

Laccase-catalyzed oxidation of Hantzsch 1,4-dihydropyridines to pyridines and a new one pot synthesis of pyridines

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Electronic Supplementary Information

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1. General remarks

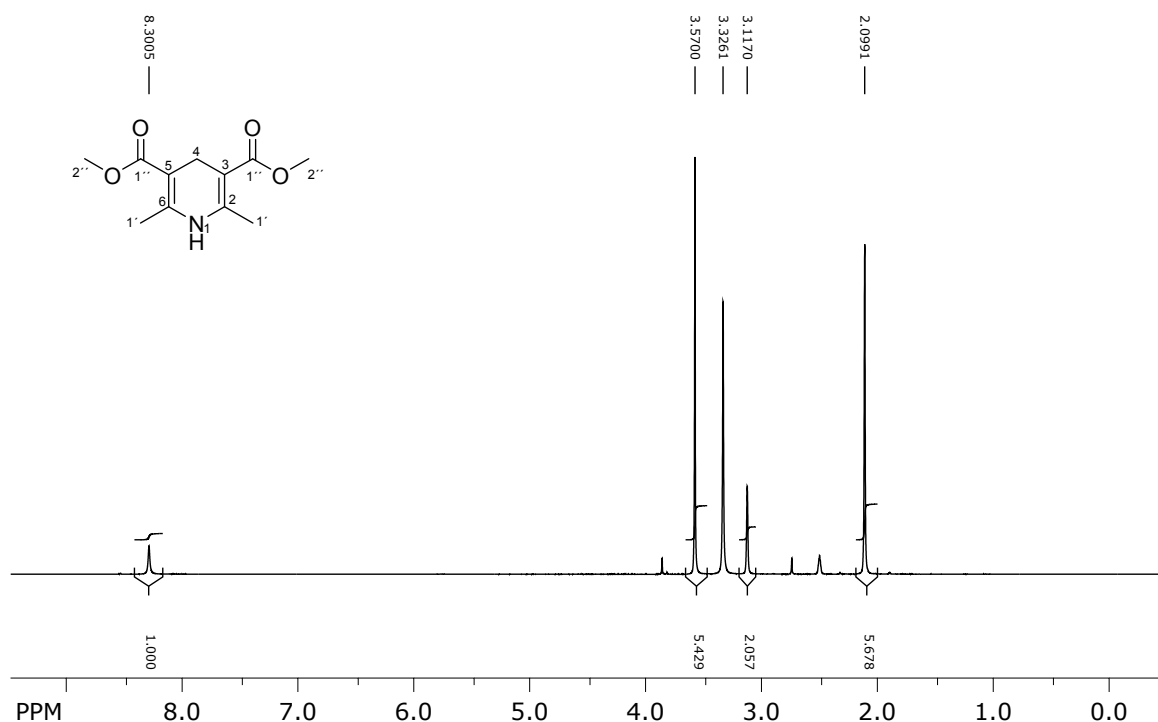
All chemicals and the laccase from *Trametes versicolor* (Fluka) were purchased from commercial suppliers. Solvents used in extraction and purification were distilled prior to use. Aldehydes and 1,3-dicarbonyl compounds used in the reactions were freshly distilled before use. The pH of the buffer was adjusted using a pH 330/SET-1 pH-meter. Reaction temperatures are reported as bath temperatures. 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 ethanolic vanillin solution followed by heating. Flash chromatography was carried out on silica gel MN 60, 0.04-0.053 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 CDCl₃ or DMSO-*d*₆. The chemical shifts were referenced to the solvent signals at $\delta_{H/C}$ 2.49 / 39.50 ppm (DMSO-*d*₆) and 7.26 / 77.00 ppm (CDCl₃) relative to TMS as internal standards. 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), sep (septet) 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 I for the synthesis of Hantzsch 1,4-dihydropyridines **4a-k**¹

An oven-dried 10 mL vial with a magnetic stirrer bar was charged with a mixture of an aldehyde **1** (1 mmol), a 1,3-dicarbonyl compound **2** (2 mmol) and ammonium acetate (**3**) (1.5 mmol). The vial was sealed and the reaction mixture was stirred at 80 °C for the time given. After completion of the reaction, the solid crude product was isolated upon addition of ice-cold water (10 mL) followed by scratching. The precipitate was filtered and further purified by recrystallization from methanol to yield the 1,4-dihydropyridines **4a-k**.

3. Synthesis and analytical data of 1,4-dihydropyridines 4a-k

3.1. Synthesis and analytical data of dimethyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (4a). According to the general procedure I, paraformaldehyde (**1a**) (30 mg, 1 mmol), methyl acetoacetate (**2a**) (232 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 10 min. Purification afforded dimethyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4a**) (160 mg, 71%) as canary yellow powder; mp 210 - 212 °C (from MeOH) (lit.,^{2,3} 208 - 210 °C); $R_f = 0.29$ (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\max}$ (atr)/ cm^{-1} 3350 (NH), 2982, 2962 and 2873 (C-H), 1698 and 1647 (C=O), 1626 (C=C), 1203 and 1073 (C-O); δ_{H} (300 MHz; DMSO- d_6) 2.10 (6H, s, 1'-H), 3.12 (2H, s, 4-H), 3.57 (6H, s, 2''-H) and 8.30 (1H, s, 1-H); δ_{C} (75 MHz; DMSO- d_6) 17.80 (C-1'), 24.78 (C-4), 50.60 and 50.62 (C-2''), 96.88 (C-3 and C-5), 146.77 (C-2 and C-6) and 167.45 (C-1''), m/z (EI, 70 eV) 225 (M^+ , 23%), 224 (50, $\text{M}^+ - 1$), 210 (100, $\text{M}^+ - \text{CH}_3$) and 194 (33).



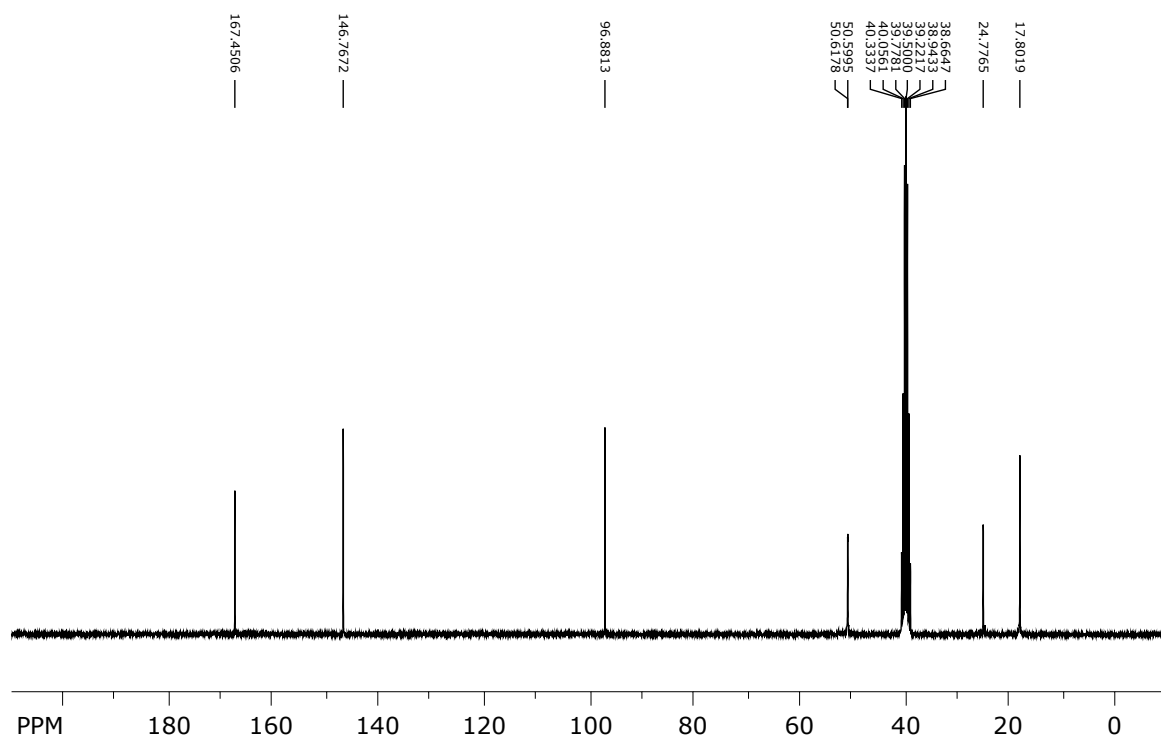


Fig. 1 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **4a** in $\text{DMSO-}d_6$.

3.2. Synthesis and analytical data of dimethyl-1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate (4b). According to the general procedure I, acetaldehyde (**1b**) (66 mg, 1.5 mmol), methyl acetoacetate (**2a**) (232 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 30 min. Purification afforded dimethyl-1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate (**4b**) (180 mg, 75%) as white crystals; mp 153 - 155 °C (from MeOH) (lit.,^{2,3} 152 - 154 °C); R_f = 0.41 (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\text{max}}(\text{atr})/\text{cm}^{-1}$ 3340 (NH), 2950 (C-H), 1677 and 1647 (C=O), 1630 (C=C), 1222 and 1054 (C-O); δ_{H} (300 MHz; CDCl_3) 0.95 (3H, d, $^3J_{1'''\text{-H}}$, $^3J_{4\text{-H}}$ 6.6 Hz, 1'''-H), 2.27 (6H, s, 1'-H), 3.71 (6H, s, 2''-H), 3.80 (1H, q, $^3J_{4\text{-H}, 1'''\text{-H}}$ 6.6 Hz, 4-H) and 5.65 (1H, s, 1-H); δ_{C} (75 MHz; CDCl_3) 19.40 (C-1'), 22.25 (C-1'''), 28.41 (C-4), 50.94 and 50.95 (C-2''), 104.41 (C-3 and C-5), 144.63 (C-2 and C-6) and 168.23 (C-1''); m/z (EI, 70 eV) 239 (M^+ , 2%), 224 (100, $\text{M}^+ - \text{CH}_3$), 208 (37, $\text{M}^+ - \text{OCH}_3$) and 192 (17).

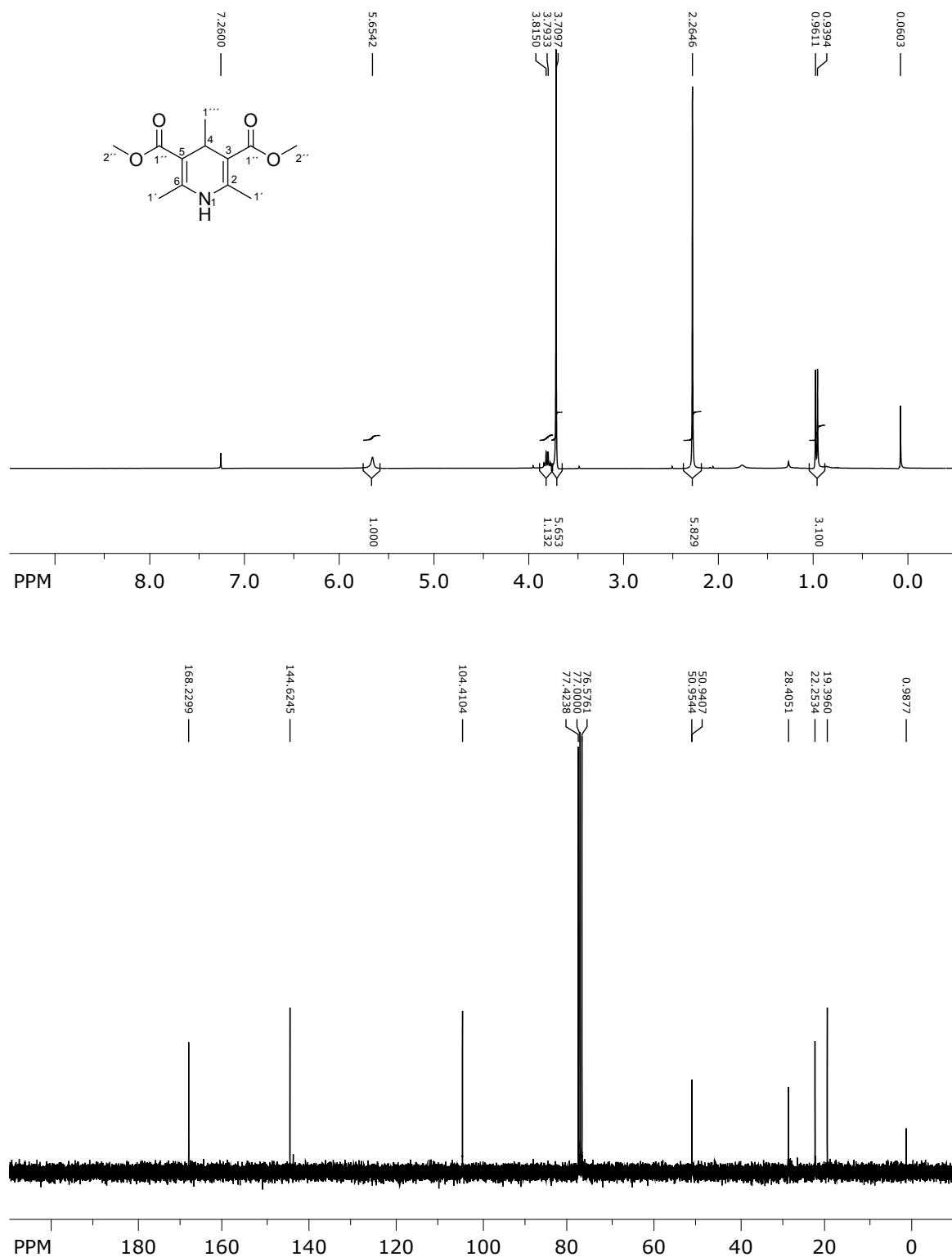
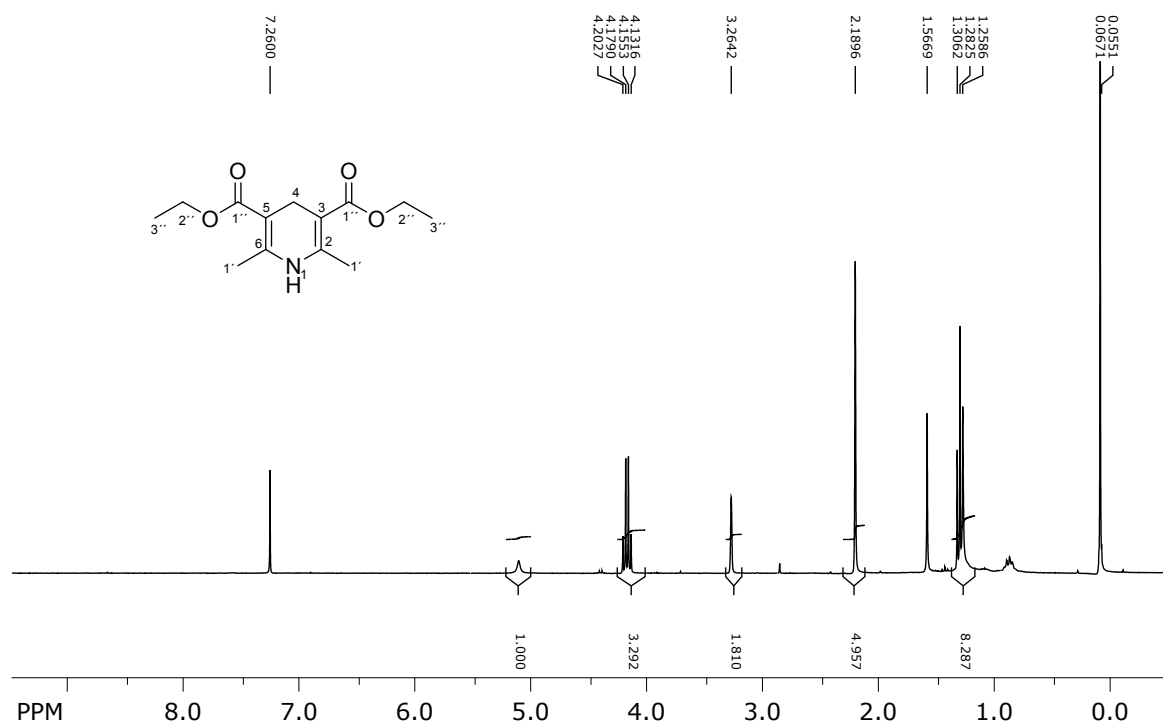


Fig. 2 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **4b** in CDCl_3 .

3.3. Synthesis and analytical data of diethyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (4c). According to the general procedure I, paraformaldehyde (**1a**) (30 mg, 1 mmol), ethyl acetoacetate (**2b**) (260 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 15 min. Purification afforded diethyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4c**) (200 mg, 79%) as canary yellow powder; mp 172 - 174 °C (from MeOH) (lit.,^{2,3} 170 - 172 °C); $R_f = 0.39$ (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 3347 (NH), 2988, 2939 and 2897 (C-H), 1691 and 1647 (C=O), 1629 (C=C), 1220 and 1055 (C-O); δ_{H} (300 MHz; CDCl_3) 1.28 (6H, t, $^3J_{3''\text{-H}, 2''\text{-H}}$ 7.2 Hz, 3''-H), 2.19 (6H, s, 1'-H), 3.26 (2H, s, 4-H), 4.17 (4H, q, $^3J_{2''\text{-H}, 3''\text{-H}}$ 7.2 Hz, 2''-H) and 5.20 (1H, s, 1-H); δ_{C} (75 MHz; CDCl_3) 14.45 (C-3''), 19.15 (C-1'), 24.78 (C-4), 59.64 (C-2''), 99.51 (C-3 and C-5), 144.78 (C-2 and C-6) and 168.05 (C-1'); m/z (EI, 70 eV) 253 (M^+ , 21%), 252 (30, $\text{M}^+ - 1$), 224 (100) and 196 (86).



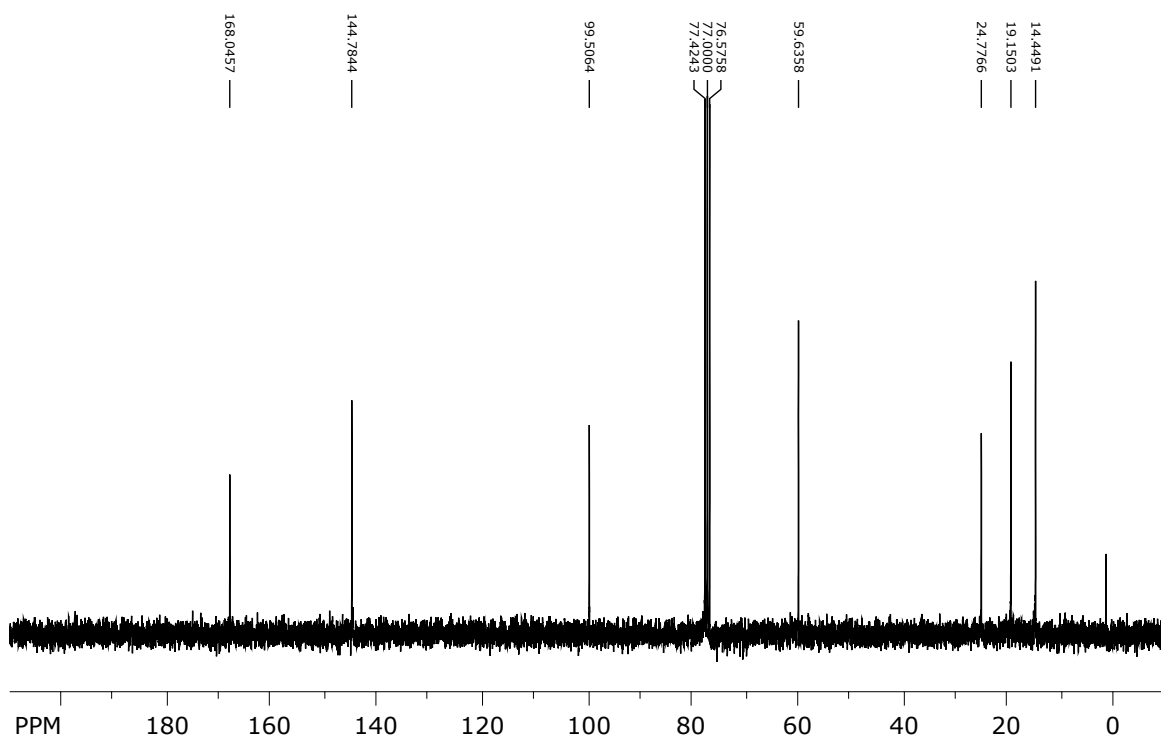


Fig. 3 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **4c** in CDCl_3 .

3.4. Synthesis and analytical data of diethyl-1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate (4d). According to the general procedure I, acetaldehyde (**1b**) (66 mg, 1.5 mmol), ethyl acetoacetate (**2b**) (260 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 15 min. Purification afforded diethyl-1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate (**4d**) (188 mg, 70%) as white crystals; mp 128 - 130 °C (from MeOH) (lit.,^{2,3} 128 - 130 °C); R_f = 0.43 (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\text{max}}(\text{atr})/\text{cm}^{-1}$ 3343 (NH), 2984, 2963 and 2905 (C-H), 1695 (C=O), 1639 (C=C), 1222 and 1056 (C-O); δ_{H} (300 MHz; CDCl_3) 0.96 (3H, d, $^3J_{1'''\text{-H}, 4\text{-H}}$ 6.6 Hz, 1'''-H), 1.29 (6H, t, $^3J_{3''\text{-H}, 2''\text{-H}}$ 7.2 Hz, 3''-H), 2.26 (6H, s, 1'-H), 3.83 (1H, q, $^3J_{4\text{-H}, 1'''\text{-H}}$ 6.6 Hz, 4-H), 4.15 (2H, dq, $^2J_{2''\text{a-H}, 2''\text{b-H}}$ 10.9 Hz, $^3J_{2''\text{a-H}, 3''\text{H}}$ 7.2 Hz, 2''a-H), 4.21 (2H, dq, $^2J_{2''\text{a-H}, 2''\text{b-H}}$ 10.9 Hz, $^3J_{2''\text{b-H}, 3''\text{H}}$ 7.2 Hz, 2''b-H) and 5.55 (1H, s, 1-H); δ_{C} (75 MHz; CDCl_3) 14.41 (C-3'), 19.48 (C-1'), 22.22 (C-1'''), 28.50 (C-4), 59.56 and 59.57 (C-2''), 104.70 (C-3 and C-5), 144.21 (C-2 and C-6) and 167.83 (C-1''); m/z (EI, 70 eV) 265 ($\text{M}^+ - 2$, 5%), 252 (100, $\text{M}^+ - \text{CH}_3$), 224 (34) and 196 (37).

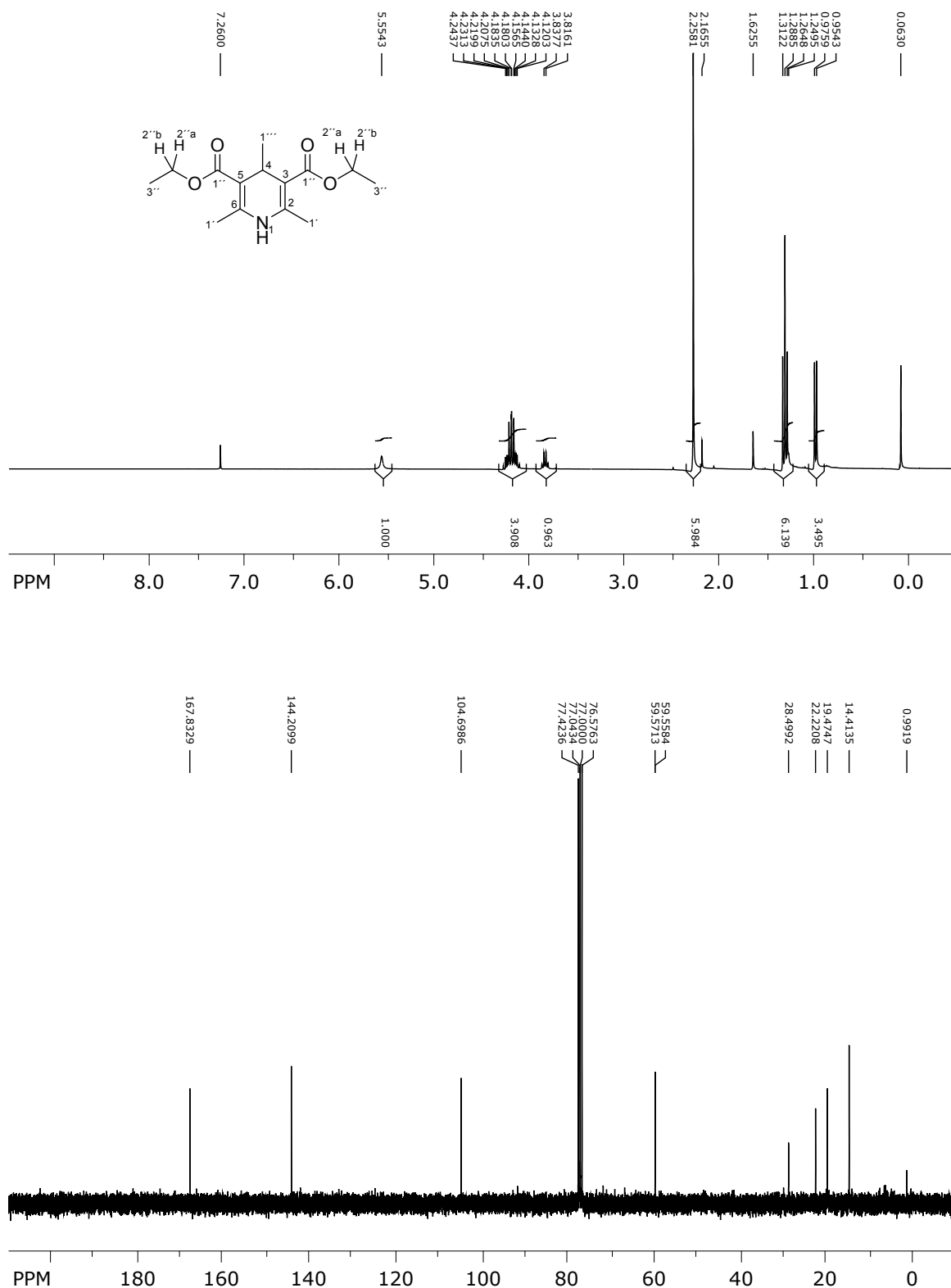
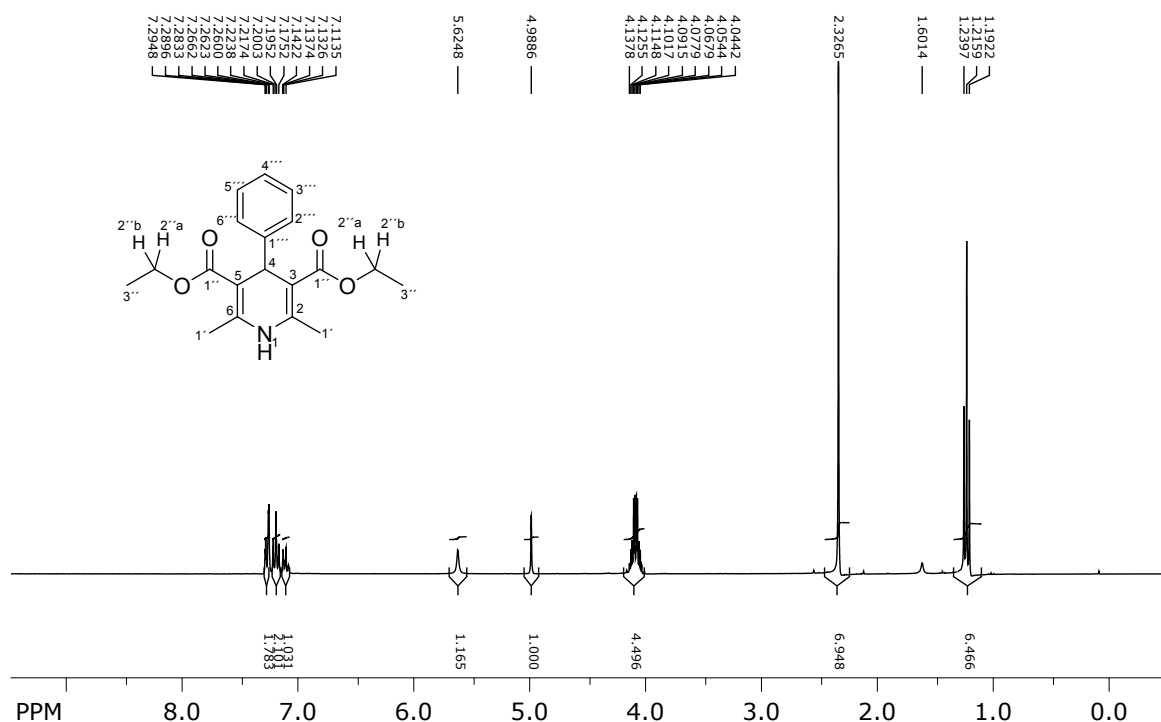


Fig. 4 ¹H (300 MHz) and ¹³C (75 MHz) NMR spectra of **4d** in CDCl₃.

3.5. Synthesis and analytical data of diethyl-1,4-dihydro-2,6-dimethyl-4-phenylpyridine-3,5-dicarboxylate (4e). According to the general procedure I, benzaldehyde (**1c**) (106 mg, 1 mmol), ethyl acetoacetate (**2b**) (260 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 180 min. Purification afforded diethyl-1,4-dihydro-2,6-dimethyl-4-phenylpyridine-3,5-dicarboxylate (**4e**) (148 mg, 45%) as white crystals; mp 160 - 162 °C (from MeOH) (lit.,^{2,3} 158 - 160 °C); $R_f = 0.49$ (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 3339 (NH), 3060 and 2982 (C-H), 1686 and 1649 (C=O), 1620 (C=C), 1207 and 1090 (C-O); δ_{H} (300 MHz; CDCl_3) 1.22 (6H, t, $^3J_{3''\text{-H}, 2''\text{-H}}$ 7.2 Hz, 3''-H), 2.33 (6H, s, 1'-H), 4.07 (2H, dq, $^2J_{2''\text{a-H}, 2''\text{b-H}}$ 10.9 Hz, $^3J_{2''\text{a-H}, 3''\text{H}}$ 7.0 Hz, 2''a-H), 4.10 (2H, dq, $^2J_{2''\text{b-H}, 2''\text{a-H}}$ 10.9 Hz, $^3J_{2''\text{b-H}, 3''\text{H}}$ 7.1 Hz, 2''b-H), 4.99 (1H, s, 4-H), 5.63 (1H, s, 1-H), 7.11-7.14 (1H, m, 4'''-H), 7.18-7.22 (2H, m, 3'''-H and 5'''-H) and 7.26-7.30 (2H, m, 2'''-H and 6'''-H); δ_{C} (75 MHz; CDCl_3) 14.22 (C-3''), 19.58 (C-1'), 39.62 (C-4), 59.70 (C-2''), 104.19 (C-3 and C-5), 126.07 (C-4'''), 127.80 (C-3''' and C-5'''), 127.99 (C-2''' and C-6'''), 143.77 (C-2 and C-6), 147.73 (C-1''') and 167.60 (C-1''); m/z (EI, 70 eV) 329 (M^+ , 12%), 300 (15, $\text{M}^+ - \text{C}_2\text{H}_5$), 284 (18, $\text{M}^+ - \text{OC}_2\text{H}_5$), 252 (100, $\text{M}^+ - \text{C}_6\text{H}_5$), 224 (36) and 196 (45).



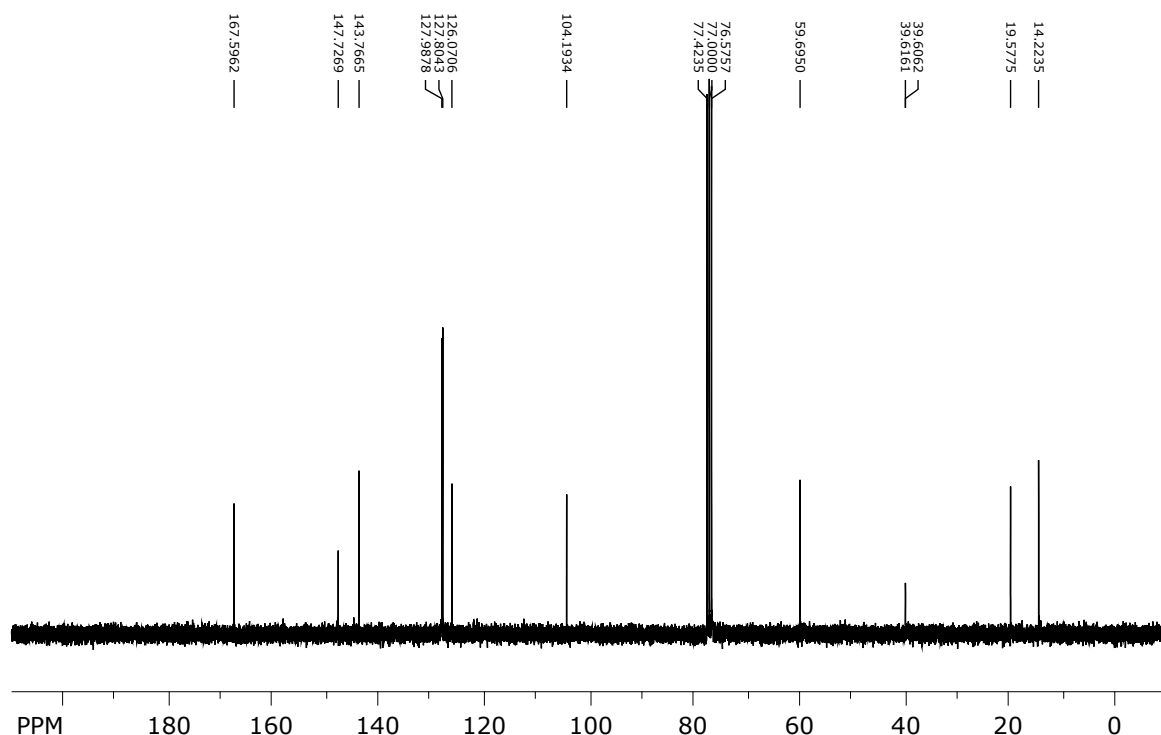


Fig. 5 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **4e** in CDCl_3 .

3.6. Synthesis and analytical data of di-isopropyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (4f). According to the general procedure I, paraformaldehyde (**1a**) (30 mg, 1 mmol), isopropyl acetoacetate (**2c**) (288 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 30 min. Purification afforded di-isopropyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4f**) (196 mg, 70%) as pale yellow crystals; mp 119 - 121 °C (from MeOH) (lit.,⁴ 123 - 125 °C); $R_f = 0.48$ (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\text{max}}(\text{atr})/\text{cm}^{-1}$ 3359 (NH), 2979, 2935 and 2878 (C-H), 1667 and 1644 (C=O), 1630 (C=C), 1232 and 1106 (C-O); δ_{H} (300 MHz; CDCl_3) 1.25 (12H, d, $^3J_{3''\text{-H}, 2''\text{-H}}$ 6.3 Hz, 3''-H), 2.18 (6H, s, 1'-H), 3.24 (2H, s, 4-H), 5.03 (2H, sep, $^3J_{2''\text{-H}, 3''\text{-H}}$ 6.3 Hz, 2''-H) and 5.06 (1H, s, 1-H); δ_{C} (75 MHz; CDCl_3) 19.23 (C-1'), 22.08 (C-3''), 24.84 (C-4), 66.78 and 66.80 (C-2''), 99.86 (C-3 and C-5), 144.36 (C-2 and C-6) and 167.63 (C-1''); m/z (EI, 70 eV) 281 (M^+ , 4%), 280 (17, $\text{M}^+ - 1$), 279 (84, $\text{M}^+ - 2$) and 28 (100).

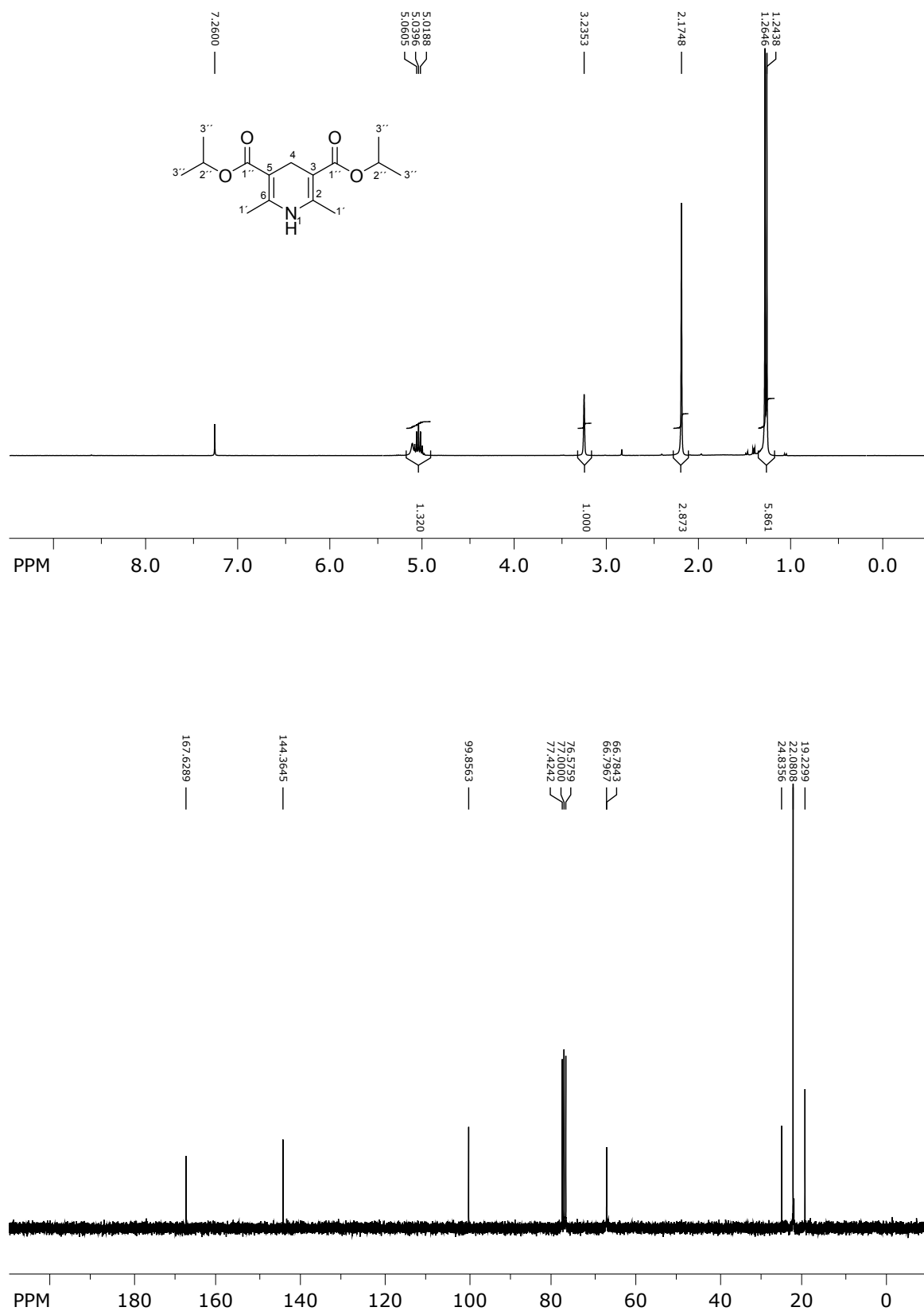
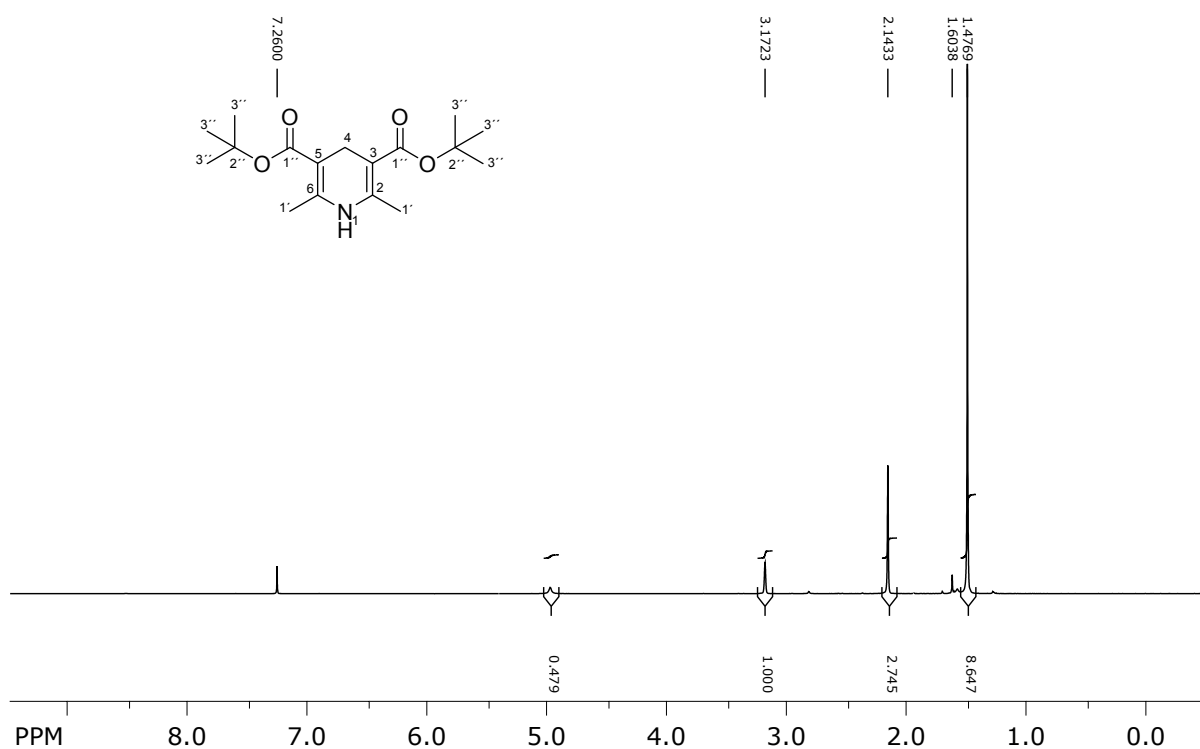


Fig. 6 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **4f** in CDCl_3 .

3.7. Synthesis and analytical data of di-*tert*-butyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (4g). According to the general procedure I, paraformaldehyde (**1a**) (30 mg, 1 mmol), *tert*-butyl acetoacetate (**2d**) (316 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 30 min. Purification afforded di-*tert*-butyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4g**) (246 mg, 80%) as white crystals; mp 151 - 153 °C (from MeOH) (lit.,⁴ 152 - 154 °C); $R_f = 0.64$ (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 3336 (NH), 2958 (C-H), 1694 and 1650 (C=O), 1639 (C=C), 1203 and 1092 (C-O); δ_{H} (300 MHz, CDCl_3) 1.48 (18H, s, 3''-H), 2.14 (6H, s, 1'-H), 3.17 (2H, s, 4-H) and 5.02 (1H, s, 1-H); δ_{C} (75 MHz, CDCl_3) 19.20 (C-1'), 25.42 (C-4), 28.19 and 28.36 (C-3''), 79.41 (C-2''), 100.89 (C-3 and C-5), 143.72 (C-2 and C-6) and 167.57 (C-1''); m/z (EI, 70 eV) 309 (M^+ , 68%), 308 (100, $\text{M}^+ - 1$), 307 (59, $\text{M}^+ - 2$) and 292 (60).



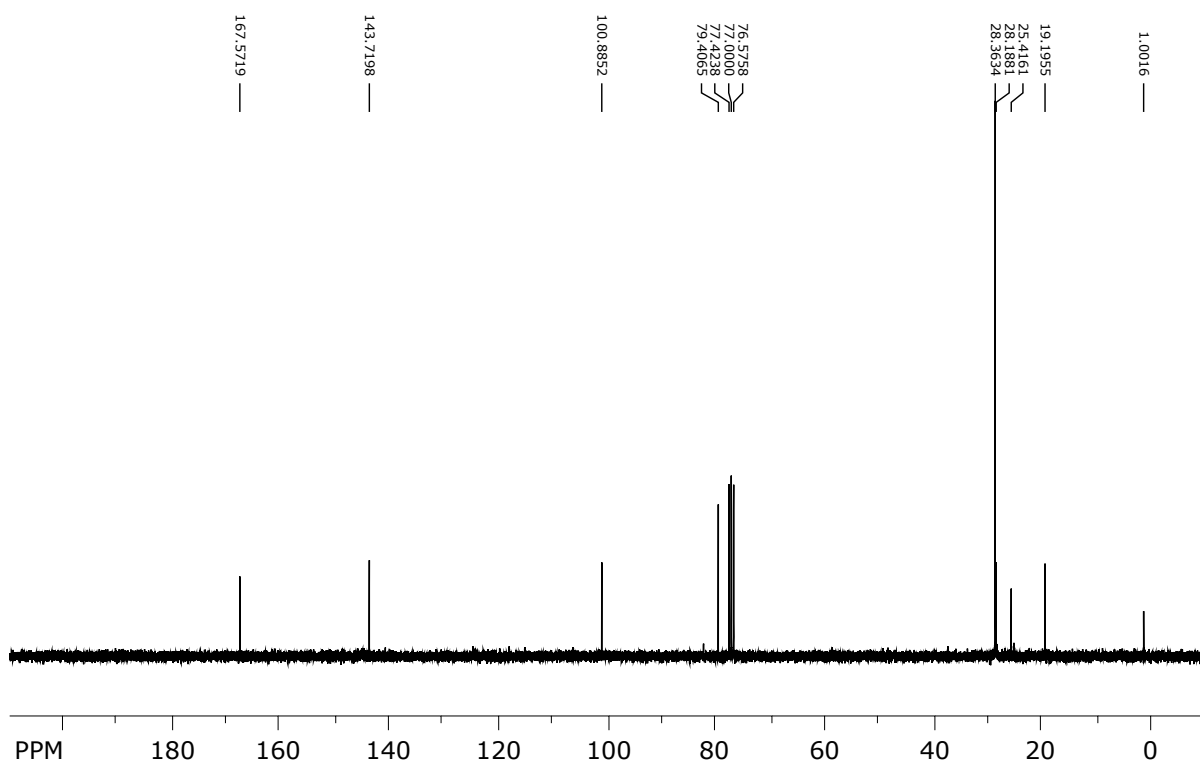


Fig. 7 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **4g** in CDCl_3 .

3.8. Synthesis and analytical data of diallyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (4h). According to the general procedure I, paraformaldehyde (**1a**) (30 mg, 1 mmol), allyl acetoacetate (**2e**) (284 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 10 min. Purification afforded diallyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4h**) (200 mg, 72%) as yellow powder; mp 164 - 166 °C (from MeOH); R_f = 0.50 (petroleum ether / EtOAc = 2:1); λ_{max} (MeCN)/nm 366 (log ϵ , 3.81) and 228 (4.18); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 3345 (NH), 3085, 2985, 2941 and 2879 (C-H), 1694 and 1655 (C=O), 1627 (C=C), 1211 and 1085 (C-O); δ_{H} (300 MHz; CDCl_3) 2.20 (6H, s, 1'-H), 3.33 (2H, s, 4-H), 4.62 (4H, dt, $^3J_{2''\text{-H}, 3''\text{-H}}$ 5.4 Hz, $^4J_{1.6}$ Hz, 2''-H), 5.20 (2H, dq, $^2J_{1.4}$ Hz, $^3J_{\text{cis } 4''\text{-a-H}, 3''\text{-H}}$ 10.5 Hz, $^4J_{1.4}$ Hz, 4''a-H), 5.31 (2H, dq, $^2J_{1.7}$ Hz, $^3J_{\text{trans } 4''\text{-b-H}, 3''\text{-H}}$ 17.4 Hz, $^4J_{1.7}$ Hz, 4''b-H) and 5.96 (2H, ddt, $^3J_{3''\text{-H}, 2''\text{-H}}$ 5.4 Hz, $^3J_{\text{cis } 3''\text{-H}, 4''\text{-a-H}}$ 10.5 Hz, $^3J_{\text{trans } 3''\text{-H}, 4''\text{-b-H}}$ 17.4 Hz, 3''-H); δ_{C} (75 MHz; CDCl_3) 19.21 (C-1'), 24.73 (C-4), 64.38 (C-2''), 99.33 (C-3 and C-5), 117.17 (C-4'), 132.92 (C-3'), 145.26 (C-2 and C-6) and 167.50 (C-1''); m/z (EI, 70 eV) 277 (M^+ , 7%), 275 (15, $\text{M}^+ - 2$), 236 (44, $\text{M}^+ - \text{CH}_2\text{CHCH}_2$), 218 (75), 191 (23), 41 (93, CH_2CHCH_2) and 28 (100); HRMS (EI, M^+) found: 277.1275 calcd. for $\text{C}_{15}\text{H}_{19}\text{NO}_4$: 277.1314.

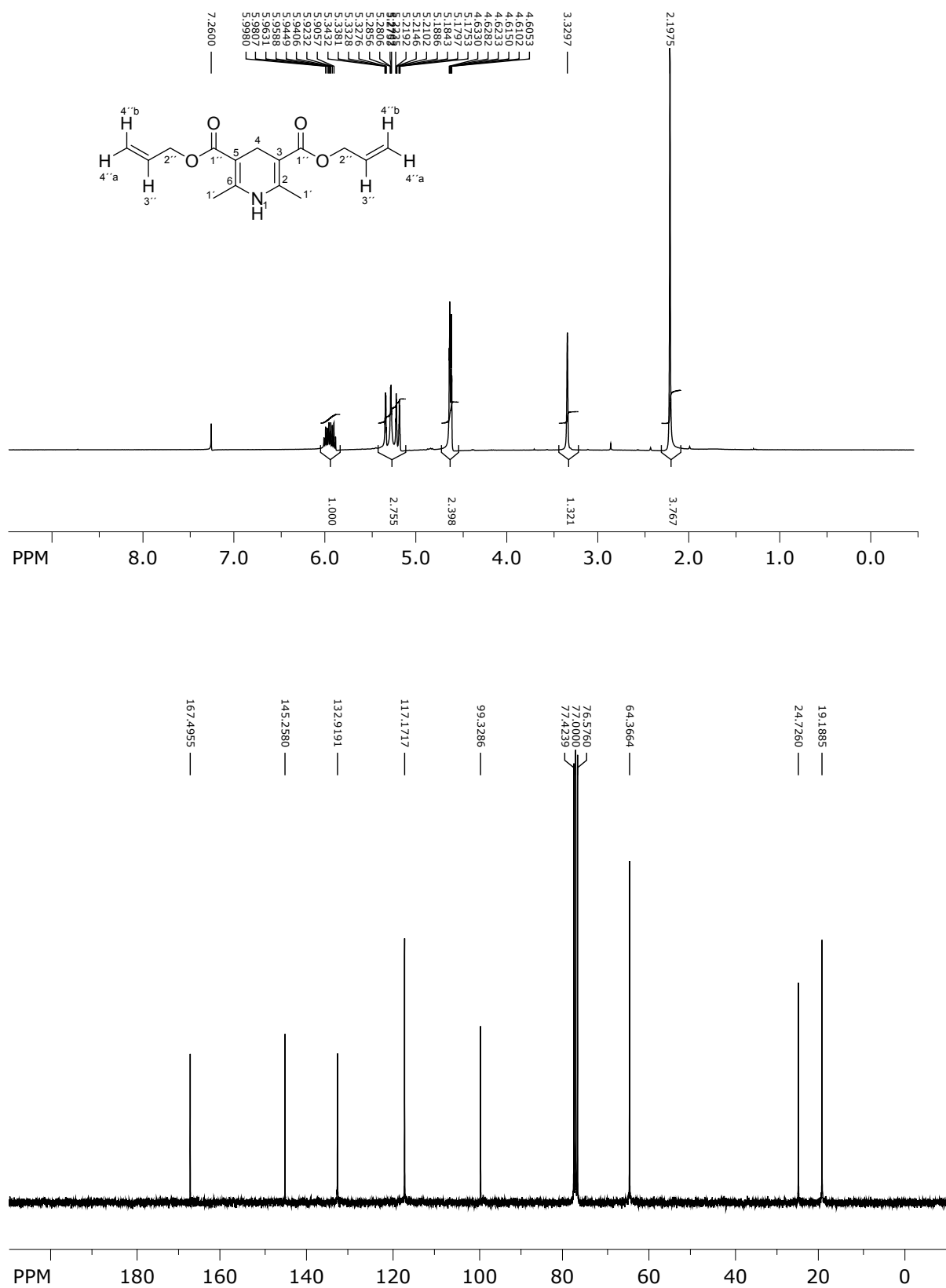
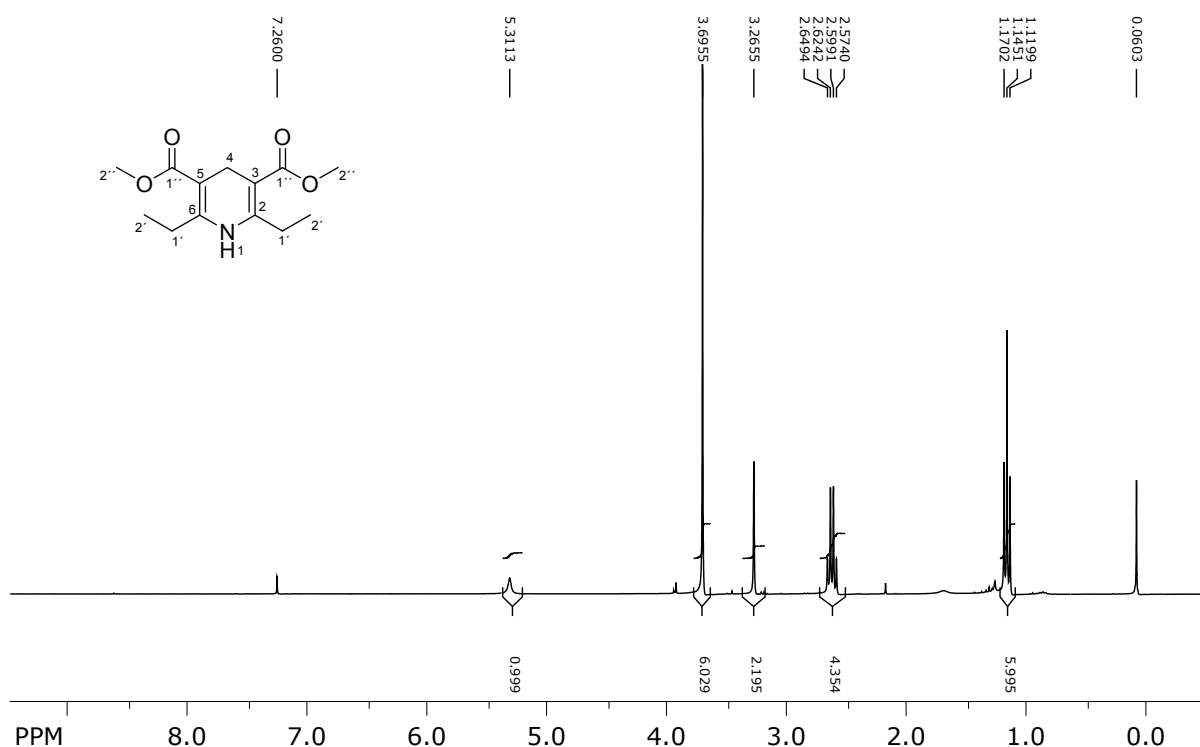


Fig. 8 ¹H (300 MHz) and ¹³C (75 MHz) NMR spectra of **4h** in CDCl₃.

3.9. Synthesis and analytical data of dimethyl-2,6-diethyl-1,4-dihydropyridine-3,5-dicarboxylate (4i). According to the general procedure I, paraformaldehyde (**1a**) (30 mg, 1 mmol), methyl propionyl acetate (**2f**) (260 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 15 min. Purification afforded dimethyl-2,6-diethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4i**) (227 mg, 90%) as yellow powder; mp 129 - 131 °C (from MeOH); $R_f = 0.64$ (petroleum ether / EtOAc = 2:1); $\lambda_{\max}(\text{MeCN})/\text{nm}$ 363 (log ϵ , 3.64), 230 (4.14) and 206 (4.14); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 3350 (NH), 2982, 2962 and 2873 (C-H), 1697 and 1646 (C=O), 1625 (C=C), 1203 and 1072 (C-O); δ_{H} (300 MHz; CDCl_3) 1.15 (6H, t, $^3J_{2\text{H}, 1\text{H}}$ 7.5 Hz, 2'-H), 2.61 (4H, q, $^3J_{1\text{H}, 2\text{H}}$ 7.5 Hz, 1'-H), 3.27 (2H, s, 4-H), 3.70 (6H, s, 2''-H) and 5.31 (1H, s, 1-H); δ_{C} (75 MHz; CDCl_3) 12.54 (C-2'), 24.86 (C-4), 25.62 (C-1'), 50.98 (C-2''), 98.21 (C-3 and C-5), 150.96 (C-2 and C-6) and 167.86 (C-1''); m/z (EI, 70 eV) 253 (M^+ , 24%), 252 (78, $\text{M}^+ - 1$), 238 (100, $\text{M}^+ - \text{CH}_3$), 222 (35, $\text{M}^+ - \text{OCH}_3$) and 194 (13, $\text{M}^+ - \text{COOCH}_3$); HRMS (EI, M^+) found: 253.1308 calcd. for $\text{C}_{13}\text{H}_{19}\text{NO}_4$: 253.1314.



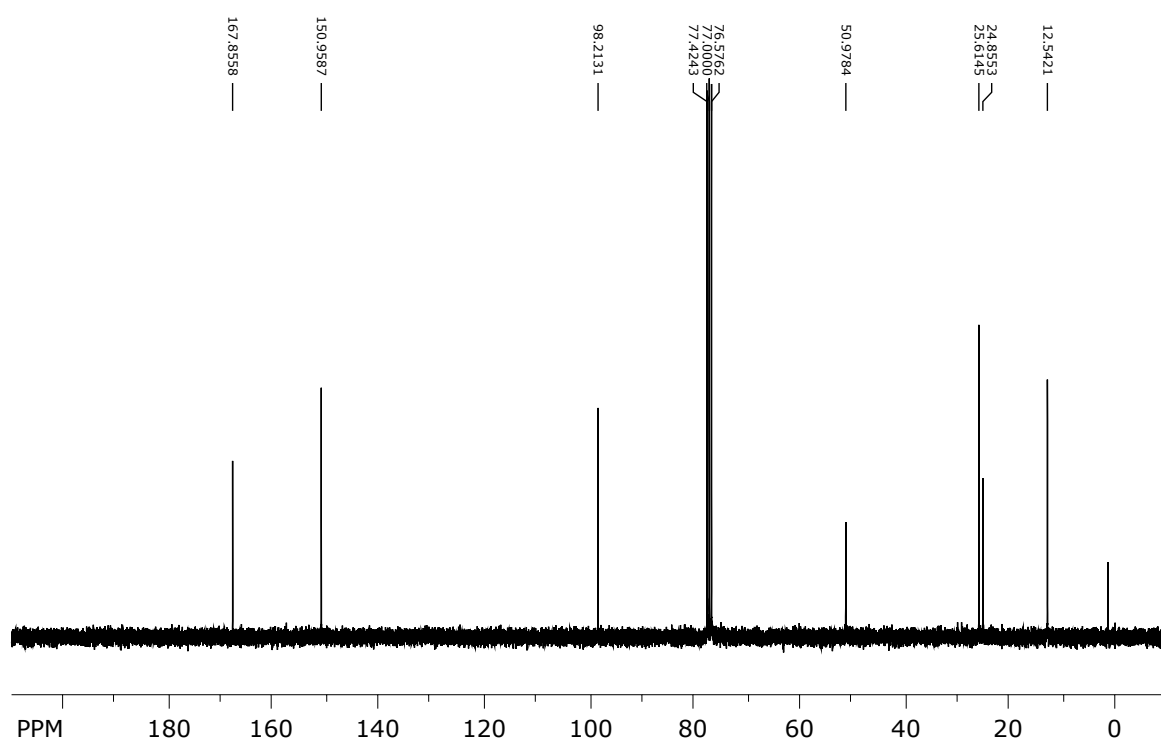


Fig. 9 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **4i** in CDCl_3 .

3.10. Synthesis and analytical data of diethyl-2,6-diethyl-1,4-dihydropyridine-3,5-dicarboxylate (4j). According to the general procedure I, paraformaldehyde (**1a**) (30 mg, 1 mmol), ethyl propionyl acetate (**2g**) (288 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 40 min. Purification afforded diethyl-2,6-diethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4j**) (196 mg, 70%) as yellow powder; mp 106 - 108 °C (from MeOH); R_f = 0.70 (petroleum ether / EtOAc = 2:1); λ_{max} (MeCN)/nm 363 (log ϵ , 3.67), 229 (4.13) and 203 (4.05); $\tilde{\nu}_{\text{max}}$ (atr)/ cm^{-1} 3344 (NH), 2980, 2935 and 2873 (C-H), 1694 and 1645 (C=O), 1625 (C=C), 1193 and 1071 (C-O); δ_{H} (300 MHz; CDCl_3) 1.14 (6H, t, $^3J_{2\text{H}, 1\text{H}}$ 7.5 Hz, 2'-H), 1.27 (6H, t, $^3J_{3\text{H}, 2\text{H}}$ 6.9 Hz, 3''-H), 2.59 (4H, q, $^3J_{1\text{H}, 2\text{H}}$ 7.8 Hz, 1'-H), 3.26 (2H, s, 4-H), 4.16 (4H, q, $^3J_{2\text{H}, 3\text{H}}$ 7.2 Hz, 2''-H) and 5.33 (1H, s, 1-H); δ_{C} (75 MHz; CDCl_3) 12.62 (C-2'), 14.39 (C-3'), 24.85 (C-4), 25.72 (C-1'), 59.58 (C-2''), 98.44 (C-3 and C-5), 150.62 (C-2 and C-6) and 167.55 (C-1'); m/z (EI, 70 eV) 281 (M^+ , 9%), 238 (48), 220 (28) and 195 (100); HRMS (EI, M^+) found: 281.1581 calcd. for $\text{C}_{15}\text{H}_{23}\text{NO}_4$: 281.1627.

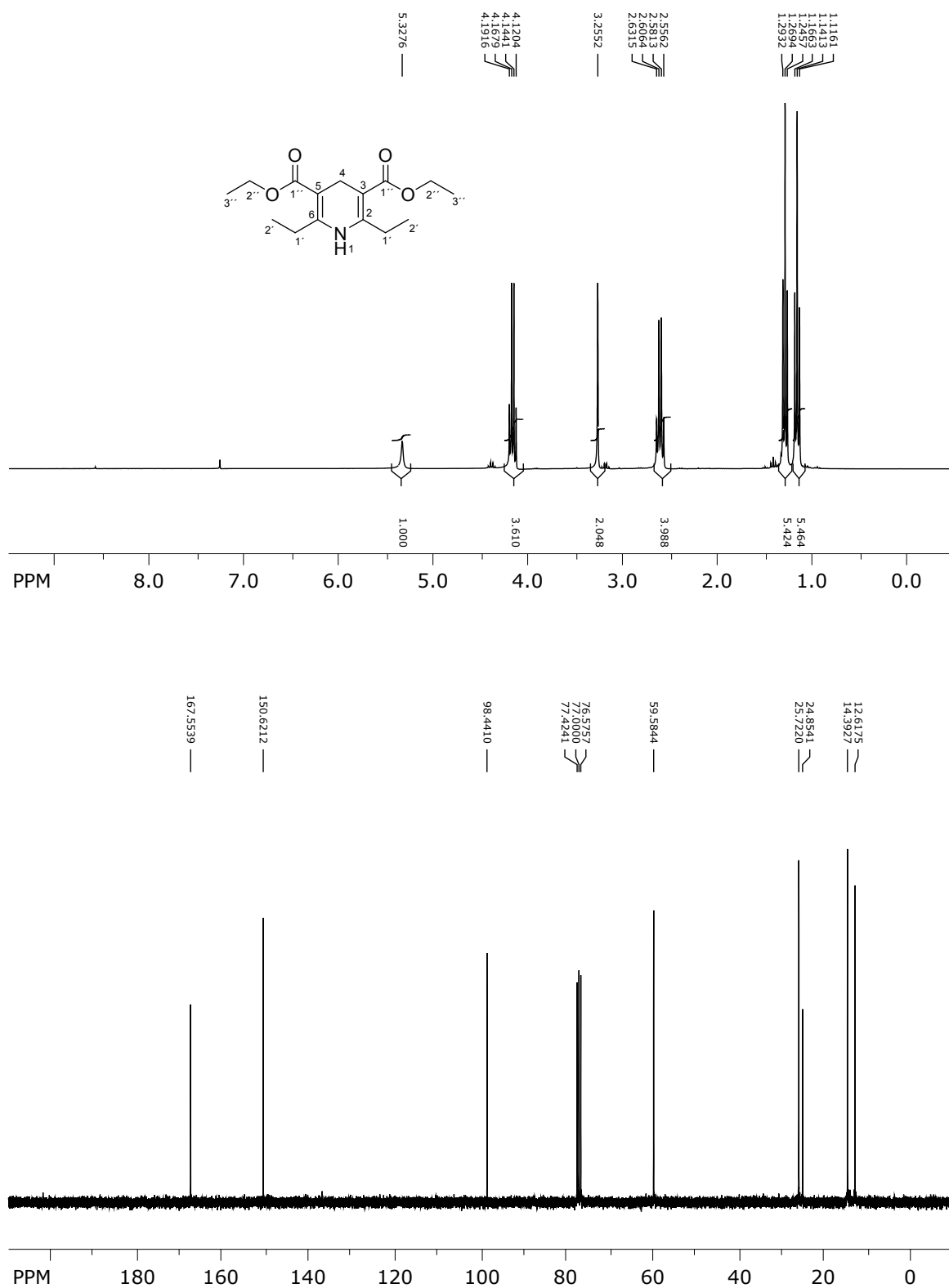
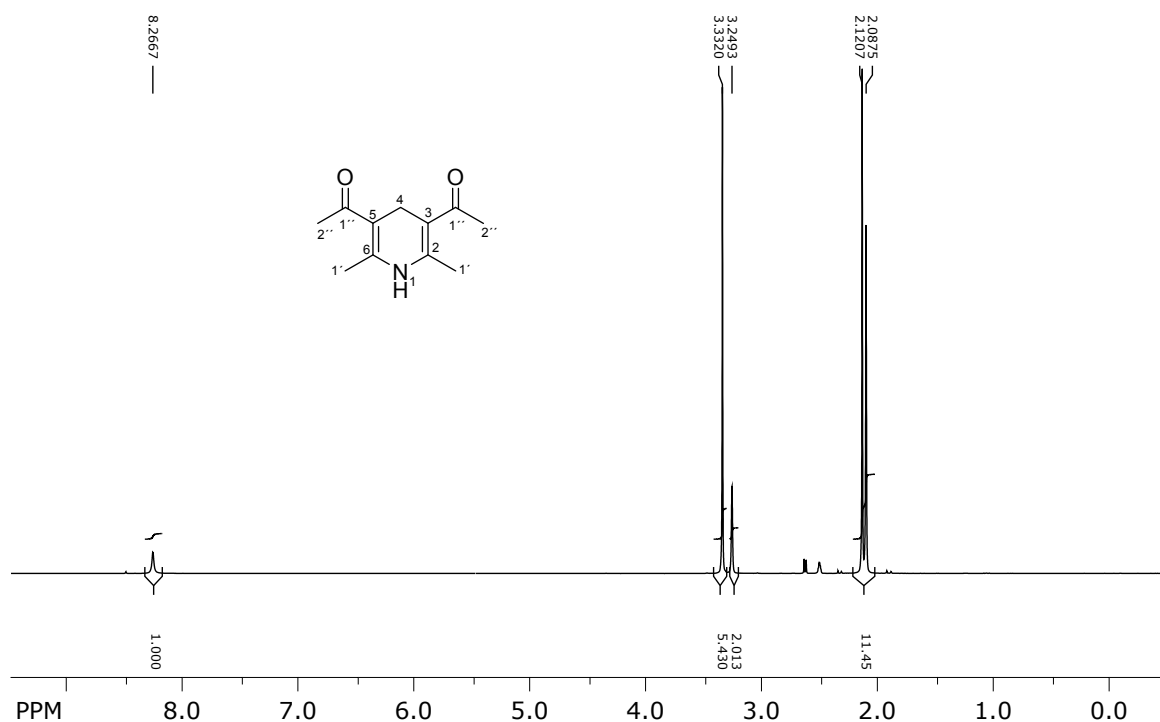


Fig. 10 ¹H (300 MHz) and ¹³C (75 MHz) NMR spectra of **4j** in CDCl₃.

3.11. Synthesis and analytical data of 2,6-dimethyl-3,5-diacetyl-1,4-dihydropyridine (**4k**).

According to the general procedure I, paraformaldehyde (**1a**) (30 mg, 1 mmol), acetylacetone (**2h**) (200 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 15 min. Purification afforded 2,6-dimethyl-3,5-diacetyl-1,4-dihydropyridine (**4k**) (145 mg, 75%) as yellow powder; mp 213 - 215 °C (from MeOH) (lit.,⁵ 216 - 220 °C); $R_f = 0.25$ (petroleum ether / EtOAc = 1:2); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 3382 (NH), 2920 (C-H), 1668 and 1643 (C=O) and 1588 (C=C); δ_{H} (300 MHz; DMSO- d_6) 2.09 (6H, s, 1'-H), 2.12 (6H, s, 2''-H), 3.25 (2H, s, 4-H) and 8.27 (1H, s, 1-H); δ_{C} (75 MHz; DMSO- d_6) 18.67 (C-1'), 26.39 (C-4), 30.10 (C-2''), 107.62 (C-3 and C-5), 145.24 (C-2 and C-6) and 196.65 (C-1''); m/z (EI, 70 eV) 193 (M^+ , 45%), 192 (63, $M^+ - 1$), 178 (50, $M^+ - \text{CH}_3$), 150 (23, $M^+ - \text{COCH}_3$), 106 (23) and 43 (38, COCH_3).



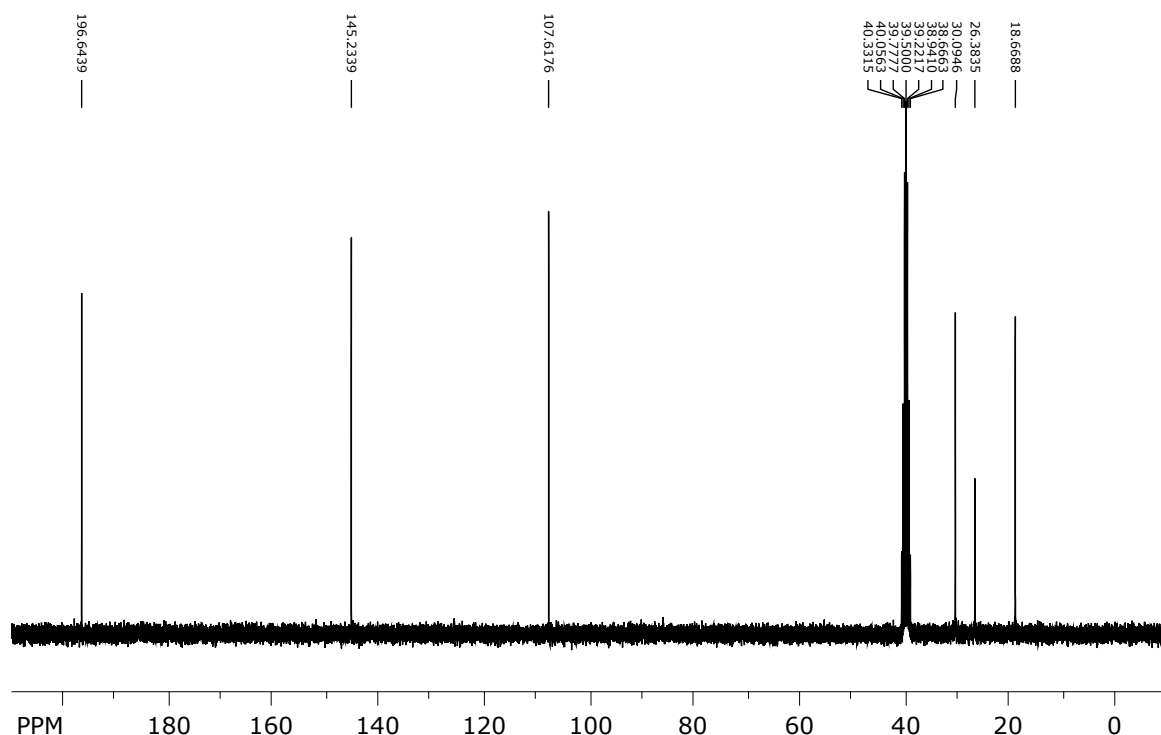


Fig. 11 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **4k** in $\text{DMSO-}d_6$.

4. General procedures II and III for the laccase-catalyzed oxidation of 1,4-dihydropyridines 4a-k to the corresponding pyridines 5a-k

4.1. General procedure II for the laccase-catalyzed oxidation of 1,4-dihydropyridines 4a-k to the corresponding pyridines 5a-k (method A)

A 100 mL round bottomed flask with a magnetic stirrer bar was charged with a solution of 1,4-dihydropyridine **4** (1 mmol) in methanol (3 - 6 mL). Acetate buffer (0.2 M, pH 4.37, 30 mL), laccase from *Trametes versicolor* (168 U, 12 mg) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were added and the reaction mixture was stirred at 50 °C for the time given. After extraction with CH_2Cl_2 (7×20 mL), the combined organic phases were dried over anhydrous Na_2SO_4 , filtered and the solvents were evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO_2 (petroleum ether / EtOAc = 6:1). It was found that 2-butanone can be also used for extraction.

4.2. General procedure III for the laccase-catalyzed oxidation of 1,4-dihydropyridines 4a-k to the corresponding pyridines 5a-k (method B)

A 100 mL round bottomed flask with a magnetic stirrer bar was charged with a solution of 1,4-dihydropyridine **4** (1 mmol) in methanol (3 - 6 mL). Acetate buffer (0.2 M, pH 4.37, 30 mL), laccase from *Trametes versicolor* (168 U, 12 mg) and ABTS diammonium salt (54.9 mg, 0.1 mmol) were added and the reaction mixture was stirred at 50 °C for the time given. After extraction with CH₂Cl₂ (7 × 20 mL), the combined organic phases were dried over anhydrous Na₂SO₄, filtered and the solvents were evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO₂ (petroleum ether / EtOAc = 6:1). It was found that 2-butanone can be also used for extraction.

5. General procedures IV and V for the one pot synthesis of pyridines 5a-k

5.1. General procedure IV for the one pot synthesis of pyridines 5a-k (method C)

A 100 mL round bottomed flask with a magnetic stirrer bar was charged with an aldehyde **1** (1 mmol), a 1,3-dicarbonyl compound **2** (2 mmol) and ammonium acetate (**3**) (1.5 mmol). The mixture was stirred at 80 °C until the starting materials were consumed (TLC). After cooling to room temperature, the mixture was dissolved in methanol (3 - 6 mL). Acetate buffer (0.2 M, pH 4.37, 30 mL), laccase from *Trametes versicolor* (168 U, 12 mg) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were added and the mixture was stirred at 50 °C for the time given. The reaction mixture was extracted with CH₂Cl₂ (7 × 20 mL) and the combined organic phases were dried over anhydrous Na₂SO₄, filtered and the solvents were evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO₂ (petroleum ether / EtOAc = 6:1). It was found that 2-butanone can be also used for extraction.

5.2. General procedure V for the one pot synthesis of pyridines 5a-k (method D)

A 100 mL round bottomed flask with a magnetic stirrer bar was charged with an aldehyde **1** (1 mmol), a 1,3-dicarbonyl compound **2** (2 mmol) and ammonium acetate (**3**) (1.5 mmol). The mixture was stirred at 80 °C until the starting materials were consumed (TLC). After cooling to

room temperature, the mixture was dissolved in methanol (3 - 6 mL). Acetate buffer (0.2 M, pH 4.37, 30 mL), laccase from *Trametes versicolor* (168 U, 12 mg) and ABTS diammonium salt (54.9 mg, 0.1 mmol) were added and the mixture was stirred at 50 °C for the time given. The reaction mixture was extracted with CH₂Cl₂ (7 × 20 mL) and the combined organic phases were dried over anhydrous Na₂SO₄, filtered and the solvents were evaporated *in vacuo*. The crude product was purified by flash chromatography on SiO₂ (petroleum ether / EtOAc = 6:1). It was found that 2-butanone can be also used for extraction.

6. Synthesis and analytical data of pyridines 5a-k

6.1. Synthesis and analytical data of dimethyl-2,6-dimethylpyridine-3,5-dicarboxylate (5a)

Synthesis of 5a

According to the general procedure II, dimethyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4a**) (225 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were reacted for 5 h. Purification afforded dimethyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5a**) (209 mg, 94%).

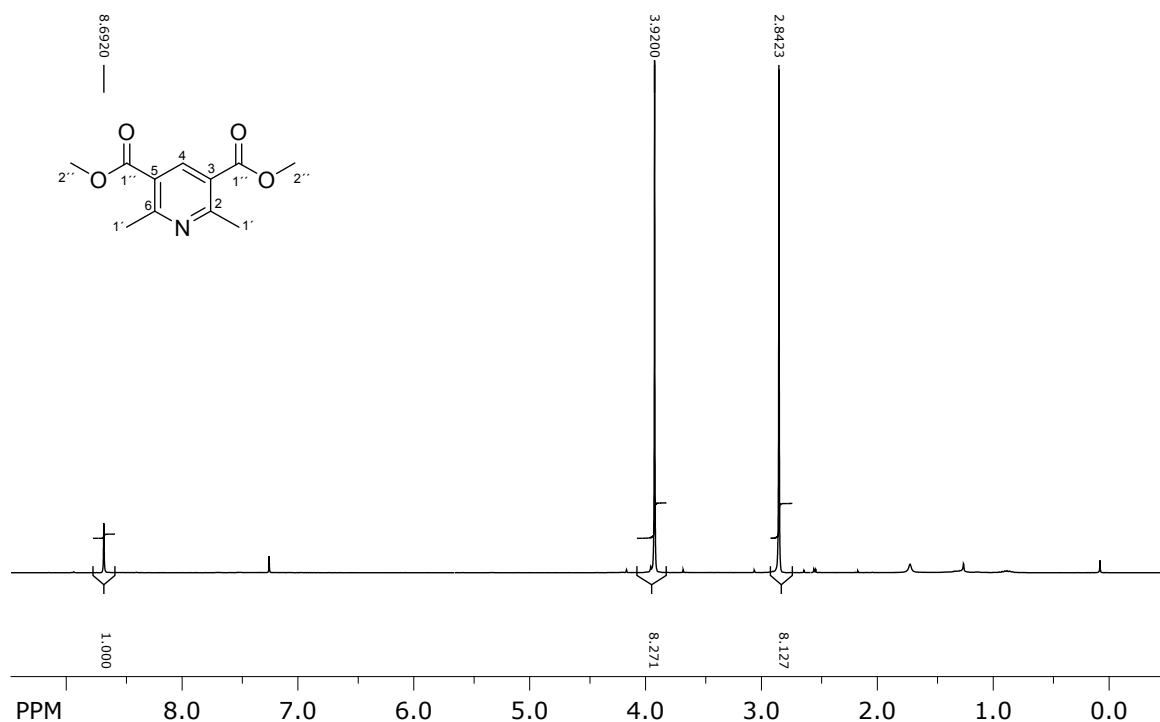
According to the general procedure III, dimethyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4a**) (225 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) were reacted for 5 h. Purification afforded dimethyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5a**) (212 mg, 95%).

According to the general procedure IV, paraformaldehyde (**1a**) (30 mg, 1 mmol), methyl acetoacetate (**2a**) (232 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 10 min. The reaction mixture was treated with methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) for 7 h. Purification afforded dimethyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5a**) (133 mg, 60%).

According to the general procedure V, paraformaldehyde (**1a**) (30 mg, 1 mmol), methyl acetoacetate (**2a**) (232 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 10 min. The reaction mixture was treated with methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) for 6 h. Purification afforded dimethyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5a**) (130 mg, 58 %).

Analytical data of **5a**

White powder; mp 98 - 100 °C (lit.,⁶ 101 - 103 °C); $R_f = 0.49$ (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 2970 (C-H), 1721 (C=O), 1595 and 1547 (C=C, C=N), 1227 and 1104 (C-O); δ_{H} (300 MHz; CDCl_3) 2.84 (6H, s, 1'-H), 3.92 (6H, s, 2''-H) and 8.69 (1H, s, 4-H); δ_{C} (75 MHz; CDCl_3) 24.92 (C-1'), 52.28 (C-2''), 122.60 (C-3 and C-5), 141.04 (C-4), 162.61 (C-2 and C-6) and 166.21 (C-1''); m/z (EI, 70 eV) 223 (M^+ , 98%), 192 (100, $\text{M}^+ - \text{OCH}_3$) and 164 (61).



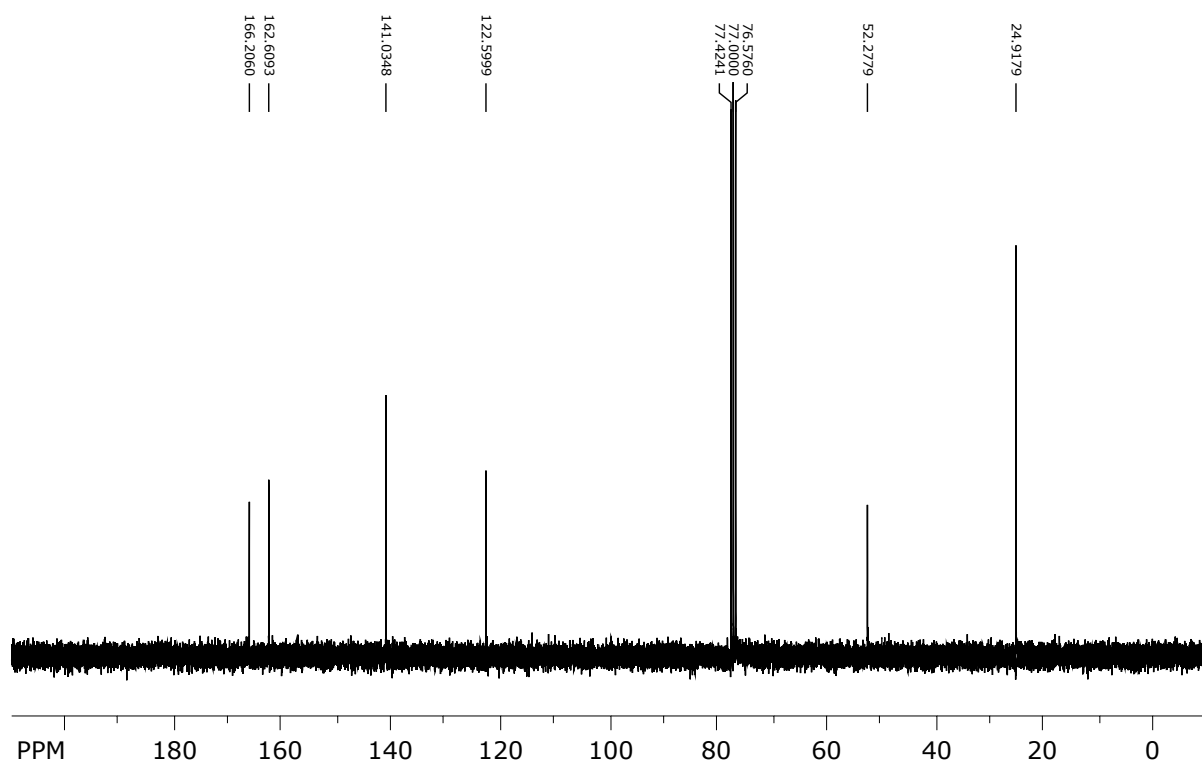


Fig. 12 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **5a** in CDCl_3 .

6.2. Synthesis and analytical data of dimethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate (**5b**)

Synthesis of **5b**

According to the general procedure II, dimethyl-1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate (**4b**) (239 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were reacted for 4 h. Purification afforded dimethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate (**5b**) (213 mg, 90%).

According to the general procedure III, dimethyl-1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate (**4b**) (239 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) were reacted for 8 h. Purification afforded dimethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate (**5b**) (189 mg, 80%).

According to the general procedure IV, acetaldehyde (**1b**) (66 mg, 1.5 mmol), methyl acetoacetate (**2a**) (232 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 30 min. The reaction mixture was treated with methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) for 7 h. Purification afforded dimethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate (**5b**) (142 mg, 60%).

According to the general procedure V, acetaldehyde (**1b**) (66 mg, 1.5 mmol), methyl acetoacetate (**2a**) (232 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 30 min. The reaction mixture was treated with methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) for 9 h. Purification afforded dimethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate (**5b**) (118 mg, 50%).

Analytical data of **5b**

White powder; mp 78 - 80 °C (lit.,⁷ 78 - 80 °C); $R_f = 0.48$ (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 2980 (C-H), 1713 (C=O), 1558 (C=C, C=N), 1220 and 1043 (C-O); δ_{H} (300 MHz; CDCl₃) 2.24 (3H, s, 1'''-H), 2.50 (6H, s, 1'-H) and 3.92 (6H, s, 2''-H); δ_{C} (75 MHz; CDCl₃) 17.11 (C-1'''), 23.01 (C-1'), 52.40 (C-2''), 127.27 (C-3 and C-5), 142.35 (C-4), 155.22 (C-2 and C-6) and 168.84 (C-1''); m/z (EI, 70 eV) 237 (M⁺, 30%), 222 (33, M⁺ - CH₃), 206 (56) and 28 (100).

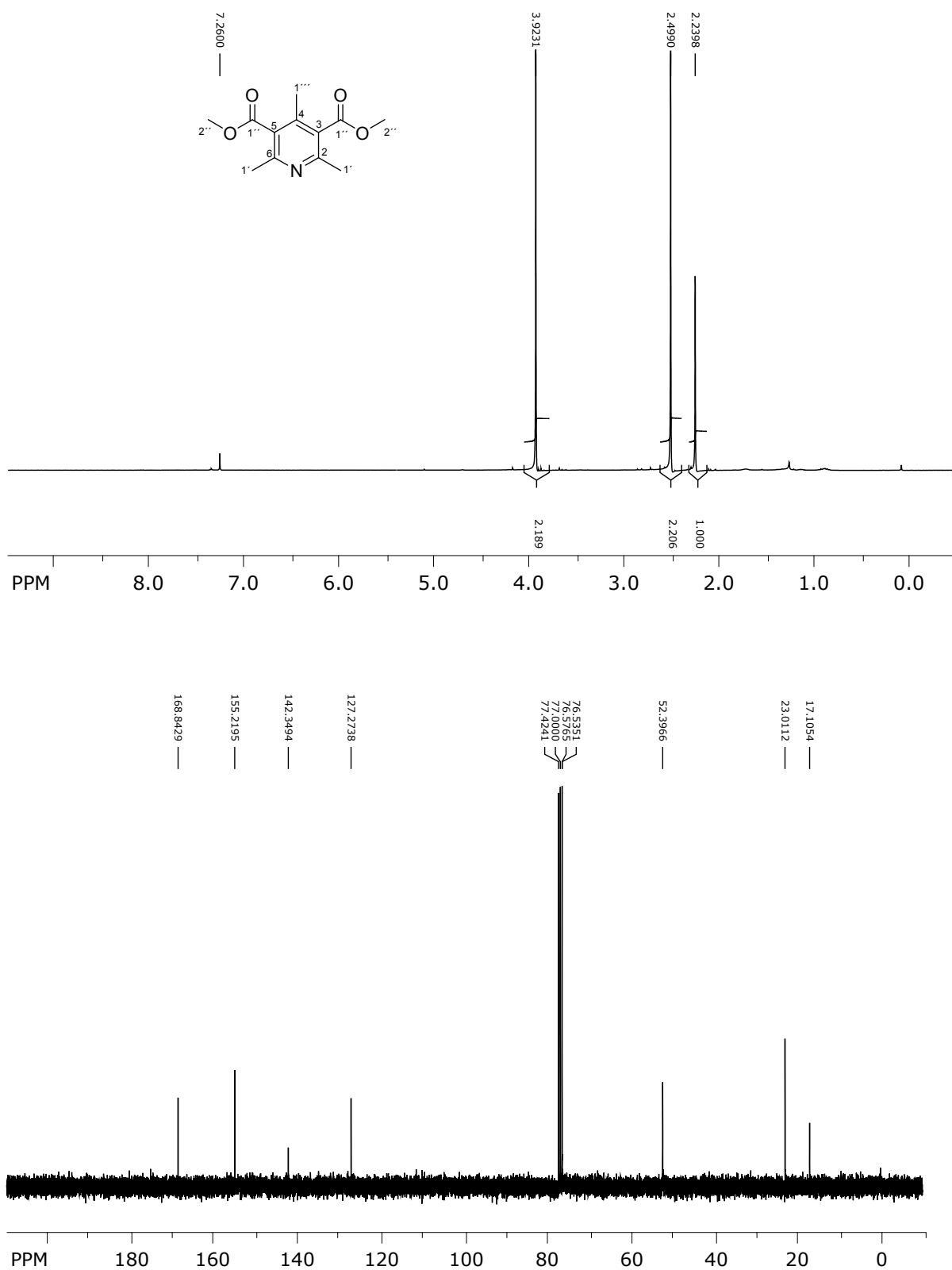


Fig. 13 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **5b** in CDCl_3 .

6.3. Synthesis and analytical data of diethyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5c**)

Synthesis of **5c**

According to the general procedure II, diethyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4c**) (253 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were reacted for 5 h. Purification afforded diethyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5c**) (208 mg, 83%).

According to the general procedure III, diethyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4c**) (253 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) were reacted for 5 h. Purification afforded diethyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5c**) (225 mg, 90%).

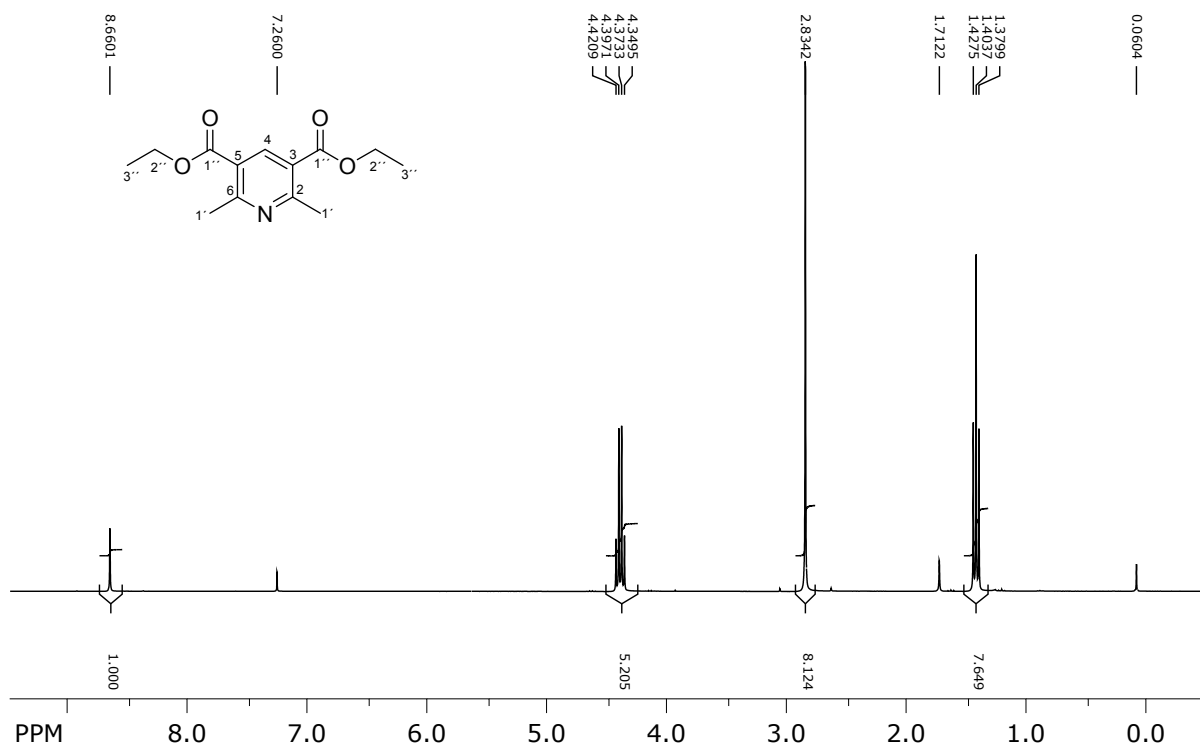
According to the general procedure IV, paraformaldehyde (**1a**) (30 mg, 1 mmol), ethyl acetoacetate (**2b**) (260 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 15 min. The reaction mixture was treated with methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) for 5 h. Purification afforded diethyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5c**) (133 mg, 53%).

According to the general procedure V, paraformaldehyde (**1a**) (30 mg, 1 mmol), ethyl acetoacetate (**2b**) (260 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 15 min. The reaction mixture was treated with methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) for 5 h. Purification afforded diethyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5c**) (168 mg, 67%).

Analytical data of **5c**

White powder; mp 69 - 71 °C (lit.,⁶ 70 - 71 °C); $R_f = 0.63$ (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 2920 (C-H), 1714 (C=O), 1580 (C=C, C=N), 1221 and 1042 (C-O); δ_{H} (300 MHz;

CDCl₃) 1.40 (6H, t, ³J_{3''-H, 2''-H} 7.2 Hz, 3''-H), 2.83 (6H, s, 1'-H), 4.38 (4H, q, ³J_{2''-H, 3''-H} 7.2 Hz, 2''-H) and 8.66 (1H, s, 4-H); δ_C (75 MHz; CDCl₃) 14.25 (C-3''), 24.94 (C-1'), 61.38 (C-2''), 123.05 (C-3 and C-5), 140.88 (C-4), 162.20 (C-2 and C-6) and 165.95 (C-1'); *m/z* (EI, 70 eV) 251 (M⁺, 82%), 206 (100) and 195 (22).



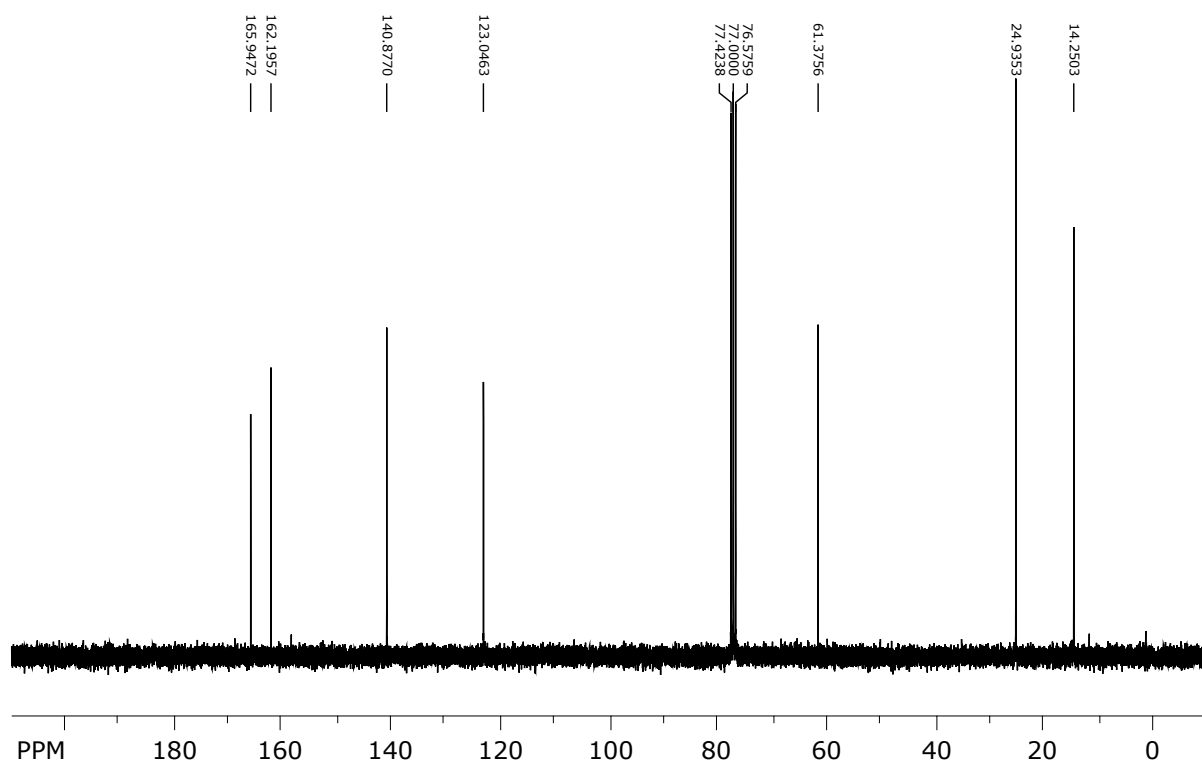


Fig. 14 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **5c** in CDCl_3 .

6.4. Synthesis and analytical data of diethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate (**5d**)

Synthesis of **5d**

According to the general procedure II, diethyl-1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate (**4d**) (267 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were reacted for 6 h. Purification afforded diethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate (**5d**) (230 mg, 87%).

According to the general procedure III, diethyl-1,4-dihydro-2,4,6-trimethylpyridine-3,5-dicarboxylate (**4d**) (267 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) were reacted for 5 h. Purification afforded diethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate (**5d**) (238 mg, 90%).

According to the general procedure IV, acetaldehyde (**1b**) (66 mg, 1.5 mmol), ethyl acetoacetate (**2b**) (260 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 15 min. The reaction mixture was treated with methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*), ABTS diammonium salt (6.9 mg, 0.0125 mmol) for 6 h. Purification afforded diethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate (**5d**) (148 mg, 56%).

According to the general procedure V, acetaldehyde (**1b**) (66 mg, 1.5 mmol), ethyl acetoacetate (**2b**) (260 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 15 min. The reaction mixture was treated with methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*), ABTS diammonium salt (54.9 mg, 0.1 mmol) for 6 h. Purification afforded diethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate (**5d**) (161 mg, 61%).

Analytical data of **5d**

Colourless oil (lit.,⁶ oil); $R_f = 0.66$ (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 2981 and 2962 (C-H), 1721 (C=O), 1565 (C=C, C=N), 1214 and 1038 (C-O); δ_{H} (300 MHz; CDCl₃) 1.36 (6H, t, $^3J_{3''\text{-H}, 2''\text{-H}}$ 7.2 Hz, 3''-H), 2.24 (3H, s, 1'''-H), 2.49 (6H, s, 1'-H) and 4.40 (4H, q, $^3J_{2''\text{-H}, 3''\text{-H}}$ 7.2 Hz, 2''-H); δ_{C} (75 MHz; CDCl₃) 14.11 (C-3''), 16.88 (C-1'''), 22.86 (C-1'), 61.50 (C-2''), 127.51 (C-3 and C-5), 141.97 (C-4), 154.86 (C-2 and C-6) and 168.33 (C-1''); m/z (EI, 70 eV) 265 (M⁺, 35%), 220 (74) and 28 (100).

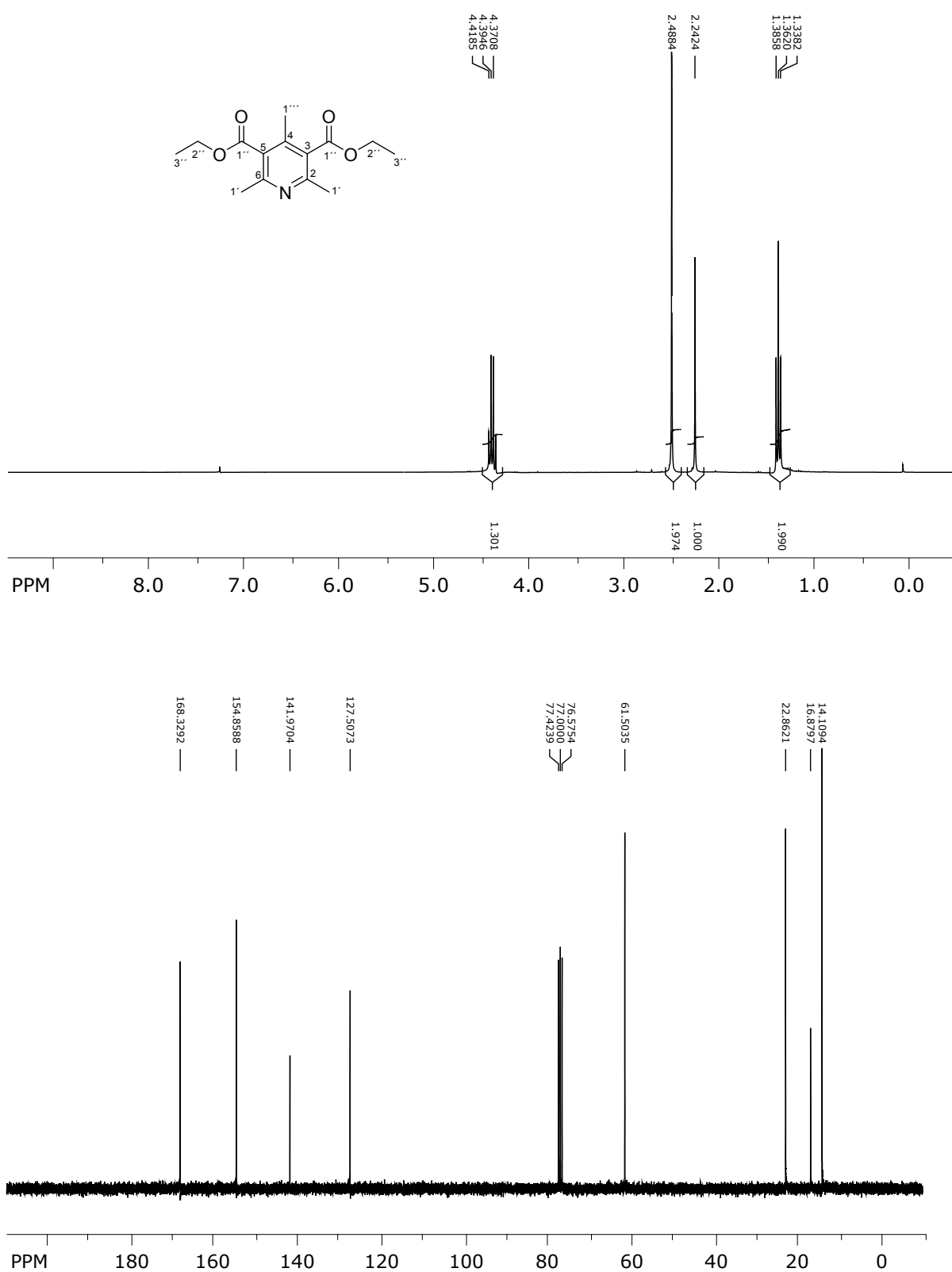


Fig. 15 ¹H (300 MHz) and ¹³C (75 MHz) NMR spectra of **5d** in CDCl₃.

6.5. Synthesis and analytical data of diethyl-2,6-dimethyl-4-phenylpyridine-3,5-dicarboxylate (**5e**)

Synthesis of **5e**

According to the general procedure II, diethyl-1,4-dihydro-2,6-dimethyl-4-phenylpyridine-3,5-dicarboxylate (**4e**) (329 mg, 1 mmol), methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were reacted for 24 h. Purification afforded diethyl-2,6-dimethyl-4-phenylpyridine-3,5-dicarboxylate (**5e**) (88 mg, 27%).

According to the general procedure III, diethyl-1,4-dihydro-2,6-dimethyl-4-phenylpyridine-3,5-dicarboxylate (**4e**) (329 mg, 1 mmol), methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) were reacted for 9 h. Purification afforded diethyl-2,6-dimethyl-4-phenylpyridine-3,5-dicarboxylate (**5e**) (261 mg, 80%).

According to the general procedure V, benzaldehyde (**1c**) (106 mg, 1 mmol), ethyl acetoacetate (**2b**) (260 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 180 min. The reaction mixture was treated with methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) for 24 h. Purification afforded diethyl-2,6-dimethyl-4-phenylpyridine-3,5-dicarboxylate (**5e**) (65 mg, 20%).

Analytical data of **5e**

White powder; mp 63 - 65 °C (lit.,⁶ 60 - 62 °C); $R_f = 0.63$ (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 2980 (C-H), 1714 (C=O), 1555 (C=C, C=N), 1226 and 1039 (C-O); δ_{H} (300 MHz; CDCl₃) 0.90 (6H, t, $^3J_{3''\text{-H}, 2''\text{-H}}$ 7.2 Hz, 3''-H), 2.60 (6H, s, 1'-H), 3.99 (4H, q, $^3J_{2''\text{-H}, 3''\text{-H}}$ 7.2 Hz, 2''-H), 7.23-7.25 (3H, m, 3'''-H, 4'''-H and 5'''-H) and 7.35-7.37 (2H, m, 2'''-H and 6'''-H); δ_{C} (75 MHz; CDCl₃) 13.50 (C-3''), 22.88 (C-1'), 61.29 (C-2''), 126.89 (C-4'''), 128.04 and 128.05 (C-3''' and C-5'''), 128.08 and 128.37 (C-2''' and C-6'''), 136.55 (C-1'''), 146.08 (C-4), 155.38 (C-2 and C-6) and 167.84 (C-1''); m/z (EI, 70 eV) 327 (M^+ , 100 %), 282 (56, $M^+ - \text{OC}_2\text{H}_5$), 236 (79) and 209 (24).

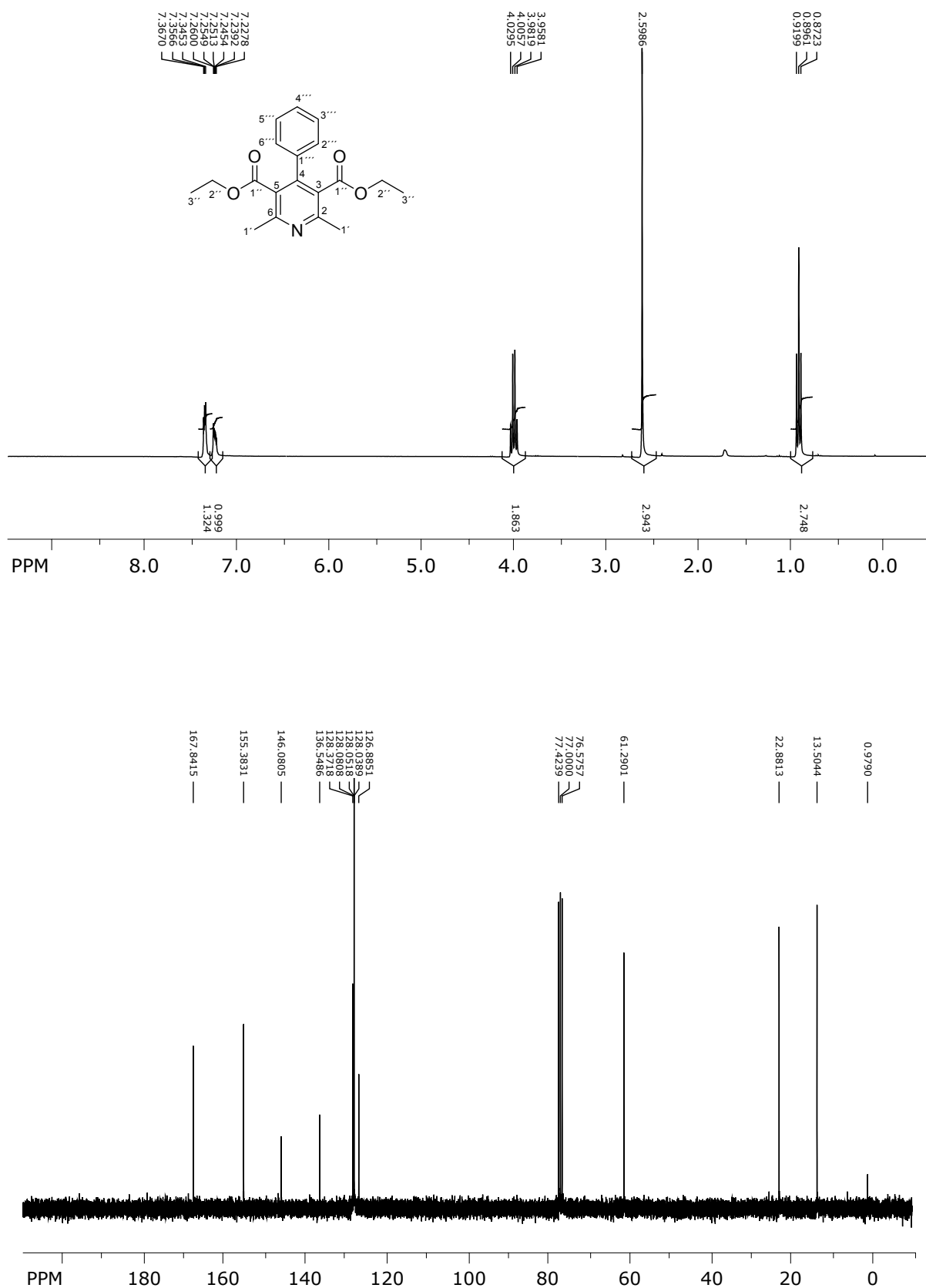


Fig. 16 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **5e** in CDCl_3 .

6.6. Synthesis and analytical data of di-isopropyl-2,6-dimethylpyridine-3,5-dicarboxylate (5f)

Synthesis of 5f

According to the general procedure II, di-isopropyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4f**) (281 mg, 1 mmol), methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were reacted for 20 h. Purification afforded di-isopropyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5f**) (226 mg, 81%).

According to the general procedure III, di-isopropyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4f**) (281 mg, 1 mmol), methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) were reacted for 8 h. Purification afforded di-isopropyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5f**) (260 mg, 93%).

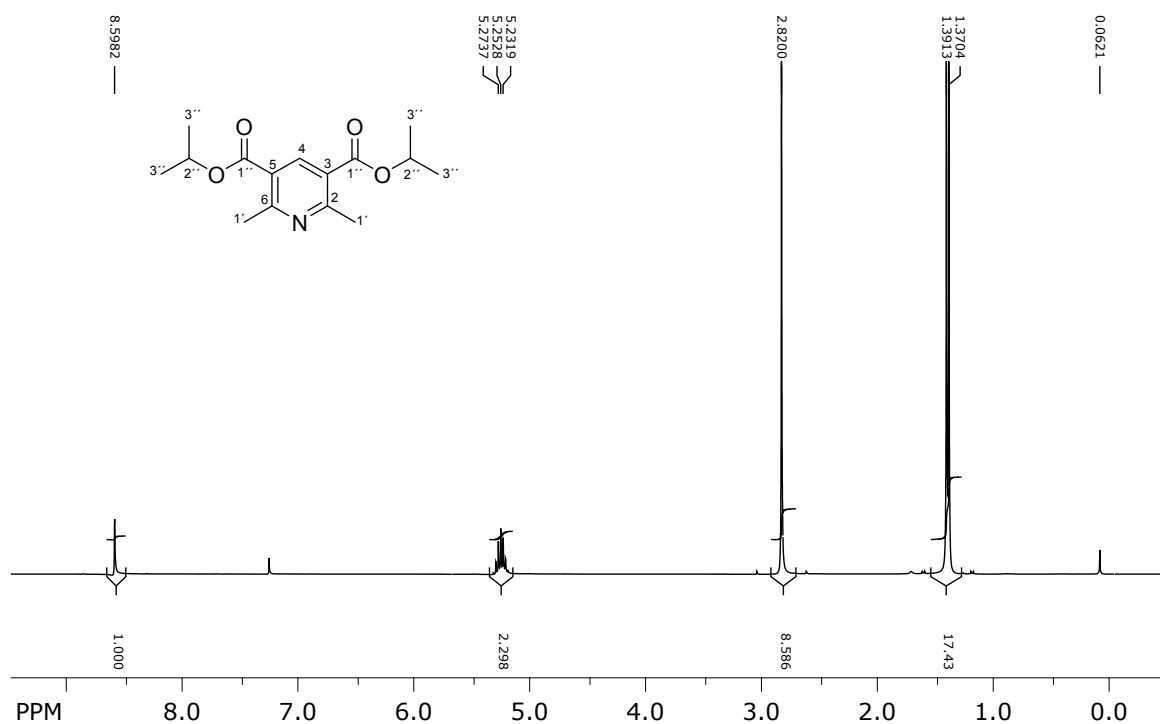
According to the general procedure IV, paraformaldehyde (**1a**) (30 mg, 1 mmol), isopropyl acetoacetate (**2c**) (288 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 30 min. The reaction mixture was treated with methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) for 21 h. Purification afforded di-isopropyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5f**) (159 mg, 57%).

According to the general procedure V, paraformaldehyde (**1a**) (30 mg, 1 mmol), isopropyl acetoacetate (**2c**) (288 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 30 min. The reaction mixture was treated with methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) for 9 h. Purification afforded di-isopropyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5f**) (189 mg, 68%).

Analytical data of 5f

White powder; mp 63 - 65 °C (lit.,⁴ 64 - 66 °C); $R_f = 0.71$ (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 3020, 2984 and 2936 (C-H), 1712 (C=O), 1591 (C=C, C=N), 1221 and 1100

(C-O); δ_{H} (300 MHz; CDCl_3) 1.38 (12H, d, $^3J_{3''\text{-H}, 2''\text{-H}}$ 6.0 Hz, 3''-H), 2.82 (6H, s, 1'-H), 5.25 (2H, sep, $^3J_{2''\text{-H}, 3''\text{-H}}$ 6.0 Hz, 2''-H) and 8.60 (1H, s, 4-H); δ_{C} (75 MHz; CDCl_3) 21.88 (C-3''), 24.96 (C-1'), 69.10 (C-2''), 123.55 (C-3 and C-5), 140.77 (C-4), 161.74 (C-2 and C-6) and 165.65 (C-1''); m/z (EI, 70 eV) 279 (M^+ , 10 %), 250 (22, $\text{M}^+ - \text{C}_2\text{H}_5$), 220 (21), 195 (55) and 31 (100); HRMS (EI, M^+) found: 279.1451 calcd. for $\text{C}_{15}\text{H}_{21}\text{NO}_4$: 279.1471.



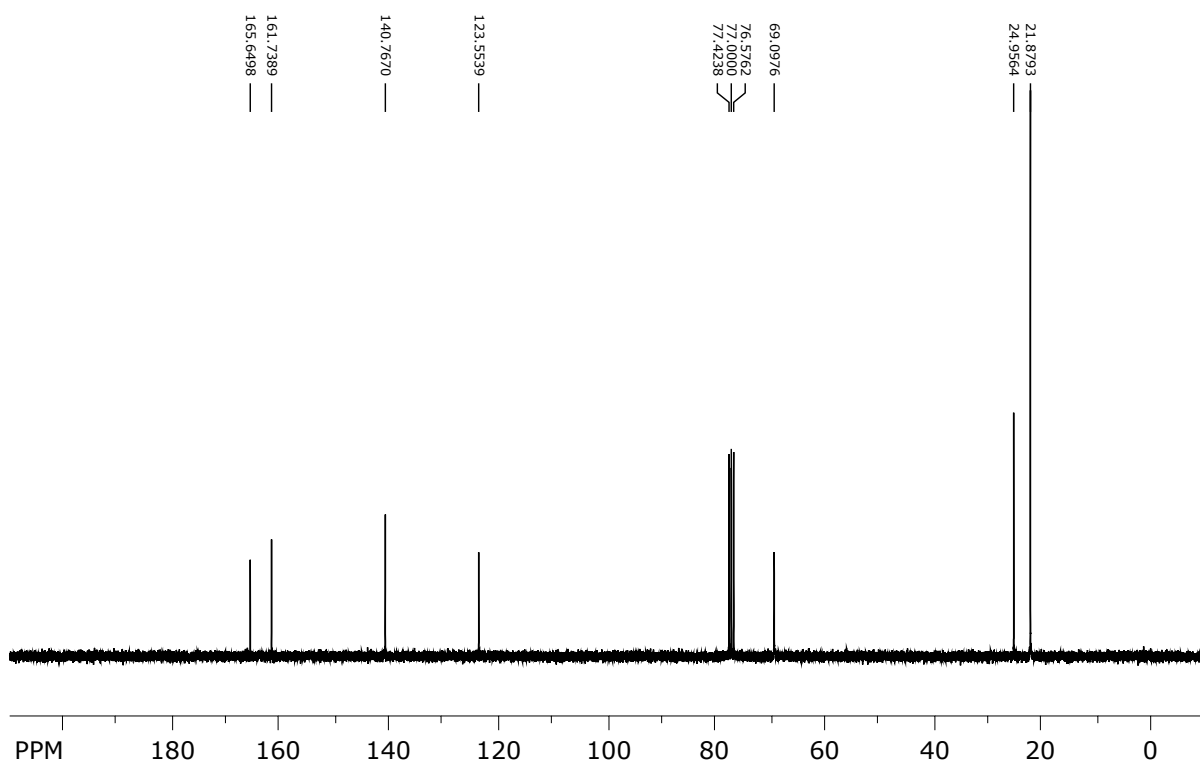


Fig. 17 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **5f** in CDCl_3 .

6.7. Synthesis and analytical data of di-*tert*-butyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5g**)

Synthesis of **5g**

According to the general procedure II, di-*tert*-butyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4g**) (309 mg, 1 mmol), methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were reacted for 15 h. Purification afforded di-*tert*-butyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5g**) (185 mg, 60%).

According to the general procedure III, di-*tert*-butyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4g**) (309 mg, 1 mmol), methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) were reacted for 7 h. Purification afforded di-*tert*-butyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5g**) (275 mg, 90%).

According to the general procedure IV, paraformaldehyde (**1a**) (30 mg, 1 mmol), *tert*-butyl acetoacetate (**2d**) (316 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 30 min. The reaction mixture was treated with methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) for 24 h. Purification afforded di-*tert*-butyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5g**) (93 mg, 30%).

According to the general procedure V, paraformaldehyde (**1a**) (30 mg, 1 mmol), *tert*-butyl acetoacetate (**2d**) (316 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 30 min. The reaction mixture was treated with methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) for 24 h. Purification afforded di-*tert*-butyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5g**) (190 mg, 62%).

Analytical data of **5g**

White powder; mp 107 - 109 °C (lit.,⁴ 108 - 109 °C); R_f = 0.80 (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 3003, 2980 and 2937 (C-H), 1709 (C=O), 1595 (C=N, C=C), 1150 and 1105 (C-O); δ_{H} (300 MHz; CDCl₃) 1.59 (18H, s, 3''-H), 2.79 (6H, s, 1'-H) and 8.51 (1H, s, 4-H); δ_{C} (75 MHz; CDCl₃) 24.95 (C-1'), 28.18 (C-3''), 82.08 (C-2''), 124.61 (C-3 and C-5), 140.74 (C-4), 161.11 (C-2 and C-6) and 165.44 (C-1''); m/z (EI, 70 eV) 279 (M^+ - C₂H₄, 23%), 264 (100) and 251 (77).

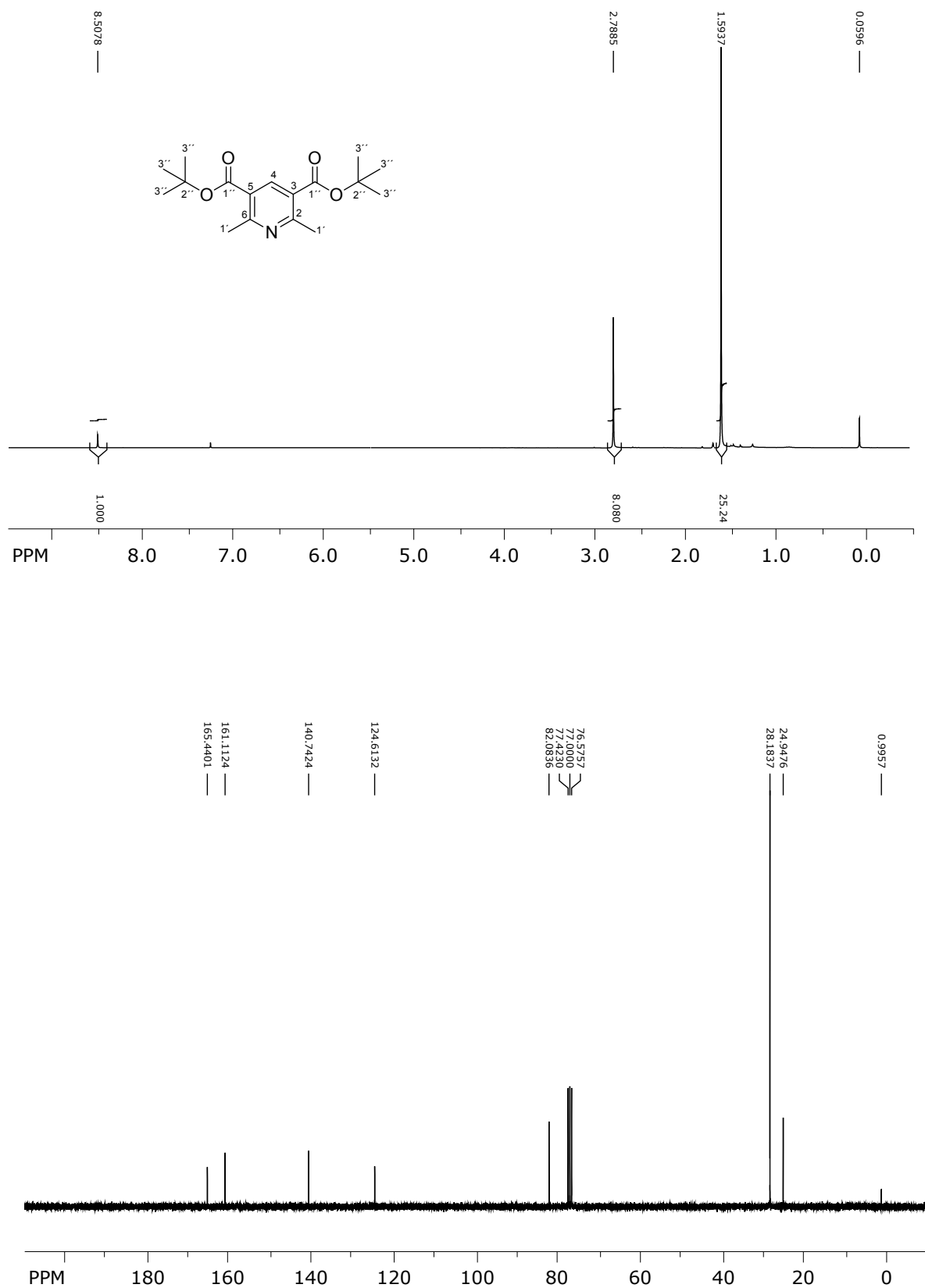


Fig. 18 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **5g** in CDCl_3 .

6.8. Synthesis and analytical data of diallyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5h**)

Synthesis of **5h**

According to the general procedure II, diallyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4h**) (277 mg, 1 mmol), methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were reacted for 7 h. Purification afforded diallyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5h**) (236 mg, 86%).

According to the general procedure III, diallyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (**4h**) (277 mg, 1 mmol), methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) were reacted for 8 h. Purification afforded diallyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5h**) (220 mg, 80%).

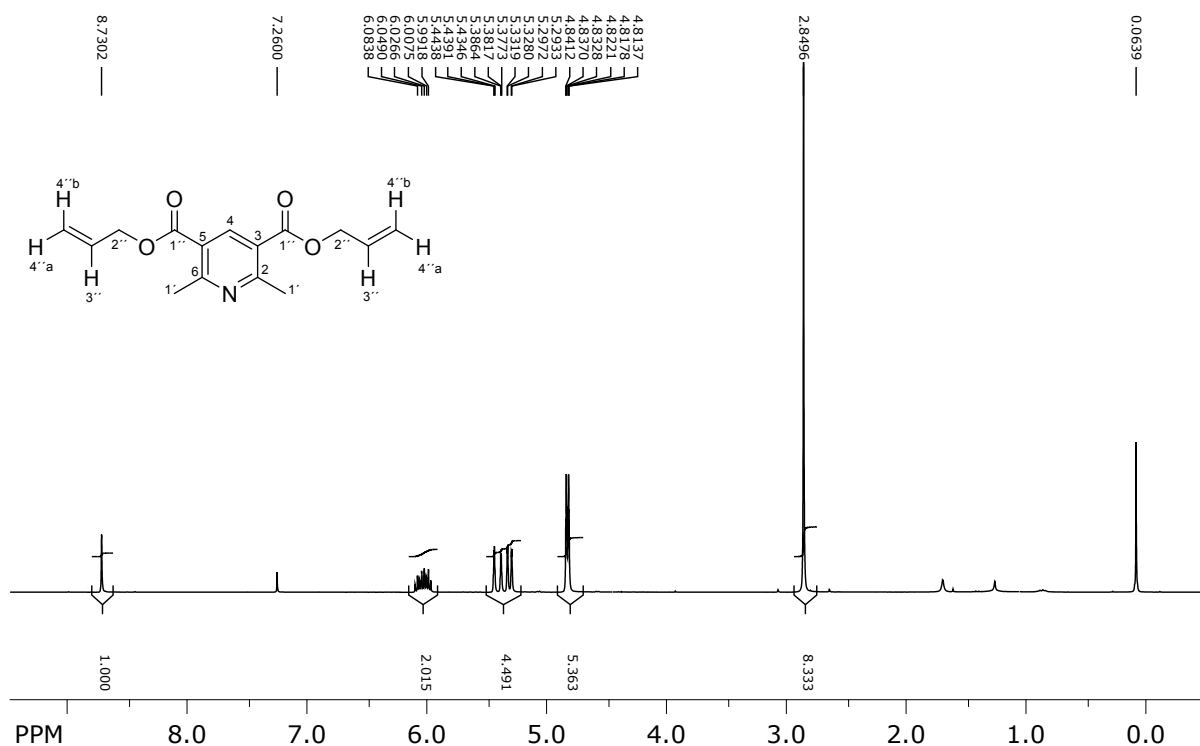
According to the general procedure IV, paraformaldehyde (**1a**) (30 mg, 1 mmol), allyl acetoacetate (**2e**) (284 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 10 min. The reaction mixture was treated with methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) for 24 h. Purification afforded diallyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5h**) (167 mg, 61%).

According to the general procedure V, paraformaldehyde (**1a**) (30 mg, 1 mmol), allyl acetoacetate (**2e**) (284 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 10 min. The reaction mixture was treated with methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) for 9 h. Purification afforded diallyl-2,6-dimethylpyridine-3,5-dicarboxylate (**5h**) (136 mg, 49 %).

Analytical data of **5h**

White powder; mp 64 - 66 °C; $R_f = 0.80$ (petroleum ether / EtOAc = 2:1); $\lambda_{\max}(\text{MeCN})/\text{nm}$ 272 (log ϵ , 3.60), 235 (4.07) and 207 (4.54); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 3091 and 2928 (C-H), 1716 (C=O), 1591 (C=N, C=C), 1219 and 1106 (C-O); δ_{H} (300 MHz; CDCl_3) 2.85 (6H, s, 1'-H), 4.83 (4H, dt, $^3J_{2'}$

H, 3''-H 5.6 Hz, 4J 1.3 Hz, 2''-H), 5.32 (2H, dq, 2J 1.3 Hz, $^3J_{cis}$ 4''a-H, 3''-H 10.5 Hz, 4J 1.3 Hz, 4''a-H), 5.40 (2H, dq, 2J 1.4 Hz, $^3J_{trans}$ 4''b-H, 3''-H 17.2 Hz, 4J 1.4 Hz, 4''b-H), 6.03 (2H, ddt, $^3J_{3''-H, 2''-H}$ 5.7 Hz, $^3J_{cis}$ 3''-H, 4''a-H 10.4 Hz, $^3J_{trans}$ 3''-H, 4''b-H 17.3 Hz, 3''-H) and 8.73 (1H, s, 4-H); δ_C (75 MHz; CDCl₃) 25.00 (C-1'), 65.99 (C-2''), 118.85 (C-4''), 122.73 (C-3 and C-5), 131.79 and 131.81 (C-3'), 141.02 (C-4), 162.60 (C-2 and C-6) and 165.47 (C-1''); m/z (EI, 70 eV) 275 (M⁺, 5%), 234 (15), 218 (33) and 41 (100); HRMS (EI, M⁺) found: 275.1146 calcd. for C₁₅H₁₇NO₄: 275.1158.



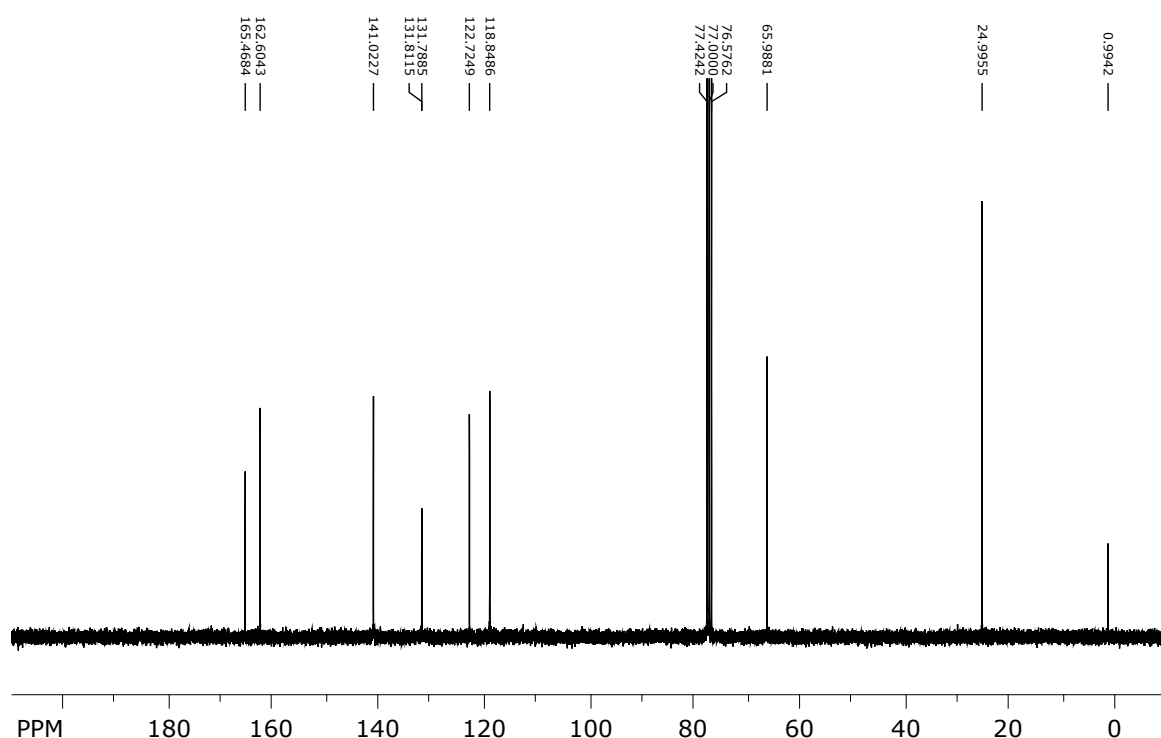


Fig. 19 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **5h** in CDCl_3 .

6.9. Synthesis and analytical data of dimethyl-2,6-diethylpyridine-3,5-dicarboxylate (**5i**)

Synthesis of **5i**

According to the general procedure II, dimethyl-2,6-diethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4i**) (253 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were reacted for 20 h. Purification afforded dimethyl-2,6-diethylpyridine-3,5-dicarboxylate (**5i**) (200 mg, 80%).

According to the general procedure III, dimethyl-2,6-diethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4i**) (253 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) were reacted for 14 h. Purification afforded dimethyl-2,6-diethylpyridine-3,5-dicarboxylate (**5i**) (179 mg, 71%).

According to the general procedure IV, paraformaldehyde (**1a**) (30 mg, 1 mmol), methyl propionyl acetate (**2f**) (260 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 15 min. The reaction mixture was treated with methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) for 26 h. Purification afforded dimethyl-2,6-diethylpyridine-3,5-dicarboxylate (**5i**) (186 mg, 74%).

According to the general procedure V, paraformaldehyde (**1a**) (30 mg, 1 mmol), methyl propionyl acetate (**2f**) (260 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 15 min. The reaction mixture was treated with methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) for 20 h. Purification afforded dimethyl-2,6-diethylpyridine-3,5-dicarboxylate (**5i**) (167 mg, 67%).

Analytical data of **5i**

White powder; mp 50 - 52 °C; $R_f = 0.84$ (petroleum ether / EtOAc = 2:1); $\lambda_{\max}(\text{MeCN})/\text{nm}$ 272 (log ϵ , 3.52), 235 (3.99) and 207 (4.47); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 2976, 2958 and 2938 (C-H), 1716 (C=O), 1593 (C=N, C=C), 1229 and 1048 (C-O); δ_{H} (300 MHz; CDCl_3) 1.30 (6H, t, $^3J_{2\text{'-H}, 1\text{'-H}}$ 7.5 Hz, 2'-H), 3.20 (4H, q, $^3J_{1\text{'-H}, 2\text{'-H}}$ 7.5 Hz, 1'-H), 3.92 (6H, s, 2''-H) and 8.63 (1H, s, 4-H); δ_{C} (75 MHz, CDCl_3) 13.69 (C-2'), 30.36 (C-1'), 52.29 (C-2''), 122.03 (C-3 and C-5), 141.22 (C-4), 166.35 (C-1'') and 167.23 (C-2 and C-6); m/z (EI, 70 eV) 251 (M^+ , 83%), 236 (100, $\text{M}^+ - \text{CH}_3$) and 220 (25, $\text{M}^+ - \text{OCH}_3$); HRMS (EI, M^+) found: 251.1157 calcd. for $\text{C}_{13}\text{H}_{17}\text{NO}_4$: 251.1158.

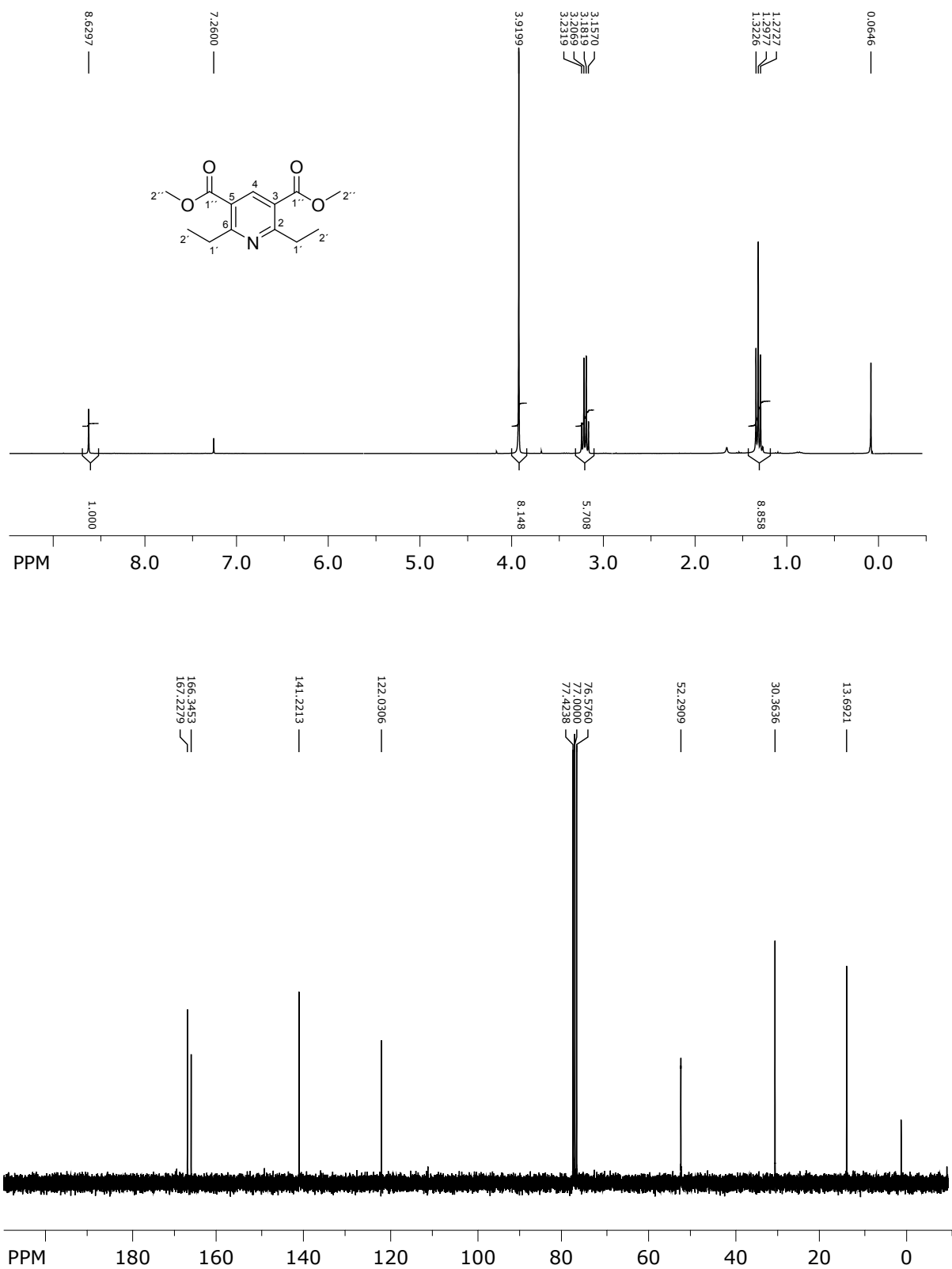


Fig. 20 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **5i** in CDCl_3 .

6.10. Synthesis and analytical data of diethyl-2,6-diethylpyridine-3,5-dicarboxylate (**5j**)

Synthesis of **5j**

According to the general procedure II, diethyl-2,6-diethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4j**) (281 mg, 1 mmol), methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were reacted for 24 h. Purification afforded diethyl-2,6-diethylpyridine-3,5-dicarboxylate (**5j**) (200 mg, 72%).

According to the general procedure III, diethyl-2,6-diethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4j**) (281 mg, 1 mmol), methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) were reacted for 9 h. Purification afforded diethyl-2,6-diethylpyridine-3,5-dicarboxylate (**5j**) (237 mg, 85%).

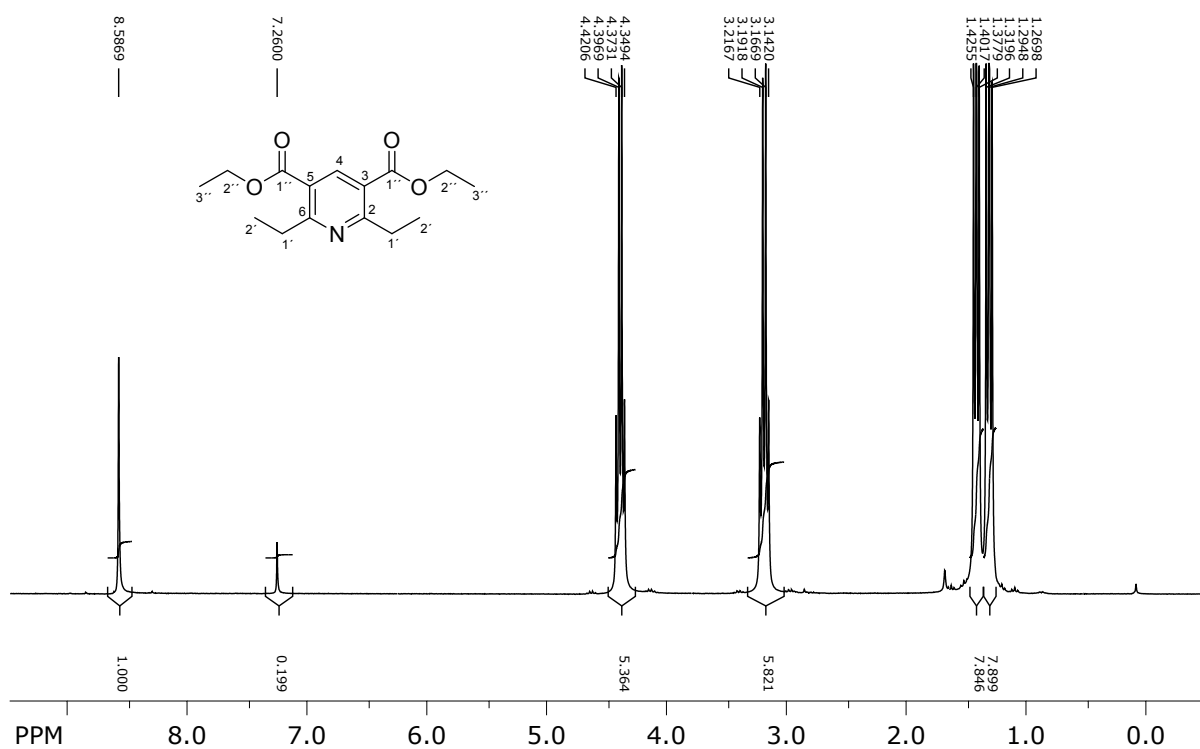
According to the general procedure IV, paraformaldehyde (**1a**) (30 mg, 1 mmol), ethyl propionyl acetate (**2g**) (288 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 40 min. The reaction mixture was treated with methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) for 24 h. Purification afforded diethyl-2,6-diethylpyridine-3,5-dicarboxylate (**5j**) (162 mg, 58%).

According to the general procedure V, paraformaldehyde (**1a**) (30 mg, 1 mmol), ethyl propionyl acetate (**2g**) (288 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 40 min. The reaction mixture was treated with methanol (6 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) for 8 h. Purification afforded diethyl-2,6-diethylpyridine-3,5-dicarboxylate (**5j**) (155 mg, 56%).

Analytical data of **5j**

Colourless solid; mp 35 - 37 °C; R_f = 0.83 (petroleum ether / EtOAc = 2:1); $\lambda_{\max}(\text{MeCN})/\text{nm}$ 272 (log ϵ , 3.58), 235 (4.05) and 206 (4.52); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 2977, 2936 and 2874 (C-H), 1717 (C=O), 1590 (C=N or C=C), 1224 and 1044 (C-O); δ_{H} (300 MHz; CDCl_3) 1.30 (6H, t, $^3J_{2\text{-H}, 1\text{'-H}}$ 7.5 Hz,

2'-H), 1.40 (6H, t, $^3J_{3''\text{-H}, 2''\text{-H}}$ 7.2 Hz, 3''-H), 3.18 (4H, q, $^3J_{1'\text{-H}, 2'\text{-H}}$ 7.5 Hz, 1'-H), 4.39 (4H, q, $^3J_{2''\text{-H}, 3''\text{-H}}$ 7.2 Hz, 2''-H) and 8.59 (1H, s, 4-H); δ_{C} (75 MHz; CDCl_3) 13.80 (C-2'), 14.22 (C-3'), 30.42 (C-1'), 61.35 (C-2''), 122.55 (C-3 and C-5), 141.02 (C-4), 166.13 (C-1'') and 166.78 (C-2 and C-6); m/z (EI, 70 eV) 279 (M^+ , 19%), 250 (100, $\text{M}^+ - \text{C}_2\text{H}_5$) and 222 (43); HRMS (EI, M^+) found: 279.1446 calcd. for $\text{C}_{15}\text{H}_{21}\text{NO}_4$; 279.1471.



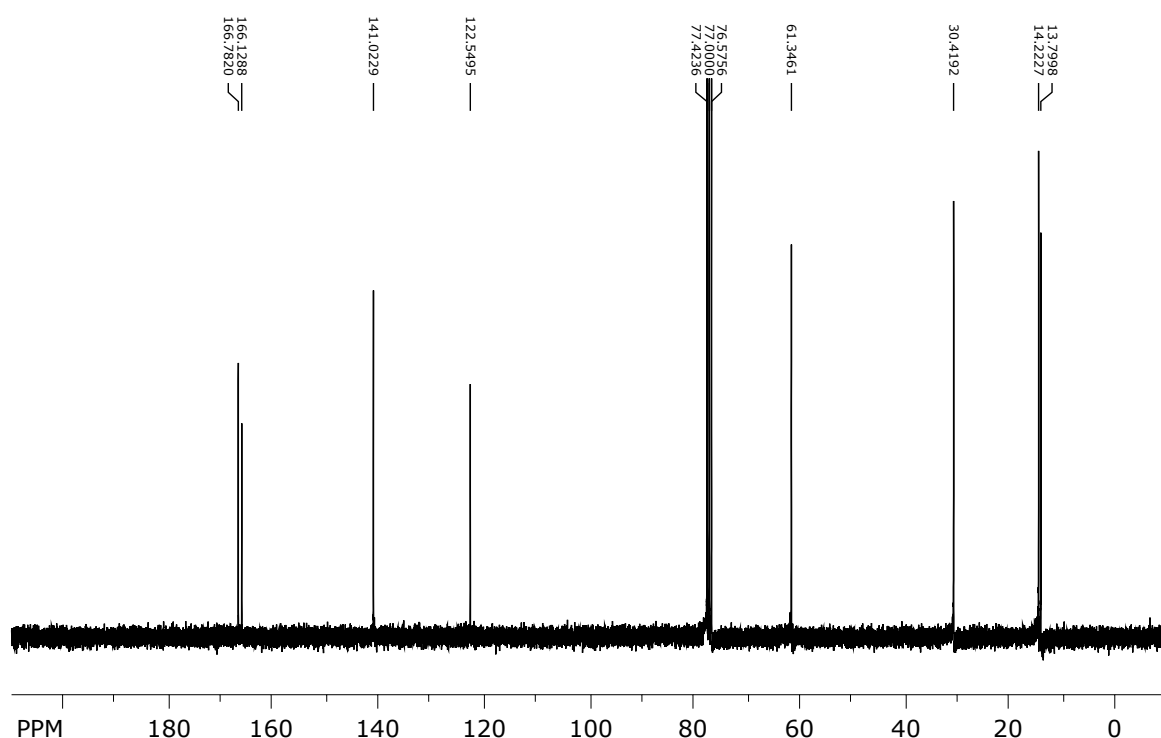


Fig. 21 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **5j** in CDCl_3 .

6.11. Synthesis and analytical data of 2,6-dimethyl-3,5-diacetylpyridine (**5k**)

Synthesis of **5k**

According to the general procedure II, 2,6-dimethyl-3,5-diacetyl-1,4-dihydropyridine (**4k**) (193 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) were reacted for 10 h. Purification by flash chromatography on SiO_2 (petroleum ether / EtOAc = 2:1) afforded 2,6-dimethyl-3,5-diacetylpyridine (**5k**) (165 mg, 86%).

According to the general procedure III, 2,6-dimethyl-3,5-diacetyl-1,4-dihydropyridine (**4k**) (193 mg, 1 mmol), methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) were reacted for 10 h. Purification by flash chromatography on SiO_2 (petroleum ether / EtOAc = 2:1) afforded 2,6-dimethyl-3,5-diacetylpyridine (**5k**) (172 mg, 90%).

According to the general procedure IV, paraformaldehyde (**1a**) (30 mg, 1 mmol), acetylacetone (**2h**) (200 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 15 min. The reaction mixture was treated with methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (6.9 mg, 0.0125 mmol) for 14 h. Purification by flash chromatography on SiO₂ (petroleum ether / EtOAc = 2:1) afforded 2,6-dimethyl-3,5-diacetylpyridine (**5k**) (112 mg, 59%).

According to the general procedure V, paraformaldehyde (**1a**) (30 mg, 1 mmol), acetylacetone (**2h**) (200 mg, 2 mmol) and ammonium acetate (**3**) (116 mg, 1.5 mmol) were reacted for 15 min. The reaction mixture was treated with methanol (3 mL), acetate buffer (0.2 M, pH 4.37, 30 mL), laccase (168 U, 12 mg, *Trametes versicolor*) and ABTS diammonium salt (54.9 mg, 0.1 mmol) for 10 h. Purification by flash chromatography on SiO₂ (petroleum ether / EtOAc = 2:1) afforded 2,6-dimethyl-3,5-diacetylpyridine (**5k**) (115 mg, 60%).

Analytical data of **5k**

White crystals; mp 65 - 67 °C (lit.,⁶ 68 - 70 °C); $R_f = 0.29$ (petroleum ether / EtOAc = 2:1); $\tilde{\nu}_{\max}(\text{atr})/\text{cm}^{-1}$ 2980 (C-H), 1673 (C=O) and 1578 (C=N, C=C); δ_{H} (300 MHz; CDCl₃) 2.61 (6H, s, 2''-H), 2.75 (6H, s, 1'-H) and 8.22 (1H, s, 4-H); δ_{C} (75 MHz; CDCl₃) 24.95 (C-1'), 29.32 (C-2''), 130.11 (C-4), 137.73 (C-3 and C-5), 160.23 (C-2 and C-6) and 199.19 (C-1''); m/z (EI, 70 eV) 191 (M⁺, 37%), 176 (77, M⁺ - CH₃), 106 (39) and 28 (100).

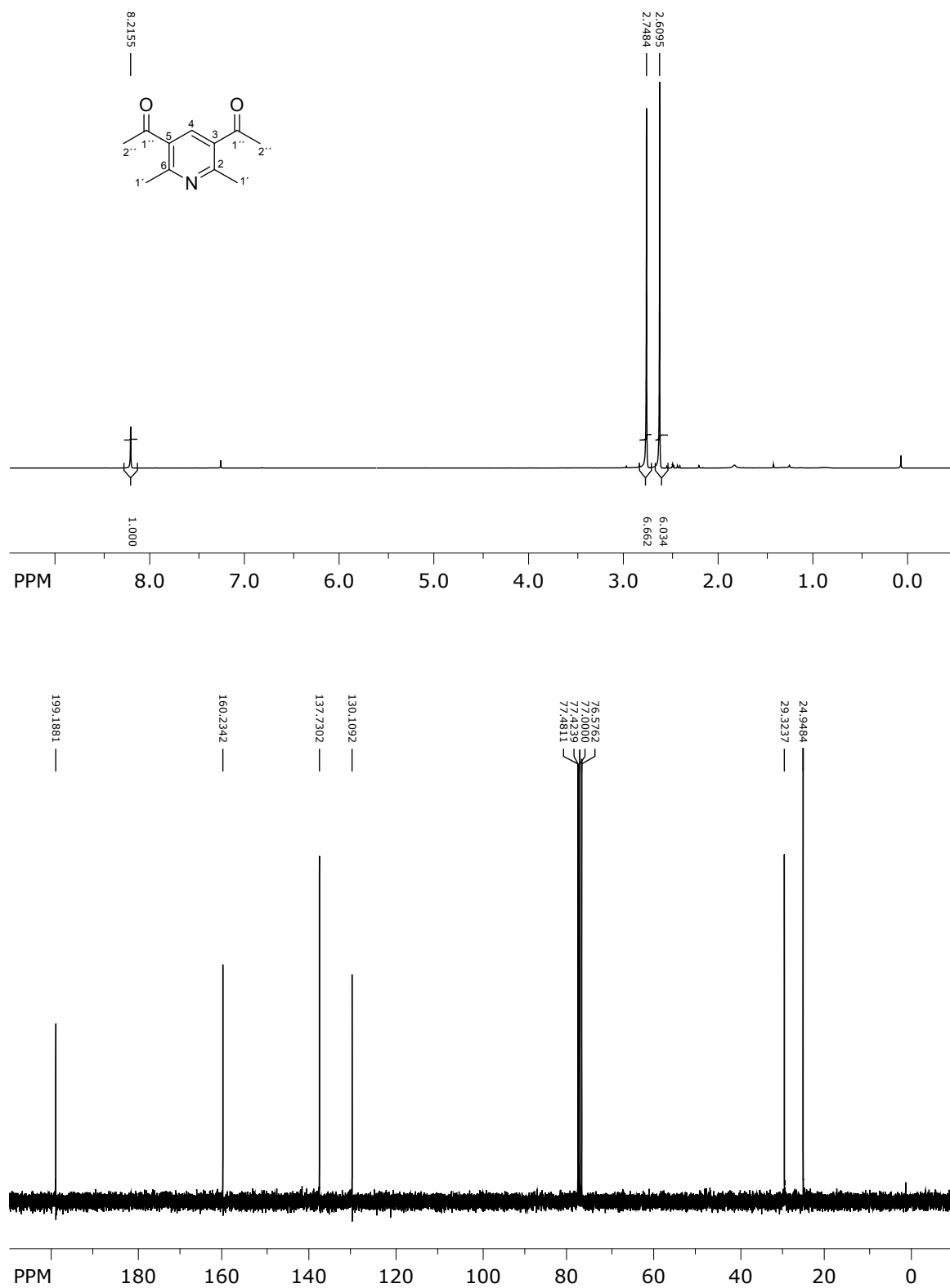


Fig. 22 ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **5k** in CDCl_3 .

7. Determination of the laccase activity⁸

A 0.1 M solution of ABTS (0.3 mL) in 0.2 M acetate buffer (pH 4.37) was diluted with 0.2 M acetate buffer (2.6 mL, pH 4.37) and treated with a solution of laccase in the same buffer (0.1 mL). The change in absorption was followed *via* UV spectroscopy ($\lambda = 414$ nm). One unit was defined as the amount of laccase (*Trametes versicolor*, Fluka) that converts 1 mmol of ABTS per minute at pH 4.37 at rt.

8. References

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