Exploring the biocatalytic scope of a bacterial flavin-containing monooxygenase

Ana Rioz-Martínez,[a] Malgorzata Kopacz,[b] Gonzalo de Gonzalo,[b,*] Daniel E. Torres Pamiño, Vicente Gotor[a] and Marco W. Fraaije[b,*]

[a] Departamento de Química Orgánica e Inorgánica, Instituto de Biotecnología de Asturias, Universidad de Oviedo, c/ Julián Clavería 8, 33006, Oviedo, Spain.

[b] Laboratory of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands. e-mail: g.de.gonzalo.calvo@rug.nl or m.w.fraaije@rug.nl.

Supporting Information

Contents

1. Experimental procedures.

2.1. Asymmetric oxidation of sulfide 8a employing the Kagan methodology.[1]

Ti(O-*i*-Pr)$_4$ (10 mmoles) and (*R*,*R*)-DET (+)-diethyl L-tartrate) (20 mmoles) were dissolved at room temperature in 50 mL of CH_2Cl_2 under nitrogen atmosphere. Distilled water (5 mmoles) was then added dropwise. Stirring was maintained until the yellow solution formed became homogeneous and sulfide **8a** was added (5 mmoles). The resulting mixture was cooled to – 20 ºC and a 5.5 M TBHP (*tert*-butyl hidroperoxide) solution in decane (5.5 mmoles) was added. After four hours, additional water was added dropwise (50 mmoles). A strong stirring was maintained for 1 hour at – 20 ºC and then for one additional hour at room temperature. The gel obtained was filtrated and washed with CH_2Cl_2 (10 mL). The filtrate was kept in the presence of a mixture of NaOH (5%) in brine (30 mL) for 1 hour and then extracted with CH_2Cl_2 (3×20 mL). The organic phase was dried over Na_2SO_4 and concentrated to give the crude product, which was purified by flash chromatography using a mixture of CH_2Cl_2 / MeOH (95:5) as eluent in order to give 4-acetylphenyl methyl sulfoxide (*R*)-**8b**.

(*R*)-4-acetylphenyl methyl sulfoxide, (*R*)-8b: Yield 40% (344 mg)**.** $[\alpha]_D^{25}$= +90.2 (*c* 1.0, acetone), *ee* = 87%.

2.2. Asymmetric oxidation of sulfide 2a employing the Kagan methodology.

p-Hydroxyphenyl methyl sulfide **2a** was acylated with acetic anhydride and pyridine in order to obtain *p*-acetoxyphenyl methyl sulfide **2c** (90% yield). This substrate was oxidised by the methodology previously described except for an acidification step with HCl 1.0 M before the extraction with CH_2Cl_2, obtaining (*R*)- *p*-acetoxyphenyl methyl sulfoxide in 16% yield and *ee*= 30% after purification by flash chromatography using a mixture of CH_2Cl_2 / MeOH (95:5) as eluent.

***p*-Acetoxyphenyl methyl sulfide (2c)**. (90% yield). White solid. Mp: 43-44ºC IR (KBr): ν 2924, 1760, 1489, 1370, 1202, 739 cm^{-1}. ^{1}H-NMR (300.13 MHz, $CDCl_3$, 25ºC): δ 2.21 (s, 3H), 2.39 (s, 3H), 6.97 (d, $^3J_{H,H}$ 6.8 Hz, 2H), 7.21 (d, $^3J_{H,H}$ 6.8 Hz, 2H). ^{13}C-NMR (75.5 MHz, $CDCl_3$, 25ºC): δ 15.6 (CH_3), 20.4 (CH_3), 121.6 ($2CH_{ar}$), 127.2 ($2CH_{ar}$), 135.2 (C_{ar}), 147.9 (C_{ar}), 168.8 (CO). (EI$^+$, *m/z*): 182 (M$^+$, 41%), 140 (100), 125 (44), 96 (13), 43 (33). HRMS (ESI$^+$): calcd. for $C_9H_{10}NaO_2S$ (M+Na)$^+$: 205.0295; found: 205.0294.

2.3. Biocatalysed sulfoxidation of thioanisole at different pHs.

Thioanisole (**1a**) (5 mM) was dissolved in a Tris-HCl 50 mM buffer (pH from 6.0 to 10.0) containing sodium phosphite (10 mM), NADPH (0.2 mM) and the self-sufficient biocatalyst PTDH-mFMO (4 μM). Reactions were stirred at 25 ºC and 250 rpm for 8 h. Crude mixtures were then stopped, extracted with EtOAc (2 x 0.5 mL), dried onto $MgSO_4$ and analyzed by GC and HPLC in order to determine the conversions and the optical purities of (*S*)-**1b**, as shown in Figure S1. Control reactions in absence of biocatalyst were performed for all substrates, not observing formation of the final products.

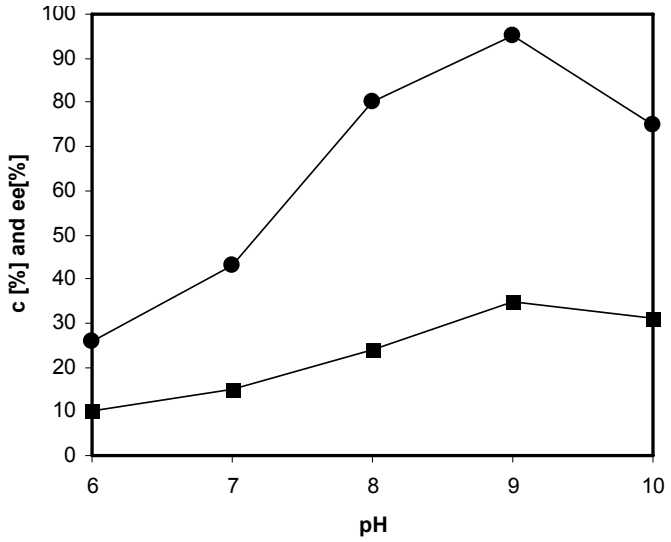


Figure S1. Effect of pH in the conversion (●) and in the enantiomeric excess (■) for the PTDH-mFMO biocatalyzed oxidation of thioanisole **1a**.

2.4. Biocatalysed sulfoxidation of thioanisole in presence of organic cosolvents.

Thioanisole (**1a**) (5 mM) was dissolved in a Tris-HCl 50 mM buffer pH 9.0 containing a 5% v v^{-1} of organic cosolvent (1.0 mL), sodium phosphite (10 mM), NADPH (0.2 mM) and the self-sufficient biocatalyst PTDH-mFMO (4 μM). Reactions were stirred at 25 ºC and 250 rpm for 8 h. Crude mixtures were then stopped, extracted with EtOAc (2 x 0.5 mL), dried onto $MgSO_4$ and analyzed by GC and HPLC in order to determine the conversions and the optical purities of (*S*)-**1b**. Control reactions in absence of biocatalyst were performed for all substrates, not observing formation of the final products.

Table S1. Effect of organic cosolvents in the biocatalysed oxidation of thioanisole employing PTDH-mFMO.

Entry	Cosolvent	*c* [%][a]	*ee* [%][b]
1	None	48	33
2	1% DMSO	95	35
3	5% DMSO	21	29
4	5% DMF	22	29
5	5% MeOH	45	18
6	5% 1,4-Dioxane	≤ 3	n.d.
7	5% *i*-PrOH	22	18
8	5% *t*-BuOMe	12	15
9	5% Toluene	9	26
10	5% 2-octanol	45	36

[a] Measured by GC. [b] Determined by HPLC. n.d. not determined

2.5. PTDH-mFMO catalyzed oxidation of racemic sulfoxides (±)-1b, (±)-4-5b and (±)-7b.

Sulfoxides (±)-**1b**, (±)-**4-5b** or (±)-**7b** (5 mM) were dissolved in a Tris-HCl buffer pH 9.0 containing 1% DMSO and the organic cosolvent when stated (final volume 1.0 mL), sodium phosphite (10 mM), NADPH (0.2 mM) and the self-sufficient biocatalyst PTDH-mFMO (4 μM). Reactions were stirred at 25ºC and 250 rpm for the times established. Reactions were then stopped, extracted with EtOAc (2 x 0.5 mL), dried onto $MgSO_4$ and analyzed by GC and/or HPLC in order to determine the conversions and the optical purities of the remaining sulfoxides. Control reactions in absence of biocatalyst were performed for all substrates, not observing formation of the final products.

2. Kinetic data.

PTDH-mFMO concentration were measured photometrically by monitoring the absorption of the FAD-cofactor at 450 nm (ε_{450} = 11.3 mM^{-1} cm^{-1}) after treating the enzyme with 0.1% SDS.

Steady-state kinetics.

The enzymatic activity of PTDH-mFMO for the oxidation of indole and its derivatives, trimethylamine, sulfides **1a**, **3a**, **5a**, **9a**, **13a**, **21a** and **24a**, coenzymes NADPH and $NADP^+$ and sodium phosphite were determined spectrophotometrically by monitoring NADPH consumption or formation at 340 nm (ε_{340} = 6.22 mM^{-1} cm^{-1}).

Stocks solutions of sulfides **1a**, **3a**, **5a**, **9a**, **13a**, **21a** and **24a** (250 mM) and indoles (1.0 M) were made in 1,4-dioxane meanwhile 10 mM $NADP^+$, NADPH, sodium phosphite and trimethylamine (500 mM) stock solutions were prepared in Tris-HCl (50 mM, 35 mM NaCl, pH 8.5) buffer. A reaction mixture (1.0 mL) usually contained Tris-HCl (25 mM, 35 mM NaCl, pH 9.0 for the sulfides and pH 8.5 for indoles, trimethylamine, NADPH and $NADP^+$), 100 μM NADPH, 1% v v^{-1} 1,4-dioxane for the measurements of the sulfides and indoles, and 0.17-1.00 μM PTDH-mFMO.

Table S2. Steady-state kinetic parameters for PTDH-mFMO.

Compound	K_M [μM]	k_{cat} (s^{-1})	k_{cat}/K_M (M^{-1} s^{-1})
$NADP^+$	19±3	6.3±0.11	330,000
NADPH	11±1	5.0±0.03	460,000
Sodium phosphite	1600±25	3.6±0.02	2,200
Trimethylamine	8±1	4.7±0.10	590,000
Thioanisole	170±8	0.45±0.01	2,600
4-Chlorothioanisole	87±6	0.25±0.02	2,900
4-Methoxythioanisole	220±15	0.23±0.01	1,000
4-Cyanothioanisole	590±10	0.29±0.01	490
Benzyl methyl sulfide	470±12	0.34±0.01	720
Cyclohexyl methyl sulfide	350±13	0.19±0.01	540
Butyl ethyl sulfide	94±3	0.62±0.02	6,600

Table S.3. Reaction rates observed at 1.0 mM concentration in the PTDH-mFMO catalysed oxidation of indole and derivatives.

Compound	K_{obs} (s^{-1})[a]
Indole	0.090
4-Chloroindole	0.061
5-Chloroindole	0.070
6-Chloroindole	0.077
7-Chloroindole	0.063
5-Bromoindole	0.066
5-Nitroindole	0.035
5-Hydroxyindole	0.042
5-Methylindole	0.042
5-Methoxyindole	0.038
2-Methylindole	0.079

[a] For all compounds, standard deviations (average 4 measures) were bellow ±5.0%.

3. GC and HPLC Analyses.

The following columns were used for the determination of conversions and enantiomeric excesses of the sulfoxides by GC: A: Restek RT-BetaDEXse (30 m x 0.25 mm x 0.25 μm, 12 psi N_2) and B: Hewlett Packard HP-1 (30m x 0.32 mm x 0.25μm, 12.2 psi N_2).

Table S.4. Determination of conversion values and enantiomeric excesses by GC.

Substrate	Program[a]	Column	t_R [min] **sulfides**	t_R [min] **sulfoxides**
1a	70/10/190	B	3.2	5.9
2a	100/5/3/200	B	8.6	19.6
3a	70/10/190	B	6.1	9.3
4a	70/0/10/190	B	4.5	7.4
5a	70/0/10/190	B	5.5	8.2
6a	70/0/10/190	B	5.5	7.4
7a	70/0/10/190	B	5.5	5.5
8a	100/5/3/130	B	12.6	19.4
9a	70/0/10/190	B	7.4	9.4
10a	70/0/10/190	B	8.9	10.6
11a	70/0/10/190	B	3.8	7.1
12a	70/0/10/190	B	4.7	8.2
13a	70/0/10/190	B	4.1	7.6
14a	70/0/10/190	B	4.4	7.7
15a	70/5/3/150	B	12.7	24.5
18a	70/5/3/200	B	5.4	12.9
19a	70/0/10/190	B	2.4	5.2
20a	70/5/3/130	B	8.7	16.6
21a	70/5/3/150	B	4.6	16.4
23a	50/5/3/200	A	12.2	31.4 (*S*), 31.8 (*R*)
24a	50/5/3/200	A	14.2	33.1(*S*), 35.4 (*R*)

[a] Program: initial T (ºC)/ time (min)/ slope (ºC/min)/T (ºC)/ time (min).

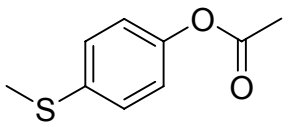
For the determination of the enantiomeric excesses of the sulfoxides by HPLC, the following columns were employed: column A: Chiralcel OB-H (0.46 cm x 25 cm), column B: Chiralcel OD (0.46 cm x 25 cm), column C: Chiralpak IA (0.46 cm x 25 cm), column D: Chiralcel OD-H (0.46 x 25 cm) and column E: Chiralcel OJ-H (0.45 cm x 25 cm), all of them are from Daicel.

Table S.5. Determination of enantiomeric excesses by HPLC.

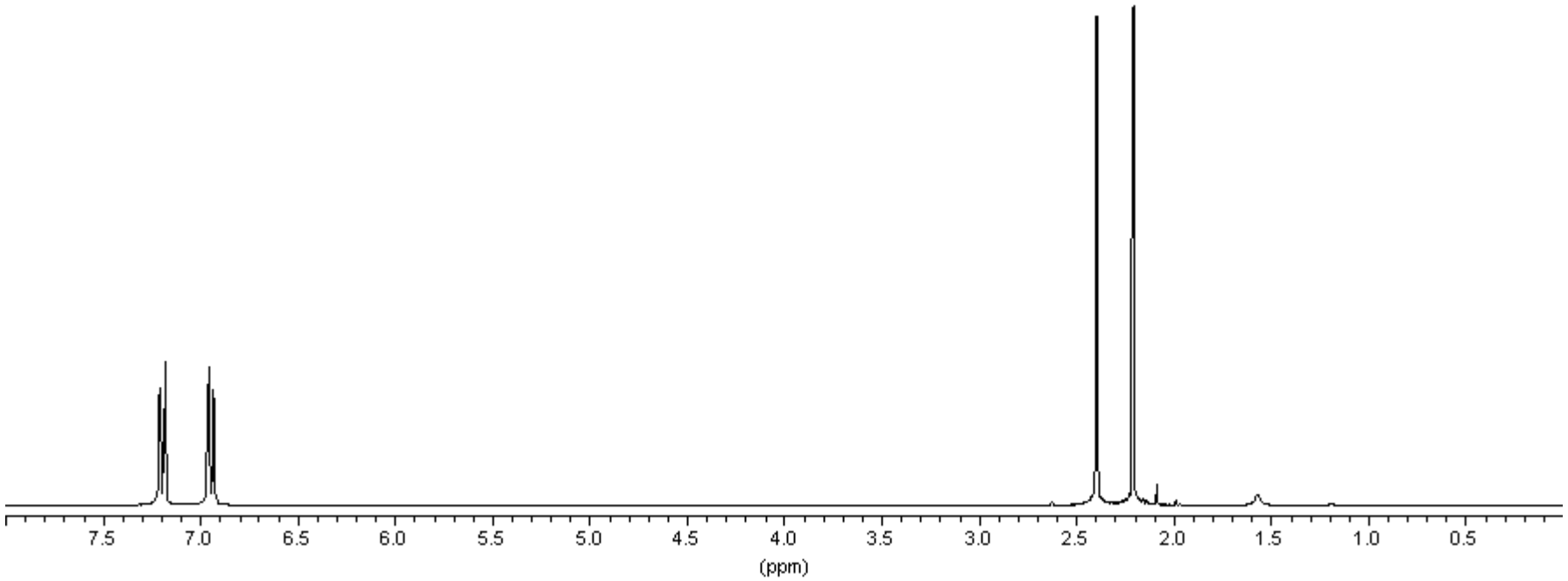
Substrate	Column	Flow rate [mL min^{-1}]	T [ºC]	Eluent[a]	Retention time (min)
1b	B	0.8	25	*n*-hexane-IPA 9:1	13.6 (*R*); 16.8 (*S*)
2b[b]	C	0.7	20	*n*-hexane-EtOH 95:5[c]	38.7 (*R*); 39.9 (*S*)
3b	D	1.0	25	*n*-hexane-IPA 9:1	15.2 (*R*); 17.1 (*S*)
4b	A	0.5	20	*n*-hexane-IPA 9:1	20.6 (*R*); 22.9 (*S*)
5b	A	0.5	20	*n*-hexane-IPA 8:2	18.5 (*S*); 28.3 (*R*)
6b	A	0.8	30	*n*-hexane-IPA 8:2	10.8 (*R*); 14.6 (*S*)
7b	A	0.5	20	*n*-hexane-IPA 8:2	17.2 (*S*); 29.6(*R*)
8b	E	0.7	30	*n*-hexane-EtOH 8:2	21.9 (*S*); 23.5 (*R*)
9b	C	1.0	30	*n*-hexane-IPA 8:2	20.7 (*R*); 23.0 (*S*)
10b	A	1.0	25	*n*-hexane-IPA 8:2	39.1 (*S*); 50.0 (*R*)
11b	B	0.5	20	*n*-hexane-IPA 9:1	18.1 (*R*); 22.2 (*S*)
12b	A	1.0	25	*n*-hexane-IPA 8:2	13.5 (*S*); 16.7 (*R*)
13b	B	0.8	25	*n*-hexane-IPA 9:1	18.3 (*R*); 19.8 (*S*)
14b	B	1.0	25	*n*-hexane-IPA 9:1	17.7 (*R*); 19.1 (*S*)
15b	A	1.0	25	*n*-hexane-IPA 8:2	40.2 (*S*); 42.7 (*R*)
18b	A	1.0	25	*n*-hexane-IPA 9:1	16.6 (*S*); 29.8 (*R*)
19b	A	1.0	25	*n*-hexane-IPA 8:2	12.6 (*S*); 14.3 (*R*)
20b	A	0.5	25	*n*-hexane-IPA 92:8	21.4 (*S*); 26.5 (*R*)
21b	A	0.7	25	*n*-hexane-IPA 8:2	8.1 (*R*); 9.7 (*S*)

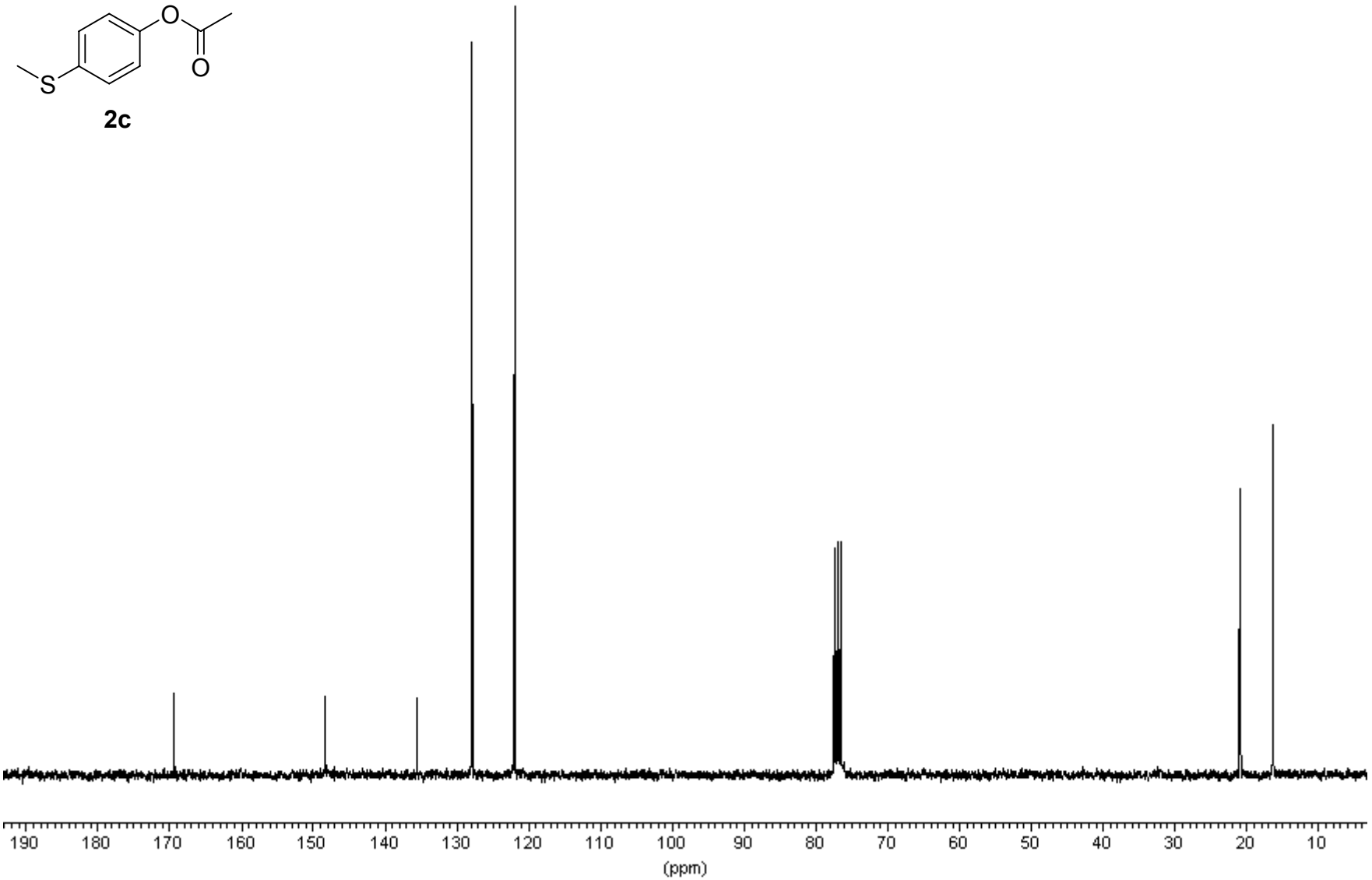
[a] Experiments were performed with isocratic eluent. [b] Compound **2b** was derivatised into the corresponding acetate, compound **2c**. [c] Starting flow 95:5 *n*-hexane-EtOH, increased polarity until 9:1 in 15 minutes and from 9:1 to 8:2 in 30 minutes.

4. NMR Spectra



2c

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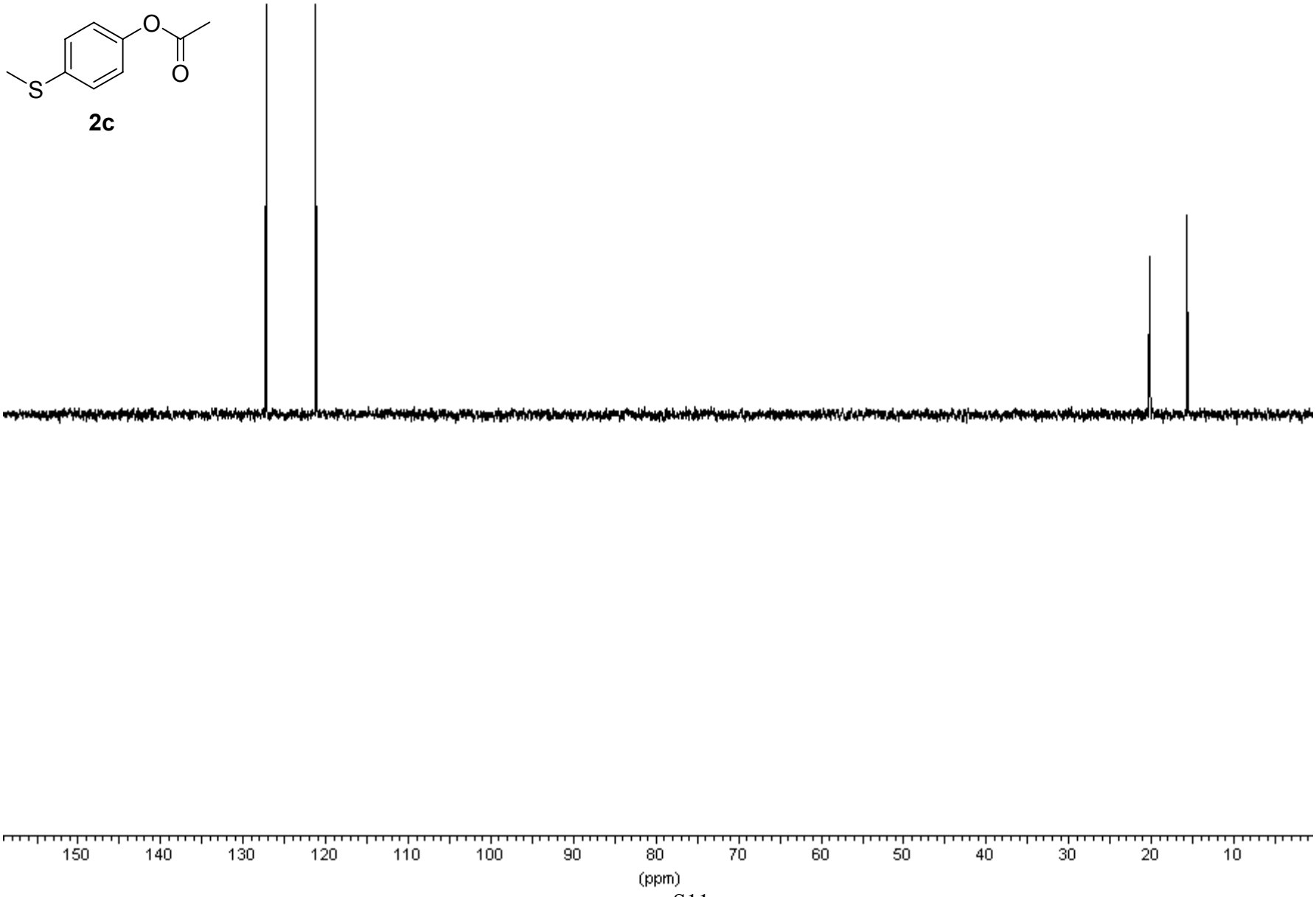
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