Supporting information

First efficient uncharged reactivators for the dephosphylation of poisoned human acetylcholinesterase

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1. General Information

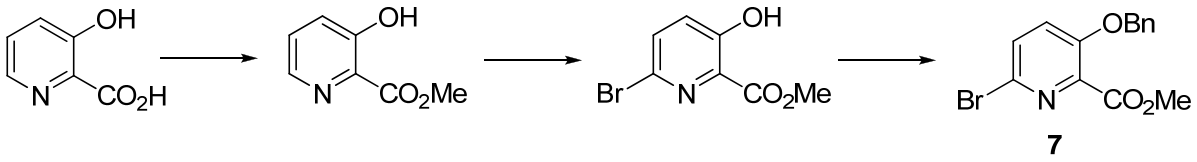
Column chromatography purifications were performed on Merck silica gel (40-63 μm). Thin-layer chromatography (TLC) was carried out on Merck DC Kieselgel 60 F-254 aluminium sheets. Compounds were visualized by illumination with a short wavelength UV lamp (λ = 254 nm). All solvents were dried following 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.

Melting points were recorded on a LEICA VMHB Kofler system at atmospheric pressure and were uncorrected. Microanalyses were carried out on Carlo-Erba 1106. ^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). ^1J 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.

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 at 254 nm.

2. Synthesis of reactivators 2 and 3

Methyl 3-Benzyloxy-6-bromopicolinate 7



7

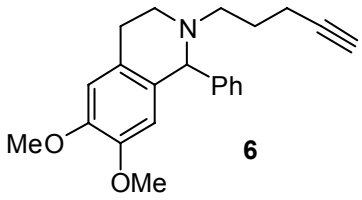
H_2SO_4 (1.8 mL, 3 equiv.) was added dropwise to a suspension of 3-hydroxypicolinic acid (1.5 g, 10.5 mmol) in MeOH (24 mL). The mixture was refluxed for 24 h. Then, the mixture was neutralized with an aqueous solution of K_2CO_3 (pH 8.5). The aqueous layer was extracted with EtOAc (thrice). The organic 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, 1 equiv.) was added portiowise (4 x 84 µL in 2h) 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). ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 4.07 (s, 3H), 7.29 (d, *J* = 8.7 Hz, 1H), 7.58 (dd, *J* = 8.7, 0.3 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, 3 equiv.) was slowly added to a mixture of previous compound (500 mg, 2.1 mmol) and K_2CO_3 (1.3 g, 4.5 equiv.) in acetone (30 mL). The solution was refluxed for 15 h. The resulting mixture was filtered and concentrated under reduce pressure. Purification by chromatography on silica gel (cyclohexane/EtOAc 9/1, v/v) gave **7** (631 mg, 93%) as a white solid. R*f* = 0.3 (cyclohexane/EtOAc 8/2, v/v). ^1H 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) $[M+H]^+$.

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

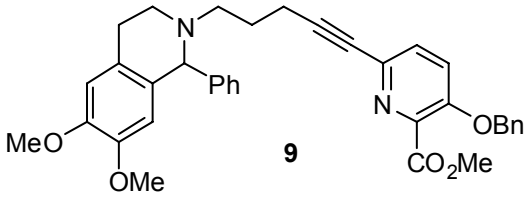
2-Pent-4-ynyl-6,7-dimethoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline 6



6

A mixture of 6,7-dimethoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline **4**[2] (1.1 g, 4.1 mmol), 4- pentynyl 4-methylbenzenesulfonate[3] (1 g, 1.1 equiv.) and K_2CO_3 (1.7 g, 3 equiv.) in dry CH_3CN (30 mL) was refluxed for 15 h. The crude mixture was filtered, concentrated under reduced pressure and purified by chromatography on silica gel (cyclohexane/EtOAc 8/2, v/v) to give **6** (982 mg, 71 %) as a white solid. R*f* = 0.3 (cyclohexane/EtOAc 8/2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.65 – 1.80 (m, 2H), 1.88 (t, *J* = 2.7 Hz, 1H), 1.97 – 2.10 (m, 1H), 2.18 – 2.31 (m, 1H), 2.37 – 2.46 (m, 1H), 2.53 – 2.65 (m, 2H), 2.78 (dt, *J* = 4.5, 16.0 Hz, 1H), 2.94 – 3.06 (m, 1H), 3.17 (dt, *J* = 4.5, 11.5 Hz, 1H), 3.61 (s, 3H), 3.87 (s, 3H), 4.50 (s, 1H), 6.19 (s, 1H), 6.62 (s, 1H), 7.24 – 7.31 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 16.2, 26.1, 28.2, 46.9, 52.9, 55.7, 55.8, 68.1, 68.2, 84.6, 110.7, 111.6, 126.9, 127.1, 128.1, 129.6, 130.1, 144.2, 147.0, 147.3. MS (ESI+): *m/z* (%): 336 (100) $[M+H]^+$.

Methyl 3-(benzyloxy)-6-(5-(6,7-dimethoxy-1-phenyl-3,4-dihydroisoquinolin-2(1H)-yl)pent-1-ynyl)picolinate 9



9

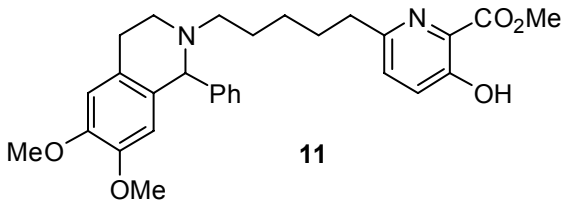
To a Schlenk tube were added **6** (335 mg, 1.0 mmol), **7** (322 mg, 1 equiv.), CuI (19 mg, 0.1 equiv.), $Pd(PPh_3)_4$ (58 mg, 0.05 equiv.) and degassed THF (10 mL) and NEt_3 (5 mL). The resulting mixture was stirred at room temperature for 15 h under argon atmosphere. After concentration under reduced pressure, the residue was purified by chromatography on silica gel (cyclohexane/EtOAc 6/4, v/v) to give **9** (429 mg, 75%) as a light brown solid. R*f* = 0.25 (cyclohexane/EtOAc 6/4, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ

[2] (a) A. Krasiński, Z. Radić, R. Manetsch, J. Raushel, P. Taylor, K. B. Sharpless, H. C. Kolb, *J. Am. Chem. Soc.* **2005**, *127*, 6686 - 6692 (b) Kumar, P.; Dhawan, K. N.; Kishor, K.; Bhargava, K. P.; Satsangi, R. K. *J. Heterocyclic Chem.* **1982**, *19*, 677-679.

[3] S. C. Zammit, A. J. Cox, R. M. Gow, Y. Zhang, R. E. Gilbert, H. Krum, D. J. Kelly, S. J. Williams, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 7003-7006.

(ppm) 1.74 – 1.80 (m, 2H), 2.15 – 2.28 (m, 1H), 2.36 – 2.48 (m, 2H), 2.52 – 2.64 (m, 2H), 2.76 (dt, *J* = 3.9, 15.8 Hz, 1H), 2.92 – 3.05 (m, 1H), 3.15 (dt, *J* = 5.2, 11.7 Hz, 1H), 3.59 (s, 3H), 3.85 (s, 3H), 3.95 (s, 3H), 4.48 (s, 1H), 5.20 (s, 2H), 6.16 (s, 1H), 6.60 (s, 1H), 7.15 – 7.28 (m, 5H), 7.30 – 7.46 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 17.1, 25.9, 28.3, 47.0, 52.7, 53.1, 55.7, 55.8, 68.1, 70.7, 79.2, 90.5, 110.8, 111.6, 121.7, 126.9, 126.9, 127.1, 128.1, 128.2, 128.7, 129.5, 130.0, 130.2, 135.5, 135.6, 139.9, 144.2, 146.9, 147.3, 152.8, 164.9. MS (ESI+): *m/z* (%): 577 (100) $[M+H]^+$.

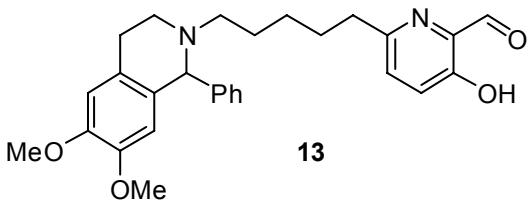
Methyl 6-(5-(6,7-dimethoxy-1-phenyl-3,4-dihydroisoquinolin-2(1H)-yl)pentyl)-3-hydroxypicolinate 11



11

To a solution of **9** (400 mg, 0.69 mmol) in degassed EtOAc (10 mL) was added Pearlman's catalyst (200 mg, 0.2 equiv., 20% Pd, moisture 50%). The solution was bubbled with H_2 and the reaction was stirred at RT under H_2 *atmosphere* (1 atm) for 15 h . The mixture was filtrated through celite and concentrated under reduce pressure to furnish **11** (310 mg, 92%) as a light brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.22 – 1.32 (m, 2H), 1.47 – 1.62 (m, 4H), 2.23 – 2.35 (m, 1H), 2.43 – 2.62 (m, 2H), 2.68 – 2.82 (m, 3H), 2.91 – 3.04 (m, 1H), 2.76 (dt, *J* = 5.1, 11.7 Hz, 1H), 3.60 (s, 3H), 3.85 (s, 3H), 4.03 (s, 3H), 4.49 (s, 1H), 6.17 (s, 1H), 6.60 (s, 1H), 7.21 – 7.30 (m, 7H), 10.55 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 26.6, 27.0, 28.1, 29.9, 37.6, 46.7, 53.2, 53.9, 55.7, 55.8, 67.9, 110.7, 111.6, 126.6, 126.9, 127.0, 128.1, 128.7, 129.2, 129.6, 130.1, 144.3, 147.0, 147.3, 154.2, 157.2, 170.2. MS (ESI+): *m/z* (%): 491 (100) $[M+H]^+$.

6-(5-(6,7-dimethoxy-1-phenyl-3,4-dihydroisoquinolin-2(1H)-yl)pentyl)-3-hydroxypicolinaldehyde 13

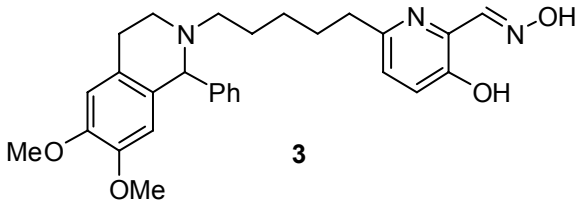


13

To a solution of **11** (290 mg, 0.60 mmol) in dry DMF (10 mL) were successively added imidazole (122 mg, 3 equiv.) and TBDMSCl (196 mg, 2.2 equiv.). The mixture was stirred at RT for 2 h under argon atmosphere. EtOAc (30 mL) was added and the organic layer was washed with water (thrice), dried over

$MgSO_4$ and concentrated under reduce pressure. To a solution of the resulting residue in dry CH_2Cl_2 (10 mL) was added dropwise DIBAL-H (1.2 mL, 1M in CH_2Cl_2, 2 equiv.) at -78 °C. Then, the reaction mixture was stirred at this temperature for 10 min. The reaction was quenched with MeOH (1.2 mL) and the mixture was allowed to warm at room temperature. The organic layer was washed with an aqueous solution of NaOH (1M), dried over $MgSO_4$ and concentrated under reduced pressure. Then, TBAF (660 µL, 1M in THF, 1.1 equiv.) was added at 0 °C to the residue in dry THF (20 mL) and the mixture was stirred for 30 min at this temperature. After concentration under reduce pressure, a chromatography on silica gel (cyclohexane/EtOAc 5/5, v/v) afforded access to compound **13** (149 mg, 53 %) as a pale yellow oil. R*f* = 0.3 (cyclohexane/EtOAc 5/5, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.20 – 1.33 (m, 2H), 1.47 – 1.66 (m, 4H), 2.25 – 2.33 (m, 1H), 2.45 – 2.60 (m, 2H), 2.69 – 2.79 (m, 3H), 2.90 – 3.02 (m, 1H), 3.13 (dt, *J* = 5.1, 11.7 Hz, 1H), 3.59 (s, 3H), 3.84 (s, 3H), 4.47 (s, 1H), 6.16 (s, 1H), 6.59 (s, 1H), 7.18 – 7.30 (m, 7H), 10.00 (s, 1H), 10.64 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 26.6, 26.9, 28.2, 29.6, 37.3, 46.8, 54.0, 55.7, 55.8, 68.0, 110.7, 111.6, 126.3, 126.9, 127.0, 128.1, 129.6, 129.8, 130.2, 135.7, 144.4, 147.0, 147.3, 155.1, 157.0, 198.8. MS (ESI+): *m/z* (%): 461 (100) $[M+H]^+$.

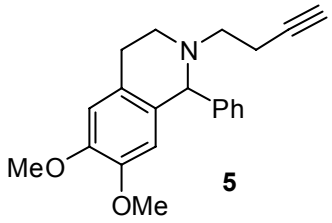
6-(5-(6,7-dimethoxy-1-phenyl-3,4-dihydroisoquinolin-2(1H)-yl)pentyl)-3-hydroxypicolinaldehyde oxime 3



3

To a solution of **13** (130 mg, 0.28 mmol) in absolute EtOH (5 mL) were added successively $HONH_2{\cdot}HCl$ (29 mg, 1.5 equiv.) and NaOAc (34 mg, 1.5 equiv.). The mixture was stirred at RT for 30 min under argon atmosphere. After concentration under reduced pressure, the residue was purified by flash chromatography on silica gel (cyclohexane/EtOAc 5/5, v/v) to give **3** (95 mg, 71%) as a white solid. R*f* = 0.2 (cyclohexane/EtOAc 5/5, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.20 – 1.35 (m, 2H), 1.53 – 1.68 (m, 4H), 2.39 – 2.51 (m, 1H), 2.52 – 2.61 (m, 1H), 2.62 – 2.71 (m, 2H), 2.72 – 2.83 (m, 1H), 2.83 – 2.95 (m, 1H), 2.95 – 3.18 (m, 1H), 2.82 (dt, *J* = 4.9, 16.2 Hz, 1H), 3.59 (s, 3H), 3.84 (s, 3H), 4.73 (s, 1H), 6.16 (s, 1H), 6.62 (s, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 7.10 – 7.29 (m, 5H), 8.36 (s, 1H), 10.74 (br s, 1H), 10.76 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 25.7, 26.9, 29.9, 36.9, 45.9, 53.9, 55.7, 55.8, 67.2, 110.7, 111.4, 123.7, 124.6, 126.0, 127.7, 128.3, 128.4, 130.0, 135.1, 141.5, 147.3, 147.8, 152.5, 152.8, 153.1. MS (ESI+) *m/z* (%): 476 (100) $[M+H]^+$. HRMS (ESI+): calcd. for $C_{28}H_{34}N_3O_4$ 476.2540; found 476.2549. HPLC : t_R = 22.7 min (purity > 95%).

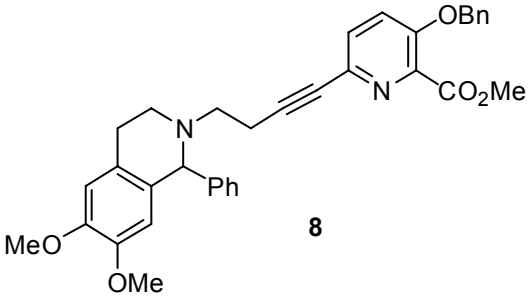
2-But-3-ynyl-6,7-dimethoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline 5



5

A mixture of 6,7-dimethoxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline **4** (0.8 g, 3.0 mmol), 3-butynyl 4-methylbenzenesulfonate[3] (686 mg, 1.1 equiv.) and K_2CO_3 (1.2 g, 3 equiv.) in dry CH_3CN (30 mL) was refluxed for 15 h. The crude mixture was filtered, concentrated under reduced pressure and purified by chromatography on silica gel (cyclohexane/EtOAc 8/2, v/v) to afford **5** (443 mg, 46 %) as a beige solid. R*f* = 0.14 (cyclohexane/EtOAc 8/2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.94 (t, *J* = 2.4 Hz, 1H), 2.26 – 2.49 (m, 2H), 2.58 – 2.86 (m, 4H), 2.95 – 3.07 (m, 1H), 3.13 – 3.21 (m, 1H), 3.62 (s, 3H), 3.87 (s, 3H), 4.60 (s, 1H), 6.19 (s, 1H), 6.62 (s, 1H), 7.24 – 7.32 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 16.9, 28.2, 47.2, 53.0, 55.7, 55.8, 67.2, 69.0, 83.0, 110.8, 111.6, 126.7, 127.3, 128.2, 129.4, 129.8, 143.9, 147.1, 147.7. MS (ESI+): *m/z* (%): 322 (100) $[M+H]^+$.

Methyl 3-(benzyloxy)-6-(4-(6,7-dimethoxy-1-phenyl-3,4-dihydroisoquinolin-2(1H)-yl)but-1-ynyl)picolinate 8

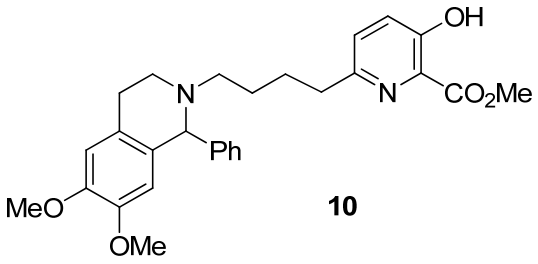


8

To a Schlenk tube were added **5** (212 mg, 0.66 mmol), **7** (212 mg, 1 equiv.), CuI (13 mg, 0.1 equiv.), $Pd(PPh_3)_4$ (38 mg, 0.05 equiv.) and degassed THF (10 mL) and NEt_3 (5 mL). The resulting mixture was stirred at room temperature for 15 h under argon atmosphere. After concentration under reduced pressure, the residue was purified by chromatography on silica gel (cyclohexane/EtOAc 6/4, v/v) to give **8** (245 mg, 66%) as a light brown solid. R*f* = 0.25 (cyclohexane/EtOAc 6/4, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 2.42 – 2.90 (m, 6H), 2.95 – 3.08 (m, 1H), 3.13 – 3.21 (m, 1H), 3.59 (s, 3H), 3.84 (s, 3H), 3.94 (s, 3H), 4.58 (s, 1H), 5.19 (s, 2H), 6.16 (s, 1H), 6.59 (s, 1H), 7.22 – 7.28 (m, 6H), 7.31 – 7.39 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 17.7, 28.4, 47.3, 52.7, 52.8, 55.7, 55.8, 67.3, 70.8, 80.0, 88.8, 110.8,

111.6, 121.7, 126.6, 126.9, 127.3, 128.2, 128.3, 128.8, 129.4, 129.9, 129.9, 135.4, 135.5, 140.0, 143.9, 147.0, 147.4, 152.9, 164.8. MS (ESI+): *m/z* (%): 563 (100) $[M+H]^+$.

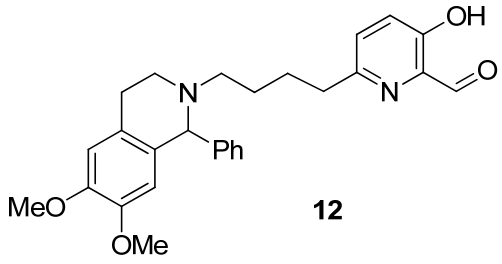
Methyl 6-(4-(6,7-dimethoxy-1-phenyl-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-3-hydroxypicolinate 10



10

To a solution of **8** (230 mg, 0.41 mmol) in degassed EtOAc (10 mL) was added Pearlman's catalyst (200 mg, 0.2 equiv., 20% Pd, moisture 50%). The solution was bubbled with H_2 and the reaction was stirred at RT under H_2 atmosphere (1 atm) for 15 h. The mixture was filtrated through celite and concentrated under reduced pressure to give **10** (310 mg, 92%) as a light brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.50 – 1.70 (m, 4H), 2.28 – 2.36 (m, 1H), 2.49 – 2.59 (m, 2H), 2.67 – 2.80 (m, 3H), 2.92 – 3.04 (m, 1H), 3.12 (dt, *J* = 5.0, 11.7 Hz, 1H), 3.59 (s, 3H), 3.84 (s, 3H), 4.03 (s, 3H), 4.47 (s, 1H), 6.16 (s, 1H), 6.59 (s, 1H), 7.21 – 7.30 (m, 7H), 10.60 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 26.4, 27.7, 28.3, 37.4, 46.9, 53.2, 53.9, 55.7, 55.8, 68.0, 110.7, 111.6, 126.6, 126.9, 127.1, 128.1, 128.7, 129.2, 129.6, 130.2, 144.3, 147.0, 147.3, 154.1, 157.2, 170.2. MS (ESI+): *m/z* (%): 477 (100) $[M+H]^+$.

6-(4-(6,7-dimethoxy-1-phenyl-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-3-hydroxypicolinaldehyde 12

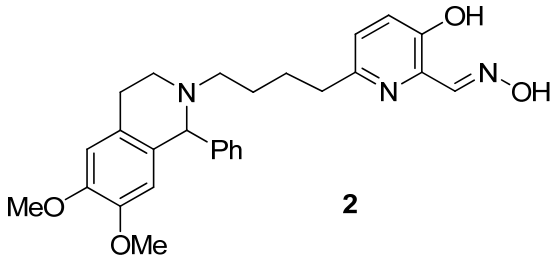


12

To a solution of **10** (180 mg, 0.38 mmol) in dry DMF (10 mL) were successively added imidazole (78 mg, 3 equiv.) and TBDMSCl (125 mg, 2.2 equiv.). The mixture was stirred at RT for 2 h under argon atmosphere. EtOAc (30 mL) was added and the organic layer was washed with water (thrice), 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 (760 µL, 1 M in CH_2Cl_2, 2 equiv.) at -78 °C. Then, the reaction mixture was stirred at this temperature for 10 min. The reaction was quenched with MeOH (760 µL) and the mixture was allowed to warm at room temperature. The organic layer was washed with an aqueous solution of NaOH (1M), dried over $MgSO_4$ and concentrated under reduced pressure. Then, TBAF (420

μL, 1.1 equiv., 1 M in THF) was added at 0 °C to the residue in dry THF (20 mL) and the mixture was stirred for 30 min at this temperature. After concentration under reduce pressure, a chromatography on silica gel (cyclohexane/EtOAc 5/5, v/v) afforded **12** (90 mg, 53 %) as a pale yellow oil. R*f* = 0.3 (cyclohexane/EtOAc 5/5, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.47 – 1.73 (m, 4H), 2.28 – 2.38 (m, 1H), 2.50 – 2.62 (m, 2H), 2.66 – 2.81 (m, 3H), 2.94 – 3.02 (m, 1H), 3.14 (dt, *J* = 4.8, 11.7 Hz, 1H), 3.59 (s, 3H), 3.84 (s, 3H), 4.47 (s, 1H), 6.16 (s, 1H), 6.60 (s, 1H), 7.17 – 7.29 (m, 7H), 10.00 (s, 1H), 10.53 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 26.3, 27.2, 28.3, 37.0, 46.9, 53.8, 55.7, 55.8, 68.2, 110.7, 111.6, 126.3, 126.9, 127.1, 128.1, 129.6, 129.8, 130.2, 135.6, 144.3, 147.0, 147.3, 154.9, 156.9, 198.8. MS (ESI+): *m/z* (%): 447 (100) $[M+H]^+$.

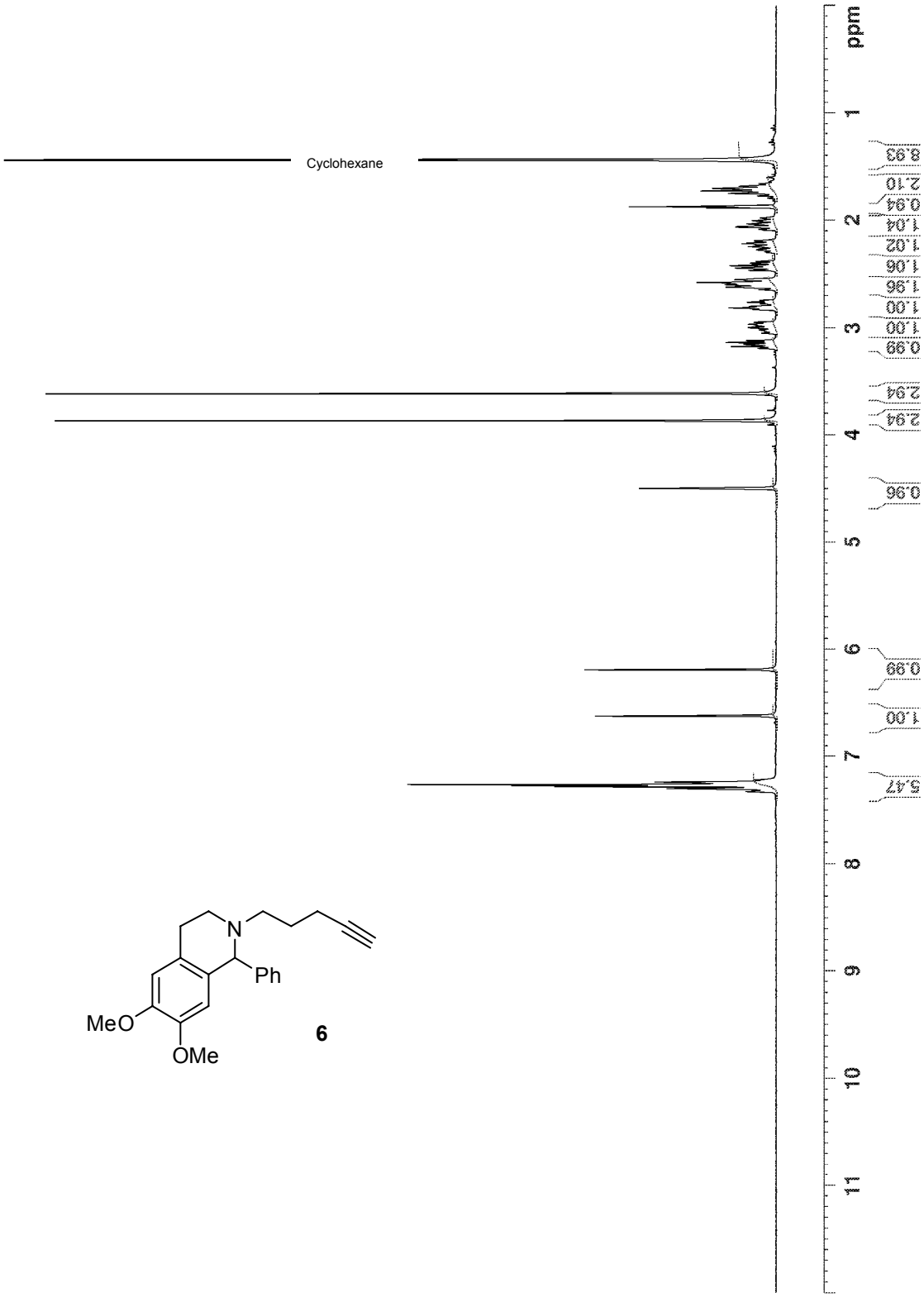
6-(4-(6,7-dimethoxy-1-phenyl-3,4-dihydroisoquinolin-2(1H)-yl)butyl)-3-hydroxypicolinaldehyde oxime 2



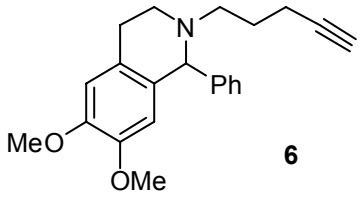
2

To a solution of **12** (85 mg, 0.19 mmol) in absolute EtOH (5 mL) were added successively $HONH_2{\cdot}HCl$ (20 mg, 1.5 equiv.) and NaOAc (24 mg, 1.5 equiv.). The mixture was stirred at RT for 30 min under argon atmosphere. After concentration under reduced pressure, the residue was purified by chromatography on silica gel (cyclohexane/EtOAc 5/5, v/v) to give **2** (77 mg, 88%) as a white solid. R*f* = 0.2 (cyclohexane/EtOAc 5/5, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.53 – 1.70 (m, 4H), 2.32 – 2.45 (m, 1H), 2.51 – 2.72 (m, 4H), 2.76 – 2.87 (m, 1H), 2.94 – 3.06 (m, 1H), 3.11 – 3.23 (m, 1H), 3.58 (s, 3H), 3.84 (s, 3H), 4.57 (s, 1H), 6.13 (s, 1H), 6.60 (s, 1H), 6.90 (d, *J* = 8.4 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 7.18 – 7.29 (m, 5H), 8.36 (s, 1H), 9.95 (br s, 1H), 11.68 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 25.7, 27.5, 27.6, 36.6, 46.6, 54.0, 55.7, 55.8, 67.9, 110.7, 111.5, 123.9, 124.6, 126.4, 127.4, 128.2, 129.3, 129.9, 135.0, 142.6, 147.1, 147.6, 152.5, 152.6 152.8. MS (ESI+) *m/z* (%): 462 (100) $[M+H]^+$. HRMS (ESI+): calcd. for $C_{27}H_{32}N_3O_4$ 462.2407; found 462.2393. HPLC : t_R = 22 min (purity > 95%).

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



6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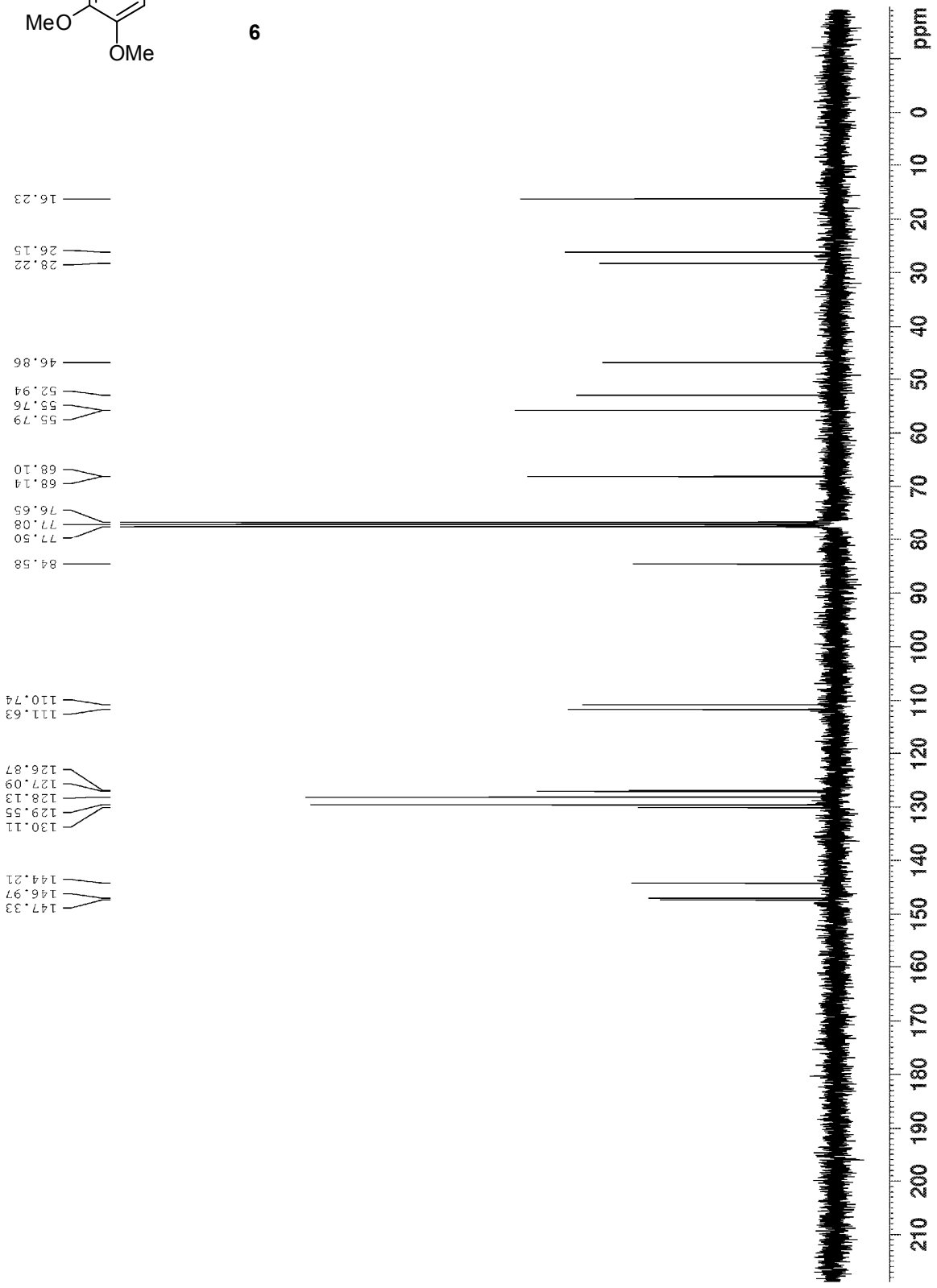
MeO
OMe

6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26.15
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68.10
68.14
76.65
77.08
77.50
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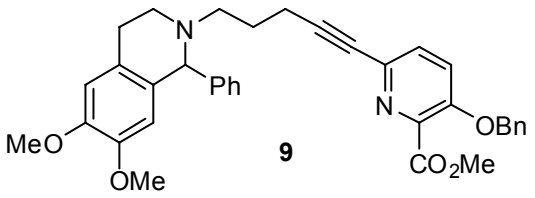
111.63
126.87
127.09
128.13
129.55
130.11
144.21
146.97
147.33
ppm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Ph
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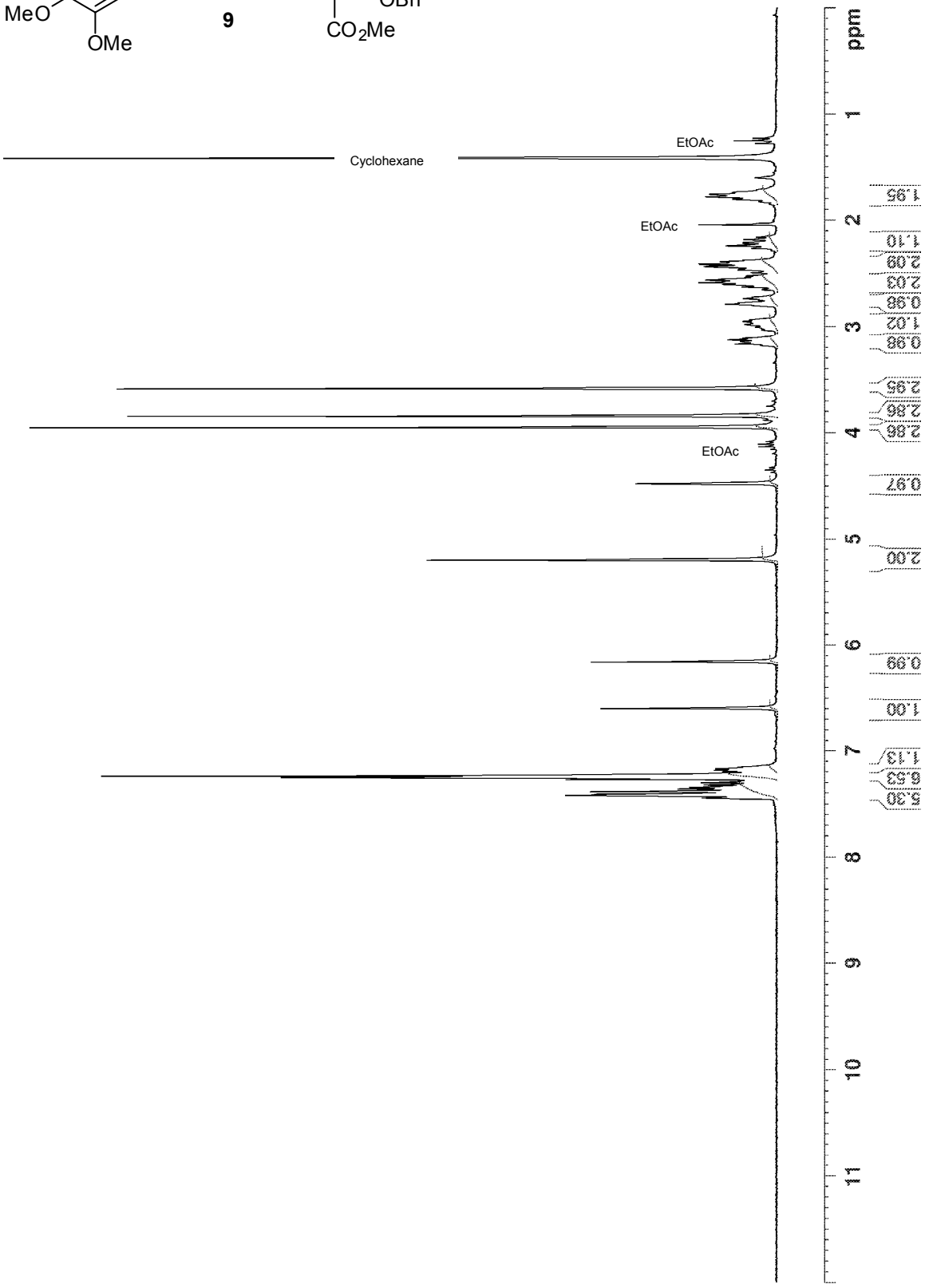
OMe
N
OBn
CO2Me

9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EtOAc
EtOAc
ppm
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2
3
4
5
6
7
8
9
10
11
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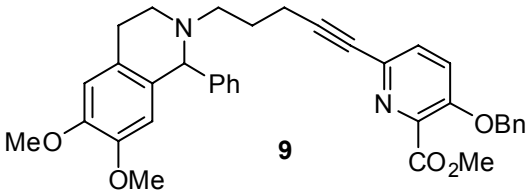
1.10
2.09
2.03
0.98
1.02
0.98
2.95
2.86
2.86
0.97
2.00
0.99
1.00
1.13
6.53
5.30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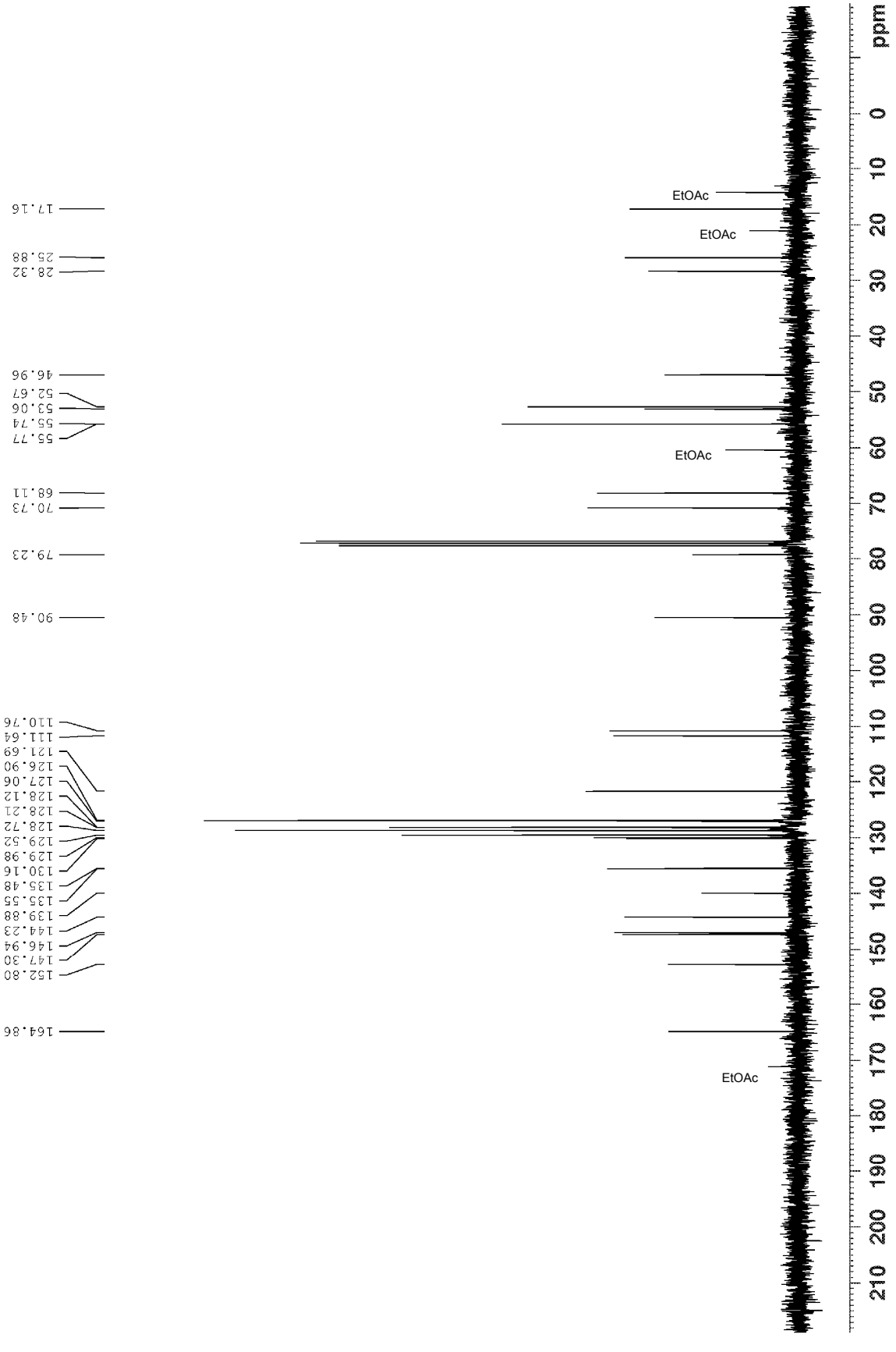
Ph
MeO
OMe
N
OBn
CO2Me

9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79.23
90.48
110.76
111.64
121.69
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127.06
128.12
128.21
128.72
129.52
129.98
130.16
135.48
135.55
139.88
144.23
146.94
147.30
152.80
164.86
EtOAc
EtOAc
EtOAc
EtOAc
ppm
0
10
20
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60
70
80
90
100
110
120
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140
150
160
170
180
190
200
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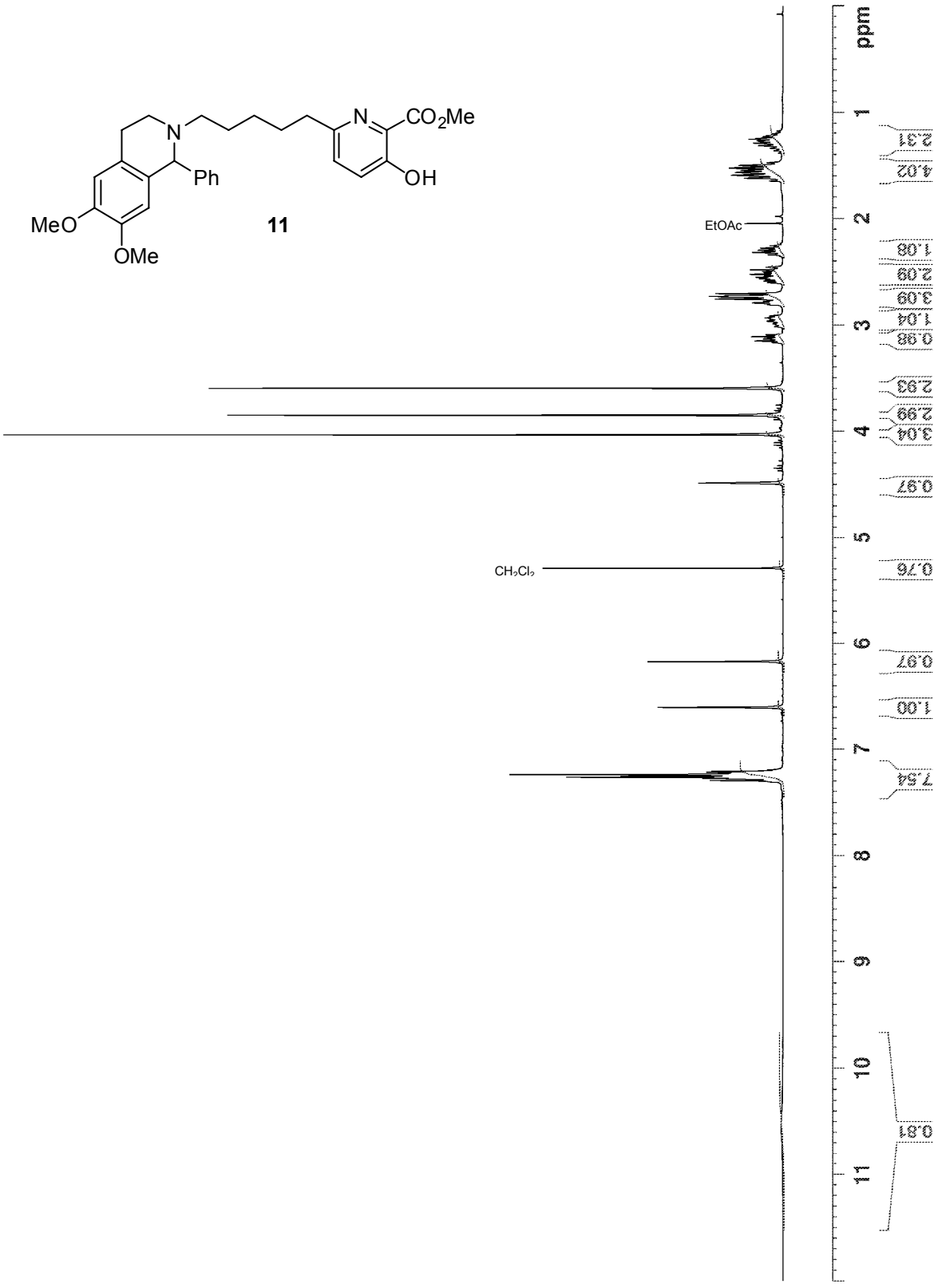
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CO2Me
N
Ph
OH
MeO
OMe
11
EtOAc
CH2Cl2
ppm
1
2
3
4
5
6
7
8
9
10
11
2.31
4.02
1.08
2.09
3.09
1.04
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3.04
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0.76
0.97
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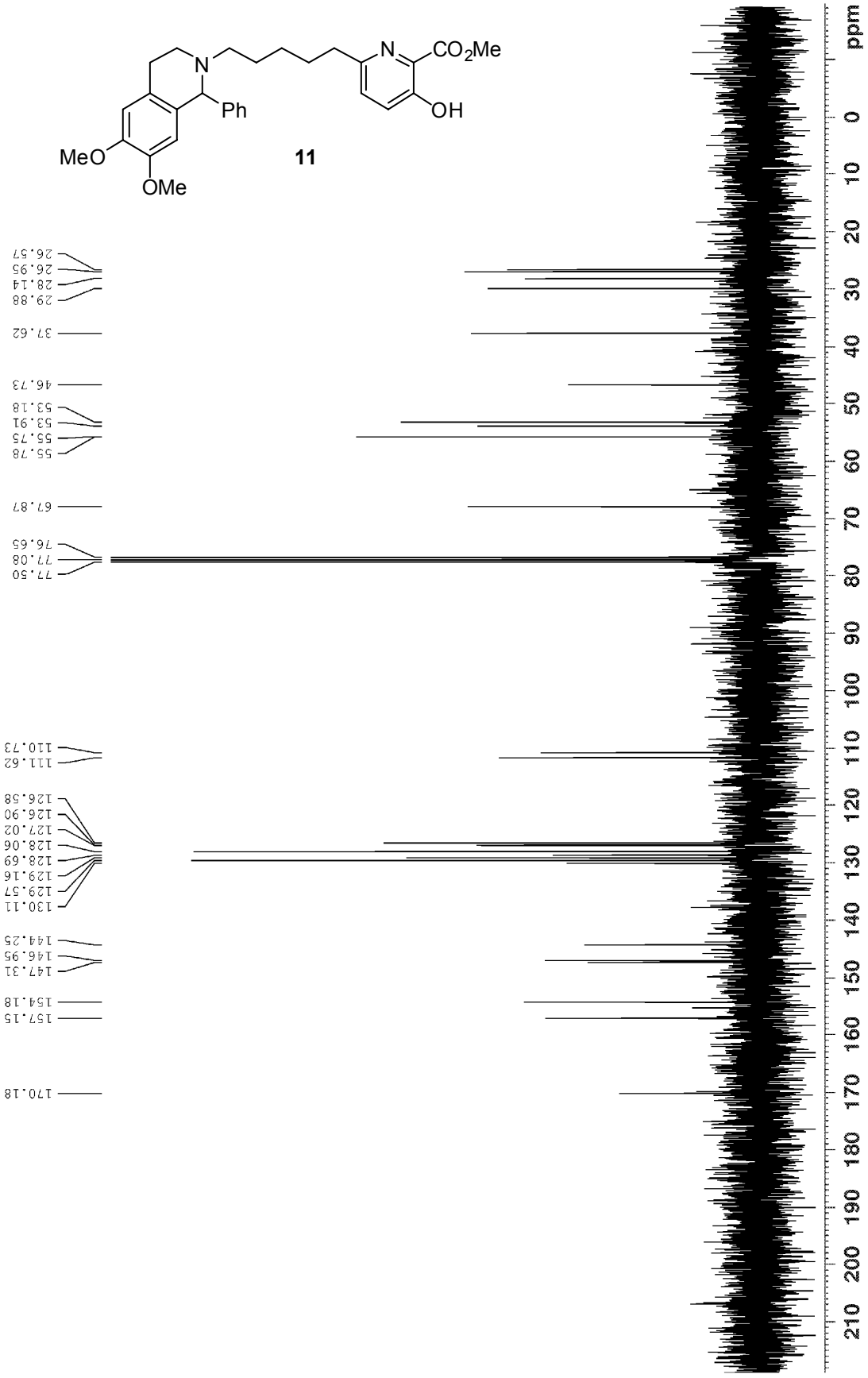
0.81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CO2Me
Ph
OH
MeO
OMe
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ppm
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20
30
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50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
26.57
26.95
28.14
29.88
37.62
46.73
53.18
53.91
55.75
55.78
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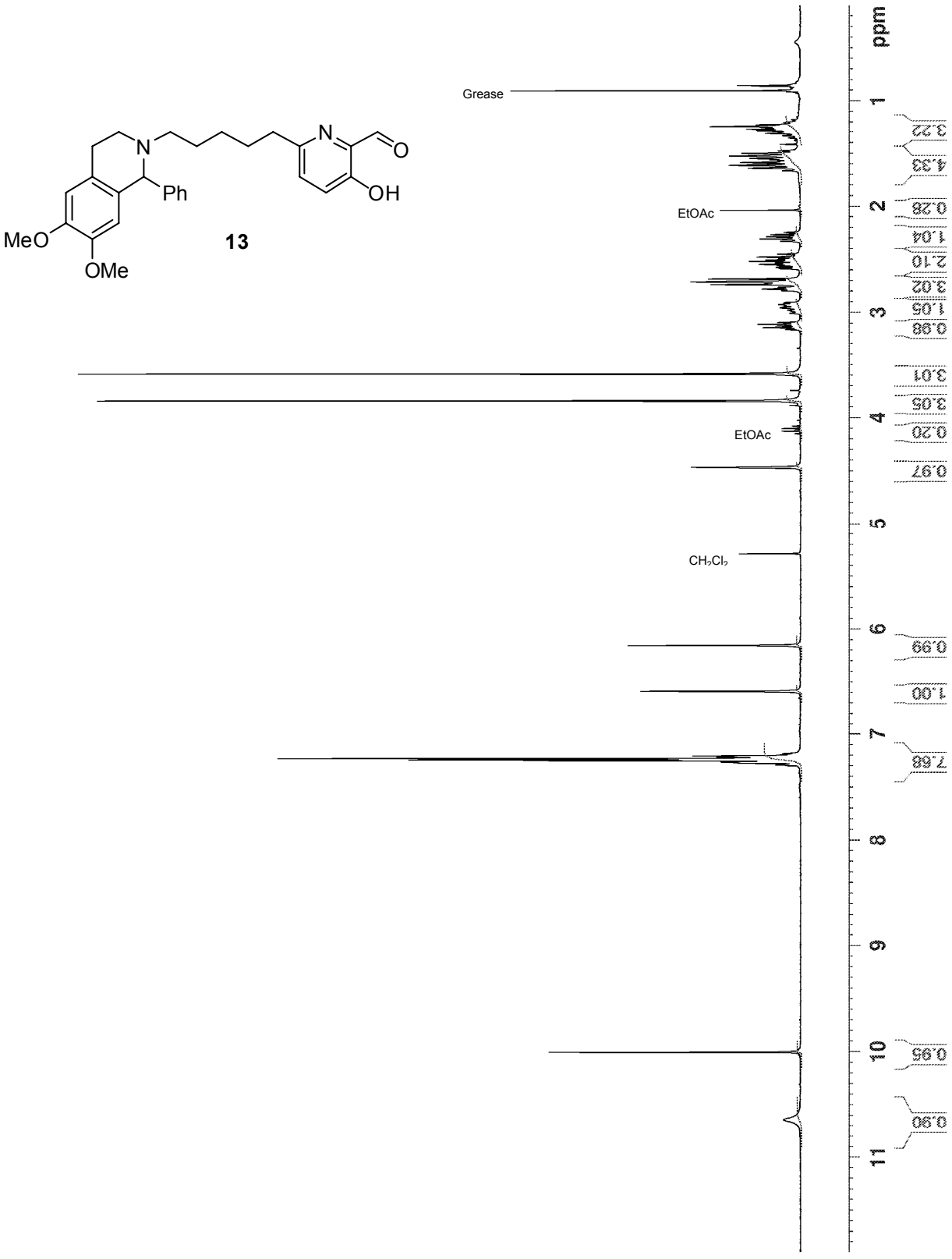
154.18
157.15
170.18


Grease
EtOAc
EtOAc
CH2Cl2
ppm
3.22
4.33
0.28
1.04
2.10
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1.05
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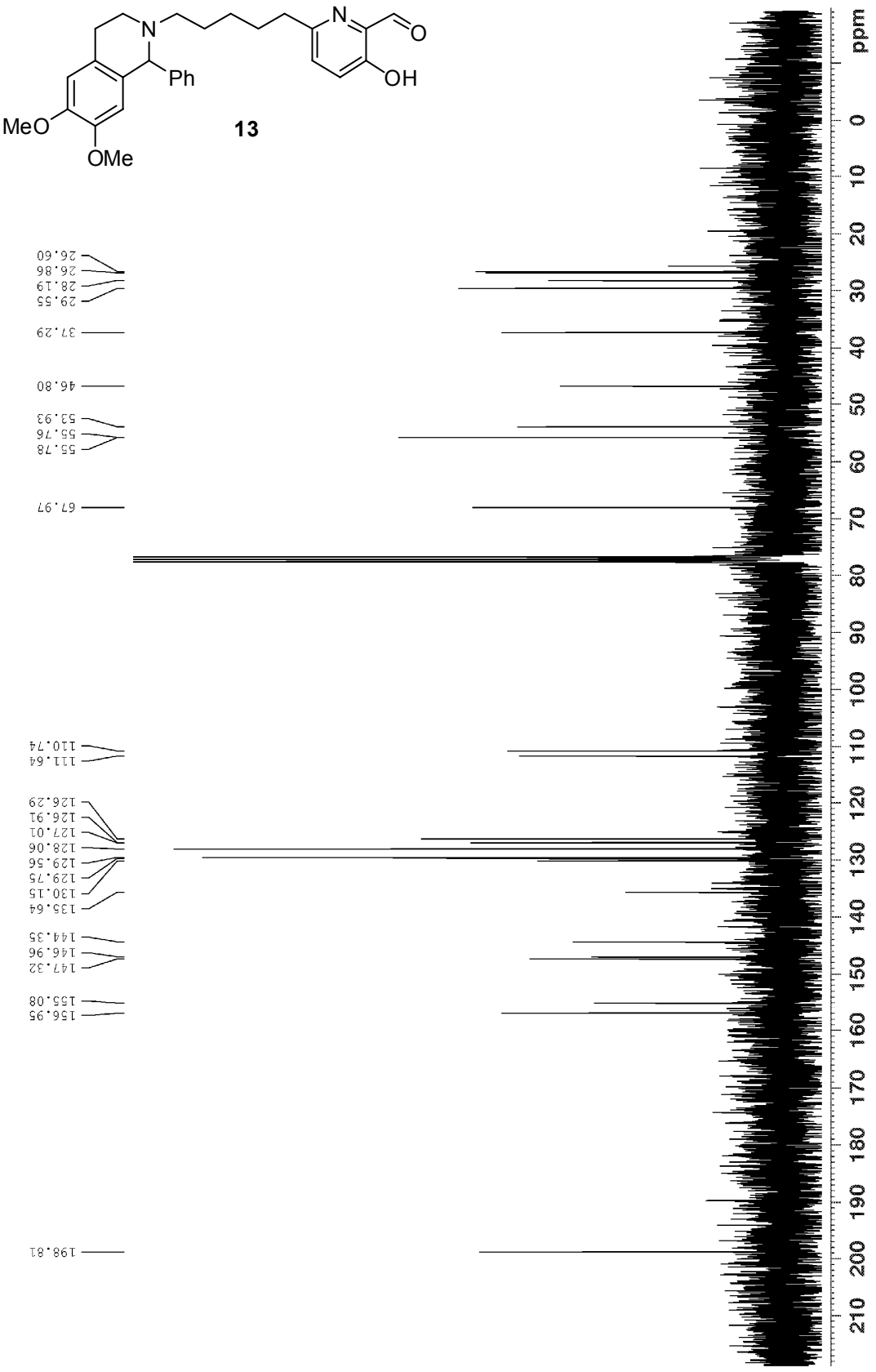
3.01
3.05
0.20
0.97
0.99
1.00
7.68
0.95
0.90

13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OMe
N
O
OH
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37.29
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55.76
55.78
67.97
110.74
111.64
126.29
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127.01
128.06
129.56
129.75
130.15
135.64
144.35
146.96
147.32
155.08
156.95
198.81
ppm
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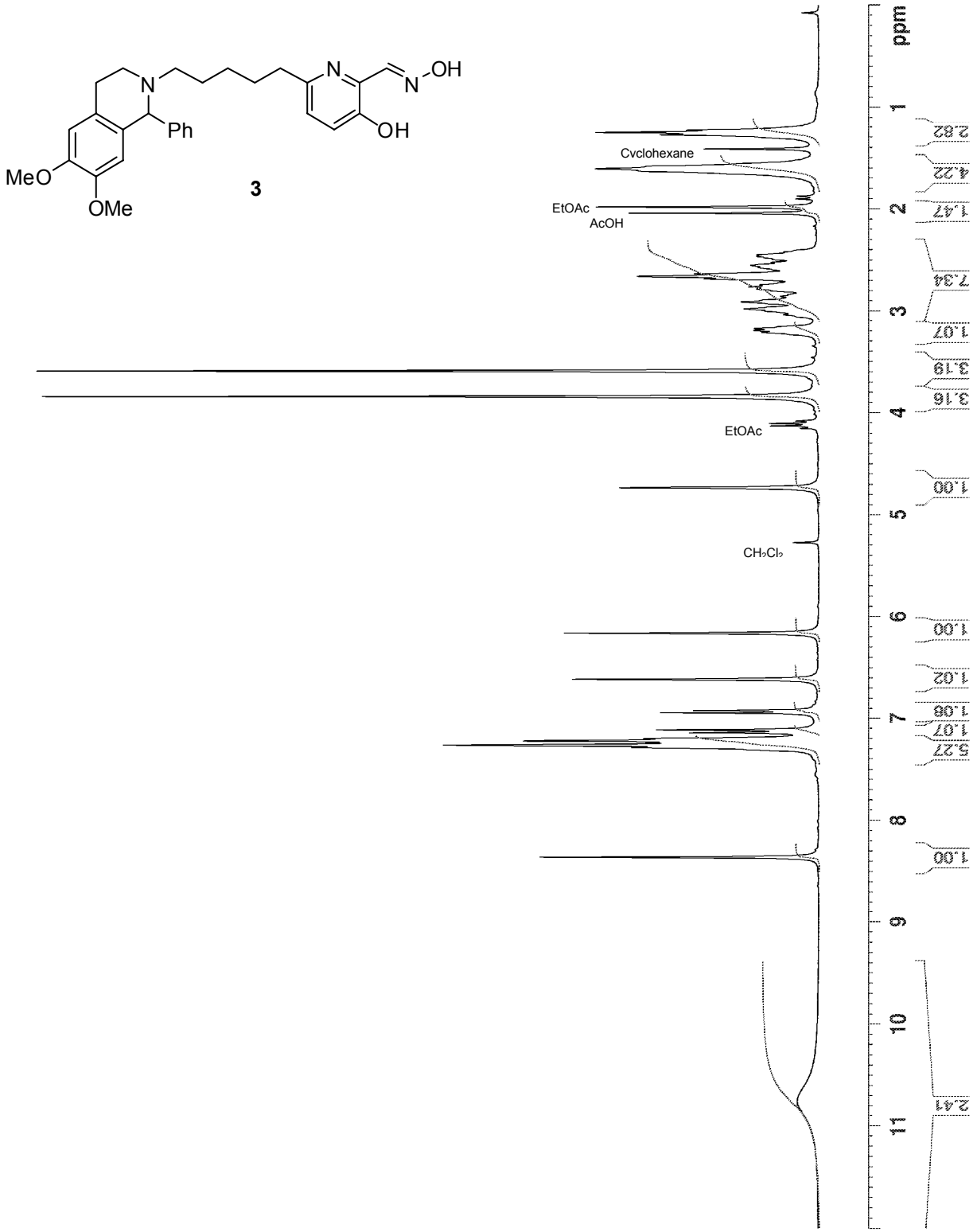
200
210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N
OH
OH
Ph
MeO
OMe
3
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EtOAc
AcOH
EtOAc
CH2Cl2
ppm
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1.08
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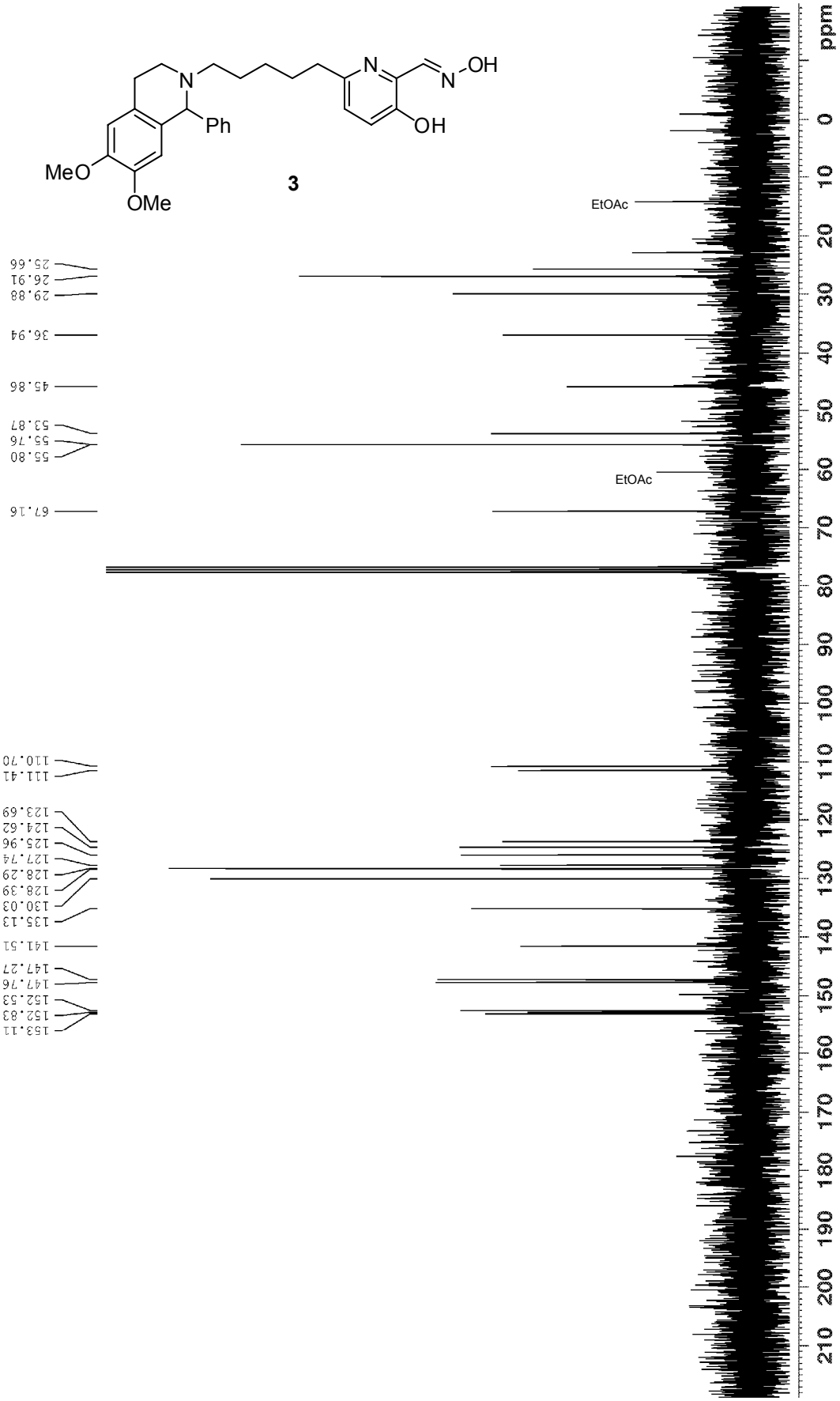
5.27
1.00
2.41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N
Ph
OH
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OMe
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EtOAc
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29.88
36.94
45.86
53.87
55.76
55.80
67.16
110.70
111.41
123.69
124.62
125.96
127.74
128.29
128.39
130.03
135.13
141.51
147.27
147.76
152.53
152.83
153.11
ppm
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70
80
90
100
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140
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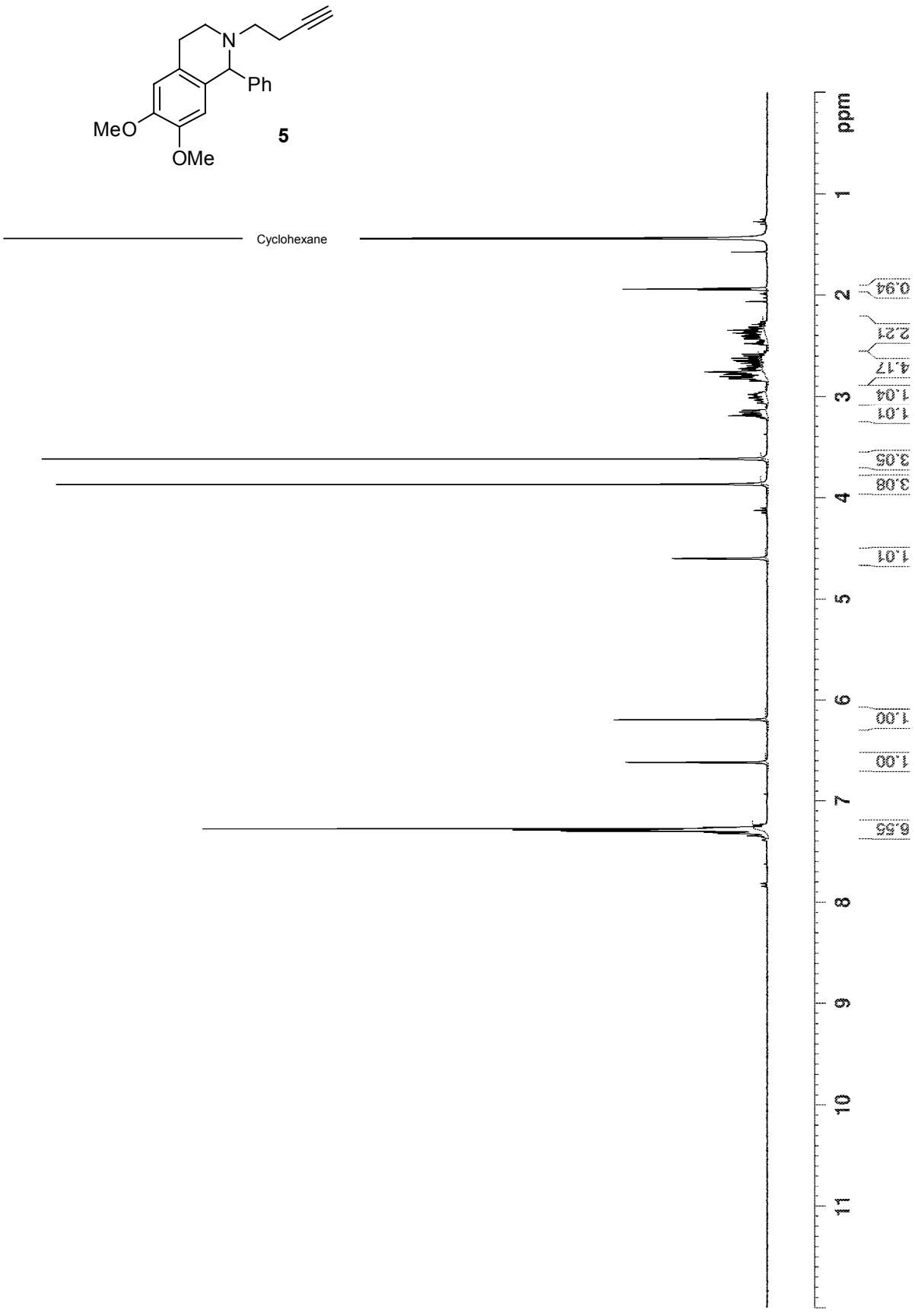
170
180
190
200
210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OMe
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ppm
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3.05
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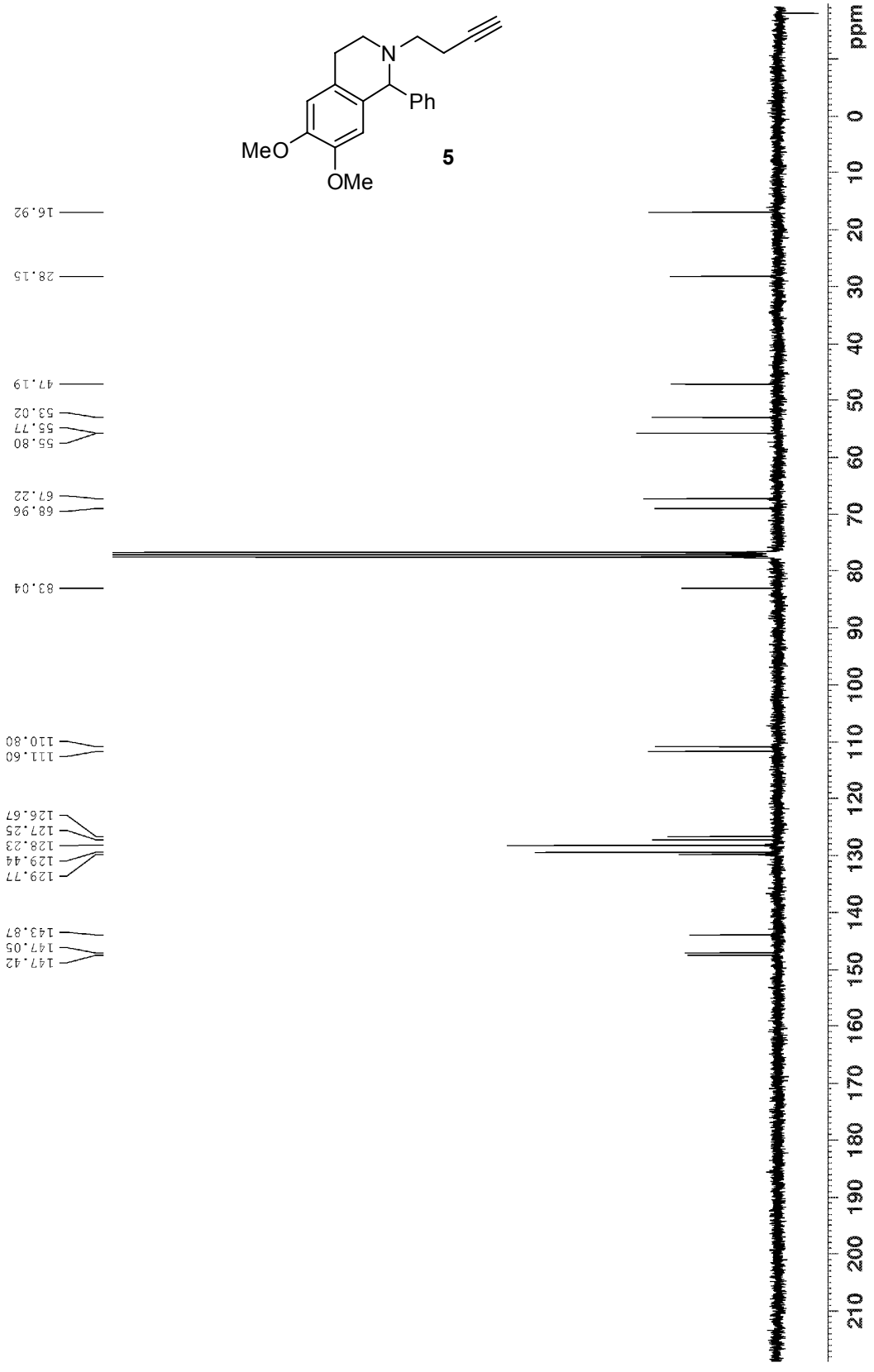
1.00
1.00
6.55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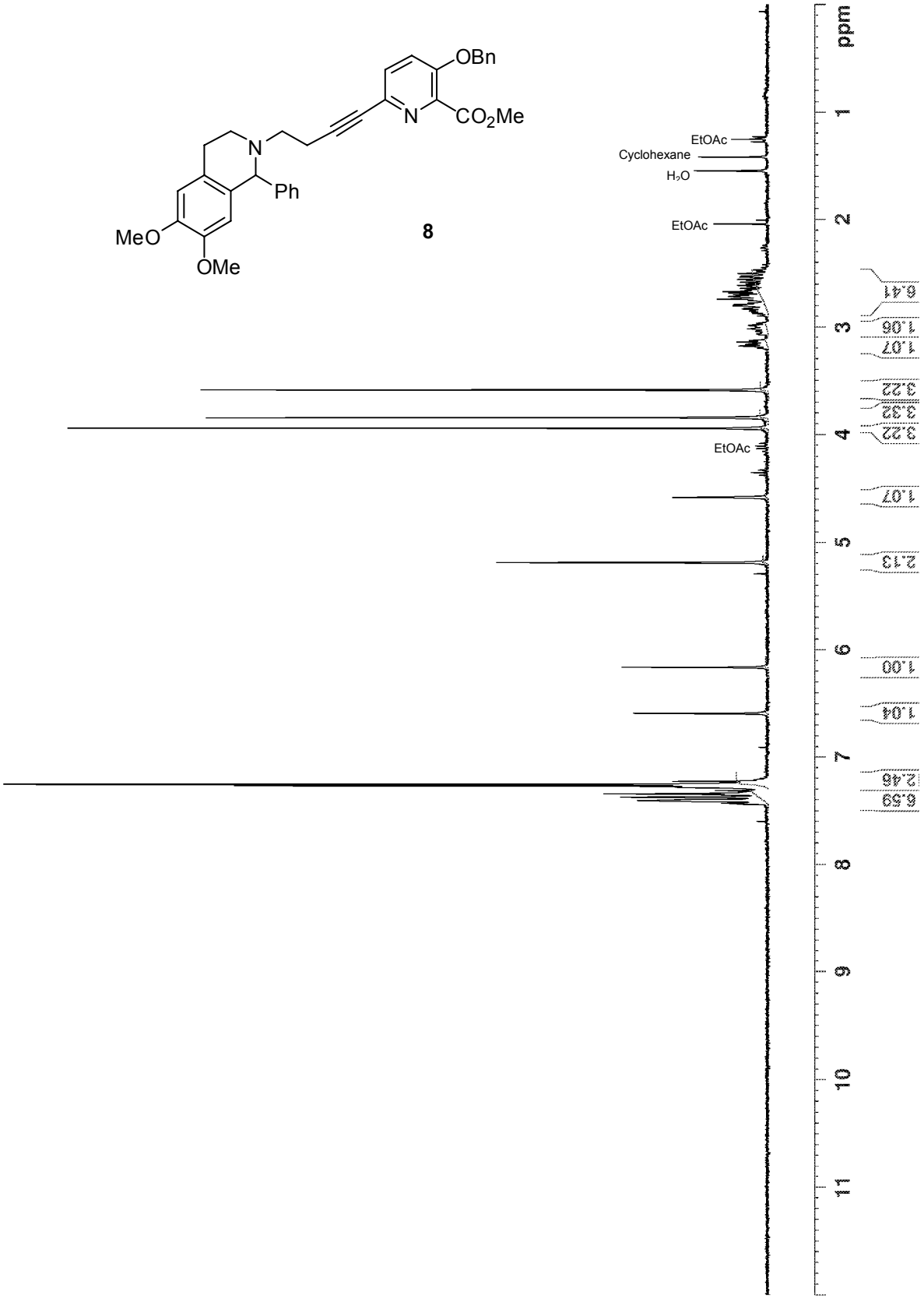
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Ph
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OMe
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EtOAc
EtOAc
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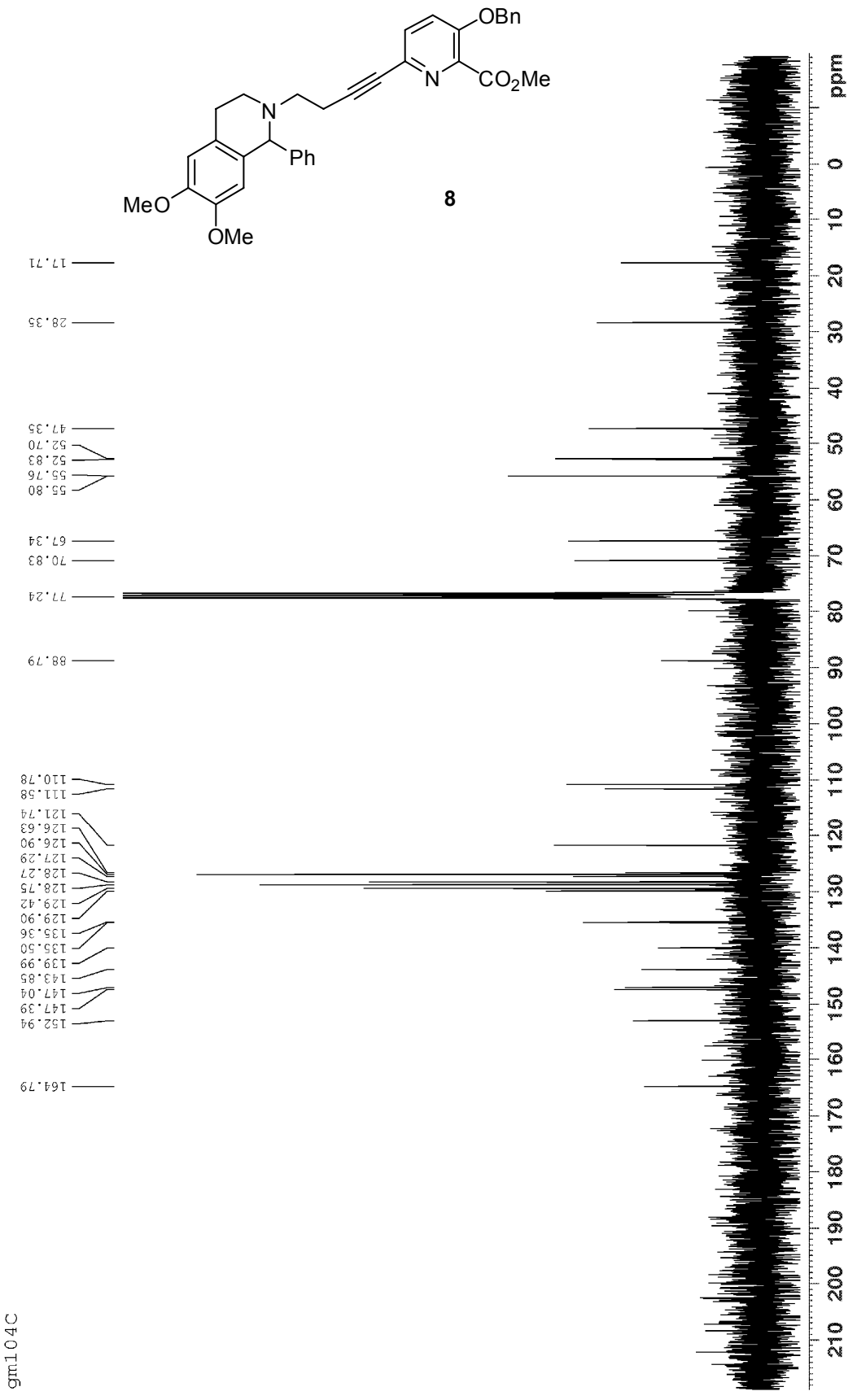
6.59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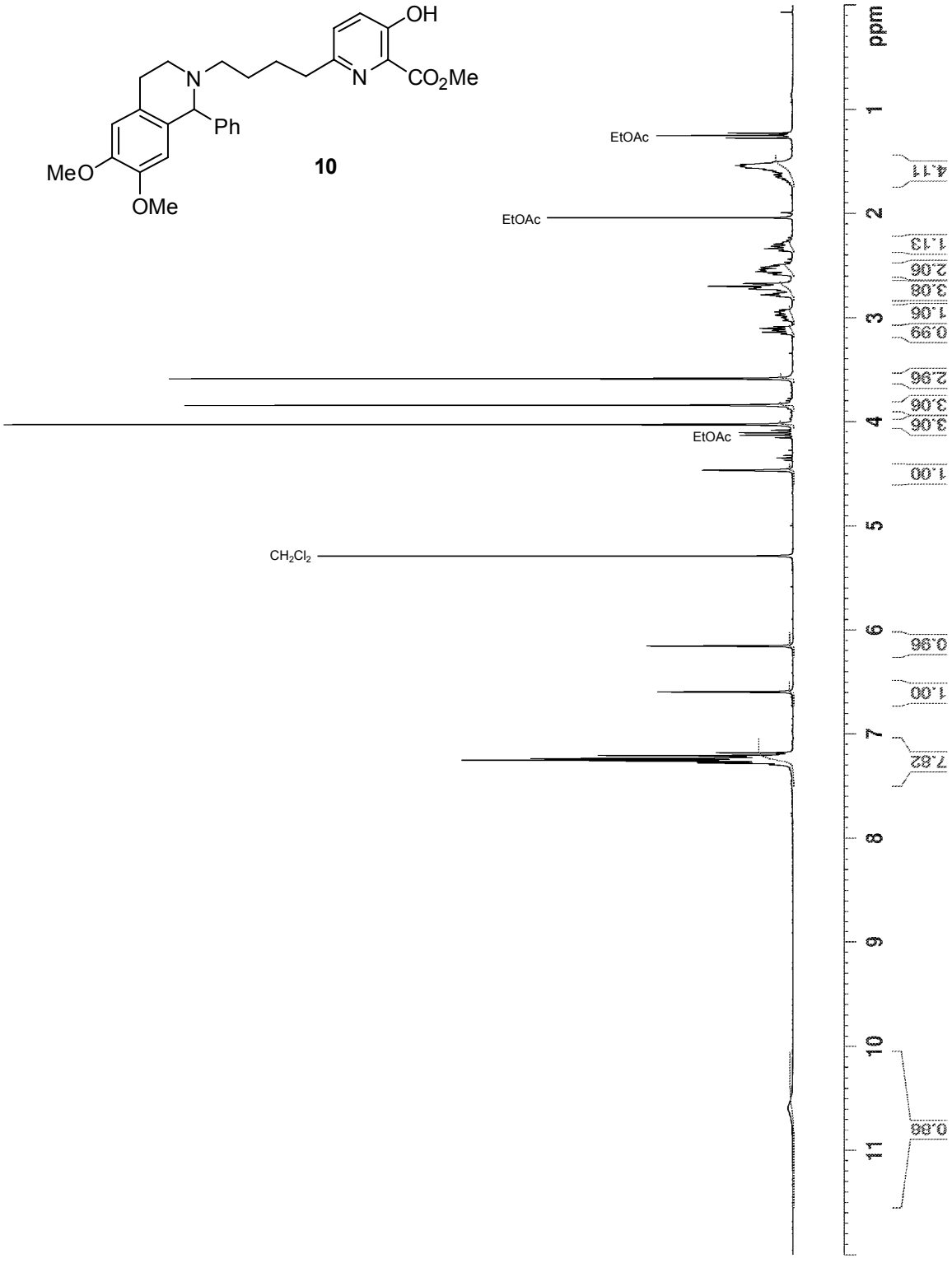
164.79
gm104C
ppm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OMe
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EtOAc
CH2Cl2
ppm
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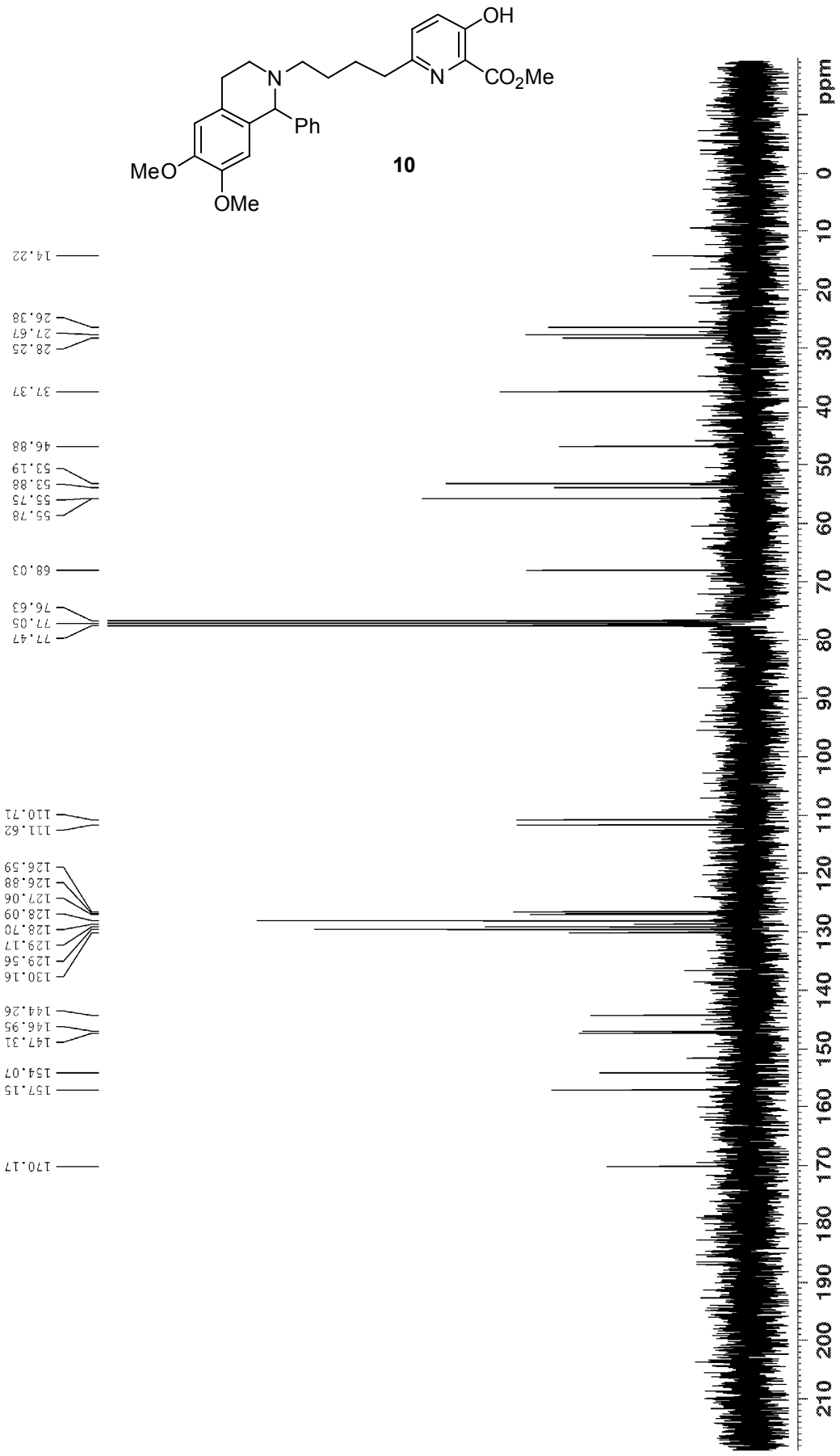
3.06
3.06
1.00
0.96
1.00
7.82
0.86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Ph
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OMe
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53.88
55.75
55.78
68.03
76.63
77.05
77.47
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111.62
126.59
126.88
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147.31
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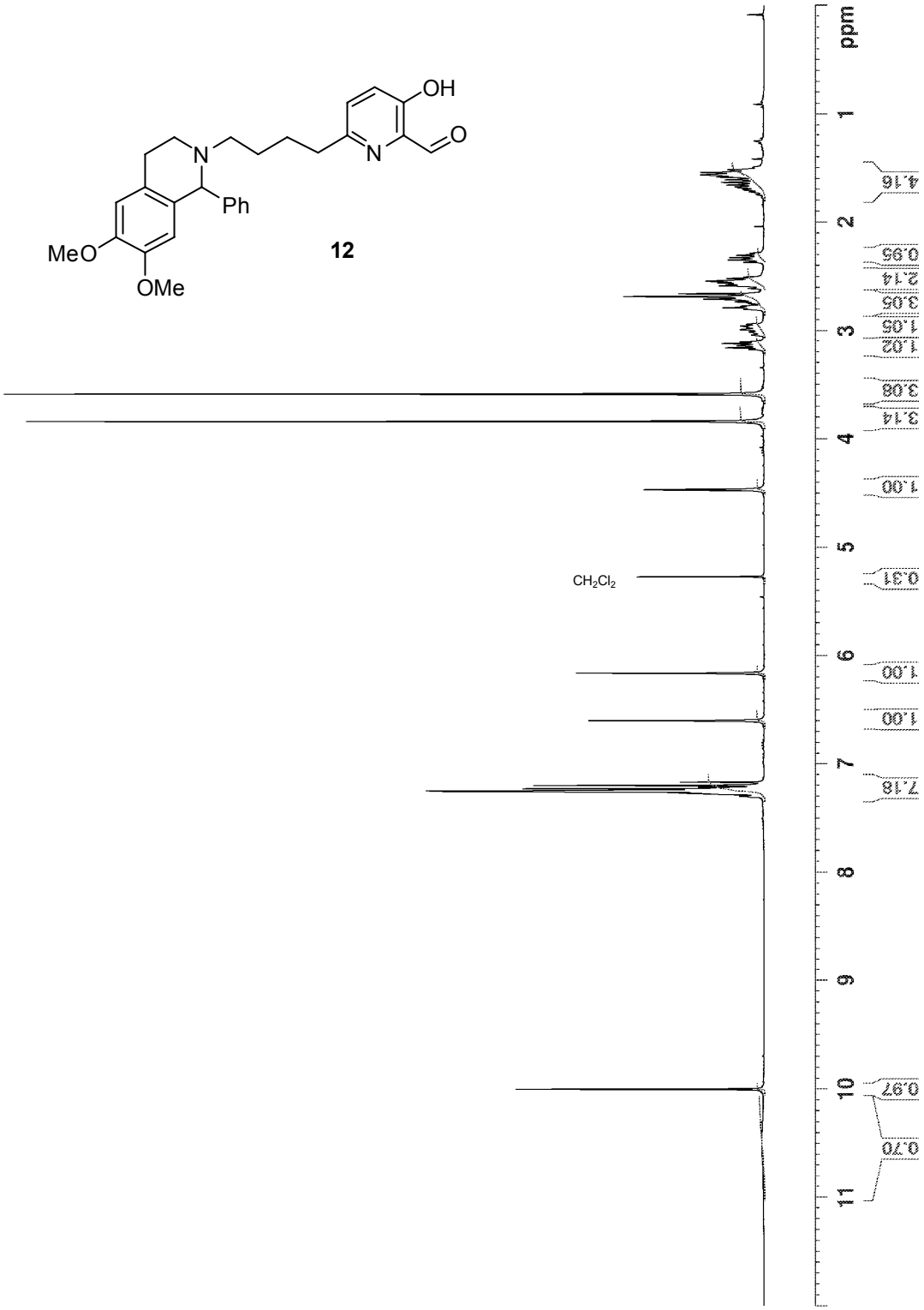
ppm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Ph
MeO
OMe
12
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ppm
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8
9
10
11
4.16
0.95
2.14
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3.08
3.14
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1.00
1.00
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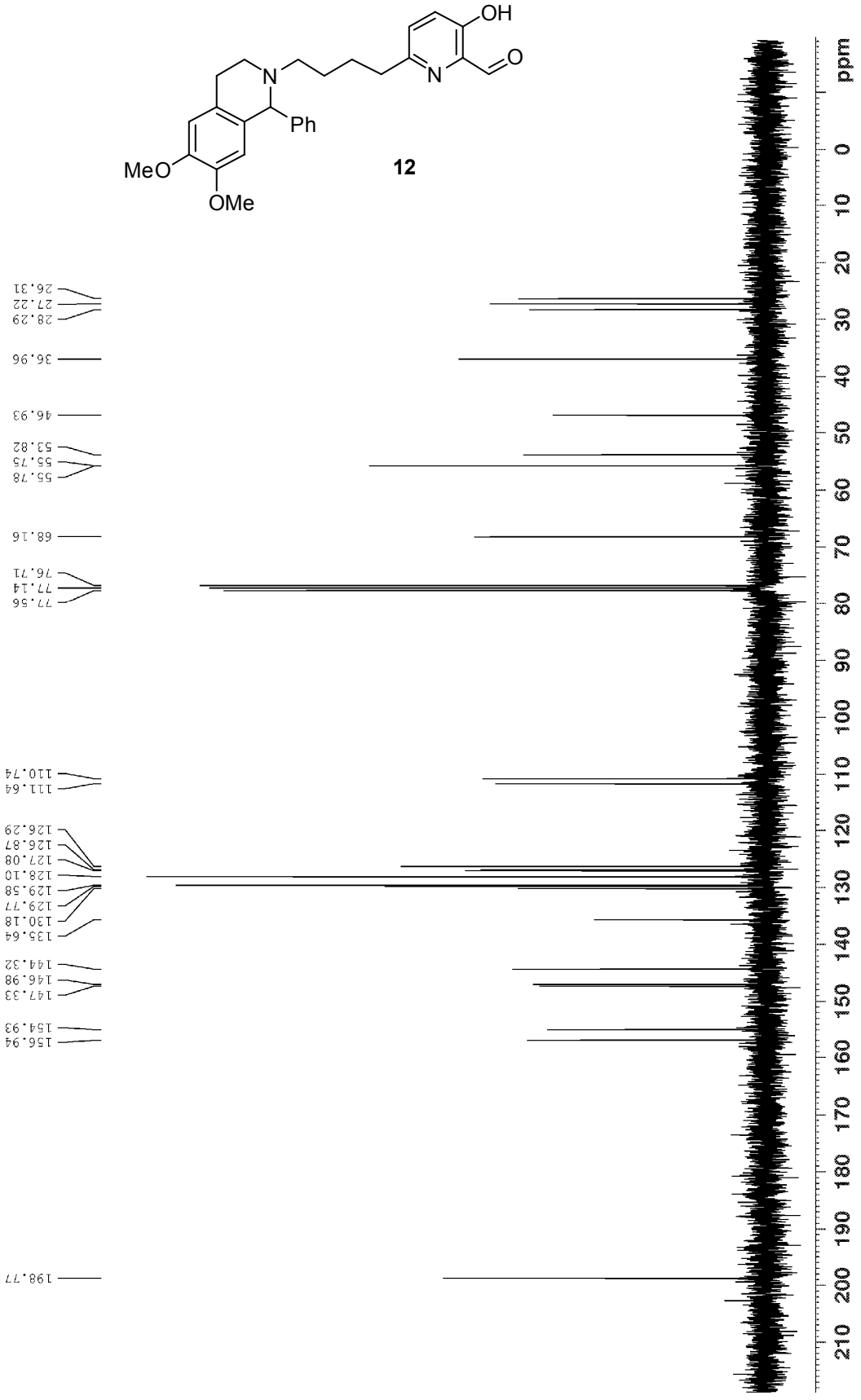
0.70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Ph
MeO
OMe
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ppm
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50
60
70
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28.29
36.96
46.93
53.82
55.75
55.78
68.16
76.71
77.14
77.56
110.74
111.64
126.29
126.87
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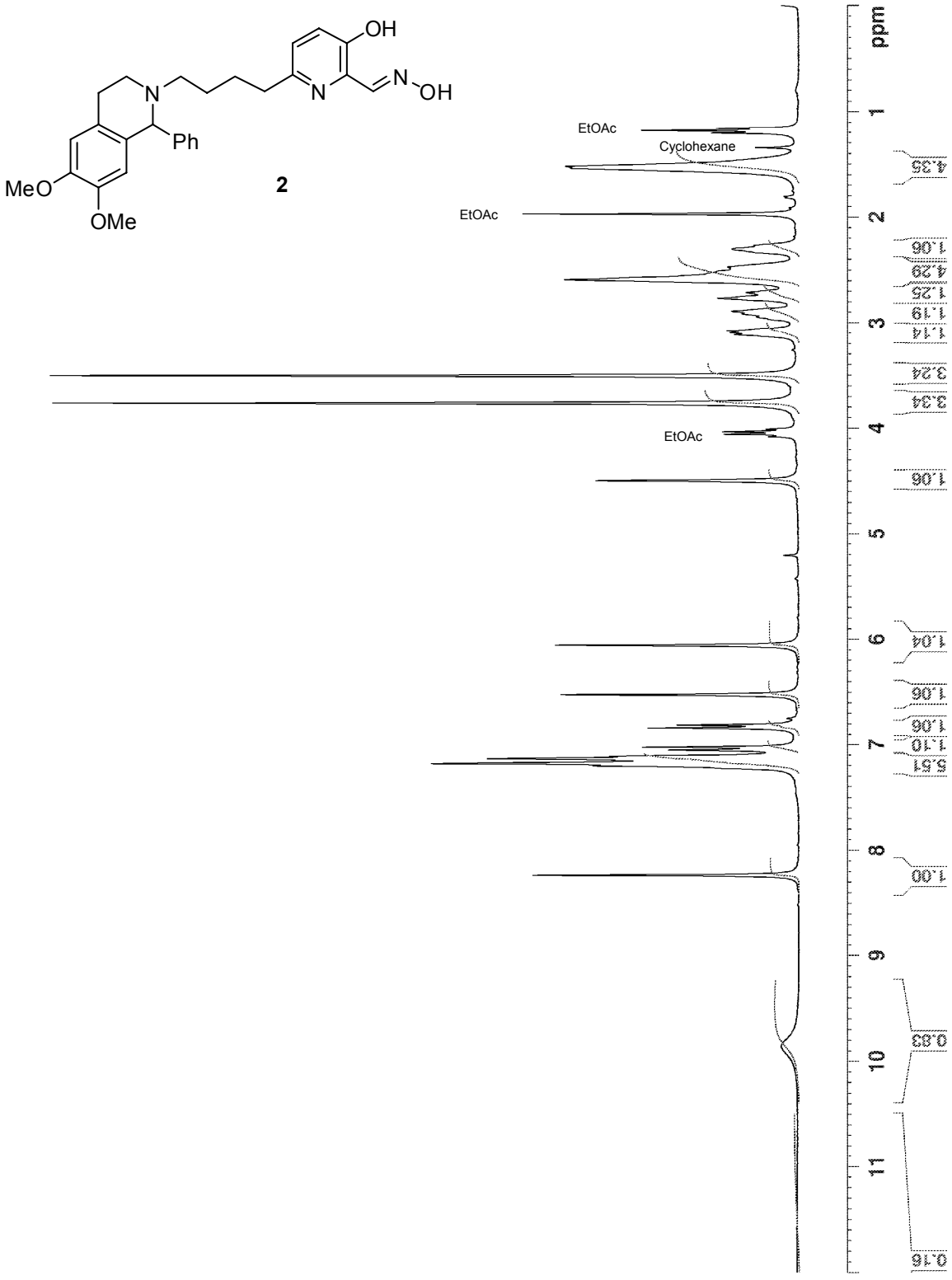
154.93
156.94
198.77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Ph
MeO
OMe
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EtOAc
EtOAc
ppm
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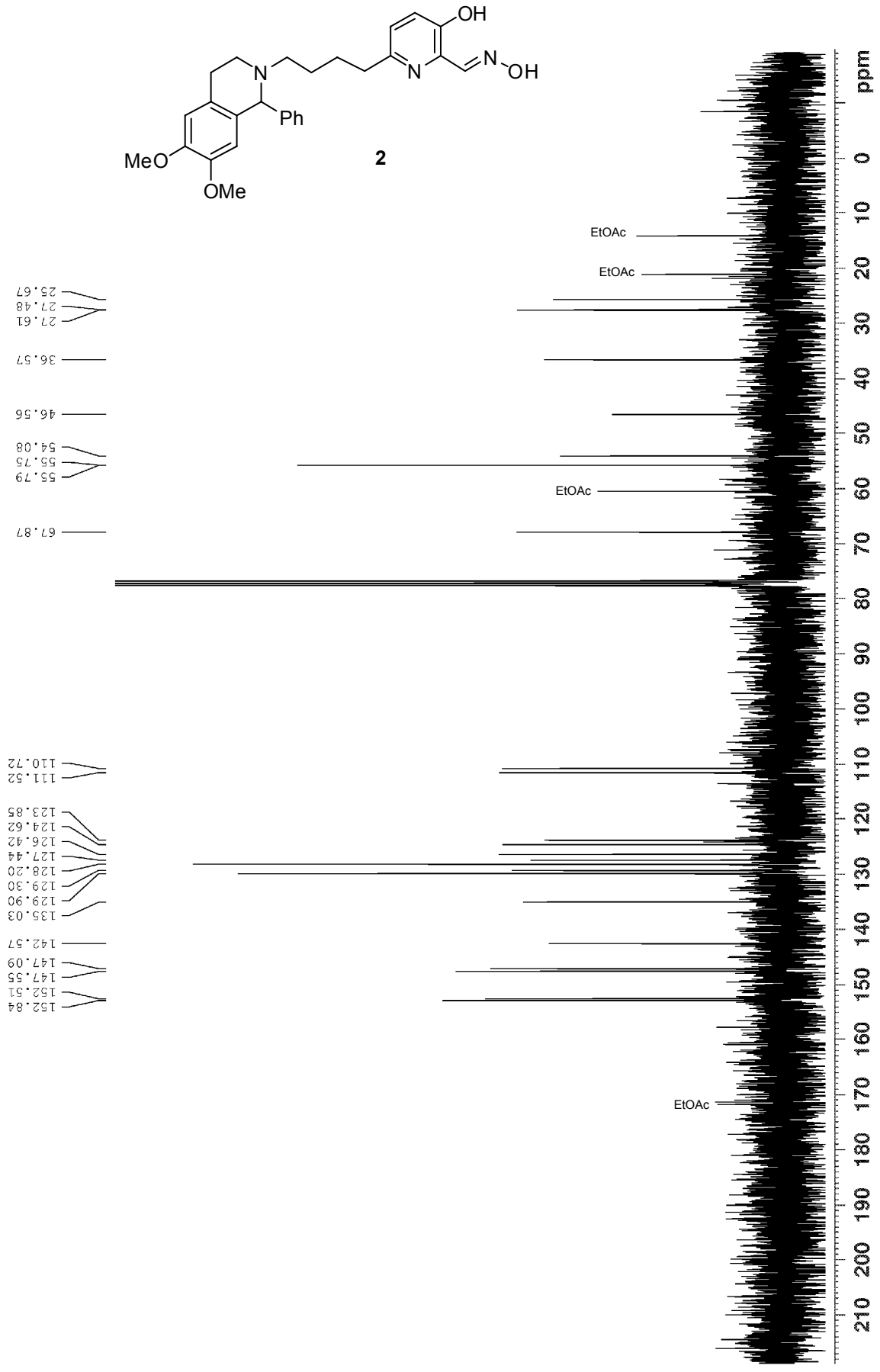
4.29
1.25
1.19
1.14
3.24
3.34
1.06
1.04
1.06
1.06
1.10
5.51
1.00
0.83
0.16


OH
N
N
N
OH
Ph
MeO
OMe
2
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EtOAc
EtOAc
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36.57
46.56
54.08
55.75
55.79
67.87
110.72
111.52
123.85
124.62
126.42
127.44
128.20
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129.90
135.03
142.57
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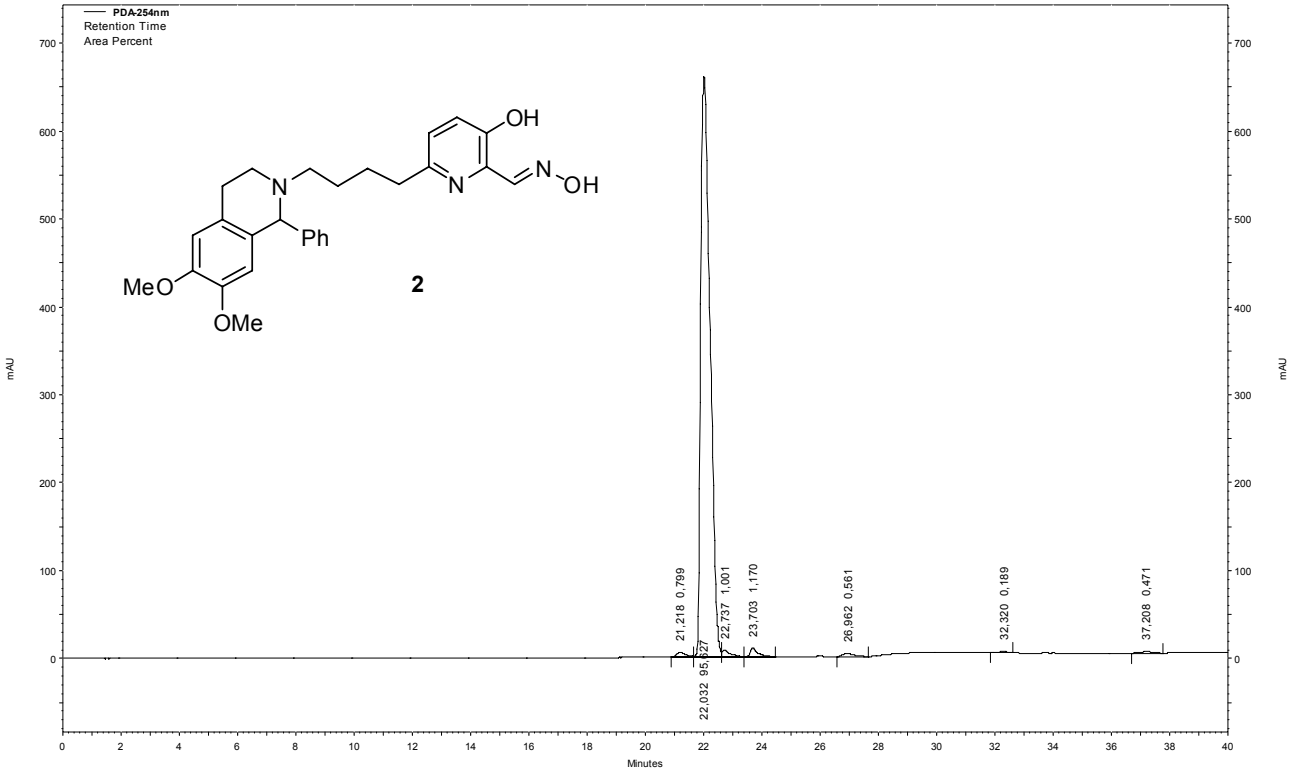
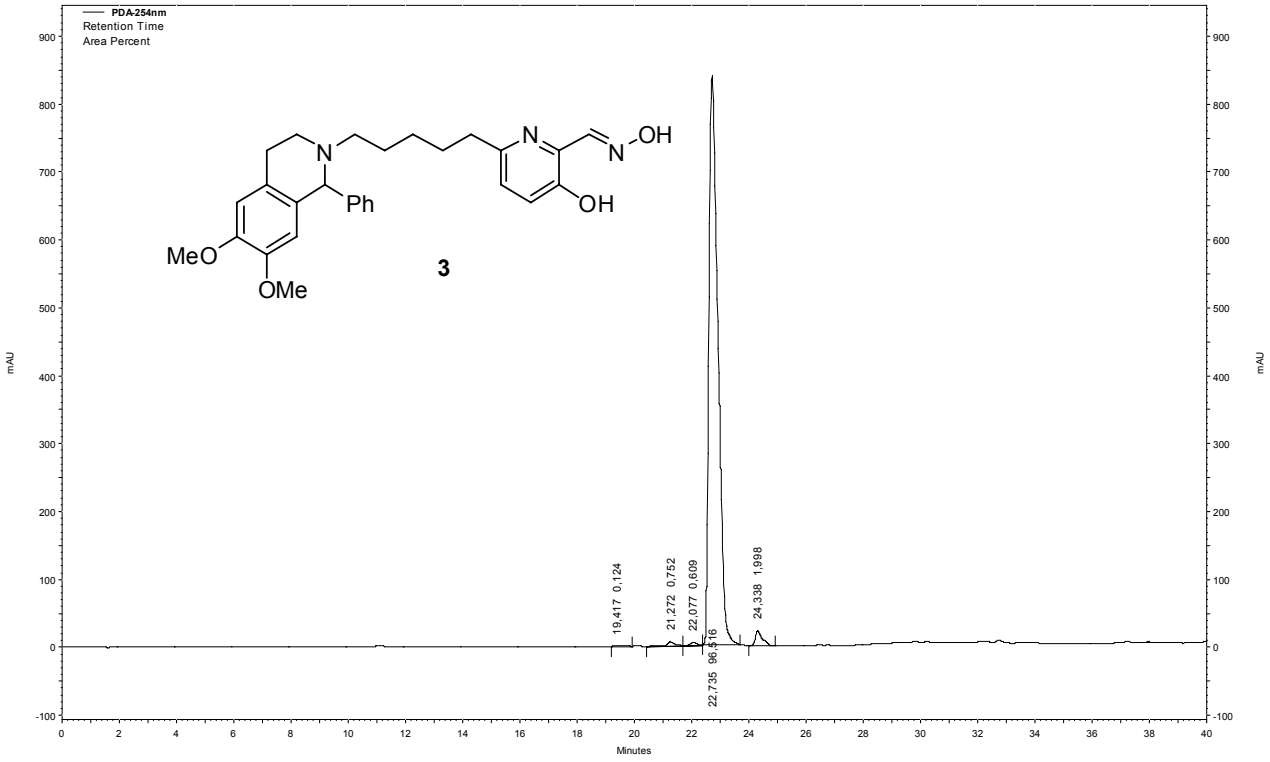
152.51
152.84
ppm

4. HLPC analysis of 2 and 3





5. Inhibition and reactivation kinetics

Recombinant hAChE was produced and purified as previously described.[4] VX and Tabun were from DGA maîtrise NRBC (Vert le Petit, France). HI-6 was from Pharmacie Centrale des Armées (Orléans, France). All other chemicals were from Sigma.

Stock solution of VX and tabun were 5 mM in isopropanol. The nerve agents were further diluted in MeOH to low µM concentrations. The inhibition of hAChE was performed in phosphate buffer (0.1 M, pH 7.4, 0.1% BSA) at 25°C. The amount of nerve agents was chosen to attain an inhibition plateau >80% in about 30 minutes while carefully avoiding 100% inhibition. Under these conditions, there is no inhibitor left that can affect the reactivation rate measurements.

OP-inhibited h-AChE was incubated at 37°C with different concentrations of oxime at 37°C in phosphate buffer (0.1 M, pH 7.4, 0.1% BSA, 5% methanol). Methanol was used for complete dissolution of the oximes; we determined the effect of MeOH on hAChE activity stabilized by BSA as a control experiment and found no significant effect up to 10% MeOH. The final concentrations of oximes used for VX-hAChE reactivation were: 1, 2, 3, 5, 10, 20, 25, 40, 50, 60, 75 µM for **2** , 0.1, 0.5, 1, 3, 5, 8, 10, 15 µM for **3**, 1, 2.5, 5, 8, 10, 12, 15, 25, 35, 50 µM for HI-6, 10, 25, 50,100, 200, 500, 1000 µM for trimedoxime, and 10, 25, 50, 75, 100, 160, 200 µM for obidoxime. The final concentrations used for tabun-hAChE reactivation were 0.5, 1, 5, 10, 25, 50, 100 µM for **2** and 2, 5, 10, 25, 50, 100 µM for **3,** 10, 50, 100, 200, 300, 400, 500, 1000 µM for trimedoxime, and 25, 50, 100, 200, 500, 700, 1000 for obidoxime. Aliquots were transferred to 1-ml cuvettes at time intervals ranging from 1 minutes to 10 minutes depending on the reactivation rate for measurement of h-AChE activity using 1 mM acetylthiocholine in Ellman's buffer (phosphate 0.1 M, pH 7.4, 0.1% BSA, 0.5 mM DTNB, 25°C).[5] The same procedure was applied to the control containing the uninhibited enzyme and oxime. 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 dissociation constant K_D of inhibited enzyme-oxime complex (E-POx) and the reactivity rate constant k_r were calculated by non-linear fit using the standard oxime concentration-dependent reactivation equation derived from the following scheme:

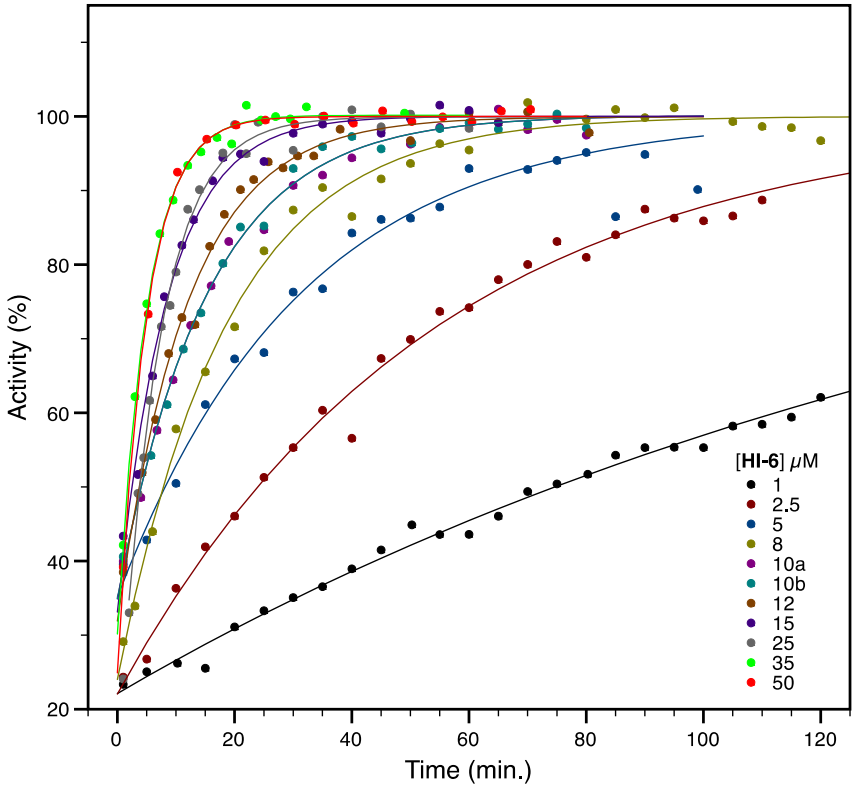
$$\text{E-P} + \text{Ox} \underset{}{\overset{K_D}{\rightleftharpoons}} \text{E-POx} \xrightarrow{k_r} \text{E} + \text{POx}$$

[4] 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.
[5] Ellman G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88-95.

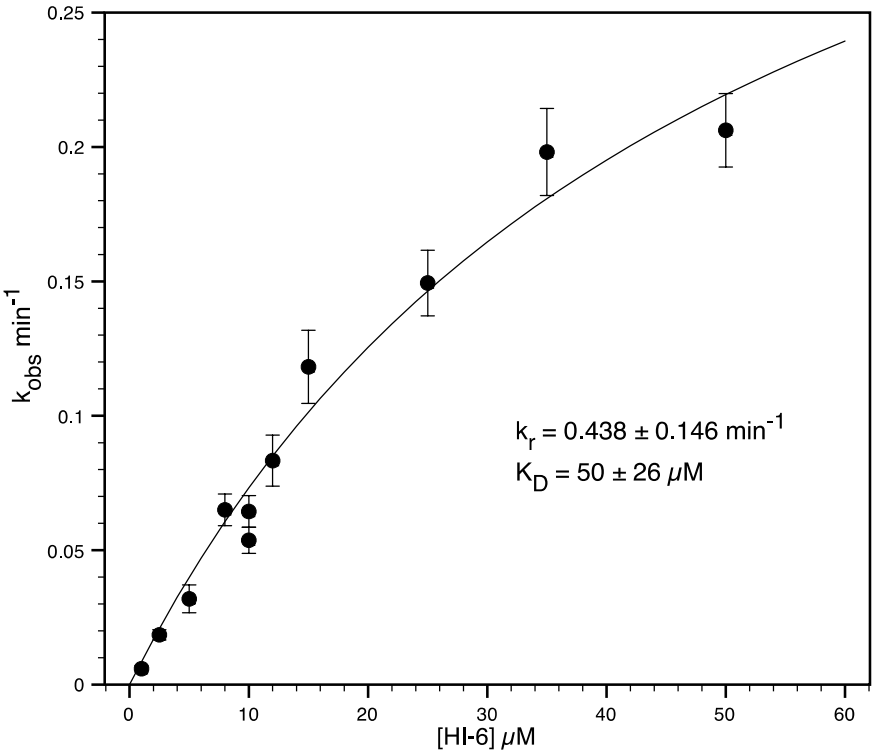
$$\%E_{react} = 100 \cdot \left(1 - e^{k_{obs} \cdot t}\right) \quad \text{and} \quad k_{obs} = \frac{k_r[Ox]}{K_D + [Ox]}$$

The inhibition potency of both oximes was determined at 25°C by measuring hAChE activity in presence of various concentrations of **2** (1 to 100 μM) and **3** (0.1 to 50 μM) using 1 mM acetylthiocholine in Ellman's buffer (phosphate 0.1 M, pH 7.4, 0.1% BSA, 0.5 mM DTNB, 25°C, 5% methanol).

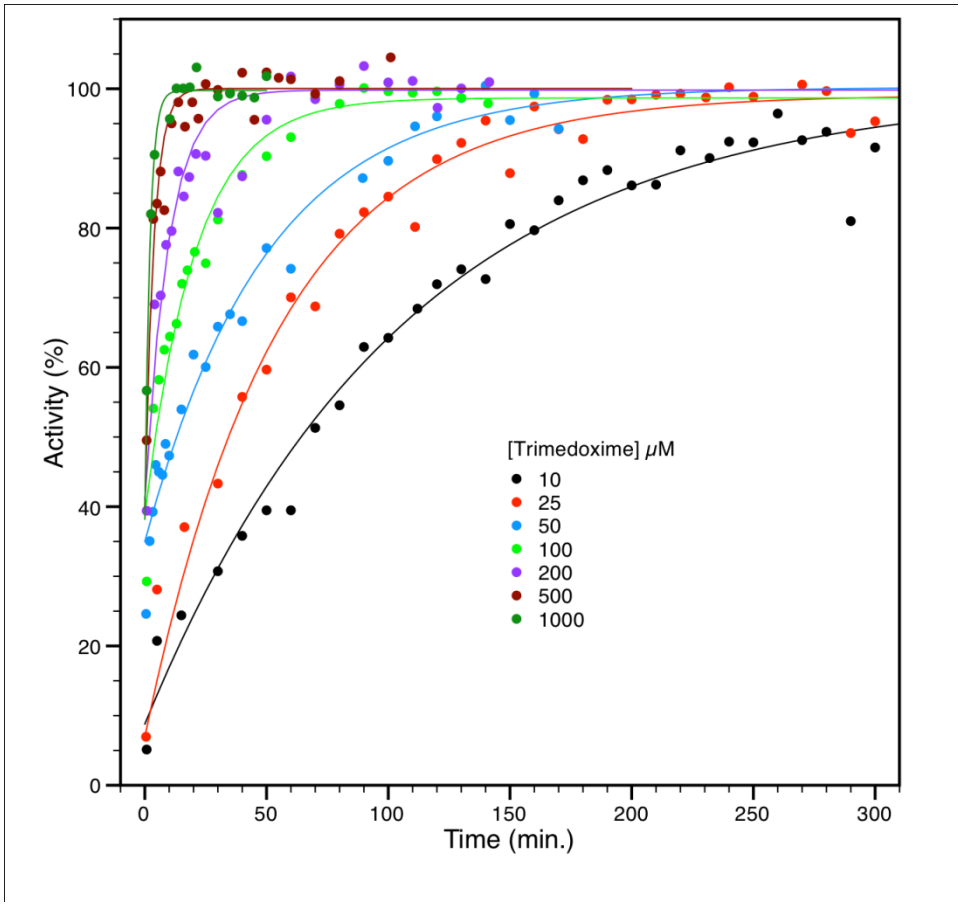
Reactivation of VX-inhibited hAChE by HI-6



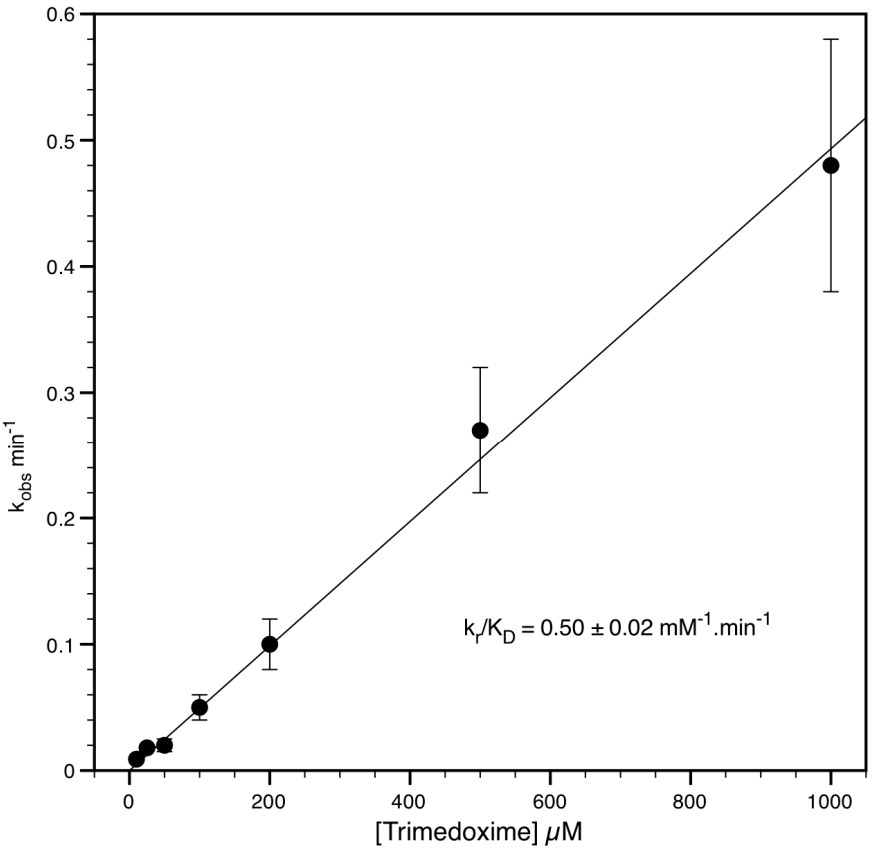
Plot of k_{obs} vs [HI-6]



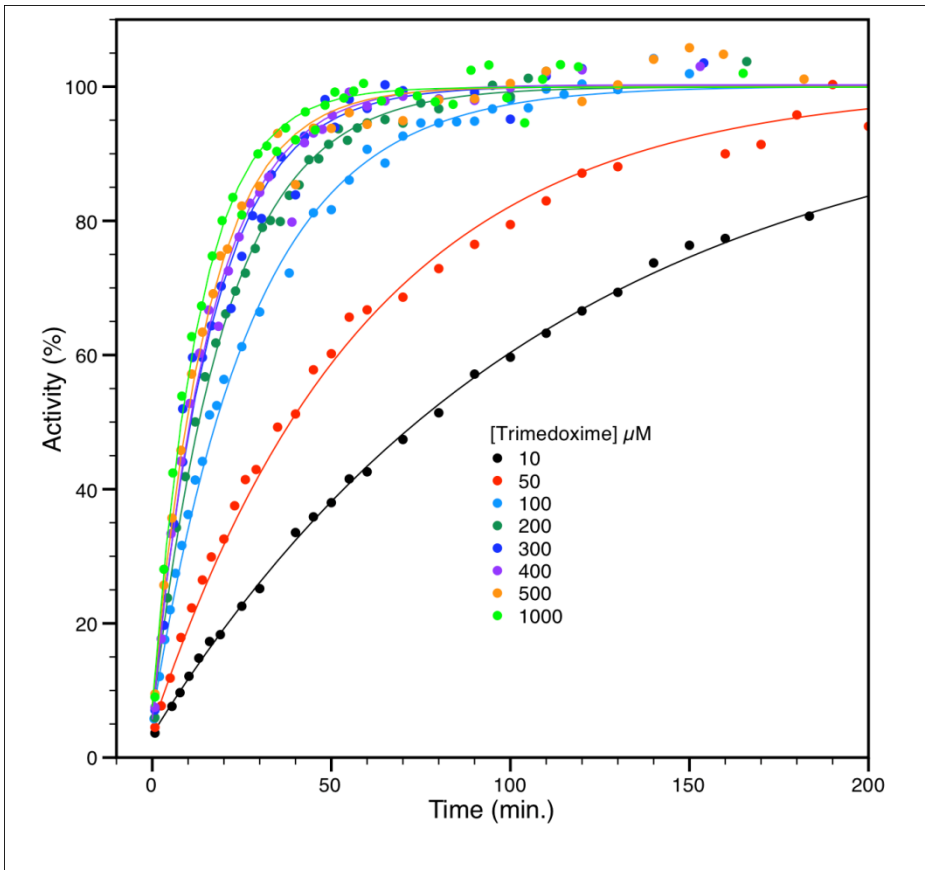
Reactivation of VX-inhibited hAChE by trimedoxime (TMB4)



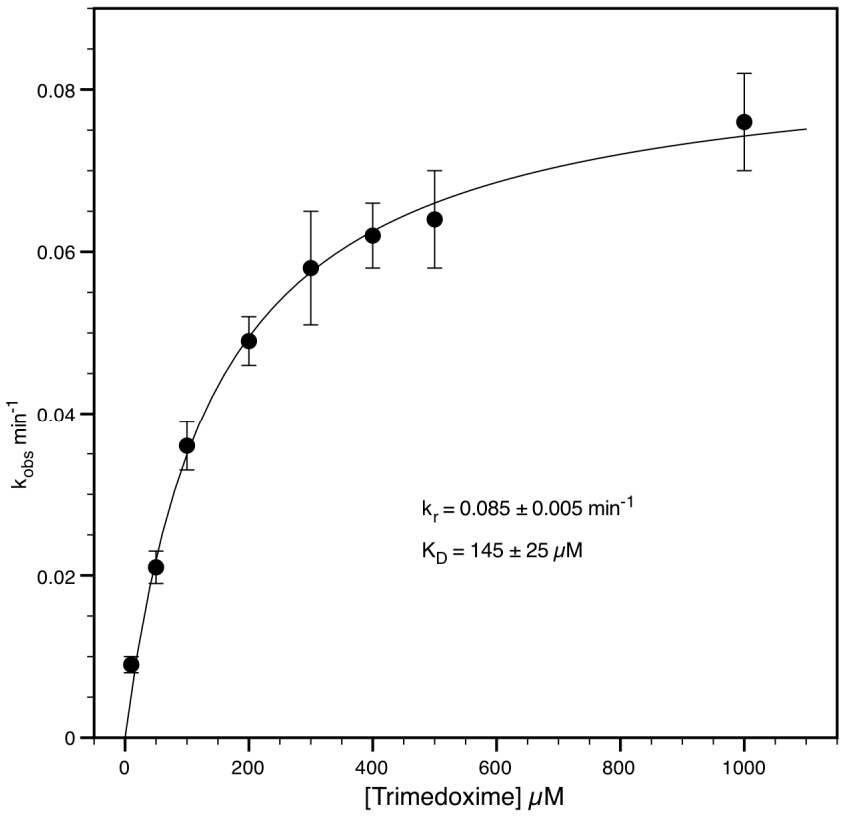
Plot of k_{obs} vs [TMB4]



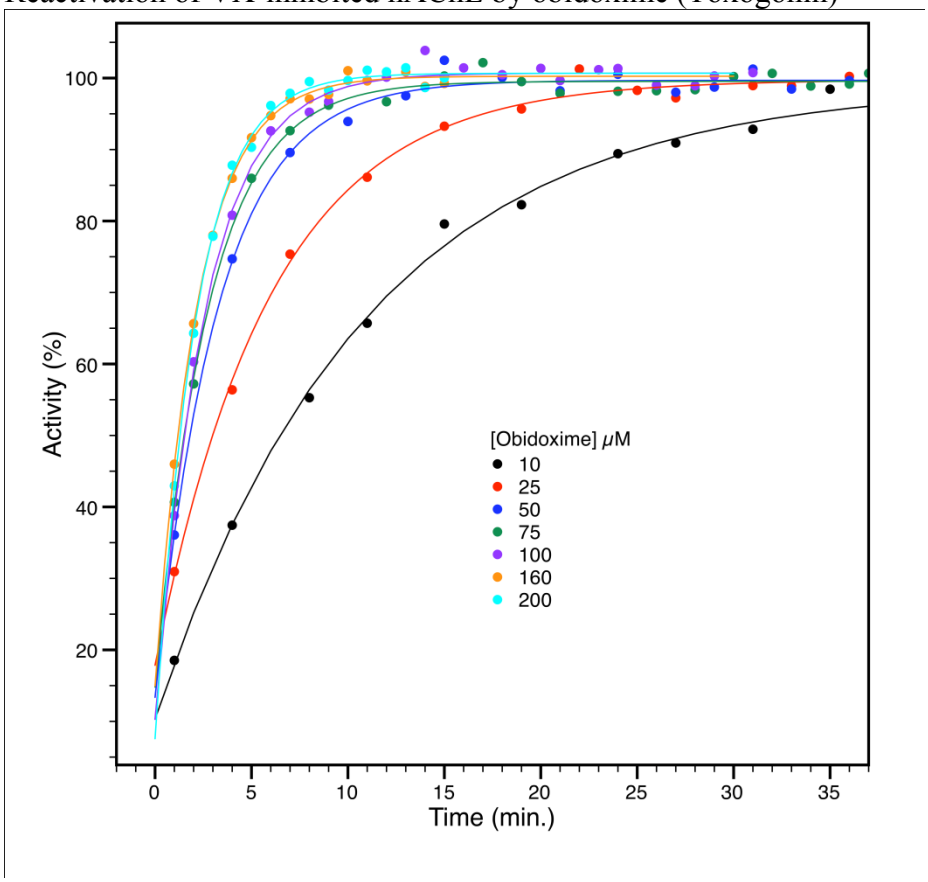
Reactivation of tabun-inhibited hAChE by trimedoxime (TMB4)



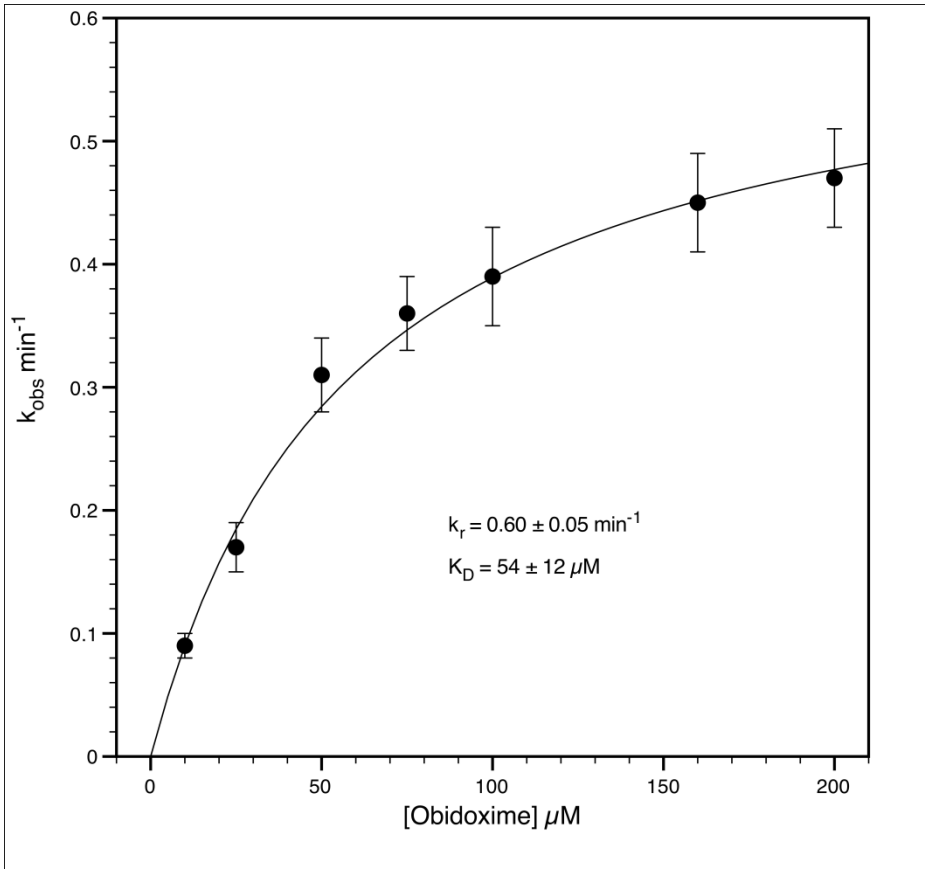
Plot of k_{obs} vs [trimedoxime]



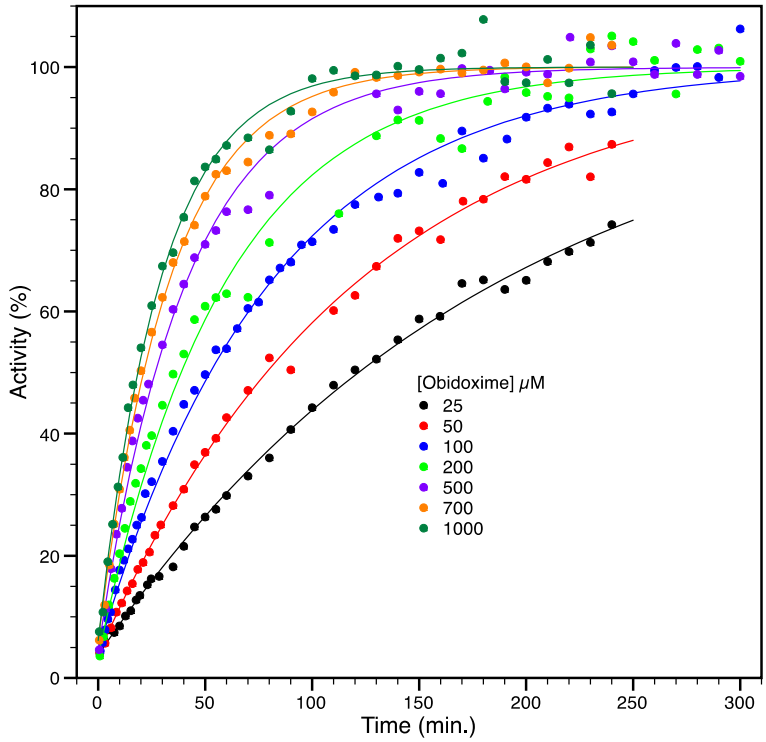
Reactivation of VX-inhibited hAChE by obidoxime (Toxogonin)



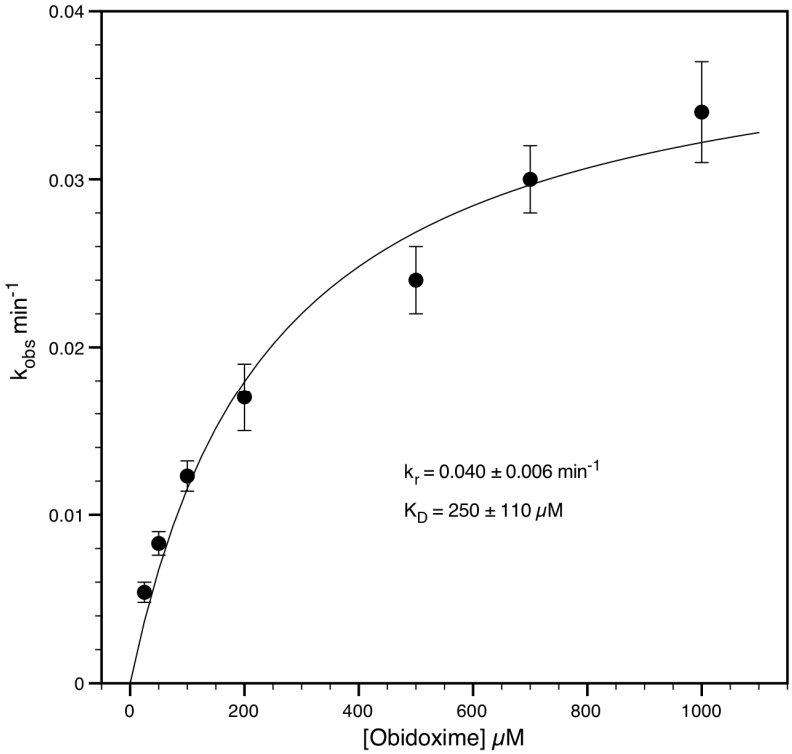
Plot of k_{obs} vs [Obidoxime]



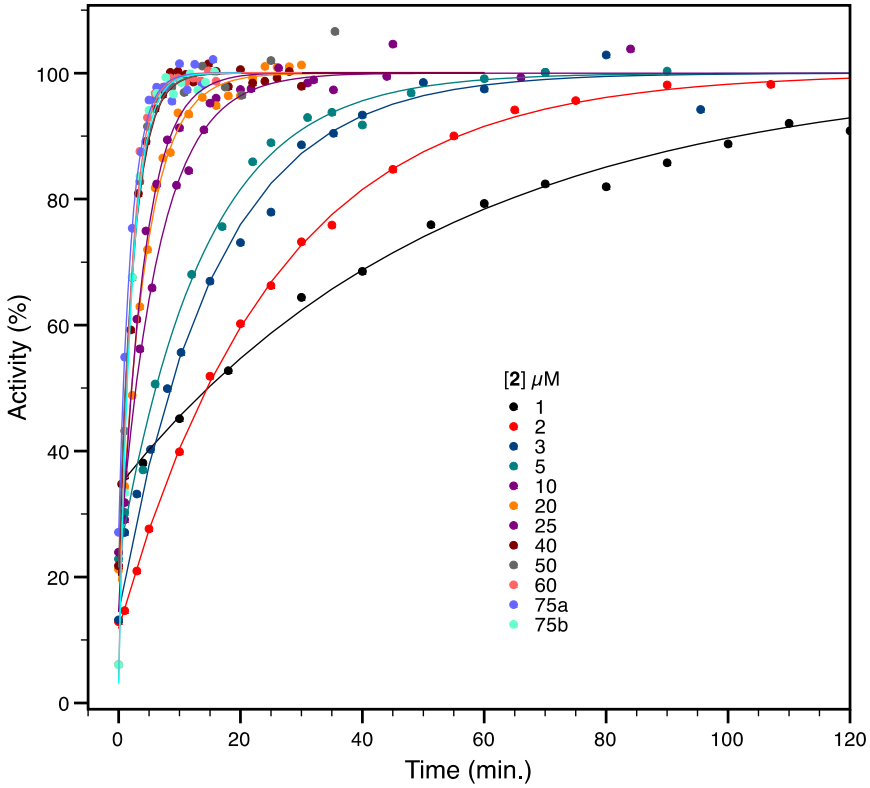
Reactivation of tabun-inhibited hAChE by obidoxime (Toxogonin)



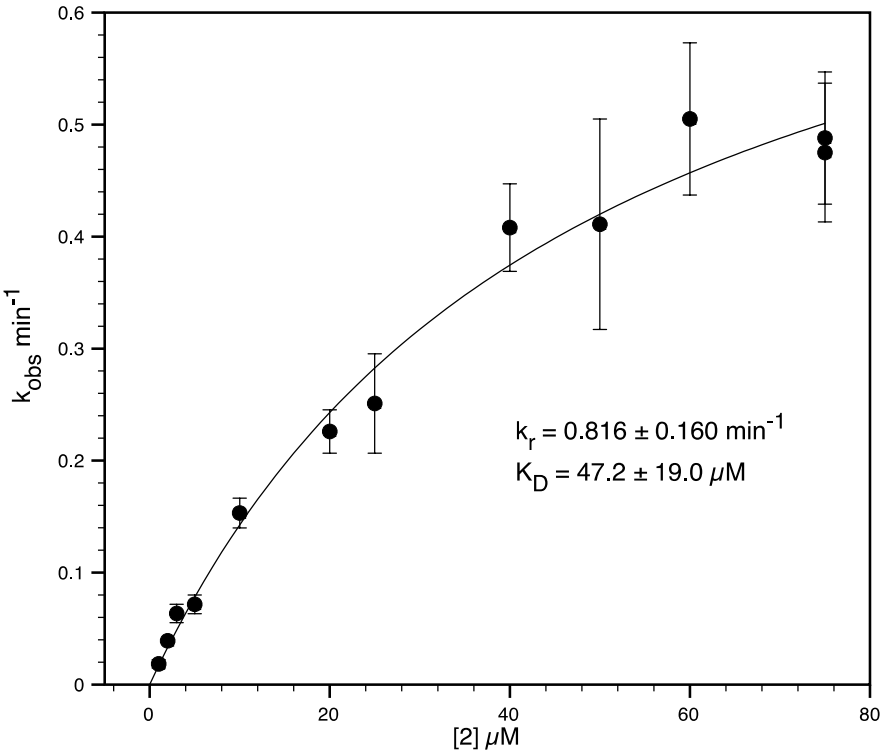
Plot of k_{obs} vs [Obidoxime]



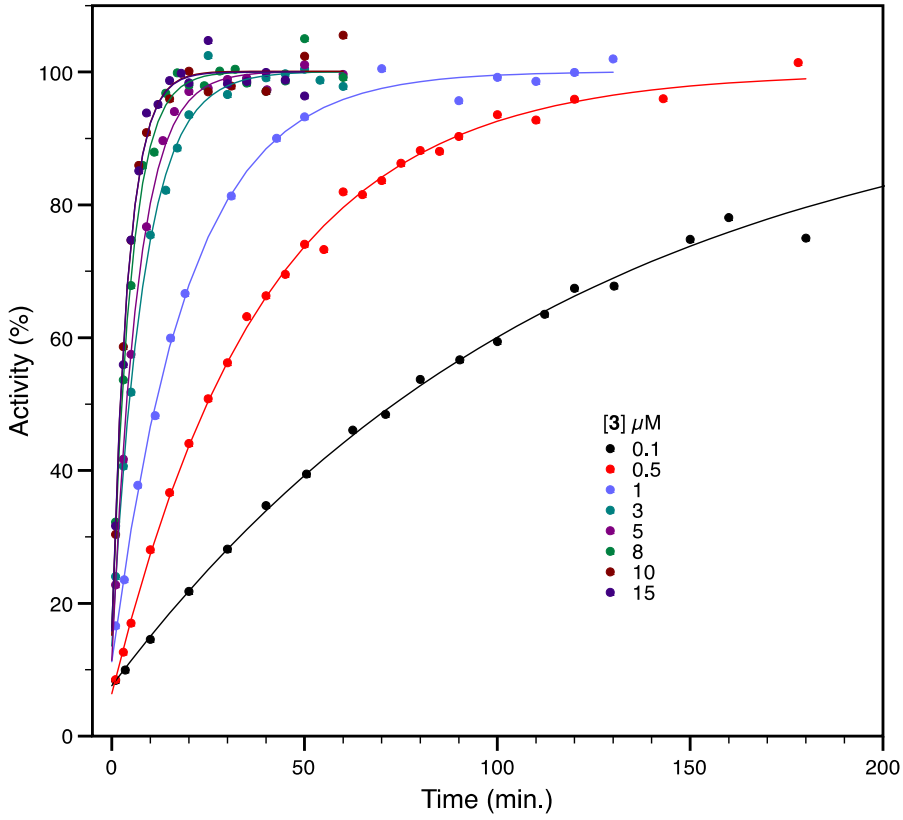
Reactivation of VX-inhibited hAChE by **2**



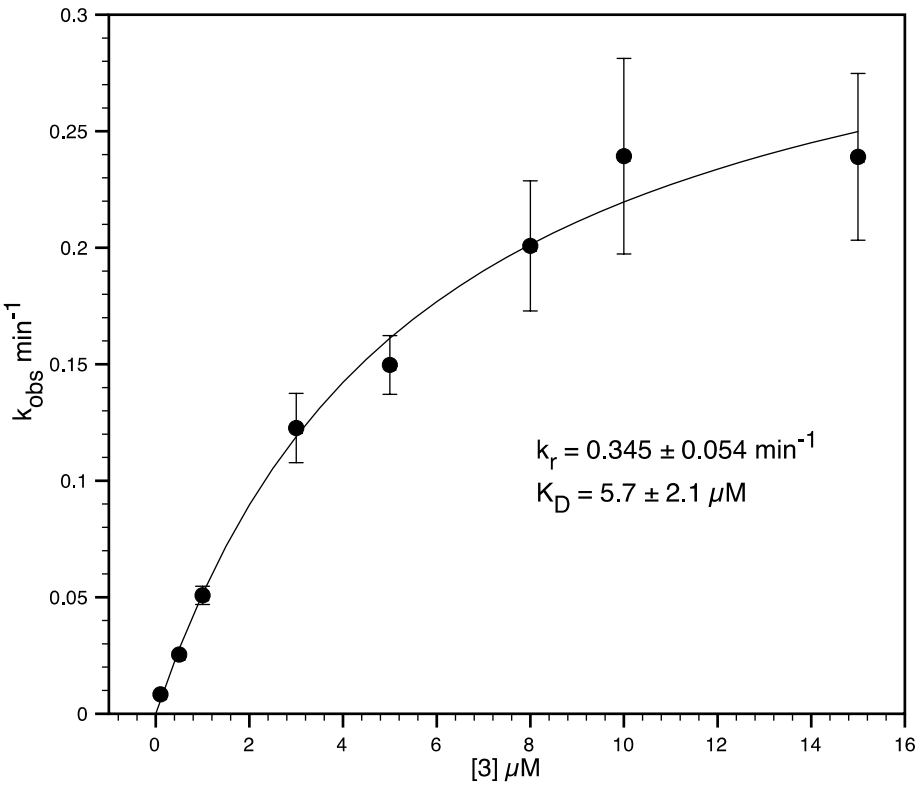
Plot of k_{obs} vs [**2**]



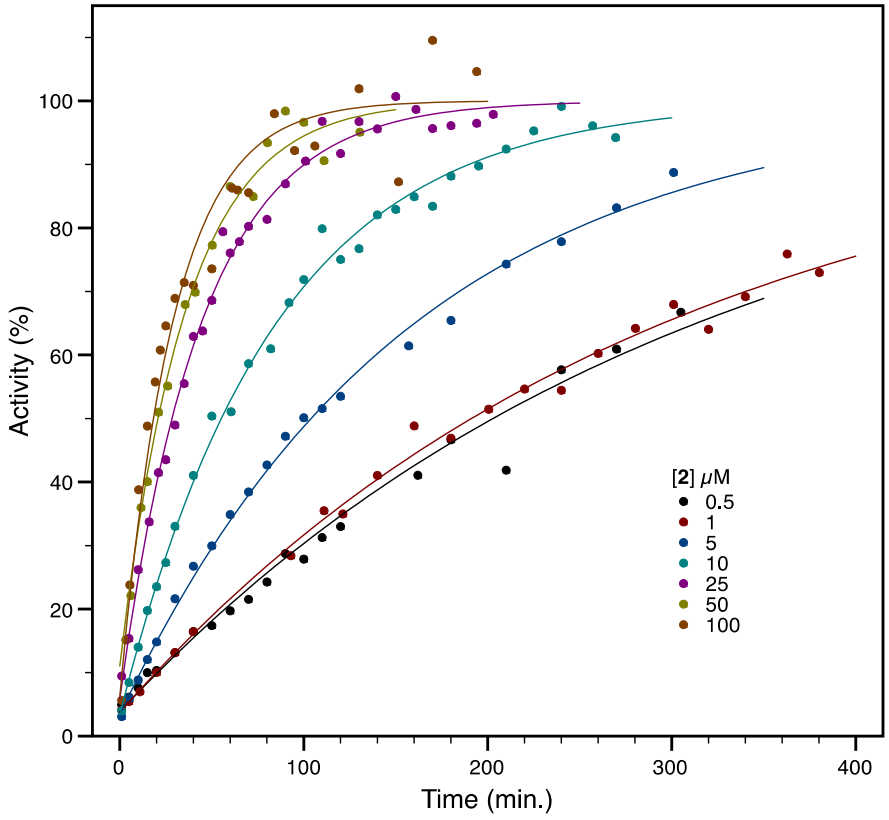
Reactivation of VX-inhibited hAChE by **3**



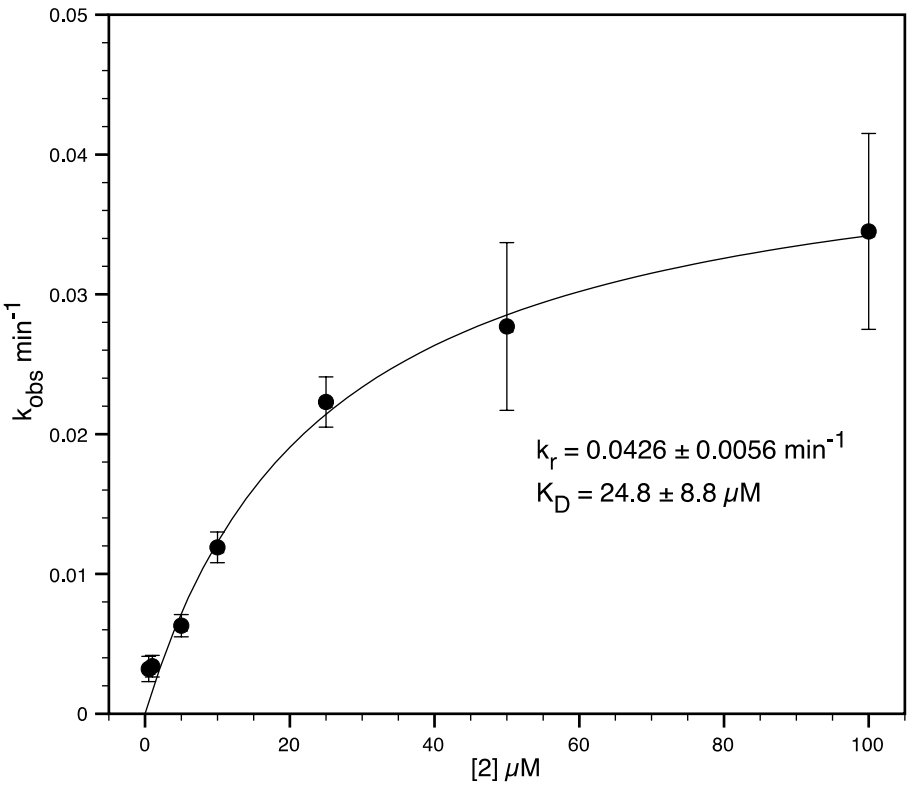
Plot of k_{obs} vs [**3**]



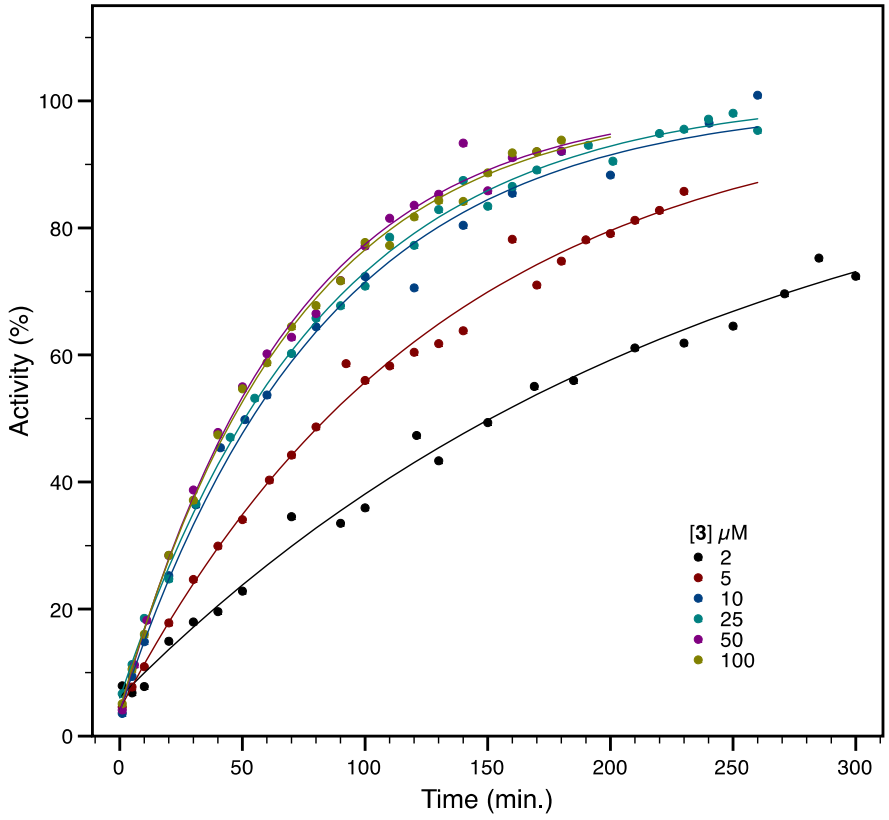
Reactivation of tabun-inhibited hAChE by **2**



Plot of k_{obs} vs [**2**]



Reactivation of tabun-inhibited hAChE by **3**



Plot of k_{obs} vs [**3**]