Pyrrolidine-2,3-diones: Heterocyclic scaffolds that inhibit and eradicate *S. aureus* biofilms

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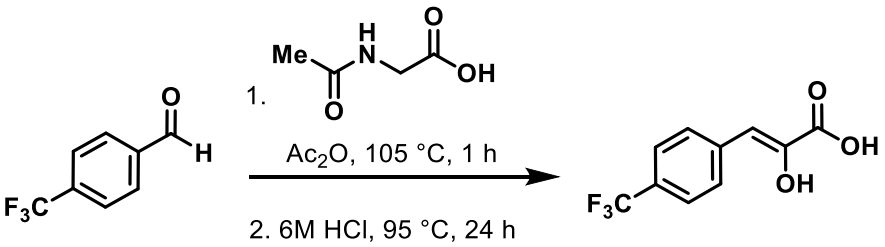
General information – Chemistry

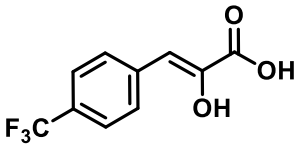
Synthesis: Tetrahydrofuran (THF) and dichloromethane (DCM) were purified using an alumina filtration system before use. Aldehydes were purchased from a commercial chemical company and used as received unless otherwise noted. Test reactions were monitored by TLC analysis (pre-coated silica gel 60 F_{254} plates, 250 mm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a $KMnO_4$ solution (1.5 g of $KMnO_4$, 10 g of K_2CO_3, and 1.25 mL of a 10% NaOH solution in 200 mL of water). Test reactions were also monitored by LC-MS (2.6 mm C18 50 x 2.10 mm column). Yields that are reported are from reactions that were not monitored by TLC or LC-MS. A Biotage® flash chromatography system was used to purify all of the compounds. Melting points were determined using a DigiMelt apparatus. Infrared spectra were determined on a Bruker Alpha spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a 500, or 600 MHz instrument in CDCl3 or DMSO-d_6 as indicated. Chemical shifts were reported as observed in parts per million with the residual solvent peak used as an internal standard ($CDCl_3$ = 7.26 ppm for ^{1}H and 77.16 ppm for ^{13}C; DMSO-d_6 = 2.50 ppm for ^{1}H and 39.52 ppm for ^{13}C). ^{1}H NMR spectra were run at 500 or 600 MHz and are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, bs = broad singlet, dt = doublet of triplet, tt = triplet of triplet), number of protons, and coupling constant(s). ^{13}C NMR spectra were run at 125 or 150 MHz using a proton-decoupled pulse sequence with a d1 of 1 second unless otherwise noted, and are tabulated by observed peak. High resolution mass spectra were obtained on an ion trap mass spectrometer using heated electrospray ionization (HESI).

Preparation of 2-oxa-3-arylpropanoic acid:



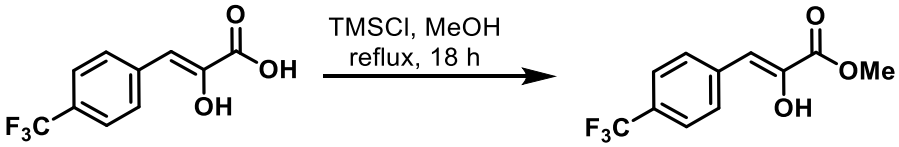
Scheme S1. Synthetic of (Z)-2-hydroxy-3-(4-(trifluoromethyl)phenyl)acrylic acid.

4-(Trifluoromethyl)benzaldehyde (5.00 g, 3.92 mL, 28.7 mmol), sodium acetate (2.59 g, 31.6 mmol), *N*-acetylglycine (3.36 g, 28.7 mmol) and acetic anhydride (8.14 mL, 86.2 mmol) were added to a 250 mL round bottom (RB) flask. The contents of the flask were stirred vigorously and refluxed at 110 °C for 1 h. The reaction mixture was pulled out of the oil bath and added 50 mL of 6 M HCl and the suspension was stirred for 24 h at 95 °C. The resulting reaction mixture was filtered and washed with small amount of cold deionized water. The resulting dark yellow solid was transferred to an Erlenmeyer flask and triturated with dichloromethane (50 mL), filtered and washed with additional dichloromethane to obtain 4.49 g of a bright yellow solid.[1] The dried solid was used without further purification.



(*Z*)-2-Hydroxy-3-(4-(trifluoromethyl)phenyl)acrylic acid: Synthesis of this starting material was performed using 3.0 g (16.9 mmol) of the 4- (trifluoromethyl) benzaldehyde and acetic anhydride (10 equiv). The product, 2.73g (75 % yield), was isolated as a bright yellow powder.

Preparation of methyl (*Z*)-2-hydroxy-3-(4-(trifluoromethyl)phenyl)acrylate:

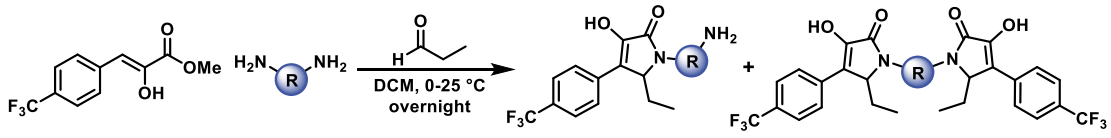


Scheme S2. Synthesis of (Z)-2-hydroxy-3-(4-(trifluoromethyl)phenyl)acrylate.

In a round bottom flask, (*Z*)-2-Hydroxy-3-(4-(trifluoromethyl)phenyl)acrylic acid (4.49 g, 19.34 mmol) was dissolved in dry methanol (65 mL) and chlorotrimethylsilane (8.87 mL, 7.59 g, 58.02 mmol) was added dropwise. The mixture was stirred and heated at 65 ºC overnight and reaction was followed by TLC. Upon reaction completion, the solvent was evaporated using a rotary evaporator and the mixture was dry loaded on silica, and the mixture was purified using flash chromatography (2-8% EtOAc:hexane). Product was obtained as a white semisolid in 97% yield (4.65 g). Analytical data was consistent with data previously reported.[2]

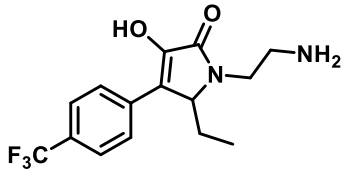
General procedure: Synthesis of 3-hydroxy-1,5-dihydro-2*H*-pyrrol-2-ones

General procedure: Synthesis of 3-hydroxy-1,5-dihydro-2*H*-pyrrol-2-ones diamine monomers and corresponding dimers



Scheme S4. Synthesis of 3-hydroxy-1,5-dihydro-2*H*-pyrrol-2-ones diamine monomers and dimers

To a solution of methyl α-oxoester (0.6 mmol) in DCM (0.5 M) at room temperature was added aldehyde (1.2 mmol), followed by diamine (1.2 mmol). The reaction was stirred for 8 to 24 hours, being monitored by HPLC-MS. Upon consumption of phenyl pyruvic methyl ester, solvent was removed in vacuo or the solids in suspension were separated via vaccum filtration. The crude products were then purified by C-18 reverse phase flash column chromatography using mobile phase comprised of acetonitrile and water with 0.1% formic acid, or methanol, water and 0.1% formic acid as well.



16

1-(2-Aminoethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (16): According to general procedure, **16** was synthesized in a yield of 127 mg (39%) as a white solid.

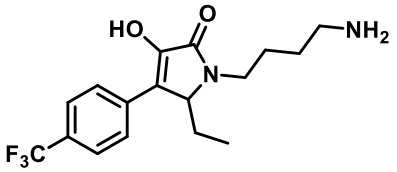
^{1}H NMR (500 MHz, Methanol-d_4) δ 7.83 (d, J = 8.1 Hz, 2H), 7.59 (d, J = 8.1 Hz, 2H), 4.69 (d, J = 3.4 Hz, 1H), 3.96 – 3.81 (m, 1H), 3.15 – 2.91 (m, 3H), 2.03 – 1.91 (m, 1H), 1.90 – 1.79 (m, 1H), 0.42 (t, J = 7.3 Hz, 3H).

^{13}C NMR (126 MHz, Methanol-d_4) δ 170.51, 149.53, 137.52, 126.25, 124.64, 124.60, 116.54, 56.86, 40.44, 39.20, 20.81, 3.99.

IR ν_{max} (cm^{-1}): 3181, 3172, 2969, 2881, 1668, 1614, 1573, 1442, 1392, 1325, 1292, 1269, 1157, 1109, 1066.

mp: decomposes at 173.0-175 °C.

HRMS (HESI) m/z calculated for $C_{15}H_{17}F_3N_2O_2$ $[M+H]^+$ 315.13149, found 315.13126.



17

1-(4-Aminobutyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (17): According to general procedure, **17** was synthesized in a yield of 283 mg (81%) as a white solid.

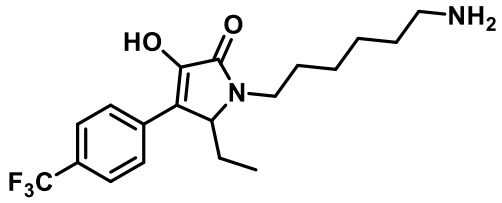
^{1}H NMR (600 MHz, Methanol-d_4) δ 8.40 (s, 1H), 7.79 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 7.5 Hz, 2H), 4.71 (s, 1H), 3.77 (m, 1H), 3.15 (m, 1H), 2.93 (m, 2H), 1.97 (m, 1H), 1.79 (m, 1H), 1.67 (m, 4H), 0.38 (t, J = 6.8 Hz, 3H).

^{13}C NMR (151 MHz, Methanol-d_4) δ 167.44, 144.86, 135.83, 127.31, 124.96, 124.94, 123.40, 119.21, 56.59, 38.86, 38.80, 24.90, 24.45, 20.84, 3.95.

IR ν_{max} (cm^{-1}): 3153, 3118, 2969, 2878, 1737, 1661, 1614, 1456, 1392, 1319, 1167, 1116, 1105, 1066, 850.

mp: 207-209 °C.

HRMS (HESI) m/z calculated for $C_{17}H_{21}F_3N_2O_2$ $[M+H]^+$ 343.16279, found 343.16263.



18

1-(6-Aminohexyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (18): According to general procedure, **18** was synthesized in a yield of 267 mg (66%) as a white solid:

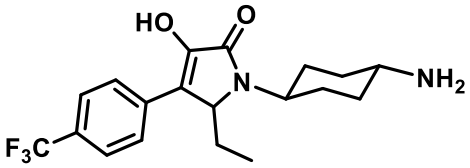
^{1}H NMR (600 MHz, Methanol-d_4) δ 8.52 (s, 1H), 7.87 (d, J = 8.2 Hz, 2H), 7.70 (d, J = 8.2 Hz, 2H), 4.76 (s, 1H), 3.83 (dt, J = 14.0, 8.0 Hz, 1H), 3.21 – 3.13 (m, 1H), 2.94 (t, J = 7.5 Hz, 2H), 2.08 – 1.99 (m, 1H), 1.91 – 1.83 (m, 1H), 1.77 – 1.63 (m, 4H), 1.55 – 1.37 (m, 4H), 0.45 (t, J = 7.3 Hz, 3H).

^{13}C NMR (151 MHz, Methanol-d_4) δ 168.31, 167.29, 145.03, 135.92, 127.29, 125.22, 124.96, 124.93, 123.42, 118.95, 56.60, 39.44, 39.12, 27.71, 27.07, 25.89, 25.50, 20.87, 3.98.

IR ν_{max} (cm^{-1}): 3064, 2969, 1668, 1614, 1575, 1456, 1392, 1319, 1107, 1064, 1010, 844.

mp: decomposes at 250 °C.

HRMS (HESI) m/z calculated for $C_{19}H_{25}F_3N_2O_2$ $[M+H]^+$ 371.19409, found 371.19445.



19

1-((1r,4r)-4-Aminocyclohexyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (19): According to general procedure, **19** was synthesized in a yield of 180 mg (50%) as a white solid.

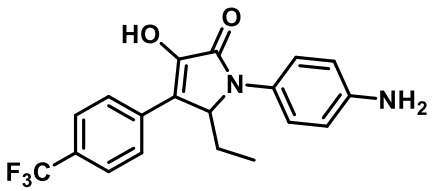
^{1}H NMR (600 MHz, Methanol-d_4) δ 7.76 (d, J = 8.0 Hz, 2H), 7.62 (d, J = 8.0 Hz, 2H), 4.70 (s, 1H), 3.60 (t, J = 12.0, 3.8 Hz, 1H), 3.12 (t, J = 11.8 Hz, 1H), 2.27 (q, 1H), 2.15 – 1.96 (m, 4H), 1.94 – 1.86 (m, 2H), 1.86 – 1.75 (m, 1H), 1.50 (q, J = 12.2 Hz, 2H), 0.42 (t, J = 7.2 Hz, 3H).

^{13}C NMR (126 MHz, Methanol-d_4) δ 168.57, 167.31, 144.93, 135.76, 127.40, 124.93, 124.90, 119.15, 57.65, 52.58, 49.02, 29.88, 29.73, 28.36, 27.50, 27.23, 21.80, 4.10.

IR ν_{max} (cm^{-1}): 3140, 3086, 2969, 2946, 2876, 1659, 1567, 1539, 1456, 1387, 1321, 1290, 1273, 1230, 1163, 1118, 1105, 1059, 1016.

mp: 166 °C.

HRMS (HESI) m/z calculated for $C_{19}H_{23}F_3N_2O_2$ $[M+H]^+$ 369.17844, found 369.17804.



20

1-(4-Aminophenyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (20): According to general procedure, **20** was synthesized in a yield of 63 mg (21%) as a dark yellow solid.

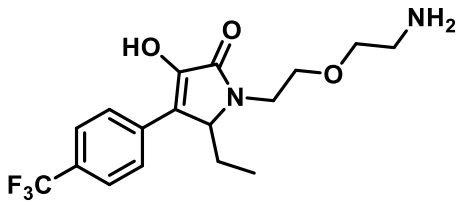
^{1}H NMR (600 MHz, DMSO-d_6) δ 10.40 (s, 1H), 7.91 (d, J = 8.2 Hz, 2H), 7.78 (d, J = 8.3 Hz, 2H), 7.26 – 7.21 (m, 2H), 6.66 – 6.61 (m, 2H), 5.42 (t, J = 3.5 Hz, 1H), 5.38 – 4.80 (m, 2H), 3.33 (s, 1H), 1.76 – 1.56 (m, 2H), 0.29 (t, J = 7.3 Hz, 3H).

^{13}C NMR (176 MHz, DMSO-d_6) δ 168.45, 164.77, 147.07, 145.99, 136.72, 131.04, 127.77, 125.79, 125.57, 125.32, 124.68, 117.76, 114.32, 102.63, 57.33, 52.39, 50.13, 21.82, 5.37.

IR ν_{max} (cm^{-1}): 3453, 3373, 3103, 2967, 2876, 1750, 1653, 1614, 1517, 1446, 1383, 1319, 1282, 1217, 1193, 1157, 1107, 1066, 850, 826.

mp: 210-216 °C.

HRMS (HESI) m/z calculated for $C_{19}H_{17}F_3N_2O_2$ $[M+H]^+$ 363.13149, found 363.13091.



21

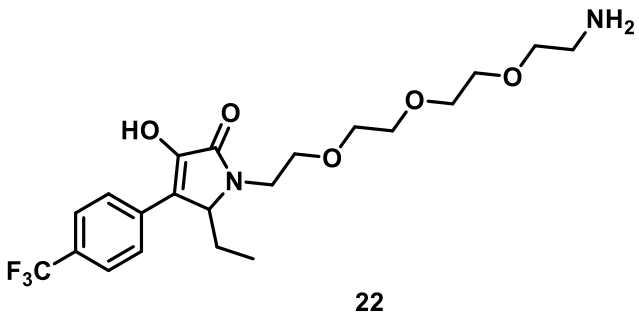
1-(2-(2-Aminoethoxy)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (21): According to general procedure, **21** was synthesized in a yield of 202 mg (69%) as a yellow film.

^{1}H NMR (500 MHz, Methanol-d_4) δ 7.85 (d, J = 8.1 Hz, 2H), 7.69 (d, J = 8.1 Hz, 2H), 4.84 (t, J = 3.5 Hz, 1H), 4.09 – 4.00 (m, 1H), 3.83 – 3.59 (m, 5H), 3.40 – 3.33 (m, 1H), 3.16 – 3.09 (m, 2H), 2.14 – 1.98 (m, 1H), 1.93 – 1.78 (m, 1H), 0.44 (t, J = 7.3 Hz, 3H).

^{13}C NMR (126 MHz, Methanol-d_4) δ 165.37, 158.84, 142.34, 133.38, 124.95, 122.57, 120.81, 117.11, 115.32, 112.99, 66.17, 63.85, 54.77, 37.03, 36.76, 18.36, 1.42.

IR ν_{max} (cm^{-1}): 3086, 2969, 2881, 1661, 1614, 1456, 1385, 1321, 1321, 1163, 1107, 1066, 1014, 848.

HRMS (HESI) m/z calculated for $C_{17}H_{21}F_3N_2O_3$ $[M+H]^+$ 359.15770, found 359.15727.



22

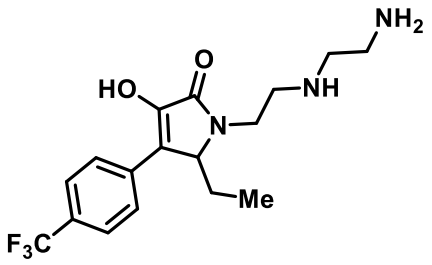
1-(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (22): According to general procedure, **22** was synthesized in a yield of 256 mg (71%) as a yellow waxy solid.

^{1}H NMR (500 MHz, Methanol-d_4) δ 7.51 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 4.52 (t, J = 3.5 Hz, 1H), 3.74 – 3.65 (m, 1H), 3.44 – 3.25 (m, 13H), 3.00 – 2.93 (m, 1H), 2.77 (t, J = 5.0 Hz, 2H), 1.79 – 1.68 (m, 1H), 1.56 – 1.44 (m, 1H), 0.08 (t, J = 7.3 Hz, 3H).

^{13}C NMR (126 MHz, Methanol-d_4) δ 167.21, 161.06, 144.62, 135.70, 128.34, 127.20, 125.22, 124.83, 123.07, 119.37, 117.58, 115.26, 69.93, 69.87, 69.71, 69.65, 68.32, 66.29, 57.30, 39.49, 39.08, 20.60, 3.78.

IR ν_{max} (cm^{-1}): 3118, 2961, 2872, 2102, 1662, 1610, 1569, 1448, 1383, 1319, 1161, 1105, 1066.

HRMS (HESI) m/z calculated for $C_{21}H_{29}F_3N_2O_5$ $[M+H]^+$ 447.21013, found 447.21000.



23

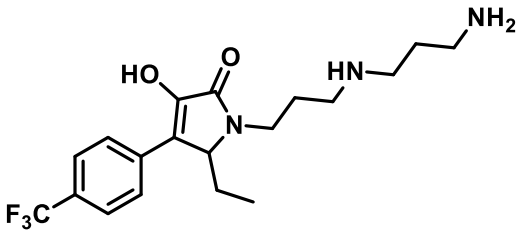
1-(2-((2-aminoethyl)amino)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (23): According to general procedure, **23** was synthesized in a yield of 56 mg (51%) as a yellow oil (obtained as a formic acid salt).

^{1}H NMR (700 MHz, Methanol-d_4) δ 8.21 (s, 2H), 7.75 (d, J = 8.1 Hz, 2H), 7.59 (d, J = 8.1 Hz, 2H), 4.72 (s, 1H), 3.93 – 3.81 (m, 1H), 3.20 (d, J = 8.7 Hz, 1H), 3.05 – 2.71 (m, 6H), 1.95 (dp, J = 11.3, 3.8 Hz, 1H), 1.77 (ddt, J = 15.0, 7.5, 4.7 Hz, 1H), 0.35 (t, J = 7.3 Hz, 3H).

^{13}C NMR (151 MHz, Methanol-d_4) δ 167.34, 146.28, 137.24, 129.88, 128.71, 126.36, 126.33, 120.86, 58.35, 48.30, 46.82, 40.58, 39.78, 22.18, 5.31.

IR ν_{max} (cm^{-1}): 3377, 2967, 2930, 2363, 1774, 1580, 1454, 1379, 1327, 1163, 1111, 1066, 850, 768

HRMS (HESI) m/z calculated for $C_{17}H_{23}F_3N_3O_2$ 358.17369 found 358.17300.



24

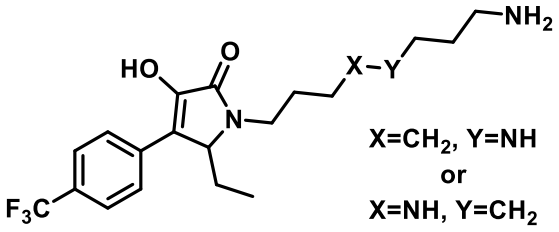
1-(3-((3-aminopropyl)amino)propyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (24): According to general procedure, **24** was synthesized in a yield of 190 mg (63%) as a yellow waxy oil. (*obtained as a diformic acid salt)

^{1}H NMR (600 MHz, Methanol-d_4) δ 8.55 (s, 2H), 7.86 (d, J = 8.2 Hz, 2H), 7.67 (d, J = 8.3 Hz, 2H), 5.61 (s, 5H), 4.80 (t, J = 3.5 Hz, 1H), 3.87 (ddd, J = 14.7, 8.2, 6.6 Hz, 1H), 3.42 – 3.34 (m, 1H), 3.19 (t, J = 7.6 Hz, 2H), 3.14 (t, J = 7.6 Hz, 2H), 3.09 (t, J = 7.5 Hz, 2H), 2.22 – 2.00 (m, 5H), 1.89 – 1.80 (m, 1H), 0.42 (t, J = 7.3 Hz, 3H).

^{13}C NMR (151 MHz, Methanol-d_4) δ 169.99, 169.48, 146.41, 137.21, 129.65 (q), 128.63, 126.26, 124.71, 121.06, 58.33, 46.38, 45.74, 38.23, 37.70, 26.44, 25.42, 22.15, 5.42.

IR ν_{max} (cm^{-1}): 3325, 2967, 2937, 2870, 2363, 1662, 1573, 1446, 1379, 1319, 1163, 1111, 1066.

HRMS (HESI) m/z calculated for $C_{19}H_{27}F_3N_3O_2$ $[M+H]^+$ 386.20499 found, 386.20480



25

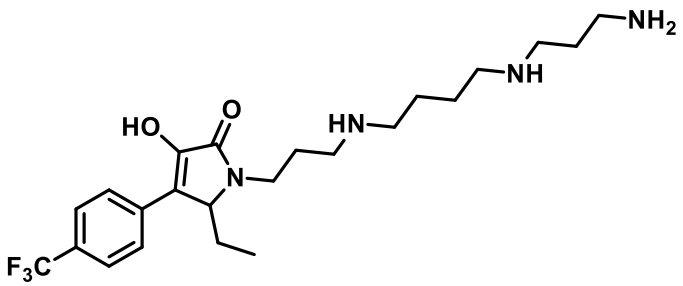
1-(3-((4-Aminobutyl)amino)propyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (25): According to general procedure, **25** was synthesized in a yield of 116 mg (36%) as a yellow oil (*obtained as a formic acid salt).

^{1}H NMR (500 MHz, Methanol-d_4) δ 8.50 (s, 2H), 7.86 (d, J = 8.1 Hz, 2H), 7.69 (dd, J = 8.5, 3.0 Hz, 2H), 4.83 – 4.72 (m, 1H), 3.91 – 3.76 (m, 1H), 3.15 – 2.89 (m, 8H), 2.13 – 1.99 (m, 3H), 1.93 – 1.63 (m, 7H), 0.47 – 0.39 (m, 3H).

^{13}C NMR (126 MHz, Methanol-d_4) δ 170.09, 169.76, 169.07, 146.60, 146.43, 137.45, 137.37, 128.86, 128.81, 126.51, 124.76, 121.27, 120.67, 58.61, 58.05, 46.46, 45.81, 40.26, 40.03, 38.29, 37.91, 26.69, 26.53, 25.73, 25.56, 24.65, 24.43, 22.36, 5.54, 5.50.

IR ν_{max} (cm^{-1}): 3330, 3047, 2965, 2935, 2784, 2220, 2067, 1670, 1577, 1448, 1377, 1321, 1163, 1114, 1066, 975, 848, 764.

HRMS (HESI) m/z calculated for $C_{20}H_{29i}F_3N_3O_2$ $[M+H]^+$ 400.22064, found 400.21990.



26

1-(3-((4-((3-Aminopropyl)amino)butyl)amino)propyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (26): According to general procedure, **26** was synthesized in a yield of 210 mg (56%) as a yellow solid (obtained as a formic acid salt).

^{1}H NMR (600 MHz, Methanol-d_4) δ 8.55 (s, 3H), 7.87 (d, J = 8.2 Hz, 2H), 7.69 (d, J = 8.3 Hz, 2H), 4.81 (t, J = 3.4 Hz, 1H), 3.91 – 3.82 (m, 1H), 3.43 – 3.35 (m, 1H), 3.16 (t, J = 7.7 Hz, 2H), 3.12 – 3.04 (m, 8H), 2.18 – 2.01 (m, 5H), 1.91 – 1.79 (m, 5H), 0.43 (t, J = 7.3 Hz, 3H).

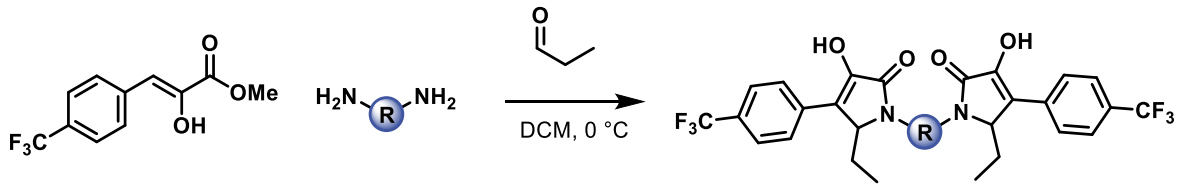
^{13}C NMR (126 MHz, Methanol-d_4) δ 170.47, 170.12, 147.12, 137.62, 129.51, 128.70, 126.47, 124.78, 120.83, 58.55, 50.00, 48.19, 46.42, 45.87, 38.27, 37.96, 26.69, 25.63, 24.49, 22.36, 5.57.

IR ν_{max} (cm^{-1}): 3276, 2932, 2859, 2658, 2093, 1735, 1653, 1577, 1508, 1457, 1411, 1319, 1159, 1105, 1064, 1018, 844.

mp: 110-113 °C.

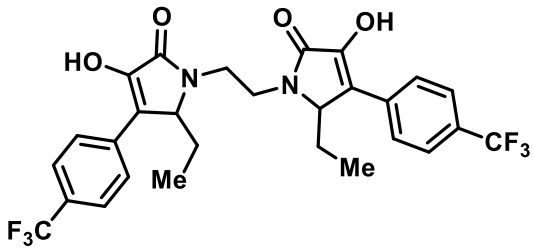
HRMS (HESI) m/z calculated for $C_{23}H_{35}F_3N_4O_2$ $[M+H]^+$ 457.27849, found 457.27748.

Preparation of 3-Hydroxy-1,5-dihydro-2H-pyrrol-2-ones dimers



General Procedure:

To a solution of methyl α-oxoester (1.1 mmol) in DCM (0.5 M) at room temperature was added aldehyde (20.0 mmol), followed by diamine (0.5 mmol). The reaction was stirred for 8 to 24 hours, being monitored by HPLC-MS. Upon consumption of phenyl pyruvic ester, solvent was removed in vacuo or the solids in suspension were separated via vaccum filtration. The crude products were then purified by C-18 reverse phase flash column chromatography using mobile phase comprised of acetonitrile and water with 0.1% formic acid, or methanol, water and 0.1% formic acid as well.



27

1,1'-(Ethane-1,2-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (27): According to general procedure, **27** was synthesized in a yield of 52 mg (50%) as a white solid.

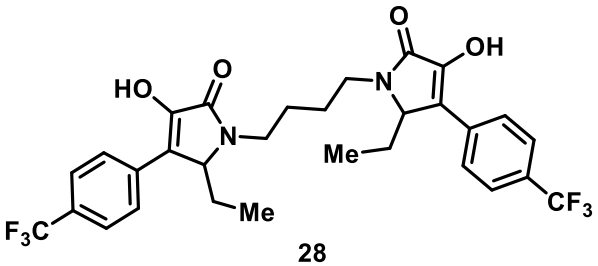
^{1}H NMR (600 MHz, DMSO-d_6, 55 °C) δ 7.88 – 7.80 (m, 4H), 7.76 – 7.62 (m, 4H), 4.89 – 4.80 (m, 2H), 4.12 – 4.02 (m, 1H), 3.98 (q, 1H), 3.32 (q, J = 5.8 Hz, 2H), 2.09 – 1.96 (m, 2H), 1.84 – 1.70 (m, 2H), 0.41 (t, J = 7.3, 2.8 Hz, 6H).

^{13}C NMR (126 MHz, Acetone-d_6) δ 167.82, 145.94, 137.69, 132.62, 128.97, 126.69 (q), 124.80, 119.95, 57.18, 38.89, 22.14, 5.98.

IR ν_{max} (cm^{-1}): 3187, 2963, 2932, 2359, 1743, 1661, 1614, 1456, 1385, 1321, 1267, 1232, 1163, 1107, 1066, 1016, 846, 794, 770.

mp: decomposes at 132 – 133 °C.

HRMS (HESI) m/z calculated for $C_{28}H_{26}F_6N_2O_4$ $[M+H]^+$ 569.18695, found 569.18592.



28

1,1'-(Butane-1,4-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (28): According to general procedure, **28** was synthesized in a yield of 124 mg (52%) as a white solid.

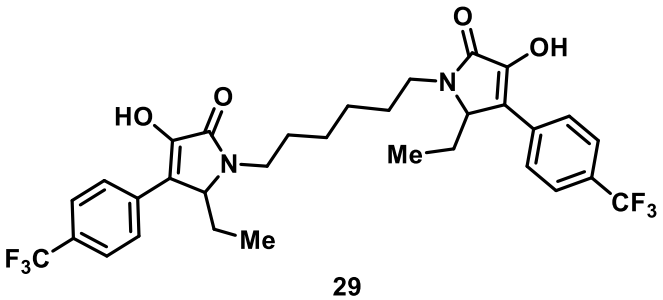
^{1}H NMR (600 MHz, Methanol-d_4) δ 7.88 (d, J = 8.2 Hz, 1H), 7.80 (d, J = 8.2 Hz, 3H), 7.71 (d, J = 8.2 Hz, 1H), 7.64 (d, J = 8.2 Hz, 3H), 4.82 (t, J = 3.5 Hz, 1H), 4.72 (t, J = 3.5 Hz, 1H), 3.94 – 3.84 (m, 2H), 3.27 – 3.19 (m, 2H), 2.10 – 2.02 (m, 2H), 1.92 – 1.82 (m, 2H), 1.82 – 1.74 (m, 2H), 1.74 – 1.64 (m, 2H), 0.52 – 0.41 (m, 6H).

^{13}C NMR (151 MHz, Methanol-d_4) δ 164.83, 161.08, 142.40, 124.79, 122.37, 116.65, 53.76, 37.00, 36.40, 22.98, 22.60, 18.30, 1.42.

IR ν_{max} (cm^{-1}): 3146, 2965, 2933, 2874, 2084, 1653, 1612, 1457, 1387, 1319, 1265, 1215, 1161, 1105, 1066, 1014, 844, 794, 768.

mp: decomposes at 151 °C.

HRMS (HESI) m/z calculated for $C_{30}H_{30}F_6N_2O_4$ $[M+H]^+$ 597.21825, found 597.21687.



29

1,1'-(Hexane-1,6-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (29): According to general procedure, **29** was synthesized in a yield of 66 mg (61%) as a white solid.

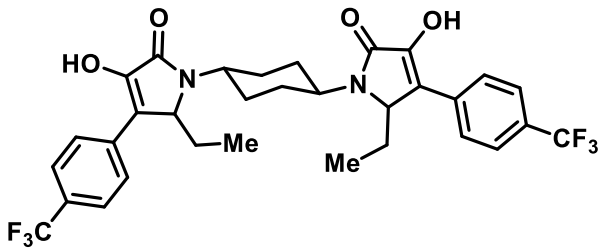
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.49 (s, 2H), 7.83 (d, J = 8.0 Hz, 4H), 7.72 (t, J = 8.6 Hz, 4H), 4.77 (m, 2H), 3.77 – 3.64 (m, 2H), 3.05 – 2.93 (m, 2H), 1.97 – 1.83 (m, 2H), 1.74 – 1.64 (m, 2H), 1.64 – 1.47 (m, 4H), 1.40 – 1.26 (m, 4H), 0.40 – 0.22 (m, 6H).

^{13}C NMR (126 MHz, DMSO-d_6, reported as observed) δ 166.02, 136.38, 127.13, 127.00, 125.42, 125.21, 123.26, 55.27, 55.23, 27.75, 27.68, 25.96, 25.89, 20.64, 5.07.

IR ν_{max} (cm^{-1}): 3107, 2965, 2933, 2859, 2084, 1659, 1614, 1456, 1388, 1319, 1290, 1265, 1163, 1122, 1109, 1066, 1014, 848, 796, 773.

mp: decomposes at 125 °C.

HRMS (HESI) m/z calculated for $C_{32}H_{35a}F_6N_2O_4$ $[M+H]^+$ 625.24955, found 625.24823.



30

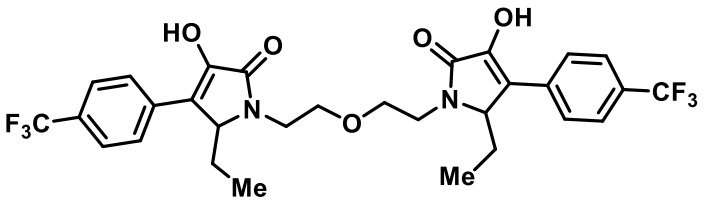
1,1'-((1r,4r)-Cyclohexane-1,4-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (30): According to general procedure, **30** was synthesized in a yield of 37 mg (39%) as a white solid.

^{1}H NMR (600 MHz, Pyridine-d_5) δ 13.84 (s, 2H), 8.11 (t, J = 7.0 Hz, 4H), 7.79 (d, J = 8.0 Hz, 4H), 4.71 (s, 2H), 3.84 (d, J = 11.8 Hz, 2H), 2.58 – 2.42 (m, 2H), 2.34 – 2.19 (m, 2H), 2.06 – 1.85 (m, 8H), 0.55 (q, J = 5.3, 3.7 Hz, 6H).

^{13}C NMR (151 MHz, Pyridine-d_5) δ 167.20, 149.27, 147.81, 137.15, 134.90, 127.65, 125.47, 122.89, 117.60, 57.33, 57.26, 53.32, 53.25, 29.80, 29.70, 29.38, 29.25, 22.50, 22.46, 5.34.

IR ν_{max} (cm^{-1}): 3185, 2965, 2937, 2876, 1659, 1614, 1454, 1385, 1325, 1165, 1122, 1068.

HRMS (HESI) m/z calculated for $C_{32}H_{33}F_6N_2O_4$ $[M+H]^+$ 623.23390, found 623.23414.



33

1,1'-(Oxybis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (33): According to general procedure, **33** was synthesized in a yield of 51 mg (48%) as a white solid.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.57 (s, 4H), 7.53 – 7.37 (m, 4H), 4.56 (s, 2H), 4.10 (brs, 2H), 3.60 (d, *J* = 67.9 Hz, 4H), 3.13 (brs, 2H), 1.87 (brs, 2H), 1.76 (brs, 2H), 0.36 (brs, 6H).

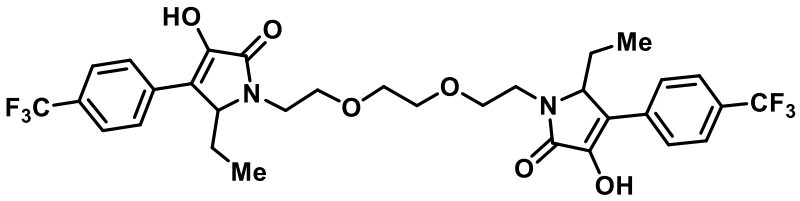
^{1}H NMR (600 MHz, DMSO-d_6, VT, 45 °C) δ 10.55 (s, 2H), 7.73 (dd, *J* = 34.8, 8.0 Hz, 4H), 7.65 – 7.40 (m, 4H), 4.72 (s, 2H), 4.08 – 3.97 (m, 1H), 3.91 (d, *J* = 14.5 Hz, 1H), 3.74 – 3.51 (m, 4H), 3.21 (dt, *J* = 15.1, 5.0 Hz, 1H), 3.17 – 3.09 (m, 1H), 2.05 – 1.86 (m, 2H), 1.74 – 1.56 (m, 2H), 0.31 (t, *J* = 7.3 Hz, 6H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 168.73, 135.58, 130.57, 128.42, 127.04, 125.31, 125.22, 123.16, 77.41, 67.55, 58.20, 39.67, 21.46, 5.31.

IR ν_{max} (cm^{-1}): 3107, 2969, 2935, 2874, 1772, 1748, 1661, 1456, 1387, 1321, 1163, 1107, 1066, 1016, 846.

mp: 110 °C.

HRMS (HESI) m/z calculated for $C_{30}H_{30}F_6N_2O_5$ $[M+H]^+$ 613.21317, found 613.21168.



34

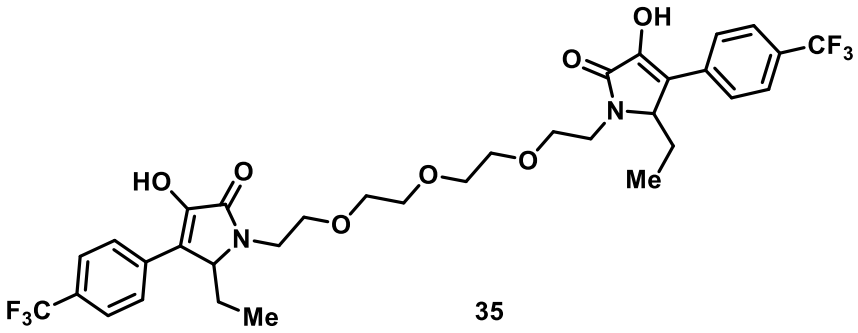
1,1'-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (34): According to general procedure, **34** was synthesized in a yield of 65 mg (50%) (50%) as a white solid.

^{1}H NMR (500 MHz, Methanol-d_4) δ 7.82 (dd, J = 14.3, 8.2 Hz, 4H), 7.69 (dd, J = 13.7, 8.2 Hz, 4H), 4.85 (d, J = 3.4 Hz, 1H), 4.82 (t, J = 3.6 Hz, 1H), 4.03 – 3.93 (m, 2H), 3.79 – 3.71 (m, 2H), 3.71 – 3.59 (m, 6H), 3.30 – 3.20 (m, 2H), 2.09 – 1.95 (m, 2H), 1.85 – 1.73 (m, 2H), 0.43 – 0.32 (m, 6H).

^{13}C NMR (126 MHz, Methanol-d_4) δ 168.61, 146.26, 137.29, 132.20, 129.83 (q), 128.67, 126.77, 126.39, 126.36, 124.62, 120.77, 71.52, 71.43, 70.21, 70.11, 59.12, 59.03, 54.19, 49.51, 41.07, 41.05, 22.11, 5.26, 5.23.

IR ν_{max} (cm^{-1}): 3109, 2967, 2937, 2669, 2363, 1655, 1610 , 1454, 1387, 1319, 1163, 1111, 1066, 1014, 842

HRMS (HESI) m/z calculated for $C_{32}H_{35}F_6N_2O_6$ $[M+H]^+$ 657.23938, found 657.23876.



35

1,1'-(((Oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (35): According to general procedure, **35** was synthesized in a yield of 42 mg (15%) as a white solid.

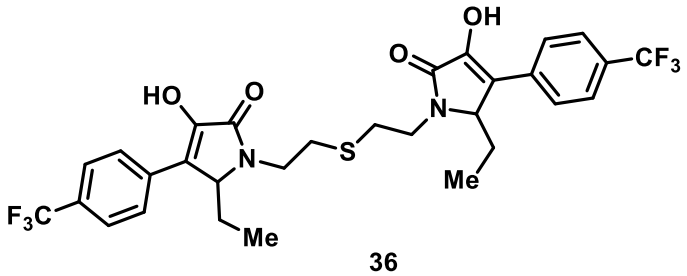
^{1}H NMR (600 MHz, Methanol-d_4) δ 7.85 (dd, J = 8.6, 3.5 Hz, 4H), 7.70 (dd, J = 8.4, 4.3 Hz, 4H), 4.91 – 4.88 (m, 2H), 4.02 – 3.95 (m, 2H), 3.73 – 3.66 (m, 4H), 3.64 – 3.57 (m, 8H), 3.30 – 3.22 (m, 2H), 2.12 – 2.01 (m, 2H), 1.89 – 1.77 (m, 2H), 0.42 (td, J = 7.3, 1.6 Hz, 6H).

^{13}C NMR reported as observed (126 MHz, Methanol-d_4) δ 168.58, 146.23, 137.31, 132.18, 129.56 (q), 128.71, 126.76, 126.36, 126.34, 124.61, 120.80, 86.27, 71.56, 71.51, 71.42, 71.41, 70.13, 59.09, 59.05, 54.28, 41.00, 40.97, 39.83, 22.10, 5.28.

IR ν_{max} (cm^{-1}): 3122, 2967, 2935, 2874, 2085, 1774, 1746, 1659, 1614, 1454, 1388, 1319, 1269, 1163, 1107, 1066, 1016, 846, 796.

mp: 105 °C.

HRMS (HESI) m/z calculated for $C_{34}H_{38}F_6N_2O_7$ $[M+H]^+$ 701.26560, found 701.26436.



36

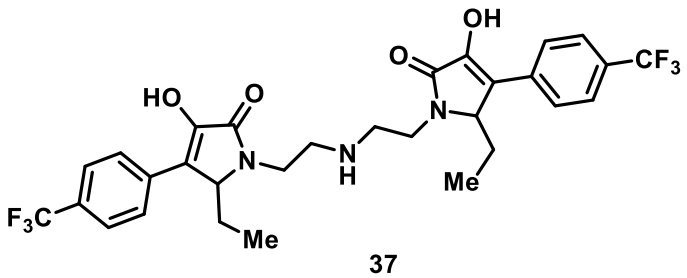
1,1'-(thiobis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (36). According to general procedure, **36** was synthesized in a yield of 42 mg (52%) as a clear wax.

^{1}H NMR (600 MHz, DMSO-d_6, VT, 45 °C) δ 7.82 (t, J = 9.1 Hz, 4H), 7.62 (t, J = 9.4 Hz, 4H), 4.74 (dt, J = 7.6, 3.5 Hz, 2H), 3.97 – 3.85 (m, 2H), 3.34 – 3.18 (m, 3H), 2.99 – 2.87 (m, 2H), 2.84 – 2.72 (m, 2H), 2.02 – 1.88 (m, 2H), 1.81 – 1.68 (m, 2H), 0.36 (q, J = 6.9 Hz, 6H).

^{13}C NMR (151 MHz, Chloroform-*d*) δ 166.20, 136.58, 136.09, 127.99, 126.79, 125.17, 123.42, 121.62, 59.17, 41.91, 29.24, 21.90, 5.86.

IR ν_{max} (cm^{-1}): 3019, 2967, 2930, 2363, 1655, 1454, 1327, 1163, 1111, 1066, 820, 537

HRMS (HESI) m/z calculated for **HRMS (HESI)** m/z calculated for $C_{30}H_{30}F_6N_2O_4SNa$ $[M+Na]^+$ 651.17227, found 651.17108; m/z calculated for $C_{30}H_{29}F_6N_2O_4S$ $[M-H]^-$ 627.17577, found 627.17514.



37

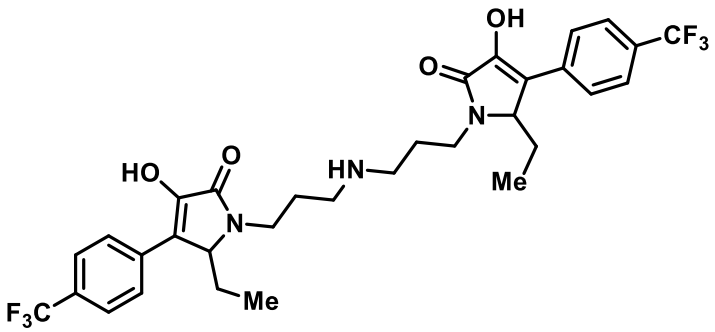
1,1'-(azanediylbis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl) phenyl)-1,5-dihydro-2H-pyrrol-2-one) (37). According to general procedure, **37** was synthesized in a yield of 13 mg (16%) as a yellow waxy solid.

^{1}H NMR (700 MHz, Methanol-d_4) δ 8.30 (s, 3H), 7.84 (d, J = 8.1 Hz, 4H), 7.68 (d, J = 8.1 Hz, 4H), 4.81 (s, 2H), 3.09 – 2.97 (m, 8H), 2.03 (dp, J = 11.3, 3.8 Hz, 2H), 1.86 (ddt, J = 15.0, 7.5, 4.7 Hz, 2H), 0.43 (t, J = 7.3 Hz, 6H).

^{13}C NMR (151 MHz, Methanol-d_4) δ 174.39, 172.65, 172.29, 146.21, 141.86, 137.03, 131.53, 128.69, 128.63, 126.45, 126.42, 126.41, 126.30, 126.28, 126.26, 57.98, 46.83, 46.81, 38.25, 24.04, 24.00, 22.36, 5.24.

IR ν_{max} (cm^{-1}): 3399, 2967, 2878, 2363 ,1677, 1588, 1439, 1387, 1319, 1215, 1163, 1111, 1066, 1014, 850.

HRMS (HESI) m/z calculated for $C_{30}H_{32}F_6N_3O_4$ $[M+H]^+$ 612.22915, found 612.22851.



38

1,1'-(azanediylbis(propane-3,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (38): According to general procedure, **38** was synthesized in a yield of 42 mg (41%) as an off-white solid.(obtained as a formic acid salt)

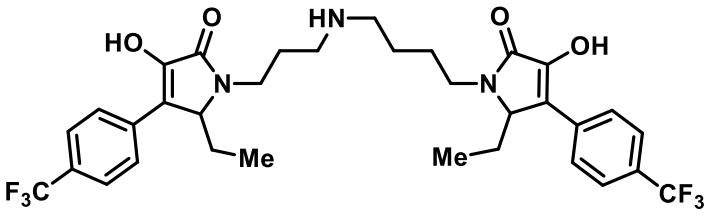
^{1}H NMR (600 MHz, Methanol-d_4) δ 8.43 (s, 1H), 7.80 (d, J = 8.0 Hz, 4H), 7.64 (d, J = 8.0 Hz, 4H), 4.75 (s, 2H), 3.86 – 3.76 (m, 2H), 3.40 – 3.31 (m, 2H), 3.03 (t, J = 7.4 Hz, 4H), 2.10 – 1.96 (m, 6H), 1.87 – 1.77 (m, 2H), 0.39 (td, J = 7.3, 2.4 Hz, 6H).

^{13}C NMR (151 MHz, Methanol-d_4) δ 169.51, 146.14, 137.14, 129.92, 128.73, 126.34, 124.77, 121.16, 58.54, 46.51, 38.22, 26.62, 22.25, 5.39.

IR ν_{max} (cm^{-1}):

mp: 109 – 111

HRMS (HESI) m/z calculated for $C_{32}H_{36}F_6N_3O_4$ $[M+H]^+$ 640.26045, found 640.26013.



39

5-Ethyl-1-(3-((4-(2-ethyl-4-hydroxy-5-oxo-3-(4-(trifluoromethyl)phenyl)-2,5-dihydro-1H-pyrrol-1-yl)butyl)amino)propyl)-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (39): According to general procedure, **39** was synthesized in a yield of 20 mg (44%) as a clear waxy oil.

^{1}H NMR (500 MHz, Methanol-d_4) δ 7.85 (d, J = 7.8 Hz, 4H), 7.70 (dt, J = 8.2, 4.3 Hz, 4H), 4.82 – 4.75 (m, 2H), 3.92 – 3.76 (m, 2H), 3.19 – 3.03 (m, 5H), 2.11 – 1.98 (m, J = 7.2 Hz, 4H), 1.93 – 1.83 (m, 2H), 1.81 – 1.66 (m, 5H), 0.45 (td, J = 7.4, 2.7 Hz, 6H).

^{13}C NMR reported as observed (126 MHz, Methanol-d_4) δ 169.72, 169.00, 168.37, 146.30, 146.01, 137.31, 137.17, 135.87, 131.50, 128.92, 128.85, 126.61 (q), 126.53 (q), 121.55, 120.82, 58.83, 58.79, 58.12, 58.10, 46.52, 46.49, 40.25, 40.22, 38.26, 38.21, 26.75, 26.47, 26.46, 24.72, 24.68, 22.38, 5.53, 5.48.

IR ν_{max} (cm^{-1}): 2965, 2932, 2874, 2093, 1661, 1614, 1456, 1383, 1319, 1265, 1161, 1107, 1066, 1014, 846.

HRMS (HESI) m/z calculated for $C_{33}H_{37}F_6N_3O_4$ $[M+H]^+$ 654.27610, found 654.27563.

40

1,1'-((Butane-1,4-diylbis(azanediyl))bis(propane-3,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (40): According to general procedure, **40** was synthesized in a yield of 50 mg (49%) as a white solid (obtained as a formic acid salt).

^{1}H NMR (600 MHz, Methanol-d_4) δ 8.44 (s, 2H), 7.83 – 7.77 (m, 4H), 7.67 – 7.61 (m, 4H), 4.75 (q, J = 3.9 Hz, 2H), 3.83 – 3.73 (m, 2H), 3.39 – 3.32 (m, 2H), 3.26 (t, J = 1.7 Hz, 2H), 3.05 – 2.96 (m, 8H), 2.06 – 1.96 (m, 6H), 1.86 – 1.76 (m, 6H), 0.40 (t, J = 7.3 Hz, 6H).

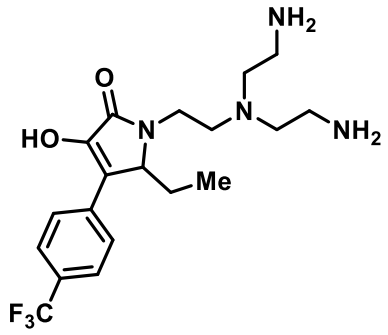
^{13}C NMR (151 MHz, Methanol-d_4) δ 169.74, 169.59, 146.26, 137.20, 129.78 (q, J = 32.6 Hz), 128.70, 126.57, 126.37, 124.77, 121.17, 58.57, 48.08, 46.32, 38.12, 26.60, 24.32, 22.23, 5.40.

IR ν_{max} (cm^{-1}): 2963, 2935, 2102, 1662, 1571, 1448, 1381, 1319, 1265, 1161, 1105, 1066, 1014, 846.

mp: 133-135 °C.

HRMS (HESI) m/z calculated for $C_{36}H_{45}F_6N_4O_4$ $[M+H]^+$ 711.33395, found 711.33212.

Synthesis of 3-Hydroxy-1,5-dihydro-2H-pyrrol-2-ones monomer, dimer and trimer:



41

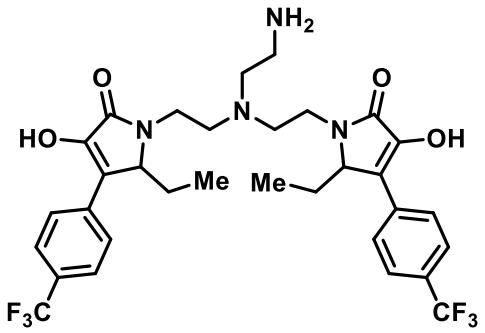
1-(2-(bis(2-aminoethyl)amino)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (41): To a solution of methyl α-oxoester (0.35 mmol) in DCM (0.5 M) at room temperature was added aldehyde (1.00 mmol), followed by diamine (0.33 mmol). The reaction was stirred for 24 hours, being monitored by HPLC-MS. Reaction mixture was filtered, and crude mixture were then purified by C-18 reverse phase flash column chromatography using mobile phase comprised of acetonitrile and water with 0.1% formic acid, or methanol, water and 0.1% formic acid as well.
Desired product **41** was synthesized in a yield of 50 mg (56%) as a dark yellow oil (obtained as a formic acid salt).

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.38 (s, 3H), 7.83 (d, J = 8.2 Hz, 2H), 7.67 (d, J = 8.3 Hz, 2H), 4.70 (t, J = 3.4 Hz, 1H), 3.78 (dt, J = 14.4, 7.5 Hz, 1H), 3.16 – 3.06 (m, 1H), 2.96 – 2.83 (m, 4H), 2.79 – 2.65 (m, 5H), 2.64 – 2.58 (m, 1H), 1.98 – 1.86 (m, 1H), 1.77 – 1.63 (m, 1H), 0.32 (t, J = 7.2 Hz, 3H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 168.30, 166.00, 138.16, 126.43, 125.89, 125.56, 124.09, 55.98, 51.61, 50.50, 37.18, 37.02, 21.19, 5.58.

IR ν_{max} (cm^{-1}): 3325, 2497, 2363, 2102, 1797, 1580, 1454, 1379, 1327, 1170, 1118, 574, 552.

HRMS (HESI) m/z calculated for $C_{19}H_{28}F_3N_4O_2$ $[M+H]^+$, 401.21589 found 401.21481.



42

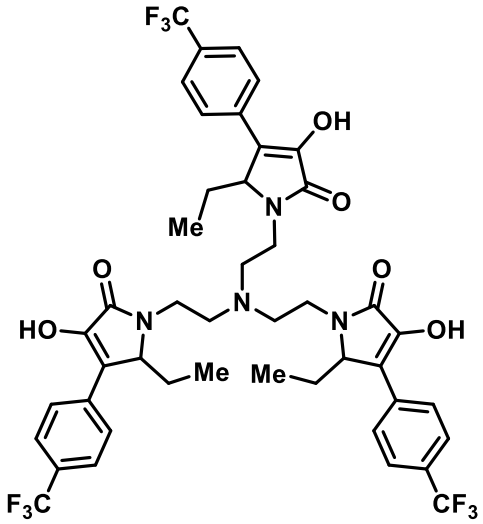
1,1'-(((2-aminoethyl)azanediyl)bis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (42): To a solution of methyl α-oxoester (0.50 mmol) in DCM (0.5 M) at room temperature was added aldehyde (3.30 mmol), followed by diamine (0.33 mmol). The reaction was stirred for 24 hours, being monitored by HPLC-MS. Reaction mixture was filtered, and filtrate was dry loaded on reverse phase silica. The crude mixture was then purified by C-18 reverse phase flash column chromatography using mobile phase comprised of acetonitrile and water with 0.1% formic acid, or methanol, water and 0.1% formic acid as well. Desired product **42** was synthesized in a yield of 65 mg (57%) as a light yellow oil (obtained as a formic acid salt).

^{1}H NMR (500 MHz, Methanol-d_4) δ 8.46 (s, 1H), 7.72 (d, J = 8.3 Hz, 1H), 7.69 (d, J = 8.2 Hz, 3H), 7.60 (d, J = 8.2 Hz, 1H), 7.54 (d, J = 8.2 Hz, 3H), 4.71 (dt, J = 17.5, 3.4 Hz, 2H), 3.86 – 3.75 (m, 2H), 3.15 – 3.03 (m, 4H), 2.90 – 2.76 (m, 6H), 2.01 – 1.89 (m, 2H), 1.78 – 1.65 (m, 2H), 0.31 (q, J = 7.7 Hz, 6H).

^{13}C NMR reported as observed (126 MHz, Methanol-d_4) δ 169.48, 169.27, 169.23, 146.37, 146.17, 137.04, 129.80, 129.55, 128.65, 126.71, 126.35, 126.25, 124.56, 120.68, 120.64, 58.17, 58.13, 55.67, 55.07, 53.21, 53.14, 40.56, 40.07, 39.10, 39.05, 22.30, 22.18, 5.19, 5.09.

IR ν_{max} (cm^{-1}): 3317, 2960, 2930, 2363, 1767, 1573, 1454, 1319, 1163, 1111, 1066

HRMS (HESI) m/z calculated for $C_{32}H_{37}F_6N_4O_4$ $[M+H]^+$, 655.27135 found 655.26959.



43

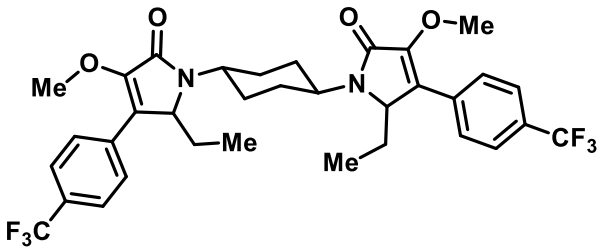
1,1',1''-(nitrilotris(ethane-2,1-diyl))tris(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (43): To a solution of methyl α-oxoester (1.00 mmol) in DCM (0.5 M) at room temperature was added aldehyde (6.68 mmol), followed by diamine (0.33 mmol). The reaction was stirred for 24 hours, being monitored by HPLC-MS. The crude products was dried under vacuum and then purified by C-18 reverse phase flash column chromatography using mobile phase comprised of acetonitrile and water with 0.1% formic acid, or methanol, water and 0.1% formic acid as well. Desired product **43** was synthesized in a yield of 28 mg (38%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.97 (s, 2H), 7.98 – 7.63 (m, 6H), 7.63 – 7.28 (m, 6H), 4.94 – 4.49 (m, 3H), 3.95 – 3.65 (m, 3H), 3.08 (s, 3H), 2.77 (s, 6H), 2.03 – 1.80 (m, 3H), 1.79 – 1.50 (m, 3H), 0.26 (s, 9H).

^{13}C NMR reported as observed (176 MHz, DMSO-d_6) δ 167.78, 166.70, 161.47, 153.78, 129.73, 126.74, 125.23, 124.87, 123.73, 56.40, 51.34, 27.04, 20.65, 9.16, 5.04.

IR ν_{max} (cm^{-1}): 2967, 2915, 2363, 1662, 1454, 1387, 1319, 1163, 1111, 1066, 1014, 850

HRMS (HESI) m/z calculated for $C_{45}H_{45}F_9N_4O_6Na$ $[M+Na]^+$, 931.30876 found 931.30609. and m/z calculated for $C_{45}H_{44}F_9N_4O_6$ $[M–H]^-$, 907.31226 found 907.31148.



48

1,1'-((1r,4r)-cyclohexane-1,4-diyl)bis(5-ethyl-3-methoxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (48) To a stirring solution of trans-cyclohexyl-dimer **30** (68 mg, 0.109 mmol) in dry DMF (2.2 mL, 0.05M) at 0 °C was added DBU (36 μL, 2.2 equiv.) and iodomethane (27.2 μL, 4.0 equiv.) dropwise. The reaction mixture was stirred for 2.5 h at 0 °C, and the reaction was followed by TLC and LCMS until full consumption of the starting material. The reaction mixture was then added in to a mixture of diethylether (5 mL) and 1M HCl (5 mL). The organic layer was separated, and aqueous solution was extracted with diethyl ether (5 mL x 3). The combined organic layers were washed with water (10 mL x2), saturated NaCl solution (10 mL x2), dried over anhydrous sodium sulfate and concentrated in vacuo. Crude was purified using normal phase flash chromatography to access the desired product **48** (51 mg, 71%) as a white solid.

^{1}H NMR (700 MHz, Chloroform-*d*) δ 7.67 – 7.64 (m, 4H), 7.64 – 7.61 (m, 4H), 4.56 (dt, *J* = 6.9, 3.6 Hz, 2H), 4.12 (s, 6H), 3.78 – 3.73 (m, 2H), 2.32 – 2.25 (m, 2H), 2.08 – 2.02 (m, 4H), 2.01 – 1.96 (m, 4H), 1.77 – 1.70 (m, 3H), 0.56 – 0.49 (m, 6H).

^{13}C NMR (176 MHz, Chloroform-*d*) δ 166.75, 147.18, 134.92, 129.83 (q), 128.10, 127.35, 125.63, 124.90, 123.35, 58.92, 57.44, 53.04, 29.50, 22.65, 5.40. (171.02, 60.31, 20.86 and 14.1 are ethyl acetate impurity peaks).

IR ν_{max} (cm^{-1}): 2937, 2766, 2356, 1677, 1319, 1163, 1118, 1066, 1014, 850, 731

HRMS (HESI) m/z calculated for C34H37F6N2O4 $[M+H]^+$ 651.26520 found 651.26409.

References:

(1) B. H. Frohock, J. M. Gilbertie, J. C. Daiker, L. V. Schnabel, J. G. Pierce, 5-Benzylidene-4-Oxazolidinones Are Synergistic with Antibiotics for the Treatment of Staphylococcus aureus Biofilms. *ChemBioChem* **2020**, *21*, 933-937.

(2) N. V. Shymanska, J. G. Pierce, Stereoselective Synthesis of Quaternary Pyrrolidine-2,3-diones and β-Amino Acids. *Org. Lett.* **2017**, *19*, 2961−2964.

(3) A. Q. Cusumano, J. G. Pierce, 3-Hydroxy-1,5-dihydro-2H-pyrrol-2-ones as novel antibacterial scaffolds against methicillin-resistant Staphylococcus aureus. Bioorg. *Med. Chem. Lett.* **2018**, *28*, 2732-2735.

General information - Biological Assays

Methicillin-resistant and methicillin sensitive Staphylococcus aureus (MRSA and MSSA respectively) strains were obtained from the Laboratory of Professor Christian Melander (NCSU) (ATCC BAA 44 and 33591) and Dr. Jessica Gilbertie (ATCC 25923). Bacteria were kept in frozen stocks on glycerol at - 80 °C until use. Bacteria was streaked onto tryptic-soy agar for colony isolation. Mueller-Hinton broth (MHB, 211443-BD), tryptic soy broth (TSB, Remel: R455052) and *D*-glucose (CAS: 492-62-6) were purchased from Fisher Scientific. Tryptic soy agar (TSA, cat. # 22091) and Linezolid (cat. # P70014) were purchased from Sigma-Aldrich. Bacteria for biofilm inhibition were cultured overnight in TSB-G (tryptic soy broth with 0.5% glucose supplement) in 96 well plates. All assays were run in triplicate and repeated at least two separate times for MIC assays and at least three separate times for biofilm inhibition. All compounds were dissolved in molecular biology grade DMSO as 10 mM stock solutions. Optical densities were measured using a Thermo Scientific Genesys 20 spectrophotometer. Data for biofilm inhibition, MBEC, and MIC assays were collected using a BioTek ELx808 Microplate Reader. All graphs were generated and analyzed using GraphPad Prism 7.

Broth microdilution method for determination of minimum inhibitory concentrations (MIC)

As prescribed by the Clinical and Laboratory Standards Institute (CLSI) M07-A8, Vol. 29 (2) MSSA (ATCC 25923) and MRSA (ATCC BAA 44 and 33591) was grown in MHB for 6-8 h; this culture was used to inoculate fresh MHB (5 x 10^5 CFU/mL). The resulting bacterial suspension was aliquoted (0.5 mL) into 1.5 mL Eppendorf tubes and compound was added from a 10 mM DMSO stock to achieve the desired initial starting concentration (typically 128 μg/mL). Linezolid (from a 10 mM DMSO stock) was used as a positive control. Inoculated media not treated with compound served as the negative control. The MIC was determined by microbroth dilution following the CLSI guidelines. The MIC was defined as the lowest concentration of antibiotic with no visible growth. The plate was sealed and incubated under stationary conditions at 37 °C. After 16 h, MIC values were recorded as the lowest concentration of compound at which no visible growth of bacteria was observed.

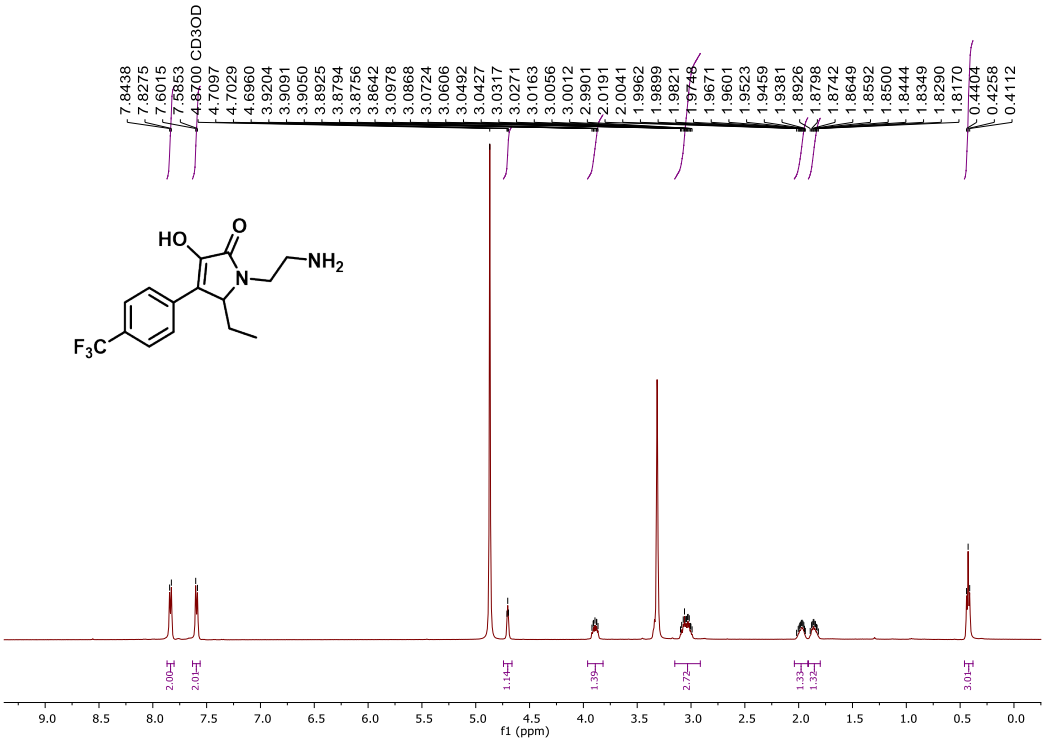
Determination of the Minimum Biofilm Eradication Concentrations (MBEC) using the Calgary Biofilm Device (CBD) on MSSA (ATCC 25923) biofilms

(H. Ceri, M. E. Olson, C. Stremick, R. R. Read, D. Morck, and A. Buret, J. Clin. Microbiol., 1999, 1771–1776.)

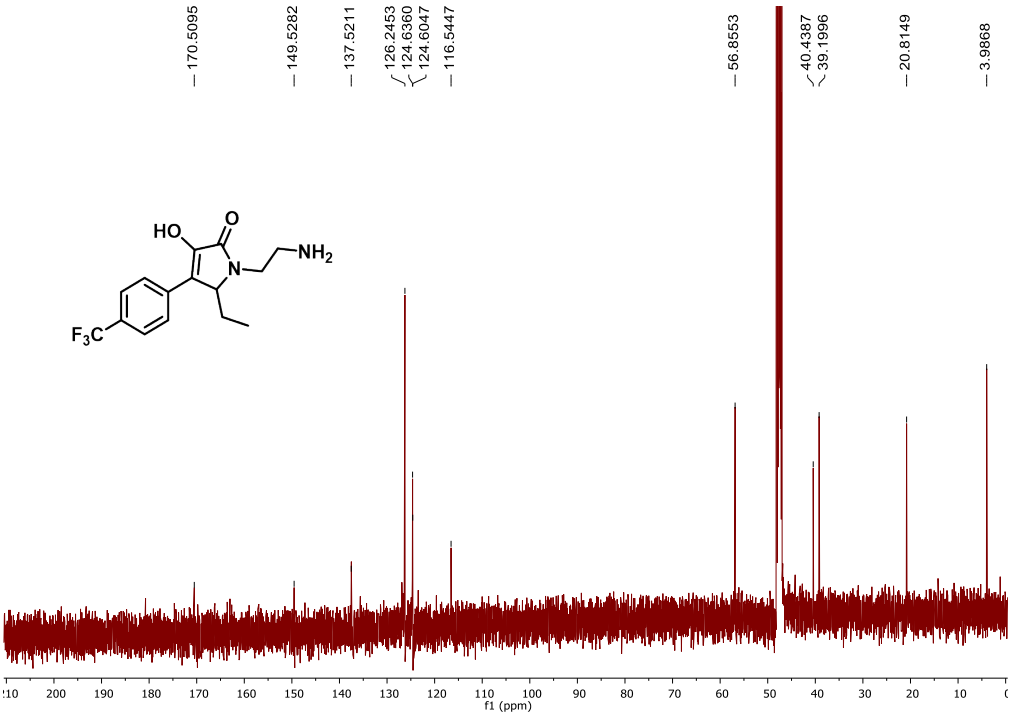
Biofilm eradication experiments were performed using MSSA (ATCC 25923) and the Calgary Biofilm Device (CBD) to determine MBEC values for various compounds of interest (Innovotech, product code: 19111). The Calgary device is a 96-well plate with a lid containing 96 pegs that sit in the media contained in the bottom well. Biofilm are established on the individual pegs. The established biofilm (contained on the individual peg) can then be transferred to a new base well for MBEC testing. For the MBEC assay, an overnight culture of MSSA (ATCC 25923) was adjusted to 0.5 McFarland in MHB-G. The CBD was inoculated with 100 µL of the 0.5 McFarland and incubated at 37 °C for 24 hours to establish biofilms. The CBD lid containing the established biofilms on individual pegs was removed, washed 3x with PBS and transferred to another 96-well plate containing serial dilutions of the test compounds (the "challenge plate") and incubated at 37 °C for 24 hours. The CBD lid was then removed from the challenge plate, washed 3x with PBS to remove any residual compound and placed into a new 96-well base plate containing fresh MHB. The plate was then sonicated for sonicated for 30 minutes to disperse biofilms on the pegs into the fresh MHB in the base well. After sonication, the plate was incubated for 24 hours at 37 °C. MBEC values were determined as the lowest test concentration that resulted in no growth in the sonicate fluid confirmed by absorbance at 600 nm in a plate reader.

^{1}H and ^{13}C NMR Data:

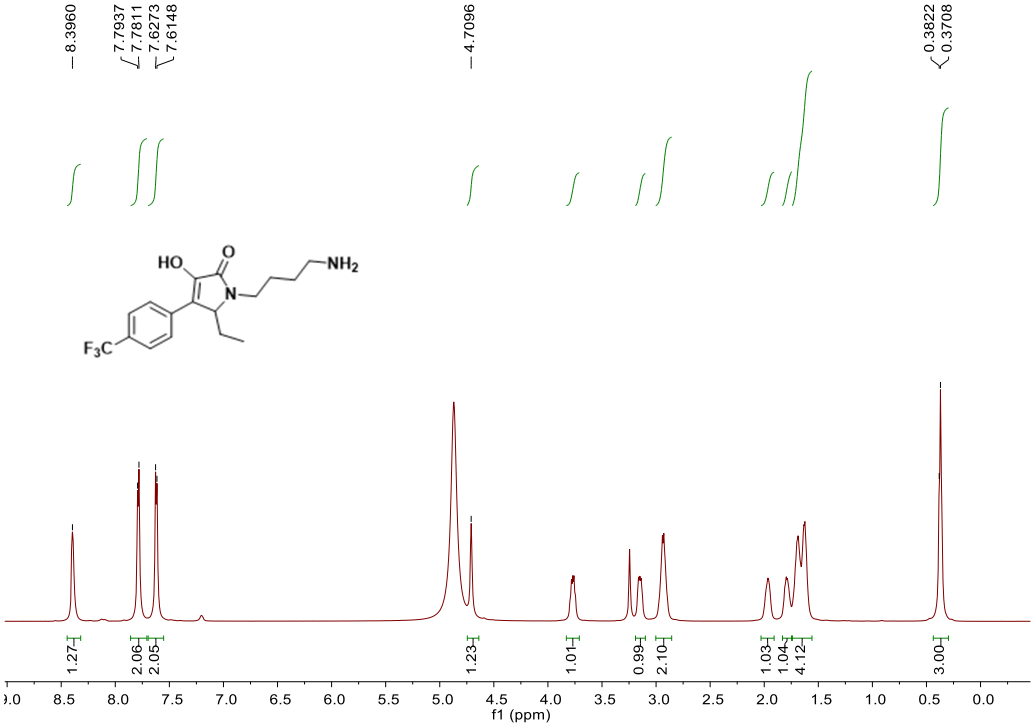
^{1}H NMR (500 MHz, Methanol-d$_4$) 1-(2-Aminoethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (16):



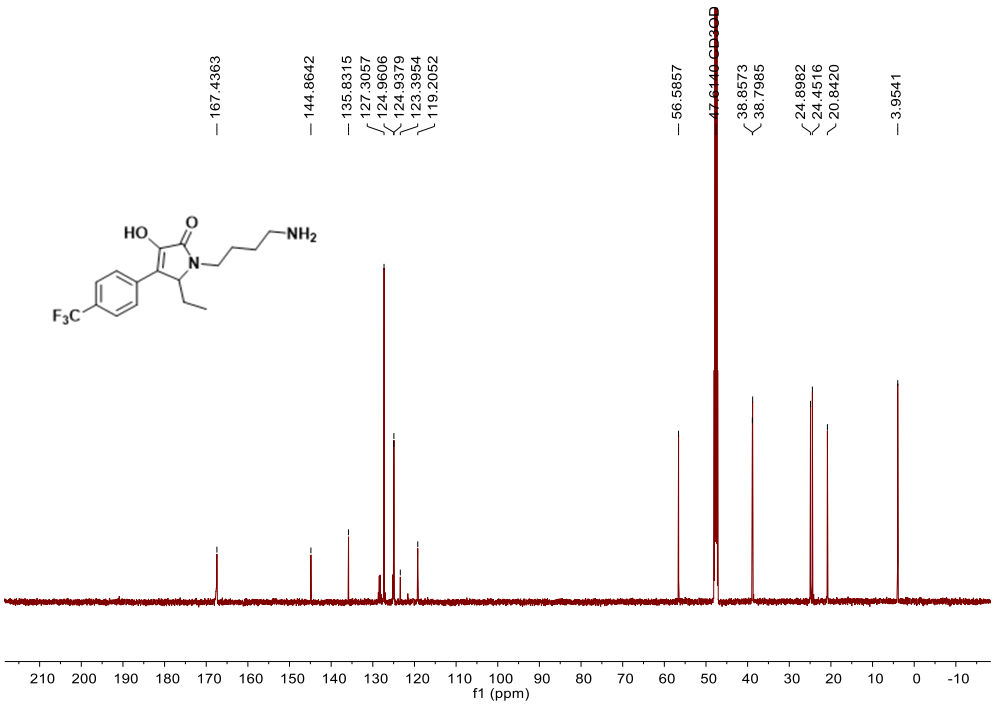
^{13}C NMR (126 MHz, Methanol-d$_4$) 1-(2-Aminoethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (16)



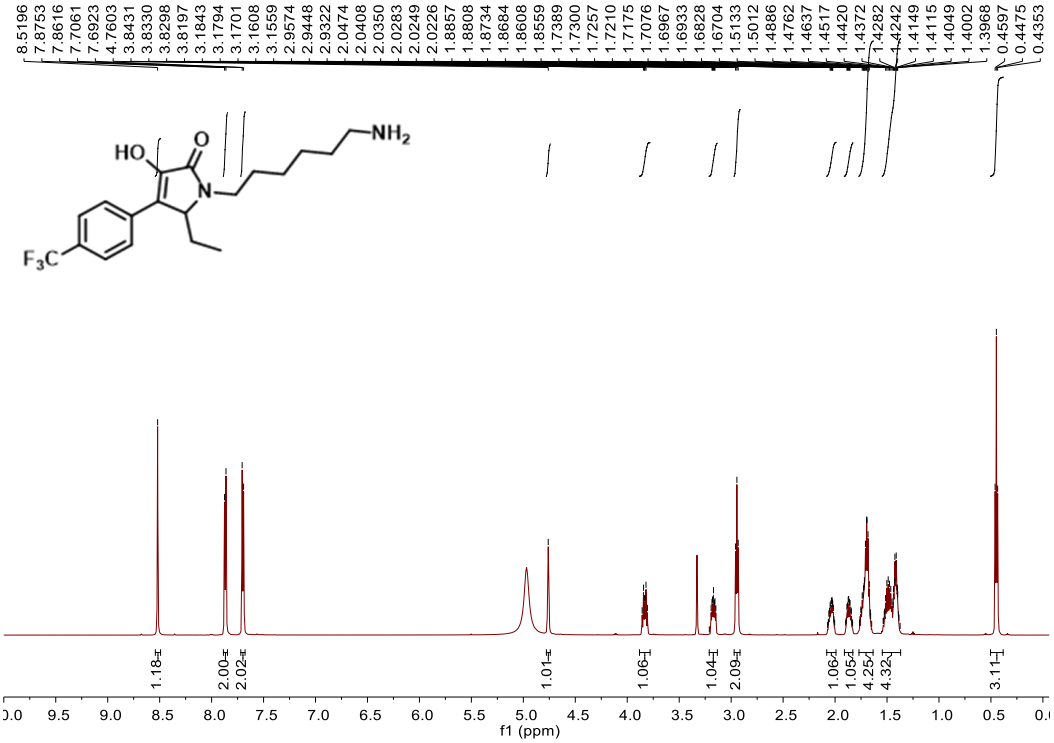
^{1}H NMR (600 MHz, Methanol-d_4) 1-(4-Aminobutyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (17)



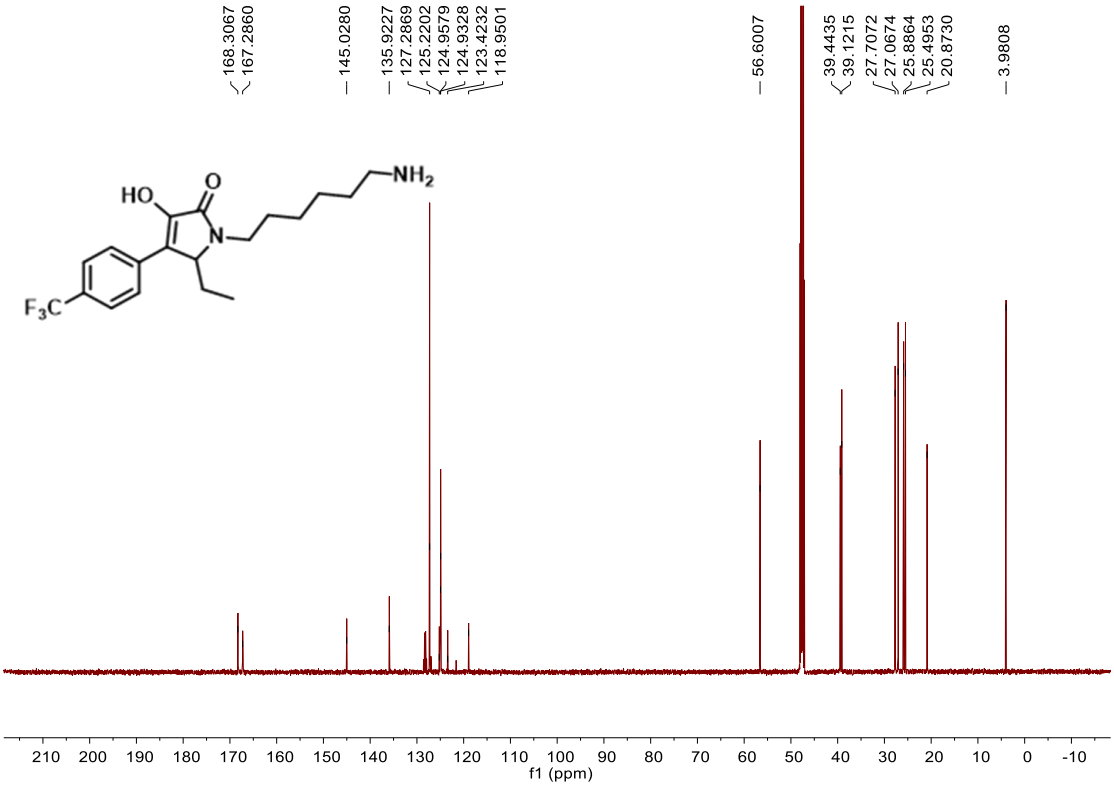
^{13}C NMR (151 MHz, Methanol-d_4) 1-(4-Aminobutyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (17)



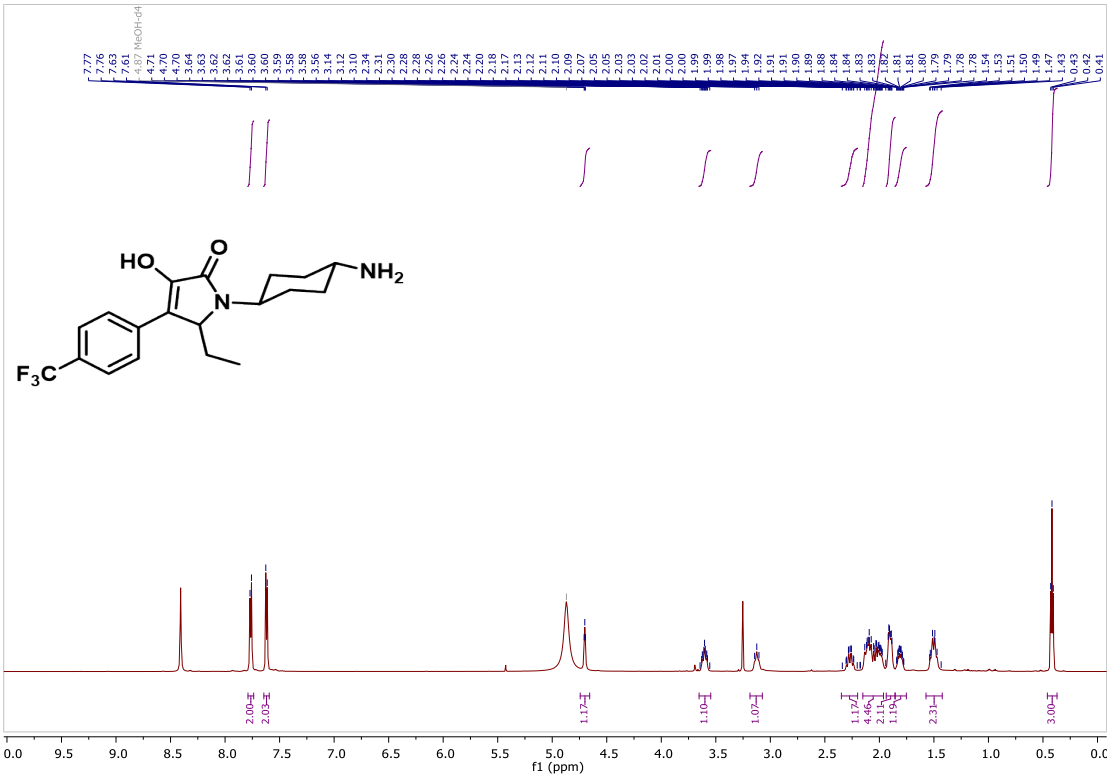
^{1}H NMR (600 MHz, Methanol-d_4) 1-(6-Aminohexyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (18)



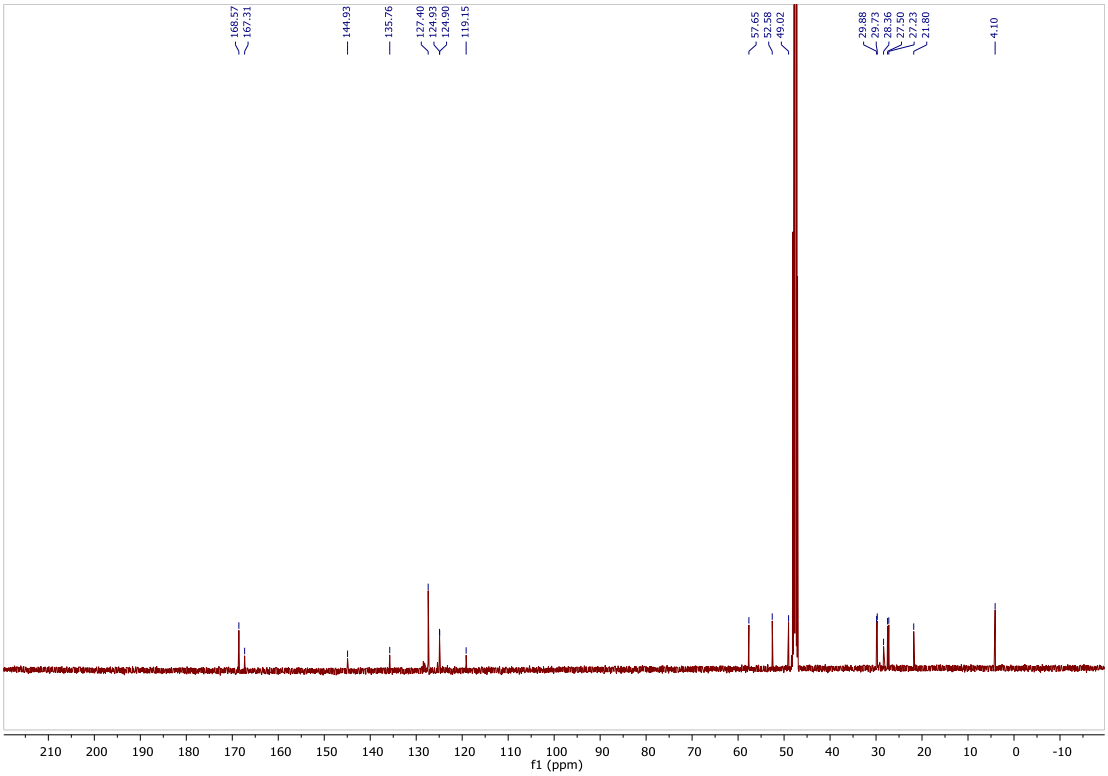
^{13}C NMR (151 MHz, Methanol-d_4) 1-(6-Aminohexyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (18)



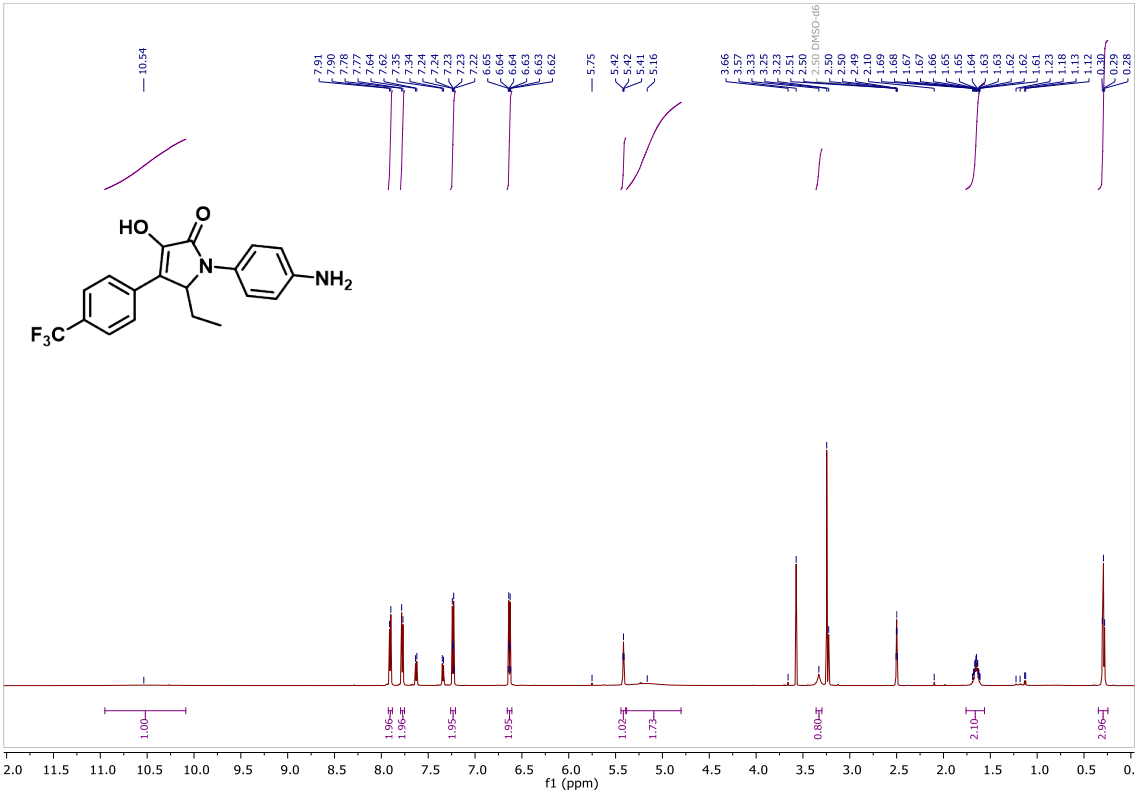
^{1}H NMR (600 MHz, Methanol-d_4) 1-((1r,4r)-4-Aminocyclohexyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (19)



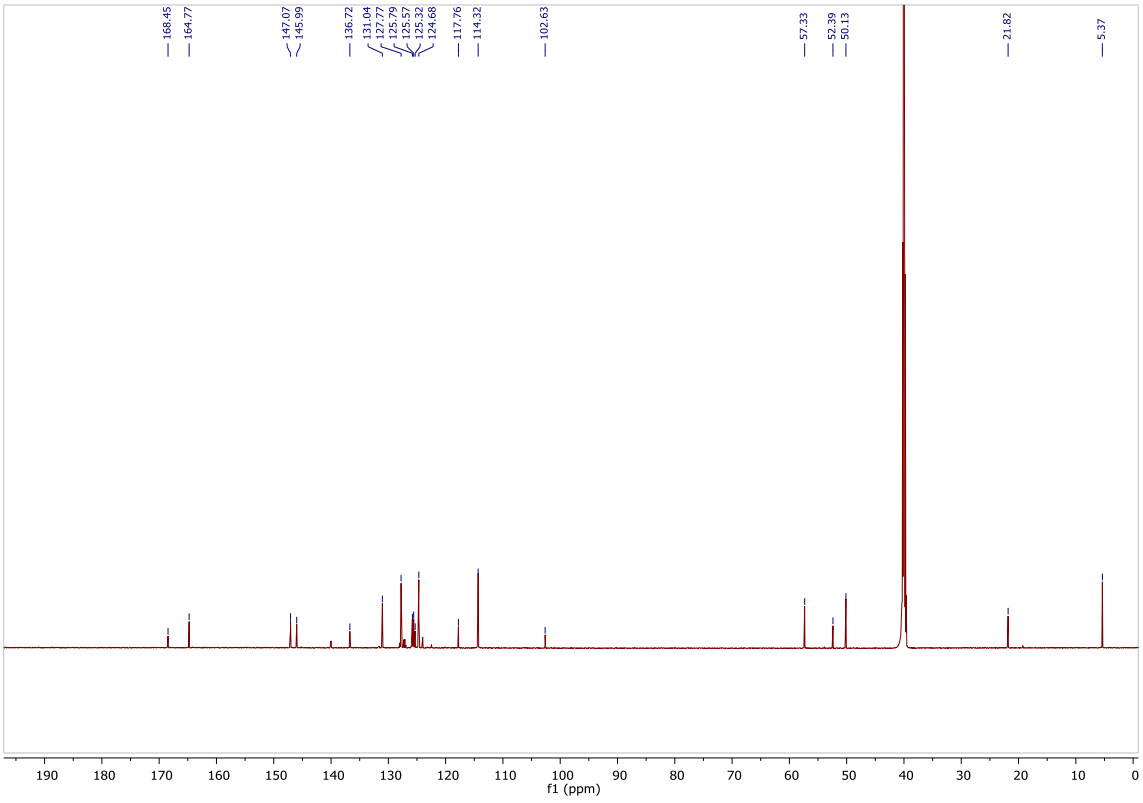
^{13}C NMR (126 MHz, Methanol-d_4) 1-((1r,4r)-4-Aminocyclohexyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (19)



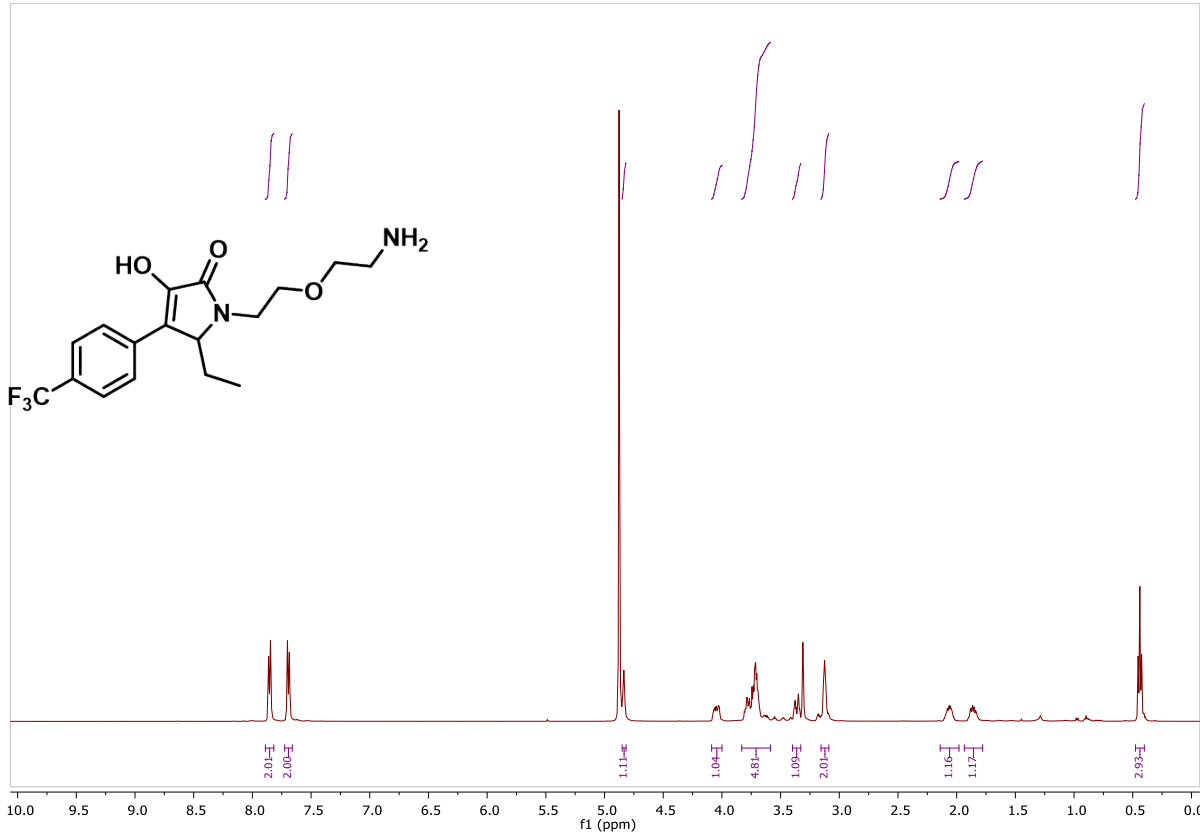
^{1}H NMR (600MHz, DMSO-*d6*) 1-(4-Aminophenyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (20)



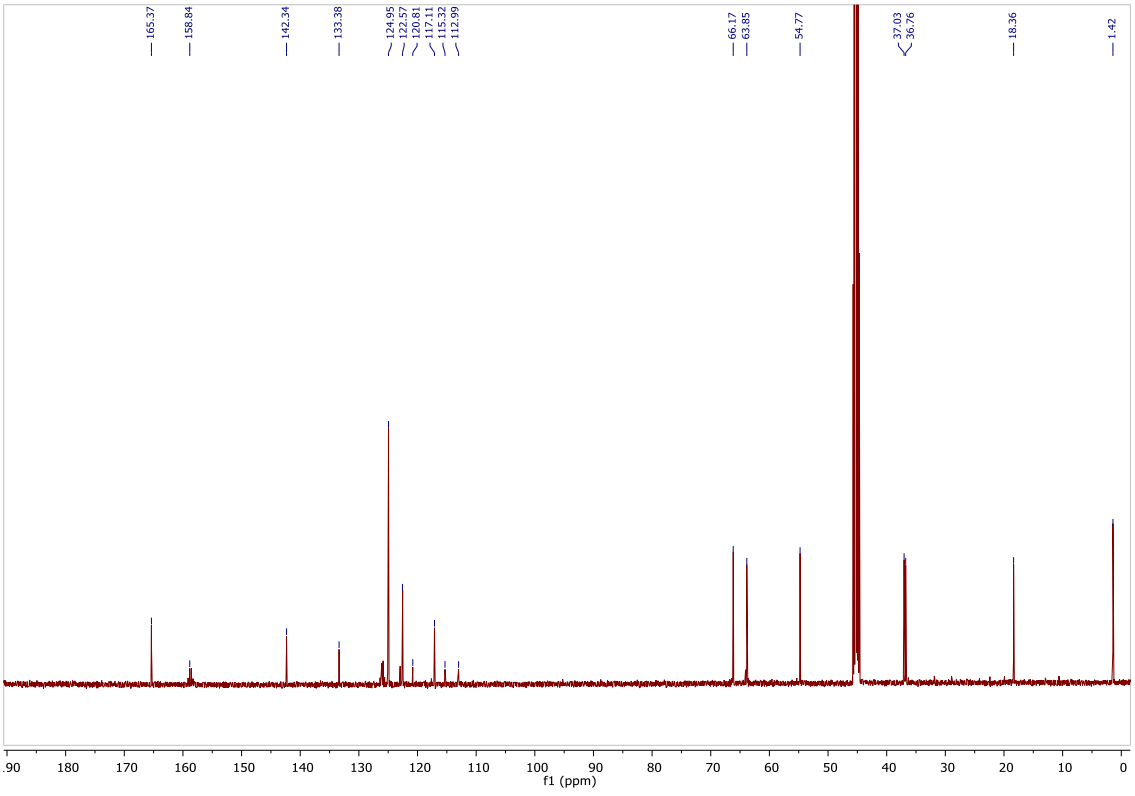
^{13}C NMR (176 MHz, DMSO-*d6*) 1-(4-Aminophenyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl) phenyl)-1,5-dihydro-2H-pyrrol-2-one (20)



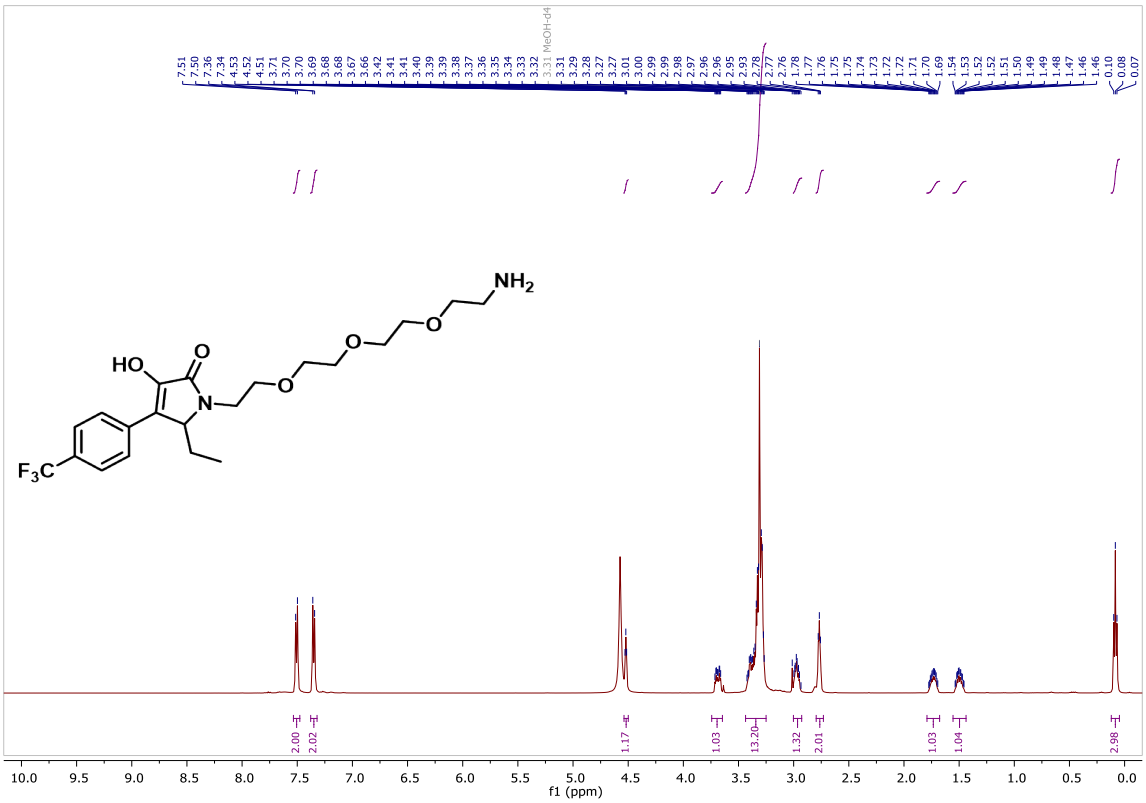
^{1}H-NMR (500 MHz, Methanol-d_4) 1-(2-(2-Aminoethoxy)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (21)



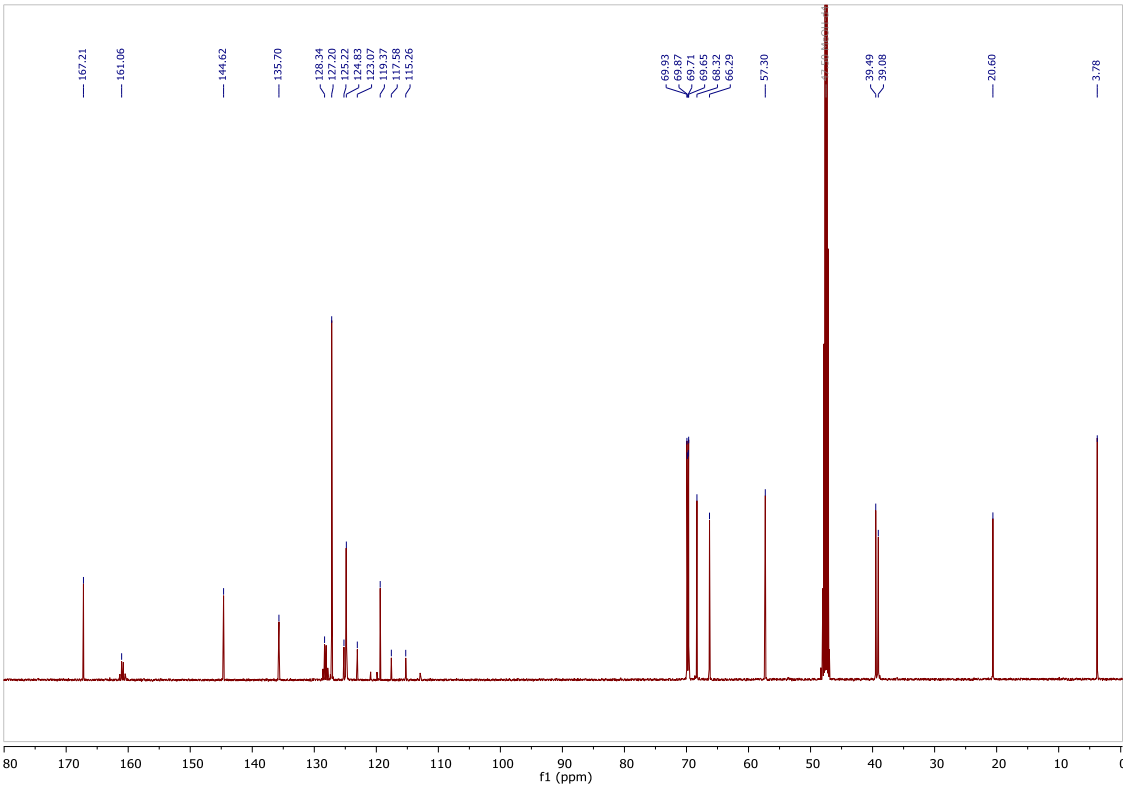
^{13}C NMR (126 MHz) 1-(2-(2-Aminoethoxy)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (21)



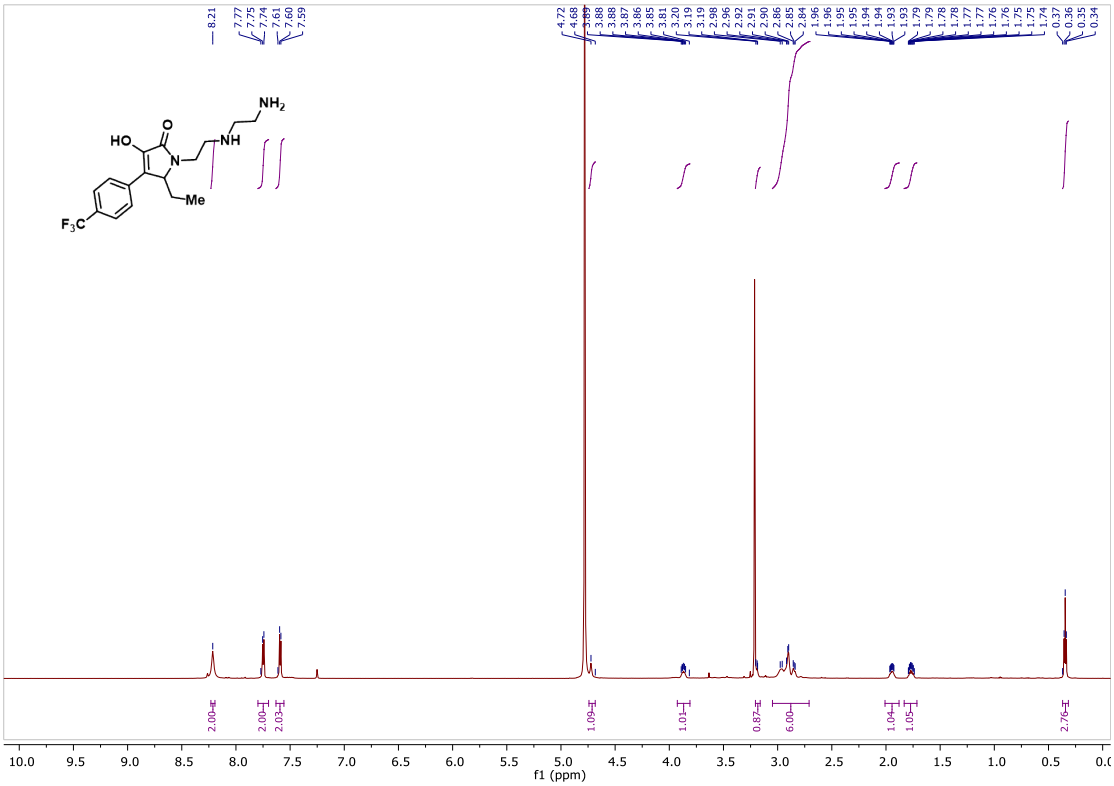
^{1}H NMR (500 MHz, Methanol-d_4) 1-(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (22)



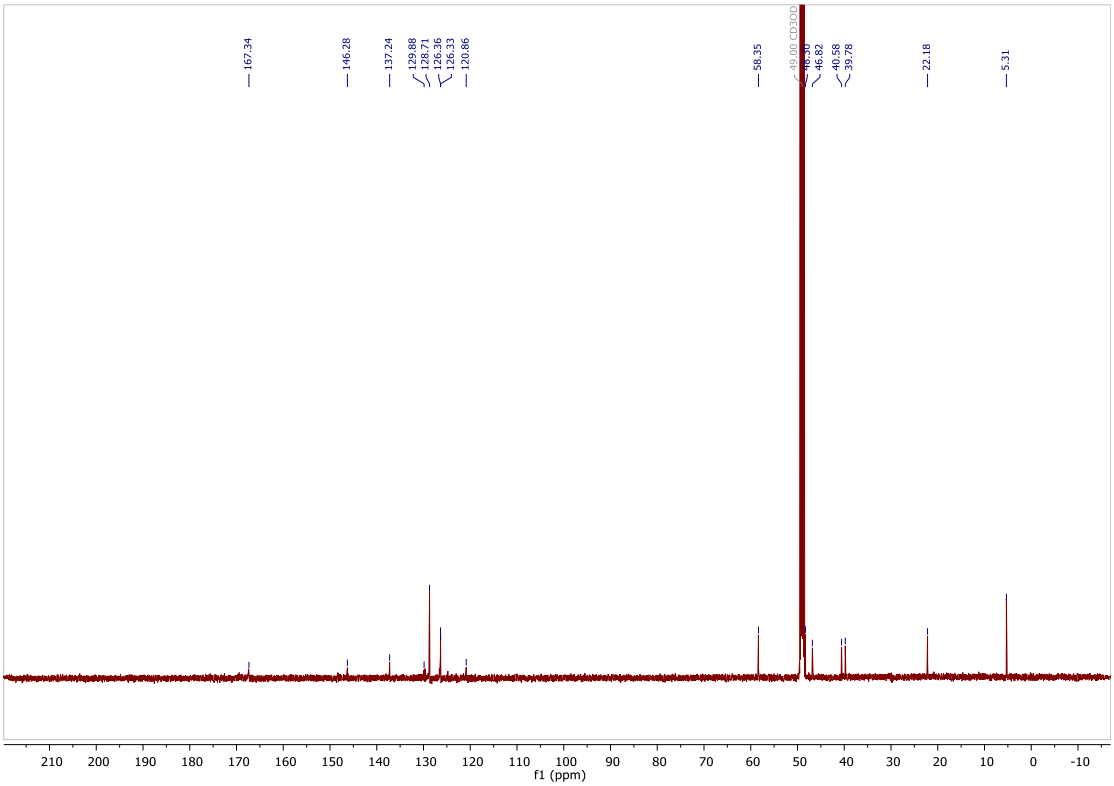
^{13}C NMR (126 MHz, Methanol-d_4) 1-(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (22)



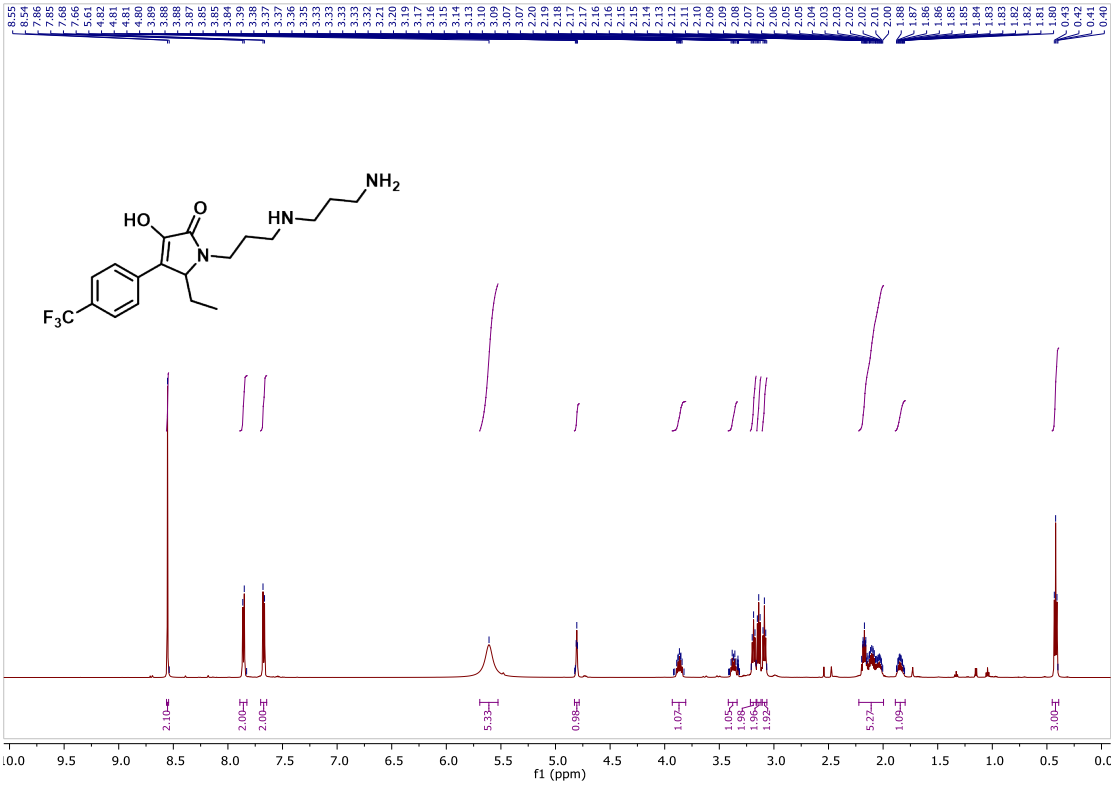
^{1}H NMR (700 MHz, Methanol-d_4) 1-(2-((2-aminoethyl)amino)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (23)



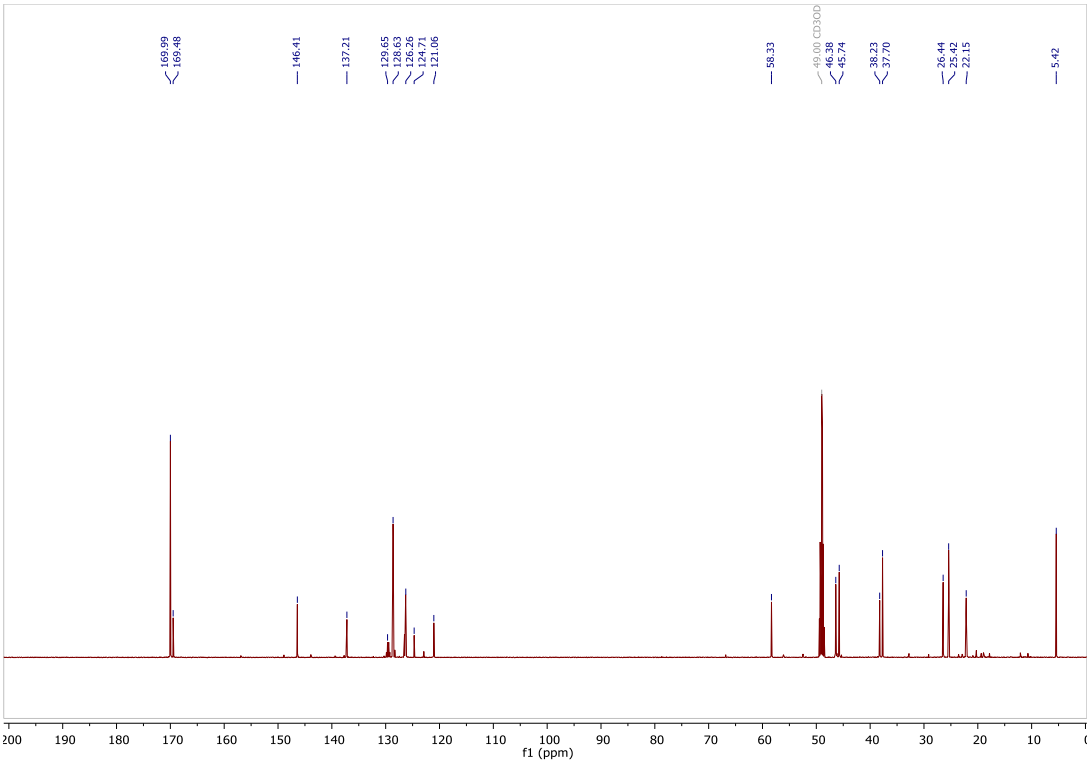
^{13}C NMR (151 MHz, Methanol-d_4) 1-(2-((2-aminoethyl)amino)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (23)



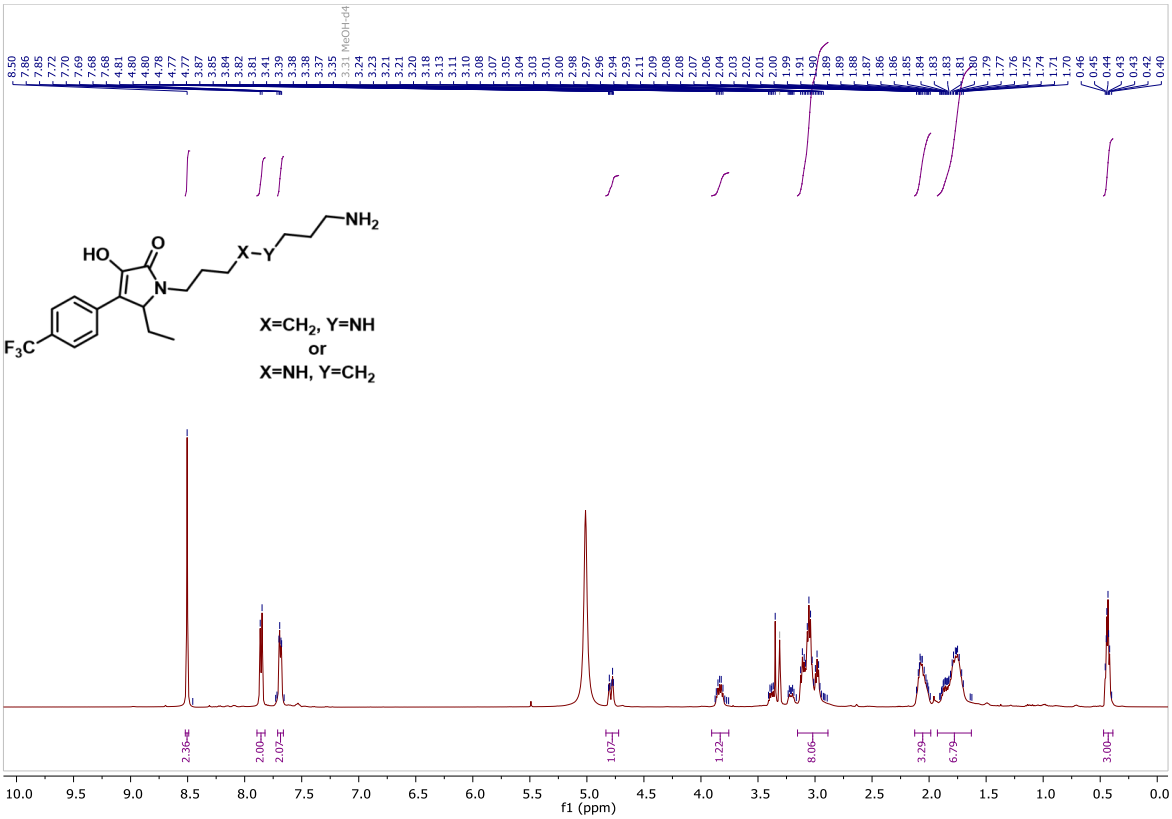
^{1}H NMR (600 MHz, Methanol-d_4) **1-(3-((3-aminopropyl)amino)propyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (24)**



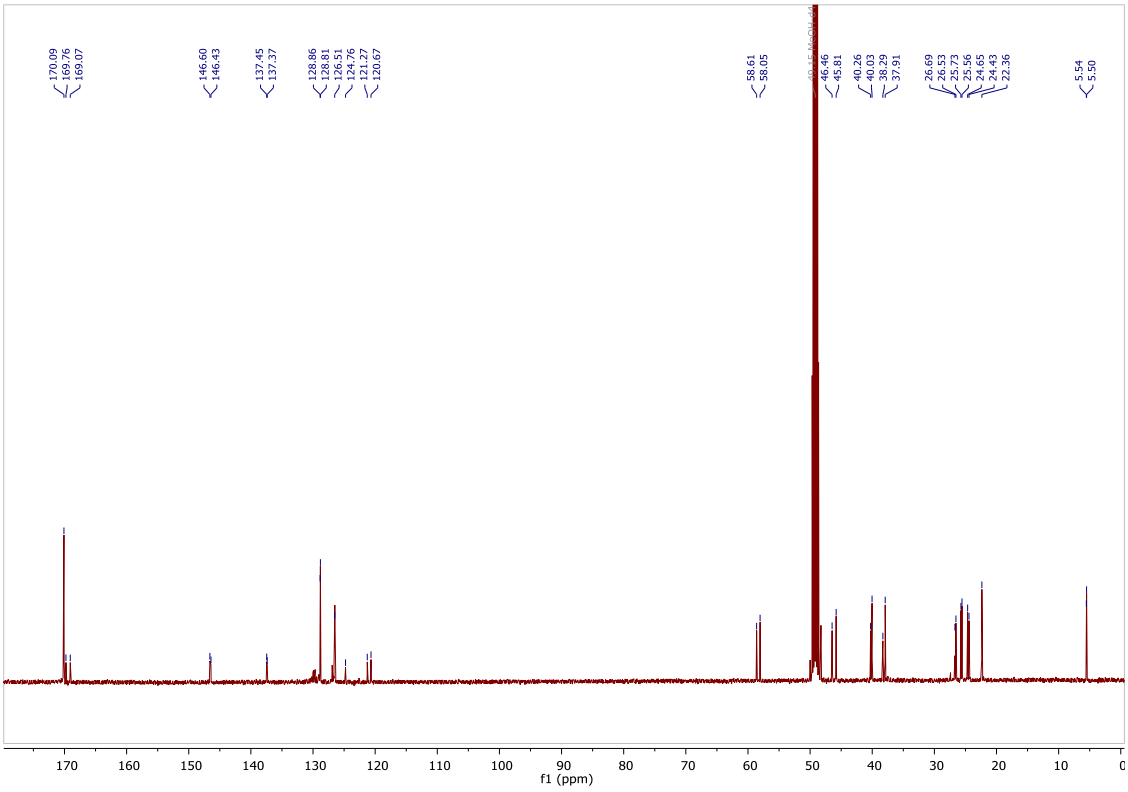
^{13}C NMR (151 MHz, Methanol-d_4) **1-(3-((3-aminopropyl)amino)propyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (24)**



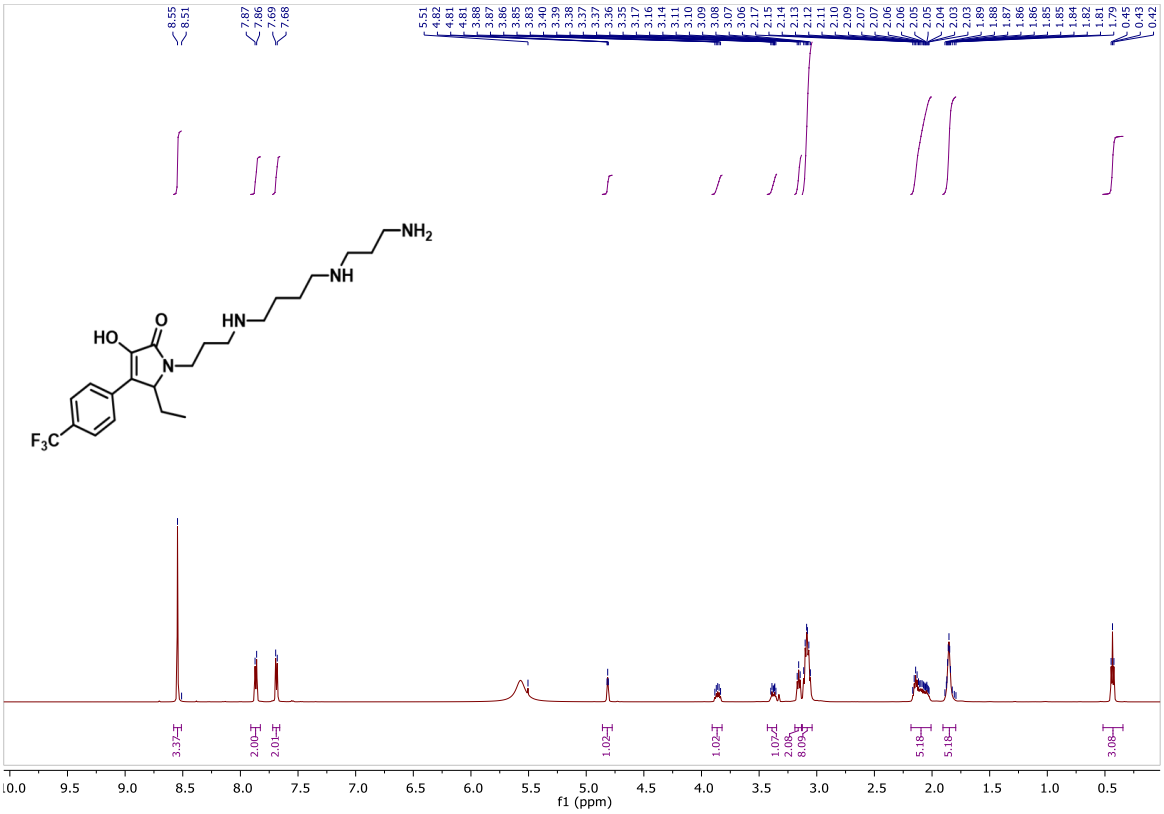
^{1}H NMR (500 MHz, Methanol-d_4) 1-(3-((4-Aminobutyl)amino)propyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (25)



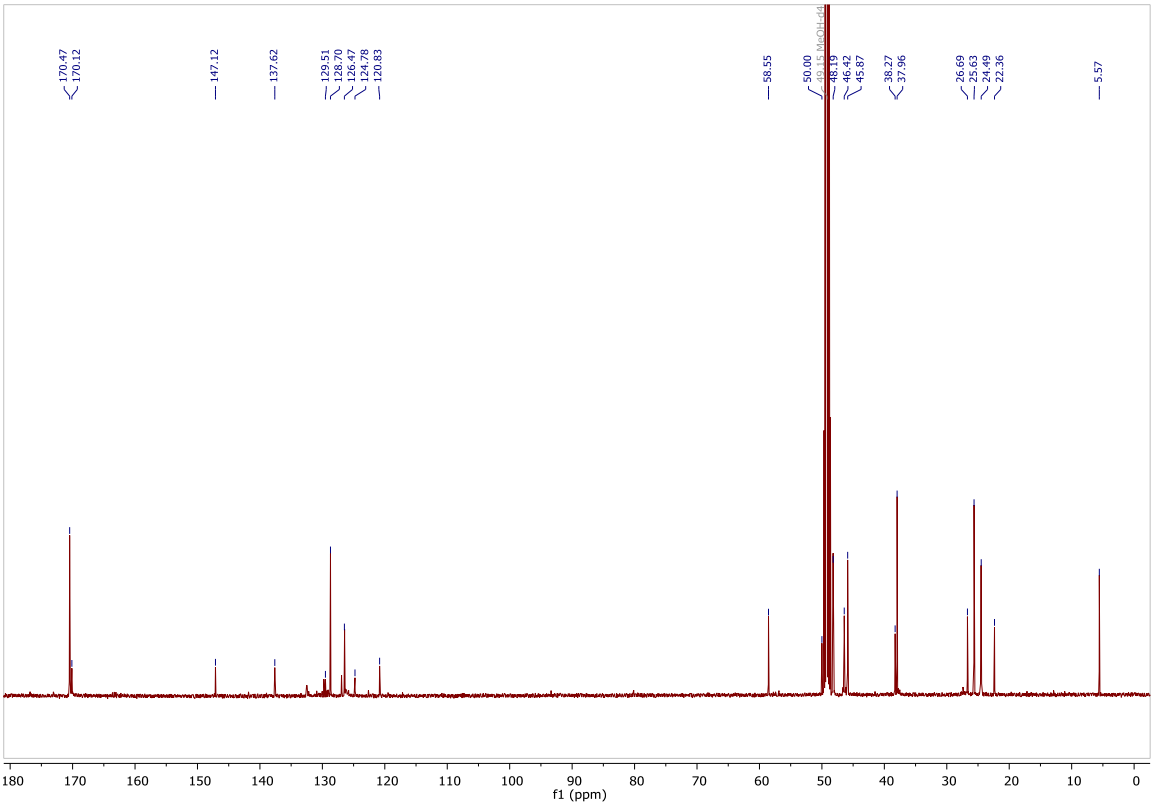
^{13}C NMR (126 MHz, Methanol-*d4*) 1-(3-((4-Aminobutyl)amino)propyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (25)



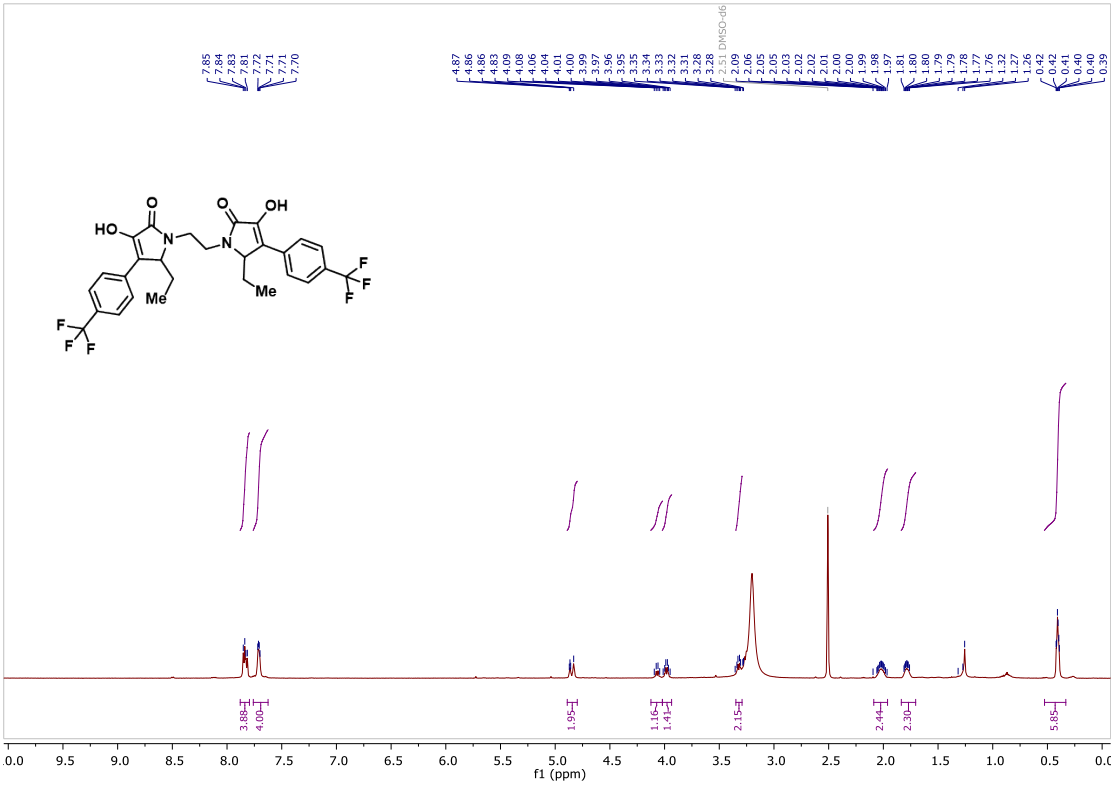
^{1}H NMR (600 MHz, Methanol-d_4) 1-(3-((4-((3-Aminopropyl)amino)butyl)amino)propyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (26)



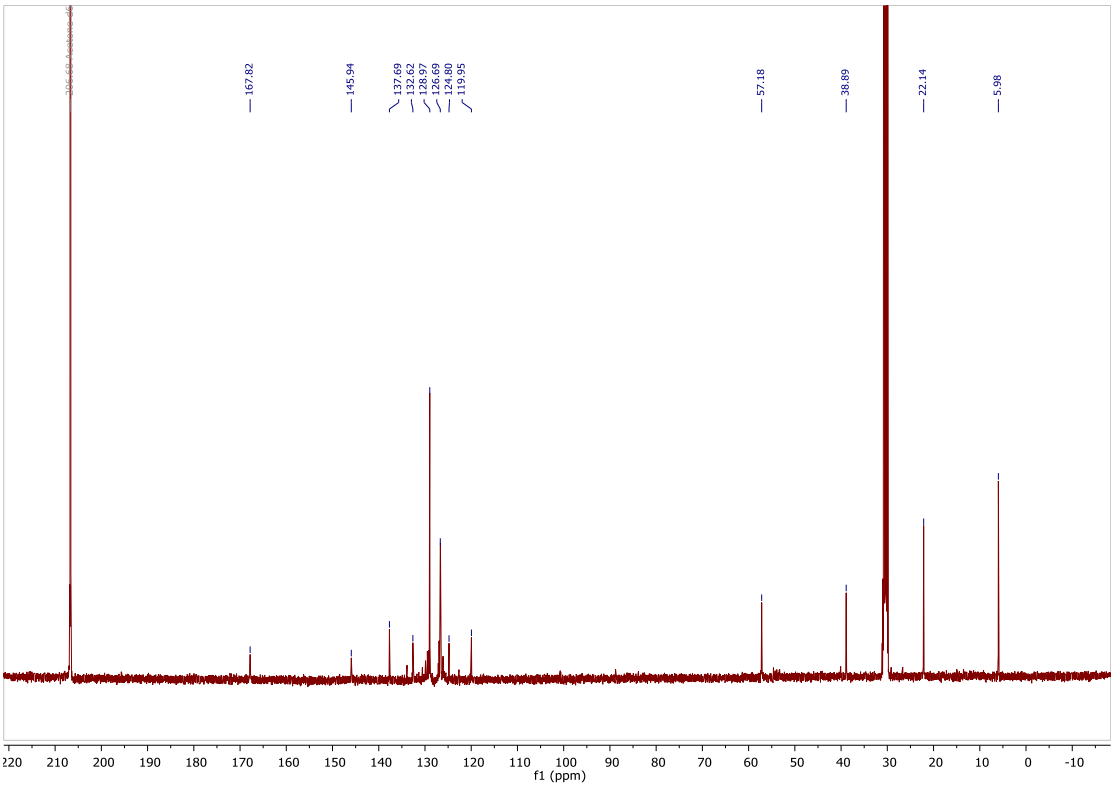
^{13}C NMR (126 MHz, methanol-*d4*) 1-(3-((4-((3-Aminopropyl)amino)butyl)amino)propyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (26)



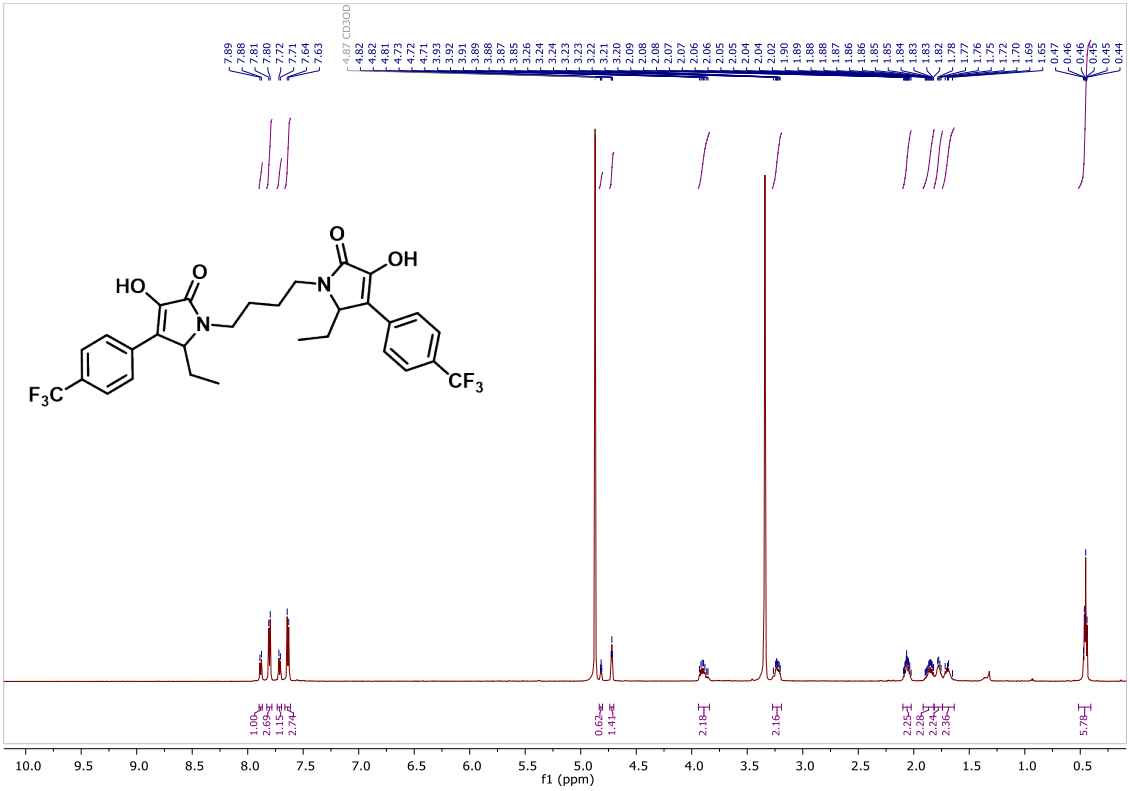
^{1}H NMR (600 MHz) 1,1'-(Ethane-1,2-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (27)



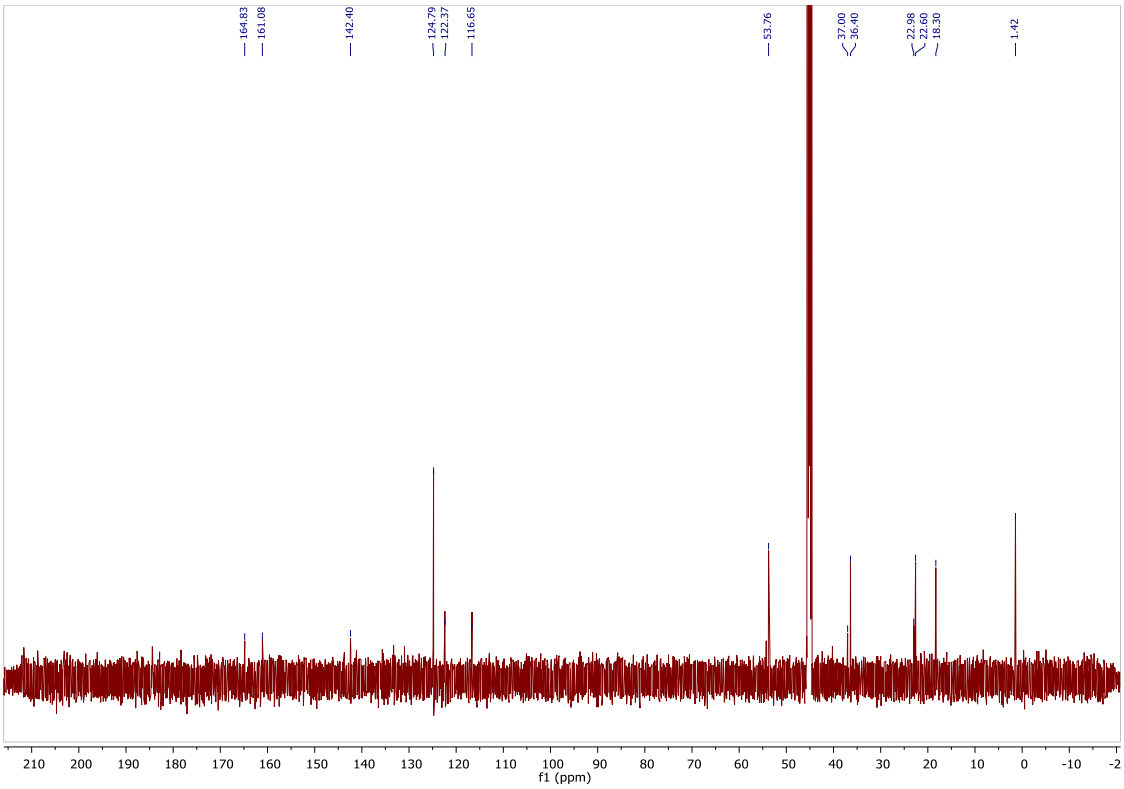
^{13}C NMR (151 MHz) 1,1'-(Ethane-1,2-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (27)



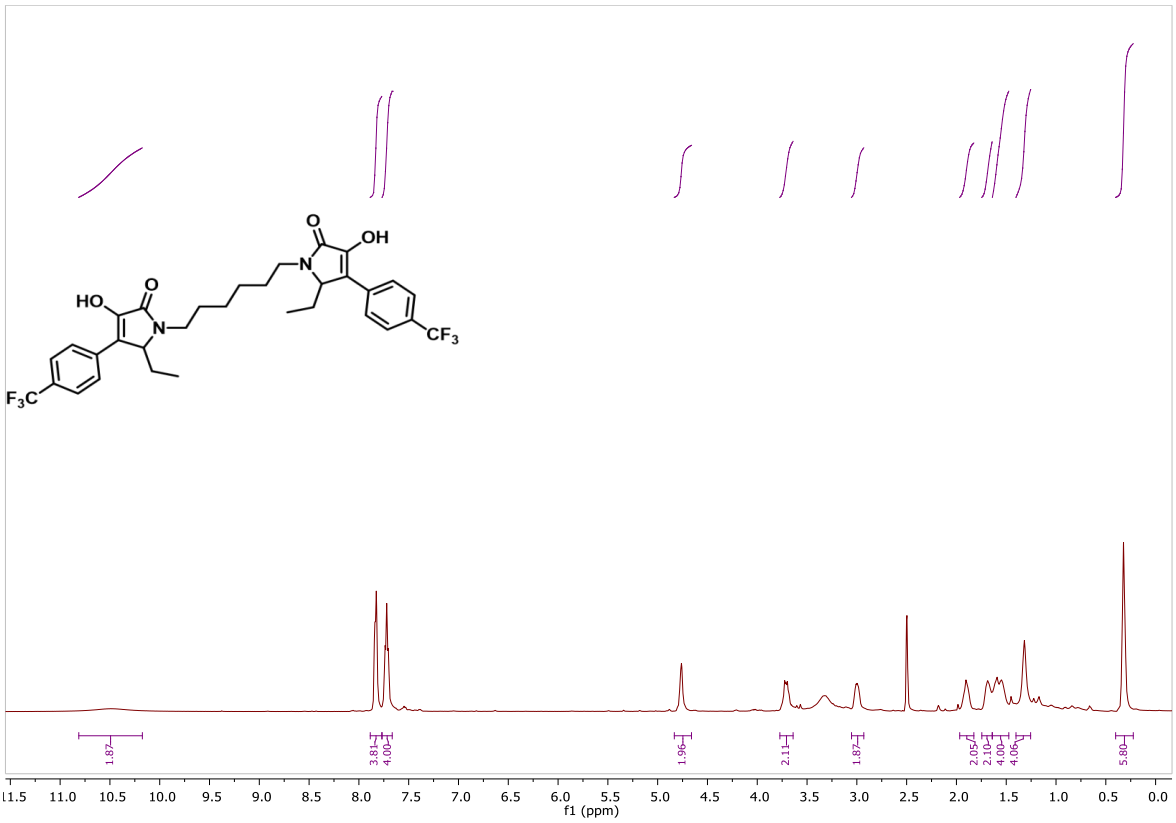
^{1}H NMR (600 MHz) 1,1'-(Butane-1,4-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (28)



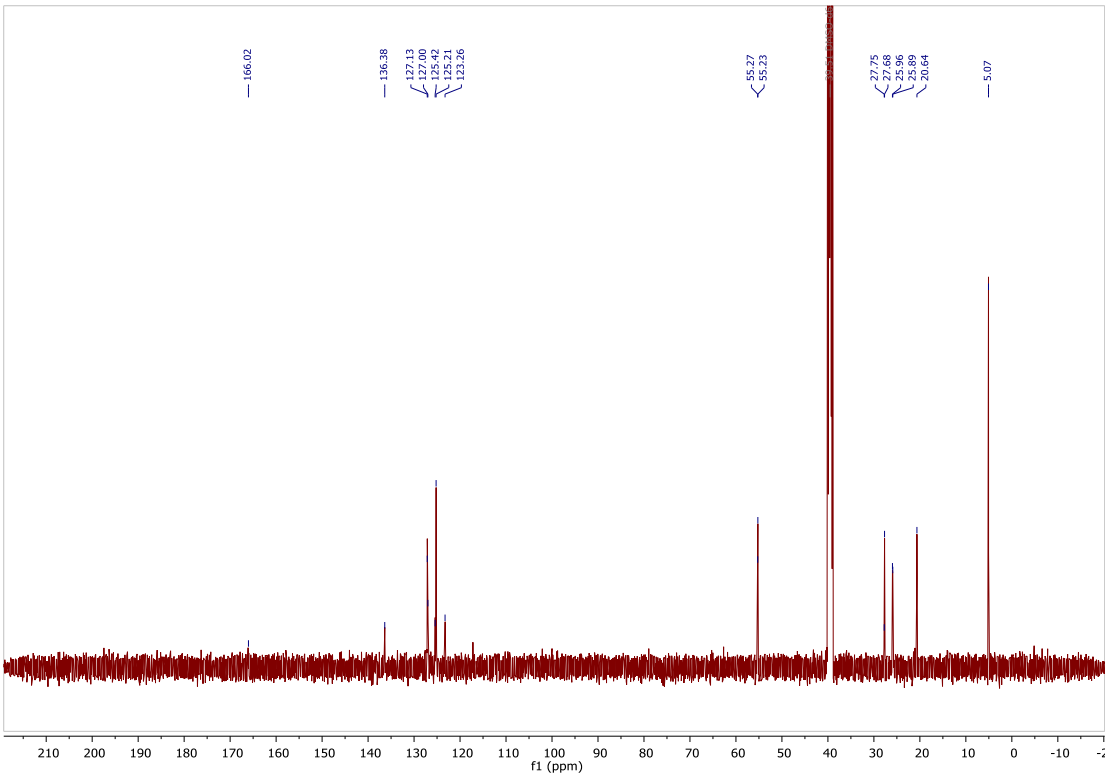
^{13}C NMR (151 MHz) 1,1'-(Butane-1,4-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (28)



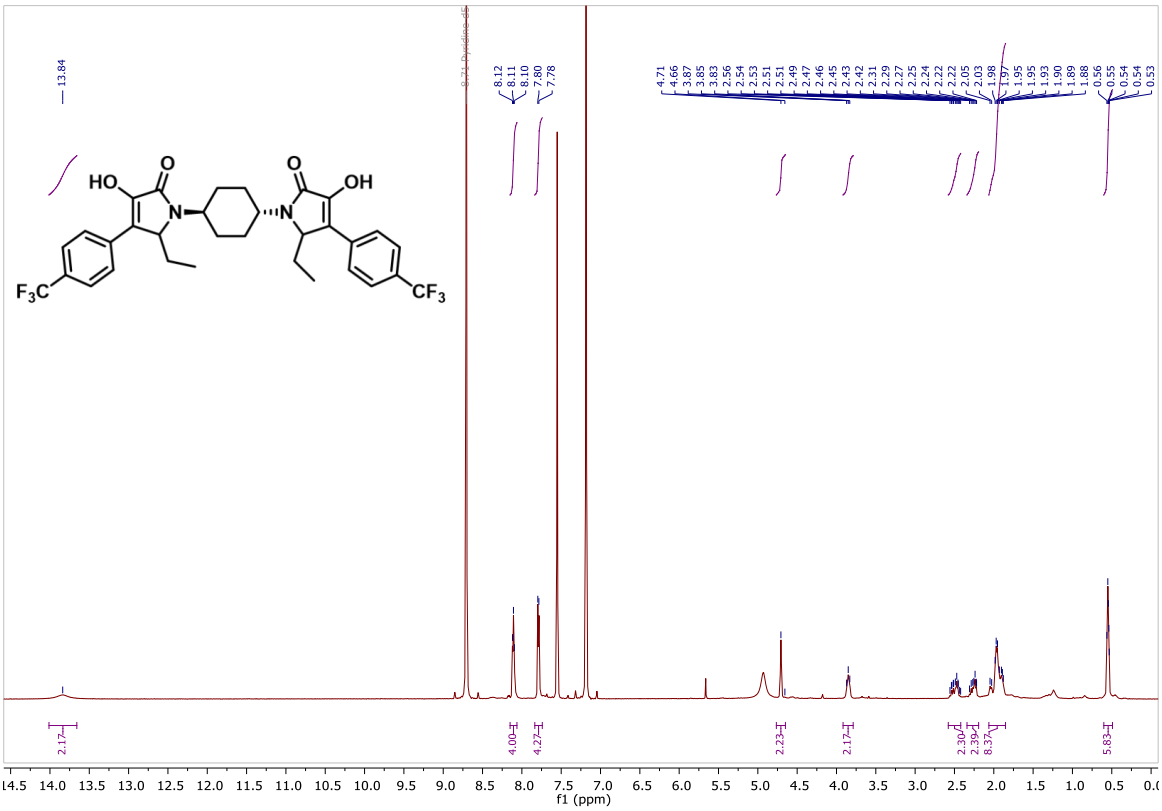
^{1}H NMR (500 MHz, DMSO-d_6) 1,1'-(Hexane-1,6-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (29)



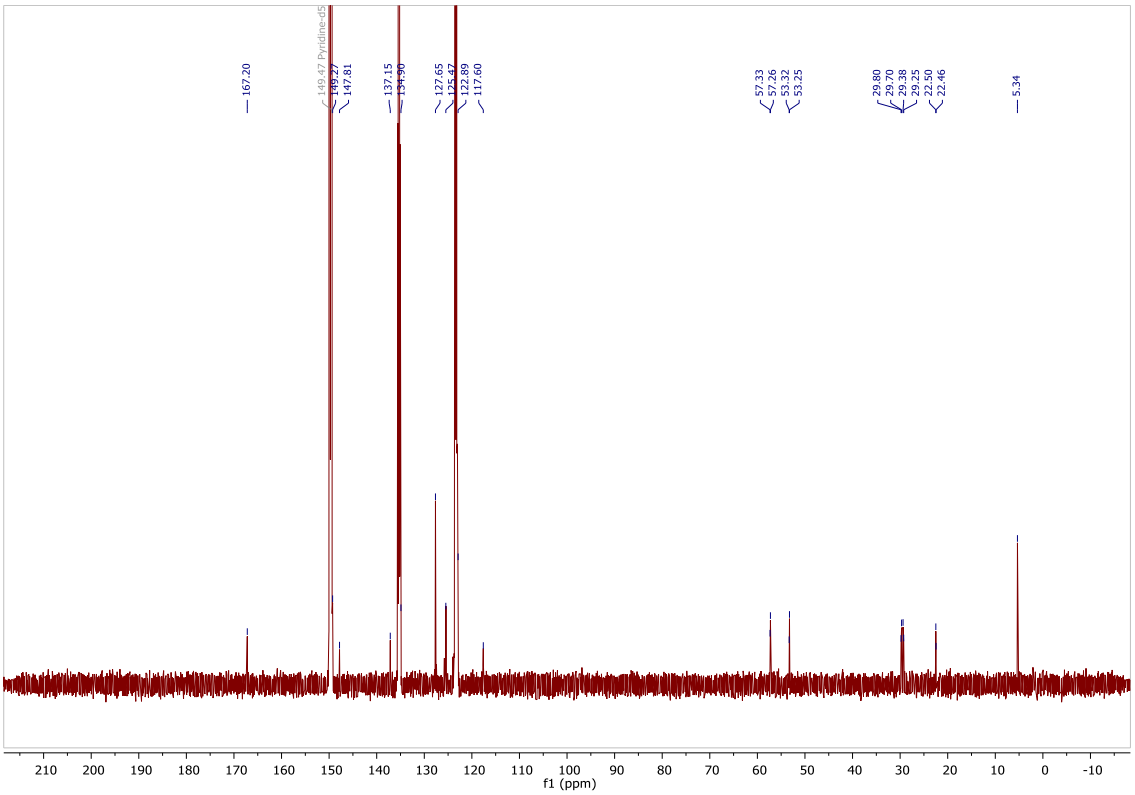
^{13}C NMR (126 MHz, DMSO-d_6) 1,1'-(Hexane-1,6-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (29)



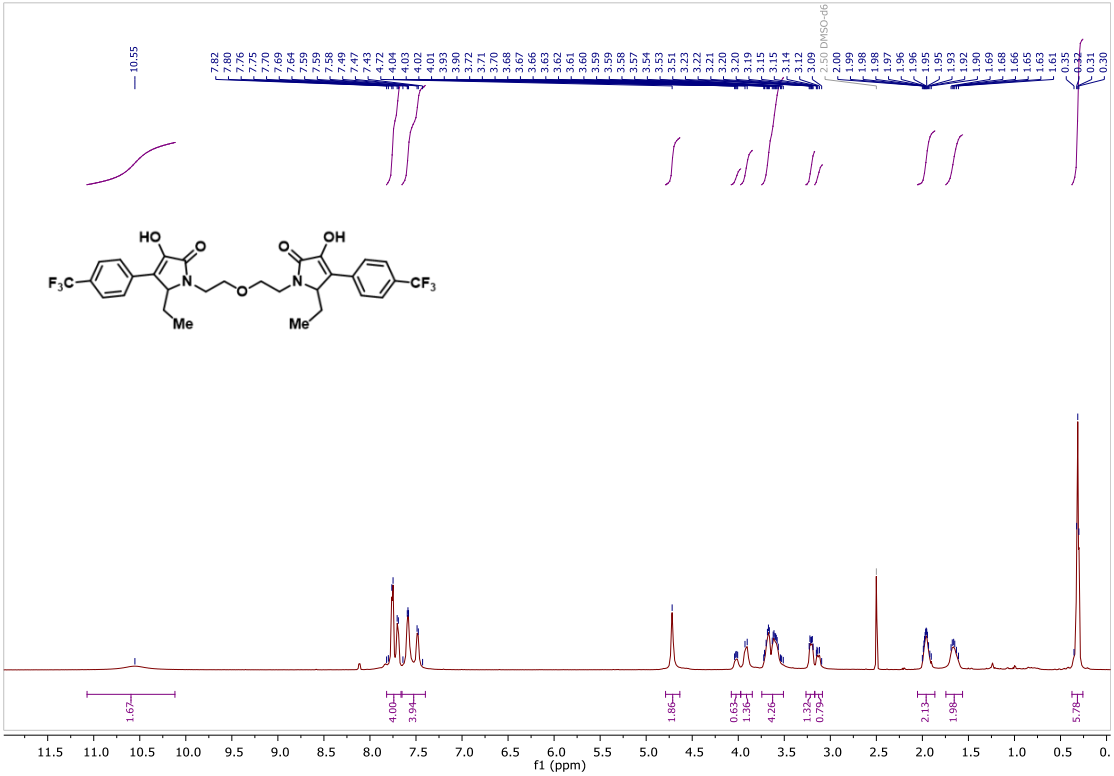
^{1}H NMR (600 MHz, Pyridine-d_5) 1,1'-((1r,4r)-Cyclohexane-1,4-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (30)



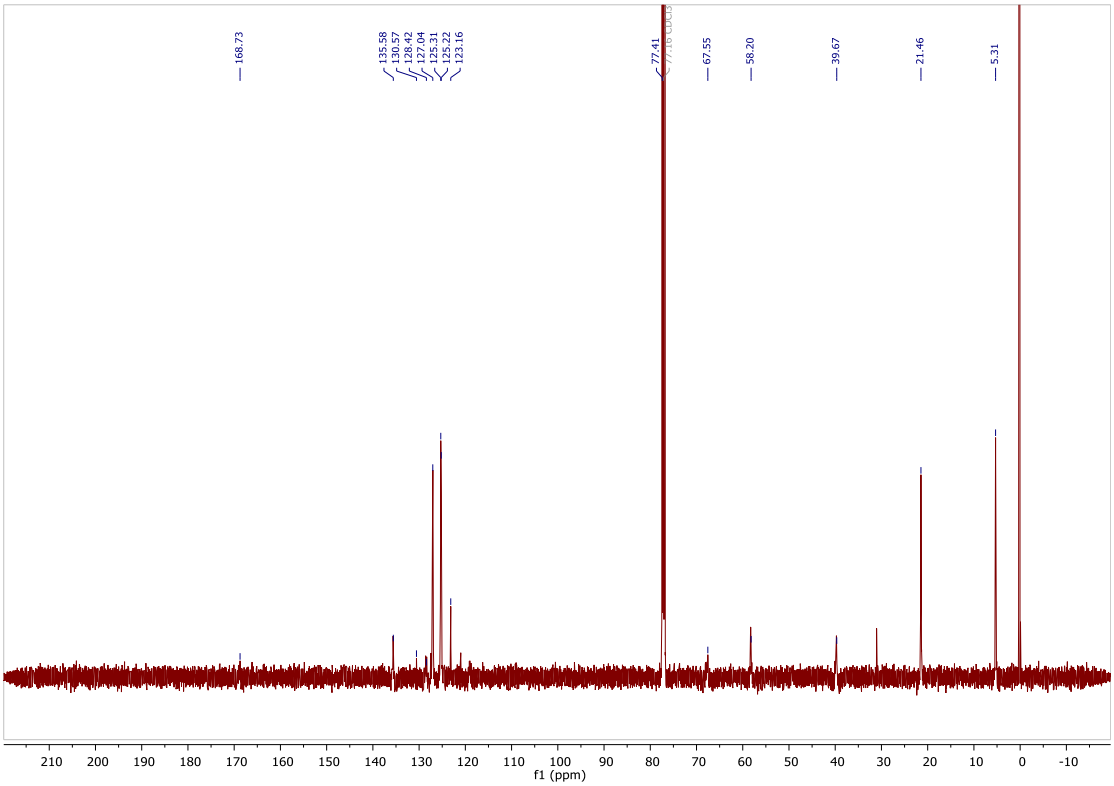
^{13}C NMR (151 MHz, Pyridine-d_5) 1,1'-((1r,4r)-Cyclohexane-1,4-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (30)



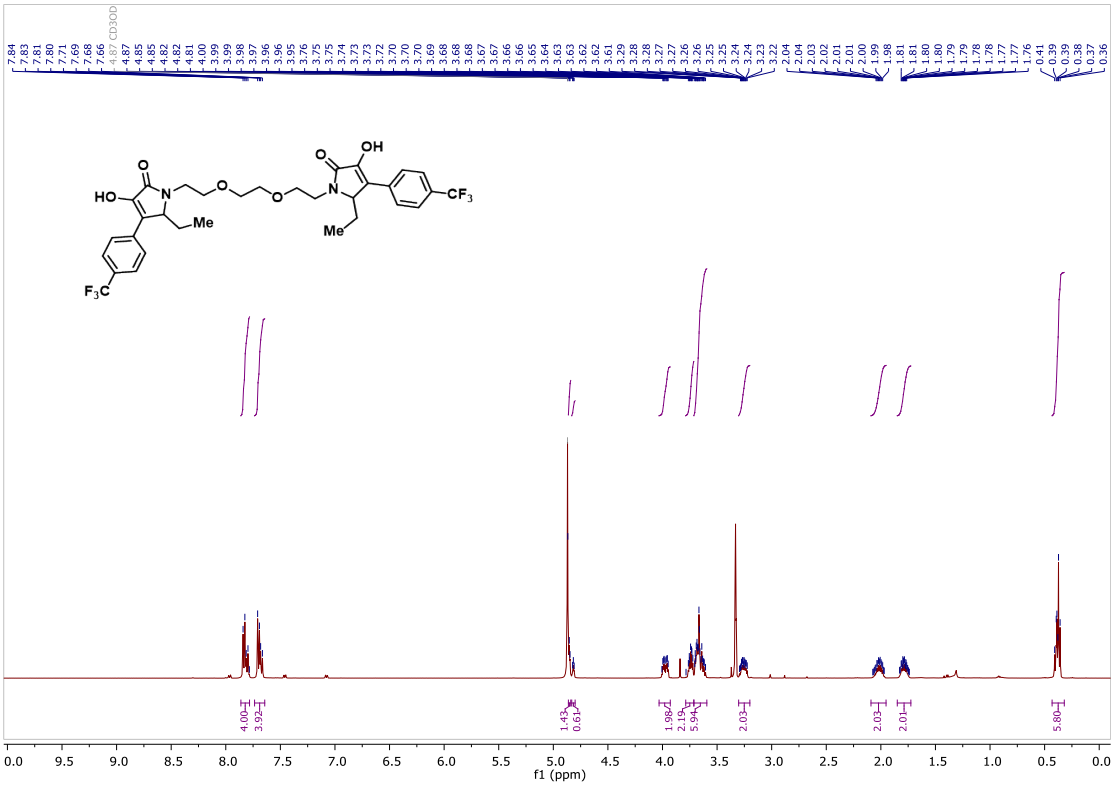
^{1}H NMR (600 MHz, DMSO-d_6) 1,1'-(Oxybis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (33)



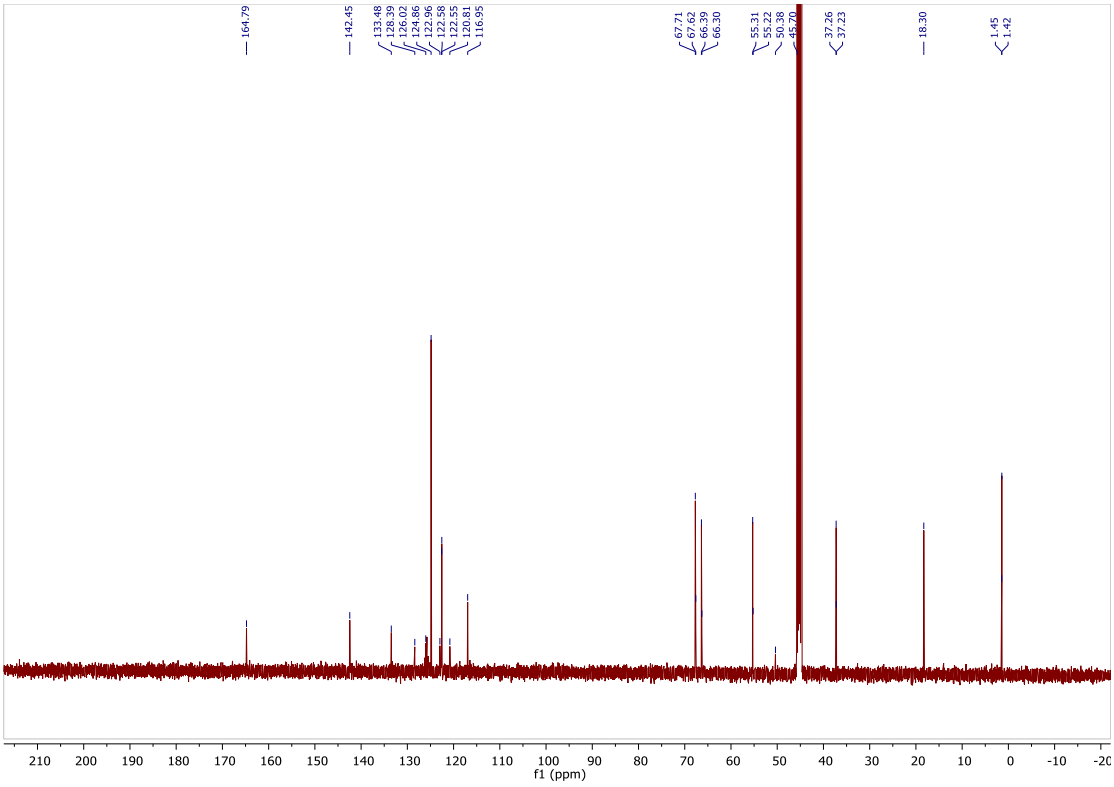
^{13}C NMR (126 MHz, Chloroform-*d*) 1,1'-(Oxybis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (33)



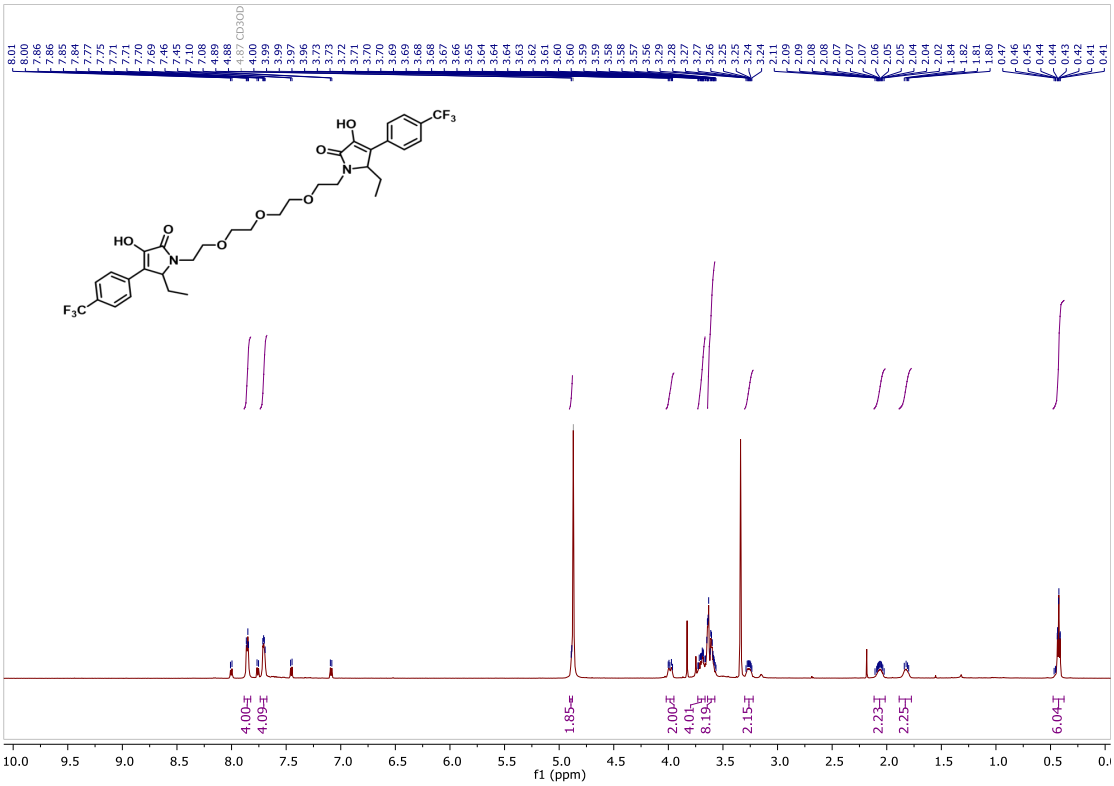
^{1}H NMR (500 MHz, Methanol-d_4) 1,1'-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (34)



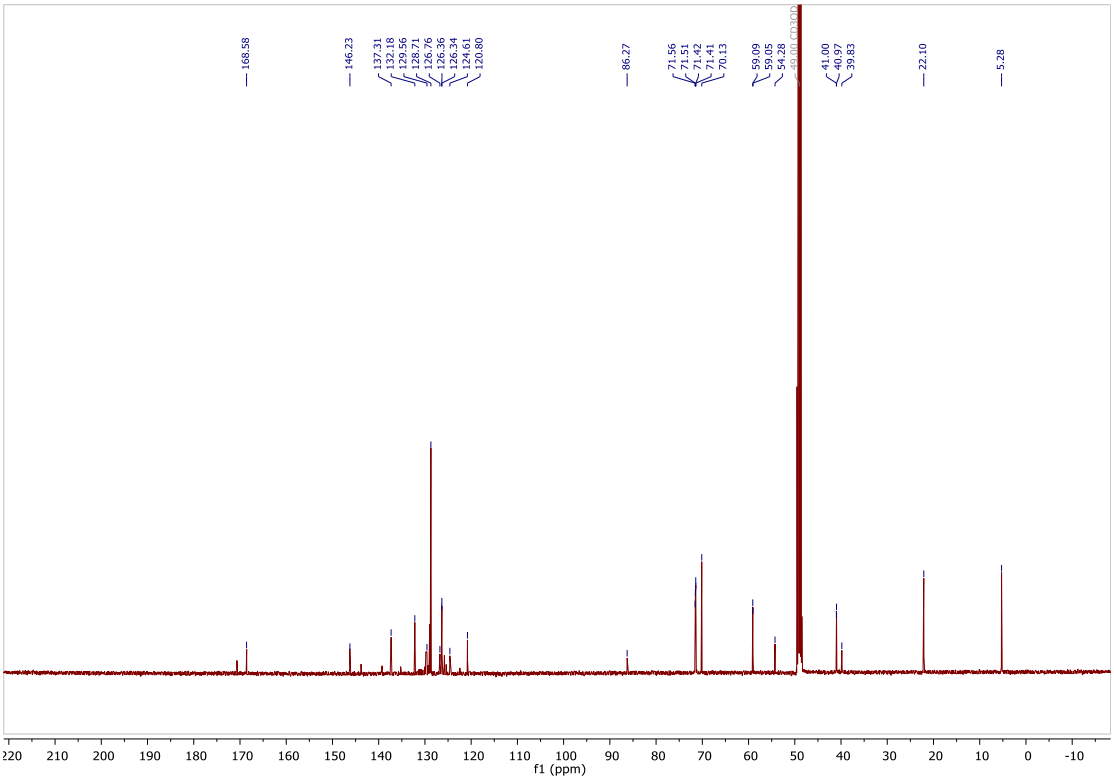
^{13}C NMR (126 MHz, Methanol-d_4) 1,1'-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (34)



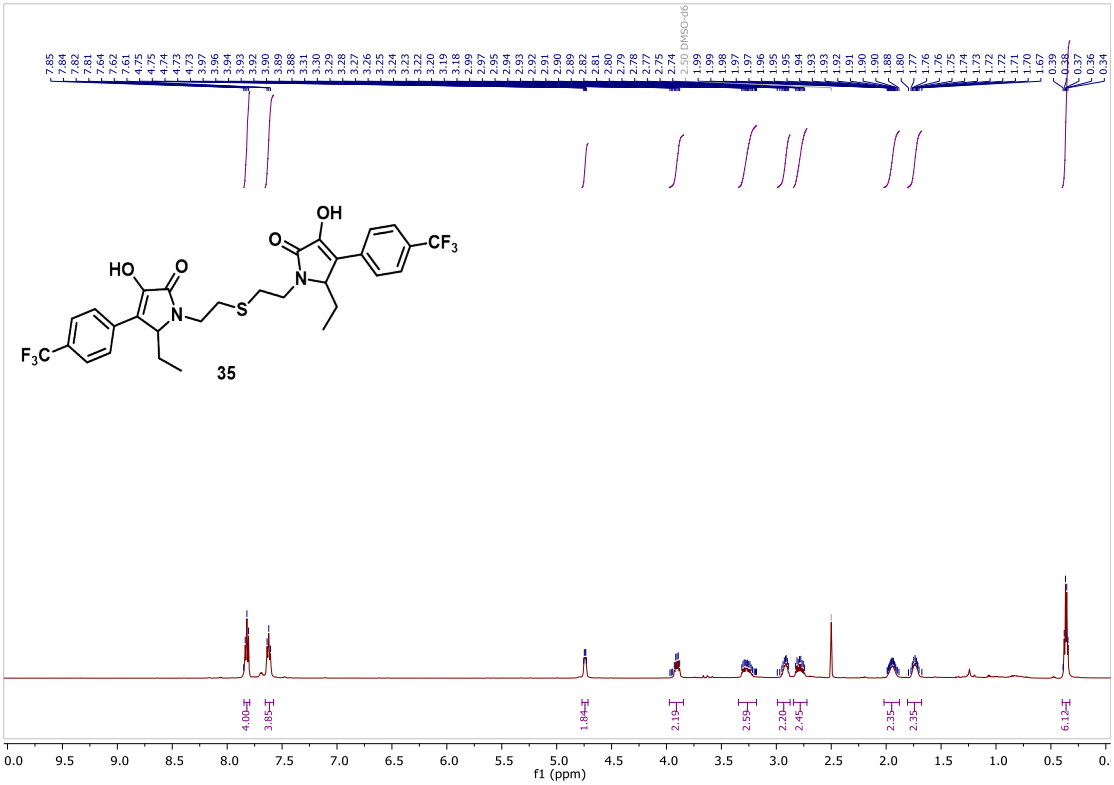
^{1}H NMR (600 MHz, Methanol-d_4) 1,1'-(((Oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (35)



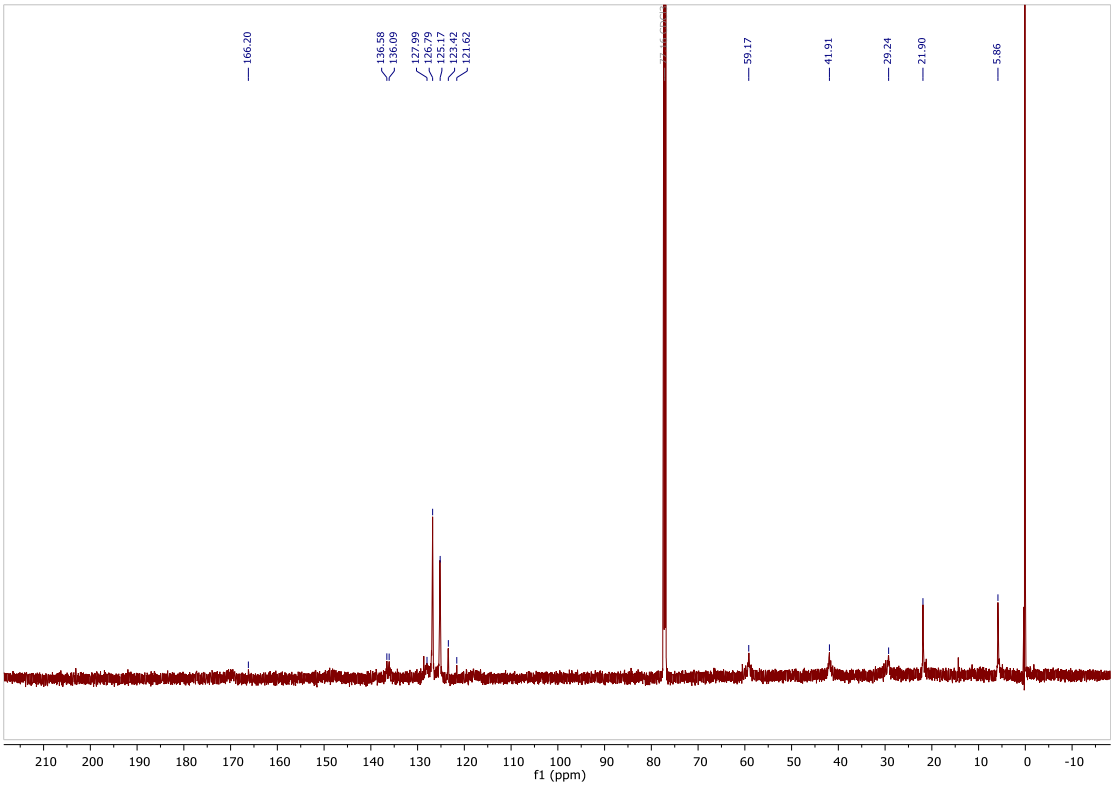
^{13}C NMR (126 MHz, Methanol-d_4) 1,1'-(((Oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (35)



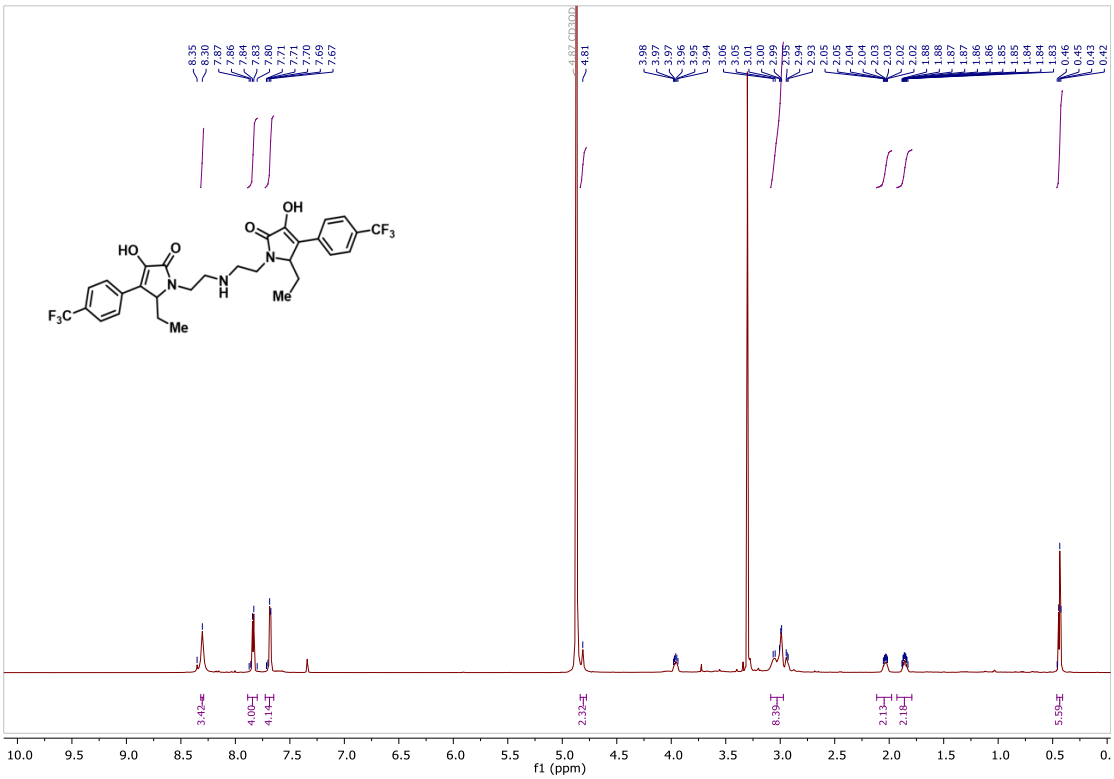
^{1}H NMR (600 MHz, DMSO-d_6, VT, 45 °C) 1,1'-(thiobis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (36)



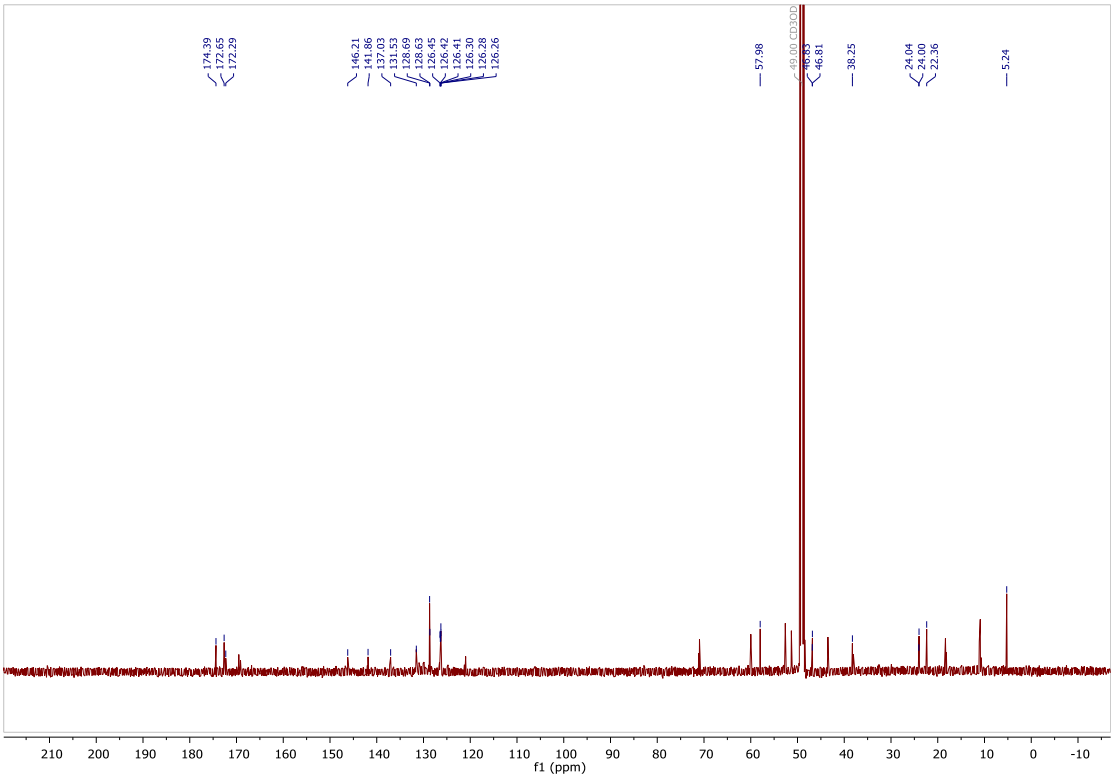
^{13}C NMR (151 MHz, Chloroform-d) 1,1'-(thiobis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (36)



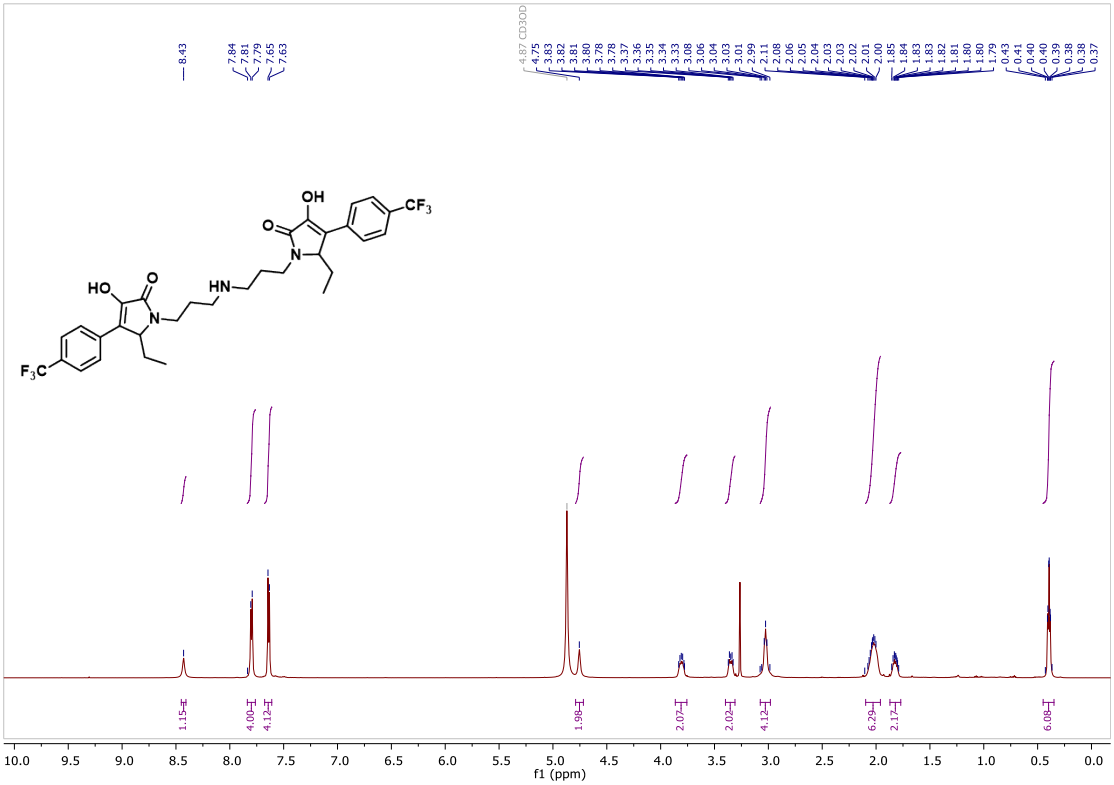
^{1}H NMR (700 MHz, Methanol-d$_4$) 1,1'-(azanediylbis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (37)



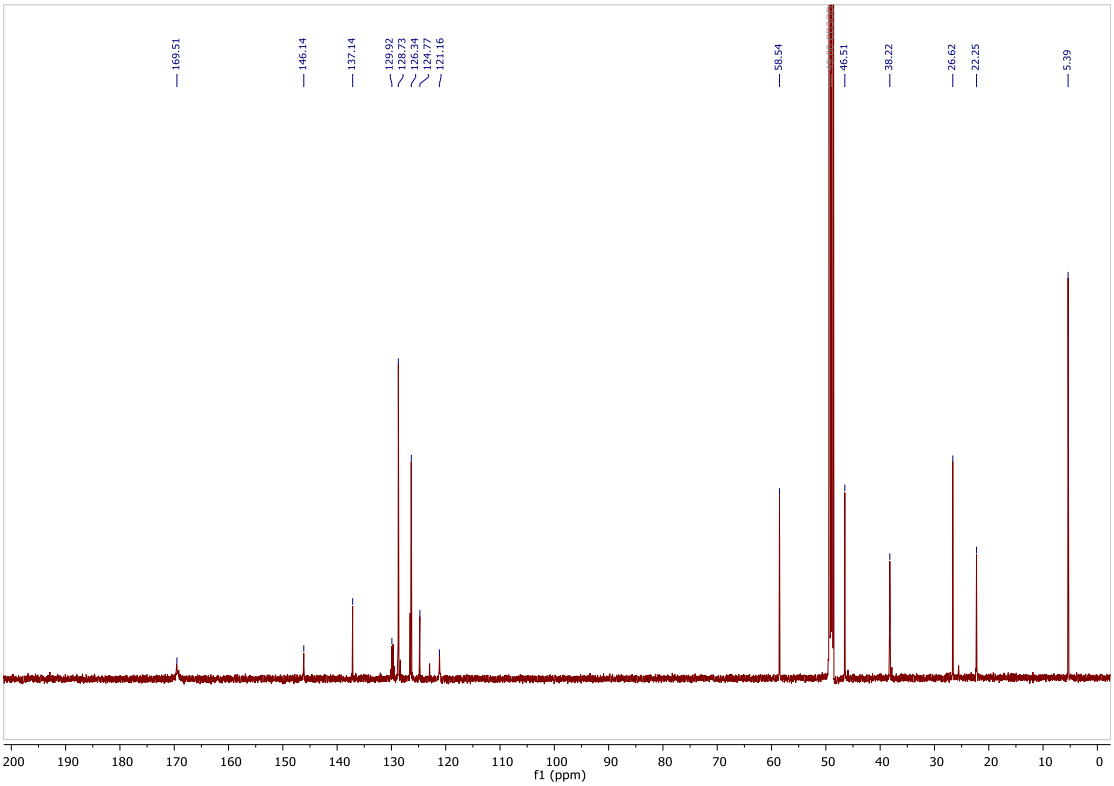
^{13}C NMR (151 MHz, Methanol-d$_4$) 1,1'-(azanediylbis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (37)



^{1}H NMR (600 MHz, Methanol-d_4) **1,1'-(azanediylbis(propane-3,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (38)**

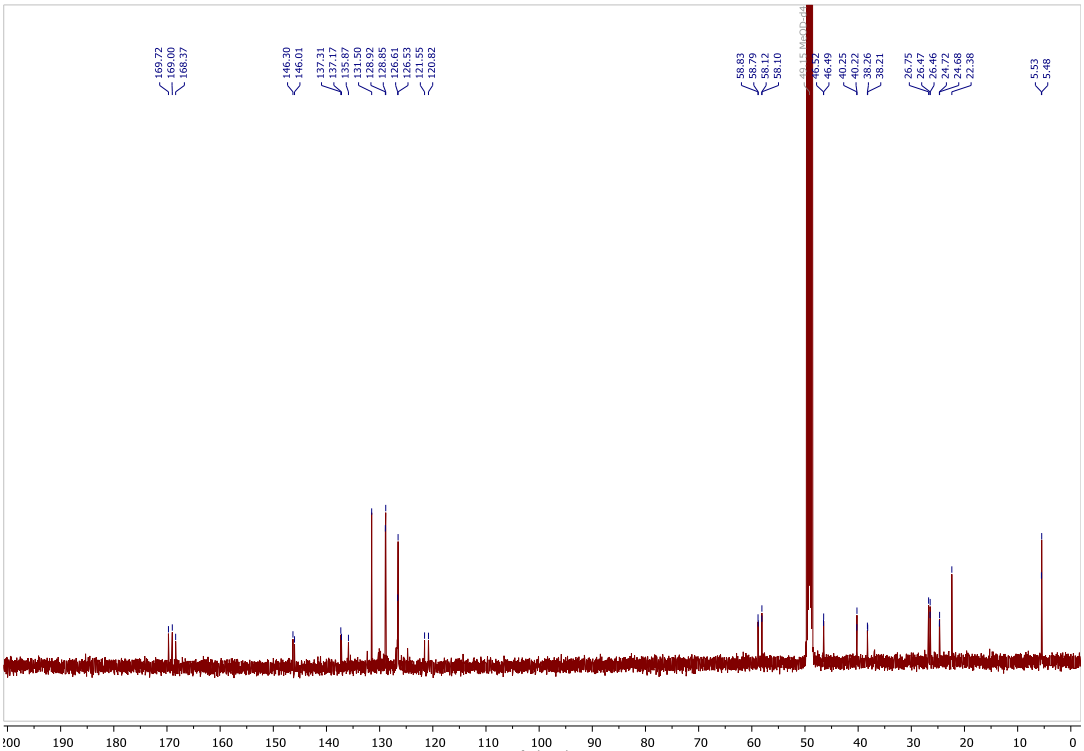


^{13}C NMR (151 MHz, Methanol-d_4) **1,1'-(azanediylbis(propane-3,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (38)**

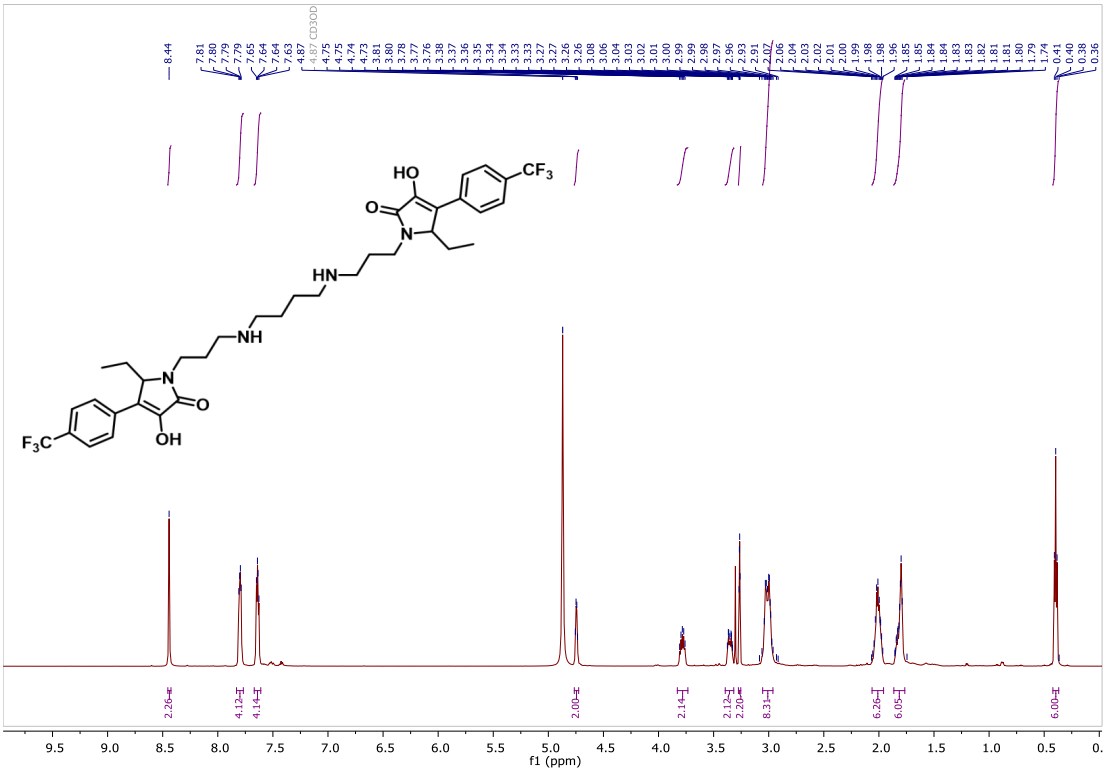


^{1}H NMR ( ) 5-Ethyl-1-(3-((4-(2-ethyl-4-hydroxy-5-oxo-3-(4-(trifluoromethyl)phenyl)-2,5-dihydro-1H-pyrrol-1-yl)butyl)amino)propyl)-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (39)

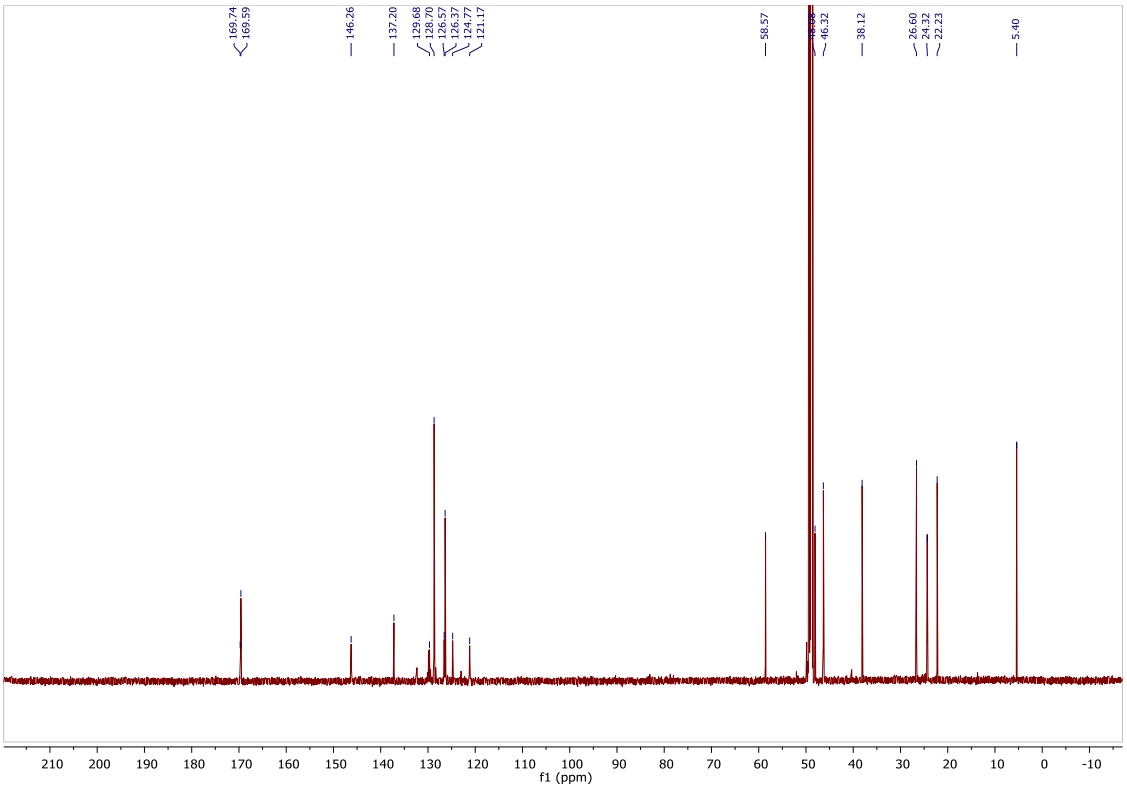
^{13}C NMR ( ) 5-Ethyl-1-(3-((4-(2-ethyl-4-hydroxy-5-oxo-3-(4-(trifluoromethyl)phenyl)-2,5-dihydro-1H-pyrrol-1-yl)butyl)amino)propyl)-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (39)



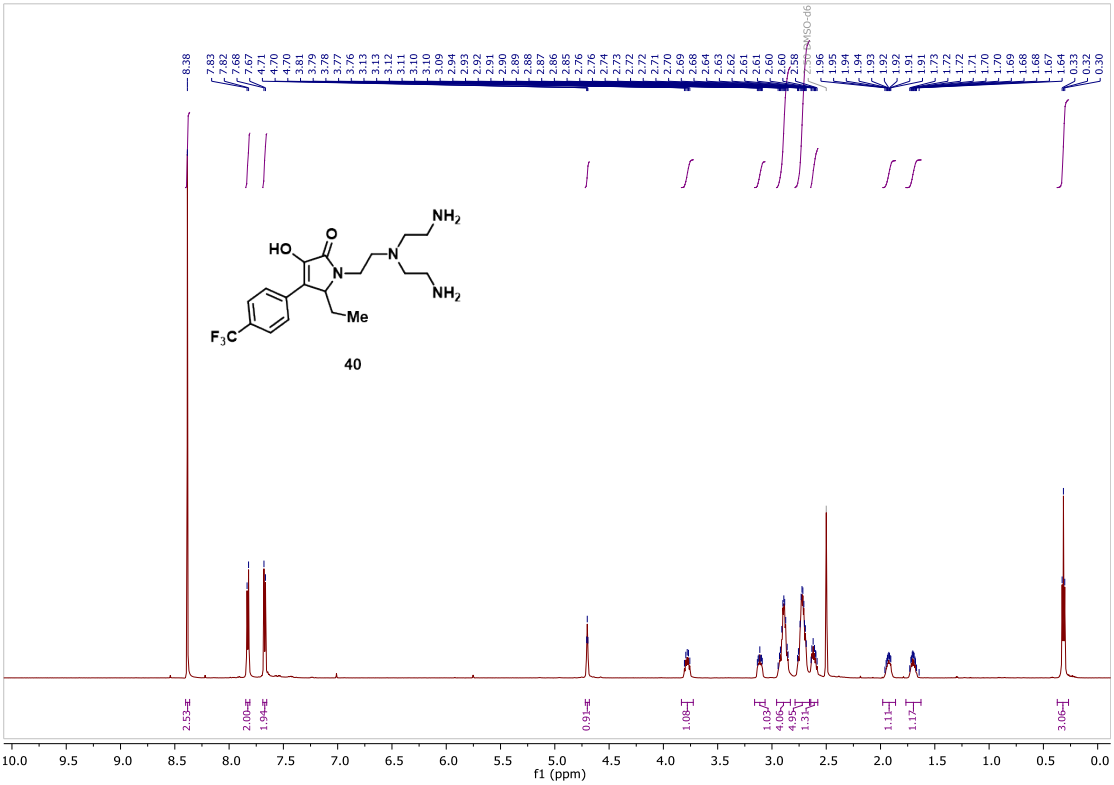
^{1}H NMR (500 MHz) 1,1'-((Butane-1,4-diylbis(azanediyl))bis(propane-3,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (40)



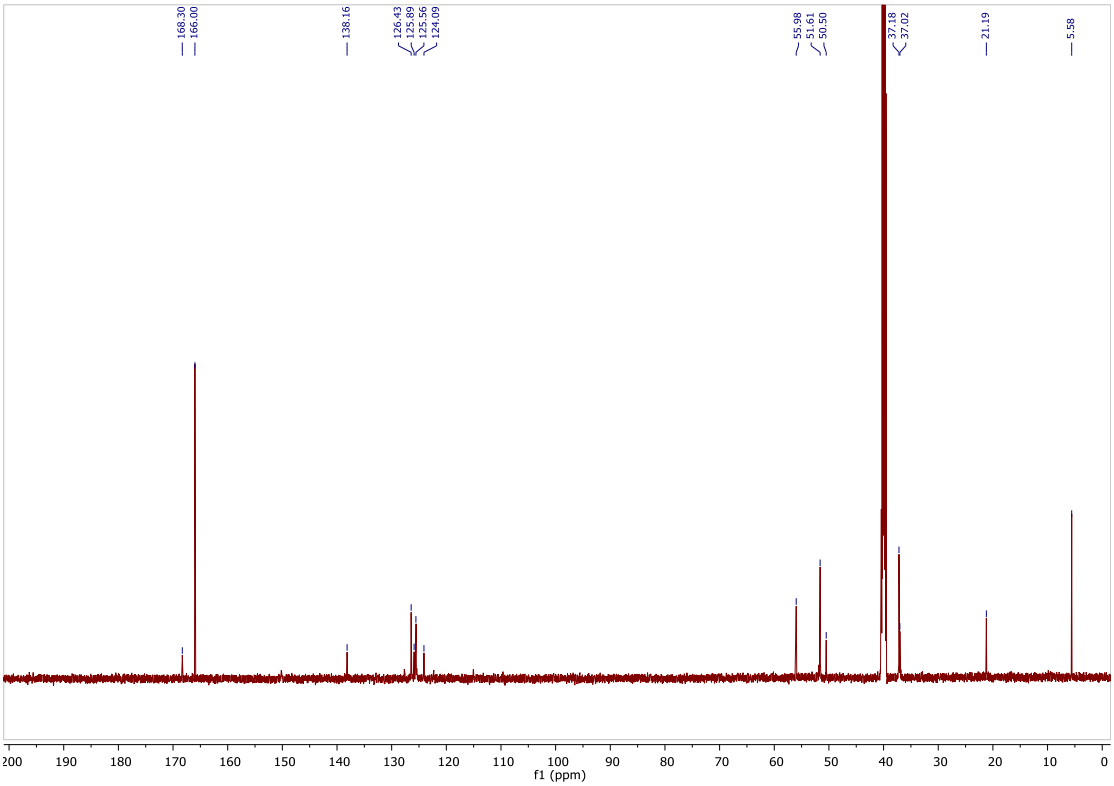
^{13}C NMR (125 MHz) 1,1'-((Butane-1,4-diylbis(azanediyl))bis(propane-3,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (40)



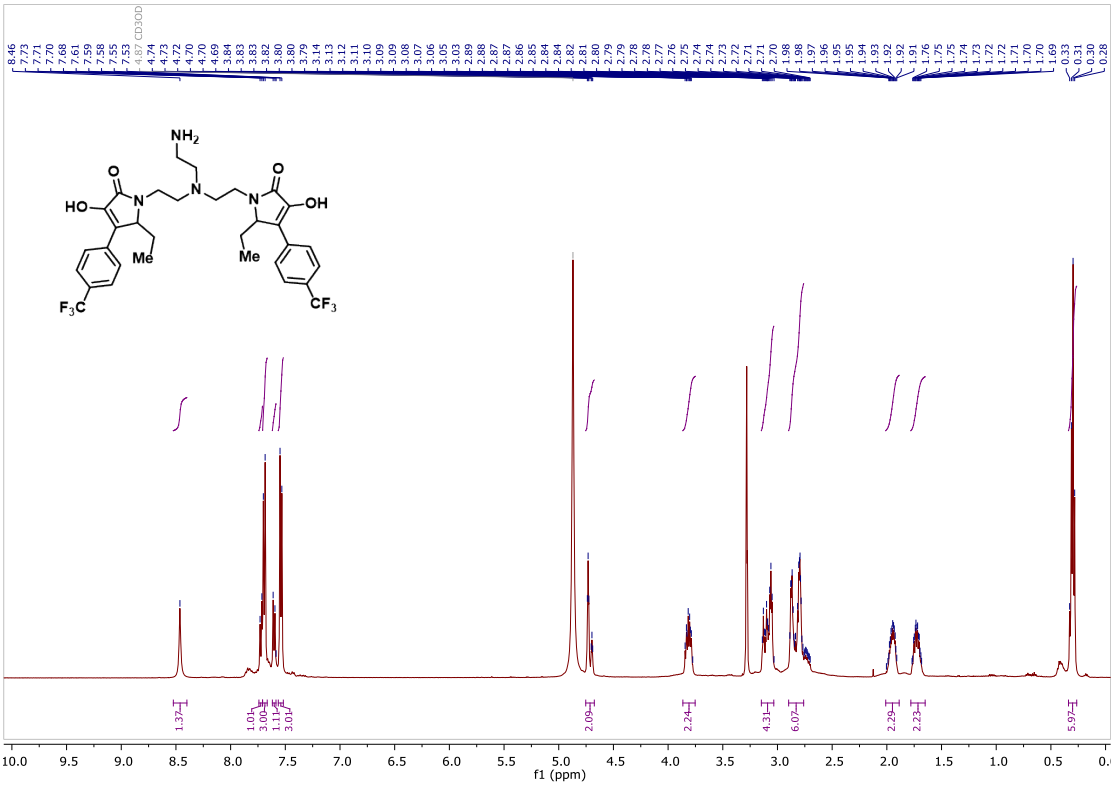
^{1}H NMR (600 MHz, DMSO-d_6) 1-(2-(bis(2-aminoethyl)amino)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (41)



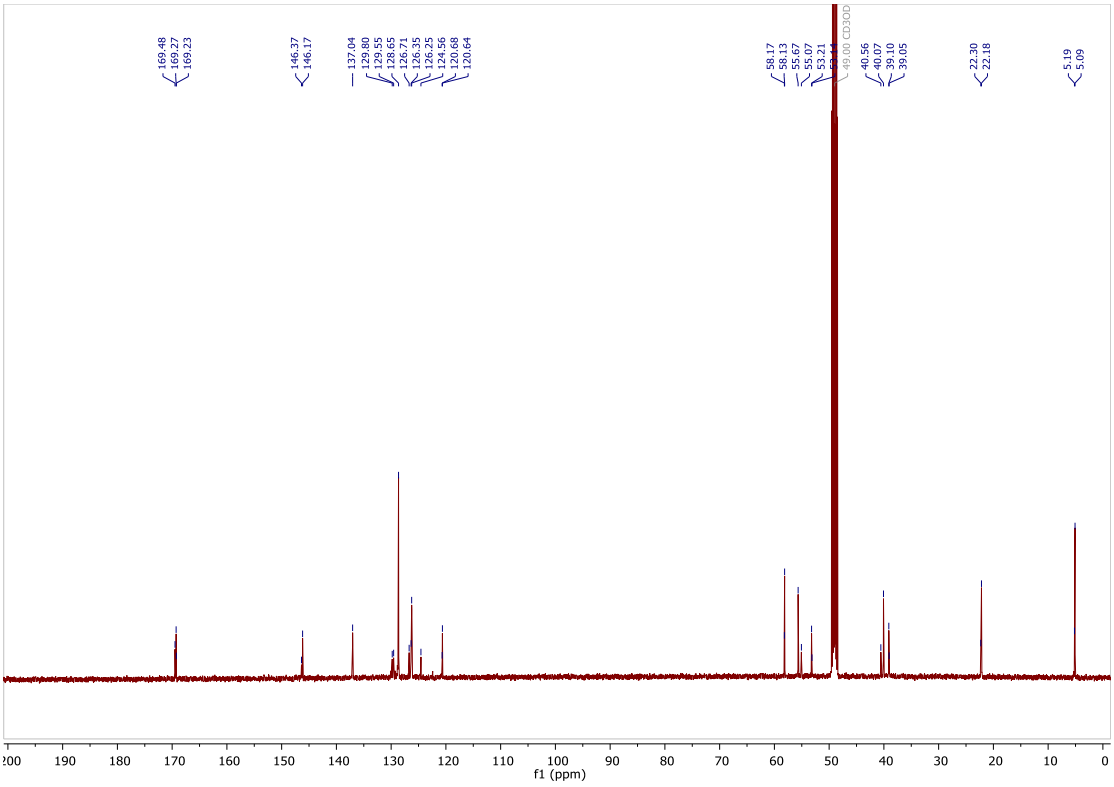
^{13}C NMR (151 MHz, DMSO-d_6) 1-(2-(bis(2-aminoethyl)amino)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (41)



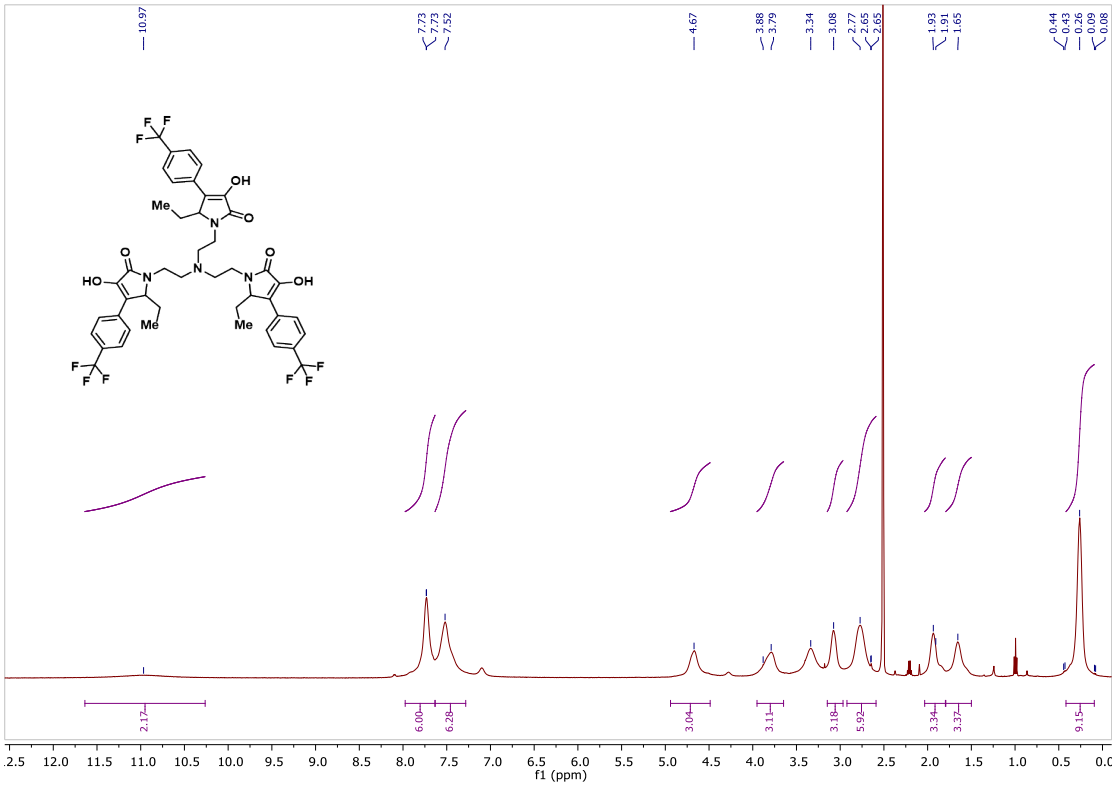
^{1}H NMR (500 MHz, Methanol-d_4) 1,1'-(((2-aminoethyl)azanediyl)bis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (42)



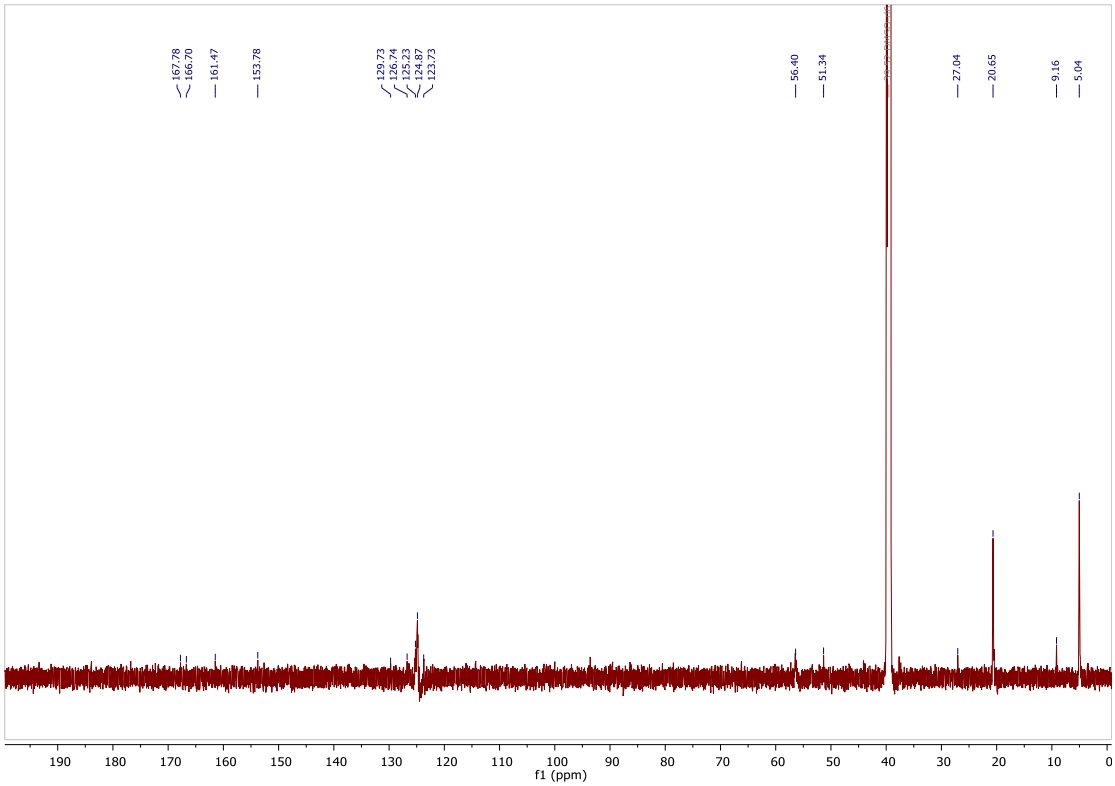
^{13}C NMR (126 MHz, Methanol-d_4) 1,1'-(((2-aminoethyl)azanediyl)bis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (42)



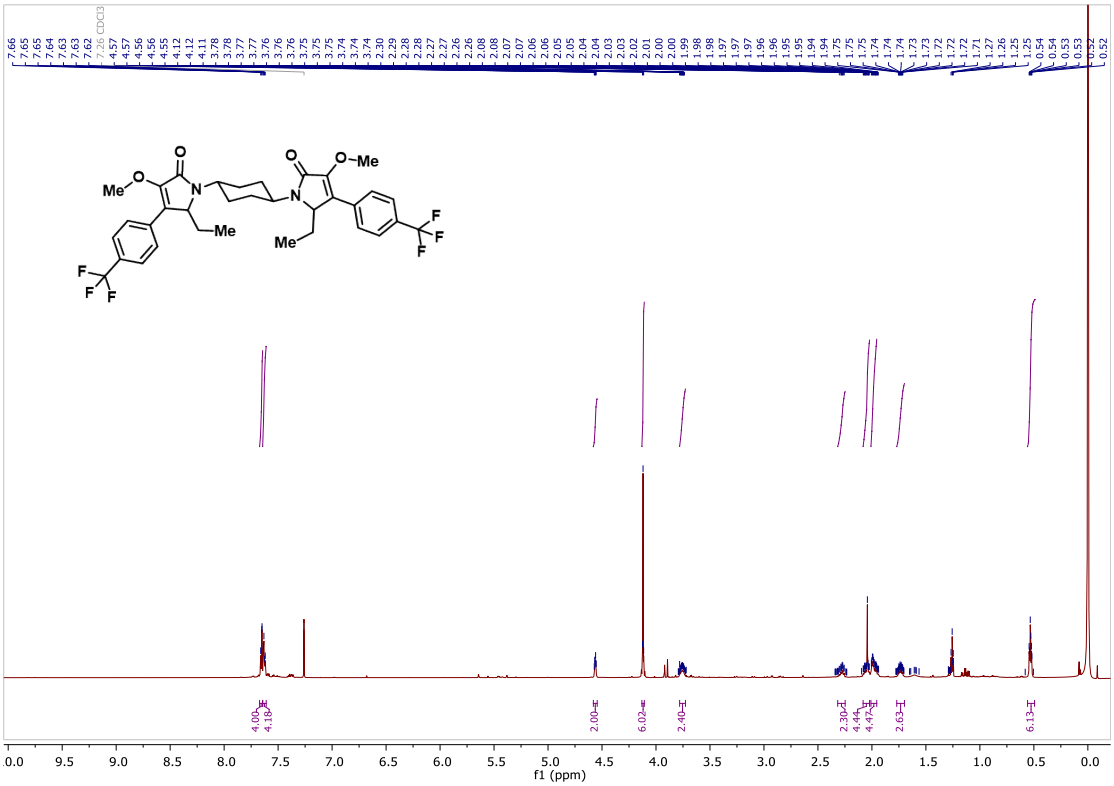
^{1}H NMR (500 MHz, DMSO-d_6) 1,1',1''-(nitrilotris(ethane-2,1-diyl))tris(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (43).



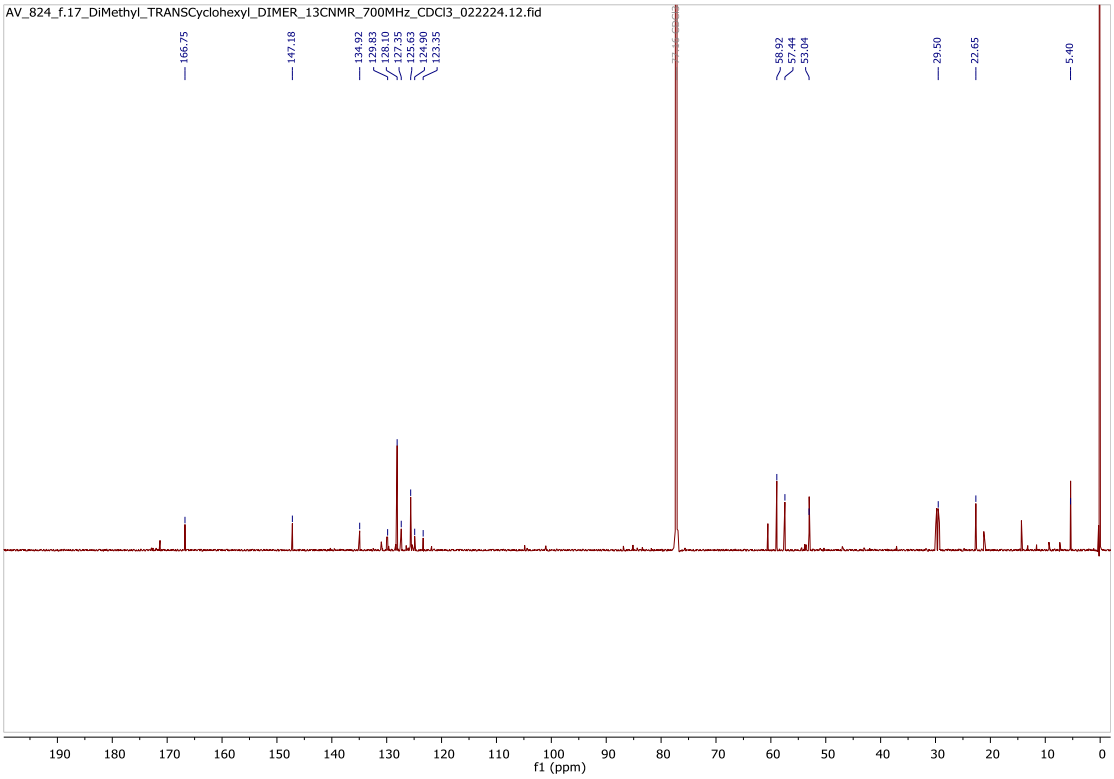
^{13}C NMR (176 MHz, DMSO-d_6 1,1',1''-(nitrilotris(ethane-2,1-diyl))tris(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (43).



^{1}H NMR (700 MHz, Chloroform-*d*) 1,1'-((1r,4r)-cyclohexane-1,4-diyl)bis(5-ethyl-3-methoxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (48)

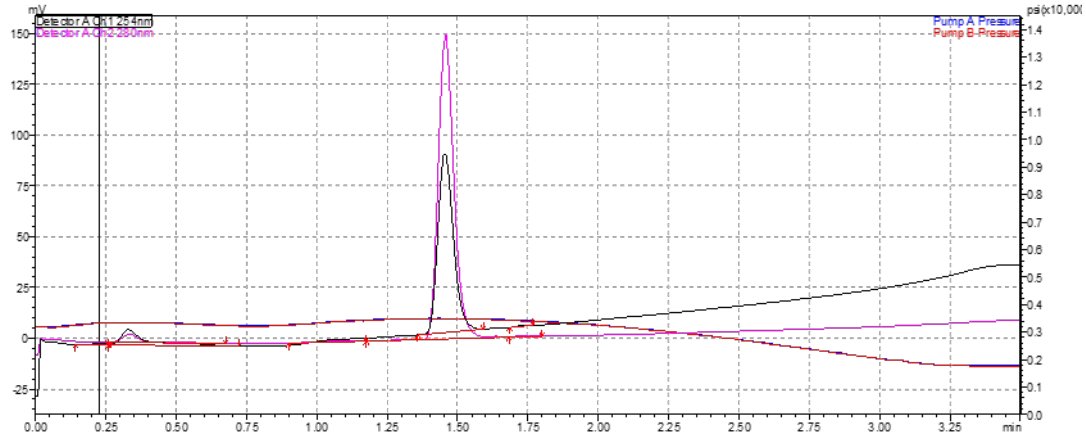


^{13}C NMR (176 MHz, Chloroform-*d*) 1,1'-((1r,4r)-cyclohexane-1,4-diyl)bis(5-ethyl-3-methoxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (48)

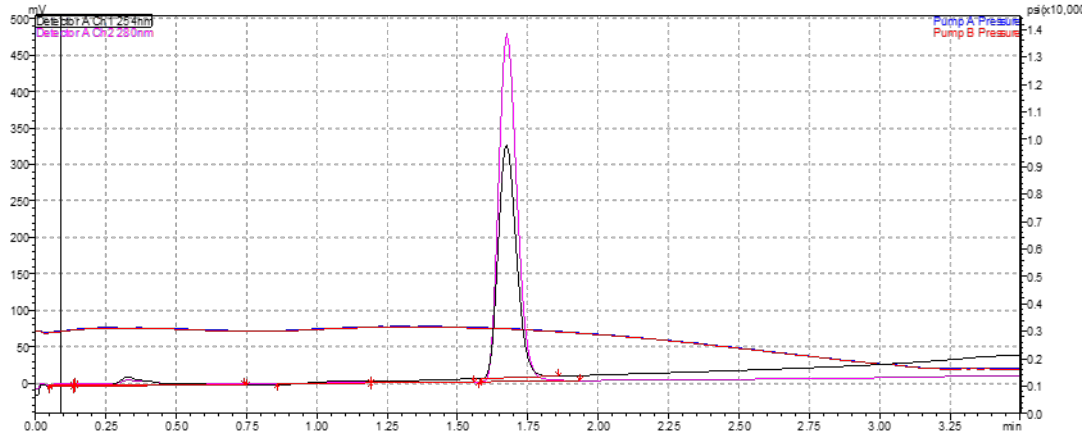


HPLC traces of microbiologically tested compounds

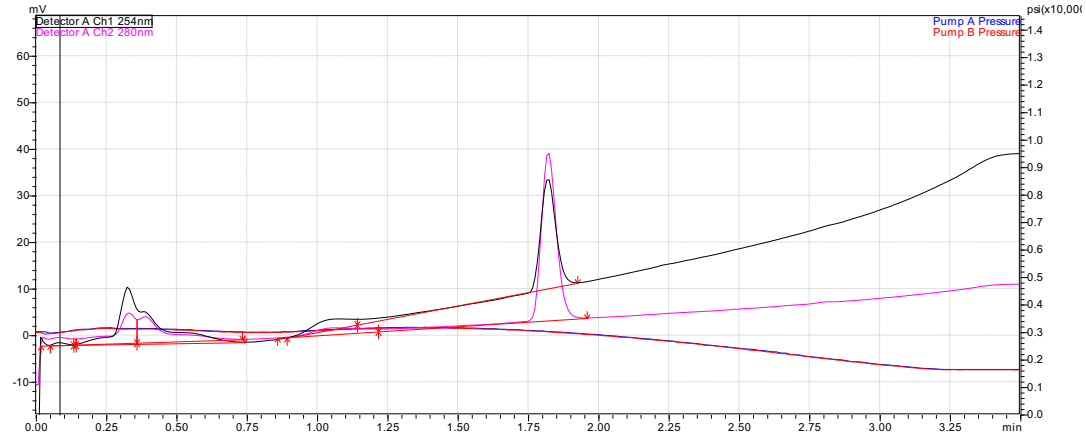
1-(2-Aminoethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (16)



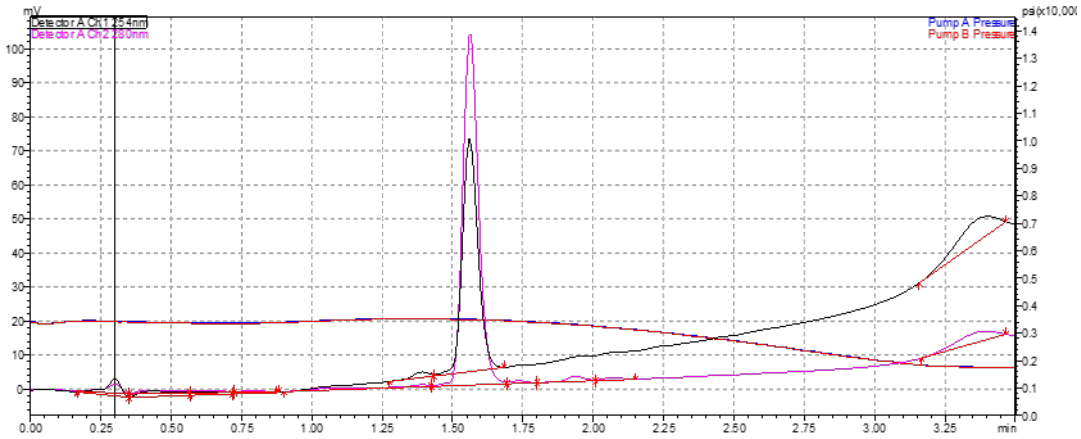
1-(4-Aminobutyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (17):



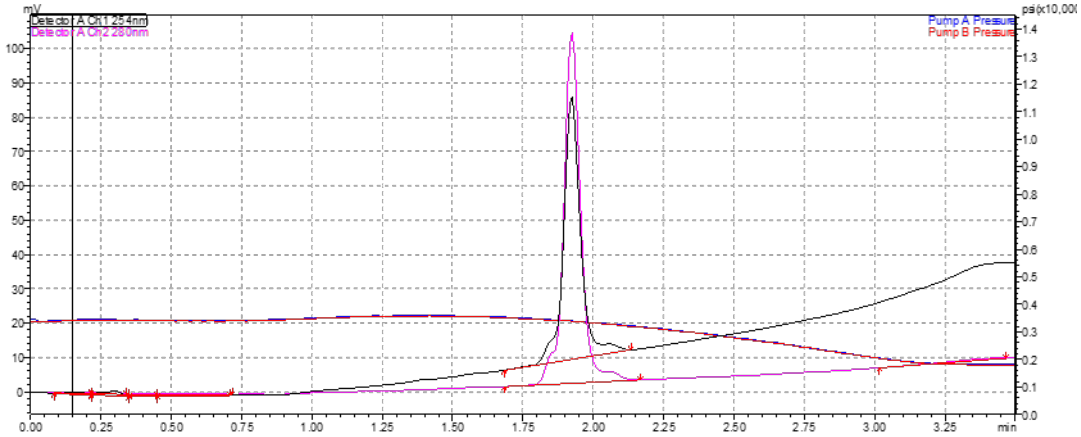
1-(6-Aminohexyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (18):



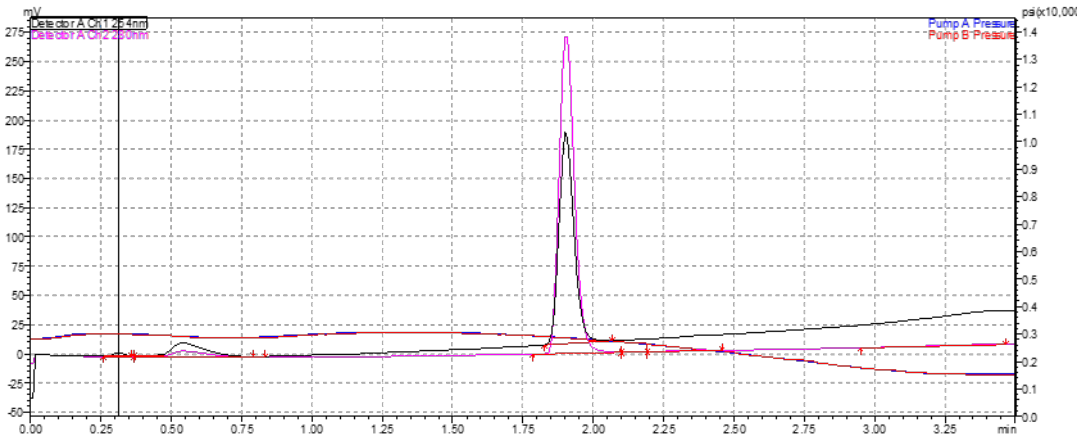
1-((1r,4r)-4-Aminocyclohexyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (19):



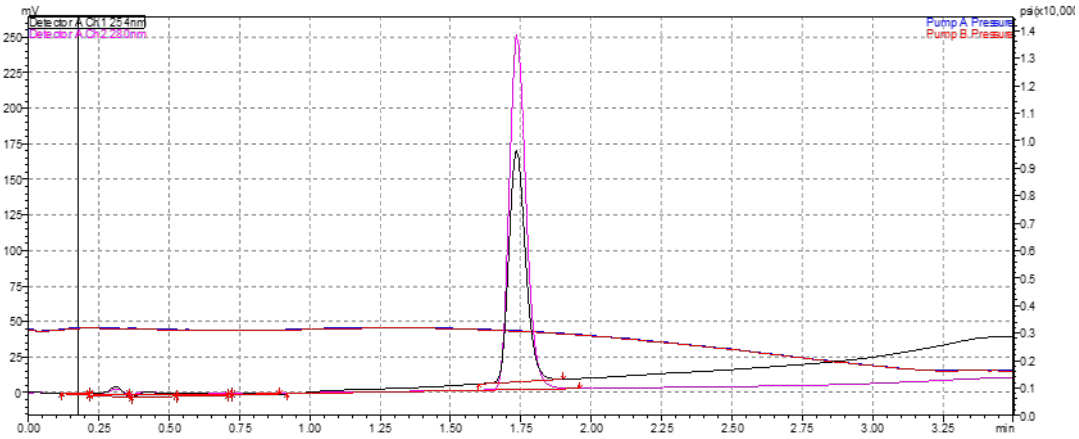
1-(4-Aminophenyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (20):



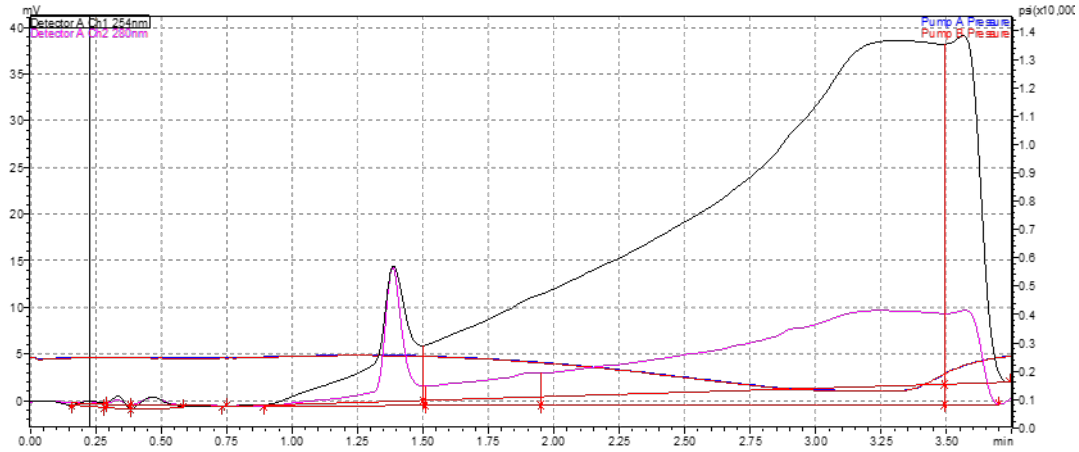
1-(2-(2-Aminoethoxy)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (21):



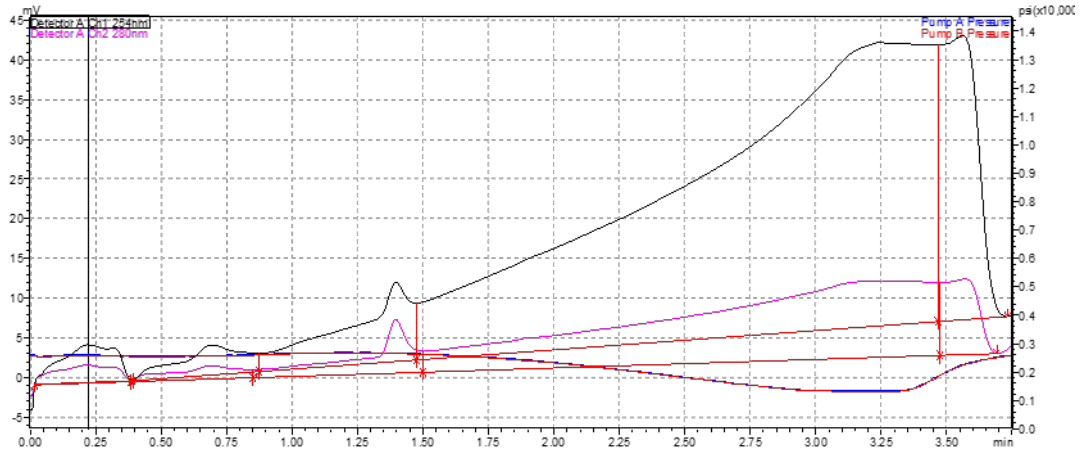
1-(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (22):



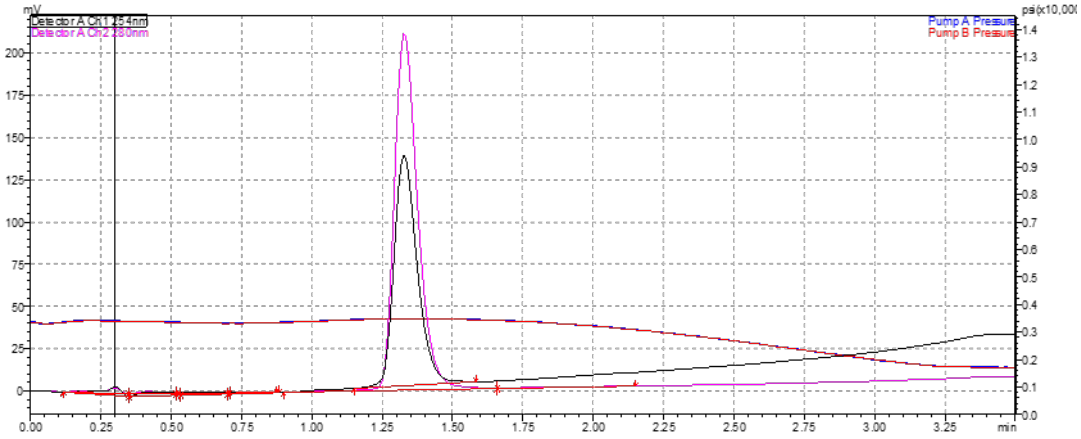
1-(2-((2-aminoethyl)amino)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (23):



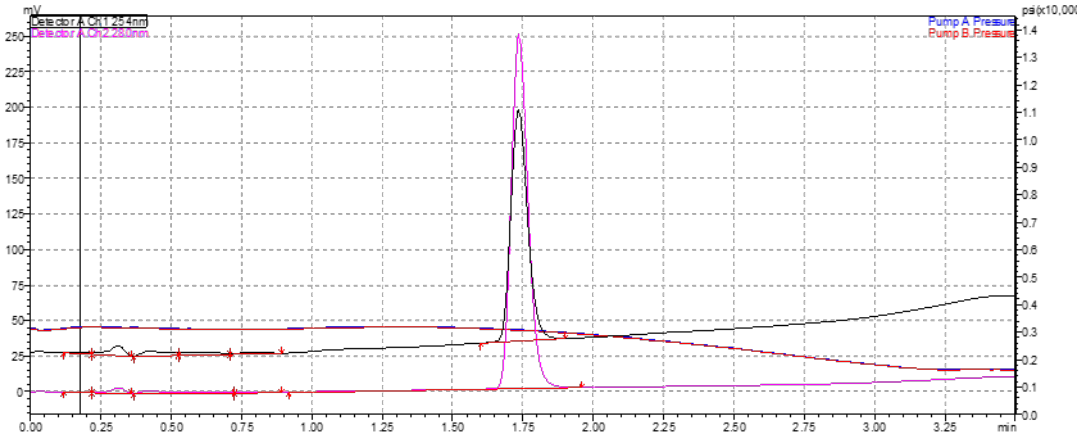
1-(3-((3-aminopropyl)amino)propyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (24):



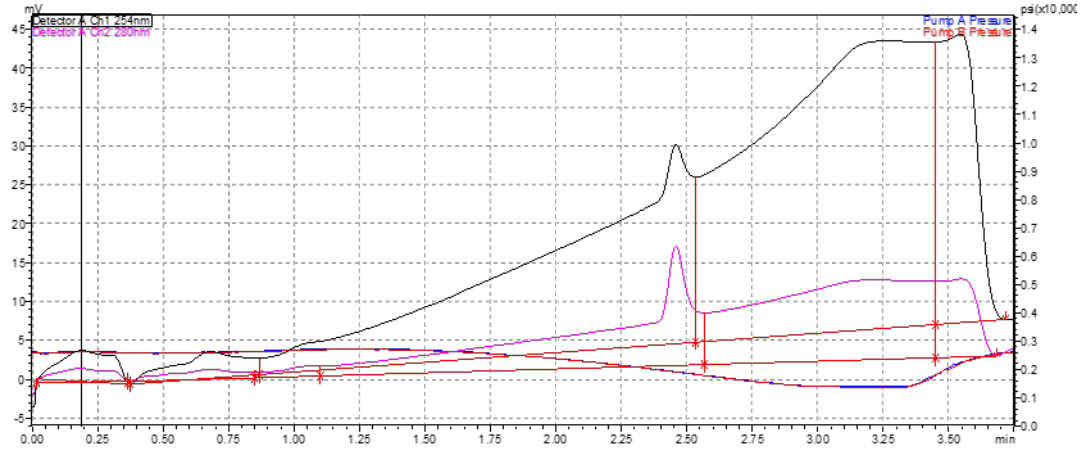
1-(3-((4-Aminobutyl)amino)propyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (25):



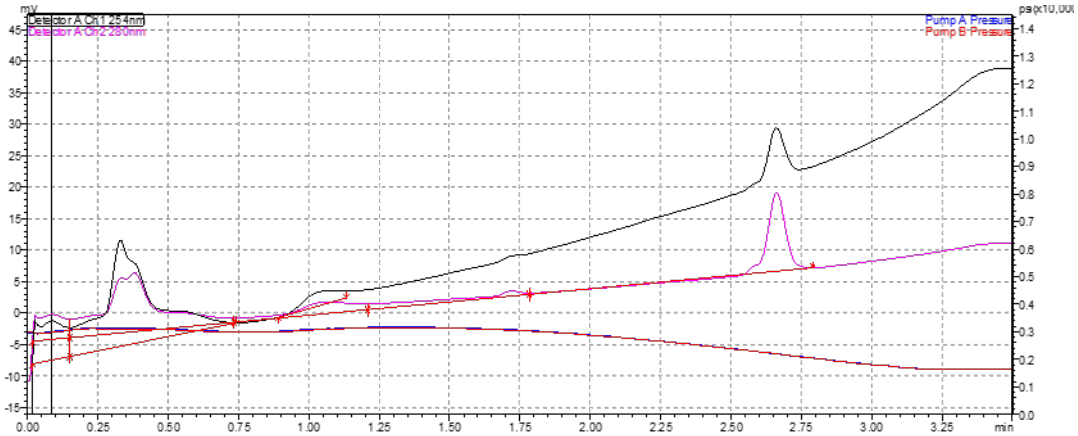
1-(3-((4-((3-Aminopropyl)amino)butyl)amino)propyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (26):



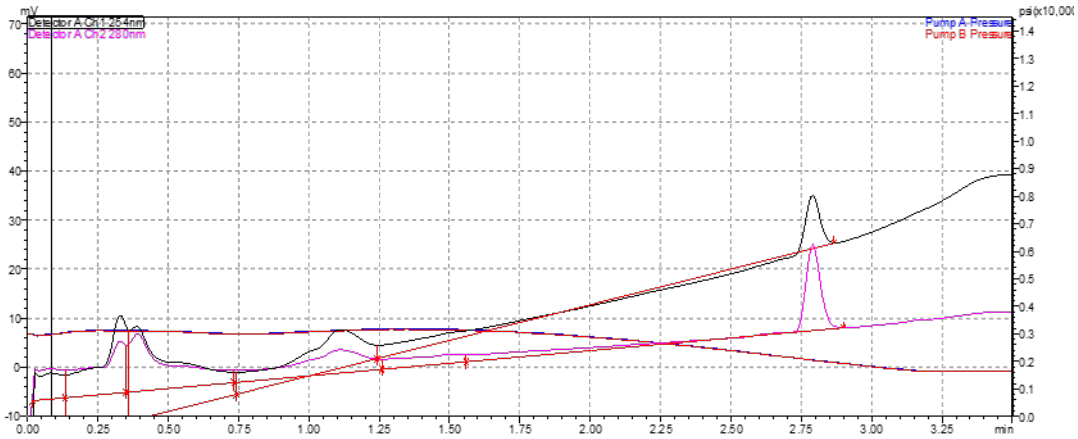
1,1'-(Ethane-1,2-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (27):



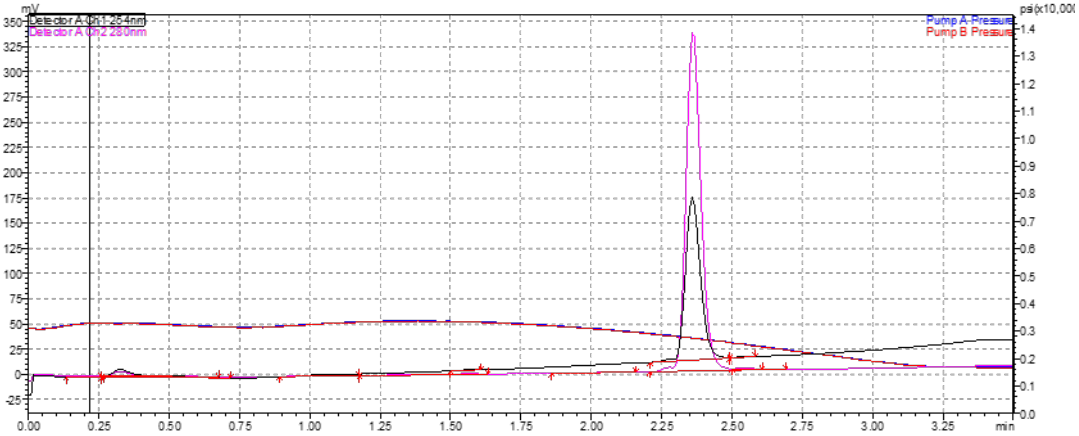
1,1'-(Butane-1,4-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (28):



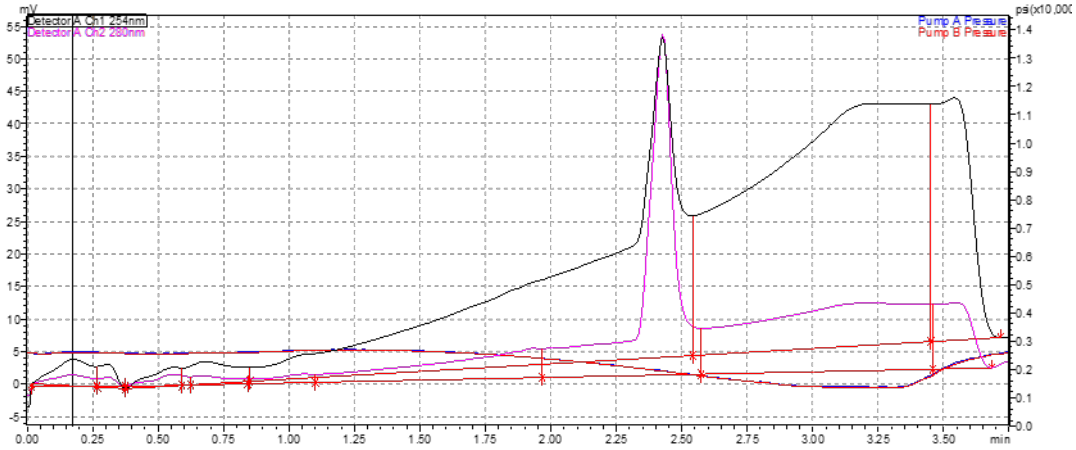
1,1'-(Hexane-1,6-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (29):



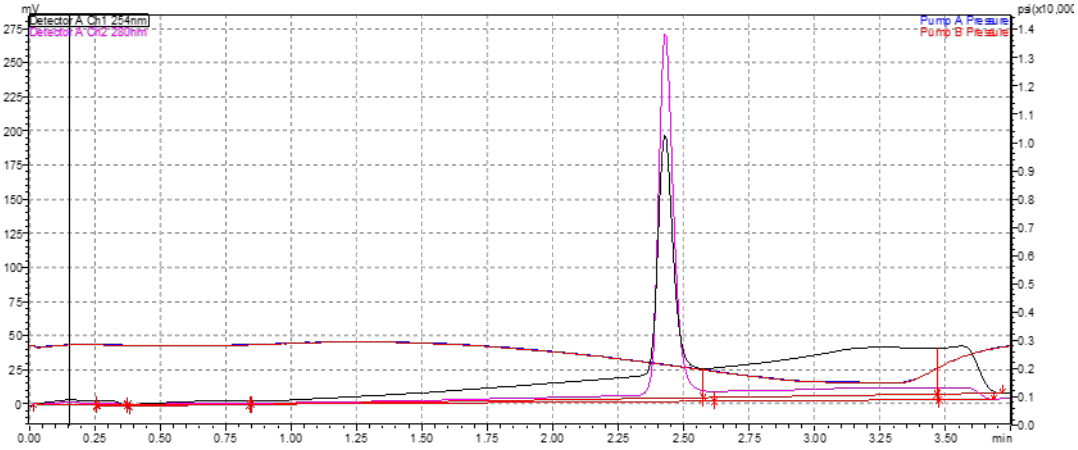
1,1'-((1r,4r)-Cyclohexane-1,4-diyl)bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (30):



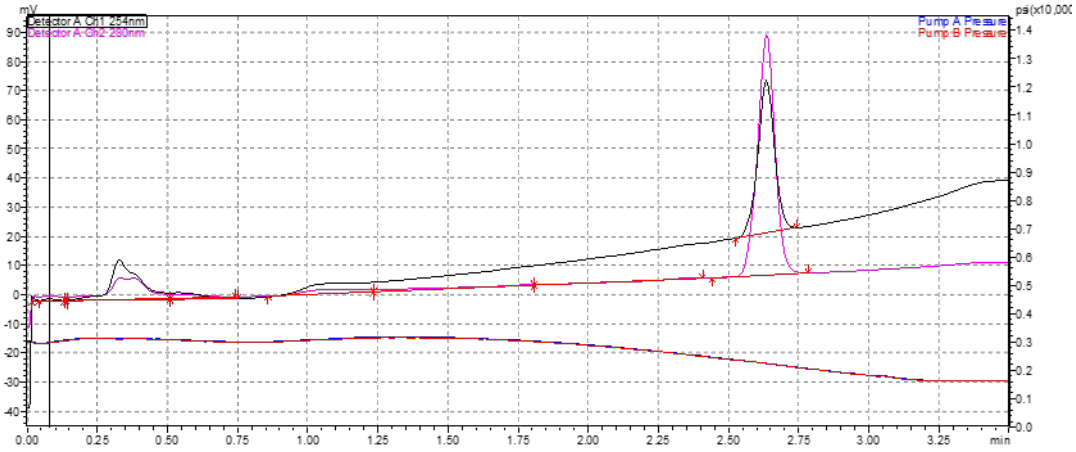
1,1'-(Oxybis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (33):



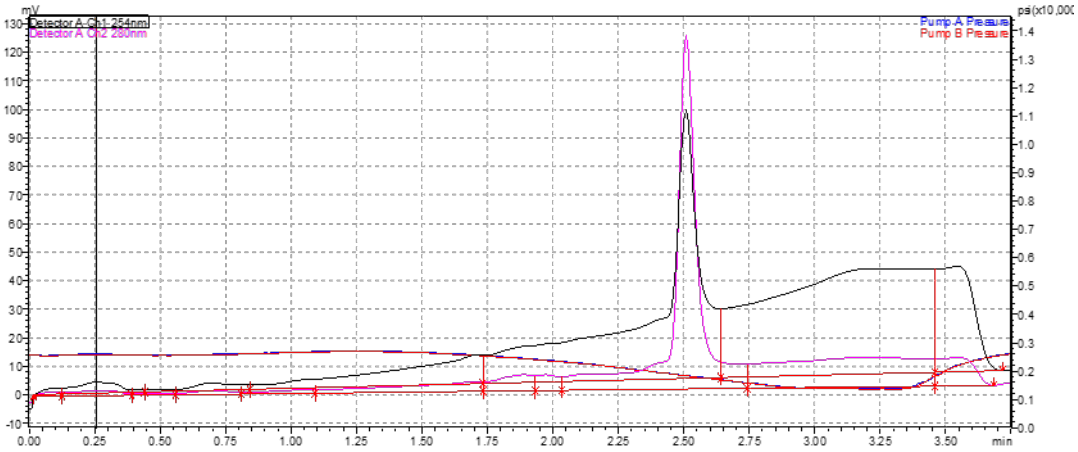
1,1'-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (34):



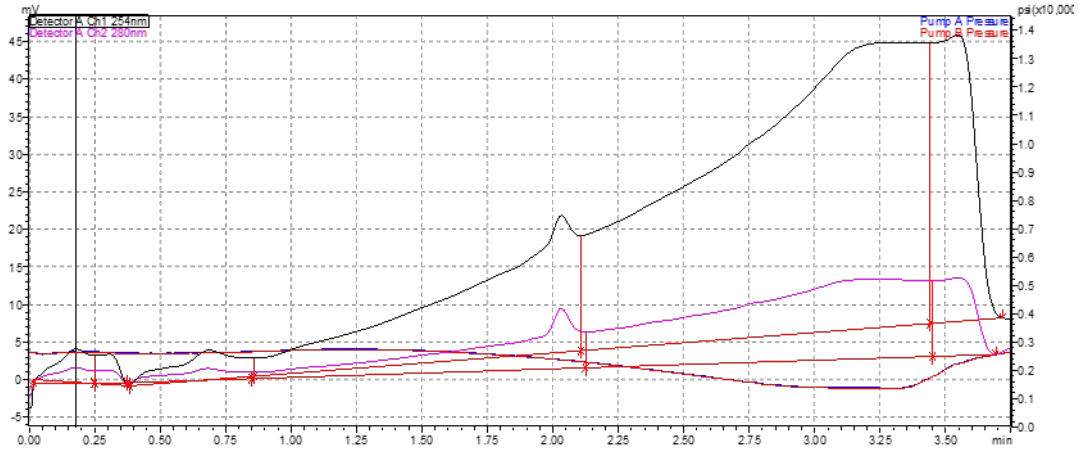
1,1'-(((Oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (35):



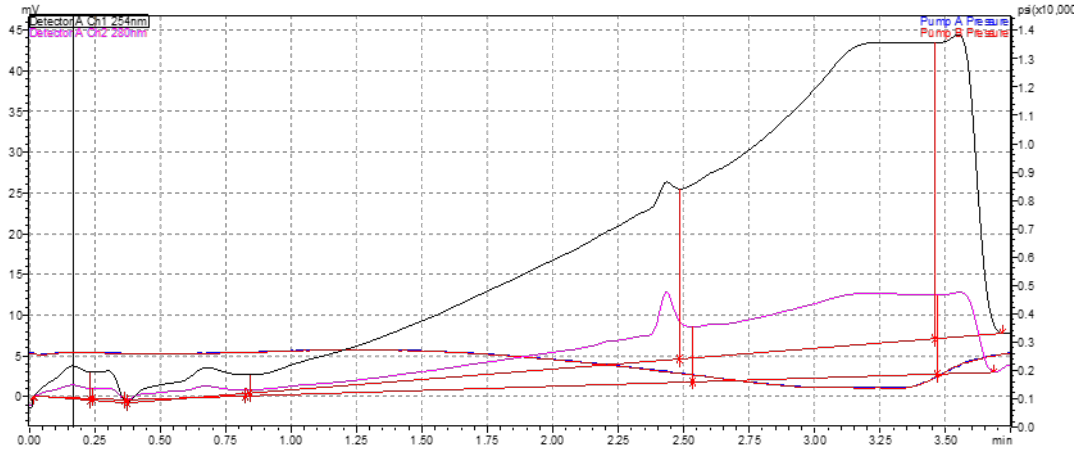
1,1'-(thiobis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (36).



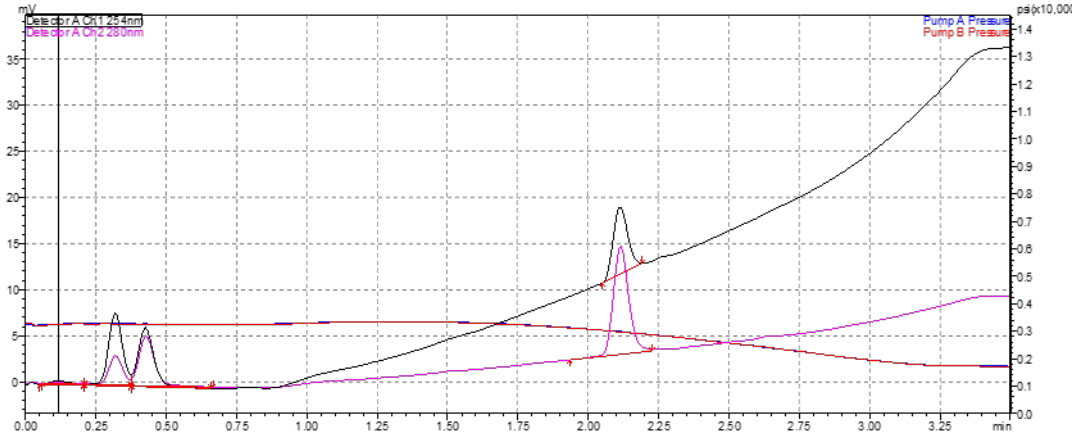
1,1'-(azanediylbis(ethane-2,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl) phenyl)-1,5-dihydro-2H-pyrrol-2-one) (37).



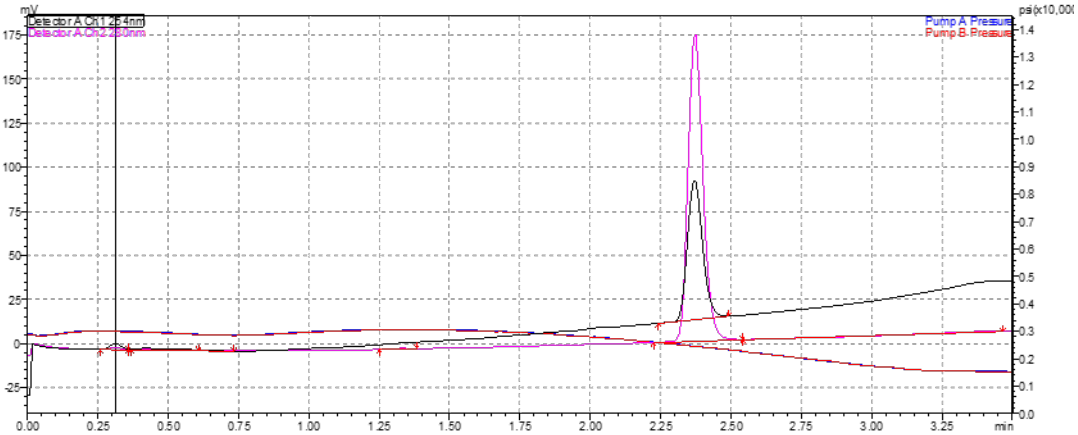
1,1'-(azanediylbis(propane-3,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (38):



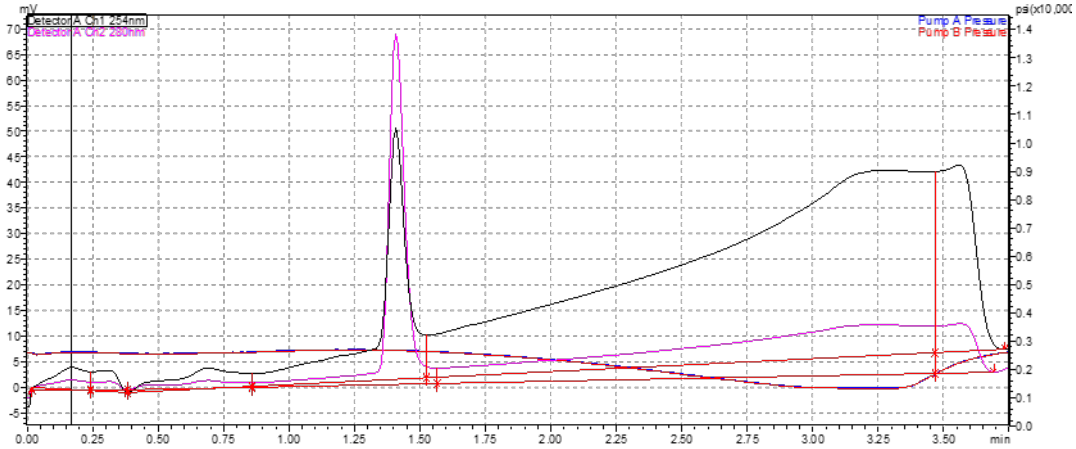
5-Ethyl-1-(3-((4-(2-ethyl-4-hydroxy-5-oxo-3-(4-(trifluoromethyl)phenyl)-2,5-dihydro-1H-pyrrol-1-yl)butyl)amino)propyl)-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (39):



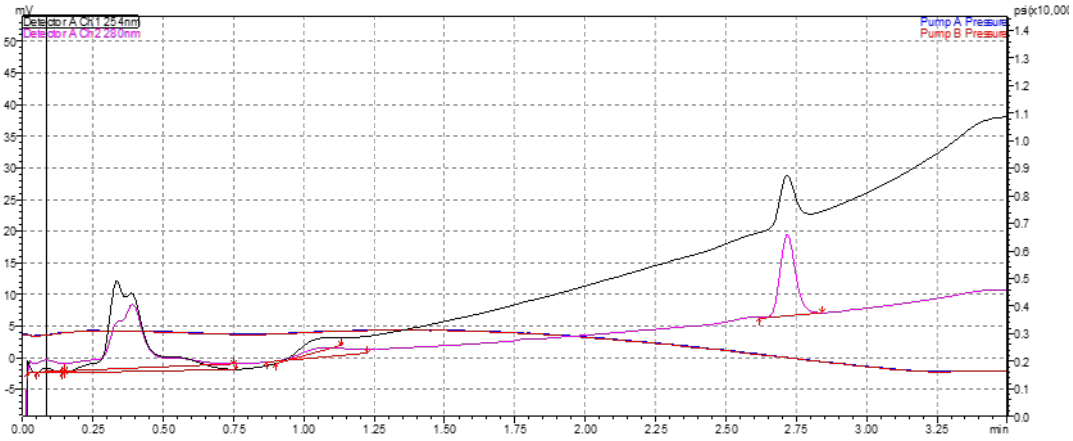
1,1'-((Butane-1,4-diylbis(azanediyl))bis(propane-3,1-diyl))bis(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (40):



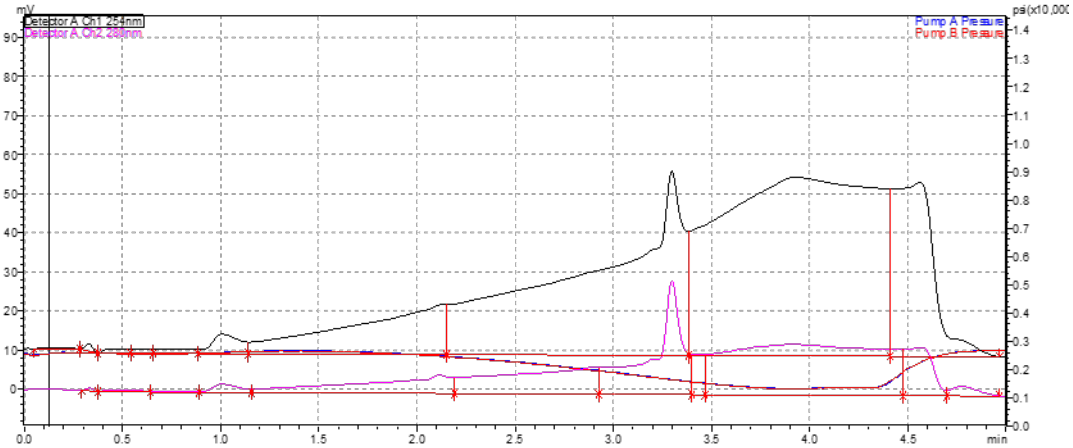
1-(2-(bis(2-aminoethyl)amino)ethyl)-5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one (41):



1,1',1''-(nitrilotris(ethane-2,1-diyl))tris(5-ethyl-3-hydroxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (43):



1,1'-((1r,4r)-cyclohexane-1,4-diyl)bis(5-ethyl-3-methoxy-4-(4-(trifluoromethyl)phenyl)-1,5-dihydro-2H-pyrrol-2-one) (48)



Aqueous solubility of selected analogs in different media

	Solubility (μg/mL)	
Pyrrolidinedione Compound	**PBS 1X**	**MHB**
6	<128	<64
7	<128	<64
8	<128	<64
16	>512	>128
21	>512	>128
24	ND	>128
27	>128	>128
33	>256	>128
35	>512	>128
39	>128	>128
41	>256	>256

ND: not determined

*These values have been determined in the aforementioned media on intervals of 16, 20, or 24 h, at 37 °C.

Figure S1: MBEC:MIC ratio of selected FDA-approved antimicrobials and most active pyrrolidine-2,3-dione dimers.

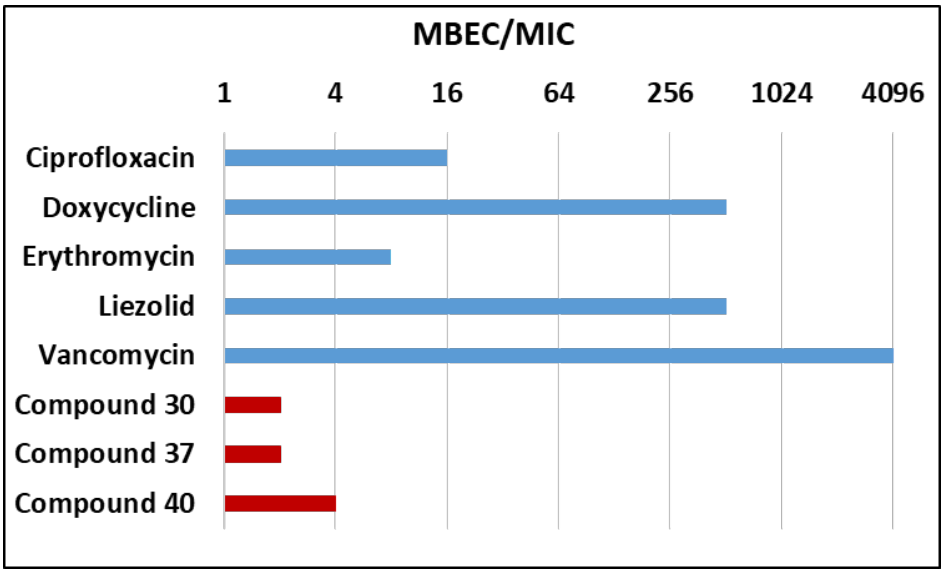
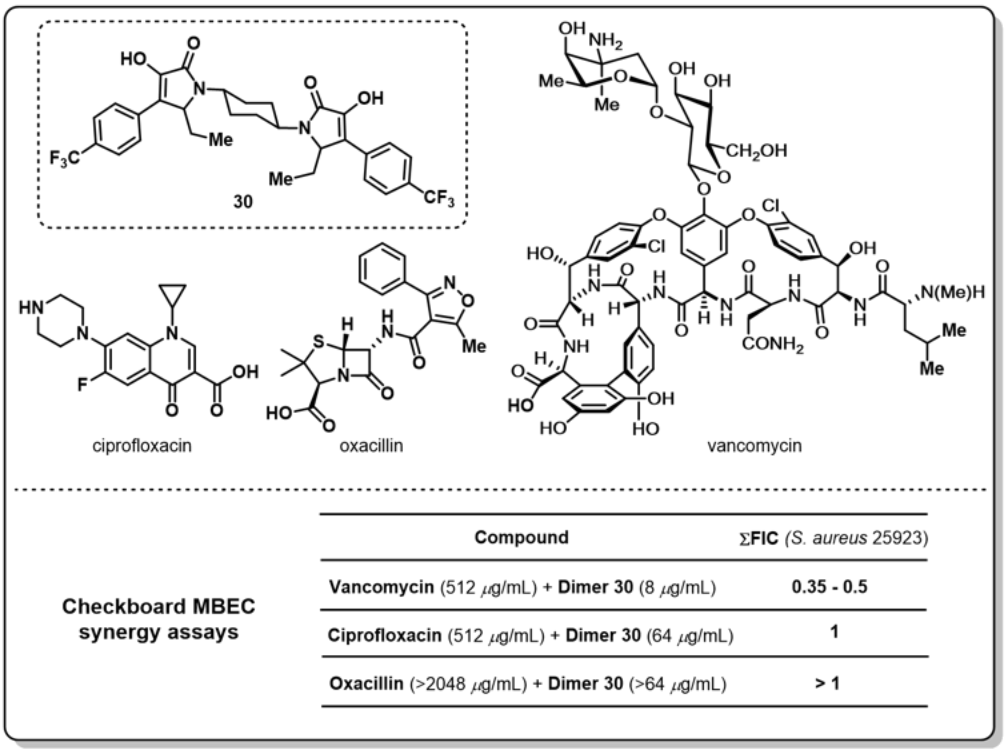


Figure S2: Minimum biofilm eradication concentration (MBEC) synergism assays between one of the 2,3-pyrrolidinedione leads and different FDA-approved antimicrobials. *ΣFIC: sum of fractional inhibitory concentration.



Checkboard MBEC synergy assays

Compound	ΣFIC (*S. aureus* 25923)
Vancomycin (512 μg/mL) + **Dimer 30** (8 μg/mL)	**0.35 - 0.5**
Ciprofloxacin (512 μg/mL) + **Dimer 30** (64 μg/mL)	**1**
Oxacillin (>2048 μg/mL) + **Dimer 30** (>64 μg/mL)	**> 1**