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COMMUNICATION

Coordination chemistry and biological activity of 5′-OH modified quinoline–B12 derivatives†

Karel Zelenka,[a] Helmut Brandl,[b] Bernhard Spingler[a] and Felix Zelder*[a]

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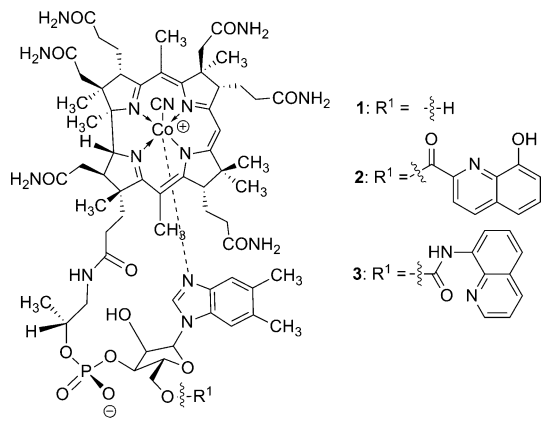
The consequences of structural modifications at the 5′-OH ribofuranotide moiety of quinoline modified B12 derivatives are discussed in regard of the coordination chemistry, the electrochemical properties and the biological behaviour of the compound.

Vitamin B12 (B12) is essential for the metabolism of mammals and most other forms of life, but only produced by few microorganisms.[1,2] Nature developed a complex, but efficient transport of B12 into the cells.[3–5] Thereabout, B12 runs through multiple constitutional and electronical changes until it is converted to the biologically active cofactors adenosylcobalamin (Ado-Cbl) and methylcobalamin (Me-Cbl), respectively.[4] These cofactors are required in humans for the enzymes methionine synthase and methylmalonylCoA mutase, respectively.[6] In both proteins, the cofactors are bound in the "base off-histidine on" form. In contrast, some bacteria such as *Lactobacillus delbrueckii* depend on AdoCbl dependent ribonucleotide reductase (RNR) with the cofactor bound in the "base-on" constitution.[7,8] Pioneering studies with modified B12 derivatives demonstrated that structural changes of B12 may have biological consequences.[9,10]

In the last years, cell delivery of therapeutic and diagnostic agents using B12-bioconjugates has regained considerable attraction.[5,11,12] Wuerges, Randaccio and co-workers suggested the 5′-OH position of B12 was best-suited for further modifications based on crystallographic studies of a transcobalamin (TC)-("base-on") cobalamin complex which was further supported by biological investigations.[13,14]

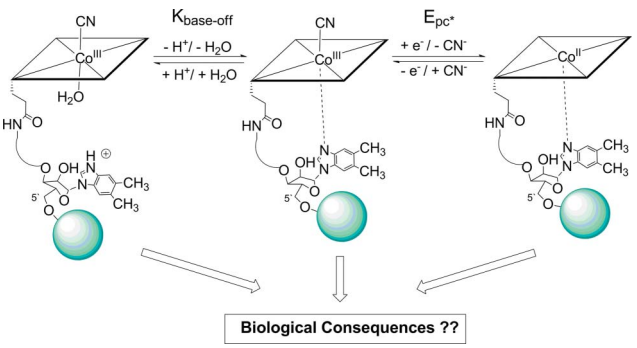
Rational approaches towards the design of B12-based cell delivery vehicles are also intensively followed by others.[5,11,15–23]

Little attention is paid in these biological studies on the evaluation of the coordination chemistry of the modified B12-derivative as well as the electronic properties at the cobalt center, although alterations of these intrinsic properties might also effect the biological behaviour. Kräutler *et al.* showed that modifications



Scheme 1 B12 (**1**) and quinoline-B12 derivatives (**2**, **3**) used in this study.

at the α-ribonucleotide moiety of B12 can destabilize the "base-on" form.[24,25] As part of our program to develop B12-derivatives for medical applications, we decided to study the consequences of structural modifications at the 5′-OH moiety of B12 in respect to the "base-on/base-off"equilibrium (i), the electronic properties at the cobalt center (ii) and the biological activity of the derivative (iii) (Scheme 2). Modifications of B12 with quinolines have been chosen. Quinoline esters have been earlier successfully applied as metal cleavable groups in amino acids or peptide nucleic acids.[26,27] This behaviour was of interest for the biological studies.



Scheme 2 Evaluation of structural modification at the 5′-OH moiety of B12 (blue ball) on the "base-on/base-off" (*left*) and Co(III)/Co(II) (*right*) equilibria as well as the biological activity of the compound in bacterial growth tests with *L. delbrueckii*.

[a]*Institute of Inorganic Chemistry, University of Zürich, Winterthurerstr. 190, 8057, Zürich, Switzerland. E-mail: zelder@aci.uzh.ch,http://www.zelder.ch.vu; Fax: +41 44 63 56802; Tel: +41 44 63 54624*
[b]*Institute of Evolutionary Biology and Environmental Studies, University of Zürich, Winterthurerstrasse 190, CH-8057, Zürich, Switzerland*

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Two related quinoline-B12 derivatives (**2**, **3**) have been synthesized attaching 8-hydroxy quinoline 2-carboxylate (HQCA) as an ester or 8-amino quinoline (8-AQ) as a carbamate to B12 according to published procedures (Scheme S1, S2†).[20,28,29,32] Co_β-cyano-O5R-[(8-hydroxyquinoline)-2-carboxyl] cobalamin (**2**) and Co_β-cyano-O5R-(8-aminoquinoline)-8-carbamoyl) cobalamin (**3**) were isolated by preparative reverse phase C18 chromatography and analysed with UV-vis, ^{1}H-NMR and MS spectrometry.[29] In the high-resolution mass spectra of **2** and **3**, $[M]^{2+}$ ions at m/z 785.78821 (m/z_{calc} 785.78894) and m/z 785.29657 (m/z_{calc} 785.29663) were observed, consistent with molecular formulas of $C_{73}H_{93}CoN_{15}Na_2O_{16}P$ and $C_{73}H_{94}CoN_{16}Na_2O_{15}P$, respectively. The UV-vis spectra of **1**–**3** were comparable and showed a characteristic red shift compared to the absorption of aqua-cyano cobinamide (Fig. S3†).[29] This indicates that **2** and **3** are available in their "base-on" forms.[29] The chemical shifts of the protons at C4R and C5R of the ribofuranosyl moiety in the ^{1}H-NMR spectra are distinct from those of B12,[30] but comparable to those of Co_β-cyano-O5R-acetyl cobalamin.[28,29,31] The structural identity of **2** was further supported by crystal structure analysis.[32] Compound **2** crystallized in the orthorhombic space group $P2_12_12_1$ and the quinoline unit was oriented underneath and parallel to the rim at the C ring of the macrocycle of **2** (Fig. 1). This is a high quality X-ray structure of a B12 derivative (the $R1$ value of the strong reflections up to 0.8 Å is 4.92%). The Co–C bond length is 1.882(2) Å, the Co–N(benzimidazole) bond length is 2.052(3) Å, the corrin fold angle is 13.4(1)° and the base tilt angle is 11.8(2)°. The overall geometry of **2** resembles that of other cobalamines.[33,34]

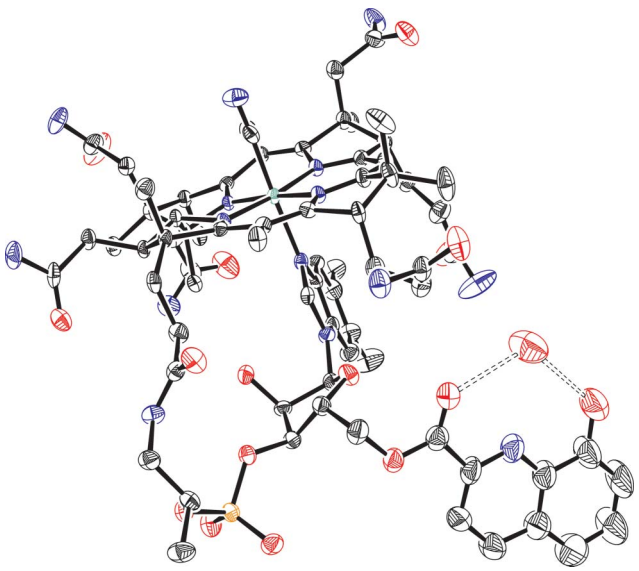


Fig. 1 X-Ray crystal structure analysis of **2**. ORTEP representation with C, N, O, P and Co colored in gray, blue, red, orange and green respectively. Hydrogen atoms and most solvent molecules have been omitted for viewing clarity. Hydrogen bonds between the HQCA moiety and a water molecule are indicated.

The "base-on/base-off" and the Co(III)/Co(II)-redox equilibria of **3** were investigated in more detail. Investigations with **2** were disclaimed because of the instability of the ester functionality under acidic conditions. Spectrophotometric titration with **3** showed that intramolecular coordination of the dmbz base to the Co(III) center was not significantly affected by the modification at the 5′-OH moiety as indicated by the same $pK_{base\text{-}off}$ value of 0.1 (Fig. S4†).[2,29]

Table 1 'Base-on/base-off' (left), Co(III)/Co(II) (right) equilibria of **1** and **3** (Scheme 2)

Entry	Compound	$pK_{base\text{-}off}$	E_{pc*} [V][a]
1	**1**	0.1[b]	−1.126
2	**3**	0.1	−1.121

[a] $E_{pc}* = E_{pc} - -E_0'$ (see Supporting Information†); [b] Ref. 2

The electrochemical reduction of the octahedral cobalt(III) to the square-pyramidal cobalt(II) center of **1** and **3** was investigated with cyclic voltammetry (CV) in water ([Tris-HCl] = 0.2 M, pH 8.0) and indicated a comparable cathodic reduction potential (E_{pc}*) (Table 1; Fig. S5†).[29]

This behaviour is in agreement with recent studies of B12 derivatives having modifications at the f-side chain showing a linear correlation between the $pK_{base\text{-}off}$ value and the Co(III)/Co(II) reduction potential.[35]

B12-dependant cell growth studies with *L. delbrueckii* showed that bacterial cell growth was indeed strongly reduced with **2**, **3** by 76 to 81% compared to **1** (Fig. 2).

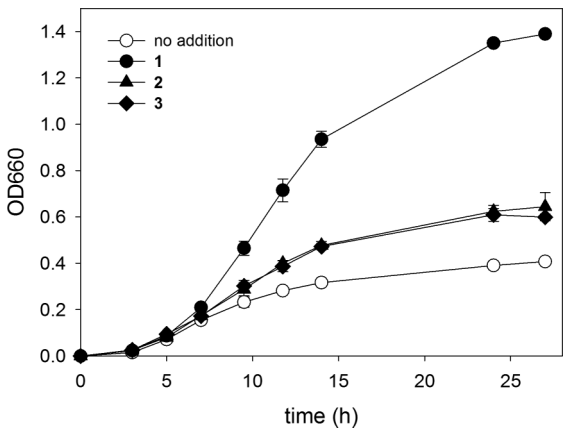
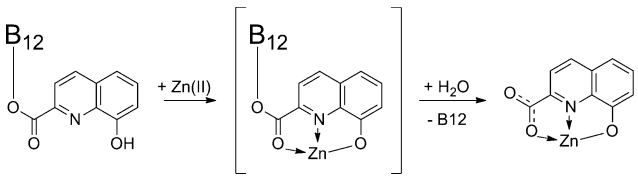


Fig. 2 Growth of *L. delbrueckii* dependent on **1**, **2**, or **3** (c = 250 pg ml^{-1}) at 37 °C (n = 3).

Of note, the carbamate linker of **3** was not hydrolysed in the assay medium as shown with HPLC (Fig. S1†).[29] This excludes the possibility that the observed activity of **3** derived from (hydrolysed) B12.

Background hydrolysis of **2** to B12 and HQCA was also negligible,[29] but the addition of Zn(II) ions (25 eq) triggered ester cleavage of **2** (130 mM) with a half-life time of 370 min following pseudo-first order kinetics (Scheme 3; Fig. S1†).[29,36] A



Scheme 3 Proposed mechanism for the zinc(II)-mediated quinoline-ester hydrolysis.

partly hydrolysed sample of **2** containing approx. one third of B12 (**1**) showed indeed an 30% increase in cell growth compared to unhydrolyzed **2** as shown in Fig. S6.† This behaviour is in good agreement with the biological activity of the individual compounds **1** and **2**.

Interestingly, when Zn^{2+} ions (1, 5, 10, 20 or 75 mg l^{-1}) were added as $ZnSO_4 \cdot 7H_2O$ at the beginning or 24 h after the start of bacterial growth with **2** (250 pg ml^{-1}), the biological activity of the modified B12-derivative was not altered (Fig. S7†). This gives evidence that **2** is not affected by zinc(II) mediated hydrolysis, most likely due to protection by bacterial cellular uptake.

Competition experiments with B12 (250 pg ml^{-1}) and increasing amounts of **3** (0.1–10 000 eq) showed that at least an 100-fold excess of **3** is necessary to obtain partial inhibition (Fig. S8†). Co-crystal structures of B12 with the Cbl dependant RNRs of *Lactobacillus delbrueckii* and *Thermotoga maritima* have been reported.[8,37] Superposition of the cofactor which is bound in the active site of the latter structure with **2** suggests no steric strain (Fig. S9†).[29] We assume therefore that the reduced biological activity of **2** is not caused by difficulties in Ado-Cbl dependant RNR. Reasons may include a disturbed metabolism to the organometallic cofactor (i) as well as lowered binding to other participating proteins (ii).

In summary, modification at the 5′-OH position of B12 with quinoline did not alter intramolecular coordination and the electronic properties at the metal centre. These properties are desirable for the development of B12-bioconjugates as cell-delivery agents. Bacterial uptake in *L. delbrueckii* was demonstrated with a B12-quinoline derivative containing a metal-cleavable linker. The strongly reduced biological activity in cell growth can therefore be assigned to the structural modification at the 5′-OH moiety. This information is important for the developments of modified B12-derivatives for biological and medical applications.

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Notes and references

1 B. Kräutler, D. Arigoni and B. Golding, *Vitamin B12 and B12-Proteins*, Wiley-VCH, Weinheim, 1998.
2 K. L. Brown, *Chem. Rev.*, 2005, **105**, 2075–2149.
3 E. Nexo, in *Vitamin B12 and B12-Proteins*, ed. B. Kräutler, D. Arigoni and B. Golding, Wiley-VCH, Weinheim, 1998, p. 461.
4 R. Banerjee, C. Gherasim and D. Padovani, *Curr. Opin. Chem. Biol.*, 2009, **13**, 484–491.
5 A. K. Petrus, T. J. Fairchild and R. P. Doyle, *Angew. Chem., Int. Ed.*, 2009, **48**, 1022–1028.
6 R. Banerjee and S. Chowdhury, in *Chemistry and Biochemistry of B12*, ed. R. Banerjee, Wiley-Interscience, New York, 1st edn, 1999, pp. 707–730.
7 J. Stubbe, D. G. Nocera, C. S. Yee and M. C. Y. Chang, *Chem. Rev.*, 2003, **103**, 2167–2201.
8 K. M. Larsson, D. T. Logan and P. Nordlund, *ACS Chem. Biol.*, 2010, **5**, 933–942.
9 J. H. Matthews, *Blood*, 1997, **89**, 4600–4607.
10 G. R. McLean, P. M. Pathare, D. S. Wilbur, A. C. Morgan, C. S. Woodhouse, J. W. Schrader and H. J. Ziltener, *Cancer Res.*, 1997, **57**, 4015–4022.
11 S. M. Clardy, D. G. Allis, T. J. Fairchild and R. P. Doyle, *Expert Opin. Drug Delivery*, 2011, **8**, 127–140.
12 S. Kunze, F. Zobi, P. Kurz, B. Spingler and R. Alberto, *Angew. Chem., Int. Ed.*, 2004, **43**, 5025–5029.
13 J. Wuerges, G. Garau, S. Geremia, S. N. Fedosov, T. E. Petersen and L. Randaccio, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 4386–4391.
14 P. Siega, J. Wuerges, F. Arena, E. Gianolio, S. N. Fedosov, R. Dreos, S. Geremia, S. Aime and L. Randaccio, *Chem.–Eur. J.*, 2009, **15**, 7980–7989.
15 L. Hannibal, C. A. Smith, D. W. Jacobsen and N. E. Brasch, *Angew. Chem., Int. Ed.*, 2007, **46**, 5140–5143.
16 A. K. Petrus, D. G. Allis, R. P. Smith, T. J. Fairchild and R. P. Doyle, *ChemMedChem*, 2009, **4**, 421–426.
17 R. P. Doyle, N. Viola-Villegas, A. E. Rabideau, M. Bartholoma and J. Zubieta, *J. Med. Chem.*, 2009, **52**, 5253–5261.
18 D. A. Collins and H. P. C. Hogenkamp, *J. Nucl. Med.*, 1997, **38**, 717–723.
19 D. R. van Staveren, S. Mundwiler, U. Hoffmanns, J. K. Pak, B. Spingler, N. Metzler-Nolte and R. Alberto, *Org. Biomol. Chem.*, 2004, **2**, 2593–2603.
20 M. Lee and C. B. Grissom, *Org. Lett.*, 2009, **11**, 2499–2502.
21 R. Mukherjee, E. G. Donnay, M. A. Radomski, C. Miller, D. A. Redfern, A. Gericke, D. S. Damron and N. E. Brasch, *Chem. Commun.*, 2008, 3783–3785.
22 P. Ruiz-Sanchez, S. Mundwiler, B. Spingler, N. R. Buan, J. C. Escalante-Semerena and R. Alberto, *J. Biol. Inorg. Chem.*, 2007, **13**, 335–347.
23 R. A. Horton, J. D. Bagnato and C. B. Grissom, *J. Org. Chem.*, 2003, **68**, 7108–7111.
24 S. Gschosser, K. Gruber, C. Kratky, C. Eichmuller and B. Kräutler, *Angew. Chem., Int. Ed.*, 2005, **44**, 2284–2288.
25 S. Gschosser and B. Kräutler, *Chem.–Eur. J.*, 2008, **14**, 3605–3619.
26 E. J. Corey and R. L. Dawson, *J. Am. Chem. Soc.*, 1962, **84**, 4899–4904.
27 F. H. Zelder, J. Brunner and R. Krämer, *Chem. Commun.*, 2004, 902–903.
28 R. B. Hannak, S. Gschosser, K. Wurst and B. Kräutler, *Monatsh. Chem.*, 2007, **138**, 899–907.
29 Supporting information.
30 P. Butler, M. O. Ebert, A. Lyskowski, K. Gruber, C. Kratky and B. Kräutler, *Angew. Chem., Int. Ed.*, 2006, **45**, 989–993.
31 X. Wang, L. Wei and L. P. Kotra, *Bioorg. Med. Chem.*, 2007, **15**, 1780–1787.
32 Crystal data for **2**: $C_{79}H_{125}CoN_{15}O_{28.5}P$, M = 1830.84 g mol^{-1}, orthorhombic, a = 15.75386(17) Å, b = 22.5570(3) Å, c = 25.5975(3) Å, V = 9096.32(19) $Å^3$, T = 183(2)K, space group $P2_12_12_1$, Z = 4, 39047 reflections measured, 18584 independent reflections (R_{int} = 0.0335). R_1 = 0.0492 for $I > 2\sigma(I)$ or 0.0707 for all data, $wR(F^2)$ = 0.1085 for $I > 2\sigma(I)$ or 0.1135 for all data. Flack parameter = –0.001(11).
33 B. Kräutler, R. Konrat, E. Stupperich, G. Faerber, K. Gruber and C. Kratky, *Inorg. Chem.*, 1994, **33**, 4128–4139.
34 N. Marino, A. E. Rabideau and R. P. Doyle, *Inorg. Chem.*, 2011, **50**, 220–230.
35 K. Zhou and F. Zelder, *Angew. Chem., Int. Ed.*, 2010, **49**, 5178–5180.
36 R. W. Hay and C. R. Clark, *J. Chem. Soc., Dalton Trans.*, 1977, 1866–1874.
37 M. D. Sintchak, G. Arjara, B. A. Kellogg, J. Stubbe and C. L. Drennan, *Nat. Struct. Biol.*, 2002, **9**, 293–300.