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Inorganic Cyanide as Protecting Group in the Stereospecific Reconstitution of Vitamin B_{12} from an Artificial Green Secocorrinoid

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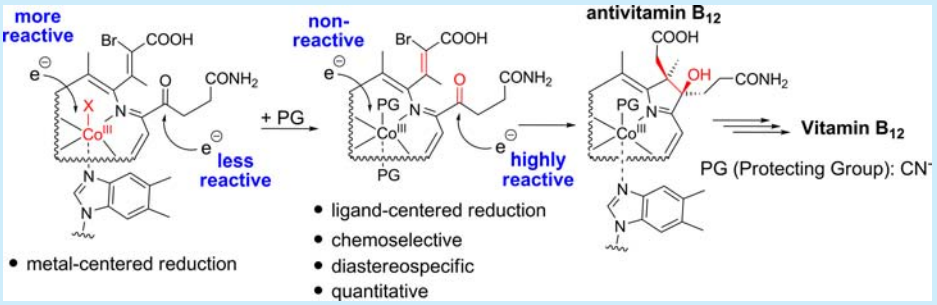
Supporting Information



ABSTRACT: The synthesis of vitamin B_{12} in four steps from an artificial green secocorrinoid is presented. The stereospecific reconstitution of the B-ring of the cobalamin involves a quantitative and rapid ligand-centered radical ring closure reaction leading first to a new B_{12} derivative with antivitamin activity that is subsequently converted to the natural product. Chemoselectivity in the one-electron reduction of the macrocycle was achieved by introducing inorganic cyanide as an axially coordinating protecting group of the otherwise reduction-sensitive Co^{III}-ion. The integrity of structure and function of the reconstituted natural product was unequivocally proven by single crystal structural analysis and a microbiological assay using *Lactobacillus leichmannii*.

Vitamin B_{12} ("B_{12}", **1**, Figure 1) and B_{12} cofactors represent the most complex nonpolymeric natural products[1,2] combining uniquely a central cobalt ion with a highly decorated corrin macrocycle.[3] Their nature, structure, chemistry, and enzymology have inspired scientists for decades, and B_{12} research contributed significantly to the advancements in natural sciences in the past century.[2,4,5]

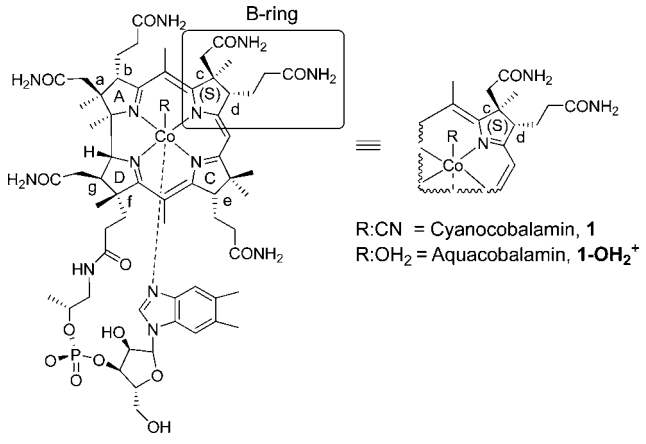


Figure 1. Structural formula of cob(III)alamins: cyanocobalamin (**1**, vitamin B_{12}, R = CN), aquacobalamin (**1-OH$_2^+$**, vitamin B_{12a}, R = H_2O) highlighting the B-ring area (in bracket) and its schematic representation.

The total synthesis of vitamin B_{12} by the groups of Eschenmoser and Woodward is considered a milestone in natural product synthesis for which the development of ingenious novel bond forming reactions and synthetic methodologies were required.[6−11] To name a few, Eschenmoser and his group developed during the first total synthesis of B_{12} the sulfide contraction reaction for joining two halves of the corrin macrocycle together in a metal-templated reaction.[12] Later, photochemical ring closure reactions were introduced for the challenging direct C−C coupling reaction between rings A and D of the corrin macrocycle.[13] Inspired by these important pioneering studies and recent progress in the field,[14] we herein report the unprecedented stereospecific reconstitution of vitamin B_{12} in four steps from an artificial 'green' secocorrinoid[15] with a C−C bond forming radical key reaction using inorganic cyanide as a metal-ion protecting group. In this reaction, the stabilization of the +III oxidation state of the cobalt center with two axially coordinating cyanide ligands was essential for achieving chemoselectivity in the ligand-centered reduction of the Co^{III}-containing octahedral complex.[16,17]

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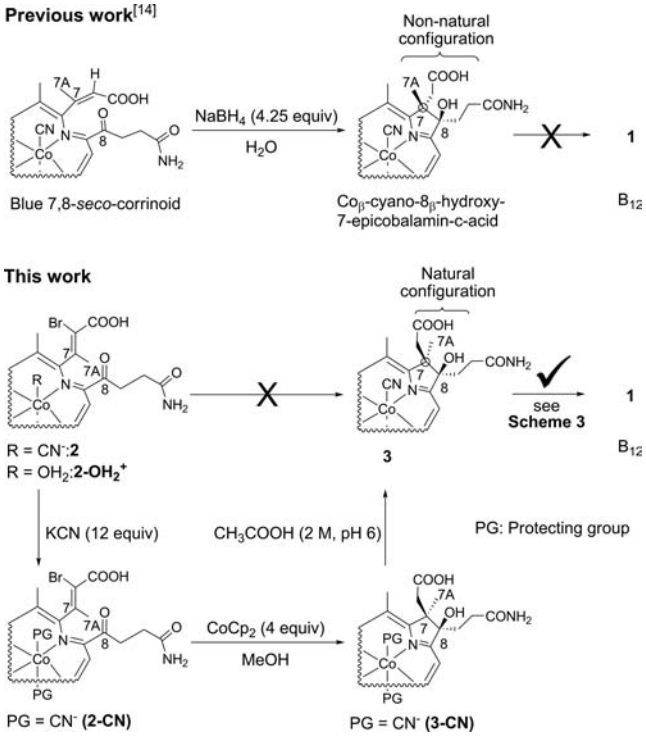
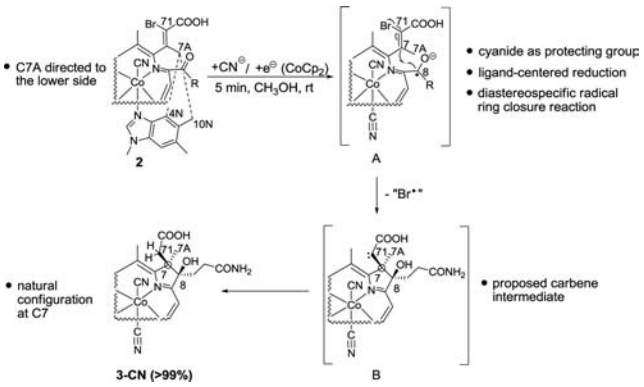
The radical C−C bond forming reaction between C8 and C7 of a secocorrinoid was first described by Kräutler and co-workers for converting a 'blue' secocorrinoid[18] into a B_{12} derivative with an intact corrin macrocycle (Scheme 1, top).[14]

Scheme 1. (Top) Schematic Representation of the Conversion of Blue 7,8-*seco*-Corrinoid to Co$_\beta$-cyano-8$_\beta$-hydroxy-7-epicobalamin-c-acid; (Bottom) Conversion of Green 7,8-*seco*-Corrinoid to Vitamin B_{12}



Unfortunately, the configuration of the c-acid side chain at C7 of the reaction product was inverted compared to B_{12}, making further transformations of the epimer to the natural product unfeasible. We speculated whether reconstitution of secocorrinoids to vitamin B_{12} would be principally possible with other derivatives and tested therefore the green 7,8-*seco*-corrinoid **2** (Scheme1, bottom).[15] We were optimistic since ^{1}H−^{1}H ROESY experiments showed correlations between H_{C7A} and both H_{C4N} and H_{C10N} of this derivative (Scheme 2), suggesting that C7A is directed to the lower (α) site of the secocorrinoid, thus representing the same orientation as observed in B_{12}.[15] For triggering the intended radical ring closure between C7 and C8 of **2**, consisting of a C−C coupling and a subsequent bromine elimination, we applied the one-electron donor cobaltocene ($CoCp_2$, E = −0.750 V vs Ag^+/AgCl in MeOH)[19] in the reaction. However, only metal-centered reduction of Co^{III} to Co^{II} was observed for **2**, leading finally to **2-OH$_2^+$** after reoxidation of the pentacoordinated cob(II)alamin with air (Scheme S1).[20] The observed metal-centered reactivity is in line with the behavior of B_{12} yielding OH_2Cbl (**1-OH$_2^+$**) under the same reaction conditions (Scheme S2). In order to achieve chemoselective reduction of the macrocyclic ligand at its C8 position, we transformed **2** with an excess of cyanide to the dicyanospecies **2-CN** (Schemes 1, 2). Advantageously, such an axially coordinating cyanide ligand is easily introduced and can also be selectively removed from the metal center under slightly acidic conditions.[21] Importantly, the dicyano-Co^{III} derivative **2-CN** contains a less reduction-sensitive Co^{III} center compared to **2**. Indeed, the strongly δ-donating cyanide ligand shifts the reduction potential of cob(III)alamins to more negative values (approximately 400 mV) and thus stabilizes the Co^{III}-ion against reduction.[17,22] This behavior should render ligand- instead of metal-centered reductions of **2-CN** more likely, but has not yet been so far exploited for synthetic purposes. In fact, control experiments revealed that violet dicyanoB_{12} (**1-CN**) did not show any color change in the presence of cobaltocene (Scheme S3). After having proven the inertness of Co^{III} in dicyanocob(III)alamins under reductive conditions, the reactivity of **2-CN** was tested in the presence of cobaltocene (Schemes 1, 2, S4). To our delight, the dark green solution turned violet within seconds. The immediate appearance of the typical color of a dicyanocob(III)alamin species suggested reconstution of the corrin macrocycle and excluded any coincidental reduction of the Co^{III} center to a brown Co^{II} derivative.[23] The UV−vis spectrum of **3-CN** closely resembled that of dicyanocob(III)alamin **1-CN**. MS analysis of the reaction mixture suggested conversion of **2-CN** to Co$_{\alpha,\beta}$-dicyano-8$_\beta$-hydroxy-cobalamin-c-acid (**3-CN**; m/z = 698.4, $[M - H]^{2-}$). ^{1}H NMR analysis of the crude base-on compound **3** after removal of the cyanide protecting group (Scheme 1, Figure 2) showed excellent agreement with the spectrum of B_{12} (**1**), exhibiting only significant differences in chemical shifts at the B-ring of the macrocycle as well as at C4N of the dimethylbenzimidazole nucleobase (Figures S4−6, Table S1). In particular, **3** lacks a signal at 3.46 ppm of the C8−H proton of **1** and the corresponding ^{13}C signal was shifted downfield by 30 ppm (88.2 ppm in **3** vs 58.2 ppm in **1**). Moreover, the ^{1}H NMR analysis univocally shows that **3** was formed as a single diastereoisomer in the reaction. The orientation of the c- and d-side chains of **3** was first tentatively assigned based on the reactivity of **3** under acidic conditions. Quantitative conversion to a new species with a pseudo-molecular ion at m/z = 1354.6 ($[M + H]^+$) was observed, that is 18 units less than the molecular mass of **3**. We assumed the formation of a c-lactone between the c-monocarboxylic acid and the 8-hydroxo group of the B-ring for explaining the structural change (Scheme 3). Importantly, such an intramolecular reaction is only possible for steric reasons if both functionalities are pointing toward the same side of the molecule.

Scheme 2. Schematic Representation of the Proposed Radical Triggered Reconstitution of the B-Ring of 3-CN Using Inorganic Cyanide As a Metal-Ion Protecting Group (Intermediate A)[a]



[a]Dotted lines in **2** indicate ^{1}H−^{1}H ROESY interactions.[15]

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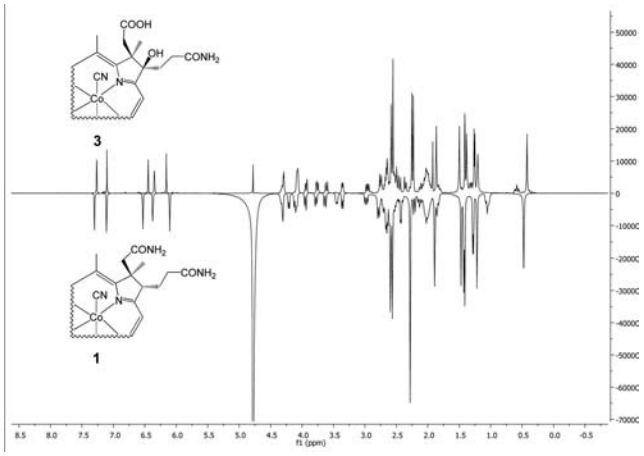
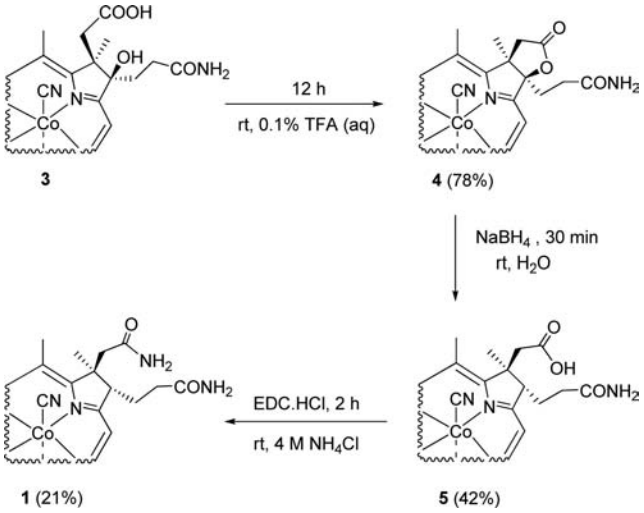


Figure 2. ^{1}H NMR spectra of Co$_{\beta}$-cyano-8$_{\beta}$-hydroxy-cobalamin-c-acid (**3**, top) and cyanocobalamin (B_{12}, **1**, bottom).

Scheme 3. Reconstitution of Vitamin B_{12} (1) from 3



To evaluate the orientation of the c-lactone moiety in the reconstituted compound, we compared its analytical data with those of CNCbl-c-lactone **4** (Figures S7–S9). The latter contains a c-lactone moiety attached to the upper side of the B-ring of the corrin macrocycle and was synthesized independently from B_{12}.[24] In brief, all analytical data for the reconstituted compound and **4** were identical, indicating strikingly that the c-lactone functionalities are directed to the same side of the molecule. In turn, this means for the reconstituted compounds **3** and **4** that the c-acetic acid functionalities at C7 are orientated upward and the propionamide moieties at C8 are orientated to the lower side of the molecule. To our delight, this behavior reveals that the side chains located at the periphery of the B-ring of the reconstituted compounds exhibit the same orientation as in B_{12}. The diastereospecific nature of the ring closure reaction is probably best rationalized by a highly preorganized arrangement of C7, C7A, and C8 in the secocorrinoid **2** for the C–C bond forming reaction (Scheme 2).[15] In particular, the reaction is initiated by the formation of a highly reactive C8 centered radical (intermediate A; Scheme 2) that combines then rapidly and diastereospecifically with the short-distanced C7 under the mild reaction conditions. The existence of intermediate A (Scheme 2) was supported by radical scavenging experiments. Indeed, only starting material was observed by UPLC-MS when the reduction of **2-CN** with cobaltocene was attemped in the presence of the radical scavenger TEMPO (see Supporting Information). However, in the absence of this inhibitor, C–C coupling occurs and it is suggested that this reaction is accompanied by the elimination of a bromine radical from A forming the carbene intermediate B. The latter incorporates finally two hydrogens from MeOH leading to reconstituted **3**.

Having CNCbl-c-lactone (**4**) in hand allowed us to devise a straightforward route to vitamin B_{12} in two steps.[25] Reductive ring opening of **4** with $NaBH_4$ afforded CNCbl-c-acid (**5**) that was finally converted with *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and ammonium chloride to CNCbl (**1**) (Scheme 3).

The analytical data of the reconstituted and microbially produced vitamin B_{12} (**1**) are identical. Single crystals of the reconstituted compounds **5** and **1** (both synthesized starting from **2**) were grown by vapor diffusion of acetone into an aqueous solution of either **1** or **5**, and X-ray analysis confirmed the natural configuration of all side chains of the reconstituted products. As the crystals were weakly diffracting on a molybdenum source and X-ray fluorescence using copper radiation was observed, we finally measured both crystals with the help of a gallium source (see Supporting Information for more details). The top portion of Figure 3 shows the overlay of

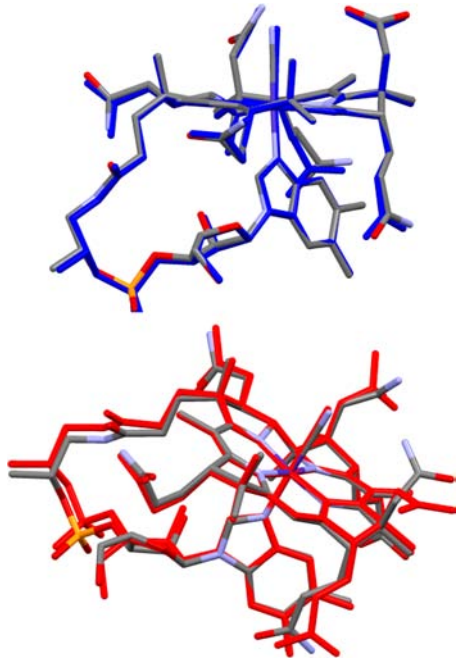


Figure 3. (Top) Overlay of the X-ray structures of **1** (blue) and **5** (normal element colors). (Bottom) Overlay of **1** (normal element colors) and a reference structure of vitamin B_{12} (red).[26]

1 (blue) and **5** (normal element colors), and the bottom portion of Figure 3 is the overlay of **1** (normal element colors) and a reference structure of vitamin B_{12} (red).[26]

With confidence established regarding the structural integrity of reconstituted **1**, its function was evaluated in a microbiological assay using *Lactobacillus leichmannii*.[27] This bacterium requires cofactor B_{12} for ribonucleotide reductase as the only B_{12} dependent enzyme.[27,28] Bacterial cell growth was strongly increased in the presence of nanomolar concentrations

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of reconstituted **1**, and its biological activity was identical to that of the microbially produced natural product (Figure S19).

In contrast, the novel reconstituted Cbl derivative **3** with an acetate functionality at C7 and an alcohol group at C8 behaves differently from B_{12} and shows antivitamin activity in the B_{12}-dependent growth of *L. Leichmannii*. In particular, 50% inhibition was observed with antivitamin **3** (1 μM) in the presence of B_{12} (0.1 nM) (Figure 4).

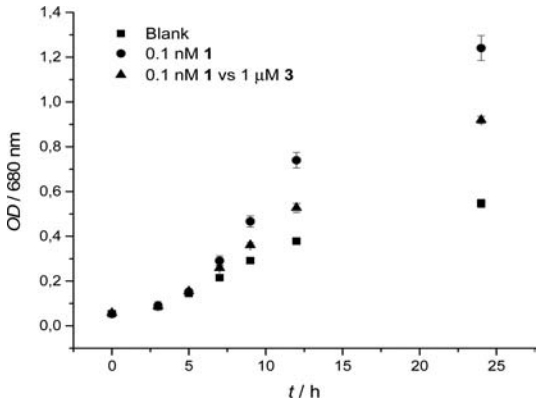

Figure 4. Growth of *Lactobacillus leichmannii* at 37 °C (n = 3) in the presence of B_{12} (0.1 nM) and in the presence and absence of antivitamin B_{12} **3** (1 μM).

These results are inspiring and suggest that this synthetic route can be probably still further exploited for synthesis of even more potent antivitamin B_{12} derivatives, a research field that currently attracts great interest in the community.[3,27,29−32]

In summary, the unprecedented reconstitution of B_{12} from an artificial green secocorrinoid is reported. The key step of the route is a stereospecific radical C−C bond formation for reconstructing the B-ring of the macrocycle. This rapid and quantitative ring closure reaction was initiated by a one-electron ligand-centered reduction of the secocorrinoid and leads first to a novel antivitamin B_{12} derivative that is subsequently converted to the final natural product. Chemoselectivity in this transformation was achieved by reversibly protecting the Co^{III} center of the precursor with inorganic cyanide against undesired metal-centered reduction.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02611.

All experimental procedures and complete analytical and biological data of new products (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Hogenkamp, H. P. C. In *Chemistry and Biochemistry of B12*; Banarjee, R., Ed.; John Wiley and Sons: New York, 1999; pp 3−8.
(2) Kräutler, B. In *Vitamin B 12 and B 12-Proteins*; Kräutler, B., Golding, B. T., Arigoni, D., Eds.; John Wiley and Sons: New York, 2008; pp 3−43.
(3) Zelder, F. *Chem. Commun.* **2015**, *51*, 14004−14017.
(4) Brown, K. L. *Chem. Rev.* **2005**, *105*, 2075−2149.
(5) ó Proinsias, K.; Giedyk, M.; Gryko, D. *Chem. Soc. Rev.* **2013**, *42*, 6605−6619.
(6) Woodward, R. B. *Pure Appl. Chem.* **1973**, *33*, 145−178.
(7) Eschenmoser, A.; Wintner, C. E. *Science* **1977**, *196*, 1410−1420.
(8) Nicolaou, K.; Sorensen, E. *Classics in Total Synthesis* **1996**, 1991.
(9) Riether, D.; Mulzer, J. *Eur. J. Org. Chem.* **2003**, *2003*, 30−45.
(10) Craig, G. W. *J. Porphyrins Phthalocyanines* **2016**, *20*, 1−20.
(11) Eschenmoser, A. *Helv. Chim. Acta* **2015**, *98*, 1483−1600.
(12) Eschenmoser, A. *Q. Rev., Chem. Soc.* **1970**, *24*, 366−415.
(13) Yamada, Y.; Wehrli, P.; Miljkovic, D.; Wild, H.-J.; Bühler, N.; Götschi, E.; Golding, B.; Löliger, P.; Gleason, J.; Pace, B.; Ellis, L.; Hunkeler, W.; Schneider, P.; Fuhrer, W.; Nordmann, R.; Srinivasachar, K.; Keese, R.; Müller, K.; Neier, R.; Eschenmoser, A. *Helv. Chim. Acta* **2015**, *98*, 1921−2054.
(14) Ruetz, M.; Fedosov, S. N.; Kräutler, B. *Angew. Chem., Int. Ed.* **2012**, *51*, 6780−6784.
(15) Oetterli, R. M.; Prieto, L.; Spingler, B.; Zelder, F. *Org. Lett.* **2013**, *15*, 4630−4633.
(16) Lexa, D.; Saveant, J. M. *Acc. Chem. Res.* **1983**, *16*, 235−243.
(17) Sonnay, M.; Fox, T.; Blacque, O.; Zelder, F. *Chem. Sci.* **2016**, *7*, 3836−3842.
(18) Fedosov, S. N.; Ruetz, M.; Gruber, K.; Fedosova, N. U.; Kräutler, B. *Biochemistry* **2011**, *50*, 8090−8101.
(19) Ruiz-Sánchez, P.; König, C.; Ferrari, S.; Alberto, R. *JBIC, J. Biol. Inorg. Chem.* **2011**, *16*, 33−44.
(20) Dereven'kov, I. A.; Salnikov, D. S.; Silaghi-Dumitrescu, R.; Makarov, S. V.; Koifman, O. I. *Coord. Chem. Rev.* **2016**, *309*, 68−83.
(21) Zhou, K.; Zelder, F. *Eur. J. Inorg. Chem.* **2011**, *2011*, 53−57.
(22) Lexa, D.; Sayeant, J. M.; Zickler, J. *J. Am. Chem. Soc.* **1980**, *102*, 2654−2663.
(23) Schindler, O. *Helv. Chim. Acta* **1951**, *34*, 1356−1361.
(24) Bonnett, R. *Chem. Rev.* **1963**, *63*, 573−605.
(25) Brown, K.; Zou, X.; Wu, G.-Z.; Zubkowski, J.; Valente, E. *Polyhedron* **1995**, *14*, 1621−1639.
(26) Mebs, S.; Henn, J.; Dittrich, B.; Paulmann, C.; Luger, P. *J. Phys. Chem. A* **2009**, *113*, 8366−8378.
(27) Zhou, K.; Oetterli, R. M.; Brandl, H.; Lyatuu, F. E.; Buckel, W.; Zelder, F. *ChemBioChem* **2012**, *13*, 2052−2055.
(28) Larsson, K.-M.; Logan, D. T.; Nordlund, P. *ACS Chem. Biol.* **2010**, *5*, 933−942.
(29) Zelder, F.; Sonnay, M.; Prieto, L. *ChemBioChem* **2015**, *16*, 1264−1278.
(30) Kräutler, B. *Chem. - Eur. J.* **2015**, *21*, 11280−11287.
(31) *Vitamin B12 und verwandte Corrinoide*; Friedrich, W., Ed.: Georg Thieme Verlag: Stuttgart, 1975.
(32) Chrominski, M.; Lewalska, A.; Gryko, D. *Chem. Commun.* **2013**, *49*, 11406−11408.

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