

Supporting information

Development and Evaluation of Two Potential 5-HT₇ Receptor PET Tracers: [¹⁸F]ENL09 and [¹⁸F]ENL10

Elina Tampio L'Estrade^{a,b,c}, Mengfei Xiong^{a,b}, Vladimir Shalgunov^b, Fraser G. Edgar^b, Balázs Volk^d, Simone L. Baerentzen^a, Mikael Palner^{a,e}, Maria Erlandsson^c, Tomas Ohlsson^c, Gitte M. Knudsen^{a,f} and Matthias M. Herth^{a,b,g,*}

a) Neurobiology Research Unit, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark

b) Department for Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

c) Radiation Physics, Nuclear Medicine Physics Unit, Skånes University Hospital, Barngatan 3, 222 42 Lund, Sweden

d) Directorate of Drug Substance Development, Egis Pharmaceuticals Plc., P.O. Box 100, H-1475 Budapest, Hungary

e) Center of Translational Neuroscience, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

f) Institute of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

g) Department of Clinical Physiology, Nuclear Medicine and PET, University Hospital Copenhagen, Rigshospitalet Blegdamsvej 9, 2100 Copenhagen, Denmark

Keywords: PET, 5-HT₇R, Fluorine-18, Fragment-based Dual-labelling, PDSP, Rat

*Corresponding Author: Tel.: +45 93565414; fax: +45 35 33 60 41; e-mail: matthias.herth@sund.ku.dk

Contents

Reference syntheses.....	2
<i>In vitro</i> affinity screening	7
Calculations of an theoretical binding ratio (tBR _{target/off-target})	7
<i>In vivo</i> evaluation in rat	9
References	11
Appendices	13

Reference syntheses

General information

The syntheses of previously presented compounds are described below. The molecular names were acquired from ChemDraw Professional

*4-(3-Hydroxyphenyl)-piperazine-1-Boc (ENL01)*¹

To a solution of *N*-(3-hydroxyphenyl) piperazine (5.00 g, 28.1 mmol) and NaHCO₃ (4.11 g, 49.3 mmol) in THF (70 mL), H₂O (70 mL) and Dioxane (70 mL); di-*tert*-butyl dicarbonate (7.32 g, 33.9 mmol) was added and stirred over night at room temperature. The solution was diluted with water and extracted using DCM, and thereafter dried with Na₂SO₄ and evaporated *in vacuo*; ENL01 was afforded as an orange/white solid (639.5 mg, 91.9%). ¹H NMR (400 MHz, chloroform-*d*) δ 7.11 (t, *J* = 8.1 Hz, 1H), 6.49 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.40 (t, *J* = 2.4 Hz, 1H), 6.34 (dd, *J* = 8.0, 2.3 Hz, 1H), 3.56 (t, *J* = 5.2 Hz, 4H), 3.16–3.08 (m, 4H), 1.48 (s, 9H). ¹³C NMR (151 MHz, chloroform-*d*) δ 156.82, 154.94, 152.88, 130.23, 109.10, 107.22, 103.62, 80.22, 49.27, 28.59. UPLC-MS (ESI): RT: 1.46 min, *m/z*: 279.1 [M⁺H]⁺ at 215-254 nm. R_f: 0.23 (Hep:EtOAc 7:3). **Appendices 1-3**

*4-(3-(2-Fluoro-ethoxy)-phenyl)-piperazine-1-Boc (ENL02)*¹

To a solution of **ENL01** (0.842 g, 3.00 mmol) in dry DMF (21 mL), sodium hydride (60%) (126.4 mg, 3.00 mmol) was added portion wise, under nitrogen and on cooling (ice-bath). Thereafter it was left stirring for another 30 min. When at 0 °C 1,2-Bromofluoroethane (0.22 mL, 3 mmol) was slowly added. This mixture was then stirred for an additional 20 h at 60 °C and then evaporated *in vacuo*. The residue was dissolved in ethyl acetate (EtOAc) and washed with water and brine and it was extracted using EtOAc. The organic layers were dried over MgSO₄. Column chromatography (CC) (Hept/EtOAc 7:3) afforded ENL02 as a yellow oil (435.7 mg, 45%). ¹H NMR (400 MHz, chloroform-*d*) δ 7.18 (t, *J* = 8.2 Hz, 1H), 6.61–6.55 (m, 1H), 6.52 (s, 1H), 6.48–6.42 (m, 1H), 4.87–4.59 (m, 2H), 4.20 (ddd, *J* = 27.8, 5.1, 3.2 Hz, 2H), 3.57 (t, *J* = 5.1 Hz, 4H), 3.14 (t, *J* = 5.2 Hz, 4H), 1.48 (s, 9H). ¹³C NMR (101 MHz, chloroform-*d*) δ 159.61, 154.86, 130.09, 110.06, 104.05, 100.13, 82.95, 81.25, 80.09, 67.25, 28.58. UPLC-MS (ESI): RT: 4.33 min, *m/z*: 325.1 [M⁺H]⁺ at 215-254 nm. R_f: 0.21 (Hept/EtOAc 7:3).

Appendices 4-6.

*1-[3-(2-Fluoro-ethoxy)-phenyl]-piperazine (ENL03)*¹

ENL02 was dissolved in 4M HCl in dioxane (20 mL) and left stirring for 90 min. It was then concentrated by rotary evaporation to a yellow solid (737.5 mg, >99%). ¹H NMR (400 MHz, chloroform-*d*) δ 7.18 (t, *J* = 8.2 Hz, 1H), 6.63–6.40 (m, 3H), 4.83–4.64 (m, 2H), 4.20 (ddd, *J* = 27.8, 5.1, 3.2 Hz, 2H), 3.57 (t, *J* = 5.1 Hz, 4H), 3.14 (t, *J* = 5.2 Hz, 4H), 1.48 (s, 9H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 159.13, 151.34, 129.91, 108.76, 105.65, 102.76, 82.17, 66.90, 45.38, 42.51. UPLC-MS (ESI): RT: 2.81 min, *m/z*: 225.2 [M⁺H]⁺ at 215-254 nm. R_f: 0.29 (CHCl₃:MeOH:Et₃N 90:9:1).

Appendices 7-9.

tert-Butyl 4-(3-(2-hydroxyethoxy)phenyl)piperazine-1-carboxylate (**ENL11**)²

A mixture of **ENL01** (400 mg, 1.44 mmol), 2-bromoethanol (0.15 mL, 2.16 mmol) and sodium carbonate (362.9 mg, 4.32 mmol) in DMF (15 mL) was heated for six hours at 90 °C. The mixture was then cooled to room temperature, followed by a dilution with water. The mixture was then further diluted with diethyl ether, and the organic layer was separated. The combined organic layers were washed with brine, dried over magnesium sulfate and concentrated *in vacuo*. The residue was purified using flash chromatography (EtOAc:Hept 1:1) to yield pure **ENL11** as a solid yellow oil (275 mg, 59%). ¹H NMR (400 MHz, chloroform-*d*) δ 7.17 (t, *J* = 8.2 Hz, 1H), 6.55 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.48 (d, *J* = 2.4 Hz, 1H), 6.44 (dd, *J* = 8.2, 2.3 Hz, 1H), 4.11–4.02 (m, 2H), 3.94 (t, *J* = 4.5 Hz, 2H), 3.56 (t, *J* = 5.2 Hz, 4H), 3.12 (t, *J* = 5.1 Hz, 4H), 1.48 (s, 9H). UPLC-MS (ESI): RT: 4.23 min, *m/z*: 323.2 [M⁺H]⁺ at 215-254 nm. R_f: 0.3 (Hep:EtOAc 1:1). **Appendices 10-11.**

tert-Butyl 4-(3-(2-(tosyloxy)ethoxy)phenyl)piperazine-1-carboxylate (**ENL04**).³

ENL11 (120.5 mg, 0.37 mmol) was dissolved in DCM (10 mL) in a round-bottom flask, to which *p*-TsCl (70.5 mg, 0.37 mmol) and DMAP (1 mg, 8.2 μmol) were added. A reflux condenser was attached and the resultant solution stirred for 10 minutes. Triethylamine (58.5 μL, 0.42 mmol) was then added dropwise to the reaction over 15 minutes, as a result the trimethylammonium salt precipitated, with the release of heat (visible condensation on the condenser). After 1 hour, the reflux condenser was removed and replaced by a stopper. The reactions progress was monitored by TLC to observe the consumption of starting materials. Following reaction completion, 1M HCl was added. The organic layer was separated, washed, dried and concentrated. The product **ENL04** was yielded as a light yellow oil and did not require further purification (134.7 mg, 76.4%). ¹H NMR (600 MHz, chloroform-*d*) δ 7.85–7.80 (m, 2H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.13 (t, *J* = 8.2 Hz, 1H), 6.54 (dd, *J* = 8.3, 2.3 Hz, 1H),

6.36 (t, $J = 2.4$ Hz, 1H), 6.31 (dd, $J = 8.2, 2.3$ Hz, 1H), 4.38–4.33 (m, 2H), 4.16–4.12 (m, 2H), 3.56 (t, $J = 5.1$ Hz, 4H), 3.11 (t, $J = 5.2$ Hz, 4H), 2.45 (s, 3H), 1.48 (s, 9H). ^{13}C NMR (151 MHz, chloroform- d) δ 159.01, 154.65, 152.57, 144.82, 132.92, 129.82, 127.97, 109.84, 105.36, 103.65, 79.88, 68.06, 65.39, 49.13, 46.19, 28.38, 21.61. UPLC-MS (ESI): RT: 3.06 min, m/z : 477.6 $[\text{M}^+\text{H}]^+$ at 215 - 254 nm. R_f : 0.64 (90:9:1 CHCl_3 :MeOH:Et $_3$ N). **Appendices 12-14**

*3-(4-Hydroxybutyl)indolin-2-one (ENL12):*⁴

A mixture of 2-oxindole (1.99 g, 15 mmol), butan-1,4-diol (30 mL, 340 mmol) and Raney® nickel (1.62 g) was heated to 200 °C in acetone in an autoclave and stirred overnight. Following cooling the reaction, the mixture was diluted with acetone, filtered over celite and the filtrate concentrated. The excess alcohol was then removed using Kugelrohr distillation. The product was purified using column chromatography (EtOAc; 846.1 mg, 27.4%). ^1H NMR (600 MHz, DMSO- d_6) δ 10.31 (s, 1H), 7.23 (d, $J = 7.3$ Hz, 1H), 7.16 (tt, $J = 7.7, 1.0$ Hz, 1H), 6.94 (td, $J = 7.5, 1.0$ Hz, 1H), 6.81 (d, $J = 7.7$ Hz, 1H), 4.32 (t, $J = 5.1$ Hz, 1H), 3.41 (t, $J = 5.9$ Hz, 1H), 3.36–3.32 (m, 2H), 1.87 (ddt, $J = 13.4, 11.0, 5.5$ Hz, 1H), 1.76 (tdd, $J = 11.3, 8.5, 5.5$ Hz, 1H), 1.42–1.33 (m, 2H), 1.24 (m, $J = 21.2, 13.2, 11.3, 5.3$ Hz, 1H). ^{13}C NMR (151 MHz, DMSO- d_6) δ 178.86, 142.74, 129.73, 127.48, 123.92, 121.17, 109.06, 60.50, 45.13, 32.53, 29.78, 21.83. UPLC-MS (ESI): RT: 1.54 min, m/z : 206.1 $[\text{M}^+\text{H}]^+$ at 215-254 nm. R_f : 0.44 (EtOAc). **Appendices 15-17.**

*4-(2-Oxindolin-3-yl)butyl methanesulfonate (ENL14):*⁴

ENL12 (358.5 mg, 1.70 mmol) was dissolved in THF (2.5 mL). Triethyl amine (0.47 mL, 3.4 mmol) was then added, and the solution cooled to -78 °C, thereafter MsCl (0.26 mL, 3.4 mmol) was added dropwise. After addition, the mixture was warmed to room temperature and stirred for a further hour. The white precipitate was filtered off, and the filtrate concentrated. The residual oil was dissolved in ethyl acetate, and extracted with 2M HCl until pH 5. The organic layer was dried over sodium sulfate, and evaporated. The product should be pure enough for direct use, alternatively product can be purified by flash chromatography (242.4 mg, 50%). ^1H NMR (600 MHz, chloroform- d) δ 7.50 (s, 1H), 7.17–7.13 (m, 2H), 6.97 (td, $J = 7.5, 1.0$ Hz, 1H), 6.81–6.78 (m, 1H), 4.15–4.11 (m, 2H), 3.42 (t, $J = 6.0$ Hz, 1H), 2.91 (s, 3H), 1.99–1.92 (m, 2H), 1.71 (dddd, $J = 12.9, 9.0, 6.5, 2.8$ Hz, 2H), 1.47–1.34 (m, 2H). ^{13}C NMR (151 MHz, chloroform- d) δ 179.10, 141.14, 129.14, 127.99, 124.13, 122.43, 109.44, 77.16,

76.95, 76.74, 69.40, 45.47, 37.34, 29.72, 28.98, 21.66. UPLC-MS (ESI): RT: 3.16 min, m/z: 382.2 [M⁺H]⁺ at 215-254 nm. R_f: 0.78 (EtOAc). **Appendices 18-20.**

General synthesis of reference compounds⁴⁻⁶

The melt of the secondary amine (0.5 mmol) and sodium carbonate (0.5 mmol) was heated to 120 °C under slow stirring. Thereafter the oxindole (0.5 mmol) was added and after 1 h reaction time, the crude mixture was cooled to ambient temperature. Ethyl acetate and water were added to the residue and the layers were separated. The organic layer was dried over MgSO₄ and evaporated *in vacuo*. CC (EtOAc) afforded the isolated product.

3-(4-(4-(3-(2-fluoroethoxy)phenyl)piperazin-1-yl)butyl)indolin-2-one (ENL20):⁴

This reference was received from Matthias Herth.

¹H NMR (600 MHz, DMSO-*d*₆) δ 10.33 (s, 1H), 7.26 (d, *J* = 7.4 Hz, 1H), 7.18 (tt, *J* = 7.7, 1.1 Hz, 1H), 7.12 (t, *J* = 8.2 Hz, 1H), 6.96 (td, *J* = 7.5, 1.0 Hz, 1H), 6.83 (d, *J* = 7.7 Hz, 1H), 6.54 (dd, *J* = 8.1, 2.3 Hz, 1H), 6.47 (t, *J* = 2.4 Hz, 1H), 6.39 (dd, *J* = 8.0, 2.3 Hz, 1H), 4.79–4.75 (m, 1H), 4.72–4.65 (m, 1H), 4.26–4.20 (m, 1H), 4.19–4.15 (m, 1H), 3.45 (t, *J* = 5.9 Hz, 1H), 3.10 (t, *J* = 5.0 Hz, 4H), 2.44 (t, *J* = 5.0 Hz, 4H), 2.27 (t, *J* = 7.1 Hz, 2H), 1.96–1.76 (m, 2H), 1.45 (h, *J* = 7.1, 6.5 Hz, 2H), 1.35–1.25 (m, 2H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 178.72, 159.07, 152.88, 142.81, 129.72, 129.62, 127.49, 123.93, 121.16, 109.07, 104.48, 101.89, 99.49, 82.20, 66.86, 57.57, 52.65, 48.06, 45.04, 29.65, 26.19, 23.12. UPLC-MS (ESI): RT: 2.93 min, m/z: 412.3 [M⁺H]⁺ at 215-254 nm. **Appendices 21-23.**

3-Ethyl-3-(4-(4-(3-(2-fluoroethoxy)phenyl)piperazin-1-yl)butyl)-5-methoxyindolin-2-one (ENL61)²:

64.4 mg, 27.4%. ¹H NMR (600 MHz, chloroform-*d*) δ 8.50 (s, 1H), 7.14 (t, *J* = 8.2 Hz, 1H), 6.81–6.78 (m, 1H), 6.74–6.70 (m, 2H), 6.53 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.47 (t, *J* = 2.4 Hz, 1H), 6.39 (dd, *J* = 8.1, 2.3 Hz, 1H), 4.78–4.74 (m, 1H), 4.70–4.67 (m, 1H), 4.22–4.19 (m, 1H), 4.17–4.14 (m, 1H), 3.80–3.77 (m, 27H), 3.14 (t, *J* = 5.0 Hz, 4H), 2.49 (t, *J* = 5.0 Hz, 4H), 2.27–2.21 (m, 2H), 1.91 (ddd, *J* = 13.5, 8.3, 6.0 Hz, 2H), 1.81–1.69 (m, 2H), 1.49–1.35 (m, 1H), 1.11 (tdt, *J* = 13.4, 10.1, 5.3 Hz, 1H), 0.95–0.86 (m, 1H), 0.63 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (151 MHz, chloroform-*d*) δ 182.46, 159.49, 155.96, 152.85, 134.91, 134.28, 129.88, 111.74, 110.65, 109.44, 104.83, 103.30, 82.67, 81.55, 67.21, 58.33, 55.85, 54.84, 53.18, 48.95, 37.77, 31.27, 27.01, 22.40, 8.70. UPLC-MS (ESI): RT: 3.22 min, m/z: 470.4 [M⁺H]⁺ at 215 - 254 nm. R_f: 0.18 (EtOAc). **Appendices 30-32.**

*3-(4-(4-(4-fluorophenyl)piperazin-1-yl)butyl)indolin-2-one (ENL21):*⁴

This reference compound was received from Balázs Volk.

¹H NMR (600 MHz, CD₃OD) δ 7.30 (dt, J = 7.4, 0.8 Hz, 1H), 7.23 (td, J = 7.7, 1.2 Hz, 1H), 7.05 (td, J = 7.5, 1.0 Hz, 1H), 7.01 – 6.96 (m, 4H), 6.92 (d, J = 7.8 Hz, 1H), 3.15 – 3.10 (m, 4H), 2.65–2.60 (m, 4H), 2.40 (td, J = 7.1, 1.5 Hz, 2H), 2.03 (m, J = 36.1, 13.7, 10.7, 5.4 Hz, 2H), 1.58 (dtd, J = 14.8, 6.8, 3.2 Hz, 2H), 1.44–1.24 (m, 3H). ¹³C NMR (151 MHz, CD₃OD) δ 182.44, 158.72 (d, J = 232.7 Hz), 149.41, 143.79, 131.12, 128.94, 125.16, 123.30, 119.27, 116.36, 116.21, 110.76, 59.36, 54.21, 50.96, 49.85, 31.01, 27.42, 24.48. UPLC-MS (ESI): RT: 2.89 min, m/z : 368.2 [M^+H]⁺ at 215-254 nm.

Appendices 33-35.

*3-(4-(4-(4-fluorophenyl)piperazin-1-yl)butyl)-3-methylindolin-2-one (ENL06):*²

63.2 mg, 33%. ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.30 (s, 1H), 7.22 (dd, J = 7.1, 1.2 Hz, 1H), 7.15 (td, J = 7.6, 1.3 Hz, 1H), 7.09–6.99 (m, 2H), 6.96 (td, J = 7.5, 1.1 Hz, 1H), 6.92–6.88 (m, 2H), 6.83 (d, J = 7.7 Hz, 1H), 2.99 (t, J = 4.9 Hz, 4H), 2.61 (p, J = 1.9 Hz, 2H), 2.40–2.36 (m, 6H), 2.19–2.12 (m, 2H), 1.73 (ddd, J = 9.6, 5.7, 3.1 Hz, 2H), 1.22 (s, 3H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 181.52, 159.84, 147.94, 141.58, 134.29, 127.43, 122.83, 121.40, 116.94, 115.17, 109.17, 57.43, 52.59, 48.90, 47.93, 37.53, 26.30, 23.77, 22.08. UPLC-MS (ESI): RT: 3.16 min, m/z : 382.2 [M^+H]⁺ at 215-254 nm. R_f : 0.26 (EtOAc). **Appendices 36-38.**

*3-ethyl-3-(4-(4-(4-fluorophenyl)piperazin-1-yl)butyl)indolin-2-one (ENL05):*⁶

109.9 mg, 55%. ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.33 (s, 1H), 7.29–7.08 (m, 2H), 7.06–6.96 (m, 3H), 6.95–6.89 (m, 2H), 6.84 (d, J = 7.7 Hz, 1H), 3.00 (t, J = 4.9 Hz, 4H), 2.55–2.53 (m, 1H), 2.40 (tdd, J = 6.5, 4.3, 2.1 Hz, 4H), 2.16 (ddd, J = 8.1, 6.5, 3.2 Hz, 2H), 1.80–1.68 (m, 4H), 1.37–1.23 (m, 2H), 1.03–0.93 (m, 1H), 0.51 (t, J = 7.4 Hz, 3H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 180.80, 155.10, 147.95, 142.47, 132.24, 127.44, 122.97, 121.39, 116.94, 115.17, 109.01, 57.44, 53.13, 52.58, 48.89, 36.88, 30.32, 26.36, 21.86, 8.39. UPLC-MS (ESI): RT: 3.13 min, m/z : 396.3 [M^+H]⁺ at 215-254 nm. R_f : 0.16 (EtOAc). **Appendices 39-41.**

*3-Ethyl-3-(4-(4-(4-fluorophenyl)piperazin-1-yl)butyl)-5-methoxyindolin-2-one (ENL60):*²

101.1 mg, 47%. ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.13 (s, 1H), 7.02 (t, J = 8.9 Hz, 2H), 6.92–6.88 (m, 2H), 6.84 (t, J = 1.5 Hz, 1H), 6.72 (d, J = 1.5 Hz, 2H), 3.69 (s, 3H), 2.99 (t, J = 5.0 Hz, 4H), 2.61 (p, J = 1.9 Hz, 1H), 2.40–2.36 (m, 4H), 2.19–2.13 (m, 2H), 1.76–1.65 (m, 4H), 1.29 (dtd, J = 34.9,

14.2, 13.3, 6.8 Hz, 2H), 0.94 (td, $J = 17.3, 16.6, 6.3$ Hz, 1H), 0.49 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (151 MHz, DMSO- d_6) δ 181.14, 155.35, 136.22, 134.20, 117.51, 115.60, 112.24, 110.75, 109.72, 55.83, 54.18, 52.99, 37.34, 30.83, 22.27, 8.91. UPLC-MS (ESI): RT: 3.21 min, m/z : 426.3 $[\text{M}^+\text{H}]^+$ at 215-254 nm. R_f : 0.12 (EtOAc). **Appendices 42-44.**

In vitro affinity screening

Supplementary Table 1: pharmacological selectivity profile of all synthesized reference compounds

ID	K _i (nM)										
	5-HT _{1A}	5-HT _{2A}	5-HT ₆	5-HT ₇	α _{1A}	α _{1B}	α _{1D}	H1	SERT	σ ₁	σ ₂
ENL20	145	162	2373	9.5	339	668	502	-	-	26	51
ENL10	72	53	422	5.6	748	825	1288	-	-	18	65
ENL09	107	160	1389	13	-	1413	-	-	2344	29	67
ENL61	389	103	-	38	878	448	1373	-	274	165	62
ENL21	288	28	-	1.5	130	228	162	-	305	8.1	2.1
ENL06	306	21	230	2.8	239	174	375	-	1712	11	3.5
ENL05	190	37	292	2.4	280	168	300	-	848	15	1.4
ENL60	267	29	389	5.6	132	208	135	-	148	39	2
ENL17	144	113	2219	8.3	40	346	33	-	1278	9.9	2.9
ENL08	38	121	685	5.2	41	263	66	-	3569	3.7	1.7
ENL07	49	100	1042	11	128	430	116	-	2477	6.8	2.5
ENL15	155	48	-	92	159	-	157	-	1280	38	1.7

Calculations of an theoretical binding ratio ($tBR_{\text{target/off-target}}$)

$$tBR_{\text{target/off-target}} = S \times D = \left(\frac{K_{D, \text{off-target}}}{K_{D, \text{target}}} \right) \times \left(\frac{B_{\text{avail, target}}}{B_{\text{avail, off-target}}} \right)$$

Example:

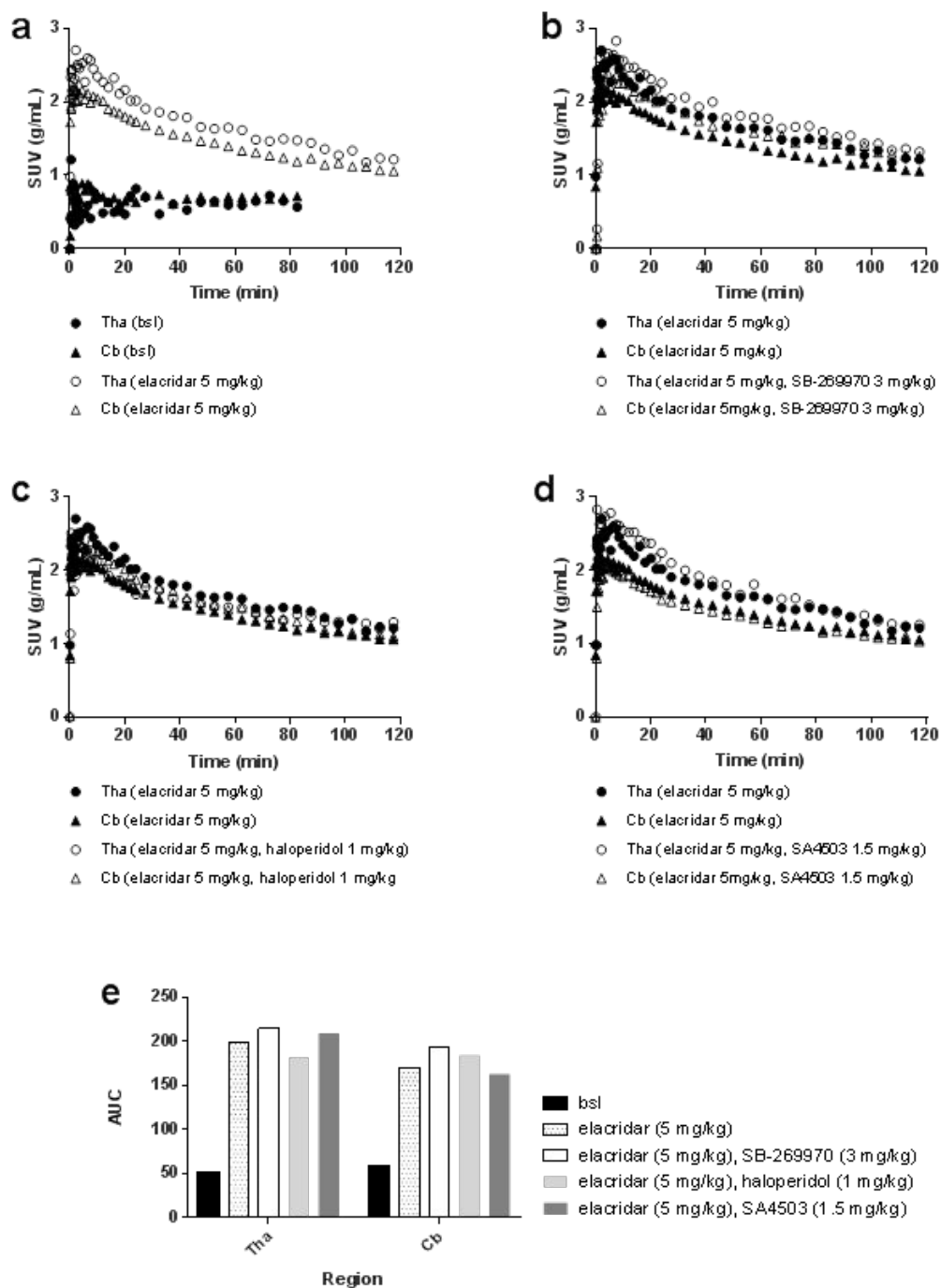
ENL10: $K_{i, 5\text{-HT}_7} = 5.6$ nM, $K_{i, \sigma_1} = 18$ nM (averaged σ -receptor affinity)

B_{\max} for the rat 5-HT₇R in thalamus: 47 fmol/mg tissue.⁷

B_{\max} for the rat σ -receptor in thalamus: 600 fmol/mg of σ -1 and 833 fmol/mg (according to the density in an adult rat, hence 3 times lower than the measured density in 1 day old rats) of σ -2 receptors gives a $(600+833)/2 \sim 700$ fmol/mg protein of the σ -receptors in rat thalamus.⁸ This can roughly be estimated to 70 fmol/mg tissue.⁹

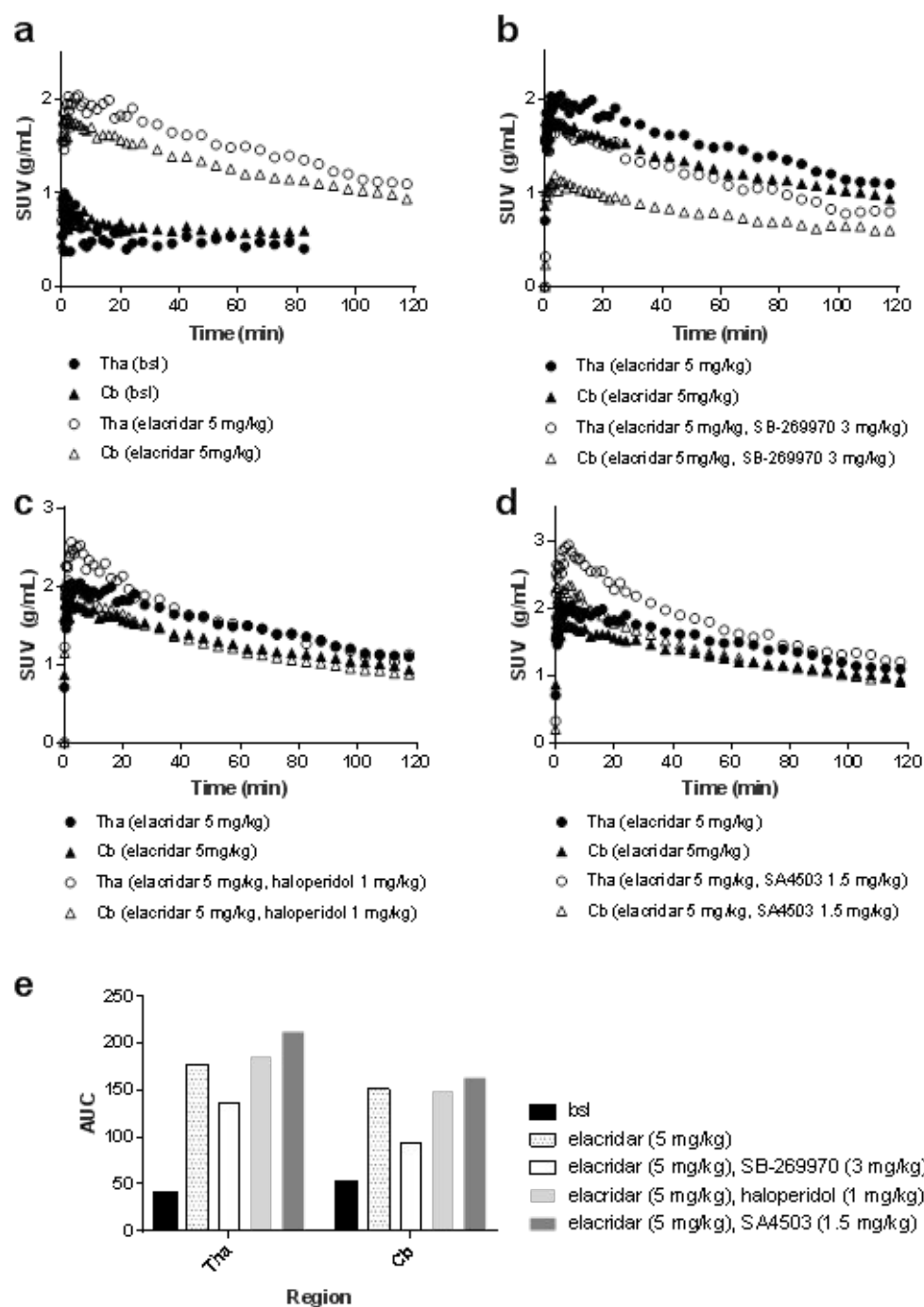
$$\mathbf{tBR}_{\text{target/off-target}} = (18 \text{ nM}/5.6 \text{ nM}) \times (47 \text{ pmol/g tissue} / 70 \text{ fmol/mg tissue}) = \underline{\underline{2.15}}$$

In vivo evaluation in rat



Supplementary Figure 1: Representative time-activity curves (TACs) of [^{18}F]ENL09 in the thalamus (circles, tha) and cerebellum (triangles, Cb) in the rat brain. a) TACs at baseline (bsl) and after pretreatment with elaclidar (5 mg/kg). b) Thalamus and cerebellum TACs after pretreatment with elaclidar and elaclidar + SB-269970 (3 mg/kg). c) Thalamus and cerebellum TACs after pretreatment with elaclidar and elaclidar + haloperidol (1 mg/kg). d) Thalamus and cerebellum TACs after pretreatment with elaclidar and elaclidar +

SA4503 (1.5 mg/kg). e) Calculated area-under-curve (AUC) for all TACs displayed as a grouped barplot. All TACs are normalized to injected radioactivity and animal weight resulting in the standard uptake value (SUV).



Supplementary Figure 2: Representative time-activity curves (TACs) of $[^{18}\text{F}]$ ENL10 in the thalamus (circles, tha) and cerebellum (triangles, Cb) in the rat brain. a) TACs at baseline (bsl) and after pretreatment with elacridar (5 mg/kg). b) Thalamus and cerebellum TACs after pretreatment with elacridar and elacridar + SB-269970 (3 mg/kg). c) Thalamus and cerebellum TACs after pretreatment with

elacridar and elacridar + haloperidol (1 mg/kg). d) Thalamus and cerebellum TACs after pretreatment with elacridar and elacridar + SA4503 (1.5 mg/kg). e) Calculated area-under-curve (AUC) for all TACs displayed as a grouped barplot. All TACs are normalized to injected radioactivity and animal weight resulting in the standard uptake value (SUV).

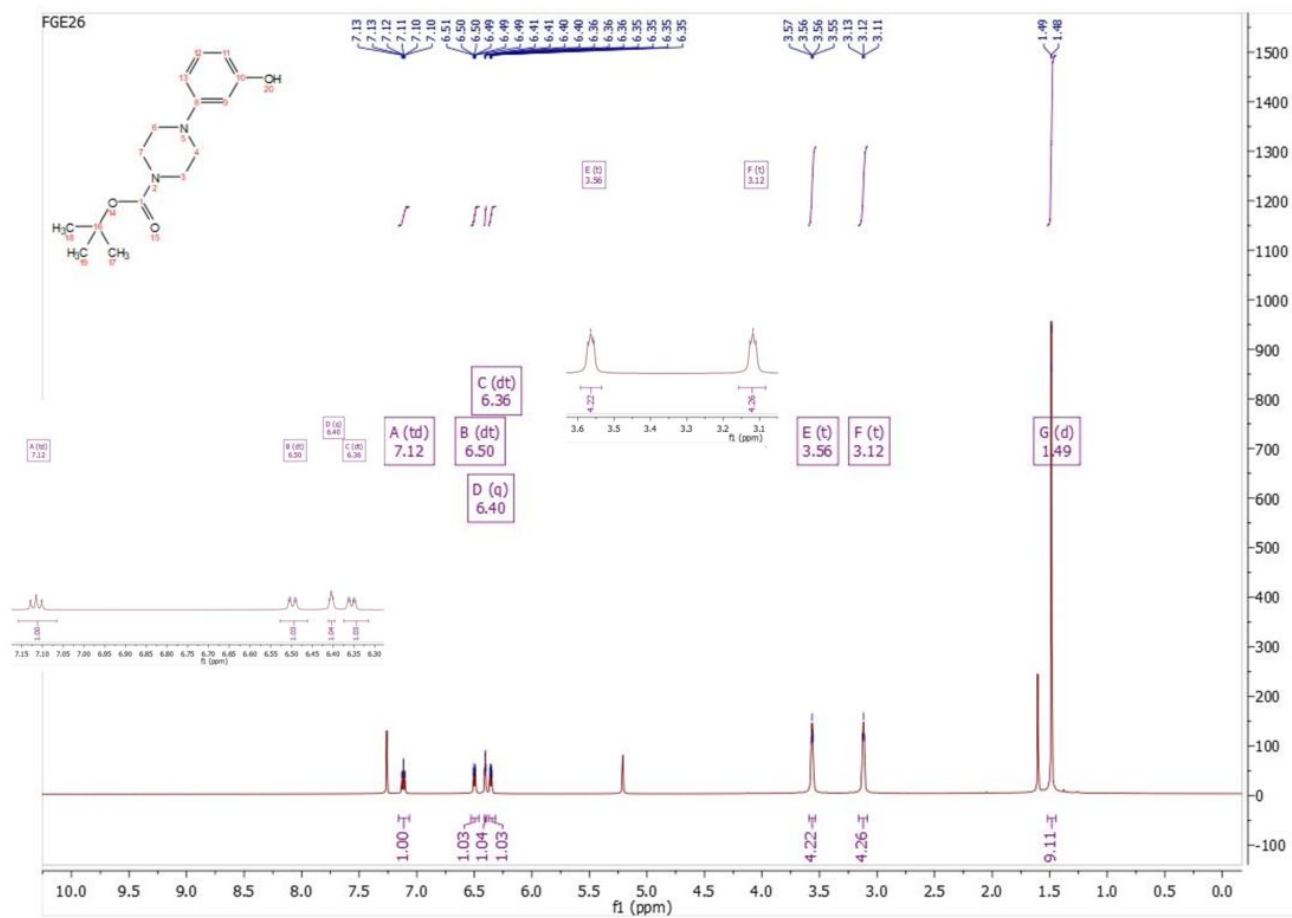
References

1. Herth, M. M.; Kramer, V.; Rösch, F., Synthesis of novel WAY 100635 derivatives containing a norbornene group and radiofluorination of [^{18}F]AH1. MZ as a serotonin 5-HT_{1A} receptor antagonist for molecular imaging. *Journal of Labelled Compounds and Radiopharmaceuticals: The Official Journal of the International Isotope Society* **2009**, 52 (6), 201-207.
2. Elina Tampio L'Estrade, S. L., Ida Nymann Petersen, Fraser G. Edgar, Balázs Volk, Maria Erlandsson, Tomas Ohlsson, Gitte M. Knudsen and Matthias M. Herth, Fragment-based Labelling to Increase Preclinical PET Throughput Screening Using Simple Condensation Reactions. *Submitted Manuscript* **2019**.
3. Prante, O.; Tietze, R.; Hocke, C.; Lober, S.; Hubner, H.; Kuwert, T.; Gmeiner, P., Synthesis, radiofluorination, and in vitro evaluation of pyrazolo[1,5-a]pyridine-based dopamine D₄ receptor ligands: discovery of an inverse agonist radioligand for PET. *J Med Chem* **2008**, 51 (6), 1800-10.
4. Herth, M. M.; Volk, B.; Pallagi, K.; Kofoed Bech, L.; Antoni, F. A.; Knudsen, G. M.; Kristensen, J. L., Synthesis and in vitro evaluation of oxindole derivatives as potential radioligands for 5-HT₇ receptor imaging with PET. *ACS Chem Neurosci* **2012**, 3 (12), 1002-7.
5. Volk, B.; Gacsályi, I.; Pallagi, K.; Poszavác, L.; Gyönös, I.; Szabó, É.; Bakó, T.; Spedding, M.; Simig, G.; Szénási, G., Optimization of (arylpiperazinylbutyl) oxindoles exhibiting selective 5-HT₇ receptor antagonist activity. *Journal of medicinal chemistry* **2011**, 54 (19), 6657-6669.
6. Volk, B.; Barkóczy, J.; Hegedus, E.; Udvari, S.; Gacsályi, I.; Mezei, T.; Pallagi, K.; Kompagne, H.; Lévy, G.; Egyed, A., (Phenylpiperazinyl-butyl) oxindoles as selective 5-HT₇ receptor antagonists. *Journal of medicinal chemistry* **2008**, 51 (8), 2522-2532.
7. Horisawa, T.; Ishiyama, T.; Ono, M.; Ishibashi, T.; Taiji, M., Binding of lurasidone, a novel antipsychotic, to rat 5-HT₇ receptor: analysis by [^3H]SB-269970 autoradiography. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **2013**, 40, 132-137.
8. McCann, D. J.; Weissman, A. D.; Su, T. P., Sigma-1 and Sigma-2 sites in rat brain: Comparison of regional, ontogenetic, and subcellular patterns. *Synapse* **1994**, 17 (3), 182-189.

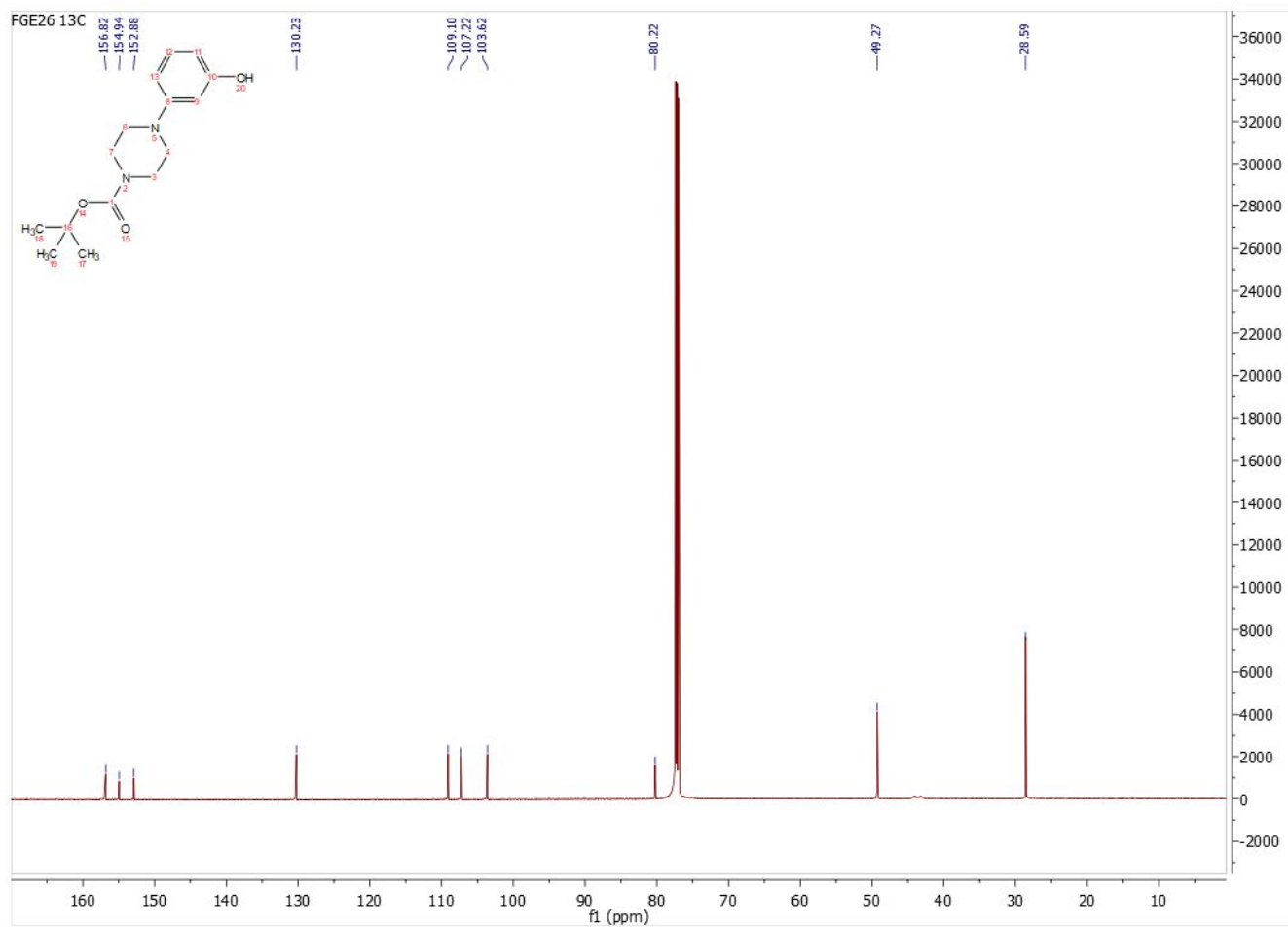
9. Cumming, P., Absolute abundances and affinity states of dopamine receptors in mammalian brain: a review. *Synapse* **2011**, 65 (9), 892-909.

Appendices

Appendix 1 – ^1H NMR of ENL01



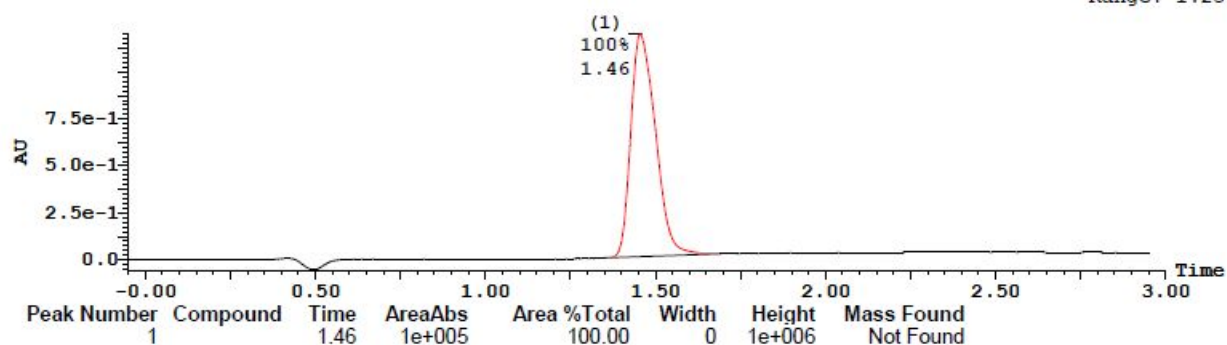
Appendix 2— ^{13}C NMR of ENL01



Appendix 3– UPLC-MS of ENL01

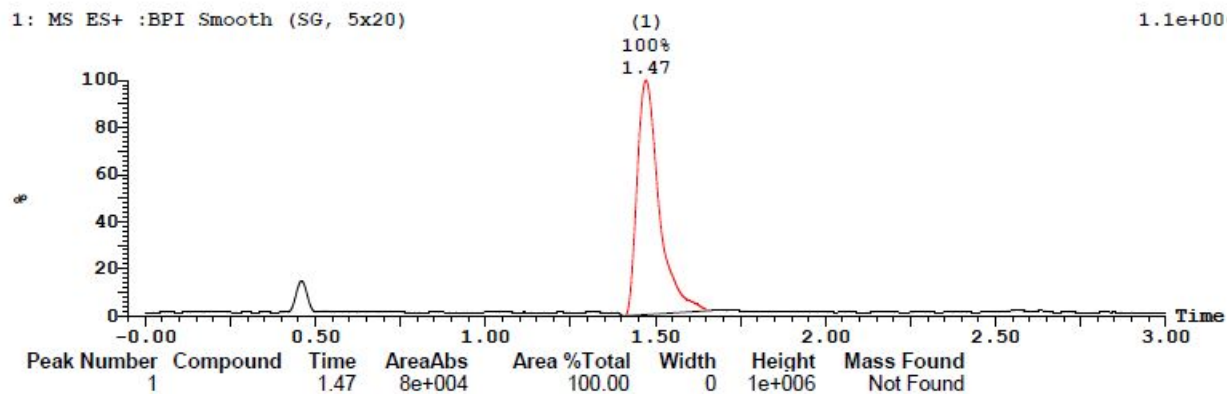
2: UV Detector: TAC :Wavelength Range: (215 - 254)

1.203
Range: 1.256



1: MS ES+ :BPI Smooth (SG, 5x20)

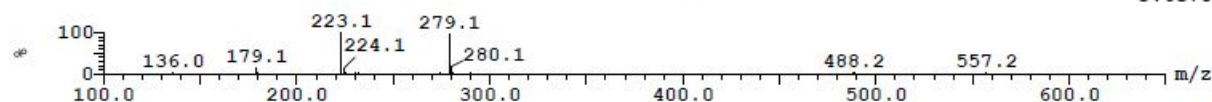
1.1e+006



Peak ID	Compound	Time	Mass Found
1		1.47	Not Found

1: (Time: 1.46) Combine (1216:1395-(1000:1089+1604:1694))

1:MS ES+
3.6e+005

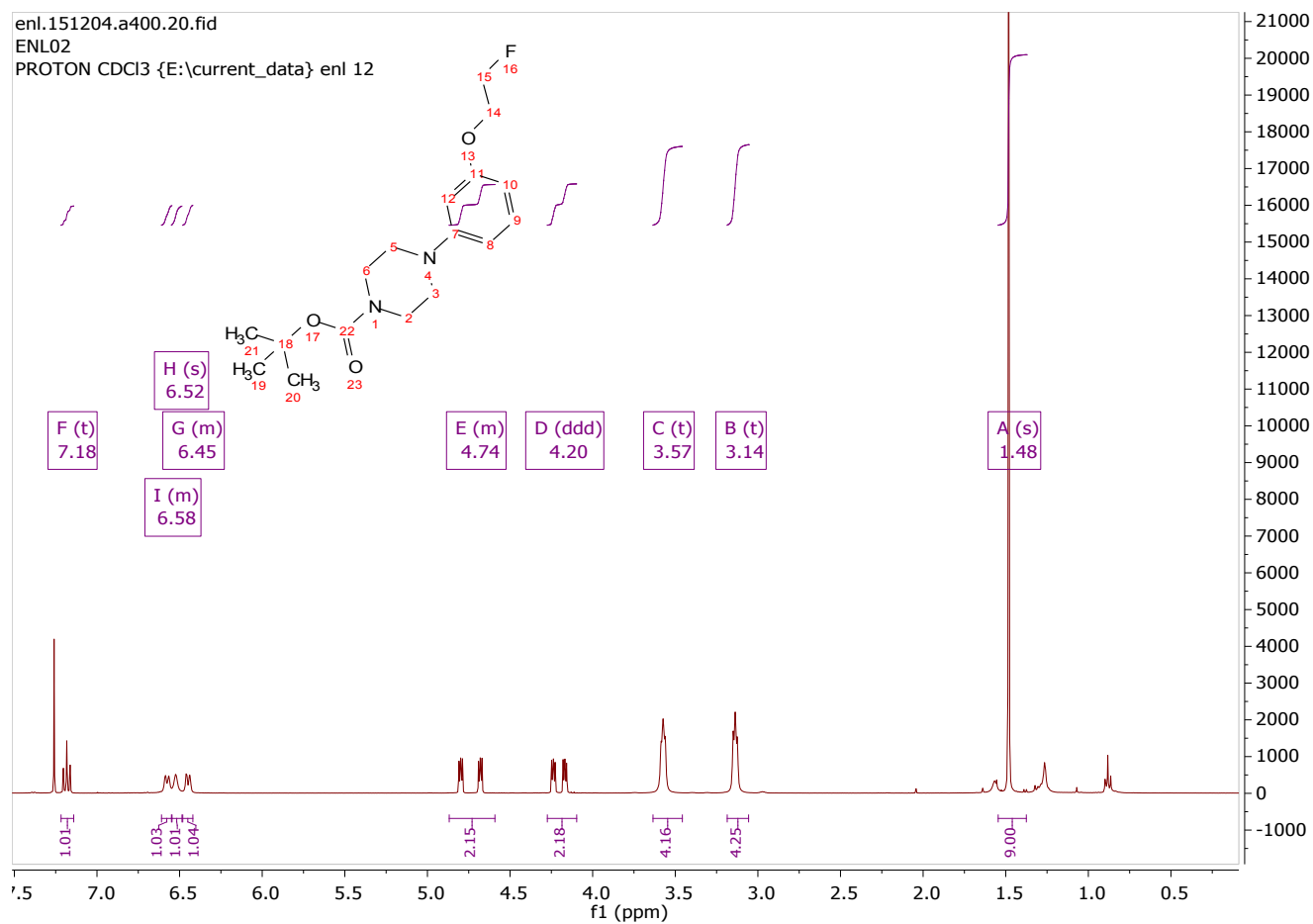


Appendix 4— ^1H NMR of ENL02

enl.151204.a400.20.fid

ENL02

PROTON CDCl₃ {E:\current_data} enl 12

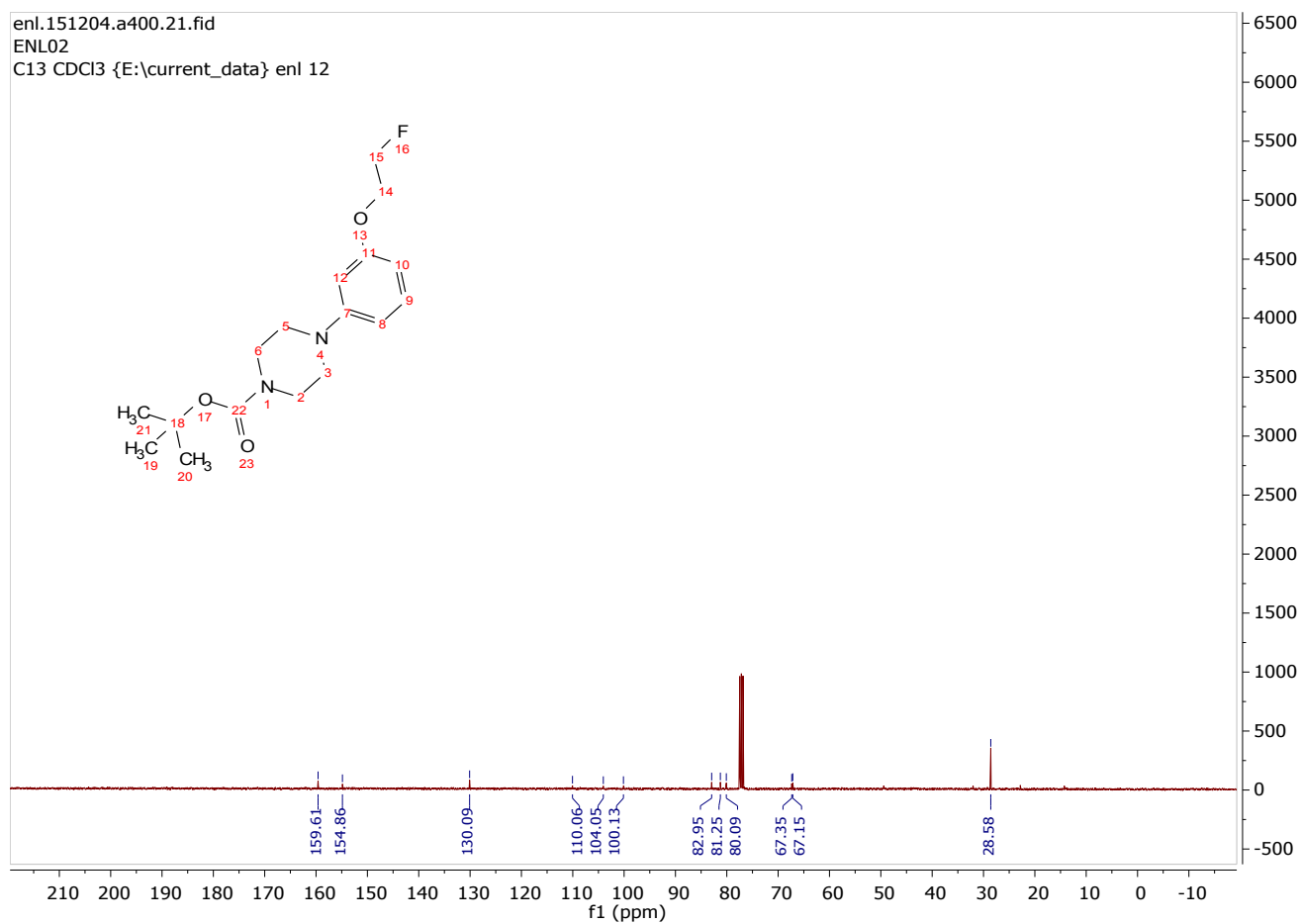


Appendix 5—¹³C NMR of ENL02

enl.151204.a400.21.fid

ENL02

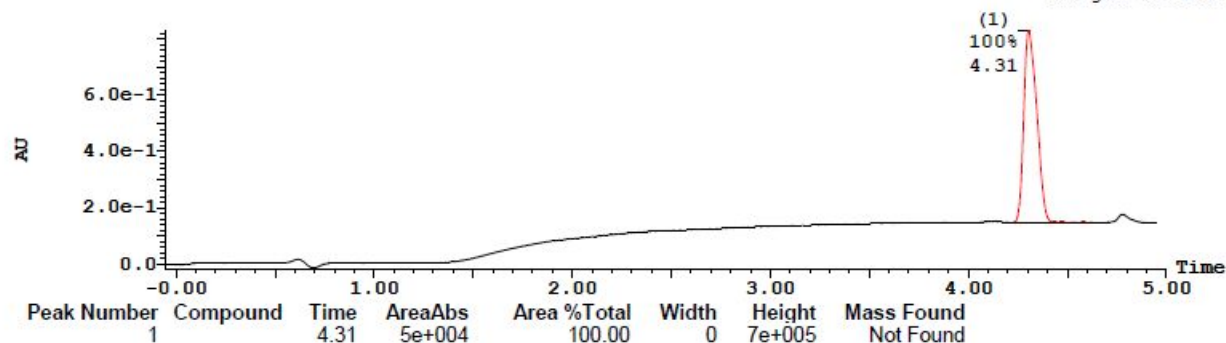
C13 CDCl3 {E:\current_data} enl 12



Appendix 6– UPLC-MS of ENL02

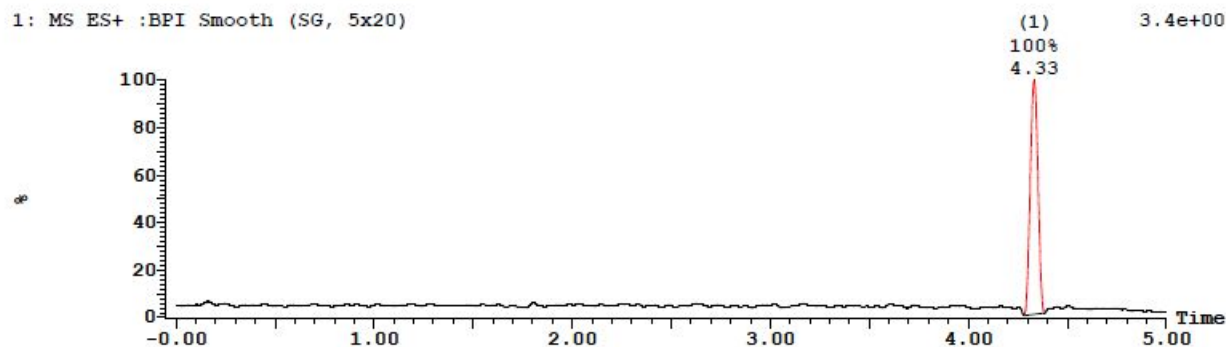
2: UV Detector: TAC :Wavelength Range: (215 - 254)

8.284e-1
Range: 8.419e-1



1: MS ES+ :BPI Smooth (SG, 5x20)

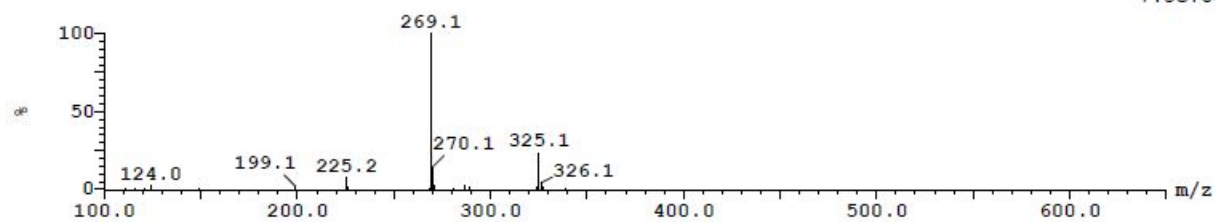
3.4e+005



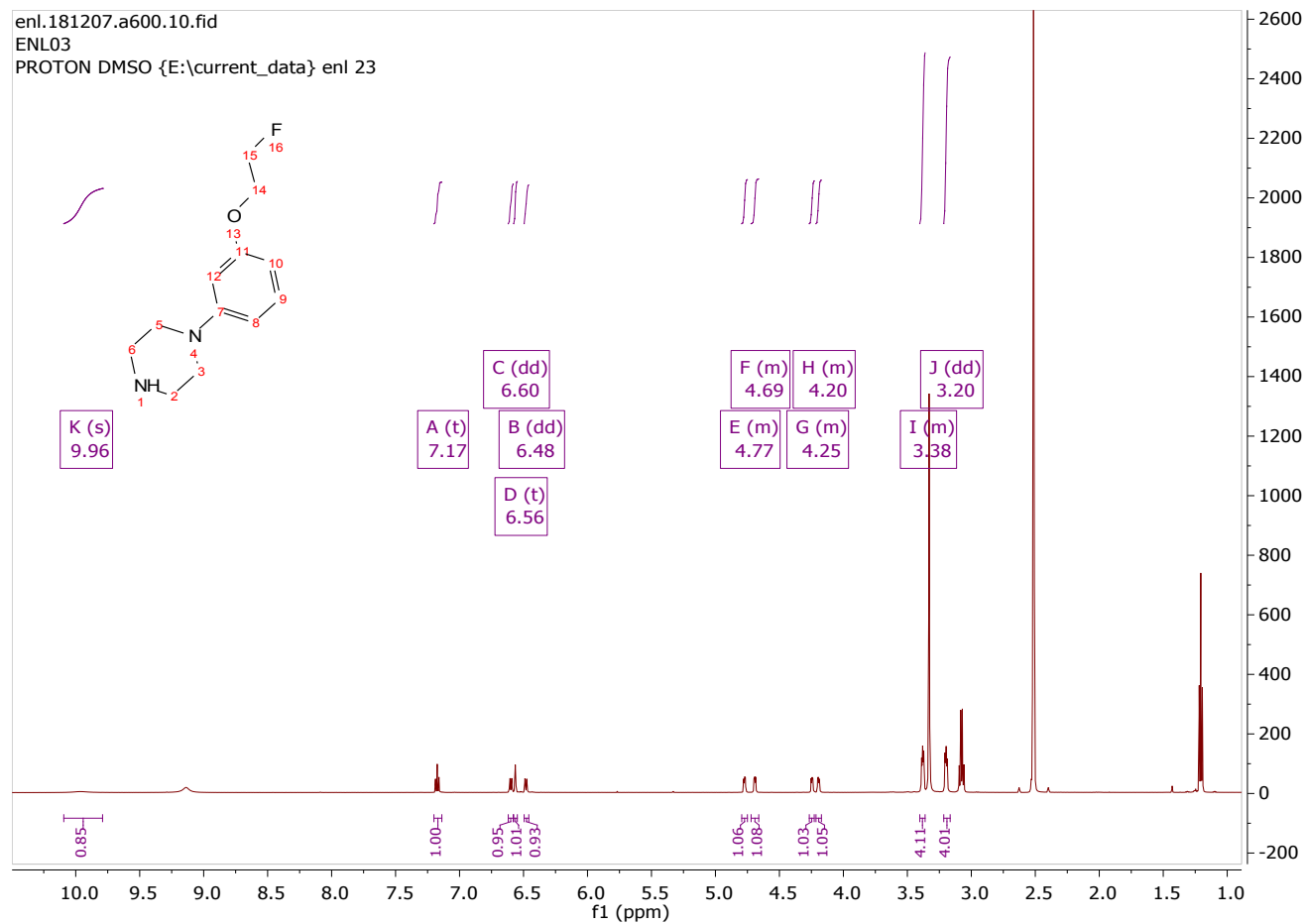
Peak ID	Compound	Time	Mass Found
1		4.33	Not Found

1: (Time: 4.31) Combine (3770:3950- (3606:3696+4271:4360))

1:MS ES+
7.5e+004



Appendix 7— ^1H NMR of ENL03

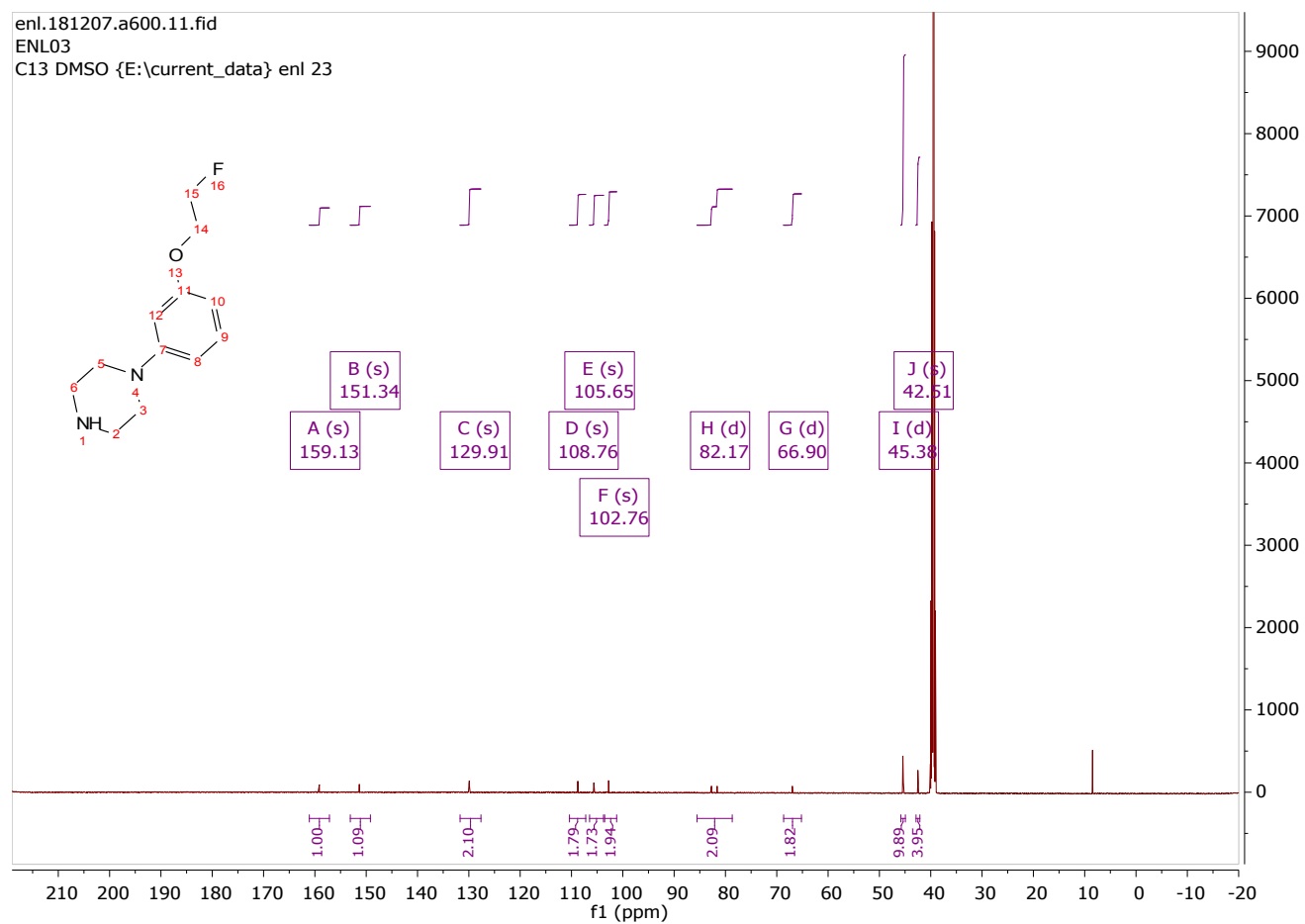


Appendix 8— ^{13}C NMR of ENL03

enl.181207.a600.11.fid

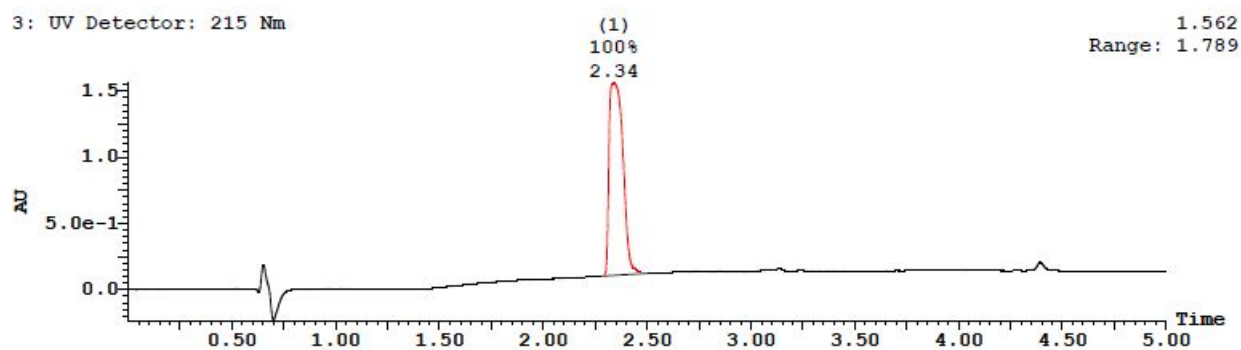
ENL03

C13 DMSO {E:\current_data\} enl 23

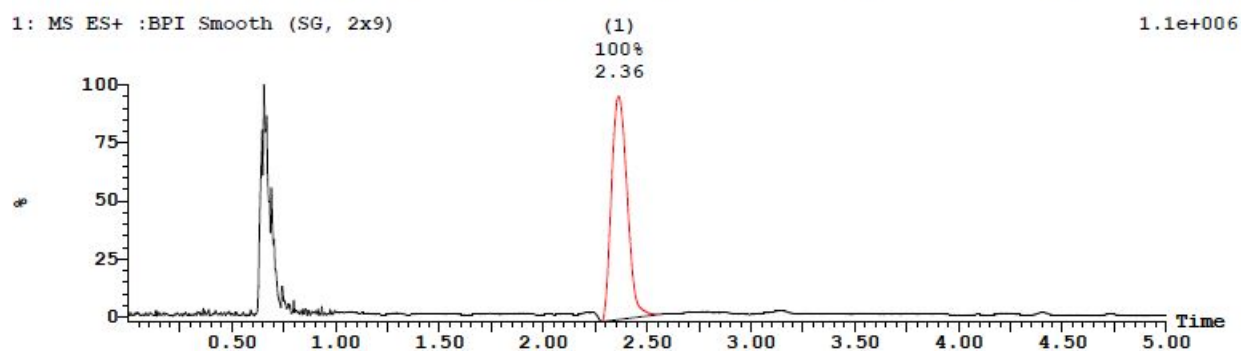


Appendix 9– UPLC-MS of ENL03

3: UV Detector: 215 Nm

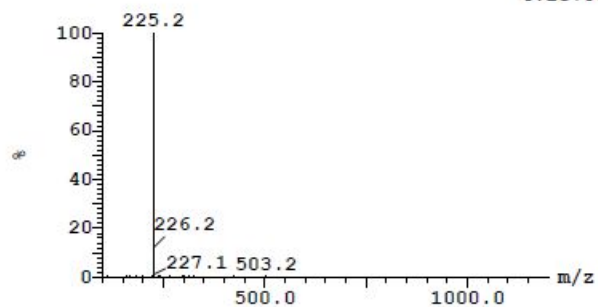


1: MS ES+ :BPI Smooth (SG, 2x9)

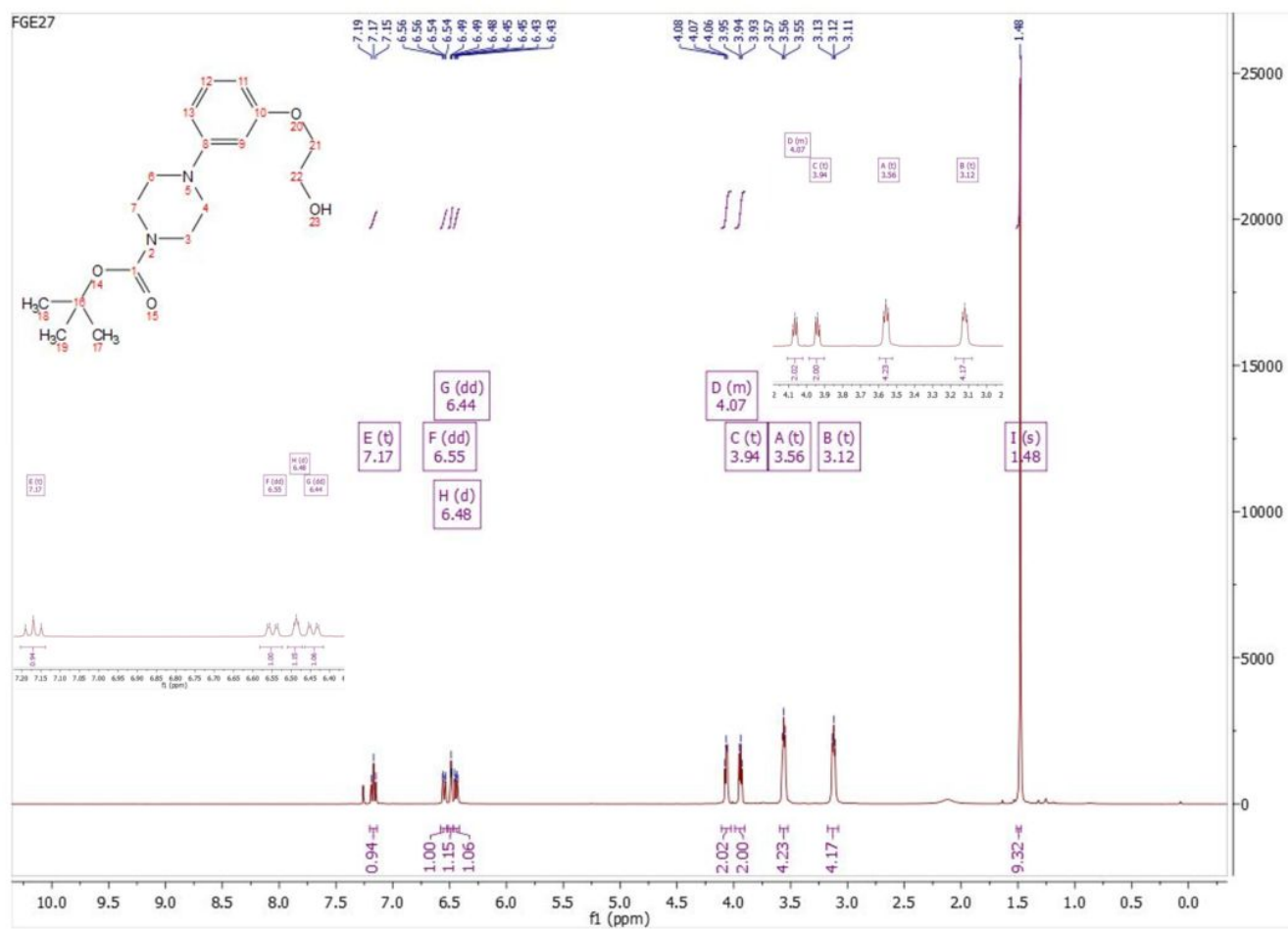


Peak ID	Compound	Time	Mass Found
1		2.36	Not Found

1:MS ES+
4.1e+005

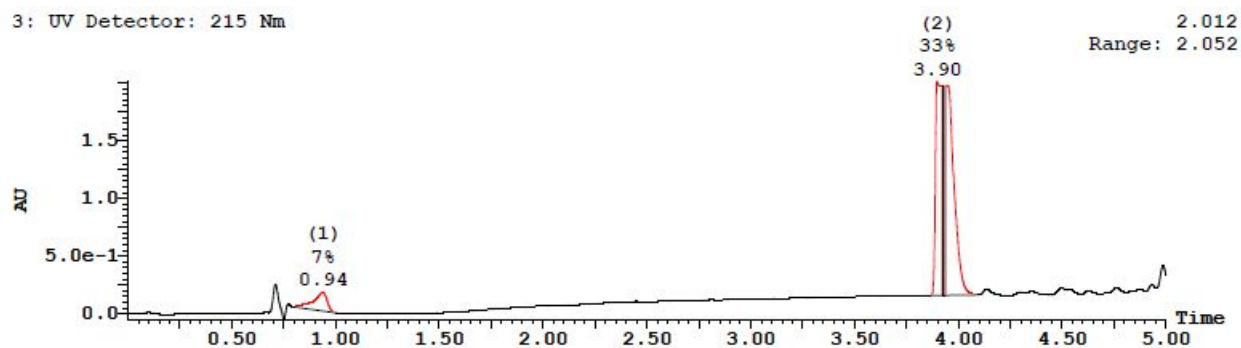


Appendix 10 – ^1H NMR of ENL11

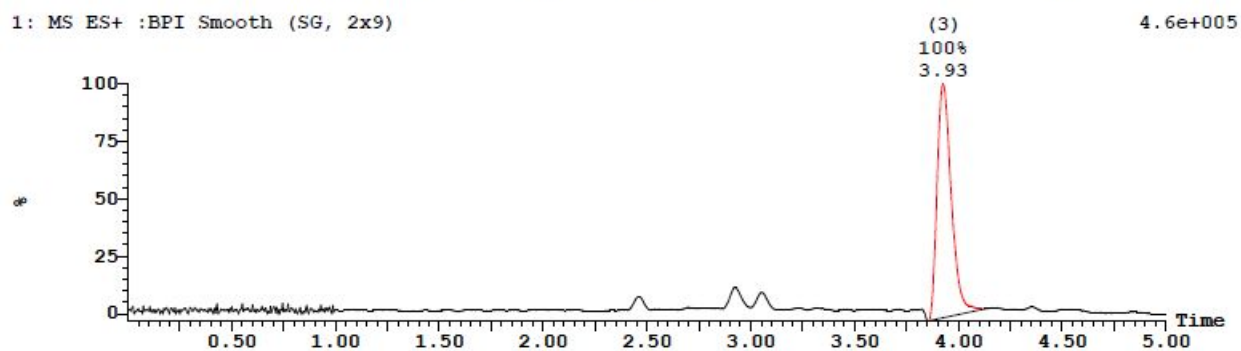


Appendix 11– UPLC-MS of ENL11

3: UV Detector: 215 Nm

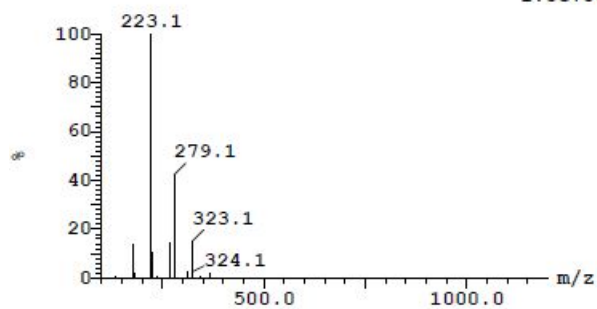


1: MS ES+ :BPI Smooth (SG, 2x9)



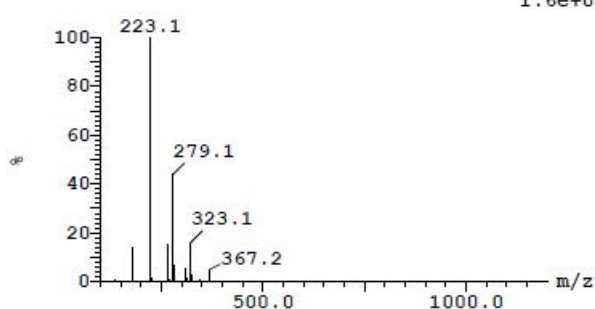
Peak ID 2 Compound Time 3.90 Mass Found Not Found

1:MS ES+
1.5e+005

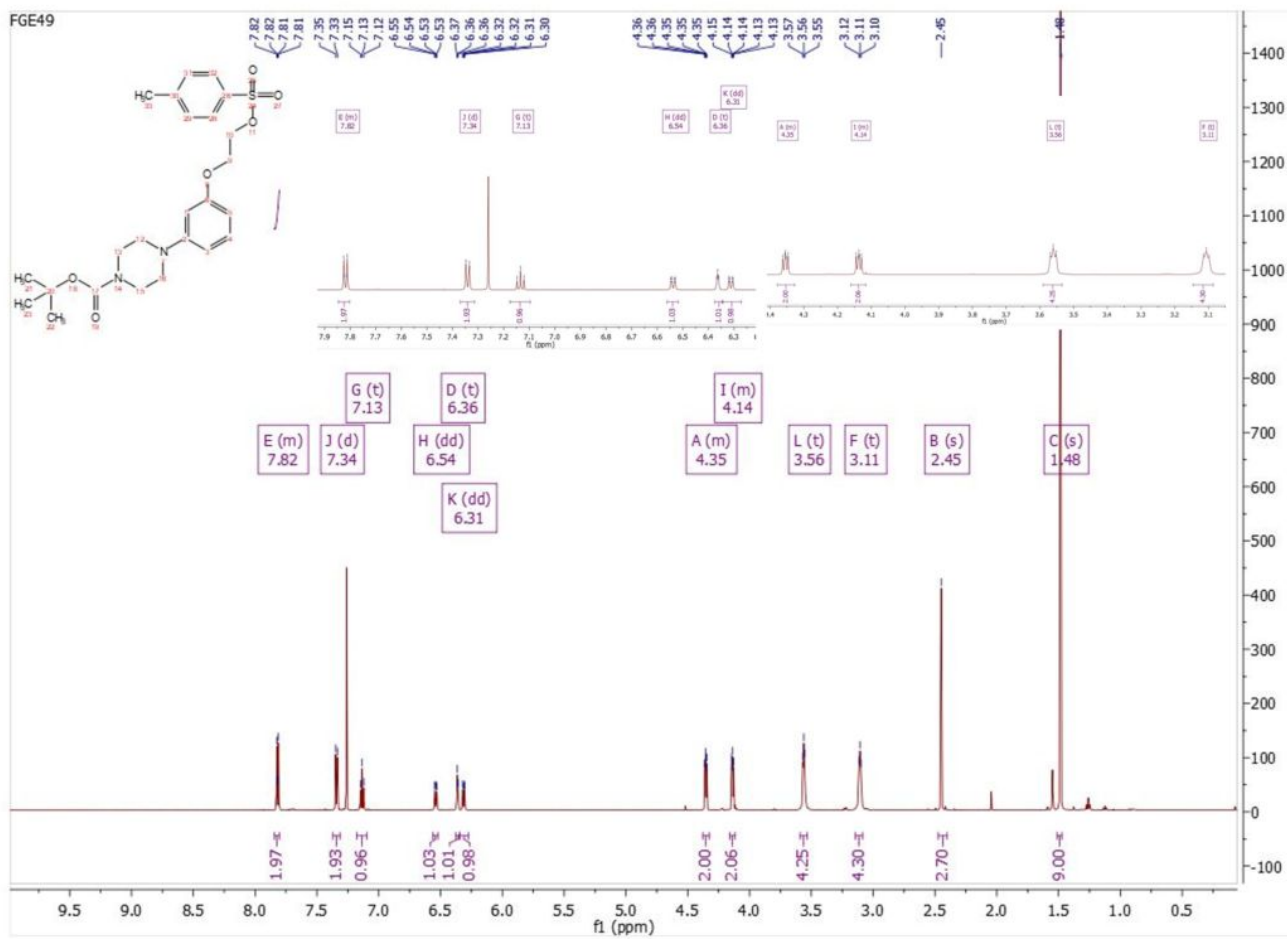


Peak ID 3 Compound Time 3.93 Mass Found Not Found

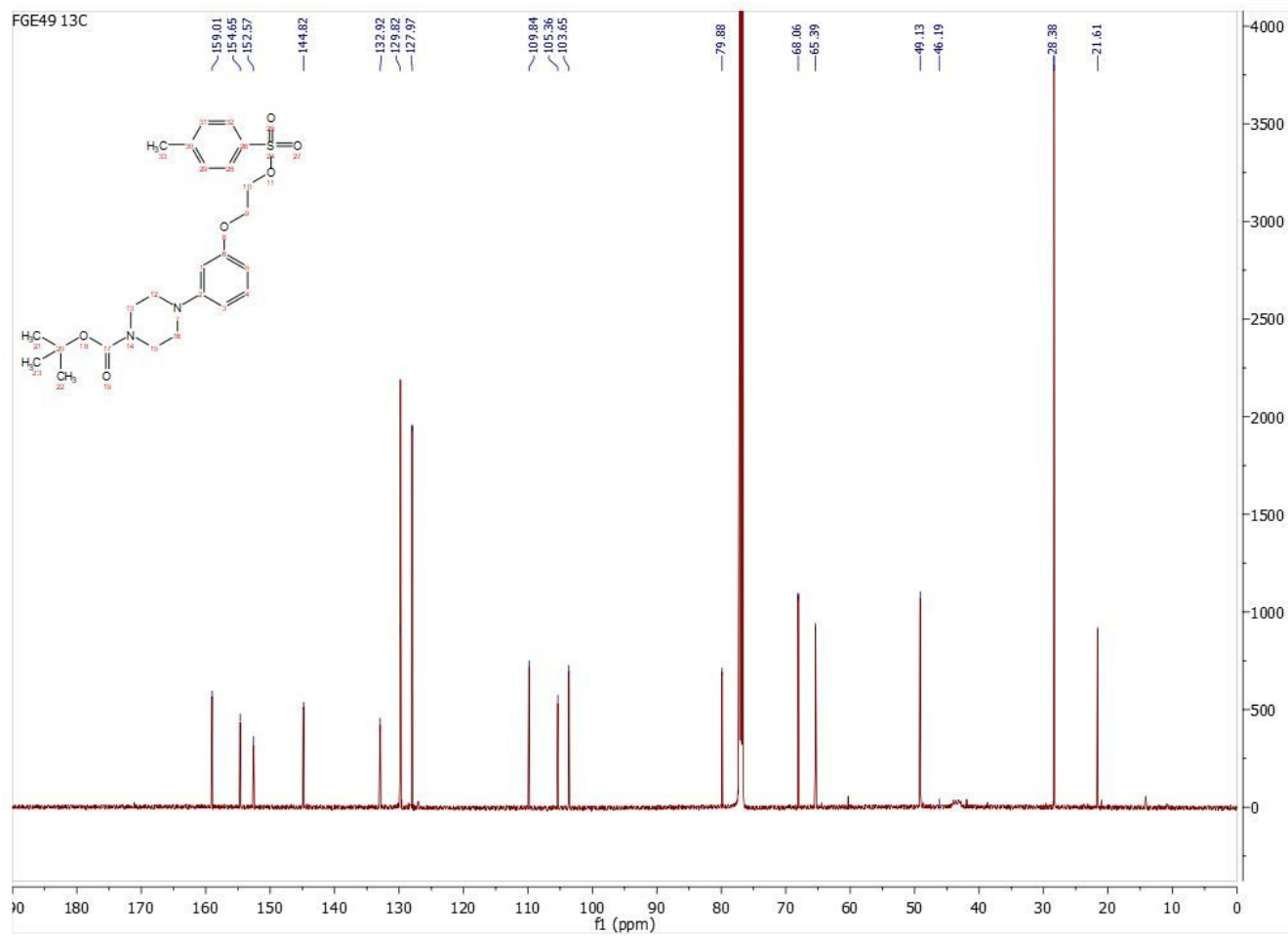
1:MS ES+
1.6e+005



Appendix 12 – ¹H NMR of ENL04

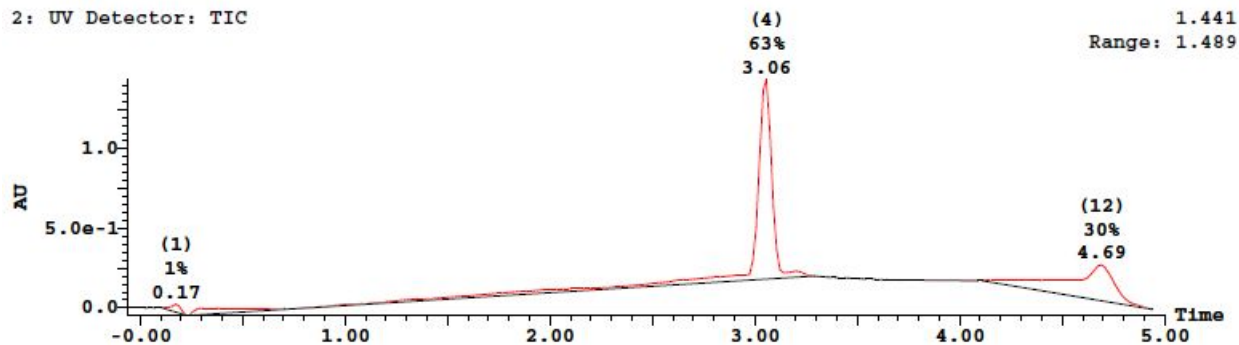


Appendix 13 – ^{13}C NMR of ENL04



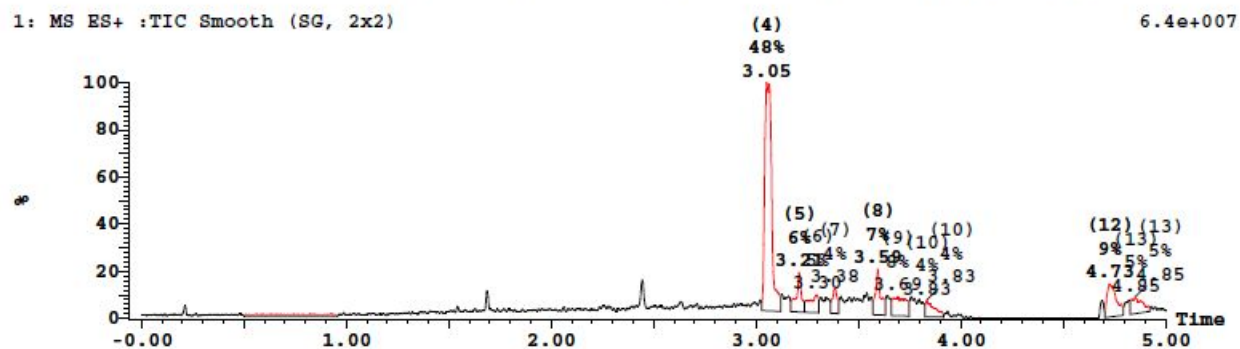
Appendix 14– UPLC-MS of ENL04

2: UV Detector: TIC



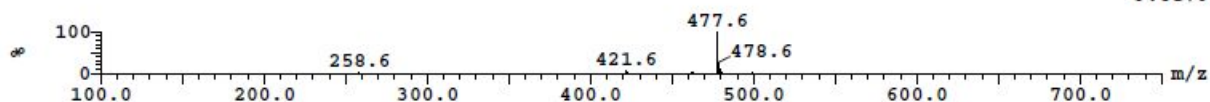
Sample 1 Vial 2:23 ID MCR_KB20-1 File MCR_KB20-1 Date 19-Jun-2018 Time 12:45:04 Description ENL04_PURE

1: MS ES+ :TIC Smooth (SG, 2x2)

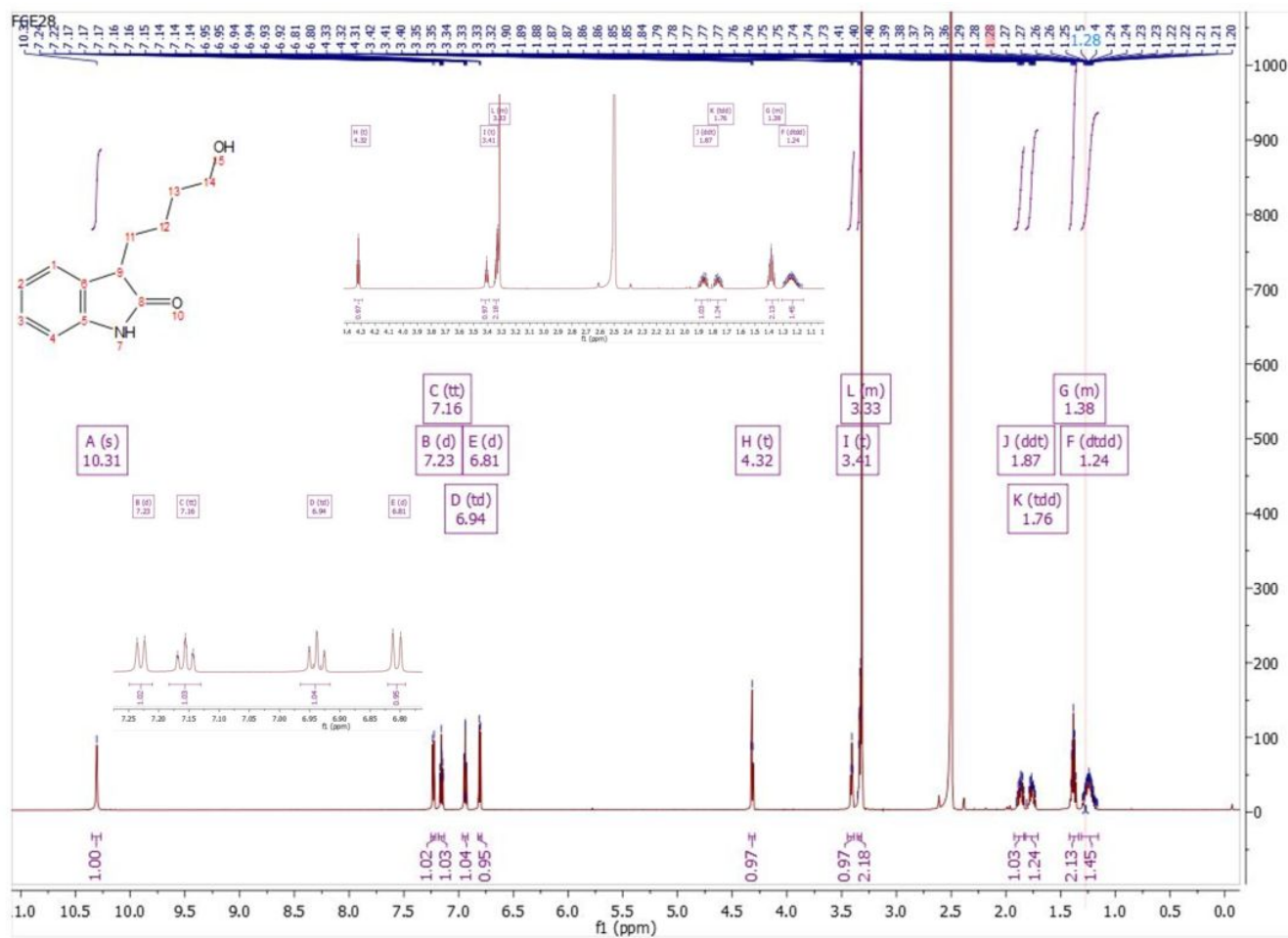


Peak ID	Compound	Time	Mass Found
4		3.05	

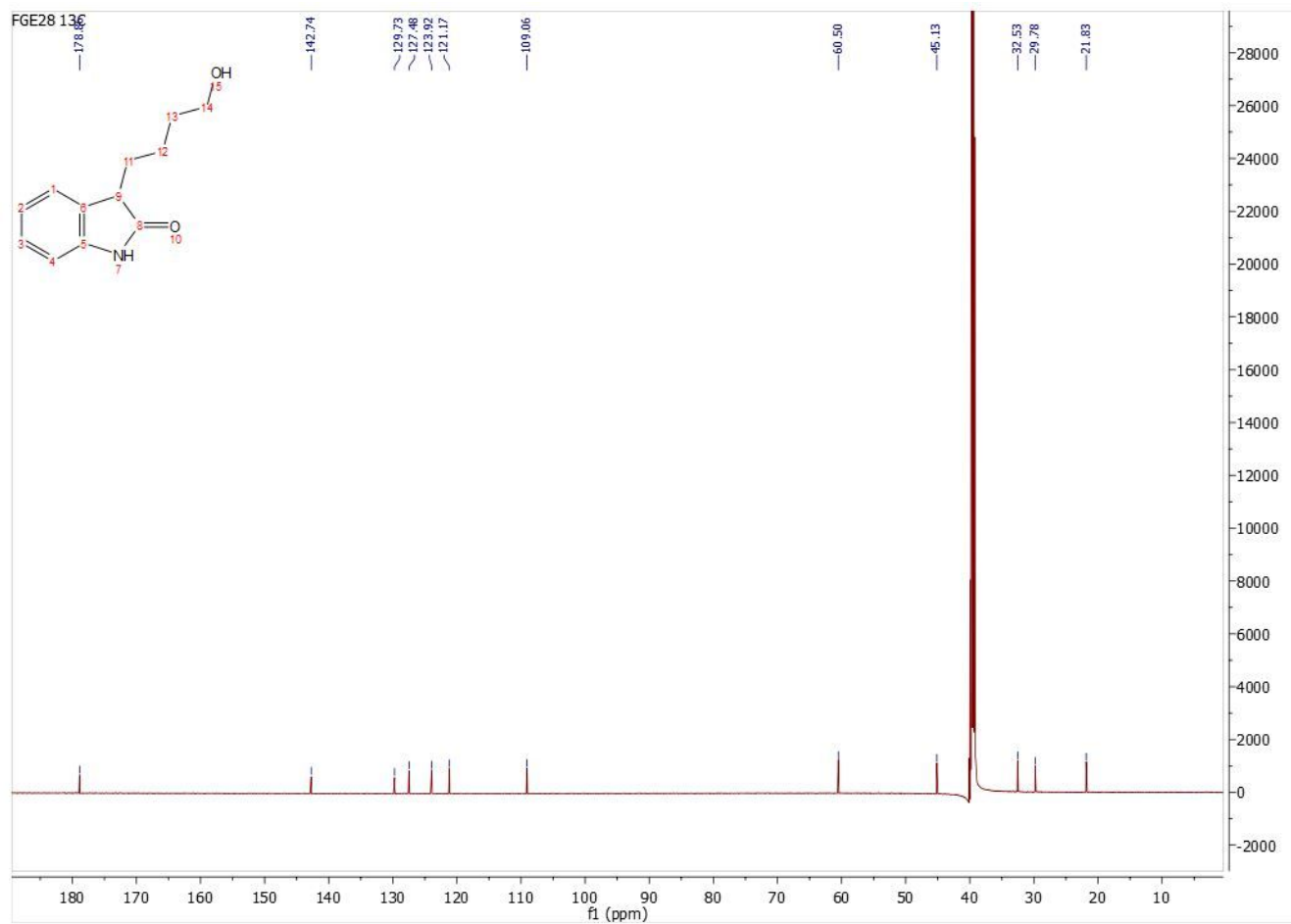
1:MS ES+
9.8e+005



Appendix 15 – ¹H NMR of ENL12



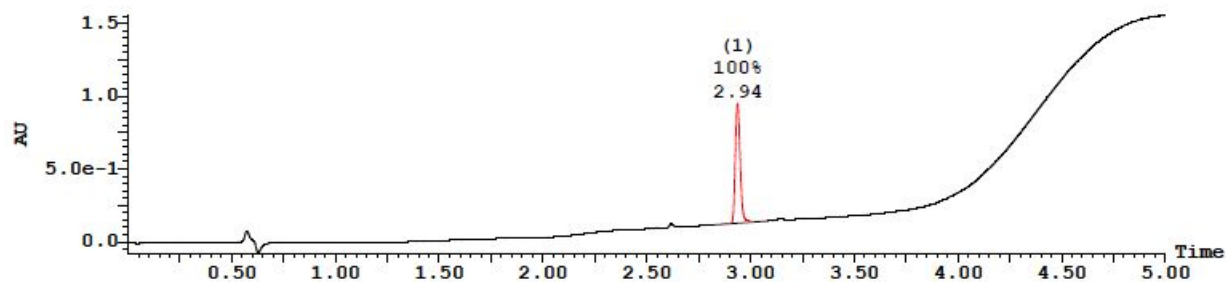
Appendix 16 – ^{13}C NMR of ENL12



Appendix 17– UPLC-MS of ENL12

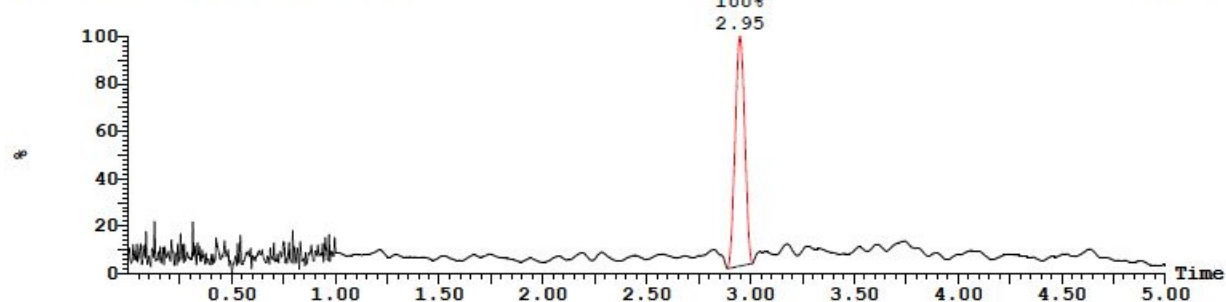
3: UV Detector: 215 Nm

1.553
Range: 1.626



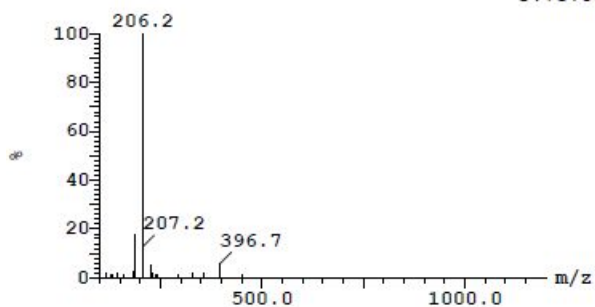
1: MS ES+ :BPI Smooth (SG, 2x9)

1.4e+005

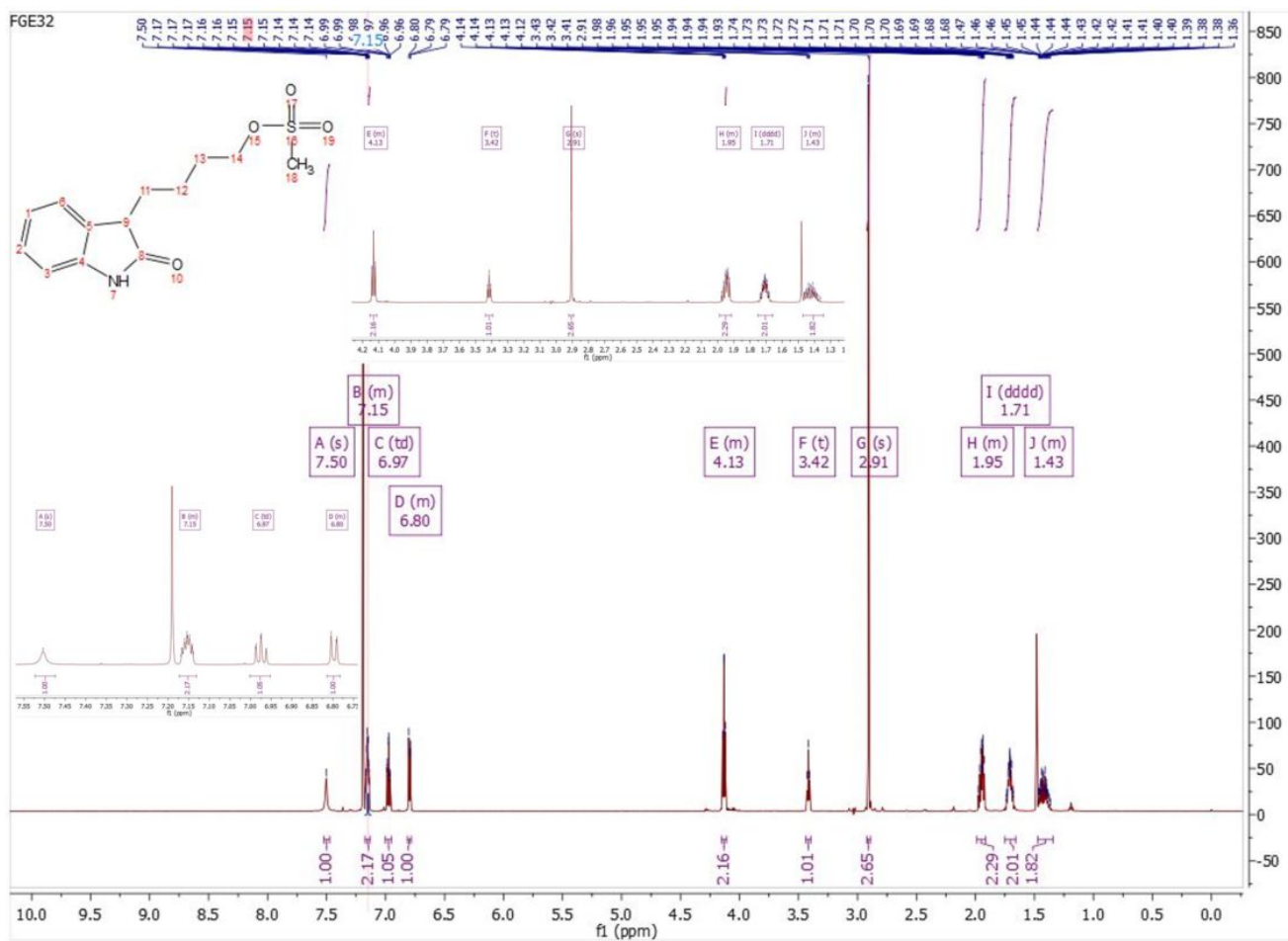


Peak ID	Compound	Time	Mass Found
1		2.95	Not Found

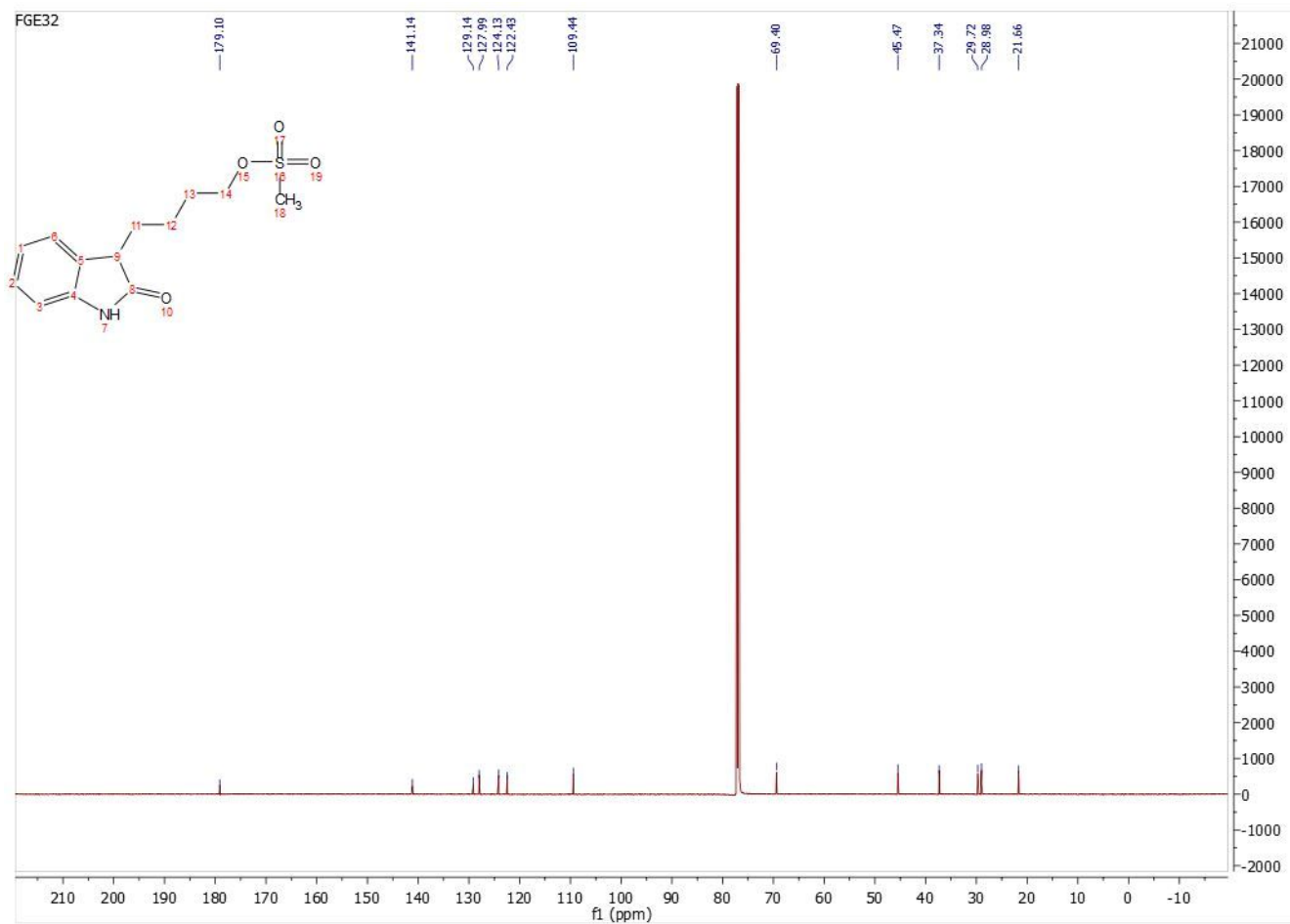
1:MS ES+
3.7e+004



Appendix 18 – ¹H NMR of ENL14



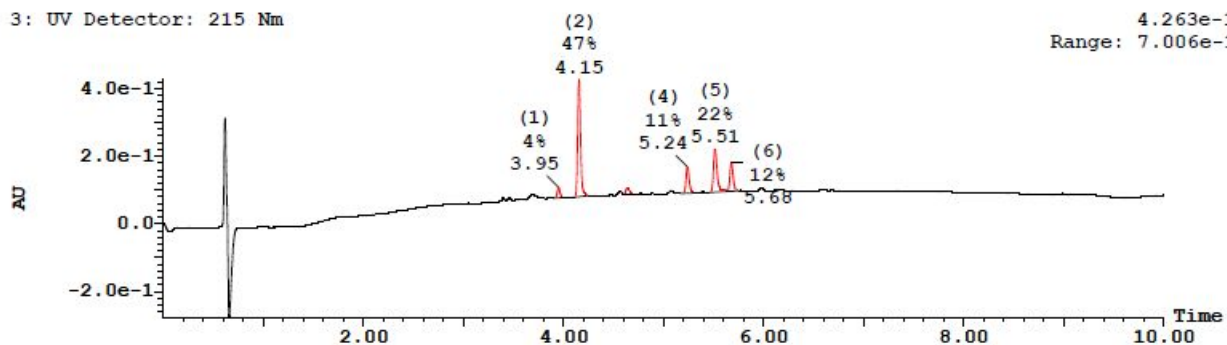
Appendix 19 – ^{13}C NMR of ENL14



Appendix 20– UPLC-MS of ENL14

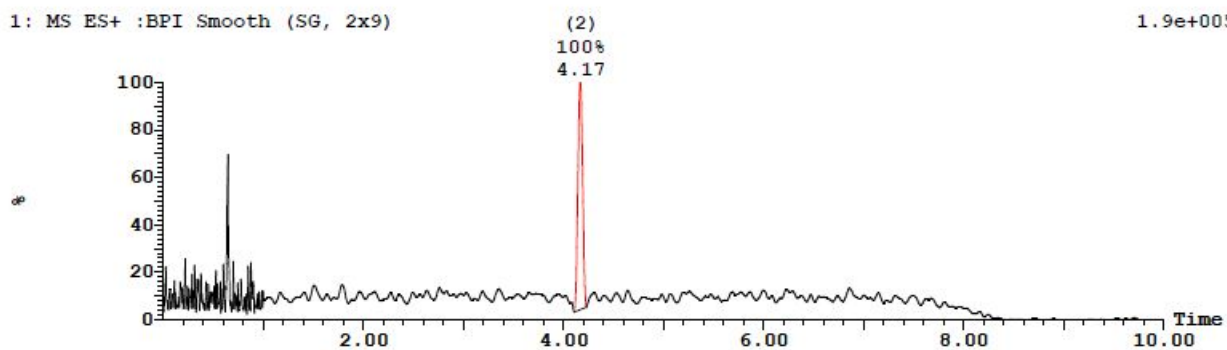
3: UV Detector: 215 Nm

4.263e-1
Range: 7.006e-1



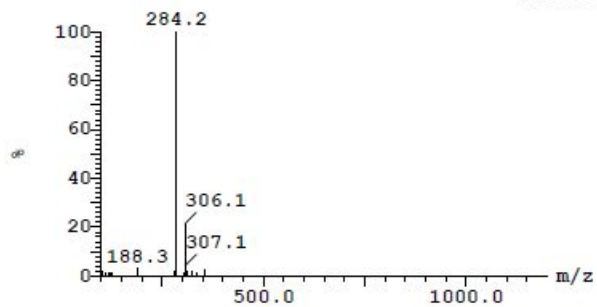
1: MS ES+ :BPI Smooth (SG, 2x9)

1.9e+005



Peak ID	Compound	Time	Mass Found
2		4.17	Not Found

1:MS ES+
4.9e+004

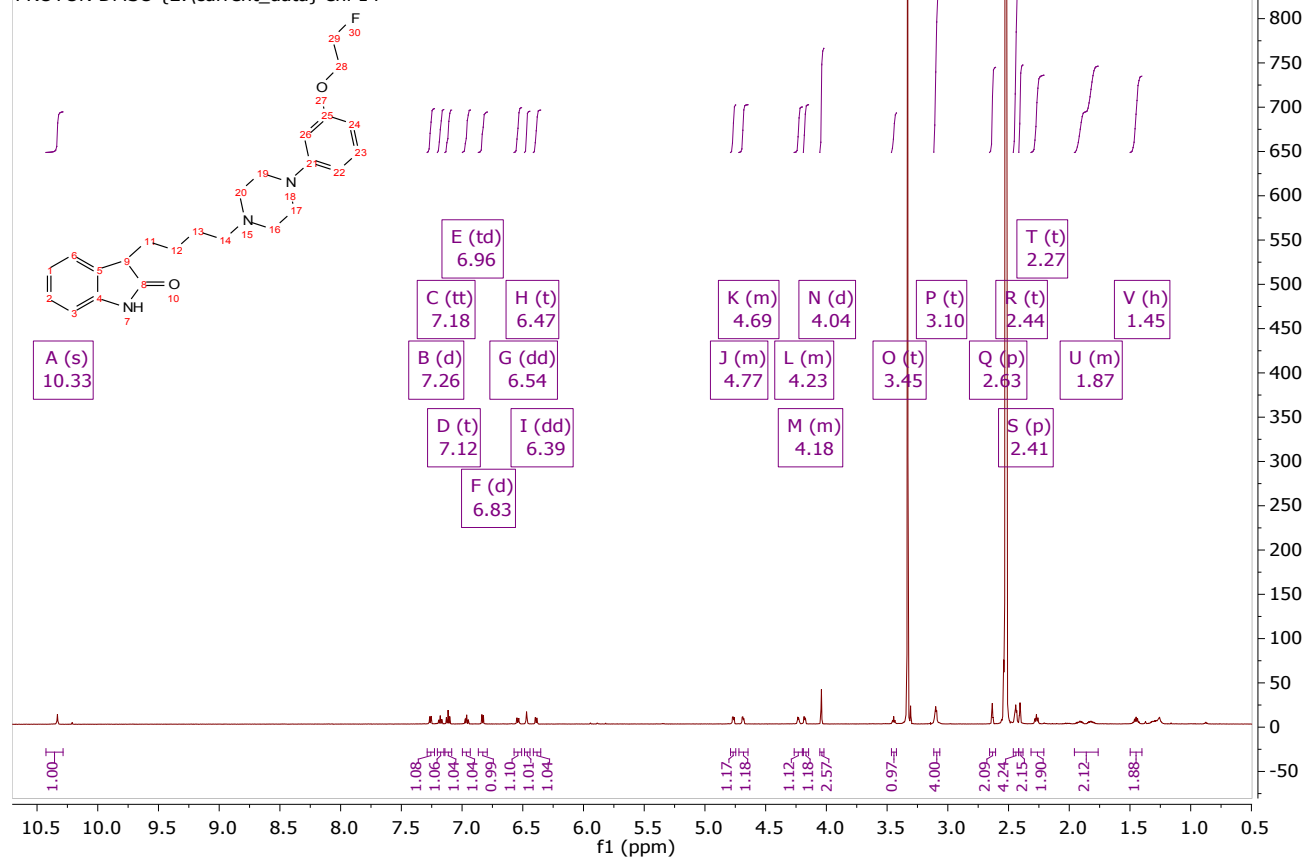


Appendix 21 – ¹H NMR of ENL20

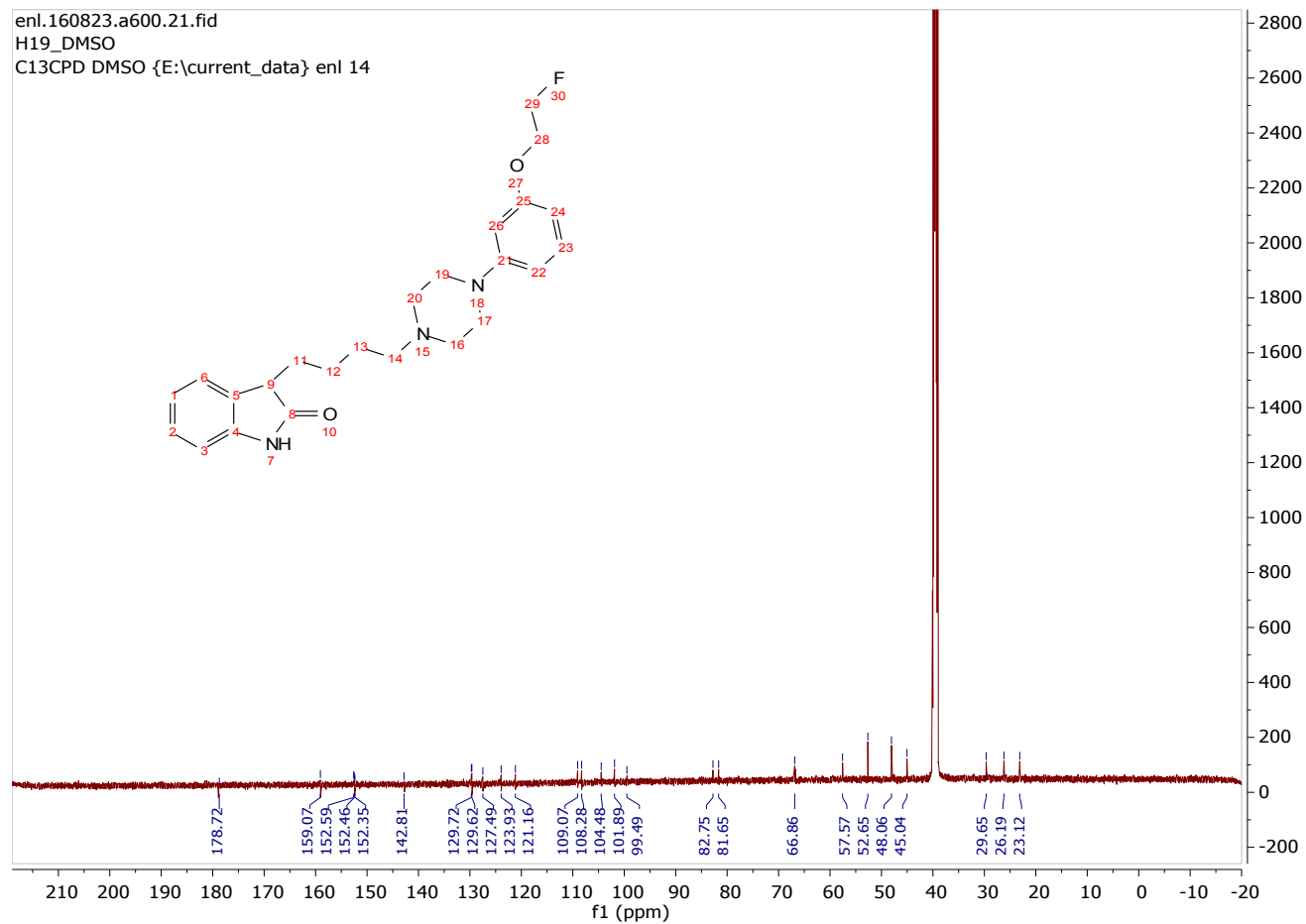
enl.160823.a600.20.fid

H19_DMSO

PROTON DMSO {E:\current_data} enl 14

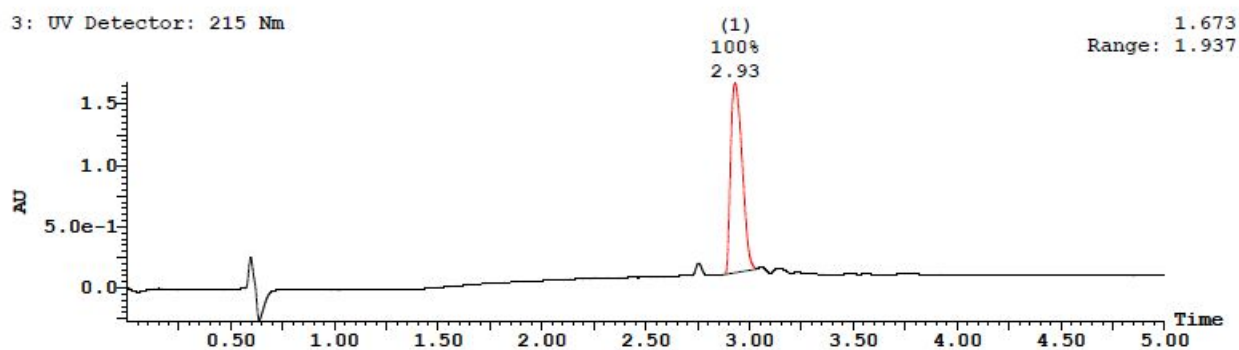


Appendix 22 – ^{13}C NMR of ENL20

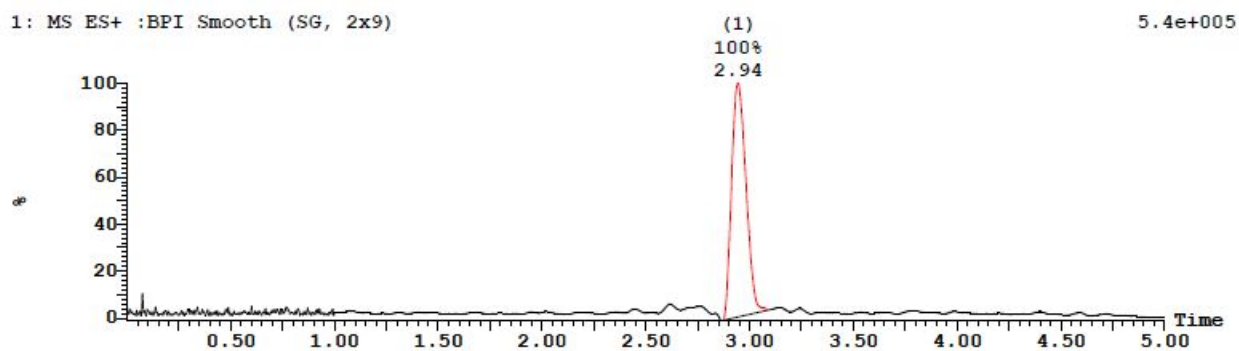


Appendix 23– UPLC-MS of ENL20

3: UV Detector: 215 Nm

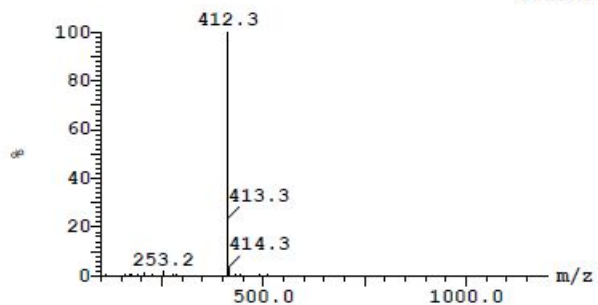


1: MS ES+ :BPI Smooth (SG, 2x9)



Peak ID	Compound	Time	Mass Found
1		2.94	Not Found

1:MS ES+
2.1e+005

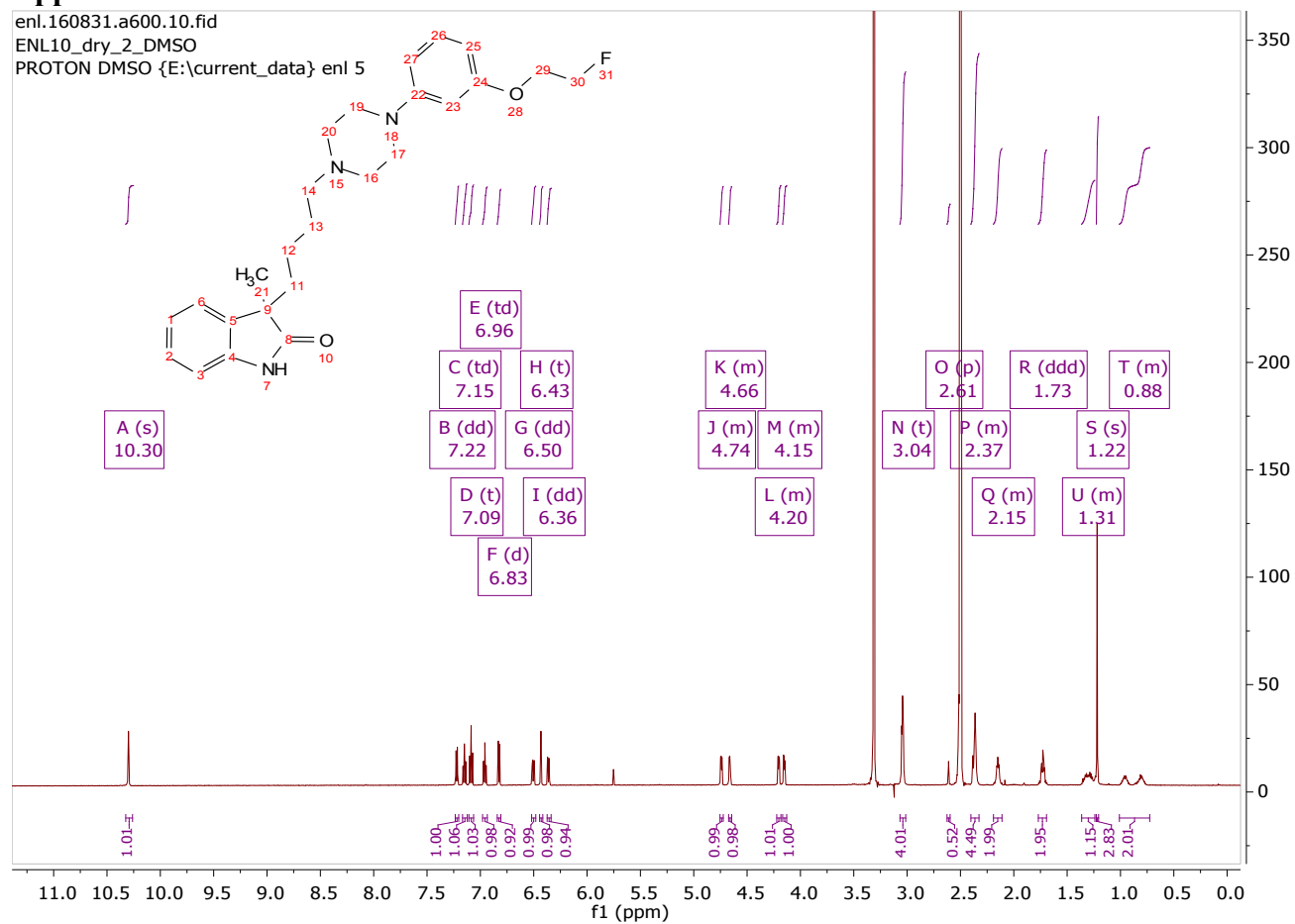


Appendix 24 – ¹H NMR of ENL10

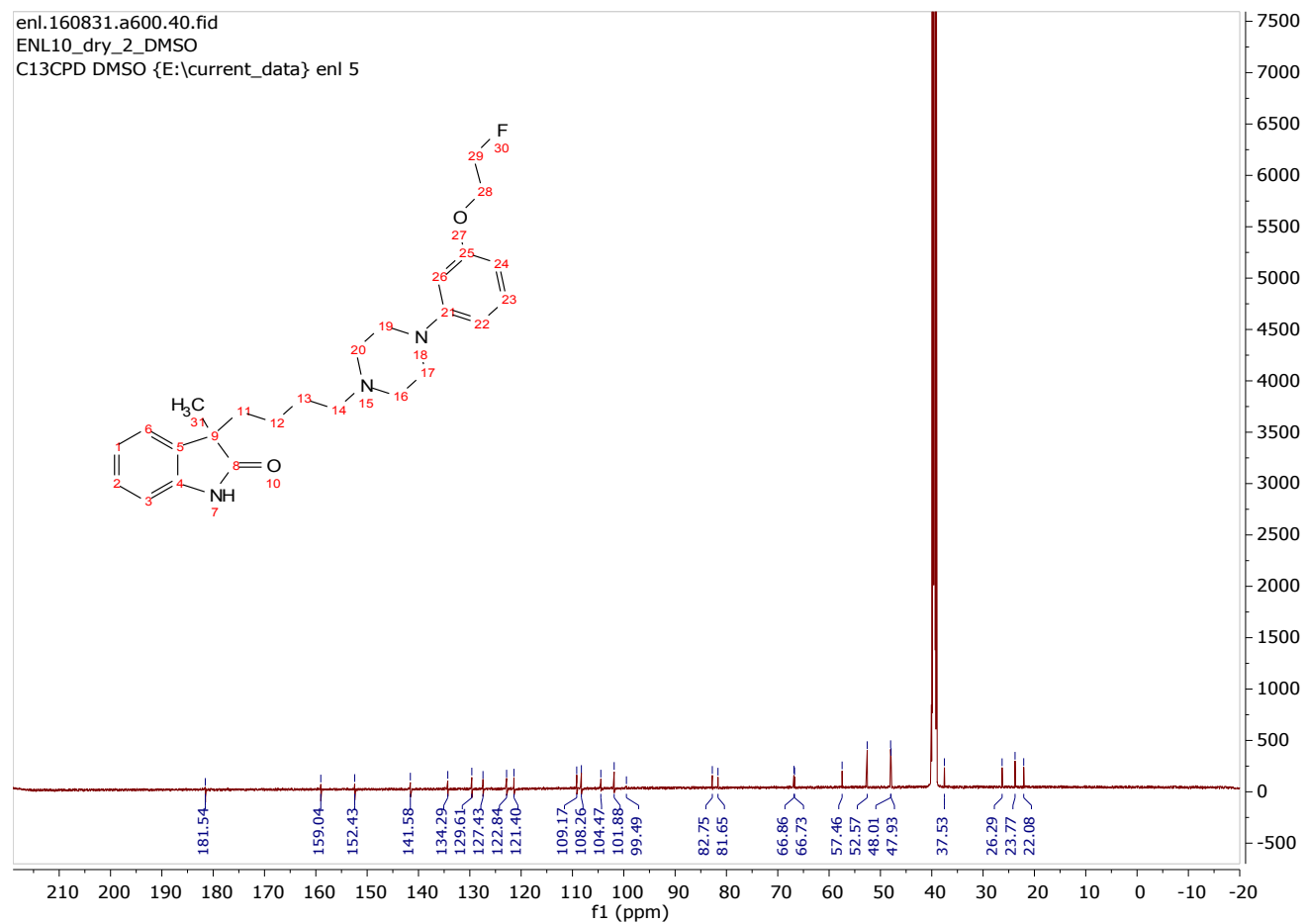
enl.160831.a600.10.fid

ENL10_dry_2_DMSO

PROTON DMSO {E:\current_data} enl 5

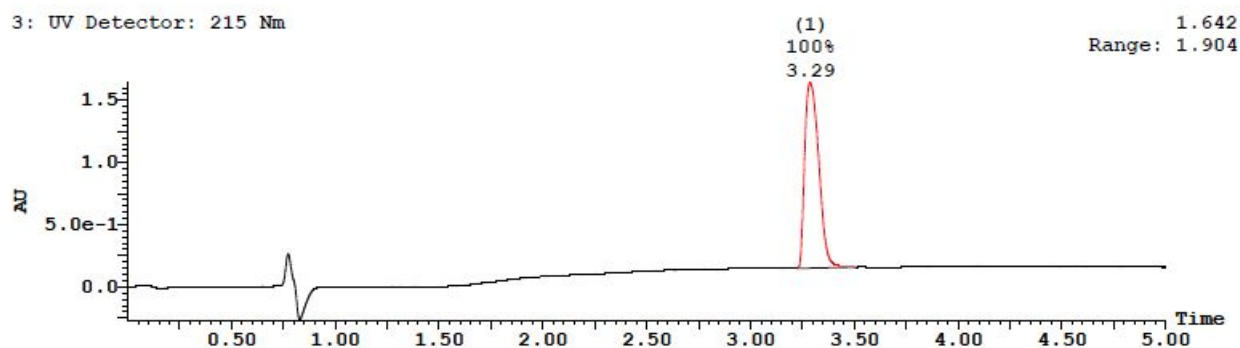


Appendix 25 – ^{13}C NMR of ENL10

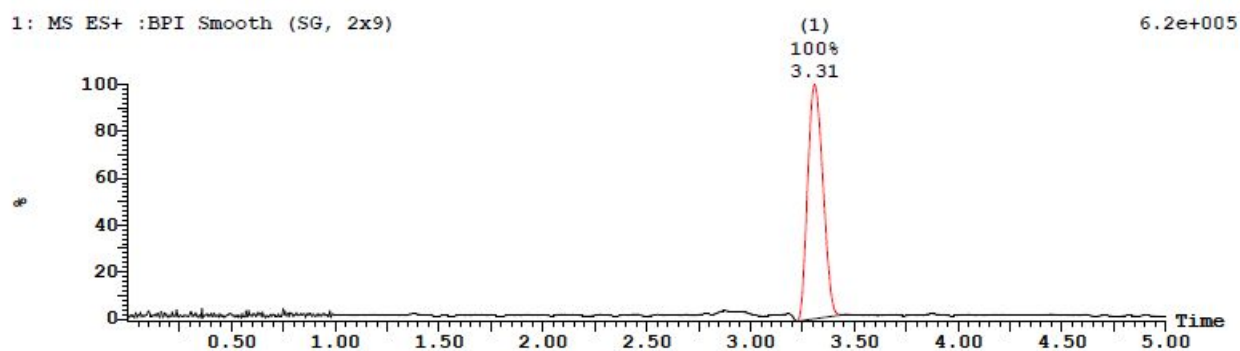


Appendix 26– UPLC-MS of ENL10

3: UV Detector: 215 Nm

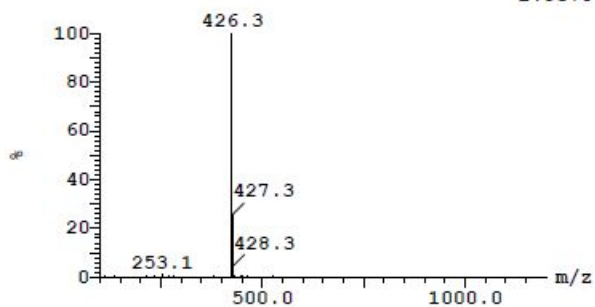


1: MS ES+ :BPI Smooth (SG, 2x9)

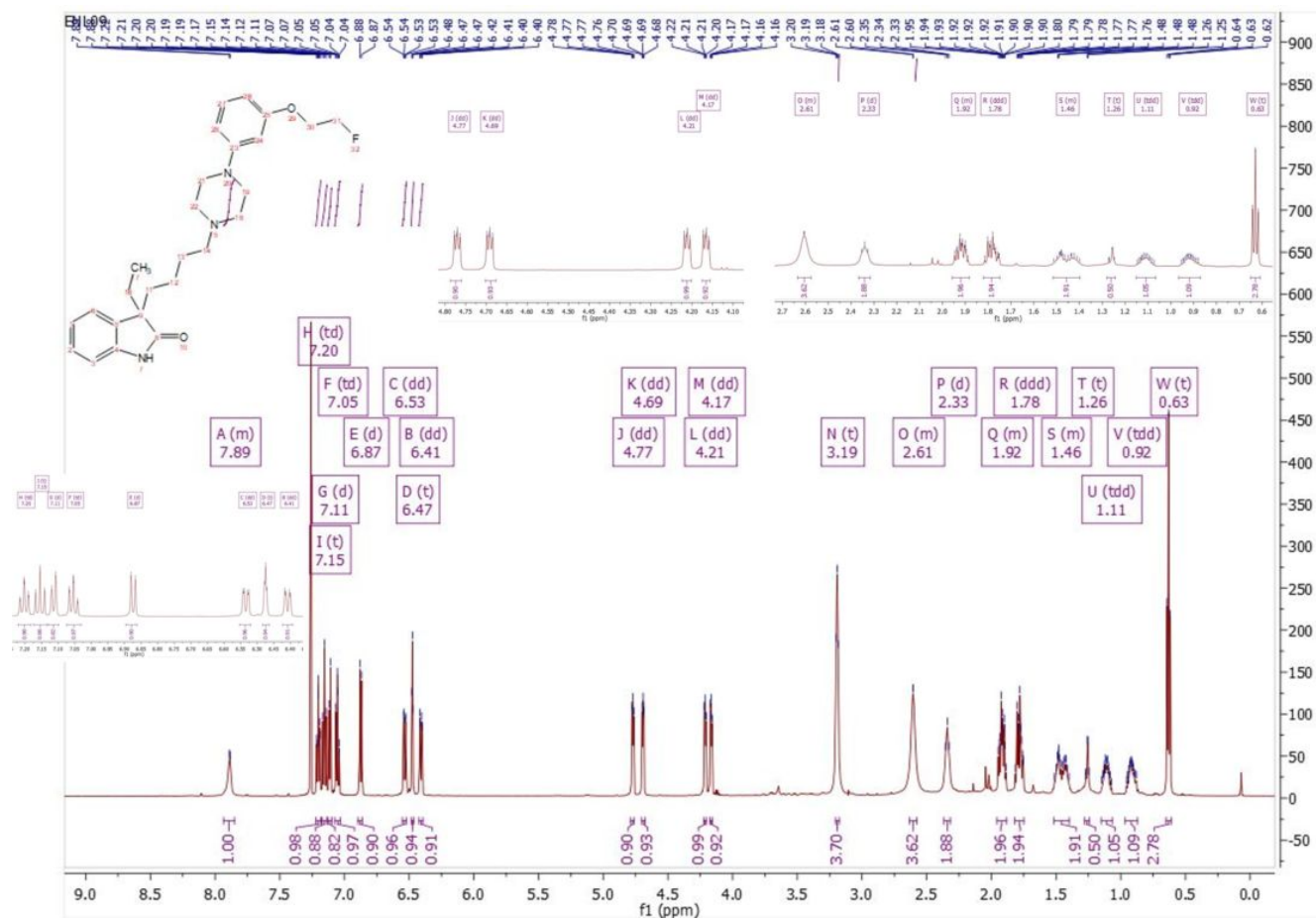


Peak ID	Compound	Time	Mass Found
1		3.31	Not Found

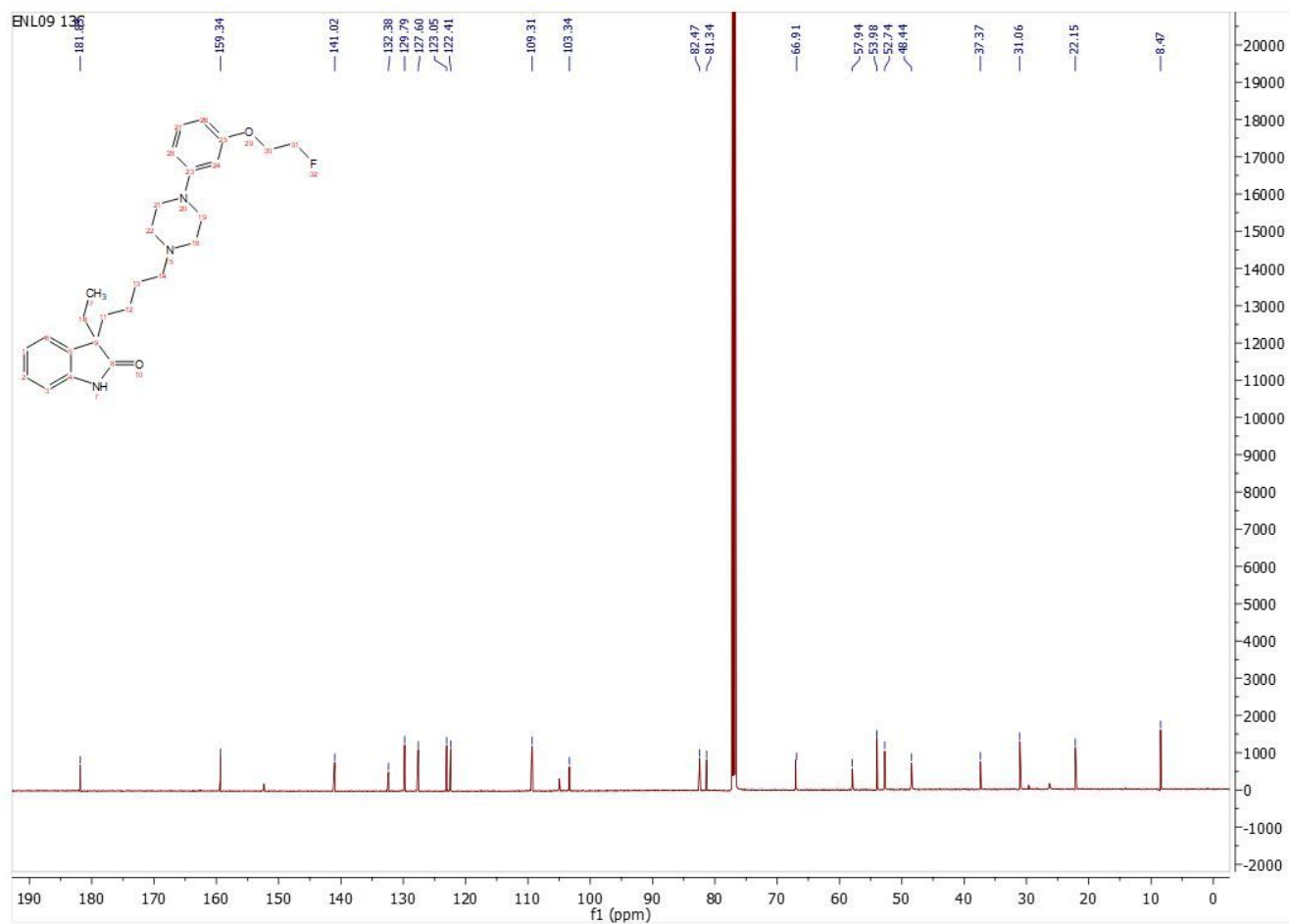
1:MS ES+
2.5e+005



Appendix 27 – ¹H NMR of ENL09

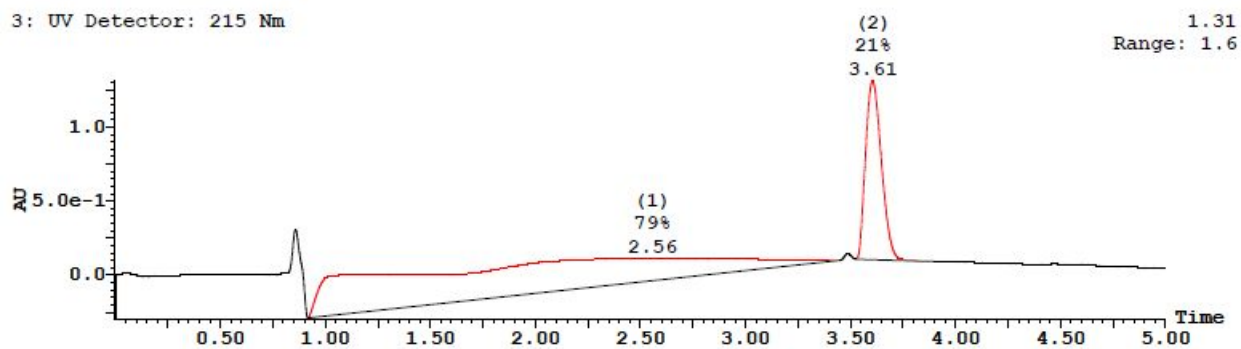


Appendix 28 – ^{13}C NMR of ENL09

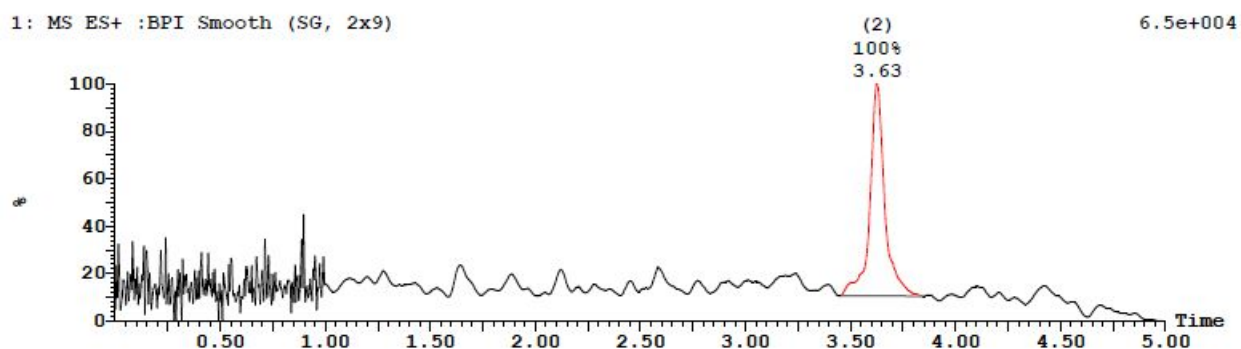


Appendix 29– UPLC-MS of ENL09

3: UV Detector: 215 Nm

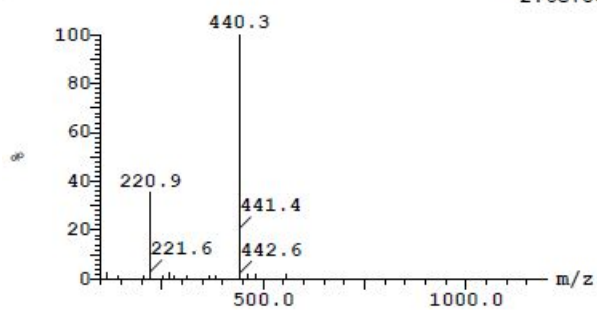


1: MS ES+ :BPI Smooth (SG, 2x9)

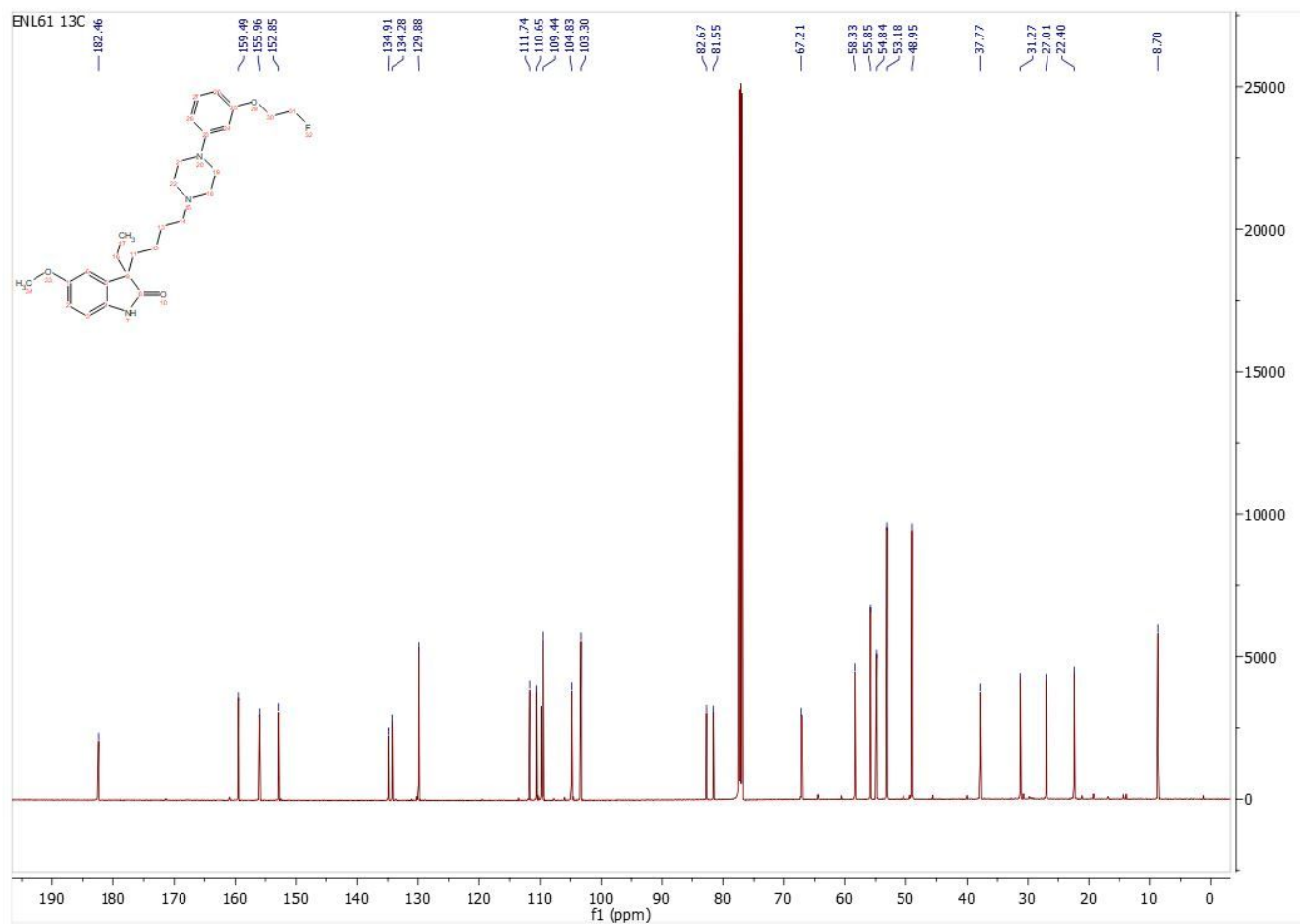


Peak ID	Compound	Time	Mass Found
2		3.63	Not Found

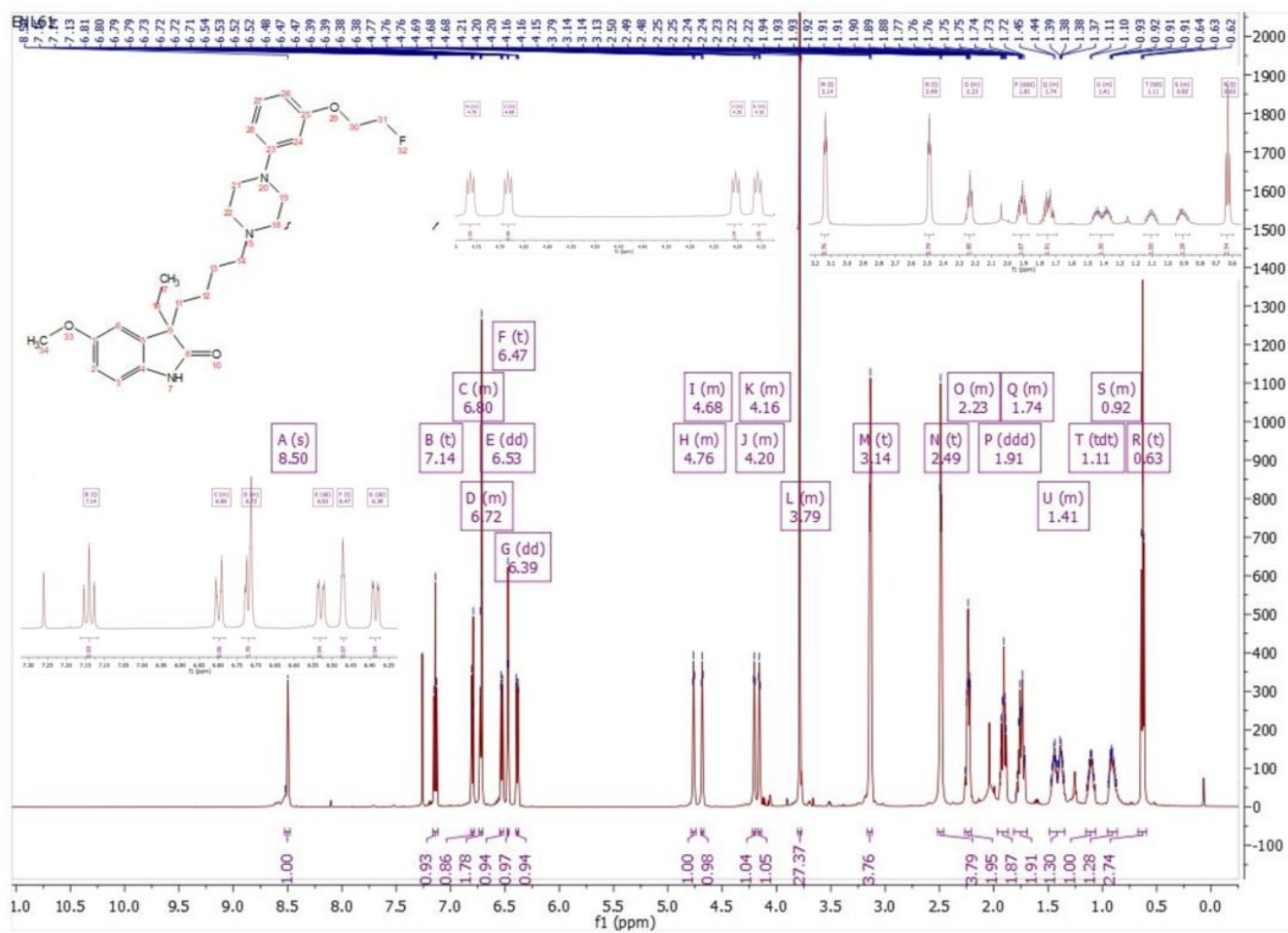
1:MS ES+
2.6e+004



Appendix 30 – ^{13}C NMR of ENL61

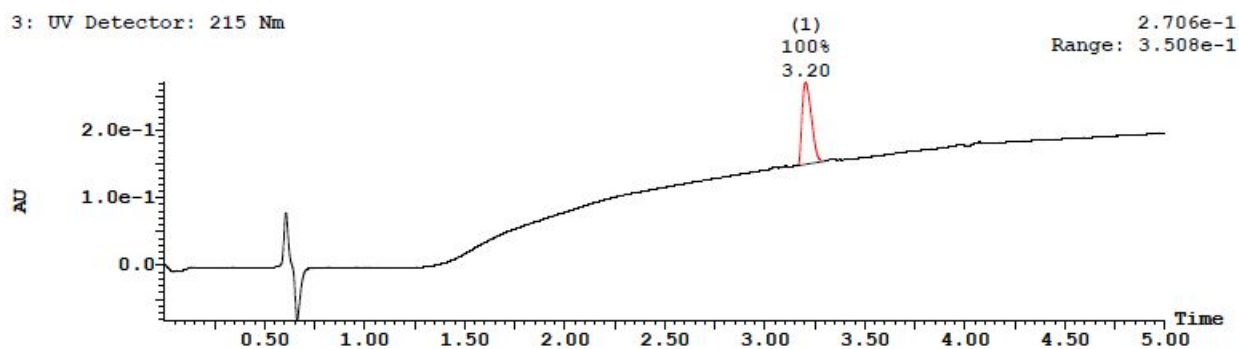


Appendix 31 – ¹H NMR of ENL61

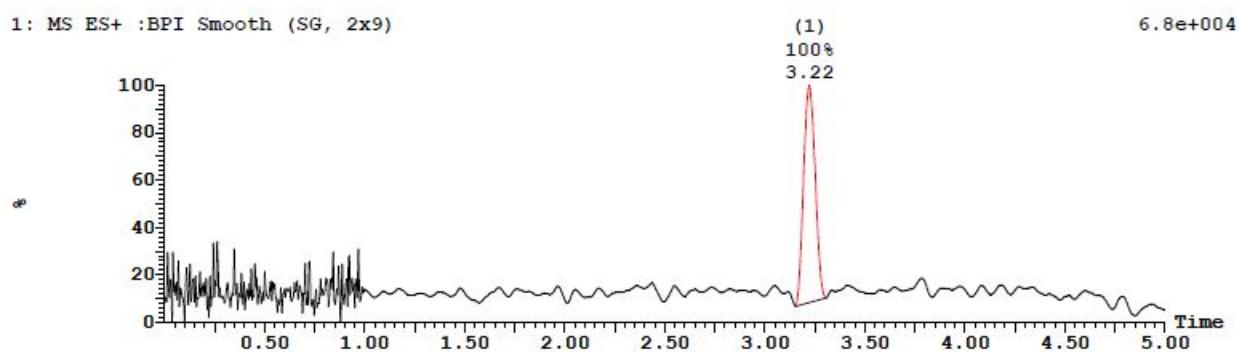


Appendix 32– UPLC-MS of ENL61

3: UV Detector: 215 Nm

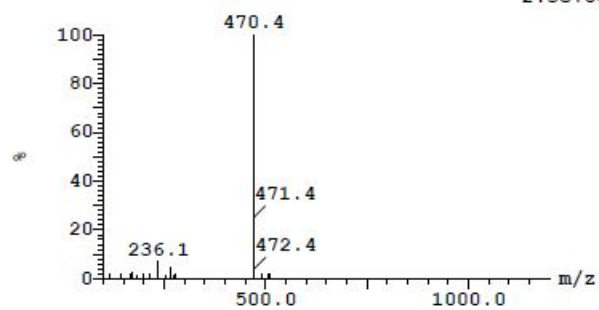


1: MS ES+ :BPI Smooth (SG, 2x9)

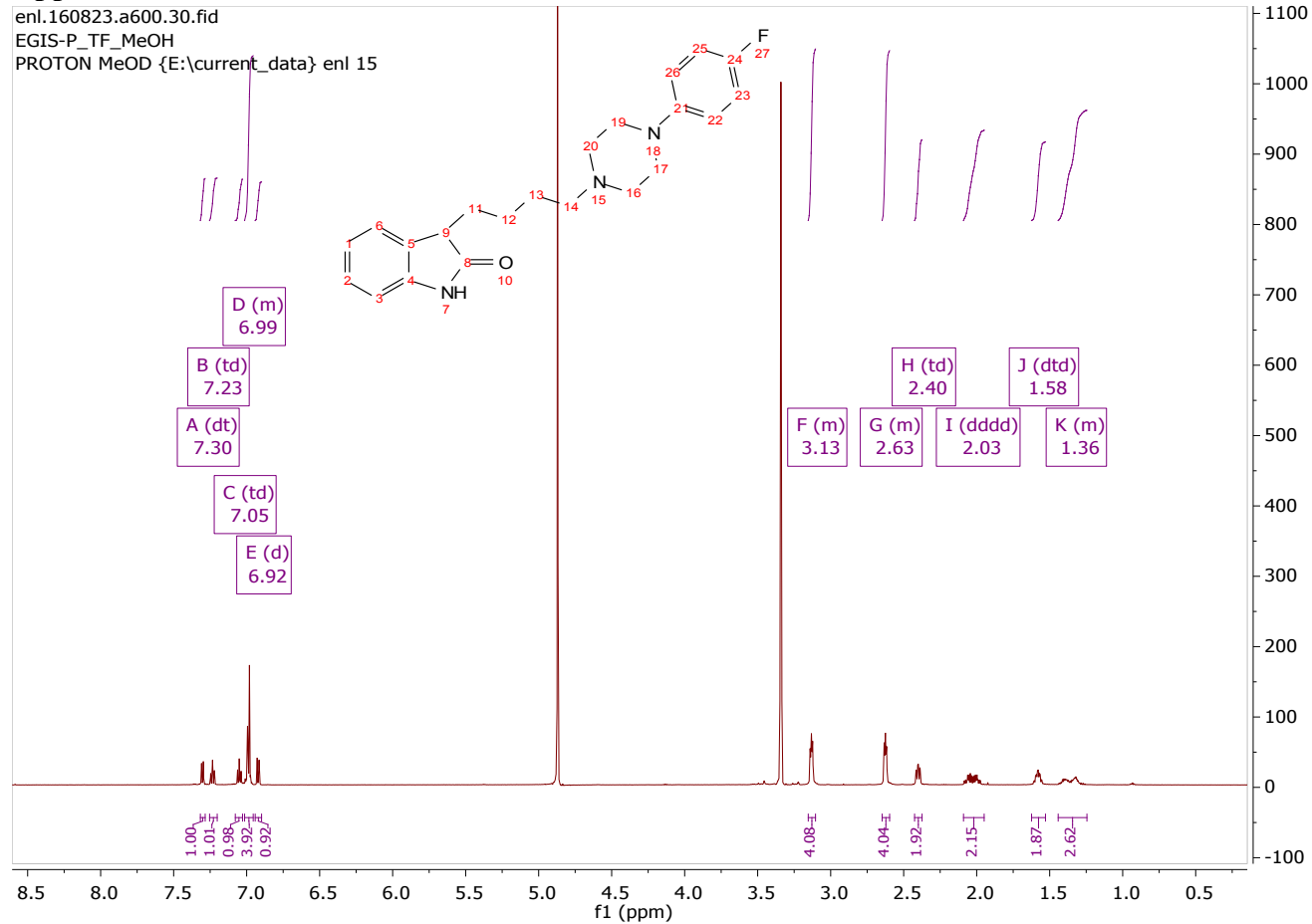


Peak ID	Compound	Time	Mass Found
1		3.22	Not Found

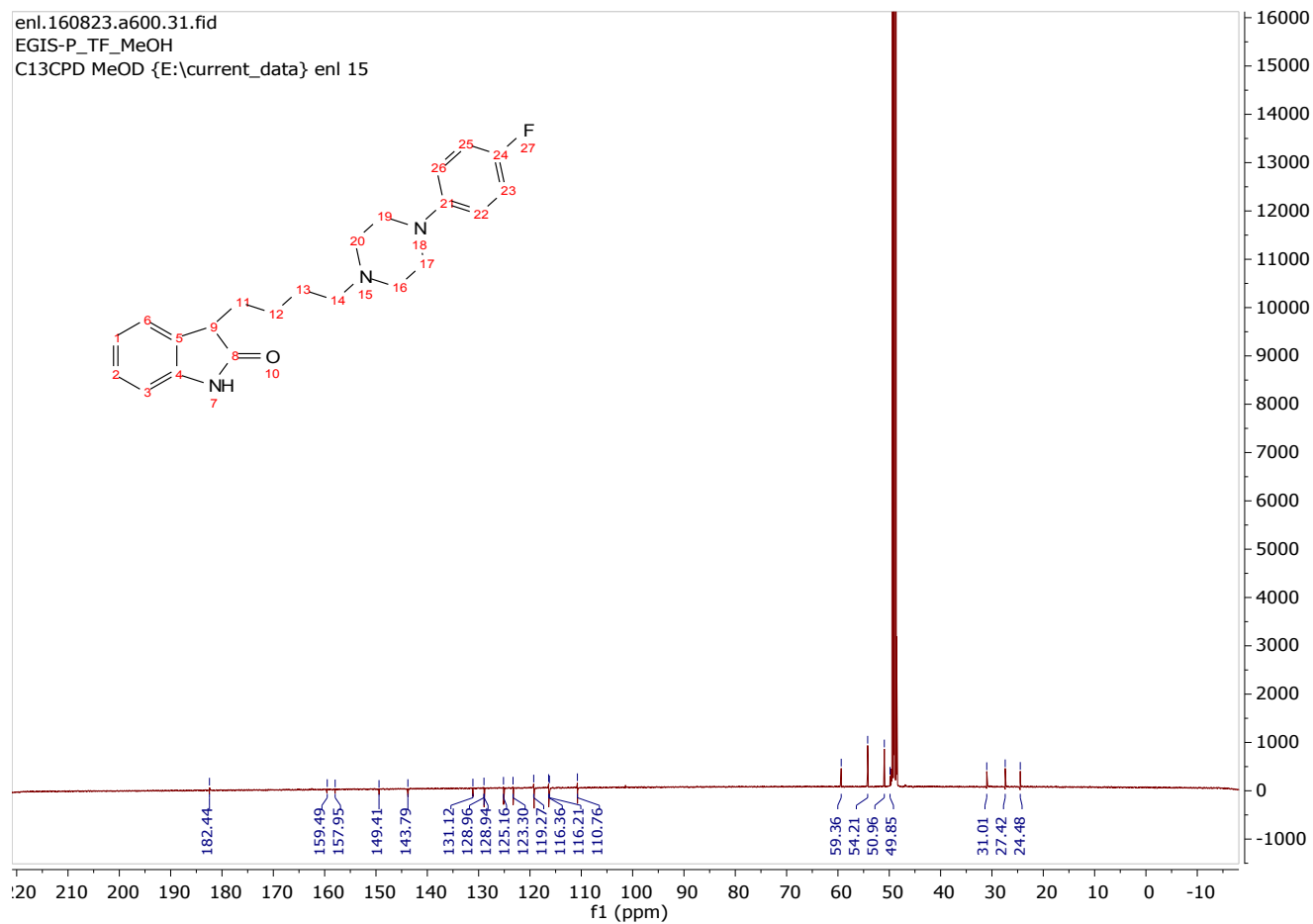
1:MS ES+
2.3e+004



Appendix 33 – ¹H NMR of ENL21

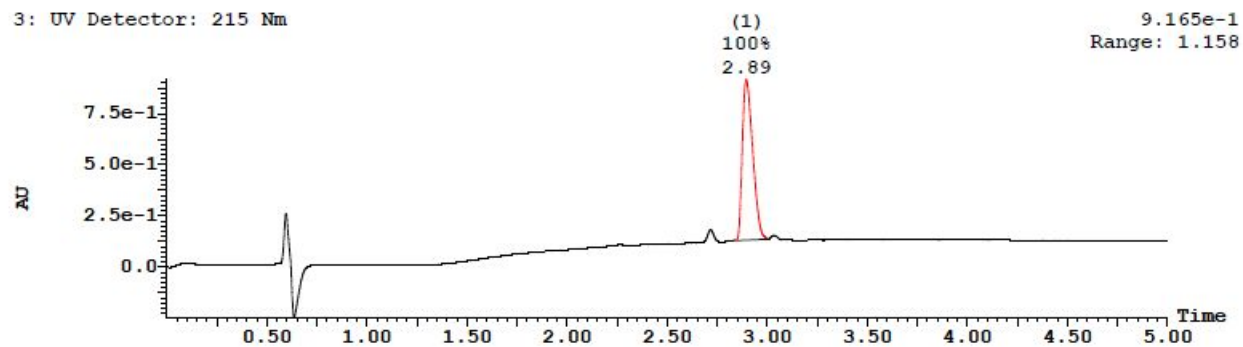


Appendix 34 – ^{13}C NMR of ENL21

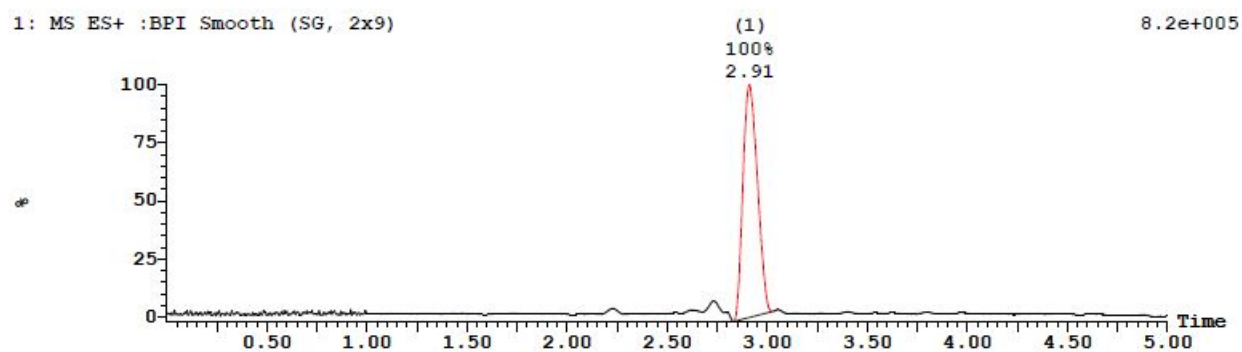


Appendix 35– UPLC-MS of ENL21

3: UV Detector: 215 Nm

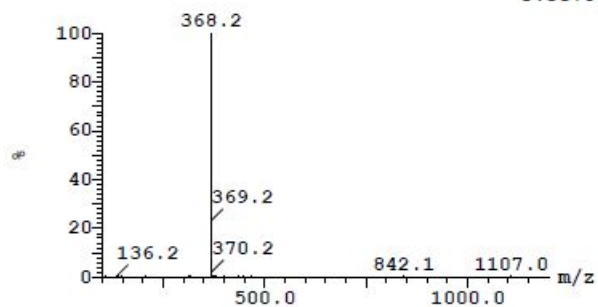


1: MS ES+ :BPI Smooth (SG, 2x9)



Peak ID	Compound	Time	Mass Found	Mass Found
1		2.91	Not Found	

1:MS ES+
3.3e+005

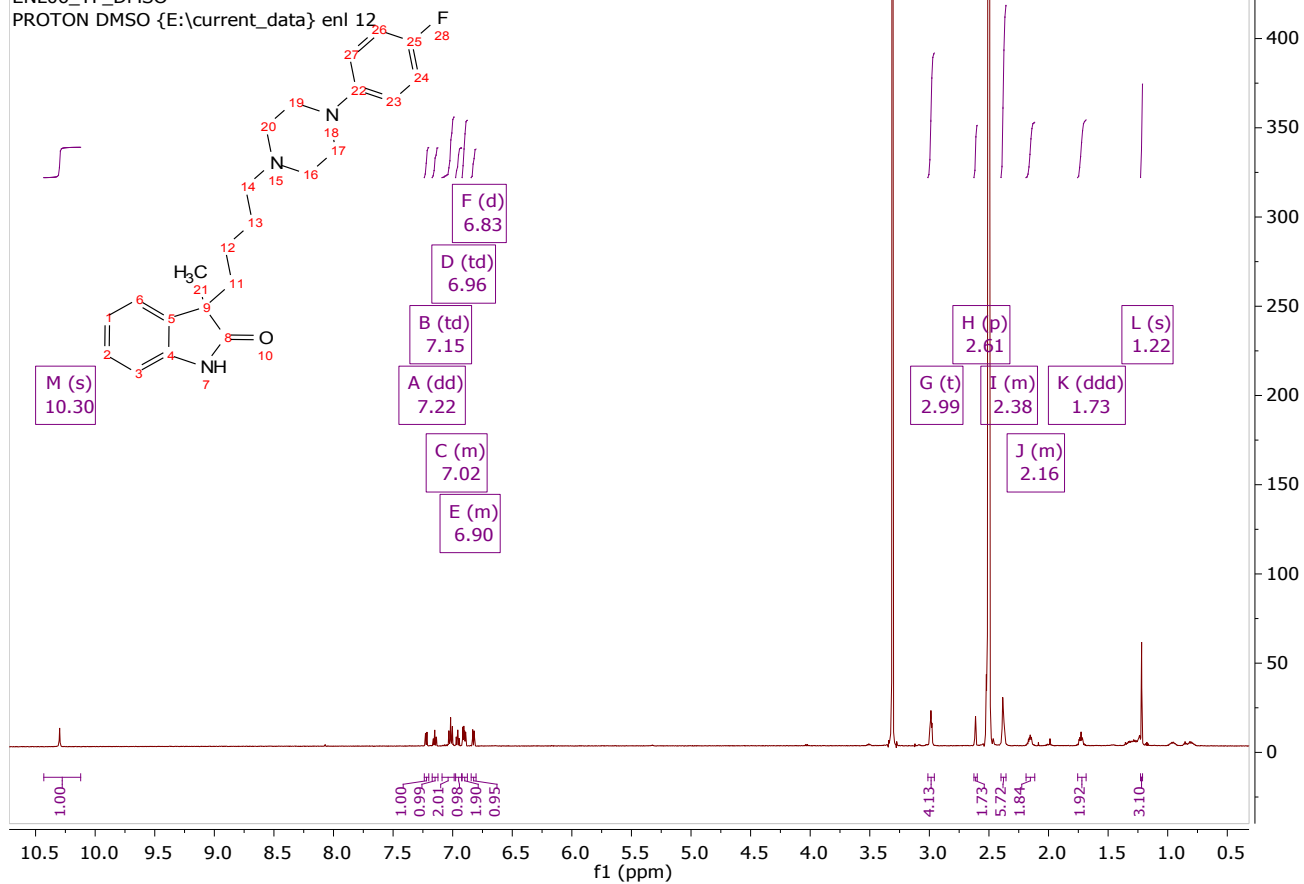


Appendix 36 – ¹H NMR of ENL06

enl.160819.a600.20.fid

ENL06_TF_DMSO

PROTON DMSO {E:\current_data} enl 12

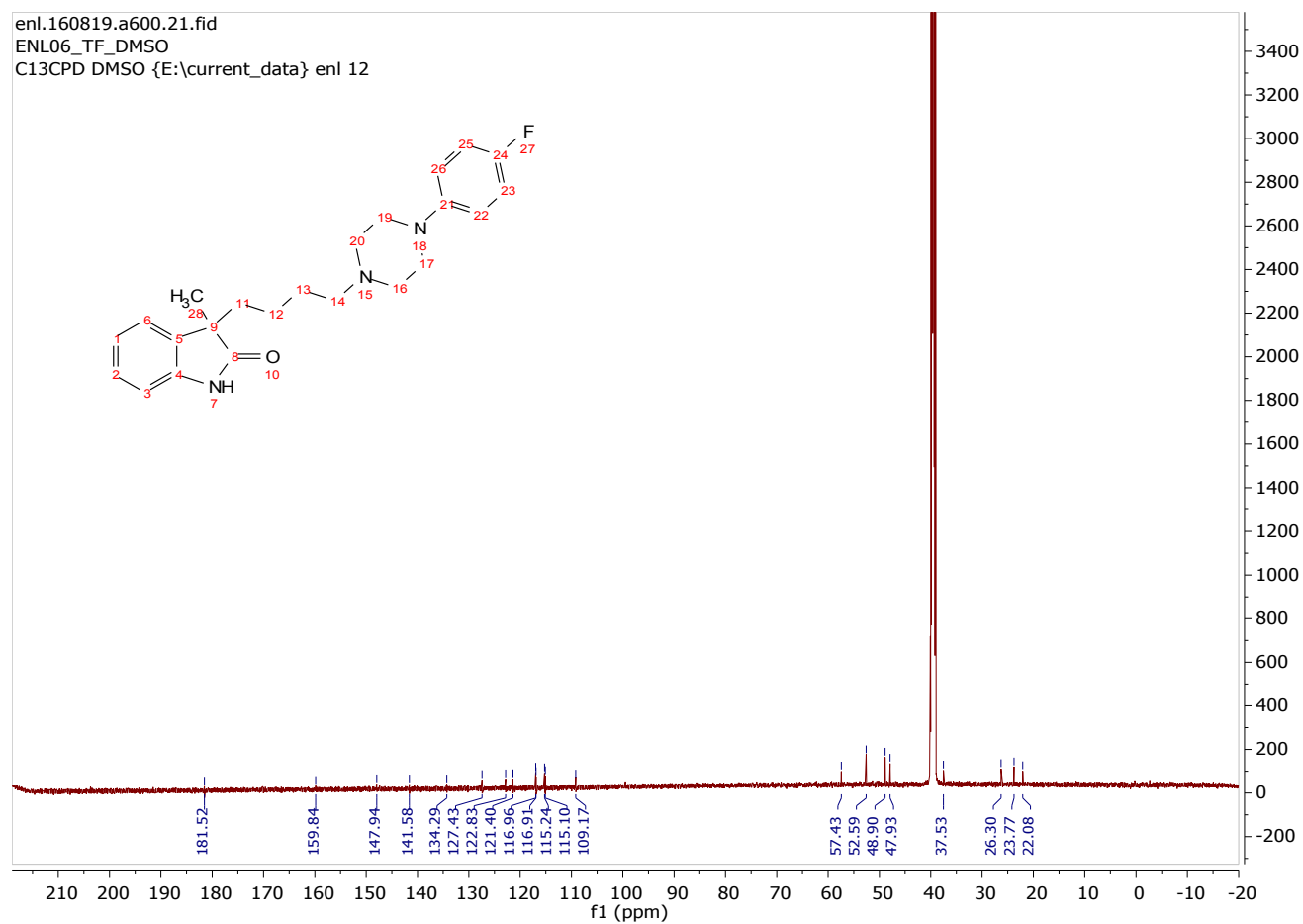


Appendix 37 – ^{13}C NMR of ENL06

enl.160819.a600.21.fid

ENL06_TF_DMSO

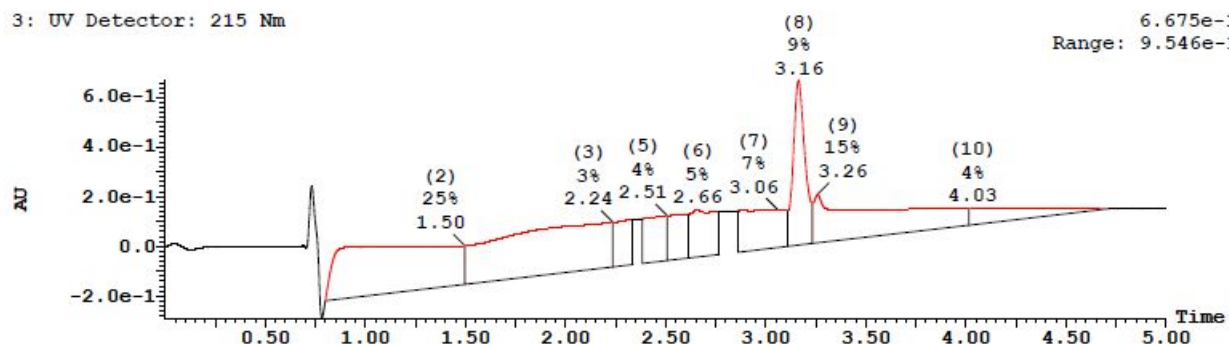
C13CPD DMSO {E:\current_data} enl 12



Appendix 38– UPLC-MS of ENL06

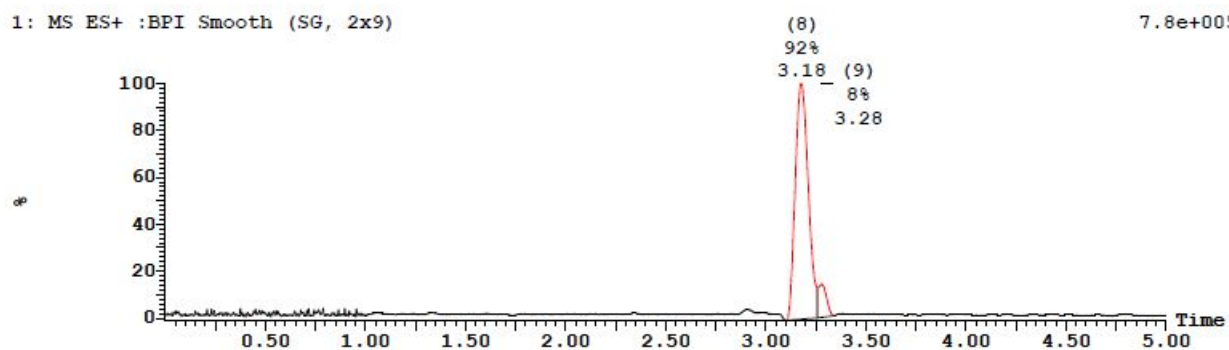
3: UV Detector: 215 Nm

6.675e-1
Range: 9.546e-1



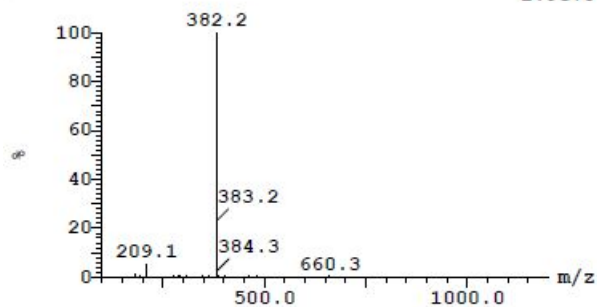
1: MS ES+ :BPI Smooth (SG, 2x9)

7.8e+005



Peak ID	Compound	Time	Mass Found	Mass Not Found
8		3.18		

1: MS ES+
2.9e+005

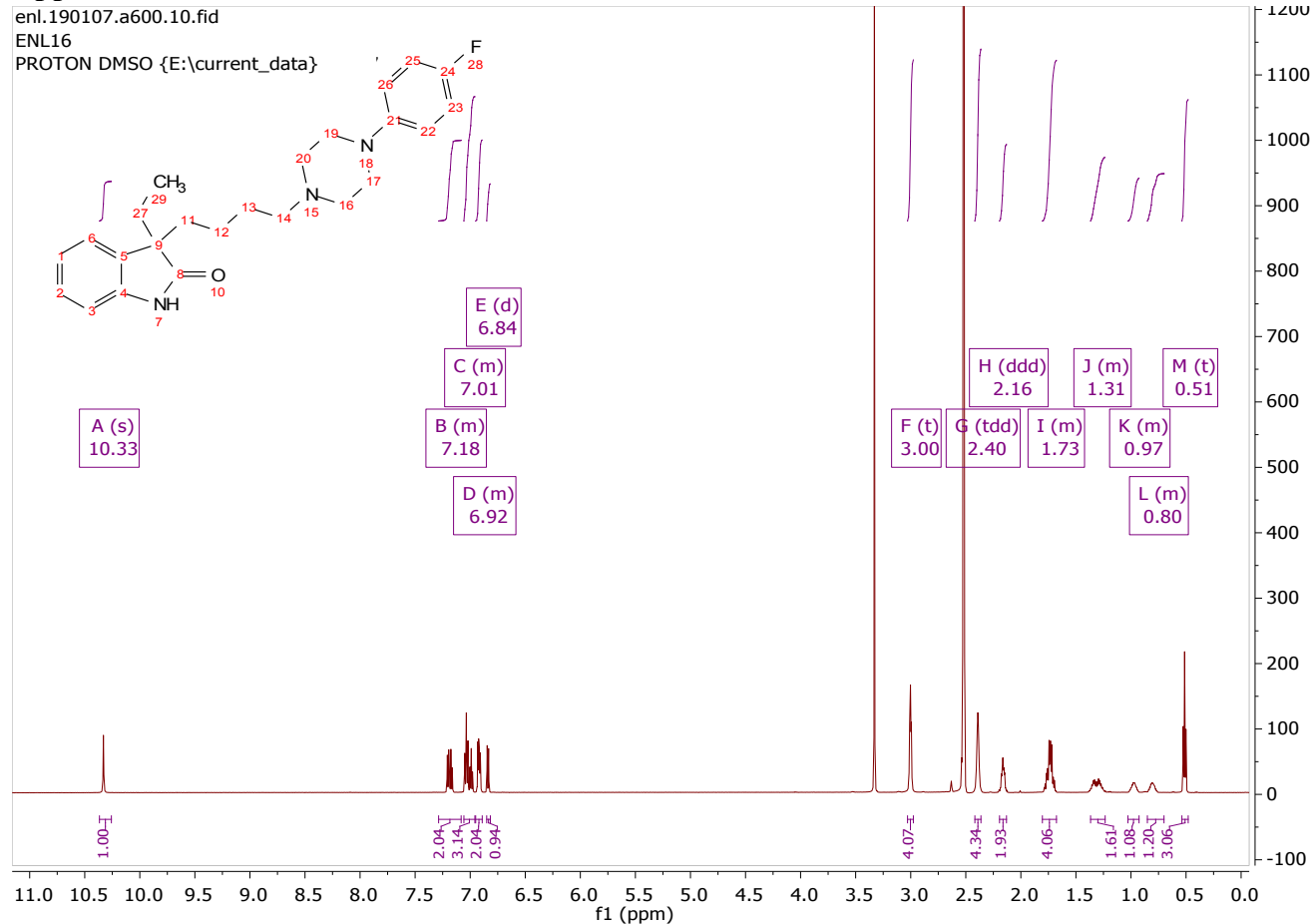


Appendix 39 – ¹H NMR of ENL05

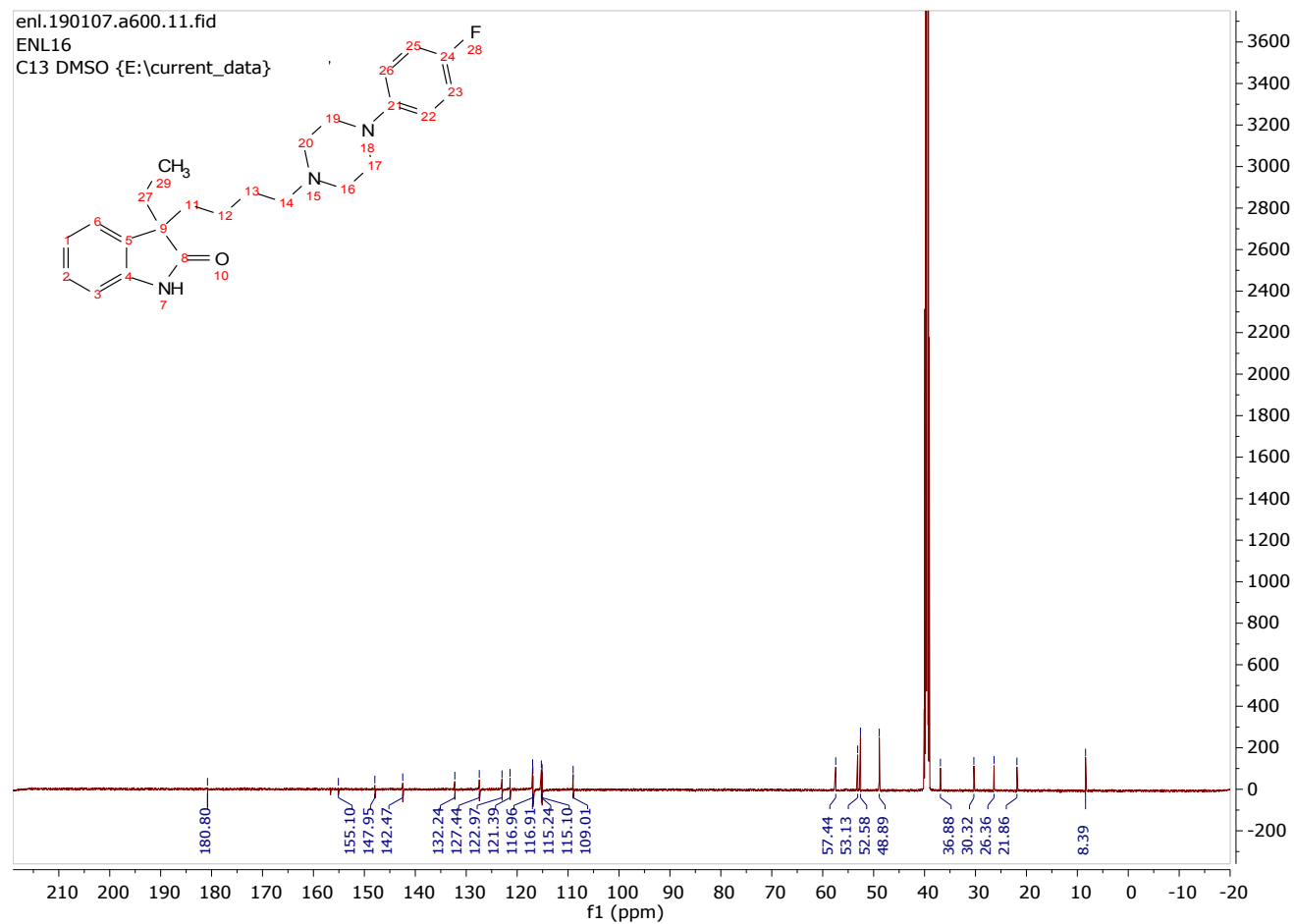
enl.190107.a600.10.fid

ENL16

PROTON DMSO {E:\current_data}

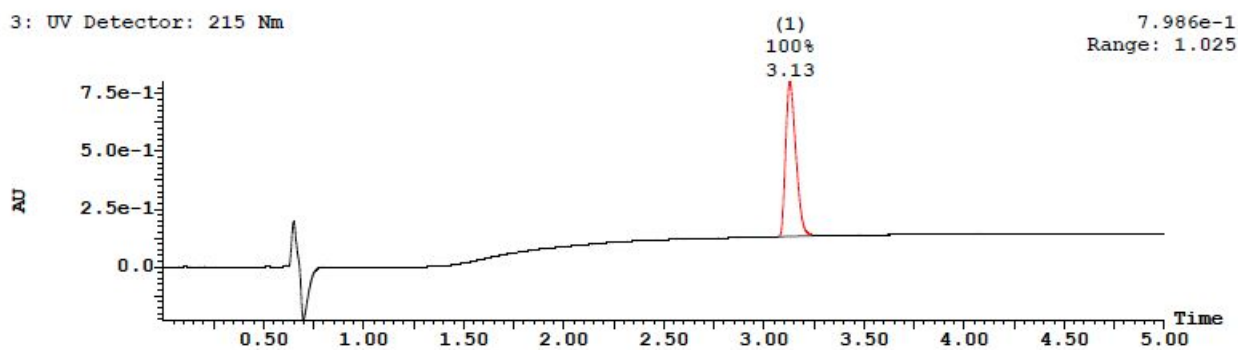


Appendix 40 – ^{13}C NMR of ENL05

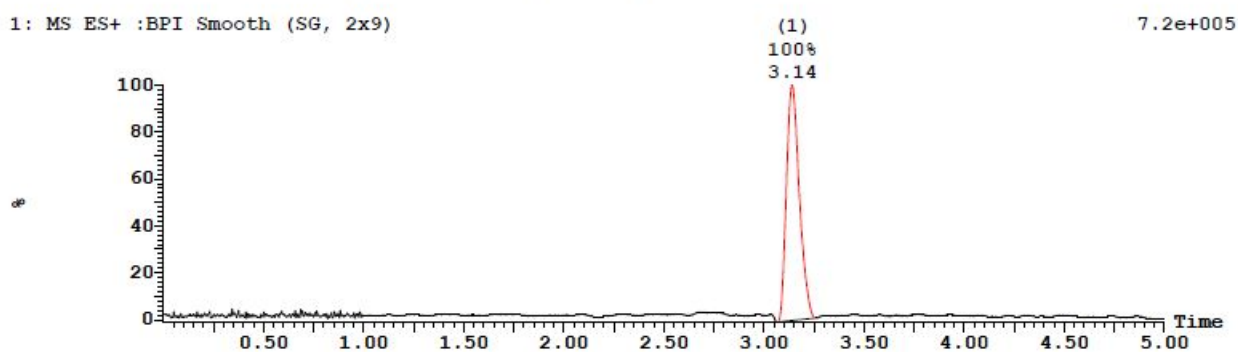


Appendix 41– UPLC-MS of ENL05

3: UV Detector: 215 Nm

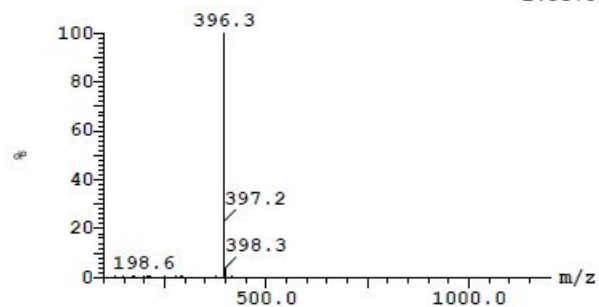


1: MS ES+ :BPI Smooth (SG, 2x9)



Peak ID	Compound	Time	Mass Found
1		3.14	Not Found

1:MS ES+
2.5e+005



Chemical Structure of Compound 10: COc1cc2c(c1)c(=O)[nH]2C(=O)OCC3=CC=CC=C3

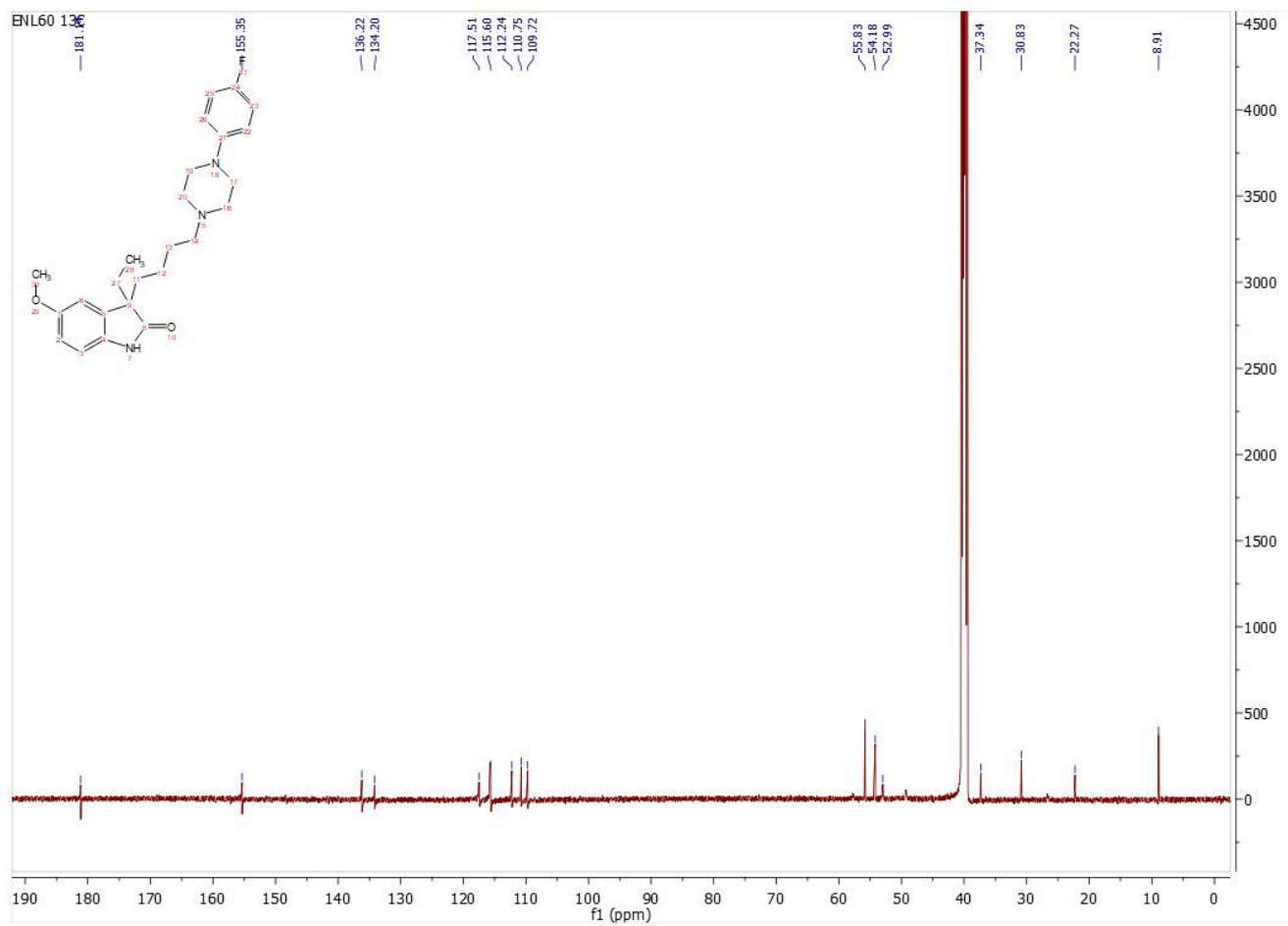
¹H NMR Spectrum (Top): The spectrum shows peaks from 0.4 to 10.6 ppm. The following table lists the peak assignments, chemical shifts, and integrations:

Assignment	Chemical Shift (ppm)	Integration
A (s)	10.13	1.00
B (t)	7.02	1.86
C (m)	6.90	1.86
D (t)	6.84	1.00
E (d)	6.72	1.63
F (t)	2.99	2.75
G (m)	2.38	3.80
H (s)	3.69	0.53
I (m)	2.16	4.18
J (m)	1.70	2.08
K (dtd)	1.29	3.70
L (td)	0.94	1.60
M (t)	0.49	0.82
N (t)	0.49	0.16
O (p)	2.61	3.06

¹³C NMR Spectrum (Bottom): The spectrum shows peaks from 1.6 to 10.6 ppm. The following table lists the peak assignments and chemical shifts:

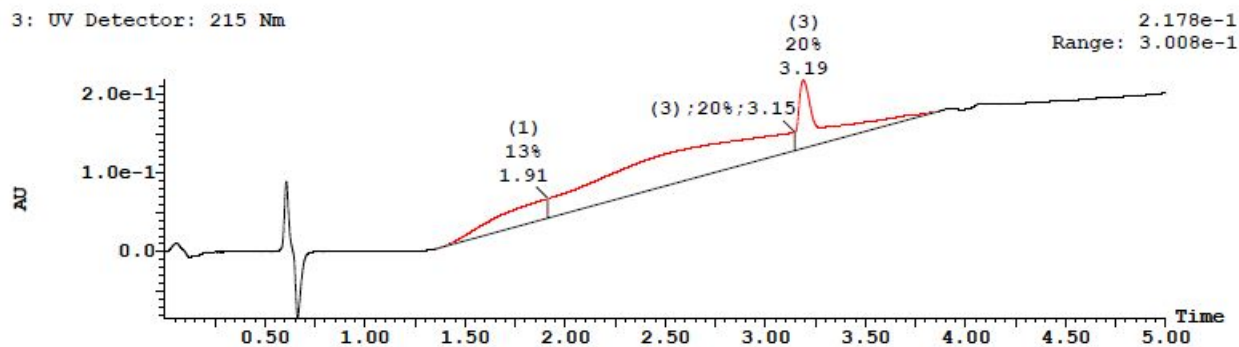
Assignment	Chemical Shift (ppm)
A (s)	10.13
B (t)	7.02
C (m)	6.90
D (t)	6.84
E (d)	6.72
F (t)	2.99
G (m)	2.38
H (s)	3.69
I (m)	2.16
J (m)	1.70
K (dtd)	1.29
L (td)	0.94
M (t)	0.49
N (t)	0.49
O (p)	2.61

Appendix 43 – ^{13}C NMR of ENL60

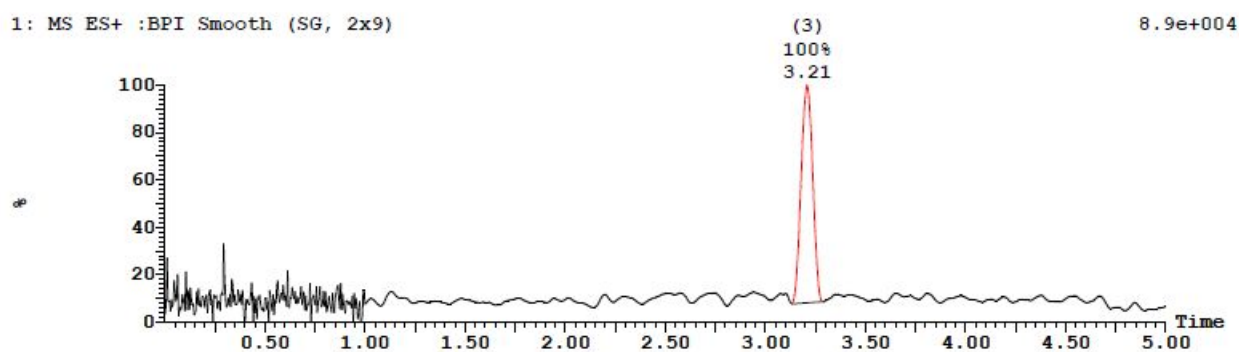


Appendix 44– UPLC-MS of ENL60

3: UV Detector: 215 Nm

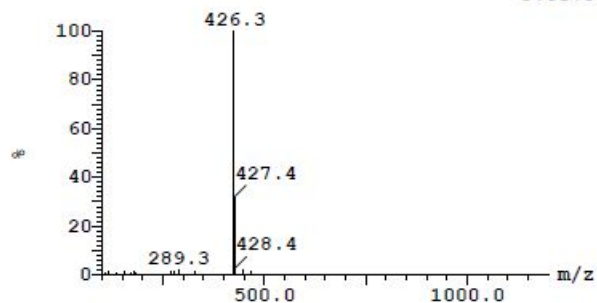


1: MS ES+ :BPI Smooth (SG, 2x9)



Peak ID	Compound	Time	Mass Found
3		3.21	Not Found

1:MS ES+
3.0e+004

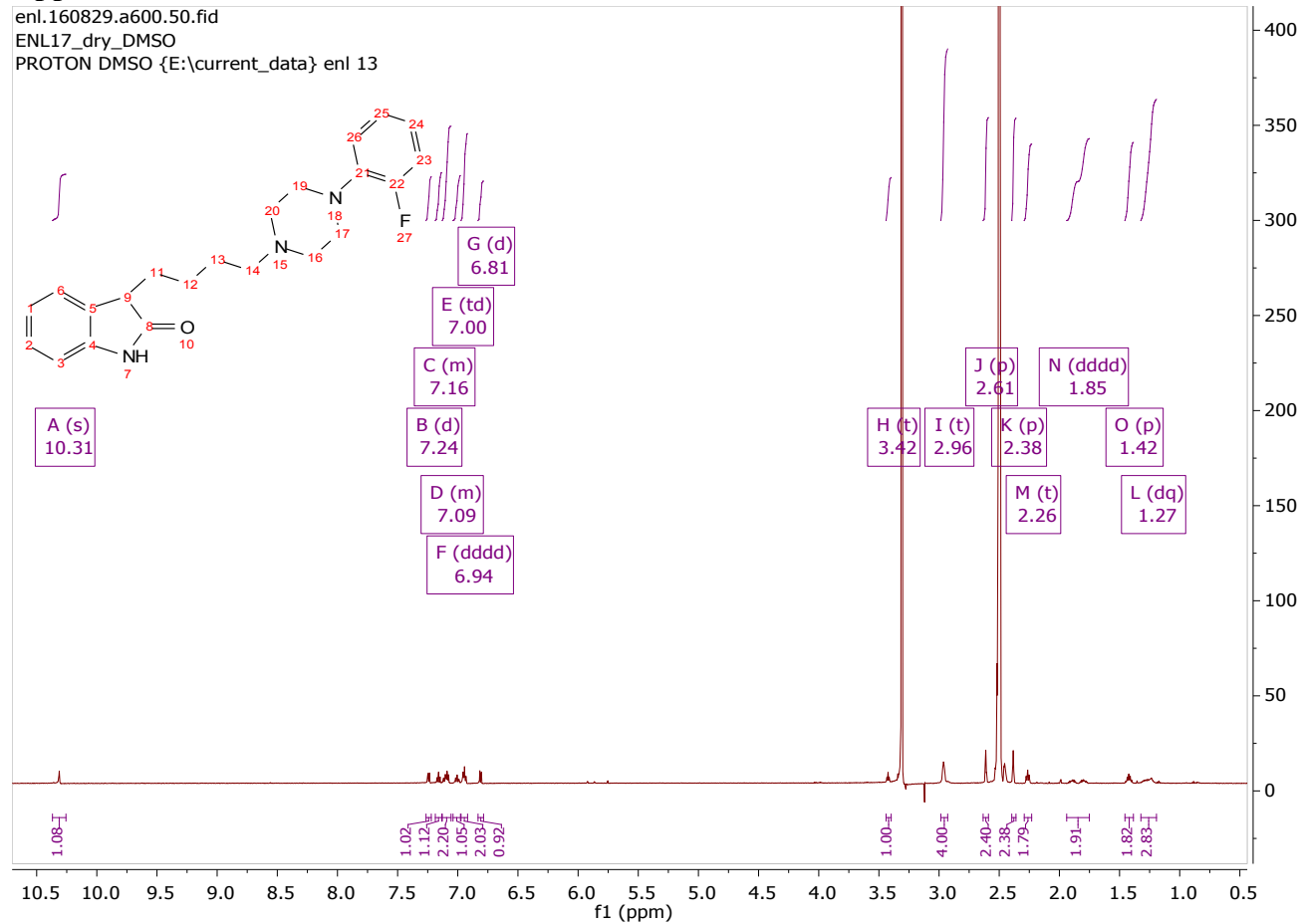


Appendix 45 – ¹H NMR of ENL17

enl.160829.a600.50.fid

ENL17_dry_DMSO

PROTON DMSO {E:\current_data} enl 13

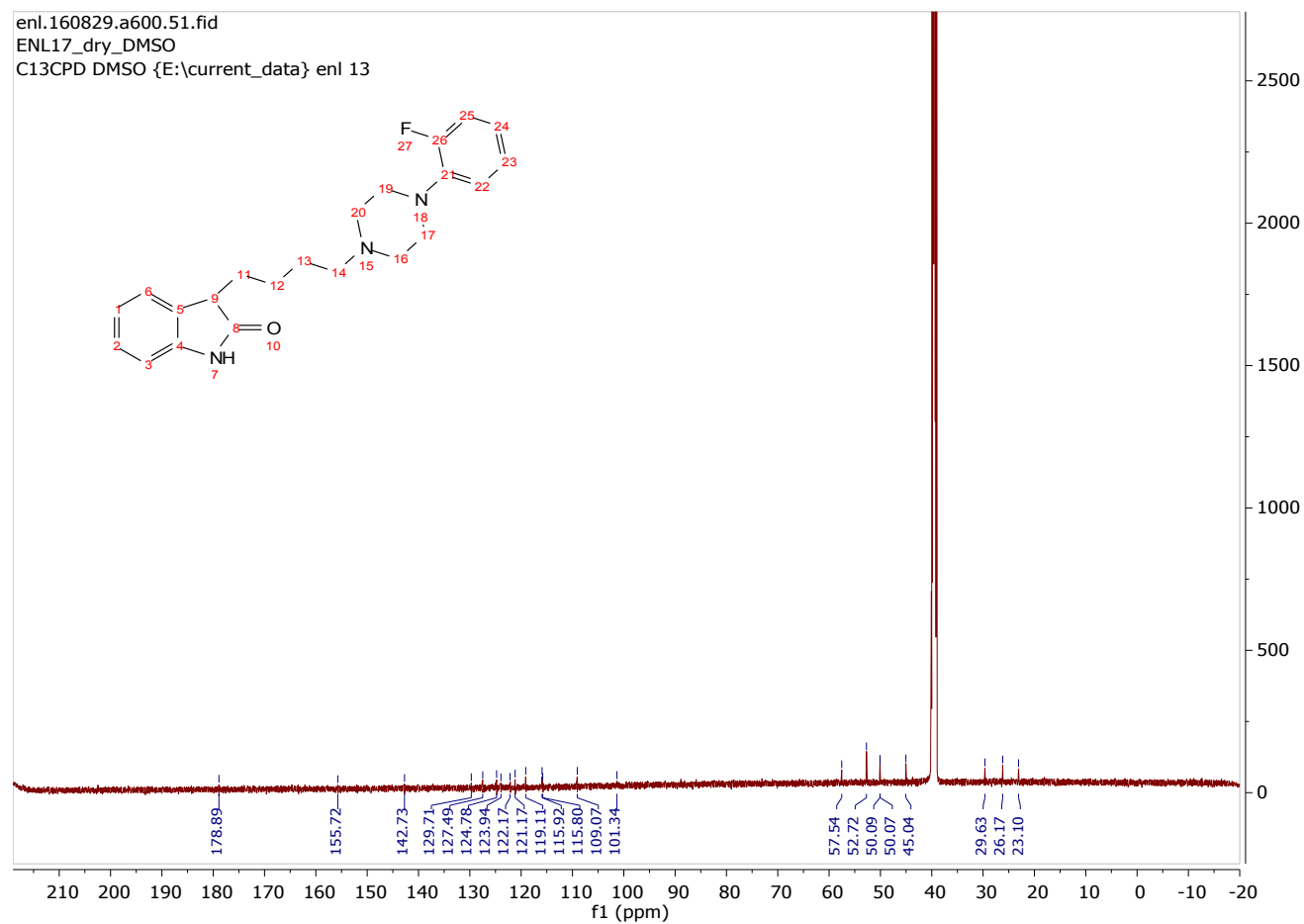


Appendix 46 – ^{13}C NMR of ENL17

enl.160829.a600.51.fid

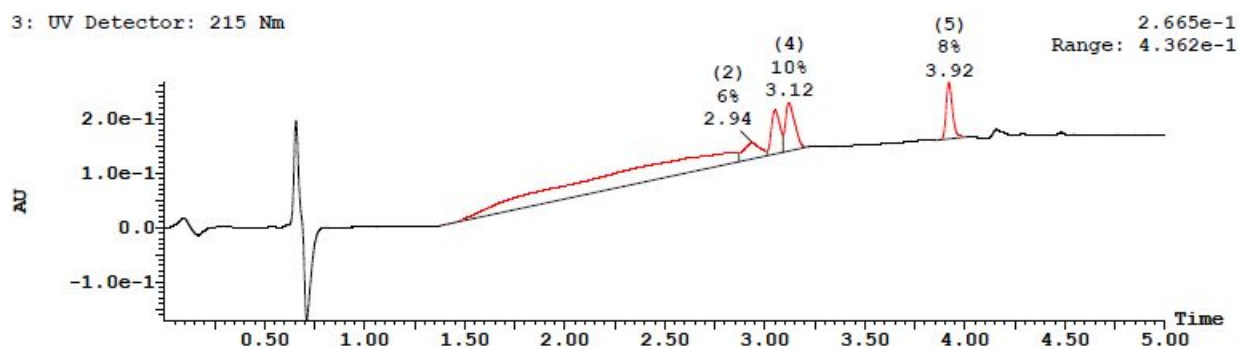
ENL17_dry_DMSO

C13CPD DMSO {E:\current_data} enl 13

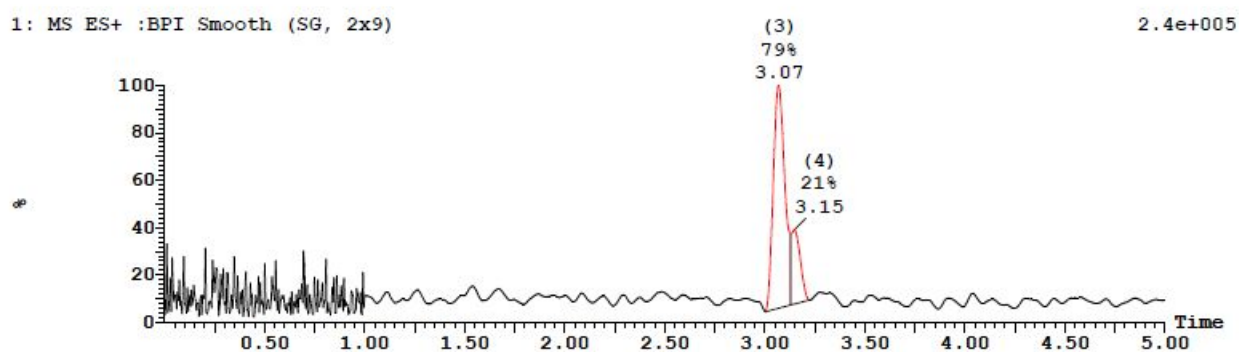


Appendix 47– UPLC-MS of ENL17

3: UV Detector: 215 Nm

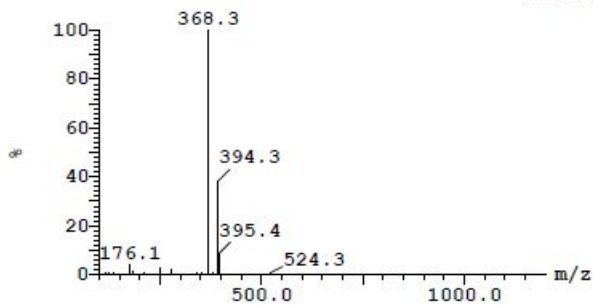


1: MS ES+ :BPI Smooth (SG, 2x9)



Peak ID	Compound	Time	Mass Found
3		3.07	Not Found

1:MS ES+
7.6e+004

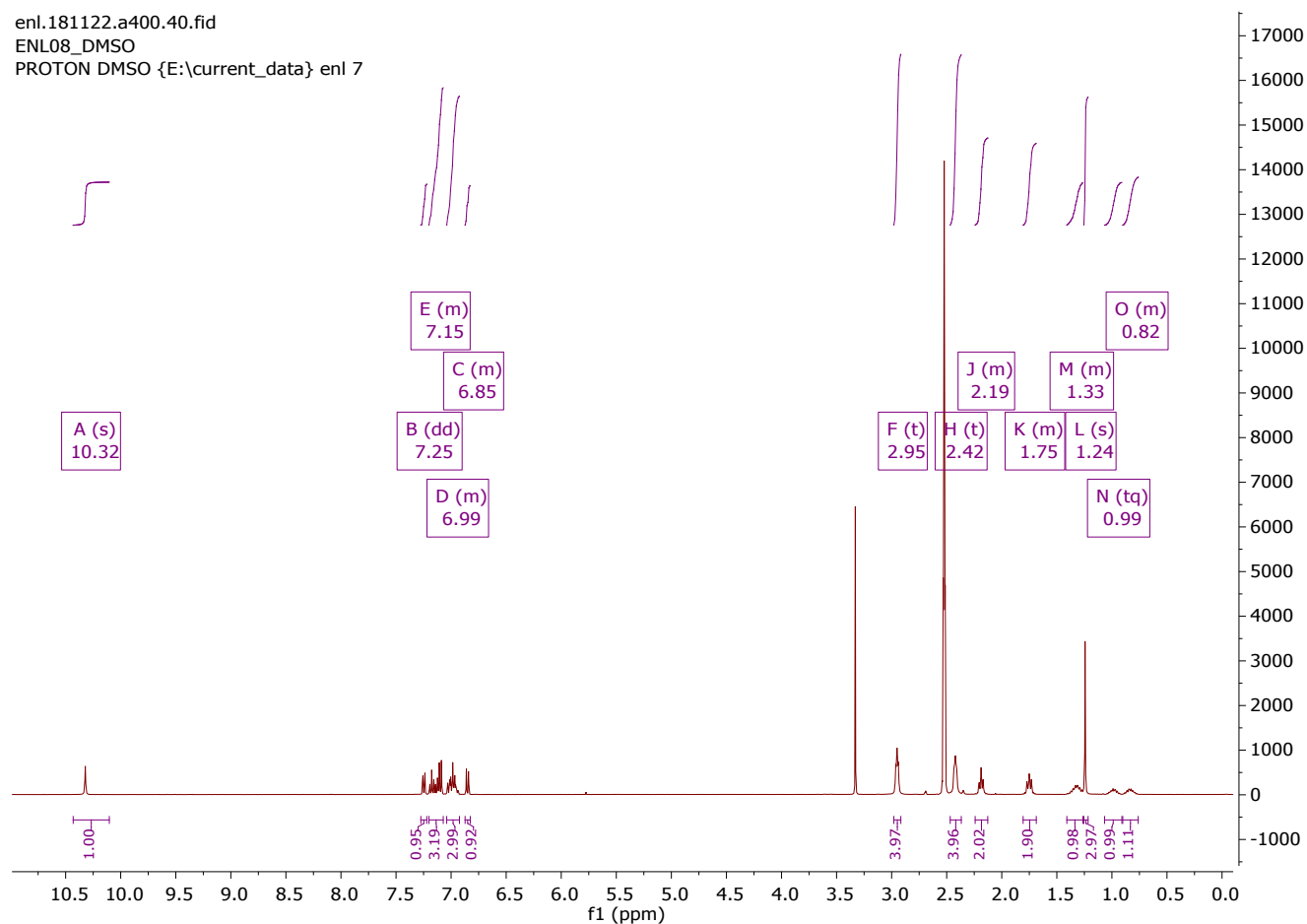


Appendix 48 – ^1H NMR of ENL08

enl.181122.a400.40.fid

ENL08_DMSO

PROTON DMSO {E:\current_data} enl 7

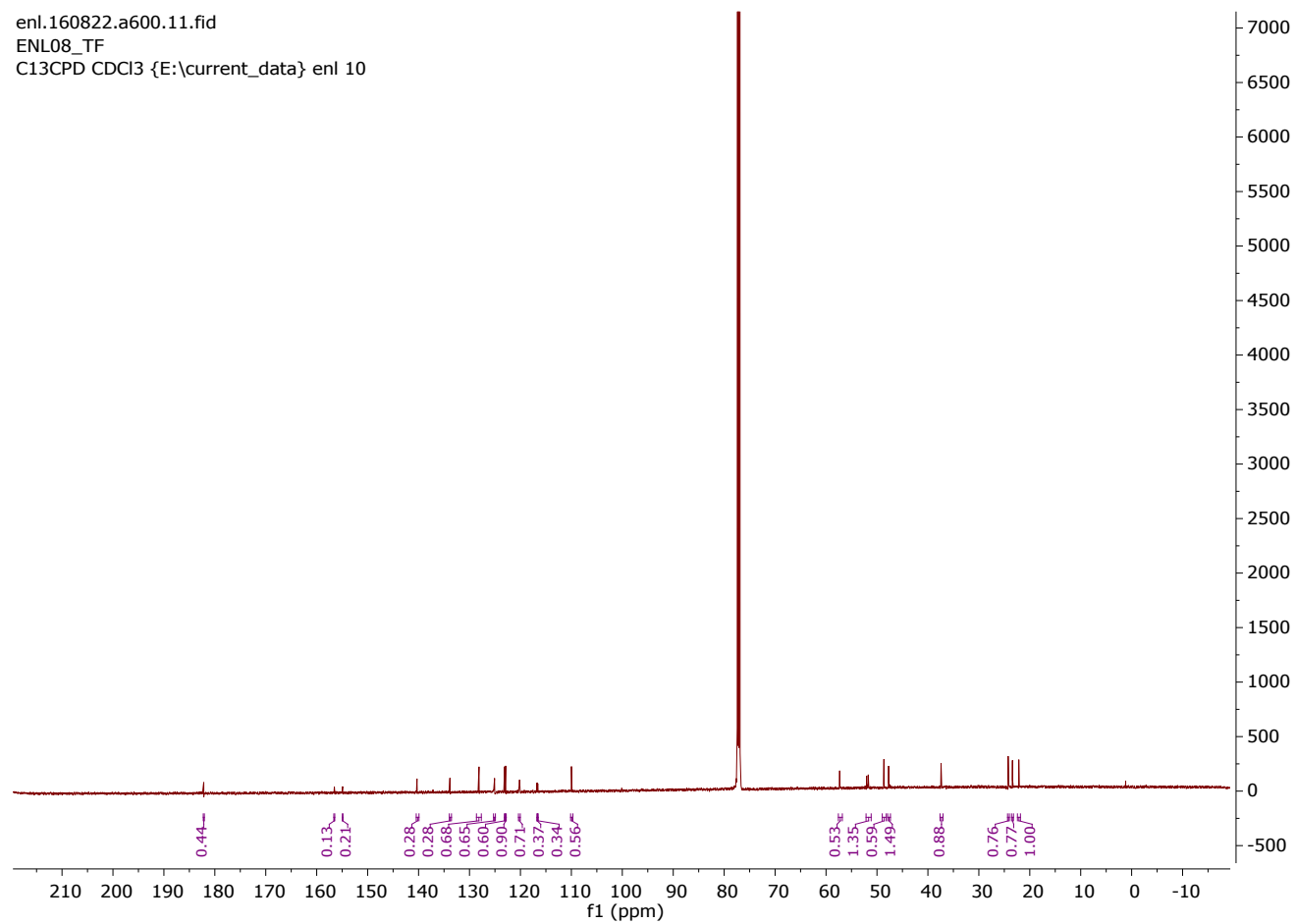


Appendix 49 – ^{13}C NMR of ENL08

enl.160822.a600.11.fid

ENL08_TF

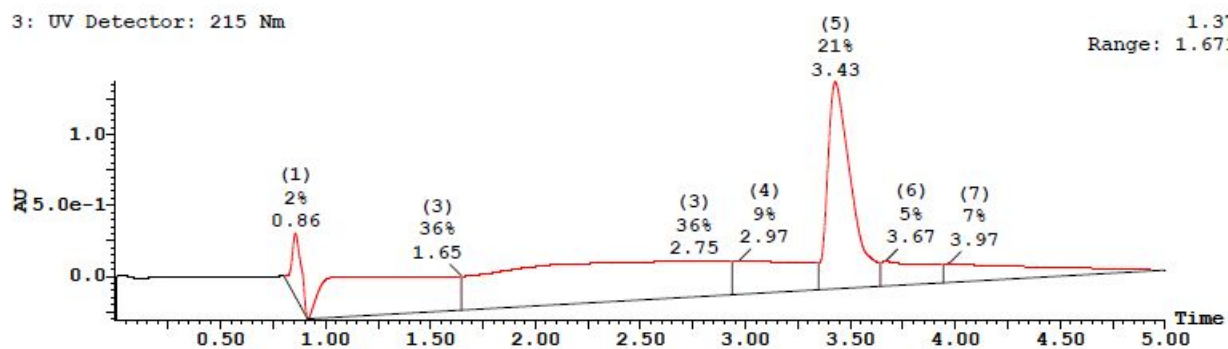
C13CPD CDCl₃ {E:\current_data} enl 10



Appendix 50– UPLC-MS of ENL08

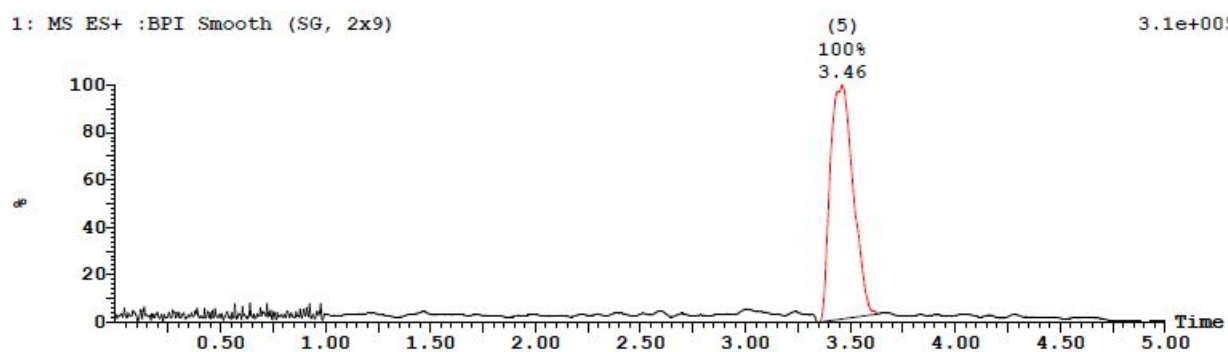
3: UV Detector: 215 Nm

1.37
Range: 1.671



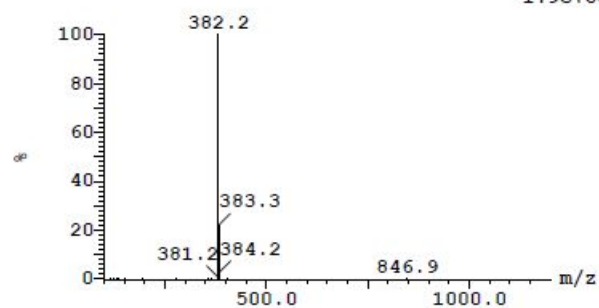
1: MS ES+ :BPI Smooth (SG, 2x9)

3.1e+005



Peak ID	Compound	Time	Mass Found
5		3.46	Not Found

1:MS ES+
1.9e+005

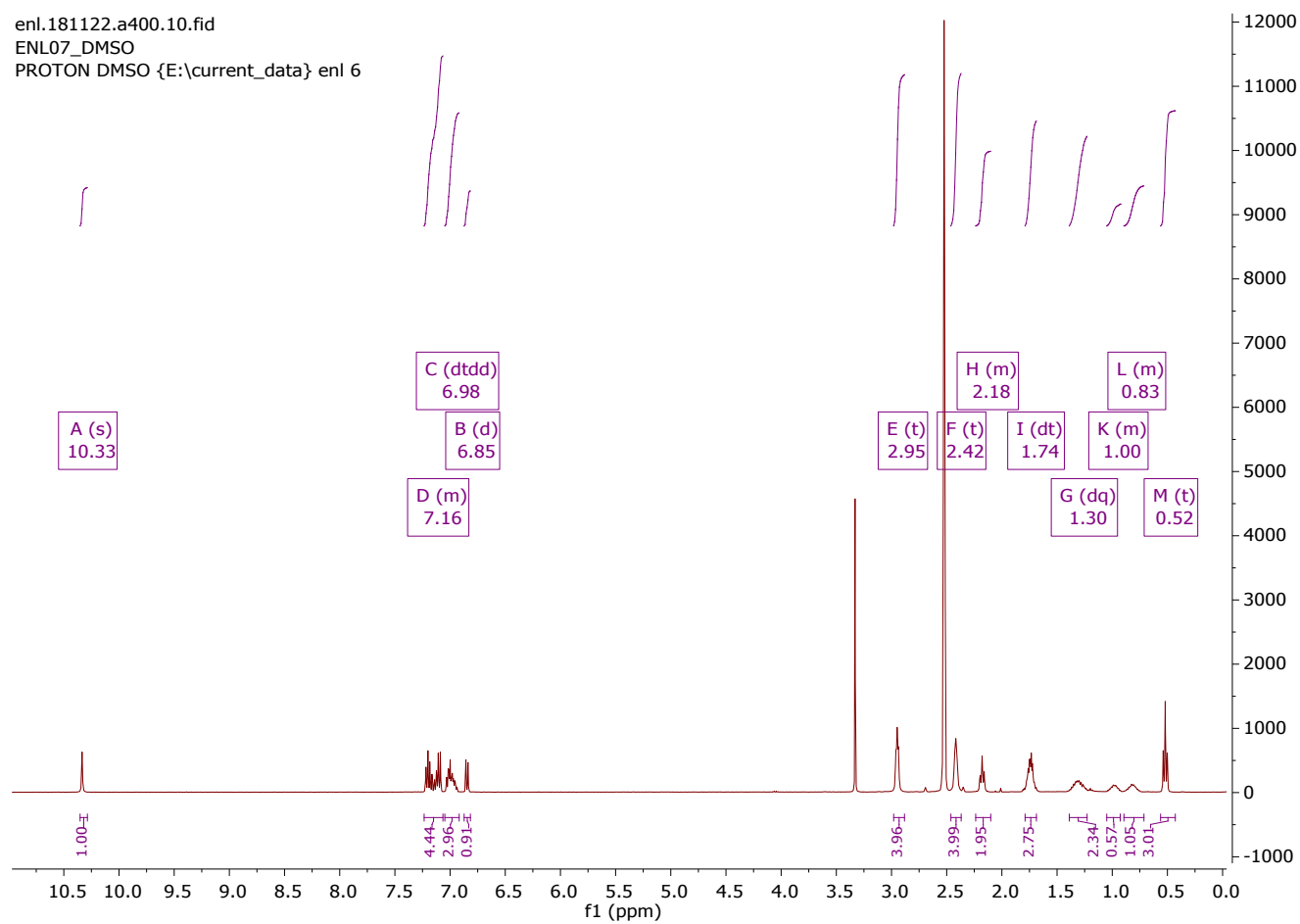


Appendix 51 – ^1H NMR of ENL07

enl.181122.a400.10.fid

ENL07_DMSO

PROTON DMSO {E:\current_data} enl 6

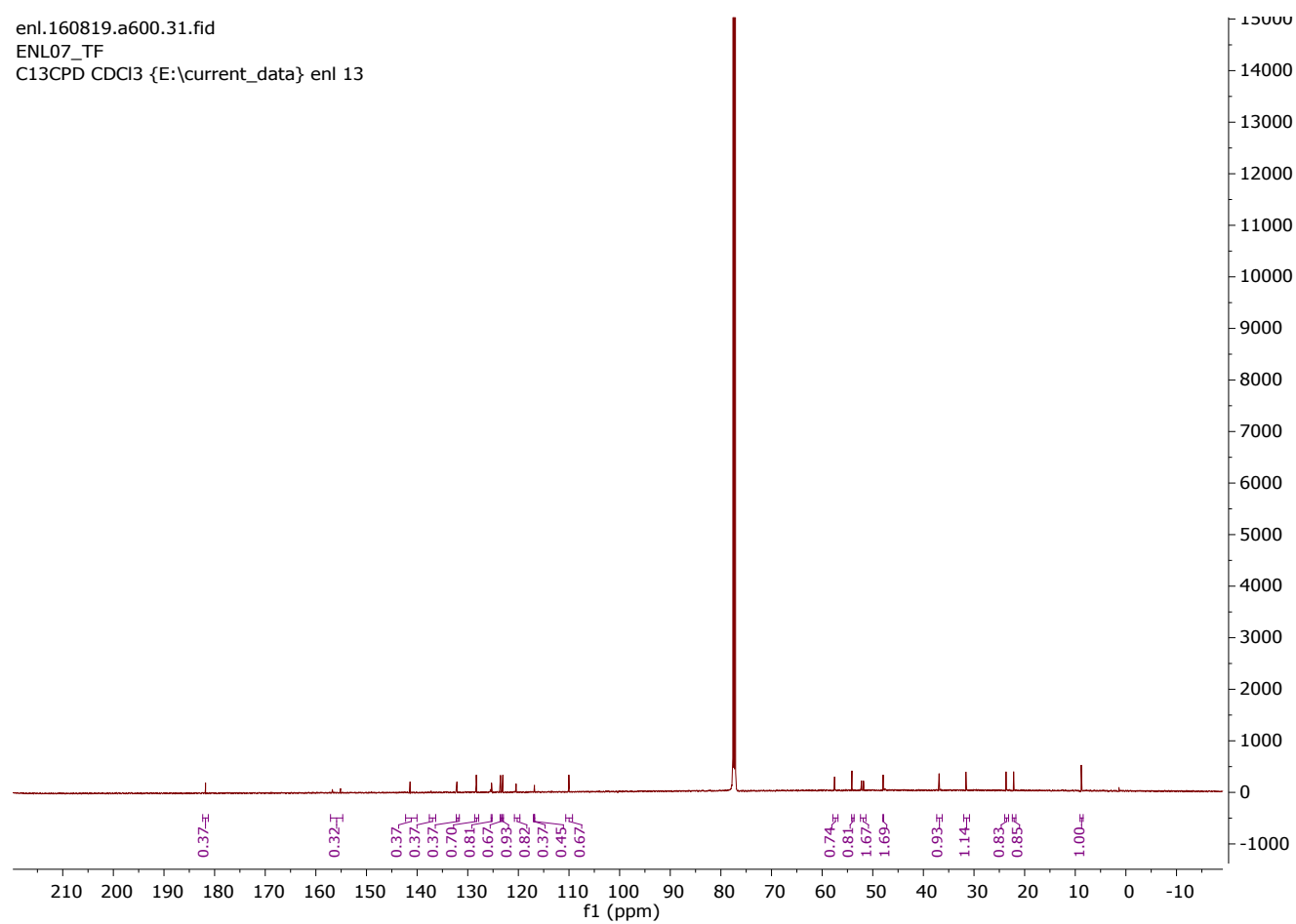


Appendix 52 – ^{13}C NMR of ENL07

enl.160819.a600.31.fid

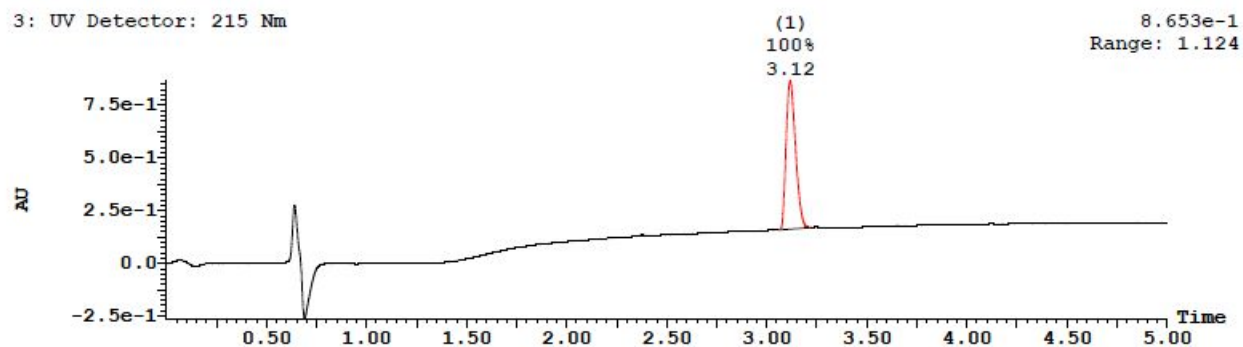
ENL07_TF

C13CPD CDCl₃ {E:\current_data\ enl 13

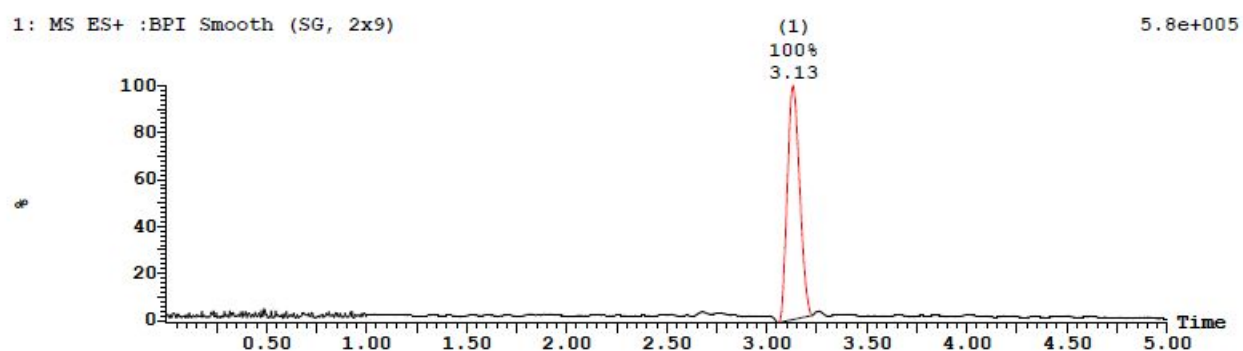


Appendix 53– UPLC-MS of ENL07

3: UV Detector: 215 Nm

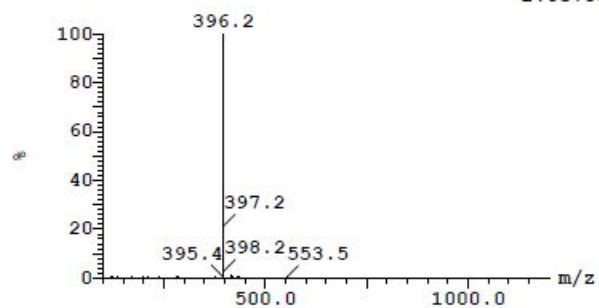


1: MS ES+ :BPI Smooth (SG, 2x9)



Peak ID	Compound	Time	Mass Found
1		3.13	Not Found

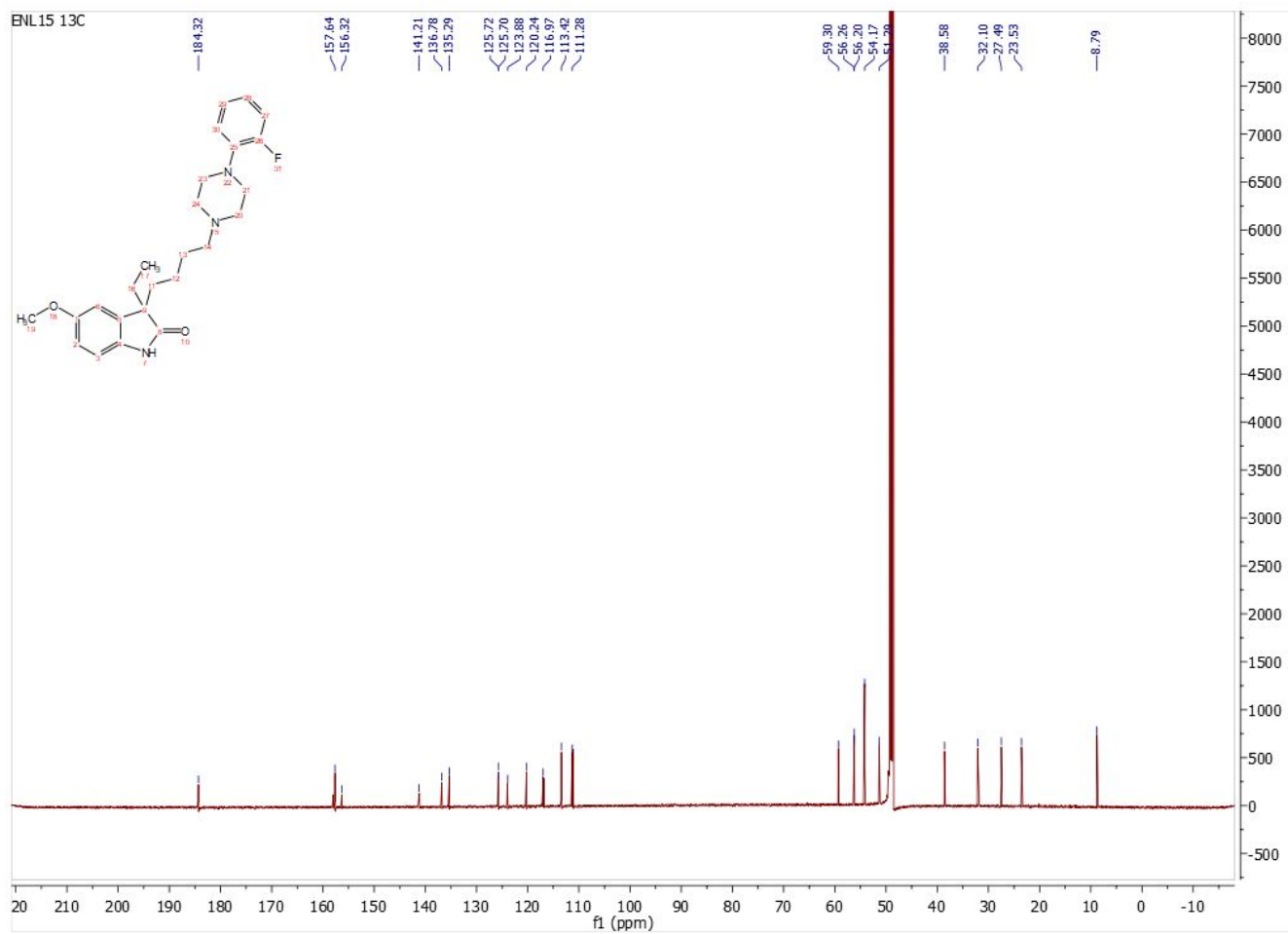
1:MS ES+
2.0e+005



Appendix 54 – ¹H NMR of ENL15

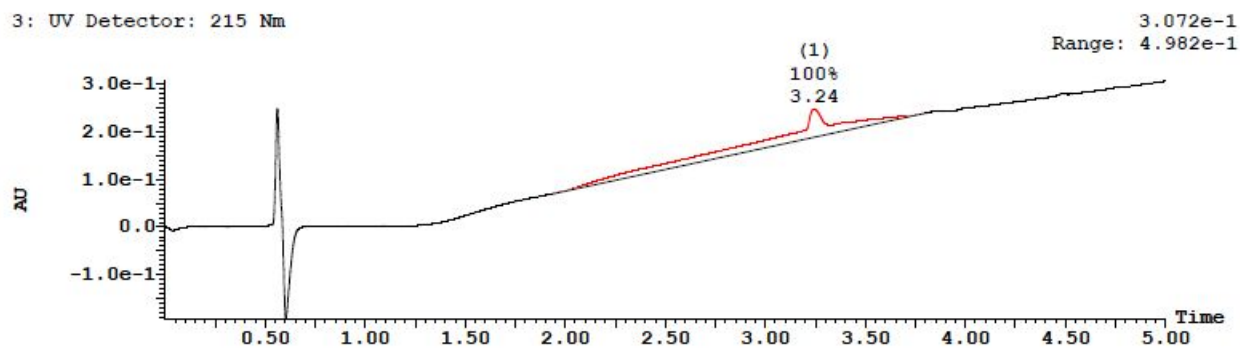


Appendix 55 – ^{13}C NMR of ENL15

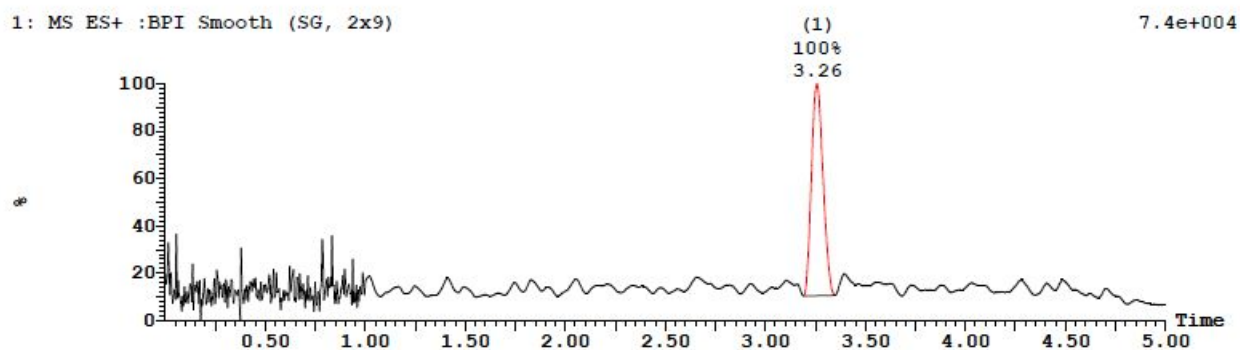


Appendix 56– UPLC-MS of ENL15

3: UV Detector: 215 Nm



1: MS ES+ :BPI Smooth (SG, 2x9)



Peak ID	Compound	Time	Mass Found
1		3.26	Not Found

1:MS ES+
2.5e+004

