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Encasing the paramagnetic copper(II)-ion by the ring-contracted corrin ligand of vitamin B₁₂†‡

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The d⁹-Cu(II)-corrin cupribyrate (**Cuby**) was synthesized in 93% crystalline yield by rapid chelation of Cu²⁺-ions by the metal-free corrin-ligand of vitamin B₁₂. 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 d¹⁰-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 B₁₂-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 B₁₂-cofactors the unique capacity for their exceptional bio-organometallic catalysis.^{7–9} The complementary fundamental question, why cobalt? in B₁₂-cofactors,^{1,3,9,10} has generated the long-standing experimental quest for non-cobalt analogues of the B₁₂-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)-complexes¹⁹ 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.

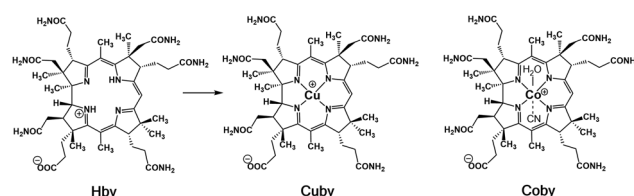
The complexation of metal-free **Hby** with Cu(II)-ions occurred readily at room temperature (RT) in a 0.25 M aqueous solution of Cu(II)-acetate at pH 6 and was practically quantitative within

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)-complex¹⁹ 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 C₄₅H₆₄CuN₁₀O₈ (see the ESI,† Fig. S1).

Glassy frozen solutions of the paramagnetic Cu(II)-corrin **Cuby** in 20% glycerol in H₂O showed the typical EPR-signature (see Fig. 2, for a spectrum at *T* = 148 K) of a roughly square-planar 4-coordinate Cu(II)-N₄-complex with an index ^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 *P*₂₁) 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 Co₂cyano, Co_{3a}quo-cobyric acid (**Coby**).

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† Dedicated to the memory of Albert Eschenmoser on the occasion of his 100th birthday.

‡ Electronic supplementary information (ESI) available. CCDC 2402239. For ESI and crystallographic data in CIF or other electronic format see DOI: <https://doi.org/10.1039/d5cc02129d>



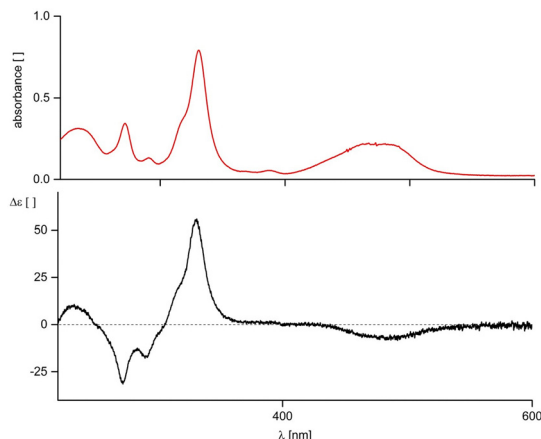


Fig. 1 UV-Vis- and CD-spectra of **Cuby** (19 μ M in 10 mM aqueous phosphate pH 7).

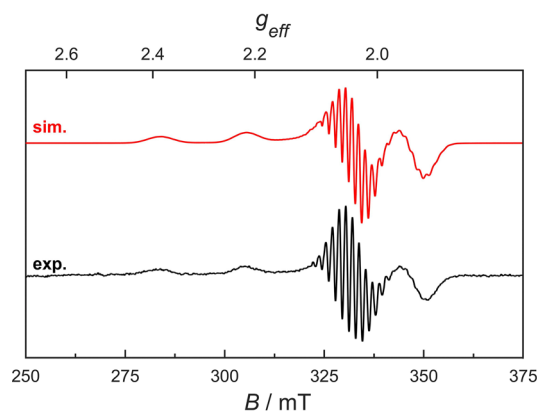


Fig. 2 EPR-spectrum of a 1.34 mM frozen solution (at $T = 148$ K) of **Cuby** in H_2O :glycerol (4 : 1) and its simulation with key parameters obtained by least square fitting (for details see the ESI,† Table S1). The spectra exhibited a significant T-dependence, with a maximum signal intensity of around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

the geometry index $\tau_4 = 0.17$.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-fold²⁶ (10.0°) and corrin helicity² (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

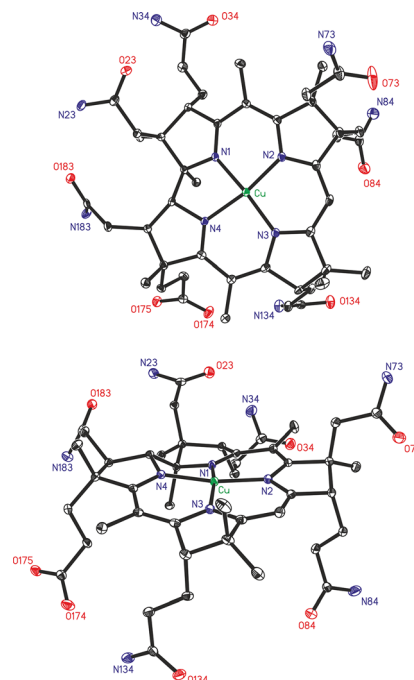


Fig. 3 X-ray crystal structure of **Cuby** in ORTEP-representations. Top: axial view from above (β -side); bottom: approximate in-plane view, revealing the slightly nonplanar 4-fold coordination of the encased Cu(II)-ion.

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**)²⁷ and cob(II)ester²⁸ and in typical Co(III)-corrins, such as coenzyme B₁₂ (**AdoCbl**)²⁹ and vitamin B₁₂.^{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

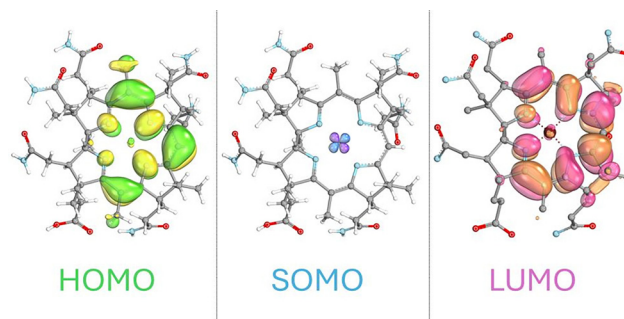


Fig. 4 Frontier molecular orbitals (FMOs) of the cupribyric acid cation (**HCuby**⁺) from the self-consistent field in gas-phase calculations. From left to right: the highest occupied MO (HOMO), a corrin π -type orbital; the $d_{x^2-y^2}$ -type Cu(II)-located singly occupied MO (SOMO); and the lowest 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 difficult³² 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 **H₂-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 -hfc's 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 **Nibly**, 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 $k^{\text{Cu(II)}} = 0.54 \pm 0.04 \text{ L mol}^{-1} \text{ min}^{-1}$, remarkably quicker by about 2×10^2 times than the binding of the biologically crucial Co(II)-ions ($k^{\text{Co(II)}} = 3 \times 10^{-3} \text{ L mol}^{-1} \text{ min}^{-1}$), and five times faster than Zn(II)-ions ($k^{\text{Zn(II)}} = 0.111 \pm 0.002 \text{ L mol}^{-1} \text{ min}^{-1}$, see the ESI†). The chelation rates of these metal ions follow the trend established with Eschenmoser's model corrin³⁵ and with a water-soluble tetra-mesopyridyl-porphyrin.⁴²

Obviously, the biological roles of $\text{Co}^{7,8,43}$ 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)-corrins¹⁸ 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 (Metbals),^{49,50} lack the precise cobalt-dependent reactivity of Cbls^{8,43} and, when mimicking Cbl-structures, may represent genuine antivitamin 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 Metbals 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* biosynthesis⁵ 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 intermediates¹⁷ may selectively inhibit bacterial growth. As mimics of enzyme-bound Cbl-structures in B_{12} -dependent enzymes at intermediate stages of catalysis, Metbals 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 products⁴⁷ as antimicrobial agents.

Synthetic, analytical and spectroscopic work: C. K. and M. W.; crystallography: C. K. and K. W.; theoretical and computational study: L. P. P., D. F. D., and K. R. L.; EPR-spectroscopy – data acquisition, supervision and data curation: D. L., M.-K. Z., M. B., and S. H.; and research conceptualization and conduction and original draft: B. K.; all authors have reviewed and contributed to the final draft.

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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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