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Structures of the b- and d-Acid Derivatives of Vitamin B_{12} and Their Complexes with $[M(CO)_3]^+$ (M = ^{99m}Tc, Re)

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The acid hydrolysis of natural vitamin B_{12} yields several products in which the acetamide or propionamide side chains on the corrin framework are converted into the corresponding acids. These acids can be derivatised with further functionalities. We have separated in particular the b- and the d-acid derivatives **1** and **2**, respectively, since functionalisation at these positions of the corrin ring generally keeps the affinity for vitamin B_{12} transport proteins intact. Although the authenticity of **1** and **2** seemed evident from ^{1}H NMR investigations, it has not been supported by X-ray structure analysis. The coupling of ethyl *N*-(3-aminopropyl)-*N*-(pyridin-2-ylmethyl)glycinate (**3**) to the carboxylate groups in **1** and **2** by peptide synthetic methods gave the vitamin B_{12} derivatives **4** and **5**, respectively. The structures of **4** and **5** could be characterised by X-ray analysis, which unambiguously confirmed the presence of the b- and d-acid in **1** and **2**, respectively. The reaction of **4** and **5** with the complex $[Re(OH_2)_3(CO)_3]^+$ in water gave the corresponding vitamin B_{12} derivatives **8** and **9**, and the aqueous reaction of **4** and **5** with the complex $[^{99m}Tc(OH_2)_3(CO)_3]^+$ afforded the ^{99m}Tc homologues **10** and **11**, respectively.

Introduction

Cyanocobalamin or vitamin B_{12} (abbreviated as B_{12} in the following) is, like most of the vitamins, not synthesised by mammals but by a few bacteria only. However, essentially all higher organisms are B_{12} dependent, and the human body in particular has developed a highly complex system for the uptake and biochemical conversion of B_{12} into the two coenzymes methyl- and adenosylcobalamin. Excellent and comprehensive reviews appeared recently covering all aspects of B_{12} chemistry, biochemistry and physiology.[1–3] B_{12} has been proposed as a Trojan horse for cyto- or radiotoxic compounds, since it is essential for the human body. Vitamin-mediated drug uptake by rapidly growing and dividing aggressive tumours having a high density of transcobalamin receptors seems to be an attractive strategy.[4] Covalent conjugation of bioactive molecules to the B_{12} molecule can be performed at several functional sites. The 5′-OH group of the α-ribofuranoside in the backloop would be such a versatile site according to structure–activity relationships. However, little functionalisation has been reported, because of the complexity of site-specific derivatisation.[5] Furthermore, the b- and d-acid sites are attractive positions for conjugation of functionalities or metal complexes. It could be shown by Hogenkamp et al. that covalent binding of a spacer with DTPA and subsequent labelling with ^{111}In led to a good uptake in the tumour cells, but the uptake was also high in the kidneys.[6,7] We have chosen the b- and d-acids for derivatisation. Although it is known that the affinity of such derivatives for transcobalamin proteins decreases, it is still strong enough for binding and transportation.[8–10] In our previous work, we attached histidine derivatives at the b- or d-acids of B_{12}.[11,12] Preliminary studies with ^{99m}Tc-labelled B_{12} derivatives led to very promising results in vitro and in vivo. Biologically active molecules have also been introduced by direct coordination to the Co^{III} atom in B_{12} or through the cyano group bound to it. Grissom et al. coupled fluorescing molecules by a Co–C bond to B_{12} and used this derivative for lymphatic mapping.[13,14] We bound several rhenium, technetium and platinum complexes to the cyano group and could confirm the authenticity of the products by X-ray structure analysis.[15,16]

The preferred site for derivatisation are the three propionamide and the two acetamide groups bound to the periphery of the corrin ring, which is, like the backloop, bound to three propionamide and two acetamide groups. Scheme 1 gives an overview of the structure of B_{12} and the relative positions of the respective groups.

Characterisation of B_{12} derivatives is mainly performed with NMR spectroscopy. NMR-restrained molecular mechanics/molecular dynamics methods have successfully been used to structurally characterise B_{12} derivatives.[17] Nevertheless, X-ray structure analysis would be preferable, but

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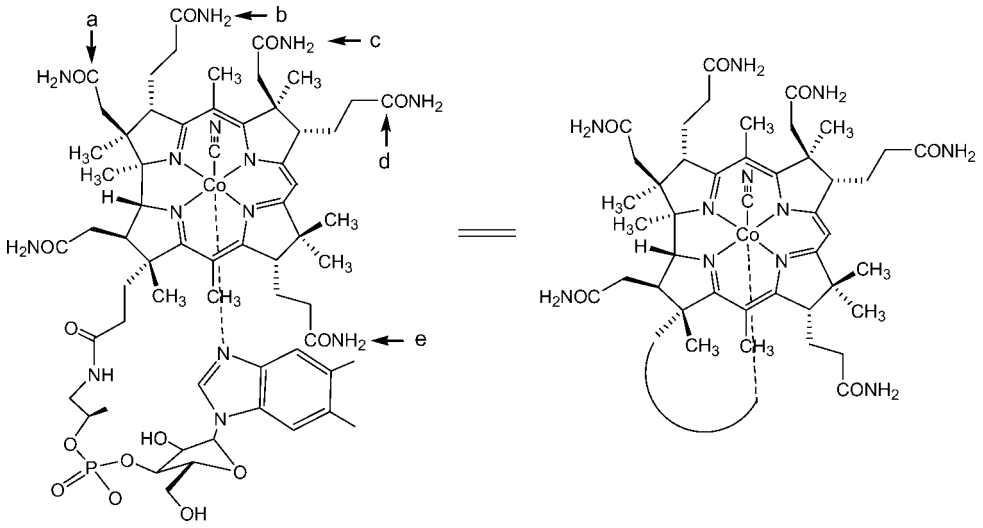


Scheme 1. Assignment of the side chains on the periphery of the corrin ring in B_{12}.

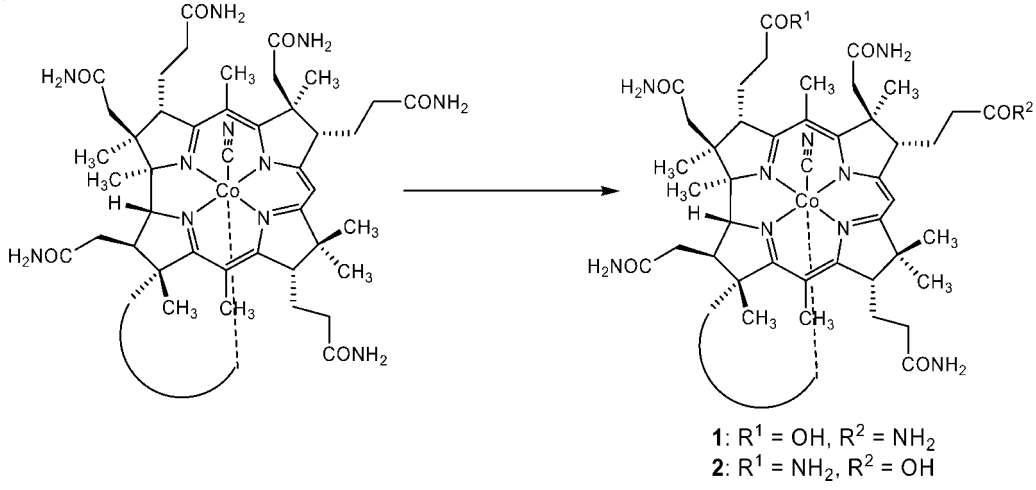
some B_{12} derivatives are very difficult to crystallise. Milestones in B_{12} structure analysis were certainly the elucidation of the structure of native B_{12} by Hodgkin et al.,[18] and those of methylcobalamin and finally adenosylcobalamin (coenzyme B_{12}).[19,20] X-ray crystallography of B_{12} and its derivatives has recently been reviewed extensively.[21–23] These reviews reveal the surprising fact that no X-ray analysis of a B_{12} derivative in which one of the amide side chains has been functionalised is available. To the best of our knowledge, the only available X-ray structure of the product of a derivatisation at the corrin ring is that of one in which the C10-H has been replaced by a chlorine atom.[24] We present in this study the synthesis and the first X-ray structures of B_{12} molecules that were derivatised at either the b- or the d-acid with a ligand for binding to transition metals. In a second step, these B_{12} derivatives were labelled with the *fac*-$[M(CO)_3]^+$ (M = Re, ^{99m}Tc) moiety.

Results and Discussion

Syntheses

Mild acid hydrolysis of the amide side chains in B_{12} yields a mixture of monocarboxylic b-, d- and e-acids. These can be separated and obtained in pure form by sequential ion-exchange chromatography and RP-HPLC methods.[8,25–27] With a slightly modified method (see Experimental Section) we obtained 280 mg (15%) of the b-acid **1** and 132 mg (7%) of the d-acid **2**, starting with 1.9 g of vitamin B_{12} (Scheme 2). These yields are consistent with the literature. However, we isolated only 94 mg (5%) of the e-acid.

The determination of the exact structures of the products of hydrolysis turned out to be difficult. Initially, the main product, compound **1**, was thought to be the e-acid because



Scheme 2. Mild hydrolysis of B_{12} yields the b- and d-acids **1** and **2**.[8,25–27]

of its ability to hydrolyse the backloop and from the results of X-ray and neutron diffraction studies.[26,28] Subsequent neutron diffraction studies indicated that it could be the b-acid, an assumption that was later confirmed by sophisticated multi-dimensional NMR measurements.[29–32] The similar diffraction power of the OH and NH_2 groups was the problem in X-ray analysis. Cobalamin crystals were rarely obtained in the quality required for an unambiguous differentiation between these two groups. An X-ray structure analysis of respective derivatives such as esters or alkylamides would solve this problem (see later). However, initially all attempts failed, because the conjugated groups prevented crystallisation or were disordered.[23,33]

Since we are interested in the introduction of coordinating ligands rather than simple organic groups, we selected the tridentate chelate *N*-(pyridin-2-ylmethyl)glycine. This basic ligand structure is known to form strong complexes with the *fac*-$[M(CO)_3]^+$ moiety that are stable in vivo.[34] To enable the conjugation of the basic ligand framework to the biomolecule, an alkyl chain with a terminal functionality was introduced to the secondary amine by adaptation of known synthetic pathways.[35] In this case, the 3-aminopropyl chain bound to the secondary amine group in the basic ligand structure gave compound **3**, which then comprised the tridentate chelate (after de-esterification) and an additional primary amine for coupling with the b- and d-carboxylic acids (Scheme 3).

The reaction of **1** and **2** in the presence of ligand **3** and coupling reagents gave the corresponding derivatives **4** (b-acid) and **5** (d-acid), respectively, after HPLC purification, in about 30% yield. In order to obtain the free tridentate ligand, the ester functionality can be removed from the ligand by alkaline hydrolysis. It turned out, however, that the hydrolysis in advance was not required, since coordination to the *fac*-$[M(CO)_3]^+$ moiety catalysed this reaction and led to immediate hydrolysis during the coordination process.

X-ray-quality crystals could be grown by very slow vapour diffusion of either acetone or acetonitrile into an aqueous solution of **4** or **5**, respectively. The crystals of **4** were of exceptional quality for B_{12} crystals, which is shown by the R_1 value of 5.9% for the strong reflections. Furthermore, more than 92% of all reflections with a resolution better than 0.75 Å had an intensity higher than 2 sigma. On the other hand, the diffraction of the crystals of **5** was not strong enough on our Mo home source; therefore, we collected synchrotron data on the F1 beamline at HASYLAB/DESY to a resolution of 0.99 Å. Both structures crystallised in the "typical" B_{12} space group $P2_12_12_1$. In the literature, the family of B_{12} crystal structures with the $P2_12_12_1$ space group is subdivided into four clusters on the basis of the ratio of the cell axes *b*/*a* and *c*/*a*.[21] Introduction of the rather large unit of **3** into the B_{12} framework apparently did not distort the normal B_{12} crystal packing. The corresponding ratios for the crystal structures of **4** and **5** are 1.40 and 1.39 for *b*/*a* and 1.61 and 1.61 for *c*/*a*, respectively. These values place **4** and **5** clearly into cluster II. Furthermore, an overlay of the two structures reveals an almost identical conformation of all substituted as well as unsubstituted amide side chains (Figure 1). The ORTEP plots of **4** and **5** are shown in Figure 2 and Figure 3, respectively. It should be noted that both ligand structures include the ethyl ester as the pendent since the coupling was performed directly with compound **3**. This was confirmed by the mass spectra of both compounds. Because of the disorder in the structure of **5**, we were only able to localise the terminal CH_3 group in **4** but not that in **5**; hence, it does not appear in the line drawings or the ORTEP drawings.

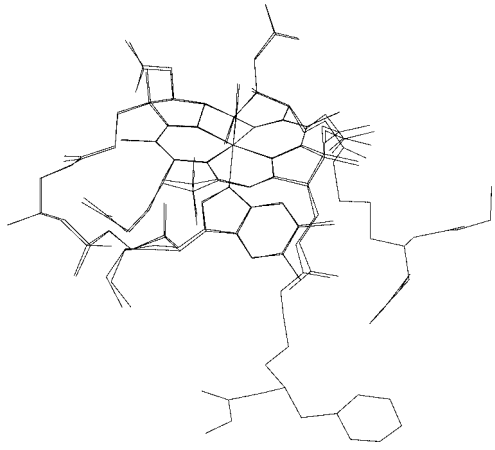
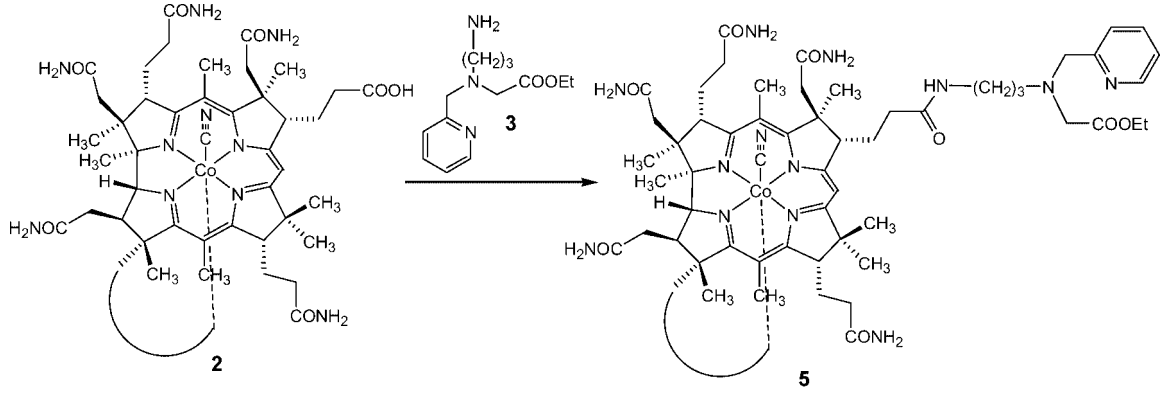


Figure 1. Overlay of the X-ray structures of **4** and **5**.



Scheme 3. Derivatisation of B_{12} d-acid **2** with ligand **3** to yield compound **5**.

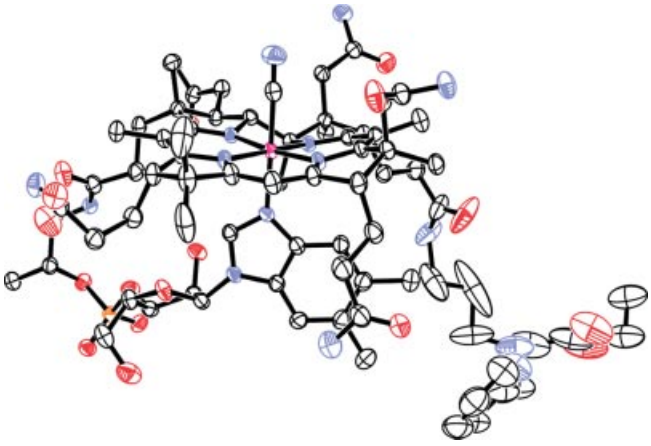


Figure 2. ORTEP plot of **4**. Hydrogen atoms and some water molecules were omitted for clarity.

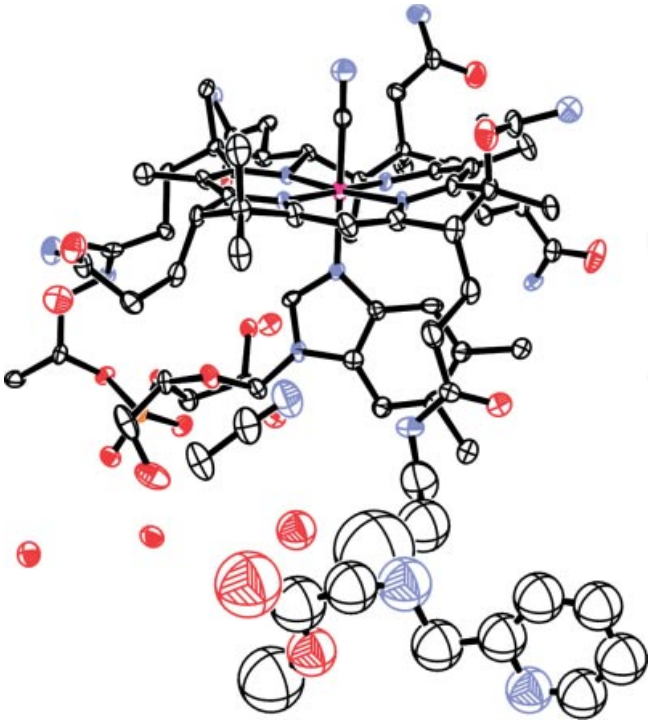
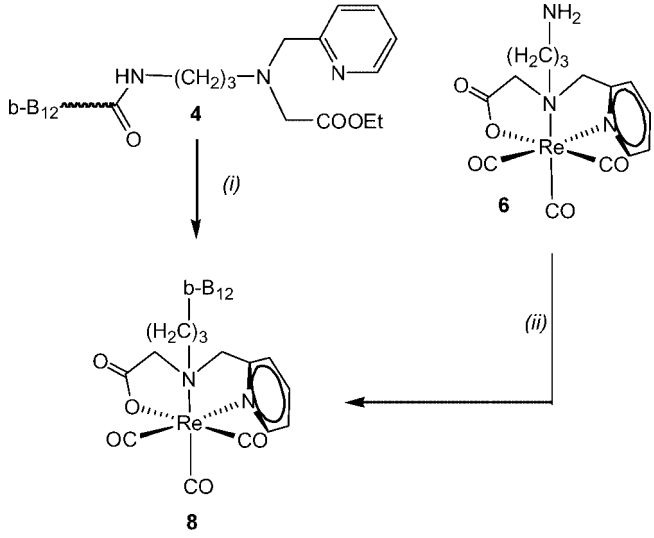


Figure 3. ORTEP plot of **5**. Hydrogen atoms and some water molecules were omitted for clarity.

The nitrogen atom of the pyridine group in **4** is hydrogen-bonded to a five-membered cluster of water molecules with extensive contact between three B_{12} molecules. The nitrogen atom of the pyridine group in **5** has two hydrogen bonds with symmetry-generated neighbouring molecules in a Y-shaped alignment of three water molecules (Table 1).

Complexation with $[Re(OH_2)_3(CO)_3]^+$ and Labelling with $[^{99m}Tc(OH_2)_3(CO)_3]^+$

In compounds **4** and **5**, the carboxylate group is still protected by an ester functionality. Mild alkaline hydrolysis of the ethyl ester over an extended period would convert the ester into the acid and render all three coordination sites available; however, decomposition products were observed during this process, presumably as a result of c-lactam formation. The formation of the c-lactam of B_{12} in alkaline media is a known reaction.[36,37] During the reaction of **4** and **5** with $[Re(OH_2)_3(CO)_3]^+$ in water, we observed that ester cleavage was catalysed by the presence of the metal complex, probably after initial coordination to one or two of the nitrogen donors. A similar observation was made with histidine complexes, in which an ester function was also converted quantitatively to the carboxylate during coordination.[12] After the reaction was completed, we found a mixture of two complexes, one of which was the desired product **8** (for the b-derivative). The highest yield for a single product, however, was obtained by an indirect pathway. The formation of the complex **6** from **3** and $[Re(OH_2)_3(CO)_3]^+$ is quantitative. Complex **6** was then coupled to acid **1** and afforded complex **8** directly in very good yield without byproducts (Scheme 4). The second strategy, as described before, refers to the prelabelling of biomolecules with ^{99m}Tc. Since yields are usually far from quantitative, prelabelling is not the method of choice for technetium. We had observed previously that ester bonds are much more efficiently cleaved in the presence of technetium than in the presence of rhenium. This observation was confirmed by treating $[^{99m}Tc(OH_2)_3(CO)_3]^+$ with **4** or **5** at moderate temperature. We observed the exclusive formation of the desired products **10** and **11** without any byproducts, even though byproducts had been observed in the corresponding reactions with $[Re(OH_2)_3(CO)_3]^+$. Comparison of the retention times of **10** and **11** with **8** and **9** confirmed the identity of the corresponding homologues. These technetium complexes are stable in human serum albumin for at least 24 h without reoxidation to $[^{99m}TcO_4]^-$. Compounds **10** and **11** therefore represent excellent radiotracers for uptake studies in cancer cells or microbial infections.



Scheme 4. Reaction pathways to **8**, (*i*) direct reaction of **4** with $[Re(OH_2)_3(CO)_3]^+$ to give **8** (postlabelling) or (*ii*) conjugation of preformed **6** to **1** (prelabelling).

Conclusions

We have synthesised two B_{12} derivatives that comprise tridentate chelators attached to the corrin periphery. For the first time, an X-ray structure analysis of a B_{12} compound with corrin side-chain derivatisation could be elucidated confirming the assignments of earlier NMR investigations. The derivatives are coordinated to $[Re(OH_2)_3(CO)_3]^+$ and to $[^{99m}Tc(OH_2)_3(CO)_3]^+$, thus representing useful radiolabelled B_{12} compounds for in vitro and in vivo studies.

Experimental Section

General Remarks: Reagent grade chemicals were obtained from Merck, Dietikon (CH), Aldrich, Fluka or Buchs (CH) and were used without further purification. HPLC analyses were performed with a Merck-Hitachi L-7000 system equipped with a EG&G Berthold LB 508 radiometric detector, using Waters XTerra RP8 columns (5-μm particle size, 1×100 mm) and a flow rate of 1 mL min^{-1}. Chromatograms were recorded at 250 and 360 nm. Sodium acetate buffer A1 was prepared by mixing acetic acid (2.9 mL) and sodium hydroxide (4.55 mL, 2 M) in water (900 mL) and methanol (100 mL). Tris buffer A2 was prepared by dissolving 2-amino-2-(hydroxymethyl)propane-1,3-diol (605 mg) in water, adding HCl (2 M) to reach a pH of 8.2, adjusting the volume to 1000 mL and adding acetonitrile (10 mL). Eluent B was always methanol. Preparative HPLC separations were performed with a Varian Prostar system equipped with two Prostar 215 pumps and a Prostar 320 UV/Vis detector using Waters XTerra Prep RP8 columns (5-μm particle size, 3×100 mm and 30×100 mm). Flow rates were 4 mL min^{-1} for the 3×100-mm column and 30 mL min^{-1} for the 30×100-mm column. UV/Vis spectra were recorded with a Varian Cary 50 spectrometer, IR spectra were recorded with a Bio-Rad FTS-45 spectrometer in KBr. ESI-MS were recorded with a Merck Hitachi M-8000 spectrometer. The values of the ^{187}Re isotope are reported for Re compounds. NMR spectra were recorded with a Bruker DRX 500 MHz spectrometer.

Cobalamin derivatives were desalted by applying an aqueous solution of the compound to a Chromafix RP18ce cartridge and then thoroughly rinsing with water. The desalted product was then eluted with methanol, the solvent was removed, and the resulting product was dried in high vacuum.

The ethyl ester of *N*-(3-aminopropyl)-*N*-(pyridin-2-ylmethyl)glycinate, compound **3**, was prepared as described for its pentyl analogue.[35] Cyclisation of the compound was observed under basic conditions; therefore, the Boc-protected precursor was stored and deprotected with TFA just prior to further functionalisation. [Re({*N*-(3-aminopropyl)*N*-pyridin-2-ylmethylamino}acetato)$(CO)_3$] (**6**) was prepared by treating the fully deprotected [*N*-(3-aminopropyl)-*N*-pyridin-2-ylmethylamino]acetic acid with $(Et_4N)_2[Re(Br)_3(CO)_3]$ in water (see below). The syntheses of the b- and d-acids **1** and **2** of B_{12} were performed according to the literature but with some slight modifications in the purification process as described below.[8]

X-ray Crystallography: Suitable crystals were covered with Paratone N oil, the mother liquor was removed, and the crystals were finally mounted with the help of the magnetic loops. Single-crystal diffraction data for **4** were collected with a Stoe IPDS diffractometer at 183(2) K by using graphite-monochromated Mo radiation (0.71073 Å). Data were corrected for Lorentz and polarisation effects as well as for absorption. Single-crystal diffraction data of **5** were collected at the F1 beamline at HASYLAB/DESY with a Huber Kappa diffractometer at 100(2) K, by using $\lambda = 0.7500$ Å radiation and a MarCCD 165 detector. Integration of intensities was performed with the XDS program.[38] A series of 190 frames with a 1.0-degree phi rotation and an exposure time of 90 s was collected. The XDS program compensated for the beam intensity loss between two refills. Both structures were solved with direct methods by using SIR97[39] and refined by full-matrix least-squares methods on F^2 with SHELXL-97.[40] Appropriate restraints had to be used to constrain the geometry of the highly flexible ligand part in **5**. The terminal carbon atom of the ethyl ester in **5** could not be localised in the electron density map. ORTEP plots were generated by the ORTEP32 software and are drawn at 30% probability. The crystal data and refinement parameters of **4** and **5** are summarised in Table 1. CCDC-630989 and CCDC-630990 contain the supple-

Table 1. Crystal and structure refinement data for **4** and **5**.

Compound No.	**4**	**5**
X-ray source	sealed Mo tube	F1 beamline at HASYLAB/DESY
Detector	Stoe IPDS	MarCCD 165
Wavelength [Å]	0.71073	0.7500
Formula[a]	$C_{79}H_{132}CoN_{16}O_{27}P$	$C_{77}H_{122}CoN_{17}O_{24}P$
M_r	1827.91	1759.82
Crystal system	orthorhombic	orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
a [Å]	15.9566(6)	15.9200(15)
b [Å]	22.3021(8)	22.1900(18)
c [Å]	25.6917(12)	25.680(3)
V [Å^3]	9142.8(6)	9071.8(15)
Z	4	4
D_c [g cm^{-3}]	1.328	1.288
Goodness-of-fit on F^2	1.046	1.138
R[b,c]	0.0594	0.0824
wR_2[b,d]	0.1600	0.1979
Flack parameter	0.009(9)	–0.06(3)
Max., min. peaks [e·Å^{-3}]	1.034, –0.815	0.698, –0.852

[a] Formulas are different since various amounts of solvent molecules were found in the structures. [b] Observation criterion: $I > 2\sigma(I)$. [c] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [d] $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$.

mentary crystallographic data for this paper. They can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Synthesis of Vitamin B_{12} b- and d-Acids 1 and 2: Vitamin B_{12} (1.88 g, 1.39 mmol) was hydrolysed in HCl (0.1 M, 190 mL) as described in literature.[8] The purification was modified in the following way. After desalting the crude reaction mixture by phenol extractions, three fractions were isolated on a Dowex column. The first fraction contained exclusively **2**, the second one a mixture of **1** and **2**, and the third one contained **2** and the e-acid. The second fraction (mixture of **1** and **2**) was separated by preparative HPLC (column: Waters XTerra Prep RP8, 5 μm, 30 × 100 mm; buffer A1; gradient: 0.5%min^{-1} starting from 100% A1). The mixture of **2** and the e-acid was separated on the same system but using the buffer A2. Compound **1** was isolated in a yield of 280.6 mg (14.9%), compound **2** in a yield of 131.5 mg (7.0%), and the e-acid was obtained in a yield of 94.26 mg (5.0%). Assignment of the isomers was carried out by comparison with the HPLC retention order given in literature.

Synthesis of 4: A solution of freshly prepared **3** (361 μmol) in water (1 mL) was added to **1** (65.0 mg, 48.1 μmol). EDC (46.1 mg, 240 μmol) was added, and the pH was adjusted to 5.5 with NaOH (0.1 M). After the mixture was stirred at room temperature for 15 h, HPLC analysis exhibited about 50% of product **4**. More EDC (46.1 mg, 240 μmol) was added, but even extended stirring at room temperature did not lead to a significant amount of formation of more **4**. The solvent was removed in vacuo, and the residue was purified by preparative HPLC (buffer A1, gradient: 0.5%min^{-1} starting from 100% A1). The main fraction was collected, the solvent was removed in vacuo, and the product was desalted to give **4** in a yield of 25.8 mg (16.2 μmol, 33.3%). R_t = 13.1 min (gradient A1/B). ^{1}H NMR (500 MHz, CD_3OD, 300 K): δ = 8.43 (d, J = 4.9 Hz, 1 H, L12), 7.77 (t, J = 7.7 Hz, 1 H, L10), 7.58 (d, J = 7.8 Hz, 1 H, L9), 7.28 (t, J = 6.9 Hz, 1 H, L11), 7.25 (s, 1 H, B7), 7.12 (s, 1 H, B2), 6.56 (s, 1 H, B4), 6.27 (d, J = 3.0 Hz, 1 H, R1), 6.04 (s, 1 H, C10) ppm. ^{13}C NMR (125.8 MHz, CD_3OD, 293 K): δ = 181.65, 180.20, 177.67, 176.73, 175.65, 175.58, 175.43, 174.72, 174.48, 174.34, 173.19, 167.22, 166.98, 160.49, 160.44, 149.67, 143.55, 138.86, 138.34, 135.72, 133.88, 131.56, 125.07, 124.04, 117.92, 112.65, 108.77, 105.21, 95.76, 88.05, 86.50, 83.74 (d, $J_{C,P}$ = 5.6 Hz), 76.40, 75.55, 73.69 (d, $J_{C,P}$ = 6.1 Hz), 71.66, 70.80, 70.56, 62.74, 61.79, 60.85, 60.45, 57.75, 56.97, 55.91, 55.67, 55.13, 53.31, 52.64, 50–49 (1 C below solvent), 46.91, 43.99, 43.16, 40.22, 38.88, 37.06, 35.43, 33.58, 33.09, 33.06, 32.42, 29.74, 28.01, 27.96, 27.52, 21.07, 20.61, 20.43, 20.26 (d, $J_{C,P}$ = 3.8 Hz), 20.02, 17.67, 17.16, 16.51, 16.21, 14.71 ppm. ESI-MS: m/z = 806.5 $[M + H + Na]^{2+}$, 795.6 $[M + 2 H]^{2+}$. UV/Vis (methanol): λ (ε, L mol^{-1} cm^{-1}) = 278.0 (8500), 361.1 (26500), 549.1 (8000) nm. A full ^{1}H NMR spectrum is shown in the Supporting Information. The ester can be hydrolysed to the acid. Because of self-catalysed cleavage processes (see above) this compound has to be labelled immediately. Labelling can be performed directly with **4** without the need for prior deesterification. R_t = 10.8 min.

Synthesis of 5: The procedure is similar to the preparation of **4**. Compound **2** (9.3 mg, 6.9 μmol) was treated with **3** (7 μmol) and EDC (6.6 mg, 34 μmol) as described for the synthesis of **4**. After HPLC purification, the product **5** was isolated in a yield of 3.6 mg (33%). R_t = 15.5 min (gradient A1/B). ^{1}H NMR (500 MHz, CD_3OD, 300 K): δ = 8.41 (d, J = 4.9 Hz, 1 H, L12), 7.79 (t, J = 7.7 Hz, 1 H, L10), 7.56 (d, J = 7.8 Hz, 1 H, L9), 7.30 (t, J = 5.6 Hz, 1 H, L11), 7.25 (s, 1 H, B7), 7.12 (s, 1 H, B2), 6.56 (s, 1 H, B4), 6.27 (d, J = 3.0 Hz, 1 H, R1), 6.05 (s, 1 H, C10) ppm. ^{13}C NMR (125.8 MHz, CD_3OD, 293 K): δ = 181.72, 180.27, 177.77, 177.64, 177.50, 175.86, 175.69, 175.47, 174.72, 174.36, 173.84, 173.05, 167.25, 167.05, 160.65, 149.63, 143.58, 138.90, 138.43, 135.60, 134.00, 131.59, 125.10, 124.06, 118.02, 112.62, 108.82, 105.24, 95.76, 88.11, 86.56, 83.84 (d, $J_{C,P}$ = 5.6 Hz), 76.49, 75.65, 73.77 (d, $J_{C,P}$ = 6.1 Hz), 70.85, 62.84, 61.75, 60.86, 60.51, 57.79, 57.02, 55.73, 55.15, 53.10, 52.62, 46.95, 44.02, 43.19, 40.25, 38.59, 36.37, 35.45, 33.78, 33.63, 33.13, 32.52, 32.45, 29.79, 28.21, 27.78, 27.57, 21.09, 20.60, 20.51, 20.33 (d, $J_{C,P}$ = 3.8 Hz), 20.09, 17.65, 17.24, 16.53, 16.25, 14.77 ppm. ^{31}P NMR (300 MHz, CD_3OD, 300 K): δ = 1.373 ppm. ESI-MS: m/z = 1612 $[M + Na]^+$, 1590 $[M + H]^+$, 806 $[M + H + Na]^{2+}$, 795.1 $[M + 2 H]^{2+}$. UV/Vis (methanol): λ (ε, L mol^{-1} cm^{-1}) = 279.0 (13400), 361.1 (23300), 549.1 (7200) nm. Free acid R_t = 10.6 min (A1/B). Full ^{1}H and ^{13}C NMR spectra are shown in the Supporting Information.

Synthesis of 6: Complex $[NEt_4]_2[ReBr_3(CO)_3]$ (75 mg, 0.09 mmol) was dissolved in water (10 mL), and a solution of fully deprotected **3** (36 mg, 0.13 mmol) in methanol was added. The solution was heated to 70–80 °C for 2 h. The proceeding of the reaction was monitored by HPLC: the peak with R_t = 6.2 min ($[Re(OH_2)_3(CO)_3]^+$) disappeared, and a new peak (product **6**) with R_t = 15.1 min appeared. After 2 h, the conversion was complete. When the reaction mixture was cooled, a white powder precipitated from solution. This precipitate was filtered, washed with small amounts of cold water and dried in vacuo. Yield: 32 mg (72%); the rest remained in the filtrate. It is important to apply temperatures above 70 °C in order to ensure ester hydrolysis during complexation. ^{1}H NMR (300 MHz, CD_3OD, 300 K): δ = 8.83 (d, J = 5.7 Hz, 1 H), 8.11 (t, J = 7.8 Hz, 1 H), 7.74 (d, J = 8.1 Hz, 1 H), 7.56 (t, 1 H, J = 6.9 Hz), 4.78 (d, 1 H, J = 15.6 Hz), 4.55 (d, 1 H, J = 15.9 Hz), 3.90 (d, 1 H, J = 17.1 Hz), 3.70 (m, 2 H), 3.53 (d, J = 16.8 Hz, 1 H), 3.04 (t, J = 7.5 Hz, 2 H), 2.18 (m, 2 H) ppm. ESI-MS: m/z = 493.80 $[M + H]^+$. $C_{12}H_{18}F_3N_3O_7Re$ (6·TFA): calcd. C 25.76, H 3.24, N 7.51; found C 25.23, H 3.21, N 7.33.

Synthesis of 8: Two synthetic procedures are possible. The direct reaction of **4** with $[Re(OH_2)_3(CO)_3]^+$ in water gives compound **8** in good yield. Alternatively, the complex **6** can be preformed by the reaction of fully deprotected **3** and $[Re(OH_2)_3(CO)_3]^+$ followed by coupling to **1** as described in the following part. Compound **1** (26.7 mg, 19.8 μmol), complex **6** (29.2 mg, 60 μmol), EDC (11.5 mg, 60 μmol) and *N*-hydroxysuccinimide (6.9 mg, 60 μmol) were dissolved in a mixture of water (5 mL) and DMSO (0.5 mL), and the pH was adjusted to 5.5. After 5 h at room temperature, HPLC analysis exhibited about 33% of product **8**. More EDC and *N*-hydroxysuccinimide were added and the stirring was continued for 3 d with addition of EDC and *N*-hydroxysuccinimide in 24-h intervals. The water was removed in vacuo, and the product was precipitated by adding diethyl ether. The oily suspension was centrifuged and decanted. Washing with diethyl ether was repeated twice (5 mL each time) until a fine precipitate formed. The crude product was dried at high vacuum, purified by preparative HPLC (A1, gradient 1%min^{-1} starting from 100% A1) and desalted to give **8** in a yield of 9.1 mg (23%). R_t = 14.2 min (gradient A1/B). IR (KBr): $\tilde{\nu}$ = 2927, 2134, 2024, 1913, 1897, 1662 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, 300 K): δ = 8.1 (d, J = 4.5 Hz, 1 H, L12), 8.10 (t, J = 8 Hz, 1 H, L10), 7.73 (m, 1 H, L9), 7.54 (m, 1 H, L11), 7.26 (s, 1 H, B7), 7.14 (s, 1 H, B2), 6.59 (m, 1 H, B4), 6.27 (s, 1 H, R1), 6.05 (s, 1 H, C10) ppm. ^{13}C NMR (125.8 MHz, CD_3OD, 300 K): δ = 198.39, 198.24, 197.46, 183.33, 181.75, 180.28, 177.72, 177.68, 176.77, 175.74, 175.69, 175.46 (2 C), 174.90, 174.76, 174.36, 167.27 (2 C), 160.90, 153.72, 143.63, 141.85, 138.39, 135.77, 133.97, 131.63, 127.20, 125.19, 117.99, 112.65, 105.25, 102.57, 95.74, 88.13, 86.59, 83.83 (d, $J_{C,P}$ = 4.5 Hz), 76.52, 75.74, 73.72 (d, $J_{C,P}$ =

5.4 Hz), 70.89, 69.44, 68.87, 62.90, 62.04, 60.55, 55.27, 55.77, 49–50 (1C below solvent), 46.93, 44.35, 43.04, 40.29, 38.01, 36.44, 35.51 (2 C), 33.66, 33.11 (2 C), 32.45 (2 C), 29.74, 27.60, 26.46, 21.13, 20.64, 20.47, 20.31 (d, $J_{C,P}$ = 3.2 Hz), 20.09, 17.65, 17.25, 16.69, 16.24 ppm. ESI-MS: m/z = 1831.7 $[M + H]^+$, 916.1 $[M + H]^{2+}$. UV/Vis (methanol): λ = 278.0, 361.1, 519.9, 551.1 nm. A full ^{1}H NMR spectrum is shown in the Supporting Information.

Synthesis of 9: Compound **9** was prepared following one of the procedures described for **8**. Full ^{1}H and ^{13}C NMR spectra are shown in the Supporting Information.

Labelling of 3 and 4 with $[^{99m}Tc(OH_2)_3(CO)_3]^+$ (10 and 11): Solutions of the precursor $[^{99m}Tc(OH_2)_3(CO)_3]^+$ were prepared from $[^{99m}TcO_4]^-$ as described in the literature or by using the Isolink® kits as commercially available from Mallinckrodt Med. B.V. Petten NL.[41] Labelling of **4** and **5** was performed in a 10-mL glass vial with a rubber stopper which was flushed with N_2 for 10 min. In this vial, a solution of the respective B_{12} derivative (20 μL, 0.001 M in water), MES buffer pH 6.2 (20 μL or 40 μL, final conc. 0.1 M) and a $[^{99m}Tc(OH_2)_3(CO)_3]^+$ solution (160 or 1940 μL) were mixed, and the reaction mixture was kept at 75 °C for 1–2 h. The final B_{12} concentration was 10^{-4} to 10^{-5} M. HPLC analysis with γ-detection was performed to verify full conversion of the ^{99m}Tc precursor. Under these conditions, the ester protecting groups of the chelators were cleaved to enable tridentate complexation. Comparison with the retention times of the corresponding rhenium complexes confirmed the authenticity of the labelled compounds. R_t (**8**) = 15.1 min, R_t (**9**) = 17.9 min. The delay is a result of the two separate detectors. Both compounds labelled with ^{99m}Tc were stable in human serum albumin for at least 24 h at 37 °C.

Supporting Information (see footnote on the first page of this article): ^{1}H NMR spectra for compounds **4**, **8**, **5** and **9** and ^{13}C NMR spectra for compounds **5** and **9**.

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[1] G. J. Russel-Jones, D. H. Alpers, *Chemistry and Biochemistry of Vitamin B_{12}*, John Wiley & Sons, New York, **1999**, pp. 411–441.
[2] B. Kräutler, D. Arigoni, B. T. Golding (Eds.), *Vitamin B_{12} and B_{12}-Proteins*, Wiley-VCH, Weinheim, **1998**.
[3] K. L. Brown, *Chem. Rev.* **2005**, *105*, 2075–2149.
[4] G. Russell-Jones, K. McTavish, J. McEwan, J. Rice, D. Nowotnik, *J. Inorg. Biochem.* **2004**, *98*, 1625–1633.
[5] R. A. Horton, J. D. Bagnato, C. B. Grissom, *J. Org. Chem.* **2003**, *68*, 7108–7111.
[6] D. A. Collins, H. P. C. Hogenkamp, *J. Nucl. Med.* **1997**, *38*, 717–723.
[7] D. A. Collins, H. P. C. Hogenkamp, M. W. Gebhard, *Mayo Clin. Proc.* **1999**, *74*, 687–691.
[8] P. M. Pathare, D. S. Wilbur, S. Heusser, E. V. Quadros, P. McLoughlin, A. C. Morgan, *Bioconjugate Chem.* **1996**, *7*, 217–232.
[9] P. M. Pathare, D. S. Wilbur, D. K. Hamlin, S. Heusser, E. V. Quadros, P. McLoughlin, A. C. Morgan, *Bioconjugate Chem.* **1997**, *8*, 161–172.
[10] D. S. Wilbur, P. M. Pathare, D. K. Hamlin, S. P. Rothenberg, E. V. Quadros, *Bioconjugate Chem.* **1999**, *10*, 912–920.
[11] D. R. van Staveren, R. Waibel, S. Mundwiler, P. A. Schubiger, R. Alberto, *J. Organomet. Chem.* **2004**, *689*, 4803–4810.
[12] D. R. van Staveren, S. Mundwiler, U. Hoffmanns, J. K. Pak, B. Spingler, N. Metzler-Nolte, R. Alberto, *Org. Biomol. Chem.* **2004**, *2*, 2593–2603.
[13] S. N. Fedosov, C. B. Grissom, N. U. Fedosova, S. K. Moestrup, E. Nexo, T. E. Petersen, *FEBS Lett.* **2006**, *273*, 4742–4753.
[14] J. M. McGreevy, M. J. Cannon, C. B. Grissom, *J. Surg. Res.* **2003**, *111*, 38–44.
[15] S. Kunze, F. Zobi, P. Kurz, B. Spingler, R. Alberto, *Angew. Chem. Int. Ed.* **2004**, *43*, 5025–5029.
[16] S. Mundwiler, B. Spingler, P. Kurz, S. Kunze, R. Alberto, *Chem. Eur. J.* **2005**, *11*, 4089–4095.
[17] C. B. Perry, K. L. Brown, X. Zou, H. A. Marques, *J. Mol. Struct.* **2005**, *737*, 245–258.
[18] D. C. Hodgkin, J. G. White, K. N. Trueblood, R. A. Sparks, J. Lindsey, *Proc. R. Soc. London, Ser. A* **1962**, *266*, 494–517.
[19] P. G. Lenhert, D. C. Hodgkin, *Nature* **1961**, *192*, 937–938.
[20] M. Rossi, J. P. Glusker, L. Randaccio, M. F. Summers, P. J. Toscano, L. G. Marzilli, *J. Am. Chem. Soc.* **1985**, *107*, 1729–1738.
[21] L. Randaccio, S. Geremia, G. Nardin, J. Wuerges, *Coord. Chem. Rev.* **2006**, *250*, 1332–1350.
[22] K. Gruber, G. Jogl, G. Klintschar, C. Kratky, "High Resolution Crystal Structures of Cobalamins" in *Vitamin B_{12} and B_{12}-Proteins* (Eds.: B. Kräutler, D. Arigoni, B. T. Golding), Wiley-VCH, Weinheim, **1998**, pp. 335–349.
[23] C. B. Perry, H. M. Marques, *S. Afr. J. Sci.* **2004**, *100*, 368–380.
[24] K. L. Brown, S. F. Cheng, X. Zou, J. D. Zubkowski, E. J. Valente, L. Knapton, H. M. Marques, *Inorg. Chem.* **1997**, *36*, 3666–3675.
[25] J. B. Armitage, J. R. Cannon, A. W. Johnson, L. F. J. Parker, E. L. Smith, W. H. Stafford, A. R. Todd, *J. Chem. Soc.* **1953**, 3849–3864.
[26] K. Bernhauer, F. Wagner, H. Beisbarth, P. Rietz, H. Vogelmann, *Biochem. Z.* **1966**, *344*, 289–309.
[27] H. M. Marques, D. C. Scooby, M. Victor, K. L. Brown, *Inorg. Chim. Acta* **1989**, *162*, 151–155.
[28] C. K. Nockolds, S. Ramasesh, D. C. Hodgkin, T. N. M. Waters, J. M. Waters, F. M. Moore, B. T. M. Willis, *Nature* **1967**, *214*, 129–130.
[29] C. E. Nockolds, S. Ramaseshan, *Proc. Indian Acad. Sci. (Chem. Sci.)* **1984**, *93*, 197–218.
[30] F. H. Moore, B. H. Oconnor, B. T. M. Willis, D. C. Hodgkin, *Proc. Indian Acad. Sci. (Chem. Sci.)* **1984**, *93*, 235–260.
[31] J. M. Waters, T. N. M. Waters, *Proc. Indian Acad. Sci. (Chem. Sci.)* **1984**, *93*, 219–234.
[32] T. G. Pagano, L. G. Marzilli, *Biochemistry* **1989**, *28*, 7213–7223.
[33] D. C. Hodgkin, *Proc. Indian Acad. Sci. (Chem. Sci.)* **1984**, *93*, 195–196.
[34] R. Alberto, R. Schibli, R. Waibel, U. Abram, A. P. Schubiger, *Coord. Chem. Rev.* **1999**, *192*, 901–919.
[35] A. Stichelberger, R. Waibel, C. Dumas, P. A. Schubiger, R. Schibli, *Nucl. Med. Biol.* **2003**, *30*, 465–470.
[36] M. Binder, J. F. Kolhouse, K. C. Vanhorne, R. H. Allen, *Anal. Biochem.* **1982**, *125*, 253–258.
[37] R. Bonnett, J. R. Cannon, V. M. Clark, A. W. Johnson, L. F. J. Parker, E. L. Smith, A. Todd, *J. Chem. Soc.* **1957**, 1158–1168.
[38] W. Kabsch, *J. Appl. Crystallogr.* **1993**, *26*, 795–800.
[39] A. Altomare, M. C. Burla, M. Camalli, G. L. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori, R. Spagna, *J. Appl. Crystallogr.* **1999**, *32*, 115–119.
[40] G. M. Sheldrick, *SHELXL97–2: Program for the Refinement of Crystal Structures*, University of Göttingen, Germany, **1997**.
[41] R. Alberto, K. Ortner, N. Wheatley, R. Schibli, A. P. Schubiger, *J. Am. Chem. Soc.* **2001**, *123*, 3135–3136.

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