SUPPORTING INFORMATION

An electroneutral macrocyclic iron(II) complex that enhances MRI contrast in vivo

Fayçal Touti, Akhilesh Kumar Singh, Philippe Maurin, Laurence Canaple, Olivier Beuf, Jacques Samarut, and Jens Hasserodt

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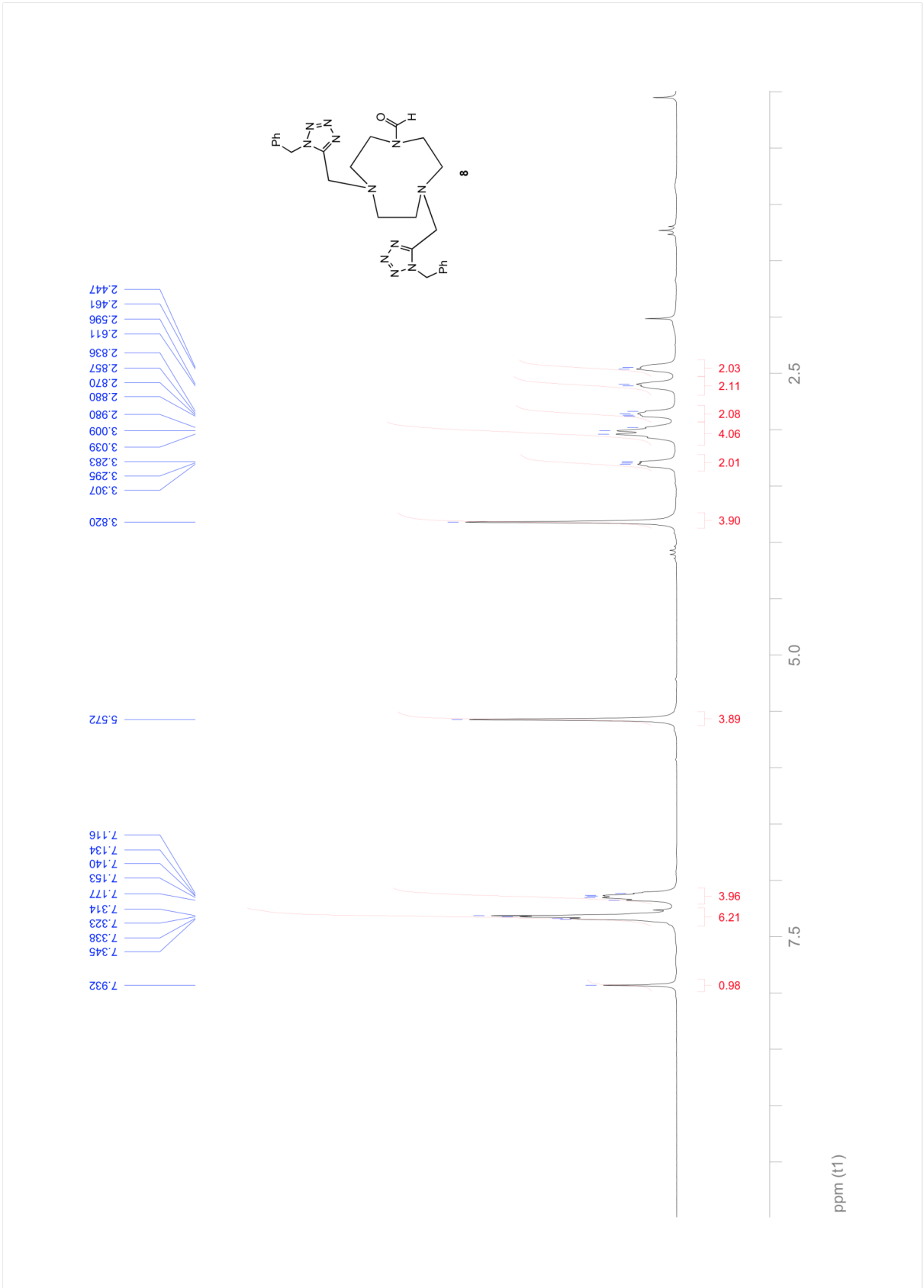
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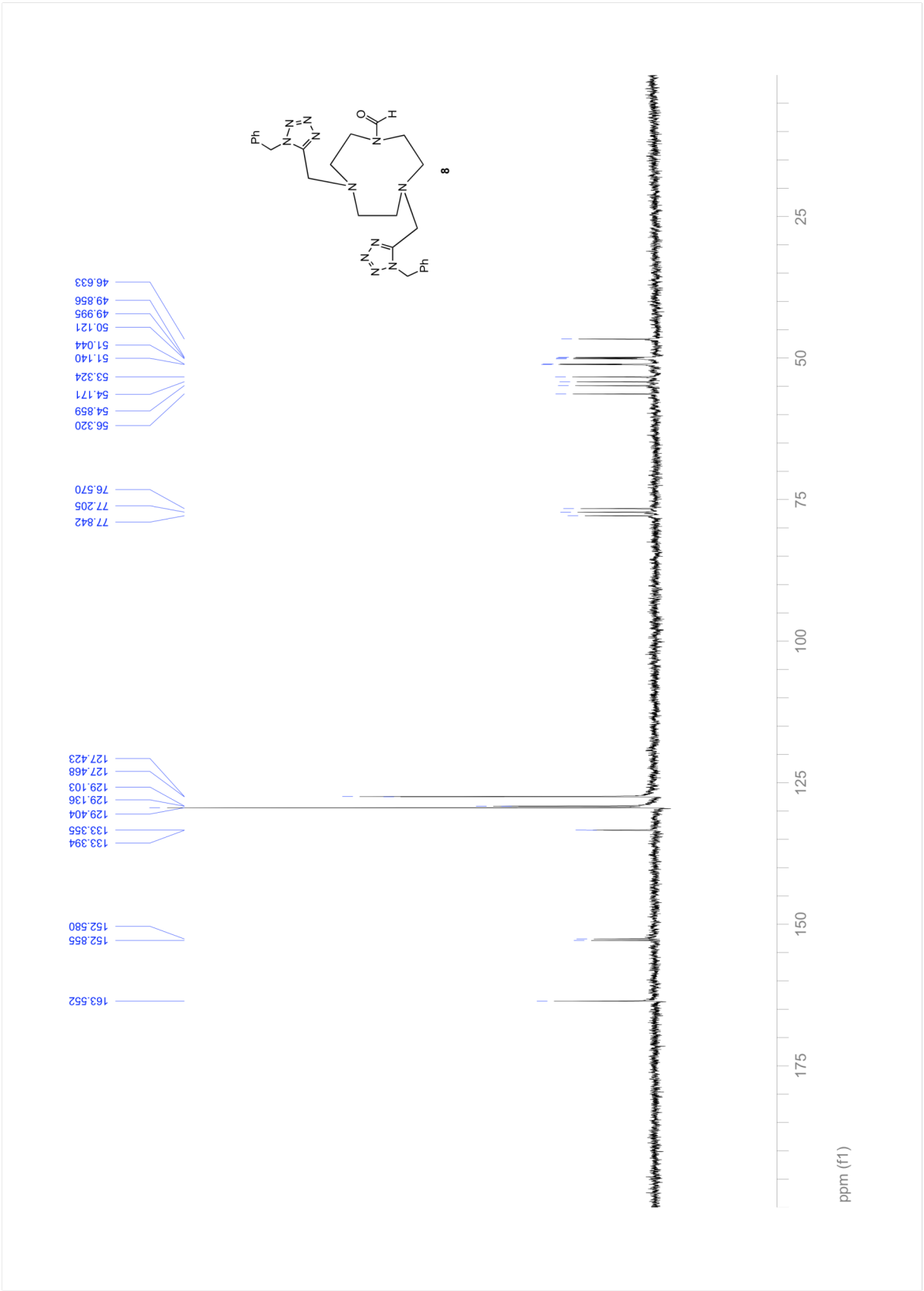
3.039
3.283
3.295
3.307
3.820
5.572
7.116
7.134
7.140
7.153
7.177
7.314
7.323
7.338
7.345
7.932
2.03
2.11
2.08
4.06
2.01
3.90
3.89
3.96
6.21
0.98
2.5
5.0
7.5
ppm (t1)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77.205
77.842
127.423
127.468
129.103
129.136
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133.394
152.580
152.855
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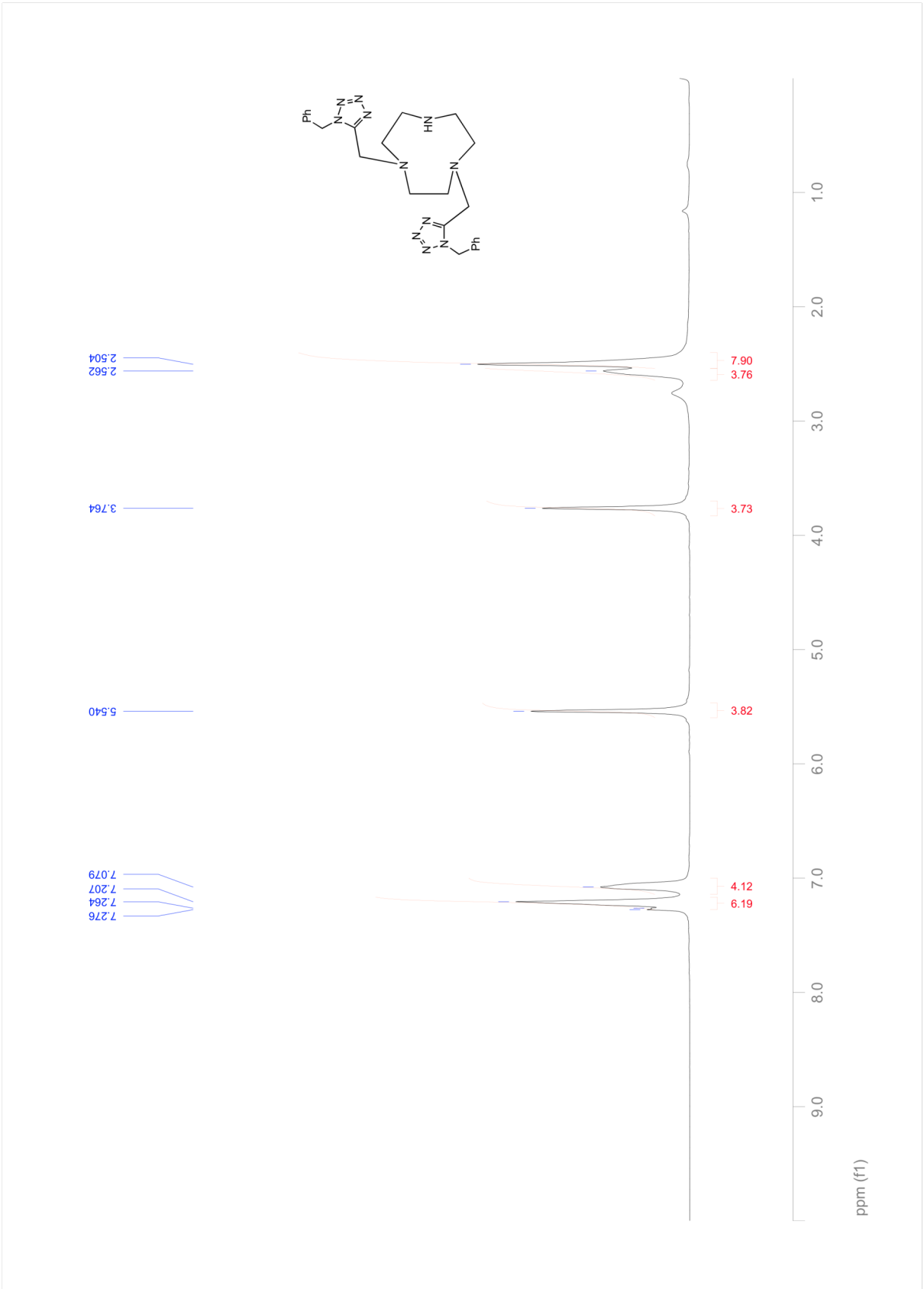
25
50
75
100
125
150
175
ppm (f1)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3.73
3.82
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2.0
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6.0
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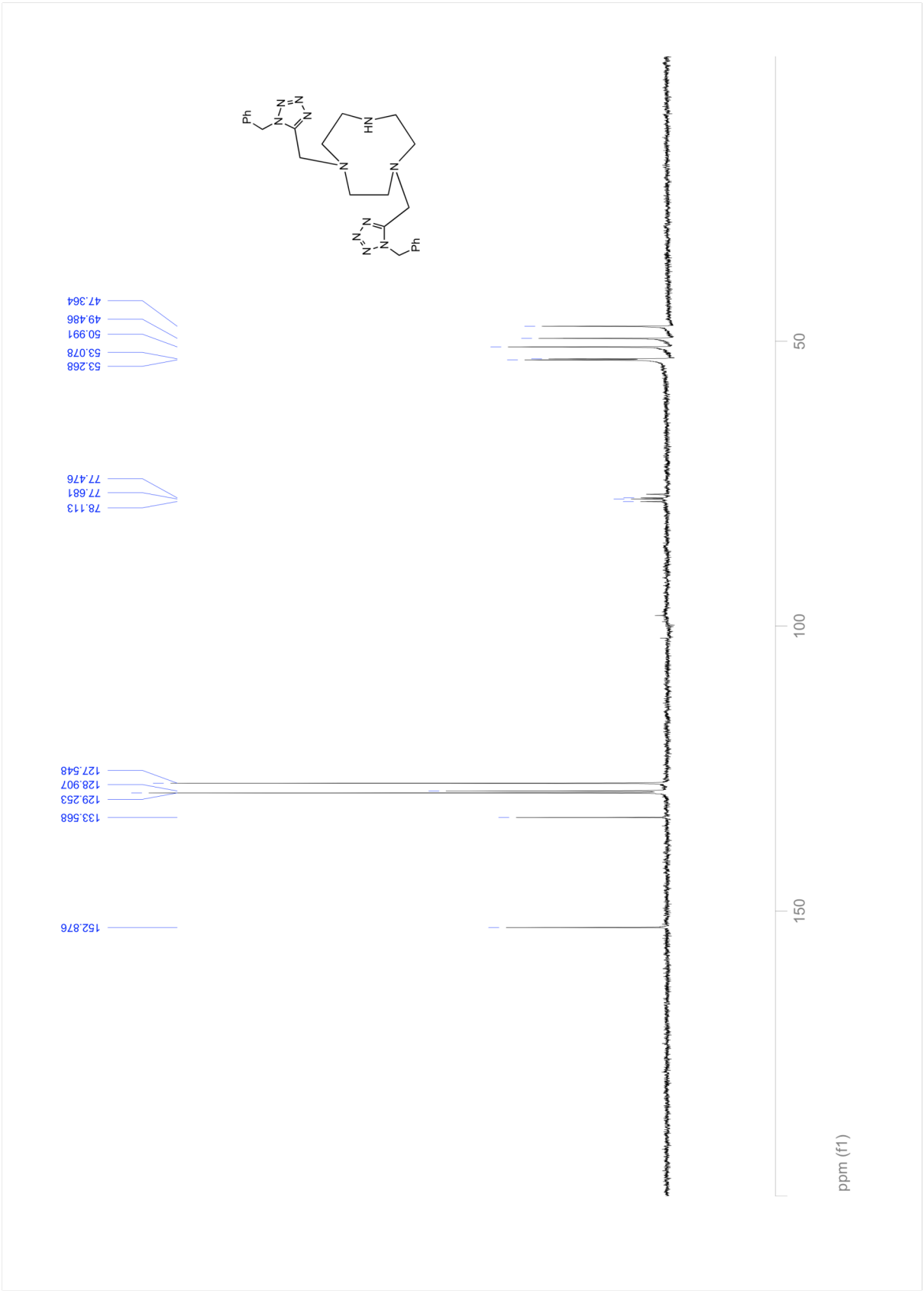
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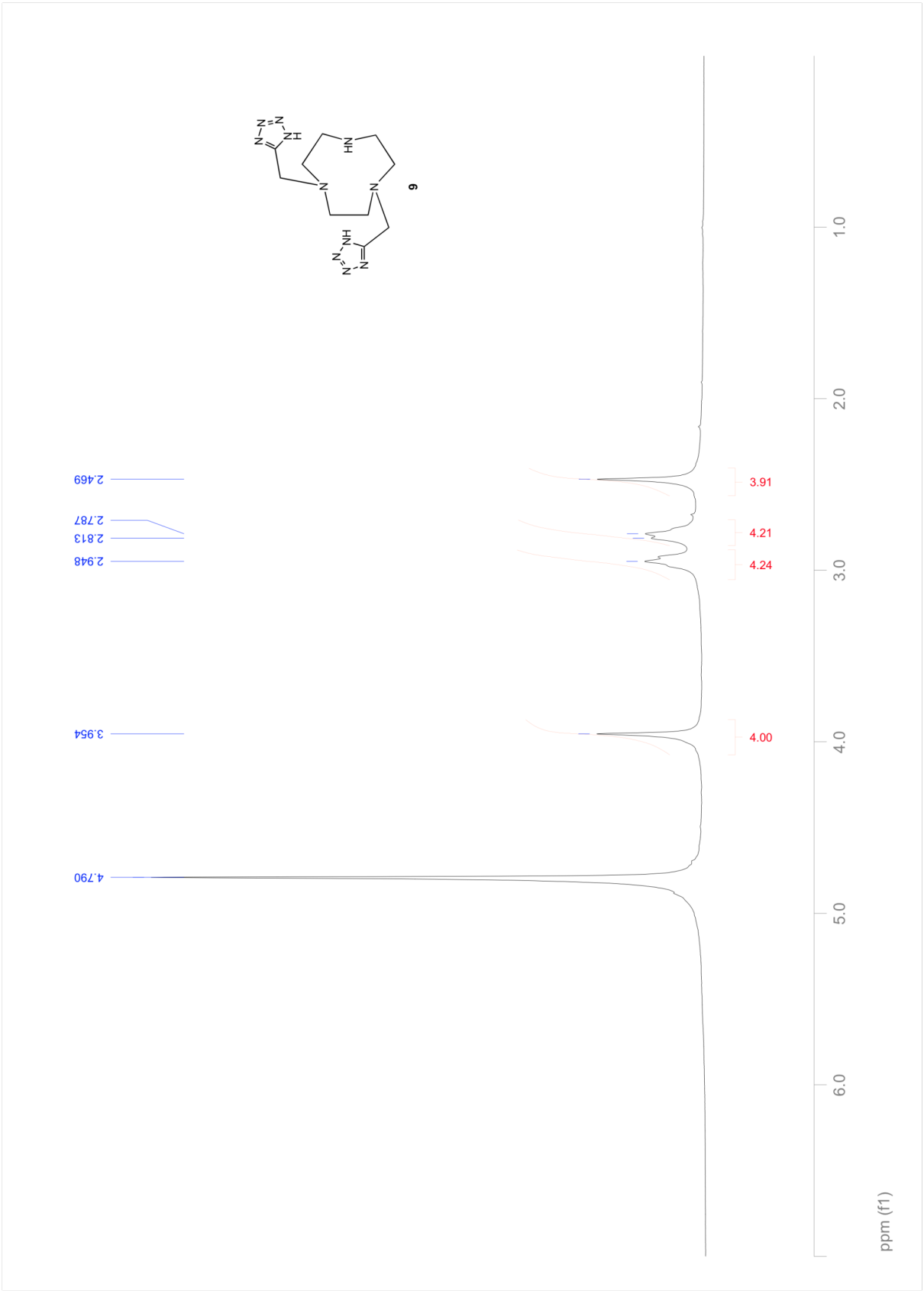
150
ppm (f1)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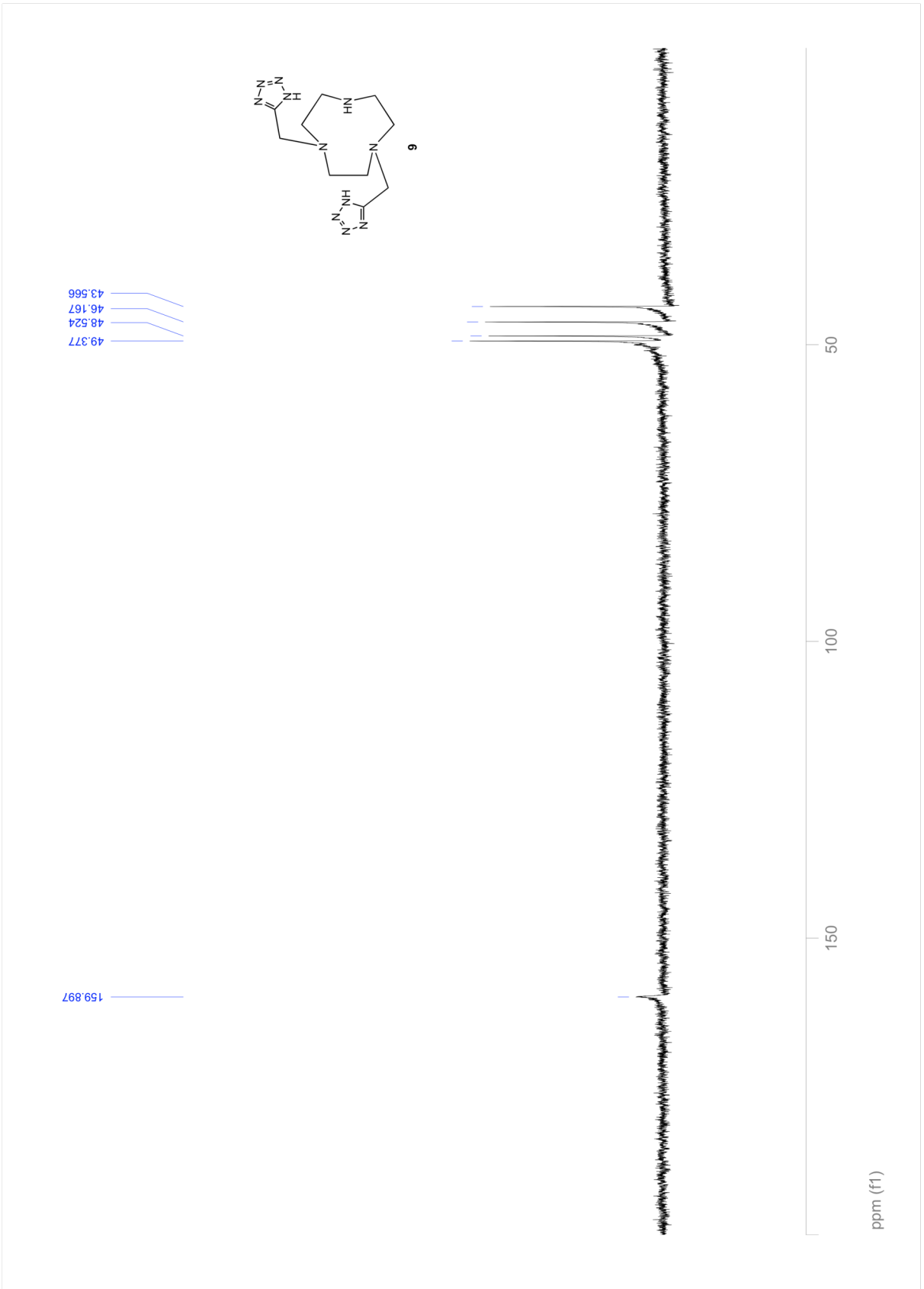
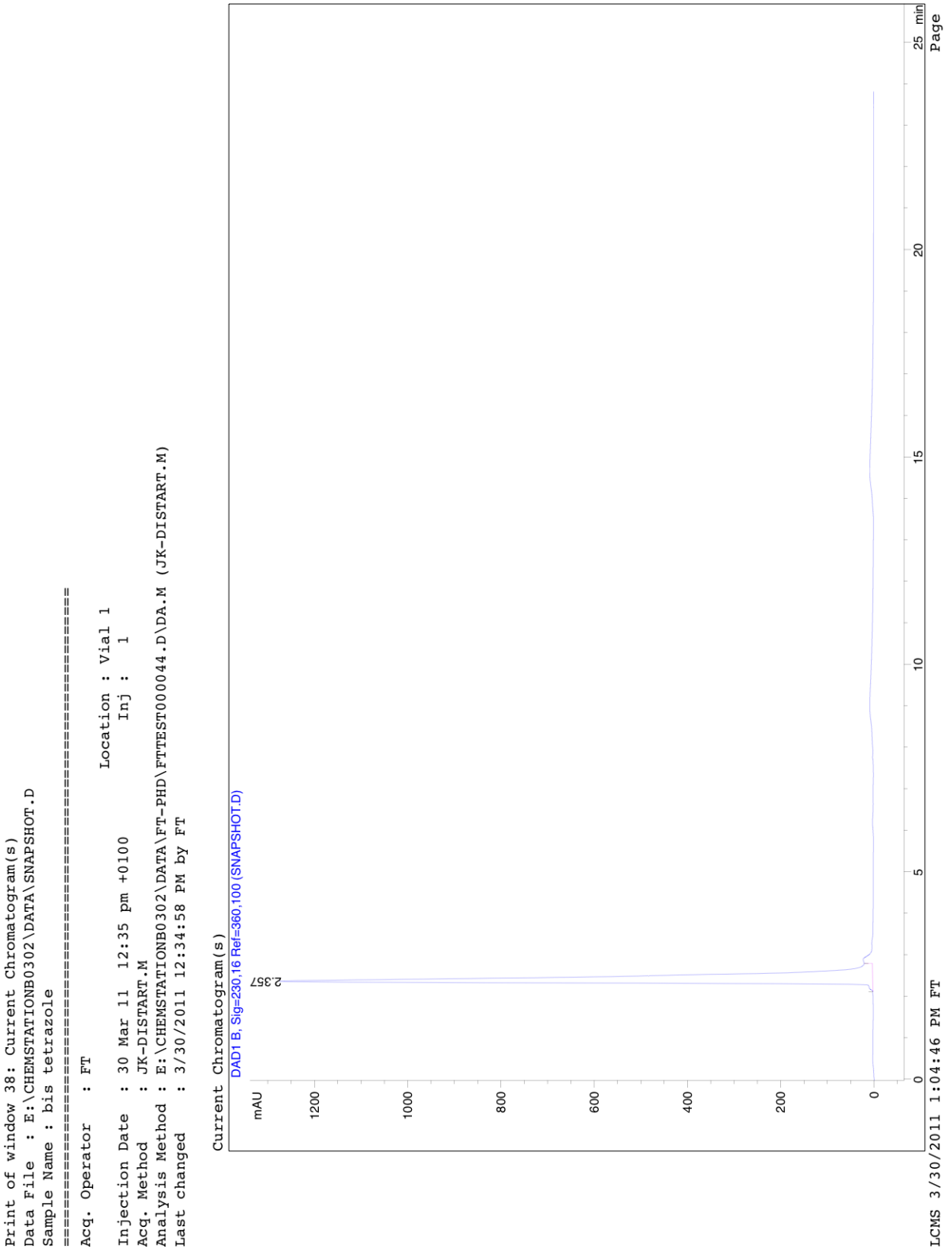
5.0
6.0
ppm (f1)


6
43.566
46.167
48.524
49.377
159.897
50
100
150
ppm (f1)

LCMS analyses of ligand **9** : (a) DAD detection; (b) mass detection by scan in positive mode; (c) mass detection by scan in negative mode. Chromatographic conditions :

Column : Zorbax Eclipse XDB-C8, 3.5µ, 150 x 3.00 mm.
Mobile phase : A = 0.25 mM ammonium formate in water; B = Acetonitrile
Isocratic: 90% A
Flow Rate: 0.5 mL/mn
Column temp: 25°C
(a) DAD: 230.16 nm, reference = 360. Ligand **9** exhibits very weak absorbance intensity.



(b) Mass analysis : API-ESI, scan mode / positive mode
Chemical formula: $C_{10}H_{10}N_{11}$ (293,18) : M+1 = 294.2 (observed); 2M +1 : 587.3 (observed)

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Injection Date  : 30 Mar 11  12:35 pm +0100          Inj :   1
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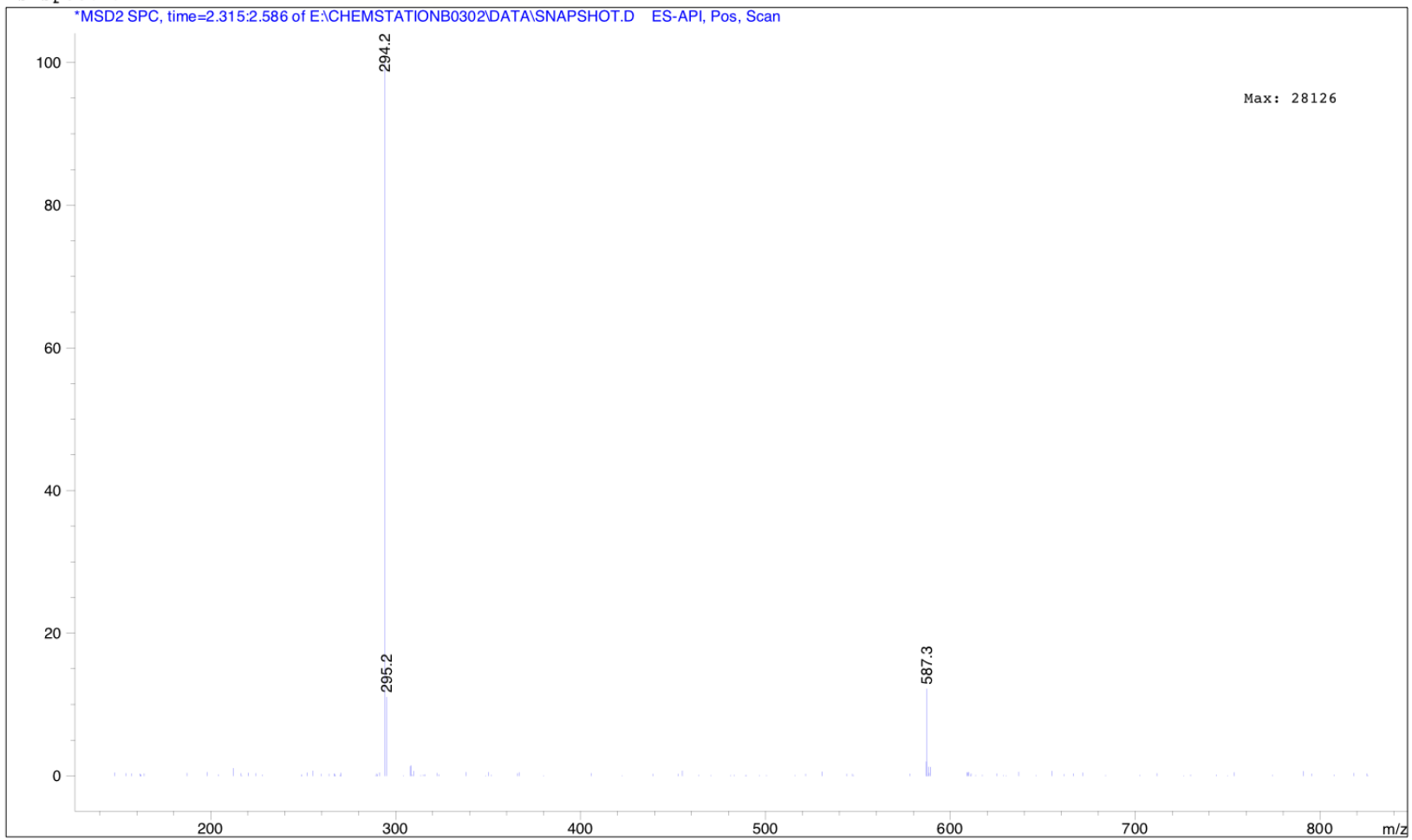
MS Spectrum



LCMS 3/30/2011 1:14:57 PM FT Page

(c) Mass analysis : API-ESI, scan mode / negative mode
Chemical formula: $C_{10}H_{10}N_{11}$ (293,18) : M-1 = 292.3 (observed); 2M -1 : 585.3 (observed)

```
Print of window 80: MS Spectrum
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Acq. Instrument : LCMS                          Location : Vial 1
Injection Date  : 3/30/2011 12:35:51 PM              Inj :   1
                                                Inj Volume : 2 µl
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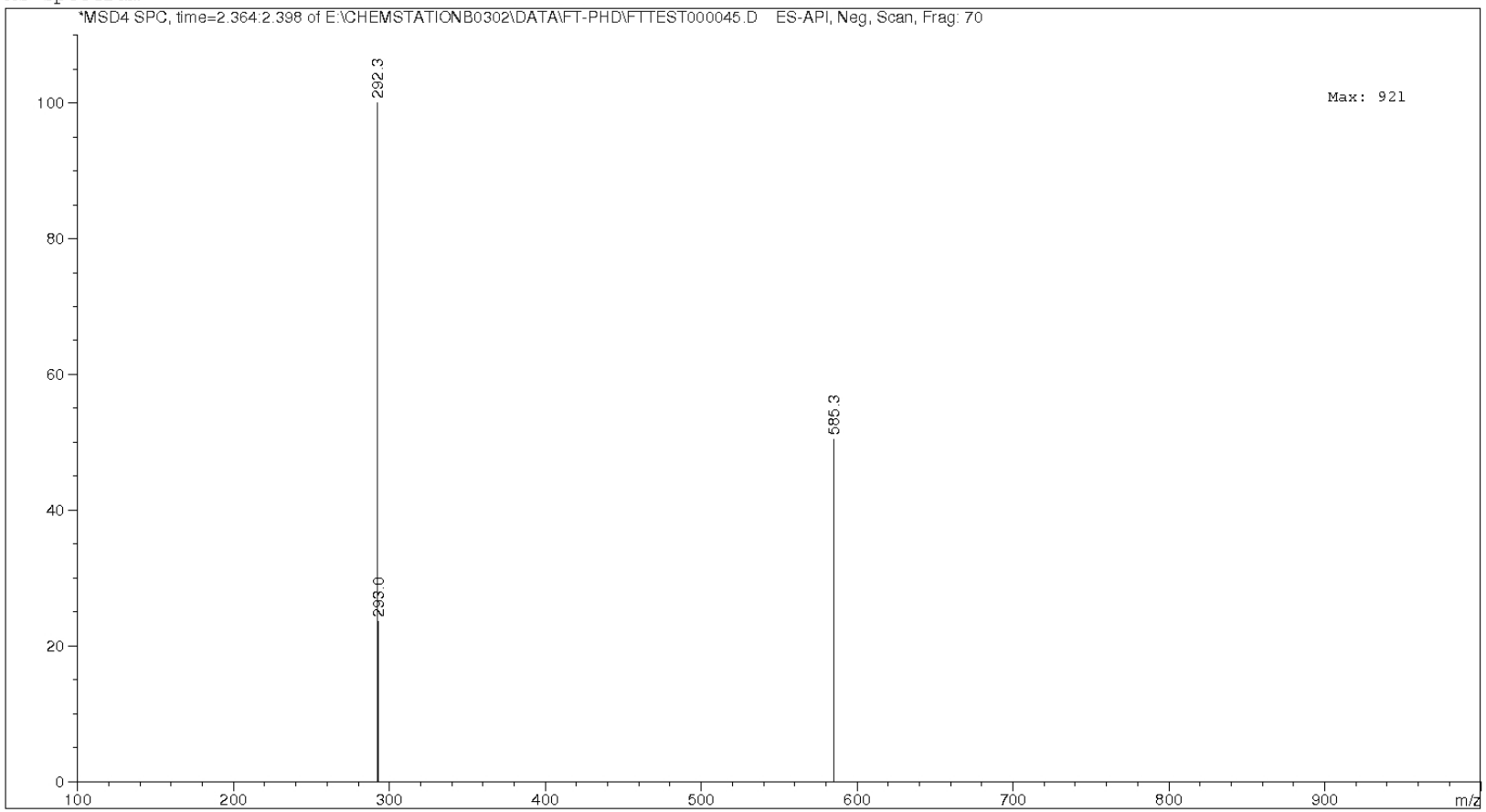
MS Spectrum



LCMS 4/4/2011 12:57:40 PM FT Page

LCMS analyses of complex **1** : (a) DAD detection; (b) mass detection by Selected Ion Monitoring (SIM) in positive mode :

Column : Zorbax Eclipse XDB-C8, 3.5µ, 150 x 3.00 mm.

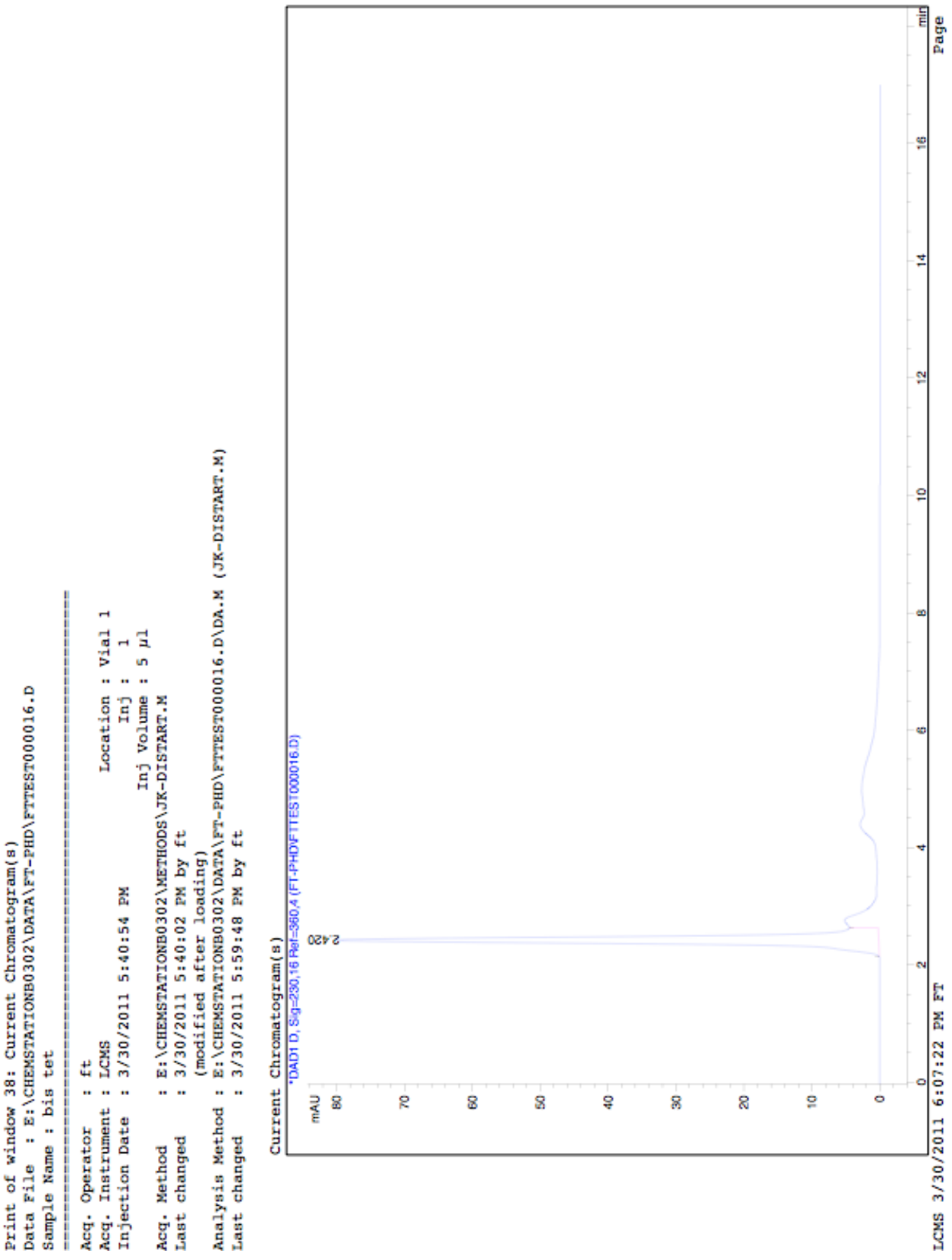
Mobile phase : A = 0.25 mM ammonium formate in water; B = acetonitrile

Isocratic: 90% A

Flow Rate: 0.5 mL/mn

Column temp: 25°C

(a) DAD: 230.16 nm, reference = 360. Complex **1** exhibits even weaker absorbance intensity compared to ligand **9**.



(b) Mass analysis : API-ESI, SIM mode / positive mode
Chemical formula: $C_{10}H_{17}FeN_{11}$ (347,16) ; M+1 = 348.1 (observed).

```
Print of window 80: MS Spectrum
Data File  : E:\CHEMSTATIONB0302\DATA\FT-PHD\FTTEST000016.D
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=====================================================================
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Acq. Instrument : LCMS                          Location : Vial 1
Injection Date  : 3/30/2011 5:40:54 PM               Inj :   1
                                                Inj Volume : 5 µl
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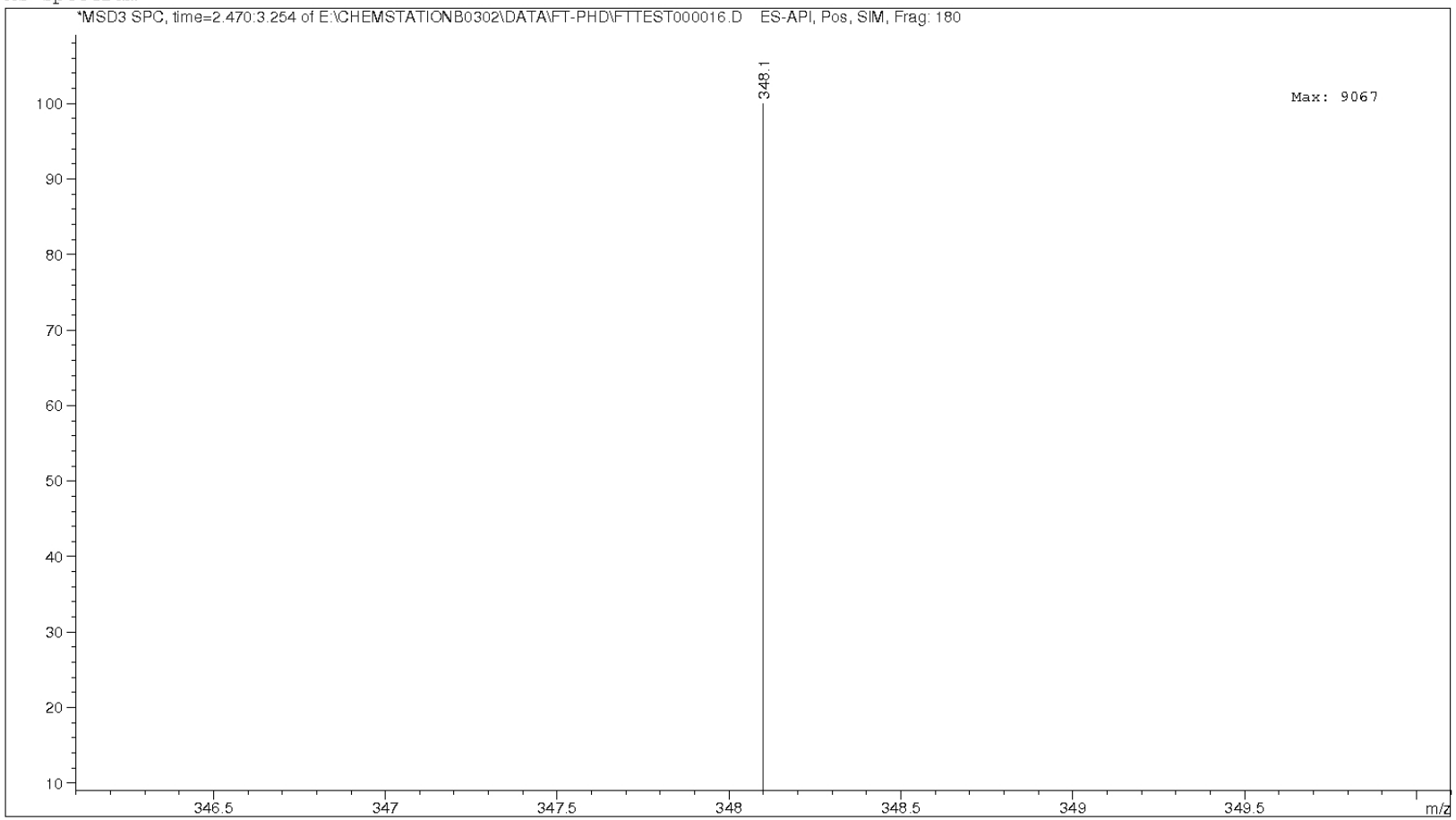
MS Spectrum



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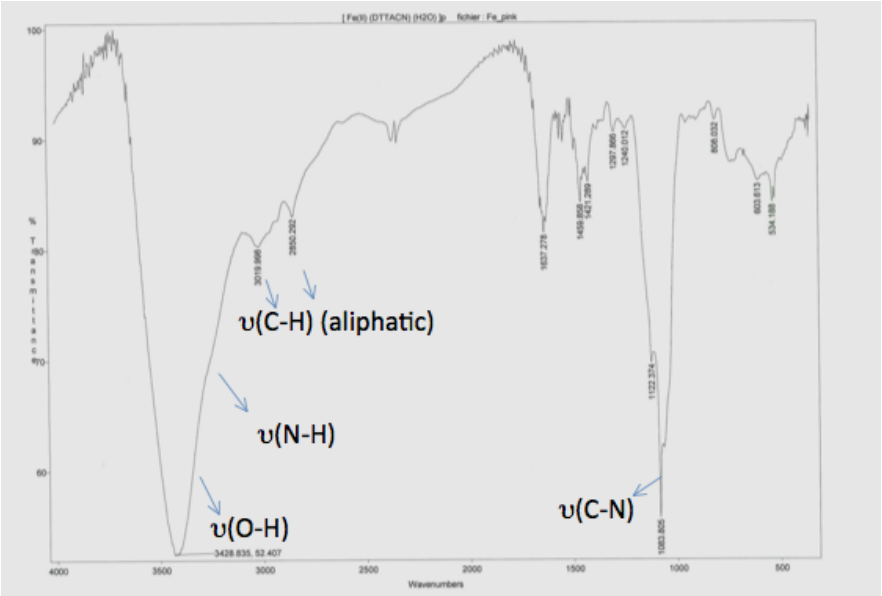
Infrared spectrum of complex **1** (KBr pellet)



Injection/electroporation protocol

Electroporation conditions : 200 V/cm, 8 pulses of 20 ms with 500 ms intervals.

All animal studies were performed in the Plateau de Biologie Expérimentale de la Souris (N° A 69 123 0303, PBES, ENS Lyon) under animal care procedures, conducted in accordance with the institutional guidelines set by the European Community Council Directives (86/609/EEC) and approved by the local ethical committee.

MRI

MR images were performed on a 7T Biospec system (Bruker Biospin, Ettlingen, Germany) using a transmit/receive 32 mm inner diameter quadrature birdcage coil (Rapid Biomedical, Würzburg, Germany).

T_1 value quantification

The longitudinal relaxation time T_1 was measured with an Inversion Recovery Fast Imaging with Steady state Precession (IR-FISP) sequence. Imaging parameters were: TR/TE = 4.2/2.1 ms, inversion times TI = (68 + i x 50) ms where i = 0, 1, …, 32. Geometric parameters were: 40 x 40 mm2 field of view (FOV), 3 mm slice thickness and 256 x 192 matrix size. T_1 values were obtained from multiple T_I images by fitting a mono-exponential function to the data.

In vivo MRI

Anesthesia was conducted with a dedicated gas anesthesia system (TEM, Bordeaux, France). Mice were first placed in an induction box with air mixed with 3 % of isoflurane gas (Nicholas Piramal limited, BLondon, UK) administrated at 0.8°L/min flow. The animals were then placed in a supine position on a plastic bed with the mouse nose placed in a face cone mask delivering the anesthetic gas (2 % isoflurane with air at 0.6 L/min flow). Circulating warm water located above the mouse was used to regulate the body temperature at 37 ± 1°C. The respiratory cycle was monitored using an air pillow connected to a Trigger Unit HSB-T (Rapid Biomedical, Würzburg, Germany).

T2-weighted turbo spin echo and a T_1-weighted spin echo sequences were systematically performed at the same location both in coronal and transverse orientation. The T2-weighted images were acquired with TR/TE 3488/55 ms; a turbo factor of eight and a number of experiments (NEX) of 2. The T_1-weighted images were acquired with TR/TE 500/12 ms and 4 NEX. For all these scans, FOV was 30 x 30 mm^2, the slice thickness was 0.8 mm and the acquisition matrix size was 256 x 192. A zero-filling was performed at reconstrution to obtain a 512 x512 matrix size.

Additionnaly, a three-dimensional (3D) Fast Low Angle Shot (FLASH) sequence was used with the following parameters: 45° flip angle, TR/TE 30/2.2 ms, 69 kHz receiver bandwidth (rbw) and 2 NEX. A 30 x 30 x 20 mm^3 FOV was acquired with a matrix size of 256 x 192 x 48. Acquisition volume was reconstructed to a 512 x 512 x 64 matrix.