# Coenzyme $B_{12}$ Chemical Precedent Studies: Probing the Role of the Imidazole Base-on Motif Found in $B_{12}$-Dependent Methylmalonyl-CoA Mutase

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**Abstract:** Adenosylcobinamide ($AdoCbi^+$) plus *N*-methylimidazole (N-MeIm), $[AdoCbi{\cdot}N\text{-}MeIm]^+BF_4^-$, has been studied with the goal of providing a chemical precedent for the benzimidazole base-off, protein histidine base-on form of adenosylcobalamin (AdoCbl, also coenzyme $B_{12}$) found in the recent X-ray crystallographic structural study of methylmalonyl-CoA (MMCoA) mutase. Specifically, the axial-base binding $K_{assoc}$ and associated $\Delta H$ and $\Delta S$ thermodynamic parameters for $[AdoCbi{\cdot}N\text{-}MeIm]^+BF_4^-$ have been obtained as well as its Co−C thermolytic cleavage products and kinetic parameters. The thermodynamic studies reveal that imidazole is unique among the aromatic nitrogenous bases tested, with a more favorable $\Delta H = -7.8 \pm 0.4$ kcal/mol but a compensatingly less favorable $\Delta S = -28 \pm 1$ eu when binding to $AdoCbi^+$. A stronger, *shorter* Co−N (N-MeIm) bond is implied for $[AdoCbi{\cdot}N\text{-}MeIm]^+$ (i.e., vs pyridine as the axial base). The product studies reveal that imidazole changes the mode of Co−C cleavage from ≥98% homolysis (for the appended 5,6-dimethylbenzimidazole in AdoCbl) to ∼50% homolysis and ∼50% abiological heterolysis for $[AdoCbi{\cdot}N\text{-}MeIm]^+$. The kinetic studies demonstrate that *both* Co−C homolysis and heterolysis are accelerated by the record amounts of 8- and 350-fold, respectively, vs the reference point of 5,6-dimethylbenzimidazole base-on AdoCbl (and by a record 870- and 30 700-fold, respectively vs the reference point of the solvent ethylene glycol as the axial base, $[AdoCbi{\cdot}ethylene\ glycol]^+$). The biological significance of these findings is discussed, notably (i) that the MMCoA mutase subclass of $B_{12}$-dependent enzymes must either (a) utilize or (b) *prevent* Co−C heterolysis and (ii) the expectation that a long, "ideal" length Co−N(imidazole) is one key way the enzyme can inhibit Co−C heterolysis as well as accelerate Co−C homolysis. Also discussed are the steric and electronic differences of imidazole vs pyridine axial-ligands, including the literature of imidazole's $\pi$-bonding interactions. Finally, a brief summary of the needed $[AdoCbi{\cdot}base]^+$ and $[Co(II)Cbi{\cdot}base]^+$ structural, computational, and other additional studies is presented.

## Introduction

The recent X-ray crystal structure of coenzyme $B_{12}$ (AdoCbl; Figure 1)[1] dependent methylmalonyl-CoA (MMCoA) mutase[2a] has launched a new era in coenzyme $B_{12}$ bioinorganic chemistry. Specifically, the MMCoA mutase structure[2a] provides the insight[3] that $AdoB_{12}$'s appended 5,6-dimethylbenzimidazole ($Me_2BzIm$) axial base is *not* bound directly to cobalt in MMCoA mutase as previously believed but, instead, serves as a "tail" by which AdoCbl is anchored to the protein (at least within the subclass of AdoCbl-dependent proteins that bind $B_{12}$ in this $Me_2$-BzIm base-off fashion,[4] *vide infra*). Hence, Marzilli's 1993 statement[5] that despite intense prior effort on the so-called axial-base problem,[5−10a] "the role of the benzimidazole ligand is the most uncertain aspect of the involvement of the cobalamin component in Co−C homolysis", is even more fitting today than it was back in 1993.

Recently, we reported an initial series of investigations,[9,10] studies necessary to begin to provide a firm chemical reference point from which to interpret the role of the axial base coordinated to $AdoB_{12}$—be it a histidine side-chain imidazole base-on form or, in the second apparent subclass of AdoCbl-dependent enzymes, the classical $Me_2BzIm$-base-on form. A critical review of the biochemical literature relevant to the axial-base problem is also available.[9]

However, the biologically relevant case of an *N*-alkylimidazole axial base in combination with $AdoCbi^+$ has not been

† Part 3 of a series.[9,10,11h]

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(1) Abbreviations used herein include: AdoCbl (coenzyme $B_{12}$, adocobalamin, 5′-deoxy-5′-adenosylcobalamin); $AdoCbi^+$ ($AdoCbi^+BF_4^-$, adocobinamide, 5′-deoxy-5′-adenosylcobinamide); cobamide (cobalamins and cobinamides); $Co(II)Cbi^+$ (cob(II)inamide); TEMPO (2,2,6,6,-tetramethylpiperidinyl-1-oxy); cyclic-Ado (8,5′-anhydroadenosine); Ado-H (5′-deoxyadenosine); $Me_2BzIm$ (5,6-dimethylbenzimidazole); py (pyridine); N-MeIm (*N*-methylimidazole); $Me_2N$-py (*p*-(*N*,*N*-dimethylamino)pyridine).

(2) (a) Mancia, F.; Keep, N. H.; Nakagawa, A.; Leadlay, P. F.; McSweeney, S.; Rasmussen, B.; Böscke, P.; Diat, O.; Evans, P. R. *Structure* **1996**, *4*, 339. (b) Since no error bars are given on the enzymatic Co−N bond length of 2.5 Å, a meaningful discussion of this bond length is somewhat problematic. However, the data presented herein agree with the statement (see p 347)[2a] that "The 2.5 Å long Co−N bond would stabilize the Co(II) species relative to Co(III) . . .".

(3) The finding that $B_{12}$'s appended 5,6-$Me_2BzIm$ axial base binds in a 5,6-$Me_2BzIm$ base-off, but histidine side-chain imidazole base-on form was first discovered for $MeB_{12}$ bound to methionine synthase: Drennan, C. L.; Huang, S.; Drummond, J. T.; Matthews, R. G.; Ludwig, M. L. *Science* **1994**, *266*, 1669. (See also the introductory comments about this landmark paper: Stubbe, J. *Science* **1994**, *266*, 1663.)

(4) (a) Two subclasses of AdoCbl-dependent enzymes now *appear* to exist, based on whether they do, or do not, have the conserved protein sequence (Asp-X-His-X-X-Gly ...(41) ...Ser-X-Leu ...(26−28) ...Gly-Gly) that identifies the 5,6-$Me_2BzIm$ base-off binding site. See the following reference (plus the discussion provided elsewhere[9]): Ludwig, M. L.; Drenan, C. L.; Matthews, R. G. *Structure* **1996**, *4*, 505. (b) Note, however, the statement for diol dehydratase, presumably a 5,6-dimethylbenzimidazole base-on AdoCbl enzyme (i.e., given that it *does not* have the conserved sequence indicative of histidine/imidazole binding), that "imidazole group-(s) of histidine were required for the coenzyme binding".[41] Toraya, T. In *Metal Ions in Biological Systems*; Sigel, H., Siegel, A., Eds.; Marcel Dekker: New York; 1994; Vol. 30, Chapter 8, p 226. (Reference 41 therein is: Kuno, S.; Fukui, S.; Toraya, T. *Arch. Biochem. Biophys.* **1990**, *277*, 211.)

(5) (a) Marzilli, L. G. In *Bioinorganic Catalysis*; Reedijk, J., Ed.; Marcel Dekker: New York, 1993; pp 227−259. (b) For a concise discussion of the possibility of an ideal Co−N bond to the axial 5,6-dimethylbenzimidazole ligand, see pp 248−249 of Marzilli's review.[a]

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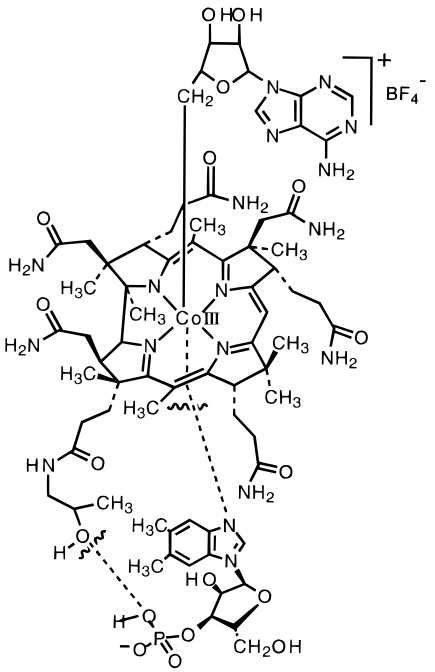


**Figure 1.** A composite representation of 5′-deoxy,-5′-adenosylcobinamide ($AdoCbi^+BF_4^-$) plus, in the lower part of the figure, the α-ribazole (1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole) fragment produced by $Ce(OH)_3$ catalyzed $H_2O$ addition across the phosphodiester bond in the synthetic precursor, adocobalamin (AdoCbl or, equivalently, coenzyme $B_{12}$). The two wavy lines locate the two chemical cleavage sites of the P−O and Co−N bonds present in the AdoCbl starting material. The 5,6-dimethylbenzimidazole nucleotide is shown with an exaggerated displacement from its normal, closer-to-cobalt orientation in base-on AdoCbl since it is, of course, completely absent in the isolated $AdoCbi^+BF_4^-$ used in the present studies.

previously studied. Important questions include the following. Does *N*-methylimidazole (N-MeIm) support primarily Co−C homolysis or heterolysis in $[AdoCbi{\cdot}N\text{-}MeIm]^+$? Is N-MeIm "special" in any identifiable way in comparison to either equal p$K_a$ pyridine axial bases or the appended 5,6-$Me_2$BzIm in AdoCbl? Alternatively, does a chemical precedent study of $[AdoCbi{\cdot}N\text{-}MeIm]^+$ serve primarily to suggest special functions of the enzyme, thereby focusing attention on additional key questions and studies with the $B_{12}$-dependent enzymes? (i.e., much in the way the enzymic $10^{12\pm1}$ activation of AdoCbl's Co−C bond was discovered via earlier chemical precedent studies of AdoCbl's Co−C homolysis,[11] a topic of increasing investigation and interpretation[11e−g]).

Herein we report chemical precedent studies for the imidazole base-on subclass of AdoCbl-dependent enzymes. Specifically, we detail our studies of $[AdoCbi{\cdot}N\text{-}MeIm]^+$ and its N-MeIm $K_{assoc}$, $\Delta H$, and $\Delta S$ values as well as studies of its Co−C thermolytic cleavage products and kinetics. Note that the *N*-alkylimidazole, N-MeIm, rather than imidazole itself, N-HIm, was chosen *deliberately* for this particular initial study in order to avoid possible complications from, for example, trace imidazolate anion, $N\text{-}Im^-$, coordination, followed by the greatly

(6) Lead references to prior work on the $B_{12}$ "axial-base problem": (a) Schrauzer, G. N.; Windgassen, R. J. *J. Am. Chem. Soc.* **1966**, *88*, 3738. (b) Thusius, D. *J. Am. Chem. Soc.* **1971**, *93*, 2629. (c) Toscano, P. J.; Marzilli, L. G. *Prog. Inorg. Chem.* **1984**, *31*, 105. (d) Bresciani-Pahor, N.; Forcolin, M.; Marzilli, L. G.; Randaccio, L.; Summers, M. F.; Toscano, P. J. *Coord. Chem. Rev.* **1985**, *63*, 1. (e) Randaccio, L; Bresciani-Pahor, N.; Zangrando, E.; Marzilli, L. G. *Chem. Soc. Rev.* **1989**, *18*, 225. (f) Grate, J. H.; Schrauzer, G. N. *J. Am. Chem. Soc.* **1979**, *101*, 4601. (g) Schrauzer, G. N.; Grate, J. H. *J. Am. Chem. Soc.* **1981**, *103*, 541. (h) Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2274. (i) Brown, K. L.; Gupta, B. D. *Inorg. Chem.* **1990**, *29*, 3854. (j) Brown, K. L.; Hakimi, J. M. *J. Am. Chem. Soc.* **1986**, *108*, 496. (k) Brown, K. L.; Satyanarayana, S. *Inorg. Chem.* **1992**, *31*, 1366. (l) Ng, F. T. T.; Rempel, G. L.; Halpern, J. *Inorg. Chim. Acta* **1983**, *77*, L165. (m) Halpern, J. *Pure Appl. Chem.* **1983**, *55*, 1059. (n) Ng, F. T. T.; Rempel, G. L.; Halpern, J. *J. Am. Chem. Soc.* **1982**, *104*, 621. (o) Ng, F. T. T.; Rempel, G. L.; Mancuso, C.; Halpern, J. *Organometallics* **1990**, *9*, 2762. (p) Chopra, M.; Hun, T. S. M.; Leung, W.-H.; Yu, N.-T. *Inorg. Chem.* **1995**, *34*, 5973.

(7) Work from the Marzill−Randaccio team: (a) Marzilli, L. G.; Toscano, P. J.; Randaccio, L.; Bresciani-Pahor, N.; Calligaris, M. *J. Am. Chem. Soc.* **1979**, *101*, 6754. (b) Randaccio, L.; Bresciani-Pahor, N.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1980**, *102*, 7372. (c) Randaccio, L.; Bresciani-Pahor, N.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1981**, *103*, 6347. (d) Bresciani-Pahor, N.; Randaccio, L.; Toscano, P. J.; Marzilli, L. G. *J. Chem. Soc., Dalton Trans.* **1982**, 567. (e) Summers, M. F.; Toscano, P. J.; Bresciani-Pahor, N.; Nardin, G.; Randaccio, L.; Marzilli, L. G. *J. Am. Chem. Soc.* **1983**, *105*, 6259. (f) Summers, M. F.; Marzilli, L. G.; Bresciani-Pahor, N. Randaccio, L. *J. Am. Chem. Soc.* **1984**, *106*, 4478. (g) Bresciani-Pahor, N.; Randaccio, L.; Zangrando, E.; Summers, M. F.; Ramsden, J. H. Jr.; Marzilli, P. A.; Marzilli, L. G. *Organometallics* **1985**, *4*, 2086. (h) Parker, W. O. Jr.; Bresciani-Pahor, N.; Zangrando, E. Randaccio, L.; Marzilli, L. G. *Inorg. Chem.* **1985**, *24*, 3908. (i) Bresciani-Pahor, N.; Randaccio, L.; Zangrando, E.; Toscano, P. J. *Inorg. Chim. Acta* **1985**, *96*, 193. (j) Marzilli, L. G.; Summers, M. F.; Bresciani-Pahor, N.; Zangrando, E.; Charland, J.-P.; Randaccio, L. *J. Am. Chem. Soc.* **1985**, *107*, 6880. (k) Mealli, C.; Sabat, M.; Marzilli, L. G. *J. Am. Chem. Soc.* **1987**, *109*, 1593. (l) Charland, J.-P.; Zangrando, E.; Bresciani-Pahor, N.; Randaccio, L.; Marzilli, L. G. *Inorg. Chem.* **1993**, *32*, 4256 (see the statement, p 4261, that "we have shown that the value of the C−N−C angle about the N bound to Co for planar ligands can be used to determine the steric effect of ligand bulk on the axial Co−N distance"[3,21]).

(8) Reports of *non-Ado* alkylcobinamide axial base $K_{assoc}$ measurements: (a) Pailes, W. H.; Hogenkamp, H. P. C. *Biochemistry* **1968**, *7*, 4160. (b) Baldwin, D. A.; Betterson, E. A.; Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1985**, 1613. (c) Brown, K. L.; Brooks, H. B. *Inorg. Chem.* **1991**, *30*, 3420. (d) Brodie, J. D. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *62*, 461. (e) Brown, K. L.; Satyanarayana, S. *Inorg. Chim. Acta* **1992**, *201*, 113−119. (f) See also ref 38 herein.

(9) Part 1: Garr, C. D.; Sirovatka, J. M.; Finke, R. G. *Inorg. Chem.* **1996**, *35*, 5912. In this first paper, a series of 14 exogenous axial-bases are studied with the 5,6-$Me_2$BzIm-free form of AdoCbl, adocobinamide ($AdoCbi^+$); also, $K_{assoc}$, $\Delta H$, and $\Delta S$ studies of $Adocbi^+$ + base ⇌ $[Adocbi{\cdot}base]^+$ are reported.

(10) (a) Part 2. Garr, C. D.; Sirovatka, J. M.; Finke, R. G. *J. Am. Chem. Soc.* **1996**, 118, 11142. In this second paper, Co−C thermolytic cleavage product and kinetic studies of $[AdoCbi{\cdot}base]^+$ were conducted (base = pyridine (py), *p*-N,N-$Me_2$N-pyridine ($Me_2$N-py), and others, but not imidazole bases). That work showed the uniqueness of the appended $Me_2$-BzIm in AdoCbl, at least in comparison to equal p$K_a$ pyridine bases, "in being able to enhance (biologically relevant) Co−C homolysis while simultaneously minimizing (abiological) Co−C heterolysis". The other most important finding is that, on going from the nonbasic solvent-based ligand $HOCH_2CH_2OH$ to the p$K_a$ 9.7 axial-base $Me_2$N-py, the rate constant for Co−C homolysis increases only 240-fold, while the Co−C heterolysis rate constant increases *8400-fold*. (b) The initially produced aldehyde, 2,3-dihydroxy-4-pentenal, has been characterized as its $BH_4^-$ reduction, triol product: Johnson, A. W.; Shaw, N.; *J. Chem. Soc.* **1962**, 4608. However, and as noted previously in footnote 31a elsewhere,[10a] the anticipated acid (or, in ROH solvents, ester) product from Co(III) oxidation of the initially produced aldehyde has never been unequivocally characterized.

(11) (a) Finke, R. G.; Hay, B. P. *Inorg. Chem.* **1984**, *23*, 3041. (b) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 4820. (c) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 8012. (d) Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1469. (e) A short review of our prior work on $B_{12}$-Based Chemical Precedent for Co−C Bond Homolysis and Other Key Elementary Steps. Finke, R. G. In *Vitamin $B_{12}$: 4th European Symposium on Vitamin $B_{12}$ and $B_{12}$ Proteins*; Kraütler, B., Ed.; Verlag Chemie, 1997; in press. This review contains, for example, a discussion of the $10^{12\pm1}$ finding for the interested reader, plus discussion of the effects of substrate C−H, or protein cysteine −S−H thiol, cleavage that more recent enzymic evidence says occurs concomitantly with AdoCbl Co−C homolysis (see also our earlier, published discussions of this point in footnote 12 elsewhere[11f] and also in footnote 34 elsewhere[11g]). (f) Waddington, M. D.; Finke, R. G. *J. Am. Chem. Soc.* **1993**, *115*, 4629. (g) Garr, C. D.; Finke, R. G. *Inorg. Chem.* **1993**, *32*, 4414. (h) Part 4. Sirovatka, J. M.; Finke, R. G. experiments in progress. Studies include imidazole and imidazolate coordination to adocobinamide under the excess imidazole conditions required to ensure formation of measurable amounts of the base-on adduct, $[AdoCbi{\cdot}base]+$ (base = Im-NH, Im-N−). Relevent p$K_a$s are ImH (p$K_a$ = 14), $[AdoCbi{\cdot}Im\text{-}NH]^+$ (p$K_a$ < 12 expected), and $[H-Im\text{-}NH]^+$ (p$K_a$ = 7.0), from which a $K_{eq} \geq 10^{-5}$ can be estimated for the possible reaction, $[AdoCbi{\cdot}Im\text{-}NH]^+$ + Im-NH ⇌ $[AdoCbi^+{\cdot}Im\text{-}N-]$ + $[H-Im\text{-}NH]^+$. Note, however, that our studies to date[9,10] show that even a relatively small fraction of $[AdoCbi^+{\cdot}Im\text{-}N^-]$ could have a kinetically dominant rate of Co−C cleavage, a key reason why we chose the simpler system of N-MeIm for these initial studies of imidazole bases.

enhanced Co−C heterolysis expected[10a] from [AdoCbi$^+$·Im-N$^-$]. Separate studies of N-HIm, N-Im$^-$, and especially of sterically bulky imidazole and pyridine bases are in progress and will be reported in due course.[11h]

## Experimental Section

Experimental details and methods are identical to those used previously[9,10a] except where otherwise noted.

**Chemicals.** AdoCbi$^+$BF$_4^-$ was prepared according to our literature procedure (98% pure by HPLC)[12] and stored below 0 °C; solutions of AdoCbi$^+$ were protected from light at all times. Adenine (Sigma) and 5′-deoxyadenosine (Sigma) were stored at 0 °C and used as received. *N*-Methylimidazole (Aldrich, redistilled by the maufacturer) and ethylene glycol (Aldrich, redistilled by the manufacturer) were stored under argon and used as received. The nitroxide TEMPO (Aldrich) was sublimed before use (mp 37−39 °C; lit.[13] 37−39 °C). All other materials were obtained as previously described.[9,10a]

**Instrumentation.** UV−visible spectra were recorded on a HP 8452A spectrophotometer equipped with a thermoelectric Peltier cell block temperature controller (25 ± 1 °C). The spectrophotometer has a dynamic range of 0.002−3.3 absorbance units at 350 nm and an accuracy of ±0.005 at 1.0 absorbance unit at 440 nm. Sample temperatures for the $K_{assoc}$ data were measured using a E. H. Sargent Hg thermometer (NBS certified, ±0.2 °C) immersed in the sample cuvette. The temperature of the oil bath (110.0 ± 0.2 °C) employed in the kinetic experiments was calibrated using an NBS-calibrated thermometer with ±0.2 °C gradations and controlled using a Barnant temperature controller. All kinetic samples were prepared in a Vacuum Atmospheres inert atmosphere glovebox ($O_2$ level ≤ 0.3 ppm) using Schlenk-type[14] UV−visible cuvettes. An HP 1050 HPLC ($\lambda$ = 260 nm detector) equipped with an Alltech C18 Versapack reversed phase column was used for product analyses. Product quantitation was accomplished using HPLC response factors generated from linear plots of peak area vs concentration of authentic product material.

**$K_{assoc}$ Measurements and Calculations.** Samples of 1 × 10$^{-4}$ M AdoCbi$^+$ in 4 mL of ethylene glycol were titrated with *N*-methylimidazole (N-MeIm, 100−800 $\mu$L, 0.5−2.0 M) at 25.1, 16.7, 40.3, and 48.5 °C (±0.1 °C), analogous to our earlier work.[9] The absorbance data at 520 nm were worked up according to the appropriate equations and via Drago's method (which graphically portrays the quality of the data and associated error bars), all exactly as detailed previously (see eqs 1 and 2 in our earlier work[9]). The resulting temperature dependent $K_{assoc}$ data were analyzed via the usual ln($K_{assoc}$) vs 1/$T$ plot to yield the $\Delta H$ and $\Delta S$ for N-MeIm binding, Figure A, Supporting Information. The results are provided in Table 1, along with selected data from our previous studies.[9] Key controls were also done to verify the reliability of the data; specifically, we repeated our published Me$_2$N-py $K_{assoc}$ data to verify the techniques and methods used herein but in a different pair of hands. The results show good agreement with our previously published data[9] (in parentheses): $K_{assoc}$ (25 °C) = 2.4 ± 0.1 (vs 2.5 ± 0.1 previously).

**[AdoCbi·N-MeIm]$^+$ Co−C Thermolytic Cleavage Product Studies**. The product studies were also performed exactly as detailed previously:[10a] solutions of ca. 1 × 10$^{-4}$ M AdoCbi$^+$, 0.85 M TEMPO (sufficient to trap caged pair intermediates[10a]), and N-MeIm (ranging from 0.1−0.6 M) in 4 mL of ethylene glycol were heated at 110 ± 0.2 °C for ∼24 h. The reaction was followed by UV−visible spectroscopy at 474 nm, and the products were analyzed (from the identical cuvettes used for the kinetic studies, *vide infra*) via HPLC: inital conditions, 95/5 water/acetonitrile, flow 1.0 mL/min, followed by a gradient ramped first to 70/30 to elute Ado-TEMPO, then to 10/90 to complete elution of the base (a broad peak; width ≈ 40 min at half-height). The observed combination of the homolysis product (Ado-TEMPO, the only homolysis product detected at high [TEMPO]) and heterolysis products (adenine, plus an adenine byproduct which results from a base-initiated degradation reaction) are shown in Scheme 2, the exact same products characterized before for AdoCbi$^+$ plus pyridine axial bases.[10] The cobalt corrin product, [Co(II)Cbi·N-MeIm]$^+$ is 100 ± 10% ($\lambda_{max}$ = 474 nm, $\epsilon_{474}$ = 1.6 × 10$^4$ M$^{-1}$ cm$^{-1}$). Note that while Co−C bond heterolysis produces Co$^{III}$ *initially*, it is known to react rapidly with the heterolysis product 2,3-dihydroxy-4-pentenal (produced stoichiometrically from the pentose sugar) to give reduced, Co$^{II}$ cobamide and an oxidized organic product.[10b,11b] Thus, the only corrin product detected is Co$^{II}$Cbi$^+$.

Again, control experiments were completed to ensure the reliability of the data, specifically product and kinetic studies for Me$_2$N-py as the axial ligand, reported originally in our earlier work,[10a] were repeated and found to be in agreement within experimental error to our previous work: the product ratio ([adenine]/[total homolysis products]) obtained herein as a control is 0.90 (vs 0.85 previously[10a]).

**Product Control Experiments: Temperature Dependence Studies.** Control studies were done to verify, as previously seen for Me$_2$N-py,[10a] that the ratio of Co−C heterolysis to homolysis products (the product ratio, eq 2, *vide infra*) is not detectably changed over the experimentally accessible temperature range in which the Co−C cleavage reaction is fast enough to be measured (i.e., from 110 °C to ca. 85 °C). Hence, the product composition at 85 °C was measured under our otherwise identical conditions used above for the 110 °C reactions (i.e., ∼1 × 10$^{-4}$ M AdoCbi$^+$BF$_4^-$, 0.6 M N-MeIm, 0.85 M TEMPO). The higher concentration of N-MeIm was used to produce as much of the base-on cobinamide as possible. The 85 °C reaction results in 33% adenine (compared to 31% adenine at 110 °C). Note that at high [N-MeIm], the base peak overwhelms and obscures the homolysis and adenine byproduct peaks, as described earlier,[10a] resulting in the necessity to follow adenine alone. Hence, this control confirms that the amount of Co−C heterolysis does not change with temperature within experimental error (±5%), over the accessible 85−110 °C range, and when employing the specific conditions cited.

**[AdoCbi·N-MeIm]$^+$ Co−C Thermolysis Kinetic Studies.** Kinetic studies of the thermolysis of AdoCbi$^+$ in the presence of 0.1−2.0 M N-MeIm were accomplished by UV−visible spectroscopy (using the same samples that were used for the product studies), again exactly analogous to our recent paper.[10a] The data were analyzed according to the mechanism established previously[10a] (see also Scheme 3 herein) and using the appropriate kinetic and product ratio equations, eqs 1 and 2, originally derived in detail in our recent paper.[10a] A control was done at the beginning of these kinetic studies to verify that the rate constants measured previously for Me$_2$N-py were repeatable ($k_{obs}$ (0.3 M, 110 °C) = 24(1) × 10$^{-5}$ s$^{-1}$ (vs 21(3) × 10$^{-5}$ s$^{-1}$ previously[10a]) adding, therefore, additional confidence in both the present and our earlier kinetic studies.

**Qualitative MO Calculations.** In order to help understand both the literature descriptions of, and the evidence uncovered herein for, imidazole's $\pi$-bonding effects, some initial, qualitative MO calculations were undertaken, mostly to gain to start a correct "pictorial" understanding of the orbitals and their overlaps. Calculations were completed using Spartan v.4.1 (Wavefunction, Inc.) on an SGI Indy computer. *N*-Methylimidazole was drawn in Spartan using the program's build subprogram; the molecule was then minimized in the build program under default parameters. To calculate the $\pi$-orbitals of *N*-methylimidazole, the molecule was optimized for geometry using Hartree−Fock theory with a 3-21G(*) basis set. Isosurfaces of the HOMO and LUMO orbitals were then calculated. The calculated MOs were used to qualitatively draw the orbitals in Figure 6 and in ref 37d.

## Results

**N-MeIm Plus AdoCbi$^+$ $K_{assoc}$, $\Delta H$ and $\Delta S$ Parameters.** The axial-base association equilibrium constants, $K_{assoc}$, for [AdoCbi]$^+$BF$_4^-$ plus N-MeIm were studied following the characteristic yellow to red color change at 450 to 520 nm that accompanies the base-off to base-on equilibrium, Scheme 1. The spectral data reveal isosbestics at 390 and 481 nm, Figure 2, confirming the two-cobalamin nature of the equilibrium under measurement, Scheme 1.

The data for N-MeIm and other selected bases from our previous studies[9] are shown in Table 1. Comparing the 25 °C

(12) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 8012.

(13) (a) Rozantzev, E. G.; Neiman, M. B. *Tetrahedron*, **1964**, *20*, 131. (b) Prisbe, E. J.; Smejkal, J.; Verheydwn, J. P. H.; Moffatt, J. G. *J. Org. Chem.* **1976**, *41*, 1836.

(14) (a) Finke, R. G.; Hay, B. P. *Inorg. Chem.* **1984**, *23*, 3041. (b) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 4820.

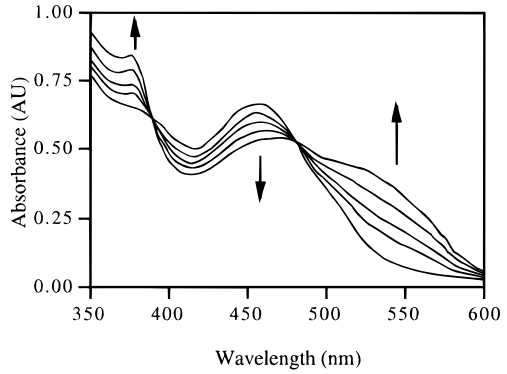
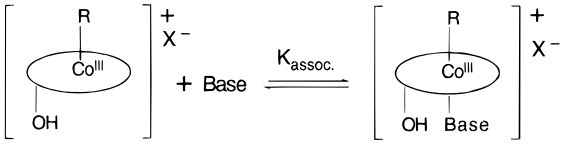



**Figure 2.** Visible spectra of ca. $1 \times 10^{-4}$ M AdoCbi$^+$ titrated with 0.5−3.0 M N-MeIm at 25 °C in ethylene glycol.

**Scheme 1.** The RCbi$^+$X$^-$ Plus Exogenous Axial-Base Equilibrium




$$K_{assoc.} \equiv \frac{[RCbi^+ \bullet Base]}{[RCbi^+]\,[Base]}$$

**Table 1.** Association Constants and Thermodynamic Parameters for AdoCbi$^+$ Plus Selected Bases (Ethylene Glycol, 25 °C)

| nitrogenous base | p$K_a$ [a] | $K_{assoc.}$ ($M^{-1}$) 25 °C | $\Delta H$ (kcal mol$^{-1}$) | $\Delta S$ (cal mol$^{-1}$ K$^{-1}$) |
|---|---|---|---|---|
| 1,5,6-Me$_3$-benzimidazole[b] | 5.6 | <0.2 | | |
| pyridine[b] | 5.2 | 1.0 ± 0.2 | −3.3 ± 0.4 | −11 ± 1 |
| *p*-Me$_2$N-pyridine[b] | 9.7 | 2.5 ± 0.1 | −6.5 ± 1.0 | −20 ± 3 |
| 1-methyl-imidazole | 7.0 | 0.5 ± 0.1 | −7.8 ± 0.4 | −28 ± 1 |
| 5,6-Me$_2$-benzimidazole in AdoCbl[c] | 5.6[d] | $K_{assoc}'$[e] 14.3 | −7.6 ± 0.2 | −20.2 ± 0.7[e] |

[a] Aqueous p$K_a$ values are taken from: Christensen, J. J.; Hansen, L. D.; Izatt, R. M. *Handbook of Proton Ionization Heats;* Wiley & Sons: New York, 1976. (Since the same relative order is seen in MeOH and EtOH, *op. cit.*, these p$K_a$ values should follow the same relative order in the closely related solvent, ethylene glycol.) [b] Taken from our earlier work.[9] [c] Data taken from ref 11d. [d] To avoid confusion, this p$K_a$ is for the free α-ribazole fragment; see Table 3 in Brown, K. L.; Hakimi, J. M. *J. Am. Chem. Soc.* **1986**, *108*, 496. The p$K_a$ of the protonated, appended 5,6-Me$_2$-benzimidazole−H$^+$ in AdoCbl−H$^+$ (i.e., for the "attached α-ribazole") is, of course, lower (p$K_a$ = 3.7; *op. cit.*) due to the added driving force of 5,6-Me$_2$-benzimidazole coordination to cobalt after it is deprotonated (Brown's $K_{co}$; *op. cit.*). [e] This *intra*molecular $K_{assoc}'$ and the corresponding entropy are not directly comparable to the other $K_{assoc}$ ($M^{-1}$) or entropy values (standard state in units of $M^{-1}$) in the table because of the different units and standard state associated with this dimensionless $K_{assoc}'$ equilibrium constant.

$K_{assoc}$ value for N-MeIm to that for the sterically more encumbered, and $\Delta$p$K_a$ = 1.4 less basic, axial-base 1,5,6-trimethylbenzimidazole reveals that N-MeIm binds ≥2.5-fold better to AdoCbi$^+$. More importantly, the thermodynamic parameters listed in Table 1 indicate that N-MeIm *is unique among the bases we have studied to date*, demonstrating more negative $\Delta H$ and $\Delta S$ values than the similar p$K_a$ pyridine ligands. The more favorable $\Delta\Delta H = -1.3$ to $-4.5$ kcal/mol, in comparison to the pyridine bases,[9] is offset by the compensatingly less favorable $\Delta\Delta S = -8$ to $-17$ cal mol$^{-1}$ K$^{-1}$, Table 1. The $K_{assoc}$, $\Delta H$, and $\Delta S$ measurements imply a stronger, *shorter Co−N bond for N-MeIm* than the pyridine bases, for example, even though N-MeIm is a $\Delta$p$K_a$ 2.7 less strong $\sigma$-donor than Me$_2$N-py, Table 1. The implied, important question addressed next is "how do these unusual binding parameters affect the cleavage of the Co−C bond, the essence of the initial function of the coenzyme".

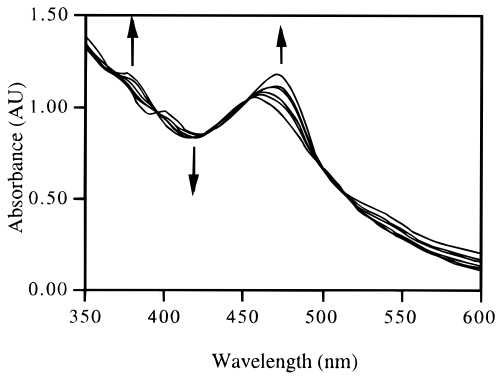



**Figure 3.** Visible kinetic trace of ca. $1 \times 10^{-4}$ M AdoCbi$^+$, 0.85 M TEMPO, and 0.4 M N-MeIm at 110 °C over 24 h in ethylene glycol. The absorbance increase at 474 nm was used to obtain the kinetic data.

**[AdoCbi·N-MeIm]$^+$ Co−C Thermolysis Product Studies.** The observed products of Co−C bond cleavage are just those we have come to expect[10,11b] for parallel paths of Co−C homolysis (leading to Ado-TEMPO) and heterolysis (leading to adenine, Co(III)Cbi$^+$ and an Ado-byproduct), Scheme 2. Note that, as seen previously[9,10a] and fortunately for the kinetic studies which follow, the only *final* cobalt product is [Co(II)-Cbi·N-MeIm]$^+$ (i.e., and no [Co(III)Cbi·N-MeIm]$^+$) due to the well-established, fast follow-up reactions that reduce any initially formed Co(III) back to Co(II).[10,11b] Note also that, although not specifically shown in Scheme 2 (but discussed in detail in Schemes 1 and 3 and footnotes 31 and 47 elsewhere[10a] as well as in an earlier paper[11b]), a H$^+$ from the glycol solvent is required for the adenine, Ad-H, formation. That is, the presence of a protic solvent such as ethylene glycol is the other well-established[10a,11b] requirement for the Co−C heterolysis component of the present Co−C cleavage rection.

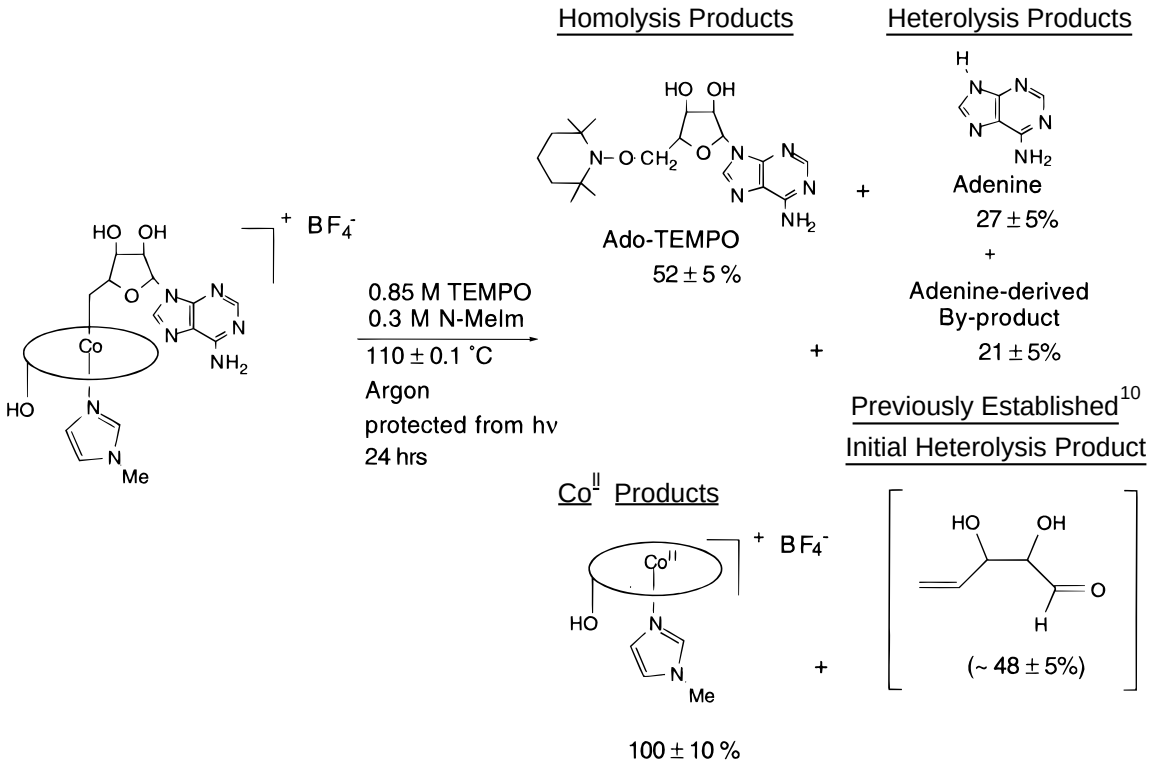
The two key results from the product studies, Scheme 2, are (i) the products of [AdoCbi·N-MeIm]$^+$ Co−C cleavage are about 50% homolysis *and 50% abiological heterolysis* and (ii) the product distribution is comparable to that of the stronger base Me$_2$N-py,[10a] despite N-MeIm's less basic nature [p$K_a$ (Me$_2$N-py) = 9.7 vs p$K_a$ (N-MeIm) = 7.0].

An additional, very important point from a control experiment is that the amount of heterolysis is *independent of temperature within experimental error* (±5%) over the accessible temperature range of 85−110 °C, a result also found previously for pyridine bases.[10a] This significant result means that the heterolysis to homolysis product ratio measured herein, and deconvolution of rate constants and other conclusions based on this product ratio (*vide infra*) are relevant to the lower, physiological temperatures of 37 °C of the B$_{12}$-dependent enzymes.

**[AdoCbi·N-MeIm]$^+$ Co−C Thermolysis Kinetic Studies.** The visible spectral data as a function of time shows isosbestics at 396, 425, and 455 nm and, thus, the quality of the data, Figure 3. The kinetic and products studies, as well as our[10a] and other[15] previous work, are consistent with and fully supportive of the minimal mechanistic scheme we presented recently for other axial bases, Scheme 3.[10a] The appropriate kinetic equation derived for this mechanism is shown in its double reciprocal,

(15) Recent mechanistic studies of Co−C heterolysis (see also ref 42a−g elsewhere[10a] for a listing of the earlier literature of Co−C heterolysis): (a) Gerards, L. E.H.; Balt, S. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 137 (the full paper). (b) Gerards, L. E. H.; Balt, S. *Recl. Trav. Chim. Pays-Bas* **1992**, *111*, 411 (the preliminary communication).

**Scheme 2.** The AdoCbi$^+$ ($\sim 1 \times 10^{-4}$ M) Plus Exogenous Axial-Base Heterolysis Plus Homolysis Stoichiometry




$1/k_{obs}$ vs 1/[base] plot form, eq 1 (derived previously elsewhere;[10a] see also the derivations leading to eq 7 herein that are supplied as part of the Supporting Information).

$$\frac{1}{k_{obsd}} = \left[\frac{1}{K\cdot(k_{on,T} - k_{off,T})}\right]\cdot\frac{1}{[base]} + \left[\frac{1}{(k_{on,T} - k_{off,T})}\right] \quad (1)$$

$$\begin{bmatrix}\text{product}\\ \text{ratio}\end{bmatrix}_t = \frac{[\text{adenine}]_t}{[\text{ado}\cdot\text{products}]_t} = \frac{k_{on,het}}{k_{on,h}} \quad (2)$$

The required kinetic data as a function of the concentration of added N-MeIm were obtained, and the appropriate double reciprocal plot according to eq 1 shows a linear fit to the data, Figure 4. The $k_{on,total}$ derived from this plot was further deconvoluted into the desired $k_{on,h}$ (the base-on homolysis rate constant) and $k_{on,het}$ (the base-on, heterolysis rate constant) using the ratio of heterolysis to homolysis products, defined as the product ratio, eq 2. Note that the use of eq 2 requires sufficient [N-MeIm] be present so that the *product ratio does not change with additional N-MeIm*—an experimental fact proved as part of these studies. (See elsewhere[10a] for the derivation of eq 2 (equals eq 11c in that work), especially eqs 8−11 and the derivation in the Supporting Information in our previous paper;[10a] see also the Supporting Information provided herewith, especially the details of the data analysis and the *ex post facto* verification that the conditions necessary for eq 2 are, in fact, fully satisfied by the conditions used and data obtained herein.)

The resultant individual rate constants, plus selected rate constants from our prior publication for comparative purposes,[10a] are summarized in Table 2. The three most important findings from the kinetic studies are the following: (i) N-MeIm is exceptional at enhancing the total rate of Co−C cleavage, accelerating significantly the rate of both homolysis and heterolysis; (ii) quantitatively speaking, N-MeIm accelerates the rate constant for Co−C homolysis by a factor of 870 (vs the $k_{on,h}$ for the baseline reference point of ethylene glycol, [AdoCbi·ethylene glycol]$^+$) and even a factor of 4 over the $\Delta pK_a$ 2.7 units more basic $Me_2N$-py; and (iii) N-MeIm accelerates the rate constant for Co−C heterolysis by a factor of 30 700-fold vs the reference point of $k_{on,het}$ for ethylene glycol as the axial ligand.[10a]

## Discussion

**Key Findings and Their Biological Implications.** The new insights from these [AdoCbi·N-MeIm]$^+$ chemical precedent studies can be summarized as follows:

(1) AdoCbi$^+$ binds N-MeIm with an unusually negative $\Delta H$ and an unusually compensatingly negative $\Delta S$, in comparison to pyridine bases spanning N-MeIm's p$K_a$ (Table 1). Compared to $Me_2N$-Py and py, the $\Delta\Delta H = -1.3$ to $-4.5$ kcal/mol and $\Delta\Delta S = -8$ to $-17$ eu for N-MeIm require a stronger, and therefore presumably shorter, Co−N axial-base bond.

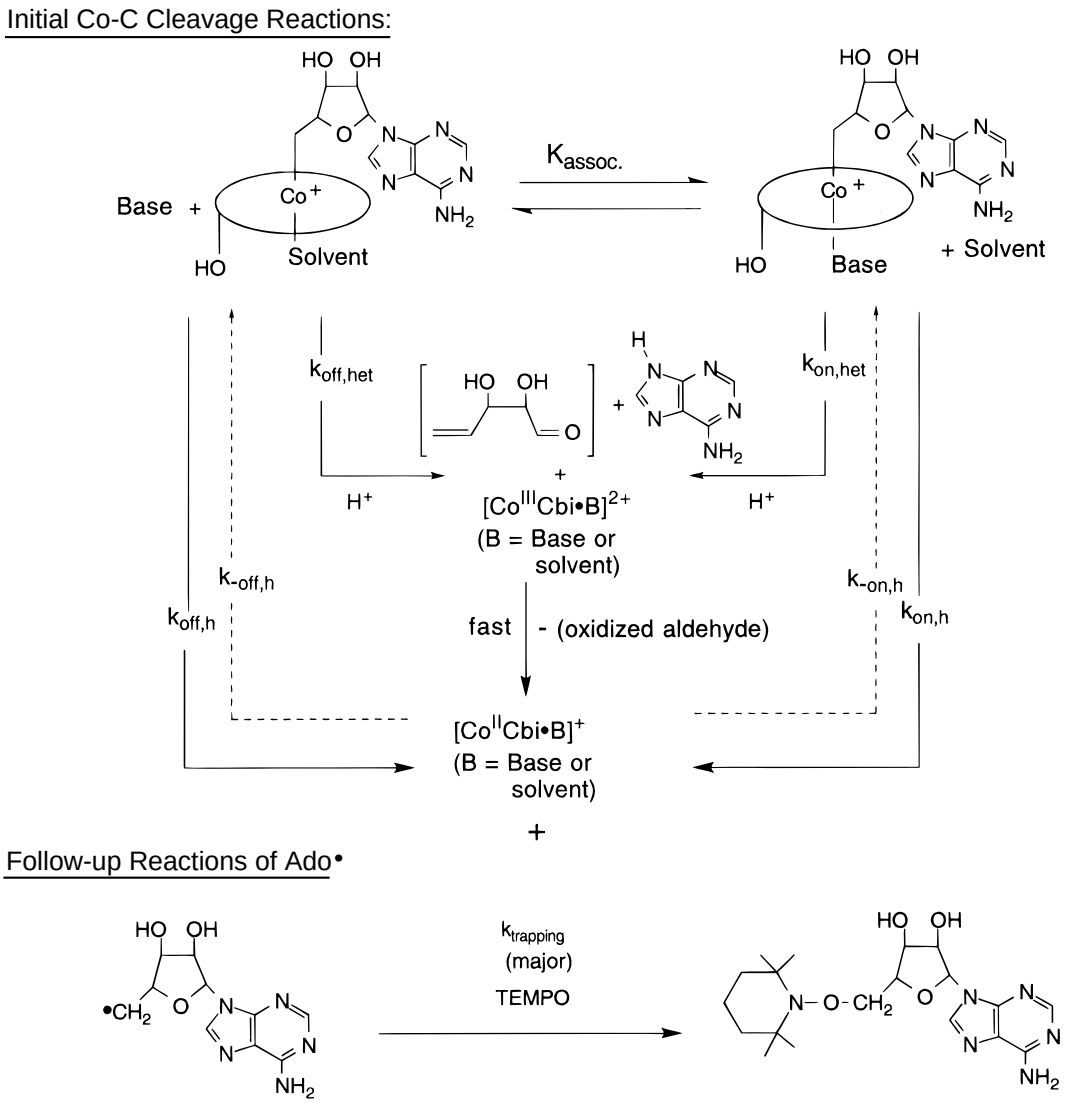
(2) This stronger and implied shorter Co−N bond in [AdoCbi·N-MeIm]$^+$ compared to pyridine, for example, leads to a significant change in the mode of Co−C cleavage, namely Co−C *heterolysis*.

(3) The N-MeIm $K_{assoc}$ $\Delta H = -7.8 \pm 0.4$ kcal/mol and $\Delta S = -28 \pm 1$ eu values for this *inter*molecular axial-base association are, however, strikingly similar to the *intra*molecular $K_{assoc}'$ (unitless) values for the appended 5,6-dimethylbenzimidazole,[11d] $\Delta H = -7.6 \pm 0.2$ kcal/mol and $\Delta S = -20.2 \pm 0.7$ eu. Note that the different units (and thus standard states) of the underlying $K_{assoc}$ ($M^{-1}$) and $K_{assoc}'$ (unitless) values means that entropies are not directly comparable.

(4) Quantitatively, Co−C heterolysis in [AdoCbi·N-MeIm]$^+$ is accelerated 30 700-fold, while Co−C homolysis is accelerated 34-fold less, by a factor of 870, vs the reference point of ethylene glycol as the base, [AdoCbi·ethylene glycol]$^+$. Compared to the alternative reference point of AdoCbl and its appended 5,6-$Me_2$BzIm, Co−C heterolysis is accelerated 350-fold and Co−C homolysis 8-fold.

(5) It follows, then, that the enzymes in the methylmalonyl-CoA mutase, imidazole base-on subclass,[4] must either (i) *prevent*

**Scheme 3.** Mechanism for Co−C Heterolysis in AdoCbi$^+$ Suggested Previously[10a] Showing the Precedented[15,18] Acceptance of a Proton by the Weakly Basic $\beta$-Oxygen of the Ado−Alkyl Group




Co−C heterolysis or (ii) *utilize* Co−C heterolysis, despite the seemingly heretical nature of this latter, logical possibility.

Since Co−C heterolysis in any AdoCbl-utilizing enzyme has never been detected to our knowledge (i.e., at least not kinetically competent Co−C heterolysis along the productive, catalytic reaction coordinate), we doubt that Co−C heterolysis is the correct conclusion, especially in the MMCoA mutase subclass of enzymes.[16] The implied conclusion, then, is that the MMCoA subclass of enzymes *probably limits* Co−C *heterolysis*.

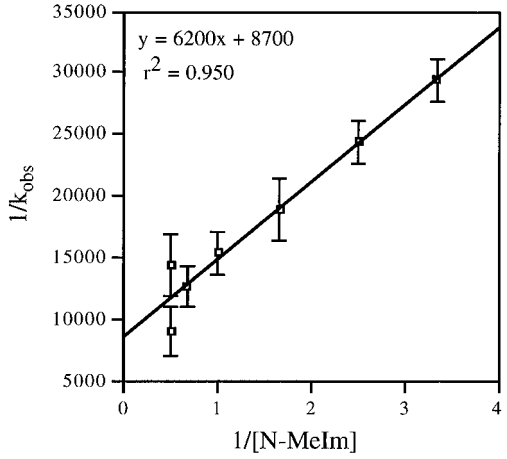



**Figure 4.** Reciprocal $1/k_{obs}$ vs 1/[N-MeIm] plot for 0.3−2.0 M N-MeIm (110 °C data).

**Known Factors Affecting Co−C Heterolysis vs Homolysis.** There are at least three main ways, based on the available chemical precedent,[15,17] that the AdoCbl-dependent enzymes might limit Co−C heterolysis. First, since facile AdoCbl Co−C heterolysis requires protonation at the $\beta$-oxygen of the Ado-group,[18] the enzyme could in principle limit the availability of any such protons. Second, Professor Kraütler and co-workers

(16) (a) Curiously, in the second subclass of enzymes, which include diol dehydratase, ethanolamine ammonia-lyase, and ribonucleotide reductase,[4] a Co−C heterolysis step is chemically much more reasonable. That is, this class of probably cysteine side-chain, (Enz−SH), utilizing enzymes could undergo AdoCbl Co−C heterolysis to give Ado−H, Co(III)Cbl$^+$ and Enz−S$^-$, followed by rapid, short-range electron transfer, Co(III)Cbl$^+$ + Enz-S$^-$ → Co(II)Cbl + Enz−S$^\bullet$, leading to products indistinguishable from Co−C homolysis (i.e., overall, AdoCbl + Enz−SH → Ado-H + Co(II)-Cbl + Enz−S$^\bullet$). However, this subclass apparently does not have the histidine imidazole base-on form, but instead is 5,6-$Me_2$Bz base-on, and this form is special in at least solution Co−C thermolysis studies[10a] in showing *less heterolysis* than any other nitrogenous base examined.[10a] Hence, chemical precedent studies do not appear to favor Co−C heterolysis for either of the two subclasses of AdoCbl-dependent enzymes. (b) Licht, S.; Gerfen, G. J.; Stubbe, J. *Science* **1996**, *271*, 477. (c) Booker, S.; Licht, S.; Broderick, J.; Stubbe, J. *Biochemistry* **1994**, *33*, 12676. (d) Booker, S.; Stubbe, J. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8352.

**Table 2.** A Comparison of the Absolute and the Relative $k_{on,h}$ and $k_{on,het}$ Rate Constants for AdoiCbi$^+$·base and AdoCbl[b]

| AdoCbi$^+$·base complex | absolute $k_{on,h}$ ($\times 10^5$) $s^{-1}$ | relative $k_{on,h}$ | absolute $k_{on,het}$ ($\times 10^5$) $s^{-1}$ | relative $k_{on,het}$ |
|---|---|---|---|---|
| AdoCbi$^+$·(ethylene glycol)[a] | 0.3(0.1) ($k_{off,h}$) | 1.0(0) ($k_{off,h}$) | 0.008 (0.003) ($k_{off,het}$) | 0.026(13) ($k_{off,het}$) |
| AdoCbl[a] | 33(14) | 110(47) | 0.69(0.3) | ≤2.3(1.0) |
| AdoCbi$^+$·py[a] | 75(26) | 250(120) | 4(1) | 13(5) |
| AdoCbi$^+$·$NMe_2$-py[a] | 73(11) | 240(40) | 67(12) | 220(40) |
| AdoCbi$^+$·N-MeIm | 260(50) | *870(170)* | 240(50) | *800(170)* |

[a] Taken from our earlier work.[10a] [b] The relative rate constants are all relative to the AdoCbi$^+$·Ethylene Glycol value of $k_{off,h} = 1$ and, therefore, are directly comparable in each case. Propagated error estimates are shown in parentheses after each entry.

have convincing evidence that, even in solution, AdoCbl is in a conformation that restricts the optimum Co−$C_\alpha$−$C_\beta$−O *trans*-antiperiplanar conformation required for the fastest Co−C heterolysis.[17] The enzyme could further control this conformation, making Co−C heterolysis in the AdoCbl·imidazole−enzyme complex even less favorable.

Third, the enzyme may *lengthen the Co−N distance (or otherwise control the Co−N bond), thereby limiting Co−C heterolysis*. This possibility is certainly supported by the long, 2.5 (± not given) Co−N(imidazole) distance found in the recent MMCoA mutase X-ray structure.[2a] However, spectroscopic studies indicate that the Co-Ado bond has been cleaved, and the CoCbl present is a mixture of mostly Co(II) and some Co(III), making the exact interpretation of the long, ca. 2.5 Å distance somewhat ambiguous.[2b] The suggestion of a long Co−N(imidazole) in the *AdoCbl·enzyme complex* and its role in controlling the mode of Co−C cleavage is consistent with an insightful MO calculation by Mealli, Sabat, and Marzilli.[19] It is also arguably consistent with the available studies of Co−N(imidazole) bond lengths,[20−22] although the ideal crystallographic reference point of a Co−N(imidazole) bond length in enzyme-free [AdoCbl·N-MeIm]$^+$ is not yet available. In addition, there is relevant crystallographic and EXAFS data in the literature on Co(III)−N and Co(II)−N distances for 5,6-dimethylbenzimidazole as the axial ligand,[23] but the interpretation and reliability of the Co(II)−N distance data require further clarification.

Summarizing, then, the available evidence suggests that the enzyme may profitably employ a *long AdoCo(III)−N(Im) bond to destabilize the AdoCo(III)Cbl·imidazole−enzyme complex* (i.e., long relative to the enzyme-free AdoCo(III)Cbi$^+$·N-MeIm bond length) *but a closer to normal Co(II)−N(Im) bond length to stabilize the Co(II)Cbl·imidazole−enzyme complex* (i.e., "normal" relative to the enzyme-free Co(II)Cbi$^+$·N-MeIm Co−N bond length). An important point is that the enzyme must accomodate the 0.12 Å larger ionic radius of low spin Co(II) in comparison to the ionic radius of low spin Co(III).[24,25] In other words, if for example the enzyme maintains a *constant*, "long" Co−N bond length, then it will have the effect of producing a near-ideal Co(II)−N bond length,[5b] which would reduce the activation energy required for Co−C bond cleavage, *vide infra*.

The above ideas are fully consistent with the recent MMCoA mutase crystallographic structure which concluded (see p 347)[2a] that "the 2.5 Å long Co−N bond would stabilize the Co(II) species relative to Co(III) . . .". Certainly, it will be most interesting to learn more about how AdoCo(III)−N and Co(II)−N imidazole bond distances within the enzyme compare to those for enzyme-free [AdoCbi·N-MeIm]$^+$ and [Co(II)Cbi·N-MeIm]$^+$ Co−N distances,[26] that is, once the needed crystal

(17) (a) These authors have observed a ca. $10^4$ faster Co−C heterolysis in 2′,3′-dideoxyadenosylcobalamin which, unlike AdoCbl, they show can achieve the *trans*−antiperiplanar Co−Cα−C*β*−O arrangement preferred for Co−C heterolysis. (b) Kraütler, B. In Organic Reactivity: Physical and Biological Aspects; Special Publication No. 148, Golding, B. T., Griffin, R. J., Maskill, H., Eds.; The Royal Society of Chemistry: 1995; pp 209−222.

(18) Brown, K. L.; Salmon, L.; Kirby, J. A. *Organometallics* **1992**, *11*, 422. See, also: the list of previous Co−C heterolyses studies (of especially *β*-alkoxy and -hydroxy $B_{12}$ and $B_{12}$-model complexes) that comprise ref 1−27 therein.

(19) Mealli, C.; Sabat, M.; Marzilli, L. G. *J. Am. Chem. Soc.* **1987**, *109*, 1593.

(20) (a) A longer Co−N(imidazole) bond length in the AdoCbl·enzyme complex (compared to the cobinamide model) is probably even consistent with Kraütler's crystal structure studies of cyano(imidazolyl)cobalamin (i.e., where the imidazole is intramolecularly appended) in which a *short* Co−N imidazole bond of 1.97(1) Å is found.[20b] This statement follows since the strong *π*-bonding (Im−Co−C≡N ↔ N-Im-Co$^+$=C=N$^-$)[21] and the electron withdrawing effect of the *β*-cyano ligand undoubtedly decrease the Co−N(Im) bond length to its relatively short 1.97(1) Å value. Supporting this interpretation is the fact that the Co−$N_{bzm}$ bond length in cyanocobalamin is 0.27 Å shorter than in coenzyme $B_{12}$.[22] (b) Kraütler, B.; Konrat, R.; Stupperich, E.; Färber, G.; Gruber, K.; Kratky, C. *Inorg. Chem.* **1994**, *33*, 4128.

(21) (a) Brown K. L.; Gupta, B. D. *Inorg. Chem.* **1990**, *29*, 3854. (b) Brown, K. L.; Hakimi, J. M. *J. Am. Chem. Soc.* **1986**, *108*, 496. (c) Brown K. L.; Satyanarayana, S. *Inorg. Chem.* **1992**, *31*, 1366.

(22) Glusker, J. P. In *$B_{12}$*; Dolphin, D., Ed.; John Wiley & Sons: New York, 1982; Vol. 1, p 45.

(23) A confusing set of structural data is the following, although it does help in revealing the needed additional structural studies of Co(II)−N distances. Recent *solution* EXAFS[23a] data (that is somewhat in question, however, *vide infra*) and X-ray edge spectroscopy on Co(II)Cbl[23b] reveal evidence for a relatively *short* 1.99 (±0.03) Å Co(II)−N axial bond length for Co(II)Cbl *in solution*, one shorter, however, than that found in the *solid-state* Co(II)Cbl X-ray structure[23f] of 2.13 Å (± not given). On the other hand, one EXAFS study finds a *longer* 2.15(3) Å distance for aquocobalamin ($H_2O$-CoCbl$^+$),[23c] one that others have redetermined (again by EXAFS) to be *shorter*, 1.90 Å and, by X-ray crystallography, to be 1.925 (2) Å).[23d,e] Hence, while a reliable Co(III)−N distance for ($H_2O$-CoCbl$^+$) seems to be in hand, the uncertainty about the solution vs solid state (and EXAFS vs crystallographic) Co(II)−N distance for Co(II)Cbl, possibly in different conformers, requires further clarification. (a) Sagi, I.; Wirt, M. D.; Chen, E.; Frisbie, S. M.; Chance, M. R. *J. Am. Chem. Soc.* **1990**, *112*, 8639. (b) Wirt, M. D.; Sagi, I.; Chen, E.; Frisbie, S. M.; Lee, R.; Chance, M. R. *J. Am. Chem. Soc.* **1991**, *113*, 5299. (c) Sagi, I.; Chance, M. R. *J. Am. Chem. Soc.* **1992**, *114*, 8061. (d) Kräutler, B.; Konrat, R.; Stupperich, E.; Fäber, G.; Gruber, K.; Kratky, C. *Inorg. Chem.* **1994**, *33*, 4128 (see p 4139). (e) Kratky, C.; Fäber, G.; Gruber, K.; Wilson, K.; Dauter, Z.; Nolting, H.-F.; Konrat, R.; Kräutler, B. *J. Am. Chem. Soc.* **1995**, *117*, 4654. (f) Kräutler, B.; Keller, W.; Kratky, C. *J. Am. Chem. Soc.* **1989**, *111*, 8936. (g) Glusker, J. P. In *$B_{12}$*; Dolphin, D., Ed.; John Wiley & Sons: New York; Vol. 1, Chapter 3.

(24) (a) Huheey, J. E. *Inorganic Chemistry, Principles of Structure and Reactivity*; Harper and Row: New York, 1972; p 74, Table 3.6, 2nd column, Co(II) and Co(III) entries. (b) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*; John Wiley & Sons: New York, 1988; p 1388.

(25) (a) The coordination number also influences this correction; see the Co(II) and Co(III) high and low spin entries in Table 3.6, pp 74−75 elsewhere.[24] And, although there is to our knowledge no evidence for a low to high spin change accompanying Co(II) formation within the AdoCbl·enzyme complex, this intriguing possibility has been discusssed by Randaccio and Marzilli[25b] as well as by Pratt.[25c] (b) Randaccio, L.; Bresciani Pahor, N.; Zangrando, E.; Marzilli, L. G. *Chem. Soc. Rev.* **1989**, *18*, 225. On p 247 Marzilli discusses the literature findings[25d,e] that suggest the possibility of a five-coordinate, high-spin form of Co(II) where the basal Co−N distances are longer but the axial Co−N distance is shorter.[25d,e] (c) Baldwin, D. A.; Betterton, E. A.; Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1985**, 1613. On p 1618 Pratt briefly suggests a five-coordinate, possibly triplet Co(II)Cbl in which cobalt is significantly out of the corrin plane. (d) Calligaris, M.; Nardin, G.; Randaccio, L. *J. Chem. Soc., Dalton* **1974**, 1903. (e) Kennedy, B. J.; Fallon, G. D.; Gatehouse, B. M. K. C.; Murray, K. S. *Inorg. Chem.* **1984**, *23*, 580.

structures and EXAFS studies of the latter two complexes become available.

Also consistent with the above AdoCo(III)Cbl destabilization, Co(II) stabilization ideas are our *preliminary* studies[27] of Co(II)Cbi$^+$ axial-base binding $K_{assoc}$, $\Delta H$, and $\Delta S$, results which indicate that *nonenzymic* Co(II)Cbi$^+$ binds axial bases *more tightly* than does nonenzymic AdoCbi$^+$. These Co(II)Cbi$^+$ plus base studies are nearing completion and will be reported elsewhere in due course.[27] Noteworthy in this context are our comments a decade ago[28] that for a ca. 31 kcal/mol endothermic Co−C homolysis step (i.e., a Co−C BDE of 31 kcal/mol[29]), tighter binding and other enzymic manipulation of Co(II)Cbl (i.e., in addition to any manipulation of the AdoCbl ground state) is what one expects. This follows (at least outside the enzyme, in solution), since Co(II)Cbl is, by Hammond's postulate, closer to the Co−C homolysis transition state than is AdoCbl. Recent resonance- and FT-Raman measurements[30] support the general conclusion of greater axial-base effects on the Co(II)Cbl complex and the Co−C cleavage transition state, [Ado−CoCbl]$^‡$, than in the ground state, especially Marzilli's work[30d,g] reaching exactly this same conclusion (others apparently agree as well[30i]).

The *heterolysis* transition state effects on Co(III)Cbi$^+$ must be even larger than the homolysis transition state effects on Co(II)Cbi$^+$. This follows since the [AdoCbi·N-MeIm]$^+$ ground state effects are necessarily the same for both heterolysis and homolysis, yet the rate of heterolysis increases faster than that of homolysis upon adding N-MeIm. We suspect that $\pi$-effects of the imidazole are important in these stabilizations, *vide infra*, and the greater effect on Co(III) logically implicates a $\pi$-*donor* effect in at least the Co(III) case (i.e., since Co(III) would be the better $\pi$-acceptor, but worse $\pi$-donor, compared to Co(II)). However, these ideas require verification or refutation (as do other interesting observations and comparisons[31,32]).

**Co−C Homolysis Reaction Coordinate Model.** All of the above evidence point toward enzymic axial-base effects that favor Co(II) over Co(III). It is helpful to view (a) what we now know about the $\Delta H$ vs reaction coordinate diagram for AdoCbl homolysis *outside of the enzyme* (i.e., based on the data herein) and, therefore, to summarize (b) what enzyme-induced changes and resultant $\Delta H$ reaction coordinate diagram one anticipates within the AdoCbl·enzyme complex, Figure 5.

The Co−C bond energy for [AdoCbi·solvent]$^+$ is $\Delta H = 34$ kcal/mol as shown in Figure 5.[12] Our equilibrium data, $\Delta H = -7.8$ kcal/mol, for N-MeIm show that in the ground state, enzyme-free (i.e., solution) AdoCbi$^+$ is stabilized by ca. 8 kcal/mol when it binds N-MeIm, forming [AdoCbi·N-MeIm]$^+$, Figure 5. This alone would *slow* the rate of homolysis. However, since our kinetic data show that $k_{on,h}$ is *faster* than $k_{off,h}$ (Table 2), the transition state of [Co$^{II}$Cbi·N-MeIm]$^+$ must be stabilized to a greater extent than the ground state (i.e., by >8 kcal/mol). The enzyme could further manipulate this picture by weakly coordinating histidine to Co$^{III}$ (in the ground state) but then strongly coordinating histidine to Co$^{II}$ *after* homolysis (keeping in mind the 0.12 Å differential in ionic radii, *vide supra*), reducing the $\Delta H^‡$ required for homolysis (the dashed line in Figure 5).

(26) The Co−N distance comparisons of interest extend to the Co−N *benzimidazole* distances in AdoCbl (2.24 Å[23g]) and in Co(II)Cbl (≤2.13 Å[23f]). It is noteworthy that the latter, *nonenzymic* Co(II)Cbl distance is ≥0.23 Å *shorter* than the predicted "ideal distance" of 2.24 + 0.12 = 2.36 Å. Exactly what this means and how to properly interpret it are not yet completely clear, although it would seem to point again[2] to enzymic control of the Co−N axial bond length. In addition, it will be of interest to see if this ≥0.23 Å shorter than predicted Co−N bond distance extends to the cobinamides [AdoCbi·N-MeIm]$^+$ and [Co(II)Cbi·N-MeIm]$^+$.

(27) Studies of Co(II)Cbi$^+$ + base $K_{assoc}$, $\Delta H$, and $\Delta S$: Sirovatka, J. M.; Garr, C. D.; Finke, R. G. Unpublished results and experiments in progress. We note, however, that a presently unresolved issue in these preliminary Co(II)Cbi$^+$ $K_{assoc}$ studies is the extent to which $\alpha,\beta$-isomerism is present in the [Co(II)Cbi·base]$^+$ complexes, and, thus to what extent, if any, this leads to the observed higher $K_{assoc}$ values.

(28) (a) See p 8018 elsewhere.[11c] (b) See p 4636 in: Waddington, M. D.; Finke, R. G. *J. Am. Chem. Soc.* **1993**, *115*, 4629.

(29) (a) Finke, R. G.; Hay, B. P. *Inorg. Chem.* **1984**, *23*, 3041. (b) Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1469. (c) The AdoCbl BDE is given in the following paper, after the necessary measurement of, and correction for, the cage-efficiency factor, $F_c$: Garr, C. D.; Finke, R. G. *Inorg. Chem.* **1993**, *32*, 4414.

(30) (a) Marzilli's findings using near-IR FT-Raman,[30f,i] as well as the Spiro and Banerjee resonance Raman results,[30k] support indirectly our conclusion of greater effects on the Co···C cleavage *transition state* since they find no major effects at the R-Co(III) ground state. However, when reading this literature one can avoid considerable confusion by substituting the term (ground-state, Hooke's-Law-based) "force constant" [or, alternatively, electronic or steric (depending upon the case) "*trans-influence*" as Marzilli uses[30i]] for each place that "bond *strength*" is mentioned. In the future, we urge a more careful use of better defined terminology. To that end, we would add that the terms bond dissociation energy (and, we and Dragol would argue, "bond strength") are well defined *thermodynamic* terms[30b] ($\Delta H_f$(products) − $\Delta H_f$(reactant)) that *should not* be confused with, nor used interchangeably with, ground-state only terms such as the *trans*-influence.[31b] Also noteworthy are Drago's cautions on BDE vs vibrational frequency correlations.[30l] We thank Professors T. Spiro, R. Banerjee, and L. Marzilli for helpful discussions of these points. (b) *The Encyclopedia of Chemistry*, 2nd ed.; Clark, G. L., Hawley, G. G. Eds.; Van Nostrand and Reinhold Co.: New York, 1996; pp 139−141. (c) Wozniak, W. T.; Spiro, T. G. *J. Am. Chem. Soc.* **1973**, *95*, 3402. (d) Galluzi, F.; Garozzo, M.; Ricci, F. F. *J. Raman Spec.* **1974**, *2*, 351. (e) Nie, S.; Marzilli, L. G.; Yu, N.-T. *J. Am. Chem. Soc.* **1989**, *111*, 9256. (f) Nie, S.; Marzilli, P. A.; Marzilli, L. G.; Yu, N.-T. *J. Am. Chem. Soc.* **1990**, *112*, 6084. This paper makes several important points, specifically: (i) Raman spectroscopy probes "soley ground state properties" and (ii) the differences in Co−C BDE are "distributed over many chemical bonds in the pertinent molecule" (see p 6090; see also Marzilli's 1996 paper[30i]); that is, a key point is that *many other modes besides the* Co−C *stretch can house the energy differences contained within a (*Co−C*) BDE*. [30k] (g) Nie, S.; Marzilli, P. A.; Marzilli, L. G.; Yu, N.-T. *J. Chem. Soc., Chem. Commun.* **1990**, 770. (h) Chopra, M.; Hun, T. S. M.; Leung, W.-H.; Yu, N.-T. *Inorg. Chem.* **1995**, *34*, 5973. (i) Puckett, J. M., Jr.; Mitchell, M. B.; Hirota, S.; Marzilli, L. G. *Inorg. Chem.* **1996**, *35*, 4656. (j) Hirota, S.; Polson, S. M.; Puckett, J. M., Jr; Moore, S. J.; Mitchell, M. B.; Marzilli, L. G. *Inorg. Chem.* **1996**, *35*, 5646 and ref 7−9 and 11−14 therein to earlier work in this series. (k) Dong, S.; Padmakumar, R.; Banerjee, R.; Spiro, T. G. *J. Am. Chem. Soc.* **1996**, *118*, 9182 ("...Co−C Stretching Frequencies Reflect Bond Strength Changes in Alkyl Cobalamins, but are Unaffected by *Trans* Ligand Substitution"). Note that the author's use of the term "bond *strength*" as in the paper's text and title is *not* intended to mean BDE. (l) Drago, R. S. *Physical Methods*; 2nd ed.; Saunders College Publishing: 1992. Drago notes in italics (see p 152) that "*...there is no simple relation between bond dissociation energy and force constant"*(e.g., as suggested by the oversimplified expression derived on p 5977[30h] and p 6090[30f] elsewhere by, ultimately, equating the bottom portions of Morse and Hooke's Law potential wells; note that this yields a conceptually confusing equation derived for only the ground state, yet which contains a term (the BDE) which requires a difference between higher energy *product*- and ground-states). Drago also uses bond strength as a $\Delta H$ term (i.e., as we suggest above) and provides comments (p 194) discouraging both the attempts themselves and the underlying assumptions behind bond strength vs vibrational frequency shift correlations.

(31) (a) It is also of some interest to note that the results for [AdoCbl·N-MeIm]$^+$ would *appear* to be opposite Professor Bürgi's $B_{12}$-model-based structural studies predicting a *slower* Co−C homolysis for the shorter Co−N bond (the interesting "anomalous trans effect").[32a] But, one must remember that this anomalous trans effect is really a ground-state (anomalous), structural *trans-influence*,[31b] so that a ground-state slowing could be overcome by a strong stabilization (and thus rate-increasing) effect *on the Co−C transition state*, leading to a net rate increase for N-MeIm *as we see.* Restated, our *cobinamide* and Professor Bürgi's *cobaloxime $B_{12}$-model* results may still be consistent with one another (and even though they rigorously need not be, since the systems are different), depending upon whether or not transition state effects overwhelm effects on the AdoCbl ground state, *as we believe they will* (*vide supra*). (b) Huheey, J. E. *Inorganic Chemistry, Principles of Structure and Reactivity*; Harper and Row: New York, 1972; p 426.

(32) (a) De Ridder, D. J. A.; Zangrando, E.; Bürgi, H.-B. *J. Mol. Struct.* **1996**, *374*, 63. (b) Although Bürgi predicts an inverse *trans*-influence in cobalamins as well as cobaloximes,[32a] he has not considered the effects of the $\beta$-ligands, nor the possibility of $\pi$ effects, in his correlations. For example, the graph on p 81 appears to be misleading, as many readers will (like us) probably interpret the "straight line" therein, instead of as a linear Co−N, Co−C bond length relationship, as a series of *different cobalamin domains*, based on the different $\beta$-ligands, of nearly perpendicular slopes to the line shown.

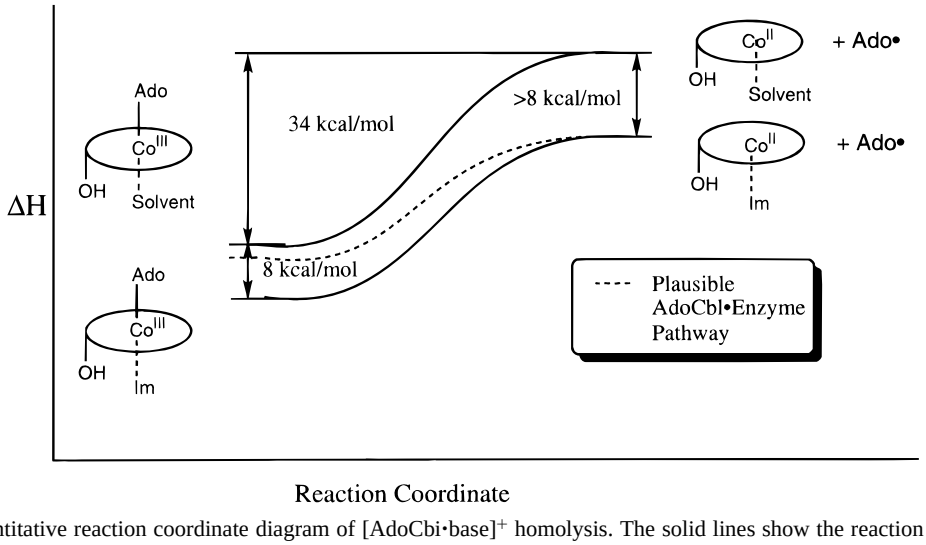



**Figure 5.** A semiquantitative reaction coordinate diagram of [AdoCbi·base]$^+$ homolysis. The solid lines show the reaction pathway for [AdoCbi·solvent]$^+$ and [AdoCbi·base]$^+$ homolysis, while the dashed line shows one plausible route the enzyme could take to lower the activation energy required for Co−C homolysis. Note that a long Co(III)−N (almost non)-bond destabilizes the ground state, while an "ideal" Co(II)−N bond stabilizes the transition state. See the discussion in the main text for why the Co(III)−Im BDE (bond dissociation energy) is >8 kcal/mol (and more like 16 kcal/mol), and thus why the enzyme could in principle manipulate the Co−N axial bond by amounts which could approach this 16 kcal/mol.

Note that the anticipated enzyme effects are, in the end, just those any enzymologist would have predicted (i.e., without any detailed knowedge of AdoCbl-dependent enzymes) in that they show a *leveling of the reaction coordinate diagram*, to one without "high hills" or "deep valleys",[33] Figure 5, dashed line, approaching the ideal case of unity $K_{eq}$ between intermediates.[34] The biochemical literature indicates that the evolutionary improvement of the catalytic efficiency of enzymes, in comparison to their nonenzymic solution analogs, can be separated into three broad types of increasingly difficult alterations to the ΔG profile: uniform binding, differential binding, and catalysis of elementary steps.[34b−d] Differential binding involves, according to Albery and Knowles,[34b] "changes in the relative stabilities of the internal intermediates, and the consequential effects on the internal transition states". The implication, then, is that MMCoA mutase is using differential binding to accelerate the rate of Co−C bond cleavage. Elsewhere we have discussed how such differential binding—the expression of intrinsic binding energy along the Co−C cleavage reaction coordinate—fits the available enzymic and chemical model data for the $B_{12}$-dependent enzymes.[9,11e]

**Further Interpretaion of the Observed Results in Terms of Steric and Electronic Effects.** For the purpose of interpreting the results presented herein as well as for understanding the effects of histidine imidazole as an axial base to cobamide-containing enzymes such as methylmalonyl Co-A mutase the question naturally arises of the different *steric* and *electronic* effects of imidazole vs, say, pyridine (pyridine being a common reference point both in the literature, *vide infra*, as well as in our prior studies[10a]). In all the discussion which follows, it is crucial to remember that the present work requires (as Figure 5 illustrates) that the observed acceleration of Co−C cleavage is due to an enhanced Co(II)−N (for homolysis) or Co(III)−N (for heterolysis) binding *at the transition states* of these parallel reactions.

Steric effects are best discussed first, given that there is an extensive literature[6−8,11f] indicating that alkylcobamide complexes can show steric accelerations of Co−C homolysis of $10^2$−$10^6$ (see elsewhere[11f] for additional lead references to the work of Schrauzer and Grate, Glusker, Marzilli and Randaccio, Halpern, Pratt, Brown, and others). A somewhat smaller steric effect and thus shorter Co−N bond for imidazole vs pyridine is what one expects given imidazole's sterically less encumbered five-membered ring vs pyridine's larger six-membered ring (and accompanying heterocyclic ring C−N−C angles of ∼105° vs ∼120°, respectively[7l]). Note that this expected smaller steric effect of imidazole should synergistically enhance imidazole's electronic effect of greater σ electron donating ability (p$K_a$ = 7.0, Table 1) vs that of pyridine (p$K_a$ = 5.2, Table 1)—that is, the closer approach[7l] of an intrinsically more basic ligand should synergistically enhance the σ donation to cobalt. Hence, an accelerated Co−C cleavage rate is expected for imidazole vs pyridine, just as we see, Table 2.

No further discussion would be needed were it not for several additional key facts: Table 2 reveals that the *less basic* N-MeIm (p$K_a$ = 7.0) gives a 3.6(±1.0)-fold enhancement of Co−C homolysis ($k_{on,h}$; Table 2) and an identical, 3.6(±1.0)-fold enhancement of Co−C heterolysis ($k_{on,het;}$ Table 2) in comparison to the Δp$K_a$ 2.7 more basic $Me_2N$−py (p$K_a$ = 9.7). Since σ-donation effects alone would give the opposite results, *some effect other than just* σ-donation (i.e., at identical Co−N distances) must be operative. Again, this could be just the effect of a shorter Co−N(Im) bond in the case of imidazole and the resultant enhanced σ-donation at a shorter Co−N distance. However, a further scrutiny of the data in Table 2 for the isosteric ligands pyridine (p$K_a$ = 5.2) and the significantly more basic $Me_2N$-pyridine (p$K_a$ = 9.7; Δp$K_a$ 4.5) reveals that these two different basicity axial bases have *identical homolysis rates*, but that the more basic $Me_2N$-py has a 17(±7)-fold faster Co−C heterolysis. This same effect can be seen in the cross comparison of the N-MeIm and the pyridine data in Table 2: only a 3.6(±1.8)-fold increase is seen in the homolysis rate constant, $k_{on,h}$, yet there is more than an order of magnitude larger, 60(±19)-fold rate increase in heterolysis, $k_{on,het}$ when comparing N-MeIm to pyridine. In short, while σ-donor ability appears (not surprisingly) to be controlling Co−C *heterolysis* via its stabilization of the "$H^+$··Ado$^-$··Co(III)$^+$−base" transition state, the enhancement of Co−C *homolysis* reaches a leveling effect with respect to increasing axial-ligand basicity and σ-donation Hence, *some other effect is controlling* Co−C

(33) (a) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman: San Francisco, 1979; pp 30−33. (b) Abeles, R. H.; Frey, P. A.; Jencks, W. P. *Biochemistry*; Jones and Bartlett: Boston, 1992; pp 146−149.

(34) (a) Albery, W. J.; Knowles, J. R. *Biochemistry* **1976**, *15*, 5627. (b) Albery, W. J.; Knowles, J. R. *Biochemistry* **1976**, *15*, 5631. (c) Knowles, J. R.; Albery, W. J. *Acc. Chem. Res.* **1977**, *10*, 105. (d) Chin, J. *J. Am. Chem. Soc.* **1983**, *105*, 6502. (e) Benner, S. A. *Chem. Rev.* **1989**, *89*, 789.

*homolysis*. Again, the shorter imidazole Co−N(Im) distance expected on the basis of steric effects could be the answer, but there is a good possibility based on the literature (*vide infra*) that the ability of imidazole to $\pi$ bond is also important in the observed effects.

Recall also that in the $RCoCbi^+$ *ground state*, the axial-base thermodynamic measurements herein showed that $\pi$-, not $\sigma$-, bonding effects must be primarily responsible for the more negative $\Delta H$ and implied stronger Co−N(Im) BDE over any of the pyridines tested, since even the $\Delta pK_a$ 2.7 *more basic* $Me_2N$-py has an up to 1.3 (±1.1) kcal/mol *less* favorable $\Delta H$ value. And, since (as previously pointed out[10a]) base binding is stronger in Co(II)−N, and since Co(II)−N is closer in energy to, and thus a better model for, the Co---C homolysis transition state (Hammond's postulate), it follows that $\pi$-bonding effects are probably also crucial in achieving the more important transition-state stabilization.

**The Literature of Imidazole $\sigma$-Donor and $\pi$-Donor or Acceptor Electronic Effects.** It occurred to us that there should be relevant bioinorganic literature on imidazole $\sigma$- and $\pi$-effects due to the Fe(porphyrin)·imidazole hemoprotein models, and indeed a literature search uncovered several especially useful papers. Work done on Fe(II) systems by Silver[35] and Scheidt and Chipman[36] provides evidence that imidazole is special among aromatic-nitrogen, donating ligands. Silver finds, through careful examination of the Soret bands of protoporhyrins with N-donor ligands, that imidazole is a somewhat stronger $\sigma$-donor than simply (un-*para*-substituted) pyridine-type ligands (as expected, given imidazole's $\Delta pK_a$ 1.8 unit greater proton basicity vs that of pyridine). In addition, he suggests based on Mössbauer spectroscopy that imidazole can be a $\pi$-accepting (backbonding) ligand via its LUMO,[37] Figure 6a, at least in combination with an Fe(II)(protoporphyrin) $\pi$-donor.[35]

Scheidt and Chipman provide charge-iterative Extended-Hückel MO calculations in support of imidazole's p-$\pi$ HOMO, Figure 6b, interacting with *p*-$\pi$ orbitals of the metal in an imidazole $\pi$-donor interaction. A series of complexes, including low spin $d^7$ Co(II)(porphyrin)(imidazole) as well as high-spin $d^5$ Fe(II)(porphyrin)(2-MeIm) and $d^6$ [Fe(III)(porphyrin)(2-MeIm)]$^+$ were examined and compared the the crystallographically determined structures of these complexes. All of their findings provide good support for an imidazole p-$\pi$ to metal p-$\pi$ bonding picture in the metalloporphyrin complexes, specifically (a) that there is a preferred rotational orientation of the imidazole about the R-metal(porphyrin)-base axis (showing a dependence on the metal's spatially oriented p orbitals) and (b) that this preferred orientation is, as expected, *independent* of

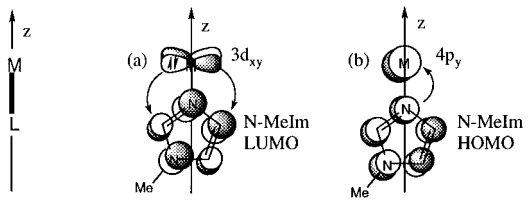



**Figure 6.** Possible $\pi$ interactions between N-MeIm's HOMO and LUMO and a metal's d and p orbitals from the Spartan MO calculation (consistent with previous literature)[35,36,37b] shown looking at the imidazole ring face-on, such that the $\pi$, p, and d orbitals extend in front of (and behind) the plane of the paper. (a) The stabilizing $\pi$-interaction ($d\pi \rightarrow p\pi$) between N-MeIm's vacant LUMO and the filled $d_{xy}$ orbital on the metal.[35] (b) The stabilizing $\pi$-interaction ($p\pi \rightarrow 4p\pi$) between N-MeIm's filled HOMO and a vacant $4p_y$ orbital on the metal.[36]

metal oxidation state, $d^n$, or the *trans*-axial ligand opposite the imidazole. In addition, Veillard[37a] discusses a *destabilizing* $\pi$ interaction between the N-MeIm HOMO and the $d_{xy}$ orbital of the metal.[37d] However, this destabilization is negated by the $d_{xy}$ − N-MeIm LUMO stabilizing interaction in Figure 6a.

This literature reveals that [AdoCbi·N-MeIm]$^+$ is an important target for additional crystallographic, computational, and other structure and bonding methods—does it exhibit a preferred orientation for imidazole? In addition, it will prove fascinating *if* any imidazole preferred orientation effects are found, to then see how they compare to (a) the orientation of the appended, and thus fixed, 5,6-dimethylbenzimidazole in AdoCbl, and (b) any putative preferred orientation that can be identified within the AdoCbl·imidazole−enzyme complex itself.

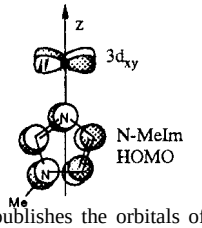
A collection of other literature relevant to imidazole as an axial ligand in $B_{12}$ or $B_{12}$-model complexes is also available.[38] One useful but not surprising observation from this data is that imidazole is typically different in its physical properties in comparison to pyridine ligands.[38e]

**Implications for Whether Alkyl-Cobamides are Five- or Six-Coordinate in Solution.** An old issue in the $B_{12}$ literature is whether or not $RCbi^+$ are five- or six-coordinate in solution[39ab] (i.e., $RCbi^+$ or [RCbi·solvent]$^+$), an issue that is an important subtlety in the proper interpretation of the axial-base binding $\Delta H$ and $\Delta S$ thermodynamic parmeters reported herein and in the literature. The observation that the $RCbi^+$ nitrogenous axial-base binding $\Delta H$ data herein, *and in the literature* (see Tables 2 and 3 elsewhere[9]), are all in the $\Delta H = 0-7.8$ kcal/mol range, *plus* the fact that even non-basic solvents such as alkanes have

(35) Al-Jaff, G.; Silver, J.; Wilson, M. T. *Inorg. Chim. Acta* **1990**, *176*, 307.

(36) Scheidt, W. R.; Chipman, D. M. *J. Am. Chem. Soc.* **1986**, *108*, 1163.

(37) (a) Basic MO theory of course dictates that the donor (or acceptor) properties of any ligand also depend on the relative energetics of the *conjugate* acceptor (or donor). (b) An early paper on conformational preferences of the axial-ligands in metalloporphyrins: Rohmer, M.-M.; Strich, A.; Veillard, A. *Theoret. Chim. Acta (Berl.)* **1984**, *65*, 219−231. (c) Hoffmann's classic MO paper on six-coordinate complexes: Hoffmann, R.; Howell, J. M.; Rossi, A. R. *J. Am. Chem. Soc.* **1976**, *98*, 2484. (d) Veillard's[37b] destabilizing filled−filled ($p\pi \leftrightarrow d\pi$) interaction:




Note that Veillard only publishes the orbitals of the α-carbons; the full molecular orbitals shown here and in Figure 6 result from the minimization in the Spartan program (as described in the Experimental Section).

(38) (a) Pahor, N. B.; Attia, W. M.; Geremia, S.; Randaccio, L. *Acta Crystallogr.* **1989**, *C45*, 561. Note the 2.083 to 2.086 Å Co(III)−N(Im) bond lengths in the two RCo(cobaloxime)(1,2-dimethylimidazole) structures determined therein. (b) Bigotto, A.; Zangrando, E.; Randaccio, L.; *J. Chem. Soc., Dalton Trans.* **1976**, 96. Note also the 0.01 Å shorter Co−N bond distance therein for N-MeIm vs pyridine as the base in MeCo(cobaloxime)(base). (c) The only Co−C BDE where imidazole is the *trans*-axial ligand is for PhCH(Me)Co(cobaloxime)(Im): Ng, F. T. T.; Rempel, G. L.; Halpern, J. *J. Am. Chem. Soc.* **1982**, *104*, 621 (see entry 6 in Table 1). (d) Marques, H. M.; Marsh, J. H.; Mellor, J. R.; Munro, O. Q. *Inorg. Chim. Acta* **1990**, *170*, 259. (e) Pratt has established, at least for non-alkyl cobamides, a linear free-energy relationship of increasing log $K$ with axial-base $pK_a$, $\log K = a(pK_a) + b$(where $a$ = positive): Hamza, M. S. A.; Pratt, J. M. *J. Chem. Soc. Dalton Trans.* **1994**, 1377 (and earlier papers in this series i.e., Part 29, p 1373). Of special interest to the present work is that distinct $b$ values (i.e., the log $K$ at a hypothetical $pK_a = 0$) are seen for pyridine vs imidazole bases. Note, however, that there are unresolved issues in this work of composition and α,β-structural isomerism in the resulting [($H_2O$, CN)Cbi·base]$^+$ product complexes.

(39) (a) See the discussion and references in footnote 25 elsewhere[12] to the work of others on this issue. (b) The finding of a six-, not five-, coordinate, solvated complex is just what organometallic chemistry has found in other systems and thus predicts, see: Strauss, S. *Chem. Rev.* **1993**, *93*, 927. (c) Collman, J. P.; Hegedus, L. S.; Norton, J. R.; Finke, R. G. *Principles and Applications of Organotransition Metal Chemistry*; University Science Books: Mill Valley, CA, 1987; see p 250 and references therein.

metal-ligand bond energies of ca. 8 ± 3 kcal/mol,[39c] means that the $\Delta H$ measured herein must be for *displacement* of a solvent molecule, that is, for the equilibrium between six-coordinate species: [AdoCbi·solvent]$^+$ + base ⇌ [AdoCbi·base]$^+$ + solvent. This means, in turn, that the true RCo(III)Cbi$^+$−base bond energies are the measured $\Delta H$ + ca. 8 kcal/mol or ca. 16 (± ca. 3) kcal/mol for AdoCbi$^+$---N-MeIm. This then implies that the enzyme AdoCbl−Im bond strength could be up to ca. 16 kcal/mol for a "normal" length Co(III)−N(Im) bond, since AdoCbl should be delivered to the enzyme as AdoCbl (and not as 5,6-$Me_2$BzIm base-off, solvent base-on, "AdoCbl·solvent"). In other words, the enzyme can, in principle, destabilize the AdoCbl ground state *by values approaching 16 kcal/mol*, that is, up to 8 kcal/mol more than the 8 kcal/mol shown back in Figure 5 (and in the limit of a very long Co−N(Im) (non-) bond, one somewhat longer than even the long Co(III)−N(Im) bond length seen in MMCoA mutase).

According to one of the referees for this paper, the above evidence for [RCbi·solvent]$^+$ "has profound effects for the interpretation of the thermodynamic values". We agree. One such issue, pointed out by the referee, is the initially surprising $\Delta S = -11$ to $-28$ eu values given in Table 1 (surprising, the referee noted, since a positive contribution to $\Delta S$ is expected for the release of bound solvent). We would add that contributions (and their arithmetic sign) to $\Delta S$ should include (but are not limited to) the loss of translational, vibrational, and rotational entropy of the added base (negative); the loss of vibrational and rotational entropy, following axial-base binding, by the otherwise highly flexible corrin (negative; and perhaps a major contributor); the entropy *gain* by the released solvent (positive); the entropy gained by release of solvent H-bonded to the unbound base (positive); and any entropy changes due to H-bond changes to the corrins side chains and the other, >16 total H-bonding sites in AdoCbl.[11e] The observed axial-base binding $\Delta S$ is, therefore, *a complicated composite*, the deconvolution of which will be required before any more insightful interpretation is possible.

## Summary

To summarize, the most important conclusions from the present work are as follows.

(1) N-MeIm binds to AdoCbi$^+$ with a $\Delta H = -7.8 \pm 0.4$ kcal/mol, a $\Delta H$ the same within experimental error of that for the intramolecularly appended 5,6-dimethylbenzimidazole in AdoCbl ($\Delta H = -7.6 \pm 0.2$ kcal/mol), and a $\Delta H$ −1.3 kcal/mol more favorable than seen for pyridines.

(2) $\pi$-, not $\sigma$-, bonding effects must be primarily responsible for this more negative $\Delta H$ and implied stronger Co−N(Im) BDE, since even the $\Delta pK_a$ 2.7 more basic $Me_2$N-py has an up to 1.3 (±1.1) kcal/mol less favorable $\Delta H$ value.

(3) N-MeIm changes the mode of Co−C cleavage in adocobamides from ≥98% homolysis in AdoCbl to 50% homolysis, 50% heterolysis in [AdoCbi·N-MeIm]$^+$.

(4) N-MeIm increases the rate of Co−C heterolysis *and* homolysis by record amounts (i.e., for bases examined to date), by 350 and 8, respectively, vs AdoCbl with its appended 5,6-dimethylbenzimidazole axial base (and by 30 700 and 870, respectively, compared to ethylene glycol as the axial base).

(5) The present chemical reference point studies reveal that enzymes in the methylmalonyl-CoA mutase, imidazole base-on subclass,[4] apparently either (i) *utilize* Co−C heterolysis or perhaps more likely (ii) *prevent* Co−C heterolysis. Three chemically precedented ways that the enzyme could prevent Co−C heterolysis were presented (restriction of the $H^+$ required for Ado-$\beta$-oxygen protonation prior to Co−C heterolysis, conformational restriction of the ribose ring; or manipulation of the Co−N(Im) bond length). However, additional studies are needed to support or refute these possibilities.

(6) The recent MMCoA mutase crystal structure and MO calculations in the literature as well as the chemical precedent studies presented herein suggest that the enzyme may lengthen the Co−N(Im) bond length for the purposes of more efficient catalysis: specifically, the enzyme may enforce a long Co−N bond length in the AdoCo(III)Cbl starting material that is, however, closer to ideal for the Co(II)−N(Im) enzymic product. Chemical models for these possibilities are also needed and are under investigation.

(7) A detailed examination of the N-MeIm vs pyridine and $Me_2$N-pyridine homolysis and heterolysis rate constant data reveal that $\sigma$-donation dominates the transition state stabilization for Co−C *heterolysis* but that other factors besides steric-size-enhanced $\sigma$-donation, notably $\pi$-effects, appear to be involved in achieving N-MeIm's record effects on Co−C *homolytic* cleavage.

Finally, many of the chemical model, enzymic structual and computational studies required to confirm or refute the ideas in Figure 5 and in points (5), (6), and (7) above have been noted, ideas that should provide fertile ground for additional research.

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**Supporting Information Available:** ln $K_{assoc}$ vs $1/T$ plot, titration of Co(II)Cbi$^+$ with N-MeIm, and key kinetic derivations and data analysis (7 pages). See any current masthead page for ordering and Internet access instructions.

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