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

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Tel: +49 931 31 83636; Fax: +49 931 31 84605

Supporting Information

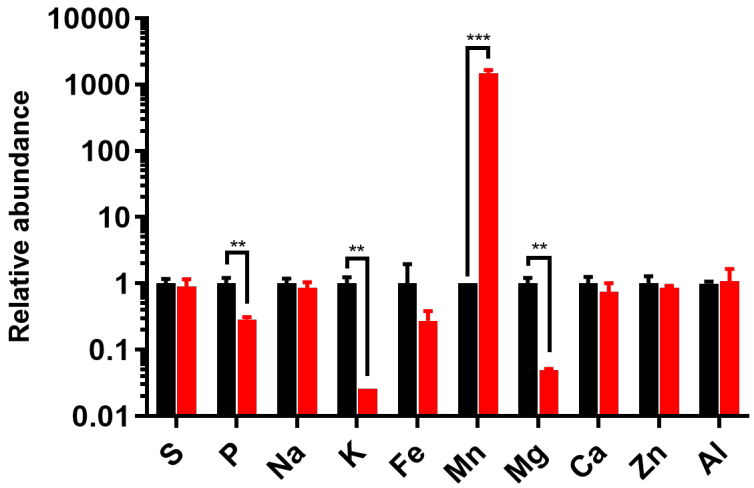


Figure S1. ICP-MS determination of the intracellular metal content of *E. coli* treated with [Mn(CO)$_3$(bqpa-κ^3*N*)]Br (red bars) relative to the metal content of control samples treated with 1% DMSO (black bars). Data shown are mean of three biological replicates and errors bars represent the standard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

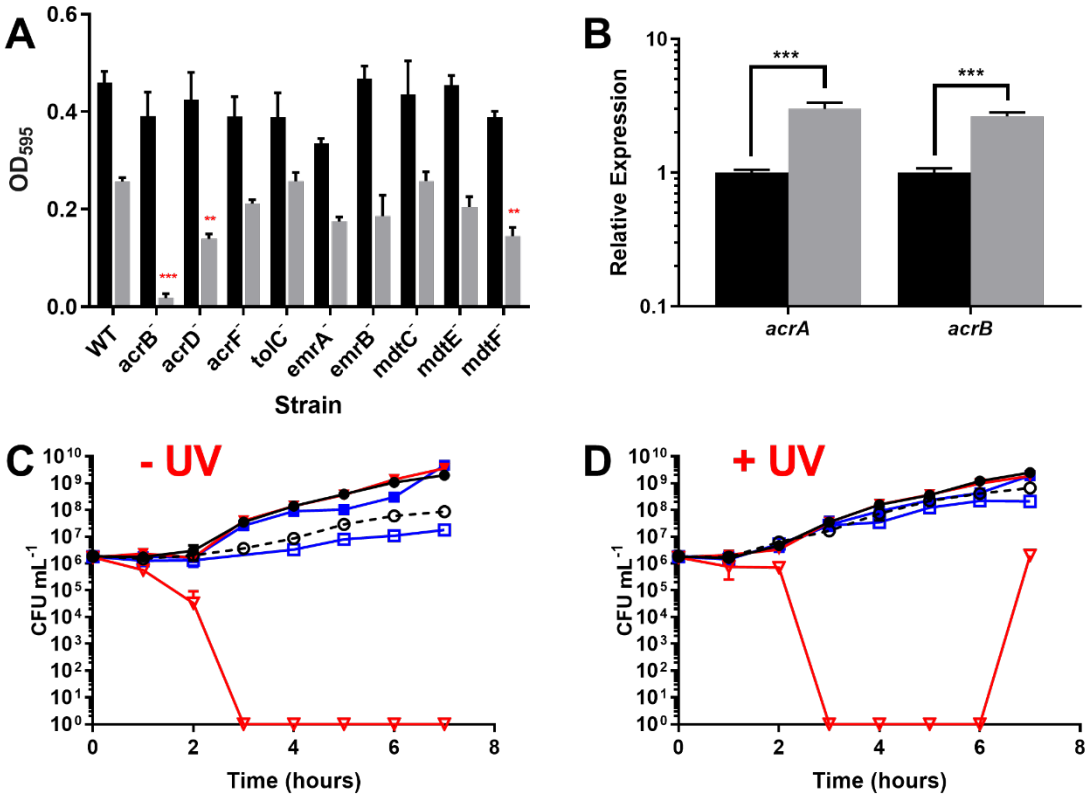


Figure S2. A) 5 h growth of *E. coli* strains in the absence (black bars) and presence (grey bars) of [Mn(CO)$_3$(bqpa-κ^3*N*)]Br. Values are means of three biological repeats with error bars indicating standard deviation. B) RT-PCR showing the relative expression of the *E. coli* genes *acrA* and *acrB* in cells grown in the absence (black bars) and presence (grey bars) of [Mn(CO)$_3$(bqpa-κ^3*N*)]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-κ^3*N*)]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-κ^3*N*)]Br. *, **, and *** denote *p*-values of 0.01–0.05, 0.001–0.01, and < 0.001 respectively, by Student's *t*-test.

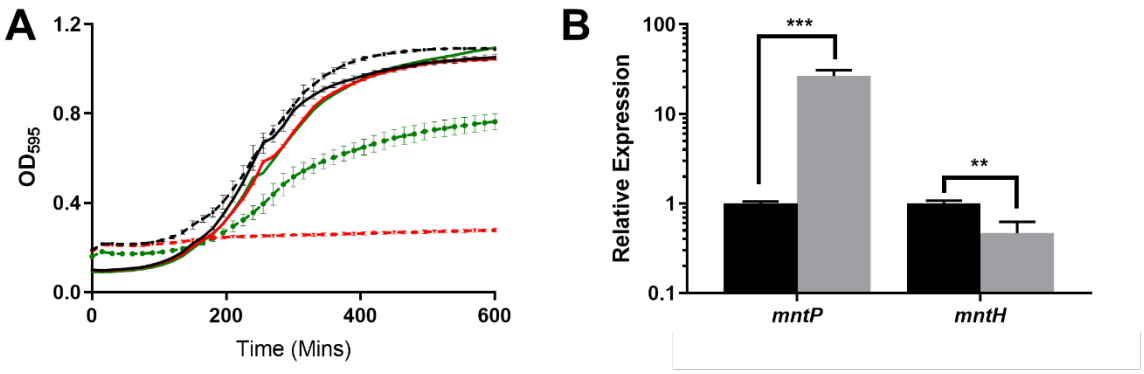
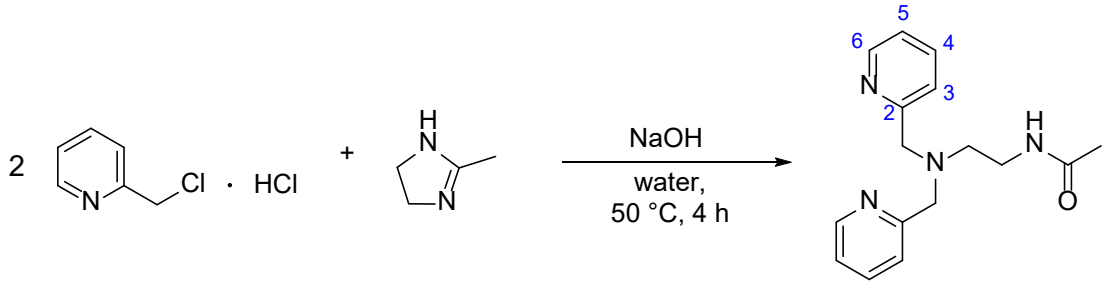


Figure S3. A) Growth curves of *E. coli* strains with (solid line) and without (dashed line) addition of [Mn(bqpa-κ^3*N*)(CO)$_3$]Br (105 μM); WT (BW25113) is represented by the black lines, *mntP*$^-$ is represented by the red lines and *mntR*$^-$ is represented by the green lines and B) RT-PCR of *mntP* 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-κ^3*N*)(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

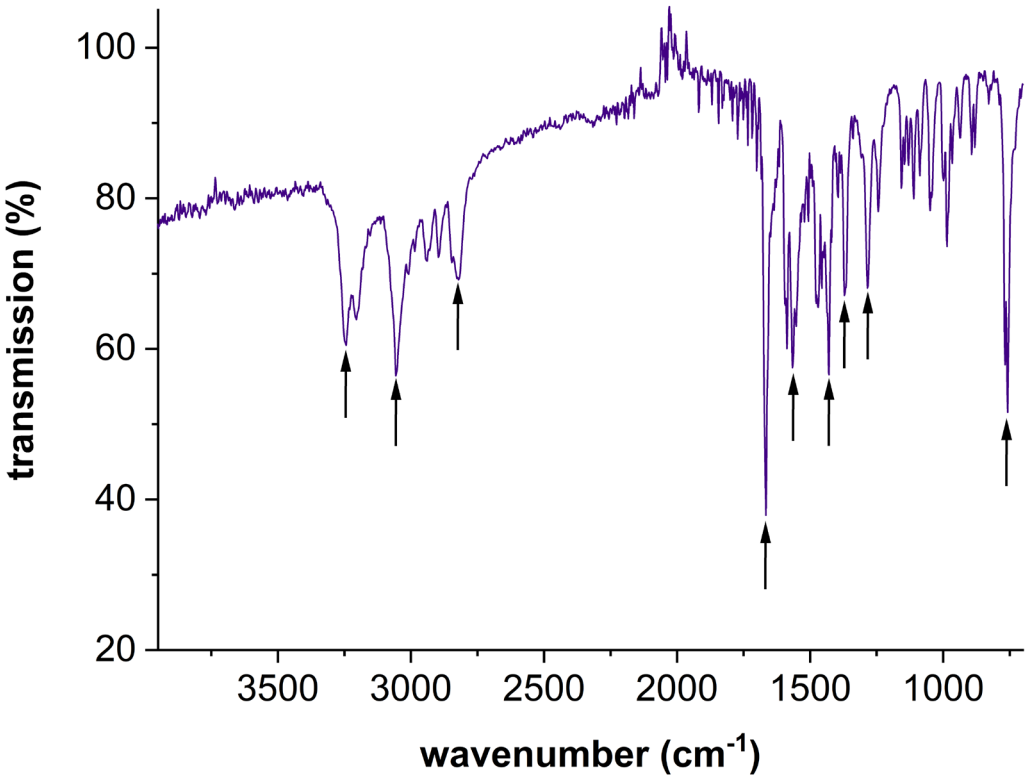


$C_6H_7Cl_2N$
164.03 g/mol

$C_4H_8N_2$
84.12 g/mol

$C_{16}H_{20}N_4O$
284.36 g/mol

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 und 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 dichlormethane (4 x 50 mL), the combined organic phases dried over sodium sulfate, the solvent removed under vacuum. The resulting dark red oil was dissolved in ethyl acetate (15 mL) and applied to a glas 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}; **^{1}H NMR** (300.18 MHz, CD_2Cl_2): δ = 8.53 (ddd, 2H, $^3J_{H6,H5}$ = 4.9 Hz, $^4J_{H6,H4}$ = 1.8 Hz, $^5J_{H6,H3}$ = 0.9 Hz, py-H6), 7.63 (dt, 2H, $^3J_{H4,H5/H3}$ = 7.7 Hz, $^4J_{H4,H6}$ = 1.8 Hz, py-H4), 7.49 (s, 1H, N*H*), 7.36 (d, 2H, $^3J_{H3,H4}$ = 7.7 Hz, py-H3), 7.16 (ddd, 2H, $^3J_{H5,H4}$ = 7.5 Hz, $^3J_{H5,H6}$ = 4.9 Hz, $^4J_{H5,H3}$ = 1.2 Hz, py-H5), 3.85 (s, 4H, py-CH_2), 3.27 (q, 2H, ^3J = 5.4 Hz, CH_2NHAc), 2.71 (t, 2H, ^3J = 5.7 Hz, (Py-CH$_2$)$_2$NCH_2), 1.95 (s, 3H, CH_3) ppm; **^{13}C NMR** (75.48 MHz, CD_2Cl_2): δ = 169.89 (*C*=O) 159.88 (py-*C*2), 149.42 (py-*C*6), 136.71 (py-*C*4), 123.45 (py-*C*3), 122.41 (py-*C*5), 60.26 (py-*C*H$_2$), 53.05 ((py-CH$_2$)$_2$N*C*H$_2$), 37.94 (*C*H$_2$NHAc), 23.34 (*C*H$_3$) ppm; **MS** (ESI$^+$, CH_3OH): *m*/*z* = 285.1694 [M+H]$^+$, 307.1513 [M+Na]$^+$; **Elemental analysis** (%) calcd. for $C_{16}H_{20}N_4O$: C 67.58, H 7.09, N 19.70; found (%): C 67.32, H 7.07, N 19.70.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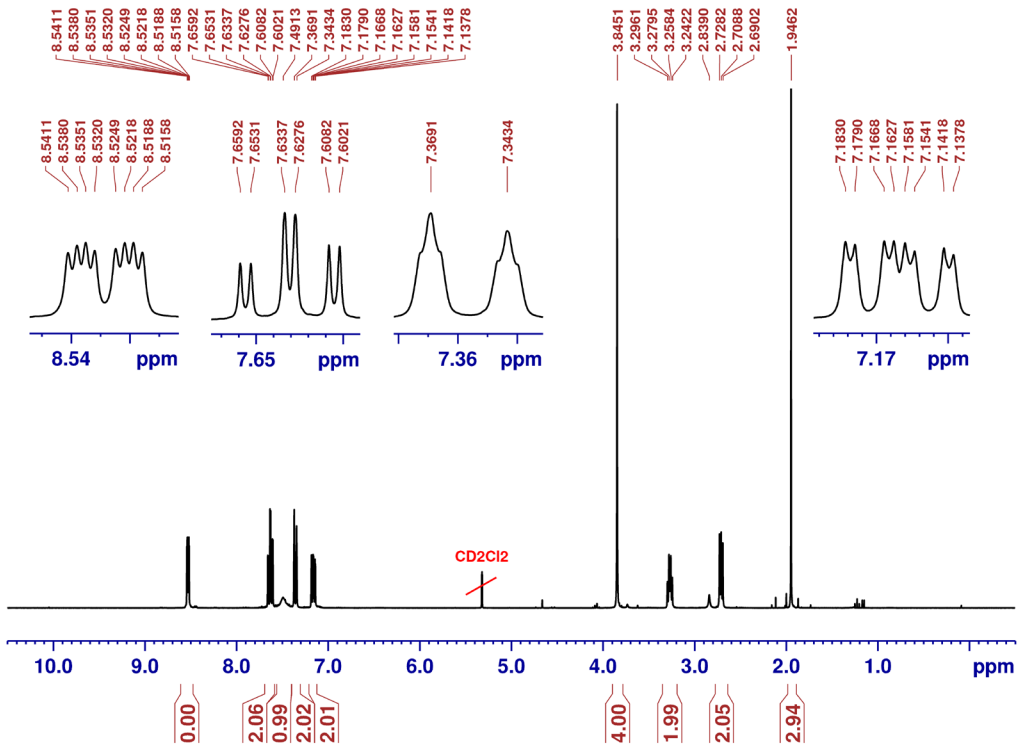
60
40
20
3500
3000
2500
2000
1500
1000
wavenumber (cm-1)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7.17 ppm
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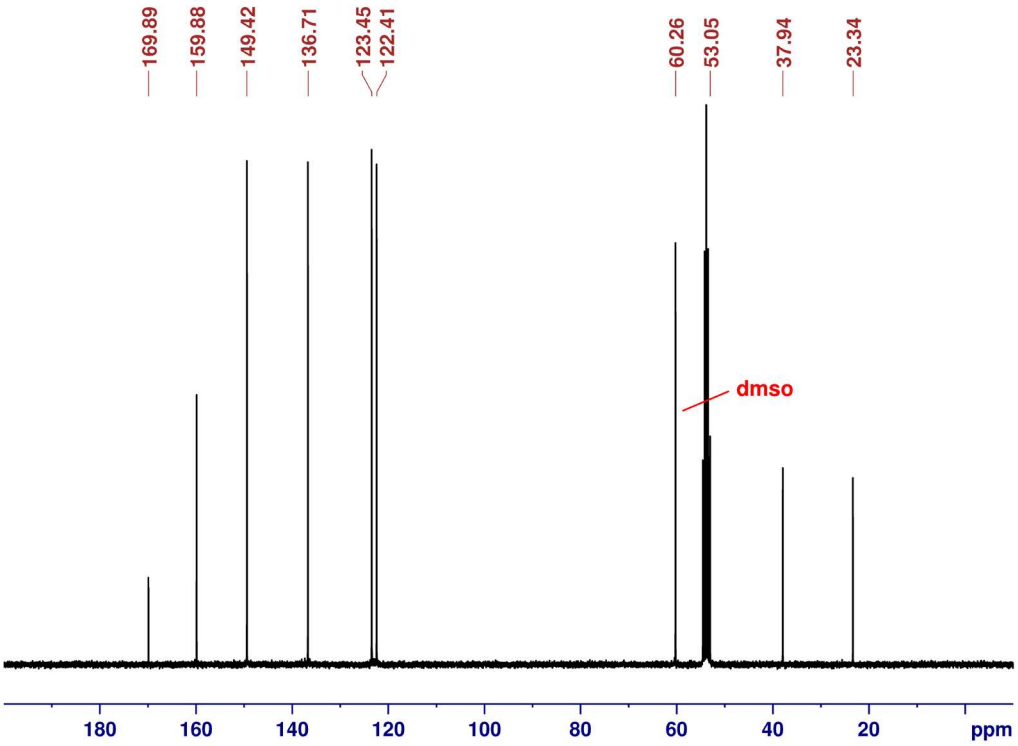
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20
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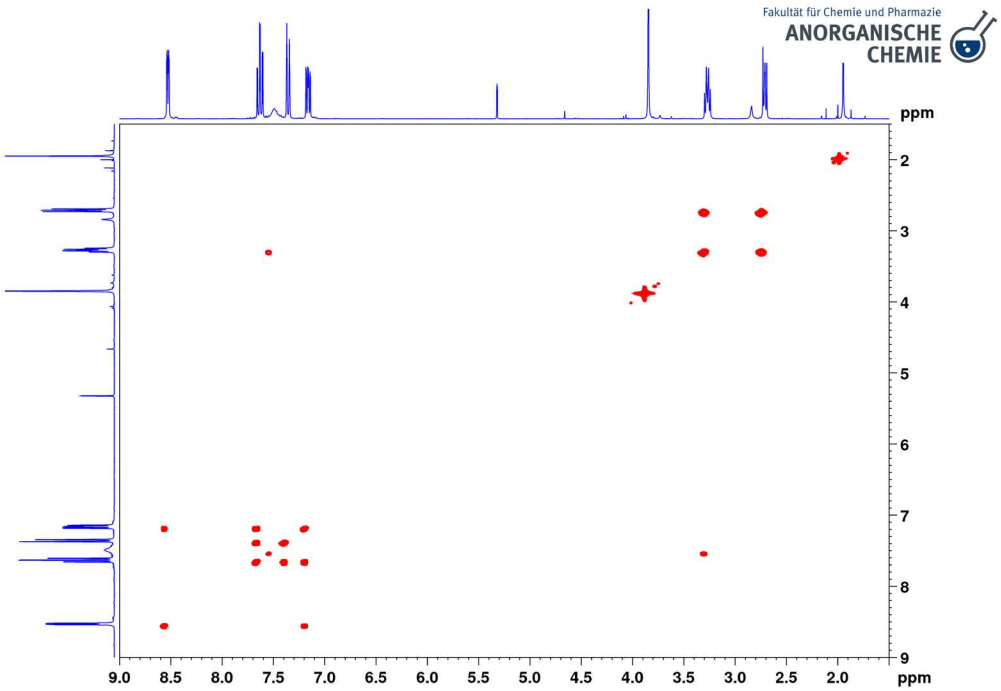
Fakultät für Chemie und Pharmazie
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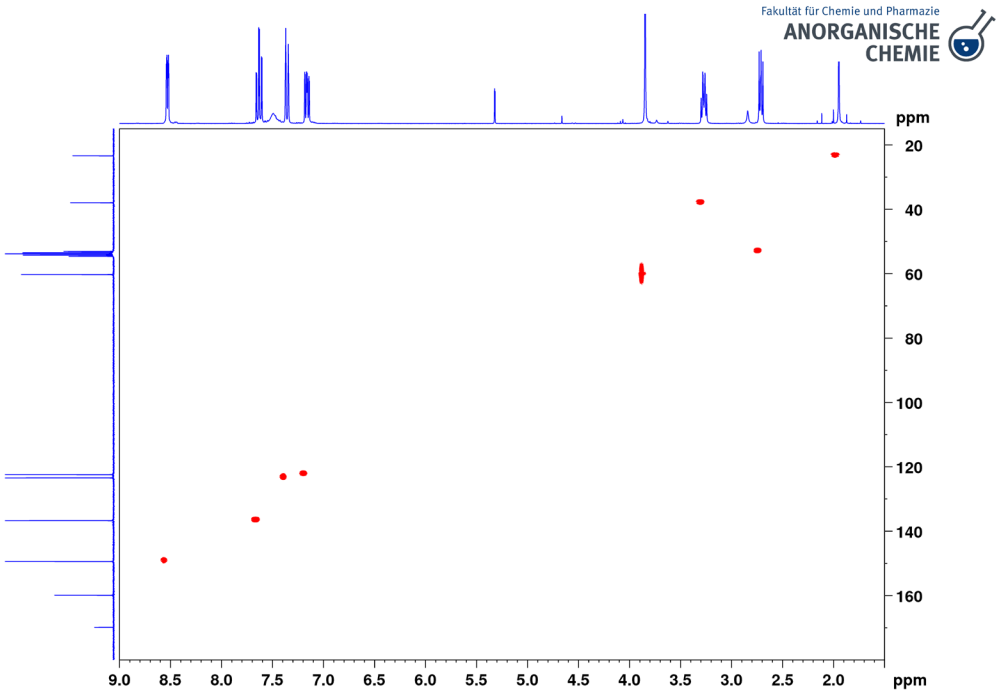
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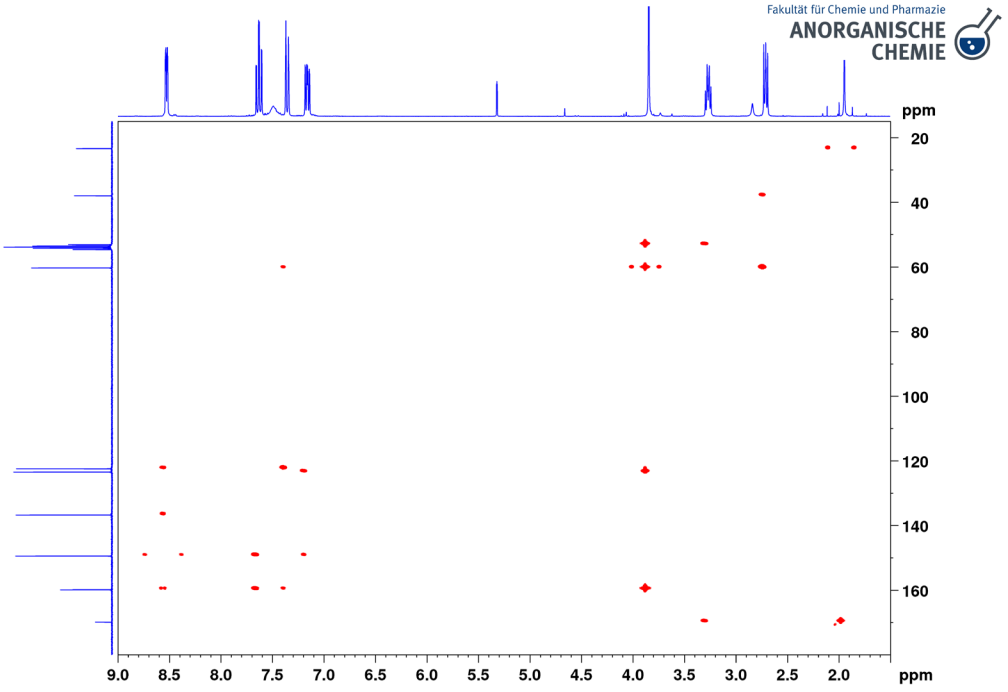
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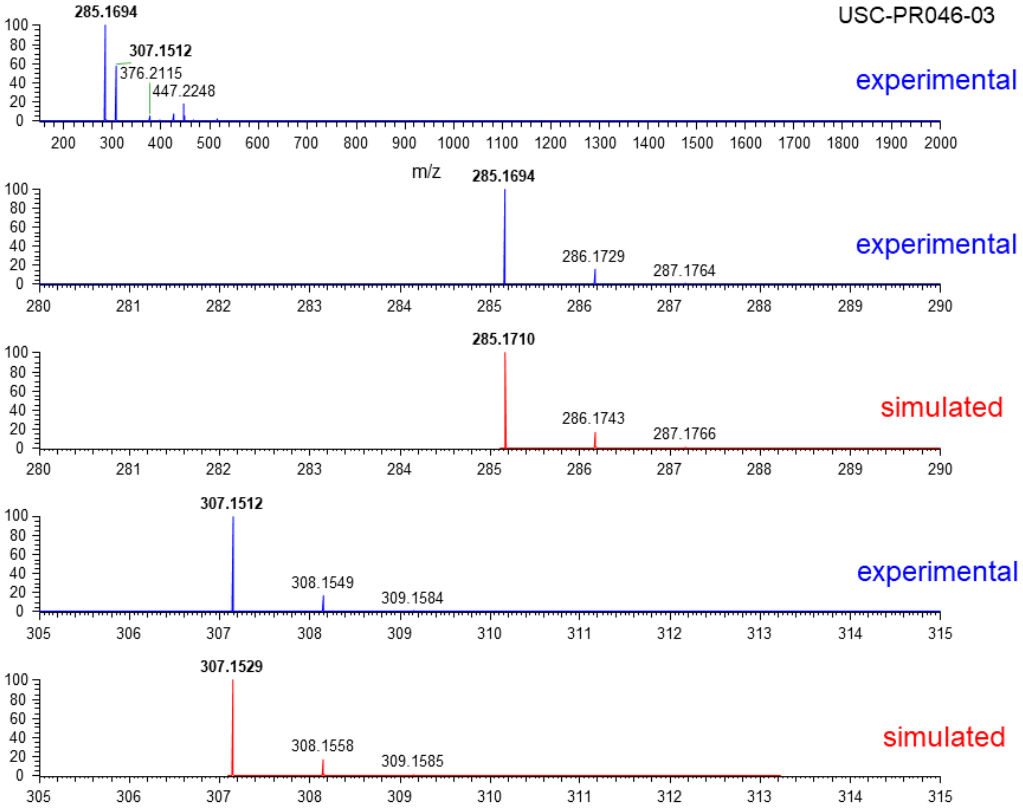
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experimental
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286.1743
287.1766
simulated
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308.1549
309.1584
experimental
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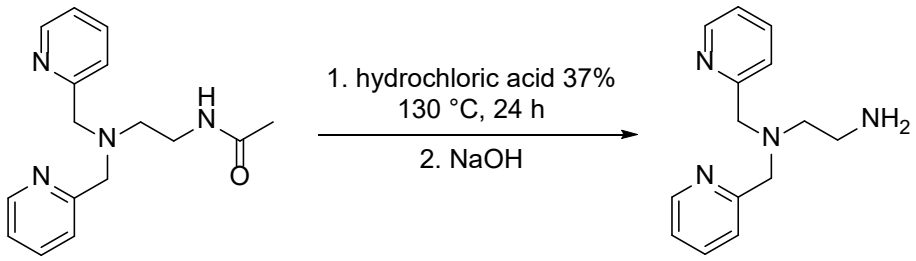
simulated

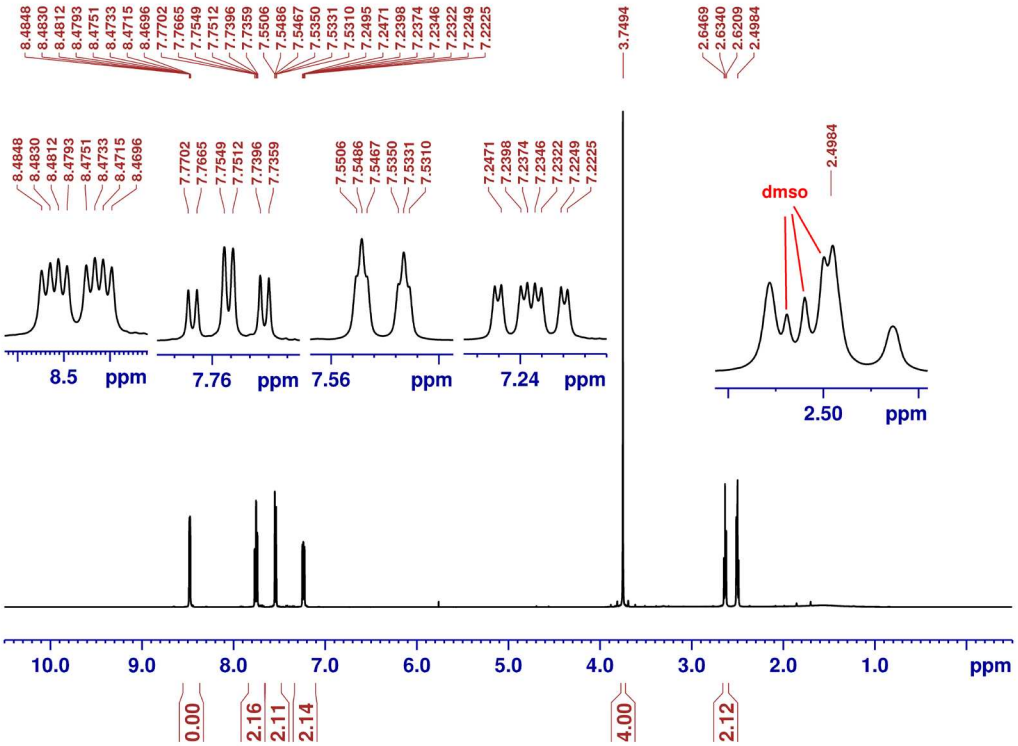
Synthesis of *N*,*N*-bis(pyridin-2-ylmethyl)ethan-1,2-diamine (bpen)[1] 4



$C_{16}H_{20}N_4O$
284.36 g/mol

$C_{14}H_{18}N_4$
242.33 g/mol

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 dichlormethane (3 x 50 mL) and the combined organic phases were dried over sodium sulfate. 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). **^{1}H NMR** (500.13 MHz, DMSO-d_6): δ = 8.48 (ddd, 2H, $^3J_{H6,H5}$ = 4.9 Hz, $^4J_{H6,H4}$ = 1.8 Hz, $^5J_{H6,H3}$ = 0.9 Hz, py-H6), 7.75 (dt, 2H, $^3J_{H4,H5/H3}$ = 7.7 Hz, $^4J_{H4,H6}$ = 1.9 Hz, py-H4), 7.54 (td, 2H, $^3J_{H3,H4}$ = 7.7 Hz, $^4J_{H3,H5}$ = 1.0 Hz, $^5J_{H3,H6}$ = 1.0 Hz, py-H3), 7.24 (ddd, 2H, $^3J_{H5,H4}$ = 7.5 Hz, $^3J_{H5,H6}$ = 4.9 Hz, $^4J_{H5,H3}$ = 1.2 Hz, py-H5), 3.75 (s, 4H, py-CH_2), 2.63 (t, 2H, ^3J = 6.5 Hz, CH_2NH$_2$), 2.50 (t, 2H, ^3J = 6.5 Hz, (py-CH$_2$)$_2$NCH_2, overlapping with solvent peak) ppm; **^{13}C NMR** (125.76 MHz, DMSO-d_6): δ = 159.52 (py-*C*2), 148.70 (py-*C*6), 136.43 (py-*C*4), 122.59 (py-*C*3), 122.01 (py-*C*5), 60.04 (py-*C*H$_2$), 57.23 ((py-CH$_2$)$_2$N*C*H$_2$), 39.40 (*C*H$_2$NH$_2$, overlapping with solvent peak) ppm.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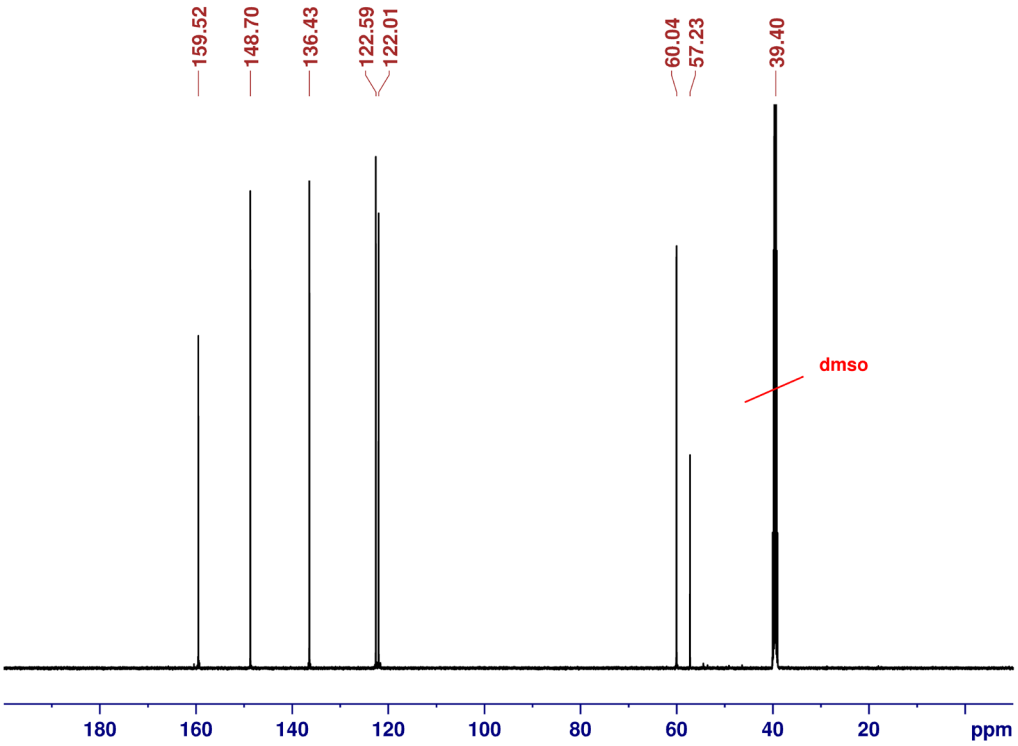
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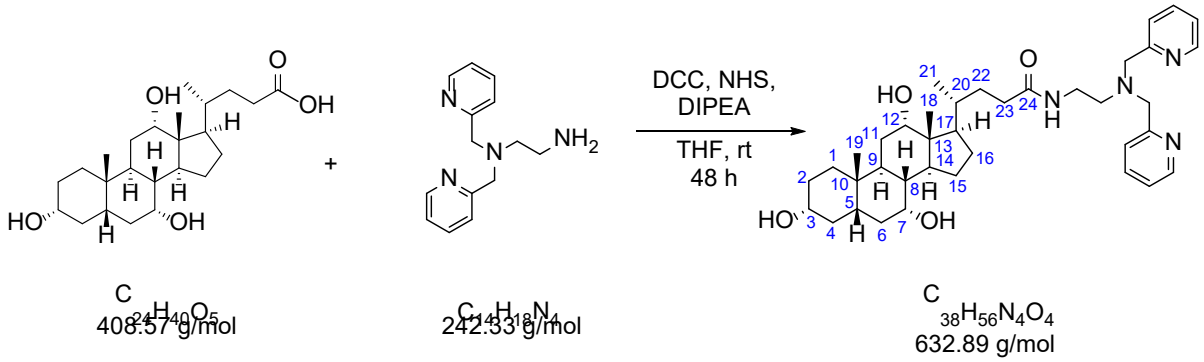
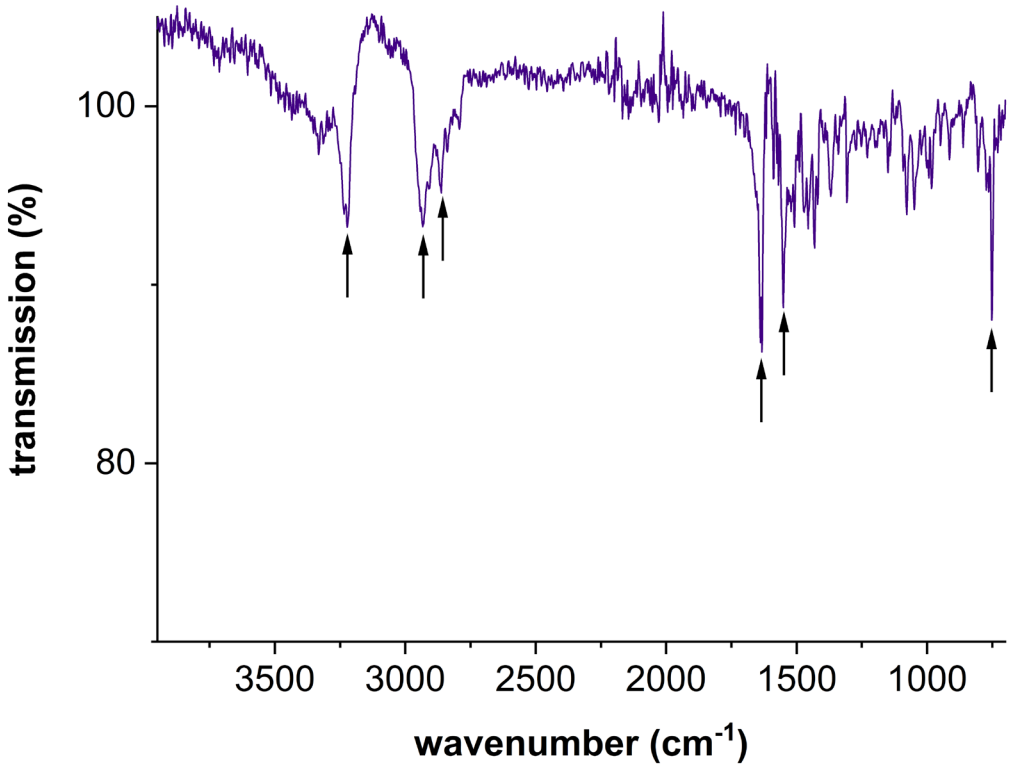
Spectroscopic data for *N*-(2-(bis(pyridin-2-ylmethyl)amino)ethyl)cholamide (bpen^{cholamid}) 6





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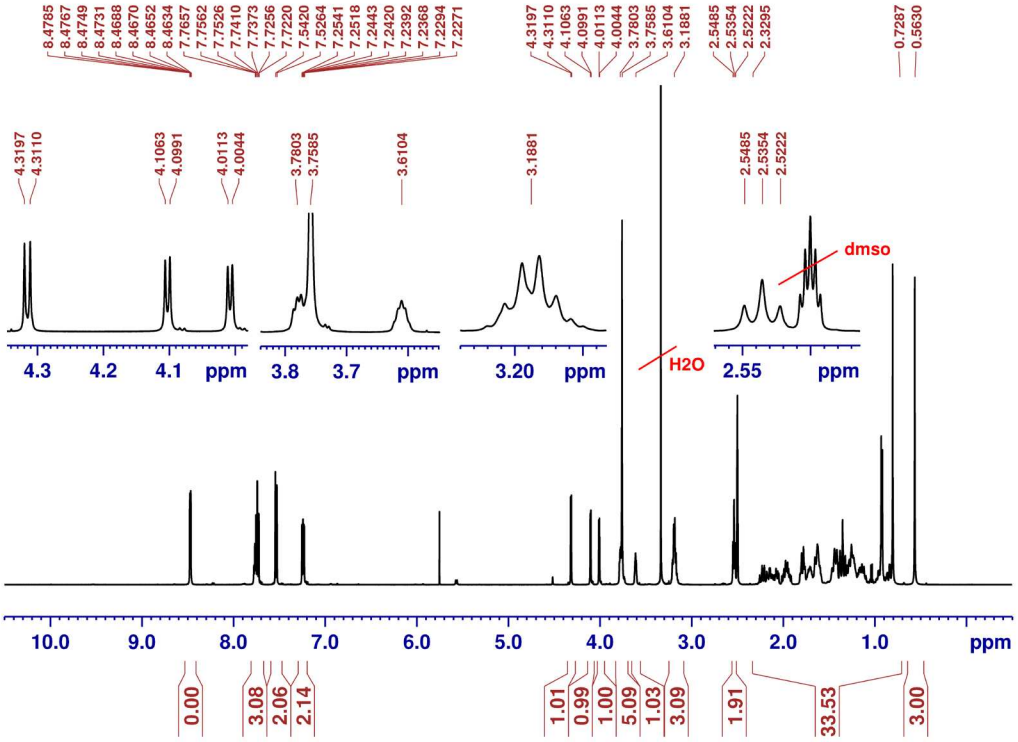
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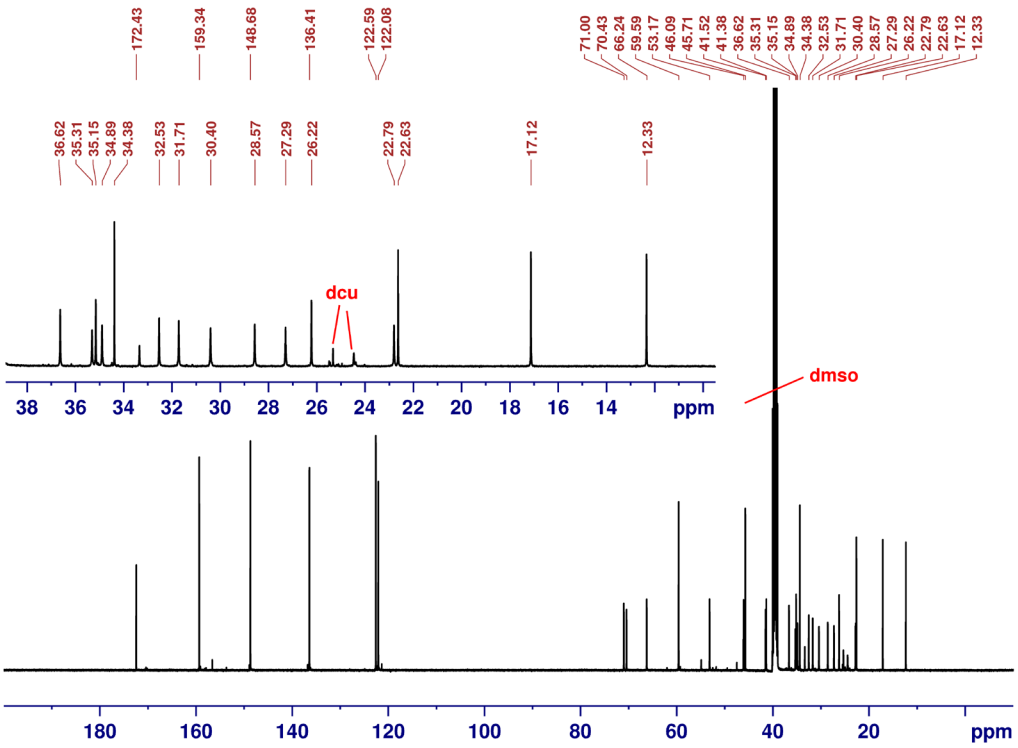
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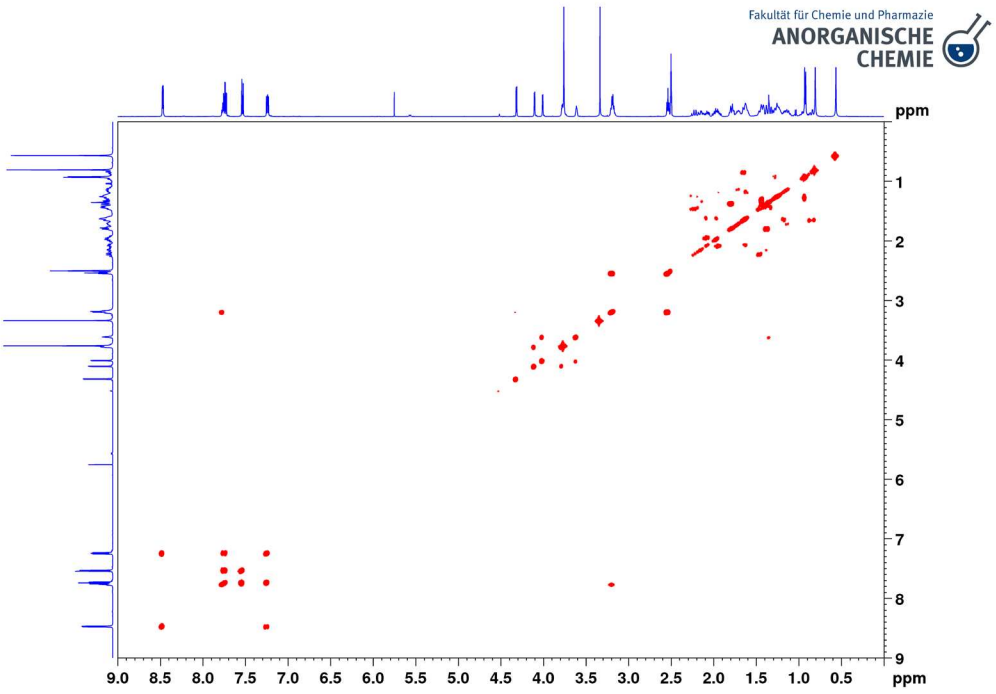
Fakultät für Chemie und Pharmazie
ANORGANISCHE CHEMIE
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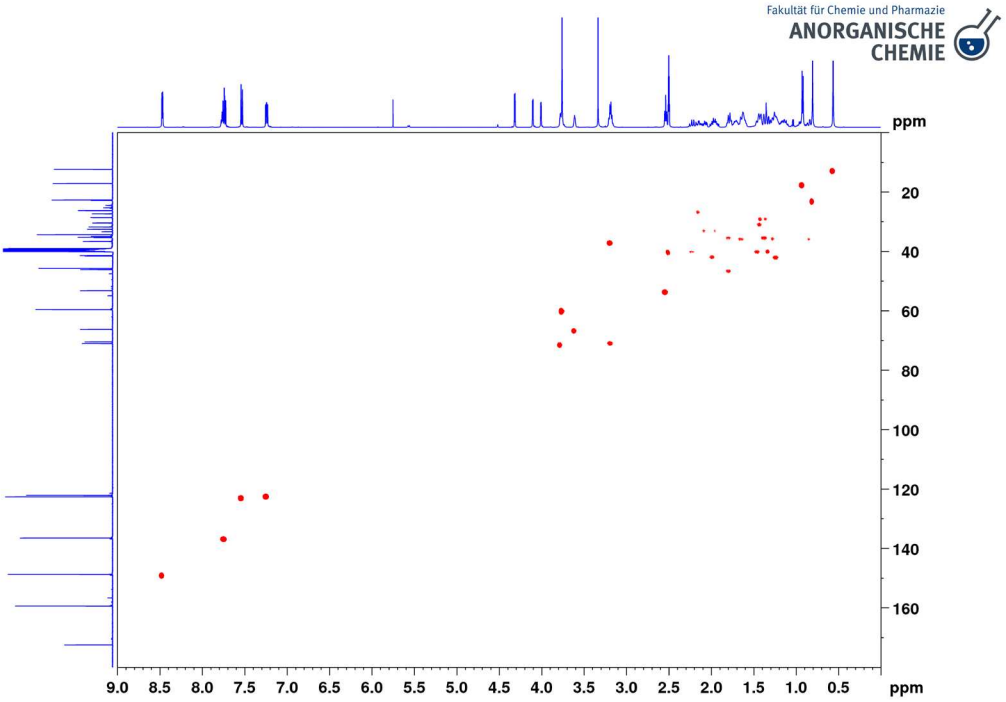
Fakultät für Chemie und Pharmazie
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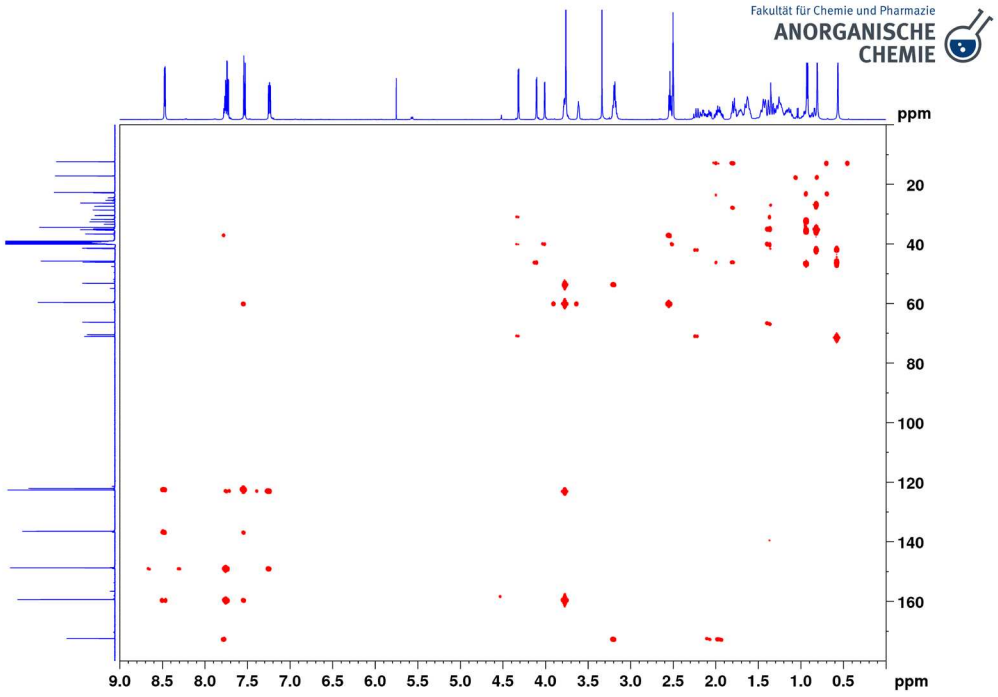
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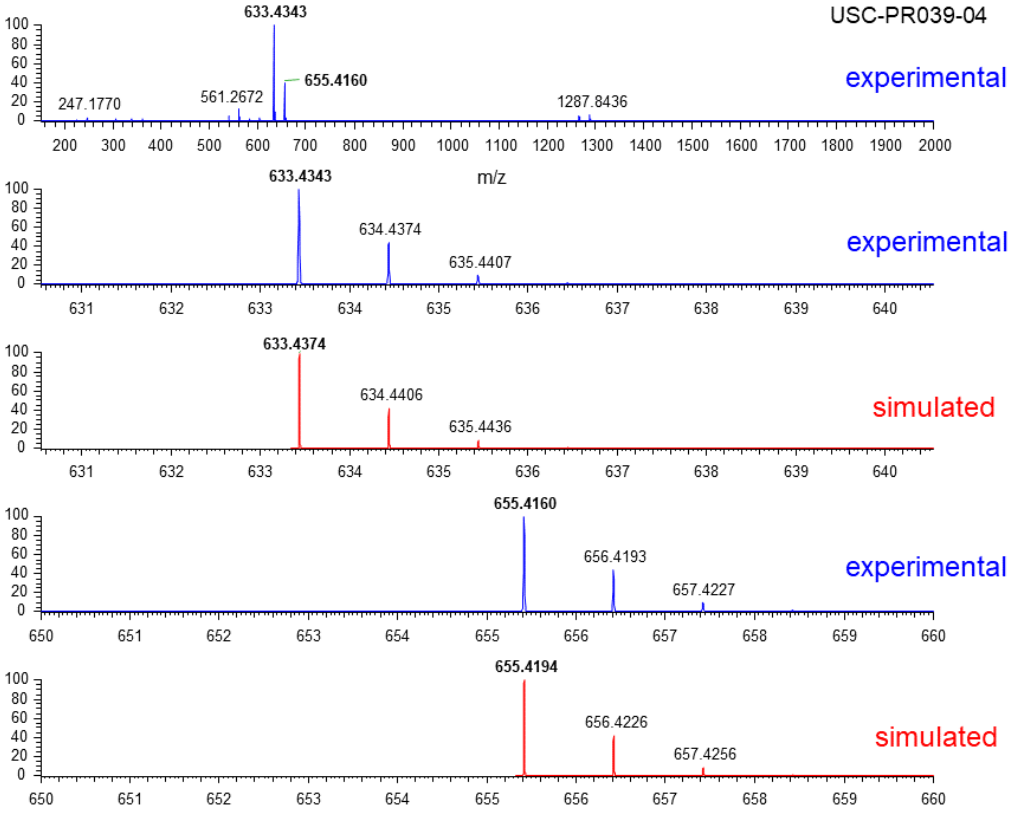
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634.4406
635.4436
simulated
655.4160
656.4193
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656.4226
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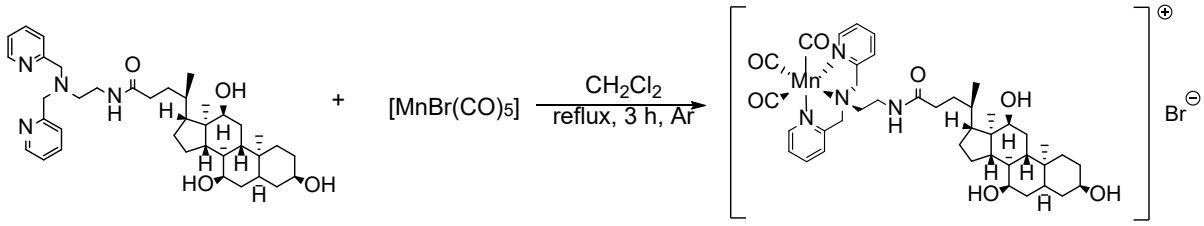
simulated

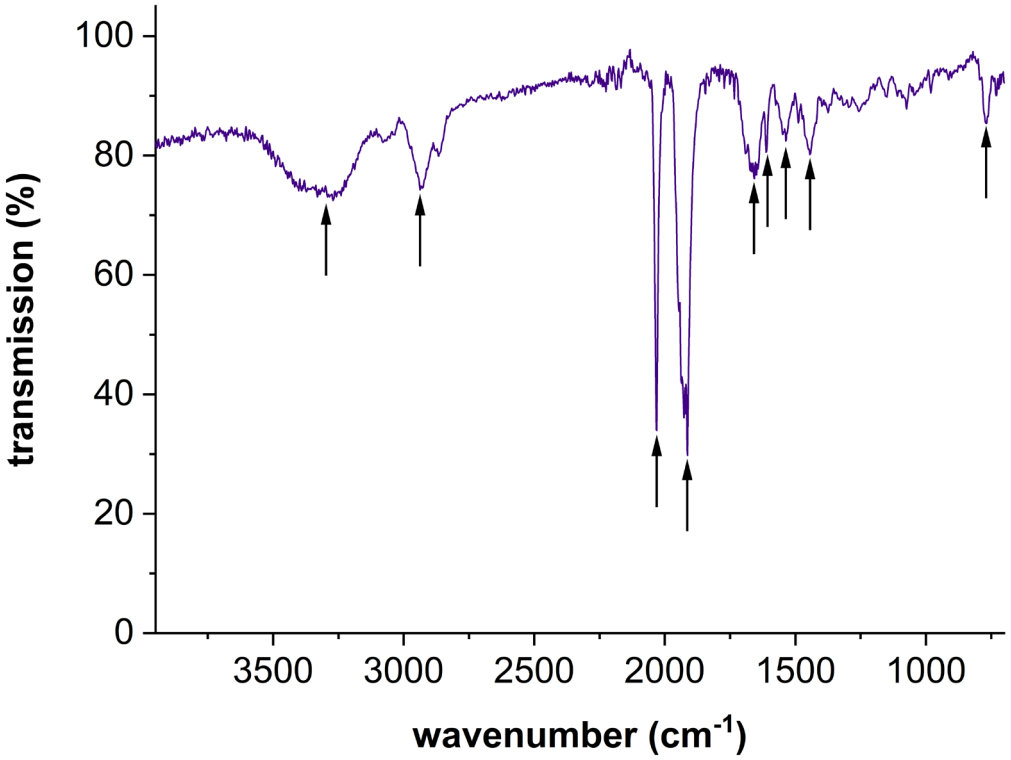
Spectroscopic data for [Mn(bpen^{cholamid}-κ^3*N*)(CO)$_3$]Br 9

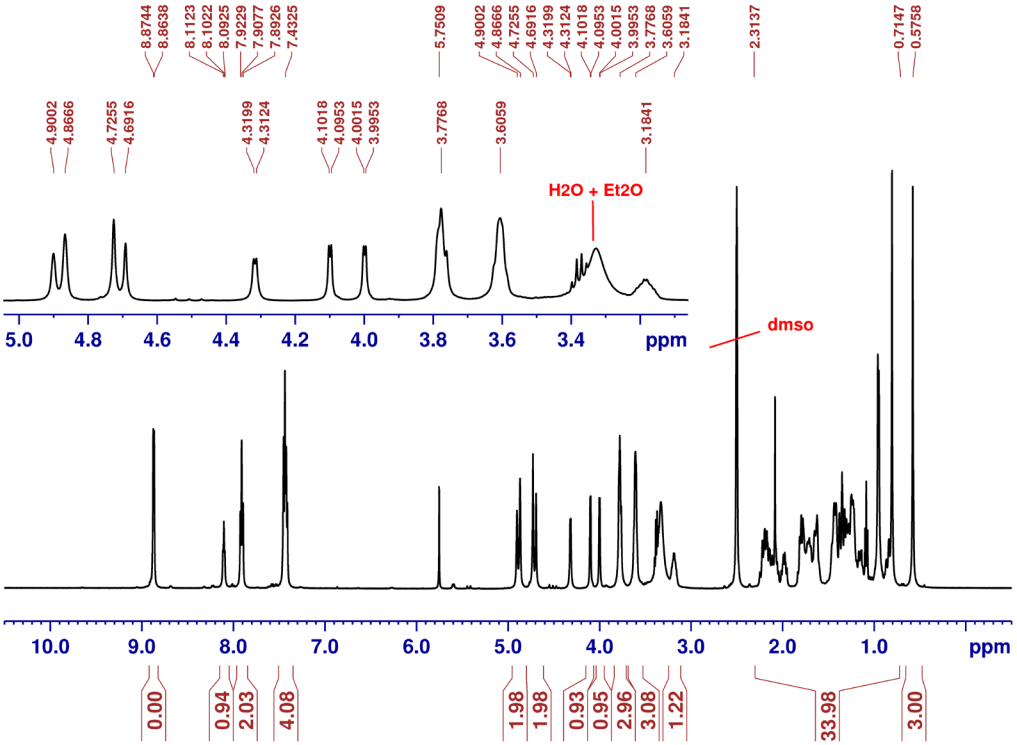


$C_{38}H_{56}N_4O_4$
632.89 g/mol

274.89 g/mol

$C_{41}H_{56}BrMnN_4O_7$
851.76 g/mol

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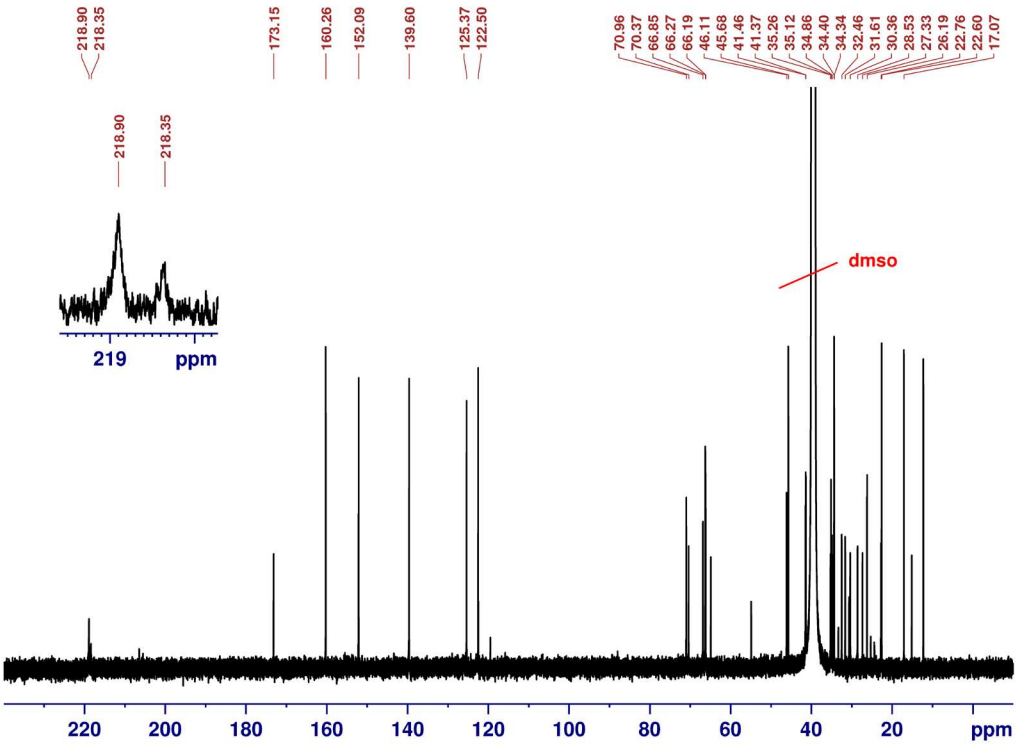
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41.37
35.26
35.12
34.86
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dmso
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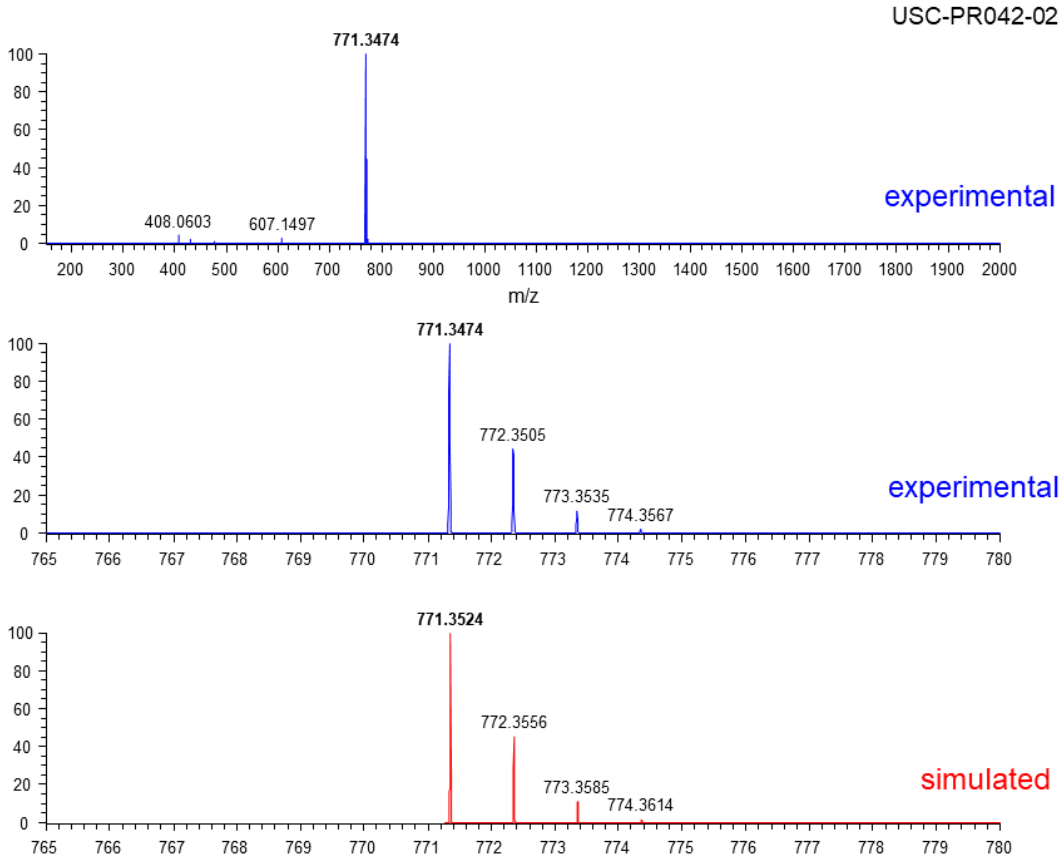
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experimental
m/z
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experimental
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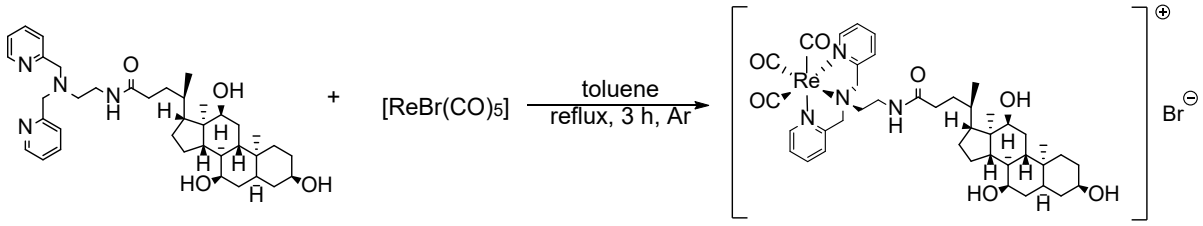
774.3614
simulated

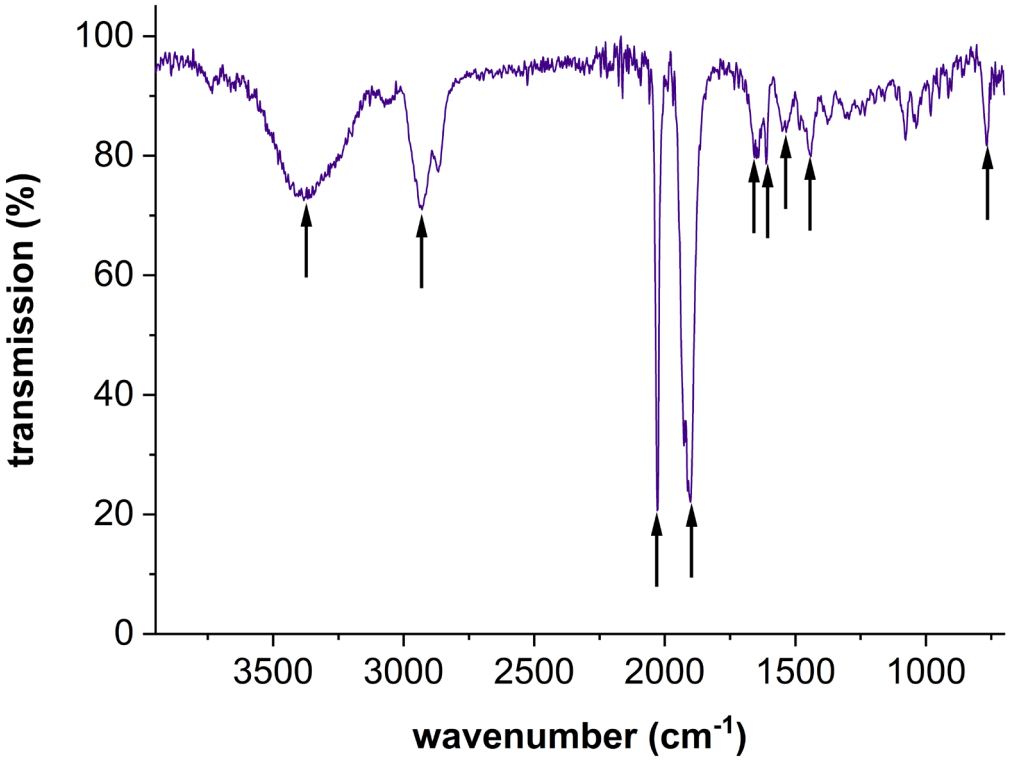
Spectroscopic data for [Re(bpen^{cholamid}-κ^3*N*)(CO)$_3$]Br 10

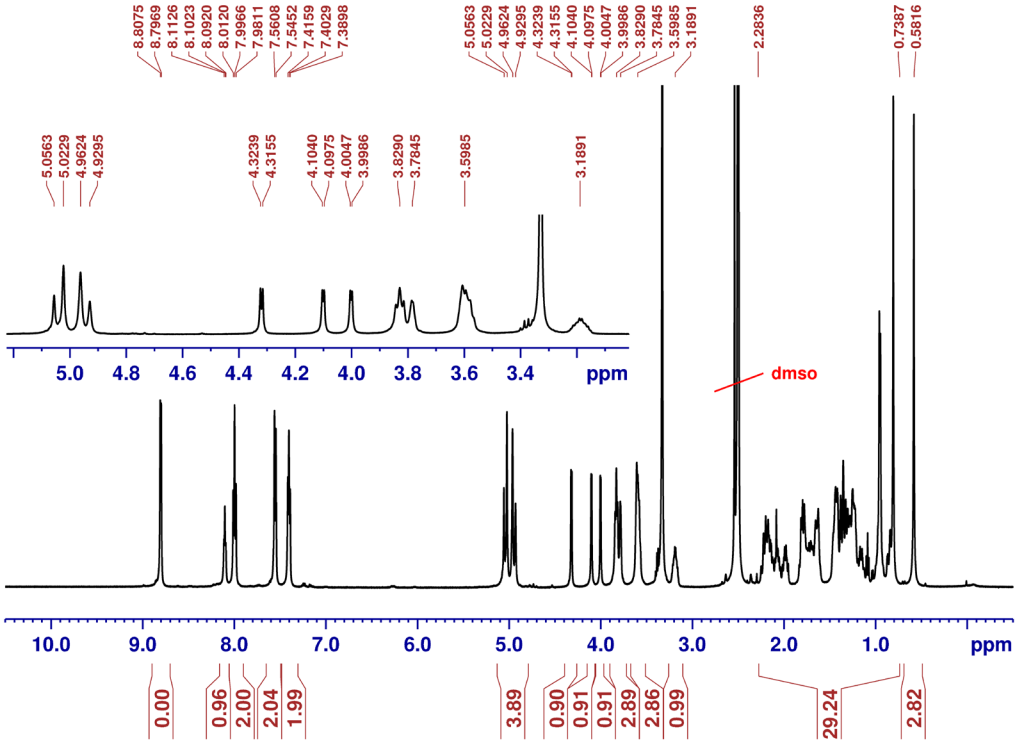


$C_{38}H_{56}N_4O_4$
632.89 g/mol

406.16 g/mol

$C_{41}H_{56}BrN_4O_7Re$
983.03 g/mol

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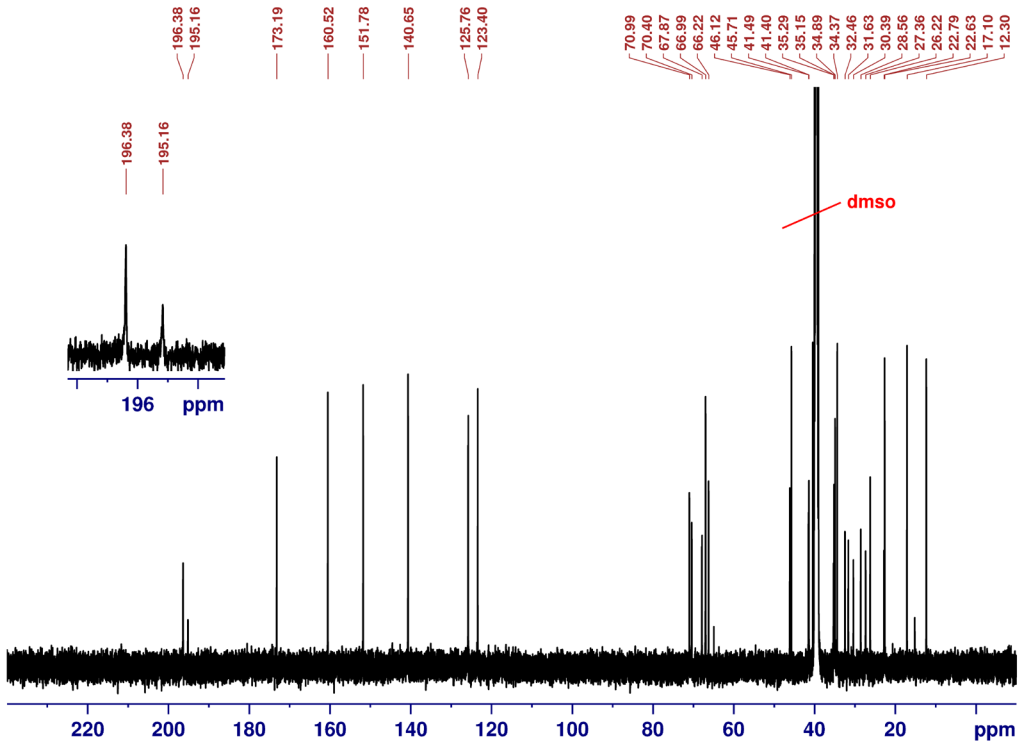
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46.12
45.71
41.49
41.40
35.29
35.15
34.89
34.37
32.46
31.63
30.39
28.56
27.36
26.22
22.79
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ppm
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40
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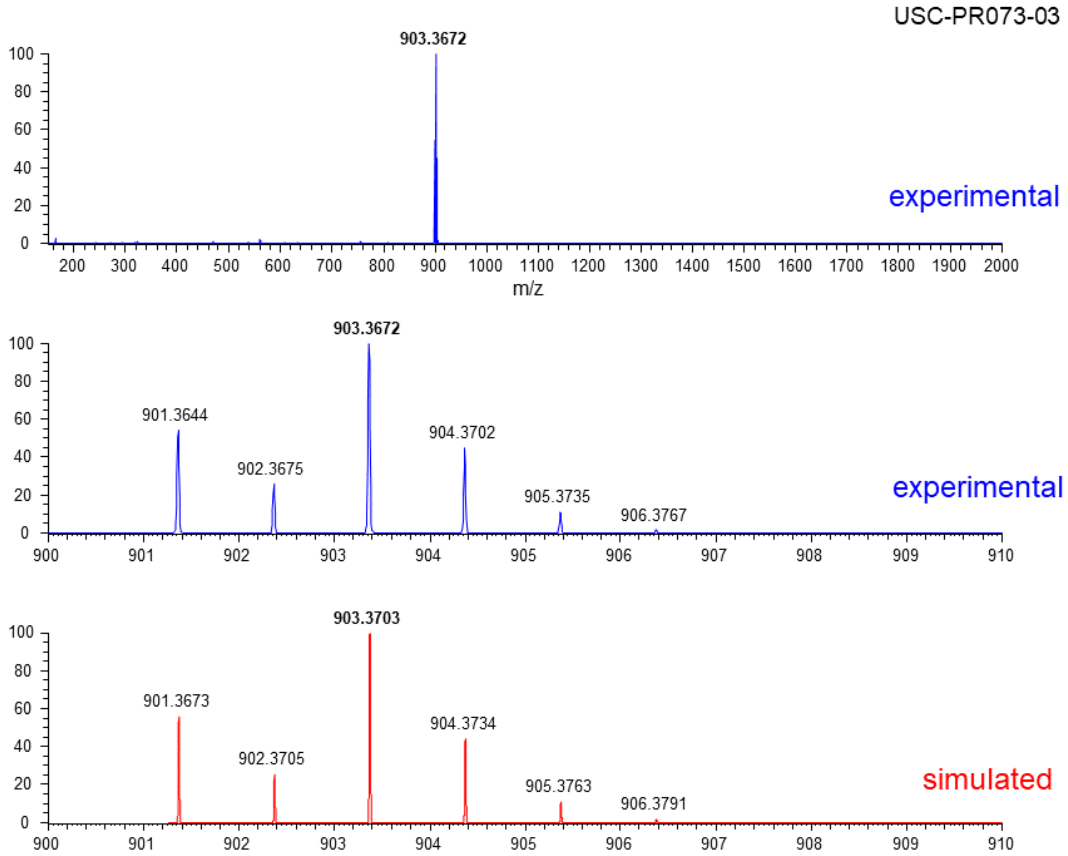

References

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