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Replacement of the Cobalt Center of Vitamin B$_{12}$ by Nickel: Nibalamin and Nibyric Acid Prepared from Metal-Free B$_{12}$ Ligands Hydrogenobalamin and Hydrogenobyric Acid

Christoph Kieninger, Klaus Wurst, Maren Podewitz, Maria Stanley, Evelyne Deery, Andrew D. Lawrence, Klaus R. Liedl, Martin J. Warren,* and Bernhard Kräutler*

Dedicated to Professor Albert Eschenmoser on the occasion of his 95th birthday

Abstract: The (formal) replacement of Co in cobalamin (Cbl) by Ni$^{II}$ generates nibalamin (Nibl), a new transition-metal analogue of vitamin B$_{12}$. Described here is Niby, synthesized by incorporation of a Ni$^{II}$ ion into the metal-free B$_{12}$ ligand hydrogenobalamin (Hbl), itself prepared from hydrogenobyric acid (Hby). The related Ni$^{II}$ corrin nibyric acid (Niby) was similarly synthesized from Hby, the metal-free cocyclic acid ligand. The solution structures of Hbl, and Niby and Nibl, were characterized by spectroscopic studies. Hbl features two inner protons bound at N2 and N4 of the corrin ligand, as discovered in Hby. X-ray analysis of Niby shows the structural adaptation of the corrin ligand to Ni$^{II}$ ions and the coordination behavior of Ni$^{II}$. The diamagnetic Niby and Nibl, and corresponding iso-electronic Co$^{II}$ corrins, were deduced to be isostructural. Nibl is a structural mimic of four-coordinate base-off Cbls, as verified by its ability to act as a strong inhibitor of bacterial adenosyltransferase.

Introduction

Biologically active vitamin B$_{12}$ derivatives exclusively utilize cobalt as their specific transition metal center, which is bound and activated exquisitely by a helical corrin macrocycle.[1] The metal-free corrin ligand of vitamin B$_{12}$, hydrogenobyric acid (Hby), has recently been made available as a consequence of engineered B$_{12}$ biosynthesis in E. coli.[2] The availability of Hby has provided an unparalleled opportunity for the effective synthesis of metal-free and transition metal analogues of the natural cobalt-corrinoids a previously intractable challenge in bioorganic and B$_{12}$ chemistry.[4] We have recently used Hby for the synthesis of the corresponding zinc-corrin zincobyric acid (Znyby) and the Zn analogue of vitamin B$_{12}$ zincobalamin (Znbl), of interest as luminescent structural B$_{12}$ mimics.[5]

Herein, we report on the first nickel-complexes of natural corrin ligands, including nibalamin (Nibl). We also describe the syntheses of crystalline nibyric acid (Niby), the novel Ni$^{II}$ complex of Hby,[3] and hydrogenobyric acid (Hbl), the metal-free complete B$_{12}$ ligand (see Scheme 1). Koppenhagen and co-workers, back in the 1970s, reported the isolation of Hbl from a Chromatium strain supplemented with 5,6-dimethylbenz-imidazole (DBM). They were able to characterize Hbl by UV/Vis-spectroscopy and demonstrated that it could be converted into vitamin B$_{12}$ by insertion of cobalt,[6,7] and later reported its mass spectrum.[8]

A Ni$^{II}$-corrin, the NiCor (see Scheme 1), was prepared in the Eschenmoser lab as the first synthetic corrin, making use of the Ni$^{II}$ ion as a “template” for the assembly of the corrin macro-ring.[9] NiCor also became the object of the first X-ray crystallographic investigation of the structure of a non-cobalt corrin.[9] Four coordinate Ni$^{II}$ complexes prefer to adopt a planar geometry and therefore are more structurally related to the corresponding Co$^{II}$ complexes.[10] Indeed, recently, there has been a resurgence in the quest for close Ni analogues of the B$_{12}$ cofactors.[11,12] The planar ligand set of Nibl potentially represents a structural B$_{12}$ mimic that is inert to the organometallic transformations typical of B$_{12}$-dependent enzymes, as suggested by its expected coordination chemistry and structural properties. Specific interest in Nibl, the Ni$^{II}$ analogue of vitamin B$_{12}$ and of other cobalamins (Cbls) (see Scheme 1), is thus a consequence not only of its chemistry, but also of its possible use as a molecular probe in B$_{12}$ biology and biomedicine, helpful for the investigation of cobalaminedependent processes and their physiological effects.[13]
Results and Discussion

Nibyric acid (Niby) was prepared by dissolving 1.40 mg (1.6 μmol) of crystalline hydrogenobyric acid (Hby) in 3.5 mL of deoxygenated 0.5 M aqueous NiII acetate, pH 6, with stirring at 90 °C for 75 min. Separation on a short reverse phase column, evaporation and crystallization from aqueous acetonitrile yielded 0.90 mg (0.97 μmol, 61%) of Niby, which was isolated as yellow crystals (see Scheme 2, Exptl. Part and Supporting Information (SI)). The UV/Vis absorption spectrum of an aqueous solution of Niby displayed bands at 464 nm (shoulder), 448 nm and 334 nm (Figure 1), and exhibited similar gross features to those observed in an absorption spectrum of the Ni-corrin NiCor (but with a slightly red-shifted maxima)\(^{[6a,c]}\). The solution structure of the diamagnetic NiII-corrin Niby (molecular formula C\(_{45}\)H\(_{64}\)N\(_{10}\)O\(_{8}\)Ni, for HR mass spectra see SI, Figure S3) was analyzed by NMR spectroscopy, providing assignment of all 52 non-exchangeable H atoms and 44 C atoms (see SI, Figure S4 and Table S1). A 500 MHz 1H NMR spectrum of Niby in D\(_2\)O displayed five high field singlets for the six methyl groups, a singlet of HC10 at 6.30 ppm, as well as several signals at intermediate field for HC19, HC3, HC8 and HC13 (see Figure 2). The data from homonuclear and heteronuclear correlations confirmed the stereostructure of Niby (see SI, Figure S5).

![Scheme 2. Preparation of the NiII-corrins Niby and Nibl from Hby.](image)

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Figure 1. Absorption spectra of aqueous solutions of metal-free B12 ligands Hby and Hbl and of their NiII-complexes Niby and Nibl at 298 K. Top: UV/Vis-absorption spectra of Hby (c = 31.3 μM, pH 5, black trace) and Niby (c = 34.5 μM, unbuffered, red trace). Bottom: UV/Vis-absorption of Hbl (pH 5, black trace) and of Nibl (unbuffered, red trace).

Figure 2. 500 MHz 1H NMR spectrum of Niby in D\(_2\)O (c = 1.9 mM, 298 K); the water signal after presaturation is marked by an X.
The complete metal-free ligand of the cobalamins, hydrogenobilamin (Hbi), was assembled by attaching the B12 nucleotide moiety[1a,12] to the propionate moiety of Hby at 0°C through application of the carbodiimide method (Scheme 2).[4a,13] In brief, an aqueous solution of 9.12 mg (10.4 μmol) of Hby and of 14.71 mg (33.4 μmol) of the B12 nucleotide was treated with 9.4 mole of HOBt and degassed. To the frozen reaction mixture 4.4 mole of EDC·HCl were added under Ar. Upon subsequent warm-up of the reaction mixture to 0°C, 16 mole EDC·HCl were added and stirring was continued for 4 d (see SI). Work-up, using RP18-chromatographic purification, precipitation with MeCN and drying, furnished 11.3 mg (8.89 μmol, 85 % yield) of Hbl as an orange powder. An aqueous solution of Hbl at pH 5 exhibited UV/Vis[4c] and CD spectral features (SI, Figure S8 and S9) similar to those of Hby.[3] The UV/Vis absorption maximum at 525 nm of the α-band of Hbl and the fluorescence emission maximum at 554 nm (SI, Figure S10) located the first excited singlet state of Hbl at Eα near 221 kJ mol−1, marginally lower than for Hby.[3] The structure of Hbl (molecular formula C62H90N13O14PNi, SI, Figure S17) was characterized in aqueous solution by heteronuclear NMR spectroscopy (600 MHz 1HNMR spectrum in Figure 3), providing assignment of 89 H atoms and of all 62 C atoms (see SI, Table S2). The two “inner” H atoms gave rise to singlets at δ = 12.32 and δ = 12.57 ppm, which were assigned to H(N4) and to H(N2), respectively, indicating a minor up-field shift of both of them when compared to Hby.[3] The methyl group singlet of H3C1 at δ = 0.81 ppm occurred at 0.47 ppm to higher field, compared to Hby, suggesting a temporary residence of the heterocorin DMB unit of Hbl near to its corrin moiety, a conclusion that was further supported by weak inter-residual correlations in the 1H,1HROESY spectra (see SI, Figure S13). However, the signals of the DMB moiety (HN2 at δ = 8.35 ppm, HN4 at δ = 7.31 ppm, HCN7 at δ = 7.30 ppm) were found at similar chemical shift values to those of the free B12 nucleotide,[12] effectively incompatible with a time-averaged positioning of the DMB part close to the corrin chromophore, as found for zincobilamin (Zahl)[9] and for typical “base-on” CoIIChb.[14]

The NiII-corrin nibalamin (Nibl) was prepared by heating a deoxygenated aqueous solution of Hbl and Ni(OAc)2 for 1 h at 90°C (Scheme 2), furnishing Nibl in 77% yield as a yellow powder. An unbuffered aqueous solution of Nibl exhibited a UV/Vis spectrum that is incompatible with coordination by the DMB base and nearly indistinguishable (at >300 nm) from the spectrum of Niby, and similar to the spectrum of the NiII-corrin NiCorII[4c] (see Figure 1). However, the absorption maxima of Nibl occurred at characteristically longer wavelengths when compared to the spectrum of the recently described vitamin B12-derived 5,6-dihydroxy-5,6-dihydroni-bilamin, which features an interrupted corrin π-system.[6] Lower pH values affected only the short wavelength part of the Nibl UV/Vis-absorption spectrum, which was altered by DMB-protonation, consistent with a pKΘ = 4.35 ± 0.06 for protonated Nibl-HII+ (see SI, Figure S20).

The structure of Nibl (molecular formula C62H88N13O14PNi, SI, Figure S17) was characterized in aqueous solution by heteronuclear NMR spectroscopy (see 500 MHz 1HNMR spectrum in Figure 3), providing assignment of all 73 non-exchangeable H atoms and of all 62 C atoms (SI, Table S3). The positions of the singlets of H3C1A (δ = 1.10 ppm), of HCN2 (δ = 8.51 ppm), HN4 (δ = 7.36 ppm) and HCN7 (δ = 7.39 ppm) of the DMB moiety all indicate a base-off form with a four-coordinate NiII center. Hence, the UV/Vis and NMR spectral features of Nibl characterize it as an isoelectronic and, roughly, isostructural analogue of the diamagnetic cob(II)alamin (Chbl), which is considered to feature a “base-off” structure with a four-coordinated CoII center.[15,16]

The nickel corrin Niby was crystallized from aqueous acetonitrile, furnishing yellow single crystals (P21/c) suitable for X-ray analysis (see Figure 4). The incorporation of a NiII ion into the corrin macrocycle of the metal-free Hby increased the effective symmetry of the corrin ligand as revealed by a comparison of the crystal structures of Niby and

![Figure 3](image-url) 500 MHz 1H NMR spectrum of Nibl in D2O (c = 1.4 mM, 298 K); residual water signal after presaturation marked by an X.

![Figure 4](image-url) Crystal structure of Niby. Left. Crystallographic model of Nibl in two projections; Right. Graphs representing the corrin core with display of N-Ni bond lengths (top) and the coordination geometry around NiII center, highlighting the arrangement of the four inner corrin N atoms in a flattened tetrahedron around the NiII center (middle and bottom).
of $\text{Hby}$ (see SI for details). Coordination of the Ni$^{\text{II}}$-ion largely equalizes the lengths of the two diagonals, whereas the N2-N4 diagonal of $\text{Niby}$ exceeds its N1-N3 counterpart by only $\Delta d = 0.047\ \text{Å}$, far less than in $\text{Hby}$ ($\Delta d = 0.297\ \text{Å}$)[3] or in $\text{Znby}$ ($\Delta d = 0.197\ \text{Å}$)[8]. The somewhat longer N2-N4 diagonals in the metal corrins $\text{Niby}$ and $\text{Znby}$ appear to reflect the preferred mode of the conformational adaptation of the coordination hole of the flexible, unsymmetrical corrin ligand to bound metal ions. The radial size of the coordination hole also shrank upon Ni$^{\text{II}}$ coordination as the average length of the N1-N3 and N2-N4 diagonals of $\text{Hby}$ ($d = 3.82\ \text{Å}$) was reduced to $d = 3.71\ \text{Å}$ in the complex $\text{Niby}$. Hence, the coordination of the Ni$^{\text{II}}$ ion in $\text{Niby}$ contracts the corrin ligand and makes it more symmetrical. This latter effect is also expressed by the regularly alternating bond lengths of the corrin $\pi$-system in $\text{Niby}$, observations that are compatible with the model Ni-corrin C$\text{biiCor}$.[9]

The four-coordinate Ni$^{\text{II}}$ ion sits very close to the plane of the four inner corrin $\text{N}$ atoms, comparable to the situation in the Ni$^{\text{II}}$-corrin C$\text{biiCor}$,[9] and in typical Co$^{\text{III}}$-corrins,[16a] but contrasting somewhat with the out of plane distance of 0.048 $\text{Å}$ of the five-coordinate Co$^{\text{II}}$ center of heptamethylcob(II)pyrinate perchlorate (Chin$^{\text{II}}$)[17] (SI, Table S5). As expected,[9,18] the metal–N bonds in $\text{Niby}$ (average Ni–N bond length $= 1.86\ \text{Å}$) are shorter than those found in the Co$^{\text{II}}$ analogue Chin$^{\text{II}}$ and in the Co$^{\text{III}}$-corrin coenzyme B$^{\text{12}}$ (AdoCbl), where average (Co$^{\text{II}}$-N) and (Co$^{\text{III}}$-N) bond lengths of 1.89 $\text{Å}$[19] and of 1.90 $\text{Å}$,[20] respectively, were observed.

The coordination of the Ni$^{\text{II}}$ ion barely affects the conformational properties of the metal-free corrin ligand (Figures 5,6). Only a slight reduction of the helicity, $h$ (Figure 7), of the inner corrin $\text{N}$ atoms from $h = 12.9^\circ$ in $\text{Hby}$ to $h = 10.1^\circ$ is seen in $\text{Niby}$. Indeed, the effect of the binding of the Ni$^{\text{II}}$ ion on the corrin helicity is comparable to the situation in the enzyme-bound four-coordinate cob(II)alamin (4c-Chin$^{\text{II}}$ (ACA)) of the adenosyltransferase ACA,[21] for which $h = 8^\circ$.[15] In contrast, in five-coordinate Co$^{\text{III}}$-corrins the corrin helicity is significantly smaller, for example, $h = 6.1^\circ$ in the Co$^{\text{III}}$-corrin Chin$^{\text{II}}$, and in typical Co$^{\text{III}}$-corrins planarization of the corrin ligand is still more pronounced, leading, for example, to $h = 3.5^\circ$ in AdoCbl.[3]

The observed lower drive of the four-coordinate d$^8$ Ni$^{\text{II}}$ ion to planarize the corrin macrocycle is similarly reflected by its own coordination geometry, which deviates strongly in $\text{Niby}$ from the coplanar arrangement of the coordinating ligand atoms in typical four-coordinate low-spin Ni$^{\text{II}}$ complexes.[9,10,18] In $\text{Niby}$ a remarkably large interplanar angle $\phi$ (Figure 7) at Ni$^{\text{II}}$ ($\phi = 11.1^\circ$) results from extensive directional coordinative adaptation of the Ni$^{\text{II}}$-center to the geometric requirements imposed by the helical corrin ligand (see Figure 6 and SI). $\phi$ is significantly larger in $\text{Niby}$ than in Co$^{\text{III}}$-corrins, which exhibit $\phi$’s around 5° or less,[3] and is comparable to the situation in five-coordinate Co$^{\text{III}}$-corrins Cbl$^{\text{II}}$ ($\phi = 12^\circ$) and Chin$^{\text{II}}$ ($\phi = 7.6^\circ$).

The corrin helicity $h$ and the inter-planar angle $\phi$ (Figure 7), were introduced recently as two complementary parameters characterizing inner conformational effects of the mutual structural adaptation of the corrin macrocycle and of the coordination geometry at the bound metal ion.[3] The so-called corrin fold of the helical corrin macrocycle,[22] a classic parameter characterizing the nonplanar corrin ring in Cbls and in other “complete” cobamides (Figure 7), was not used in this current study. Conceived as a measure of the major conformational adaptation of the corrin ring to the cobalt coordination of the (bulky) DMB moiety in “base-on” Cbls, it runs roughly along the Co-C10 (east-west) axis.[22] However, in four- and five-coordinate metal-corrins lacking the DMB unit, like Chin$^{\text{II}}$, $\text{Niby}$ and $\text{Znby}$, the calculated corrin-fold is dominated by the effects of the corrin helicity and the intersection between the two relevant planes adheres to a north-south direction (see SI Table S5 and Figure S22).

DFT calculations were carried out to test further the proposed close structural similarity between Ni$^{\text{II}}$-corrins and their analogues with four-coordinate cobalt centers (see Figure 8 and SI for further details). In order to minimize the relevance of peripheral H-bonds between the amide functions in the implicit solvent calculations, the five-coordinate lip-
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its low-spin state. In contrast, in the porphyrinoid B$_{12}$-related nickel complex coenzyme F$_{450}$[18, 26], the 16-membered porphyrinoid macrocycle is a key player in the active, specific adjustment of the spin state and coordinative activity of the nickel center to its function in the enzyme catalyzed methane formation.[27] Indeed, the discovery of coenzyme F$_{450}$ provoked an entirely new look at the structural effect of the tetrpyrrolic macrocycle on the coordination chemistry of bound first-row transition metals.[18]

A common feature of the valence shell of the low spin states of the transition metal ions Ni$^{II}$, Co$^{II}$, Co$^{III}$, and Co$^{I}$ is their unoccupied d$_{x^2-y^2}$ orbital, a key factor responsible for their strong sigma bonding interactions with the four inner corrin N atoms, leading to similar radial characteristics of their corrin complexes. A differing number of valence shell electrons in Ni$^{II}$-, Co$^{II}$-, Co$^{III}$-, and Co$^{I}$-corrins is transduced primarily into characteristically different reactivity of the metal centers in the axial direction, strongly affecting their potential binding sites there. Consequently, Ni$^{II}$-corrins are to be considered particularly well-suited structural mimics of Co$^{II}$-corrins, the critical intermediates in heterolytic organometallic transitions in B$_{12}$-dependent enzymes.[28] Crystallographic insights and DFT-based structure calculations also indicate a structural similarity between Ni$^{II}$-corrins and the exceptional four-coordinate Co$^{II}$-corrins. This result contrasts strikingly with the mutually different structures of the typical five-coordinate Co$^{III}$-corrins and their Zn$^{II}$-analogues[5] with similarly sized metal ions[29] that differ by the number of electrons in the valence shell.

The structural analysis of the Nibyrinates predicts that the constitutively robust Nibi would likely be an excellent redox-resistant structural mimic for the elusive cob(I)alamin (AdoCbl), a highly reactive redox-active intermediate[30] that is found in B$_{12}$-dependent methyl group transferases, such as methionine synthase,[31, 32] as well as in the biosynthesis of AdoCbl from Cbl$^{II}$ (via Cbl$^{III}$) by Cbl-adenosyltransferases.[23, 24] Nibi may, likewise, act as a good structural mimic of the recently described natural four-coordinate Co$^{III}$-corrins, proposed as key intermediates in the enzymatic transformations catalyzed by the vitamin B$_{12}$ tailoring enzyme CblC,[31] in corrinoid dehalogenases,[29] or as substrates for the reduction to Co$^{II}$-species in enzymatic cobalt alkylation.[14, 13] Indeed, as verified here, Nibi is a very effective inhibitor of the bacterial Cbl-adenosyltransferase BtuR.

We have developed a rational and direct synthetic path from hydroenoboric acid (H$_{2}$B) via hydrogenobalamin (Hbl) to nibalamin (Nibi), a novel transition-metal analogue of the Cbls. Our recent studies with the Rh$^{III}$ analogue AdoRhbl of AdoCbl,[33] with the Zn$^{II}$ analogue Zabbl of Cbl$^{III}$,[31] and now the Ni$^{II}$ analogue Nibi of Cbl$^{III}$, have furnished a valuable suite of cobalamin mimics for use in the study of B$_{12}$-dependent enzymatic processes,[18, 28, 30, 34, 35] and in B$_{12}$-dependent biological regulation.[36] Well-characterized and adequately accessible transition metal analogues (Metbls) of the Cbls provide a promising small-compound platform that may contribute significantly to the ongoing quest for innovative B$_{12}$-based biological and biomedical applications.[17, 37] Along these lines, some Metbls may find applications as effective antivitamins B$_{12}$[44, 11]. The availability of selected Metbls and of related metal corrins (MetCor) will also allow more detailed experimental investigations into the chemical relevance of the coordination of transition metal ions by the uniquely skewed, strongly helical and unsymmetric natural corrin ligands.[33] Such studies will endow a more informed understanding of the specific evolutionary selection of cobalt rather than any other transition metal[11] for the task of complex organometallic catalysis achieved by the B$_{12}$ cofactors.

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**Conflict of interest**

The authors declare no conflict of interest.

**Keywords:** cobalamin - porphyrinoids - transition metals - crystal structures - vitamins
Nibalamin (Nibl), the novel Ni\textsuperscript{II} analogue of vitamin B\textsubscript{12}, was synthesized from the metal-free B\textsubscript{12} ligand hydrogenobalamin, prepared from biosynthetic hydrogenobyric acid (Hby). An X-ray crystal analysis of nibyric acid, the Ni\textsuperscript{II} complex of Hby, revealed the first structural details of a nickel complex of a natural corrin ligand. Nibl is a structural mimic of the corresponding four-coordinate cobalamins, which are of interest in biological investigations.