Adocobinamide, the Base-off Analog of Coenzyme B_{12} (Adocobalamin). 2.[1] Probing the "Base-on" Effect in Coenzyme B_{12} via Cobalt−Carbon Bond Thermolysis Product and Kinetic Studies as a Function of Exogenous Pyridine Bases

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Abstract: The thermolysis of the Co−C bond in adocobinamide ($AdoCbi^+BF_4^-$) in anaerobic ethylene glycol has been studied as a function of a series of *para*-substituted pyridine axial bases using the TEMPO radical-trapping method. In contrast to the slower rates of Co−C cleavage previously found for benzylcobinamide, neopentylcobinamide, and the (α-phenylethyl)cobaloxime coenzyme B_{12} models, for $AdoCbi^+$ the rate of total Co−C cleavage becomes *faster* as the *para*-substituted pyridines become more electron-donating. Specifically, the 110 °C k_{obsd} for $AdoCbi^+BF_4^-$ *total* Co−C cleavage increased 23-fold on going from 1 M pyridine (py) to 1 M *p*-(dimethylamino)-pyridine (Me_2N-py). However, HPLC product studies reveal that the percentage of abiological Co−C *heterolysis* increases (to a limiting value); that is, Co−C heterolysis is a major reason for the observed rate increase seen for Me_2N-py. Deconvolution of the k_{obsd} rate constant into its heterolysis and homolysis components yields values of the 110 °C $k_{heterolysis}$ and $k_{homolysis}$ for $AdoCbi^+$·base for Me_2N-py and pyridine. These data in turn reveal that the $AdoCbi^+BF_4^-$ base-on homolysis rate constant *does not* increase within experimental error as one goes from py to the more basic Me_2N-py ($k_{on,h} = 8(3) \times 10^{-4}$ and $7(1) \times 10^{-4}$ s^{-1}, respectively), but that the base-on heterolysis rate constant changes by 17-fold ($k_{on,het} = 0.4(0.1) \times 10^{-4}$ and $7(1) \times 10^{-4}$ s^{-1} for py and Me_2N-py, respectively). The plausible biological significance of these results is then discussed, notably the heretofore unsubstantiated idea, first suggested by Mealli, Sabat, and Marzilli, that one major evolutionary pressure for selecting and appending the 5,6-dimethylbenzimidazole axial base in coenzyme B_{12} is because this base *limits Co−C bond heterolysis*, thereby promoting the biologically relevant Co−C cleavage reaction, Co−C homolysis.

Introduction

Coenzyme B_{12} (or adocobalamin, AdoCbl)[2] contains an intramolecularly appended 5,6-dimethylbenzimidazole base which can exist in either an α-axial base-on or α-axial base-off form, Figure 1.[3] In a preceding paper[1b] we presented (i) experimental K_{assoc}, and ΔH, and ΔS (where measurable) for a series of 14 exogenous axial bases and (ii) a critical analysis of

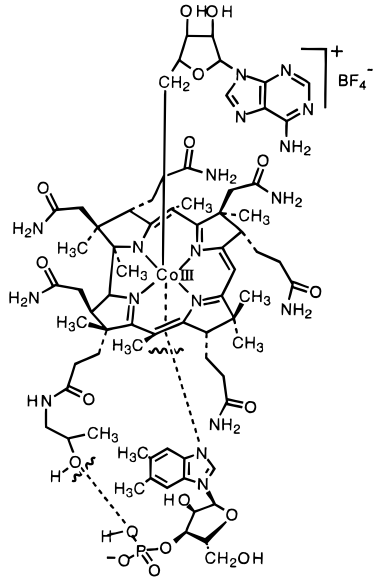

Figure 1. A composite representation of (5′-deoxy-5′-adenosyl)-cobinamide ($AdoCbi^+BF_4^-$) plus, in the lower part of the figure, the α-ribazole (1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole) fragment produced by $Ce(OH)_3$-catalyzed H_2O addition across the phosphodiester bond in the adocobalamin (AdoCbl or, equivalently, coenzyme B_{12}) synthetic precursor. 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.

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(1) (a) This paper is based in part on the following dissertation: Garr, C. D. Adocobinamide (Axial Base-off Coenzyme B_{12}) Equilibria and Co-C Bond Cleavage Kinetic Studies: Mechanistic Probes into the Function of Coenzyme B_{12}'s Axial Base. Ph.D. Dissertation, February 1993. (b) Part 1: Garr, C. D.; Sirovatka, J. M.; Finke, R. G. *Inorg. Chem.* **1996**, *35*, 5912. (c) A list of lead references is given in the first of these back-to-back publications and ref 4 therein.[1b] (c) Studies of $Co^{II}Cbi^+$ + base K_{assoc}, ΔH, and ΔS: Sirovatka, J. M.; Garr, C. D.; Finke, R. G. Unpublished results and experiments in progress. (d) Part 3: Sirovatka, J. M.; Finke, R. G. Studies of the K_{assoc}, ΔH, ΔS and Co-C product and kinetic studies of the N-Me-imidazole adduct of $AdoCbi^+$, $[AdoCbi^+(N\text{-}Me\text{-}Im)]^+$. Submitted for publication.

(2) 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 (5′,8-anhydroadenosine); Ado-H (5′-deoxyadenosine); py (pyridine); *p*-CN-py (4-cyanopyridine); *p*-Me-py (4-methylpyridine); *p*-NH_2-py (4-aminopyridine); *p*-Me_2N-py [4-(dimethylamino)-pyridine].

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the previously confusing literature concerning the biological role(s) of the nucleotide loop and its appended 5,6-dimethylbenzimidazole axial base in AdoCbl.[4−6] Concisely put, a summary of the biochemical literature of the coenzyme B_{12} "axial-base problem" [1] suggests that the nucleotide loop and its appended 5,6-dimethylbenzimidazole (i) are crucial for tight binding of the cofactor to both the B_{12}-dependent rearrangement and the B_{12} transport enzymes and (ii) are pivotal in maintaining the correct protein folding conformations necessary to accomplish tight binding and control the radical intermediates (thereby preventing destructive side reactions during the catalytic cycle, at least for the enzyme diol dehydratase[7]), yet (iii) *direct coordination* of the 5,6-dimethylbenzimidazole base is not *absolutely* required for the initial Co−C homolysis in either of the two subclasses[6c] of AdoB_{12}-dependent enzymes. Hence, this biochemical literature suggests that the 5,6-dimethylbenzimidazole is less crucial than previously thought for the Co−C homolysis step even in the subclass of B_{12}-dependent enzymes in which 5,6-dimethylbenzimidazole is believed to be directly coordinated. However, the question of whether or not a coordinated 5,6-dimethylbenzimidazole is in some way crucial for the Co−C homolysis step (i.e., in the 5,6-dimethylbenzimidazole base-on subclass of B_{12}-dependent enzymes) was, ultimately, left unanswered by the biochemical investigations. It is, therefore, the specific question that is the focus of the present chemical precedent studies.

The recent X-ray crystal structure of AdoB_{12}-dependent (methylmalonyl)CoA mutase[6d]—the first single-crystal structure determination of an *Ado*B_{12}-dependent enzyme—also raises several unanswered questions. The key finding is that the 5,6-dimethylbenzimidazole is *not* coordinated to cobalt but instead has been replaced by a histidine side-chain imidazole,[6d] a finding first demonstrated for MeB_{12}-dependent methionine synthase.[6e] This raises further questions of the role(s) of *imidazole* in the Co−C homolysis step (and in any other B_{12}-dependent steps). Those questions, and the needed studies of [AdoCbi·(*N*-methylimidazole)]$^+$, have just been completed and will be reported elsewhere.[1d]

The Base-on Effect in Cobalamins and Cobinamides. The general role of the axial 5,6-dimethylbenzimidazole base in AdoCbl, both in the AdoCbl·enzyme complex and in solution, has been lumped somewhat imprecisely under the term the "base-on effect".[8] As early as 1969, Hill, Pratt, and Williams proposed[9] that corrin ring distortions enhance steric interactions with the adenosyl ligand, thereby promoting Co−C homolysis ("the butterfly effect").[10] This idea sat dormant until nearly a decade later, when Schrauzer and Grate expanded on the original idea by coining the term "mechanochemical trigger", thereby suggesting that compression of the Co-N (α-axial base) bond "triggered" corrin ring−adenosyl steric interactions.[11] Other contributions quickly followed, with contributions from the laboratories of Glusker, Marzilli and Randaccio, Pratt, Brown, Halpern, and others, including our own contributions.[4−6,8,10,11b−h] Our measurement in 1984 of the AdoCbl Co−C homolysis rate outside the enzyme (i.e., in solution) revealed the remarkable $10^{12\pm1}$ enzymic acceleration of Co−C homolysis;[12] this in turn led to enhanced interest in the butterfly effect or mechanochemical trigger possibilities, as well as interest in the possible role of the axial 5,6-dimethylbenzimidazole base in these proposed mechanisms.[13] Indeed, the exact explanation for the *enzymic* $10^{12\pm1}$ acceleration of AdoB_{12}'s Co−C cleavage has become one of the most important questions in mechanistic coenzyme B_{12} science.

In a subsequent study,[12b] the axial base in AdoCbl was chemically removed and the resultant base-off AdoCbi$^+$ was shown to have a Co−C homolysis rate *only* 10^2 *less* in comparison to base-on AdoCbl; that is, the demonstrated rate effect of the 5,6-dimethylbenzimidazole (at least outside the enzyme) is 10^2 and not anything like the enzymic 10^{12}. Consistent with the above findings, Toraya and co-workers' recent contributions[7] (discussed in detail in a preceding paper[1b]) show that Co−C homolysis still occurs in at least one 5,6-dimethylbenzimidazole base-free analog of AdoCbl, adenosylcobinamide methyl phosphate, AdoCbi-P-Me (i.e., in the methylated phosphate, but 5,6-dimethylbenzimidazole free, derivative of AdoCbl). This is *not* to say, however, that an *enzyme-induced* butterfly effect is ruled out; indeed, the literature indicates that most current B_{12} scientists believe that an *enzyme-induced* corrin ring distortion is a key to triggering Co−C homolysis in the AdoCbl·enzyme complex. What has been missing until now has been a study of Co−C thermolysis of *Ado*Cbi$^+$·base as a function of different axial bases: Just what, if anything, is special about 5,6-dimethylbenzimidazole?

Other important work includes findings by the Randaccio and Marzilli team indicating that the axial 5,6-dimethylbenzimidazole serves to keep Co^{III} in the plane of the corrin ring and can stabilize Co^{II}Cbl (the product of Co−C homolysis).[14] Elsewhere[1b] we have directly confirmed this latter point by demonstrating that Co^{II}Cbi$^+$ axial-base K_{assoc} constants are *larger* than those

(4) See refs 4−8 elsewhere[1b] and references therein.

(5) See refs 9−13 elsewhere[1b] and references therein.

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for AdoCo^{III}Cbi$^+$.[1d] Other important literature includes Marzili's review,[6] a key paper by Brown and co-workers thermolizing benzyl- and neopentyl-Cbi$^+$ in the presence of exogenous axial bases,[8] a paper which provides an enzyme-free chemical model exhibiting 10^6 of the enzymic 10^{12} acceleration,[15] and the use of EXAFS[16a] and X-ray edge spectroscopy[16b] (see rebuttals to some of this work, however[16c,d]) to further probe the role of the axial base in AdoCbl.

Non-Ado Alkylcobamide Kinetic Studies as a Function of Exogenous Axial Base. There are a few related RCbl and RCbi$^+$·base studies (R ≠ Ado) that constitute important background material, especially since rather different results will be uncovered herein for AdoCbi$^+$. Methyl-,[17] cyclohexyl-,[18a] and neopentyl*cobalamins*[19] show an increase in their *photolytic* Co−C homolysis rates in the presence of 0.5 M imidazole (Im); no explanation for this unexpected finding (for these already *5,6-dimethylbenzimidazole base-on* cobalamins) has been reported. [It could conceivably be the effect of forming the imidazole base-on alkyl-Cbl·Im or, more likely, the effect of radical-cage pair trapping of Co^{II}Cbl by Im (i.e., forming Im·Co^{II}Cbl), *vide infra*.] Likewise, methyl-Cbi$^+$ shows an increased rate of photolysis in the presence of cyanide or pyridine,[17] isopropyl-Cbi$^+$ shows a rate increase of ca. 10^2 in the presence of imidazole or 5,6-dimethylbenzimidazole,[18b] but methyl-Cbi$^+$ photolyzes more slowly (to incompletely characterized products) in the presence of imidazole.[17]

Brown and Brooks[8] examined the thermal Co−C bond kinetics of neopentyl- and benzylcobinamide Co−C homolyses in H_2O in the presence of a variety of exogenous bases. Both benzyl- and neopentyl-Cbi$^+$ showed *enhancements* in their Co−C bond homolysis rates, in comparison to the base-free RCbi$^+$, at least in the presence of pyridine, *p*-Me-py, *p*-NH_2-py, and imidazole, and in the interesting case of azide. The authors concluded that this aspect of the base-on effect is primarily steric in origin.[8] However, for these two alkylcobinamide, [RCbi·base]$^+$, complexes, the more electron-donating the axial base, the *slower* the observed rate of *thermal* Co−C homolysis.

B_{12} Model Co−C Bond Cleavage Kinetic Studies as a Function of Exogenous Base. Many kinetic studies of Co−C bond cleavage have focused on alkylcobaloximes as "B_{12} model" compounds.[13b,20,23] Unfortunately, the most desirable model studies are missing, those probing the rate of Co−C homolysis within five-coordinate, base-off *Ado*B_{12} model compounds, plus their comparison to the (also missing) base-on *Ado*B_{12} model (i.e., reference) compound. The available studies do show that, as the steric bulk of the alkyl ligand increases, concomitant increases in the Co−C bond length,[21] and decreases in the Co−C bond dissociation energy, are observed.[13b,20a] Near infrared FT-Raman spectroscopic data show that the steric nature of the trans ligand influences the Co−C bond stretching frequency by up to 31 cm^{-1}.[22] Overall, a recurring trend is firmly established *in at least these B_{12} model complexes*: steric effects of the *trans*-alkyl and axial-base ligands dominate, with the bulkier either ligand, the weaker the Co−C bond.[23]

Electronic effects of the axial ligands on the rate of Co−C bond homolysis in (α-phenylethyl)(L)cobaloximes (where L is a *para*-substituted pyridine) have been examined by Halpern and co-workers in an already classic, often cited study.[20c,d] By using a series of electronically different but isosteric *para*-substituted pyridines, ranging from the electron-withdrawing *p*-CN-py to the electron-donating *p*-NH_2-py, these authors convincingly demonstrate that, as the electron-donating ability of the base increases, the rate of Co−C homolysis *decreases*. These observations were rationalized in terms of R−Co^{III} ground-state stabilization (i.e., rather than any effect on the R···Co^{II} homolysis transition state). However, an experimental test of whether or not these B_{12} *model* trends would translate to the $AdoB_{12}$ cofactor itself had not been carried out until now.

Present Studies of AdoCbi$^+$·Base. Herein we report thermal Co−C bond cleavage kinetic studies of adocobinamide in the presence of a series[20] of electronically different, but isosteric, *para*-substituted pyridines. Complete product analyses demonstrate a rather different base-on effect for AdoCbi$^+$; in particular, *Co−C heterolysis dominates for the more basic pyridine axial bases tested*. Moreover, for [AdoCbi·base]$^+$ the Co−C heterolysis rate *increases* rather than decreases as the base is changed along an increasingly electron-donating series of *para*-substituted pyridine axial bases. The present studies, plus those of the biologically relevant *N*-methylimidazole congener, [AdoCbi·(*N*-MeIm)]$^+$, available elsewhere,[1d] provide the first firm chemical precedent and reference point from which to interpret the role of the axial 5,6-dimethylbenzimidazole (or the histidine *N*-MeIm[1d]) in the biological cofactor itself, *Ado*Cbl.

Experimental Section

Chemicals. AdoCbl (98%, Sigma), adenine (Sigma), and 5-deoxyadenosine (Sigma) were stored at 0°C and used as received. 5′-Deoxy-5′-TEMPO-adenosine (99% by HPLC) and 5′,8-anhydroadenosine (98% by HPLC) were obtained as described previously.[24] 4′,5′-Didehydroadenosine (97% pure by HPLC) was a gift from Syntex Discovery Research. 1,8-Bis(dimethylamino)napthalene (Proton Sponge; Aldrich; mp 48−51 °C, lit.[26c] mp 49−51 °C) was used as received. Reagent grade pyridine (MCB) and ethylene glycol (Baker) were freshly vacuum distilled from 4 Å molecular sieves, while *p*-(dimethylamino)pyridine (Aldrich) was used as received. The nitroxide TEMPO (Aldrich) was sublimed before use (mp 38−39 °C, lit.[25] mp 37−39 °C). All other materials were obtained as previously described.[1b]

Instrumentation. UV−vis spectra were recorded on a Beckman DU-7 spectrophotometer equipped with a built-in thermoelectric Peltier

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cell block temperature controller (25 ± 1 °C). The temperature in the oil bath (110.0 ± 0.2 °C) employed in the kinetic experiments was measured using an NBS-calibrated thermometer with ±0.2 °C gradations and controlled by a Pt thermocouple (Omega) attached to a Variac. All samples were prepared in a Vacuum Atmospheres inert atmosphere glovebox using Schlenk-type[12] UV−vis cuvettes. A Waters HPLC (λ = 260 nm detector), equipped with a PRP-1 semipreparative C_{18} column (Hamilton), 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.

Product Studies. Nucleoside products were determined by HPLC, as previously described,[11e,12,24,28] *using the exact same samples as used for the individual kinetic runs* (unless noted otherwise). Conditions employed were 10% CH_3CN/90% H_2O at 2 mL/min flow, for all nucleosides (except 5′-deoxy-5′-TEMPO-adenosine, which is retained on the column under these conditions, and hence was eluted using 30% CH_3CN/70% H_2O). Unless otherwise noted, nucleosides were identified and quantitated by comparison to authentic samples and their independently determined HPLC detector response factors; the latter were obtained from linear plots of peak area vs nucleoside concentration (1×10^{-3} to 5×10^{-5} M).

Since the PRP-1 HPLC column is stable from pH 1 to pH <13, only solutions containing [Me_2N-py] ≤ ca. 0.3 M (and thus where the solution pH ≤ ca. 9.4) were analyzed for nucleoside products (Me_2N-py has a pK_a of 9.7[26a]). Similarly, when pyridine was the base, product analyses were performed only for 0.3 M (or less) pyridine in order to provide product data obtained under comparable conditions.

Product Control Experiments. To test for any possible effect of 0.3 M Me_2N-py on the stability of TEMPO in ethylene glycol, separate ethylene glycol solutions of 0.3 M Me_2N-py and 0.3 M Me_2N-py with 2×10^{-2} M TEMPO were prepared. Each sample was examined by HPLC before and after ca. 8 h of heating at 110 °C. As anticipated, no new peaks appeared in either sample.

Similar controls examined the effect of 0.3 M Me_2N-py and Me_2N-py with added TEMPO[27] on the nucleoside products, specifically the four AdoCbi$^+$ Co−C bond homolysis products[28] (5′,8-anhydroadenosine, 5′-deoxyadenosine, 4′,5′-didehydroadenosine 5′-deoxy-5′-TEMPO-adenosine) and the Co−C heterolysis product[12,28] (adenine). For these controls, ethylene glycol solutions containing ca. 1×10^{-4} M authentic nucleoside plus 0.3 M Me_2N-py were prepared with and without 2×10^{-2} M TEMPO. Each sample was examined by HPLC before and after heating for ca. 6 h at 110 °C. These proved to be important control experiments, as it was found that adenine (retention time 5.9 min) reacts with Me_2N-py to give 35−45% of a new peak (retention time 6.6 min; the sum of the adenine and the new peak accounted for 100 ± 5% of the adenine, assuming that the new peak had a HPLC response factor equal to adenine). In a similar control experiment, 4′,5′-didehydroadenosine had decomposed by 31−44%, but no new peak was detected employing 10% CH_3CN/90% H_2O. All other nucleosides were unaffected by the presence of 0.3 M Me_2N-py.

A control experiment was also done to see if the adenine byproduct (formed in the presence of base) could be recovered by the addition of acid (HOAc). The answer is "no"; in fact the addition of 0.3 M HOAc and heating at 110 °C (as one of the final, harsher treatments after lesser acid or no heating failed to recover any adenine) resulted in a ca. 20% *decrease* in adenine. Further details of this control experiment are available in the Supporting Information.

Product Control Experiment: Temperature Dependence Studies. The details of this experiment for a solution of 0.3 M Me_2N-py, 2×10^{-2} M TEMPO, and 1×10^{-4} M AdoCbi$^+$ are provided in the Supporting Information. The results show that the amount of heterolysis products at 110 °C (45%), 95 °C (35%), and 85 °C (40%) do not increase (within ±5%) over the accessible, 25 °C temperature range evaluated.

Product Control Experiments: Experimental Demonstration That the Heterolysis to Homolysis Product Ratio Is Invariant at High [Base]. Equation 3, one required for the deconvolution of the k_{obs} into the desired $k_{on,het}$ and $k_{on,h}$ (see Scheme 2 for a definition of these rate constants), requires measurement of the product ratio (defined as the ratio of heterolysis to homolysis products, eq 3) under conditions where it is *independent of the concentration of added base* (see eq 3 and especially its derivation provided in the Supporting Information). In order to verify this, a series of control experiments for both Me_2-N-py and py were performed, looking at the final heterolysis product values with the [base] ranging from 0.1 to 0.6 M [0.6 M base is the maximum where the HPLC conditions still allow the products to be analyzed since, at high [base], the large base peak overwhelms, broadens, and otherwise interferes with the homolysis product peaks (see Table 1), rendering their quantitation unreliable].

The control experiments which follow were done by J.M.S. after our move from Oregon, so that the HPLC, column, and conditions used were different from those detailed above. Specifically, a Hewlett-Packard Model Ti-1050 HPLC, equipped with a λ = 260 nm detector and a Alltech C18 Versapack reversed phase column, was employed. Product analysis was accomplished by eluting at 1.0 mL/min with an initial 95/5 water/acetonitrile gradient, ramping to a 70/30 water/acetonitrile gradient to elute Ado-TEMPO homolysis product, and then ramping to 10/90 water/acetonitrile to ensure complete elution of the base. (Since the column degrades at pH > 7.5, extra care was taken to ensure that all the base had eluted off the column.) In the case of Me_2N-py, 0.85 M TEMPO was used to ensure the highest mass balance (see the text and Table 1). Product quantitation was accomplished using HPLC response factors generated using authentic products.

The results, summarized in Table A in the Supporting Information (an expanded version of Table 1 containing all the product studies from this work), reveal that the percent heterolysis products (51 ± 7%) for Me_2N-py as the exogenous base *did not change within experimental error over the 0.1−0.6 M range*, indicating that eq 3 is valid. For the pyridine experiments, we used 2×10^{-2} M TEMPO, since complete product stoichiometry can be obtained with the lower amount of radical trap. These experiments were allowed to run for 5 days (87% completion), with the results again confirming that the product ratio is invariant within experimental error (5 ± 2% heterolysis; see Table A, Supporting Information, for full details).

Kinetic Studies. Samples prepared for kinetic runs were ca. 1×10^{-4} M AdoCbi$^+$ in ethylene glycol and contained [TEMPO] ranging from 0 to 0.85 M and [base] ranging from 0 to 2 M. Kinetic runs were carried out in general as previously reported:[11e,12,24,28] the cuvettes were immersed in a 110.0 ± 0.2 °C oil bath for predetermined time intervals, transferred after a prechosen time to a 20 °C bath for rapid thermal quenching to stop the reaction, and then equilibrated for >10 min at 25 °C in the UV−vis holding cell prior to obtaining the desired spectrum. (Note that these types of kinetic experiments, in which the temperature control (±0.2 °C) is accomplished *outside* of the UV−vis spectrophotometer, *do not* have any of the temperature control errors described recently by Brown and co-workers.[29]) Scans from 600 to 330 nm were recorded at the wavelengths of maximum absorbance for AdoCbi$^+$·base (520 nm), Co^{II}Cbi$^+$·base (474 nm), Co^{II}Cbi$^+$ (468 nm), and AdoCbi$^+$ (380 nm) [as reference points, the absorbance maxima of the analogous base-on cobalamins are (AdoCbl) 520 nm and (Co^{II}-Cbl) 474 nm.] Kinetic plots for the first-order appearance of total Co^{II}-cobamide (i.e., base-on plus base-off Co^{II}), d{[Co^{II}Cbi$^+$·base] + [Co^{II}Cbi$^+$]}/dt, were constructed by following the absorbance *increase* at 474 nm (see the derivation of kinetic equations in the Supporting Information) and proved of the highest precision and otherwise the most satisfactory (i.e., vs the absorbance changes at either 520 or 380 nm which were also surveyed). Note that the above expression simplifies to d([Co^{II}Cbi$^+$·base])/dt for the experiments at ≥1 M base. [The pyridine and Me_2N-py respective Co^{II}Cbi$^+$ K_{assoc} values at 25 °C (the temperature at which the visible spectra were obtained) are 23-(±4) and 166(±62),[1b] from which it is readily calculated that the percentage base-on Co^{II}Cbi$^+$·base is 70% (at 0.1 M py), 96% (at 1 M py), 94% (at 0.1 M Me_2N-py), and 99.4% (at 1 M Me_2N-py).] In all

(27) It has previously been demonstrated[12] that the presence of TEMPO does not effect authentic nucleosides; however, to duplicate exactly the reaction conditions (other than the presence of AdoCbi$^+$), the addition of TEMPO and Me_2N-py was employed in this control experiment.

(28) (a) Garr, C. D.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 10440. (b) Garr, C. D.; Finke, R. G. *Inorg. Chem.* **1993**, *32*, 4414.

(29) Brown, K. L.; Evans, D. E. *Inorg. Chem.* **1994**, *33*, 6380. See also ref 33 therein in which Brown confirms the fact that the kinetic methods used in the present paper (and, indeed, those used for all our previous AdoCbl and related kinetic studies) *do not* suffer from the type of temperature measurement errors Brown and Evans describe (because of the completely different type of kinetic experiment and temperature control employed both in our previous work and herein).

Table 1. Nucleoside Products Formed during the Thermolysis (110 °C; Ethylene Glycol) of AdoCbi$^+$ (ca. 1×10^{-4}) as a Function of [Base] and [TEMPO][a]

added base	[base] (M)	[TEMPO] (M)	% Ado-TEMPO	% cyclic-Ado	% Ado-H	% 4′,5′-didehydro-Ado[b]	% adenine[d]	total (mass balance)
none	0	0	0	46	16	33	2	97
none	0	2×10^{-2}	73	1	0	24	2	100
none	0	0.85	91	0	0	7	3	101
Proton Sponge[e]	7.5×10^{-3} [e]	0	0	8	2	8	47 (52)[d]	65 (70[d])[c]
Me_2N-py	0.1	0	0	23	6	5[c]	17 (29)[d]	51 (63[d])[c]
Me_2N-py	0.3	0	0	12	3	3[c]	36 (52)[d]	54 (70[d])[c]
Me_2N-py	0.3	2×10^{-2}	30	0	0	2[c]	30 (45)[d]	62 (77[d])[c]
Me_2N-py	0.3	0.85	53	0	0	0	25 (45)[d]	78 (98)
py	0.15	2×10^{-2}	74	1	0	20	3	98
py	0.3	2×10^{-2}	76	0	0	18	7	101

[a] An expanded version of this table, one including the product studies done to confirm that the product ratio (see eq 3) is invariant at high [base], is available as Table A, Supporting Information. [b] 4′,5′-Didehydroadenosine has been shown to be an *in-cage homolysis (disproportionation) product*.[28] [c] The controls presented in the Experimental Section demonstrate that 4′,5′-didehydroadenosine decomposes in the presence of Me_2N-py, thereby accounting for the missing nucleoside. [d] Control experiments described in the Experimental Section demonstrate that adenine and the added Me_2N-py react (presumably via an initial deprotonation) to yield a new product with a retention time of ca. 6.6 min under our HPLC conditions; the values in parentheses are the % nucleoside with this adenine byproduct figured in (assuming a detector response equal to adenine). [e] Proton Sponge (i.e., 1,8-bis(dimethylamino)napthalene) was used to generate the 6.5×10^{-3} M ethylene glycol monoalkoxide, $HOCH_2CH_2O^-$, to test for its involvement in accelerating Co−C cleavage.

experiments, a reference cuvette was employed in the Beckman DU-7 spectrophotometer, one containing a solution of TEMPO and the chosen base in ethylene glycol at concentrations identical to those in the sample cuvette. Isosbestic points at 587, 490, 395, and 337 nm were maintained throughout the concentration ranges examined (0.3−2.0 M Me_2N-py and 2×10^{-2} M TEMPO); representative spectra are available as Supporting Information. First-order rate constants were obtained by the usual linear regression of the Co^{II}-cobamide $\ln[Abs_\infty/(Abs_\infty - Abs_t)]$ appearance vs time data. (Note that the Abs_∞ end points *were not* obtained by photo*homolysis* as before,[28a] since it is important to obtain the accurate ratio of Co−C thermal homolysis *and heterolysis* products in each kinetic experiment. A single exception here is AdoCbi$^+$ without added base which is both a slow and predominately homolysis reaction, and thus photolysis was done at the end of the run to check on the final Abs_∞ value.)

Control Experiments Testing for Alkoxide Involvement in the Observed Rate Acceleration of Co−C Cleavage. The details of these experiments are provided in the Supporting Information. The results show that the observed AdoCbi$^+$ Co−C cleavage rate was in fact ca. 8-fold faster in the presence of ca. 6.5×10^{-3} M monoalkoxide (compared to AdoCbi$^+$ in ethylene glycol with no base added), Table 2, entry 3.

A second control experiment was also done to establish the (50-fold greater, *vide infra*) rate effect due to the pyridine axial ligand. A 110 °C thermolysis of AdoCbi$^+$ was run in *neat pyridine* (i.e., in the complete absence of an alkoxide source) following the same procedure as described above in the Kinetics Studies section, the results of which are presented in Table 2, entry 2.

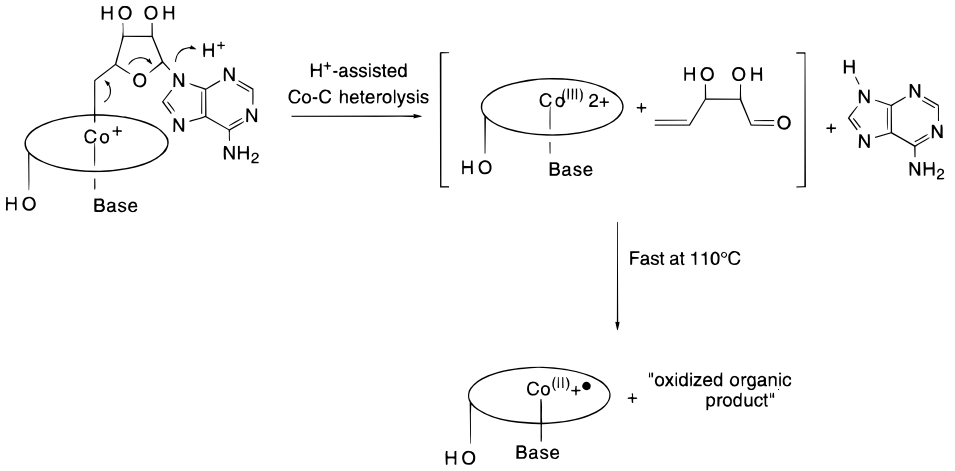
Results

Nucleoside Products in the Absence of Added Base. One of the most telling parts of these studies proved to be the quantitative determination of the nucleoside products by HPLC, Table 1. The required reference point is the established[28] AdoCbi$^+$ thermolysis product studies in the absence of either base or TEMPO radical trap (entry 1, Table 1); the products demonstrate 97% Co−C homolysis to produce three major nucleoside products: 5′,8-anhydroadenosine (cyclic-Ado), 5′-deoxyadenosine (Ado-H), and 4′,5′-didehydroadenosine (4′,5′-didehydro-Ado). A small amount (2%) of the Co−C heterolysis product, adenine, accounts for the rest of the starting AdoCbi$^+$ in the thermolysis reference point of (base-free and TEMPO-free) AdoCbi$^+$.

When TEMPO is added but exogenous base is still absent, entries 2 and 3 in Table 1, the HPLC results demonstrate the absence of cyclic-Ado and Ado-H (products formed from freely diffusing Ado$^\bullet$ radical[28]); the expected concomitant increase in Ado-TEMPO is also observed.[24,28] Only at high 0.85 M TEMPO is the 4′,5′-didehydroadenosine olefin product diverted to Ado-TEMPO, entry 3, Table 1; note that this product is known to be formed *within the solvent cage*[28] by β-H elimination from the caged [Ado$^\bullet$ $^\bullet Co^{II}$Cbi$^+$] radical pair. Note also that the 2% adenine product is unaffected by the presence of even this 0.85 M TEMPO, a result which provides excellent, confirming evidence *that adenine is a Co−C heterolysis product* (and not a Co−C homolysis or radical-cage product).[12,28]

Nucleoside Products in the Presence of *p*-(Dimethylamino)pyridine. The AdoCbi$^+$ thermolysis products in the presence of added, exogenous axial base proved to be quite different from those in the absence of base. Entries 5 and 6 in Table 1 show that thermolysis of AdoCbi$^+$ in the presence of 0.1 and 0.3 M Me_2N-py gave, respectively, only 34% and 18% *total* homolysis products (homolysis equals the sum of cyclic-Ado, Ado-H, and 4′,5′-didehydro-Ado), *plus 29% and 52% heterolysis products* (heterolysis equals the sum of adenine plus its decomposition product). Note that the total mass balance in these experiments is lower (63% and 70%, respectively). The reason for the lower mass balance became clear from product studies in the presence of TEMPO radical trap. When sufficient TEMPO to trap only the freely diffusing Ado$^\bullet$ radical is added (i.e., 2×10^{-2} M TEMPO), the ratio of homolysis to heterolysis products, and the "missing" nucleoside, remain approximately the same. However, under 0.85 M TEMPO conditions, where Ado$^\bullet$ trapping within the cage has been demonstrated,[28] *98% of the nucleoside is accounted for* (and the yield of adenine is unchanged, as expected for a nonhomolysis/noncage product). Because our previous work demonstrated that 4′,5′-didehydroadenosine is an in-cage β-H elimination product, one which can be diverted by caged-pair trapping with 0.85 M TEMPO,[28] and given the control reaction (provided in the Experimental Section) demonstrating that 4′,5′-didehydroadenosine is decomposed by Me_2N-py under our reaction conditions, it follows that the missing nucleoside product is just the expected additional

Scheme 1. Ado-cobamide Co−C *Overall* Heterolysis Reaction Followed by the Known[12b,31,32] Subsequent Redox Reaction Which Converts Co^{III} to Co^{II} (See Scheme 3 for a More Detailed Mechanism for This Overall Reaction)



4′,5′-didehydroadenosine (which decomposes to an HPLC nondetectable byproduct in the presence of added bases such as *p*-(dimethylamino)pyridine).

The data in Table 1 (e.g., entries 5 and 6 at 0.1 and 0.3 M Me_2N-py), plus the data in Table A, Supporting Information (entry 11, at high, 0.6 M Me_2N-py), show that the percent adenine is *invariant* within experimental error as a function of the [Me_2N-py]. This important result provides the heterolysis to homolysis product ratio (eq 3, *vida infra*) needed to deconvolute the kinetic data for Me_2N-py.

Nucleoside Products in the Presence of Alkoxide. A simple equilibrium calculation, employing the pK_a values[26] of Me_2N-py (9.7) and of the ethylene glycol solvent (14.8), reveals that, with 0.3 M added Me_2N-py, 6.5×10^{-3} M monoalkoxide, [$HOCH_2CH_2O^-$] [Me_2N-Py·H^+], will be present. Hence, to test for the possible involvement of the monoalkoxide, $HOCH_2$-CH_2O^-, in accelerating Co−C heterolysis a control experiment was performed consisting of the thermolysis of $AdoCbi^+$ in the presence of 6.5×10^{-3} M monoalkoxide, Table 1, entry 4. The HPLC product studies do indeed exhibit a significant increase in the percentage of Co−C heterolysis, 52% vs only 3% heterolysis for $AdoCbi^+$ with no added alkoxide or pyridine base, Table 1, entries 1−3. In addition, in the presence of the monoalkoxide $HOCH_2CH_2O^-$ the percentage of missing nucleoside is ca. 30% (by mass balance), a value somewhat greater than that seen for $AdoCbi^+$ with Me_2N-py, Table 1, entries 5 and 6. However, HPLC control experiments described in the Experimental Section reveal that the missing nucleoside must in fact be additional 4′,5′-didehydroadenosine (the in-cage β-elimination product) since authentic 4′,5′-didehydroadenosine is decomposed by the monoalkoxide, [$HOCH_2CH_2O^-$] [Me_2N-py·H^+] (see the Experimental Section).

Nucleoside Products in the Presence of Pyridine. Product analyses of $AdoCbi^+$ thermolyzed in the presence of TEMPO plus 0.1, 0.15, 0.3, or 0.6 M pyridine were run for comparison to the analogous reactions with the more electron-donating base Me_2N-py. The results, Table 1 (last two entries, 0.15 and 0.3 M py), and the data obtained more recently by J.M.S., Table A, Supporting Information (entries 12 and 13, 0.1 and 0.6 M py), again show that the percent adenine (equals the percent heterolysis) and thus the product ratio (eq 3, *vide infra*) is *invariant within experimental error* as a function of added [pyridine] (the percent adenine is 5, 3, 7, and 7% for 0.1, 0.15, 0.3, and 0.6 M pyridine, respectively). However, this 5 ± 2% adenine heterolysis product is an order of magnitude less than the 51 ± 7% seen for the more basic Me_2N-py [for the same 0.1−0.6 M concentration range of Me_2N-py, Table A, Supporting Information, entries 7−11]. Note that, for pyridine, *complete nucleoside mass balance is achieved* [i.e., the sum of the homolysis (Ado-TEMPO and 4′,5′-didehydroadenosine) plus heterolysis (adenine) products accounts for 100 ± 2% of the starting Ado group]. The lower pK_a of 5.2 for pyridine means that much less $HOCH_2CH_2O^-$ will be generated; this result confirms the suggestion (above) that $HOCH_2CH_2O^-$ is the culprit in degrading the sensitive 4′,5′-didehydroadenosine olefin product.

Corrin Products. Anaerobic 110 °C thermolysis in ethylene glycol of $AdoCbi^+$ in the presence of *p*-(dimethylamino)pyridine base and 2×10^{-2} M TEMPO exhibited visible spectra closely resembling those of base-on Co^{II}Cbl.[30] This is as expected, since the 25 °C K_{assoc} for $Co^{II}Cbi^+$ binding of Me_2N-py to yield base-on $AdoCbi^+$·py-NMe_2 is 170 ± 60 M^{-1},[1c] which corresponds to a calculated 94% base-on Co(II)Cbi·$base^+$ at even the lowest (0.1 M) concentration of Me_2N-py used in these studies.

What was also expected and observed is that 100% (±10%) of the *final* corrin product is *Co^{II}*Cbi^+, despite the fact that Co−C heterolysis necessarily produces Co^{III} *initially*. However, it is known that the initial Co^{III}-corrin and 2,3-dihydroxy-4-pentenal *aldehyde* heterolysis products (the latter being produced in stoichiometric amounts from the pentose sugar) rapidly react further at 110 °C to give reduced Co^{II}-corrin and oxidized organic product (see Scheme 1).[12b,31,32] Hence, the expected and observed corrin product is the previously demonstrated,[12b] final product of $AdoCbi^+$ Co−C heterolysis, $Co^{II}Cbi^+$. Note,

(30) (a) That is, the final state of cobalt is nitrogenous base-on Co^{II}-Cbi^+·base based on the fact that the final visible spectra are the same as Co^{II}Cbl (known to be benzimidazole base-on; H_2O, pH > 5, K_{eq}(25 °C) = 59),[30c] except for a slight shoulder at ca. 350 nm. (b) As discussed elsewhere,[1b] there is the possibility that the "unnatural" β-isomer of Co^{II}-Cbi^+·base is formed *after* the rate-determining Co−C cleavage step, although this possibility has no implication for the present studies since the α-base- and β-Ado-containing $AdoCbi^+$·base starting material necessarily ensures that the Co−C thermal cleavage transition state of interest involves only the "natural" α-base complex. (c) Lexa, D.; Saveant, J.-M. *Acc. Chem. Res.* **1983**, *16*, 235.

(31) (a) The oxidized organic product, presumably the corresponding acid or ester derived from the aldehyde being oxidized to yield 2 e^- and 2 H^+, has never been unequivovcally characterized. (b) There is also a reasonably facile "self-reduction" reaction of the corrin involving the c-side chain being converted to an internal corrin lactone or lactam (depending upon the pH), with concomitant reduction of the Co^{III}cobamide to the Co^{II}-cobamide; see ref 36 in an earlier paper[12b] as well as elsewhere[32] for additional details.

(32) (a) Bonnett, R. In *B_{12}*; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, p 225. (b) Bonnett, R.; Cannon, J. R.; Clark, V. M.; Johnson, A. W.; Parker, L. F. J.; Smith, E. L.; Todd, A. *J. Chem. Soc.* **1957**, 1158. (c) Gossauer, A.; Heiss, K. P.; Lass, H.; Inhoffen, H. H. *Liebigs Ann. Chem.* **1976**, 1150.

Table 2. Kinetic Results for Co−C Thermolysis of AdoCbi$^+$·Base Complexes in Anaerobic, 110 °C Ethylene Glycol Containing ca. 2×10^{-2} M TEMPO

added base[a]	[base] (M)	pK_a[b]	110 °C K_{assoc}[c] (M^{-1})	k_{obsd} ($s^{-1} \times 10^5$)	$k_{on,T}$[d] ($s^{-1} \times 10^5$)	$k_{off,T}$[d] ($s^{-1} \times 10^5$)	$k_{on,h}$[d] ($s^{-1} \times 10^5$)	$k_{on,het}$[d] ($s^{-1} \times 10^5$)
				Control Experiments				
none	0			0.32(0.10)	NA[e]	0.32(0.10)	NA[e] [$k_{off,h}$ = $0.3(0.1) \times 10^{-5}$ s^{-1}]	NA[e] [$k_{off,het}$ = $0.008(0.003) \times 10^{-5}$ s^{-1}]
py	neat (12.4 M)	5.2	0.27(0.1)	100(30)[f]				
Proton Sponge[g]	7.5×10^{-3}	12.4		2.5(0.1)	>2.2(0.1)		≥1.0(0.1)	≥1.2(0.1)
N,*N*-dimethylaniline	1.0	5.1	0.5(0.1)	0.8(0.2)				
				Co−C Cleavage Rates at 1.0 M Axial Base				
py	1.0	5.2	0.27(0.1)	2.2(0.2)				
Me-py	1.0	6.0	0.3(0.1)	4.5(0.2)				
H_2N-py	1.0	9.2	0.4(0.2)	35 (1)				
Me_2N-py	1.0	9.7	0.5(0.2)	51 (3)				
				Co−C Cleavage Rates as a Function of [Base]				
				K'_{assoc}[h] = AdoCbi$^+$·base/AdoCbi$^+$				
py	0.15	5.2	0.04 = 4/96[h]	0.35(0.10)				
py	0.3	5.2	0.08 = 8/92[h]	0.46(0.09)	79(26)[i]		75(26)[i]	4(1)[i]
Me_2N-py	0.1	9.7	0.05 = 5/95[h]	12 (2)				
Me_2N-py	0.3	9.7	0.15 = 13/87[h]	21 (3)	140(20)[i]		73(11)[i]	67(12)[i]

[a] A legend for the base abbreviations shown is provided elsewhere.[2] [b] Conjugate acid, base·H^+, pK_a values.[26] [c] Calculated values from extrapolation of ln K_{assoc} vs $1/T$ (K) plots available elsewhere[1b] (see also the Supporting Information associated with the present paper); errors are derived from linear regression. In the case of the Me_2N-py K_{assoc}, the 0.5(0.2) value is the average of the K_{assoc} values derived from the thermodynamic and kinetic data. [d] For the derivation of the kinetic equations employed, and for the detailed deconvolution of the k_{obsd}, see the Supporting Information. The $k_{on,h}$ are actually $k_{on,h}$(apparent) as they contain a component due to Co−C homolysis with $HOCH_2CH_2O^-$ as the axial base; see the text and ref 38. [e] Not applicable, due to the absence of an exogenous nitrogen base. [f] The short, ca. 11 min half-life for this reaction limits the number of data points and their quality. [g] 7.5×10^{-3} M Proton Sponge was used to independently generate the 6.5×10^{-3} M ethylene glycol monoalkoxide calculated to be present when 0.3 M Me_2N-py is the added base (see the text and Experimental Section for further details). [h] $K'_{assoc} = K_{assoc}$[base] = the AdoCbi$^+$·base/AdoCbi$^+$ ratio. [i] These values are derived from a reciprocal $1/k_{obsd}$ vs 1/[base] plot of the additional k_{obsd} vs [base] data in Table 1 in the Supporting Information, plus the applicable [product ratio] data, all as detailed in the Supporting Information.

therefore, that one must use the nucleoside organic product (i.e., the adenine), and not the (misleading) corrin product, to distinguish initial Co−C heterolysis from Co−C homolysis. Failure to do so has already caused one error in the literature[13a] (see elsewhere for a correction[12b]).

Axial-Base K_{assoc} Equilibrium Binding Constants. In order to design and then perform the Co−C cleavage kinetic studies under conditions where a known amount of the AdoCbi$^+$ is in the base-on form, the axial-base K_{assoc} binding constants are needed. The required equilibrium K_{assoc} binding constants, plus their associated ΔH and ΔS values, are reported in a preceding paper,[1b] and the ones that are directly revelant to the kinetic studies reported herein are summarized as part of Table 2, column 4.

Kinetic Studies: Base-Free AdoCbi$^+$·Solvent Thermolysis and Other Control Experiments. Several control experiments (Table 2, section 1) which proved necessary will be presented briefly first, since these controls are required to understand and to interpret the kinetic data summarized in Table 2, sections 2 and 3.

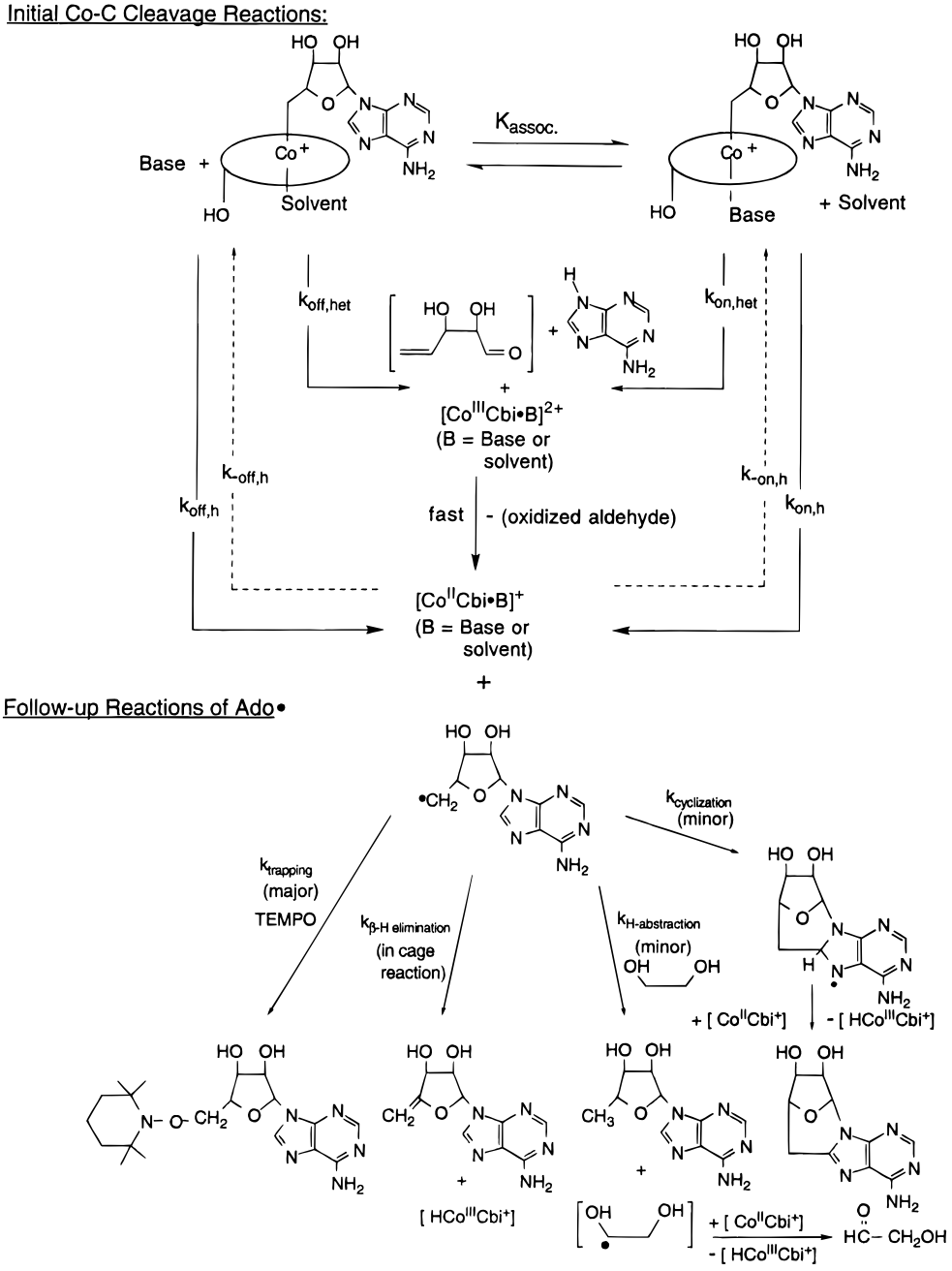
AdoCbi$^+$·Solvent (Solvent = Ethylene Glycol). In the first control experiment, the 110 °C Co−C thermolysis rate constant for AdoCbi$^+$ in the absence of any added base was observed to be $k_{obsd} = 0.32 \times 10^{-5}$ s^{-1} (entry 1, Table 2), a value identical within experimental error to that from our previous work.[28] This control experiment (i) demonstrates the reproducibility of our earlier results, (ii) provides greater confidence in the precision and accuracy of the kinetic results described below, and (iii) yields the necessary reference point rate constants shown in Table 2 (entry 1, last two columns) for base-free AdoCbi$^+$· solvent (solvent = ethylene glycol) of $k_{off,het} = 0.008 \times 10^{-5}$ s^{-1} and $k_{off,h} = 0.3 \times 10^{-5}$ s^{-1}. (For details, see the kinetic derivations and data analysis, eqs 14a−e, Supporting Information.) Note that, *in the absence of a nitrogenous axial base*, the background heterolysis is very slow, 375-fold slower than the background homolysis. (A full mechanistic scheme, along with a pictorial definition of $k_{off,het}$, $k_{off,h}$, and the other required rate constants, is provided in Scheme 2, *vide infra.*)

AdoCbi$^+$·Pyridine. Next, two control experiments were done to test whether or not any part of the Co−C cleavage rate enhancement seen in Table 2 (i.e., as axial pyridine bases are added) is due to the monoalkoxide $HOCH_2CH_2O^-$. In the first control experiment, neat pyridine was used as a solvent (i.e., a case where no ethylene glycol is present and hence involvement by its alkoxide is impossible). The results, entry 2, Table 2, show a large (100/0.32 = 312-fold) rate enhancement over the baseline case of no added axial base (entry 1, Table 2). This control demonstrates that RO^- is *not* required for the *largest* Co−C cleavage rate acceleration seen in these studies.[33] This is excellent evidence against RO^-, and thus for the added pyridine base as the *primary* source of observed Co−C cleavage rate enhancements.

AdoCbi$^+$·$^-$OCH$_2$CH$_2$OH. In the third control experiment, pyridine was omitted, but the *maximum amount* of $HOCH_2CH_2O^-$ produced (i.e., by the strongest of the bases examined, Me_2N-py) was independently generated using the noncoordinating base, Proton Sponge (see the Experimental Section for additional details). The result, entry 3, Table 2, shows there is (only) an 8-fold increase of k_{obsd} in the presence of 6.5×10^{-3} M monoalkoxide (vs no added base, Table 1, entry 1). Hence, even though RO^- does increase the Co−C bond cleavage rate, it is only responsible for ca. 12% of the rate increases documented in Table 2 (i.e., entry 3, section 1, and entry 4,

(33) (a) The $k_{obsd} = 100 \times 10^{-5}$ s^{-1} in neat pyridine[33b] (entry 2, Table 2) can be used to estimate a $k_{on,h}$ = ca. 130×10^{-5} s^{-1} (from eq 7c in the Supporting Information, K(py; 100 °C) = 0.27 M^{-1}, and [py](neat) = 12.4 M, plus the fact, from Table 2, that $k_{off,T} \ll k_{obsd}$). While this value is probably equivalent with the true experimental error of the $k_{on,h} = 75(26) \times 10^{-5}$ s^{-1} (Table 2, entry 10),[33b] we expected the $k_{on,h}$ in neat pyridine to be slightly larger than that in ethylene glycol due to pyridine's lower solvent viscosity (vs than that of ethylene glycol),[35] and thus the resultant decreased solvent-cage efficiency (less primary recombination; faster k_{obsd} and $k_{on,h}$) in pyridine. (Elsewhere we have shown that AdoCbi$^+$ in 110 °C ethylene glycol has a strong solvent cage, $0.94 \le F_c \le 1.0$,[28a] as does base-on AdoCbl, $0.4 \le F_c \le 1.0$.[28b])

Scheme 2. Minimum Mechanistic Scheme Necessary To Account for the [AdoCbi·Base]$^+$ Thermolysis Product and Kinetic Data (at 110 °C, in Ethylene Glycol with TEMPO Radical Trap)[a]



[a] By "follow-up reactions" we mean reactions after, and faster at 110 °C than, the rate-determining Co−C cleavage.

section 3, both in comparison to entry 1, section 1; all in Table 2). Again, the $k_{on,het}$ and $k_{on,h}$ rate constants for alkoxide base-on AdoCbi$^+$·$^-$OCH$_2$CH$_2$OH were obtained, Table 2, entry 3 (last two columns), by using the homolysis/heterolysis product ratio (see the Supporting Information for details). Note that from this and from the AdoCbi$^+$·ethylene glycol values just discussed (Table 2, entry 1, last two columns), the ratio of heterolysis rate constants, $k_{on,het}$, for AdoCbi$^+$·$^-$OCH$_2$CH$_2$OH vs AdoCbi$^+$·ethylene glycol is $\geq 1.2/0.008 = 150$. *This shows that the amount of Co−C heterolysis in [AdoCbi·base]$^+$ is very sensitive to increases in the the basicity of the trans-axial ligand.*

Controls for Py vs −NH$_2$ Amine Nitrogen Coordination and Hence Kinetic Contributions to Co−C Cleavage: AdoCbi$^+$·Py-NMe$_2$ vs AdoCbi$^+$·Py-NH$_2$ vs AdoCbi$^+$·N(Me$_2$)-Ph. In a final series of control experiments, 1 M H$_2$N-py, Me$_2$N-py, the much bulkier, inherently more poorly coordinating, *N*,*N*-dimethylaniline, and Me$_2$N-Ph were used to determine whether coordination of the pyridine or the amine nitrogen[13d,34] (or both) is responsible for the Co−C cleavage rate accelerations seen with these potentially ambidentate ligands. The results, entries 4, 7, and 8, Table 2, show that 1 M H$_2$N-py or Me$_2$N-py accelerates Co−C cleavage by 110- and 160-fold, respectively (Table 2, entries 7 and 8, vs no added base, entry 1). The fact that these two rate accelerations vary *inversely* with their coordinating ability—but in the order expected for their pyridine nitrogen basicity—is consistent only with coordination of pyridine nitrogen in the kinetically dominant form. Confirming this conclusion, the addition of even 1 M *N*,*N*-dimethylaniline

barely accelerates Co−C cleavage, by only 2.5-fold (Table 2, section 1, entry 4 vs entry 1). It is also noteworthy that the final $Co^{II}Cbi^+$ visible spectrum for the 1 M *N*,*N*-dimethylaniline experiment is identical to that of *base-off* $Co^{II}Cbi^+$, indicating that *N*,*N*-dimethylaniline does not bind well to $Co^{II}Cbi^+$ [1b] (a result which argues against this 2.5 rate acceleration being due to the possibility of cage-pair trapping of $Co^{II}Cbi^+$ under these high, 1 M *N*,*N*-dimethylaniline conditions). Rather clearly, then, the observed rate accelerations are the result of coordination of only the more basic pyridine nitrogen.

Kinetics in the Presence of 1 M *para*-Substituted Pyridine Axial Bases. The kinetics of AdoCbi$^+$·base Co−C bond thermolysis were determined in the presence of a 1 M concentration of a series of increasingly electron-donating, yet isosteric, *para*-substituted pyridine axial bases, Table 2, section 2. The high, 1 M conditions were chosen to enforce a reasonable percentage of the base-on form, for example, 21% py base-on (AdoCbi$^+$·py) and 33% Me_2N-py base-on (AdoCbi$^+$·Me_2N-py); these high [base] also ensure a predominance of base-on $Co^{II}Cbi^+$·base, 96% for pyridine, 99.4% for Me_2N-py at 25 °C. A marked effect on the observed rate of *net Co−C bond cleavage* is seen during 110 °C thermolysis of an ethylene glycol solution of ca. 1×10^{-4} M AdoCbi$^+$ in the presence of 1 M exogenous base and ca. 2×10^{-2} M TEMPO. The greatest rate increase in k_{obsd} is 160-fold for the most electron-donating pyridine tested, Me_2N-py (entry 8 vs entry 1, Table 2).[35] In contrast, pyridine itself exhibited a rate increase in k_{obsd} of only 7-fold over the base-off AdoCbi$^+$ baseline (entry 5 vs entry 1, Table 2). The intermediate cases of *p*-Me-py and *p*-NH_2-py showed 14- and 110-fold rate increases, respectively (Table 2, entries 6 and 7). Note that *these k_{obsd} values are composites*, reflecting both the percent base-off/on and the heterolysis vs homolysis components. Moreover, since the products with even 0.3 M added exogenous base include a significant percentage of Co−C *heterolysis*, it follows that these rate increases contain a significant component due to a *heterolytic Co−C cleavage process.*

Additional AdoCbi$^+$·Base Thermolyses at Higher [Base] (Base = Pyridine and *p*-(Dimethylamino)pyridine). Some additional kinetic studies were performed for pyridine and *p*-Me_2N-py. The concentrations examined ranged from 0.82 to 2.1 M for pyridine, and from 0.59 to 2.1 M for *p*-Me_2N-py. A table and plots of k_{obsd} vs [base] are available as Supporting Information, Table 1 and Figure B (the py data) and Figure C (the Me_2N-py data). We will return to these data in an upcoming section in which the composite k_{obsd} values will be deconvoluted into their base-on and base-off, and heterolysis and homolysis, components via a $1/k_{obsd}$ vs 1/[base] plot.

Proposed Mechanistic Scheme. The mechanistic scheme necessary to account for the product studies reported, and required to be consistent with the now sizable relevant literature (i.e., of AdoCbi$^+$ or AdoCbl thermolyses in ethylene glycol,[12,15,24,28] and the base-off/on K_{assoc} of AdoCbi$^+$ [1b]), is shown in Scheme 2. This scheme summarizes the parallel reactions (and associated rate constant definitions) of (i) base-on heterolysis ($k_{on,het}$), (ii) base-on homolysis ($k_{on,h}$), (iii) base-off heterolysis ($k_{off,het}$), (iv) base-off homolysis ($k_{off,h}$), and also (v) the definition of the AdoCo(III)Cbi$^+$ base-off, base-on K_{assoc} equilibrium constant. Note that the specific kinetic contributions of an axial $HOCH_2CH_2O^-$ base have not been separately shown in Scheme 2, since the "AdoCbi$^+$·base" therein covers all types of bases. In addition, the radical cage known to exist for both AdoCbi$^+$ and AdoCbl in ethylene glycol[28] has also been necessarily omitted for the sake of clarity, and so that we can focus on the axial-base study and associated issues at hand. However, we note in this regard that any interpretations of experiments at axial-base concentrations approaching or above 1 M will have to consider possible radical-cage effects.[28]

Kinetic Equations Derived for Scheme 2. Working from Scheme 2 and under conditions where Co−C cleavage is irreversible [i.e., sufficient TEMPO trap that Co−C homolysis is irreversible, and assuming that Co−C heterolysis is irreversible (as seems likely; there is no evidence to the contrary)], the kinetic expressions necessary to deconvolute the k_{obsd} data into its $k_{on,T}$ and $k_{off,T}$ components (where T = total), and then to further deconvolute $k_{on,T}$ into its $k_{on,h}$ and $k_{on,het}$ parts, are readily derived, eqs 1−3. Subtracting $k_{off,T}$ from both sides of eq 1

$$k_{obsd} = \frac{k_{off,T} + k_{on,T}K[\text{base}]}{1 + K[\text{base}]} \qquad (1)$$

$$K = K_{assoc};\ k_{on,T} = k_{on,h} + k_{on,het};\ k_{off,T} = k_{off,h} + k_{off,het}$$

$$\text{T} = \text{total, h} = \text{homolysis, het} = \text{heterolysis}$$

and inverting yields eq 2, after appropriate algebraic manipulation (and under sufficient added base so that $k_{obsd} \gg k_{off,T}$; see the Supporting Information for the full details of the derivations of eqs 1−3 and the subsequent data analysis). The 110 °C

$$\frac{1}{k_{obsd}} = \left[\frac{1}{K(k_{on,T} - k_{off,T})}\right]\frac{1}{[\text{base}]} + \left[\frac{1}{k_{on,T} - k_{off,T}}\right] \qquad (2)$$

equilibrium (K_{assoc}) constants necessary to deconvolute the 110 °C kinetic data according to eqs 1 and 2 were derived from linear extrapolation of our previously measured ln K_{assoc} vs $1/T$ plots,[36,37a] data available elsewhere[1b] (the appropriate plots and extrapolations are provided in the Supporting Information, Figure E). The resultant K_{assoc} values (110 °C) have already been summarized as column 4 in Table 2 along with their estimated error bars.

The one other relevant type of data that we have (i.e., with which to deconvolute the k_{obsd} rate constants) is the ratio of Co−C heterolysis vs homolysis products, hereafter defined as the product ratio, eq 3. This ratio can in turn be shown to equal

$$[\text{product ratio}]_t = \frac{[\text{adenine}]_t}{[\text{Ado·products}]_t} = \frac{k_{on,het}}{k_{on,h}} \qquad (3)$$

$k_{on,het}/k_{on,h}$ (eq 3) *under conditions where the predominant fraction of the products comes from the base-on AdoCbi$^+$·base form* (or, mathematically, when $k_{on,h}K[\text{base}] \gg k_{off,h}$ and when $k_{on,het}K[\text{base}] \gg k_{off,het}$; see eq 11c and its derivation in the Supporting Information). Fortunately,[37b] *ex post facto* analysis

(34) (a) This control is still required, even though we have shown elsewhere[1b] that the primary mode of binding is through the pyridine nitrogen and not the amine $-NH_2$ nitrogen,[34b] since there is always the possibility that a trace amount of the $-NH_2$ coordinated, base-on amine species could have a surprisingly high *kinetic* reactivity. (b) Neither aniline (conjugate acid pK_a = 9.2, Table 2) nor *N*,*N*-dimethylaniline (conjugate acid pK_a = 9.7, Table 2) exhibits detectable binding[1b] to AdoCbi$^+$, even at 25 °C, as expected on the basis of their pK_a values and thus lower basicity in comparison to pyridine (conjugate acid pK_a = 5.2, Table 2). Note also that, at the higher, 110 °C thermolysis temperature, even less axial-base binding is expected due to the invariably negative ΔH for the axial-base binding equilibrium. (c) Brown and Brook performed a similar control in their recent publication, and they, too, found no evidence for the amine nitrogen binding.[8]

(35) (a) The 312-fold rate increase for neat pyridine (entry 2 vs entry 1, Table 2, control section) cannot be directly compared to the [base] = 1 M data due to the lower viscosity of pyridine (0.97 cP at 25 °C[35b]) vs ethylene glycol (19.9 cP at 25 °C[35c]). We have shown elsewhere that the observed rate for AdoCbi$^+$ Co−C bond thermolysis is directly related to the solvent viscosity, due to solvent-cage effects.[28,33a] (b) *CRC Handbook of Chemistry and Physics*; Weast, R. C., Astle, M. J., Eds.; CRC Press: Boca Raton, FL, 1981. (c) Thomas, L. H.; Meatyard, R.; Smith, H. Davis, G. H. *J. Chem. Eng. Data* **1979**, *24*, 161.

(36) Representative examples of these ln K_{assoc} vs $1/T$ plots are available as Supporting Information.

of the deconvoluted rate constants obtained using eq 3 reveals that the above assumptions are, in fact, valid under our conditions by ≥10-fold (see the *ex post facto* checks in the Data Analysis section of the Supporting Information). In addition, recall that the product study data already discussed independently confirmed *experimentally* the prediction of eq 3—that is, that the heterolysis to homolysis product ratio is *independent* of the [base] for both py and Me_2N-py.

Kinetics as a Function of Base Concentration: Deconvolution of k_{obsd} Into Its Heterolysis and Homolysis Components, and into $k_{on,h}$ and $k_{on,het}$. With eqs 1 and 2 in hand, the two most extensive sets of data, the Me_2N-py and the pyridine k_{obsd} vs [base] data (Table 1, Supporting Information) were deconvoluted into $k_{on,T} - k_{off,T}$ (see eq 2, and its implied slope and intercept). For the full details of the data analysis which follows, see eqs 14a−e and 15a−i in the Supporting Information.

Me_2N-py Data. The appropriate $1/k_{obsd}$ vs 1/[base] reciprocal plot (Figure D, Supporting Information) exhibited excellent linearity for the Me_2N-py data, from which a slope of 1.4(0.1) × 10^3 s·M^{-1} = $1/[K(k_{on,T} - k_{off,T})]$ (i.e., following eq 2) was obtained. The slope and intercept of the reciprocal plot yield K = 0.6(±0.1), in good agreement with the independently measured K = 0.3(±0.2).[1b] The two equilibrium values were averaged to yield K = 0.5(±0.2), the value used for further deconvolution. Using the average K(110 °C; Me_2N-py) = 0.5-(±0.2), a value of $k_{on,T} - k_{off,T}$ = 1.5(0.7) × 10^{-3} s^{-1} results. The reciprocal plot (eq 2) also yields an intercept of 8(1) × 10^2 s = $1/(k_{on,T} - k_{off,T})$, which in turn provides a second determination of $k_{on,T} - k_{off,T}$ = 1.3(0.2) × 10^{-3} s^{-1}, one in excellent agreement with the 1.5(0.7) × 10^{-3} s^{-1} value determined above from the slope and the K_{assoc}. These two determinations were averaged to yield the value listed in Table 2, $k_{on,T} - k_{off,T}$ = 1.4(0.2) × 10^{-3} s^{-1}. *The close agreement between the two, independent determinations offers strong support for the methods, the experimental results, and the metrical details of data deconvolution.*

The $k_{on,T} - k_{off,T}$ = 1.4(0.2) × 10^{-3} s^{-1} was, in turn, deconvoluted further as follows. By independent determination using AdoCbi$^+$·solvent (i.e., without any added base), $k_{off,T}$ = 0.3(0.1) × 10^{-5} s^{-1} as detailed earlier, Table 2 (entry 1, column 7). This can be subtracted from $k_{on,T} - k_{off,T}$ to yield an effectively unchanged $k_{on,T}$ = 1.4(0.2) × 10^{-3} s^{-1}, listed in Table 2 (as 140(20) × 10^{-5} s^{-1}, last entry, column 6). Next, using the product ratio in Table 1 (entry 8), $k_{on,h}$ = 73(11) × 10^{-5} s^{-1} and $k_{on,het}$ = 67(12) × 10^{-5} s^{-1} were obtained, Table 2 (last entry, column 7 and eqs 15g−i in the Supporting Information).

These results reveal two interesting findings: (i) that the base-on homolysis rate constant, $k_{on,h}$, for AdoCbi$^+$·py-NMe_2 is *240-fold larger* than the base-off rate constant, $k_{off,h}$, for AdoCbi$^+$·solvent (solvent = ethylene glycol) and (ii) that the base-on rate heterolysis rate constant, $k_{off,het}$, for AdoCbi$^+$·py-NMe_2 is *8400-fold larger* than the base-off heterolysis rate constant, $k_{off,het}$, for AdoCbi$^+$·solvent. This latter result both demonstrates and quantitates for the first time *the dramatic enhancement of AdoCbi$^+$ Co−C heterolysis by trans-axial electron-donating bases*.

Pyridine Data. The AdoCbi$^+$ k_{obsd} vs [pyridine] data were deconvoluted similarly (see the data analysis and eqs 16a−h, Supporting Information). The results, while not as precise as the Me_2N-py data, yielded estimates for $k_{on,h}$ = 8(3) × 10^{-4} s^{-1} and $k_{on,het}$ = 4(1) × 10^{-5} s^{-1}. Note that the $k_{on,h}$ value is the same, within experimental error, as that seen for Me_2N-py, indicating an insensitivity of the $k_{on,h}$ for *Ado*Cbi$^+$ to the electron-donating ability of the axial pyridine base—results which would *seem* to contrast those for alkylcobaloxime B_{12} models[20c,d]—and also to those for RCbi$^+$·base (R = benzyl, neopentyl) complexes with these same pyridine bases.[8] However, a closer look at the data reveals that the true $k_{h,on}$ values probably decrease by ≤8-fold on going from pyridine to the more electron-donating Me_2N-py once the hidden effect of changing pH (and the contribution to $k_{on,h}$ of the alkoxide-containing [AdoCbi·$^-OCH_2CH_2OH$]) is taken into account.[38] These AdoCbi$^+$·py $k_{h,on}$(apparent) and $k_{on,het}$ results are listed in Table 2 (entry 10, last 2 columns) and, as relative rate constants, in Table 3.

Thermolysis of AdoCbi$^+$ with RS^-/RSH Present. Because a cysteinethiolate has been suggested in the literature as a conceivable axial ligand for enzyme-bound AdoCbl,[39] we briefly examined the products and kinetics of AdoCbi$^+$ thermolysis at 100 °C in phosphate-buffered, pH 7.2 H_2O with the dithiol dithioerythritol (DTE; 25 °C, pK_{a_1} = 9.2, pK_{a_2} = 9.9;[40a] 100 °C, pK_{a_1} = ca. 6.8, pK_{a_2} = ca. 7.5[40b]). The major product, and thus the major mode, of Co−C cleavage is *heterolysis* (77% at 0.2 M DTE), with a ca. 20-fold rate enhancement of Co−C cleavage (k_{obsd} = 6 × 10^{-5} s^{-1}) over that for AdoCbi$^+$ without added axial base. The Co^{II}Cbi$^+$ is *base-off* (isosbestic points

(37) (a) The Co^{II}Cbi$^+$ with Me_2N-py and pyridine association constants for 110 °C ethylene glycol solutions are Me_2N-py $K_{Co^{II},assoc}$ = 9.9 and pyridine $K_{Co^{II},assoc}$ = 1.9. The plots of ln $K_{Co^{II},assoc}$ vs $1/T$ used to derive these values are presented as Supporting Information, and additional $K_{Co^{II},assoc}$ values and discussion will be available elsewhere.[1c] (b) During the reviewing process, one of the three reviewers expressed confusion over why the product ratio is constant at conditions where only a small percentage of AdoCbi$^+$ is in the base-on form, [AdoCbi·base]$^+$, stating erroneously that the ratio of homolysis to heterolysis "*must* change smoothly with increasing [L] until [L] is sufficiently high that the only significant species present is [AdoCbi·base]$^+$". That this statement is incorrect is proven *experimentally* by the fact that the product ratio is constant, for Me_2N-py for example, from 0.1 to 0.6 M Me_2N-py, conditions where the K_{assoc} (110 °C) = 0.5 M^{-1} (Table 2) reveals that there is only 5−23% base-on form present. Yet the k_{obsd} does increase as a function of added [base], Figures C and D, Supporting Information. These results are easily understood if one does not confuse the *product* vs the k_{obsd} dependencies on the added [base]. As soon as there is sufficient base present to ensure that all the (homolysis plus heterolysis) products come from the (faster) reaction of the base-on, [AdoCbi·base]$^+$ form (i.e., when $k_{on,h}K$[base] ≫ $k_{off,h}$ and when $k_{on,het}K$[base] ≫ $k_{off,het}$; see eq 11c and its derivation in the Supporting Information), then the product ratio becomes invariant of [base]. This happens for Me_2N-py after ≤5% is in the base-on form (e.g., as the data cited above demonstrates). On the other hand, the k_{obsd} will increase with increasing [base] until all the AdoCbi$^+$ is in the (faster) [AdoCbi·base]$^+$ form. We hope this brief explanation helps avoid future confusion over these points.

(38) (a) The $k_{on,h}$ results for neopentyl- and benzyl-Cbi$^+$·base (designated as k_L elsewhere)[8] decrease with increasing electron donation, from py to H_2N-py (Table 2, 35 °C data, p 3424 elsewhere[8]), by 2.3-fold (for $PhCH_2$-Cbi$^+$) to 8-fold (for NpCbi$^+$). There are, however, sufficient differences between Brown's and the present studies so that the exact origins of the different response to increasingly electron axial pyridine bases are not immediately identifiable. Specific differences in the two studies include the key R = Ado vs benzyl and Np difference, the H_2O vs ethylene glycol solvent difference, and the 1.0 M ionic strength and variable buffers (from pH 7.5 to pH 11.2) in Brown and co-workers' study vs the choice not to add buffers or ionic strength additives in the present studies. Despite these differences, one reasonable guess as to why our $k_{on,h}$ values do not slow by, say, 3−10-fold with the more electron-donating pyridines is due to alkoxide, $HOCH_2CH_2O^-$, involvement in the present (unbuffered) solutions, established herein to be an accelerating factor of ca. 8 (Table 2, entry 3 vs entry 1). A second possible explanation is that different RCbi$^+$·base conformers exist for Ado vs neopentyl or benzyl alkylcobamides as one proceeds along the productive Co−C homolysis reaction coordinate, and then, one must postulate in this hypothetical explanation that these conformers respond differently to increasingly electron-donating pyridine bases.

(39) Yakusheva, M. I.; Poznanskaya, A. A.; Pospelova, T. A.; Rudakova, I. P.; Yurkevich, A. M.; Yakovlev, V. A. *Biochim. Biophys. Acta* **1977**, *484*, 216.

(40) (a) Houk, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1987**, *109*, 6825. (b) Estimated 100 °C pK_a values using an (endothermic) RSH heat of ionization[40c] of 7 kcal/mol and the readily derived equation $\ln(K_1/K_2) = -\Delta H/R(1/T_1 - 1/T_2)$. (c) Christenson, J. J.; Hansen, L. D.; Izatt, R. M. *Handbook of Proton Ionization Heats*; Wiley & Sons: New York, 1976. See also: Torchinsky, Y. M. *Sulfur in Proteins*; Pergamon Press: Oxford, 1981.

Table 3. A Comparison of the Relative AdoiCbi$^+$·Base and AdoCbl (Coenzyme B_{12}; Appended 5,6-Dimethylbenzimidazole Base) $k_{on,h}$ and $k_{on,het}$ Rate Constant[a]

AdoCbi$^+$·base complex	relative $k_{on,h}$	relative $k_{on,het}$
AdoCbi$^+$·ethylene glycol	1.0(0) ($k_{off,h}$)	0.026(13) ($k_{off,het}$)
AdoCbi$^+$·$^-$OCH$_2$CH$_2$OH	≥3.3(1.1)	≥4.0(1.4)
AdoCbl	110(47)[b]	≤2.3(1.0)[b]
AdoCbi$^+$·pyridine	250(120)	13(5)
AdoCbi$^+$·py-NMe$_2$	240(88)	220(83)

[a] The rate constatnts 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. [b] Computed using a 112 °C $k_{on,T} = 3.4(5) \times 10^{-4}$ s^{-1}, a product ratio = [adenine]/[Ado·products] ≤ 2/98 (=0.020(5)) in ethylene glycol (from Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1469; see p 1478), and thus $k_{on,h} = 33(9) \times 10^{-5}$ s^{-1} and $k_{on,het} = 0.7(2) \times 10^{-5}$ s^{-1}. Note that, in pH 7 H_2O, the product ratio = 3/97 = 0.031.[12b]

at 405 and 350 nm), indicating that even Co^{II}Cbi$^+$ has a low affinity for RS$^-$/RSH, fully consistent with our findings[1b] that the AdoCbi$^+$ precursor to Co^{II}Cbi$^+$ showed no detectable binding of RS$^-$/RSH. Additional details are available as Supporting Information, but the take-home message is clear: the presence of RS$^-$/RSH axial ligands leads to predominately Co−C heterolysis in AdoCbi$^+$, *even under conditions where no RS$^-$/RSH binding to AdoCbi$^+$ can be detected*.

Discussion

Key Findings: A Comparison of Relative Rate Constants. As noted in the Introduction, the exact role(s) of the intramolecularly appended, 5,6-dimethylbenzimidazole axial base in AdoCbl has remained uncertain despite considerable prior investigation. Indeed, the recent finding that (methylmalonyl)-CoA mutase binds a protein side-chain histidine imidazole,[6d] rather than the intramolecularly appended 5,6-dimethylbenzimidazole, raises more questions than it answers.

A comparison of the deconvoluted rate constants that are a result of the studies here is provided in Table 3. The results both reveal and quantitate five previously unavailable findings: (i) For AdoCbi$^+$ plus a series of *para*-substituted pyridine bases, and for RSH/RS$^-$, a sizable to dominant Co−C cleavage heterolysis reaction[41] is introduced by adding these ligands. (ii) The rate of Co−C homolysis increases 240-fold on going from solvent (ethylene glycol) to Me_2N-py, but (iii) $k_{on,h}$(apparent) does not increase on going from py to the more basic Me_2N-py (and, probably, the true $k_{h,on}$ actually decreases by ≤8-fold[38]); (iv) The rate constants for Co−C heterolysis increase *much faster* with increasingly basic axial ligands, increasing by 8400-fold on going from ethylene glycol (solvent) to Me_2N-py.[42] Lastly, (v) the intramolecularly appended 5,6-dimethylbenzimidazole in AdoCbl is *unique, at least in comparison to the pyridine bases examined, in being able to enhance (biologically relevant) Co−C homolysis while simultaneously minimizing (abiological) Co−C heterolysis*,[43] its $k_{on,h}/k_{on,het}$ ratio being >48 whereas that for Me_2N-py is 19.

The present work provides, therefore, the first experimental test of, and support for, Mealli, Sabat, and Marzilli's theoretical studies,[6,14b] suggesting that the weakly donating 5,6-dimethylbenzimidazole base in AdoCbl aids in preventing Co−C bond heterolysis, in part due to its longer, highly evolved and thus "nearly ideal" Co−N distance.[6]

Recent Literature Addressing Co−C Heterolysis. Valuable studies by Brown[41m] and co-workers, and by Gerards and Balt,[44] show that—*under strongly acidic conditions such as H_2SO_4*—it is the β-oxygen (pK_a ≤ ca. −3)[41m] in β-oxoalkyl complexes that is protonated prior to the entry of a H_2O or another nucleophile which is necessary for the fastest rate of Co−C heterolytic cleavage; that is, an A_2 or, equivalently, an I_d mechanism obtains, *again, at least under strongly acidic conditions*.[44] Confirming this is our unpublished study of Co−C thermolysis in the AdoCbl derivative N^1-methyl-AdoCbl, work which demonstrates that the presence of a "Me$^+$" (and thus presumably a H$^+$) at the most basic position (N1) of adenine *does not* lead to any rate enhancement nor any additional Co−C heterolysis.[45a]

However, the conditions of the present study are *basic*, not acidic (e.g., with ca. 6.5×10^{-3} M [Me_2N-pyH$^+$][HOCH$_2$-

(41) Co−C heterolyses to form Co^{III} and "R$^-$" is well precedented in cobalt complexes which have β-hydroxy, β-alkoxy, or β-acyloxy alkyl groups (see also ref 42 for a rewiew and ref 44 for more recent work). (a) Hogenkamp, H. P. C.; Rush, J. E.; Swenson, C. A. *J. Biol. Chem.* **1965,** *240*, 3641. (b) Schrauzer, G. N.; Windgassen, R. J. *J. Am. Chem. Soc.* **1967**, *89*, 143. (c) Golding, B. T.; Holland, H. L.; Horn, U.; Sakrikar, S. *Angew Chem., Intl. Ed. Engl.* **1970**, *9*, 959. (d) Brown, K. L.; Ingraham, L. L. *J. Am. Chem. Soc.* **1974**, *96*, 7681. (e) Vickrey, T. M.; Katz, R. N.; Schrauzer, G. N. *J. Am. Chem. Soc.* **1975**, *97*, 7284. (f) Silverman, R. B.; Dolphin, D. *J. Am. Chem. Soc.* **1976**, *98*, 4633. (g) Vickrey, T. M.; Wright, E. E.; Kok, R. A. *Inorg. Nucl. Lett.* **1975**, *15*, 317. (h) Espenson, J. H.; Wang, D. M. *Inorg. Chem.* **1979**, *18*, 2853. (i) Curzon, E. H.; Golding, B. T.; Wong, A. K. *J. Chem. Soc., Chem. Commun.* **1982**, 63. (j) Brown, K. L.; Zahonyi-Buda, E. *J. Am. Chem. Soc.* **1982**, *104*, 4117. (k) Brown, K. L.; Ramamurthy, S. *Organometallics* **1982**, *1*, 413. (l) Brown, K. L.; Ramamurthy, S.; Marynick, D. S. *J. Organomet. Chem.* **1985**, *287*, 377−394. (m) Brown, K. L.; Salmon, L.; Kirby, J. A. *Organometallics* **1992**, *11*, 422. See also the list of previous Co−C heterolysis studies (of especially β-alkoxy and -hydroxy B_{12} and B_{12} model complexes) that comprise refs 1−27 therein. (n) The heterolytic Co−C cleavage under *basic* conditions in (β-ROCH$_2$CH$_2$)cobaloxime complexes (R = R, H) has been the focus of a series of papers. For two lead references see: Mock, W. L.; Bieniarz, C. *Organometallics* **1985**, *4*, 1917−1925. Brown, K. L.; Lessmann, E.; Evans, D. R. *Organometallics* **1992**, *11*, 1655−1665.

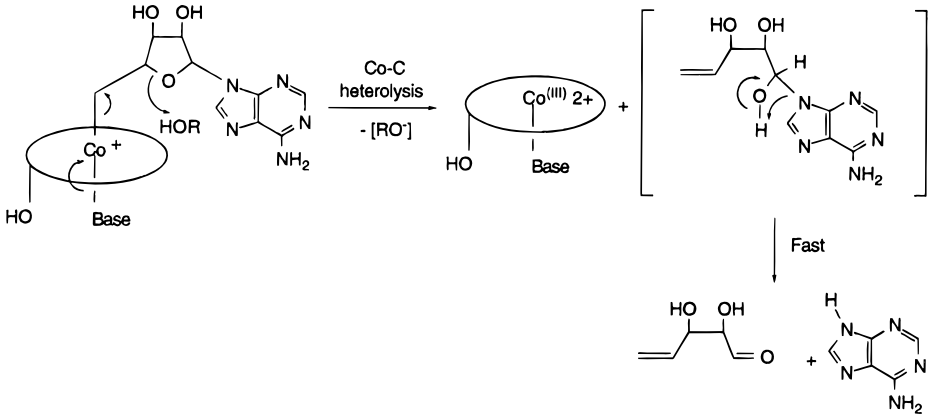
(42) (a) An early but still quite valuable review of Co−C homolysis and heterolysis: Hogenkamp, H. P. C., In *B_{12}*; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 1, Chapter 9 and references therein. (b) Schrauzer, G. N.; Katz, R. N.; Grate, J. H.; Vickrey, T. M. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 170. (c) Hogenkamp, H. P. C. *Ann. N. Y. Acad. Sci.* **1964**, *112*, 552. (d) Johnson, A. W.; Shaw, N. J. *J. Chem. Soc.* **1962**, 4608. (e) An important previous study demonstrates CN$^-$ axial-base-assisted Co−C heterolysis in AdoCbl via a two-step mechanism, one in which CN$^-$ first displaces 5,6-dimethylbenzimidazole, and then Co−C heterolysis proceeds from the 5,6-dimethylbenzimidazole base-off, but CN$^-$ base-on, [AdoCbl-CN]$^-$ intermediate: Rudakova, I. P.; Pospelova, T. A.; Borodulino-Shvets, V. I.; Kurganov, B. I.; Yurkevich, A. M. *J. Organomet. Chem.* **1973**, *61*, 389. (f) There is some previous, albeit generally qualitative and nondefinitive, evidence in B_{12} model complexes that Co−C heterolytic cleavage is enhanced by more electron-donating trans-axial ligands, specifically (i) (β-hydroxyethyl)cobaloximes cleave heterolytically to ethylene, H_2O, and Co^{III}cobaloxime only slowly in $HClO_4$, faster in H_2SO_4, and very fast in dilute HCN[42g] and (ii) (formylmethyl)cobaloxime is decomposed 90% in 6 h in 0.1 M HBr, but only 21% in 1 h in 0.1 M H_2SO_4.[42h] We note, however, that trans effects are of course well established both for such square-planar ligand systems and also specifically within B_{12} and its models.[47] Also noteworthy is Espenson's quantitation of the small but measureable (25%) effect of Cl$^-$ on the rate of (β-hydroxyalkyl)-cobaloxime decomposition in H$^+$.[41h] (f) Schrauzer, G. N.; Windgassen, R. J. *J. Am. Chem. Soc.* **1967**, *89*, 143. (g) Silverman, R. B.; Dolphin, D. *J. Am. Chem. Soc.* **1975**, *97*, 2924. See also: Silverman, R. B.; Dolphin, D. *J. Organomet. Chem.* **1975**, *101*, C14.

(43) The fact that Co−C cleavage requires temperatures outside of the enzyme which approach 100 °C means that the 110 °C results and findings presented herein are not directly comparable to physiological, 37 °C temperatures. *However*, the control studies of the ratio of homolysis to heterolysis products reported in the Experimental Section indicate that the product percentages, and thus this ratio, are invariant (±5%), at least over the experimentally accessible 25 °C (110−85 °C) temperature range.

(44) (a) Gerards, L. E. H.; Balt, S. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 137−144 (the full paper). (b) Gerards, L. E. H.; Balt, S. *Recl. Trav. Chim. Pays-Bas* **1992**, *111*, 411 (the preliminary communication). (c) Note that any kinetically important protonation at the β-oxygen in AdoCbl was deliberately omitted in the construction of Scheme 1, since the purpose of that particular scheme is to show the *overall reaction stoichiometry* leading to Co^{II}Cbi$^+$.

(45) (a) Fleming, P. E.; Daikh, B. E.; Finke, R. G. Manuscript in preparation (The Coenzyme B_{12} Derivative N^1-methyl-5′-deoxyadenosyl-cobalamin: Synthesis, Characterization, and Co-C Thermolysis Products and Kinetic Studies). (b) Kraütler, B. In *Organic Reactivity: Physical and Biological Aspects*; Golding, B. T., Griffin, R. J., Maskill, H., Eds.; Special Publication No. 148; The Royal Society of Chemistry: Cambridge, 1995; pp 209−222.

Scheme 3. One Plausible Intimate Mechanism for Co−C Heterolysis in AdoCbi$^+$ Showing the Precedented[44] Acceptance of a Proton by the Weakly Acidic β-Oxygen



CH_2O^-] present). Using the published rate constants for Co−C heterolysis of a β-oxygen-protonated Ado(H^+)-B_{12}, it appears that this β-oxygen-protonation mechanism is $\gg 10^{10}$ *too slow* to account for our results.[46] Hence, the unproved *suggestion* is that a glycol solvent ROH is the proton donor *under our conditions*, as shown in Scheme 3, and that *the nucleophile attacks from the "underneath", trans-axial position* (and not from the Ado or "top" side, as indicated by others[44]). Such a suggestion is both intuitively appealing and consistent with the body of literature of significant trans effects in B_{12} chemistry and B_{12} models[47] (see also Bürgi's work on the "anomalous structural trans effect" in coenzyme B_{12} model complexes[47i]).

Also noteworthy is Professor B. Kraütler's documentation that the preferred transition state for Co−C heterolysis likely has the anticipted trans-antiperiplanar Co−C_a−C_b−O arrangement.[45b] Consistent with this, these authors have observed a ca. 10^4 faster Co−C heterolysis in (2′,3′-dideoxyadenosyl)-cobalamin which, unlike AdoCbl,[45b] can achieve this preferred conformation.

Biological Implications. The studies presented herein and previously[1b] are consistent with, and highly supportive of, the notion that it is indeed 5,6-dimethylbenzimidazole base-on AdoCbl which undergoes Co−C homolysis, that is, at least within the subclass of AdoCbl·enzymes that are believed to exist in the 5,6-dimethylbenzimidazole base-on form. Apparently, and as suggested by Mealli, Sabat, and Marzilli on the basis of EHMO theory calculations,[14b] a long Co−N bond (ca. 2.24 Å[14b]) to the α-axial position in AdoCbl prevents a close Co−N contact which would otherwise favor Co−C heterolysis, a Co−axial-base interaction that is presumably close to the ideal length to stabilize the $Co^{II}Cbi^+$ Co−C homolysis product.[6,7,14b] We caution, however, that these ideas are just the *chemical precedent-derived suggestions* of what to look for in the enzymic systems themselves; confirmation (or refutation) of these ideas will require X-ray crystallographic and other studies of the AdoCbl-dependent enzymes.[48] Also needed are the X-ray structures of $Co^{II}Cbi^+$·base complexes, and a comparison of their Co−N bond lengths to those in AdoCbl and Co^{II}Cbl. Does a change in axial base alter only the Co−N length, or is the *corrin conformation* also changed as one might guess? Additional computation studies addressing these points would be welcome too.

Implications for the Corrin "Butterfly Conformational Distortion" Theory. This widely cited theory suggests that an upward, "butterfly" corrin conformational distortion, with its resultant steric interactions between the corrin and the 5′-deoxyadenosyl group, is a key to the observed 10^{12} enzymic activation of AdoCbl's Co−C bond (see our preceding paper for additional discussion and a summary of key references[1b]). However, the previous demonstration that the removal of the 5,6-dimethybenzimidazole in AdoCbl resulted in only a 10^2 slowing of Co−C homolysis[24]—and nothing approaching a 10^{12} effect—provided highly suggestive evidence that, contrary to previous thinking, the axial 5,6-dimethylbenzimidazole *is not* the cricial element in achieving the 10^{12} rate enhancement.[24] The present work provides additional evidence for this conclusion: *if* a closer corrin-to-benzimidazole contact, and thus a shorter Co−N distance, were the case, *then* enhanced Co−C *heterolysis* should occur. Hence, and instead, it appears that the B_{12}-binding enzymes are using the known[49,52] H-bonding contacts between the upper Ado group and the corrin side chains to achieve any upward corrin butterfly conformation that may exist in the B_{12}·enzyme complex. Restated, the "mechano-chemical" theory of Co−C labilization, in which the axial base

(46) A crude but telling "back of the envelope calculation" goes as follows. For the protonation equilibrium AdoCbi$^+$ + $HOCH_2CH_2OH$ ⇌ AdoCbi-H^{2+} + $HOCH_2CH_2O^-$, a rough K_{eq} of 10^{-18} is calculated from the glycol pK_a of ca. 15 and for a β-oxygen-protonated AdoCbi-H^{2+} of estimated pK_a^{41m} = ca. −3. The observed k_{het} appears to be no larger than 10^{-1} (i.e., from the literature[41m] "$k_{off}/K_{BH}{}^+$" = ca. $10^{-4}/10^{-3} = 10^{-1}$ s^{-1}, and assuming that the units of k_{off} of s^{-1} given elsewhere[44a] are in error and should be s^{-1}·M), so that $k_{calc} \approx K_{eq}k_{het} = 10^{-18} \times 10^{-1} = 10^{-19}$, which is 10^{15} different from the actual $k_{obsd} \approx 10^{-4}$ s^{-1} we generally report herein. Hence, even with allowances for the 110 °C vs the ca. 25 °C data used in this very rough calculation, the differences are likely $\gg 10^{10}$. (Indeed, even if k_{het} is assumed to be 10^{13} s^{-1}, that is, at its vibrational time scale absolute limit, the $k_{calc} \approx K_{eq}k_{het} = 10^{-18} \times 10^{13} = 10^{-5}$ s^{-1}, is still 10^1 slower than the k_{obsd} of 10^{-4} s^{-1}.)

(47) Lead references to the trans (and cis) influence and effect in B_{12} and B_{12} models: (a) Hayward, G. C.; Hill, H. A. O.; Pratt, J. M.; Vanston, N. J.; Williams, R. J. P. *J. Chem. Soc.* **1965**, 6485. (b) Firth, R. A.; Hill, H. A. O.; Mann, B. E.; Pratt, J. M.; Thorp, R. G.; Williams, R. J. P. *J. Chem. Soc. A* **1968**, 2419. (c) Firth, R. A.; Hill, H. A. O.; Pratt, J. M.; Thorp, R. G.; Williams, R. J. P. *J. Chem. Soc. A* **1968**, 2428. (d) In an important 1970 paper, Holmes and Hogenkamp concluded that "the nature of the axial ligands in B_{12} profoundly influences the reactivity of the cobalt atom": Hogenkamp, H. P. C. ; Holmes, S. *Biochemistry* **1970**, *9*, 1886. (e) Hill, H. A. O. In *Inorganic Biochemistry*; Eichorn, G. L., Ed.; Elsevier: New York, 1973; Vols. I and II, p 1079. (f) Pratt, J. M. *Inorganic Chemistry of Vitamin B_{12}*; Academic Press: New York, 1972; pp 165−169. (g) Endicot, J. F.; Lilie, J.; Kuszaj, J. M.; Ramaswamy, B. S.; Schmonsees, W. G.; Simic, M. G.; Glick, M. D.; Rillema, D. P. *J. Am. Chem. Soc.* **1977**, *99*, 429. (h) Kraütler, B. *Helv. Chim. Acta* **1987**, *70*, 1268. (i) Bürgi and co-workers' recent work on the "anomolous structural trans effect" (i.e., where trans ligands in B_{12} model complexes simultaneously lengthen or shorten): De Ridder, D. J. A.; Zangrando, E.; Bürgi, H.-B. *J. Mol. Struct.* **1996**, *374*, 63−83.

(48) (a) For example, it is conceivable that the enzyme prevents Co−C heterolysis by, for instance, having a −X−H group[48b] present to intercept the incipient Ado• radical, or by preventing the availability of the proton required for the optimum A_2 or I_a elimination reaction.[44b] (b) See the discussion and references in the following, especially to the seminal work of Cleland, Stubbe, McGee, and Richards, Babior, and others in regards to the "protein −XH side-chain" problem: Finke, R. G. In *Molecular Mechanisms in Bioorganic Processes*; Bleasdale, C., Golding, B. T., Eds.; The Royal Society of Chemistry: Cambridge, England, 1990; pp 271−274 and references therein.

is the key, is almost surely *not* the major source of the *enzymic* Co−C labilization.[11] Instead, the implication is that, as Marzilli and co-workers have suggested, an "ideal Co−N distance" [6b] is maintained while the enzyme otherwise distorts the corrin and the associated Co−C bond.

Summary

The community of scientists interested in B_{12} chemistry and biochemistry has long been interested in understanding the three key regions and components of coenzyme B_{12} (i.e., AdoCbl) shown in Figure 1: the upper Ado group, the middle corrin and its associated side chains, and the appended axial 5,6-dimethylbenzimidazole. The understanding at this time is arguably as follows: the upper Ado group provides multiple H-bonding interaction sites to bind to the various B_{12} enzymes, interactions crucial for activcation of the Co−C bond.[49] It is also "important (for the Ado alkyl) to be ribosylfuranose" as well, as Grissom and co-workers have shown, since the ribosyl ring oxygen is crucial for a high rate of cage recombination (at least in solution, outside of the enzyme).[50]

The central corrin is quite different from a porphyrin in that the corrin has greater flexibility,[51] and its formally uninegative charge is probably central to making available all three of the known oxidation states (Co^{III}, Co^{II}, and Co^{I}) and to stabilizing Co^{II} relative to the other two. The side chains appended to the corrin are also not surprisingly known to be crucial, especially in achieving Co−C homolysis,[52] probably (given Brown and co-workers' recent studies[53]) by lowering the *enthalpy*[53c] of Co−C homolysis in the AdoCbl·enzyme complex.

Last but not least is the 5,6-dimethylbenzimidazole base in coenzyme B_{12}, one appended in a nearly strain-free 19-membered ring conformation[54] (at least at the preferred Co−N bond length[54b]). The present results demonstrate how the 5,6-dimethylbenzimidazole, with its particular basicity and optimized Co−N distance,[6] supports facile Co−C homolysis while avoiding abiological Co−C heterolysis, an impressive example, apparently, of the fine-tuning possible via evolution. Equally if not more important is the role, identified via the compelling evidence of Toraya and Ishida, where "...the bulky dimethylbenzimidazole moiety..." plays "...an indispensable role in preventing the reactive intermediates from undesired side reactions, and thus in keeping the holoenzyme active during catalysis...", at least in diol dehydratase.[7] Hence, it may be fair to say that the axial-base chapter of the fascinating chemistry of coenzyme B_{12} is becoming better understood. We do not know, however, the precise role(s) of the cobalt-coordinated side-chain imidazole (Im) present in the (methylmalonyl)CoA mutase subclass of B_{12} enzymes.[6c,d] Elsewhere we detail the results of our studies of $[AdoCbi{\cdot}N\text{-MeIm}]^+$, its K_{assoc} and unusual ΔH and ΔS values, and product and kinetic studies of its Co−C thermolysis, work which addresses the needed chemical precedent for this second subclass of $AdoB_{12}$-dependent enzymes.[1d]

Also not yet available is a detailed understanding of the likely fascinating protein biochemistry and enzymology of the *full range* of B_{12}-dependent enzymes, an understanding that will require additional X-ray crystallographic structural determinations[6d,e] of active, B_{12}-dependent holoenzyme complexes. Certainly, definition of the full and exact roles of the upper Ado, the middle corrin, and even of the lower 5,6-dimethylbenzimidazole (i.e., when it is in its base-off, but side-chain imidazole base-on, form[6]) will require additional X-ray crystallographic and other biochemical investigations of at least a couple of members of each subclass[6c] of AdoCbl·enzyme complexes. Efforts toward this challenging goal are continuing.

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Supporting Information Available: Table A, the expanded version of Table 1 which includes the product control studies at high [base]; a control experiment attempting to recover the adenine byproduct by an acid quench; control experiments testing the temperature dependence of the Co−C cleavage products; control experiments testing for alkoxide involvement in the observed rate acceleration of Co−C cleavage; UV−vis overlaid scans of $AdoCbi^+$ thermolysis in the presence of 1.0 M Me_2N-py and 2×10^{-2} M TEMPO (110 °C; anaerobic ethylene glycol); data tables; plots of k_{obsd} vs [base] and $1/k_{obsd}$ vs 1/[base] for Me_2N-py and pyridine; $AdoCbi^+$ and base ln K vs $1/T$ plots, where base = Me_2N-py and pyridine; $Co^{II}Cbi^+$ with Me_2N-py and pyridine ln K vs $1/T$ plot; results of $AdoCbi^+$ thermolysis in the presence of RSH/RS^-; and a complete derivation of kinetic equations and associated deconvolution of k_{obsd} (23 pages). See any current masthead page for ordering and Internet access instructions.

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(49) For example see: (a) Zagalak, B.; Pawelkiewicz, J. *Acta Biochim. Palon.* **1965**, *12*, 219. (b) Sando, G. N. ; Blakley, R. L.; Hogenkamp, H. P. C.; Hoffman, P. J. *J. Biol. Chem.* **1975**, *250*, 8774. (c) Sando, G. N.; Grant, M. E.; Hogenkamp, H. P. C. *Biochem. Biophys. Acta* **1976**, *428*, 228. (d) Yakusheva, M. I.; Poznaskaya, A. A.; Pospeloova, T. A.; Rudakova, I. P.; Yurkevich, A. M.; Yakovlev, V. A. *Biochem. Biophys. Acta* **1977**, *484*, 216−235 (see also pp 236−243). (e) Reviews to Toraya and co-workers' extensive series of papers: Fukui, S; Toraya, T. In *Progress in Bioorganic Chemistry and Molecular Biology*; Ovchimmikov, Y. A., Ed.; Elsevier: Amsterdam, 1984, pp 487−493, especially Table II and references therein. See also Fukui, S; Toraya, T. In *Vitamin B_{12}*; Zagalak, B., Fredrich, Eds.; Walter de Gruyter: Berlin, 1979, p 413. (f) Toraya, T.; Matsumoto, T.; Ichikawa, M.; Itoh, T.; Sugawara, T.; Mizuno, Y. *J. Biol. Chem.* **1986**, *261*, 9289. (g) Toraya, T.; Watanabe, N.; Ichikawa, M.; Matsumoto, T.; Ushio, K.; Fukui, S. *J. Biol. Chem.* **1987**, *262*, 8549. (h) Ichikawa, M.; Toraya, T. *Biochem. Biophys. Acta* **1988**, *952*, 191−200. (i) Toraya, T.; Ishida, A. *Biochemistry* **1988**, *27*, 7677−7681. (j) Krouwer, J. S.; Holmquist, B.; Kipnes, R. S.; Babior, B. M. *Biochem. Biophys. Acta* **1980**, *612*, 153.

(50) Lott, W. B.; Chagovetz, A. M.; Grissom, C. B. *J. Am. Chem. Soc.* **1995**, *117*, 12194.

(51) Geno, M. K.; Halpern, J. *J. Am. Chem. Soc.* **1987**, *109*, 1238.

(52) (a) Toraya, T.; Fukui, S. In *B_{12}*; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 2, Chapter 9; see Table 2, p 247. (b) Toraya, T.; Krodel, E.; Mildvan, A. S.; Ables, R. H. *Biochemistry* **1979**, *18*, 417. (c) Toraya, T.; Ables, R. H. *Biochem. Biophys. Acta* **1980**, *203*, 174.

(53) (a) Brown, K. L.; Hakimi, J. M.; Nuss, D. M.; Montejano, Y. D.; Jacobsen, D. W. *Inorg. Chem.* **1984**, *23*, 1463. (b) Brown, K. L.; Zou, X.; Evans, D. R. *Inorg. Chem.* **1994**, *33*, 5713. (c) Brown, K. L.; Cheng, S.; Marques, H. M. *Inorg. Chem.* **1995**, *34*, 3038. (d) See the arguments presented in ref 56 elsewhere.[1b] (e) Brown, K. L.; Evans, D. R.; Cheng, S.; Jacobsen, D. W. *Inorg. Chem.* **1996**, *35*, 217. In this work, neopenytlcobalamin derivatives are found to be stabilized by a $\Delta G^{\ddagger}$(25 °C) of 2.8 ± 1.0 kcal/mol to Co−C homolysis upon complexation to haptocorrin, a net effect that is 67(±38)% enthalpic (the expected radical-cage effect by the haptocorrin) and 33(±10)% entropic at 25 °C.

(54) (a) Eschenmosher, A. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 5; *Angew. Chem.* **1988**, *100*, 5. (b) Brown has argued, on the basis of NMR studies, that some strain is present in the nucleotide loop as the Co−N bond distance varies from 2.19 Å: Brown, K. L. *Inorg. Chem.* **1986**, *25*, 3111 (see the statement on p 3113). Brown, K. L.; Evans, D. R. *Inorg. Chem.* **1993**, *32*, 2544 (see the statement about the "progressive strain in the nucleotide loop" on p 2546).