Synthesis, Spectral Characterization and Crystal Structure of Chlororhodibalamin: A Synthesis Platform for Rhodium Analogues of Vitamin B₁₂ and for Rh-Based Antivitamins B₁₂

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Abstract Chlororhodobilamin (ClRhbl), a rhodium analogue of vitamin B₁₂ (cyanocobalamin), was prepared in 84% yield by metalation of the metal-free B₁₂ ligand hydrogenobalamin using the RhI-complex [Rh(CO)₂Cl]₂. ClRhbl was identified and characterized by UV/Vis, circular dichroism, high-resolution mass and heteronuclear NMR spectra. The Rh₃-corrin ClRhbl features the ‘base-on’ architecture of vitamin B₁₂. X-ray analysis of single crystals of ClRhbl have revealed its detailed 3D-geometry and close structural similarity to the Co₃-analogue chlorocobalamin (CICbl). 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 B₁₂, adenosylcobalamin and methylcobalamin.

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

The vitamin B₁₂ cofactors are unique cobalt complexes of the structurally intricate and highly substituted natural corrin ligand. The biological partnership of cobalt and of the natural corrin ligands is an intriguing feature of the natural B₁₂ cofactors and coenzymes that has provoked the questions ‘why corrin’ and ‘why cobalt’. It also generated a heightened interest in transition-metal analogues of the vitamin B₁₂ derivatives. As the closest group IX homologue of cobalt, rhodium is in prime position in this latter respect, although Rh is not considered a ‘bio-metal’ and has no known natural biological use. Rh₃- and Co₃-corrins are expected to have similar structures, but to differ significantly in their reactivity. As non-functional structural cobalamin (Cbl) mimics, the corresponding rhodibalamin (Rhbls) have been proposed to specifically qualify as potential ‘antivitamins B₁₂’.

We describe here a concise synthesis and detailed structural analysis of chlororhodobilamin (ClRhbl), the Rh₃-analogue of the vitamin B₁₂ 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. For their work, the metal-free B₁₂-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). More recently, a bioengineered specific biosynthetic production of the metal-free corrin hydrogenoboric acid (Hby) has opened up a rational entry to the synthesis of transition-metal analogues of vitamin B₁₂, first realized with Zn. 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 B₁₂-ligand via a chemical-biological path, in order to make Hbl available as a versatile starting material for the direct generation of a range of transition-metal analogues of the cobalamin. So far, we have...
used such semisynthetic Hbl for the synthesis of the previously unknown Ni-analogue of vitamin B$_{12}$, named nibalamin.$^{17}$

As described herein, the semisynthetic metal-free corrin Hbl$^{17}$ also served as the starting material in a high-yielding, one-step synthesis of CIRhbl. The orange-yellow Rh$^{	ext{III}}$-corrin CIRhbl was prepared by the reaction of Hbl with an excess of μ-dichlorotetracarbonyldirhodium(I) ([Rh(CO)$_2$Cl]$_2$)$^{18–20}$ This substitution labile dimeric Rh$^{	ext{II}}$ 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 CIRhbl)$^{12}$ which were based on the original method developed by the Eschenmoser group for the synthesis of a model dicyano-Rh$^{	ext{III}}$-corrin.$^{20,21}$ Work-up of the raw CIRhbl in the presence of air, purification by preparative HPLC and crystallization from aqueous acetonitrile furnished crystalline CIRhbl in 84% yield (see experimental section).

An aqueous solution of CIRhbl 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 CIRhbl 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 CIRhbl 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 CIRhbl as C$_{62}$H$_{88}$ClN$_{13}$O$_{14}$PRh. A 500 MHz $^1$H NMR spectrum of the diamagnetic CIRhbl in D$_2$O (see Figure 2) revealed the characteristic set of four singlets at low field that arise from the aromatic DMB-protons and from HC$_1$R. 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 and from the amine protons and from HC$_{1}$R. 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 and from the amine protons and from HC$_{1}$R. 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 and from the amine protons and from HC$_{1}$R.
min. Extensive $^1$H,$^1$H-homonuclear (COSY and ROESY) as well as $^1$H,$^{13}$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.

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,\textsuperscript{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$^{III}$ form (e.g., by sodium borohydride, see below).\textsuperscript{12} ClRhbl crystallized from aqueous acetonitrile, furnishing single crystals (orthorhombic space group P2$_1$2$_1$2$_1$) suitable for analysis by X-ray crystallography.\textsuperscript{23} The highly resolved crystal structure confirmed the NMR-derived ‘base-on’ nature of ClRhbl, as well as the presence of a chloride ion as axial ligand at the ‘upper’ β-face of the Rh$^{III}$ centre (Figure 3). Furthermore, it provided detailed insights into the molecular structure of ClRhbl (see Table S2 in the SI), revealing it as isostuctural to the cobalt analogue CICbl (see Figures S3, S4 in the SI).\textsuperscript{24}

When comparing the structures of ClRhbl and of CICbl\textsuperscript{24} (or of a more recently analyzed crystallized ester derivative of CICbl\textsuperscript{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.\textsuperscript{26} Likewise, the lengths of the axial bonds in ClRhbl, observed as Rh–Cl$\beta$ = 2.352(3) Å and Rh–N$_\alpha$ = 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. Interestingly, the order of the relative lengths of the axial bonds is inverted in both of the ‘inorganic’ chloro complexes, CI-Cbl (Cl-CoIII > CoIII-Nr) and CI-Rhbl (Cl-RhIII > RhIII-Nr), when compared to the analogous organometallic pair adenosylcobalamin (AdoCbl) and its Rh-analogue adenosylrhodibalamin (AdoRhbl), where Ado-CoIII < CoIII-Nr and Ado-RhIII < RhIII-Nr. Remarkably, these observations indicate a quantitatively comparable (structural) trans-influence of the axial ligands in the rhodibalamins CI-Rhbl and AdoRhbl and in the cobalamins CI-Cbl and AdoCbl. A roughly similar geometric behaviour of the CoIII- and RhIII-ions in the respective chloro-corrins is further supported by the insignificantly different corrin fold in CI-Cbl and in CI-Rhbl, with fold angles of 17.8° and 17.4°, respectively. Hence, the previously derived suggestions, based on the detailed structures of the organometallic homologues AdoCbl and AdoRhbl, that the larger RhIII-ions show a similar (but apparently slightly better) fit for the natural corrin ligand compared to CoIII-ions, and that corresponding CoIII- and RhIII-corrins are probably isostructural, are verified here for the analogous pair of the ‘inorganic’ Cl-CoIII- and Cl-RhIII-corrins CI-Cbl and CI-Rhbl (see Figure S5 in the SI).

Herein, a high-yield, one-step partial synthesis of crystalline CI-Rhbl 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. One of these, adenosylrhodibalamin (AdoRhbl), the rhodium analogue of coenzyme B12, was recently prepared by an intricate combination of biological and chemical synthetic steps. 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 CI-Rhbl 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-idoadenosine (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 B12-cofactor MeCbl, and the preparation of ‘inorganic’ analogues of CI-Rhbl, such as iodorhodibalamin (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 RhIII-corrin CI-Rhbl 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 CI-Rhbl itself may also be of specific interest in view of recent insights into the widespread bacterial B12-dependent reductive dehalogenases, where the formation of a cobalt–halogen bond has been proposed to represent the mechanistically critical step of the dehalogenation reaction in some (but not all) 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. The de-
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 anticambios B12-based chemical biology and (bio)medicine.10,15 Thus, open up a path to a new class of potentially highly effective inhibitors of AdoCbl- or MeCbl-dependent enzymes, respectively. In consequence, AdoRhbl and MeRhbl may act as specific B12 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 B12-based chemical biology and (bio)medicine.10,15

Experimental section

5'-Iodo-5'-deoxy-adenosine was prepared as described.38 Water was deionized using Eurep, Barnstead Co.; acetic acid was distilled over P2O5 prior to use; acetonitrile and methanol HPLC gradient grade were from BDH Prolabo; μ-dichloro-tetracarbonyldiiodoruthenium(I) ([Rh(CO)2Cl]2), methyl tosylate, potassium dihydrogen phosphate, di-sodium hydrogen phosphate, sodium borohydride, purum, ethanediol 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, λmax in nm (log ε); CD spectra were recorded with a JASCO-J-715 spectrometer (λmax, λmin and λnm in nm (Δε)).1,1H and 13C NMR spectra were recorded with a 500 MHz Varian Unity Inova instrument, equipped with 5 mm triple-resonance probe with z-gradients, in D2O, 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 95% methanol. 1H and 13C NMR spectra are provided in the SI.

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 H2O. Aqueous solutions of the samples were loaded on the cartridge, which was then washed with 20 mL of H2O. 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-tetracarbonyldiiodoruthenium(I) ([Rh(CO)2Cl]2) 6.89 mg, 17.7 μmol) was dissolved in 1 mL ethylene glycol under a carbon monoxide atmosphere. Hbl (6.2 mg, 5.1 μmol) 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 deionized water (5 mL) was added. The red-orange reaction mixture was filtered and desalted with 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 acetoneitrile gave pure yellow-orange ClRhbl (4.5 μmol, 84% yield).23

UV/Vis (H2O, 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).

H2O, c = 0.16 mM: λmax (Δεmin) = 508 (–0.6), 484 (–0.2), 472 (–0.2), 458 (–0.1), 442 (0.8), 400 (0.4), 389 (0.7), 382 (0.5), 363 (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); λmin; 429, 342, 277, 251 (Figure 1).

1H NMR (500 MHz, D2O, 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, HC1O), 4.71 (m, 1 H, HC3O), 4.32/4.26/4.26 (m, 3 H, 3J = 18 Hz, 2 H, H2C21), 1.8 – 2.3 (m, H2C31, H2C71, H4C71, H4C11, H2C17, H2C12, H4C71, H4C12) superimposed by 2.70 (s, HC151) and 2.65 (s, HC51), in total 15 H, 4.23/2.36 (AB-system, J = 18 Hz, 2 H, HC21), 1.8 – 2.3 (m, H2C31, H2C71, H2C17, H2C12) superimposed by 2.27 (s, H2C1O), 2.24 (s, HC11N) and 1.97 (s, HC7A), in total 19 H, 1.75 (m, 1 H, HC8B2), 1.56 (3 H, HC12A), 1.51 (3 H, HC2A), 1.4–1.1 (m, H2C17, H2C8B, H2C8) superimposed by 1.35 (s, HC17B), 1.28 (s, HC12B), in total 10 H, 1.08 (m, 1 H, HC8B1), 0.84 (3 H, 3 H, HC1A) (see Figure 2 and the SI).

13C NMR: indirect detection of signals and assignment from heteronuclear H1-C-HSQC and H1,C-HMBC spectra measured at 500 MHz (see Figures S4, S5 and Table S1 in the SI).

HRMS (ESI pos, LTQ-Orbitrap, MeOH/H2O (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)+ calculated for C16H15N7O2P2Rh+: 1408.5128; found: 1408.5104 (see Figure S1 in the SI).

Adenosyorthodibalamin (AdoRhbl)

In a small glass tube, 5'-ido-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, NaBH4 (2.9 μg, 77 μmol) was dissolved in methanol (0.29 mL) and deoxygenated for 15 minutes. After stirring the solution for 6 min in the dark, the air-protected methanolic solution of 5'-ido-5'-deoxyadenosine was added and, after 4 minutes, the pH of the solution was adjusted to pH 5 with acetic acid. After 90 minutes, 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-rodibalamin (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\(^\text{10}\) of UV/Vis, \(^1\text{H}\) NMR and HR-EI-MS-spectra (see the SI).

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**Supporting Information**

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**References**

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(23) X-ray crystal data of ClRhbl have been deposited at the Cambridge Crystallographic Data Centre. CCDC 2017112 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/getstructures. See the Supporting Information for the CIF file with respect to the structure analysis of ClRhbl.
(35) Interest in antivitamins \(B_{12}\) (see ref. 33) has induced the Zelder group\(^26\) to explore a remarkable alternative approach to transition-metal analogues of vitamin \(B_{12}\), starting with a 5,6-seco-5,6-dioxo-cobalamin from photooxygcnolysis of vitamin \(B_{12}\).\(^27\) In their approach, chemical extrusion of cobalt from the 5,6-seco-5,6-dioxo-cobalamin, followed by reductive ring closure, gave a first 5,6-dihydroxy-5,6-dihydrohydroxobalamin, which was metallated using NiSO\(_4\), furnishing a 5,6-dihydroxy-5,6-dihydrohydroxobalamin.