Electronic Supplementary Material (ESI) for Dalton Transactions.

Supporting Information for

Stabilizing Intramolecular Cobalt-Imidazole Coordination with a Remote Methyl Group in the Backbone of a Cofactor B_{12}-Protein Model

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Contents

Materials and Methods

All chemicals were of reagent grade and used without further purification. Deuterated solvents were obtained from *Armar Chemicals* (Döttingen, Switzerland). Solvents for HPLC and UPLC were of the corresponding grade. Compounds **4**$^{+}$, **6** and **7** were synthesized as described elsewhere.[S1, 2] Compounds **4**$^{+}$ and **5**$^{+}$ were obtained as CF_3COO^- (TFA) salts after preparative HPLC. The dicyano derivatives **4-CN** and **5-CN** were obtained by addition of 1 eq. of KCN after purification of the compounds.

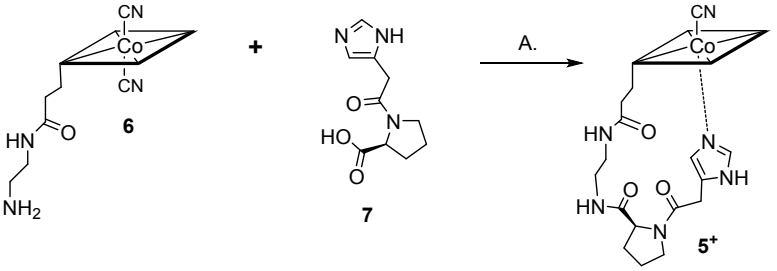
HPLC solvents were of an aq. soln. of CF_3COO^- (0.1%; A) and CH_3OH (B). UPLC solvents were of an aq. soln. of $HCOO^-$ (0.1%; C) and CH_3CN (D). Analytical HPLC analyses were performed on a *Merck-Hitachi L-7000* system equipped with a diode array UV-Vis spectrometer. *Macherey Nagel Nucleosil C18ec RP* column (5 µm particle size, 100 Å pore size, 250 x 3 mm. Flow rate: 0.5 mL min^{-1}) was used as column. UPLC-MS spectra were recorded on an *Acquity Waters* system equipped with a PDA detector and an autosampler using an *ACQUITY UPLC BEH C18 Gravity* 1.7 µm (2.1 mm × 50 mm) reverse phase column. The UPLC system was connected to a *Bruker Daltonics HCT ESI-MS* spectrometer. A total volume of 1 µL of a sample soln. was analysed. UPLC gradient was: 0.0-0.5 min 5% D *vs.* C. 0.5-2.0 min 5-30% D *vs.* C. 2.0-4.0 min 30-100% D *vs.* C. Preparative HPLC separations were performed on a *Varian Prostar* system equipped with two *Prostar 215* pumps, a *Prostar 320* UV/Vis detector and *Macherey Nagel Nucleosil C-18ec RP* column (7 µm particle size, 100 Å pore size, 250 x 21 mm. Flow rate: 18 mL min^{-1}) was used as column. Preparative gradient was: 0-3 min 25% B *vs.* A. 3-30 min 25-100%B *vs.* A.

NMR spectra were recorded on a *Bruker AV-500* spectrometer (Karlsruhe, Germany). For ^{1}H-NMR, the chemical shifts are given in ppm relative to the signal from the deuterated solvent. Coupling constants *J* are given in Hz. For ^{13}C-NMR, the chemical shifts are given in ppm relative to (trimethylsilyl)propionic acid sodium salt.

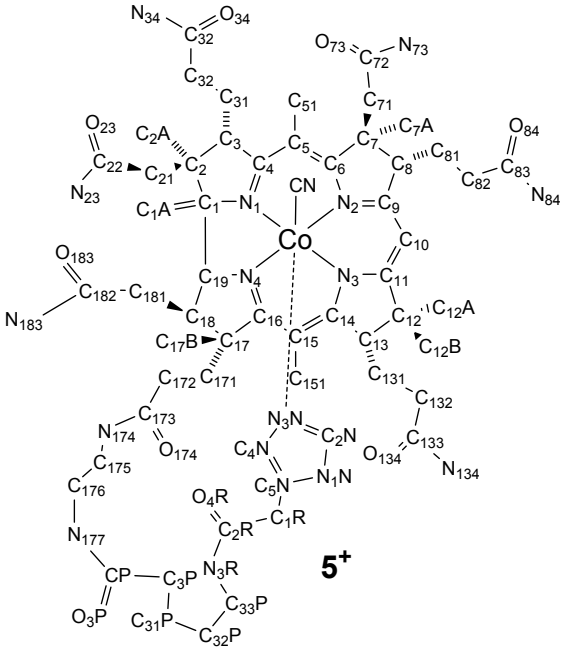
High-resolution electrospray mass spectra were recorded on a *Bruker maXis QTOF-MS* instrument (*Bruker Daltonics* GmbH, Bremen, Germany). The samples were dissolved in CH_3OH and analyzed via continuous flow injection at 3 µL/min. The mass spectrometer was operated in positive ion mode with a capillary 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 (H_2O (500 µL): iPrOH (500 µL): HCOOH (20 µL): an aq. soln. of NaOH (0.1 M; 20 µL). 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.

Scheme S1. Synthesis of **5⁺** (A. DMAP, EDC, DMF, 0°C).



Scheme S2. Atom Numbering of **5⁺**.



Experimental Procedures:

Compound 5^+ (as CF_3COO^- salt): Compound **7**[S2] (19.7 mg, 88.0 µmol) was dissolved in DMF (5 mL) and cooled down to 0°C. DMAP (10.7 mg, 88.0 µmol) was then added. After 10 min, the soln. was added to a soln. of **6** [S1] (20 mg, 17.6 µmol) in dry DMF (8 mL). After 30 min, EDC (13.7 mg, 88.0 µmol) was added. The mixture was let to react for 18h at 24°C. It was quenched with H_2O (15 mL) and washed with 3 portions of CH_2Cl_2 (20 mL). Purification of the crude mixture was performed with preparative HPLC
to afford the product as a mixture of **5^+** and **5-OH_2^+** (6.7 mg, yield: 29 %). To obtain the base-on form **5^+**, the solid was dissolved in H_2O (6 mL) and converted with KCN to the corresponding dicyano-derivative **5-CN**. The pH of the soln. was then lowered to 3.8 with an aq. soln. of HCl (1 M) and the reaction was stirred at 24°C for 24h. The solvent was then removed under reduced pressure to afford a pink powder.

HR-MS: $[M+H]^{2+}$ calculated for $C_{58}H_{83}CoN_{16}O_9$: 603.29250, found 603.29267
UV-Vis: (0.2 M KCl) λ_{max} / nm (log ε) = 555 (3.95); 523 (3.92); 411 (3.56); 362 (4.46); 321 (3.93); 306 (4.00); 278 (4.09)
UPLC-MS: m/z = 1205.7 $[M]^+$ 603.8 $[M+H]^{2+}$, R_t = 1.9 min.

^{1}H-NMR of **5-CN**: (500 MHz, D_2O) δ / ppm = 7.73 (*s*, 1H), 7.05 (*s*, 1H), 5.92 (*s*, 1H), 4.43 (*dd*, *J* = 8.7, 4.1 Hz, 1H), 3.86 (*d*, *J* = 8.6 Hz, 1H), 3.79 (*s*, 2H), 3.78-3.64 (*m*, 3H), 3.43 (*dd*, *J* = 7.1, 4.9 Hz, 1H), 3.41 – 3.26 (*m*, 5H), 2.90 (*s*, 1H) , 2.78 - 2.69 (*m*, 2H), 2.65 - 2.39 (*m*, 5H), 2.29 - 2.31 (*m*, 4H), 2.31 - 2.23 (*m*, 9H), 2.23 – 2.05 (*m*, 4H), 2.05 - 1.92 (*m*, 5H), 1.91 - 1.74 (*m*, 3H), 1.71 (*s*, 3H), 1.55 (*s*, 3H), 1.49 (*s*, 3H), 1.46 (*s*, 3H), 1.33 (*s*, 3H), 1.21 (*s*, 3H).

^{13}C-NMR of **5-CN:** (126 MHz, D_2O) δ / ppm = 181.6, 181.5, 181.1, 180.0, 180.5, 180.0, 179.6, 178.8, 175.3, 175.2, 166.5, 166.1, 165.9, 165.8, 139.0, 134.6, 121.9, 119.9, 118.2, 115.9, 110.8, 108.1, 94.8, 86.1, 77.8, 63.6, 61.2, 59.1, 57.8, 56.0, 51.1, 51.0, 49.8, 49.3, 46.5, 45.0, 41.7, 41.6, 41.4, 37.6, 35.8, 35.4, 35.2, 34.7, 34.6, 34.4, 33.0, 32.6, 29.4, 28.4, 27.6, 27.3, 23.4, 21.5, 20.1, 19.2, 17.7, 17.4.

Table S1: Comparison of NMR chemical shift values for **4-CN**[S2] and **5-CN**.[a]

	4-CN		**5-CN**	
	^{1}H	^{13}C	^{1}H	^{13}C
C1		85.9		86.1
C1A	1.49	24.5	1.49	23.4
C2		49.1		49.3
C2A	1.55	19.3	1.55	19.2
C3	3.87	59.2	3.86	59.1
C4		179.7		179.6
C5		108		108.1
C6		166		166.1
C7		49.0		51.0
C7A	1.72	21.5	1.71	21.5
C8	3.41	59.1	3.43	57.8
C9		174.9		175.2
C10	5.93	93.9	5.92	93.8
C11		180.4		180.5
C12		49.5		
C12A	1.46	21.3	1.46	21.5
C12B	1.22	33.2	1.21	33.0
C13	3.32	56	3.32	56.0
C14		166.3		165.9
C15		105.9		108.1
C16		180.1		180.0
C17		61.6		61.2
C17B	1.33	20.2	1.32	20.1
C18	2.89	41.7	2.9	41.7
C19	3.74	77.9	3.75	77.8
C21	2.32/2.26	45	2.32/2.28	45.0
C31	2.24/1.98	27.7	2.29/1.99	27.6
C32	2.55/2.46	37.7	2.57/2.49	37.6
C51	2.25	18	2.28	17.7
C71	2.58/2.27	46.5	2.60/2.29	46.5
C81	2.12/1.79	29.4	2.13/1.83	29.4
C82	2.26/1.93	32.7	2.28/1.95	32.6
C131	2.08/1.86	28.4	2.13/1.88	28.4
C132	2.16/2.09	24.6	2.30/2.20	34.6

C151	2.27	17.6	2.33	17.4
C172	2.44/1.85	35.5	2.44/1.87	35.4
C175	3.32	46.4	3.36	41.4
C176	4.05	48.4	3.36	41.6
C177	1.12	19.7		
C181	2.73	35.2	2.74	35.2
C3P	4.64/4.40	63.8	4.65/4.41	63.6
C31P	2.28	34.7	2.39/2.30	34.7
C32P	1.97	27.1	2.00	27.6
C33P	3.76 /3.70	51.1	3.71	51.1
C1R	3.80	35.6	3.79	35.8
C2R		175.2		175.3
C2N	7.75	139	7.74/7.73	139.9
C4N	7.06/7.04	119.8	7.05/7.02	119.9
C5N		134.2		134.6

[a]Assignment by ^{1}H-NMR, ^{13}C-NMR, ^{1}H,^{1}H ROESY, ^{1}H,^{13}C-HSQC correlation and comparison with **4**$^{+\cdot}$ [S2]

References

S1. K. Zhou and F. Zelder, *Angew. Chem. Int. Ed.*, 2010, **49**, 5178-5180.
S2. M. Sonnay, T. Fox, O. Blacque and F. Zelder, *Chem. Sci.*, 2016, **7**, 3836-3842.