Neopentylcobalamin (NeopentylB_{12}) Cobalt–Carbon Bond Thermolysis Products, Kinetics, Activation Parameters, and Bond Dissociation Energy: A Chemical Model Exhibiting 10^6 of the 10^{12} Enzymic Activation of Coenzyme B_{12}'s Cobalt–Carbon Bond

Mark D. Waddington[†] and Richard G. Finke*

Contribution from the Department of Chemistry, University of Oregon, Eugene, Oregon 97403

Received March 23, 1992

Abstract: A quantitative study of the thermolysis of neopentylcobalamin (NpB_{12}) in ethylene glycol is reported, studies aimed at providing a well-defined chemical model for the 10^{12} enzymic rate acceleration observed for $AdoB_{12}$'s (coenzyme B_{12}'s) Co–C bond homolysis. First, the mild 25–45 °C thermolysis of >98% pure NpB_{12} in anaerobic ethylene glycol solutions containing ≥1 equiv of the nitroxide free-radical trap TEMPO (2,2,6,6,-tetramethylpiperidinyl-1-oxy) is reported, a clean reaction (5 UV–visible isosbestic points) which proceeds quantitatively to 98 ± 2% neopentyl-TEMPO (trapped neopentyl radicals) and 99 ± 2% $Co(II)B_{12r}$. Kinetic studies establish the full rate law including an inverse, $[Co(II)B_{12r}]^{-1}$, dependence at low TEMPO concentrations, thereby unequivocally demonstrating the reversible homolysis of NpB_{12} to Np• and •$Co(II)B_{12r}$ under the conditions employed. Homolysis rate constants ($k_{h,obsd}$), determined under excess TEMPO conditions where the homolysis step is rate-determining, yield precise activation parameters for NpB_{12} Co–C bond homolysis in ethylene glycol, $\Delta H^{\ddagger}_{h,obsd}$ = 28.5 ± 0.3 kcal/mol and $\Delta S^{\ddagger}_{h,obsd}$ = 18.3 ± 1.1 cal/mol·K. Axial-base, off-on equilibrium parameters were obtained through independent measurements, ΔH = –4.7 ± 0.2 kcal/mol and ΔS = –17.8 ± 0.9 cal/mol·K, and then used to deconvolute the NpB_{12} activation parameters (for the temperature-dependent shift in the base-on to base-off equilibrium); the resultant activation enthalpy and entropy for NpB_{12} Co–C homolysis in ethylene glycol are $\Delta H^{\ddagger}_{h,on}$ = 32.2 ± 0.6 kcal/mol and $\Delta S^{\ddagger}_{h,on}$ = 33 ± 2 cal/mol·K. Key results are then discussed, specifically: the finding that NpB_{12} exhibits 10^6 of the 10^{12} enzymic rate acceleration of coenzyme B_{12}'s Co–C bond homolysis; the proper radical-cage chemistry corrections which yield a base-on NpB_{12} Co–C bond dissociation energy (BDE) estimate of 28 ± 2 kcal/mol; a comparison of the results for the three alkylcobalamins now studied by the TEMPO trapping method (adenosyl-, methyl-, and neopentylcobalamin); plus the implications of these results on current models of enzyme-accelerated Co–C bond homolysis.

Introduction

Coenzyme B_{12}, also known as adenosylcobalamin (abbreviated $AdoB_{12}$), is an essential cofactor in twelve unusual enzyme-catalyzed rearrangement reactions,[1,2] in B_{12}-dependent ribonucleotide reductase reactions,[3] and in the recent discovered epoxyqueosine reductase reaction.[3b] Mechanistic studies of these intriguing reactions and of the B_{12} cofactor's role(s) have taken on an increased importance recently due to the realization that certain key features of the B_{12} reactions and mechanism, such as the initial Co–C homolysis of $AdoB_{12}$ and possibly a radical-chain mechanism,[2b] are most likely widespread throughout a number of different, non-B_{12}-dependent enzymes; a more detailed discussion of these points is available.[2] For these reasons, the thermal Co–C homolysis in B_{12} model complexes,[4] and more recently and perhaps more significantly in alkylcobalamins themselves[5–8,11] (Figure 1), has been the subject of investigation in several laboratories.

The central (and slow) step to any careful and quantitative studies of Co–C homolyses has proved to be the development of the needed radical-trapping methodology, for example the early and important efforts by Halpern and co-workers.[4a,b] Also noteworthy is the nitroxide radical trapping method,[4d,e,5] which have proved valuable for studying alkylcobalamin and cobinamide Co–C homolyses as well as some other non-corrin M–C homolyses.[5,6c,8a,c] The nitroxide-trapping method has been suc-

[†] NIH Postdoctoral Fellow, 1985–1986. Current address: Eastern Montana College, 1500 North 30th St., Billings, MT 59101.

(1) Lead reviews include (see also ref 2 below): (a) *B_{12}*; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vols. I and II. (b) Vitamin B_{12}. Proceedings of the 3rd European Symposium on Vitamin B_{12} and Intrinsic Factor; Zagalak, B., Friedrich, W., Eds.; Walter de Gruyter: New York, 1979. (c) Babior, B. M.; Krouwer, J. S. *Crit. Rev. Biochem.* **1979**, *6*, 35. (d) Abeles, R. H.; Dolphin, D. *Acc. Chem. Res.* **1976**, *9*, 114. (e) Golding, B. T. In ref 1a, Vol. II, Chapter 15, p 543. (f) Pratt, J. M. *Chem. Soc. Rev.* **1985**, *14*, 161. (g) Halpern, J. *Science (Washington, D.C.)* **1985**, *227*, 869. (h) Finke, R. G.; Schiraldi, D. A.; Mayer, B. J. *Coord. Chem. Rev.* **1984**, *54*, 1. (i) For a listing of reviews prior to 1983, see refs 1–17 in: Finke, R. G.; McKenna, W. P.; Schiraldi, D. A.; Smith, B. L.; Pierpont, C. *J. Am. Chem. Soc.* **1983**, *105*, 7592.

(2) (a) A recent review of work done at Oregon: Finke, R. G. In *Molecular Mechanisms of Bioorganic Processes*; Bleasdale, C., Golding, B. T., Eds.; The Royal Society of Chemistry: Cambridge, England, 1990. (b) For a discussion of the evidence for a radical-chain mechanism and for a listing of Stubbe's insightful reviews on this subject, see ref 3a.

(3) (a) Stubbe, J. *J. Biol. Chem.* **1990**, *265*, 5329 and references therein. (b) Frey, B.; McCloskey, J.; Kersten, W.; Kersten, H. *J. Bacteriol.* **1988**, *170*, 2078.

(4) Alkyl radical scavengers used in B_{12}-model studies include Co(II),[4a] n-C_3H_7SH,[4b] H_2O_2 or Cr^{2+},[4c] and the nitroxide method for studying Co–C homolyses[4d,6c] developed at Oregon by Smith,[4c] as well as studies which, unfortunately, fail to use a trap[4f] (and other related work[4h]): (a) Halpern, J.; Ng, F. T. T.; Rempel, G. L. *J. Am. Chem. Soc.* **1979**, *101*, 7124. See also: Ng, F. T. T.; Rempel, G. L.; Halpern, J. *Inorg. Chim. Acta* **1983**, *77*, L165. (b) Ng, F. T. T.; Rempel, G. L.; Halpern, J. *J. Am. Chem. Soc.* **1982**, *104*, 621. (c) Gjerde, H. B.; Espenson, J. H. *Organometallics* **1982**, *1*, 435. Bakac, A.; Espenson, J. H. *Inorg. Chem.* **1987**, *26*, 4305. (d) Finke, R. G.; Smith, B. L.; Mayer, B. J.; Molinaro, A. A. *Inorg. Chem.* **1983**, *22*, 3677. (e) Smith, B. L. Ph.D. Dissertation, University of Oregon, 1982. (f) Ogho, Y.; Orisaku, K.; Hasegawa, E.; Takeuchi, S. *Chem. Lett.* **1986**, 27. Ogho, Y.; Wada, H.; Othera, C.; Ikarashi, M.; Baba, S.; Takeuchi, S. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 2656. (g) Ng, F. T. T.; Rempel, G. L.; Mancuso, C.; Halpern, J. *Organometallics* **1990**, *9*, 2762. (h) Seyler, J. W.; Fanwick, P. E.; Leidner, C. R. *Inorg. Chem.* **1992**, *31*, 3699. This study demonstrates, not surprisingly, that TEMPO *radical* is an unsuitable trap for *paramagnetic*, d^5 Ru(OEP)-CH_3. Bimolecular reactions with TEMPO or other "traps" are one reason we insist on Co–C bond dissociation energy kinetic measurements under conditions where TEMPO is zero-order yet TEMPO appears in the product, demanding an intermediate step; one can then test for reversible Co–C homolysis (as done herein) via an inverse Co(II) dependence in the rate law.[5]

0002-7863/93/1515-4629$04.00/0

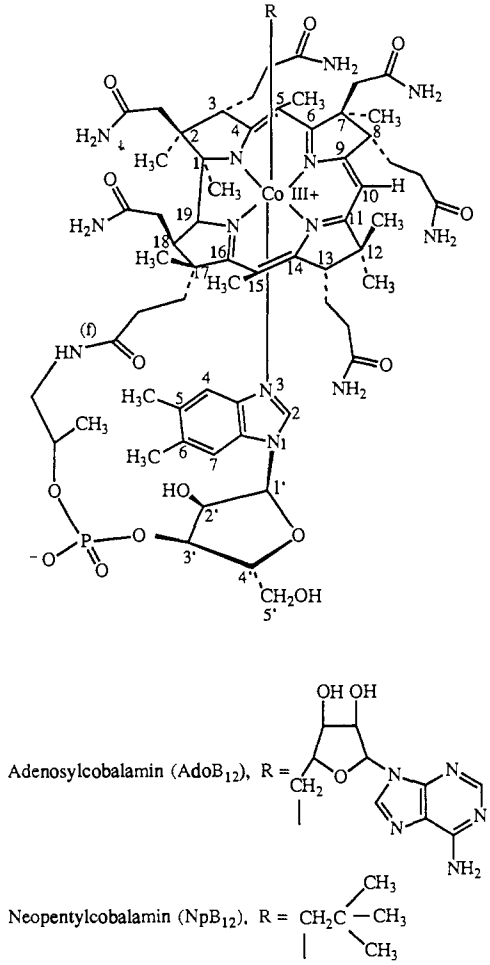


Figure 1. General alkylcobalamin (R-B_{12}) structure and specific alkyl (R) groups of adenosylcobalamin ($AdoB_{12}$) and neopentylcobalamin (NpB_{12}).

cessfully applied to[5a] $AdoB_{12}$ and[5e] MeB_{12} [the two naturally occurring, biologically significant alkylcobalamins], and to the axial-benzimidazole-base-free $AdoB_{12}$ derivative Adocobinamide ($AdoCbi^+$).[5c] Also now in hand is the proper radical-cage chemistry formalism for the conceptually correct conversion of Co–C (and other metal–ligand, M–L) bond homolysis activation parameters into Co–C (or M–L) bond dissociation energies.[9] In very recent work, evidence has been uncovered for unprecedented solvent-cage effects in the thermolysis of base-off Adocobinamide,[10] work that raises the possibility that B_{12} proteins may also be exploiting such (protein) radical-cage effects.[10]

One key finding from the above studies is the quantitation of the 10^{12} enzymic acceleration of Co–C bond cleavage in the holoenzyme complex, $AdoB_{12}$-enzyme, in comparison to that of enzyme-free $AdoB_{12}$.[5a,c,d] In light of this, our interests have naturally focused on providing a chemical precedent for the possible factors which might enhance Co–C homolysis, including steric, electronic, axial-base,[5c] or protein radical-cage effects;[9,10,11] the possibility of a radical-chain mechanism,[3b,12] or even the intriguing idea of electron-transfer-catalysis activation of Co–C homolysis.[5e,f] The most widely discussed explanation for the enzyme-accelerated Co–C homolysis in $AdoB_{12}$ is the enzyme-induced "methanochemical"[7] or "butterfly"[13] corrin conformation, steric distortion theory.[14,15]

Neopentylcobalamin has risen through a number of prior[6b,7,8d,11b,13] studies[16,17] as the prototype non-enzymic candidate

(5) $AlkylB_{12}$ studies using the nitroxide method.[4a-g,6] (a) $AdoB_{12}$ Co–C homolysis (in ethylene glycol, a substrate of B_{12}-dependent diol dehydratase): [2d] Finke, R. G.; Hay, B. P. *Inorg. Chem.* **1984**, *23*, 3041; **1985**, *24*, 1278. (b) $AdoB_{12}$ in H_2O including pH 7 studies where net heterolysis[6a] is not a problem: Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 4820. (c) Adocobinamide in H_2O: Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 8012. (d) $AdoB_{12}$ in ethylene glycol, full paper, in a minisymposium in print on M–L BDEs: Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1469. (e) MeB_{12}: Martin, B. D.; Finke, R. G. *J. Am. Chem. Soc.* **1990**, *112*, 2419. Full paper: Martin, B. D.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 585. (f) $AdoB_{12}$ and the possibility of electron-transfer-catalyzed Co–C cleavage: Martin, B. M.; Finke, R. G. *J. Inorg. Biochem.* **1990**, *40*, 19. (g) NeopentylB_{12} (the present work): A preliminary account of this work was first disclosed at the 41st Northwest Regional ACS Meeting, June 16–18, 1986, abstract #149. (h) See also the $AdoCbi^+$ studies in ref 10.

(6) (a) A second, preliminary study of $AdoB_{12}$ in pH 4.3 H_2O has subsequently been shown[4c] to lead to different net Co–C heterolysis products, findings[4c] which have since been supported by an independent study:[6b] Halpern, J.; Kim, S.-H.; Leung, T. W. *J. Am. Chem. Soc.* **1984**, *106*, 8317; **1985**, *107*, 2199. (b) Kim, S.-H.; Chen, H. L.; Feilchenfeld, N.; Halpern, J. *J. Am. Chem. Soc.* **1988**, *110*, 3120. (c) For interesting results in the related alkylporphyrins, obtained using the nitroxide method,[4d,e,5] see: Geno, M. K.; Halpern, J. *J. Am. Chem. Soc.* **1987**, *109*, 1238.

(7) Some of the earliest work providing evidence for the "methanochemical" activation of B_{12} Co–C bonds is Grate's work using O_2 as a trap: (a) Grate, J. H.; Schrauzer, G. N. *J. Am. Chem. Soc.* **1979**, *101*, 4601. (b) Schrauzer, G. N.; Grate, J. H. *J. Am. Chem. Soc.* **1981**, *103*, 541.

(8) The nitroxide-[8a] and O_2-[8b,d,11b] trapping methods have also been applied to benzylcobalamins, and the nitroxide method is part of work in water and water/glycerol for adenosylcobamides:[8c] (a) Blau, R. J.; Espenson, J. H. *J. Am. Chem. Soc.* **1985**, *107*, 3530. (b) Nome, F.; Rezende, M. C.; Saboia, C. M.; Da Silva, A. C. *Can. J. Chem.* **1987**, *65*, 2095. (c) Gerards, L. E. H.; Bulthuis, H.; de Bolster, M. W. G.; Balt, S. *Inorg. Chim. Acta* **1991**, *190*, 47. (d) A study of benzyl- and neopentylcobamides by the O_2-trapping method: Brown, K. L.; Brooks, H. B. *Inorg. Chem.* **1991**, *30*, 3420. This very valuable paper contains, however, three confusing statements which obscure the important findings therein. Hence, a brief discussion of those parts is provided in the supplementary material, along with a brief discussion of how possibly large cage effects[10] not accounted for in the comparisons made may alter some of the conclusions.

(9) (a) Koenig, T. W.; Finke, R. G. *J. Am. Chem. Soc.* **1988**, *110*, 2657. (b) Koenig, T. W.; Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1499.

(10) (a) Cage effect studies of $AdoCbi^+$ in ethylene glycol using the nitroxide-trapping method: Garr, C. D.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 10 440. (b) Cage effect studies of AdoCbl in ethylene glycol using the nitroxide-trapping method, plus presentation of a hypothetical role of AdoCbl binding proteins as "Ultimate Radical Cages and Ultimate Radical Traps": Garr, C. D.; Finke, R. G. *Inorg. Chem.*, submitted.

(11) There is some evidence (specifically a sizeable resistance to photohomolysis[11a] and a slowing of thermal Co–C cleavage) for what one could term protein-cage effects in B_{12}-dependent[11a] or transport (haptocorrin)[11b] enzymes (see also elsewhere[10b]): (a) Toraya, T.; Ishida, A. *Biochemistry* **1988**, *27*, 7677. (b) Brown, K. L.; Brooks, H. B.; Behnke, D.; Jacobson, D. W. *J. Biol. Chem.* **1991**, *266*, 6737 (work aimed at establishing the influence of B_{12}-binding proteins on Co–C cleavage rates).

(12) (a) Note that the presence of a radical-chain mechanism doesn't invalidate the 10^{12} finding, as this results from a direct comparison[5a,c,d] of the rate of $Co(II)B_{12r}$ formation inside vs outside the enzyme (and not, say, from a comparison to the rate of product turnover). Proof of the presence of a radical-chain mechanism would, however, *influence one's perspective*, as the 10^{12} finding would then become a less unexpected, "more natural" finding if each act of enzyme-accelerated Co–C cleavage were to lead to, say, $>10^6$ turnovers. (b) In addition, a likely possibility is that a "hidden" part of the 10^{12} is contained within the comparison of a (unimolecular) $AdoB_{12}$ homolysis in solution to the possible step (inherently bimolecular in solution but unimolecular in the enzyme) of *concerted* $AdoB_{12}$ + HX–protein to give AdoH + $Co(II)B_{12r}$ + 'X–protein, a step we are actively investigating[12c] (see ref 2a for further discussion of this possibility). (c) Garr, C. D.; Skaugset, A. E.; Finke, R. G. Unpublished results and experiments in progress.

(13) Pratt,[13a] Glusker,[14] Marzilli and Randaccio,[21] and Brown[8d,13c] have expanded on Grate and Schrauzer's early contribution[7] by providing insights that form the basis for the current "butterfly" corrin conformational distortion theory. (a) Chemalay, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2274. (b) Pratt, J. M. *Chem. Soc. Rev.* **1985**, 161. (c) Brown's recent work emphasizing the importance of entropic and corrin side-chain effects also deserves mention in this regard.[8d]

(14) The X-ray structural basis (from corrins) for the butterfly conformational distortion theory is detailed in: Glusker, J. P. In *B_{12}*; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 1, Chapter 3.

(15) For further discussion and additional references see pp 8017–8018 of: Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 8012. Included therein are references to axial-base and Co–C–C angle effects (work of Marzilli, Randaccio, and co-workers[21] and Golding and co-workers[22a-e]), to MO theory studies by several groups, and to extensive enzymic studies of now more than 70 modified alkylcobalamins and -cobinamides and their interactions with the B_{12}-dependent enzymes.

(16) Of the two NpB_{12} thermolysis studies prior to Halpern's recent work[17] and the present studies[19] (plus one more recent study[8d,11b]), two used oxygen[7,8d,11b] as a radical trap while the other[13a] used oxygen, alcohols, or thiols as traps.

(17) A more recent NpB_{12} study[6b] does report complete product studies and good evidence for reversible homolysis (a $[Co(II)B_{12r}]^{-1}$ rate dependence). However, given the problems these workers encountered previously[6a] with the "Co(II)[DMG]$_2$" trap used for NpB_{12},[6b] it is important to compare that work with results obtained by the proven nitroxide radical trapping method, studies that have been completed and will be reported elsewhere.[34c]

to date[18] to model and study the steric distortion and labilization theory. However, there is no prior study of neopentylcobalamin by the preferred nitroxide-trapping method, no study which accurately measures the axial-base equilibrium parameters in ethylene glycol (data *required* to deconvolute the Co–C activation parameters into the desired Co–C homolysis activation parameters),[19] no study which uses the proper cage chemistry formalism and associated equations[9] to correctly obtain a reliable NpB_{12} Co–C BDE, no prior report or discussion of the fact that NpB_{12} exhibits 10^6 of the 10^{12} enzymic activation of $AdoB_{12}$'s Co–C bond, and no prior report where NpB_{12}'s thermolysis has been studied under conditions where *all* of the following are available: full product studies (and complete mass balance), independent evidence[17] for Co–C homolysis (as opposed to heterolysis, for example), and where a preferred trap[4,5] has been used that has also been demonstrated to be reliable in other alkylcobalamins such as[5a,d] $AdoB_{12}$ and[5e] MeB_{12}. The present study[19] fills these gaps.

Structural Studies of NpB_{12} or NpB_{12} Models. Prior to presenting the Results section, it is useful to summarize the structural data available demonstrating conformational or other distortions in NpB_{12}. Unfortunately, no X-ray crystallographic, 2D NMR, EXAFS, or molecular modeling investigations are available for NpB_{12} itself, although we are actively pursuing these needed studies.[20]

However, now classic X-ray crystallographic investigations[21] of B_{12} models and notably of neopentylcobaloxime[21c,d] by Marzilli, Randaccio, and co-workers (Figure 2) provide hints about what *might* be found in the sterically more demanding cobalamin NpB_{12}. Their pioneering structural work convincingly shows that Co–C bonds in even the sterically less encumbered cobaloxime B_{12} models are highly susceptible to steric effects imposed by bulky axial alkyl ligands (notably the neopentyl[21c,d,h] and isopropyl[21a–c,e,i] complexes), especially if bulky axial bases are also present. The results document a lengthening of the axial bonds, bending of the equatorial ligand "downward" (away from the neopentyl group), and distortions of the Co–C(α)–C(β) bond angle significantly away from the typical 109° sp^3 carbon center (and even away from the perhaps *normal*[22] 124° value in $AdoB_{12}$). The profound effect of having *both* the neopentyl ligand[23] *and* a bulky, trans-axial PPh_3 (cone angle = 145°)[24] is demonstrated by the structure of the neopentyl(cobaloxime)PPh_3 complex shown in Figure 2A. An *upward* butterfly or "mechanochemical"[7] folding of the equatorial ligand is apparent and contrasts the

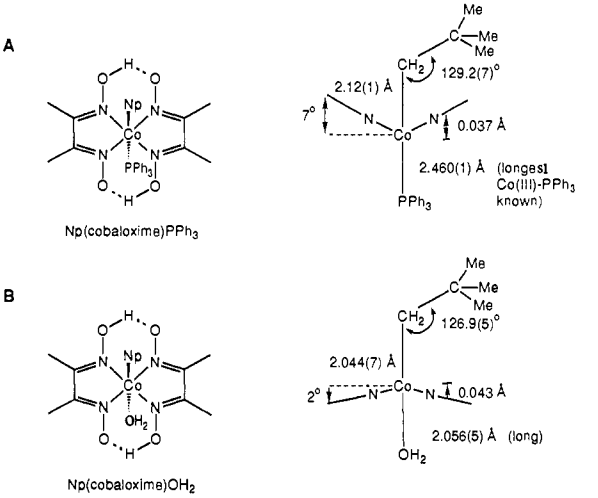


Figure 2. Structures, plus side views of key crystallographic results, from Marzilli and Randaccio's classic structural studies of (A) Np(cobaloxime)-PPh_3 and (B) Np(cobaloxime)OH_2.

downward folding present in the corresponding neopentyl(cobaloxime)H_2O complex[21d] (Figure 2B). The axial Co–PPh_3 bond length of 2.460(1) Å is the longest Co(III)–P bond known, and the lengthening of the Co–C bond to 2.12(1) Å and opening of the Co–C(α)–C(β) angle to 129.2(7)° are also evident (Figure 2A). (It is worth noting here that Marzilli has proposed enzymic control of the axial 5,6-dimethylbenzimidazole-to-cobalt bond length as a key to enzymic control of the Co–C homolysis rate.[21j] However, convincing evidence for this interesting, largely B_{12}-model-based structural hypothesis will require studies of the axial-base-to-Co bond length in the AdoCbl·protein holoenzyme complex.)

Finally, it is useful to mention an important paper by Brown[8d] which calls into question the largely B_{12}-model-based "mechanochemical" Co–C labilization theory. Specifically, Brown's quantitative comparison of the Co–C homolysis kinetics of benzyl- and neopentyl- (base-off) alkylcobinamides (vs their corresponding base-on alkylcobalamins) demonstrates that the so-called base-on effect is *entropic* in origin, at least for R = benzyl and neopentyl (the key case of Ado *is different*, however; see the Discussion section). Brown insightfully suggests corrin side-chain entropy as a key component of the entropic rate effect; note that none of the available "B_{12} models" have such side chains [nor the other complexities of the coenzyme B_{12} (AdoCbl) cofactor].

Results

A. Synthesis and Characterization of >98% Pure NpB_{12}.[19] The literature preparation[7b] of NpB_{12} (oxidative addition of

(18) (a) However, other possibly better models are conceivable such as the presently unknown adamantylcobalamin (adamantyl*cobaloxime* is known, however).[18b] (b) Bresciani-Pahor, N.; Randaccio, L.; Zangrando, E.; Summers, M. F.; Ramsden, J. H., Jr.; Marzilli, P. A.; Marzilli, L. G. *Organometallics* **1985**, *4*, 2086.

(19) This work, begun in 1983, was first disclosed in preliminary form in 1986.[5g] It was delayed, early on, by our desire to only work with as pure as possible (and fully characterized) NpB_{12} and, more recently, by the difficult task of obtaining *accurate and reliable* axial-base equilibrium parameters in cases like NpB_{12}.

(20) (a) Marzilli, L.; Waddington, M. D.; Finke, R. G. Experiments in progress. (b) Anderson, O.; Miller, S.; Waddington, M. L.; Finke, R. G. Experiments in progress. (c) Garr, C. D.; Finke, R. G. Unpublished results and experiments in progress. (d) Chance, M.; Waddington, M. L.; Finke, R. G. Experiments in progress.

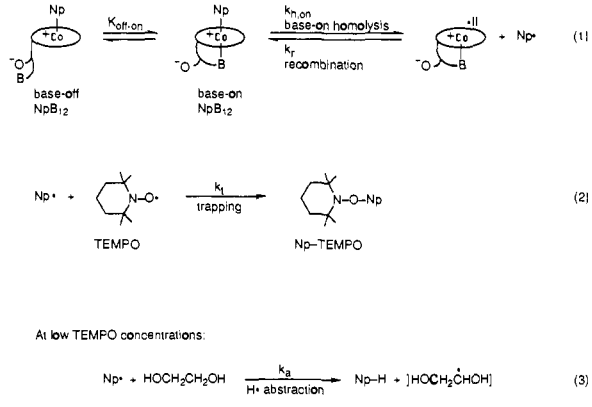
(21) (a) Marzilli, L. G.; Toscano, P. J.; Randaccio, L.; Bresciana-Pahor, N.; Calligani, M. *J. Am. Chem. Soc.* **1979**, *101*, 6754. (b) Randaccio, L.; Bresciana-Pahor, N.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1980**, *102*, 7372. (c) Randaccio, L.; Bresciana-Pahor, N.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1981**, *103*, 6347. (d) Bresciana-Pahor, N.; Randaccio, L.; Toscano, P. J.; Marzilli, L. G. *J. Chem. Soc., Dalton Trans.* **1982**, 567. Bresciani-Pahor, N.; Calligaris, M.; Nardin, G.; Randaccio, L. *J. Chem. Soc., Dalton Trans.* **1982**, 2549. (e) Summers, M. F.; Toscano, P. J.; Bresciana-Pahor, N.; Nardin, G.; Randaccio, L.; Marzilli, L. G. *J. Am. Chem. Soc.* **1983**, *105*, 6259. (f) Summers, M. F.; Marzilli, L. G.; Bresciana-Pahor, N.; Randaccio, L. *J. Am. Chem. Soc.* **1984**, *106*, 4478. (g) Bresciana-Pahor, N.; Randaccio, L. J.; Zangrando, E.; Summers, M. F.; Ramsden, J. H.; Toscano, P. J.; Marzilli, P. A.; Marzilli, L. G. *Organometallics* **1985**, *4*, 2086. (h) Parker, W. O., Jr.; Bresciani-Pahor, N.; Zangrando, E.; Randaccio, L.; Marzilli, L. G. *Inorg. Chem.* **1985**, *24*, 3908. (i) Bresciani-Pahor, N.; Randaccio, L.; Zangrando, E.; Toscano, P. J. *Inorg. Chim. Acta* **1985**, *96*, 193. (j) Marzilli, L. In *Bioinorganic Catalysis*; Reedijk, J., Ed.; Marcel Dekker, Inc.: New York, in press.

(22) In alkylcobalamins, Golding's most recent evidence[22a–c] is that the ribosyl component[22c] of the adenosyl group in $AdoB_{12}$ may be even more important than the β-substitution at carbon in causing the large Co–C(α)–C(β) angle of 124° seen in the coenzyme relative to other alkylcobalamins. Hence, Golding notes that this angle is perhaps *normal* for *non-enzyme-bound*, "*ground-state, stable* $AdoB_{12}$. If so, then this is further evidence[5c] that it is of importance to do structures[20] of *non-$AdoB_{12}$-like, less-stable* alkylcobalamins, such as NpB_{12}, that are *much less stable* and thus should not have a $AdoB_{12}$-like, 124° Co–C(α)–C(β) angle, "ground-state"[5c] structure. (a) Dixon, R. M.; Golding, B. T.; Mwesigye-Kibende, S.; Ramakrishna Rao, D. N. *Philos. Trans. R. Soc. London* **1985**, *B311*, 531. (b) Alcock, N. W.; Dixon, R. M.; Golding, B. T. *J. Chem. Soc., Chem. Commun.* **1985**, 603. (c) Bleasdale, C.; Clegg, W.; Ellwood, S. B.; Golding, B. T. *Acta Crystallogr., Sect. C* **1991**, *47*, 550.

(23) (a) Substitution at the β carbon also affects the position of the axial-base equilibria between red, six-coordinate, axial-base-coordinated forms and the corresponding yellow, axial-base-off forms. For example, increasing the number of β methyl groups in the alkyl ligand series *n*-propyl, isobutyl, and neopentyl results[23b] in a decrease in the axial-base $K_{off\text{-}on}$ = [base-on]/[base-off] from 5 to 3.4 and 1.5, respectively. A corresponding increase for the series in the observed pK_a for protonation of the axial bases (protonated base-off $\rightleftarrows$ H^+ + base-off + base-on), from 3.84[7a] to 4.27[7a] and 4.55,[7b] is found. (b) Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2274. Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2259. Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2267.

(24) Tolman, C. A. *Chem. Rev.* **1977**, *77*, 314.

Scheme I



neopentyl iodide to Co(I)B_{12s}) involves synthesis and workup under acidic conditions, followed by phenol extraction and subsequent aerobic workup, to give the protonated, base-off salt NpB_{12}·H^+Cl^-. [The protonated cobalamin product is considerably more stable (less prone to Co–C cleavage) in the protonated, base-off form.] Commercial neopentyl iodide must be avoided, otherwise the MeI impurity therin results in a substantial contamination of NpB_{12} with MeB_{12}.

In order to acquire NpB_{12} of the highest purity, several modifications of the literature procedure proved necessary (details are provided in the Experimental Section). The modified procedure reported provides NpB_{12} of >98% purity.

Since it had not been fully characterized when these studies began, the NpB_{12} product was further characterized by ^1H NMR and FAB mass spectroscopy in addition to HPLC and UV–visible spectroscopy;[25] details are provided in the Experimental Section. (See also Brown's recent work.[8d]) Two-dimensional NMR studies are of interest, due to the expectation of a distorted corrin structure, and therefore are in progress.[20a]

B. Product Studies: NpB_{12} Thermolysis in Ethylene Glycol. The >98% pure NpB_{12}·HCl produced by the above synthesis was placed in anaerobic ethylene glycol solution containing excess TEMPO plus 0.050 F Na_2HPO_4/NaH_2PO_4 buffer (0.020 F $Na_2HPO_4·H_2O$ + 0.030 F Na_2HPO_4), the buffer serving to deprotonate the axial base and thereby initiate the thermolysis reaction. (A control showed that the thermolysis results were not buffer dependent, since spectra and rates of decomposition were the same in 0.050 M NaOH/ethylene glycol.) The reaction was monitored by UV–visible spectroscopy and proceeds cleanly at 25–45 °C according to Scheme I, yielding the homolysis[5] products Co(II)B_{12r} (by UV–visible) and the TEMPO-trapped neopentyl radical, TEMPO–$CH_2C(CH_3)_3$ (by NMR and GLC; see below). Without TEMPO, neopentane and (initially) $HOCH_2CH(OH)$• are formed, the expected H• abstraction products from the reaction of neopentyl radicals with ethylene glycol.[26]

With more than 1 equiv of TEMPO present per mole of NpB_{12},[26] the temporal progression of spectral changes at 25.7 °C occurs with isosbestic points[27] at 336, 406, 424, 441, 490, and 555 nm, giving 99 ± 2% Co(II)B_{12r} as the sole cobalamin product.[28]

The neopentyl radical products and yields from NpB_{12} and >1 equiv of TEMPO in 25 °C ethylene glycol were determined by gas chromatographic analysis of completed (30 h) reactions. The plot of percent yield of neopentyl-TEMPO (determined in comparison to independently synthesized, authentic material) vs

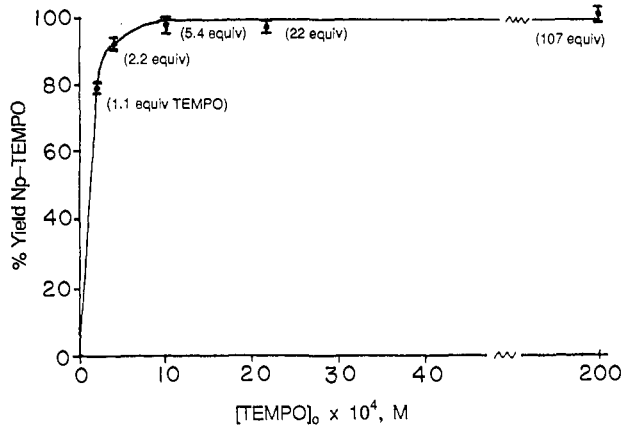


Figure 3. Percent yield of Np-TEMPO vs [TEMPO] for the 25.7 °C anaerobic thermolysis of 1.87 × 10^{-4} M NpB_{12} in ethylene glycol and buffer (0.020 F NaH_2PO_4 and 0.030 F Na_2HPO_4). The error bars represent the ~2% experimental error.

initial [TEMPO] (Figure 3) shows TEMPO to be a very effective trap for neopentyl radicals, with only 10 × 10^{-4} M (5.4 equiv) being required to trap 98 ± 2% of the freely diffusing neopentyl radicals. As the initial [TEMPO] is increased, decreasing amounts of neopentane are detected by GLC, until no neopentane remains once 21.5 equiv of TEMPO has been added.[29] The key feature illustrated by the plot in Figure 3 is that *all* of the neopentyl radicals can be trapped by TEMPO.[30,31] This result, where TEMPO is incorporated into the neopentyl-containing product under conditions where the rate law is found to be zero-order in TEMPO (vide infra), *demands* that the reaction proceed via at least one intermediate. The kinetic studies detailed below (especially the inverse $[Co(II)B_{12r}]^{-1}$ dependence) provide compelling evidence that the intermediates in *ethylene glycol* are those expected from NpB_{12} Co–C homolysis, •Co(II)B_{12r} and Np• radicals (as previously demonstrated in H_2O[6b,17]).

C. Kinetic Studies. The kinetics of the conversion of NpB_{12} to B_{12r} were determined by monitoring the increase in absorbance at the 473-nm λ_{max} of the Co(II)B_{12r} product. The results (Table I) are consistent with the kinetic expressions (eqs 1a–c and 2a,b, vide infra) derived for Scheme I, where homolysis of the Co–C

(25) (a) When our spectra lacked the reported[7b] 326-nm maxima in the UV region, we checked with an author of that paper,[7b] who informed us that this maxima is apparently an artifact of their instrument (Grate, J. H. Personal communication). (b) The small discrepancy in the extinction coefficients may reflect our conversion of NpB_{12} to dicyanocobalamin[25c,d] to accurately determine the cobalamin concentration (the preferred method, according to the literature); alternatively, it may simply reflect the extent of drying (i.e. the variable residual lattice water) in NpB_{12}·XH_2O samples made in different laboratories. (c) Barker, H. A.; Smyth, R. D.; Weissbach, H.; Toohey, J. I.; Ladd, J. N.; Volcani, B. E. *J. Biol. Chem.* **1960**, *235*, 480. (d) Hill, J. A.; Pratt, J. M.; Williams, R. J. P. *J. Chem. Soc.* **1964**, 5149.

(26) (a) At least 1 equiv of TEMPO is required for clean isosbestic points throughout the kinetic analysis.[26b] (b) With <1 equiv of TEMPO present, the spectral changes are more complex and isosbestic points are not present until much later due to a subsequent, slow reaction (half-life of 100 h at ambient temperature) which occurs with isosbestic points at 332, 386, 490, and 590 nm, eventually leading to solely Br_{12r}. Experiments using authentic formylmethylcobalamin[26] indicate that it is this cobalamin which is formed, and then slowly decomposes, when NpB_{12} is thermolyzed without TEMPO present. The details of that work, which is of relevance to the mechanism of the B_{12}-dependent enzyme dioldehydratase and the so-called cobalt participation question,[26c] are under investigation.[26d] (c) Wang, Y.; Finke, R. G. *Inorg. Chem.* **1989**, *28*, 983 and references therein. (d) Waddington, M. D.; Wang, Y.; Finke, R. G. Unpublished results and experiments in progress.

(27) At 39.8 °C the isosbestic points are 336, 444, 491, and 545 nm.

(28) Strict exclusion of trace oxygen and the usage of cuvettes containing only glass or Teflon surfaces are required for reproducible results (see the Experimental Section, Section G.3. Additional Precautions).

(29) In the absence of TEMPO, a maximum of 52% yield of neopentane is detected (quantitated by GLC vs the authentic hydrocarbon).[26]

(30) (a) It is also possible from this limited data to estimate[30b] an upper limit for the ratio of 25.7 °C rate constants for TEMPO trapping (k_t) to that for H• abstraction (k_a) from ethylene glycol (hereafter Etgly) as $k_t/k_a \geq 5 \times 10^5$. Again using the recently measured value[31j] of $k_t \simeq 1.7 \times 10^8$ M^{-1} s^{-1} (in MeOH at 18 °C), k_a becomes ≤340 M^{-1} s^{-1} at 25.7 °C. For comparison, the 110 °C k_t/k_a ratio for the 5′-deoxyadenosyl radical is 5.7 × 10^4 and, employing a k_t (110 °C; temperature corrected[31j] from 1.7 × 10^8 M^{-1} s^{-1})[31j] of ≈ 3 × 10^8 M^{-1} s^{-1}, k_a = 5300 M^{-1} s^{-1} at 110 °C.[5d] Both of these k_a values lie within the range of rate constants (10^2–10^4 M^{-1} s^{-1}) found for H• abstraction from alcohols by methyl radical.[30c] (b) The details are as follows. From eqs 1 and 2 of Scheme I we can obtain $-d[Np\text{-}TEMPO]/dt = k_t[TEMPO]$ and $-d[neopentane]/dt = k_a[Etgly]$. At constant [TEMPO] and [Etgly], integration and combination of the expressions yields $k_t[TEMPO]/k_a[Etgly]$ = % yield of Np-TEMPO/% yield of neopentane. Taking a minimum of 96% yield of Np-TEMPO at 10.1 × 10^{-4} M (5.4 equiv) initial [TEMPO] (median concentration during reaction = 9.2 × 10^{-4} M) and [Etgly] = 17.9 M gives $k_t(9.2 \times 10^{-4})/(k_a(17.9))$ = >96/4 and thus (k_t/k_a) as >5 × 10^5. (c) Thomas, J. K. *J. Phys. Chem.* **1967**, *71*, 1919.

Table I. Rate Constants for the Thermolysis of NpB_{12} in Ethylene Glycol/TEMPO Solution[a]

temp (°C)	$[NpB_{12}]_0 \times 10^4$ (M)	$[Co(II)B_{12r}] \times 10^4$ (M)	$[TEMPO]_0 \times 10^4$ (M)	equiv of TEMPO	$k_{obsd} \times 10^5$ (s^{-1})	% reaction[b]
25.7	1.75	0	3.0	1.7	8.00	5–40
					7.50	50–90
			8.0	4.6	8.15	50–90
			15	8.6	8.25	5–40
					8.07	50–90
			86	49	8.35	5–90
	1.04	0	0.50	0.48	7.73	5–30
		0.52			6.95	5–30
		1.02			6.28	5–30
30.4	1.75			49	18.4	ca. 10–90
35.1					39.3	
39.8					78.6	
44.5					152	

[a] In anaerobic ethylene glycol containing 0.030 F Na_2HPO_4 and 0.020 F NaH_2PO_4. Rate constants ±3%. [b] Portion of the reaction used to obtain k_{obsd}.

bond in base-on NpB_{12} is followed by three competing reactions: $Co(II)B_{12r}$ plus Np• recombination, TEMPO trapping of Np•, and (at low TEMPO concentrations) $HOCH_2CH_2OH$ trapping of Np• by H• abstraction. Scheme I correctly illustrates Co–C homolysis occurring from the base-on form only, since a control experiment showed that the spectrum of protonated (0.1 M H_3PO_4/ethylene glycol) and thus base-off neopentylcobalamin is unchanged after 24 h at 25 ± 2 °C, consistent with the literature.[7b,8d] This demonstration of the expected stability of the base-off form in ethylene glycol (as had previously been demonstrated in water)[7b,8d] is important, as it allows for considerable simplification in the kinetic equations and studies.

Kinetic results were obtained as follows. With >10 equiv (>4 mM) of TEMPO present, first-order plots of the growth of the $Co(II)B_{12r}$ product were linear within ±3% over 5 half-lives to 97% reaction and gave correlation coefficients of 0.9995 or better. At lower initial TEMPO concentrations, where the TEMPO concentration did not remain effectively constant (conditions non-zero-order in TEMPO), first-order plots showed the curvature predicted by the k_{obsd} expression in eq 1 of Scheme I (see the data as a function of the extent of reaction shown in Table I, where, for example, the first two entries show a 6% decrease in k_{obsd} from the initial to the latter stages of the reaction when only 1.2 equivs of TEMPO are present). (Representative kinetic plots are shown in Figures A1–A3, Supplementary Material.) Such nonlinearity at low [TEMPO] is the expected result as the $Co(II)B_{12r}$ concentration, and thus the $k_r[Co(II)B_{12r}]$ recombination term, increases with time to become large with respect to the radical-trapping terms k_t[TEMPO] and $k_a[HOCH_2CH_2OH]$ in eq 1a. In order to determine experimentally the minimum amount of TEMPO required to achieve zero-order [TEMPO] conditions even when $Co(II)B_{12r}$ is accumulating throughout the reaction, the plot of the observed rate constant, k_{obsd}, was determined at 25.7 °C using data for the latter half (ca. 50–90%) of the reaction (in order to *maximize* the observation of any $Co(II)B_{12r}$ rate inhibition). The resulting plot of k_{obsd} vs initial TEMPO concentration (Figure 4) shows that the limiting rate constant of 8.35×10^{-5} s^{-1} is achieved at only 15.0×10^{-4} M (8.6 equiv) TEMPO. Under these rate-limiting (excess TEMPO) conditions,

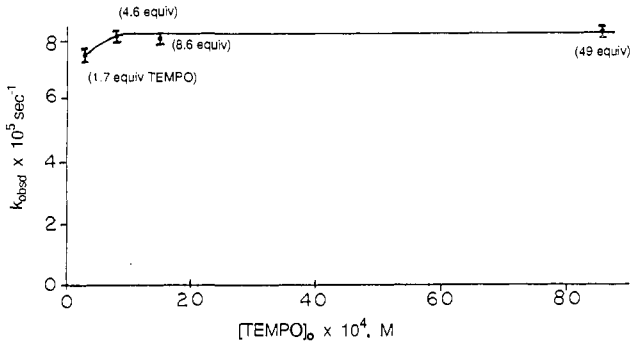


Figure 4. Observed (apparent) rate constant, k_{obsd}, vs [TEMPO] for the 25.7 °C anaerobic thermolysis of 1.75×10^{-4} M NpB_{12} in ethylene glycol and buffer (0.020 F NaH_2PO_4 and 0.030 F Na_2HPO_4); the k_{obsd} values were determined for data over the last 50–90%, and the error bars show the ±3% in k_{obsd}. Note the rigorous zero-order dependence, when [TEMPO] > 10×10^{-4} M, demonstrated by this data.

the rate constants are those for the observed homolysis step, $k_{h,obsd} = F_{base\text{-}on}k_{h,on}$ (eq 1b,c). Note that the limiting value (zero-order

$$\frac{-d[NpB_{12}]}{dt} = \frac{-d[Co(II)B_{12r}]}{dt} = \left\{[K_{off\text{-}on}/(K_{off\text{-}on} + 1)]k_{h,on}\right\}\left\{\frac{k_t[TEMPO] + k_a[HOCH_2CH_2OH]}{k_r[TEMPO] + k_a[HOCH_2CH_2OH] + k_r[Co(II)B_{12r}]}\right\}[NpB_{12}] \quad \text{(eq 1a)}$$

$$= \left\{F_{base\text{-}on}k_{h,on}\right\}\left\{\frac{k_t[TEMPO] + k_a[HOCH_2CH_2OH]}{k_r[TEMPO] + k_a[HOCH_2CH_2OH] + k_r[Co(II)B_{12r}]}\right\}[NpB_{12}] \quad \text{(eq 1b)}$$

[Where the fraction base-on, $F_{base\text{-}on}$, equals $K/(K + 1)$]

$$= k_{obsd}[NpB_{12}] \quad \text{(eq 1c)}$$

$$1/k_{obsd} = \frac{k_r}{F_{base\text{-}on}k_{h,on}}\left\{\frac{[Co(II)B_{12r}]}{k_t[TEMPO] + k_a[HOCH_2CH_2OH]}\right\} + 1/(F_{base\text{-}on}k_{h,on}) \quad \text{(eq 2a)}$$

or at constant [TEMPO] and $[HOCH_2CH_2OH]$

$$1/k_{obsd} = C[Co(II)B_{12r}] + 1/(F_{base\text{-}on}k_{h,on}) \quad \text{(eq 2b)}$$

$$\left(\text{where } C = \frac{k_r}{F_{base\text{-}on}k_{h,on}}\left\{\frac{1}{k_t[TEMPO] + k_a[HOCH_2CH_2OH]}\right\}\right)$$

in [TEMPO]) reached by k_{obsd} rules out any contribution from a bimolecular TEMPO-trap-induced reaction with NpB_{12} and also establishes the [TEMPO] conditions under which Np• trapping is fast so that Co–C cleavage is rate-determining (ignoring for the moment any radical-cage processes).

Demonstration of the putative homolysis step in ethylene glycol was also possible via the expected inverse dependence,[6b] $1/k_{obsd}$ vs $[Co(II)B_{12r}]$ (eq 2a,b). However, Figure 5 shows that $Co(II)B_{12r}$ formation reduces k_{obsd} by only about 10% (to 7.5×10^{-5} s^{-1}) at even the lowest initial TEMPO concentration used (3.0×10^{-4} M, 1.7 equiv). Hence, demonstration and quantitation of the relatively small rate inhibition by added authentic $Co(II)B_{12r}$ required use of the lowest possible initial TEMPO concentration. The results (Figure 5) were obtained using 1.04×10^{-4} M initial NpB_{12} concentration, 0.50×10^{-4} M (0.48 equiv) TEMPO, and analyzing the absorbance data for just the initial

(31) Early work using nitroxides as radical traps: (a) Brownlie, I. T.; Ingold, K. U. *Can. J. Chem.* **1967**, *45*, 2427. Chateauneuf, J.; Lusztyk, J.; Ingold, K. U. *J. Org. Chem.* **1988**, *53*, 1629. (b) Hill, C. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1974**, *96*, 870. (c) Nigam, S.; Asmus, K. D.; Willson, R. L. *J. Chem. Soc., Faraday Trans. 1* **1976**, 2342. (d) Schmid, P.; Ingold, K. U. *J. Am. Chem. Soc.* **1978**, *100*, 2493. (e) Kinney, R. J.; Jones, W. D.; Bergman, R. G. *J. Am. Chem. Soc.* **1978**, *100*, 7902. (f) Sheats, J. R.; McConnell, H. M. *J. Am. Chem. Soc.* **1977**, *99*, 7091. Sheats, J. R.; McConnell, H. M. *Proc. Natl. Acad. Sci.* **1978**, *75*, 4661. (g) See also the discussion on p 5406 and references 2–6 and 43 in: Root, K. S.; Hill, C. L.; Lawrence, L. M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 5405. (h) Espenson, J. H.; McDowell, M. S. *Organometallics* **1982**, *1*, 1514. (i) Beckwith, A. L. J.; Bowry, V. W.; O'Leary, M.; Moad, G.; Rizzardo, E.; Soloman, D. H. *J. Chem. Soc., Chem. Commun.* **1986**, 1003. (j) Recent work on the solvent and structural effects on nitroxide radical trapping rate constants: Beckwith, A. L. J.; Bowry, V. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1992**, *114*, 4983 [k_t for TEMPO plus Np• in alcohol (MeOH) is taken from Table IV, entry 35]. Bowry, V. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1992**, *114*, 4992.

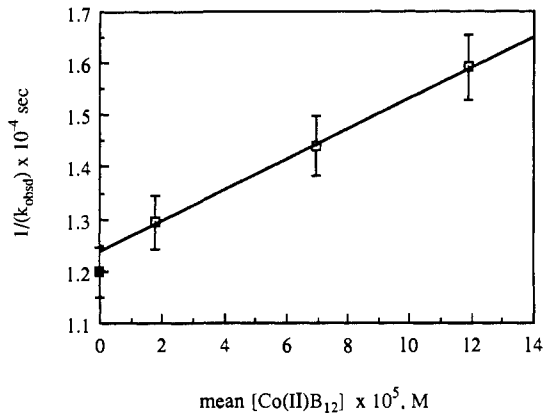


Figure 5. Plot of $1/k_{obsd}$ vs total (mean) [Co(II)B_{12r}] for the 25.7 °C anaerobic thermolysis of 1.04 × 10^{-4} M NpB_{12} in ethylene glycol and buffer (0.020 F NaH_2PO_4 and 0.030 F Na_2HPO_4) in the presence of 0.05 × 10^{-4} M TEMPO. The □ data are determined for 5–30% reaction of NpB_{12} (the mean Co(II)B_{12r} is computed at 17% reaction), the error bars show the ~4% error in k_{obsd}, and the ■ (solid-square) is the expected intercept corresponding to $k_{h,obsd}$ = 8.35 × 10^{-5} s^{-1} obtained under rate-limiting conditions with excess (50 equiv) TEMPO.

stages of the thermolysis where isosbestic points were largely maintained.[26] The intercept of the plot, $1/k_{obsd}$ (eq 2a,b and Figure 5), gives a value for $k_{h,obsd}$ of 8.1 × 10^{-5} s^{-1}, which is identical within experimental error to the value determined with the rate-limiting concentration of TEMPO without added Co(II)B_{12r}, 8.35 × 10^{-5} s^{-1} (the solid square in Figure 5). This agreement provides excellent evidence for the validity of the kinetic methods and for the reliability and precision of the resultant k_{obsd} and $k_{h,obsd}$ values. From the slope of the plot we can estimate[32] k_r/k_t at 25.7 °C as less than 0.2 and, upon using Beckwith, Bowry, and Ingold's recent measurement[31j] for Np• of k_t (in ROH, at 18 °C) of 1.7 × 10^8 M^{-1} s^{-1}, k_r becomes ~3 × 10^7 M^{-1} s^{-1}. This first *estimate* of the Np• plus Co(II)B_{12r} recombination rate constant is just slightly below the lower limit of the range of known[33] Co(II)B_{12r} + R• recombination rate constants of (5–200) × 10^7 M^{-1} s^{-1}, a finding that is readily explicable in terms of the steric bulk of the Np•.

Next, NpB_{12} rate constants for the homolysis step, $k_{h,obsd}$, were measured as a function of temperature using excess TEMPO (8.6 × 10^{-3} M; 49 equiv) at 5-deg intervals between 25 and 45 °C. Observed activation parameters $\Delta H^{\ddagger}_{h,obsd}$ and $\Delta S^{\ddagger}_{h,obsd}$ of 28.5 ± 0.3 kcal/mol and 18.3 ± 1.1 cal/mol·K were calculated from a linear plot of $\ln(k_{h,obsd}/T)$ vs $1/T$.

These values *do not* agree within experimental error with those measured by a different group using a different, less well-established trapping method (Co(II)[DMG]$_2$ as a trap),[6b] $\Delta H^{\ddagger}_{h,obsd}$ = 26.8 ± 0.3 kcal/mol and $\Delta S^{\ddagger}_{h,obsd}$ = 13 ± 1 cal/mol·K. It is interesting and important to note, however, that the latter activation numbers[6b] were in fact at one time equivalent within experimental error to our earliest (incorrect; nonpublished) activation parameters, work that was discovered[34a] to contain a small but non-negligible *systematic error* in temperature measurement in the Peltier temperature control device of the UV–visible spectrophotometer used in the kinetic studies.[34] (We will be reporting additional collaborative studies on systematic errors in alkylcobamide activation parameter determinations.[34c]) This gives us confidence that our final values reported herein of $\Delta H^{\ddagger}_{h,obsd}$ and $\Delta S^{\ddagger}_{h,obsd}$ in ethylene glycol (i.e. of 28.5 ± 0.3 kcal/mol and 18.3 ± 1.1 cal/mol·K, respectively) are in fact the correct numbers.

D. Temperature Dependence of the Axial-Base Coordination Equilibrium in Ethylene Glycol. The measurement of reliable axial-base equilibrium parameters as a function of temperature is one of the more difficult aspects of alkylB_{12} thermolysis investigations. In fact, of the prior NpB_{12} studies,[6b,7a,8d,13] only two[6b,8d] provide estimates for the axial-base ΔH and ΔS values (and only one in ethylene glycol,[6b] the solvent of interest to this work), parameters required to deconvolute the composites $k_{h,obsd}$, $\Delta H^{\ddagger}_{h,obsd}$, and $\Delta S^{\ddagger}_{h,obsd}$ into their corresponding base-on values, $k_{h,on}$, $\Delta H^{\ddagger}_{h,on}$, plus $\Delta S^{\ddagger}_{h,on}$ (and then finally the $\Delta H^{\ddagger}_{h,obsd}$ into the NpB_{12} Co–C BDE). The one study which does report, in a full paper,[6b] "preliminary measurements" in ethylene glycol includes unstated assumptions[35] and cites axial-base off-on thermodynamic parameters of ΔH = −10 ± 1 kcal/mol and ΔS = −34 ± 5 eu. These values will prove to be quite different from those[19a] reported herein.

Our own efforts to measure reliable axial-base parameters for NpB_{12} *in ethylene glycol* uncovered several fundamental limitations. In particular, the following *preclude* the measurement of UV–visible absorbance vs temperature data over *a sufficiently wide temperature range* for NpB_{12} in ethylene glycol: the reactivity of unprotonated base-on NpB_{12} in ethylene glycol at even moderate temperatures; the relatively small $K_{on\text{-}off}$ (i.e. relatively low % of base-on form) even at room temperature; and the inability to employ significantly lower temperatures due to the relatively high freezing point (−10 °C) for this solvent. The last two limitations mean that one cannot obtain the type of sigmoidal-shaped absorbance vs temperature plot that is optimum for successful "no assumptions", ab initio curve-fitting determinations of ΔH and ΔS (i.e. where ideally one achieves both limiting forms, 100% base-off at higher temperature and 100% base-on at sufficiently low temperatures).[36] Our independent measurement of the absorbance vs temperature plot for NpB_{12} in ethylene glycol and its comparison to the (linear and also somewhat different) literature data (Figure B, Supplementary Material) demonstrate that even if one invokes the assumptions used by others,[35a] there is still too little sigmoidal curvature in the temperature-dependent visible spectra of NpB_{12} in ethylene glycol over the small, 10–30 °C temperature range reported[6b] to reliably obtain the needed ΔH and ΔS values. (See the Experimental Section, Section K, for a control experiment documenting this point.)

Fortunately, we have been able to develop an alternative approach based on seven points: (i) the use of ^{1}H NMR rather than UV–visible spectroscopy to monitor the position of the axial-base equilibrium as a function of temperature (advantages of

(32) Using slope = $(k_r)/[(k_{h,obsd})(k_t[\text{TEMPO}] + k_a[\text{Etgly}])]$ = 3.0 × 10^7 s M^{-1}, $k_{h,obsd} = F_{base\text{-}on}k_{h,on}$, substituting $1/k_{h,obsd} = 1/(8.35 \times 10^{-5}\ s^{-1})$, $k_a < [k_t/(5 \times 10^5)]$ from product studies, and [Etgly] = 17.9 M and fixing [TEMPO] < 0.50 × 10^{-4} M gives $k_r/k_t < 0.2$.

(33) (a) Roche, T. S.; Endicott, J. F. *Inorg. Chem.* **1974**, *13*, 1575. (b) Endicott, J. F.; Ferraudi, G. J. *J. Am. Chem. Soc.* **1977**, *99*, 243. (c) Mok, C. Y.; Endicott, J. F. *J. Am. Chem. Soc.* **1977**, *99*, 1276. (d) Mok, C. Y.; Endicott, J. F. *J. Am. Chem. Soc.* **1978**, *100*, 123. (e) Tait, A. M.; Hoffman, M. Z.; Hayon, E. *Int. J. Radiat. Phys. Chem.* **1976**, *8*, 691. (f) Mulac, W. A.; Meyerstein, D. *J. Am. Chem. Soc.* **1982**, *104*, 4124. (g) Elroi, H.; Meyerstein, D. *J. Am. Chem. Soc.* **1978**, *100*, 5540.

(34) (a) We thank Professor Ken Brown for exchanging unpublished information with us and especially for his suggestion that such temperature errors might be the reason for small but non-negligible differences between activation parameters measured in different groups. (b) A comparison of our NpB_{12} thermolysis rates and activation parameters with those from Professor Jack Halpern's group at the Portland, Oregon, ACS Regional meeting in June 1986 indicated that the (axial-base uncorrected) $\Delta H^{\ddagger}_{h,obsd}$ and $\Delta S^{\ddagger}_{h,obsd}$ values, for both ethylene glycol and water, were in close agreement at that time.[5g] However, the small systematic temperature measurement error discussed in the text and in the Experimental Section was subsequently discovered;[34c] this is suggestive (but not definitive) evidence that similar systematic temperature measurement errors may exist in literature data.[6b] (c) Brown, K. L.; Evans, D. R.; Waddington, M. D.; Finke, R. G. Unpublished results on the thermolysis of NpB_{12} in H_2O.

(35) (a) The reported literature determination in ethylene glycol[6b] requires two assumptions to obtain the parameters it reports: the assumption that the absorbance of the base-off form is the same as that of the protonated form and the assumption that there is negligible temperature dependence of the extinction coefficients[35b] (this assumption is not stated, and apparently was not realized, despite Brown's[35c] caution about it and despite literature proving that it is generally not true[35d] (except if one picks wavelengths that are *isosbestic* points for the temperature-dependent UV–visible spectrum[35b])). (b) Further details and discussion of the temperature-dependent extinction coefficients are provided as supplementary material. (c) Brown, K. L.; Peck-Siler, S. *Inorg. Chem.* **1988**, *27*, 3548. (d) 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.

NMR[37] include that it extends the available lower temperature limit and avoids problems of temperature-dependent UV–visible extinction coefficients[8d]); (ii) some unpublished work with coenzyme $AdoB_{12}$, demonstrating that $K_{off\text{-}on}$ for the axial-base off-on equilibrium in $AdoB_{12}$ is dependent primarily on the solvent polarity[38] (and thus presumably is very similar in similar solvents with similar polarities, other factors being held the same);[38d] (iii) the control experiment described below *proving* that at least $AdoB_{12}$'s axial-base parameters do not change in ethylene glycol vs methanol [MeOH has a polarity within 2% of that of ethylene glycol[38c] and has a much better low-temperature range (freezing point –60 °C) where NpB_{12} is stable]; (iv) important prior work by Brown[35c] using ^{13}C NMR to obtain axial-base parameters, work that provides the proper equations *including the inherent temperature dependence of the chemical shifts* (m^{on} and m^{off} in his eqs 5 and 10);[35c] (v) the key choice of the C_{10}-hydrogen (C_{10}-H; see Figure 1) to be monitored in the ^1H NMR based on prior work showing that the chemical shift of *this hydrogen* is both sensitive to axial-base changes[35d,39] yet *inherently temperature insensitive otherwise*[35d] (constant to ±0.04 ppm for aquocyanocobinamide from –50 to +30 °C *in* CD_3OD and to ±0.02 ppm for this same cobinamide in D_2O; see also the other examples in reference 35d); (vi) a NMR control experiment confirming that the C_{10}-H chemical shift *in protonated, base-off* NpB_{12}·H^+Cl^- is also constant to a precision of 10% (±0.02 ppm) from –45 to +40 °C. (When examined in higher precision NMR experiments, we find that it is actually a linear function of temperature as Brown's findings and equations require.[35c] However, even then its total chemical-shift change is only 10% of the larger change due to the NpB_{12} axial-base equilibrium over the same –45 to +40 °C temperature range.);[40] and (vii) literature evidence that the so-called "tuck-in"[23,41] axial-base form for a certain class of alkylcobalamins, including NpB_{12},[8d,23b,41] is not a predominant form[8d] and hence is assumed[42] to have a negligible effect on the thermolysis rate of NpB_{12}.

The key control experiment with $AdoB_{12}$, which allows us to use methanol in place of ethylene glycol, proceeded as follows. The chemical shift of the C_{10}-H of $AdoB_{12}$ in CD_3OD (Me_4Si internal standard) was determined at 5-deg intervals from 20 to 60 °C. Curve fitting, using the C_{10}-H shift under acidic conditions as a fixed end point[43] for the base-off form (Figure C, Supplementary Material), gave the precise values $\Delta H = -7.9 \pm 0.2$ kcal/mol and $\Delta S = -22.1 \pm 0.4$ cal/mol·K. Significantly, these values are in *excellent agreement*[43b] with $\Delta H = -7.6 \pm 0.2$ kcal/mol and $\Delta S = -20.2 \pm 0.7$ cal/mol·K determined earlier for $AdoB_{12}$ from 10 to 80 °C by UV–visible spectroscopy in ethylene glycol.[5a,d] Hence there is every reason to believe that the NMR method *in MeOH* will provide a more than adequate estimate of the axial-base parameters for NpB_{12} *in ethylene glycol*.

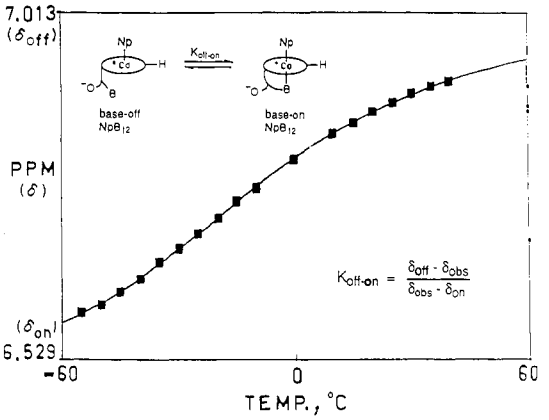


Figure 6. NpB_{12} C_{10}-H chemical shift vs temperature for 10 mM NpB_{12} in 40 mM $NaOCD_3$ in CD_3OD. The solid squares are the data, and the solid line is the nonlinear least-squares curve fit from which the axial-base ΔH, ΔS, δ_{off}, and δ_{on} are determined. (For a curve fit of this data by Brown's more exact equations[35c] which, however, yields the same results as reported in the text to within experimental error, see Section and Figure D, Supplementary Material.)

Returning to the NpB_{12} system, C_{10}-H chemical shifts were determined at 5-deg intervals over a 75-deg range from –45 to 40 °C in CD_3OD (with 8 equiv of $NaOCD_3$ added to ensure deprotonation of the NpB_{12}·H^+Cl^-). The resultant data and curve fit[44] (Figure 6, squares and solid line, respectively) gave $\Delta H = -4.7 \pm 0.2$ kcal/mol and $\Delta S = -17.8 \pm 0.9$ cal/mol·K, values considerably different from (and which should replace) the literature "preliminary estimates"[6b] of $\Delta H = -10 \pm 1$ kcal/mol and $\Delta S = -34 \pm 5$ cal/mol·K. Our values yield an equilibrium constant of $K_{off\text{-}on} = 0.36$ at 25 °C; that is, only 26% of NpB_{12} is base-on at 25 °C with even less, 18%, base-on at 45 °C.[44] [We have also recently extended our curve-fitting procedure to Brown's equations[35c] (see Section and Figure D, Supplementary Material). The results are equivalent within experimental error, $\Delta H = -4.6$ kcal/mol, $\Delta S = -16.6$ eu.]

Finally, using our thermodynamic parameters for the NpB_{12} axial-base equilibrium to compute the needed $K_{off\text{-}on}$ values as a function of temperature, values[45,46] for $k_{h,on}$ were calculated from $k_{h,on} = (k_{h,obsd})(K_{off\text{-}on} + 1)/(K_{off\text{-}on})$ (eq 1); for example at 25.7

(36) Our previous and successful use of this method for $AdoB_{12}$ in ethylene glycol and other solvents[5a,b,d] confirms these points, as there is a greater percentage of base-on form for the less bulky Ado alkyl group. Hence sigmoidal-shaped curvature is obtainable in the absorbance vs temperature data, and curve fittings proceed smoothly to yield precise equilibrium ΔH and ΔS parameters. In the case of smaller alkyls exemplified by MeB_{12},[5c] one has the opposite problem, since only the base-on form is present except at very high temperatures.

(37) NMR analysis also has the advantage of being less sensitive (in the observed chemical shift of the equilibrating base-off-on forms) to partial Co–C bond cleavage. That is, even small amounts of Co(II)B_{12r} or (in air) Co(III)B_{12a} Co–C cleavage products have absorbances in the UV–visible that overlap with NpB_{12} absorbances; in the NMR, these products do not interfere with the chemical shift analysis (at least for NpB_{12}).

(38) (a) For 25 °C $K_{off\text{-}on}$ data determined by UV–visible spectroscopy in H_2O, ethylene glycol, and DMSO, plots[38b] of $\ln(K_{off\text{-}on})$ vs Dimroth's E_T or Kosower's Z parameters[38c] gave correlation coefficients of 0.97.[38d] (b) Hay, B. P.; Finke, R. G. Unpublished experiments. (c) Reichardt, C. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 29. Dimroth's E_T for ethylene glycol and methanol, therein, are 56.3 and 55.5, respectively; Kosower's Z values are 85.1 and 83.6, respectively. (d) Restated, ΔG is dependent on the solvent polarity.

(39) Data for the temperature-dependent shifts of coenzyme B_{12},[39a,b] MeB_{12},[39c] and a number of cobinamides[35d] have appeared. The most recent and most detailed study is Brown's work with ^{13}C-enriched alkylcobinamides.[35c] (a) Cockle, S. A.; Hensens, O. D.; Hill, H. O. A.; Williams, R. J. P. *J. Chem. Soc., Dalton Trans.* **1975**, 2633. (b) Brodie, J. D.; Poe, M. *Biochemistry* **1972**, *11*, 2534. (c) Hensens, O. D.; Hill, H. A. O.; Thorton, J.; Turner, A. M.; Williams, R. J. P. *Philos. Trans. R. Soc. London* **1976**, *B273*, 353.

(40) Note that a similar, largely temperature-independent *inherent* chemical shift for the C_{10}-H *in* $AdoB_{12}$ is indicated by the agreement, *within experimental error* (±2.6% in ΔH and ±5% in ΔS; see the text), between the UV–visible (in ethylene glycol) and NMR (in CD_3OD) determinations of the axial-base ΔH and ΔS for $AdoB_{12}$.[5a,d] Restated, this is a rare experimental confirmation of the validity (i.e. to within the stated error bars) of both the UV–visible and NMR methods and any assumptions therein.

(41) The axial-base "tuck-in" form is part of Brown's "complete scheme"[35c] for treating cobalamin axial-base equilibria. The important point here is that two limiting classes of alkylcobalamins have been identified:[23b] (i) those with hindered alkyls like neopentyl, cyclohexyl, or isopropyl that appear to show little tuck-in form (Brown's recent estimate is that K_H for formation of tuck-in NpB_{12} is 0.65)[8d] and (ii) those like $AdoB_{12}$ or MeB_{12} that probably have most of their base-off form present with the axial-base "tucked-in" under, and H-bonded to, the corrin.

(42) The probably correct (but unproven) assumption here is that any tuck-in form[41] has a base-off-like rate of homolysis (i.e. negligibly slow relative to base-on homolysis). Neither the possibility of the tuck-in form nor this implicit assumption about it was previously noted.[6b]

(43) (a) While this assumption is admittedly no better a priori than the analogous assumption made in the UV–visible method, the *agreement between the independently determined NMR and UV–visible results* for $AdoB_{12}$ provides strong ex post facto justification for this point.[40] (b) Note that the agreement of the NMR and UV–visible-obtained parameters for $AdoB_{12}$ does not, by itself, justify the assumption[35] of equivalent limiting absorbances (i.e. extinction coefficients) of the protonated and neutral base-off forms, since this assumption *is not made in our* UV–visible method[5a,d] and nearly ab initio fitting procedure.

(44) (a) δ_{off} and δ_{on}, both obtained from the curve fitting, are assumed to be independent of temperature. (b) As a check, the use of the experimentally determined value for δ_{off} in acidic methanol as a fixed point in the curve fitting yields the satisfying results of similar values: $\Delta H = -4.4 \pm 0.1$ kcal/mol, $\Delta S = -16.7 \pm 0.2$ cal/mol·K, and $K_{off\text{-}on} = 0.38$ (20% base-on) at 25 °C. (c) For a curve fit according to Brown's equations,[35c] see the supplementary material, Section and Figure D.

Table II. Co–C Bond Homolysis Kinetics and Activation Parameters for Base-On RB_{12} Complexes in Ethylene Glycol

compound	$k_{h,on}$ (s^{-1}) at 25 °C[a]	$\Delta H^{\ddagger}_{h,on}$ (kcal/mol)	$\Delta S^{\ddagger}_{h,on}$ (eu)	Co–C BDE (kcal/mol)
MeB_{12}	9×10^{-13}	41 ± 3	24 ± 6	37 ± 3
$AdoB_{12}$	4×10^{-10}	34.5 ± 0.8	14 ± 1	30 ± 2
NpB_{12}	3×10^{-4}	32.2 ± 0.6	33 ± 2	28 ± 2

[a] Calculated from listed $\Delta H^{\ddagger}_{h,on}$ and $\Delta S^{\ddagger}_{h,on}$ values.

°C, $k_{h,on} = 3.2 \times 10^{-4}$ s^{-1} ($t_{1/2}$ = 36.5 min). Similarly, a plot of $\ln[(k_{h,on})/T]$ vs $1/T$ gave the precise[45,46] $\Delta H^{\ddagger}_{h,on} = 32.2 \pm 0.6$ kcal/mol and a rather positive entropy of activation, $\Delta S^{\ddagger}_{h,on} = 33 \pm 2$ cal/mol·K, for the Co–C bond homolysis of base-on NpB_{12} in ethylene glycol.

The entropy is especially interesting, being 19 ± 34 eu more positive than that for $AdoB_{12}$ in the same solvent.[5d] This almost surely reflects the relief of ground-state strain (present in the sterically crowded NpB_{12}) in the homolysis transition state; this finding is consistent with and supportive of Brown's suggestion[8d] (cited in the Introduction) that much of such entropy is probably associated with the degree of flexibility (or inflexibility) of the corrin ring side chains in B_{12}[8d] (i.e. their rotational and vibrational degrees of freedom). These results lead, in turn, to the unanswered question (previously raised by Brown[8d]) of whether or not $AdoB_{12}$-binding enzymes employ similar steric and entropic components (plus possibly enzymic Co–N axial-base distance compression[21j]) to achieve the 10^{12} acceleration of Co–C bond cleavage seen in $AdoB_{12}$·enzyme complexes.

Discussion

Neopentylcobalamin is currently the prototype of a sterically hindered alkylcobalamin. The most interesting findings from the present Co–C thermolysis studies, results obtained under conditions demonstrating both reversible homolysis *and* complete product studies with full mass balance, are summarized below.

NpB_{12} Exhibits 10^6 of the 10^{12} Enzymic Co–C Bond Activation. An important, previously unreported finding is that the rate of NpB_{12} homolysis at 25 °C in ethylene glycol, $k_{h,on} = 3 \times 10^{-4}$ s^{-1}, is roughly 10^6 faster than the rate of $AdoB_{12}$ homolysis at 25 °C,[5a,c,d] $k_{h,on} \simeq 4 \times 10^{-10}$ s^{-1} (see Table II). That is, NpB_{12} is as good a non-enzymic, chemical model as exists to date, *exhibiting roughly half of 10^6 of the 10^{12} enzymic acceleration* that we previously identified.[5a,c,d] This finding establishes *base-on* and *base-off* NpB_{12} as the most important currently unstudied candidate for 2D NMR,[20a] X-ray crystallographic,[20b] molecular modeling,[20c] and EXAFS[20d] investigations, efforts that will reveal whether or not the case of NpB_{12} supports the upward corrin "butterfly" conformational distortion theory for activating Co–C bonds.

NpB_{12} Co–C Bond Dissociation Energy. Another goal of such thermolysis studies is the estimation of Co–C BDEs from the homolysis activation enthalpy. Elsewhere we have provided the radical-cage formalism which allows the proper correction of solution activation parameters once the fractional-cage efficiency, Fc, is known:[9] Co–C BDE $\simeq \Delta H^{\ddagger}$(soln) – Fc·$\Delta H^{\ddagger}\eta$. (Previously, others[6b] have applied an improper gas-phase reaction coordinate as discussed elsewhere.[9]) The radical-cage formalism emphasizes that, at the minimum, if the required Fc is not available, *the assumed Fc* needs to be explicitly stated.[9]

Only a single reliable Fc has been determined for any alkylcobamide at this time, the recent determination of $0.94 < Fc \leq 1.0$ for axial-base-free Adocobinamide.[10] (For $AdoB_{12}$, Fc has been estimated by direct, albeit imprecise, observation as[47] between 0.2–1.0.) For NpB_{12}, Fc is unknown for ethylene glycol (or any other solvent); in principle it can vary from 0 to 1 (but the steric inhibition to Np· and ·Co(II)B_{12r} recombination suggests that Fc for NpB_{12} is less than 1). For the purposes herein, we *assume* that Fc for NpB_{12} is 0.75 ± 0.25 in 25–45 °C ethylene glycol (note that this *initial estimate* ignores the expected decrease of Fc with increasing temperature[9]).

Next, a value for $\Delta H^{\ddagger}\eta$ for ethylene glycol is needed. The absolute viscosity, as well as its change over the temperature range used, is well-known to be larger for ethylene glycol vs, for example, water, as others[48] and we[5b,d,e] have previously noted (19.94 cp at 20 °C and 7.68 cp at 45 °C for ethylene glycol[49a] in comparison to 1.002 cp at 20 °C and 0.4665 at 60 °C for water[49b]). Similarly, the calculated enthalpy for viscous flow in glycol is $\Delta H^{\ddagger}\eta = 6.3 \pm 0.1$ kcal/mol (3.2 kcal larger than it is for water, 3.1 ± 0.10 kcal/mol).[50] Using these values, the assumed Fc values, and our solution $\Delta H^{\ddagger}_{h,on}$ values for NpB_{12} yields a base-on Co–C BDE in ethylene glycol of 28 ± 2 kcal/mol.

Comparison of Co–C Thermolysis Data for Three Key Alkylcobalamins. NpB_{12} is only the third alkylcobalamin (along with adenosylcobalamin[5a,d] and methylcobalamin;[5e] see also ref 8a) for which kinetic Co–C bond homolysis data has been determined by the TEMPO method *in the same solvent* (ethylene glycol) and under conditions fully demonstrating the validity of the kinetic results. It is therefore of interest to compare the TEMPO-derived data in ethylene glycol for these three alkylcobalamin systems (Table II).

Comparing the most stable alkylcobalamin (MeB_{12}) and the least stable one (NpB_{12} shows that, at 25 °C, NpB_{12} undergoes Co–C homolysis 3×10^8 faster, has a ca. 9 kcal/mol or 22% lower $\Delta H^{\ddagger}$, and has a ca. 9 kcal/mol or 24% lower Co–C BDE than MeB_{12}. In comparison to $AdoB_{12}$, NpB_{12} homolyzes ca. 10^6 faster at 25 °C, as already discussed, with about one-quarter of this 25 °C difference in the enthalpy of activation and three-fourths due to a more favorable entropy of activation. (Note that *both* enthalpic and entropic components are important, although the entropy term dominates.)

Implications for the Corrin Butterfly Conformation Steric Distortion *Model* and the Base-On Effect Therein. As discussed in the Introduction, the early "mechanochemical" distortion model[7] has been extended by several groups[13] to its present form, known as the corrin butterfly conformational (steric) distortion theory. This theory, currently the most cited *chemical model explanation* for how B_{12}-dependent enzymes achieve the large 10^{12} enzymic activation of *$AdoB_{12}$'s* Co–C bond, is inherently limited, however (and as discussed elsewhere[5c]), as (i) it has developed to be largely a *ground-state* distortion theory only[5c] (while enzymic tight binding of something resembling *distorted* Co(II)B_{12r} is anticipated[5c]), (ii) it is largely a "B_{12}-model"-based theory, one which lacks good structural precedent for these steric distortions in *alkyl*cobalamin complexes (the bulk of the structural data for corrin complexes is for *non-alkyls*),[14,15] (iii) it is perhaps likely to be only one component of how the Co–C bond in AdoCbl is activated (e.g. Brown's side-chain entropic effects[8d] and protein "Ultimate Radical Cage and Ultimate Radical Trap" effects[10b] may also be important), and (iv) only after crystallographic (plus

(45) Error bars shown for $\Delta H^{\ddagger}_{h,on}$ reflect the proper uncertainties imposed (i.e. propagated) by $\Delta H^{\ddagger}_{h,obsd}$, $\Delta S^{\ddagger}_{h,obsd}$, and ΔH and ΔS. Error bars in $\Delta S^{\ddagger}_{h,on}$ are estimated.

(46) The NpB_{12} $k_{h,on}$, $\Delta H^{\ddagger}_{h,on}$, and $\Delta S^{\ddagger}_{h,on}$ values obtained herein should supplant the reported values[6b] (e.g. $\Delta H^{\ddagger}_{h,on} = 33 \pm 2$ kcal/mol and $\Delta S^{\ddagger}_{h,on} = 35 \pm 10$ eu) because they include a contribution from the inaccurate axial-base thermodynamic parameters.

(47) (a) Rigorously, the data placed the $AdoB_{12}$ Fc in 25 °C H_2O at 0.7 ± 0.5 (i.e. in the range 0.2–1.0): Endicott, J. F.; Netzel, T. L. *J. Am. Chem. Soc.* **1979**, *101*, 4000. (b) An abstract reports data implying a Fc of 0.79 for $AdoB_{12}$ in H_2O and at ambient temperature, but a final value for this valuable constant is not yet available:[47c] Chen, E.; Chance, M. R. *J. Inorg. Biochem.* **1989**, *36*, 264; abstract H070. (c) Chen, E.; Chance, M. R. *J. Biol. Chem.* **1990**, *265*, 12987.

(48) Geno, M. K.; Halpern, J. *J. Chem. Soc., Chem. Commun.* **1987**, 1052.

(49) (a) Viswanath, D. S.; Natarajan, G. *Data Book on the Viscosity of Liquids*; Hemisphere Publishing Corp.: New York, 1989. (b) *CRC Handbook of Chemistry and Physics*, 59th ed.; Chemical Rubber Co.: West Palm Beach, FL, 1978; p F-55.

(50) (a) $\Delta H^{\ddagger}\eta$ values are calculated in the usual way[50b] from the slopes of plots of ln(viscosity) vs $1/T$ for the solvent and temperature ranges used. (b) Glasstone, S.; Laidler, K. J.; Eyring, H. *Theory of Rate Processes*; McGraw-Hill: New York, 1941.

additional kinetic and spectroscopic) investigations of enzyme–B_{12} complexes become available, will we have a firmer idea of how $AdoB_{12}$'s Co–C bond is actually activated.[5c]

Rigorously speaking, the closest chemical (i.e. enzyme-free) model for enzyme-bound $AdoB_{12}$ might have been anticipated to be where the alkyl group is always Ado but the axial base has been changed to try and find evidence for an axial-base-induced butterfly-type distortion—for example, studies of the thermolysis of[5a,b,d] base-on $AdoB_{12}$ and[5d] axial-benzimidazole-base-free $AdoCbi^+$. The results of those specific studies show, however, only a ca. 100-fold rate increase in Co–C homolysis at 25 °C (in H_2O) due to the presence of the axial benzimidazole; this 10^2 *base-on effect* in benzimidazole-base-free $AdoCbi^+$ (in comparison to base-on AdoCbl) is due[5a,b,d] to a lower, more favorable enthalpy in the base-on form ($\Delta\Delta H^\ddagger = -5.7 \pm 1.4$ kcal/mol) working *against* a less positive entropy ($\Delta\Delta S^\ddagger = -9 \pm 3$ eu). Hence, the base-on effect is *apparently* not entirely entropic, as has been suggested,[8d] at least for $AdoB_{12}$, although sizeable radical-cage effects[10] remain to be deconvoluted out of this comparison (and out of the comparisons made by others[8d]).

Alternatively, the 10^6 rate acceleration seen for NpB_{12} in comparison to $AdoB_{12}$ at 25 °C in ethylene glycol is again the result of both enthalpic and entropic effects, this time due to a somewhat more favorable enthalpy of activation for NpB_{12} ($\Delta\Delta H^\ddagger = -2.3 \pm 1.0$ kcal/mol), but especially due to the *considerably more favorable entropy of activation* ($\Delta\Delta S^\ddagger = 19 \pm 2$ eu; $T\Delta\Delta S^\ddagger = 5.6$ kcal/mol at 25 °C).

Overall, three points seem clear: (i) Sterically bulky alkyls like neopentyl give the largest (nonredox)[5e,f] Co–C homolysis enhancements seen to date (Brown's recent work probing both steric and electronic axial-base effects also emphasizes steric effects).[8d] (ii) *Both* enthalpic and entropic components generally seem to be important in achieving enhanced rates of Co–C homolysis (outside the enzyme, at least; intuitively, it seems that the enzyme would evolve to exploit *both* enthalpic and entropic routes to accelerate AdoCbl's Co–C cleavage). And (iii) there is a pressing need to obtain accurate solution and structural data on *alkyl*cobalamins, both free of and bound to the enzyme (and with both R = Ado and non-Ado alkyls, such as base-on *and* base-off NpB_{12}). Only with such studies (a) will a more rigorous structural basis *in cobalamin complexes* for or against the butterfly conformational distortion theory be apparent and (b) will it prove possible to more rigorously connect (or discard) the base-on effect to that theory. Efforts toward the needed structural studies of NpB_{12} and of B_{12}-enzyme complexes are continuing.[20]

Experimental Section

A. General Methods. 1. Methods for Handling Air- and Light-Sensitive Compounds. Solutions of alkylcobalamins were handled under dim light or under a photographic red light and were further protected from light by wrapping flasks, syringes, and other equipment with aluminum foil. A Vacuum Atmospheres inert atmosphere (N_2) glovebox (registering ≤2 ppm O_2) was used for the storage and preparation of reaction solutions. Manipulations done on the bench employed Schlenk techniques.[51] House N_2 was further purified by passage through a Linde 4-Å molecular-sieve column and a heated 20 × 1.5 in. glass column of BASF R3-11 oxygen scavenger in the black (reduced) form. Solvents and solutions were degassed by bubbling with a fast flow of box gas for 15 min. Manipulations of small volumes of solvents and solutions were accomplished using gas-tight μL and mL syringes. Stock solutions of alkyl-B_{12}·HCl complexes, hydroxo-B_{12}·HCl, and Co(II)B_{12r} (<1–6 mM) were prepared in the glovebox (unless otherwise indicated), and 10–250 μL aliquots were removed by syringe for use. For air-free UV–visible work, 4-mm bore high-vacuum Teflon stopcock valves were glass-blown onto standard 3-mL volume, 1-cm path length Pyrex cuvettes and modified to include a small side-arm chamber (of about 250-μL volume) above the optical path, but below the sealing stopcock. Concentrated stock solutions could then be placed in the side chamber without exposure to the remaining components in the bulk solution of the cell. The cell could then be removed from the glovebox to the UV–visible spectrometer for thermal equilibration of the bulk of the cell solution prior to the initiation of a kinetic process by mixing. Analysis of gaseous products was determined by using cuvettes fitted instead with air-tight, Teflon-surfaced septa and screw caps.

Anaerobic samples for NMR analysis were prepared in septum-capped tubes in the glovebox except as otherwise noted.

2. Materials. Adenosylcobalamin and hydroxocobalamin hydrochloride (Sigma) were used as received except as stipulated. Hydroxocobalamin was prepared by passing an aqueous solution of the hydrochloride down a column of Mallinckrodt Amberlite IRA-400 anion-exchange resin in the basic form followed by recrystallization from water/acetone and drying overnight at 0.005 mm Hg. TEMPO (Aldrich) was purified by sublimation at 35 °C using aspirator vacuum, mp 39 °C (lit[52] 37–39 °C). Crystalline Co(II)B_{12r} and MeB_{12} were prepared as described in the literature.[53] Neopentyl iodide was prepared from the tosylate[54] (see the Experimental Section, Section C, on the preparation of NpB_{12}·HCl); commercial NpI *cannot* be used due to its MeI impurity.[55] Neopentane was from a lecture bottle (Pfalz and Bauer #44390). CD_3OD (99.5% D) and D_2O (99.9% D) were from Cambridge, and 20% DCl/D_2O (99+% D) was from Aldrich. Basic solutions of deuterated solvents were prepared by addition of sodium metal to the neat solvents. Dry Et_2O was obtained from a solvent still after overnight reflux under N_2 over CaH_2. Reagent-grade ethylene glycol was distilled from 4-Å molecular sieves under high vacuum. Buffered ethylene glycol was prepared by stirring sufficient anhydrous Na_2HPO_4 and crushed $NaH_2PO_4·H_2O$ with ethylene glycol overnight in the glovebox in order to give solutions that were 0.030 F in Na_2HPO_4 and 0.020 F in NaH_2PO_4 and H_2O. Stock solutions of basic and acidic ethylene glycol were prepared either by diluting concentrated (85%) H_3PO_4 or by stirring crushed NaOH in the solvent overnight followed by filtration. Aqueous solutions were prepared using house-distilled water.

All other solvents and reagents were of reagent grade or better and were used without further purification.

3. Equipment. NMR spectra of solutions in 5 mm o.d. sample tubes were recorded using Nicolet NT-360 or G.E. QE-300 spectrometers at ambient temperatures (ca. 20 °C) or (when indicated) at temperatures held within 0.1 °C of those cited by means of constant-temperature accessories. The *actual* NMR probe temperatures, believed accurate to ±0.5 °C, were measured using the standard MeOH temperature-dependent chemical-shift method well established in the literature.[56] Numerical simulations (done as controls), using hypothetical temperatures varying as much as even 2 °C from the actual temperatures employed in the axial-base curve fitting (Section J), resulted in values well within the error limits cited for ΔH and ΔS. Hence, it did not prove necessary, *at least for these specific experiments with NpB_{12}*, to measure the sample temperature with greater precision (e.g. as Brown and co-workers have done[8d]).

Chemical shifts were recorded vs internal TMS except for D_2O solutions, when internal DSS was used. Ultraviolet–visible (UV–visible spectra for the wavelength region 315–600 nm were recorded using a Beckman DU-7 spectrometer with cell temperatures maintained at ±0.1 °C from the desired temperature (25.7 °C except where otherwise specified) via a Peltier accessory. *It is important to note that the digital readout temperature of the Peltier accessory differed from the true temperatures reported herein* (by +0.7 at 25 °C to +0.1 at 35 °C to −0.5 at 45 °C);[34] *actual cell temperatures were determined using a 0.1 mm diameter microthermocouple (Omega) calibrated at the freezing point and at the atmospheric-pressure-corrected boiling point of water.* A Varian Series 2700 gas chromatograph was used for preparative work, and a Hewlett Packard 5790A instrument equipped with FID detectors, nitrogen carrier gas, and a Hewlett Packard 3390 integrator was used for analytical samples. A capillary column (cross-linked dimethylsilicone, 12.5 m × 0.2 mm i.d. H.P. part #19091-60312) operated in the split

(51) Shriver, D. F.; Drezdzon, M. A. *The Manipulation of Air-Sensitive Compounds*, 2nd ed.; J. Wiley and Sons: New York, 1986.

(52) Rozantzev, E. G.; Neiman, M. B. *Tetrahedron* **1964**, *20*, 131.

(53) (a) Blazer, H. U.; Halpern, J. *J. Am. Chem. Soc.* **1980**, *102*, 1684. (b) Dolphin, D. *Methods in Enzymology*; Academic Press: New York, 1971; Vol. XVIII, p 34.

(54) Neopentyl iodide synthesis starting from neopentyl tosylate: (a) Tipson, R. S. *J. Org. Chem.* **1949**, *9*, 235. (b) Roberts, D. D.; Snyder, R. C., Jr. *J. Org. Chem.* **1980**, *45*, 4052. (c) Winstein, S.; Morse, B. K.; Grunwald, E.; Schreiber, K. C.; Corse, J. *J. Am. Chem. Soc.* **1952**, *74*, 1113.

(55) Commercial neopentyl iodide (97%, Columbia) contained 1.5 mol % of methyl iodide (a reactant in a common synthetic route[55a]) according to NMR. Resonances attributable to a number of other alkyl iodide impurities[55b,c] present at less than the 1% level were also detected. (a) Landauer, S. R.; Rydon, H. N. *J. Chem. Soc.* **1953**, 2224. (b) Use of the following literature purification method also gave 99% pure NpI in our hands: Kornblum, N.; Iffland, D. C. *J. Am. Chem. Soc.* **1955**, *77*, 6653.

(56) (a) Van Geet, A. L. *Anal. Chem.* **1968**, *40*, 2227. (b) Van Geet, A. L. *Anal. Chem.* **1970**, *42*, 679.

mode (50:1 split ratio) was used for analytical applications with liquid samples. Confirmation of the identities of reaction products was obtained by co-injection of authentic material. HPLC analysis was done using a Waters Lambda-Max Model 481 instrument equipped with an Alltech 300 × 4.1 mm Versipac C-18 column with detection at 260 nm. Photolysis was accomplished using a 250-W tungsten flood lamp positioned 10–30 cm from the Pyrex cuvettes containing the sample.

B. Determination of Extinction Coefficients and Solution Concentrations. Extinction coefficients for B_{12r} in buffered ethylene glycol were determined by first measuring the spectrum of a 1.425×10^{-4} M anaerobic solution of $AdoB_{12}$ ($\epsilon_{520} = (7.78 \pm 0.06) \times 10^3$ M^{-1} cm^{-1})[57] containing 10 equiv of TEMPO and then measuring the spectrum again after photolyzing for 40 min. The final spectrum was exactly superimposable upon that of authentic $Co(II)B_{12r}$ and gave the extinction coefficients (wavelength in nm, $\epsilon \times 10^{-3}$ M^{-1} cm^{-1}): 404m (6.88), 472.5m (8.67). Similarly, extinction coefficients for NpB_{12} in buffered ethylene glycol were determined from initial spectra plus the determination of cobalamin concentrations from the $Co(II)Br_{12r}$ reaction products. Beer's Law behavior for all cobalamins was checked and obeyed for the wavelength and concentration ranges used in the kinetic analysis (to 2.0×10^{-4} M). Dissolution of TEMPO in ethylene glycol in a volumetric flask gave $\epsilon =$ 12.0 M^{-1} cm^{-1} at the 442.5 nm maximum, and Beer's Law behavior was obeyed for the $(0.27–1.09) \times 10^{-2}$ M concentrations used.

Extinction coefficients for NpB_{12} in aqueous solution were obtained by the literature method;[25c] specifically, the cobalamin concentration of a stock NpB_{12} solution was determined by photolyzing an aliquot of that solution to dicyanocobalamin ($\epsilon_{367} = 3.04 \times 10^4$ M^{-1} cm^{-1})[25c] in pH 10 H_2O containing 0.10 M KCN. (A control showed that the addition of a further 10% of KCN did not change the visible spectrum.)

C. Preparation and Purification of >98% Pure Neopentylcobalamin Hydrochloride. $NpB_{12}{\cdot}H^+Cl^-$ was prepared with some modifications of the literature method.[7b] First of all, all steps of both the synthesis *and* workup were carried out under a nitrogen atmosphere. Full protection against light-induced Co–C cleavage was employed at all times (see A. 1. "Methods for Handling Air- and Light-Sensitive Compounds"). Furthermore, a previously unreported problem is the presence of substantial amounts (ca. 0.1–1.0 equiv) of phenol by ^1H NMR in samples prepared according to the literature synthesis. For this reason, our $NpB_{12}{\cdot}H^+Cl^-$ preparation employed reprecipitation from anaerobic MeOH by the addition of anaerobic Et_2O, as detailed below. Also, it is crucial (as indicated in the literature[7b]) to use neopentyl iodide prepared from the tosylate;[54] commercial samples of the iodide were found to contain methyl iodide,[55] which, due to the faster rate of MeI vs NpI oxidative addition, leads to intolerable amounts of MeB_{12} as an impurity. [**Note Added in Proof**: We thank Professor K. Brown for the suggestion that an alternative way to proceed here is to use NpBr, as they have never seen any MeBr contamination by NMR of NpBr, presumably due to the volatility of MeBr (5 °C; a bp 100 °C below that of NpBr), and thus they do not see any MeB_{12} contamination of NpB_{12} prepared from NpBr.]

A 10-mL solution of 260 mg (0.193 mmol) of hydroxocobalamin in 5% NH_4Br/MeOH was placed in a septum-capped 12-mL centrifuge tube containing 1.0 g of zinc dust. After a 2-min purge with box atmosphere (via needles; to remove H_2 pressure build up) and occasional shaking, the solution was greenish-black, indicative of $Co(I)B_{12s}$. Upon the addition of 75 μL (112 mg, 0.566 mmol, 2.6 equiv) of *freshly prepared* (noncommercial; MeI free)[54,55] neopentyl iodide and shaking, the solution immediately changed to the color (orange-red) of the base-off, protonated $NpB_{12}{\cdot}HCl$ product. The solution was purged with box atmosphere for 2 min and shaken, and then the process was repeated once more. The zinc was allowed to settle, the supernatent was immediately filtered (medium-frit sintered-glass funnel) and poured into a 250-mL separatory funnel containing 100 mL of 1.0 M HCl (aq), and the cobalamin was extracted into 50 mL of degassed 50/50 (w/v) phenol/$CHCl_3$.

After separation of the organic layer, the $CHCl_3$ was removed in vacuo at room temperature, the cobalamin was precipitated from the solid phenol by the addition of 50 mL of dry Et_2O, and the resulting mixture was shaken and poured into two 50-mL centrifuge tubes. After centrifugation and discarding of the supernatents, the tubes were filled with Et_2O, shaken to disperse the insoluble $NpB_{12}{\cdot}HCl$, and centrifuged and the supernatent was discarded. After this Et_2O washing procedure was repeated three times, the crude yellow-orange solid was isolated by filtration (medium-frit sintered-glass funnel), washing with Et_2O on the filter, crushing into a powder, and drying overnight at room temperature and 0.01 mm Hg: crude yield 188 mg (0.131 mmol), 68%. Because ^1H NMR analysis showed 0.1–1.0 equiv of phenol in the product, the following procedure was adapted as a modification of the literature workup.[7b] The crude solid was dissolved in 5 mL of absolute methanol under nitrogen with stirring, filtered (medium frit), and precipitated by the addition with stirring of 30 mL of Et_2O. After filtration and drying as described above, a 62% net yield (172 mg) of yellow-orange product was obtained and stored at −30 °C in the glovebox freezer. While exhaustive drying in vacuo did remove all residual ether, less stringent drying resulted in even the highly volatile Et_2O (10–40 mol % by NMR) still being trapped in the solid product (this was confirmed by GLC).

No cobalamin or other non-cobalamin components are detectable by ^1H NMR in 10 mM solutions of our $NpB_{12}{\cdot}H^+Cl^-$ product in D_2O, where most of the cobalamin is in the protonated, base-off form ($NpB_{12}{\cdot}HCl$; $pK_a = 5.18$ at 1.0 M ionic strength;[8d] pK_a reported as 4.55 at an unspecified ionic strength;[7b] pK_a reported as 5.07 ± 0.03[6b] at an unspecified ionic strength and obtained by an unspecified method). Since little of the more reactive, base-on cobalamin is present under the above conditions, no detectable (≤1%) reaction to aquo-$Co(III)B_{12a}$ is observed in aerobic D_2O during the time required for spectroscopic analysis of freshly prepared solutions. Nor are any impurities detectable by ^1H NMR in D_2O with ca. 3 equiv of Na^+OD^- added (where the cobalamin exists as an equilibrium mixture of base-off and base-on forms); control experiments showed that even 2% MeB_{12} would have been detected. Moreover, no detectable paramagnetic $Co(II)Br_{12r}$ cobalamin is present, since exposure of the $NpB_{12}{\cdot}HCl$ solution to air did not result in any detectable $Co(III)B_{12a}$ aquocabalamin, easily identifiable by its unique ^1H NMR resonances in the 6–8 ppm region. (Solutions of $NpB_{12}{\cdot}HCl$ and $Co(II)B_{12r}$, deliberately produced by partial anaerobic photolysis in an NMR tube, showed the immediate production of aquocobalamin on exposure to air.) HPLC analysis, of a freshly prepared 2.0×10^{-4} M buffered ethylene glycol solution and using 30/70 acetonitrile/pH 5.00 0.010 M aqueous potassium phosphate buffer as eluent, showed a single alkylcobalamin peak at retention time 28 min (some of the non-alkyl, aerobic decomposition or trace light photolysis product, aquocobalamin, is also seen at 7.1-min retention time). Any contaminating methylcobalamin, a side product identified in early preparations using impure, MeI-contaminated commercial neopentyl iodide[55] as alkylating agent, would have been detected at the 2% level via its retention of 5.1 min under these conditions.

Although the UV–visible spectra we measured in acidic and neutral solutions showed good agreement with the reported[7b] wavelength, our $NpB_{12}{\cdot}HCl$ extinction coefficients (determined by the standard method of conversion to dicyanocobalamin)[25] were *repeatably* 9 ± 2% *lower* than the literature values.[7b]

Further characterization of $NpB_{12}{\cdot}HCl$: FAB-MS (dithioerythritol/dithiothreotol matrix) shows the expected molecular ion corresponding to the protonated cobalamin, $NpB_{12}{\cdot}HCl$, along with the expected higher (isotopic) peaks exhibiting the correct intensities. Calcd mass (after loss of Cl^-) for $C_{67}H_{100}O_{14}PCo^+$, 1400.66; found *m/e* 1400.66. Isotopic peaks (*m/e* values with relative intensities in parentheses): found 1400.66 (100), 1401.63 (86), 1402.66 (40), 1403.60 (14), and 1404.61 (4); calculated 1400.61 (100), 1401.66 (83), 1402.66 (36), 1403.67 (11), 1404.67 (3). Selected ^1H NMR (360 MHz) for 1 mM solution in D_2O at 20 °C: δ 9.27 (s, 1 H, B2-H), 7.55 (s, 1 H, B7-H), 7.47 (s, 1 H, B4-H), 7.04 (s, 1 H, C10-H), 6.54 (d, 1 H, R1-H), −0.11 (s, 9 H, neopentyl methyl H). In the crude product, phenol appeared at δ 7.29 (t, 2 H), 6.98 (t, 1 H), and 6.91 (d, 2 H) and Et_2O at 1.20 (t, 6 H) and 3.58 (q, 4 H). UV–visible [wavelength in nm, extinction coefficient × (10^{-3} M·cm)]: in buffered ethylene glycol 386m (8.46), 437.5m (6.96), 472.5 (5.80), 490m (5.91); in 0.10 M H_3PO_4/ethylene glycol 389m (8.76), 437m (8.20); in pH 7.0 0.10 M sodium phosphate(aq) 388m (8.71), 438m (6.99), 491m (6.18) [literature[7b,25c] 326m (15.5), 388m (9.46), 439m (7.52), 487m (6.78)]; in 0.10 M H_3PO_4(aq) 389.5m (9.14), 437m (8.24), [literature[7b,25c] 326m (15.3), 387m (10.2), 437m (9.05)].

D. $NpB_{12}{\cdot}HCl$ Stability Control Experiments. Several control experiments were also performed in order to compare the stability and reactivity of our NpB_{12} to the literature; the goal here was to facilitate our subsequent studies (e.g. to avoid premature thermal or photochemical decomposition of the NpB_{12} when undertaking quantitative thermolysis product and kinetic studies). As expected, UV–visible (315–600 nm) spectra in anaerobic or aerobic aqueous 0.01 M H_3PO_4 (where all of the cobalamin is in a protonated, base-off form) were unchanged (less than 2% reaction) after 15 h at 25 °C. In anaerobic pH 7.0 H_2O solution, slightly more reaction occurred during this time (ca. 5% by UV–visible). In *aerobic* pH 7.0 H_2O solution, oxidation to $Co(III)B_{12a}$ occurred with the reported isosbestic points[7b] (at 335, 371, and 474 nm); when further thermalized to completion, first-order kinetic plots of $-\ln((A - A_\infty)/(A_0 - A_\infty))$ vs time were linear to 97% reaction, yielding a 25.7 °C rate constant of 1.9×10^{-4} s^{-1} and a half-life of 62 min, in rough agreement

(57) Hay, B. P. Ph.D. Dissertation, University of Oregon, 1986.

with the literature half-lives at 25 °C of[13a] 60 min, 75 min,[7b] and 96 min[8d] (the discrepancy between these thermolysis results *in* H_2O is under investigation and will be reported elsewhere[34c]). Subsequent photolysis led to a very small (≤1%) additional change in absorbance which can be ascribed to minor cobalamin impurities (below the detection levels of our characterization methods) to yield spectra which were exactly superimposible upon that of the authentic cobalamin product. Superior kinetic plots (linear first-order plots over 5 half-lives, 97% reaction) were obtained when the thermal, rather than photochemical, end points were used.

E. Preparation of 1-(2,2-Dimethylpropanoxy)-2,2,6,6-tetramethylpiperidine (Np-TEMPO). We previously cited[4d] the isolation and NMR characteristics of this compound but have not previously reported the synthetic details. Neopentyl magnesium iodide was prepared under N_2 by the dropwise addition (by syringe over 2.5 h) of 2.2 mL (0.017 mol) of neopentyl iodide to 0.24 g (0.010 mol) of Mg ribbon contained in 70 mL of refluxing Et_2O. Dropwise addition of a degassed solution of 3.1 g (0.020 mol) of TEMPO in 6 mL of Et_2O to the stirring, 0 °C, Grignard solution over 5 min was accomplished by syringe. After extracting the mixture at room temperature with 40 mL of 25% NH_4Cl(aq), washing with water, and drying ($MgSO_4$), the solvent was removed first in vacuo (water aspirator) and then by simple distillation (aspirator vacuum) using a maximum bath temperature of 90 °C. The orange 2.4-g pot residue was chromatographed under 5 psi of N_2 pressure using a 2.5 × 30 cm column of EM Merck GF-254 (Type 60) silica gel and eluting with 40:1 pentane/Et_2O. The first colorless fractions contained the product according to GLC; they were combined and reduced by rotary evaporation at room temperature to a volume of 850 μL. Preparatory GLC using a 5 ft × $^1/_4$ in. 1.5% OV-101 on 100/120 chromosorb HP column with temperature programming gave 585 mg (2.58 mmol) of Np-TEMPO, at 26% yield (61% based on TEMPO consumed in the formation of the Grignard reagent). Anal. Calcd for $C_{14}H_{29}NO$: C, 73.95; H, 12.86; N, 6.16. Found (Galbraith Laboratories): C, 74.04; H, 12.94; N, 6.12. The ^{1}H NMR ($CDCl_3$) shows two distinct peaks for the ring methyl groups[4d] at 25 °C, a result of a slow nitroxide-ring-inversion process imposed by the bulky neopentyl alkyl group, but these collapse to a single resonance at 55 °C: at 25 °C δ 0.95 (s, 9 H), 1.11 (s, 6 H), and 1.14 (s, 6 H) (s, 12 H at 55 °C), 1.29 (br d, 1 H), 1.35–1.60 (m, 5 H), 3.44 (s, 2 H).

F. Quantitation of Neopentyl Radical Products Neopentane and Np-TEMPO by GLC. Appropriate quantities of stock TEMPO/ethylene glycol and buffered ethylene glycol sufficient to make 2.90 mL of solution were added to septum-capped vials (5.00 ± 0.05 mL volume) followed by 100 μL of freshly prepared stock NpB_{12}·HCl/ethylene glycol. An additional vial was prepared with neopentane gas (added via gas-tight syringe) and Np-TEMPO, each at the quantities expected for 100% yield from 1.87 × 10^{-4} M neopentyl radical. [No attempt was made to more fully quantitate the yield of the volatile neopentane product (i.e. better than the semiquantitation that follows), as it is not of direct importance to the present studies (which emphasize the reaction in the presence of excess TEMPO where the yield of neopentane is zero).] After 30 h of reaction in the glovebox at 25 ± 3 °C, 50 μL of gas was removed with a 100-μL syringe from the headspace of each vial for analysis of neopentane (retention time 5.5 min) using a 6 ft × $^1/_8$ in. Porapak column at 120 °C with an N_2 flow rate of 60 mL/min. The ≤40 mol % Et_2O present in the solid cobalamin (confirmed by co-injection of ether vapor) eluted at 9.8 min under these conditions. In the glovebox, aliquots of the vials were transferred to UV–visible cells for determination of the cobalt products. Next, (2.0 ± 0.05) μL of the solutions was analyzed for Np-TEMPO by capillary GLC using a column flow rate of 1 mL/min at 40 °C and a column temperature program (50 °C for 2 min then 30–200 °C for 3.8 min). Np-TEMPO eluted at 6.2 min (after both ethylene glycol and TEMPO) and was quantitated vs authentic material. Owing to the limited solubility of Np-TEMPO in ethylene glycol, standard stock solutions of less than 3 × 10^{-4} M Np-TEMPO were made by adding the neat compound to the required large volume of solvent (rather than by serial dilution). A standard curve showed linearity for Np-TEMPO in the concentration range used for analysis of the reaction samples. Yields of Np-TEMPO from duplicate injections agreed within 3%. The relative percent yields of neopentane shown below are based on 100% yield in the absence of TEMPO;[29] data are given as initial [TEMPO] (initial equivalents of TEMPO vs NpB_{12}), % yield of Np-TEMPO, % yield of neopentane: 0 (0), 0, 100; 2.01 × 10^{-4} M (1.07 equiv of TEMPO), 79, 16; 4.02 × 10^{-4} M (2.15), 92, 6.4; 10.1 × 10^{-4} M (5.4), 98, 1.2; 40.2 × 10^{-4} M (21.5) 98, 0; 201 × 10^{-4} M (107), 100, 0.

G. Kinetic Studies. 1. Kinetics in Anaerobic Ethylene Glycol. Appropriate quantities (5–200 μL) of stock TEMPO/ethylene glycol and Co(II)B_{12r}/buffered glycol were placed in reaction cuvettes containing 2.5–3.0 mL of acidic, basic, or buffered ethylene glycol solvent followed by the careful placement of 50–200 μL of reactant cobalamin/ethylene glycol solution into the isolated sidearm chamber of the cells. After the Teflon stoppers were affixed, the cells were removed from the glovebox to the cell compartment of the UV–visible instrument and equilibrated for 10–15 min at the reaction temperature. (Temperatures in the UV–visible cell were determined using a microthermocouple (Omega) as described in section A. 3. "Equipment".) Good mixing of the cell's contents and initiation of the reactions was accomplished by inverting the cells several times before replacing them into the UV–visible instrument for the kinetic measurements. A temperature re-equilibration time (i.e. following mixing) of about 5 min occurred at the higher temperatures, and hence kinetic data for this period were not included in the analysis.

Reported rate constants (Table I) were determined from 20–35 (absorbance at 473 nm, time) points taken at equal intervals over 4 half-lives using the kinetics mode of the instrument and correcting absorbance values for instrument drift. Except as noted in the Results section, first-order plots of $-\ln[(A_t - A_\infty)/(A_0 - A_\infty)]$ vs time were linear (less than 4% deviation in slope of the portion of the reaction followed) and gave correlation coefficients R = 0.9995 or better. Slopes and the standard deviations of the slopes and intercepts of all plots (including Eyring plots) were determined by standard least-squares methods. Duplicate runs, made using the wavelength-scan mode of the instrument, maintained isosbestics during the portion of the reaction studied (and when analyzed gave rate constants from data at several wavelengths agreeing within 10%) but displayed more scatter than those using the kinetics mode. At the lower temperatures, the cell temperatures of reaction mixtures containing at least 1 equiv of TEMPO were increased after 5 half-lives of reaction in order to drive the reactions to thermal completion. This was followed by cooling to the reaction temperature for determination of the final absorbance values (A_∞). When appropriately scaled, final spectra run at 25.7 °C were superimposable upon those obtained starting with other reactant concentrations and temperatures, both before and after photolysis.

For the study of the [Co(II)B_{12r}] rate dependence, in which less than 1 molar equiv of TEMPO per mole of neopentylcobalamin was initially present, final absorbances could not be easily determined experimentally.[26] A_∞ values were calculated by adding the initial absorbance for each reaction mixture to the absorbance change ($A_\infty - A_0$) for a reaction run with excess [TEMPO] but with the same initial [NpB_{12}].

2. Additional Precautions. As we have noted before,[4d,5d] strict exclusion of trace oxygen and the usage of cuvettes containing only glass or Teflon surfaces are required for reproducible results using these air-sensitive complexes. When such precautions are not taken (such as when using septa without the Teflon liners or if stock solutions are injected by syringe into septum cap cuvettes outside of the drybox), a slow reaction characterized by increases in absorbance at about 373 and 530 nm and a decrease at 473 nm sometimes occurs which disrupts the isosbestic points, alters the calculated rate constants, and then makes the final spectra non-superimposable upon that of authentic B_{12r}. The nature of this irreproducable reaction, which does not have the same absorbance changes as the oxidation of Co(II)Br_{12r} to Co(III)B_{12a}, is unknown.

H. Temperature Dependence of the Axial-Base Equilibria for $AdoB_{12}$ in Methanol (for Comparison to Ethylene Glycol) as a Control. The ^{1}H NMR chemical shift (±0.001 ppm) of the C_{10}-hydrogen of a 1.0-mL saturated solution of $AdoB_{12}$ in CD_3OD/TMS was determined at 5-deg intervals from 20 to 60 °C. The chemical shift δ_{off} was determined at 25 °C after acidifying the solution with 10 μL of 20% DCl/D_2O. In analogy to our earlier work,[5a,d] the equation $(1/T) = (-R/\Delta H)\{\ln[(\delta_{off} - \delta_{obs})/(\delta_{obs} - \delta_{on})] - (\Delta S/R)\}$ was fit to the (δ_{obs}, T) data by variation of the parameters ΔH, ΔS, and δ_{on} with use of standard nonlinear-regression methods (the term $(\delta_{off} - \delta_{obs})/(\delta_{obs} - \delta_{on})$ in the equation equals $K_{off\text{-}on}$). Note that δ_{off} and δ_{on} are assumed to be temperature-independent during this treatment. During this curve fitting, the following physically valid constraints were imposed: ΔH and $\Delta S \leq 0$ and $\delta_{on} \leq$ the smallest δ_{obs} value. Results gave ΔH = −7.9 ± 0.2 kcal/mol and ΔS = −22.1 ± 0.4 cal/mol·K, in substantial agreement with ΔH = −7.6 ± 0.2 kcal/mol and ΔS = −20.2 ± 0.7 cal/mol·K, determined earlier from 10 to 80 °C by UV–visible spectroscopy in ethylene glycol.[5a,d]

I. Control Establishing the Small, Linear Temperature Dependence of the C_{10}-H of Protonated, Base-Off NpB_{12}. The C_{10}-H chemical shift of 10 mM NpB_{12}·H^+Cl^- in aerobic CD_3OD (±0.001 ppm vs TMS internal standard) was determined at 5-deg intervals from −40.0 to +35.0 °C. A plot according to Brown's equation[35c] of $\delta_{off(obsd)} = m_{off}T(K) + \delta_{off}(0\ K)$ was linear with slope m_{off} = (−4.81 ± 0.09) × 10^4 ppm/K and intercept δ_{off}(0 K) = 7.175 ± 0.003 ppm with r = −0.999.

J. Temperature Dependence of the Axial-Base Equilibria for NpB_{12}. The C_{10}-H chemical shift (vs internal standard) of 10 mM solutions of

NpB_{12} was determined at 5-deg intervals in 80 mM $NaOCD_3/CD_3OD$ with 10 mM TMS standard (−45 to 40 °C). Several tubes containing identical solutions were required for data acquisition owing to partial Co–C bond thermolysis of the samples at the higher temperatures, but the extent of reaction did not alter the chemical shifts of any NpB_{12} resonances. Curve fitting was accomplished as described in part H above (except that δ_{off} was also varied in the fitting procedure). Plots of both experimental and calculated δ_{obsd} vs T appear in Figure 6.

The NMR spectra of NpB_{12} in $MeO^-/MeOH$ exhibited some decomposition at the higher temperatures used, but the chemical shifts of the NpB_{12} resonances at 0 °C were the same before and after the sample temperature was raised to 40 °C for 15 min. This is taken as evidence that negligible destruction of the NpB_{12} occurs during the course of these variable-temperature NMR experiments in $MeO^-/MeOH$.

Following the appearance of Brown's "complete-scheme" paper,[35c] we also curve fit our NMR data to his more exact equations. The results, however, were unchanged within experimental error (see Section and Figure D, supplementary material).

K. Absorbance of NpB_{12} Solutions as a Function of Temperature. To test whether or not $K_{off\text{-}on}$ equilibrium constants in ethylene glycol could be determined reliably by UV–visible over the small temperature range (10–30 °C) used by others,[6b] the following experiment was carried out. The results demonstrate that 10–30 °C is too small a temperature range for a reliable determination of $K_{off\text{-}on}$.

In the drybox, 2.5 mL of buffered ethylene glycol was added to a cuvette fitted with a gas-tight, Teflon-lined injection cap. After the cuvette was allowed to reach thermal equilibrium in the UV–visible spectrophotometer, 100 μL of a concentrated $NpB_{12}{\cdot}H^+Cl^-$ solution in anaerobic ethylene glycol was injected via a gas-tight syringe. After mixing, several spectra were obtained over a period not exceeding 8 min to minimize NpB_{12} thermolysis (controls showed that thermal re-equilibration required 6 min after mixing). Concentrations after mixing were 1.5×10^{-4} M NpB_{12}, 0.0020 F NaH_2PO_4, and 0.0030 F Na_2HPO_4. After the last spectra were obtained, 10.0 μL CF_3CO_2H was injected by syringe (to convert the NpB_{12} to the much less reactive, base-off form); the cuvette (now containing 0.05 M CF_3CO_2H) was allowed to re-equilibrate to 25.7 °C, and a final spectrum was recorded. This entire process was repeated in order to obtain spectral data at 11.6, 16.2, 21.0, 25.7, and 30.4 °C; the final spectra in acid solution were analyzed to confirm the absence of NpB_{12} thermal decomposition.

The A_{520} data were corrected then in two ways, (a) first for small ca. 2% differences in the amount of injected $NpB_{12}{\cdot}H^+Cl^-$ (correction was accomplished by multiplying the absorbance by a small factor (0.98–1.02) to bring each final A_{520} to 0.548 for each final, protonated $NpB_{12}{\cdot}H^+Cl^-$ spectrum) and (b) second by correcting solvent volumes for temperature (by multiplying A_{520} by the appropriate factor derived from the ratio of ethylene glycol's density at the set temperature vs 25 °C). The corrected A_{520} values thus correspond to the same [NpB_{12}] and are plotted vs temperature in Figure B, supplementary material.

In order to evaluate the shape of the plot in Figure B, a shape determined by the ΔH and ΔS for the base-off to base-on equilibrium, the following equation is of use:

$$A_T = F_{base\text{-}on}(A_{on(calc)} - A_{off}) + A_{off}$$
$$= [K_{off\text{-}on}/(K_{off\text{-}on} + 1)](A_{on(calc)} - A_{off}) + A_{off}$$

where $F_{base\text{-}on}$ is the fraction base-on, $K_{off\text{-}on} = e^{[(\Delta S - \Delta H/T)/R]}$, A_T is the calculated absorbance as a function of temperature, A_{off} is the limiting absorbance (taken here as 0.548) when all the NpB_{12} is in the base-off form, and A_{on} is experimentally unobtainable. Hence, the replacement $A_{on,calc}$ is the calculated value (1.1081) obtained from the above expression by substituting our A_{off} = 0.548 and A(25 °C) = 0.796 and using the claimed[6b] ΔH = −10 kcal/mol, ΔS = −34 eu, and T = 298.16 to give $K_{off\text{-}on}$. The above expression then allows the A, T points (Figure B) to be determined for the temperature range in question, 10–30 °C.

This analysis shows that the plot of A vs T is essentially linear over 10–30 °C. That is, even with the experimentally unattainable A_{on} estimated by our $A_{on(calc)}$, there is none of the sigmoidal curvature needed for a successful curve-fitting determination of ΔH and ΔS.

Acknowledgment. Support from NIH Grant DK-26214, and an NIH postdoctoral fellowship to M.D.W., is gratefully acknowledged. We thank Dr. John Grate, of Catalytica Inc., for helpful discussions about the synthesis of alkylcorrins and other issues and Professor Ken Brown for valuable comments on the penultimate draft of the manuscript.

Supplementary Material Available: Figures A1–A3, representative first-order kinetic plots (ethylene glycol data); Figure B, UV–visible absorbance vs temperature data for NpB_{12} in ethylene glycol; Figure C, $AdoB_{12}$ in CD_3OD C_{10}-H ^1H NMR chemical shift vs temperature data and curve fit; Figure and Section D, a curve fit and explanation of the ^1H NMR chemical shift vs temperature data according to Brown's equations;[35c] Figure E, visible spectra exhibiting six isosbestic points during the 25.7 °C anaerobic thermolysis of NpB_{12} in ethylene glycol; a section titled Further Discussion of the Temperature-Dependent Extinction Coefficient Problem; and a section titled Discussion of Three Confusing Statements from the Recent Literature (9 pages).[8d] Ordering information is given on any current masthead page.