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Encasing the paramagnetic copper(II)-ion by the ring-contracted corrin ligand of vitamin B_{12}†‡

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The d^9-Cu(II)-corrin cupribyrate (Cuby) was synthesized in 93% crystalline yield by rapid chelation of Cu^{2+}-ions by the metal-free corrin-ligand of vitamin B_{12}. Single crystals of the EPR-active Cuby allowed for the first X-ray structure determination of a Cu-corrin. SCF-calculations provided insights complementary to the experimental data of Cuby and indicated an out-of-plane displacement of the reduced d^{10}-Cu(I)-ion, consistent with the observed reductive activation of Cuby towards loss of its Cu-center.

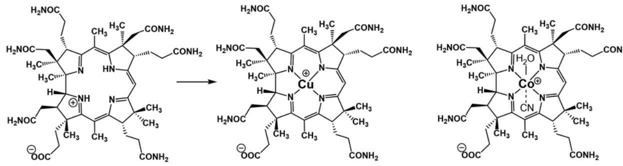
The ring-contracted natural corrin ligand of the B_{12}-derivatives is a uniquely skewed, helical environment[1,2] that binds cobalt-ions very tightly.[3,4] This biosynthetically costly ligand for cobalt[5,6] represents a precisely evolved entatic state module,[2] giving B_{12}-cofactors the unique capacity for their exceptional bio-organometallic catalysis.[7–9] The complementary fundamental question, why cobalt? in B_{12}-cofactors,[1,3,9,10] has generated the long-standing experimental quest for non-cobalt analogues of the B_{12}-derivatives,[11,12] a challenge met by newly developed synthetic approaches.[2,13] We have, thus, prepared Rh(III)-,[13–17] Ni(II)-[18] and Zn(II)-complexes[19] of natural corrin ligands for studies of their structures and reactivity. Here, we report on cupribyrate (**Cuby**) (Scheme 1), the Cu(II)-complex of hydrogenobyric acid (**Hby**),[2] including the first Cu-corrin X-ray crystal structure.

The complexation of metal-free **Hby** with Cu(II)-ions occurred readily at room temperature (RT) in a 0.25 M aqueous solution of Cu(II)-acetate at pH 6 and was practically quantitative within 90 min (see the ESI‡). It did not require the reported strong heating ('brief boiling').[11,20] Crystallization of the raw **Cuby**-isolate from water/acetonitrile mixtures furnished **Cuby** in >93% yield.

The UV/Vis-spectrum (Fig. 1) of an aqueous solution of **Cuby** exhibits a corrin-type and is comparable to the earlier reported spectra of partially characterized Cu(II)-corrins.[20,21] UV/Vis- and CD-spectra of **Cuby** show remarkably similar features to the corresponding spectra of the Zn(II)-complex[19] of **Hby**, consistent with the dominating role of the corrin chromophore for the spectral signature in the UV- and Vis-range. A HR-ESI mass spectrum of **Cuby** confirmed the calculated molecular formula of $C_{45}H_{64}CuN_{10}O_8$ (see the ESI,‡ Fig. S1).

Glassy frozen solutions of the paramagnetic Cu(II)-corrin **Cuby** in 20% glycerol in H_2O showed the typical EPR-signature (see Fig. 2, for a spectrum at T = 148 K) of a roughly square-planar 4-coordinate Cu(II)-N_4-complex with an index[22,23] g^{II}/A^{II} = 98.2 cm, assigning an exceptionally low value to the encasement of the Cu(II)-ion by the corrin ligand (see the ESI‡ for further details).

The neutral cupribyrate **Cuby** crystallized from an aqueous solution upon addition of acetonitrile. The monoclinic crystals (space group $P2_1$) contain two **Cuby** molecules per unit cell, as well as molecules of water and acetonitrile (ordered near the **Cuby**-carboxylate). The Cu(II)-center of the **Cuby** molecule sits only +0.033 Å above the mean plane of the four 'inner' corrin N-atoms, which span an unsymmetrical and nearly planar coordination pattern (see Fig. 3), as reflected by the value of



Scheme 1 Structure-based outline of the synthesis of cupribyrate (**Cuby**) from hydrogenobyric acid (**Hby**) (see the ESI‡) and the structural formula of Co_αcyano, Co_βaquo-cobyric acid (**Coby**).

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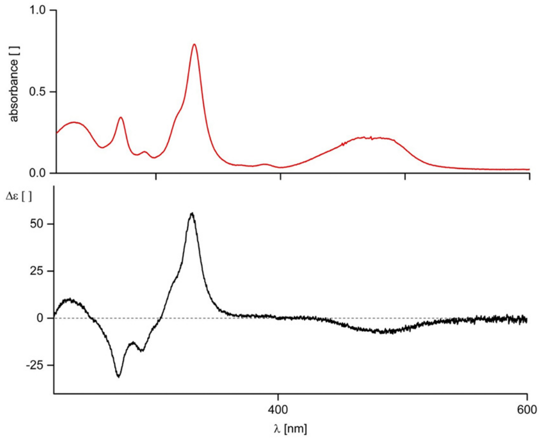

Fig. 1 UV/Vis- and CD-spectra of **Cuby** (19 μM in 10 mM aqueous phosphate pH 7).

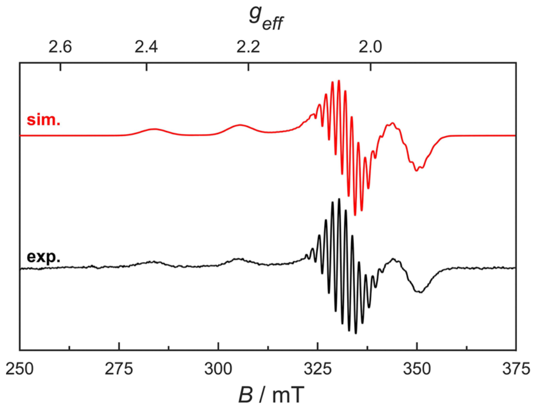

Fig. 2 EPR-spectrum of a 1.34 mM frozen solution (at T = 148 K) of **Cuby** in H_2O:glycerol (4 : 1) and its simulation with key parameters obtained by least square fitting (for details see the ESI,‡ Table S1). The spectra exhibited a significant T-dependence, with a maximum signal intensity of around 200 K and continuous decrease at lower temperatures (see the ESI,‡ Fig. S4).

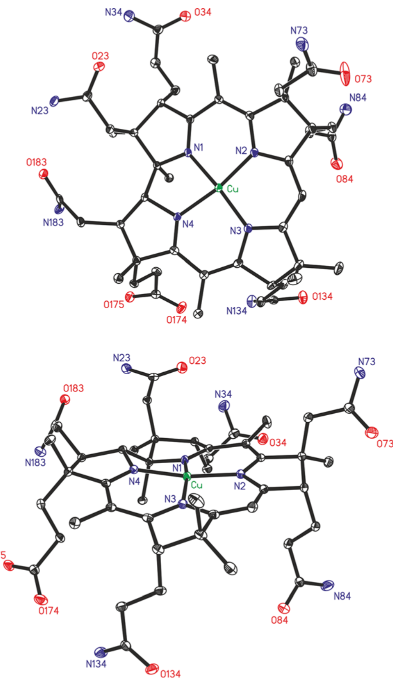

Fig. 3 X-ray crystal structure of **Cuby** in ORTEP-representations. Top: axial view from above (β-side); bottom: approximate in-plane view, revealing the slightly nonplanar 4-fold coordination of the encased Cu(II)-ion.

the geometry index τ_4 = 0.17.[24] However, **Cuby** exhibits a less planar arrangement around its 4-coordinate d^9 Cu(II)-center, than in the Ni(II)-corrin nibyrate (**Niby**),[18] which experiences a better fit of its 4-coordinate low-spin d^8 Ni(II)-ion (see the ESI,‡ Table S3). The average Cu–N distance in **Cuby** amounts to 1.91 Å, merely 0.05 Å longer than in **Niby**, in which the 0.08 Å smaller low spin d^8-ion Ni(II)[25] induced a slight contraction.[18] In fact, binding of the d^9 Cu(II)-ion largely retains the architecture of the coordination hole of the metal-free corrin ligand **Hby**, expanded by two 'inner' protons.[2] In **Cuby**, the critical angle parameters corrin-fold[26] (10.0°) and corrin helicity[2] (12.4°) are also similar to those of the ligand **Hby**,[2] but remarkably larger than in **Niby**. Likewise, the angle between the planes N1–Cu–N2 and N3–Cu–N4 (roughly 13.6°) relating to the inner coordination-sphere around the Cu(II)-center (see the ESI,‡ Table S3) is close to the value derived for **Hby**.[2] Interestingly, the N1–M–N3 pseudo-diagonal in **Cuby** was roughly 0.07 Å shorter than the N2–M–N4 counterpart, thus displaying a larger difference of the distances across these pseudo-diagonals than in **Niby**. This desymmetrization of the corrin core in **Cuby** also goes along the one observed in **Hby** and its Zn(II)-complex,[19] but is insignificant in the Co(II)-corrins Co(II)-cobalamin (**Cbl^{II}**)[27] and cob(II)ester[28] and in typical Co(III)-corrins, such as coenzyme B$_{12}$ (**AdoCbl**)[29] and vitamin B$_{12}$.[30,31]

Our self-consistent field (SCF) in the gas-phase calculation of cupribyric acid (**HCuby+**), the cationic carboxylate-protonated form of **Cuby**, used the atomic coordinates of the **Cuby** crystal structure. In this model, large artefactual electron density contributions of the carboxylate function of **Cuby** to occupied MOs were lacking, consistent with the experimental absence of such interactions. The so-derived computational

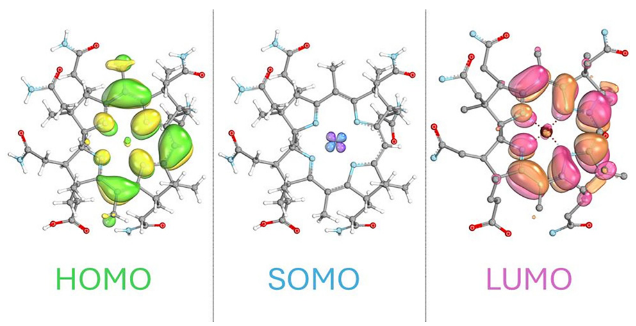

Fig. 4 Frontier molecular orbitals (FMOs) of the cupribyric acid cation (**HCuby$^+$**) from the self-consistent field in gas-phase calculations. From left to right: the highest occupied MO (HOMO), a corrin π-type orbital; the d$_{x^2-y^2}$-type Cu(II)-located singly occupied MO (SOMO); and the lowest unoccupied MO (LUMO), a corrin π-type orbital. Orbitals are seen from the upper side.

insights into the bonding interactions of a d^9-Cu(II)-corrin were fully consistent with separately located calculated frontier molecular orbitals, either as π-type corrin ligand MOs or as the singly occupied $d_{x^2-y^2}$-type orbital on the Cu(II)-center (see Fig. 4 and ESI‡ Fig. S13). We also tested computed models of cuprobyric acid (Cu(I)by) to shed light on the difficult[32] one-electron reduction to a d^{10}-Cu(I)-corrin (for details, see the ESI,‡ Fig. S14). The calculations suggest a large upper axial out of plane movement of the Cu(I)-ion, comparable to the position of the rather weakly bound Zn(II)-center in zincobyric acid (**Znby**).[19] Indeed, the iso-electronic nature of the closed shell d^{10}-ions Cu(I) and Zn(II) suggested the likelihood of the complete removal of a Cu(I)-ion from reduced **Cuby** in a weakly acidic aqueous medium. In an exploratory experiment, **Cuby** was treated with Zn-powder in an aqueous NH_4Cl solution, leading to the effective replacement of the Cu-center of **Cuby** by Zn(II), furnishing **Znby**,[19] and its tentatively (by mass- and UV/Vis-spectroscopy) characterized dihydro-form **H_2-Znby**, an unprecedented ring-reduced yellow corrinoid[33,34] (see ESI,‡ Scheme S1). We ascribe the observed formation of **Znby** from **Cuby** to a transient generation of an exchange-labile d^{10}-Cu(I)-center by the Zn-reduction, thus strategically circumventing Eschenmoser's postulate that a B-type transition metal could not be removed without destruction of the corrin-ligand.[35]

The replacement, by copper, of the biologically selected cobalt-center of a corrinoid B_{12}-derivative[1,36,37] erases its fundamental organometallic redox-reactivity.[7] The single unpaired electron of the paramagnetic **Cuby** does not contribute any (cobalt-mimetic) radical reactivity, but is 'buried' in a $d_{x^2-y^2}$ orbital of its d^9 Cu(II)-center. Consistent with the EPR-spectral fingerprint of **Cuby** and its large ^{14}N-hfcs with the four inner corrin N-atoms, in particular, the unpaired spin is located in a $d_{x^2-y^2}$ orbital of an effectively antibonding type with respect to the coordinating corrin N-atoms (Fig. 4). Compared to the d^8 Ni(II)-ion in **Niby**, the Cu(II)–N bonds in **Cuby** are, indeed, longer. Copper complexes of the superficially similar corroles represent a remarkably more complex situation:[38,39] there 'non-innocence' of the corrole ligand is caused by its extended π-system, assisting an intramolecular electron-shift and stabilizing the copper center in a higher oxidation state.[40]

The chelation of the fluorescent metal-free corrin **Hby**[2,41] by Cu(II)-ions in aqueous solution occurs cleanly at ambient temperature at pH 5. The Cu(II)-ions chelate **Hby** with a rate $k^{\mathrm{Cu(II)}} = 0.54 \pm 0.04$ L mol^{-1} min^{-1}, remarkably quicker by about 2×10^2 times than the binding of the biologically crucial Co(II)-ions ($k^{\mathrm{Co(II)}}$ of about 3×10^{-3} L mol^{-1} min^{-1}), and five times faster than Zn(II)-ions ($k^{\mathrm{Zn(II)}} = 0.111 \pm 0.002$ L mol^{-1} min^{-1}, see the ESI‡). The chelation rates of these metal ions follow the trend established with Eschenmoser's model corrin[35] and with a water-soluble tetra-mesopyridyl-porphyrin.[42]

Obviously, the biological roles of Co[7,8,43] and Cu[44–47] do not match. However, the 4-coordinate Cu(II)-complexes of natural corrin ligands may serve as structural mimics of reduced B_{12}-derivatives. In concert with the divergent reactivity profiles of copper- and cobalt-corrins, biologically interesting applications are likely. **Cuby** is structured similar to the corrin-core of enzyme-activated 4-coordinate Co(II)-cobamides, first characterized in an ATP:Co(I)-corrinoid adenosyltransferase that generates **AdoCbl** from 4-coordinate Co(II)-Cbl.[48] With their largely inert 4-coordinate d^9- and d^8-metal-centers, respectively, Cu(II)- and Ni(II)-corrins[18] may effectively mimic the structures of the highly activated 4-coordinate Co(II)- and Co(I)-corrins. Indeed, nibalamin (**Nibl**), the diamagnetic Ni(II)-analogue of 'base-off' Co(II)-Cbl, was shown to be an effective inhibitor of the corrinoid adenosyltransferase BtuR from *Brucella melitensis*.[18] The crystal structure of **Cuby** qualifies Cu(II)-containing B_{12}-derivatives, such as cupribalamin (**Cubl**), for similar inhibitory effects.

Transition metal analogues of vitamin B_{12} and other cobalamins (Cbls), also classified as metbalamins (Metbls),[49,50] lack the precise cobalt-dependent reactivity of Cbls[8,43] and, when mimicking Cbl-structures, may represent genuine antivitamins B_{12}.[50,51] This is the case for rhodibalamins (Rhbls), the Rh(III)-homologues of Cbls. Surprisingly, their Rh(III)-center has even been revealed to experience a slightly better fit to the corrin ligand than the naturally selected Co(III)-ions.[13–15] Whereas uptake and physiological activity of Metbls with stable 4-coordinate corrin-bound metal centers are still unknown in humans and animals, microorganisms are typically more structure-promiscuous for B_{12}-import, satisfying their supply with cobamides by *de novo* biosynthesis[5] or by partial assembly from salvaged natural corrinoids.[52,53] As deduced for some Rhbls[13,15] and for **Nibl**,[18] transition metal-based structural mimics of B_{12}-cofactors or of corrinoid B_{12}-biosynthesis intermediates[17] may selectively inhibit bacterial growth. As mimics of enzyme-bound Cbl-structures in B_{12}-dependent enzymes at intermediate stages of catalysis, Metbls may specifically act as very effective enzyme inhibitors. The Cu(II)-analogues of natural B_{12}-derivatives are, hence, EPR-active candidates for their applications as B_{12}-antimetabolites for B_{12}-dependent microorganisms, an expansion of the toolbox of Cu-coordinating natural products[47] as antimicrobial agents.

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Conflicts of interest

There are no conflicts to declare.

Data availability

See the ESI.‡ Crystallographic data for cupribyrate (**Cuby**) have been deposited at the Cambridge Crystallographic Data Center

(CCDC) and are available under accession number CCDC-2402239.

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