ORGANIC LETTERS

2013
Vol. 15, No. 18
4630–4633

Synthesis of a B Ring Opened 7,8-*seco*-Vitamin B_{12} Derivative with Grob Fragmentation

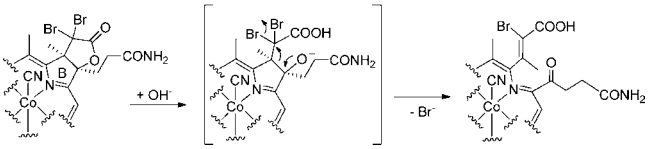
René M. Oetterli, Lucas Prieto, Bernhard Spingler, and Felix Zelder*

Institute of Inorganic Chemistry, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

zelder@aci.uzh.ch

Received June 4, 2013

ABSTRACT



A synthetic route toward B ring opened *7,8-seco*-cyanocobalamins is described. Hydrolysis of a novel *c*-lactone vitamin B_{12} (B_{12}) derivative generates a cobalamin (Cbl) with a β-bromo alcoholate subunit that reacts *in situ* via Grob fragmentation to the secocorrin.

In 2011, Fedosov, Kräutler and co-workers reported a remarkable new 'blue corrinoid' that was produced by hydrolysis of vitamin B_{12} (B_{12}) with aqueous bicarbonate (Scheme 1A).[1] This 7,8-*seco*-cyanocobalamin (7,8-*s*CNCbl) has large parts of homology with its natural precursor, but exhibits an unprecedented structural change at the B-ring of the pseudotetrapyrrolic cobalt(III) corrin macrocycle. Thorough NMR and crystallographic studies revealed cleavage of the peripheral C−C bond at positions 7 and 8 and the formation of a double bond (C7, C71) as well as a keto functionality (C8). The severe structural change in the chromophoric region compared to B_{12} explains the red shift in the absorption spectrum as well as the altered binding to B_{12} transporting proteins.[1] Therapeutic applications as a drug carrier molecule have been proposed for B ring modified B_{12} derivatives,[1,2] but new opportunities as biomimetic catalysts or chemosensors can also be envisaged.[3] However, access toward this new class of compounds is mainly limited by its low yield (3−6%) and long reaction time (3 weeks). The underlying mechanism of this "puzzling partial degradation"[4] is also not yet understood.

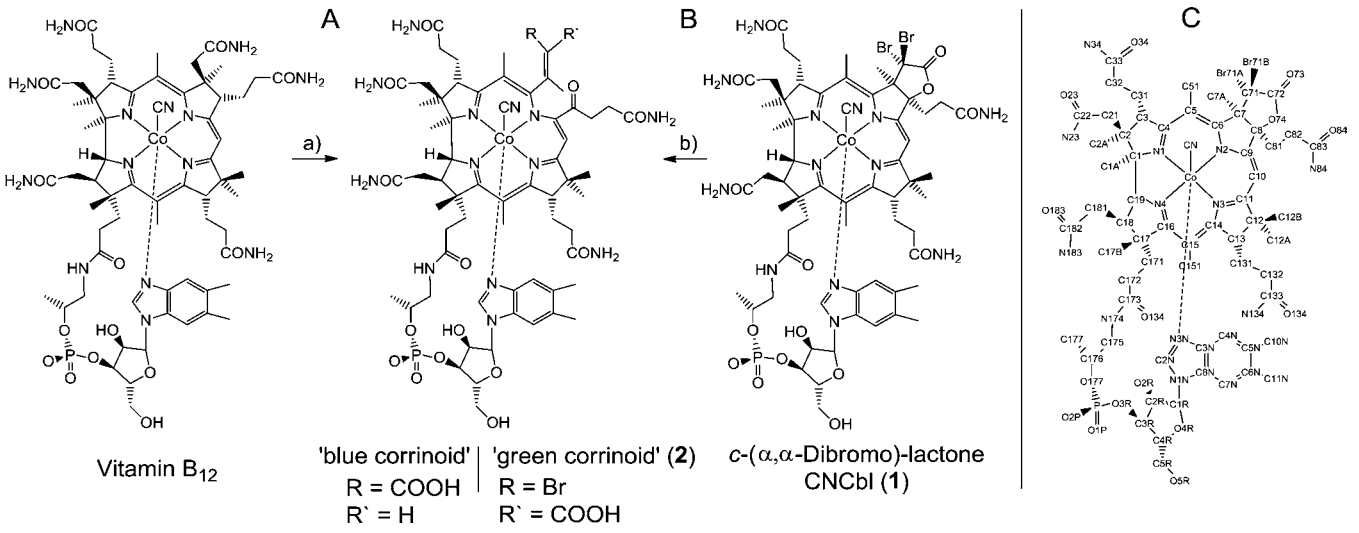
We report in this communication a concise and straightforward synthetic route toward this class of corrinoids using Grob fragmentation.[5] A *c*-(α,α-dibromo)-lactone cyanocobalamin (**1**) converts under basic conditions quantitatively and within seconds to the novel 7,8-*s*CNCbl **2** (Scheme 1B). As outlined in Scheme 2, our retrosynthetic analysis of **2** connects C7 and C8 and introduces a β-bromo alcoholate subunit between positions C8 of the B ring and C71 of the *c*-side chain of intermediate **3**. This structural feature is required for the proposed Grob fragmentation of **3** to **2**. We assumed that intermediate **3** is easily accessible by hydrolysis of the double brominated *c*-lactone **1**.

Cyclization at the B ring of B_{12} under formation of a *c*-lactone subunit can be achieved by the treatment of B_{12} with *N*-bromosuccinimide (NBS) at room temperature.[6] We expected to realize concurrent double bromination in the α-position of the carbonyl moiety by slight modifications of this procedure considering the studies by Movassaghi and Jacobsen.[7]

(1) Fedosov, S. N.; Ruetz, M.; Gruber, K.; Fedosova, N. U.; Kräutler, B. *Biochemistry* **2011**, *50*, 8090.
(2) (a) Kurcon, S.; ó Proinsias, K.; Gryko, D. *J. Org. Chem.* **2013**, *78*, 4115. (b) Zelder, F.; Zhou, K.; Sonnay, M. *Dalton Trans.* **2013**, *42*, 854.
(3) (a) Shimakoshi, H.; Abiru, M.; Izumi, S.; Hisaeda, Y. *Chem. Commun.* **2009**, 6427. (b) Männel-Croise, C.; Zelder, F. *Anal. Methods* **2012**, *4*, 2632. (c) Zelder, F.; Alberto, R. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Elsevier Science: San Diego, 2012; pp 83−130.
(4) Ruetz, M.; Fedosov, S. N.; Kräutler, B. *Angew. Chem., Int. Ed.* **2012**, *51*, 6780.
(5) Eschenmoser, A.; Frey, A. *Helv. Chim. Acta* **1952**, *35*, 1660.
(6) Wagner, F. *Proc. Roy. Soc.* **1965**, *A288*, 344.
(7) Movassaghi, M.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2002**, *124*, 2456.

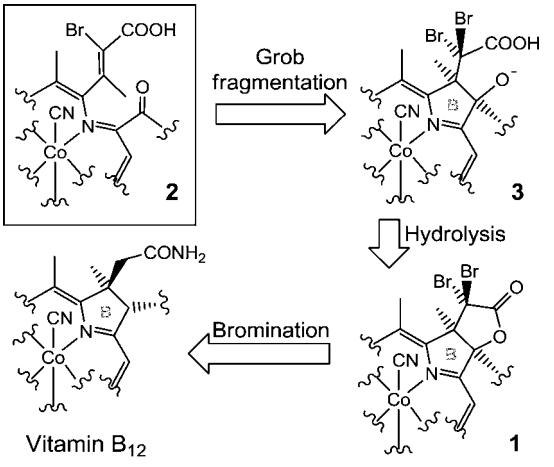
10.1021/ol401572j
Published on Web 08/30/2013

Scheme 1. (A) Synthesis of 'Blue Corrinoid' from Vitamin B_{12} as Reported in ref 1; (B) Fragmentation of **1** to 'Green Corrinoid' (**2**); (C) Atom Numbering for **1**[a]



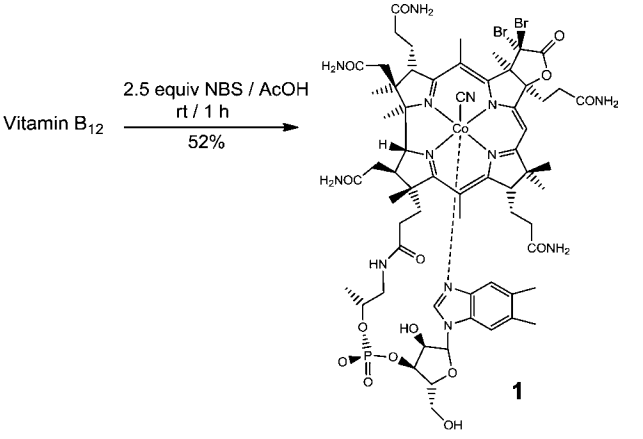
[a] (a) 0.2 M $NaHCO_3$ at pH 9, 50 °C, 3 weeks, 3−6%. (b) pH 11.5, rt, 3 s, 100%.

Scheme 2. Retrosynthetic Analysis of 71-Bromo-7,8-*s*CNCbl (**2**)[a]



[a] Only the region of the B ring subunit of the B_{12} framework is shown.

Scheme 3. Synthesis of **1**



After optimization of the reaction conditions, the synthesis of **1** was performed by treatment of B_{12} with 2.5 equiv of NBS in acetic acid (Scheme 3).[8]

In the high resolution mass spectrum of **1**, an $[M + 2Na]^{2+}$ ion was observed at m/z 778.66763 (m/z_{calc} 778.66762) corresponding to a molecular formula of $C_{63}H_{83}Br_2CoN_{13}O_{15}PNa_2$. This value and the observed isotopic pattern were consistent with the proposed double bromination at C71 of derivative **1**.[8]

The structure of **1** was fully elaborated with two-dimensional homo- and heteronuclear NMR analyses as described in the Supporting Information.[8] Comparison of the ^1H and ^{13}C NMR signals with those of B_{12} indicated diagnostic differences in the B ring area of the corrin macrocycle.[8] The signals in this region show a tendency to shift downfield, which is particularly pronounced for C71 ($\Delta\delta$ = +21.0 ppm) and C8 ($\Delta\delta$ = +37.3 ppm). This behavior is rationalized by the strong deshielding effect of the two electron withdrawing bromo-substituents at C71 as well as the ester functionality at C8, respectively.

The structural identity of **1** was further supported by a crystal structure analysis (Figure 1).[8] Compound **1** crystallized in the orthorhombic space group $P2_12_12_1$ and reassembled those of other Cbl's.[9]

Incubation of **1** for 1 h at pH 9.0 (0.2 M $NaHCO_3$) and 50 °C led to a change of color from red to green and the

(8) Supporting Information.

(9) (a) Zelenka, K.; Brandl, H.; Spingler, B.; Zelder, F. *Dalton Trans.* **2011**, *40*, 9665. (b) Marino, N.; Rabideau, A. E.; Doyle, R. P. *Inorg. Chem.* **2011**, *50*, 220.

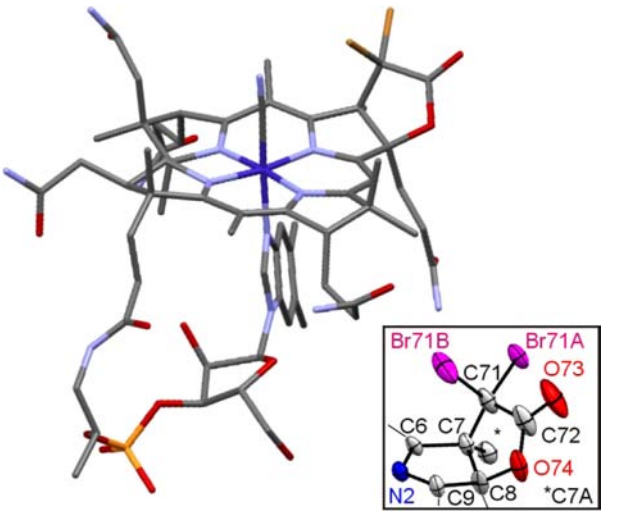


Figure 1. Crystal structure of **1**. *Insert*: details of the B ring and *c*-lactone as ORTEP representation at 50% probability.

quantitative formation of a novel 'green corrinoid' with an absorption maximum (λ_{max}) at 640 nm (Table 1). The red shift ($\Delta\lambda_{max}$ = 60 nm) compared to **1** indicates an alteration of the chromophoric π-system of the corrin macrocycle. A similar trend was observed during the preparation of the 'blue corrinoid' from B_{12}.[1] Control experiments with the unmodified *c*-lactone B_{12} derivative **4** (Table 1; see Scheme S2 for structure)[8] indicated the importance of the bromo leaving group for this type of conversion (Table 1). The kinetics of fragmentation was also followed at pH 10.2 with UV−vis spectroscopy (Figure 2). The conversion of **1** to the 'green corrinoid' obeyed pseudo-first-order kinetics with a rate constant (k_{obs}) of 0.166 min^{-1}.[8] The most convenient approach for the quantitative synthesis of **2** included the conversion of **1** at pH 11.5 within seconds followed by purification with C18-solid phase extraction.

Table 1. Comparison of Rate and Yield of the Fragmentation of *c*-Lactone-B_{12} (**4**)[8] and *c*-(α,α-Dibromo)-lactone-B_{12} (**1**)

entry	*c*-lactone (**4**)	*c*-(α,α-dibromo)-lactone-B_{12} (**1**)
0.2 M $NaHCO_3$ pH 9/50 °C	1d/0%[a]	1 h/100%
pH 11.5/23 °C	1d/0%[a]	3 s/100%

[a] Evaluation of LCMS data indicated quantitative hydrolysis to the corresponding carboxylic acid. For the structure of **4** see Scheme S2.[8]

In addition to UV−vis spectroscopy, the 'structure' of the 'green corrinoid' was fully elucidated as 71-bromo-7,8-*s*CNCbl (**2**) by two-dimensional homo- and heteronuclear NMR analyses, high-resolution mass spectroscopy, and crystal structure analysis.

In the high resolution mass spectrum of **2**, an $[M-H+3Na]^{2+}$ ion was observed at m/z 757.70075 (m/z_{calc} 757.70079) corresponding to a molecular formula of $C_{63}H_{83}BrCoN_{13}O_{16}PNa_3$. This value and the observed isotopic pattern were consistent with the formation of **2** and confirmed the removal of one of the two bromo-substituents at C71 during fragmentation.

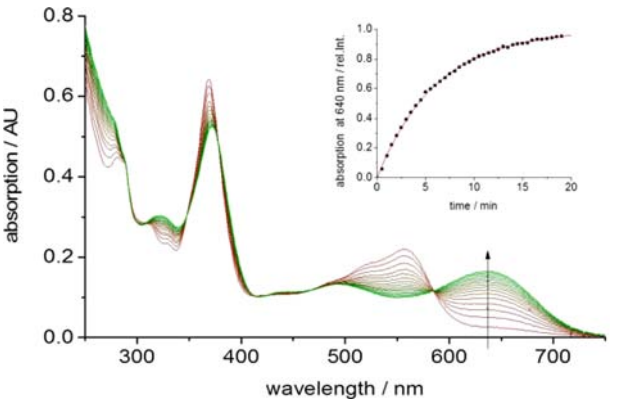


Figure 2. Fragmentation of **1** ($c = 3.99 \times 10^{-5}$ M) at pH 10.2 and 25 °C followed by UV−vis spectroscopy. *Insert:* Change in absorbance at 640 nm for the corresponding reaction.

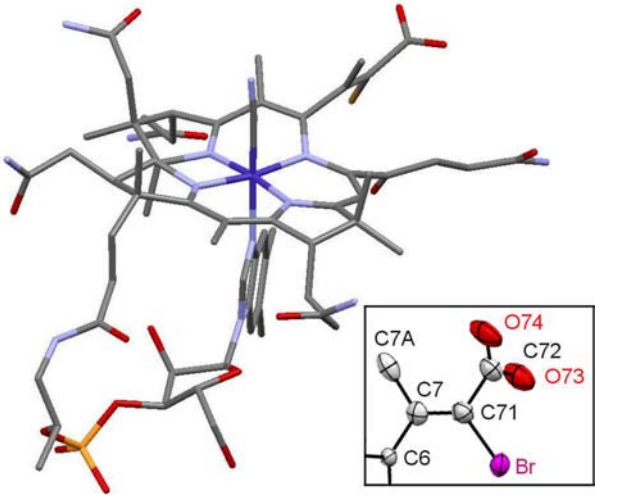


Figure 3. Crystal structure of 'green corrinoid' (**2**). *Insert*: ORTEP representation at 50% probability. Only the major isomer is shown for clarity.

The complete assignments of signals of ^1H and ^{13}C spectra of 'green corrinoid' (**2**) were obtained from $^1H-^1H$-homonuclear (COSY, NOESY) and $^1H-^{13}C$-heteronuclear (HSQC, HMBC) correlations.

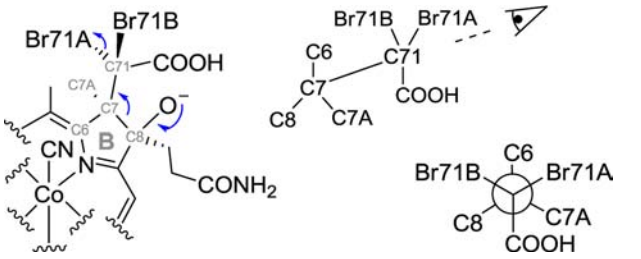
Evaluation of the spectra were conducted by comparison with the 'blue corrinoid'[1] as described in the Supporting Information.[8] In brief, the chemical shifts of the green fragmentation product **2** showed the same tendencies as observed for the 'blue corrinoid'[1] under consideration of the residual 71-bromo-substituent and indicated the B ring as the center of structural modification. $^1H-^{13}C$-heteronuclear

multiple bond correlations (HMBC) between H7A and C8 were present in the dibrominated educt **1**, but lacking in the 'green corrinoid' (**2**). This behavior can be explained with the scission of the C7−C8 σ-bond. Furthermore and in agreement with the structure of **2**, the signals of H81 and H82 were downfield shifted. The structural elucidation of the 'green corrinoid' as derivative **2** was further supported by crystal structure analysis[8] and confirmed (i) cleavage of the C7,C8 σ-bond; (ii) formation of a *Z*-configured double bond between C7 and C71 (1.32(1) Å); and (iii) the formation of a keto functionality at C8. Interestingly, the *c*-carboxylic acid functionality showed a strong intermolecular hydrogen bond to a neighboring phosphate group.[8] Derivative **2** crystallized in the uncommon orthorhombic space group $P2_12_12$ with cell dimensions of a = 11.52310(10) Å, b = 52.2227(3) Å, and c = 15.51610(12) Å (Figure 3). There is only one other cobalamin structure known so far that crystallized in the same space group, but with very different cell dimensions of a = 27.2778(17) Å, b = 24.9345(6) Å, and c = 14.0940(14) Å.[10]

Some mechanistic insights into the mode of fragmentation have been obtained from structural comparison between intermediate **3** and product **2**. Based on the *Z*-configuration of the double bond in derivative **2**, we propose Br71A as the leaving group of **3** because of its *anti* orientation to the scissible C7−C8 bond as shown in Scheme 4.[5,11]

In summary we report a concise and convenient two-step procedure for the preparation of 7,8-*seco*-cyanocobalamins. Straightforward synthetic access toward this interesting new class of B_{12} derivatives will facilitate potential applications in the near future.

Scheme 4. Different Views on the C8, C7, C71 Region of the β-Bromo Alcoholate Subunit of Intermediate **3**



Acknowledgment. This work was financially supported by the Forschungskredit of the University of Zürich. Support by R. Alberto (University of Zurich) and the Institute of Inorganic Chemistry of the University of Zurich is acknowledged. The authors are thankful to L. Bigler (University of Zurich) for recording of the HR-ESI-MS spectra and to DSM Nutritional Products AG (Basel/Switzerland) for a generous gift of vitamin B_{12}.

Supporting Information Available. Preparative procedures and characterizations. NMR assignments of compounds **1** and **2**. Crystal data and structure refinement of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(10) Mundwiler, S.; Spingler, B.; Kurz, P.; Kunze, S.; Alberto, R. *Chem.—Eur. J.* **2005**, *11*, 4089.
(11) Prantz, K.; Mulzer, J. *Chem. Rev.* **2010**, *110*, 3741.