Electronic Supplementary Material (ESI) for RSC Chemical Biology.

In vivo Active Organometallic-containing Antimycotic Agents

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Supporting information contains:

1. General

1.1 Materials. All chemicals were of reagent grade quality, obtained from commercial suppliers unless otherwise stated and used without further purification. Solvents were dried over molecular sieves. All preparations were carried out using standard Schlenk techniques, unless otherwise stated.

1.2 Instrumentation and Methods. Evaporation of the solvents *in vacuo* was done with a rotary evaporator at 40°C. Thin-layer chromatography (TLC) was performed using silica gel 60 F-254 (Merck) plates with detection of spots being achieved by exposure to UV light. ^{1}H and ^{13}C NMR spectra were recorded in deuterated solvents on Bruker AV-400 (^{1}H, 500 MHz; ^{19}F, 376 MHz) and AV-500, AV-501 (^{1}H, 500 MHz; ^{13}C, 126 MHz) spectrometers at room temperature. The chemical shifts, δ, are reported in ppm (parts per million). The residual solvent peaks have been used as an internal reference. The abbreviations for the peak multiplicities are as follows: s (singlet), d (doublet), t (triplet), m (multiplet). Elemental microanalyses were performed on a LECO TruSpec Micro elemental analyser. The UPLC-MS spectra were measured on an Acquity from Waters system equipped with a PDA detector and an auto sampler using an Agilent Zorbax 300SB-C18 analytical column (3.5 µm particle size, 300 Å pore size, 150 x 4.6 mm). This UPLC was coupled to an Esquire HCT apparatus from Bruker for the MS measurements. The UPLC run (flow rate 0.3 mL min-1) was performed with a linear gradient of A (distilled water containing 0.1% v/v formic acid) and B (acetonitrile, Sigma-Aldrich HPLC grade): t = 0 min, 0% B; t = 1 min, 0% B; t = 20 min, 66% B. Infrared spectra were recorded on a Bruker Vertex 70 FTIR spectrometer.

1.3 Yeast Culture. Wild type *S. cerevisiae* was freshly inoculated and cultured in autoclaved YPD buffer containing 2% bacto peptone, 1% bacto yeast extract and 2% glucose anhydrous at 30°C (Kuehner shaker Labtherm, Kuehner Switzerland).

1.4 Cell Culture. Human fibroblast (MRC-5) and retinal pigment epithelial (RPE) cell lines were cultured in F-10 medium supplemented with 10% fetal calf serum (FCS, Gibco), 100 U/mL penicillin, 100 µg/mL streptomycin or DMEM medium (Gibco) supplemented with 10% fetal calf serum (FCS, Gibco), 100 U/mL penicillin, 100 µg/mL streptomycin, respectively, at 37 °C and 6% CO_2. RPE and MRC-5 human cell lines were obtained from the Institute Curie in Paris and the University of Zurich, respectively.

1.5 Enzyme Inhibition Studies. Enzyme inhibition studies were performed on complex **2a** and Fluconazole by mean of the service provider Cyprotex GmbH, taking advantage protected

developed assay. More information about the assay can be found on https://www.cyprotex.com/admepk/in-vitro-metabolism/cytochrome-p450-inhibition/.

1.6 Uptake Studies.

Sample Preparation for ICP-MS. *S. cerevisiae* colony were inoculated in YPD medium in 5 mL culture tubes and cultured one day before treatment. Cultures were normalized to an OD of 0.1 and incubated with the target complexes (previously dissolved in DMSO as vehicle, v/v < 0.1%) at a concentration of 5 μM for 2 hours. Working concentration and incubation time have been chosen in order to avoid extended yeast mass lost due to the high cytotoxicity (which could cause bias) of the complexes but taking into account a Fe final amount that could afford a significant determination of the metal content. OD for any culture has been recalculated, the yeast centrifuged at 1500 g for 10 minutes and the supernatant was transferred to a fresh tube. The isolated samples were then lyophilized on an Alpha 2-4 LD plus (CHRIST). The resulting samples underwent chemical digestion with 5 mL of a 20% nitrohydrochloric acid solution for 24 h. The resulting solutions were filtered on 0.20 μm non-pyrogenic sterile Filtropur filters (Sarstedt) and added with 2% HNO_3 up to 10 mL. The obtained samples were injected in ICP-MS. All the samples were performed in triplicates. A negative control with the yeast cells added of DMSO (but without complex) has been performed in order to defined the basal values.

ICP-MS Studies. ICP-MS measurements were performed on an Agilent QQQ 8800 Triple quad ICP-MS spectrometer (Agilent Technologies) with a ASX200 autosampler (Agilent Technologies), equipped with standard nickel cones and a “micro-mist” quartz nebulizer fed with 0.3 ml/min analytic flow (as a 2% HNO_3 aqueous solution). Rhenium was measured against a Fe single element standard and verified by a control (Agilent5188-6524 PA Tuning 2). Iron content of the samples was determined by means of a 9-step serial dilution in the range between 0 and 300 ppb in Fe (R>0.99) with a background equivalent concentration of BEC: 5.3 ppt and a detection limit of DL: 1.9 ppt. The isotope Fe^{54} (5.85% abundance) Fe^{56} (91.75% abundance) was evaluated in “no-gas” mode and He-gas mode. Spiking the samples with

untreated negative controls (to account for eventual carbon content from the biological samples) resulted in equivalent values within error ranges. A solution of Indium (500 ppb) and Tungsten (500 ppb) was used as internal standard. The results are expressed as ng Fe ppb / OD, as mean ± standard deviation error of different independent experiments.

2. Chemical Synthesis

1-((2-(2,4-difluorophenyl)oxiran-2-yl)methyl)-1H-1,2,4-triazole (6). Compound **6** was synthesized following an adapted literature procedure.[1] In a 250 mL round-bottom flask, NaH (0.02 g, 0.79 mmol) was dispersed in dry DMSO (10 mL) and trimethylsulfoxonium iodide (0.17 g, 0.77 mmol) was added. The suspension was stirred at room temperature for 20 min, while the mixture became clear. 1-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-yl)ethan-1-one (0.14 g, 0.64 mmol) in dry DMSO (20 mL) was added and the reaction mixture was heated to 85°C for 5 h. After cooling the solution to room temperature, the reaction mixture was poured into cold H_2O (80 mL, 10°C). The product was extracted with EtOAc (2 x 50 mL) and the combined organic phases were washed with H_2O (50 mL), brine (50 mL), dried over $MgSO_4$, filtered and evaporated to give 1-((2-(2,4-difluorophenyl)oxiran-2-yl)methyl)-1H-1,2,4-triazole (**6**) as an orange oil, which was stored at -20°C and further used for the next reaction step within the next 16 h. Yield: 76% (0.12 g, 0.51 mmol). The spectroscopic data matched those reported.[1]

Ferrocenecarbaldehyde oxime (9a). The target compound was synthesized following an adapted literature procedure.[2] In a 50 mL round-bottom flask, ferrocene carboxaldehyde (0.30 g, 1.40 mmol), NaOH (0.34 g, 8.5 mmol) and hydroxylamine hydrochloride (0.39 g, 5.61 mmol) were dissolved in EtOH (15 mL). The solution was refluxed for 3 h and then cooled to room temperature. After dilution with H_2O (35 mL), the mixture was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phases were dried over $MgSO_4$, filtered and evaporated to obtain ferrocenecarbaldehyde oxime (**9a**) as an orange amorphous solid. Yield: 81% (260 mg, 1.13 mmol). The spectroscopic data matched those previously reported.[2]

Ferrocenylmethanamine (5a). Compound **5a** was synthesized following an adapted literature procedure.[2] In a 50 mL round-bottom flask, $LiAlH_4$ (0.23 g, 6.06 mmol) was dissolved in THF (8 mL). A solution of crude ferrocenecarbaldehyde oxime (0.26 g, 1.14 mmol) in THF (8 mL) was added dropwise and the mixture was refluxed for 3 h. The reaction was quenched with H_2O (25 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated to give ferrocenylmethanamine (**7a**) as a red oil. Yield: 89% (0.22 g, 1.01 mmol). The spectroscopic data matched those previously reported.[2]

1-Ferrocenyl-N-methylmethanimine (9b). Compound **9b** was synthesized following an adapted literature procedure.[2] In a 25 mL round-bottom flask, ferrocene carboxaldehyde (0.25 g, 1.17 mmol) was dissolved in 40% aqueous methylamine solution (4.8 mL, 55.45 mmol). After stirring at room temperature for 2 h, the product was extracted with Et_2O (2 x 30 mL). The combined organic phases were washed with H_2O (20 mL), dried over $MgSO_4$, filtered and

evaporated to give 1-ferrocenyl-N-methylmethanimine (**9b**) as a red crystalline solid, which was used for the next reaction step without further purification. Yield: 74% (0.25 g, 1.10 mmol). The spectroscopic data matched those previously reported.[2]

1-Ferrocenyl-*N*-methylmethanamine (5b). Compound **5b** was synthesized following an adapted literature procedure.[2] In a 50 mL round-bottom flask crude 1-ferrocenyl-*N*-methylmethanimine (0.25 g, 1.10 mmol) was dissolved in EtOH (30 mL). $NaBH_4$ (0.42 g, 11.10 mmol) was added, and the suspension stirred for 2 h at room temperature. After evaporation of the solvent, the residue was dissolved in Et_2O (40 mL) and the solution was washed with H_2O (30 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford 1-ferrocenyl-*N*-methylmethanamine (**5b**) as a red oil, which was used for the next reaction step without further purification. Yield: 76% (0.19 g, 0.83 mmol). The spectroscopic data matched those previously reported.[2]

1-Ferrocenyl-N-ethylmethanimine (7c). Compound **7c** was synthesized following an adapted literature procedure.[2] In a 50 mL round-bottom flask ferrocene carboxaldehyde (0.25 g, 1.17 mmol) was dissolved in 70% aqueous ethylamine solution (4.5 mL, 56.60 mmol). After stirring at room temperature for 3 h, the solvent was evaporated and the residue was dissolved in Et_2O (80 mL). After washing the solution with H_2O (2 x 30 mL), the organic phase was dried over MgSO4, filtered and evaporated to give 1-ferrocenyl-*N*-ethylmethanimine (**7c**) as a red crystalline solid, which was used for the next reaction step without further purification. Yield: 90% (0.25 g, 1.04 mmol). The spectroscopic data matched those previously reported.[2]

1-Ferrocenyl-*N*-ethylmethanamine (5c). Compound **5c** was synthesized following an adapted literature procedure.[2] In a 50 mL round-bottom flask, crude 1-ferrocenyl-*N*-ethylmethanimine (0.25 g, 1.04 mmol) was dissolved in EtOH (13 mL). $NaBH_4$ (0.42 g, 11.10 mmol) was added, and the suspension stirred for 2 h at room temperature. After evaporation of the solvent, the residue was dissolved in Et_2O (80 mL) and the solution was washed with H_2O (2 x 30 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford 1-ferrocenyl-*N*-ethylmethanamine (**5c**) as a red oil which was used for the next reaction step without further purification. Yield: 67% (0.17 g, 0.70 mmol). The spectroscopic data matched those previously reported.[2]

1-Ferrocenyl-N-isopropylmethanamine (5d). Compound **5d** was synthesized following an adapted literature procedure.[2] In a 50 mL round-bottom flask, crude 1-ferrocenyl-*N*-isopropylmethanimine (0.27 g, 1.08 mmol) was dissolved in EtOH (13 mL). $NaBH_4$ (0.42 g, 11.10 mmol) was added, and the suspension stirred for 2 h at room temperature. After

evaporation of the solvent, the residue was dissolved in Et_2O (60 mL) and the solution was washed with H_2O (2 x 30 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated to afford 1-ferrocenyl-*N*-isopropylmethanamine (**5d**) as a red oil which was used for the next reaction step without further purification. Yield: 89% (0.24 g, 0.96 mmol). The spectroscopic data matched those previously reported.[2]

1-((Ferrocenylmethyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (1).

A solution of 1-((2-(2,4-difluorophenyl)oxiran-2-yl)methyl)-1H-1,2,4-triazole (423 mg, 1.79 mmol), ferrocenylmethanamine (324 mg, 1.49 mmol) and Et_3N (0.42 mL, 3.00 mmol) in dry THF (15 mL) was refluxed for 7.5 h under nitrogen atmosphere. The solvent was evaporated, and the residue was purified by flash chromatography on silica with EtOAc:DCM:MeOH (40:1:1) as eluent system (R_f = 0.24, EtOAc:DCM:MeOH (40:1:1)) to obtain 1-((ferrocenylmethyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol **1** as an orange oil (293 mg, 0.64 mmol, 43%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (s, 1H), 7.82 (s, 1H), 7.59 – 7.52 (m, 1H), 6.87 – 6.77 (m, 2H), 4.63 (d, *J* = 14.3 Hz, 1H), 4.53 (d, *J* = 14.3 Hz, 1H), 4.09 – 4.05 (m, 4H), 4.02 (s, 5H), 3.39 (d, *J* = 13.4 Hz, 1H), 3.36 (d, *J* = 13.4 Hz, 1H), 3.25 (dd, *J* = 12.4, 1.5 Hz, 1H), 2.86 (dd, *J* = 12.4, 1.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.9 (dd, *J* = 249.9, 12.2 Hz), 159.1 (dd, *J* = 247.2, 11.7 Hz), 151.4, 144.8, 130.1 (dd, *J* = 9.1, 6.2 Hz), 125.2 (dd, *J* = 13.4, 3.5 Hz) 111.7 (dd, *J* = 20.6, 2.8 Hz), 104.4 (t, *J* = 26.5 Hz), 86.4, 73.5, 68.5, 68.3, 68.0, 56.0 (d, *J* = 4.9 Hz), 54.2 (d, *J* = 3.8 Hz), 49.2. ^{19}F NMR (376.5 MHz, $CDCl_3$): δ -108.92 (d, *J* = 7.9 Hz), -110.41 (d, *J* = 7.7 Hz).

1-((ferrocenylmethyl)(methyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (2).

A solution of 1-((2-(2,4-difluorophenyl)oxiran-2-yl)methyl)-1H-1,2,4-triazole (417 mg, 1.76 mmol), 1-ferrocenyl-*N*-methylmethanamine (443 mg, 1.94 mmol) and Et_3N (0.86 mL, 6.16 mmol) in dry EtOH (9.3 mL) was refluxed for 7 h, and the solvent was evaporated. The residue was purified by flash chromatography on silica with EtOAc:hexane:MeOH (22:22:1) as the eluent system (R_f = 0.22, EtOAc:hexane:MeOH (22:22:1)) to obtain 1-((ferrocenylmethyl)(methyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol **2**as an orange oil (481 mg, 1.03 mmol, 58%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.09 (s, 1H), 7.76 (s, 1H), 7.58 – 7.53 (m, 1H), 6.84 – 6.77 (m, 2H), 4.45 (d, *J* = 14.2 Hz, 1H), 4.40 (d, *J* = 14.3 Hz, 1H), 4.08 – 4.06 (m, 2H), 4.02 – 4.01 (m, 1H), 4.01 – 3.99 (m, 5H), 3.96 – 3.95 (m, 1H), 3.26 (d, *J* = 13.2 Hz, 1H), 3.14 (d, *J* = 13.2 Hz, 1H), 2.95 (dd, *J* = 13.5, 1.7 Hz, 1H), 2.67 (d, *J* = 13.5 Hz, 1H), 1.96 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 162.8 (dd, *J* = 249.5, 11.1 Hz), 159.0 (dd, *J* = 246.8, 10.8 Hz), 151.0, 144.8, 129.7, 126.6 (d, *J* = 11.4 Hz), 111.5 (d, *J* = 20.1

Hz), 104.2 (t, *J* = 26.2 Hz), 82.4, 71.9, 70.2, 69.7, 68.6, 68.3, 60.4, 58.3, 56.4, 43.5. ^{19}F NMR (376.5 MHz, $CDCl_3$): δ 108.49 (d, *J* = 7.4 Hz), -110.93 (d, *J* = 7.3 Hz).

1-((ferrocenylmethyl)(ethyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (3).

A solution of 1-((2-(2,4-difluorophenyl)oxiran-2-yl)methyl)-1H-1,2,4-triazole (90 mg, 0.38 mmol), 1-ferrocenyl-*N*-ethylmethanamine (85 mg, 0.35 mmol) and Et_3N (0.15 mL) in dry EtOH (4 mL) was refluxed for 7.5 h, and the solvent was evaporated. The residue was purified by flash chromatography on silica with EtOAc:cyclohexane:MeOH (22:22:1) as the eluent system (R_f = 0.33, EtOAc:cyclohexane:MeOH (22:22:1)) to obtain 1-((ferrocenylmethyl)(ethyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol **3** as an orange oil (64 mg, 0.133 mmol, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.79 (s, 1H), 7.65 – 7.56 (m, 1H), 6.91 – 6.79 (m, 2H), 4.48 (d, *J* = 14.2 Hz, 1H), 4.40 (d, *J* = 14.2 Hz, 1H), 4.07 (s, 2H), 4.02 (s, 1H), 3.97 (s, 5H), 3.91 (s, 1H), 3.20 (d, *J* = 13.7 Hz, 1H), 3.11 (d, *J* = 13.7 Hz, 1H), 3.06 (d, *J* = 13.8 Hz, 1H), 2.68 (d, *J* = 13.8 Hz, 1H), 2.26 (q, *J* = 8.0, 2H), 0.78 (t, *J* = 8.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.9 (dd, *J* = 249.7, 12.0 Hz), 159.1 (dd, *J* = 246.7, 11.6 Hz), 151.0, 145.0 , 129.8 (dd, *J* = 8.8, 6.4 Hz), 127.1, 111.6 (d, *J* = 20.3 Hz), 104.3 (t, *J* = 26.3 Hz), 82.4, 71.3, 70.7, 70.3, 69.7, 68.6, 68.2, 56.8 (dd, *J* = 50.1, 3.1 Hz), 54.0, 48.2, 41.1, 12.1. ^{19}F NMR (376.5 MHz, $CDCl_3$): δ -108.48, -110.90.

1-((ferrocenylmethyl)(isopropyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (4).

A solution of 1-((2-(2,4-difluorophenyl)oxiran-2-yl)methyl)-1H-1,2,4-triazole (90 mg, 0.38 mmol), 1-ferrocenyl-*N*-isopropylmethanamine (214 mg, 0.96 mmol) and Et_3N (0.32 mL) in dry EtOH (5.0 mL) was refluxed for 7.5 h. The solvent was evaporated, and the residue was purified by flash chromatography on silica with EtOAc:hexane (1:1) as the eluent system (R_f = 0.38, EtOAc:hexane (1:1)) to obtain 1-((ferrocenylmethyl)(isopropyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol **4** as an orange oil (70 mg, 0.14 mmol, 37%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.79 (s, 1H), 7.67 – 7.63 (m, 1H), 6.91 – 6.82 (m, 2H), 5.55 (br, 1H), 4.46 (d, *J* = 14.2 Hz, 1H), 4.36 (d, *J* = 14.2 Hz, 1H), 4.05 – 4.02 (m, 3H), 3.92 (s, 5H), 3.05 (s, 2H), 2.95 (dd, *J* = 13.9, 1.2 Hz, 1H), 2.71 (d, *J* = 14.0 Hz, 1H), 2.66 (sept, *J* = 6.4 Hz, 1H), 0.77 (d, *J* = 6.5 Hz, 3H), 0.71 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.8 (dd, *J* = 249.5, 12.1 Hz), 159.1 (dd, *J* = 246.8, 11.6 Hz), 151.0, 145.1, 129.7 (dd, *J* = 9.1, 6.1 Hz), 111.4 (dd, *J* = 20.5, 2.2 Hz), 104.3 (t, *J* = 26.3 Hz), 84.0, 70.7 (d, *J* = 5.6 Hz), 70.3, 69.2, 68.7, 68.6, 67.9, 56.5 (d, *J* = 4.6 Hz), 52.8, 50.9, 49.7, 20.5, 15.8. ^{19}F NMR (376.5 MHz, $CDCl_3$): δ -108.4, -111.0.

***N*-(ferrocenylmethyl)-2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propan-1-aminium chloride (1a).** In a 20 mL vial, 1-((ferrocenylmethyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (0.11 g, 0.24 mmol) was dissolved in acetone (4 mL) and 32% aq. HCl (26.5 µL, 0.27 mmol) was added. After 3 h of stirring, an orange precipitate formed which was separated from the supernatant by centrifugation and decantation. The orange powder was washed with acetone, Et_2O, and dried on high vacuum to obtain *N*-(cyclopentylmethyl)-2-(2,4-difluorophenyl)-2-hydroxy-3-(1H-1,2,4-triazol-1-yl)propan-1-aminium chloride (**1a**) as a yellow amorphous solid. Yield: 82 % (0.10 g, 0.20 mmol). IR (cm-1): 3105w, 1615m, 1500m, 1395w, 1270m, 1140s, 970m, 850s, 780w, 675s, 445s. ^{1}H NMR (400 MHz, MeOD): δ 8.29 (s, 1H), 7.90 (s, 1H), 7.65 – 7.56 (m, 1H), 7.10 – 6.96 (m, 2H), 4.69 (q, *J* = 14.6 Hz, 1H), 4.63 (d, *J* = 14.6 Hz, 1H), 4.39 – 4.28 (m, 4H), 4.20 (s, 5H), 4.02 (s, 2H), 3.56 (d, *J* = 13.1 Hz, 1H)., 3.32 (d, *J* = 13.1 Hz, 1H, overlapping with solvent residual peak identified in the HSQC spectrum).
^{13}C NMR (101 MHz, $CDCl_3$): δ 165.1 (dd, *J* = 248.8, 12.5 Hz), 161.0 (dd, *J* = 246.3, 12.5 Hz), 152.1, 146.4, 131.4 (dd, *J* = 10.0, 5.0 Hz), 130.0 (dd, *J* = 12.5, 3.8 Hz), 112.9 (dd, *J* = 20.0, 2.5 Hz). 105.9, 76.3, 73.6, 71.8, 71.0, 70.1, 56.5 (d, *J* = 5.0 Hz), 52.3 (d, *J* = 5.0 Hz).
^{19}F NMR (376 MHz, MeOD): δ -109.2 (d, *J* = 7.6 Hz), -110.9 (s). ESI-MS: m/z (%) = 452.1 ([M-HCl]+, 100), 198.9 ($C_{11}H_{10}Fe^+$, 25), 255.1 ($C_{11}H_{13}F_2N_4O^+$, 20). Elemental Analysis: calcd. for $C_{22}H_{23}N_4OF_2ClFe$ = C, 54.07; H, 4.74; N, 11.46. Found = C, 53.81; H, 4.71; N, 11.50.

***N*-(ferrocenylmethyl)-2-(2,4-difluorophenyl)-2-hydroxy-N-methyl-3-(1H-1,2,4-triazol-1-yl)propan-1-aminium chloride (2a).** In a 20 mL vial, 1-((ferrocenylmethyl)(methyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (0.14 g, 0.29 mmol) was dissolved in acetone (4 mL) and 32% aq. HCl (26.5 µL, 0.31 mmol) was added. After 3 h of stirring an orange precipitate formed which was separated from 32the supernatant by centrifugation and decantation. The residue was washed with acetone, Et2O and dried on high vacuum to obtain *N*-(ferrocenylmethyl)-2-(2,4-difluorophenyl)-2-hydroxy-Nmethyl-3-(1H-1,2,4-triazol-1-yl)propan-1-aminium chloride (**2a**) as a yellow-orange amorphous solid. Yield: 82 % (0.11 g, 0.22 mmol). IR (cm-1): 3105w, 2350w, 1615m, 1500m, 1425m, 1270m, 1210w, 1135m, 1020m, 965m, 845s, 675m, 485s. *A 1:1 mixture of conformers is observed leading to all signals in ^{1}H, ^{13}C and ^{19}F analyses to be doubled.* ^{1}H NMR (400 MHz, MeOD): δ 8.34 (s, 1H), 8.29 (s, 1H'), 7.94 (s, 1H), 7.92 (s, 1H'), 7.61 (td, *J* = 9.1, 6.3 Hz, 1H+1H'), 7.17 – 6.97 (m, 2H+2H'), 4.71 (d, *J* = 14.7 Hz, 1H), 4.68 (d, *J* = 14.8 Hz, 1H), 4.63 (d, *J* = 14.7 Hz, 1H'), 4.49 (d, *J* = 14.8 Hz, 1H'), 4.41 – 4.11 (m, 11H + 11H'), 4.01 – 3.94 (m, 2H), 3.72 (d, *J* = 13.9 Hz, 1H), 3.53 (d, *J* = 13.6 Hz, 1H), 3.46 – 3.42 (m, 2H), 2.85 (s, 3H), 2.48 (s, 3H'). ^{13}C NMR (101 MHz, MeOD): δ 165.0 (dd, *J* = 250.3, 12.4 Hz), 160.6 (dd, *J* = 247.2, 12.1 Hz), 152.3, 146.7, 131.1 (d, *J* = 34.5 Hz), 123.7 (d, *J* = 93.3 Hz), 113.2, 106.1, 74.8, 74.0, 73.3, 72.8, 72.3, 71.8, 71.5, 70.2,

60.6, 60.3, 59.6, 56.8, 44.5, 43.0. ^{19}F NMR (376 MHz, MeOD): δ -107.7, -108.9, -110.4, -110.6. The compound ESI-MS: m/z (%) = 467.1 ([M-Cl]$^+$, 100), 198.9 ($C_{11}H_{10}Fe^+$, 47), 269.1 ($C_{12}H_{14}F_2N_4O^+$, 20). Elemental Analysis: calcd. for $C_{23}H_{25}N_4OF_2ClFe \cdot H_2O$ = C, 53.05; H, 5.23; N, 10.76. Found = C, 52.87; H, 5.65; N, 10.33.

***N*-(ferrocenylmethyl)-2-(2,4-difluorophenyl)-2-hydroxy-N-ethyl-3-(1H-1,2,4-triazol-1-yl)propan-1-aminium chloride (3a).** In a 20 mL vial, 1-((ferrocenylmethyl)(ethyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (0.12 g, 0.25 mmol) was dissolved in acetone (4 mL) and 32% aq. HCl (25.4 µL, 0.26 mmol) was added. After 3 h of stirring, an orange precipitate formed which was separated from the supernatant by centrifugation and decantation. The orange powder was washed with acetone, Et_2O and dried on high vacuum to obtain syn/anti- N-(ferrocenylmethyl)-2-(2,4-difluorophenyl)-2-hydroxyN-ethyl-3-(1H-1,2,4-triazol-1-yl)propan-1-aminium chloride (**3a**) as a yellow amorphous solid. Yield: 93 % (0.11 g, 0.21 mmol). IR (cm-1): 3040w, 1615w, 1500m, 1415m, 1255w, 1145m, 970m, 850m, 640s, 500s. *A 1:1 mixture of conformers is observed leading to all signals in ^{1}H, ^{13}C and ^{19}F analyses to be doubled.* ^{1}H NMR (400 MHz, MeOD): δ 8.50 – 8.33 (brs, 2H), 7.94 (s, 1H+1H'), 7.76 – 7.60 (m, 1H+1H'), 7.24 – 7.02 (m, 2H+2H'), 4.83 – 3.57 (m, 17H), 3.22 – 2.86 (m, 2H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.07 (t, *J* = 7.1 Hz, 3H'). ^{13}C NMR (101 MHz, MeOD): δ 165.2 (dd, *J* = 250.5, 12.1 Hz), 160.6 (dd, *J* = 247.0, 12.3 Hz), 152.4, 147.0, 131.2 (m), 124.2 (m), 113.3 (d, *J* = 21.2 Hz), 106.07 (q, *J* = 26.9 Hz), 74.5, 74.1, 73.8, 73.4, 72.9, 72.6, 72.0, 71.5, 71.3, 71.3, 70.3, 70.1, 57.6, 56.6, 55.7, 51.8, 51.4, 24.2, 8.9. ^{19}F NMR (376 MHz, MeOD): δ -107.62 (d, *J* = 9.3 Hz), -108.70 (d, *J* = 9.7 Hz), -110.25.
ESI-MS: m/z (%) = 481.1 ([M-Cl]+, 100), 198.9 ($C_{11}H_{10}Fe^+$, 43), 283.1 ($C_{13}H_{17}F_2N_4O^+$, 16). Elemental Analysis: calcd. for $C_{24}H_{27}N_4OF_2ClFe \cdot 2\ H_2O$ = C, 52.14; H, 5.65; N, 10.13. Found = C, 52.00; H, 5.06; N, 9.90.

***N*-(ferrocenylmethyl)-2-(2,4-difluorophenyl)-2-hydroxy-N-isopropyl-3-(1H-1,2,4-triazol-1-yl)propan-1-aminium chloride (4a).** In a 20 mL vial, 1-((ferrocenylmethyl)(isopropyl)amino)-2-(2,4-difluorophenyl)-3-(1H-1,2,4-triazol-1-yl)propan-2-ol (0.09 g, 0.18 mmol) was dissolved in acetone (4 mL) and 32% aq. HCl (19.1 µL, 0.19 mmol) was added. After 3 h of stirring an orange precipitate formed which was separated from the supernatant by centrifugation and decantation. The orange powder was washed with acetone, Et_2O and dried on high vacuum to obtain *N*-(ferrocenylmethyl)-2-(2,4-difluorophenyl)-2-hydroxy-N-isopropyl-3-(1H-1,2,4-triazol-1-yl)propan-1-aminium chloride (**4a**) as a yellow amorphous solid. Yield: 77 % (0.07 g, 0.13 mmol). IR (cm-1): 3010w, 2255w, 1615w, 1495m, 1395m, 1270m, 1140s, 1090m, 970m, 830w, 680m, 495s. *A 1:1 mixture of conformers is observed leading to all signals in ^{1}H, ^{13}C and ^{19}F analyses to be doubled.* ^{1}H NMR (400 MHz, MeOD): δ 8.43 (s, 1H), 8.34 (s, 1H'), 7.95 (s, 1H),

7.89 (s, 1H'), 7.74 (td, *J* = 9.0, 6.1 Hz, 1H), 7.66 – 7.51 (m, 1H'), 7.32 – 7.16 (m, 2H), 7.14 – 6.95 (m, 2H'), 4.92 – 4.41 (m, 2H+2H'), 4.41 – 3.88 (m, 9H+9H'+2H+2H'), 3.78 – 3.37 (m, 1H+1H'+2H+2H'), 1.41 – 1.01 (m, 6H+6H'). ^{13}C NMR (101 MHz, MeOD): δ 165.2 (dd, *J* = 250.2, 12.2 Hz), 160.8 (dd, *J* = 224.1, 12.0 Hz), 152.3, 146.9, 131.57 (dd, *J* = 9.7, 5.1 Hz), 131.42 – 130.88 (m), 124.9 – 124.7 (m), 124.6 – 124.2 (m), 123.0 – 122.7 (m), 113.4 (d, *J* = 22.1 Hz), 112.9 (d, *J* = 21.7 Hz), 106.2 (q, *J* = 26.5 Hz), 105.7 (q, *J* = 26.5 Hz), 75.4, 74.5, 73.8 (d, *J* = 35.9 Hz), 73.2, 72.7 (d, *J* = 27.1 Hz), 72.2, 71.9, 71.4, 71.2 (d, *J* = 5.4 Hz), 70.2 (d, *J* = 17.0 Hz), 58.2, 56.5, 55.6, 54.7 (d, *J* = 33.1 Hz), 53.9, 53.0, 50.8, 18.6 (d, *J* = 11.9 Hz), 18.3, 16.7, 14.8. ^{19}F NMR (376.5 MHz, MeOD) δ -107.5 (q, *J* = 10.2 Hz), -108.5 (q, *J* = 10.8, 10.2 Hz), -109.4 (q, *J* = 10.2 Hz), -110.0 (t, *J* = 8.3 Hz), -110.4 (t, *J* = 8.2 Hz), -111.0 (p, *J* = 8.1 Hz). ESI-MS: m/z (%) = 495.1 ([M-Cl]+, 100), 198.9 (C11H10Fe+, 40), 297.2 ($C_{14}H_{19}F_2N_4O^+$, 17). Elemental Analysis: calcd. for $C_{25}H_{29}N_4OF_2ClFe$ = C, 56.57; H, 5.51; N, 10.55. Found = C, 56.43; H, 5.61; N, 10.61.

3. Biological studies

3.1 Toxicity towards Fungi. The activity of compounds **1a-4a** on *S. cerevisiae* was evaluated via a newly established colony formation assay.[3] Briefly, one day before treatment an aliquot of wild type *S. cerevisiae* was inoculated in 5 mL YPD buffer solution and incubated overnight at 30°C. The fungal proliferation was then quantified at 600 nm in a Cary60 UV/Vis (Agilent Technologies). The SC was then seeded at different concentrations of the yeast (representing OD of 0.2, 0.02, 0.002 and 0.0002 for different colony growth rate) in 24-wells plates containing 2 mL growing terrain. The growing terrain was composed by YPD containing 2% of agar, autoclaved and kept at 50°C (water bath, Kotterman AG). Just before use, the growing terrain was treated with increasing concentrations of the new ferrocenyl complexes or with fluconazole (used for comparison) and poured in the different wells, before solidification. The treated plates were loaded with *S. cerevisiae* and incubated for 24 h at 30°C. The fungal colony formation was monitored with Alpha Digitec camera (Bucher Biotec) and the colony density was calculated with AlphaImager software (v1.3.0.7) with multiplex band analysis mode / single tool. A series of blanks (with the terrain not seeded with *S. cerevisiae*), negative controls (with the terrain seeded with *S. cerevisiae* and treated just DMSO as vehicle) and Clotrimazole as a positive control were performed. The results expressed as mean ± standard error of independent experiments.

3.2 ROS Level Determination. The evaluation of intracellular ROS levels was performed following a method recently established by our group and re-adapted for fungal cultures.[3] The ROS determination was detected by the use of 2′,7'-dichlorofluorescin diacetate (H_2DCF-DA,

Sigma-Aldrich), a cell-permeable non-fluorescent probe, which is hydrolyzed in cell and, which upon oxidation, turns to highly fluorescent 2′,7’-dichlorofluorescein.[3] Briefly, an aliquot of SC was inoculated in 5 mL YPD buffer one day before treatment and incubated overnight at 30 °C under gentle shaking (180 rpm) in a Kuehner shaker Labtherm incubator (Kuehner, Switzerland). The culture was then diluted to an OD of 0.005, added with 10 µM of **1a – 4a** and incubated further for different time frame (1 h, 6 h and 18 h). The YPD medium was then replaced with fresh medium containing H_2DCF-DA (final concentration 20 µM) and further incubated for 1 h at 30 °C under gentle shaking. The OD of the fungal growth was re-measured, the suspension was plated at different dilutions in duplicate in a 96-wells plate and fluorescence was quantified at 530 nm emission with 488 nm excitation wavelength in a SpectraMax M5 microplate Reader. The results, expressed as mean and standard error of different independent experiments, were corrected for the fungal population. Fluconazole (50 µM) was used for comparative purposes and H_2O_2 at final concentration of 10 µM was taken as positive control.

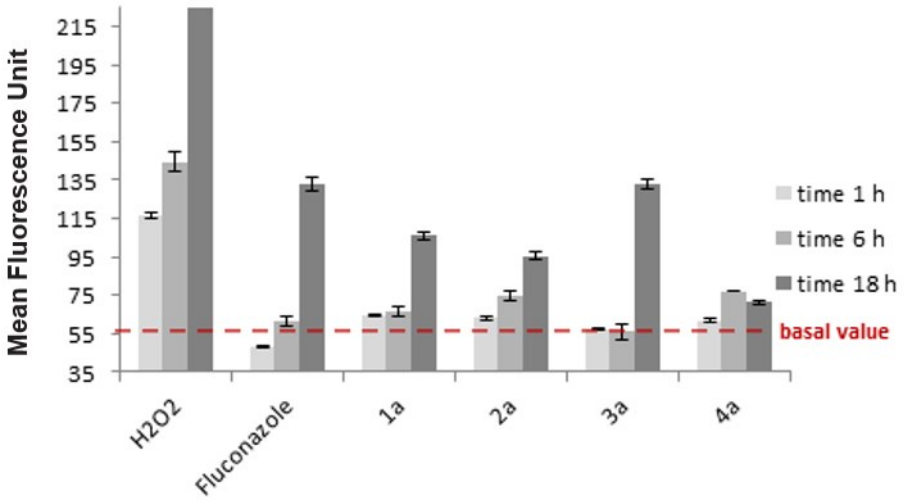


Figure S1. ROS levels in *S. cerevisiae* colonies upon treatment with the new fluconacenes. Fluconazole and H_2O_2 were used as positive controls. The basal value is the ROS level value present in the untreated population, which produces some endogenous ROS.

3.3 *In vitro* antifungal susceptibility assay. Clinical isolates of *Candida* strains (*C. albicans* MFB005FS3, *C. albicans* SC5314, *C. albicans* YMS 102_2, *C. albicans* YMS 102-6, *C. parapsilosis* MFB005FS5, *C. parapsilosis* MFB011 N1, *C. glabrata* MFB004, *C. glabrata* RTT99_3 and *C. tropicalis* RTT35-1, *C. tropicalis* RTT35-3, *Penicillium paneum* MFB042 N1, *Aspergillus glaucus* MFB027 N1, *Trichosporon asahii* MFB034 N1) were tested for their susceptibility to compound **2a** and fluconazole.[4] Yeasts were grown on Sabouraud agar medium (Sigma Aldrich, St. Louis, MO, USA) for 48 h at 30°C and resuspended in distilled water at a concentration of 1-5 x 10^5 CFU/ml before testing. Minimal inhibitory concentration

(MIC) values for compound **2a** and fluconazole were determined following the European Committee for Antimicrobial Susceptibility Testing protocol (EUCAST Definitive Document EDef 7.2 Revision, 2012)[5] as described by Tocci *et al*.[6] Briefly, cells were grown in RPMI-1640 medium supplied with 2.0% glucose, counted and inoculated at a concentration of 1-5 x 10^5 CFU/ml. MIC_{50} values were detected using a spectrophotometer (at 530 nm) after 48 h of incubation, as the lowest concentration of the drug that resulted in a ≥ 50% inhibition of growth, relative to the control. For compound **2a**, 10 dilution series, ranging from 0 μM to 1 μM were tested. Fluconazole was tested in the range of 0 μM to 100 μM.

Growth curve assay.

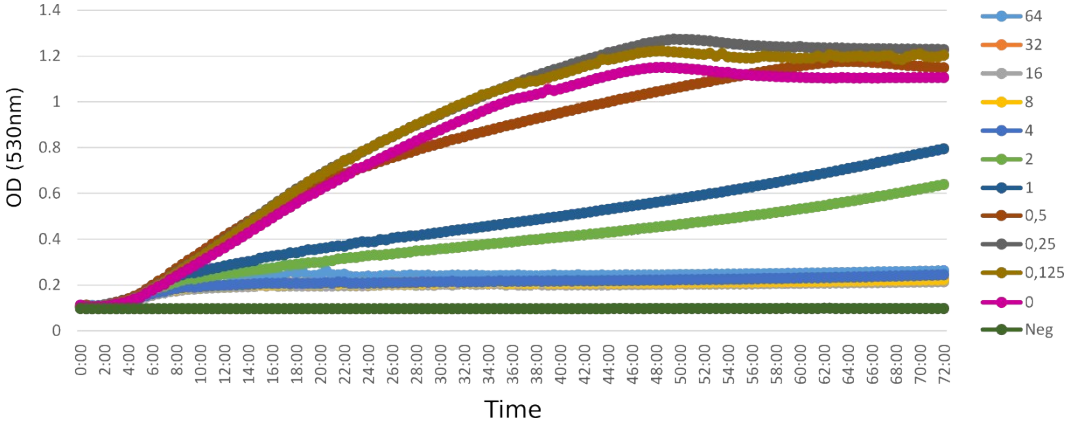


Figure S2. Growth curves of the *C. albicans* reference strain SC5314. Cells (inoculum 1-5 x 105 CFU/ml) were grown at 30°C for 72h in RPMI-1640 medium supplied with 2.0% glucose in 10 dilution series with increasing concentrations of compound **2a**, ranging from 0,125μg/ml to 64μg/ml. The curve corresponding to 50% of growth inhibition after 48hours (1μg/ml) did not reach a plateau but linearly increased growth till 72h, indicating a fungistatic effect of compound **2a** at MIC_{50} concentration for the strain SC5314.

3.4 Infection and treatment. *Candida albicans* strain (SC-5314) was grown in Sabouraud medium (0.2% yeast extract, 2% glucose, 0.2% bovine serum albumin). Female C57BL/6 mice were infected intravenously (i.v.) with 1×10^6 yeasts and monitored for fungal growth, tissue pathology and cytokine production. Fluconazole at the dose of 10 mg/kg and compound 2a, diluted in 5% dimethyl sulfoxide in PBS, at the doses of 1 and 10 mg/kg, were administered intragastrically (i.g.) for three consecutive days, beginning the day of the infection until the sacrifice of the animals (4 days after the infection). Controls received the diluent alone. The efficacy of the treatment was evaluated in terms of fungal growth in the target organs (kidneys and liver), inflammatory pathology in kidney and colon and inflammatory cytokines levels in kidney homogenates. To this end, the kidney and the liver were aseptically removed and homogenized in sterile PBS using a tissue homogenizer (IKA T18, Ultra Turrax). Quantification of fungal growth (colony forming units, CFU, per organ) was done by serially diluting

homogenates and plating them in triplicate in Sabouraud agar. For histology, sections (3–4 μm) of paraffin-embedded tissues were stained with periodic acid-Schiff (PAS) reagent. Kidneys were assessed for the presence of inflammatory cells including neutrophils and lymphocytes/plasma cells and scored as follows: score 0 = no inflammation, score 1 = < 3 foci of inflammation, score 2 = 4 to 6 foci of inflammation, score 3 = > 6 foci of inflammation, but less than 25% of kidney affected, score 4 = > 25% of kidney affected. Scoring was performed in a blinded fashion. Four histological components were assessed in the colon: 'inflammation extent', 'damage in crypt architecture', 'hyperemia/edema', 'grade of accumulation with inflammatory cells'. The colonic sections were scored from 0 to 3 points for each parameter. The total histological score, ranging from 0 to 12, was obtained by summing the four histological components scores. The concentration of cytokines was measured in kidney homogenates using commercially available ELISAs (R&D systems) according to the protocols supplied by the manufacturer. Statistical analyses were performed with one-way ANOVA. Data, from three experiments using 7 mice/group, were expressed as mean ± SD and were analyzed in triplicate using GraphPad Prism Software. Mouse experiments were performed according to Italian Approved Animal Welfare Authorization 360/2015-PR and Legislative Decree 26/2014 regarding the animal license obtained by the Italian Ministry of Health lasting for 5 years (2015–2020). Animals were treated as the guidelines of EAMC.

3.5 Chemogenomic Studies. Frozen aliquots of Yeast Deletion Heterozygous Diploid Pools (Cat. no.95401.H4Pool) were recovered for 10 generations and logarithmically growing cells were diluted to a final OD600 of 0.06 (=10^5 cells/ml) in YPD containing 1% DMSO or compound. The compound was applied at a dose of 1 μM corresponding to 10%–20% wild-type growth inhibition. Cells were grown in a Synergy 2 Multimode Microplate reader (BioTek), harvested after 20 generations of growth and frozen at -20°C for subsequent preparation of DNA. Genomic DNA was purified by Phenol:Chloroform: Isoamylalcohol extraction (including RNASe digestion). DNA quality was assessed via agarose gel electrophoresis and UV-Vis spectroscopy. For each condition (control or compound) 4 biological replicates were generated.

A two-step PCR protocol for efficient multiplexing of Bar-seq libraries was applied as previously described with the following modifications.[7] In a first step, UPTAGs and DNTAGs from a single sample were amplified using the primers Illumina UPTAG Index and Illumina UPkanMX and Illumina DNTAG Index and Illumina DNkanMX in separate PCR reactions. Illumina UPTAG and Illumina DNTAG primers contain a 5-bp sequence that uniquely identifies the sample. A complete list of primer sequences is provided in Table S2. Genomic DNA was normalized to 10ng/ml and 100ng were used as template for amplification of barcodes using FastStart High Fidelity PCR system (Roche Diagnostics), applying the following PCR program: 4 min at 95°C

followed by 30 cycles of 30 sec at 95°C, 30 sec at 50°C, 30 sec at 72°C, and a final extension step of 7 min at 72°C. PCR products were confirmed on 2% agarose gels and purified using the Agencourt AMPure XP system (Beckman Coulter). Purified PCR products were quantified using the Quant-iT dsDNA kit (Invitrogen) and 60ng from each of the 4 different UPTAG libraries and, in a separate tube, 60 ng from each of the 4 different DNTAG libraries were combined. The multiplexed UPTAG libraries were then amplified using the primers P5 and Illumina UPkanMX, and the combined DNTAG libraries were amplified using the P5 and IlluminaDNkanMX primers (Table S2) and the PCR program: 4 min at 95°C followed by 25 cycles of 10 sec at 95°C, 10 sec at 50°C, 10 sec at 72°C, and a final extension step of 3 min at 72°C. The 140-bp UPTAG and DNTAG libraries were purified using the Agencourt AMPure XP system, quantified using the Quant-iT dsDNA kit and combined in equimolar amounts. The library was sequenced on an Illumina HiSeq 2000 using standard methods, including the use of the standard Illumina sequencing primer (5'-ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT-3'). The qseq files for each of the 4 samples are available from the European Nucleotide Archive (ENA) with the accession number PRJEB27820.

Read matching was performed using an in-house developed Python script, which is available on Github (https://github.com/marcomoretto/Bar-seq). A Levenshtein distance of 2 was used to allow mismatches between reads. Reads perfectly matching multiple barcodes were removed from the analysis. The final count matrix has been obtained summing up the UPTAG and DNTAG, removing barcodes with zeros across all samples and matches with low-count since they are more likely due to sequencing noise. The statistical analysis was performed with the voom transformation package[8] that estimates the mean–variance relationship of log counts, generating a precision weight for each observation that is fed into the limma empirical Bayes analysis pipeline.[9] A double threshold based on both P-value (<0.01) and expression log2 fold change (>1.5) was imposed to identify barcodes differentially abundant through pairwise comparison (Figure 3).

Table S1. Top ten sensitive and resistant deletion mutants.

Gene	Chr	logFC	P.Value	Annotation
Sensitive mutants				
ERG11	8	-5.4	2.79E-07	Lanosterol 14-alpha-demethylase; catalyzes C-14 demethylation of lanosterol to form 4,4''-dimethyl cholesta-8,14,24-triene-3-beta-ol in ergosterol biosynthesis pathway; transcriptionally down-regulated when ergosterol is in excess; member of cytochrome P450 family; associated and coordinately regulated with the P450 reductase Ncp1p; human CYP51A1 functionally complements the lethality of the erg11 null mutation
GSP1	12	-4.4	0.0001	Ran GTPase; GTP binding protein (mammalian Ranp homolog) involved in the maintenance of nuclear organization, RNA processing and transport; regulated by Srm1p, Rna1p, Yrb1p, Yrb2p, Yrp4p, Yrb30p, Cse1p and Kap95p; GSP1 has a paralog, GSP2, that arose from the whole genome

				duplication
MED4	15	-3.8	0.0003	Subunit of the RNA polymerase II mediator complex; associates with core polymerase subunits to form the RNA polymerase II holoenzyme; essential for transcriptional regulation
SET6	16	-3.5	2.36E-08	SET domain protein of unknown function; deletion heterozygote is sensitive to compounds that target ergosterol biosynthesis, may be involved in compound availability
PDR16	14	-3.3	4.46E-07	Phosphatidylinositol transfer protein (PITP); controlled by the multiple drug resistance regulator Pdr1p; localizes to lipid particles and microsomes; controls levels of various lipids, may regulate lipid synthesis; homologous to Pdr17p; protein abundance increases in response to DNA replication stress
PDR5	15	-2.8	1.04E-05	Plasma membrane ATP-binding cassette (ABC) transporter; multidrug transporter actively regulated by Pdr1p; also involved in steroid transport, cation resistance, and cellular detoxification during exponential growth; PDR5 has a paralog, PDR15, that arose from the whole genome duplication
HXT12	9	-2.8	0.0003	Possible pseudogene in strain S288C; YIL170W/HXT12 and the adjacent ORF, YIL171W, together encode a non-functional member of the hexose transporter family
SLD5	4	-2.6	0.0002	Subunit of the GINS complex (Sld5p, Psf1p, Psf2p, Psf3p); complex is localized to DNA replication origins and implicated in assembly of the DNA replication machinery
RPS5	10	-2.5	0.039	Protein component of the small (40S) ribosomal subunit; least basic of non-acidic ribosomal proteins; phosphorylated in vivo; essential for viability; homologous to mammalian ribosomal protein S5 and bacterial S7
ARC1	7	-2.5	0.0094	Protein that binds tRNA and methionyl- and glutamyl-tRNA synthetases; involved in tRNA delivery, stimulating catalysis, and ensuring localization; also binds quadruplex nucleic acids; protein abundance increases in response to DNA replication stress; methionyl-tRNA synthetase is Mes1p; glutamyl-tRNA synthetase is Gus1p
Resistant mutants				
PMP1	3	4.3	0.004	Regulatory subunit for the plasma membrane H(+)-ATPase Pma1p; small single-membrane span proteolipid; forms unique helix and positively charged cytoplasmic domain that is able to specifically segregate phosphatidylserines; PMP1 has a paralog, PMP2, that arose from the whole genome duplication
IOC2	12	3.9	0.00088	Subunit of the Isw1b complex; exhibits nucleosome-stimulated ATPase activity and acts within coding regions to coordinate transcription elongation with termination and processing; contains a PHD finger motif; other complex members are Isw1p and Ioc4p
HEH2	4	3.8	0.0012	Inner nuclear membrane (INM) protein; contains helix-extension-helix (HEH) motif, nuclear localization signal sequence; targeting to the INM requires the Srp1p-Kap95p karyopherins and the Ran cycle; HEH2 has a paralog, SRC1, that arose from the whole genome duplication
DSD1	7	3.3	0.0098	D-serine dehydratase (aka D-serine ammonia-lyase); converts D-serine to pyruvate and ammonia by a reaction dependent on pyridoxal 5'-phosphate and zinc; may play a role in D-serine detoxification; L-serine is not a substrate
TPS1	2	3.2	0.0054	Synthase subunit of trehalose-6-P synthase/phosphatase complex; synthesizes the storage carbohydrate trehalose, which is critically important for survival of long-term desiccation; also found in a monomeric form; expression is induced by the stress response and repressed by the Ras-cAMP pathway; protein abundance increases in response to DNA replication stress and in response to prolonged exposure to boric acid
MSP1	7	2.9	0.014	Highly-conserved N-terminally anchored AAA-ATPase; distributed in the mitochondrial outer membrane and peroxisomes; involved in mitochondrial protein sorting; functions as an extraction engine in local organelle surveillance to remove and initiate degradation of mistargeted proteins, ensuring fidelity of organelle-specific localization of tail-anchored proteins; contains an N-terminal transmembrane domain and C-terminal cytoplasmic ATPase domain
MUB1	13	2.8	0.01	MYND domain-containing protein; component of the Mub1p-Ubr2p-Rad6p ubiquitin ligase complex, required for ubiquitination and degradation of Rpn4p; interacts with Ubr2p (E3) and indirectly with Rad6p (E2); short-lived protein degraded in a Ubr2p/Rad6p dependent manner; proposed to function as both a partner and substrate of the Ubr2p/Rad6p ubiquitin ligase; similar to the A. nidulans samB gene

NEL1	8	2.8	0.052	Activator of Sar1p GTPase activity; paralog of Sec23 but does not associate with the COPII components; not an essential gene
CTS1	12	2.7	0.073	Endochitinase; required for cell separation after mitosis; transcriptional activation during the G1 phase of the cell cycle is mediated by transcription factor Ace2p
RMD5	4	2.7	0.027	Component of GID Complex that confers ubiquitin ligase (U3) activity; necessary for polyubiquitination and degradation of the gluconeogenic enzyme fructose-1,6-bisphosphatase; forms dimer with Fyv10p that is then recruited to GID Complex by Gid8p; also required for sporulation; conserved protein that has a degenerate RING finger domain

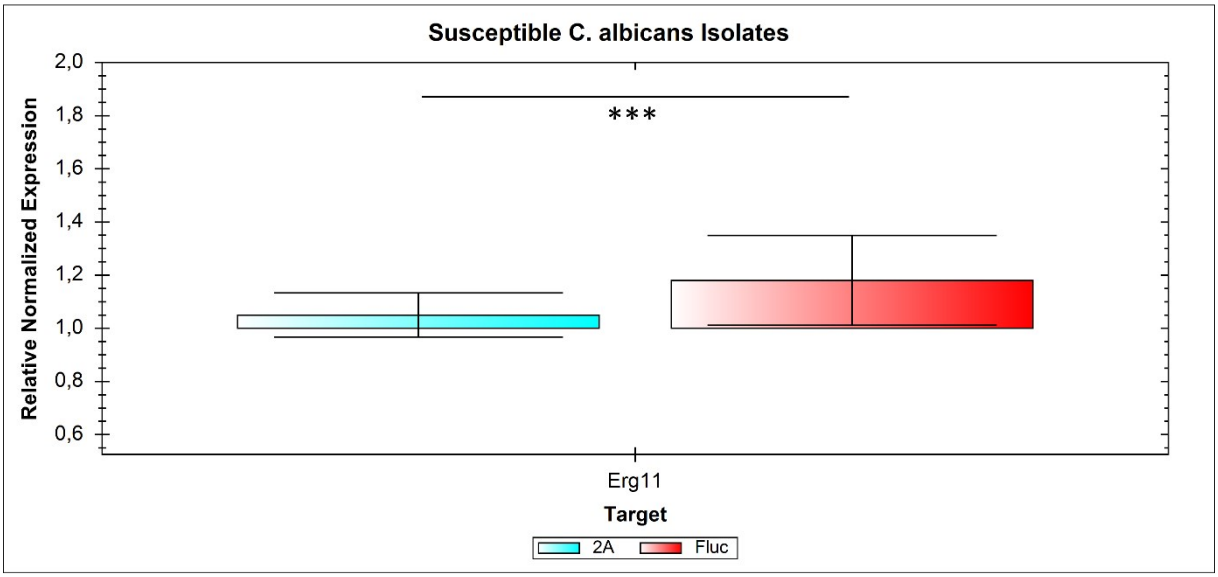
Table S2: Complete list of primer sequences

oligoname	**Sequence 5' - 3'**
UPTAG Index 1	ACG CTC TTC CGA TCT ATACC GTC CAC GAG GTC TCT
UPTAG Index 2	ACG CTC TTC CGA TCT TCTAG GTC CAC GAG GTC TCT
IUPTAG Index 3	ACG CTC TTC CGA TCT GCAGC GTC CAC GAG GTC TCT
UPTAG Index 4	ACG CTC TTC CGA TCT CCGAG GTC CAC GAG GTC TCT
DNTAG Index 1	ACG CTC TTC CGA TCT ATACC GTG TCG GTC TCG TAG
DNTAG Index 2	ACG CTC TTC CGA TCT TCTAG GTG TCG GTC TCG TAG
DNTAG Index 3	ACG CTC TTC CGA TCT GCAGC GTG TCG GTC TCG TAG
DNTAG Index 4	ACG CTC TTC CGA TCT CCGAG GTG TCG GTC TCG TAG
UPTAG_Index_9	ACG CTC TTC CGA TCT AGACA GTC CAC GAG GTC TCT
UPTAG_Index_10	ACG CTC TTC CGA TCT TGTTC GTC CAC GAG GTC TCT
UPTAG_Index_11	ACG CTC TTC CGA TCT GCCGG GTC CAC GAG GTC TCT
UPTAG_Index_12	ACG CTC TTC CGA TCT CGTAG GTC CAC GAG GTC TCT
DNTAG_Index_9	ACG CTC TTC CGA TCT AGACA GTG TCG GTC TCG TAG
DNTAG_Index_10	ACG CTC TTC CGA TCT TGTTC GTG TCG GTC TCG TAG
DNTAG_Index_11	ACG CTC TTC CGA TCT GCCGG GTG TCG GTC TCG TAG
DNTAG_Index_12	ACG CTC TTC CGA TCT CGTAG GTG TCG GTC TCG TAG
UPkanMX	CAA GCA GAA GAC GGC ATA CGA GAT GTC GAC CTG CAG CGT ACG
DNkanMX	CAA GCA GAA GAC GGC ATA CGA GAT ACG AGC TCG AAT TCA TCG
P5	A ATG ATA CGG CGA CCA CCG AGA TCT ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT

3.7 Real-time quantitative PCR. For gene expression analysis overnight cultures of *C. albicans* strains (SC5314, MFB005FS3, YMS 102-6 and YMS 102_2) were diluted to an OD530 of 0.1 and grown in SD medium at 30°C to mid-exponential-phase in absence or presence of drug (Fluconazole or compound **2a** at 0.1µM). RNA was extracted from mid-exponential-phase cells using the hot phenol method and resuspended in RNase-free water. Total RNA was treated with Turbo DNAse (ThermoFisher Scientific) and RNA quantity and integrity were assessed by UV/Vis spectrometry (NanoDrop; ThermoFisher Scientific) and with the Agilent Tape Station System (Agilent Technologies) respectively. cDNA synthesis from 500ng total RNA was performed using the Applied BiosystemsTM High-Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific) according to manufacturer's recommendations. PCR reactions were carried out on a CFX96 Touch Real time system (Bio-Rad) using the qPCRBIO SyGreen Mix (PCR Biosystems). Each reaction was run in triplicate. The PCR conditions consisted of an initial step of 95°C for 3 min followed by 40 cycles of 95°C

for 5 s, 58°C for 5 s, 60°C for 20 s and 76°C for 10 s. The reaction was completed with 98°C for 30 s and followed by a melting curve analysis going from 65°C to 95°C in 0.5°C increments. The CFX Manager Software V3.0 (Bio-Rad) was used to perform efficiency-corrected quantification and to calculate normalized expression relative to the reference gene *ACT1* ($2^{-\Delta\Delta Cq}$). Primer sequences of *ACT1* and *ERG11* were taken from Chau et al.[10]

A)



B)

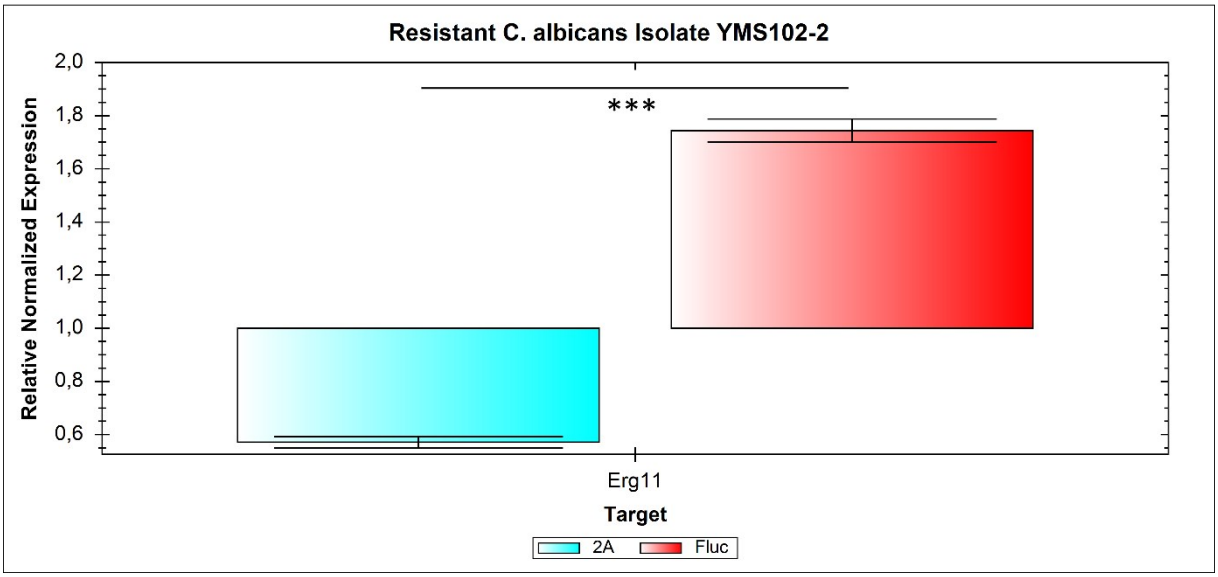


Figure S3. Expression levels of *ERG11* in *C. albicans* isolates. Relative gene expression of *ERG11* in *C. albicans* cells grown to mid-log-phase in presence of drug (concentration: 0.1µM; 'Fluc' = Fluconazole; '2A' = compound 2a) normalized to the untreated control ($2^{-\Delta\Delta Cq}$). The expression level of the untreated control is set to 1. Fluconazole led to a significant higher *ERG11* gene expression when compared to compound 2a in both susceptible ((**A**) FC=1.73;

P= 0.00016) and resistant ((**B**) FC=3.05; *P*= 0.000002) *C. albicans* isolates. All gene expression levels were measured in triplicate. Error bars show standard error of the mean.

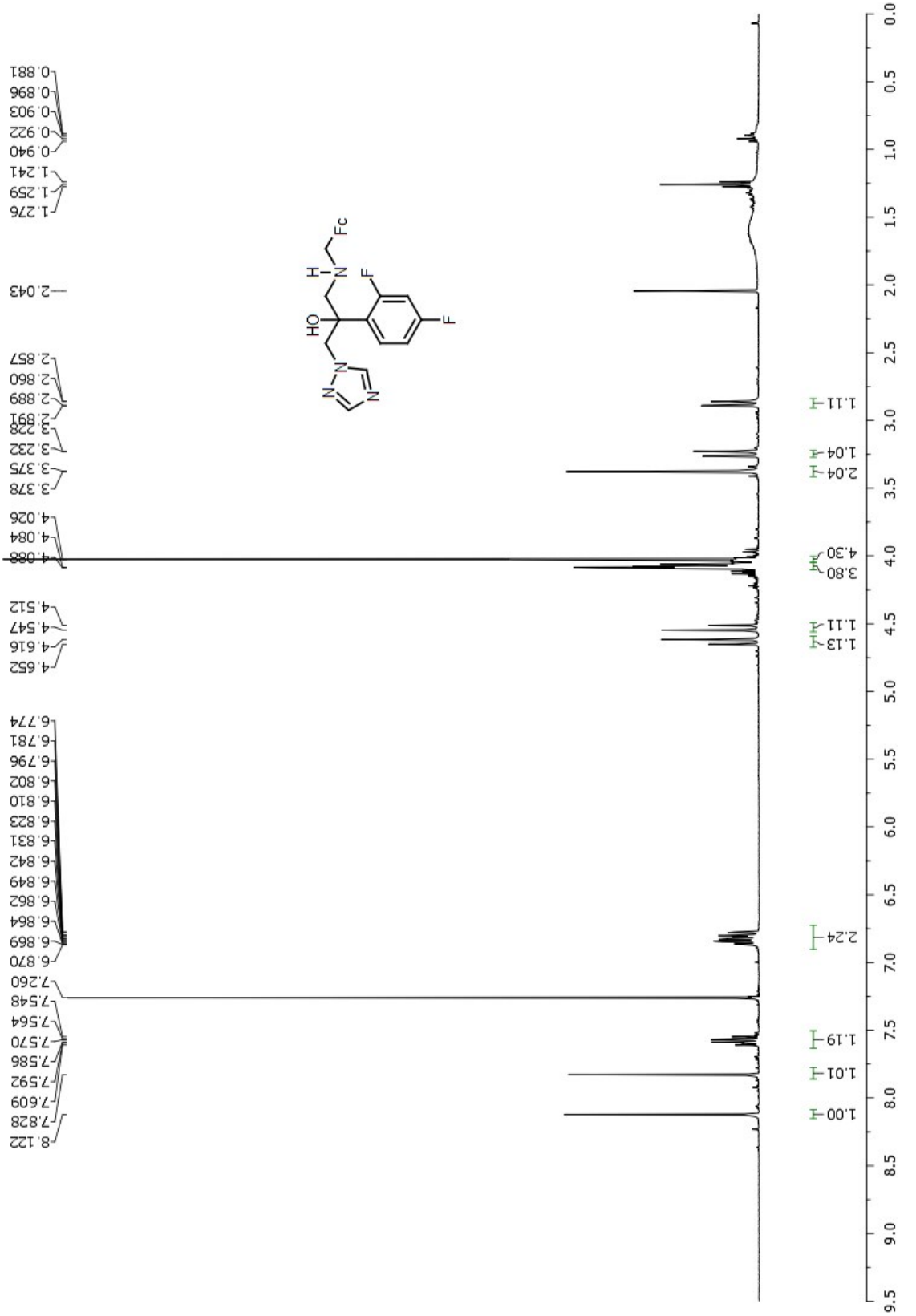
3.8 Transmission electron microscopy Studies. *S. cerevisiae* cultures were inoculated in a 5 mL culture tube in presence of YPD buffer and grown overnight. The obtained colony was spotted at OD of 0.05, transferred in a fresh tube supplemented with YPD buffer containing 5 µM of the target drug (or parent drug Fluconazole) and incubated at 30 °C at different time frames (1, 6, 18 h). The colony was then centrifuged, washed with PBS and the obtained pellet was resuspended in a small amount of PBS, sucked into cellulose capillary tubes, immersed in 1-hexadecene, cut in pieces and high-pressure frozen in a 150 µm well of a 6 mm aluminium specimen carrier covered with a flat 6 mm aluminium specimen carrier. All samples were frozen in an HPM 100 high-pressure freezing machine (Leica Microsystems, Vienna, Austria). Subsequently, the samples were freeze-substituted in water-free acetone containing 1% of OsO4 in an EM AFS2 unit (Leica Microsystems) for 8 h at -90°C, 8 h at -60°C, 8 h at -30°C, 1 h at 0°C and 1 h at 24°C with periodic temperature transition gradients of 30°C/h. Afterwards, samples were rinsed three times with water-free acetone, incubated for 1 hour in 1% uranyl acetate in anhydrous acetone at 4°C, rinsed again three times with water-free acetone and finally embedded in Epon/Araldite as follows: 33% and 66% Epon/Araldite in water-free acetone at 4°C overnight each. 100% Epon/Araldite 1h, polymerisation at 60°C for 28 hours. Ultrathin sections of 50 nm of all specimens were contrasted with lead citrate and analysed in a Tecnai G2 Spirit or CM 100 transmission electron microscope (Thermo Fisher, Eindhoven, The Netherlands) using an ORIUS 1000 CCD camera (Gatan, Munich, Germany).

3.9 SQR and AOX inhibition assays. *E. coli* membrane samples containing the either the *C. auris* AOX or the *C. albicans* AOX were synthesised and obtained as has been described previously.[12] Rat liver mitochondria were harvested and isolated in accordance to Home office guidelines, using the method described previously.[13]. Inhibitors were tested in a 96-well plate format using a Thermoscientific TM Multiskan Sky and packaged SkanIt 5.0 software, with dose response curves generated using the least squared method in GraphPad PRISM version 7.0. All data are presented as an average of 3 biological replicates ± SEM. Conditions used for each sample were as follows: AOX membrane samples were diluted to ~ 60 µg ml-1 in 65 mM MOPS pH 7.5 containing 1 mM KCN and 10mM GMP. Respiration was initiated upon addition of 300 µM NADH, with the subsequent reaction followed at 340 nm for 10 minutes. Rat liver mitochondria were diluted to ~ 300 µg ml-1 in mitochondrial media containing 200 mM sucrose, 25 mM KCl, 10 mM $MgCl_2$, 5 mM KH_2PO_4, 5 mM MOPS pH 7.4. 1mM KCN, 1mM ATP, 1mM Rotenone and 64 µM Cytochrome C was added to the mitochondrial sample, and

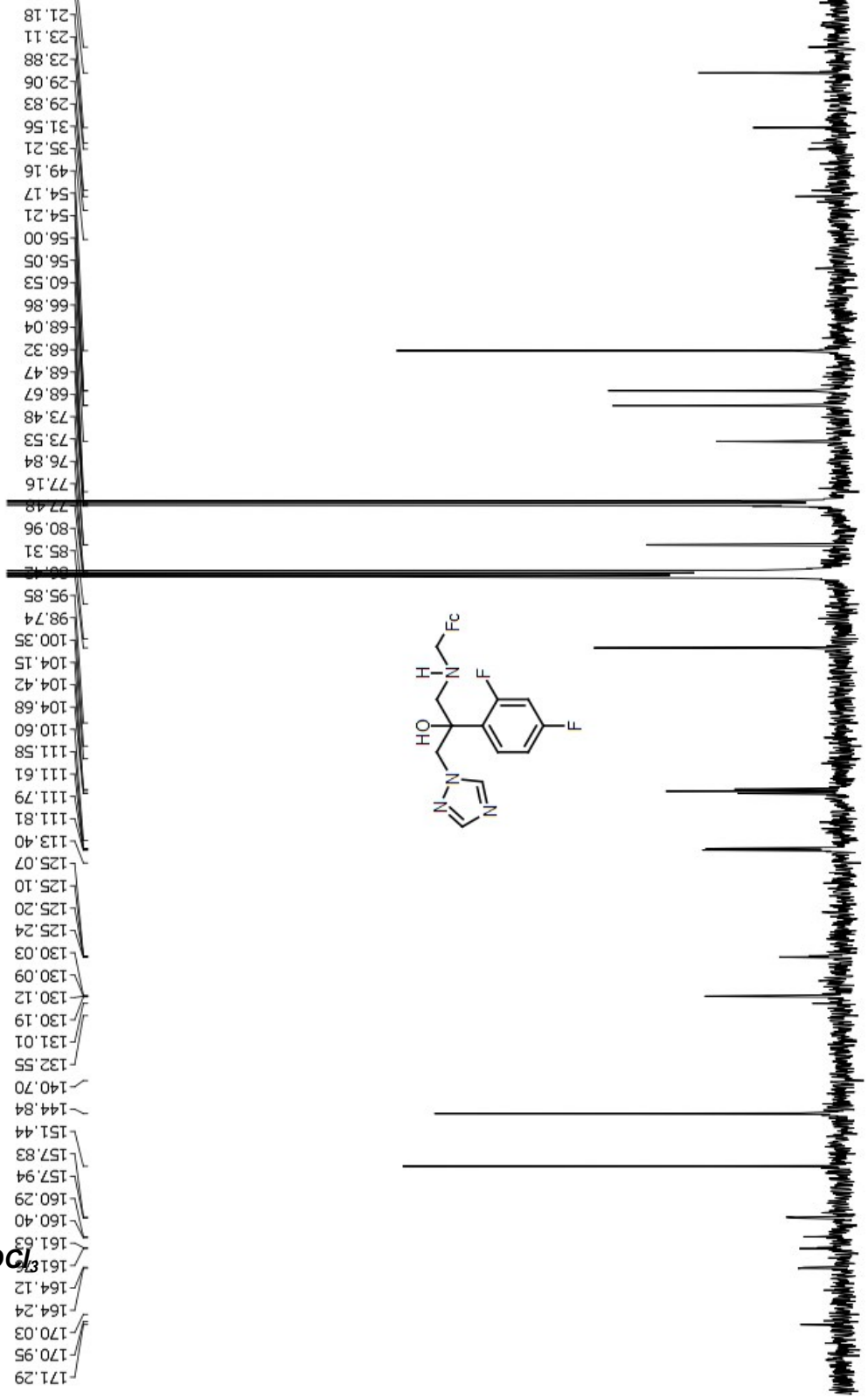
the reaction was initiated with 6mM Succinate. The reduction of cytochrome C was followed at 550 nm for 10 minutes.

4. Copies o

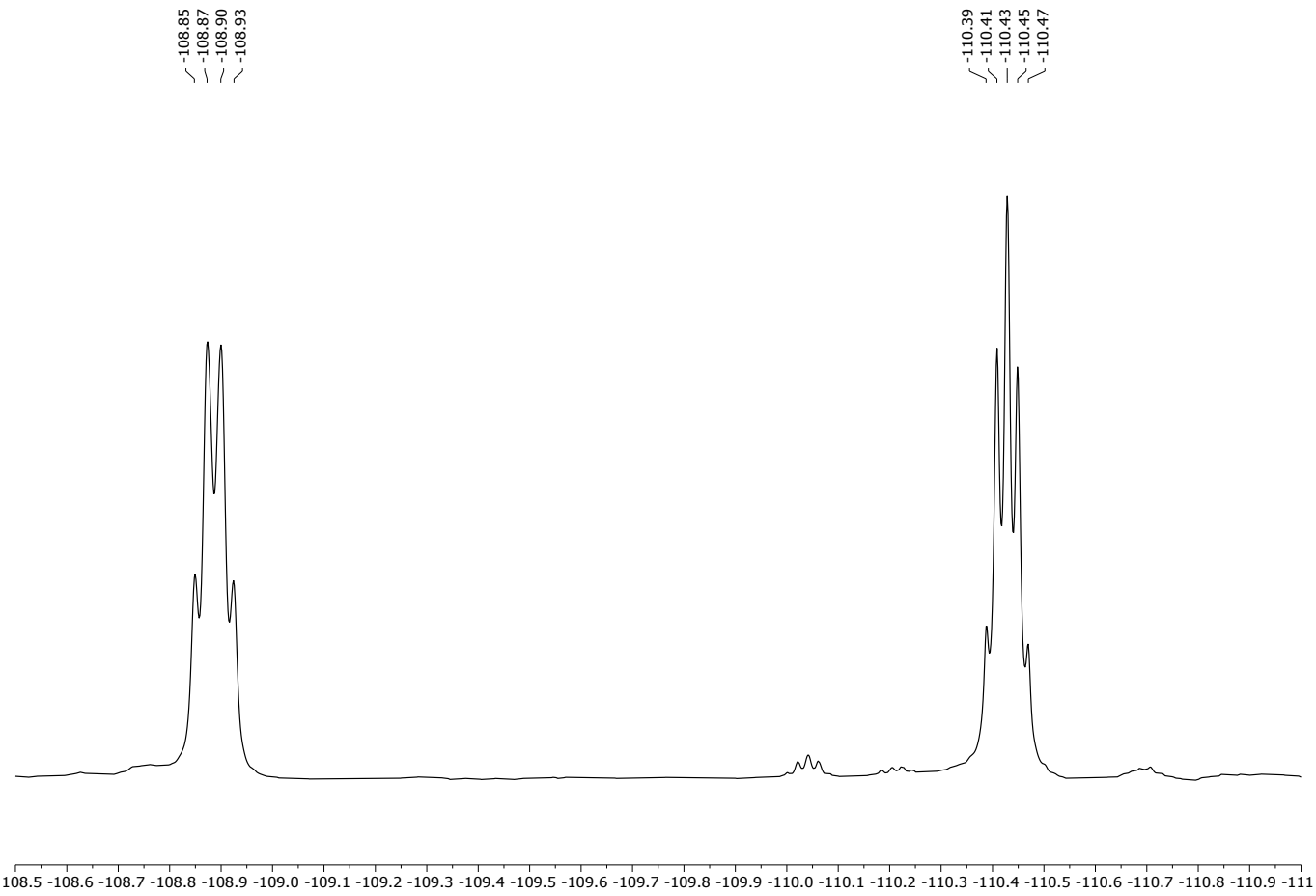
analysis



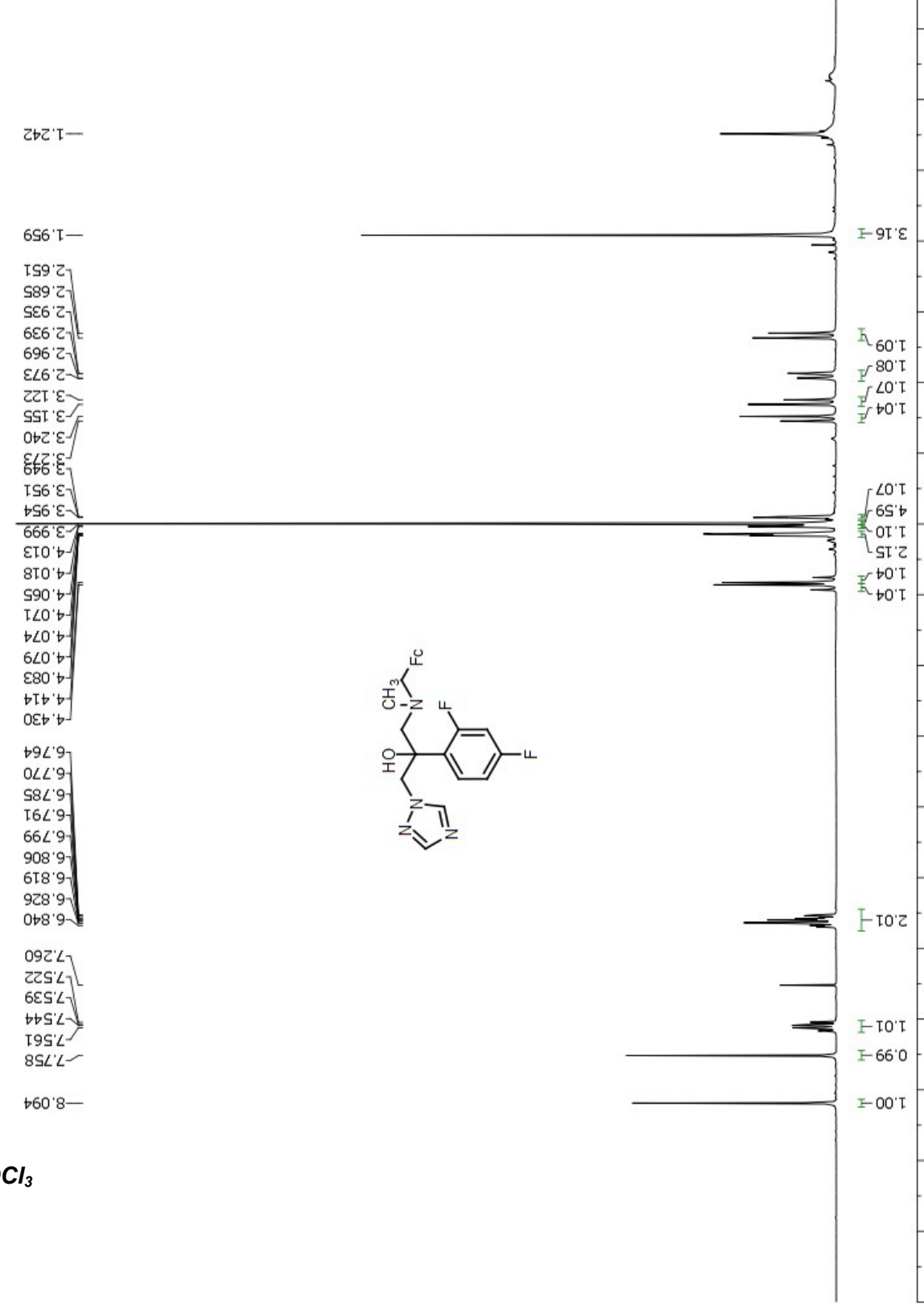
^{1}H NMR of 1 *in* $CDCl_3$



^{13}C NMR of 1 *in CDCl$_3$*

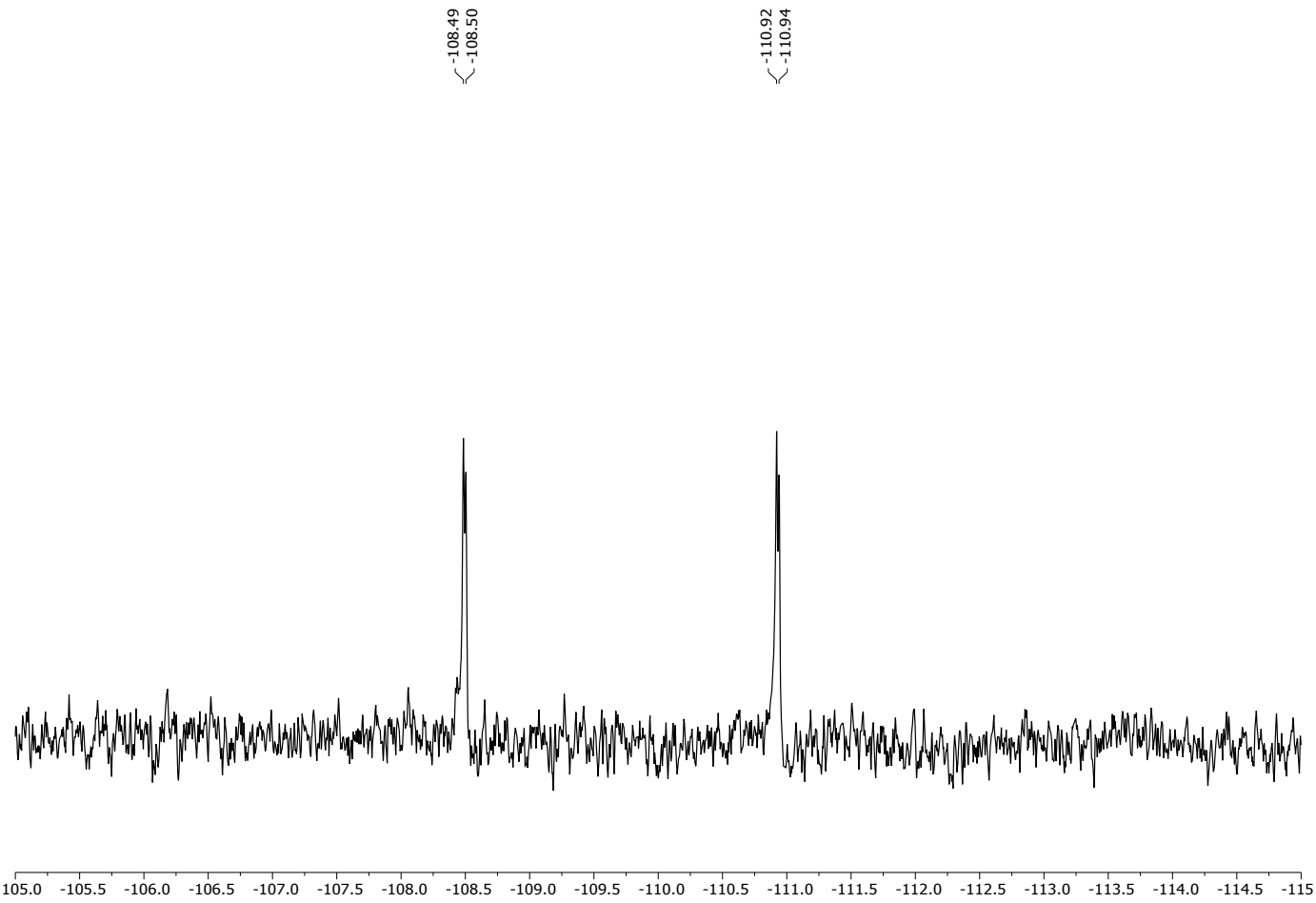


^{19}F NMR of 1 *in $CDCl_3$*



^{1}H NMR of 2 *in* $CDCl_3$

^{13}C NMR of 2 *in* $CDCl_3$



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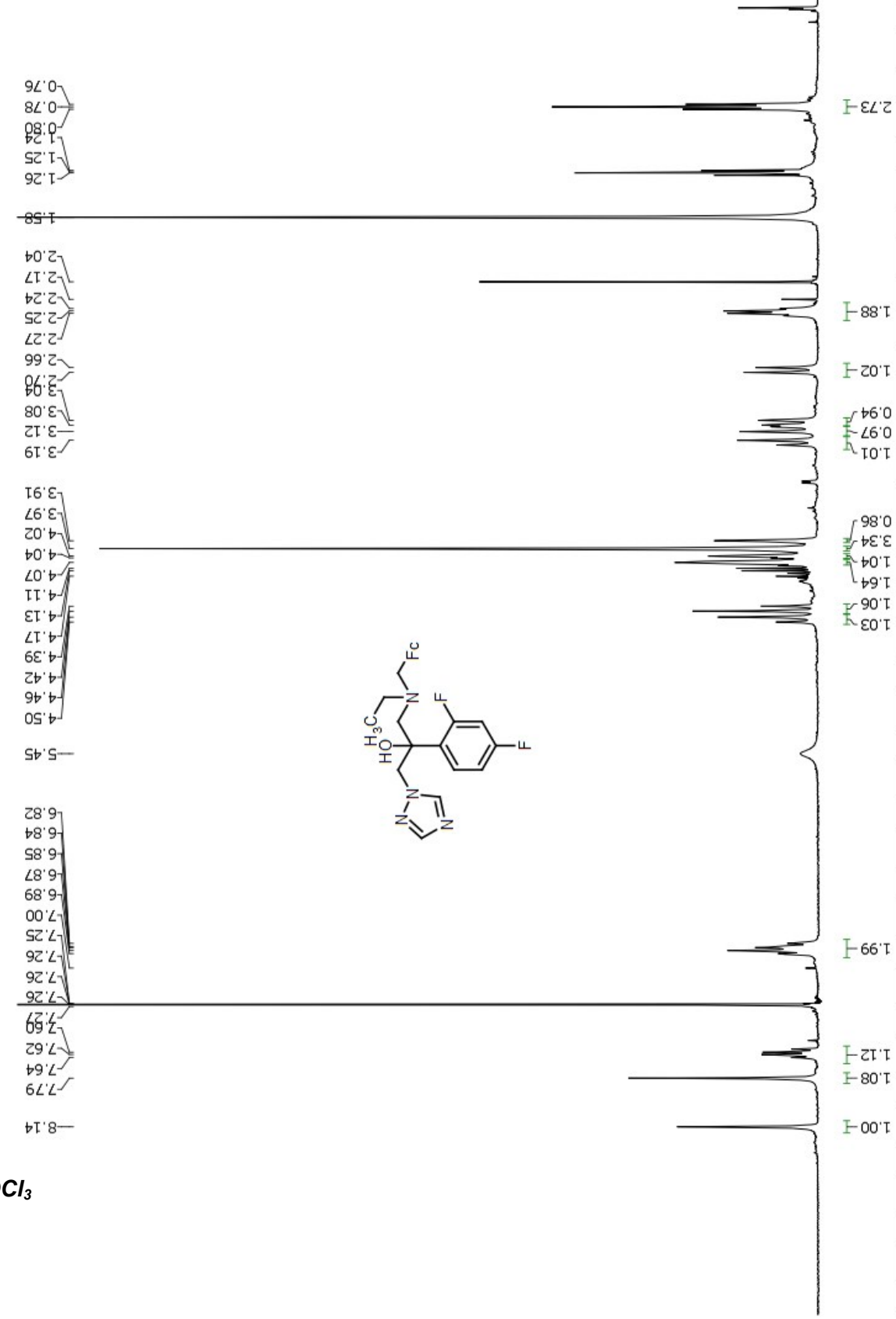

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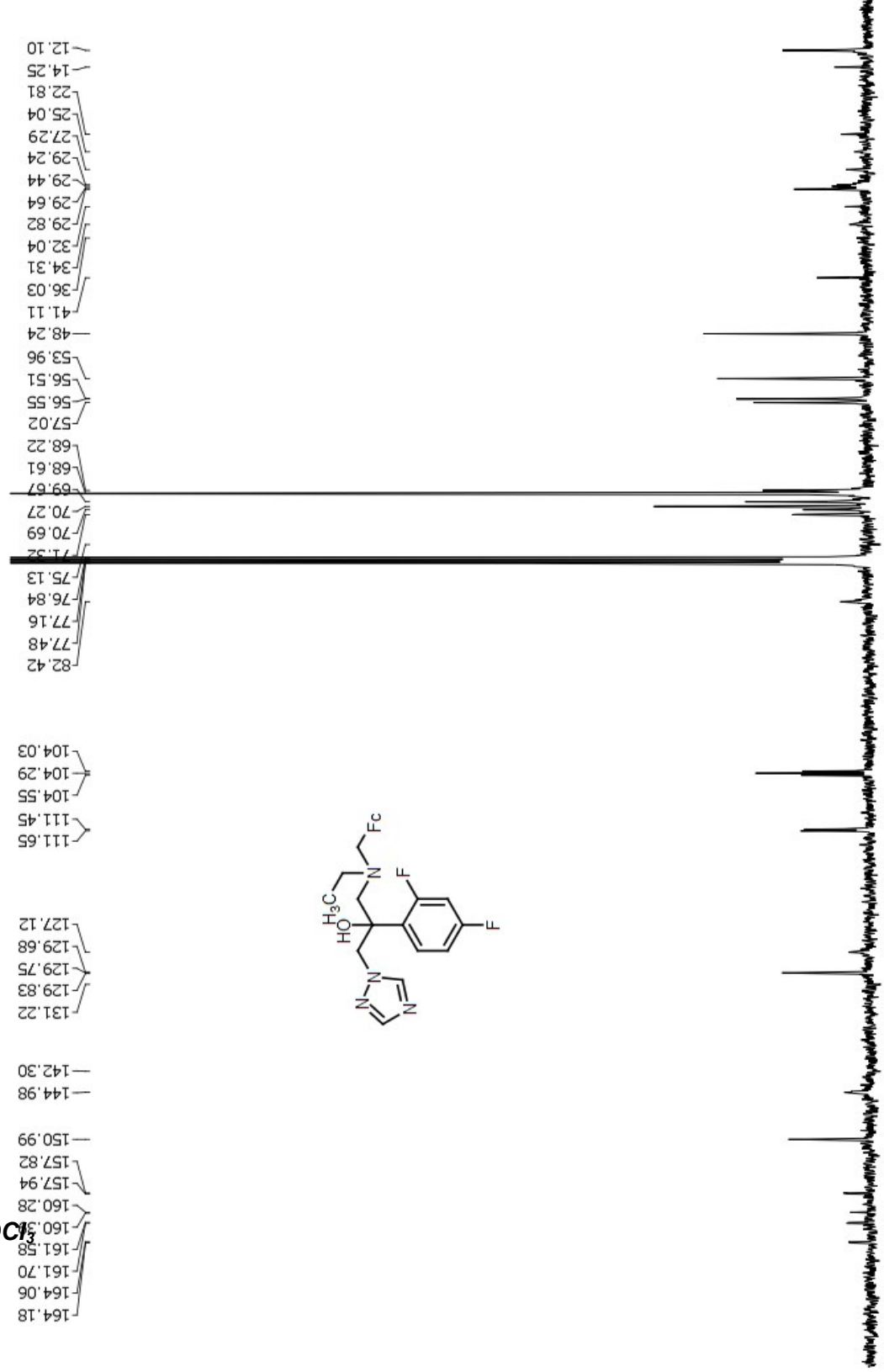

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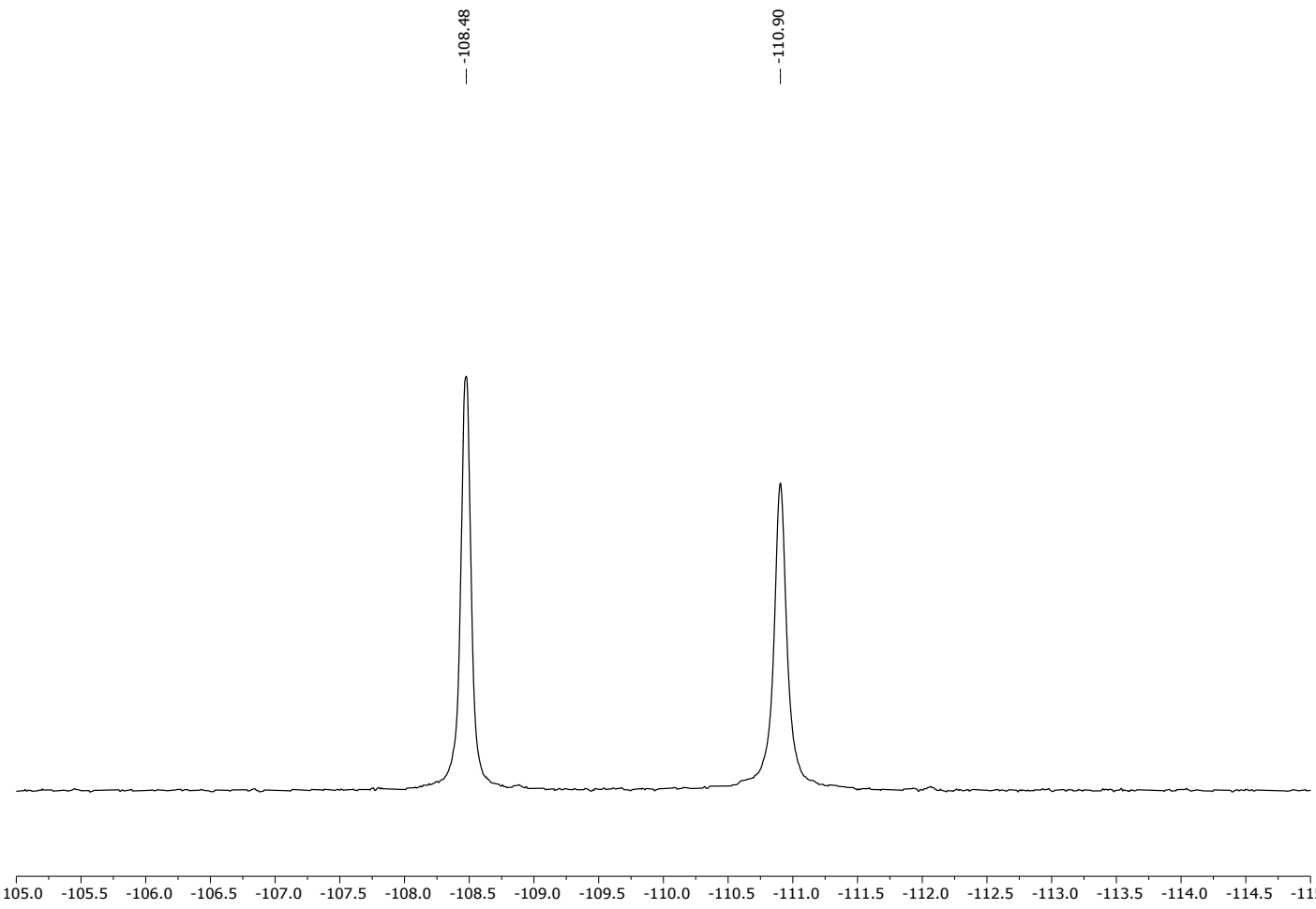
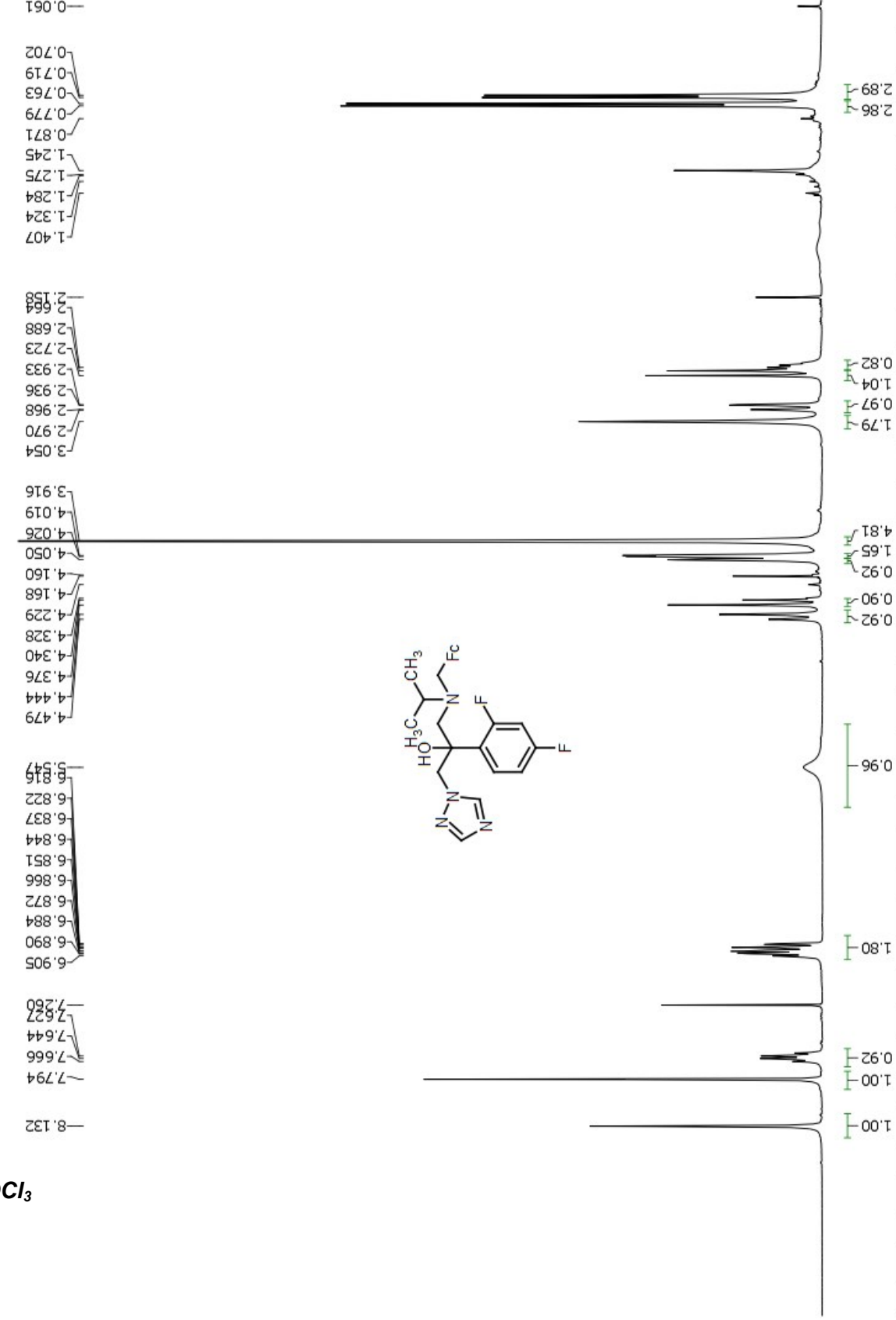


^{19}F NMR of 3 *in* $CDCl_3$



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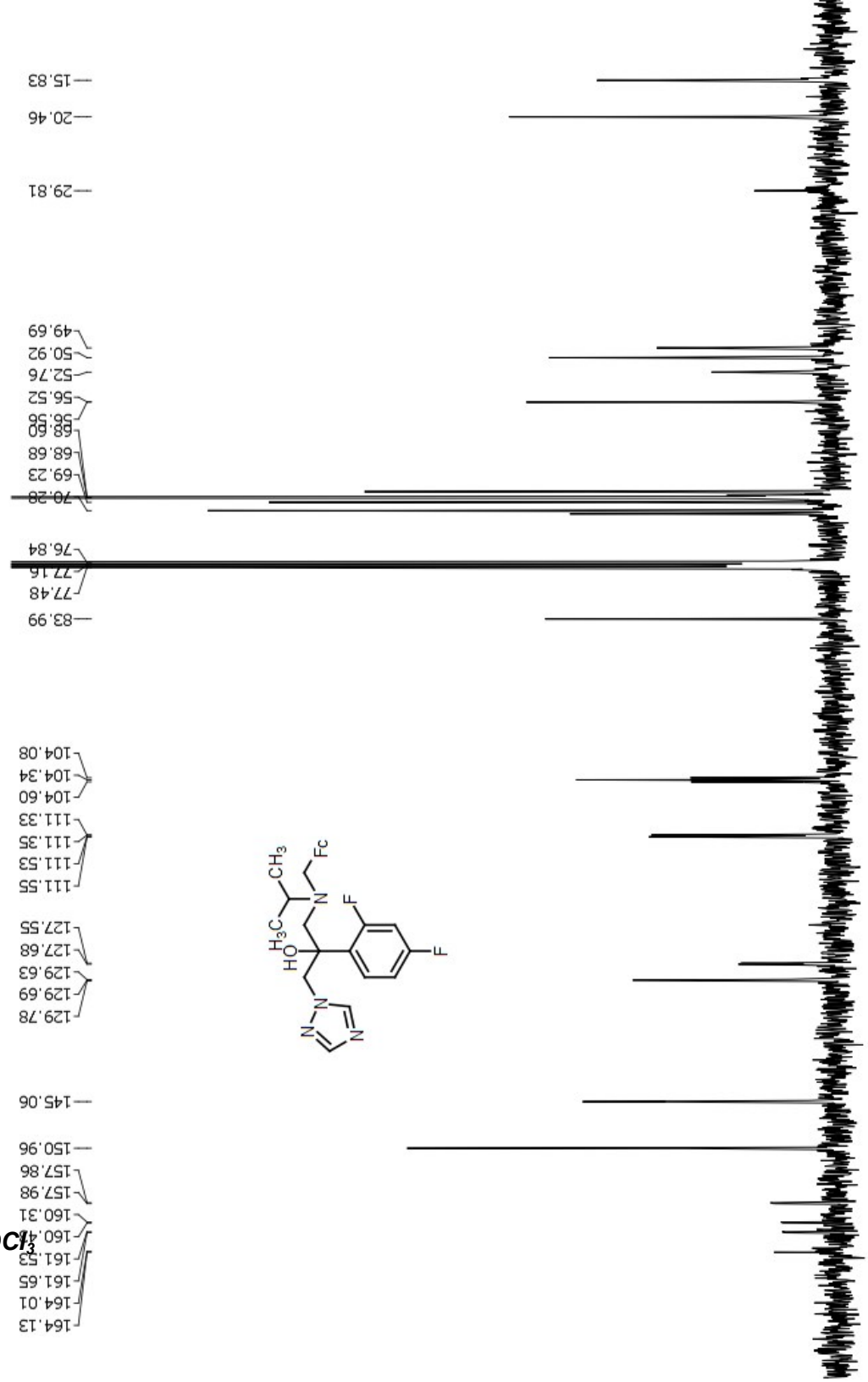

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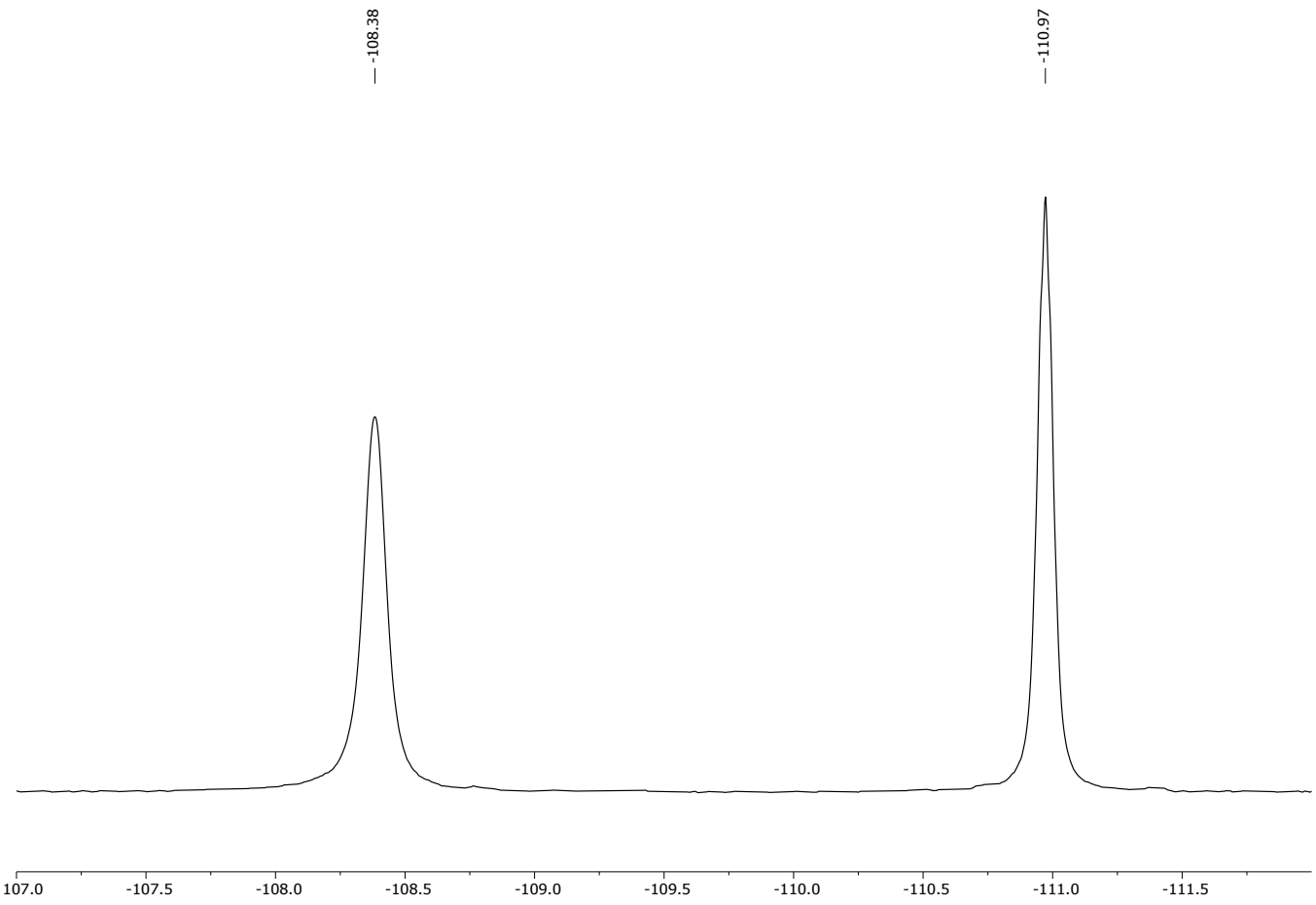
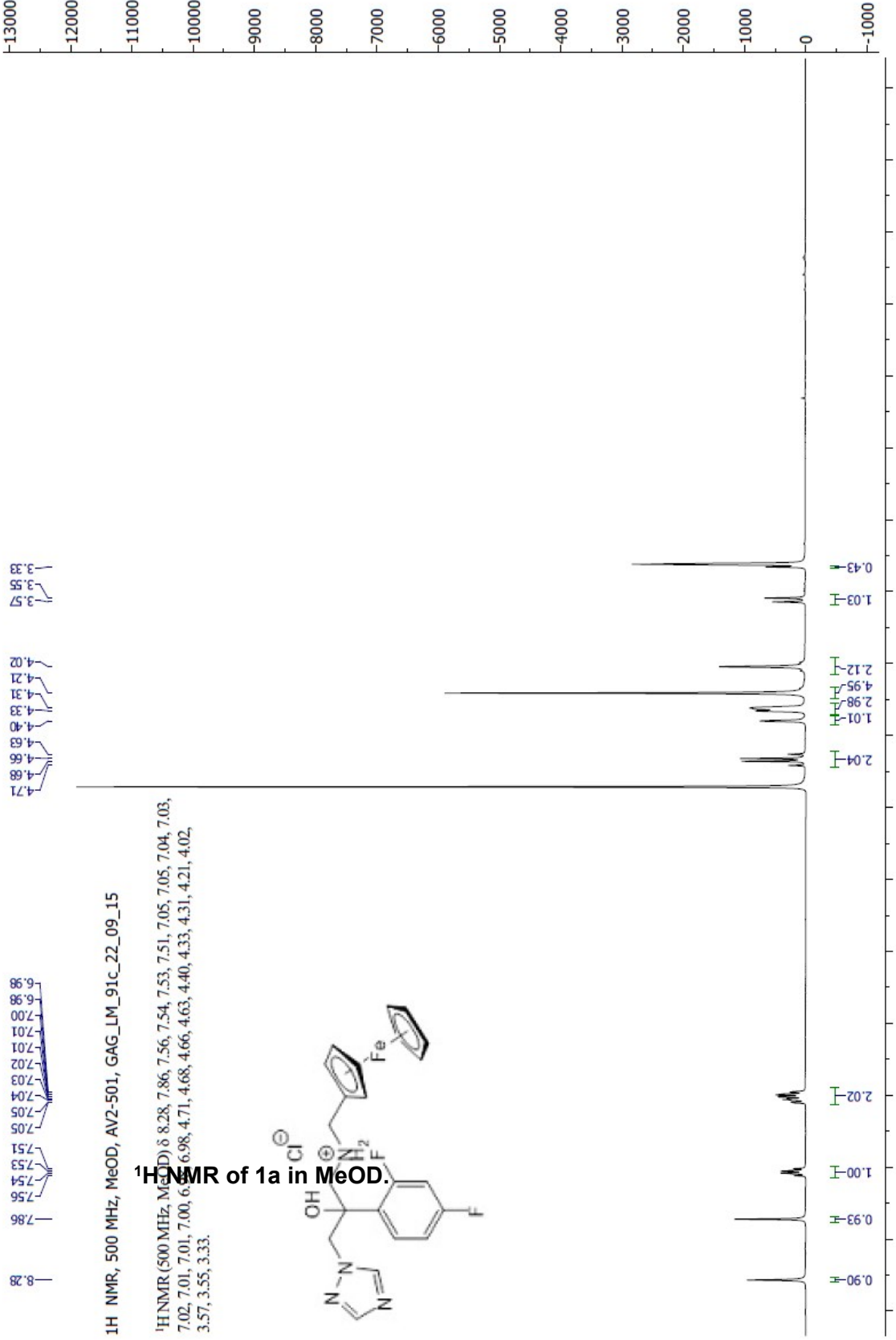
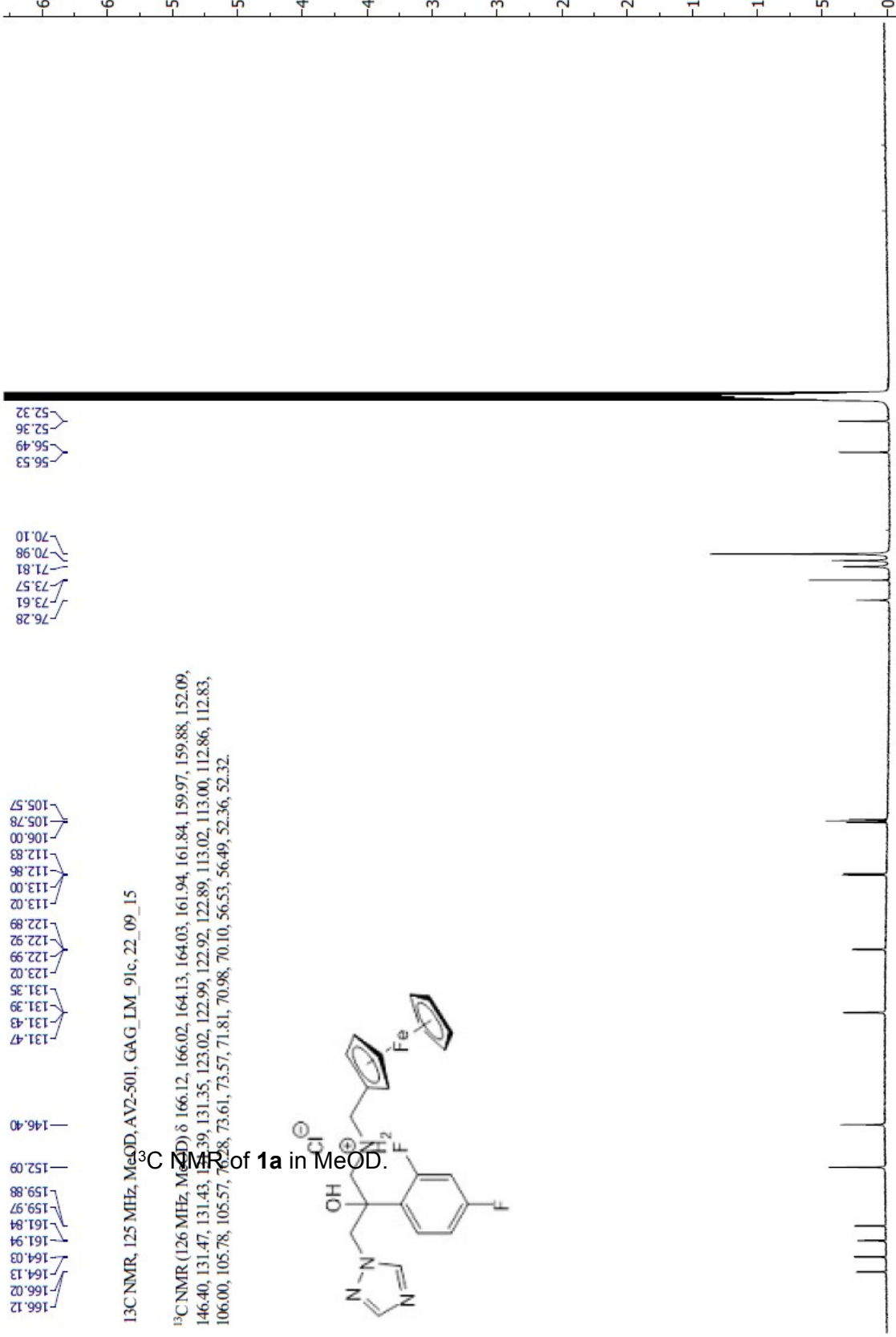


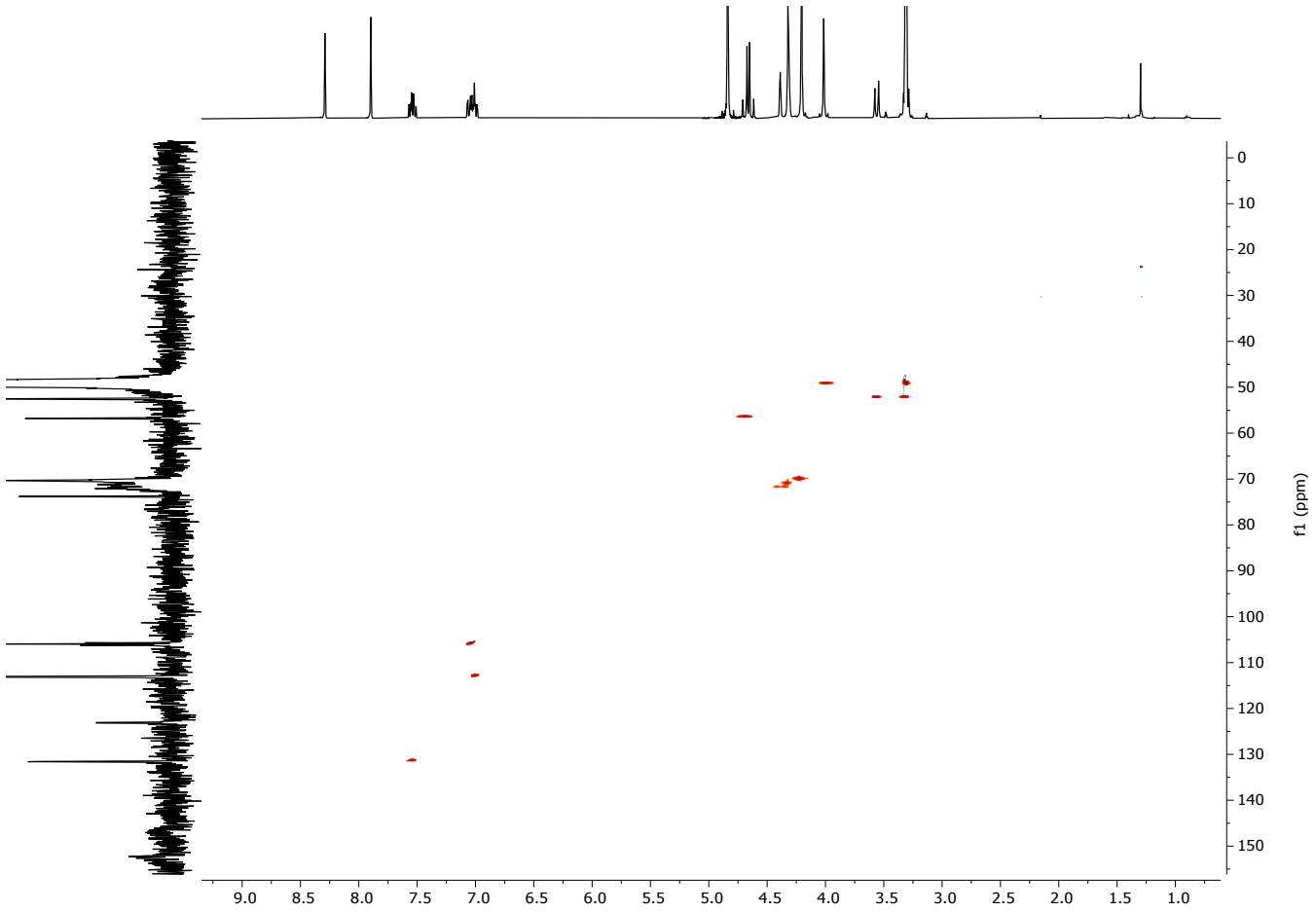
^{19}F NMR of 4 *in* $CDCl_3$



^{1}H NMR of 1a in MeOD.



^{13}C NMR of **1a** in MeOD.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8.0
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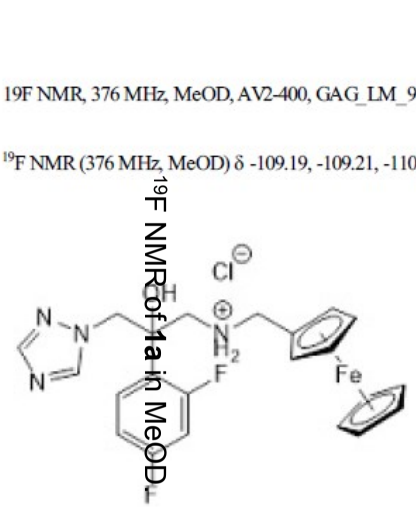
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

HSQC of 1a

19F NMR, 376 MHz, MeOD, AV2-400, GAG_LM_91c, 16_11_15

^{19}F NMR (376 MHz, MeOD) δ -109.19, -109.21, -110.87, -110.90.

^{19}F NMR of **1a** in MeOD.



Generic Display Report

Analysis Info

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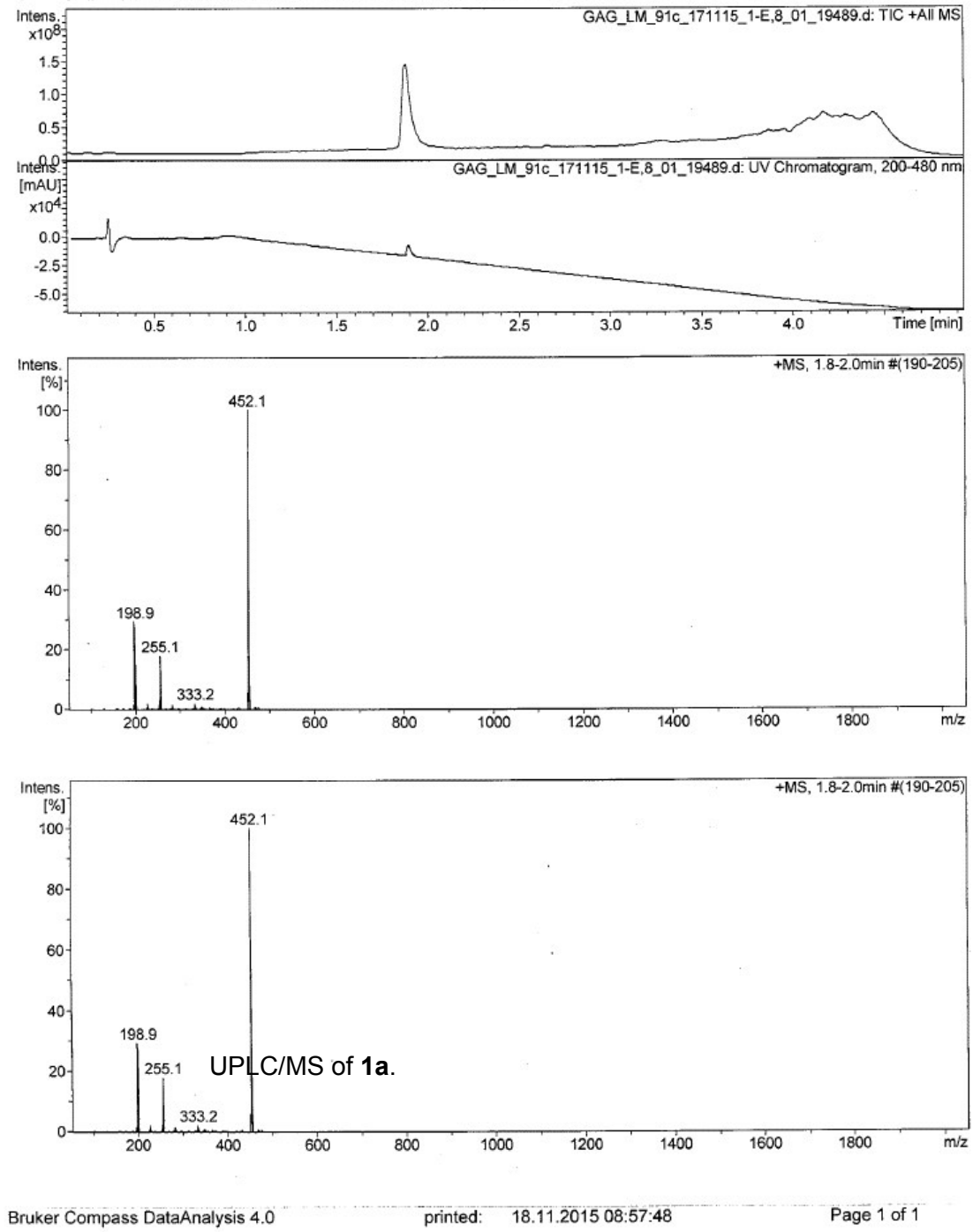
Comment			



UPLC/MS of **1a**.

Eidgenössische Technische Hochschule Zürich

Laboratorium für Organische Chemie

Vladimir-Prelog-Weg 3 HCI E304 8093 Zürich Tel: 044/633 43 58

Mikroelementaranalyse

Name: Mastrobuoni Luciano Gruppe: Gasser UNIZH
Labor: Y34H40 Tel: 044/635 46 86

Substanz:GAG-LM-91c
Molekularformel: C22 H23 N4 O F2 Cl Fe Mr = 488,75 g/mol

Schmelzpunkt: Sublimationspunkt:
gereinigt: wie? getrocknet: wie?

Bestimmungen: C H N

Eingang: 30.09.15 Ausgang: 01.10.15

M-160952 Operator: PK

Berechnete Gewichtsanteile:

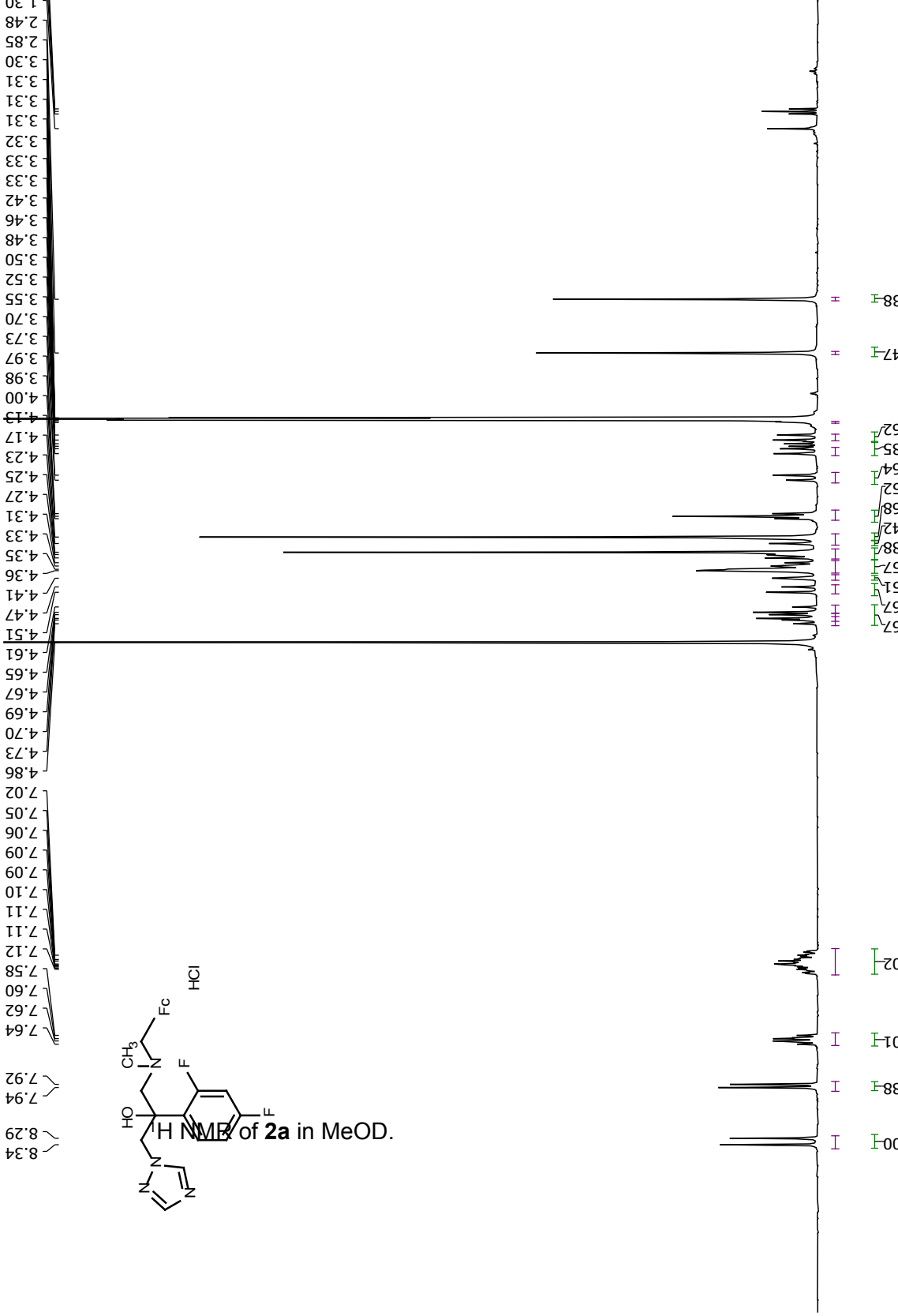
[C]	54,07%	[H]	4,74%	[N]	11,46%	[O]	3,27%	[F]	7,77%
[Cl]	7,25%	[Fe]	11,43%						

Gefundene Gewichtsanteile:

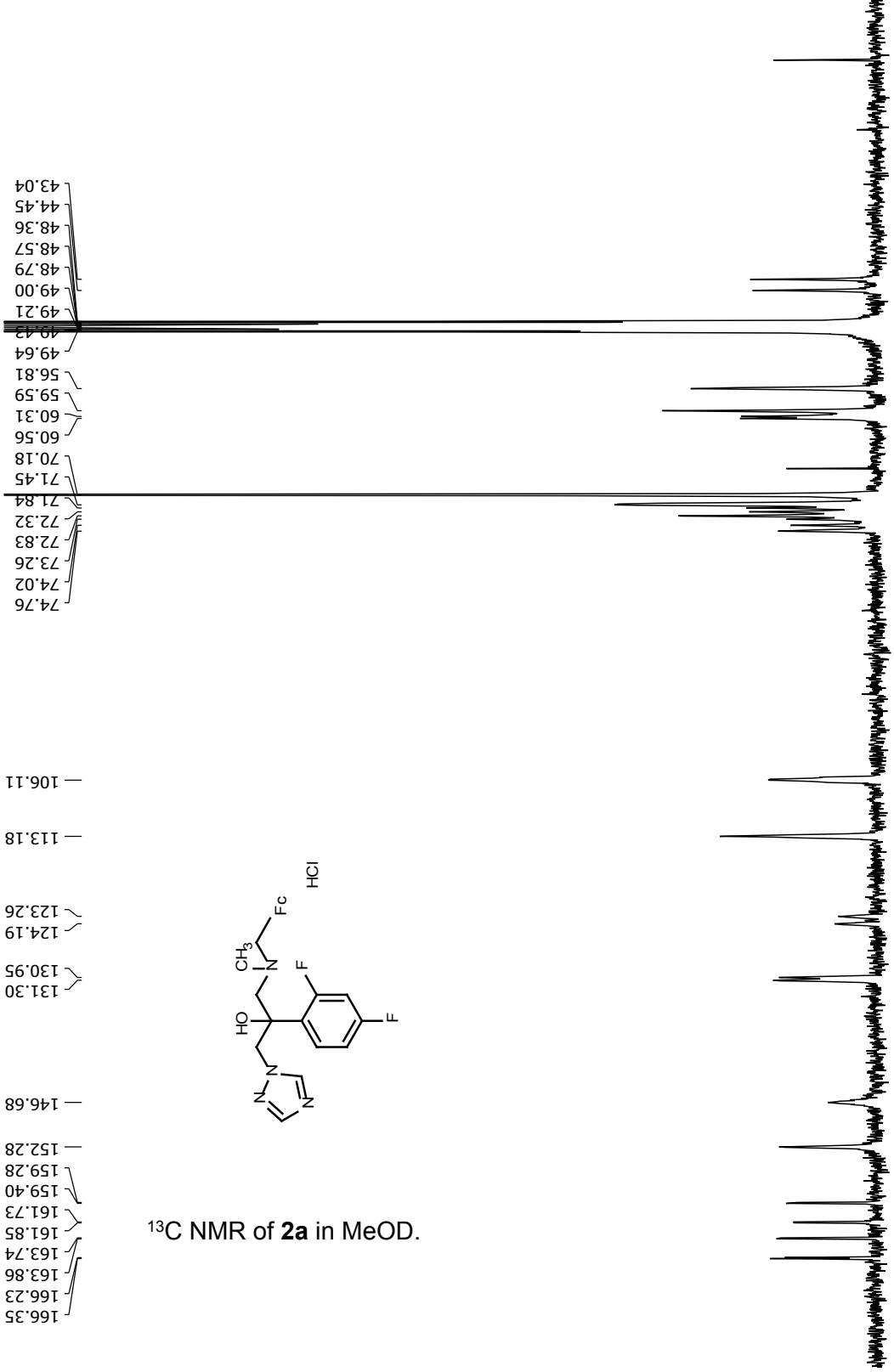
Einwaage: 1,194mg LECO TruSpec Micro

[C] 53,81% [H] 4,71% [N] 11,50% 01.10.15

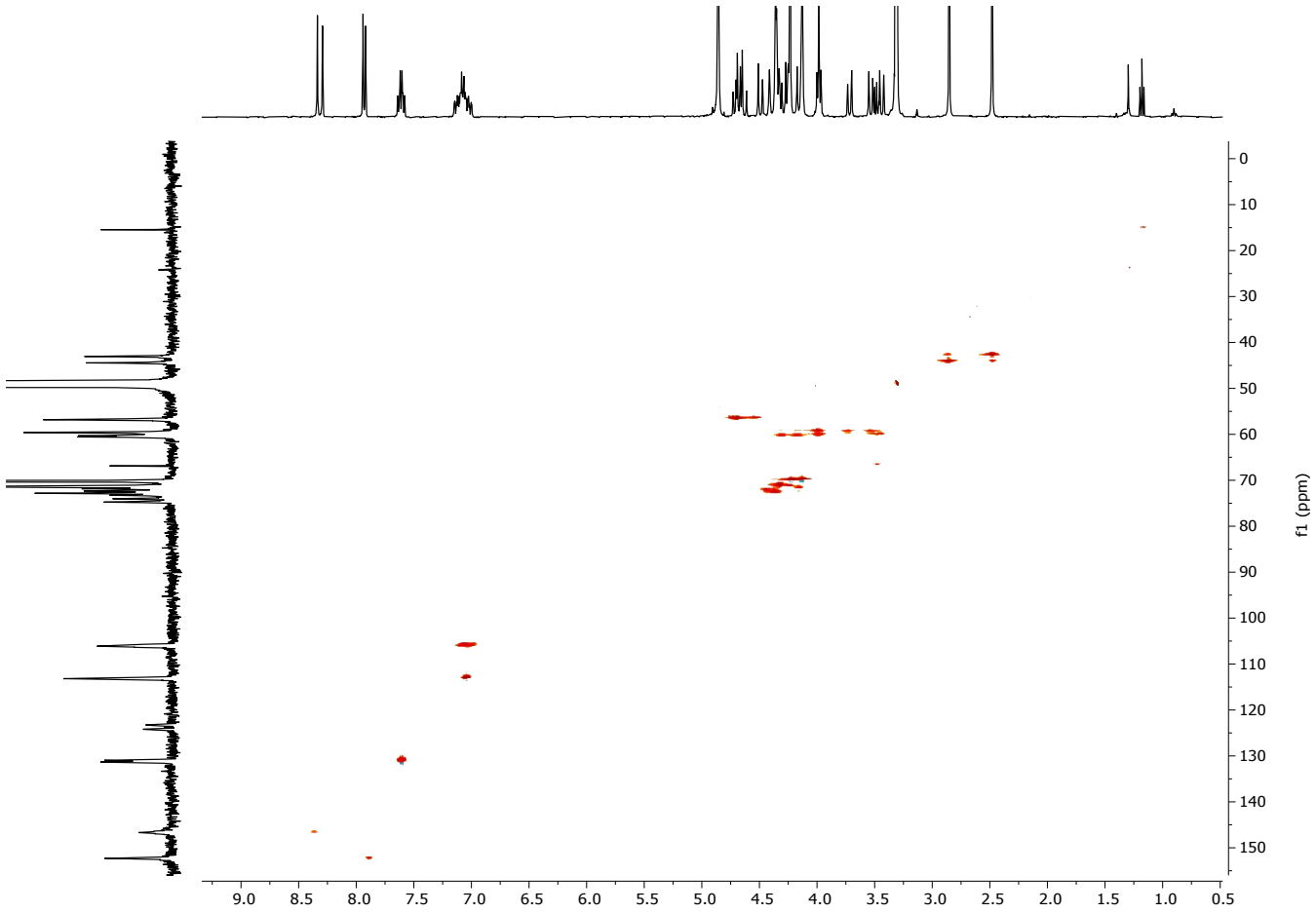
Elemental Analysis of **1a**.



^{1}H NMR of **2a** in MeOD.



^{13}C NMR of **2a** in MeOD.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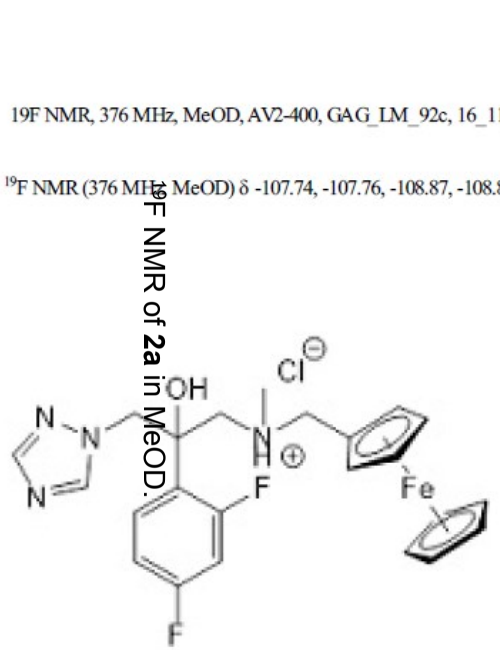
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

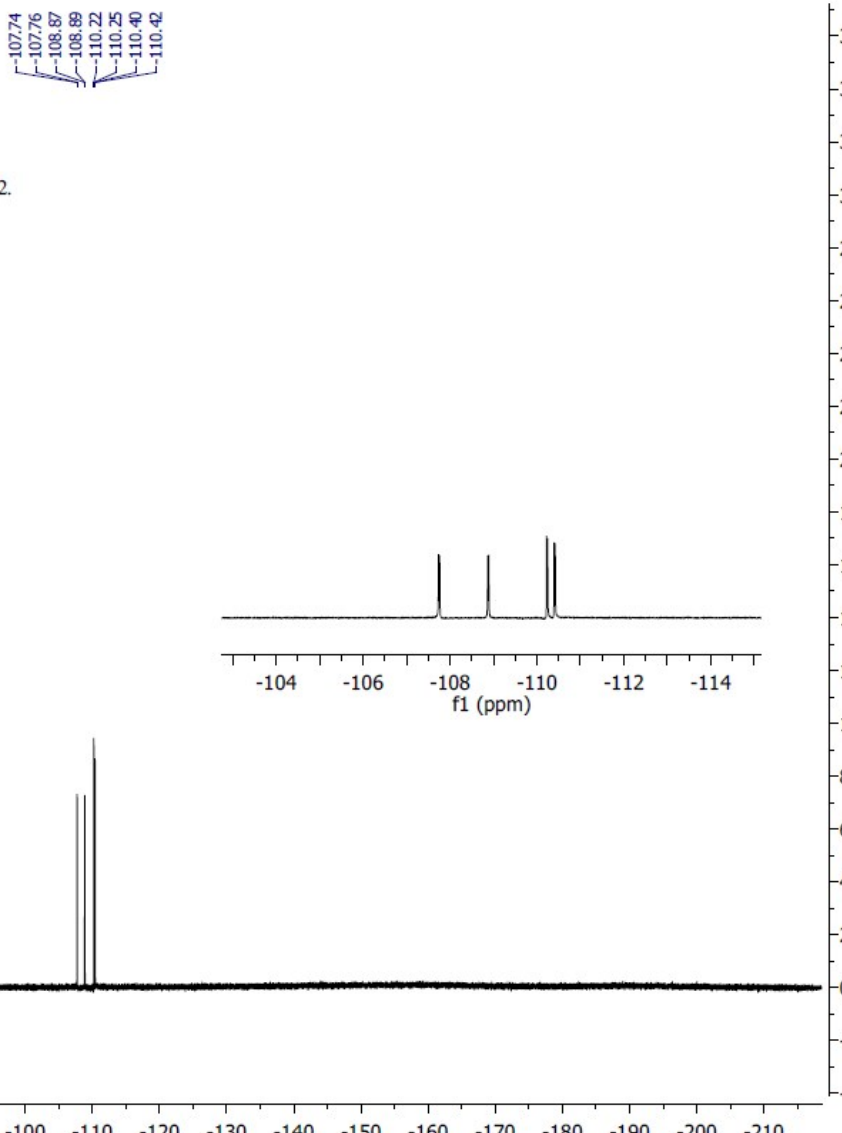
HSQC of 2a

19F NMR, 376 MHz, MeOD, AV2-400, GAG_LM_92c, 16_11_15

^{19}F NMR (376 MHz, MeOD) δ -107.74, -107.76, -108.87, -108.89, -110.22, -110.25, -110.40, -110.42.

^{19}F NMR of **2a** in MeOD.





Generic Display Report

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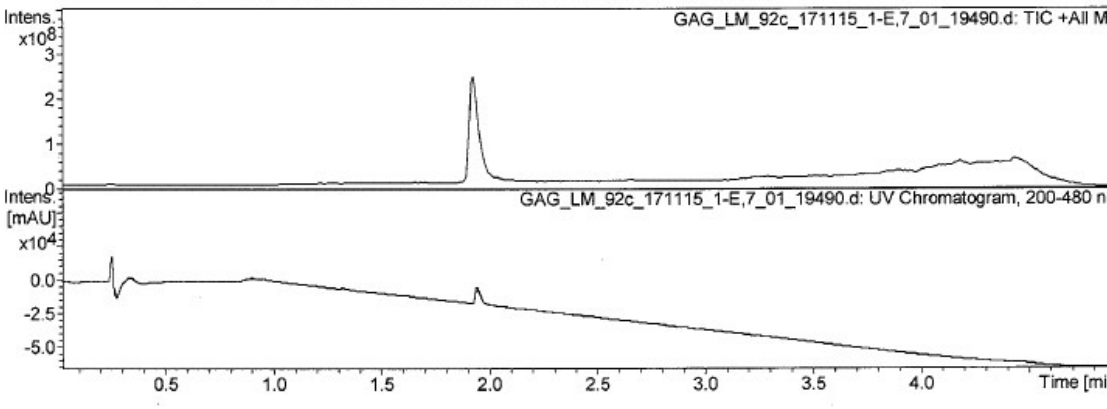
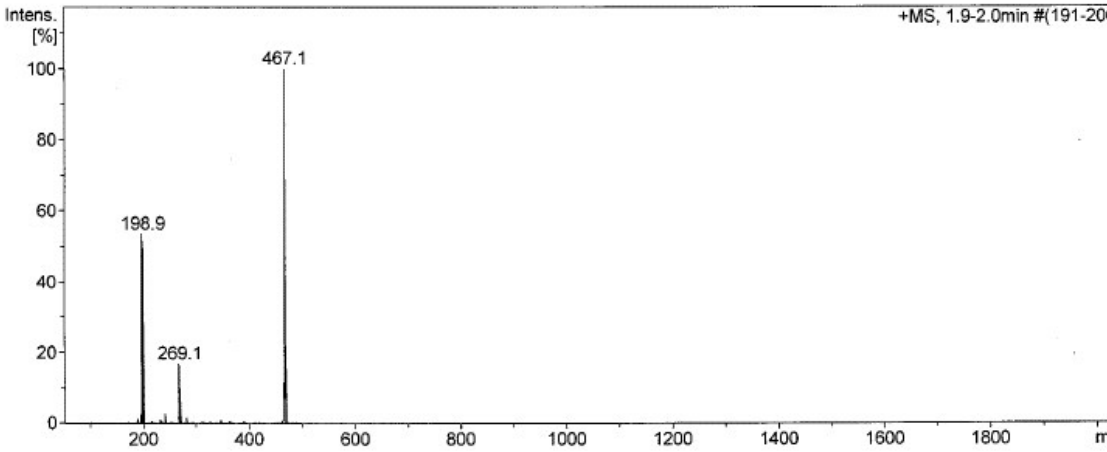
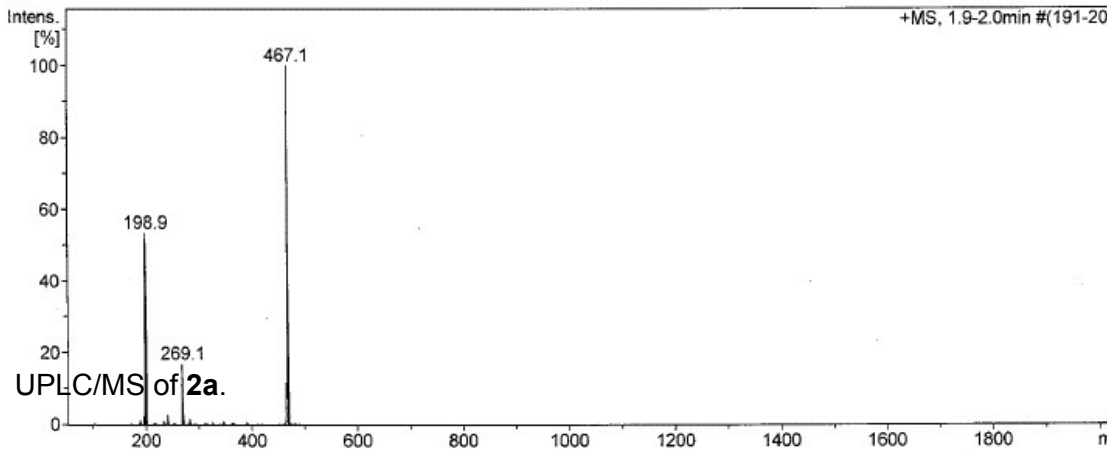
Sample Name	GAG_LM_92c_171115	Instrument	HCT
Comment			







UPLC/MS of **2a**.

Bruker Compass DataAnalysis 4.0 printed: 18.11.2015 08:58:13 Page 1 of 1

Eidgenössische Technische Hochschule Zürich
Laboratorium für Organische Chemie
Vladimir-Prelog-Weg 3 HCI E304 8093 Zürich Tel: 044/633 43 58
Mikroelementaranalyse

Name: Mastrobuoni Luciano
Labor: Y34H40
Gruppe: Gasser UNIZH
Tel: 044/635 46 86

Substanz:GAG-LM-92c
Molekularformel: C23 H25 N4 O F2 Cl Fe
Mr = 502,77 g/mol

Schmelzpunkt: ?
gereinigt: wie?
Sublimationspunkt:
getrocknet: wie?

Bestimmungen: C H N

Eingang: 29.09.15
Ausgang: 30.09.15

M-160940
Operator: PK

Berechnete Gewichtsanteile:

[C]	54,95%	[H]	5,01%	[N]	11,14%	[O]	3,18%	[F]	7,56%
[Cl]	7,05%	[Fe]	11,11%						

Gefundene Gewichtsanteile:

Einwaage: 1,170mg
LECO TruSpec Micro
[C] 52,87% **[H] 5,65%** **[N] 10,33%** 30.09.15

Einwaage: 1,190mg
LECO TruSpec Micro
[C] 52,79% **[H] 5,71%** **[N] 10,28%** 30.09.15

Elemental Analysis of **2a**.

^{1}H NMR of **3a** in MeOD.

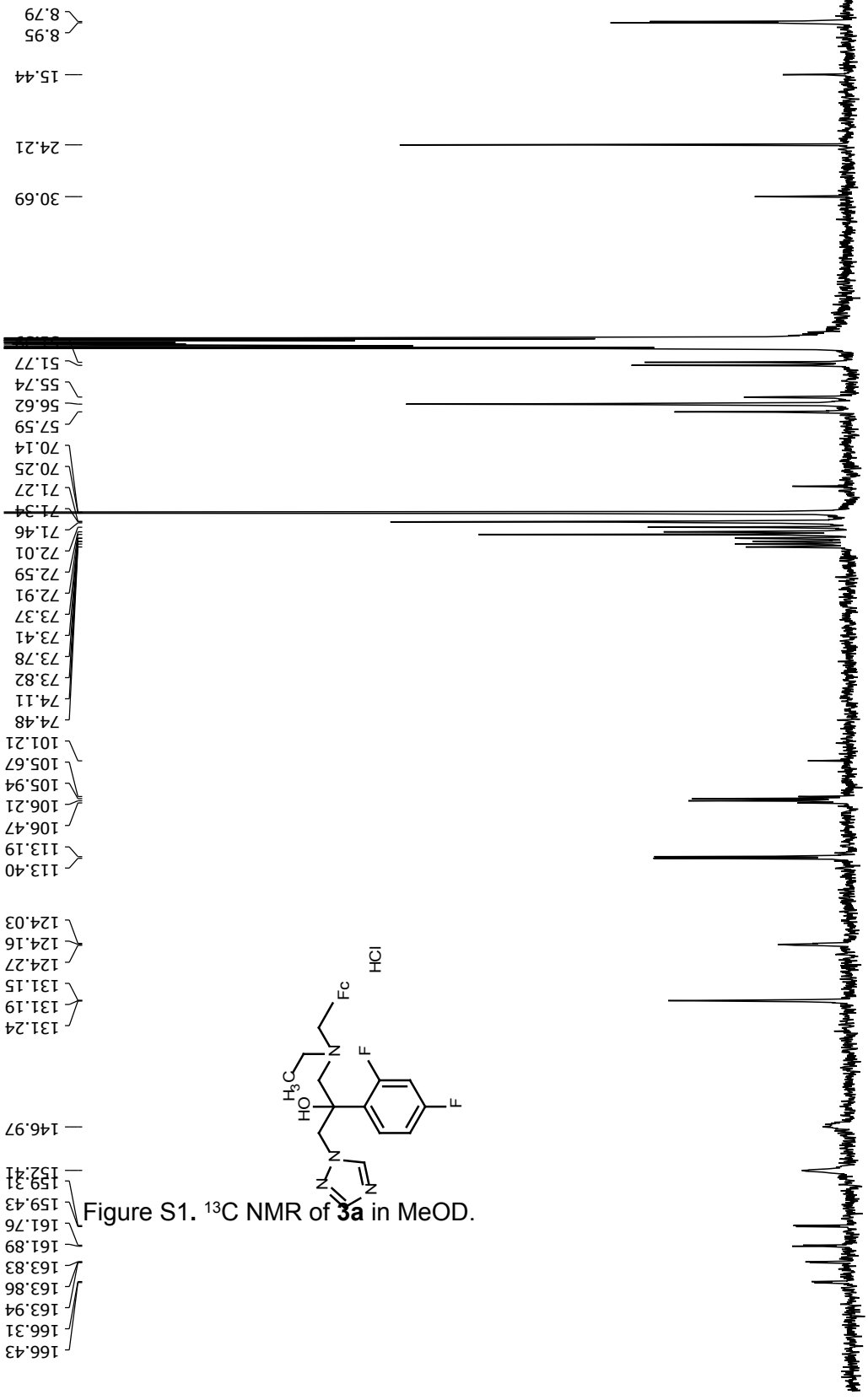
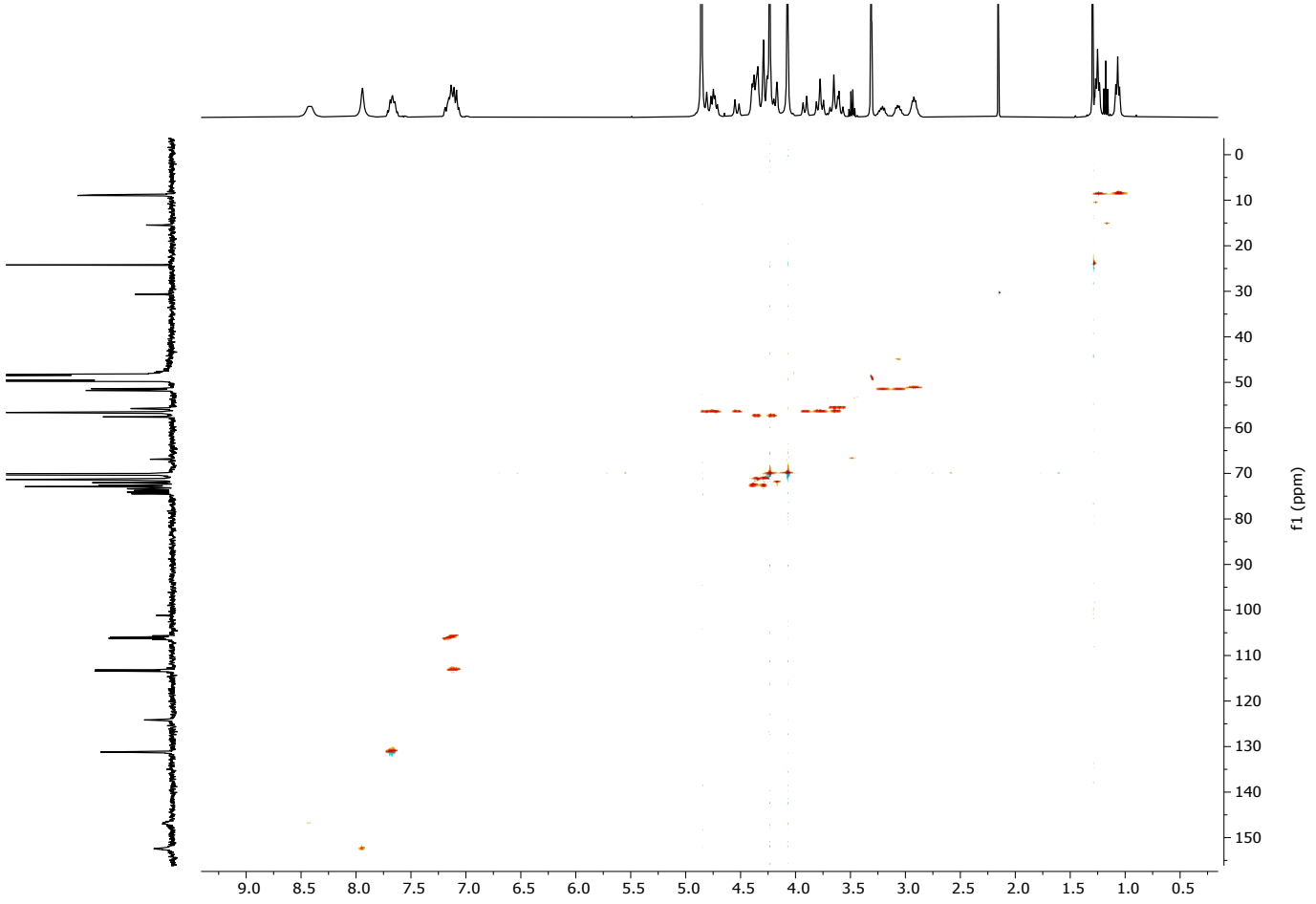


Figure S1. ^{13}C NMR of **3a** in MeOD.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HSQC of 3a

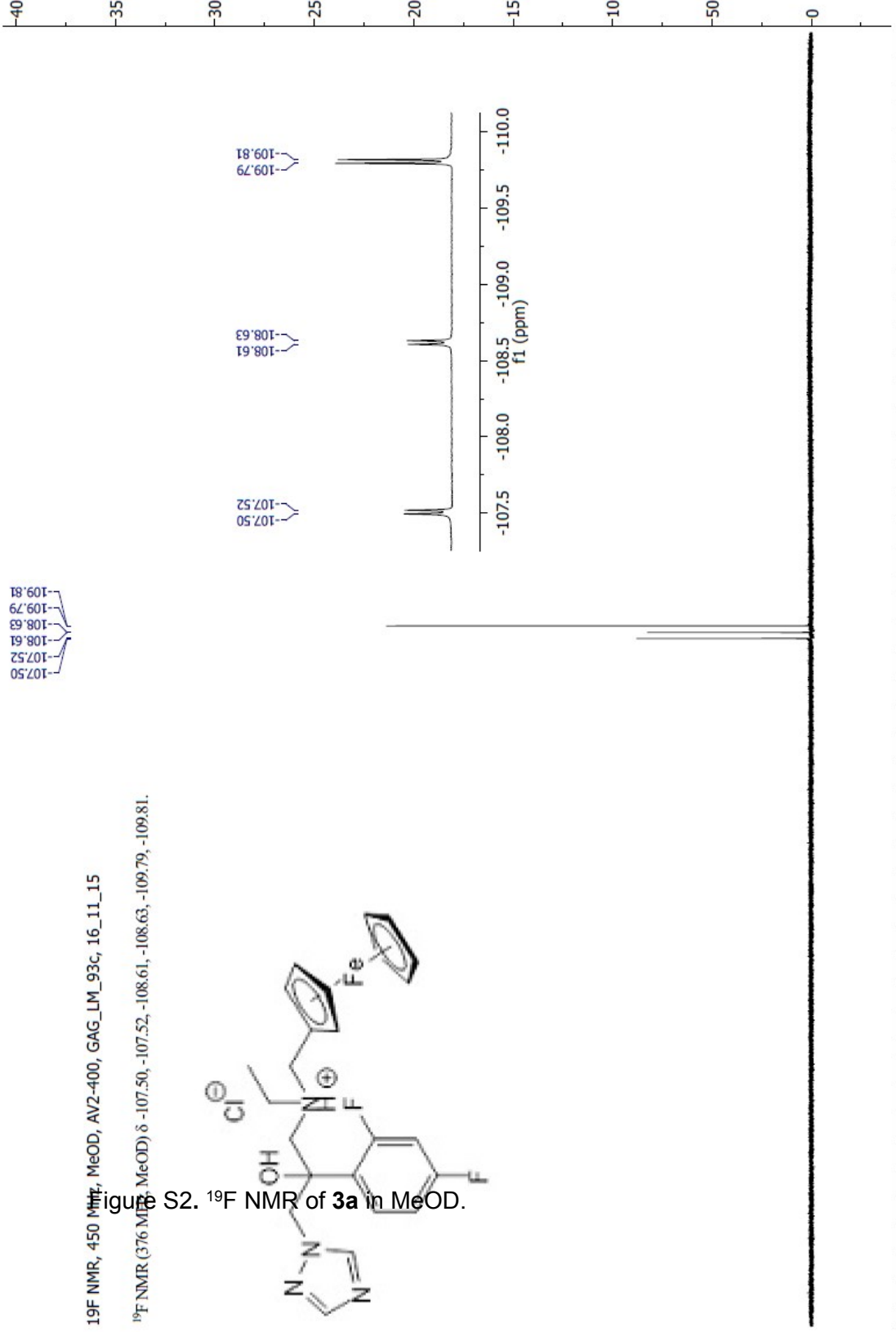

Figure S2. ^{19}F NMR of **3a** in MeOD.

Generic Display Report

Analysis Info

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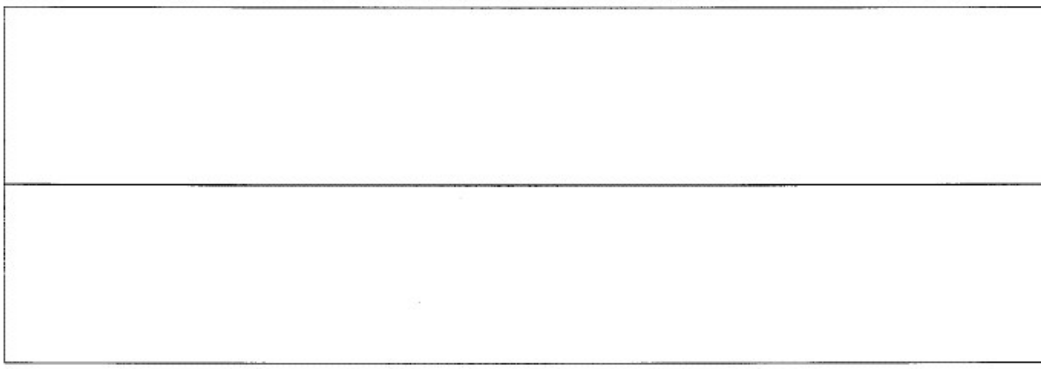
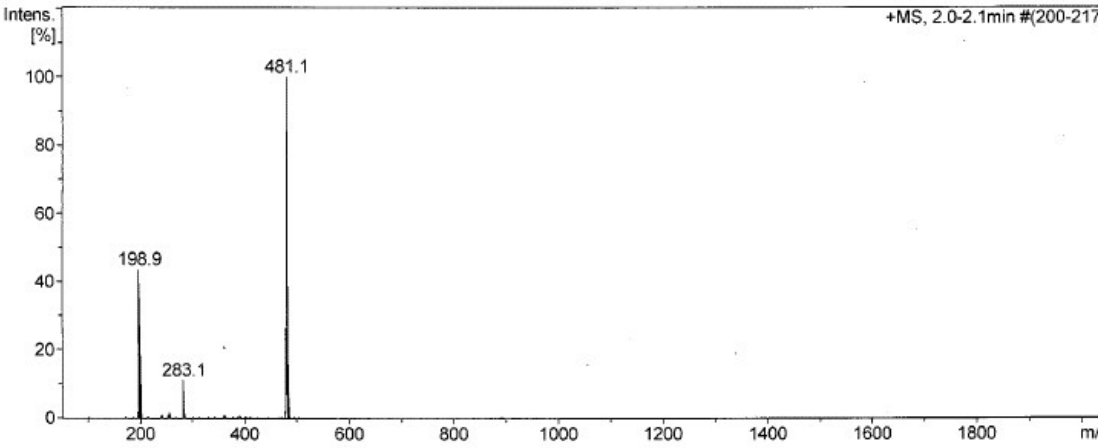
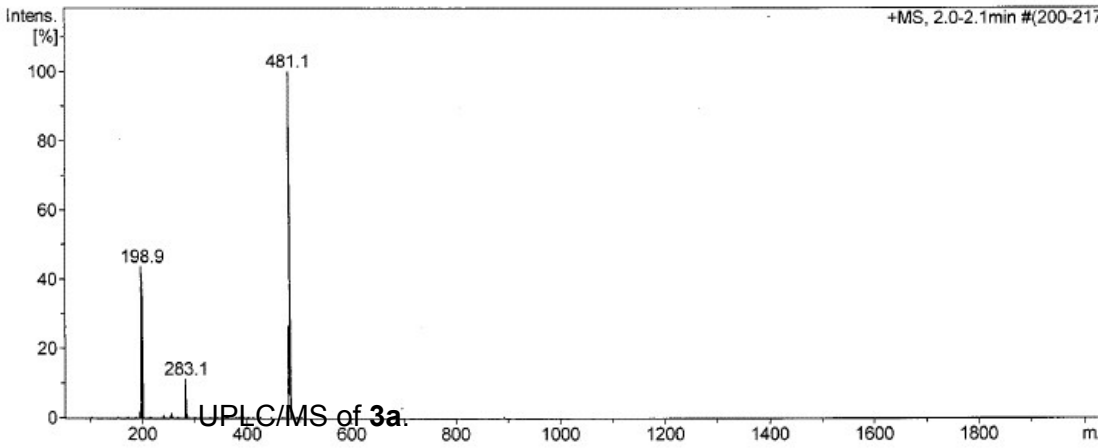
Sample Name	GAG_LM_93c_171115	Instrument	HCT
Comment			







UPLC/MS of **3a**.

Bruker Compass DataAnalysis 4.0 printed: 18.11.2015 09:04:17 Page 1 of 1

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Laboratorium für Organische Chemie
Vladimir-Prelog-Weg 3 HCI E304 8093 Zürich Tel: 044/633 43 58
Mikroelementaranalyse

Name: Mastrobuoni Luciano Gruppe: Gasser UNIZH
Labor: Y34H40 Tel: 044/635 46 86

Substanz:GAG-LM-93c
Molekularformel: C24 H27 N4 O F2 Cl Fe Mr = 516,80 g/mol

Schmelzpunkt: Sublimationspunkt:
gereinigt: wie? getrocknet: wie?

Bestimmungen: C H N

Eingang: 01.10.15 Ausgang: 01.10.15

M-160953 Operator: PK

Berechnete Gewichtsanteile:

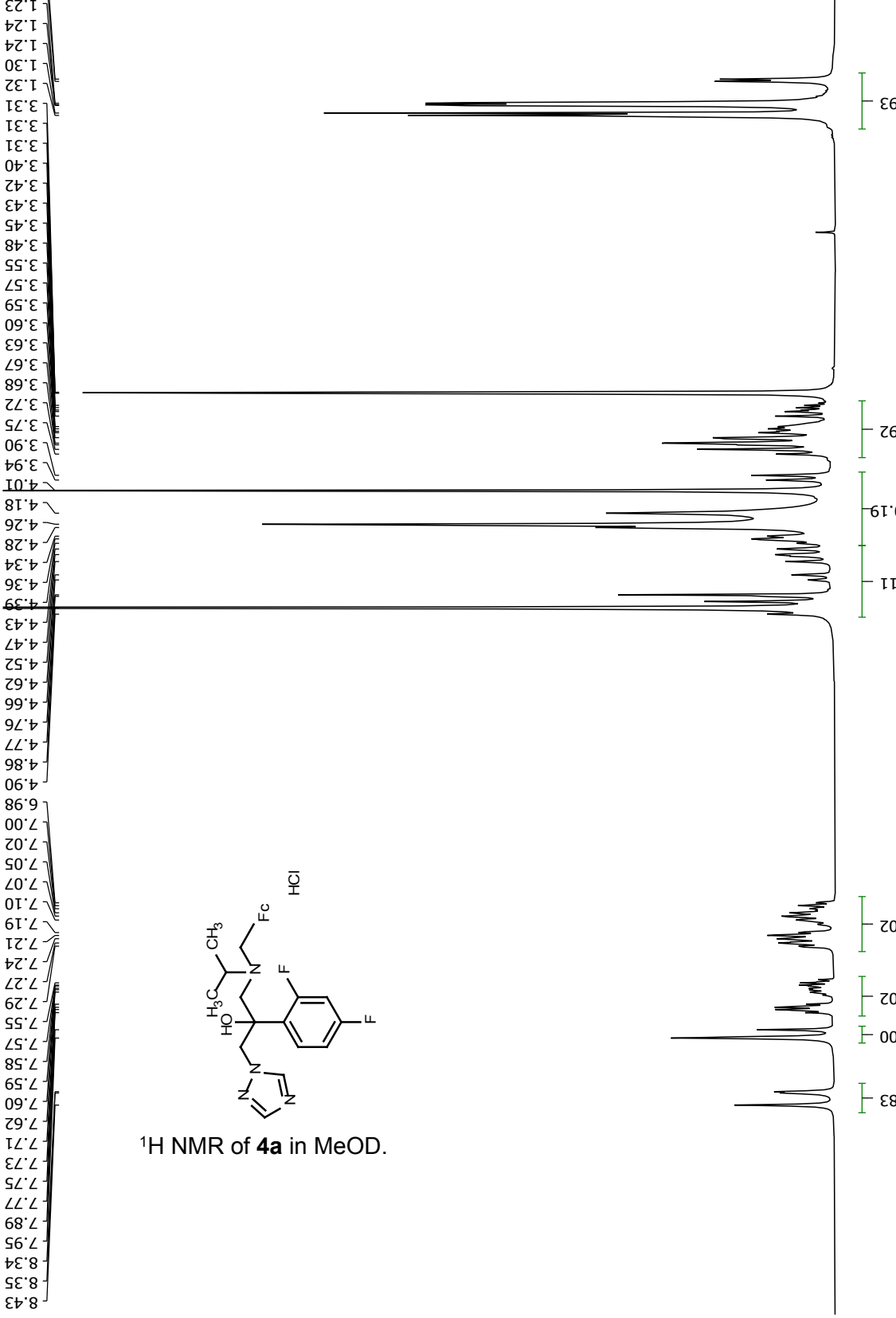
[C]	55,78%	[H]	5,27%	[N]	10,84%	[O]	3,10%	[F]	7,35%
[Cl]	6,86%	[Fe]	10,81%						

Gefundene Gewichtsanteile:

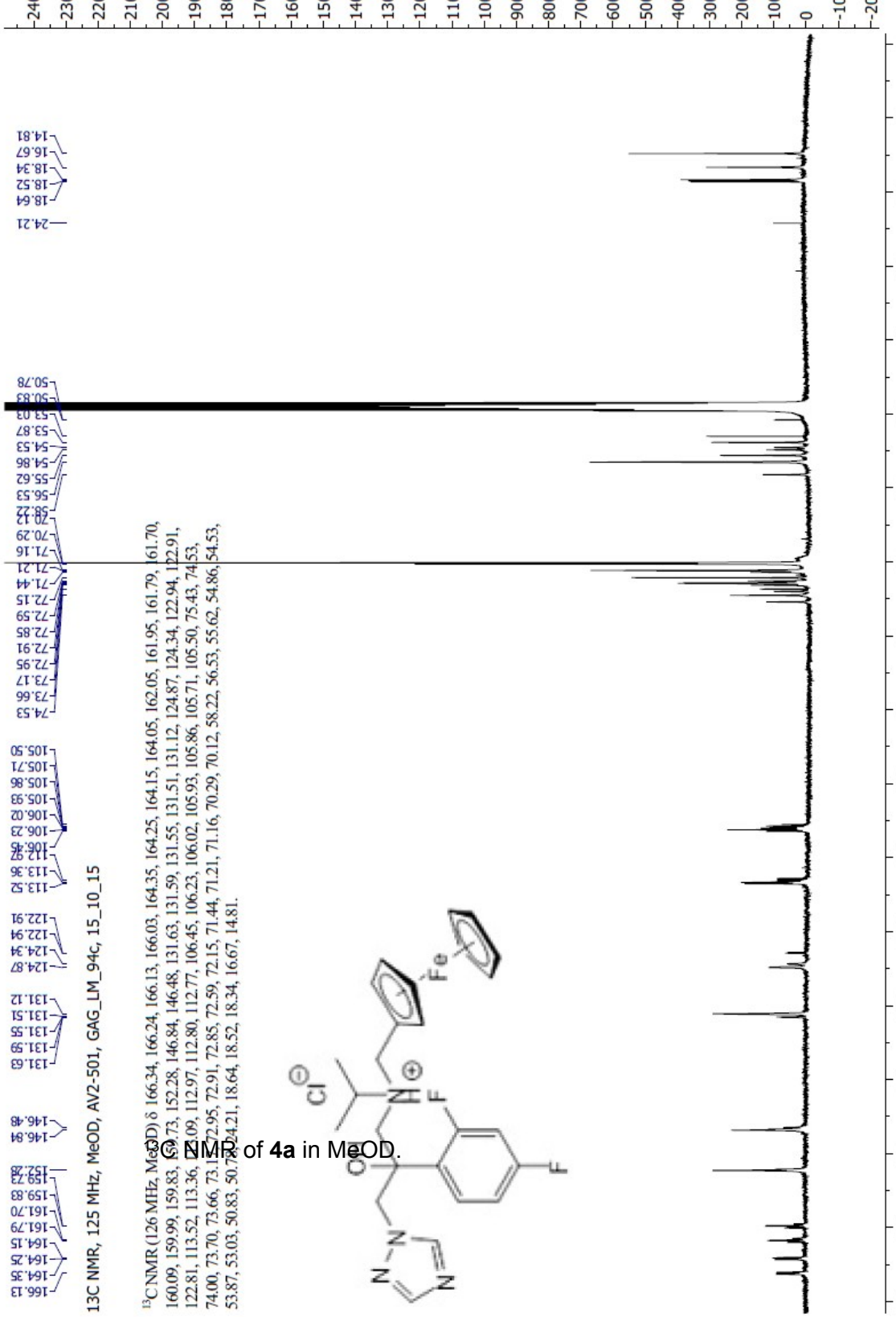
Einwaage: 1,149mg LECO TruSpec Micro
[C] 52,00% [H] 5,06% [N] 9,90% 01.10.15

Einwaage: 1,162mg LECO TruSpec Micro
[C] 51,93% [H] 5,01% [N] 9,90% 01.10.15

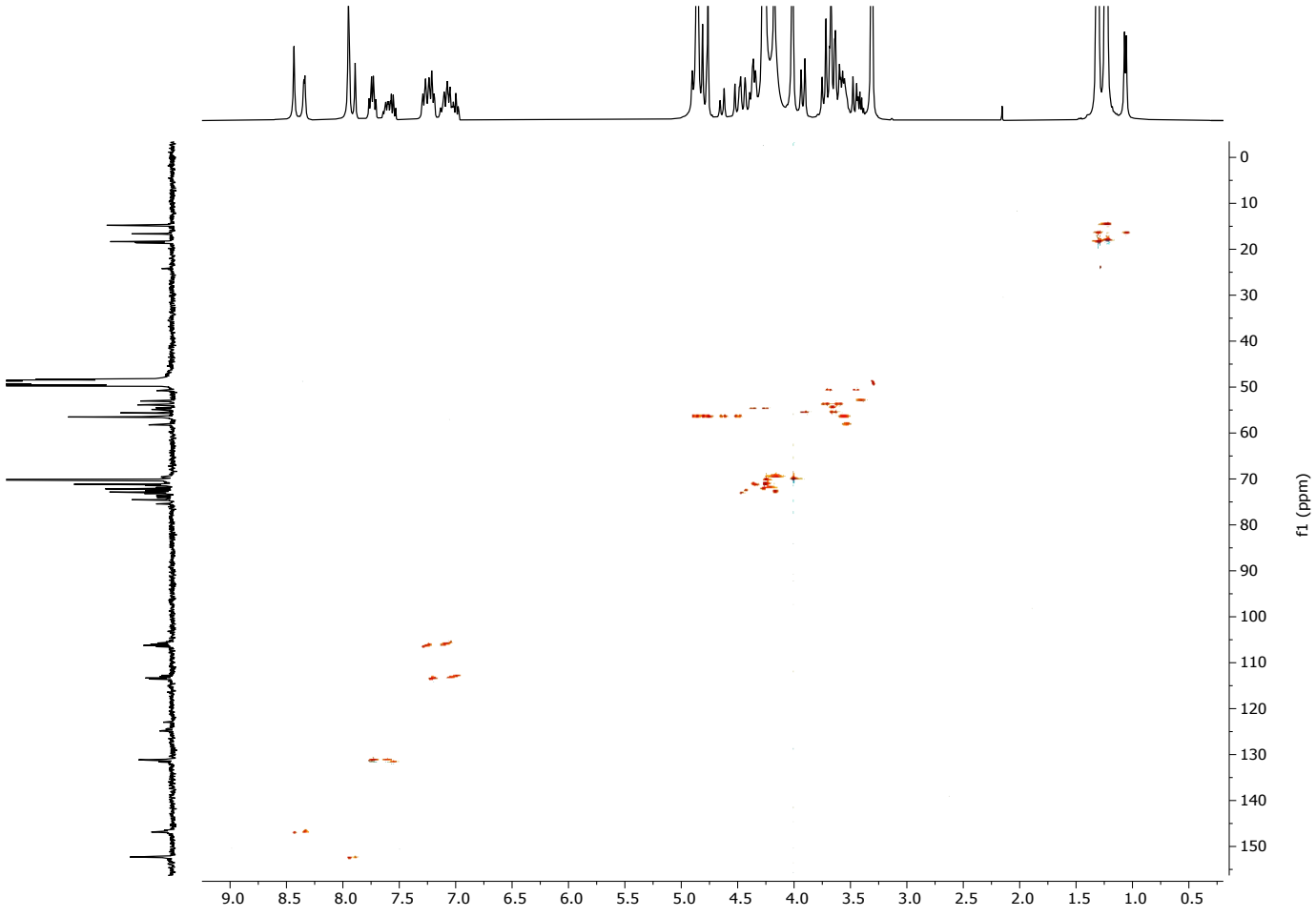
Elemental Analysis of **3a**.



^{1}H NMR of **4a** in MeOD.

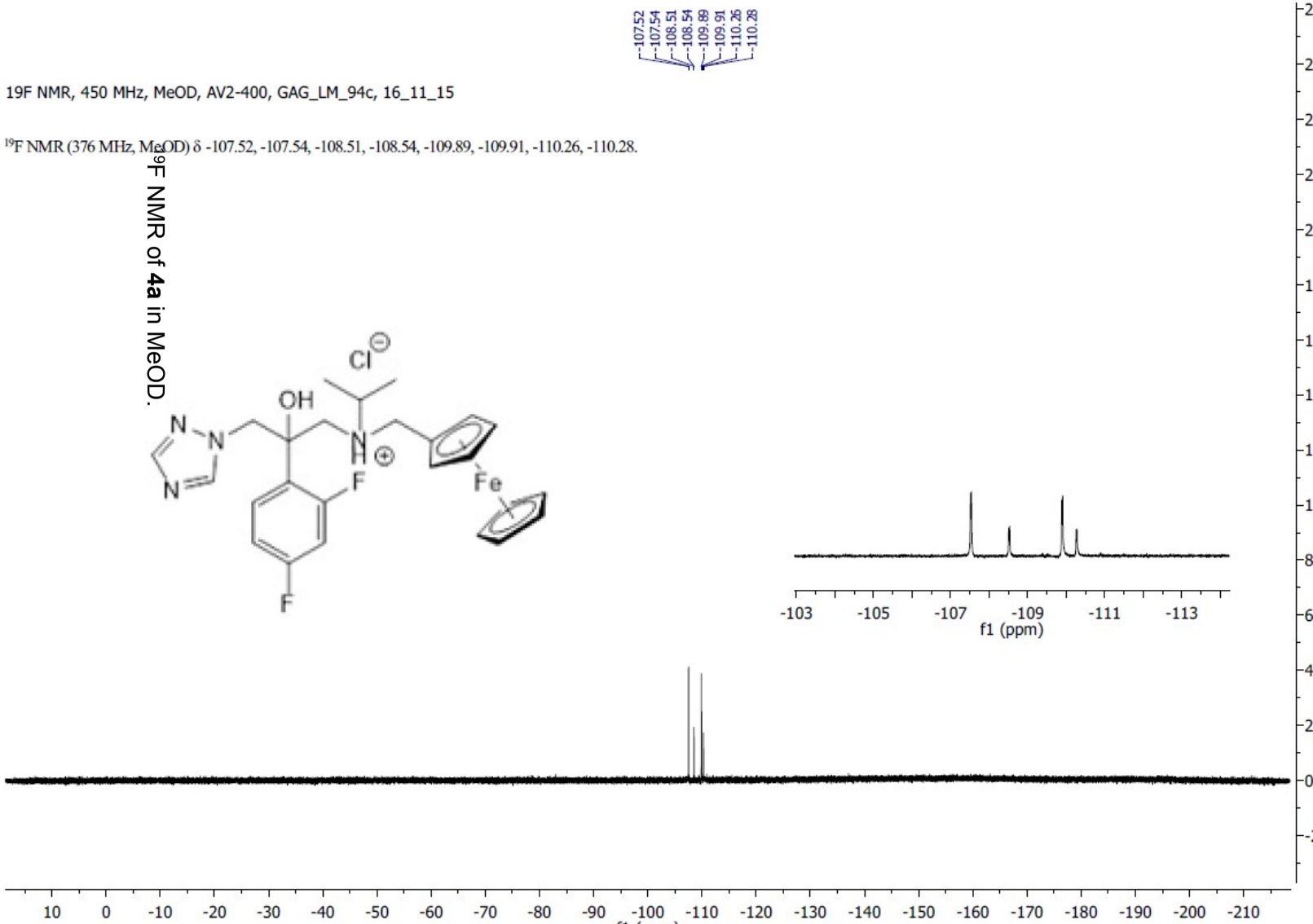


^{13}C NMR of **4a** in MeOD.


0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

HSQC of 4a

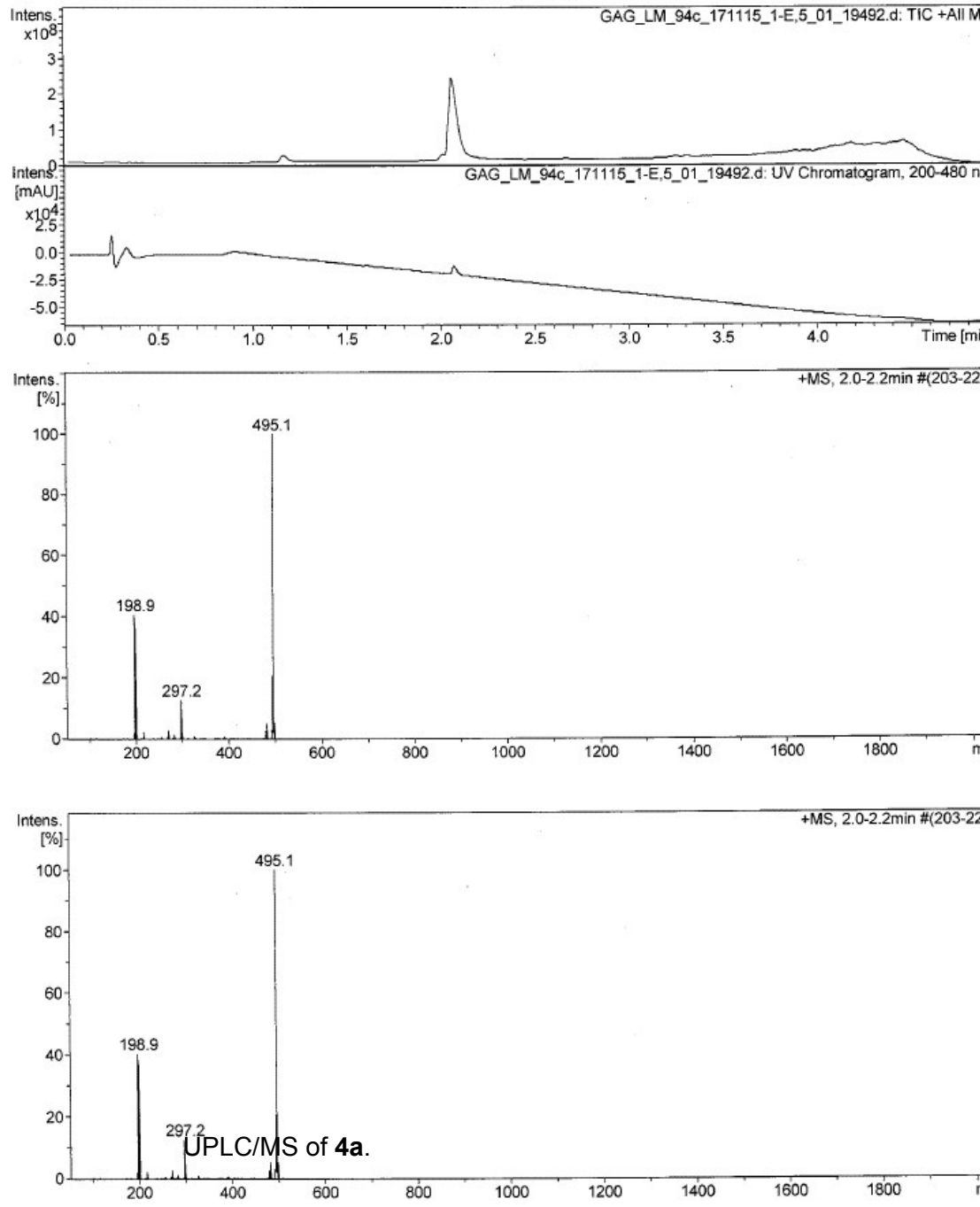
^{19}F NMR of **4a** in MeOD.



Generic Display Report

Analysis Info

Analysis Name	D:\data\Luciano Mastrobuoni\GAG_LM_94c_171115_1-E,5_01_19492.d	Acquisition Date	17.11.2015 18:51:37
Method	5-100 uplc col4 600-5min-lowmass_2015_19492.m	Operator	meola
Sample Name	GAG_LM_94c_171115	Instrument	HCT
Comment			



UPLC/MS of **4a**.

Bruker Compass DataAnalysis 4.0 printed: 18.11.2015 09:04:52 Page 1 of 1

Eidgenössische Technische Hochschule Zürich
Laboratorium für Organische Chemie
Vladimir-Prelog-Weg 3 HCI E304 8093 Zürich Tel: 044/633 43 58
Mikroelementaranalyse

Name: Mastrobuoni Luciano Gruppe: Gasser UNIZH
Labor: Y34H40 Tel: 044/635 46 86

Substanz:GAG-LM-94c
Molekularformel: C25 H29 N4 O F2 Cl Fe Mr = 530,83 g/mol

Schmelzpunkt: ? Sublimationspunkt:
gereinigt: wie ? getrocknet: wie ?
hygroskopisch

Bestimmungen: C H N

Eingang: 19.10.15 Ausgang: 19.10.15

M-161071 Operator: SM

Berechnete Gewichtsanteile:

[C] 56,57% [H] 5,51% [N] 10,55% [O] 3,01% [F] 7,16%
[Cl] 6,68% [Fe] 10,52%

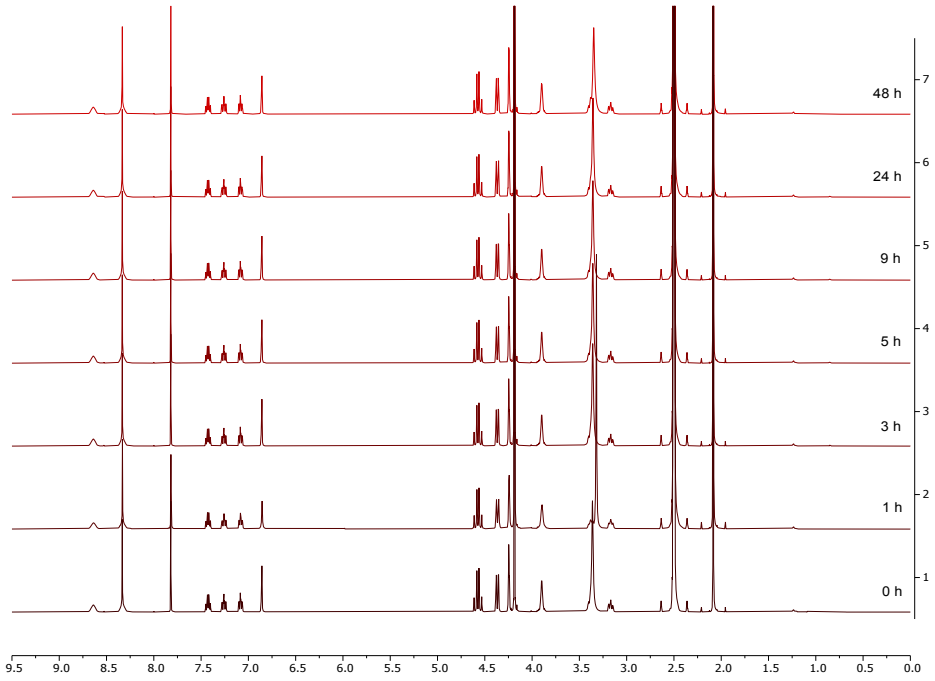
Gefundene Gewichtsanteile:

Einwaage: 1,346mg LECO CHN-900
[C] 56,43% [H] 5,61% [N] 10,61% 19.10.15

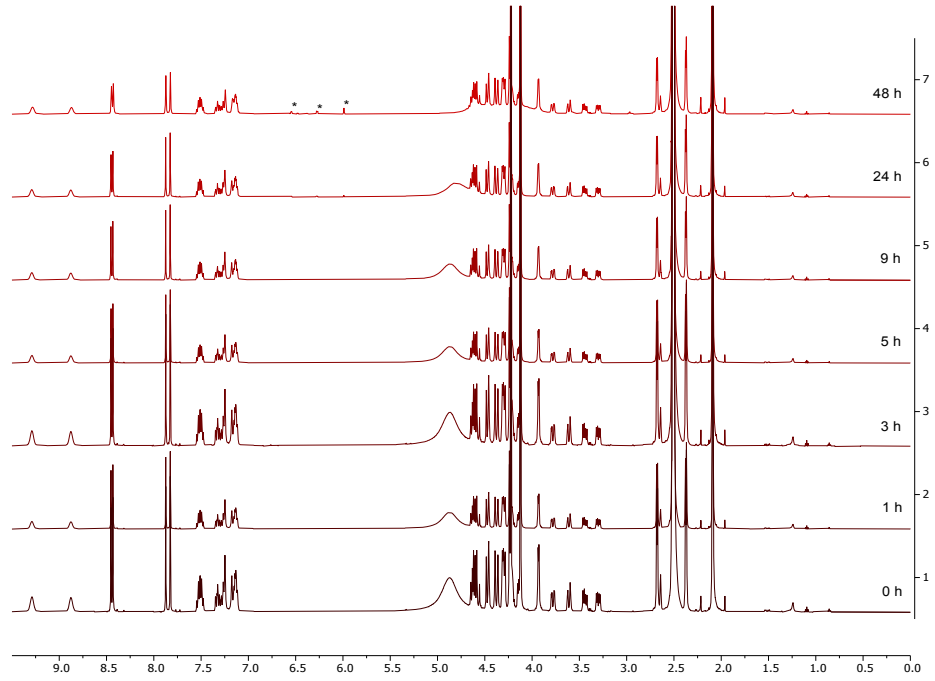
Elemental Analysis of **4a**.

5. Stability Assays: NMR spectra

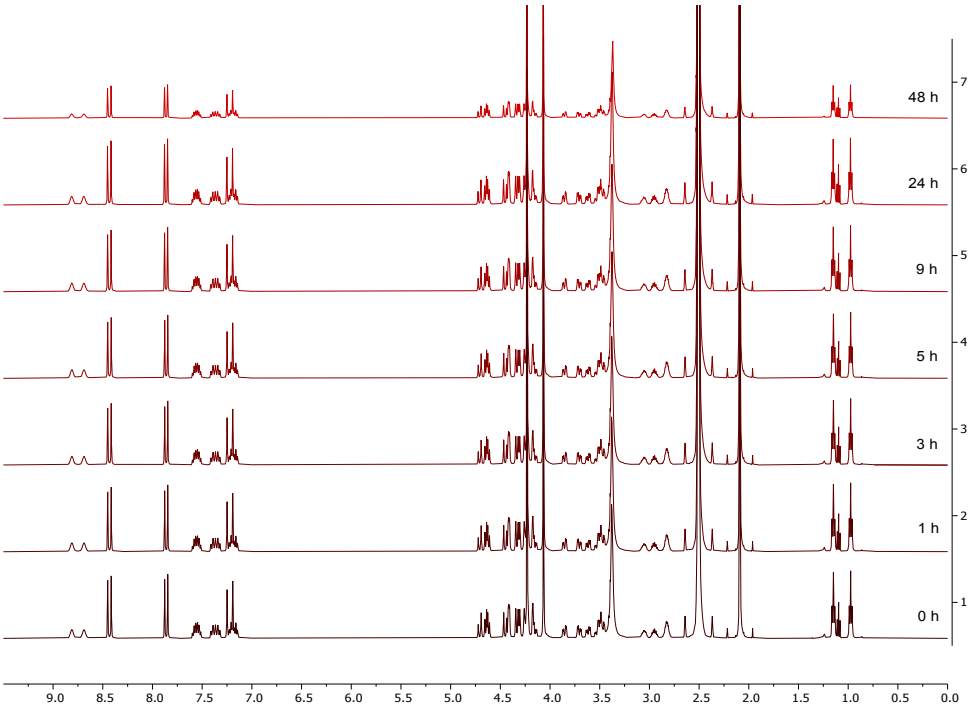
5.1 Stability of the compounds in DMSO



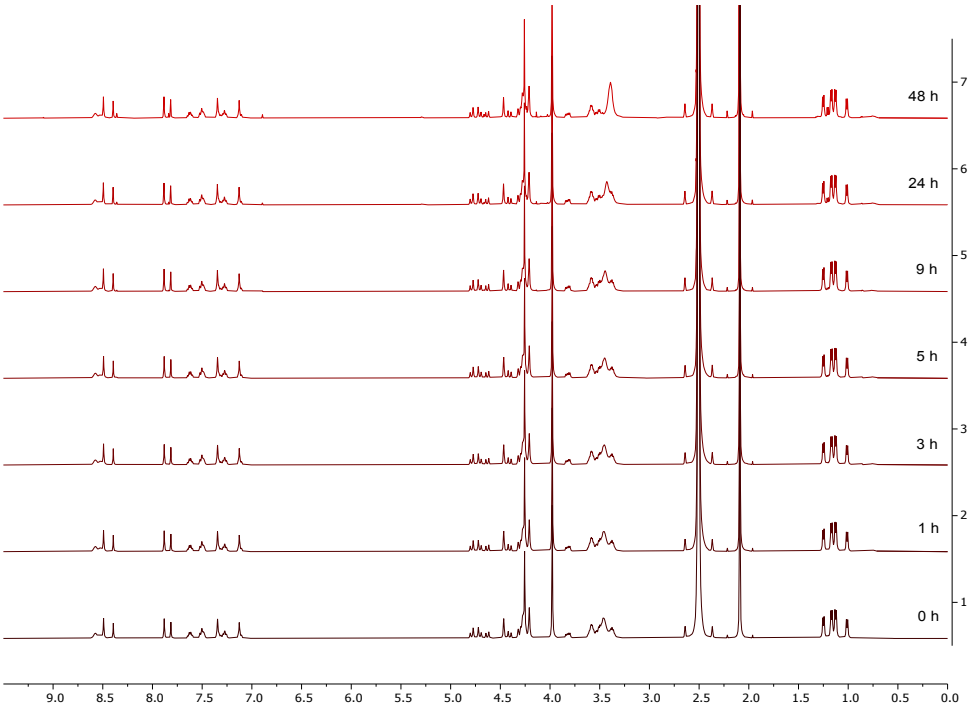
^{1}H NMR (500 MHz, DMSO-d_6); stability of 1a in DMSO over 48 h



^{1}H NMR (500 MHz, DMSO-d_6); stability of 2a in DMSO over 48 h

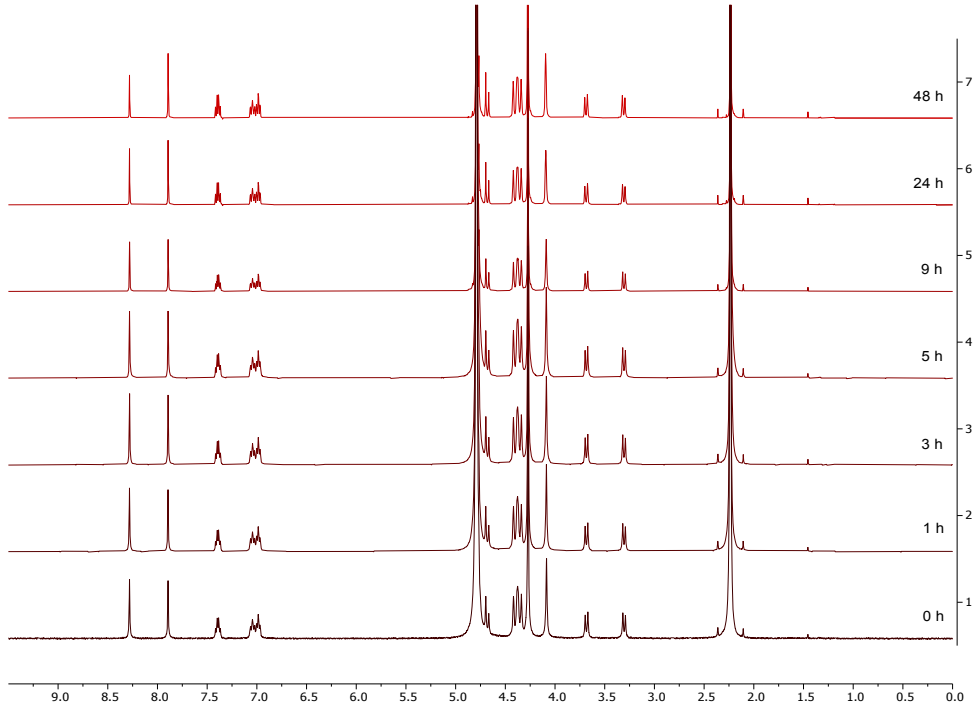


^{1}H NMR (500 MHz, DMSO-d_6); stability of 3a in DMSO over 48 h

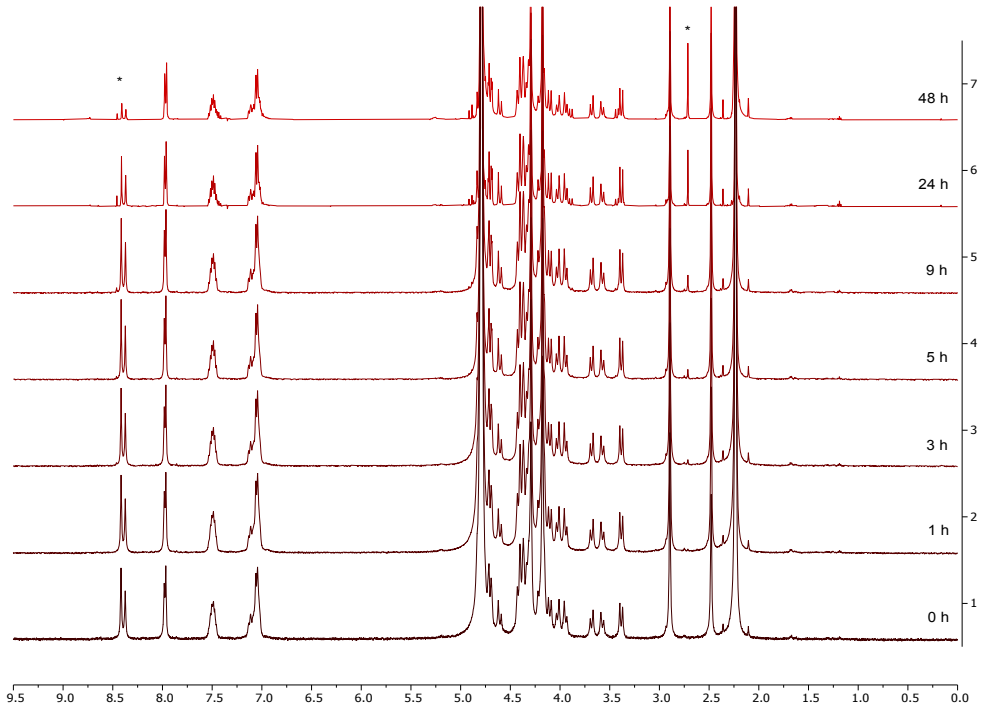


^{1}H NMR (500 MHz, DMSO-d_6); stability of 4a in DMSO over 48 h

5.2 Stability of the compounds in water

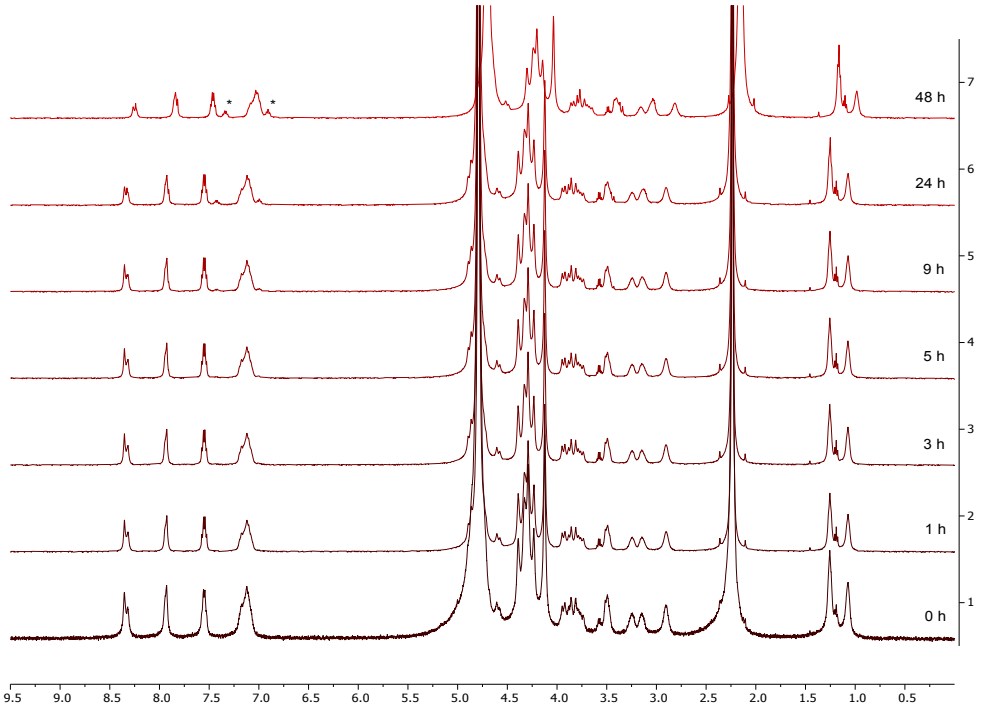


^{1}H NMR (500 MHz, D_2O); stability of 1a in D_2O over 48 h

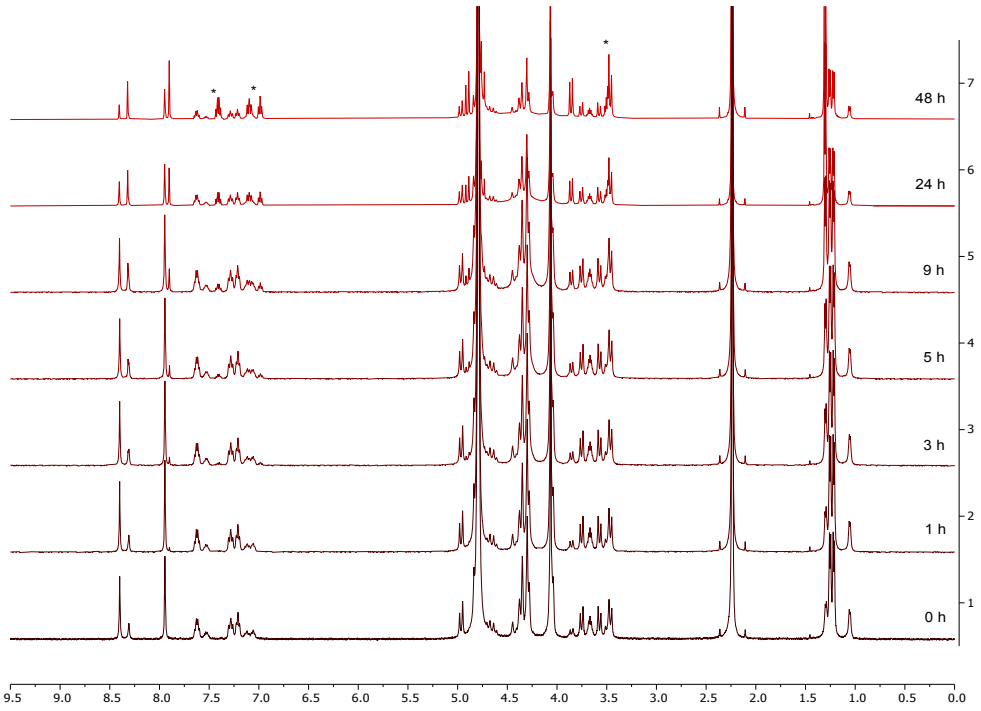


The gradual disappearance of the signal at 8.4 ppm was attributed with a slow H-D exchange

^{1}H NMR (500 MHz, D_2O); stability of 2a in in D_2O over 48 h



^{1}H NMR (500 MHz, D_2O); stability of 3a in in D_2O over 48 h



^{1}H NMR (500 MHz, D_2O); stability of 4a in in D_2O over 48 h

6. References

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