Bioinorganic Chemistry

Vitamin B12 as a Ligand for Technetium and Rhenium Complexes**

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Vitamin B12 is a biomolecule that is fundamental for most living organisms despite being produced by only a few bacteria. It plays a key role in enzymatic processes in the

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mitochondria, cell nucleus, and cytoplasm. Its uptake in humans is very complex and requires at least three different transport proteins: intrinsic factor (IF), transcobalamin, and haptocorrin. The human organism uses vitamin B12 very efficiently: the daily requirement is only about 10 μg. Its chemistry, biochemistry, and biology has been comprehensively reviewed.[1–4]

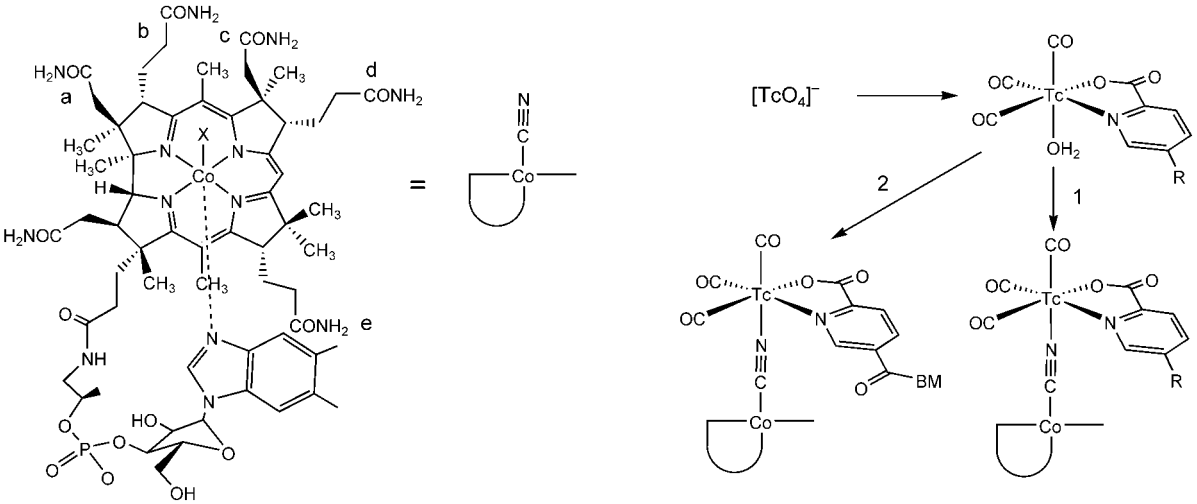
The demand for vitamin B12 (abbreviated as "B12") is concentrated at sites of enhanced proliferation, in particular in cancer cells or at sites of bacterial infections. An organism's need for B12 makes it an attractive targeting agent. Applications of B12 to the delivery of radioisotopes[5,6] or as various cytotoxic agents to cancer cells, for which it can be used as a Trojan horse, have been the most actively studied areas of research.[7–9] Both of these strategies require derivatization of B12, and so the introduction of ligands or receptor-binding molecules has been reported.[5,6,9,10] Chelators for radiometals have been conjugated to peripheral acid groups (prepared by controlled amide hydrolysis) and coordinated to the 5′-OH group in the ribose ring of the backloop. Alternatively, a Co^{III} center can be reduced to Co^{I} and a molecule introduced by oxidative alkylation.[8,11]

The only functionality in B12 that has not received much attention is the Co^{III}-coordinated cyanide group. It is well established that M–CN moieties tend to bridge two metal centers to form a M-C-N-M′ unit. Numerous examples have been published and reviewed to date,[11] but examples with porphyrin-like systems are rare[12–14] and, to the best of our knowledge, unknown for B12. The reverse situation in which $[Fe(CN)_6]^{3-}$ or nitroprusside $[Fe(NO)(CN)_5]^{-}$ are coordinated to Co^{III} or Co^{II} centers of aqua–cobalamin has been studied in detail.[15–19]

It is intriguing to use the cyanide anion in B12 as a ligand and to introduce a metal complex either by direct coordination to B12 or by conjugation of an organic molecule through mediation by a metal complex (pathways 1 and 2 in Scheme 1). Since Co-CN-M′ is not very stable, M′ represents an inert complex fragment.

Our interest lies in radiopharmaceuticals containing the *fac*-$[^{99m}Tc(CO)_3]^+$ moiety.[20–22] The water ligands are readily exchanged in $[^{99m}Tc(OH_2)_3(CO)_3]^+$, to yield complexes of high kinetic stability even with monodentate donors.[23] The concept of our study involved B12 acting as a monodentate ligand while the other two sites of *fac*-$[M(CO)_3]^+$ are bound to a bidentate ligand L^2. The complex $[^{99m}Tc(OH_2)(L^2)(CO)_3]$ is then coordinated to B12. The ligand L^2, introduced prior to B12 coordination, is variable and allows the biological authenticity of the final conjugate to be fine-tuned. The use of a bidentate and a monodentate ligand on the *fac*-$[^{99m}Tc(CO)_3]^+$ moiety is consistent with a mixed ligand approach.[24]

The reaction of $[M(OH_2)_3(CO)_3]^+$ (M = ^{99}Tc, **1**; Re, **2**) in water with the monoanionic ligands (L^2), imidazolecarboxylic acid (Himc), picolinic acid (Hpic), 2,4-dipicolinic acid (Hdipic) or serine (Hser), and *N*,*N*-dimethylglycine (Hdmg) yields $[M(L^2)(OH_2)(CO)_3]$. The corresponding ^{99m}Tc complexes are prepared directly from $[^{99m}TcO_4]^-$. The bidentate ligand is tightly bound but the remaining water ligand can be replaced by the nitrogen atom from the cyanide anion in B12. $[M(L^2)(OH_2)(CO)_3]$ exhibits a strong affinity for imidazole-type ligands but coordination to intermediately released benzimidazole from B12 was not observed. The reaction of $[Re(imc)(OH_2)(CO)_3]$ (**3**), $[Re(dipic)(OH_2)(CO)_3]$ (**4**), $[Re(ser)(OH_2)(CO)_3]$ (**5**), $[Re(dmg)(OH_2)(CO)_3]$ (**6**), or their ^{99m}Tc analogues with B12 in methanol or water afforded [(**3**–B12)] (**7**), [(**4**–B12)] (**8**), [(**5**–B12)] (**9**), and [(**6**–B12)] (**10**). High-performance liquid chromatographic (HPLC) analysis after coordination of Re or $^{99(m)}$Tc centers gave only two well-separated signals in a ratio of about 1:1. The comparable retention times from the UV/Vis analysis of, for example, **7** and the corresponding radioactivity trace of the ^{99m}Tc complex indicate the identity of the Re and Tc complexes (Figure 1). The signals can be understood by the two possible orientations adopted by the bidentate N,O ligand L^2 relative to the corrin ring ($[M(L^2)(OH_2)(CO)_3]$ complexes are racemic) and, thus, the presence of two diastereomers. The



Scheme 1. a) Structure of B12 with assignment of the side arms. b) Coordination of $[^{99(m)}Tc(OH_2)(L^2)(CO)_3]$ to B12: 1) for radiopharmaceuticals and 2) metal-mediated coupling of biomolecules (BM).

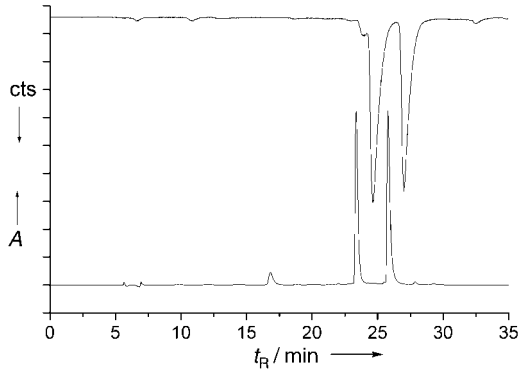


Figure 1. HPLC traces of the reaction between B12 and **3** showing the two diastereomers. Lower trace: UV/Vis spectrum at $\lambda = 360$ nm, upper trace: γ-detection of the ^{99m}Tc analogue of **7**.

two compounds could be separated by preparative HPLC and showed the same mass ($m/z = 869.1$ [M^{2+}]; $m/z = 1736.9$ [M^{+}]) in ESI-MS; ^{1}H, ^{13}C, and ^{31}P NMR spectroscopic analysis confirmed the proposed composition (see Supporting Information).

The orientation of B12 relative to the bidentate ligand was investigated by ROESY experiments, as recently performed for a (methoxycarbonyl)methyl derivative.[25] The spectra did not show cross peaks even at low-temperature, thus indicating fast rotation of the complex. Since the equatorial planes of the complexes are relatively far away from the corrin framework, they are not locked in a fixed position. For all the complexes **7**–**10**, the two isomers could be readily separated by preparative HPLC. X-ray quality crystals for one of the isomers of both **7** and **10** could be grown, and so their structures could be elucidated (Figure 2).[26]

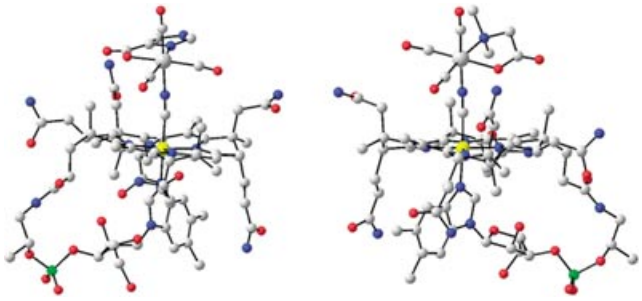


Figure 2. X-ray crystal analysis of **7** (left) and **10** (right). Important bond lengths [Å] and angles [°] are for **7**: Co-CN: 1.846(13), Co-N(benzimidazole): 2.010(8), C-N(cyanide): 1.113(14), CN-Re1A: 2.171(9), Co-C-N: 169(1), C-N-Re1A: 166(1); and for **10**: Co-CN: 1.90(1), Co-N(benzimidazole): 2.032(7), C-N(cyanide): 1.180(12), CN–Re: 2.14(1), Co-C-N: 167.7(9) , C-N-Re: 169.2(9).

The relative orientation of the N,O ligands L^2 imc in **7** and dmg in **10** are reversed for the two structures. Both show a hydrogen bridge between the terminal carbonyl oxygen O74 in **7** and the coordinating O72 atom in **10** to the amide nitrogen atom of the a-side chain in B12 (Figure 3). The bridge is shorter in **7** than in **10** and probably determines the orientation of the Re complex in the solid state. We assume

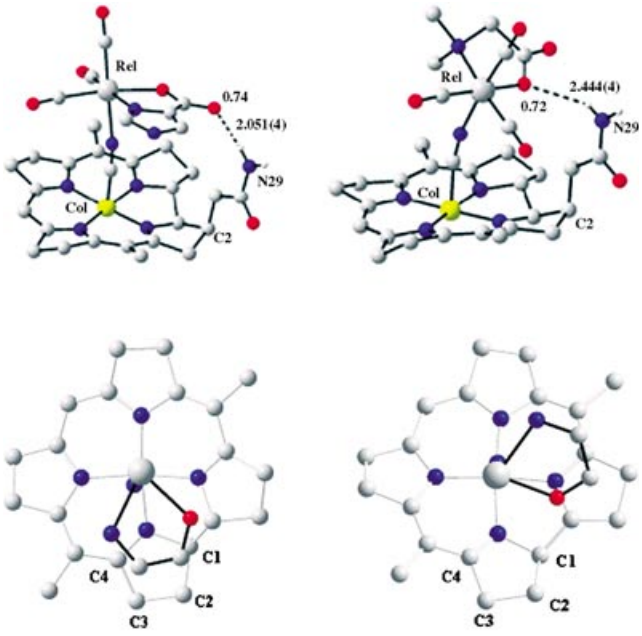


Figure 3. Hydrogen-bonding interactions in **7** and **10** (above) and orientation of the imc and dmg ligands relative to the corrin ring (below). C2 represents the carbon atom to which the a-side chain is attached.

that the respective diastereomer of each complex might be represented by the orientations as observed for **7** and **10**.

We observed that the diastereomerically pure complexes [$M(L^2)(L'^1)(CO)_3$] with one labile ligand (for example, L-serine (L^2) and guanine L'^1) always return to equilibrium in water within hours, probably through formation of a five-coordinate intermediate by dissociation of the monodentate ligand. The pure diastereomers of **7**–**10** do not interconvert into each other even after days in water at room temperature, which confirms the high kinetic stability of the monodentately bound B12. Acetonitrile is a strong ligand for complexes [$Re(L^2)(OH_2)(CO)_3$]. Dissolving **7** or **9** in a water/acetonitrile mixture did not result in cleavage of **3** from B12 in **7** and only about a 10% cleavage of **9** from **10**, which confirms again the stability of the Co-CN-Re(Tc) bonds.

The electronic spectra of the two diastereomers of **10** both have essentially the shape of B12, but the shoulder at about 482 nm in the spectrum of B12 becomes a distinct peak at 475 nm (see Suppporting Information). Furthermore, B12 and **7** behave different electrochemically. Whereas B12 exhibits a reduction wave ($E_{1/2} = -670$ mV versus Ag^+/AgCl) with about 60% reversibility, the same process with **7** ($E_{1/2} = -625$ mV) is about 80% reversible (Figure 4). The Co^{III} center in **7** is easier to reduce since coordination of the cyanide group through the nitrogen atom to the rhenium center reduces the electron density at the Co^{III} center. The increased ease of the reversibility can be understood by the more facile accommodation of the two additional electrons in the Co→C backbond. Complex **7** can be considered as an (inorganic) isocyanide complex of a Co^{III} center. Since isocyanides bind to low-oxidation states better than the cyanide anion, the Co^{I} center becomes stabilized.

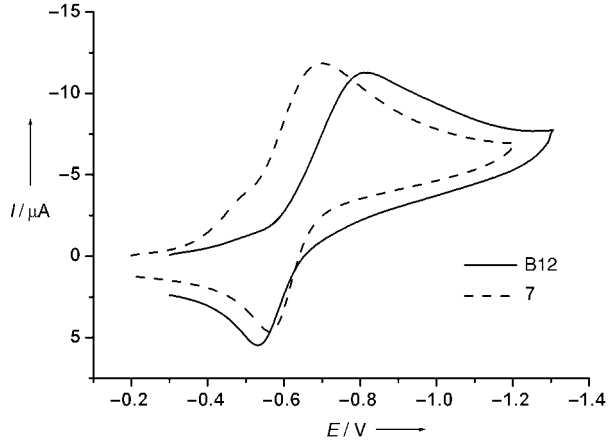


Figure 4. Cyclovoltamograms of complex **7** and B12. The shoulder results from the reduction of imc in **7**.

Biological applications of these compounds require them to be stabile in serum and have an affinity for the various B12-binding proteins. It is known that haptocorrin recognizes derivatives of B12 whereas the transcobalamins are more sensitive towards structural changes.[27–29] Derivatizations at the Co^{III} center are more readily tolerated by both proteins and direct labeling of B12 with $[^{99m}Tc(L^2)(OH_2)(CO)_3]$ is feasible. Complexes $[^{99m}Tc(L^2)(OH_2)(CO)_3]$ coordinated quantitatively to B12 between 10^{-2} and 10^{-3} M within 60 minutes at 37 °C. Once formed and separated by HPLC, the isomers were stable for at least 24 hours at 37 °C. Complex **10** was treated with human serum albumin at 37 °C (1 % in phosphate buffer) but no transmetalation to proteins could be observed. This opens a convenient way for studying the biological behavior of labeled native B12 by varying the nature of L^2. The intracellular B12-dependent enzymes might not recognize these derivatives anymore but then the radionuclide has already reached its target. We emphasize that the Re center is likely to mediate L^2-coupled biologically active molecules and B12 (for example, through the free carboxylic acid functionality in dipic).

In conclusion, we have shown that CN^- in B12 bridges to Re^I and Tc^I centers to yield robust complexes with the central structural feature {Co-CN-Re(Tc)}. This concept allows direct labeling of B12 with complexes $[^{99m}Tc(OH_2)(L^2)(CO)_3]$ for radiodiagnosis or with rhenium as a mediator between B12 and additional biomolecules. The observed kinetic stability implies that coordination of other fragments with the d^6 or d^8 configurations is also possible. The use of B12 as an enantiomerically pure and stereochemically demanding ligand provides water solubility, which could be useful for enantioselective synthesis with an appropriate catalyst. Binding studies with different B12 transporters and the coordination of other metal complexes are currently under investigation.

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Keywords: bridging ligands · cyanides · radiochemistry · technetium · vitamins

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[26] Crystallographic analysis: **7** ($C_{70}H_{106}CoN_{16}O_{28}PRe$), red plates, $0.57 \times 0.15 \times 0.04$ mm^3, orthorhombic, space group $P2_12_12_1$, $a =$ 15.958(1), $b = 21.2328(12)$, $c = 27.9776(13)$ Å, $V = 9479.6(9)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.328$ g cm^{-3}, $R_1(I \geq 2\sigma(I)) = 0.0990$, $wR_2(F^2,\ I \geq 2\sigma(I)) = 0.2370$ for 16557 independent data (10604 observed $(I \geq 2\sigma(I))$), 1026 parameters, 40 restraints, numerical absorption correction, $\mu = 1.545$ mm^{-1}, $T_{min} = 0.5508$, $T_{max} = 0.9331$, *Flack parameter* $= -0.007(11)$. **10** ($C_{73}H_{112.6}CoN_{15}O_{27.3}PRe$), red needles, $0.46 \times 0.08 \times 0.07$ mm^3, orthorhombic, space group $P2_12_12_1$, $a = 15.8758(7)$, $b = 21.845(1)$, $c = 26.3673(14)$ Å, $V =$ 9144.4(8) Å^3, $Z = 4$, $\rho_{calcd} = 1.390$ g cm^{-3}, $R_1(I \geq 2\sigma(I)) = 0.0662$, $wR_2(F^2,\ I \geq 2\sigma(I)) = 0.1588$ for 68628 collected data (17853 independent), 1071 parameters, 2 restraints, numerical absorption correction, $\mu = 1.601$ mm^{-1}, $T_{min} = 0.7060$, $T_{max} = 0.9257$, *Flack parameter* $= -0.014(8)$. CCDC-240399 (for **7**) and CCDC-240398 (for **10**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from www.ccdc.cam.ac.uk/conts/retrieving.html (or from

the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).
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