

Synthesis of disulfides by laccase-catalyzed oxidative coupling of heterocyclic thiols

Heba T. Abdel-Mohsen, Kavitha Sudheendran, Jürgen Conrad and Uwe Beifuss*

Bioorganische Chemie, Institut für Chemie, Universität Hohenheim

Garbenstr. 30, D-70599 Stuttgart, Germany

Fax: (+49) 711 459 22951; Tel. (+49) 711 459 22171

E-mail: ubeifuss@uni-hohenheim.de

Electronic Supplementary Information

Table of contents

1. General remarks	2
2. General procedure for the laccase-catalyzed oxidative coupling of heterocyclic thiols	2
3. Synthesis and analytical data of disulfides	3
4. Computational studies of compounds 2a , 2c and 2f	22
5. The greenness of the laccase-catalyzed oxidative coupling of thiols 1 to disulfides 2 according to the twelve principles of green chemistry	26
6. Calculation of TON, TOF and STY for the oxidative coupling of 1m to 2m	28
7. Determination of the activity of laccase from <i>Trametes versicolor</i>	29
8. References	29

1. General remarks

All chemicals were purchased from commercial suppliers. Laccase from *Trametes versicolor* was purchased from Sigma Aldrich and laccase from *Agaricus bisporus* was purchased from ASA Spezialenzyme. Solvents used in extraction and purification were distilled prior to use. The pH of the buffer was adjusted using a pH 330/SET-1 pH-meter. Analytical thin layer chromatography (TLC) was performed on precoated silica gel 60 F₂₄₅ aluminium plates (Merck) with visualization under UV light and by immersion in permanganate solution followed by heating. Flash chromatography was carried out on silica gel MN 60, 0.04-0.05 mm (Macherey & Nagel). Melting points were determined on a Büchi melting point apparatus B-545 with open capillary tubes and are uncorrected. UV/VIS spectra were recorded with a Varian Cary 50. IR spectra were measured on a Perkin-Elmer *Spectrum One* (FT-IR-spectrometer). ¹H and ¹³C NMR spectra were recorded at 300 (75) MHz on a Varian ^{Unity}Inova using acetone-*d*₆, CDCl₃, DMSO-*d*₆ or pyridine-*d*₅. The chemical shifts were referenced to the solvent signals at $\delta_{H/C}$ 2.05 / 29.80 ppm (acetone-*d*₆), 7.26 / 77.00 ppm (CDCl₃), 2.49 / 39.50 ppm (DMSO-*d*₆) and 8.74 / 150.35 ppm (pyridine-*d*₅) relative to TMS as internal standards. HSQC, HMBC and COSY spectra were recorded on a Varian ^{Unity}Inova spectrometer (300 MHz). Coupling constants *J* [Hz] were directly taken from the spectra and are not averaged. Splitting patterns are designated as s (singlet), d (doublet), t (triplet), q (quartet), sex (sextet) and m (multiplet). Low resolution electron impact mass spectra (EI-LRMS) and exact electron impact mass spectra (HRMS) were recorded at 70 eV on a Finnigan MAT 95 instrument. The intensities are reported as percentages relative to the base peak (*I* = 100%).

2. General procedure for the laccase-catalyzed oxidation of heterocyclic thiols **1** to the corresponding disulfides **2**

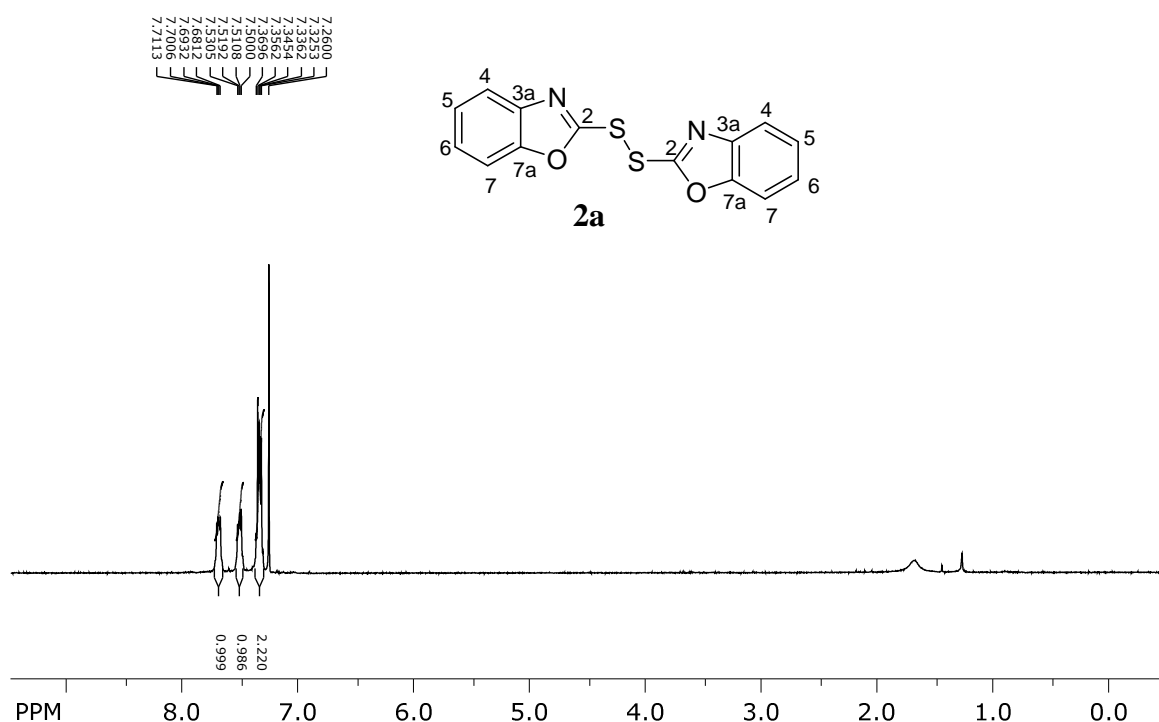
A 50 or 100 mL round bottomed flask with a magnetic stirrer bar was charged with a solution or suspension of heterocyclic thiol **1** (1 mmol) in methanol (3-6 mL). Acetate buffer (0.2 M, pH 4.4, 20-30 mL), laccase from *Trametes versicolor* (200 U, 10 mg) and ABTS diammonium salt (13.7 mg, 0.025 mmol) were added and the reaction mixture was stirred at rt for the time given. After extraction with EtOAc (3 × 30 mL) or CH₂Cl₂ (3 × 30 mL), the combined organic phases were dried over anhydrous Na₂SO₄, filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO₂. The extraction

could be also performed with less EtOAc (3 × 10 mL, 3 × 5 mL, 3 × 3 mL) and without any significant loss of yield.

3. Synthesis and analytical data of disulfides

3.1. Synthesis and analytical data of bis(2-benzoxazolyl) disulfide (**2a**)¹

According to the general procedure, 2-mercaptobenzoxazole (**1a**) (151 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.4, 27 mL), laccase (200 U, 10 mg, *Trametes versicolor*) and ABTS diammonium salt (13.7 mg, 0.025 mmol) were reacted for 30 h. After extraction with CH₂Cl₂ (3 × 30 mL), the combined organic phases were dried over anhydrous Na₂SO₄, filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO₂ (petroleum ether / EtOAc = 20:1) to give bis(2-benzoxazolyl) disulfide (**2a**) as white powder (89 mg, 59%); mp 94-96 °C (lit.,¹ 92-94 °C); *R*_f = 0.63 (petroleum ether / EtOAc = 5:1); δ_H (300 MHz; CDCl₃) 7.33-7.37 (4H, m, 5-H and 6-H), 7.50-7.53 (2H, m, 4-H) and 7.68-7.71 (2H, m, 7-H); δ_C (75 MHz; CDCl₃) 110.60 (C-7), 119.84 (C-4), 124.93 (C-5 or C-6), 125.43 (C-5 or C-6), 141.84 (C-3a), 152.58 (C-7a) and 159.89 (C-2); *m/z* (EI, 70 eV) 300 (M⁺, 100%), 268 (M⁺ - S, 5), 151 (C₇H₅NOS, 100), 122 (44) and 91 (13).



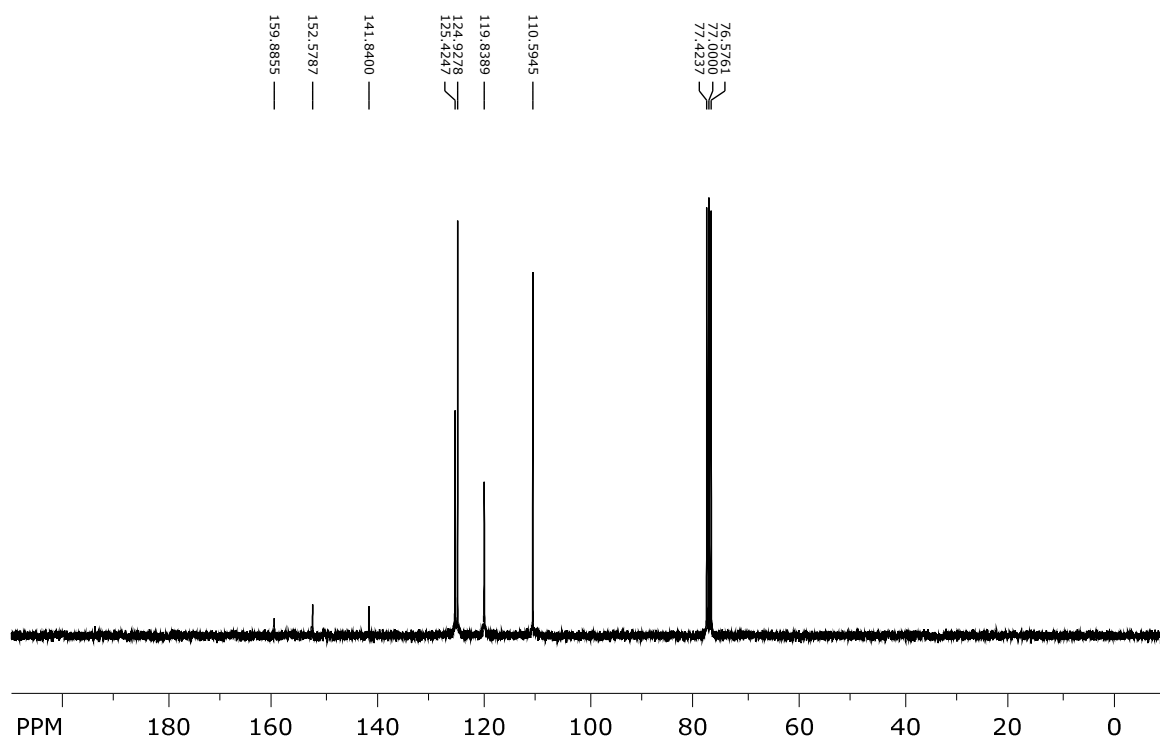


Fig. 1 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **2a** in CDCl_3

3.2. Synthesis and analytical data of bis(5-chloro-2-benzoxazolyl) disulfide (**2b**)

According to the general procedure, 5-chloro-2-mercaptobenzoxazole (**1b**) (92.8 mg, 0.5 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.4, 20 mL), laccase (100 U, 5 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were reacted for 24 h. After extraction with EtOAc (3×30 mL), the combined organic phases were dried over anhydrous Na_2SO_4 , filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO_2 (petroleum ether / EtOAc = 20:1) to give bis(5-chloro-2-benzoxazolyl) disulfide (**2b**) as white powder (46 mg, 50%); mp 130-132 °C; R_f = 0.82 (petroleum ether / EtOAc = 5:1); λ_{max} (MeCN)/nm 293 (log ϵ , 4.24), 248 (4.29) and 203 (4.66); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 1494, 1443, 1137, 911 and 815; δ_{H} (300 MHz; acetone- d_6) 7.46 (2H, dd, $^3J_{6\text{-H},7\text{-H}}$ 8.8 Hz, $^4J_{4\text{-H},6\text{-H}}$ 1.9 Hz, 6-H), 7.70 (2H, d, $^3J_{6\text{-H},7\text{-H}}$ 8.8 Hz, 7-H) and 7.77 (2H, d, $^4J_{4\text{-H},6\text{-H}}$ 1.9 Hz, 4-H); δ_{C} (75 MHz; acetone- d_6) 112.77 (C-7), 120.27 (C-4), 126.74 (C-6), 131.04 (C-5), 143.85 (C-3a), 152.14 (C-7a) and 162.46 (C-2); m/z (EI, 70 eV) 368 (M^+ , 38%), 336 ($\text{M}^+ - \text{S}$, 4), 184 ($\text{M}^+ - \text{C}_7\text{H}_3\text{ClNOS}$, 100) and 156 (28); HRMS (EI, M^+) found: 367.9248 calcd for $\text{C}_{14}\text{H}_6\text{Cl}_2\text{N}_2\text{O}_2\text{S}_2$: 367.9248.

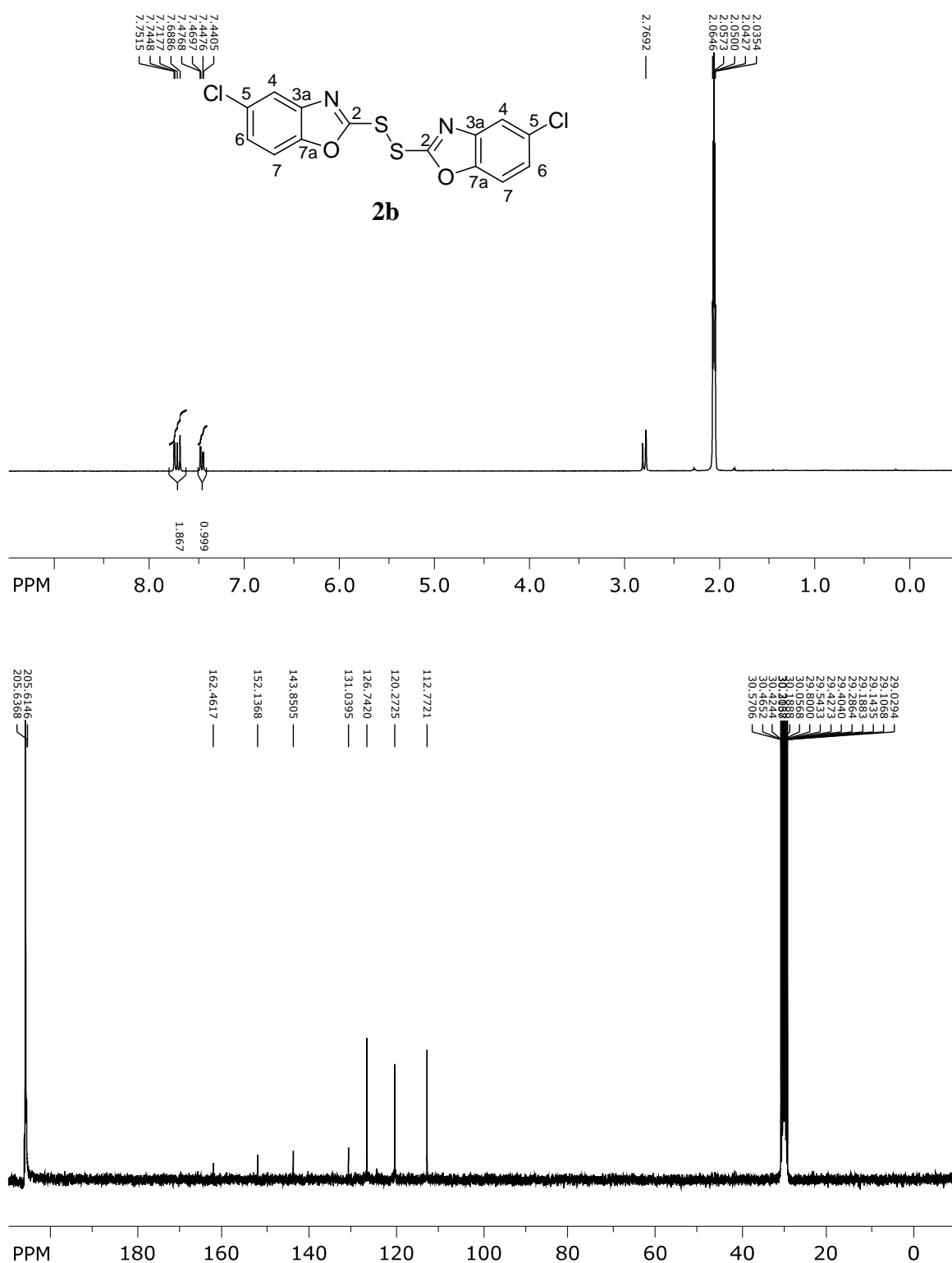
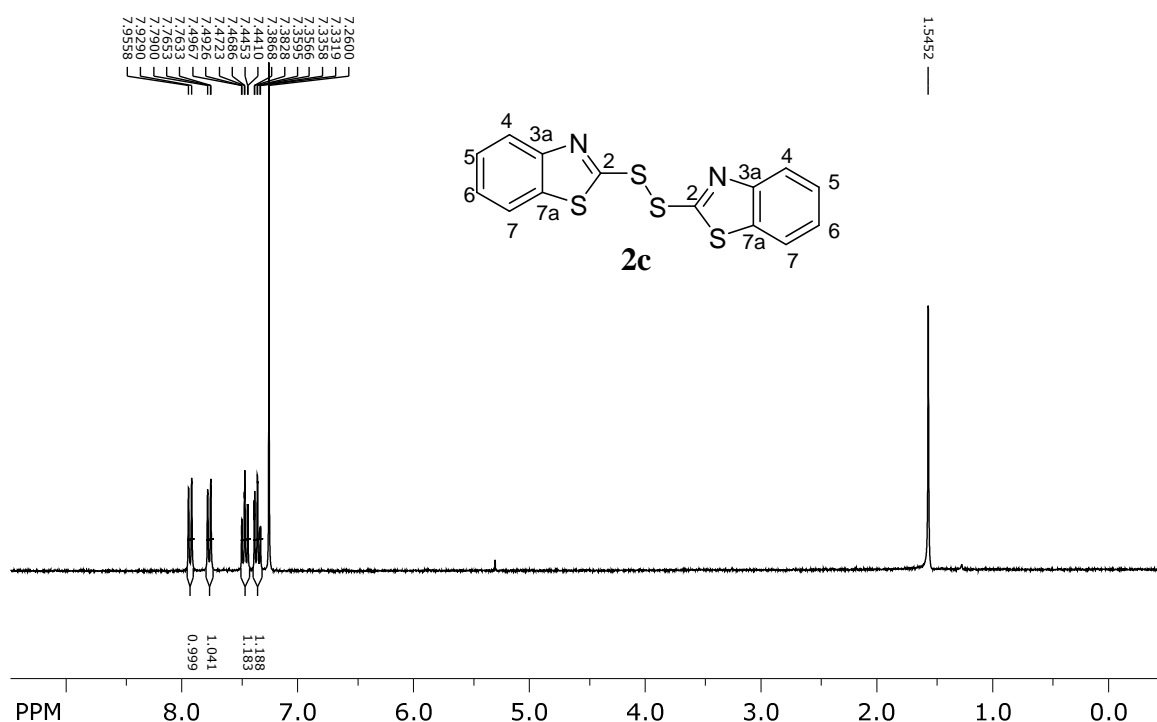


Fig. 2 ¹H (300 MHz) and ¹³C (75 MHz) NMR spectra of **2b** in acetone-*d*₆

3.3. Synthesis and analytical data of bis(2-benzothiazolyl) disulfide (**2c**)¹

According to the general procedure, 2-mercaptobenzothiazole (**1c**) (167 mg, 1 mmol), methanol (6 mL), acetate buffer (0.2 M, pH 4.4, 30 mL), laccase (200 U, 10 mg, *Trametes versicolor*) and ABTS diammonium salt (13.7 mg, 0.025 mmol) were reacted for 24 h. After extraction with EtOAc (4 × 30 mL), the combined organic phases were dried over anhydrous Na₂SO₄, filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO₂ (petroleum ether / EtOAc = 5:1 to 2:1) to give bis(2-benzothiazolyl) disulfide (**2c**) as white powder (129 mg, 78%); mp 176-178 °C (lit.,¹ 178-179 °C); *R*_f = 0.61 (petroleum ether / EtOAc = 5:1); δ_H (300 MHz; CDCl₃) 7.36 (2H, ddd, ³*J*_{5-H,6-H} 7.5 Hz or 8.3 Hz, ³*J*_{6-H,7-H} 7.5 Hz or 8.3 Hz, ⁴*J*_{4-H,6-H} 1.2 Hz, 6-H), 7.47 (2H, ddd, ³*J*_{4-H,5-H} 7.2 Hz or 8.4 Hz, ³*J*_{5-H,6-H} 7.2 Hz or 8.4 Hz, ⁴*J*_{5-H,7-H} 1.2 Hz, 5-H), 7.78 (2H, dd, ³*J*_{4-H,5-H} 8.0 Hz, ⁴*J*_{4-H,6-H} 1.2 Hz, 4-H) and 7.94 (2H, dd, ³*J*_{6-H,7-H} 7.9 Hz, ⁴*J*_{5-H,7-H} 1.2 Hz, 7-H); δ_C (75 MHz; CDCl₃) 121.29 (C-4), 122.68 (C-7), 125.28 (C-6), 126.57 (C-5), 136.13 (C-7a), 154.54 (C-3a) and 167.83 (C-2).



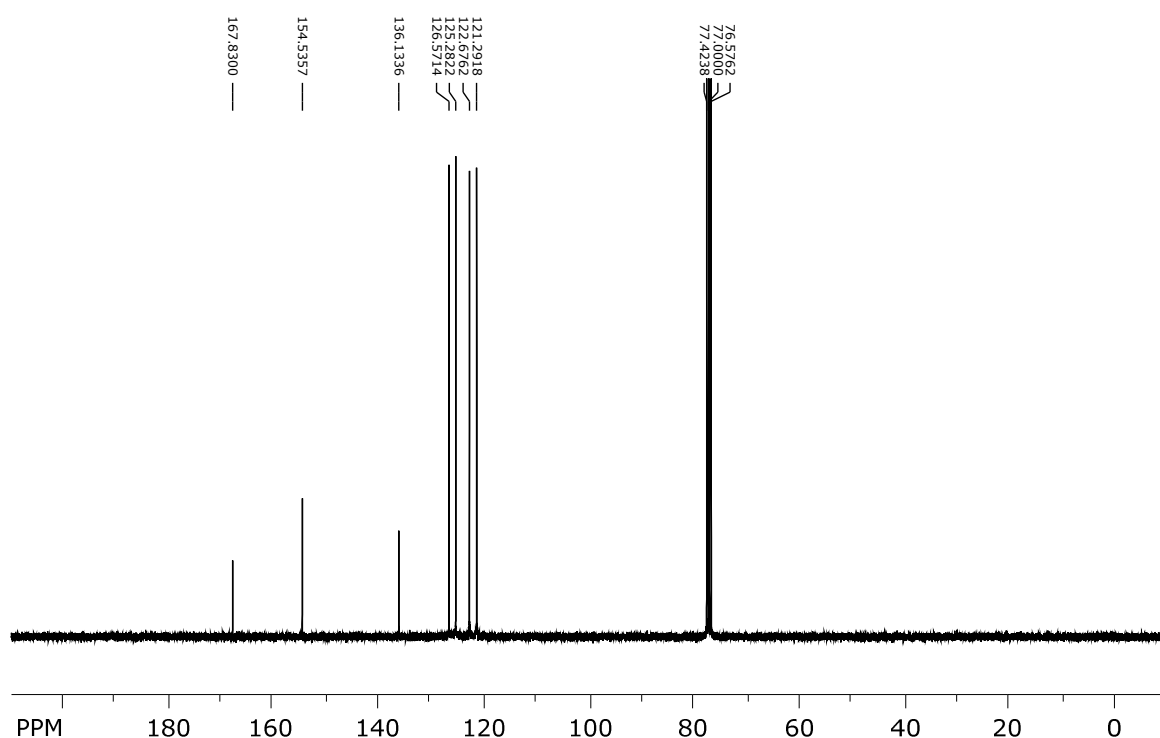


Fig. 3 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **2c** in CDCl_3

3.4. Synthesis and analytical data of bis(5-methoxy-2-benzothiazolyl) disulfide (**2d**)

According to the general procedure, 2-mercapto-5-methoxybenzothiazole (**1d**) (98.6 mg, 0.5 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.4, 30 mL), laccase (100 U, 5 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were reacted for 24 h. After extraction with EtOAc (3×30 mL), the combined organic phases were dried over anhydrous Na_2SO_4 , filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO_2 (petroleum ether / EtOAc = 5:1 to 2:1) to give bis(5-methoxy-2-benzothiazolyl) disulfide (**2d**) as white powder (89 mg, 91%); mp 169-171 °C; R_f = 0.42 (petroleum ether / EtOAc = 5:1); λ_{max} (MeCN)/nm 336 (log ϵ , 4.02), 267 (3.99), 228 (4.35) and 205 (4.45); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 1517, 1493, 1452, 1217 and 1097; δ_{H} (300 MHz; pyridine- d_5) 3.74 (6H, s, 1'-H), 7.14 (2H, dd, $^3J_{6\text{-H},7\text{-H}}$ 8.9 Hz, $^4J_{4\text{-H},6\text{-H}}$ 2.5 Hz, 6-H), 7.69 (2H, d, $^4J_{4\text{-H},6\text{-H}}$ 2.5 Hz, 4-H) and 7.81 (2H, d, $^3J_{6\text{-H},7\text{-H}}$ 8.9 Hz, 7-H); δ_{C} (75 MHz; pyridine- d_5) 56.08 (C-1'), 106.44 (C-4), 116.16 (C-6), 122.85 (C-7), 128.99 (C-7a), 156.82 (C-3a), 160.24 (C-5) and 169.12 (C-2); m/z (EI, 70 eV) 392 (M^+ , 100%), 361 (M^+ - OCH_3 , 3), 328 (M^+ - S_2 , 13) and 196 (96); HRMS (EI, M^+) found: 391.9782 calcd for $\text{C}_{16}\text{H}_{12}\text{N}_2\text{O}_2\text{S}_4$: 391.9782.

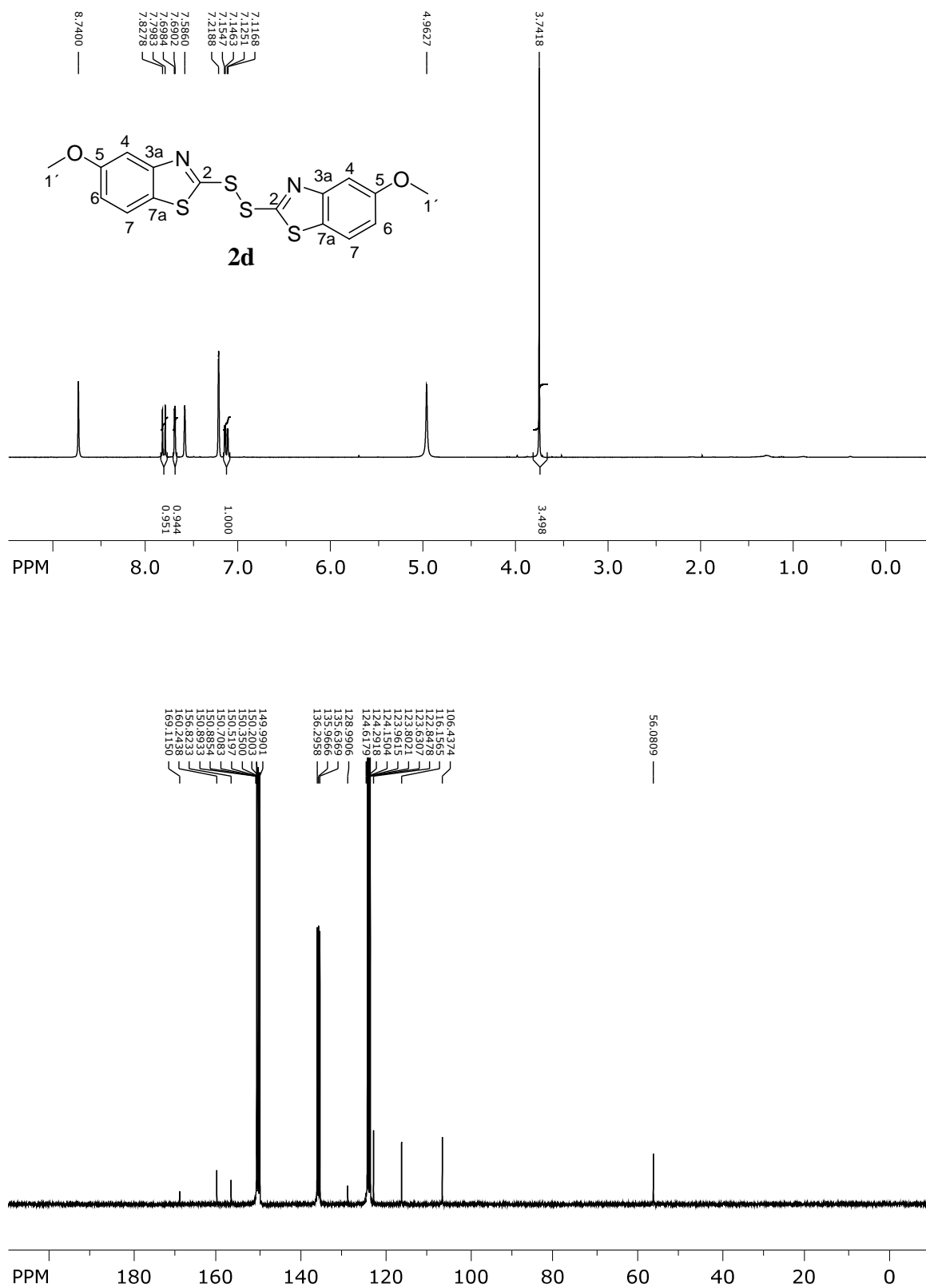
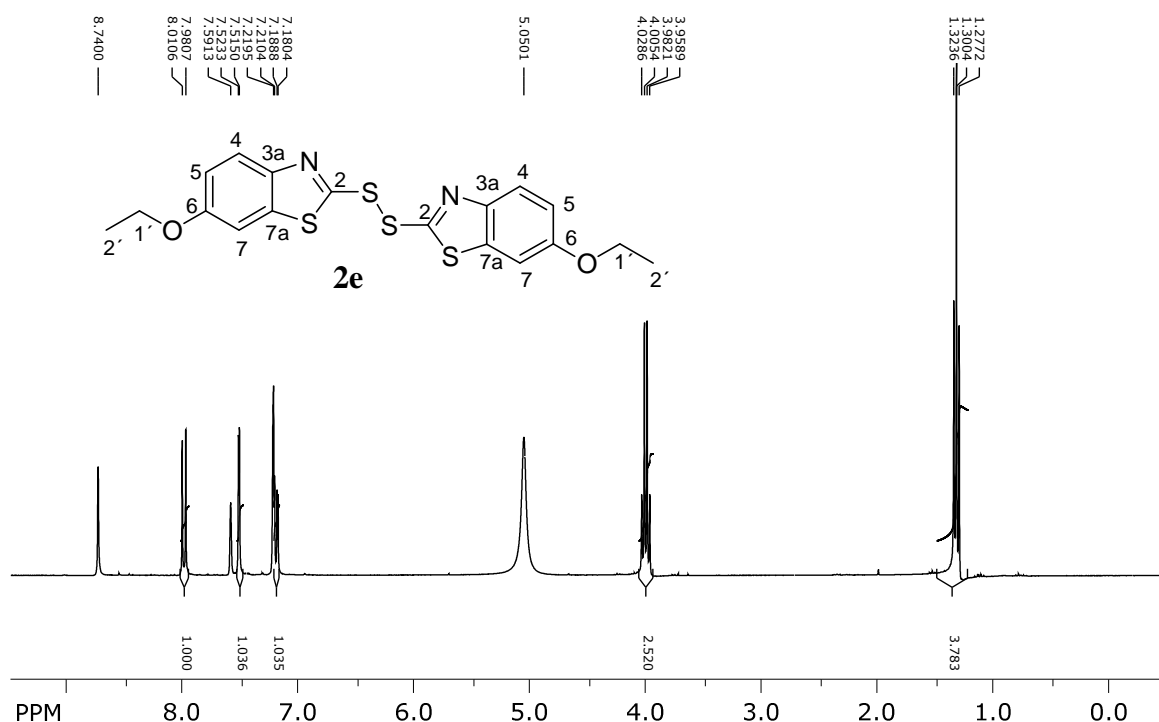


Fig. 4 ¹H (300 MHz) and ¹³C (75 MHz) NMR spectra of **2d** in pyridine-*d*₅

3.5. Synthesis and analytical data of bis(6-ethoxy-2-benzothiazolyl) disulfide (**2e**)

According to the general procedure, 6-ethoxy-2-mercaptobenzothiazole (**1e**) (211 mg, 1 mmol), methanol (6 mL), acetate buffer (0.2 M, pH 4.4, 30 mL), laccase (200 U, 10 mg, *Trametes versicolor*) and ABTS diammonium salt (13.7 mg, 0.025 mmol) were reacted for 12 h. After extraction with EtOAc (4 × 30 mL), the combined organic phases were dried over anhydrous Na₂SO₄, filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO₂ (petroleum ether / EtOAc = 5:1 to 2:1) to give bis(6-ethoxy-2-benzothiazolyl) disulfide (**2**) as white powder (187 mg, 89%); mp 136-138 °C; *R*_f = 0.36 (petroleum ether / EtOAc = 10:1); λ_{max}(MeCN)/nm 327 (log ε, 4.15) and 212 (3.43); ν_{max} (atr)/cm⁻¹ 2972 and 2870 (C-H), 1557, 1445, 1209 and 1006 (C-O); δ_H (300 MHz; pyridine-*d*₅) 1.30 (6H, t, ³*J*_{1'-H,2'-H} 7.0 Hz, 2'-H), 3.99 (4H, q, ³*J*_{1'-H,2'-H} 7.0 Hz, 1'-H), 7.20 (2H, dd, ³*J*_{4-H,5-H} 9.0 Hz, ⁴*J*_{5-H,7-H} 2.5 Hz, 5-H), 7.52 (2H, d, ⁴*J*_{5-H,7-H} 2.5 Hz, 7-H) and 8.00 (2H, d, ³*J*_{4-H,5-H} 9.0 Hz, 4-H); δ_C (75 MHz; pyridine-*d*₅) 15.23 (C-2'), 64.83 (C-1'), 105.90 (C-7), 117.30 (C-5), 123.91 (C-4), 138.97 (C-7a), 149.73 (C-3a), 158.18 (C-6) and 164.45 (C-2); *m/z* (EI, 70 eV) 420 (M⁺, 100%), 387 (M⁺ - SH, 2), 356 (M⁺ - S₂, 6), 210 (M⁺ - C₉H₈NOS₂, 69) and 182 (C₇H₄NOS₂, 69); HRMS (EI, M⁺) found: 420.0095 calcd for C₁₈H₁₆N₂O₂S₄: 420.0095.



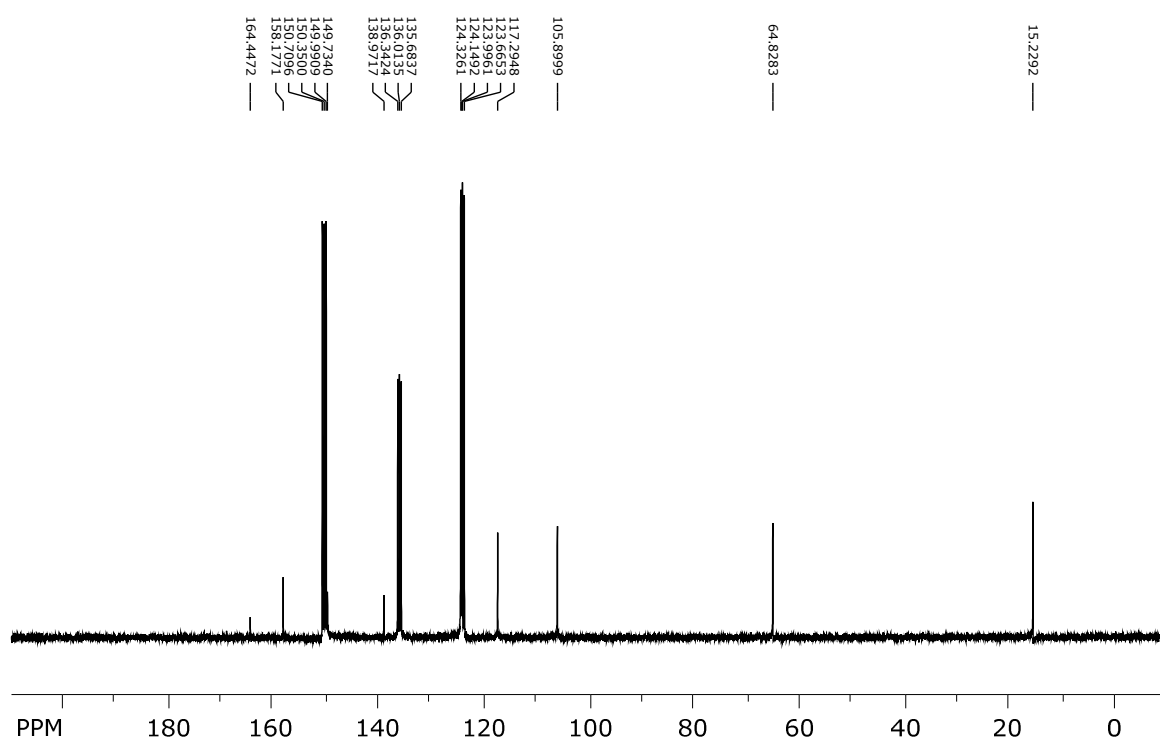


Fig. 5 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **2e** in pyridine- d_5

3.6. Synthesis and analytical data of bis(2-thiazolyl) disulfide (**2f**)²

According to the general procedure, 2-mercaptothiazole (**1f**) (117 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.4, 20 mL), laccase (200 U, 10 mg, *Trametes versicolor*) and ABTS diammonium salt (13.7 mg, 0.025 mmol) were reacted for 8 h. After extraction with EtOAc (3 × 30 mL), the combined organic phases were dried over anhydrous Na_2SO_4 , filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO_2 (petroleum ether / EtOAc = 5:1) to give bis(2-thiazolyl) disulfide (**2f**) as white powder (94 mg, 81%); mp 77-79 °C (lit.,² 79-80 °C); R_f = 0.25 (petroleum ether / EtOAc = 5:1); δ_{H} (300 MHz; CDCl_3) 7.40 (2H, d, $^3J_{4\text{-H},5\text{-H}}$ 3.3 Hz, 5-H) and 7.78 (2H, d, $^3J_{4\text{-H},5\text{-H}}$ 3.3 Hz, 4-H); δ_{C} (75 MHz; CDCl_3) 122.41 (C-5), 144.32 (C-4) and 164.80 (C-2).

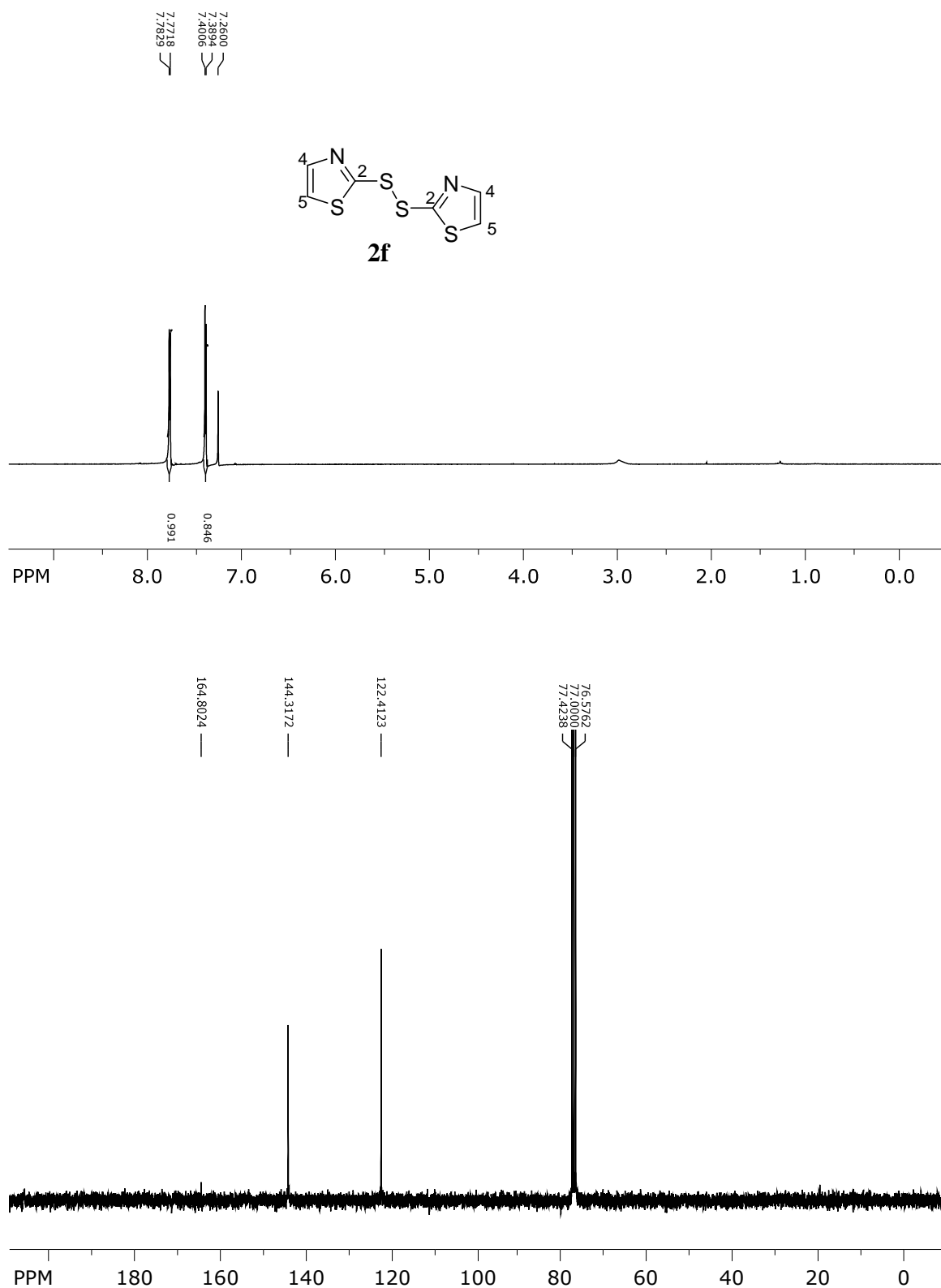
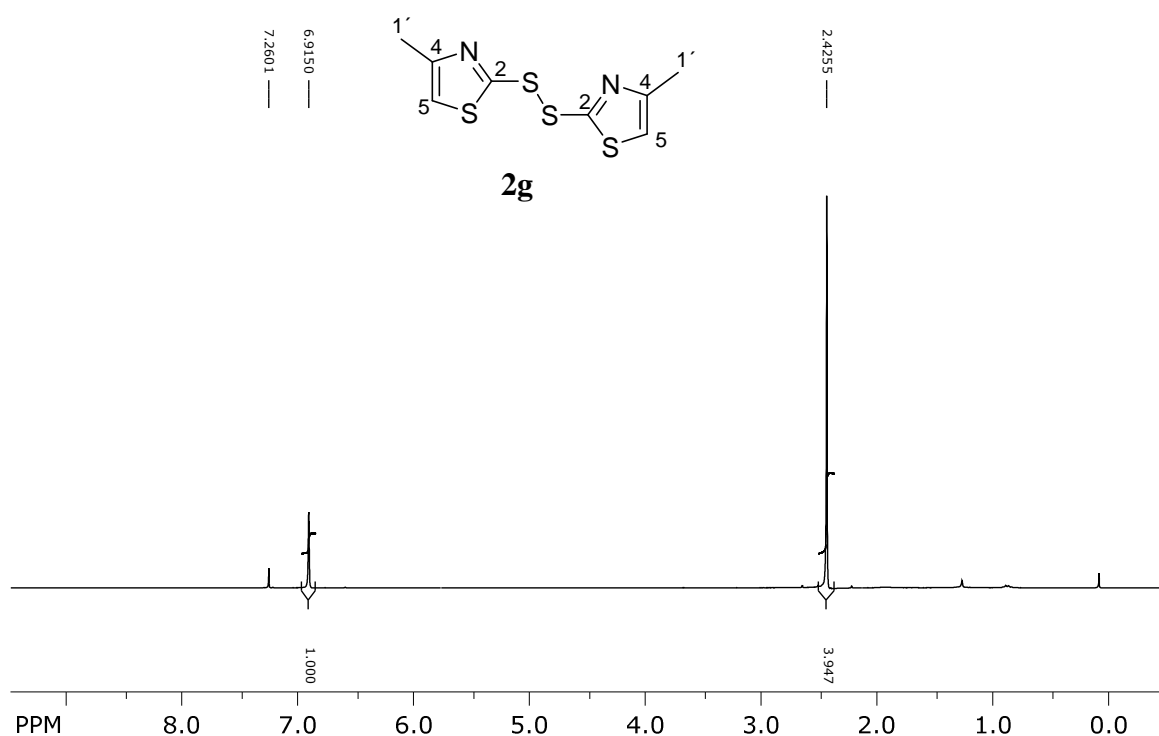


Fig. 6 ¹H (300 MHz) and ¹³C (75 MHz) NMR spectra of **2f** in CDCl₃

3.7. Synthesis and analytical data of bis(4-methylthiazolyl)-2-disulfide (**2g**)³

According to the general procedure, 2-mercapto-4-methylthiazole (**1g**) (131 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.4, 27 mL), laccase (200 U, 10 mg, *Trametes versicolor*) and ABTS diammonium salt (13.7 mg, 0.025 mmol) were reacted for 8 h. After extraction with EtOAc (3 × 30 mL), the combined organic phases were dried over anhydrous Na₂SO₄, filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO₂ (petroleum ether / EtOAc = 5:1) to give bis(4-methylthiazolyl)-2-disulfide (**2g**) as white powder (86 mg, 66%); mp 62-63 °C (lit.,³ 61-61.5 °C); *R*_f = 0.44 (petroleum ether / EtOAc = 5:1); δ_H (300 MHz; CDCl₃) 2.43 (6H, s, 1'-H) and 6.92 (2H, s, 5-H); δ_C (75 MHz; CDCl₃) 17.24 (C-1'), 116.87 (C-5), 154.75 (C-4) and 163.81 (C-2).



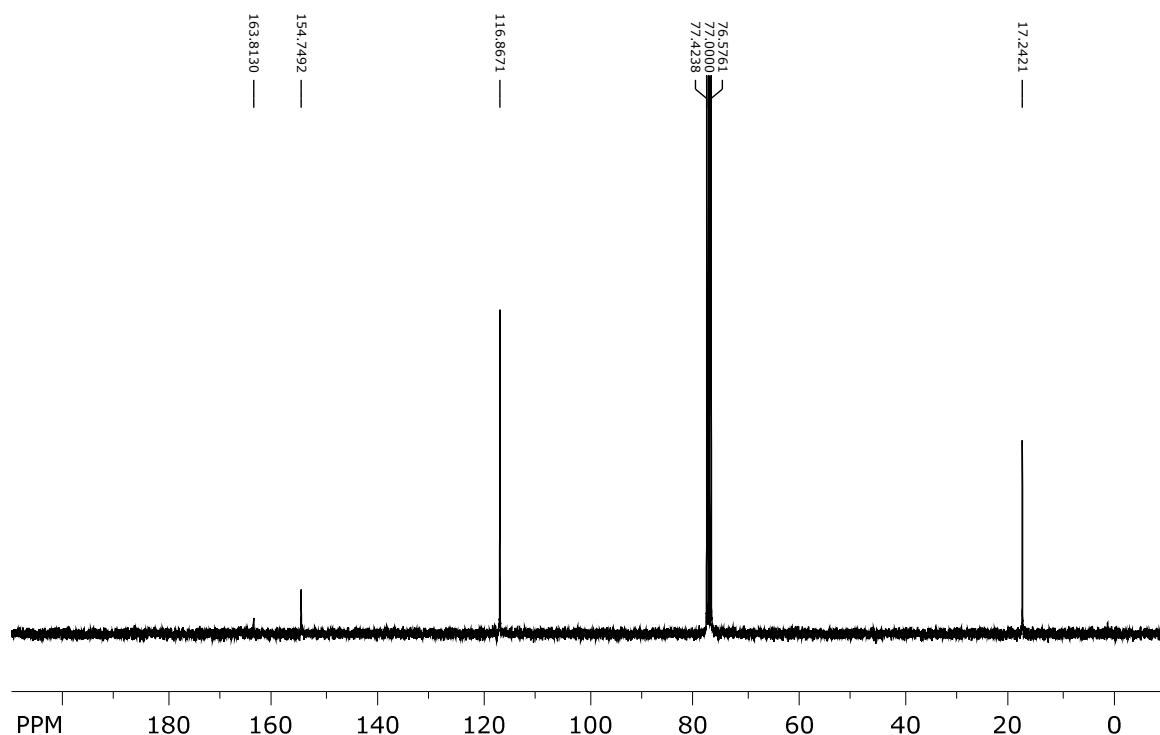


Fig. 7 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **2g** in CDCl_3

3.8. Synthesis and analytical data of bis(4-phenylthiazolyl)-2-disulfide (**2h**)

According to the general procedure, 2-mercapto-4-phenylthiazole (**1h**) (193 mg, 1 mmol), methanol (6 mL), acetate buffer (0.2 M, pH 4.4, 30 mL), laccase (200 U, 10 mg, *Trametes versicolor*) and ABTS diammonium salt (13.7 mg, 0.025 mmol) were reacted for 8 h. After extraction with EtOAc (3×30 mL), the combined organic phases were dried over anhydrous Na_2SO_4 , filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO_2 (petroleum ether / $\text{CH}_2\text{Cl}_2 = 1:1$) to give bis(4-phenylthiazolyl)-2-disulfide (**2h**) as white powder (177 mg, 92%); mp 79-81 °C; $R_f = 0.44$ (petroleum ether / $\text{CH}_2\text{Cl}_2 = 1:1$); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 3024, 2956 and 2929 (C-H), 1575, 1542, 1386 and 1152; δ_{H} (300 MHz; $\text{DMSO}-d_6$) 7.36-7.47 (6H, m, 3'-H, 4'-H and 5'-H), 7.91-7.93 (4H, m, 2'-H and 6'-H) and 8.26 (2H, s, 5-H); δ_{C} (75 MHz; $\text{DMSO}-d_6$) 118.28 (C-5), 125.98 (C-2' and C-6'), 128.54 (C-4'), 128.90 (C-3' and C-5'), 133.22 (C-1'), 155.81 (C-4) and 163.55 (C-2); m/z (EI, 70 eV) 384 (M^+ , 100%) and 320 ($\text{M}^+ - \text{S}_2$, 40); HRMS (EI, M^+) found: 383.9911 calcd for $\text{C}_{18}\text{H}_{12}\text{N}_2\text{S}_4$: 383.9883.

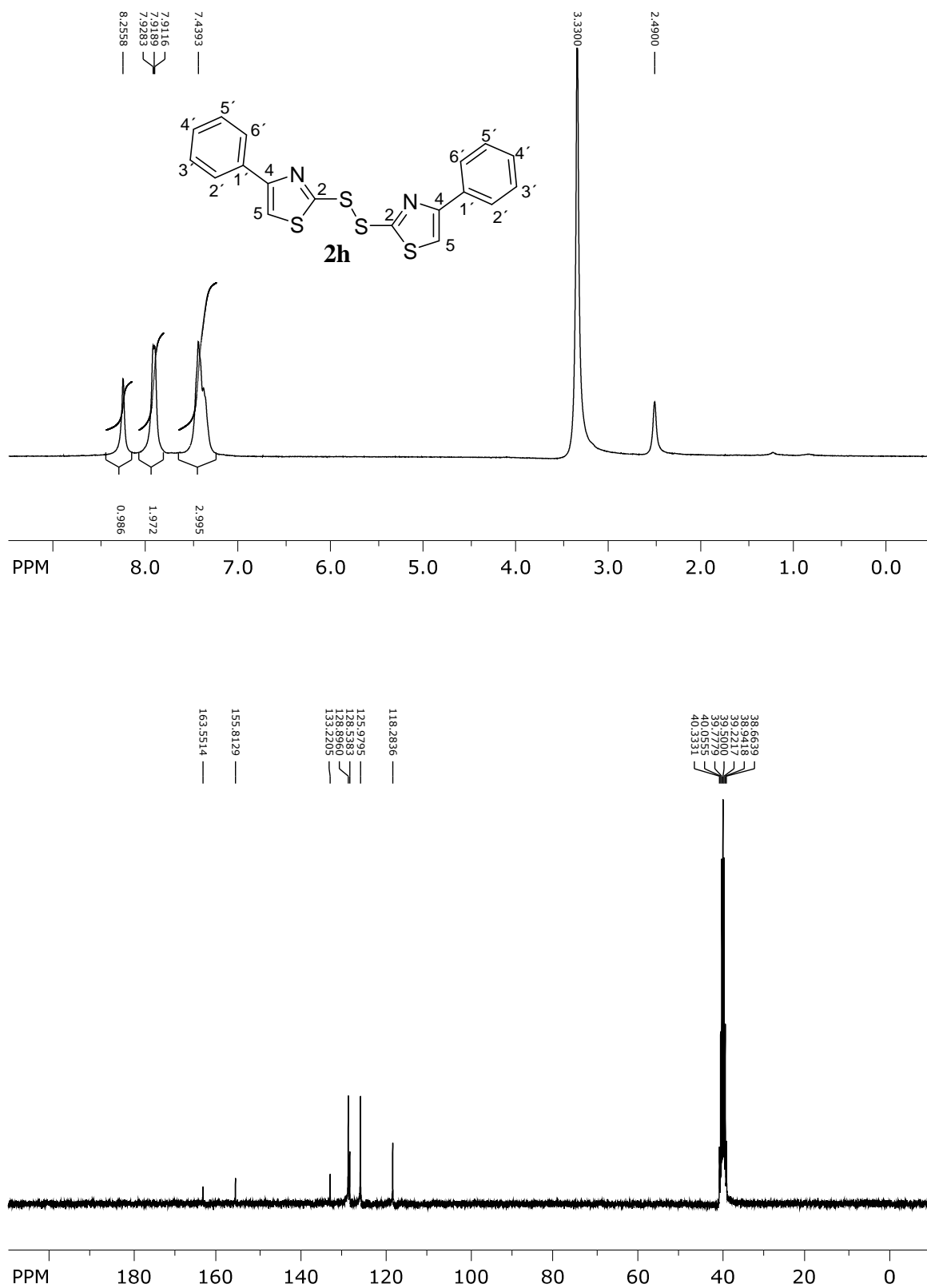
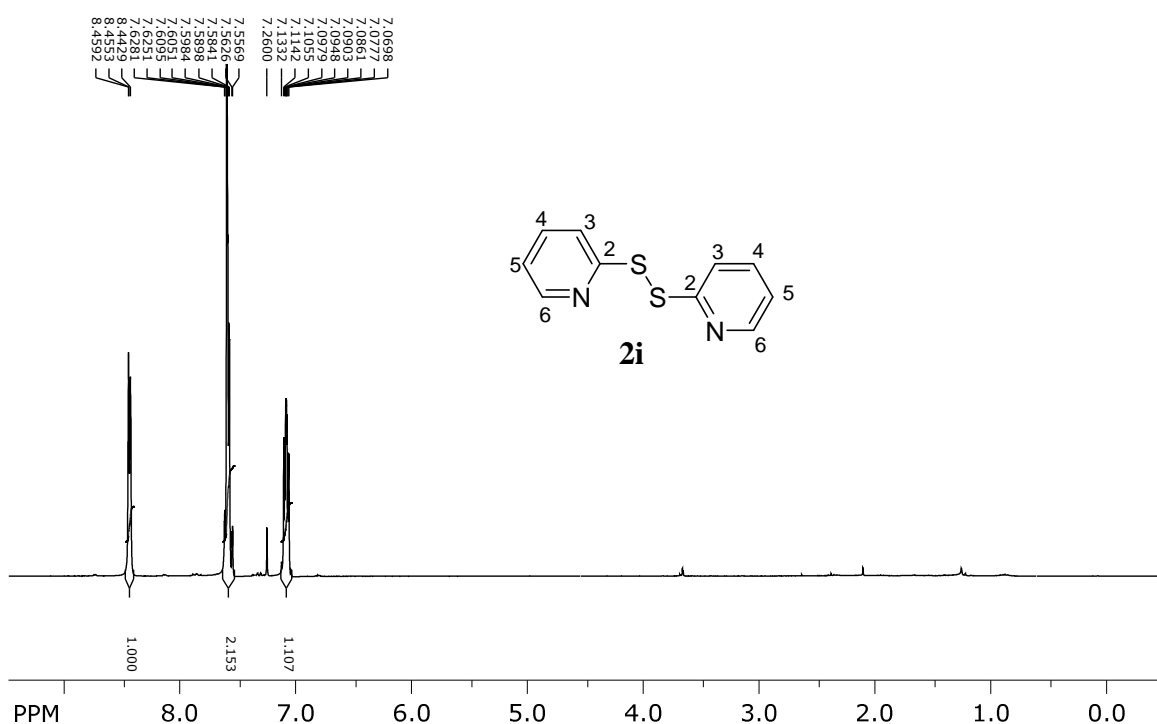


Fig. 8 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **2h** in $\text{DMSO-}d_6$

3.9. Synthesis and analytical data of bis(2-pyridinyl) disulfide (**2i**)¹

According to the general procedure, 2-mercaptopyridine (**1i**) (111 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.4, 20 mL), laccase (200 U, 10 mg, *Trametes versicolor*) and ABTS diammonium salt (13.7 mg, 0.025 mmol) were reacted for 5 h. After extraction with CH₂Cl₂ (3 × 30 mL), the combined organic phases were dried over anhydrous Na₂SO₄, filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO₂ (CH₂Cl₂ / EtOAc = 5:1) to give bis(2-pyridinyl) disulfide (**2i**) as pale yellow solid (90 mg, 82%); mp 55-57 °C (lit.,¹ 55-56 °C); *R*_f = 0.31 (petroleum ether / EtOAc = 5:1); δ_H (300 MHz; CDCl₃) 7.07-7.13 (2H, m, 5-H), 7.56-7.59 (2H, m, 4-H), 7.60-7.63 (2H, m, 3-H) and 8.46 (2H, ddd, ³*J*_{5-H,6-H} 4.8 Hz, ⁴*J*_{4-H,6-H} 1.3 Hz or 1.8 Hz, ⁵*J*_{3-H,6-H} 1.3 Hz or 1.8 Hz, 6-H); δ_C (75 MHz; CDCl₃) 119.62 (C-3), 121.05 (C-5), 137.34 (C-4), 149.51 (C-6) and 158.89 (C-2).



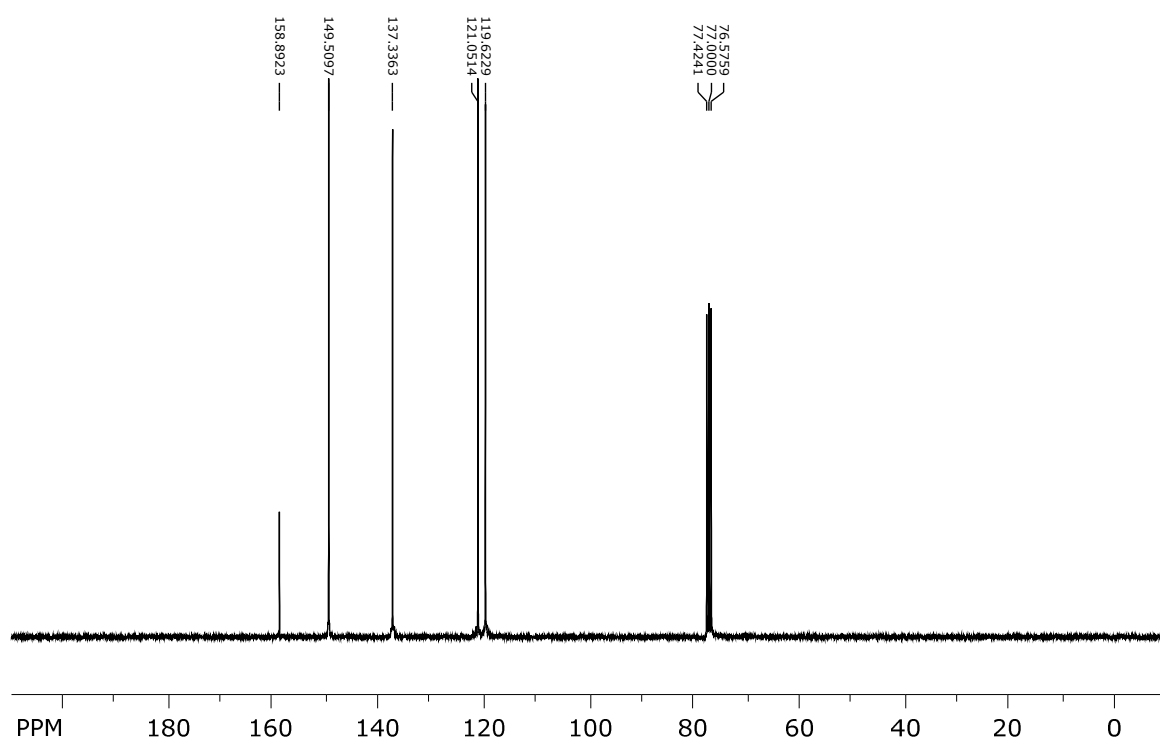


Fig. 9 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **2i** in CDCl_3

3.10. Synthesis and analytical data of bis(2-pyrimidinyl) disulfide (**2j**)¹

According to the general procedure, 2-mercaptopyrimidine (**1j**) (112 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.4, 20 mL), laccase (200 U, 10 mg, *Trametes versicolor*) and ABTS diammonium salt (13.7 mg, 0.025 mmol) were reacted for 5 h. After extraction with EtOAc (3×30 mL), the combined organic phases were dried over anhydrous Na_2SO_4 , filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO_2 (CH_2Cl_2 / EtOAc = 5:1) to give bis(2-pyrimidinyl) disulfide (**2j**) as white powder (100 mg, 90%); mp 140-142 °C (lit.,¹ 143-145 °C); R_f = 0.38 (CH_2Cl_2 / EtOAc = 5:1); δ_{H} (300 MHz; CDCl_3) 7.35 (2H, t, $^3J_{4\text{-H},5\text{-H}}$ 4.9 Hz, $^3J_{5\text{-H},6\text{-H}}$ 4.9 Hz, 5-H) and 8.70 (4H, d, $^3J_{4\text{-H},5\text{-H}}$ 4.8 Hz, $^3J_{5\text{-H},6\text{-H}}$ 4.8 Hz, 4-H and 6-H); δ_{C} (75 MHz; CDCl_3) 119.11 (C-5), 158.53 (C-4 and C-6) and 167.83 (C-2); m/z (EI, 70 eV) 222 (M^+ , 100%), 158 ($\text{M}^+ - \text{S}_2$, 97) and 112 ($\text{C}_4\text{H}_4\text{N}_2\text{S}$, 24).

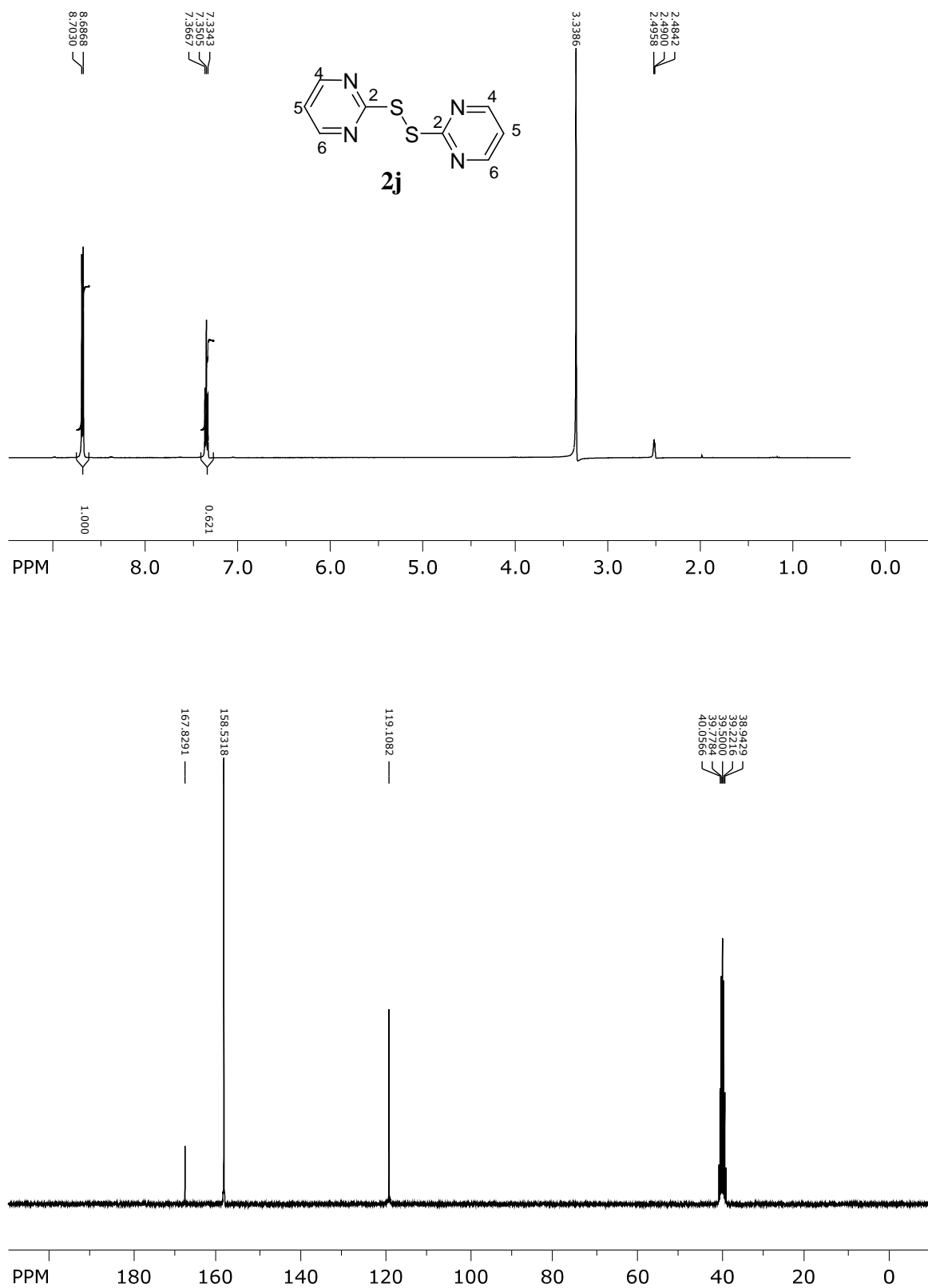
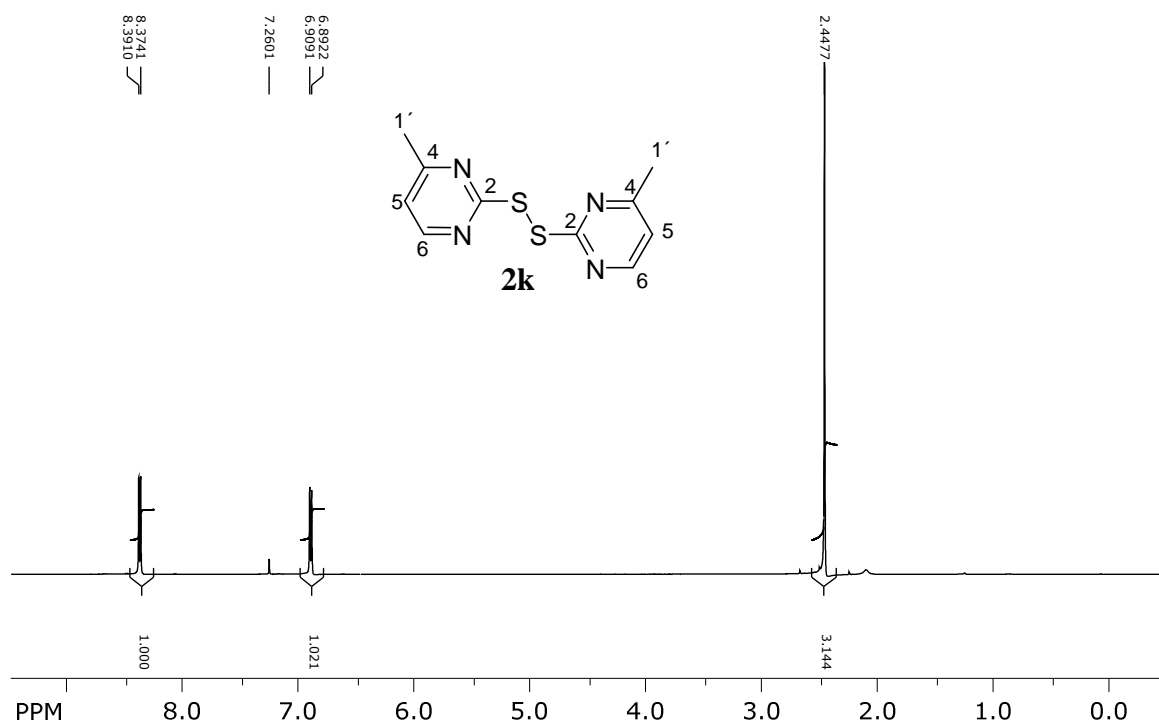


Fig. 10 ¹H (300 MHz) and ¹³C (75 MHz) NMR spectra of **2j** in DMSO-*d*₆

3.11. Synthesis and analytical data of bis(4-methyl-2-pyrimidinyl) disulfide (**2k**)⁴

According to the general procedure, 2-mercapto-4-methylpyrimidine (**1k**) (126 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.4, 20 mL), laccase (200 U, 10 mg, *Trametes versicolor*) and ABTS diammonium salt (13.7 mg, 0.025 mmol) were reacted for 5 h. After extraction with EtOAc (3 × 30 mL), the combined organic phases were dried over anhydrous Na₂SO₄, filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO₂ (CH₂Cl₂ / EtOAc = 5:1) to give bis(4-methyl-2-pyrimidinyl) disulfide (**2k**) as white powder (109 mg, 87%); mp 109-111 °C (lit.,⁴ 110 °C); *R*_f = 0.32 (CH₂Cl₂ / EtOAc = 5:1); δ_H (300 MHz; CDCl₃) 2.45 (6H, s, 1'-H), 6.90 (2H, d, ³*J*_{5-H,6-H} 5.1 Hz, 5-H) and 8.38 (2H, d, ³*J*_{5-H,6-H} 5.1 Hz, 6-H); δ_C (75 MHz; CDCl₃) 23.97 (C-1'), 117.76 (C-5), 157.37 (C-6), 168.21 (C-4) and 169.18 (C-2); *m/z* (EI, 70 eV) 250 (M⁺, 100%), 217 (M⁺ - HS, 5), 126 (C₅H₆N₂S, 11) and 93 (22).



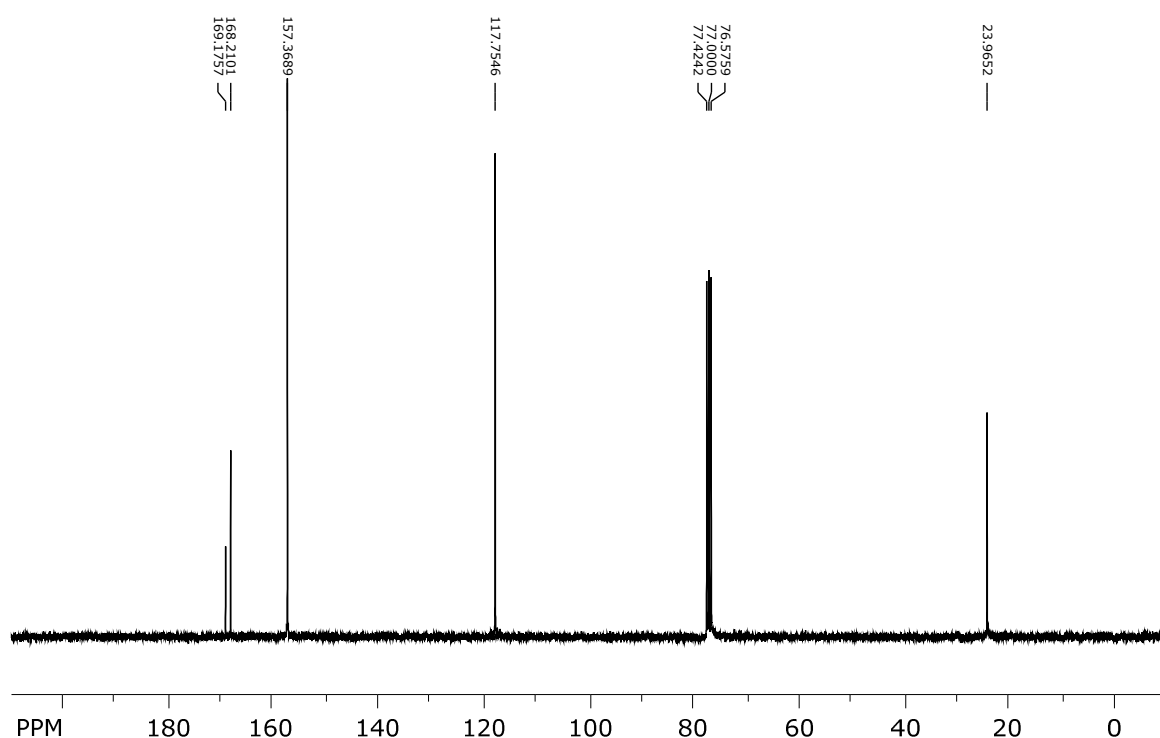


Fig. 11 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **2k** in CDCl_3

3.12. Synthesis and analytical data of bis(5-propyl-2-pyrimidinyl) disulfide (**2l**)

According to the general procedure, 2-mercapto-5-propylpyrimidine (**1l**) (154 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.4, 20 mL), laccase (200 U, 10 mg, *Trametes versicolor*) and ABTS diammonium salt (13.7 mg, 0.025 mmol) were reacted for 6 h. After extraction with EtOAc (3×30 mL), the combined organic phases were dried over anhydrous Na_2SO_4 , filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO_2 (CH_2Cl_2 / EtOAc = 10:1) to give bis(5-propyl-2-pyrimidinyl) disulfide (**2l**) as white powder (130 mg, 85%); mp 71-73 °C; R_f = 0.58 (CH_2Cl_2 / EtOAc = 10:1); λ_{max} (MeCN)/nm 273 (log ϵ , 4.43) and 243 (4.46); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 2959 (C-H), 1575, 1541 and 1383; δ_{H} (300 MHz; CDCl_3) 0.92 (6H, t, $^3J_{2'-\text{H},3'-\text{H}}$ 7.4 Hz, 3'-H), 1.60 (4H, sex, $^3J_{1-\text{H},2'-\text{H}}$ 7.3 Hz, $^3J_{2'-\text{H},3'-\text{H}}$ 7.3 Hz, 2'-H), 2.50 (4H, t, $^3J_{1'-\text{H},2'-\text{H}}$ 7.8 Hz, 1'-H) and 8.39 (4H, s, 4-H and 6-H); δ_{C} (75 MHz; CDCl_3) 13.43 (C-3'), 23.83 (C-2'), 31.74 (C-1'), 131.96 (C-5), 157.77 (C-4 and C-6) and 166.86 (C-2); m/z (EI, 70 eV) 306 (M^+ , 100%), 242 ($\text{M}^+ - \text{S}_2$, 32) and 154 ($\text{C}_7\text{H}_{10}\text{N}_2\text{S}$, 20); HRMS (EI, M^+) found: 306.0972 calcd for $\text{C}_{14}\text{H}_{18}\text{N}_4\text{S}_2$: 306.0973.

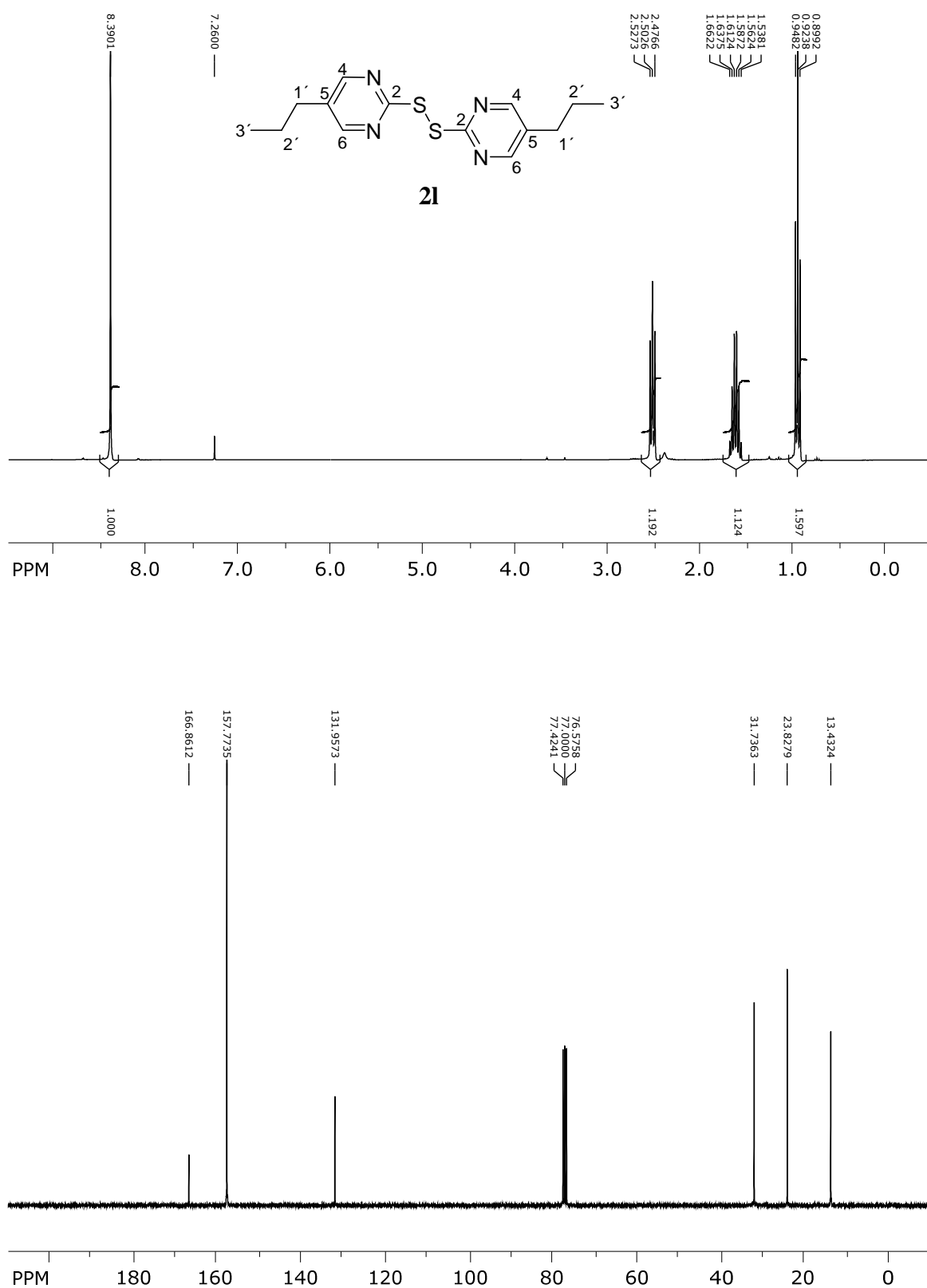
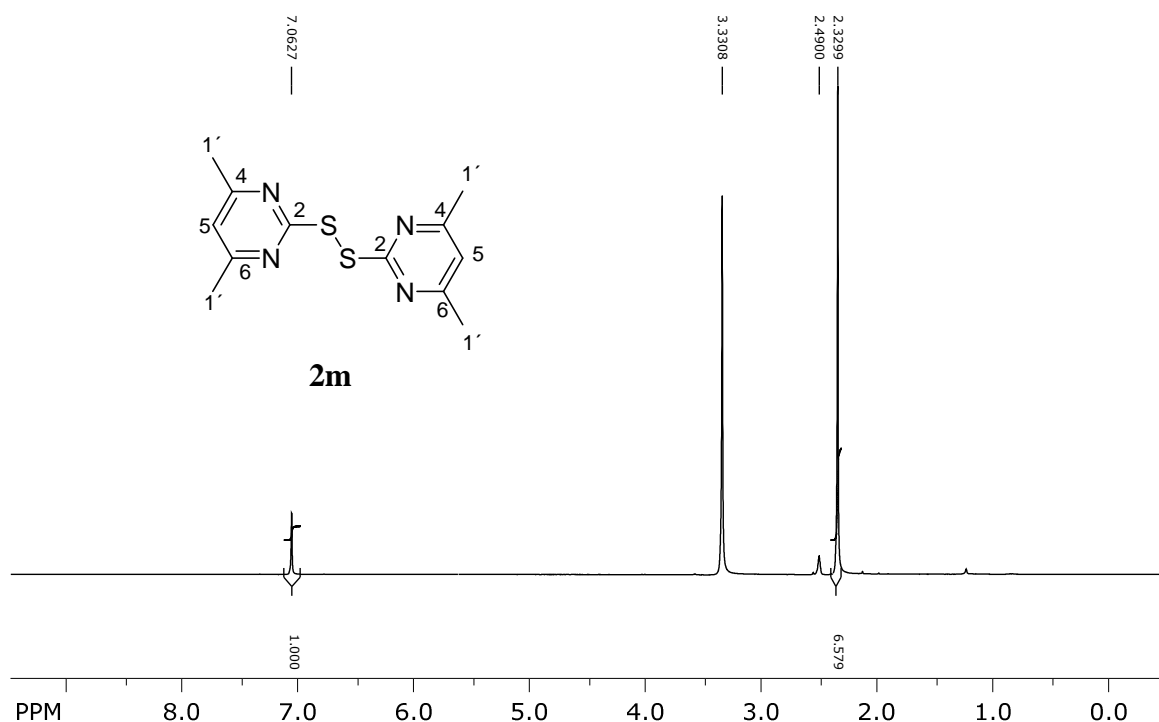


Fig. 12 ¹H (300 MHz) and ¹³C (75 MHz) NMR spectra of **2I** in CDCl₃

3.13. Synthesis and analytical data of bis(4,6-dimethyl-2-pyrimidinyl) disulfide (**2m**)⁵

According to the general procedure, 4,6-dimethyl-2-mercaptopyrimidine (**1m**) (140 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.4, 20 mL), laccase (200 U, 10 mg, *Trametes versicolor*) and ABTS diammonium salt (13.7 mg, 0.025 mmol) were reacted for 8 h. After extraction with EtOAc (3 × 30 mL), the combined organic phases were dried over anhydrous Na₂SO₄, filtered and evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO₂ (CH₂Cl₂ / EtOAc = 5:1) to give bis(4,6-dimethyl-2-pyrimidinyl) disulfide (**2m**) as white powder (132 mg, 95%).

When 1 mmol **1m** was reacted in methanol (1 mL) and acetate buffer (0.2 M, pH 4.4, 10 mL) with laccase (200 U, 10 mg, *Trametes versicolor*) and ABTS diammonium salt (13.7 mg, 0.025 mmol) for 8 h and the crude product was extracted with a) 3 × 3 mL, b) 3 × 5 mL and c) 3 × 10 mL EtOAc, compound **2m** was isolated in a) 94%, b) 96% and c) 95% yield after flash chromatography on SiO₂ (CH₂Cl₂ / EtOAc = 5:1); mp 166-168 °C (lit.,⁵ 167-169 °C); *R*_f = 0.65 (CH₂Cl₂ / EtOAc = 5:1); δ_H (300 MHz; DMSO-*d*₆) 2.33 (12H, s, 1'-H) and 7.06 (2H, s, 5-H); δ_C (75 MHz; DMSO-*d*₆) 23.35 (C-1'), 117.66 (C-5), 167.13 (C-2) and 167.81 (C-4 and C-6); *m/z* (EI, 70 eV) 278 (M⁺, 92%), 244 (56), 212 (84), 140 (100) and 112 (60).



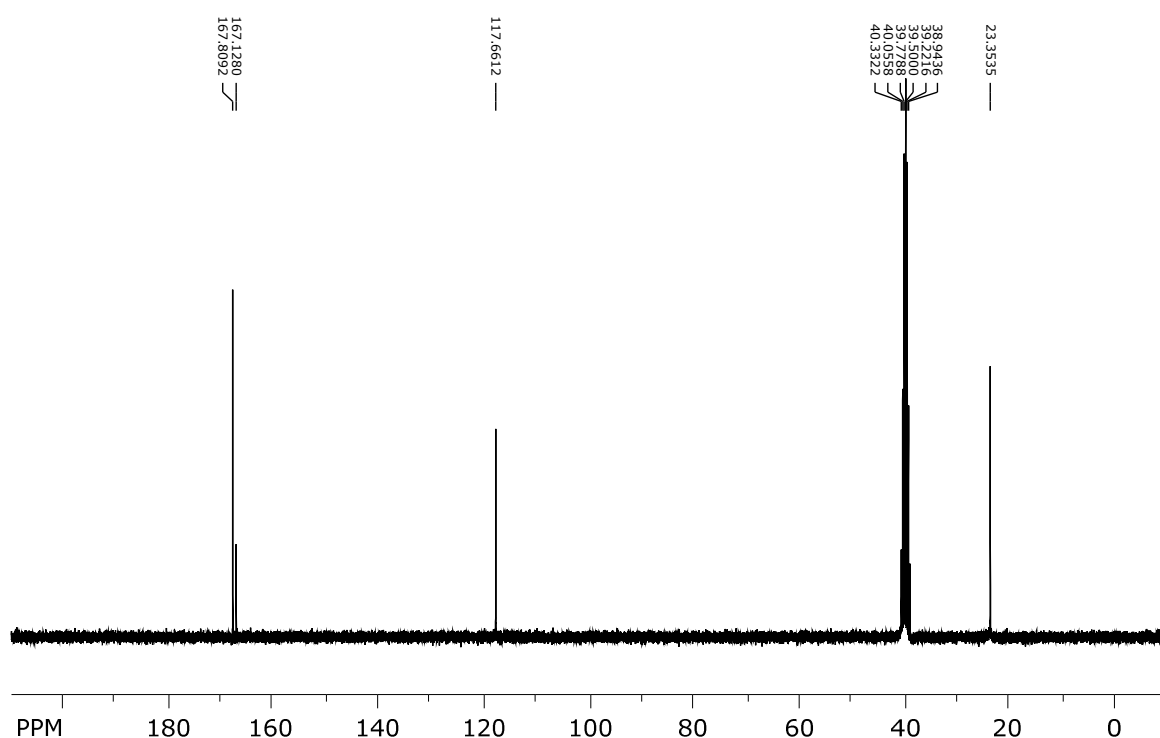


Fig. 13 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **2m** in $\text{DMSO-}d_6$

4. Computational studies of compounds **2a**, **2c** and **2f**

All the calculations reported in this paper were performed within Density Functional Theory, using the Gaussian 03 package.⁶ ^{13}C NMR chemical shifts of selected compounds **2a**, **2c** and **2f** were calculated as follows: the structures were optimized with the MM2 force field implemented in Chem3D Pro.⁷ In the second step, the optimized structures were subsequently reoptimized at the UFF, AM1 level followed by the RHF/3-21G level and finally by B3LYP/6-31G(d) level of theory within the Gaussian 03 package. In the final step, the ^{13}C NMR chemical shielding of the reoptimized geometries were computed once at the mPW1PW91/6-311+G(2d,p)//mPW1PW91/6-31G(d) level of theory in the gas phase.⁶ The references TMS and benzene for the MSTD approach according to Sarotti and Pellegrinet⁸ were computed in the same manner as for **2a**, **2c** and **2f**. For comparison with the experimental ^{13}C NMR chemical shifts the computationally derived ^{13}C NMR chemical shifts were calculated as follows:

$$\delta_a = \sigma_{\text{ref gas phase}} - \sigma_a \text{ gas phase} + \delta_{\text{ref}}$$

where σ_{ref} and σ_a are the calculated NMR isotropic magnetic shielding tensors of the reference compound and carbon a of the compound of interest: $\sigma_{\text{TMS}} = 185.81$ ppm and

$\sigma_{\text{benzene}} = 54.41$ ppm at the mPW1PW91/6-311+G(2d,p)// mPW1PW91/6-31G(d) level gas phase; δ_{ref} represents the chemical shift of the reference compound in deuterated DMSO: $\delta_{\text{TMS}} = 0$ ppm; $\delta_{\text{benzene}} = 128.27$ ppm. An HP Compaq with a 2.39 GHz processor and 2 GB RAM was used for the calculations.

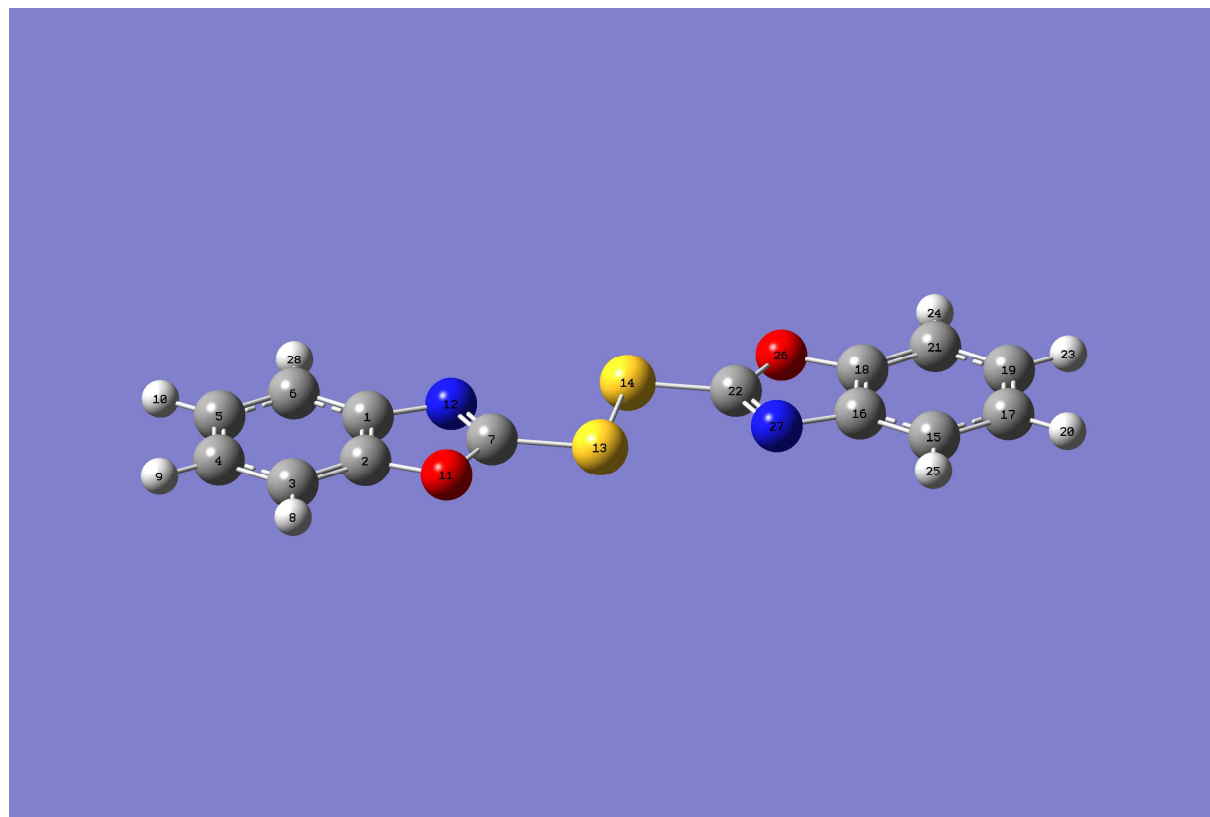


Fig. 14 3D structure of **2a**

4.1. Cartesian of 2a

Row	Symbol	X	Y	Z
1	C	-3.8899810	0.6172160	0.0000250
2	C	-4.1764250	-0.7495250	-0.0000140
3	C	-5.4535860	-1.2743820	-0.0000270
4	C	-6.4852440	-0.3369730	0.0000030
5	C	-6.2270130	1.0407840	0.0000420
6	C	-4.9294790	1.5428570	0.0000540
7	C	-2.0586290	-0.4223890	-0.0000080
8	H	-5.6388040	-2.3416450	-0.0000570
9	H	-7.5127440	-0.6844200	-0.0000050
10	H	-7.0630060	1.7321210	0.0000640
11	O	-2.9782730	-1.4221400	-0.0000360
12	N	-2.5063680	0.7856320	0.0000260
13	S	-0.4002870	-0.9718020	-0.0000230
14	S	0.4002870	0.9718020	-0.0000270
15	C	4.9294790	-1.5428570	0.0000580
16	C	3.8899810	-0.6172160	0.0000260

17	C	6.2270130	-1.0407840	0.0000440
18	C	4.1764250	0.7495250	-0.0000180
19	C	6.4852440	0.3369730	0.0000000
20	H	7.0630060	-1.7321210	0.0000680
21	C	5.4535860	1.2743820	-0.0000330
22	C	2.0586290	0.4223890	-0.0000120
23	H	7.5127440	0.6844200	-0.0000100
24	H	5.6388040	2.3416450	-0.0000670
25	H	4.7263870	-2.6073000	0.0000920
26	O	2.9782730	1.4221400	-0.0000420
27	N	2.5063680	-0.7856320	0.0000290
28	H	-4.7263870	2.6073000	0.0000840

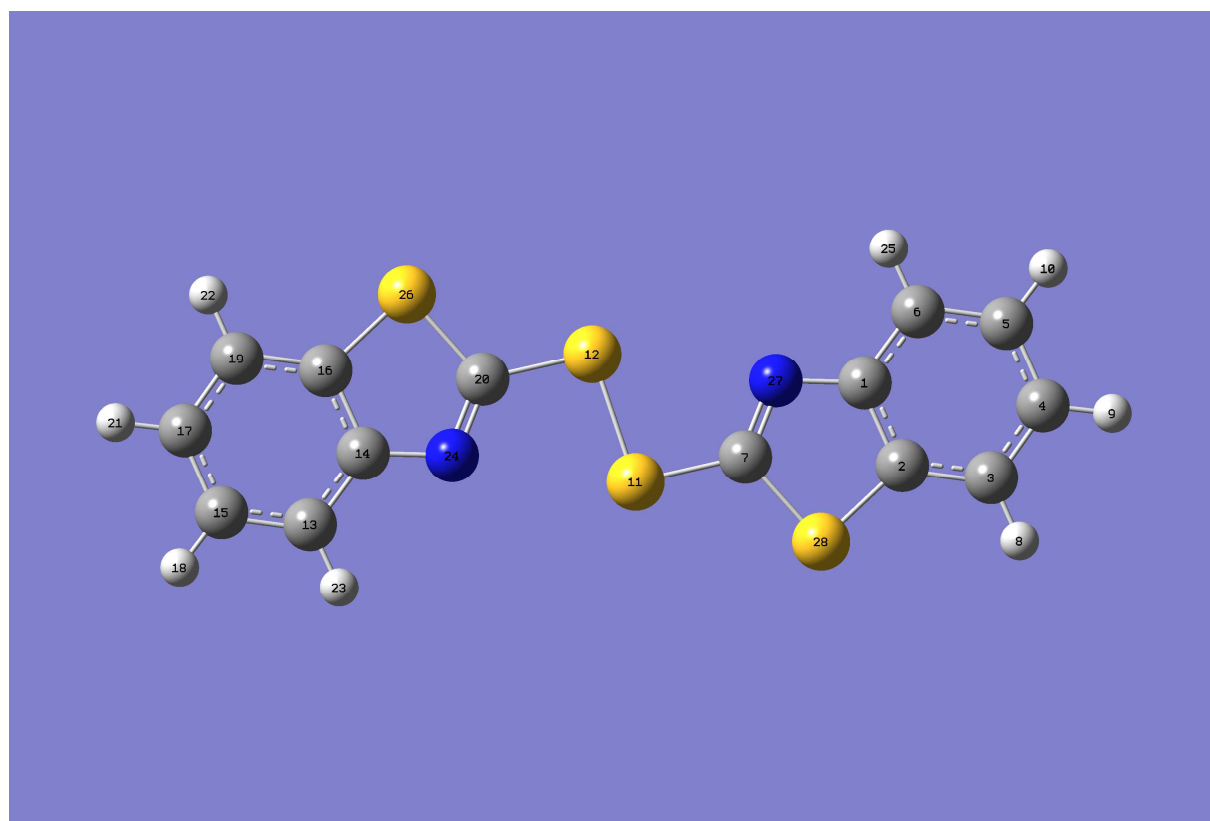


Fig. 15 3D structure of **2c**

4.2. Cartesian of **2c**

Row	Symbol	X	Y	Z
1	C	3.8918510	0.6066700	0.0003040
2	C	4.4792050	-0.6755270	0.0003600
3	C	5.8604740	-0.8452800	0.0004610
4	C	6.6556770	0.2940300	0.0005040
5	C	6.0848170	1.5728820	0.0004490
6	C	4.7086140	1.7400370	0.0003500
7	C	2.0414960	-0.5787340	0.0001610
8	H	6.3056820	-1.8339270	0.0005090
9	H	7.7352940	0.1876330	0.0005850

10	H	6.7297460	2.4451810	0.0004850
11	S	0.3406400	-0.9954270	0.0000560
12	S	-0.3406400	0.9954270	-0.0000560
13	C	-4.7086140	-1.7400370	-0.0003500
14	C	-3.8918510	-0.6066700	-0.0003040
15	C	-6.0848170	-1.5728820	-0.0004490
16	C	-4.4792050	0.6755270	-0.0003600
17	C	-6.6556770	-0.2940300	-0.0005040
18	H	-6.7297460	-2.4451810	-0.0004850
19	C	-5.8604740	0.8452800	-0.0004610
20	C	-2.0414960	0.5787340	-0.0001610
21	H	-7.7352940	-0.1876330	-0.0005850
22	H	-6.3056820	1.8339270	-0.0005090
23	H	-4.2523680	-2.7232600	-0.0003050
24	N	-2.5093990	-0.6215080	-0.0001990
25	H	4.2523680	2.7232600	0.0003050
26	S	-3.2239580	1.8906090	-0.0003030
27	N	2.5093990	0.6215080	0.0001990
28	S	3.2239580	-1.8906090	0.0003030

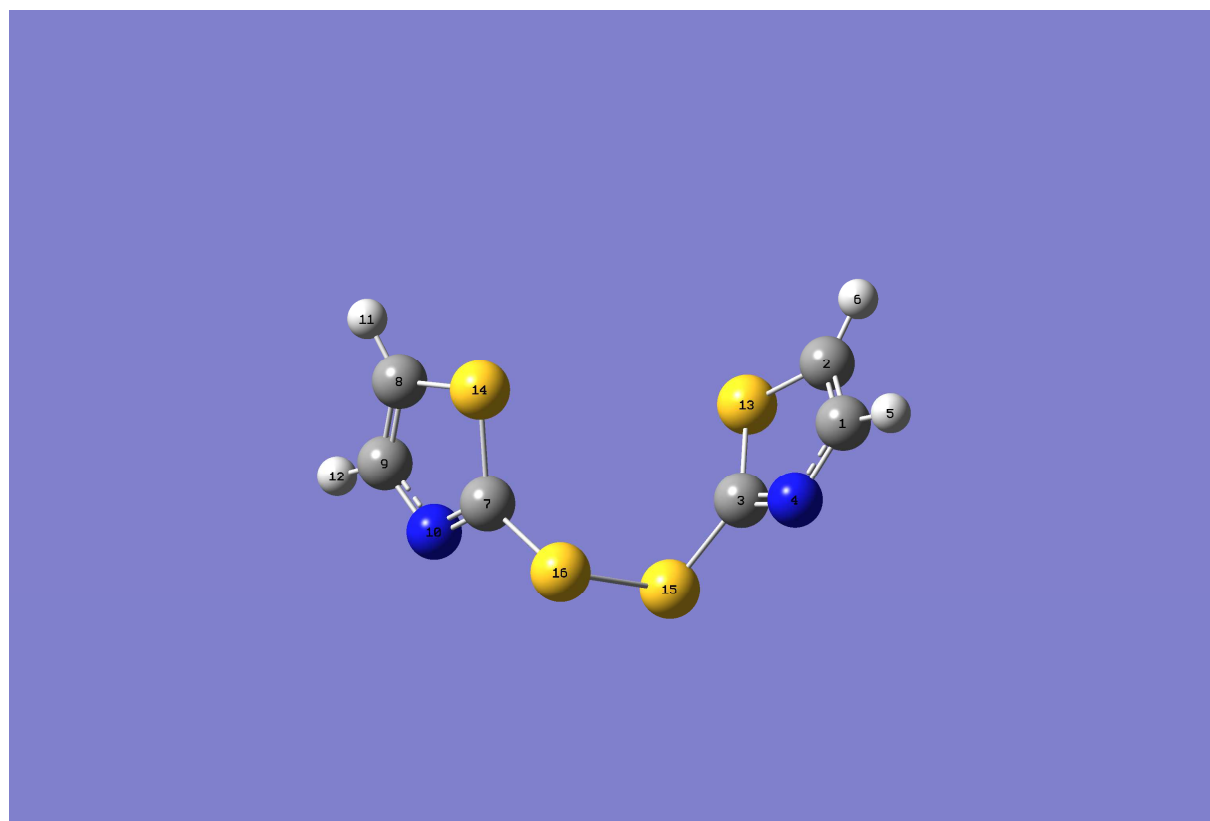


Fig. 16 3D structure of **2f**

4.3. Cartesian of **2f**

Row	Symbol	X	Y	Z
1	C	3.5820570	-0.4600380	-0.8260130
2	C	3.2398290	-1.5452280	-0.0659100

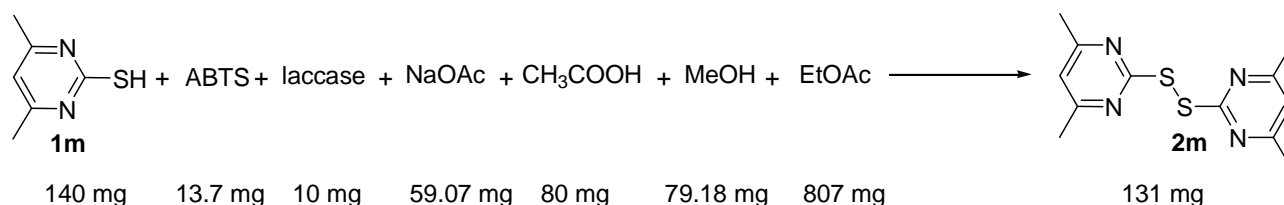
3	C	1.8857750	0.4447360	0.2268400
4	N	2.8215120	0.6566200	-0.6538050
5	H	4.4041910	-0.4346450	-1.5302840
6	H	3.7139440	-2.5157100	-0.0331280
7	C	-1.8857760	0.4447360	-0.2268420
8	C	-3.2398330	-1.5452260	0.0659040
9	C	-3.5820580	-0.4600390	0.8260120
10	N	-2.8215110	0.6566190	0.6538060
11	H	-3.7139510	-2.5157080	0.0331170
12	H	-4.4041920	-0.4346470	1.5302820
13	S	1.8870560	-1.1623720	0.9105350
14	S	-1.8870510	-1.1623740	-0.9105300
15	S	0.7155490	1.6446980	0.7644130
16	S	-0.7155510	1.6446980	-0.7644130

5. The greenness of the laccase-catalyzed oxidative coupling of thiols **1** to disulfides **2** according to the twelve principles of green chemistry⁹

Principle 1 - waste prevention instead of remediation.

This reaction is a highly selective enzyme-catalyzed process that allows for a substantial reduction of waste. The oxidative dimerization of thiols **1** delivers analytically pure disulfides **2** in up to 95% yield. No toxic byproducts are formed. Molecular oxygen as the oxidant is converted into nontoxic water. Using the oxidative coupling of **1m** to **2m** as an example, the E-factor^{9c,d,10} (kg waste per kg product) of the overall process (under the assumption that 10% of the solvent used was lost) amounts to **8.08 kg kg⁻¹**. This value compares well with the E-factors of other synthetic methods for the synthesis of disulfides.

Calculation of the E-factor of the oxidative dimerization of **1m** to **2m** in a mixture of 10 mL acetate buffer and 1 mL MeOH using 3 × 3 mL of EtOAc for work up. The isolated yield of pure **2m** was 94%: (For details see page 21).



Total amount of the reactants (taking into account a loss of 10% of the solvent used) = 140 mg + 13.7 mg + 10 mg + 59.07 mg + 80 mg + 79.18 mg + 807 mg = 1188.95 mg.

Amount of the final product = 131 mg.

Amount of waste = 1188.95 - 131 = 1057.95 mg

E-factor = Amount of waste [kg]/Amount of product [kg] = 1057.95/131 = **8.08 kg kg⁻¹**.

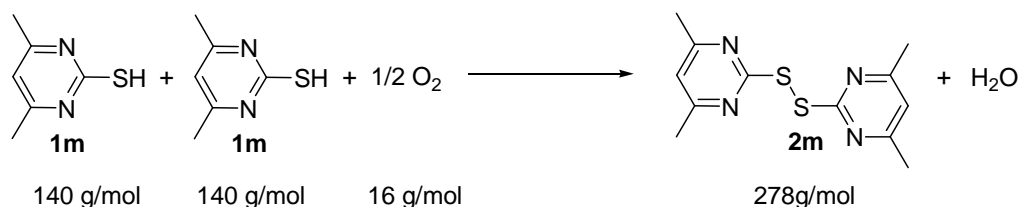
Principle 2 - atom economy.

The atom economy¹¹ of the process is very high; it amounts to **94%**.

The atom economy of the reaction was calculated according to the following equation:

$$\% \text{ Atom economy} = 100 \times \frac{\text{Molecular weight of the desired product}}{\text{Molecular weight of all reactants}}$$

Calculation of the atom economy of the oxidative dimerization of **1m** to **2m**.



Atom economy = $100 \times 278/296 = \mathbf{94\%}$

Principle 3 - less hazardous/toxic chemicals.

In comparison to most of the developed procedures, our procedure avoids the use of any toxic reagents like hazardous heavy metal catalysts or oxidants. Instead totally safe laccase was used as catalyst.

Principle 4 - Safer products by design.

Does not apply to the developed method.

Principle 5 - Innocuous solvents and auxiliaries.

Acetate buffer was always used as the solvent and methanol as a cosolvent. Both are safe and environmentally preferred solvents. In addition, we used ethyl acetate which is a preferred green solvent in nearly all cases for the extraction of the product.

Principle 6 - Energy efficient by design.

The laccase-catalyzed oxidative coupling was carried out at room temperature, under air at atmospheric pressure and at pH 4.4.

Principle 7 - Preferable renewable raw materials.

The laccase is isolated from renewable raw materials and is completely biodegradable. The same holds true for the acetic acid of the acetate buffer. We assume that the buffer waste from the reaction is suitable for biotreatment.

Principle 8 - Shorter syntheses (avoid derivatization).

Does not apply to the developed reaction.

Principle 9 - Catalytic rather than stoichiometric reagents.

The laccase-catalyzed oxidative coupling of thiols using aerial oxygen as an oxidant is a highly efficient biocatalytic transformation. It is characterized by high turnover numbers. Using the dimerization of **1m** to **2m** as an example, the TON of the process amounts to **9024**. This value confirms the high catalytic efficiency of the process. The turnover frequencies of the process are also high; in the above mentioned example the TOF is **1128 h⁻¹**. These values compare well with TONs and TOFs of other synthetic methods for the synthesis of disulfides.

Principle 11 - Analytical methodologies for pollution prevention and

Principle 12 - Inherently safer processes.

The reactions are run at room temperature, under aerial oxygen, at atmospheric pressure in an aqueous system at pH 4.4. No toxic byproducts are produced during the reaction, the only byproduct formed is water. The process developed is inherently safe and there is no need for analytical methodologies for pollution prevention.

6. Calculation of TON, TOF and STY for the oxidative coupling of 1m to 2m

Yield of **2m** = 94% (for details see page 21).

6.1. Calculation of TON

Molecular weight of laccase from *Trametes versicolor* = 96 000 g/mol.

Specific activity of the laccase = 20 U/mg.

200 U Laccase corresponds to 10 mg, ie 1.0417×10^{-7} mol = 1.0417×10^{-4} mmol.

TON = Amount of the substrate consumed [mmol] / Amount of catalyst [mmol].

TON for the oxidative coupling of **1m** = $0.94 / 1.0417 \times 10^{-4} = \mathbf{9024}$.

6.2. Calculation of TOF

$$\text{TOF} = \frac{\text{TON}}{\text{Time}}$$

TOF for **1m** = $9024 / 8 \text{ h} = \mathbf{1128 \text{ h}^{-1}}$.

6.3. Calculation of the space time yield

STY = Desired product quantity [Mol] / Volume [L] × Time [h].

STY = $0.00047 \text{ [mol]} / 0.011 \text{ [L]} \times 8 \text{ [h]} = \mathbf{0.005 \text{ Mol} \times \text{L}^{-1} \times \text{h}^{-1}}$.

7. Determination the activity of laccase from *Trametes versicolor*¹²

A 0.1 M solution of ABTS (0.3 mL) in 0.2 M acetate buffer (pH 4.4) was diluted with 0.2 M acetate buffer (2.6 mL, pH 4.4) and treated with a solution of laccase in the same buffer (0.1 mL). The change in absorption was followed *via* UV-Vis spectroscopy ($\lambda = 414 \text{ nm}$). One unit was defined as the amount of laccase (*Trametes versicolor*) that converts 1 μmol of ABTS per minute at pH 4.4 at rt.

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