Electronic Supplementary Material (ESI) for Catalysis Science & Technology

Substrate Promiscuity of Cytochrome P450 RhF

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General Experimental

All chemicals were purchased from Sigma-Aldrich and Fisher Scientific. *Escherichia coli* BL21(DE3) were purchased from Invitrogen (Carlsbad, CA). Cells were routinely grown in baffled flasks. Cells expressing the P450 RhF gene were harvested at 6000 rpm for 15 mins. Biotransformations were carried out in 48-well plates purchased from Fisher Scientific. GC-MS spectra were recorded on a Hewlett Packard HP 6890 equipped with a HP-1MS column, a HP 5973 Mass Selective Detector and an ATLAS GL FOCUS sampling robot. GC-FID analysis was performed on Agilent 6850 equipped with a Gerstel Multipurposesampler MPS2L and a HP-1 column. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer (400.1 MHz for ^{1}H and 100.6 MHz for ^{13}C) in $CDCl_3$ without additional standard.

Growth and Expression Conditions

A single colony of *E. coli* BL21(DE3) cells containing plasmid P450 RhF/LIC (see below for full gene/vector sequence) was picked from overnight plates grown at 37°C and used to inoculate 5 mL LB medium supplemented with 100 μg of ampicillin/mL. The culture was grown overnight at 37°C/250 rpm. These cells were used to inoculate 500 mL of M9 medium containing 50 μg kanamycin/mL, 0.4% of glucose, 0.05% of $FeCl_3$, 1mM $MgSO_4$, 1mM $CaCl_2$ in a 2 L baffle flask. Cells were grown at 37°C to an optical density (OD_{600}) of 0.8. β-D-thiogalactopyranoside (IPTG) (0.4 mM) and 5-aminolevulinic acid hydrochloride (ALA) (0.5 mM) were added and the cells were grown for a further 16 h at 20°C. Expression of P450 RhF was confirmed by analysing cell extracts with SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

General procedure for Whole Cell Biotransformations

After overnight growth, cells were harvested by centrifugation (6000 rpm, 15 min, 4 °C) and the wet cells were resuspended in 100 mM potassium phosphate buffer (pH = 7.8, 180 mg wet cells / mL of phosphate buffer) containing 0.4% of glycerol. Cells harbouring the LICRED vector[1] (expressing the reductase domain) were used as a control. Reactions were performed in a 48 well plates containing 1 mL of re-suspended cells and 1mM substrate (20 μL of a 50 mM stock solution in DMSO). and the biotransformation with **4c** was also carried out at30 mM substrate concentration (30 μL of a 1M stock solution in DMSO). The plate was incubated at 20°C/250 rpm for 48 hours. 300 μL aliquots of the reaction mixture were and extracted with 500 μL of ethyl acetate. The organic phase was analysed by GC-MS, GC-FID or HPLC. Commercially available standards or synthetic standards were used to confirm the identity of all oxidation products. Aromatic hydroxylation products **3b** and **3d** were isolated from the biotransformation and characterised by NMR.

Cytochrome P450 RhF Gene Sequence

CCATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGAAGTTCTGTT
CCAGGGACCAGCAATGAGTGCATCAGTTCCGGCGTCGGCGCCGGCGTGTCCCGT
CGACCACGCGGCCCTGGCGGGCGGCTGCCCGGTGTCGGCGAACGCCGCGGCGTT
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CGCGACGAGGAGCCGGTGTTCTACAGCCCCGAACTCGGCTACTGGGTCGTCACC
CGGTACGAGGATGTGAAGGCGGTGTTCCGCGACAACATCCTGTTCTCGCCGGCG
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CGGTACGACTACGCCATGGCCCGGACCCTCGTGAACGAGGACGAGCCCGCCCAC
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CACGAGGCGATGGTGCGACGGCTCACGCGCGAATACGTCGACCGCTTCGTCGAA
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GCGACTGCGAGGAAGGCCTCTGCGGCTCGTGCGAGGTCGCCGTCCTCGACGGCG

AGGTCGACCATCGCGACACGGTGCTGACCAAGGCCGAGCGGGCGGCGAACCGGC
AGATGATGACCTGCTGCTCGCGTGCCTGTGGCGACCGGCTGGCCCTGCGACTCTG
ACGCGCCTTCTCCTCACATATG

Figure S1. RhF in the pET-YSBLIC 3C vector: NcoI also contains the start codon (green) and NdeI sites are underlined. The start codon for the natural RhF gene is in blue and the stop codon is in red. The rest of the sequence is as for the pET-28a(+) vector.

Additional Compounds Screened

Compounds **6**, **7** and **9** were substrates for RhF but only low conversions (< 5%) were achieved (dealkylation products). 50% of nitrile derivative **18** (mixture of *cis* and *trans* isomers) was oxidized providing the dealkylation product. The enzymes selectivity for the *cis/trans* isomers was not investigated but it is likely that only the *trans* isomer was a substrate suggesting 100% of this isomer was oxidised. However, this has not been confirmed. Benzyl sulfide **24**, which has a p-OMe group was an excellent substrate. 100% of the material was converted to two major oxidation products; the demethylation product and an uncharacterized oxidation product.

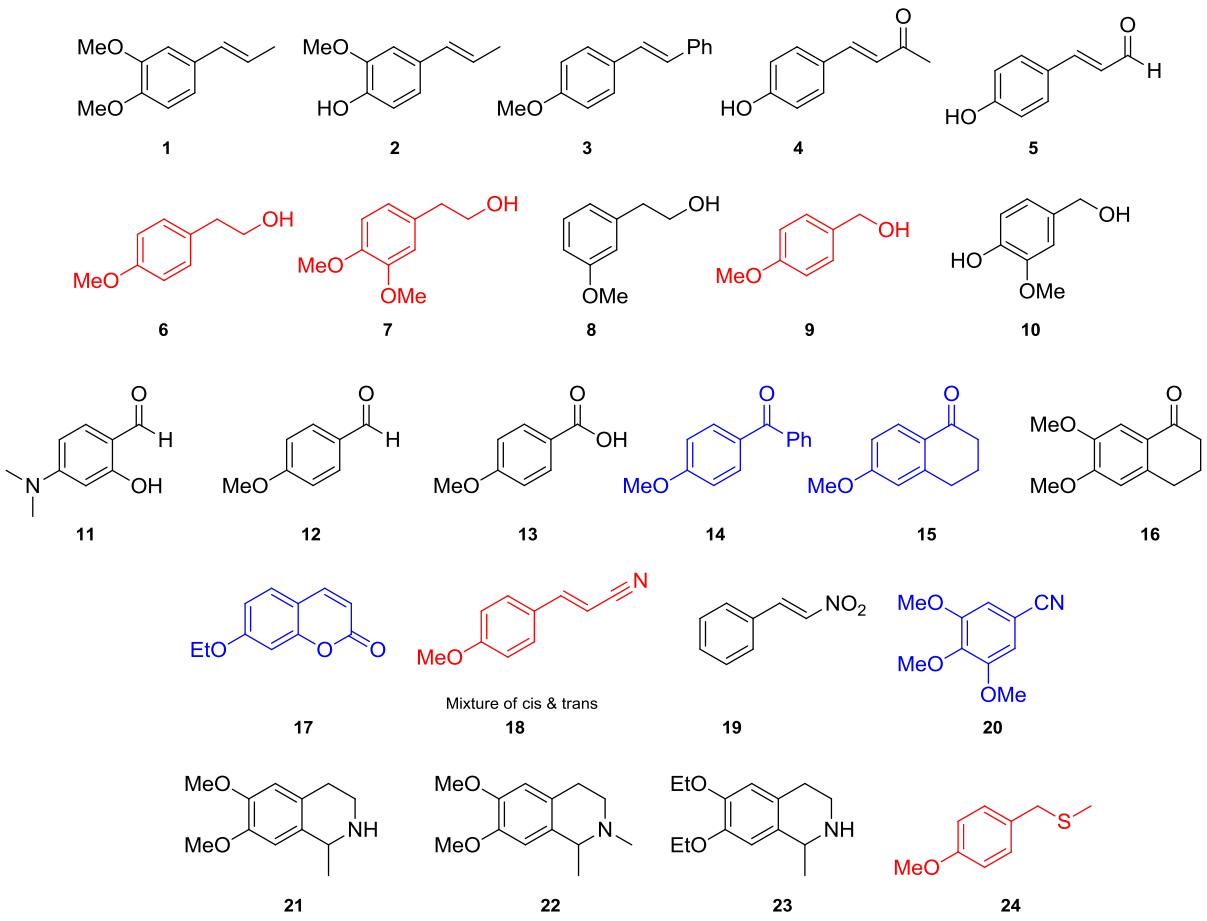


Figure S2. A list of compounds which do not feature in the main text. Structures in blue are compounds for which activity has previously been reported. Compounds in red are new substrates. No activity was detected for compounds shown in black.

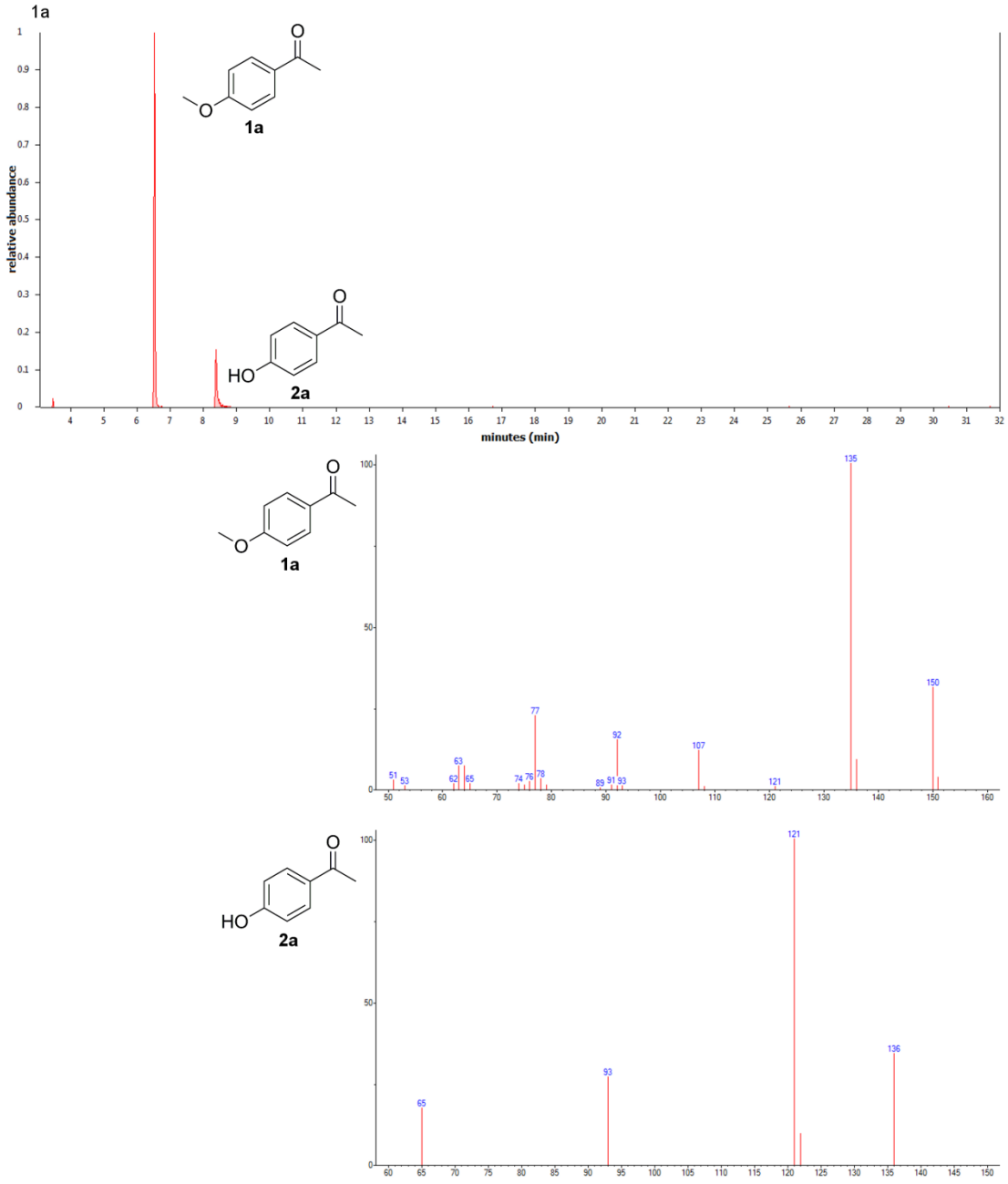
Determination of Enantiomeric Excess

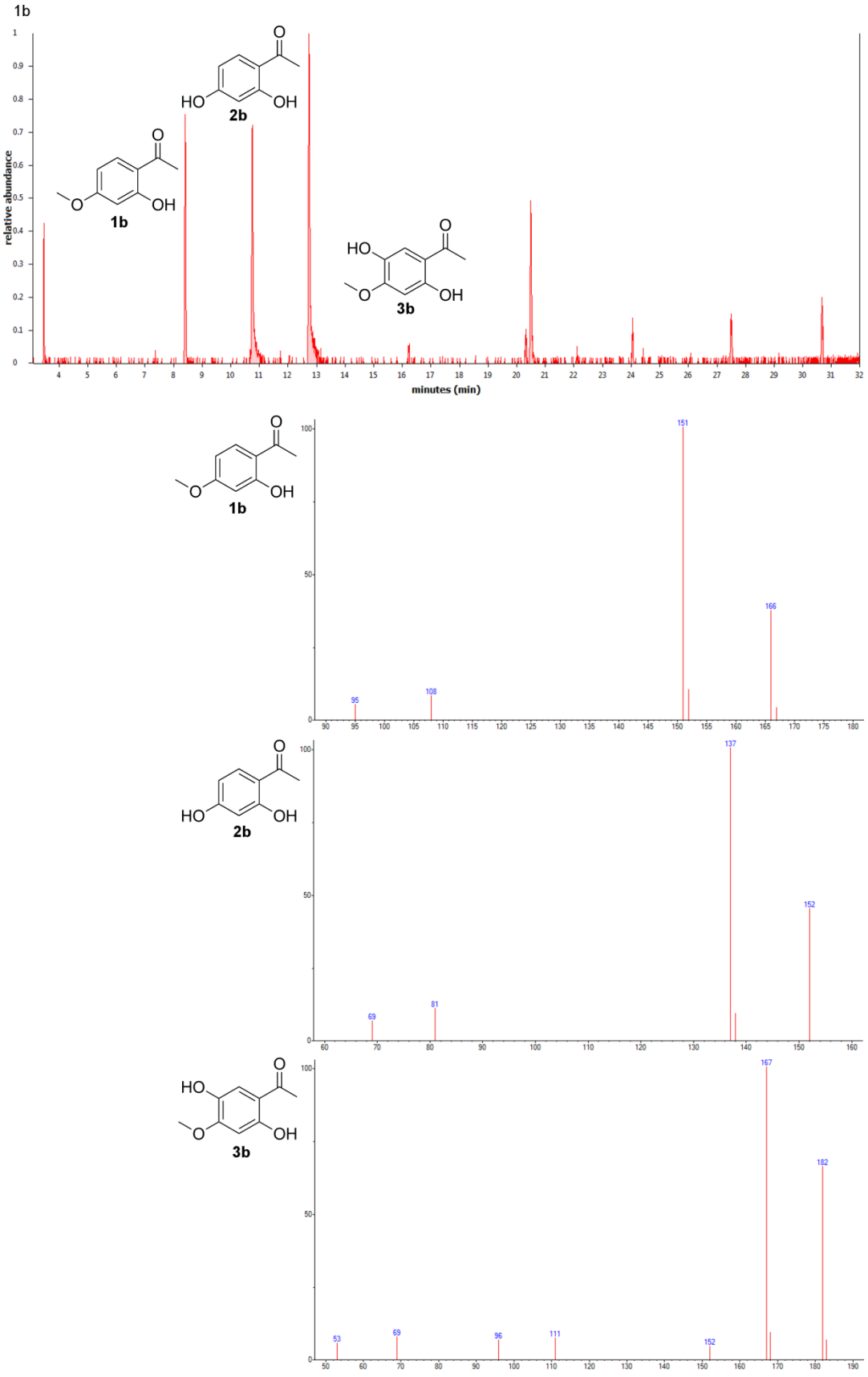
meta-Chloroperoxybenzoic acid (mCPBA) (2 mmol, 0.95eq) was added to a stirring solution of sulfide (2 mmol, 1 eq) in dichloromethane (10mL). The reaction was stirred at room temperature for 3 hours. Aqueous $NaHCO_3$ (20mL) was added, the organic layer was separated and the aqueous layer extracted with DCM (3 x 15mL). The racemic sulfoxides were purified by silica gel column chromatorgraphy (EtOAc:cyclohexane, 4:1). ^{1}H and ^{13}C data were consistent with literature: **8a-8d** & **8f**.[2]

Sulfoxide biotransformations were analysed on a Chiracel OD-H column (**8a-c**, **8e-f**) or a Chiracel OJ-H column (**8d**) and compared to synthesised racemic standards. Literature HPLC conditions were used to assign absolute stereochemistry where possible. For HPLC conditions see the following references: **8a** & **8b**,[3] **8c**.[4]

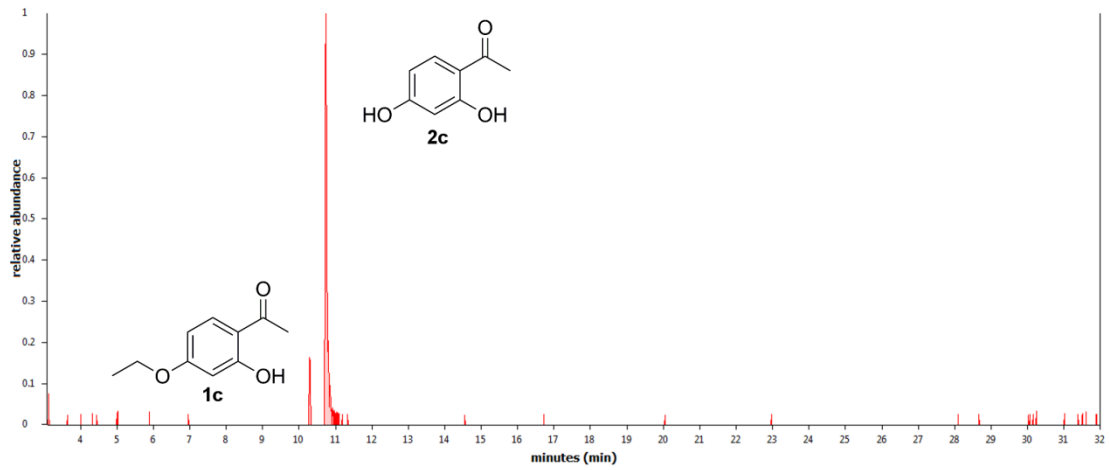
GC analysis.

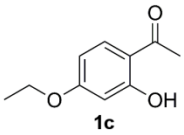
Analysis were performed on a Hewlett Packard GC/MS (HP 6890 Series/HP 5973) or on an Agilent 6850 GC/FID. Helium was used as the carrier gas at flow rate of 1.7 mL/min for GC-MS. GC-FID was equipped with an Agilent HP-1 column (30m, 0.32 mm i.d, 0.25 μm film thickness) and GC-MS with an Agilent HP-1MS column of the same dimensions. The inlet temperature was 270°C. The initial temperature was 100°C with a hold time of 1 minute followed by a 4°C/minute gradient until a temperature of 220°C was reached. The temperature was then increased by 30°C /minute until it reached 250°C. 2 μL of the biotransformation extract was injected.

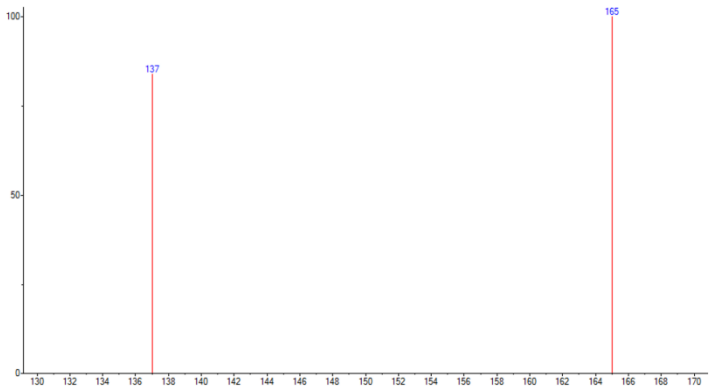


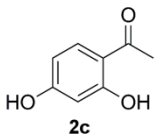


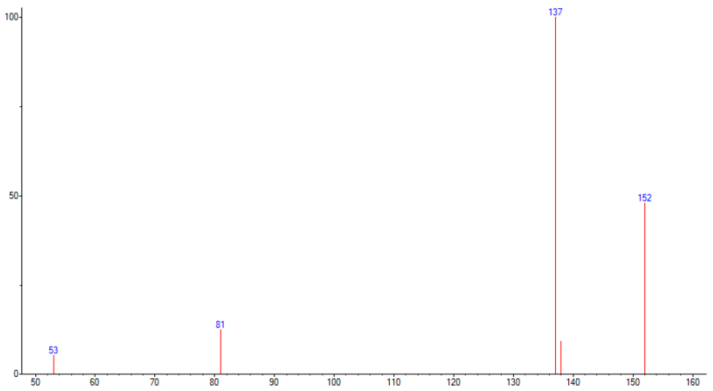
1c











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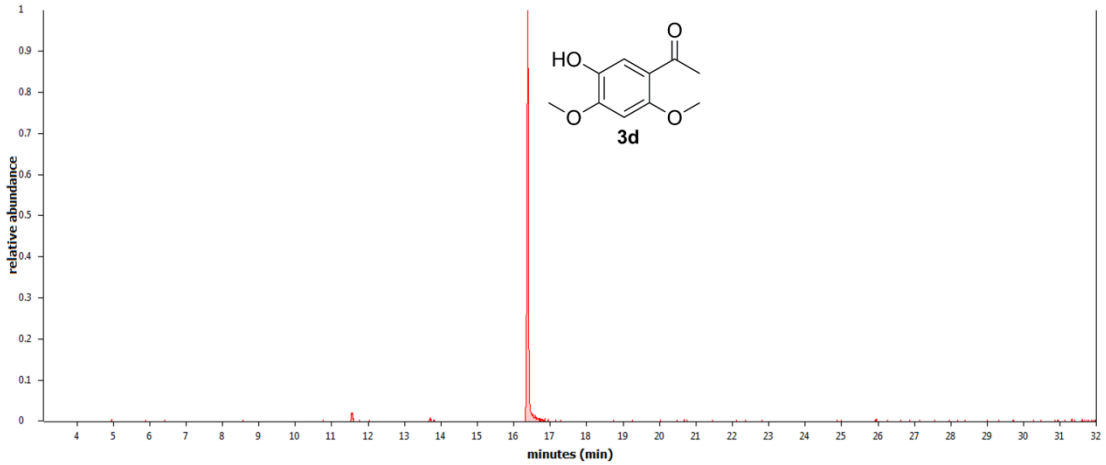
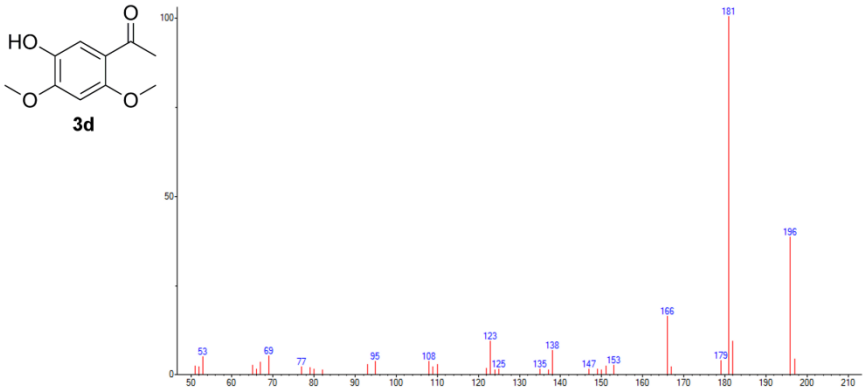
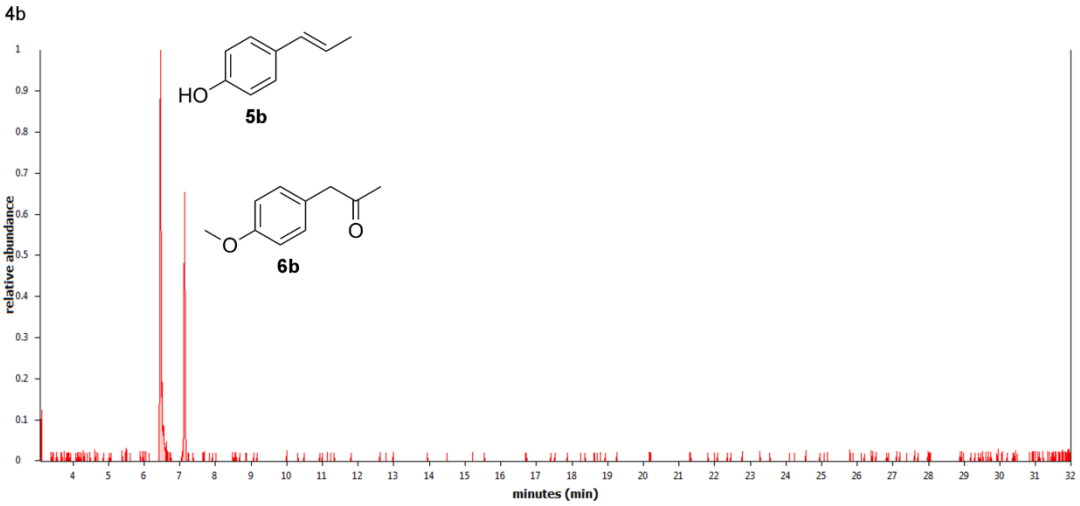
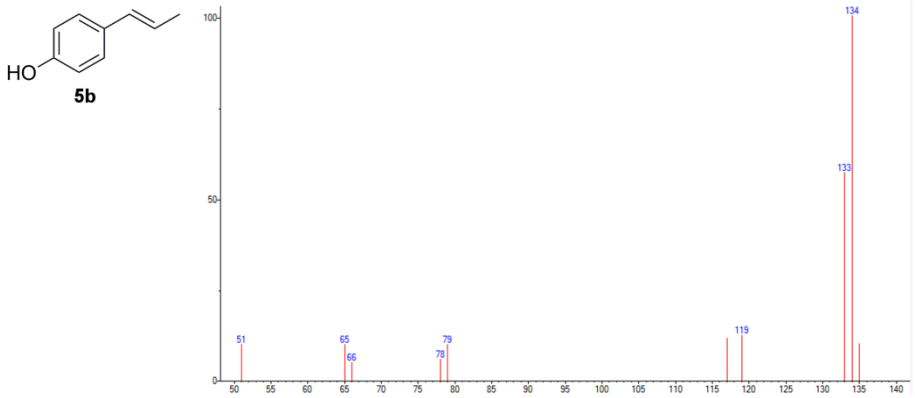
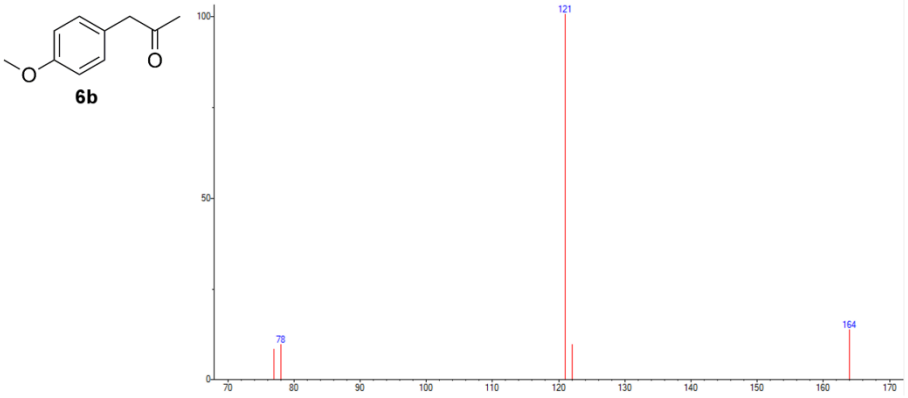


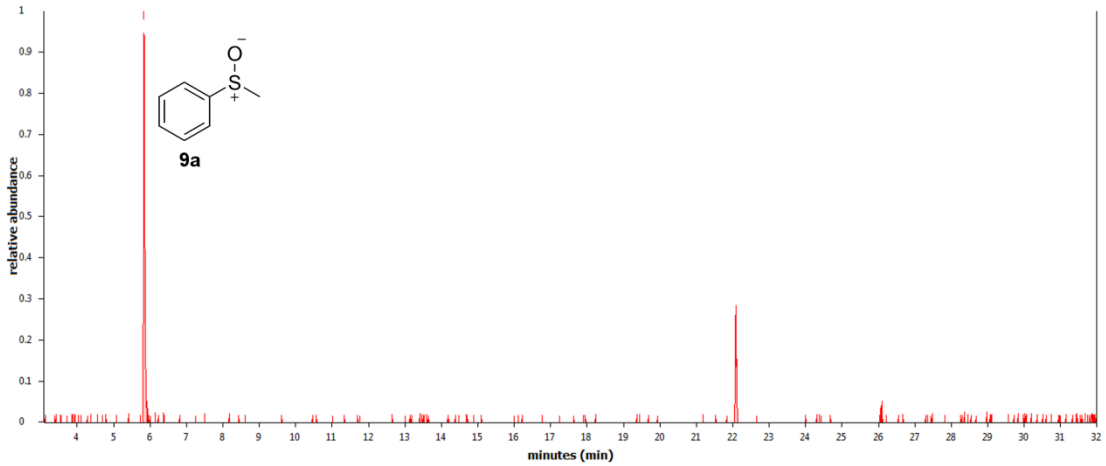


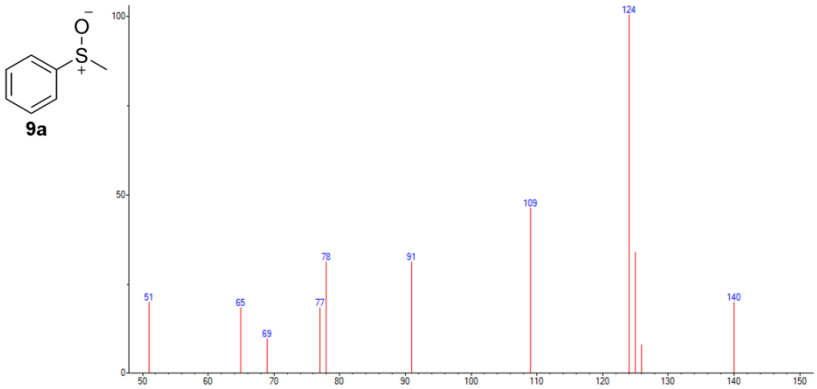






8a





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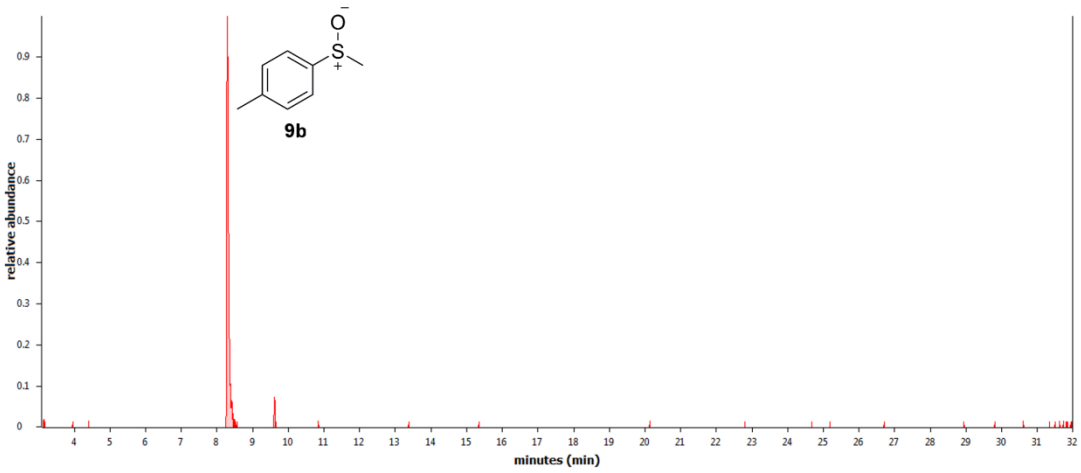

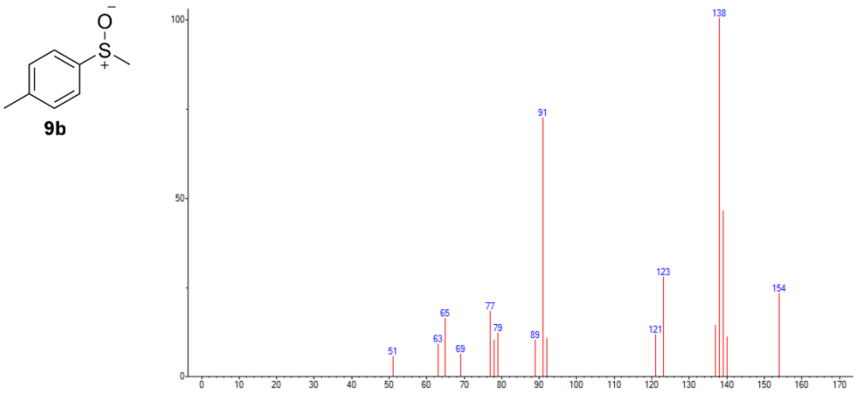

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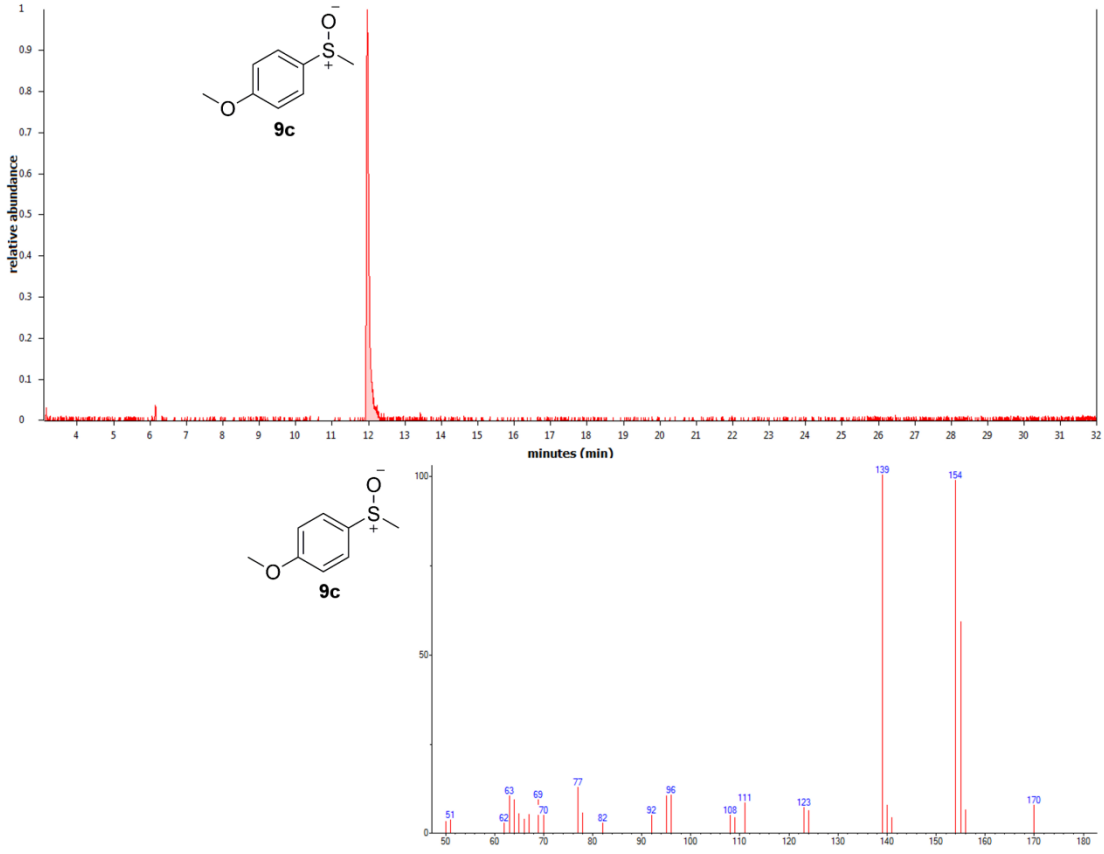
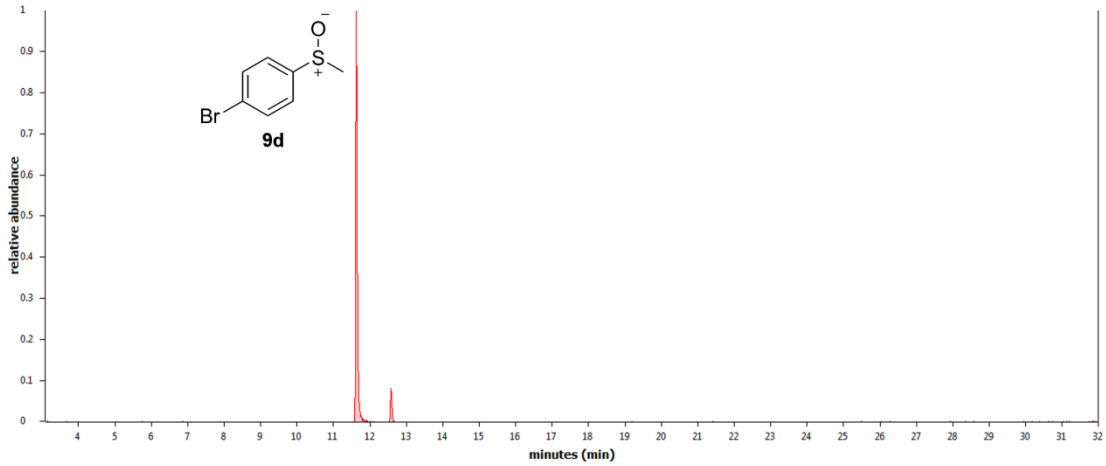
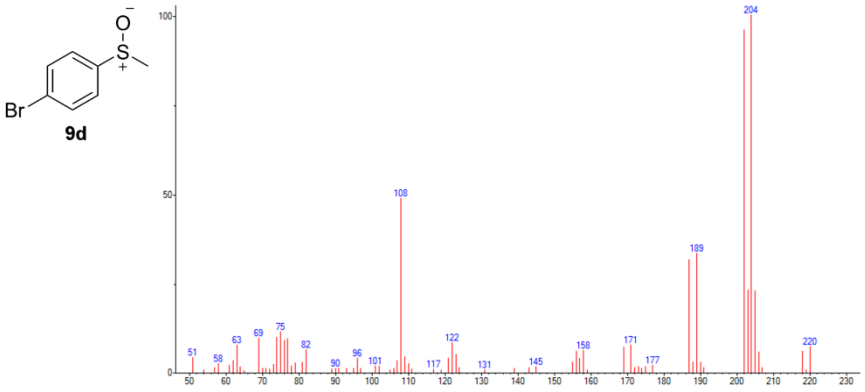
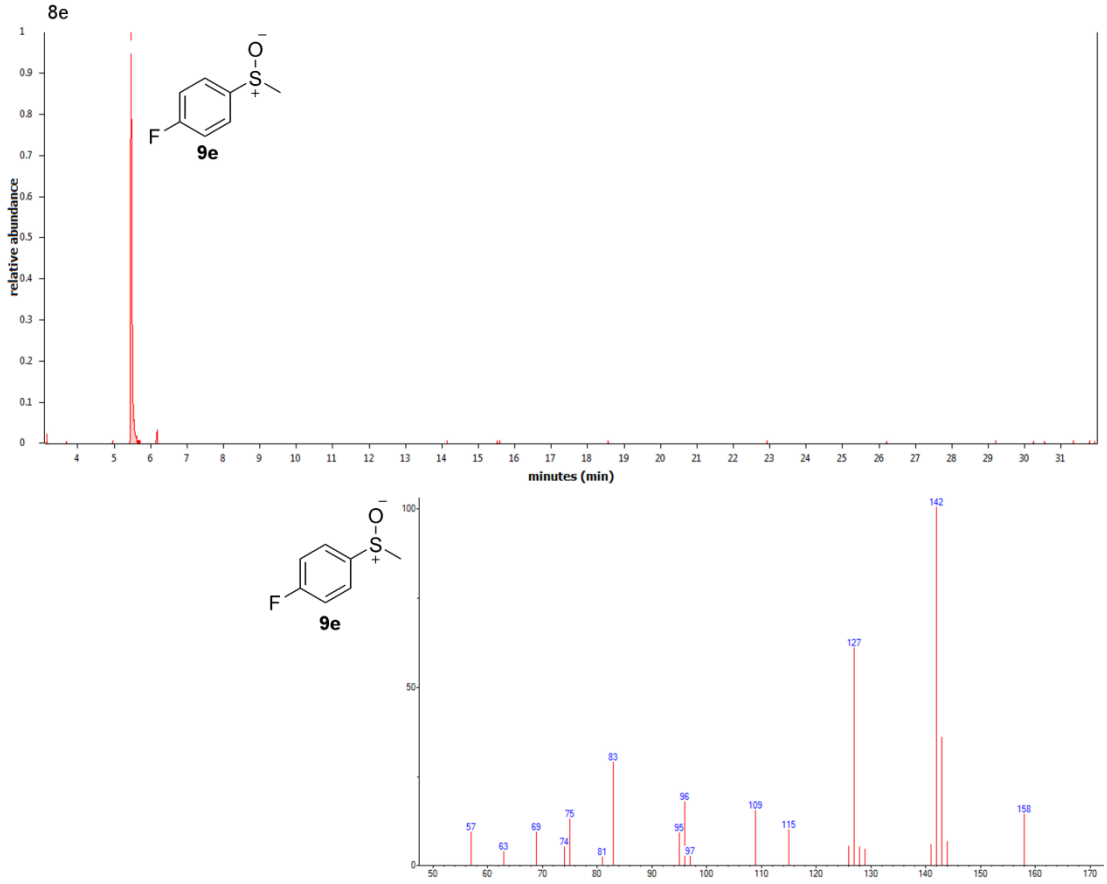
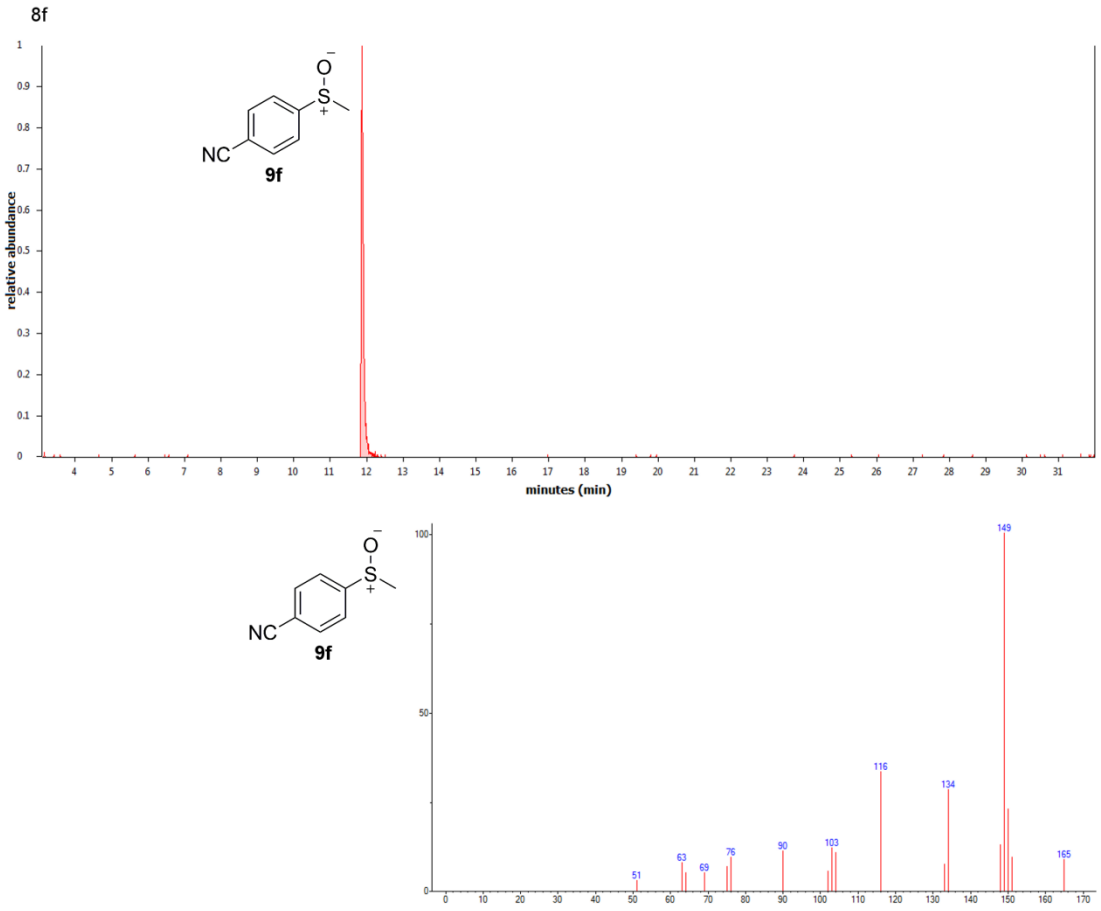


8d









1-(2,5-dihydroxy-4-methoxyphenyl)ethanone 3b

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.49 (s, 1H), 7.20 (s, 1H), 6.44 (s, 1H), 5.23 (s, 1H), 3.93 (s, 3H), 2.53 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.67, 158.88, 153.65, 137.94, 113.91, 112.47, 99.72, 56.20, 26.50.

1-(5-hydroxy-2,4-dimethoxyphenyl)ethanone 3d

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (s, 1H), 6.48 (s, 1H), 5.22 (s, 1H), 3.96 (s, 3H), 3.89 (s, 3H), 2.57 (s, 1H), 1.42 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.62, 154.68, 151.03, 139.28, 120.38, 115.72, 95.77, 56.29, 56.08, 31.89.

[1] F. Sabbadin, R. Hyde, A. Robin, E. M. Hilgarth, M. Delenne, S. Flitsch, N. Turner, G. Grogan, N.C. Bruce, *ChemBioChem,* **2010**, 11, 987; [2] Y. Yuan, X. Shi and W. Liu, *Synlett* 2011, **4**, 559; [3] P. Le Maux and G. Simonneaux, *Chem. Commun.* 2011, **47**, 6957. [4] Y.-C. Jeong, E. J. Kang and K.-H. Ahn, *Bull. Kor. Chem. Soc.* 2009, **30**, 2795.