Electronic Supplementary Material (ESI) for Metallomics.

A mitochondria-targeting fluorescent probe for detection of mitochondrial labile Fe(II) ion

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Contents

1. Steady state absorption and fluorescence measurements

The UV-vis absorption spectra were recorded on an Agilent 8453 photodiode array UV-vis spectrometer. Fluorescence spectra were recorded using a JASCO FP6600 with a slit width of 5 nm and 6 nm for excitation and emission, respectively. The path length was 1 cm with a cell volume of 3.0 mL. Excitation was provided at 510 nm.

For all the fluorescence measurements, MtFluNox was used at a final concentration of 2 μM (from 1 mM stock solution in DMSO) in 50 mM HEPES buffer (pH 7.4).

Quantum yields were measured in 50 mM HEPES buffer (pH 7.4) by using a Quantaurus-QY absolute photo-luminescence quantum yields measurement system (C11347-01, Hamamatsu Photonics).

Fluorescence responses of MtFluNox to various metal ions were measured as follows. An aqueous solution of transition metal ion species (stock solutions: 10 mM for $MnSO_4$, $CoSO_4$, $NiSO_4$, $FeSO_4$, $FeCl_3$, $CuSO_4$, and $ZnSO_4$; 1 M for NaCl, KCl, $MgCl_2$, and $CaCl_2$), or $[Cu(CH_3CN)_4]PF_4$ (from 10 mM stock solution in MeCN) was added to give the final concentrations of 1 mM for Na(I), K(I), Mg(II), and Ca(II) and 20 μM for other metal ion species. In the case of Cu(I), MtFluNox was reacted with Cu(I) in the presence of 200 μM glutathione. The mixtures were kept at room temperature for 1 h and then applied to fluorescence measurement. Selectivity for reductants, reactive oxygen species, transferrin, and effect of chelator were tested under the conditions as follows.

$Na_2S_2O_3$	: 100 μM from 100 mM stock solution in water
Sodium ascorbate	: 1 mM from 100 mM stock solution in water
Cysteine	: 1 mM from 100 mM stock solution in water
Glutathione	: 1 mM from 100 mM stock solution in HEPES buffer (pH was adjusted to 7.4)
$NaNO_2$	: 100 μM from 100 mM stock solution in water
$O_2^{\bullet -}$	: 100 μM from saturated KO_2 solution in DMSO (ca. 1 mM)[1)]
H_2O_2	: 100 μM from 100 mM stock solution in water
•OH	: 200 μM H_2O_2 and 20 μM $FeSO_4$ (1 mM stock solution of MtFluNox in DMF was used instead of DMSO)
NaOCl	: 100 μM from 100 mM stock solution in water
NO	: 100 μM NOC-5 from 10 mM stock solution in 0.1 M NaOH aq.
hemin	: 20 μM hemin from 5 mM stock solution in DMSO.
Fe(II)+Bpy	: 20 μM $FeSO_4$ in the presence of 200 μM 2,2'-bipyridyl (Bpy) form 100 mM stock solution in DMSO.
Fe(II)+DFO	: 20 μM $FeSO_4$ $FeSO_4$ in the presence of 200 μM deferroxamine (DFO) form 100 mM stock solution in water.

MtFluNox (2 μM) was incubated under each condition in 50 mM HEPES buffer (pH 7.4, 0.2% DMF as co-solvent) for 1 h, and then fluorescence spectra were measured, respectively.

2. Product analysis

To a solution of a probe (100 μM) in 50 mM HEPES buffer (pH 7.4, 5%DMSO as co-solvent) was added a solution of $FeSO_4$ (final, 1 mM). The mixture was kept for 1 h under an ambient condition. The products were analyzed with LC-MS system (Chromaster®5110, Hitachi High-tech) equipped with a photodiode-array detector (Chromaster®5430, Hitachi High-tech) and a mass spectrometer (Chromaster®5610 MS Detector, Hitachi High-tech) and with Waters symmetry C_{18} column (3.5 μm, 4.6 × 75 mm) eluted with a gradient system consisting of H_2O (solvent A) and CH_3CN (solvent B) containing 0.05% formic acid; 20%B to 70%B over 20 min. The retention times were compared with those of the parent dyes in 50 mM HEPES buffer (pH 7.4, 5%DMSO as co-solvent). Assignments of the compounds were based on the observed *m*/*z* values at each peak.

Reference

1) A. R. Lippert, E. J. New and C. J. Chang, *J. Am. Chem. Soc.*, 2011, **133**, 10078–10080.

3. Supporting figures

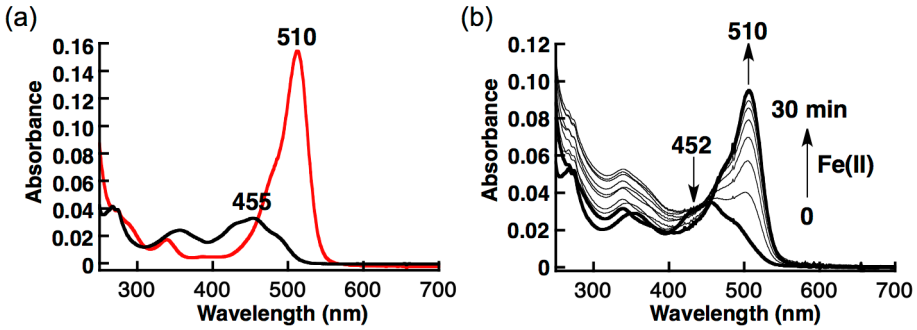


Figure S1 (a) UV-vis absorption spectra of MtFluNox (2 μM, black line) and MtRhodol (2 μM, red line). (b) UV-vis absorption change of MtFluNox (2 μM) upon the addition of Fe(II) (20 μM; as $FeSO_4$). Each spectrum was recorded every 5 min. All the spectra were obtained in HEPES buffer (50 mM, pH 7.4, 0.2%DMSO as co-solvent).

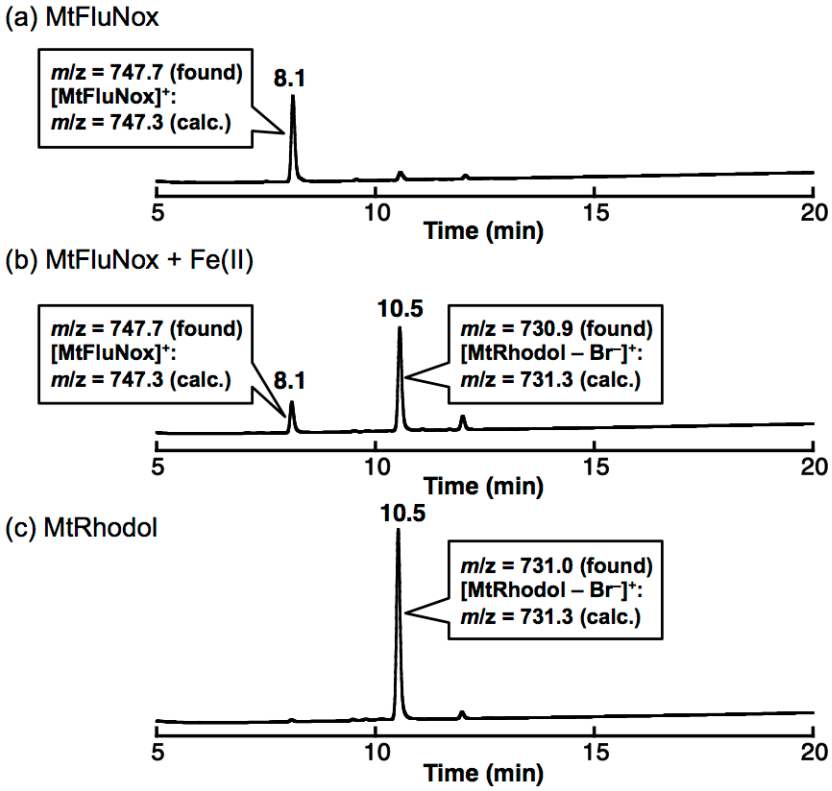


Figure S2 HPLC-mass monitoring of the reaction between MtFluNox (100 µM) and Fe(II) ion (1 mM). (a) MtFluNox, (b) the reaction mixture of MtFluNox and Fe(II), and (c) MtRhodol (as the parent fluorophore). The detailed LC condition is described in the experimental section 3. Product analysis.

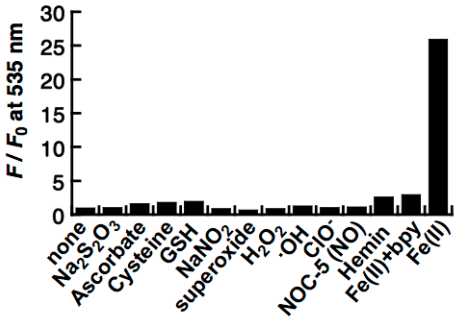


Figure S3 Fluorescence response of MtFluNox against various reductants, reactive oxygen species. Bars represent relative fluorescence intensities at 535 nm.

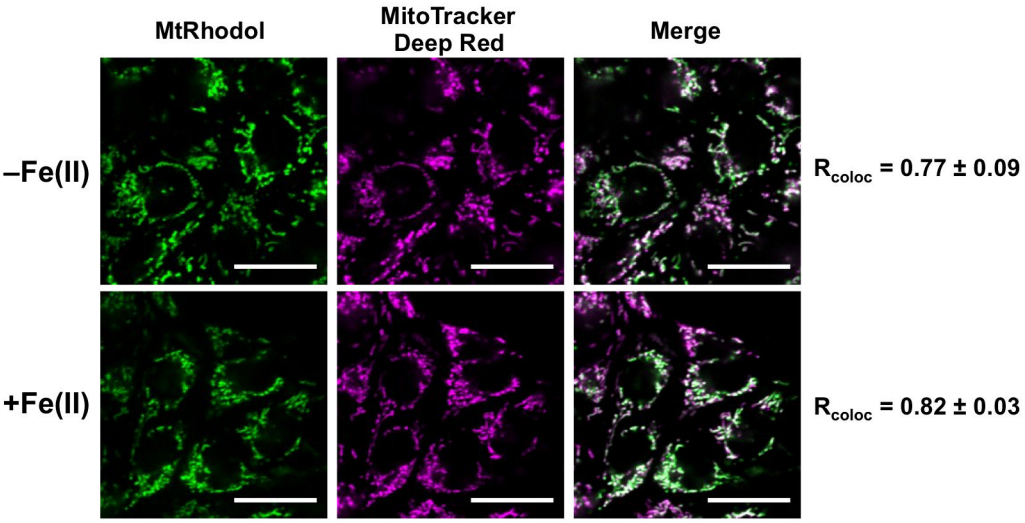


Figure S4 (a) Colocalization assay of MtFluNox and MitoTracker® Deep Red FM in HepG2 cells in the absence or presence of Fe(II). (b) Colocalization assay of MtRhodol and MitoTracker® Deep Red FM in HepG2 cells in the absence or presence of Fe(II). Pearson's correlation values (R_{coloc}) for each experiment are shown (n = 5). Scale bars indicate 25 μm.

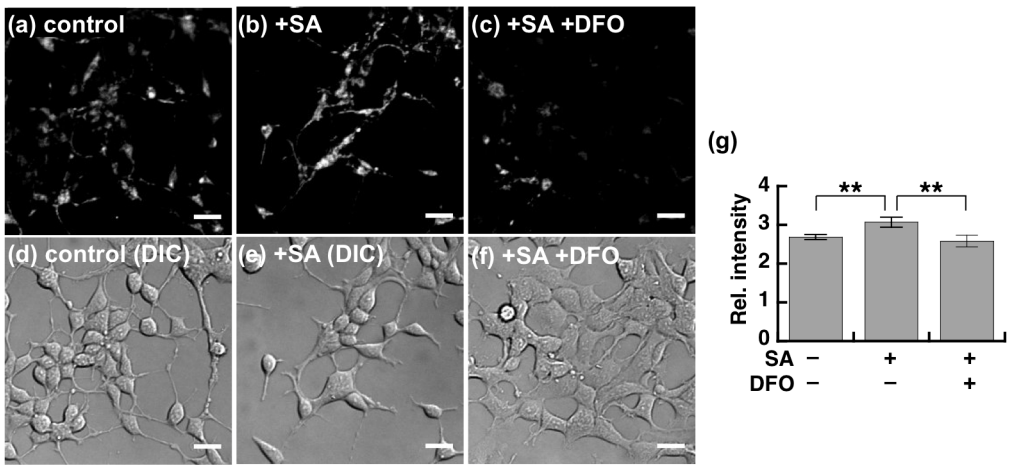
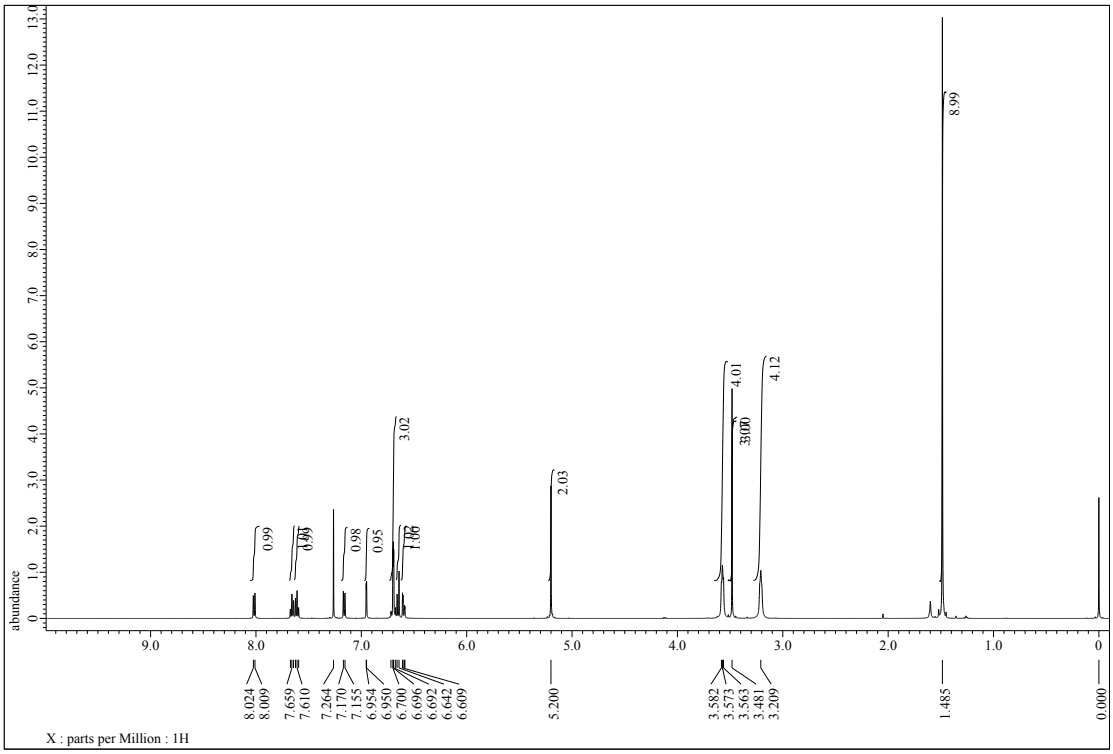


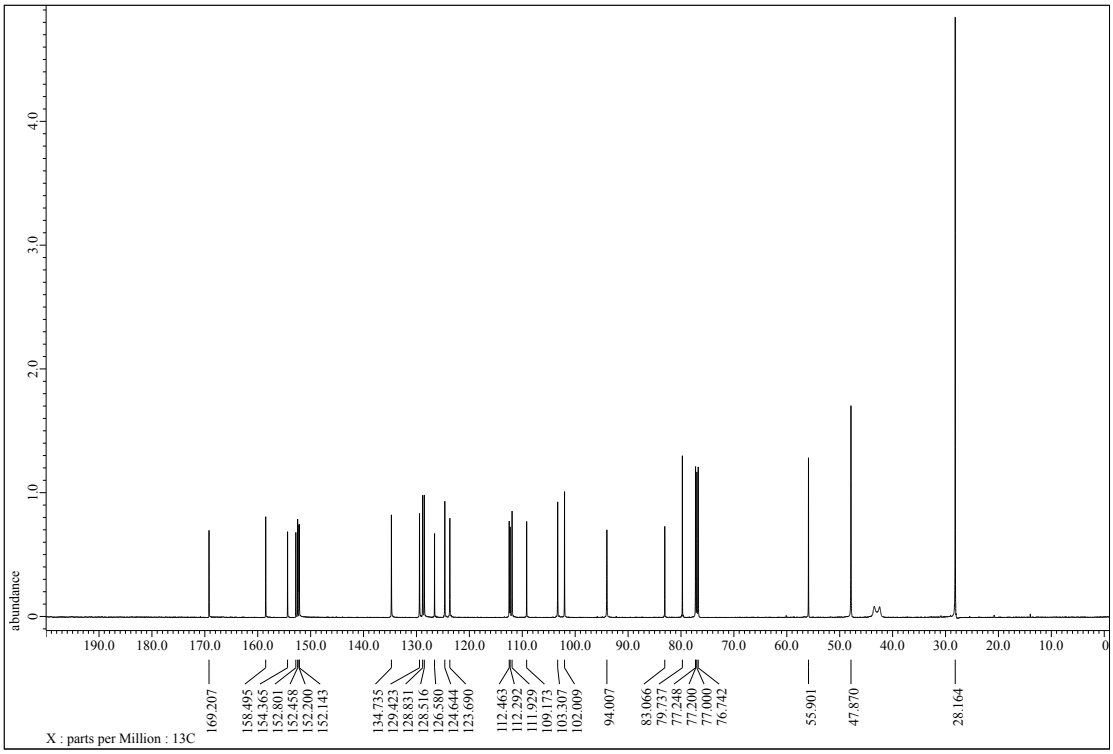
Figure S5 Fluorescence microscopic images of mitochondrial labile Fe(II) in HEK293 cells treated with (a) vehicle, (b) succinylacetone (SA; 1 mM), or (c) SA in the presence of DFO (100 μM). The cells were stained with Ac-MtFluNox (1 μM, 1 h). (d–f) DIC images of the same fields of view of (a–c),

respectively. Scale bars indicate 25 μm. (g) Quantification of the images taken under each condition (n = 10). Statistical analysis was performed by Student's *t*-test. $^{**}P < 0.05$ (n = 6). Error bars indicate ±S.E.M.

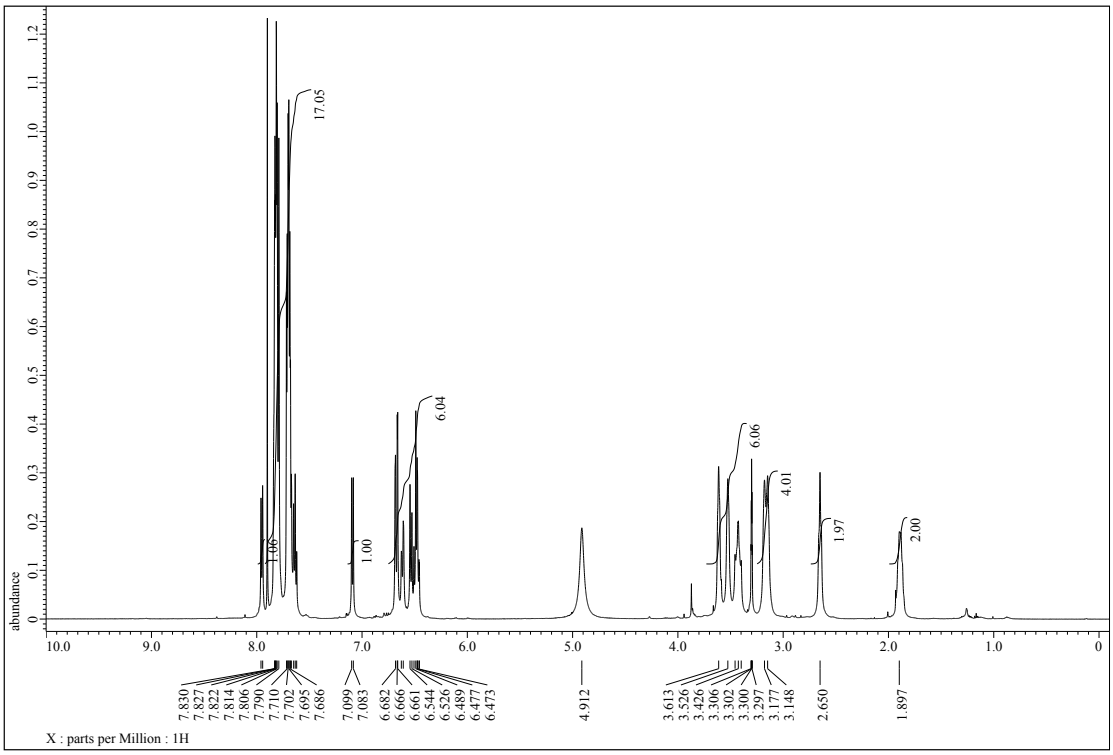
4. ^{1}H- and ^{13}C-NMR spectra of the newly synthesized compounds

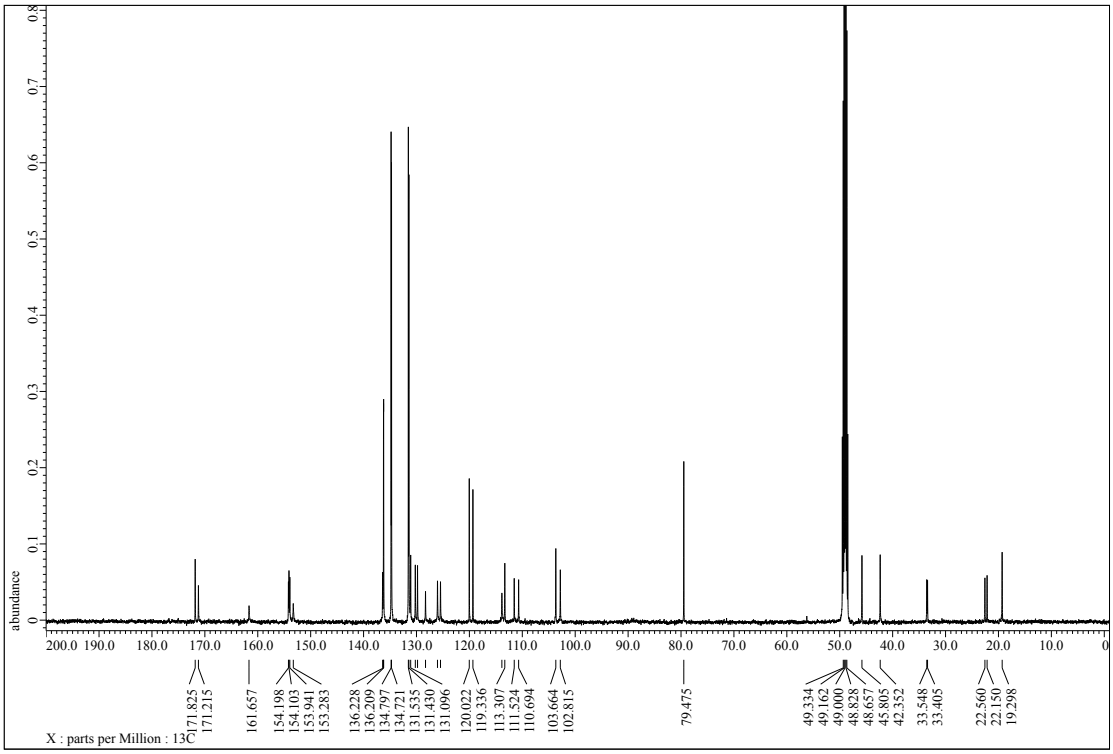
3-*O*-Methoxymethyl-6-(4-*tert*-butoxycarbonylpyperazin-1-yl)rhodol (Boc-piperazinylrhodol, 2)



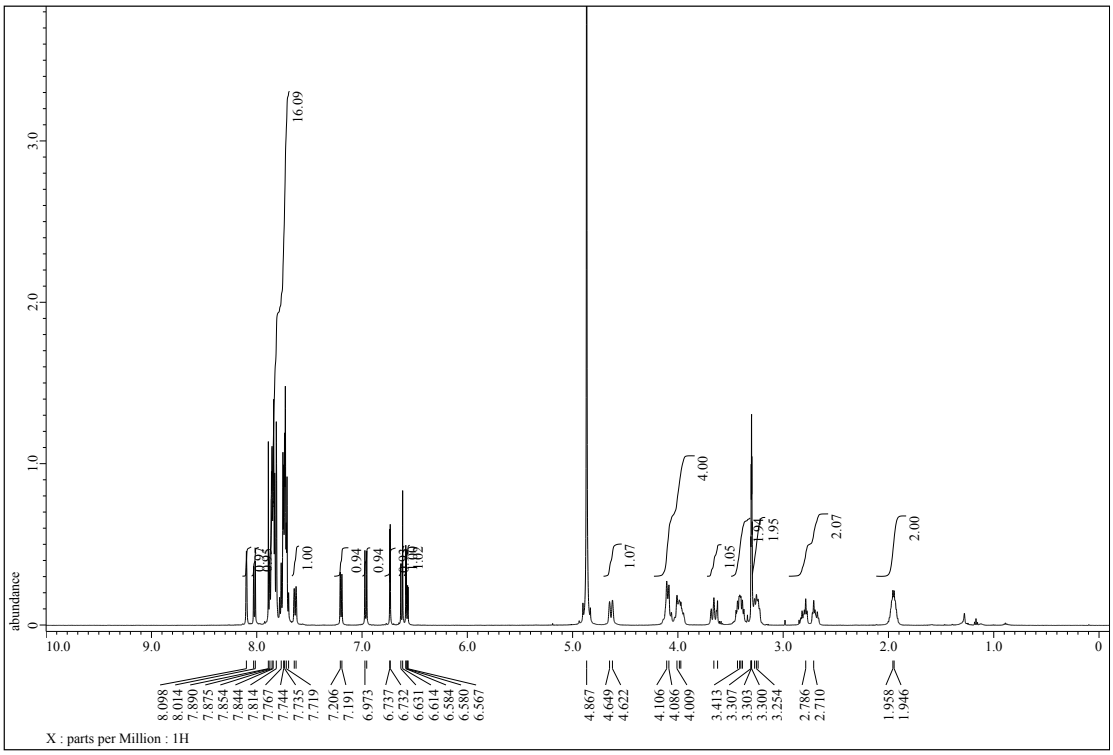


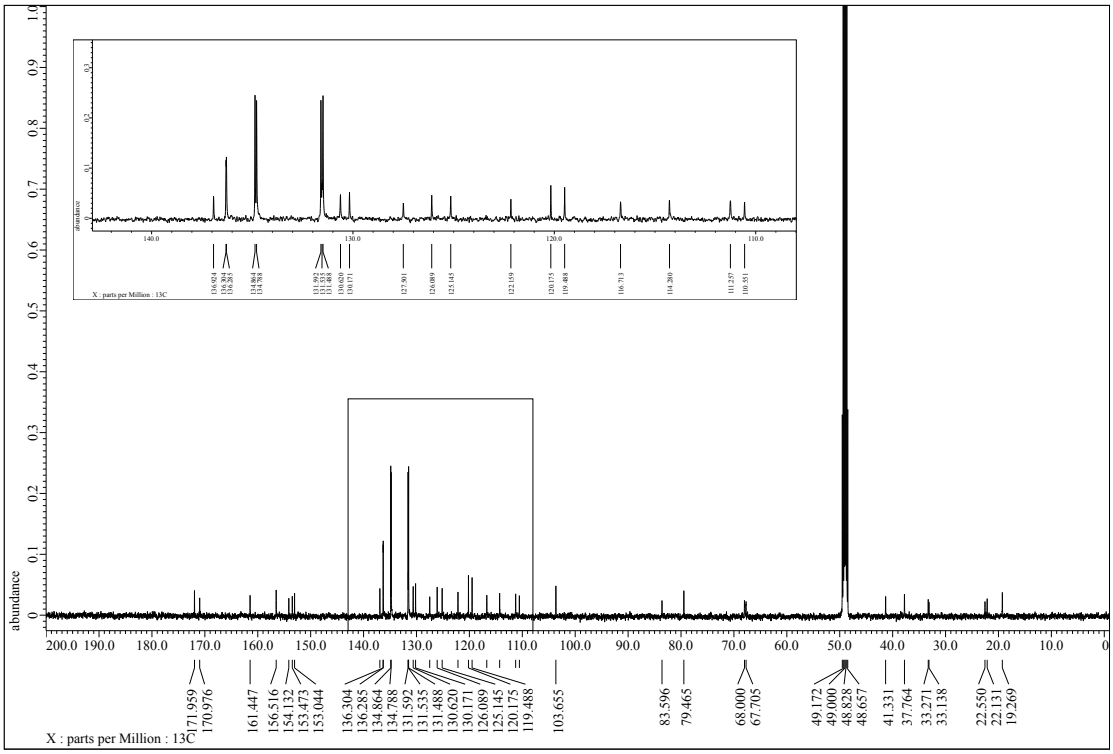
Piperazinylrhodol-triphenylphosphonium bromide salt (MtRhodol, 4)



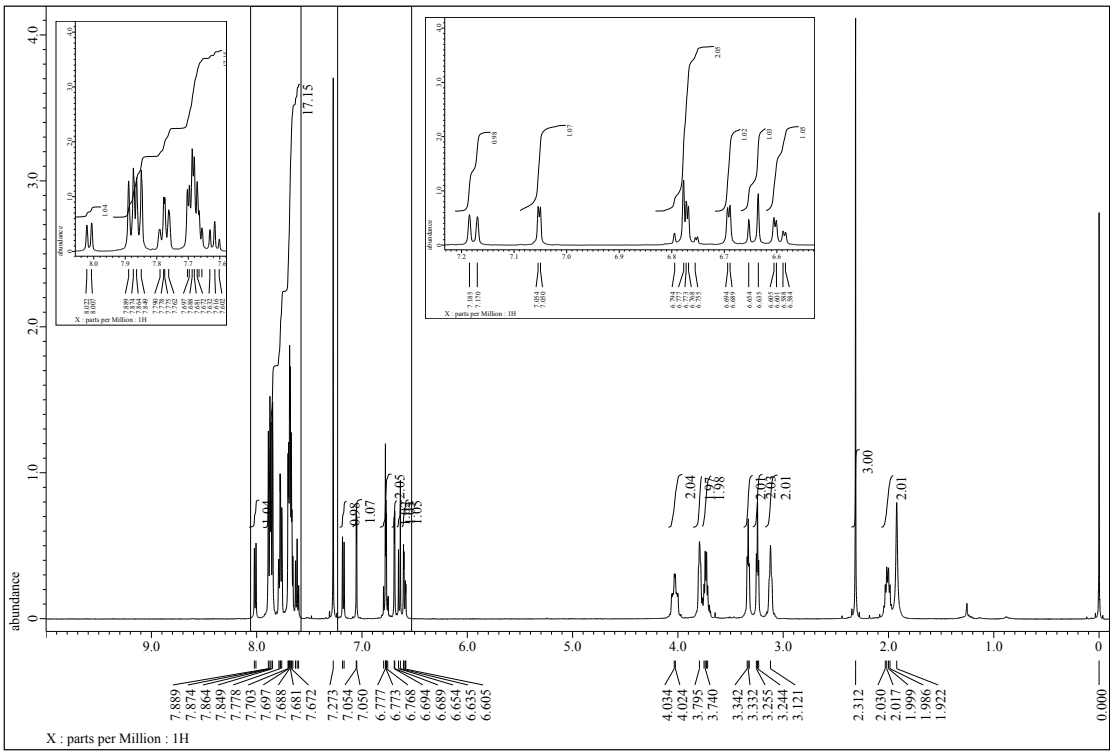


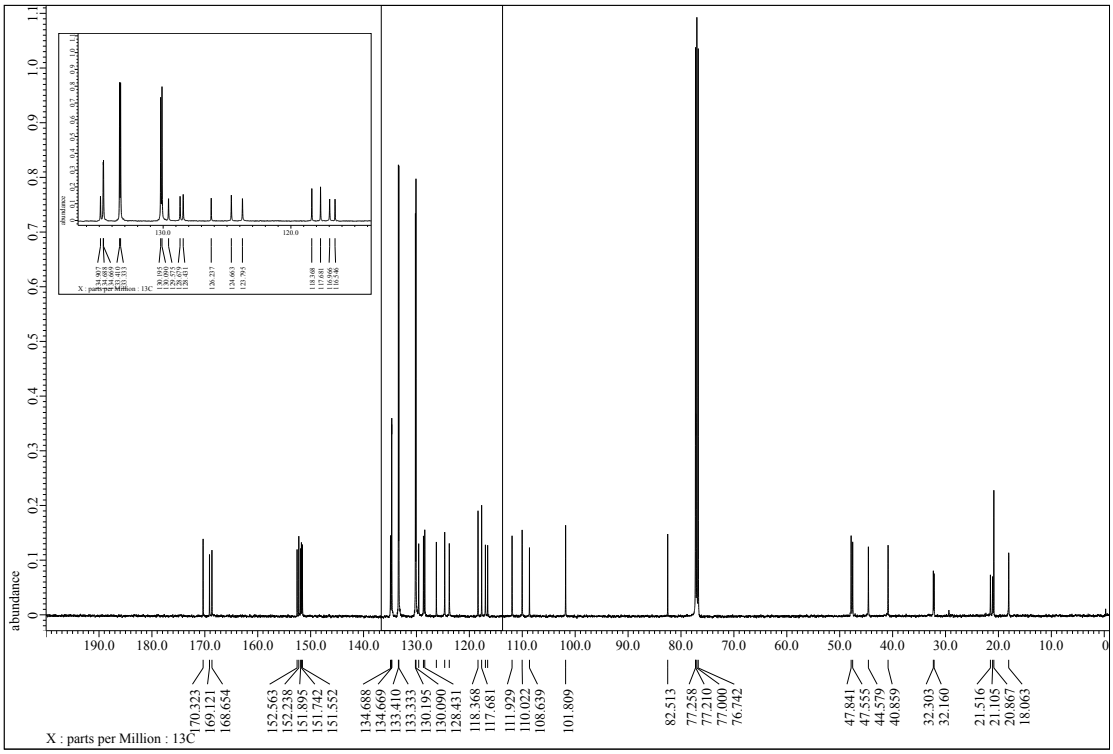
MtFluNox•PF_6 salt



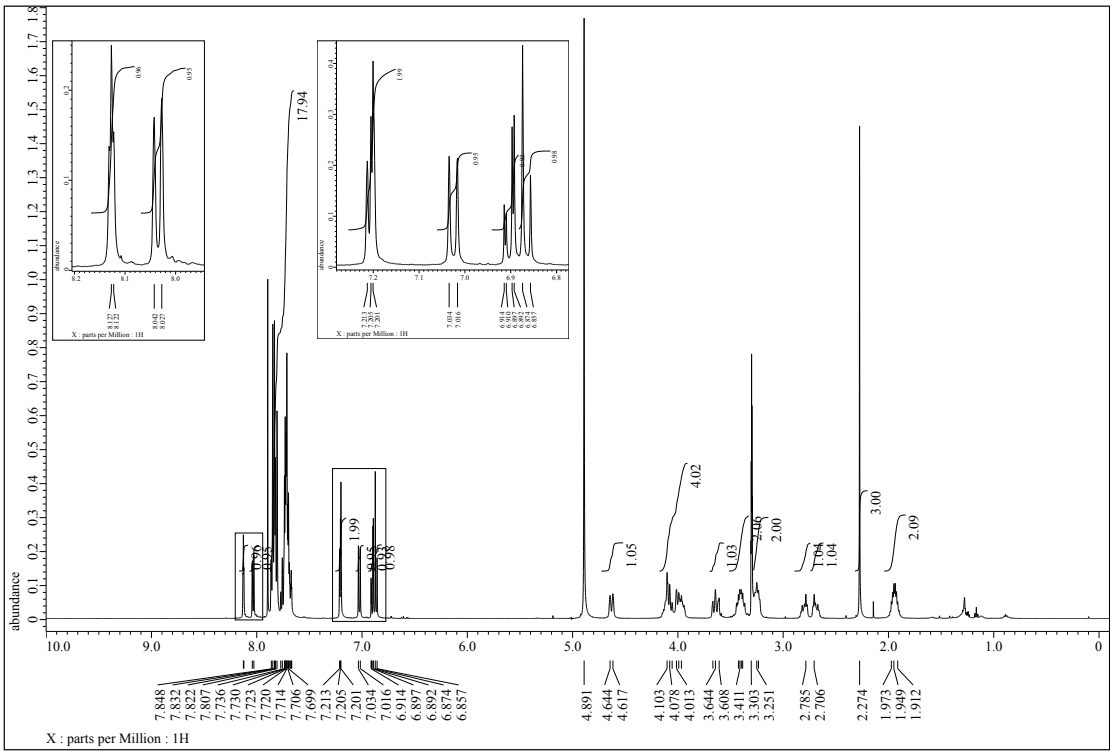


Ac-MtRhodol (5)





Ac-MtFluNox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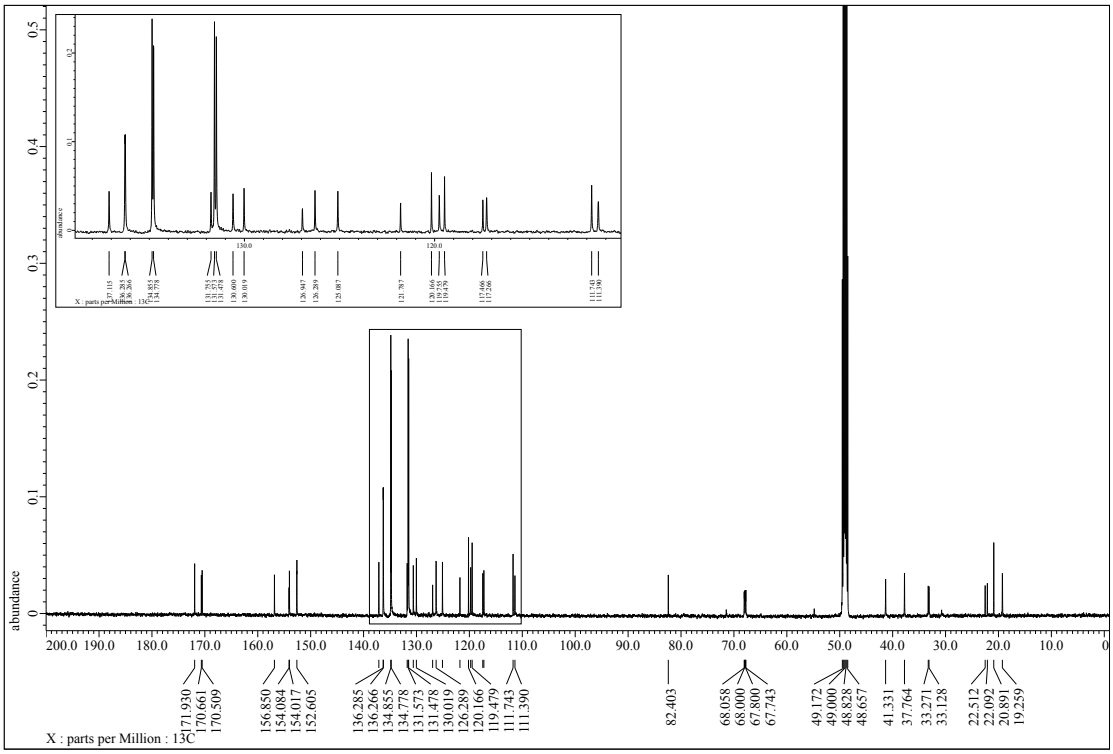
abundance
X : parts per Million : 1H


abundance
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