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Axial Base $K_{assoc.}$ Values for Coenzyme B_{12} "Models".

Many axial base $K_{assoc.}$ are available for Coenzyme B_{12} "model" complexes, particularly for the well studied alkylcobaloximes.[1] For example, methylcobaloxime has a pyridine base binding constant[1f,g] of ca. 2×10^3 M^{-1} and a *p*-NH_2-pyridine binding constant[1f] of ca. 1×10^4 M^{-1} (25 °C; H_2O, ionic strength 1.0 M). Even for the sterically bulky alkyl isoproplycobaloxime, the pyridine binding constant is 484 ± 18 M^{-1}.[1f] Tertiary phosphines are also well known to bind tightly to alkylcobaloximes[1d] [results which contrast dramatically the lack of binding to $AdoCbi^+$ reported herein of even the smallest and most basic phosphines, i.e., PMe_3]. In other words, the cobaloxime B_{12} "models" are very poor quantitative mimics of coenzyme B_{12}'s axial-base binding equilibria, often exhibiting $K_{assoc.}$ too large by at least 10^2.[1h] (Additional cobaloxime axial-base and related studies are available to the interested reader.[1j-r])

We note, however, that the Co(SALEN) or Co(SALOPH) B_{12} models exhibit axial-base properties more like those of B_{12}, for instance axial-base association constants that are weaker than those exhibited by cobaloximes and thus more B_{12}-like (a rare 5 coordinate alkylCo(SALEN) has even been crystallographically characterized[2]). Also noteworthy is the ca. 10^{11} higher substitution lability of Co(SALEN) than that seen for the analogous cobaloximes,[2] another "more B_{12} -like" property. Lippard's recently reported 5 coordinate RCo(tropocoronand) B_{12} models[1i] are very intriguing in this regard and merit axial-base $K_{assoc.}$ studies, and further studies of their apparent stereochemical control of R-Co and Co-B

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[1] (a) Bresciani-Pahor, N.; Forcolin, M.; Marzilli, L. G.; Randaccio, L.; Summers, M. F.; Toscano, P. J. *Coord. Chem. Rev.* **1985**, *63*, 1. (b) Nie, S.; Marzilli, L. G.; Yu, N.-T. *J. Am. Chem. Soc.* **1989**, *111*, 9256. (c) Nie, S.; Marzilli, P. A.; Marzilli, L. G.; Yu, N. -T. *J. Chem. Soc., Chem. Commun.* **1990**, 770. (d) Ng, F. T. T.; Rempel, G. L.; Halpern, J. *Inorg. Chim. Acta* **1983**, *77*, L165; Halpern, J. *Chem Soc. Jpn.*, **1988**, *61*, *13* [see Table I therein for studies of RCo(cobaloxime)(PR_3) complexes in toluene, acetone, and ethylene glycol (although $K_{assoc.}$ constants for phosphines are not reported)]. (e) Geno, M. K.; Halpern, J. *J. Am. Chem. Soc.* **1987**, *109*, 1238. (f) Brown, K. L.; Lyles, D.; Pencovici, M.; Kallen, R. G. *J. Am. Chem. Soc.* **1975**, *97*, 7338. (g) Brown, K. L.; Chernoff, D.; Keljo, D. J.; Kallen, R. G. *J. Am. Chem. Soc.* **1972**, *94*. 6697. (h) This conclusion is consistent with our earlier findings; see the data in Table III in: Finke, R. G.; Smith, B. L.; McKenna, W. A.; Christian, P. A. *Inorg. Chem.* **1981**, *20*, 687. (i) Jaynes, B. S.; Ren, T.; Masschelein, A.; Lippard, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 5589. (j) Brown, K. L.; Satyanarayana, S. *J. Am. Chem. Soc.* **1992**, *114*, 5674. (k) Brown K. L.; Yang, T.-F. *Inorg. Chem.* **1987**, *26*, 3007. (l) Brown, K. L.; Ngamelue, M. *J. Organomet. Chem. 1983*, *243*, 339. (m) Brown, K. L.; Zahonyi-Budo, E. *J. Am. Chem. Soc.* **1982**, *104*, 4117. (n) Brown K. L.; Hessley, R. K. *Inorg. Chim. Acta* **1981**, *53*, L115. (o) Brown, K. L.; Zahonyi-Budo, E. *Inorg. Chem.* **1981**, *20*, 1264. (p) Brown, K. L.; Lu, L.-Y. *Inorg. Chem.* **1981**, *20*, 4178. (q) Brown, K. L.; Awtrey, A. W. *J. Organomet. Chem. 1980*, *195*, 113. (r) Brown, K. L.; Awtrey, A. W. *Inorg. Chem.* **1978**, *17*, 111.

[2] Marzilli, L. G.; Summers, M. F.; Bresciani-Pahor, N.; Zangrando, E.; Charland, J.-P.; Randaccio, L. *J. Am. Chem. Soc.* **1985**, *107*, 6880.

labilities and bond energies. Furthermore, and as Marzilli has noted,[3] the interesting *high-spin* forms of 5 coordinate Co(II)(SALEN or SALOPH) [and possibly of Co(II)(tropocoronands) (?)][1i] are potentially very significant and worthy of greater study.[4] It remains, however, to be seen if the Co out-of-plane distortions and associated high spin Co(II) form *in B_{12} models* can be analogously demonstrated in *cobalamin* chemistry. *If* so, then they become very interesting candidates to possibly help explain the enzyme's 10^{12} acceleration of the AdoCbl Co-C homolysis step.[5] In addition, as also noted in the main text, quantitative $K_{assoc.}$, ΔH, and ΔS measurements in ethylene glycol for the following *Ado-* alkyl complexes would be of value and would permit a more quantitative comparison to the $AdoCbi^+$ studies provided in the main text: *Ado*cobaloxime, *Ado*Co(SALEN/SALOPH), and *Ado*Co(tropocoronand). Also of general interest would be studies measuring axial-base $K_{assoc.}$, ΔH, and ΔS values for Co(II)cobaloxime, Co(II)(SALEN/SALOPH), and Co(II)(tropocoronand).

Attempted Studies of $K_{assoc.}$ for $AdoCbi^+$ Plus Common Phosphines

Experimental details, plus a brief discussion of the results, are provided in the main text. The key result, however, is that in no case was phosphine binding detectable.

Table A. $K_{assoc.}$ (25 °C) Upper Limits for $AdoCbi^+$ Plus Phosphines in Ethylene Glycol.

Phosphine (PR_3)	[Phosphine] (M)	Equivalents of phosphine	$K_{assoc.}$[a] (M^{-1}) 25 °C	Cone angle,[b] (degrees)
P(cyclohexyl)$_3$	1.0×10^{-2}	188	<5.0	>170
P(*i*-propyl)$_3$	3.0×10^{-2}	206	<2.2	160
P(Ph)$_2$(*n*-butyl)	3.0×10^{-2}	165	<1.8	135
P(*n*-butyl)$_3$	3.0×10^{-2}	173	<1.8	132
P(ethyl)$_3$	1.6×10^{-1}	1000	<0.3	132
P(methyl)$_3$	3.0×10^{-1}	1562	<0.2	118

(a) *Maximum* 25 °C $K_{assoc.}$ values, calculated as described in the Experimental Section.

(b) Tolman, C. A. *Chem. Rev.* **1977**, *77*, 212.

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[3] For a concise discussion of the possibility of an "ideal" Co-N bond to the axial 5,6-dimethylbenzimidazole ligand see pp 248-249 of: Marzilli, L. In *Bioinorganic Catalysis;* Reedijk, J. Ed.; Marcel Dekker, Inc.: New York, 1993, p. 227-259.

[4] (a) Calligaris, M.; Nardin, G.; Randaccio, L. *J. Chem. Soc., Dalton* Trans. **1974**, 1903. (b) Kennedy, B. J.; Fallon, G. D.; Gatehouse, B. M. K. C.; Murray, K. S. *Inorg. Chem.* **1984**, *23*, 580.

[5] (a) Finke, R. G.; Hay, B. P. *Inorg. Chem.* **1984**, *23*, 3041. (b) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 4820. (c) Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1469.

Overlays of AdoCbi$^+$ UV-visible spectra with differing concentrations of base.

As described in the Experimental section of the main text, the $K_{assoc.}$ constants were determined by measuring the absorbance as a function of base concentration and temperature. Each scan was run on a separate cuvette containing nearly identical concentrations of AdoCbi$^+$ (the slight deviance in isosbestic points in some of the runs is due to a slightly different corrin concentration in the different cuvettes). Base concentrations ranged from 250 to 10,000 equivalents (relative to the [corrin]). Representative examples of the spectra are provided below. (Note that, in order to have maximal Δ absorbance changes at the wavelengths followed [520, 455 and, where possible as a check, 415 and 380 nm], the asorbance at < 330 nm is deliberately in a unreliable absorbance range of > 1.5.)

Figures A-E. Overlaid spectra of AdoCbi$^+$ (ca. 2 to 2.5 x 10^{-2} M) in ethylene glycol (25 °C) as a function of added base.

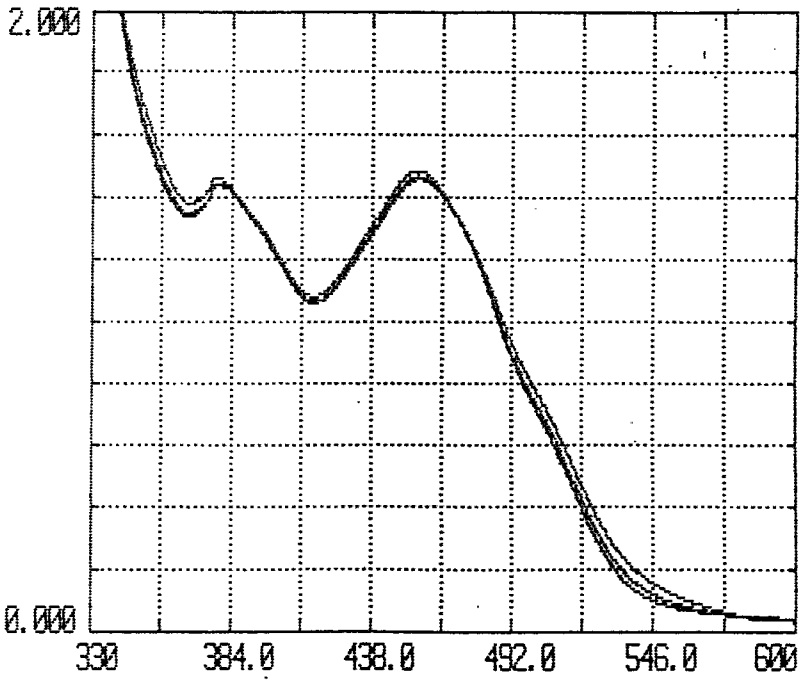

Figure A. AdoCbi$^+$ plus *p*-CN-pyridine (2.0 to 0.5 M).

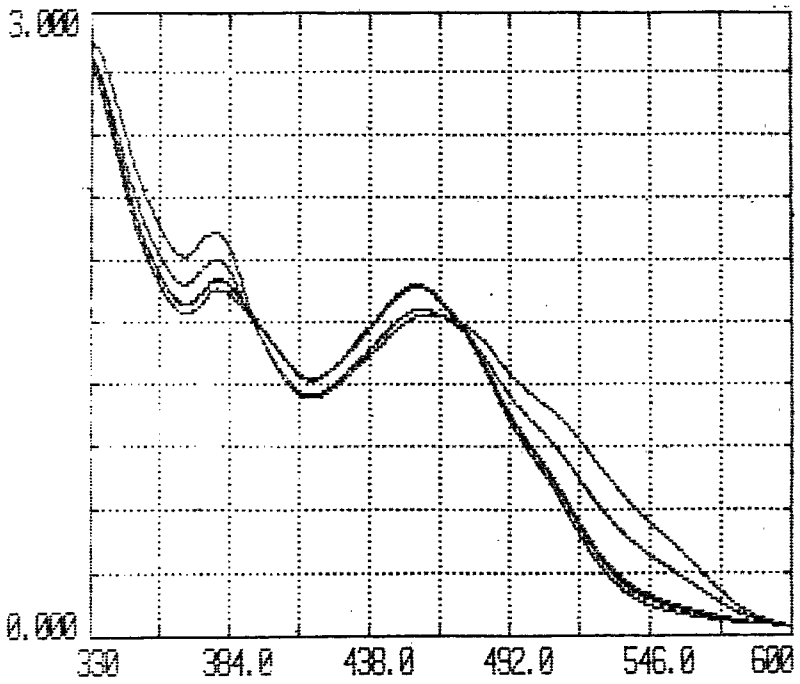

Figure B. AdoCbi$^+$ plus pyridine (2.0 to 0.25 M).

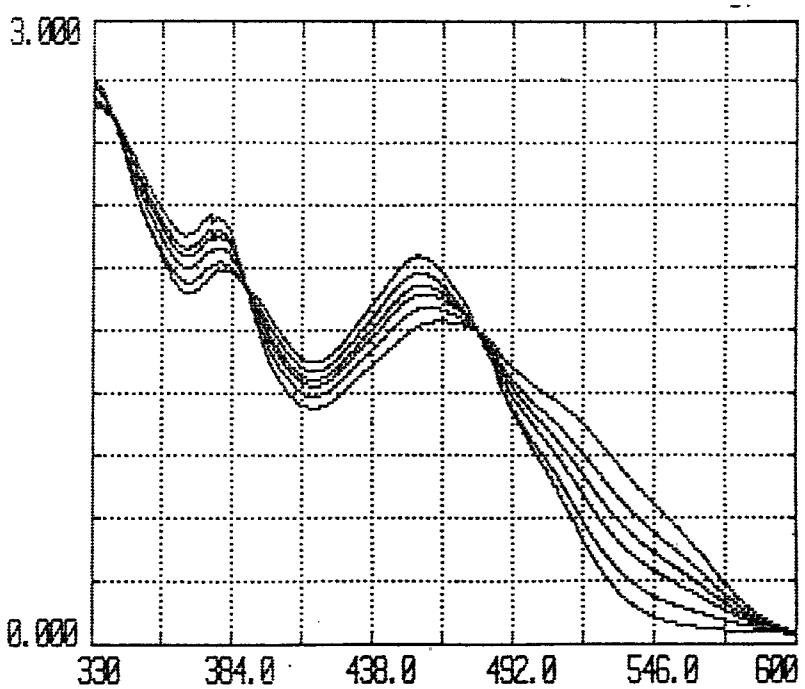

Figure C. AdoCbi$^+$ plus *p*-CH_3-pyridine (2.0 to 0.125 M).

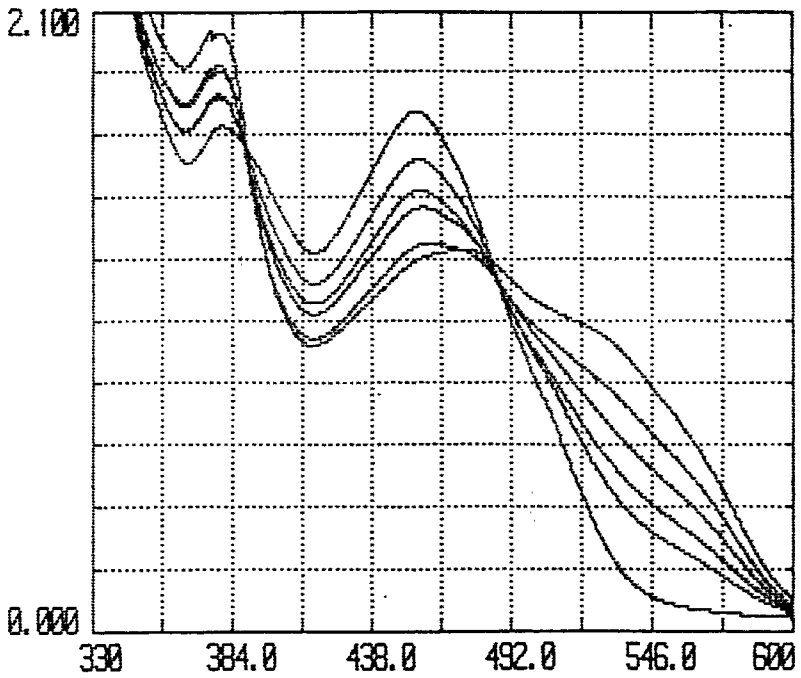

Figure D. AdoCbi$^+$ plus *p*-NH_2-pyridine (2.0 to 0.125 M).

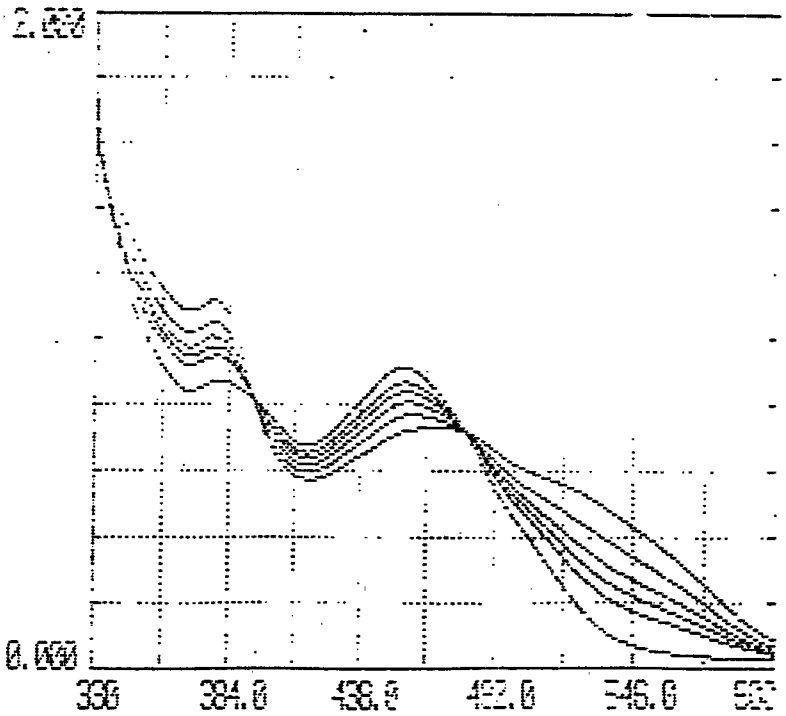

Figure E. AdoCbi$^+$ plus *p*-Me_2N-pyridine (1.0 to 6.25 x10^{-2} M).

Representative plots using equations 1 or 2.

For an equilibria of the form $A + B \rightleftharpoons C$,

where A = AdoCbi$^+$, B = Base, C = [AdoCbi•base$^+$]:

$$(1) \quad Abs = \frac{-(Abs-Abs_o)}{[B_o]} \bullet \frac{1}{K_{assoc}} + Abs_\infty$$

$$(2) \quad K^{-1} = \frac{(Abs-Abs_o)}{(\varepsilon_c-\varepsilon_a)} - [A_o] - [B_o] + \frac{([A_o][B_o](\varepsilon_c-\varepsilon_a))}{(Abs-Abs_o)}$$

Using equation 1 (for a derivation, see reference 21b provided in the main text), a plot of (Abs-Abs$_o$) / [B$_o$]) vs. Abs is made; the slope = K^{-1} and the y intercept = Abs$_\infty$, the all-base-on absorbance. The only limitation to this equation is that the [Base] must be greater than the [AdoCbi$^+$].

Another method for deriving the desired $K_{assoc.}$ is Drago's method,[6] eq. 2. The data is plotted by estimating the extinction coefficient of the base-on AdoCbi$^+$ and solving the equation for K^{-1} at a given concentration of base. Three or more ε_c estimates yields a straight line. This process is repeated at three or more [Base] values, resulting in a graph consisting of three intersecting lines. K^{-1} and ε_c are extrapolated from the center of the triangle formed, and error bars are estimated from the triangle's extremes.

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[6]Drago, R. S. *Physical Methods in Chemistry*; W. B. Saunders: Philadelphia, 1977; p 90.

Representative examples of the various plots are provided below.

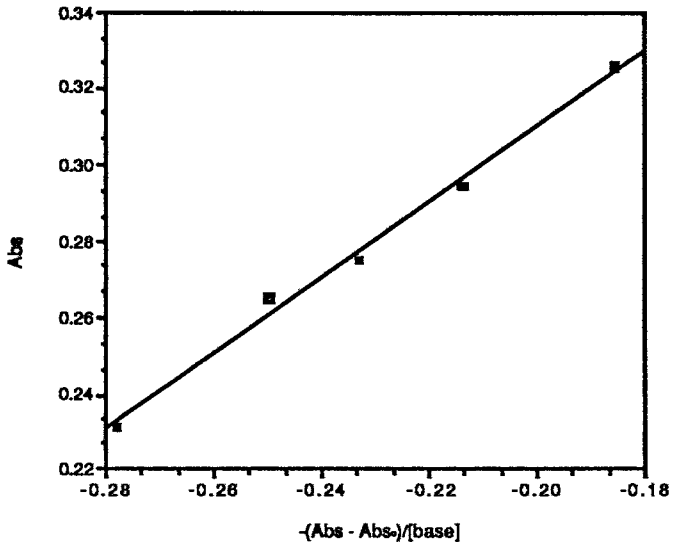
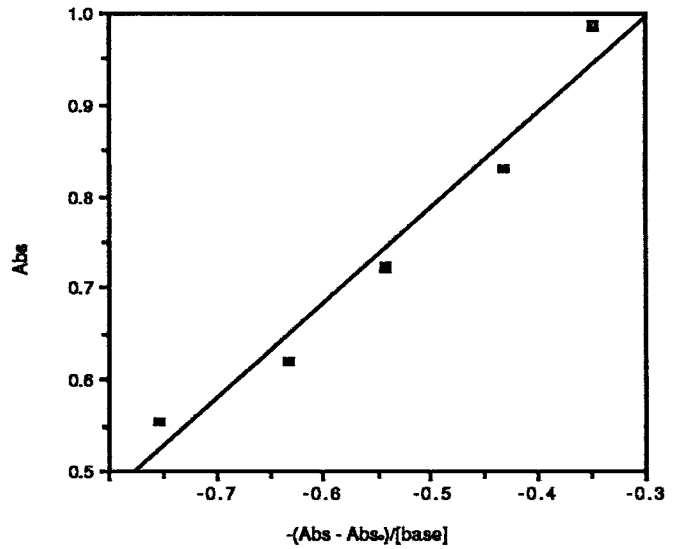


Figure F. AdoCbi$^+$ plus pyridine (25 °C; 520 nm data), Abs vs. -(Abs - Abs_0)/[base] plot; y = 0.99x + 0.51, R^2 = 0.992. Data collected at 380 or 455 nm gave the same results within experimental error.



Figures G. AdoCbi$^+$ plus CH_3-pyridine (25 °C, 520 nm data) linear regression of Abs vs. -(Abs - Abs_0)/[base]; y = 1.05x + 1.3, R^2 = 0.96. Data collected at 380, 415, or 455 nm gave the same results within experimental error, as did "Drago plots" of the 380, 455 or 520 nm data.

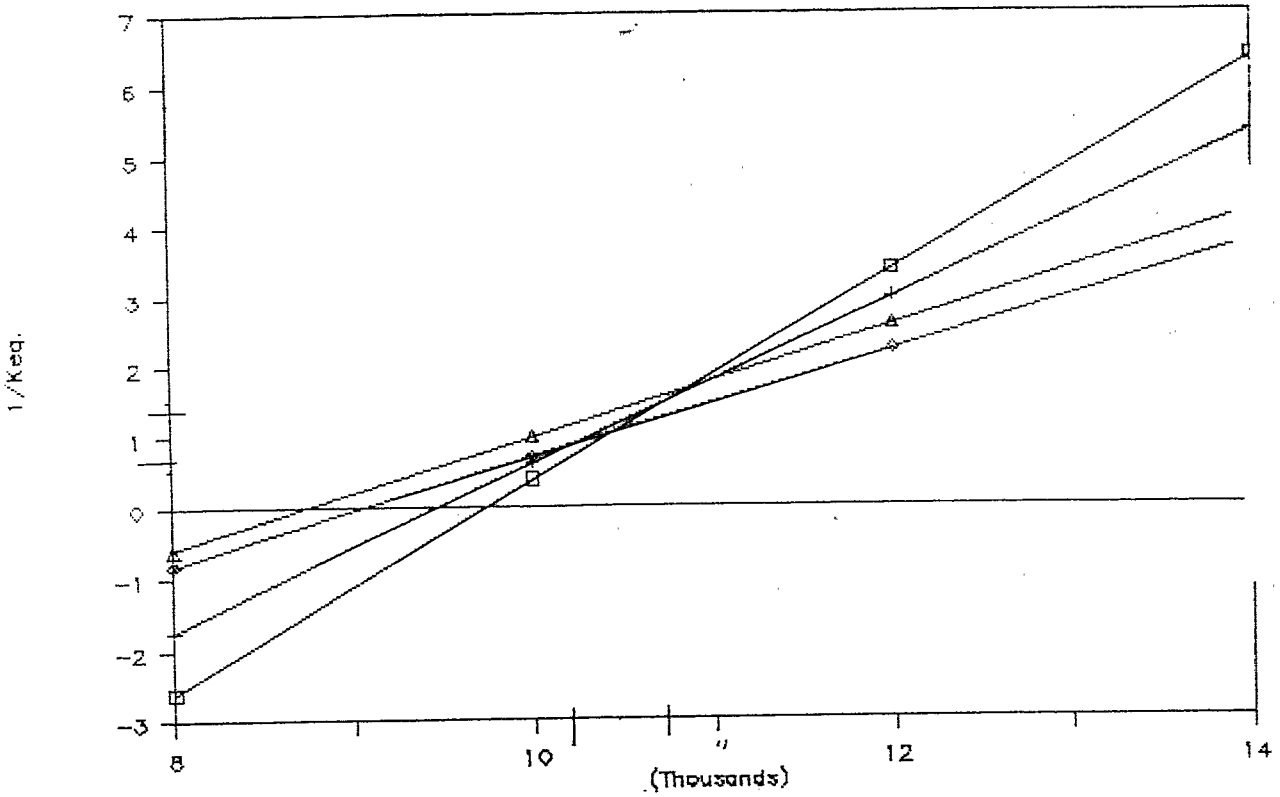


Figure H. AdoCbi$^+$ plus CH_3-pyridine "Drago plot" of the 380 nm data. The center of the resultant triangle yields $K^{-1} = 1$, while the extremes of the triangle yielded the error estimates ± 0.33.

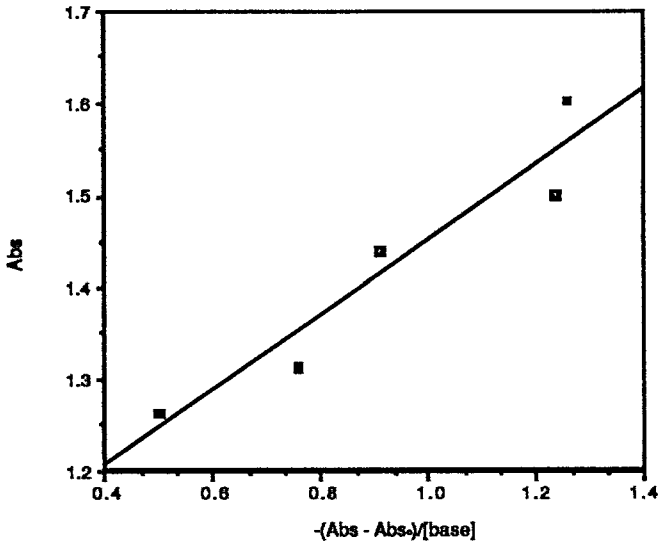


Figure I. AdoCbi$^+$ plus NH_2-pyridine (25 °C, 455 nm data) Abs vs. -(Abs - Abs_o/[base] plot; y = 0.408x + 1.04, R^2 = 0.911. Data collected at 380, 415, or 520 nm gave the same results within experimental error, as did "Drago plots" of the 415 or 520 nm data.

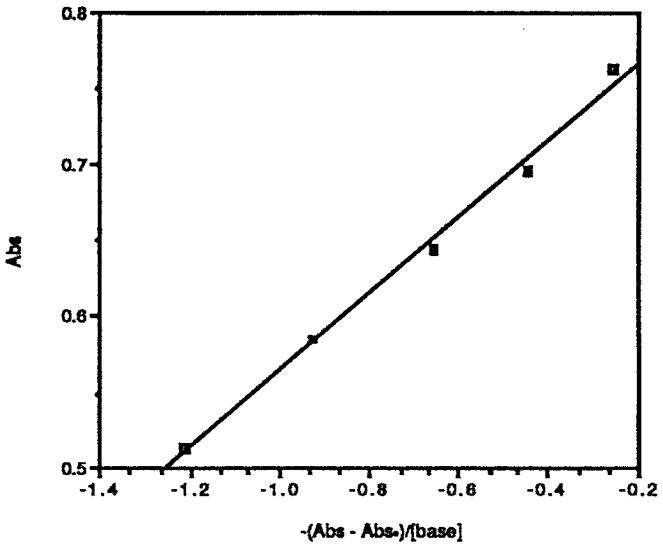


Figure J. AdoCbi$^+$ plus Me_2N-pyridine (10°C; 520 nm data), Abs vs. -(Abs - Abs_0)/[base] plot; y = 0.252x + 0.82, R^2 = 0.993. Data collected at 380 or 455 nm gave the same results within experimental error.

ln $K_{assoc.}$ vs. 1/T plots.

From the $K_{assoc.}$ values measured at several different temperatures, the thermodynamic parameters ΔH and ΔS were obtained in the usual fashion by plotting ln $K_{assoc.}$ vs. 1/T, where the slope yields $-\Delta H/R$ and the y-intercept yields $\Delta S/R$. All plots are provided below.

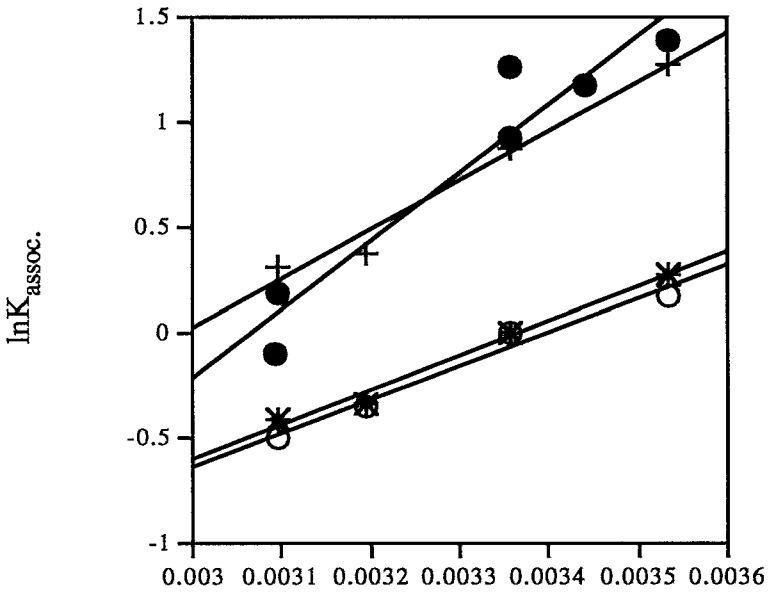


Figure K. ln $K_{assoc.}$ vs. 1/T plots of AdoCbi$^+$ plus pyridine [O; y = (1.64 x 10^3) x – 5.57, R^2 = 0.976]; plus CH_3-pyridine [*; y = (1.67 x 10^3) x – 5.65, R^2 = 0.993]; plus NH_2-pyridine [+; y = (2.28 x 10^3) – 6.78, R^2 = 0.977], or plus Me_2N-pyridine [● ; y = (3.25x 10^3) x – 9.97, R^2 = 0.914].

Attempted AdoCbi$^+$ $K_{assoc.}$ Studies with RSH/RS$^-$

See the main text for experimental details and for a brief discussion of these results. However, the key conclusion from the following table of results is that, at least for each case and under the conditions tested, no thiol or thiolate α-axial binding could be detected.

Table B. $K_{assoc.}$ Upper Limits for AdoCbi$^+$ Plus RSH or RS$^-$ at 25 °C in Ethylene Glycol.

RSH (or thiolate conjugate base)[a]	pK_a [b]	solvent; pH	25 °C $K_{assoc.}$ (M^{-1})
β-mercaptoethanol	9.5	14.3 M (neat) β-mercaptoethanol	<0.01
β-mercaptoethanol	9.5	ethylene glycol	<0.53
β-mercaptoethanol	" "	pH 7.2 aqueous[c]	<0.47
β-mercaptoethanol [a]	" "	pH 10 aqueous[c]	<0.28
dithioerythritol	$pK_a1 = 9.2$ $pK_a2 = 9.9$	pH 7.2 aqueous	<0.53
dithioerythritol [a]	" " "	pH 10 aqueous	<0.26
glutathione	9.4	pH 7.2 aqueous	<0.19
glutathione [a]	" "	pH 10 aqueous	<0.21

(a) Thiolate (or, for dithioerythritol, dithiolate) form of the corresponding thiol (or dithiol). (b) pK_a values are from Houk, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1987**, *109*, 6825. (c) Aqueous solutions were prepared by degassing (via 3-freeze-pump-thaw cycles) deionized H_2O, then buffering with 0.05 M phosphate. Any necessary slight pH adjustments were then made by the dropwise addition of 10% NaOH.