Citation for published version


DOI

https://doi.org/10.1002/anie.201908428

Link to record in KAR

https://kar.kent.ac.uk/76274/

Document Version

Author's Accepted Manuscript

Copyright & reuse

Content in the Kent Academic Repository is made available for research purposes. Unless otherwise stated all content is protected by copyright and in the absence of an open licence (eg Creative Commons), permissions for further reuse of content should be sought from the publisher, author or other copyright holder.

Versions of research

The version in the Kent Academic Repository may differ from the final published version. Users are advised to check http://kar.kent.ac.uk for the status of the paper. Users should always cite the published version of record.

Enquiries

For any further enquiries regarding the licence status of this document, please contact: researchsupport@kent.ac.uk

If you believe this document infringes copyright then please contact the KAR admin team with the take-down information provided at http://kar.kent.ac.uk/contact.html
Zinc Substitution of Cobalt in Vitamin $B_{12}$: Zincobyric acid and Zincobalamin as Luminescent Structural $B_{12}$-Mimics

Christoph Kieninger*, Joseph A. Baker*, Maren Podewitz*, Klaus Wurst, Steffen Jockusch, Andrew D. Lawrence, Evelyne Deery, Karl Gruber, Klaus R. Liedl, Martin J. Warren,* and Bernhard Kräutler*

Dedicated to Professor Dieter Jahn on the occasion of his 60th birthday

Abstract: Replacing the central cobalt ion of vitamin $B_{12}$ by other metals has been a long-held aspiration within the $B_{12}$-field. Herein, we describe the synthesis from hydrogonyric acid of zincobyric acid ($Znby$) and zincobalamin ($Znbl$), the transition-metal analogues of the natural cobalt-corrins cobyric acid and vitamin $B_{12}$, respectively. The solution structures of $Znby$ and $Znbl$ were studied by NMR-spectroscopy. Single crystals of $Znby$ were produced, providing the first X-ray crystallographic structure of a zinc corrin. The structures of $Znby$ and of computationally generated $Znbl$ were found to resemble the corresponding $Co^{II}$-corrins, making such Zn-corrins potentially useful for investigations of $B_{12}$-dependent processes. The singlet excited state of $Znby$ had a short life-time, limited by rapid intersystem crossing to the triplet state. $Znby$ allowed the unprecedented observation of a corrin triplet ($E^T = 190 \text{kJ mol}^{-1}$) and was found to be an excellent photo-sensitizer for $^{1}O_{2}$ ($\Phi_d = 0.70$).

The biological use of cobalt as the specific transition metal center in natural $B_{12}$-cofactors and the interaction between cobalt and the corrin ligand raise intriguing questions concerning the origins of its natural selection. Engineered $B_{12}$-biosynthesis has opened up a preparative route to transition-metal analogues of the natural cobalt-corrins. $Zn^{II}$-analogues of natural corrinoids have hardly been explored but are attractive, as $Zn$- and low-spin $Co^{II}$-centers exhibit similar structural properties in small complexes and in metalloproteins.

Fischli and Eschenmoser reported the synthesis and characterization of the first $Zn$-corrin ($ZnCor$), when exploring the synthesis and chemistry of corrins in model studies towards the total synthesis of vitamin $B_{12}$. Indeed, in the Eschenmoser[7] and Woodward labs[8] a 5,15-nor-zincobyric acid of zincobyric acid ($ZnBy$), the $Zn$-analogues of vitamin $B_{12}$ ($CNcbI$) and cobyric acid ($Cby$) (Scheme 1), were first reported by Koppenhagen and Pfiffner. Herein, we delineate an effective synthesis of $Znby$ and of $Znbl$, starting from crystalline $Hby$[3] describe the pertinent spectroscopic and structural properties of these luminescent

Scheme 1. Formulae of metal-free, cobalt- and zinc-corrinoids. Left: General formula of the cobalamins vitamin $B_{12}$ ($R = \text{CN, CNcbI}$), coenzyme $B_{12}$ ($R = S'\text{-deoxyadenosyl, AdoCbl}$), cob(II)alamin ($R = e^-, Cbl^-$) Center: Formulae of hydrogonyric acid ($Hby$), $Co^{II}$-cobyric acid ($Cby$) and zincobyrate ($Znby$), where the axial solvent ligands for both the $Zn$ and $Co$ have been omitted. Right: formula of zincobalamin ($Znbl$) in its “base-on” form.
B12-derivatives and report a kinetic study of the binding of ZnII-ions to Hby. Znby was prepared at room temperature in 83% yield from Hby and ZnII-acetate (see Scheme 2 and the Supporting Information). ZnII-ions bound to Hby readily under these conditions (Supporting Information, Figure S4), and over 20 times faster than CoII-ions. Znby was resistant to removal of the ZnII-ion in acidic aqueous solution, and Hby could not be efficiently (re)generated from Znby.

The UV/Vis spectrum of Znby in aqueous buffer, pH 5, displayed absorption maxima at 335 nm, 493 nm, and 518 nm (see Figure 1) and showed similar basic features as those recorded for ZnCor and for a 5,15-nor-zincobyrinate, but with maxima at roughly 20 nm longer wavelengths. The aqueous solution of Znby fluoresced with a maximum emission at 552 nm.

The solution structure of Znby (molecular formula C62H88N13O14PZn, see Supporting Information, Table S1) was characterized by NMR spectroscopy, providing assignment of 52 H-atoms and of all C-atoms (Supporting Information, Figure S3). A 500 MHz 1H-NMR spectrum of Znby in D2O right: UV/Vis absorption (black trace), CD (red trace), and fluorescence emission (blue trace) of Znbl in 10 mM Na-phosphate buffer, pH 5 (see the Supporting Information for details).

Scheme 2. Preparation of Znby and Znbl from Hby. i) 2 mg Hby in 2.3 mL aq. 0.5 mM Zn(OAc)2 at pH 6, 80 min, room temperature; ii) 5 mg Znby, 3.3 meq B12-nucleotide moiety, 20 meq HOBt and 23 meq EDC•HCl in 1.9 mL H2O, 4 h, 0°C (see the Supporting Information for details).

Figure 1. Absorption and fluorescence spectra of Znby and Znbl at 298 K. Left: UV/Vis absorption (black trace), CD (red trace), and fluorescence emission (blue trace) of Znby in H2O. Right: UV/Vis absorption (black trace), CD (red trace), and fluorescence emission (blue trace) of Znbl in 10 mM Na-phosphate buffer, pH 5 (see the Supporting Information for details).

Figure 2. 500 MHz 1H-NMR spectra of Znby and Znbl (in D2O, 298 K). Top: Znby (ε = 1.1 mm). Bottom: Znbl (ε = 7.2 mm); residual water signal after pre-saturation marked by an X.
The fluorescence of Znby in EtOH at 296 K showed an emission maximum at 548 nm and an energy of the lowest singlet excited state of Znby of 225 kJ mol\(^{-1}\), close to the value observed with the metal-free Hby (\(E^F = 223 \text{ kJ mol}^{-1}\)).[3] Hence, the closed shell Zn-ions do not appear to significantly perturb the \(\pi,\pi^*\)-transitions of the corrin ligand. However, the fluorescence of Znby (fluorescence lifetime \(\tau_f < 0.4\) nsec) decayed about an order of magnitude more rapidly at 23°C than that of Hby (\(\tau_f = 3.3\) nsec), exhibiting a correspondingly lower quantum yield \(\Phi_f = 0.025\) (for Hby \(\Phi_f = 0.18\)). The short fluorescence lifetime of photo-excited Znby at 296 K is due to the efficient singlet-triplet intersystem crossing with an estimated rate of more than \(2 \times 10^9\) sec\(^{-1}\), boosted by the coordination of the Zn-ion.[15] At 77 K the solution of Znby in EtOH displayed an absorption maximum at 523 nm, and emitted both fluorescence (first maximum at 628 nm, Figure 5, see the Supporting Information for details). Hence, at 77 K the lowest triplet state of Znby occurred at \(E^T = 190 \text{ kJ mol}^{-1}\), furnishing the first such benchmark value for a natural corrin ligand. The phosphorescence of photo-excited Znby decayed with a lifetime of 13 ± 1 msec at 77 K. Znby sensitized the formation of \(^1\text{O}_2\) with a quantum yield \(\Phi_1 = 0.70\). The Zn-corrin ZaCor[6] emitted fluorescence with a maximum at 573 nm (\(\Phi_1 = 0.09\)) at room temperature in EtOH,[16] and was an efficient triplet sensitizer in the legendary photo-induced A/D-secocorrin to corrin cycloisomerization.[6,7,16]

Znby (Supporting Information, Table S4). The Zn–N bonds in Znby (average length = 2.03 Å) are longer than those found in Chin\(^{III}\) (average Co–N bond length = 1.90 Å). Likewise, the axial displacement of the metal-ion from the mean plane through the four corrin N-atoms in the Zn-corrinate Znby (0.624 Å) is palpably greater than that of the Co\(^{III}\)-center of Chin\(^{III}\) (0.048 Å). In Znby and Chin\(^{III}\), an axial ligand is bound at the \(\beta\)-face with a long metal-oxygen bond, and the four corrin N-atoms are displaced slightly from a planar to a squashed tetrahedral arrangement (Supporting Information, Table S4). However, whereas the core of the corrin ligand is made nearly C2-symmetrical by the coordination of a Co\(^{III}\)-center, in Znby the N2–N4 diagonal remains remarkably longer than its N1–N3 counterpart, with \(\Delta d = 0.186\) Å. Hence, about 60 % of \(\Delta d = 0.297\) Å in Hby are retained in the corrin ligand of Znby. This feature of Znby reflects a preferred mode of the conformational adaptation of the coordination hole of the flexible, unsymmetrical corrin ligand to the 5-coordinate closed-shell Zn-ion. The “helicity” \(h(\text{Znby}) = 8.0^\circ\) is in line with a small directional effect of Zn\(^{II}\), compared to Co\(^{III}\), or Co\(^{III}\)-binding, where \(h = 6.1^\circ\) in Chin\(^{II}\) and \(h = 4.1^\circ\) in CNChl[3] In Znby, the corrin ligand adapts to the skewed pyramidal arrangement around the Zn\(^{II}\)-center by an unprecedented conformational “doming” of the corrin ligand (Figure 4). Consequently, the corrin-based inter-planar angle \(\phi(\text{Znby}) = 50.2^\circ\) far exceeds \(\phi(\text{Chin}^{III}) = 7.6^\circ\) and \(\phi(\text{CNChl}) = 4.6^\circ\).[3]

The fluorescence of Znby in EtOH at 296 K showed an emission maximum at 548 nm and the energy of the lowest singlet excited state of Znby of 225 kJ mol\(^{-1}\), close to the value observed with the metal-free Hby (\(E^F = 223 \text{ kJ mol}^{-1}\)).[3] Hence, the closed shell Zn-ions do not appear to significantly perturb the \(\pi,\pi^*\)-transitions of the corrin ligand. However, the fluorescence of Znby (fluorescence lifetime \(\tau_f < 0.4\) nsec) decayed about an order of magnitude more rapidly at 23°C than that of Hby (\(\tau_f = 3.3\) nsec), exhibiting a correspondingly lower quantum yield \(\Phi_f = 0.025\) (for Hby \(\Phi_f = 0.18\)). The short fluorescence lifetime of photo-excited Znby at 296 K is due to the efficient singlet-triplet intersystem crossing with an estimated rate of more than \(2 \times 10^9\) sec\(^{-1}\), boosted by the coordination of the Zn-ion.[15] At 77 K the solution of Znby in EtOH displayed an absorption maximum at 523 nm, and emitted both fluorescence (first maximum at 628 nm, Figure 5, see the Supporting Information for details). Hence, at 77 K the lowest triplet state of Znby occurred at \(E^T = 190 \text{ kJ mol}^{-1}\), furnishing the first such benchmark value for a natural corrin ligand. The phosphorescence of photo-excited Znby decayed with a lifetime of 13 ± 1 msec at 77 K. Znby sensitized the formation of \(^1\text{O}_2\) with a quantum yield \(\Phi_1 = 0.70\). The Zn-corrin ZaCor[6] emitted fluorescence with a maximum at 573 nm (\(\Phi_1 = 0.09\)) at room temperature in EtOH,[16] and was an efficient triplet sensitizer in the legendary photo-induced A/D-secocorrin to corrin cycloisomerization.[6,7,16]
To shed further light on the structure of Znby, the gas-phase structure of the hypothetical 4-coordinate analogue Znby(4) was calculated, using DFT, from the crystal structures of Hby, as well as of the heptamethyl ester Chin[4], the latter providing computational Znby models in which the polar side chain functions are replaced by methyl ester groups (for details, see the Supporting Information). Ligation of an acetate ligand at the “upper” (β) or at the “lower” (α) side of the latter Znby(4) structure, furnished models of Znby and of its coordination isomers Znby(α). The calculated structure of Znby closely reflected the observed crystallographic structural peculiarities of Znby, such as the longer N2–N4 diagonal (Δd_{N2–N4} = 0.22 Å), the long Zn–N-bonds (Zn–N_{av} = 2.06 Å), the out-of-plane position of the 5-coordinate Zn-ion (0.65 Å), and the doming of the corrin ligand. In Znby(α), the calculations generated a model with comparably long Zn–N-bonds (Zn–N_{av} = 2.06 Å), an N2–N4 diagonal shorter than N1–N3 (Δd_{N2–N4} = -0.10 Å), a profound out-of-plane position of the 5-coordinate Zn-ion (–0.62 Å) and an “inverted” doming of the corrin ligand. A structure of Znbl was calculated (Figure 6) starting from a previously optimized gas-phase structure of Chb[4]. It showed a pronounced out-of-plane movement of the 5-coordinate Zn-ion (–0.46 Å), exceeding that of Co[4] in Chb[4] (–0.13 Å), but compensated in part by the slightly shorter Zn–N_{DMB}-bond (2.07 Å) in Znbl than the Co–N_{DMB} bond (2.11 Å) in Chb[4]. The structure of Znbl showed a downward movement of the DMB-base, compared to Chb[4], but was similar in its overall architecture. Hence, Znbl can be considered as a good structural mimic of the non-luminescent Chb[4].

As an iso-structural analogue of some Chbs that is inactive in the organometallic processes typical of B12-dependent enzymes, Znbl may represent an “antivitamin B12”[37] and be a useful fluorescent molecular probe in B12-biology and biomedicine.[34] The structure analysis of Znby has indicated that the closed shell d⁹- ion of Zn⁴⁺ lacks the precise fit of the similarly sized low-spin Co⁴⁺-centers (d⁶-ions),[39] where an empty d⁴–2⁰-s-orbital provides an excellent electronic complement for the four corrin N-atoms.[20] Hence, the basic fit of low spin Co⁴⁺ and diamagnetic Co⁴⁺-ions to the ring size of the corrin ligand[35] is not extended to the 5-coordinate Zn⁴⁺-ion. A similar (but less pronounced) difference is seen in Zn⁵⁺ and Co⁵⁺-porphyrins, where porphyrin “doming” and axial displacement of 5-coordinate Zn⁵⁺-centers towards the axial ligand exceed the effect of the 5-coordinate Co⁵⁺-ions.[21]

The lack of out-of-plane displacement of the 5-coordinate Co⁵⁺-centers in Co⁵⁺-corrins appears to be a consequence of the partially occupied valence shell of this electronically adaptable d⁶-ion. Indeed, the 15-membered equatorial perimeter of the “ring contracted” corrin ring is able to accommodate the size of both low-spin Co⁵⁺ and diamagnetic Co⁴⁺-ions, which have the capacity to fit their electronic configuration to favorable interactions with the ligand.[5a,5b,22] In contrast, when binding a 5-coordinate closed shell d⁹ Zn²⁺-ion, the corrin ligand undergoes doming and further conformational relaxations. In spite of the structural differences between Znby and Chin[4], as well as those deduced for Znbl and Chb[4], the redox-inactive Zn-complexes of natural corrins may be useful as luminescent (inactive) mimics of corresponding B12-derivatives.

The work reported here describes a rational avenue to the construction and characterization of Znbl, promising to be useful in biological and biomedical experiments. Significantly, the engineering of bacterial strains for the production Hby[3] has unlocked the gateway to the direct generation of a range of other Mebls and Mebys, the transition-metal analogues of the Chbs and Chys, respectively. The helical, un-symmetric natural corrin-ligand is a unique binding partner for transition-metal ions, providing an exciting opportunity to construct a diverse range of metal analogues of vitamin B12, investigate their structural behavior, examine their reactivity, and to test biological effects.

**Experimental Section**

**Crystallographic Data.** X-ray crystal data of Znby have been deposited at the Cambridge Crystallographic Data Centre (CCDC) under the reference number CCDC 1921462.

**Acknowledgements**

Supported by grants from the Biotechnology and Biological Sciences Research Council (BBBSC; BB/L010208/1, BB/K009249/1 and BB/S002197/1) to M.J.W., and from the Austria Science Fund (F.W.F., P-28892) to B.K. and Lise Meitner Fellowship (M-2005) to M.P., who is also grateful to the Tyrolean Science Fund (TWF, UNI-0404/1980), the Vienna Scientific Cluster (VSC3) and the University of Innsbruck HPC infrastructure for support of the computational work.

**Conflict of interest**

The authors declare no conflict of interest.

**Keywords:** cobalamins · photochemistry · transition metal · vitamin · X-ray structure
Zinc Substitution of Cobalt in Vitamin B₁₂: Zincobyric acid and zincobalamin as Luminescent Structural B₁₂-Mimics

Zincobyric acid and zincobalamin, the Zn-analogues of cobyric acid and of vitamin B₁₂, were efficiently prepared from the metal-free corrin, hydrogenobyric acid. The crystal structure of the zincobyrate, the first of a Zn-corrin, revealed a significant misfit of the closed shell Zn²⁺-ion to the corrin ligand. The luminescent Zn-mimics of vitamin B₁₂ do not support organometallic B₁₂-enzyme reactions and may be useful enzyme inhibitors.