327: 5'-GCATCT**GCGGCCGC**ATTAGCTAAATGTACACCAAGGGGTTGC-3'

The plasmid product, pET-41a(+)-β4GALT7$_{aa82}$, was amplified (Library Efficiency DH5α bacteria, Invitrogen), purified (HiSpeed Plasmid Midi Kit, Qiagen) and sequenced (Eurofins MWG Operon). BL21(DE3) *E. Coli* bacteria (Merck Millipore) were transformed with pET-41a(+)-β4GALT7$_{aa82}$ and grown on LB agar containing kanamycin (30 mg/ml). A resistant colony was transferred to selective LB medium and grown at 37 °C with shaking at 250 rpm to OD_{600}≈0.5. Expression was induced at 32 °C with IPTG (0.56 mM, Sigma) and culture was left shaking at 250 rpm over night. Cells were pelleted and then lysed with Cellytic (1X, Sigma) in Tris (20 mM, pH 7.6), NaCl (500 mM), Benzonase (50 U/ml, Sigma), lysozyme (0.2 mg/ml, Sigma) and PMSF (1 mM) for 20 min at 22 °C. Clarified lysate was applied to an equilibrated HisTrap HP column (GE Healthcare) at a flowrate of 1 ml/min. The column was washed with a phosphate buffer (20 mM, pH 7.4) containing NaCl (500 mM) and imidazole (40 mM), after which the protein was eluted by an imidazole gradient (40-250 mM). Protein purity for each eluted fraction was analyzed by SDS-PAGE on a Bis-Tris 4-12 % gel in MOPS buffer (Invitrogen), visualized by Brilliant Blue G colloidal (Sigma). Fractions containing pure protein were pooled and buffer exchanged into TBS (20 mM, pH 7.9) on PD-10 columns (GE Healthcare).

[1]H-NMR Phenyl β-D-xylopyranoside **(3)**



^{13}C-NMR Phenyl β-D-xylopyranoside **(3)**



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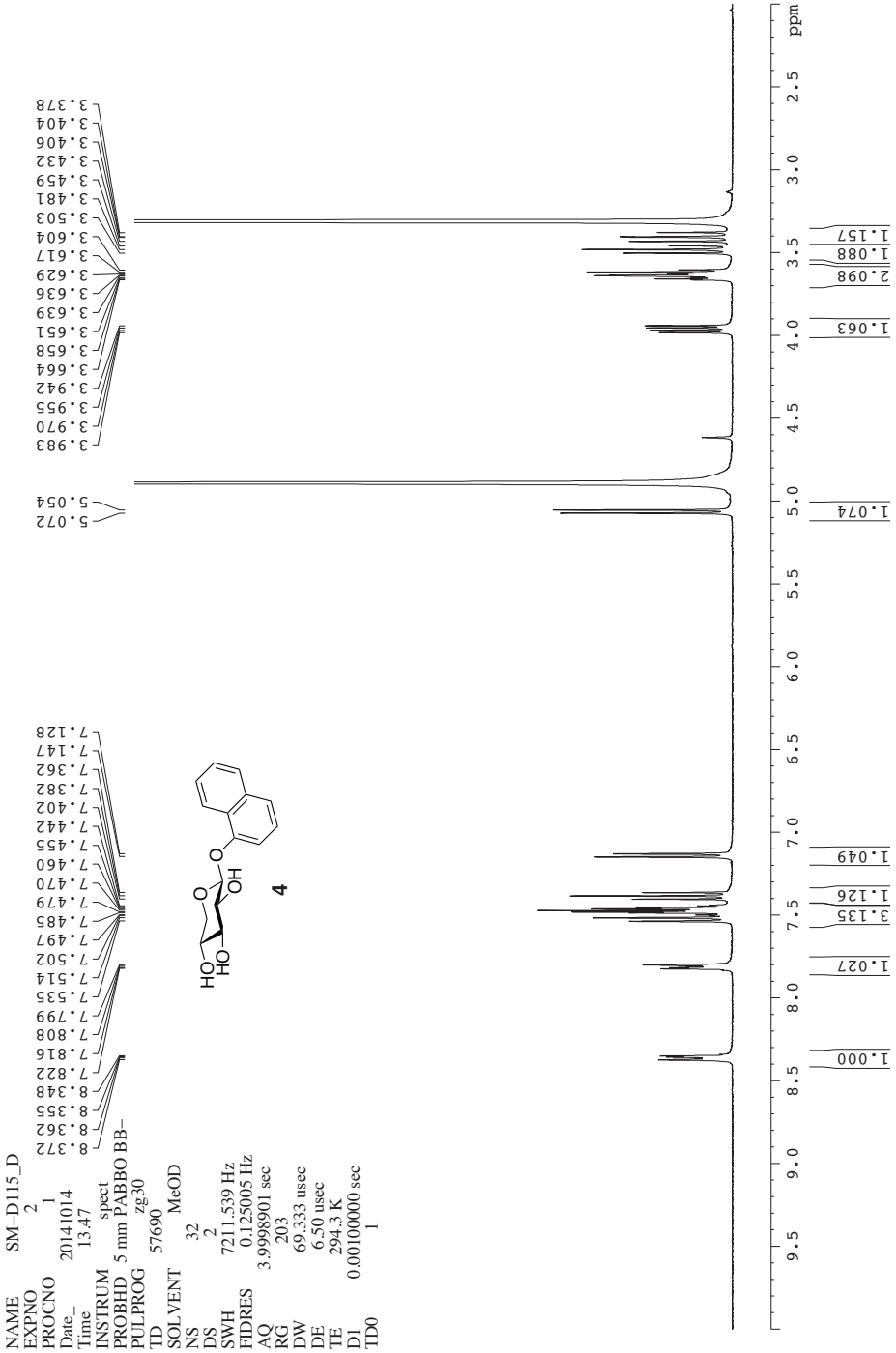

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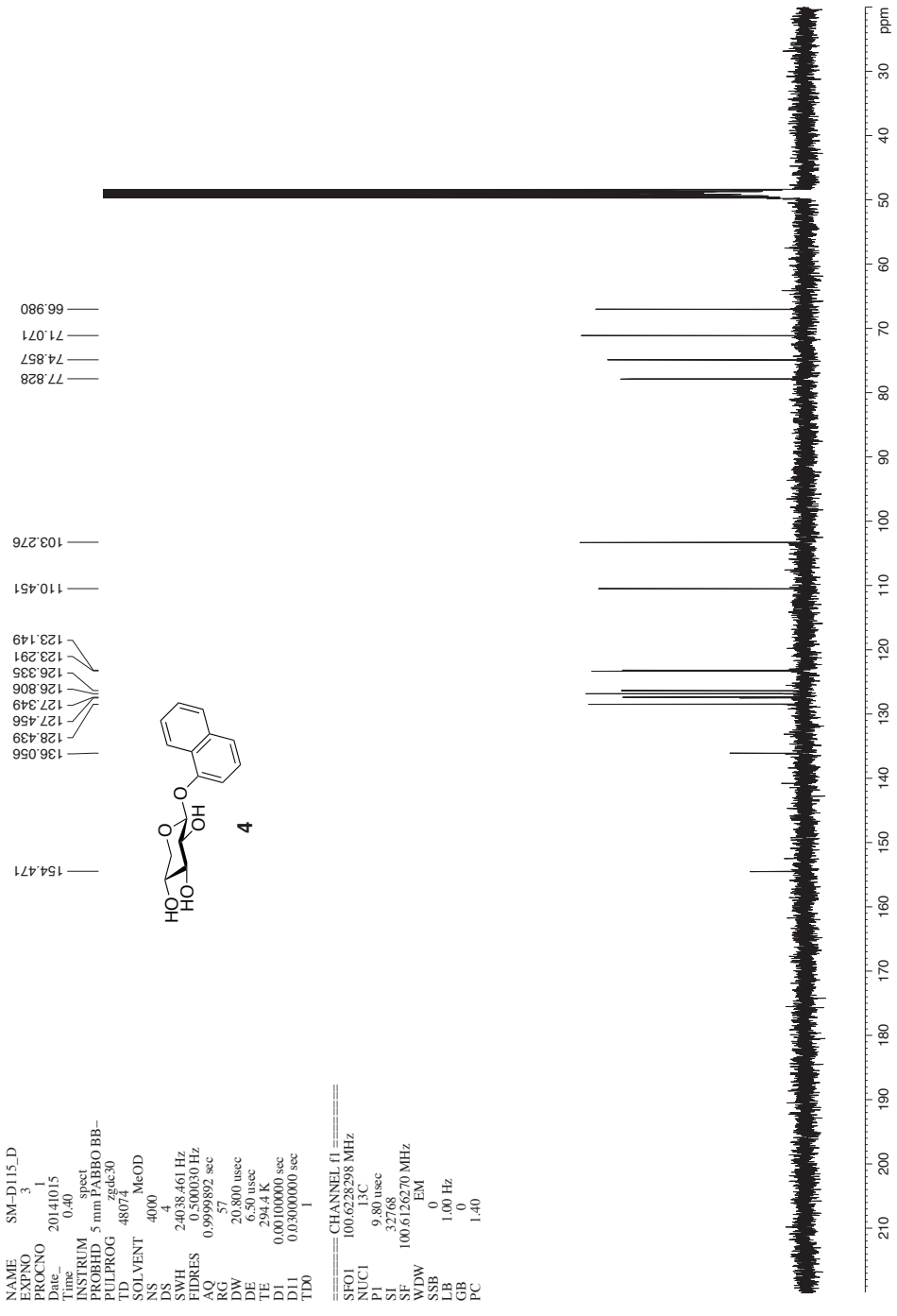

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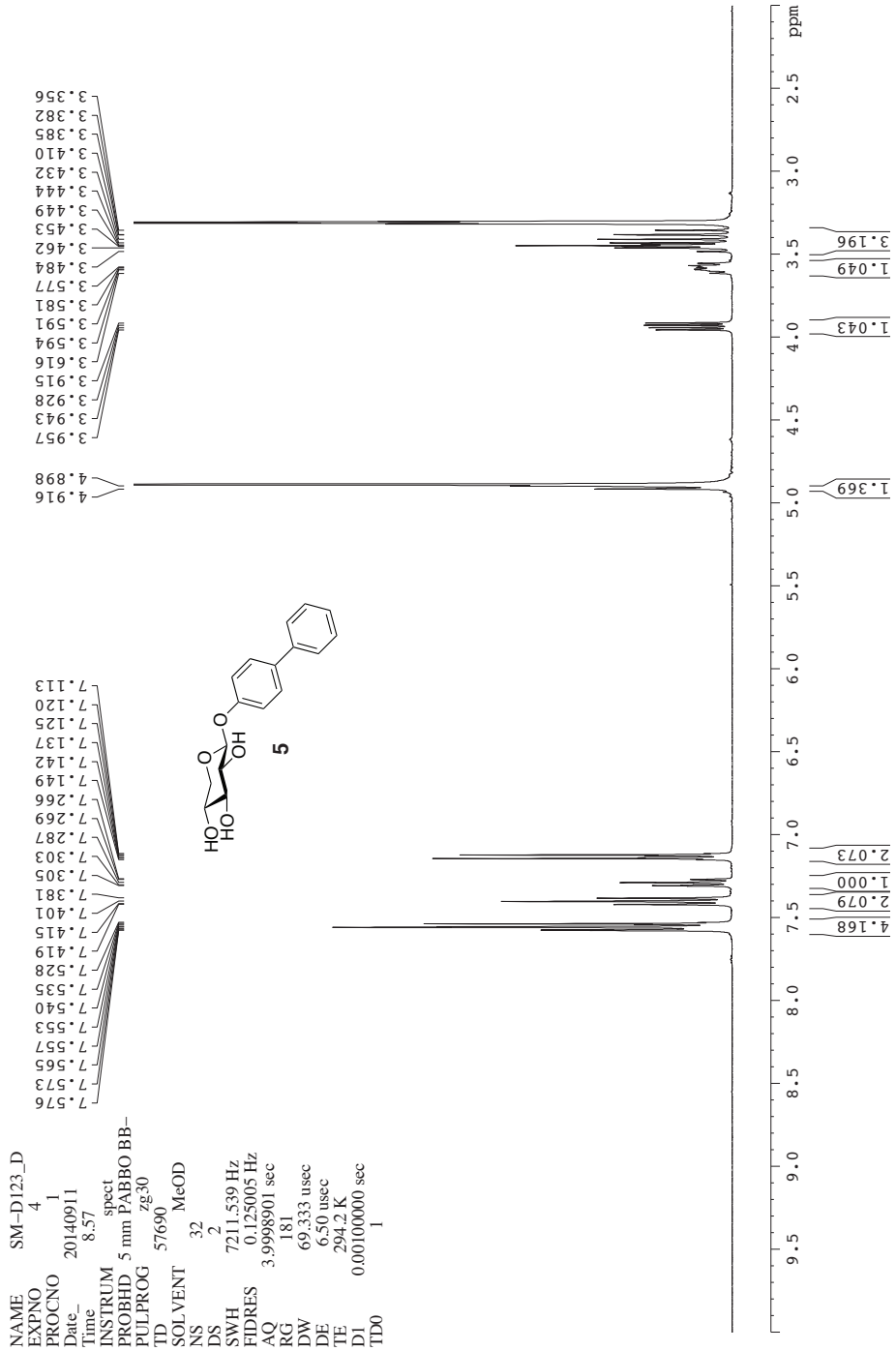

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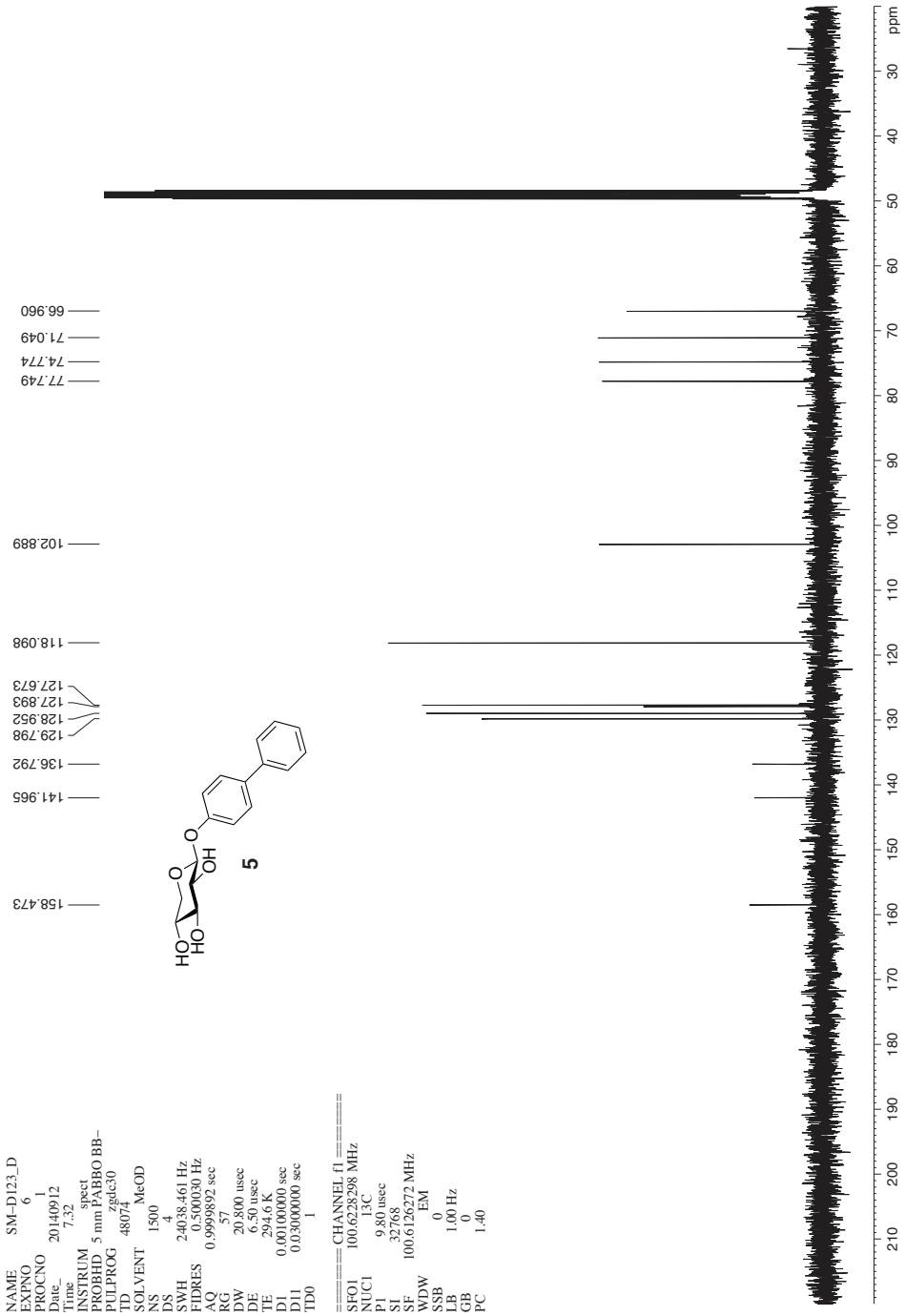

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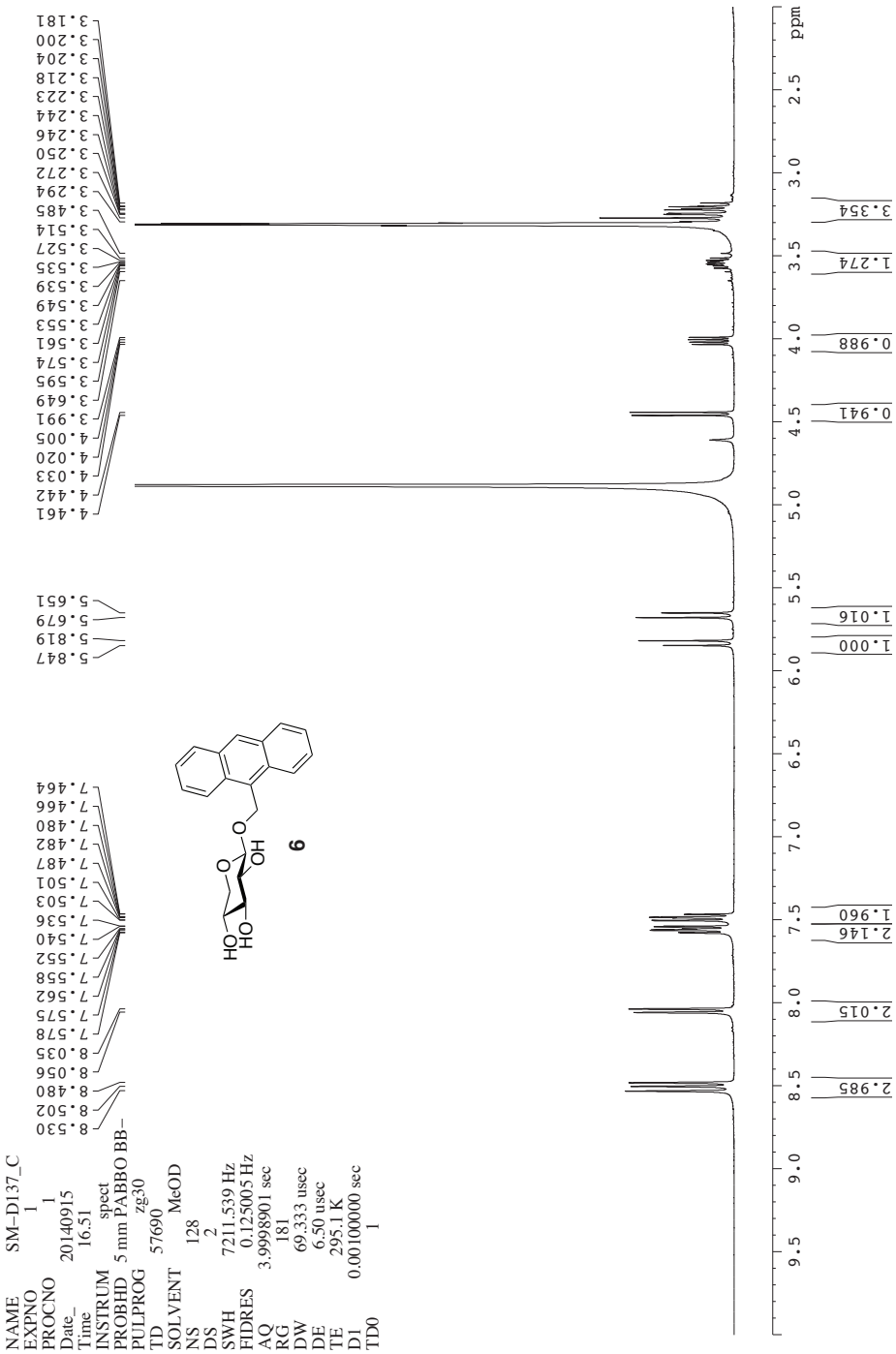

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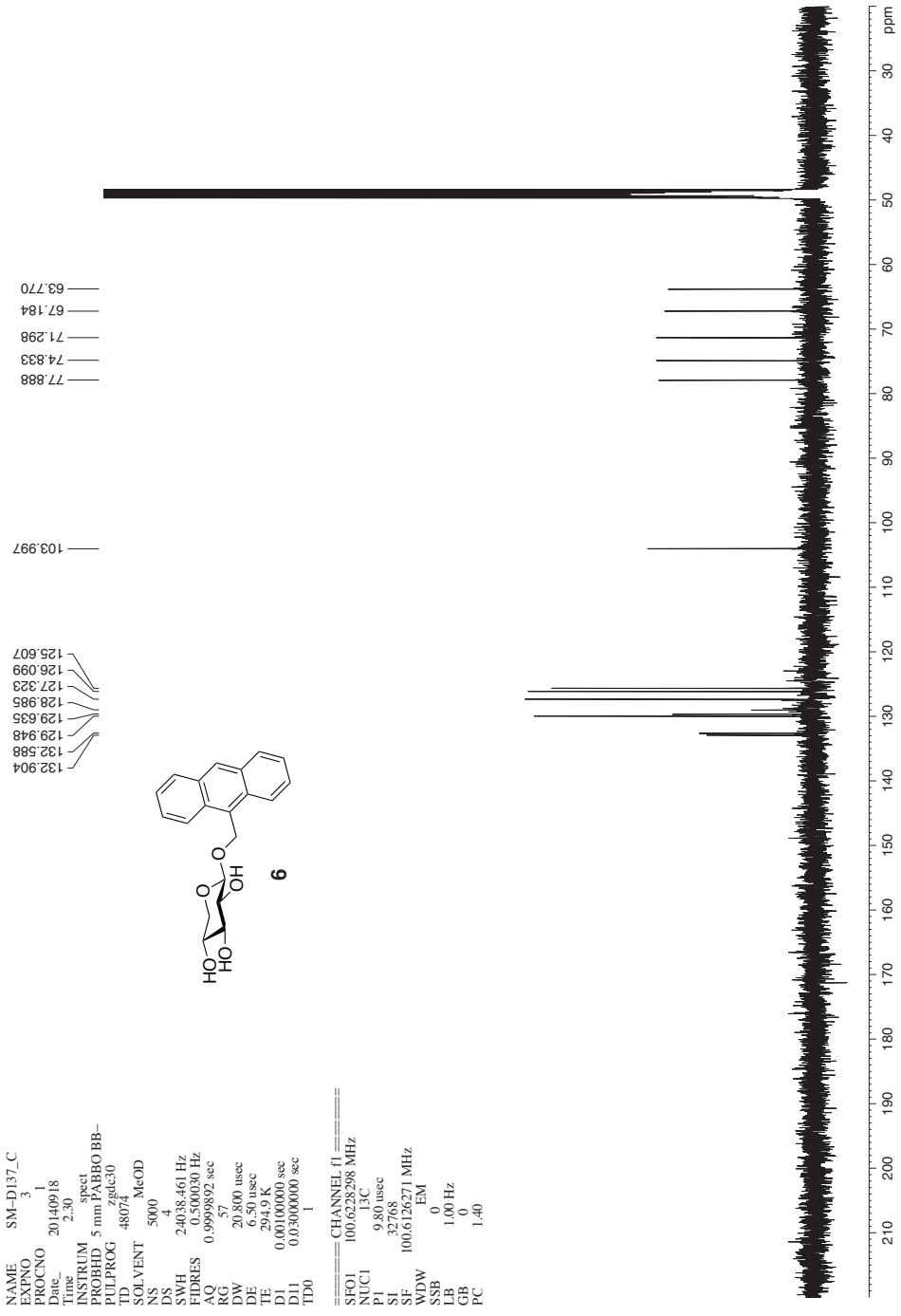

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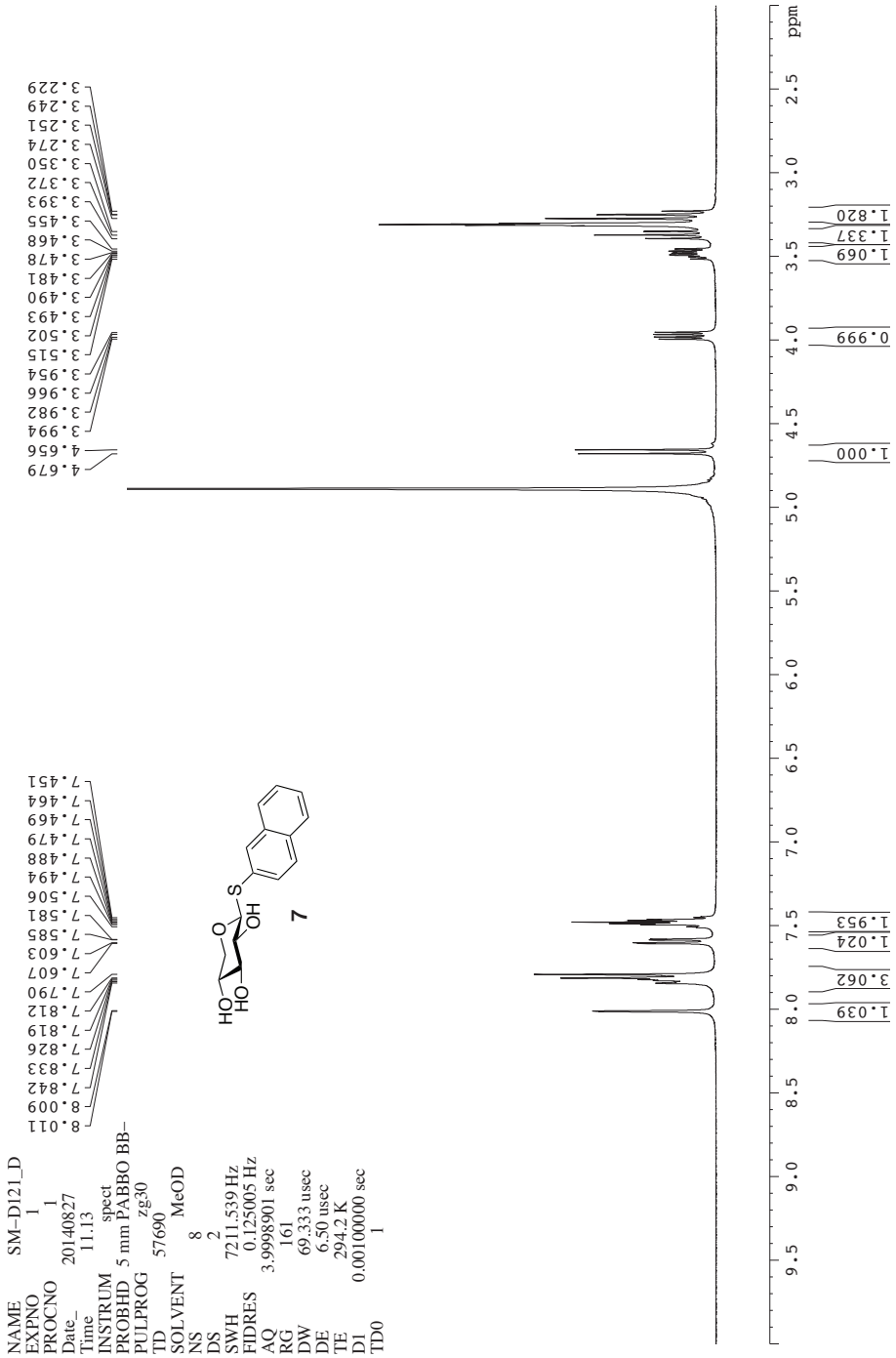

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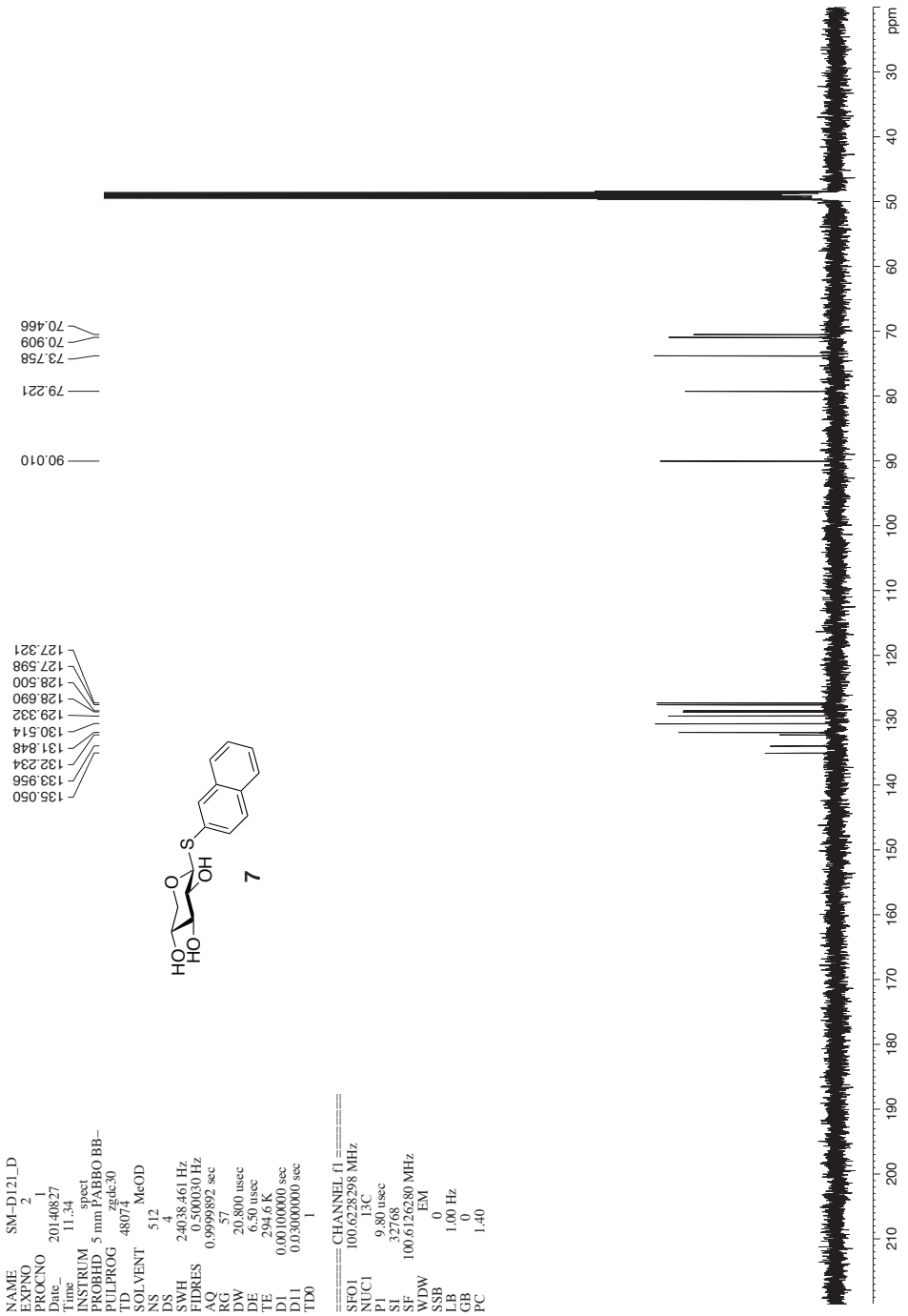

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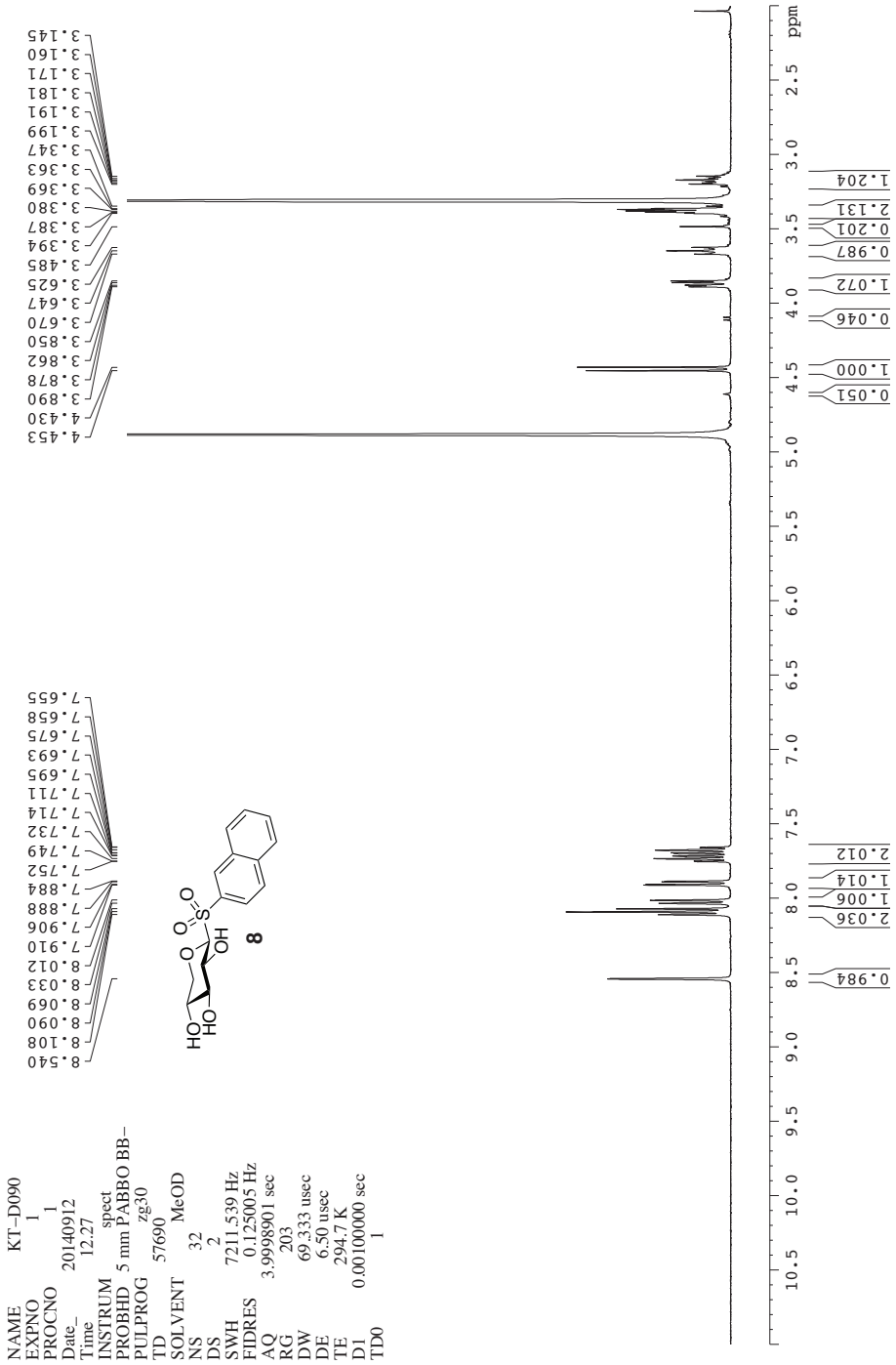

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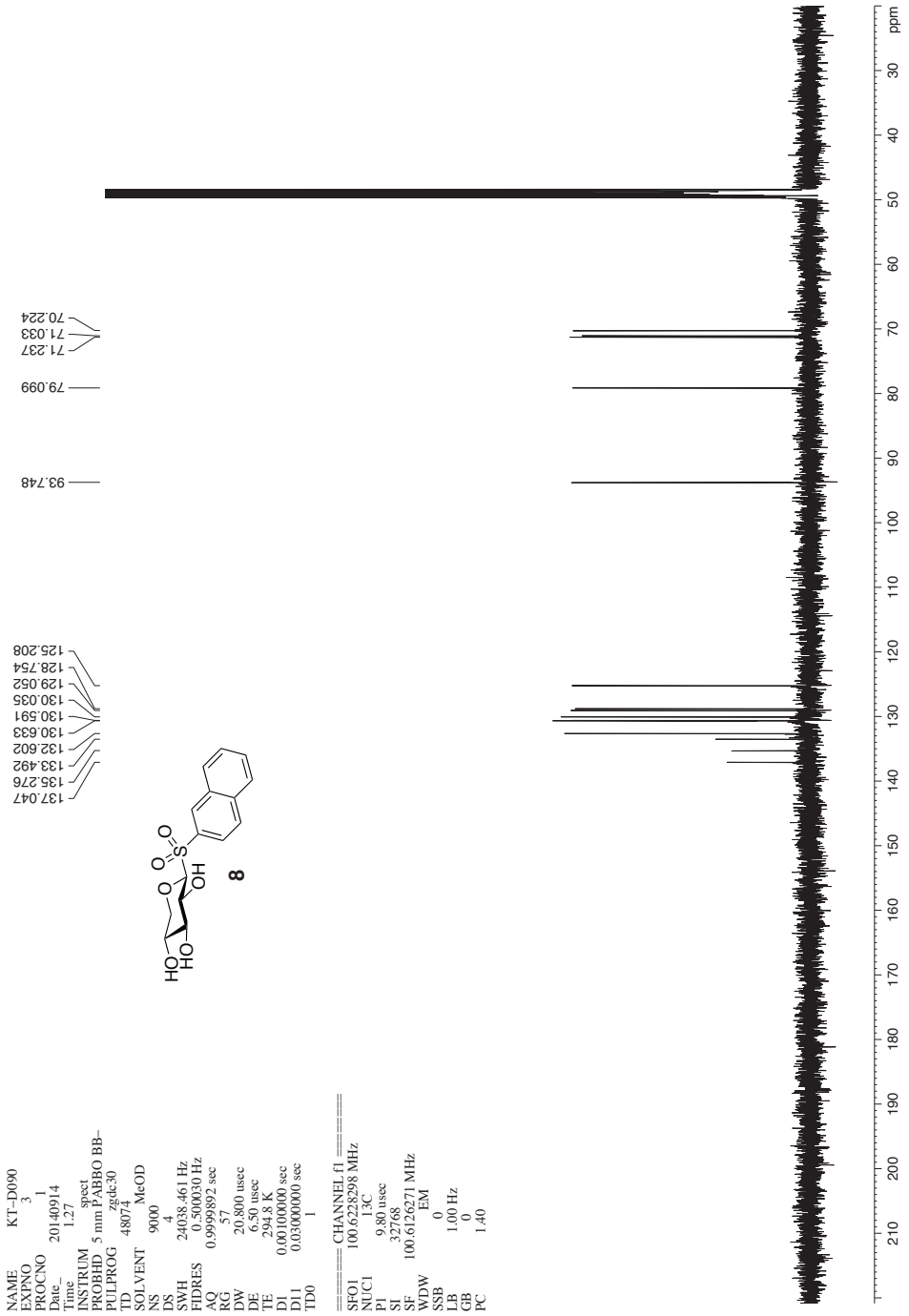

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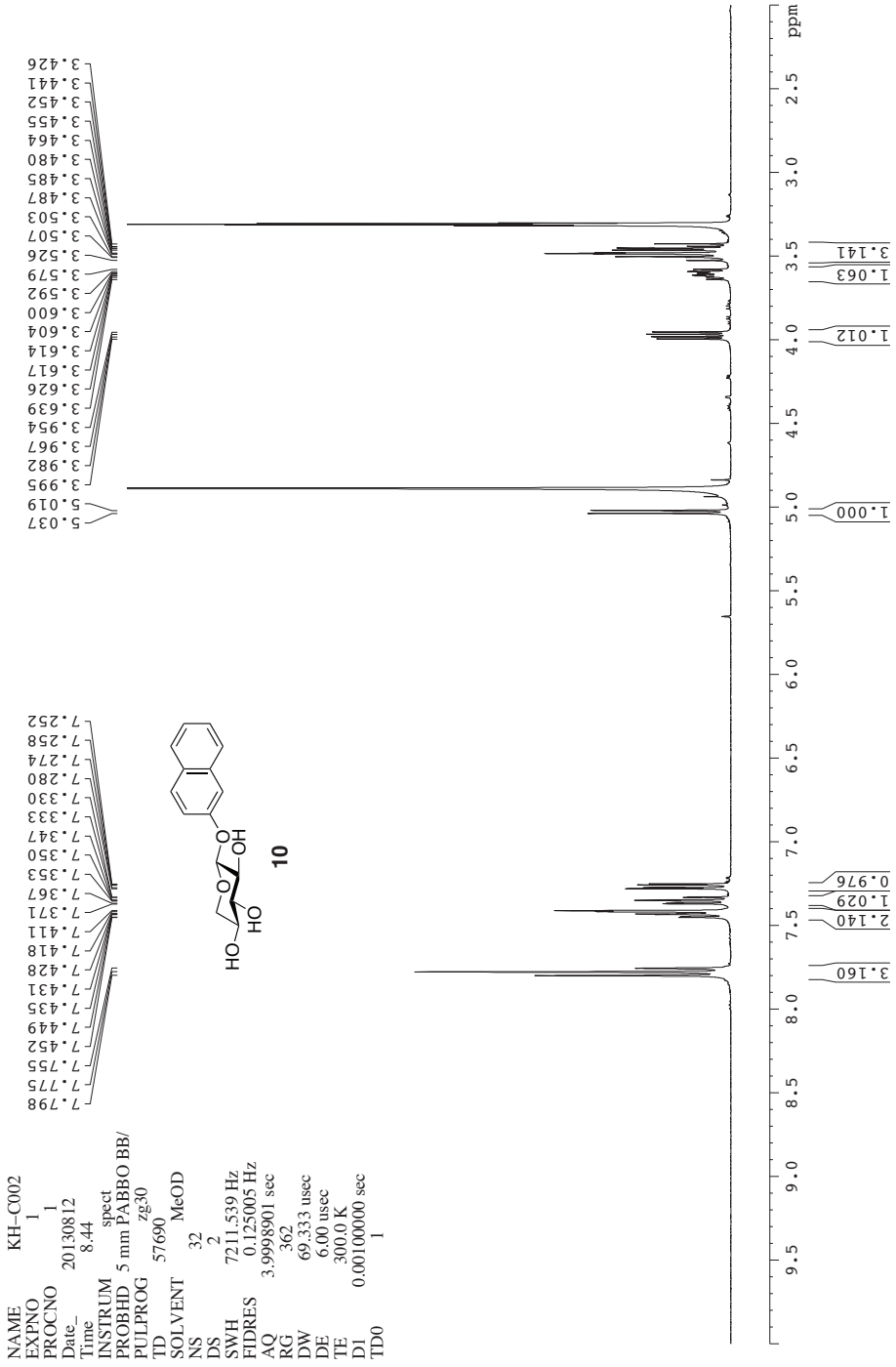

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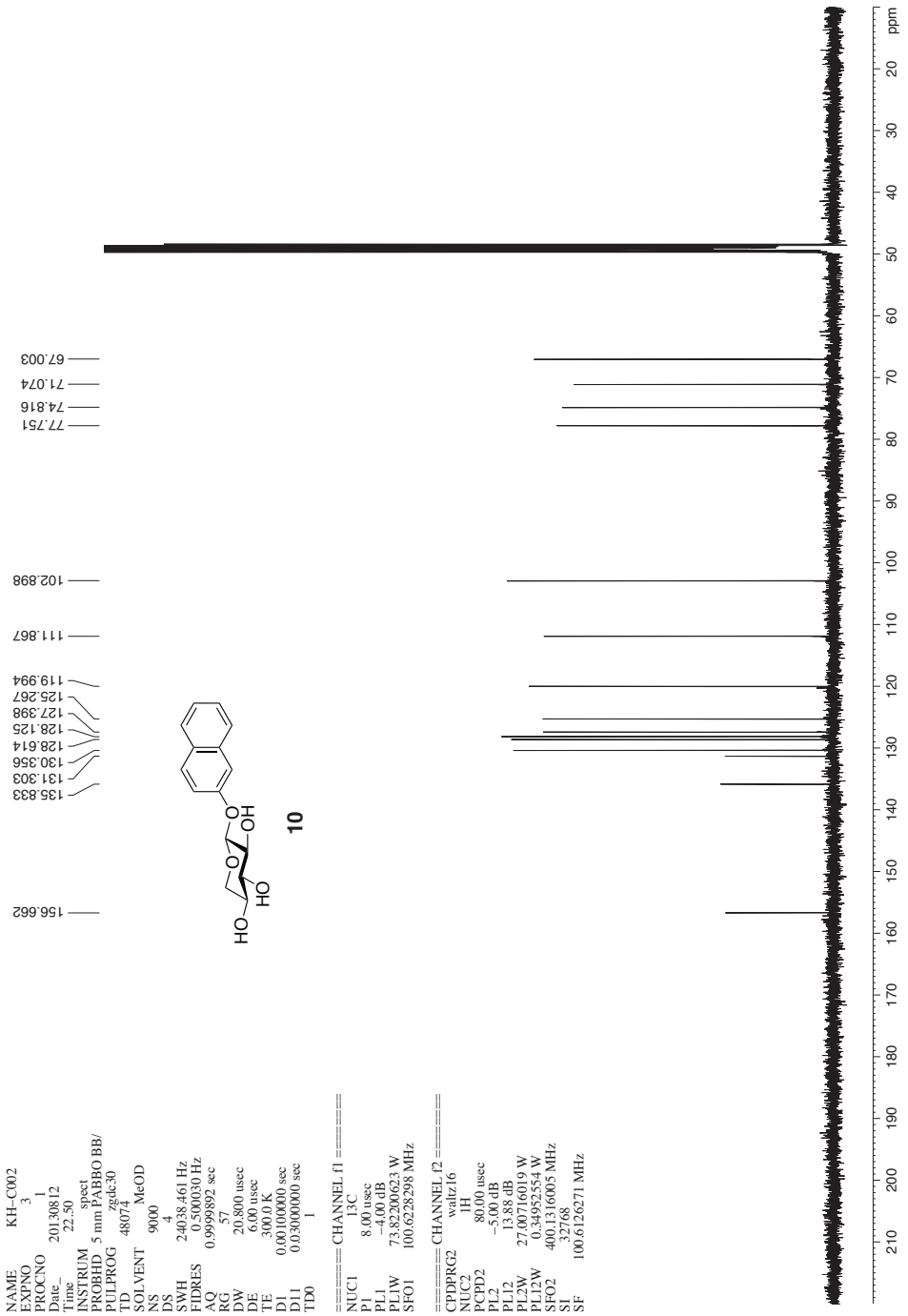

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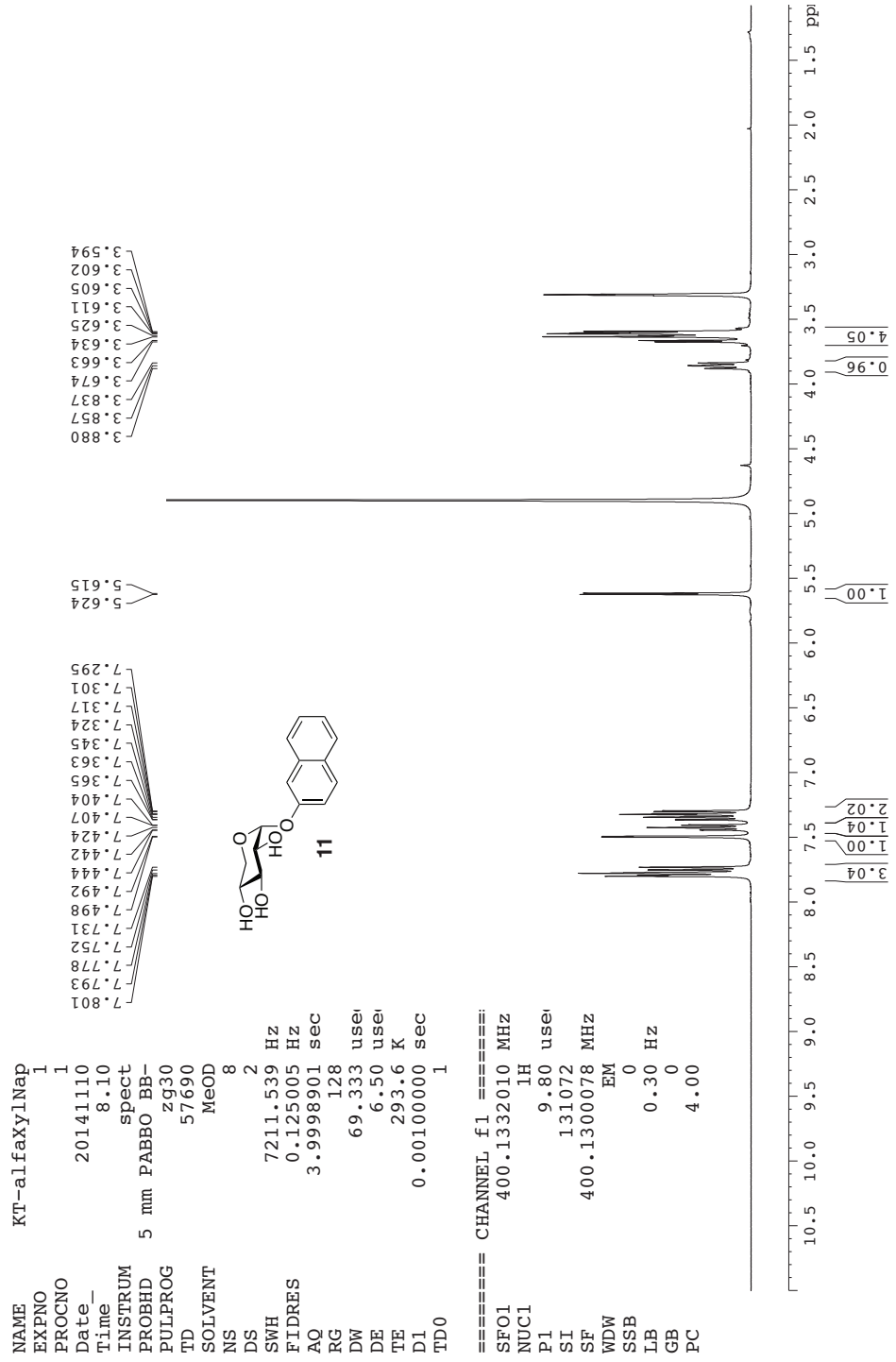

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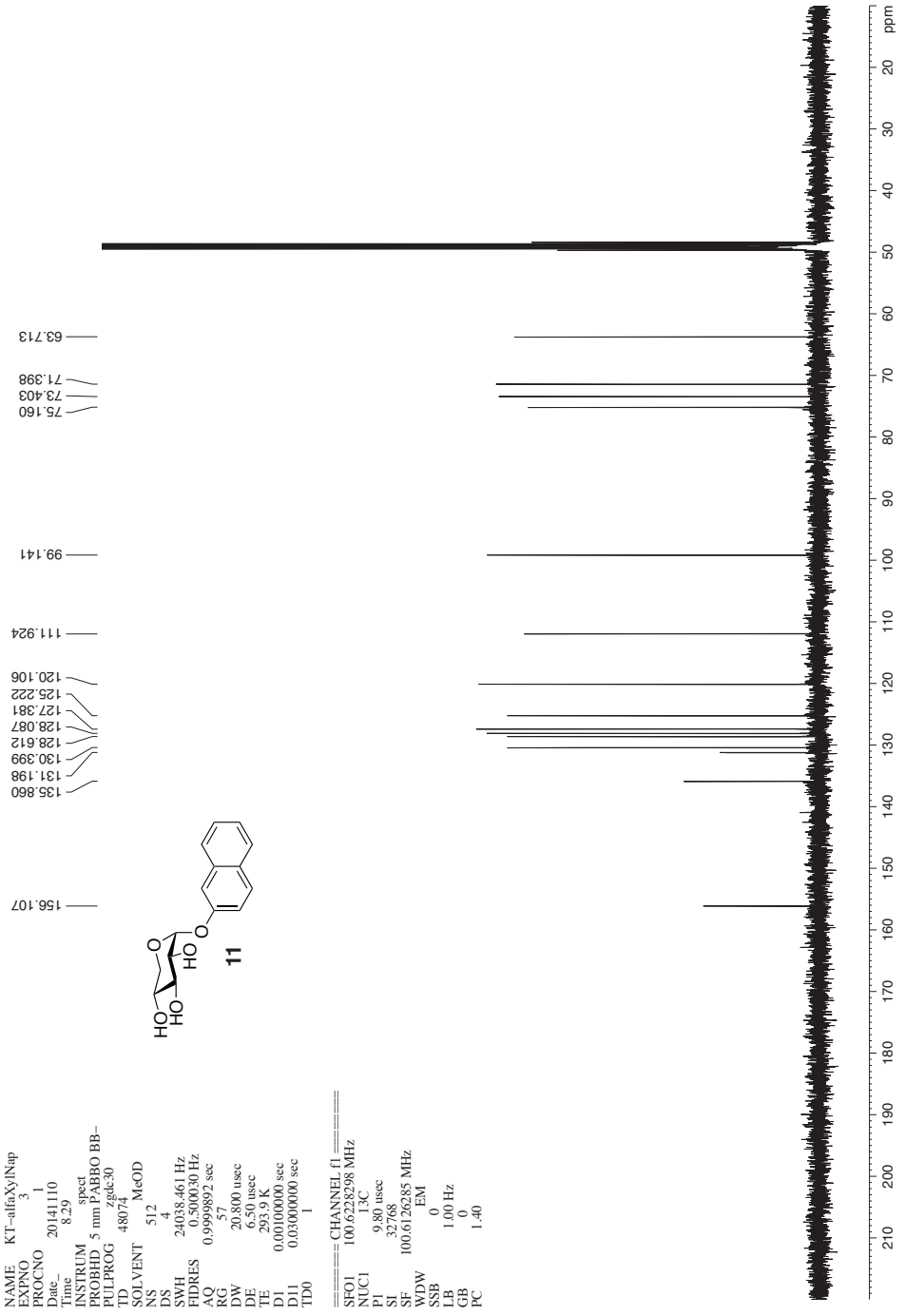
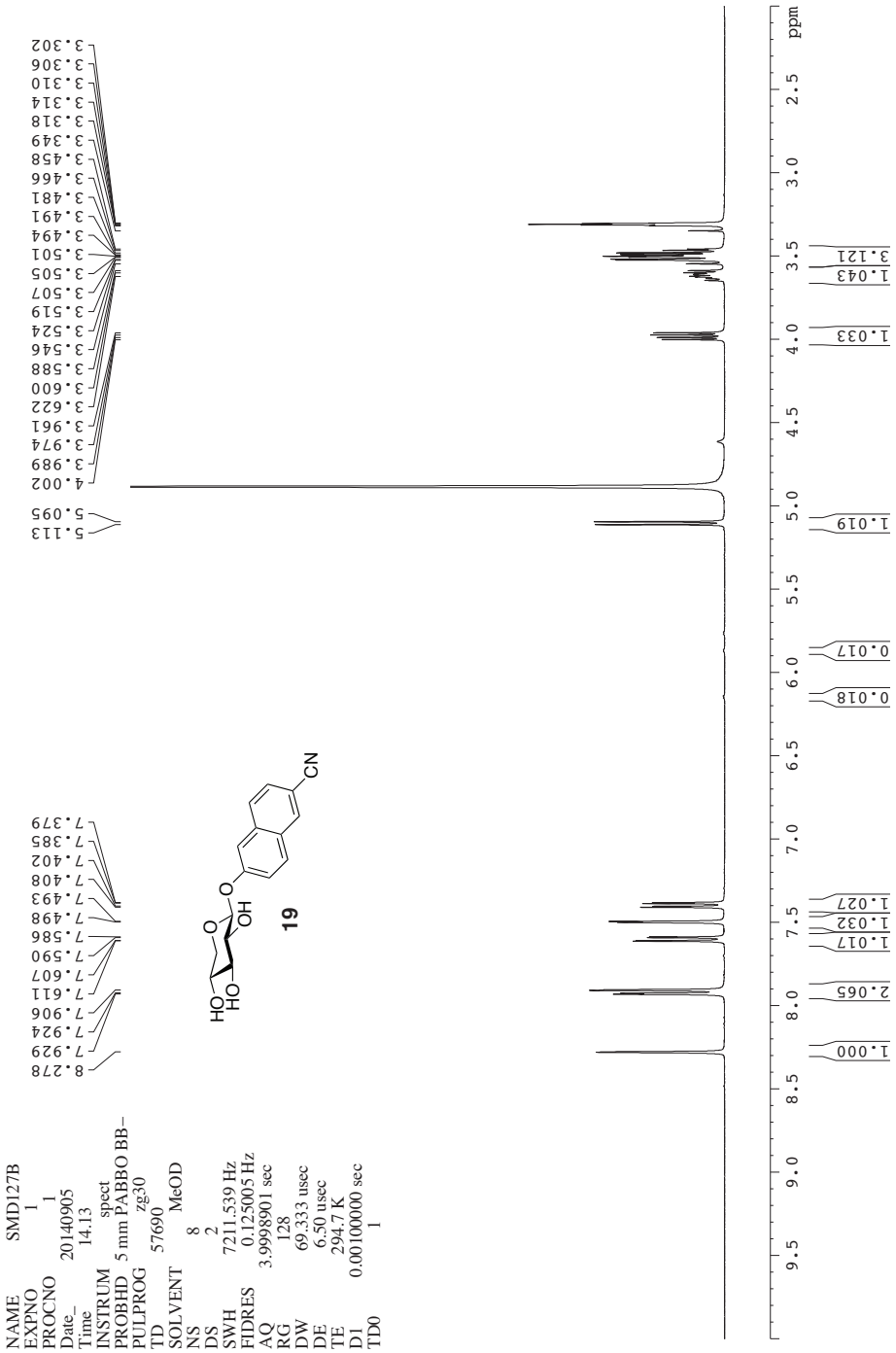
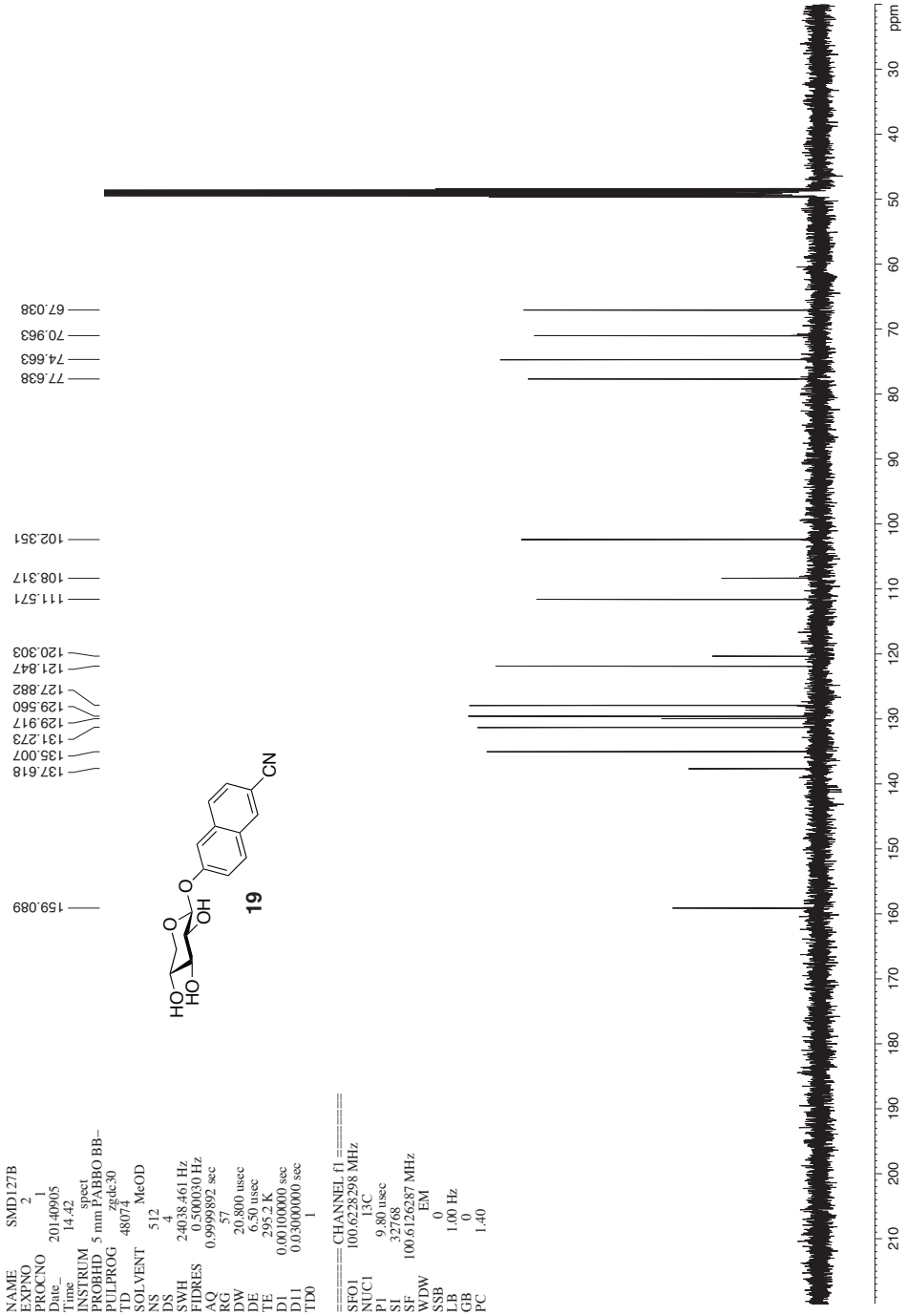


[1]H-NMR 2-(6-Cyano-naphthyl) β-D-xylopyranoside **(19)**



^{13}C-NMR 2-(6-Cyano-naphthyl) β-D-xylopyranoside **(19)**



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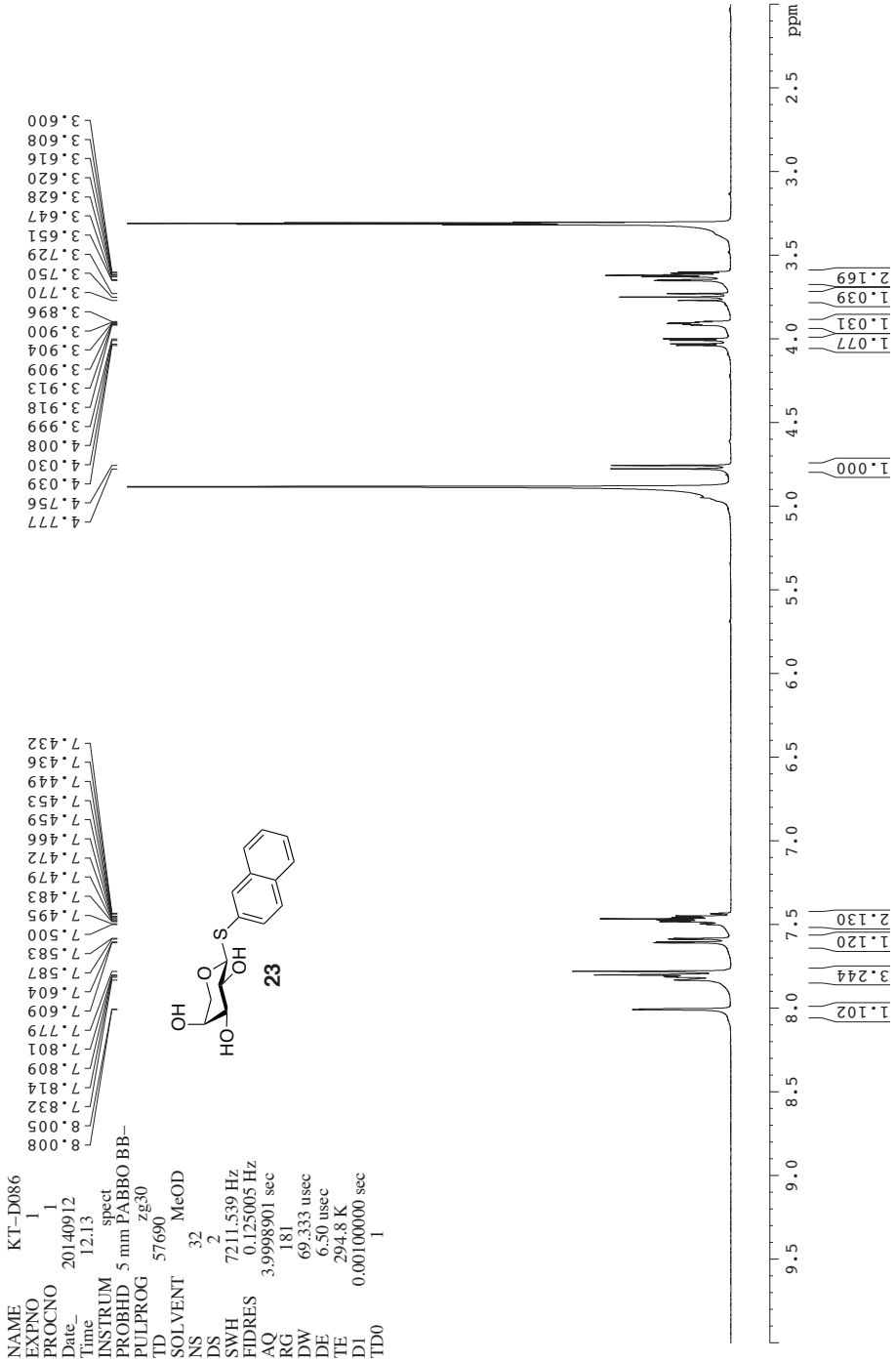

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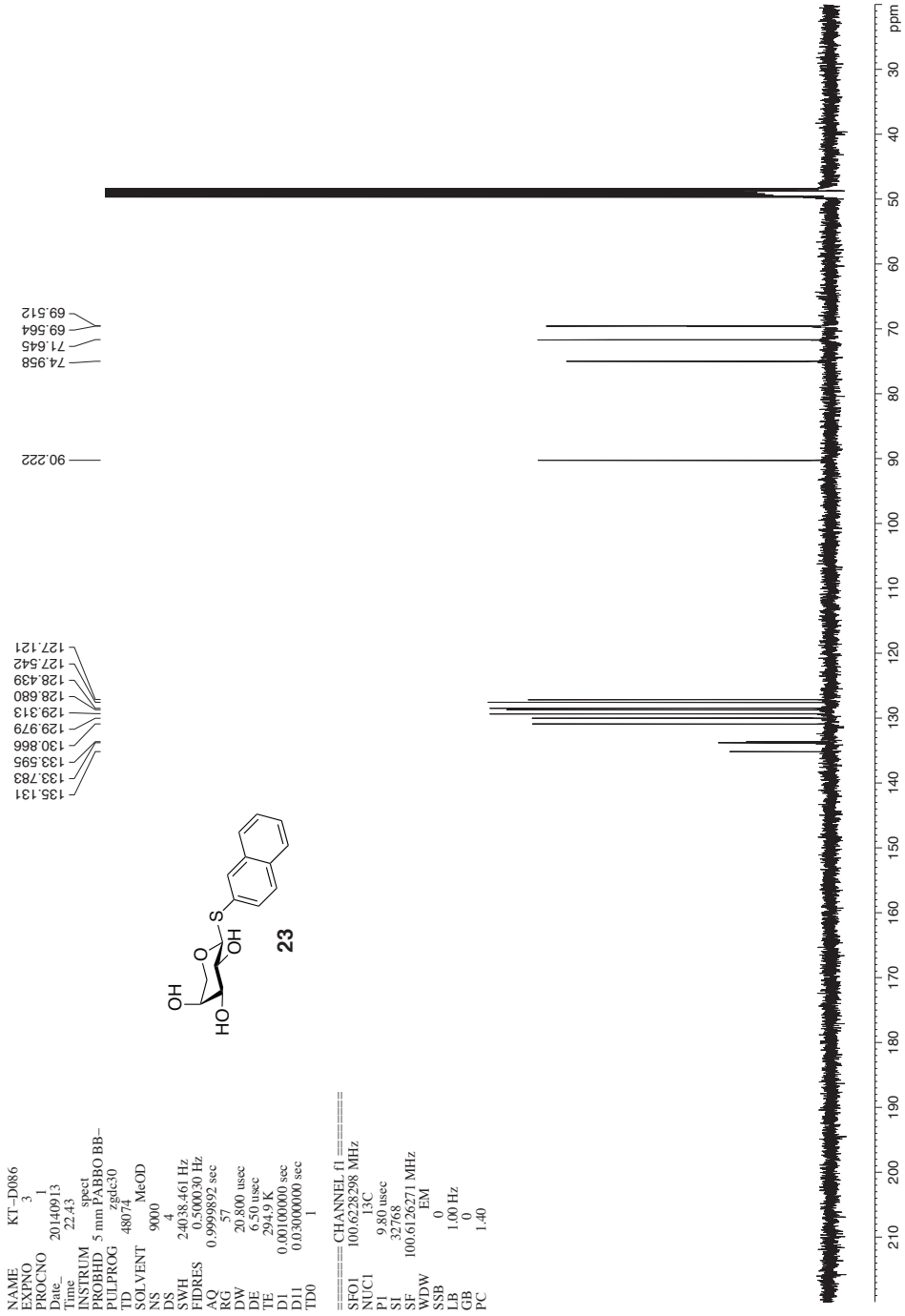
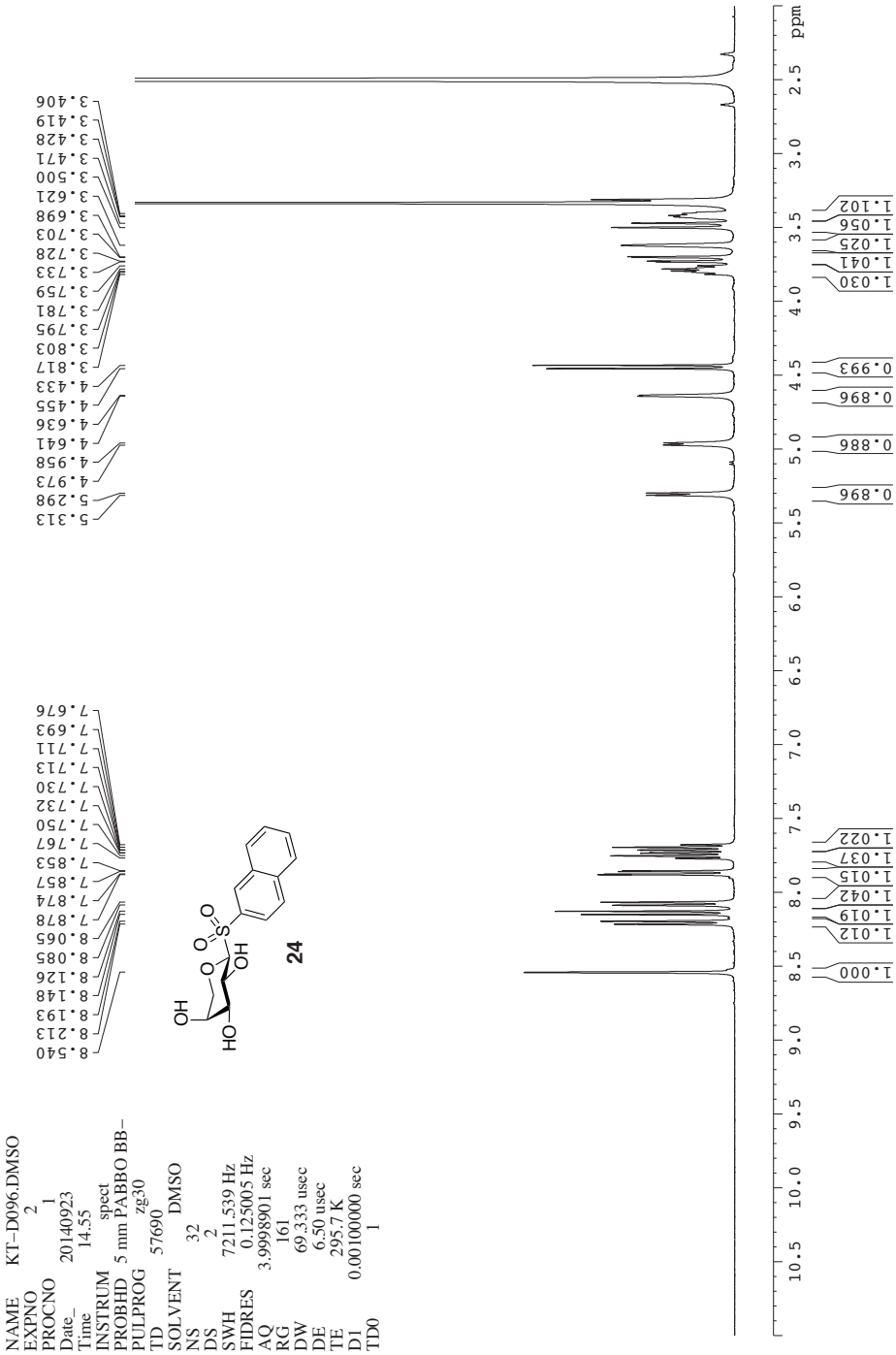
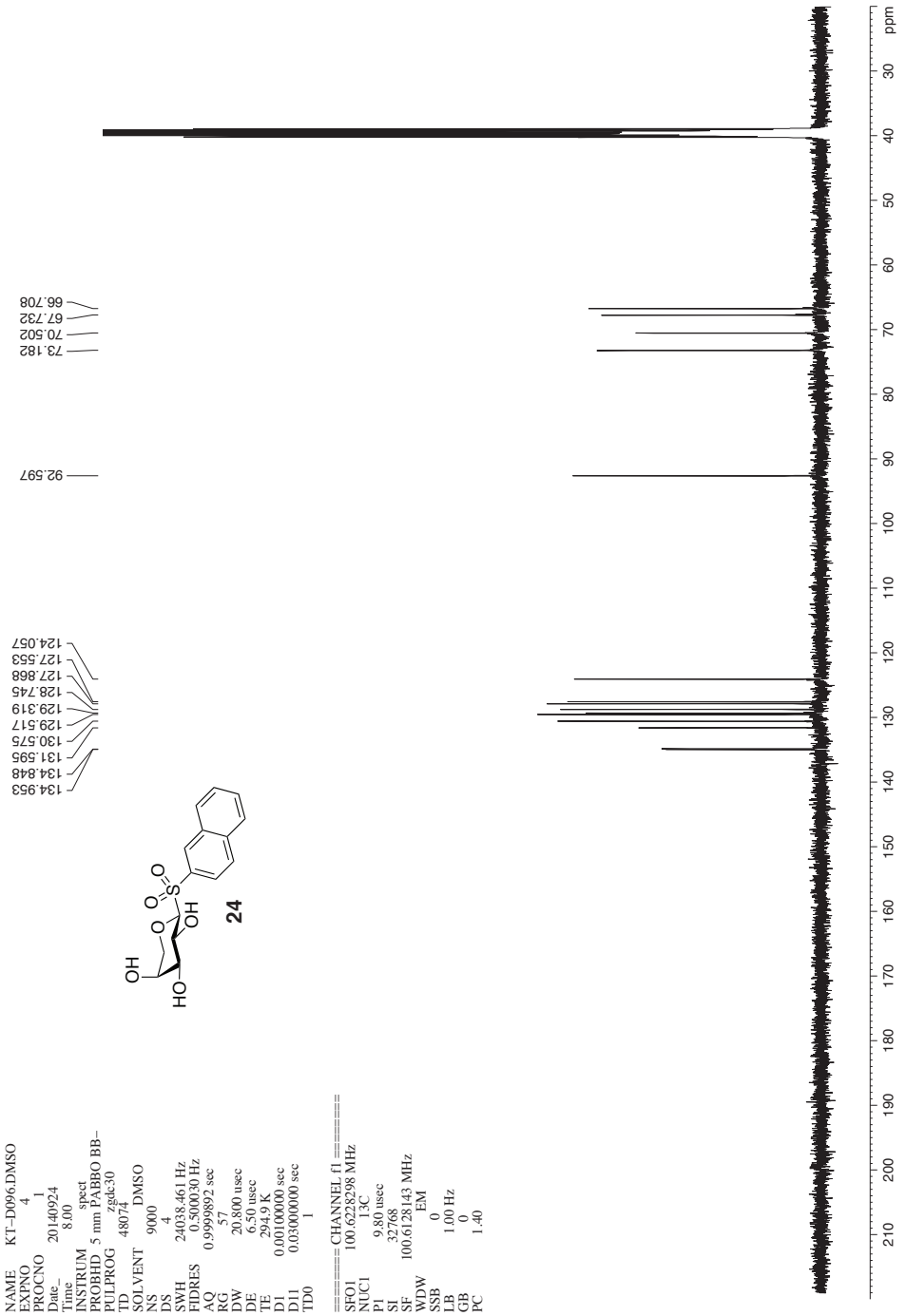


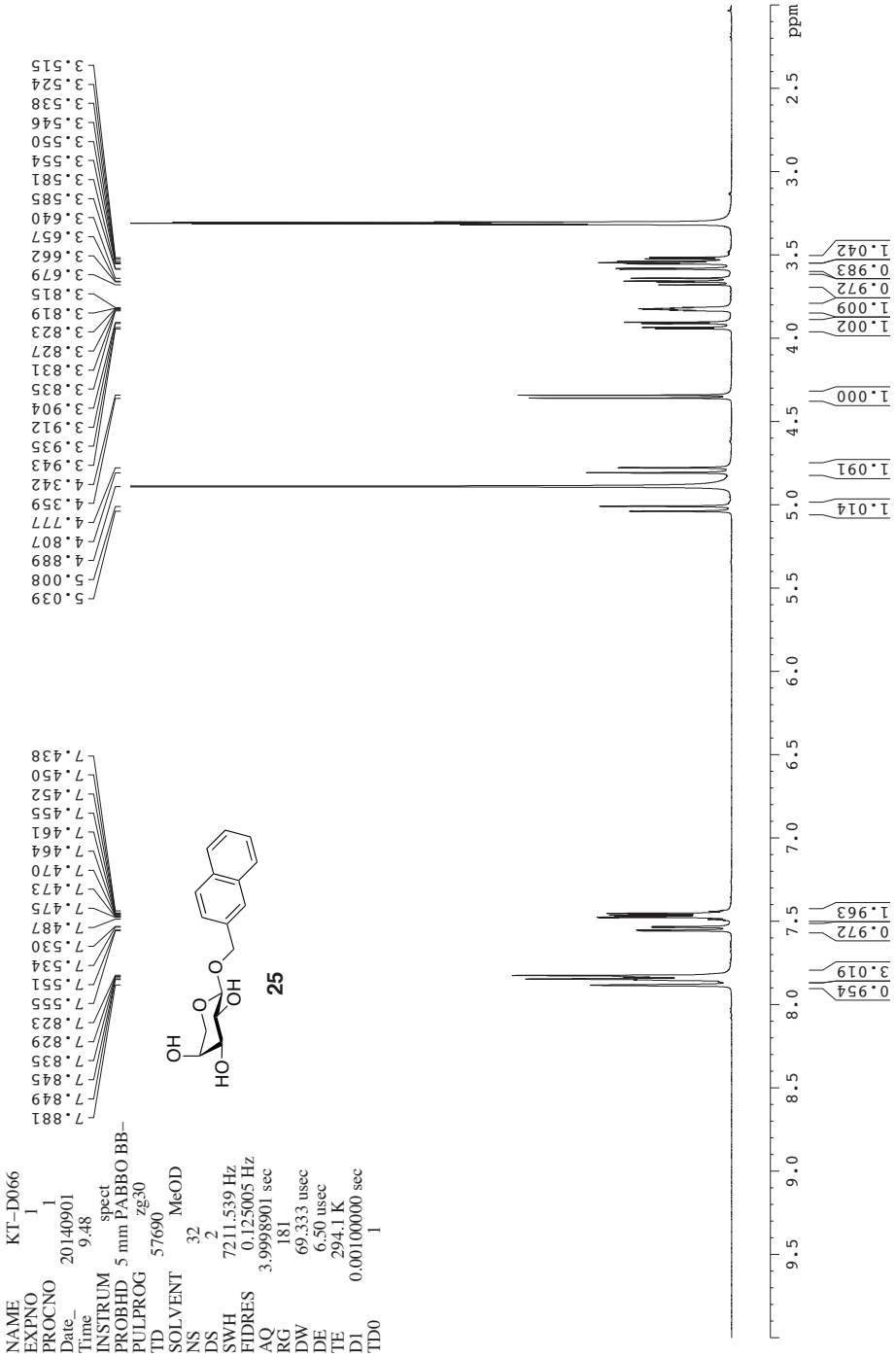
[1]H-NMR α-L-Arabinopyranoside 2-naphthyl sulfone **(24)**



^{13}C-NMR α-L-Arabinopyranoside 2-naphthyl sulfone **(24)**



^{1}H-NMR (2-Naphthyl)-methyl α-L-arabinopyranoside **(25)**



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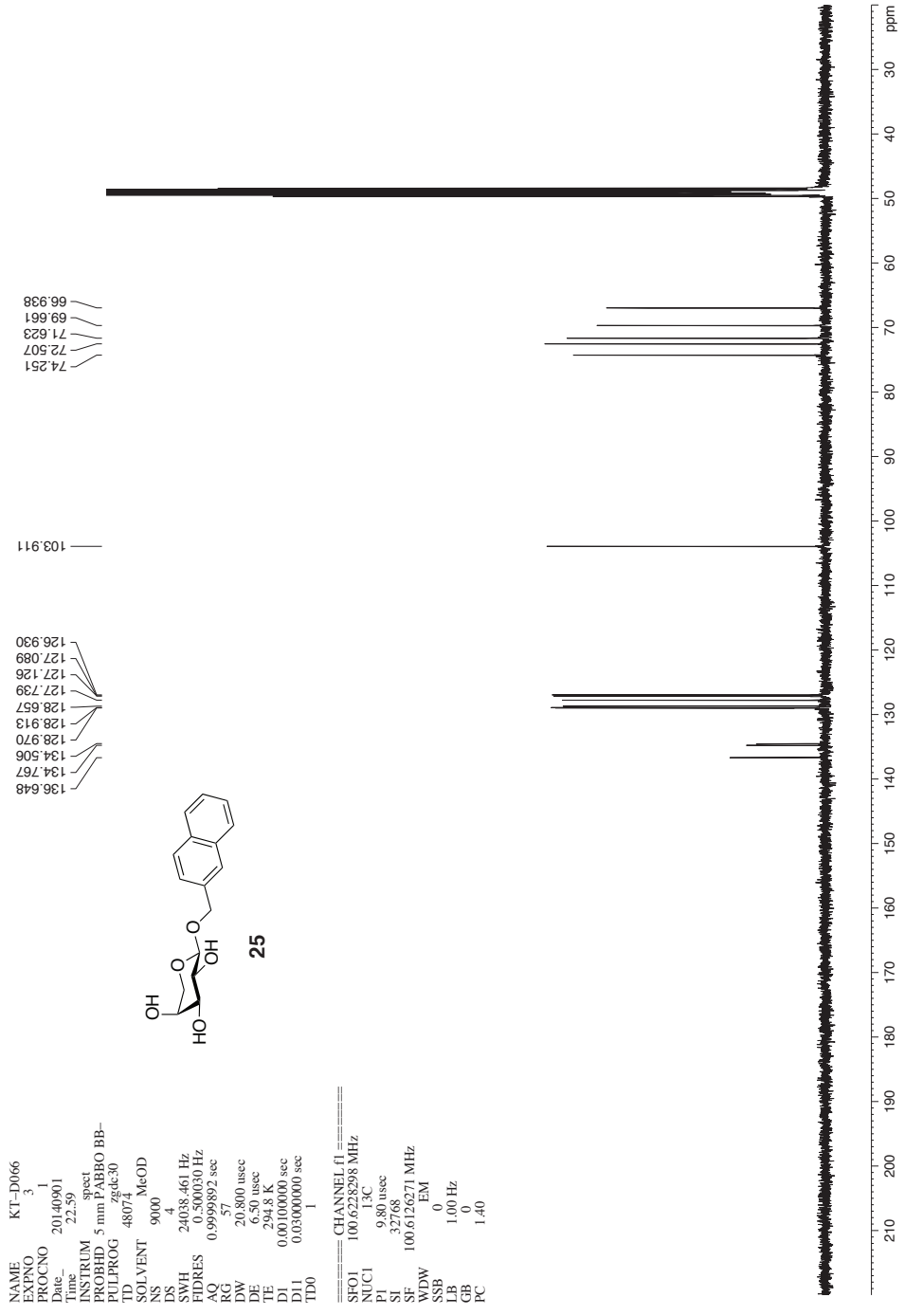
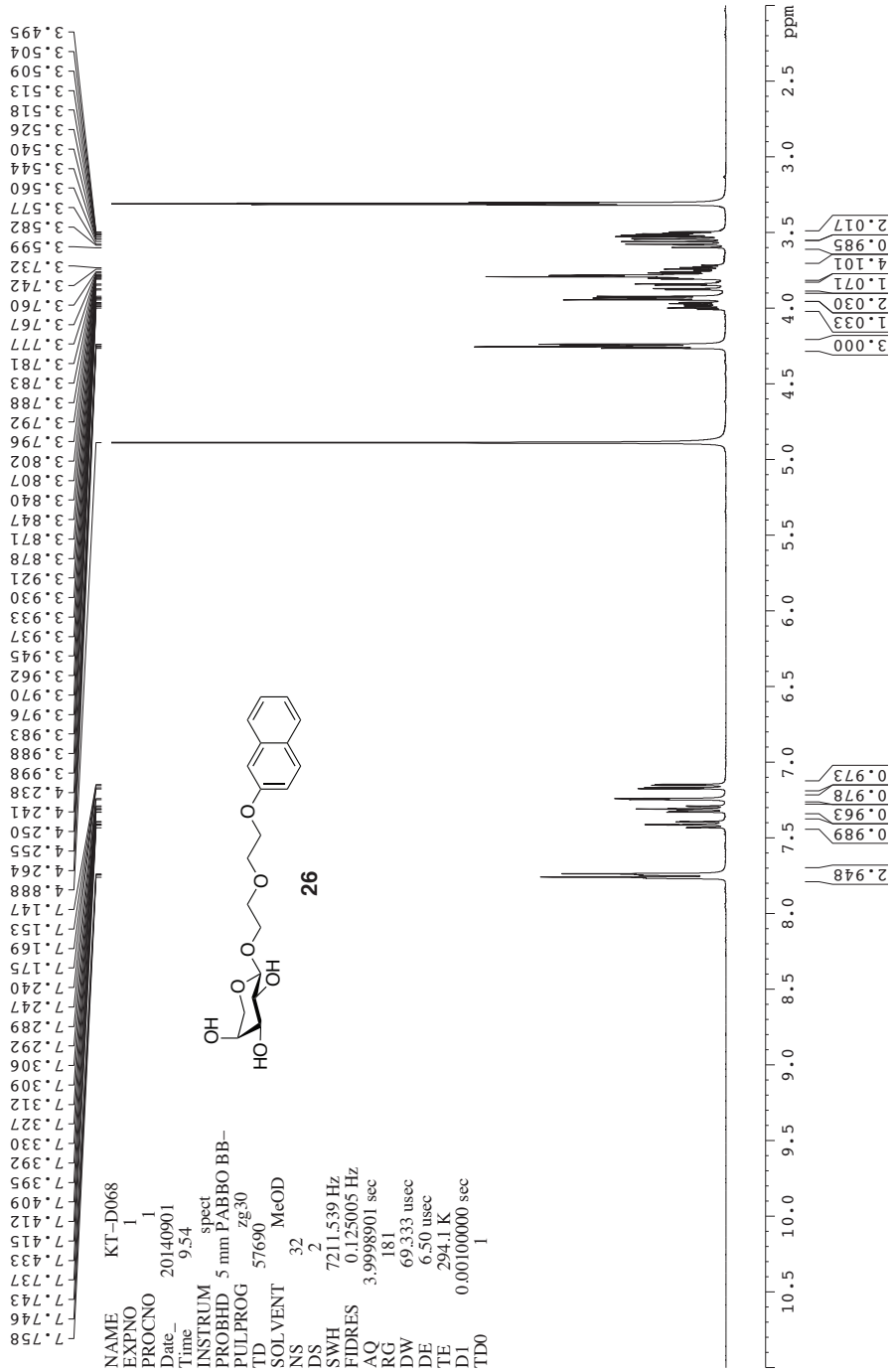


^{1}H-NMR 5-(2-Naphthoxy)-3-oxo-1-pentyl α-L-arabinopyranoside **(26)**



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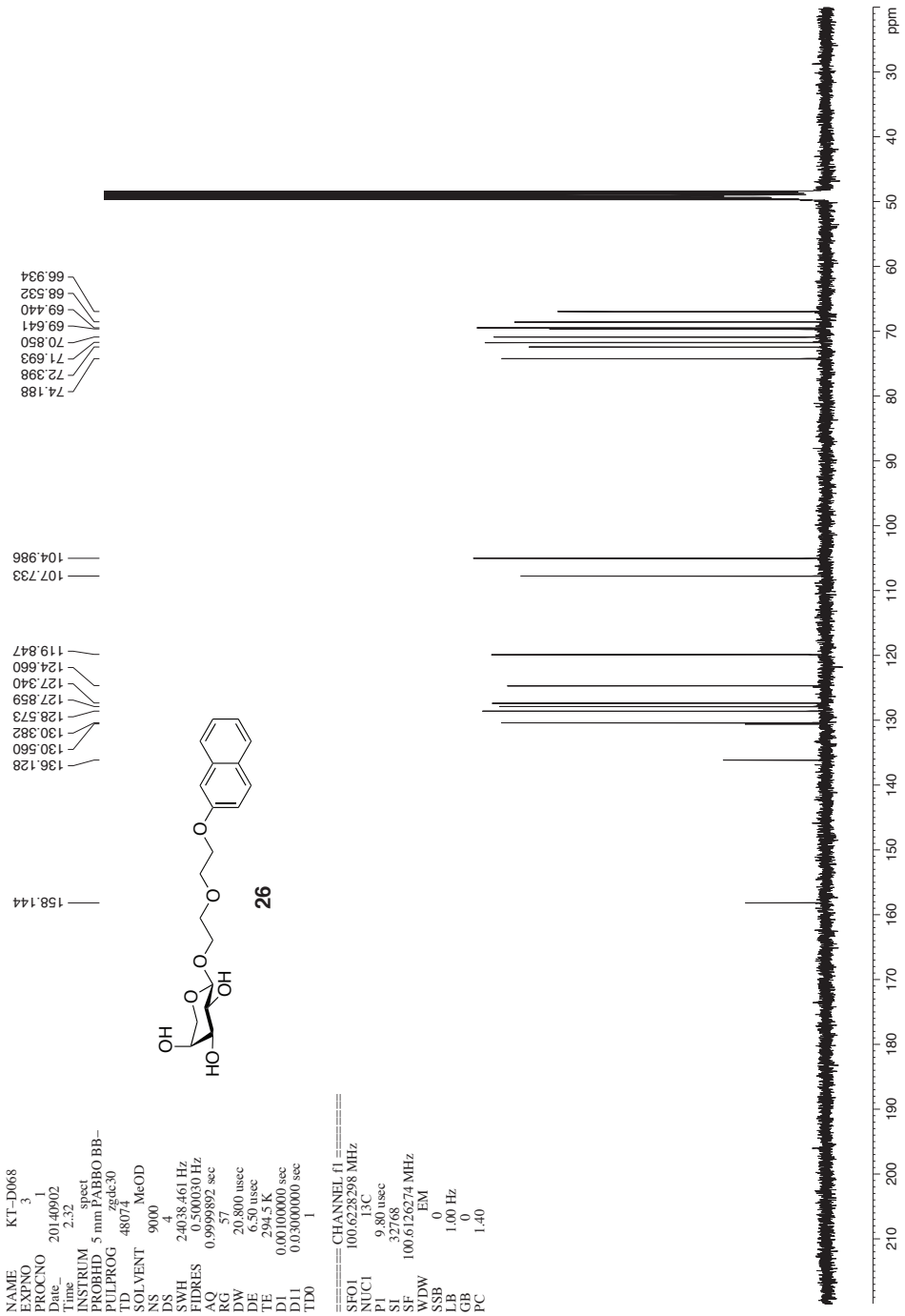
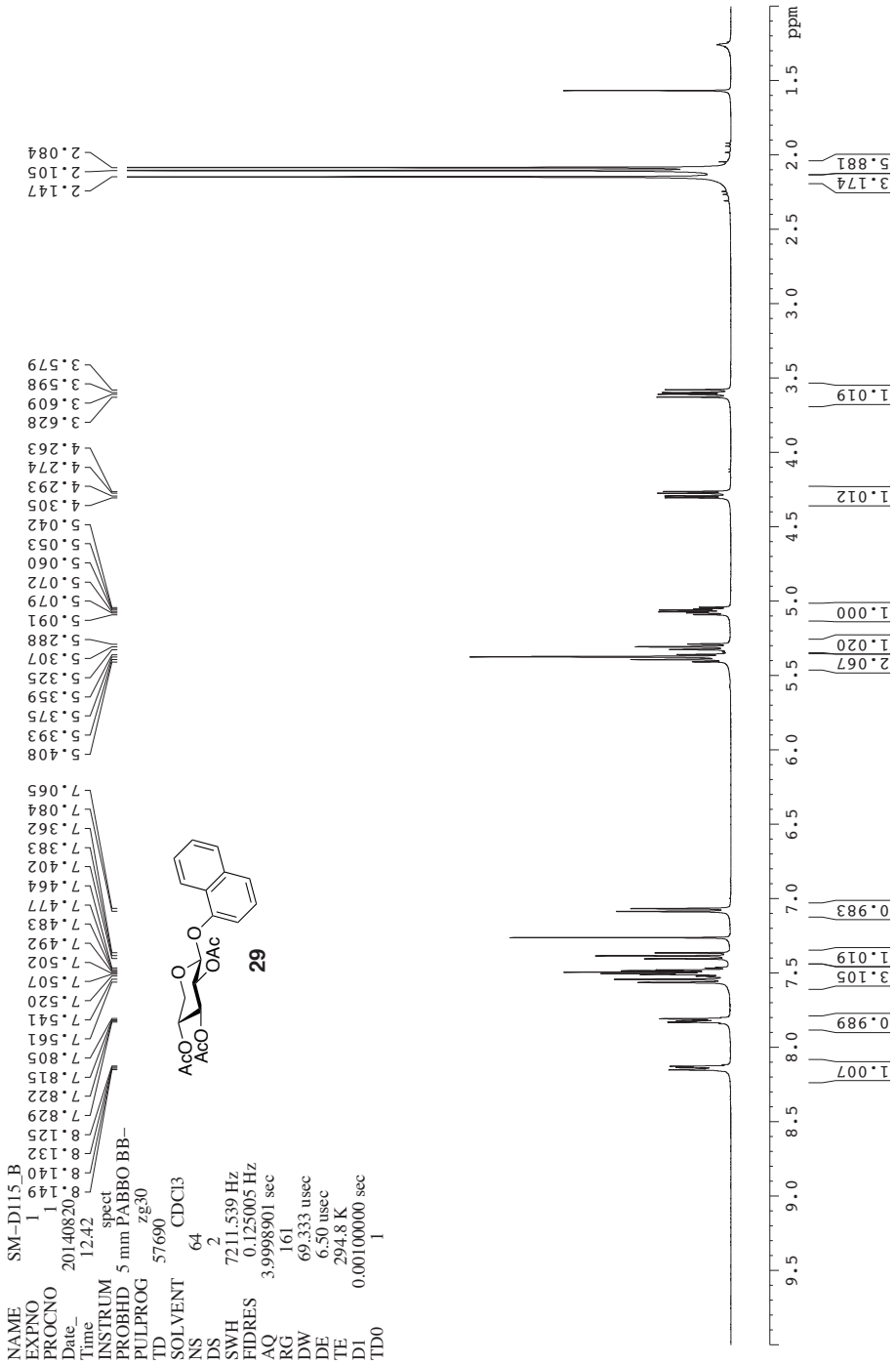


[1]H-NMR 1-Naphthyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside **(29)**



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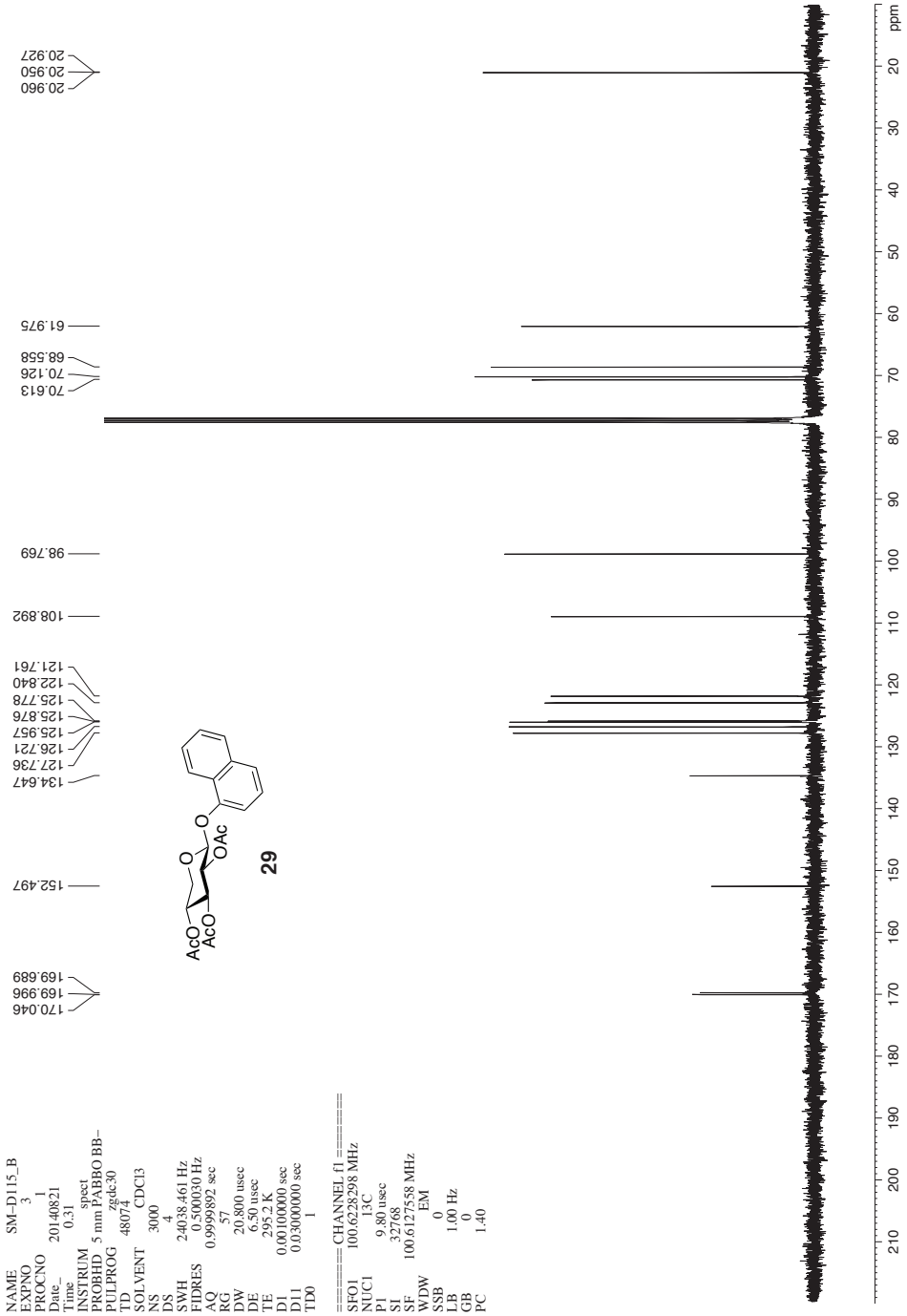

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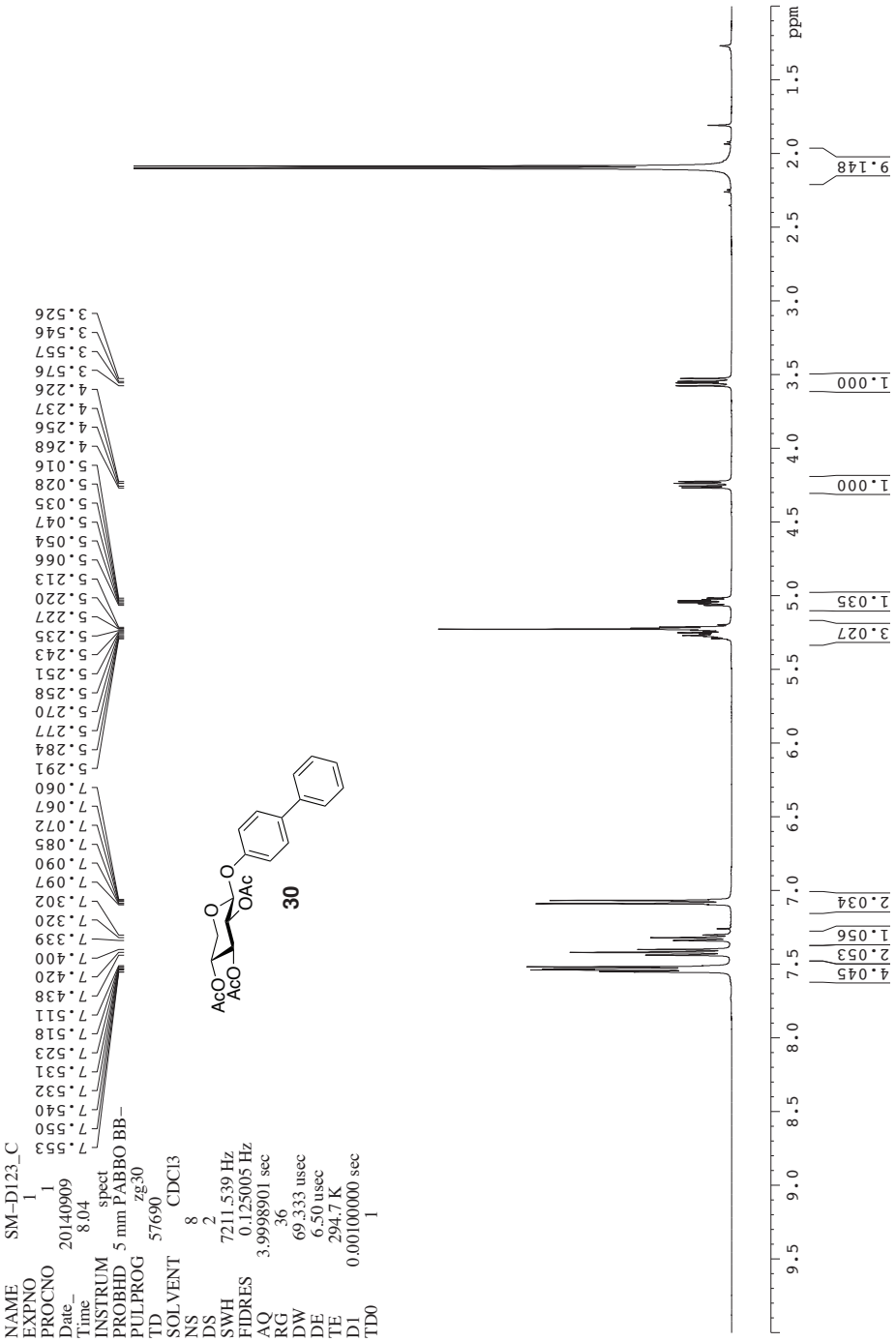

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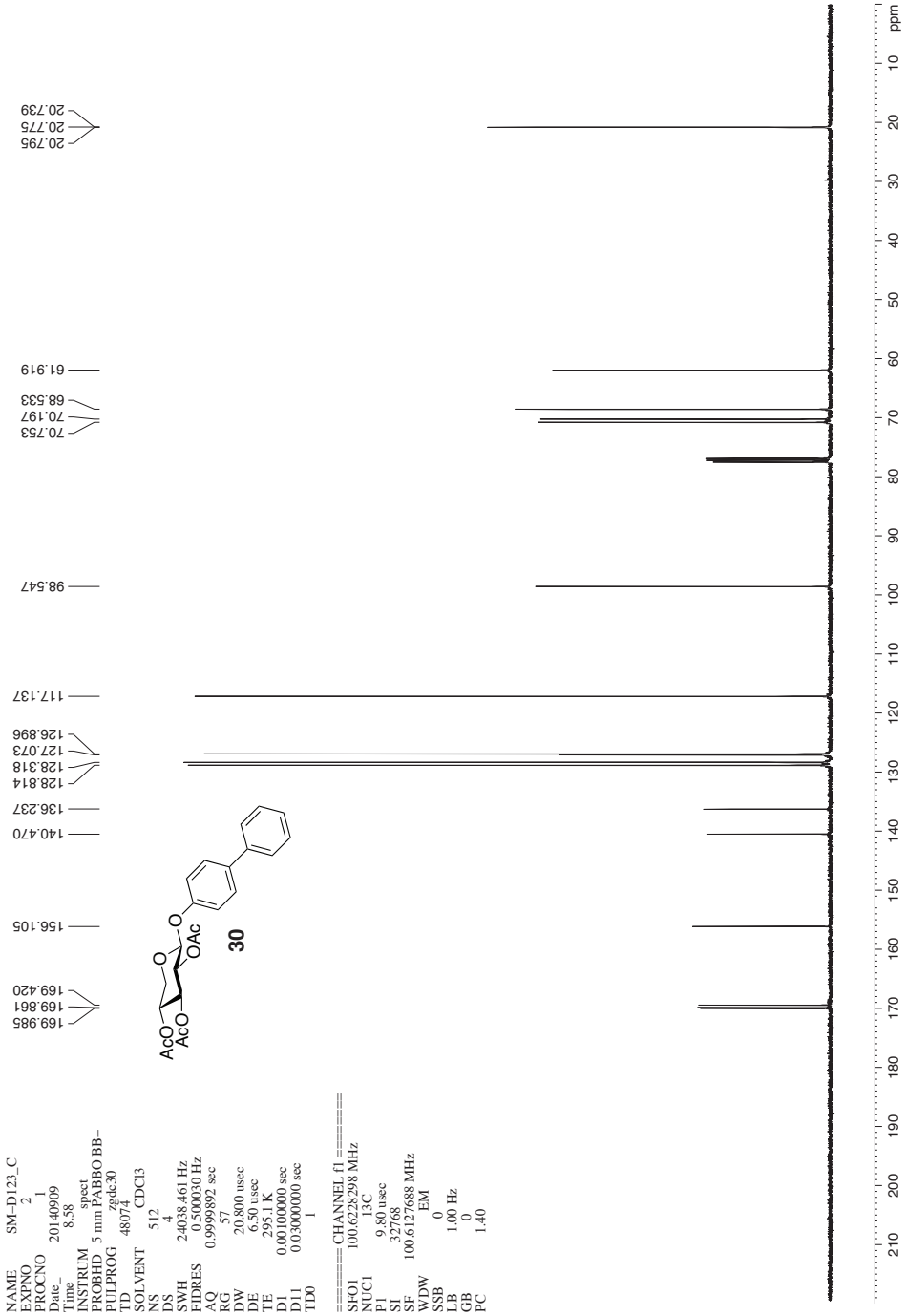

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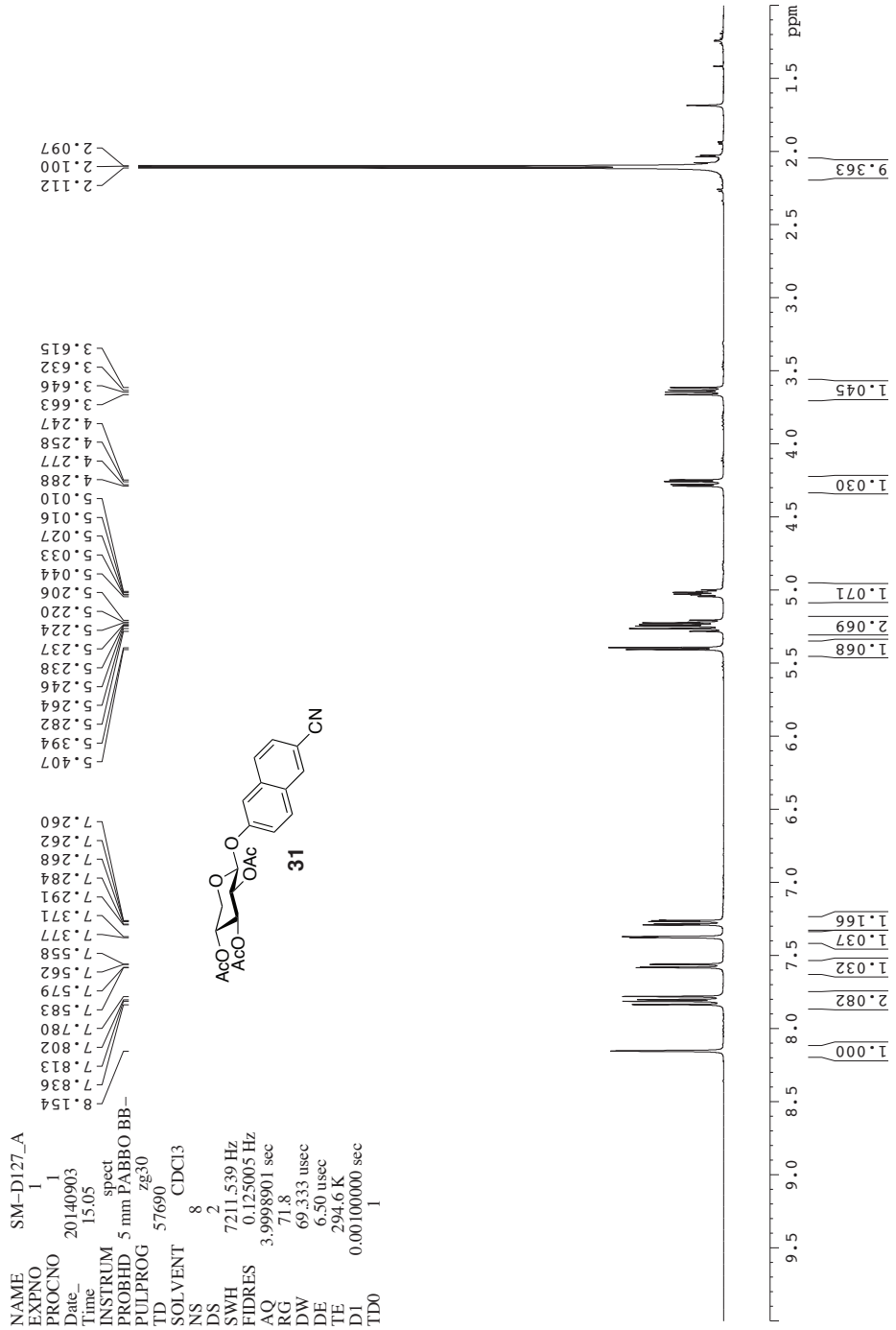
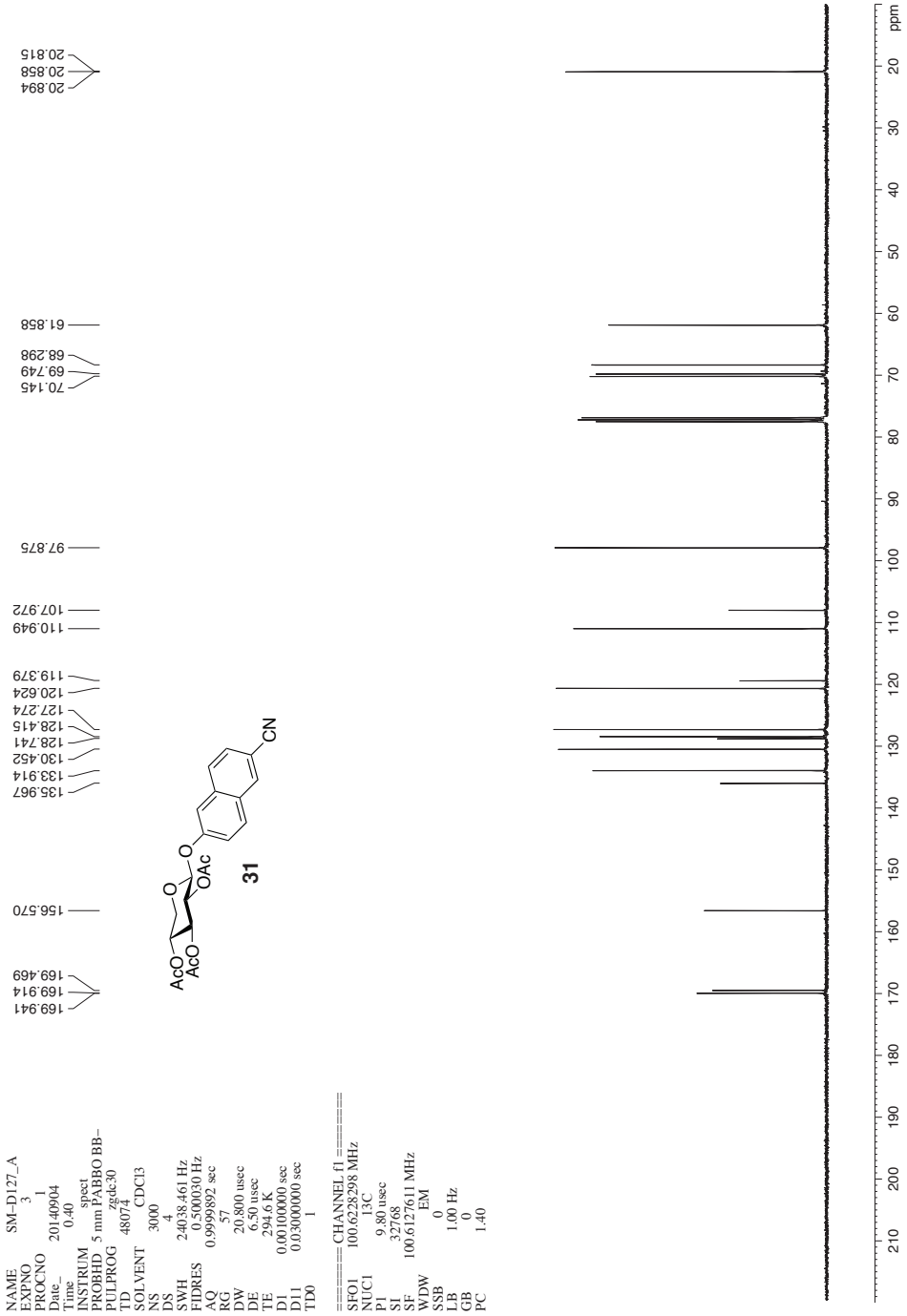
[1]H-NMR 2-(6-Cyano-naphthyl) 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside **(31)**



^{13}C-NMR 2-(6-Cyano-naphthyl) 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside **(31)**



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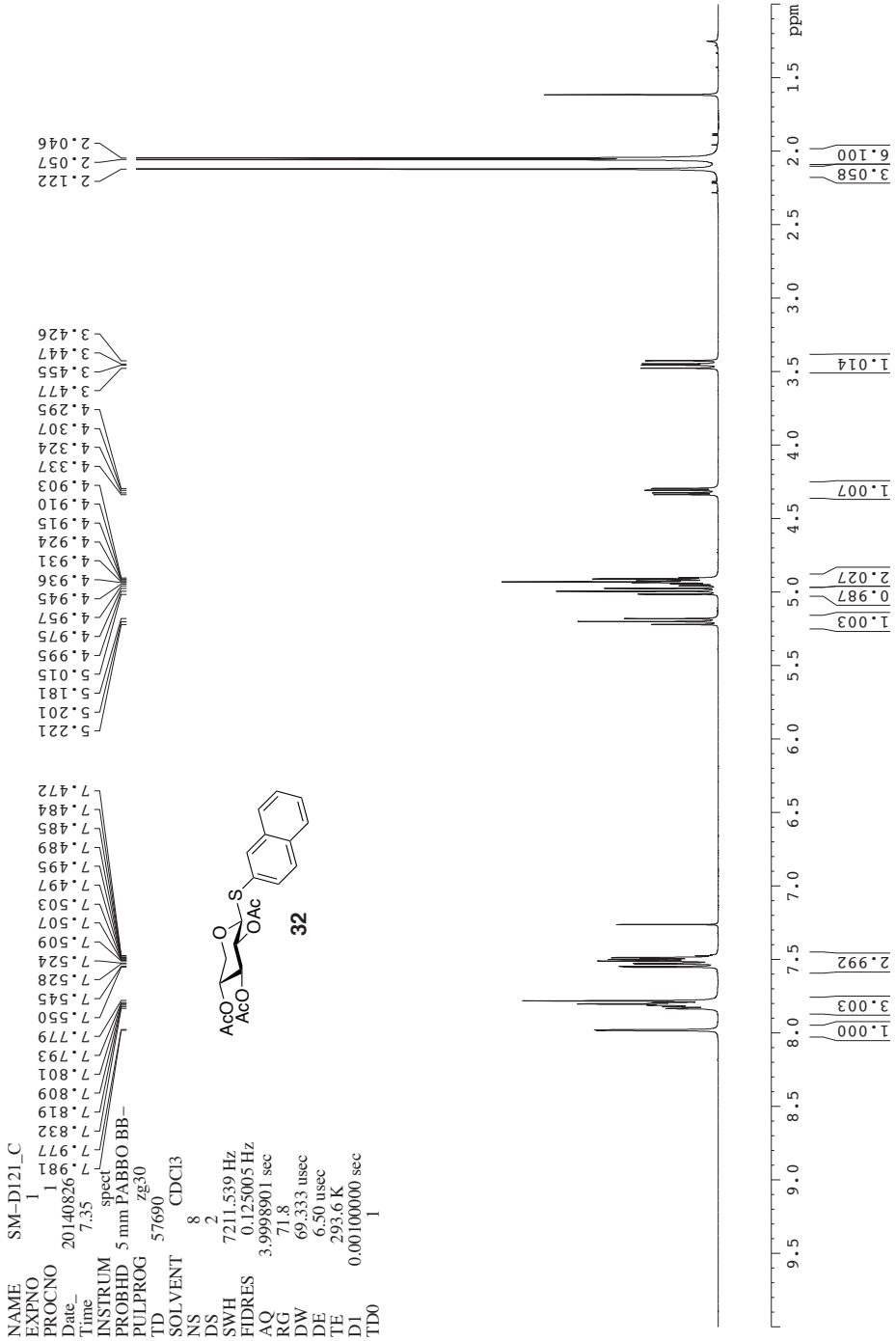

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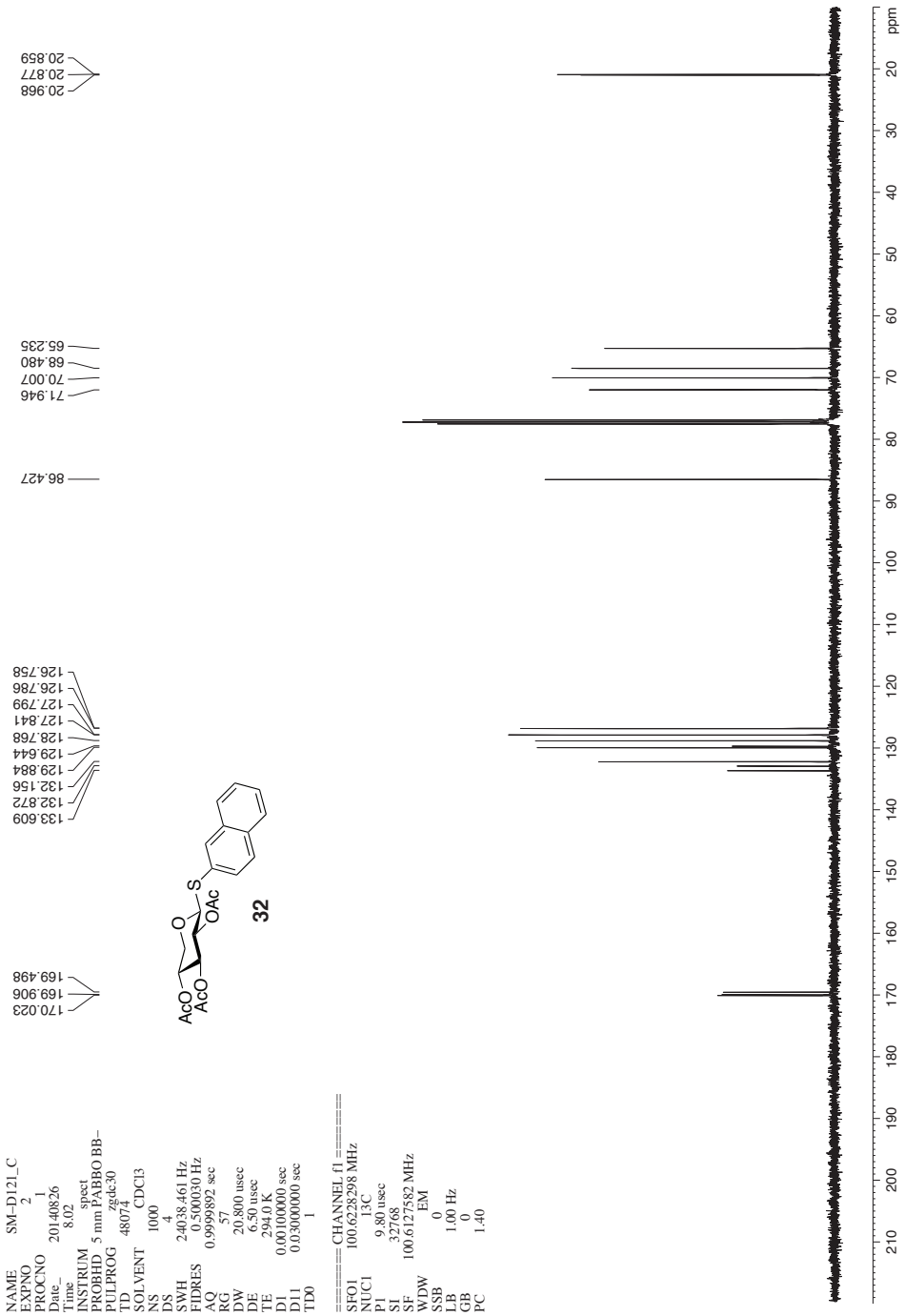
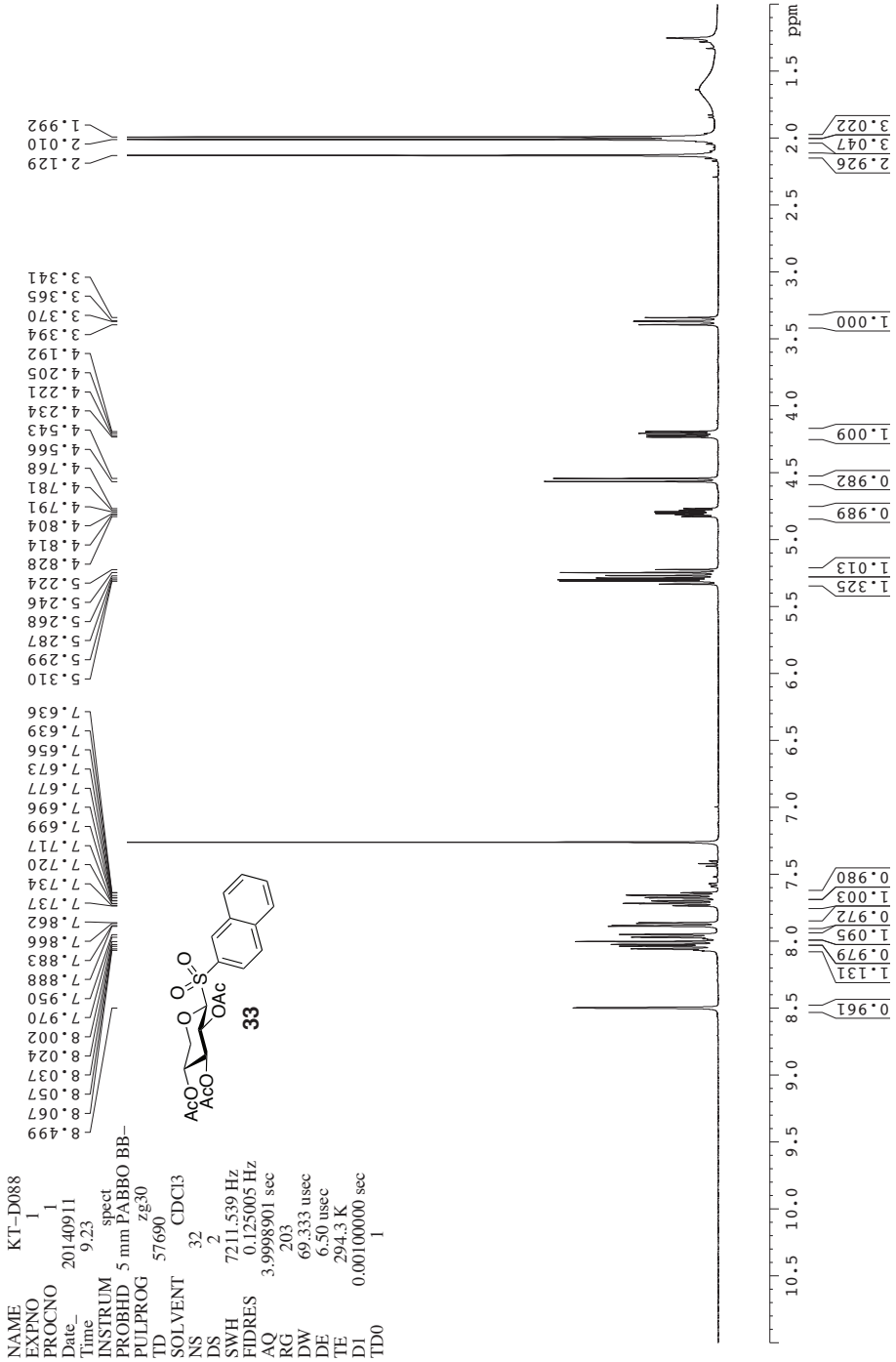


[1]H-NMR 2-Naphthyl 2,3,4-tri-*O*-acetyl β-D-xylopyranosyl sulfone **(33)**



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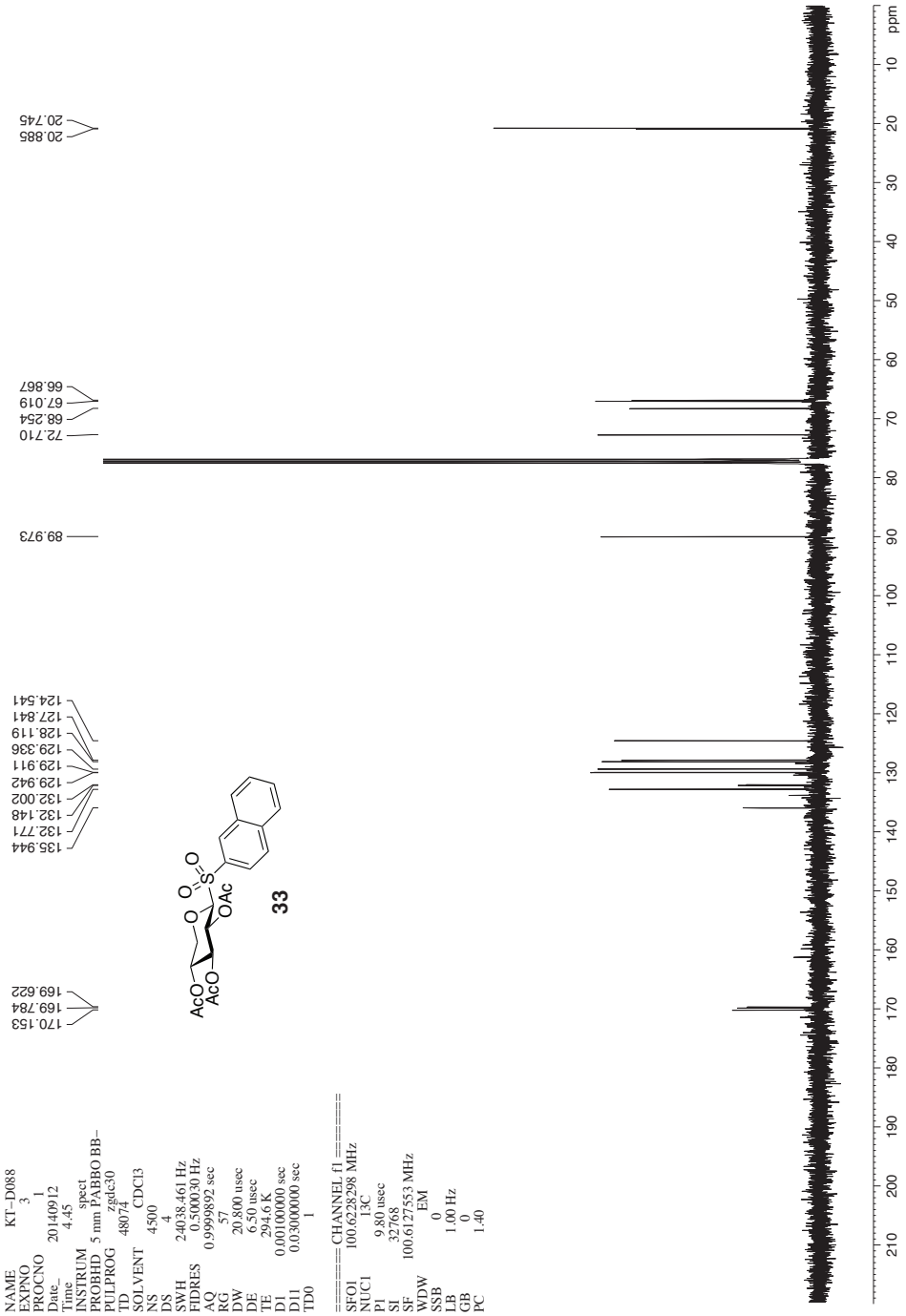

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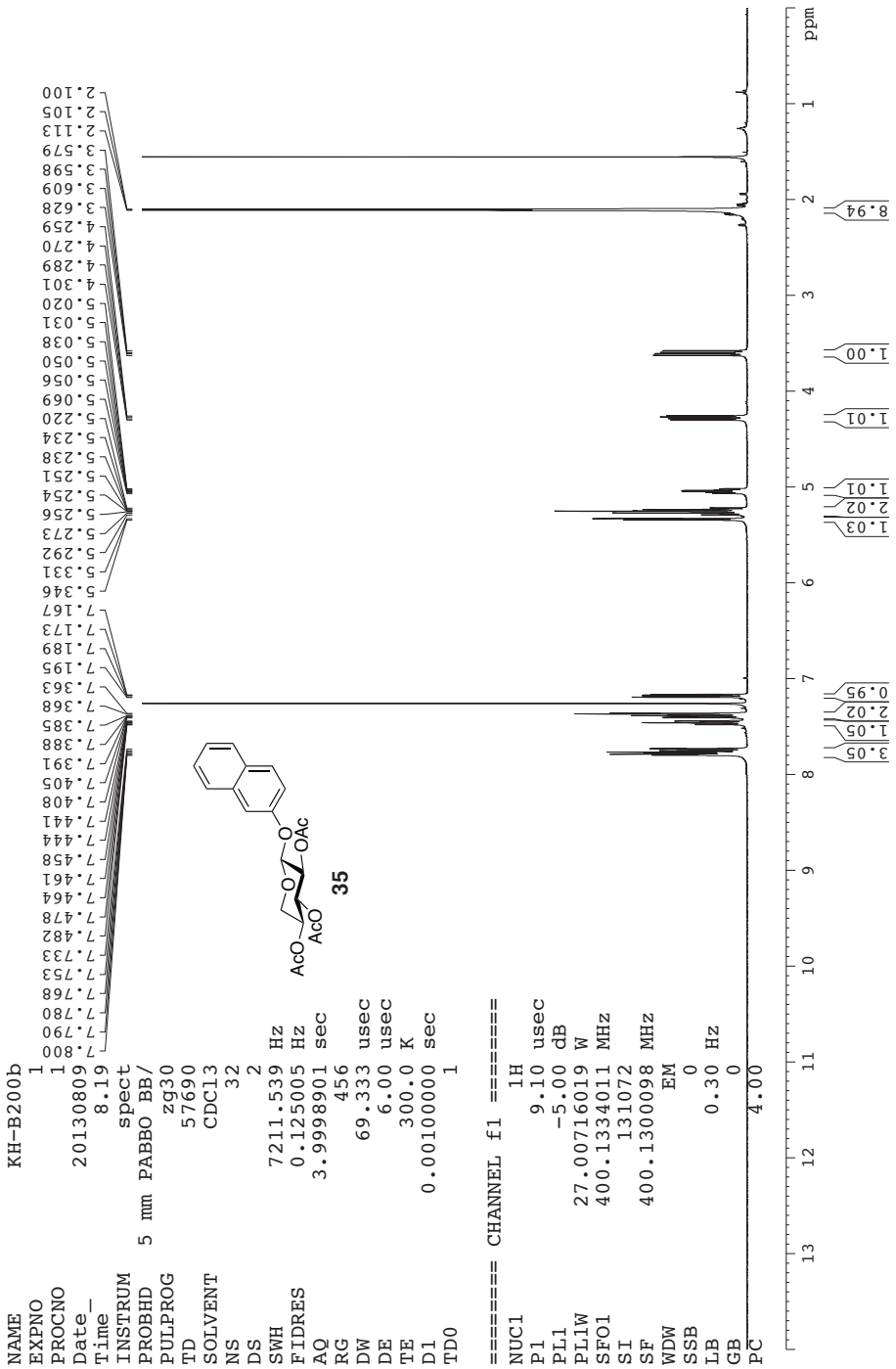
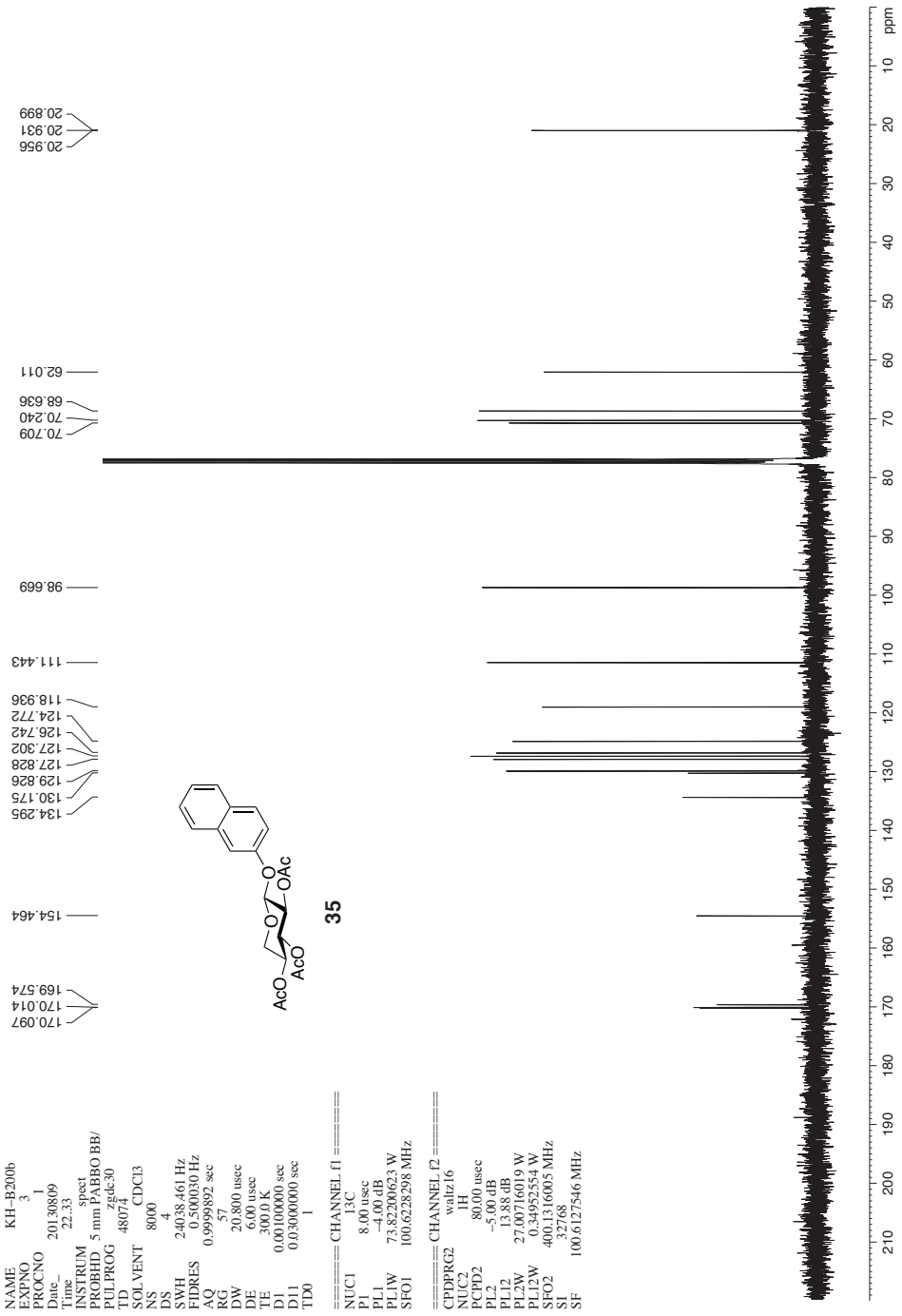


^{13}C-NMR 2-Naphthyl 2,3,4-tri-*O*-acetyl β-L-xylopyranoside **(35)**



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DE 6.00 usec
TE 300.0 K
D1 0.00100000 sec
D11 0.03000000 sec
TD0 1
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P1 8.00 usec
PL1 4.00 dB
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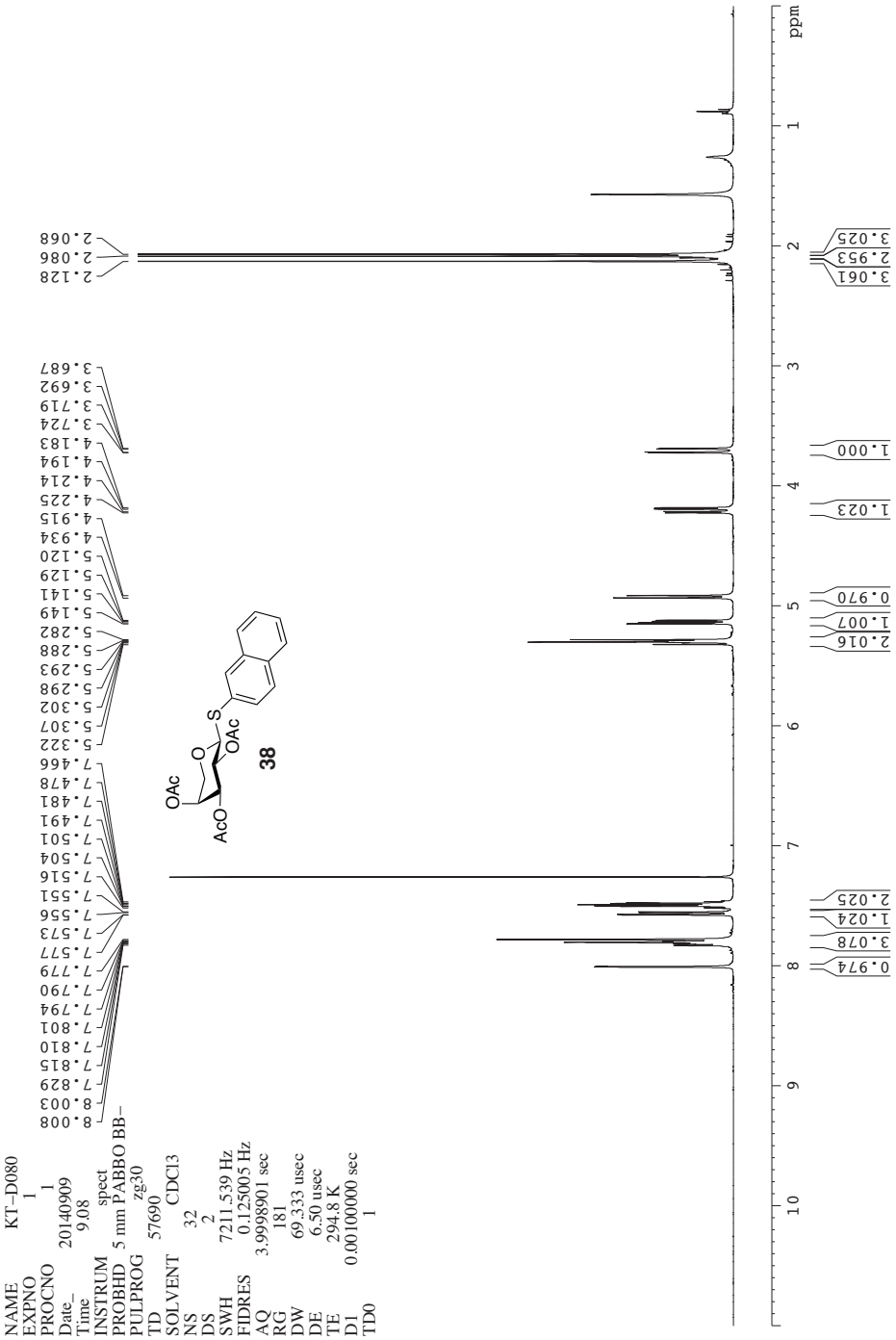
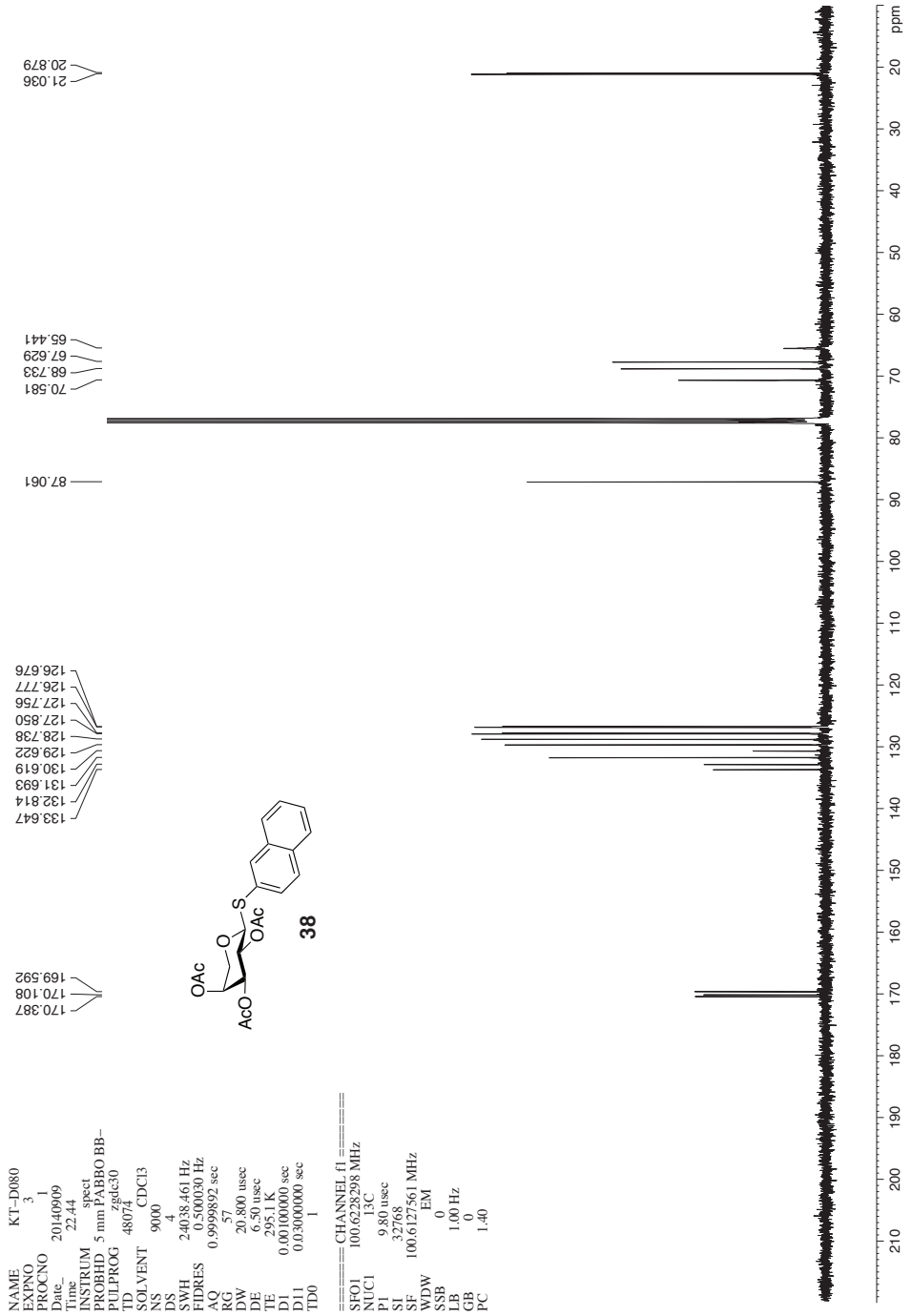
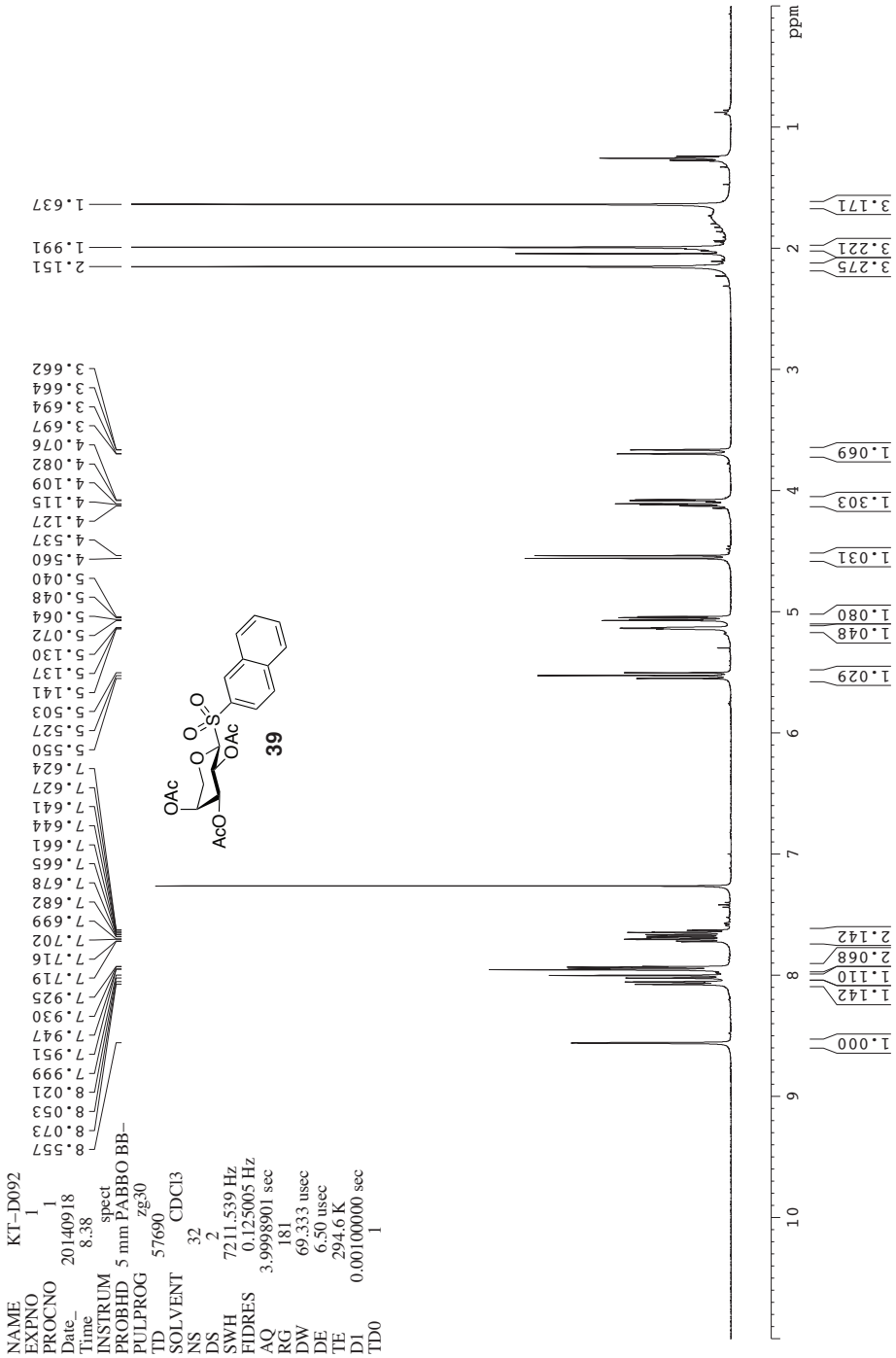
[1]H-NMR 2-Naphthyl 2,3,4-tri-*O*-acetyl 1-thio-α-L-arabinopyranoside **(38)**



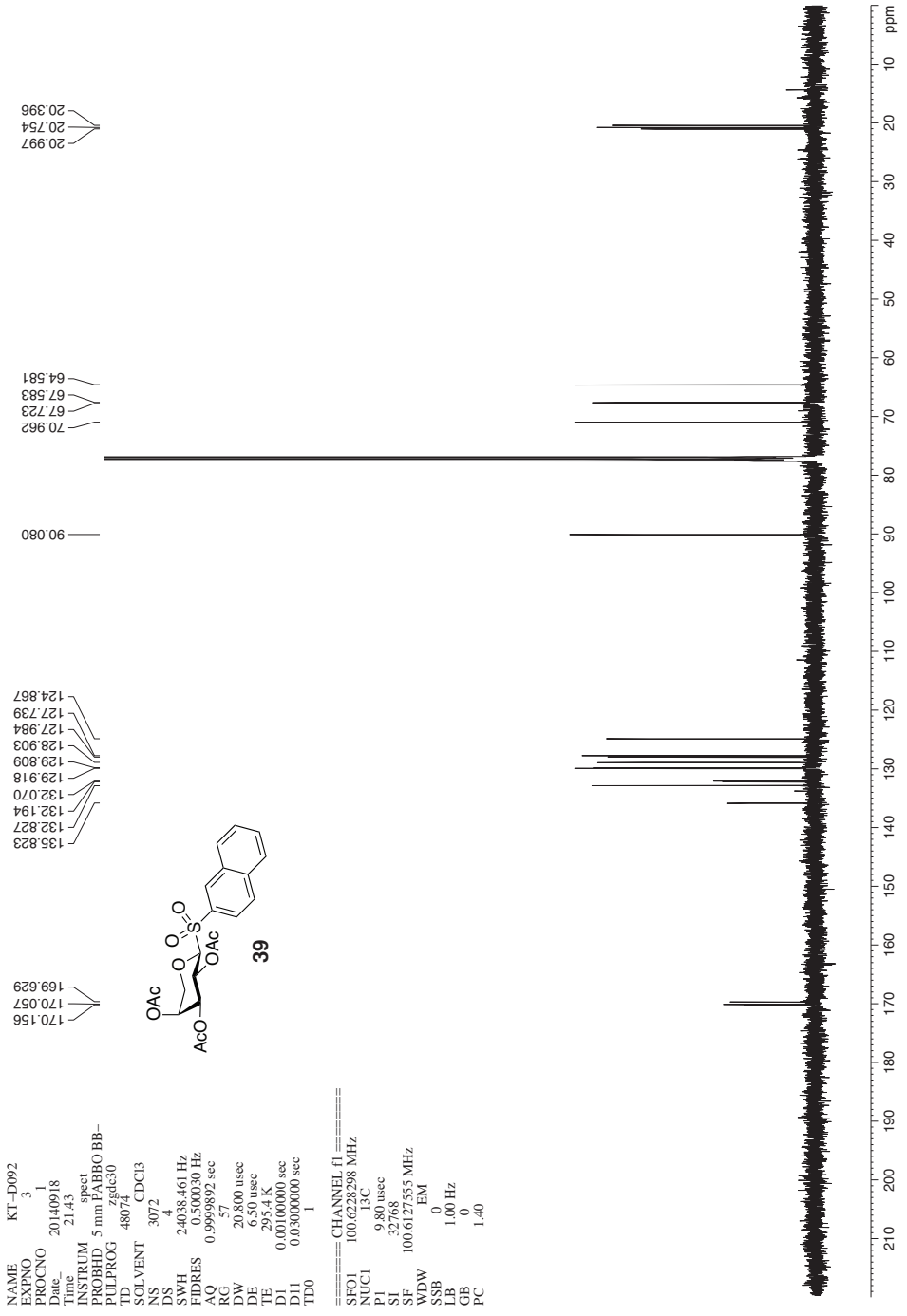
^{13}C-NMR 2-Naphthyl 2,3,4-tri-*O*-acetyl 1-thio-α-L-arabinopyranoside **(38)**



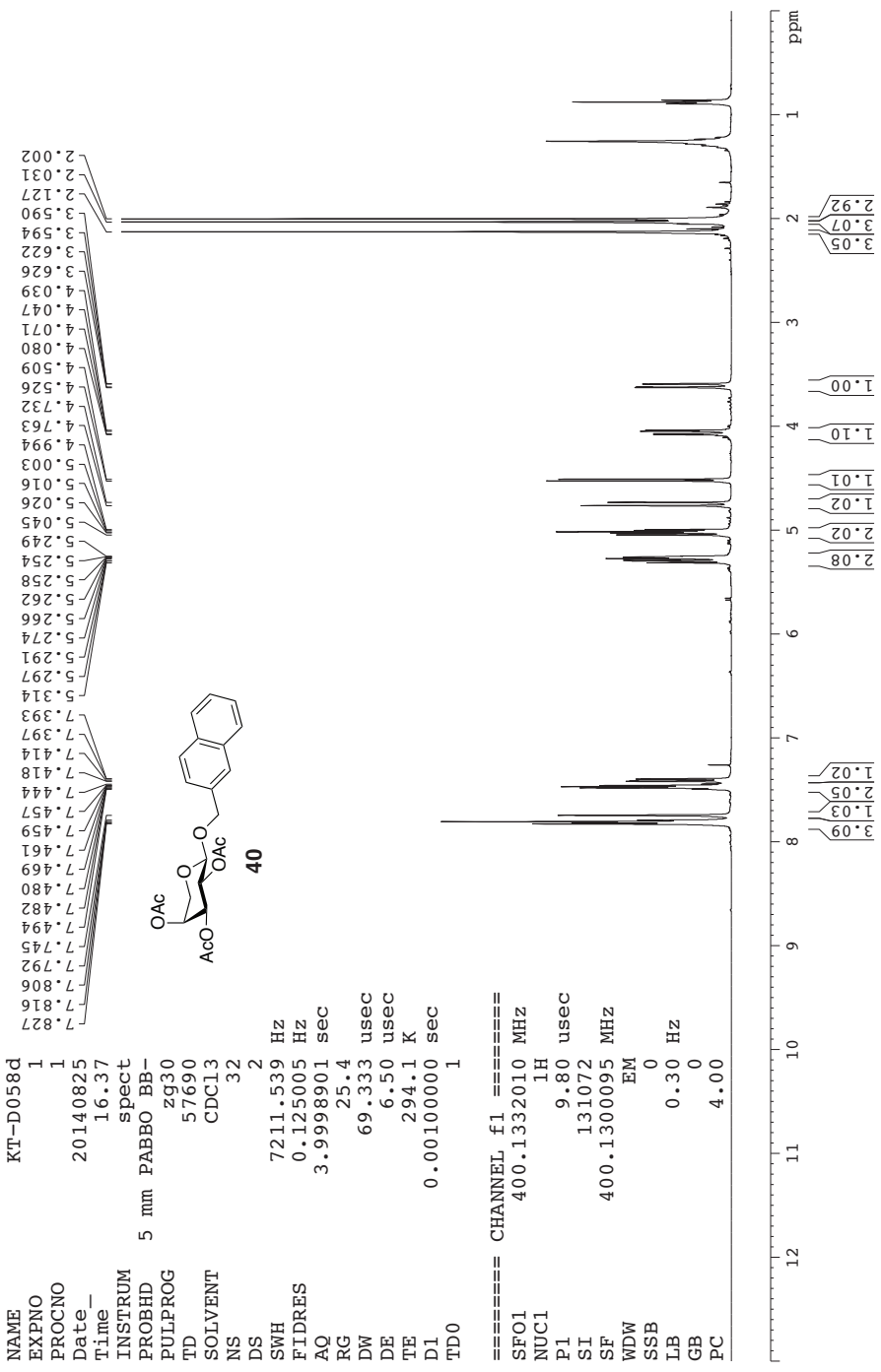
^{1}H-NMR 2-Naphthyl 2,3,4-tri-*O*-acetyl α-L-arabinopyranosyl sulfone **(39)**



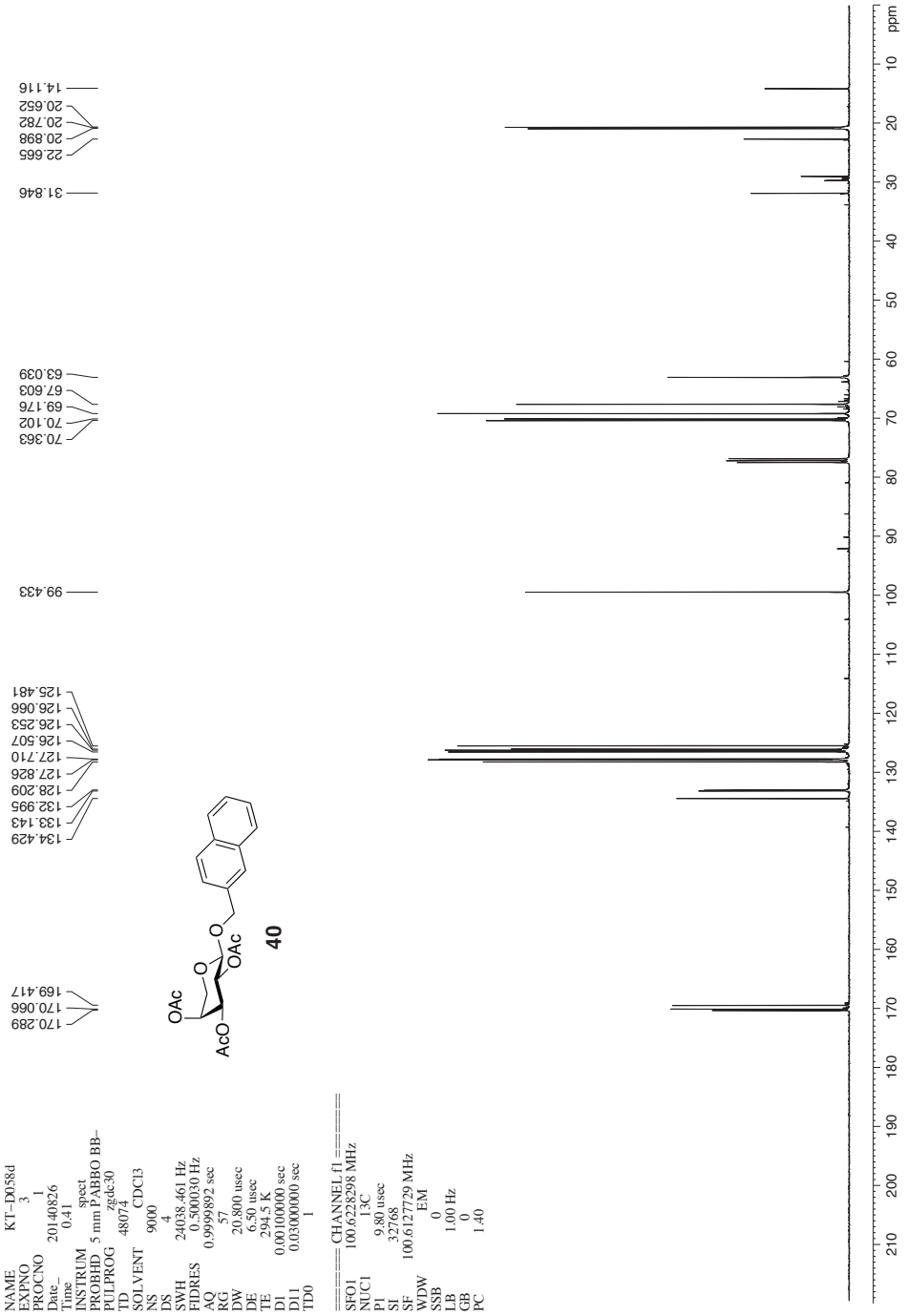
^{13}C-NMR 2-Naphthyl 2,3,4-tri-*O*-acetyl α-L-arabinopyranosyl sulfone **(39)**



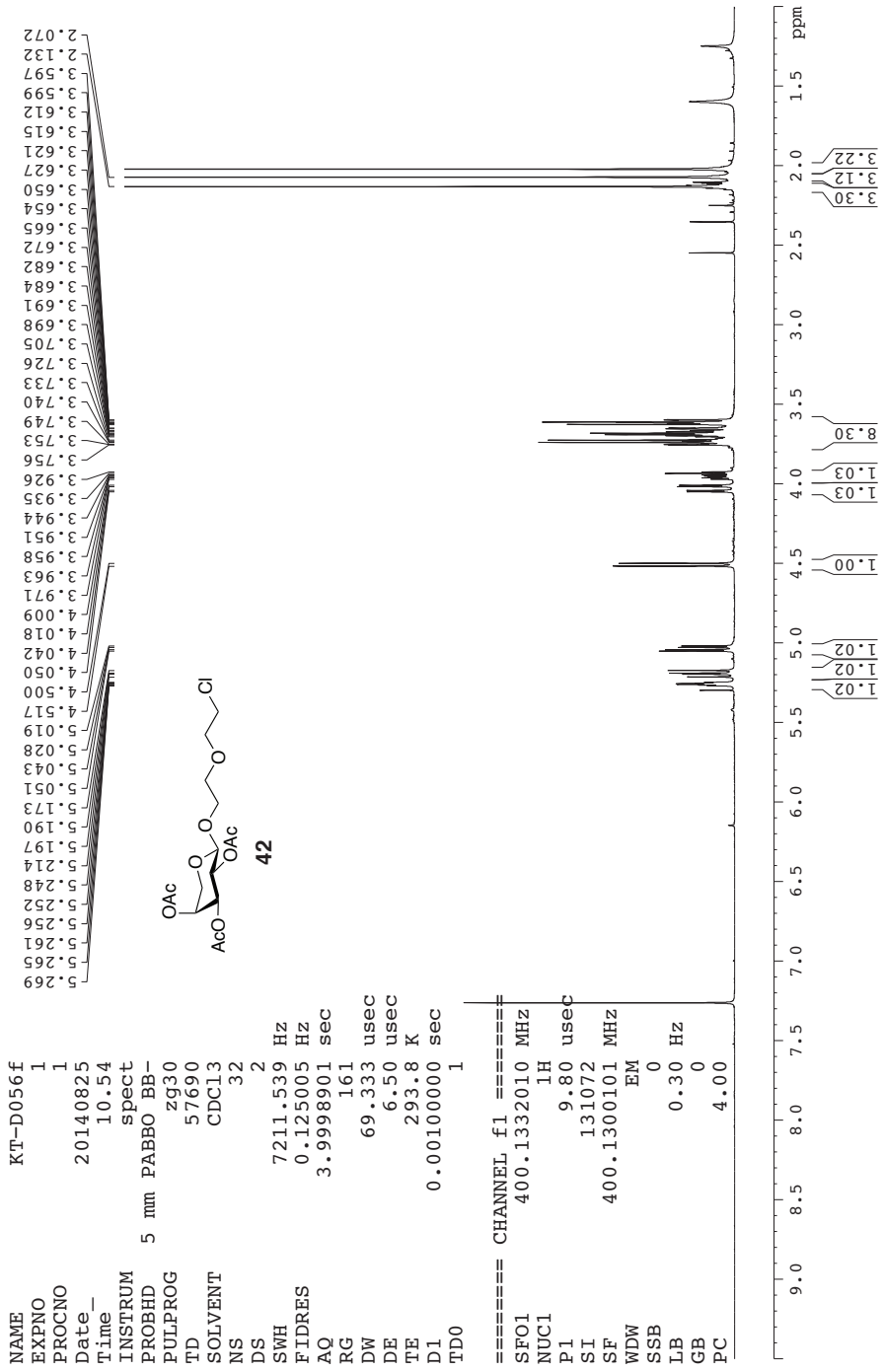
[1]H-NMR (2-Naphthyl)-methyl 2,3,4-tri-*O*-acetyl α-L-arabinopyranoside **(40)**



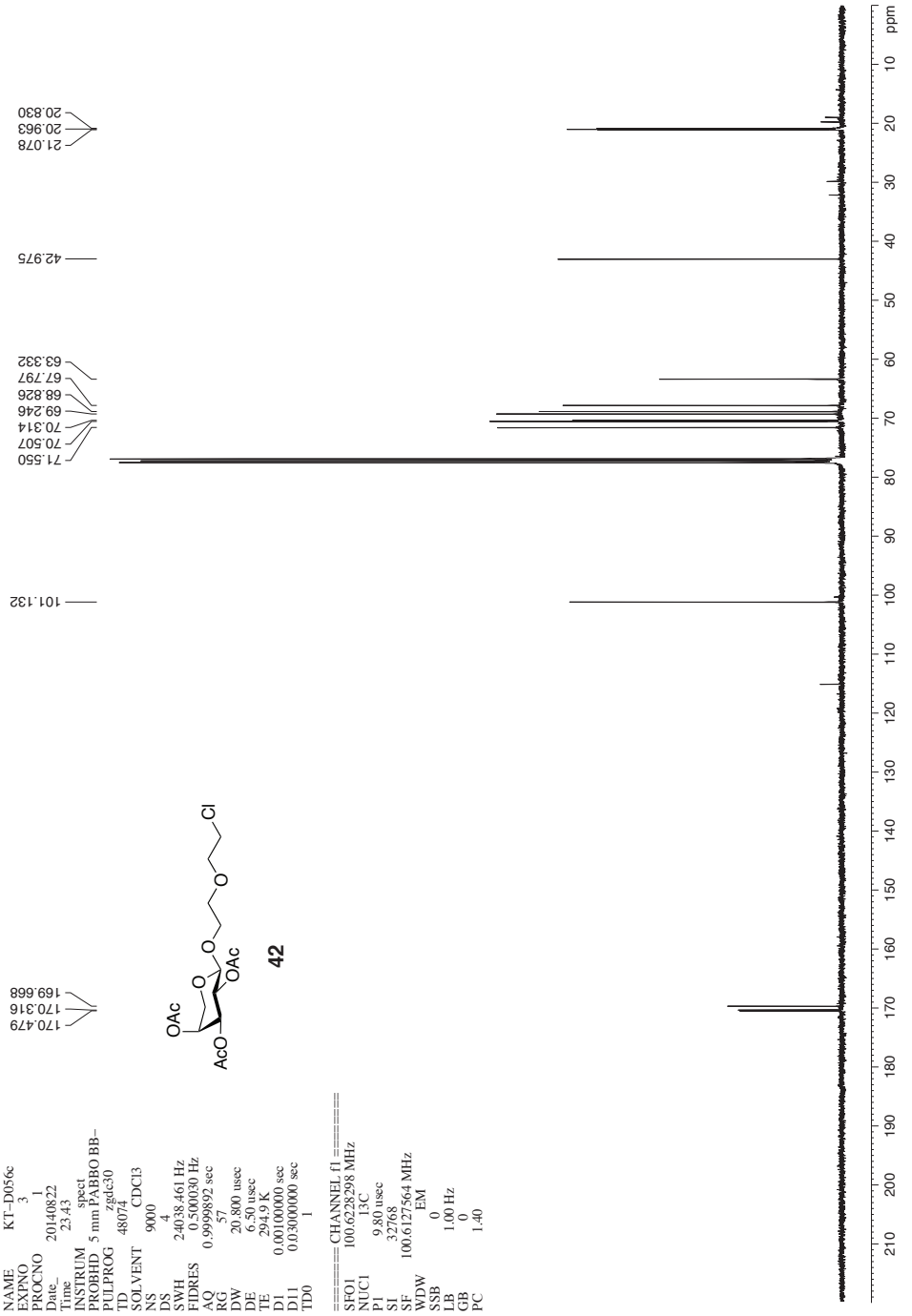
^{13}C-NMR (2-Naphthyl)-methyl 2,3,4-tri-*O*-acetyl α-L-arabinopyranoside **(40)**



[1]H-NMR 5-Chloro-3-oxo-1-pentyl 2,3,4-tri-*O*-acetyl-α-L-arabinopyranoside **(42)**



^{13}C-NMR 5-Chloro-3-oxo-1-pentyl 2,3,4-tri-*O*-acetyl-α-L-arabinopyranoside **(42)**



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Time 23.43
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NS 9000
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FIDRES 0.500030 Hz
AQ 0.9999892 sec
RG 57
DW 20.800 usec
DE 6.50 usec
TE 294.9 K
D1 0.00100000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
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NUC1 13C
P1 9.80 usec
SI 32768
SF 100.6127564 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
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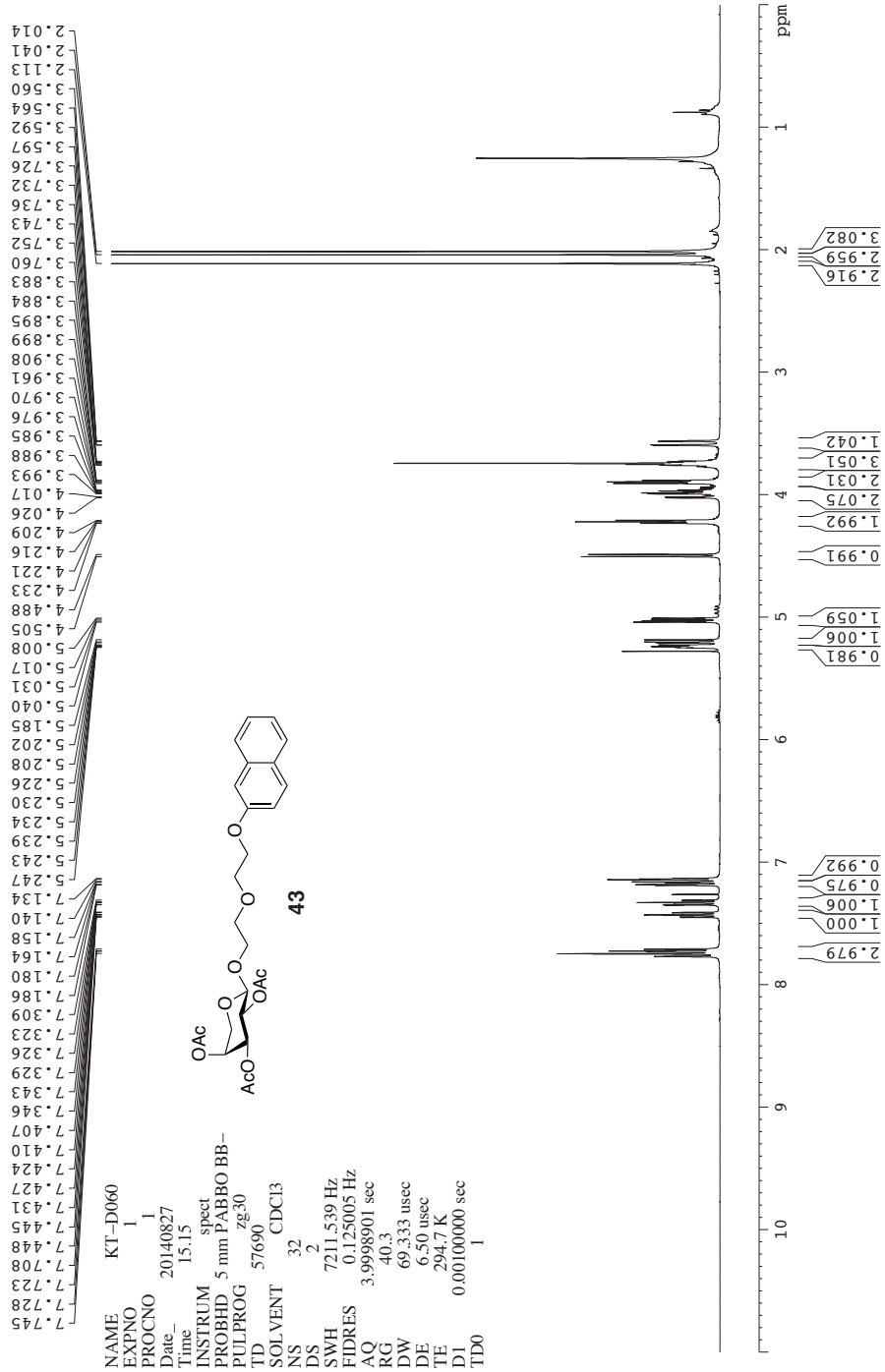
[1]H-NMR 5-(2-Naphthoxy)-3-oxo-1-pentyl 2,3,4-tri-*O*-acetyl-α-L-arabinopyranoside **(43)**



^{13}C-NMR 5-(2-Naphthoxy)-3-oxo-1-pentyl 2,3,4-tri-*O*-acetyl-α-L-arabinopyranoside **(43)**