Supporting Information

Modified Biovectors for the Tuneable Activation of Anti-platelet Carbon Monoxide Release

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Contents

Materials and Methods

Materials: Chemicals were of reagent grade quality or better, obtained from commercial suppliers and used without further purification. Vitamin B_{12} was a generous gift from DSM Nutritional products AG (Basel/Switzerland) and Prof. B. Jaun (retired ETH Zurich). All solvents were of reagent, analytical, HPLC or LC-MS grade respectively and obtained from commercial suppliers. Bidistilled water was used in all reactions.

Analytical HPLC: Spectra were recorded on an Acquity Waters system equipped with a PDA detector and an autosampler using a Nucleosil C18 250/3 column from Macherey-Nagel. A gradient (0-5 min 25% A; 5-30 min 25-100% A) of methanol (solvent A) versus bidistilled water containing 0.1% trifluoroacetic acid (solvent B) was applied using a flow rate of 0.3 mL/min.

Preparative HPLC: Separations were conducted on a *VWR LaPrep* system equipped with a PDA detector and a Nucleosil C18 250/40 column from Macherey-Nagel. A gradient (0-3 min 5% C; 3-3.20 min 25% C; 3.20-30 min 25-33% C) of acetonitrile (solvent C) versus bidistilled water containing 0.1% trifluoroacetic acid (solvent B) was applied using a flow rate of 40 mL/min.

ESI-MS: Spectra were recorded on a Bruker Daltonics HTC ESI-MS operated in the positive or negative mode. Injection rate 3 µL/min. Nebulizer P =10 psi, dry gas flow rate 5 L/min, gas T = 350 ºC. All solvents used were of LCMS grade.

ICP/OES: Inductively coupled plasma/optical emission spectrometry (ICP/OES) measurements were performed on a Perkin Elmer Optima 7300 V HF ICP-OES Spectrometer.

HR-ESI-MS: Spectra were recorded on a Bruker maXis QTOF-MS instrument (Bruker Daltonics GmbH, Bremen, Germany). The samples were dissolved in MeOH and analyzed via continuous flow injection at 3 µL/min. The mass spectrometer was operated in positive ion mode with a capillary voltage of 4 kV, an endplate offset of –500 V, nebulizer pressure of 5.8 psig, and a drying gas flow rate of 4 L/min at 180°C. The instrument was calibrated with a sodium formate solution (500µl H_2O: 500µl iPrOH: 20µl HCOOH: 20µl 0.1 M $NaOH_{aq}$). The

resolution was optimized at 30'000 FWHM in the active focus mode. The accuracy was better than 2 ppm in a mass range between *m/z* 118 and 1600.

Spectroscopy: UV-Vis spectra were recorded on a *Varian Cary 50* using quartz cells with a path length of 1 cm. Citation of λ_{max} (log ε) in nm. For platelet studies, spectra were recorded on a Perkin Elmer double beam spectrophotometer Lambda 950 using quartz cells with a 1 cm path length. The kinetics of carbon monoxide release from CO donor compounds were studied by recording spectra at the Soret band region (λ_{max} = 424 nm) and observing the conversion from deoxyMb to MbCO at room temperature. Solutions of Mb (Myoglobin from equine heart, SIGMA) were prepared fresh by dissolving the protein in deionized water in such amount to obtain final concentrations 10 or 100 μM. Sodium dithionite (1 mg/ml, eq. 0.1%) was added just before measurements to convert myoglobin to deoxyMb. Before every experiment the reference spectra of deoxygenated protein were recorded. Solutions of CO donor compounds dissolved in DMSO:PBS (50:50) were added to the quartz cuvettes with deoxyMb in such amounts to give final concentrations of 10 or 100 μM. All solutions were always overlaid with mineral oil (0.5 cm^3) to prevent CO escaping and myoglobin being oxygenated. ^{1}H- and ^{13}C-NMR as well as 2D-NMR spectra were recorded on a 500 MHz *Oxford NMR AS 500* using a QNP probe head and *MestReNova 6.0.2* as evaluation tool. All spectra were recorded in D_2O at 300 K and TSP was used as a reference for all ^{13}C-NMR experiments.

Cyclic voltammetry: Cyclic voltammograms were obtained on a Metrohm 757 VA Computrace System. Measurements were performed using a glassy carbon electrode (working electrode) and an Ag/AgCl electrode (reference electrode). Samples were dissolved in 2 mL of 0.1 M TRIS buffered at pH 8. Hexacyanoferrate (0.5 mM) was used as an internal reference. Samples were purged with N_2 (g) for 5 minutes prior to each measurement. The measurement was accepted if $E_{K3[Fe(CN)6]}$ was found between +179 and +186 mV.

Solid Phase Extraction: Chromafix C18ec columns were applied for solid phase extraction (SPE). The compounds were dissolved in water, transferred to the adsorbent, washed with water and eluted with MeOH.

Experimental procedures

Cyanocobalamin-c-lactone (2). The synthesis was performed according to a procedure reported by Bonnet et al.[S1] Vitamin B_{12} (100 mg 74 μmol, 1 equiv) was dissolved in 1 M HCl (10 mL) and H_2O (100 mL). Chloramin-T (25.2 mg, 110 μmol 1.5 equiv) was dissolved in H_2O (50 mL) and was added drop wise over a period of 60 minutes. The pink solution was desalted over Amberlite XAD-2. Purification by preparative HPLC and lyophilization afforded **2** in good yields (82 mg, 60.6 μmol, 82%) **UV-Vis** (c = $1.66 \cdot 10^{-5}$ M) λ/nm (log ε) = 278 (4.0), 289 (3.9), 306 (4.1), 359 (4.5), 409 (3.4), 523 (3.7), 551 (3.7). **HPLC** t_R = 14.9 min. **ESI-MS** (H_2O:MeOH 1:1) m/z = 677.9 [100%; $M+2H]^{2+}$, 1354.5 [95%; $M+H]^+$ (m/z_{calc} for $C_{63}H_{86}BrCoN_{13}O_{15}P$: 1354.5). **CV** (0.1 M TRIS pH 8, $K_3[Fe(CN)_6]$) E_{red} = -929 mV. **^{1}H NMR** (D_2O; 500 MHz; 300K) δ/ppm = 7.32 (s, 1H), 7.12 (s, 1H), 6.44 (s, 1H), 6.37 (d, J = 3.2 Hz, 1H), 6.09 (s, 1H), 4.29 (m, 2H), 4.20 (dd, J = 8.9, 1.9 Hz, 1H), 4.09 (m, 2H), 3.93 (dd, J = 12.9, 2.4 Hz, 1H), 3.75 (dd, J = 12.9, 4.0 Hz, 1H), 3.61 (d, J = 14.4 Hz, 1H), 3.38 (d, J = 9.8 Hz, 1H), 3.30 – 3.26 (m, 1H), 2.99 – 2.92 (m, 2H), 2.85-1.15 (m, 35H), 2.61 (s, 3H), 2.57 (s, 3H), 2.29 (s, 3H), 2.27 (s, 3H), 1.93 (s, 3H), 1.49 (s, 3H), 1.42 (s, 3H), 1.38 (s, 3H), 1.26 (d, J = 6.3 Hz, 3H), 1.17 (s, 3H), 0.50 (s, 3H).

10-bromo-cyanocobalamin (3). The synthesis was performed by a modified procedure of Wagner.[S2] Vitamin B_{12} (100 mg, 74 μmol, 1 equiv) was dissolved in glacial AcOH (3 ml) and NBS (13 mg, 74 μmol, 1 equiv) was added in small portions (~0.5 mg) over a period of 3 h. The resulting dark purple solution was desalted with solid phase extraction (SPE) and the solvents were removed under reduced pressure. Purification by preparative HPLC and lyophilization afforded **3** in quantitative yields (105 mg, 73 μmol, 99 %) as a purple powder. **UV-vis** (c = $6.05 \cdot 10^{-5}$ M; H_2O) λ/nm (log ε) = 283 (3.9), 289 (3.9), 365 (4.3), 415 (3.3), 550 (3.7), 576 (3.7). **HPLC** t_R = 16.5 min. **ESI-MS** (H_2O:MeOH 1:1) m/z = 718.2 [100%; $M+2H]^{2+}$, 1435.5 [70%; $M+H]^+$ (m/z_{calc} for $C_{63}H_{88}BrCoN_{14}O_{14}P$: 1435.48); **HR-ESI-MS** (MeOH, NaI) m/z = 740.2279 [100%; $M+2Na]^{2+}$ (m/z_{calc} for $C_{63}H_{87}BrCoN_{14}O_{14}PNa_2$: 740.2281). **CV** (0.1 M TRIS pH 8, $K_3[Fe(CN)_6]$) E_{red} = -798 mV. **^{1}H NMR** (500 MHz, D_2O) δ/ppm = 7.31 (s, 1H), 7.13 (s, 1H), 6.52 (s, 1H), 6.37 (d, *J* = 3.1 Hz, 1H), 4.43 – 4.39 (m, 1H), 4.33 – 4.31 (m, 2H), 4.25 (d, *J* = 8.4 Hz, 1H), 4.07 (t, *J* = 8.6 Hz, 3H), 3.96 – 3.92 (m, 1H), 3.78 (dd, *J* = 12.9, 3.8 Hz, 2H), 3.69 – 3.56 (m, 3H), 3.40 (d, *J* = 8.8 Hz, 1H), 2.99 (dd, *J* = 14.4, 9.3 Hz, 1H), 2.80-1.10 ppm (m, 36H), 2.61 (s, 3H), 2.58 (s, 3H), 2.29 (s, 3H), 2.28 (s,

3H), 1.93 (s, 3H), 1.83 (s, 3H), 1.41 (s, 3H), 1.39 (s, 3H), 1.34 (s, 3H), 1.28 (d, *J* = 6.2 Hz, 3H), 0.39 (s, 3H).

10-chloro-cyanocobalamin (4). To vitamin B_{12} (100 mg, 74 μmol, 1 equiv) dissolved in glacial AcOH (3 ml) NCS (10 mg, 73 μmol, 1.0 equiv) was added over a period of 3 h. The resulting dark purple solution was desalted with solid phase extraction (SPE) and the solvents were removed under vacuum. Purification by preparative HPLC and lyophilization afforded **4** (75 mg, 55 μmol, 75%) as a purple powder. **UV-vis** (c = $4.75 \cdot 10^{-5}$M; H_2O) λ/nm (log ε) = 282 (3.9), 289 (3.9), 364 (4.2), 408 (3.3), 551 (3.6), 574 (3.7); **HPLC** t_R = 16.0 min; **ESI-MS** (H_2O:MeOH 1:1) m/z = 695.3 [100%; $M+2H]^{2+}$, 1389.5 [70%; $M+H]^+$ (m/z_{calc} for $C_{63}H_{88}ClCoN_{14}O_{14}P$: 1389.53); **HR-ESI-MS** (MeOH, NaI) m/z = 717.2531 [100%; $M+2Na]^{2+}$ (m/z_{calc} for $C_{63}H_{87}ClCoN_{14}O_{14}PNa_2$: 717.2534).). **CV** (0.1 M TRIS pH 8, $K_3[Fe(CN)_6]$ E_{red}= -810 mV . **^{1}H NMR** (500 MHz, D_2O) δ/ppm = 0.40 (s, 3H), 1.28 (d, *J* = 6.3 Hz, 3H), 1.36 (s, 3H), 1.39 (s, 3H), 1.42 (s, 3H), 1.79 (s, 3H), 1.93 (s, 3H), 2.27 (s, 3H), 2.29 (s, 3H), 2.58 (s, 3H), 2.61 (s, 3H), overlapped by 1.09-2.80 (m, 38H), 3.00 (dd, *J* = 14.4, 9.2 Hz, 1H), 3.39 (d, *J* = 9.1 Hz, 1H), 3.65 (d, *J* = 14.3 Hz, 1H), 3.79 (dd, *J* = 12.9, 3.7 Hz, 1H), 3.94 (d, *J* = 10.6 Hz, 1H), 4.06 – 4.11 (m, 2H), 4.24 (dd, *J* = 9.3, 5.9 Hz, 3H), 4.30 – 4.35 (m, 2H), 6.38 (d, *J* = 3.0 Hz, 1H), 6.51 (s, 1H), 7.11 (s, 1H), 7.32 (s, 1H).

10-chloro-cyanocobalamin-c-lactone (5). Vitamin B_{12} (200 mg, 148 μmol, 1 equiv) was dissolved in glacial acetic acid (3 mL) and NCS (20 mg, 150 μmol, 1.0 equiv) was added over a period of 3 h. The resulting dark purple solution was purified with solid phase extraction (SPE) and preparative HPLC. Solvents were removed under vacuum. **5** (14 mg, 10 μmol, 7%) was obtained as a purple powder. **UV-vis** (c = $1.80 \cdot 10^{-5}$M; H_2O) λ/nm (log ε) = 281 (3.2), 289 (3.2), 363 (4.6), 421 (3.4), 551 (3.6), 577 (3.6); **HPLC** t_R = 17.45 min; **ESI-MS** (H_2O:MeOH 1:1) m/z = 694.7 [100%; $M+2H]^{2+}$, 1388.2 [70%; $M+H]^+$ (m/z_{calc} for $C_{63}H_{85}CoN_{13}O_{15}PCl$: 1388.50); **HR-ESI-MS** (MeOH NaI): m/z = 694.75596 $[M+2H]^{2+}$ (m/z_{calc} for $C_{63}H_{85}CoN_{13}O_{15}PCl$: 694.75568) **CV** (0.1 M TRIS buffer at pH 8, $K_3[Fe(CN)_6]$ E_{red}= -784 mV; **^{1}H NMR** (500 MHz, CD_3OD, TMS) δ/ppm = 0.40 (s, 3H), 1.25 (d, 3H), 1.29 (s, 3H), 1.79 (s, 3H), 2.00 (s, 3H), 2.29 (s, 3H), 2.31 (s, 3H), 2.62 (s, 3H), 2.63 (s, 3H), overlapped by 1.79-2.87 (m, ~46H), 3.39 (d, *J* = 9.4, 1H), 3.67 (d, *J* = 14.0 Hz, 1H), 3.76-3.92 (m, 1H), 4.08 (m, 1H), 4.20 (m, 1H), 4.54 (m, 1H), 4.62 (m, 1H), 6.32 (d, *J* = 2.9 Hz, 1H), 6.46 (s, 1H), 7.14 (s, 1H), 7.34 (s, 1H).

c-(α,α-dibromo)-lactone-cyanocobalamin (6): The synthesis was performed according to recent literature procedures.[S3]

c-(α,α-dichloro)-lactone-cyanocobalamin (7): To vitamin B_{12} (100 mg, 74 µmol, 1 equiv) dissolved in glacial AcOH (3 ml), NCS (99 mg, 744 µmol, 10 equiv) was added in one portion. The dark red solution was stirred at rt for 1 h. The resulting dark violet solution was desalted with solid phase extraction (SPE) and the solvents were removed under vacuum. Purification by preparative HPLC and lyophilization afforded **3** (58 mg, 41 µmol, 55%) as a violet powder. **UV-vis** (c = $4.21 \cdot 10^{-5}$M; H_2O) λ/nm (log ε) = 279 (4.0), 288sh (3.9), 310 (3.8), 363 (4.2), 412 (3.3), 531 (3.7), 554 (3.7); **HPLC** (Method 1) t_R = 19.0 min; **ESI-MS** (H_2O:MeOH 1:1) m/z = 712.7 [100%; $M+2H]^{2+}$, 1424.4 [85%; $M+H]^+$ (m/z_{calc} for $C_{63}H_{84}CoN_{13}O_{15}PCl_2$: 1424.4); **HR-ESI-MS** (MeOH, NaI): m/z = 711.73666 (m/z_{calc} for $C_{63}H_{85}CoN_{13}O_{15}PCl_2$: 711.73619) **CV** (0.1 M TRIS pH 8, $K_3[Fe(CN)_6]$ E_{red}= -715 mV; **^{1}H-NMR** (500 MHz, 300 K, D_2O) δ/ppm = 0.54 (s, 3H), 1.16 (s, 3H), 1.26 (d, $J_{H,H}$ = 6.5 Hz, 3H), 1.41 (s, 3H), 1.47 (s, 3H), 1.54 (s, 3H), 2.10 (s, 3H), 2.29 (s, 3H), 2.33 (s, 3H), 2.63 (s, 3H), 2.74 (s, 3H), overlapped by 1.21-2.80 (m, ~36H), 2.96-3.00 (m, 1H), 3.39 (t, $J_{H,H}$ = 5.5 Hz, 1H), 3.61 (d, $J_{H,H}$ = 14.5 Hz, 1H), 3.76 (dd, $J_{H,H}$ = 4.0 Hz, 13.0 Hz, 1H), 3.94 (dd, $J_{H,H}$ = 2.5 Hz, 13.0 Hz, 1H), 4.07-4.09 (m, 1H), 4.18 (d, $J_{H,H}$ = 8.5 Hz, 1H), 4.23 (d, $J_{H,H}$ = 9.5 Hz, 1H), 4.29-4.35 (m, 2H), 6.12 (s, 1H), 6.39 (d, $J_{H,H}$ = 4.5 Hz, 1H), 7.10 (s, 1H), 7.34 (s, 1H). **^{13}C-NMR** (126 MHz, 300 K, CD_3OD) δ/ppm= 182.2,181.0,180.0, 177.6, 177.4, 175.5, 175.3, 175.3, 174.5, 167.5, 166.6, 166.3, 158.6, 143.4, 132.2, 134.3, 131.6, 127.1, 116.8, 113.1, 110.8, 107.7, 93.3., 92.2, 88.2, 86.7, 84.9, 76.6, 75.2, 73.6, 70.5, 65.2, 62.3, 60.6, 57.6, 55.0, 50.3, 46.7, 43.0, 40.1, 40.1, 36.1, 35.3, 32.2, 31.9, 31.8, 31.6, 30.1, 29.7, 29.5, 27.5, 25.00, 24.00, 21.1, 20.6, 20.3, 20.1, 19.2, 17.6, 17.5, 16.7, 16.3.

Cyanocobalamin-µ-CN-[$Re(CO)_2Br_2(CH_3OH)$] (8). The synthesis was performed by modifications of a reported procedure.[S4] Vitamin B_{12} (100 mg, 74 µmol, 1 equiv) and **1** (114 mg, 164 µmol, 2.2 equiv) were stirred in MeOH (60 mL) at 50 ºC for 90 minutes. The solvent was subsequently removed and the resulting red powder was washed several times with dichloromethane (20 mL) and acetone (20 mL). **8** (105.9 mg, 59 µmol, 80%) was obtained as a red microcrystalline powder. **UV-vis** (c = $1.81 \cdot 10^{-4}$ M; H_2O) λ/nm (log ε) = 276 (3.9), 323 (3.9), 361 (4.3), 407 (3.3), 518 (3.7), 547 (3.7); **ESI-MS** (H_2O:MeOH 1:1) m/z = 865.6 [100%; $M+2H]^{2+}$, 1757.7 [70%; $M+H]^+$ (m/z_{calc} for $C_{63}H_{88}CoN_{14}O_{14}PReC_2O_2Br_2$: 1755.35).

IR (solid state, KBr, cm^{-1}) $\nu_{C\equiv N}$ = 2184, $\nu_{C\equiv O}$ =1989, 1839. **ICP/OES** measurements of Re content by relative weight: calcd 10.41; found 9.88 ± 0.05.

Cyanocobalamin-c-lactone-μ-CN-[Re(CO)$_2$Br$_2$(CH$_3$OH)] (9). 2 (15.9 mg, 12 μmol, 1 equiv) and **1** (20 mg, 29 μmol, 2.4 equiv) were dissolved in methanol (10 mL) and stirred at 50ºC for 90 minutes. The solvent was subsequently removed and the resulting red powder was washed several times with dichloromethane (20 mL) and acetone (20 mL). **9** was obtained as a red microcrystalline powder (16.5 mg, 9 μmol, 75%). **UV-vis** (c = $1.81 \cdot 10^{-4}$ M; H_2O) λ/nm (log ε) = 276 (4.0), 323 (3.7), 361 (4.3), 407 (3.4), 518 (3.6), 547 (3.6); **HR-ESI-MS** (H_2O:MeOH 1:1) m/z = 894.3 [100%; M+2H]$^{2+}$, 1757.3235 [100%; M+H]$^+$ (m/z_{calc} for $C_{63}H_{86}CoN_{13}O_{15}PReC_2O_2Br_2$: 1757.3236). **IR** (solid state, KBr, cm^{-1}) $\nu_{C\equiv N}$ 2190, $\nu_{C\equiv O}$ 1989, 1841, 1789 ($\nu_{C=O}$ lactone). **ICP/OES** measurements of Re content by relative weight: calcd 10.41; found 9.89 ± 0.32.

10-bromo-cyanocobalamin-μ-CN-[Re(CO)$_2$Br$_2$(CH$_3$OH)] (10). 3 (8 mg, 5.5 μmol, 1 equiv) and **1** (10 mg, 14 μmol, 2.5 equiv) were dissolved in methanol (7 ml) and stirred for 30 minutes at 50 ºC. The solvent was subsequently removed and the resulting red powder was washed several times with dichloromethane (20 mL) and acetone (20 mL). **10** was obtained as a dark red microcrystalline powder (7.5 mg, 4 μmol, 66%). **UV-vis** (c = $1.89 \cdot 10^{-4}$ M; H_2O) λ/nm (log ε) = 279 (3.9), 366 (4.3), 411 (3.3), 550 (3.6), 574 (3.6); **HR-ESI-MS** (H_2O:MeOH 1:1) m/z = 1836.2687 [100%; M+H]$^+$ (m/z_{calc} for $C_{63}H_{88}CoN_{14}O_{14}PReC_2O_2Br_3$: 1836.2647). **IR** (solid state, KBr, cm-1) $\nu_{C\equiv N}$ = 2176, $\nu_{C\equiv O}$ = 1992, 1845. **ICP/OES** measurements of Re content by relative weight: calcd 9.96; found 9.33 ± 0.12.

10-chloro-cyanocobalamin-μ-CN-[Re(CO)$_2$Br$_2$(CH$_3$OH)] (11). 4 (20 mg, 14 μmol, 1equiv) and **1** (34 mg, 48 μmol, 3.5 equiv) were dissolved in methanol (12 mL) and stirred for 30 minutes at 50 ºC. The solvent was subsequently removed and the resulting red powder was washed several times with dichloromethane (20 mL) and acetone (20 mL). **11** was obtained as a purple microcrystalline powder (10.5 mg, 6 μmol, yield 85%). **UV-vis** (c = $1.84 \cdot 10^{-4}$ M; H_2O) λ/nm (log ε) = 279 (3.9), 287 (3.9), 363 (4.2), 415 (2.3), 545 (3.6), 570 (3.6); **HR-ESI-MS** (H_2O:MeOH 1:1) m/z = 1792.3110 [100%; M+H]$^+$ (m/z_{calc} for $C_{63}H_{88}CoN_{14}O_{14}PClReC_2O_2Br_2$: 1792.3156). **IR** (solid state, KBr, cm^{-1}) $\nu_{C\equiv N}$ = 2178, $\nu_{C\equiv O}$

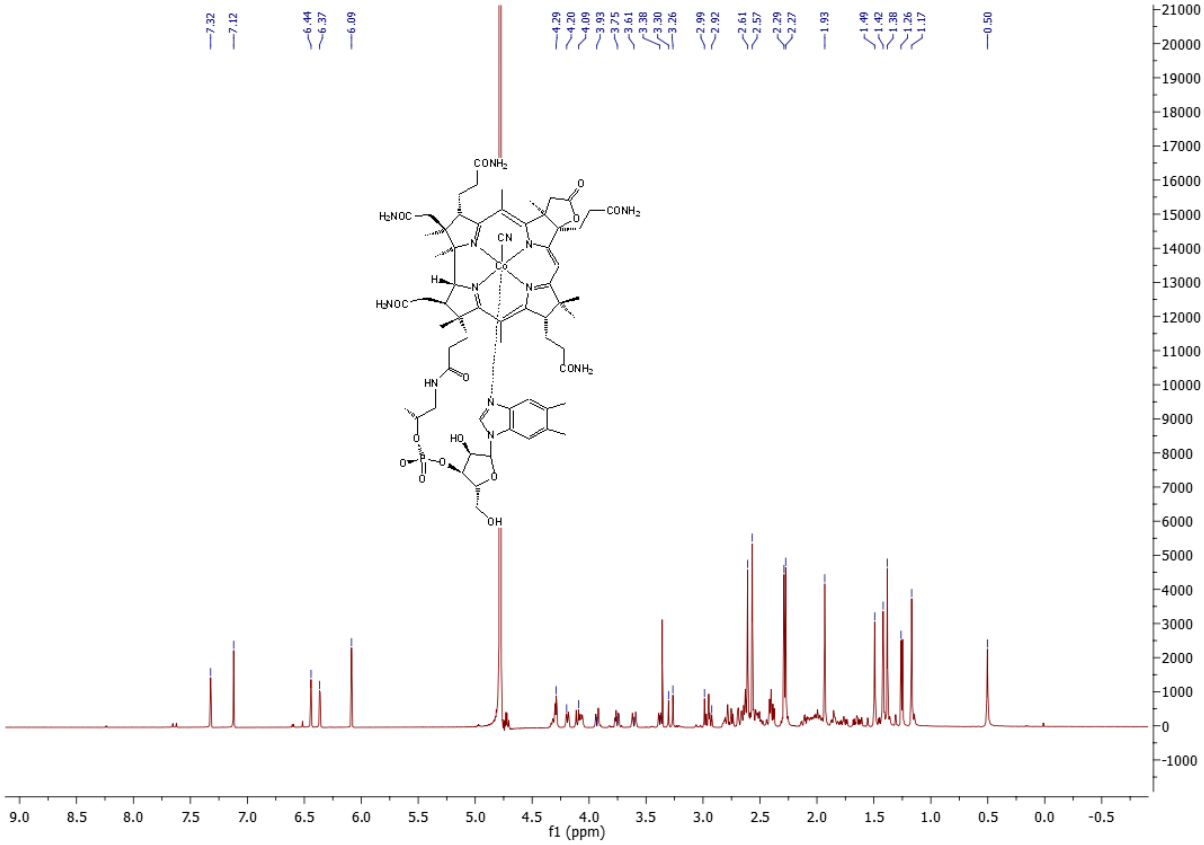
=1973, 1841. **ICP/OES** measurements of Re content by relative weight: calcd 10.20; found 9.79 ± 0.23.

10-chloro-cyanocobalamin-c-lactone-μ-CN-[Re(CO)$_2$Br$_2$(CH$_3$OH)] (12). 5 (6.5 mg, 5 μmol, 1 equiv) and **1** (8 mg, 11 μmol, 2.2 equiv) were dissolved in methanol (6 mL) and the mixture was stirred for 20 minutes at 50 ºC. The solvent was subsequently removed and the resulting red powder was washed several times with dichloromethane (15 mL) and acetone (15 mL). **12** was obtained as a dark purple microcrystalline powder (7.9 mg, 4 μmol, 80%). **UV-vis** (c = $1.84 \cdot 10^{-4}$ M; H_2O) λ/nm (log ε) = 277 (3.2), 285 (3.2), 362 (4.2), 417 (3.4), 545 (3.6), 570 (3.6); **ESI-MS** (H_2O:MeOH 1:1) m/z = 895.3 [100%; $M+2H]^{2+}$, 1790.6 [70%; $M+H]^+$ (m/z_{calc} for $C_{63}H_{86}ClCoN_{13}O_{15}PReC_2O_2Br_2$: 1790.29). **IR** (solid state, KBr, cm^{-1}) $\nu_{C\equiv N}$ = 2124, $\nu_{C\equiv O}$ = 1988, 1840, 1783. **ICP/OES** measurements of Re content by relative weight: calcd 10.20; found 9.75 ± 0.36.

c-(α,α-dibromo)-lactone-cyanocobalamin-μ-CN-[Re(CO)$_2$Br$_2$(CH$_3$OH)] (13). 6 (10.5 mg, 7 μmol, 1equiv) and **1** (12 mg, 17 μmol, 2.4 equiv) were dissolved in methanol (5 ml) and stirred for 30 minutes at 50 ºC. The solvent was subsequently removed and the resulting red powder was washed several times with dichloromethane (20 mL) and acetone (20 mL).**13** was obtained as a dark purple microcrystalline powder (10 mg, 5.2 μmol, 75%). **UV-vis** (c = $1.63 \cdot 10^{-4}$ M; H_2O) λ/nm (log ε) = 278 (4.1), 311 (3.9), 363 (4.2), 413 (3.7), 529 (3.6), 558 (3.6); **HR-ESI-MS** (H_2O:MeOH 1:1) m/z = 1915.1439 [100%; $M+H]^+$ (m/z_{calc} for $C_{63}H_{84}Br_2CoN_{13}O_{15}PReC_2O_2Br_2$: 1915.1426). **IR** (solid state, KBr, cm^{-1}) $\nu_{C\equiv N}$ = 2181, $\nu_{C\equiv O}$ = 1987, 1841, 1794. **ICP/OES** measurements of Re content by relative weight: calcd 9.56; found 9.14 ± 0.05.

c-(α,α-dichloro)-lactone-cyanocobalamin-μ-CN-[Re(CO)$_2$Br$_2$(CH$_3$OH)] (14). 7 (10 mg, 7 μmol, 1equiv) and **1** (12 mg, 17 μmol, 2.4 equiv) were dissolved in methanol (7 ml) and stirred for 25 minutes at 50 ºC. The solvent was subsequently removed and the resulting red powder was washed several times with dichloromethane (20 mL) and acetone (20 mL).**14** was obtained as a dark purple microcrystalline powder (10 mg, 5.2 μmol, 77%). **UV-vis** (c = $1.81 \cdot 10^{-4}$ M; H_2O) λ/nm (log ε) = 278 (3.6), 288 (3.5), 310 (3.4), 362 (4.2), 413 (3.1), 527 (3.6), 553 (3.6); **HR-ESI-MS** (H_2O:MeOH 1:1) m/z = 1825.2319 [100%; $M+H]^+$ (m/z_{calc} for $C_{63}H_{84}Cl_2CoN_{13}O_{15}PReC_2O_2Br_2$: 1825.2445). **IR** (solid state, KBr, cm^{-1}) $\nu_{C\equiv N}$ = 2185, $\nu_{C\equiv O}$ =

1988, 1838, 1808. **ICP/OES** measurements of Re content by relative weight: calcd 10.02; found 9.67 ± 0.10.



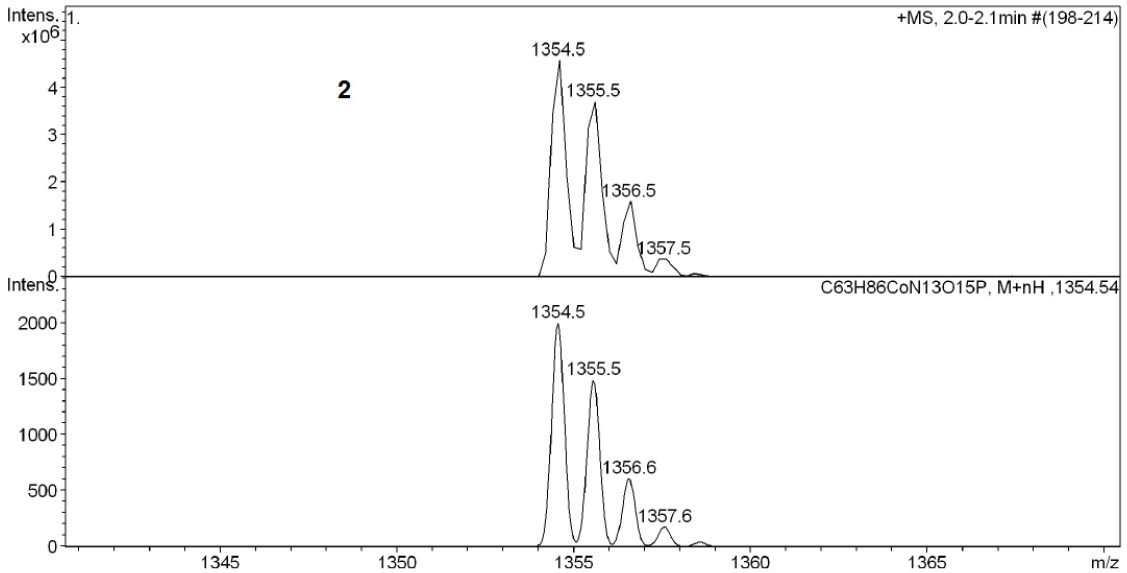


Figure S1. Top: ^{1}H-NMR spectrum of cyanocobalamin-c-lactone (**2**) recorded in D_2O [500 MHz]. Bottom: measured (*top row*) and calculated (*bottom row*) ESI-MS spectra of **2**.

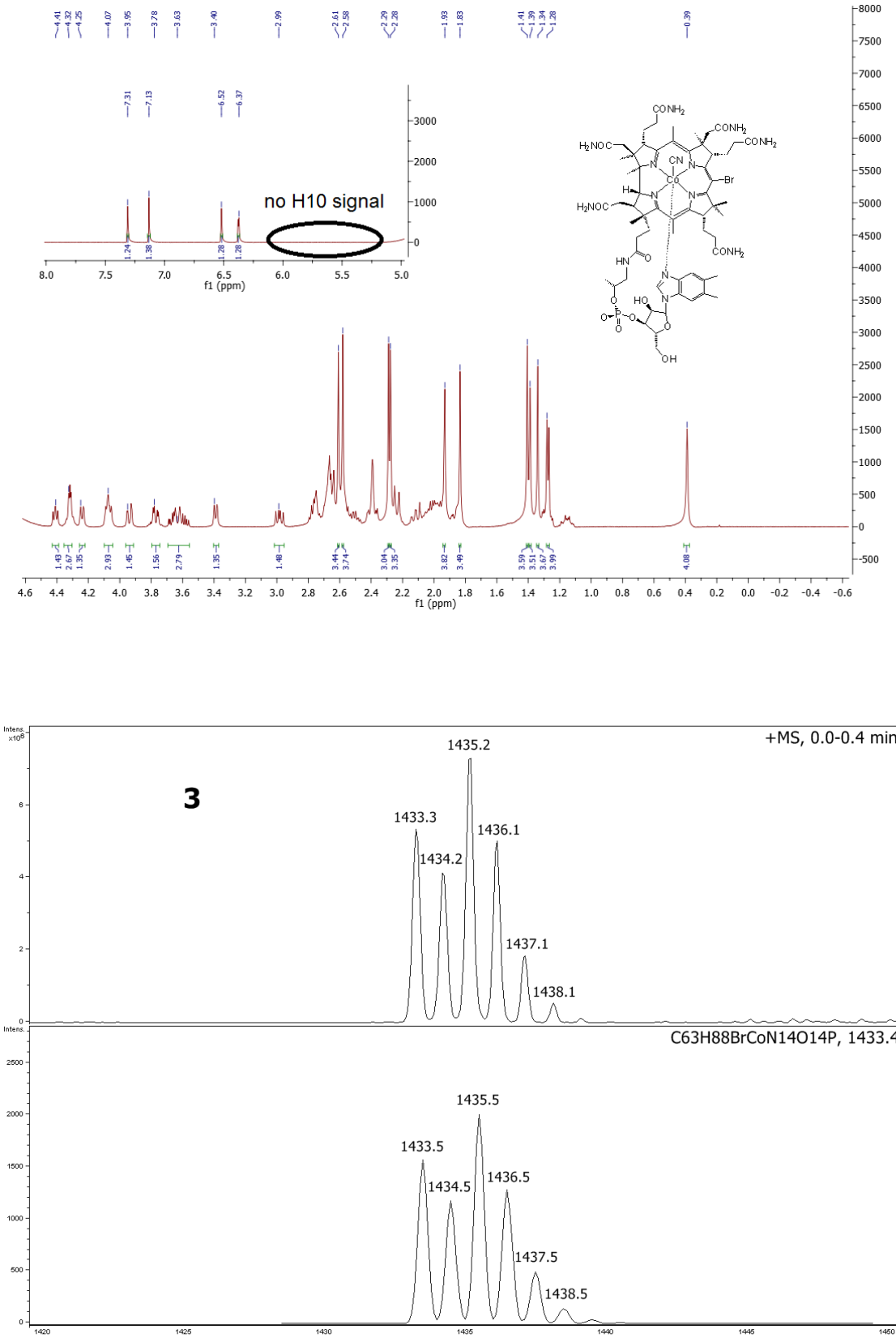


Figure S2. Top: ^{1}H-NMR spectrum of 10-bromo-cyanocobalamin (**3**) recorded in D_2O [500 MHz]. Region of missing H10 signal is assigned. Bottom: measured (*top row*) and calculated (*bottom row*) ESI-MS spectra of **3**.

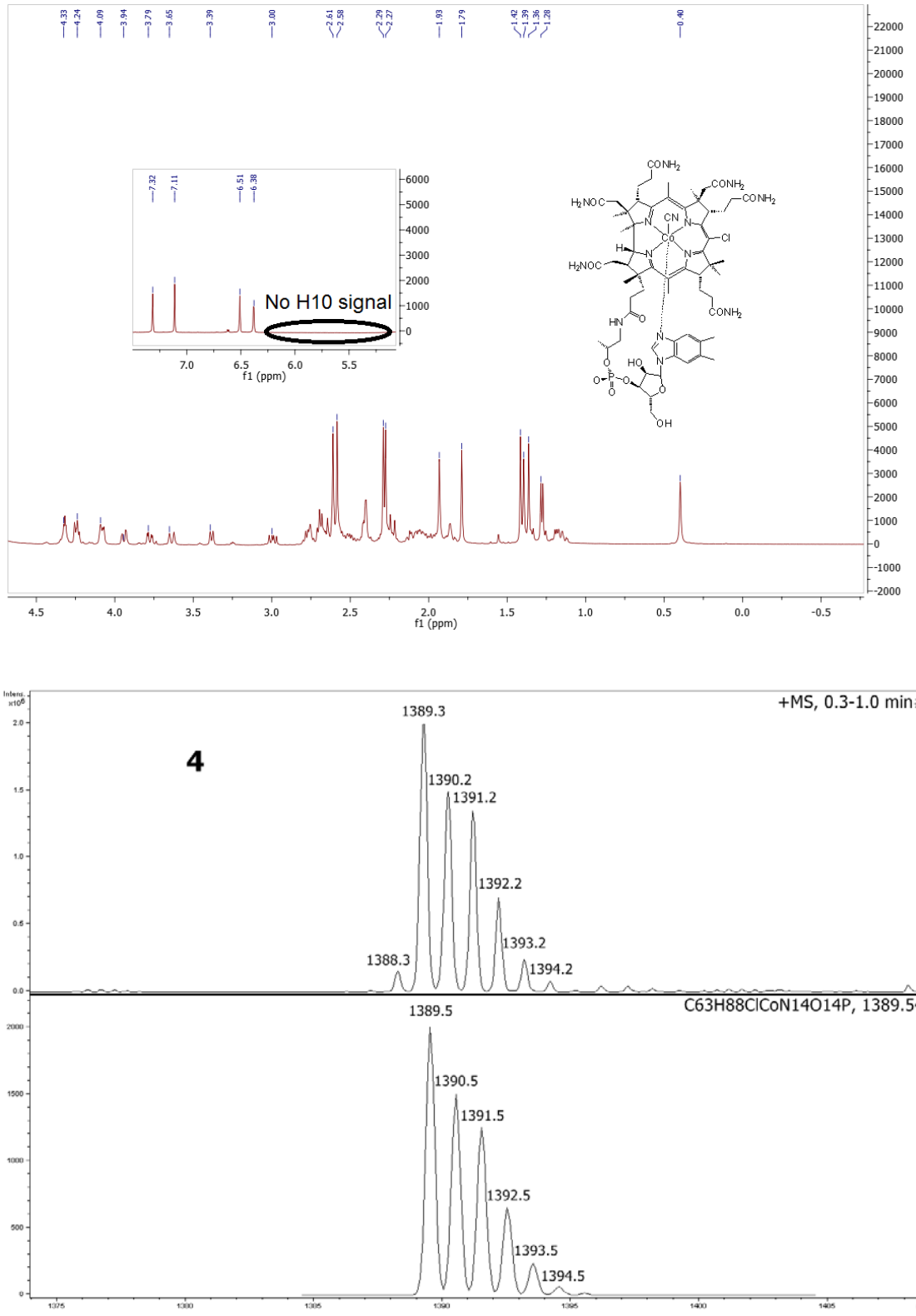


Figure S3. Top: ^{1}H-NMR spectrum of 10-chloro-cyanocobalamin (**4**) recorded in D_2O [500 MHz]. Region of missing H10 signal is assigned. Bottom: measured (*top row*) and calculated (*bottom row*) ESI-MS spectra of **4**.

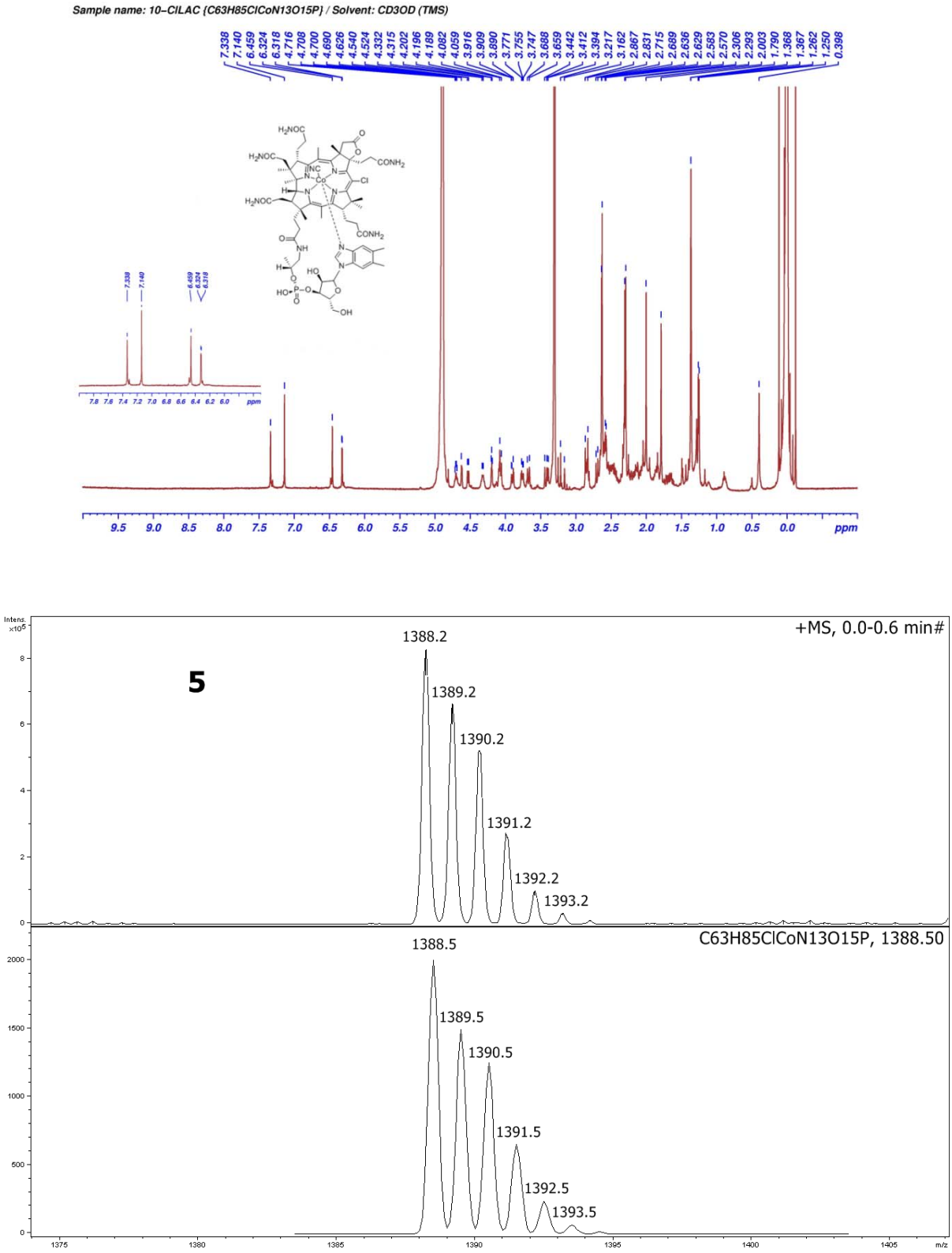
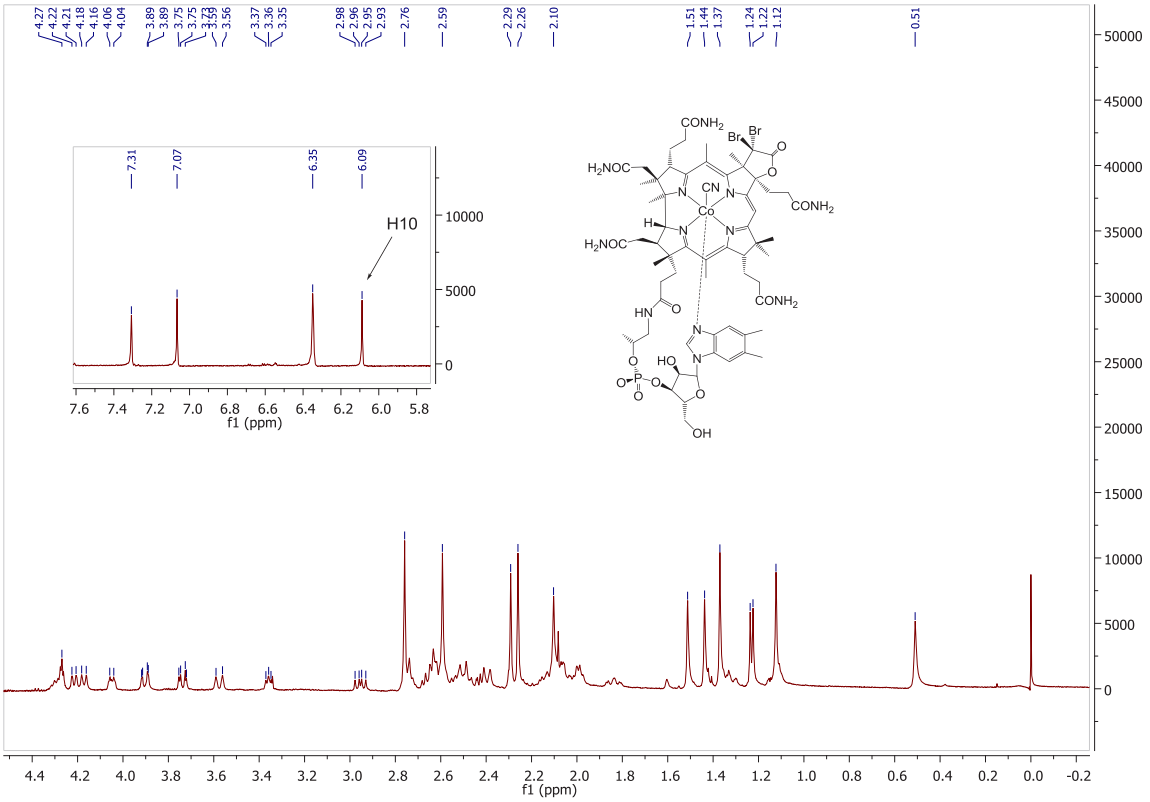


Figure S4. Top: ^{1}H-NMR spectrum of 10-chloro-lactone cyanocobalamin (**5**) recorded in CD_3OD/TMS [500 MHz]. Region of missing H10 signal is assigned. Bottom: measured (*top row*) and calculated (*bottom row*) ESI-MS spectra of **5**.



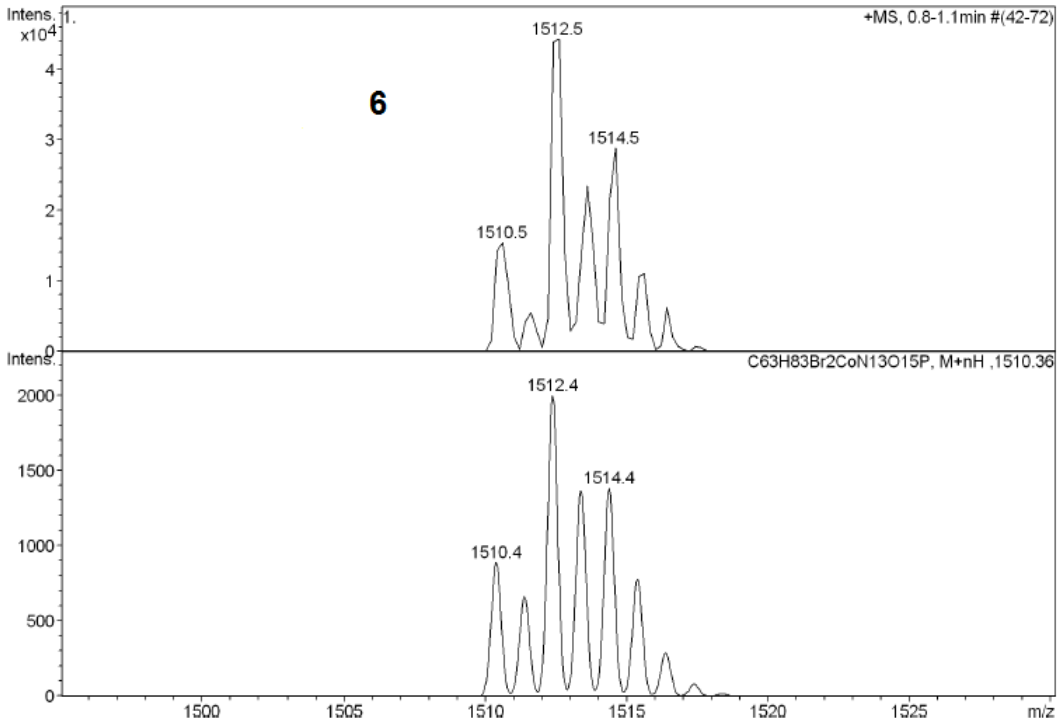


Figure S5. Top: ^{1}H-NMR spectrum of c-(α,α-dibromo)-lactone-cyanocobalamin (**6**) recorded in D_2O//TMS [500 MHz]. H10 signal is assigned. Bottom: measured (*top row*) and calculated (*bottom row*) ESI-MS spectra of **6**.

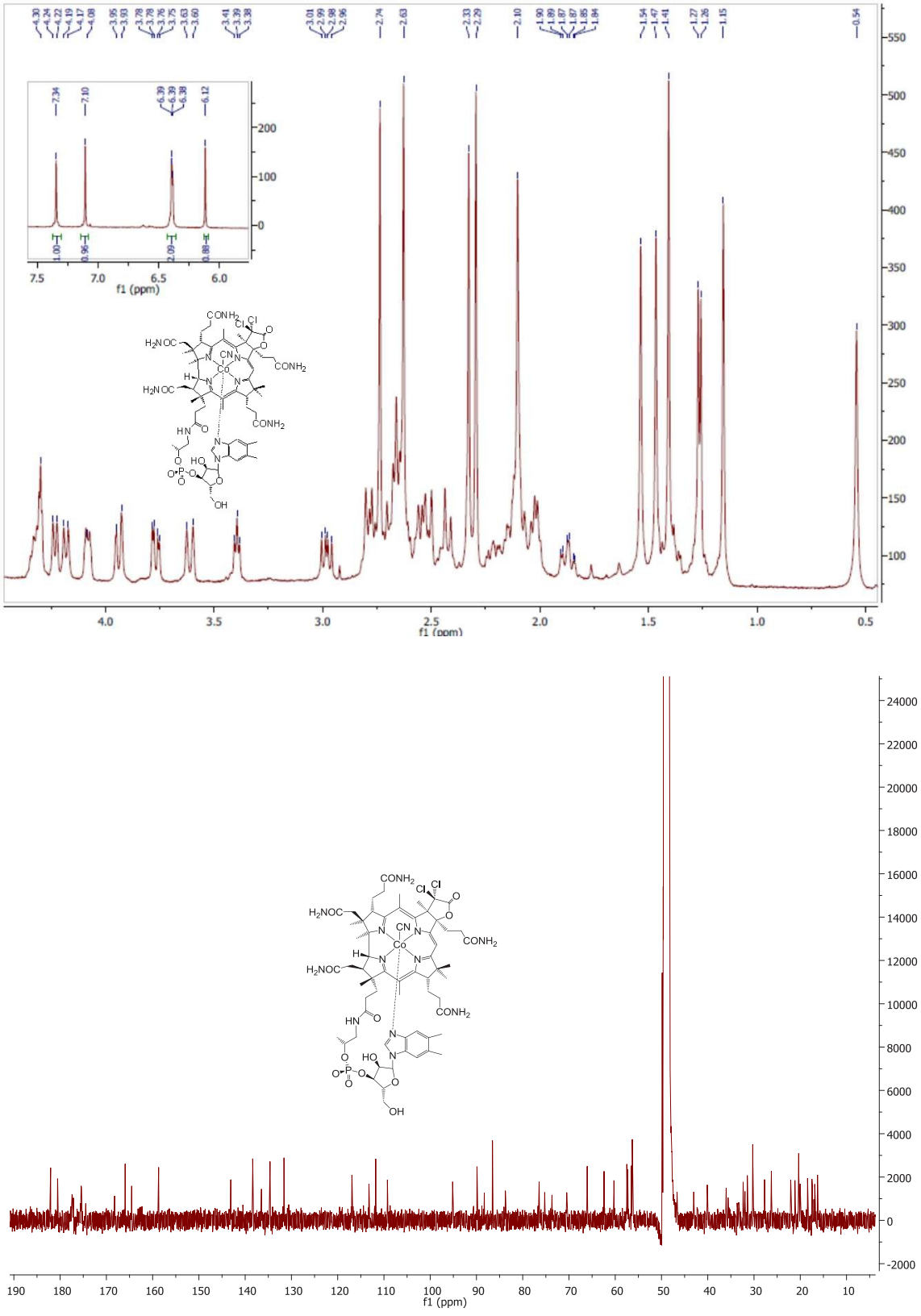


Figure S6. Top: ^{1}H-NMR spectrum of *c*-(α,α-dichloro)-lactone-cyanocobalamin (**7**) recorded in D_2O//TMS [500 MHz]. H10 signal is assigned. Bottom: ^{13}C-NMR spectrum of **7** recorded in CD_3OD [126 MHz].

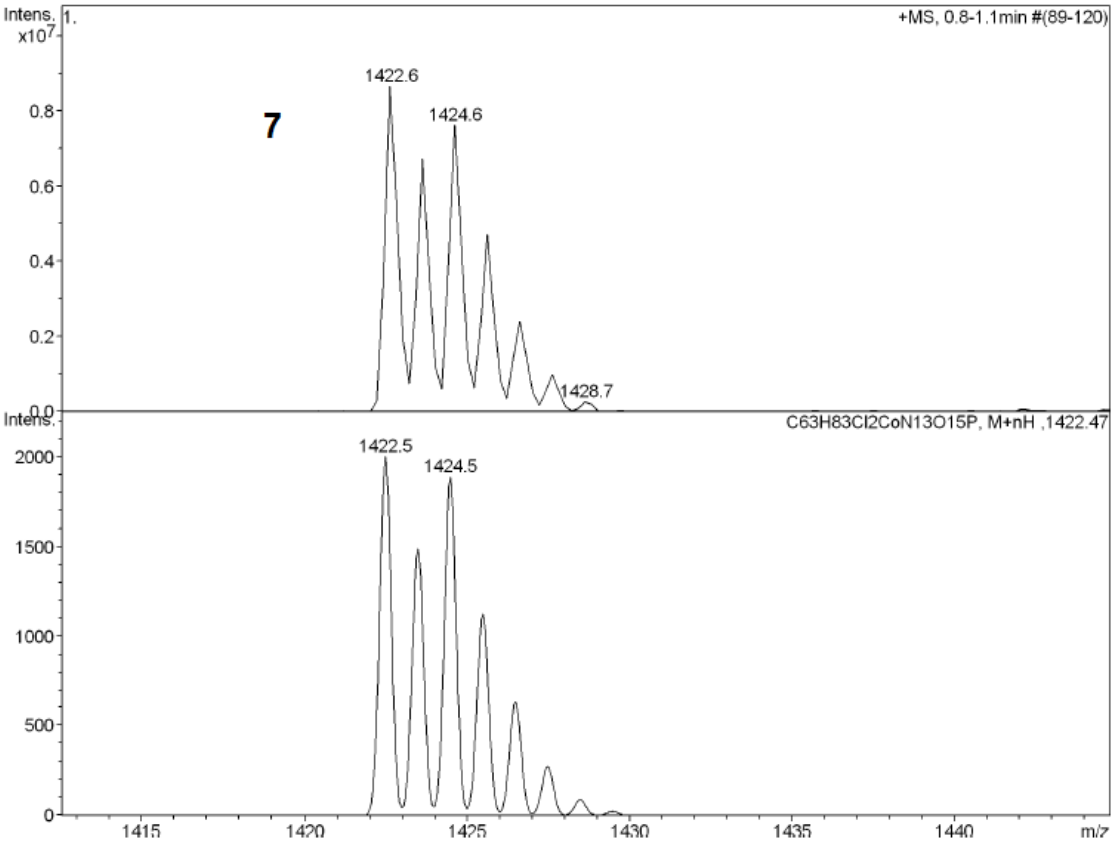


Figure S7. Measured (*top row*) and calculated (*bottom row*) ESI-MS spectra of **7**.

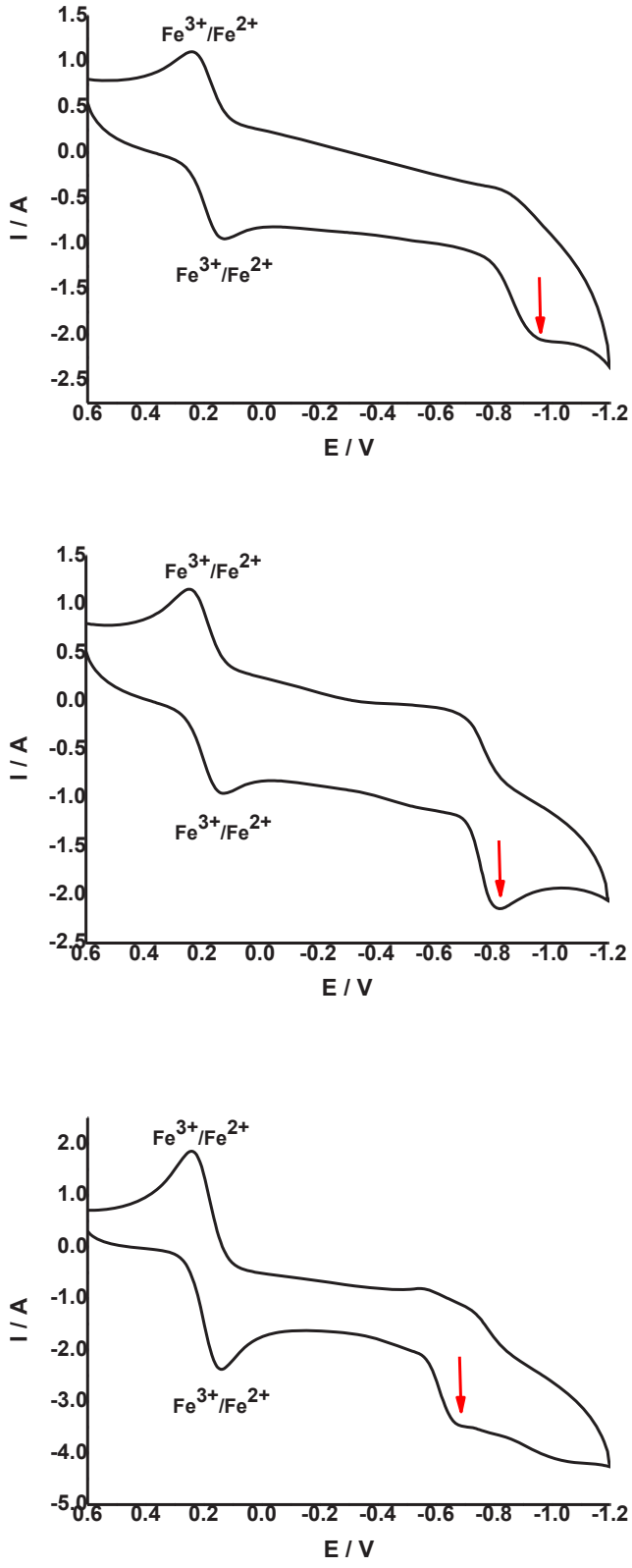


Figure S8. Cyclovoltammograms of (from top to bottom) **2**, **4** and **7**.*Red/solid arrow:* reduction potential of $Co^{III} > Co^{I}$.

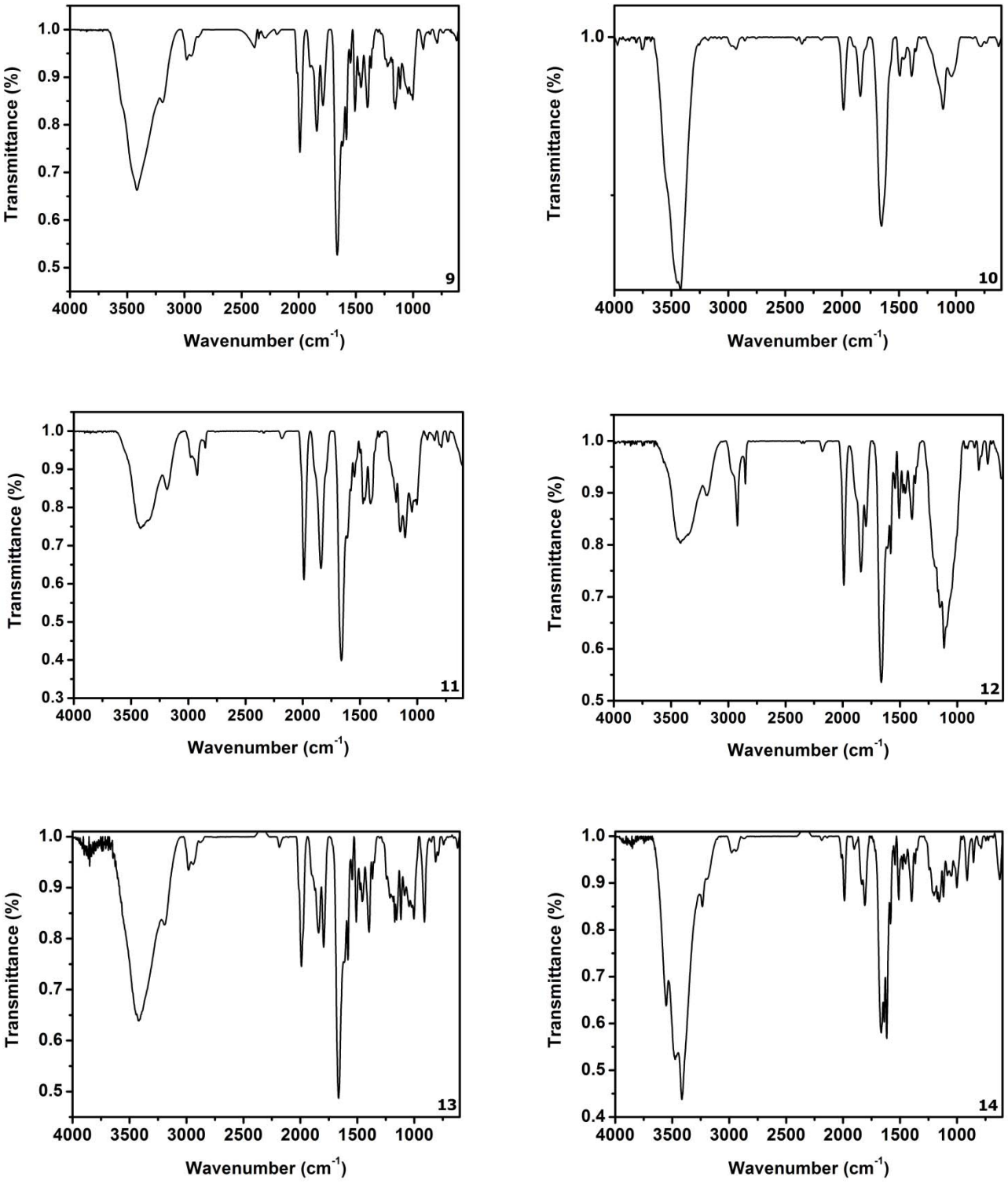


Figure S9. IR spectra of **9**, **10**, **11**, **12**, **13** and **14**.

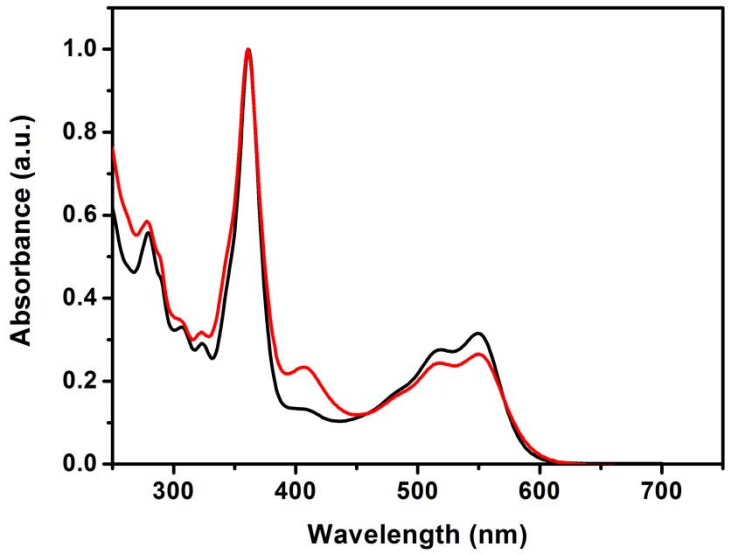


Figure S10. UV-visible spectra of an unbuffered aqueous solution of B_{12} (black line) and **8** (red line).

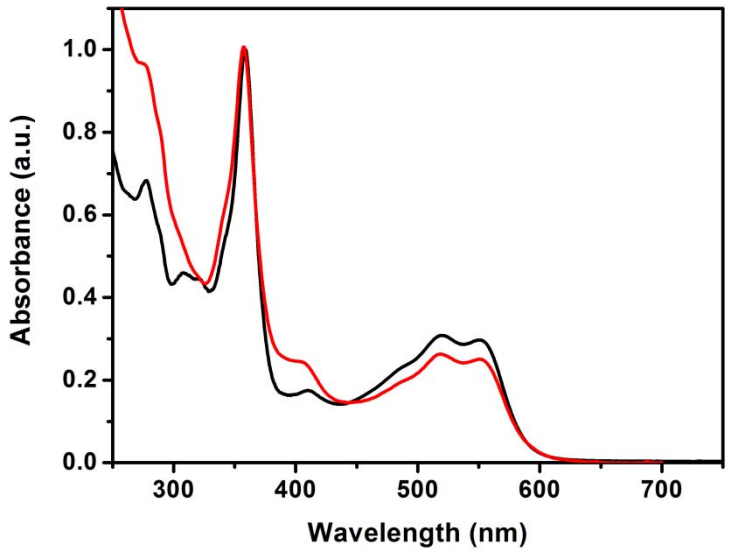


Figure S11. UV-visible spectra of an unbuffered aqueous solution of **2** (black line) and **9** (red line).

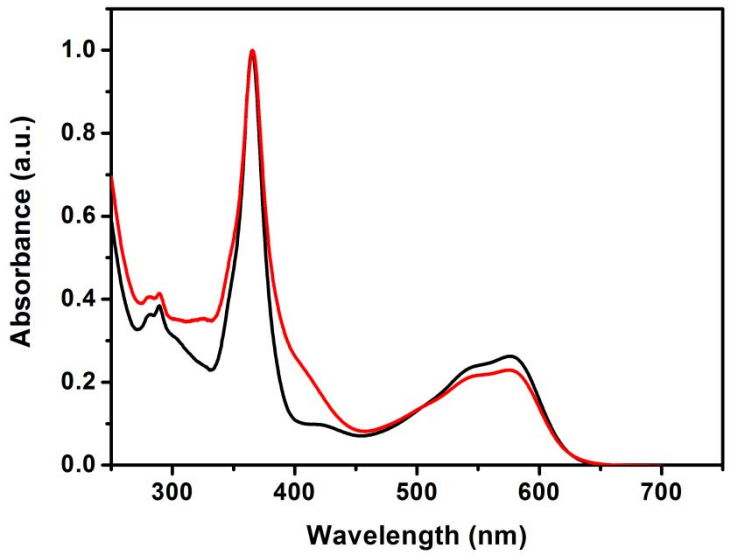


Figure S12. UV-visible spectra of an unbuffered aqueous solution of **3** (black line) and **10** (red line).

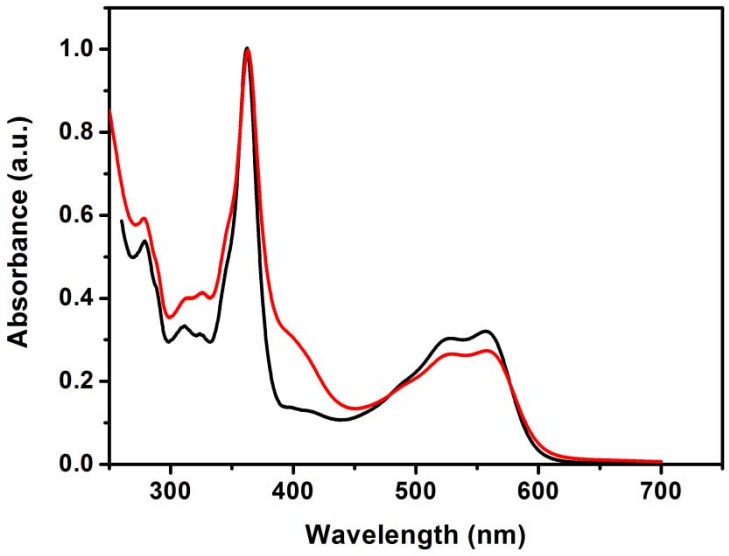


Figure S13. UV-visible spectra of an unbuffered aqueous solution of **6** (black line) and **13** (red line).

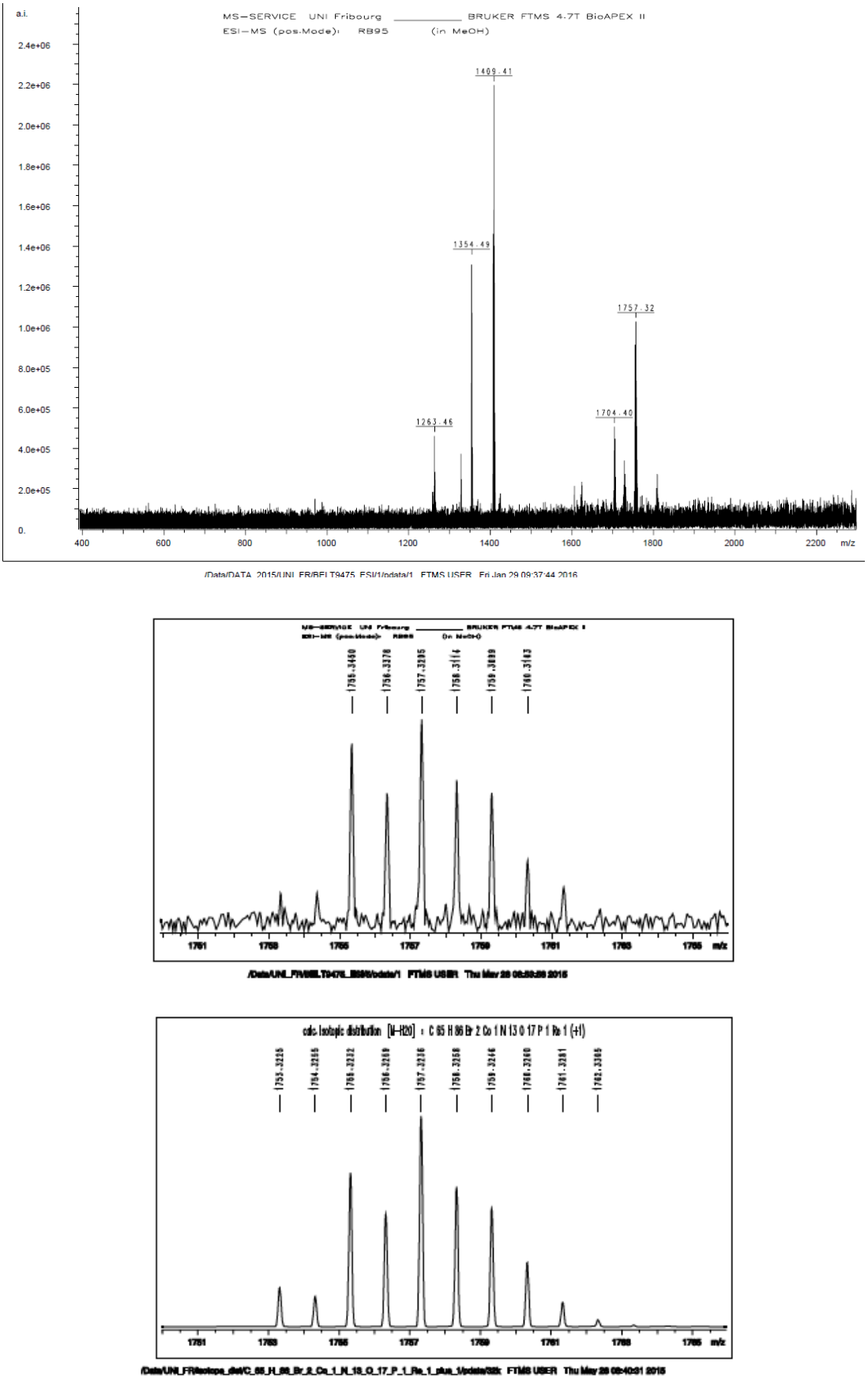


Figure S14. HR-ESI-MS (H_2O:MeOH 1:1) spectrum of **9**.

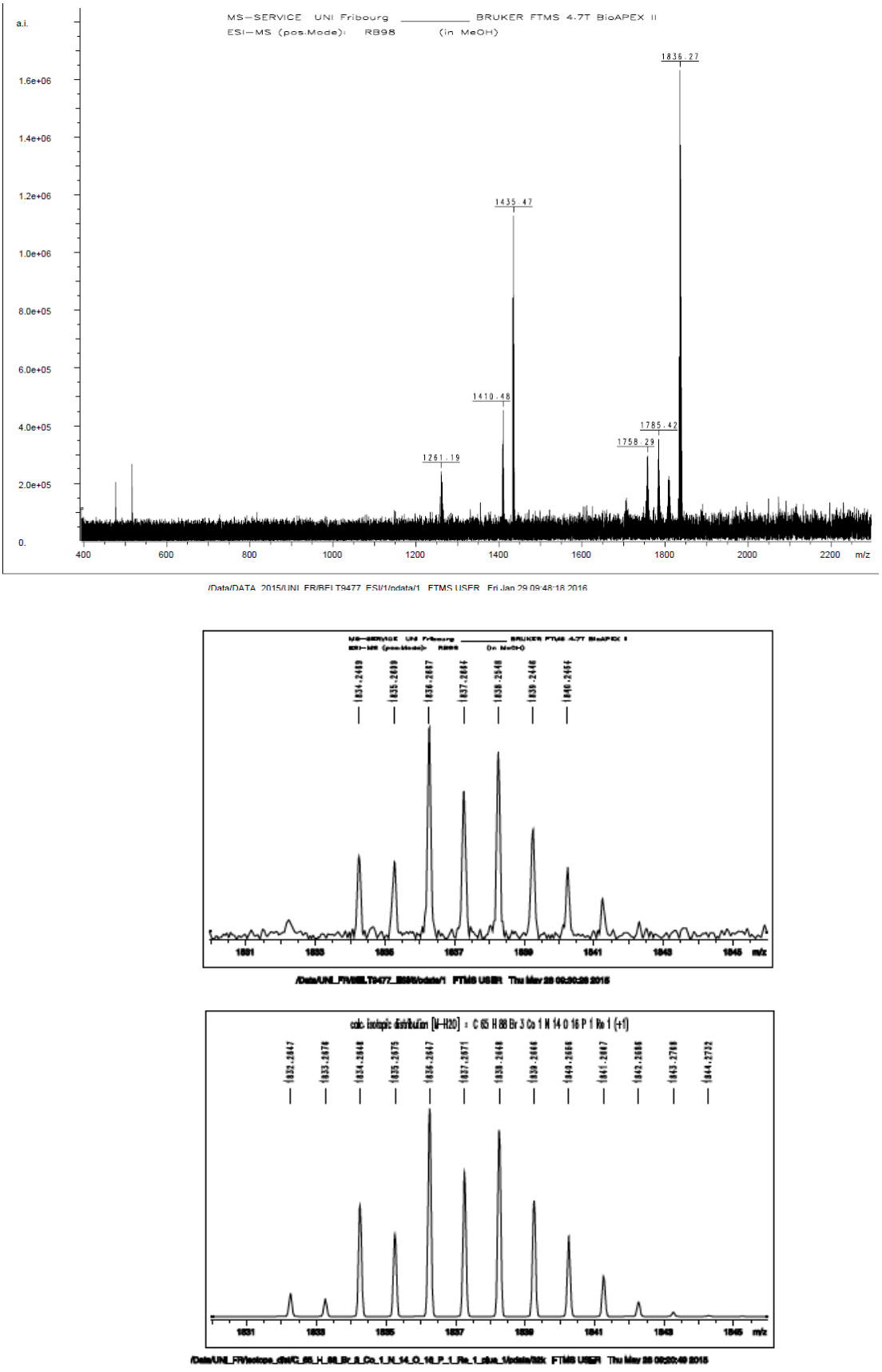
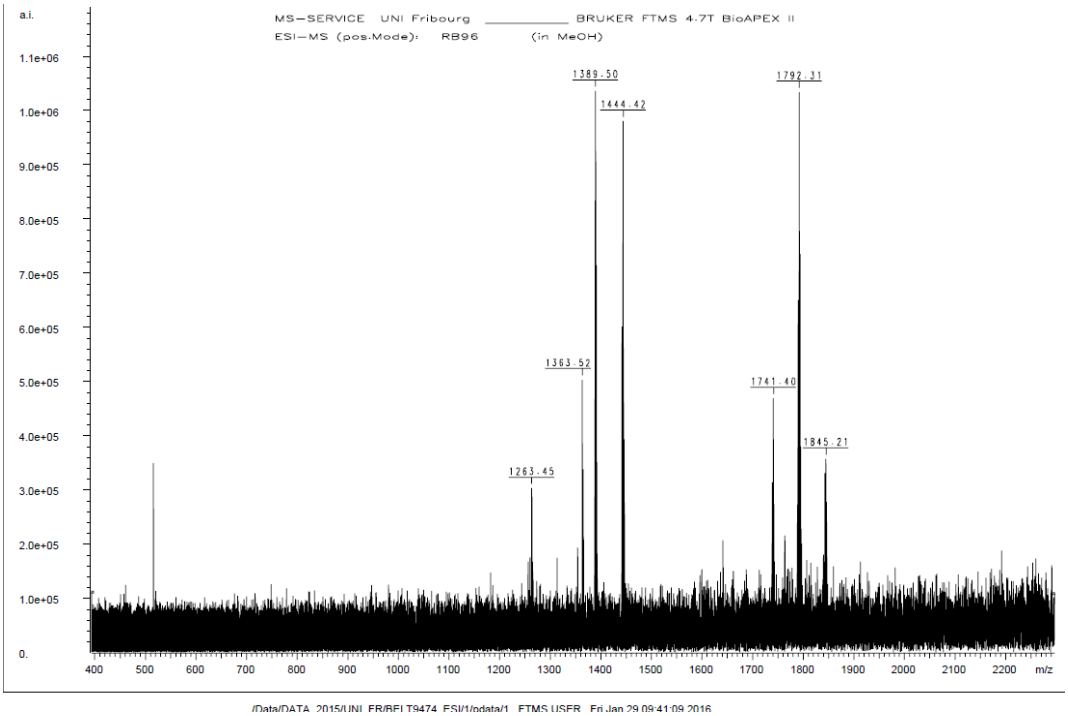
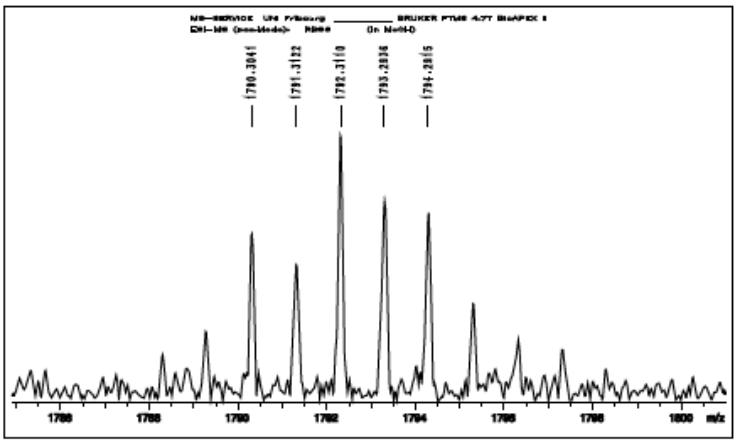


Figure S15. HR-ESI-MS (H_2O:MeOH 1:1) spectrum of **10**.





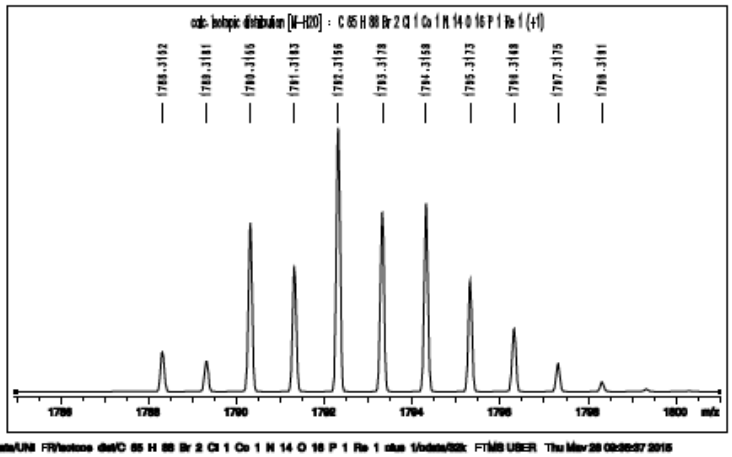


Figure S16. HR-ESI-MS (H_2O:MeOH 1:1) spectrum of **11**.

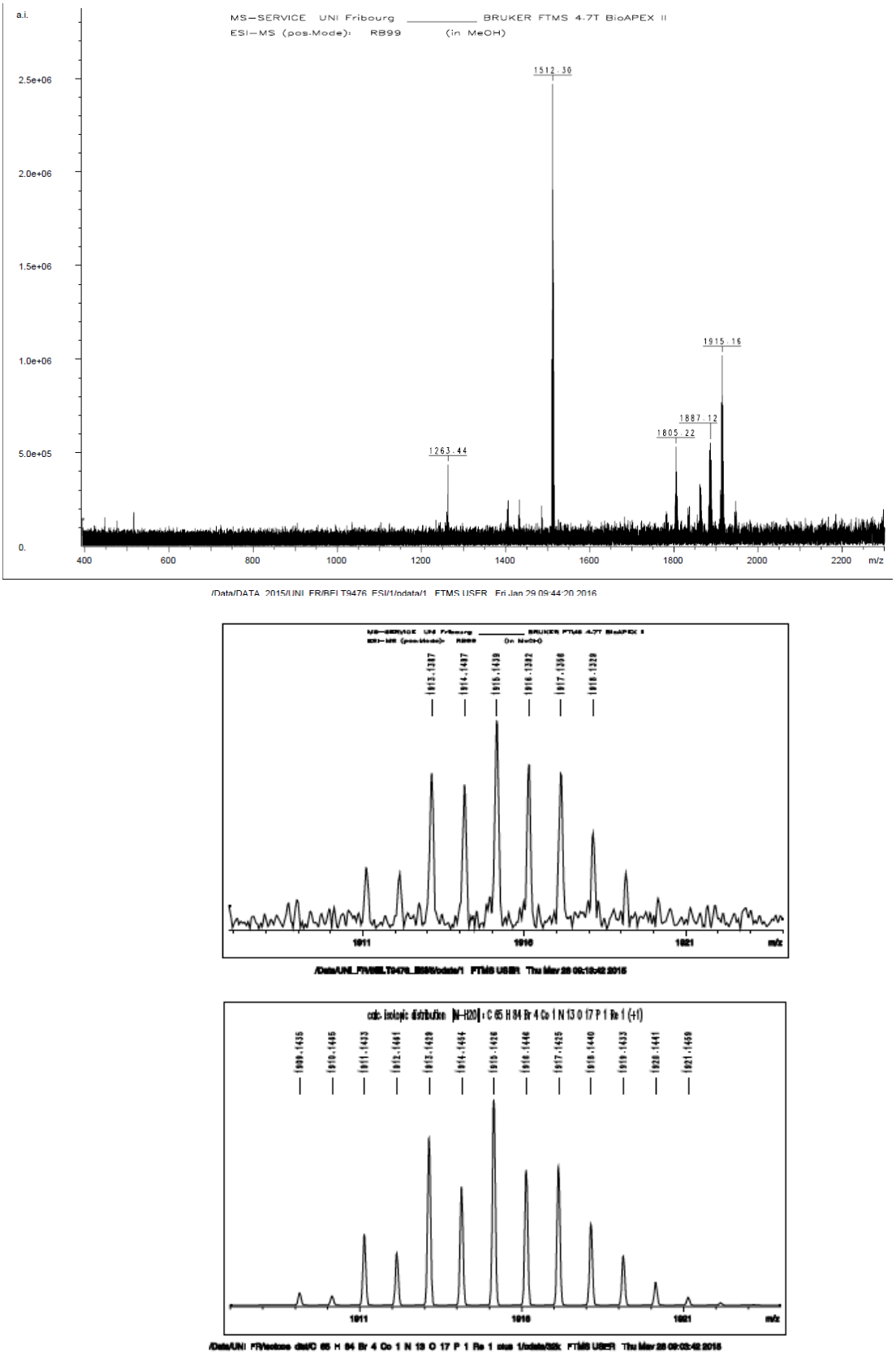


Figure S17. HR-ESI-MS (H_2O:MeOH 1:1) spectrum of **13**.

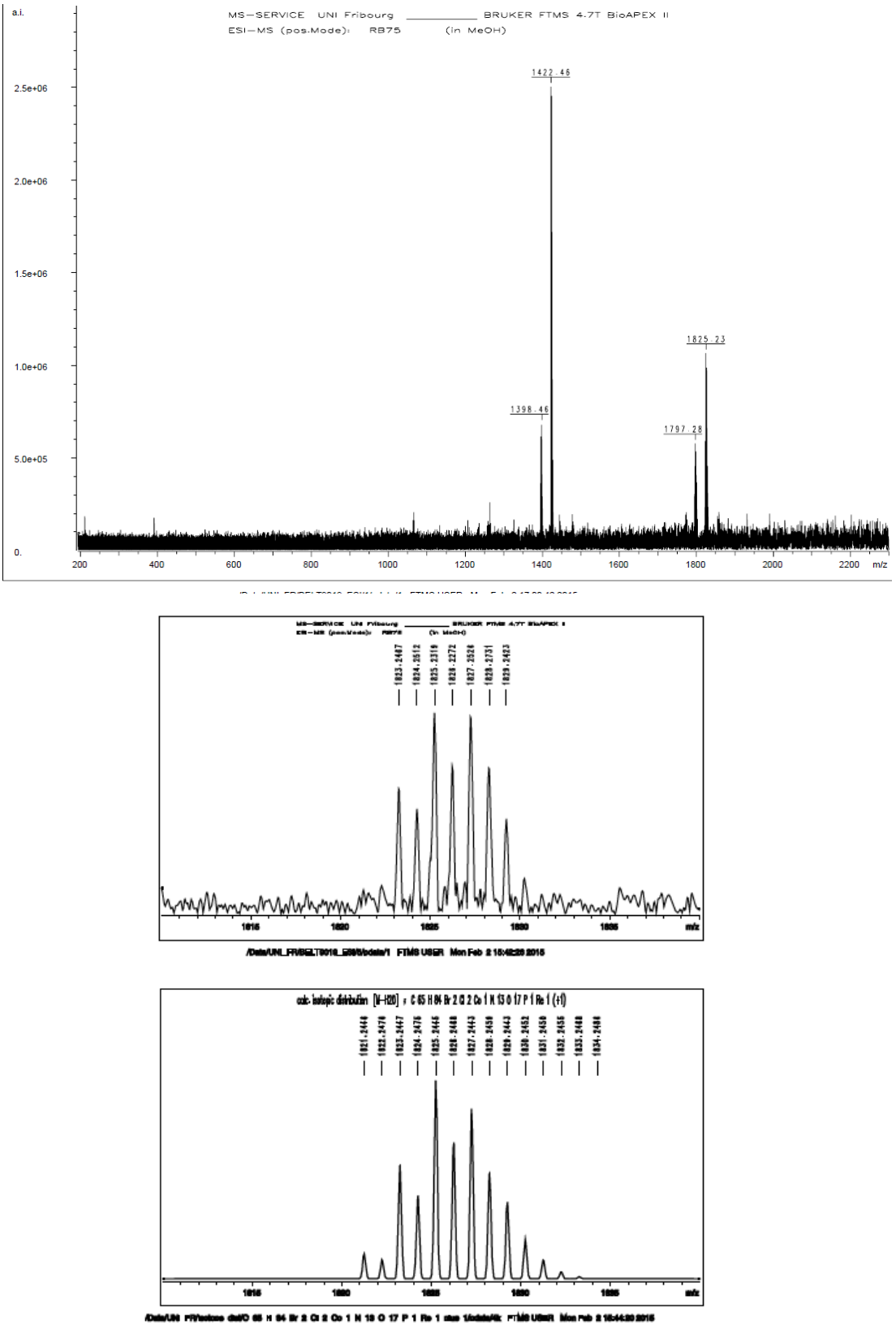


Figure S18. HR-ESI-MS (H_2O:MeOH 1:1) spectrum of **14**.

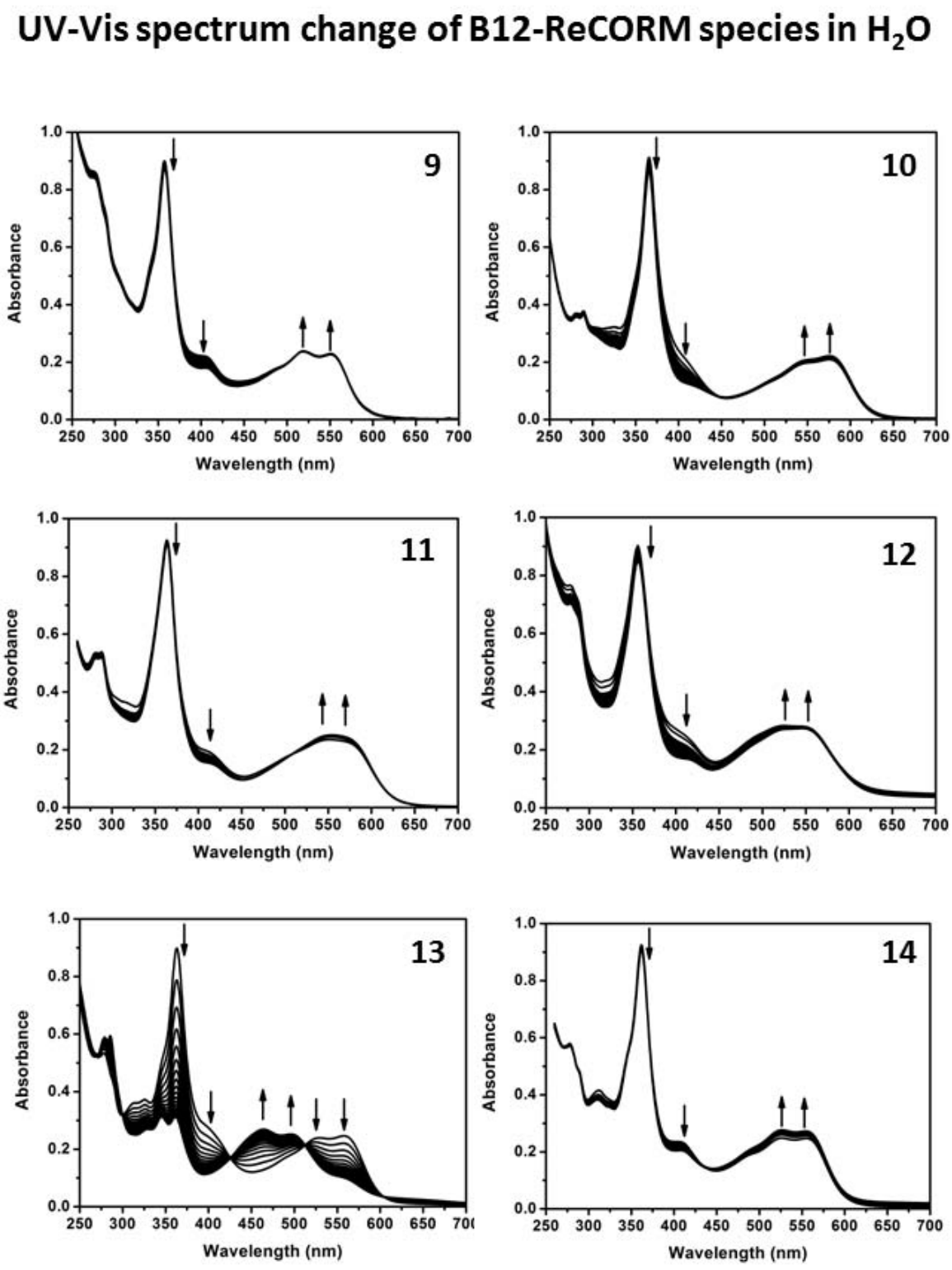


Figure S19. Changes in the UV-visible spectrum of an unbuffered aqueous solution of selected B12-ReCORM species. Spectra were recorded at fixed time intervals at 25 °C.

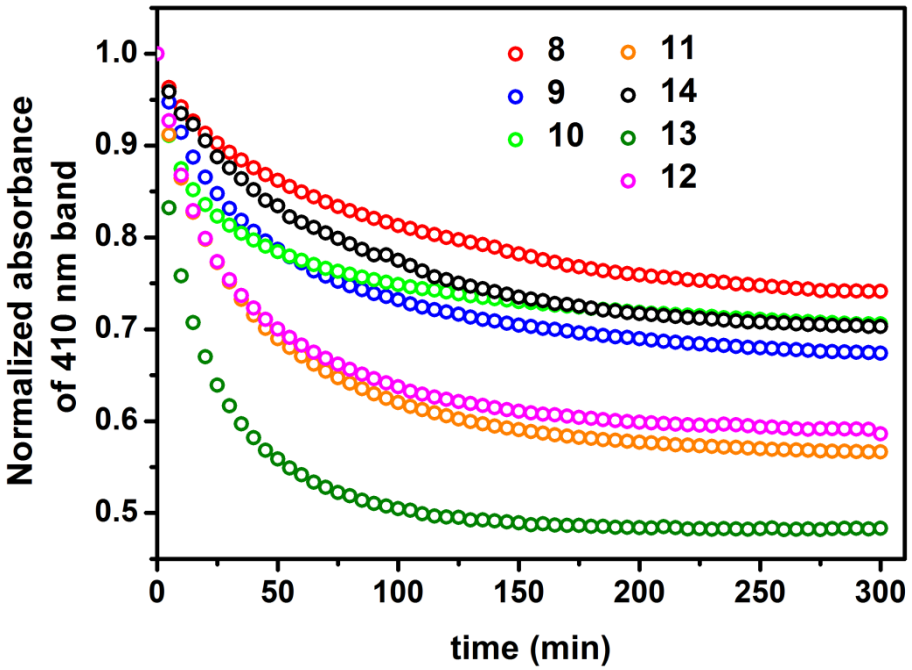


Figure S20. Normalized exponential hypochromic shift of 410 nm band in the UV-Vis spectrum of compounds **8-14** in H_2O.

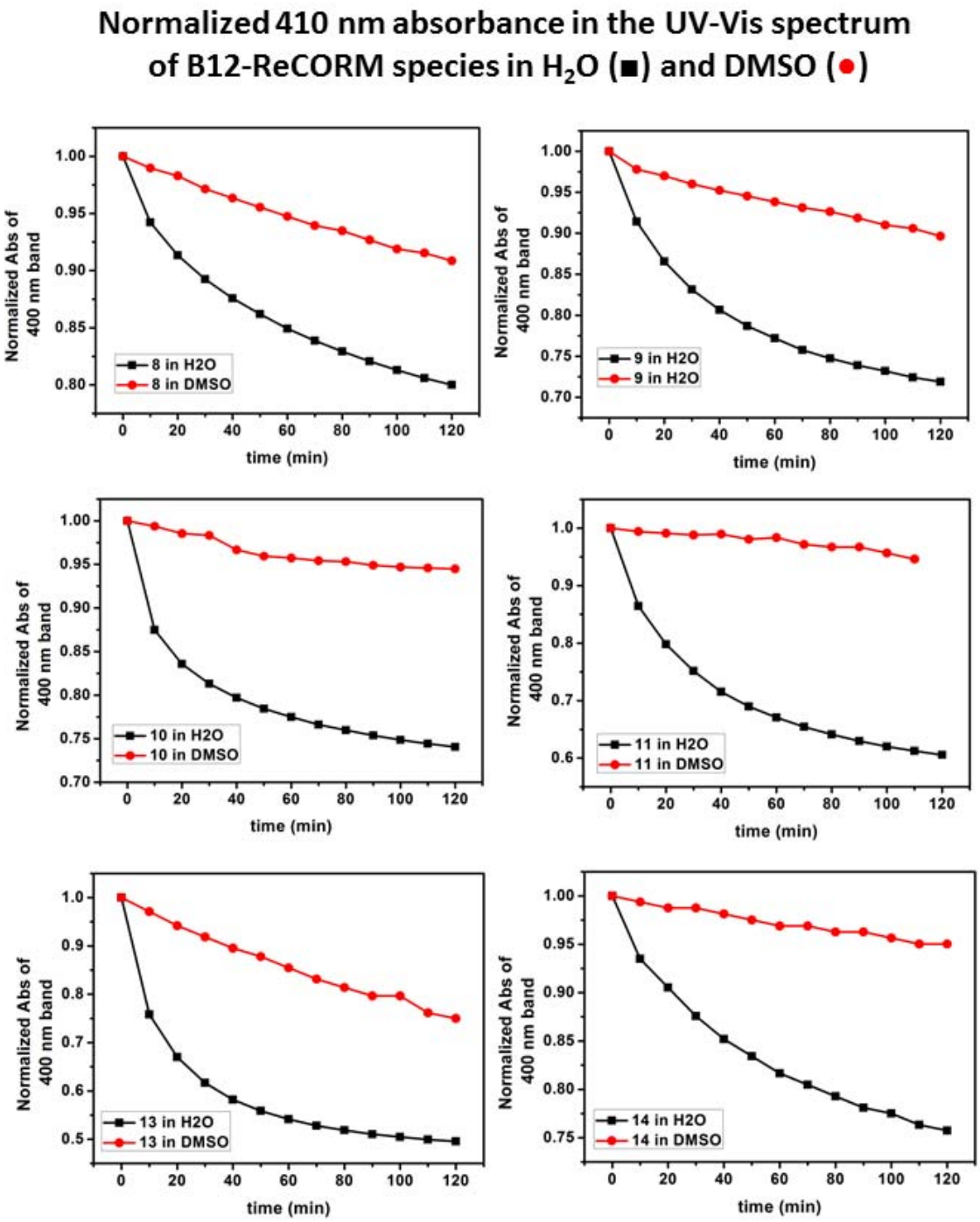


Figure S21. Normalized exponential hypochromic shift of 410 nm band in the UV-Vis spectrum of compounds **8-14** in DMSO.

Table S1. Half-life (t½) of stability of species **8-14** in DMSO.

Compound	$t_{½}$ hypochromic shift of 410 nm band in DMSO [a]
8	2.3 ± 0.3
9	1.6 ± 0.6
10	0.99 ± 0.2
11	> 3
12	n. d.
13	1.9 ± 0.4
14	> 3

[a] Assuming a first order exponential decay (hours).

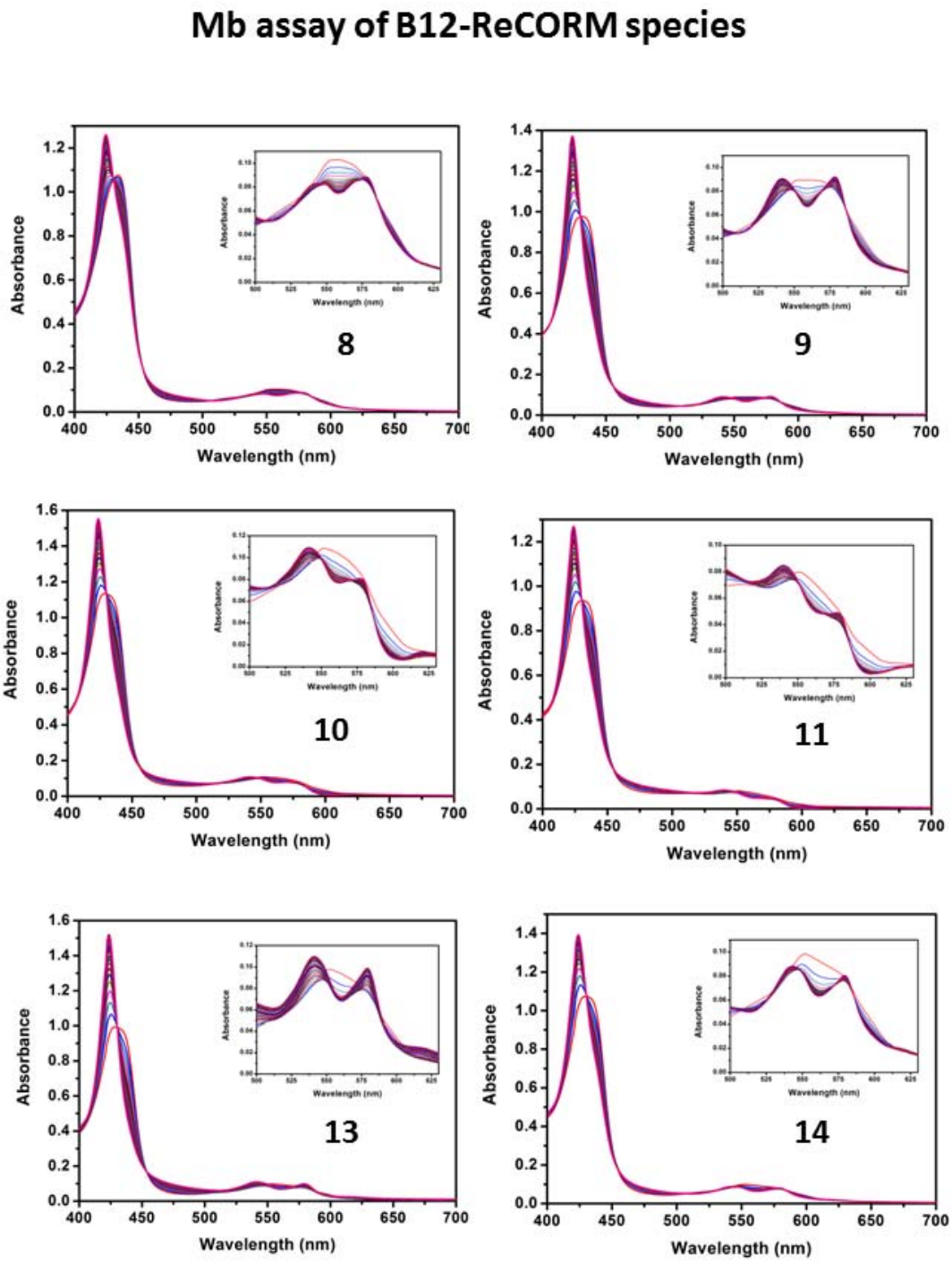


Figure S22. Spectrum changes (5 min intervals) of a solution of deoxy-myoglobin (Mb, 20 μM, phosphate buffer, pH = 7.4) solution after addition of 1 equivalent of species **8-14**.

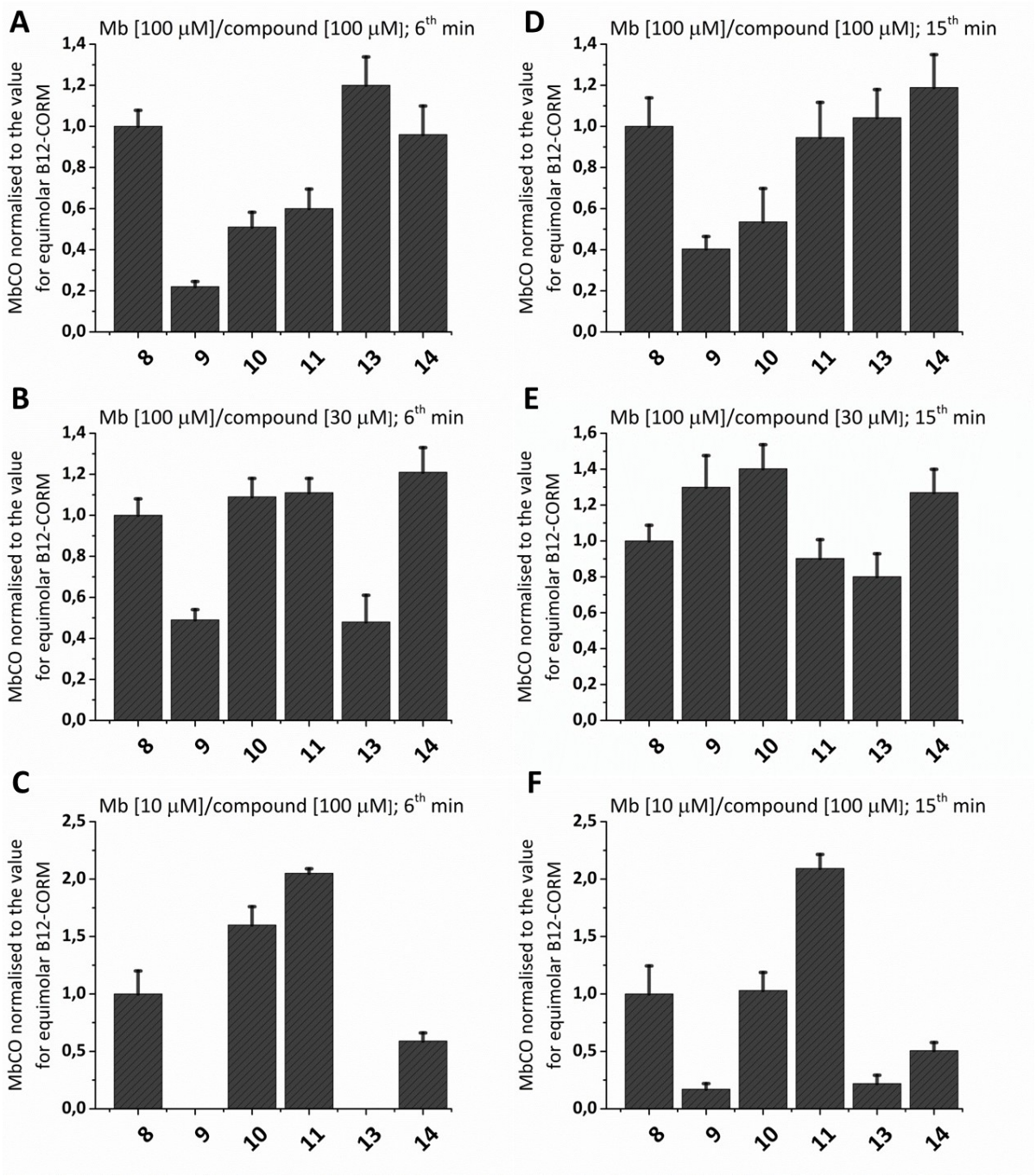


Figure S23. The formation of MbCO in sixth (A-C) and fifteenth (D-F) minutes of B_{12}-ReCORM complexes **8-14** incubation with Mb (mean and standard deviation of N = 4-5) normalized to MbCO formation for B_{12}-ReCORM.

S1. Bonnett, R.; Cannon, J.; Johnson, A.; Todd, A., 226. Chemistry of the vitamin B 12 group. Part IV. The isolation of crystalline nucleotide-free degradation products. *J. Chem. Soc.* **1957**, 1148-1158.

S2. Wagner, F., Vitamin B and Related Compounds. *Ann. Rev. Biochem.* **1966,** *35* (1), 405-434.

S3. Oetterli, R. M.; Prieto, L.; Spingler, B.; Zelder, F., Synthesis of a B Ring Opened 7,8-seco-Vitamin B12 Derivative with Grob Fragmentation. *Org. Lett.* **2013,** *15* (18), 4630-4633.

S4. Zobi, F.; Blacque, O.; Jacobs, R. A.; Schaub, M. C.; Bogdanova, A. Y., 17 e− rhenium dicarbonyl CO-releasing molecules on a cobalamin scaffold for biological application. *Dalton Trans.* **2012,** *41* (2), 370-378.