

**Antibacterial activity of Mn(I) and Re(I) tricarbonyl complexes conjugated
to a bile acid carrier molecule**

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Supporting Information

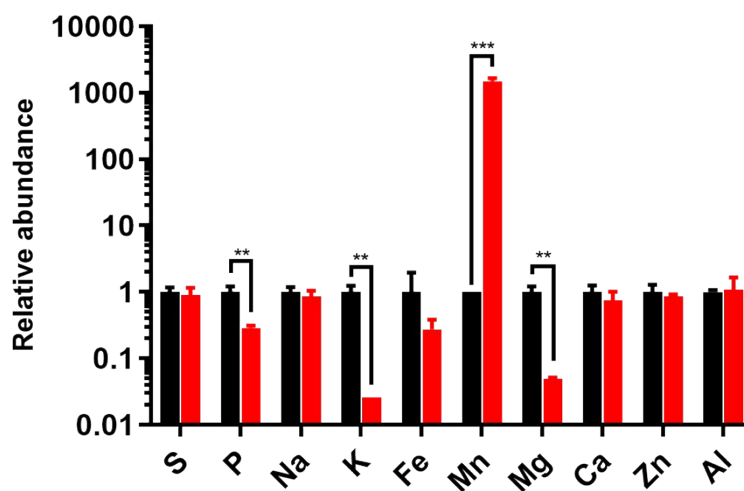


Figure S1. ICP-MS determination of the intracellular metal content of *E. coli* treated with $[\text{Mn}(\text{CO})_3(\text{bqpa-}\kappa^3\text{N})]\text{Br}$ (red bars) relative to the metal content of control samples treated with 1% DMSO (black bars). Data shown are mean of three biological replicates and errors bars represent the standard deviation. *, **, and *** denote p -values of 0.01–0.05, 0.001–0.01, and < 0.001 respectively, by Student's t -test.

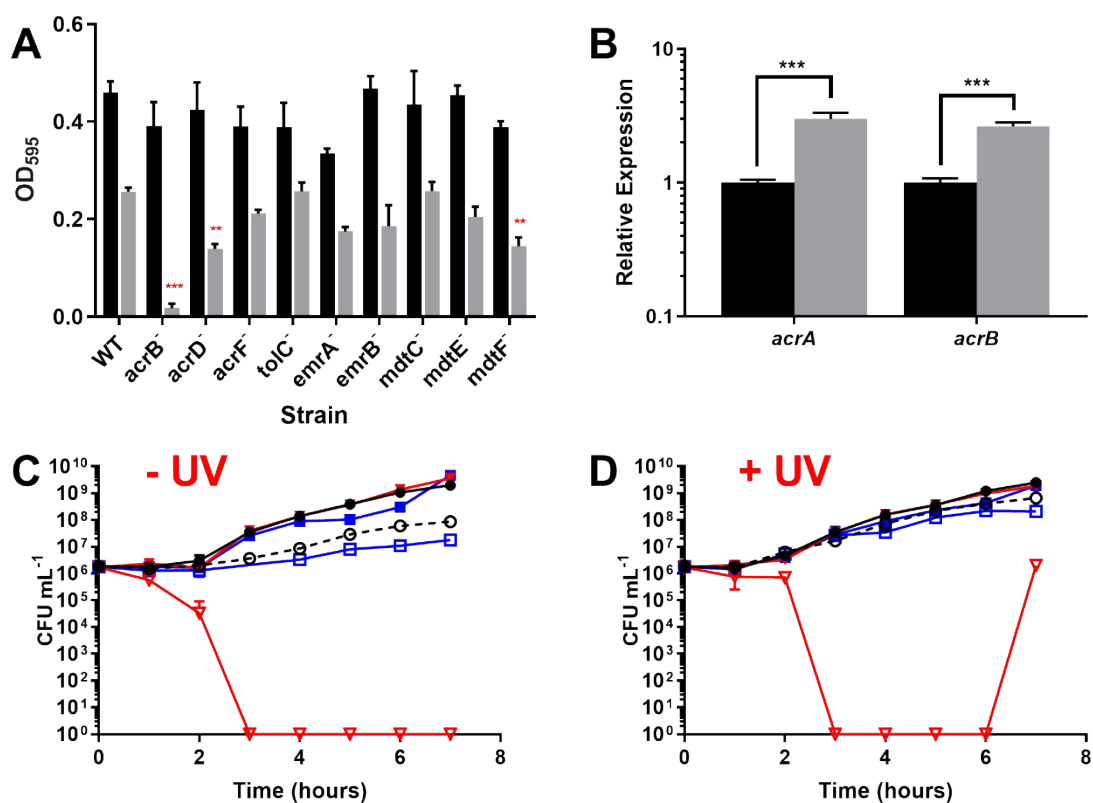


Figure S2. A) 5 h growth of *E. coli* strains in the absence (black bars) and presence (grey bars) of [Mn(CO)₃(bqpa-κ³N)]Br. Values are means of three biological repeats with error bars indicating standard deviation. B) RT-PCR showing the relative expression of the *E. coli* genes *acrA* and *acrB* in cells grown in the absence (black bars) and presence (grey bars) of [Mn(CO)₃(bqpa-κ³N)]Br. Expression levels are reported relative to the levels determined for untreated cells and normalized to the *rrsA* housekeeping gene. Data are plotted as means of results from three biological replicates (each consisting of three technical replicates) with standard deviations shown as error bars. C) Growth of *E. coli* without and D) with UV activation of [Mn(CO)₃(bqpa-κ³N)]Br. Black lines represent wildtype (BW25113), red lines represent *acrB*⁻ and blue line represent *acrD*⁻; solid points indicate the 1% DMSO control and unfilled points indicate cells treated with 105 μM [Mn(CO)₃(bqpa-κ³N)]Br. *, **, and *** denote *p*-values of 0.01–0.05, 0.001–0.01, and < 0.001 respectively, by Student's *t*-test.

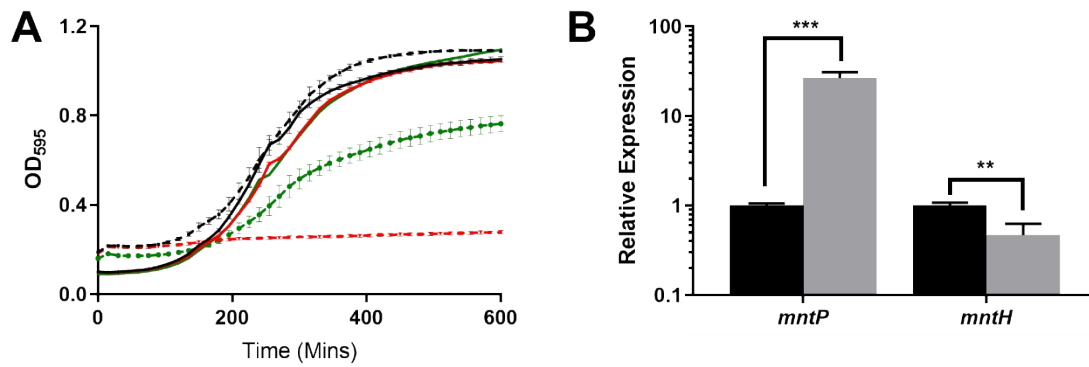
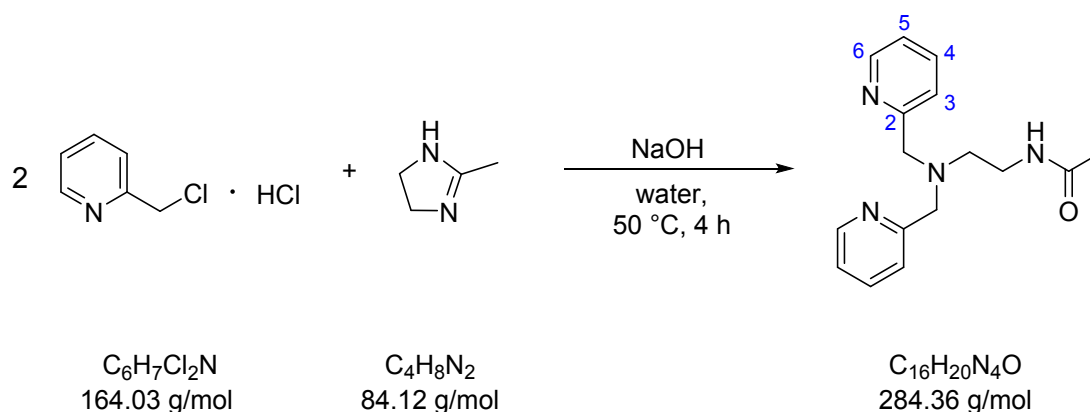


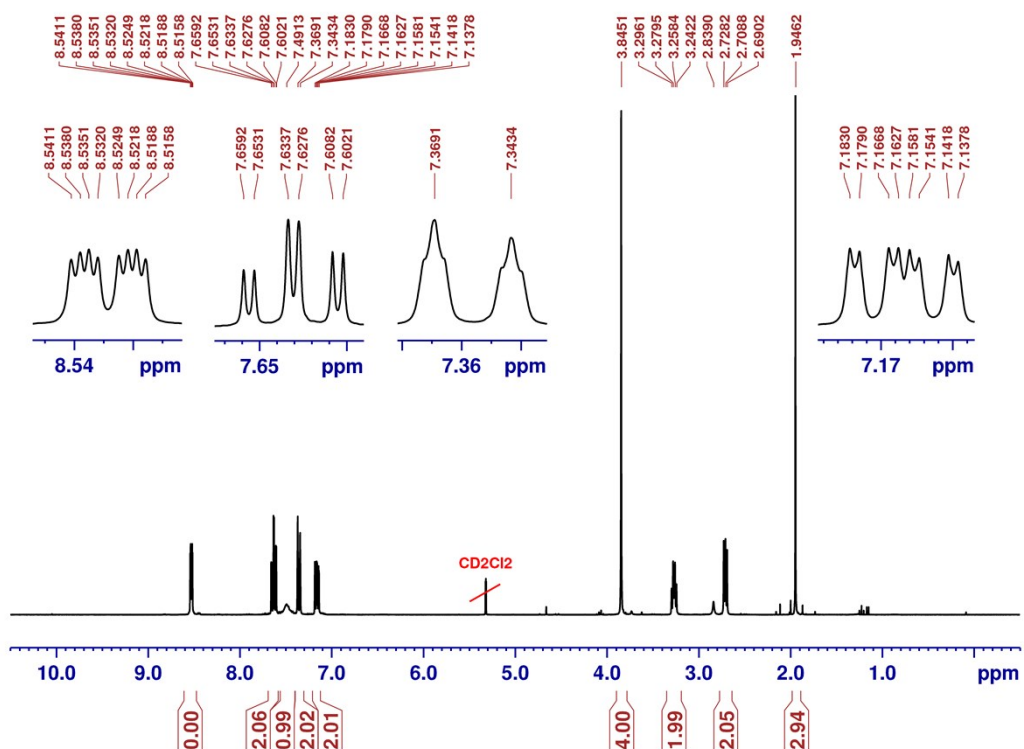
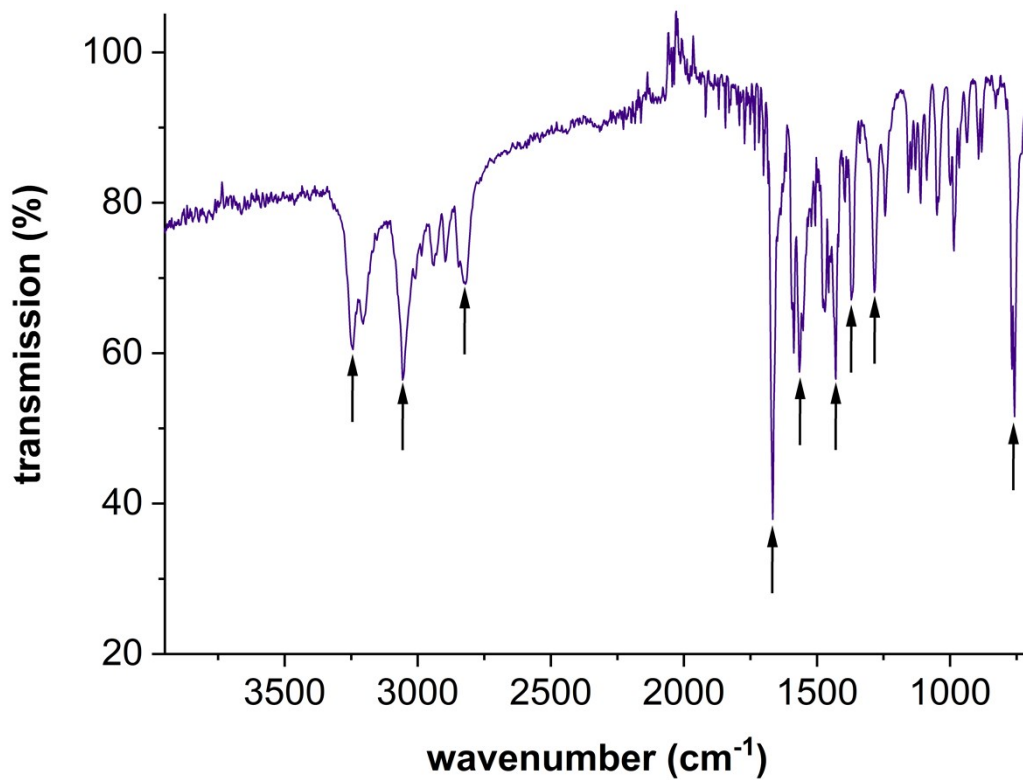
Figure S3. A) Growth curves of *E. coli* strains with (solid line) and without (dashed line) addition of [Mn(bqpa-κ³N)(CO)₃]Br (105 μM); WT (BW25113) is represented by the black lines, *mntP*⁻ is represented by the red lines and *mntR*⁻ is represented by the green lines and B) RT-PCR of *mntP* and *mntH* indicates activation of the Mn regulator MntR, as shown by the increased in expression of *mntP* and the decrease in expression of *mntH* in response to treatment with [Mn (bqpa-κ³N)(CO)₃]Br (grey) compared to the 1% DMSO control (black).

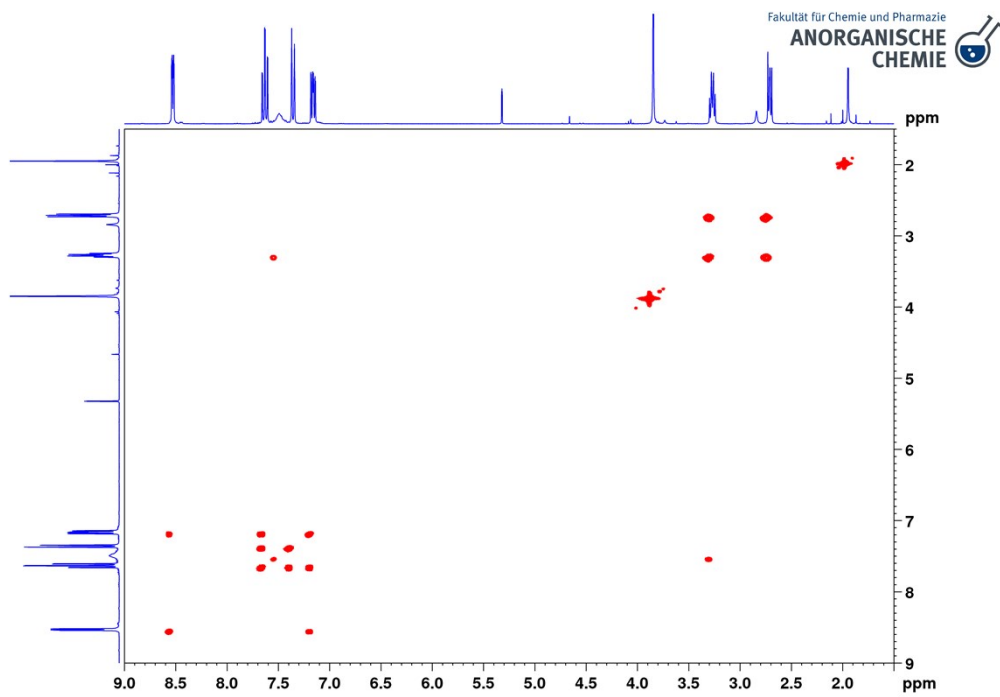
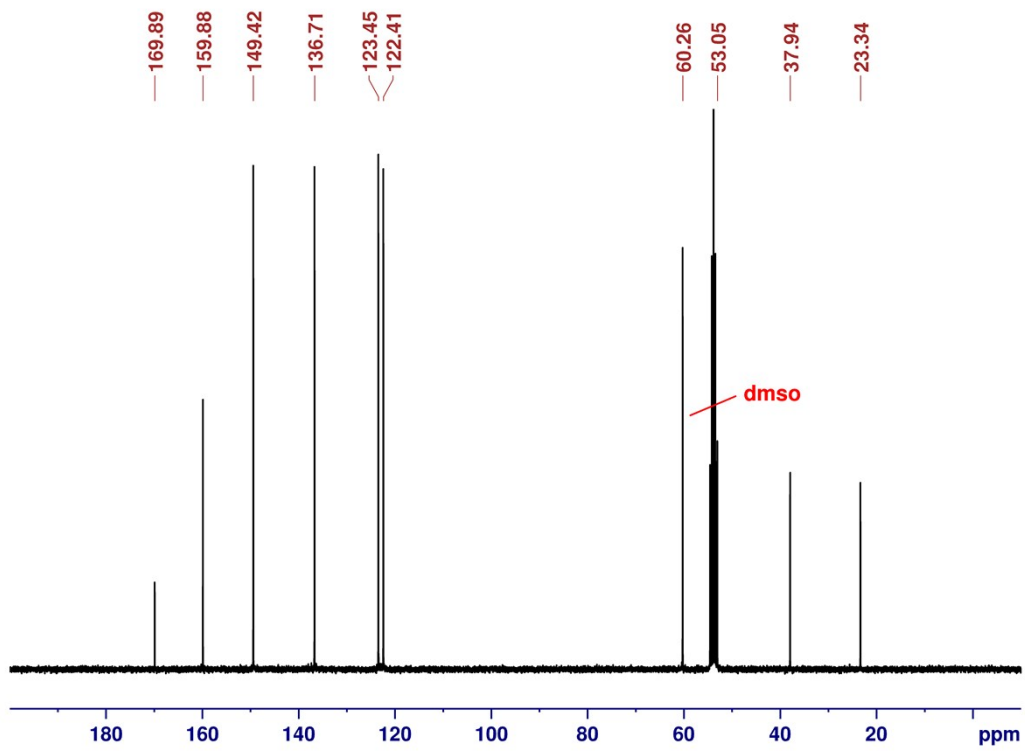
Ligand synthesis

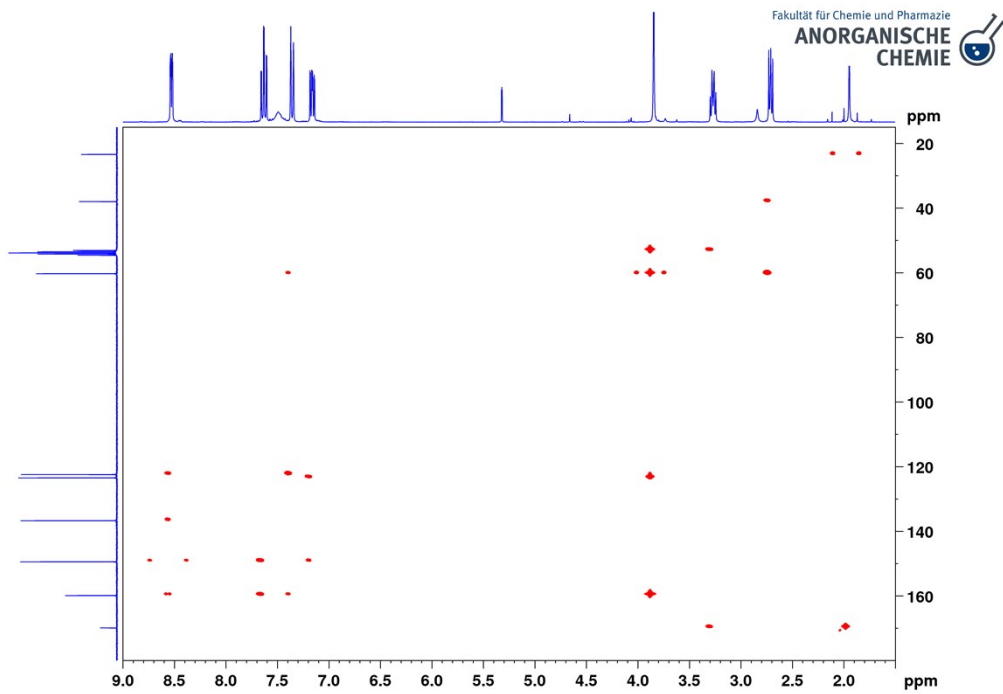
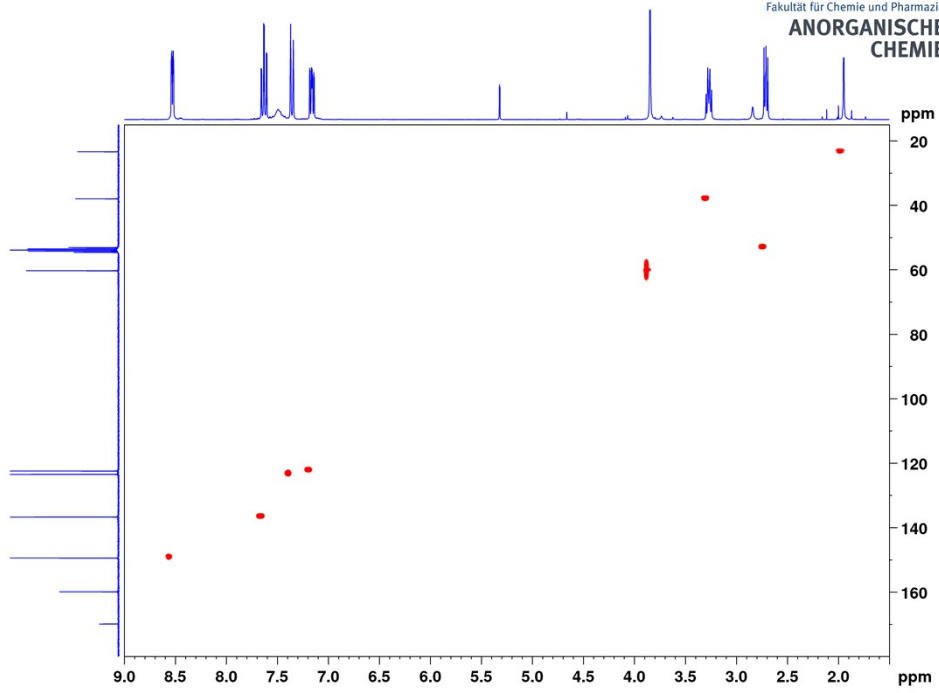
Synthesis of *N*-(2-(bis(pyridin-2-ylmethyl)amino)ethyl)acetamide (**bpen**^{COCH₃})¹ **3**

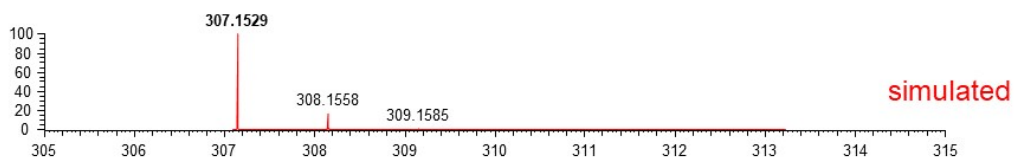
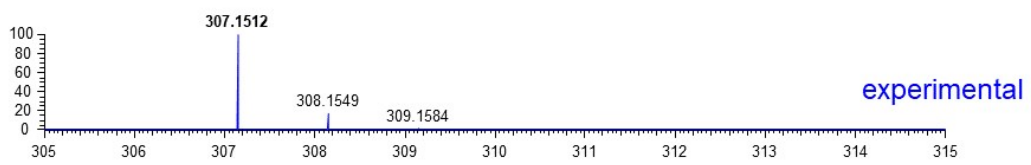
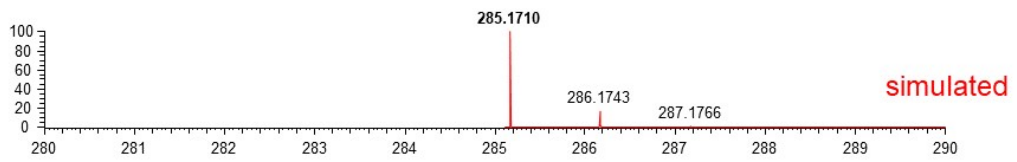
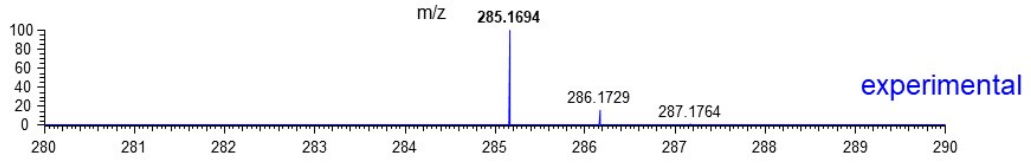
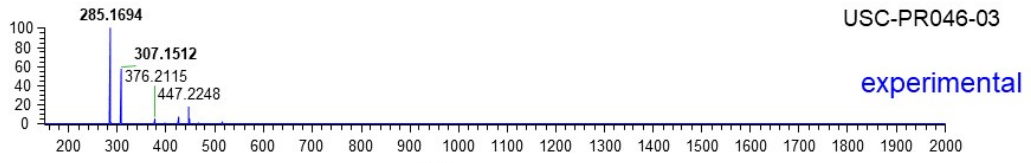


2-Methyl-4,5-dihydro-1*H*-imidazole (5.14 g, 61.1 mmol) was dissolved in water (5 mL) and heated to 75 °C for 2 h. The resulting yellow solution was cooled to room temperature and added to a solution of 2-(chloromethyl)pyridine hydrochloride (20.05 g, 122.2 mmol) in water (50 mL). The mixture was heated to 50 °C and then, with vigorous stirring, 10 M aqueous sodium hydroxide (24.5 mL, 245 mmol) was slowly added over 2 h, resulting in a colour change from light brown to violet-red. After complete addition, heating was continued for another 2 h and the reaction mixture then cooled to room temperature. The solution was extracted with dichloromethane (4 x 50 mL), the combined organic phases dried over sodium sulphate, the solvent removed under vacuum. The resulting dark red oil was dissolved in ethyl acetate (15 mL) and applied to a glass frit filled with neutral aluminium oxide. The yellow-orange product was eluted with ethyl acetate (approx. 300 mL) and the solvent removed under vacuum to obtain the product as a beige solid. Yield: 50% (8.70 g, 30.6 mmol). **IR** (ATR): $\tilde{\nu} = 3244$ (m), 3056 (m), 2821 (w), 1666 (s), 1566 (m), 1430 (m), 1370 (w), 1284 (w), 758 (s) cm^{-1} ; **¹H NMR** (300.18 MHz, CD₂Cl₂): $\delta = 8.53$ (ddd, 2H, ³*J*_{H₆H₅} = 4.9 Hz, ⁴*J*_{H₆H₄} = 1.8 Hz, ⁵*J*_{H₆H₃} = 0.9 Hz, py-H6), 7.63 (dt, 2H, ³*J*_{H₄H₅/H₃} = 7.7 Hz, ⁴*J*_{H₄H₆} = 1.8 Hz, py-H4), 7.49 (s, 1H, NH), 7.36 (d, 2H, ³*J*_{H₃H₄} = 7.7 Hz, py-H3), 7.16 (ddd, 2H, ³*J*_{H₅H₄} = 7.5 Hz, ³*J*_{H₅H₆} = 4.9 Hz, ⁴*J*_{H₅H₃} = 1.2 Hz, py-H5), 3.85 (s, 4H, py-CH₂), 3.27 (q, 2H, ³*J* = 5.4 Hz, CH₂NHAc), 2.71 (t, 2H, ³*J* = 5.7 Hz, (Py-CH₂)₂NCH₂), 1.95 (s, 3H, CH₃) ppm; **¹³C NMR** (75.48 MHz, CD₂Cl₂): $\delta = 169.89$ (C=O) 159.88 (py-C2), 149.42 (py-C6), 136.71 (py-C4), 123.45 (py-C3), 122.41 (py-C5), 60.26 (py-CH₂), 53.05 ((py-CH₂)₂NCH₂), 37.94 (CH₂NHAc), 23.34 (CH₃) ppm; **MS** (ESI⁺, CH₃OH): *m/z* = 285.1694 [M+H]⁺, 307.1513 [M+Na]⁺; **Elemental analysis** (%) calcd. for C₁₆H₂₀N₄O: C 67.58, H 7.09, N 19.70; found (%): C 67.32, H 7.07, N 19.70.

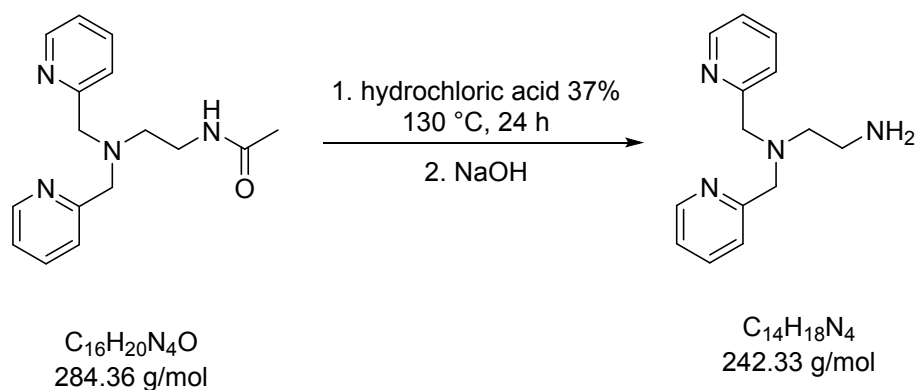




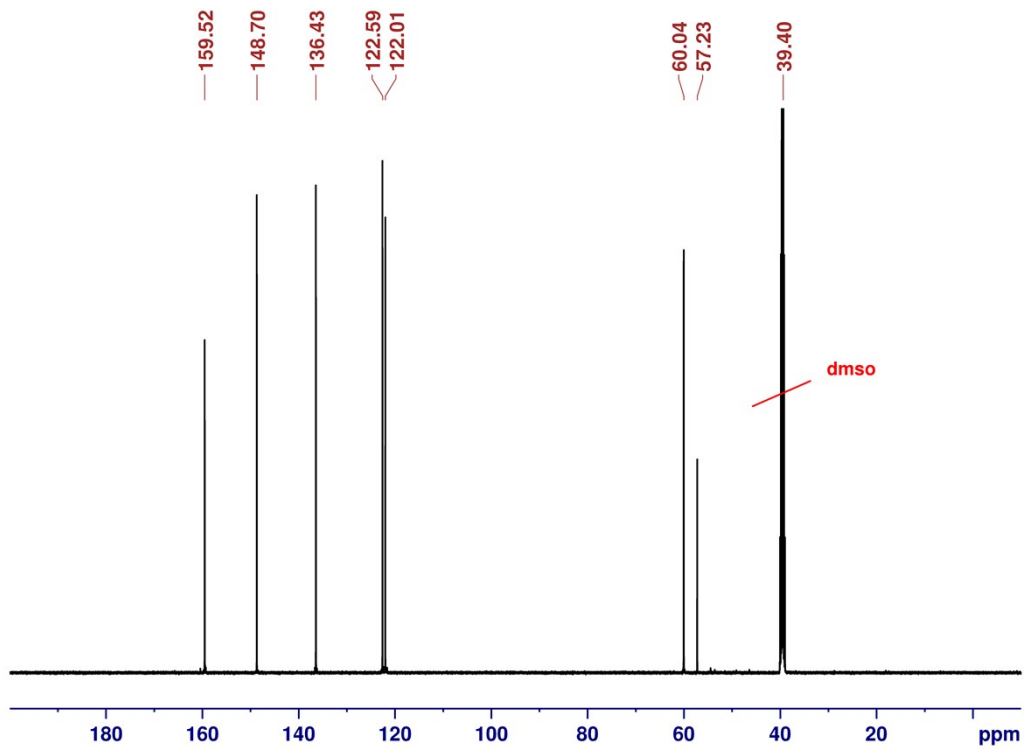
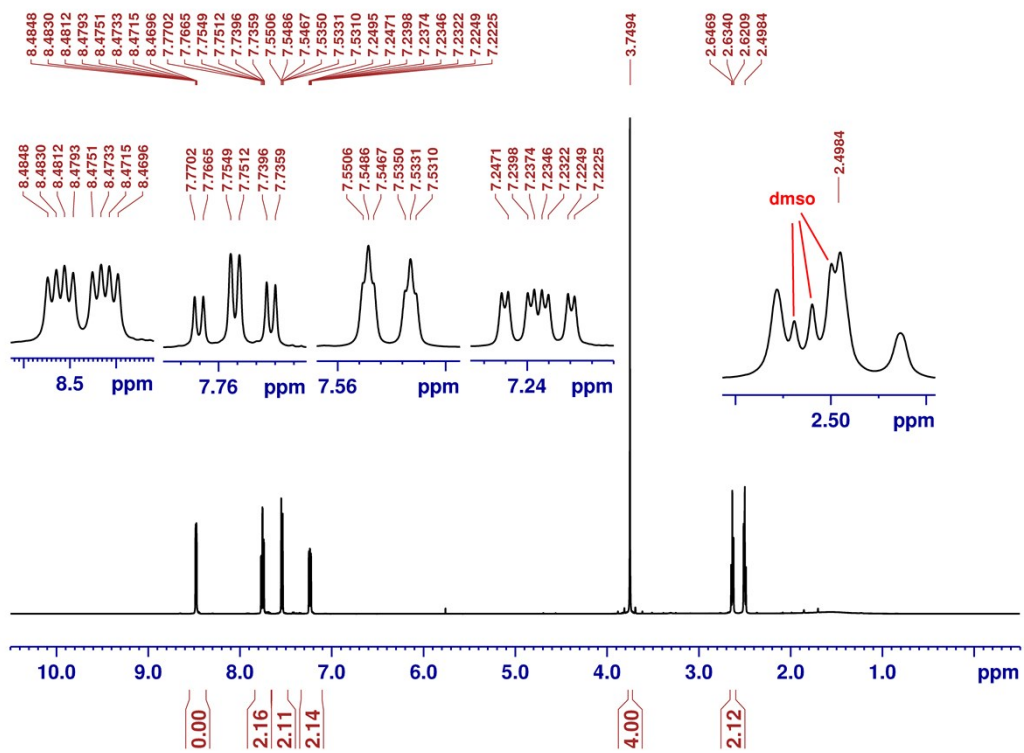




Synthesis of *N,N*-bis(pyridin-2-ylmethyl)ethan-1,2-diamine (bpen)¹ 4

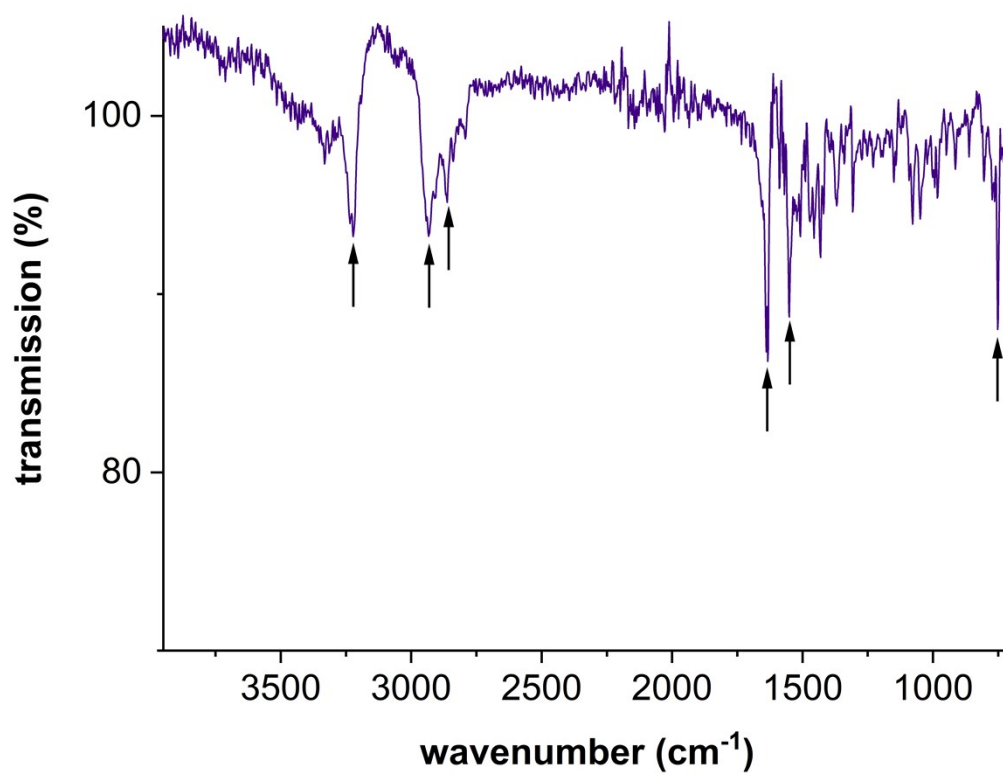
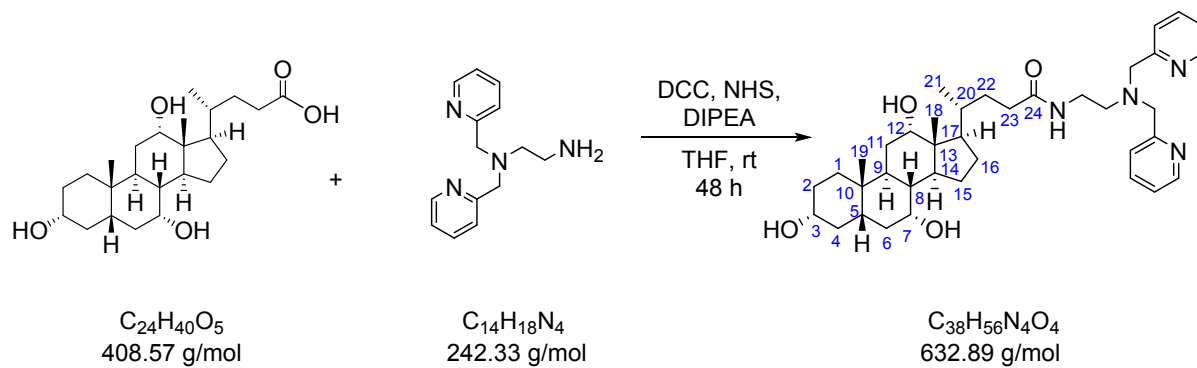


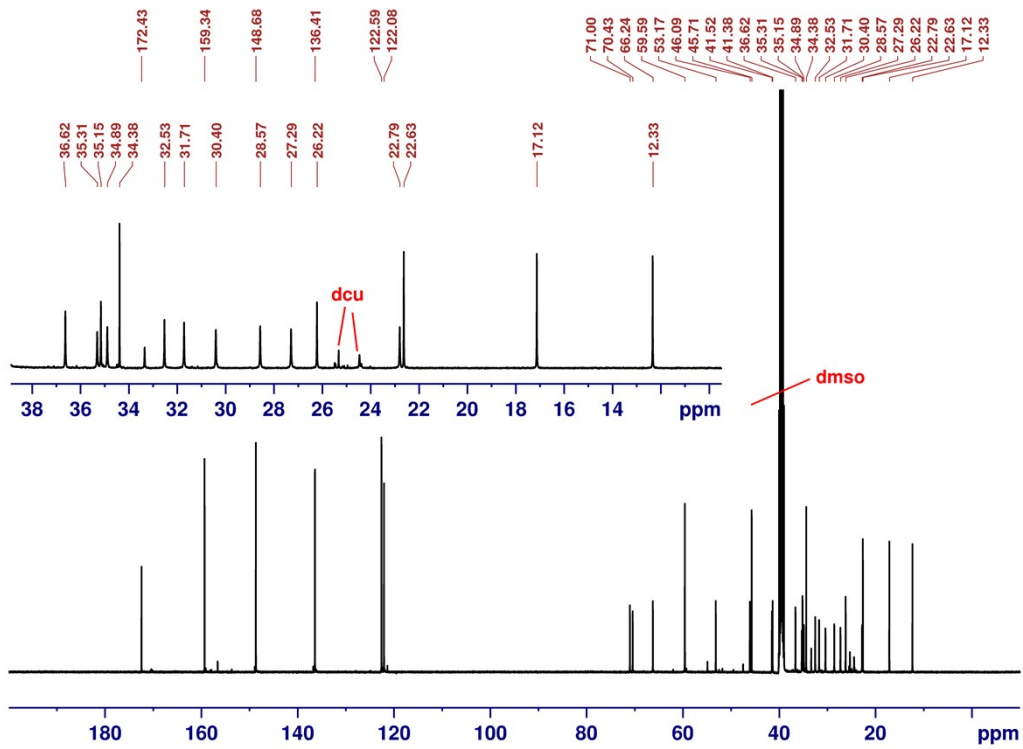
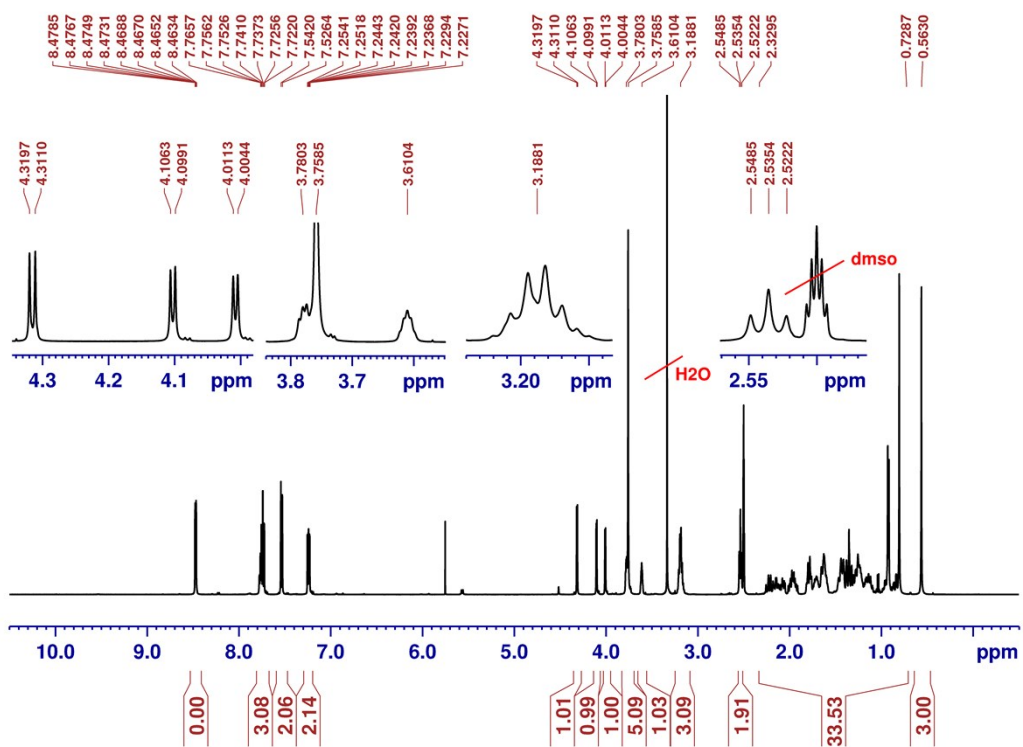
N-(2-(Bis(pyridin-2-ylmethyl)amino)ethyl)acetamide (4.73 g, 16.6 mmol) was dissolved in concentrated hydrochloric acid (50 mL) and then heated to 130 °C for 24 h. The resulting pale-yellow solution was carefully diluted with water (150 mL) and then solid sodium hydroxide was added to adjust to pH 10. The aqueous solution was extracted with dichloromethane (3 x 50 mL) and the combined organic phases were dried over sodium sulphate. After removal of the solvent under vacuum, the product was obtained as a pale-yellow oil. Since the compound is prone to decomposition, it was directly used in the next step with only limited characterization. Yield: 96% (3.89 g, 16.0 mmol). ¹H NMR (500.13 MHz, DMSO-*d*₆): δ = 8.48 (ddd, 2H, ³*J*_{H₆,H₅} = 4.9 Hz, ⁴*J*_{H₆,H₄} = 1.8 Hz, ⁵*J*_{H₆,H₃} = 0.9 Hz, py-H6), 7.75 (dt, 2H, ³*J*_{H₄,H₅/H₃} = 7.7 Hz, ⁴*J*_{H₄,H₆} = 1.9 Hz, py-H4), 7.54 (td, 2H, ³*J*_{H₃,H₄} = 7.7 Hz, ⁴*J*_{H₃,H₅} = 1.0 Hz, ⁵*J*_{H₃,H₆} = 1.0 Hz, py-H3), 7.24 (ddd, 2H, ³*J*_{H₅,H₄} = 7.5 Hz, ³*J*_{H₅,H₆} = 4.9 Hz, ⁴*J*_{H₅,H₃} = 1.2 Hz, py-H5), 3.75 (s, 4H, py-CH₂), 2.63 (t, 2H, ³*J* = 6.5 Hz, CH₂NH₂), 2.50 (t, 2H, ³*J* = 6.5 Hz, (py-CH₂)₂NCH₂, overlapping with solvent peak) ppm; ¹³C NMR (125.76 MHz, DMSO-*d*₆): δ = 159.52 (py-C2), 148.70 (py-C6), 136.43 (py-C4), 122.59 (py-C3), 122.01 (py-C5), 60.04 (py-CH₂), 57.23 ((py-CH₂)₂NCH₂), 39.40 (CH₂NH₂, overlapping with solvent peak) ppm.

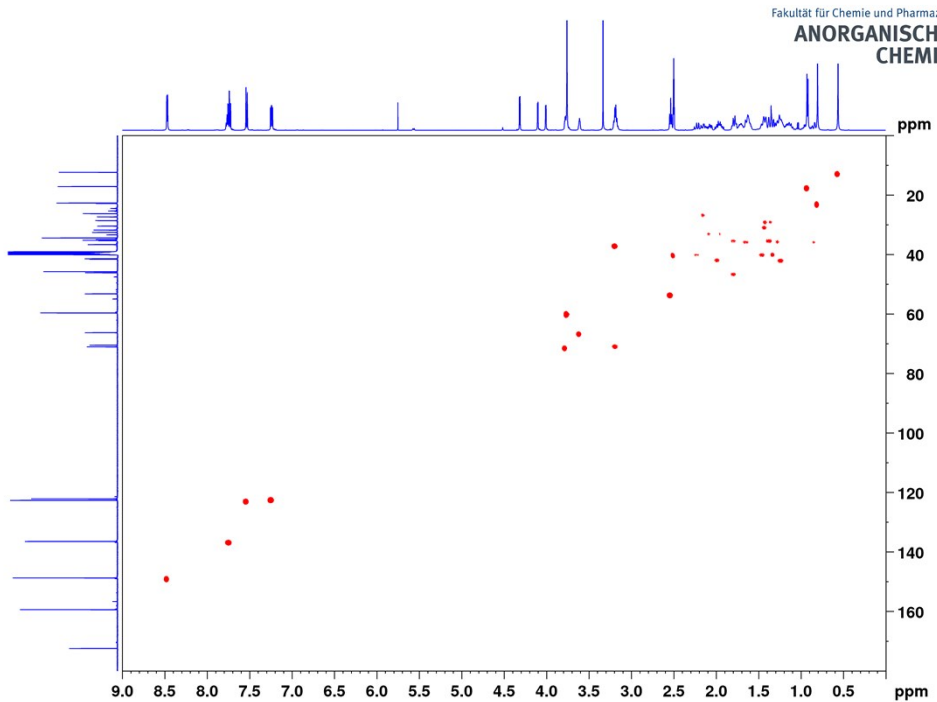
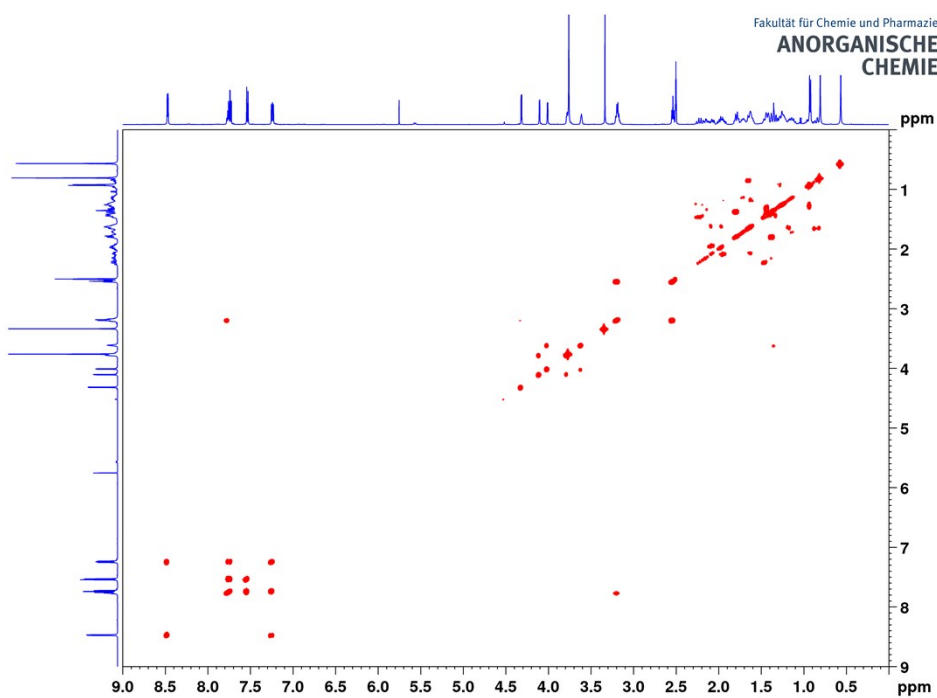


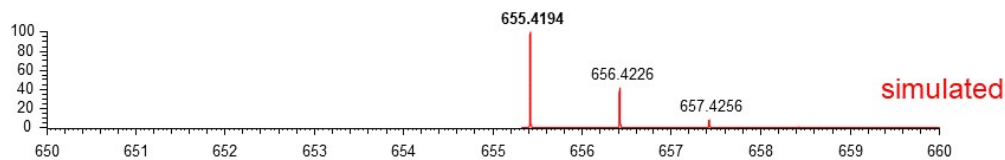
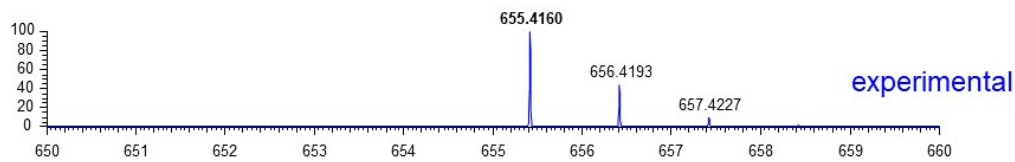
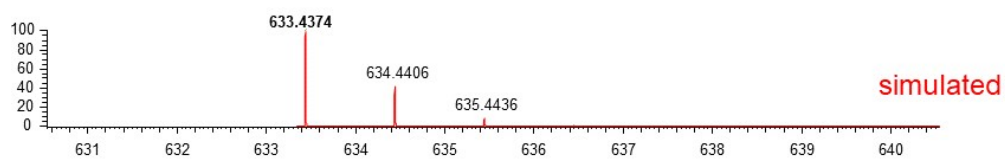
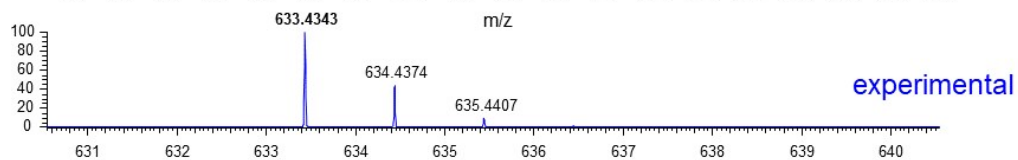
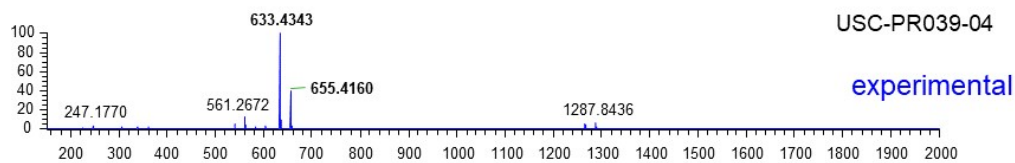
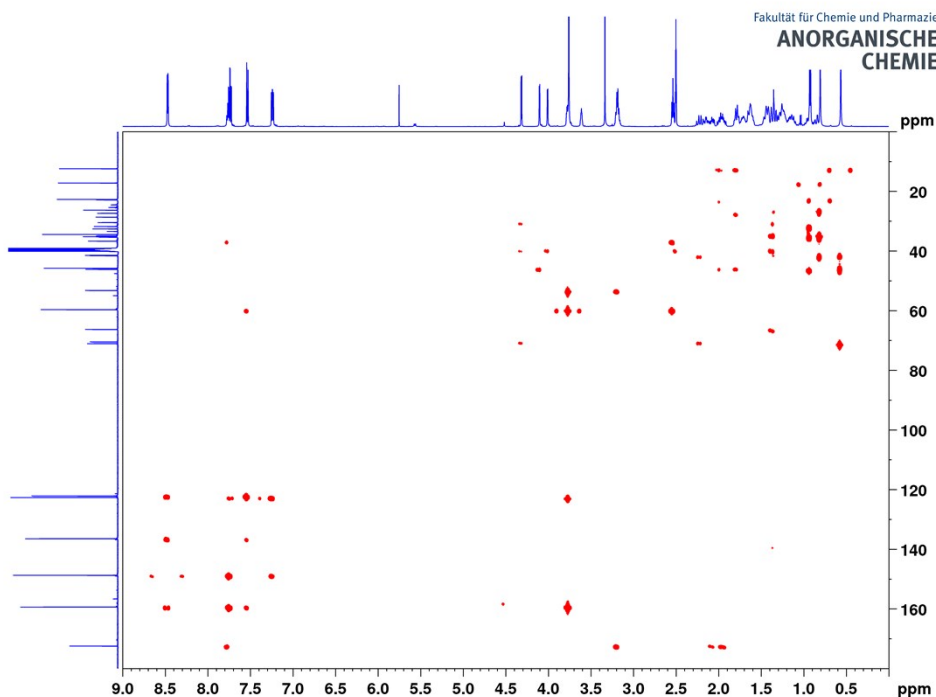
Spectroscopic data for *N*-(2-(bis(pyridin-2-ylmethyl)amino)ethyl)cholamide

(bpen^{cholamid}) **6**

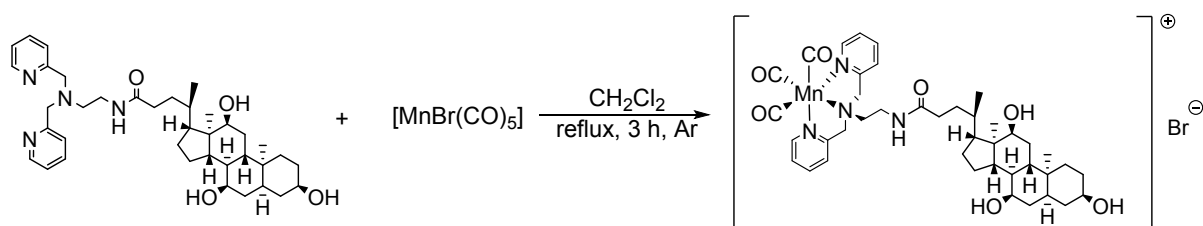








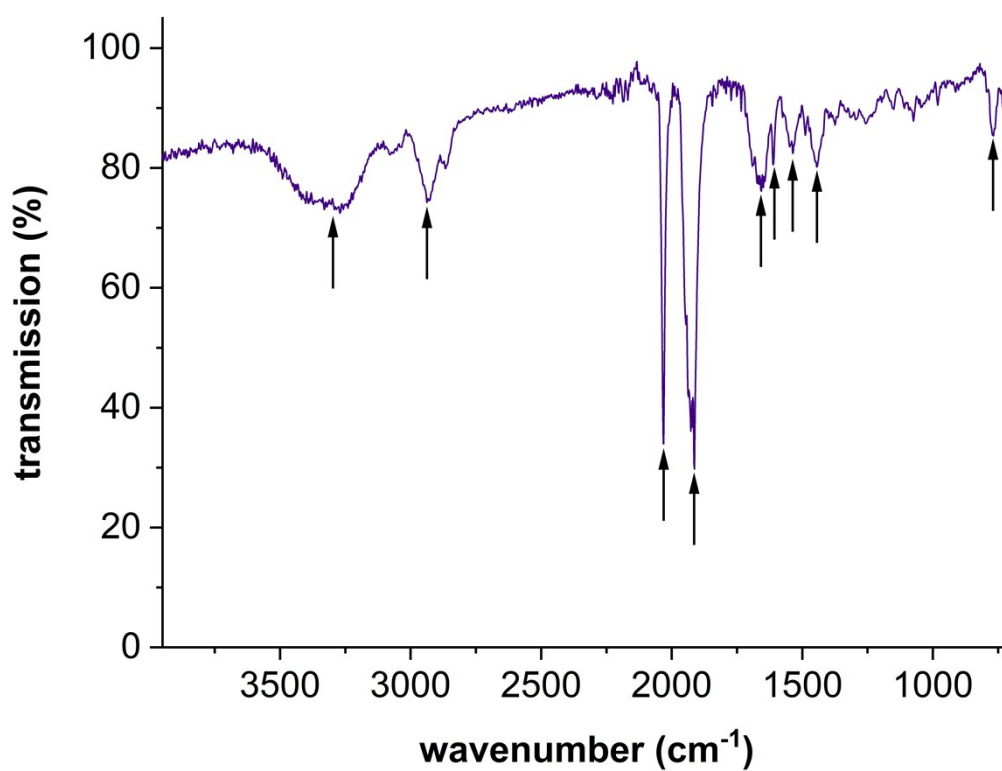
Spectroscopic data for $[\text{Mn}(\text{bpen}^{\text{cholamid}}-\kappa^3\text{N})(\text{CO})_3]\text{Br}$ 9

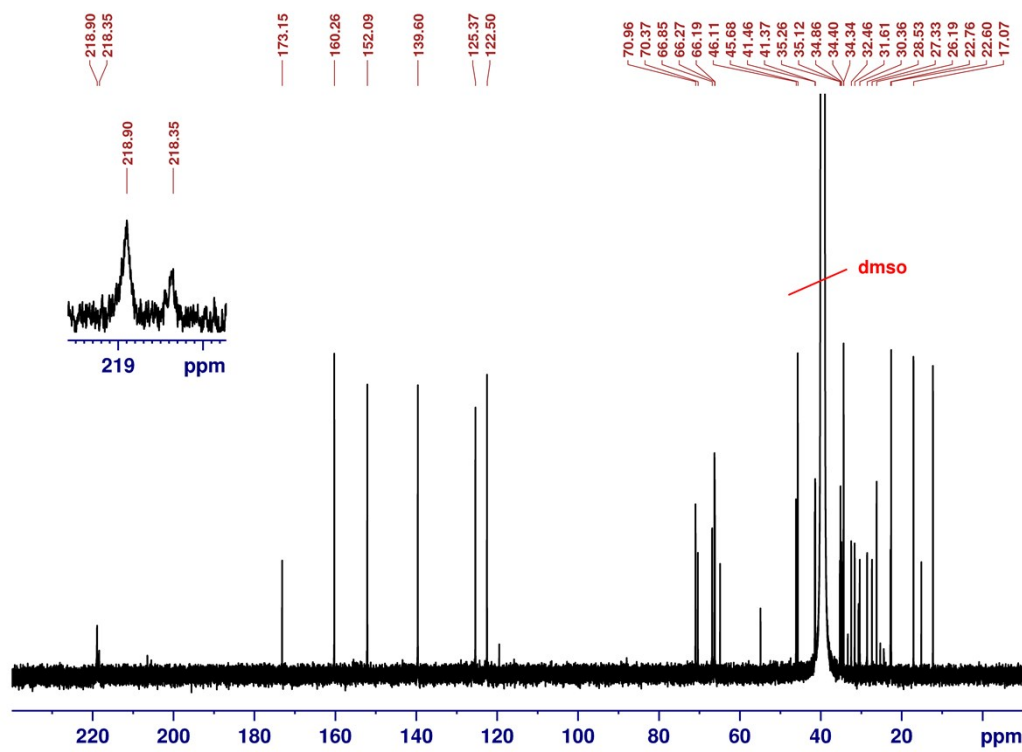
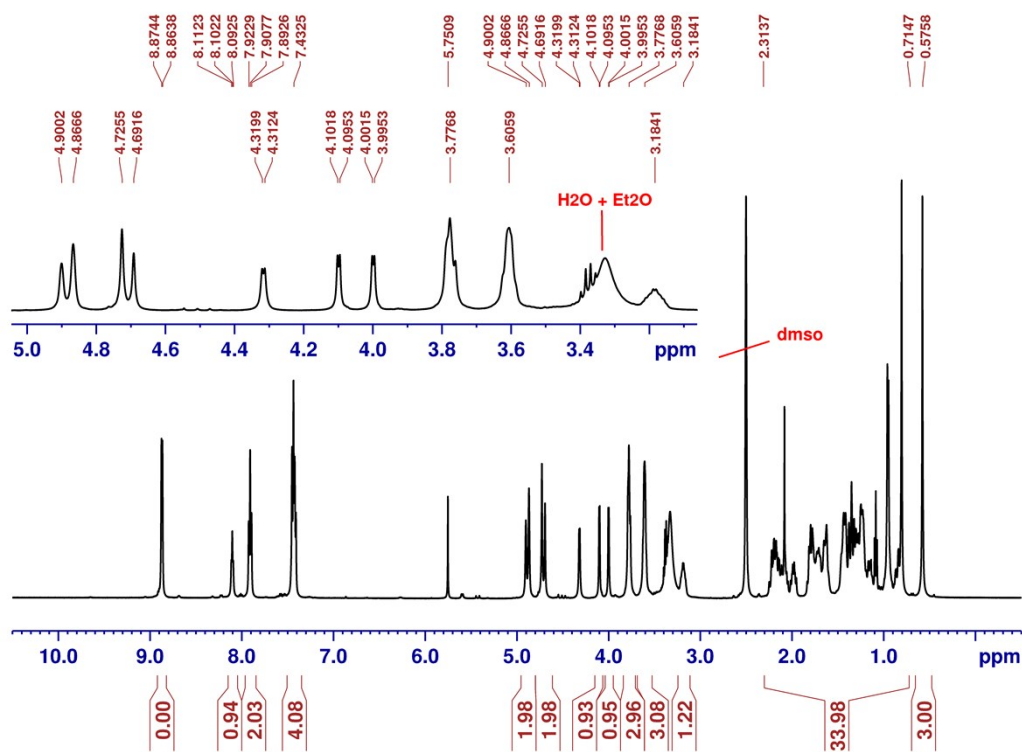


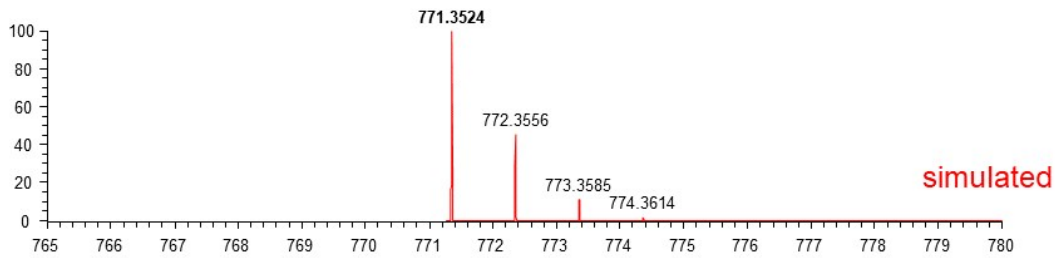
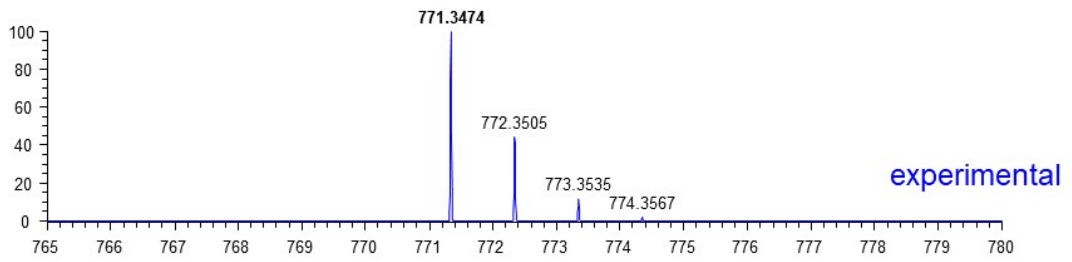
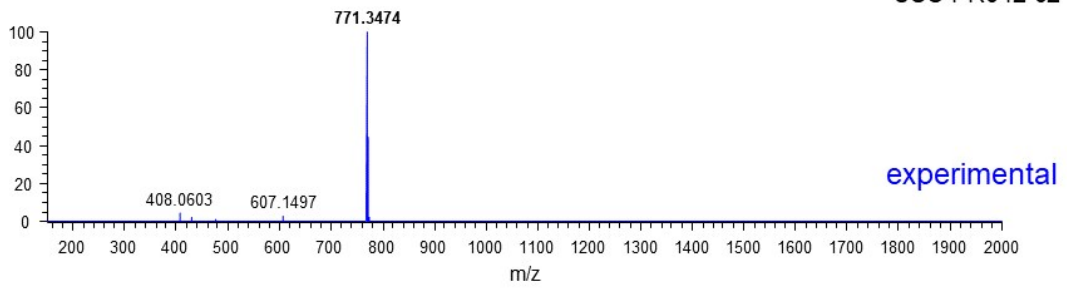
$\text{C}_{38}\text{H}_{56}\text{N}_4\text{O}_4$
632.89 g/mol

274.89 g/mol

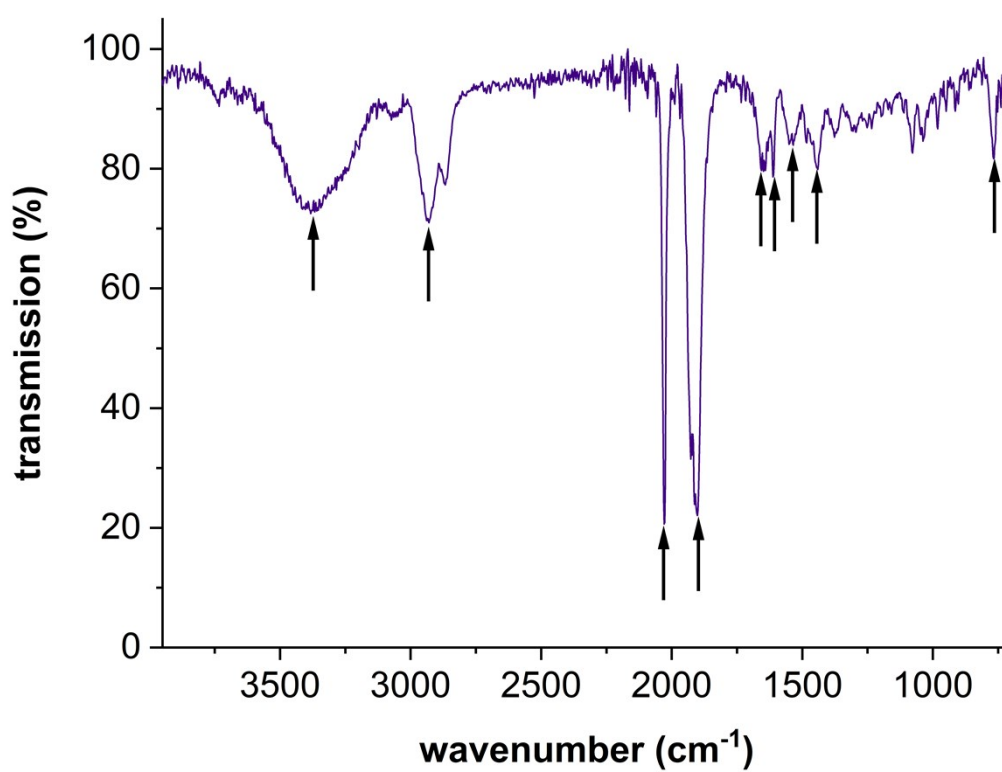
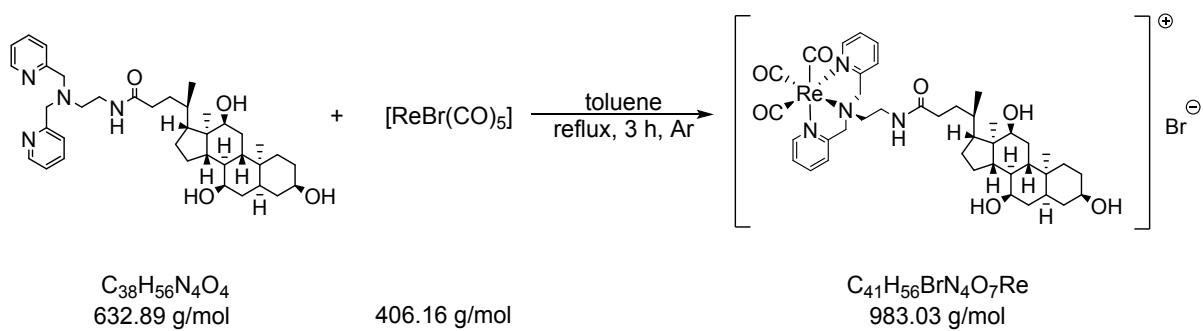
$\text{C}_{41}\text{H}_{56}\text{BrMnN}_4\text{O}_7$
851.76 g/mol

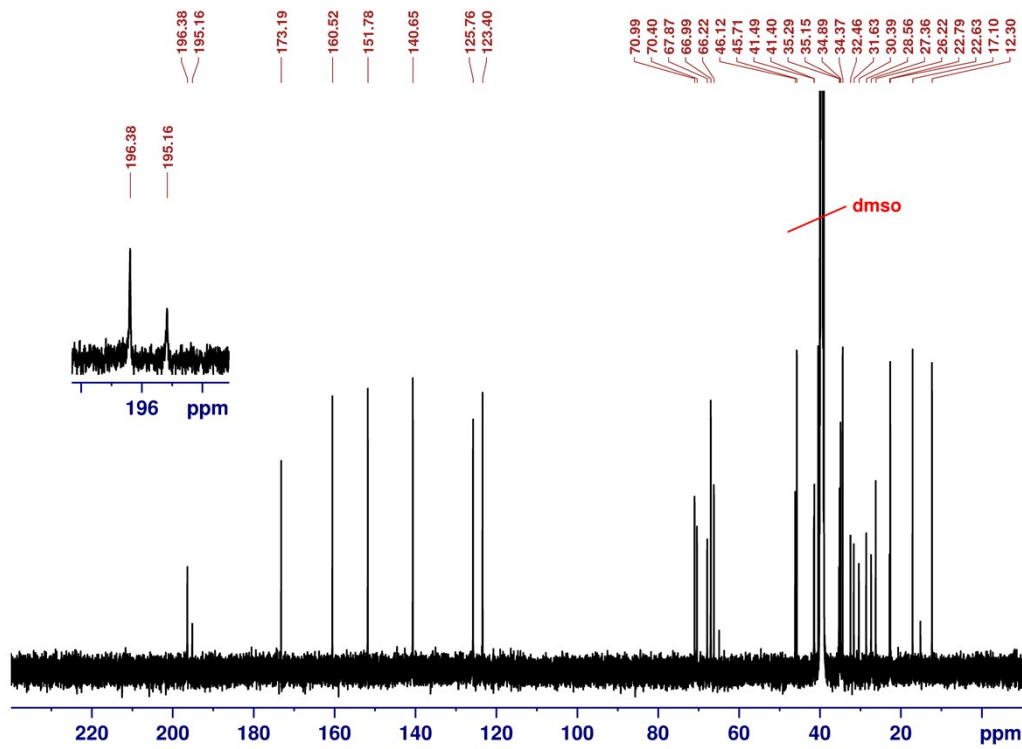
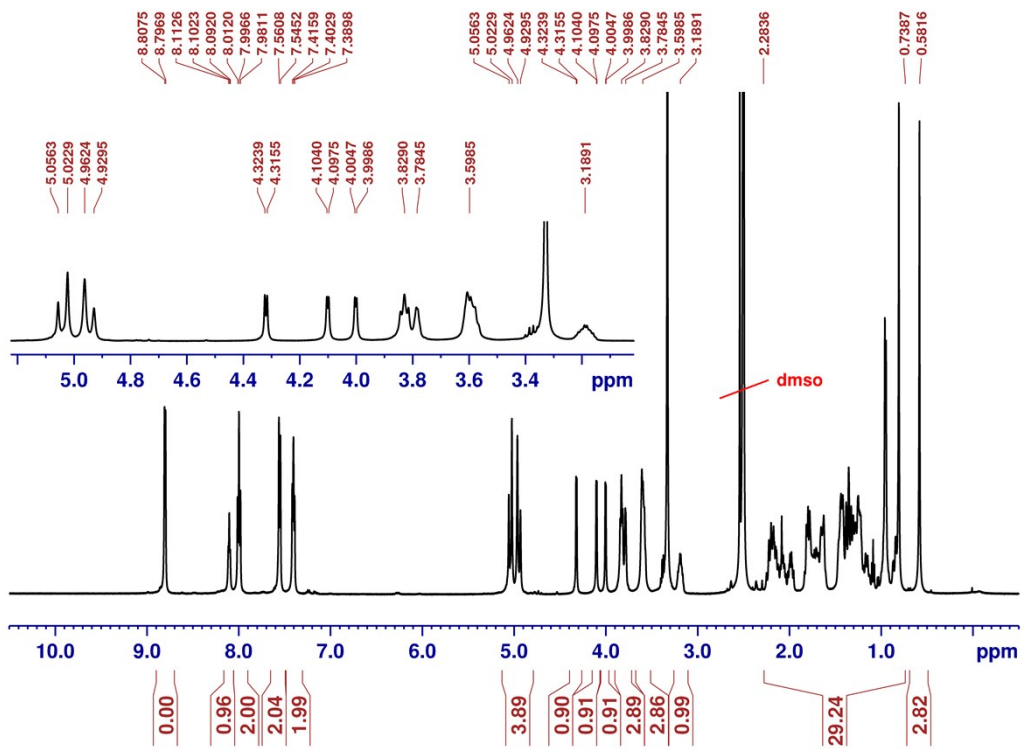


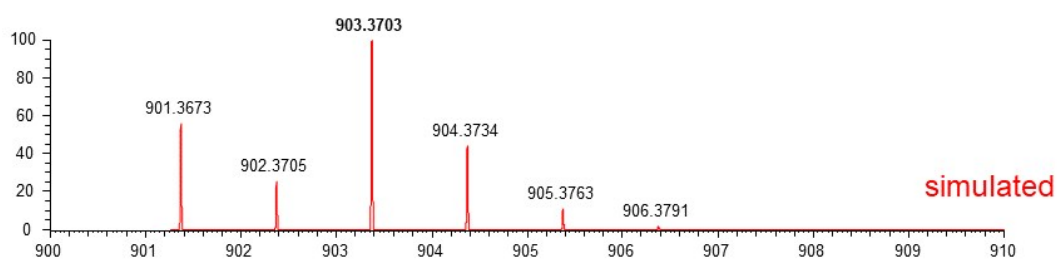
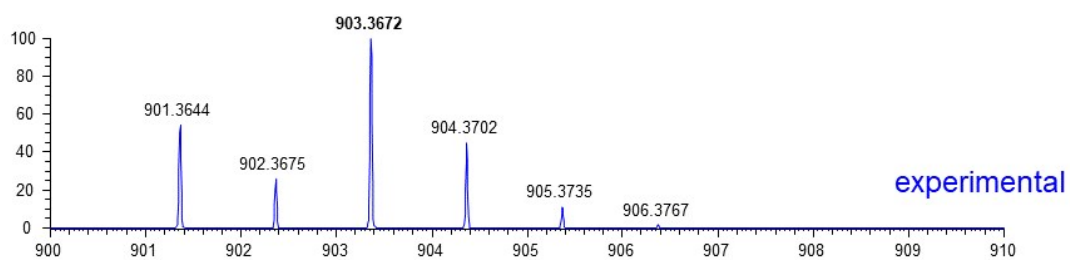
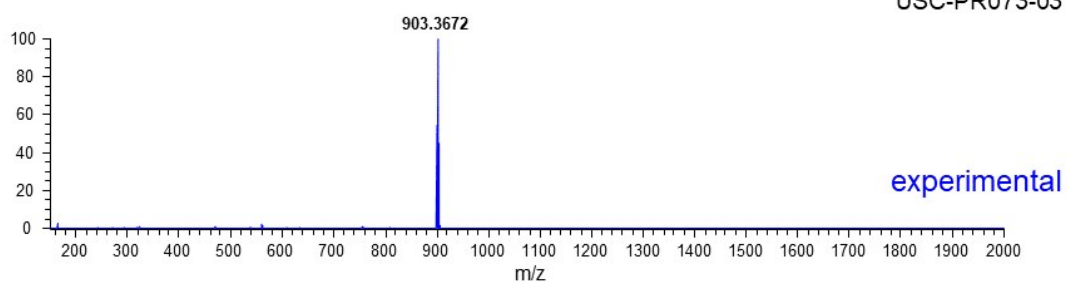




Spectroscopic data for $[\text{Re}(\text{bpen}^{\text{cholamid}}-\kappa^3\text{N})(\text{CO})_3]\text{Br}$ 10







References

- 1 Y.-H. Chiu and J. W. Canary, *Inorg. Chem.*, 2003, **42**, 5107.