Unexpected degradation of bisphosphonate P-C-P bridge under mild conditions

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General experimental details: ^{1}H, ^{31}P and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer operating at 500.1, 202.5 and 125.8 MHz, respectively. TMS or TSP (for D_2O solutions) was used as an internal standard for ^{1}H and ^{13}C measurements, and 85% H_3PO_4 was used as an external standard for ^{31}P measurements. The $^{3}J_{HH}$ couplings are indicated by the letter "*J*". The $^{n}J_{HP}$ couplings were calculated from proton spectra and all *J* values are given in Hz. The $^{n}J_{CP}$ couplings were calculated from proton decoupled carbon spectra with the coupling constants given in parenthesis as hertz. The purity of the compound **1a** was determined from ^{1}H and ^{31}P NMR spectra and was ca. 96 %. Electronspray ionization mass spectra were acquired by an LCQ quadrupole ion trap mass spectrometer with an electronspray ionization source.

Procedure for the preparation of etidronate derivative 1a: Acetylated etidronic acid (**5**) (150 mg, 0.60 mmol), Na_2CO_3 (388 mg, 3.66 mmol) and ethyl chloroformate (3 ml) was refluxed for overnight under the nitrogen atmosphere. Reaction mixture was evaporated to dryness, diethyl ether (8 ml) was added and reaction mixture was stirred for 15 minutes before the precipitate was removed by centrifugation. The remaining solution was evaporated to dryness and compound **1a** was obtained as colorless oil in 55% yield.

[1-(diethoxycarbonyloxy)phosphoryl-1-acetyloxyethyl]-1-(ethoxycarbonyloxy)phosphonic acid ethyl ester (1a): Pair of diastereomers (ratio 50:50). ^{1}H NMR ($CDCl_3$): δ 4.49-4.41 (m, 2H), 4.36-4.18 (m, 6H), 2.14 (s, 3H), 1.973 (dd, $^{3}J_{HP}$ = 15.4, $^{3}J_{HP'}$ = 17.0) and 1.965 (dd, $^{3}J_{HP}$ = 15.5, $^{3}J_{HP'}$ = 17.0, 3H), 1.41-1.34 (m, 12H). ^{13}C NMR ($CDCl_3$) δ 168.91 (dd, $^{3}J_{CP}$ = 7.8, $^{3}J_{CP'}$ = 8.9), 168.76 (dd, $^{3}J_{CP}$ = 7.0, $^{3}J_{CP'}$ = 8.8), 147.67 (d, $^{3}J_{CP}$ = 7.0), 147.66 (d, $^{3}J_{CP}$ = 7.3), 147.59 (d, $^{3}J_{CP}$ = 7.3), 78.79 (dd, $^{1}J_{CP}$ = 156.1, $^{1}J_{CP'}$ = 157.4), 78.71 (dd, $^{1}J_{CP}$ = 155.2, $^{1}J_{CP'}$ = 159.5), 66.27 (d, $^{2}J_{CP}$ = 7.6), 66.14 (d, $^{3}J_{CP}$ = 7.6), 65.82, 65.81, 64.33 (d, $^{4}J_{CP}$ = 6.9), 64.29 (d, $^{4}J_{CP}$ = 7.0), 64.23 (d, $^{4}J_{CP}$ = 7.2), 63.91 (d, $^{4}J_{CP}$ = 7.5), 21.26, 21.24, 18.61 (t, $^{2}J_{CP}$ = 2.8), 18.18 (t, $^{2}J_{CP}$ = 2.0), 16.44 (d, $^{5}J_{CP}$ = 1.8), 16.39 (d, $^{5}J_{CP}$ = 2.6), 16.39 (d, $^{5}J_{CP}$ = 2.6), 16.34 (d, $^{5}J_{CP}$ = 2.6), 15.99 (d, $^{4}J_{CP}$ = 6.7), 13.98, 13.97. ^{31}P NMR ($CDCl_3$) δ 13.19 d ($^{2}J_{PP}$ = 22.5) 10.28 and 13.12 d ($^{2}J_{PP}$ = 21.0) 10.23 d. ESI-MS: 515.3 (M + Na; 100%).

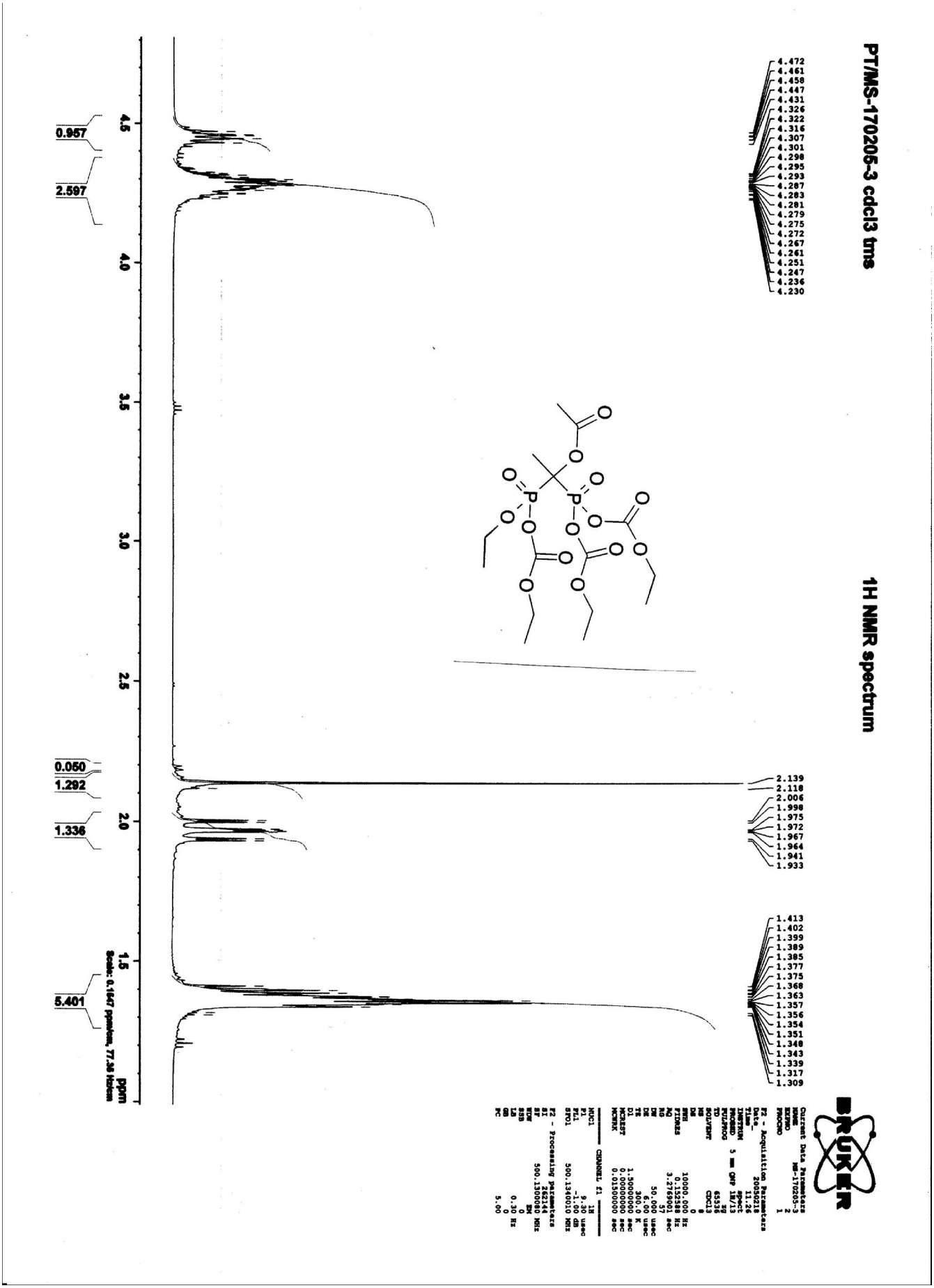
Typical example of 1a degradation experiment: Compound **1a** (70.5 mg, 0.143 mmol) was dissolved in MeOH (2 ml), 40% NaOH (58 µl, 4 eq) was added and the reaction mixture was stirred for 0.5 h at room temperature. Reaction mixture was evaporated to dryness and dried in vacuo. Product (ca. 63 mg) was obtained as white powder and contained mixture of acetate and phosphites **2-4** as identified from ^{1}H and ^{31}P NMR spectra (see pages S12-13).

Typical example of 1b degradation experiment: Compound **1b** (50 mg) was dissolved in H_2O (1 ml), pH was adjusted to ≥ 11 with 6 M NaOH (this needed only 1 pasteur pipette drop) and the reaction mixture was stirred for 1 h at room temperature before evaporating it to dryness in vacuo. Product was obtained as white powder and contained mixture of acetate and phosphite **4** as

identified from ^{1}H and ^{31}P NMR spectra (see pages S14-15; in ^{31}P NMR spectrum the remaining *H*-P coupling can be seen because of insufficient decoupling power).

Typical example of 1b degradation experiment in triethyl amine: Compound **1b** (50 mg) was dissolved in H_2O (1 ml), triethyl amine (122 µl, 5 eq) was added and the reaction mixture was stirred for 1 h at 60 ºC before evaporating it to dryness in vacuo. Product was obtained as slightly yellow solid and contained mixture of acetylphosphonate **7** and phosphite **3** as identified from ^{1}H and ^{31}P NMR spectra (see pages S16-17; in ^{31}P NMR spectrum the remaining *H*-P coupling can be seen for the compound **3** because of insufficient decoupling power).

Compound **1a**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4.316
4.307
4.301
4.298
4.295
4.293
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4.236
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1.972
1.967
1.964
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1.933
1.413
1.402
1.399
1.389
1.385
1.377
1.375
1.368
1.363
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1.356
1.354
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1.343
1.339
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2.0
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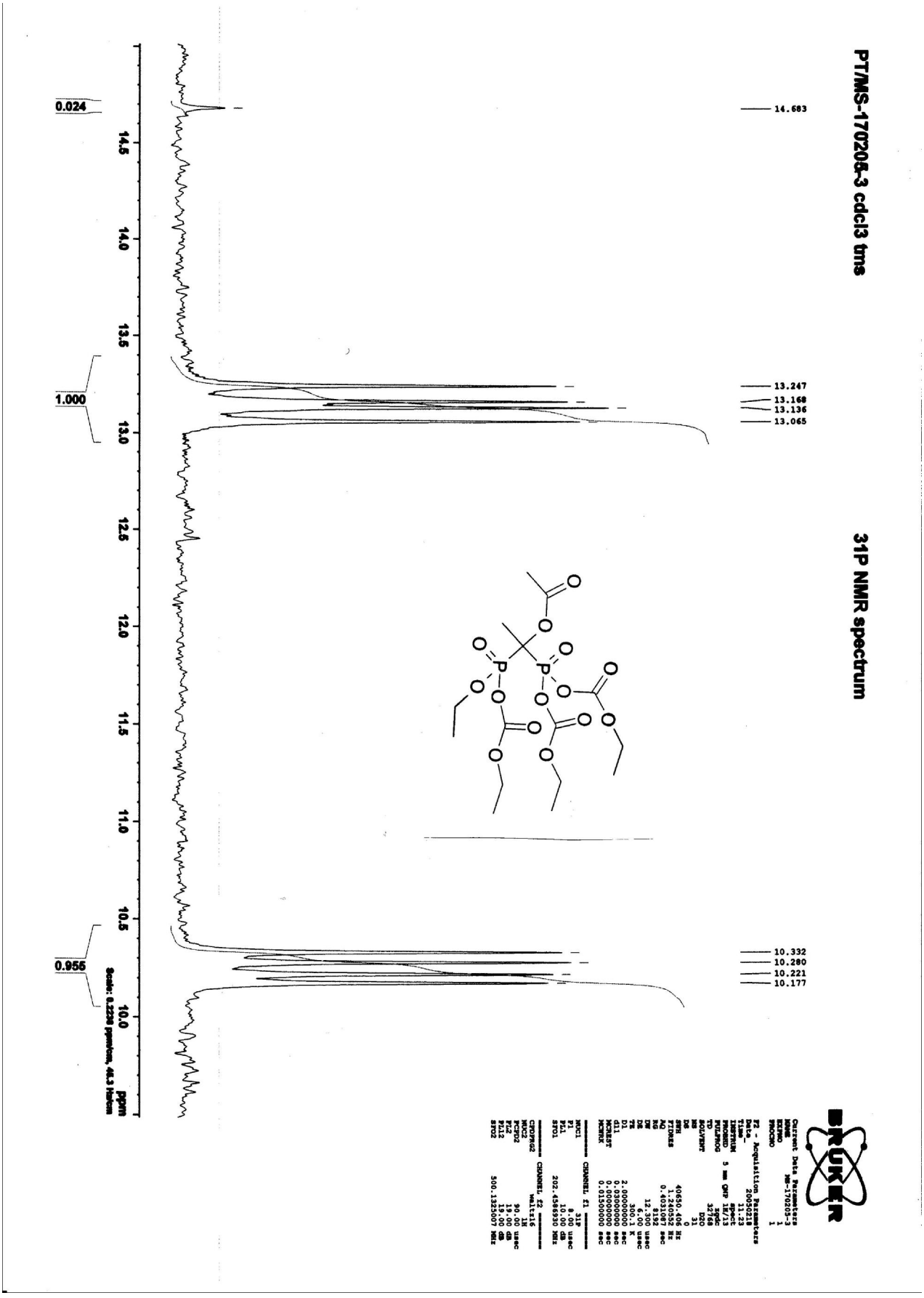
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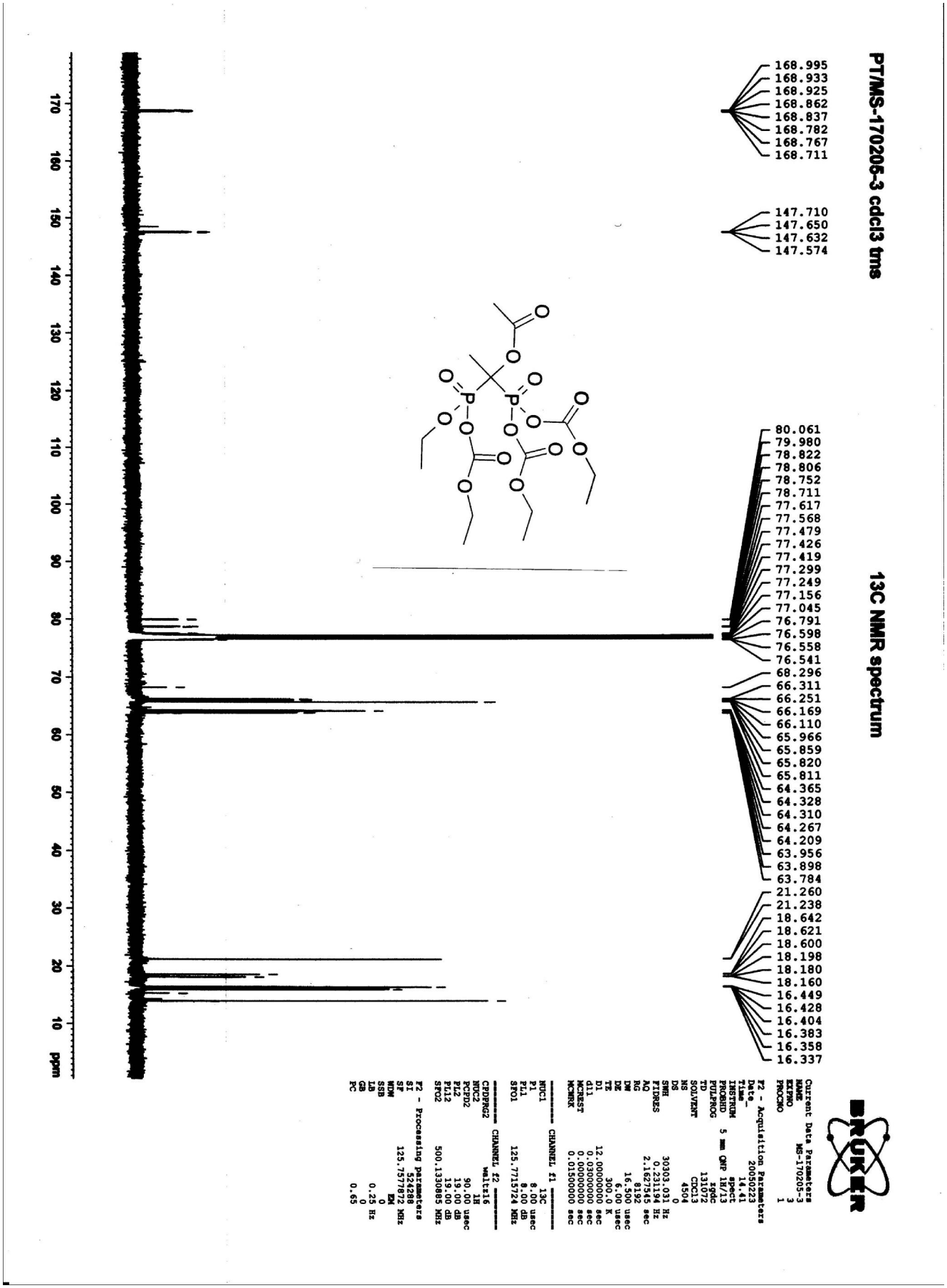
BRUKER

Compound **1a**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64.209
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16.383
16.358
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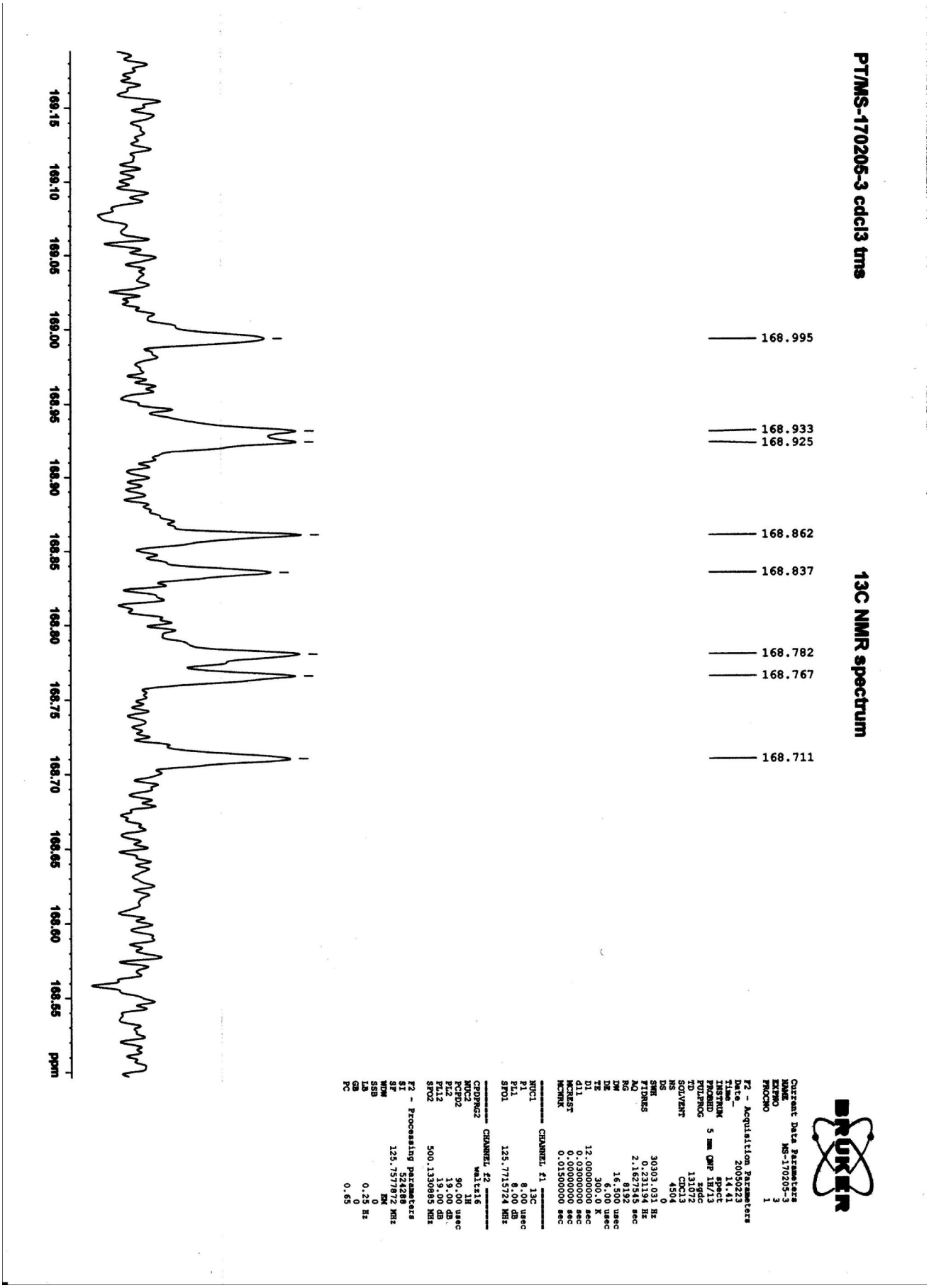
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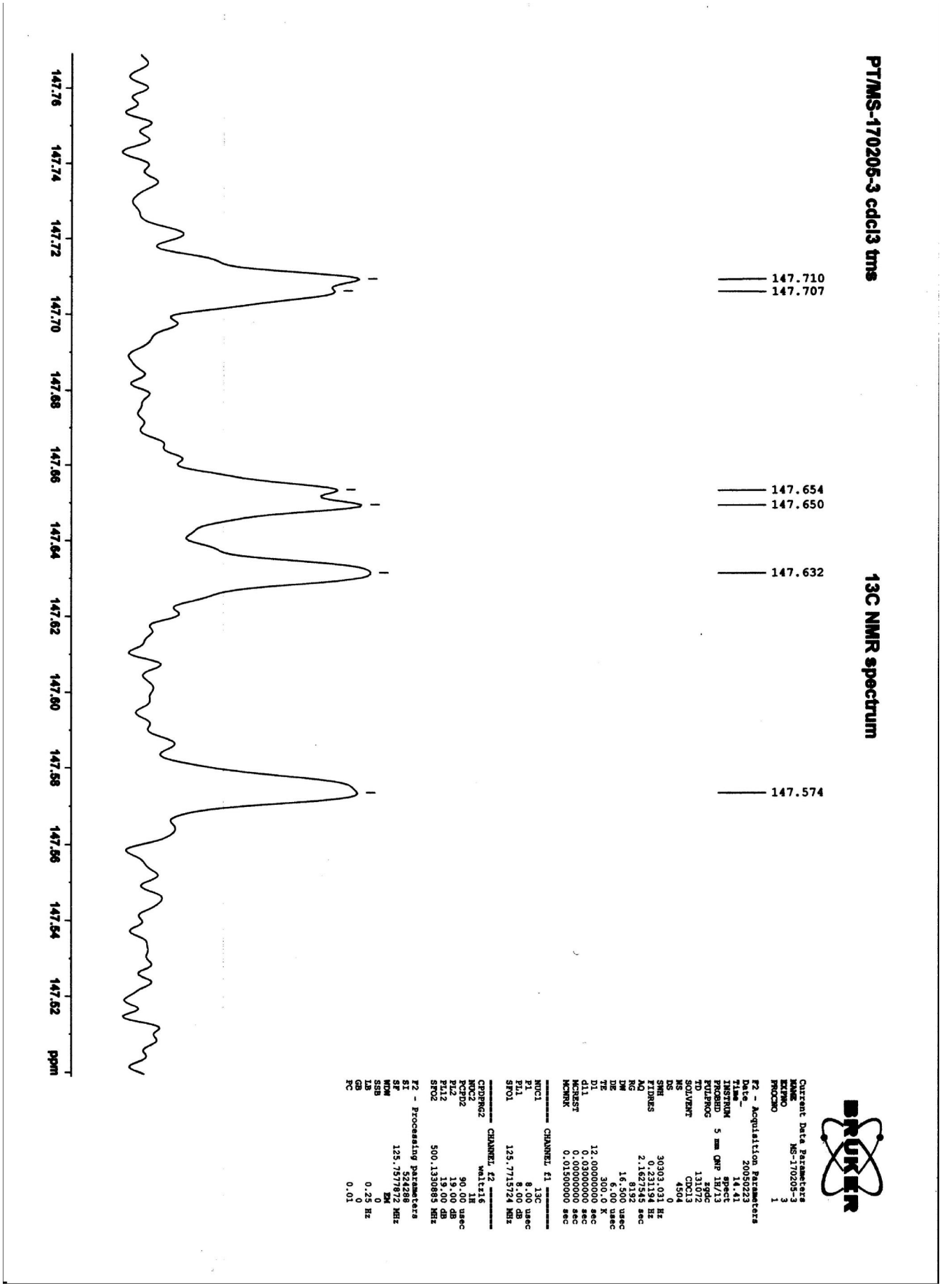
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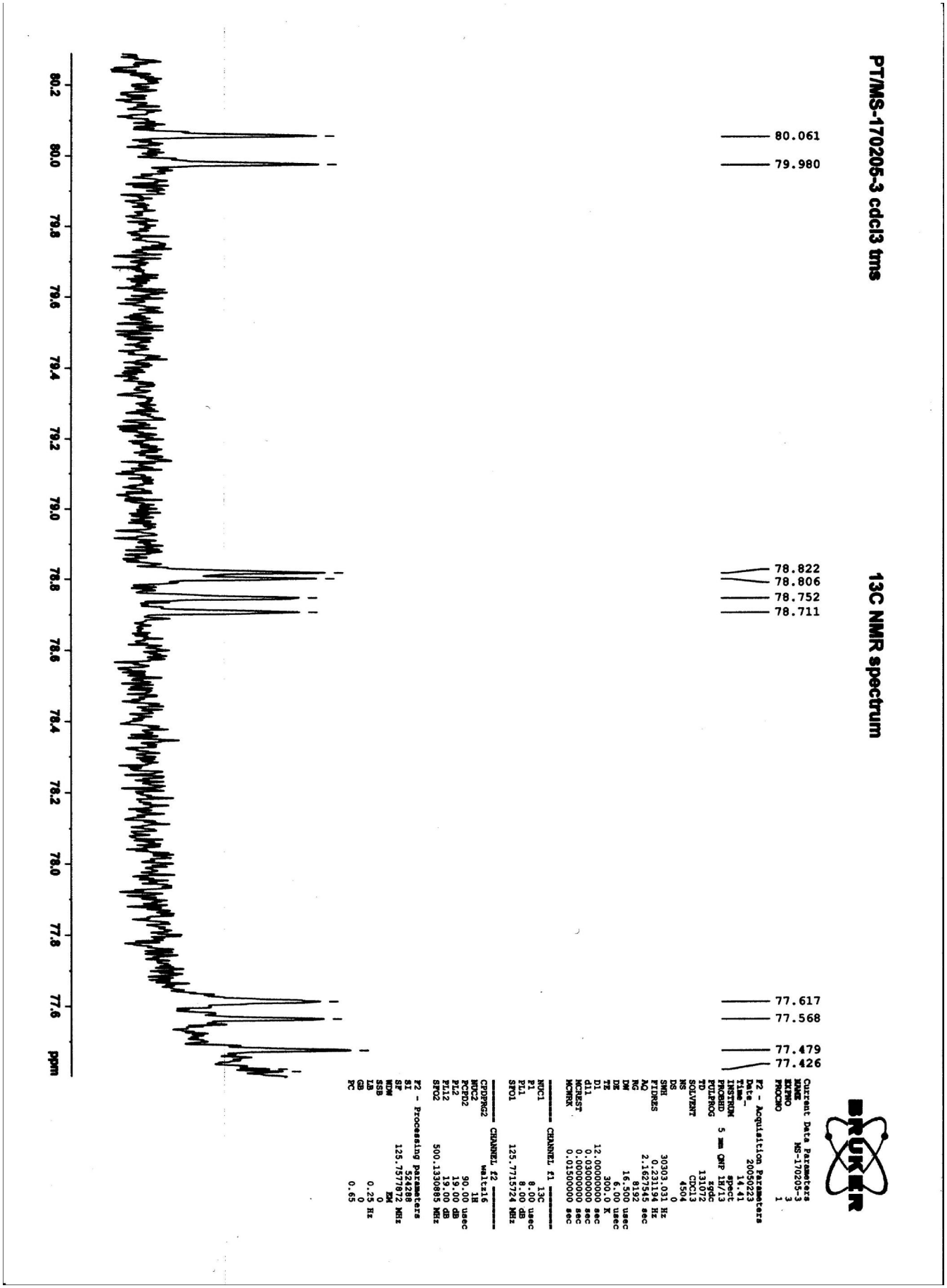
Compound **1a**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80.2
80.0
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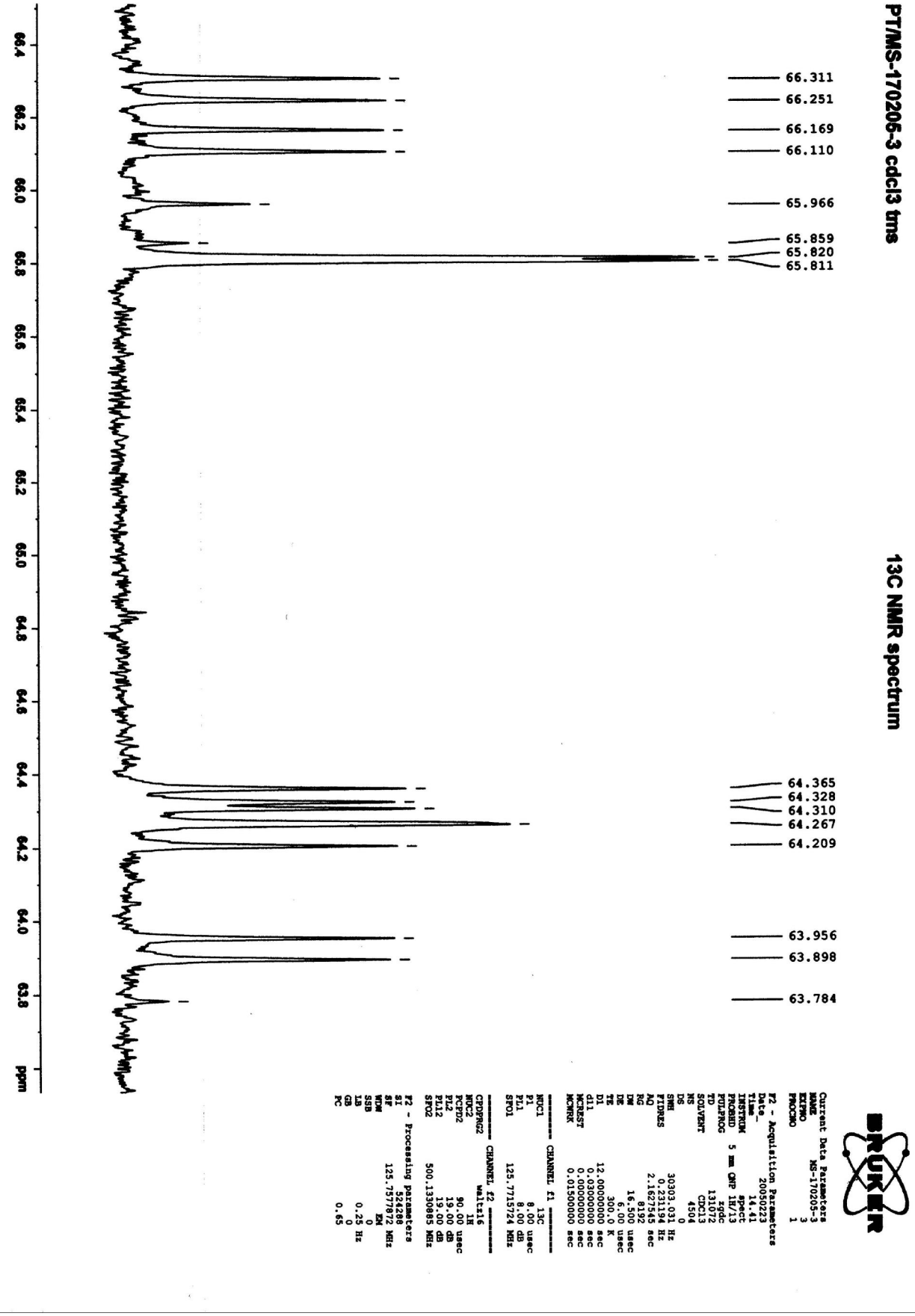
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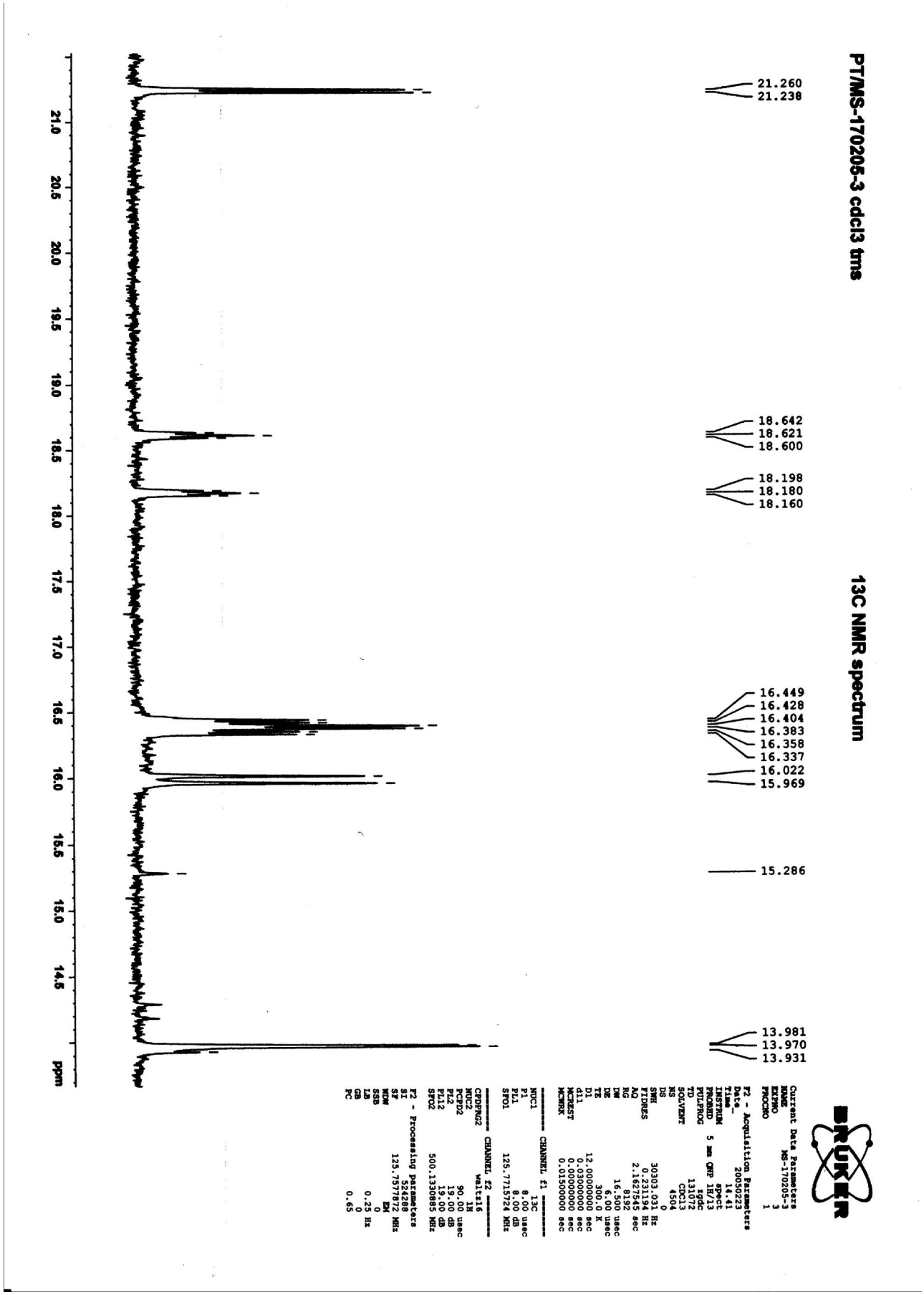
Compound **1a**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16.383
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CHANNEL f2 1H
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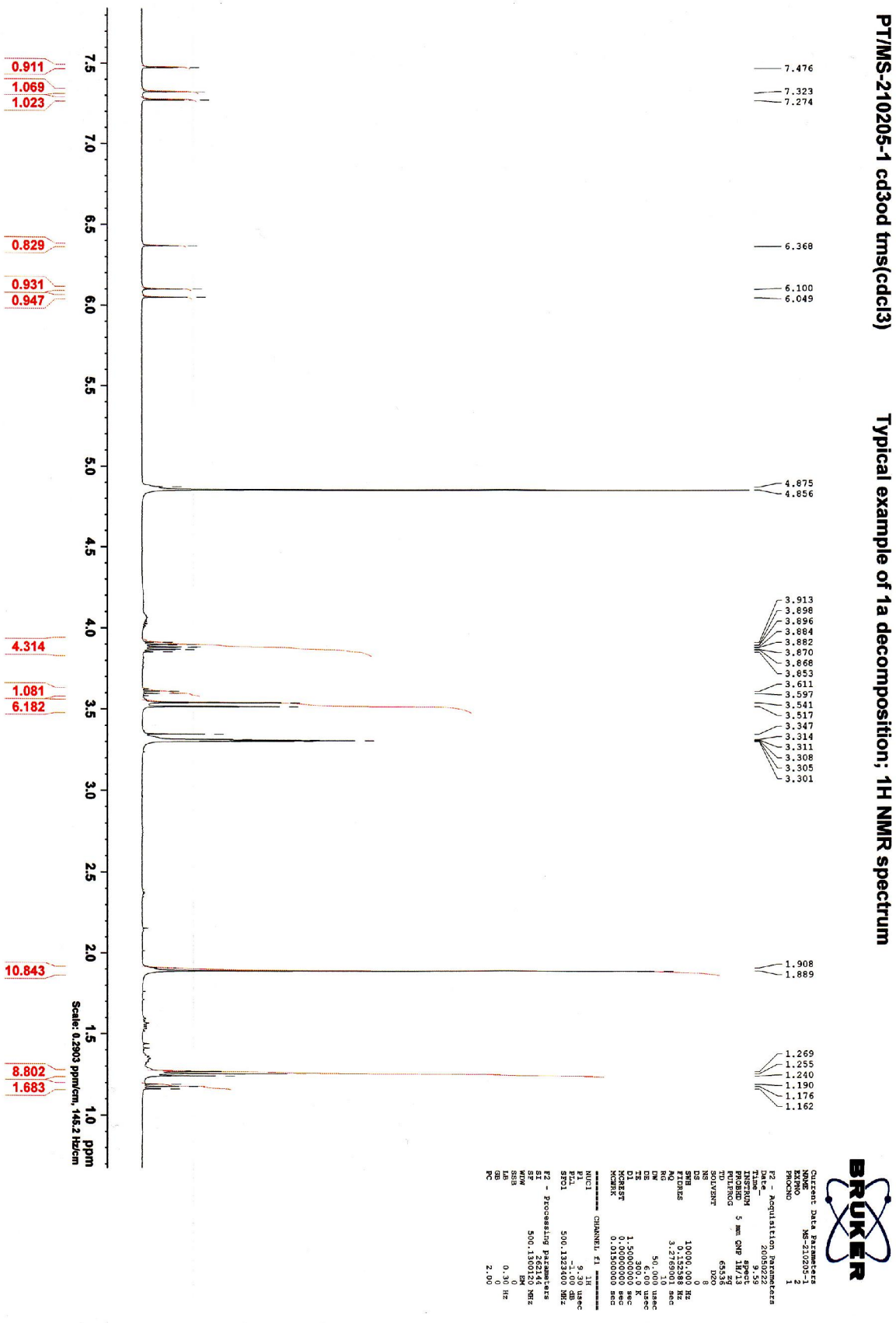
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Typical example of 1a decomposition; 1H NMR spectrum
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7.323
7.274
6.368
6.100
6.049
4.875
4.856
3.913
3.898
3.896
3.884
3.882
3.870
3.868
3.853
3.611
3.597
3.541
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3.311
3.308
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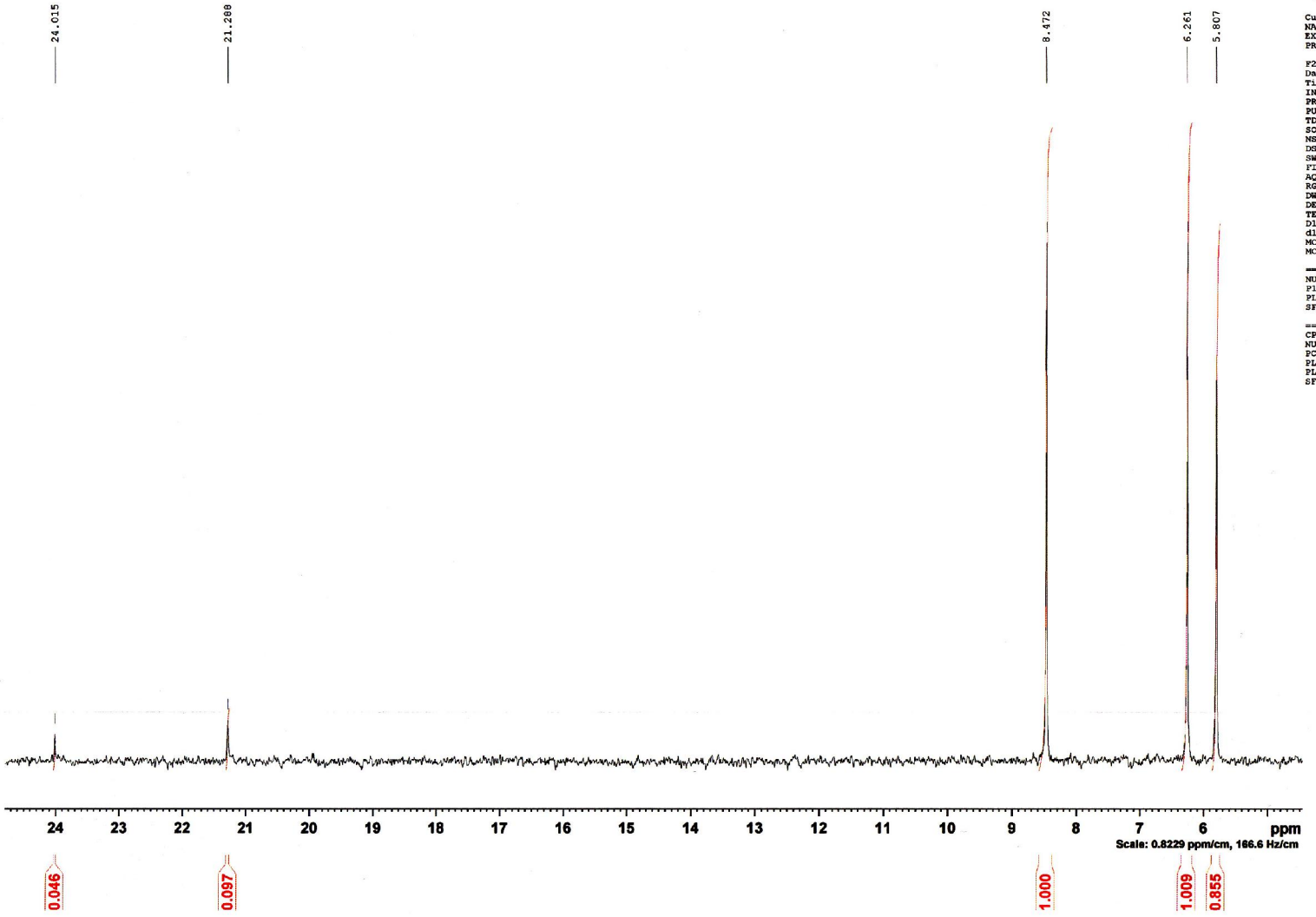
Typical example of 1a decomposition; 31P NMR spectrum



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MCWRK          0.01500000 sec

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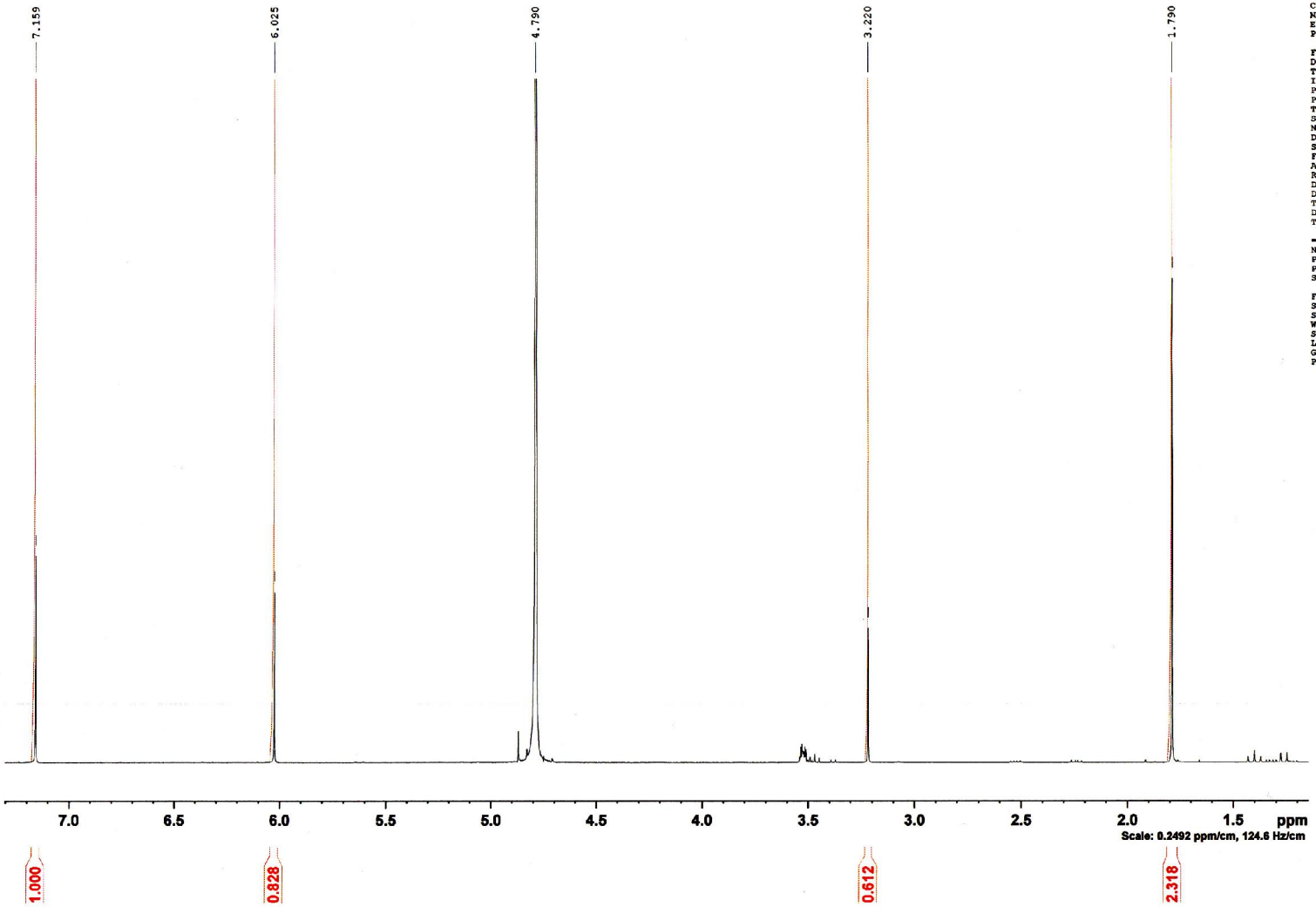

BRUKER

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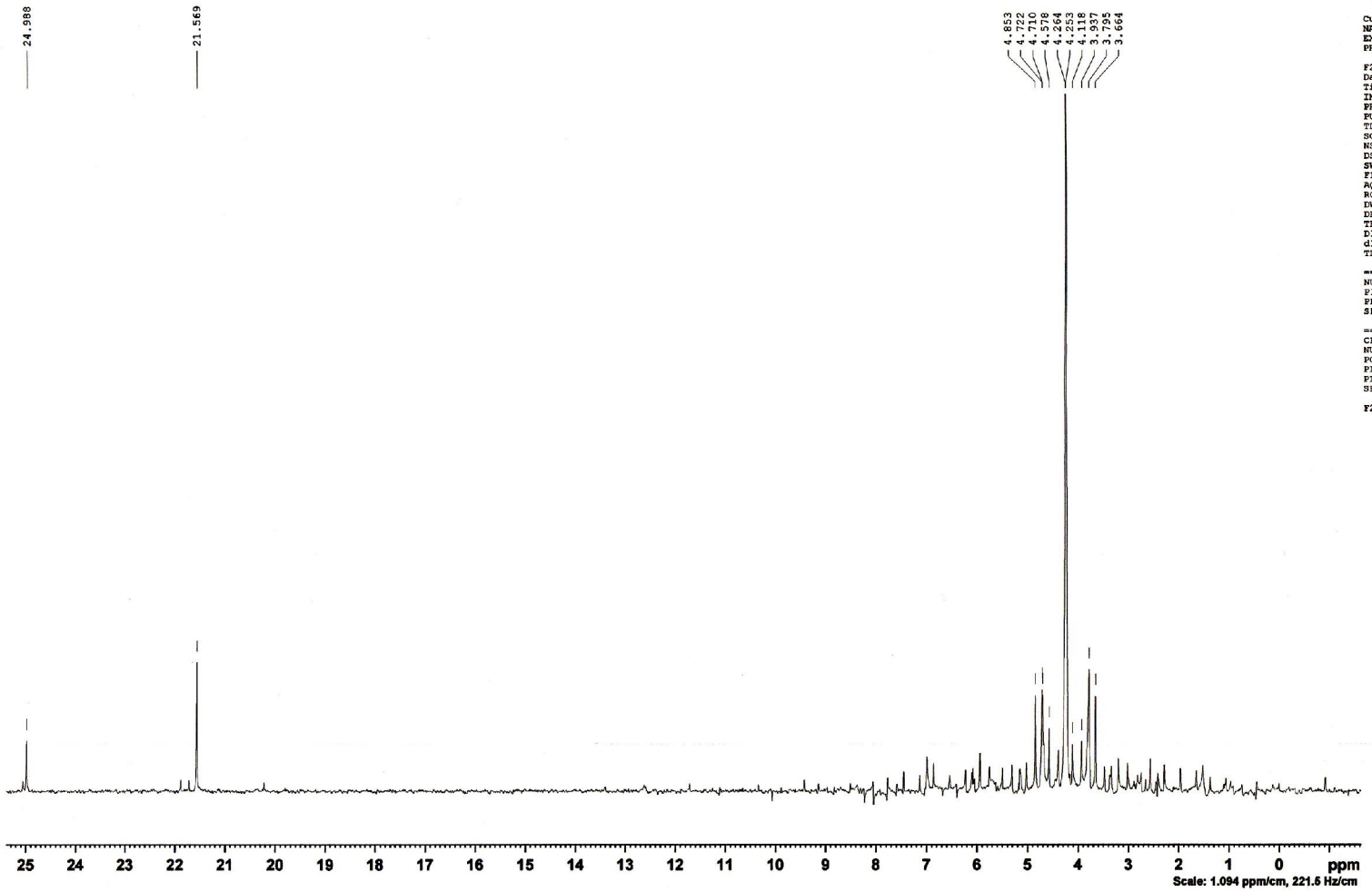
PT/MS-190906-2b d2o Typical example of 1b decomposition; 31P NMR spectrum



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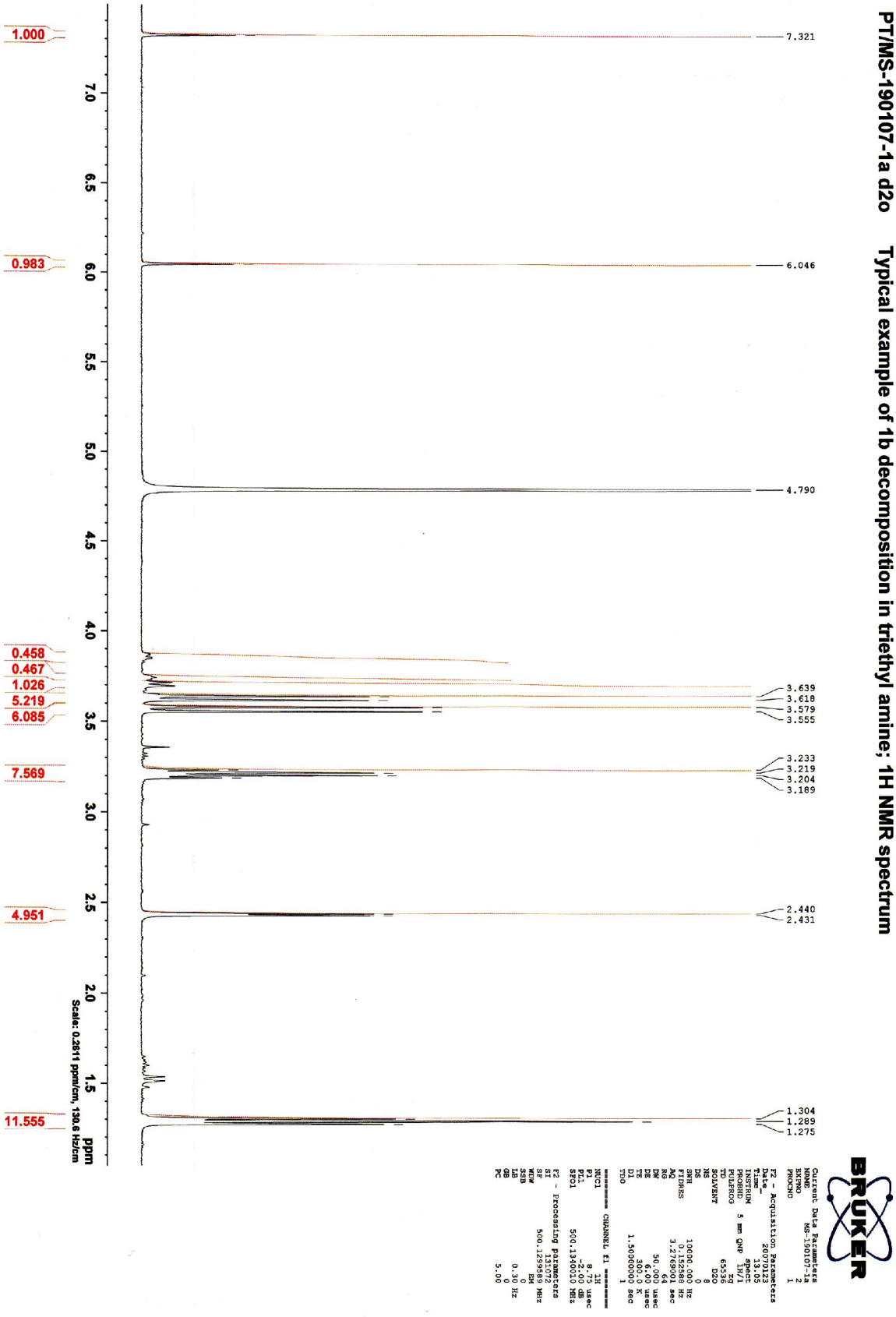
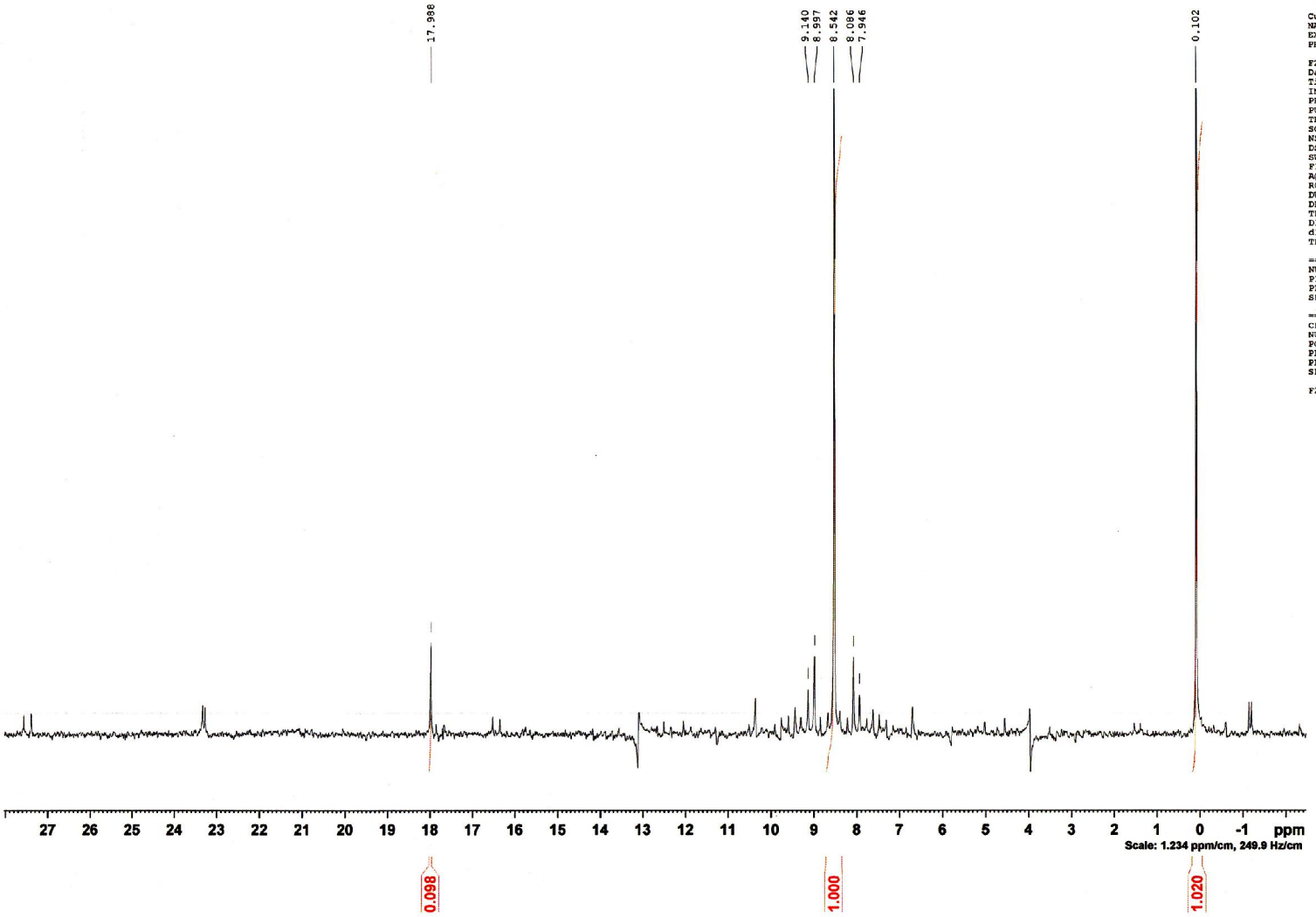
PL1               10.00 dB
SFO1        202.4586930 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             90.00 usec
PL2               24.00 dB
PL12              24.00 dB
SFO2        500.1325007 MHz

F2 - Processing parameters
```


PT/MS-190107-1a d2o Typical example of 1b decomposition in triethyl amine; 1H NMR spectrum
7.321
6.046
4.790
3.639
3.618
3.579
3.555
3.233
3.219
3.204
3.189
2.440
2.431
1.304
1.289
1.275
1.000
0.983
0.458
0.467
1.026
5.219
6.085
7.569
4.951
11.555
ppm
Scale: 0.2611 ppm/cm, 130.6 Hz/cm
BRUKER

PT/MS-190107-1a d2o **Typical example of 1b decomposition in triethyl amine; 31P NMR spectrum**

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Current Data Parameters
NAME          MS-190107-1a
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20070122
Time                 10.54
INSTRUM              spect
PROBHD   5 mm QNP  1H/1
PULPROG              zgdc
TD                  65536
SOLVENT               D2O
NS                     16
DS                      0
SWH           40650.406 Hz
FIDRES        0.620276 Hz
AQ            0.8061551 sec
RG                   2048
DW               12.300 usec
DE                6.00 usec
TE                300.1 K
D1            2.00000000 sec
d11           0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                  31P
P1                 8.50 usec
PL1               10.00 dB
SFO1        202.4586930 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             90.00 usec
PL2               24.00 dB
PL12              24.00 dB
SFO2        500.1325007 MHz

F2 - Processing parameters
```