Electronic and Steric Influence of Trans Axial Base on the Stereoelectronic Properties of Cobalamins

Pawel M. Kozlowski*,† and Marek Z. Zgierski‡

Department of Chemistry, University of Louisville, Louisville, Kentucky 40292, and Steacie Institute for Molecular Science, National Research Council of Canada, Ottawa, Ontario, Canada K1A OR6

Received: May 20, 2004

Density functional theory (DFT) has been applied to investigate the relationship between steric and electronic properties of the trans axial base and energetics of Co−C bond cleavage in models of coenzyme B_{12}. By using structurally reliable six-coordinate models, B-[Co^{III}(corrin)]-R^+, it was shown that for a given base (B) the energy of homolytic cobalt−carbon bond cleavage correctly follows the Co−C_R bond lengthening. For a given axial ligand (R) the dissociation energy is very weakly dependent on the trans axial base and correlates with its basicity. Analysis of the five-coordinate homolysis products, B-[Co^{II}(corrin)]$^+$, shows that the Co^{II}−N_B bond length is in the range of ∼2.2 Å, slightly shorter in comparison to six-coordinate analogues, while five-coordinate heterolysis products, B-[Co^{III}(corrin)]$^{+2}$, have the Co^{III}−N_B bond significantly shorter ∼1.9 Å. This noticeable difference suggests that controlling the Co−N_B bond length could be an effective way to promote biologically important homolysis of the Co−C bond in B_{12}-dependent enzymes over abiological heterolysis.

Introduction

The origin of the enormous catalytic activity of coenzyme B_{12}-dependent enzymes continues to be an outstanding problem in bioinorganic chemistry.[1−9] During enzymatic catalysis the Co−C bond of coenzyme B_{12} (AdoCbl; see Figure 1) is cleaved homolytically, leading to the formation of the 5′-deoxyadenosyl radical and cob(II)alamin.[10] The rate of enzymatically accelerated homolytic cobalt−carbon bond cleavage of AdoCbl exceeds the rate observed in aqueous solution by about 12 orders of magnitude[11−13] as a consequence of the coenzyme interaction with the substrate in the presence of apoenzyme. Among factors affecting the catalytic activity of coenzyme B_{12}-dependent enzymes, involvement of the axial 5,6-dimethylbenzimidazole (DBI) base continues to be a subject of intriguing debate.[2,5,7,14−19] One of the most frequently advanced hypotheses, known as the mechanochemical corrin conformational effect theory,[20−23] recognizes the axial base as the key structural element involved in the enzymatic activation of the Co−C bond. The central point of this hypothesis is the suggestion that the enzyme induces an upward folding ["butterfly" bending] of the corrin ring via an appended bulky DBI base through compression of the Co−N_{DBI} bond. Consequently, the "butterfly" bending of the corrin ring leads to Co−C bond elongation and subsequent weakening. Despite the conceptual simplicity and popularity of the mechanochemical triggering hypothesis, there is a growing skepticism about its validity based on crystallographic, spectroscopic, and classical (i.e. purely steric) molecular mechanics calculations.[24,25]

In the free coenzyme the axial base is weakly coordinated to the cobalt, and the Co−N_{DBI} bond length is 2.214 or 2.237 Å, respectively, according to available X-ray data.[26,27] The appended axial base can be cleaved chemically at the phosphodiester group to form structurally similar adenosylcobinamide (AdoCbi$^+$) with the ability to test the binding characteristic of other structurally and electronically different ligands. Kinetic studies have demonstrated that Co−C bond homolysis of AdoCbi$^+$ is slowed only by a factor of 10^2 in comparison to AdoCbl.[14] More importantly, replacement of the axial base leads to a second competing reaction involving an internal fragmenta-

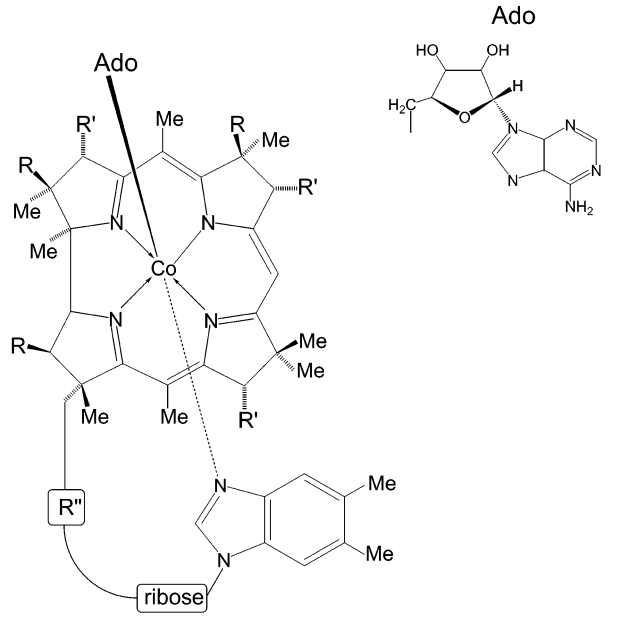


Figure 1. Molecular structure of coenzyme B_{12} (AdoCbl; 5′-deoxy-5′-adenosyl-cobalamin).

* Address correspondence to this author. Phone: (502) 852-6609. Fax: (502) 852-8149. E-mail: pawel@louisville.edu.
† University of Louisville.
‡ National Research Council of Canada.

10.1021/jp040373c CCC: $27.50
Published on Web 08/21/2004

tion of the adenosyl ligand and heterolysis of the Co−C bond.[17,18,28] The heterolysis of the Co−C bond in adenosylcobalamine is an abiological site reaction contrary to a physiological heterolysis of Co−C bond with a Co^{I} intermediate in methyl-cobalamin-dependent enzymes.[29,30] Chemical precedent studies have shown that different exogenous bases promote abiological heterolysis differently and that the axial base in the free coenzyme has a greater effect on accelerating Co−C bond heterolysis rather than homolysis.[16−18] Variations in the axial base most likely introduce changes to the $Co-N_B$ bond length, which, in response, modulates properties of the Co center. As a consequence of this change, heterolysis of Co−C is promoted over homolysis. Elucidation of how the kinetics of Co−C bond cleavage is dependent upon electronic and steric properties of the axial base, and hence the $Co-N_B$ bond length, has been an important goal in bioinorganic research of coenzyme B_{12}.[15−18,31−33] Unfortunately, a lack of crystallographic structures corresponding to corrin-based models of free B_{12} cofactor with different axial ligands renders it difficult to establish a direct correlation between kinetic data and structural parameters. Most of the information comes from spectroscopic studies, mainly UV or EPR, and thus changes observed in the $Co-N_B$ bond length rely on interpretation of these spectroscopic data. The fact that the weak $Co-N_B$ axial bond cannot be probed directly unavoidably leads to conflicting results as a consequence of the interpretation of spectroscopic data.[18,19]

When the coenzyme is bound to the apoenzyme, the status of the axial base is quite different. According to crystallographic data in a certain subclass of B_{12} enzymes, as for example in methylmalonyl CoA mutase,[34,35] the DBI base is replaced by histidine while in another subclass of B_{12} enzymes, such as diol dehydratase,[36,37] the DBI base is retained as the lower axial ligand. Furthermore, the $Co-N_{His}$ length was found to be very long (∼2.5 Å) in methylmalonyl CoA mutase,[34] and a similar bond length of 2.5 Å for $Co-N_{DBI}$ was also reported for diol dehydratase.[36] The functional significance of this trans bond lengthening is currently uncertain. It was suggested that the long $Co-N_B$ bond might stabilize the Co^{II} species relative to Co^{III}, thus favoring adenosyl radical formation. Stated differently, the goal of utilizing a weakly bound axial ligand may be to limit internal fragmentation of the adenosyl and Co−C bond heterolysis (which has never been detected in AdoCbl-utilizing enzymes) and promote the more biologically relevant homolysis of Co−C. On the other hand, limited crystallographic resolution, the mixed Co^{III}/Co^{II} states, and the mixture of conformers observed in crystal structures make interpretations of these structural data uncertain. An extended X-ray absorption fine structure (EXAFS) study showed a similar bond length of 2.45 ± 0.05 Å for methylmalonyl-CoA mutase,[38] but the accuracy of this method is limited by the presence of four other nitrogens coordinated to cobalt, which can interfere with this measurement.[39]

Involvement of DBI represents a complex problem and additional studies are needed to elucidate how electronic and steric properties of the trans axial base influence the $Co-N_B$ bond length and how variation in the cobalt−nitrogen distance, as well as displacement of the axial base, tunes the catalytic activity of coenzyme B_{12}. Since the axial $Co-N_B$ bond length is difficult to probe directly by most of the currently available experimental techniques, computational modeling, in particular density functional theory (DFT), can be useful to gather insight into the nature of cobalt−N(axial base) binding. The growing interest in modeling the structure and electronic properties of cobalamins[40−49] has demonstrated that DFT could be an important part of coenzyme B_{12} research.[50] This work represents a theoretical effort directed toward establishing the relationship between electronic and steric properties of the trans axial base and stereoelectronic properties of cobalamins. The six-coordinate models of coenzyme B_{12}, denoted as B-[Co^{III}(corrin)]-R^+ (Figure 2), have been successfully used in previous studies[40,41,43] and are employed in the current work. All reported calculations were carried out with the GAUSSIAN 98[51] suite of programs for electronic structure calculations, using the Becke−Lee−Yang−Parr composite exchange correlation functional (B3LYP) level of theory with 6-31G(d) [for H, C, and N] and Ahlrich's VTZ [for Co][52] basis sets.

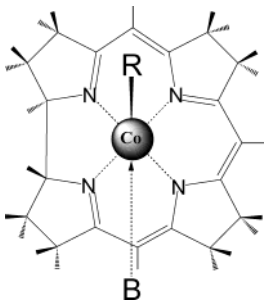


Figure 2. Molecular structure of B-[Co^{III}(corrin)]-R^+ models of coenzyme B_{12}.

Results and Discussion

1. Homolytic Co−C Bond Energy Dissociation in Cobalamins. The elucidation of factors which influence the Co−C bond dissociation energy (BDE) in coenzyme B_{12}-dependent enzymes has been one the most important aspects of B_{12} bioinorganic research. Determination of cobalt−carbon dissociation energies has been the subject of intense experimental scrutiny mainly in the Halpern[10,31−33,53−55] and Finke[12,14,56,57]-laboratories, but only recently has this issue been addressed quantum mechanically.[43,45,47−49] Computationally, the energy of homolytic cleavage of the $Co-C_R$ bond in B-[Co^{III}(corrin)]-R^+ models of coenzyme B_{12} was estimated as

$$\mathrm{BDE} = \text{B-[Co}^{\mathrm{III}}\text{(corrin)]-R}^{+}{}_{\mathrm{opt}} - \text{B-[Co}^{\mathrm{II}}\text{(corrin)]}^{+}{}_{\mathrm{opt}} - {}^{\bullet}\mathrm{R}_{\mathrm{opt}} \quad (1)$$

where the subscript "opt" denotes the energy of the optimized structure. In the present work two different trans axial bases of biological importance (B = DBI or Im) were considered. Each base was combined with five different β axial ligands (R) and for each B−R combination the $Co-C_R$ BDE energy was computed according to the above equation. The results of these calculations indicate that the DBI ↔ Im interchange of the trans axial base has a very small influence on the energy of the $Co-C_R$ bond homolysis (Table 1). Interestingly, these DFT calculations show a small but consistent increase of $Co-C_R$ BDE in Im-[Co^{III}(corrin)]-R^+ relative to DBI-[Co^{III}(corrin)]-R^+, suggesting the possibility of a small steric effect of the axial base on the homolytic dissociation of the cobalt−carbon bond. For example, in the case of Me, the difference between Im and DBI is 2.4 kcal/mol while in the case of Rib the difference is only 1 kcal/mol.

The lowering of the dissociation energy correctly follows the $Co-C_R$ bond lengthening. These energies diminish in the order Me > Rib > Et > iProp > tBut, consistent with the changes in the $Co-C_R$ bond length, and the correlation is essentially linear (Figure 3). To simplify computational effort the 5′-deoxyadeosyl group (Ado) was replaced by 5′-deoxy-

TABLE 1: DFT-Optimized Co−C_R and Co−N_B Bond Lengths (Å), Total Energies (au), and Co−C_R BDEs (kcal/mol) of B-[Co^{III}(corrin)]-R^+

B	R	Co−C_R	Co−N_B	B-[Co^{III}(corrin)]-R^+	B-[Co^{II}(corrin)]$^+$	Co−C_R BDEs[a,b]
Im	Me	1.962	2.218	−2604.515610	−39.837518	28.0
	Rib	1.989	2.237	−2986.181047	−421.508382	24.6
	Et	1.989	2.261	−2643.824691	−79.156385	21.9
	iProp	2.033	2.325	−2683.131915	−118.475958	14.1
	tBut	2.103	2.418	−2722.434337	−157.795393	3.4
DBI	Me	1.962	2.293	−2836.791675	−39.837518	25.6
	Rib	1.989	2.341	−3218.459372	−421.508382	23.6
	Et	1.990	2.358	−2876.100752	−79.156385	19.5
	iProp	2.027	2.586	−2915.410071	−118.475958	13.0
	tBut	2.091	2.841	−2954.713741	−157.795393	3.1

[a] DFT energy of optimized Im-[Co^{II}(corrin)]$^+$ = −2564.633477 au. [b] DFT energy of optimized DBI-[Co^{II}(corrin)]$^+$ = −2796.913370 au.

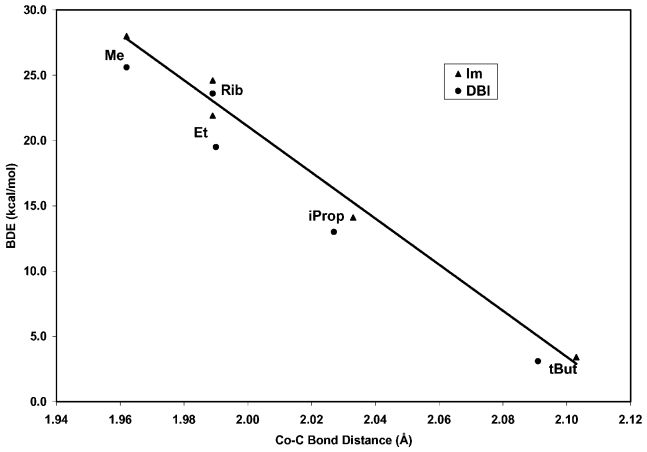


Figure 3. Theoretically determined BDEs plotted as a function of the Co−C_R bond length.

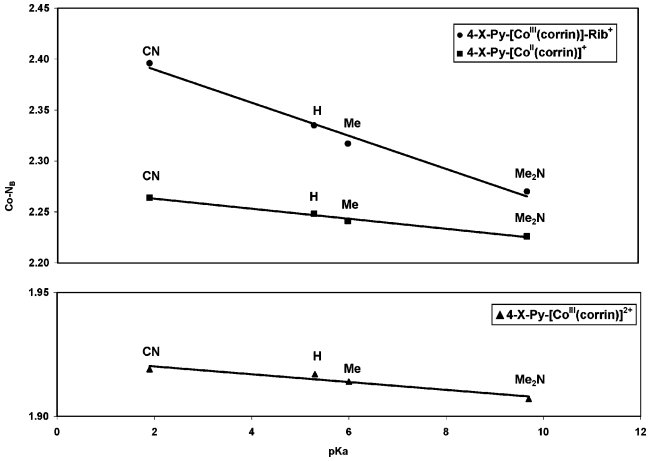


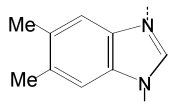
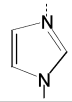
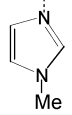
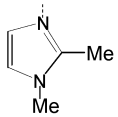
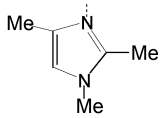
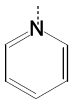
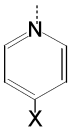
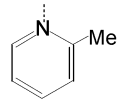
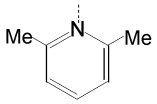
Figure 4. Theoretically determined Co−N_B bond distances plotted as a function of pK_a.

ribosyl (Rib). As previously shown, the use of Rib as a model for Ado is reasonable[45−47] and will be used throughout the present study. Although the trend shown in Figure 3 is correctly captured by DFT calculations, computed energies of homolytic Co−C_R bond cleavage are too low in comparison with experiment.[43] For MeCbl a theoretical value of 25.6 kcal/mol was found in comparison to experimental values of 37 ± 3 kcal/mol[56,57] or 36 kcal/mol[58] whereas for AdoCbl, an experimental energy of 31.4 ± 1.5 kcal/mol[13] was estimated at 23.6 kcal/mol computationally (Table 1). The underestimation of the Co−C_R BDEs based upon a DFT approach was also found in other theoretical studies[45,47,49] and the source of the discrepancy between theoretical and experimental values for the Co−C_R bond dissociation energy is still not fully understood. In recent work Jensen and Ryde[48] investigated why previously published B3LYP estimations of the BDE for methylcobalamin have given such poor results and provided systematic analysis of possible BDE underestimation including factors such as zero point energy vibrational correction (Δ_{ZPE}), basis set superposition error (Δ_{BSSE}), solvent, or relativistic effects. They concluded that these factors have only secondary importance and that the problem seems to be the B3LYP functional. Clearly this issue still requires further analysis since the B3LYP functional is widely recognized as one of the most accurate for structures, energies, and frequencies as has been demonstrated for many models of metalloproteins.[59−61]

2. Electronic and Steric Factors Influencing the Co−N_B Bond Length. To elucidate how electronic and steric factors of the trans axial base (B) affect the Co−N_B bond length, eleven different complexes of B-[Co^{III}(corrin)]-Rib^+ were fully optimized and the results scrutinized with respect to the optimized structural parameters. The α-trans ligand was modeled by a series of bases ranging from DBI through different imidazoles and pyridines including sterically encumbered bases as well as electronically different para-substituted pyridines, while the β ligand was kept unchanged (Rib). Among the eleven investigated bases four of them do not form a stable bond with cobalt (Table 2). These four bases are sterically encumbered axial ligands and include 1,2-dimethylimidazole (1,2-Me_2Im), 1,2,4-trimethylimidazole (1,2,4-Me_3Im), 2-methylpyridine (2-MePy or 2-picoline), and 2,6-dimethylpyridine (2,6-Me_2Py or 2,6-lutidine). This DFT prediction is consistent with K_{assoc} measurements carried out with use of visible spectroscopy.[18] The low values reported for three sterically hindered bases (1,2-Me_2Im, 2-MePy, and 2,6-Me_2Py) were indicative that none of them bind to $AdoCbi^+$ at 25 °C to a detectable degree.

The remaining seven bases coordinate to the cobalt ion and the axial Co−N_B bond length correlates with pK_a (Table 2). The immediate conclusion that can be reached from the results of these calculations is that the stronger the basic character of the trans base, the stronger the bond it forms with the corrin. This is particularly evident for series of isosteric, but electronically different, para-substituted pyridines. A wide range of investigated pyridines with different basicity, starting from weak 4-CN-Py (pK_a = 1.9), through Py and 4-Me-Py, with pK_a values of 5.3 and 6.0, respectively, to strong 4-Me_2N-Py (pK_a = 9.7), demonstrated a linear correlation between the pK_a and the Co^{III}−N_B bond length (Figure 4). The Co^{III}−N_{4-X-Py} bond length decreases in 4-X-Py-[Co^{III}(corrin)]-Rib^+ models as the donor ability of 4-X-Py increases in the order 4-CN-Py < Py < 4-Me-Py < 4-Me_2N-Py. The line describing this correspondence has a slope of -1.612×10^{-2} and an intercept of 2.422 Å, with a correlation factor being equal to 0.9932. Nearly the same bond length is predicted for Co−N_{DBI} (2.341 Å) and Co−N_{Py} (2.341 Å) consistent with their basicity since both bases

TABLE 2: DFT-Optimized Co−N_B Bond Lengths in B-[Co^{III}(corrin)]-Rib^+ (Å) together with Corresponding Five-Coordinate Homolysis and Heterolysis Products, Respectively. NB Indicates That Trans Axial Base Is Not Part of the Co Coordination Sphere

B		pK_a	B-[Co^{III}(corrin)]-Rib^+	AdoCo(III)Cbi[a,b]	B-[Co^{II}(corrin)]$^+$	B-[Co^{III}(corrin)]$^{2+}$	Co(III)Cbi[a]
		5.6	2.341	(2.240)	2.251	1.908	—
		7.2	2.237	—	2.213	1.892	—
		7.3	2.223	2.098 (2.22)	2.212	1.890	2.090
		7.9	NB	2.132 (2.25)	(2.329)		2.129
			NB	2.19	NB	NB	—
		5.3	2.335	2.114 (2.23)	2.248	1.917	2.111
	X = CN	1.9	2.396	—	2.264	1.919	—
	X = Me	6.0	2.317	—	2.241	1.914	—
	X = NMe_2	9.7	2.270	—	2.226	1.907	—
		6.0	NB	2.163 (2.29)	NB	NB	2.150
		6.6	NB	2.233 (2.37)	NB	NB	2.193

[a] UFF/MM optimized Co−N(axial base) bond lengths from Sirovatka, J. M.; Finke, R. G. *Inorg. Chem.* **1999**, *38*, 1697. [b] In parentheses, UFF/MM optimized Co−N(axial base) bond lengths from Sirovatka, J. M.; Rappe, A. K.; Finke, R. G. *Inorg. Chem. Acta* **2000**, *300*−*302*, 545.

have similar pK_a values, despite the fact that both ligands are sterically quite different. These bond lengths are approximately 0.1 Å longer in comparison to imidazole (Co−N_{Im} = 2.237 Å) or *N*-methylimidazole (Co−N_{N-MeIm} = 2.223 Å), reflecting their higher pK_a values of 7.2 and 7.3, respectively.

The available crystallographic data for AdoCbl[27] allow us to make direct comparison only for the DBI-[Co^{III}(corrin)]-Rib^+ model. The DFT-optimized Co−N_{DBI} bond length of 2.341 Å is about 0.1 Å longer than the experimental value of 2.237 Å. Other authors have noticed similar overestimations of this parameter in studies employing DFT approaches: Jensen et al.[42] obtained 2.335 Å, while Dölker et al.[45] found even a longer value of 2.447 Å. The difference with experiment may be related to the fact that these models do not possess a sidearm, but a more plausible source of discrepancy most likely reflects the shallowness of the potential energy surface associated with the Co−N_{DBI} bond. One has to remember that these models were optimized in vacuo and compared to crystallographic data, which were obtained in the presence of crystal packing forces.

To examine how the change of the cobalt oxidation state from Co^{III} to Co^{II} affects the Co−N_B bond and how structures of homolysis and heterolysis five-coordinate species differ in comparison to six-coordinated analogues, we carried out similar structural analyses for B-[Co^{II}(corrin)]$^+$ and B-[Co^{III}(corrin)]$^{2+}$ models using the same set of axial bases (B). The results of geometry optimization were consistent with six-coordinate B-[Co^{III}(corrin)]-Rib^+ models (with 1,2-Me_2Im being an exception) as summarized in Table 2. The resulting Co^{III}−N_B bond lengths computed for B-[Co^{III}(corrin)]$^{2+}$ were found to be significantly shorter, in the range of 1.9 Å, consistent with recent theoretical work of Dölker et al.[47] The consequence of these findings will be discussed separately in a following section. The DFT-optimized Co−N_B bond lengths are different from UFF molecular mechanics (UFF/MM) calculations reported by Finke

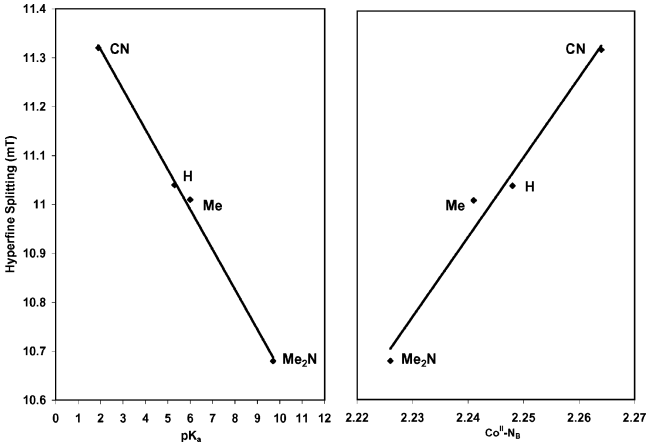


Figure 5. Plot of EPR hyperfine splitting as a function of $Co^{II}-N_B$. The values of hyperfine splitting (left panel) were taken from ref 19. Correlation with cobalt(II)−nitrogen distances (right panel) is based on present work.

and co-workers[18,24] for [AdoCbl·bulky base]$^+$ systems. The current DFT analyses do not predict binding nor the presence of a long $Co-N_B$ bond for any sterically hindered bases, contrary to the UFF/MM approach where computed bond lengths are in the range of 2.1322−2.233 Å (Table 2). In terms of absolute values the DFT-optimized $Co-N_{DBI}$ bond length is 2.341 Å and longer than the UFF/MM $Co-N_{DBI}$ predicted bond length of 2.233 Å. In general, the UFF/MM values for Co−N(axial base) bond distances are too short in comparison to those resulting from DFT analyses.

According to B3LYP calculations, four sterically hindered ligands do not bind to the corrin while the remaining seven possess shorter $Co^{II}-N_B$ bond lengths, ∼2.2 Å on average, and the difference is in the range of 0.01−0.1 Å in comparison to $Co^{III}-N_B$ analogues. The exception is the 1,2-Me_2Im base for which a much longer bond of 2.329 Å was found. As one might expect, the $Co^{II}-N_B$ lengths are shorter in comparison to $Co^{III}-N_B$ due to the absence of the β ligand. This is consistent with crystallographic data for cob(II)alamin.[62,63] The range of $Co^{II}-N_B$ bond lengths is not as large as these for $Co^{III}-N_B$, but a similar dependence on basicity is maintained. This is best illustrated by the series of electronically different para-substituted pyridines where correlation between pK_a and $Co^{II}-N_B$ is linear. As for the six-coordinate analogues, the $Co^{II}-N_{4-X-Py}$ bond length decreases in 4-X-Py-[Co^{II}(corrin)]$^+$ as the donor ability of 4-X-Py increases (Table 2). The line describing this correspondence for 4-X-Py-[Co^{II}(corrin)]$^+$ has a lower slope of -4.53×10^{-3} in comparison to 4-X-Py-[Co^{II}(corrin)-Rib$^+$, with an intercept of 2.270 Å and a correlation factor of 0.9941 (Figure 4). These findings for $Co^{II}-N_B$ bond lengths agree nicely with recent electron paramagnetic resonance spectroscopy results for cobinamide B_{12} models published by Marzilli and co-workers.[19] These authors have shown that no formation of adducts, and hence no presence of a hyper-long $Co^{II}-N$ bond, took place for 2-picoline and 2,6-lutidine. They demonstrated that hyperfine splitting of 4-X-PyCo(II)Cbi$^+$ behaves as a linear function of pK_a, which increases as the donor ability of 4-X-Py decreases in the following order: 4-Me_2N-Py > 4-Me-Py > Py > 4-CN-Py. This correlation between the axial ligand pK_a and the Co^{II} hyperfine coupling constant reflects the $Co^{II}-N_{4-X-Py}$ bond length dependence as a function of pK_a (Figure 5a). The knowledge of these two correlations allows for the expression of hyperfine splitting solely as a function of $Co^{II}-N_{4-X-Py}$ distance as shown on Figure 5b. According to this graph the decrease of hyperfine splitting by 0.6 mT could be

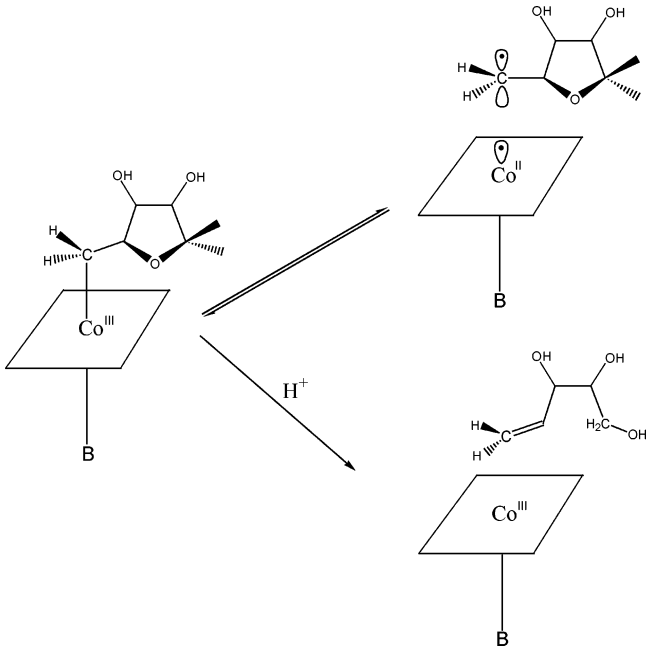


Figure 6. Computer model of homolytic vs heterolytic cleavage of the Co−C bond.

associated with a rather small $Co^{II}-N$ bond length change of approximately 0.04 Å.

3. Structural Changes Associated with Co−C Bond Homolysis and Heterolysis. Considerable experimental efforts has been directed toward the understanding of how coenzyme B_{12}-dependent enzymes limit Co−C bond heterolysis, and promote the more biologically relevant homolysis in enzymatic reactions. As pointed out in the Introduction, the heterolysis of the Co−C bond in adenosyl-cobalamine is an abiological site reaction contrary to a physiological heterolysis of the Co−C bond with a Co^I intermediate in methyl-cobalamin-dependent enzymes. The heterolysis of the Co−C bond, which involves an internal fragmentation of the Ado ligand leaving the Co^{III}Cbl center, has never been detected in enzymes employing coenzyme B_{12} contrary to the free cofactor, where both reactions take place in a competing manner.[17] The illumination of structural factors that prevent heterolysis and those that promote homolysis is an important goal in the field of B_{12} chemistry.[17−19]

For the purpose of computational studies the homolytic vs heterolytic cleavage of the $Co-C_{Rib}$ bond was defined as the process shown in Figure 6. A point of particular interest is the change of the cobalt−nitrogen bond length upon homolytic cleavage,

$$\Delta(\text{Co-N}_B) \text{ for B-[Co}^{III}\text{(corrin)]-Rib}^+ \rightarrow \text{B-[Co}^{II}\text{(corrin)]}^+ \quad (2)$$

as well as upon heterolytic cleavage,

$$\Delta(\text{Co-N}_B) \text{ for B-[Co}^{III}\text{(corrin)]-Rib}^+ \rightarrow \text{B-[Co}^{III}\text{(corrin)]}^{+2} \quad (3)$$

To illustrate the magnitude of these changes we calculated the cobalt−nitrogen bond length differences in both cases (bond length compression) with respect to the corresponding length in six-coordinate models (Figure 7). In the case of homolytic cleavage the change is 0.01−0.13 Å, consistent with the available X-ray data for cob(II)alamin,[62,63] where the length of the axial $Co-N_{DBI}$ (2.13 Å) is shorter by ∼0.1 Å in comparison

TABLE 3: DFT-Optimized Co−N_B and Co−C_{Rib} Bond Lengths (Å), Total Energies (au), and Co−C_{Rib} BDEs (kcal/mol) of B-[Co^{III}(corrin)]-Rib^+

	B	$Co^{III}-N_B$	$Co-C_{Rib}$	B-[Co^{III}(corrin)]-Rib^+	$Co^{II}-N$	B-[Co^{II}(corrin)]$^+$	$Co-C_{Rib}$ BDE[a]
1	N-MeIm	2.223	1.991	−3025.494561	2.212	−2603.946164	25.1
2	Im	2.237	1.989	−2986.181047	2.213	−2564.633477	24.6
3	4-Me_2N−Py	2.270	1.984	−3142.222999	2.226	−2720.676315	24.0
4	4-Me-Py	2.317	1.990	−3047.565747	2.241	−2626.019307	23.9
5	Py	2.335	1.988	−3008.245321	2.248	−2586.698934	23.9
6	DBI	2.341	1.989	−3218.459372	2.251	−2796.913370	23.6
7	4-CN-Py	2.396	1.984	−3100.474087	2.264	−2678.928357	23.4

[a] DFT energy of optimized 5′-deoxyribosyl radical •Rib = −421.508382014 au.

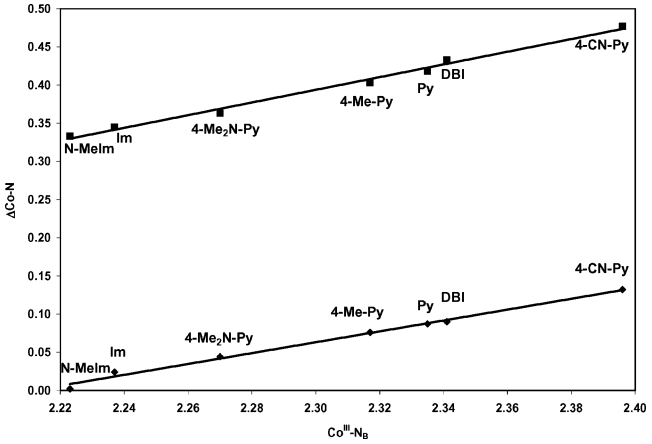


Figure 7. Theoretically determined changes in Co−N_B bond lengths during homolytic cleavage (lower line, eq 2) and heterolytic cleavage (upper line, eq 3).

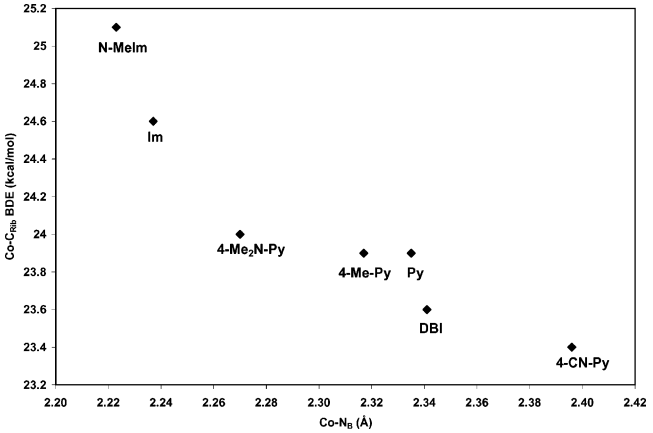


Figure 8. Theoretically determined BDEs plotted as a function of the $Co^{III}-N_B$ bond length.

to that of coenzyme B_{12} (2.237 Å).[27] The difference increases for longer bonds since the axial base becomes more tightly bonded to corrin, but does not exceed a value of 0.1 Å. The most interesting observation is the significant change of the Co−N_B bond length in the case of heterolytic cleavage. DFT calculations predict that the magnitudes of these changes are around 0.33−0.46 Å. The longer the bond in six-coordinate species, the more the shortening is observed upon heterolysis of the Co−C_{Rib} bond. These two lines are nearly parallel but shifted by about 0.32 Å with respect to each other. Lack of structural data for heterolysis intermediates prevents a direct comparison of calculated values with experiment.

4. Theoretical Analysis of Axial Base Influence on Co−C Bond Homolysis. The optimized structural models of coenzyme B_{12} (Table 2) allow for a more thorough theoretical analysis of Co−C BDEs, particularly with respect to changes in the Co−N_B bond length. Seven structural models were selected from the previous analysis (Table 2) and used to compute energetics of homolytic cobalt−carbon bond cleavage according to eq 1. The results of these calculations are listed in Table 3 where the computed Co−C_{Rib} BDEs have been arranged according to increasing values of the Co−N_B length. Consistent with the previous analysis (see Section 1), variation of the trans axial base has very little effect on the Co−C_{Rib} bond length. The plot of BDEs as a function of Co−N_B shows monotonic decay, which levels off for longer cobalt−nitrogen distances (Figure 8). This trend is consistent with the expectation that the axial base should have noticeable influence when the cobal−N(axial base) distances are short but negligible influence with increased bond length. Indeed, the biggest change, which does not exceed 1.2 kcal/mol, is observed in the 2.22−2.30 Å range and is only 0.4 kcal/mol for longer 2.30−2.40 Å distances. The small decrease of dissociation energies reflects the effect of the trans axial base, and it cannot be associated with the change in the cobalt−carbon bond length (Table 3). Interestingly, the arrangement of the DBI ligand (entry 6 in Table 3) with respect to both Py and 4-CN-Py is indicative that electronic effects dominate rather than steric effects. Thus, the small lowering of the dissociation energy is mainly due to the electronic influence of the trans axial base.

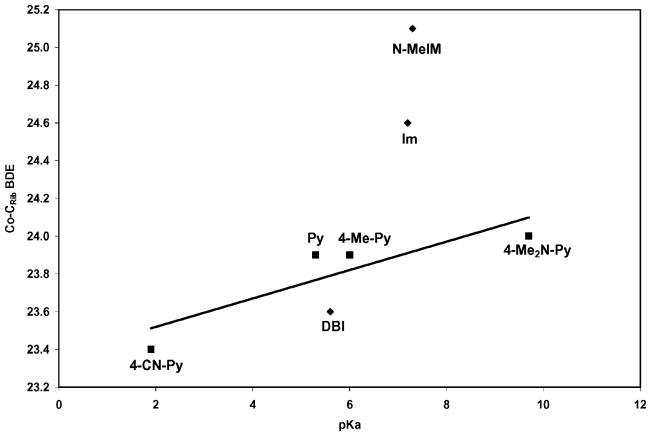


Figure 9. Theoretically determined BDEs plotted as a function of pK_a. The linear correlation is only observed for isosteric para-substituted pyridines.

To further investigate how these dissociation energies depend on the axial base we plotted Co−C_{Rib} BDEs as function of pK_a (Figure 9). The increase of Co−C_{Rib} BDEs correlates with the basicity (pK_a), as is particularly evident for the series of electronically different but isosteric para-substituted pyridines for which the correlation is nearly linear. If one excludes the N-MeIm, Im, and DBI points from Figure 9, the dependence of the Co−C_{Rib} bond dissociation energy of B-[Co^{III}(corrin)]-Rib^+ on the pK_a of B is the same one found by Halpern and co-workers[31,55] in their studies of ligand effects on transition-metal-alkyl bond dissociation energies. Although they studied a series of α-phenylethyl(L)cobaloxomines (where L is a para-substituted pyridine), the correlation found in the present studies

is of the same nature including even the deviation of BDE for B = Im from line correlation describing isosteric pyridines.[31]

Summary and Conclusions

This work represents a computational effort directed toward the elucidation of the relationship between the N-donor axial base and the catalytic activity of coenzyme B_{12}. Using structurally reliable models of coenzyme B_{12}, B-[Co^{III}(corrin)]-R^+, we have shown that for a given B the energy of homolytic cobalt−carbon bond cleavage correctly follows the $Co-C_R$ bond lengthening, while for a given R the dissociation energy is very weakly dependent on the trans axial base and correlates with its basicity.

The most important result of present DFT analysis is the finding that the five-coordinate homolysis products, B-[Co^{II}(corrin)]$^+$, have the $Co^{II}-N_B$ bond length in the range of ∼2.2 Å, slightly shorter in comparison to six-coordinate analogues, while five-coordinate heterolysis products, B-[Co^{III}(corrin)]$^{+2}$, have the $Co^{III}-N_B$ bond significantly shorter, ∼1.9 Å. These predicted changes in $Co-N_B$ bond lengths are in agreement with other theoretical studies,[46,47] though different strategy was applied to obtain these results. The noticeable difference in the $Co-N_{axial}$ bond lengths between homolysis and heterolysis products points out that controlling the $Co-N_{axial}$ length may be important primarily to inhibit the Co−C bond heterolysis in AdoCbl-dependent enzymes. The protein may inhibit a heterolytic pathway by restraining the $Co-N_{axial}$ bond to be longer than the optimum distance. This hypothesis is consistent with a "long" $Co-N_{axial}$ bond reported in recent crystallographic studies of coenzyme B_{12}-dependent enzymes.[34−37] However, our recent combined DFT/MM calculations do not support the existence of a "long" $Co-N_{axial}$ bond but rather predict a "normal" bond, which is labilized.[64] Whether the labilization of the $Co-N_{axial}$ bond has a functional implication to inhibit abiological heterolysis requires more advanced computational modeling in which the presence of the protein environment needs to be taken into account.

Note Added in Proof. An updated version of ref 18 entitled "Adenosylcobinamide Plus Exogenous, Sterically Hindered, Putative Axial Bases: A Reinvestigation into the Cause of Record Levels of Co−C Heterolysis" by K. M. Doll and R. G. Finke (*Inorg. Chem.* **2004**, *43*, 2611−2623) has appeared while this paper was in press. One of us (P.M.K.) would like to acknowledge Prof. R. G. Finke for personal communication regarding this new work.

References and Notes

(1) Dolphin, D., Ed. *B_{12}*; Wiley-Interscience: New York, 1982.
(2) Marzilli, L. G. In *Bioinorganic Catalysis*; Reedijk, J., Ed.; Marcel Dekker: New York, 1993; pp 227−259.
(3) Banerjee, R. *Chem. Biol.* **1997**, *4*, 175.
(4) Ludwig, M. L.; Matthews, R. G. *Annu. Rev. Biochem.* **1997**, *66*, 269.
(5) *Vitamin B_{12} and B_{12} Proteins*; Kräutler, B., Arigoni, D., Golding, B. T., Eds.; Wiley-VCH: New York, 1998.
(6) Banerjee, R. *Chemistry and Biochemistry of B_{12}*; John Wiley & Sons: New York, 1999.
(7) Banerjee, R. *Biochemistry* **2001**, *40*, 6191.
(8) Toraya, T. *Chem. Rev.* **2003**, *103*, 2095.
(9) Banerjee, R. *Chem. Rev.* **2003**, *103*, 2083.
(10) Halpern, J. *Science* **1985**, *227*, 869.
(11) Finke, R. G.; Hay, B. P. *Inorg. Chem.* **1984**, *23*, 3031.
(12) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 4820.
(13) Finke, R. G. In *Vitamin B_{12} and B_{12} Proteins*, Lectures presented at the 4th European Symposium on Vitamin B_{12} and B_{12} Proteins; Kraütler B., Arigoni, D., Golding, B. T., Eds.; Wiley-VCH: Weinheim, Germany, 1998; Chapter 25.
(14) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 8012.
(15) Garr, C. D.; Sirovatka, J. M.; Finke, R. G. *Inorg. Chem.* **1996**, *35*, 5912.
(16) Garr, C. D.; Sirovatka, J. M.; Finke, R. G. *J. Am. Chem. Soc.* **1996**, *118*, 11142.
(17) Sirovatka, J. M.; Finke, R. G. *J. Am. Chem. Soc.* **1997**, *119*, 3057.
(18) Sirovatka, J. M.; Finke, R. G. *Inorg. Chem.* **1999**, *38*, 1697. Sirovatka, J. M.; Finke, R. G. *Inorg. Chem.* **2001**, *40*, 1082.
(19) Trommel, J. S.; Warncke, K.; Marzilli, L. G. *J. Am. Chem. Soc.* **2001**, *123*, 3358.
(20) Grate, J. H.; Schrauzer, G. N. *J. Am. Chem. Soc.* **1979**, *101*, 4601.
(21) Schrauzer, G. N.; Grate, J. H. *J. Am. Chem. Soc.* **1981**, *103*, 541.
(22) Marzilli, L. G.; Toscano, P. J.; Rndaccio, L.; Bresciani-Pahor, N.; Calligaris, M. *J. Am. Chem. Soc.* **1979**, *101*, 6754.
(23) Bresciani-Pahor, N.; Forcolin, M.; Marzilli, L. G.; Randaccio, L.; Summers, M. F.; Toscano, P. J. *Coord. Chem. Rev.* **1985**, *63*, 1.
(24) Sirovatka, J. M.; Rappé, A. K.; Finke, R. G. *Inorg. Chim. Acta* **2000**, *300*−*302*, 545.
(25) Brown, K. L.; Marques, H. M. *J. Inorg. Biochem.* **2001**, *83*, 121.
(26) Lenhert, P. G. *Proc. R. Soc. A* **1968**, *45*, 303.
(27) Ouyang, L.; Rulis, P.; Ching, W. Y.; Nordin, G.; Randaccio, L. *Inorg. Chem.* **2004**, *43*, 1235.
(28) Jensen, J. M.; Halpern, J. *J. Am. Chem. Soc.* **1999**, *121*, 2181.
(29) Mathews, R. G. *Acc. Chem. Res.* **2001**, *34*, 681.
(30) The focus in this paper is only on abiological heterolysis of the Co−C bond relevant to adenosyl-cobalamine dependent enzymes.
(31) Ng, F. T. T.; Rempel, G. L.; Halpern, J. *J. Am. Chem. Soc.* **1982**, *104*, 621.
(32) Geno, M. K.; Halpern, J. *J. Am. Chem. Soc.* **1987**, *109*, 1238.
(33) Ng, F. T. T.; Rempel, G. L.; Mancuso, C.; Halpern, J. *Organometallics* **1990**, *9*, 2762.
(34) Mancia, F.; Keep, N. H.; Nakagawa, A.; Leadlay, P. F.; McSweeny, S.; Rasmussen, B.; Bösecke; Diat, O.; Evans, P. R. *Structure* **1996**, *4*, 339.
(35) Mancia, F.; Evans, P. R. *Structure* **1998**, *6*, 711.
(36) Shibata, N.; Masuda, J.; Tobimatsu, T.; Toraya, T.; Suto, K.; Morimoto, Y.; Yasuoka, N. *Structure* **1999**, *7*, 997.
(37) Masuda, J.; Shibata, N.; Morimoto, Y.; Toraya, T.; Yasuoka, N. *Structure* **2000**, *8*, 775.
(38) Scheuring, E.; Padmakumar, R.; Banerjee, R.; Chance, M. R. *J. Am. Chem. Soc.* **1997**, *119*, 12192.
(39) Champloy, F.; Jogl, G.; Reitzer, R.; Buckel, W.; Bothe, H.; Beatrix, B.; Broeker, G.; Michalowicz, A.; Meyer-Klaucke, W.; Kratky, C. *J. Am. Chem. Soc.* **1999**, *121*, 11780.
(40) Andruniow, T.; Zgierski, M. Z.; Kozlowski, P. M. *Chem. Phys. Lett.* **2000**, *331*, 509.
(41) Andruniow, T.; Zgierski, M. Z.; Kozlowski, P. M. *J. Phys. Chem. B* **2000**, *104*, 10921.
(42) Jensen, K. P.; Sauer, S. P. A.; Liljefors, T.; Norrby, P.-O. *Organometallics* **2001**, *20*, 550.
(43) Andruniow, T.; Zgierski, M. Z.; Kozlowski, P. M. *J. Am. Chem. Soc.* **2001**, *123*, 2679.
(44) Rovira, C.; Kunc K.; Hutter J.; Parrinello M. *Inorg. Chem.* **2001**, *40*, 11.
(45) Dölker, N.; Maseras, F.; Lledos A. *J. Phys. Chem. B* **2001**, *105*, 7564.
(46) Jensen, K. P.; Ryde, U. *J. Mol. Struct. (THEOCHEM)* **2002**, *585*, 239.
(47) Dölker, N.; Maseras, F.; Lledos, A. *J. Phys. Chem. B* **2003**, *107*, 306.
(48) Jensen, K. P.; Ryde, U. *J. Phys. Chem. A* **2003**, *107*, 7539−7545.
(49) Dölker, N.; Maseras.; Siegbahn, P. E. M. *Chem. Phys. Lett.* **2004**, *386*, 174−178.
(50) Kozlowski, P. M. *Curr. Opin. Chem. Biol.* **2001**, *5*, 736.
(51) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Johnson, P. B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, Revision A.7; Gaussian, Inc.: Pittsburgh, PA, 1998.
(52) Schaefer, A.; Horn, H.; Ahlrichs, R. *J. Chem. Phys.* **1992**, *97*, 2571.
(53) Halpern, J.; Ng F. T. T.; Rempel G. L *J. Am. Chem. Soc.* **1979**, *101*, 7124.
(54) Halpern, J.; Kim, S.-H.; Leung, T. W. *J. Am. Chem. Soc.* **1984**, *106*, 8317.
(55) Halpern, J. *Polyhedron* **1988**, *7*, 1483.
(56) Martin, B. M.; Finke, R. G. *J. Am. Chem. Soc.* **1990**, *112*, 2419.
(57) Martin, B. M.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 585.

(58) Hung, R. R.; Grabowski, J. J. *J. Am. Chem. Soc.* **1999**, *121*, 1359.
(59) Siegbahn, P. E. M.; Blomberg, M. R. A. *Chem. Rev.* **2000**, *100*, 421.
(60) Blomberg, M. R. A.; Siegbahn, P. E. M. *J. Phys. Chem.* **2001**, *105*, 9375.
(61) Himo, F.; Siegbahn, P. E. M. *Chem. Rev.* **2003**, *103*, 2421.
(62) Kräutler, B.; Keller W.; Kratky, C. *J. Am. Chem. Soc.* **1989**, *111*, 8936.
(63) Kräutler, B. In *Vitamin B_{12} and B_{12} Proteins*; Kräutler, B., Arigoni, D., Golding, B. T., Eds.; Wiley-Interscience: New York, 1998; p 12.
(64) Freindorf, M.; Kozlowski, P. M. *J. Am. Chem. Soc.* **2004**, *126*, 1928.