Preparation and Characterization of Alkyl(3,5,6-trimethylbenzimidazolyl)cobamides, Analogs of Base-Off Alkylcobalamins: Products with Defined Stereochemistry via Direct Methylation of Alkylcobalamins[1]

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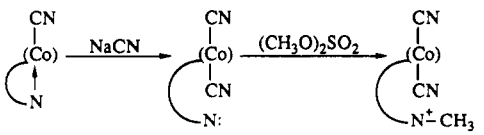
Received September 16, 1992

Reductive alkylation of cyano(3,5,6-trimethylbenzimidazolyl)cobamide ($CNMe_3BzmCba$), an analog of cyanocobalamin in which the pendant axial 5,6-dimethylbenzimidazole ligand is uncoordinated and N-methylated, in zinc/aqueous methanol with various alkylating agents (CH_3I, CH_3CH_2I, $NCCH_2Br$, CF_3CH_2I, and CF_3Br) has been shown to lead to pairs of diastereomeric α- and β-alkyl(3,5,6-trimethylbenzimidazolyl)cobamides ($RMe_3BzmCba$'s), in which the organic ligand occupies the "lower" or "upper" axial ligand position, respectively. In the case of CF_3Br, reductive alkylation of $CNMe_3BzmCba$ in zinc/aqueous acetic acid also leads to a pair of diastereomeric α- and β-$CF_2HMe_3BzmCba$'s due to the reductive defluorination of the $CF_3Me_3BzmCba$'s formed in situ. The ratio of the diastereomers formed varies widely with R from 2:98 (α:β) for R = CH_3CH_2 to 93:7 for R = CF_3. In order to provide a stereocontrolled route to the β-$RMe_3BzmCba$'s, which serve as convenient models of the base-off species of β-alkylcobalamins (β-RCbl's), direct N-methylation of β-RCbl's with dimethyl sulfate in excess cyanide has been investigated. For those organocobalt corrinoids which are stable in excess cyanide (R = CH_3CH_2, $NCCH_2$, CF_3, and CF_2H) this route provides uniquely the β-$RMe_3BzmCba$ in yields of 76-86%. For β-CH_3Cbl, which undergoes partial decomposition in cyanide, the yield was only 20%, while for β-CF_3CH_2Cbl, which undergoes massive decomposition in cyanide, no β-$CF_3CH_2Me_3BzmCba$ could be obtained by this route. The latter compound, as well as β-$CH_3CH_2Me_3BzmCba$, was obtained, in 20% yield, by N-methylation of β-CF_3CH_2Cbl or β-CH_3CH_2Cbl in the absence of cyanide in refluxing methanol.

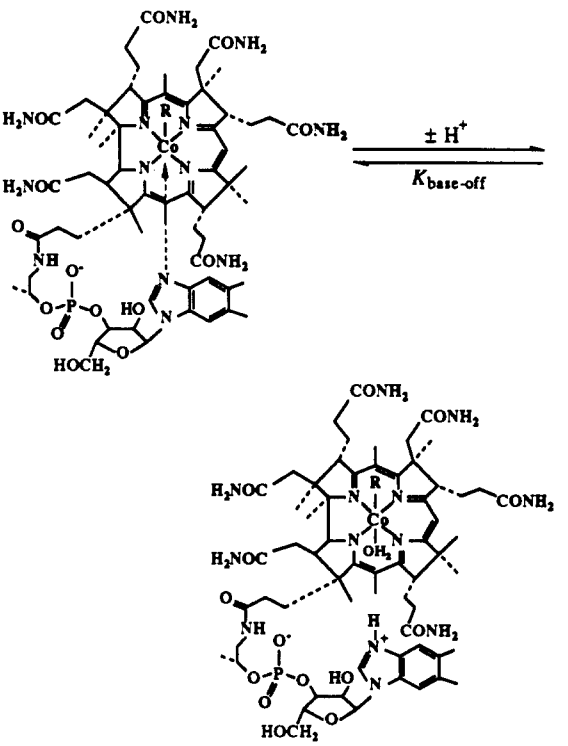
Introduction

The (3,5,6-trimethylbenzimidazolyl)cobamides ($Me_3BzmCba$'s[1]), analogs of cobalamins in which the axial 5,6-dimethylbenzimidazole ligand is N-methylated (Figure 1), were first described by Friedrich and Bernhauer (as the so-called factor B_{12} Nm) in 1956.[4] These authors synthesized the N-methylated derivative of CNCbl, $CNMe_3BzmCba$,[1,3] by reaction of CNCbl with dimethyl sulfate in excess cyanide (eq 1), since under these conditions the axial 5,6-dimethylbenzimidazole nucleotide of CNCbl is displaced by cyanide to form $(CN)_2Cbl$, thus freeing the coordinating nitrogen nucleophile for methylation.



(1)

The $Me_3BzmCba$'s serve as excellent models of the "base-off" forms of cobalamins in which the axial 5,6-dimethylbenzimidazole nucleotide is uncoordinated and protonated (eq 2). Depending on the nature of the trans



(2)

(or β) axial ligand, the value of $pK_{base\text{-}off}$ (eq 2) can vary from 4.16 to -2.13.[5-8] Thus, the $Me_3BzmCba$'s can mimic

(1) IUPAC-IUB[2] nomenclature is used throughout. Abbreviations: $CNMe_3BzmCba$, monocyanomonoaquo(3,5,6-trimethylbenzimidazolyl)-cobamide;[3] β-$RMe_3BzmCba$, Coα-(α-3,5,6-trimethylbenzimidazolyl)-Coβ-alkylcobamide; α-$RMe_3BzmCba$, Coα-alkyl-Coβ-aquo(α-3,5,6-trimethylbenzimidazolyl)cobamide; β-RCbl, β-alkylcobalamin; α-RCbl, α-alkylcobalamin; β-AdoCbl, 5'-deoxyadenosylcobalamin; β-RCbi, β-alkylcobinamide; α-RCbi, α-alkylcobinamide; H_2OCbl, aquocobalamin; β-Ado-$Me_3BzmCba$, Coα-(α-3,5,6-trimethylbenzimidazolyl)-Coβ-5'-deoxyadenosylcobamide; CNCbl, cyanocobalamin.

(2) *Biochemistry* **1974**, *13*, 1555.

(3) $CNMe_3BzmCba$ is a mixture of diastereomeric α-CN-β-$(H_2O)Me_3BzmCba$ and α-H_2O-β-$(CN)Me_3BzmCba$.

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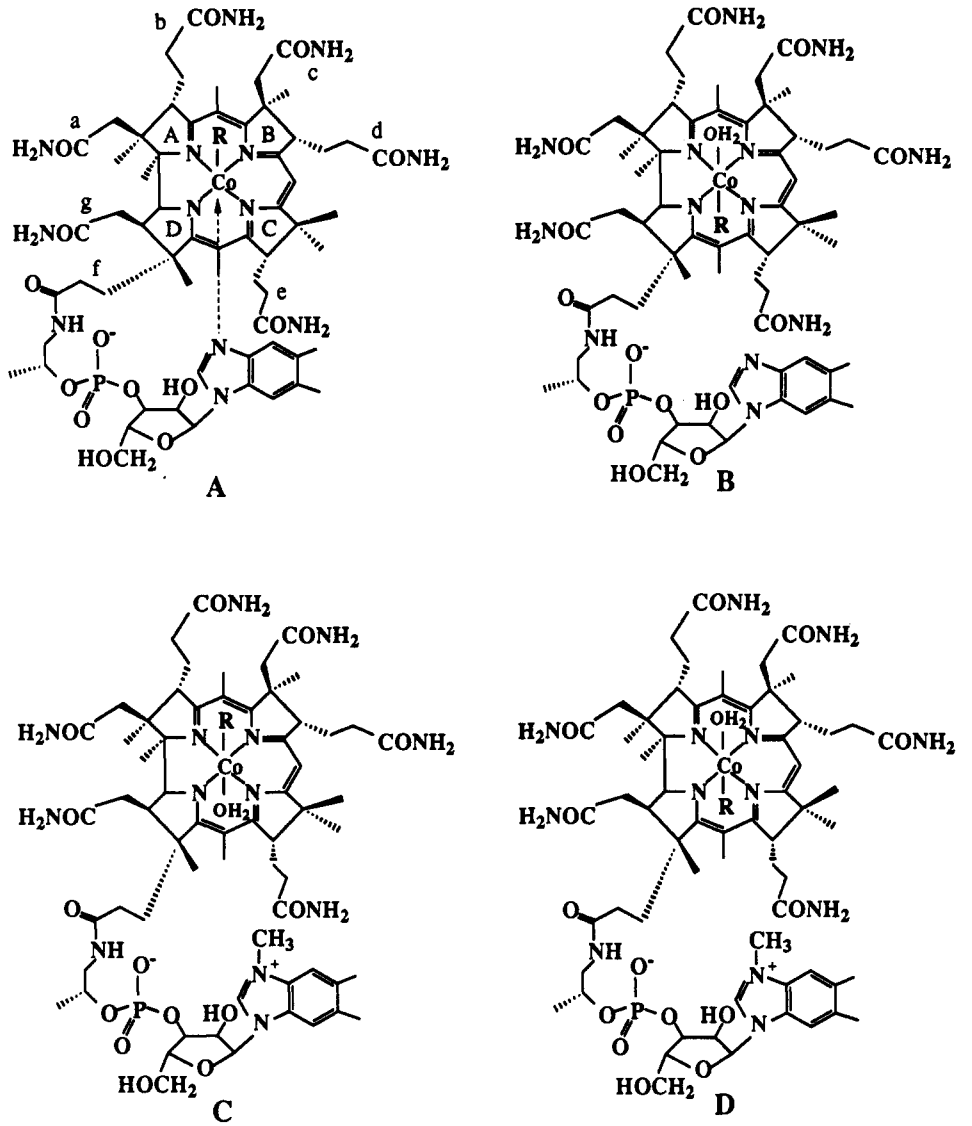

Figure 1. Structure of a base-on β-RCbl (A), an α-RCbl (B), a β-RMe$_3$BzmCba (C), and an α-RMe$_3$BzmCba (D). In a base-off β-RCbl, the axial 5,6-dimethylbenzimidazole ligand is uncoordinated and protonated (see eq 2).

the properties of base-off cobalamins in neutral solution, avoiding the sometimes destructive necessity of lowering the pH sufficiently to trap the dissociated 5,6-dimethylbenzimidazole nucleotide by protonation. As such, they have been used to make NMR assignments and to probe the solution structure of base-off alkylcobalamins,[9,10] to probe the structural requirements of the coenzyme form of vitamin B_{12}, 5′-deoxyadenosylcobalamin, for binding to the enzyme glycerol dehydratase,[11] and to probe the mechanism and determine the kinetic parameters for the dealkylation of methylcobalamin by iridium, iron, gold, mercury, and platinum electrophiles.[12-17] Despite this utility, only a few alkyl derivatives of Me$_3$BzmCba seem to have been reported, namely, the methyl,[9] carboxymethyl,[9] and 5′-deoxyadenosyl[11] derivatives.

Recent work[18-23] has shown that α diastereomers of alkylcobalt corrinoids, in which the organic ligand occupies the "lower" axial ligand position (Figure 1), once thought to be rare,[7,23-31] in fact occur quite generally when reductive alkylation is performed under conditions (or with cor-

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rinoids) in which the "lower", or α, axial ligand position is free. For instance, when cobinamides, derivatives of vitamin B_{12} in which the axial 5,6-dimethylbenzimidazole nucleotide has been chemically removed, are reductively alkylated, mixtures of α- and β-RCbi's[1] are obtained in which the diastereomeric ratio can vary from <2:98 (α:β) to 98:2.[18,19,21,22] Similarly, when H_2OCbl is reductively alkylated under sufficiently acidic conditions so that the product β-RCbl is primarily base-off, significant amounts of α-RCbl's can also be obtained.[18-22] We thus anticipated that reductive alkylation of $Me_3BzmCba$ should, in general, produce pairs of diastereomeric α- and β-$RMe_3BzmCba$'s. As detailed below, this is indeed the case. However, β-$RMe_3BzmCba$'s of defined stereochemistry can, in many cases, be obtained by direct methylation of β-RCbl's.

Experimental Section

Materials. Aquocobalamin was from Roussell, and alkyl halides (CH_3I, CH_3CH_2I, $NCCH_2Br$, CF_3CH_2Br, CF_3Br) were from Aldrich. Dimethyl sulfate was from Kodak, and Amberlite XAD (type 2, particle size 0.05–0.1 mm) was from Serva. $CNMe_3BzmCba$[1,3] was prepared by a modification of the method of Friedrich and Bernhauer[4] as follows. CNCbl (1.00 g, 0.738 mmol), $NaCO_3$ (132.5 g, 1.25 mol), and NaCN (2.00 g, 40.8 mmol) were dissolved in 1.0 L of water. At 40 °C and with mechanical stirring, dimethyl sulfate (100 mL, 1.06 mol) was added dropwise over a period of 4 h. After an additional 1 h of stirring, the reaction mixture was loaded onto a 2 × 13 cm column of XAD-2 for desalting.[5] The column was washed with water (200 mL), 0.01 M HCl (150 mL), and then water (150 mL), and the corrinoids were eluted with 50% aqueous CH_3CN. The CH_3CN was removed by rotary evaporation, and the unreacted CNCbl was separated by chromatography on SP-Sephadex[32,33] (Na^+ form), eluting with water (181 mg, 18% recovered CNCbl). The $Me_3BzmCba$ was eluted with 0.1 M NaCl. It was desalted by chromatography on Amberlite XAD-2, as above, quantitated as described below, and stored frozen in aqueous solution (yield, 628 mg, 61%).

The reductive alkylation of $CNMe_3BzmCba$ with liquid alkyl halides was carried out as follows. In a hood in the dark, $CNMe_3BzmCba$ (8–18 mg, 5.76–13.0 μmol) in 4 mL of 75% (v/v) methanol/water was deoxygenated by argon purge for 1 h. Zinc wool (0.25–0.6 g), freshened by brief stirring in 1 M HCl followed by water washing, was added and reduction of the corrinoid was allowed to proceed for 1–1.5 h. A 225-fold molar excess of liquid alkyl halide was then added by syringe, and alkylation was permitted to proceed for 30 min. The reaction mixture was removed from the flask by cannula (under argon pressure), filtered, and desalted by chromatography on Amberlite XAD-2, as above. After removal of the CH_3CN and concentration by rotary evaporation, the products were separated by semipreparative HPLC as described below. The isolated $RMe_3BzmCba$'s were desalted by chromatography on Amberlite XAD-2, concentrated, quantitated, and stored frozen in aqueous solution. Total yields (both diastereomers) varied from 59% to 92% by HPLC, while isolated yields varied from 39 to 76%.

The diastereomers of $CF_3Me_3BzmCba$ were prepared similarly, except that the gaseous alkylating agent (CF_3Br) was introduced by bubbling into the reaction mixture for 5 min, after which the reaction mixture was removed by cannula. After purification, as above, 50% alkylated corrinoid (isolated yield) was obtained. Under these conditions, unlike those which follow, defluorination of the (trifluoromethyl)cobalt corrinoids to (difluoromethyl)-cobalt corrinoids[19,34] does not occur.

The diastereomers of $CF_2HMe_3BzmCba$ were obtained similarly, except that the medium was 10% aqueous acetic acid. After CF_3Br was introduced into the reaction mixture by bubbling for 5 min, the solution was further stirred for 50 min under argon purge to permit defluorination of the $CF_3Me_3BzmCba$'s to $CF_2HMe_3BzmCba$'s. After purification, as above, the diastereomeric $CF_2HMe_3BzmCba$'s were obtained in 23% yield while the diastereomeric $CF_3Me_3BzmCba$'s were obtained in 9% total yield (5% α diastereomer and 4% β diastereomer).

Direct methylation of the β-RCbl's (R = CH_3, CH_3CH_2, $NCCH_2$, CF_2H, CF_3) was carried out as follows. In a 15-mL round-bottomed flask, $NaHCO_3$ (50 mmol) or $NaCO_3$ (25 mmol), NaCN (10 mmol), and β-RCbl (25–30 mg, 16.7–21.4 μmol) were mixed with 10 mL H_2O and warmed to 40 °C. With mechanical stirring, 5.25–4.0 mL (34–42 mmol) dimethyl sulfate was added periodically, 150 μL at a time using a syringe, at 10-min intervals over a period of 4–5 h. *Caution, dimethyl sulfate is a highly toxic, cancer suspect agent and must be handled appropriately.* After the addition of dimethyl sulfate was complete, the reaction mixture was stirred at 40 °C for an additional 1 h, filtered, and loaded onto a 1 × 3 cm column of Amberlite XAD-2 for desalting. The column was washed with 300 mL H_2O, 100 mL 0.1 M HCl, and another 100 mL H_2O. The corrinoids were eluted with 50% (v/v) aqueous CH_3CN. After concentration and removal of the CH_3CN by rotary evaporation, the product was purified by HPLC as described below. The isolated yields of β-$RMe_3BzmCba$ were 55–70%, except for R = CH_3, where the yield was 25%, and 45% unreacted β-CH_3Cbl was recovered.

Methods. UV–visible spectra were obtained on a Cary 219 recording spectrophotometer. Cobalt corrinoids were quantitated spectrophotometrically by conversion to their dicyano derivatives by aerobic photolysis in excess cyanide, using $\epsilon_{368} = 3.04 \times 10^4$ M^{-1} cm^{-1}.[35,36]

Analytical HPLC was performed on a 0.46 × 25 cm Beckman C_8 Ultrasphere column using a 50 mM aqueous ammonium phosphate buffer, pH 3.0 (solvent A) and acetonitrile (solvent B) and a flow rate of 2 mL/min. The solvent program used was 5% B for 0.5 min, followed by a linear increase to 20% B over 1 min, then isocratic elution at 20% B for 5.1 min, followed by a linear increase to 30% B over 1 min. Elution at 30% B was continued for 3.4 min and then starting conditions were reestablished by a linear gradient to 5% B over 1 min. The HPLC yields and diastereomer ratios were determined from integration of the chromatogram at 254 and 350 nm, using the molar absorptivities determined from the isolated compounds. Mobilities (T_R) are reported as retention times relative to that of CNCbl (4.60 min). Semipreparative HPLC was performed similarly on a 1 × 25 cm Beckman C_8 Ultrasphere column at a flow rate of 6 mL/min. The solvents and solvent program used were the same as those for analytical HPLC except that the content of acetonitrile at the 5.1-min isocratic elution stage was adjusted to between 18% and 23%.

FAB-MS was performed on a Kratos MS80RFA mass spectrometer with an Ion Tech FAB 11NF FAB source using 5–6 kV argon bombardment. Samples contained 10 μg/μL organocobalt corrinoid in a *m*-nitrobenzyl alcohol matrix.[37,38] ^{19}F NMR spectra were obtained on a GE QE300 NMR spectrometer operating at 282.894 MHz. Samples were dissolved in 30% D_2O, and chemical shifts were referenced to external fluorobenzene.

Results and Discussion

Reductive Alkylation of $CNMe_3BzmCba$. As anticipated from previous work with cobinamides,[18-22] re-

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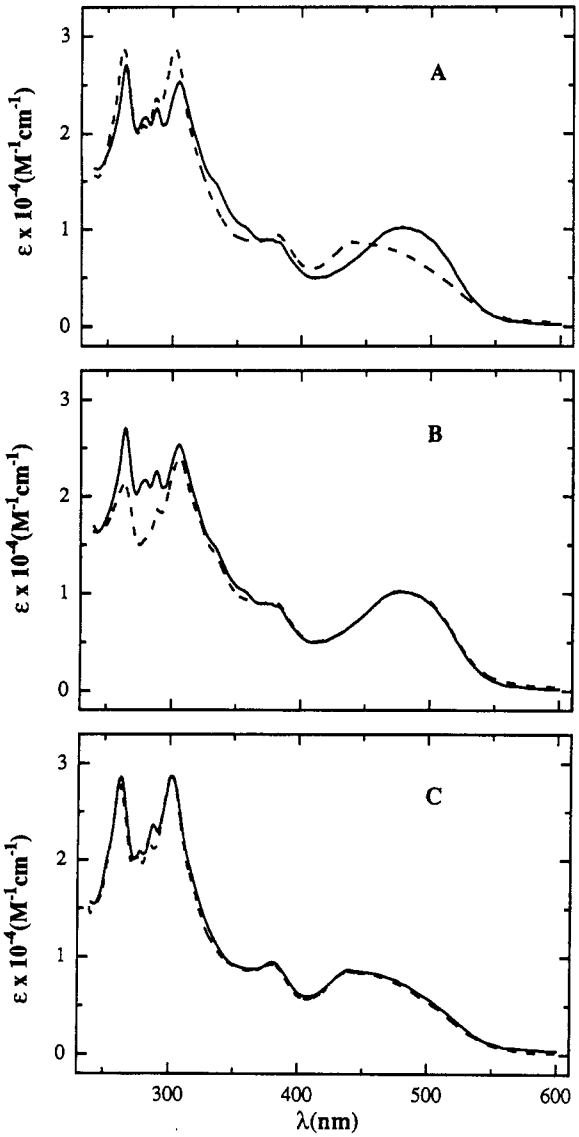
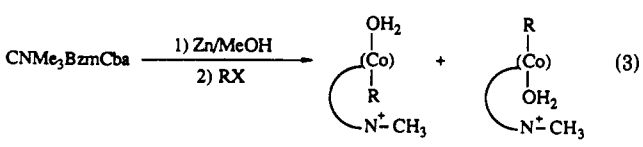


Figure 2. UV–visible spectra of (A) α-$CH_3CH_2Me_3BzmCba$ (solid line) and β-$CH_3CH_2Me_3BzmCba$ (dashed line) in 0.1 M potassium phosphate buffer, pH 7.0, (B) α-$CH_3CH_2Me_3$-BzmCba in 0.1 M potassium phosphate buffer (solid line) and α-CH_3CH_2Cbl in neutral aqueous solution (dashed line), (C) β-$CH_3CH_2Me_3BzmCba$ in 0.1 M potassium phosphate buffer, pH 7.0 (solid line), and β-CH_3CH_2Cbl in 0.2 M HCl (dashed line).

ductive alkylation of $CNMe_3BzmCba$ in Zn/methanol, in general, yielded two photolabile organocobalt corrinoids which could be positively identified (vide infra) as the α and β diastereomers (Figure 1) of $RMe_3BzmCba$ (eq 3).



(3)

The products were readily separable by semipreparative HPLC and could be characterized by uv-visible spectroscopy. As an example, Figure 2A shows the spectra of the two products obtained from reductive alkylation of $CNMe_3BzmCba$ with CH_3CH_2I. The spectral relationship between the two compounds is the same as that between a pair of α- and β-RCbi's; i.e., the spectra are quite similar in that most of the transitions occur at the same (or nearly the same) wavelengths except for the longest wavelength (or α) band, which is red-shifted by 34 nm in the complex which had the shorter HPLC retention time. This is the same red shift previously seen for the α band of α-CH_3CH_2Cbi relative to that of β-CH_2CH_2Cbi.[39] The spectral relationship in neutral H_2O between the putative α-$CH_3CH_2Me_3BzmCba$ and α-CH_3CH_2Cbl[39] is shown in Figure 2B. The spectra are essentially identical above 300 nm and the spectral differences in the UV are in accord with the difference between the spectrum of the detached 5,6-dimethylbenzimidazole nucleoside, α-ribazole, and the spectrum of its conjugate acid.[5] Indeed, the spectrum (not shown) of α-CH_3CH_2Cbl in 0.01 M HCl, where the pendant nucleotide is protonated, is virtually identical to that of the putative α-$CH_3CH_2Me_3BzmCba$. Figure 2C shows that the other organocobalt corrinoid product from the reductive alkylation of $CNMe_3BzmCba$ has the same spectrum as β-CH_3CH_2Cbl in 0.1 M HCl, where the latter exists entirely as the protonated, base-off species (eq 2). This permits identification of the second product as β-$CH_3CH_2Me_3BzmCba$, in which the coordination of the 5,6-dimethylbenzimidazole ligand is prevented by its N-methylation.

The identities and relationship of the two $CH_3CH_2Me_3BzmCba$'s were further confirmed by FAB MS. As is the case for the RCbi's,[18,19,21,40] the cationic $RMe_3BzmCba$'s are pentacoordinate in positive ion FAB mass spectra and thus occur at $M^+ - H_2O$. The two $CH_3CH_2Me_3BzmCba$'s had parent ions at *m/e* 1373.5 (α) and 1373.6 (β), while the calculated value for $M^+ - H_2O$ is 1373.5. These results confirm both the identity of the two products as $CH_3CH_2Me_3BzmCba$'s and the isomeric relationship between them.

Similar results were obtained when $CNMe_3BzmCba$ was reductively alkylated with CH_3I, $NCCH_2Br$, CF_3CH2I, and CF_3Br. Yields, diastereomer ratios, HPLC retentions, FAB MS parent ions, and the wavelengths and molar absorptivities of the α band of these products are given in Table I. Only in the case of CF_3Br do the results differ appreciably from those previously obtained for the reductive alkylation of cobinamides.[18,19] We have previously reported[19,34] that reductive alkylation of cobinamide with CF_3I of CF_3Br in Zn/10% acetic acid leads to mixtures of the diastereomeric α- and β-CF_3Cbi's and the α- and β-CF_2HCbi's. The formation of the latter has been shown to be due to reductive defluorination of the CF_3Cbi's in Zn/acid media, a process which appears to proceed through a two-electron-reduced CF_2–Co carbenoid-type intermediate.[34] The current results show that this defluorination of CF_3–cobalt corrinoids does not occur when the reducing agent is Zn/methanol. Thus, reductive alkylation with CF_3Br under these conditions leads exclusively to the diastereomeric $CF_3Me_3BzmCba$'s. In order to prepare the $CF_2HMe_3BzmCba$'s, reductive alkylation of $CNMe_3BzmCba$ was carried out in Zn/10% acetic acid. After the alkylating agent was bubbled into the reaction mixture for 5 min, the addition of CF_3Br was stopped, and the reaction mixture was stirred for an additional 50 min to permit reductive defluorination of the initially generated $CF_3Me_3BzmCba$'s to occur. Unfortunately, as (trifluoromethyl)cobalt corrinoids also undergo reductive dealkylation in Zn/acid media,[34] the competition between dealkylation and de-

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Table I. Yields, Diastereomer Ratios, HPLC Retention Times, and Spectral Properties of the Alkyl(3,5,6-dimethylbenzimidazolyl)cobamides Obtained by Reductive Alkylation of CNMe$_3$BzmCba[a]

RX	products	total yield (%)[b]	α:β ratio[b]	T_R[c]	m/e (calc)[d]	λ_{max} (nm)[e] ($10^4\epsilon$, M^{-1} cm^{-1})
CH_3I	α-CH_3Me$_3$BzmCba, β-CH_3Me$_3$BzmCba	92	16:84	1.49	1359.0 (1359.5)	492 (1.11)
				2.27	1359.3 (1359.5)	460 (0.97)
CH_3CH_2I	α-CH_3CH_2Me$_3$BzmCba, β-CH_3CH_2Me$_3$BzmCba	91	2:98	1.74	1373.5 (1373.5)	476 (1.01)
				2.29	1373.6 (1373.5)	442 (0.87)
$NCCH_2Br$	α-$NCCH_2$Me$_3$BzmCba, β-$NCCH_2$Me$_3$BzmCba	82	71:29	1.24	1384.2 (1384.4)	488 (0.96)
				1.86	1384.8 (1384.4)	480 (0.88)
CF_3CH_2I	α-CF_3CH_2Me$_3$BzmCba,[f] β-CF_3CH_2Me$_3$BzmCba[g]	59	75:25	2.16	1427.6 (1427.4)	484 (1.00)
				2.42	1427.4 (1427.4)	454 (0.92)
CF_3Br	α-CF_3Me$_3$BzmCba,[h] β-CF_3Me$_3$BzmCba[i]	50	93:7	1.41	1413.7 (1413.4)	510 (0.98)
				2.43	1413.2 (1413.4)	510 (0.88)
CF_3Br[j]	α-CF_2HMe$_3$BzmCba,[k] β-CF_2HMe$_3$BzmCba[l]	41	67:33	1.33	1395.3 (1395.4)	485 (0.98)
				2.33	1395.3 (1395.4)	454 (1.05)

[a] Reductive alkylation with RX in Zn/75% methanol, except as noted. [b] Yields and diastereomer ratios determined from integrations of HPLC chromatograms at 254 and 350 nm using the molar absorptivities of the isolated complexes. Isolated yields were somewhat lower (see Experimental Methods). [c] HPLC retention time relative to that of CNCbl (4.60 min). [d] FAB-MS parent ion m/e calculated for loss of the (presumed) axial water ligand, i.e., $M^+ - H_2O$. [e] Wavelength and molar absorptivity of the lowest energy, or α, band of the UV–visible spectrum. [f] ^{19}F NMR: t, 59.9 ppm (relative to fluorobenzene), $^3J_{HF}$ = 14.6 Hz. [g] ^{19}F NMR: t, 57.5 ppm (relative to fluorobenzene), $^3J_{HF}$ = 14.6 Hz. [h] ^{19}F NMR: s, 90.7 ppm (relative to fluorobenzene). [i] ^{19}F NMR: s, 86.5 ppm (relative to fluorobenzene). [j] Reductive alkylation performed in Zn/10% acetic acid. See text. [k] ^{19}F NMR: A-B q of d's, 23.2 and 26.0 ppm (relative to fluorobenzene), $^2J_{F_AF_B}$ = 158.9 Hz and $^2J_{HF}$ = 54.4 Hz. [l] ^{19}F NMR: A-B q of d's, 24.9, 27.0 ppm (relative to fluorobenzene), $^2J_{F_AF_B}$ = 149.2 Hz and $^2J_{HF}$ = 53.8 Hz.

fluorination limits the yield of the CF_2HMe$_3$BzmCba's.[41] Data for these compounds are also given in Table I.

Each of the pairs of diastereomeric RMe$_3$BzmCba's had the same spectral relationship as that shown in Figure 2 for the CH_3CH_2Me$_3$BzmCba's. In each case, the spectra of the α-RMe$_3$BzmCba differed from that of the analogous α-RCbl only in the UV and was essentially identical to that of the α-RCbl in acid. The spectrum of the β-RMe$_3$BzmCba was identical to that of the protonated, base-off β-RCbl. In addition, for all R except CF_3, the lowest energy band of the UV–visible spectrum of the α-RMe$_3$BzmCba (Table I) is red-shifted relative to that of the β-RMe$_3$BzmCba by 8–34 nm, depending on R, as previously seen for the α- and β-RCbi's.[19] The HPLC retention time (Table I) for each α-Me$_3$BzmCba is also shorter than that of its β diastereomer, as is the case for the α- and β-RCbi's. All of the RMe$_3$BzmCba's had positive ion FAB MS parent ions within acceptable experimental error of the mass calculated for the five-coordinate species, $M^+ - H_2O$. These FAB parent ion masses further confirm the isomeric relationship between each pair of α- and β-RMe$_3$BzmCba's.

The fluoromethyl(3,5,6-trimethylbenzimidazolyl)cobamides were further characterized by ^{19}F NMR (chemical shifts reported relative to external fluorobenzene). The ^{19}F NMR resonances of the α- and β-CF_3Me$_3$BzmCba's were each singlets with the resonance of the α diastereomer (90.3 ppm) appearing nearly 4 ppm downfield from that of the β diastereomer (86.5 ppm). The ^{19}F chemical shifts for the α- and β-CF_3Cbi's were 90.7 and 86.6 ppm, respectively.[19] As was also the case for the CF_2HCbi's, the CF_2HMe$_3$BzmCba ^{19}F resonances were A–B quartets of doublets reflecting the diastereotopic nature of the two fluorine atoms and their coupling to the alkyl ligand hydrogen. For the α diastereomer, the fluorine resonances occurred at 23.2 and 26.0 ppm, with $^2J_{F_AF_B}$ = 158.9 Hz and $^2J_{HF}$ = 54.4 Hz. The analogous values for the β diastereomer were 24.9 and 27.0 ppm, with $^2J_{F_AF_B}$ = 149.2 Hz and $^2J_{HF}$ = 53.8 Hz. All of these values are quite close to those for α- and β-CF_2HCbi.[19] The ^{19}F NMR resonances of α- and β-CF_3CH_2Me$_3$BzmCba were triplets at 59.9 ppm ($^3J_{HF}$ = 14.6 Hz) and 57.5 ppm ($^3J_{HF}$ = 14.6 Hz), respectively, virtually identical to those of α- and β-CF_3CH_2Cbi.[19]

The ratio of diastereomers obtained upon reductive alkylation of CNMe$_3$BzmCba (Table I) varies widely with R, with as little as 2% or as much as 93% of the product being α diastereomer. The diastereomer ratios obtained here for the RMe$_3$BzmCba's are quite similar to those previously obtained for reductive alkylation of cobinamide,[18,19,21] the only significant exception being for the CH_3 complex, for which 16% of the product was α diastereomer in the Me$_3$BzmCba's while only 4% α diastereomer was obtained in the Cbi system. In general, the factors controlling the diastereomer ratio during reductive alkylation are not understood, except that it is now clear that the CH_3Cbi's reach equilibrium during synthesis, apparently via cobalt-to-cobalt methyl group transfer to either cob(I)inamide or cob(II)inamide.[48] Thus, the difference in diastereomer ratio for the CH_3Me$_3$BzmCba's and the CH_3Cbi's could reflect a medium effect on the equilibrium constant for diastereomerism since the CH_3Cbi synthesis was done in Zn/10% acetic acid while the CH_3Me$_3$BzmCba synthesis was done in Zn/75% methanol. However, it is clear that equilibrium is not attained for any of the other pairs of RCbi diastereomers during reductive alkylation.[48] Instead, these products reach a nonequilibrium steady state. While the factors controlling the composition of this steady state are not yet completely clear, the similarity of the diastereomer ratios for the RMe$_3$BzmCba's and the RCbi's suggests that the same factors are controlling the outcome of reductive alkylation in both systems.

Direct Methylation of β-RCbl's. Although the yields from the reductive alkylation of CNMe$_3$BzmCba are, in most cases, quite good, for many R groups the α diastereomer is the predominant product, sometimes substan-

(41) Many organocobalt corrinoids undergo reductive dealkylation due to homolysis of the Co–C bond of reduced R-Co^{II} species.[18-20,42-47] The (difluoromethyl)cobalt corrinoids, however, are stable to zinc reductants, BH_4^-, and controlled-potential reduction at potentials as low as −1.2 V.[34]

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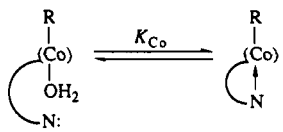
Table II. Conditions and Yields of β-RMe$_3$BzmCba's from the Direct Methylation of β-RCbl's[a]

RCbl	molar excess $(CH_3O)_2SO_2$	product	K_{CN} (M^{-1})[b]	yield (%)[c]	red. alkylation yield (%)[d]
β-CH_3CH_2Cbl	2050	β-$CH_3CH_2Me_3$BzmCba	0.665	86 (55)	74
β-$NCCH_2$Cbl	1780	β-$NCCH_2Me_3$BzmCba	63.8	80 (65)	20
β-CF_3Cbl	825	β-CF_3Me_3BzmCba		85 (70)	4
β-CF_2HCbl	1725	β-CF_2HMe_3BzmCba	3.27	76 (66)	7
β-CH_3Cbl	2230	β-CH_3Me_3BzmCba		25 (25)[e]	60
β-CF_3CH_2Cbl	15,450	*f*		0 (0)	

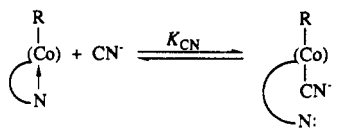
[a] In aqueous solution (10 mL) containing 10 mmol NaCN and either 25 mmol Na_2CO_3 or 50 mmol $NaHCO_3$. [b] Equilibrium constant for the displacement of the pendant 5,6-dimethylbenzimidazole ligand from the β-RCbl by cyanide (eq 5). Values from ref 50. [c] By HPLC. Isolated yields in parentheses. [d] Isolated yield of the β-RMe$_3$BzmCba from reductive alkylation of CNMe$_3$BzmCba. [e] 18.5% CNMe$_3$BzmCba was recovered from the reaction mixture. [f] After 1 h only CNMe$_3$BzmCba was present.

tially so (Table I). Since it is the β-RMe$_3$BzmCba's which are of the most interest as models of base-off β-RCbl's, we have attempted the direct N-methylation of the axial 5,6-dimethylbenzimidazole ligand of β-RCbl's as a stereospecific route to the β-RMe$_3$BzmCba's. As the equilibrium constants for the binding of the pendant, free base 5,6-dimethylbenzimidazole ligand to the cobalt atom of the β-RCbl's, K_{Co} (eq 4), are in general fairly large (K_{Co} = 20



(4)

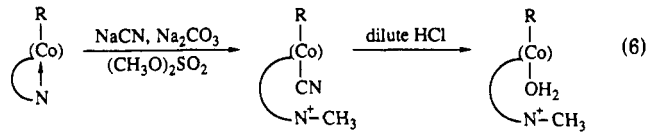
to 1.32 × 10^4 for various R),[8] the availability of the free base nucleotide for N-methylation in neutral solution is quite poor. However, cyanide ion is known to bind reasonably tightly trans to the organic ligand in RCbi's (K_{eq} = (4.3–2.8) × 10^5 M^{-1}).[49] This binding is, in many cases, sufficiently strong to displace the axial 5,6-dimethylbenzimidazole ligand of β-RCbl's, values of K_{CN} (eq 5) varying from 0.665 to 63.8 M^{-1} for various R.[50]



(5)

Unfortunately, a number of organocobalt corrinoids, including β-AdoCbl and other 5′-deoxyadenosylcobalt corrinoids,[51-53] (carbomethoxymethyl)-, (carbomethoxyethyl)-, and (2-cyanoethyl)cobalamins,[54,55] and α- and β-CF_3CH_2Cbi, as well as others,[49] are labile toward dealkylation by cyanide in the dark. However, other complexes, including β-CH_3CH_2Cbl, β-$NCCH_2$Cbl, and β-CF_2HCbl,[50] are known to be sufficiently stable toward cyanolysis to permit the possibility of direct methylation of the pendant 5,6-dimethylbenzimidazole moiety in excess cyanide.

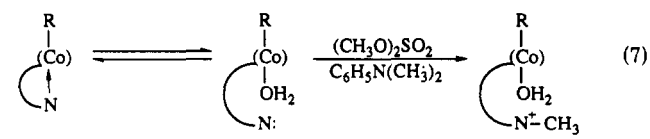
The direct methylation of a number of β-RCbl's was thus attempted as shown in eq 6. Since most of the values of K_{CN} (eq 5) are quite small, these methylations were carried out in 1.0 M NaCN. When a 800–2200-fold excess of dimethyl sulfate was added to β-RCbl (R = CH_3CH_2, $CNCH_2$, CF_3, CF_2H) at 40 °C in 1.0 M aqueous NaCN



(6)

with buffering $NaHCO_3$ or Na_2CO_3 (to prevent the reaction mixture from going acidic due to dimethyl sulfate hydrolysis) over a period of 4–5 h, the respective β-RMe$_3$BzmCba was obtained in yields (by HPLC) of 76–86% (Table II). Although isolated yields were somewhat lower (Table II), they are clearly adequate to make this a valuable synthetic procedure. Most importantly, isolated yields of ≥65% could be obtained for R = $NCCH_2$, CF_3, and CF_2H, for which the isolated yields of the β-RMe$_3$BzmCba from the reductive alkylation of CNMe$_3$BzmCba were only 20%, 4%, and 7%, respectively. For β-CH_3Cbl, the yield of β-CH_3Me_3BzmCba by direct methylation (eq 6) was poor (25%), apparently due to dealkylations by cyanide, as substantial CNMe$_3$BzmCba was obtained in the reaction mixture. As expected, direct methylation of β-CF_3CH_2Cbl failed to produce any product due to the known lability of this complex toward cyanolysis in the dark.[49,50]

In order to attempt to provide a direct, stereocontrolled route to the β-RMe$_3$BzmCba's for complexes which are labile toward cyanolysis, attempts were also made to carry our direct methylation of the axial nucleotide of β-RCbl's in the absence of cyanide. These were based on the fact that although the equilibrium governed by K_{Co} (eq 4) is displaced well toward the base-on species, the base-off, but benzimidazole deprotonated, species of β-RCbl does exist in neutral solution and is favored at higher temperatures (i.e., $\Delta H_{Co} < 0$).[5,8] Thus, direct methylation in the absence of cyanide (eq 7) was attempted for β-CF_3CH_2-



(7)

Cbl. When this complex as treated with a 4900-fold excess of dimethyl sulfate in refluxing methanol containing 6 M *N,N*-dimethylaniline as a buffer over 3 h, a 20% yield of β-$CF_3CH_2Me_3$BzmCba was obtained. Unfortunately, this barely exceeded the 16% isolated yield of this complex from the reductive alkylation of CNMe$_3$BzmCba with CF_3CH_2I. A similar attempt to methylate β-CH_3CH_2Cbl via eq 7 also gave a 20% yield, far below the 74% isolated yield from ethylation of CNMe$_3$BzmCba.

In summary, reductive alkylation of CNMe$_3$BzmCba with various alkyl halides has been shown to produce mixtures of diastereomeric α- and β-RMe$_3$BzmCba's, as was shown to be the case with the reductive alkylation of cobinamides in earlier work.[18,19,21] The ratio of diaster-

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eomers depends strongly on R, and in some cases very little of one of the two diastereomers is obtained. Direct methylation of β-RCbl's in the presence of excess cyanide does provide a stereocontrolled route to β-RMe_3BzmCba's for those complexes which are stable toward cyanolysis and in several cases provided a much better yield of the β-RMe_3BzmCba than reductive alkylation of $CNMe_3$-BzmCba. Direct methylation of β-RCbl's in refluxing methanol in the absence of cyanide is also feasible but provides β-RMe_3BzmCba's only in low yield.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society (grant no. 23783-AC), for the support of this research.

OM920566Z