Alkyl Radical Geometry Controls Geminate Cage Recombination in Alkylcobalamins

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Received September 20, 1994. Revised Manuscript Received May 16, 1995[⊗]

Abstract: The radical pair that results from photolysis of adenosylcob(III)alamin ($AdoCbl^{III}$) undergoes primary geminate recombination with a first-order rate constant of $1 \times 10^9\ s^{-1}$. In contrast, methylcob(III)alamin (CH_3Cbl^{III}) and aristeromicylcob(III)alamin ($AriCbl^{III}$, the carbocyclic analogue of $AdoCbl^{III}$ in which the ribofuranose ring oxygen has been replaced with a methylene group) does not undergo primary geminate recombination. The ribofuranose group enables a high rate of geminate recombination in the [Ado• Cbl^{III}] radical pair. This may be due to a stereoelectronic (β-anomeric) effect that maintains a pyramidal geometry at the 5′-carbon of the 5′-deoxyadenosyl radical, or it may be due to hindered rotation about the $C_{4'}-C_{5'}$ bond such that β-elimination to the olefin is prevented. Recombination in the geminate singlet radical pair is in competition with diffusive escape to form a solvent-separated radical pair. Hyperfine coupling from Co^{II} promotes intersystem crossing to the triplet radical pair (Chagovetz, A. M.; Grissom, C. B. *J. Am. Chem. Soc.* **1993**, *115*, 12152). Recombination of the [CH_3• Cbl^{II}] radical pair is not prevented by a lack of intersystem crossing, as neither unlabeled or ^{13}C-labeled CH_3Cbl^{III} undergoes geminate recombination. There is only a small difference in the rate of diffusive recombination in the solvent cage for $AdoCbl^{III}$, $AriCbl^{III}$, and CH_3Cbl^{III} following photolysis: $2.01 \times 10^4\ s^{-1}$, $2.20 \times 10^4\ s^{-1}$, and $1.16 \times 10^4\ s^{-1}$. The rate of diffusive recombination is limited by productive collisions and not by radical geometry or intersystem crossing. The CF_3• radical that results from photolysis of (trifluoromethyl)cob(III)alamin (CF_3Cbl^{III}) maintains its pyramidal geometry and undergoes faster diffusive recombination in the solvent cage at $51 \times 10^4\ s^{-1}$. The C−Co bond dissociation enthalpy in $AriCbl^{III}$ is 37 ± 1.4 kcal/mol. The profound difference in geminate recombination rates for $AdoCbl^{III}$ and CH_3Cbl^{III} is consistent with their different biological roles as enzymatic cofactors: $AdoCbl^{III}$ is an initiator of radical chain chemistry in the active site, whereas CH_3Cbl^{III} is a methyl group donor in an S_N2-type process.

Introduction

The remarkable chemistry afforded by the B_{12} cofactor is ascribed to the unusually weak C−Co bond. The bond dissociation energy (BDE) of adenosylcob(III)alamin[1-3] ($AdoCbl^{III}$) is estimated to be 31 kcal/mol, whereas the BDE for methylcob(III)alamin[4] (CH_3Cbl^{III}) is 37 kcal/mol. Another unusual observation is the high efficiency of radical pair (RP) recombination following C−Co bond homolysis (>94% in the axial-base-off 5′-deoxyadenosylcobamide).[5] The observations of a low BDE and efficient RP recombination are not strictly contradictory, although chemical intuition suggests that an inherently weak bond has a low electron density between the bonding partners and this should produce a low rate of radical pair recombination following dissociation.

Both $AdoCbl^{III}$ and CH_3Cbl^{III} undergo photohomolysis of the C−Co bond to produce cob(II)alamin (Cbl^{II}) and the corresponding alkyl radical. The continuous-wave quantum yields (ϕ_{CW}) at 440−540 nm are 0.20 ± 0.03 and 0.35 ± 0.03, respectively.[6-8] Curiously, ϕ_{CW} is 1.75-fold lower for $AdoCbl^{III}$ photolysis than for CH_3Cbl^{III} photolysis, even though CH_3Cbl^{III} has a significantly stronger C−Co bond, and the $\pi \rightarrow \pi^*$ transition near 520 nm that leads to photohomolysis has a similar extinction coefficient.[7b,9] The developing consensus among the biochemical community is that transient C−Co bond homolysis occurs as the first step in many enzymatic $AdoCbl^{III}$ dependent 1,2-migration reactions, while CH_3Cbl^{III} seldom reacts via C−Co homolysis in enzymatic reactions, but instead reacts through 2-electron chemistry via S_N2 (or similar) processes.[4b,5,6c,d,8,10-12]

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[⊗] Abstract published in *Advance ACS Abstracts*, December 1, 1995.

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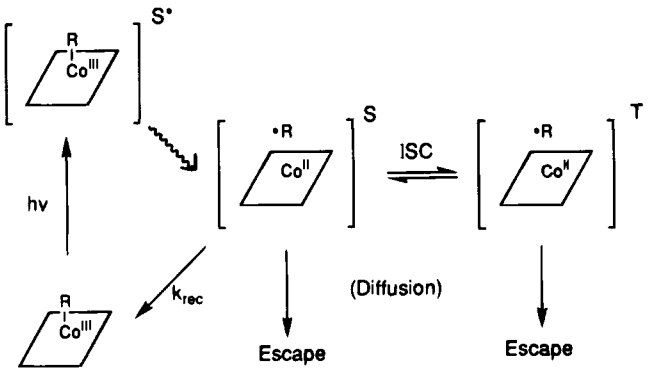
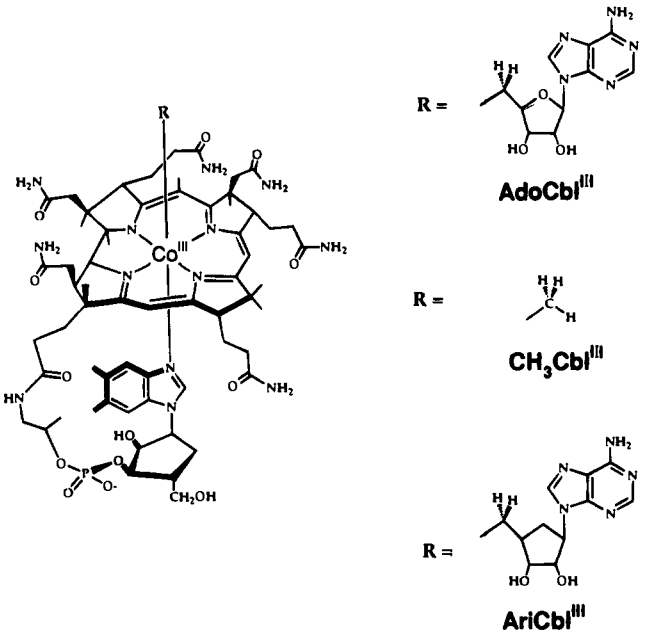
Scheme 1



Photodissociation of AdoCbl^{III} produces 5′-deoxyadenosyl radical and cob(II)alamin as the singlet radical pair {Ado• Cbl^{II}} (curved brackets indicate a geminate radical pair) that undergoes recombination[8,13] (Scheme 1). Similarly, CH_3Cbl^{III} exhibits a magnetic field dependent quantum yield under conditions of continuous-wave illumination.[14] In AdoCbl^{III}, the first-order rate constant for *geminate* RP recombinations is 1×10^9 s^{-1} and this rate is independent of solvent viscosity.[8] A four-fold increase in the rate of geminate RP recombination is observed at 0.05 T.[8] This is consistent with the RP being formed in the singlet spin state with a magnetic field dependent increase in recombination arising from a decrease in hyperfine coupling that partially populates the triplet state at the expense of the singlet population at zero field.[8,13,15] We suggested the high efficiency and unusually fast rate of geminate recombination may be enabled by the pyramidal geometry of the 5′-deoxyadenosyl radical.[8] We further suggested that the pyramidal geometry of the radical center is influenced by a stereoelectronic (β-anomeric) effect from the lone pair electrons of the ribofuranose ring oxygen[8] (Chart 1).

To explore this hypothesis, we have synthesized the carbocyclic analogue of AdoCbl^{III}, aristeromicylcob(III)alamin (AriCbl^{III}; Chart 1) and compared its recombination kinetics to that of authentic coenzyme B_{12}.[16] We have verified this hypothesis and extended our transient kinetics study to other alkylcob(III)alamins (AlkylCbl^{III}) with radical pair elements of varying pyramidal geometry. We have demonstrated the remarkable *unimportance* of geminate recombination in (CH_3Cbl^{III}).[17] This is consistent with the planar geometry of the methyl radical (CH_3•). The profound difference in geminate recombination rates for AdoCbl^{III} and CH_3Cbl^{III} is consistent with their putative biological roles as enzymatic cofactors: AdoCbl^{III} as initiator of radical chain chemistry in the active site, and CH_3Cbl^{III} as donor of methyl-cation from CH_3Cbl.[4b,5,6c,d,8,11-13,18]

Chart 1



Experimental Section

General. AdoCbl^{III}, CH_3Cbl^{III}, and hydroxocob(III)alamin were purchased from Sigma. All other chemicals were of reagent grade or better. All solvents were distilled and dried according to established procedures.[19] All glassware used for the picosecond and nanosecond kinetic studies was pyrolyzed at 600 °C to remove contaminating chromophores.

Synthesis of Alkylcob(III)alamins. Formation of the C−Co bond in alkylcob(III)alamins is accomplished by reduction of hydroxocob(III)alamin to cob(I)alamin with $NaBH_4$, followed by addition of the appropriate electrophile (typically, an alkyl halide).[20,21] (Trifluoromethyl)cob(III)alamin (CF_3Cbl^{III}) was synthesized from CF_3Br according to literature methods.[21] Our preparation of CF_3Cbl^{III} was characterized by a comparison to known spectral data.[21] Synthetic alkylcob(III)alamins were desalted on an amberlite XAD 2 column and further purified by ion exchange chromatography on SP Sephadex C-25.[21]

Aristeromicylcob(III)alamin, 9-[2′,3′-Dihydroxy-4′-(hydroxymethyl)cyclopentyl]adenylcob(III)alamin. AriCbl^{III} was synthesized from aristeromicin and hydroxocob(III)alamin by a modification of literature methods.[22]

9-[2′,3′-Di-*O*-isopropylidene-4′-(hydroxymethyl)cyclopentyl]-adenine. Aristeromicin, 9-[2′,3′-dihydroxy-4′(hydroxymethyl)cyclopentyl]adenine (200 mg, 0.79 mmol; obtained from Glaxo, U.K.) was dried at 100 °C overnight under vacuum with P_2O_5 and dissolved in 50 mL anhydrous acetone under N_2. Anhydrous toluenesulfonic acid (1.5 g, 7.9 mmol) was added with vigorous stirring. After 30 min at 25 °C, the solution turned pale yellow. After a total of 2.5 h, the

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(16) A preliminary report of this work was presented at the 208th National Meeting of the American Chemical Society, Washington, DC, August 23, 1994.

(17) A preliminary report of the absence of geminate recombination in the RP produced by photolysis of CH_3Cbl^{III} was included in ref 13, Figure 16, but insufficient data to analyze this result was available at the time of publication.

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solution was cooled to 0 °C, and 20 mL of cold 1 M $NaHCO_3$ was added with stirring. After 10 min, all solvent was removed by rotary evaporation. The solid residue was extracted with 3 × 20 mL portions of anhydrous acetone. The acetone extracts were combined, and the acetone was removed by rotary evaporation. The solid residue was dissolved in 10 mL of nearly-boiling water and concentrated under vacuum to a total volume of 3 mL. The supersaturated solution was left to crystallize at 0 °C overnight to give 125 mg of pale yellow needles (60%). ^{1}H NMR ($CDCl_3$/DMSO-d_6) δ 7.79 (1H, s), 7.58 (1H, s), 6.20 (2H, br s), 4.58 (1H, t), 4.30−4.41 (1H, m), 4.20−4.29 (1H, dd), 3.25 (2H, br s), 2.78 (1H, br s), 1.85−2.08 (3H, m), 1.10 (3H, s), 0.86 (3H, s).

9-[2′,3′-Di-*O*-isopropylidene-4′-[[(*p*-tolylsulfonyl)oxy]methyl]-cyclopentyl]adenine. A slight modification of the literature procedure was employed.[22d] The acetonide (110 mg, 0.375 mmol) was dried overnight at 100 °C under vacuum over P_2O_5 and partially dissolved in 2 mL of freshly distilled pyridine. The solution was cooled to 0 °C, and anhydrous toluenesulfonyl chloride (120 mg, 0.630 mmol) was added with stirring. The reaction mixture was stirred at 0 °C for 10 min during which time the solution changed to yellow-brown. The solution was stirred at 25 °C overnight. Pyridine was removed under vacuum at 25 °C, and the solid residue was dissolved in 2 mL of $CHCl_3$ and 3 mL of 3 N H_2SO_4 at 0 °C. After separation of the solvents on ice, the H_2SO_4 layer was decanted and the $CHCl_3$ solution was washed once again with 3 mL of 3 N H_2SO_4 at 0 °C. After removing the acid phase, the $CHCl_3$ phase was washed with 4 mL of H_2O at 25 °C, followed by two washes with 2 × 4 mL saturated $NaHCO_3$. The aqueous washes were discarded, and the $CHCl_3$ was removed under vacuum. The solid residue was stored at −20 °C (yield: 139 mg, 0.311 mmol). The tosylate residue was used without further purification. ^{1}H NMR ($CDCl_3$/DMSO-d_6) δ 8.25 (1H, s), 7.80 (1H, s), 7.77 (2H, d), 7.31 (2H, d), 5.75 (2H, br s), 5.09 (1H, t), 4.71 (1H, m), 4.55 (1H, dd), 4.19 (2H, m), 2.45 (3H, m), 2.40 (3H, s), 1.51 (3H, s), 1.22 (3H, s).

Aristeromicylcob(III)alamin, 9-[2′,3′-Dihydroxy-4′-(hydroxymethyl)cyclopentyl]adenylcob(III)alamin. The procedure of Brown and Peck was used to form the C−Co bond.[21] Starting with hydroxocob(III)alamin (500 mg, 0.36 mmol) in 25 mL of H_2O, 185 mg (0.12 mmol) of aristeromicylcob(III)alamin was recovered before purification. The protecting group was removed by stirring a solution of the aristeromicylcob(III)alamin acetonide in 0.1 M HCl for 18 h. The acidic solution was neutralized with 10% $NaHCO_3$, and the solution was concentrated under vacuum. The concentrate was desalted on an Amberlite XAD column eluted with water, followed by 50% acetonitrile. The solvent was removed under vacuum, and the residue was applied to an SP sephadex C-25 ion exchange column. The free aristeromicylcob(III)alamin was eluted from the column with water. The aristeromicylcob(III)alamin was recrystallized from acetone/water. The deprotection was followed by the disappearance of the acetonide methyl groups in the ^{1}H NMR spectrum. UV-vis λ_{max} = 358 nm, 512 nm. ^{1}H NMR (D_2O) δ 7.68 (1H, d), 7.35 (1H, d), 6.05−7.25 (3H, m), 4.15−4.40 (2H, m), 3.82−4.13 (1H, m), 3.45−3.80 (1H, m), 3.29−3.43 (1H, m), 1.1−2.8 (m).

Continuous Photolysis Kinetics. Continuous photolysis kinetic studies were carried out as described previously.[8] Alkylcob(III)alamins have characteristic absorbtion maxima at 365−375 nm and 520−535 nm.[9,23] Cob(II)alamin has a distinct absorbance at 476 nm.[9,23] An aqueous solution of the alkylcob(III)alamin was prepared with 50 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (Hepes), pH 7.0. The solution was deaerated in a sealed 1 cm quartz cuvette by purging with Ar immediately prior to photolysis. Continuous-wave visible light irradiation was accomplished at 514 nm with an Ar^+ laser. Absorption spectra were recorded in 1 s with a diode array spectrophotometer at time intervals of 10 s to 1 min. Exposure to light during analysis was kept to a minimum. The plot of [AlkylCbl^{III}] vs time (t) appeared zero-order in substrate in all cases and dA/dt was linearly proportional to laser fluence (W/cm^2).

Nanosecond Photolysis Kinetics. Nanosecond laser flash photolysis kinetic studies were carried out at the NIH Center for Fast Kinetics Research at the University of Texas, Austin. Photolysis at 532 nm was initiated with a frequency-doubled Nd:YAG Q-switched laser with a pulse length of 10 ns and a total power of 100 mJ per pulse (attenuated with neutral density filters to either 8, 20, or 40 mJ, as specified). The concentration of cob(II)alamin was monitored continuously by difference absorption spectroscopy at 476 nm using a high pressure Xe lamp, monochromator, and photomultiplier tube. Photolysis by the interrogating light source was minimized by a shutter that was opened immediately prior to laser triggering and closed immediately after data collection. The absorbance reading was balanced to zero before the flash, and data were recorded on a digital storage oscilloscope for a period of 450 μs following the flash. Typically, ten transient measurements were averaged. A solution of the indicated alkylcob(III)alamin was purged with Ar for 40 min immediately prior to and during photolysis. The sample solution was pumped through a 1 cm path length 2 mL volume quartz flow cuvette at 10 mL/min without recycle. The absolute absorbance was ≈0.8 at 532 nm, which corresponds to [AlkylCbl^{III}] of 100−150 μM. The rate of disappearance of Cbl^{III} vs t was first-order in appearance, and the data were fitted to [Cbl^{III}] = [Cbl^{II}]$_0$[exp(-$k_{rec}t$)] by nonlinear methods. Since this is a difference spectroscopy measurement, the concentration of Cbl^{II} was determined using ϵ_{476} = 9.2 × 10^3 M^{-1} cm^{-1} and alkylCbl^{III} ϵ_{476} = 6.5 × 10^3 M^{-1} cm^{-1} which gives $\Delta\epsilon_{476}$ = 2.7 × 10^3 M^{-1} cm^{-1} to correct for the presence of [Cbl^{III}] by the method of Chance.[6d]

Picosecond Photolysis Kinetics. Picosecond laser flash photolysis kinetic studies were carried out at the NIH Center for Fast Kinetics Research at the University of Texas−Austin as described previously.[8] Preparation of CH_3Cbl^{III} and AriCbl^{III} photolysis solutions was exactly as described for AdoCbl^{III}.[8] [Cbl^{II}] was monitored at 476 nm with the specified extinction coefficients.[6d,8]

Bond Dissociation Energy of AriCbl^{III}. The bond dissociation energy of AriCbl^{III} was estimated by the method of Finke.[1,4] The base-on/base-off equilibrium parameters for AriCbl^{III} were determined by monitoring the absorbance of a degassed 100 μM solution of AriCbl^{III} at 520 nm as a function of temperature from 5−80 °C where thermolysis is slow. The measurements were taken at various temperatures and in random order to ensure that the base-on/base-off equilibrium was reversible and that no significant thermolysis occurred. Quartz cuvettes fitted with Pyrex extension tubes were charged with 2.2 mL of 100 μM AriCbl^{III} and 50 mM 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO) in ethylene glycol. The samples were degassed via three freeze-pump-thaw cycles and sealed under vacuum. The samples were thermolyzed as a function of temperature from 90−120 °C. The rate of the reaction for each isotherm was determined by following the first-order disappearance of the absorbance at 510 nm ascribed to AriCbl^{III}. The first-order rate constants were corrected for the base-on/base-off equilibrium and then used to construct an Eyring plot[1] (see supporting information). The temperature dependent change in cage efficiency of ethylene glycol was corrected by subtracting 4 kcal/mol from the apparent value of ΔH.[1a,4b] The observed activation enthalpy was calculated according to the method of Finke.[1a,4b] The entire procedure was also carried out with AdoCbl^{III} as a test of our implementation of the Finke method of calculating BDE values in alkylcobalamins.

Results and Discussion

C−Co Bond Dissociation Energy in AriCbl^{III}. Our experiments indicate that for the base-on form of AriCbl^{III}, the C−Co BDE is 37 ± 1 kcal/mol. This value was determined by the TEMPO trapping method of Finke.[1] An Eyring plot of the axial, base-on corrected, rate constants give the homolysis activation parameters $\Delta H^{\ddagger}_{h,on}$ = 41 ± 1 kcal/mol and $\Delta S^{\ddagger}_{h,on}$ = 13 ± 2 cal/(mol K) (data in supporting information). The recombination barrier of ≈4 kcal/mol imposed by ethylene glycol is subtracted from this value to give an estimate of 37 ± 1 for the bond dissociation energy of AriCbl^{III}.

As a validation of our methodology, we redetermined the BDE of AdoCbl to be 31 ± 1 kcal/mol by the method described above. This value is in remarkable agreement with Finke's published value of 31 kcal/mol.[1c,g] The reader is referred to a recent summary[1a] for a complete discussion of the uncertainties

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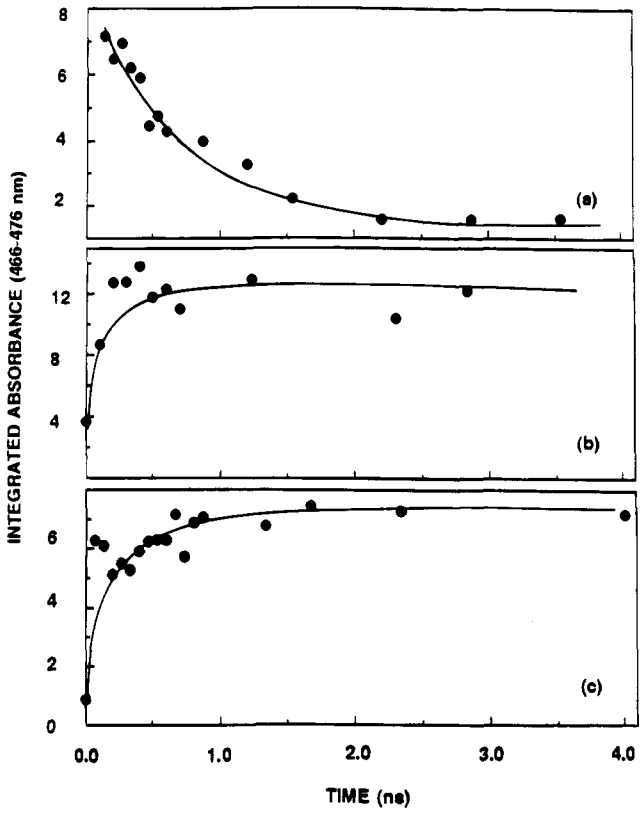

Figure 1. Picosecond photolysis. Kinetic trace of [cob(II)alamin] after a 30 ps 532 nm photolysis pulse. [Cob(II)alamin] was determined by integrating the transient absorbance centered at 471 nm. Conditions are 20 °C, 200 μM alkylcob(III)alamin and 50 mM Hepes, pH 7.0. (A) Adenosylcob(III)alamin (data reprinted by permission from ref 8). The line is the result of fitting the data to the first order rate equation with $k_{rec} = 1 \times 10^{-9}$ s^{-1}.[8] (B) Methylcob(III)alamin. (C) Aristeromicylcob(III)alamin. With CH_3Cbl^{III} and $AriCbl^{III}$, no decrease in [Cob(II)alamin] is observed in the range 0.1−3.5 ns. This places a lower limit of 1.7×10^8 s^{-1} on k_{rec} for CH_3Cbl^{III} and $AriCbl^{III}$.

associated with the determination of C−Co BDE values.[1,2,24] Regardless of any uncertainty that exists in the absolute value of the BDE, the *relative* values within a closely homologous series of alkylcobalamins should be useful for comparing *relative* bond dissociation energies. Within this caveat, the C−Co BDE in AriCbl is 6 ± 1.4 kcal/mol higher than in AdoCbl.

Picosecond Laser Flash Photolysis at 532 nm. For *net* photolysis of CH_3Cbl^{III}, $\phi_{CW} = 0.35$ and for $AdoCbl^{III}$, $\phi_{CW} = 0.20$.[6-8] The quantum yield for CH_3Cbl^{III} is 1.75-fold higher than ϕ_{CW} for net photolysis of AdoCbl. If ϕ_{CW} is high, geminate recombination must be low.[6c,d] Figure 1 shows the kinetic trace from 0.1−3.5 ns after photolysis for (A) $AdoCbl^{III}$ (data reprinted from ref 8 by permission); (B) CH_3Cbl^{III}; and (C) $AriCbl^{III}$. As previously reported,[8] the {Ado• Cbl^{II}} RP produced by photolysis of $AdoCbl^{III}$ recombines with $k_{rec} = 1 \times 10^9$ s^{-1}. This exceptionally fast and viscosity-independent rate can only represent *primary geminate* recombination that occurs without molecular correlation or interaction with solvent. In marked contrast, the {CH_3• Cbl^{II}} and {Ari• Cbl^{II}} radical pairs do not recombine on the geminate time scale, since the absorbance at 476 nm does not decrease following photolysis. The recombination rate constant for {CH_3• Cbl^{II}} and {Ari• Cbl^{II}}, as observed by picosecond laser flash photolysis, must be considerably lower than k_{rec} for $AdoCbl^{III}$ photolysis. Data points were collected out to 4.0 ns, as given by a variable optical delay line limit of 1.2 m. Since no recombination of {CH_3• Cbl^{II}} or {Ari• Cbl^{II}} was observed during this time interval, the rate of geminate recombination must be decreased by at least six-fold, to a value that is less than 1.7×10^8 s.$^{-1}$ [25]

The picosecond photolysis data of Endicott and Netzel[6a] clearly showed recombination of {Ado• Cbl^{II}}, but the data for {CH_3• Cbl^{II}} recombination were ambiguous because of the poor signal-to-noise ratio.[6a] Their use of a Nd:glass laser source, while having an extremely short 6 ps pulse, limited photolysis repetition to approximately 1 shot per min because of slow heat dissipation. Because of this technical limitation, they incorrectly concluded that the recombination rates for {CH_3• Cbl^{II}} and {Ado• Cbl^{II}} must be similar. Their recombination rate for the radical pair generated by AdoCbl photolysis is in exact agreement with our value. Neither study showed evidence for short-lived intermediates other than Cbl^{II}. The temporal resolution in our experiment was ≈3 ps, beginning within a few tens of ps after the 30 ps photolyzing laser pulse.

Because the singlet RP is produced, the intrinsic rate of {Ado• Cbl^{II}} recombination is not somehow limited by ISC. To show that recombination of {CH_3• Cbl^{II}} is not limited by the absence of ISC, $^{13}CH_3Cbl^{III}$ was synthesized to promote rapid ISC via nuclear-electronic hyperfine coupling. No additional recombination was observed with {$^{13}CH_3$• Cbl^{II}} (data not shown). This shows that the lack of recombination in either CH_3Cbl^{III} or $AriCbl^{III}$ photolysis is not due to inefficient ISC from a triplet precursor.[26]

The marked difference in recombination rates may be partially attributed to the smaller size, lower τ_c, and faster diffusion rate for CH_3•. However, the 5′-deoxyaristeromicyl radical should be so similar to the 5′-deoxyadenosyl radical that these differences can be discounted when comparing the recombination behavior of $AdoCbl^{III}$ and $AriCbl^{III}$. The few water molecules that are hydrogen-bonded to the ribofuranose ring oxygen should be inconsequential when compared to the overall similar structure of the 5′-deoxyaristeromicyl and 5′-deoxyadenosyl radicals. A more significant factor contributing to the difference in k_{rec} for {CH_3• Cbl^{II}} and {Ado• Cbl^{II}} is the planar geometry of CH_3•. Soon after homolysis, the methyl radical reaches a geometry that is no greater than 10° off planarity (Figure 2).[27] This makes CH_3• sufficiently less reactive such that primary recombination is disfavored, the diffusive escape occurs. In alkyl radicals with significant σ-acceptor or π-donor ligands, a pyramidal geometry is maintained and recombination is energetically more favorable.[28] In contrast to the methyl radical, the 5′-deoxyadenosyl radical may maintain a pyramidal geometry for a sufficiently long time, so as to favor recombination. This hypothesis will be developed further in the following sections.

Nanosecond Laser Flash Photolysis. Irradiation with a 10 ns laser pulse and interrogation on the 1−500 μs time scale allows us to probe diffusive recombination that occurs in the bulk solvent or solvent cage, where recombination is limited by the rate of diffusive encounter. The nanosecond transient

(24) Gerards, L. E. H.; Bulthuis, H.; de Bolster, W. G.; Balt, S. *Inorg. Chim. Acta* **1991**, *190*, 47.

(25) This is a conservative calculation that assumes a 50% decrease in [Co^{II}] could be detected with greater than a 95% confidence limit. The $T_{1/2}$ for a first-order rate of 1.67×10^8 s^{-1} would be 4.2×10^{-9} s.

(26) The continuous-wave photolysis and thermolysis of [5′-^{13}C]adenosylcob(III)alamin and [$^{13}CH_3$]cob(III)alamin has previously been shown to be qualitatively similar to unlabeled material (Hogenkamp, H. P. C.; Vergamini, P. J.; Matwiyoff, N. A. *J. Chem. Soc.* **1975**, 2628).

(27) (a) Karplus, M.; Fraenkel, G. K. *J. Chem. Phys.* **1961**, *35*, 1312. (b) Herzberg, G. *Proc. R. Soc.* **1961**, *A262*, 291. (c) Kaplan, L. In *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. II, Chapter 18. (d) Pacansky, J. *J. Phys. Chem.* **1982**, *86*, 485.

(28) Avila, D. V.; Ingold, K. U.; Lusztyk, J.; Dolbier, Jr., W. R.; Dan, H.-Q.; Muir, M. *J. Am. Chem. Soc.* **1994**, *116*, 99 (and references therein).

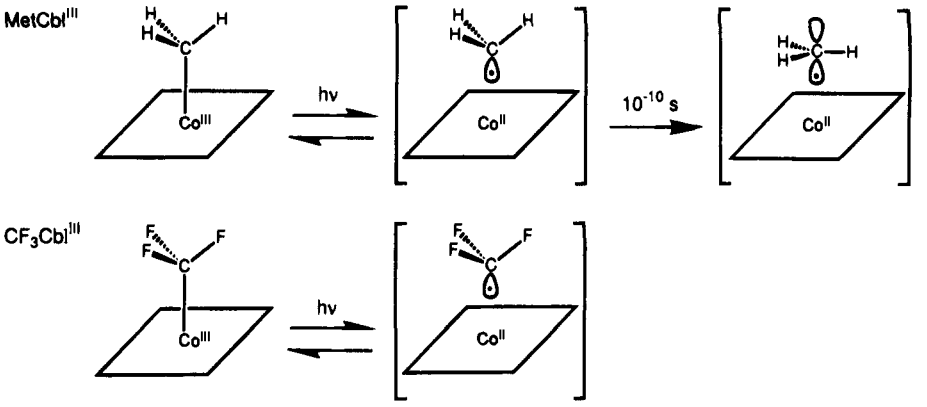

Figure 2. The methyl radical reaches planarity within 10^{-10} s after C−Co homolysis, whereas the trifluoromethyl radical maintains a pyramidal geometry.

Table 1. Rate of Secondary Solvent Cage Recombination[a]

cobalamin	solvent	rate constant, k_{rec} ($\times 10^4$ s^{-1})
CH_3	H_2O	2.20[b]
CH_3	CH_2OHCH_2OH	0.296
$^{13}CH_3$	H_2O	2.48
$^{13}CH_3$	CH_2OHCH_2OH	0.159
adenosyl	H_2O	2.01
adenosyl	CH_2OHCH_2OH	0.295
aristeromicyl	H_2O	1.16
aristeromicyl (prot)[c]	H_2O	1.76
CF_3	H_2O	50.9
CF_3	CH_2OHCH_2OH	1.64

[a] Determined by nanosecond laser flash photolysis initiated by a 10 ns 532 nm pulse. [Cob(II)alamin] was followed at 476 nm. Assay conditions: aqueous solutions were buffered with 50 mM Hepes, pH 7.0, 20 °C; or ethylene glycol, 20 °C. [b] Rates are the average of two determinations at 20 mJ and 40 mJ per pulse. The standard error from averaging the two measurements is less than 2% in all cases. The standard error from fitting each kinetic trace to the first-order rate equation is less than 0.5% of the reported value in all cases. [c] Protected aristeromicylcob(III)alamin is 9-[2′,3′-di-*O*-isopropylidene-4′-(hydroxymethyl)cyclopentyl]adenylcob(III)alamin.

absorption studies of $AdoCbl^{III}$ by Chance[6c] showed that recombination also occurs in the bulk solvent [Ado•//Cbl^{II}] (square brackets and hash marks indicate solvent separated RP). We have extended these studies to include the solvent cage recombination kinetics of the RP produced by the photolysis of CH_3Cbl^{III}, $AriCbl^{III}$, $^{13}CH_3Cbl^{III}$, and CF_3Cbl^{III}, as well as $AdoCbl^{III}$. A representative kinetic trace following a 10 ns pulse is shown in supporting information. Table 1 shows the first-order rate constants for RP recombination in the solvent cage. The rate constants for [CH_3•//Cbl^{II}] and [$^{13}CH_3$•//Cbl^{II}] recombination are nearly identical at 2.20×10^4 s^{-1} and 2.48×10^4 s^{-1}, indicating that ISC has only a small importance for RP recombination in bulk solvent of low viscosity ($\eta_{H_2O} \approx 1$ cP).[29] The radical pairs resulting from photolysis of $AdoCbl^{III}$ and $AriCbl^{III}$ combine with only slightly different rate constants of 2.01×10^4 s^{-1} and 1.16×10^4 s^{-1}. The RP resulting from photolysis of the acetonide of $AriCbl^{III}$ [9-[2′,3′-di-*O*-isopropylidene-4′-[[(p-tolylsulfonyl)oxy]methyl]cyclopentyl]-adeninylcob(III)alamin] also combines at a similar rate of 1.76×10^4 s^{-1} in the solvent cage. A two-fold (or less) variation in the rate of recombination for all of these compounds (vide infra) was observed. This suggests that the difference in $AdoCbl^{III}$, CH_3Cbl^{III}, and $AriCbl^{III}$ RP recombination rates observed on the geminate time scale does not matter on the slower time scale of recombination in the solvent cage.

(29) All measured rate constants have standard errors of 1−2% and are the average of at least two measurements at 20% and 40% laser intensity.

The viscous solvent, ethylene glycol, slows the rate of diffusion and decreases the rate of secondary solvent cage recombination by 1 order of magnitude. This supports the role of solvent in limiting diffusive recombination. Our results are in agreement with the nanosecond transient absorption measurements of Chen and Chance.[6c] Although their focus was not to measure the kinetics of recombination of the [Ado•//Cbl^{II}] RP, they estimated the rate of diffusional recombination to be $\approx 1 \times 10^8$ M^{-1} s^{-1}. This is in good agreement with our measurement of the *apparent* first-order recombination rate of $\approx 1 \times 10^4$ s^{-1} for a starting alkylcobalamin concentration of ≈100 μM.

(Trifluoromethyl)cob(III)alamin Photolysis. The RP resulting from CF_3Cbl^{III} photolysis is the only alkylcob(III)alamin that was observed to have a markedly faster rate of recombination (50.9×10^4 s^{-1}).[29] This may be the result of several factors, as described below.

Perfluoroalkyl radicals similar to CF_3• tend to have a pyramidal geometry at the radical center (Figure 2).[30] Thus, perfluoroalkyl radicals do not have to pay the energy cost of ≈ 2.6 kcal/mol to reorient from a planar to a pyramidal geometry.[27d,28] The 23-fold difference in rate constant for CF_3Cbl^{III} and CH_3Cbl^{III} RP recombination reflects approximately 1.9 kcal/mol difference in reactivity. Furthermore, electron withdrawal by fluorine enhances the electrophilicity of perfluoroalkyl radicals and increases the rate of addition to olefins.[28] A similar effect of electron demand probably exists for the addition of a perfluoroalkyl radical to Cbl^{II}.

A significantly stronger C−Co bond also results from substitution of F for H in alkylcob(III)alamins. Attempts to estimate the C−Co bond dissociation energy for CF_3Cbl^{III} by the method of Finke[1,4] were unsuccessful, as high temperatures were required for homolysis and complex kinetic behavior was observed. Further investigation will be necessary to estimate the bond dissociation energy of CF_3Cbl^{III}.

In spite of the low rate of geminate pair recombination for {CH_3• Cbl^{II}}, a modest magnetic field dependence of the net rate of photolysis was observed in our previous investigation of CH_3Cbl^{III} steady-state photolysis at 248 nm in solvents of increased viscosity.[14] The continuous-wave quantum yield for CH_3Cbl^{III} photolysis at 248 nm in 75% glycerol ($\eta/\eta_0 = 30$) decreases 60% at 0.05 T. This is close to the decrease in the continuous-wave quantum yield for $AdoCbl^{III}$ at 0.04 T. Because CH_3Cbl^{III} does not undergo geminate recombination, yet the quantum yield in 75% glycerol is magnetic field dependent under conditions of continuous irradiation, the

(30) (a) Krusic, P. J.; Bingham, R. C. *J. Am. Chem. Soc.* **1976**, *98*, 230. (b) Chen, K. S.; Krusic, P. J.; Meakin, P.; Kochi, J. K. *J. Phys. Chem.* **1974**, *78*, 2014.

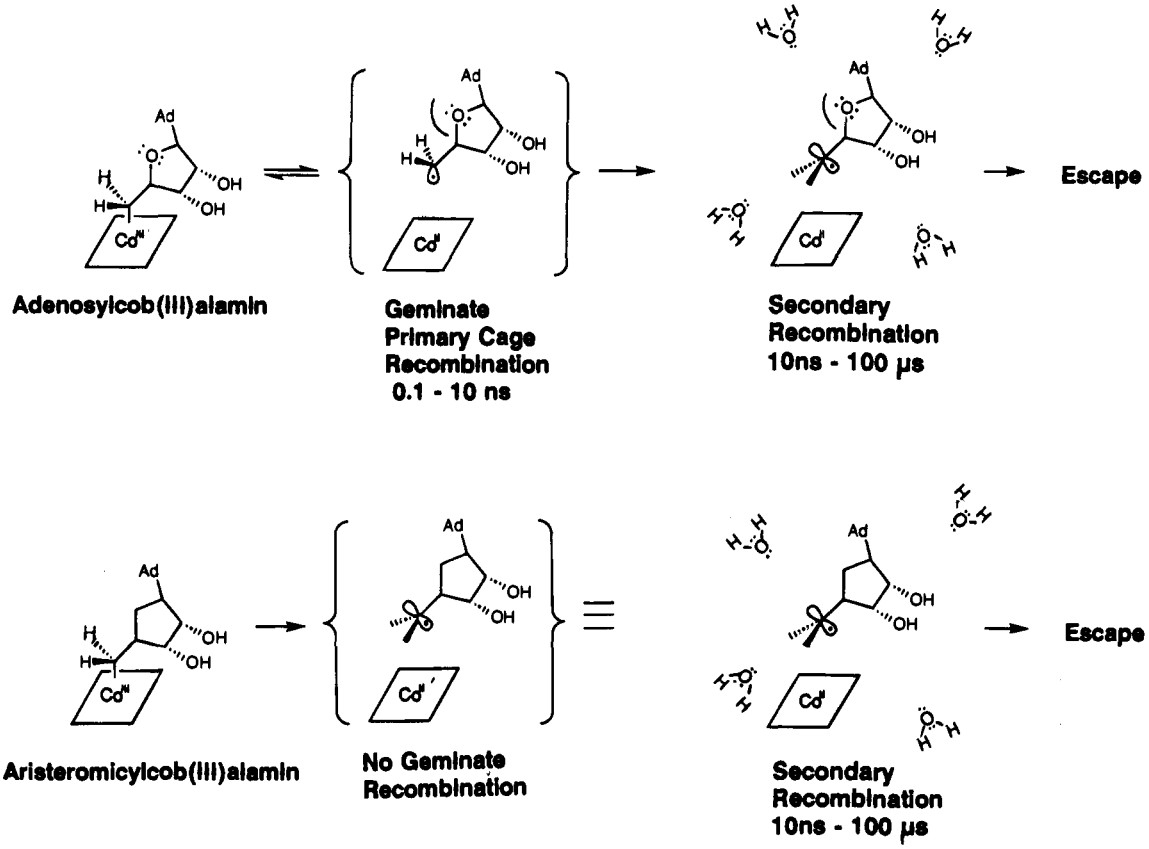

Figure 3. Time domains for radical pair recombination following photolysis. The geminate radical pair {Ado• Cbl^{II}} undergoes efficient primary geminate recombination by virtue of the pyramidal geometry at $C_{5'}$, whereas {Ari• Cbl^{II}} does not undergo primary geminate recombination because the radical at $C_{5'}$ reaches a planar geometry within 10^{-10} s after homolysis.

magnetic field sensitive step must be diffusive recombination with an average RP lifetime that is enhanced by the viscous medium.

There is precedent for magnetic field induced changes in the recombination rates of diffusing radical pairs (so-called F-pairs).[31] Singlet F-pairs that come together in a collision complex will undergo recombination with unit efficiency that is decreased only by the fraction of the molecules that don't present their reactive cross sections. In contrast, triplet radical pairs are prevented from reacting until an ISC event converts the RP to the singlet state. If the energy difference between the singlet and triplet RP states is nearly 0 (the case for a weakly-coupled RP), then the limiting fraction of singlet and triplet F-pairs will be 25:75. A radical pair encounter that is prolonged by a medium of increased viscosity (with a corresponding decrease in the rate of diffusional separation) will allow more of the triplet F-pair population to undergo ISC to the reactive singlet state. This is probably the basis for the magnetic field dependence that is seen in the continuous-wave irradiation kinetics for [CH_3•//Cbl^{II}] in viscous solvents (75% glycerol $\eta/\eta_0 = 30$).[8,14] In the absence of viscosigen, ($\eta_0 = 1$) diffusive separation of the triplet F-pair occurs before ISC.

Temporal Constraints on Alkylcob(III)alamin Recombination. There are at least two time domains for RP recombination in photochemically-produced alkylcob(III)alamin radical pairs: (1) *geminate* recombination that occurs in the radical pair prior to significant molecular rotation or translation and has a recombination rate constant of 1×10^9 s^{-1} for $AdoCbl^{III}$; and (2) *diffusive* recombination that occurs in the bulk solvent and has a recombination rate constant of $\approx 1 \times 10^8$ M^{-1} s^{-1} (Figure 3). Whatever causes the difference in recombination rates for [Ado• Cbl^{II}], and [CH_3• Cbl^{II}] or [Ari• Cbl^{II}] on the geminate time scale, these differences are not important determinants of diffusive recombination rates in bulk solvent.

Importance of Ribofuranose. The data in Figure 1 show that the ribofuranose ring oxygen plays an important role in the recombination kinetics of {Ado• Cbl^{II}}. Several hypotheses can be put forth to explain this observation.

1. Gauche Effect. The gauche effect arises from the observation that compounds with vicinal electronegative substituents show a preference for a conformation in which they adopt a gauche relationship, rather than an antiperiplanar orientation. This effect stems from a preference for an electronegative substituent to attain an antiperiplanar relationship with a good electron-donating group, usually a C−H bond, rather than with another electronegative substituent.

In the case of $AdoCbl^{III}$ homolysis, the 5′-deoxyadenosyl radical is vicinal to the ribofuranose ring oxygen. The preferred rotational geometry about the $C_{4'}-C_{5'}$ bond will be with the ribofuranose ring oxygen antiperiplanar to a C−H bond, thereby creating a pseudogauche orientation with the singly-occupied orbital (Figure 4). Our semiempirical calculations estimate this to present a 2.9 kcal/mol barrier to rotation about the $C_{4'}-C_{5'}$ bond, with the energy minimum occurring when the ribofuranose ring oxygen is antiperiplanar to the C−H bond, as expected.[32] In contrast, the analogous radical that is produced from the homolysis of $AriCbl^{III}$ shows a rotational energy barrier of less than 0.3 kcal/mol.[32]

In the absence of a radical trapping agent, the alkyl radicals from homolysis of the C−Co bond in $AdoCbl^{III}$ and $AriCbl^{III}$

(31) (a) Cozens, F. L.; Scaiano, J. C. *J. Am. Chem. Soc.* **1993**, *115*, 5204. (b) Margulis, L. A.; Khudyakov, I. V.; Kuzmin, V. A. *Chem. Phys. Lett.* **1986**, *124*, 483.

(32) Semiempricial calculations with AM1 (Dewar, A. M. J.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902).

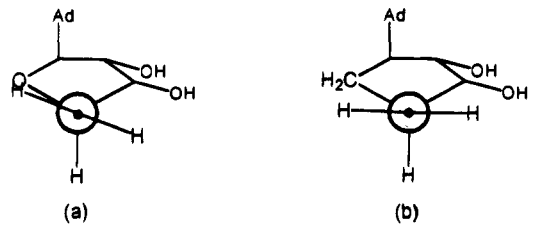

Figure 4. Newman projections along the $C_{4'}-C_{5'}$ bond: (a) 5′-deoxyadenosyl radical; (b) 5′-deoxyaristeromicyl radical (Ad is the adenine moiety). There is a 2.9 kcal/mol barrier to rotation about the $C_{4'}-C_{5'}$ bond, with the energy minimum occurring when the ribofuranose ring oxygen is antiperiplanar to the C−H bond. There is less than a 0.3 kcal/mol rotational barrier in b. Rotamer a is the preferred conformation for the 5′-deoxyadenosyl radical, and rotamer b is the preferred conformation for the 5′-deoxyadenosyl radical. Rotamer b is required for the elimination of the 4′-hydrogen atom. The exocyclic olefin produced from the elimination of the 4′-hydrogen atom is only observed in the photolysis of aristeromicylcob(III)alamin (see Figure 5).

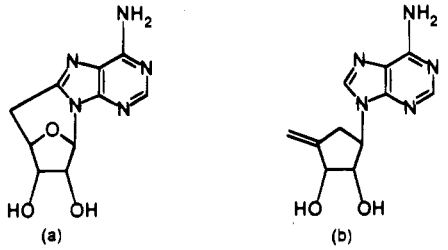

Figure 5. Major intramolecular products of the anaerobic photolysis of (a) adenosylcob(III)alamin and (b) aristeromicylcob(III)alamin in the absence of a radical trap.[22]

produce different intramolecular products (Figure 5). This observation supports the gauche effect, as described below. The 5′-deoxyadenosyl radical undergoes an intramolecular cyclization with the adenine moiety, while the aristeromicyl radical eliminates a hydrogen atom to produce a $C_{4'}-C_{5'}$-exocyclic double bond. The ribofuranose ring oxygen in the 5′-deoxyadenosyl radical inhibits the formation of the olefinic product by restricting rotation to a conformer that is conducive to elimination. The aristeromicyl radical is more free to rotate about the $C_{4'}-C_{5'}$ bond and adopt the proper geometry for elimination. The argument is analogous to the rotational correlation argument for $CH_3^{\bullet}$ from CH_3Cbl^{III} photolysis. If the $C_{4'}-C_{5'}$ bond rotates more freely in the aristeromicyl radical, then it is more likely to rotate away from a favorable geometry for geminate RP recombination with its Cbl^{II} bonding partner. The ribofuranose ring oxygen in the 5′-deoxyadenosyl radical imposes a transient barrier to rotation away from this favorable geometry, and geminate radical recombination is observed.

2. Pyramidal Radical Geometry. An alternative hypothesis can be put forth to account for the difference in geminate recombination between [Ado$^{\bullet}$ Cbl^{II}], and [$CH_3^{\bullet}$ Cbl^{II}] or [Ari$^{\bullet}$ Cbl^{II}]. A stereoelectronic (β-anomeric) interaction form the ribofuranose ring oxygen may transiently enforce a pyramidal geometry at the 5′-carbon of the 5′-deoxyadenosyl radical.[33,34] Within the geminate radical pair, a pyramidal carbon-centered radical will be more reactive toward recombination than a planar radical that has unpaired electron density that is distributed both above and below the plane that contains the carbon atom(s) and hydrogen atoms.[28]

Aristeromicylcob(III)alamin as Enzymatic Cofactor. Abeles and co-workers were the first to test the competency of $AriCbl^{III}$ as an enzymatic cofactor.[22a] They reported it supports the diol dehydrase reaction at about one-third the rate with $AdoCbl^{III}$, and they interpreted this result as being inconsistent with any mechanism that might involve protonation of the 5′-deoxyribosyl moiety.[22a] Indeed, the overall enzymatic reaction with $AriCbl^{III}$ must be very similar to the normal enzymatic reaction with $AdoCbl^{III}$, as similar deuterium kinetic isotope effects and spectral changes were observed.[22a] However, the differences between $AdoCbl^{III}$ and $AriCbl^{III}$ in solution cannot be denied. The data of Abeles suggest these differences are minimized in the enzyme active site (at least for diol dehydrase). The enzyme must exert an additional measure of control on the alkyl radical to prevent β-scission to the 4′-olefin. Additionally, it is not known if increased enzyme inactivation occurs with $AriCbl^{III}$.

Transition State for C−Co Bond Homolysis. Although the absolute value of the BDE still contains small uncertainties associated with solvent cage effects,[1,2,4,5,11,12] the relative values for close structural analogues should be informative and reliable. Finke and co-workers have estimated the C−Co bond dissociation energies of $AdoCbl^{III}$ and CH_3Cbl^{III} as 31 and 37 kcal/mol, respectively. They suggest this 6 kcal/mol difference is due to a difference in the transition state structures for $AdoCbl^{III}$ and CH_3Cbl^{III} homolysis at the elevated temperatures required for thermolysis. This difference must be in spite of similar ground states observed in the X-ray crystal structures of these compounds. The length of the C−Co bond in $AdoCbl^{III}$ and CH_3Cbl^{III} is 2.00 Å.[35] According to this observation, any difference in the observed bond dissociation energies must be reflected in the transition state, since the bond dissociation energies for these compounds are equal to the activation enthalpies for C−Co bond homolysis. Any strain imposed by the steric demand of the adenosyl moiety might be released in the transition state. However, we have measured a C−Co bond dissociation energy of 37 kcal/mol for $AriCbl^{III}$, and the transition state structure for $AriCbl^{III}$ homolysis should not differ significantly from the transition state for $AdoCbl^{III}$ homolysis *unless that difference arises directly from the ribofuranose ring oxygen of* $AdoCbl^{III}$. Since homolysis of the C−Co bond in alkylcob(III)alamins is expected to proceed through a late (productlike) transition state, the transition state structure should closely resemble the structure of the radicals produced. The Cbl^{II} species is spectroscopically identical in all cases; therefore, any significant difference should be manifested in the departing alkyl radical. In short, the ribofuranose ring oxygen of $AdoCbl^{III}$ provides a 6 kcal/mol thermodynamic stabilization of the alkyl radical produced by homolysis, while also introducing a

(33) (a) Kirby, A. J.; Williams, N. H. In *The Anomeric Effect and Associated Stereoelectronic Effects*; Thatcher, G. R. J., Ed.; American Chemical Society: Washington, D.C., 1993; Chapter 4. (b) Pinto, B. M.; Leung, Y. N. *Ibid.* Chapter 8. (c) Kirby, A. J. *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*; Springer-Verlag: Berlin, 1983; p 128.

(34) An anonymous referee has pointed out the enhanced reactivity of carbon-centered radicals with β-oxygen atoms (Barton, D. H. R.; Hartwig, W.; Motherwell, W. B. *J. Chem. Soc., Chem. Commun.* **1982**, 447). Barton suggests "...the stereochemical (orbital) arrangement of the β-atom [oxygen] will be of importance". Furthermore, he suggests "The geometry of β-substituted radicals may show preferred rotational conformers, but without significant angular deformation". Both of these hypotheses are reflected in our proposals that the gauche effect (i.e. "...preferred rotational conformers...") or the pyramidal radical geometry (i.e. "...stereochemical (orbital) arrangement...") may be important. Arguing against Barton's explanation, a re-examination of the radical stabilization imparted by a β-oxygen in the Barton deoxygenation reaction ascribes the effect purely to relaxed steric constraints when oxygen is present and not to stereoelectronic effects (Crich, D.; Beckwith, A. L. J.; Chen, C.; Yao, Q.; Davison, I. G. E.; Longmore, R. W.; de Parrodi, C. A.; Quintero-Cortes, L.; Sandoval-Ramirez, J. *J. Am. Chem. Soc.* **1995**, *117*, 8757).

(35) (a) Randaccio, L.; Pahor, N. B.; Zangrando, E.; Marzilli, L. G. *Chem. Soc. Rev.* **1989**, *18*, 225. (b) Rossi, M.; Glusker, J. P.; Randaccio, L.; Summers, M. F.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 1729. (c) Savage, H. F. J.; Lindley, P. F.; Finney, J. L.; Timmins, P. A. *Acta Crystallogr. Sect. B* **1987**, *43*, 280.

geometric barrier that kinetically favors geminate radical pair recombination.

Implications for the Biochemical Roles for AdoCbl^{III} and CH$_3$Cbl^{III}. The fact that the RP produced by photolysis of AdoCbl^{III} undergoes fast recombination, but the RP from CH$_3$Cbl^{III} photolysis does not recombine, is consistent with the accepted biological roles for the cofactors: AdoCbl^{III} is an initiator of radical pair chemistry in enzymatic reactions, whereas CH$_3$Cbl^{III} transfers a methyl group through an S_N2-like process.

The generally accepted proposal is that adenosylcob(III)-alamin will be bound in the enzyme active site so as to weaken the C−Co bond.[1b,5c,6c,11,12,36] Hydrogen bonds from the enzyme to the corrin ring and the adenosine moieties provide "excess" binding energy to stretch and weaken the C−Co bond.[37] The C−Co bond may break transiently, but fast geminate recombination will reform the C−Co bond unless the 5′-deoxyadenosyl radical is intercepted in the presence of the substrate that is to be acted upon. Adenosylcob(III)alamin thus becomes a "safe" initiator of radical chemistry,[5c,6c,11] The 5′-deoxyadenosyl radical can abstract H$^\bullet$ from substrate directly, or it can abstract H$^\bullet$ from a protein side chain and thereby activate the enzyme for catalysis.[11,38] In the latter case, subsequent catalytic cycles can occur without reforming the C−Co bond and create a radical chain reaction.[11,38,39] The 5′-deoxyadenosyl radical can only abstract H$^\bullet$ from the side opposite to the corrin ring. In order to accomplish this, a pyramidal 5′-deoxyadenosyl radical either has to rotate (unlikely) or flatten to a planar structure such that the SOMO is accessible from the side opposite the corrin ring (likely). In this scheme, relaxation to planarity requires time and only occurs when organic substrate is bound.

Efficient geminate recombination in the enzyme-bound cofactor makes AdoCbl^{III} a "safe" and "transient" radical initiator. The 5′-deoxyadenosyl radical is intercepted only when substrate is present. When the catalytic cycle is complete and additional substrate molecules are no longer present, the highly reactive 5′-deoxyadenosyl radical can be "holstered" until needed.[39] If CH$_3$Cbl^{III} were to undergo homolysis in the enzyme active site, the reactivity of the resulting CH$_3^\bullet$ radical would be difficult to control and could lead to indiscriminate and "dangerous" side reactions in the active site. The profound difference in geminate recombination rates for AdoCbl^{III} and CH$_3$Cbl^{III} may speak to the inherent difference in biological function for these B_{12} cofactors.

Acknowledgment. This work was supported by grant ES05728 from the National Institute of Environmental Health Sciences. The transient kinetics laser flash photolysis experiments were carried out at the Center for Fast Kinetics Research (CFKR) whose facilities were supported by the University of Texas−Austin and grant RR00886 from the Biomedical Research Techniques Program of the Division of Research Resources of NIH. We thank Dr. Donald O'Connor and the staff of CFKR for assistance with the fast kinetics measurements. We thank Dr. W. Blaine Knight and Glaxo U.K. for the generous gift of aristeromicin.

Supporting Information Available: An example kinetic trace following nanosecond laser flash photolysis with CH$_3$Cbl^{III}; and Eyring plot and equations for calculating the BDE of AriCbl^{III} by the method of Finke (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet instructions.

Registry Numbers supplied by the author: Adenosylcob(III)alamin, 13870-90-1; methylcob(III)alamin, 13422-55-4; glycerol, 56-81-5; CH$_3^\bullet$, 2229-07-4 (all registry numbers supplied by author).

JA943109D

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