Electronic Supplementary Information

Stabilization of the base-off forms of vitamin B_{12} and coenzyme B_{12} by complexation of the α-axial 5,6-dimethylbenzimidazole ligand with cucurbit[7]uril

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Materials and Methods

The vitamin B12, vitamin B12a, coenzyme B12, and 5,6-dimethylbenzimidazole were used as received (Sigma-Aldrich). The 1,5,6-trimethylbenzimidazole was prepared by reacting 5,6-dimethylbenzimidazole with methyl iodide.[S1] The cucurbit[7]uril was prepared and characterized by the method of Day *et al*.[S2] The UV-visible spectra were recorded on a Hewlett-Packard 8452A diode-array spectrometer in 1.00 cm quartz cells. The ^{1}H NMR spectra were obtained on Bruker Avance 400 and 500 instruments in D_2O. The host-guest stability constants were determined using ^{1}H NMR competitive binding measurements with 3-trimethylsilyl-propionic-2,2,3,3-d_4 acid ($K_{CB[7]}$ = (1.82 ± 0.22) x 10^7 dm^3 mol^{-1}) at pD = 4.75 (0.05 mol dm^{-3} sodium acetate/acetic acid).[S3] The electrospray ionization mass spectra were obtained in aqueous solution employing Applied Biosystems/MDS Sciex QSTAR XL QqTOF and Waters ZQ Single Quad ESI-MS mass spectrometers.

References

S1. L. Pappalardo, J. Elguerro and A. Fruchier, *Anal. Quim.* 1975, **71**, 598.

S2. A. Day, A.P.Arnold, R. J. Blanch and B. Snushall, B. *J. Org. Chem.* 2001, **66**, 8094**.**

S3. S. Liu, C. Ruspic, P. Mukhopadhyay, S. Chakrabarti and P. Y. Zavalij, L. Isaacs, *J. Am. Chem.*
Soc. 2005, **127**, 15959.

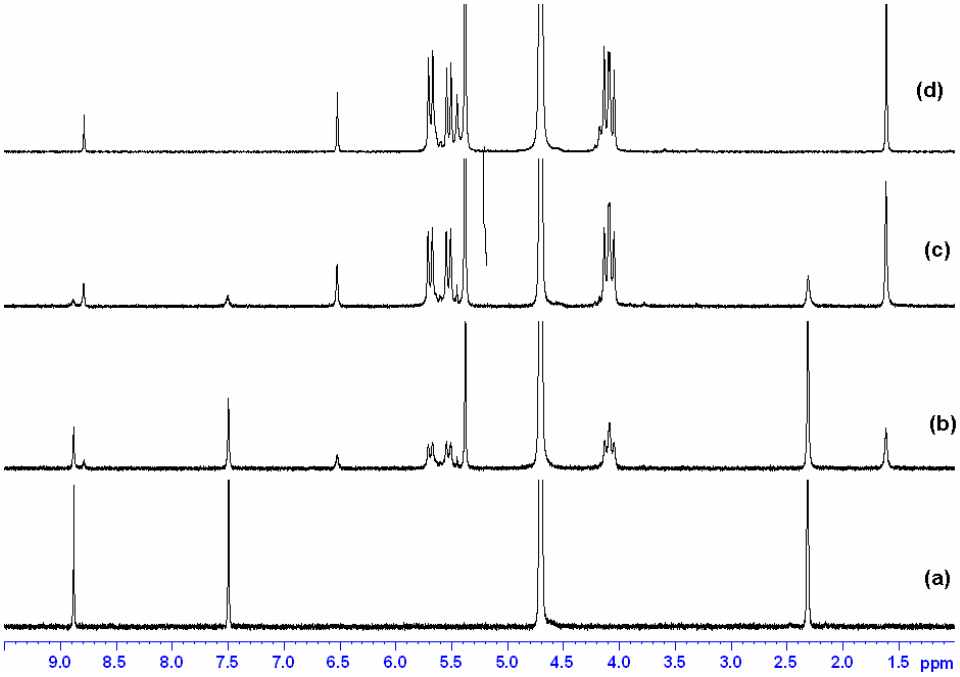

Figure S1. ^{1}H NMR spectra (400 MHz, D_2O) of protonated 5,6-dimethylbenzimidazole ($HDMB^{+}$) in the (a) absence and in the presence of (b) 0.3, (c) 0.8 and (d) 1.3 equivalents of CB[7].

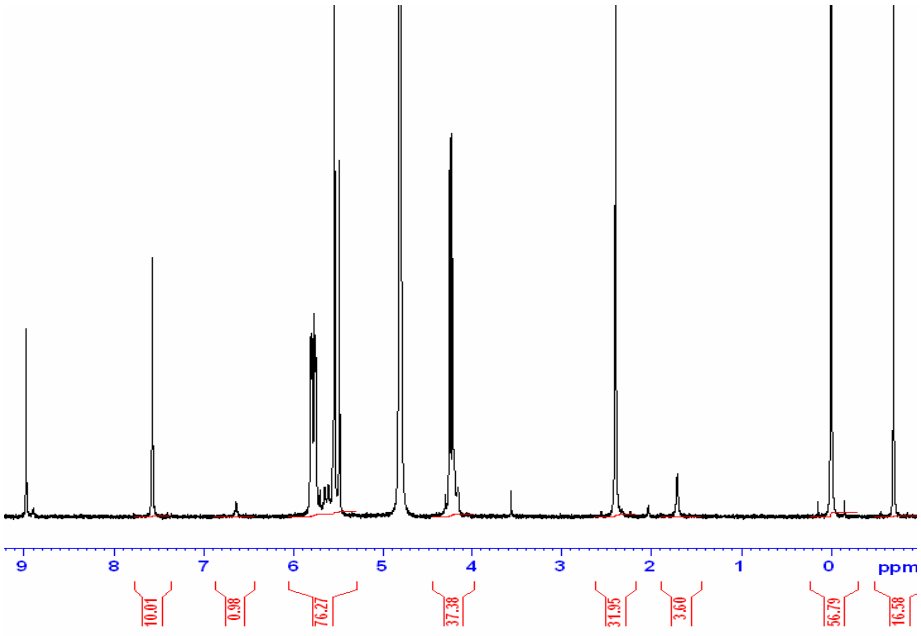

Figure S2. The ^{1}H NMR spectrum (400 MHz, D_2O, acetate buffer) of the competitive binding of CB[7] by protonated 5,6-dimethylbenzimidazole ($HDMB^{+}$) and TSP. ($K_{CB[7]} = 6.65 \times 10^{6}$ dm^{3} mol^{-1})

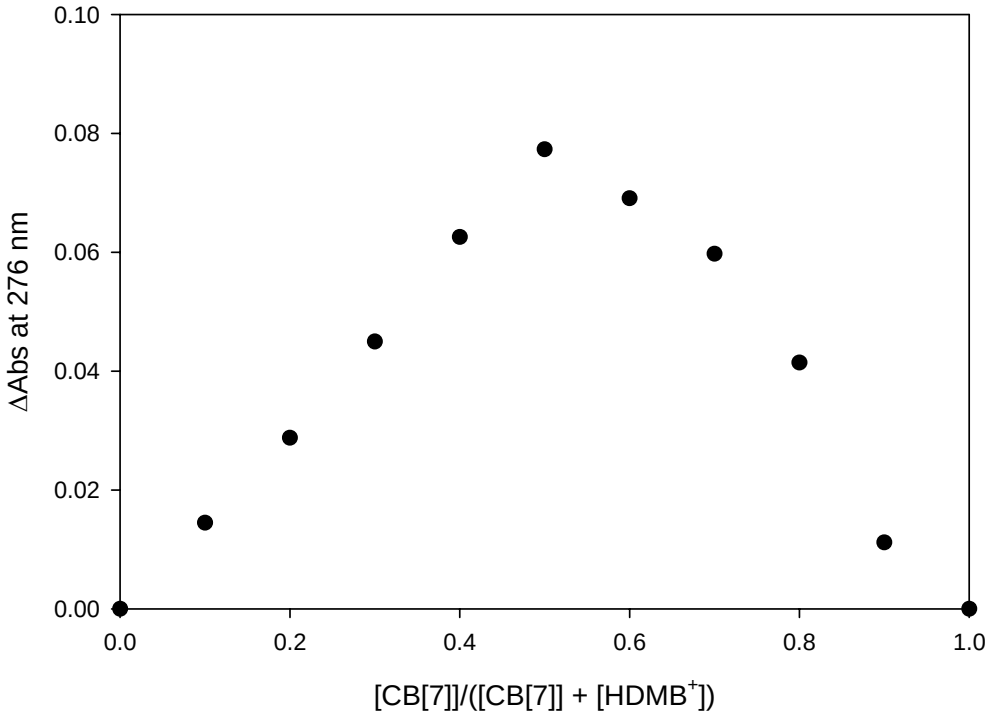

Figure S3. The Job's plot of $HDMB^{+}$ and CB[7], with the total concentration maintained at 0.10 mmol dm^{-3}.

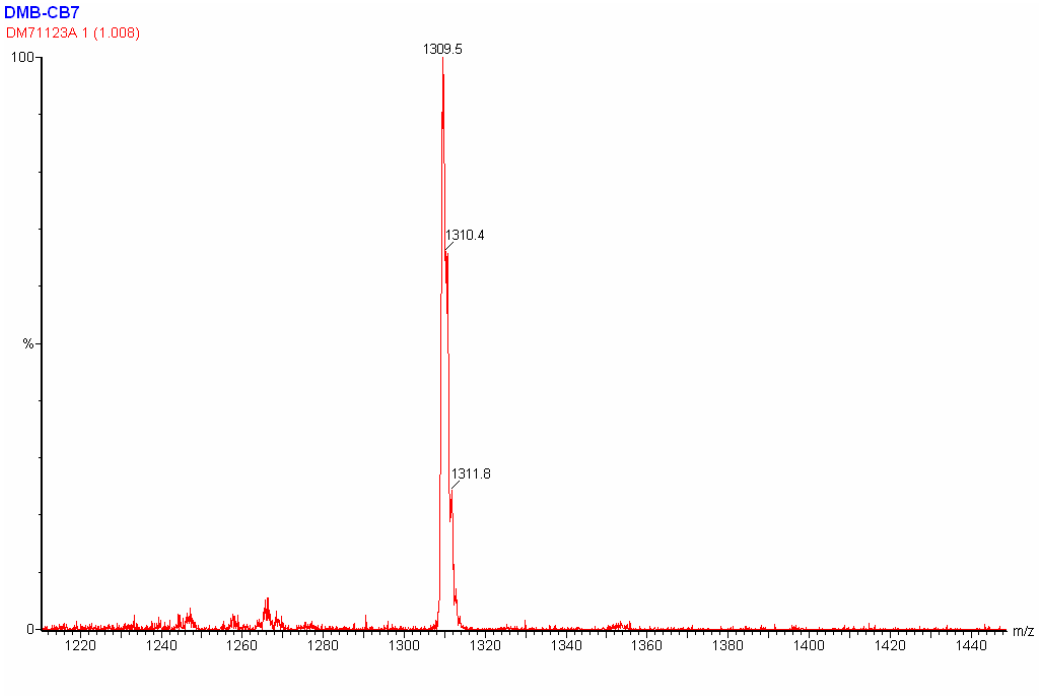

Figure S4. The electrospray mass spectrum of $\{HDMB•CB[7]\}^{+}$

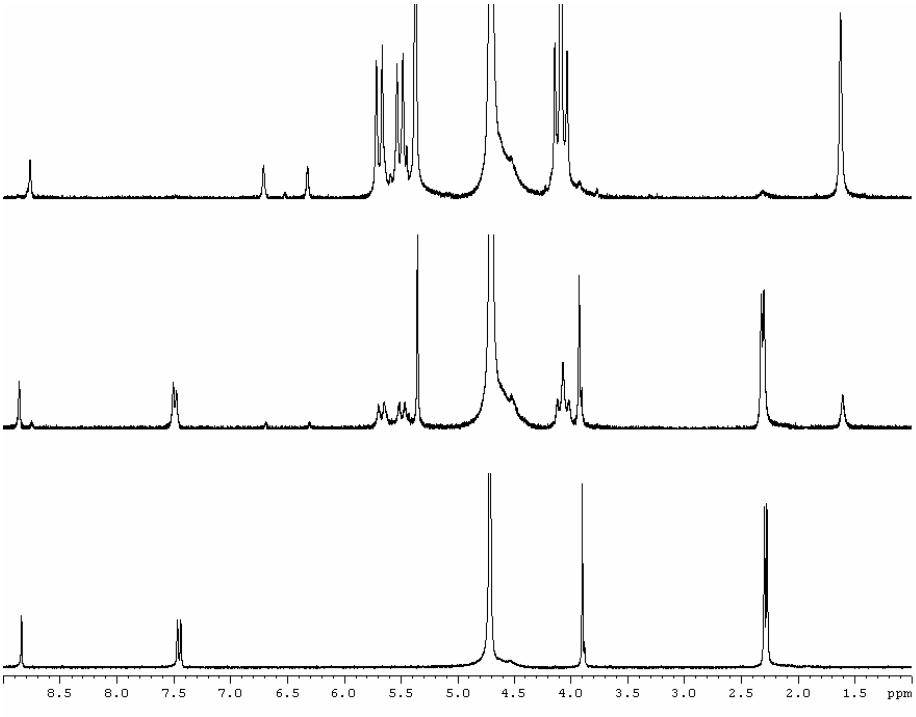

Figure S5. ^{1}H NMR spectra (400 MHz, D_2O) of protonated 1,5,6-trimethylbenzimidazole ($HTMB^+$) in the absence (bottom) and in the presence of 0.2 (middle) and 1.3 equivalents (top) of CB[7].

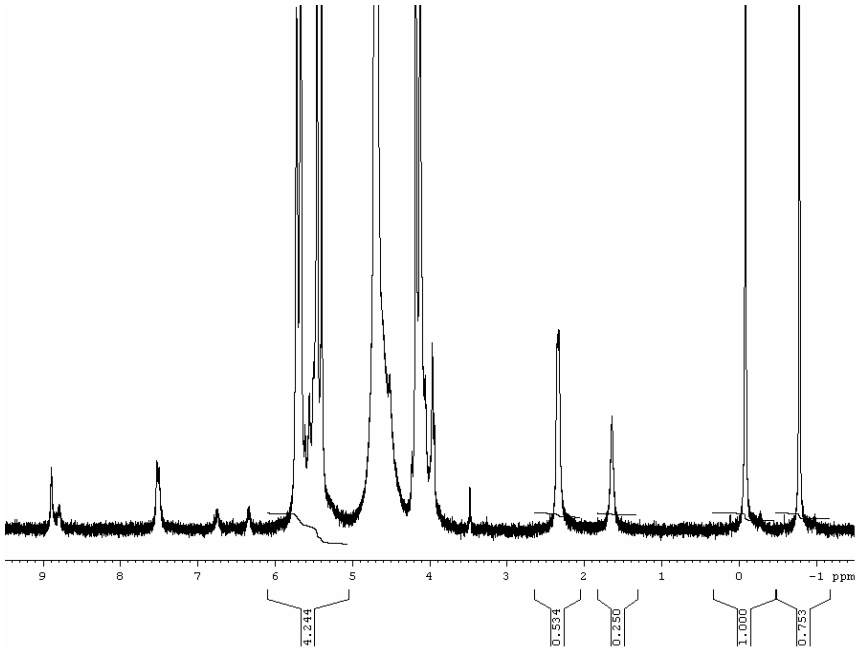

Figure S6. The ^{1}H NMR spectrum (400 MHz, D_2O, 0.050 M CH_3COONa/H) of competitive binding of CB[7] between 1,5,6-trimethylbenzimidazole and TSP ($K_{CB[7]} = (1.13 \pm 0.82) \times 10^7$ $dm^3\ mol^{-1}$).

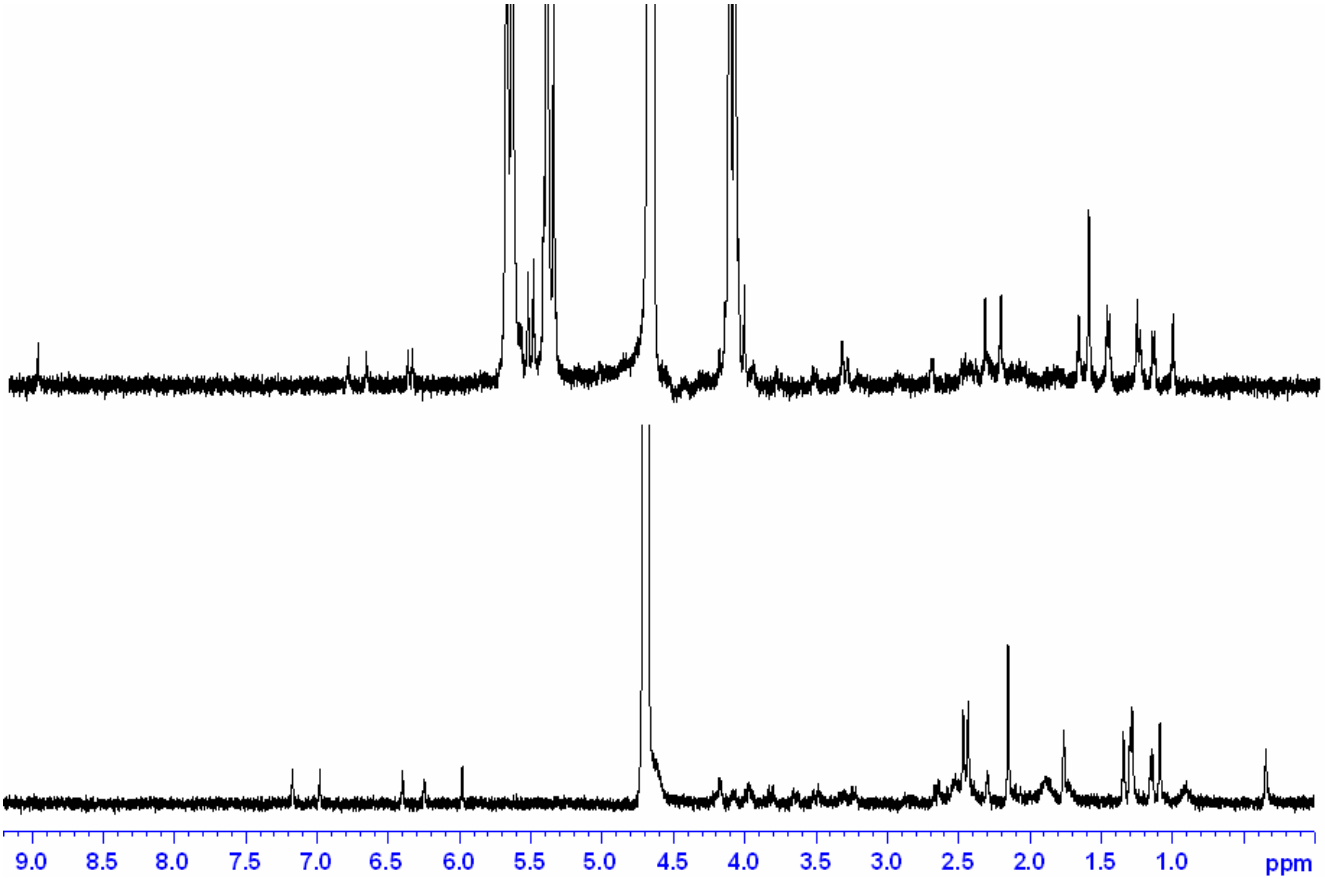

Figure S7. ^{1}H NMR spectra of CNCbl (bottom) and {CNCbl•CB[7]}$^{+}$ (2 equivalents of CB[7], top) in D_2O at pD 2.

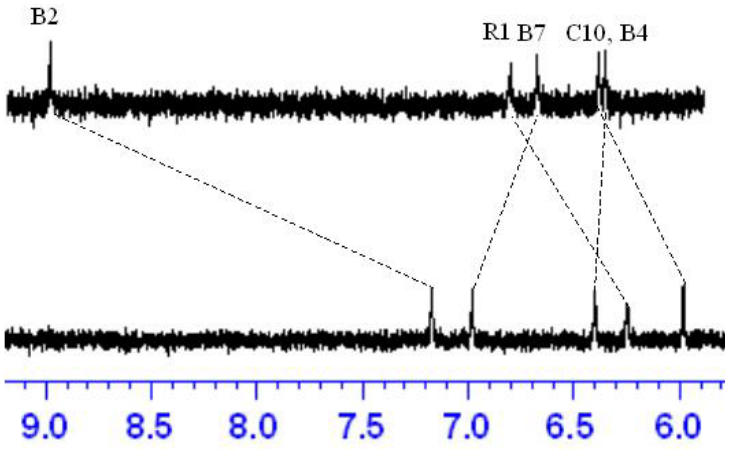

Figure S8. The aromatic portion of the ^{1}H NMR spectra (400 MHz, D_2O) of CNCbl (pD = 2) in the absence (lower spectrum) and in the presence of 2 equivalents of CB[7] (upper spectrum).

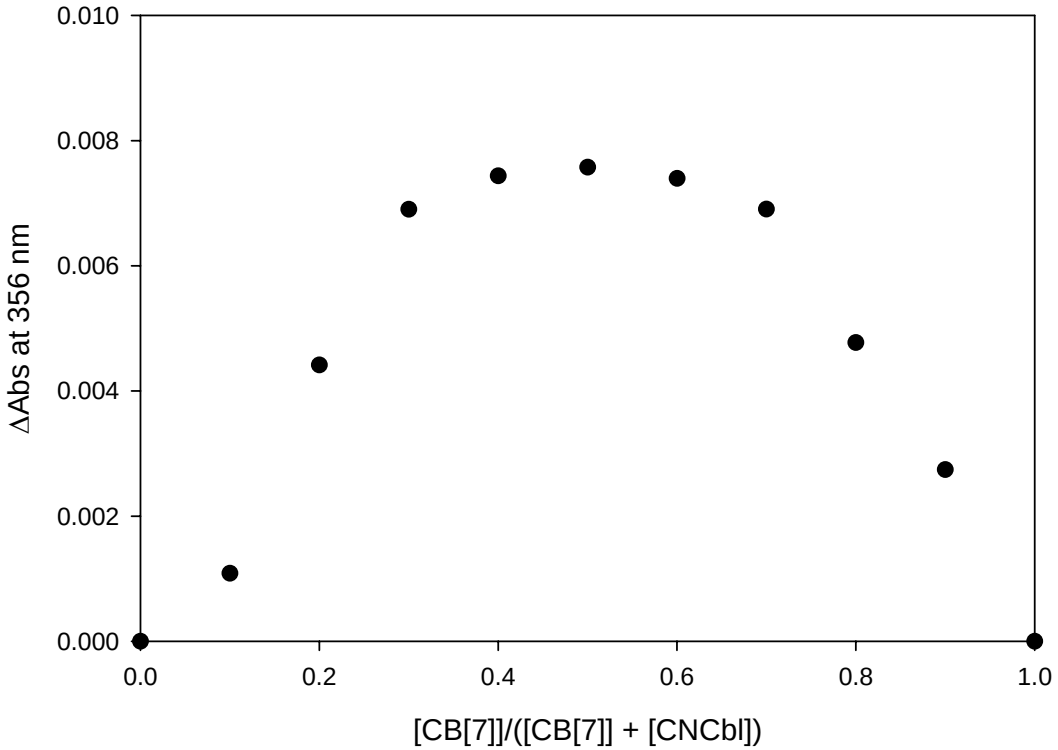

Figure S9. The Job's plot of CNCbl and CB[7], with the total concentration maintained at 18 μmol dm^{-3}.

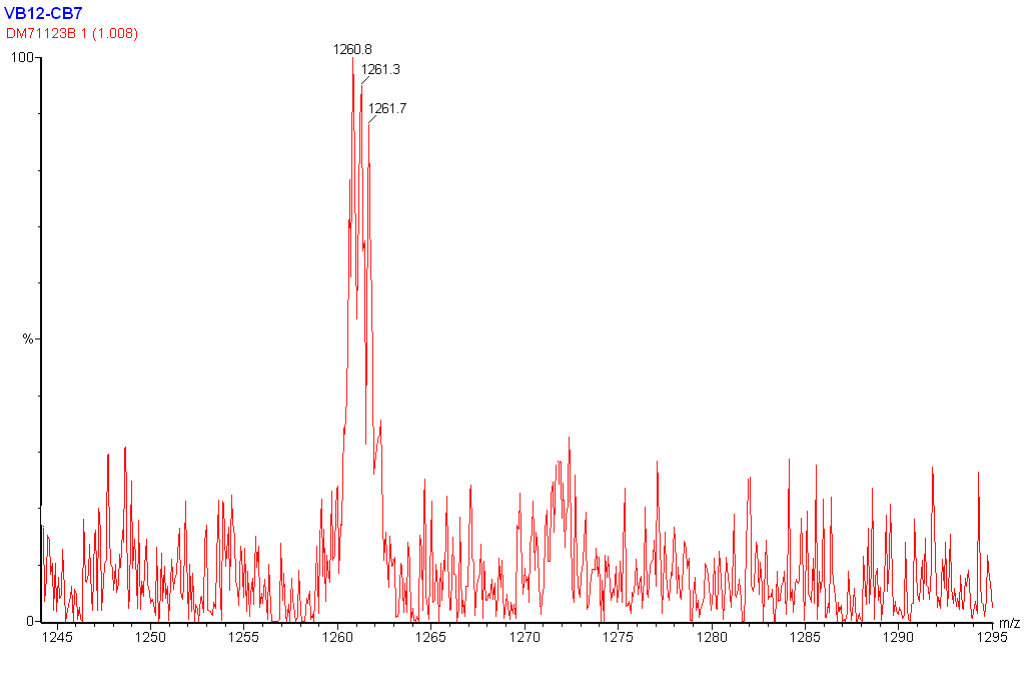

Figure S10. The electrospray mass spectrum of $\{CNCbl•CB[7]\}^{2+}$

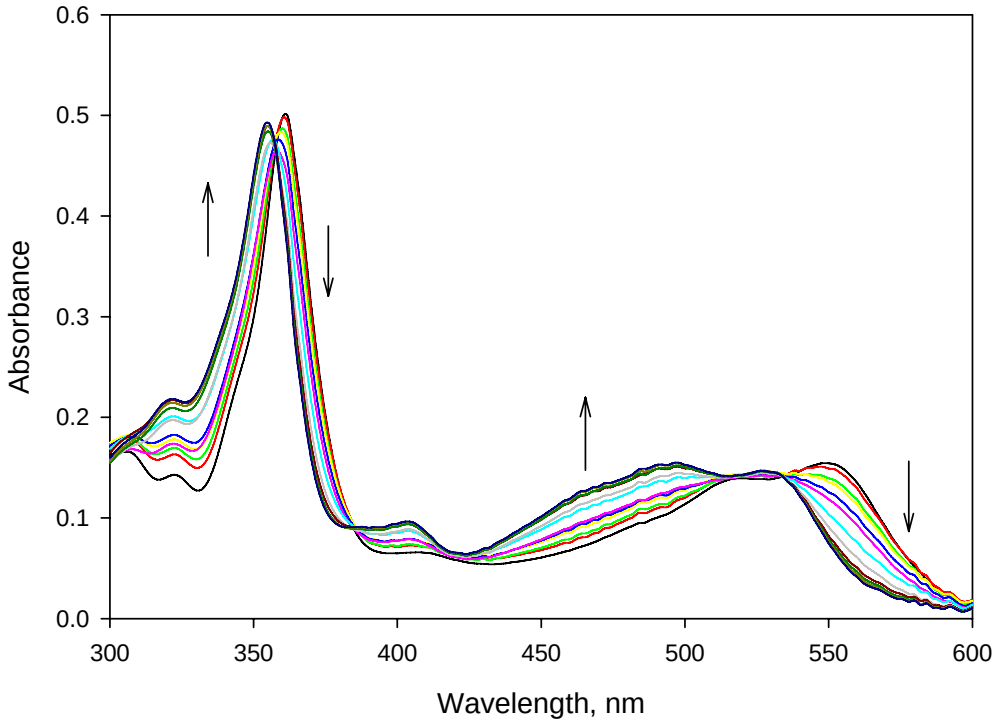

Figure S11. The UV-visible titration of CNCbl (20 μM) at pH=2 ($HClO_4$) with CB[7] (up to 2.5 equivalents).

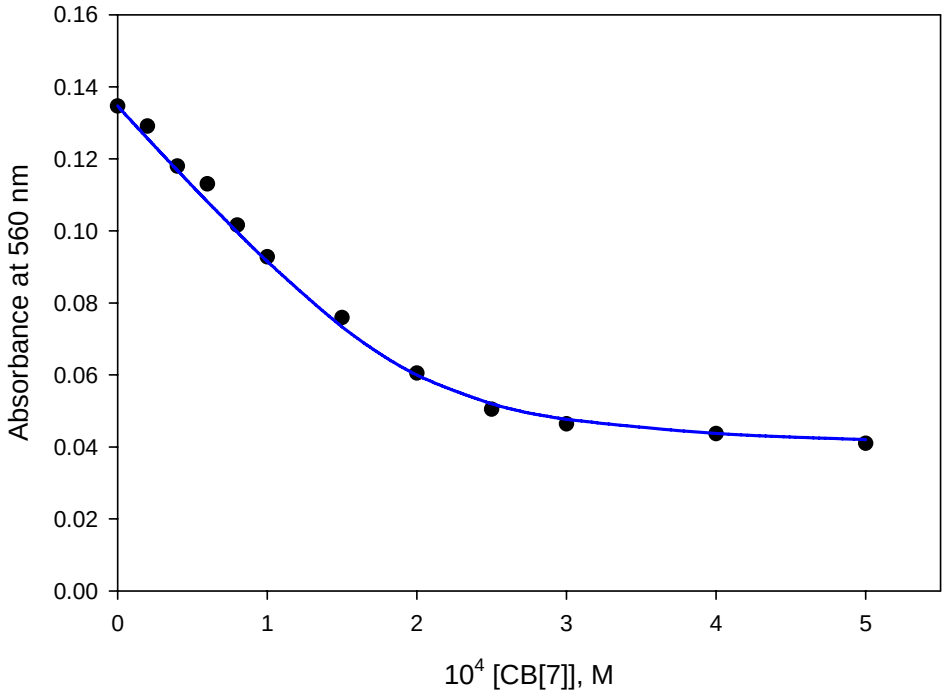

Figure S12. The titration of CNCbl with CB[7] (pH = 2), monitored at 560 nm ($K_{CB[7]}$ = 7.5 × 10^4 dm^3 mol^{-1}).

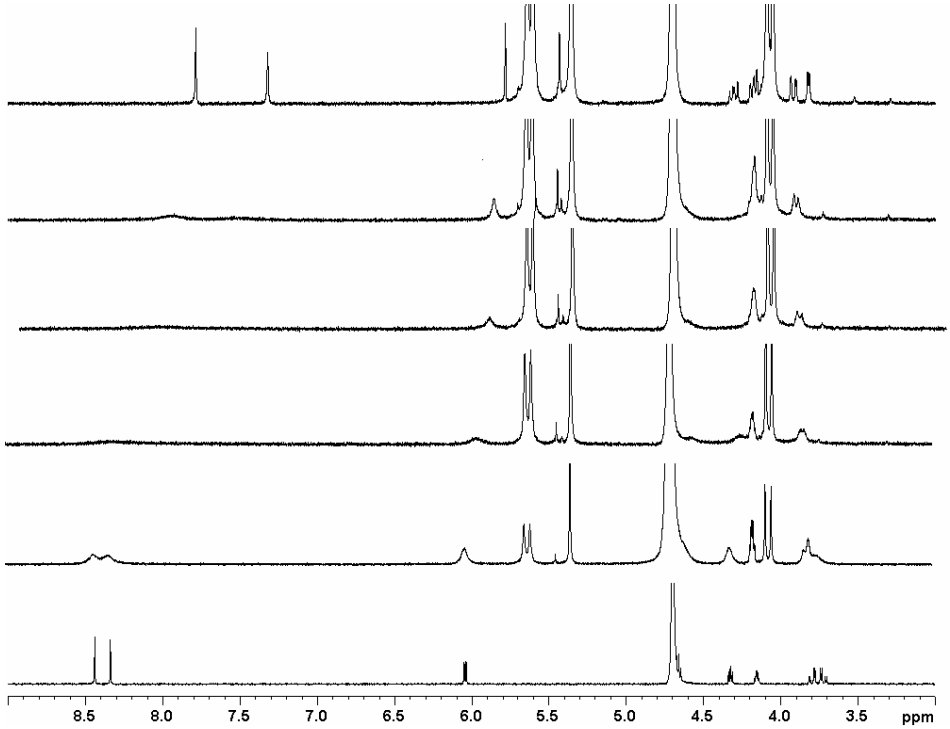

Figure S13. The ^{1}H NMR spectra (400 MHz, D_2O), bottom to tope, of adenosine (pD = 2) in the absence and in the presence of 0.2, 0.7, 1.0, 1.2 and 1.4 equivalents of CB[7].

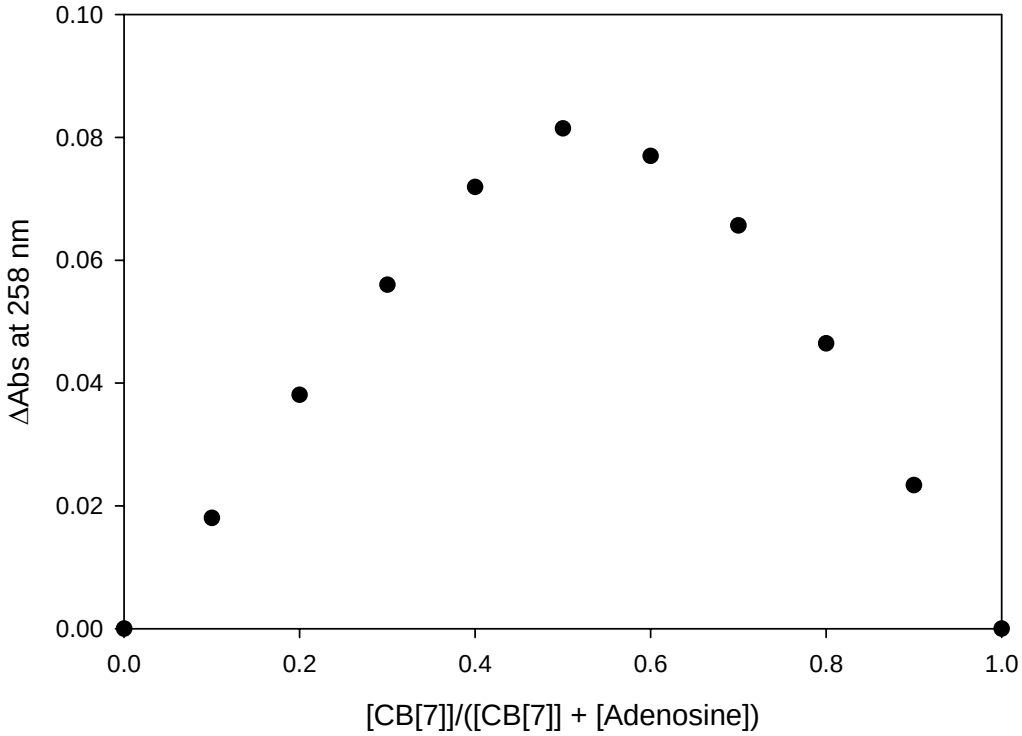

Figure S14. The Job's plot of protonated adenosine and CB[7], keeping the total concentration at 50 μmol dm^{-3}.

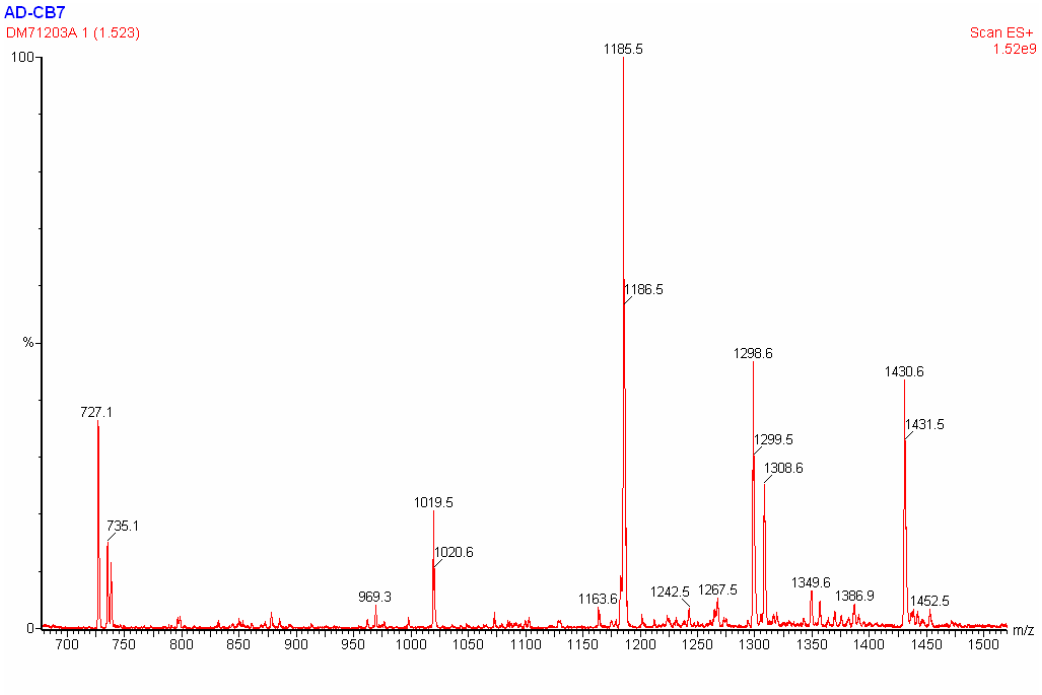

Figure S15. The electrospray mass spectrum of adenosine with CB[7].

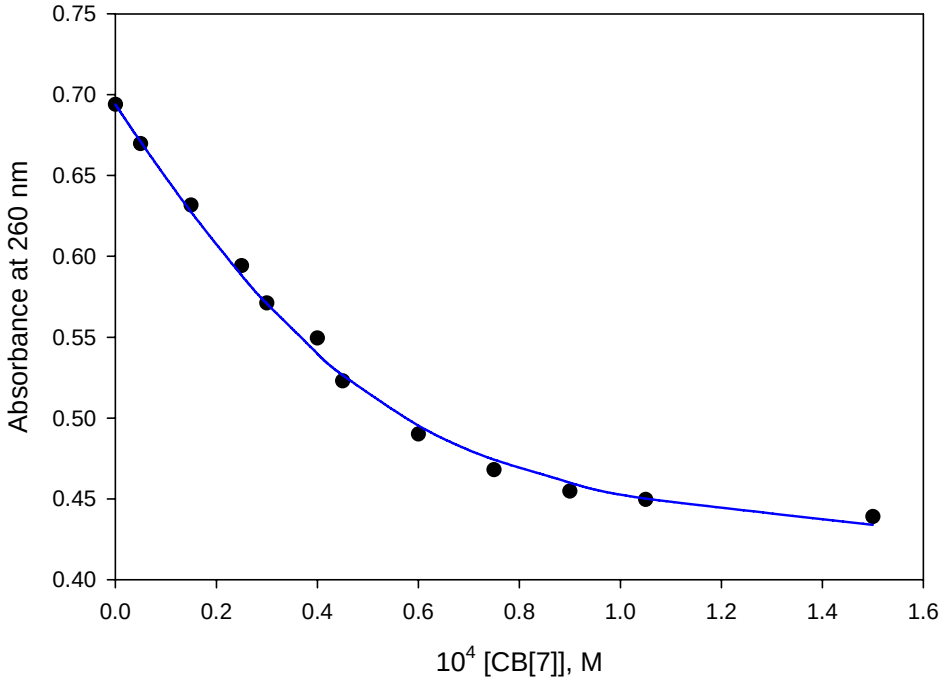

Figure S16. The titration of protonated adenosine (pH = 2) with CB[7]. $K_{CB[7]} = 8.6 \times 10^4\ M^{-1}$.

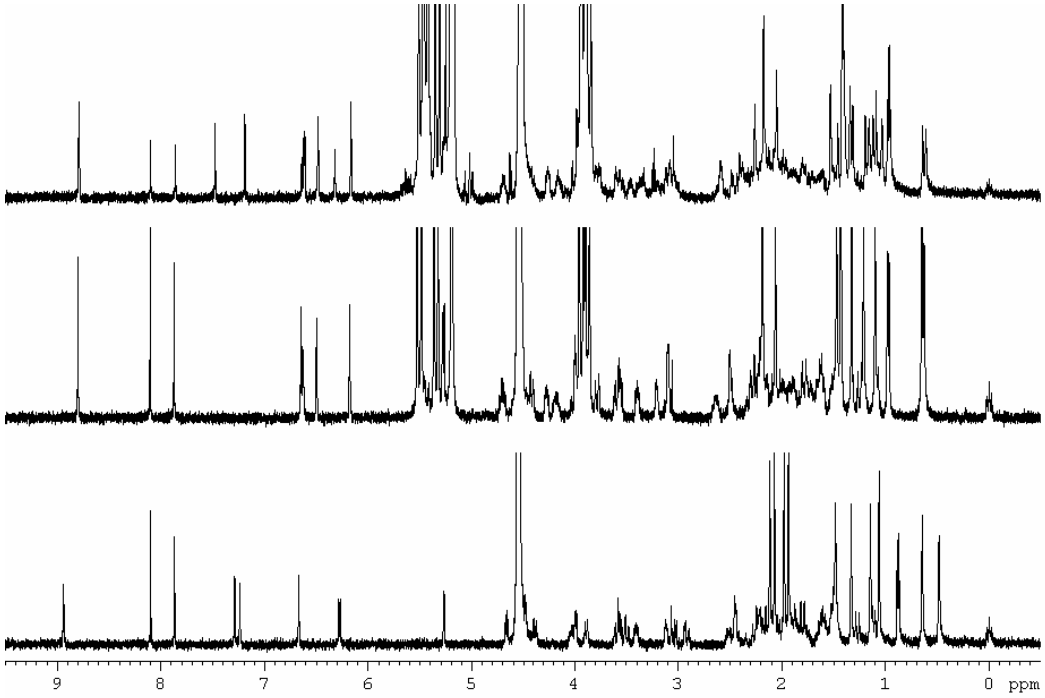

Figure S17. The ^1H NMR spectra of AdoCbl in the absence (bottom) and presence of 1.2 equivalents (middle) and 4.2 equivalents (top) of CB[7] in D_2O.

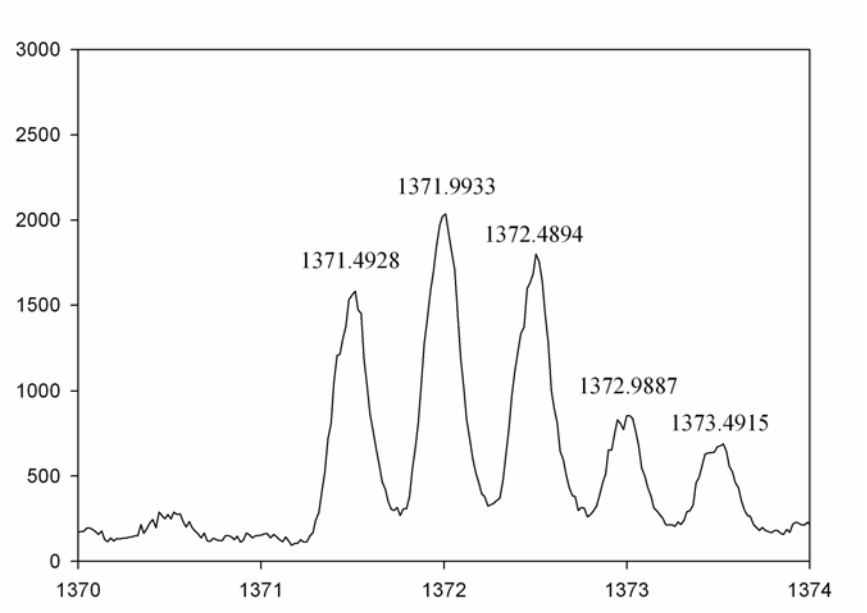

Figure S18. High resolution electrospray TOF mass spectrum of the$\{Ado{\bullet}CB[7] + 2H\}^{2+}$ species.

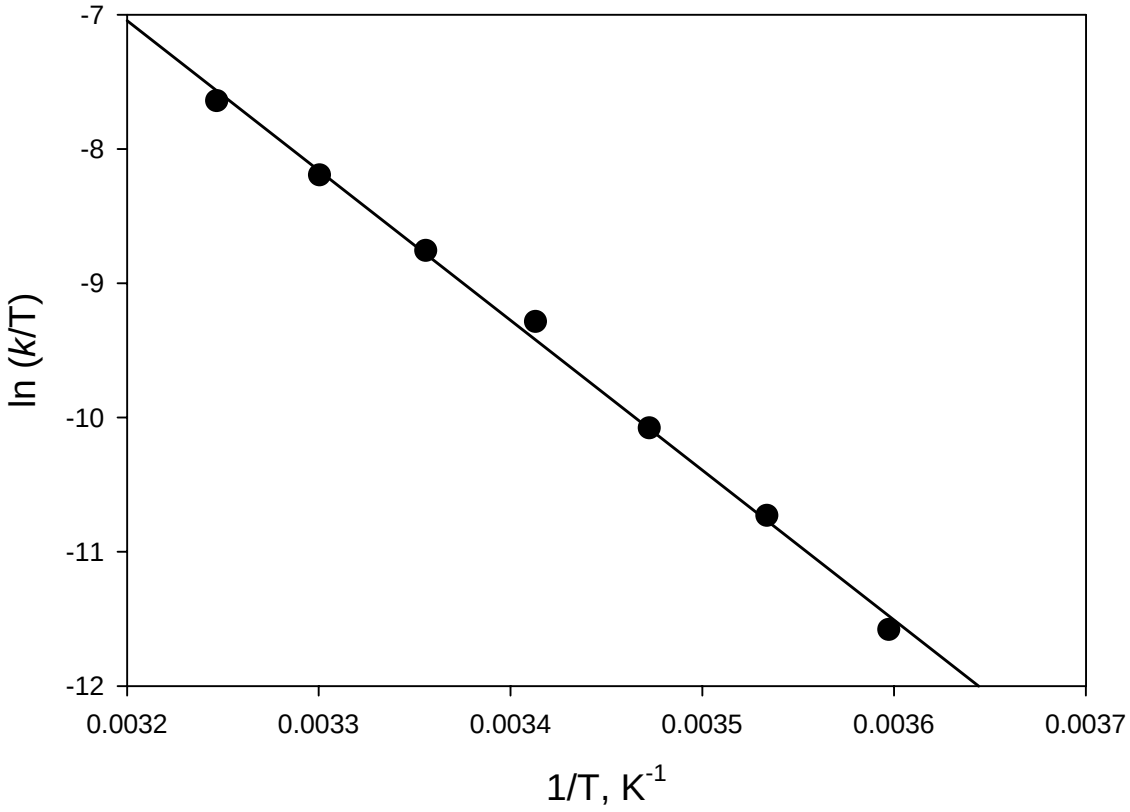

Figure S19. Erying plot for the reaction of CNCbl with CB[7] at pH 2.

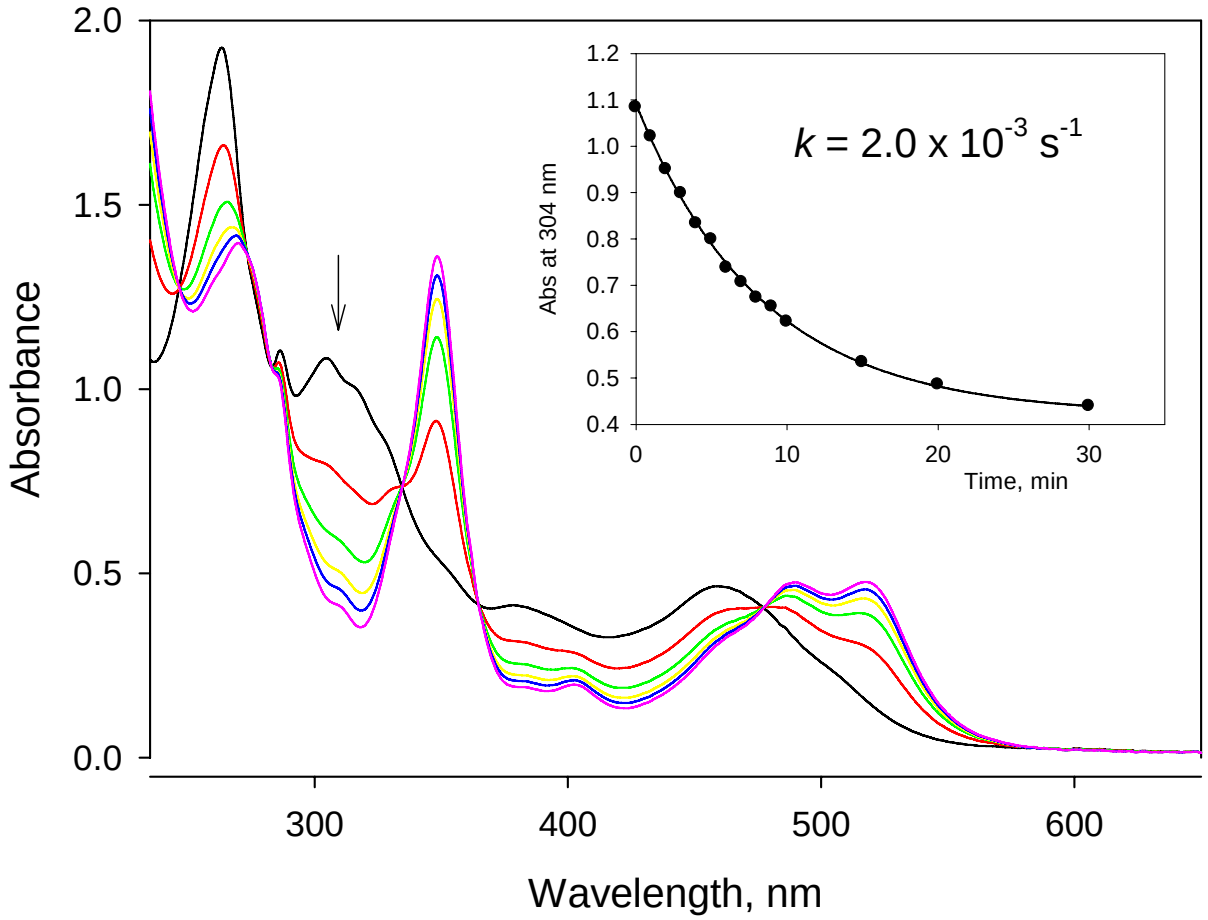

Figure S20. UV-visible spectra of the heterolytic photochemical cleavage of the Co-C bond of AdoCbl (5.0 x 10^{-5} mol dm^{-3}) in the presence of 7.5 x 10^{-5} mol dm^{-3} CB[7]. Inset: plot of the absorbance at 304 nm against time, fit with k = 2.0 x 10^{-3} s^{-1}.