Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Tryptoline-3-hydroxypyridinaldoxime conjugates as efficient reactivators of phosphorylated human acetyl and butyrylcholinesterases

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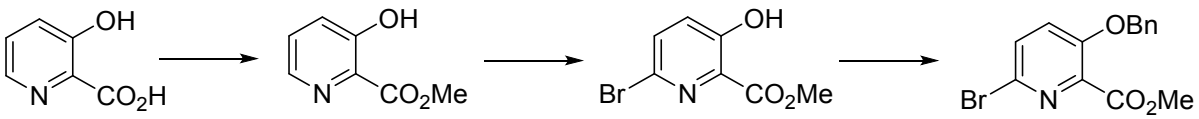
1. Chemistry

General. Column chromatography purifications were performed on Merck silica gel (40-63 µm). Thin-layer chromatography (TLC) was carried out on Merck DC Kieselgel 60 F-254 aluminium sheets. Compounds were visualized by illumination with a short wavelength UV lamp (λ = 254 nm). All solvents were dried following standard procedures (CH_2Cl_2, CH_3CN: distillation over P_2O_5, DMF: distillation over BaO under reduced pressure, THF: distillation over Na/benzophenone). Triethylamine was distilled from CaH_2 and stored over BaO or KOH. ^1H and ^{13}C NMR spectra were recorded on a Bruker DPX 300 spectrometer (Bruker, Wissembourg, France). Chemical shifts are expressed in parts per million (ppm) from $CDCl_3$ (δ_H= 7.26, δ_C= 77.16), MeOD (δ_H= 3.31, δ_C= 49.00) or DMSO (δ_H= 2.50, δ_C= 39.52).[1] *J* values are expressed in Hz. Residual solvents contained in NMR sample were indicated on spectra. Mass spectra were obtained with a Finnigan LCQ Advantage MAX (ion trap) apparatus equipped with an electrospray source. All analyses were performed in the positive mode.

All final oximes were confirmed to be ≥ 95% purity based on HPLC analysis. Analytical HPLC was performed on a Thermo Electron Surveyor instrument equipped with a PDA detector under the following conditions: Thermo Hypersil GOLD C18 column (5 µm, 4.6 x 100 mm) with MeOH and 0.1% aq. trifluoroacetic acid (TFA) as eluents [0.1% aq. TFA/MeOH (90/10) (5 min), followed by linear gradient from 10% to 100% of MeOH (45 min)] at a flow rate of 1.0 mL/min and UV detection Max Plot 220-360 nm.

Methyl 3-benzyloxy-6-bromopicolinate 6



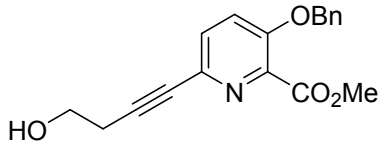
6

H_2SO_4 (1.8 mL, 31.5 mmol, 3 equiv.) was added dropwise to a suspension of 3-hydroxypicolinic acid (1.5 g, 10.5 mmol) in MeOH (24 mL). The mixture was refluxed for 24 h. Then, the mixture was neutralized with an aqueous solution of K_2CO_3 (pH 8.5). The aqueous layer was extracted with EtOAc (thrice). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give methyl 3-hydroxypicolinate (1.28 g, 80%) as a white solid. ^1H and ^{13}C NMR data *were* in agreement with *those* given in the *literature.*[1]

At 0°C, Br_2 (335 µL, 6.5 mmol) was added portionwise (4 x 84 µL in 2 h) to a suspension of previous methyl ester (1 g, 6.5 mmol) in water (40 mL). The mixture was stirred at 0 °C for 2 h then 15 h at rt. The solution was extracted with dichloromethane (thrice). The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. Purification by chromatography on silica gel (cylohexane/EtOAc 8/2, v/v) afforded methyl 6-bromo-3-hydroxypicolinate (796 mg, 53%) as a white powder. R*f* = 0.45 (cylohexane/EtOAc 8/2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.07 (s, 3H), 7.29 (d, *J* = 8.7 Hz, 1H), 7.58 (dd, *J* = 0.3, 8.7 Hz, 1H), 10.72 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 53.5 (CH_3), 129.5 (CH), 130.0 (C), 130.7 (C), 134.5 (CH), 158.5 (C), 169.1 (C). MS (ESI+): *m/z* (%): 234 (85) and 232 (100) $[M+H]^+$.

Benzyl bromide (770 µL, 6.3 mmol, 3 equiv.) was slowly added to a mixture of previous compound (500 mg, 2.1 mmol) and K_2CO_3 (1.3 g, 10.8 mmol, 4.5 equiv.) in acetone (30 mL). The solution was refluxed for 15 h. The resulting mixture was filtered and concentrated under reduced pressure. Purification by chromatography on silica gel (cyclohexane/EtOAc 9/1, v/v) gave **6** (631 mg, 93%) as a white solid. R*f* = 0.3 (cyclohexane/EtOAc 8/2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 3.98 (s, 3H), 5.22 (s, 2H), 7.25 (d, *J* = 8.7 Hz, 1H), 7.46-7.34 (m, 5H), 7.51 (d, *J* = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 52.9 (CH_3), 71.2 (CH_2), 125.0 (CH), 126.9 (2 x CH), 128.4 (CH), 128.8 (2 x CH), 131.2 (C), 131.4 (CH), 135.2 (C), 139.8 (C), 154.0 (C), 164.0 (C). MS (ESI+): *m/z* (%): 324 (85) and 322 (100) $[M+H]^+$.

Methyl 3-(benzyloxy)-6-(4-hydroxybut-1-ynyl)picolinate 9



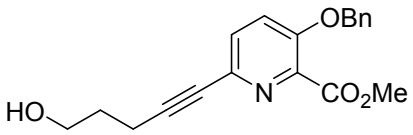
9

To a solution of methyl 3-(benzyloxy)-6-bromopicolinate **6** (800 mg, 2.48 mmol) in CH_2Cl_2 (25 mL) and Et_3N (13 mL) was added 3-butyn-1-ol (174 mg, 2.48 mmol). The resulting mixture was degassed for 15 min. CuI (47 mg, 0.25 mmol, 0.1 equiv.) and $Pd(PPh_3)_4$ (143 mg, 0.12 mmol, 0.05 equiv.) were then poured and the solution was stirred under argon at rt overnight. The reaction mixture was concentrated under reduced pressure. Purification by chromatography on silica gel (cyclohexane/EtOAc 4/6 to 3/7, v/v) afforded the desired

[1] Louise-Leriche, L.; Paunescu, E.; Saint-André, G.; Baati, R.; Romieu, A.; Wagner, A.; Renard, P.-Y., *Chem. Eur. J.* **2010,** *16*, 3510-3523.

product **9** as a yellow solid (770 mg, 99%). R*f* = 0.15 (cyclohexane/EtOAc 4/6, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 2.66 (t, J = 6.6 Hz, 2H), 3.00 (br s, 1H), 3.82 (t, J = 6.6 Hz, 2H), 3.95 (s, 3H), 4.29-4.35 (m, 1H), 5.18 (s, 2H), 7.28-7.44 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.8 (CH_2), 52.7 (CH_3), 60.7 (CH_2), 70.9 (CH_2), 80.6 (C), 87.4 (C), 121.9 (CH), 126.9 (2 x CH), 128.3 (CH), 128.8 (2 x CH), 130.1 (CH), 135.0 (C), 135.5 (C), 139.9 (C), 153.2 (C), 164.8 (C). MS (ESI+): *m/z* (%): 312 (100) $[M+H]^+$.

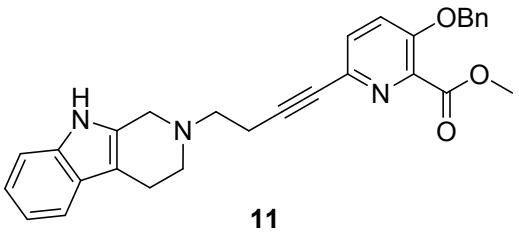
Methyl 3-(benzyloxy)-6-(5-hydroxypent-1-ynyl)picolinate 10



10

To a solution of methyl 3-(benzyloxy)-6-bromopicolinate **6** (800 mg, 2.48 mmol) in CH_2Cl_2 (25 mL) and Et_3N (13 mL) was added 4-pentyn-1-ol (208 mg, 2.48 mmol). The resulting mixture was degassed for 15 min. CuI (47 mg, 0.25 mmol, 0.1 equiv.) and $Pd(PPh_3)_4$ (143 mg, 0.12 mmol, 0.05 equiv.) were then poured and the solution was stirred under argon at rt overnight. The reaction mixture was concentrated under reduced pressure. Purification by flash chromatography (cyclohexane/EtOAc 5/5 to 4/6, v/v) afforded the desired product **10** as a yellow solid (800 mg, 99%). R*f* = 0.16 (cyclohexane/EtOAc 4/6, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.86 (qt, J = 6.4 Hz, 2H), 2.38 (br s, 1H), 2.54 (t, J = 7.0 Hz, 2H), 3.78 (t, J = 6.2 Hz, 2H), 3.96 (s, 3H), 5.20 (s, 2H), 7.30-7.45 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.9 (CH_2), 31.0 (CH_2), 52.7 (CH_3), 61.4 (CH_2), 70.9 (CH_2), 79.6 (C), 89.8 (C), 121.9 (CH), 127.0 (2 x CH), 128.3 (CH), 128.8 (2 x CH), 130.1 (CH), 135.4 (C), 135.6 (C), 140.0 (C), 153.0 (C), 164.9 (C). MS (ESI+): *m/z* (%): 326 (100) $[M+H]^+$, 348 (95) $[M+Na]^+$ and 673 (100) $[2M + Na]^+$.

Methyl 3-(benzyloxy)-6-(4-(3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)but-1-ynyl)picolinate 11

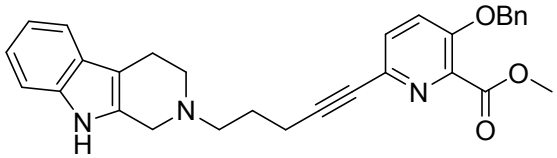


11

To a mixture of methyl 3-(benzyloxy)-6-(4-hydroxybut-1-ynyl)picolinate **9** (771 mg, 2.5 mmol) in dry CH_2Cl_2 (25 mL) was added Et_3N (1 mL, 7.5 mmol, 3 equiv.) and MsCl (290 µL, 3.8 mmol, 1.5 equiv.). The mixture was stirred at reflux for 4h. The resulting solution was cooled to rt and filtrated under Celite.

The crude product was dissolved in CH_3CN (30 mL) with 2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole **5** (430 mg, 2.5 mmol, 1 equiv.) and K_2CO_3 (1 g, 7.5 mmol, 3 equiv.). The solution was heated under reflux for 24 h and then cooled at rt. Salts were filtrated. Concentration under reduced pressure and purification by flash chromatography (cyclohexane/EtOAc 1/9, v/v) afforded the desired product **11** as a brown solid (389 mg, 34%). R*f* = 0.21 (cyclohexane/EtOAc 1/9, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 2.71 (t, J = 14.7 Hz, 2H), 2.81-2.84 (m, 2H), 2.91-2.95 (m, 4H), 3.82 (s, 2H), 3.97 (s, 3H), 5.20 (s, 2H), 7.05-7.15 (m, 2H), 7.25-7.48 (m, 9H), 8.00 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.6 (CH_2), 21.2 (CH_2), 50.0 (CH_2), 51.1 (CH_2), 52.8 (CH_3), 55.6 (CH_2), 71.0 (CH_2), 80.3 (C), 88.7 (C), 108.2 (C), 110.9 (CH), 118.0 (CH), 119.3 (CH), 121.4 (CH), 121.9 (CH), 127.0 (2 x CH), 127.3 (C), 128.4 (CH), 128.9 (2 x CH), 130.1 (CH), 131.7 (C), 135.4 (C), 135.6 (C), 136.2 (C), 140.0 (C), 153.2 (C), 165.0 (C). MS (ESI+): *m/z* (%): 466 (100) $[M+H]^+$, 931 (33) $[2M + H]^+$.

Methyl 3-(benzyloxy)-6-(5-(3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)pent-1-ynyl)picolinate 12

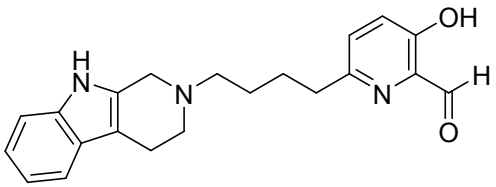


12

To a mixture of methyl 3-(benzyloxy)-6-(5-hydroxypent-1-ynyl)picolinate **10** (800 mg, 2.5 mmol) in dry CH_2Cl_2 (25 mL) was added Et_3N (1 mL, 7.5 mmol, 3 equiv.) and MsCl (290 µL, 3.8 mmol, 1.5 equiv.). The mixture was stirred at reflux for 4 h. The resulting solution was cooled to rt and filtrated under Celite.

The crude product was placed in CH_3CN (30 mL) with 2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole **5** (430 mg, 2.5 mmol, 1 equiv.) and K_2CO_3 (1 g, 7.5 mmol, 3 equiv.). The solution was heated under reflux for 24 h and then cooled at rt. Salts were removed by filtration. Concentration under reduced pressure and purification by flash chromatography (cyclohexane/EtOAc 1/9, v/v to 100% EtOAc) afforded the desired product **12** as orange oil (458 mg, 38%). R*f* = 0.14 (cyclohexane/EtOAc 1/9, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.81 (qt, J = 6.9 Hz, 2H), 2.45 (t, J = 7.2 Hz, 2H), 2.63 (t, J = 6.9 Hz, 2H), 2.78-2.81 (m, 4H), 3.49 (s, 2H), 3.93 (s, 3H), 5.13 (s, 2H), 7.05-7.10 (m, 2H), 7.16-7.43 (m, 9H), 8.62 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.4 (CH_2), 21.4 (CH_2), 25.9 (CH_2), 50.1 (CH_2), 51.2 (CH_2), 52.8 (CH_3), 56.7 (CH_2), 70.7 (CH_2), 77.6 (C), 90.3 (C), 107.9 (C), 110.9 (CH), 117.9 (CH), 118.6 (CH), 119.0 (CH), 121.1 (CH), 121.9 (CH), 126.9 (2 x CH), 127.2 (C), 128.3 (CH), 128.8 (2 x CH), 130.2 (CH), 132.2 (C), 135.4 (C), 135.5 (C), 136.2 (C), 139.8 (C), 153.0 (C), 165.0 (C). MS (ESI+): *m/z* (%): 480 (100) $[M+H]^+$.

6-(4-(3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)butyl)-3-hydroxypicolinaldehyde 13



13

Methyl 3-(benzyloxy)-6-(4-(3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)but-1-ynyl)picolinate **11** (389 mg, 0.84 mmol) was dissolved in degassed mixture of MeOH (40 mL) and EtOAc (20 mL). Pearlman's catalyst (480 mg, 0.19 mmol, 0.4 equiv., 20% Pd, moisture 50%) was added and the solution was bubbled with H_2 and the reaction was stirred at rt under H_2 *atmosphere* (1 atm) for 24 h. The mixture was filtrated through Celite and concentrated under reduced pressure.

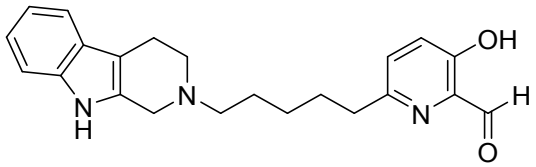
To a solution of crude product in dry CH_2Cl_2 (6 mL) were successively added 2,6-lutidine (209 µL, 1.8 mmol, 3 equiv.) and TBDMSOTf (413 µL, 1.8 mmol, 3 equiv.). The mixture was stirred at rt for 4 h under argon atmosphere. The mixture was washed with NaCl sat., dried over $MgSO_4$ and concentrated under reduced pressure.

To a solution of the resulting residue in dry CH_2Cl_2 (6 mL) was added dropwise DIBAL-H (1.8 mL, 1M in CH_2Cl_2, 1.8 mmol, 3 equiv.) at -78°C. Then, the reaction mixture was stirred

at this temperature for 12 min. The reaction was quenched with MeOH (1.8 mL) and the mixture was allowed to warm at rt. The organic layer was washed with an aqueous solution of NaOH (1M), dried over $MgSO_4$ and concentrated under reduced pressure.

Then, TBAF (650 µL, 1M in THF, 0.65 mmol, 1.1 equiv.) was added at 0 °C to the residue in dry THF (6 mL) and the mixture was stirred for 2 h at this temperature. After concentration under reduced pressure, a chromatography on silica gel (EtOAc/MeOH 9/1, v/v) afforded **13** as a yellow solid (60 mg, 29%). R*f* = 0.58 (EtOAc/MeOH 9/1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.61-1.66 (m, 2H), 1.75-1.80 (m, 2H), 2.57 (t, J = 7.2 Hz, 2H), 2.78-2.87 (m, 6H), 3.53 (s, 2H), 7.03-7.12 (m, 2H), 7.22-7.26 (s, 3H), 7.44 (d, J = 7.5 Hz, 1H), 8.34 (br s, 1H), 10.02 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2 (CH_2), 26.9 (CH_2), 27.6 (CH_2), 37.1 (CH_2), 50.3 (CH_2), 51.0 (CH_2), 57.4 (CH_2), 108.2 (C), 110.8 (CH), 118.0 (CH), 119.3 (CH), 121.3 (CH), 126.5 (CH), 127.2 (C), 129.8 (CH), 131.7 (C), 135.7 (C), 136.2 (C), 154.7 (C), 157.1 (C), 198.7 (C). MS (ESI+): *m/z* (%): 350 (100) $[M+H]^+$.

6-(5-(3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)pentyl)-3-hydroxypicolinaldehyde 14



14

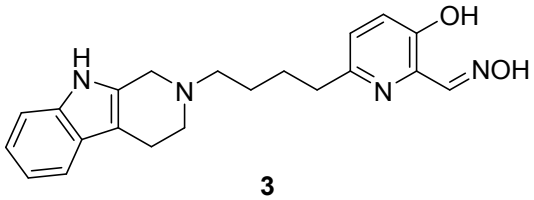
Methyl 3-(benzyloxy)-6-(5-(3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)pent-1-ynyl)picolinate **12** (458 mg, 0.96 mmol) was dissolved in degassed mixture of MeOH (50 mL) and EtOAc (25 mL). Pearlman's catalyst (270 mg, 0.19 mmol, 0.2 equiv., 20% Pd, moisture 50%) was added and the solution was bubbled with H_2 and the reaction was stirred at rt under H_2 *atmosphere* (1 atm) for 2 h. The mixture was filtrated through Celite and concentrated under reduced pressure.

To a solution of crude product in dry CH_2Cl_2 (10 mL) were successively added 2,6-lutidine (334 µL, 2.9 mmol, 3 equiv.) and TBDMSOTf (666 µL, 2.9 mmol, 3 equiv.). The mixture was stirred at rt for 4 h under argon atmosphere. The mixture was washed with NaCl sat., dried over $MgSO_4$ and concentrated under reduced pressure.

To a solution of the resulting residue in dry CH_2Cl_2 (10 mL) was added dropwise DIBAL-H (2.9 mL, 1M in CH_2Cl_2, 2.9 mmol, 3 equiv.) at -78°C. Then, the reaction mixture was stirred at this temperature for 12 min. The reaction was quenched with MeOH (2.9 mL) and the mixture was allowed to warm at room temperature. The organic layer was washed with an

aqueous solution of NaOH (1 M), dried over $MgSO_4$ and concentrated under reduced pressure. Then, TBAF (1.1 mL, 1M in THF, 1.1 mmol, 1.1 equiv.) was added at 0 °C to the residue in dry THF (10 mL) and the mixture was stirred for 16 h at this temperature. After concentration under reduced pressure, a chromatography on silica gel (EtOAc/MeOH 95/5, v/v) afforded access to **14** as a yellow solid (119 mg, 34%). R*f* = 0.68 (EtOAc/MeOH 8/2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.35-1.41 (m, 2H), 1.58-1.63 (m, 2H), 1.70-1.77 (m, 2H), 2.48 (t, J = 7.2 Hz, 2H), 2.76-2.83 (m, 6H), 3.48 (s, 2H), 7.04-7.12 (m, 2H), 7.20-7.26 (m 3H), 7.42-7.45 (m, 1H), 8.43 (br s, 1H), 10.04 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2 (CH_2), 22.7 (CH_2), 27.0 (CH_2), 27.2 (CH_2), 29.7 (CH_2), 37.3 (CH_2), 50.3 (CH_2), 50.9 (CH_2), 57.6 (CH_2), 108.1 (C), 110.8 (CH), 117.9 (CH), 119.2 (CH), 121.2 (CH), 126.5 (CH), 127.2 (C), 129.8 (CH), 131.7 (C), 135.7 (C), 136.1 (C), 154.9 (C), 157.1 (C), 198.7 (C). MS (ESI+): *m/z* (%): 364 (100) $[M+H]^+$.

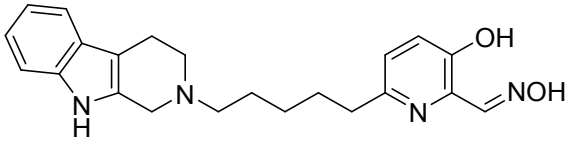
6-(4-(3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)butyl)-3-hydroxypicolinaldehyde oxime 3



3

To a solution of 6-(4-(3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)butyl)-3-hydroxypicolinaldehyde **13** (60 mg, 0.17 mmol) in dry EtOH (5 mL) were successively added $NH_2OH.HCl$ (15 mg, 0.21 mmol, 1.2 equiv.) and NaOAc (19 mg, 0.22 mmol, 1.3 equiv.). The mixture was stirred at rt for 3 h under argon atmosphere. After concentration under reduced pressure, a chromatography on silica gel (EtOAc/MeOH 95/5, v/v) afforded **3** as a white solid (14 mg, 22%). R*f* = 0.49 (EtOAc/MeOH 9/1, v/v). ^{1}H NMR (300 MHz, DMSO) δ (ppm) 1.55-1.59 (m, 2H), 1.64-1.76 (m, 2H), 2.51-2.57 (m, 2H), 2.65-2.72 (m, 6H), 3.56 (s, 2H), 6.90-7.02 (m, 2H), 7.15 (d, J = 8.4 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 7.6 Hz, 1H), 8.28 (s, 1H), 10.11 (br s, 1H), 11.82 (br s, 1H). ^{13}C NMR (75 MHz, DMSO) δ 21.2 (CH_2), 26.4 (CH_2), 27.3 (CH_2), 36.4 (CH_2), 50.1 (CH_2), 50.9 (CH_2), 57.1 (CH_2), 106.5 (C), 110.8 (CH), 117.3 (CH), 118.2 (CH), 120.2 (CH), 123.8 (CH), 124.1 (CH), 126.7 (C), 132.9 (C), 135.4 (C), 135.8 (C), 151.1 (C), 151.4 (C), 152.9 (CH). MS (ESI+): *m/z* (%): 365 (100) $[M+H]^+$. HPLC: t_R = 23.02 min (purity = 97.4%). HRMS (ESI+): *m/z* calcd for $C_{21}H_{25}N_4O_2$ 365.1978; found: 365.1970.

6-(5-(3,4-dihydro-1*H*-pyrido[3,4-b]indol-2(9*H*)-yl)pentyl)-3-hydroxypicolinaldehyde oxime 4



4

To a solution of 6-(5-(3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)pentyl)-3-hydroxypicolinaldehyde **14** (119 mg, 0.33 mmol) in dry EtOH (5 mL) were successively added $NH_2OH.HCl$ (27 mg, 0.39 mmol, 1.2 equiv.) and NaOAc (35 mg, 0.43 mmol, 1.3 equiv.). The mixture was stirred at rt for 1 h under argon atmosphere. After concentration under reduced pressure, chromatography on silica gel (EtOAc/MeOH 9/1, v/v) afforded **4** as a white solid (31 mg, 25%). R*f* = 0.68 (EtOAc/MeOH 9/1 v/v). ^{1}H NMR (300 MHz, MeOD) δ (ppm) 1.43 (dt, J = 7.4, 15.0 Hz, 2H), 1.67-1.82 (m, 4H), 2.71-2.85 (m, 4H), 2.91 (t, J = 5.8 Hz, 2H), 3.10 (t, J = 5.8 Hz, 2H), 3.92 (s, 2H), 6.95-7.10 (m, 2H), 7.17 (d, J = 8.5 Hz, 1H), 7.28 (dd, J = 3.1, 8.2 Hz, 2H), 7.40 (d, J = 7.6 Hz, 1H), 8.30 (s, 1H).

^{13}C NMR (75 MHz, MeOD) δ 21.4 (CH_2), 26.9 (CH_2), 27.9 (CH_2), 31.0 (CH_2), 37.7 (CH_2), 51.0 (CH_2), 52.2 (CH_2), 58.4 (CH_2), 107.6 (C), 112.0 (CH), 118.6 (CH), 119.9 (CH), 122.3 (CH), 125.4 (CH), 126.1 (CH), 127.9 (C), 130.8 (C), 136.2 (C), 138.0 (C), 152.7 (2 x C), 154.5 (CH). MS (ESI+): *m/z* (%): 379 (100) $[M+H]^+$. HPLC: t_R = 24.88 min (purity = 96.0%). HRMS (ESI+): *m/z* calcd for $C_{22}H_{26}N_4O_2$ 379.2134; found: 379.2133.

2. Biological assays

Inhibition of *hAChE* and *h*BChE by OPNAs. Recombinant *h*AChE and *h*BChE were produced and purified as previously described.[2,3] VX and tabun were from DGA maîtrise NRBC (Vert le Petit, France). Paraoxon-ethyl was purchased from Sigma-Aldrich. HI-6 was from Pharmacie Centrale des Armées (Orléans, France). All other chemicals including paraoxon were from Sigma. Stock solution of VX and tabun were 5 mM in isopropanol. The inhibition of 120 µM *h*AChE or 100 µM of *h*BChE is realized with a 5-fold excess of OPNAs

[2] Carletti, E.; Li, H.; Li, B.; Ekström, F.; Nicolet, Y.; Loiodice, M.; Gillon, E.; Froment, M. T.; Lockridge, O.; Schopfer, L. M.; Masson, P.; Nachon, F., *J. Am. Chem. Soc.* **2008,** *130*, 16011-16020.
[3] Brazzolotto, X.; Wandhammer, M.; Ronco, C.; Trovaslet, M.; Jean, L.; Lockridge, O.; Renard, P.-Y.; Nachon, F., *FEBS J.* **2012,** *279*, 2905-2916.

and was performed in tris buffer (20 mM, pH 7.4, 0.1% BSA) at 25 °C. After a 20-minute incubation, inhibited *h*AChE or *h*BChE was desalted on PD-10 column (GE Healthcare).

Reactivation of *h*AChE and *h*BChE inhibited by OPNAs. OPNA-inhibited *h*AChE was incubated at 37 °C with different concentrations of oxime in 0.1 M phosphate buffer, pH 7.4, 0.1% BSA, 5% methanol (See table below). Methanol was used for complete dissolution of the oximes. 50-µl aliquots of mix was transferred to 1-mL cuvettes at time intervals ranging from 1 to 10 minutes depending on the reactivation rate, for measurement of *h*AChE activity (1 mM acetylthiocholine) or *h*BChE activity (1 mM butyrylthiocholine), in Ellman's buffer (phosphate 0.1 M, pH 7.4, 0.1% BSA, 0.5 mM DTNB, 25 °C). The increase in absorbance at 412 nm was followed on a Uvikon 943 spectrophotometer.

The enzyme activity in the control remained constant during the experiment. The percentage of reactivated enzyme ($\%E_{react}$) was calculated as the ratio of the recovered enzyme activity and activity in the control. The apparent reactivation rate k_{obs} for each oxime concentration, the dissociation constant K_D of inhibited enzyme-oxime complex (E-POx) and the maximal reactivation rate constant k_r, were calculated by non-linear fit using the standard oxime concentration-dependent reactivation equation derived from the following scheme:

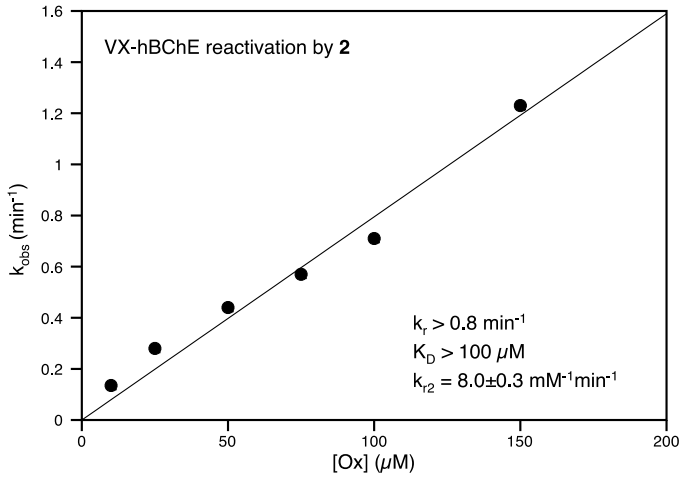
$$\text{E-P} + \text{Ox} \underset{}{\overset{K_D}{\rightleftharpoons}} \text{E-POx} \xrightarrow{k_r} \text{E} + \text{POx}$$

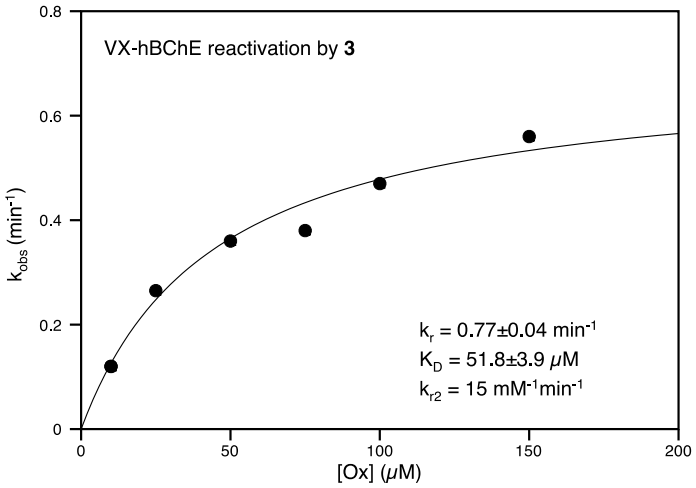
$$\%E_{react} = 100 \cdot (1-e^{k_{obs} \cdot t}) \quad \text{and} \quad k_{obs} = \frac{k_r[Ox]}{K_D + [Ox]}$$

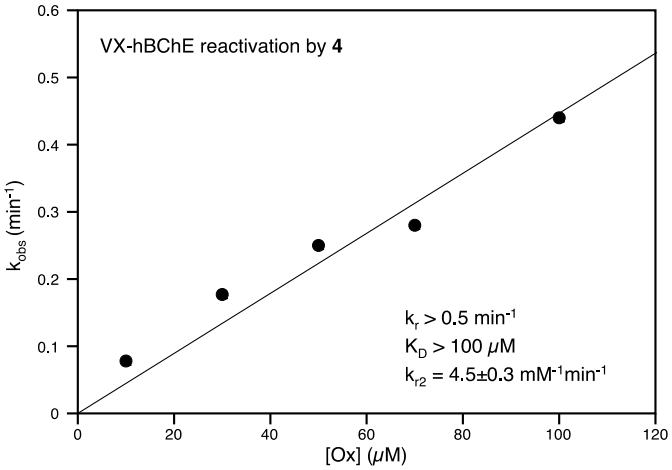
Concentration of oximes **2-4** (µM) used to determine the concentration dependence of the reactivation rate k_{obs} for reactivation of VX-*h*BChE and VX-, tabun- and paraoxon-inhibited *h*AChE.

	VX-*h*BChE	VX-*h*AChE	tabun-*h*AChE	paraoxon-*h*AChE
2	10-25-50-75 100-150	-	-	-
3	10-25-50-75 100-150	10-25-50-75-100 120-150-200-250	15-35-50-75-100 150-200	10-25-50-75-100 150-200
4	10-30-50-70 100	10-25-35-50-100 150-200-300	20-50-70-100	10-25-50-80-100 120-150

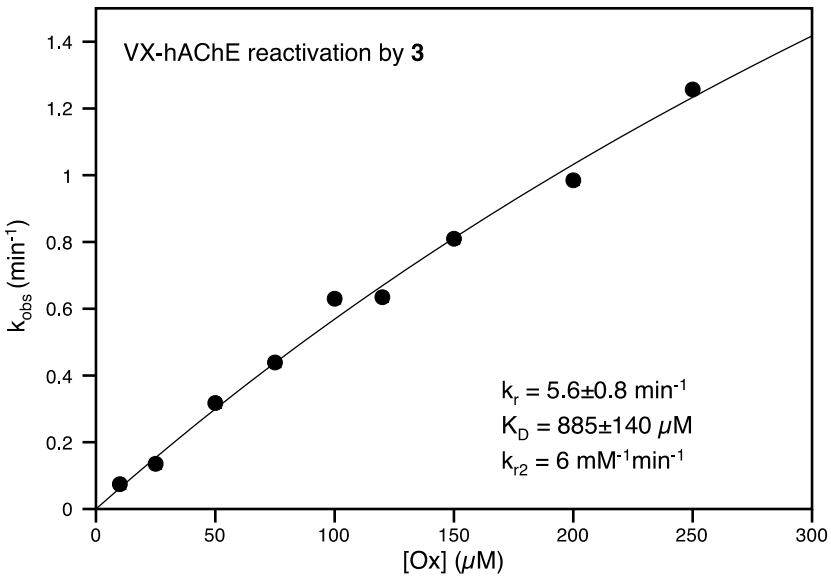
Reactivation of VX-inhibited hBChE ; Plot of k_{obs} vs [**2**] or [**3**] or [**4**]

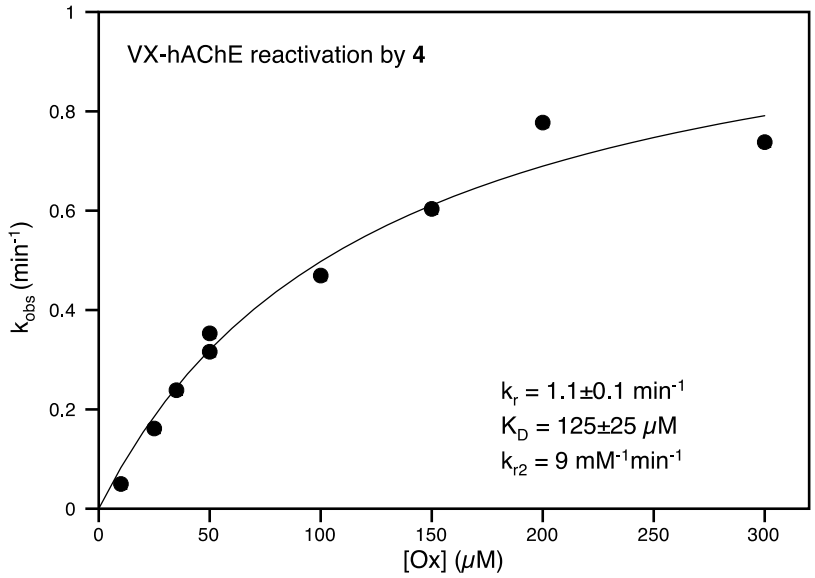




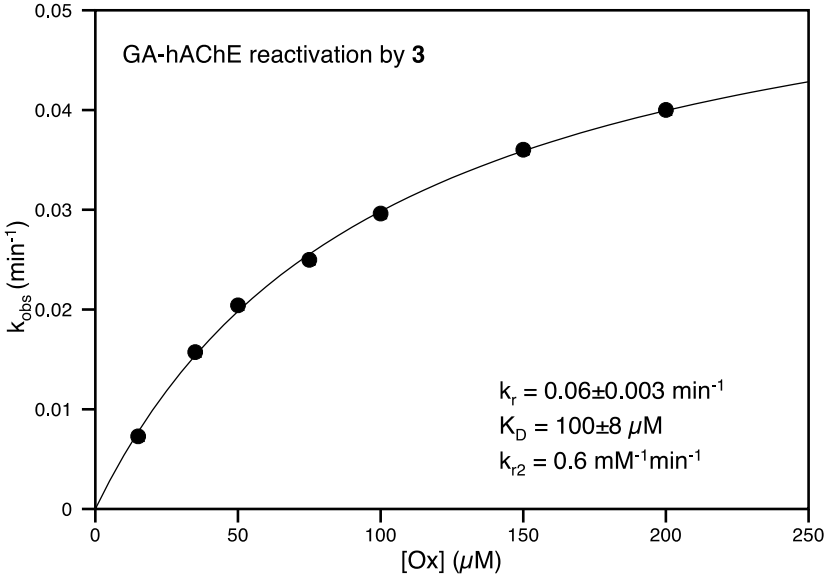


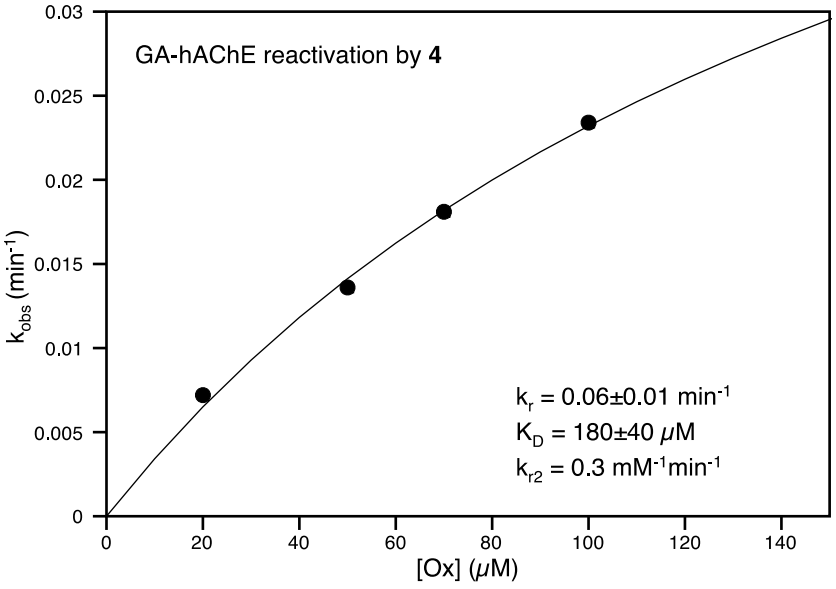
Reactivation of VX-inhibited hAChE ; Plot of k_{obs} vs [**3**] or [**4**]



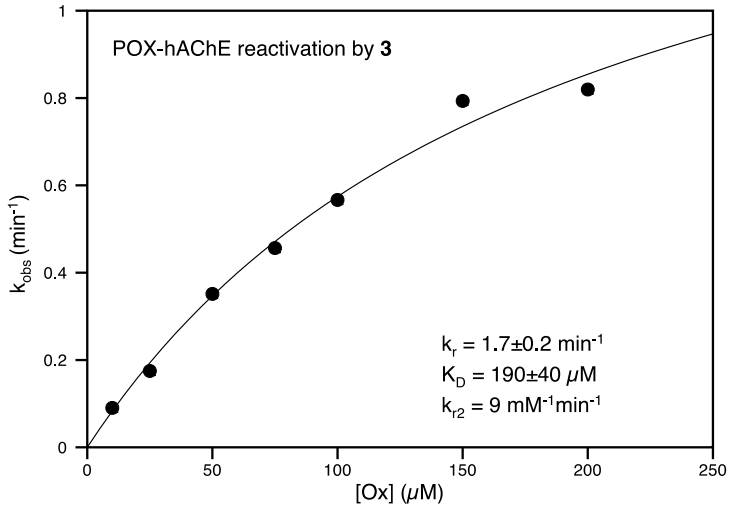


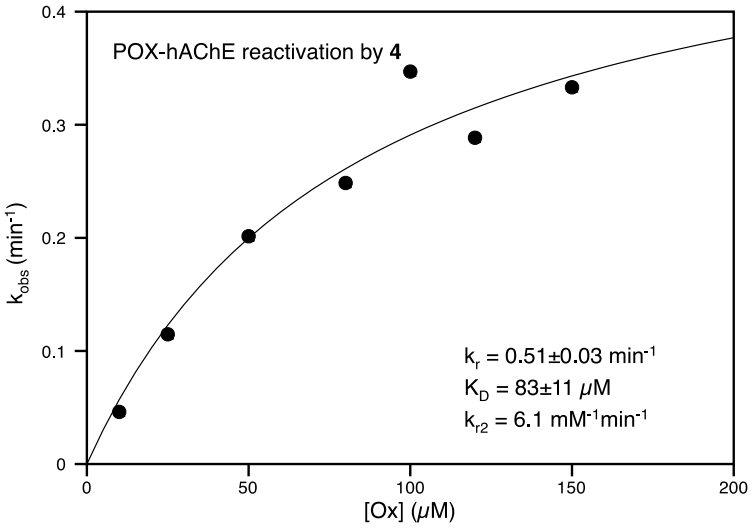
Reactivation of tabun-inhibited hAChE ; Plot of k_{obs} vs [**3**] or [**4**]



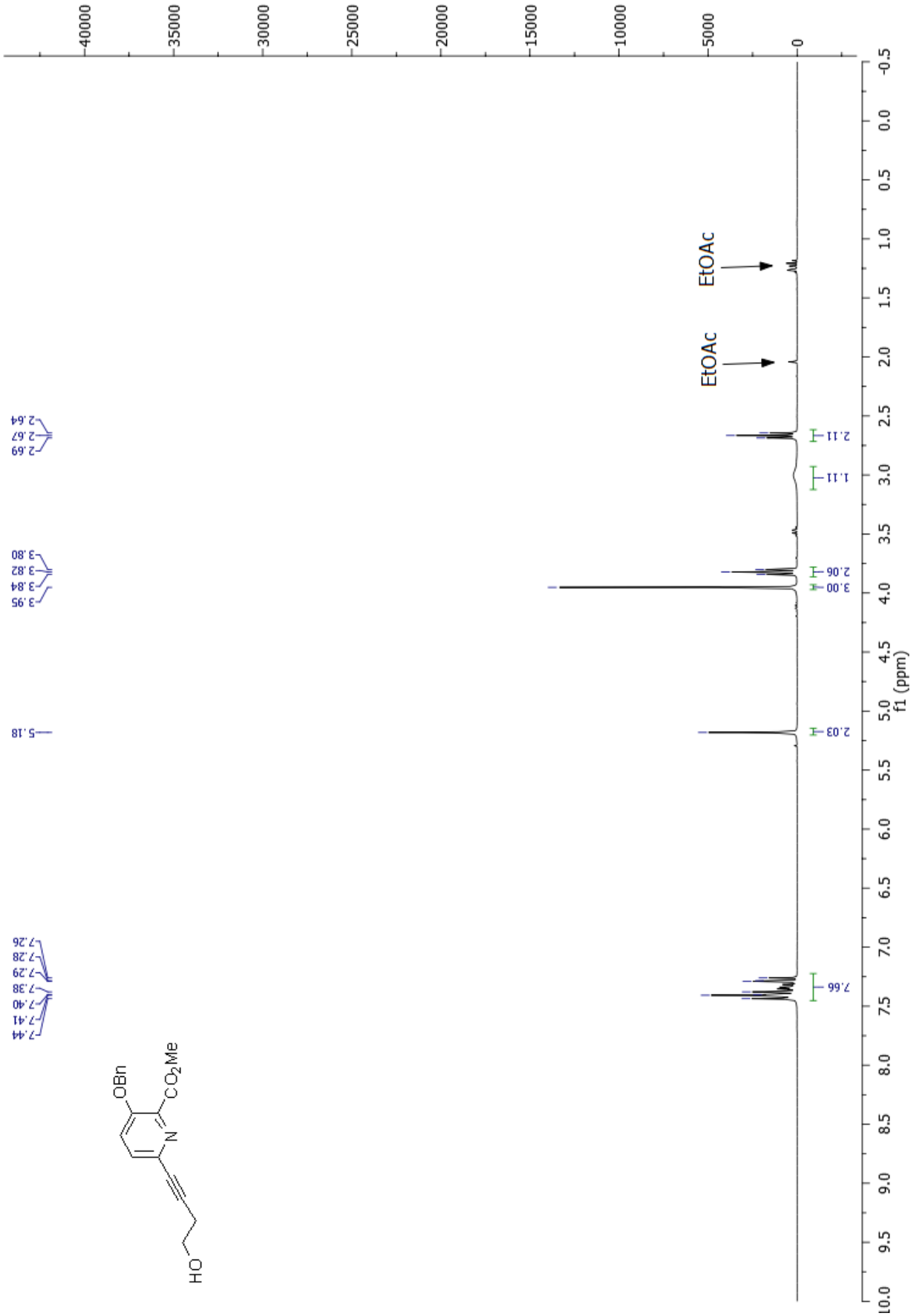


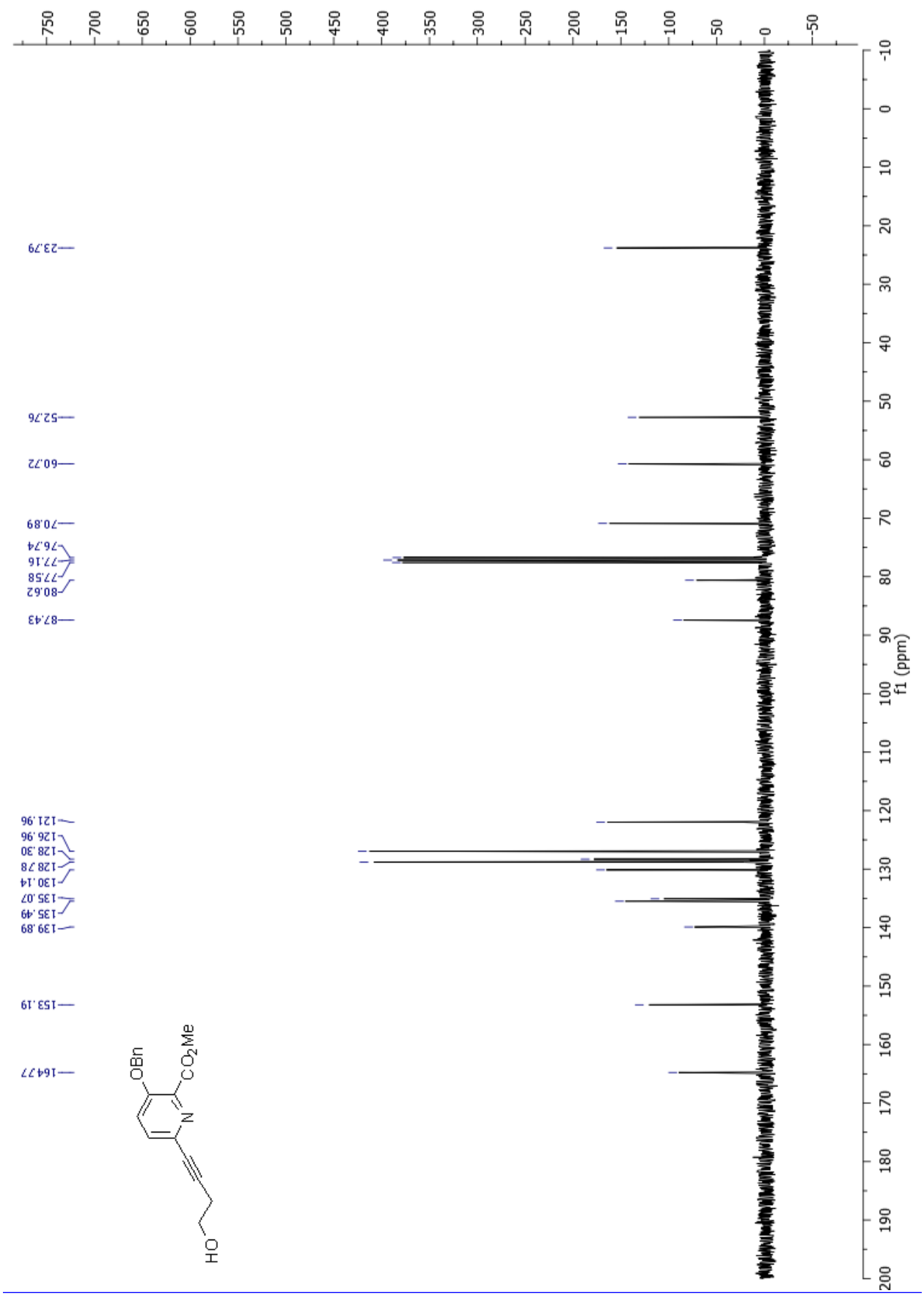
Reactivation of Paraoxon-inhibited hAChE ; Plot of k_{obs} vs [**3**] or [**4**]





3. ^{1}H and ^{13}C NMR Spectra

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128.78
128.30
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121.96
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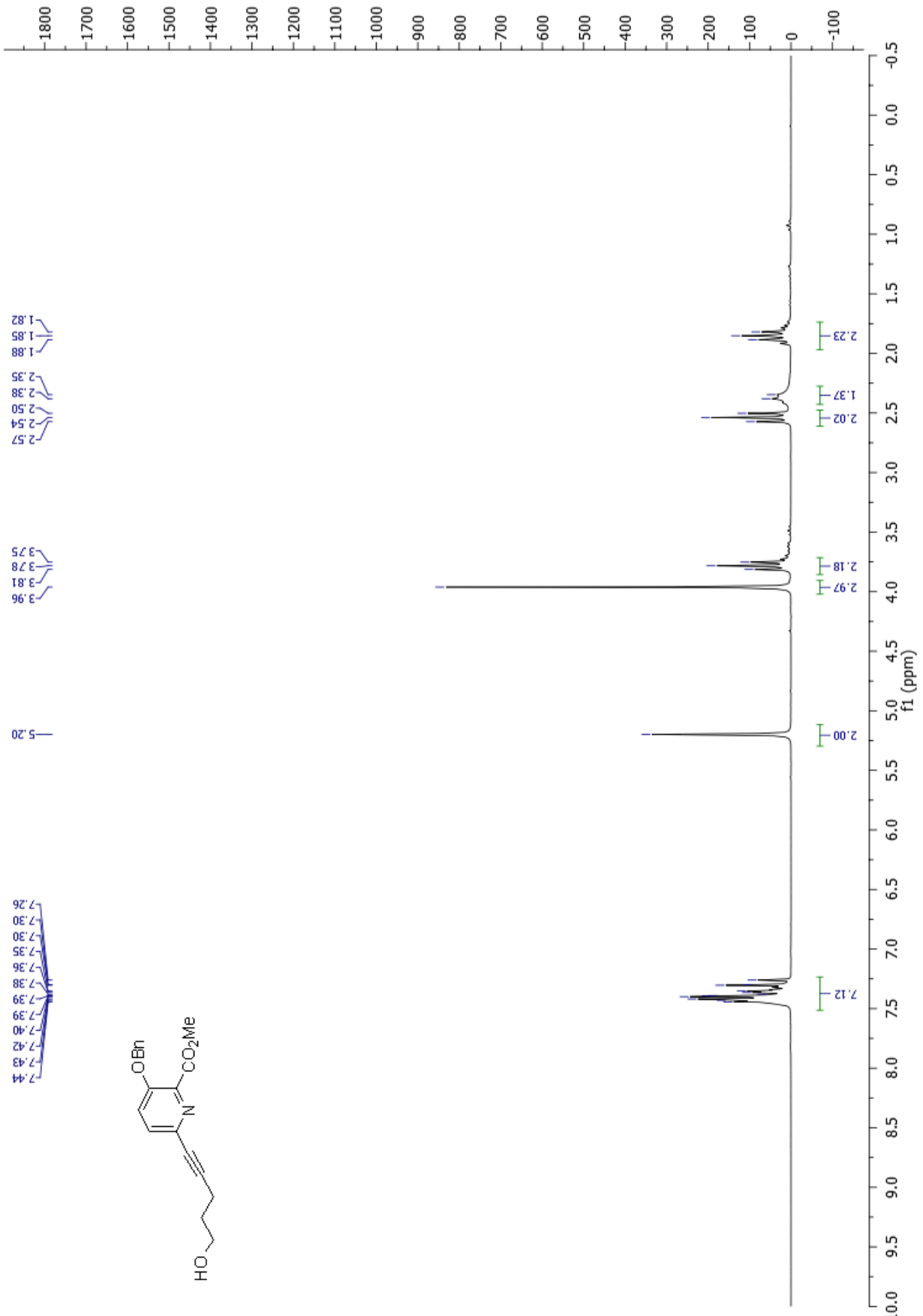
70.89
60.72
52.76
23.79
OBn
CO2Me
N
HO
f1 (ppm)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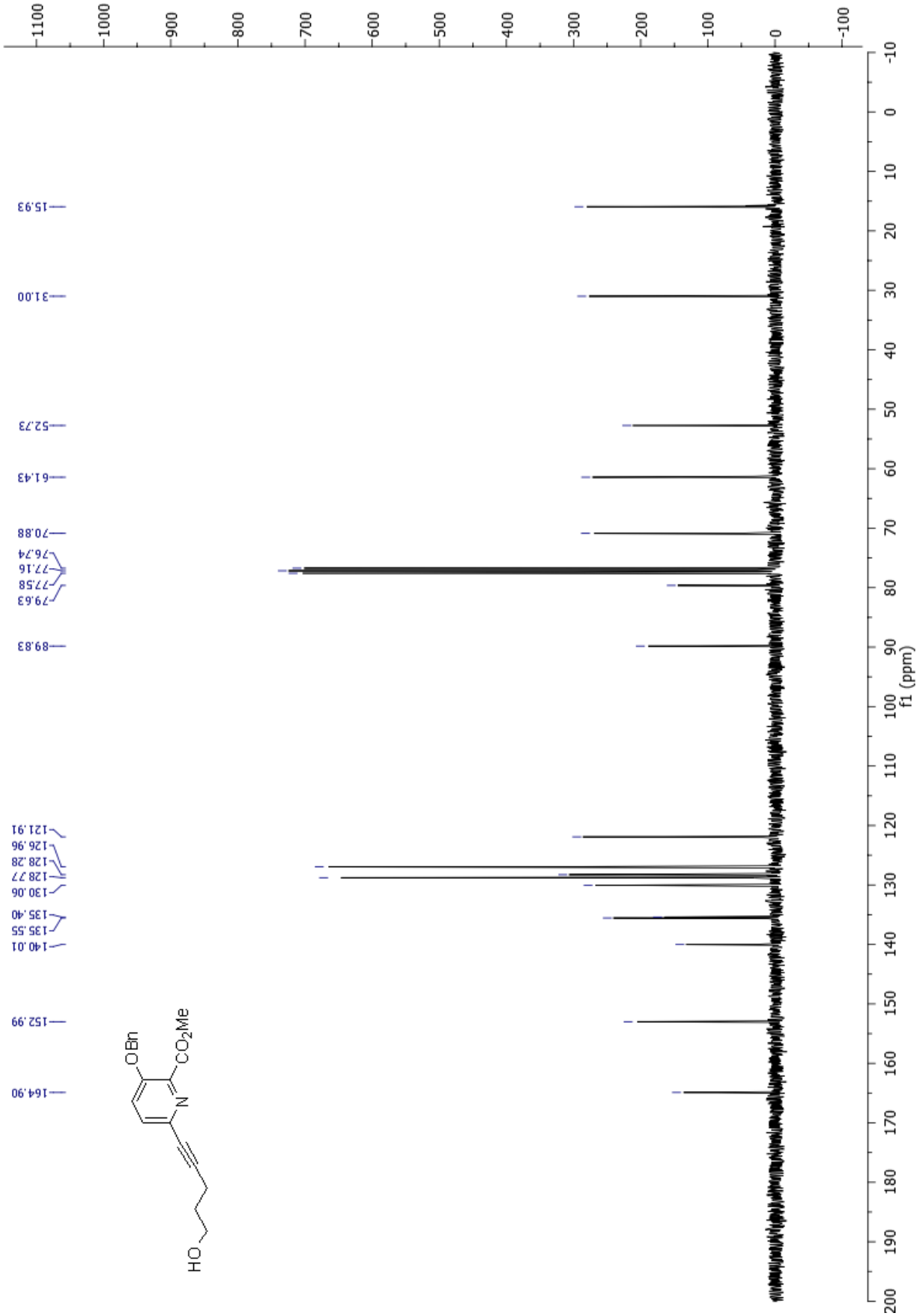
N
HO
f1 (ppm)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77.58
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121.91
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135.40
135.55
140.01
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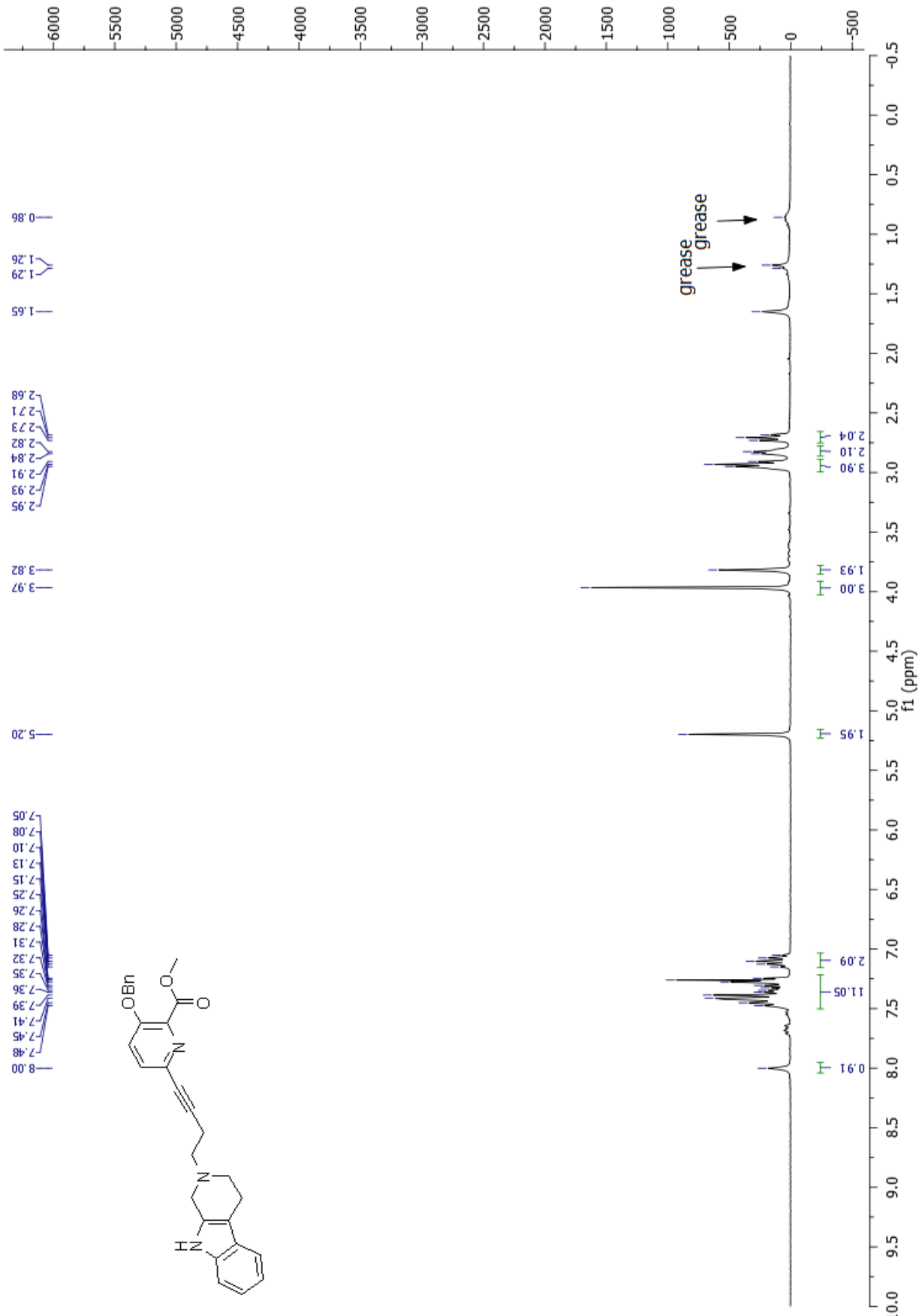
164.90
OBn
CO2Me
N
HO
f1 (ppm)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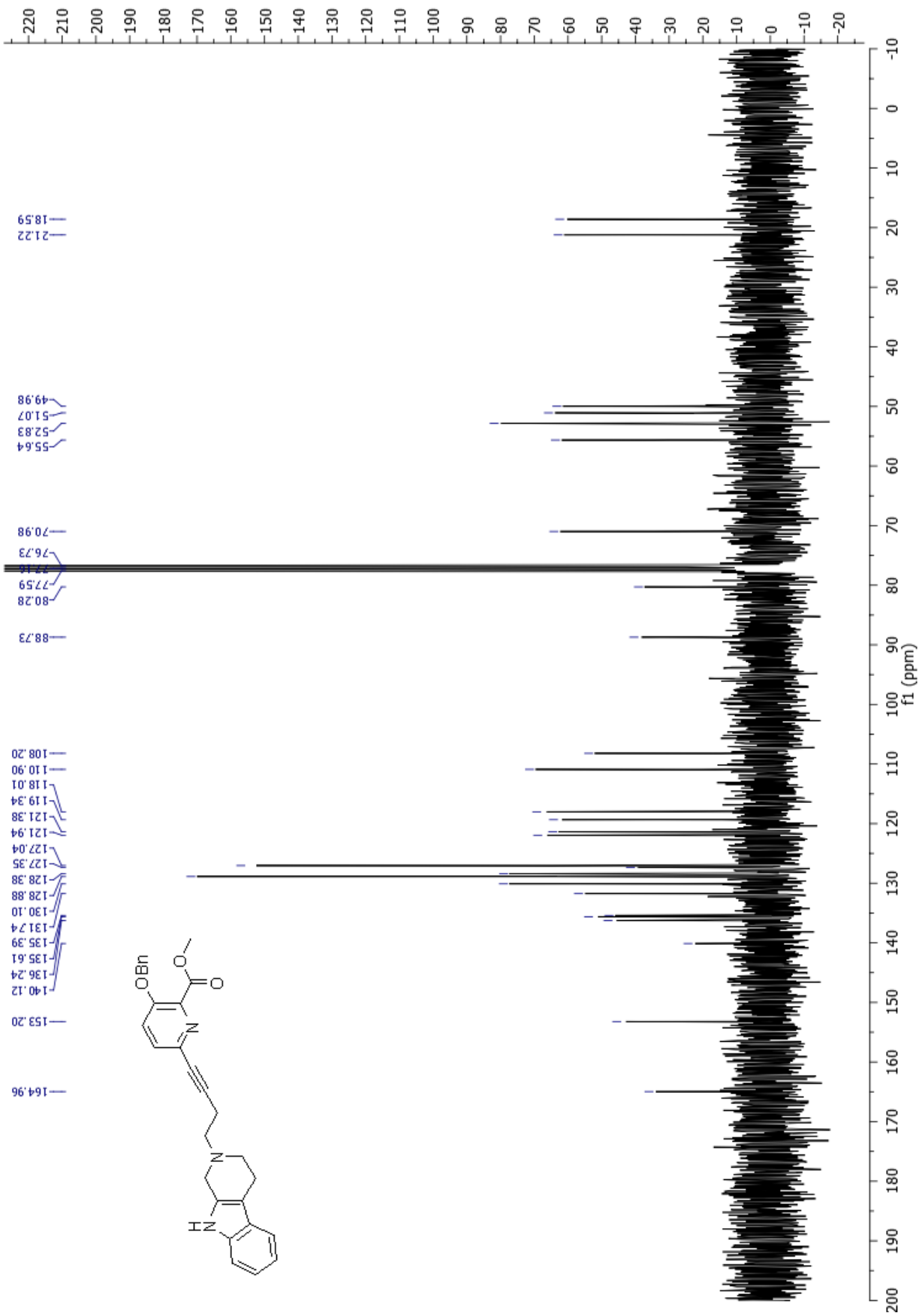
grease
f1 (ppm)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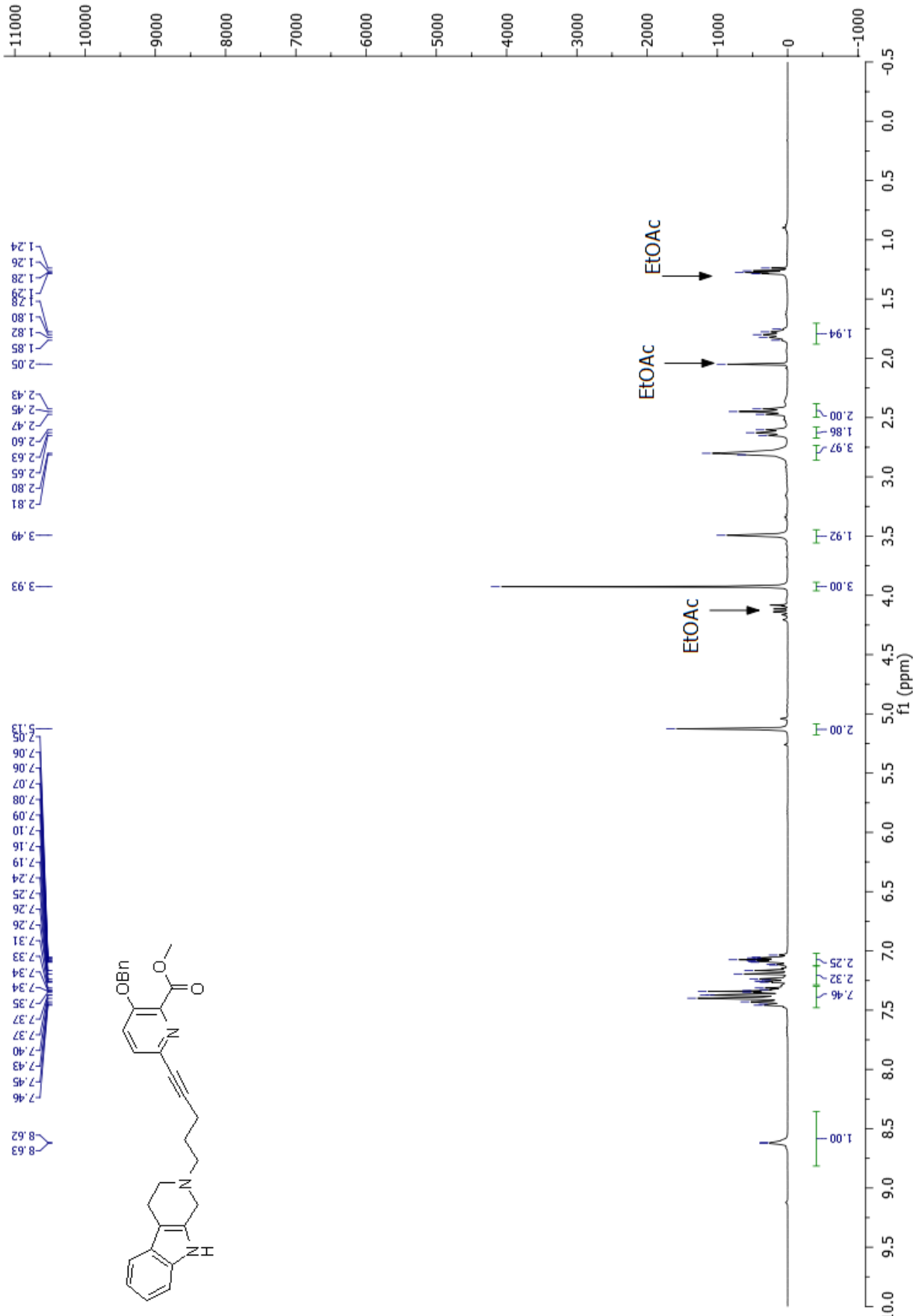
OBn
O
O
N
N
H
f1 (ppm)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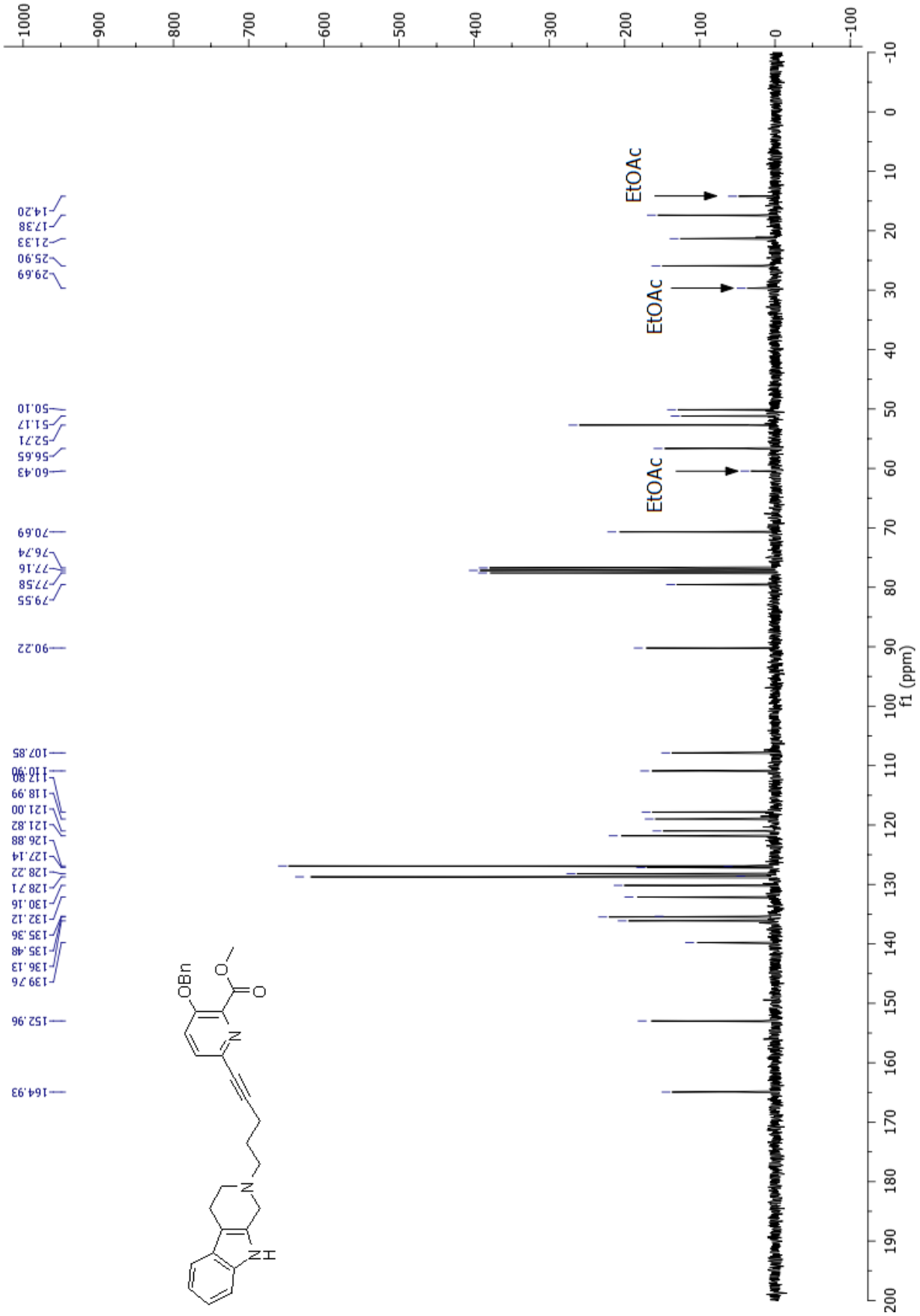
EtOAc
EtOAc
f1 (ppm)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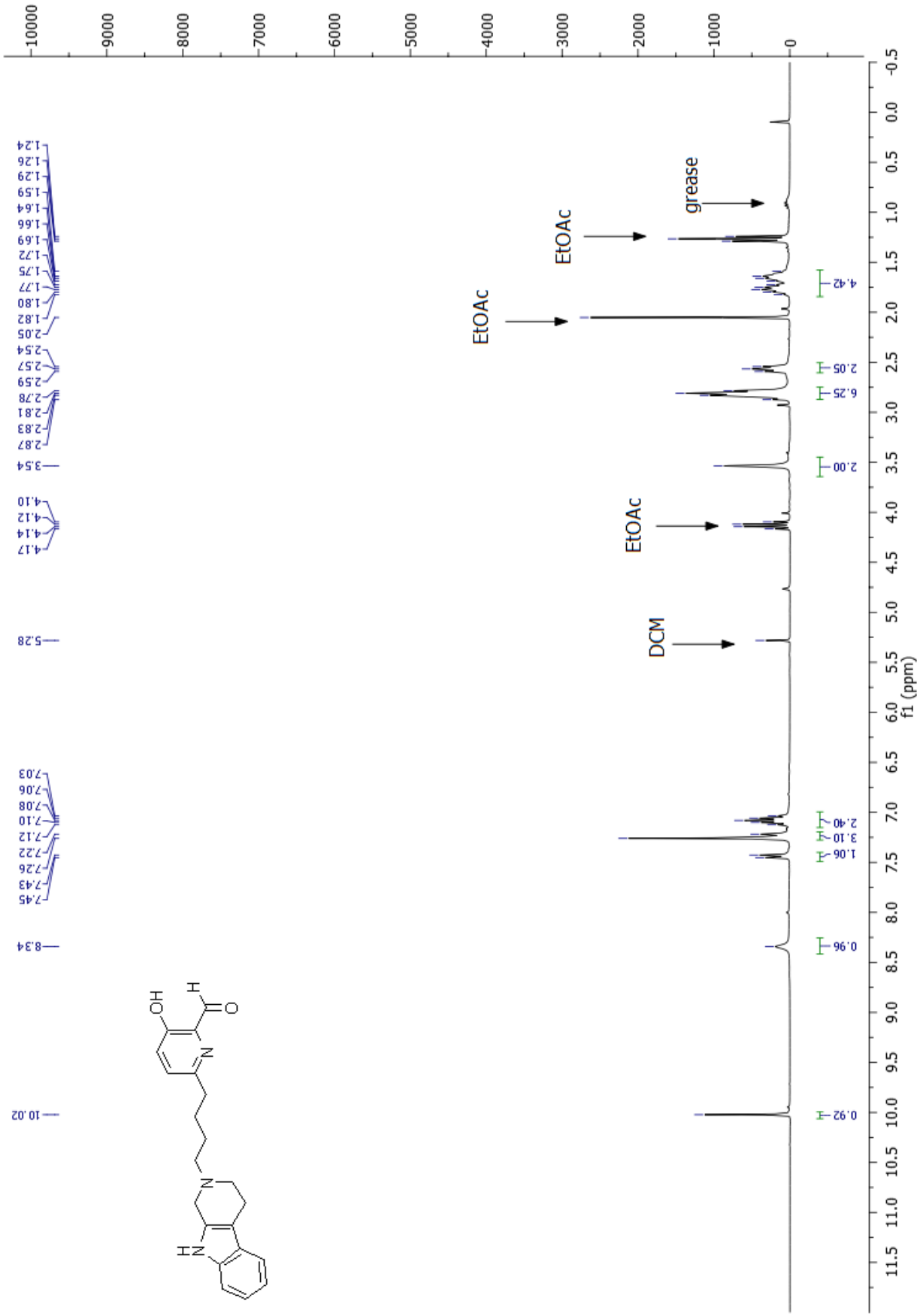
EtOAc
EtOAc
OBn
O
O
N
N
H
f1 (ppm)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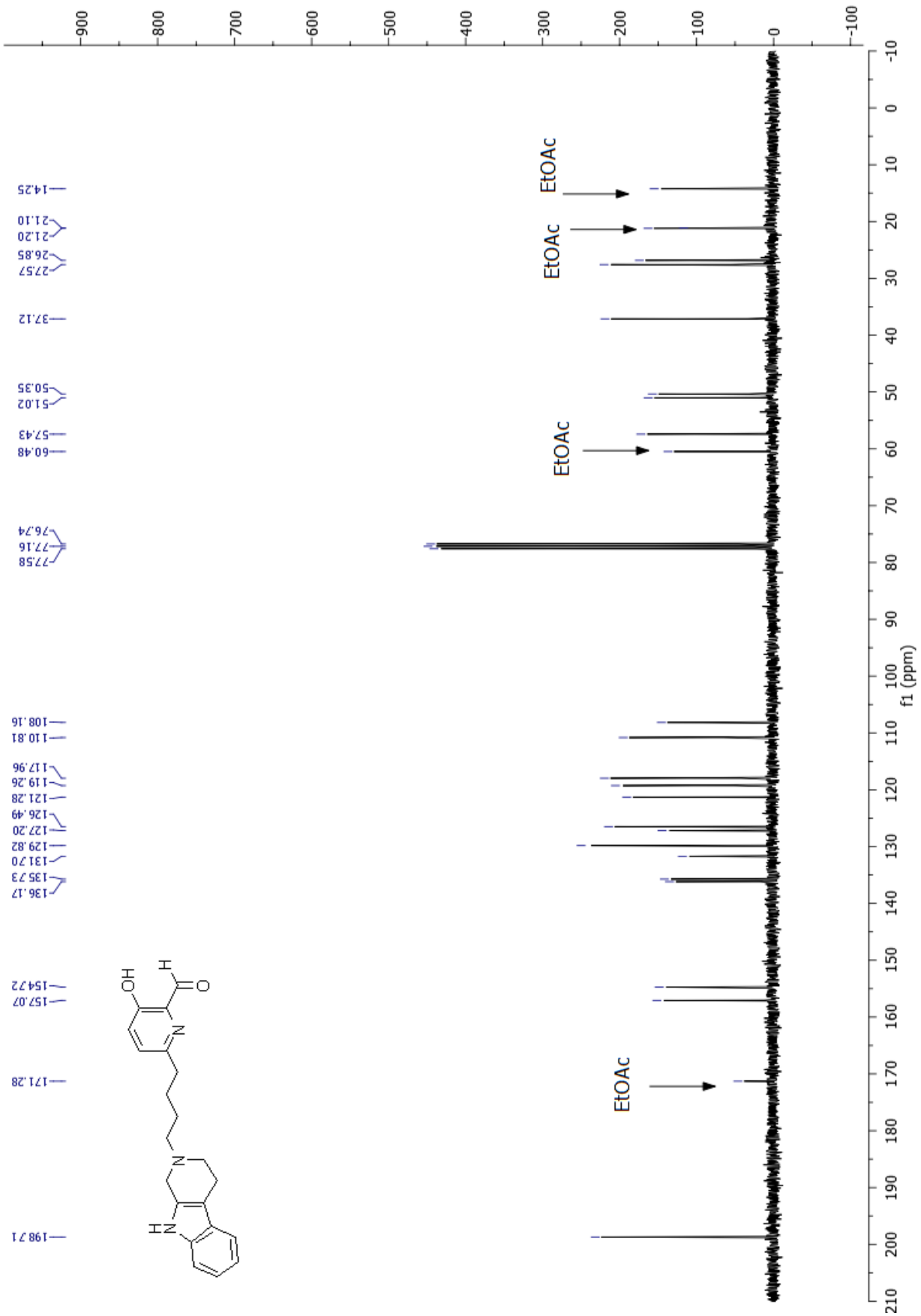
EtOAc
EtOAc
EtOAc
DCM
f1 (ppm)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EtOAc
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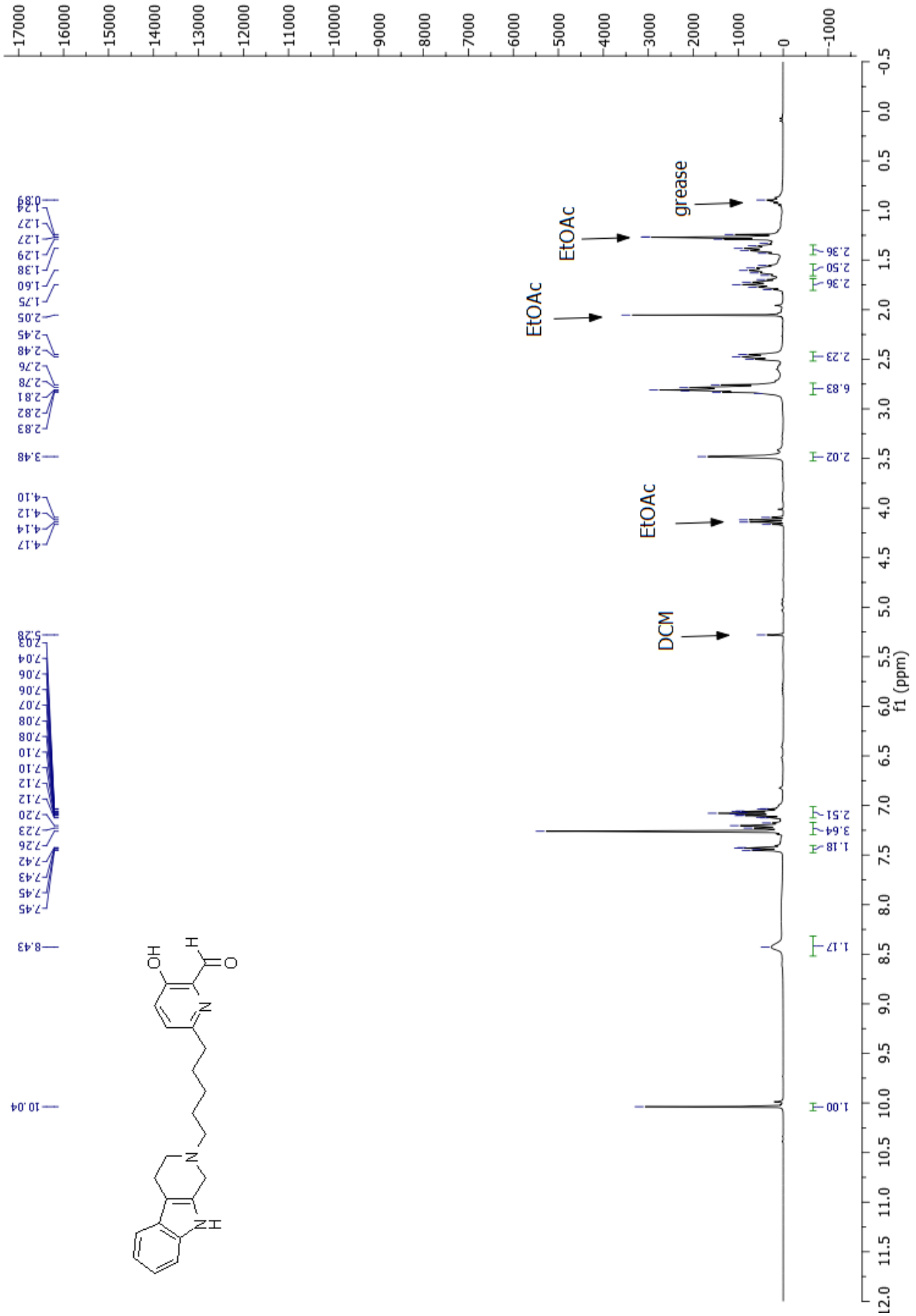
EtOAc
f1 (ppm)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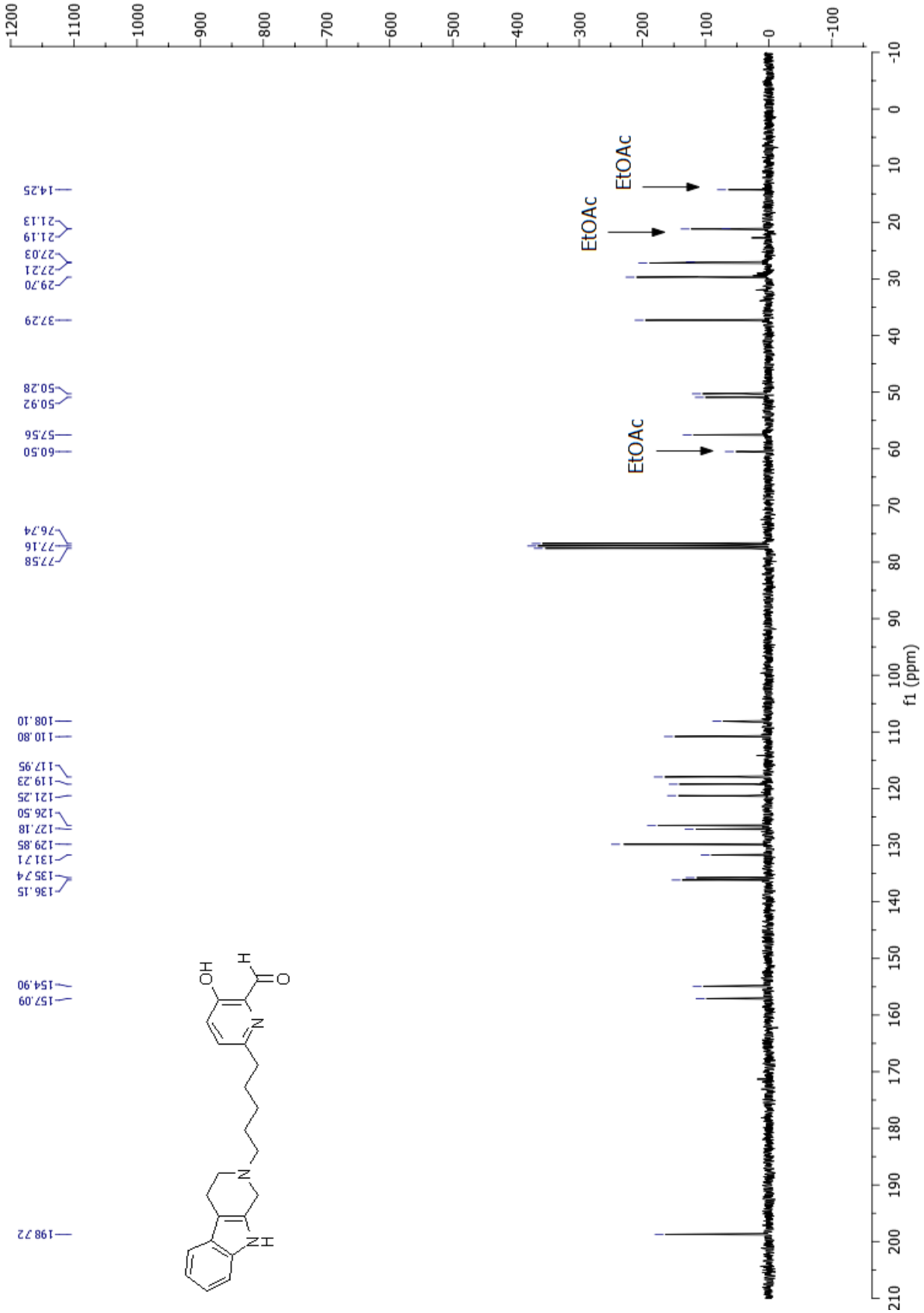
EtOAc
EtOAc
DCM
f1 (ppm)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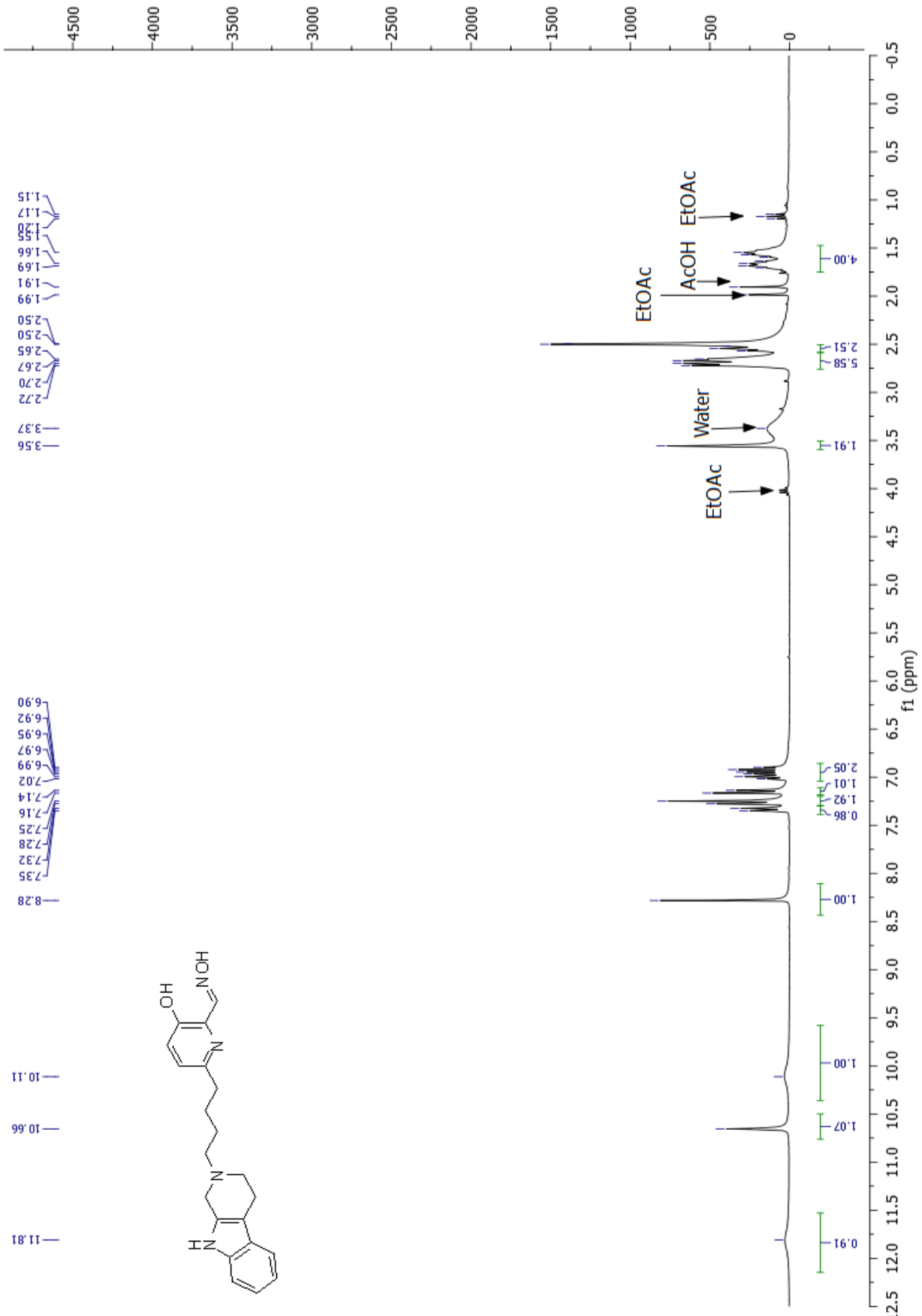
EtOAc
EtOAc
f1 (ppm)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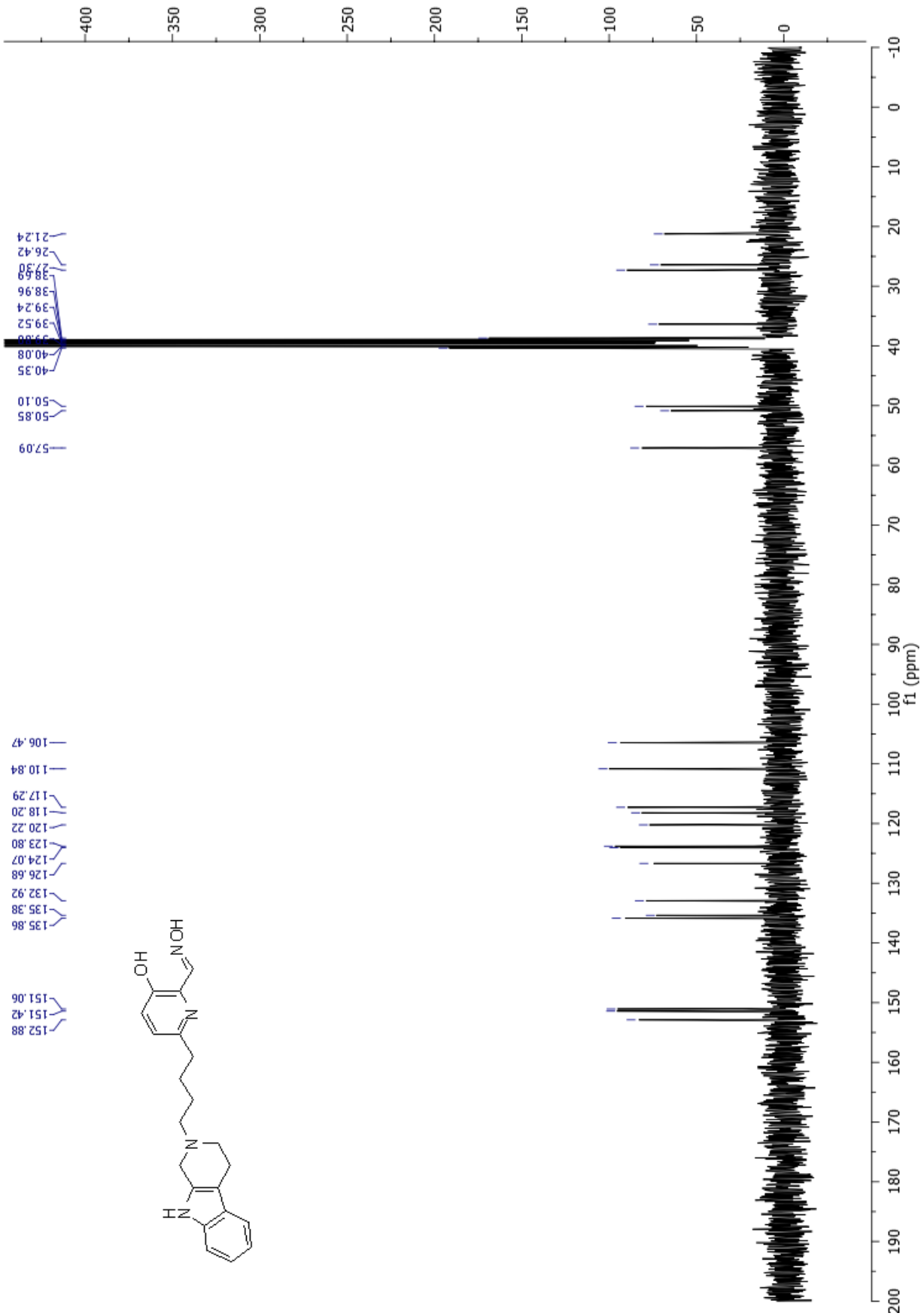
AcOH
EtOAc
Water
EtOAc
f1 (ppm)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110.84
117.29
118.20
120.22
123.80
124.07
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151.06
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OH
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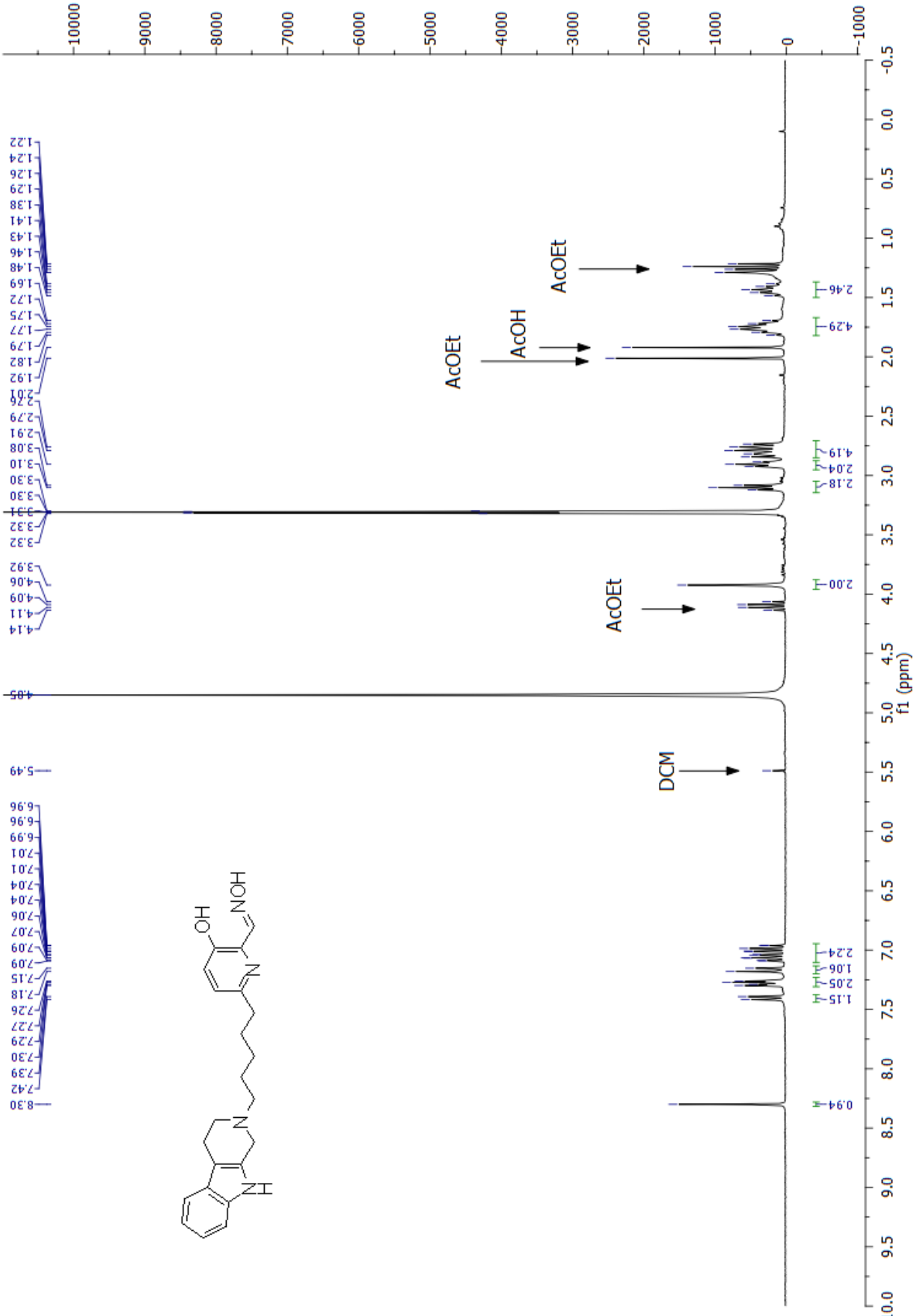
OH
N
N
H
f1 (ppm)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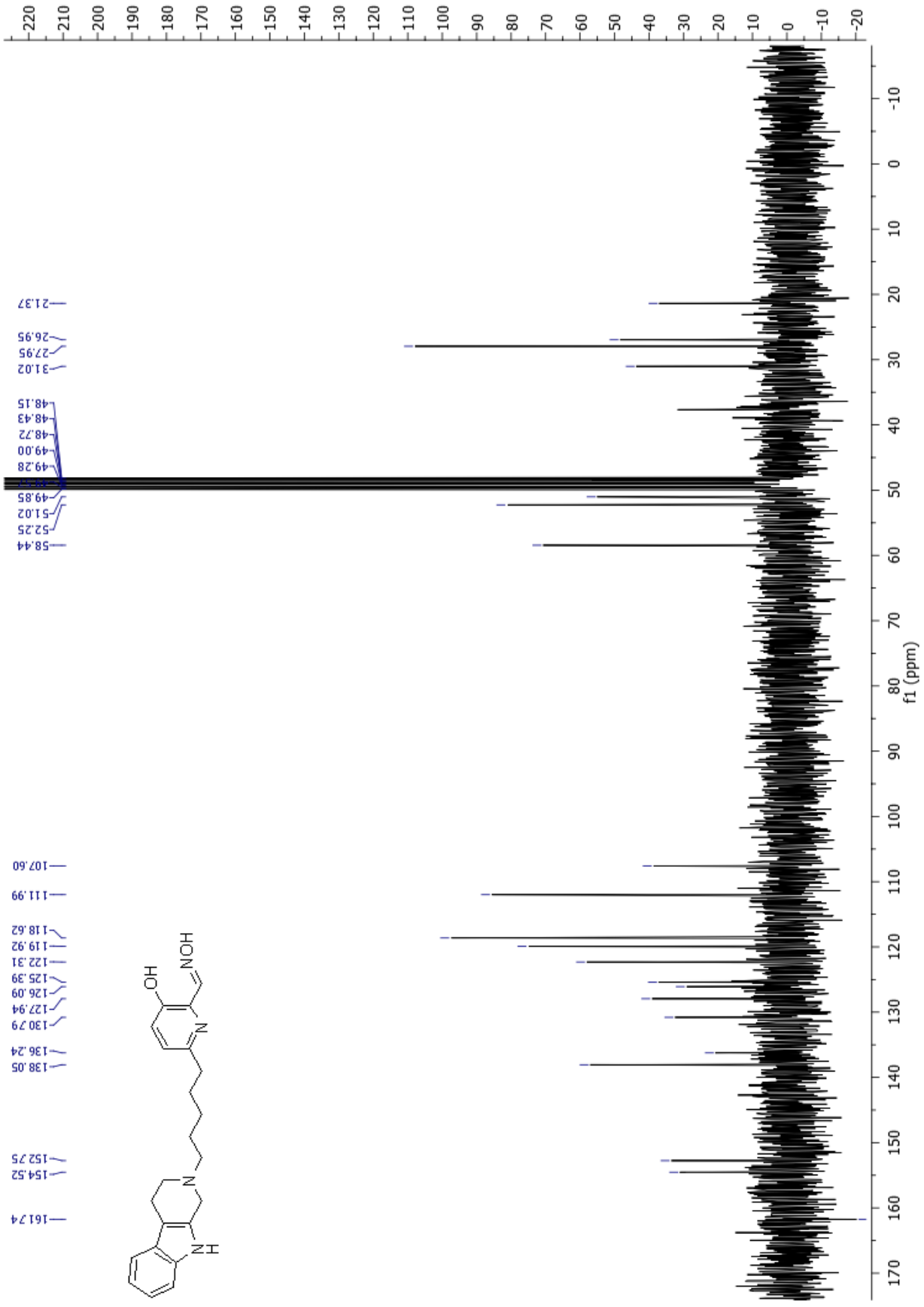
f1 (ppm)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51.02
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118.62
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122.31
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126.09
127.94
130.79
136.24
138.05
152.75
154.52
161.74
OH
NOH
N
N
H
f1 (ppm)