Electronic Supplementary Material (ESI) for Green Chemistry.

Laccase-catalysed biotransformation of collismycin derivatives. A novel enzymatic approach for the cleavage of oximes

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SUPPORTING INFORMATION (page 1 of 22 pages)

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1. General procedures

1.1 Laccase-catalysed biotransformation of the collismycin derivatives

T. versicolor laccase (2 mg, 1.84 U) and TEMPO (1.5 mg, 15% w/w, 0.25 equiv) were added to a 10 mg (~ 0.04 mmol) suspension of the corresponding collismycin derivative (**1a**-**6a**) in water (1 mL) and acetonitrile (100 μL), and the mixture was stirred vigorously in an open-to-air vial at room temperature 12 or 24 h. The reaction progress was monitored by HPLC. Once completed, the mixture was diluted with 200 mL of distilled water and the resulting solution was then applied to a solid-phase extraction cartridge (Sep-Pak C18, Waters). The cartridge retained some impurities of the enzymatic system and the decolorised solution containing the product was concentrated under reduced pressure. A final lyophilisation step led to the corresponding collismycin derivative as a white powder in good yield respect to the measured conversion.

If necessary, for incomplete reactions the crude mixture was purified by reverse phase preparative HPLC (XBridgeTM Prep C18, 5 μm, 30 x 150 mm, Waters) with isocratic elution using MeCN and 0.1% TFA in water (40:60 or 50:50, depending on the product), at 20 ml/min. The peaks were collected on 0.1 M potassium phosphate buffer (pH 7.0) and diluted four-fold with water. The resulting solution was applied to a solid-phase extraction cartridge (Sep-Pak C18, Waters), washed with 0.1% ammonia in water to remove the TFA bound to collismycins and, after further washing with water, the retained compounds would be eluted with methanol and lyophilised.

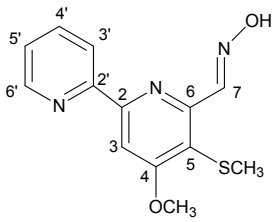


Figure S1. Typical numeration for the collismycin A molecule.

(*E*)-4-Methoxy-5-(methylthio)-[2,2'-bipyridine]-6-carboxaldehyde oxime (Collismycin A) (1a)

White solid; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 2.35 (s, 3H, SCH_3), 4.07 (s, 3H, OCH_3), 7.50 (dd, 1H, J = 7.8, 4.2 Hz, H-5'), 7.98 (dt, 1H, J = 7.8, 1,2 Hz, H-4'), 8.02 (s, 1H, H-3), 8.40 (d, 1H, J = 7.8 Hz, H-3'), 8.72 (d, 1H, J = 4.2 Hz, H-6'), 8.74 (s, 1H, H-7), 11.77 (brs, 1H, OH); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 18.3 (CH_3, SCH_3), 56.8 (CH_3, OCH_3), 103.4 (CH, C-3), 121.3 (CH, C-3'), 121.6 (C, C-5), 125.2 (CH, C-5'), 137.9 (CH, C-4'), 147.3 (CH, C-7), 149.7 (CH, C-6'), 153.1 (C, C-6), 154.9 (C, C-2'), 156.5 (C, C-2), 167.3 (C, C-4).

4- Methoxy-5-(methylthio)-[2,2'-bipyridine]-6-carboxylic acid (1b)

White solid; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 2.33 (s, 3H, SCH_3), 4.06 (s, 3H, OCH_3), 7.49 (dd, 1H, J = 7.8, 4.1 Hz, H-5'), 7.96 (t, 1H, J = 7.8 Hz, H-4'), 8.00 (s, 1H, H-3), 8.32 (d, 1H, J = 7.8 Hz, H-3'), 8.70 (d, 1H, J = 4.1 Hz, H-6'); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 17.9 (CH_3, SCH_3), 56.8 (CH_3, OCH_3), 103.5 (CH, C-3), 117.0 (C, C-5), 121.3 (CH, C-3'), 125.2 (CH, C-5'), 138.0 (CH, C-4'), 149.7 (CH, C-6'), 154.6 (C, C-2'), 156.4 (C, C-2), 159.0 (C, C-6), 167.1 (C, C-4), 169.2 (C, C=O).

4- Hydroxy-5-(methylthio)-[2,2'-bipyridine]-6-carboxylic acid (2b)

White solid; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 2.36 (s, 3H, SCH_3), 7.47 (dd, 1H, J = 7.8, 4.2 Hz, H-5'), 7.94 (t, 1H, J = 7.8 Hz, H-4'), 7.96 (s, 1H, H-3), 8.29 (d, 1H, J = 7.8 Hz, H-3'), 8.68 (d, 1H, J = 4.2 Hz, H-6'), 11.7 (s, 1H, OH); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 17.7 (CH_3, SCH_3), 108.4 (CH, C-3), 116.1 (C, C-5), 121.2 (CH, C-3'), 125.1 (CH, C-5'), 137.9 (CH, C-4'), 149.8 (CH, C-6'), 154.5 (C, C-2'), 156.0 (C, C-2), 157.6 (C, C-6), 166.7 (C, C-4), 168.4 (C, C=O).

4-Methoxy-4'-methyl-5-(methylthio)-[2,2'-bipyridine]-6-carboxylic acid (3b)

White solid; ^{1}H NMR (600 MHz, DMSO-d_6): δ = 2.35 (s, 3H, SCH_3), 2.46 (s, 3H, 4'-CH_3), 4.08 (s, 3H, OCH_3), 7.42 (d, 1H, J = 4.7 Hz, H-5'), 8.01 (s, 1H, H-3), 8.30 (s, 1H, H-3'), 8.59 (d, 1H, J = 4.7 Hz, H-6'), 11.70 (OH); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 18.6 (CH_3, SCH_3), 21.8 (CH_3, 4'-CH_3), 57.1 (CH_3, OCH_3), 104.0 (CH, C-3), 122.3 (C, C-5), 122.6 (CH, C-3'), 126.2 (CH, C-5'), 148.4 (CH, C-6'), 151.5 (C, C-6) 153.0 (C, C-2'), 154.0 (C, C-2), 155.5 (C, C-4'), 167.0 (C, C-4), 168.7 (C, C=O).

1.2 Synthesis of aldoximes and ketoximes

To a solution of the corresponding aldehyde or ketone (2 mmol) in ethanol (10 mL) were added hydroxylamine hydrochloride (4 mmol) and pyridine (6 mmol). The reaction mixture was stirred at room temperature for aldehydes or heated to reflux for ketones. The reaction progress was monitored by thin-layer chromatography (hexane/ethyl acetate or ethyl acetate/methanol mixtures). Once completed, the reaction mixture was evaporated under reduced pressure and the resulting crude purified by flash chromatography (hexane/ethyl acetate or ethyl acetate/methanol mixtures) leading to a mixture of *Z*- and *E*-isomers. The spectroscopical data for the resulting oximes **7-10** and **12** are in good agreement with those reported in the literature. Aldoxime **11** is commercially available.

Acetophenone oxime (7)

White solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.35 (s, 3H), 7.40-7.45 (m, 3H), 7.65-7.70 (m, 2H), 9.69 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.42 (CH_3), 126.1 (CH), 128.6 (CH), 129.3 (CH), 136.5 (C), 156.1 (C).

1-Phenylbutan-2-one oxime (8)

White solid; ^{1}H NMR (300 MHz, $CDCl_3$) showed a mixture of *Z*- and *E*-isomers (1:1). Compound *Z*-**7**: δ = 1.10 (t, 3H, *J* = 7.5 Hz), 2.23 (q, 2H, *J* = 7.5 Hz), 3.80 (s, 3H), 7.20-7.40 (m, 5H) and compound *E*-**7**: δ = 1.04 (t, 3H, *J* = 7.5 Hz), 2.36 (q, 2H, *J* = 7.5 Hz), 3.56 (s, 3H), 7.20-7.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): Compound *Z*-**7**: δ = 10.1 (CH_3), 27.1 (CH_2), 33.5 (CH_2), 126.4 (CH), 128.6 (CH), 136.8 (C), 160.6 (C) and compound *E*-**7**: δ = 10.7 (CH_3), 20.6 (CH_2), 40.1 (CH_2), 126.8 (CH), 129.1 (CH), 136.7 (C), 162.0 (C)

Benzophenone oxime (9)

White solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35-7.55 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 127.9 (CH), 128.3 (CH), 128.4 (CH), 129.2 (CH), 129.3 (CH), 129.6 (CH), 132.7 (C), 136.2 (C), 158.0 (C).

Picolinaldehyde oxime (11)

White solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.32 (ddd, 1H, *J* = 1.5, 4.8 and 7.5 Hz), 7.75 (td, 1H, *J* = 1.5 and 7.5 Hz), 7.84 (d, 1H, *J* = 7.5 Hz), 8.34 (s, 1H), 8.66 (d, 1H, *J* = 4.8 Hz), 9.3 (brs, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 121.0 (CH), 124.1 (CH), 136.8 (CH), 149.5 (CH), 150.5 (CH), 151.7 (C).

2-Phenylpropanal oxime (12)

White solid; ^{1}H NMR (300 MHz, $CDCl_3$) showed a mixture of *Z*- and *E*-isomers. Compound *Z*-**12**: δ = 1.50 (d, 3H, *J* = 7.2 Hz), 3.72 (quintuplet, 1H, *J* = 7.2 Hz), 7.20-7.40 (m, 5H), 7.57 (d, 1H, *J* = 6.4 Hz), 8.82 (s, 1H, OH) and compound *E*-**12**: δ = 1.47 (d, 3H, *J* = 7.2 Hz), 4.50 (quintuplet, 1H, *J* = 7.2 Hz), 6.86 (d, 1H, *J* = 7.5 Hz), 7.20-7.40 (m, 5H), 9.19 (s, 1H, OH); ^{13}C NMR (150 MHz, $CDCl_3$): Compound *E*-**12**: δ = 18.8 (CH_3), 40.4 (CH), 127.0 (CH), 127.5 (CH), 128.7 (CH), 141.9 (C), 154.7 (C) and compound *Z*-**12**: δ = 18.4 (CH_3), 35.0 (CH), 126.8 (CH), 127.3 (CH), 128.7 (CH), 142.2 (C), 155.3 (CH).

1.3 Laccase-catalysed biotransformation of aldoximes and ketoximes

T. versicolor laccase (16.6 U) and TEMPO or ABTS (10% mol) were added to a suspension of the corresponding aldo- or ketoxime **7-12** (0.5 mmol) in citrate buffer 50 mM at pH 5.0 (10 mL) and acetonitrile (1 mL), and the mixture was stirred vigorously in an open-to-air vial at room temperature overnight. The reaction progress was monitored by HPLC to establish the conversion rate.

2. Analytical data

2.1 HPLC Analyses for determination of conversion

Method HPLC-1: Analyses were carried out in an Agilent RR1200 HPLC system, using a reversed phase column (Zorbax Eclipse XDB-C18, RR, 1.8 μm, 4.6 x 50 mm, Agilent) with acetonitrile and water (0.1% TFA) as solvent. Samples were eluted with three linear gradients from 10% to 60% MeCN during 5.70 min, followed by another from 60% to 100% MeCN during 0.5 min and a third gradient from 100% to 10% MeCN during 1.90 min, at flow rate of 2 ml/min. Detection and spectral characterisation of peaks (UV absorption maxima (from HPLC-diode array) at 220 and 324 nm) were performed with a diode array detector and ChemStation Rev.B.03.01 software (Agilent).

Method HPLC-2: Analyses were carried out in an Agilent RR1200 HPLC system, using a semi-preparative reversed phase column (Zorbax Eclipse XDB-C18, RR, 5.0 μm, 9.4 x 250 mm, Agilent) with acetonitrile and water (0.1% TFA) as solvent. An isocratic elution was applied with 45% MeCN during 20 min, at flow rate of 5 ml/min. Detection and spectral characterisation of peaks (UV absorption maxima (from HPLC-diode array) at 220 and 324 nm) were performed with a diode array detector and ChemStation Rev.B.03.01 software (Agilent).

Table S1. HPLC analyses data.

Substrate	**Method**	**Retention time (min)**	
		Substrate	Product
(*E*)-4-Methoxy-5-(methylthio)-[2,2'-bipyridine]-6-carboxaldehyde oxime (**1a**)	HPLC-1	**1a** (2.4)	**1b** (1.3)
(*E*)-4-Hydroxy-5-(methylthio)-[2,2'-bipyridine]-6-carboxaldehyde oxime (**2a**)	HPLC-1	**2a** (1.3)	**2b** (0.7)
(*E*)-4-Methoxy-4'-methyl-5-(methylthio)-[2,2'-bipyridine]-6-carboxaldehyde oxime (**3a**)	HPLC-1	**3a** (2.2)	**3b** (1.7)
(4-Methoxy-5-(methylthio)-[2,2'-bipyridin]-6-yl)methanol (**4a**)	HPLC-1	**4a** (1.8)	**1b** (1.3)
6-(Hydroxymethyl)-5-(methylthio)-[2,2'-bipyridin]-4-ol (**5a**)	HPLC-1	**5a** (1.8)	**2b** (0.7)
(4-Methoxy-4'-methyl-5-(methylthio)-[2,2'-bipyridin]-6-yl)methanol (**6a**)	HPLC-1	**6a** (1.9)	**3b** (1.7)
Acetophenone oxime (**7**)	HPLC-1	**7** (3.1)	**13** (2.9)
1-Phenylbutan-2-one oxime (**8**)	HPLC-2	**8** (5.8, 6.1; isomers)	**14** (7.8)
Benzophenone oxime (**9**)	HPLC-2	**9** (9.3)	**15** (14.5)
Benzaldehyde oxime (**10**)	HPLC-1	**10** (2.5)	**16** (2.2)
Picolinaldehyde oxime (**11**)	HPLC-1	**11** (0.7)	**17** (0.6)
2-Phenylpropanal oxime (**12**)	HPLC-1	**12** (2.8)	**18** (3.0)

2.2 Copy of ^{1}H and ^{13}C spectra

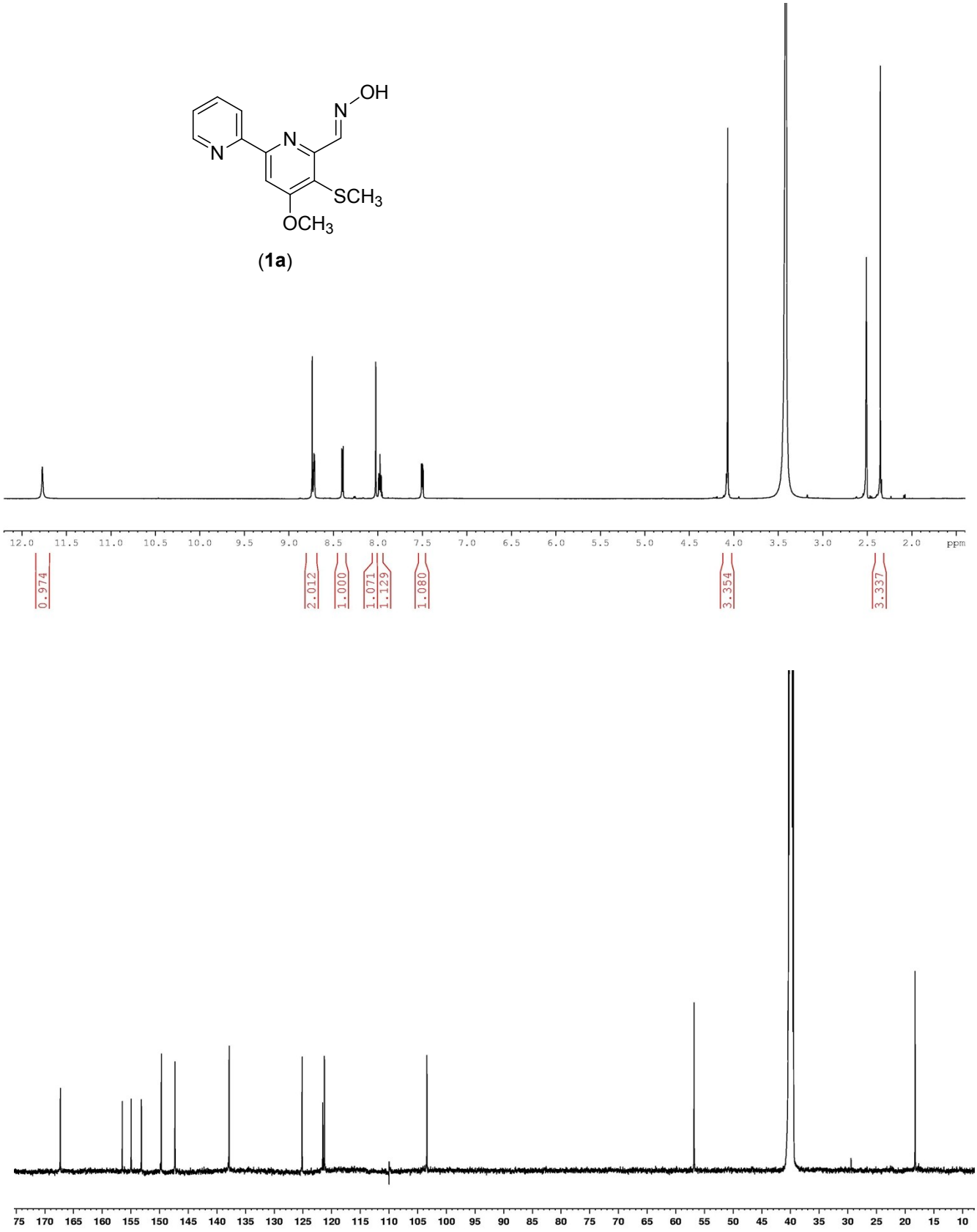


Figure S2. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR full charts for collismycin A (**1a**) in DMSO-*d*6.

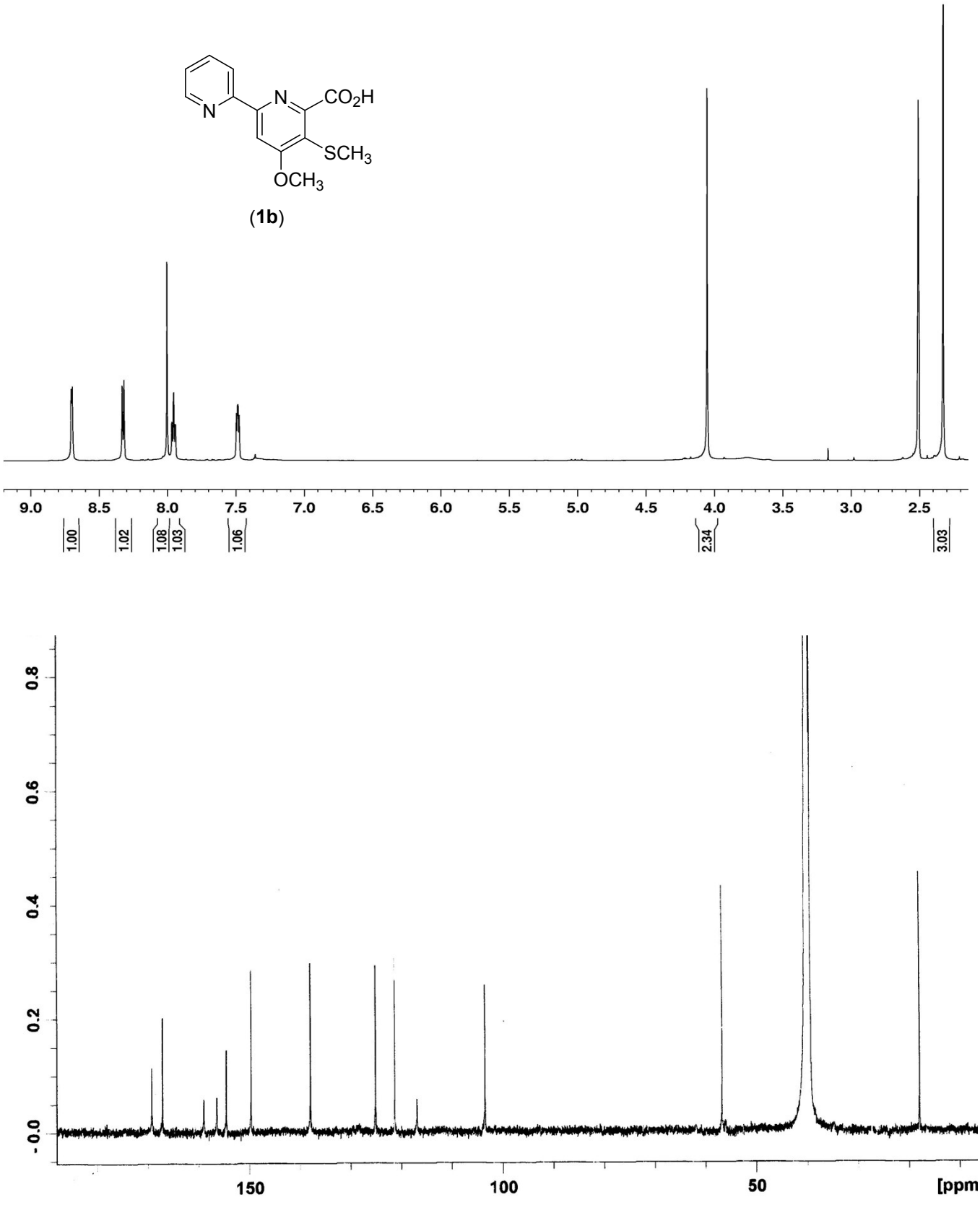
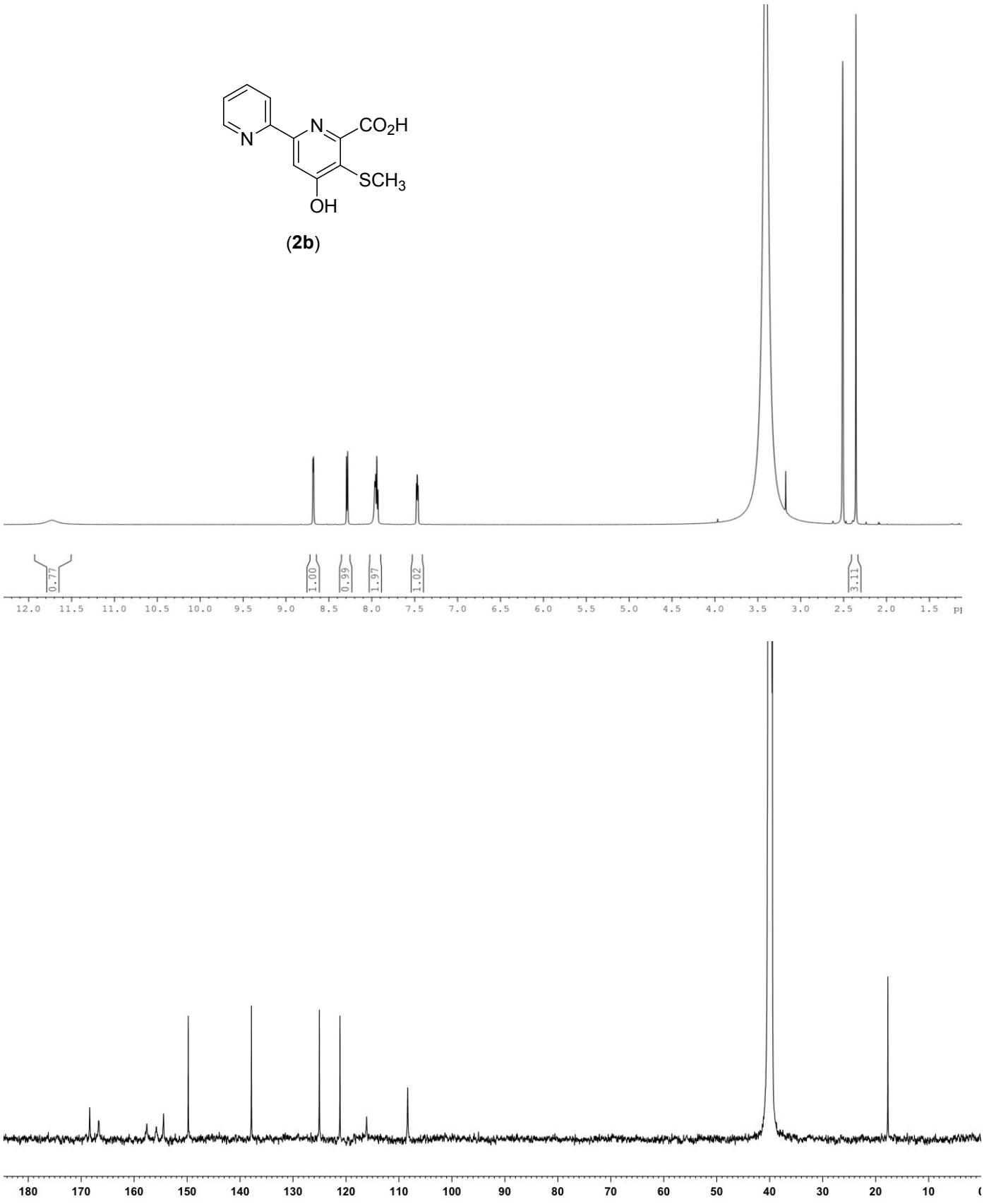


Figure S3. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR full charts for **1b** in DMSO-*d*6.



(**2b**)

Figure S4. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR full charts for **2b** in DMSO-*d*6.

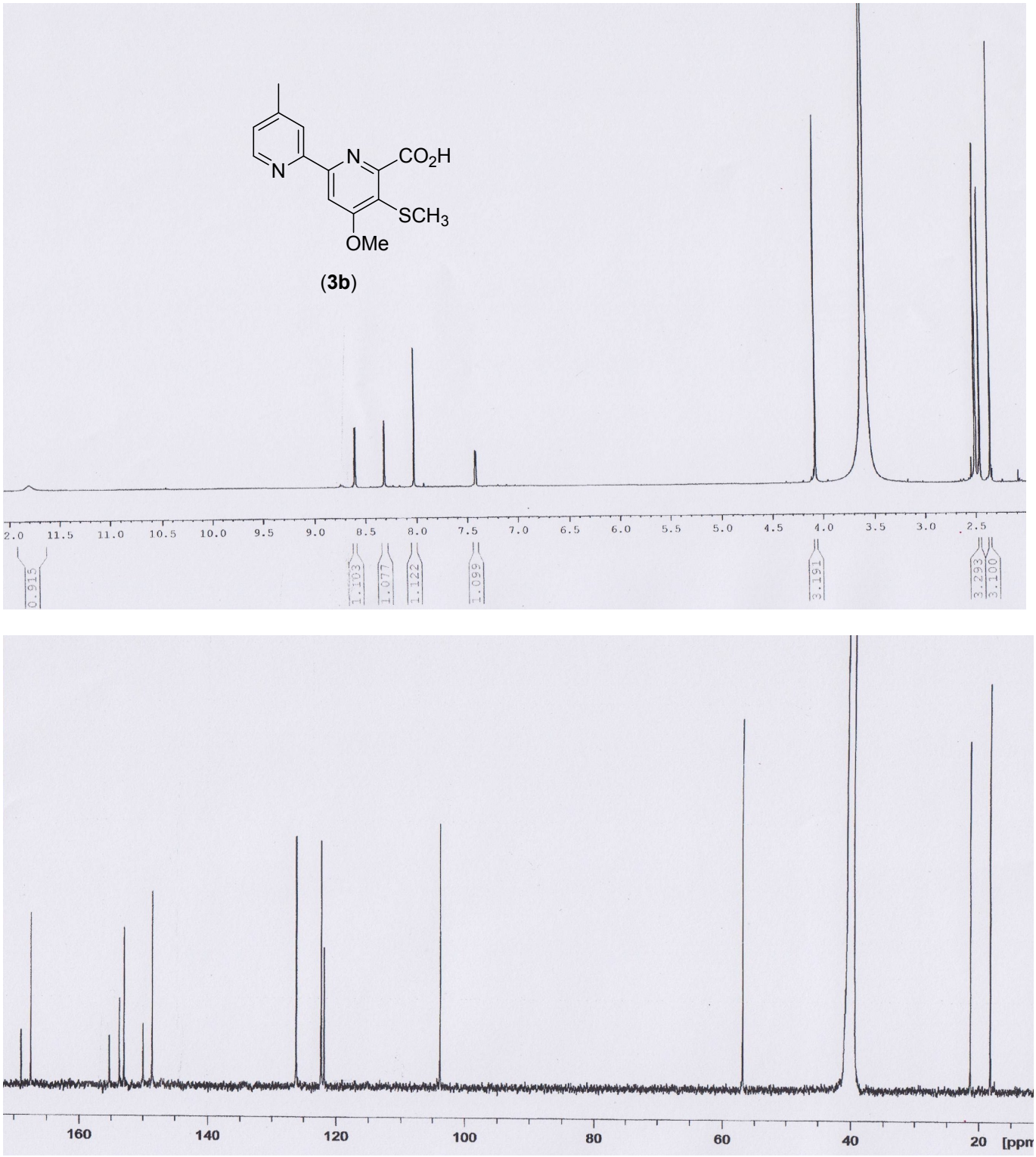


Figure S5. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR full charts for **3b** in DMSO-*d*6.

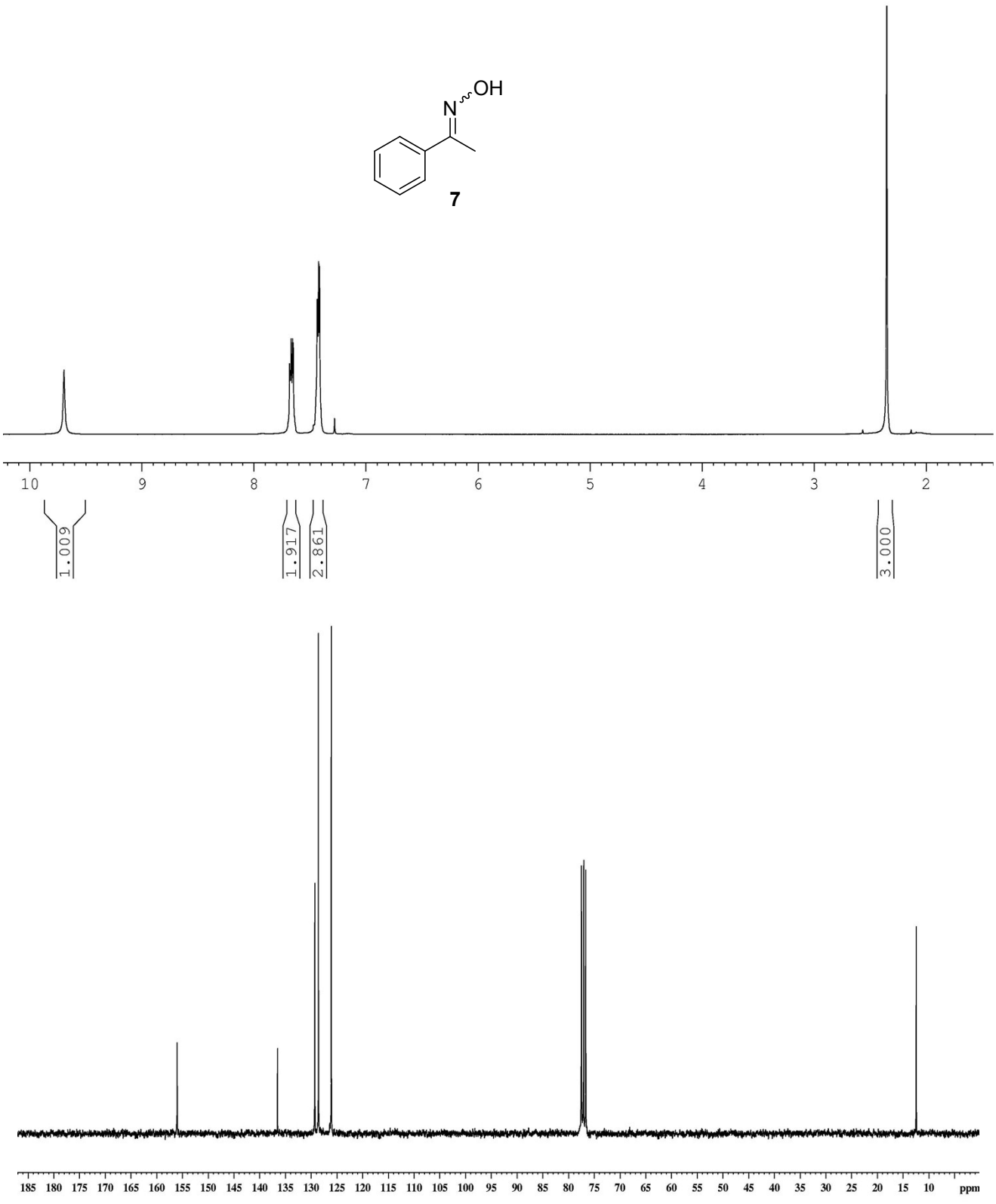


Figure S6. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR full charts for **7** in $CDCl_3$.

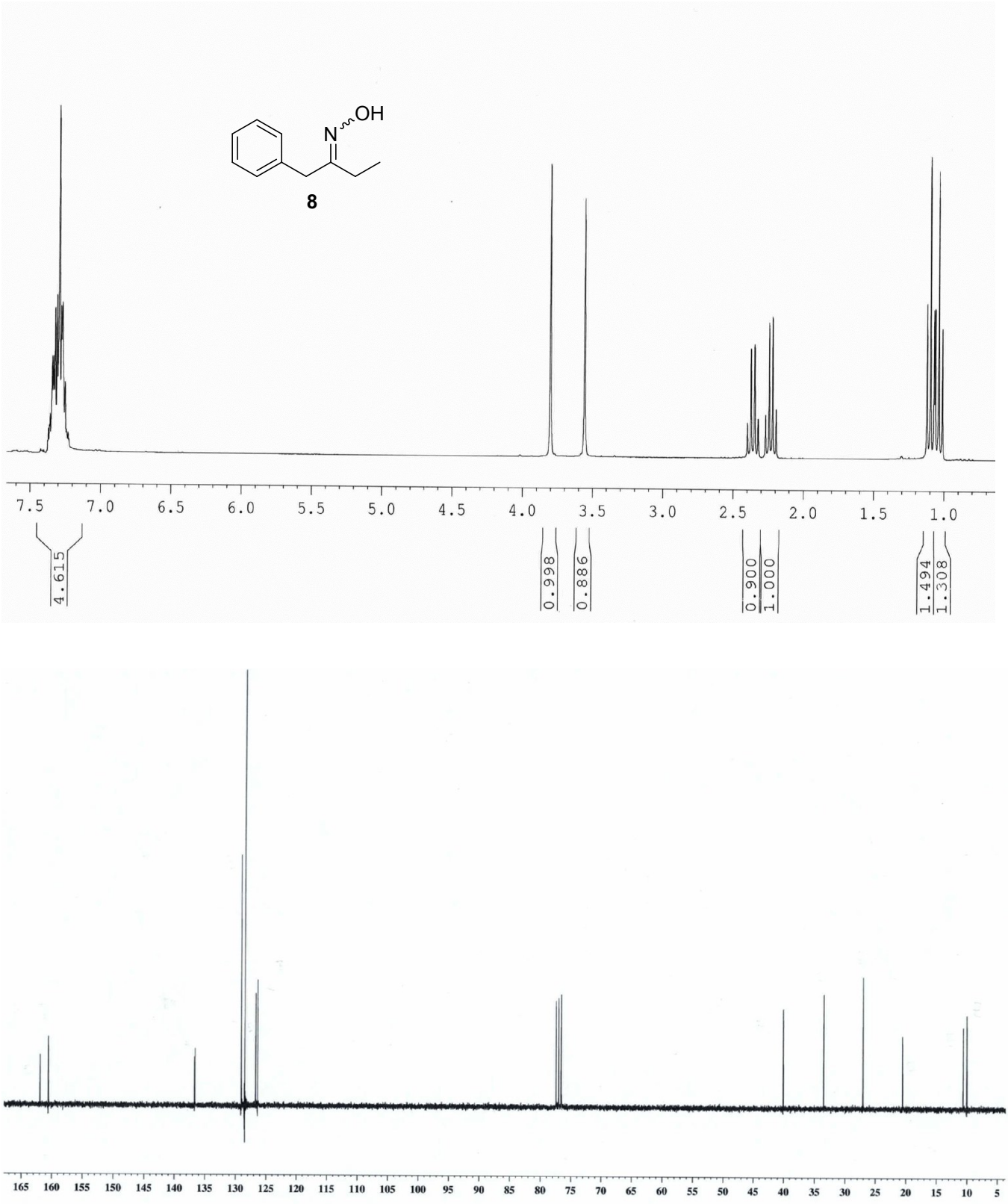


Figure S7. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR full charts for **8** in $CDCl_3$. (300 MHz)

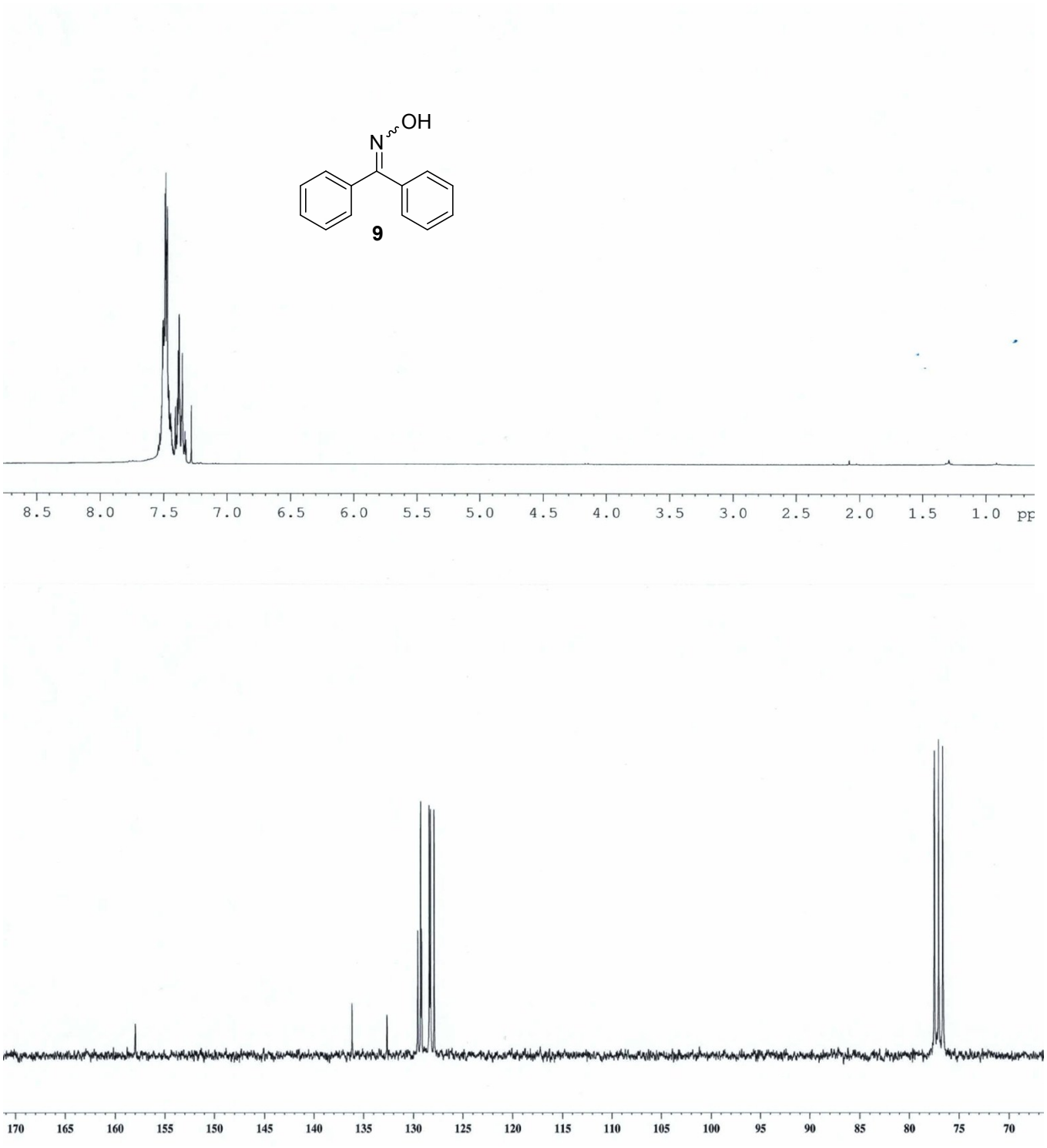


Figure S8. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR full charts for **9** in $CDCl_3$. (300 MHz)

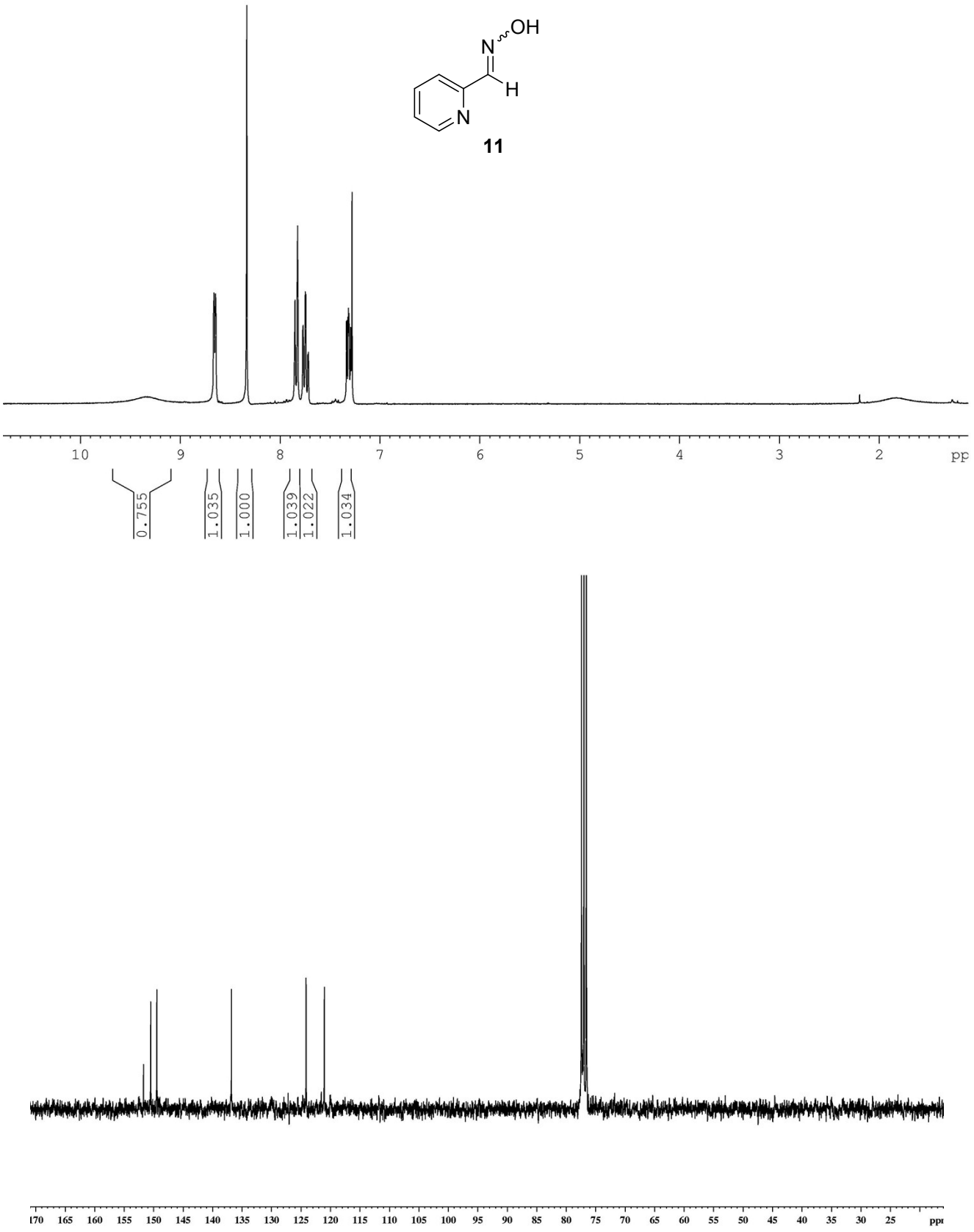


Figure S10. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR full charts for **11** in $CDCl_3$. (300 MHz)

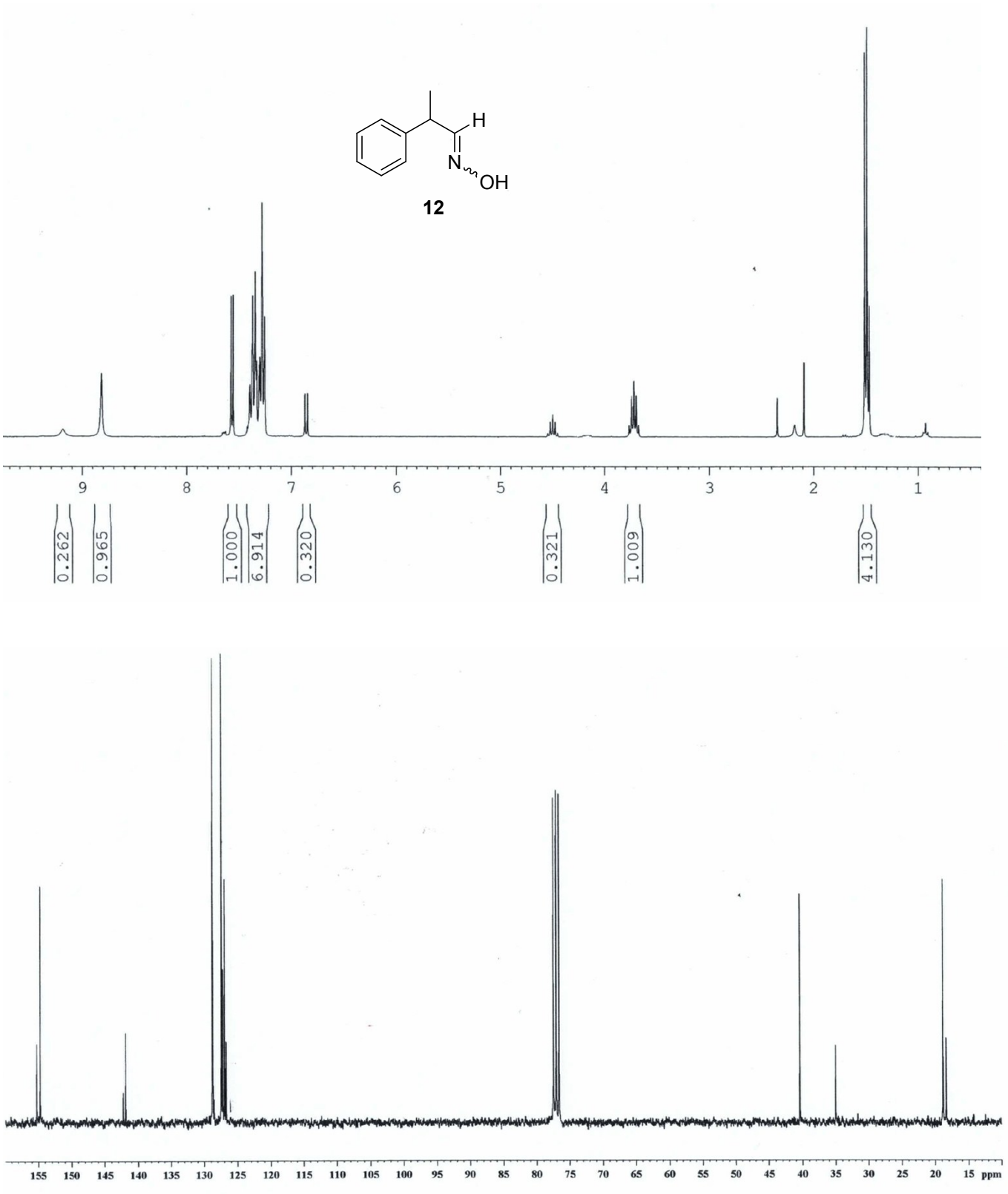
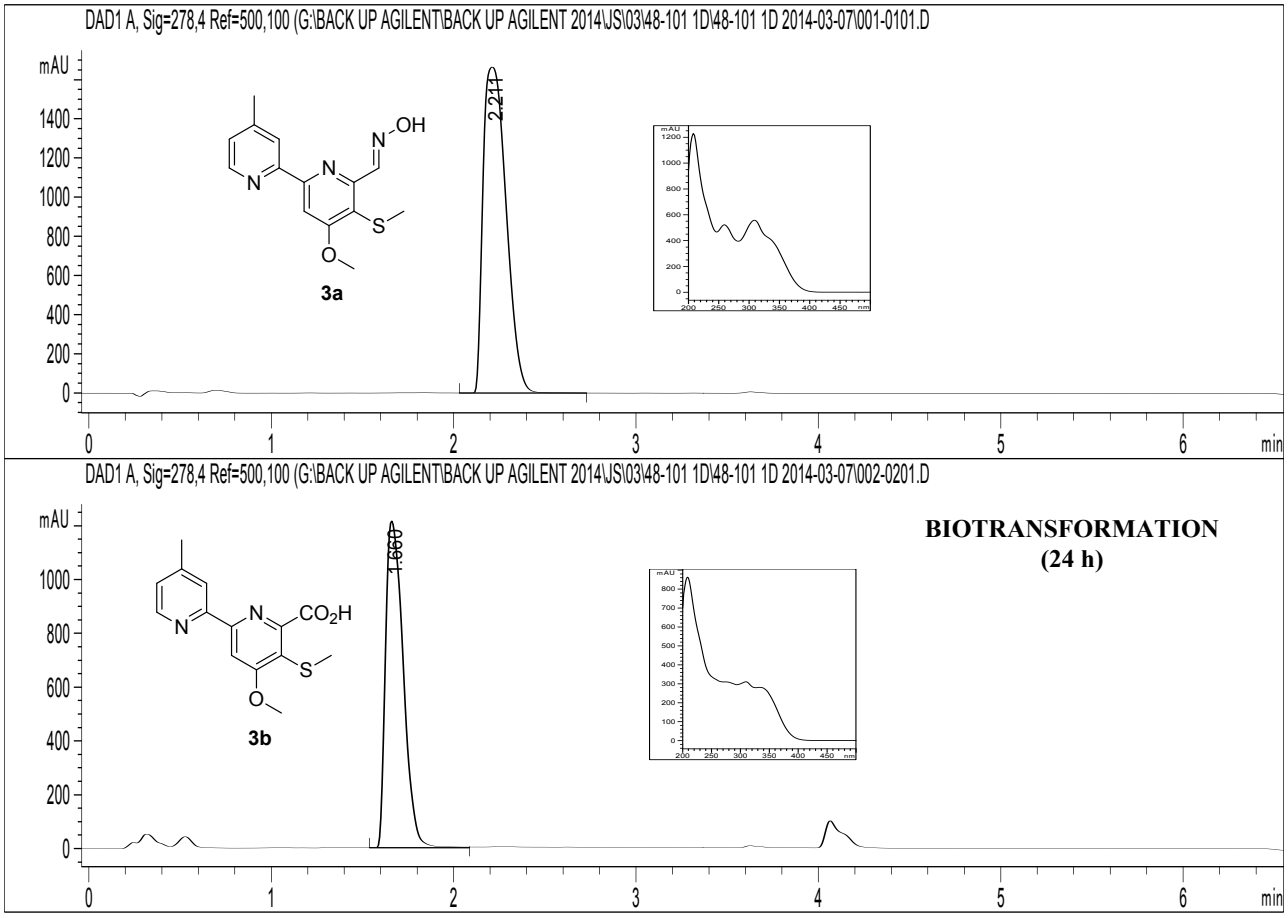


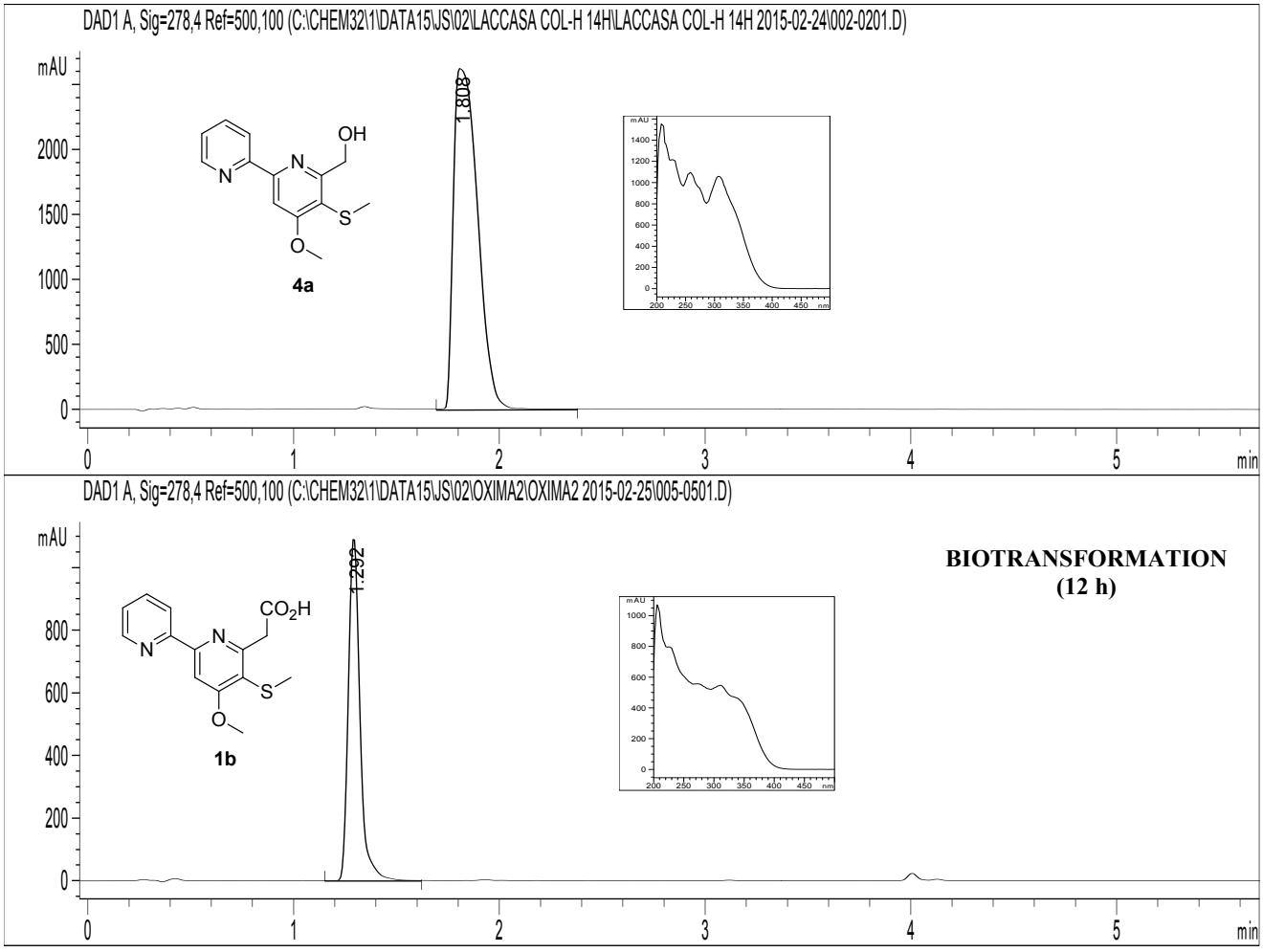
Figure S11. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR full charts for **12** in $CDCl_3$. (300 MHz)

3. Examples of in process HPLC monitoring reactions

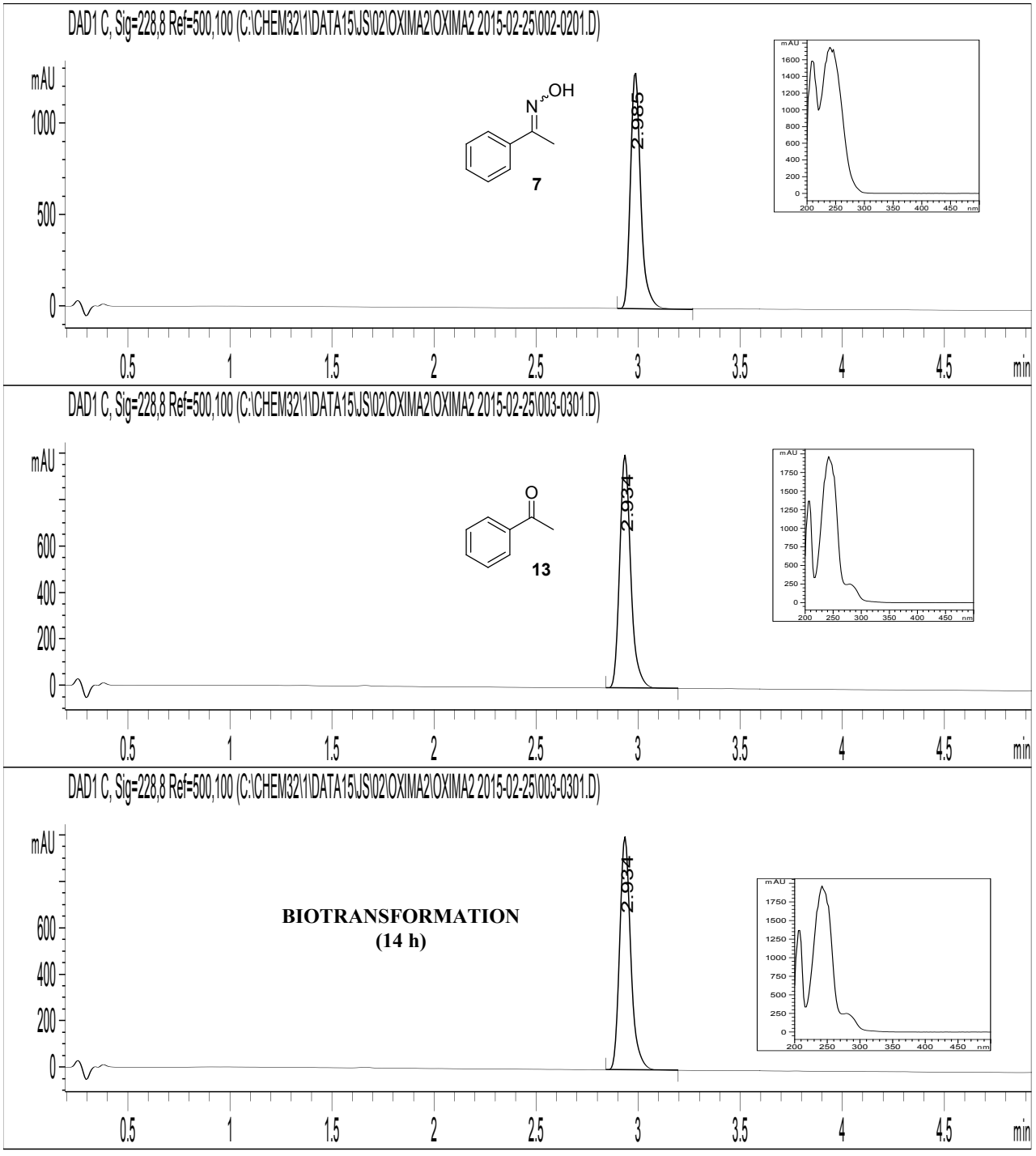
3.1 In process HPLC monitoring for the biotransformation of 3a (Table 1, entry 3).



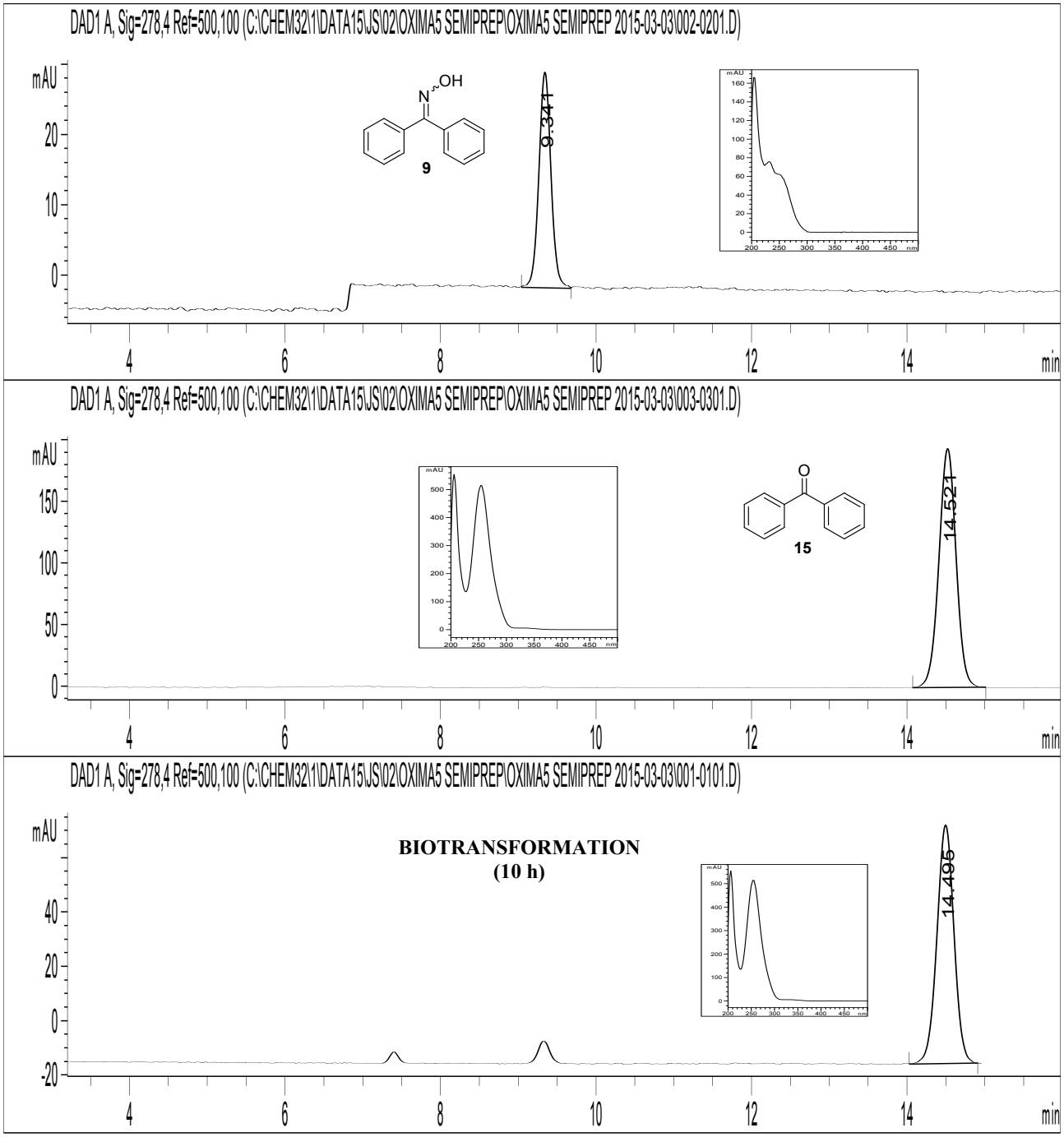
3.2 In process HPLC monitoring for the biotransformation of 4a (Table 1, entry 4).



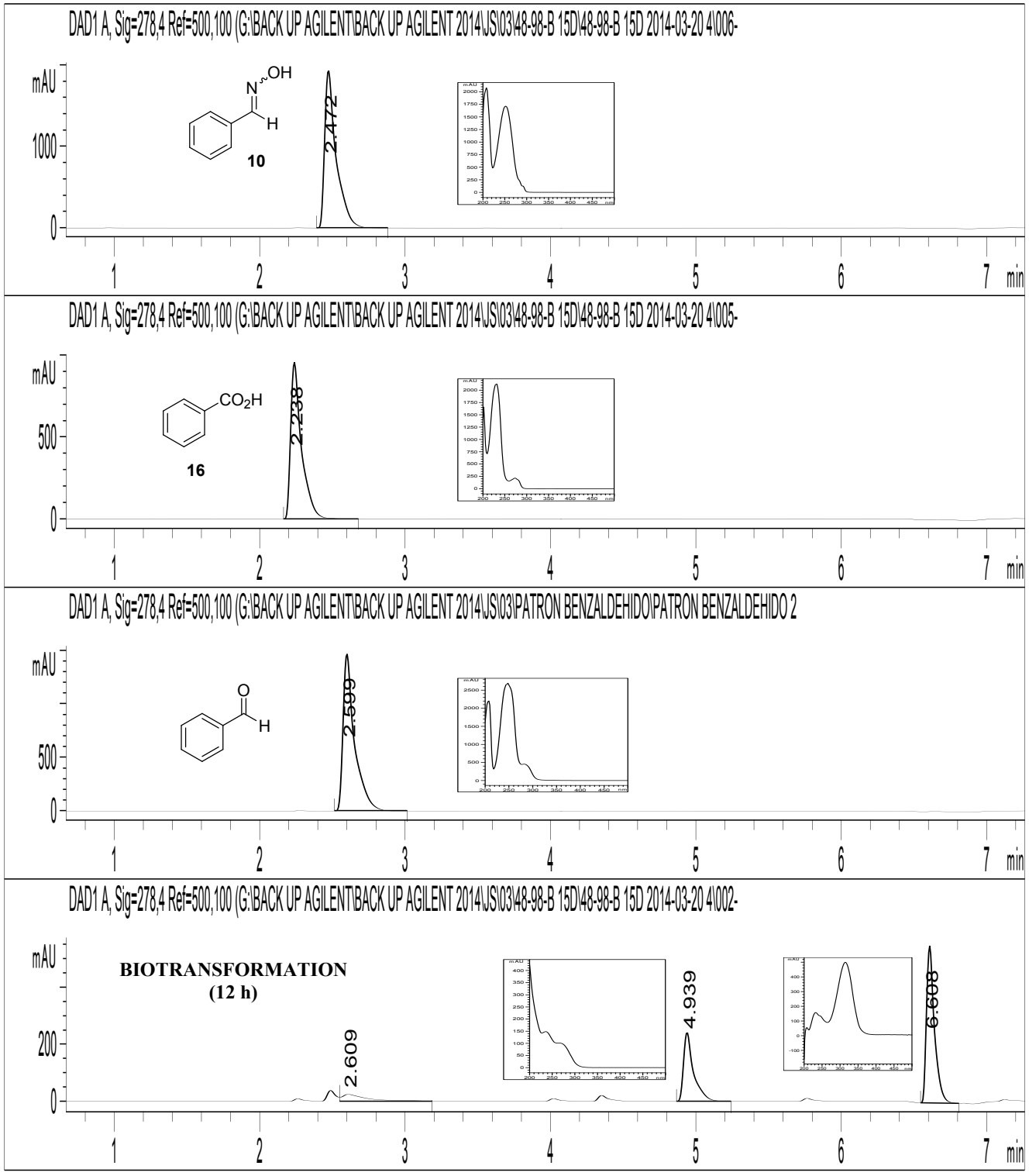
3.3 In process HPLC monitoring for the biotransformation of 7 (Table 3, entry 1).



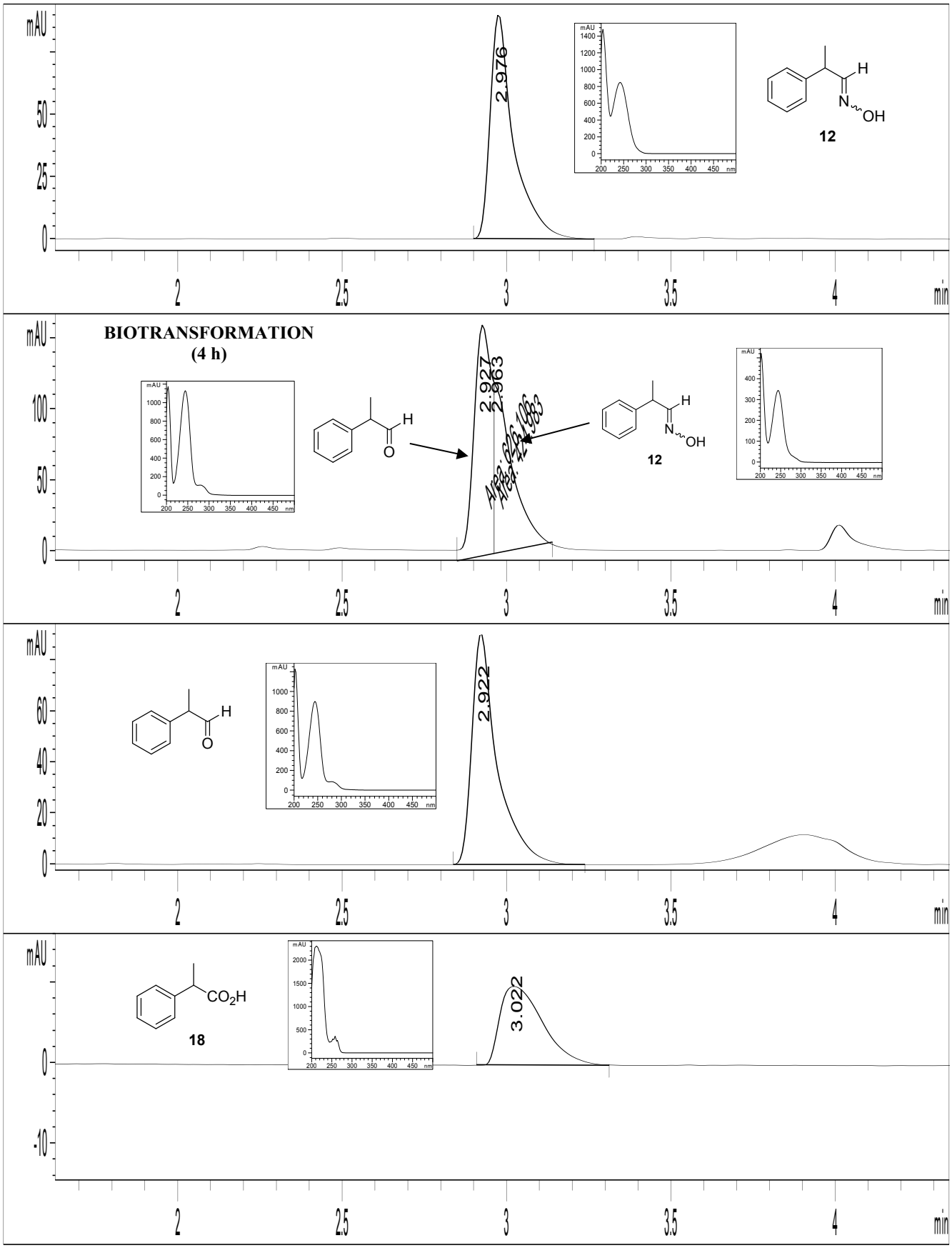
3.4 In process HPLC monitoring for the biotransformation of 9 (Table 3, entry 3).



3.5 In process HPLC monitoring for the biotransformation of 10 (Table 3, entry 4).



3.6 In process HPLC monitoring for the biotransformation of 12 (Table 3, entry 6).



4. Environmental assessment using EATOS

A simplified environmental impact analysis was performed using the *E*-factor concept (*Chem. Ind*. **1992**, 903-906). Thus, an *E*-factor of 8.3 (excluding solvents) was obtained for the laccase-catalysed deoximation of acetophenone oxime (**7**) using the EATOS tool (*Chem. Eur. J*. **2002**, *8*, 3580-3585). This value has not significance without a comparison with other methods, but it was impossible to obtain full data of the downstream processing in the previous reported publications. Nonetheless, it was comparable to a recently reported laccase/TEMPO system to deprotect *N*-benzylated amines (*Green Chem*. **2015**, *17*, 2794-2798).

Laccase/TEMPO

acetophenone

yield: 95%

Material	(Non)volatile [mg]	(Non)volatile [E]	Solvent [mL]
Starting Materials			
oxime	67,5		
water (as reagent)	9,0		
laccase from *T. versicolor*	9,0	0,158	
TEMPO	7,8	0,137	
sodium citrate	12,9	2,263	
acetonitrile			1
water (as solvent)			10
By-Products			
hydroxylamine		0,275	
Remaining oxime + other		0,067	
Auxiliaries (work-up)			
Na_2SO_4	300,0	5,263	
EtOAc			15

E factor:	
reaction	2,9
work-up	5,3
purification	*na*
total	**8,2**

Environmental assessment of chemoenzymatic deoximation

General remarks:

The *E*-factor was calculated for non-volatiles, gases and volatile compounds constituting final products. Solvents were treated separately

			E-factor				Waste classification			
method	**yield [%]**	**by-products [%]**	**overall**	**reaction**	**isolation**	**solvent demand [L/g product]**	**inorg. salts**	**organics**	**by-products**	**others**
Laccase/TEMPO	95	<3	8,2	2,9	5,3	0,46	5,3	2,6	0,3	0,1