a final 16s13p5d/10s9p5d set yielding a CHF polarizability of 1455.7 au. Since the Na^- polarizability is dominated by the contribution of the two 3s electrons which acquire a small admixture of p symmetry on introduction of the terms describing interaction with a uniform electric field, it is not surprising that the addition of d type functions hardly affects the polarizability predicted at the CHF level. However, even in the absence of the terms describing the uniform electric field, a correlated wavefunction for Na^- will have mixed into the $3s^2$ ground state a significant admixture of the $3p^2$ configuration whose distortion by a uniform electric field will contain two electron states built from orbitals of d symmetry. This argument for the necessity of including d functions in the basis is corroborated by the result[61] that for Li^- the polarizability including the important correlation contribution cannot properly be described by a basis lacking such functions.

Our best prediction for the polarizability of an isolated Na^- ion is 1090.2 au, derived using the 16s13p5d/10s9p5d basis and the coupled electron pair approach,[35,38] which previous evidence has shown to be capable of predicting the polarizabilities of small atoms and molecules to within 2%.[20,35] There are only two other calculations whose accuracy might be comparable with the CEPA result, both of these being photodetachment computations[19] in which electron correlation was properly considered. These two computations differed only in whether the dipole-length or dipole-velocity forms were used for the oscillator strength, use of the former predicting an Na^- polarizability of 989 au, and use of the latter, a polarizability of 1058 au. For the case of the Li^- ion, where photodetatchment computations using the dipole-length and dipole-velocity forms predict[19] polarizabilities of 832 and 798 au, respectively, the reliability of the predictions can be assessed by comparing with a more trustworthy result. The best value for the Li^- polarizability currently available is 798 ± 5 au predicted[61] from a computation using full configuration interaction for the two valence electrons and an extensive basis including functions of d symmetry. This result[61] is more accurate than either that of 650 au[62] from a full configuration interaction computation using a less large basis or that of 570 au derived from a variety of coupled-cluster or polarization propagator methods.[63] The extremely close agreement between the prediction of the photodetachment calculation using the dipole-velocity form of the oscillator strength with the full configuration interaction computation shows that the results derived[19] using the dipole-velocity form for the oscillator strength are to be preferred over those predicted with the dipole-length form. Hence the 989-au prediction for the Na^- polarizability should be discounted compared with that of 1058 au predicted using the dipole-velocity operator. The close agreement between this result and our completely independent CEPA prediction of 1090 au provides strong evidence that both the CEPA and ipole-velocity photodetatchment results are trustworthy.

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Heteronuclear NMR Studies of Cobalt Corrinoids. 13. Amide ^1H and ^{15}N NMR Studies of Diastereomerism and the Base-On/Base-Off Reaction in 5′-Deoxyadenosylcobalt Corrinoids[1,2]

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Abstract: Inverse detected ^1H, ^{15}N HMQC spectroscopy in DMSO-d_6 has been used to observe the ^1H and ^{15}N NMR resonances and establish the H–N connectivities of the peripheral amides of a series of five 5′-deoxyadenosylcobalt corrinoids including the β cobalamin (i.e., coenzyme B_{12}) and its α diastereomer (in which the 5′-deoxyadenosyl ligand is in the "lower" axial ligand position), the diastereomeric α- and β-5′-deoxyadenosylcobinamides (in which the axial 5,6-dimethylbenzimidazole nucleotide has been removed by phosphodiester hydrolysis), and the base-off analogue of the coenzyme, in which the coordinating nitrogen of the axial nucleotide is methylated. The ^{15}N resonances were assigned to the various side-chain amides by observation of through-space connectivities of the amide protons with other protons on the complexes via NOESY spectra in DMSO-d_6. The latter could be completely assigned by analogy to the NOESY spectra of these complexes in D_2O since the ^1H spectra of the 5′-deoxyadenosylcobalt corrinoids in D_2O have been previously assigned. The ^{15}N resonances of the unsubstituted amides are found to occur in the order *d*, *b*, *e*, *g*, *c*, *a*, regardless of the nature of the axial ligands. Conversion of the β diastereomer of the cobinamide to the α diestereomer causes a 2.7-ppm upfield shift of the *c* amide resonance suggesting that this amide interacts with the 5′-deoxyadenosyl ligand in the β diastereomer. In the base-on β diastereomer of the cobalamin (i.e., coenzyme B_{12}), the syn and anti protons of the *d* amide are chemical shift equivalent. Upon conversion to the base-off species, the resonance of one of these protons shifts downfield by 0.7 ppm and the ^{15}N resonance shifts downfield by 1.7 ppm. Amide proton chemical shift thermal gradients support the possibility of an intramolecular hydrogen bond involving the *d* amide in the base-on species. Based on models, this hydrogen bond is postulated to involve the noncoordinating nitrogen of the axial 5,6-dimethylbenzimidazole nucleotide as the acceptor.

Introduction

While the α diastereomers of organocobalt corrinoids, in which the organic ligand occupies the "lower" axial ligand position (Figure 1), were once thought to be rare,[4-8] recent work[9] has shown that, in general, reductive alkylation of cobinamide,[10] and of cobalamin under appropriate conditions, leads to mixtures of

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(2) IUPAC–IUB[3] nomenclature is used throughout. Abbreviations: β-AdoCbl, β-5′-deoxyadenosylcobalamin; β-AdoCbi$^+$, β-5′-deoxyadenosylcobinamide: α-AdoCbl, α-5′-deoxyadenosylcobalamin; α-AdoCbi$^+$, α-5′-deoxyadenosylcobinamide; β-AdoMe$_3$BzmBca$^+$, Coα-(α-3,5,6-trimethylbenzimidazolyl)-Coβ-5′-deoxyadenosylcobamide; H_2OCbl^+, aquocobalamin; $(CN)_2Cbl^-$, dicyanocobalamin.

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diastereomeric α- and β-alkylcobalt corrinoids.[11] Most recently,[13] we have found that the coenzyme form of vitamin B_{12} (i.e., 5′-deoxyadenosylcobalamin) is also subject to such diastereomerism. Thus, reductive alkylation of cobalt corrinoids with 5′-deoxy-5′-haloadenosines under appropriate conditions also leads to mixtures of diastereomeric 5′-deoxyadenosylcobalt corrinoids. Unlike the case of other reductive alkylations, however, the stereochemical course of the reductive adenosylation of cobalt corrinoids can be largely controlled by choice of alkylating agent and reaction conditions.[13] Both the α diastereomers, α-AdoCbl and α-AdoCbi, have now been characterized by complete assignment of their ^1H and ^{13}C NMR spectra using a variety of homonuclear and heteronuclear 2-D NMR techniques.[13] While such spectral assignments of cobalt corrinoids[1,4,14-16] are extremely useful for characterization, interpretation of the effects of structural variations and environmental influences on such chemical shifts has proved difficult. ^{15}N NMR, which has been little used as a structural tool or probe of intramolecular and intermolecular interactions in cobalt corrinoids,[1,17-20] has great promise despite its inherent lack of sensitivity. In particular, the chemical shifts of the amide nitrogens (and hydrogens) of the peripheral side chains of the corrin macrocycle, three of which (the *a*, *c*, and *g* acetamides) project above the corrin ring (toward the β face) and four of which (the *b*, *d*, and *e* propionamides and the *f* (nucleotide loop) side chain) project below the corrin ring (toward the α face), hold promise of being sensitive to the nature of the cobalt axial ligands and hence to the stereochemistry of diastereomeric alkylcobalt corrinoids. In addition, both amide ^{15}N[20b,21-25] and ^1H[26-28] chemical shifts are known to be sensitive to changes in hydrogen bonding and, thus, ^{15}N NMR can potentially provide a valuable probe of corrinoid side-chain hydrogen bonding interactions.

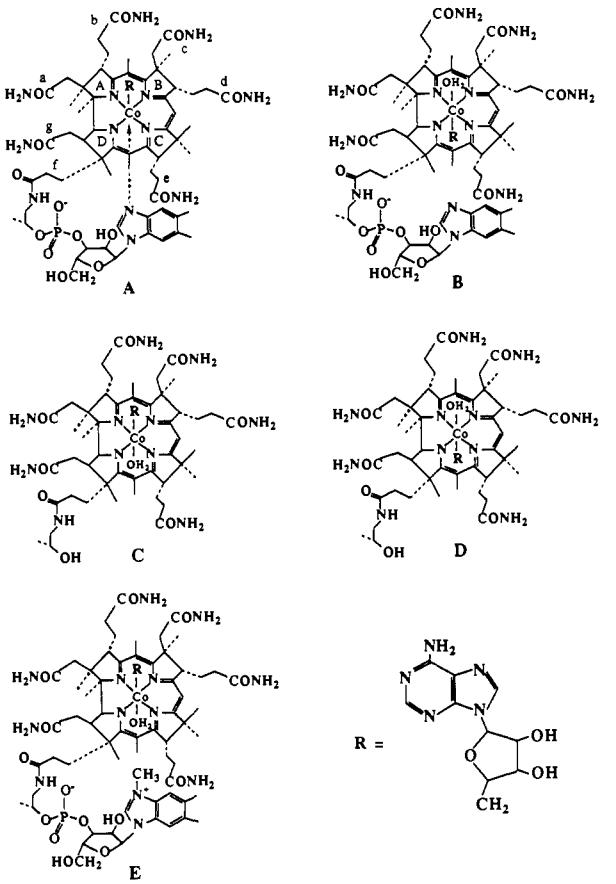

Figure 1. Structure of (A) base-on β-AdoCbl, (B) α-AdoCbl, (C) β-AdoCbi$^+$, (D) α-AdoCbi$^+$, and (E) β-AdoMe$_3$BzmCba$^+$.

The following report details the observation and assignment of the side-chain amide ^{15}N and ^1H NMR resonances of β-AdoCbl (coenzyme B_{12}), its base-off analogues β-AdoCbi$^+$ and β-AdoMe$_3$BzmCba$^+$, and the α diastereomers, α-AdoCbl and α-AdoCbi$^+$. As such it represents the first observation and assignment of any of the ^{15}N NMR resonances of coenzyme B_{12}, as well as the first opportunity to determine the effects of the base-on/base-off transition of an alkylcobalt corrinoid, and the effects of α/β diastereomerism on corrin side-chain ^{15}N and ^1H resonances. It also represents the necessary background for future studies of the interactions of 5′-deoxyadenosylcobalt corrinoids with proteins by ^{15}N and ^{15}N isotope edited ^1H NMR.

Experimental Section

H_2OCbl·OAc was from Roussell. Factor B (a mixture of the diastereomeric α-CN-β-(H_2O)-Cbi and α-H_2O-β-(CN)Cbi) was prepared by a modification[29] of the method of Renz,[30] and 5′-chloro-5′-deoxyadenosine was prepared by the method of Kikugawa[31] and characterized by NMR as described previously.[13] CNMe$_3$BzmCba$^+$ (also a mixture of diastereomeric α-CN-β-(H_2O)Me$_3$Bzm-Cba$^+$ and α-H_2O-β-(CN)-Me$_3$BzmCba$^+$) was prepared by methylation of $(CN)_2Cbl^-$ with dimethyl sulfate by a modification[12] of the procedure of Friedrich and Bernhauer.[32] α- and β-AdoCbl and α- and β-AdoCbi$^+$ were prepared by reductive adenosylation of H_2OCbl or factor B, respectively, and characterized as described previously.[13]

β-AdoMe$_3$BzmCba$^+$ was prepared as follows. CNMe$_3$BzmCba$^+$ (100 mg, 72.9 μmol) was dissolved in 100 mL of 10% acetic acid and purged with argon for 1 h. Zinc wool (2 g, 31 mmol), briefly freshened with 2

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(10) Cobinamides are derivatives of vitamin B_{12} from which the 5,6-dimethylbenzimidazole nucleotide has been removed by phosphodiester hydrolysis (Figure 1) and the vacated axial ligand position is, presumably, occupied by water.

(11) Recent work[12] has shown that reductive alkylation of cyano(aquo)-3,5,6-trimethylbenzimidazolylcobamide also leads to mixtures of diastereomers α- and β-RMe$_3$BzmCba$^+$'s.[2]

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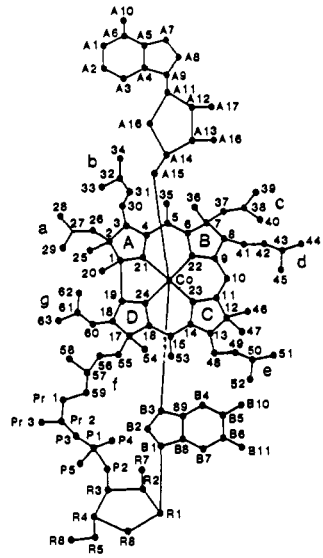

Figure 2. Numbering scheme for the 5′-deoxyadenosylcobalt corrinoids.

N HCl, was added and the reduction was allowed to proceed for 40 min. A solution of 0.5 g (1.75 mmol) of 5′-chloro-5′-deoxyadenosine in 10 mL of 10% acetic acid, which had been previously deoxygenated by argon purge, was introduced into the reaction mixture through a cannula. The progress of the reaction was monitored by periodic HPLC analysis of small samples removed by syringe. As previously observed for the reductive adenosylation of cobinamide under these conditions,[13] only a single diastereomer of $AdoMe_3BzmCba^+$ was formed in over 90% yield (by HPLC) after 120 min. The reaction was stopped by removal of the zinc by filtration and was desalted by chromatography on Amberlite XAD-2. The product was purified by semipreparative HPLC and obtained in 73% yield (86 mg). As anticipated,[13] the single diastereomer obtained under these conditions proved to be β-$AdoMe_3BzmCba^+$, as determined by the virtual identity of its UV/visible spectrum (λ_{max} (log ϵ): 455 (3.977), 376 (3.934), 303 (4.335), 286 (4.335), 263 (4.593)) to that of protonated, base-off β-AdoCbl and by ^1H NMR (vide infra).

Two-dimensional NMR spectra were obtained at 30 °C on a Bruker AMX300 NMR spectrometer using an inverse broad band probe and operating at 300.136 MHz (^1H) and 30.415 MHz (^{15}N). Samples, 20–45 mM in adenosylcobalt corrinoid, were dissolved in DMSO-d_6 and contained TSP as an internal ^1H reference. ^{15}N chemical shifts were referenced to external CH_3NO_2 but are reported relative to NH_3(l) ($\delta_{CH_3NO_2}$ = 380.23 ppm[33]). Inverse detected ^1H, ^{15}N HMQC[34,35] spectra were collected into 512 × 256 data matrices over 3623.2 Hz (^1H) and 3042 Hz (^{15}N) sweep widths; 1026 scans were collected per t_1 increment of 138 μs. The data were processed using Gaussian multiplication with −5 Hz line broadening in both dimensions. NOESY spectra were collected into 2048 × 256 data matrices with a sweep width of 3623.2 Hz in both dimensions; 128 scans were collected per t_1 increment and the total mixing time was 600 ms. The data were processed with a shifted sine bell function of 90°. Amide ^1H chemical shift thermal gradients were determined relative to internal TSP from one-dimensional ^1H observations at 5 °C increments between 20 and 55 °C on a GE QE300 NMR spectrometer operating at 300.669 MHz.

Results

The cobalt corrinoids of interest here (i.e., cobalamins and cobinamides) are characterized by seven amide functionalities on the corrin ring side chains (Figures 1 and 2). Since these nitrogens are all protonated, their ^{15}N resonances can be observed[18,20b] by the distortionless enhancement by polarization transfer sequence (DEPT),[36] but only in DMSO-d_6, as the amide protons rapidly exchange with solvent in D_2O. However, since it is an inverse detection method, the ^1H, ^{15}N heteronuclear multiple-quantum coherence method provides a substantial advantage in terms of sensitivity, particularly if performed using an inverse probe. In addition, the HMQC experiment also permits determination of

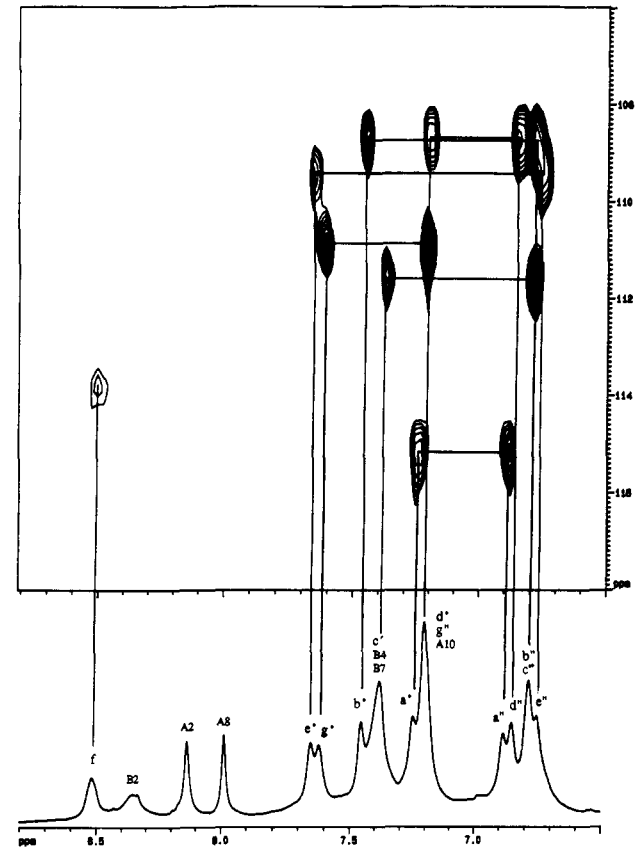

Figure 3. A portion of the ^1H, ^{15}N HMQC spectrum of α-AdoCbl in DMSO-d_6. The connectivities between the amide ^{15}N resonances and their attached protons are shown using the accompanying downfield portion of the 1-D ^1H NMR spectrum of the sample. The amide protons are labeled by their side-chain designations (Figures 1 and 2) and designated prime for the downfield (or syn) amide proton and double prime for the upfield (or anti) proton. Other protons resonating in this area of the 1-D spectrum are also labeled. The 5′-deoxyadenosyl ligand exocyclic amine group (A10) cross peak ($\delta_{^{15}N}$ = 80.6 ppm, $\delta_{^1H}$ = 7.17 ppm) is not shown.

connectivity between the amide nitrogens and their attached protons. An example is shown in Figure 3, a portion of the ^1H, ^{15}N HMQC spectrum of α-AdoCbl. The analogous regions of the ^1H, ^{15}N HMQC spectra of α-$AdoCbi^+$, β-$AdoCbi^+$, and β-$AdoMe_3BzmCba^+$ are shown in Figures S1–S3, respectively, available as supplementary material. In each case, an additional cross peak occurred outside the ^{15}N spectral window shown (at $\delta_{^{15}N}$ = 80.7 ± 0.4 ppm and $\delta_{^1H}$ = 7.21 ± 0.04 ppm), which could be assigned to the exocyclic amino group (A10) of the 5′-deoxyadenosyl ligand.

The amide region of the ^1H, ^{15}N HMQC spectrum of α-AdoCbl is typical of that of cobalt corrinoids[20b] in that the seven ^{15}N resonances are resolved and all but one show cross peaks to two ^1H resonances, i.e., those belonging to the syn and anti protons[37,38] of the six unsubstituted amides (*a*, *b*, *c*, *d*, *e*, and *g*). The seventh amide ^{15}N resonance, which has only a single cross peak, is immediately assignable to the *f* amide nitrogen as this side chain contains the only substituted amide in the molecule (Figure 1).

In order to assign the other amide resonances, advantage has been taken of the nuclear Overhauser effect, which can be observed between the amide protons and protons elsewhere in the molecule in DMSO-d_6 solution.[1] A NOESY spectrum with a long mixing time (600 ms) was generally found sufficient to provide enough cross peaks to the amide protons to allow their unambiguous assignment, and, hence, the assignment of the ^{15}N resonances. The amide region of such a NOESY map for α-AdoCbl is shown

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Table I. ^{1}H NMR Assignments for the 5′-Deoxyadenosylcobalt Corrinoids in DMSO-d_6

	$\delta_{^1H}$, ppm				
atom	α-AdoCbl	α-AdoCbi	β-AdoCbl	β-AdoCbi	β-AdoMe$_3$BzmCba[a]
A15	0.47, 1.98	0.43, 2.21	0.54, 1.46	−0.12, 0.81	−0.21, 0.88
C53	2.38	2.36	2.45	2.32	2.30
C35	2.36	2.37	2.42	2.38	2.38
C25	1.44	1.48	1.24	1.37	1.36
C54	1.13	1.07	1.28	1.20	1.29
Pr3	1.23	1.33	1.07	1.04	1.09
B11	2.37		2.18		2.43
C47	1.36	1.31	1.19	1.50	1.47
B10	2.40		2.18		2.46
C36	1.29	1.30	1.68	1.69	1.69
C20	1.63	1.75	0.40	0.86	0.88
C30	2.15, 2.45	2.12, 2.36	1.77, 1.80	1.83, 1.99	2.00, 2.05
C41	1.54, 2.07	1.63, 2.15	1.10, 1.77	1.70, 2.13	1.67, 2.20
C48	1.37, 1.63	1.44, 1.64	2.15, 2.25	1.75, 2.10	1.79, 2.02
C46	1.38	1.35	0.98	0.57	0.50
C56	2.02, 2.66	1.98, 2.70	1.03, 2.05	2.22	2.37
C60	2.45	2.68	2.50	2.52, 2.56	2.60, 2.68
C49	2.03, 2.10	2.17	2.35	2.30	2.33
C42	2.47	2.03	1.42, 1.75	2.27	2.22
C55	1.78, 2.40	1.97, 2.35	1.80, 2.46	1.83, 2.30	1.80, 2.29
C31	2.37	2.37	2.25	2.37	2.45
C18	2.97	3.03	2.75	2.88	2.90
C26	1.75, 2.02	1.63, 2.09	2.41	2.55	2.60
Pr1	3.05, 3.42	3.21, 3.26	2.93, 3.50	3.00, 3.04	3.13
C37	2.52, 2.70	2.63, 2.75	1.70, 2.18	1.70, 2.46	1.70, 2.47
C13	3.32	3.24	3.08	3.10	3.03
C8	3.76	3.79	3.83	4.04	4.03
C3	4.12	4.15	4.47	4.56	4.45
R5	3.60, 3.64		3.39, 3.57		3.60
R2	b		4.03		4.66
Pr2	4.30	4.15	4.17	3.67	4.15
A12	4.23	4.23	4.60	4.22	4.22
A13	3.64	3.52	3.10	3.46	3.43
R3	4.18		4.55		4.63
C19	3.77	3.80	4.20	4.67	4.62
R4	4.63		3.98		4.59
R1	6.30		6.20		6.60
A14	1.60	1.83	2.12	2.20	2.26
A11	5.50	5.42	5.43	5.48	5.47
C10	6.40	6.39	6.00	6.77	6.73
B7	7.39		7.24		7.78
B4	7.43		6.42		7.73
A8	8.00	8.00	8.09	8.00	7.98
A2	8.13	8.15	8.13	8.17	8.13
B2	8.35		7.25		9.64
N29 (*a*)	6.86, 7.27	6.84, 7.38	7.15, 7.76	7.06, 8.09	7.00, 8.26
N33 (*b*)	6.77, 7.46	6.82, 7.50	6.77, 7.36	6.84, 7.43	6.83, 7.43
N40 (*c*)	6.77, 7.37	6.80, 7.41	6.95, 7.54	6.98, 7.68	6.97, 7.70
N45 (*d*)	6.83, 7.21	6.82, 7.29	6.58	6.72, 7.33	6.71, 7.27
N52 (*e*)	6.73, 7.65	6.80, 7.40	6.86, 7.52	6.62, 7.12	6.83, 7.34
N59 (*f*)	8.51	8.07	7.83	7.73	8.01
N63 (*g*)	7.19, 7.64	7.08, 7.77	7.18, 7.59	7.12, 7.81	7.18, 7.97
A10 (NH_2)	7.17	7.20	7.23	7.23	7.23

[a] The *N*-methyl resonance occurred at $\delta_{^1H}$ = 1.67 ppm and $\delta_{^{13}C}$ = 33.88 ppm. [b] Not observed.

in Figure 4, and a similar portion of the NOESY spectra of α-AdoCbi$^+$, β-AdoCbl, β-AdoCbi$^+$, and β-AdoMe$_3$BzmCba$^+$ is shown in Figures S4–S7, respectively, available as supplementary material. In almost all cases, far fewer cross peaks were obtained for the propionamide amide protons (*b*, *d*, and *e* side chains) than for the acetamide amide protons (*a*, *c*, and *g* side chains). In those cases (such as for α-AdoCbl, Figure 4) where the NOESY spectrum provided too few cross peaks to permit the unambiguous assignment of the propionamide protons, additional cross peaks to these protons could always be found in a spin-locked NOE (or ROESY[39]) spectrum of the sample. Thus, for α-AdoCbl, the ROESY spectrum has cross peaks between the *e* amide protons and the C49 methylene, and *b* amide protons and the C31 methylene, and the *d* amide protons and the C41 methylene. Together with the correlations observed in the NOESY spectrum (Figure 4), these correlations permitted the unambiguous assignment of the amide proton resonances.

In order to assign the amide proton resonances from their NOE cross peaks, it is, of course, necessary to completely assign the NOESY map of each compound. This is readily accomplished since the complete proton assignments of β-AdoCbl,[16a] β-AdoCbi$^+$,[16c] α-AdoCbl, and α-AdoCbi$^+$ [13] in D_2O are known. Thus, comparison of the NOESY map of each of the compounds in DMSO-d_6 to the assigned NOESY map of the same compound in D_2O generally allowed complete assignment of the former spectrum. In cases where any ambiguity arose owing to differences in the ^{1}H chemical shifts in DMSO-d_6 and in D_2O, the observation of a COSY spectrum and a ^{1}H, ^{13}C HMQC spectrum (not shown) permitted resolution of any ambiguities. For β-AdoMe$_3$BzmCba$^+$, the proton spectrum was very similar to that of the protonated, base-off species of β-AdoCbl,[16b] and, in fact, the NOESY map of this compound in DMSO-d_6 was essentially identical with that

(39) (a) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811. (b) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207.

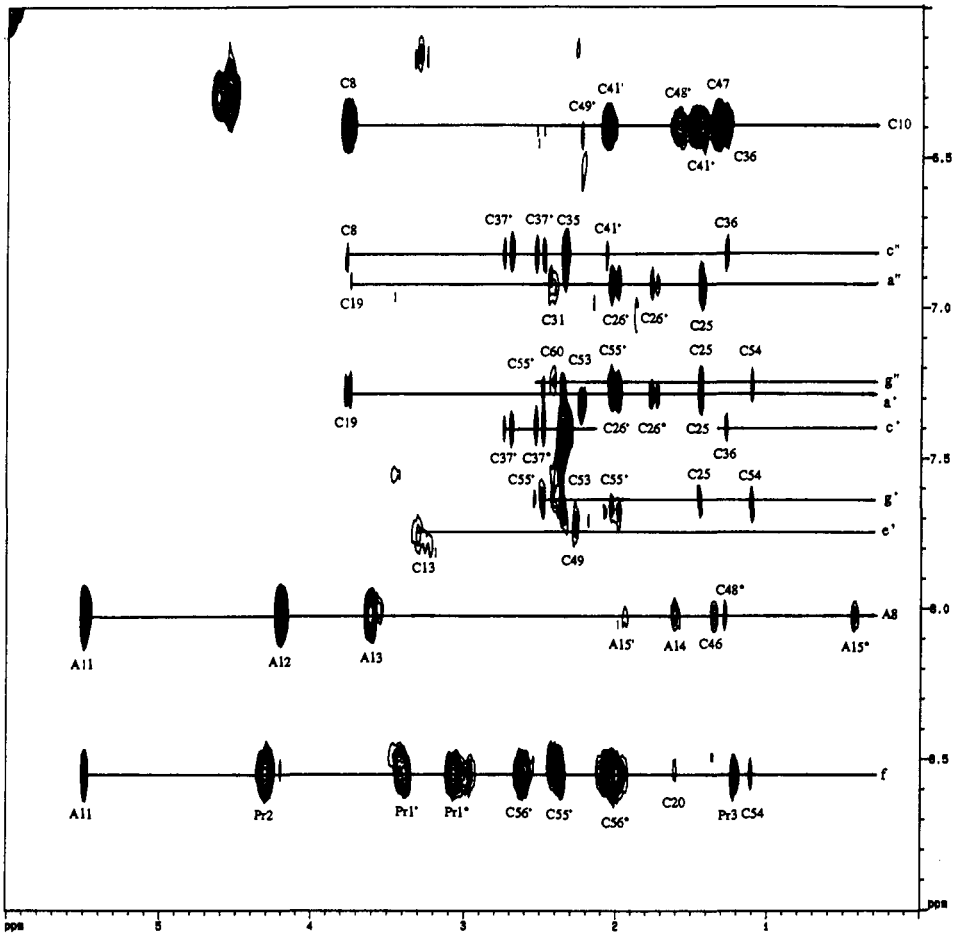

Figure 4. Downfield portion of the NOESY spectrum (mixing time 600 ms) of α-AdoCbl in DMSO-d_6 showing the cross peaks used to assign the amide proton resonances.

of β-AdoCbi$^+$, except that the former contained cross peaks due to the benzimidazole nucleotide. A complete, and, because of the long mixing time, very extensive NOE correlation table for all five compounds is given in Table SI, available as supplementary material, and the ^{1}H NMR assignments of each of the five 5′-deoxyadenosylcobalt corrinoids are given in Table I.

Once the NOESY maps were completely assigned, unambiguous assignment of the amide ^{1}H resonances was straightforward. Even, the *a* and *g* amide protons, which potentially have cross peaks to many of the same protons, were always readily distinguishable. For example, in β-AdoCbi$^+$, the *a* amide protons had NOE's to the C19, C20, C25, C26′, C26″, C30′, and C35 protons, while the *g* amide protons NOE's were to the C18, C20, C25, C53, C54, C55′, C55″, C60′, and C60″ protons. The final assignments of the ^{15}N resonances of all seven amides for all five compounds are given in Table II.

In addition to permitting assignment of the amide ^{1}H and ^{15}N resonances, the complete assignment of the NOESY spectra also permitted unambiguous assignment of the stereochemistry of the product obtained by reductive adenosylation of CNMe$_3$BzmCba$^+$ with 5′-chloro-5′-deoxyadenosine. Previously it had been shown[13] that reductive alkylation of cobinamide with 5′-deoxy-5′-haloadenosines produces mixtures of diastereomeric α- and β-AdoCbi$^+$. However, the ratio of diastereomers was found to be time dependent and sensitive to the nature of the halide leaving group and the molar excess of alkylating agent employed. Under the conditions employed here for the synthesis of AdoMe$_3$BzmCba$^+$ (i.e., alkylation with 5′-chloro-5′-deoxyadenosine employed in large (24-fold) excess), only the β diastereomer of AdoCbi$^+$ was obtained.[13] As anticipated, reductive alkylation of CNMe$_3$BzmCba$^+$ under these conditions also yielded only a single product. That this product is expected β diastereomer is confirmed by the observation of NOE's between adenosyl ligand protons and C19, C37′, C37″, C46, and C54 (Table SI), all of which are on the β face of the corrin ring. In contrast, the two α-5′-deoxyadenosylcobalt corrinoids had NOE's between their adenosyl ligands and α face protons C18, C47, C48′, C48″, C55′, and Pr3, thus confirming their stereochemistry.

Discussion

In general, the ^{1}H NMR chemical shifts of the 5′-deoxyadenosylcobalt corrinoids in DMSO-d_6 (Table I) are quite similar to those in D$_2$O.[13,16a,c] This, in fact, makes it possible to assign the NOESY spectra in DMSO-d_6 based primarily on the knowledge of the chemical shifts in D$_2$O. There are, however, significant differences in ^{1}H chemical shifts in the two solvents including some in the side chains (the C37 and C42 methylenes in α-AdoCbl; the C26, C30, C37, C49, and D56 methylenes in α-AdoCbi$^+$; the C31 and C55 methylenes in β-AdoCbl; and the C31, C41, and C49 methylenes and Pr1 in β-AdoCbi$^+$). There are also significant ^{1}H chemical shift differences (≥0.25 ppm) in the two solvents at several ring carbons (C3 in all compounds, C13 in β-AdoCbi$^+$), particularly at C8 in β-AdoCbl, where the ^{1}H resonance occurs 0.54 ppm upfield in DMSO-d_6 relative to its position in D$_2$O. The only methyl group showing a significant solvent shift is C46, which resonates 0.35 ppm upfield in DMSO-d_6. While there are also some significant differences at some of the adenosyl and benzimidazole nucleoside carbons, the largest solvent-dependent ^{1}H chemical shift difference is at A15, where the upfield member of the diastereotopic methylene pair in DMSO-d_6 resonates 0.55 ppm upfield of its position in D$_2$O. While some of these ^{1}H chemical shift differences can undoubtedly be attributed to solvation effects, the possibility that these complexes adopt significantly different conformations in DMSO-d_6 and D$_2$O cannot be excluded.

The data in Table II show that the ^{15}N resonances of the unsubstituted amides of all five 5′-deoxyadenosylcobalt corrinoids occur in the order *d*, *b*, *e*, *g*, *c*, *a*, the same order previously

Table II. Amine ^{15}N and ^{1}H NMR Chemical Shift Assignments and ^{1}H Chemical Shift Thermal Gradients for the 5′-Deoxyadenosylcobalt Corrinoids[a]

	β-AdoCbl[b,c]		β-AdoCbi[b,d]		β-AdoMe$_3$BzmCba[b,e]		α-AdoCbl[f]		α-AdoCbi[g]	
amide	δ_{15N}, ppm	δ_{1H}, ppm	δ_{15N}, ppm	δ_{1H}, ppm	δ_{15N}, ppm	δ_{1H}, ppm	δ_{15N}, ppm	δ_{1H}, ppm	δ_{15N}, ppm	δ_{1H}, ppm
d	106.7	6.58	108.5	6.72	108.4	6.71	108.5	6.83	108.8	6.82
		(−2.45 ± 0.21)		(4.96 ± 0.10)		(5.31 ± 0.03)				
		6.58		7.33		7.29		7.21		7.29
		(3.56 ± 0.10)		(4.38 ± 0.02)		(4.62 ± 0.05)				
b	108.0	6.77	108.7	6.84	108.5	6.83	108.6	6.77	108.8	6.82
		(4.56 ± 0.05)		(5.33 ± 0.07)		(5.77 ± 0.10)				
		7.36		7.43		7.43		7.46		7.50
		(3.35 ± 0.18)		(3.91 ± 0.02)		(3.81 ± 0.03)				
e	110.0	6.86	109.7	6.62	109.6	6.83	109.3	6.73	109.5	6.80
		(5.88 ± 0.04)		(4.84 ± 0.05)		(5.57 ± 0.10)				
		7.52		7.12		7.34		7.65		7.40
		(3.77 ± 0.19)		(3.52 ± 0.12)		(3.72 ± 0.04)				
g	110.4	7.18	110.6	7.12	111.2	7.18	110.7	7.19	110.9	7.08
		(5.10 ± 0.04)		(4.79 ± 0.15)		(4.57 ± 0.09)				
		7.59		7.81		7.97		7.64		7.77
		(4.96 ± 0.05)		(3.83 ± 0.05)		(4.18 ± 0.32)				
f	112.3	7.83	113.4	7.73	112.0	8.01	114.4	8.51	114.0	8.07
		(−1.44 ± 0.12)		(5.06 ± 0.02)		(3.42 ± 0.04)				
c	114.3	6.95	114.2	6.98	114.2	6.97	111.5	6.77	111.5	6.80
		(4.73 ± 0.05)		(5.63 ± 0.03)		(5.86 ± 0.05)				
		7.54		7.68		7.70		7.37		7.41
		(4.83 ± 0.14)		(3.63 ± 0.04)		(3.39 ± 0.09)				
a	116.1	7.15	115.8	7.06	116.3	7.00	115.1	6.86	115.6	6.84
		(5.10 ± 0.04)		(4.79 ± 0.15)		(4.57 ± 0.09)				
		7.76		8.09		8.26		7.27		7.38
		(4.96 ± 0.05)		(3.83 ± 0.05)		(4.18 ± 0.32)				

[a] In DMSO-d_6, 30 °C. ^{1}H chemical shifts were determined relative to internal TSP. ^{15}N chemical shifts were determined relative to external CH_3NO_2, but are reported relative to $NH_3(l)$ ($\delta_{CH_3NO_2}$ = 380.23 ppm[33]). [b] Amide proton chemical shift gradient, $-(\Delta\delta/\Delta T) \times 10^3$, ppm/°C, in parentheses. [c] The 5′-deoxyadenosyl exocyclic amino resonance (A10) occurred at δ_{15N} = 80.6 ppm, δ_{1H} = 7.23 ppm. [d] The 5′-deoxyadenosyl exocyclic amino resonance (A10) occurred at δ_{15N} = 81.1 ppm, δ_{1H} = 7.23 ppm. [e] The 5′-deoxyadenosyl exocyclic amino resonance (A10) occurred at δ_{15N} = 80.4 ppm, δ_{1H} = 7.23 ppm. [f] The 5′-deoxyadenosyl exocyclic amino resonance (A10) occurred at δ_{15N} = 80.6 ppm, δ_{1H} = 7.17 ppm. [g] The 5′-deoxyadenosyl exocyclic amino resonance (A10) occurred at δ_{15N} = 80.9 ppm, δ_{1H} = 7.20 ppm.

determined for those of $(CN)_2Cbl^-$ and $(CN)_2Cbi$.[1,20b] The three propionamide ^{15}N resonances (*b*, *d*, and *e*) are clustered in the more upfield region, between 106.7 and 110.0 ppm. With the exception of base-on β-AdoCbl, these resonances cluster in the much narrower region of 108.4 to 110.0 ppm, while the three acetamide resonances (*a*, *c*, *g*) are more spread out, in the more downfield region between 110.4 and 116.3 ppm. The substituted amide in the *f* side chain of all of the β-5′-deoxyadenosylcobalt corrinoids occurs between the *g* and *c* resonances, as was the case with $(CN)_2Cbl^-$ and $(CN)_2Cbi$,[1,20b] but falls between the *c* and *a* resonances for the α diastereomers.

These data allow an assessment of the effect of the base-on/base-off transition on the amide ^{15}N resonances by a comparison of the shifts of β-AdoCbl and β-AdoMe$_3$BzmCba$^+$, an excellent mimic for the monocationic, benzimidazole protonated base-off species. Among the propionamides, only the *d* amide shows a significant change and is shifted 1.7 ppm downfield in the base-off species. This shift is similar to, but larger than the 1.1-ppm downfield shift of the *d* amide ^{15}N resonance when CNCbl is converted to $(CN)_2Cbl^-$.[20b] That this change in the *d* amide ^{15}N chemical shift is due to the removal of the benzimidazole nucleotide from the α face is clearly shown by the fact that the chemical shifts of the *d* amide of β-AdoCbi$^+$ and β-AdoMe$_3$BzmCba$^+$ are essentially identical. This chemical shift difference between the base-on and base-off species suggests an interaction of the *d* amide in the base-on species which is absent (or altered) in the base-off species, as further discussed below. Among the acetamides, only the *g* amide ^{15}N resonance undergoes a significant shift, being 0.8 ppm downfield in β-AdoMe$_3$BzmCba$^+$ relative to β-AdoCbl. However, this difference disappears when β-AdoCbl and β-AdoCbi$^+$ are compared. The nucleotide loop (*f*) amide ^{15}N chemical shift is unchanged during the base-on/base-off transition, but undergoes a 1.1-ppm downfield shift when the nucleotide is removed (i.e., in β-AdoCbi$^+$), possibly the result of a conformational change in the *f* side chain due to the loss of the bulky nucleotide substituent. However, this effect is absent in the α diastereomers where the *f* amide chemical shifts of (necessarily) base-off α-AdoCbl and baseless α-AdoCbi$^+$ are nearly identical.

These data also show the effects of diastereomerism on the side-chain amide ^{15}N resonances as the organic ligand is moved from the β (in β-AdoCbi$^+$) to the α position (in α-AdoCbi$^+$). While there is a slight (0.6 ppm) downfield shift of the *f* amide resonances, by far the largest change is a 2.7-ppm upfield shift of the *c* acetamide resonance as a result of the β to α transition. This suggests an interaction of the *c* amide with the adenosyl ligand which is missing in one of the diastereomers. Since the *c* acetamide projects toward the β face, it seems unlikely that it interacts with the organic ligand when the latter is in the α position, although such an interaction is possible. The more likely possibility, i.e., that the interaction is between the *c* amide and the 5′-deoxyadenosyl ligand in the β diastereomer, is surprising in that in the crystal structure of β-AdoCbl[40-42] the 5′-deoxyadenosyl ligand lies over the "southern" hemisphere of the corrin, between the C46 and C54 methyls, and is thus quite far from the *c* amide. This suggests the possibility that either the (unknown) conformation of the base-off analogue of coenzyme B_{12} (i.e., β-AdoCbi$^+$) has a different conformation than the base-on coenzyme, or that, in solution, there is conformational flexibility with respect to the disposition of the adenosyl ligand.[16] Either of these possibilities is sufficiently intriguing to stimulate further investigation.

The HMQC map of base-on β-AdoCbl (Figure 5) is unique in that the upfield-most ^{15}N resonance (the *d* amide resonance) appears to correlate to a single ^{1}H resonance at 6.6 ppm. Since the integral of the latter represents two protons, it is clear that the syn and anti protons of the *d* amide are chemical shift equivalent in β-AdoCbl. In all of the six other known cases, the chemical shifts of the *d* amide protons differ by 0.4 to 0.6 ppm

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(41) Savage, H. F. J.; Lindley, P. F.; Finney, J. L. *Acta Cryst.* **1987**, *B43*, 280.
(42) Glusker, J. P. In *B_{12}*; Dolphin, D., Ed., Wiley: New York, 1982; Vol. 1, Chapter 3.

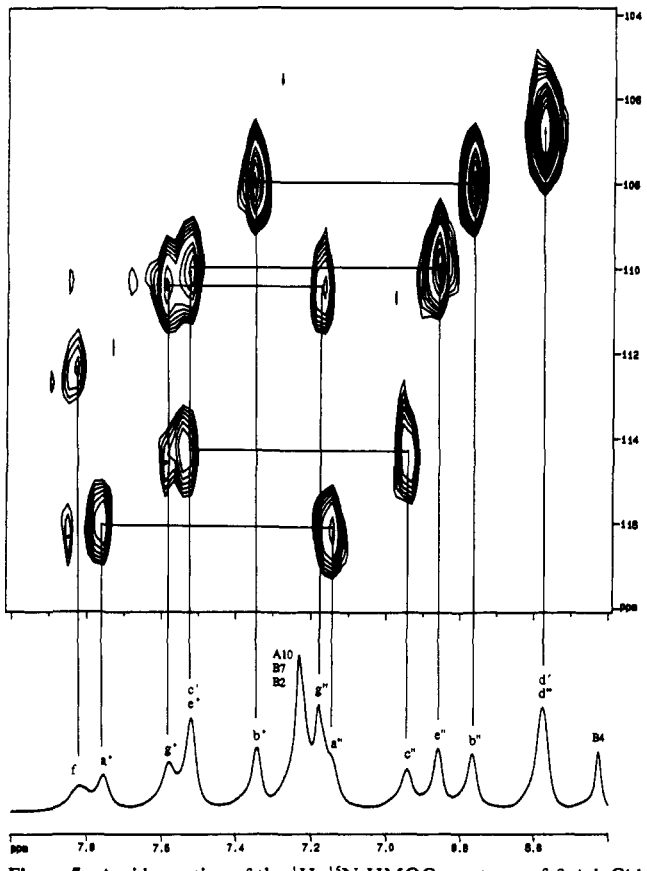

Figure 5. Amide portion of the ^1H, ^{15}N HMQC spectrum of β-AdoCbl in DMSO-d_6. The connectivities between the amide ^{15}N resonances and their attached protons are shown using the accompanying downfield portion of the 1-D ^1H NMR spectrum of the sample. The amide protons are labeled by their side-chain designations (Figures 1 and 2) and designated prime for the downfield (or syn) amide proton and double prime for the upfield (or anti) proton. Other proton resonating in this area of the 1-D spectrum are also labeled. The most upfield ^{15}N resonance (the *d* amide) is shown to be correlated to a single ^1H resonance which integrates to two protons relative to the B4 resonance.

(Table II and ref 20b). In the transition to the base-off analogues (β-AdoCbi$^+$ or β-AdoMe$_3$BzmCba$^+$), one of these protons remains at a very similar chemical shift (6.7 ppm) while the other moves downfield by 0.7 ppm, mirroring the relatively large (1.7 ppm) downfield shift of the *d* amide ^{15}N resonance. Since both amide ^{15}N[20b,21-25] and ^1H[26-28] chemical shifts are sensitive to changes in hydrogen bonding, one possibility is that there is a difference in hydrogen bonding to the *d* amide in the base-on and base-off species. Another possibility is that the chemical shifts of the *d* amide nitrogen and one of its protons are significantly affected by the magnetic anisotropy of the heterocyclic nucleotide in the base-on species. In order to attempt to distinguish these possibilities, the amide proton chemical shift thermal gradients, $-(\Delta\delta/\Delta T)$ of β-AdoCbl, β-AdoCbi$^+$, and β-AdoMe$_3$BzmCba$^+$, were measured and are listed in Table II. Normally, amide protons hydrogen bonded to solvent in DMSO undergo upfield shifts with increasing temperature of the order of 4–10 × 10^{-3} ppm/°C, while intramolecular amide hydrogen bonding in DMSO substantially reduces the amide proton chemical shift gradient.[26,27] In a previous study of $(CN)_2Cbl^-$ and $(CN)_2Cbi$,[20b] all but one of the amide proton chemical shift gradients fell in the range of 3.39–6.16 × 10^{-3} ppm/°C. As seen in Table II, all of the amide proton chemical shift thermal gradients of the three β-5′-deoxyadenosylcobalt corrinoids save two fall in the similar range of 3.35–5.88 × 10^{-3} ppm/°C. The two exceptions are the *f* amide proton and one of the two *d* amide protons which consequently lose their chemical shift equivalence at temperatures other than 30 °C. Both of these chemical shift gradients are slightly negative; i.e., these resonances move downfield on heating, consistent with these protons being shielded from solvent by intramolecular hydrogen bonding. While the neutron diffraction structure of β-AdoCbl[41] shows numerous intramolecular hydrogen bonds involving the side-chain amides, the *f* amide proton is involved in a hydrogen bond with a water of crystallization. Inspection of semi-space-filling models shows that, in solution, the *f* amide could be hydrogen bonded to either the *g* or the *e* amide carbonyls. Presumably, a change in *f* side-chain conformation in the base-off (i.e., β-AdoMe$_3$BzmCba$^+$) and cobinamide species disrupts this intramolecular hydrogen bond, returning the *f* amide proton chemical shift thermal gradient to the normal range. In the crystal structure,[41] one of the *d* amide protons is involved in a hydrogen bond with the *a* amide carbonyl. However, it is hard to see how the base-on/base-off transition might disrupt this interaction, nor is there any change in ^{15}N chemical shift of the *a* amide which would be expected to accompany the loss of a hydrogen bond to its carbonyl.[22-25] However, in semi-space-filling models of base-on β-AdoCbl, the *d* side-chain amide can adopt a conformation which brings one of its protons in close proximity to the *N*-glycoside nitrogen (i.e., B1) of the benzimidazole nucleotide. Such a hydrogen bond would explain why removal of the benzimidazole from the α face (either by uncoordination and N-methylation in β-AdoMe$_3$BzmCba$^+$ or by hydrolysis of the phosphodiester and removal of the nucleotide in β-AdoCbi$^+$) would disrupt the hydrogen bond and restore the *d* amide proton chemical shift thermal gradient to the normal range. We consequently propose that in the base-on species of coenzyme B_{12}, at least in DMSO solution, there is a hydrogen bond between the *d* amide and the *N*-glycoside nitrogen of the axial benzimidazole nucleotide. Experiments are in progress to see if such hydrogen bonding occurs in other base-on β-RCbl's as well.

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Supplementary Material Available: Table SI showing the NOE connectivities for the 5′-deoxyadenosylcobalt corrinoids, Figures S1–S3 showing the assigned ^1H, ^{15}N HMQC spectra of α-AdoCbi$^+$, β-AdoCbi$^+$, and β-AdoMe$_3$BzmCba$^+$, respectively, and Figures S4–S7 showing the amide region of the NOESY spectra of α-AdoCbi$^+$, β-AdoCbl, β-AdoCbi$^+$, and β-AdoMe$_3$BzmCba$^+$, respectively (17 pages). Ordering information is given on any current masthead page.