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A ^{13}C-NMR study of azacryptand complexes

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An azacryptand has been solubilised in aqueous media containing 50% (v/v) dimethyl sulphoxide. ^{13}C-NMR has been used to determine how the azacryptand is affected by zinc binding at pH 10. Using ^{13}C-NMR and ^{13}C-enriched bicarbonate we have been able to observe the formation of 4 different carbamate derivatives of the azacryptand at pH 10. The azacryptand was shown to solubilise zinc or cadmium at alkaline pHs. Two moles of zinc are bound per mole of azacryptand and this complex binds 1 Mole of carbonate. By replacing the zinc with cadmium-113 we have shown that the ^{13}C-NMR signal of the ^{13}C-enriched carbon of the bound carbonate is split into two triplets at 2.2˚C. This shows that two cadmium complexes are formed and in each of these complexes the carbonate group is bound by two magnetically equivalent metal ions. It also demonstrates that these cadmium complexes are not in fast exchange. From temperature studies we show that in the zinc complexes both complexes are in fast exchange with each other but are in slow exchange with free bicarbonate. HOESY is used to determine the position of the carbonate carbon in the complex. The solution and crystal structures of the zinc-carbonate-azacryptand complexes are compared.

The seas take up about 9 billion tons of carbon dioxide per year, about a third of the total 30 billion tons of carbon dioxide produced each year. As a result the pH of the seas is expected to drop. This will lower the amount of carbon dioxide dissolved in the oceans which will have serious consequences for the marine ecosystem, e.g. killing fish, inhibiting the growth of shellfish and corals [1, 2]. It has been estimated that there must be at least a 36% decrease in anthropogenic carbon dioxide emissions to stabilise the atmospheric carbon dioxide concentrations in 2100[3]. It is clear therefore that it is important to develop methods of reducing carbon dioxide levels in both gaseous and aqueous environments.

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† Electronic supplementary information (ESI) available: Experimental conditions for Figures 1-4, pH titration of R3Bm, linewidth data and a HOESY spectrum.

The insertion of carbon dioxide into the M-OH bonds of transition metal complexes to form carbonates has been widely established as a viable approach for the fixation of carbon dioxide[4-12]. Likewise, in nature the secondary amines of the imidazole groups of histidine residues are widely utilised for binding metal ions such as zinc in metalloenzymes. The metal usually has a catalytic role though it can also have a non-catalytic role such as helping to stabilize the protein structure[13].

Nelson and co-workers have developed azacryptands that also utilize secondary amines to bind a range of metal ions[14-18]. Using X-ray crystallography[14] it has been shown that these azacryptands can bind two metals which can then bind anions between them by cascade coordination (Scheme 1). Such complexes can bind carbonate and can also activate the carbonate, enabling it to react with other molecules. This is similar to carbonic anhydrase which uses a single zinc atom to catalyse the conversion of carbon dioxide to bicarbonate[13, 19]. Cryptates tethered to mesoporous silicate have recently been shown to activate atmospheric carbon dioxide [20]. In this study we utilize ^{13}C-NMR and ^{13}C-enriched bicarbonate to determine how carbonate reacts with an azacryptand in aqueous media. We determine how zinc or cadmium affect the binding of carbonate at different temperatures. This allows us to probe the kinetics of this process. We also examine the solution structures of the complexes formed.

Experimental

Materials

$^{113}CdCl_2$ (95.32 atm %) was obtained from Goss Scientific Instruments Limited, Gresty Lane, Shavington, Crewe, Cheshire, CW2 5DD, UK. $NaH^{13}CO_3$ (99.0 atm %) was obtained from Cortecnet, 15/17 Rue de Tilleuls, 78960 Voisins le Bretonneux, France. All other chemicals were obtained from Sigma-Aldrich Chemical Company, Gillingham, Dorset, UK.

Synthesis of R3Bm

R3Bm(1,4,12,15,18,26,31,39-octaazapentacyclo [13.13.13.1(6,10).1(20,24).1(33,37)]-tetratetracontane-6,8,10,20,22,24,33,35,37-nonaene) was synthesised from isophthalaldehyde as described earlier [15].

Quantification of the R3Bm

R3Bm (12.9 mg) was dissolved in 1 ml of d_6-DMSO and 9.1 µl of 3M tetrahydrofuran in d_6-DMSO. 0.5 ml samples were quantified using ^{1}H-NMR by comparing the integrated intensities of the aromatic protons of R3Bm at ~7 ppm with the area of the tetrahydrofuran signal at 1.77 ppm. The batches of R3Bm used were 90-98% by weight.

NMR spectroscopy

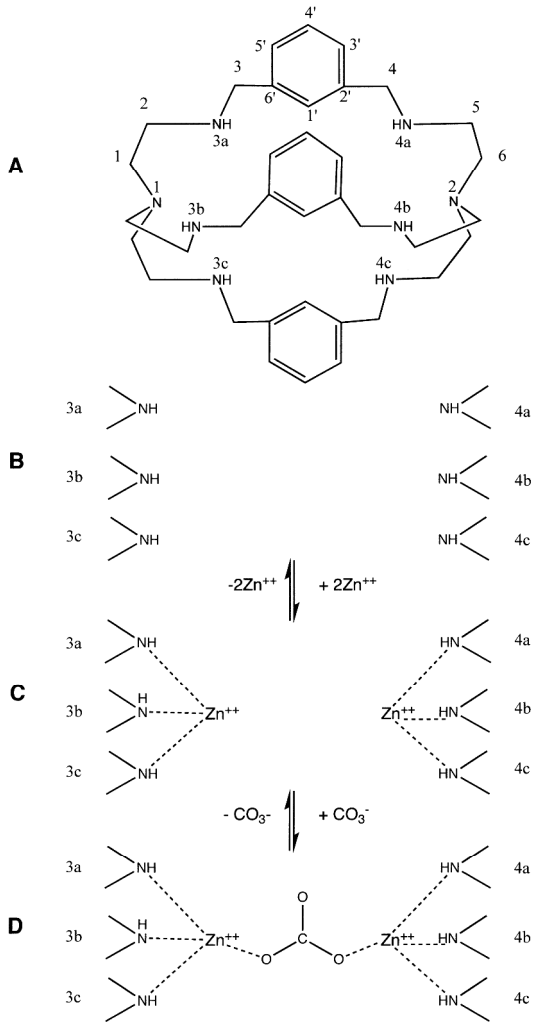
NMR spectra at 11.75 T were recorded with a Bruker Avance DRX 500 standard-bore spectrometer operating at 125.7716 MHz for ^{13}C-nuclei. 5 or 10 mm-diameter sample tubes were used for ^{13}C-NMR spectroscopy. The ^{13}C-NMR spectral and experimental conditions are given in the appropriate figure legends in the ESI. Both ^{1}H and ^{13}C chemical shifts are quoted relative to tetramethylsilane at 0.00 ppm. In aqueous solutions the chemical shift of d_6-dimethyl sulphoxide at 38.7 ppm was used as a secondary reference. NMR spectra were simulated using the dynamic NMR module written by Dr Janos Rohonczy (Eotvos Lorand University, Hungary) in Bruker Topspin 2.1.

Results

The azacryptand R3Bm (Scheme 1A) could be solubilised in 50% (v/v) dimethyl sulphoxide, 40% (v/v) ^1H_2O and 10% (v/v) ^2H_2O to give stable concentrations of ~10 mM at 25˚C.

R3Bm at pH 9.90

R3Bm contains 36 carbon atoms (Scheme 1A) and its ^{13}C-NMR spectrum shows 7 signals (Fig. 1a). There are four signals with chemical shifts at 138.63(C2' and C6'), 129.47(C4'), 128.42(C1') and 128.37 ppm (C3' and 5') due to the 18 aromatic carbons, and three signals due to the 18 aliphatic carbons 53.78 (C3), 52.34 (C1) and 46.77 ppm (C2) at 25 ˚C (Fig. 1a). It is clear therefore that the three carbons 1, 2 and 3 are magnetically equivalent to carbons 4, 5 and 6 in each of the 3 repeating units (-$CH_2CH_2NHCH_2C_6H_6CH_2NHCH_2CH_2$-) of R3Bm (Scheme 1A). This is consistent with R3Bm having a symmetrical structure in aqueous media though it does not exclude a non-symmetrical structure provided this does not significantly affect the magnetic equivalence of the carbons giving the NMR signals.



Scheme 1 Structure of R3Bm and its binding to zinc and its binding of both zinc and carbonate

Binding of zinc to the R3Bm at pH 10.0

On adding 20mM zinc chloride to 10 mM R3Bm at 25 ˚C (Fig. 1a) the seven azacryptand signals were broadened (Fig. 1b). Lowering the temperature to 2˚C led to further signal broadening (Fig.1c) while increasing the temperature to 50 ˚C led to sharpening of the 7 signals (Fig. 1d) suggesting that there is a fast exchange process occuring when zinc is added to the azacryptand. This could be explained if there are two zinc complexes in rapid exchange with each other (see later for details). As only seven signals were observed (Fig. 1d), it is clear that adding zinc has not destroyed the magnetic equivalence of the aliphatic and aromatic carbon groups in the azacryptand.

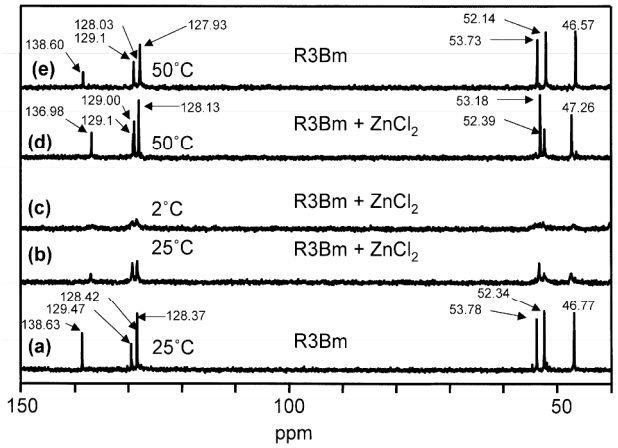


Fig. 1 Effect of 20 mM $ZnCl_2$ and temperature on the ^{13}C-NMR spectrum of 10 mM R3Bm.

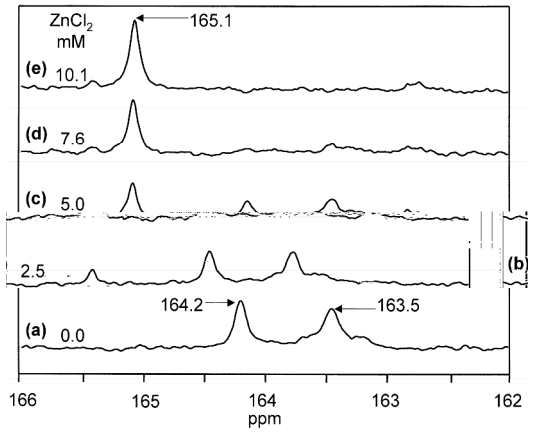


Fig. 3 ^{13}C-NMR spectra of the titration of carbamylated R3Bm with zinc chloride.

As separate signals were observed for R3Bm with and without bound zinc, then this confirms that the zinc is bound tightly to R3Bm and that zinc binding is a slow exchange process. Adding more than 20 mM zinc chloride to the R3Bm (10 mM) resulted in a precipitate of zinc hydroxide. This demonstrates that two moles of zinc are bound per mole of R3Bm.

Titration of R3Bm with $NaH^{13}CO_3$ at pH 10

On adding increasing amounts of $NaH^{13}CO_3$ (the $NaH^{13}CO_3$ signal at 159.6 ppm is not shown) to R3Bm (Fig. 2) there was a progressive increase in the intensity of the two new signals observed at 163.5 ppm and 164.2 ppm (Fig. 2a-d). However, at the higher concentrations of 41.7 and 79.0 mM $NaH^{13}CO_3$ additional signals were observed (Figs 2c,d). Computer fitting (Figs 2e,f) showed that these additional signals were at ~163.6 and 163.3 ppm.

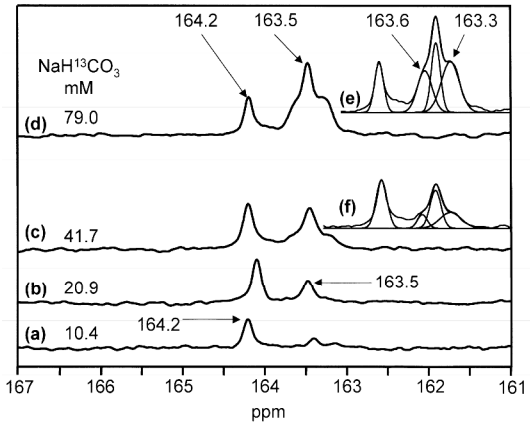


Fig. 2 ^{13}C-NMR spectra of the titration of R3Bm with $NaH^{13}CO_3$.

At 20.9 mM $NaH^{13}CO_3$ 98 % of the R3Bm had been modified (Table S1†) while at 79 mM $NaH^{13}CO_3$ there is 183% modification of R3Bm. This clearly demonstrates that with large excesses of $NaH^{13}CO_3$ more than one 1 Mole of carbonate reacts per mole of R3Bm (Table S1†).

Stoichiometry of binding of zinc with R3Bm in the presence of an excess of sodium bicarbonate at pH 10.0

When R3Bm (5.4 mM) was incubated with an ~8 fold excess of $NaH^{13}CO_3$ at pH 10.0 a large signal at 159.7 ppm due to the free bicarbonate carbon was observed (signal not shown). Two new signals were observed, one at 163.5 ppm and the other at 164.2 ppm (Figure 3a). On adding increasing amounts of zinc chloride the intensity of the signals at 163.5 and 164.2 ppm decreased and there was a concomitant increase in intensity of a new signal at 165.1 ppm (Figure 3b-e).

If more than 10 mM zinc chloride was added a white precipitate formed. This demonstrated that the azacryptand was solubilising the zinc and from the amount of zinc solubilised (10 mM) by R3Bm (5.5 mM) the stoichiometry was 1.8 moles of zinc bound per mole of R3Bm. Therefore we conclude that ~2 Moles of zinc are bound by 1 Mole of R3Bm in the presence of excess sodium bicarbonate. This is in good agreement with our results obtained without added bicarbonate. The signal at 165 ppm had a T_1 value of 5.2 ± 0.4 s and from the area of this signal we calculated that there was 0.82 moles of carbonate bound per mole of R3Bm. These results confirm that in aqueous solution the stoichiometry of the carbonate:zinc: R3Bm complex is 1:2:1 as observed in the crystalline state [14].

Binding of carbonate/bicarbonate (0.5-40 mM) with R3Bm (0.5-5.0 mM) in the presence of a ~2 fold excess of zinc chloride relative to R3Bm at pH 10.0

When $NaH^{13}CO_3$ is added to the 2:1 zinc:R3Bm complex at pH 10.0 two ^{13}C-NMR signals are observed, one at 159.7 ppm due to free bicarbonate and the other at 165.1 ppm due to carbonate in the zinc-R3Bm-carbonate complex. An apparent stability constant (K_{1app} =[Species at 165 ppm] / [$(Zn^{++})_2$R3Bm][HCO_3]) was calculated (log K_{1app} = 2.85 ± 2.80, mean of 10 determinations) using the areas of the signals at 159.6 and

165.1 ppm. From the area of the signal at 165.1 ppm and the total concentration of R3Bm the stoichiometry of binding was calculated to be 0.78 ± 0.07 % (Mean of 5 determinations) moles of carbonate per mole of $(Zn^{++})_2$R3Bm. This is in good agreement with the value determined in the previous section.

In a fully aqueous solvent, bicarbonate has a pK_a of 9.99 ± 0.01, Fig.S1A†. However in 50% v/v d_6-DMSO its pK_a was raised to 13.13 ± 0.03, Fig.S1B†. Therefore if one mole of carbonate binds to one mole of the $(Zn^{++})_2$-R3Bm complex then the stability constant (K_1) of the $(Zn^{++})_2$-R3BmCO_3^{2-} complex generating the signal at 165 ppm is given by the expression below

$$K_1 = \frac{[\text{Species at 165 ppm}]}{\left[\left(Zn^{++}\right)_2\text{-R3Bm}\right]\left[CO_3^{2-}\right]} = \frac{[\text{Species at 165 ppm}]}{\left[\left(Zn^{++}\right)_2\text{-R3Bm}\right]\left[\frac{[HCO_3^-]}{1+\frac{[H]}{K_a}}\right]}$$

Using this expression and a pK_a value of 13.13 the log of stability constant (K_1) for the binding of carbonate by $(Zn^{++})_2$-R3Bm was 6.01 ± 5.98 (Mean of 10 determinations).

Solution structure of the R3Bm complex with zinc and carbonate

On examining the orthorhombic and triclinic crystalline structure [14] it can be seen that the carbonate carbon is closest (3.16 ± 0.17 Å) to the H1' protons of two of the benzene rings of R3Bm (Scheme 1A) while the H3' and H4' protons of the benzene rings and all other carbon bound protons are at least 1.2 Å further away. As carbonate does not have directly bonded protons we cannot use conventional NOESY experiments to locate protons in its immediate vicinity. However, the HOESY experiment measures heteronuclear NOE and the connectivity between protons and quaternary carbons[21, 22]. Therefore it can be used to detect NOE interaction between the carbonate carbon and nearest protons on R3Bm. Using HOESY with the 1:2:1 R3Bm:zinc:carbonate complex we found one NOE interaction between the free bicarbonate carbon at 159.7 and the water protons and one other NOE interaction between the carbonate carbon at 165.2 ppm and the aromatic proton at 6.59 ppm (Fig. S2†) which is due to the H1' benzene protons of R3Bm (Scheme 1A). This proton is the closest to the carbonate carbon in the crystalline structure of the complex confirming that the solution and crystalline structures must be very similar.

Temperature effects

A mixture of 5 mM R3Bm, 10 mM $ZnCl_2$ and 10 mM $H^{13}CO_3^-$ gave two ^{13}C-NMR signals, one at ~160 ppm due to the excess free bicarbonate and the other one at ~165 ppm due to the 1:2:1 R3Bm:zinc:$^{13}CO_3^-$ complex. On increasing the temperature, the line width of the signal at ~165 ppm decreased showing that this signal was undergoing fast exchange, Table S2†. However, the line width of the signal at ~160 ppm increased on increasing the temperature showing that this signal was undergoing slow exchange, Table S2†.

As there is tight binding (log K_1 =6.01) of carbonate by the R3Bm-Zn^{++} complex, carbonate and bicarbonate are expected

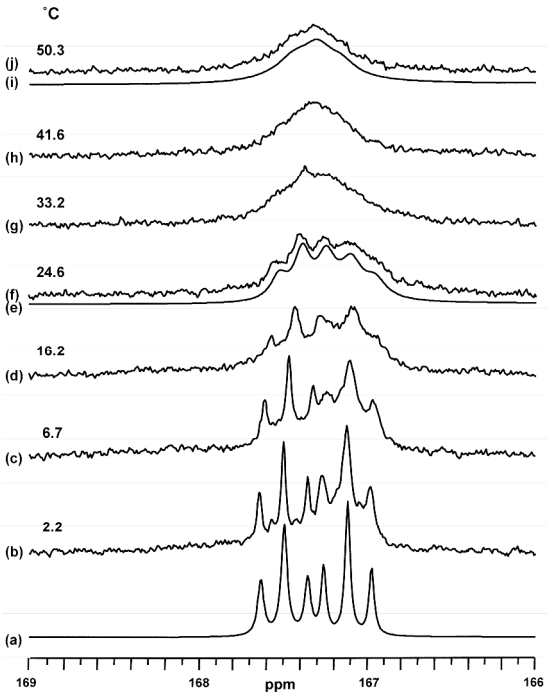
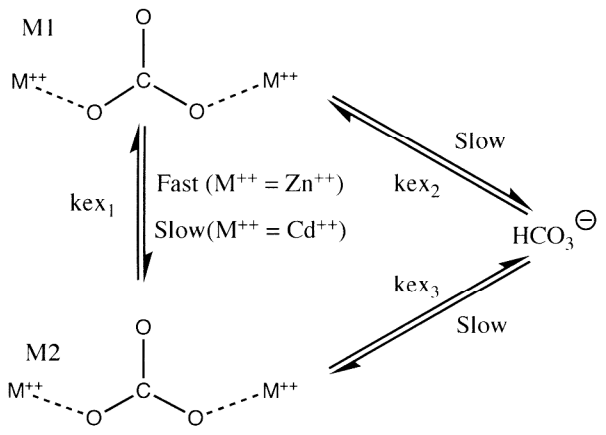


Fig. 4 Experimental and simulated ^{13}C-NMR spectra of the ^{13}C-enriched carbonate carbon of the R_3Bm-$(Cd^{++})_2$-$^{13}CO_2$ complexes at different temperatures. Spectra (a), (e) and (i) were calculated according to Scheme 2 using the Bruker dynamic NMR module of Topspin and the exchange rates kex_1, kex_2 and kex_3 in Fig. 4a,e,i were; (a) 0,140, 120 ; (e) 0, 300, 400 (i) 0, 950, 500 s^{-1} respectively.

to be in slow exchange with the R3Bm-Zn^{++} complex. However, the fast exchange broadening of the signal at 165 ppm is not so easily explained. This signal is due to the carbonate carbon (~165 ppm) bound to the R3Bm-$(Zn^{++})_2$ complex. Replacing zinc with cadmium-113 which has a spin of 0.5 should allow us to detect directly bonded ligands by their coupling to the cadmium. This approach has been successfully used to investigate metal binding and coordination in proteins such as alkaline phosphatase[23] and Bleomycin[24]. Cadmium is regarded as a valid probe of zinc-containing enzymes as it usually maintains both the structure and activity of zinc enzymes [25, 26].

When the zinc was replaced by ^{113}Cd (9.8 mM R3Bm, 19.6 mM $^{113}CdCl_2$ and 20.2 mM $H^{13}CO_3^-$) at ~25°C there was a signal at ~160 ppm (signal not shown) due to free ^{13}C-enriched bicarbonate and a broad signal at ~167.3 ppm (Fig. 4f) due to ^{13}C-enriched carbonate bound to the R_3Bm-$(Cd^{++})_2$ complex. However, at 2.2 °C a pair of triplets (J_{CCd} = 18 Hz) was observed (Fig. 4b). On increasing the temperature, these signals coalesced to give a broad signal at ~167.3 ppm (Fig. 4f-j)). In the crystal structure the carbonate group is located equidistant between two zinc atoms bound to R3Bm (Scheme 1D) and so our results suggest that the solution structure is very similar to the crystal structure. However, unlike zinc, cadmium-113 has a spin of 0.5 and so if the ^{13}C-enriched carbonate carbon is

between two cadmium atoms and both are magnetically equivalent then we expect to observe a single triplet. The fact that two triplets are observed shows that there must be two metal complexes present. These complexes are in slow exchange with the free bicarbonate in solution (Scheme 2). The fact that two triplets are observed and not two pairs of doublets



Scheme 2 Kinetic Scheme for formation of zinc or cadmium complexes with carbonate and R3Bm.

shows that in each complex both metals are in magnetically equivalent environments. However, as the chemical shifts of the carbonate carbons differ in these complexes it is clear that the magnetic environments of the carbonate carbons are not equivalent in these complexes.

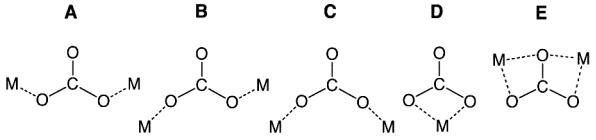
All attempts to simulate the experimental spectrum (Fig. 4f) assuming only exchange between the two triplets failed. If it is assumed that each complex can exchange with free bicarbonate (Scheme 2) then it is possible to simulate the spectra if it is assumed that the exchange rate (kex1) between the complexes M1 and M2 does not make a significant contribution to the line width. The spectra at 2.2 ˚C (Fig. 4b), 24.6 ˚C (Fig. 4 f) and 50.3 ˚C (Fig. 4j) were all calculated (Figs, 4a,e,i) assuming J_{CCd} = 18 Hz, kex1 = 0 and that there was slow exchange between free bicarbonate and the complexes M1 and M2 (Scheme 2). There was 18% more of the M2 complex (59%) than of the M1 complex (41%). Also the exchange rates of bicarbonate with the M2 (kex3) complex were larger than the exchange rates (kex2) of the M1 complex (Fig. 4). The fact that the signals due to the M1 and M2 complex did not coalesce with the signal due to free bicarbonate at ~160 ppm from 2.2-50.3˚C (Fig. 4) confirmed that the free bicarbonate was in slow exchange with the M1 and M2 complexes from 2.2 to 50.3˚C (Fig. 4).

Discussion

In the absence of zinc, bicarbonate reacts with the secondary amines ($(R)_2NH$) of R3Bm to form carbamates ($(R)_2N\text{-}COO^-$). The fact that with bicarbonate concentrations equimolar or double that of R3Bm the two carbamate signals had chemical shifts of 163.5 ppm and 164.2 ppm, demonstrates that the six secondary amines of R3Bm do not react to form equivalent carbamates (Fig. 2a,b). At higher bicarbonate concentrations new signals at 163.3 and ~163.5 ppm are formed (Fig. 2e, f). Therefore it appears that there are two forms of mono-carbamylated R3Bm with signals at 163.5 and 164.2 ppm and also two forms of dicarbamylated R3Bm with signals at 163.3 and 163.6 ppm (Table S1†).

The fact that in the zinc complex there is a decrease in line width of the signal at 165 ppm as the temperature increases (Table S2†) shows that in this case there is a fast exchange process occurring between the M1 and M2 complexes in addition to the slow exchange of each complex with free bicarbonate (Scheme 2). Therefore in aqueous solutions there are two forms of the zinc complex, which can rapidly interconvert. However, in the cadmium complex two complexes are also present but they do not interconvert by fast exchange though both complexes exchange free and bound carbonate in a slow exchange reaction (Scheme 2).

The coordination mode (Scheme 3) of the bridging carbonate in R3Bm metal complexes depends on the metal used [14]. Therefore if coordination modes of the bridging carbonate in the zinc and cadmium complexes are different (Scheme 3) in solution, then this could help explain why they exchange at different rates.



Scheme 3 Carbonate bridging coordinate modes

The fact that in the absence of added bicarbonate, zinc binding is shown to be a slow exchange process while the signals from the azacryptand carbons with zinc bound still undergo temperature dependent fast exchange broadening (Fig. 1), shows that at least two different zinc-R3Bm complexes are formed in the absence of carbonate which are in fast exchange with each other. This demonstrates that the binding of carbonate is not required for the formation of the two or more zinc-R3Bm complexes.

Conclusions

In this paper, we have studied the azacryptand R3Bm in aqueous solutions containing 50% (v/v) dimethyl sulphoxide at pH 10. Using ^{13}C-NMR we show that on adding $H^{13}CO_3^-$ the azacryptand can form four different carbamates. One mole of R3Bm solubilises two moles of zinc and the resulting complex can bind one mole of carbonate with a log K_1 of 6.01. The carbonate in this complex is in slow exchange with free bicarbonate. When the zinc in this complex is replaced by cadmium-113, two complexes are observed which are not in fast exchange with each other but are in slow exchange with

excess bicarbonate. Temperature studies show that the two analogous zinc complexes are in fast exchange with each other but are in slow exchange with free bicarbonate. Our results do not show any significant differences between the solution and crystal structures of the zinc-carbonate-R3Bm complexes.

Acknowledgements

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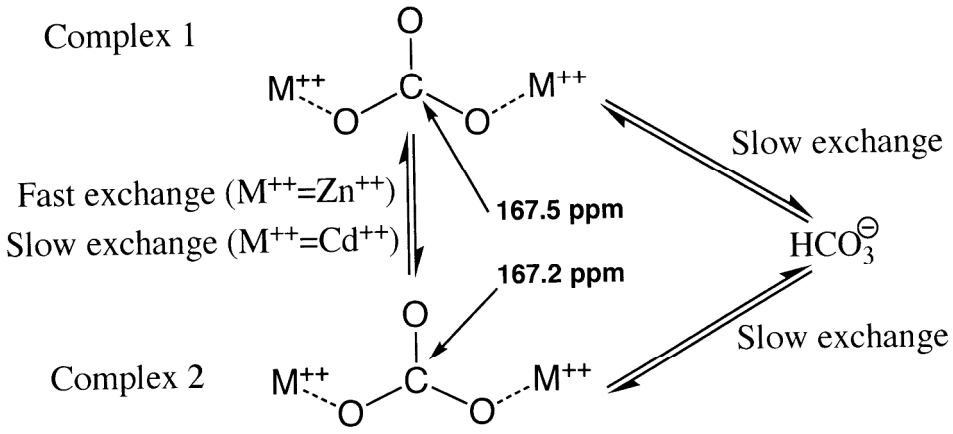

Two complexes are formed when the azacryptand R3Bm binds a metal and carbonate. These complexes interchange slowly when the metal is cadmium but rapidly when the metal is zinc.