Synthesis, Spectral Characterization and Crystal Structure of Chlororhodibalamin: A Synthesis Platform for Rhodium Analogues of Vitamin B_{12} and for Rh-Based Antivitamins B_{12}

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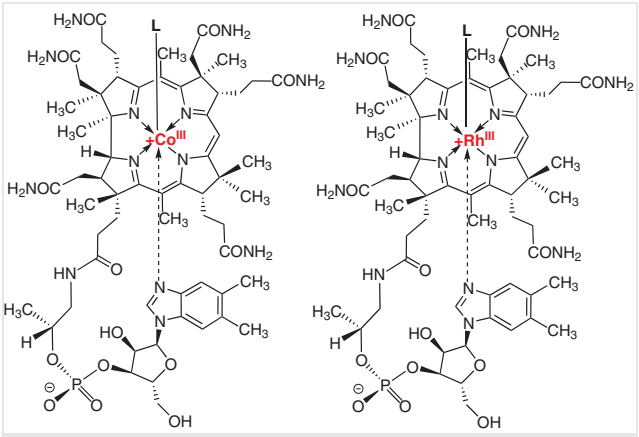
Abstract Chlororhodibalamin (ClRhbl), a rhodium analogue of vitamin B_{12} (cyanocobalamin), was prepared in 84% yield by metalation of the metal-free B_{12} ligand hydrogenobalamin using the Rh^{I}-complex $[Rh(CO)_2Cl]_2$. ClRhbl was identified and characterized by UV/Vis, circular dichroism, high-resolution mass and heteronuclear NMR spectra. The Rh^{III}-corrin ClRhbl features the 'base-on' architecture of vitamin B_{12}. X-ray analysis of single crystals of ClRhbl have revealed its detailed 3D-geometry and close structural similarity to the Co^{III}-analogue chlorocobalamin (ClCbl). ClRhbl is a versatile starting material for the preparation of other rhodibalamins, among them the organometallic derivatives adenosylrhodibalamin and methylrhodibalamin, the Rh analogues of the important coenzyme and cofactor forms of B_{12}, adenosylcobalamin and methylcobalamin.

Key words rhodium, transition metals, vitamins, porphyrins, natural products, antivitamin, metalation, inhibitors

The vitamin B_{12} cofactors are unique cobalt complexes of the structurally intricate and highly substituted natural corrin ligand.[2–7] The biological partnership of cobalt and of the natural corrin ligands is an intriguing feature of the natural B_{12} cofactors and coenzymes that has provoked the questions 'why corrin' and 'why cobalt'.[2,8] It also generated a heightened interest in transition-metal analogues of the vitamin B_{12} derivatives.[9–11] As the closest group IX homologue of cobalt, rhodium is in prime position in this latter respect,[10,12,13] although Rh is not considered a 'bio-metal' and has no known natural biological use.[14] Rh^{III}- and Co^{III}-corrins are expected to have similar structures, but to differ significantly in their reactivity.[10] As non-functional structural cobalamin (Cbl) mimics, the corresponding rhodibalamins (Rhbls) have been proposed to specifically qualify as potential 'antivitamins B_{12}'.[10,15]

We describe here a concise synthesis and detailed structural analysis of chlororhodibalamin (ClRhbl), the Rh^{III}-analogue of the vitamin B_{12} derivative chlorocobalamin (ClCbl) (Scheme 1). Incompletely characterized ClRhbl was reported in the 1970s by Koppenhagen and co-workers, who also used their ClRhbl preparations as starting materials for the synthesis of other partially characterized rhodibalamins.[12] For their work, the metal-free B_{12}-ligand hydrogenobalamin (Hbl) was produced (among other isolates) from a laborious guided biosynthesis employing a *Chromatium* strain grown in the absence of cobalt but supplemented with 5,6-dimethylbenzimidazole (DMB).[12] More recently, a bioengineered specific biosynthetic production of the metal-free corrin hydrogenobyric acid (Hby)[16] has opened up a rational entry to the synthesis of transition-metal analogues of vitamin B_{12}, first realized with Zn.[11] Subsequently, a high-yielding, one-step partial synthesis of Hbl from Hby has also been developed for the rational alternative preparation of this complete metal-free B_{12}-ligand via a chemical-biological path,[17] in order to make Hbl available as a versatile starting material for the direct generation of a range of transition-metal analogues of the cobalamins. So far, we have

used such semisynthetic Hbl for the synthesis of the previously unknown Ni-analogue of vitamin B_{12}, named nibalamin.[17]

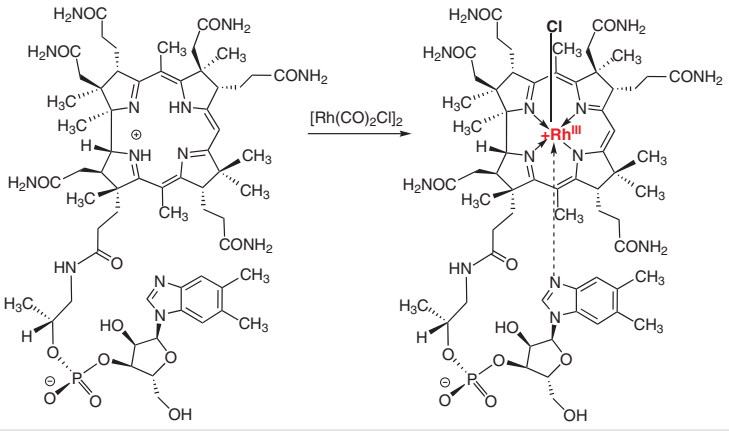


Scheme 1 General structural formula of cobalamins and of rhodibalamins. Left: cobalamins vitamin B_{12} (L = CN, cyanocobalamin, CNCbl), chlorocobalamin (L = Cl, ClCbl), coenzyme B_{12} (L = 5′-deoxy-5′-adenosyl, adenosylcobalamin, AdoCbl), methylcobalamin (L = methyl, MeCbl). Right: rhodibalamins chlororhodibalamin (L = Cl, ClRhbl), adenosylrhodibalamin (L = 5′-deoxy-5′-adenosyl, AdoRhbl), methylrhodibalamin (L = methyl, MeRhbl).

As described herein, the semisynthetic metal-free corrin Hbl[17] also served as the starting material in a high yielding, one-step synthesis of ClRhbl. The orange-yellow Rh^{III}-corrin ClRhbl was prepared by the reaction of Hbl with an excess of μ-dichloro-tetracarbonyl-dirhodium(I) ($[Rh(CO)_2Cl]_2$).[18–20] This substitution labile dimeric Rh^{I} reagent was suitable for the kinetically slow metalation of the ring-contracted corrin present in the zwitterionic metal-free B_{12} ligand Hbl, which undergoes epimerization and tautomerization reactions readily. The reaction in a deoxygenated solution in ethylene glycol, heated at 100 °C (see Scheme 2 and below for experimental details) made use of optimized preparative conditions modified from those used by Koppenhagen and co-workers (ca. 46% estimated yield of ClRhbl),[12] which were based on the original method developed by the Eschenmoser group for the synthesis of a model dicyano-Rh^{III}-corrin.[20,21] Work-up of the raw ClRhbl in the presence of air, purification by preparative HPLC and crystallization from aqueous acetonitrile furnished crystalline ClRhbl in 84% yield (see experimental section).

An aqueous solution of ClRhbl exhibited a UV/Vis-absorption spectrum with characteristic strong maxima at 512 and 485 nm (α- and β-bands) and at 344 nm (γ-band), as similarly reported by Koppenhagen and co-workers (see Figure 1A).[12] The CD spectrum of ClRhbl was well structured and featured a sequence of bands with positive and negative signs typical of the natural corrinoids, and as also observed for AdoRhbl, the Rh-analogue of coenzyme B_{12} (Figure 1B).[10]

In a high-resolution ESI mass spectrum of ClRhbl the pseudo-molecular ion $[M + H]^+$ generated the signal of its base peak at m/z 1408.5104 [see the Supporting Information (SI), Figure S1], confirming the molecular formula of ClRhbl as $C_{62}H_{88}ClN_{13}O_{14}PRh$. A 500 MHz ^1H NMR spectrum of the diamagnetic ClRhbl in D_2O (see Figure 2) revealed the characteristic set of four singlets at low field that arise from the aromatic DMB-protons and from HC10, as well as of a doublet (J = 3 Hz) associated with the anomeric ribose proton HC1R. In the high-field part of the NMR spectrum, ten singlets and a doublet were observed, and these were assigned to the eleven methyl groups attached at the corrin ligand at the benzimidazole pseudo-nucleotide and at the isopropanolamine linker group, respectively. The characteristic high-field shift to 0.64 ppm of the singlet of the methyl group H_3C1A (for atom numbering see Figure S2 in the SI) gave evidence for shielding by the neighbouring DMB moiety in the 'base-on' structure of this 'inorganic' rhodibala-



Scheme 2 Partial synthesis of chlororhodibalamin (ClRhbl) from hydrogenobalamin (Hbl) by addition of a deoxygenated solution of Hbl in ethylene glycol to a carbon monoxide saturated solution of $[Rh(CO)_2Cl]_2$ (4 equiv) in ethylene glycol, heating the air-protected mixture with stirring to 100 °C for one hour and aqueous work-up in the presence of air.

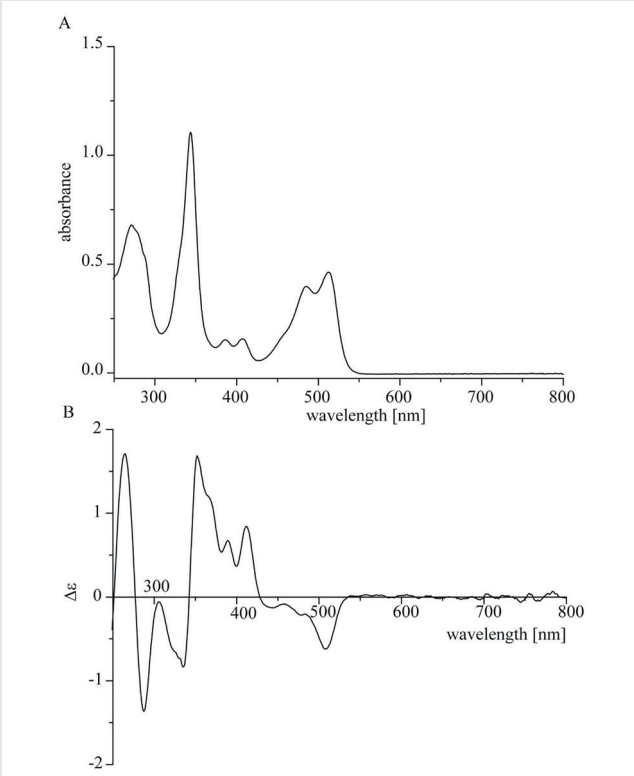


Figure 1 (A) UV/Vis-spectrum of ClRhbl in H_2O (c = 0.032 mM); (B) CD spectrum of ClRhbl in H_2O (c = 0.16 mM).

min. Extensive $^1H,^1H$-homonuclear (COSY and ROESY) as well as $^1H,^{13}C$-heteronuclear (HSQC, HMBC) spectra allowed identification and assignment of the signals of the 73 exchange-resistant protons of ClRhbl and of all of its 62 carbons (see Figures S2–6 and Table S1 in the SI). The NMR spectral information established the basic three-dimensional structure of ClRhbl in aqueous solution.

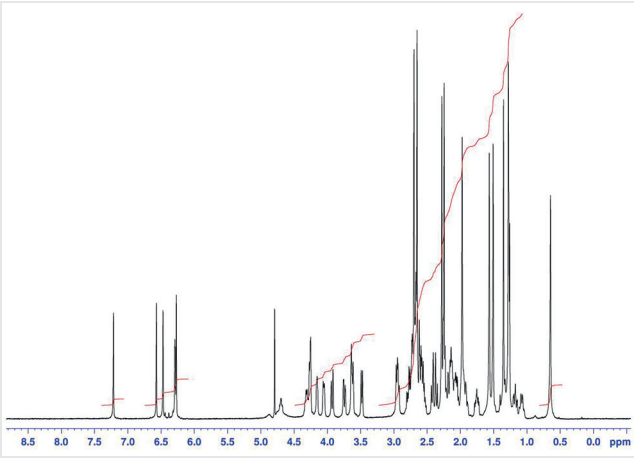


Figure 2 500 MHz ^1H NMR spectrum of ClRhbl in D_2O (c = 4.2 mM, D_2O, 298 K, with suppression of HDO-signal).

Interestingly, whereas the Co^{III}-analogue ClCbl hydrolyses and loses its chloride ion readily (and reversibly in the presence of a high chloride concentration) in aqueous solution,[22] the analogous hydrolysis of ClRhbl was not observed at room temperature. The removal of the chloride ion of ClRhbl can be induced by $AgNO_3$ or by hydride reduction of ClRhbl to the analogous Rh^I form (e.g., by sodium borohydride, see below).[12] ClRhbl crystallized from aqueous acetonitrile, furnishing single crystals (orthorhombic space group $P2_12_12_1$) suitable for analysis by X-ray crystallography.[23] The highly resolved crystal structure confirmed the NMR-derived 'base-on' nature of ClRhbl, as well as the presence of a chloride ion as axial ligand at the 'upper' β-face of the Rh^{III} centre (Figure 3). Furthermore, it provided detailed insights into the molecular structure of ClRhbl (see Table S2 in the SI), revealing it as isostructural to the cobalt analogue ClCbl (see Figures S3, S4 in the SI).[24]

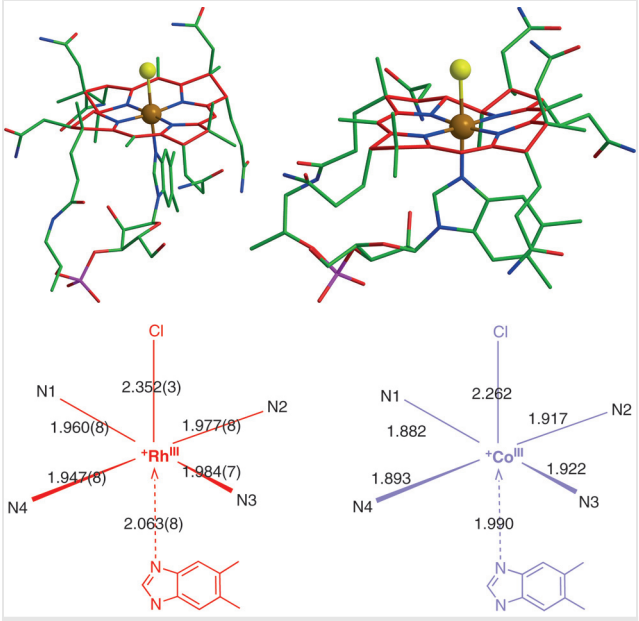


Figure 3 (Top) Structure of ClRhbl from X-ray crystal analysis shown as stick model in two projections, where C-atoms in the core of the corrin moiety are coloured red, and those of the sidechains and of the nucleotide group are coloured green (Rh- and Cl-atoms are highlighted as brown and yellow spheres, respectively). (Bottom) Crystallographic lengths (in Å) of the bonds around the corrin-bound homologous d^6-ions Rh^{III} and Co^{III} in the structures of ClRhbl (left) and chlorocobalamin (ClCbl)[24] (right), respectively.

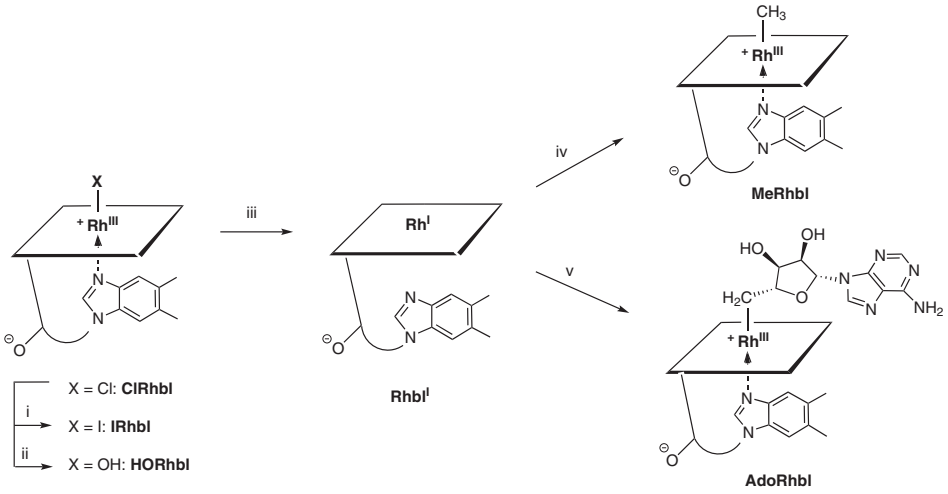
When comparing the structures of ClRhbl and of ClCbl[24] (or of a more recently analyzed crystallized ester derivative of ClCbl[25]) the four equatorial bonds were longer by an average of about 0.06 Å in the Rh^{III}-corrin ClRhbl, as roughly expected, based on the larger size of low-spin Rh^{III} centres compared to Co^{III} ions.[26] Likewise, the lengths of the axial bonds in ClRhbl, observed as Rh-Cl_β = 2.352(3) Å and Rh-N_α = 2.063(8) Å, were longer by about 0.08 Å. Similarly, longer axial and equatorial bonds had also been observed when

comparing the crystal structures of the organometallic pair AdoRhbl and AdoCbl.[10] Interestingly, the order of the relative lengths of the axial bonds is inverted in both of the 'inorganic' chloro complexes, ClCbl (Cl-Co^{III} > Co^{III}-N_α)[24] and ClRhbl (Cl-Rh^{III} > Rh^{III}-N_α), when compared to the analogous organometallic pair adenosylcobalamin (AdoCbl) and its Rh-analogue adenosylrhodibalamin (AdoRhbl), where Ado-Co^{III} < Co^{III}-N_α and Ado-Rh^{III} < Rh^{III}-N_α.[10] Remarkably, these observations indicate a quantitatively comparable (structural) *trans*-influence[27] of the axial ligands in the rhodibalamins ClRhbl and AdoRhbl and in the cobalamins ClCbl and AdoCbl. A roughly similar geometric behaviour of the Co^{III}- and Rh^{III}-ions in the respective chloro-corrins is further supported by the insignificantly different corrin fold[28] in ClCbl and in ClRhbl, with fold angles of 17.8°[27,29] and 17.4°, respectively. Hence, the previously derived suggestions, based on the detailed structures of the organometallic homologues AdoCbl and AdoRhbl, that the larger Rh^{III}-ions show a similar (but apparently slightly better) fit for the natural corrin ligand compared to Co^{III} ions, and that corresponding Co^{III}- and Rh^{III}-corrins are probably isostructural,[10] are verified here for the analogous pair of the 'inorganic' Cl-Co^{III}- and Cl-Rh^{III}-corrins ClCbl and ClRhbl (see Figure S3 in the SI).

Herein, a high-yield, one-step partial synthesis of crystalline ClRhbl is reported that opens up a door for the direct preparation of a range of rhodibalamins (Rhbls), as previously explored in part by Koppenhagen and co-workers in the 1970s.[12] One of these, adenosylrhodibalamin (AdoRhbl), the rhodium analogue of coenzyme B_{12}, was recently prepared by an intricate combination of biological and chemical synthetic steps.[10] A more direct alternative route to AdoRhbl has been explored here in a preliminary form via the reduction of a deoxygenated (Ar saturated) solution of ClRhbl in 20% aqueous MeOH (6 min), with an excess of sodium borohydride, and subsequent treatment of the yellow solution, with an excess of 5-desoxy-5-iodoadenosine (4 min) at room temperature, allowing for the preparation of crystalline AdoRhbl in 63% isolated yield (Scheme 3; see experimental section and the SI). Along these lines, a high-yield synthesis of methyl-rhodibalamin (MeRhbl), the Rh-analogue of the B_{12}-cofactor MeCbl, and the preparation of 'inorganic' analogues of ClRhbl, such as iodorhodiblamin (IRhbl), have also been explored and will be delineated in due course, together with the full characterization of the spectroscopic and structural properties of these Rhbls.

The herein fully characterized Rh^{III}-corrin ClRhbl promises to constitute a general and efficient synthesis platform to a variety of 'inorganic' and 'organometallic' Rhbls via formal ligand substitution, opening the field for more extensive studies of the chemistry of Rhbls. However, the biological chemistry and activity of ClRhbl itself may also be of specific interest in view of recent insights into the widespread bacterial B_{12}-dependent reductive dehalogenases,[30] where the formation of a cobalt–halogen bond has been proposed to represent the mechanistically critical step of the dehalogenation reaction in some[31] (but not all[32]) of these enzymes.

Preliminary findings suggest a significantly different chemical reactivity of Rhbls from that of the corresponding Cbls. Hence, the Rhbls MeRhbl and AdoRhbl are analogues of MeCbl and of AdoCbl, respectively, yet lacking the specific reactivity of these latter organometallic Cbls. Furthermore, as discussed here, the corresponding Rhbls and Cbls should have similar structures, as was first proposed with the organometallic pair AdoRhbl and AdoCbl.[10] The de-



Scheme 3 ClRhbl as synthesis platform for re-functionalized Rhbls. Schematic outline of the partial synthesis at room temperature of 'inorganic' and 'organometallic' Rhbls from ClRhbl by formal ligand substitution, either (*i*) KI in H_2O, or (*ii*) $AgNO_3$ in H_2O,[12] or (*iii*) by reduction to the presumed (still minimally characterized) Rh^I-form of Rhbl ($Rhbl^I$) with $NaBH_4$ in 20% aq MeOH, followed by (*iv*) methylation with either MeI[12] to generate MeRhbl, or (*v*) adenosylation with 5-desoxy-5-iodoadenosine to prepare AdoRhbl.

duced chemical relationships between corresponding Cbls and Rhbls may be considered to represent a reliable foundation for the suggested, rather general suitability of Rhbls as potential antivitamins B_{12},[15,33] to be analyzed biochemically and in further biological and biomedical tests.[15,34–37] Indeed, AdoRhbl and MeRhbl, the organometallic Rh-analogues of AdoCbl and of MeCbl, were shown,[10] or are presumed,[9,34] to represent specific inhibitors of AdoCbl- or MeCbl-dependent enzymes, respectively. In consequence, AdoRhbl and MeRhbl may act as specific B_{12} antimetabolites in a range of organisms that use adenosyl- or methylcobamides for a functioning metabolism and gene regulation. The rational entry to a variety of Rhbls via ClRhbl may, thus, open up a path to a new class of potentially highly effective antibiotics and anticancer agents, of particular interest in B_{12}-based chemical biology and (bio)medicine.[10,15]

Experimental section

5′-Iodo-5′-deoxy-adenosine was prepared as described.[38] Water was deionized using Epure, Barnstead Co.; acetic acid was distilled over P_2O_5 prior to use; acetonitrile and methanol HPLC gradient grade were from BDH Prolabo; μ-dichloro-tetracarbonyldirhodium(I) ($[Rh(CO)_2Cl]_2$), methyl tosylate, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium borohydride, purum, ethane-1,2-diol were all from Sigma Aldrich. 1 g Sep-Pak-C18 Cartridges were purchased from Waters Associates. LiChroprep RP-18 (25–40 μm) and TLC aluminium sheets, silica gel 60 RP-18 F254S were from Merck.

UV/Vis spectra were recorded with a Hitachi-U3000, λ_{max} in nm (log ε); CD spectra were recorded with a JASCO J-715 spectrometer (λ_{max}, λ_{min} and λ_o in nm, (Δε)). ^1H and ^{13}C NMR spectra were recorded with a 500 MHz Varian Unity Inova instrument, equipped with 5 mm triple-resonance probe with z-gradients, in D_2O, 298 K, δ(HDO) = 4.79 ppm, coupling constants *J* in Hz. ESI-HR-MS were recorded with an LTQ-Orbitrap (Thermo-Scientific) positive-ion mode, spray voltage 4.5 kV, in MeOH/H_2O 9:1 (v/v), *m*/*z* (relative intensity in % in reference to basis signal), signals >5% are listed. A Hitachi HPLC system with manual sampler was used for chromatography, L-2130 pump, online degasser and diode array detector L2130 (online-UV/Vis Spectra), reverse phase C18 column (YMC, RP18, 250 × 4.6 mm), flow rate of 1.0 mL min^{-1}; solvent composition 10 mM ammonium acetate (pH7), methanol, linear increase in 40 min from 8 to 95% methanol.

Purification of the Rhodibalamin Samples

Desalting of aqueous solutions was performed using Waters Sep-Pak Plus RP-18 cartridges, which had been conditioned with 10 mL MeOH followed by an equilibration/wash with 10 mL of H_2O. Aqueous solutions of the samples were loaded on the cartridge, which was then washed with 20 mL of H_2O. The purified samples were eluted with 5–10 mL of MeOH (until all coloured material was completely eluted). HPLC conditions were: RP18 Phenomenex 250 × 4.6 mm, flow 1.0 mL min^{-1}, phosphate buffer pH 7 (10 mM), MeOH, linear gradient 2–40% MeOH in 20 min, online UV/Vis detection at 350 nm.

Chlororhodibalamin (ClRhbl)

μ-Dichloro-tetracarbonyldirhodium(I) ($[Rh(CO)_2Cl]_2$ 6.89 mg, 17.7 μmol) was dissolved in 1.5 mL ethylene glycol under a carbon monoxide atmosphere. Hbl (6.2 mg, 5.1 μmol)[17] was dissolved in ethylene glycol (3.5 mL) and the solution was degassed three times by freeze-pump-thaw with argon. After addition of the solution of $[Rh(CO)_2Cl]_2$ to the Hbl solution under protection from air, the stirred red-orange reaction mixture was heated to 100 °C and stirring was continued for one hour. The red-orange reaction solution was cooled to r.t. and deionised water (5 mL) was added. The red-orange reaction mixture was filtered and desalted with a 1 g RP-18 cartridge, followed by removal of the solvents by evaporation under reduced pressure on a rotary evaporator. The brown-red residue was dissolved in deionized water and purified by preparative HPLC. Methanol was removed on a rotary evaporator and ClRhbl was isolated from the remaining yellow-orange aqueous solution by desalting with a 1 g RP-18 cartridge and removal of solvents. Crystallization from water and acetonitrile gave pure yellow-orange ClRhbl (4.5 μmol, 84% yield).[23]

UV/Vis (H_2O, 0.032 mM): λ (nm) (log ε) = 512 (4.16), 485 (4.09), 407 (3.69), 386 (3.68), 344 (4.54), 271 (4.33) (Figure 1).

CD (H_2O, *c* = 0.16 mM): λ_{max} (Δε), λ_{min} (Δε) = 508 (–0.6), 484 (–0.2), 478 (–0.2), 458 (–0.1), 442 (–0.1), 412 (0.8), 400 (0.4), 389 (0.7), 382 (0.5), 366sh (1.2), 352 (1.7), 335 (–0.8), 329 (–0.7), 323 (–0.6), 306 (–0.1), 287 (–1.4), 265 (1.7), 242 (–0.8); λ_o: 429, 342, 277, 251 (Figure 1).

^1H NMR (500 MHz, D_2O, 298 K): δ = 7.20 (s, 1 H, HC7N), 6.59 (s, 1 H, HC2N), 6.47 (s, 1 H, HC4N), 6.29 (d, *J* = 3 Hz, 1 H, HC1R) superimposed by 6.27 (s, 1 H, HC10), 4.71 (m, 1 H, HC3R), 4.32/4.26/4.26 (m, 3 H, HC176, HC19, HC2R), 4.16 (m, 1 H, HC3), 4.05 (m, 1 H, HC4R), 3.92/3.74 (AB-system, *J* = 13 Hz, 2 H, H_2C5R), 3.65–3.60 (m, 2 H, HC8, H_BC175), 3.47 (m,1 H, HC13), 2.95–2.90 (m, 2 H, HC18, H_AC175), 2.8–2.5 (m, H_2C171, H_2C181, H_2C132, H_2C32, H_BC71) superimposed by 2.70 (s, H_3C151) and 2.65 (s, H_3C51), in total 15 H, 2.43/2.36 (AB-system, *J* = 18 Hz, 2 H, H_2C21), 1.8 – 2.3 (m, H_2C31, H_AC71, H_2C172, H_2C131) superimposed by 2.27 (s, H_3C10N), 2.24 (s, H_3C11N) and 1.97 (s, H_3C7A), in total 19 H, 1.75 (m, 1 H, H_BC82), 1.56 (s, 3 H, H_3C12A), 1.51 (s, 3 H, H_3C2A), 1.4–1–1 (m, H_2C177, H_AC82, H_BC81) superimposed by 1.35 (s, H_3C17B), 1.28 (s, H_3C12B), in total 10 H, 1.08 (m, 1 H, HAC81), 0.64 (s, 3 H, H_3C1A) (see Figure 2 and the SI).

^{13}C NMR: indirect detection of signals and assignment from heteronuclear ^1H,^{13}C-HSQC and ^1H,^{13}C-HMBC spectra measured at 500 MHz (see Figures S4, S5 and Table S1 in the SI).

HRMS (ESI pos, LTQ-Orbitrap, MeOH/H_2O (9:1)): *m*/*z* (%) = 1433.4939 (10), 1432.4937 (25), 1431.4963 (37), 1430.4928 (44, $[M + Na]^+$), 1411.5133 (27), 1410.5120 (61), 1409.5135 (82), 1408.5104 (100, $[M + H]^+$).

HRMS: *m*/*z* $[M + H]^+$ calcd for $C_{62}H_{89}ClN_{13}O_{14}PRh^+$: 1408.5128; found: 1408.5104 (see Figure S1 in the SI).

Adenosylrhodibalamin (AdoRbl)

In a small glass tube, 5′-iodo-5′-deoxyadenosine (1.47 mg, 4 μmol) was dissolved in methanol (0.29 mL) and deoxygenated for 15 minutes with a stream of argon. ClRhbl (0.5 mg, 0.36 μmol) was dissolved in aqueous methanol (1.47 mL 20% v/v) and degassed three times by freeze-pump-thaw with argon in a Schlenk flask. To the air-protected ice-water-cooled orange solution of ClRhbl, $NaBH_4$ (2.9 mg, 77 μmol) was added. After stirring the solution for 6 min in the dark, the air-protected methanolic solution of 5′-iodo-5′-deoxyadenosine was added and, after 4 minutes, the pH of the solution was adjusted to pH 5 with acetic acid. After 90 min, the solvent was evaporated on a rotary evaporator. The residue was dissolved in deionized water and purified by preparative HPLC, separating the reaction mixture of 63% AdoRhbl, 11% ClRhbl, 4% hydroxo-rhodibalamin (HORhbl) and 5% iodo-rhodibalamin (IRhbl) as well as about 15% of less polar rhodibalamin side products. The four defined Rhbl fractions (AdoRhbl, HORhbl, ClRhbl and IRhbl) were each isolated raw by desalting with a 1 g RP-18 cartridge and removal of solvents and then tentatively identified by their mass spectral properties. AdoRhbl (0.36 mg, 0.22 μmol,

63%) was crystallized from deionized water and acetonitrile and was identified by comparison with authentic material[10] of UV/Vis, ^{1}H NMR and HR-ESI-MS-spectra (see the SI).

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0040-1707288.

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