Facile α/β Diastereomerism in Organocobalt Corrinoids. Synthesis, Characterization, and Thermolysis of α-Neopentylcobalt Corrinoids

Xiang Zou and Kenneth L. Brown*

Contribution from the Department of Chemistry, Box CH, Mississippi State University, Mississippi State, Mississippi 39762

Received February 8, 1993

Abstract: Coenzyme B_{12} analogs α-neopentylcobinamide (α-NpCbi$^+$) and α-neopentylcobalamin (α-NpCbl), where the bulky organic ligand is in the "lower" (α) axial ligand position, have been synthesized by reductive alkylation of cyanoaquocobinamide and aquocobalamin, respectively, with neopentyl bromide at ~0 °C, and characterized by their UV–visible spectra, GC/MS analysis of their anaerobic solid-state pyrolysis products, and thermal and photolytic conversion to corresponding β diastereomers. The thermolyses of α-NpCbi$^+$ and α-NpCbl were studied in aerobic aqueous solution, and the rate constants for Co–C bond homolysis were found to be 5.3×10^3 and 3.4×10^3 times larger at 35 °C and 2.3×10^4 and 1.1×10^4 times larger at 5 °C than those for β-NpCbi$^+$ and base-off β-NpCbl, respectively. As expected, product analysis under anaerobic conditions using HTEMPO as a free radical trap and under aerobic conditions using O_2 as a free radical trap showed that thermolysis of these α-neopentylcobalt corrinoids involves homolytic fission of the Co–C bond. The activation parameters were determined from Eyring plots (α-NpCbi$^+$, $\Delta H^\ddagger = 21.4 \pm 0.4$ kcal mol^{-1}, $\Delta S^\ddagger = 0.9 \pm 1.3$ eu; α-NpCbl, $\Delta H^\ddagger = 22.5 \pm 0.2$ kcal mol^{-1}, $\Delta S^\ddagger = 3.1 \pm 0.1$ eu). Both $\Delta H^\ddagger$ and $\Delta S^\ddagger$ are significantly smaller than the values previously obtained for β-NpCbi$^+$ and base-off β-NpCbl. Thus, although steric interactions between the neopentyl group and the more numerous and larger downward projecting corrin side chains would be expected to weaken the α Co–C bond relative to the β Co–C bond, there also seems to be an electronic destabilization of the Co–C bond in the α diastereomers. This, in turn, suggests that the steric congestion at the α face may be relieved by an upward puckering of the corrin ring, leading to a weakened α Co–C bond.

It is generally accepted that the unique Co–C bond homolysis in coenzyme B_{12} is the first step in the coenzyme B_{12}-dependent enzymatic rearrangement reactions.[1] One plausible hypothesis for Co–C bond activation in B_{12}-dependent enzymes requires a flexible corrin ring that is upwardly distorted to labilize the carbon–cobalt bond in the enzyme–substrate–coenzyme complex,[2] suggesting that the tremendous rate enhancement ($\sim 10^{12}$)[3] for the Co–C bond homolysis by such enzymes is mostly due to steric effects. This theory has received some support from studies of thermolysis of β-5′-deoxyadenosylcobalamin (coenzyme B_{12}, β-AdoCbl)[3–6] and numerous other model complexes, especially those with bulky organic ligands that have no β hydrogen atoms and thus must undergo Co–C bond fission by homolysis.[7–14] The latter complexes decompose more rapidly than those with sterically less demanding alkyl groups, and the activation parameters and bond dissociation energies for thermolytic Co–C bond homolysis of a number of these complexes have been determined in aqueous solution as well as in other solvents.[7–14]

Relatively little is known, however, about the stability of the Co–C bond in α-alkylcobalt corrinoids, in which the alkyl group is coordinated at the lower (or α) axial ligand position of the corrinoid (Figure 1).[4] Sterically, the corrin ring is believed to be more crowded at the α face, which bears the b, d, and e propionamide side chains and the substituted propionamide f side chain (i.e., the nucleotide loop side chain), than the β face, which bears only three acetamide side chains (Figure 1). Only with the recent synthesis and characterization of a series of stable α-alkylcobalt corrinoids has the formation of these α diastereomers been considered to be a general phenomenon.[15–18] For the thirteen known examples of α-alkylcobalt corrinoids that have been characterized so far, all but one (R = CH_2CH_3) are obtained by standard synthetic procedures (i.e., reductive alkylation of a

(1) (a) Halpern, J. *Science* **1985**, *227*, 869. (b) Dolphin, D., Ed. *B_{12}*; Wiley: New York, 1982.

(2) (a) Schrauzer, G. N.; Grate, J. H. *J. Am. Chem. Soc.* **1981**, *103*, 541. (b) Grate, J. H.; Schrauzer, G. N. *J. Am. Chem. Soc.* **1979**, *101*, 4601.

(3) (a) Finke, R. G.; Martin, B. D. *J. Inorg. Biochem.* **1990**, *40*, 19. (b) Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1469. (c) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 4820. (d) Finke, R. G.; Hay, B. P. *Inorg. Chem.* **1984**, *23*, 3041.

(4) Abbreviations: Ado, 5′-deoxyadenosyl; Np, neopentyl; HTEMPO, 4-hydroxy-2,2,6,6-tetramethyl-piperidinyloxy; Np-HTEMPO, 1-(2,2-dimethylpropoxy)-2,2,6,6-tetramethyl-4-hydroxypiperidine, TEMPO, 2,2,6,6-tetramethylpiperidinyloxy; β-RCbl, β-alkylcob(III)alamin; α-RCbl, α-alkylcob(III)alamin; β-RCbi, β-alkylcob(III)inamide; α-RCbi, α-alkylcob(III)inamide; H_2OCbl, aquocob(III)alamin; $(H_2O)_2$Cbi, diaquocob(III)inamide. In α-alkylcobalt corrinoids, the organic ligand occupies the "lower" axial ligand position, while in β-alkylcobalt corrinoids, the organic ligand is in the "upper" ligand position. See Figure 1 for structures.

(5) (a) Halpern, J.; Kim, S.-H.; Leung, T. W. *J. Am. Chem. Soc.* **1985**, *107*, 2199. (b) Halpern, J.; Kim, S.-H.; Leung, T. W. *J. Am. Chem. Soc.* **1984**, *106*, 8317.

(6) Gjerde, H. B.; Espenson, J. H. *Organometallics* **1982**, *1*, 435.

(7) Brown, K. L.; Brooks, H. B. *Inorg. Chem.* **1991**, *30*, 3420.

(8) (a) Halpern, J. *Bonding Energetics in Organometallic Compounds*; ACS Symposium Series 428; Marks, T. J. Ed.; American Chemical Society: Washington, DC, 1990; p 100. (b) Halpern, J. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 13. (c) Kim, S.-H.; Chen, H. L.; Feilchenfeld, N.; Halpern, J. *J. Am. Chem. Soc.* **1988**, *110*, 3120. (d) Geno, M. K.; Halpern, J. *J. Am. Chem. Soc.* **1987**, *109*, 1238. (e) Tsou, T.-T.; Loots, M.; Halpern, J. *J. Am. Chem. Soc.* **1982**, *104*, 623. (f) Halpern, J.; Ng, F. T. T.; Rempel, G. L. *J. Am. Chem. Soc.* **1979**, *101*, 7124.

(9) Martinho Simões, J. A.; Beauchamp, J. L. *Chem. Rev.* **1990**, *90*, 629.

(10) Nome, F.; Rezende, M. C.; Sabóia, C. M.; Da Silva, A. C. *Can. J. Chem.* **1987**, *65*, 2095.

(11) Blau, R. J.; Espenson, J. H. *J. Am. Chem. Soc.* **1985**, *107*, 3530.

(12) Baldwin, D. A.; Betterton, E. A.; Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1985**, 1613.

(13) (a) Martin, B. D.; Finke, R. G. *J. Am. Chem. Soc.* **1990**, *112*, 2419. (b) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 8012. (c) Finke, R. G.; Smith, B. L.; Mayer, B. J.; Molinero, A. A. *Inorg. Chem.* **1983**, *22*, 3677.

(14) (a) Ng, F. T. T.; Rempel, G. L. *Inorg. Chim. Acta* **1983**, *77*, L165. (b) Ng, F. T. T.; Rempel, G. L. *J. Am. Chem. Soc.* **1982**, *104*, 621.

(15) Alelyunas, Y. W.; Fleming, P. E.; Finke, R. G.; Pagano, T. G.; Marzilli, L. G. *J. Am. Chem. Soc.* **1991**, *113*, 3781.

(16) (a) Brown, K. L.; Salmon, L.; Kirby, J. A. *Organometallics* **1992**, *11*, 422. (b) Brown, K. L.; Zou, X.; Salmon, L. *Inorg. Chem.* **1991**, *30*, 1949. (c) Brown, K. L.; Evans, D. R. *Inorg. Chem.* **1990**, *29*, 2559.

(17) Brown, K. L.; Zou, X. *Inorg. Chem.* **1992**, *31*, 2541.

(18) Brown, K. L.; Zou, X. *J. Am. Chem. Soc.* **1992**, *114*, 9643.

0002-7863/93/1515-6689$04.00/0

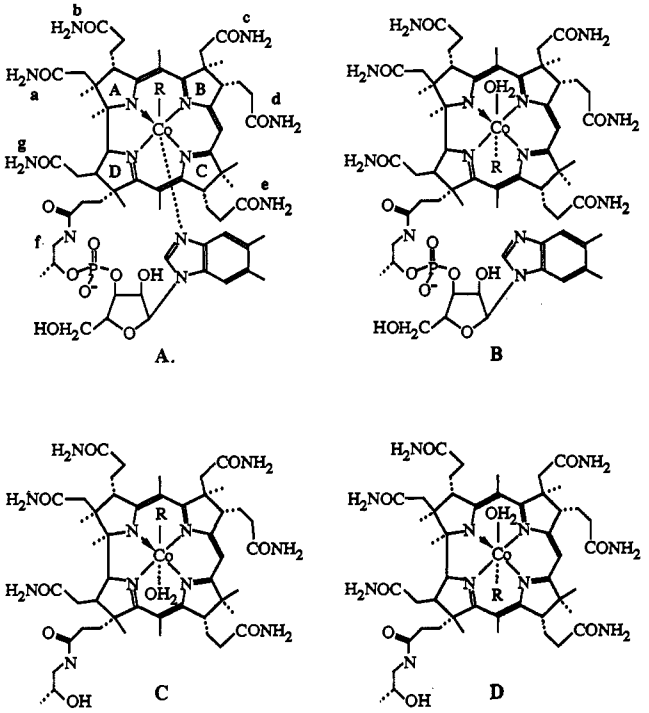


Figure 1. Structure of (A) base-on β-RCbl, (B) α-RCbl, (C) β-RCbi, and (D) α-RCbi.

cobalt(III) corrinoid), the α-alkylcobalt corrinoids being formed concurrently with the β-alkyl diastereomers, with the α diastereomers ranging from 4% to 98% of the total alkylated products. In order to obtain any α-alkylcobalamin at all, the pH of the reaction media needs to be lower than the base-on/base-off pK_a of the β-alkylcobalamin product.[16,19] Preliminary studies[17] have shown that most of these α-alkylcobalt corrinoids, including the recently characterized α-AdoCbl (the α diastereomer of coenzyme B_{12}) and α-AdoCbi,[18] are quite stable at room temperature. However, these α-alkylcobalt corrinoids can be converted thermally (and photolytically) to their corresponding β diastereomers under anaerobic conditions but do so only slowly at elevated temperatures, a process that surely involves breaking and regenerating of the Co–C bond. Starting from either diastereomer, thermal rearrangements at 70–110 °C gave mixtures of α- and β-alkylcobalt corrinoids under anaerobic conditions. Mixtures of α and β diastereomers were also obtained by anaerobic photolysis, starting from either α or β diastereomer.[17,20] In general, the Co–C bond in α-alkylcobalt corrinoids appears to be more labile than that in the β diastereomers;[17,20] however, quantitative investigations of α-alkylcobalt corrinoids thermolysis have not yet been reported.

With the synthesis and characterization of these once-rare α-alkylcobalt corrinoids, the question of how large a difference in steric congestion actually exists between the two faces of the corrin ligand can now begin to be addressed by studies of Co–C bond homolysis in α-alkylcobalt corrinoids for comparison to that in the β diastereomers. An understanding of the factors contributing to the homolysis of the α Co–C bond may also shed some light on the important question of what factors contribute to the activation of the β Co–C bond in coenzyme B_{12}, which is weakened dramatically by the B_{12}-requiring enzymes in the enzymatic rearrangement reactions.[3c,d,21]

In search of suitable candidates for such studies, neopentylcobalt corrinoids are of interest since β-NpCbl and β-NpCbi$^+$ are known to decompose homolytically[2a,8c,e,12] at conveniently measurable rates below 100 °C, and activation parameters for the thermolysis of these compounds have been extensively studied in different solvents, with and without an added radical trap.[2a,7,8c,e] In a previous paper[7] we reported that the reductive alkylation of factor B[22] and H_2OCbl^+ with 1-bromo-2,2-dimethylpropane (neopentyl bromide) gave only β-neopentylcobalt corrinoids. However, a reexamination of the course of these syntheses has shown that the α diastereomers are indeed formed during the reductive alkylation reaction, but their lability prevents them from being isolated unless special precautions are taken. We now report the first successful synthesis and characterization of α-NpCbi$^+$ and α-NpCbl and, more importantly, studies of their Co–C bond thermolysis and quantitative analysis of the thermolysis products in aqueous solution. This study allows the first detailed comparison of the relative stabilities of the Co–C bonds in a pair of diastereomeric α- and β-alkylcobalt corrinoids.

Experimental Section

Materials. H_2OCbl^+·OAc was from Roussell. 4-Hydroxy-2,2,6,6-tetramethylpiperidinyloxy (HTEMPO)[4] and 1-bromo-2,2-dimethylpropane (NpBr) were from Aldrich. Trimethylacetaldehyde (pivalaldehyde) was purchased from Pfaltz and Bauer. Molecular sieves (4 Å) were obtained from Fisher and were activated at 150 °C for 3 h before use. Factor B[22] was prepared by a modification[23] of the method of Renz.[24] Buffer components (K_2HPO_4 and KH_2PO_4) were obtained in the highest purity commercially available and were used without further purification. Glass distilled water was used throughout. α-Neopentylcobalt corrinoids, 1-(2,2-dimethylpropoxy)-2,2,6,6-tetramethyl-4-hydroxypiperidine (Np-HTEMPO), and *N*-(2,2-dimethylpropylidene)benzenamine were prepared as described below.

α-Neopentylcobalamin. H_2OCbl^+·OAc (100 mg, 63 μmol) was dissolved in 5% phosphoric acid (50 mL) and deoxygenated by argon purge for 1 h. Zinc wool (1 g, 15.3 mmol), freshened with 2 N HCl and washed with water, was added to the solution, and the reduction of the cobalt corrinoid was allowed to proceed for 20 min. The solution was then cooled in an ice-water bath, and NpBr (2 mL, 16 mmol) was added by syringe. Stirring was continued for 3 h at ~0 °C. After removal of the residual zinc by filtration, the solution was neutralized to pH 6.5 in an ice-water bath with 50% NaOH. The precipitate ($Zn_3(PO_4)_2$) that formed was filtered and discarded, and the filtrate was immediately frozen and stored at –20 °C. The total yield of NpCbl's was 40% with 25% α-NpCbl and 15% β-NpCbl by HPLC.

α-Neopentylcobinamide. α-NpCbi$^+$ was prepared similarly from factor B and NpBr, except that 10% acetic acid was used in place of phosphoric acid, and the alkylation reaction was allowed to proceed for only 15 min. After neutralization with NaOH and filtration to remove the precipitate that formed, the crude reaction mixture was stored at –20 °C for future use. The total yield of NpCbi's was 60% with 32% α-NpCbi$^+$ and 28% β-NpCbi$^+$ by HPLC.

Since α-neopentylcobalt corrinoids undergo slow thermal decomposition even at –20 °C, pure α-NpCbl and α-NpCbi$^+$ were prepared just before use from the frozen reaction mixtures by semipreparative HPLC at ~0 °C with ammonium phosphate or ammonium acetate buffer (25 mM) as the aqueous solvent (solvent A) and acetonitrile as the organic solvent (solvent B). HPLC solvent gradients used for separations were as follows: initial condition at 95% A/5% B for 1 min, increasing to 70% A/30% B in 7 min, and at 12 min changing to 95% A/5% B in 1 min. Caution was taken to cool the HPLC column and the solvents in ice-water baths to ~0 °C before and during the separation to minimize decomposition ($t_{1/2}$ ~ 11 min for α-NpCbl and ~ 6 min for α-NpCbi$^+$ at 25 °C). With these precautions, α-NpCbl and α-NpCbi$^+$ could be obtained in over 95% purity, free from nonalkylated corrinoids as determined by UV–visible spectroscopy and HPLC.

α-NpCbi$^+$ and α-NpCbl samples for thermal decomposition studies were purified just before use as described above. Because of the extreme labilities of these complexes, HPLC purified stock solutions were not desalted and thus contained 17.5 mM ammonium phosphate buffer and 30% acetonitrile from the HPLC separation. The final reaction solutions for kinetic experiments, after addition of the stock solution to the reaction media, contained 3% acetonitrile and 1.75 mM ammonium phosphate.

(19) Brown, K. L.; Zou, X. *Inorg. Chem.* **1991**, *30*, 4185.
(20) Zou, X.; Brown, K. L.; Vanghn, C. *Inorg. Chem.* **1992**, *31*, 1552.
(21) Finke, R. G.; Hay, B. P. *Inorg. Chem.* **1985**, *24*, 1278.

(22) Factor B is a mixture of the diastereomeric cyanoaquocobinamides, α-CN-β-(H_2O)Cbi and α-H_2O-β-(CN)Cbi.[4]
(23) Brown, K. L.; Hakimi, J. M.; Nuss, D. M.; Montejano, Y. D.; Jacobsen, D. W. *Inorg. Chem.* **1984**, *23*, 1463.
(24) Renz, P. *Methods Enzymol.* **1971**, *18*, 82.

Samples for anaerobic solid-state pyrolysis were purified in a similar fashion. The α-NpCbi$^+$ or α-NpCbl solution collected from HPLC was immediately frozen in dry-ice/2-propanol, lyophilized to dryness, and purged with argon for 20 min in an ice-water bath before being pyrolyzed at 230 °C as described previously.[16b,23] α-NpCbl for ^{31}P NMR experiments was separated from the frozen reaction mixture by semi-preparative HPLC using NH_4OAc buffer as the aqueous solvent to avoid the large residual ^{31}P NMR peak due to phosphate buffer. The α-NpCbl solution collected was lyophilized to dryness at ~0 °C and was used immediately for the NMR experiment.

1-(2,2-Dimethylpropoxy)-2,2,6,6-tetramethyl-4-hydroxypiperidine (Np-HTEMPO).[25] Under a nitrogen atmosphere, neopentyl bromide (0.76 g, 5 mmol) was added to refluxing diethyl ether (35 mL) containing Mg (0.12 g, 5 mmol) over 30 min. The solution was then cooled in an ice-water bath, and HTEMPO[4] (0.43 g, 2.5 mmol) in 3 mL of diethyl ether was added to the Grignard reagent over 5 min. Stirring was continued for 10 min after the addition. At the end of the reaction, the ether solution was washed with 15% NH_4Cl (2 × 30 mL) and water (5 × 30 mL) and dried over anhydrous Na_2SO_4. After removal of the solvent under vacuum, Np-HTEMPO was crystallized as a white solid (98 mg, 16%). Analytical GC showed a single peak with a retention time of 11.4 min (R_t = 8.8 min for H-TEMPO; see Methods below) and over 98% purity. ^{1}H NMR in $CDCl_3$: δ (TMS), 0.95 (9H, s), 1.18 (6H, s), 1.19 (6H, s), 1.46 (2H, t), 1.77 (2H, q), 3.45 (2H, s), 3.93 (1H, m). GC-MS: m/e = 243 (M^+), m/e = 228 ($M^+ - CH_3$), m/e = 158 ($M^+ - C_5H_9O$).

***N*-(2,2-Dimethylpropylidene)benzenamine.** The Schiff's base of trimethylacetaldehyde (pivalaldehyde) with aniline was prepared as follows. Trimethylacetaldehyde (10 mg, 0.12 mmol) was cooled to 0 °C in an ice-water bath, and aniline (200 mg, 2.2 mmol) was added over 5 min. The reaction was warmed to room temperature and stirred for 3 h. At the end of the reaction, hexane (10 mL) was added. The reaction mixture was washed with water (6 × 15 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness to give 18 mg (93%) of the product. NMR ($CDCl_3$): δ 7.68 (s, 1H, neopentylidene), δ 7.32 (t, 2H, phenyl), δ 7.16 (t, 1H, phenyl), δ 7.00 (t, 2H, phenyl), 1.19 (s, 9H, *tert*-butyl). Analytical GC (see method below; R_t = 6.6 min) showed the product to be over 97% pure. MS: m/e = 161 (M^+), m/e = 146 ($M^+ - CH_3$), m/e = 104 ($M^+ - C_4H_9$), m/e = 77 ($M^+ - C_5H_{10}N$).

Methods. Solid-state pyrolysis products of neopentylcobalt corrinoids were identified by GC/MS[17] on a Finnigan 4500 GC/MS instrument equipped with a 6 in. × 2 mm ID Carbopak B/1% SP1000 column or on a Finnigan INCOS 500 GC/MS instrument with a Restek 60 m DB5 column. GC analysis was carried out on a Varian 3700 GC instrument with a Restek 30 m DB5 column and an FID detector with 50:1 sample splitting. The following temperature programs were used for GC analyses: for Np-HTEMPO, 1 min at 100 °C, increasing to 240 °C at a rate of 10 °C/min followed by 1 min at 240 °C; for *N*-(2,2-dimethylpropylidene)benzenamine, 1 min at 100 °C, increasing to 200 °C at a rate of 10 °C/min followed by 2 min at 200 °C. ^{1}H and ^{31}P NMR experiments were performed on a GE QE 300 NMR spectrometer. Chemical shifts were measured relative to external 85% phosphoric acid for ^{31}P and to internal TMS for ^{1}H. UV–visible spectroscopy and kinetic measurements were performed on a Cary 219 recording spectrophotometer equipped with a thermostated cell block and a Neslab circulating water bath (RTE-220) as described previously.[26] The temperature of the reaction solutions was measured inside a dummy cuvette with appropriate buffer solution, while the sample cuvettes were in the cell block, using an YSI 700 series thermistor and a signal conditioner, the combination of which had previously been calibrated against NBS calibrated thermometers. All cobamides were quantitated by conversion to their dicyano derivatives (ϵ = 3.04 × 10^4 M^{-1} cm^{-1}) by aerobic photolysis in excess potassium cyanide.[27] Electronic spectra of α-NpCbi$^+$ and α-NpCbl were recorded at 5.0 °C to minimize decomposition.

The kinetics of thermal decomposition of α-NpCbi$^+$ and α-NpCbl were monitored at 348.0 and 351.6 nm, respectively, in pH 7.0 potassium phosphate buffer (0.1 M) with ionic strength adjusted to 1.0 M with KCl. In a typical experiment, the thermolysis reaction was initiated by addition of a fresh, HPLC purified solution (300 μL) of α-NpCbi$^+$ or α-NpCbl that had been temperature-equilibrated in a water bath for 2 min to a thermostated buffer solution (2.7 mL) in a 3-mL quartz cuvette. The absorbance change was followed for at least 5 half-lives (>96% completion). For α-NpCbi$^+$, the kinetic data were fitted to a simple first-order equation by a curve-fitting routine using a simplex algorithm. For α-NpCbl, the situation was more complicated in that the initial fast thermolysis of the Co–C bond was followed by a slow secondary reaction. The rate of the second phase of the reaction, however, was at least 30 times slower, and the spectral changes for which were at least 10 times smaller than the primary thermolysis reaction (see Results below). The data for about 9 half-times of α-NpCbl thermolysis were thus fitted to an exponential equation with a linear term for the secondary reaction and in all cases gave good fits for the primary thermolysis reaction. Each rate constant reported here represents the average value of at least three runs.

Product Analyses for Thermolysis of α-Neopentylcobalt Corrinoids. For product analysis, anaerobic thermolysis of α-neopentylcobalt corrinoids was carried out under an argon atmosphere using HTEMPO as the radical scavenger as follows. HTEMPO (60 mM, 1 mL) in aqueous potassium phosphate buffer solution (0.6 M) was degassed with argon for 30 min in a serum-stoppered vial in an ice-water bath. HPLC purified α-NpCbl (~5 mL) was directly introduced into the HTEMPO solution from the HPLC column through a needle fitted to the end of the column outlet. The final concentration of HTEMPO was ~10 mM (ca. 160-fold excess over α-NpCbl). The mixture was further degassed for 30 min at ~0 °C. After the solution had been warmed up to room temperature, thermolysis was allowed to proceed for 120 min (ca. 10 half-times). A portion of the reaction solution (1 mL) was cannula-transferred to a serum-stoppered cuvette under argon pressure, and the electronic spectrum was recorded to determine the concentration of cob(II)alamin species. This solution was then air-oxidized and converted to $(CN)_2$Cbl by addition of excess KCN to determine the concentration of total corrinoids present, using 3.04 × 10^4 M^{-1} cm^{-1} as the molar absorbtivity of $(CN)_2$Cbl at 368 nm.[27] The extinction coefficient of cob(II)alamin at 470 nm (ϵ = 7.78 × 10^3 M^{-1} cm^{-1}) was determined by reduction of a H_2OCbl solution with formate as described previously.[19,28]

The remainder of the thermolysis reaction solution was extracted with pentane (5 × 8 mL), and the combined extracts were dried over anhydrous Na_2SO_4 and concentrated to dryness on a rotary evaporator. An appropriate volume of diethyl ether was added to dissolve the solid residue, and the solution was analyzed by gas chromatography. The molar amount of Np-HTEMPO was determined by area integration of the gas chromatographic peak and was compared to a calibration curve prepared previously using authentic Np-HTEMPO synthesized separately as described above. The identity of Np-HTEMPO extracted from the reaction solution was also examined by GC/MS analysis and was found to be the same as the synthetic material. No other organic products were detected in the ether extract (limit of detection, ~2%). Analyses of the thermolysis products of α-NpCbi$^+$ were carried out similarly.

Aerobic thermolysis of α-neopentylcobalt corrinoids is expected to produce pivalaldehyde and neopentanol.[7,11] It proved to be technically difficult, however, to quantitate the small amount of the organic products (~5 × 10^{-7} mol) that formed from aerobic thermolysis. Consequently, pivalaldehyde was derivatized with excess aniline in the presence of molecular sieves (4 Å, as drying agent), and the Schiff's base product was quantitated by gas chromatography. The following procedure applies to product analysis for both α-NpCbl and α-NpCbi$^+$. A fresh, HPLC purified aqueous solution of α-neopentylcobalt corrinoid (~5 × 10^{-7} mol in ca. 5 mL) was introduced to a potassium phosphate buffer solution (0.5 M, pH 7.0) in a serum-stoppered vial and was allowed to stand at room temperature for 4 h. At the end of the thermolysis reaction, the solution was extracted with diethyl ether (5 × 0.5 mL). Aniline (10 mg, 0.11 mmol) and molecular sieves (4 Å) were added to the ether solution. After being stirred at room temperature for 5 h, the volume of the solution was reduced to about 0.2 mL. The quantity of *N*-(2,2-dimethylpropylidene)benzenamine was determined by GC peak integrations and compared with a standard. The identity of the Schiff's base separated from the thermolysis reaction was also confirmed by GC/MS analysis, and it was found to be identical to authentic material prepared as described above. The cobalt corrinoid product was converted to dicyanocobalt corrinoid by addition of KCN to determine the total amount of starting α-neopentylcobalt corrinoid. The yield of pivalaldehyde from the thermolysis was derived from the molar ratio of the Schiff's base to the cobalt corrinoid.

Results

Synthesis and Characterization of α-Neopentylcobalt Corrinoids. Analysis of the reaction mixture resulting from reductive

(25) Waddington, M. D.; Finke, R. G. *J. Am. Chem. Soc.*, in press.
(26) Marques, H. M.; Brown, K. L.; Jacobsen, D. W. *J. Biol. Chem.* **1988**, *263*, 12378.
(27) Barker, H. A.; Smyth, R. D.; Weissbach, H.; Toohey, J. I.; Ladd, J. N.; Volcani, B. E. *J. Biol. Chem.* **1960**, *235*, 480.
(28) Linn, D. E., Jr.; Gould, E. S. *Inorg. Chem.* **1988**, *27*, 1625.

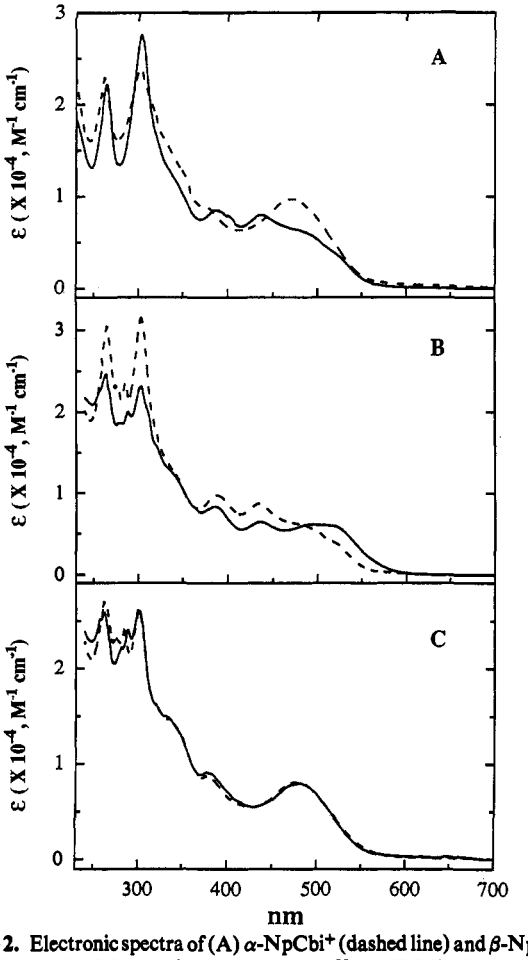


Figure 2. Electronic spectra of (A) α-NpCbi⁺ (dashed line) and β-NpCbi⁺ (solid line) in 0.1 M potassium phosphate buffer (pH 7.0), (B) β-NpCbl, solid line, in 0.1 M potassium phosphate buffer (pH 7.0), and dashed line, base-off in 0.5 M HCl, and (C) α-NpCbl, solid line, in 0.1 M potassium phosphate buffer (pH 7.0), and dashed line, in 0.5 M HCl. All spectra were recorded at 5.0 °C.

alkylation of factor B[22] with neopentyl bromide at ~0 °C showed two photolabile alkylcorrinoids in the HPLC chromatogram. One of these compounds was identical to the previously reported[7] β-NpCbi⁺ by its UV–visible spectrum, HPLC co-injection with authentic β-NpCbi⁺ prepared by the reported method,[7] and GC-MS analysis of its solid-state pyrolysis product (neopentane). The second compound, which eluted slightly earlier than β-NpCbi⁺, was readily identified as the previously unknown α-NpCbi⁺ on the following bases: (1) GC-MS analysis of its anaerobic solid-state pyrolysis gave only neopentane, the same as that from β-NpCbi⁺.[7] (2) Like other known α-alkylcobalt corrinoids,[17,20] anaerobic photolysis in solution converted this new compound to a mixture of α- and β-NpCbi⁺. (3) The electronic spectrum of the new compound showed a diagnostic red shift[16] of the longest wavelength visible (or α) band (λ ($\log_{10}\epsilon$): 471 (3.99), 374 (3.96, sh), 302 (4.37), 262 (4.36)) compared to that of β-NpCbi⁺ (Figure 2A). (4) The UV–visible spectral relationships between α- and β-NpCbi⁺ and α- and β-NpCbl (see below) are the same as those previously reported for other pairs of α- and β-RCbi's and α- and β-RCbl's (vide infra).[16] It should be pointed out that α-NpCbi⁺ is extremely thermally labile. Great care must be taken to keep reaction solutions, HPLC columns, and solvents well below ambient temperature in order to be able to prepare the compound. Consistent with our earlier report,[7] several preparations at room temperature produced less than 5% α-NpCbi⁺ (due to its thermal decomposition), which had been apparently overlooked as an impurity in the earlier work. In three separate preparations under optimized conditions (see Experimental Section), the average yield for α-NpCbi⁺ was 30% by HPLC. The electronic spectrum of α-NpCbi⁺ is shown in Figure 2A, along with a spectrum of β-NpCbi⁺ for comparison.

We have previously shown[19] that reductive alkylation of H_2OCbl produces both the β-RCbl and its α diastereomer at pH's sufficiently below the base-on/base-off pK_a of the β-RCbl product. Using the same strategy, reduction of H_2OCbl with zinc in 5% H_3PO_4 (pH = 1.2) and alkylation with neopentyl bromide also produced two alkylcobalt corrinoids. One of these compounds was the previously known β-NpCbl.[2,7] The new material, which had slightly shorter retention time than β-NpCbl in the HPLC chromatogram, did not undergo the typical base-on/base-off UV-visible transition in neutral and acidic solutions (Figure 2C), instead, showing only minor spectral changes in the UV region due to protonation of the pendent, but uncoordinated, benzimidazole nucleotide.[16] This compound had essentially the same electronic spectrum (λ ($\log_{10}\epsilon$): 473 (3.90), 376 (3.96, sh), 302 (4.36)) above 300 nm as that of the compound assigned as α-NpCbi⁺. In addition, anaerobic solid-state pyrolysis of this new species gave neopentane as the only detectable product, and it could be converted by anaerobic photolysis or thermolysis to β-NpCbl in neutral solution, as is common for other α-RCbl's.[15,16] We thus conclude that this new species is the elusive α-NpCbl. Again, this material could only be obtained if the alkylation and all the purification steps were carried out at near 0 °C.

Reductive alkylation of factor B with longer reaction times produced only the β diastereomer, apparently due to the differential rates of thermal decomposition (and possibly also to the differential rates of reductive dealkylation by zinc[16c,19,29-34]) of the two diastereomers. At ~0 °C, α-NpCbi⁺ was the predominant alkylation product (30%) when $NaBH_4$ was used as reductant. However, no α-NpCbl was detectable when $NaBH_4$ was the reductant as was previously shown to be the case for other RCbl's.[16]

In a recent study of the thermolysis of β-NpCbl in ethylene glycol,[25] Waddington and Finke found that the use of commercial neopentyl iodide for the synthesis of NpCbl unavoidably led to formation of CH_3Cbl due to contamination of the commercial reagent with CH_3I. The problem is avoided by using NpBr as the alkylating agent since this reagent contains no CH_3Br (bp = 4 °C) and produces no methylcobalt corrinoids.

Anaerobic Photolysis and Thermolysis of α-Neopentylcobalt Corrinoids. We have previously demonstrated that anaerobic photolysis starting from either α- or β-RCbi's affords a mixture of the two diastereomers, which contains about 25–30% of the α diastereomer.[17,20] Anaerobic photolysis starting from either β-NpCbi⁺ or α-NpCbi⁺ also gave a mixture of the two diastereomers, but in this case only 10–15% α-NpCbi⁺ was obtained. Interestingly, α-NpCbi⁺ seems to be more stable toward photolysis, compared to other α-RCbi's, although it is much more thermally labile. Prolonged irradiation (>1 h) was required to induce the interconversion at the same level of irradiation (3V tungsten lamp) that previously was found to induce the more rapid interconversion of other α- and β-RCba's.[17,20] Similarly, α-NpCbl and β-NpCbl could also be interconverted photolytically in acidic anaerobic solution where the axial nucleotide is protonated. At the photolytic stationary state, approximately 5–10% α-NpCbl was obtained. Unfortunately, the exact stationary-state positions for these photolytic interconversions of the α-NpCbi's and the α-NpCbl's could not be determined because

(29) Robinson, K. A.; Itabashi, E.; Mark, H. B. *Inorg. Chem.* **1982**, *21*, 3571.
(30) Kim, M.-H.; Birke, R. L. *J. Electroanal. Chem. Interfacial Electrochem.* **1983**, *144*, 331.
(31) (a) Lexa, D.; Savéant, J. M. *Acc. Chem. Res.* **1983**, *6*, 235. (b) Lexa, D.; Savéant, J. M. *J. Am. Chem. Soc.* **1970**, *100*, 3220.
(32) Zhou, D.-L.; Tinembart, O.; Scheffold, R.; Walder, L. *Helv. Chim. Acta* **1990**, *73*, 2225.
(33) Shepherd, R. E.; Zhang, S.; Dowd, P.; Choi, G.; Wilk, B.; Choi, S.-C. *Inorg. Chim. Acta* **1990**, *174*, 249.
(34) Brown, K. L.; Zou, X.; Richardson, M.; Henry, W. P. *Inorg. Chem.* **1991**, *30*, 4834.

Table I. Rate Constants for the Thermal Homolysis of α-Neopentylcobalt Corrinoids

	k_{obs}, 10^4 s^{-1} [a]	
T, °C	α-NpCbi$^+$	α-NpCbl
30.1	35.3 ± 1.4	18.9 ± 0.1
25.0	19.4 ± 0.3	10.3 ± 0.4
24.9[b]	18.8 ± 0.6	9.8 ± 0.2
20.0	9.61 ± 0.11	5.03 ± 0.25
14.9	5.28 ± 0.32	2.57 ± 0.05
10.0	2.50 ± 0.08	1.27 ± 0.05
5.0	1.34 ± 0.09	0.60 ± 0.01

[a] In 0.1 M potassium phosphate buffer, ionic strength 1 M (KCl) with 3% acetonitrile. [b] Anaerobic thermolysis in potassium phosphate buffer (0.1 M, pH 7.0) with HTEMPO (10 mM).

of the thermal labilities of these complexes. The anaerobic photolytic process could not be cleanly separated from simultaneous untrapped thermolysis in the time domain required to induce a complete reaction under the conditions used since the two processes occur on the same time scale.

As is the case with other α-alkylcobalamins,[17,20] α-NpCbl was converted completely to β-NpCbl upon anaerobic thermolysis at ambient temperature and neutral pH where the latter is base-on. Anaerobic thermolysis of α-NpCbi$^+$ at ambient temperature but in the absence of a radical trap also converted this material to its β diastereomer, but the rate ($t_{1/2} > 3$ h) was extremely slow compared to that of a trapped thermolysis ($t_{1/2} \sim 6$ min, Table I). It has been recently shown that the cage efficiency for a radical pair in an alkylcorrinoid system can be very high ($F_c \geq 0.94$).[35] The slow anaerobic thermal conversion from α-NpCbi$^+$ to β-NpCbi$^+$ compared to that of the trapped thermal decomposition of α-NpCbi$^+$ may be a cage effect since the trap prevents the recombination of the caged radical pair. The implication of this finding is currently under further investigation.

Product Analyses for Thermolysis of α-Neopentylcobalt Corrinoids. In order to confirm that the thermolysis of α-NpCbl follows a homolytic cleavage pathway as does that of β-NpCbl, the products of anaerobic thermolysis of α-NpCbl in the presence of HTEMPO (10 mM) were analyzed by spectrophotometric and gas chromatographic methods. Finke and co-workers[25] have studied the dependence of the yield of the trapped alkyl radical on nitroxide trap concentration in ethylene glycol and found that as little as 1 mM of TEMPO was adequate to trap 98% of the free neopentyl radical in β-NpCbl thermolysis and that 10 mM TEMPO trapped 100% of the alkyl radical. This is likely to be true for HTEMPO as well, and so 10 mM of HTEMPO in a reaction solution should be sufficient to trap the free alkyl radical formed from α-NpCbl thermolysis.

As expected for thermal Co–C bond homolysis, anaerobic thermolysis of α-NpCbl in the presence of 10 mM HTEMPO produced nearly quantitative yields of Np-HTEMPO (92 ± 6%) as analyzed by gas chromatography. Furthermore, cob(II)alamin was produced in 102 ± 2% yield by spectrophotometric determination. Similar experiments were also performed for α-NpCbi$^+$. However, because of the thermal lability of α-NpCbi$^+$, the amount of Np-HTEMPO being trapped by this method (see Experimental Section) may be expected to be somewhat depressed due to decomposition of α-NpCbi$^+$ during the latter stage of the HPLC purification process and transfer to the reaction solution. In this case, the yields of Np-HTEMPO and cob(II)inamide were 70 ± 3% and 75 ± 1%, respectively, the other 25–30% being cob(III)inamide, formed by unavoidable premature aerobic decomposition prior to establishment of the anaerobic condition, as evidence by a peak at ca. 350 nm (due to contaminating cob(III)inamide) in the cob(II)inamide spectrophotometric quantitation. Nevertheless, the nearly quantitative yields of cob(II)alamin and Np-HTEMPO for α-NpCbl thermolysis, and the close to 75% and nearly 70% yields of cob(II)inamide and Np-HTEMPO for α-NpCbi$^+$ thermolysis, respectively, strongly suggest that homolytic cleavage is the predominant mechanism for the α Co–C bond thermolysis, as is the case for the β diastereomers.[7,8c]

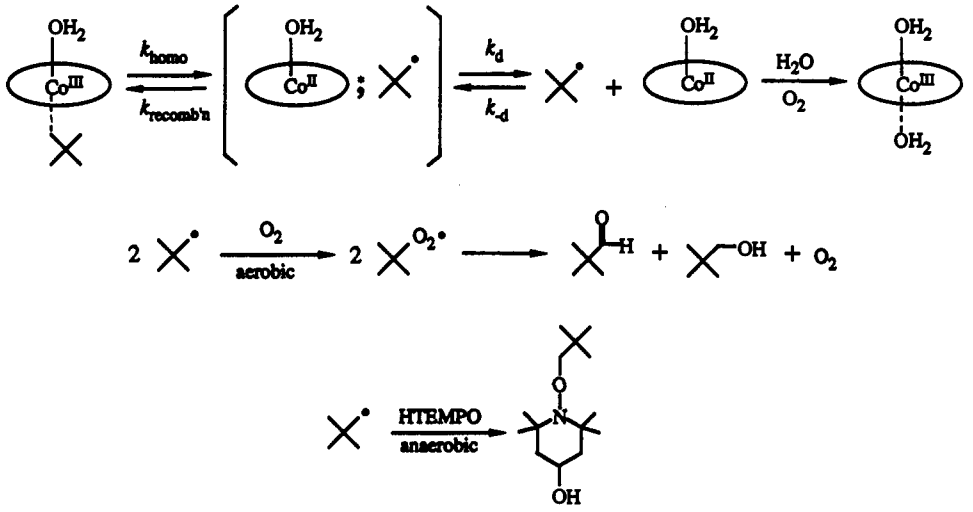
Product analysis was also attempted for aerobic thermolysis of the α-neopentylcobalt corrinoids. In this case, the neopentyl radical is trapped by O_2 to form the neopentylperoxy radical that subsequently decomposes to pivalaldehyde and neopentanol (Scheme I).[7,11] To identify and quantitate these products, we attempted direct analysis by gas chromatography but failed, due to technical difficulties in separating the pivalaldehyde (bp 74 °C) and neopentanol from the solvent, given the small quantities of products involved. Consequently, pivalaldehyde was derivatized to form its Schiff's base with aniline in the presence of molecular sieves (4 Å) as a drying agent. The Schiff's base is stable in organic solvents and can be easily quantitated by gas chromatography. By this method, the yields of pivalaldehyde were found to be 53 ± 6% for α-NpCbl and 59 ± 3% for α-NpCbi$^+$ thermolysis, in substantial agreement with the 50% yield predicted from trapping of the neopentyl radical by O_2 (Scheme I). The Schiff's base product from thermolysis of α-neopentylcobalt corrinoids was also analyzed by GC/MS and was found to be identical to synthetic material. Both product analyses show that Scheme I is the apparent mechanism, i.e., a homolytic Co–C bond cleavage leading to a neopentyl radical, for the thermal decomposition of α-neopentylcobalt corrinoids. The organic free radical product (neopentane free radical) from Co–C bond homolysis is either trapped by HTEMPO (anaerobic) or O_2 (aerobic) after escaping from the solvent cage.

Thermal Homolysis of α-NpCbi$^+$. Repetitive UV–visible scans of an aerobic solution of α-NpCbi$^+$ (5×10^{-5} M) in potassium phosphate buffer (pH 7.0, 0.1 M) showed first-order spectral changes with isosbestic points at 482, 368, and 333 nm (Figure 3). The kinetics of this reaction were conveniently followed spectrophotometrically at 348 nm where the largest spectral change occurred. Rate constants for the thermolysis were determined at temperatures ranging from 5 to 30 °C at approximately 5 °C intervals. Above 30 °C, the reaction was too fast to reestablish the target temperature rapidly after addition of the α-NpCbi$^+$ stock solution to provide accurate rate measurements. The rate constants thus obtained are collected in Table I, and an Eyring plot of these data is shown in Figure 4. The enthalpy and entropy of activation derived from the Eyring plot are 21.4 ± 0.4 kcal mol^{-1} and 0.9 ± 1.3 cal mol^{-1} K^{-1} (Table II), respectively. Anaerobic thermolysis of α-NpCbi$^+$ in the presence of 10 mM HTEMPO at 24.9 °C showed that the rates of thermolysis were almost identical (Table I), using HTEMPO (anaerobic) or O_2 (aerobic) as the radical scavenger.

Thermal Homolysis of α-NpCbl. Aerobic thermolysis of α-NpCbl was studied at pH 7.0 in potassium phosphate buffer. Repetitive scans of an α-NpCbl solution showed spectral changes consistent with loss of the alkyl group, forming a cobalt(III) corrinoid (i.e., after air oxidation of the cob(II)alamin product). However, the thermolysis was somewhat more complicated than that of α-NpCbi$^+$, and slow secondary spectral changes, though small, were observed toward the end of the reaction. These slow spectral changes were accompanied by a slow γ band shift, after the primary thermolysis, from 356 to 358 nm with an isosbestic point at about 351.5 nm. The final spectrum was that of H_2OCbl. We suspected that this slower reaction represented the formation of the base-on species of H_2OCbl from the base-off cob(III)alamin species formed after rapid air oxidation of the base-off cob(II)alamin product (Scheme II). In α-NpCbl, the alkyl group prevents the looped nucleotide from coordinating to the cobalt. After air oxidation of the initial cob(II)alamin

(35) Garr, C. D.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 10440.

Scheme I



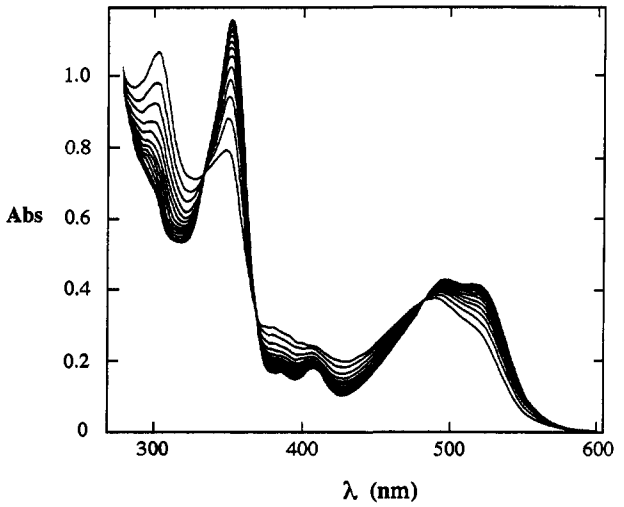


Figure 3. Repetitive spectral scans (every 4 min) of the aerobic thermolysis of α-NpCbi$^+$ in potassium phosphate buffer (pH 7.0) at 25 °C. The last two scans were at 12-min intervals.

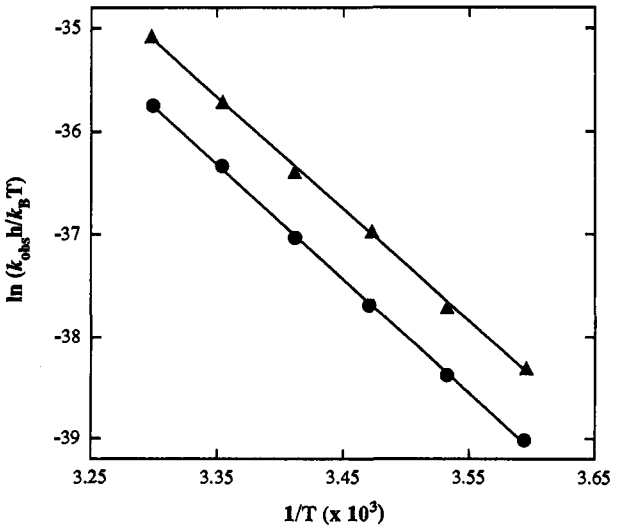


Figure 4. Eyring plots for the thermolysis of α-neopentylcobalt corrinoids ($\ln(k_{obs}h/k_BT)$ versus $1/T$, where h is Planck's constant and k_B is Boltzmann's constant): (●) α-NpCbl; (▲) α-NpCbi$^+$.

thermolysis product, formation of the base-on species of H_2OCbl is strongly thermodynamically favored ($\Delta G° = -10.5$ kcal mol^{-1}).[36] While formation of the base-on species from the free base, base-off species of β-RCbl's (and β-CNCbl) is fast,[36] Hayward et al.[37] reported from spectrophotometric observations in H_2SO_4/H_2O mixtures that the equilibration of the base-on and base-off forms of H_2OCbl is achieved "only slowly". Similarly, one of us[36] also observed this slow equilibration process for base-on and base-off H_2OCbl by ^{31}P NMR. As ligand substitutions at cobalt corrinoids are known to be dissociatively dominated,[26,38-40] the slow dissociation of water from the α-position of base-off H_2OCbl is the rate-limiting step.

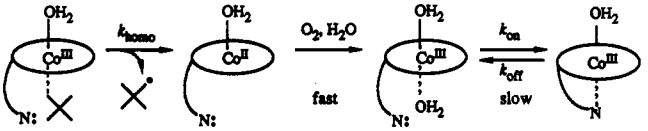
In order to tell if slow formation of base-on H_2OCbl might be the cause of the secondary spectral changes complicating the aerobic thermolysis of α-NpCbl, the spectrum of H_2OCbl was

Table II. Enthalpy and Entropy of Activation for Thermolysis of Neopentylcobalt Corrinoids

compd	$\Delta H^‡$, kcal mol^{-1}	$\Delta S^‡$, cal mol^{-1} K^{-1}	ref
α-NpCbl	22.5 ± 0.2[a]	3.1 ± 0.1[a]	this work
β-NpCbl, base-off	30.0 ± 0.8	11.5 ± 2.6	7
β-NpCbl, base-on[b]	28 ± 1[a]	21 ± 1[a]	7
	26.7 ± 1.2[c]	15 ± 5[c]	8c
	23.4 ± 0.2[d,e]	2.6 ± 0.1[d,e]	2a
	33 ± 2[f]	35 ± 10[f]	8c
	32.2 ± 0.6[f]	33 ± 2[f]	25
α-NpCbi$^+$	21.4 ± 0.4[a]	0.9 ± 1.3[a]	this work
β-NpCbi$^+$	29.7 ± 1.3[a]	10.8 ± 3.9[a]	7
	32.1 ± 0.1[d]	17.3 ± 0.4[d]	2a

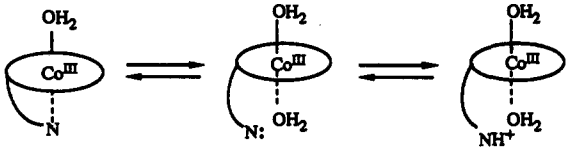
[a] In 0.1 M potassium phosphate buffer, pH 7.0, ionic strength 1 M (KCl). [b] Corrected values for base-on β-NpCbl unless otherwise indicated. [c] In potassium phosphate buffer, pH 6.8. [d] In sodium phosphate buffer, pH 7.0. [e] Not corrected for base-on/base-off equilibrium. [f] In ethylene glycol.

Scheme II



(36) (a) Brown, K. L.; Hakimi, J. M.; Jacobsen, D. W. *J. Am. Chem. Soc.* **1984**, *106*, 7894. (b) Brown, K. L.; Hakimi, J. M. *Inorg. Chem.* **1984**, *23*, 1756.
(37) Hayward, G. C.; Hill, H. A. O.; Pratt, J. M.; Vanston, N. J.; Williams, R. J. P. *J. Chem. Soc.* **1965**, 6485.
(38) Reenstra, W. W.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 5780.
(39) Randall, W. C.; Alberty, R. A. *Biochemistry* **1966**, *5*, 3189.
(40) (a) Marques, H. M.; Bradley, J. C.; Campbell, L. A. *J. Chem. Soc., Dalton Trans.* **1992**, 2019. (b) Marques, H. M.; Breet, E. L. J.; Prinsloo, F. F. *J. Chem. Soc., Dalton Trans.* **1991**, 2941. (c) Marques, H. M. *J. Chem. Soc., Dalton Trans.* **1991**, 304. (d) Marques, H. M.; Mgan, T. J.; Marsh, J. H.; Munro, O. Q. *Inorg. Chim. Acta* **1989**, 166, 249. (e) Bradley, J. C.; Marques, H. M.; Brown, K. L.; Brooks, H. B. Manuscript submitted to *J. Chem. Soc., Dalton Trans.*

recorded as a function of time in 6.5 M aqueous H_2SO_4, in which solution it is *ca.* 90% base-off at equilibrium.[36] The reaction being spectrally interrogated is thus the base-on to base-off transition of H_2OCbl (eq 1), in which the first step, formation



(1)

of the base-off species, is rate limiting. This step is the reverse of the final reaction in Scheme II in which the base-off H_2OCbl product, formed by rapid air oxidation of the direct homolysis product, base-off cob(II)alamin, is converted to the base-on species. In this H_2SO_4 solution, slow spectral changes were observed which were similar to, but in the opposite direction of, the slow spectral change following the aerobic decomposition of α-NpCbl in neutral aqueous solution. In addition, when α-NpCbl was thermolyzed anaerobically in the presence of HTEMPO at 25 °C, strictly first-order kinetics were observed and there was no slow secondary spectral change. This is expected since, in anaerobic solution, cob(II)alamin persists, and its base-off to base-on conversion ($pK_{base\text{-}off} = 3.10$)[19] is expected to be extremely fast.

Since base-on and base-off H_2OCbl's have different ^{31}P NMR chemical shifts (0.14 and –0.42 ppm, respectively),[36] the base-on/base-off transition of H_2OCbl can also be monitored by ^{31}P NMR. Thus, ^{31}P NMR studies of seven known base-off alkylcobalt corrinoids in D_2O, including α-RCbl's (R = Ado, CH_3CH_2, CH_3, CF_3, CF_3CH_2), α- and β-CF_3CH_2-(trimethylbenzimidazolyl)cobamides,[41] and $(CN)_2Cbl$, showed that all such base-off species have chemical shifts ($\delta = -0.47 \pm 0.16$ ppm) similar to that of base-off H_2OCbl and other protonated, base-off β-RCbl's that have been studied ($\delta = -0.45 \pm 0.03$ ppm).[36] Consistent with these values, α-NpCbl has a ^{31}P chemical shift at –0.48 ppm (at ~5 °C). The chemical shift of base-on H_2OCbl, on the other hand, was found to vary from 0.07 to 0.14 ppm, depending on the concentration (1.5–20 mM). In order to obtain ^{31}P spectra of freshly purified α-NpCbl, crude α-NpCbl was purified by HPLC using ammonium acetate as the solute in the aqueous solvent (see Experimental Section) instead of the usual ammonium phosphate buffer to avoid the occurrence of a large inorganic phosphate resonance in the ^{31}P spectra. In the same ammonium acetate containing solution, the ^{31}P chemical shift of H_2OCbl was found to be –0.03 ppm, presumably due to formation of β-NH_3Cbl. At 25 °C, the ^{31}P NMR spectrum of a fresh sample of α-NpCbl (1.4 mM, in *ca.* 0.2 M NH_4OAc), obtained as soon as possible after make up (*ca.* 7 min), showed three peaks attributable to α-NpCbl ($\delta = -0.48$ ppm), unavoidable contamination with H_2OCbl ($\delta = 0.07$ ppm), and the β-NH_3Cbl ($\delta = -0.03$ ppm) formed from the contaminating H_2OCbl (Figure 5A).[42] When the thermolysis of the starting α-NpCbl ($t_{1/2}$ = 11.2 min; vide infra) was ~81% complete (27 min, Figure 5B), a large resonance[43] persisted at –0.48 ppm that must be attributed to base-off H_2OCbl, i.e., the air oxidation product of the immediate product of Co–C homolysis, base-off cob(II)alamin. This resonance disappeared only *slowly*, with a half-time of 385 min, with the concomitant growth of the resonance at –0.03 ppm, due to β-NH_3Cbl (formed from the final H_2OCbl product in 0.2 M NH_4OAc). This half-time agrees well with a value of 362 min obtained from kinetic analysis of the slow UV–visible spectral change during aerobic thermolysis of α-NpCbl (vide infra). Taken together with the fact that the slow secondary spectral changes were absent when α-AdoCbl was decomposed anaerobically with HTEMPO (since ligand substitution in cob(II)alamin must be fast), these experiments satisfactorily demonstrate that the reaction observed in aqueous solutions of α-NpCbl is adequately described by Scheme II.

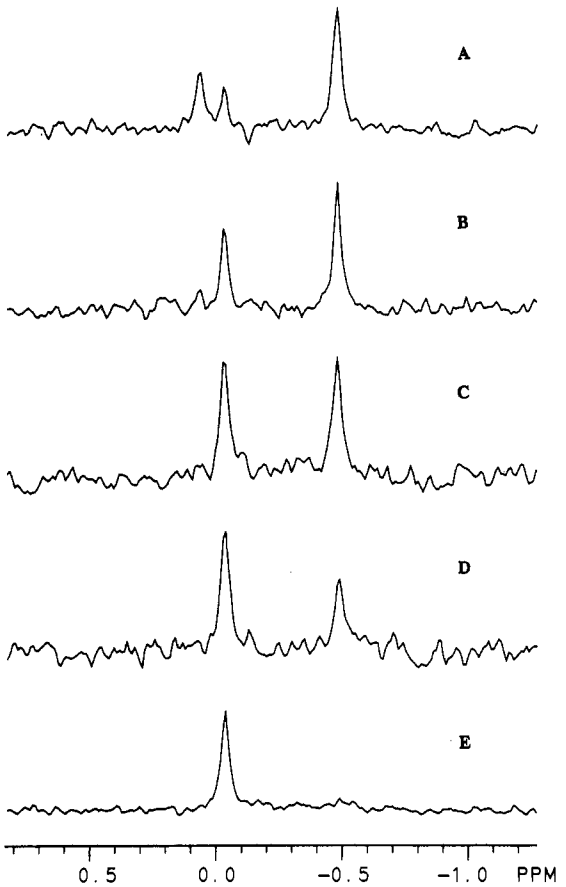


Figure 5. ^{31}P NMR spectra of α-NpCbl (1.5 mM) in ~0.2 M NH_4OAc at 25 °C: (A) 7 min; (B) 27 min; (C) 72 min; (D) 207 min; (E) 1068 min. The upfield-most peaks are due to α-NpCbl (A) and base-off H_2OCbl (C, D), as all base-off cobamides studied to date have ^{31}P chemical shifts of -0.46 ± 0.16 ppm. The peak at 0.07 ppm (A) is due to base-on H_2OCbl, and the peak at –0.03 ppm is due to NH_3Cbl, unavoidably formed from H_2OCbl in the NH_4OAc containing solvent (see text).

Although the absorbance change due to the slow formation of the base-on species was noticeable at the end of the thermolysis of α-NpCbl, its effect on the analysis of the kinetics is minimal since the base-on/base-off reaction was at least 30-fold slower than that of the primary thermolysis reaction, and the spectral change associated with formation of base-on/base-off reaction was much smaller than that of the thermolysis. Furthermore, the kinetic data for thermolysis of α-NpCbl were collected at 351.6 nm, near the isosbestic point of the slower reaction. At this

(41) Brown, K. L.; Wu, G. Z. *Organometallics* **1993**, *12*, 496.

(42) In the first obtainable ^{31}P spectrum of α-NpCbl (Figure 5A), the two peaks at 0.07 and –0.03 ppm are attributed to contaminating H_2OCbl and β-NH_3Cbl. The peak at 0.07 ppm is due to H_2OCbl, as confirmed by observation of an authentic sample of H_2OCbl in D_2O at the same concentration. The second peak at –0.03 ppm was shown to be base-on β-NH_3Cbl by control experiments in which samples of H_2OCbl (1.5 mM) were dissolved in NH_4OAc and NaOAc solutions (both 0.2 M). In NaOAc, H_2OCbl has a chemical shift of 0.07 ppm (i.e., the same shift it has in D_2O), while in NH_4OAc, the chemical shift was –0.03 ppm. Evidently, the exchange between NH_3 and H_2O in the inert cobalt complex was slow enough for H_2OCbl to appear in the first spectrum that was recorded at 7 min after making up the sample. The base-on H_2OCbl peak at 0.07 ppm disappeared in the second spectrum (Figure 5B), taken at 27 min, because all H_2OCbl had been converted to NH_3Cbl. The sample was prepared from a lyophilized solution of α-NpCbl by HPLC separation. A preparation of the scale necessary for ^{31}P NMR normally would take a minimum of 3–4 h. Apparently, about 25% of the α-NpCbl had decomposed by the time the sample was ready, in spite of the care taken to keep the purified solution cold at all times.

(43) α-NpCbl and base-off H_2OCbl (the intermediate of the faster thermolysis reaction) have the same ^{31}P chemical shift at –0.48 ppm. This is as expected as all base-off β-RCbl's and α-RCbl's have essentially the same ^{31}P chemical shift.

wavelength, the overall spectral change for the secondary reaction was at least 10 times smaller than that for the thermolysis reaction, further minimizing the influence of the secondary reaction. Consequently, the kinetic data for α-NpCbl thermolysis were successfully fitted to an equation consisting of an exponential term for the primary thermolysis reaction and a linear term for the slower secondary reaction as shown in eq 2, restricting the

$$A = C_1(1 - \exp(-k_{obs}t)) + C_2t + C_3 \quad (2)$$

data set to about 9 half-times of the faster kinetic phase. The observed rate constants thus obtained for α-NpCbl thermolysis are collected in Table I. An Eyring plot from these data is shown in Figure 4, and the activation parameters from the Eyring plot are given in Table II. The rate constants are smaller at all temperatures than those for α-NpCbi$^+$. A rate constant for anaerobic thermolysis of α-NpCbl in the presence of HTEMPO is also given in Table I for comparison.

In order to permit evaluation of the rate constant for the slow secondary spectral change and comparison to the results of the ^{31}P NMR experiment discussed above, the UV–visible spectral changes for thermolysis of α-NpCbl were also followed to completion at 358 nm, 25 °C, for both phases of the reaction. In this case, a double exponential equation was used to obtain rate constants for both phases of the reaction as shown in eq 3, where

$$A = C_1(1 - \exp(-k_1t)) + C_2(1 - \exp(-k_2t)) + C_3 \quad (3)$$

k_1 is the rate constant for the faster thermolysis reaction and k_2 is for the slower base-on reaction. The rate constant, k_1, thus obtained from eq 3 agreed well with those for the primary thermolysis reaction obtained by fitting truncated data sets to eq 2, and the value of k_2 agreed well with that obtained from the ^{31}P NMR experiment as discussed above.

Figure 6S (available as supplemental material) shows such a set of kinetic data for the thermolysis of α-NpCbl that was followed to completion for both the thermolysis and the slow base-on reaction at 358 nm. These data were fitted, respectively, to a double exponential equation (eq 3), to a single exponential equation for the entire data set, and to eq 2 for the first 110 min. These comparisons clearly show that eq 2 can give accurate rate constant for the thermolysis reaction, and the effect of the secondary reaction on the thermolysis is minimal.

Discussion

We have reported previously,[7] as have other groups,[2a,8c] that reductive alkylation of factor B or H_2OCbl with neopentyl halides produces only a single alkylcobalt corrinoid, β-NpCbi$^+$ or β-NpCbl, respectively. The current study shows, however, that substantial amounts of α-NpCbi$^+$ and α-NpCbl can be produced by reductive alkylation with NpBr below ambient temperature. Apparently, the failure to observe the formation of α diastereomers in earlier studies is due to the extreme lability of the α-neopentylcobalt corrinoids. Normal workup procedures at room temperature would have clearly lead to the destruction of these elusive complexes since they decompose rapidly (Table I).

When we started this project, our initial plan was to attempt the preparation of α-NpCbi$^+$ by anaerobic photolysis of β-NpCbi$^+$, a method that has been successfully used to prepare α-alkylcobalt corrinoids that were only minor products or were unobtainable by reductive alkylation.[20] As expected, anaerobic photolysis of β-NpCbi$^+$ produced α-NpCbi$^+$ in 10–15% yield, by HPLC. It quickly became clear to us, however, that the newly formed α-NpCbi$^+$ was quite unstable at room temperature and that workup and HPLC procedures needed to be carried out at ~0 °C to prevent massive decomposition. This finding prompted us to reexamine the reductive alkylation reactions of factor B and H_2OCbl with NpBr. Indeed, when the reaction and purification steps were carried out at lower temperatures (~0 °C), both α-NpCbi$^+$ and α-NpCbl were positively identified in and separated from reaction mixtures resulting from the reductive alkylation of factor B and H_2OCbl, respectively, with NpBr. In fact, more α diastereomer was produced than β diastereomer in both cases under appropriate conditions (see Experimental Section). Reductive alkylation has been consequently used throughout this study for the preparation of α-neopentylcobalt corrinoids because of the high yields and overall convenience of the preparations. However, due to the lability of the α-neopentylcobalt corrinoids, purification immediately before use was necessary in order to provide samples of sufficient purity (>95%).

The high yields of α-neopentylcobalt corrinoids by reductive alkylation and the recent synthesis of α-adenosylcobalt corrinoids[18] seem to suggest that the stereochemical outcome of the reaction, i.e., the ratio of α- and β-alkylcobalt corrinoids, does not depend on the size of the bulkiness of the organic ligand involved. Understanding the factors that control the stereochemistry of reductive alkylation reactions remains an important objective that is under study in our laboratory.

Unlike β-AdoCbl and β-AdoChi,[13b] in which Co–C bond heterolysis competes with homolysis due to the presence of the β oxygen atom in the organic ligand, the mechanism of thermolysis of β-NpCbl and β-NpCbi$^+$ has been firmly established to be strictly Co–C bond homolysis.[7,8c] Co–C bond fission by heterolysis is not likely to compete with the homolysis because of the lack of a β heteroatom in the neopentyl ligand. In addition, the lack of any β hydrogens in this ligand also precludes any possible β elimination reactions. The activation enthalpies (ΔH^*) and entropies (ΔS^*) for the thermolysis of base-on β-NpCbl and β-NpCbi$^+$ in water have been reported,[2a,7,8c] as well as values in other solvents.[8c,25] Thus, the thermolysis of β-neopentylcobalt corrinoids is well studied and documented, although there are some discrepancies in the reported activation parameters (Table II).

Thermolyses of α-NpCbl and α-NpCbi$^+$ are expected to follow the same mechanism, i.e., Co–C bond homolysis. However, there is no prior study in the literature on the mechanism of an α Co–C bond cleavage. Evidence to support the homolytic mechanism in the Co–C bond cleavage in the α-neopentylcobalt corrinoids was thus sought by anaerobic thermolysis of α-NpCbl (and α-NpCbi$^+$) in the presence of a nitroxide free radical trap, HTEMPO. After completion of the thermolysis at 25 °C (~120 min, >10 half times), the only product detectable by GC/MS in the pentane extract of the reaction mixture was Np-HTEMPO as identified by comparison of its mass spectrum to that of independently synthesized authentic Np-HTEMPO. This indicates that the neopentyl ligand formed a free radical during the thermolysis. By GC quantitation, the yield of Np-HTEMPO was found to be 92 ± 6% for α-NpCbl and 70 ± 3% for α-NpCbi$^+$. In addition, the corrinoid after HTEMPO-trapped anaerobic thermolysis was spectrally identical to cob(II)alamin (or cob(II)inamide) with an absorbance maximum at 470 nm,[44] and the yields of the cobalt(II) corrinoids were found to be 102 ± 2% and 78 ± 1% for α-NpCbl and α-NpCbi$^+$, respectively. The reduction of the yields of Np-HTEMPO and cob(II)inamide for α-NpCbi$^+$ below 100% is attributed to unavoidable aerobic decomposition of the α-NpCbi$^+$ during purification, as described above. These results established that the mechanism of thermolysis of α-NpCbl and α-NpCbi$^+$, like their β diastereomers, is homolytic cleavage of the Co–C bond (Scheme I).

Evidence to support a homolytic mechanism was also obtained by product analyses of aerobic thermolysis of α-neopentylcobalt corrinoids. Alkyl radicals rapidly react with oxygen[45,46] to form

(44) Beaven, G. H.; Johnson, E. A. *Nature* **1955**, *176*, 1264.
(45) Mallard, B.; Ingold, K. U.; Scaiano, J. C. *J. Am. Chem. Soc.* **1983**, *105*, 5095.
(46) Marchaj, A.; Kelley, D. G.; Bakac, A.; Espenson, J. H. *J. Phys. Chem.* **1991**, *95*, 4440.

alkylperoxy radicals that subsequently decompose to an equimolar mixture of the alkyl-derived aldehyde and alcohol (eq 4).[7,11] After

$$RCH_2^{\bullet} + O_2 \rightarrow RCH_2OO^{\bullet} \rightarrow {}^1/_2RCHO + {}^1/_2RCH_2OH + {}^1/_2O_2 \quad (4)$$

conversion to its Schiff's base with aniline (i.e., *N*-(2,2-dimethylpropylidene)benzenamine), the anticipated aldehyde product from the neopentyl radical was detected among the aerobic thermolysis reaction products of both α-NpCbi$^+$ and α-NpCbl by GC and GC/MS. Careful GC quantitation of the yields of the Schiff's base from pivalaldehyde, using a standard curve constructed with the authentic, independently synthesized Schiff's base, showed that the aldehyde was produced in slightly greater than 50% yield from both α-NpCbi$^+$ and α-NpCbl. These results are consistent with the persistence of a Co–C homolysis mechanism for the aerobic thermolysis and validate Scheme I for these decompositions.

Different radical scavengers have been used in studies of carbon–cobalt bond homolysis reactions.[6,8f,14] Some of the most widely used compounds are nitroxide derivatives, such as TEMPO.[3a,c,d,13c] An alternative is to use O_2 as a trap. Oxygen in air-saturated water has been shown to be an efficient scavenger for a wide variety of alkyl free radicals with second-order rate constants in excess of 1×10^9 M^{-1} s^{-1}.[45,46] For thermolysis of α-neopentylcobalt corrinoids in this study, oxygen-saturated water was used because of the labilities of the compounds being studied and the difficulties involved in achieving strictly anaerobic condition without some degree of prior decomposition. Furthermore, control experiments showed that thermolysis of α-neopentylcobalt corrinoids in O_2-saturated water and in anaerobic solution containing excess HTEMPO (10 mM) had essentially the same rates for both α-NpCbl and α-NpCbi$^+$ thermolysis (Table I).

There have been some discrepancies in the literature regarding the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for β-NpCbi$^+$ and β-NpCbl thermolyses in aqueous solution (Table II).[2a,7,8c] These discrepancies are most likely due to varying methods employed for temperature measurements by different researchers as well as other technical difficulties associated with such measurements.[47] In the case of β-NpCbl, additional disagreement occurs because different methods have been used to evaluate the amount of base-on species present at each temperature and hence to correct the observed rate constants for the base-on/base-off equilibrium.[2a,7,8c] In the following analysis, the values from our previous report[7] are used since these data were obtained under the same conditions as those for the α-neopentylcobalt corrinoid thermolyses in this study.

The enthalpy and entropy of activation for the thermolysis of α-NpCbi$^+$ were found to be 21.4 ± 0.4 kcal mol^{-1} and 0.9 ± 1.3 eu, respectively, from the Eyring plot (Table II). This enthalpy of activation is about 8 kcal smaller than that for β-NpCbi$^+$ homolysis under identical reaction conditions. In addition, although the entropy of activation of α-NpCbi$^+$ remains positive, it is reduced by some 10 eu from that of β-NpCbi$^+$. With extrapolation of the data of α-NpCbi$^+$ to 35 °C, the lowest temperature for which data are available for β-NpCbi$^+$,[7] α-NpCbi$^+$ was found to be 5.3×10^3-fold more reactive than the β diastereomer. This results from 8.3 kcal of activation by the reduction is $\Delta H^{\ddagger}$, countered by 3.0 kcal of stabilization due to the reduction of $\Delta S^{\ddagger}$. At 5 °C, after extrapolating the data for β-NpCbi$^+$, the ratio of the rate constants for α-NpCbi$^+$ and β-NpCbi$^+$ was 2.3×10^4. Some of this difference in the observed rate constants for trapped homolysis of the carbon–cobalt bonds of the neopentylcobinamide diastereomers may be due to differential cage effects in the two isomers.[48] However, it seems unlikely to us that much of this large observed difference in reactivity can be attributed to cage effects given the similarity of the caged pair which must be generated from α- and β-NpCbi$^+$.

It has been suggested,[7,49] that in base-on β-RCbl's with bulky organic ligands, thermal homolysis is driven by steric interactions of the bulky organic ligand with the upward projecting a, c, and g acetamide side chains. This steric effect is expressed as an elevated entropy of activation for Co–C bond homolysis since in the ground state, the steric interactions between the side chains and the organic ligand reduce the freedom of rotational motion about the side chain C–C bonds, while in the transition state this motional restriction is significantly relieved by the incipient separation of the corrinoid and organic ligand fragments. The fact that the base-off species of such β-RCbl's (and the analogous β-RCbi's) are ~10^3-fold more stable than the base-on β-RCbl's has been attributed to a downward folding of the corrin ring in these species (originally suggested by Schrauzer and Grate[2]), which reduces the steric interference with side-chain motion in the ground state, hence reducing the entropic driving force for reaction.[7] This release of ground-state steric interactions is seen in the differential activation parameters for base-on β-NpCbl and the base-off species ($\Delta\Delta H^{\ddagger} = -2 \pm 2$ kcal mol^{-1}, but $\Delta\Delta S^{\ddagger} = 10.2 \pm 4.9$ eu).[7] If this interpretation of the reaction energetics of organocobalt corrinoid Co–C bond homolysis is correct, one would anticipate that α-NpCbi$^+$ would substantially more labile than the β diastereomer (as is the case) since the downward projecting corrin ring side chains (the b, d, e propionamides and the f side-chain secondary amide) are both more numerous and longer than the upward projecting side chains. However, in this relatively simple picture of Co–C bond activation, the increase in reactivity of the α diastereomer would be expected to be found mostly in the entropy of activation.[50] The results for α-NpCbi$^+$ show that just the opposite is the case; there is a loss of entropic driving force relative to the β diastereomer, and the enthalpy of activation is lowered by 8.3 kcal mol^{-1}. If our picture of the energetics of Co–C bond homolysis is substantially correct, then these results suggest that, as is the case with the β-RCbi relative to the base-on β-RCbl, there is a flexing of the corrin ring, this time in the upward direction, which tends to relieve the steric restriction of side-chain motion in the ground state. If this is the case, the upward corrin flex in α-NpCbi$^+$ must be more extreme than the downward corrin flex in β-NpCbi$^+$ since a large reduction in $\Delta S^{\ddagger}$ is observed rather than the expected increase due to the fact that the downward projecting side chains are more numerous and larger. Such an accentuated upward flex could conceivably lead to a sufficiently distorted ground-state coordination geometry to alter (weaken) the orbital overlap in the Co–C bond and produce the observed lowering of $\Delta H^{\ddagger}$. It should be pointed out that the capability of the corrin to undergo such an upward flex (for which there is no prior evidence) could be extremely important in the "activation" of AdoCbl for Co–C bond homolysis by AdoCbl-requiring enzymes. The possibility that the α and β diastereomers of NpCbi$^+$ adopt two very different corrin ring conformations is consistent with recent NMR evidence that suggests that the corrin ring conformations of β-AdoCbi and α-AdoCbi are significantly different.[18,52] This possibility is currently being further studied.

Another possible contributor to the very low $\Delta S^{\ddagger}$ for α-NpCbi$^+$ is the very length of the downward projecting b, d, e, and f side

(47) Private communication with Professor R. G. Finke of the University of Oregon.

(48) Koenig, T. W.; Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1499.

(49) Brown, K. L.; Brooks, H. B.; Behnke, D.; Jacobsen, D. W. *J. Biol. Chem.* **1991**, *266*, 6737.

(50) In the ground state, the β-RCbl's are favored over the α-RCbl's by about 17:1 or about 1.7 kcal.[17] Binding constants for exogenous ligands to RCbi's are 10% to 90% higher for β-RCbi's than the α diastereomers (i.e., β coordination is favored by 0.05 to 0.38 kcal).[51] However, the current results represent the first example of kinetic comparison of α and β diastereomers and so it is not apparent how different $\Delta H^{\ddagger}$ for Co–C homolysis is likely to be.

(51) Brown, K. L.; Satyanarayana, S. *Inorg. Chim. Acta* **1992**, *201*, 113.

(52) Brown, K. L.; Zou, X. *J. Am. Chem. Soc.* **1993**, *115*, 1478.

chains. Thus, if the length of the breaking Co–C bond in the homolytic transition state is similar in the α and β diastereomers, the resulting geometry may free the upward projecting side chains completely or nearly completely from motional restrictions by the departing Np ligand in the β transition state but not in the α transition state since the side chains involved are longer. Thus, any relief of steric restrictions on side-chain mobility may be much smaller in the α transition state than in the β transition state leading to a small $\Delta S^{\ddagger}$ for the α diastereomer. The relative importance of this effect and the presumed upward corrin flex remain to be elucidated.

The enthalpy and entropy of activation for α-NpCbl thermolysis are statistically indistinguishable from those for α-NpCbi$^+$. This suggests that the presence of the uncoordinated benzimidazole nucleotide in α-NpCbl has little or no steric consequences for the homolysis reaction, perhaps because it is partially (or mostly) "tucked-in" (i.e., the benzimidazole B3 nitrogen is hydrogen bonded to the g side-chain amide),[53] as is the case for other α-RCbl's.[54] The nearly identical enthalpies and entropies for α-NpCbi$^+$ and α-NpCbl seem to support, retrospectively, the conclusion that Co–C bond homolysis and subsequent formation of the base-on species of H_2OCbl in α-NpCbl thermolysis could be treated, for practical purposes, as two separate reactions (Scheme II).

Comparison could also be made between the rates of thermolysis of α-NpCbl and β-NpCbl. However, such a comparison is not apt, since coordination of the axial nucleotide in the β diastereomer has been shown to cause a 10^2–10^3-fold rate enhancement in base-on over base-off β-RCbl's.[2,3b,7] A more meaningful comparison would be that between α-NpCbl and base-off β-NpCbl (Table II). The ratio of rate constants between the latter two complexes is 3.4×10^3 at 35 °C (after extrapolating the data for α-NpCbl to 35 °C, the lowest temperature at which data for base-off β-NpCbl were obtained[7]) in favor of α-NpCbl. At 5 °C, the ratio of the rate constants is 1.1×10^4. The differences in enthalpies and entropies ($\Delta\Delta H^{\ddagger} = 7.5$ kcal mol^{-1} and $\Delta\Delta S^{\ddagger} = 8.4$ eu) are almost the same as the differences between the NpCbi$^+$ pair. Again, both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ have lower values. Presumably, similar factors responsible for the effects in the diastereomeric NpCbi's are involved here as well.

In conclusion, this study shows that α-NpCbi$^+$ and α-NpCbl can be prepared by reductive alkylation of factor B and H_2OCbl with NpBr or by anaerobic photolysis from their corresponding β diastereomers. Like the β diastereomers, thermolysis of α-NpCbi$^+$ and α-NpCbl leads to Co–C bond homolysis, but the rates of homolysis of α-NpCbi$^+$ and α-NpCbl are more than 10^3–10^4 times faster than those of the corresponding β diastereomers. The differences in the rates of homolysis are due to a reduction in enthalpies of activation in the α diastereomers despite a decrease in the (positive) entropies of activation as compared to β-neopentylcobalt corrinoids. It is suggested that the α-alkylcobalt corrinoids may adopt corrin ligand conformations involving an upward corrin ligand flex as opposed to β-NpCbi$^+$, which presumably displays a downward corrin flex.

Acknowledgment. This research was supported by the National Science Foundation (Grant No. CHE 89-96104), the NSF EPSCoR program (Grant EHR 91-08767), the State of Mississippi, and Mississippi State University. The authors are grateful to Professor Earl G. Alley of this department for assistant with GC/MS analysis and to Professor R. G. Finke and M. D. Waddington for communication of their results prior to publication.

Supplementary Material Available: Figure 6S, showing the treatment of the kinetic data for aerobic thermolysis of α-NpCbl, and text giving a brief description of the methodology (2 pages). Ordering information is given on any current masthead page.

(53) (a) Brown, K. L.; Brooks, H. B.; Gupta, B. D.; Victor, M.; Marques, H. M.; Scooby, D. C.; Goux, W. J.; Timkovich, R. *Inorg. Chem.* **1991**, *30*, 3430. (b) Brown, K. L.; Brooks, H. B.; Zou, X.; Victor, M.; Ray, A.; Timkovich, R. *Inorg. Chem.* **1990**, *29*, 4841. (c) Brown, K. L.; Peck-Siler, S. *Inorg. Chem.* **1988**, *27*, 3548.

(54) Brown, K. L.; Evans, D. R. *Inorg. Chim. Acta* **1992**, *197*, 101.