Electronic Supplementary Material (ESI) for Dalton Transactions.

Supporting information

Synthesis, Characterisation and Antimicrobial Activity of Supramolecular Cobalt-Peptide Conjugates

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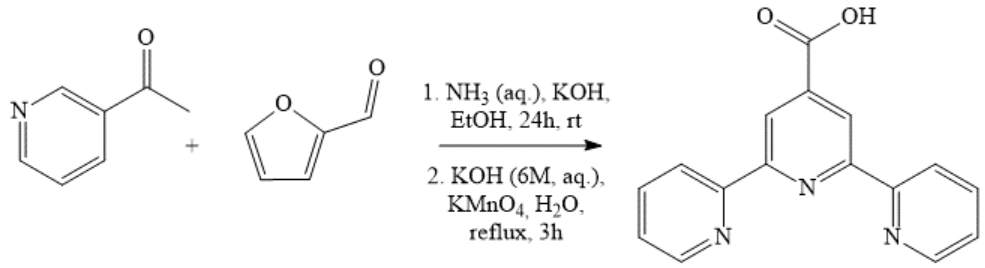
1. Synthesis of [2,2′:6′,2″-Terpyridine]-4′-carboxylic acid ligand (L)



L
$C_{16}H_{11}N_3O_2$
277.09 g/mol

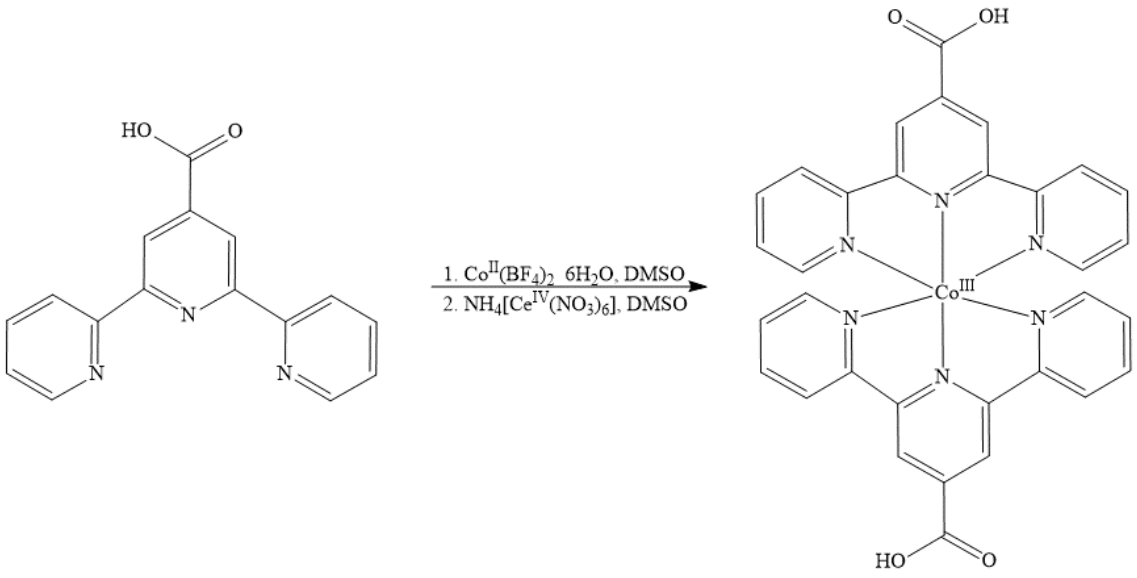
Following a modified synthetic procedure of J. Husson et al.[38], 2 g of furfural (20,8 mmol), 5 g of 2-Acetylpyridine (2 eq., 41.6 mmol) were added to ethanol (50 ml) and subsequently 3.4 g of KOH (3 eq., 62.4 mmol), and aqueous ammonia (25%, 20 ml) were added. The reaction mixture was stirred for 24h at ambient temperature. The resulting precipitate was obtained by filtration and washed with cold ethanol, and dried *in vacuo* (4 g, 12.5 mmol, 60%). The obtained white solid was dissolved in water (80 ml) and the pH was adjusted to 10 by addition of 6M KOH solution. Subsequently, 3.95 g of $KMnO_4$ (2 eq., 25 mmol) were added and the reaction mixture was refluxed for 3h. After cooling down the precipitate was removed, and the filtrate was acidified to pH 5 by addition of 6M HCl solution. The precipitated carboxylic acid was filtered off and washed three times with 30 ml of water. The desired product was obtained as a white solid and dried *in vacuo*. The analytical data was found to be in good agreement to the literature values.[38]

Yield: 1.7g (6.3 mmol, 50%)

Analysis:

^{1}H NMR (300 MHz, DMSO-d^6) δ: 7.51-7.57 (m, 2H), 8-8.08 (td, 2H), 8.64-8.68 (d, 2H), 8.75-8.77 (d, 2H), 8.87 (s, 2H).

1.1Synthesis of $[CoIII(L)_2]^{3+}$



L
$C_{16}H_{11}N_3O_2$
277.09 g/mol

$[Co^{III}\text{-}L_2]^{3+}$
$C_{32}H_{22}CoN_6O_4$
613.10 g/mol

10 mg of **L** (36 µmol) were dissolved in 2 ml of DMSO. Afterwards, 6.2 mg of $Co^{II}(BF_4)_2 \cdot 6H_2O$ (0.5 eq. 18 µmol) dissolved in 2 ml of DMSO were added to the solution of **L**, which immediately turned dark red. Then, 9.8 mg of $NH_4[Ce^{IV}(NO_3)_6]$ (0.5 eq. 18 µmol) were dissolved in 1 ml of DMSO and added to the red **Co^{II}-L_2** solution. After addition, the color of the solution changed immediately to orange. Subsequently, the product was precipitated by addition of Et_2O/EtOAc 1:1 (20 ml) to the cobalt-bis(tpy-COOH) solution. The peptide precipitate was obtained by centrifugation (8000 rpm, 8 min, 3 times) and washed two times with 10 ml of Et_2O/EtOAc 1:1, followed by separation from the supernatant by centrifugation (8000 rpm, 8 min, 3 times). The crude product was dissolved again in DMSO (2 ml) and the precipitation procedure was carried out again. Afterwards, the crude product was dissolved in H_2O (3-5 ml) and lyophilized which yielded the product as a red solid.

Yield: 8 mg (13 µmol, 72%).

Analysis:

MS (MALDI TOF [+], H_2O/ACN [1:1]): 613.25 $[M]^+$

^{1}H NMR (200 MHz, DMSO-d^6) δ: 7.71 – 7.29 (m, 8H, aromatic protons), 8.30 (t, 4H, aromatic protons), 9.15 (d, 4H, aromatic protons), 9.62 (s, 4H, aromatic protons).

^{11}B NMR (80 MHz, DMSO-d^6) δ: -1.3 (s, BF_4^-)

^{19}F NMR (235 MHz, DMSO-d^6) δ: -148.2 (s, BF_4^-)

2. Synthesis of peptides coupled with ligands

General procedure:

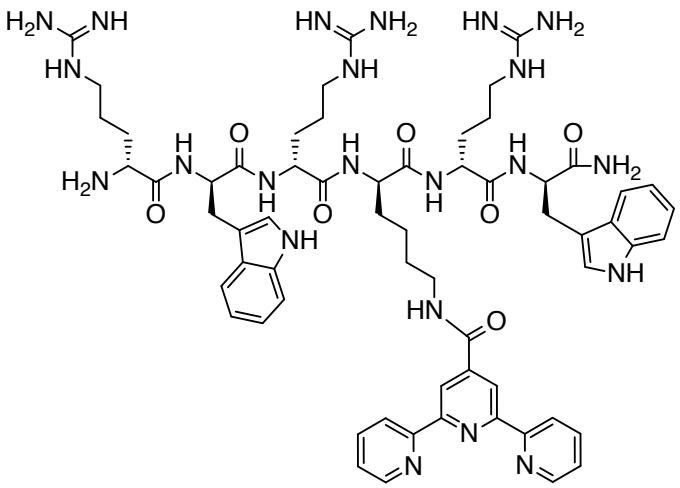
The peptides coupled with 2,2´:6´,2”-terpyridine ligand (L) were prepared by the SPPS which was preformed manually at room temperature using Fmoc protection strategy on the Rink Amide resin.

200 mg of the Rink amide resin with a loading of 0.74 $mmol \cdot g^{-1}$ (148 µmol) was filled into a 10 ml syringe with filter and swelled by shaking it five times two minutes in DCM (5 ml) and afterwards one time for one hour in DMF (5 ml). The solution was pushed out and Fmoc deprotection was carried out by shaking the resin for 20 min in 20% of piperidine in DMF (4 ml). For the further Fmoc deprotection of amino acids the same conditions were used. After deprotection, the solution was pushed out and the resin was washed five times with DMF (8 ml). After washing, the success of deprotection was tested by Kaiser test. Therefore, a drop of each of the following solutions was added to some representative resin beads: 1: 80% phenol in ethanol, 2: 0.1 mM KCN in H_2O/pyridine and 3: 6% ninhydrin in ethanol. After heating the resulting suspension for 5 min at 95°C, the successful deprotection was detected by a blue color of the resin beads and supernatant. If negative, Fmoc deprotection was repeated in the same conditions. If positive, the procedure was followed by amino acid coupling. Therefore, the coupling mixture of 4 eq of Fmoc-aa-OH, 4 eq of TBTU, and 4 eq of DIPEA dissolved in DMF (5 ml) per mmol of resin loading was added to the resin. After shaking for 1.5-3 h, the resin was washed five times with DMF (8 ml) and the Kaiser test was again performed to confirm successful coupling, which was revealed by no color change of the beads and the supernatant. If a blue color could be detected, the coupling reaction with the respective amino acid was repeated according to the description above. After the successful coupling, the unreacted amino termini were capped by acetylation by shaking the resin with a mixture of acetic anhydride/pyridine 3:2 (5 ml) over 30 min. After washing, the Kaiser test was again performed to reveal success of the acetylation reaction, which showed no color change. Following this scheme, all amino acids were coupled consecutively.

After preparation of peptides, the deprotection of Lys side chain was carried out by adding a solution of 30% TFA in DCM to the resin. After shaking for 30 min at room temperature, the resin was washed two times with DCM (8 ml) and three times with DMF (8 ml). Afterwards, the Kaiser test was carried out and showed successful Boc-deprotection of Lys (slightly blue color of beads and no color change of supernatant). Therefore, the coupling reaction was carried out by addition of a coupling mixture of 4 eq of L_1, 4 eq of DIC, and 4 eq of HOBt dissolved in 8 ml of DMF/DCM (1:1) per mmol of resin loading, whereby the coupling mixture required pre-activation time between 20-30 min. After shaking for 18 h at room temperature, the resin was washed two times with DCM (8 ml) and four times with DMF (8 ml). Afterwards, the Kaiser test was performed and showed successful coupling of L_1 with the peptide (no color change of resin beads and supernatant). Then, Fmoc-deprotection of the N-terminal amino acid of the peptides was carried out. After washing, the Kaiser test was again performed to reveal the success of the Fmoc deprotection. Subsequently, the cleavage from the resin and deprotection of the protected side chains were carried out simultaneously by addition of a

cleavage mixture of TFA/H_2O/TIS 95:2.5:2.5 (5 ml) to the resin and shaking it for 7 h at room temperature. Afterwards, the resin was filtered off and the peptide coupled with L_1 was precipitated by addition of Et_2O to the filtrate. After cooling the filtrate, the peptide precipitate was obtained by centrifugation (8000 rpm, 8 min, 3 times) and washed two times with 10 ml of Et_2O, followed by separation from the supernatant by centrifugation (8000 rpm, 8 min, 3 times). Subsequently, the peptide precipitate was dissolved in ACN/H_2O (3-5 ml) and lyophilized. The purification was carried out by reverse-phase HPLC (ACN/H_2O 5:95 + 0.1% TFA $\rightarrow$ ACN/H_2O 95:5 + 0.1 % TFA) and the peptide was obtained as a white solid.

2.1 RWRK(L)RW-NH_2 (P1L_1)



$C_{62}H_{84}N_{22}O_7$
1244.66 g/mol

Yield: 10 mg (8.03 µmol, 4%)

Analysis:

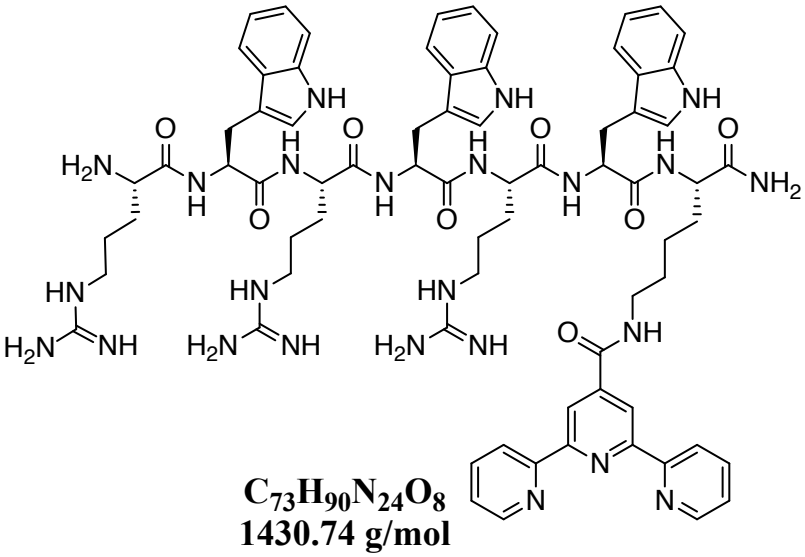
MS (MALDI TOF [+], H_2O/ACN [1:1]): 1245.75 $[M+H]^+$

Retention time semi-preparative HPLC (ACN/H_2O 5:95 + 0.1% TFA -> ACN/H_2O 95:5 + 0.1% TFA): 11.7 min

Diffusion coefficient in DMSO-d^6: $8.81 \cdot 10^{-11}$ $m^2 \cdot sec^{-1}$.

^{1}H NMR (400 MHz, DMSO-d^6) δ: 1.24 (s), 1.86 – 1.33 (m), 1.91-2.02 (m), 2.98 (m), 3.87 (m), 4.04 (m), 4.48 (d), 5.32 (t), 6.65 (m), 7.15 – 6.80 (m), 7.20 (d, *J* = 9.8 Hz), 7.33 (dd, *J* = 17.4, 9.6 Hz), 7.63 – 7.47 (m), 7.74 – 7.58 (m), 7.83 (dd), 8.11 – 7.92 (m), 8.18 – 8.05 (m), 8.44 (d), 8.52-8.53 (m), 8.66 (d, *J* = 7.9 Hz), 8.75 (d, *J* = 3.9 Hz), 8.83 (s), 9.11 (t), 10.74-10.84 (d, *J* = 26.1 Hz).

2.2 RWRWRWK(L)-NH_2 ($P2L_1$)



Yield: 10 mg (8.4 µmol, 4%)

Analysis:

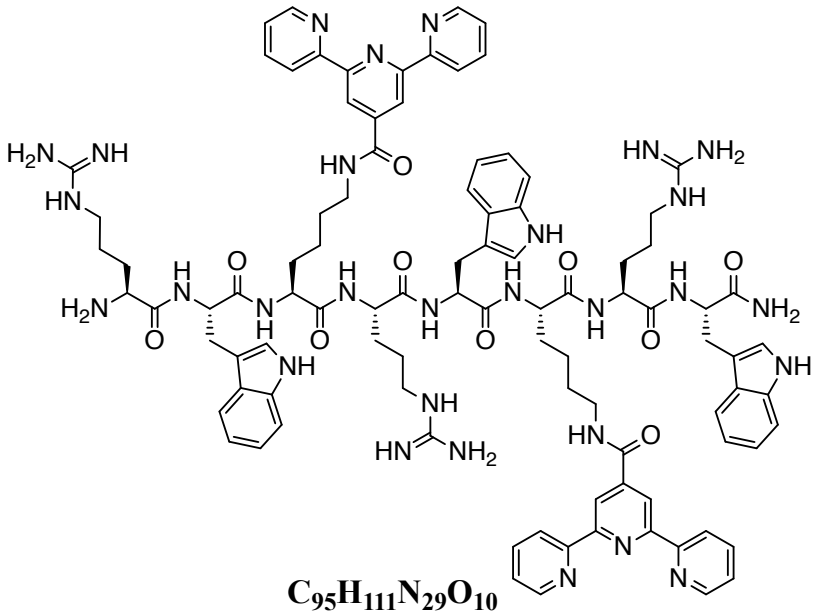
MS (MALDI TOF [+], H_2O/ACN [1:1]): 1431.7 $[M+H]^+$

Retention time analytical HPLC (ACN/H_2O 5:95 + 0.1% TFA -> ACN/H_2O 95:5 + 0.1% TFA): 7.4 min

Diffusion coefficient in DMSO-d^6: $8.5 \cdot 10^{-11}$ $m^2 \cdot sec^{-1}$.

^{1}H NMR (400 MHz, DMSO-d^6) δ: 1.30 (d, *J* = 6.7 Hz), 1.61 – 1.37 (m), 1.78 – 1.61 (m), 2.00 (q, *J* = 7.3 Hz), 3.21 – 2.80 (m), 3.34 – 3.21 (m), 4.19 (t, *J* = 6.9 Hz), 4.36 – 4.25 (m), 4.62 (p, *J* = 7.5, 7.1 Hz), 5.32 (s), 6.96 – 6.75 (m), 7.02 (dt, *J* = 12.3, 8.7 Hz), 7.23 – 7.08 (m), 7.31 (t, *J* = 8.3 Hz), 7.54 (tq, *J* = 13.0, 7.4, 6.0 Hz), 7.66 (d, *J* = 8.1 Hz), 7.96 – 7.83 (m), 8.16 – 7.96 (m), 8.16 – 7.96 (m), 8.30 – 8.15 (m), 8.36 (d, *J* = 7.9 Hz), 8.53 (d, *J* = 7.3 Hz), 8.66 (d, *J* = 8.0 Hz), 8.79 (d, *J* = 26.4 Hz), 9.11 (t, *J* = 5.6 Hz), 10.86 – 10.57 (m).

2.3 RWK(L)RWK(L)RW-NH_2 (P3L_2)



$C_{95}H_{111}N_{29}O_{10}$
1817.91 g/mol

Yield: 9.4 mg (5.17 µmol, 3%)

Analysis:

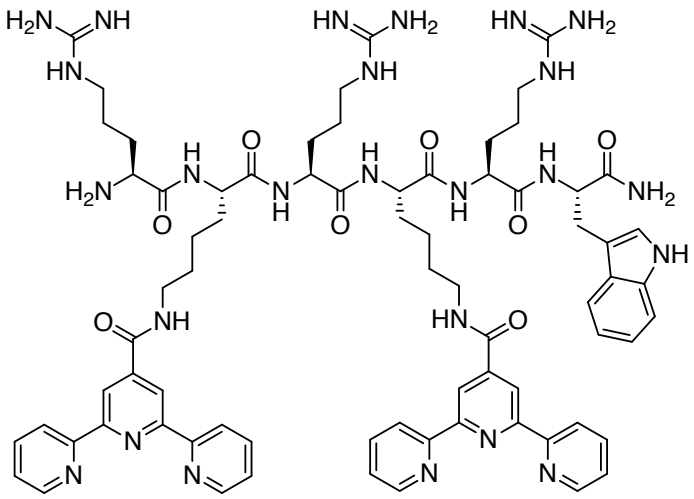
MS (MALDI TOF [+], H_2O/ACN [1:1]): 1818.17 $[M+H]^+$

Retention time analytical HPLC (ACN/H_2O 5:95 + 0.1% TFA -> ACN/H_2O 95:5 + 0.1% TFA): 5.6 min

Diffusion coefficient in DMSO-d^6: $7.78 \cdot 10^{-11}$ $m^2 \cdot sec^{-1}$.

^{1}H NMR (400 MHz, DMSO-d^6) δ: 1.24 (s), 1.34 (s), 1.51 (d, *J* = 42.9 Hz), 1.73 (d, *J* = 28.7 Hz), 1.85 (s), 2.13 – 1.90 (m), 2.99 (d, *J* = 7.6 Hz), 3.11 (d, *J* = 6.7 Hz), 3.30 (s), 3.79 (s), 4.48 (t, *J* = 7.0 Hz), 4.64 (s), 5.33 (s), 6.83 (s), 6.94 (q, *J* = 7.3, 6.6 Hz), 7.03 (td, *J* = 14.9, 7.9 Hz), 7.15 (d, *J* = 15.8 Hz), 7.25 (s), 7.38 – 7.27 (m), 7.44 (s), 7.66 – 7.46 (m), 7.71 (d, *J* = 8.2 Hz), 7.91 (d, *J* = 7.9 Hz), 8.04 (t, *J* = 7.9 Hz), 8.14 (s), 8.40 (d, *J* = 7.8 Hz), 8.65 (d, *J* = 8.0 Hz), 8.78 – 8.68 (m), 8.82 (d, *J* = 2.9 Hz), 9.27 – 8.96 (m), 10.83 (d, *J* = 17.0 Hz).

2.4 RK(L)RK(L)RW-NH_2 (P4L_2)



$C_{73}H_{91}N_{25}O_8$
1445.75 g/mol

Yield: 20 mg (13.84 µmol, 7%)

Analysis:

MS (MALDI TOF [+], H_2O/ACN [1:1]): 1446.91 $[M+H]^+$

Retention time analytical HPLC (ACN/H_2O 5:95 + 0.1% TFA -> ACN/H_2O 95:5 + 0.1% TFA): 5.4 min

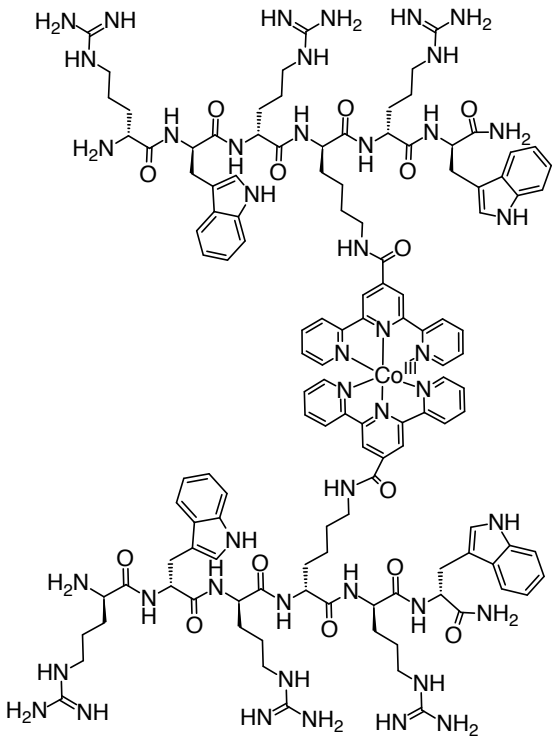
Diffusion coefficient in DMSO-d^6: $7.86 \cdot 10^{-11}$ $m^2 \cdot sec^{-1}$.

^{1}H NMR (700 MHz, DMSO-d^6) δ: 1.24 (s), 1.37 (q, *J* = 7.6, 6.9 Hz), 1.65 – 1.48 (m), 1.81 – 1.65 (m), 2.68 (t, *J* = 2.0 Hz), 1.95 (s), 3.11 (dq, *J* = 14.1, 7.0 Hz), 3.86 (q, *J* = 6.0 Hz), 4.30 (dtd, *J* = 25.6, 14.8, 13.8, 7.3 Hz), 4.48 (q, *J* = 7.0 Hz), 6.94 (t, *J* = 7.4 Hz), 7.02 (t, *J* = 7.6 Hz), 7.08 (s), 7.11 (d, *J* = 2.3 Hz), 7.31 (d, *J* = 8.1 Hz), 7.45 (s), 7.51 (dd, *J* = 7.6, 4.9 Hz), 7.57 (q, *J* = 7.9, 6.2 Hz), 7.68 (s), 7.76 (d, *J* = 6.0 Hz), 7.87 (d, *J* = 7.7 Hz), 8.02 (tt, *J* = 7.9, 2.1 Hz), 8.12 (d, *J* = 7.7 Hz), 8.18 (d, *J* = 5.2 Hz), 8.26 (d, *J* = 7.6 Hz), 8.60 (dd, *J* = 8.0, 2.8 Hz), 8.73 – 8.68 (m), 8.74 (s), 8.76 (d, *J* = 1.6 Hz), 8.81 (t, *J* = 8.8 Hz), 9.13 – 9.05 (m), 10.79 (d, *J* = 2.4 Hz).

^{19}F NMR (235 MHz, DMSO-d^6) δ: -74.6 (s, –CF_3 of TFA^-)

3. Synthesis of Cobalt-Peptide Conjugates

3.1 Synthesis of $[Co^{III}(P1L_1)_2]^{3+}$



$C_{124}H_{160}CoN_{44}O_{14}$
2548.25 g/mol

5 mg of **$P1L_1$** (4 µmol) were dissolved in 0.5 ml of DMSO-d^6. Afterwards, 7 mg of $Co^{II}(BF_4)_2 \cdot 6H_2O$ were dissolved in 1 ml DMSO-d^6 and a stoichiometric amount (0.1. ml, 0.5 eq., 2 µmol) was added to the peptide solution, which got immediately red. Then, 6.9 mg of $NH_4[Ce^{IV}(NO_3)_6]$ were dissolved in 1 ml DMSO-d^6 and a stoichiometric amount (0.1 ml, 0,5 eq., 2 µmol) was added to the red Co^{II}-peptide solution. After addition, the color of the solution changed immediately to orange and ^{1}H NMR was recorded. Subsequently, the product was precipitated by addition of Et_2O/EtOAc 1:1 (20 ml) to the cobalt-peptide solution. The peptide precipitate was obtained by centrifugation (8000 rpm, 8 min, 3 times) and washed two times with 10 ml of Et_2O/EtOAc 1:1, followed by separation from the supernatant by centrifugation (8000 rpm, 8 min, 3 times). The crude product was dissolved again in DMSO (2 ml) and the precipitation procedure was carried out again. Afterwards, the crude product was dissolved in H_2O (3-5 ml) and lyophilized which yielded the product as a red solid.

Yield: 4.5 mg (1.8 µmol, 90%)

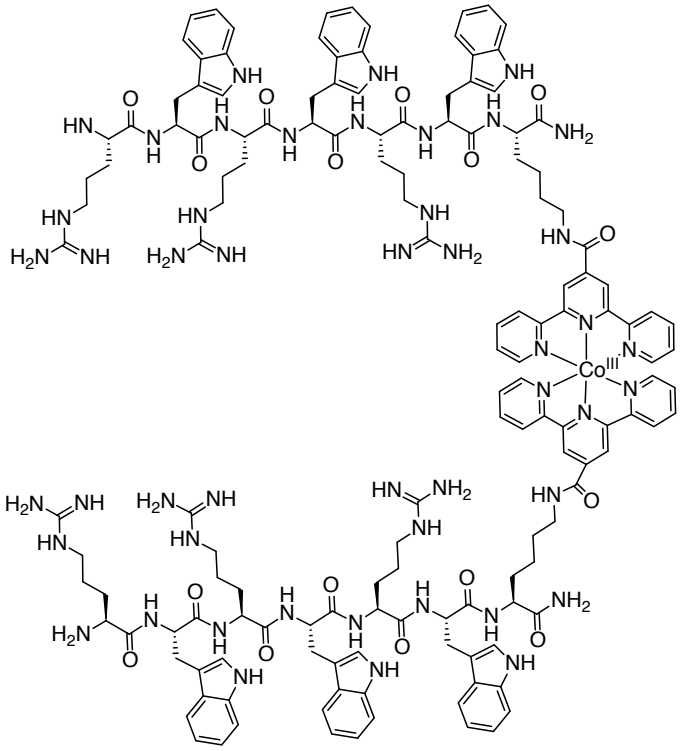
Analysis:

MS (MALDI TOF [+], H_2O/ACN [1:1]): 1303.84 [$Co^{III}(P1L_1)$]$^+$; 1245.88 [$P1L_1$+H]$^+$

Diffusion coefficient in DMSO-d^6: $6.45 \cdot 10^{-11}$ $m^2 \cdot sec^{-1}$.

^{1}H NMR (400 MHz, DMSO-*d*^{6}) δ: 1.40 (s), 1.71 – 1.47 (m), 1.94 (s), 2.19 (p, *J* = 1.8 Hz), 2.53 (p, *J* = 1.9 Hz), 2.91 – 2.78 (m), 3.01 – 2.91 (m), 3.03 (s), 3.37 (s), 3.69 (d, *J* = 33.9 Hz), 4.16 (d, *J* = 6.8 Hz), 4.22 (s), 4.39 – 4.30 (m), 4.68 – 4.44 (m), 6.80 (s), 6.83 (d, *J* = 7.8 Hz), 6.91 – 6.85 (m), 6.93 (s), 6.96 (s), 7.00 (d, *J* = 2.4 Hz), 7.05 (s), 7.08 (d, *J* = 2.4 Hz), 7.12 (s), 7.24 – 7.15 (m), 7.36 – 7.28 (m), 7.39 (d, *J* = 5.3 Hz), 7.45 (d, *J* = 8.1 Hz), 7.55 (t, *J* = 8.9 Hz), 7.78 (d, *J* = 7.6 Hz), 7.98 (d, *J* = 27.1 Hz), 8.21 (t, *J* = 7.7 Hz), 8.33 (s), 8.42 (d, *J* = 7.1 Hz), 8.80 (d, *J* = 8.2 Hz), 9.35 (s), 9.52 (d, *J* = 9.7 Hz), 10.70 (d, *J* = 29.1 Hz).

3.2 Synthesis of $[Co^{III}(P2L_1)_2]^{3+}$



$C_{146}H_{180}CoN_{48}O_{16}$

5 mg of **$P2L_1$** (3.5 µmol) were dissolved in 1 ml of DMSO-d^6. Afterwards, 5.83 mg of $Co^{II}(BF_4)_2 \cdot 6H_2O$ were dissolved in 1 ml of DMSO-d^6 and a stoichiometric amount (0.1 ml, 0.5 eq., 1.75 µmol) was added to the peptide solution, which got immediately red. Then, 9.4 mg of $NH_4[Ce^{IV}(NO_3)_6]$ were dissolved in 1 ml of DMSO- d^6 and a stoichiometric amount (0.1 ml, 0.5 eq., 1.75 µmol) was added to the red Co^{II}-peptide solution. After addition, the color of the solution changed immediately to orange and ^{1}H NMR was recorded. Subsequently, the

product was precipitated by addition of Et_2O/EtOAc 1:1 (20 ml) to the cobalt-peptide solution. The peptide precipitate was obtained by centrifugation (8000 rpm, 8 min, 3 times) and washed two times with 10 ml of Et_2O/EtOAc 1:1, followed by separation from the supernatant by centrifugation (8000 rpm, 8 min, 3 times). The crude product was dissolved again in DMSO (2 ml) and the precipitation procedure was carried out again. Afterwards, the crude product was dissolved in H_2O (3-5 ml) and lyophilized which yielded the product as a red solid.

Yield: 4.7 mg (1.5 µmol, 93%)

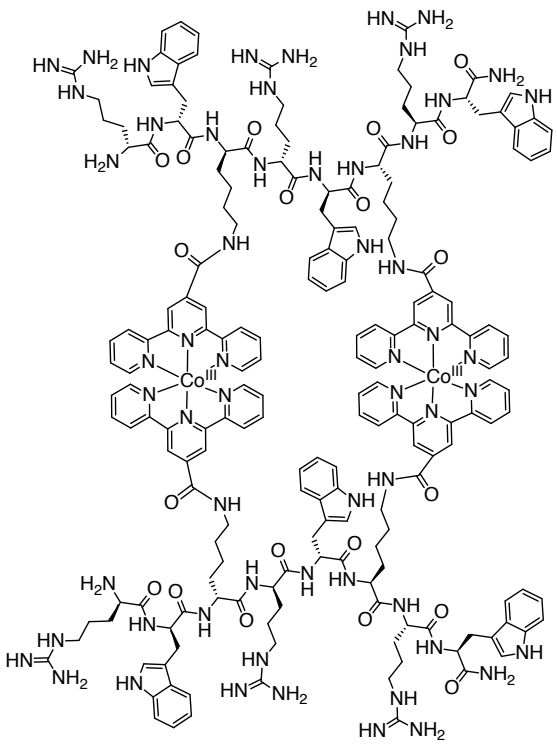
Analysis:

MS (MALDI TOF [+], H_2O/ACN [1:1]): 1489.37 [$\mathbf{Co^{III}P2L_1}$]$^+$; 1431.8 [$\mathbf{P2L_1}$+H]$^+$;

Diffusion coefficient in DMSO-d^6: $6.14 \cdot 10^{-11}$ $m^2 \cdot sec^{-1}$.

^{1}H NMR (400 MHz, DMSO-d^6) δ: 1.23 (s), 1.51 (s), 1.69 (s), 1.99 (s), 2.18 (s), 2.96 – 3.14 (m), 3.52 (s), 3.75 (s), 4.03 (q, *J* = 7.1 Hz), 4.24 (s), 4.32 (s), 4.47 (s), 4.64 (s), 4.84 (s), 5.04 (s), 5.86 (s), 6.52 (s), 6.86 – 6.99 (m), 7.02 (s), 7.06 (s), 7.09 (s), 7.13 (s), 7.21 (s), 7.32 (s), 7.41 (s), 7.49 (s), 7.54 (s), 7.59 (d, *J* = 7.6 Hz), 7.66 (d, *J* = 7.6 Hz), 7.93 (s), 8.05 (s), 8.27 (s), 8.36 (s), 8.52 (s), 8.59 – 8.87 (m), 9.01 (d, *J* = 7.9 Hz), 9.62 (s), 9.77 (s), 10.77 (d, *J* = 22.2 Hz).

3.3 Synthesis of $[Co_2^{III}(P3L_2)_2]^{6+}$



$C_{190}H_{223}Co_2N_{58}O_{20}$
3753.68 g/mol

5 mg of **$P3L_2$** (2.8 µmol) were dissolved in 1 ml DMSO-d^6. Afterwards, 8.8 mg of $Co^{II}(BF_4)_2 \cdot 6H_2O$ were dissolved in 1 ml of DMSO-d^6 and a stoichiometric amount (0.1 ml, 1 eq. 2.8 µmol) was added to the peptide solution, which got immediately red. Then, 14 mg of $NH_4[Ce^{IV}(NO_3)_6]$ were dissolved in DMSO- d^6 and a stoichiometric amount (0.1 ml, 1 eq. 2,8 µmol) was added to the red Co^{II}-peptide solution. After addition, the color of the solution changed immediately to orange and ^{1}H NMR was recorded. Subsequently, the product was precipitated by addition of Et_2O/EtOAc 1:1 (20 ml) to the cobalt-peptide solution. The peptide precipitate was obtained by centrifugation (8000 rpm, 8 min, 3 times) and washed two times with 10 ml of Et_2O/EtOAc 1:1, followed by separation from the supernatant by centrifugation (8000 rpm, 8 min, 3 times). The crude product was dissolved again in DMSO (2 ml) and the precipitation procedure was carried out again. Afterwards, the crude product was dissolved in H_2O (3-5 ml) and lyophilized which yielded the product as a red solid.

Yield: 4.5 mg (1.5 µmol, 90%)

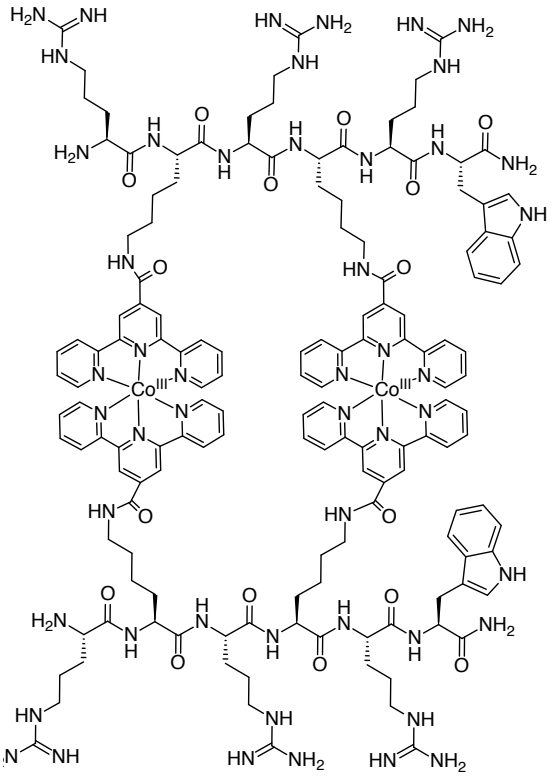
Analysis:

MS (MALDI TOF [+], H_2O/ACN [1:1]): 1935.07 [$Co_2^{III}P3L_2$]$^+$; 1876.15 [$Co^{III}P3L_2$+H]$^+$; 1818.15 [$P3L_2$+H]$^+$;

Diffusion coefficient in DMSO-d^6: $5.42 \cdot 10^{-11}$ $m^2 \cdot sec^{-1}$.

^{1}H NMR (400 MHz, DMSO-*d*^{6}) δ: 1.22 (d, *J* = 18.2 Hz), 1.72 (s,), 1.56 (s), 1.90 (s), 3.17 – 3.00 (m), 3.80 (s), 4.33 (s), 4.51 (s), 4.71 (s), 6.53 (s), 7.03 (s), 7.16 (s), 7.35 (d, *J* = 8.4 Hz), 7.61 – 7.41 (m), 7.71 (d, *J* = 8.1 Hz), 7.98 (s), 8.09 (s), 8.47 – 8.14 (m), 8.53 (s), 8.92 (d, *J* = 22.6 Hz), 9.40 (s), 9.63 (d, *J* = 15.5 Hz), 10.84 (d, *J* = 17.3 Hz).

3.4 Synthesis of $[Co_2^{III}(P4L_2)_2]^{6+}$



$C_{146}H_{182}Co_2N_{50}O_{16}$
3009.36 g/mol

5 mg of **$P4L_2$** (3.4 µmol) were dissolved in 0.5 ml DMSO-d^6. Afterwards, 11.8 mg of $Co^{II}(BF_4)_2 \cdot 6H_2O$ were dissolved in 1ml of DMSO-d^6 and a stoichiometric amount (0.1 ml, 1 eq. 3.4 µmol) was added to the peptide solution, which got immediately red. Then, 18.9 mg of $NH_4[Ce^{IV}(NO_3)_6]$ were dissolved in 1 ml of DMSO-d^6 and a stoichiometric amount (0.1 ml, 1 eq. 3.4 µmol) was added to the red Co^{II}-peptide solution. After addition, the color of the solution changed immediately to orange and ^{1}H NMR was recorded. Subsequently, the product was precipitated by addition of Et_2O/EtOAc 1:1 (20 ml) to the cobalt-peptide solution. The peptide precipitate was obtained by centrifugation (8000 rpm, 8 min, 3 times) and washed two times with 10 ml of Et_2O/EtOAc 1:1, followed by separation from the supernatant by

centrifugation (8000 rpm, 8 min, 3 times). The crude product was dissolved again in DMSO (2 ml) and the precipitation procedure was carried out again. Afterwards, the crude product was dissolved in H_2O (3-5 ml) and lyophilized which yielded the product as a red solid.

Yield: 4.6 mg (1.5 µmol, 92%)

Analysis:

MS (MALDI TOF [+], H_2O/ACN [1:1]): 1563.7 [$\mathbf{Co_2^{III}P4L_2}$]$^+$; 1505.05 [$\mathbf{Co^{III}P4L_2}$+H]$^+$; 1446.97 [$\mathbf{P4L_2}$+H]$^+$;

Diffusion coefficient in DMSO-d^6: $5.56 \cdot 10^{-11}$ $m^2 \cdot sec^{-1}$.

^{1}H NMR (700 MHz, DMSO-d^6) δ: 0.85 (t, *J* = 7.0 Hz), 1.30 – 1.21 (m), 1.53 (d, *J* = 52.9 Hz), 1.72 (s), 1.90 (d, *J* = 10.0 Hz), 3.13 (dt, *J* = 40.5, 11.8 Hz), 3.95 – 3.75 (m), 4.55 – 4.16 (m), 4.61 (s), 6.83 (s), 7.10 – 6.90 (m), 7.25 – 7.12 (m), 7.32 (d, *J* = 8.2 Hz), 7.64 – 7.37 (m), 8.02 (d, *J* = 55.2 Hz), 8.32 (s), 8.24 – 8.03 (m), 8.46 (s), 8.68 – 8.50 (m), 8.99 – 8.75 (m), 9.43 – 9.33 (m), 9.61 (t, *J* = 10.7 Hz), 10.83 (d, *J* = 28.4 Hz).

^{11}B NMR (80 MHz, DMSO-d^6) δ: -1.3 (s, BF_4^-)

^{19}F NMR (235 MHz, DMSO-d^6) δ: -148.2 (s, BF_4^-)

4. Determination of Minimal Inhibitory Concentration (MIC)

Compounds were tested against the Gram-negative strains of *Escherichia coli* DSM 30083, *Acinetobacter baumannii* DSM 30007, *Pseudomonas aeruginosa* DSM 50071 as well as against the Gram-positive strains *Bacillus subtilis* DSM 402, *Staphylococcus aureus* DSM 20231, and *Staphylococcus aureus* ATCC 43300 (MRSA) and against the yeast *Candida albicans* DSM 1386 in a microtiter plate assay according to CLSI guidelines, as described in the study of B. Albada et. al.[21]

E. coli, *A. baumannii*, *S. aureus*, *B. subtilis* and *C. albicans* were grown in Mueller Hinton broth, whereas *P. aeruginosa* was grown in cation-adjusted Mueller Hinton II broth. Stock solution of the compounds in DMSO were prepared with concentrations of 1.25, 2.5, 5 or 10 mg/mL. Serial dilution in culture media were prepared with the Tecan Freedom Evo 75 liquid handling workstation (Tecan, Männedorf, Switzerland). Dilutions, starting from a 10 mg/mL stock solution, typically covered a range from 512 to 0.5 µg/mL. Subsequently, the serial dilutions of the compound were inoculated with $5 \cdot 10^5$ bacteria/mL taken from late exponential cultures grown in the same media, whereby the assay volumes were 200 µL per well. Afterwards, cells were incubated for 16-18 hours at 37 °C and the lowest compound concentration inhibiting visible bacterial growth was recorded as MIC.

5. Results of DOSY NMR Experiments

Table S1. Results from the DOSY NMR experiments of $P1L_1$ and $P2L_1$, and their Co-complexes.

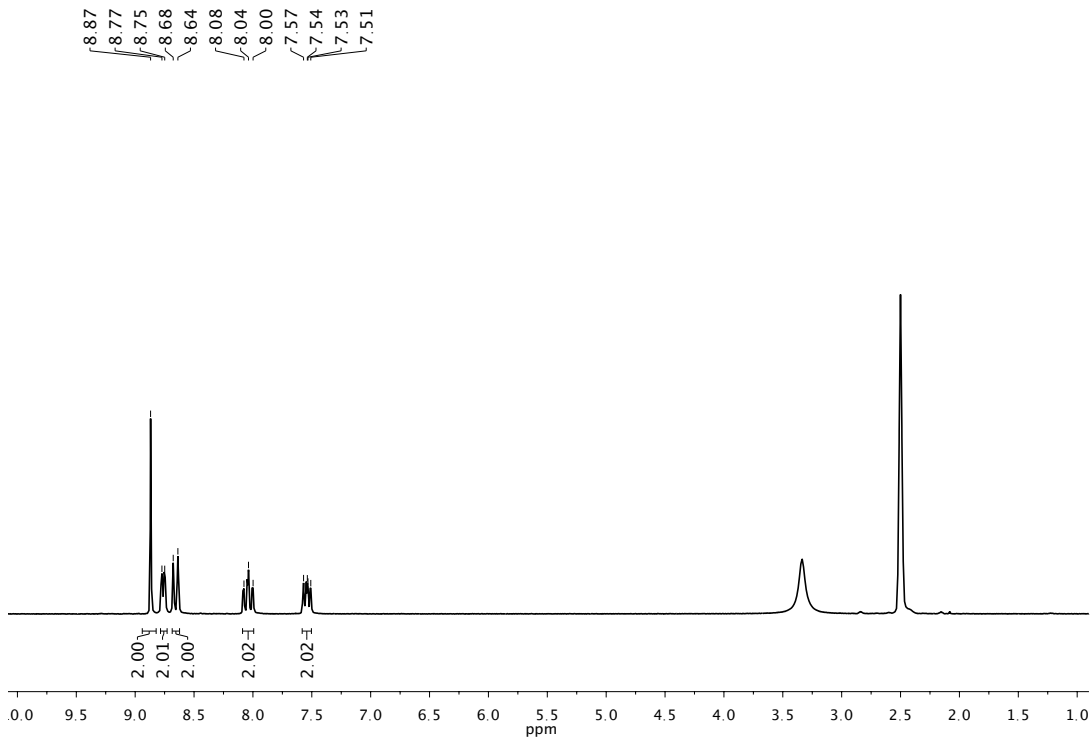
$P1L_1$		**$[Co^{III}(P1L_1)_2]^{3+}$**		**$P2L_1$**		**$[Co^{III}(P2L_1)_2]^{3+}$**	
δ/ppm	$D \cdot 10^{-11}$ (m^2/sec)	δ/ppm	$D \cdot 10^{-11}$ (m^2/sec)	δ/ppm	$D \cdot 10^{-11}$ (m^2/sec)	δ/ppm	$D \cdot 10^{-11}$ (m^2/sec)
10.76	8.19	10.66	5.7	10.78	8.18	10.81	5.7
10.89	6.83	10.74	5.5	10.74	7.9	10.75	5.86
8.75	8.78	8.5	6.59	9.1	8.81	9	5.9
8.43	8.47	7.16	5.91	8.65	8.19	8.83	7.89
7.43	7.35	7.94	8.81	7.11	8.15	7.42	6.59
6.99	6.13	7.08	8.49	6.97	8.17	7.31	6.1
7.52	7.88	6.95	7.62	3	8.78	7.14	5.54
6.95	10.6	6.87	4.93	2.98	8.45	4.32	5.91
7.2	13.1	6.93	3.97	1.82	9.82	4.23	5.5
4.25	12.2	6.91	5.3	1.77	9.13	3.09	6.36
4.46	9.17	4.68	6.13	1.54	7.91		
1.69	7.08	2.93	8.19				
Average	**8.81**		**6.42**		**8.5**		**6.14**
Stokes radius (Angstrom, Å)	**12.2**		**16.7**		**12.6**		**17.5**
Volume ($Å^3$)	**7590.61**		**19629.26**		**8469**		**22516.46**
$\frac{V_{Co-peptide}}{V_{peptide}}$	**2.58**			**2.66**			

Table S2. Results from the DOSY NMR experiments of $P3L_2$ and $P4L_2$, and their Co-complexes.

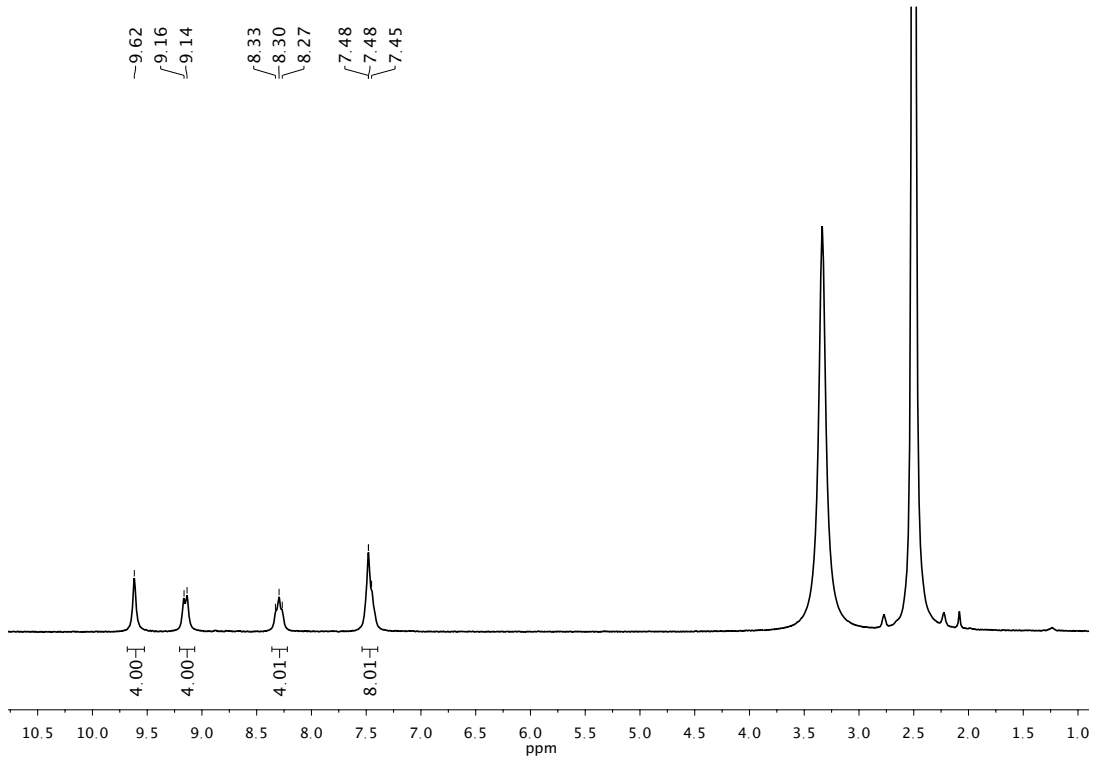
$P3L_2$		**$[Co_2^{III}(P3L_2)_2]^{6+}$**		**$P4L_2$**		**$[Co_2^{III}(P4L_2)_2]^{6+}$**	
δ/ppm	$D \cdot 10^{-11}$ (m^2/sec)	δ/ppm	$D \cdot 10^{-11}$ (m^2/sec)	δ/ppm	$D \cdot 10^{-11}$ (m^2/sec)	δ/ppm	$D \cdot 10^{-11}$ (m^2/sec)
10.81	7.62	10.82	5.11	10.8	7.9	10.82	5.5
10.85	7.37	8.93	5.3	7.24	7.36	9.62	5.7
7.31	7.9	8.2	6.12	7.04	7.89	8.32	5.88
7.07	7.61	7.46	4.79	7.02	8.81	7.55	6.13
6.83	8.81	7.16	4.75	4.45	7.78	7.15	5.11
4.49	7.63	4.34	4.93	2.98	7.62	7.04	5.1
4.64	7.35	3.13	6.13	2.96	7.61	3.9	4.75
4.48	7.89	1.56	5.91	1.94	7.56	3.53	4.76
1.46	7.82	1.72	5.75	1.35	7.35	1.9	7.34
						1.56	5.91
Average	**7,78**		**5.42**		**7.86**		**5.56**
Stokes radius (Angstrom, Å)	**13,8**		**19.8**		**13.7**		**19.34**
Volume ($Å^3$)	**11050.4**		**32634.64**		**10683**		**30317**
$\frac{V_{Co-peptide}}{V_{peptide}}$	**2.95**			**2.83**			

6. Spectra and chromatograms

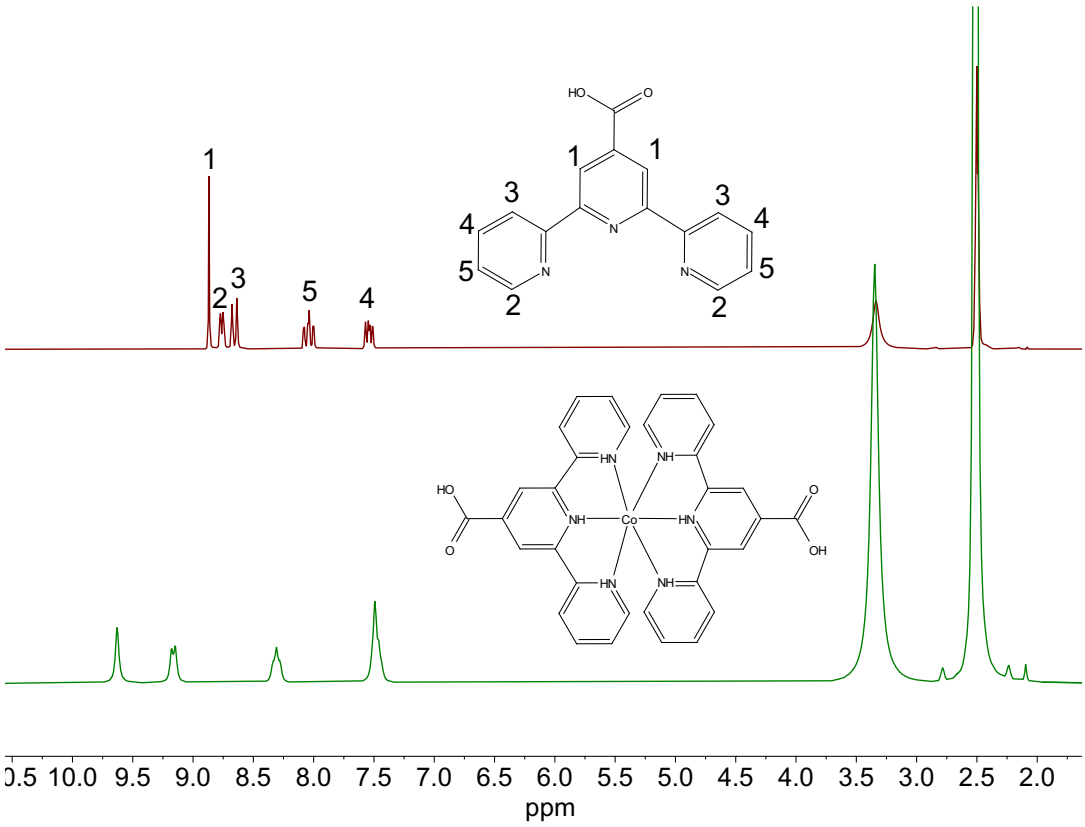
7.1 ^{1}H NMR



***Supplementary figure S1**. ^{1}H NMR of Ligand (**L**) in DMSO-d^{6} (2.5 ppm, H_2O: 3.33 ppm) at 300 MHz.*

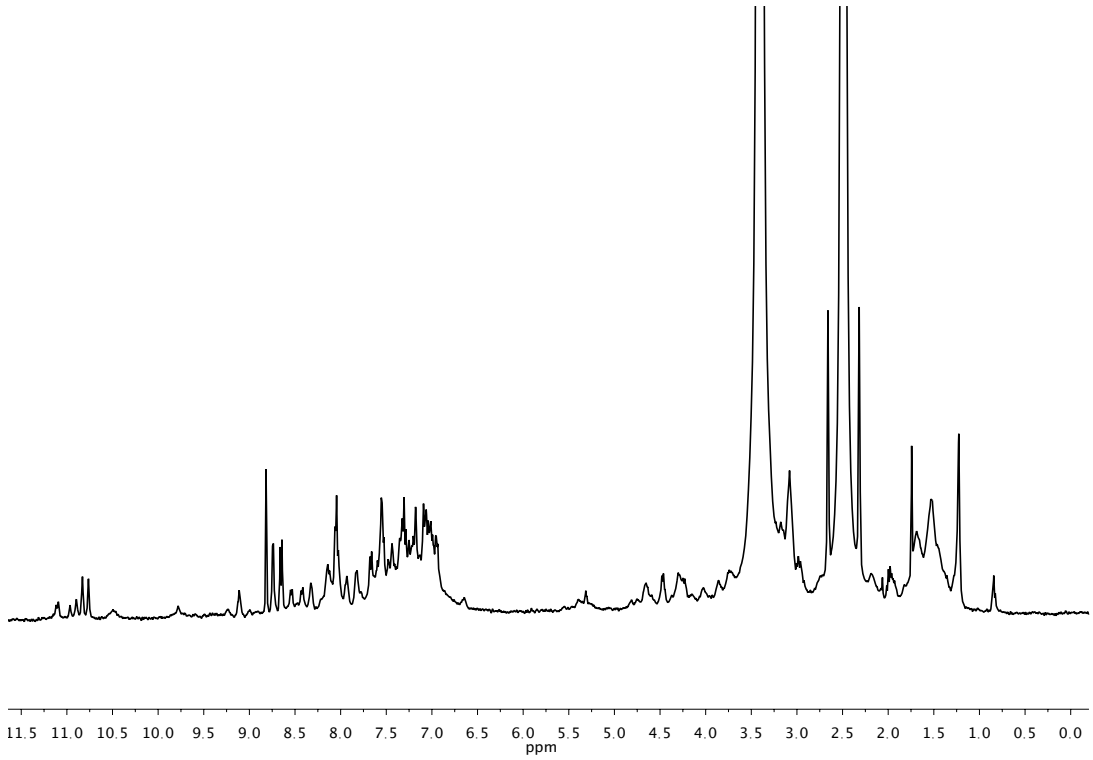


Supplementary figure S2. *^{1}H NMR of cobalt complex $[Co^{III}L_2]^{3+}$ in DMSO-d^6 (2.5 ppm, H_2O: 3.33 ppm) at 300 MHz.*

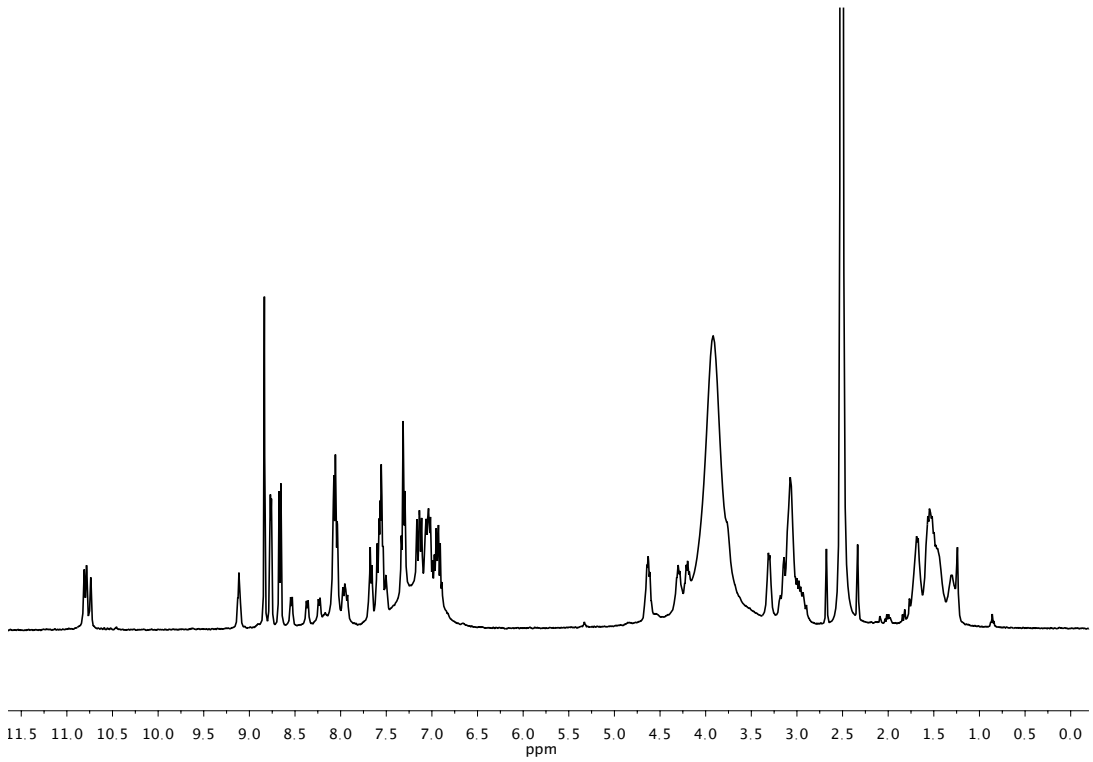


Supplementary figure S2-1. *Stacked ^{1}H NMR spectra of tpy-COOH ligand and cobalt complex $[Co^{III}L_2]^{3+}$ in DMSO-d6 (2.5 ppm, H2O: 3.33 ppm) at 300 MHz.*

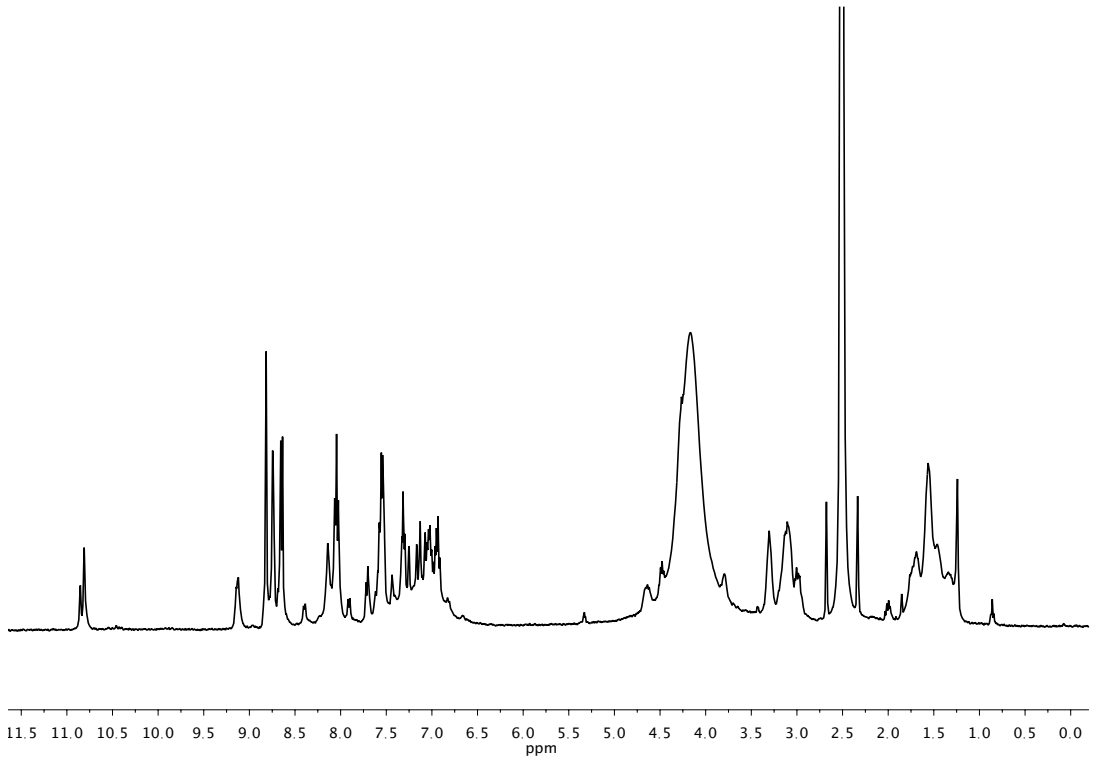
.



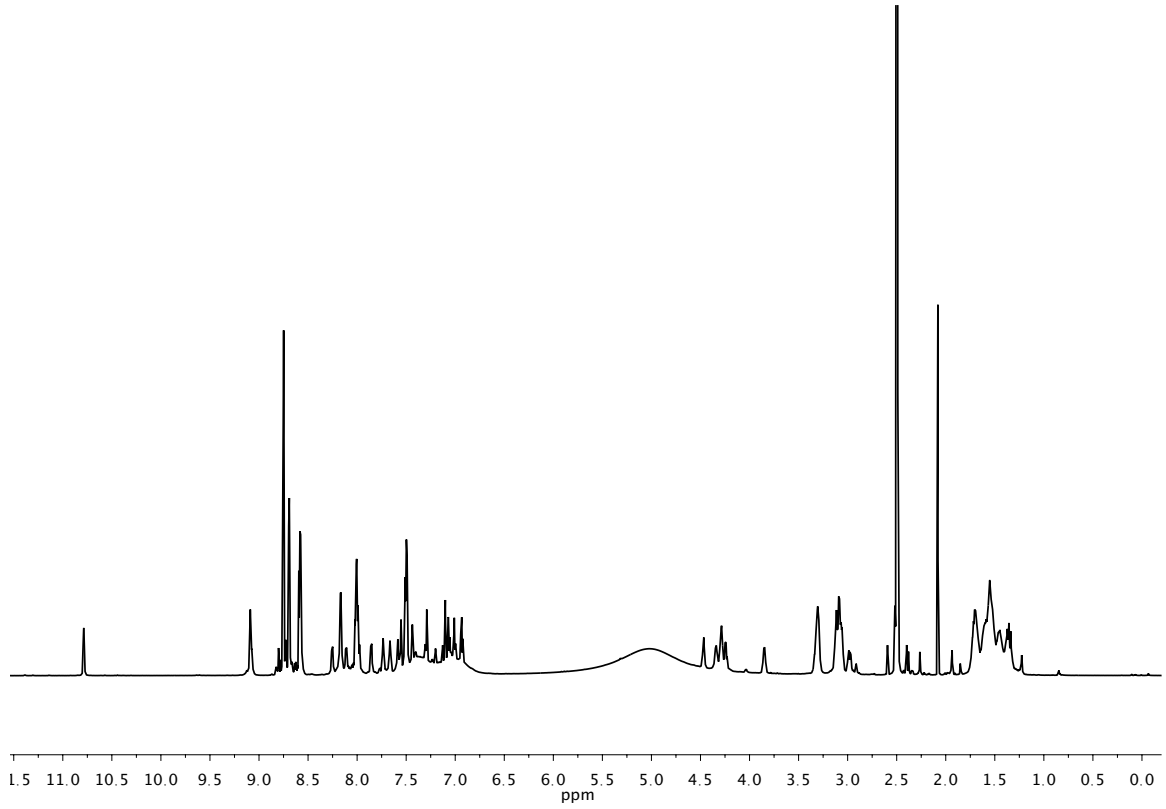
Supplementary figure S3. *^{1}H NMR of peptide **P1L$_1$** in DMSO-d6 (2.5 ppm, H2O: 3.4 ppm) at 400 MHz.*



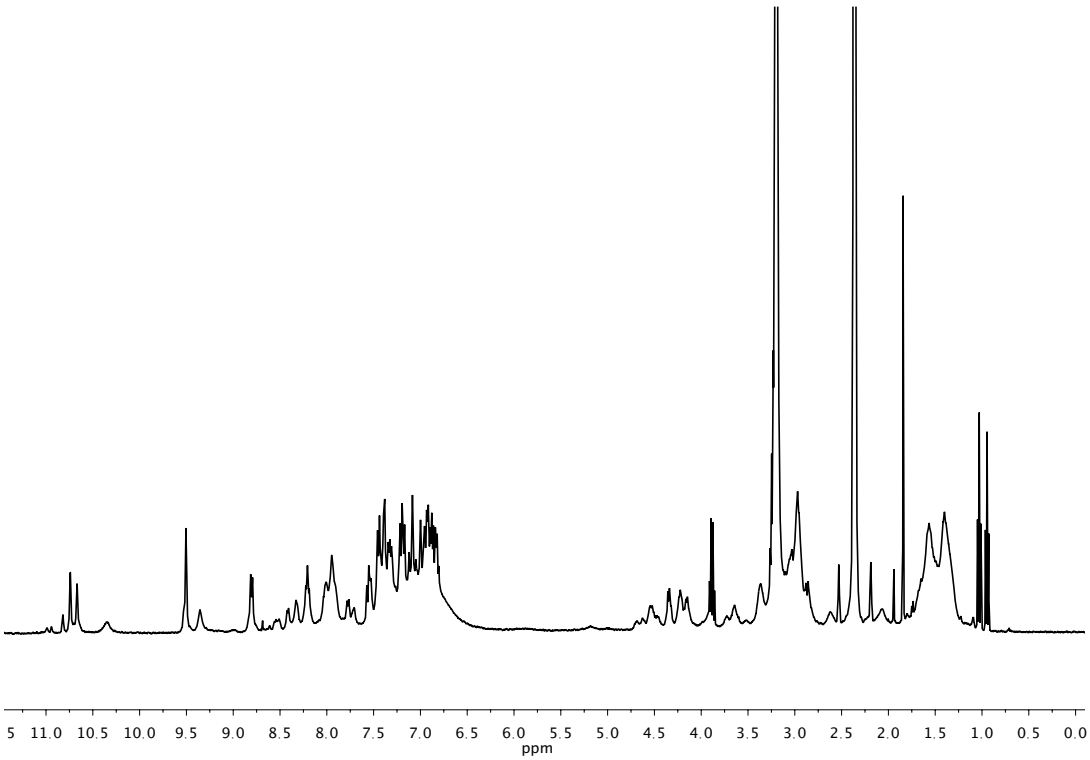
Supplementary figure S4. *^{1}H NMR of peptide **P2L$_1$** in DMSO-d6 (2.5 ppm, H_2O: 3.8 ppm) at 400 MHz.*



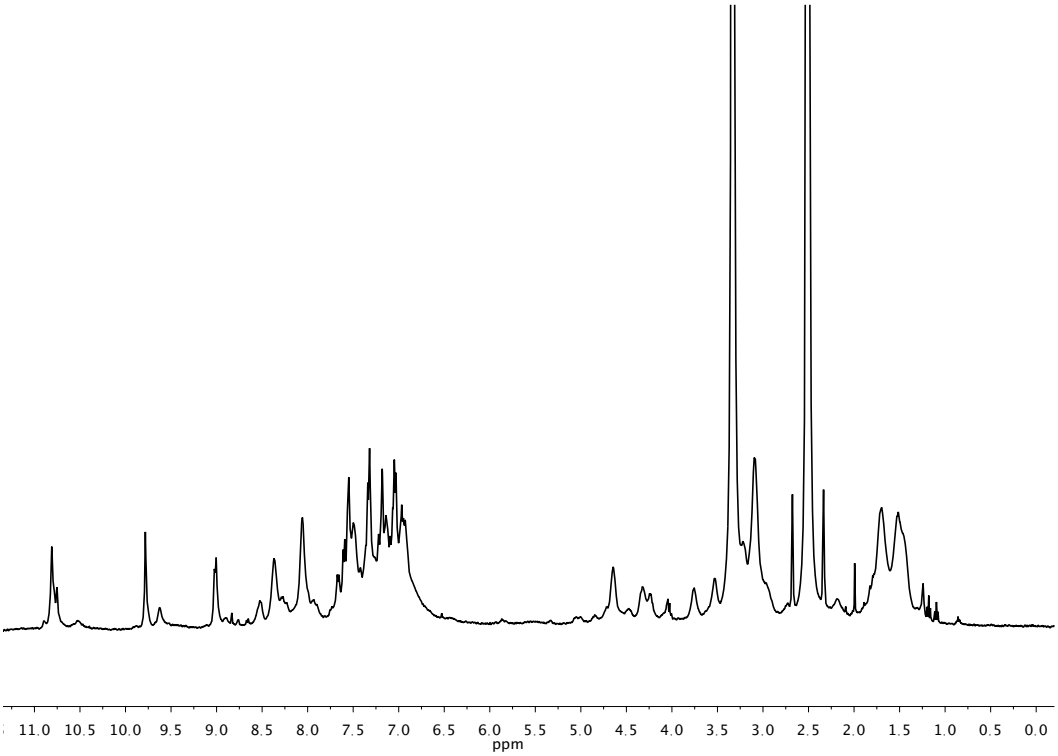
Supplementary figure S5. *^{1}H NMR of peptide **P3L$_2$** in DMSO-d6 (2.5 ppm, H_2O: 4.3 ppm) at 400 MHz.*



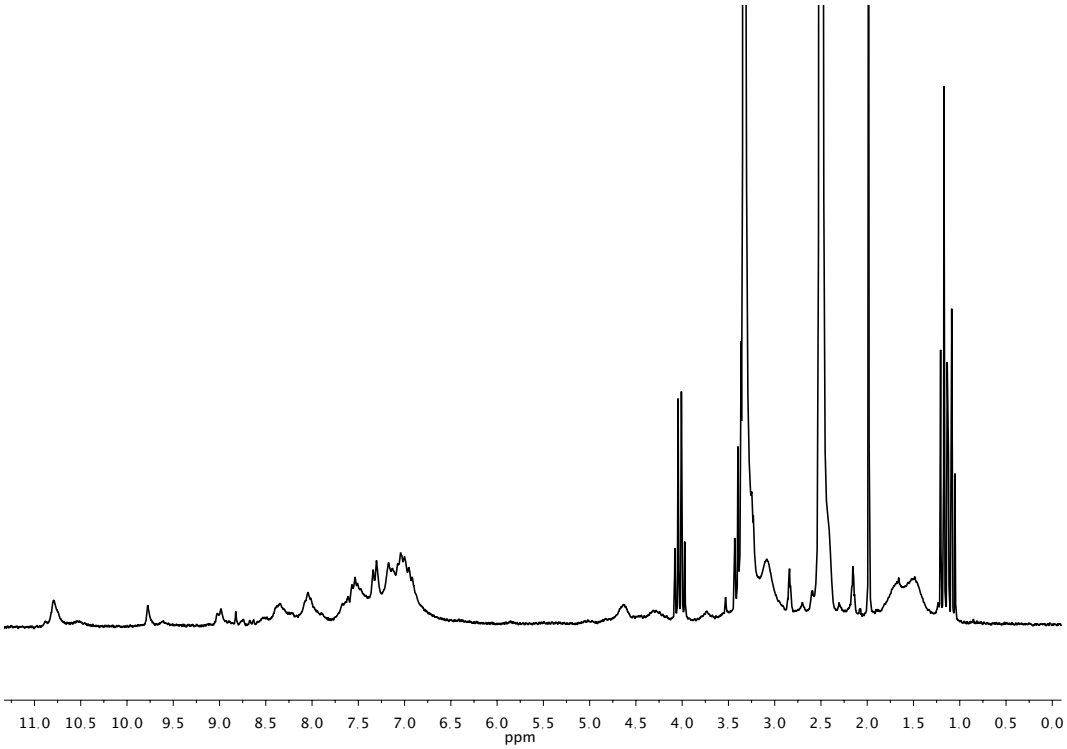
Supplementary figure S6. *^{1}H NMR of peptide **P4L$_2$** in DMSO-d6 (2.5 ppm) at 700 MHz.*



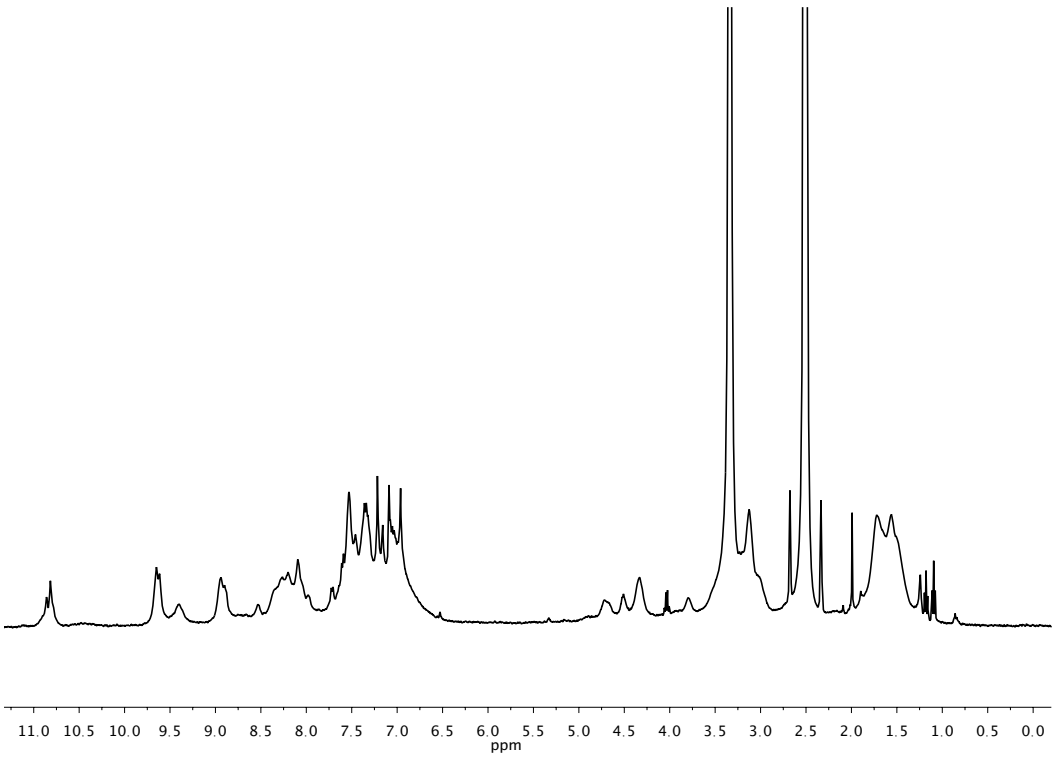
Supplementary figure S7. *^{1}H NMR of cobalt-peptide conjugate* ***$[Co^{III}(P1L_1)_2]^{3+}$*** *without the oxidation agent in DMSO-d^6 (2.5 ppm, H_2O: 3.33 ppm) at 400 MHz.*



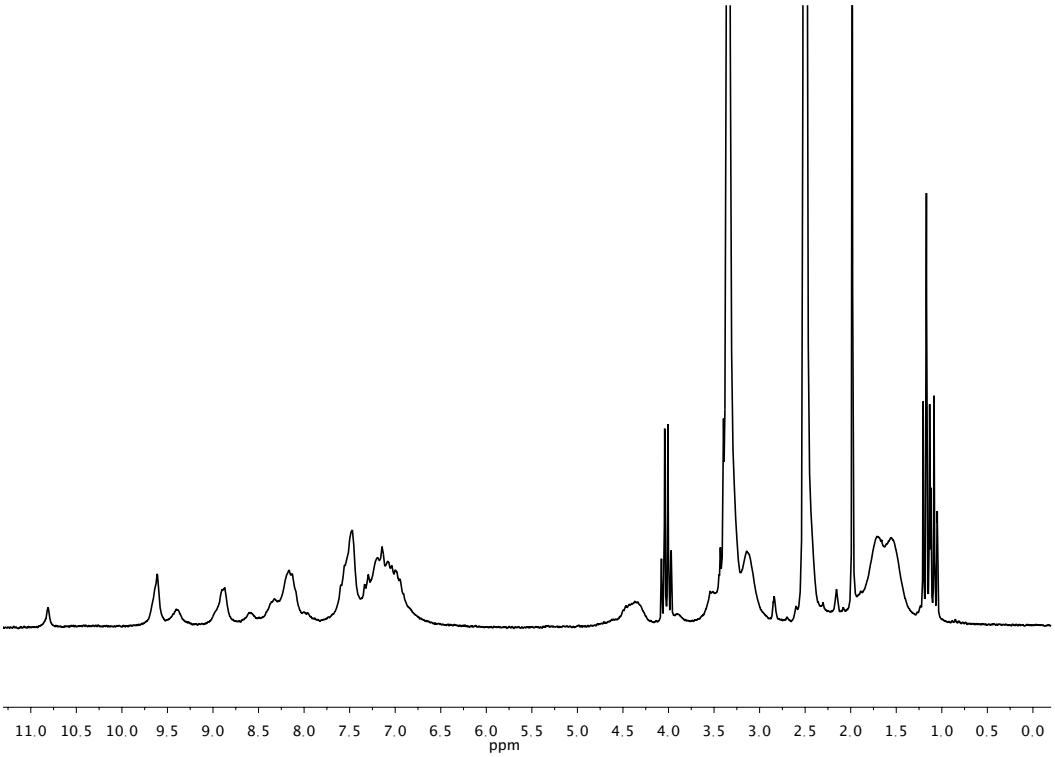
Supplementary figure S8. *^{1}H NMR of cobalt-peptide conjugate* ***$[Co^{III}(P2L_1)_2]^{3+}$*** *directly after the oxidation reaction in DMSO-d^6 (2.5 ppm, H_2O: 3.33 ppm) at 400 MHz.*



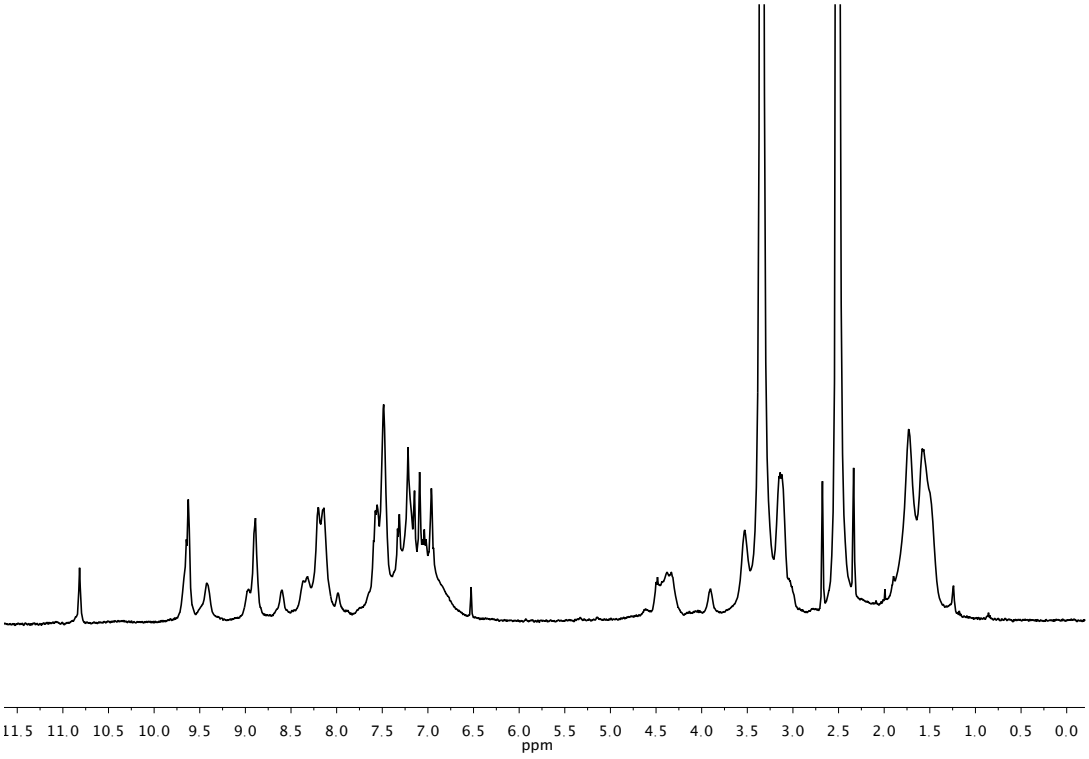
Supplementary figure S9. *^{1}H NMR of cobalt-peptide conjugate* ***$[Co^{III}(P2L_1)_2]^{3+}$*** *without the oxidation agent in DMSO-d^6 (2.5 ppm, H_2O: 3.33 ppm) at 300 MHz.*



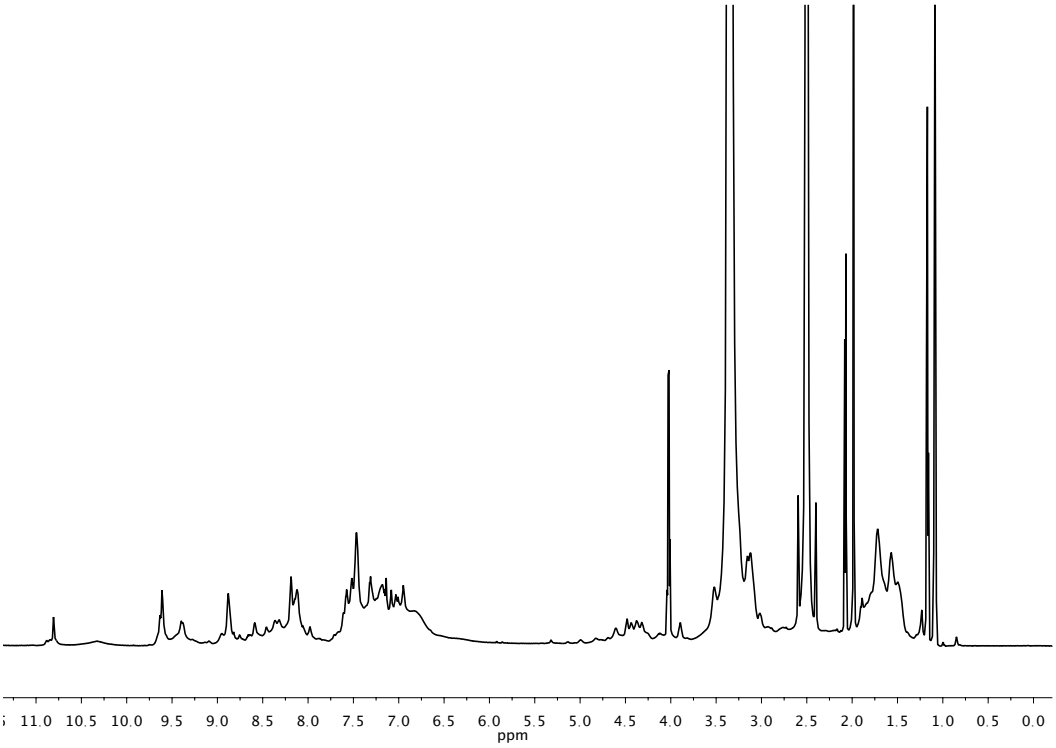
Supplementary figure S10. *^{1}H NMR of cobalt-peptide conjugate* ***$[Co_2^{III}(P3L_2)_2]^{6+}$*** *directly after the oxidation reaction in DMSO-d6 (2.5 ppm, H_2O: 3.33 ppm) at 400 MHz.*



Supplementary figure S11. *^{1}H NMR of cobalt-peptide conjugate* ***$[Co_2^{III}(P3L_2)_2]^{6+}$*** *without the oxidation agent in DMSO-d^6 (2.5 ppm) at 300 MHz.*

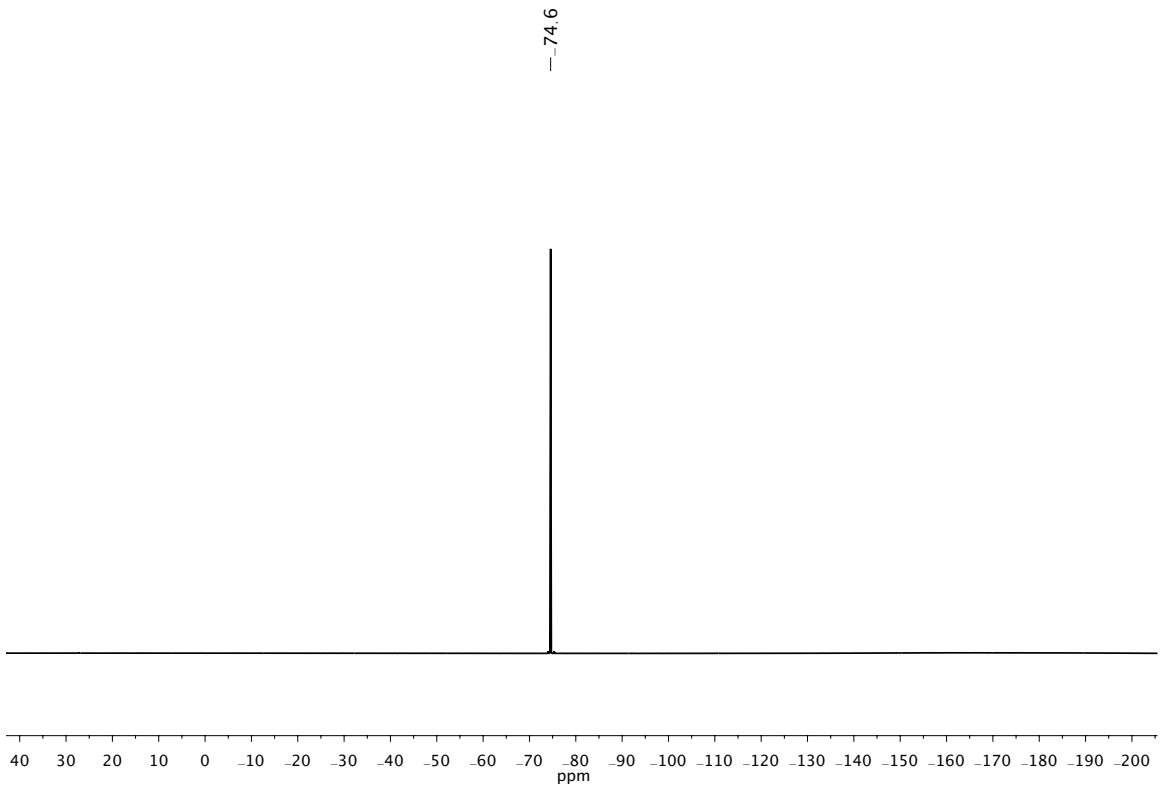


Supplementary figure S12. *^{1}H NMR of cobalt-peptide conjugate* ***$[Co_2^{III}(P4L_2)_2]^{6+}$*** *directly after the oxidation reaction in DMSO-d6 (2.5 ppm, H_2O: 3.33 ppm) at 400 MHz.*

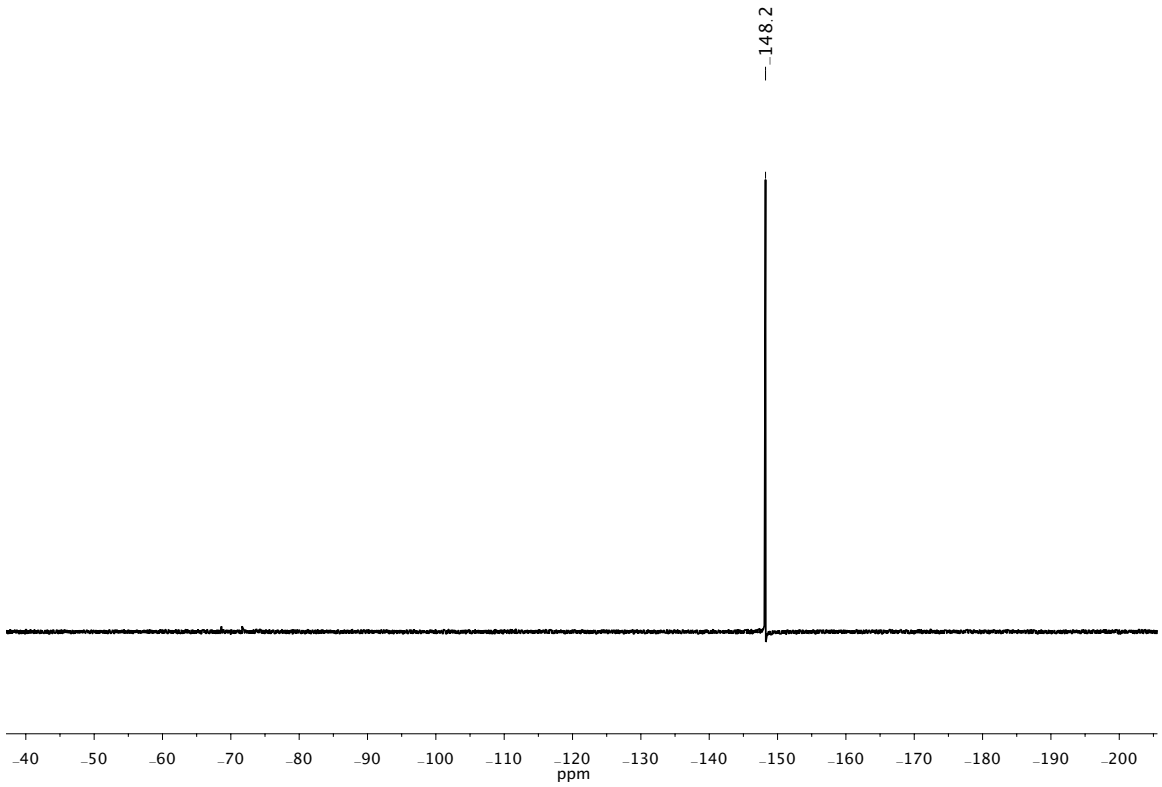


Supplementary figure S13. *^{1}H NMR of cobalt-peptide conjugate* ***$[Co_2^{III}(P4L_2)_2]^{6+}$*** *without the oxidation agent in DMSO-d^6 (2.5 ppm, H_2O: 3.33 ppm) at 700 MHz.*

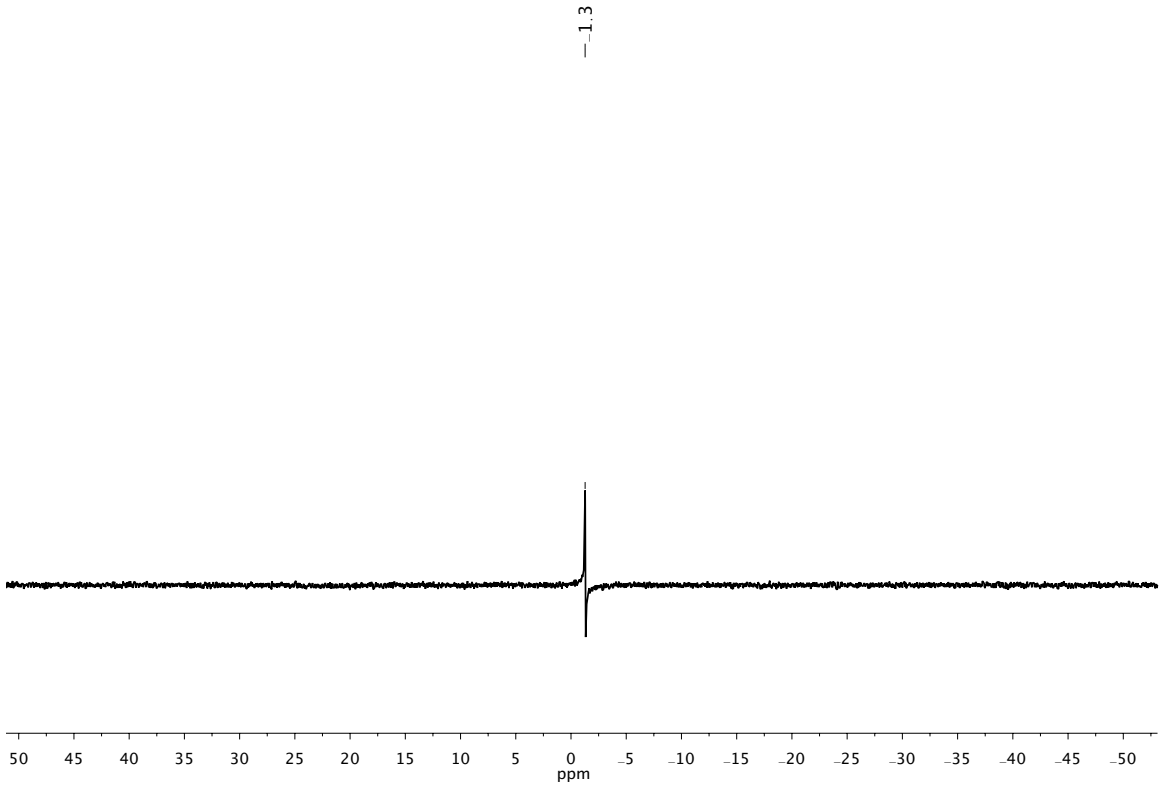
6.1 ^{19}F and ^{11}B NMR



Supplementary figure S14. *^{19}F NMR of peptide **P4L$_2$** with TFA$^-$ counter ion in DMSO-d^6 at 235 MHz.*

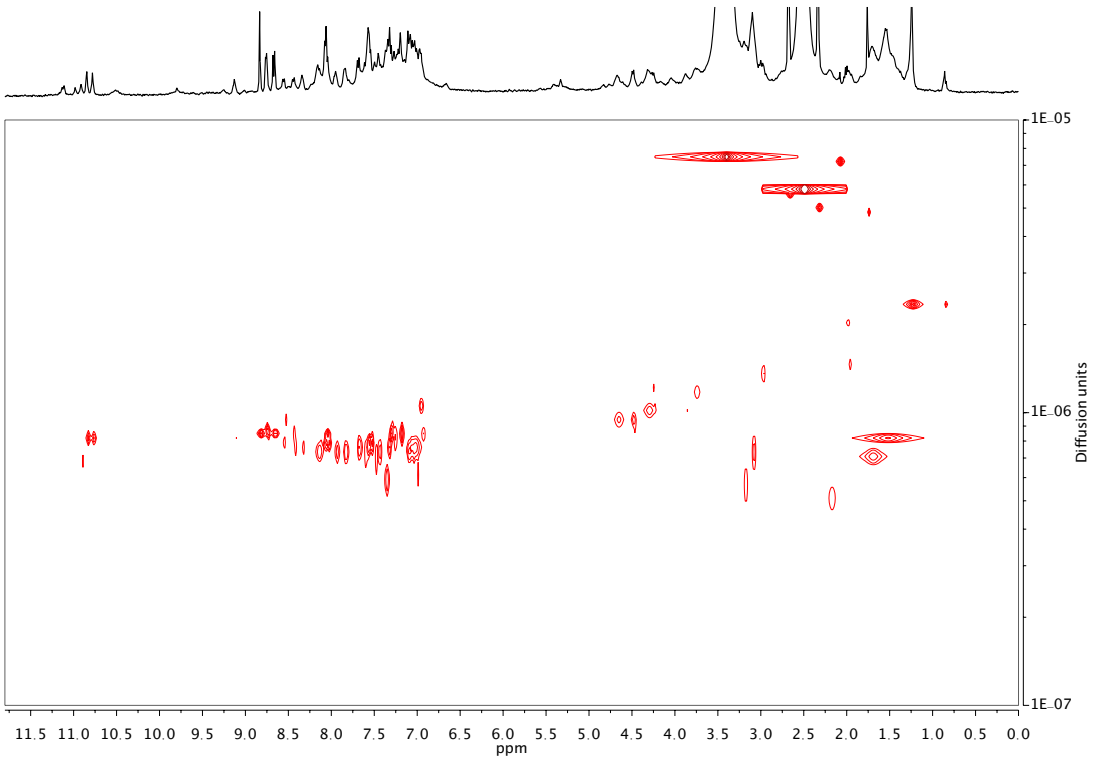


Supplementary figure S15. *^{19}F NMR of cobalt-peptide conjugate **[Co$_2^{III}$(P4L$_2$)$_2$]$^{6+}$** with BF$_4^-$ counter ion in DMSO-d^6 at 235 MHz.*

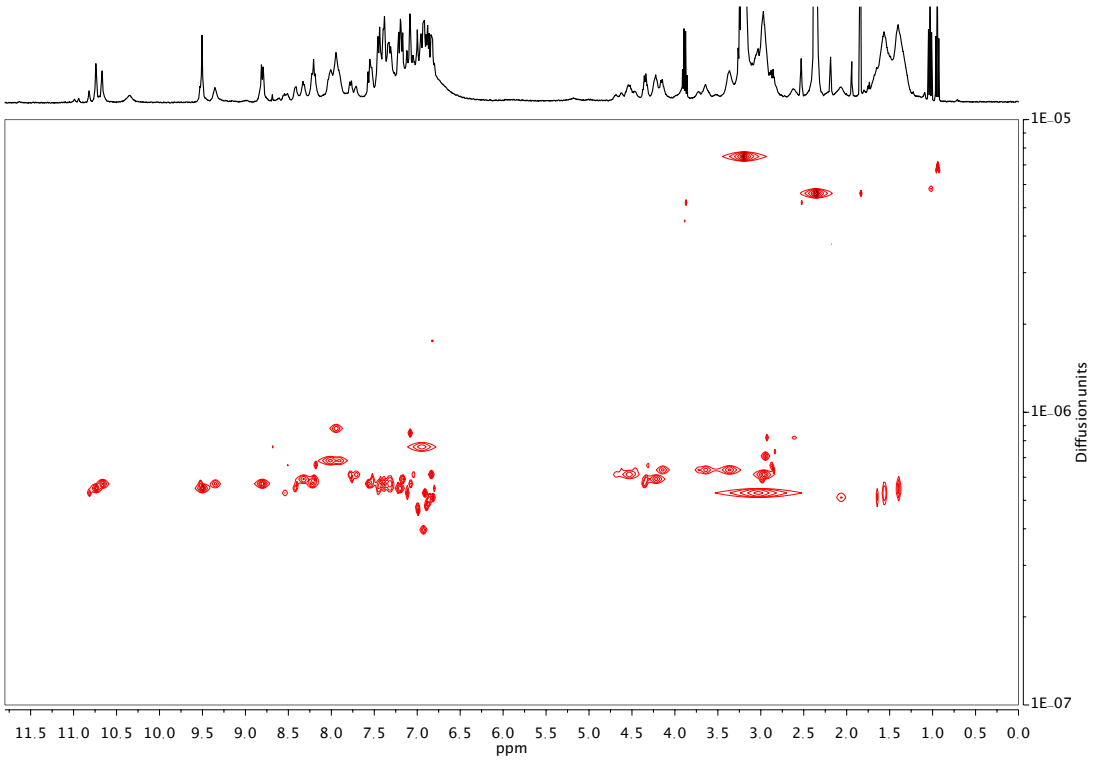


Supplementary figure S16. *^{11}B NMR of cobalt-peptide conjugate* ***$[Co_2^{III}(P4L_2)_2]^{6+}$*** *with BF_4^- counter ion in DMSO-d^6 at 80 MHz.*

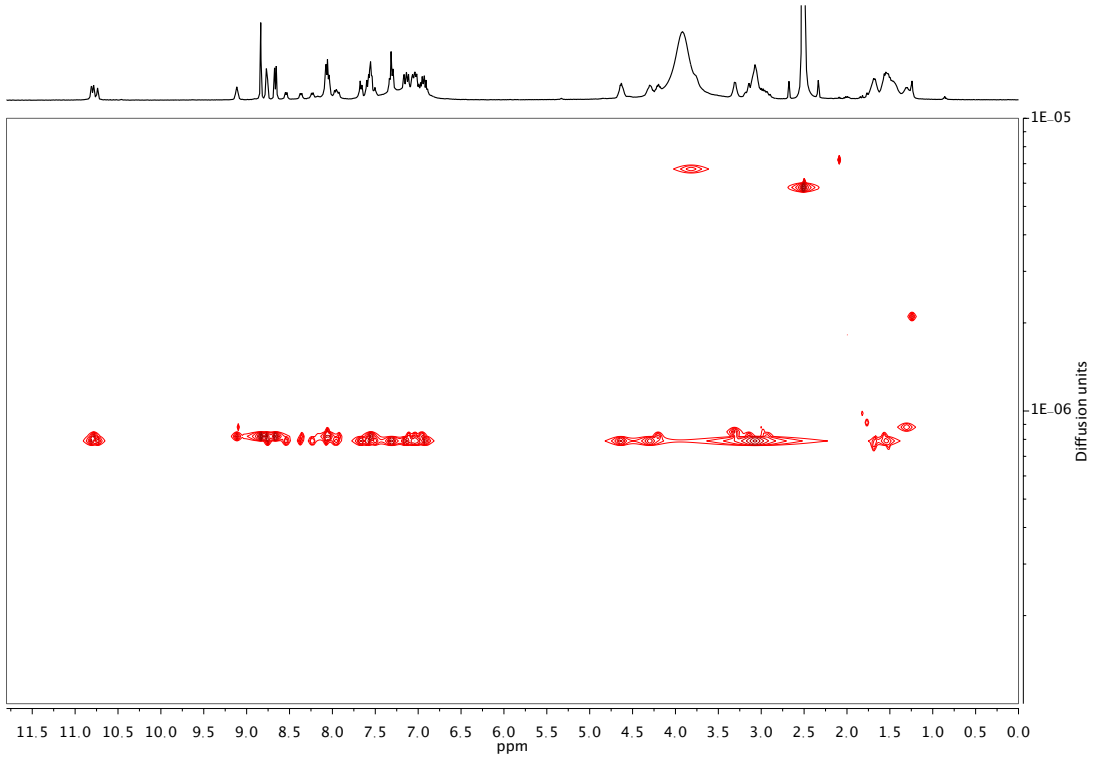
6.2 DOSY NMR



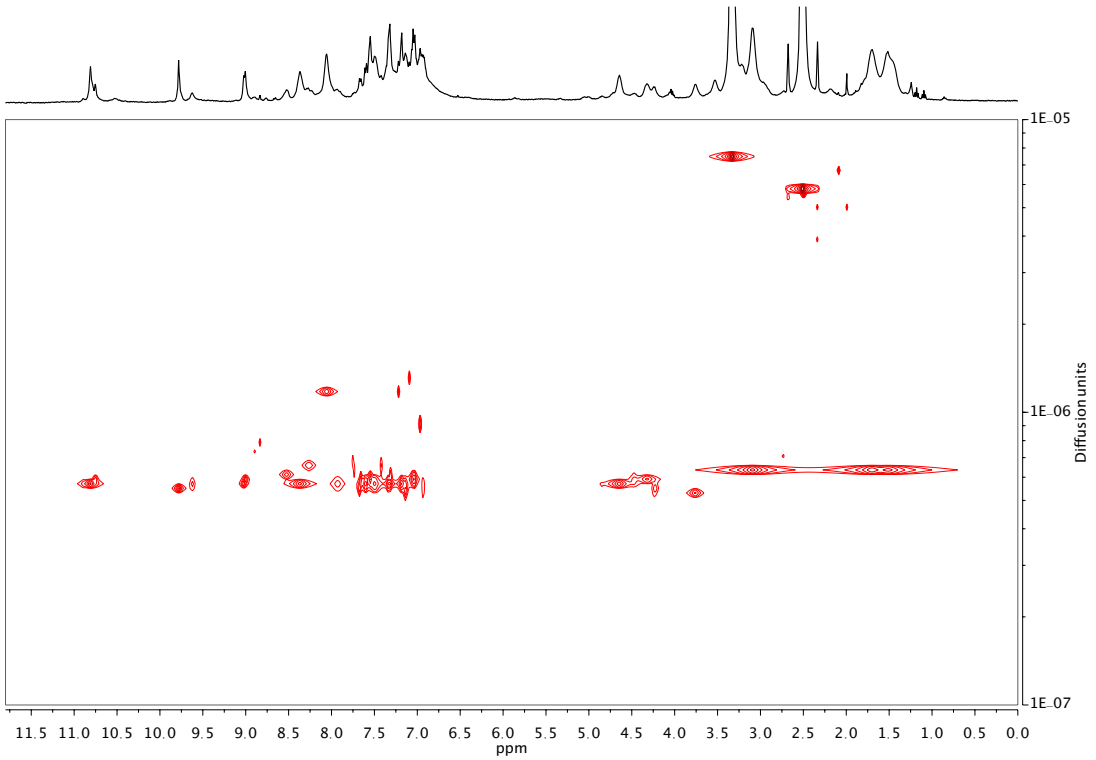
Supplementary figure S17. *2D DOSY NMR (400 MHz) spectrum of* ***P1L₁*** *with a diffusion coefficient of* $8.81 \cdot 10^{-11}$ $m^2 \cdot sec^{-1}$ *in DMSO-*d^6 *(2.5 ppm,* H_2O*: 3.33 ppm) at 25°C.*



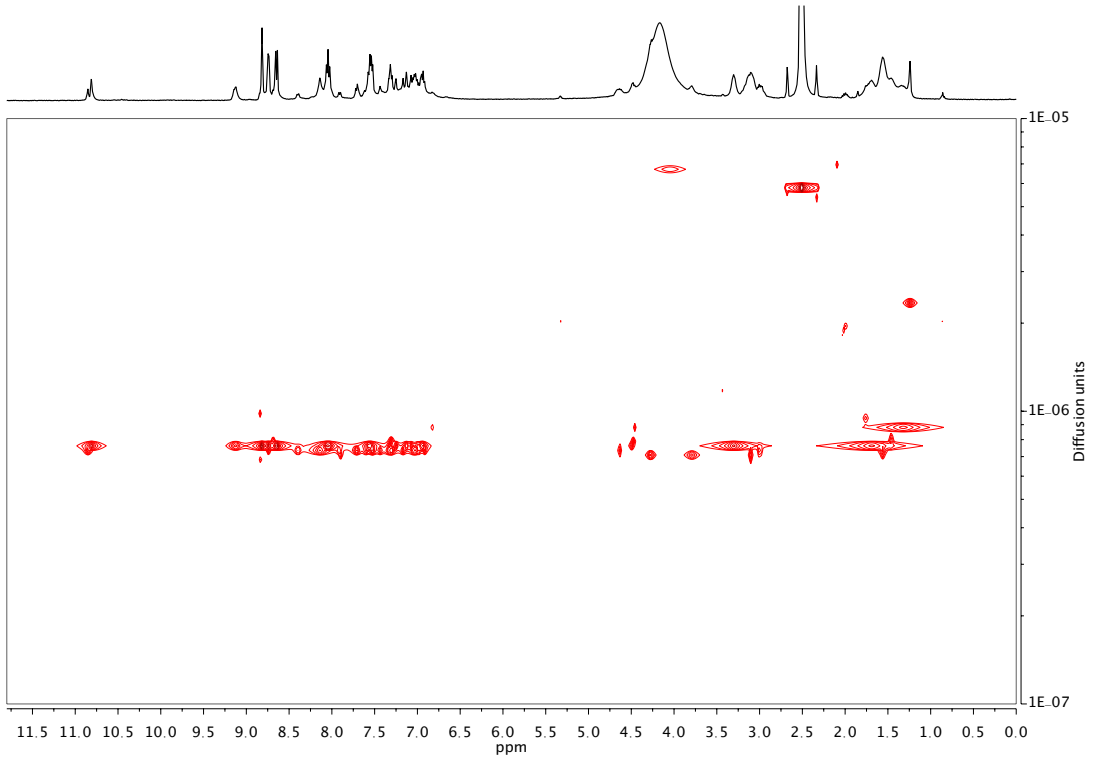
Supplementary figure S18. *2D DOSY NMR (400 MHz) spectrum of* ***[Co^{III}(P1L$_1$)$_2$]$^{3+}$*** *with a diffusion coefficient of 6.45·10^{-11} m^2·sec^{-1} in DMSO-d^6 (2.5 ppm, H$_2$O: 3.33 ppm) at 25°C, directly after the oxidation reaction.*



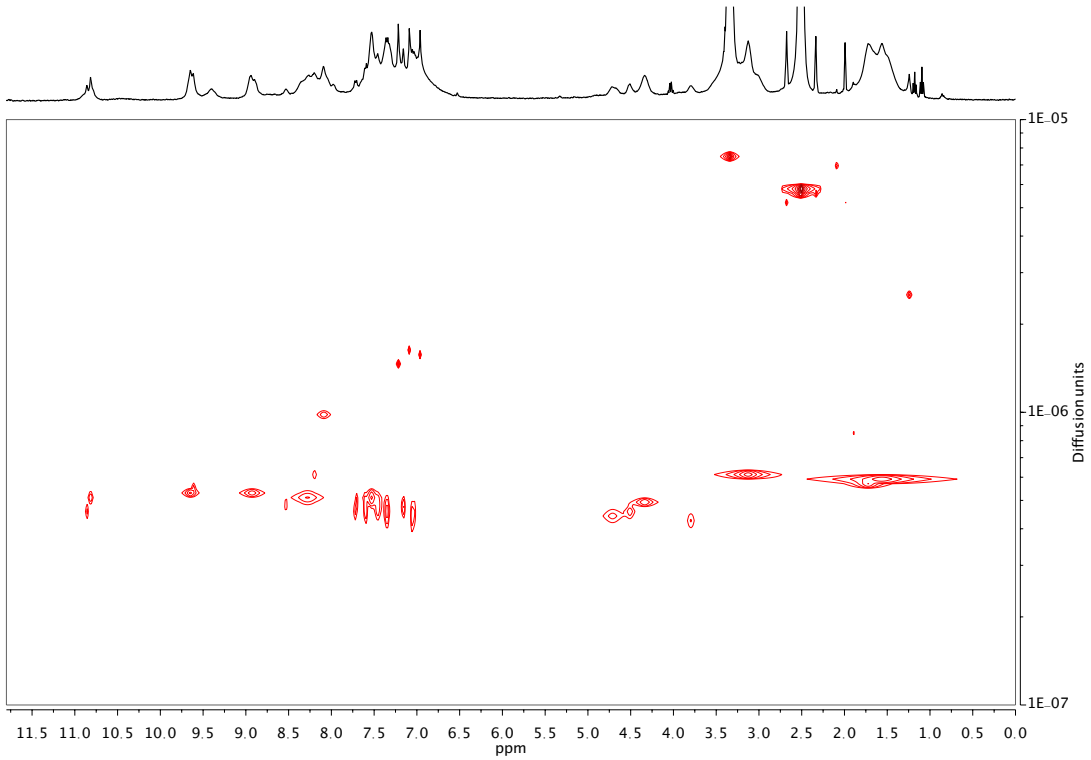
Supplementary figure S19. *2D DOSY NMR (400 MHz) spectrum of* ***P2L$_1$*** *with a diffusion coefficient of 8.5·10^{-11} m^2·sec^{-1} in DMSO-d^6 (2.5 ppm, H$_2$O: 3.33 ppm) at 25°C.*



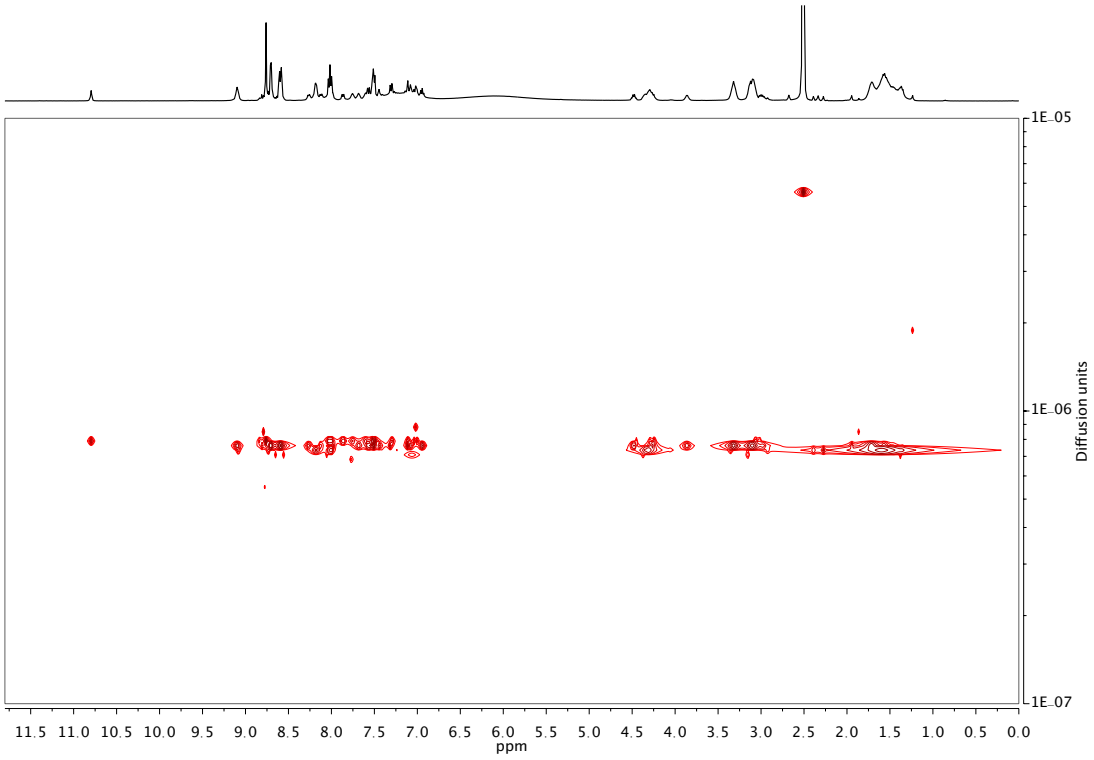
Supplementary figure S20. *2D DOSY NMR (400 MHz) spectrum of* ***[Co^{III}(P2L$_1$)$_2$]$^{3+}$*** *with a diffusion coefficient of 6.14·10^{-11} m^2·sec^{-1} in DMSO-d^6 (2.5 ppm, H$_2$O: 3.33 ppm) at 25°C, directly after the oxidation reaction.*



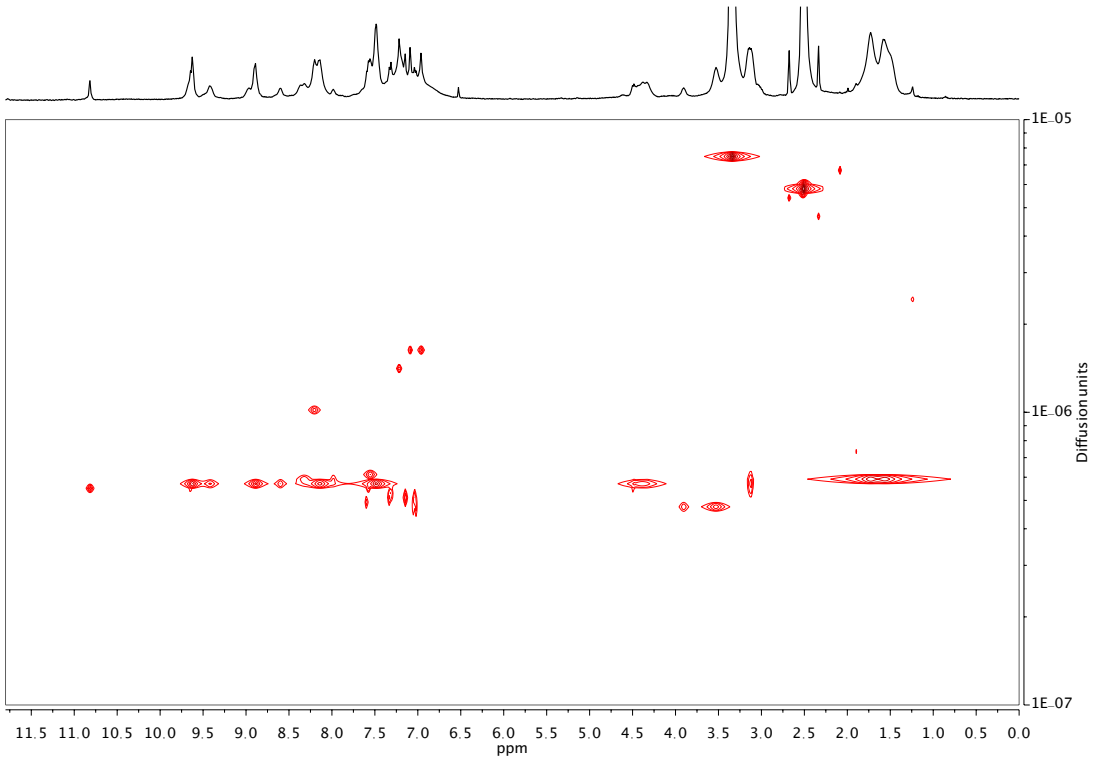
Supplementary figure S21. *2D DOSY NMR (400 MHz) spectrum of* ***P3L$_2$*** *with a diffusion coefficient of 7.78·10^{-11} m^2·sec^{-1} in DMSO-d^6 (2.5 ppm, H$_2$O: 3.33 ppm) at 25°C.*



Supplementary figure S22. *2D DOSY NMR (400 MHz) spectrum of* ***[Co$_2^{III}$(P3L$_2$)$_2$]$^{6+}$*** *with a diffusion coefficient of 5.42·10^{-11} m^2·sec^{-1} in DMSO-d^6 (2.5 ppm, H$_2$O: 3.33 ppm) at 25°C, directly after the oxidation reaction.*

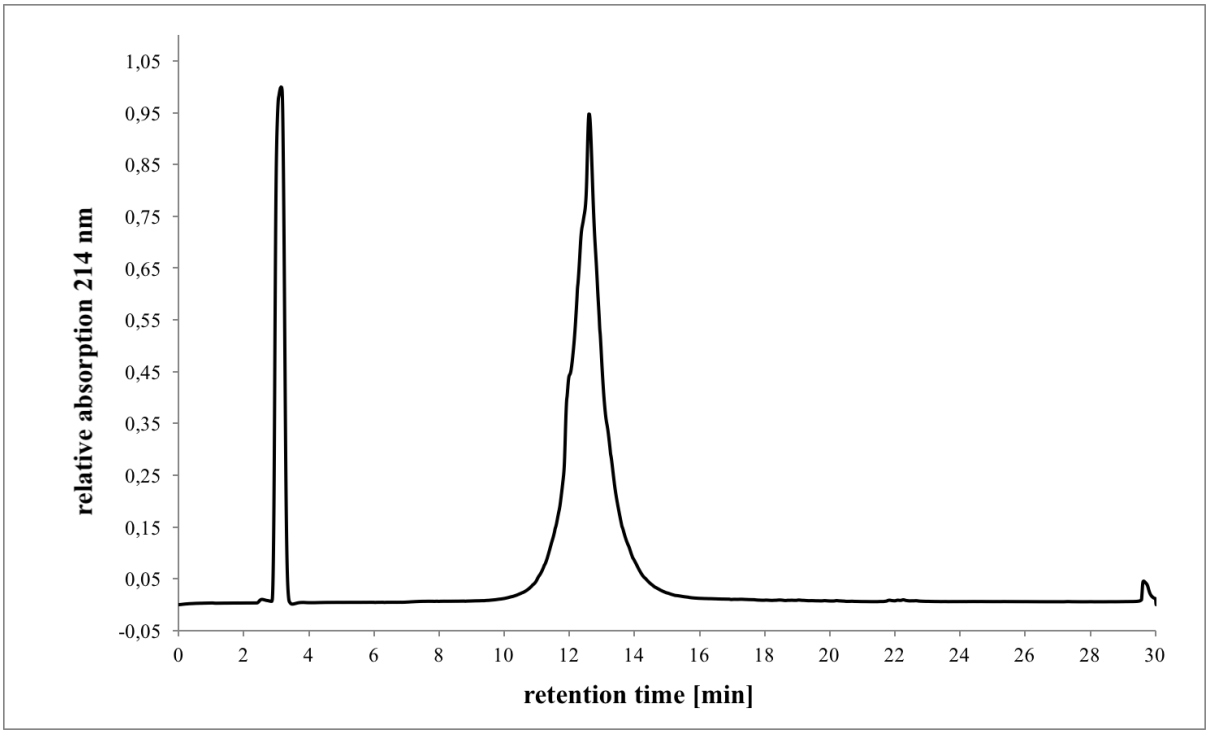


Supplementary figure S23. *2D DOSY NMR (400 MHz) spectrum of* ***P4L$_2$*** *with a diffusion coefficient of 7.86·10^{-11} m^2·sec^{-1} in DMSO-d^6 (2.5 ppm, H$_2$O: 3.33 ppm) at 25°C.*

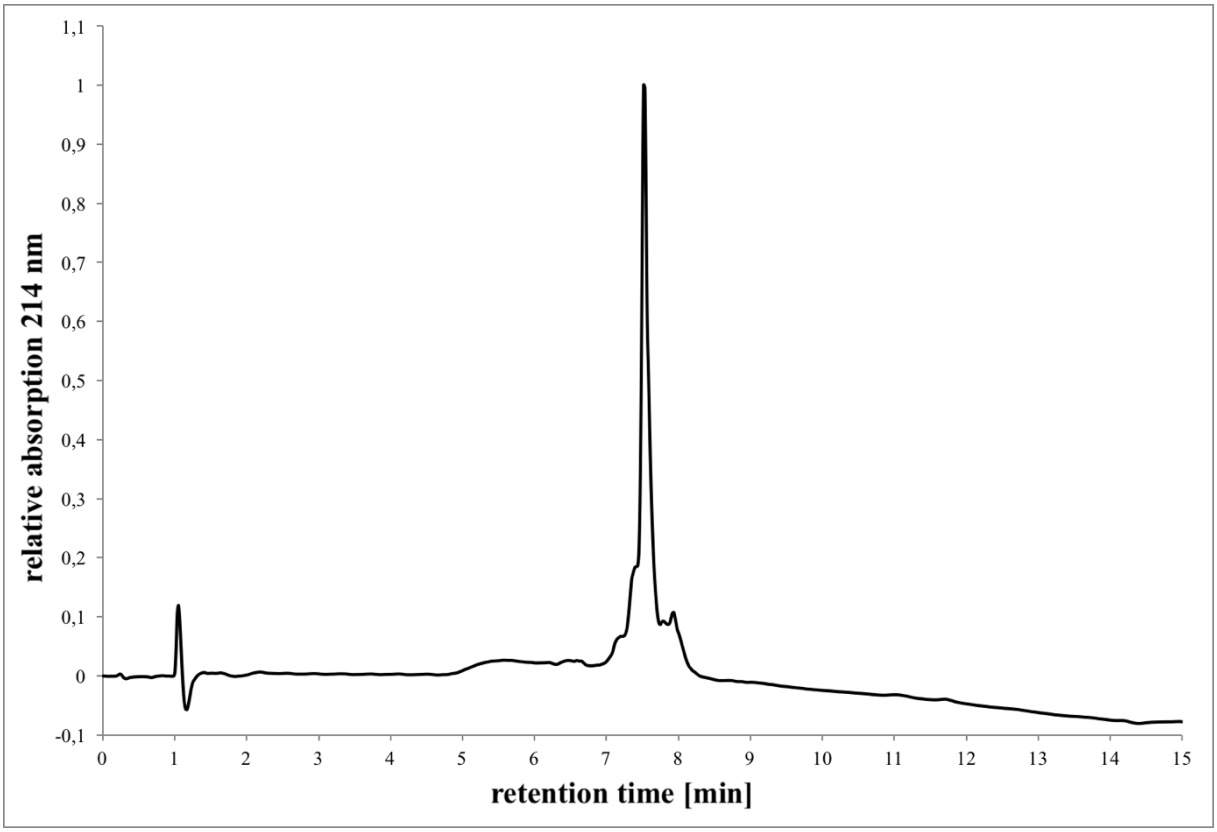


Supplementary figure S24. *2D DOSY NMR (400 MHz) spectrum* ***$[Co_2^{III}(P4L_2)_2]^{6+}$*** *with a diffusion coefficient of $5.56 \cdot 10^{-11}$ $m^2 \cdot sec^{-1}$ in DMSO-d^6 (2.5 ppm, H_2O: 3.33 ppm) at 25°C, directly after the oxidation reaction.*

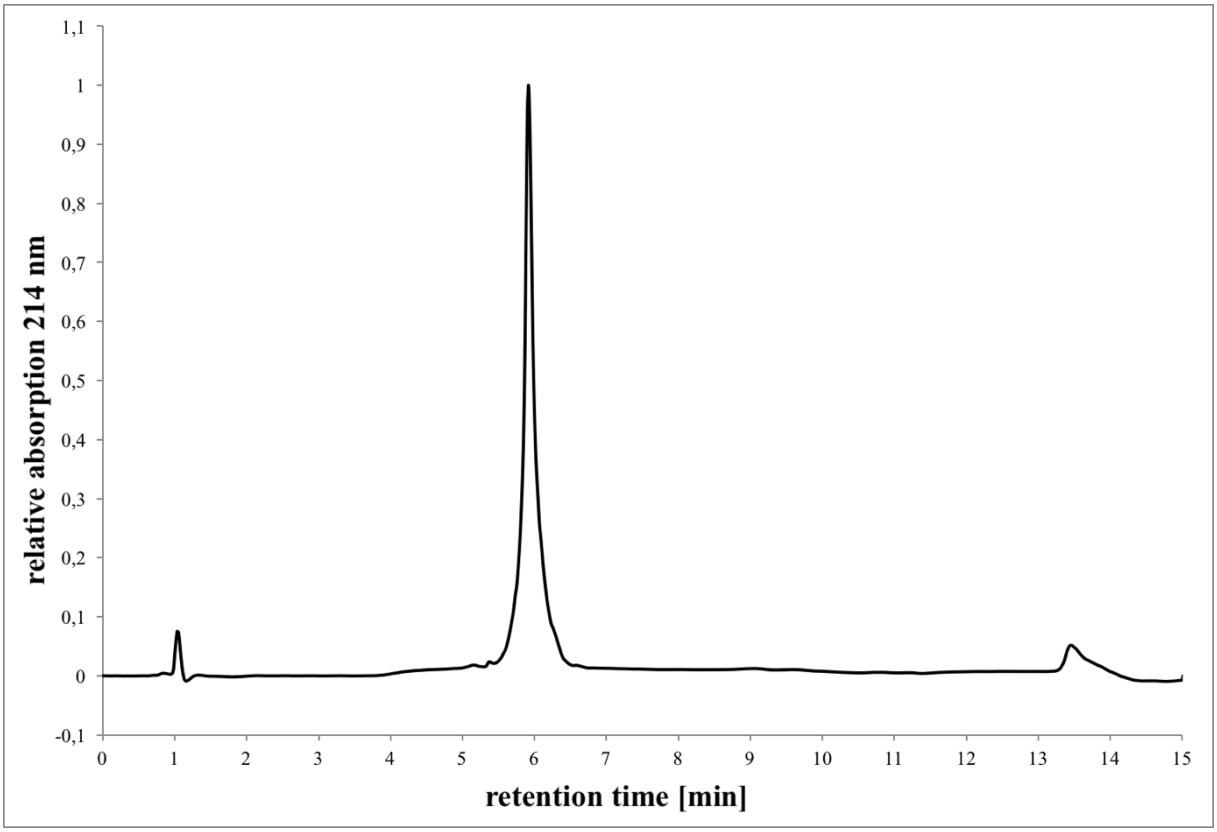
7. HPLC Chromatograms of Peptides



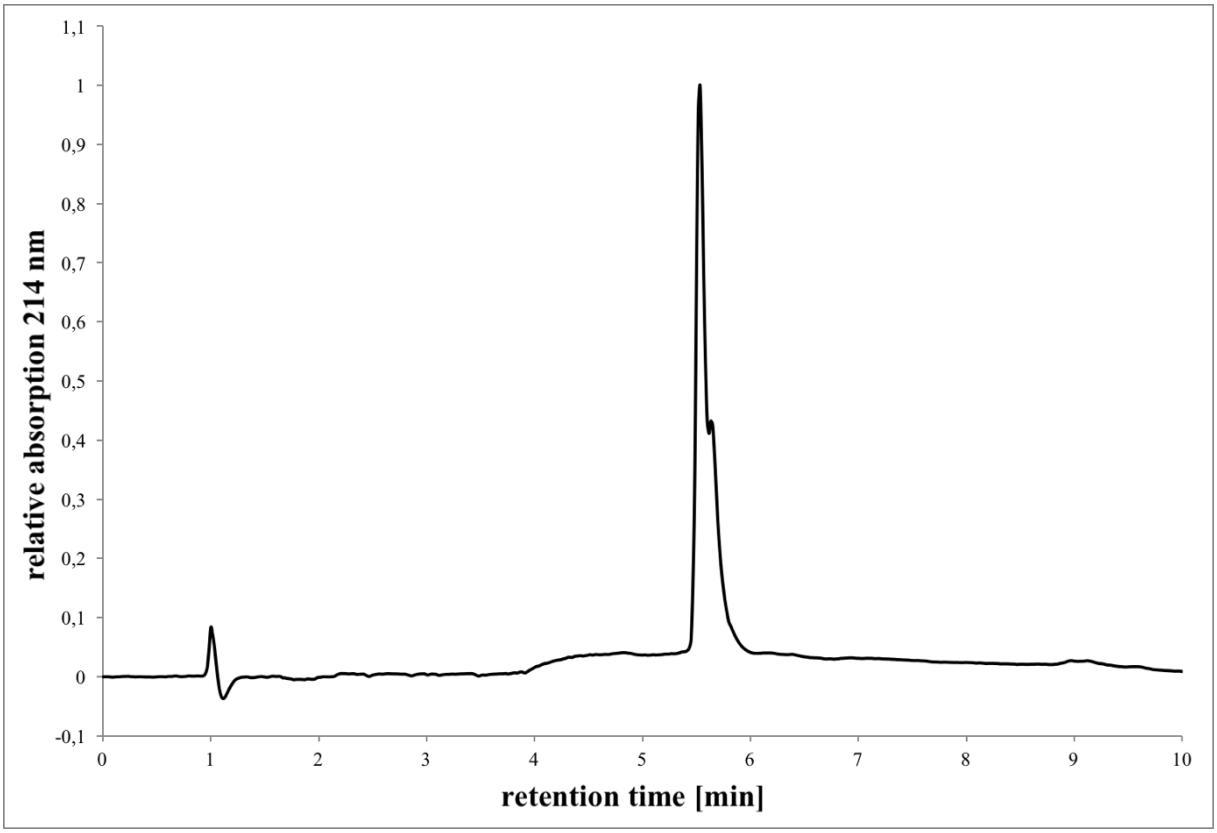
Supplementary figure S25. *HPLC chromatogram of peptide* ***P1L₁*** *upon purification by semi-preparative HPLC (C18, 250 x 10 mm; ACN/H_2O 5:95 + 0.1 % TFA → ACN/H_2O 95:5 + 0.1 % TFA) with a retention time of 11.7 min.*



Supplementary figure S26. *HPLC chromatogram of peptide* ***P2L₁*** *upon purification by analytic HPLC (C18, 250 x 4 mm; ACN/H_2O 5:95 + 0.1 % TFA → ACN/H_2O 95:5 + 0.1 % TFA) with a retention time of 7.4 min.*

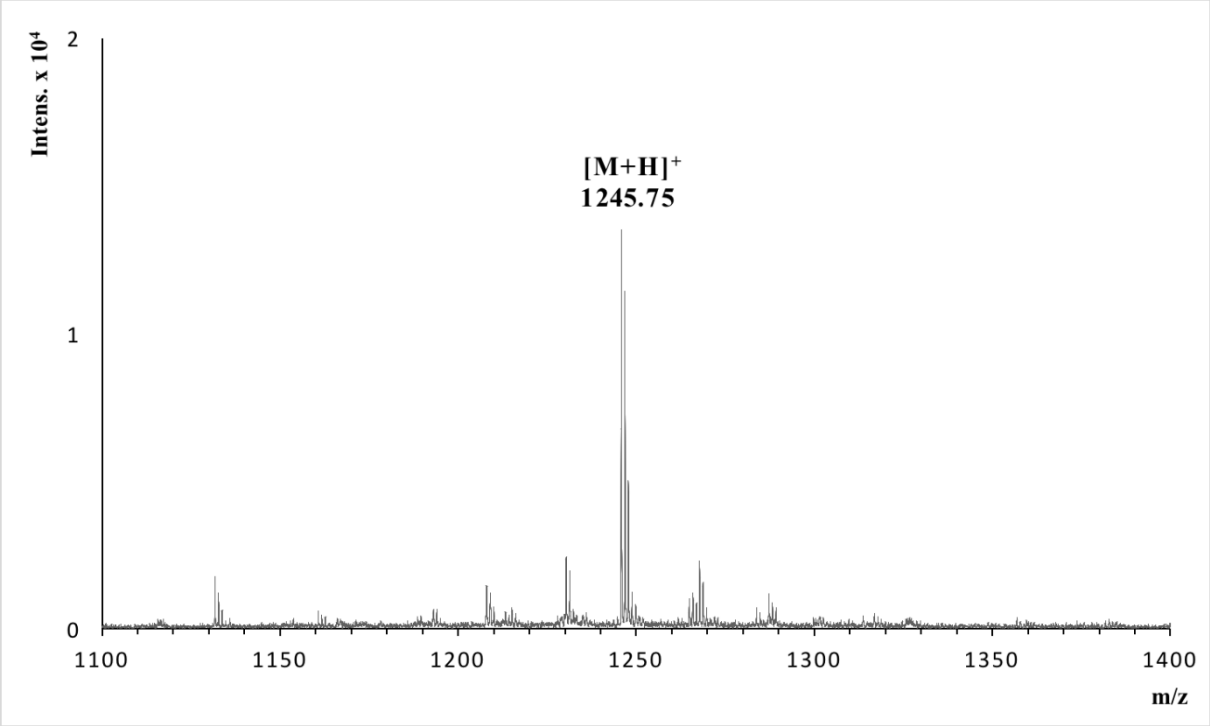


Supplementary figure S27. *HPLC chromatogram of peptide* ***P3L₂*** *upon purification by analytic HPLC (C18, 250 x 4 mm; ACN/H_2O 5:95 + 0.1 % TFA → ACN/H_2O 95:5 + 0.1 % TFA) with a retention time of 5.6 min.*

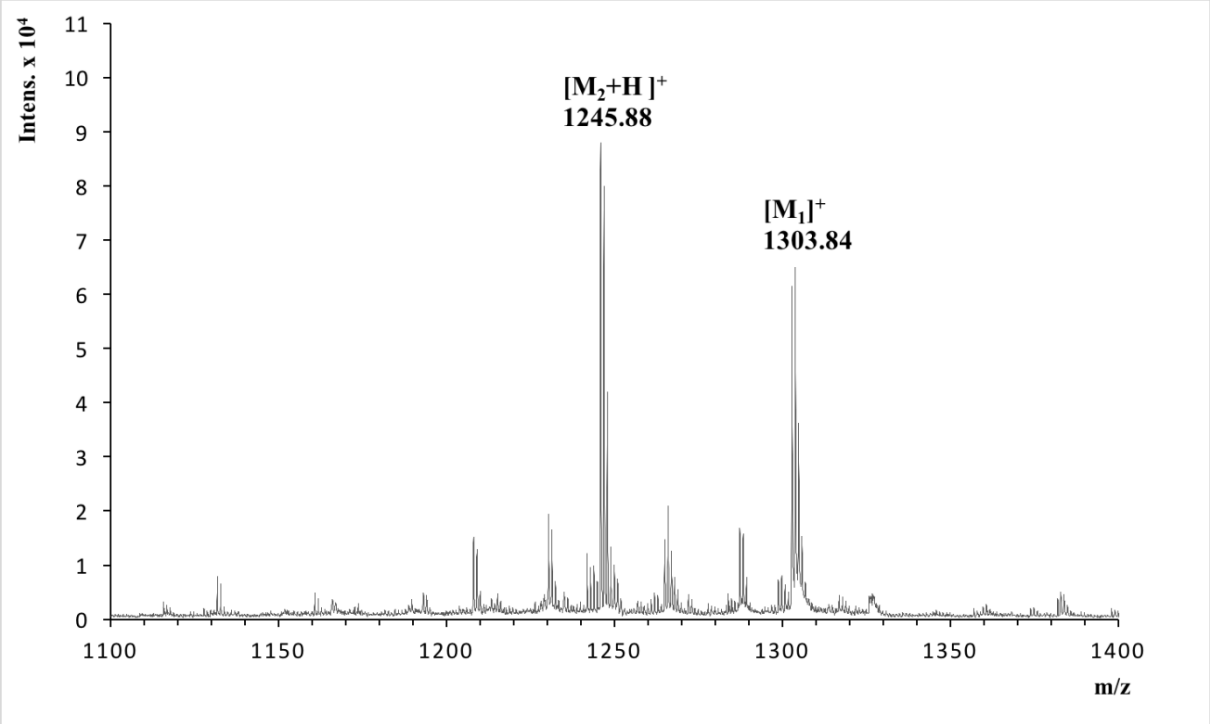


Supplementary figure S28. *HPLC chromatogram of peptide* ***P4L₂*** *upon purification by analytic HPLC (C18, 250 x 4 mm; ACN/H_2O 5:95 + 0.1 % TFA → ACN/H_2O 95:5 + 0.1 % TFA) with a retention time of 5.4 min.*

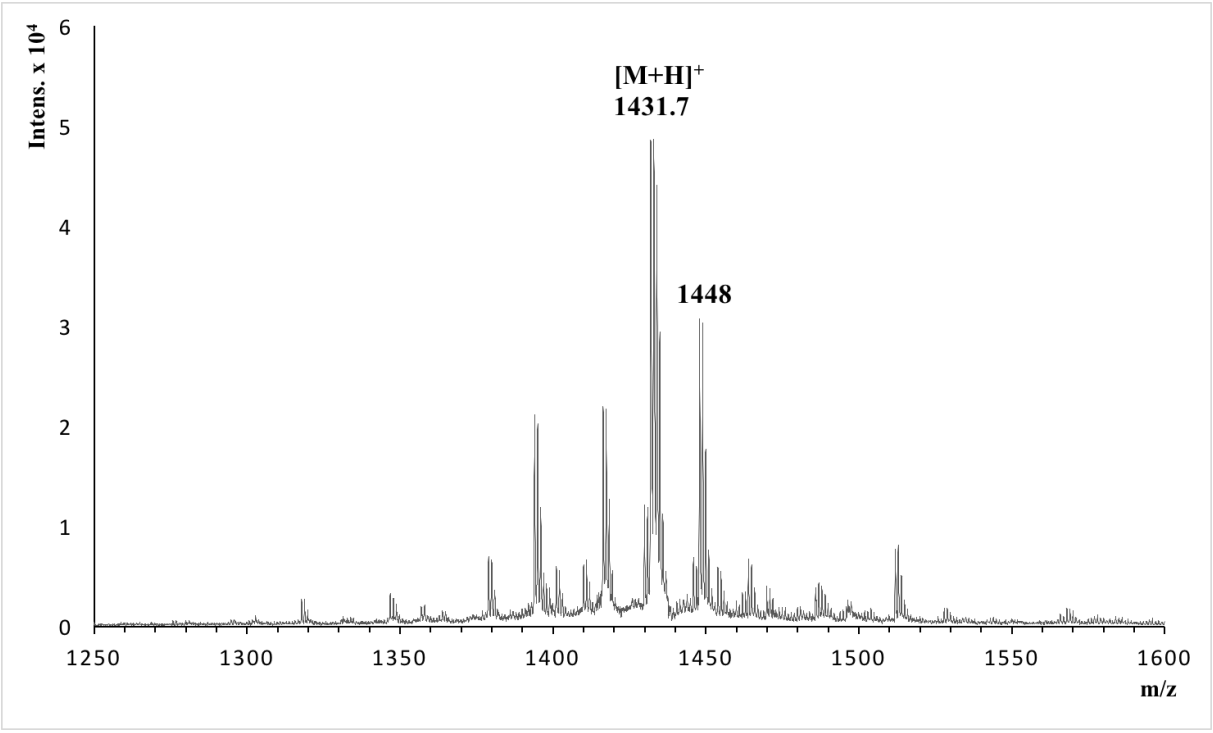
8. Mass Spectra



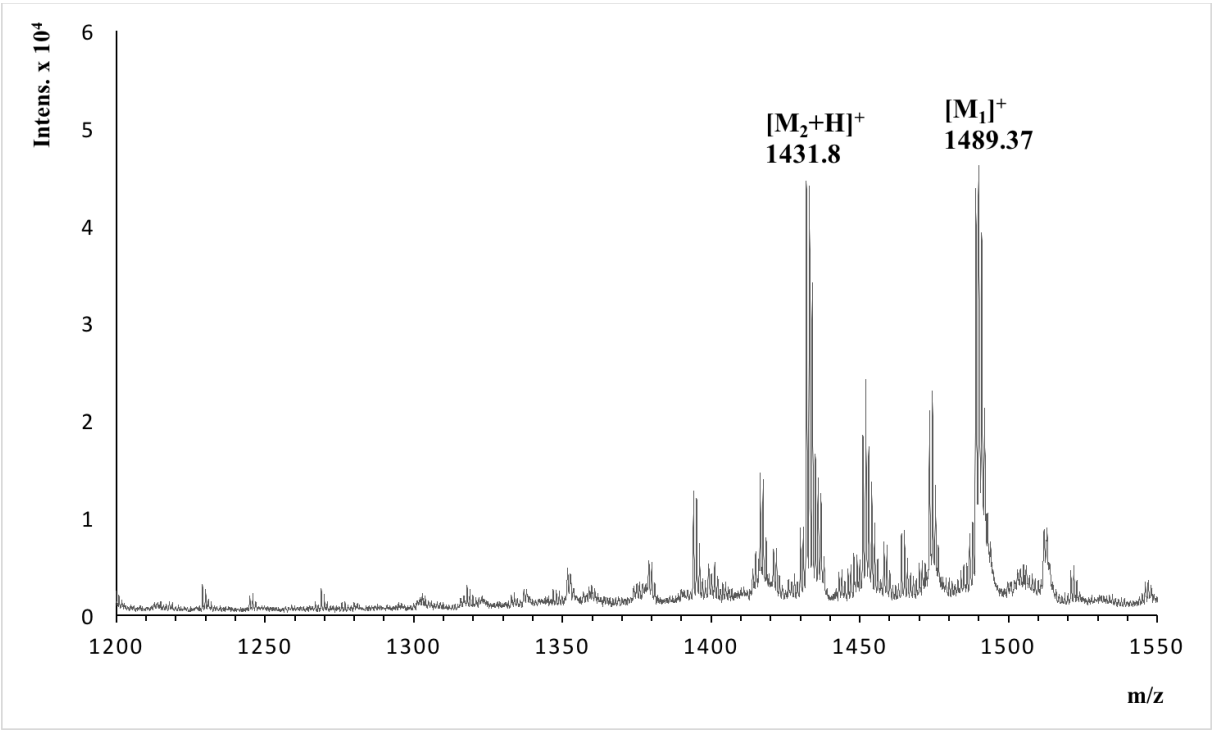
Supplementary figure S29. *MALDI TOF spectrum of peptide* ***P1L$_1$****.*



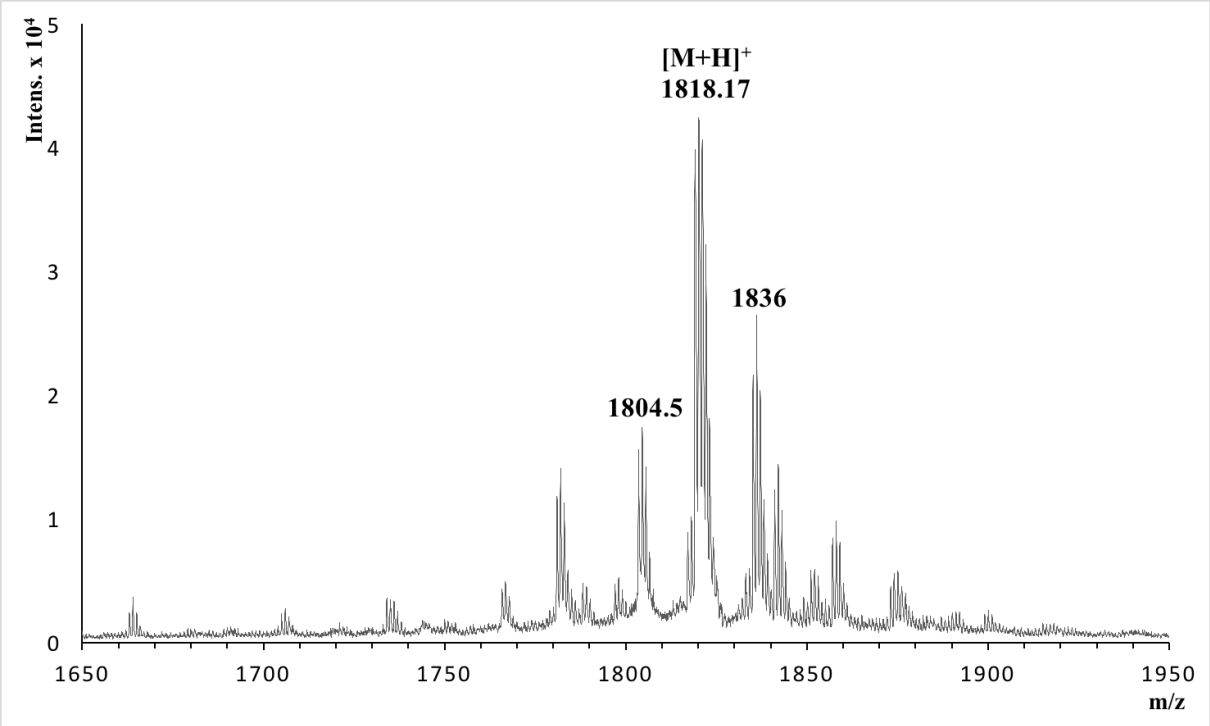
Supplementary figure S30. *MALDI TOF spectrum of cobalt-peptide conjugate* ***[Co^{III}(P1L$_1$)$_2$]$^{3+}$*** *(M_1=mass of* ***Co^{III}(P1L$_1$)****; M_2= mass of* ***P1L$_1$****).*



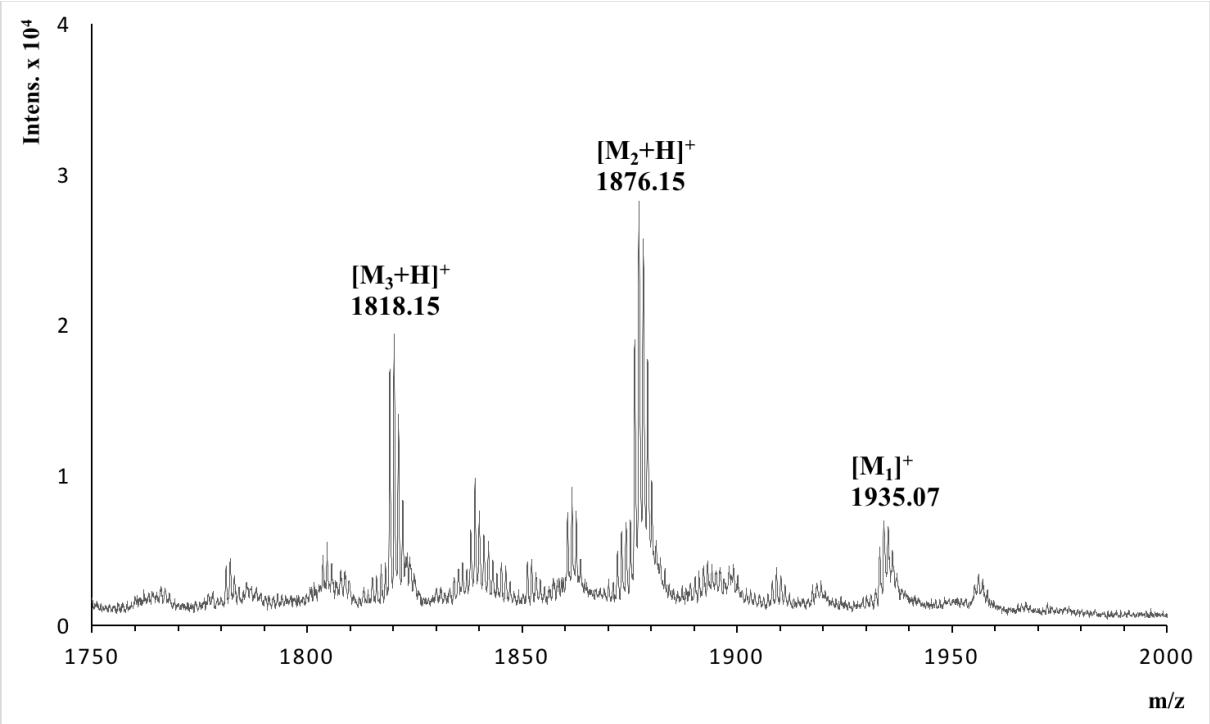
Supplementary figure S31**. MALDI TOF spectrum of peptide **P2L$_1$.



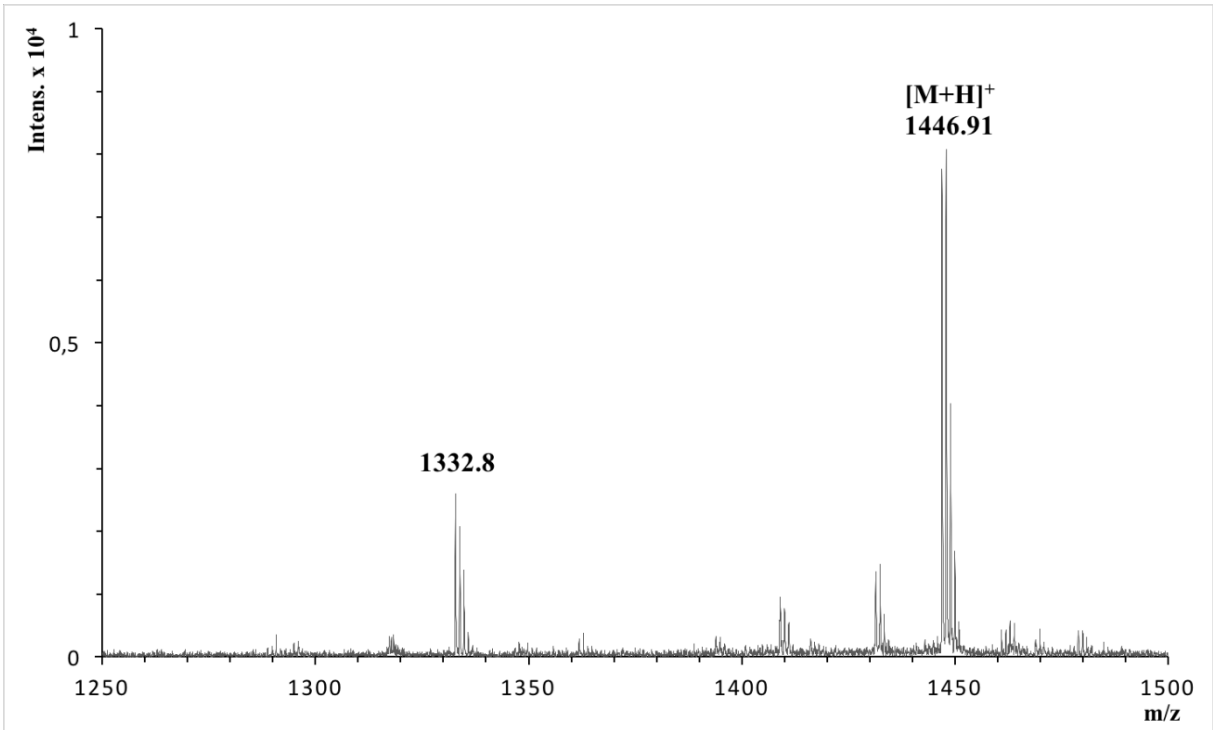
***Supplementary figure S32.** MALDI TOF spectrum of cobalt-peptide conjugate **[Co^{III}(P2L$_1$)$_2$]$^{3+}$** (M$_1$=mass of **Co^{III}(P2L$_1$)**; M$_2$= mass of **P2L$_1$**).*



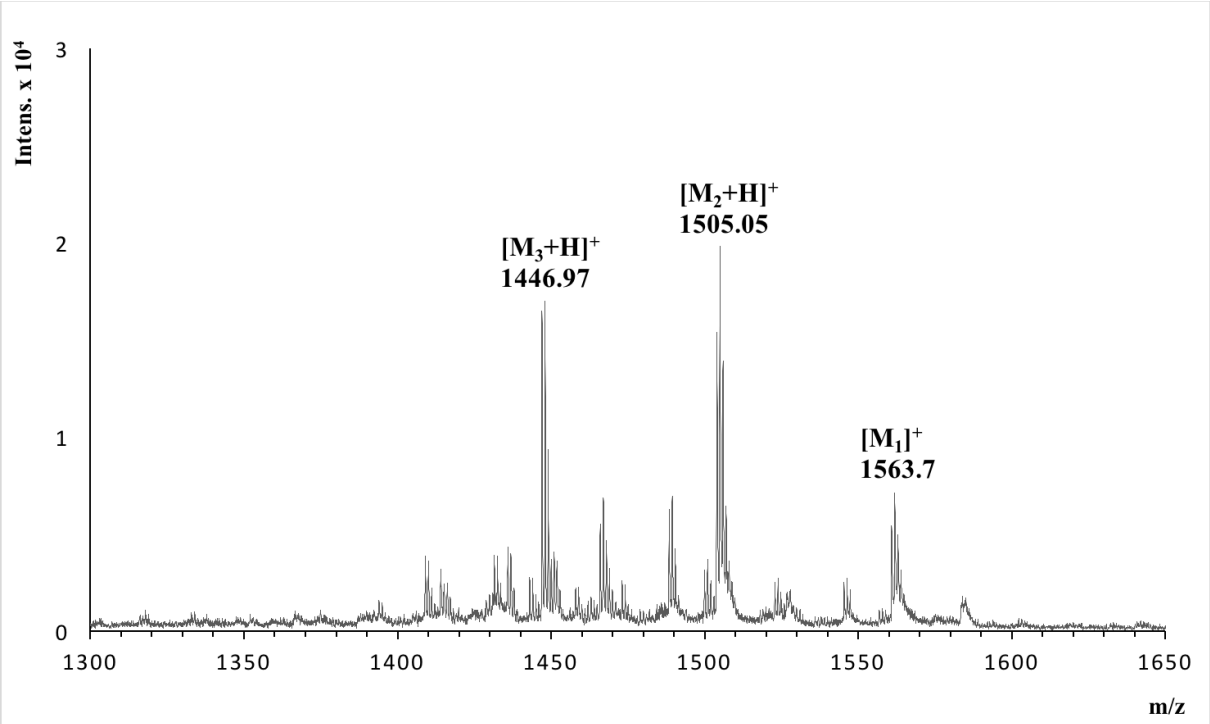
Supplementary figure S33. *MALDI TOF spectrum of peptide* ***P3L₂.***



Supplementary figure S34. *MALDI TOF spectrum of cobalt-peptide conjugate* ***$[Co_2^{III}(P3L_2)_2]^{6+}$*** *($M_1$=mass of* ***$Co_2^{III}(P3L_2)$****; M_2= mass of* ***$Co^{III}(P3L_2)$****; M_3=mass of* ***$P3L_2$****).*



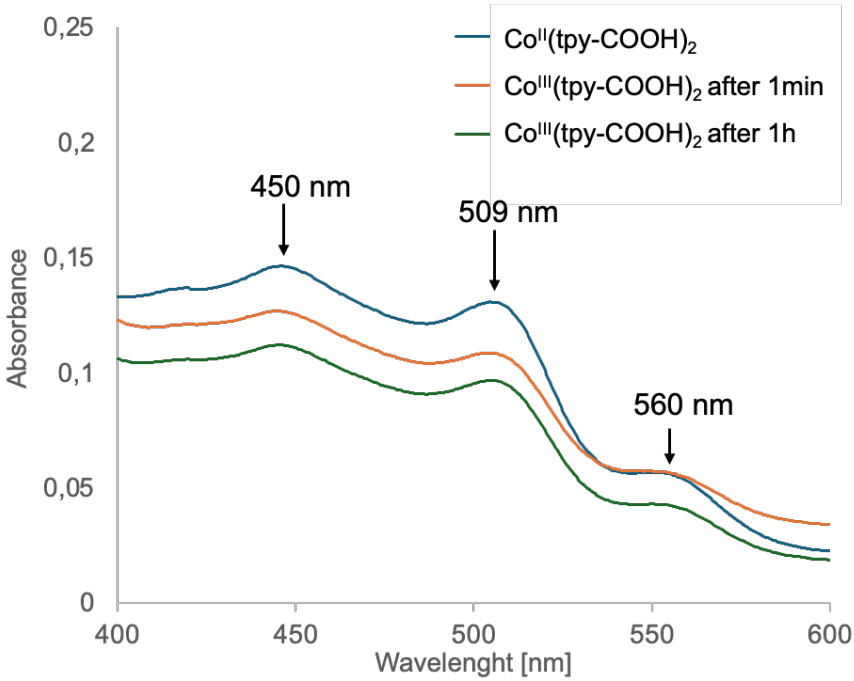
Supplementary figure S35. *MALDI TOF spectrum of peptide* ***P4L₂.***



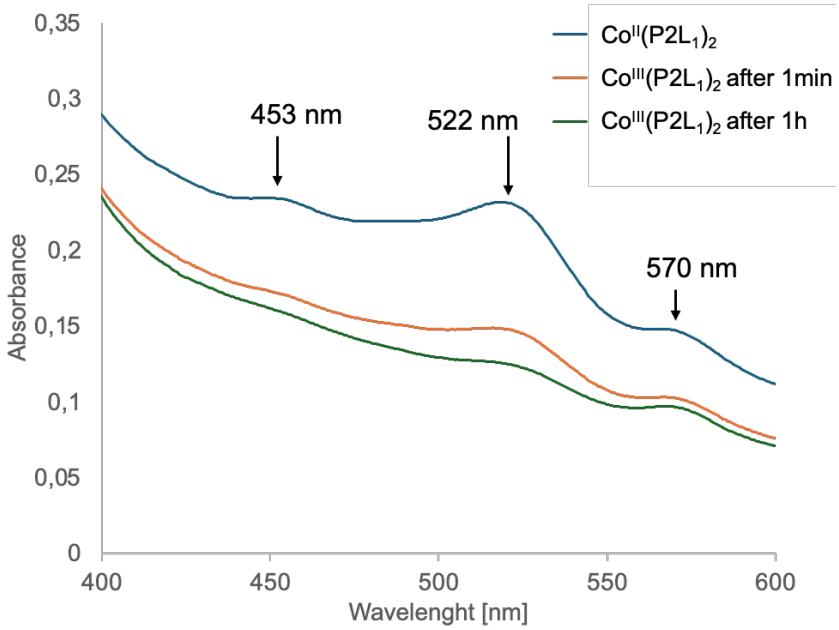
Supplementary figure S36. *MALDI TOF spectrum of cobalt-peptide conjugate* ***$[Co_2^{III}(P4L_2)_2]^{6+}$*** *($M_1$=mass of* ***$Co_2^{III}(P4L_2)$****; M_2= mass of* ***$Co^{III}(P4L_2)$****; M_3=mass of* ***$P4L_2$****).*

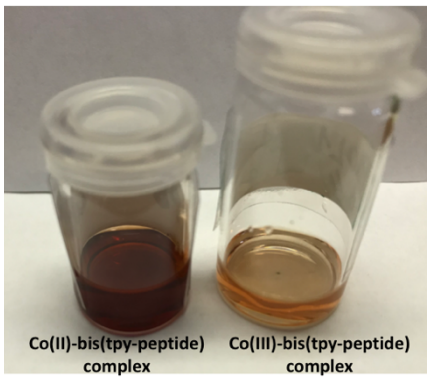
9. UV/Vis Spectra

The visible spectra for the oxidation of Co^{II} complexes to Co^{III} complexes were recorded in water. The stock solution of Co^{II} complexes was prepared in DMSO. The oxidation was carried out by adding aq.H_2O_2 (30%) to the aqueous solution of Co^{II} complex in a 10 mm QS cuvette and the visible spectra were recorded after 1 minute and 1 hour after oxidation.



Supplementary figure S37. *Visible spectra for $Co^{II}(tpy\text{-}COOH)_2$ and $Co^{III}(tpy\text{-}COOH)_2$: before oxidation (blue) and 1 min after oxidation by addition of aq.H_2O_2 (orange), and 1h after oxidation (green). Absorption maxima are shown in the spectra.*





Supplementary figure S38. *Visible spectra for $Co^{II}(P2L_1)_2$ and $Co^{III}(P2L_1)_2$: before oxidation (blue) and 1 min after oxidation by addition of aq. H_2O_2 (orange), and 1h after oxidation (green). Absorption maxima are shown in the spectra.*