Facile α/β Diastereomerism in Organocobalt Corrinoids: Synthesis, Characterization, and Complete ^1H and ^{13}C NMR Assignments of α-5′-Deoxyadenosylcobinamide and α-5′-Deoxyadenosylcobalamin

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Abstract: Reductive alkylation of cobinamide in zinc/acetic acid with ether 5′-deoxy-5′-iodoadenosine or 5′-bromo-5′-deoxyadenosine has been found to produce a pair of diastereomeric 5′-deoxyadenosylcobinamides in which the deoxyadenosyl ligand is in the "upper" (or β) axial ligand position (β-AdoCbi) or in the "lower" (or α) axial ligand position (α-AdoCbi). Unlike the case with other alkyl halides, the ratio of product diastereomers has been found to depend on time, the halide leaving group, and the concentration of the 5′-deoxy-5′-haloadenosine. Thus, reductive alkylation with 5′-chloro-5′-deoxyadenosine gave only β-AdoCbi. While the diastereomers of AdoCbi proved to be equally labile toward reductive dealkylation by zinc/acetic acid (as is the case for other pairs of α- and β-RCbi's), α-AdoCbi was dealkylated rapidly by borohydride while the β diastereomer was stable. As a result, reductive adenosylation with borohydride as the reducing agent leads exclusively to β-AdoCbi regardless of the alkylating agent. The α diastereomer of 5′-deoxyadenosylcobalamin (α-AdoCbl) has also been synthesized in zinc/phosphoric acid media. The novel α diastereomers of the 5′-deoxyadenosylcobalt corrinoids have been characterized by UV–visible spectroscopy, HPLC, and FAB-MS. In addition, the ^1H and ^{13}C NMR spectra of both diastereomers have been completely assigned using a combination of homonuclear (COSY, ROESY, and HOHAHA) and inverse detected heteronuclear (HMQC and HMBC) 2-D NMR methodologies. These assignments permit the first comparison of the NMR spectra of pairs of diastereomeric alkylcobalt corrinoids as the ^1H and ^{13}C NMR spectra of β-AdoCbi and base-off β-AdoCbl have previously been assigned by others. Significant differences in chemical shift between the pairs of diastereomers at peripheral corrin substituents are most likely attributable to differences in the α- and β-face environments in the absence or presence of a 5′-deoxyadenosyl moiety at that face. However, the largest chemical shift differences occur in the corrin ring and suggest that the diastereomeric 5′-deoxyadenosylcobalt corrinoids may have significantly different corrin ring conformations.

Introduction

It is generally accepted that the α face of the corrin ring is sterically more hindered, with its downward projecting *b*, *d*, and *e* propionamides and the secondary amide *f* side chain, than the β face, which has only three upwardly projecting *a*, *c*, and *g* acetamide side chains. Recent work has shown, however, that α-alkylcobalt corrinoids (Figure 1),[1] where alkyl = 2-oxo-1,3-dioxolan-4-yl, CH_3, CH_3CH_2, CF_2H, CF_3, $NCCH_2$, CF_3CH_2, and $ROCH_2CH_2$,[2-5] can be obtained from reductive alkylation of cobalt corrinoids with alkyl halides in yields of 2% to 83%, and that the α diastereomer can represent anywhere from 3–4% to 98% of the organocobalt corrinoid product.[4,5] However, α diastereomers of alkylcobalt corrinoids with bulky alkyl groups have not been reported, and reductive alkylation of factor B[6] with benzyl bromide or neopentyl iodide has failed to produce any detectable α-alkylcobinamides.[1,7] In addition, although the partial chemical synthesis of the coenzyme form of vitamin B_{12} (5′-deoxyadenosylcobalamin, AdoCbl[1]) was first reported some 30 years ago,[8,9] it remains unclear if α/β diastereomerism can occur in

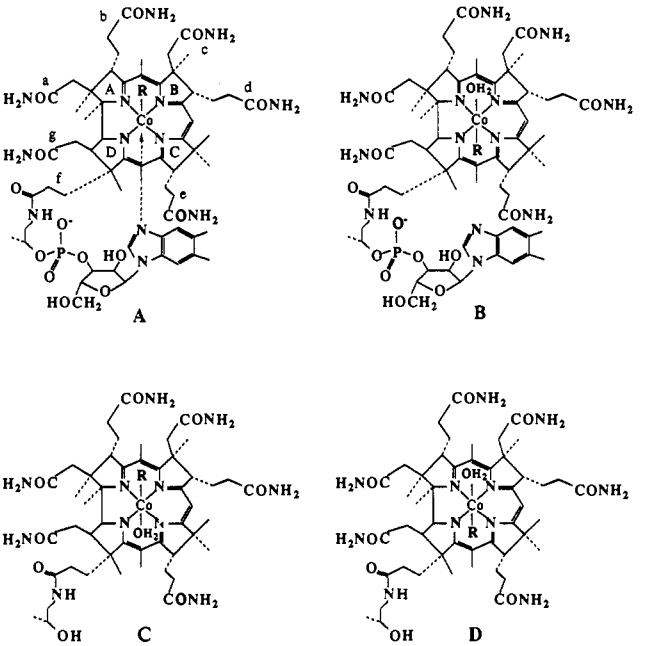

Figure 1. (A) Structure of a base-on β-alkylcobalamin (β-RCbl); (B) structure of an α-alkylcobalamin (α-RCbl); (C) structure of a β-alkylcobinamide (β-RCbi); (D) structure of an α-alkylcobinamide (α-RCbi). In the base-off structure of a β-RCbl, the dimethylbenzimidazole nucleotide is not coordinated and is protonated.

5′-deoxyadenosylcobalt corrinoids.

While the mechanisms that control the ratio of α and β diastereomers formed during reductive alkylation of cobalt corrinoids are still not clear,[2-4,10] the synthesis of β-AdoCbl has been reported

(1) 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" position. Cobinamides are derivatives of cobalamin in which the axial nucleotide has been removed by phosphodiester hydrolysis. See Figure 1 for structures. Abbreviations: $(H_2O)_2$Cbi, diaquacob(III)inamide; α-RCbi, α-alkylcob(III)inamide; β-RCbi, β-alkylcob(III)inamide; β-RCbl, β-alkylcob(III)alamin; α-RCbl, α-alkylcob(III)alamin; Ado, 5′-deoxyadenosine; H_2OCbl, aquacob(III)alamin; CNCbl, cyanocob(III)alamin; HMPA, hexamethylphosphoramide.

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by reductive alkylation of aquocobalamin[8a,9,11-19] or cyanocobalamin,[20-24] using $NaBH_4$,[8a,11,12,14-21] zinc/acid,[8b] zinc/NH_4Cl,[22-24] or thiol[13] reductants, and 2',3'-hydroxyl-protected 5'-tosyladenosine,[8a,11-16,21] unprotected 5'-tosyladenosine,[17] 5'-chloro-5'-deoxyadenosine,[18,20-24] or 5'-deoxy-5'-iodoadenosine[19] as alkylating agent. In every instance, no formation of α-AdoCbl has been reported, although it must be pointed out that prior to routine use of HPLC for analysis of such reaction mixtures, the formation of an α diastereomer in such reactions could easily have been overlooked. Similarly, the synthesis of β-AdoCbi has been reported by reductive alkylation of $(H_2O)_2Cbi$[25] or factor B,[6,24,26,27] using 5'-tosyladenosine,[25] 2',3'-isopropylidene-5'-tosyladenosine,[26] or 5'-chloro-5'-deoxyadenosine[24] as alkylating agent, and via direct hydrolysis of the phosphodiester of β-AdoCbl catalyzed by cerous hydroxide.[28] Again, formation of the α diastereomer has not been reported. More recently, the complete ^1H and ^{13}C assignments of β-AdoCbl, in both the base-on[29a] and base-off[29b] forms, and β-AdoCbi,[30] produced by phosphodiester hydrolysis of β-AdoCbl and hence of defined stereochemistry, have been reported. The X-ray crystal[31a,b] and neutron diffraction[31c] structures of β-AdoCbl have also been determined. Hence, the β diastereomers of these compounds are both well known and well characterized.

In light of our recent observations regarding the stereoisomeric outcome of reductive alkylation of factor B and H_2OCbl,[3,4,32] and the interconversion of α- and β-RCbi's upon anaerobic photolysis,[10] we have reexamined the reductive alkylation of these species with 5'-deoxy-5'-haloadenosine alkylating agents and the anaerobic photolysis of 5'-deoxyadenosylcobalt corrinoids. We now report the first successful synthesis of the α diastereomers of 5'-deoxyadenosylcobinamide and 5'-deoxyadenosylcobalamin, and the characterization of these complexes including the complete assignment of their ^1H and ^{13}C NMR spectra. To our knowledge, α-AdoCbi and α-AdoCbl represent the first example of formation of stable α-alkylcobalt corrinoids with bulky organic alkyl ligands.

Experimental Section

CNCbl was from Sigma, H_2OCbl·OAc was from Roussell, and 5'-deoxy-5'-iodoadenosine, iodo-, bromo-, and chloroacetonitriles, and thionyl chloride and thionyl bromide were from Aldrich. 5'-Chloro-5'-deoxyadenosine was prepared from adenosine and thionyl chloride by the method of Kikugawa[33] (yield, 90%) and was identical with commercial product (Sigma). ^1H NMR (DMSO-d_6), δ (TSP) 3.88 (C5'H_A, $J_{4'\text{-}5'A}$ = 6.4 Hz), 3.98 (C5'H_B, $J_{4'\text{-}5'B}$ = 5.0 Hz, $J_{5'A\text{-}5'B}$ = 11.6 Hz), 4.12 (C4'H, $J_{3'\text{-}4'}$ = 3.8 Hz), 4.26 (C3'H, $J_{2'\text{-}3'}$ = 4.8 Hz), 4.79 (C2'H, $J_{1'\text{-}2'}$ = 5.6 Hz), 5.49 (C3'OH, $J_{3'\text{-}3'OH}$ = 3.4 Hz), 5.63 (C2'OH, $J_{2'\text{-}2'OH}$ = 4.3 Hz), 5.97 (C1'H), 7.35 (NH_2), 8.18 (C2H), 8.37 (C8H). ^{13}C NMR (DMSO-d_6) δ (TSP) 44.82 (C5'), 71.26 (C3'), 72.65 (C2'), 83.65 (C4'), 87.44 (C1'), 119.12 (C5), 139.74 (C8), 149.43 (C4), 152.72 (C2), 156.08 (C6). MS, *m/e* 285.1 (15.5%, M^+), 250.1 (31.2%, M^+ − Cl), 164.1 (100%, $M^+ - C_4H_6O_2Cl$), 135.1 (98.9%, $M^+ - C_5H_6O_2Cl$). The ^{13}C NMR spectrum was in excellent agreement with the literature.[34] 5'-Bromo-5'-deoxyadenosine was synthesized analogously, using thionyl bromide. After purification by ion exchange chromatography,[33] the product could not be crystallized. It was purified by flash chromatography on Amberlite XAD, eluting with 50% aqueous acetonitrile after thorough water washing and isolated as an oil (yield, 15%). ^1H NMR, (DMSO-d_6), δ (TSP) 3.74 (C5'H_A, $J_{4'\text{-}5'A}$ = 6.7 Hz), 3.87 (C5'H_B, $J_{4'\text{-}5'B}$ = 5.0 Hz, $J_{5'A\text{-}5'B}$ = 10.6 Hz), 4.15 (C4'H, $J_{3'\text{-}4'}$ = 3.1 Hz), 4.27 (C3'H, $J_{2'\text{-}3'}$ = 4.9 Hz), 4.81 (C2'H, $J_{1'\text{-}2'}$ = 5.4 Hz), 5.67 (C3'OH), 5.82 (C2'OH), 5.98 (C1'H), 7.38 (NH_2), 8.20 (C2H), 8.41 (C8H). ^{13}C NMR (DMSO-d_6), δ (TSP) 33.96 (C5'), 72.23 (C3'), 72.91 (C2'), 83.67 (C4'), 87.71 (C1'), 119.24 (C5), 139.99 (C8), 149.52 (C4), 152.77 (C2), 156.17 (C6). Factor B[6] was prepared by a modification[35] of the method of Renz.[36] Alkylcobalt corrinoids were prepared by reductive alkylation with appropriate 5'-deoxy-5'-haloadenosine in zinc/acetic acid or zinc/5% H_3PO_4 as described previously,[3-5] and the diastereomers were separated by semipreparative HPLC.[4,5,24] The progress of alkylation reactions was followed by analytical HPLC. Reaction products were quantitated by integration of the chromatograms obtained by UV detection at 350 nm after correction for differing molar absorptivities as described previously.[4,5]

UV–visible spectra were obtained on a Cary 219 recording spectrophotometer using samples purified by semipreparative HPLC. Alkylcobalt corrinoids were quantitated by UV–visible spectroscopy after conversion to the dicyano species (ϵ_{368} = 3.04 × 10^4 M^{-1} cm^{-1})[37] by aerobic photolysis in excess KCN. Anaerobic photolysis of organocobalt corrinoids was carried out in a cylindric quartz cell equipped with a water circulation jacket and thermostatted at 25.0 ± 0.1 °C by a circulating water bath. The light source was a low-level 3-V tungsten lamp as described previously.[10]

α-AdoCbi. Since the α/β isomeric ratio of the alkyl products varies with alkylation time as well as the alkylating agent (see discussion below), different strategies were used in the synthetic reaction, depending on which isomer was desired. The following synthetic conditions gave the maximum yield of α-AdoCbi.

Factor B (10 mg, 9.7 μmol) in 10 mL of 10% acetic acid was purged with argon for 1 h. Zinc wool (0.01 mol), briefly freshened with 2.0 N HCl, was added, and the reduction was allowed to proceed for 30 min. 5'-Deoxy-5'-iodoadenosine (10 mg, 30 μmol), which had been previously degased in 10 mL of methanol, was introduced through a cannula into the reaction mixture. The alkylation reaction was allowed to proceed for 4 min and was quenched by removal of the zinc residue by filtration. The crude reaction mixture, which contained ~31% α-AdoCbi, 39% β-AdoCbi, and 26% unalkylated corrinoids by HPLC, was desalted by chromatography on Amberlite XAD-2,[35] and the isomeric AdoCbi's were separated by semipreparative HPLC. In three separate preparations, the average recovered yields were 3.5 mg of β-AdoCbi (2.8 μmol, 35 ± 5%) and 3.6 mg of α-AdoCbi (2.9 μmol, 36 ± 5%).

β-AdoCbi. For the maximum yield of β-AdoCbi, the procedure was similar to that described above except that 33 mg (97 μmol) of 5'-deoxy-5'-iodoadenosine was used, and the alkylation was allowed to continue for 30 min. After separation by HPLC, the recovered yields

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were 80% β-AdoCbi and 10% α-AdoCbi (90% total yield).

α-AdoCbl. The adenosylation of aquocobalamin was much slower than that of factor B. The procedure used was similar to that described above for α-AdoCbi except that 5% H_3PO_4 was used in place of 10% acetic acid as the reaction media,[4,5] and the alkylation was allowed to proceed for 60 min. In two separate preparations, the average total recovered yield of AdoCbl's was 44%, with 23% α-AdoCbl and 21% β-AdoCbl.

FAB–MS was performed on a Kratos MS80 RFA mass spectrometer with an Ion Tech FAB 11 NF FAB source using 5–6-kV argon bombardment and a *m*-nitrobenzyl alcohol matrix.[38]

One-dimensional ^1H and ^{13}C NMR spectra of the 5′-deoxyadenosylcobalt corrinoids were obtained in D_2O on a GE QE 300 NMR spectrometer operating at 300.669 MHz and 75.61 MHz, respectively, with TSP as an internal reference. Two-dimensional NMR experiments were performed on a Bruker AMX 300 NMR spectrometer equipped with a 5-mm inverse detection probe. Exchangeable protons were deuterated by dissolving the complex in 99.9% D_2O and evaporating to dryness three times. The residue was dissolved in "100%" D_2O (Aldrich) to provide a final concentration of 10 to 30 mM. For the homonuclear *J*-correlated experiment (COSY),[39] data were collected into a 1024 × 512 data matrix with 128 scans, preceded by 4 dummy scans, per t_1 value, and a 1-s presaturation delay between scans. Data were collected over a 3164-Hz sweep width in both dimensions and processed a 90° shifted sine-squared apodization function. The HOHAHA spectra[40] resulted from a 1024 × 256 data matrix with 128 scans, preceded by 2 dummy scans, per t_1 value and a 1-s presaturation delay between scans. The sweep width was 3164 Hz in both dimensions, the mixing time was 70 ms, and the data were processed with a 90° shifted sine-squared apodization function. The spin-locked NOE (or rotating-frame Overhauser enhancement spectroscopy, ROESY)[41] experiments utilized a 1024 × 256 data matrix, 256 scans, preceded by 2 dummy scans, per t_1 value, and a 1-s presaturation delay between scans. The sweep width was 3164 Hz in both dimensions and the mixing time was 200 ms. The data were processed with a 90° shifted sine-squared apodization function. The spectra were plotted in two colors to permit distinction of positive contours (direct NOEs) from negative contours (relayed NOEs and/or Hartmann–Hahn artifacts). For the ^1H-detected heteronuclear multiple-quantum coherence (HMQC)[42] experiments, data were collected into a 1024 × 256 data matrix using 128 scans, preceded by 4 dummy scans, per t_1 value and a 500-ms presaturation delay. The sweep widths were 3164 Hz in the ^1H dimension and 22 642.5 Hz in the ^{13}C dimension. The data were processed using Gaussian line broadening of −5 Hz. The ^1H-detected multiple-bond heteronuclear multiple-quantum coherence (HMBC)[29a,43] spectra resulted from a 1024 × 512 data matrix with 256 scans, preceded by 4 dummy scans, per t_1 value and a 500-ms presaturation delay between scans. The ^1H sweep width was 3164 Hz and the ^{13}C sweep width was 22 642.5 Hz. A 50-ms delay was used for the evolution of long-range coupling and the data were processed with Gaussian line broadening of −5 Hz.

Results and Discussion

Synthesis of Adenosylcobalt Corrinoids. The synthetic strategies for AdoCbi fall into two major categories: (1) direct hydrolysis of β-AdoCbl with cerous hydroxide to remove the 5′,6′-dimethylbenzimidazole nucleotide,[28] and (2) reductive alkylation of factor B, or $(H_2O)_2$Cbi, using 5′-tosyladenosine, with or without the 2′,3′-hydroxyl protection, or 5′-deoxy-5′-haloadenosines, as alkylating reagents.[24-27] In both cases, β-AdoCbi has been reported to be the sole product.[24-28] The first method, which starts from β-AdoCbl, necessarily produces an AdoCbi product of known stereochemistry (i.e., β-AdoCbi). However, reductive alkylation of factor B or $(H_2O)_2$Cbi clearly has the potential to produce both

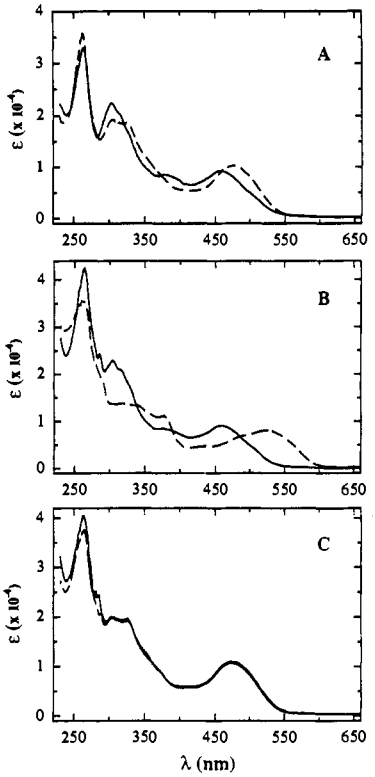

Figure 2. (A) Electronic spectra of the 5′-deoxyadenosylcobinamides in 0.2 M potassium phosphate buffer (pH 7.0): dashed line, α-AdoCbi; solid line, β-AdoCbi. (B) Electronic spectra of β-5′-deoxyadenosylcobalamin: solid line, base-off β-AdoCbl in 0.5 M HCl; dashed line, base-on β-AdoCbl in 0.2 M potassium phosphate buffer (pH 7.0). (C) Electronic spectra of α-5′-deoxyadenosylcobalamin: solid line, α-AdoCbl in 0.5 M HCl; dashed line, α-AdoCbl in 0.2 M potassium phosphate buffer (pH 7.0).

diastereomers of AdoCbi.[3-5] Work reported in the earlier literature,[25-27] prior to the widespread use of HPLC as an analytical tool in this area, could easily have missed the formation of minor amounts of α-AdoCbi. As alkylating agents, the 5′-deoxy-5′-halonucleosides are clearly most convenient since they do not require the extra protecting and deprotecting steps used for 2′,3′-hydroxyls of 5′-tosyladenosine, and thus avoid reaction conditions which may be unintended consequences elsewhere in the product.

Jacobsen et al.[24] have reported obtaining a single diastereomer of AdoCbi (by HPLC analysis), upon reductive alkylation of factor B with 5′-chloro-5′-deoxyadenosine. When we used 5′-chloro-5′-deoxyadenosine in 100- to 300-fold excess as an alkylating agent in Zn/acetic acid–methanol, a 70% yield of alkylated product and a single diastereomer was obtained, as determined by HPLC. This product could be definitely assigned as the β-diastereomer, since its ^1H and ^{13}C NMR spectra were identical with the published spectra of authentic β-AdoCbi, obtained by cerous hydroxide-catalyzed phosphodiester linkage hydrolysis of β-AdoCbl.[28] In contrast, when 5′-deoxy-5′-iodoadenosine was used as the alkylating agent in Zn/acetic acid, two product peaks appeared in the HPLC chromatogram. One of them, on the bases of its UV–visible absorption spectrum,[28] ^1H and ^{13}C NMR spectra,[30] and FAB–MS (m/e = 1240.0 obs, 1240.4 calc[44]) was the previously known β-AdoCbi. The second peak, which was eluted slightly earlier, had a visible spectrum red shifted from that of β-AdoCbi by 17 nm at the longest wavelength visible band (the α band), and had the typical strong absorption band at 263 nm

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(44) As previously observed,[3,5,28b] the parent ion in positive ion FAB–MS of cationic RCbi's is pentacoordinate (i.e., m/e = M^+-H_2O). However, the parent ion for the zwitterionic RCbl's contains a proton from the matrix and thus occurs at m/e = MH^+ for β-RCbl's. For α-RCbl's, which presumably have water coordinated at the β position, the parent ion is again pentacoordinate and occurs at MH^+-H_2O.

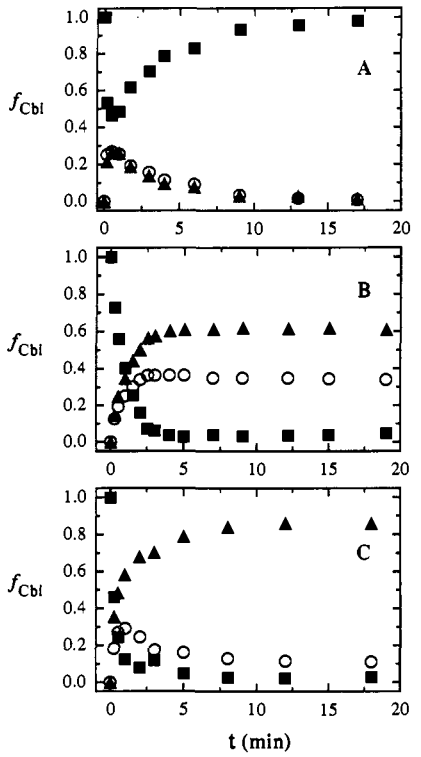

Figure 3. Plots of f_{CbI}, the fraction of total cobinamide species as α-AdoCbi (○), β-AdoCbi (▲), or as non-alkylated cobinamide (■), versus time for the alkylation of factor B in Zn/acetic acid with a 2-fold molar excess (A), a 5-fold molar excess (B), and a 30-fold molar excess (C) of 5′-deoxy-5′-iodoadenosine.

due to the adenosine nucleoside (λ (log ε) 474 (4.02), 32[illegible]4.28), 304 (4.29), 263 (4.52); Figure 2A). The red shift of the α band is characterized of the α-RCbi's and has become diagnostic of the stereochemistry.[3-5] A FAB mass spectrum of the new material had a parent ion at m/e = 1240.2 (calc 1240.4[44]), thus confirming the isomeric relationship between the products. After further characterization by ^1H and ^{13}C NMR (see below), we conclude that this new compound is α-AdoCbi.

A closer study of the product composition of the reductive akylation of factor B (0.64 mM) with 5′-deoxy-5′-iodoadenosine at various concentrations in Zn/acetic acid–methanol showed that the ratio of the two diastereomers was time dependent (Figure 3), at least when the alkylating agent was employed at relative high excess. In the presence of a 2:1 molar excess of 5′-deoxy-5′-iodoadenosine, adenosylated products accumulated rapidly and reached a maximum at about 30 s, at which time the net yield of AdoCbi's was 54% with essentially equal amounts of each diastereomer (Figure 3A). Thereafter, the concentration of both diastereomers declined steadily until there were no adenosylated products (at about 15 min), apparently the result of reductive dealkylation by the Zn/acetic acid reducing agent (vide infra). When the alkylating agent was employed in a 5:1 molar excess (Figure 3B), the total yield of adenosylated products increased over the first 3–4 min, but the ratio of diastereomers steady changed from 46:54 (α:β) to 38:42. Thereafter, both the total yield of products (≥95%) and the α/β ratio remained constant for the duration of the experiment (18 min). When the alkylating agent was employed in a significantly larger molar excess (30:1), the outcome was substantially different (Figure 3C). The yield of α-AdoCbi increased to a maximum (29%) over the first 1.5 min, but then steadily declined to 11%. In contrast, the yield of β-AdoCbi increased monotonically to 86%. Thus, while the total yield of adenosylated products was nearly quantitative (>95%) at t > 10 min, the ratio of diastereomers was only 11:89 (α:β). The best yield of α-AdoCbi was thus obtained using 5′-deoxy-5′-iodoadenosine at a 3:1 molar excess over factor B and terminating the alkylation reaction after 4–5 min as described in the Experimental Section.

The stark difference in the outcome of the alkylation of reduced cobinamide with 5′-chloro-5′-deoxyadenosine and with 5′-deoxy-5′-iodoadenosine prompted us to examine the reductive alkylation of factor B with 5′-bromo-5′-deoxyadenosine. In this case, the outcome was very similar to that with 5′-deoxy-5′-iodoadenosine, except that the rate of accumulation of adenosylated products was somewhat slower (data not shown). At a low molar excess of alkylating agent (3:1), the maximum yield of adenosylated products (42.5%) was achieved in about 2 min, but the ratio of diastereomers was 56:44 (α:β). Thereafter, the yield of both diastereomers declined to zero by 20 min. When 5′-bromo-5′-deoxyadenosine was employed in a 25:1 molar excess, α-AdoCbi reached a maximum yield (36%) at 2.5 min, but steadily declined thereafter to 9%. The yield of β-AdoCbi steady increased to 87% at 12 min. Thereafter, the total yield and diastereomeric ratio remained constant for the duration of the experiment (20 min) at 96% and 9:91, respectively.

These differences in the outcome of the reductive alkylation of factor B with different 5′-deoxy-5′-haloadenosines suggest that the halide leaving group may play a role in determining the product stereochemistry, a possibility not appreciated in earlier work. In order to determine if the stereoisomeric outcome of reductive alkylation of factor B with alkyl halides in general is affected by the halide of the alkylating agent, the synthesis of the diastereomeric $NCCH_2Cbi$'s (previously reported using only $NCCH_2Br$ as the alkylating agent[4]) was repeated using $NCCH_2I$, $NCCH_2Br$, and $NCCH_2Cl$ as alkylating agents. The ratio of diastereomers obtained from each of these alkylating agents was found to be time independent, and was the same for all three halides, i.e., α/β = 73:27, essential identical with the results previously obtained with $NCCH_2Br$.[4] Thus, the halide of the alkylating agent does not affect the diastereomeric outcome of this reductive alkylation, although the net rate of alkylation increased significantly in the order of Cl < Br < I.

Organocobalt corrinoids are well known to be labile toward reductive dealkylation,[3,32,45-50] apparently due to homolysis of the Co–C bond of the one-electron-reduced $RCo^{II\bullet}$ species. Thus, turnover of the alkylated products due to reductive dealkylation causes reductive alkylation reaction mixtures to achieve a steady state in products when there is a sufficient excess of alkylating agent. The stereoisomeric outcome of such reductive alkylations could, in principle, be affected by differential lability of the diastereomers toward reductive dealkylation. We have previously shown[32] that for diastereomeric α- and β-RCbi's (R = CF_3, CF_3CH_2, and $NCCH_2$), the rate of reductive dealkylation of each member of a pair of diastereomers by Zn/acetic acid was identical, apparently limited by the rate of diffusion to the reductant surface. The data in Figure 3A suggest that the same is true of the diastereomeric AdoCbi's. In order to be certain that differential lability toward reductive dealkylation does not influence the ratio of diastereomers in the reductive adenosylation of factor B, α- and β-AdoCbi were treated independently, and together, with Zn/10% acetic acid. In each case the rates of dealkylation of the two diastereomers were the same, consistent with our earlier results with other pairs of diastereomers. However, when the diastereomeric AdoCbi's were treated with a modest excess of $NaBH_4$, β-AdoCbi proved to be stable, while α-AdoCbi was rapidly dealkylated. Consistent with this observation, reductive alkylation of factor B with 5′-deoxy-5′-iodoadenosine using $NaBH_4$ as a reductant produced only β-AdoCbi. Thus, earlier reports of the synthesis of AdoCbi in which $NaBH_4$ was the reductant or 5′-

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chloro-5′-deoxyadenosine was the alkylating agent most likely produced only the β diastereomer.

It thus remains unclear why the stereoisomeric outcome of the reductive adenosylation of factor B with 5′-deoxy-5′-haloadenosines varies with the halide leaving group, the alkylation time, and with the concentration of the alkylating agent. None of these phenomena has been observed in other reductive alkylations of factor B. However, it also remains unclear exactly what controls the stereochemistry in such reactions in general. Recent results[10b] have suggested that the diastereomeric products of the reductive alkylation of factor B are under neither kinetic nor thermodynamic control. If this is the case, some reaction must occur under reductive alkylation conditions which interconverts the diastereomers but fails to come to equilibrium. Thus, the different behavior of reductive adenosylation and reductive alkylation with other alkyl halides may reflect differences in this as yet unknown reactivity. More concrete conclusions regarding the outcome of reductive adenosylation must consequently await a better understanding of stereochemical control in reductive alkylation in general.

The possibility of the synthesis of the α diastereomer of AdoCbl has also been investigated. Recent work has shown that the stereochemical outcome of the reductive alkylation of H_2OCbl with alkyl halides is strongly pH dependent.[32] For those alkyl halides which produce significant amounts of α-RCbi's when reacted with reduced cobinamides, reductive alkylation of H_2OCbl at low pH also produces significant amounts of the α-RCbl. In contrast, reductive alkylation at neutral pH yields only β-RCbl's.[32] The pH dependence of the stereochemical outcome is now known to be controlled by the pK_a for the base-on/base-off equilibrium of the product β-RCbl. Thus, significant amounts of the α-RCbl can only be obtained at pH's at which the β-RCbl is significantly base-off. This suggested that the α diastereomer of AdoCbl could also be obtained under appropriate conditions. When reductive alkylation of H_2OCbl was carried out in Zn/5% H_3PO_4 (pH = 0.9) using 5′-chloro-5′-deoxyadenosine as the alkylating agent, only β-AdoCbl was obtained in agreement with the results obtained with factor B. In contrast, when 5′-deoxy-5′-iodoadenosine was the alkylating agent in Zn/5% H_3PO_4, two products were identified in the HPLC chromatogram. One of these products co-migrated with authentic β-AdoCbl, had a UV–visible spectrum[18] identical with authentic β-AdoCbl, and underwent the typical base-on/base-off spectral transition when acidified (Figure 2B). The other material, which had a slightly longer retention time than β-AdoCbl, was photolabile, had ^1H and ^{13}C NMR resonances indicative of the presence of an adenosyl moiety (see below), had an electronic spectrum identical with that of α-AdoCbi above 300 nm (Figure 2), and showed only UV spectral changes indicative of protonation of a pendent, but uncoordinated benzimidazole moiety[35] (and, presumably, an adenosyl moiety) upon acidification (Figure 2C:λ (log ε), pH 7.0, 474 (4.03), 327 (4.28), 304 (4.32), 264 (4.58); pH 0.30, 472 (4.05), 325 (4.31), 304 (4.32), 263 (4.62)). The positive ion FAB–MS parent ion for this material (obs 1580.1, calc 1580.6[44]) was identical with that for β-AdoCbl (obs 1580.1), confirming the isomeric relationship. We conclude that this compound is α-AdoCbl. As anticipated,[32] and in agreement with other results,[8a,11,12,14-19] reduction of H_2OCbl with $NaBH_4$ in neutral solution, followed by alkylation with 5′-deoxy-5′-iodoadenosine, produced only β-AdoCbl.

Photolysis. Like all other organocobalt corrinoids, both α-AdoCbi and α-AdoCbl are photolabile. Under aerobic conditions, both were readily converted to aquocobalt corrinoids and to dicyanocobalt corrinoids after photolysis in excess KCN. Recent results have shown that irradiation of either α- or β-RCbi's with visible light under strictly anaerobic conditions leads to a mixture of the diastereomers, in which the β diastereomer predominates by 3–4:1, frequently with little or no net dealkylation.[10] Experiments with a nitroxide radical trap, which prevents the interconversion of the diastereomers and leads to net dealkylation only, suggest that free radicals are involved in this isomerization. Thus, in the absence of scavengers for R· and/or cob(II)inamide, recombination of these species produced by photolytic homolysis of the Co–C bond results in isomerization. A similar photo-induced isomerization has now also been observed for the AdoCbi's. However, the 5′-deoxyadenosyl radical is known to undergo rapid cyclization to yield 8,5′-dehydroadenosine,[28b,51,52] effectively providing a pathway for self-trapping.[28b,52] Thus, anaerobic photolysis of either diastereomer of AdoCbi does lead to isomerization but also to substantial dealkylation. When α-AdoCbi was irradiated anaerobically with a low level of visible light,[10] conversion to β-AdoCbi occurred with a maximum of 25% β diastereomer formed at 400 min. However, 40% net dealkylation had occurred by that time. Similarly, anaerobic irradiation of β-AdoCbi also produced the α diastereomer but with substantial decomposition. As previously observed for other α-RCbl's,[2,10] irradiation of anaerobic, neutral solutions of α-AdoCbl also led to base-on β-AdoCbl, but again, with considerable net dealkylation. Thus, unlike the situation for other RCbi's,[10] anaerobic photolysis is not a satisfactory synthetic method for obtaining the α diastereomer, because of self-trapping of the 5′-deoxyadenosyl radical by cyclization.

NMR. In order to further characterize the α-5′-deoxyadenosylcobalt corrinoids, the ^1H and ^{13}C NMR spectra of α-AdoCbi and α-AdoCbl were completely assigned by use of 2-dimensional homonuclear (COSY, HOHAHA, and ROESY) and heteronuclear (HMQC and HMBC) NMR spectroscopy. As was the case for β-AdoCbl,[29a] the cross peaks in absorption mode NOE spectra were extremely weak, apparently because the rotational correlation time for these molecules is very close to the Larmor frequency. The spin-locked NOE experiment (ROESY), however, which produces cross peaks that are always positive and increase in intensity with slower molecular tumbling,[53] gave excellent spectra. The assignment strategy used was similar to that previously followed for other cobalt corrinoid NMR assignments.[2,29,30,54-56] Spin-coupled protons were assigned from direct and relayed connectivities observed in the COSY and HOHAHA spectra, respectively. Protons not spin-coupled to other protons were assigned from their through-space NOE interactions, observed in the ROESY spectra, which also served to confirm the assignments of other protons from their spin-coupled interactions. Carbon resonances were then assigned from their C–H cross peaks in the heteronuclear multiple-quantum coherence experiments HMQC and HMBC, for protonated and unprotonated carbons, respectively. The observed connectivities are summarized in Table I for α-AdoCbi and in Table II for α-AdoCbl, and the COSY, HOHAHA, ROESY, and HMBC spectra are shown in Figures S1–S4 and S5–S8 (available as supplemental material) for α-AdoCbi and α-AdoCbl, respectively. The final ^1H and ^{13}C assignments of both complexes are shown in Table III, along with a comparison of the chemical shifts to those of β-AdoCbi[30] and the base-off species of β-AdoCbl.[29b]

As shown in the correlation tables for α-AdoCbi (Table I) and α-AdoCbl (Table II), a number of ROE cross peaks are evident between protons of the adenosyl moieties and those on the corrin and its peripheral substituents. Many of these confirm the location of the adenosyl group in the α axial position. For instance, in both compounds, several ROE's are seen between adenosyl protons and the downward projecting methyl substituents, C36 and C47 (Figures 1 and 4). In addition, when ROE cross peaks are seen between adenosyl protons and side chain methylene protons (e.g., A8 with C48′, C48″, and C49″, and A2 and A11 with C48″ in α-AdoCbi (Table I), and A15″ with C42 and C48″, A2 with C48′, and A8 with C48′ in α-AdoCbl, (Table II)), they invariably involve downward projecting propionamide side chains. Taken together with the UV–visible spectra in Figure 2 and the identity of the

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Table I. Correlation Table for NMR Connectivities of α-5′-Deoxyadenosylcobinamide Observed by Homonuclear J-Correlation (COSY), Spin-Locked NOE (ROESY), Homonuclear Hartmann–Hahn (HOHAHA), and Heteronuclear Multiple-Bond Correlation (HMBC) Methodologies[a]

^{1}H signal	COSY	HOHAHA	ROESY[b]	HMBC
A15″	A15′, A14	A11, A12, A13, A14, A15′	A11, A12, A13, A14, A15′, C35, C36, C54	A14
A15′	A15″	A12, A14, A15″	A2, A11, A12, A13, A15″, C36	A11, A13, A15
C53			C13, C48′, C49′, C54, C55″	C14, C15, C16
C35			C31, C36	C4, C5, C6
C25			C3, C18, C20, C26′, C30′	C1, C2, C3, C26
C54			C19, C48″, C53, C55″, C60′	C16, C17, C18, C57, C60
Pr3	Pr2	Pr1′, Pr1″, Pr2	Pr1′, Pr1″, Pr2	Pr1′, Pr1″, Pr2
C47			C13, C46, C49″, A2, A11, A14	C11, C12, C13, C46
C36			C8, C31, C35, C37′, C37″	C6, C7, C8, C37
C20			C18, C19, C25, C30′, C30″	C1, C2, C19
C30″	C30′	C3, C30′	C20, C31	C3, C26, C30, C32, C35
C30′	C3, C30″	C3, C30″	C3	C2, C26
C41″	C41′, C42	C8, C41′	C8, C41′, C42	C7, C8
C41′	C8, C41″, C42	C8, C41″	C41″	C41
C48″	C48′, C49′, C49″	C48′, C49′, C49″	C13, C48′, C49′, A2, A8, A11, A14	C11, C14
C48′	C48″, C49′, C49″	C48″, C49′, C49″	C13, C46, C48″, C49′, C49″, A8	
C46			C13, C47, C49′	C12, C13
C42	C41′	C8, C41′	C8, C36, C41″	C7
C60″	C18	C18, C19	C54, C55″	C17, C54
C60′	C18	C18, C19	C54, C55′	C17, C18, C54
C56″	C55′	C55′, C55″	C18, C53	Pr1, C17, C18
C56′	C55′	C55′, C55″	C18, C53	Pr1, C17
C49″	C48′, C48″, C49′	C13, C48′, C48″	C13, C49′	C13, C47
C49′	C48′, C48″, C49″	C13, C48′, C48″	C13, C47, C49″	C12
C55″	C55′, C56′, C56″	C55′, C56′, C56″	C18, C53, C54, C55′, C55″	C54, C56
C55′	C55″, C56′, C56″	C55″, C56′, C56″	C55″	C18, C56
C31	C30″	C3, C30″	C3, C26′, C30″	C2, C3, C30
C18	C19, C60′, C60″	C19, C60′, C60″	C25, C26′, C55″, C56, C60′, C60″	C17, C20, C60
C26″	C26′	C26′	C18, C19, C30′, C31	C2, C20, C25, C26, C27, C31
C26′	C26″	C26″	C30″, C31	C2, C3, C27
C37″	C37′	C37′	C8, C37′	C6, C8, C35, C37, C38
C37′	C37″	C37″	C8, C35, C37″	C7, C8, C36, C37, C38
Pr1″	Pr2	Pr2, Pr3	Pr1″, Pr2, Pr3	Pr1
Pr1′	Pr2	Pr2, Pr3	Pr1′, Pr2, Pr3	Pr1, Pr2, C57
C13	C48′, C48″	C48′, C48″, C49′, C49″	C46, C47, C48′, C48″, C49′, C49″	C12, C16, C49
C8	C41′	C41′, C41″, C42	C36, C37′, C37″, C41″	C6, C9, C36, C37, C42
C3	C30′, C30″	C30′, C30″, C31	C25, C35, C30′, C31	C1, C4, C6, C30, C31
Pr2	Pr1′, Pr1″, Pr3	Pr1′, Pr1″, Pr3	Pr1′, Pr1″, Pr3	
A13	A12, A14	A11, A12, A14, A15′, A15″	A11, A12, A14, A15′, A15″	
A12	A11, A13	A11, A13, A14	A11, A13, A14, A15′, A15″	
C19	C18	C18, C60′, C60″	C18, C20, C26′, C26″, C55′, C56	C1, C16, C20, C60, C61
A14	A13, A15′, A15″	A11, A12, A13, A15″	A12, A13, A15′, A15″, C47, C48″	A11, A13
A11	A12	A12, A13, A14, A15″	A12, A13, A15′, A15″, C47, C48″	
C10			C8, C41′, C46, C47	C8, C9, C11, C12
A8			A11, A12, A13, A15″, C10, C47, C48″, C48′, C49″	A2, A4, A5
A2			A15′, C47, C48″	A4, A6, A8

[a] Primes and double primes indicate downfield and upfield signals, respectively, of diastereotopic methylene groups. [b] Only direct NOEs are listed.

synthesis product assigned to β-AdoCbi to authentic β-AdoCbi from phosphodiester cleavage of β-AdoCbl,[28] these data provide unequivocal evidence that the products characterized here are indeed the α diastereomers of AdoCbi and AdoCbl.

The final NMR assignment (Table III) permits the first direct comparison of the NMR spectra of an α-AdoCbi and the analogous α-AdoCbl in which the assignments are known absolutely. Almost all of the significant differences in ^{13}C and ^{1}H chemical shifts occur in the nucleotide loop and in the adjacent *g* acetamide side chain on the D ring (Figure 4), and can be logically attributed to the deletion of the axial nucleotide in the cobinamide. Thus, the Pr3 and Pr1 carbons are shifted 0.63 and 1.25 ppm downfield, respectively, in α-AdoCbi relative to α-AdoCbl, while the Pr2 carbon is shifted 6.08 ppm upfield. Both the C56 methylene carbon (1.38 ppm downfield in α-AdoCbi) and the C57 carbonyl carbon of the *f* side chain (1.13 ppm upfield in α-AdoCbi) also show significant shifts, while the C55 methylene carbon is essentially unchanged. Both the C60 methylene carbon and the C61 carbonyl carbon in the adjacent *g* side chain show significant downfield shifts in α-AdoCbi (0.59 and 0.54 ppm, respectively). Inexplicably, the only other carbons which show significant chemical shift differences are the C46 and C47 methyls and the C42 methylene, which are 1.48 ppm downfield, 0.42 ppm upfield, and 1.28 upfield, respectively, in α-AdoCbi relative to α-AdoCbl.

Most importantly, the complete NMR assignments of α-AdoCbi and α-AdoCbl permit the first comparison of the NMR spectra of pairs of α and β diastereomers of organocobalt corrinoids. These comparisons are shown in Table III as the difference in chemical shift ($\Delta\delta$) between α-AdoCbi and β-AdoCbi,[30] and between α-AdoCbl and base-off β-AdoCbl.[29b] In the latter case, the base-off form of β-AdoCbl was studied at pH 2.1, where the axial benzimidazole nucleotide is protonated at B3 and the adenosyl ligand is protonated at A10 (Figure 4). These protonations prevent the comparison of the NMR characteristics of both the axial nucleotide and the adenosyl moiety in the pair of diastereomeric AdoCbl's. However, as seen in Table III, the comparison (i.e., $\Delta\delta$ values) for the rest of the molecule is quite similar to the comparison of the diastereomeric AdoCbi's and so the following discussion will focus on this latter comparison.

The most striking difference between α- and β-AdoCbi occurs at the α carbon of the organic ligand, i.e., A15. In the α diastereomers, the A15 carbon resonance is shifted 3.9 ppm upfield relative to the β diastereomer. This shift is in the same direction and at the upper end of the range of upfield shifts (1.9 to 4.1 ppm) previously seen in the comparison of the α carbon ^{13}C resonances of six pairs of α- and β-alkoxyethylcobinamides.[5] There are also

Table II. Correlation Table for NMR Connectivities of α-5′-Deoxyadenosylcobalamin Observed by Homonuclear J-Correlation (COSY), Spin-Locked NOE (ROESY), Homonuclear Hartmann–Hahn (HOHAHA), and Heteronuclear Multiple-Bond Correlation (HMBC) Methodologies[a]

^{1}H signal	COSY	HOHAHA	ROESY[b]	HMBC
A15″	A15′, A14	A13, A14, A15′	A8, A11, A12, A13, A14, A15′, C42, C48″	A14
A15′	A15″, A14	A14, A15″	A2, A11, A12, A13, A14, A15″	A13, A14
C53			C13, C48′, C48″, C49′, C54, Pr3	C14, C15, C16, C54
C35			C3, C36, C37′, C37″	C4, C5, C6
C25			C3, C18, C20, C26′, C26″, C31	C1, C2, C3, C20
C54			C18, C19, C48′, C53, C55′, C55″, C56′, C56″, C60′, C60″	C16, C17, C18, C19, C57
Pr3	Pr2	Pr1′, Pr1″, Pr2	Pr1′, Pr1″, Pr2, C48′, C53, C55	Pr1′, Pr1″, Pr2
B11		B7	R1	B5, B6, B7, B8
C47			C46, C48′, A2, A11	C11, C12, C13, C46
B10		B4		B4, B5, B6
C36			C8, C35, C37′, C37″, A12	C6, C7, C8, C37
C20			C18, C19, C30′, C30″, C31	C1, C2, C19, C25
C30″	C30′	C3, C30′	C3, C25, C26′, C30′, C31	C3, C31, C32
C30′	C3, C30″, C31	C3, C30″	C3, C20, C25, C26, C30″	C3, C4, C31
C41″	C8, C41′, C42	C8, C41′, C42	C8, C36, C41′, C42	C8, C9, C42
C41′	C41″, C42	C8, C41″	C8, C36, C37″	C8, C9, C42
C48″	C13, C48′, C49′	C13, C48′, C49′, C49″	C13, C46, C48′, C49′, C49″, A2, A8, A15″	C49
C48′	C13, C48″, C49″	C13, C48″, C49′, C49″	C13, C46, C47, C48″, C54, A2	C13, C49
C46			C47, C48′, C48″, C49′	C11, C12, C13, C47
C56″	C55″, C56′	C55″, C56′	C48″, C53, C54, C55′, C56′	C17, C54, C55, C57
C56′	C55″, C56″	C55″, C56″	C54, C55′, C55″, C56″	C17, C54, C55
C60″	C18	C18, C19	C18, C26′, C26″, C54	C17, C18, C61
C60′	C18	C18, C19	C18, C26′, C26″, C54	C17, C18, C54
C49″	C48′	C13, C48′, C48″, C49″	C13, C48′, C48″, C49′	C48
C49′	C48″	C13, C48′, C48″, C49″	C13, C48′, C48″, C49″	C13, C48, C50
C42	C41″	C8, C41″	C8, C41″, A15″	C8, C41
C55″	C55′, C56′, C56″	C56′, C56″	C53, C54, C56′	C16, C17, C56, C57
C55′	C55″	C56′, C56″	C54, C55″, C56′, C56″, Pr3	C17, C18, C54, C56
C31	C30″	C3, C30′	C3, C20, C25, C30″	C30
C18	C19, C60′, C60″	C19, C60′, C60″	C25, C26′, C26″, C54	C16, C60
C26″			C3, C18, C19, C26′, C60′, C60″	C2, C3, C27
C26′			C3, C18, C19, C25, C26″	C1, C2, C25, C27
Pr1″	Pr2	Pr2, Pr3	Pr2, Pr3	Pr2, Pr3, C57
Pr1′	Pr2	Pr2, Pr3	Pr2, Pr3	Pr2, Pr3, C57
C37″			C35, C36, C37′	C6, C7, C8, C36, C38
C37′			C35, C36, C37″	C6, C7, C8, C36, C38
C13	C48′, C48″	C46, C48′, C48″, C49′, C49″	C48′, C48″, C49′, C49″, C53, A2	C11, C12
C8	C41″	C41′, C41″	C10, C36, C37′, C37″, C41′, C41″	C6, C7, C9
C3	C30′	C30′, C30″, C31	C25, C26′, C26″, C31, C35	C1, C2, C4, C30, C31
R5″	R4, R5′	R3, R4		R2
R5′	R4, R5″	R3, R4		R3, R4
R2	R1		R1	R1, R4
Pr2	Pr1′, Pr1″, Pr3	Pr1′, Pr1″, Pr3	Pr1′, Pr1″, Pr3	
A12	A11, A13	A11, A13	A14, A15′, A15″, C36	
A13	A12, A14	A12, A14, A15′, A15″	A8, A14, A15′, A15″	A12, A14, A15′
R3		R5′, R5″		R1
C19	C18	C18, C60′, C60″	C18, C20, C26′, C26″, C54	C1, C16, C18, C20, C60
R4	R5′, R5″	R5′, R5″	R1	R5
R1	R2	R2, R3, R4	R2, R4, B11	R2, R3
A14	A13, A15′, A15″	A13, A15′, A15″	A11, A12, A13, A15′, A15″	A11, A13
A11	A12	A12, A13	A14, A15′, A15″, C47	A14
C10			C8, C46, C47	C8, C9, C12
B7		B11	R1	B6
B4		B10		B9
A8			A11, A12, A13, A15″, C48	A4, A5
A2			A12, A15′, C13, C47, C48′	A4, A6, A8

[a] Primes and double primes indicate downfield and upfield signals, respectively, of diastereotopic methylene groups. [b] Only direct NOEs are listed.

substantial differences in the ^{1}H chemical shifts of the diastereotopic A15 methylene protons of α- and β-AdoCbi. In β-AdoCbi, these resonances appear at 0.43 and 0.74 ppm,[30,57] while in α-AdoCbi, they appear at 0.24 and 2.23 ppm. The latter, extremely large diastereotopic shift difference (ca. 2 ppm) is much larger than that previously observed for the α methylene protons of α- and β-$HOCH_2CH_2Cbi$ (ca. 1.1 ppm).[5] Inspection of models shows that a conformation can be adopted in which one of the A15 protons is reasonably close to, and approximately in the plane of the six-membered ring of the adenosine moiety. As such, this proton could be significantly deshielded by the adenine ring current. This conformation also places this proton in reasonably close proximity to the A2 proton. The ROESY map of α-AdoCbi does indeed show the expected cross peak between the A2 proton and the more downfield of the two A15 protons, suggesting that this conformation may well be a significant contributor to the solution structure of α-AdoCbi.

There are also a number of significant ($\Delta\delta > 0.5$ ppm) ^{13}C chemical shift differences between α- and β-AdoCbi among corrin ring substituents. These include substituents on the α face (C36, C20, C26, C37) and on the β face (C30, C41, C48, C46, C55, C56, C49) and are evenly distributed between upfield and downfield shifts (for α-AdoCbi relative to β-AdoCbi) regardless of the orientation of the substituent. Of these chemical shift differences, ten fall in the range $0.5 \leq \Delta\delta \leq 1.5$ ppm, and only

(57) Inexplicably, these protons resonate at 0.38 and 1.46 ppm in base-off β-AdoCbl.[29b]

Table III. Final ^1H and ^{13}C NMR Assignments for α-5′-Deoxyadenosylcobinamide and α-5′-Deoxyadenosylcobalamin[a]

	α-AdoCbl		α-AdoCbi	
atom	$\delta_{^{13}C}$ ($\Delta\delta_{^{13}C}$),[b] ppm	$\delta_{^1H}$ ($\Delta\delta_{^1H}$),[b] ppm	$\delta_{^{13}C}$ ($\Delta\delta_{^{13}C}$),[c] ppm	$\delta_{^1H}$ ($\Delta\delta_{^1H}$),[c] ppm
A15	18.11 (−4.2)	0.24, 2.23 (−0.14, 0.78)	17.68 (−3.9)	0.25, 2.20 (−0.18, 1.46)
C53	18.26 (−0.1)	2.25 (−0.21)	18.18 (0.0)	2.37 (−0.09)
C35	18.57 (0.2)	2.42 (−0.02)	18.37 (0.2)	2.41 (−0.05)
C25	19.73 (0.3)	1.52 (0.04)	19.59 (0.4)	1.60 (0.08)
C54	21.24 (0.4)	1.04 (−0.36)	21.07 (0.5)	1.14 (−0.10)
Pr3	21.50 (0.00)	1.26 (0.03)	22.13 (−0.2)	1.15 (−0.06)
B11	22.53 (−0.2)	2.20 (−0.03)		
C47	22.71 (0.1)	1.42 (−0.35)	22.29 (0.0)	1.47 (−0.19)
B10	22.75 (0.2)	2.23 (−0.03)		
C36	22.78 (1.0)	1.37 (−0.45)	22.65 (0.7)	1.36 (−0.51)
C20	25.65 (−1.1)	1.66 (0.95)	25.36 (−1.3)	1.73 (0.82)
C30	28.28 (−0.7)	2.21, 2.54 (0.24, 0.45)	28.10 (0.2)	2.20, 2.61 (0.17, 0.43)
C41	28.28 (−1.1)	1.47, 2.21 (−0.28, 0.00)	28.10 (−1.0)	1.48, 2.20 (−0.44, −0.34)
C48	29.82 (0.9)	1.33, 1.74 (−0.59, −0.47)	29.82 (1.1)	1.37, 1.83 (−0.79, −0.59)
C46	33.20 (−1.2)	1.29 (0.29)	34.68 (0.6)	1.35 (0.43)
C56	34.92 (0.5)	2.20, 2.60 (0.15, 0.29)	36.30 (2.0)	2.15, 2.22 (0.31, −0.29)
C60	35.53 (0.3)	2.56, 2.36 (−0.23, −0.15)	36.12 (0.9)	2.58, 2.68 (−0.05, −0.05)
C49	36.17 (1.2)	2.12, 2.37 (0.16, 0.26)	36.54 (2.5)	2.22, 2.51 (0.39, 0.25)
C42	36.69 (1.5)	2.18 (−0.17)	35.41 (1.4)	2.09, 2.14 (−0.24, −0.29)
C55	36.74 (1.5)	1.87, 2.23 (0.02, −0.28)	36.54 (2.2)	1.94, 2.51 (0.10, 0.14)
C31	38.17 (0.3)	2.56 (0.01)	38.02 (0.2)	2.57 (−0.05)
C18	41.75 (−0.05)	3.01 (0.26)	41.59 (−0.4)	3.08 (0.16)
C26	45.05 (−1.2)	1.95, 2.13 (−0.51, −0.47)	45.01 (−0.7)	2.03, 2.19 (−0.39, −0.59)
Pr1	47.57 (−1.3)	3.27, 3.31 (−0.06, −0.11)	48.82 (−0.2)	3.17, 3.26 (−0.11, −0.04)
C37	47.98 (2.4)	2.27, 3.03 (0.63, 0.42)	47.83 (2.1)	2.78, 3.07 (0.99, 0.75)
C2	49.59 (2.2)		49.47 (1.0)	
C12	50.35 (0.8)		50.22 (1.1)	
C7	50.52 (−2.7)		50.36 (−2.6)	
C13	56.49 (1.1)	3.28 (0.15)	56.30 (1.2)	3.42 (−0.08)
C8	57.94 (−0.2)	3.54 (−0.19)	57.81 (−0.3)	3.55 (−0.32)
C3	59.76 (1.7)	3.80 (−0.43)	59.59 (1.6)	3.81 (−0.51)
C17	60.62 (−1.2)		60.39 (−1.3)	
R5	63.35 (−0.7)	3.77, 3.90 (−0.07, −0.04)		
R2	74.33 (−0.2)	4.77 (−0.20)		
Pr2	74.99 (−0.2)	4.38 (0.02)	68.91 (−0.2)	3.90 (−0.08)
A12	75.83 (−0.2)	4.33 (−0.01)	75.69 (0.3)	4.39 (−0.11)
A13	75.97 (1.1)	3.63 (−0.27)	75.62 (0.7)	3.72 (−0.15)
R3	77.22 (−0.4)	4.83 (0.00)		
C19	79.61 (2.0)	3.64 (−1.06)	79.46 (1.9)	3.76 (−1.01)
R4	86.52 (−2.7)	4.59 (−0.20)		
C1	86.78 (−3.0)		86.62 (−3.2)	
R1	88.62 (−1.6)	6.33 (−0.23)		
A14	88.82 (−0.1)	1.78 (−0.20)	88.81 (0.2)	1.88 (−0.17)
A11	89.58 (−0.6)	5.45 (−0.16)	89.34 (−0.9)	5.53 (−0.18)
C10	96.55 (−3.9)	6.33 (−0.64)	96.48 (−3.7)	6.39 (−0.67)
C15	108.47 (−1.3)		108.46 (−1.6)	
C5	108.85 (−2.4)		108.72 (−2.3)	
B7	113.89 (−1.8)	7.23 (−0.32)		
B4	121.38 (4.2)	7.26 (−0.18)		
A5	121.38 (−0.1)		121.35 (−0.2)	
B8	134.29 (2.2)			
B6	135.09 (−4.5)			
B5	135.91 (−3.7)			
A8	142.40 (−3.1)	7.71 (−0.50)	142.40 (−0.8)	7.85 (−0.28)
B9	142.40 (9.8)			
B2	145.20 (3.8)	8.26 (−0.9)		
A4	151.44 (0.4)		151.57 (0.0)	
A2	155.47 (7.1)	8.04 (−0.39)	155.57 (0.2)	8.18 (−0.13)
A6	158.15 (4.7)		158.30 (0.2)	
C14	165.64 (−1.1)		165.47 (−0.2)	
C6	166.70 (0.5)		166.34 (0.1)	
C9	173.88 (−1.2)		173.55 (−1.2)	
C61	178.07 (−1.0)		178.61 (−0.1)	
C4	178.50 (−0.9)		178.61 (−0.5)	
C16	178.71 (−0.7)		178.68 (−0.2)	
C38	178.71 (1.3)		178.33 (0.7)	
C11	178.87 (−0.1)		178.96 (0.0)	
C27	179.02 (−0.1)		179.12 (0.1)	
C57	179.22 (0.9)		178.09 (−0.1)	
C32	180.66 (−0.4)		180.53 (−0.6)	
C50	180.96 (0.0)		180.91 (−0.2)	
C43	181.02 (0.1)		181.17 (−0.7)	

[a] In D_2O, chemical shifts relative to internal TSP. Primes and double primes indicate downfield and upfield shifts, respectively, of diastereotopic methylene groups. [b] Difference in chemical shift between α-AdoCbl and base-off β-AdoCbl (ref 29b). Note that in the base-off β-AdoCbl spectra (pH 2.1), the axial benzimidazole ligand is protonated at B3 and the adenosyl ligand is protonated at A10, preventing direct comparison of the

chemical shifts of the axial ligands with those of α-AdoCbl. [c] Difference in chemical shift between α-AdoCbi and β-AdoCbi (ref 30).

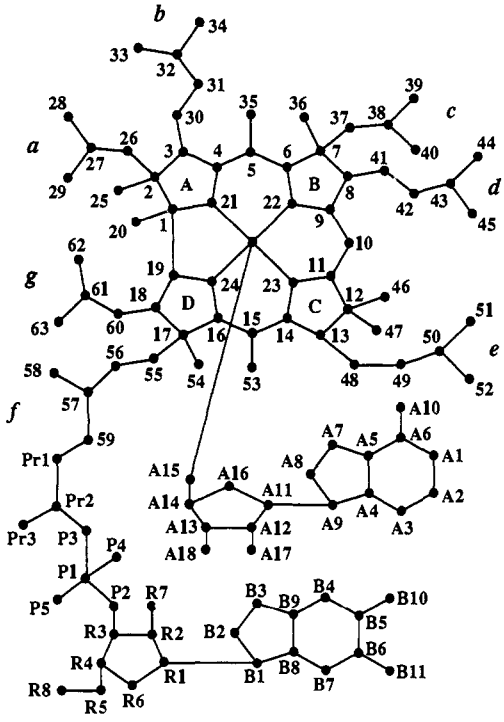
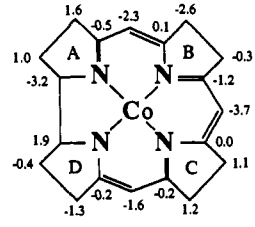


Figure 4. Numbering scheme for the α-5′-deoxyadenosylcobalt corrinoids.

one exceeds 2.0 ppm. These may simply be due to the difference in α- and β-face environment depending on which axial ligand position is occupied by the adenosyl ligand.

Far more striking are the ^{13}C chemical shift differences in the corrin ring. These differences (as $\Delta\delta = \delta_{\alpha\text{-AdoCbi}} - \delta_{\beta\text{-AdoCbi}}$ are shown in the corrin ring structure below. Of the 19 ring carbons, 13



show chemical shift differences of at least 1.0 ppm, 7 of these exceed 1.5 ppm, and four (C1, C5, C7, C10) exceed 2.2 ppm. These major changes in corrin ring carbon chemical shift are accompanied by large changes in ^1H chemical shift (i.e., at C3H, C8H, and C10H) and suggest a significant difference in corrin conformation between the α and β diastereomers. By far, the largest ^{13}C chemical shift differences in the entire molecules occur at C1 and C10 ($\Delta\delta = -3.2$ and -3.7 ppm, respectively), giving rise to the possibility of a differential "fold" of the corrin ring along the C1–C10 axis.

Conclusion

Although the mechanisms that control the diastereomeric outcome of reductive alkylation of cobalt corrinoids are still not clear,[2,10] existing evidence suggest that the diastereomeric ratio is under neither thermodynamic control nor kinetic control.[10] Nevertheless, there seems to be a trend in which reductive alkylation favors the α diastereomer when the alkyl group is electron withdrawing (R = CF_2H, CF_3, $NCCH_2$, CF_3CH_2), while the β diastereomer is favored for electron donating groups (R = CH_3, CH_3CH_2, and $ROCH_2CH_2$).[3-5] The 5′-AdoCbi's seem to fall between the two categories, producing, at least under the most favorable conditions, similar proportions of both diastereomers.

The most intriguing result of this study is that α-AdoCbi and α-AdoCbl could be produced in such high yields under reductive alkylation conditions given the fact that alkylation with other bulky alkylating agents, such as benzyl bromide and neopentyl iodide, fails to produce detectable α-RCbi's.[7] Even with the dimethylbenzimidazole nucleotide attached to the downward projecting *f* side chain in cobalamin, the net steric effect of the downward projecting side chains is still not sufficient to prevent the formation of α-AdoCbl. However, the comparison of the NMR spectra of α- and β-AdoCbi suggests a significantly different corrin ring conformation in the two diastereomers. Perhaps the corrin ring is sufficiently flexible to adopt a conformation in α-alkylcobalt corrinoids in which upward flexing of the corrin relieves the steric congestion of the *b*, *d*, *e*, and *f* side chains at the α face. If this is true, preparation of the α diastereomers of other organocobalt corrinoids with bulky alkyl groups should be possible using suitable synthetic approaches.[10] Attempts to do so, as well as to further understand steric effects on the α/β diastereomerism in organocobalt corrinoids are currently in progress.

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Supplementary Material Available: Figures S1–S4 showing the COSY, HOHAHA, ROESY, and HMBC spectra for α-AdoCbi, respectively, and Figures S5–S8 showing the COSY, HOHAHA, ROESY, and HMBC spectra for α-AdoCbl, respectively (8 pages). Ordering information is given on any current masthead page.