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Supporting Information

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Magnetogenesis under Physiological Conditions with Probes that Report on (Bio-)Chemical Stimuli**

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anie_201208848_sm_miscellaneous_information.pdf

Synthesis

General notes

Acetonitrile (HPLC grade) and benzene were dried for 24 h on thermally-activated molecular sieves (3Å, sieve activation by 24 h heating at 315°C).. Unless stated otherwise, all reactions were performed with unpurified, undried, non-degassed solvents and analytical grade reagents, used as obtained, under a protective atmosphere of argon. Yields were optimized for compound **1** and the synthesis was started from dissymmetric dptacn (Stavila, V., et al. (2008). *New J Chem*, *32*, 428–435). All compounds are characterized by ^{1}H-NMR spectra (see annex below). All spectra were acquired on a Bruker AVANCE (500 MHz) as indicated, at 293 K. All HPLC analyses of complexes reported below were executed using the following chromatographic conditions: column : Zorbax Eclipse XDB-C8, 3.5 µm, 3.5 x 150 mm. Mobile phase: isocratic 20% A and 80% B; A = 0.10 mM ammonium formate in water; B = acetonitrile. Flow Rate: 0.45 mL/min. Column temp. : 25°C
Unit mass measurements were performed on an "AGILENT 1100 SL" LCMS system with direct injection of the sample in ESI mode.

Synthetic protocols for complexes **1**, **2**, **5**, **6**, and **7**.

Complex 1:

[Iron(II) (1-((4-nitrophenyl)(2*H*-1,2,3-triazol-2-yl)methyl)-4,7-bis(pyridin-2-ylmethyl)-1,4,7-triazacyclononane] $(BF_4)_2$

A single-necked flask (50 mL) containing a solution of 0.8 g of freshly synthesized dptacn (?) in benzene (25 mL) is treated with 0.38 g (1.5 eq) of p-nitrobenzaldehyde and 0.145 mL (1.5 eq) of 1,2,3-triazol. A Dean-Stark apparatus with an additional narrow siphon installed below the habitual barrier between the flask neck and the reservoir is placed on the flask before the contents are refluxed for 18 h. The mixture is placed under argon and cooled to room temperature. An anhydrous acetonitrile solution of ([$Fe(BF_4)_2$ 6 MeCN] is now added in a titration-like manner, and the formation of complex **1** monitored by LCMS. The resulting solution is stirred for another 15 min. before being filtered over dry paper filter. The filtrate is concentrated under vacuum and the residue dissolved in a minimal volume of water/MeCN (95/5 v/v). This solution is applied to a reversed-phase cartridge (C18; 20 mL of immobile phase) that has been pre-conditionned with two cartridge volumes of MeCN followed by two volumes of water. After elution with pure water (250 mL) a gradient of water / MeCN (99/1 to 90/10, v/v) is applied that results in the collection of several intensely red fractions. These fractions are analysed by LCMS and the ones corresponding to **1** united. Evaporation of the solvents yields a red resin that is taken up in MeCN and recrystallized by ether diffusion at 4°C in pyrex tubes (50 mL). After several days burgundy-red crystals are harvested to yield 0.55 g (29 %) of a micro-crystalline powder.

The same procedure has been applied for all other complexes.

Complex 2:

[Iron(II) ((1-((4-phenylacetamidophenyl)(2*H*-1,2,3-triazol-2-yl)methyl)-4,7-bis(pyridin-2-ylmethyl)-1,4,7-triazacyclononane)] $(BF_4)_2$

Starting material N-(4-Formylphenyl)-2-phenylacetamide was synthesized according to a literature procedure in high yields (S. A. Nunez et al., *J. Org. Chem*. 2011, 76, 10099−10113).

0.21 g (19%) of red powder, compound was purified to homogeneity by RP chromatography on a C18 cartridge.

Complex 5: [Iron(II) (1-((4-nitrophenyl)(1*H*-pyrazol-1-yl)methyl)-4,7-bis(pyridin-2-ylmethyl)-1,4,7-triazacyclononane] $(BF_4)_2$

0.110 (9%) of burgundy-red crystals.

Complex 6: [Iron(II) ((1-((4-phenylacetamidophenyl)(1*H*-pyrazol-1-yl)methyl)-4,7-bis(pyridin-2-ylmethyl)-1,4,7-triazacyclononane)] $(BF_4)_2$.

0.163 g (12%) of burgundy-red crystals. Here, wet, commercial quality of $Fe(BF_4)_2 \bullet 6H_2O$ sufficed.

Complex 7: [Iron(II) (1-((4-nitrophenyl)(1*H*-1,2,3-benzotriazol-1-yl)methyl)-4,7-bis(pyridin-2-ylmethyl)-1,4,7-triazacyclononane] $(BF_4)_2$

0.282 g (25%, homogeneous quality) of red powder.

Compound Characterization

Structural Analyses by X-ray diffraction

Figure S1 : Grown crystals of ferrous complex **1**

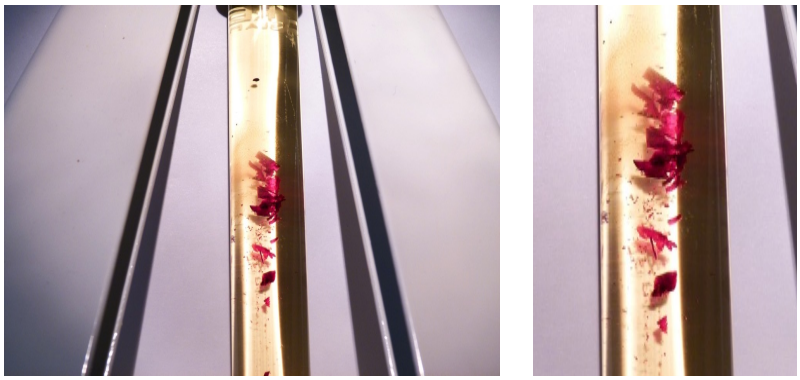
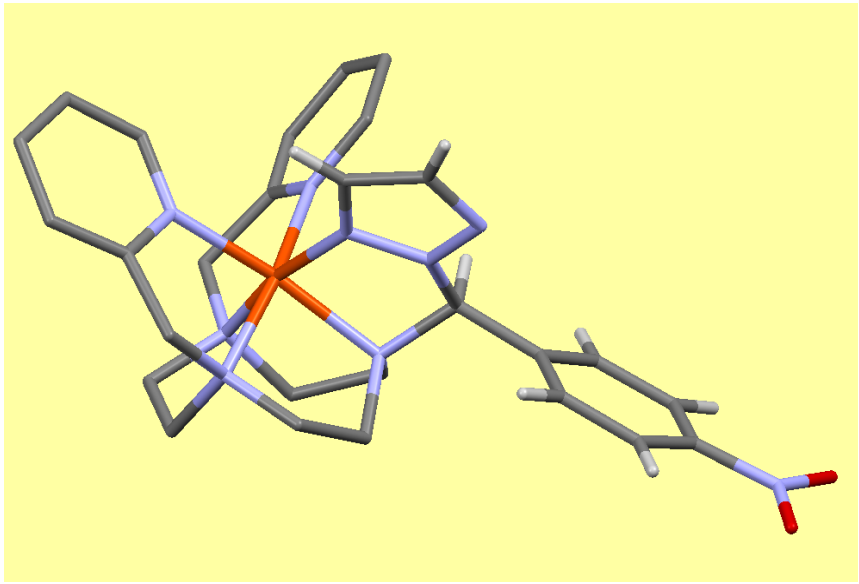


Figure S2 : X-ray structural analysis of **1**

(macrocycle and picolyl hydrogens omitted for clarity)



Summary of data CCDC 902789

Formula: C31 H33 B2 F8 Fe1 N9 O2
Unit cell parameters: a 10.599(2) b 16.1450(10) c 20.264(2)
beta 100.520(10)
space group P21/c

Figure S3 : Grown crystals of ferrous complex **5**.

Crystals grown by diethylether diffusion into acetonitrile solution; macrocycle and picolyl hydrogens omitted for clarity.

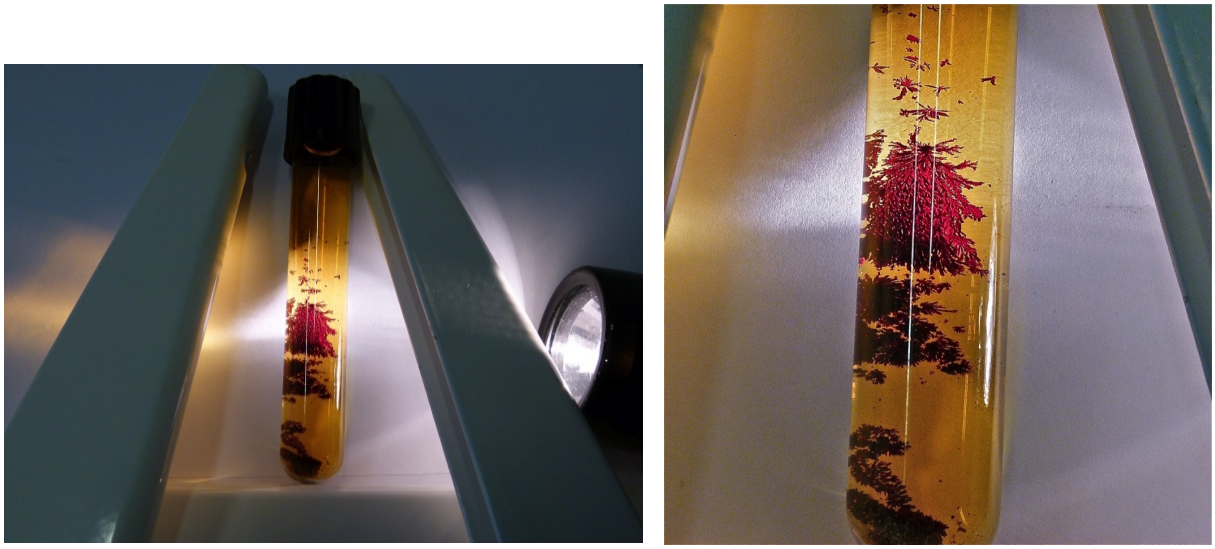
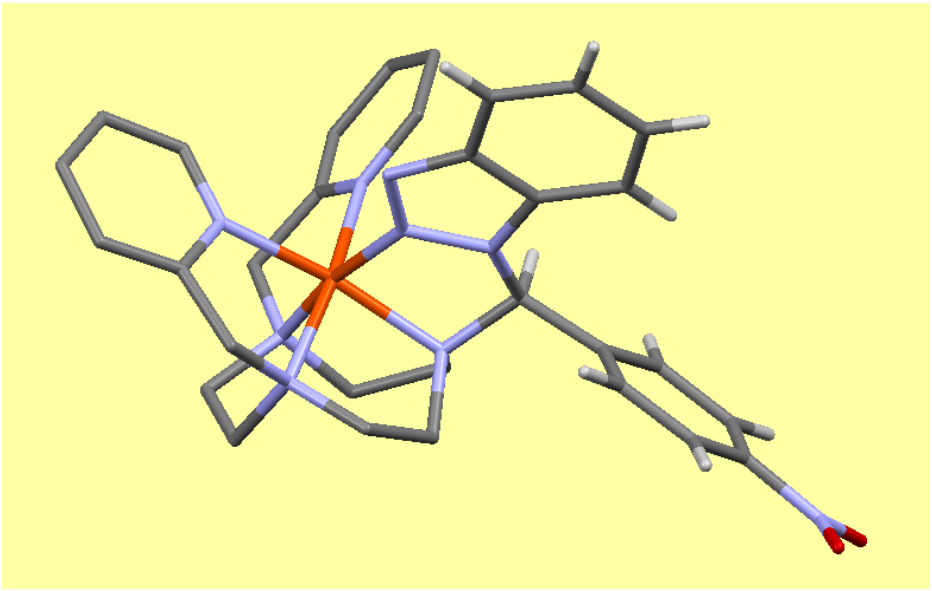


Figure S4 : X-ray structural analysis of 5

(macrocycle and picolyl hydrogens omitted for clarity)



Summary of data CCDC 902790

Formula: C31 H30 B2 F8 Fe1 N9 O2

Unit cell parameters: a 9.1444(6) b 10.5370(7) c 20.1930(10)
alpha 78.459(5) beta 89.809(5) gamma 66.744(6)
space group P-1

Figure S5 : Grown crystals of ferrous complex 6

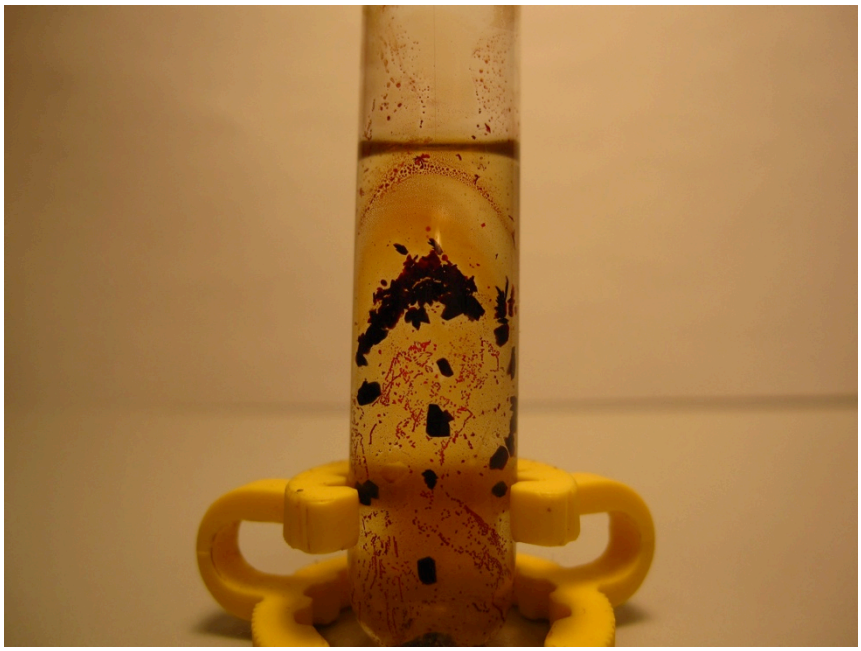
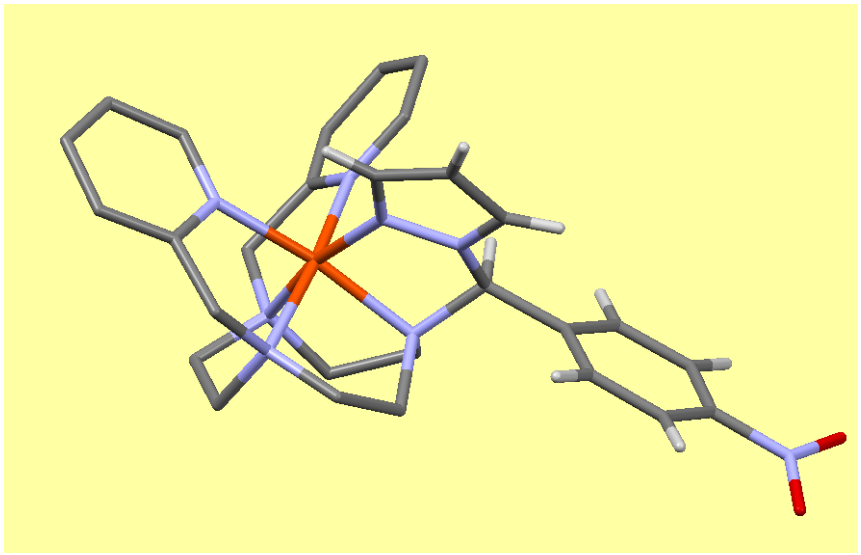


Figure S6 : X-ray structural analysis of 6

(macrocycle and picolyl hydrogens omitted for clarity)



Summary of data CCDC 902791

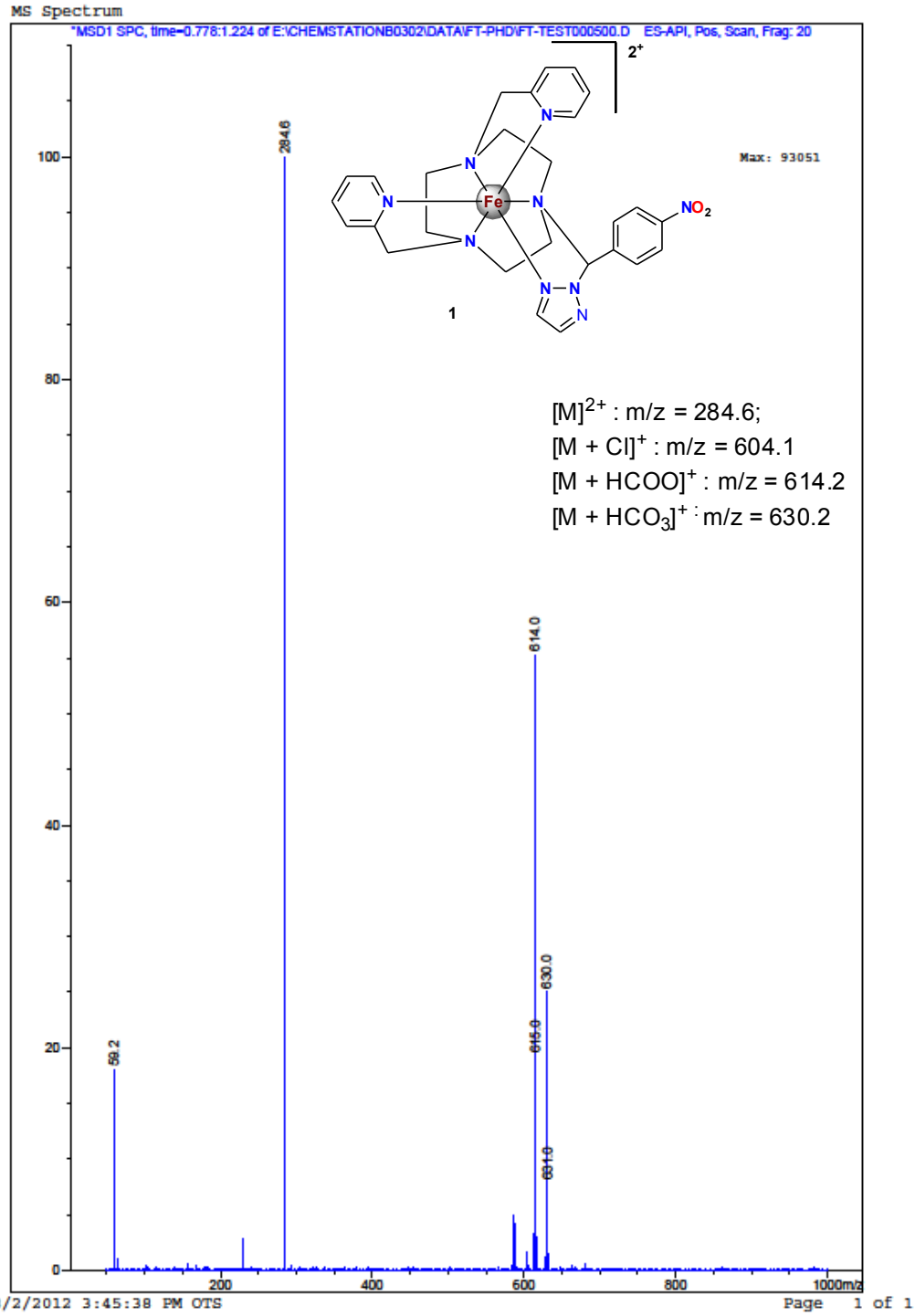
Formula: C32 H38 Cl2 Fe1 N10 O10

Unit cell parameters: a 12.7583(7) b 12.7820(8) c 13.7071(6)
alpha 65.170(5) beta 76.373(4) gamma 61.484(6)
space group P-1

Mass spectral characterization of complexes 1, 2, 5, 6, and 7.

Mass analysis complex **1** (dissolved crystals)

Print of window 80: MS Spectrum



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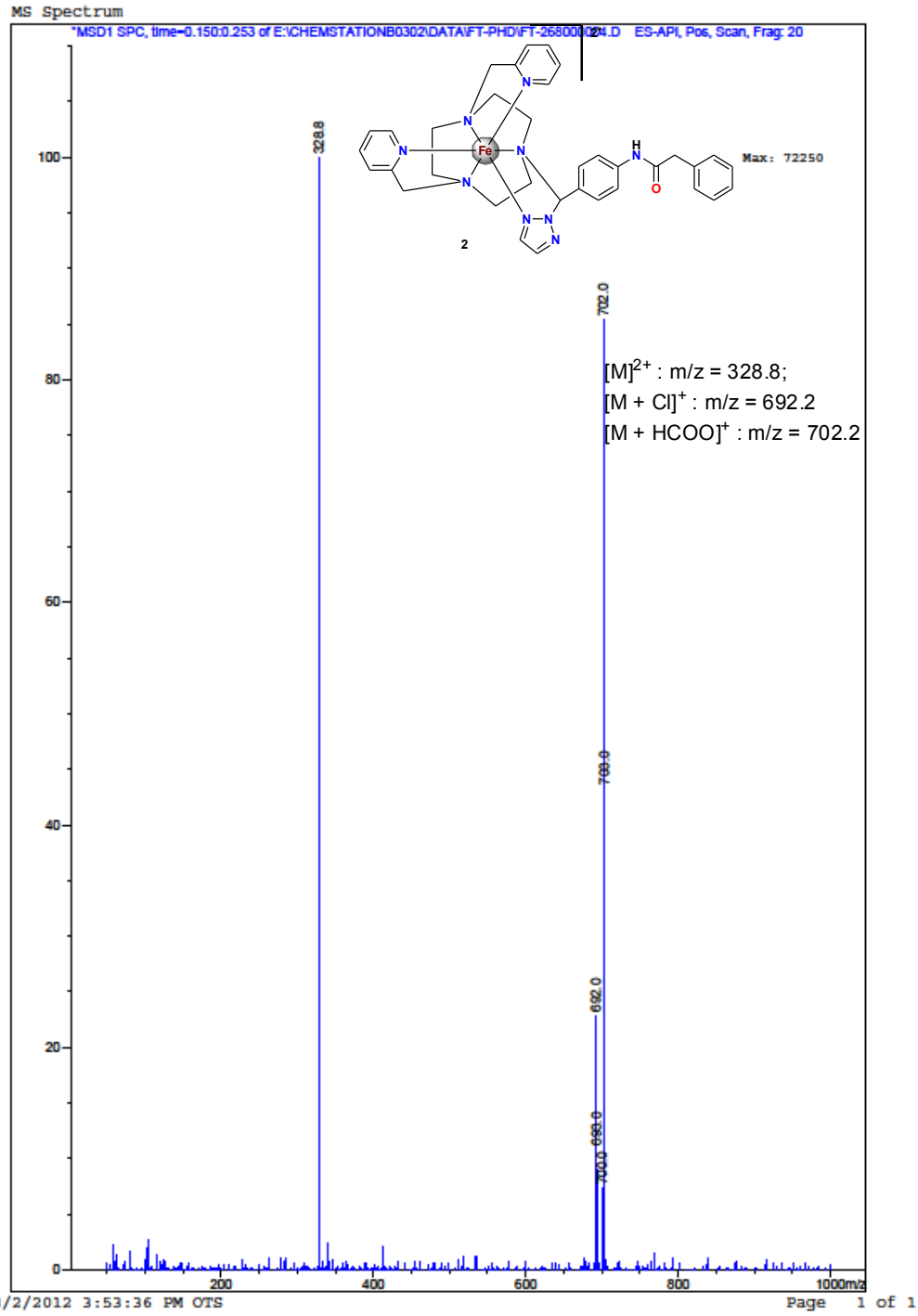
Mass analysis complex 2 (dissolved powder):

Print of window 80: MS Spectrum



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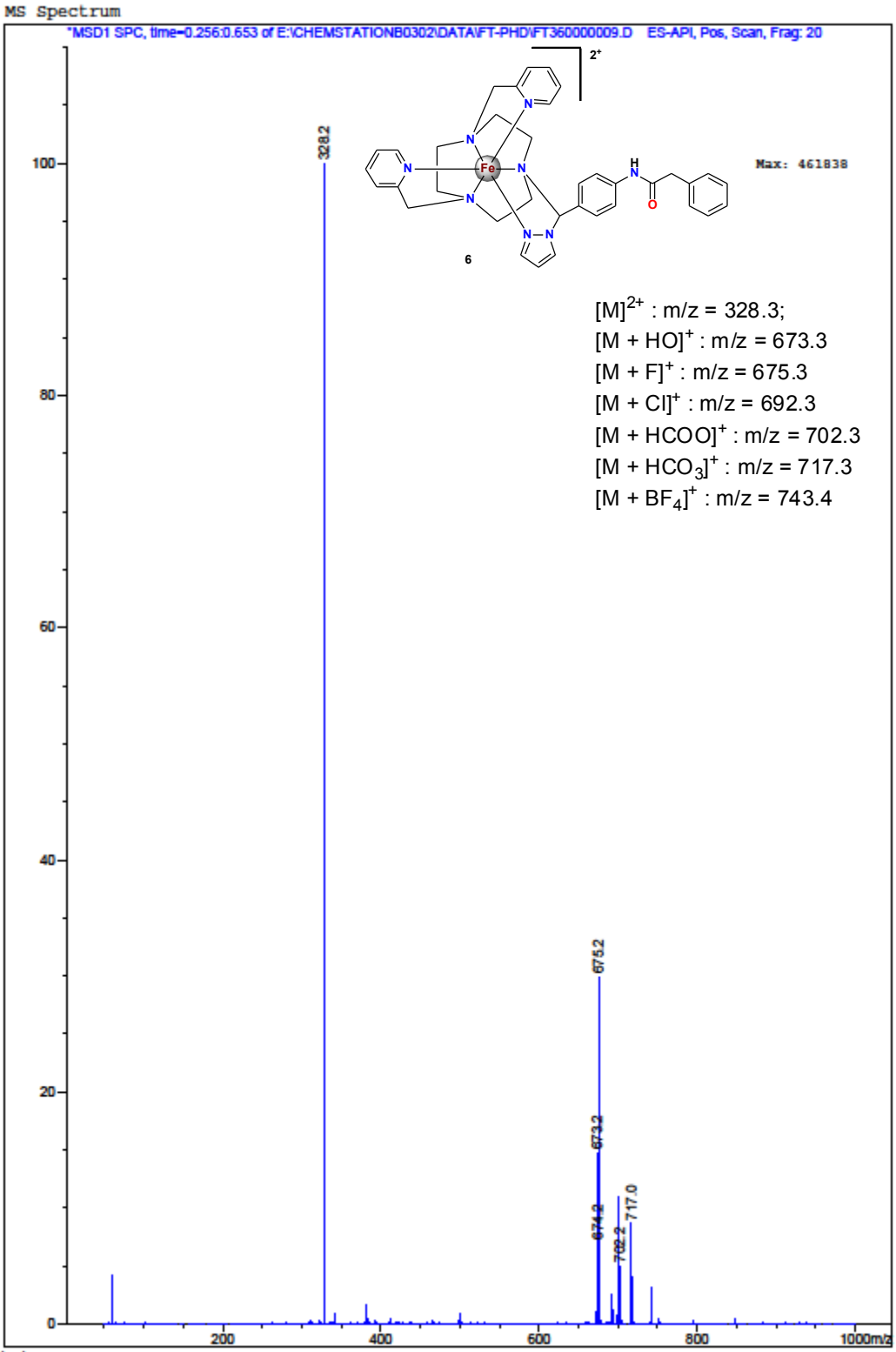
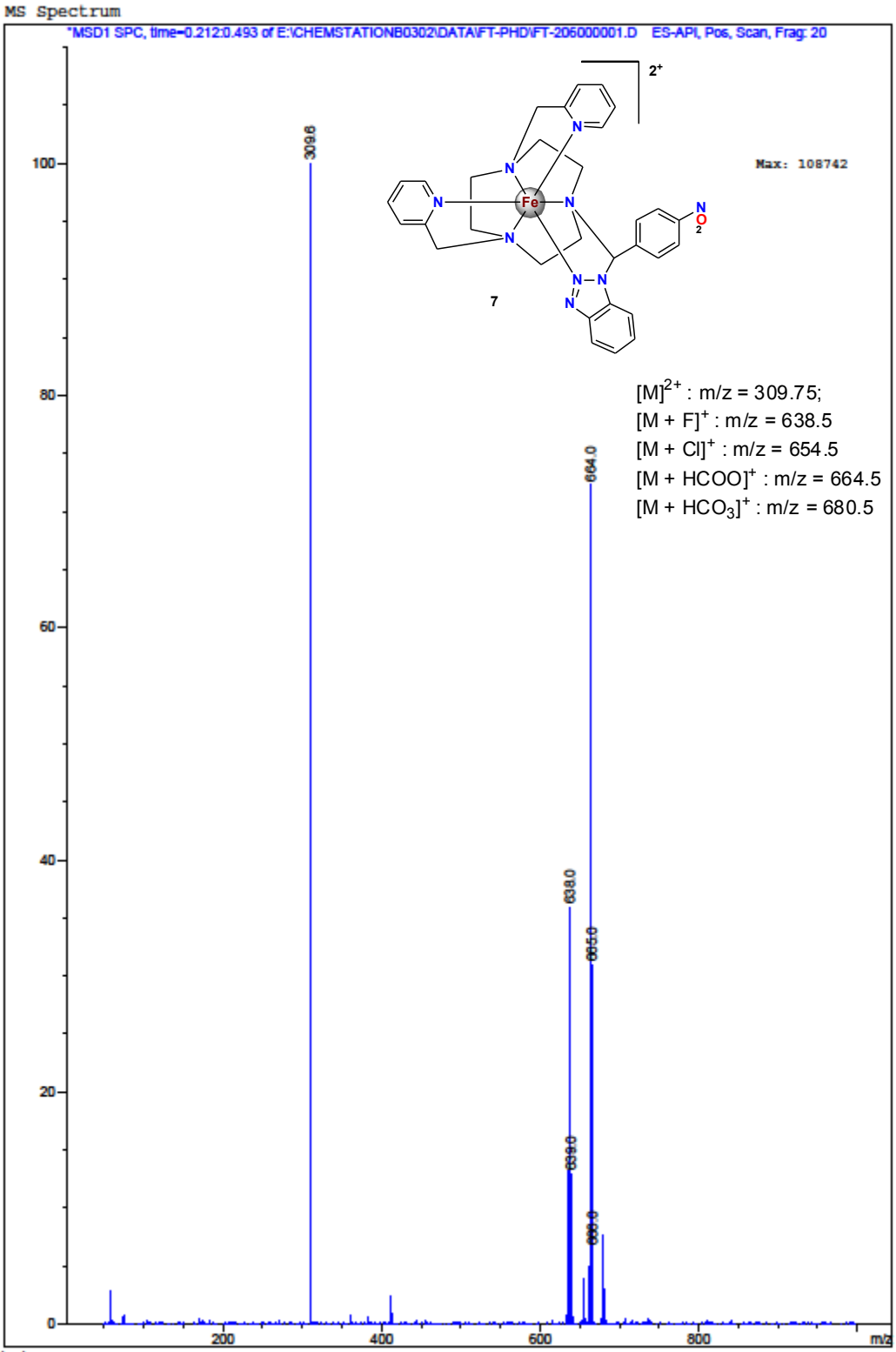
Mass analysis complex 6 (dissolved powder):

Print of window 80: MS Spectrum



Mass analysis complex 7 (dissolved crystals):

Print of window 80: MS Spectrum



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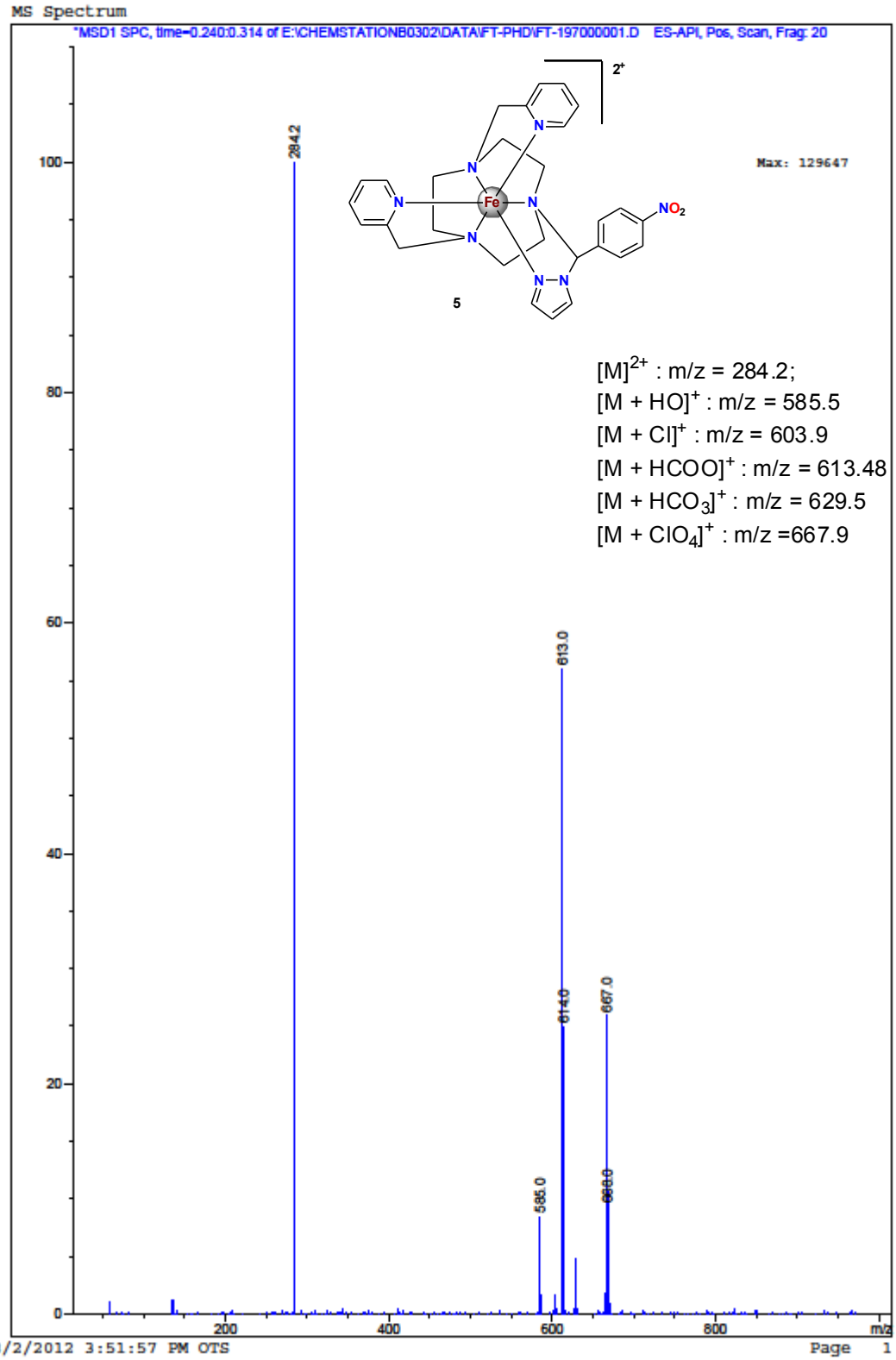
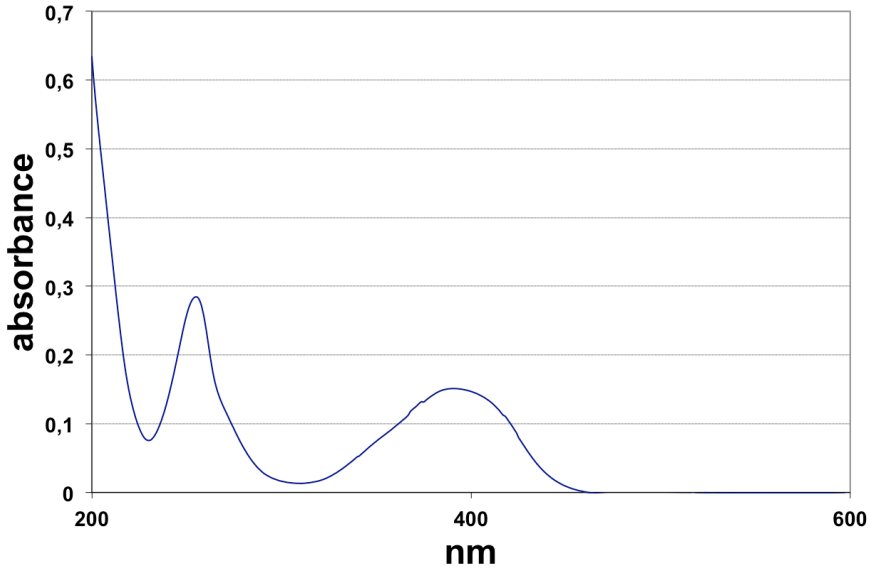
Mass analysis complex 5 (dissolved crystals):

Print of window 80: MS Spectrum

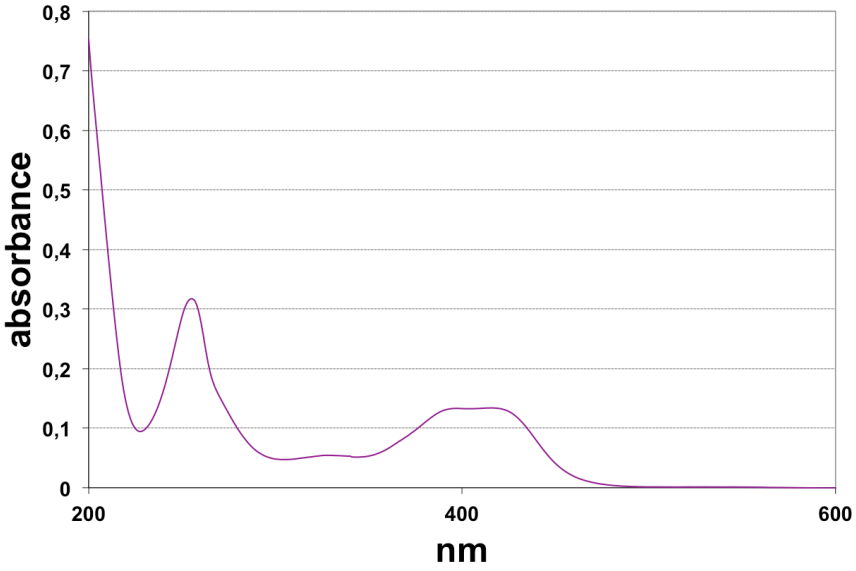


UV spectra for complexes **1**, **5**, and **7** (0.02 mM in 50 mM PB).

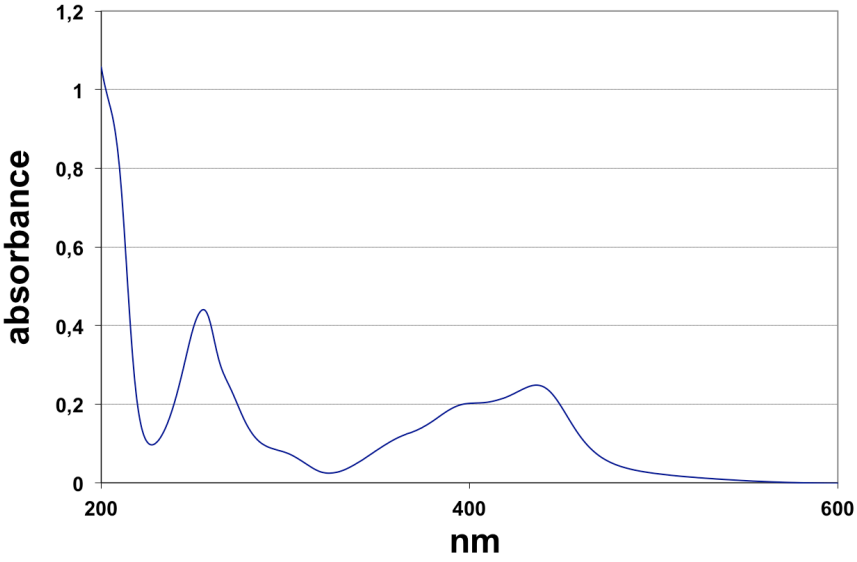
Triazol (1):



Pyrazol (5):



Benzotriazol (7):



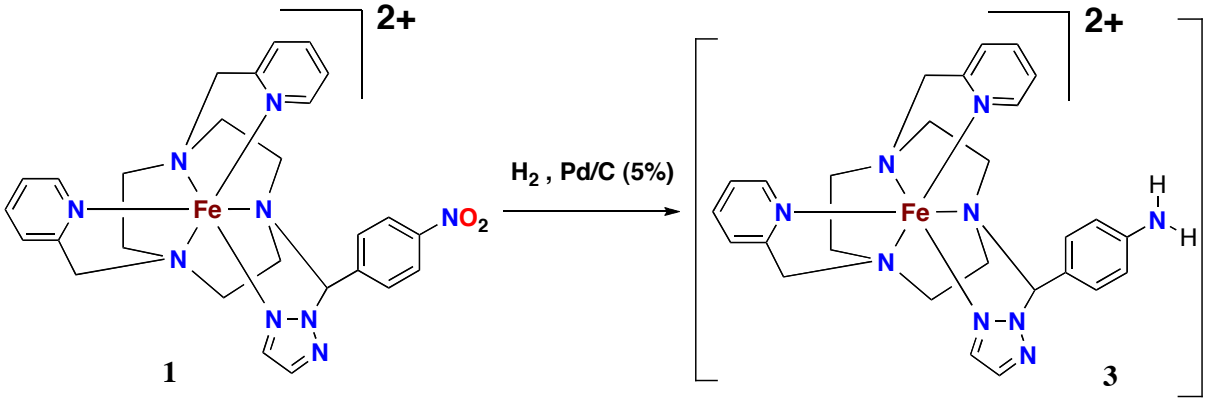
Monitoring of activation of nitro derivatives initiated by hydrogenation

Typical protocol for initiation by hydrogenation (pH 7.4; 4 mM of **1**).

To a PB (50 mM; 1 mL;) solution of complex **1** (3.0 mg; 4 mM) is added 10 mg Pd/C (5 or 10 % w/w). Hydrogen gas is bubbled via a needle through the suspension for 5-10 min. before total reduction of **1** is confirmed by mass analysis. The solution is then filtered, and the pH of 7.4 confirmed before being brought to 37°C. At this temperature the already ongoing immolation reaction is monitored at regular intervals by mass analysis or T1 measurement.

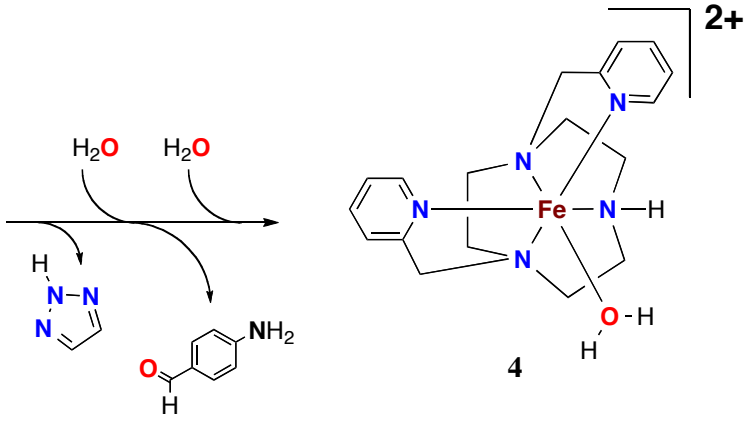
Figure S7 : Mass spectral monitoring of activation of **1** (direct injection).

Mass spectral analysis was carried out by direct injection of a sample into the mass spectrometer set to the "scan" and positive ESI modes. The following scheme associates the observed signals with the corresponding structure.



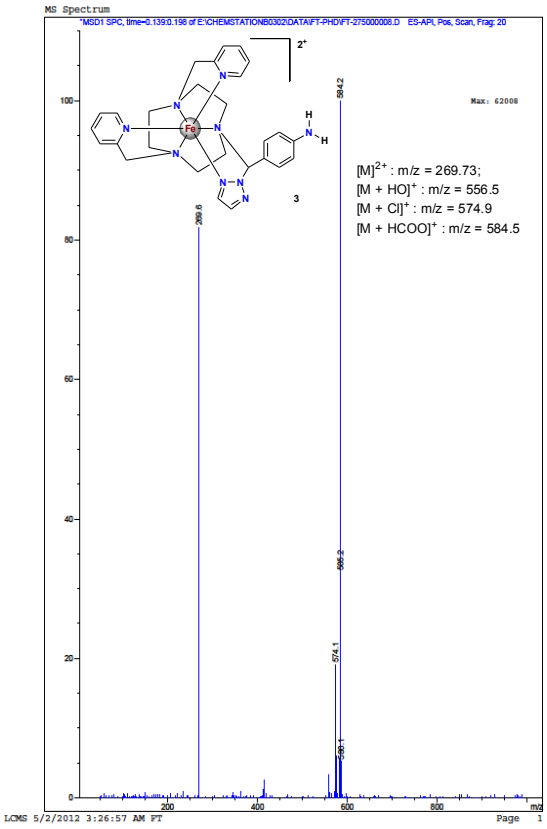
$[M]^{2+}$: m/z = 284.6;
$[M + Cl]^{+}$: m/z = 604.0
$[M + HCOO]^{+}$: m/z = 614.0

$[M]^{2+}$: m/z = 269.6 ;
$[M + Cl]^{+}$: m/z = 574.1
$[M + HCOO]^{+}$: m/z = 584.2

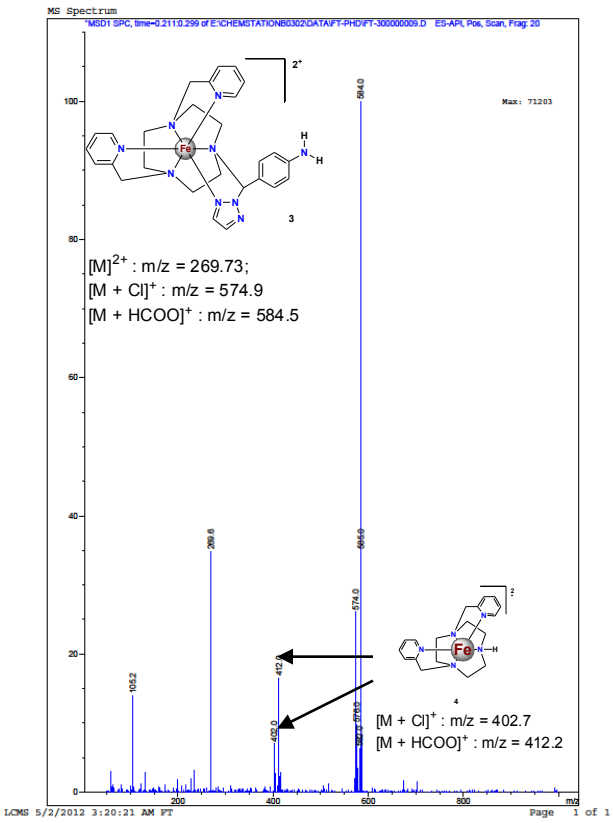


[M + Cl]+ : m/z = 402.0;
[M + HCOO]+ : m/z = 412.0.

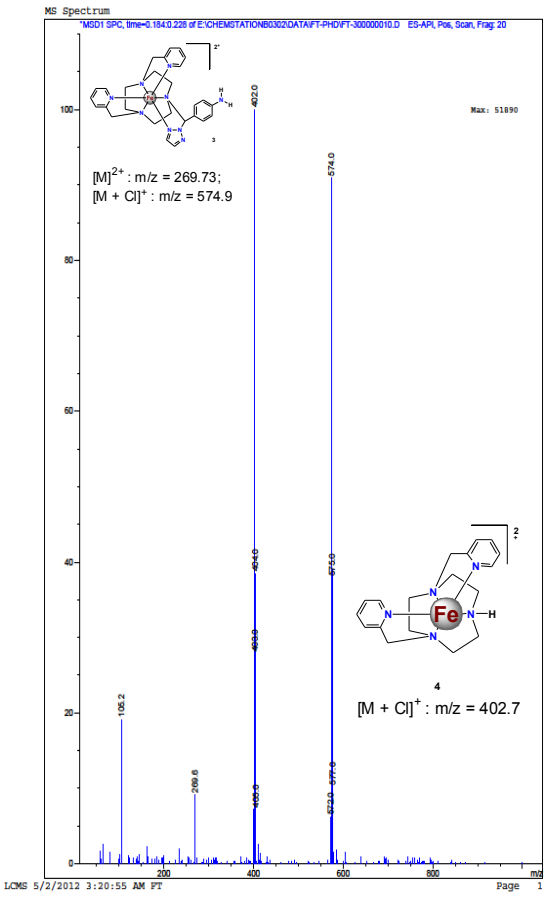
t = 5min



t = 15min.



t = 85min.



t = 125min.

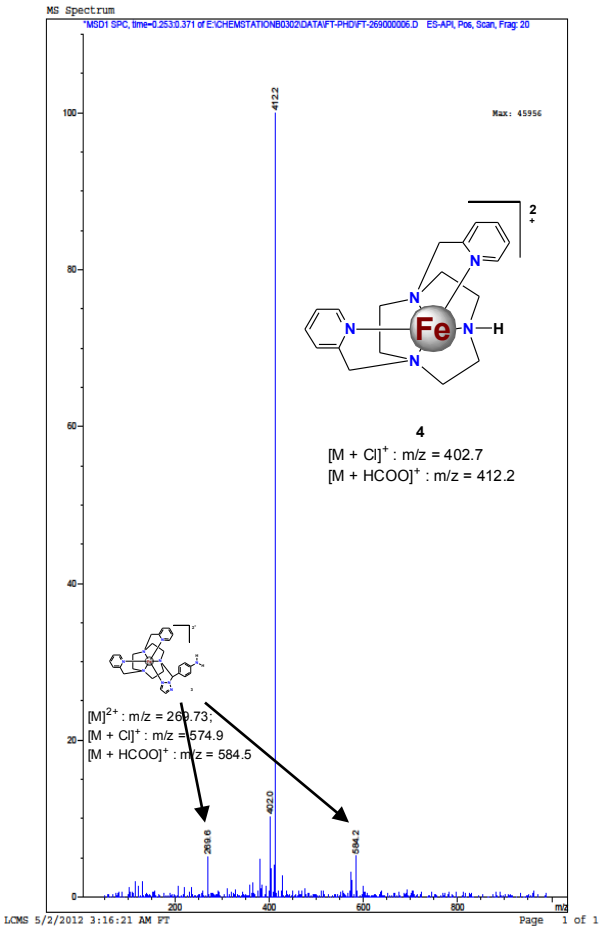
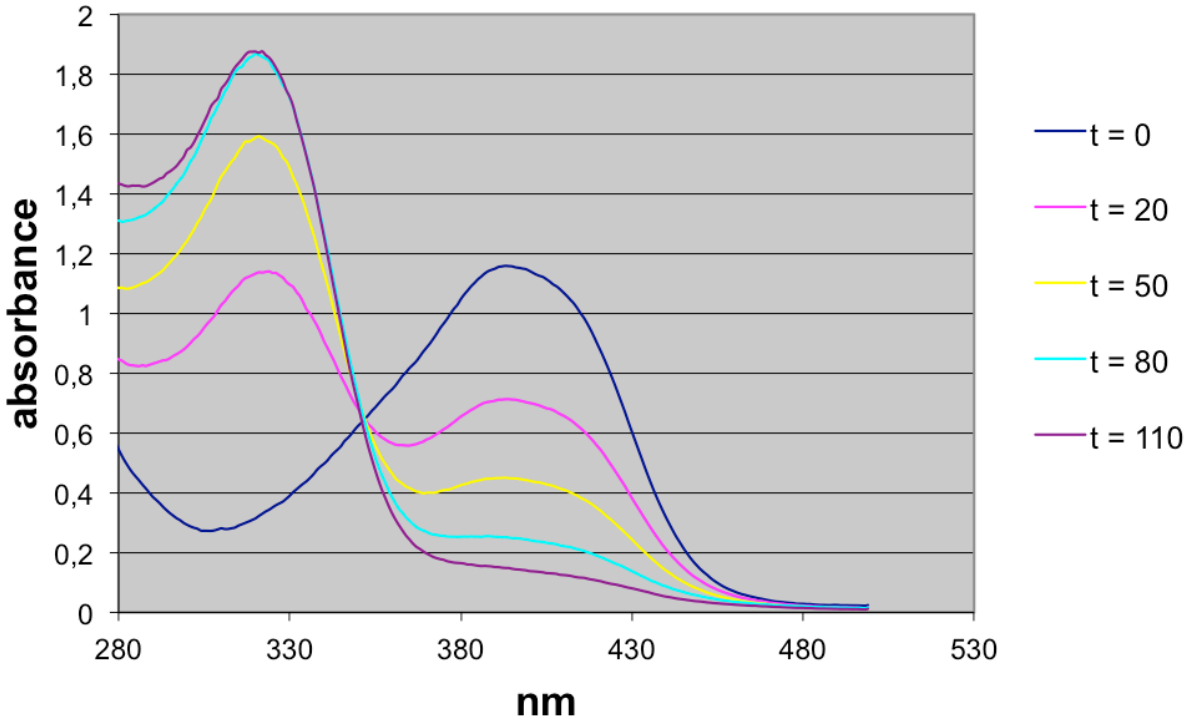


Figure S8 : UV-vis monitoring of fragmentation of **1** upon hydrogenation (0.2 mM)



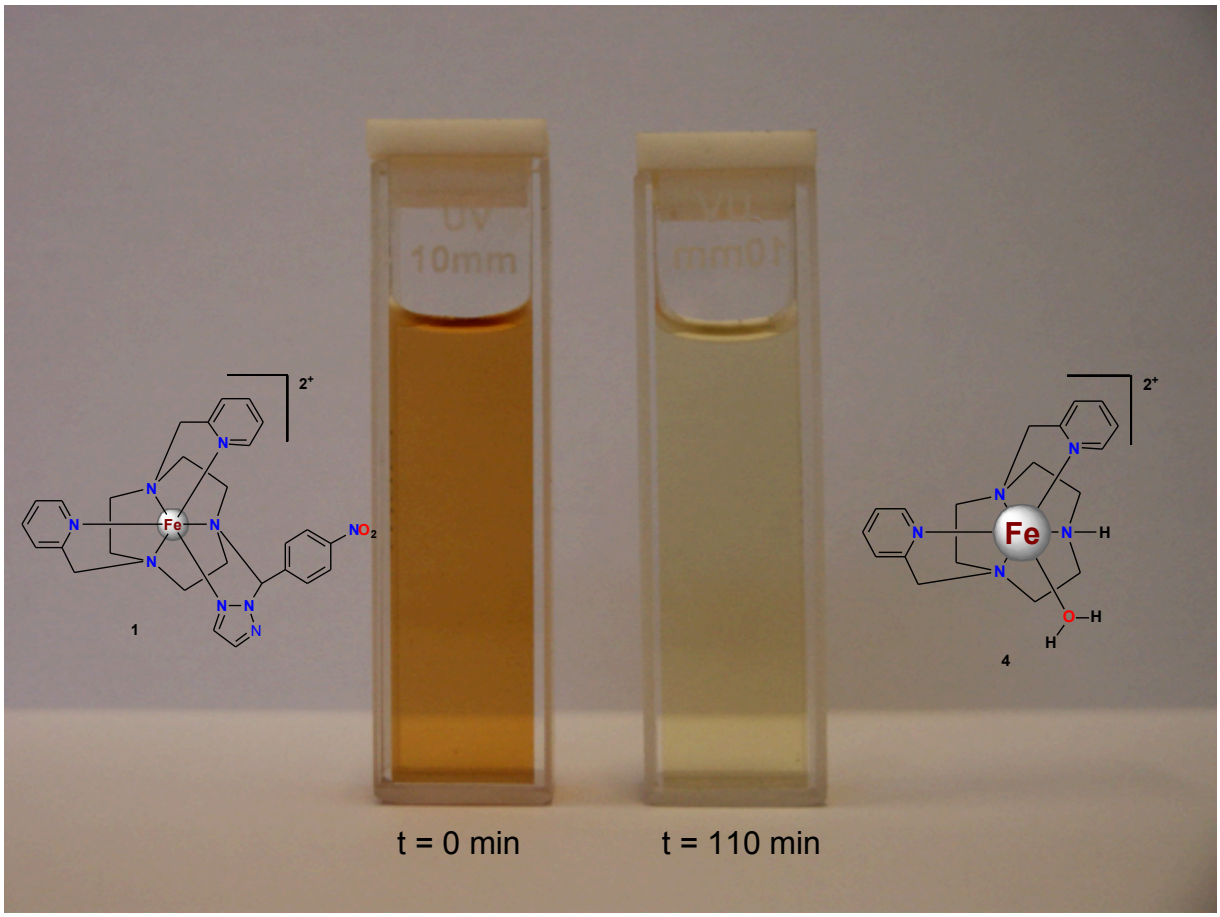
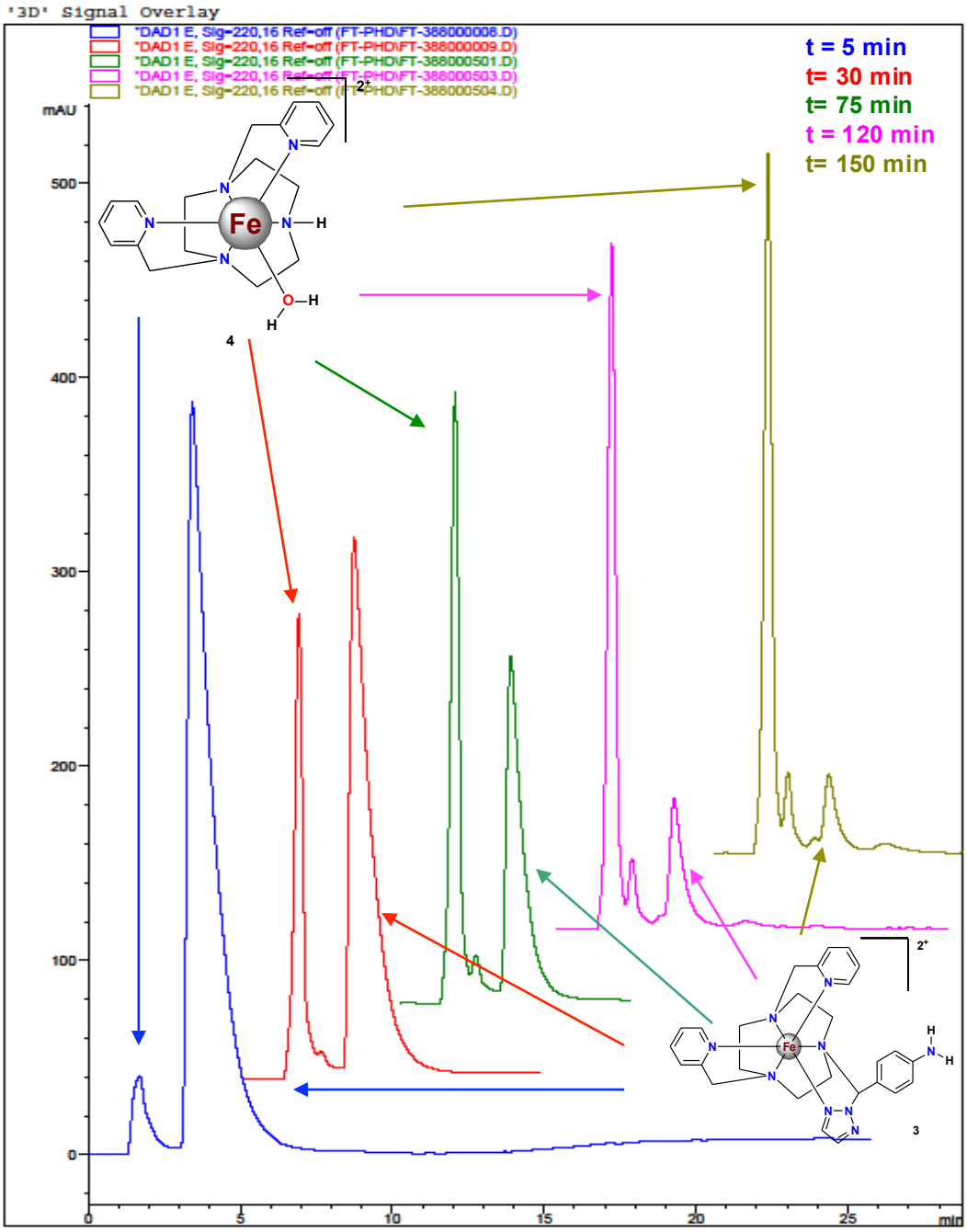


Figure S9 : HPLC monitoring of activation of 1

HPLC chromatography monitored by DAD analysis

Print of window 47: '3D' Signal Overlay



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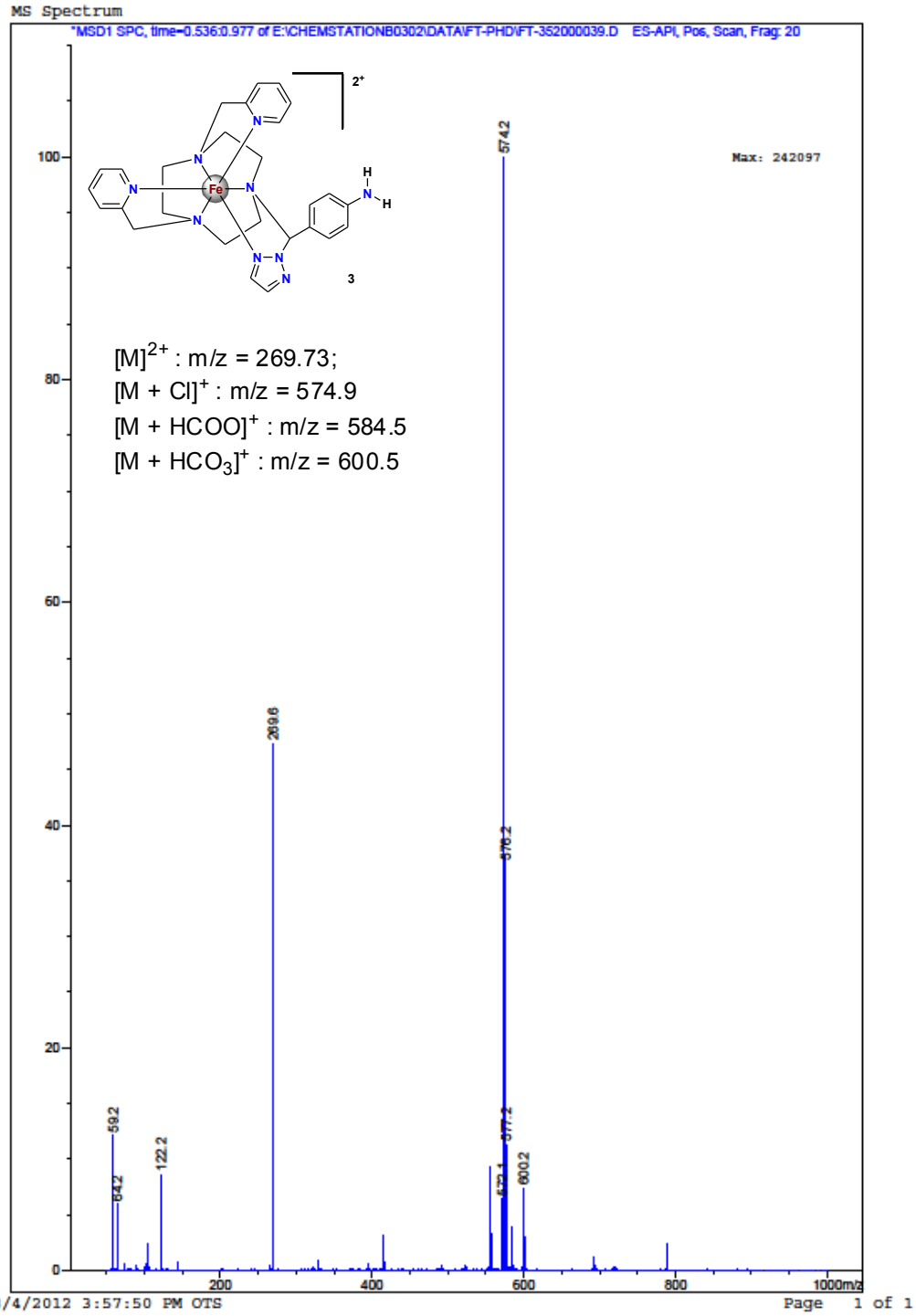
Mass spectrum by direct injection into mass spectrometer after catalytic hydrogenation for 5 minutes and filtration and corresponding to the t = 5 min (first chromatogram above):

Print of window 80: MS Spectrum



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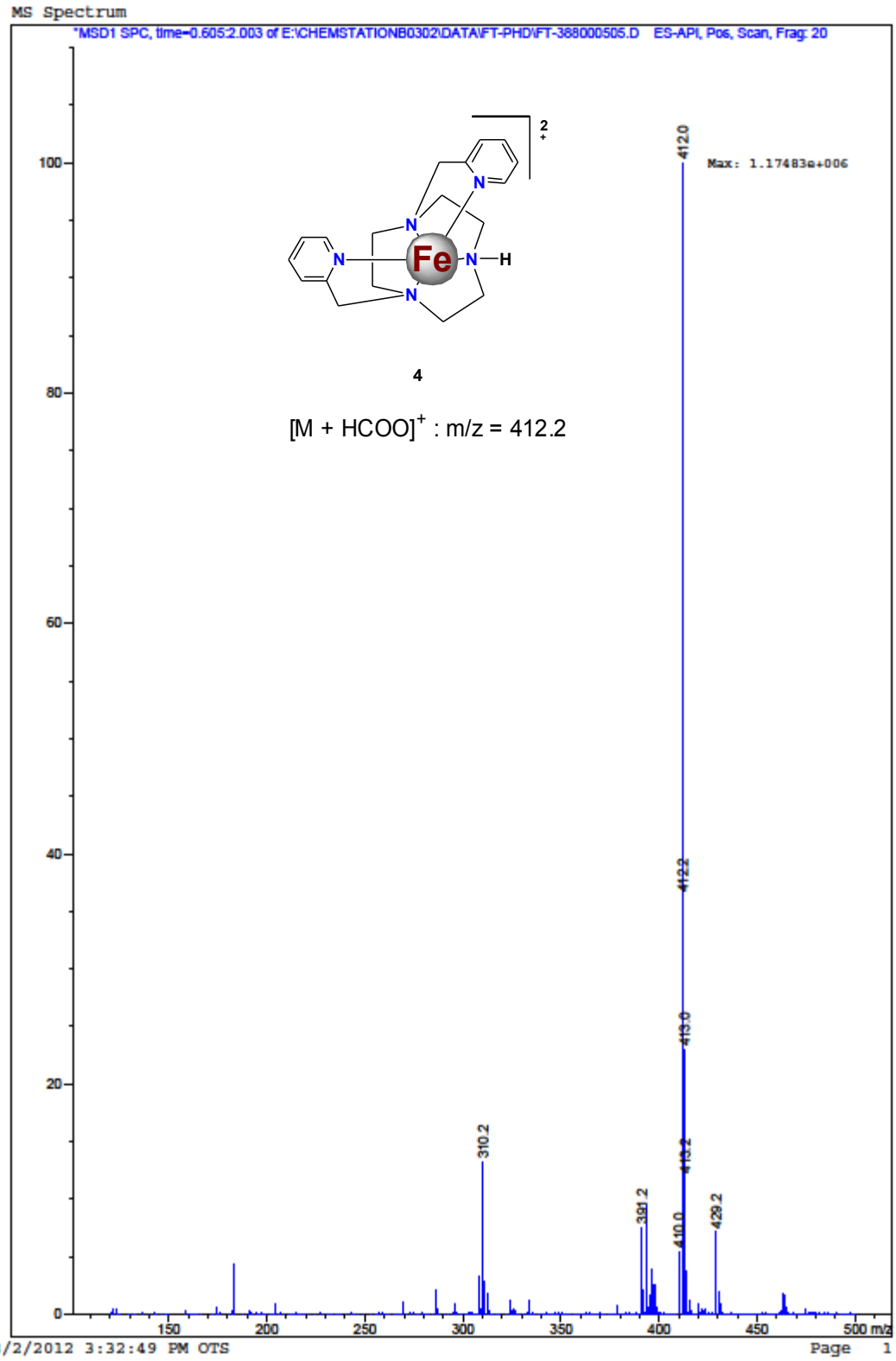
The following mass spectrum has been obtained by direct injection of the final HPLC sample (t = 150 min) into the mass spectrometer so as to be able to observe the signal for compound **4** and thus demonstrate that the final HPLC chromatogram corresponds truly to total conversion to **4** :

Print of window 80: MS Spectrum



4

$[M + HCOO]^+$: m/z = 412.2

Figure S10 : Robustness of **1** demonstrated by HPLC/UV monitoring

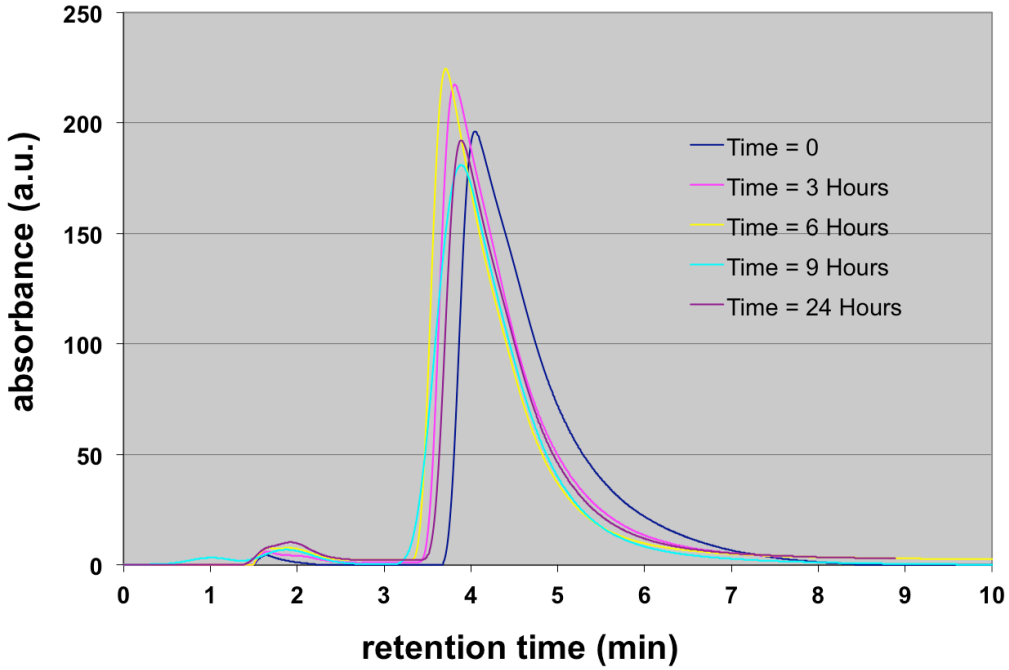
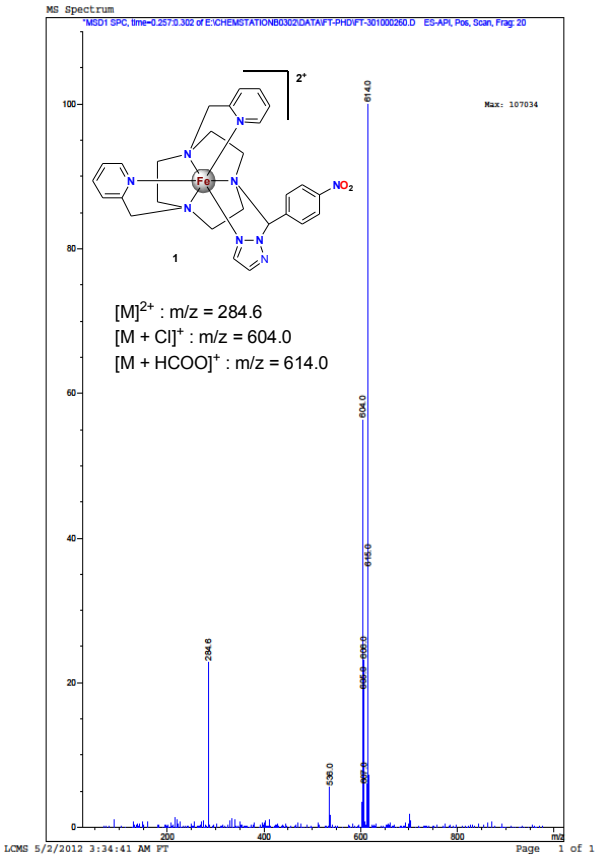
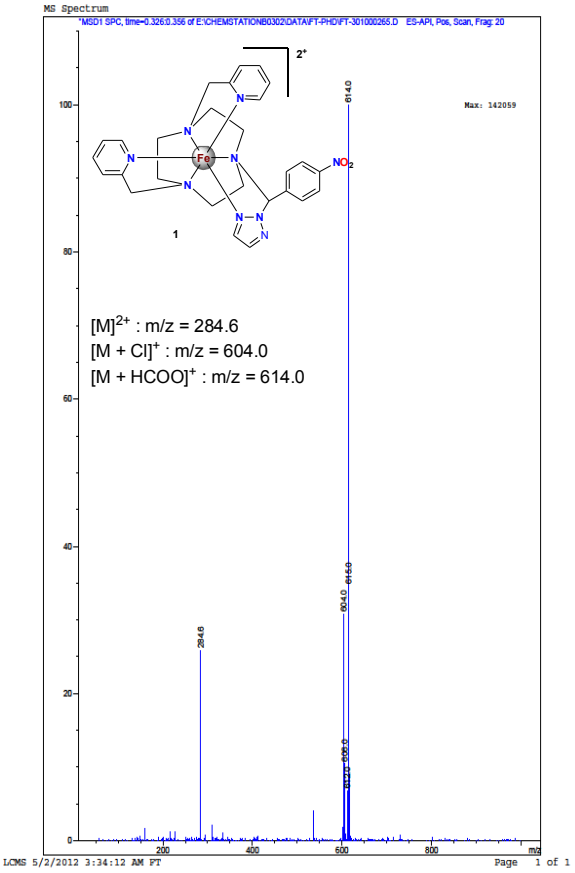


Figure S11 : Robustness of **1** demonstrated by mass spectral monitoring (direct injection)

t = 4h



t = 24h



The peak at retention time 3.8 min and t = 24 h is caused only by the untouched probe **1**, as evidenced by the corresponding mass spectrum:

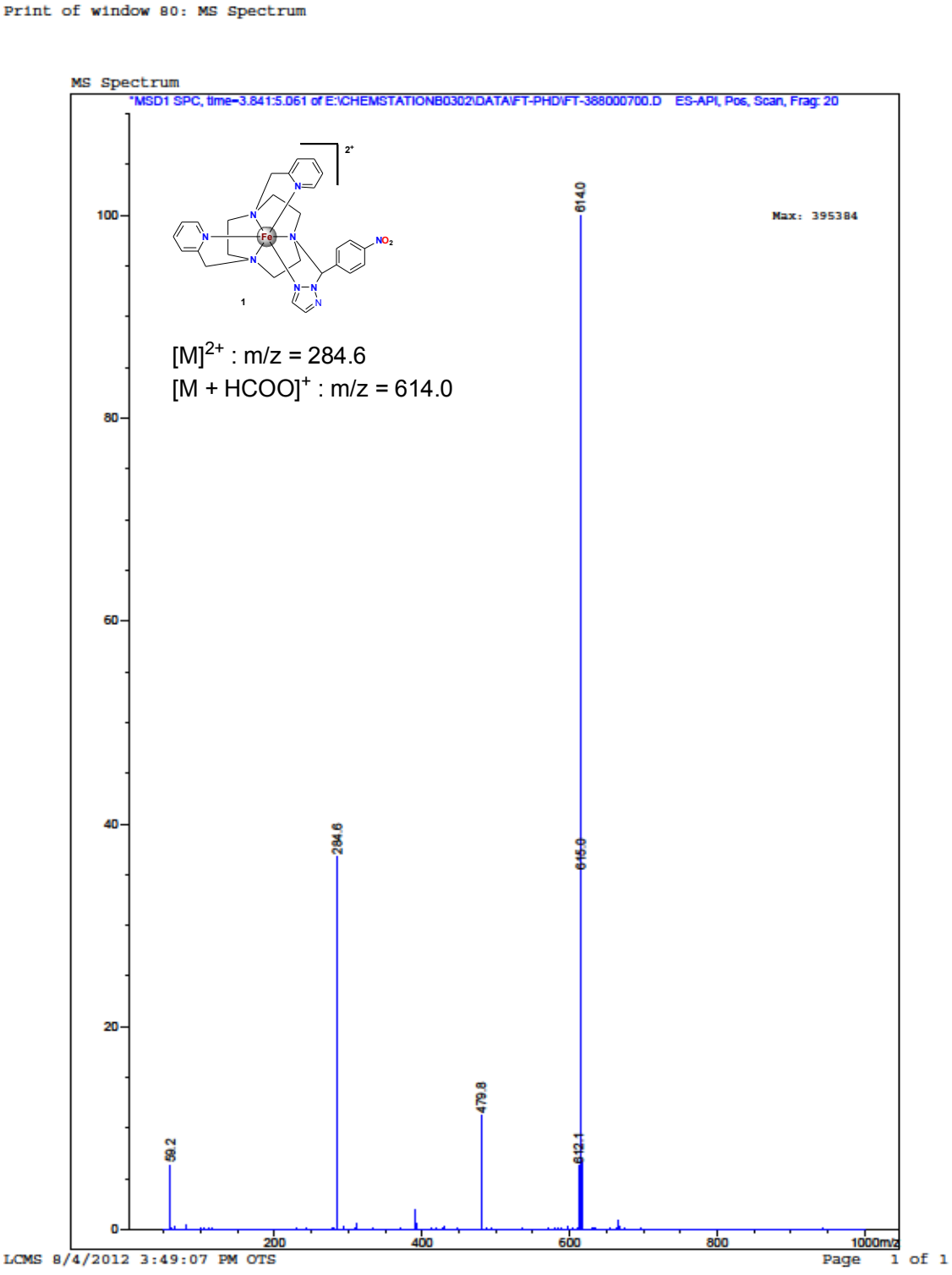
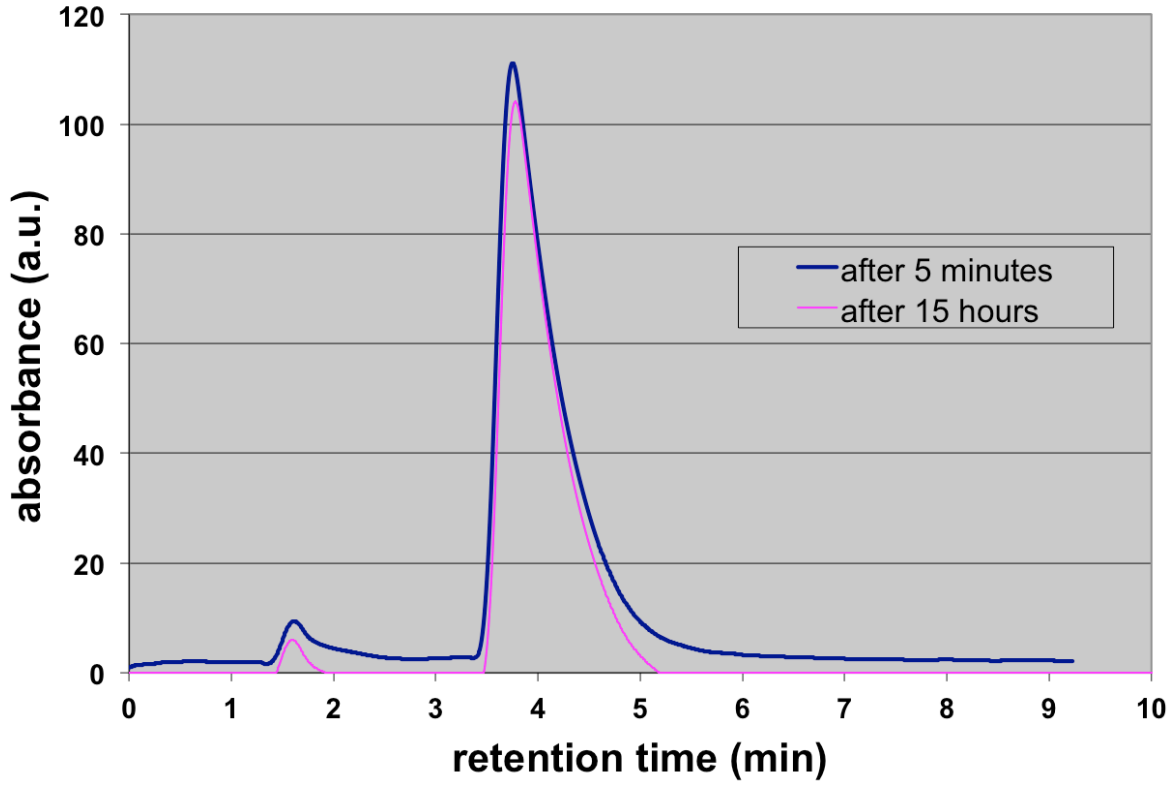


Figure S12 : Absence of fragmentation of pyrazol complex **5** (negative control)



Mass spectrum (direct injection) of sample corresponding to HPLC chromatogram above at 15 hours. Spectrum proves absence of any traces of fragmentation of aniline derivative of **5** towards **4** :

Print of window 80: MS Spectrum

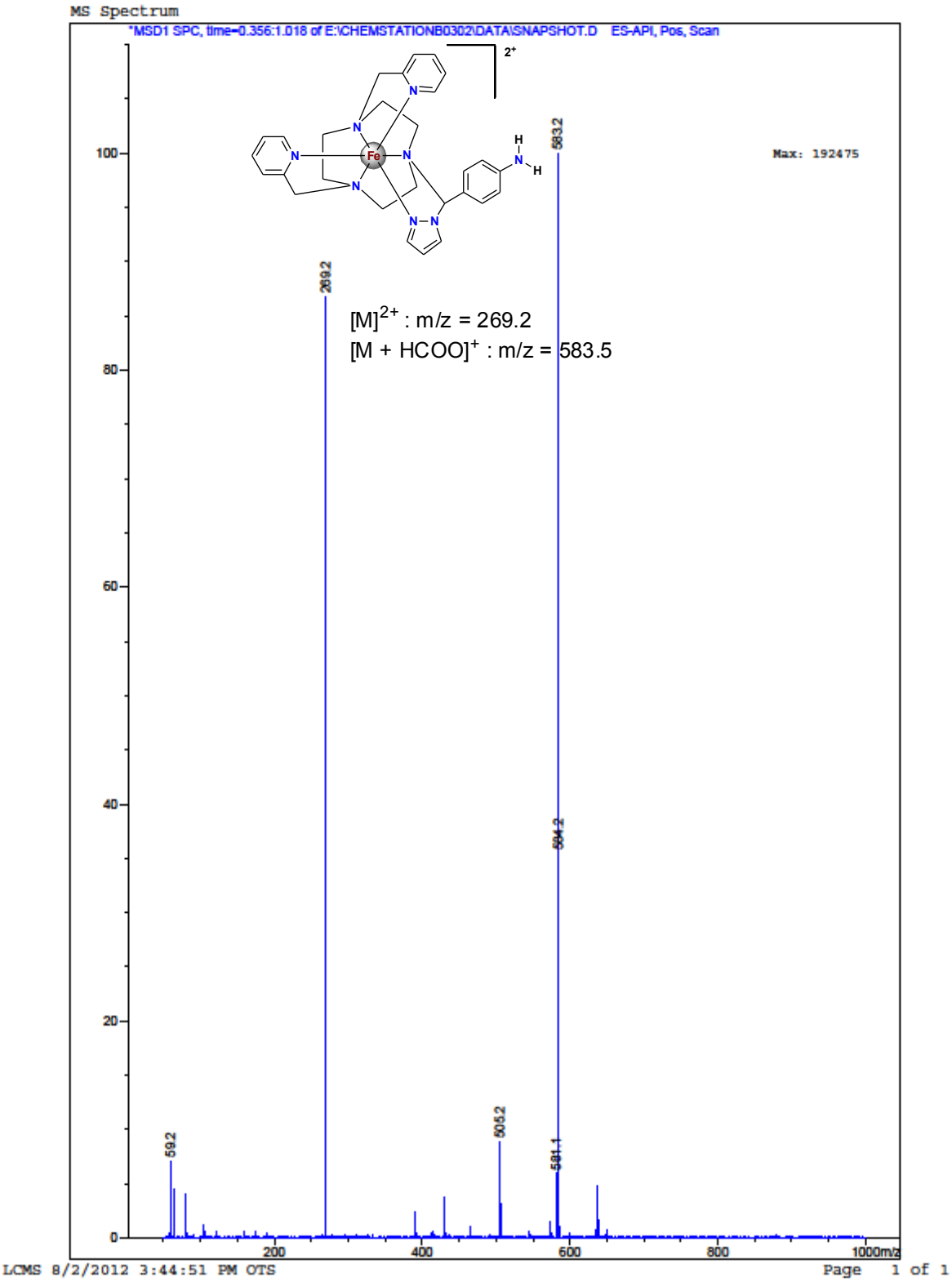
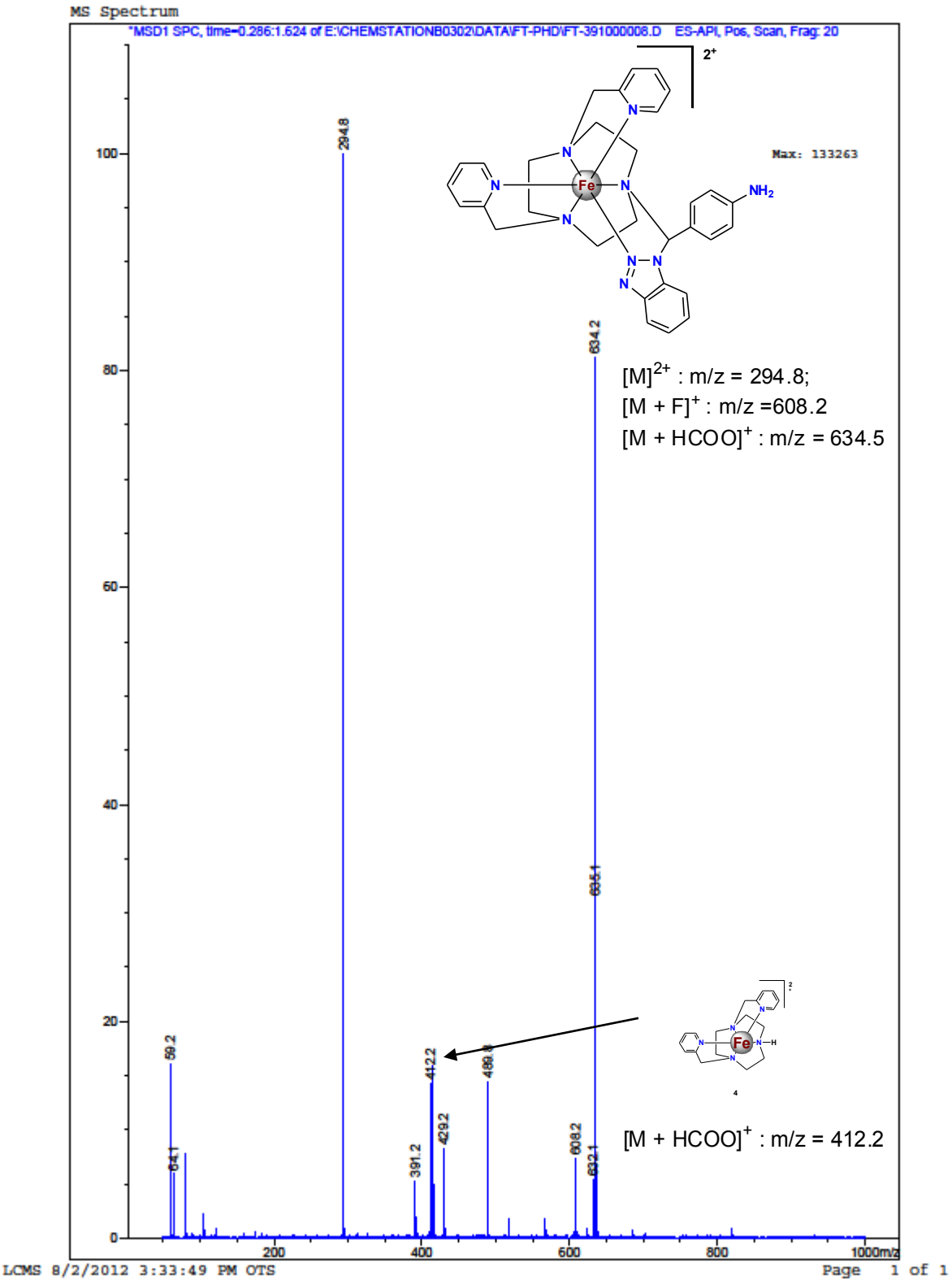


Figure S13 : Mass spectral monitoring of activation of **7** (direct injection)

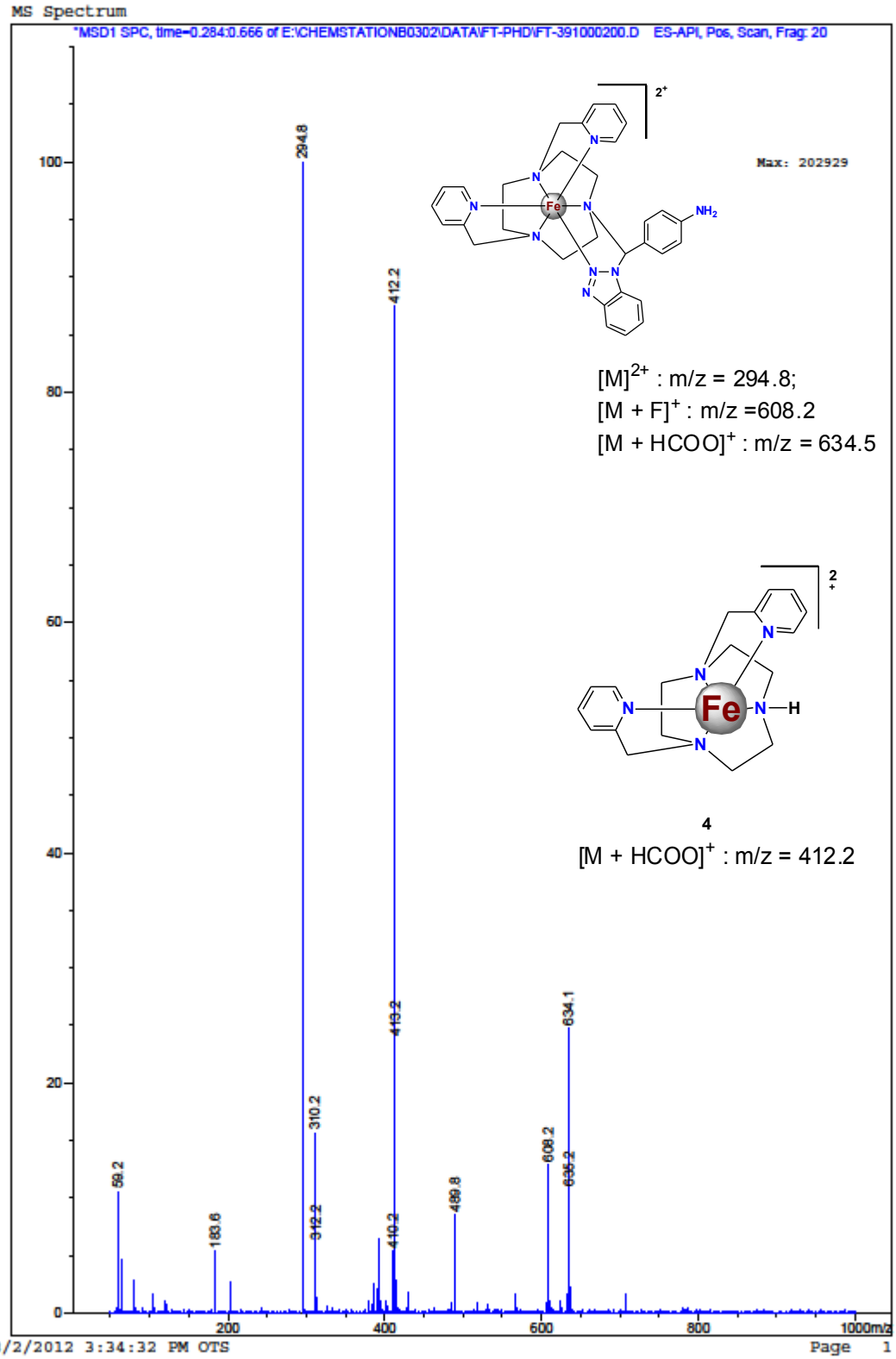
t =5 min

Print of window 80: MS Spectrum



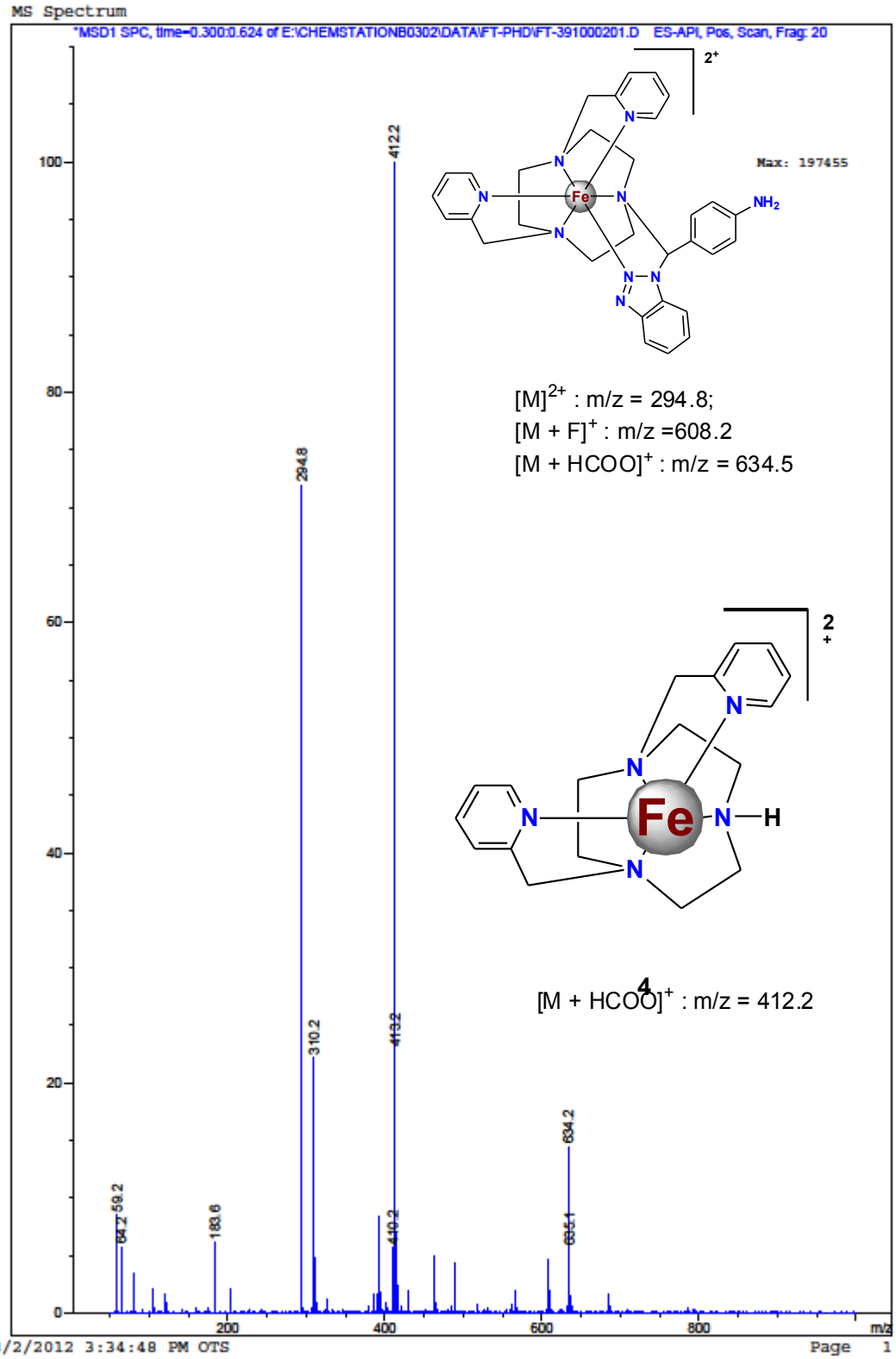
t = 65 min

Print of window 80: MS Spectrum



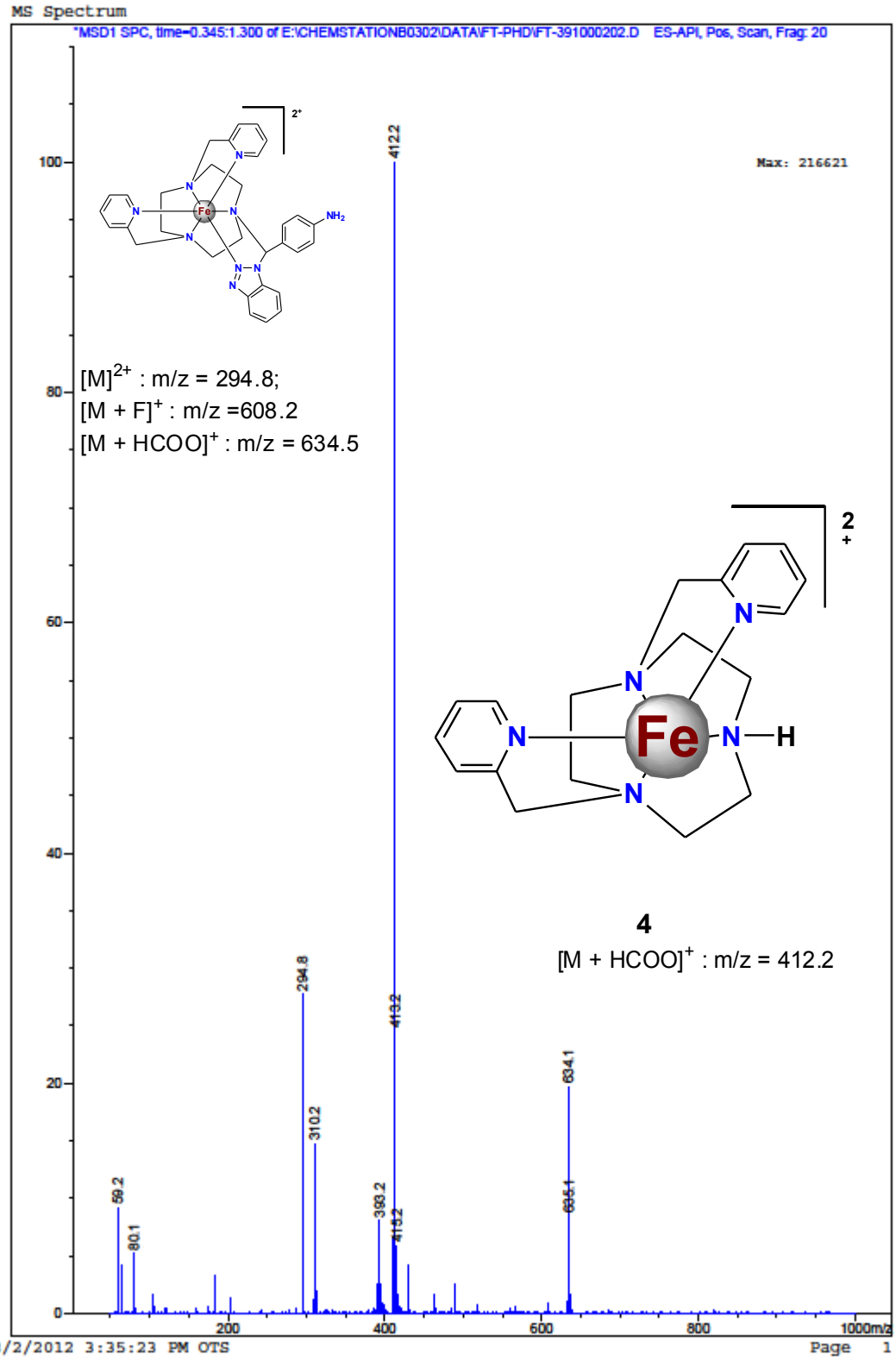
t = 125 min

Print of window 80: MS Spectrum



t = 180 min

Print of window 80: MS Spectrum



t =270 min

Print of window 80: MS Spectrum

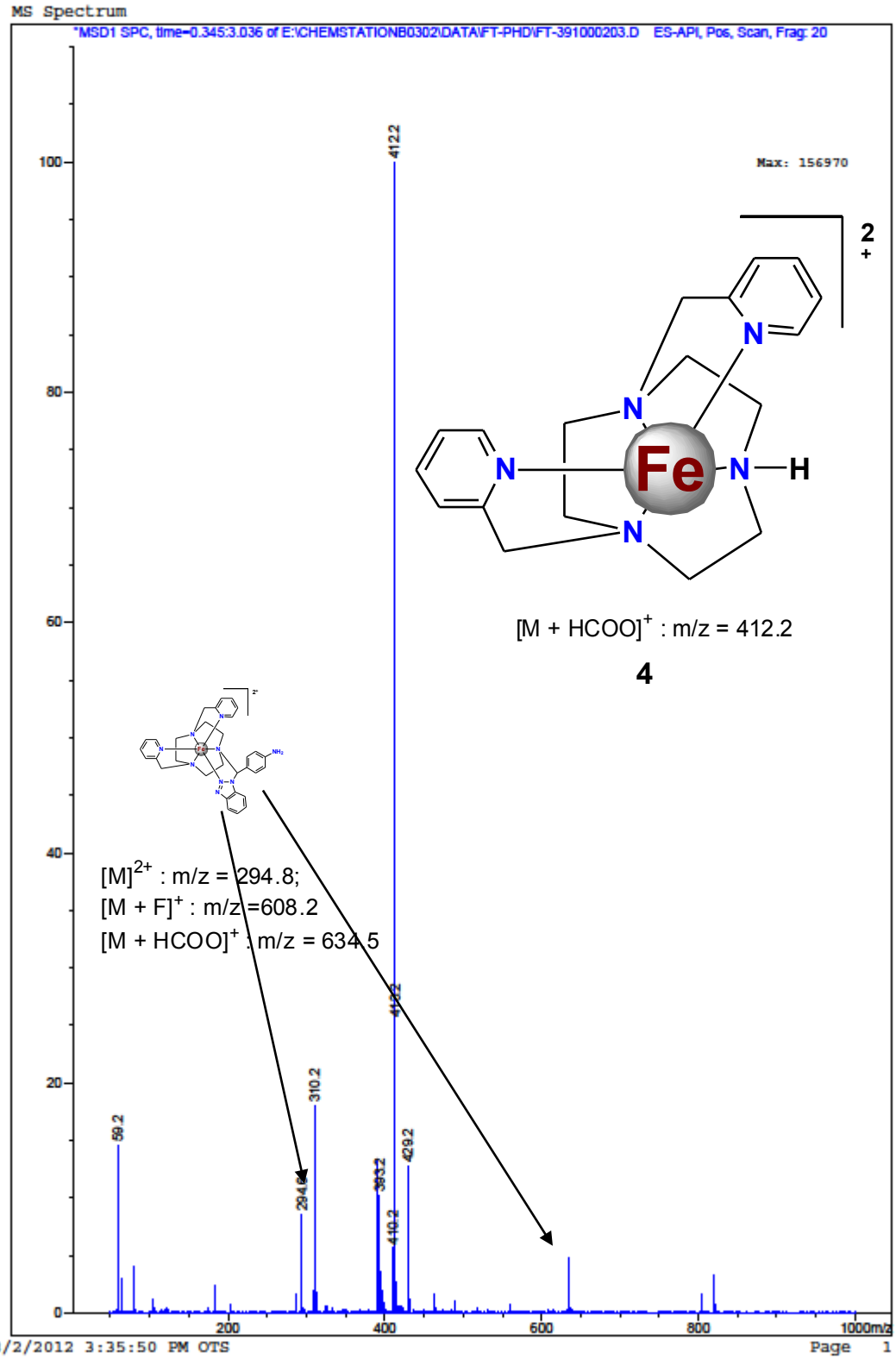


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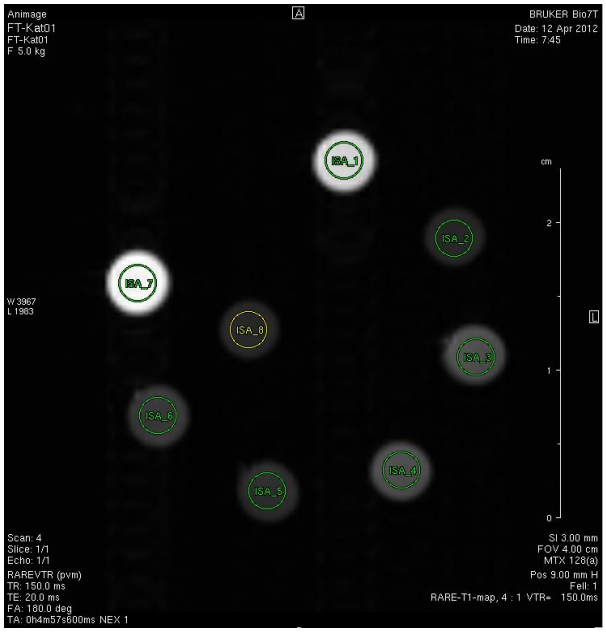
Figure S14 : *T*1 monitoring by MRI (phantom images at 7T)

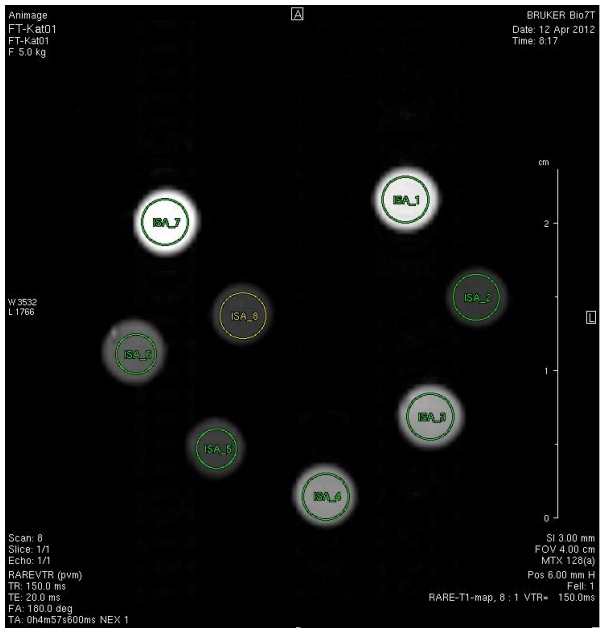
Figure 3 (main article) contains parts of the same data.

The sequence VTR allows the acquisition of multiple *T*1-weighted MR images with variable TR. From this saturation-recuperation sequence, *T*1 values can be estimated assuming a mono-exponential signal recovery.

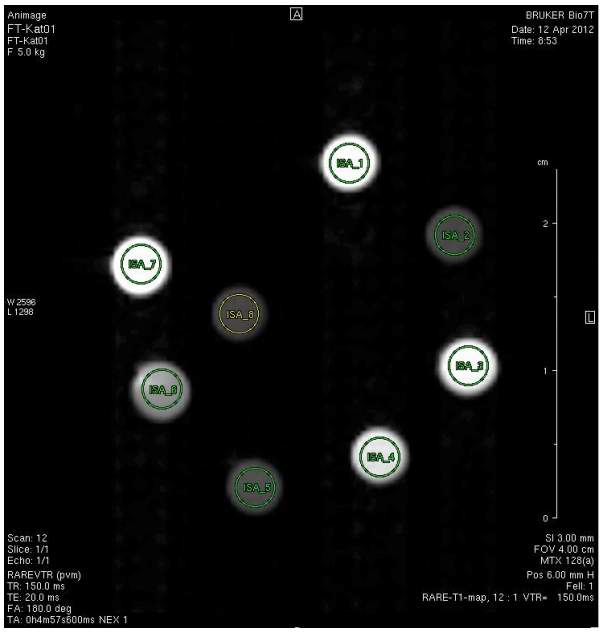
t = 20 min.



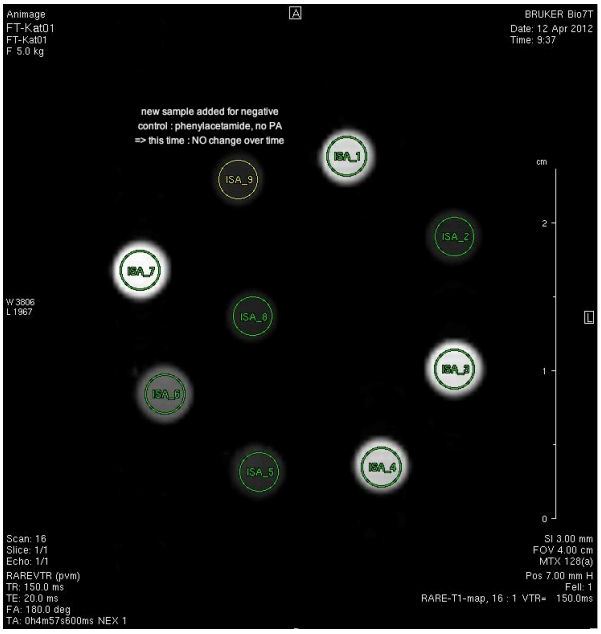
t = 50 min.



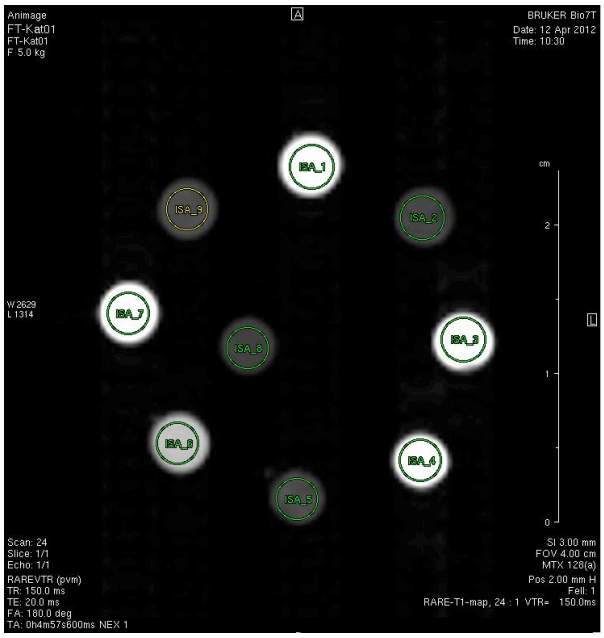
t = 1.5 h



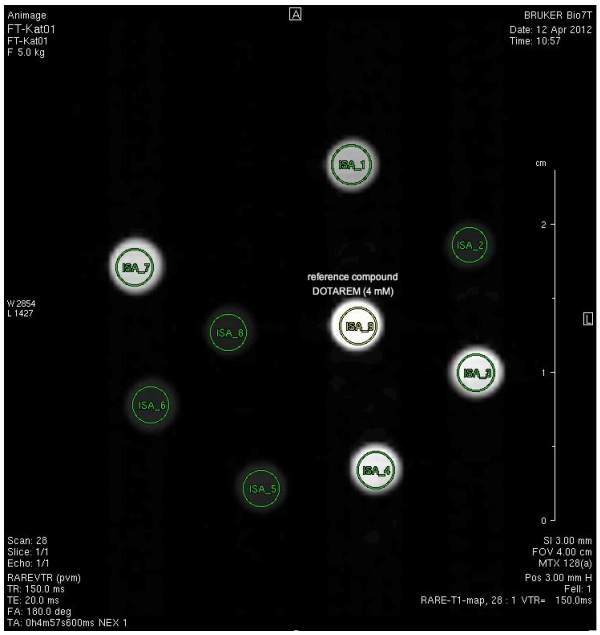
t = 2 h



t = 3 h



t = 3.5 h



Legend :

For t = 20, 50, 90 min

ISA-1 = positive control, **1** + chemical stimulus 4 mM, image 24 hours later (PBS)

ISA-3 = **1** + chemical stimulus 4 mM, t = 20, 50, 90 min after induction

ISA-5 = **1** + no chemical stimulus 4 mM, t = 20, 50, 90 min

ISA-7 = **2** + PA 4 mM, image 24 hours later

ISA-8 = PBS

For t = 120, 180 min

ISA-1 = positive control, **1** + chemical stimulus 4mM, image 24 hours later (PBS)

ISA-3 = **1** + chemical stimulus 4 mM, t = 120 and 180 min after induction

ISA-5 = **1** + no chemical stimulus 4 mM, t = 120 min and 180 min

ISA-7 = **2** + PA 4 mM, image 24 hours later

ISA-8 = PBS

ISA-9 = **2** + No PA 4 mM, t = 30 min

For t = 210 min

ISA-1 = positive control, **1** + chemical stimulus 4 mM, image 24 hours later (PBS)

ISA-3 = **1** + chemical stimulus 4mM, t = 210 min after induction

ISA-5 = **1** + no chemical stimulus 4mM, t = 210 min

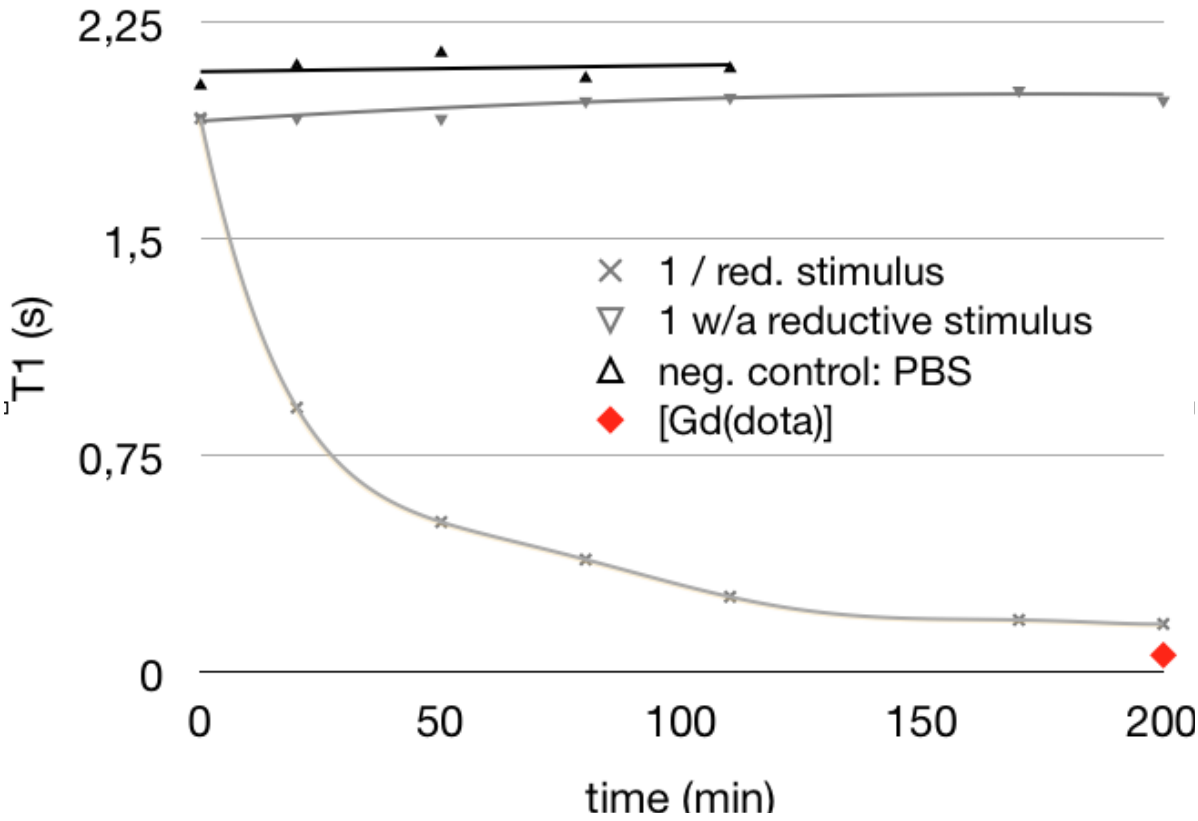
ISA-6 = **2** + no PA 4 mM, t = 110 min

ISA-7 = **2** + PA 4 mM, image 24 hours later

ISA-8 = PBS

ISA-10 = [Gd(ddota)] at 4 mM (commercial source)

T1 values corresponding to phantom images above :



Monitoring of incubation of 2 with penicillin amidase PA

Figure S15 : UV monitoring : **2** / PA (0.2 mM in 10 % serum)

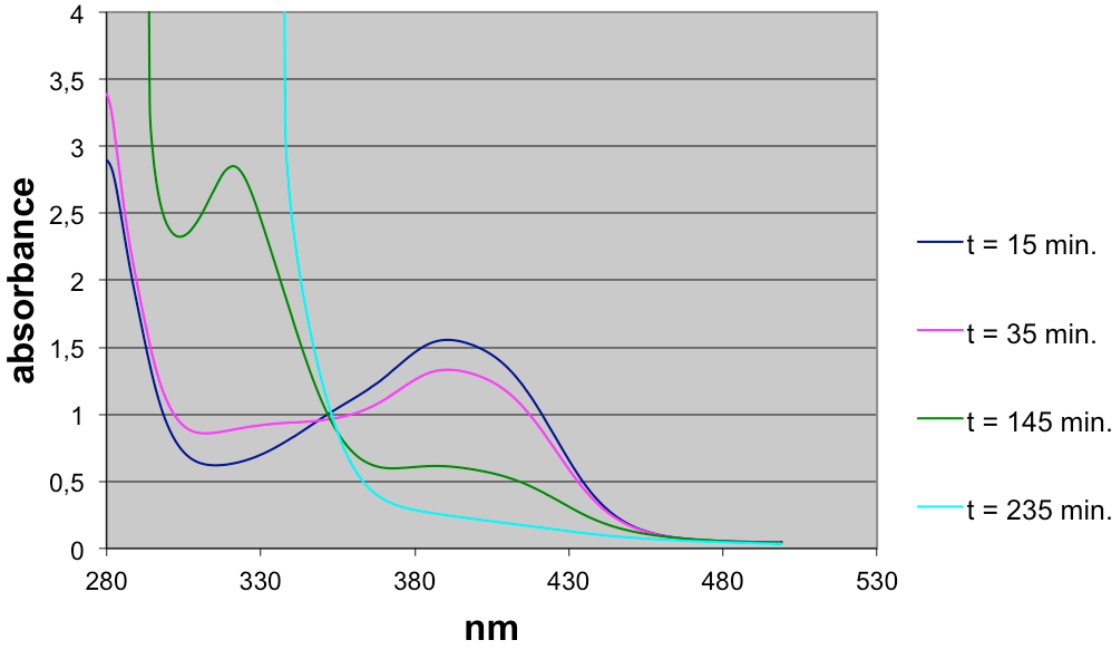
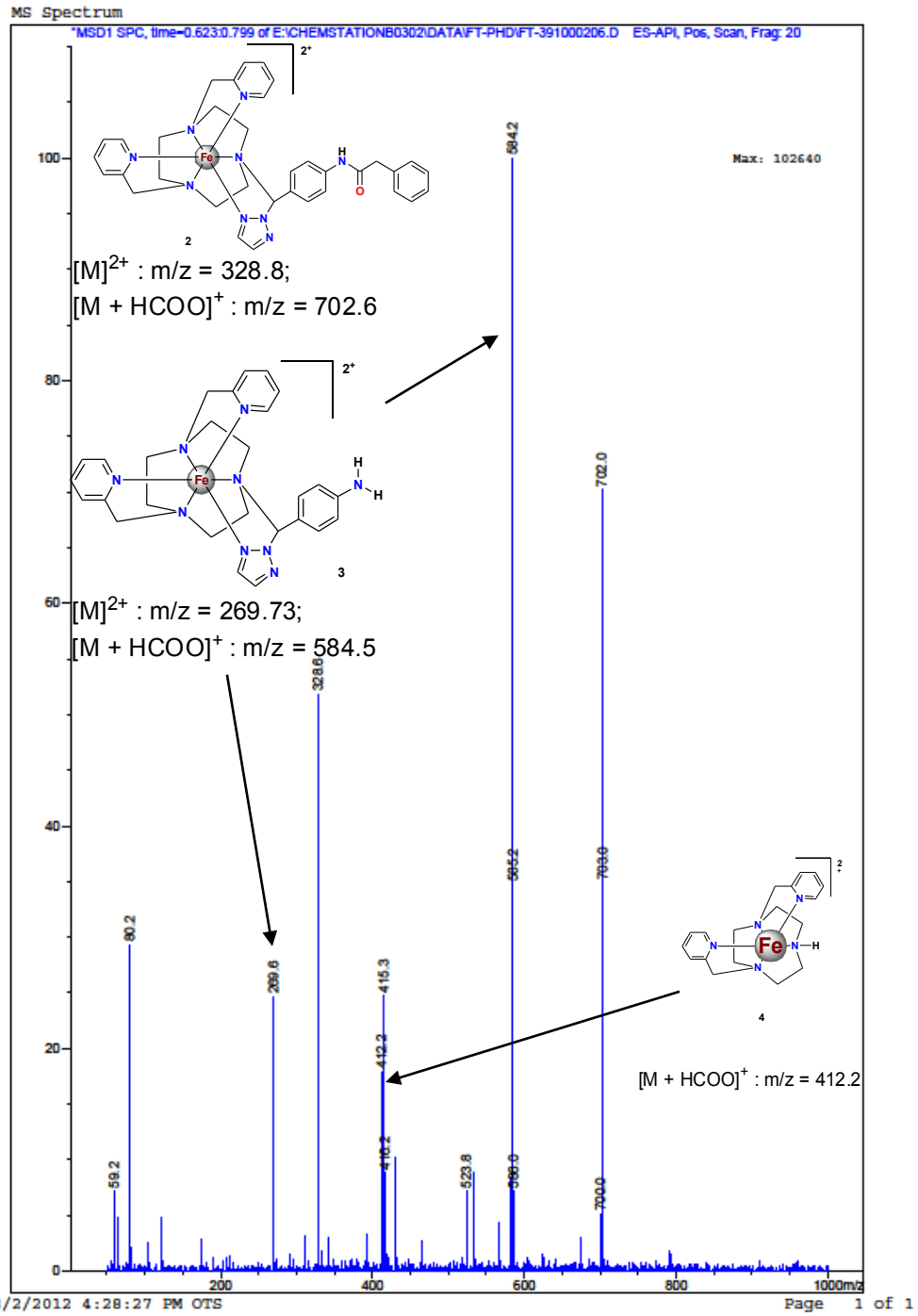


Figure S16 : Mass spectral monitoring : **2** / PA

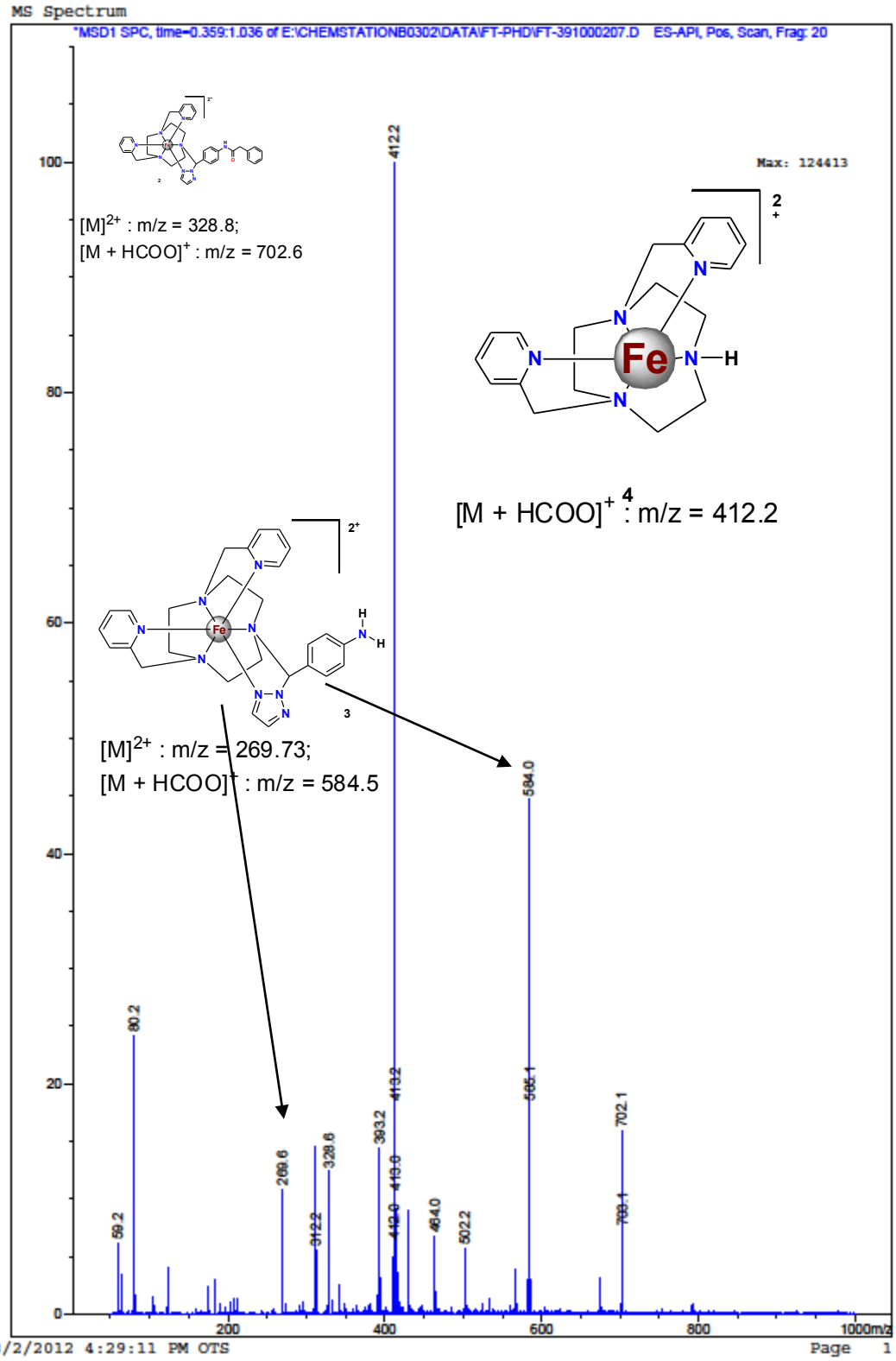
t = 30 min

Print of window 80: MS Spectrum



t = 120 min

Print of window 80: MS Spectrum



t = 200 min

Print of window 80: MS Spectrum

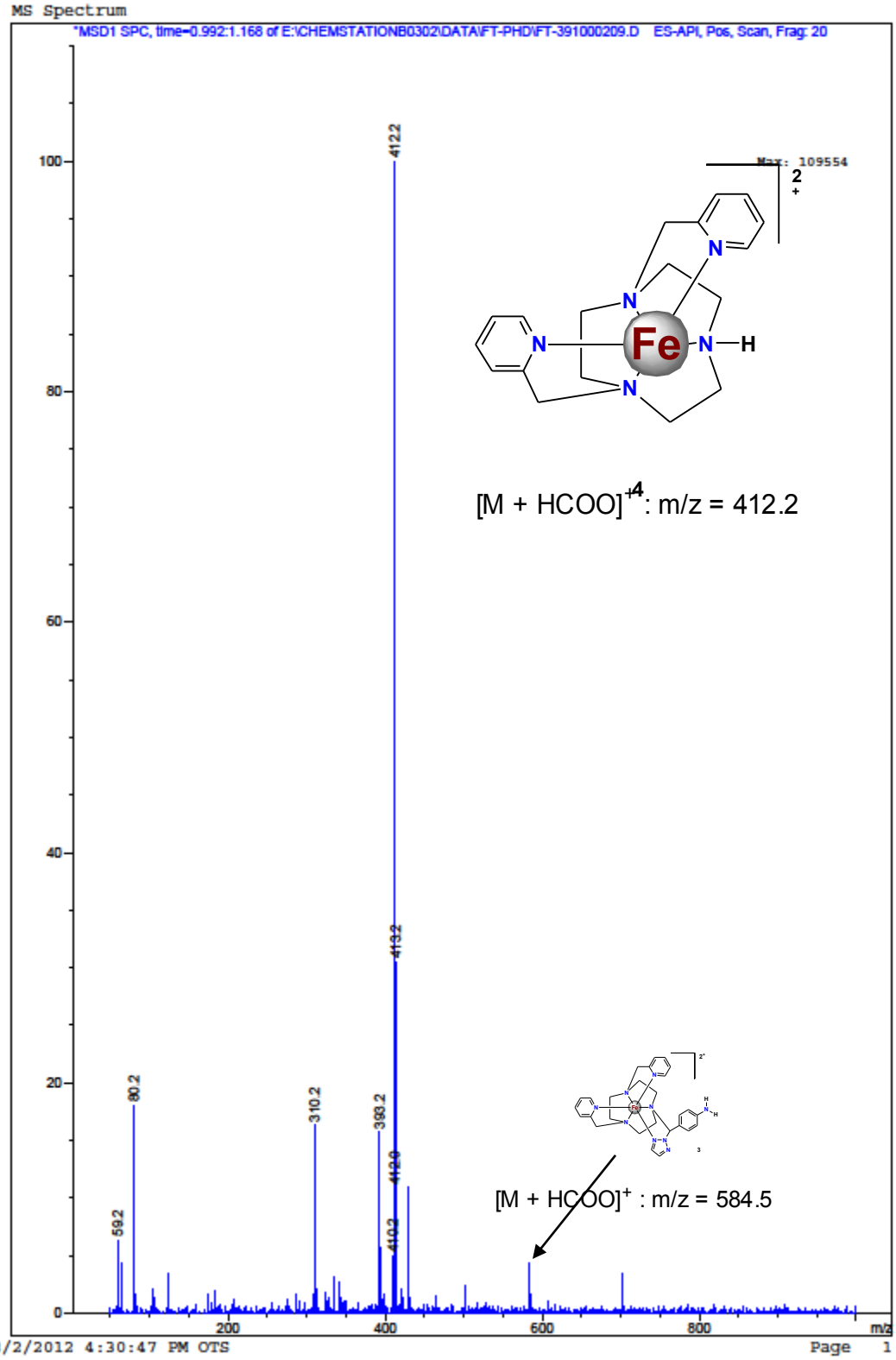
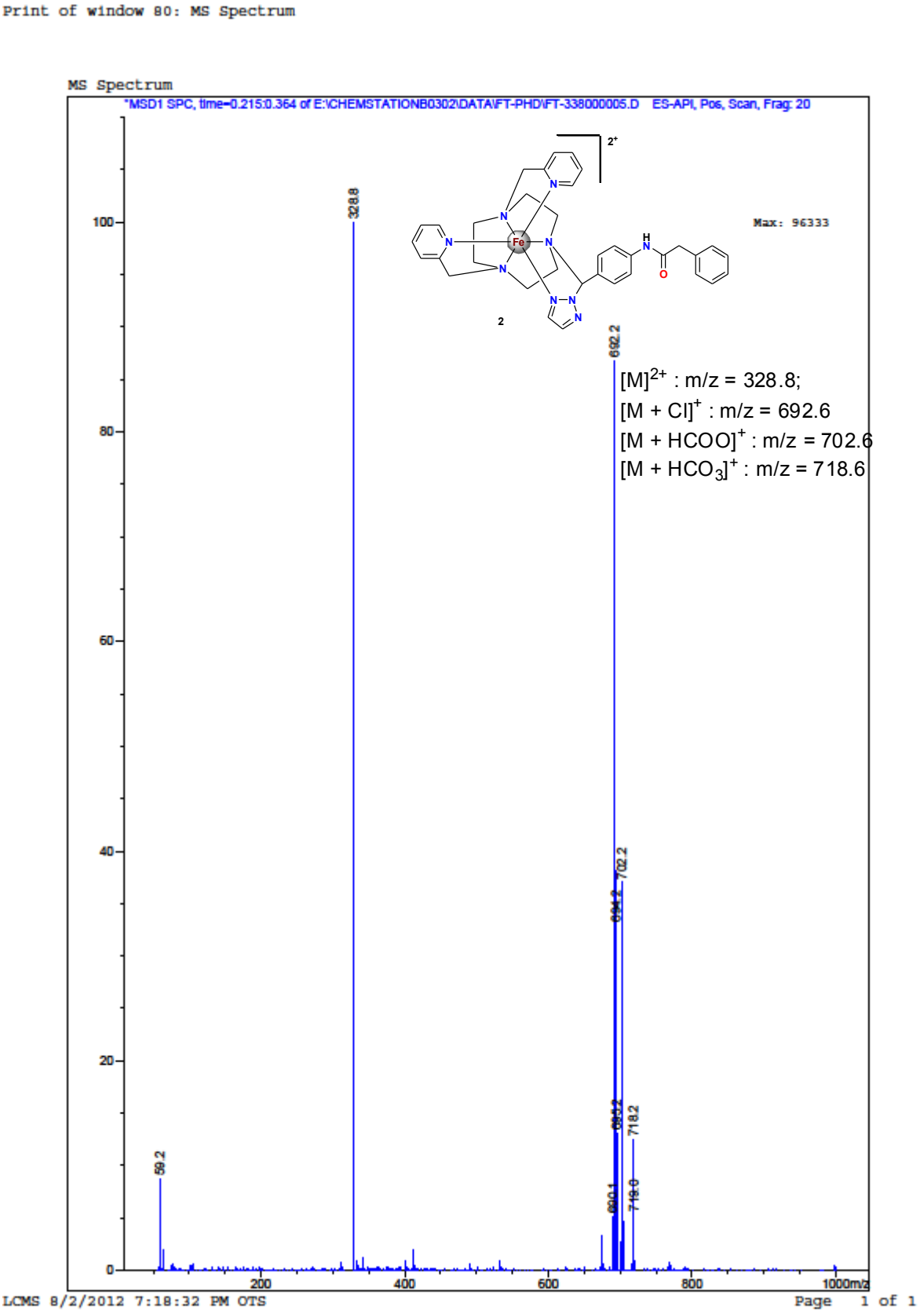


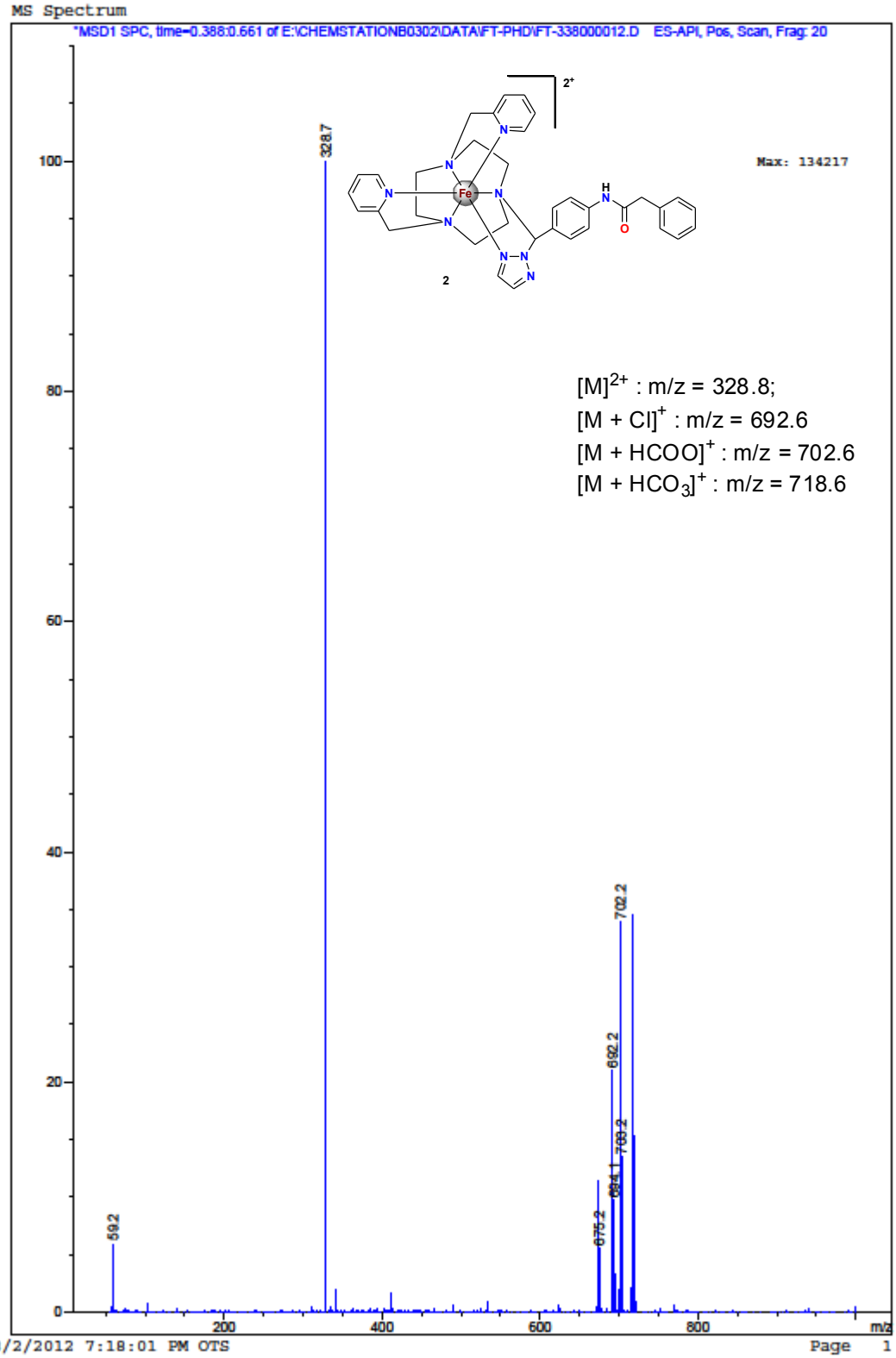
Figure S17 : Demonstration of robustness of **2** w/a PA (negative control)

t = 1 hour



t = 4 hours

Print of window 80: MS Spectrum



t = 15 hours

Print of window 80: MS Spectrum

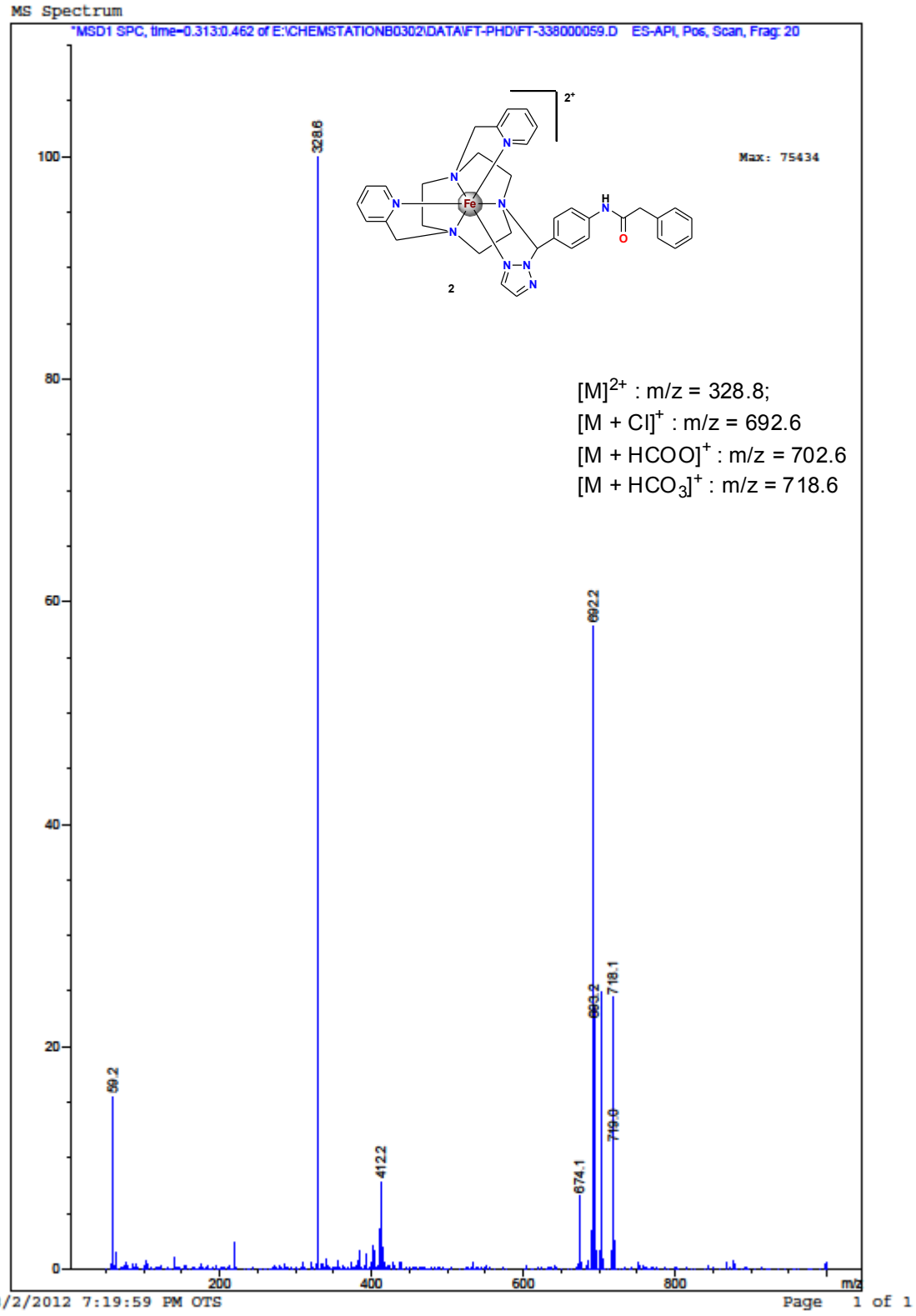
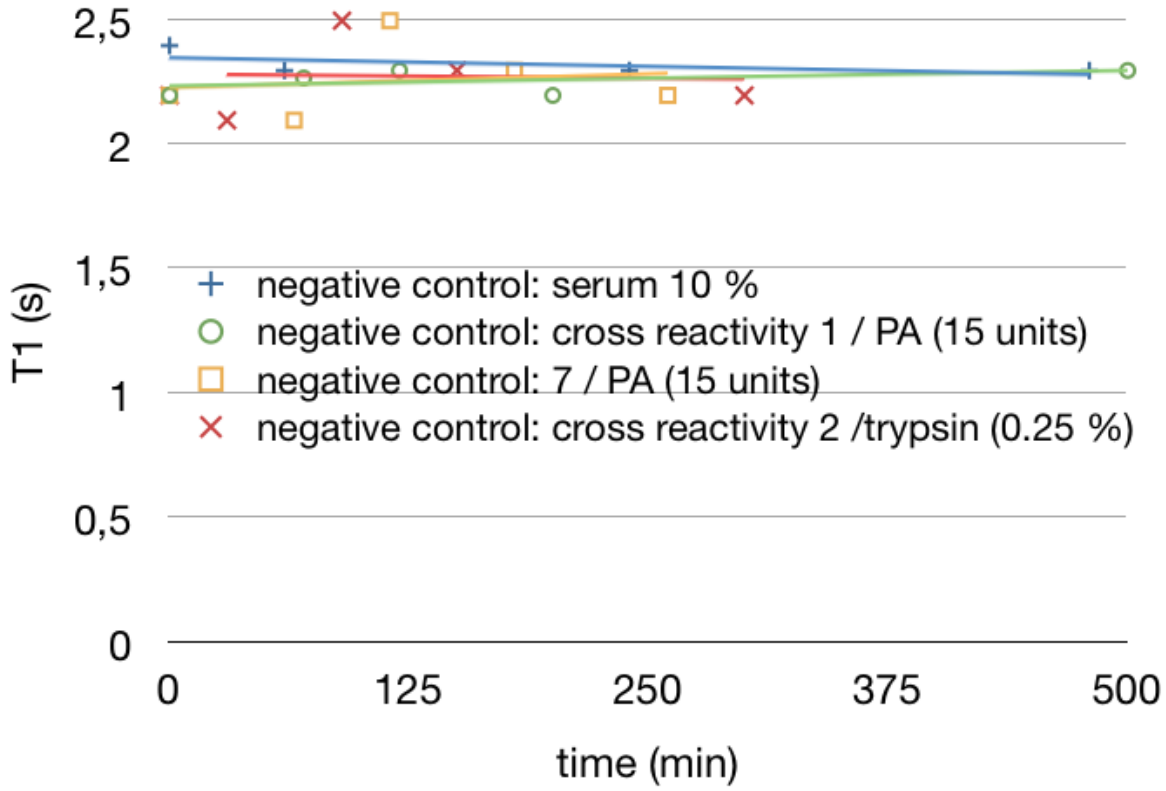


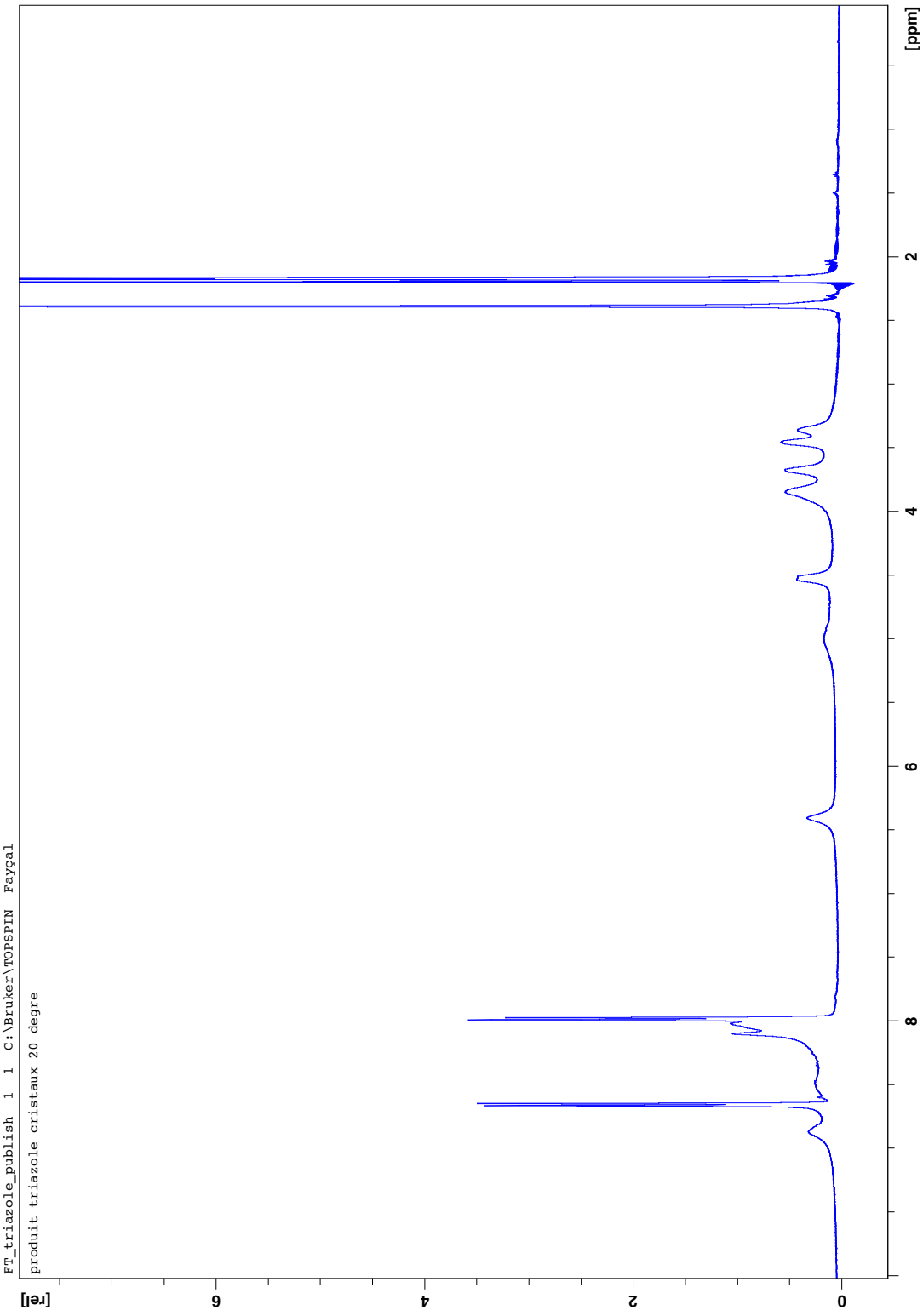
Figure S18. Proof of specificity of probe **2** for penicillin amidase (T1 monitoring).



NMR Appendix

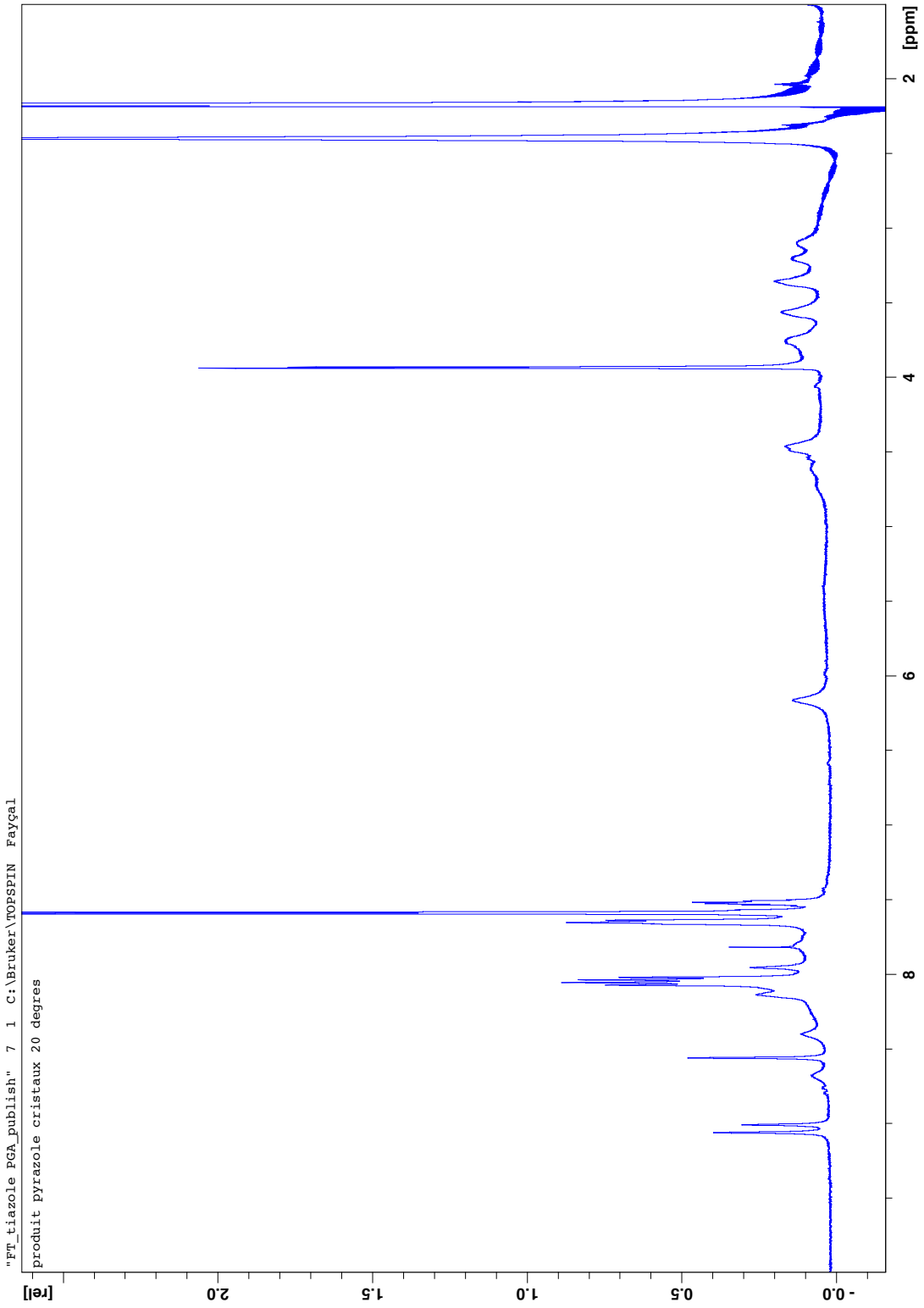
1: ^{1}H-NMR spectrum (500 MHz, CD_3CN)

dissolved crystals



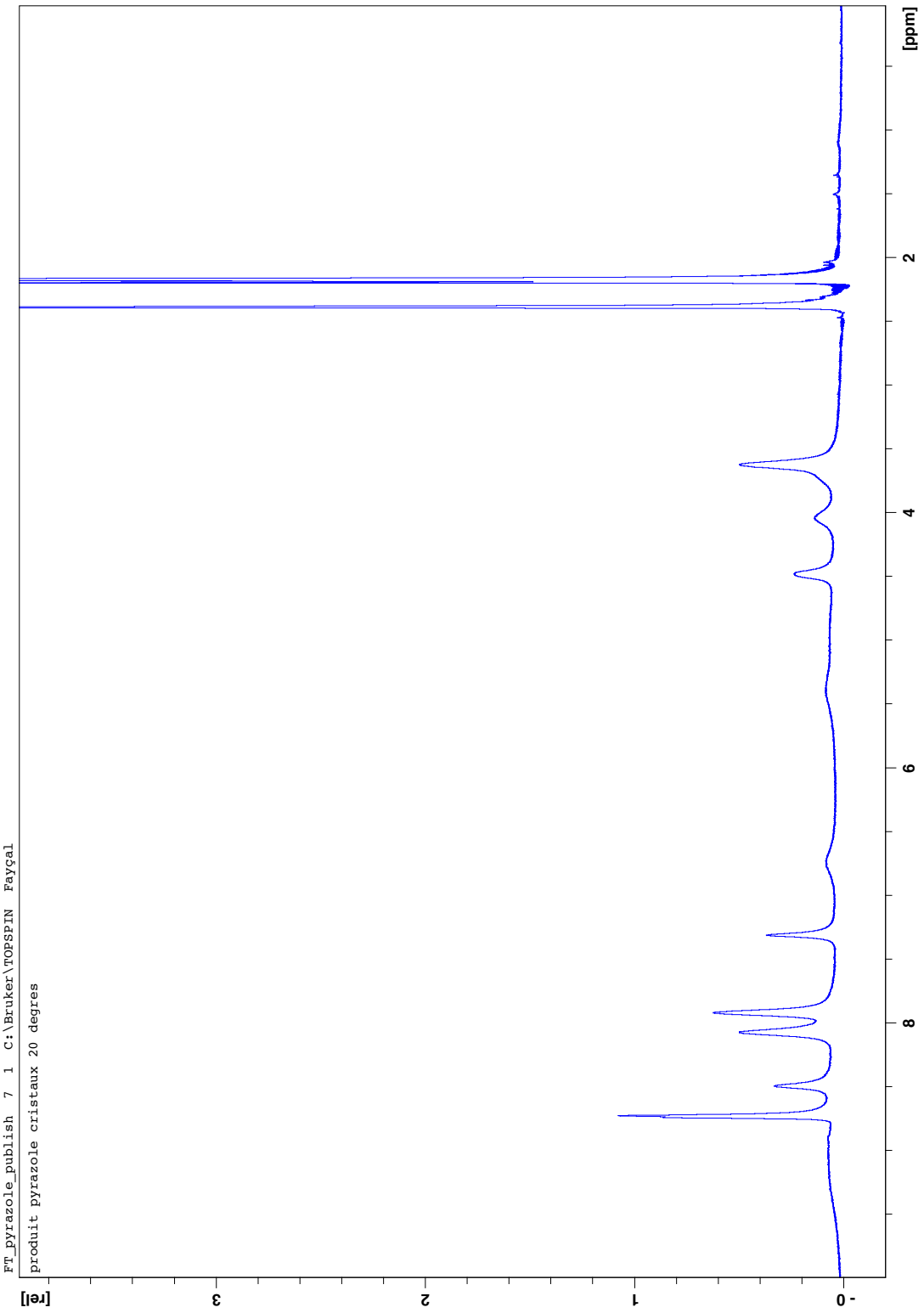
2: ^{1}H-NMR spectrum (500 MHz, CD_3CN)

(large high-field peaks : H_2O, MeCN); dissolved powder



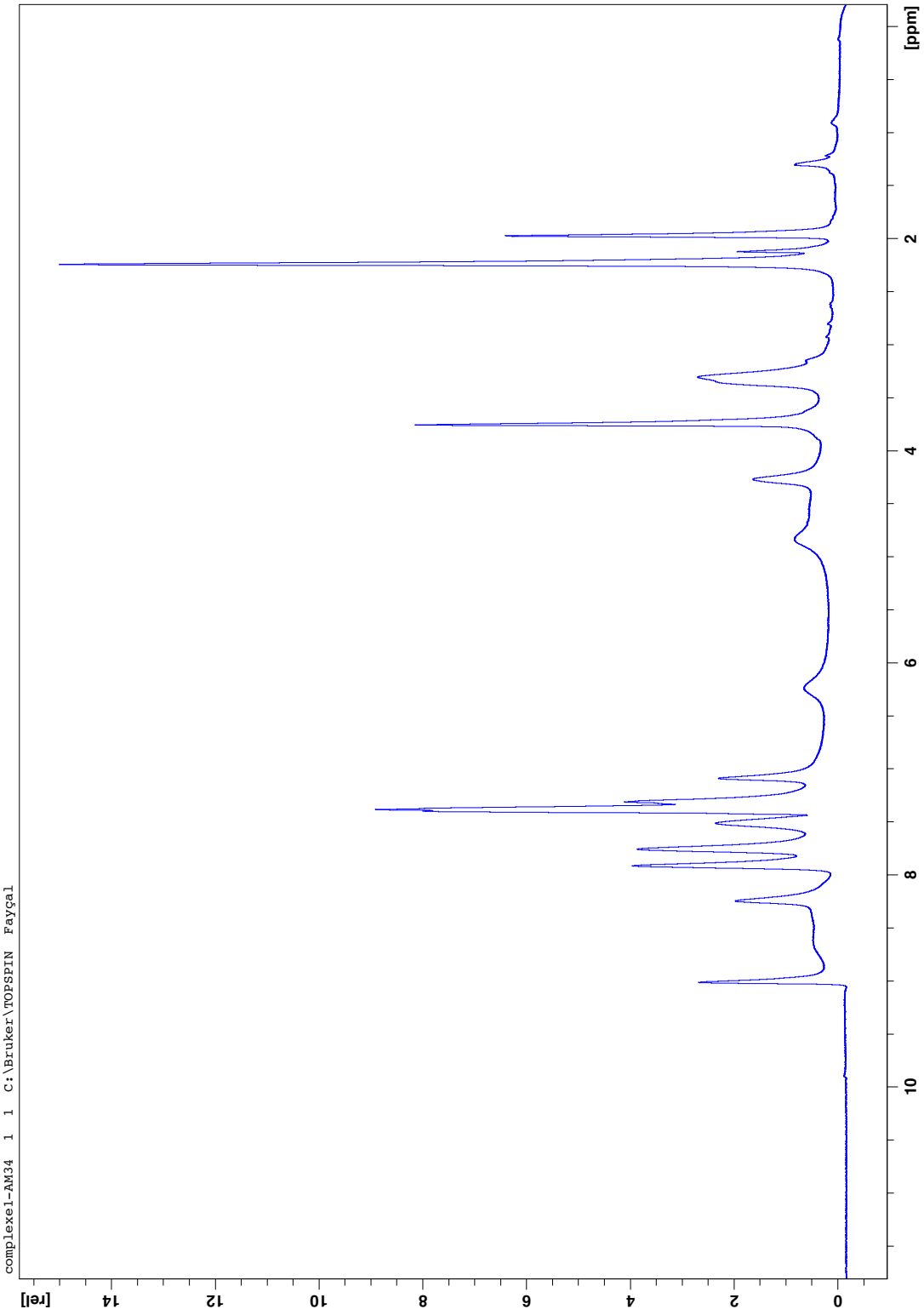
5: ^{1}H-NMR spectrum (500 MHz, CD_3CN)

(nitro –pyrazole) dissolved crystals



6: ^{1}H-NMR spectrum (500 MHz, CD_3CN)

(PGA –pyrazole) dissolved powder



7: ^{1}H-NMR spectrum (500 MHz, CD_3CN)

(nitro –Bt) dissolved crystals