

Hydrogenobinamide and nibinamide - Metal-free ligand and Ni(II)-analogue of the vitamin B₁₂ precursor cobinamide

Christoph Kieninger^a, Evelyne Deery^b, Andrew D. Lawrence^b, Martin J. Warren^{b,c,◊}
and Bernhard Kräutler^{a,◊,*}

^aInstitute of Organic Chemistry and Center for Molecular Biosciences (CMBI), University of Innsbruck, 6020 Innsbruck, Austria

^bSchool of Biosciences, University of Kent, Canterbury, CT2 7NJ. U.K

^cFood Innovation and Health Programme, Quadram Institute of Bioscience, Norwich Research Park, NR4 7UQ. U.K

Received 10 December 2022

Accepted 31 January 2023

Dedicated to Prof. Tomás Torres on the occasion of his 70th birthday

ABSTRACT: The replacement of cobalt in vitamin B₁₂ derivatives by other transition metals is a formal path to non-natural corrins. Here, we describe nibinamide (**Nibi**), the novel Ni(II)-analogue of the natural B₁₂-derivative cobinamide (**Cbi**), and its synthesis from the metal-free ligand of **Cbi**, hydrogenobinamide (**Hbi**), both isolated as tetrafluoroborate salts. Aqueous solutions of the metal-free corrin **Hbi** are strongly fluorescent, whereas its Ni(II)-complex **Nibi** is non-luminescent. The solution structures of **Hbi** and of **Nibi** were characterized by hetero-nuclear NMR-spectroscopy. The Ni(II)-corrin **Nibi** was deduced to be roughly *iso*-structural to cob(I)inamide (**Cbi**^I) and to house a diamagnetic d⁸-metal-ion *iso*-electronic to Co^I in **Cbi**^I. The chemically robust **Nibi** is, thus, a structural mimic of enzyme-activated and reduced biosynthetic precursors of vitamin B₁₂ and a B₁₂-antimetabolite potentially functioning as a specific inhibitor of B₁₂-biosynthesis.

KEYWORDS: antivitamin B₁₂, B₁₂-antimetabolite, corrin, tetrapyrrole, transition metal, vitamin B₁₂.

INTRODUCTION

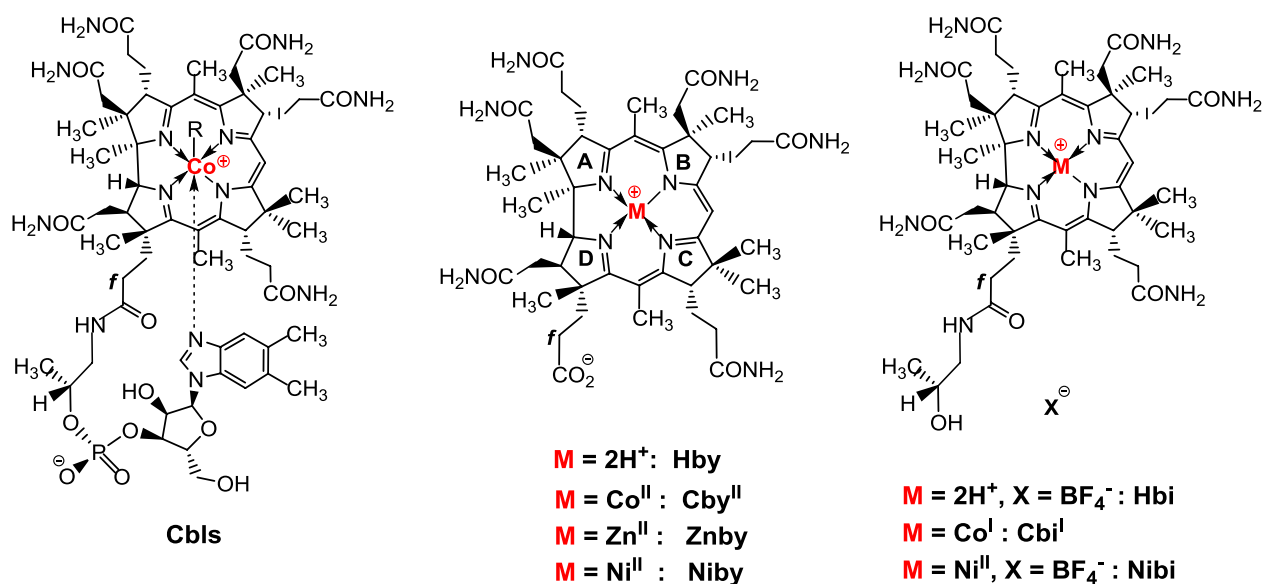
The pre-eminent biological use of the corrin ligand in the natural vitamin B₁₂-derivatives, and of cobalt as their specific transition metal center, poses the intriguing problem as to why this particular partnership has evolved

for providing the unique biochemical reactivity of the B₁₂-cofactors [1–5]. The specific chemistry of cobalt and other transition metals, when bound by the helical ‘ring-contracted’ natural corrin ligand, is the subject of fundamental questions [1, 2, 6, 7]. Indeed, the synthesis of transition metal analogues of the natural cobalt-corrinoids has been a longstanding ‘holy grail’ in the B₁₂-field [8–11]. Fortunately, the elucidation of the biochemical B₁₂-biosynthesis paths [12, 13], coupled with bioengineering approaches [14], has opened up direct preparative access to hydrogenobyric acid (**Hby**) [7], the metal-free corrin ligand of vitamin B₁₂ (**CNCbi**). The biosynthetic availability of **Hby** has generated a consummate opportunity for the synthesis and characterization of transition metal corrins, including zincobyric acid (**Znby**) [15] and nibyric acid (**Niby**) [16], the Zn(II)- and Ni(II)-complexes of **Hby**, respectively. Furthermore, **Znby** and **Hby** served as rational precursors for the preparation of the ‘complete’

◊SPP full member in good standing.

*Correspondence to: Bernhard Kräutler, e-mail. bernhard.kraeutler@uibk.ac.at, Institute of Organic Chemistry and Center of Molecular Biosciences, University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria, Tel: +43-664-8125275, Fax: +43-512-507-57799

This is an open access article published by World Scientific Publishing and distributed under the terms of the Creative Commons Attribution (CC BY) 4.0 License, which permits use, distribution and reproduction in any medium, provided the original author(s) and source are credited.



Scheme 1. Formulae of cobalt, zinc, nickel and metal-free corrinoids. Left: General formula of the cobalamins (CbIs) vitamin B₁₂ (R = CN, CNCbI), coenzyme B₁₂ (R = 5'-deoxyadenosyl, AdoCbI), methylcobalamin (R = CH₃, MeCbI) and cob(II)alamin (R = e⁻, CbII) Center: Formula of the metal-free corrin hydrogenobyrates (Hby), of Co(II)-cobyrates (CbyII), zincobyrates (Znby), with the omission of the β-axial ligands at Co(II) and Zn(II), and of the Ni(II)corrin nibrates (Niby). Right: formula of metal-free hydrogenobinamides (Hbi), of cob(I)inamide (CbiI) and of nibrates (Nibi).

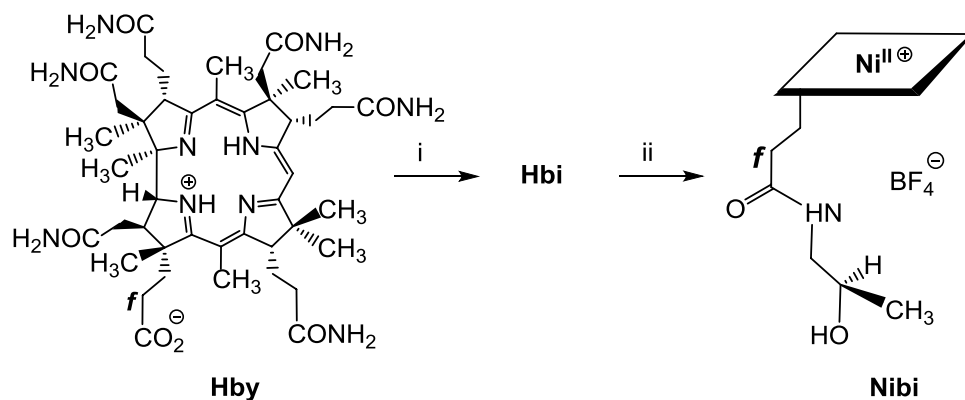
vitamin B₁₂ analogues zincobalamin (**Znbi**) and nibrates (**Nibi**), both also structurally characterized [15, 16].

In the Eschenmoser labs [17, 18], a nickel corrin was constructed in the 1960s as the first synthetic corrin, also allowing for an unprecedented X-ray crystallographic investigation of the structure of a non-cobalt corrin [19]. In more recent times, the quest for Ni-analogues of the B₁₂-cofactors as structural B₁₂-mimics has resurged [10, 11, 16], as Ni(II)-analogues of the cobalamins (**CbIs**) are predicted to reveal interesting coordination chemical features and could represent 'antivitamins B₁₂' [20–23]. The structural elucidation of the related natural porphyrinoid nickel-cofactor F₄₃₀ [24] and its complex biological chemistry [25, 26] have strongly boosted interest in the basic coordination chemistry of tetrapyrrolic nickel-complexes [27–29]. Herein, we report on the metal-free

cobinamide ligand hydrogenobinamide (**Hbi**) and its Ni(II)-complex nibrates (**Nibi**) (see Scheme 1), and their first synthesis.

RESULTS AND DISCUSSION

The metal-free Cbi-ligand **Hbi** was prepared from **Hby** by attaching the (*R*)-isopropanolamine moiety to its carboxylic acid group, using an established carbodiimide method [30]. In brief, an aqueous solution of 2.92 mg (3.33 μmol) of **Hby** and of 20 moleq N-hydroxybenzotriazole (HOBT), was treated with 7.96 mg (106 μmol) of (*R*)-isopropanolamine in 200 μl of 1 M HCl. The solution was degassed and frozen with external dry ice, and 2 mg (4 moleq) of EDC·HCl was added under Ar. The reaction



Scheme 2. Outline of the synthesis of **Hbi** and **Nibi** from **Hby**. i) (*R*)-1-amino-2-propanol; HOBT, EDC·HCl, in H₂O; ii) Ni(OAc)₂ in H₂O, 90 °C.

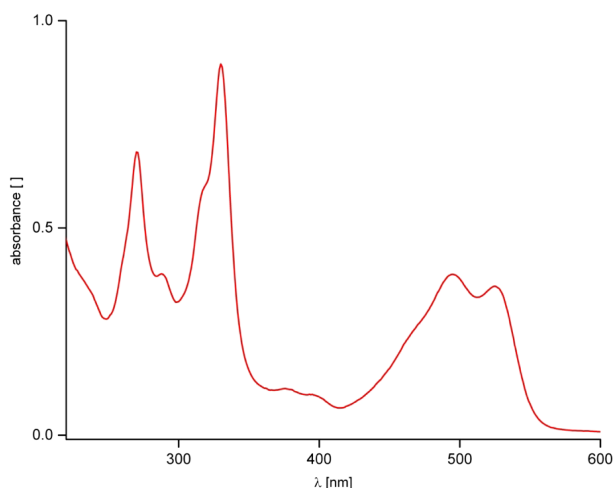


Fig. 1. UV/Vis-absorption spectrum of **Hbi** ($c = 19 \mu\text{M}$ in 10 mM aq. Na-phosphate pH5, 298K).

mixture was warmed up to 0 °C and its pH was adjusted to 6.4. Further addition of six portions of about 3 moleq EDC·HCl each at 0 °C over the course of 168 h, isolation of the product using RP18 solid phase extraction and lyophilization furnished 2.88 mg (2.82 μmol , 85% yield) of pure **Hbi**-BF₄ as an orange powder (see Scheme 2).

An aqueous solution of **Hbi** at pH 5 exhibited UV/Vis- and CD-spectral features (see Fig. 1 and Fig. S2), as well as strong fluorescence with maxima at 609 nm and 553 nm (see Fig. S3), all similar to the corresponding spectra of **Hby** [7]. A high-resolution mass spectrum of **Hbi** confirmed the expected molecular formula C₄₈H₇₄N₁₁O₈ (see Fig. S4). The data from hetero-nuclear NMR-spectra allowed for the assignment of 73 of the 74 H-atoms and of all 48 C-atoms (see Table S2), which

allowed the structure of **Hbi** in an aqueous solution to be elucidated. Two ‘inner’ H-atoms were detected that gave rise to singlets at $\delta = 12.44$ and $\delta = 12.68$ ppm in the ¹H-NMR spectrum (see Fig. 2). Using the critical HMBC and NOE correlations (see e.g. [31–33]), the two singlets were assigned to H(N4) and H(N2), respectively (see Fig. S5), in line with the corresponding assignments for **Hby** [7] and for the complete metal-free B₁₂-ligand hydrogeno-balamin (**Hbl**) [16], but (formally) contrasting the position of the ‘inner’ H-atoms in Eschenmoser’s metal-free model corrin [34]. Only small shifts to the lower field by 0.01 ppm (¹H) and by roughly 3ppm (¹⁵N) were indicated for the inner NH-groups in the spectrum of **Hbi**, when compared to **Hby** (see Tables S1 and S2). The detailed data from homo-nuclear and hetero-nuclear correlations (see Fig. S6) were consistent not only with the established diagonal arrangement of two ‘inner’ protons in metal-free corrins [7, 16, 34], but also with the intact stereo-structure of **Hbi**.

With pure samples of metal-free **Hbi** in hand, the one-step synthesis of transition metal analogues of the cobalt-containing cobinamides, metbinamides (**Metbis**), has become a realistic target, providing access to a still unexplored area in the wider B₁₂-field. Thus, the Ni(II)-corrin nibinamide (**Nibi**) was prepared by heating a deoxygenated aqueous solution of **Hbi** and Ni(OAc)₂ pH 6 for 1 h at 90°C (Scheme 2), furnishing pure **Nibi**-BF₄ (56% yield) as a yellow powder. An aqueous solution of **Nibi** buffered to pH 5 exhibited UV/Vis- and CD-spectra very similar to the corresponding spectra of **Niby** (see Fig. 3 and Fig. S8).

The solution structure of **Nibi**, molecular formula C₄₈H₇₂BF₄N₁₁O₈Ni from a high-resolution ESI-MS spectrum (see Fig. S9), was determined by hetero-nuclear

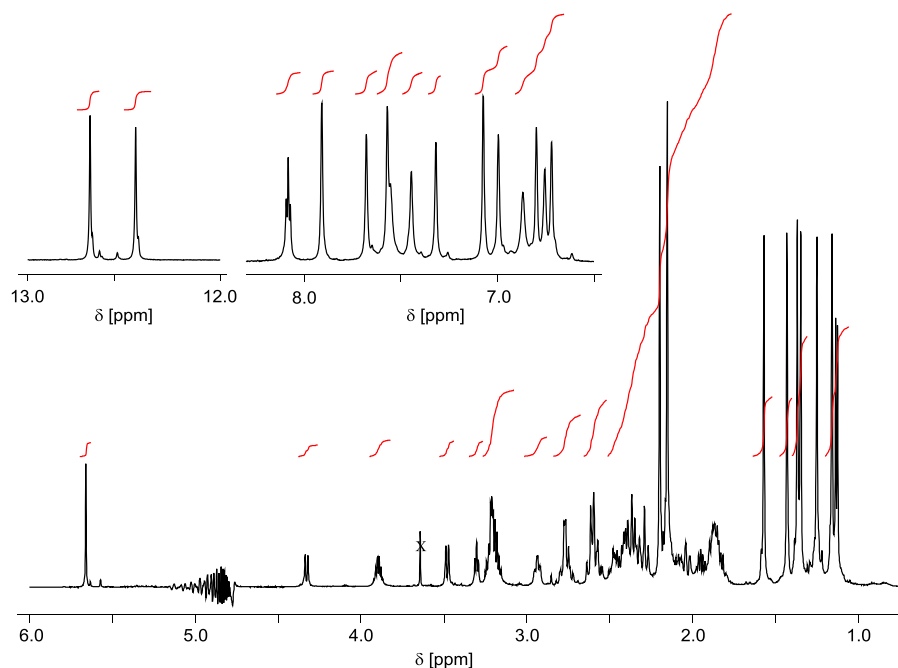


Fig. 2. 600MHz ¹H-NMR spectrum of **Hbi** ($c = 2 \text{ mM}$) in H₂O/D₂O (9:1) at pH5 and 298K.

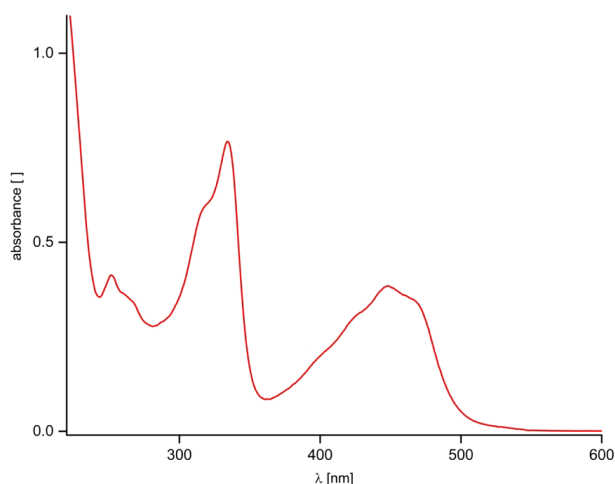


Fig. 3. UV-Vis spectrum of **Nibi** ($c=44 \mu\text{M}$) in 10 mM aqueous Na-phosphate buffer pH 5 (298K).

high field NMR-spectroscopy (see Fig. 4 for a 500 MHz ^1H -NMR spectrum), providing assignment of all 58 non-exchangeable H-atoms and all 48 C-atoms (see Table S3). The NMR-data for the Ni(II)-complex **Nibi** featured very similar characteristics to the ones described for **Niby**. Compared to the spectrum of metal-free **Hbi**, similar shift differences were determined from the NMR-data for **Nibi**, as had been observed earlier for the corresponding pair **Hby** and **Niby** [16]. The set of homo-nuclear and hetero-nuclear correlations from the NMR spectra of **Nibi** in D_2O also confirmed its expected stereo-structure (see Fig. S10). ^1H , ^1H -NOE-correlations between the isopropanol terminus of the modified f-side chain and C151, as well as the neighboring ring D moiety of the corrin ring, support a time-averaged position of this rather lipophilic terminal group near the lower face of the corrin. In fact, the acquired spectral data suggest the diamagnetic Ni(II)-corrin **Nibi** be an *iso*-electronic and roughly

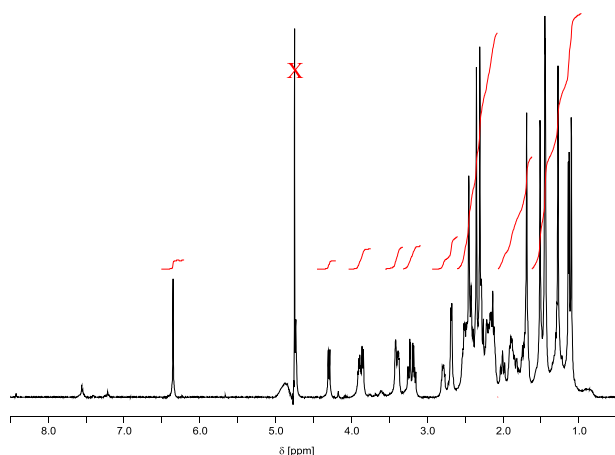


Fig. 4. 500 MHz ^1H -NMR-spectrum of **Nibi** ($c=2.6 \text{ mM}$ in D_2O , HDO pre-saturated, 298K) X= residual HDO signal.

iso-structural mimic of the strongly reducing [35] and highly nucleophilic, but structurally less characterized Co(I)-corrin cob(I)inamide (**Cbi^I**).

The major structural effects of the formal replacement of cobalt by nickel in vitamin B_{12} derivatives were revealed by the X-ray crystal structure analysis of **Niby**, finding the 4-coordinate diamagnetic Ni(II)-ion of **Niby** located close (at 0.025 \AA) to the best plane through the four inner corrin N-atoms [16]. The 4-coordinate Ni(II)-ion is bound with short average Ni-N bond lengths of 1.86 \AA in **Niby** [16]. This crystallographic finding, and a complementary one with the Rh(III)-corrin adenosylrhodibalamine (**AdoRhbl**) [9], support the suggestion that the coordination hole of the ‘ring contracted’ corrin ligand is still too large for strain-free binding of a low-spin d^8 -Ni(II)-center. However, the Ni(II)-ion of **Niby** was deduced to coordinate the corrin ligand in a similar way [16] as the 4-coordinate Co(II)-center of a protein-bound Co(II)corrin [36], or as the 5-coordinate Co(II)-centers in the crystalline Co(II)-corrins **Cbi^{II}** [37] and cob(II)yrinic acid heptamethyl ester [38].

We have developed here a rational, direct synthesis path to the polar metal-free Cbi-ligand **Hbi** and to its diamagnetic Ni(II)-complex nibinamide **Nibi** (the Ni(II)-analogue of **Cbi**). Both of these novel corrins reveal key structural features of **Cbis**, the major ‘incomplete’ natural cobalt-corrins. This work extends our recent studies with **AdoRhbl** [9], **CIRhbl** [39] and zincobalamin (**Znbl**) [15], Rh(III)- and Zn(II)- analogues of 6- and 5-coordinate ‘base-on’ **Cbis**, resp., as well as with nibalamin (**Nibl**) [16], the Ni(II)-analogue of (‘complete’) four coordinate ‘base-off’ **Cbi**-forms including **Cbi^I**. The Ni(II)-corrin **Nibi** is presented here as an excellent redox-stable structural mimic for the corresponding natural 4-coordinate ‘incomplete’ **Cbi^{II}**- and **Cbi^I**-species. Such activated reduced **Cbi^{II}**- and **Cbi^I**-species play the roles of highly reactive intermediates in basic B_{12} -biosynthetic enzyme processes in microorganisms that generate coenzyme B_{12} (**AdoCbl**) from externally supplied and actively imported **Cbis** [40] via cobalt-adenosylation and subsequent ‘completion’ to Ado-cobamides [13, 41–45]. As a stable **Cbi**-mimic, **Nibi**, thus, may represent a B_{12} -antimetabolite with the potential of selectively impairing the B_{12} -biosynthetic capacity of bacteria. Like the cobinamides [41, 46], **Nibi** would be predicted to possess the little capacity to downregulate the expression of the bacterial B_{12} -uptake systems as ligands of the B_{12} -riboswitches. **Nibi** would, furthermore, not be expected to find a ready cellular import in humans and animals via their B_{12} -uptake system [47–48], contrasting with the behavior of genuine ‘antivitamins B_{12} ’ [20–22, 49]. As a consequence, **Nibi** represents a novel antibiotic candidate, selectively targeting microorganisms. Accordingly, studies with the ‘incomplete’ Ni(II)-corrin **Nibi** and with other suitably structured transition metal analogues of the **Cbis** are clearly worthwhile.

EXPERIMENTAL

General

Materials

Methanol (MeOH), acetonitrile (MeCN), HiPerSolv Chromanorm, and acetic acid (HOAc) p.A., 1-hydroxybenzotriazole (HOBt), sodium hydroxide (NaOH) p.A. were from VWR chemicals; R(-)-1-amino-2-propanol from Fluka; N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC*HCl), nickel acetate trihydrate (Ni(OAc)₂*3 H₂O) p.A.; tetrafluoroboric acid 48% in H₂O; sodium tetrafluoroborate, p.A., sodium acetate (NaOAc); sodium dihydrogenphosphate (NaH₂PO₄), disodium hydrogenphosphate (Na₂HPO₄) from Sigma Aldrich; water (H₂O) deionized, was purified by reversed osmosis via MilliQ academic system; D₂O 99.96%D from Eurisotop; *Spectra*: UV-Vis: Agilent Cary 60. CD: Jasco J-715 or Jasco J-1500-150 CD spectropolarimeters, spectra were recorded at 298K. NMR: 500 MHz Varian Unity Inova, 5mm triple-resonance probe with z-gradients, pulse sequences from VNMR J-ChemPak 4.1; 600 MHz Bruker Avance II+ with Prodigy TCI™ probe; ¹H reference to δ(HDO) = 4.75 ppm, signal assignments were based on ¹H, (¹H,¹H)-COSY, (¹H,¹³C)-HSQC, (¹H,¹³C)-HMBC and (¹H,¹H)-ROESY spectra. ESI-HR-MS: Thermo Scientific LTQ-Orbitrap XL, (+)-ion mode, 4.5 kV in MeOH. *Chromatography*: HPLC using Hitachi Elite LaChrom, L2130 pump, L245 diode array detector; Dionex Ultimate 3000, variable wavelength detector; column: YMC-Triart –C18, 250x4.7 mm, S-5 μm, 12 nm; solvent composition: A: 10 mM aqueous NH₄OAc pH 7, B= MeOH; 8% to 95%B 0–40 min, 95% B 40–44 min, 95% to 8%B 44–45 min, flow= 1 mL/min. RP18-MPLC: Büchi C-605 pump module (binary) flow≈10 mL/min, home-packed RP18 column (l=230 mm, Ø=26 mm, column volume (cv) = 122 mL) using about 100 g LiChroprep RP18. Sep-Pak® C18 cartridges (various sizes, from Waters) were conditioned with 20 mL MeOH and 60 mL H₂O prior to use.

Synthesis of hydrogenobinamide tetrafluoroborate (Hbi)

In a 20 mL 2-necked round bottom flask 2.92 mg (3.33 μmol) **Hby** [7], 8.88 mg (65.7 μmol, 20 eq) 1-hydroxybenzotriazole (HOBt) were dissolved in 3.2 mL H₂O. A solution of 7.96 mg (106 μmol, 32eq) R(-)-1-amino-2-propanol in 100 μL 1 M HCl was added and the mixture was deoxygenated by 3 freeze/vacuum/thaw cycles. The solution was frozen and 2.0 mg (10.6 μmol, 4eq) N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC*HCl) were added under Ar. The solution was thawed and the pH was adjusted to pH 6.4 with ~40 μL 1 M NaOH under vigorous stirring. The reaction was kept on ice for 7d and ~1.5–2 mg (~3eq) EDC*HCl were

added once per day in Ar counter flux. After 168 h the orange reaction solution was diluted with H₂O to ~10ml and loaded on a Sep-Pak® plus long cartridge. The adsorbate was washed with 20 mL H₂O, 20 mL 100 mM aqueous NaBF₄ pH 6, and a further 20 mL H₂O. The **Hbi** was eluted with 3 mL 100 μM NaBF₄ in MeOH. The eluate was frozen and lyophilized under HV. The residue was dissolved in 1ml H₂O and lyophilized again. 2.88 mg (2.82 μmol, 85%) of powdery orange **Hbi** (a tetrafluoroborate) were obtained (for HPLC see Fig. S1). UV/Vis (c=18.9 μM in 10 mM aq. Na-phosphate pH 5, RT): λ^{max} [nm] (lg ε) = 525 (4.28), 495 (4.31), 472 (sh, 4.16), 3.93 (3.71), 377 (3.77), 330 (4.67), 320 (sh, 4.51), 288 (4.31), 270 (4.56) (see Fig. 1). CD (c=42.4 μM in 10 mM aq. Na-phosphate pH 5, 293K): λ^{max/min} [nm] (± Δε [l*mol⁻¹*cm⁻¹]) = 522 (-3.5), 501 (-2.7), 394 (0.4), 328 (15.2), 270 (-7.9), 232 (3.1); λ⁰ [nm] = 425, 367, 296, 242, 222 (see Fig. S2). Fluorescence (c=7.62 μM in 10 mM aq. Na-phosphate pH 5): emission spectrum (λ^{exc} = 500 nm): λ^{max} [nm] (rel. int.) = 609 (406), 553 (216); excitation spectrum (λ^{em} = 609 nm): λ^{max} [nm] (rel. int.) = 526 (477), 502 (413), 393 (70), 376 (81), 330 (580), 320 (439), 305 (285), 270 (394), 262 (258) (see Fig. S3). ¹H-NMR spectra were measured at 600 MHz (c(Hbi) = 1.96 mM in 10 mM Na-phosphate in 10% D₂O, at 298K, see Fig. 2), ¹H- ¹³C- and ¹⁵N-signal assignments from 2D homo- and hetero-nuclear spectra, see Figs. S5 and S6, Tables S1 and S2). HR-ESI-MS (MeOH): 934.579 (12), 933.575 (57), 932.572 (100, [C₄₈H₇₄N₁₁O₈]⁺ ≡ [M]⁺); 486.269 (5), 485.767 (10, [M+K]²⁺); 478.783 (16), 478.281 (56), 477.780 (98, [M+Na]²⁺); 467.291 (9), 466.789 (16, [M+H]²⁺) (see Fig. S4).

Synthesis of nibinamide tetrafluoroborate (Nibi)

In a 15 mL Schlenk tube equipped with a reflux condenser 2 mL 0.5 M Ni(OAc)₂ pH were degassed by 5 freeze/vacuum/thaw cycles. 1.70 mg (1.82 μmol) **Hbi** were added and the mixture was degassed by further 3 freeze/vacuum/thaw cycles. The apparatus was pressurized with Ar and the brown solution was heated to 90°C for 1h. After cooling to room temperature the apparatus was aerated and the green solution was diluted with H₂O to 20 mL. The solution was loaded on a Sep-Pak® C18 Classic cartridge. The adsorbate was washed with 20 mL H₂O followed 20 mL 100 mM NaBF₄ pH 6 and a further 20 mL H₂O. The crude nibinamide (**Nibi**) was eluted with 3 mL 100 μM NaBF₄ in MeOH. The solvents were evaporated on the rotary evaporator (55°C), and the residue was dissolved in 10 mM NaOAc pH 6 and loaded on the MPLC column. The crude **Nibi** was purified using 1 L portions of 10%, 12%, 13%, 14%, 15%, and 16% MeCN in 10 mM Na(OAc)₂. The Nibi-containing fraction was concentrated to ~40 ml on the rotary evaporator (50°C) and loaded on a Sep-Pak® C18 Classic cartridge. The adsorbate was washed with 80 mL 100 mM NaBF₄ pH 6, 20 mL 50 mM NaBF₄, and 20 mL H₂O. The fraction

with **Nibi** was eluted with 2 mL 100 μ M NaBF₄ in MeOH and evaporated on the rotary evaporator. The residue was dissolved in 1 mL of H₂O and the sample was lyophilized overnight. 1.10 mg (1.02 μ mol, 56%) of yellow powdery **Nibi** were isolated pure (for HPLC see Fig. S7). UV-Vis (c=44.1 μ M in 10 mM Na-phosphate pH 5, RT): λ^{\max} [nm] (lg ϵ) = 465 (3.90), 448 (3.94), 430 (sh, 3.86), 404 (sh, 3.69), 334 (4.24), 321 (sh, 4.14), 262 (sh, 3.91), 252 (3.97) (see Fig. 3). CD (c=44.1 μ M in 10 mM Na-phosphate pH 5, 293 K): $\lambda^{\max/\min}$ [nm] ($\pm \Delta \epsilon$ [$l \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$]) = 457 (-0.79), 413 (0.53), 326 (sh, 3.35), 315 (4.94), 255 (-5.86); λ^0 [nm] = 431, 393, 342, 289, 226 (see Fig. S8). ¹H-NMR spectra were measured at 500 MHz (c(Nibi) = 2.55 mM in D₂O at 298 K, see Fig. 4), ¹H- and ¹³C-signal assignments from 2D homo- and hetero-nuclear spectra, see Fig. S10 and Table S3). HR-ESI-MS (MeOH): m/z (%) = 992.944 (2), 991.490 (20), 990.488 (34), 989.495 (55), 988.492 (100, [C₄₈H₇₂N₁₁NiO₈]⁺ \equiv [M]⁺); 513.726 (5, [M+K]²⁺); 507.740 (3), 507.239 (17), 506.738 (32), 506.21 (42), 505.739 (76, [M+Na]²⁺); 496.248 (7), 495.747 (15), 495.250 (20), 494.749 (37, [M+H]²⁺) (see Fig. S9).

Acknowledgments

This work was supported by grants from the Austria Science Fund (FWF, P-28892 and P33059) to BK and from the Biotechnology and Biological Sciences Research Council (BBSRC) BB/S002197/1 to MJW.

Supporting information

Figures of CD-, fluorescence and mass spectra, and NMR-data are given in the supplementary material. This material is available free of charge via the Internet at <https://www.worldscientific.com/doi/suppl/10.1142/S1088424623500463>

REFERENCES

1. Eschenmoser A. *Angew. Chem. Int. Ed.* 1988; **27**: 5–39.
2. Pratt JM. *Inorganic Chemistry of Vitamin B₁₂*, Academic Press, New York, 1972.
3. da Silva JJRF and Williams RJP. *The Biological Chemistry of the Elements*, Clarendon Press, Oxford, 1991.
4. Gruber K, Puffer B and Kräutler B. *Chem. Soc. Rev.* 2011; **40**: 4346–4363.
5. Osman D, Cooke A, Young TR, Deery E, Robinson NJ and Warren MJ. *Biochim. Biophys. Acta* 2021; **1868**: 118896.
6. Blaser HU, Winnacker EL, Fischli A, Hardegger B, Bormann D, Hashimoto N, Schossig J, Keese R and Eschenmoser A. *Helv. Chim. Acta* 2015; **98**: 1845–1920.
7. Kieninger C, Deery E, Lawrence AD, Podewitz M, Wurst K, Nemoto-Smith E, Widner FJ, Baker JA,

- Jockusch S, Kreutz CR, Liedl KR, Gruber K, Warren MJ and Kräutler B. *Angew. Chem. Int. Ed.* 2019; **58**: 10756–10760.
8. Copenhagen VB. *Metal-Free Corrinoids and Metal Insertion*, in: D. Dolphin (Ed.) B₁₂, John Wiley & Sons, 1982, pp. 105–150.
9. Widner FJ, Lawrence AD, Deery E, Heldt D, Frank S, Gruber K, Wurst K, Warren MJ and Kräutler B. *Angew. Chem. Int. Ed.* 2016; **55**: 11281–11286.
10. Holze G and Inhoffen HH. *Angew. Chem. Int. Ed.* 1985; **24**: 867–869.
11. Brenig C, Prieto L, Oetterli R and Zelder F. *Angew. Chem. Int. Ed.* 2018; **57**: 16308–16312.
12. Battersby AR. *Nat. Prod. Rep.* 2000; **17**: 507–526.
13. Bryant DA, Hunter CN and Warren MJ. *J. Biol. Chem.* 2020; **295**: 6888–6925.
14. Deery E, Schroeder S, Lawrence AD, Taylor SL, Seyedarabi A, Waterman J, Wilson KS, Brown D, Geeves MA, Howard MJ, Pickersgill RW and Warren MJ. *Nat. Chem. Biol.* 2012; **8**: 933–940.
15. Kieninger C, Baker JA, Podewitz M, Wurst K, Jockusch S, Lawrence AD, Deery E, Gruber K, Liedl KR, Warren MJ and Kräutler B. *Angew. Chem. Int. Ed.* 2019; **58**: 14568–14572.
16. Kieninger C, Wurst K, Podewitz M, Stanley M, Deery E, Lawrence AD, Liedl KR, Warren MJ and Kräutler B. *Angew. Chem. Int. Ed.* 2020; **59**: 20129–20136.
17. Bertele E, Boos H, Dunitz JD, Elsinger F, Eschenmoser A, Felner I, Gribo HP, Gschwend H, Meyer EF, Pesaro M and Scheffold R. *Angew. Chem. Int. Ed. Engl.* 1964; **3**: 390–496.
18. Bertele E, Scheffold R, Gschwend H, Pesaro M, Fischli A, Roth M, Schossig J and Eschenmoser A. *Helv. Chim. Acta* 2015; **98**: 1755–1844.
19. Dunitz JD and Meyer jun. EF, *Helv. Chim. Acta* 1971; **54**: 77–89.
20. Kräutler B. *Chem. Eur. J.* 2015; **21**: 11280–11287.
21. Kräutler B. *Chem. Eur. J.* 2020; **26**: 15438–15445.
22. Kräutler B. *Antivitamins B₁₂*, in: Litwack G (Ed.) *Vitamins and Hormones*, Academic Press, Cambridge, Mass., 2022, pp. 221–240.
23. Zelder F, Sonnay M and Prieto L. *ChemBioChem* 2015; **16**: 1264–1278.
24. Pfaltz A, Jaun B, Fässler A, Eschenmoser A, Jaenchen R, Gilles HH, Diekert G and Thauer RK. *Helv. Chim. Acta* 1982; **65**: 828–865.
25. Jaun B and Thauer RK. *Nickel-Alkyl Bond Formation in the Active Site of Methyl-Coenzyme M Reductase*, in: Sigel A, Sigel H and Sigel RKO (Eds.) *Metal-Carbon Bonds in Enzymes and Cofactors*, Royal Society of Chemistry, Cambridge, 2009, pp. 115–132.
26. Scheller S, Goenrich M, Boecher R, Thauer RK and Jaun B. *Nature* 2010; **465**: 606–609.

27. Kratky C, Waditschatka R, Angst C, Johansen JE, Plaquevent JC, Schreiber J and Eschenmoser. *Helv Chim Acta* 1985; **68**: 1312–1337.
28. Kraatz HB and Metzler-Nolte N. *Concepts and Models in Bioinorganic Chemistry*, Wiley VCH, Weinheim, 2006.
29. Wicht R, Bahnmüller S, Brandhorst K, Schweyen P and Bröring M. *Chem. Sci.* 2016; **7**: 583–588.
30. Widner FJ, Gstrein F and Kräutler B. *Helv. Chim. Acta* 2017; **100**: e1700170
31. Tollinger M., Konrat R and Kräutler B. *Helv. Chim. Acta* 1999; **82**: 1596–1609.
32. Summers MF, Marzilli LG and Bax A. *J. Am. Chem. Soc.* 1986; **108**: 4285–4294.
33. Bax A, Marzilli LG and Summers MF. *J. Amer. Chem. Soc.* 1987; **109**: 566–574.
34. Edmond ED and Hodgkin DC. *Helv. Chim. Acta* 1975; **58**: 641–654.
35. Lexa D and Savéant JM. *Acc. Chem. Res.* 1983; **16**: 235–243.
36. Kräutler B, Keller W and Kratky C. *J. Am. Chem. Soc.* 1989; **111**: 8936–8938.
37. St Maurice MS, Mera P, Park K, Brunold TC, Escalante-Semerena JC and Rayment I. *Biochemistry* 2008; **47**: 5755–5766.
38. Kräutler B, Keller W, Hughes M, Caderas C and Kratky C. *J. Chem. Soc., Chem. Comm.* 1987; 1678–1680.
39. Widner FJ, Kieninger C, Wurst K, Deery E, Warren MJ and Kräutler B. *Synthesis* 2021; **53**: 332–337.
40. Bradbeer C. *Cobalamin Transport in Bacteria*, in: Banerjee R (Ed.) *Chemistry and Biochemistry of B₁₂*, John Wiley & Sons, New York, 1999, pp. 489–506.
41. Kennedy KJ, Widner FJ, Sokolovskaya OM, Innocent LV, Procknow RR, Mok KC and Taga ME. *mBio* 2022; <https://doi.org/10.1128/mbio.01121-22>.
42. Escalante-Semerena JC, Woodson JD, Buan NR and Zayasm CL. *Conversion of Cobinamide into Coenzyme B₁₂*, in Warren MJ and Smith AG (Eds.) *Tetrapyrroles: Birth, Life and Death*, Landes Bioscience, Austin, Texas, 2008, pp. 298–314.
43. Mok KC, Sokolovskaya OM, Nicolas AM, Hallberg ZF, Deutschbauer A, Carlson HK and Taga ME. *mBio*, 2020; **11**: e02507–20.
44. Gude S, Pherribo GJ and Taga ME. *Msystems* 2022; **7**: 00288–00222.
45. Jeter VL, Mattes TA, Beattie NR and Escalante-Semerena JC. *Biochemistry* 2019; **58**: 951–964.
46. Gallo S, Oberhuber M, Sigel RKO and Kräutler B. *ChemBioChem* 2008; **9**: 1408–1414.
47. Fedosov SN, Fedosova NU, Kräutler B, Nexo E and Petersen TE. *Biochemistry* 2007; **46**: 6446–6458.
48. Ruetz M, Gherasim C, Fedosov SN, Gruber K, Banerjee R and Kräutler B. *Angew. Chem. Int. Ed.* 2013; **52**: 2606–2610.
49. Mutti E, Ruetz M, Birm H, Kräutler B and Nexo E. *Plos One* 2013; **8**: e75312.