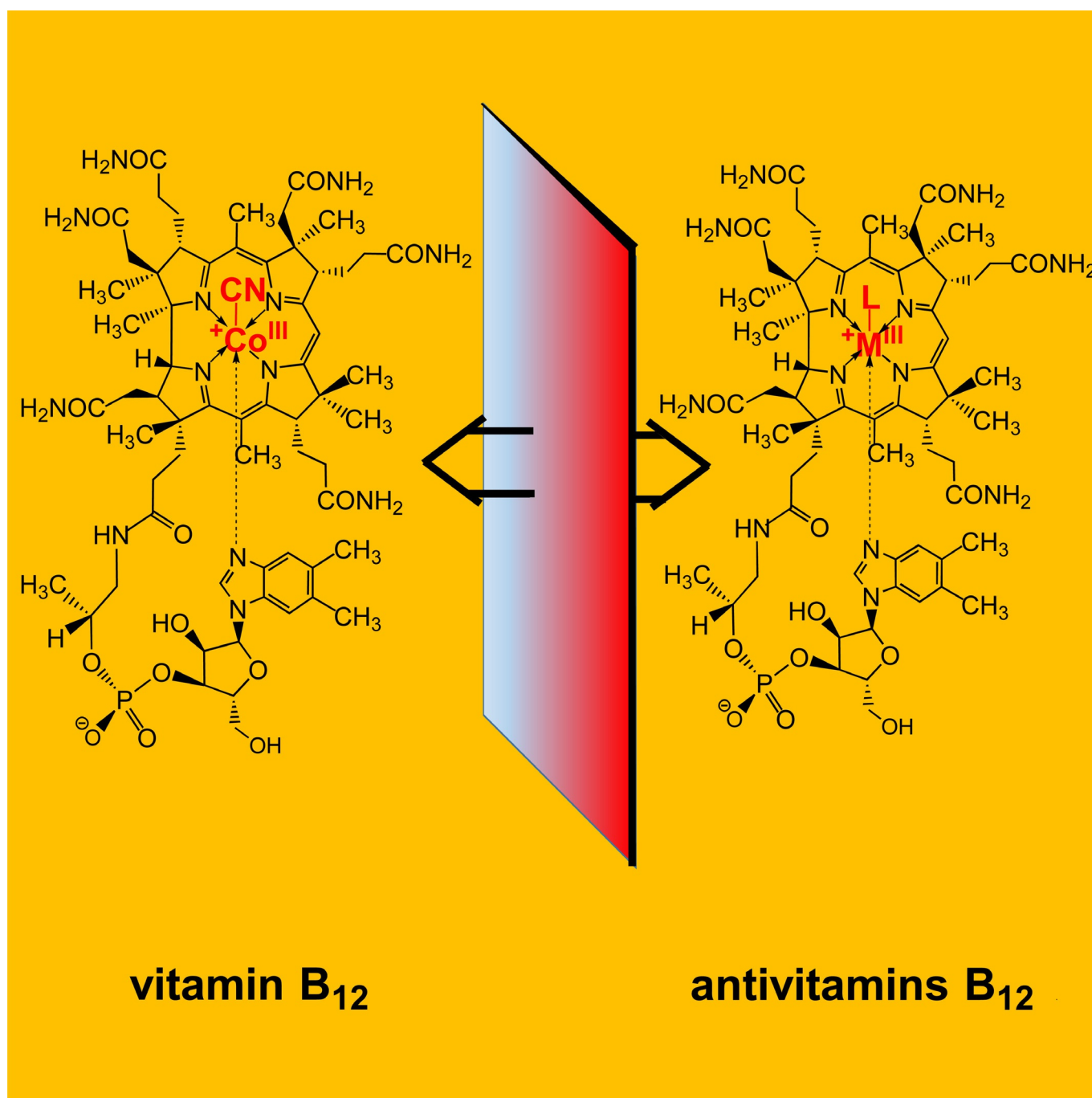


■ Vitamins | *Reviews Showcase* |🏆 Antivitamins B₁₂—Some Inaugural Milestones

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Bernhard Kräutler*^[a]*Dedicated to the memory of Professor Duilio Arigoni*

Abstract: The recently delineated structure- and reactivity-based concept of antivitamin B₁₂ has begun to bear fruit by the generation, and study, of a range of such B₁₂-dummies, either vitamin B₁₂-derived, or transition metal analogues that also represent potential antivitamin B₁₂ or specific B₁₂-antimetabolites. As reviewed here, this has opened up new research avenues in organometallic B₁₂-chemistry and bioinorganic coordination chemistry. Exploratory studies with anti-

vitamin B₁₂ have, furthermore, revealed some of their potential, as pharmacologically interesting compounds, for inducing B₁₂-deficiency in a range of organisms, from hospital resistant bacteria to laboratory mice. The derived capacity of antivitamin B₁₂ to induce functional B₁₂-deficiency in mammalian cells and organs also suggest their valuable potential as growth inhibitors of cancerous human and animal cells.

Introduction

Vitamin B₁₂, the Co^{III}-corrin cyanocobalamin (CNCbl), is a most fascinating and intriguing natural product,^[1] that was discovered as the original isolation form of the life-saving ‘extrinsic’ anti-pernicious anemia factor.^[2] An exceptional 5,6-dimethylbenzimidazole pseudonucleotide appendage to the corrin core coordinates to the cobalt-centre of CNCbl, establishing the unique and characteristic three-dimensional architecture of the cobalamins (CbIs). CbIs belong to the larger family of the cobamides (CbAs), also including the related natural ‘complete’ corrinoids^[3] with other pseudonucleotide heterocycles^[3a,4] or linker units.^[5] These complex cobalt-corrins are all generated in Nature by intricate B₁₂-biosynthetic paths,^[6] an exclusive capacity of some bacterial prokaryotes and archaea.^[6b] Indeed, according to Eschenmoser’s proposal, the natural B₁₂-derivatives may originate from structurally simpler cobalt-corrinoid precursors, presumed to have developed in early forms of life.^[7]

In spite of many years of intense medicinal,^[8] molecular biological and biochemical^[9] research, new physiological roles of B₁₂ in humans keep emerging,^[8e,10] while some further Cbl-related medical findings remain puzzling,^[11] so that B₁₂ has been classified as a ‘moonlighting’ vitamin.^[12] The association of the B₁₂’s own cobalt with a ‘Kobold’, the German word for goblin, appears to fit the occasionally puzzling situation. In fact, vitamin B₁₂ (CNCbl) itself is not a directly physiologically active vitamin in humans and other mammals.^[8e,13] In order to set free its functional capacity, CNCbl needs to be converted by the mammalian metabolism,^[10c,13] into the organometallic B₁₂-cofactors methylcobalamin (MeCbl) and coenzyme B₁₂ (adenosyl-

cobalamin, AdoCbl).^[3c] CNCbl has, thus, the role of a ‘provitamin’.^[14] In fact, various CbIs, more directly functional physiologically than CNCbl, among them AdoCbl, are preferred B₁₂-vitamers for the treatment of some patients (Figure 1).^[15]

The possible physiological effects of artificial intact CbIs designed to closely mimic the molecular shape of vitamin B₁₂ and to resist metabolic conversion into the B₁₂-cofactors, have begun to attract our interest.^[16] The highly efficient and complex B₁₂-uptake and transport system in humans^[17] and higher animals^[18] should bind such inactive vitamin B₁₂ analogues rather indiscriminately (as would, typically, also be the case for B₁₂-using bacteria^[19]), with the consequence of the cellular import of (inactive) B₁₂-dummies competing with the natural cobalamins and effectively impairing B₁₂-metabolism. In consequence, B₁₂-analogues designed according to these criteria, would act as antivitamin B₁₂ that induce functional Cbl-deficiency in humans and other mammals *in vivo*; a concept presented in this Journal about 5 years ago.^[16] Antivitamin B₁₂ relate to the broader class of the B₁₂-antimetabolites and were discussed in this context.^[14,20] Typical B₁₂-based antimetabolites, which are not covered in this Minireview, are CbIs (or other CbAs), modified at their periphery, that counteract, or fail to fulfil adequately, the physiological roles of natural B₁₂-derivatives in various B₁₂-dependent organisms, including many microorganisms. B₁₂-deficiency deprives some bacteria, animal

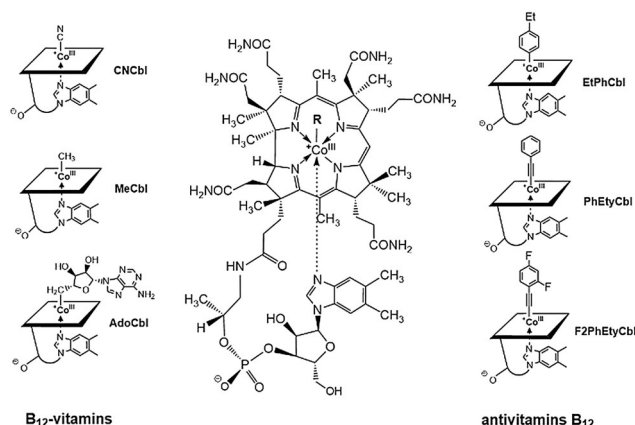


Figure 1. General structural formula of the cobalamins (centre), symbolic formulae of some important B₁₂-vitamins (left: vitamin B₁₂ (CNCbl), methylcobalamin (MeCbl) and coenzyme B₁₂ (AdoCbl)), and of (potential) Cbl-based antivitamin B₁₂ (right: the aryl-Cbl EtPhCbl and the alkynyl-CbIs PhEtyCbl and F2PhEtyCbl).

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The ORCID identification number(s) for the author(s) of this article can be found under:
<https://doi.org/10.1002/chem.202003788>.

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and human cells of vital metabolic processes, which is a desirable consequence of the administration of metabolism based antibiotics and anti-cancer agents.^[14a,16,20a] Hence, broad biological^[3] and biomedical research interests^[8,12,14,16,20,21] are exploring means of inducing (functional) B₁₂-deficiency and are devoted to studies of its pathological effects.^[8]

From vitamin B₁₂ to antivitamin B₁₂—the cobalamin strategy

In line with the original concept,^[16] the complete Cbl-scaffold of vitamin B₁₂ was used as starting point for a (most efficient) preparation of antivitamin B₁₂. The aryl-Cbl 4-ethylphenyl-cobalamin (EtPhCbl), a novel type of organometallic B₁₂-derivative, was generated as a first such Cbl-based antivitamin B₁₂ (Figure 1).^[22] The critical design criteria for EtPhCbl were (i) its predicted (and verified) structural similarity with for example, CNCbl and (ii) its expected resistance against the metabolic removal of its aromatic capping group by the cellular 'B₁₂-tailoring' enzyme CblC,^[10c] thus inhibiting a later conversion into the organometallic B₁₂-cofactors.^[16,22] The aryl-Cbl EtPhCbl bound well to the human B₁₂-transporter proteins, intrinsic factor, transcobalamin and holocobalamin, and was resistant against its tailoring by the enzyme CblC, as postulated.^[22] Most critically, EtPhCbl also led to functional Cbl-deficiency in experiments with laboratory mice.^[23] However, while fulfilling the criteria of an antivitamin B₁₂, EtPhCbl is photosensitive and visible light degrades it into the B₁₂-vitamer hydroxocobalamin (HOCbl),^[22] although with a low quantum yield.^[24] Hence, since EtPhCbl has the (often undesirable) property of a 'photo-conditional antivitamin B₁₂',^[24] our interest has turned to light stable Cbl-based B₁₂-dummies. Suitable variants of the barely explored alkynylcobalamins^[25] with a strong organometallic Co—C_{sp} bond appeared attractive as presumed light stable potential antivitamin B₁₂.^[26] The previously unknown phenylethyne-cobalamin (PhEtyCbl) was prepared, which turned out to be slightly hydrolysis-sensitive, but was light stable and thermally robust and exhibited similar binding-affinity as CNCbl for the human proteins of B₁₂-transport.^[26a] Furthermore, the fluorinated 2,4-difluorophenyl-derivative F2PhEtyCbl was not only light-stable,^[27] but also rather inert against acid-induced hydrolytic cleavage of its Co—C bond, as expected.^[26b] F2PhEtyCbl bound and inhibited the holoenzyme CblC loaded with the co-substrate glutathione, allowing for a first crystal-structure analysis of fully assembled human CblC.^[26b] Investigations, not only from our laboratory, but also from the Gryko group,^[28] have meanwhile expanded the methodology for the preparation of organometallic alkynyl-cobalt-corrinoids. Indeed, the robust alkynyl-Cbls have become attractive potential cellular import vehicles ('Trojan Horses') with a range of biological and biomedical applications.^[28b,29]

Engineered B₁₂-biosynthesis opens direct non-cobalt synthesis-paths to antivitamin B₁₂

The possible conversion of aryl- and alkynyl-Cbls into the B₁₂ vitamers hydroxocobalamin (HOCbl) or aquocobalamin

(H₂Ocbl), by light or acid, respectively, was seen as a drawback as to their use as antivitamin B₁₂.^[22,26a] prompting us to look out for strategic alternatives. Indeed, our simple structure-based design criteria for the antivitamin B₁₂, that is, structural similarity with CNCbl and resistance against metabolic tailoring by the enzyme CblC,^[16] would not only be an inbuilt feature of some inert Cbls, but a select and suitably designed group of metbalamins (Metbals),^[14b,30] transition metal analogues of the Cbls, might also serve this purpose. In this respect, rhodium, the group IX homologue of cobalt, appeared to offer a most promising access to effective potential antivitamin B₁₂, by furnishing rhodibalamins (Rhbls), the Rh-based Cbl-analogues, presumed to be largely iso-structural to corresponding Cbls.^[16] (Figure 2).

In the 1970s Koppenhagen and co-workers reported the preparation of partially characterized Rhbls.^[30] In their exploratory tests with microorganisms and human cell cultures, adenosylrhodibalamin (AdoRhbl),^[31] the Rh-homologue of AdoCbl, was indicated to behave as a B₁₂ antimetabolite.^[32] We have recently developed an intricate chemical-biological total synthesis of AdoRhbl in a team with the Warren group in Canterbury (UK). AdoRhbl was first synthesized using the biotechnologically prepared natural metal-free B₁₂-ligand hydrogenobyrinic acid

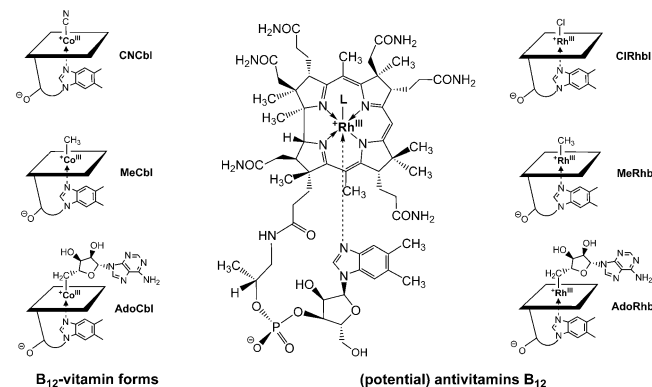


Figure 2. Left: Symbolic formulae of some important B₁₂-vitamers: vitamin B₁₂ (CNCbl), methylcobalamin (MeCbl) and coenzyme B₁₂ (AdoCbl); centre: General structural formula of the rhodibalamins; right: Symbolic formulae of three rhodibalamins as (potential) antivitamin B₁₂: chloro-Rhbl (ClRhbl), methyl-Rhbl (MeRhbl) and adenosyl-Rhbl (AdoRhbl).

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a,c-diamide (Hbad)^[6b,33] as starting material, followed by an adequate cocktail of further chemical and enzymatic transformations of Hbad.^[34] AdoRhbl was fully characterized in detail as a close structural, non-functional AdoCbl mimic that efficiently inhibited an AdoCbl-dependent enzyme diol dehydratase, as well as the growth of the bacterial pathogen *Salmonella enterica*.^[34] In an additional welcome contrast to the antivitamin B₁₂ EtPhCbl and to the coenzyme AdoCbl, the related AdoRhbl proved stable when irradiated with sunlight.^[31,34]

As RhbIs, the Rh-analogues of the CbIs, appeared to constitute a group of promising antivitamins B₁₂, a systematic and more direct synthesis methodology of RhbIs was developed. Its basis was a newly bioengineered preparative route to the now thoroughly characterized metal-free B₁₂-ligand hydrogenobyric acid (Hby).^[35] The metal-free Hby also constituted an excellent basis for the partial synthesis of hydrogenobalamin (Hbl), the complete metal-free ligand of the CbIs (Figure 3).^[36] The metal-free Hbl, in turn, is a rational general starting material for the synthesis of specific MetbIs, a long-standing dream and topical subject in the B₁₂-field,^[14b,30,37] and in bioinorganic chemistry.^[38] The biosynthetically available Hbl has meanwhile served in our hands for the one-step synthesis of chlororhodibalamin (ClRhbl),^[39] and from there, of methylrhodibalamin (MeRhbl),^[31,39] that is, of the Rh-analogues of chlorocobalamin (ClCbl)^[40] and of MeCbl,^[41] respectively (see Figure 2). As revealed by the crystal structures of the organometallic AdoRhbl^[34] and of the 'inorganic' ClRhbl^[39] Rh^{III}-corrins and Co^{III}-corrins are closely isostructural and the slightly larger Rh^{III}-ion appears to fit strikingly better into the corrin ligand of the CbIs than the 'natural' Co^{III}-ions.^[34,39]

The metal-free B₁₂-ligands Hby and Hbl are starting materials, not only for the syntheses of RhbIs, but, obviously, also of other MetbIs. So far, we have reported on the synthesis and on the detailed structural characterization of zincobalamin (Znbl), the Zn^{II}-analogue of vitamin B₁₂,^[42] and of the novel Ni^{II}-analogue, nibalamin (Nibl)^[36] (see Figure 3). According to detailed structural and computational studies, the redox-inactive penta-coordinate ('base-on') Znbl constitutes a luminescent structural mimic^[42] of the penta-coordinate 'base-on' Co^{II}-cobalamin (CbI^{II}).^[43] The tetra-coordinate diamagnetic 'base-off' Ni^{II}-corrin Nibl represents a largely redox-inactive structural mimic of the

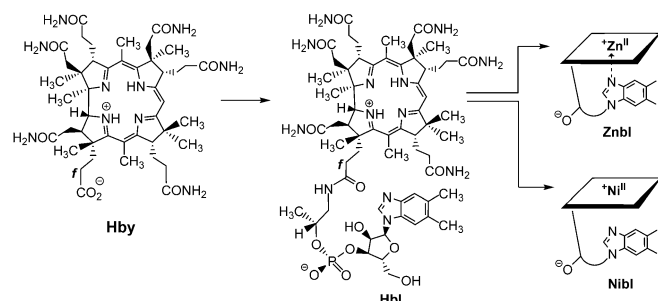


Figure 3. Biosynthetic hydrogenobyric acid (Hby) is starting material for the partial synthesis of hydrogenobalamin (Hbl), a direct synthesis platform for transition metal analogues of vitamin B₁₂ (MetbIs), such as zincobalamin (Znbl) and nibalamin (Nibl).

highly activated tetra-coordinate 'base-off' Co^{II}- and Co^I-CbIs.^[36,45] The reduced CbIs represent the often cryptic high-energy intermediates in many Cbl-dependent enzymatic reactions,^[3c,44,45] as well as in some essential B₁₂-biosynthetic organometallic transformation, for example, as catalysed by adenosyl transferases.^[46]

Together with the newly available hexa-coordinate RhbIs, penta-coordinate ('base-on') Znbl and tetra-coordinate ('base-off') Nibl constitute a complete suite of structural transition metal mimics of the CbIs in their biologically accessible redox states, that is, hexa-coordinate 'base-on' Co^{III}-CbIs, penta-coordinate 'base-on' Co^{II}- and tetra-coordinate 'base-off' Co^{II}- or Co^I-CbIs, providing us with a structurally 'complete' small set of biochemically inactive B₁₂-antimetabolites, inhibitors of B₁₂-enzymes and (some of them) potential antivitamins B₁₂.^[36] (Figure 4). The 'base-on' MetbIs RhbIs and Znbl are likely to function as genuine antivitamins B₁₂, the 'base-off' Ni^{II}-analogue Nibl as a B₁₂-antimetabolite that inhibits some B₁₂-dependent enzymes but may not be bound well by the mammalian B₁₂-transporter proteins. Hence, in order to clarify the capacity of MetbIs to serve as antivitamins B₁₂ according to our concept,^[16] their ability to mimic the CbIs with respect to high-affinity binding to the very structure-selective B₁₂-uptake and transport system of humans (and other mammals) needs to be analysed.

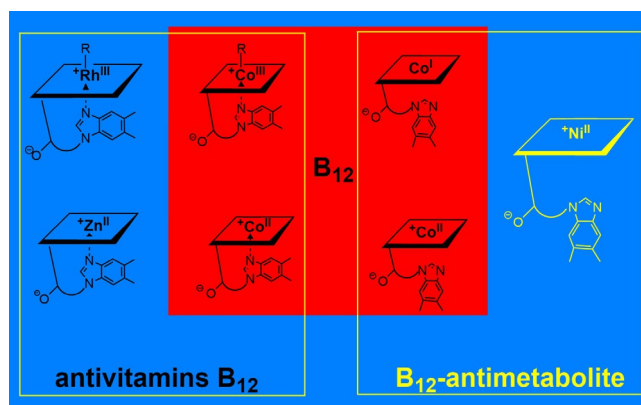


Figure 4. The MetbIs RhbIs, Znbl and Nibl (blue field) are (largely) inert structural mimics of Co^{III}-, Co^{II}- and Co^I-cobalamins (red background), and are efficient inhibitors of B₁₂-dependent enzymes useful for basic biochemical studies.

Application of antivitamins B₁₂ induces functional B₁₂-deficiency

As delineated above, antivitamins B₁₂ are structural Cbl-mimics designed to counteract the effect of CNCbl (and of its B₁₂ vitamers forms) in humans and animals by causing (functional) B₁₂-deficiency upon their cellular uptake,^[16] a deadly metabolic defect. Such an uptake of antivitamins B₁₂ leads, first of all, to the inactivity of the mammalian B₁₂-dependent enzymes methionine synthase (Meth)^[47] and methylmalonyl-CoA-mutase (MCM)^[44,48] due to functional B₁₂-deficiency, detectable in the accumulation of homocysteine and methylmalonic acid, two

biomarkers of B₁₂-deficiency.^[10d,49] Functional B₁₂-deficiency, induced by antivitamins B₁₂ in humans and in other mammals, results, on the one hand, from the inability of these B₁₂-dummies to assume the specific 'canonical' roles of the B₁₂-cofactors of MetH and MCM, which are based on the organometallic reactivity of MeCbl and of AdoCbl, respectively.^[3c,44,45] However, antivitamins B₁₂ will, on the other hand, extensively mimic the (merely) structure-based ('non-canonical') regulatory functions of the Cbls, giving fake signals for the availability of genuine B₁₂-cofactors by imitating effectively their binding capacity to natural bio-macromolecular targets, such as B₁₂-responsive regulatory proteins and RNA.^[16,34] As described below, a multitude of gene-regulatory roles of the natural B₁₂-cofactors have been discovered in microorganisms.^[50] However, so far, in humans only two such bio-macromolecular binding interactions have been detected.^[10a,51] Further 'non-canonical' roles of Cbls in humans and in other mammals are suggested, for example, by the observation of a cytokine and growth-factor imbalance in the central nervous system in laboratory rats due to Cbl-deficiency,^[8d,12] as well as of irregular melanocyte homeostasis induced by B₁₂-deficiency in human cell cultures.^[52] Antivitamins B₁₂ may be particularly helpful in imitating and identifying such puzzling roles, as well as in discovering new 'non-canonical' ones.

Antivitamins B₁₂ as molecular probes

A range of remarkable recent discoveries in the B₁₂-field has put Vitamin B₁₂ in the spotlight again.^[53] Indeed, B₁₂-derivatives play essential roles as organometallic biocatalysts,^[45] not only in humans, animals, bacteria and archaea but, surprisingly, in a range of algae, as well.^[54] Some forms of bacterial photo-regulation involve natural cobamides,^[55] as do critical steps of the biosynthesis of photosynthetic tetrapyrroles^[6b] and of other complex metabolites,^[56] including the anaerobic metabolism of hydrocarbons.^[56d] Mechanistic insights into the exceptional biochemistry of the involved B₁₂-dependent enzyme reactions or means of the B₁₂-based control of essential cellular processes are areas of continuous interest. Studies with antivitamins B₁₂ and other structurally characterized Metbls may potentially contribute to this subject,^[36] relying on two key structure-based factors: (i) By imitating the structures of the B₁₂-cofactors or of reactive intermediate B₁₂-species in the course of enzyme reactions, suitably structured (inactive) B₁₂-mimics have an excellent capacity to inhibit the corresponding enzymatic steps. Hence, for example, the Ni^I-analogue of the cryptic intermediate Co^I-form cob(I)alamin inhibits an AdoCbl-generating Ado-transferase in an in vitro study^[36] (see above for corresponding pertinent findings with the alkynyl-Cbl F2PhEtyCbl^[26b] and with AdoRhbl^[34]). (ii) By mimicking the structures of the B₁₂-type ligands in B₁₂-dependent regulatory functions in various organisms, antivitamins B₁₂ are, on the other hand, presumed to simulate the availability of the corresponding physiologically active B₁₂-derivatives, for example, via B₁₂-riboswitches^[57] and in B₁₂-responsive regulatory proteins.^[51,58] The observed strong growth-inhibition of *Salmonella enterica* by AdoRhbl was, hence, ascribed to its specific binding to the *BtuB* B₁₂-ribo-

switch as a structural AdoCbl-mimic, inhibiting the expression of a B₁₂-uptake protein in this microorganism.^[34] Similar further in vitro and in vivo experiments with AdoRhbl and some Cbl-based antivitamins B₁₂ have recently been carried out,^[59] signifying the ability of structurally competent antivitamins B₁₂ to simulate the presence of physiologically functional Cbls. Indeed, as long as the cellular and organismal import of antivitamins B₁₂ and of other Metbls by the natural pathways would be feasible, as expected, their capacity for generating functional B₁₂-deficiency should also be maintained in vivo, even in living animals.^[23]

Antivitamins B₁₂ as antibiotics and as cellular growth-inhibitors for human and animals

Antivitamins B₁₂^[16,60] and other B₁₂-antimetabolites^[14,20,61] may function as B₁₂-dummies and act as inhibitors of B₁₂-dependent enzymes, impairing the growth and reproduction of bacteria and of other microorganisms. This early explored effect of modified vitamin B₁₂-derivatives as B₁₂-antimetabolites (see for example^[3a,20]) could recently be extended to the critical case of hospital-resistant Gram-negative bacteria, where the broad antibiotic activity of sulfonamides was boosted decisively by the addition of the antivitamin B₁₂ EtPhCbl to the bactericidal sulfonamide cocktail.^[60] Addition of the antivitamin B₁₂ was proposed to result in an effective methylfolate trap,^[60] by blocking the formation of free tetrahydrofolate by methionine synthase. In addition to their proposed role in impairing the biosynthetic formation and in reducing the cellular availability of the (active) B₁₂-cofactors,^[16,22,23,60] antivitamins B₁₂ may also intercept the uptake of the essential B₁₂-derivatives by B₁₂-dependent microorganisms due to their B₁₂-mimetic regulatory activity as ligands of (for example) B₁₂-riboswitches.^[59] Indeed, the response of B₁₂-regulatory elements to binding of a B₁₂-type ligand is expected not to differentiate between the functional classification of the latter as 'vitamin' or as 'antivitamin'. In consequence, both the 'canonical' bio-catalytic and the 'non-canonical' B₁₂-regulatory roles played by the natural cobamides bestow antivitamins B₁₂ with a potentially very effective two-pronged bactericidal activity, as verified recently with AdoRhbl, the rhodium analogue of AdoCbl.^[34]

Since the deactivation of the B₁₂-dependent enzymatic processes in humans and other mammals leads to an impaired metabolism, disrupting physiological function^[8a,21a,62] and also causing fundamental neuropathological deficiencies,^[63] regular cellular growth is inhibited as consequence of a (functional) B₁₂-deficiency. Antivitamins B₁₂ may, hence, be useful as anti-cancer agents.^[14b,16] As already explored in early in vitro investigations, B₁₂ rhodium analogues were observed to inhibit as diversely active B₁₂-antimetabolites, the growth of human normo- and megaloblastic bone marrow cells.^[30,32] It will be of interest to learn more about the diagnostic and therapeutic applications of well-characterized, pure antivitamins B₁₂ as agents for anti-cancer diagnosis and treatment in humans and other mammals. Indeed, suitably fluorescence labelled, radiolabelled and other bio-conjugated B₁₂-derivatives have proved useful, over the recent years, as 'Trojan Horses' for the cellular

import of diagnostic loads and for targeted drug delivery,^[20a,64] helpful in inhibiting the growth and the detection of malignant cells,^[64a,65] and useful for a range of other biomedical applications.^[66]

Summary and Outlook

Our original interest in the subject of antivitamins B₁₂ was kindled by the expectation that these B₁₂-dummies would offer insights into functional B₁₂-deficiency in animals by an effective alternative methodology^[23] replacing total gastrectomy.^[67] This work has led to fruitful research collaborations, discovering new organometallic Cbl-chemistry, photochemistry and biochemistry.^[22,24,68] It has, likewise, opened up new avenues in the field of the fascinating transition metal analogues of the Cbls and of other natural corrinoids.^[34,36,39,42] The helical, ring-contracted natural corrin ligand has been characterized as an exceptional 'Procrustean Bed' for bound transition metal ions, important for tightly binding and specifically activating the bound cobalt-ions in their low-spin states.^[35] As discovered with synthetic Ni^{II}-corrins,^[69] the natural corrin ligand also imposes the diamagnetic low-spin state on bound Ni^{II}-ions,^[36] contrasting with the situation in related porphyrin-type Ni^{II}-corphinoids.^[7,70] Interestingly, the 5,6-dihydroxy-corrin variant of a 'B₁₂-type' Ni^{II}-complex, recently prepared and studied in the Zelder group, also features a low-spin 4-coordinate Ni^{II}-centre.^[37b]

Cbl-based antivitamins B₁₂ promise to represent exceptional antibiotics,^[60] an important area to be developed further in view of the acute problem of hospital-resistant bacteria. As some bacteria use preferentially cobamides (CbAs) other than Cbls,^[71] the eventual adaptation of the methodology for the synthesis of Cbl-based antivitamins B₁₂ to the generation of corresponding Cba-forms is expected to enhance their selective bacterial import as antibiotics, while simultaneously reducing the likelihood of the undesired uptake in human cells by their B₁₂-transporters.^[17a,72] In ongoing collaborative studies, antivitamins B₁₂ and some other metbalamins are used as specifically targeted B₁₂-antimetabolites, under investigation with respect to their capacity to serve as, for example, enzyme inhibitors, as ligands of regulatory proteins and of B₁₂-riboswitches, as antibiotics, and as potentially useful anti-cancer agents. Having now set up some inaugural milestones, a broad further impact of studies on antivitamins B₁₂ and (further) B₁₂-transition metal analogues in the bio-structural, biological and biomedical fields can be foreseen.

Acknowledgements

I would like to thank my former PhD and postdoctoral students on the antivitamin B₁₂ project, Christoph Kieninger, Florian Widner and Markus Ruetz, as well as many other co-workers and collaborators, for their essential contributions. I owe many thanks to Martin Warren and his group in Canterbury, UK, for teaming up in the synthesis quest for metal-free B₁₂-derivatives and their transition metal complexes. I am very grate-

ful to Christoph Kreutz, Thomas Müller, Klaus Wurst and Maren Podewitz for their spectroscopic, crystallographic and computational support. Our work in the B₁₂-area has been supported generously by the Austrian Science Fund (FWF), notably by the two recent and ongoing projects P-28892 and P-30359.

Conflict of interest

The author declares no conflict of interest.

Keywords: antibiotic · cobalamin · growth inhibitor · transition metal · vitamin

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Manuscript received: August 14, 2020

Accepted manuscript online: September 21, 2020

Version of record online: November 3, 2020