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The potential for the cage complexes in biology[1]

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Contents

Abstract

This paper describes aspects of the synthesis of cage complexes in relation to their prospects for use in biology. The means of attaching peptides, cholestane, paraffin tails and aromatic residues are outlined. Also, the properties of some of these molecules, especially a new class of detergents, a new polymer able to capture metal ions, the selectivity of the cages for metal ions and the DNA intercalating properties of aromatic residues attached to the cages are discussed.

Keywords: Cage complex; Cage polymer; Cage surfactant; Detergent

1. Introduction

This paper addresses some prospects of the sar-, diamsar- and aminocapten-type ligands shown in Fig. 1 for use in biologically based experiments. The syntheses of these molecules have been carried out by template methods [1–3] and an example is given in Fig. 2. One simply commences with the kinetically inert tris-ethylenediamine cobalt(III) complex as the template and uses formaldehyde and a nucleophile such as ammonia or nitromethane in basic conditions to do the synthesis

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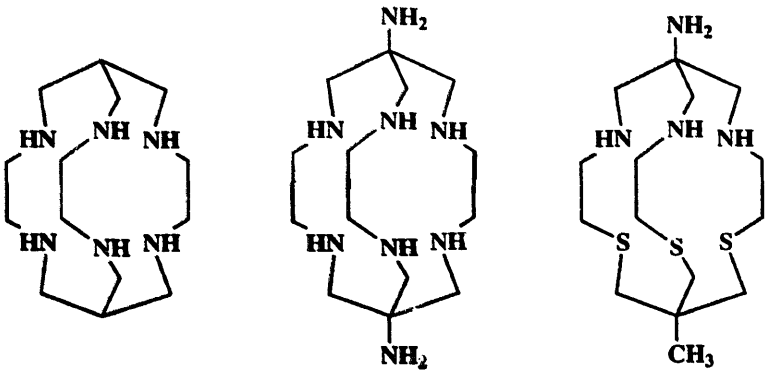


sar diamsar aminocapten

Fig. 1.

at room temperature in one step [1]. The molecules can be made cheaply and in high yield by this route. The synthesis is also stereospecific and produces only one of the possible 16 isomers from one configuration of the chiral tris-ethylenediamine template. The molecules are characterized by very high stability in both the kinetic and the thermodynamic sense. However, in the form of cobalt(II) ion the cobalt can be removed using cyanide ion or concentrated boiling HBr [3]. In this way the free ligands are obtainable and a variety of other complexes may be made from them [4–7]. Once coordinated, even the zinc and magnesium complexes are kinetically inert in these macrobicyclic ligands. This comes as no surprise, because even if one nitrogen atom dissociates, it never gets far from the metal ion and the return rate is therefore rapid [8,9]. Naturally they are very good ligands for transition metals but they do not bind alkali or alkali earth ions in aqueous solution. In this respect they are complementary to the polyether cryptands, which bind the main group elements well but are not such good ligands for the transition metal ions [10].

The periphery of the N_6 cage can be derivatized easily and aspects of this are displayed in Fig. 3. There the diazotization of the amine group on the periphery of the cage leads to substitution of chloride ion or hydroxyl ion and a variety of nucleophiles can be added in this way [1].

In Fig. 4 a variation of the template strategy is displayed. Here the imine complex pyruvato bisethylenediamine cobalt(III) is used as the nucleophile [11]. The methyl group of the pyruvate imine bound to cobalt(III) is quite acidic and the resulting nucleophile reacts quite readily with the coordinated imine derived from formaldehyde. On reduction, this dimeric metal complex not only loses one of the cobalt atoms but also leads to a glycolic acid residue or a glycine residue attached to the apical carbon of the bridgehead. It is obvious therefore that these two types of derivatives can then be extended readily to give a peptide or a depsipeptide. Potentially, such peptide derivatives could be used to target a particular site on a protein or on a DNA segment. It is also possible to tie a cholestane derivative to the bridgehead using the diazotization strategy [12]; see Fig. 5. Binding the cobalt cage to the cholestane renders the cholestane water soluble, which is quite an

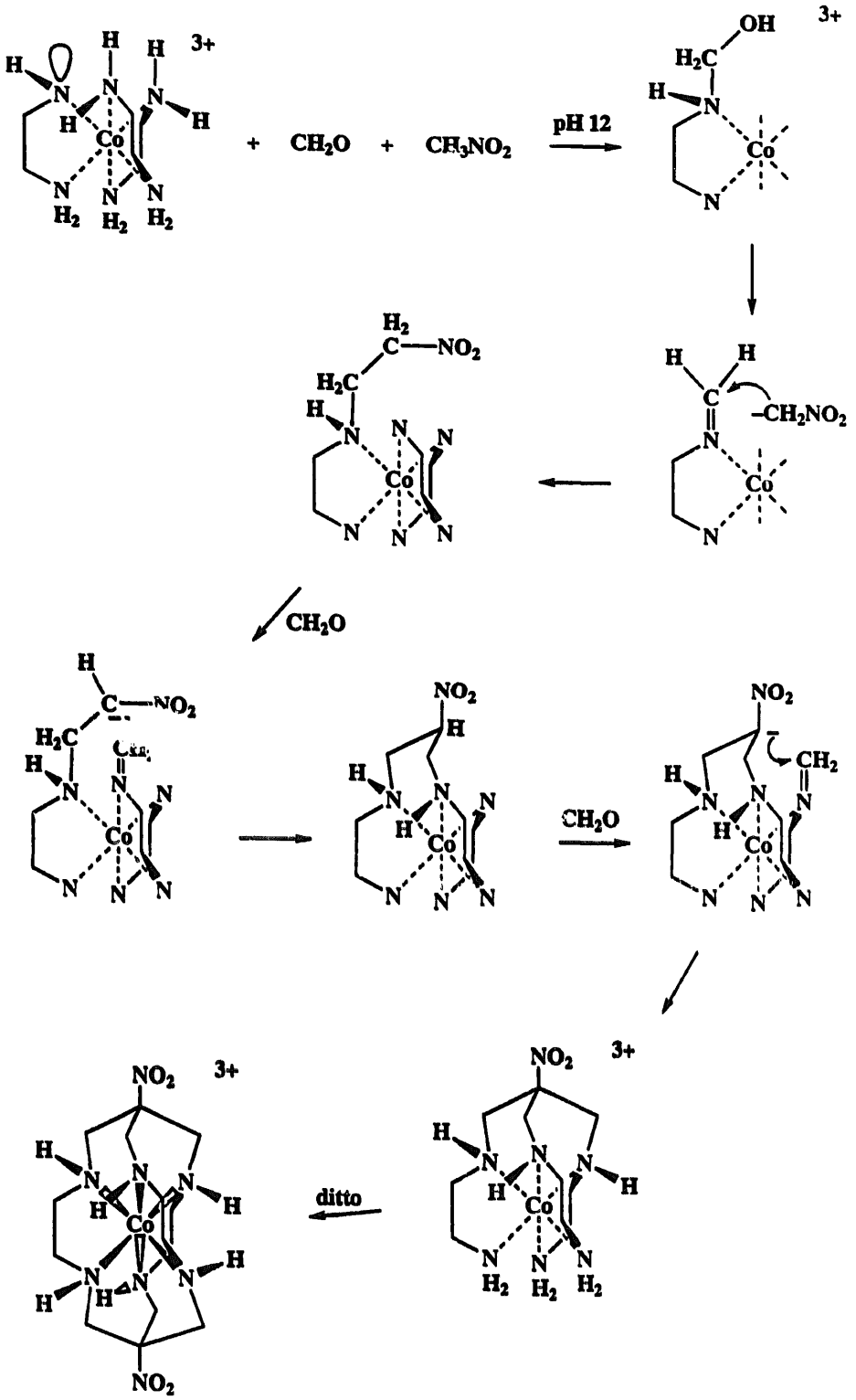


Fig. 2. Template synthesis of $[Co((NO_2)_2\text{-sar})]^{3+}$ ion.

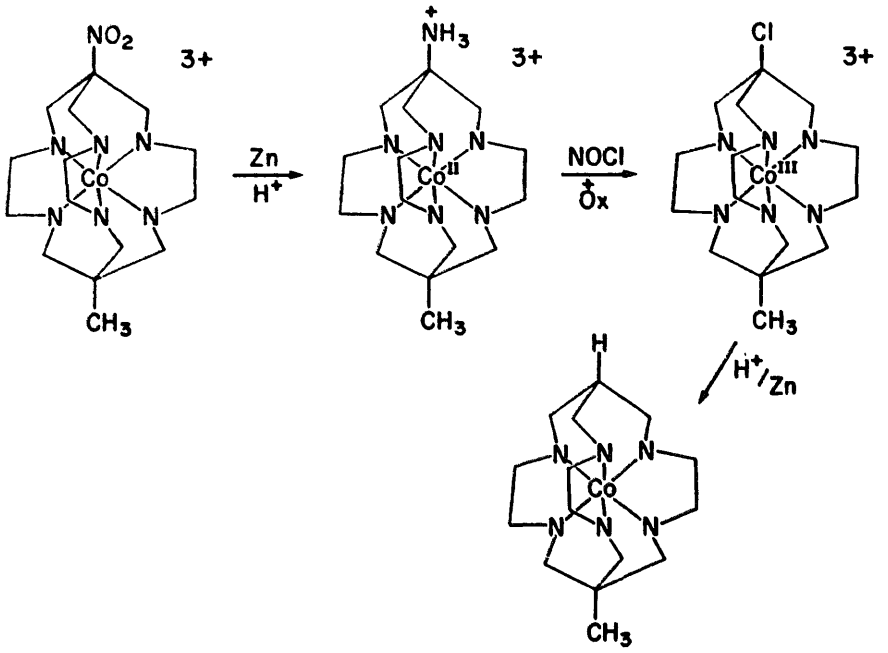


Fig. 3. Variation in substituents for sar-type cages.

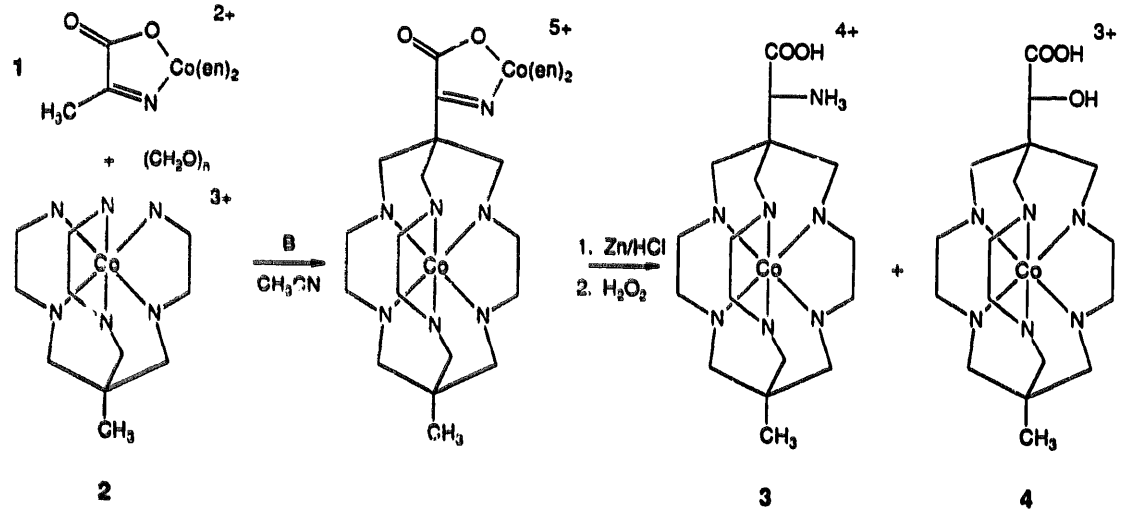


Fig. 4. Template cage synthesis using a chelated glycine precursor.

important effect in itself. Such molecules could be used, for example, to target cholesterol plaques. They might also display interesting liquid crystal phenomena and be able to insert themselves in biological membranes.

2. Detergents

A new class of detergents has also been generated by tying a paraffin tail to the cage either by the reductive alkylation strategy or by the diazotization strategy; see

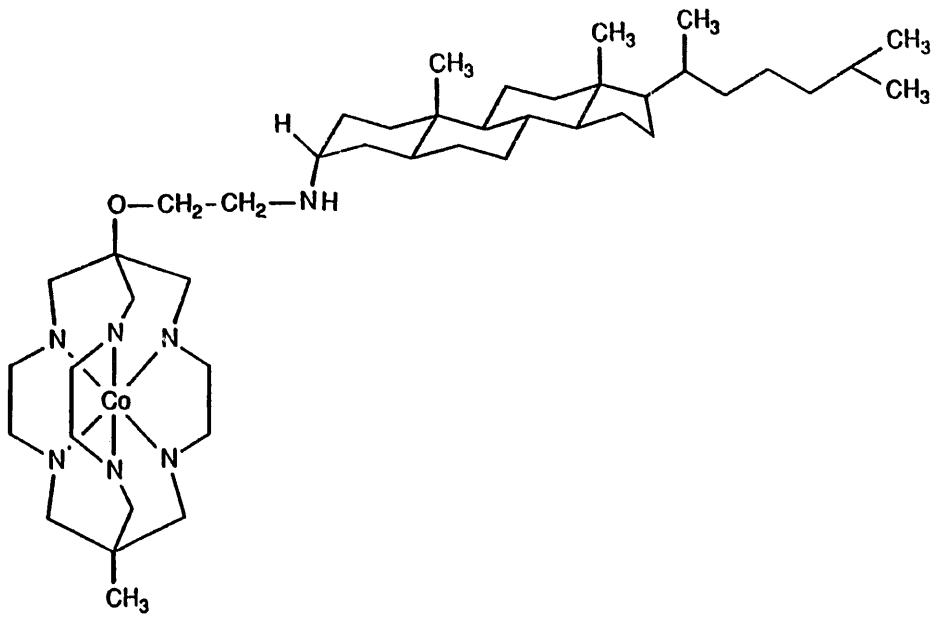
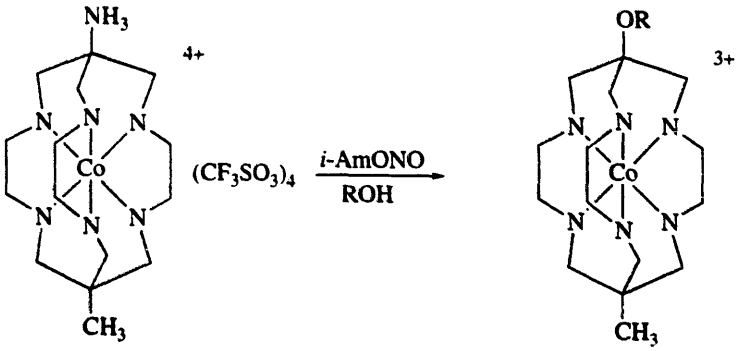


Fig. 5. Cage substituted with a choloestane residue.

Schemes 1 and 2 [12]. Examples of these cages with the paraffin tails are shown in Fig. 6. This new class of detergents has unusual qualities. They are very stable, they have a large head group and the cobalt(III) complex has a 3+charge in neutral aqueous solution. They can have a variety of chromophoric centres and can be made in chiral forms. Depending on the metal, they can also be redox active. Very few, if any, classes of detergents have this spectrum of qualities.

Recently a new strategy has been developed to make these detergents which is even more direct and gives more stable molecules [13]. The process is depicted in Fig. 7. It involves condensation of the cobalt template with a paraffin aldehyde and formaldehyde in basic conditions. This brings the paraffin tail directly into the bridgehead and after reduction of the imine an extremely stable detergent results. Despite their unusual features, these molecules have some characteristic properties. In aqueous solution they foam much as an ordinary detergent. For a C_{10} chain the critical micelle concentration (CMC) is approximately 10^{-3} M. The micelle, however, appears to be ill defined. It can be detected by conductance and surface tension measurements but not by low angle X-ray scattering or microcalorimetry. It is clear from the size of the head group relative to the tail that packing to form the micelle would be difficult, but they do have a large effect on the surface tension of water and reduce it to about the level of common organic solvents. These molecules should be interesting for a number of reasons. They could be used to detect electron transfer at interfaces, for example. The long paraffin tails could be oriented on liquid surfaces to give non-linear optical effects. One important aspect is the possibility of inserting the paraffin tail into membranes. We therefore looked for an appropriate biological problem.

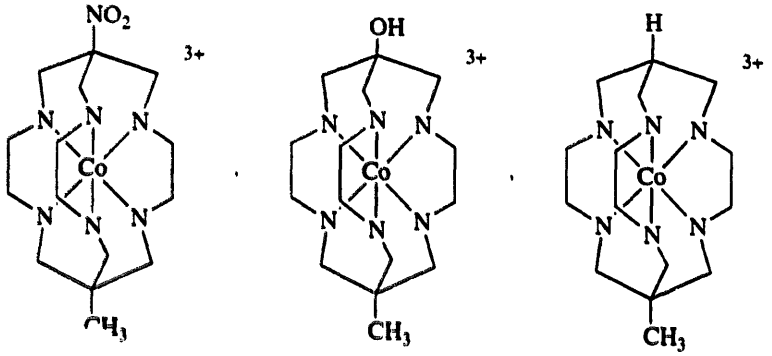
Australia has many worm infestations in animals and the parasites have to be



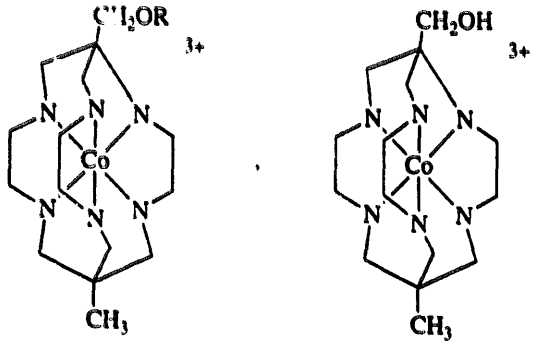
R = CH_3 (1), $-C(CH_3)_3$ (2), $HOCH_2CH_2-$ (3), $1-C_6H_{13}-$ (4),

$1-C_{10}H_{21}-$ (5), $1-C_{15}H_{31}-$ (6), $C_6H_5CH_2-$ (7), $C_6H_5CH_2CH_2-$ (8)

minor products



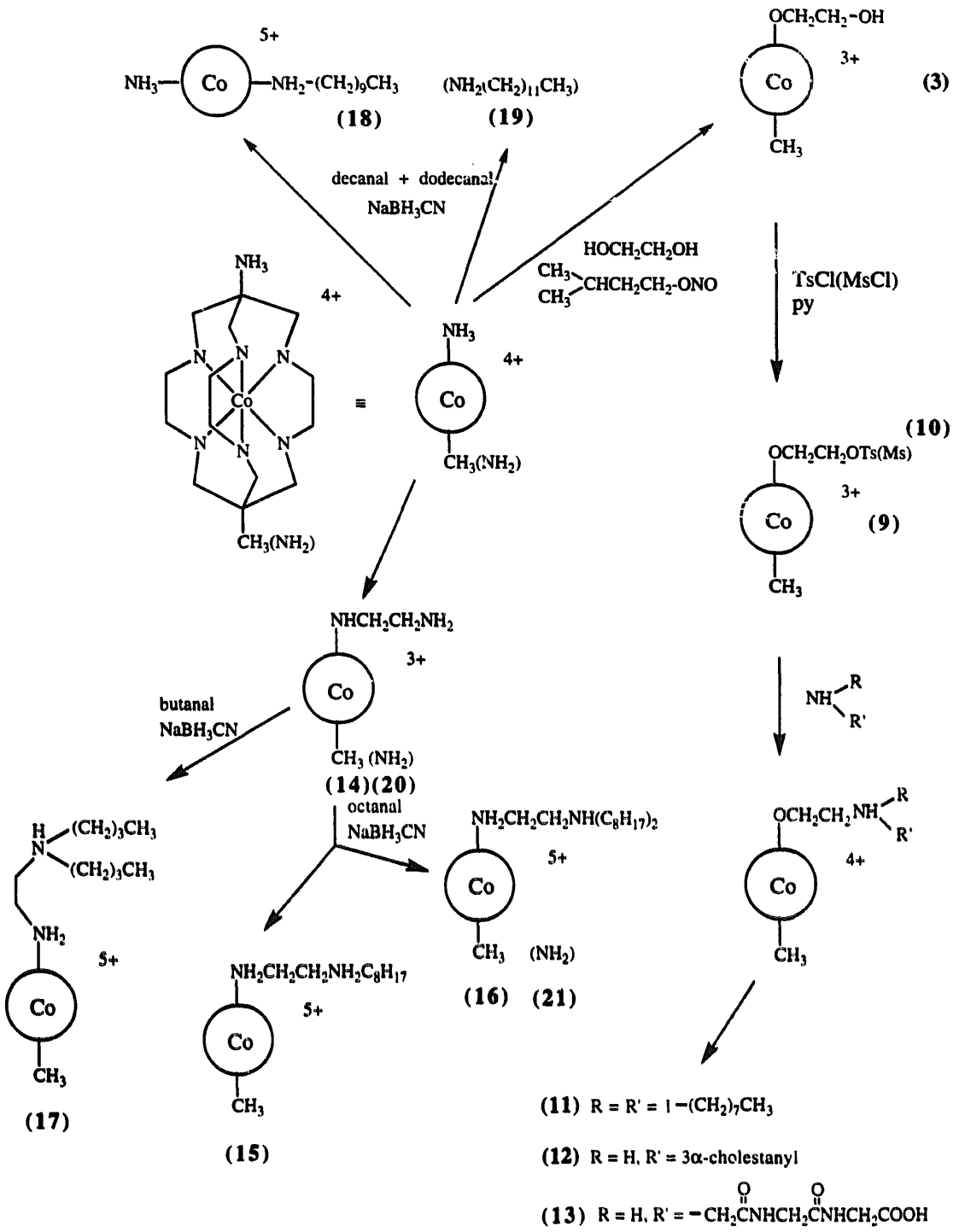
sar complexes



absar complexes

Scheme 1.

treated regularly to keep the animals in good condition. Such parasites have a characteristic lipid membranous coat through which they absorb nutrients. Hexaamine cobalt(III) complexes are also mild cholinesterase inhibitors. We thought therefore that the paraffin tail might be an effective way to get the reagent into the



Scheme 2.

organism and persuaded a parasitologist, Dr C.A. Behm, to do some experiments with these molecules, with rather striking results. Fig. 8 displays the consequences of treating parasites, namely rat tapeworms, with one of the new cobalt cage

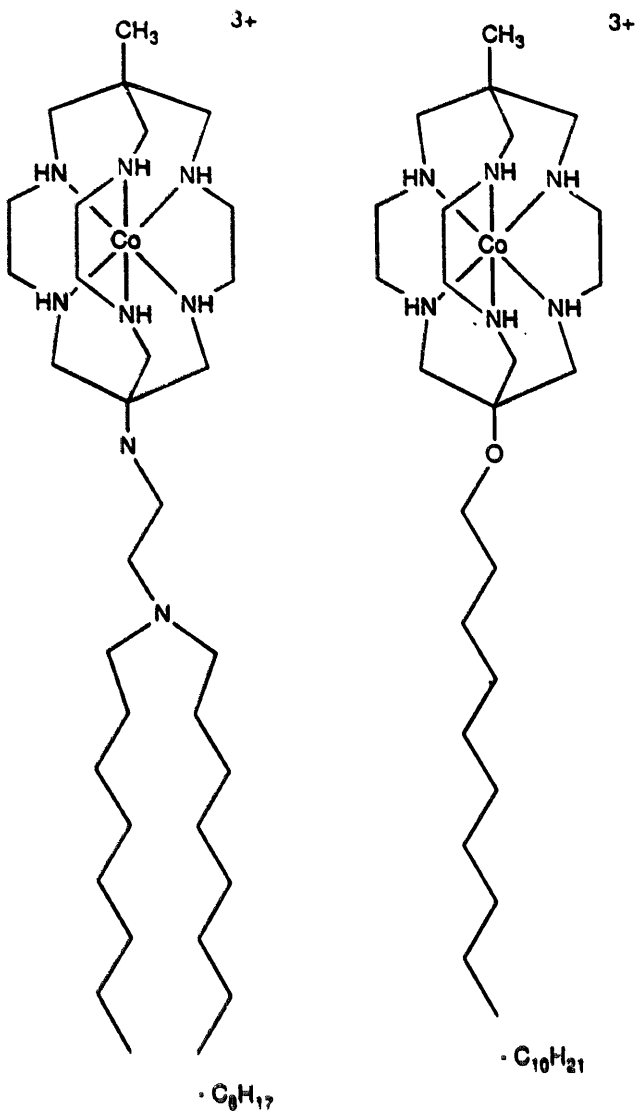


Fig. 6. Examples of cage surfactants.

detergents [12,13]. Plate 1 of Fig. 8 shows the organism untreated in a buffered saline medium. Plate 2 of Fig. 8 shows the organism 30 s after treatment with the cobalt complex. It is now slightly yellow and has obviously taken up the cobalt complex. The organism clearly does not relish the molecule. After 7–8 min (Plate 3 of Fig. 8) it is dead and part of its membranous coat is fragmented in the solution. This is more evident in Plate 4 of Fig. 8, which shows a magnified version of the dismemberment of the membrane. These and subsequent experiments indicate that the action is purely physical. Presumably the large charge and size of the head group change the curvature of the membrane and destabilize it. A similar effect was observed with synthetic vesicles.

The organic cation detergent cetylpyridiumchloride (CPC) also displays similar results and a variety of organisms and molecules were tested to establish their

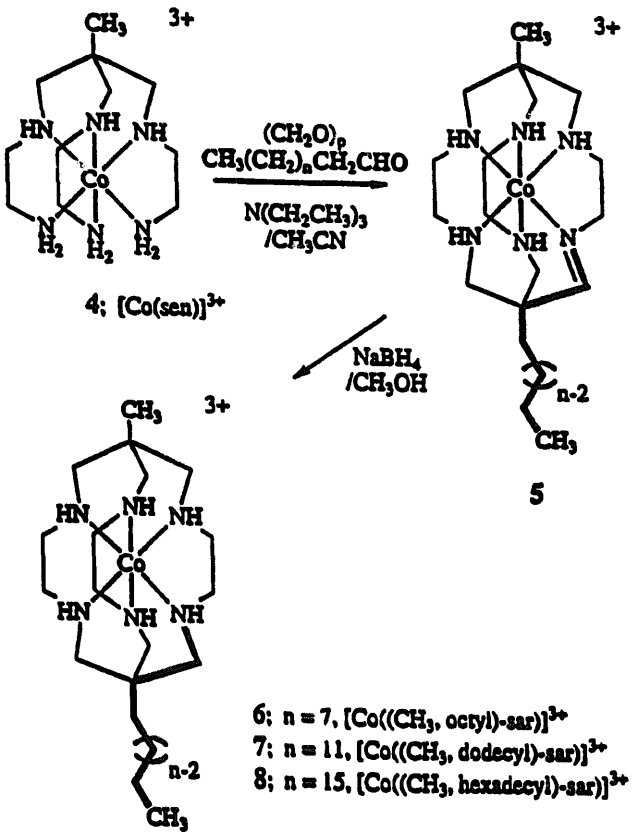


Fig. 7. New synthesis of cage surfactants.

sensitivity and efficacy respectively; see Schemes 1 and 2 and Table 1. For some molecules the experiments were so striking that it was necessary to try a reagent in vivo. Rats were then filled with tapeworms (a known number) and the paraffin tail complex **16** and CPC were fed to them orally. After an interval the rats were sacrificed and the numbers of live tapeworms were counted. In these experiments the untreated rats were compared with those treated with the cage complex and with cetylpyridiumchloride. The cage complex largely killed the tapeworms in the starved rats, but in the cetylpyridiumchloride-treated rats the worms grew larger. This was a rather puzzling result and the answer to the problem has come from a number of sources, but it is important to note here that the rats were not distressed by the treatment with the cage complexes.

Biodistribution studies were an essential part of the project [12]. Rats were treated with a ^{57}Co-labelled detergent complex (**18**). They were then sacrificed and examined in detail. The results of the biodistribution study are displayed in Table 2. A very large proportion of the complex remains in the gastrointestinal tract. Very little is absorbed systemically. Only traces were observed in skin, muscle, liver, bladder and urine. The complex is held in the stomach for a rather long time. Presumably it is bound in the membranes of the food but slowly it all comes out over about 3–4 days. Similar studies carried out with the tritiated cage complex show essentially the same pattern; see Table 3. The implication is therefore that the cobalt ion and the

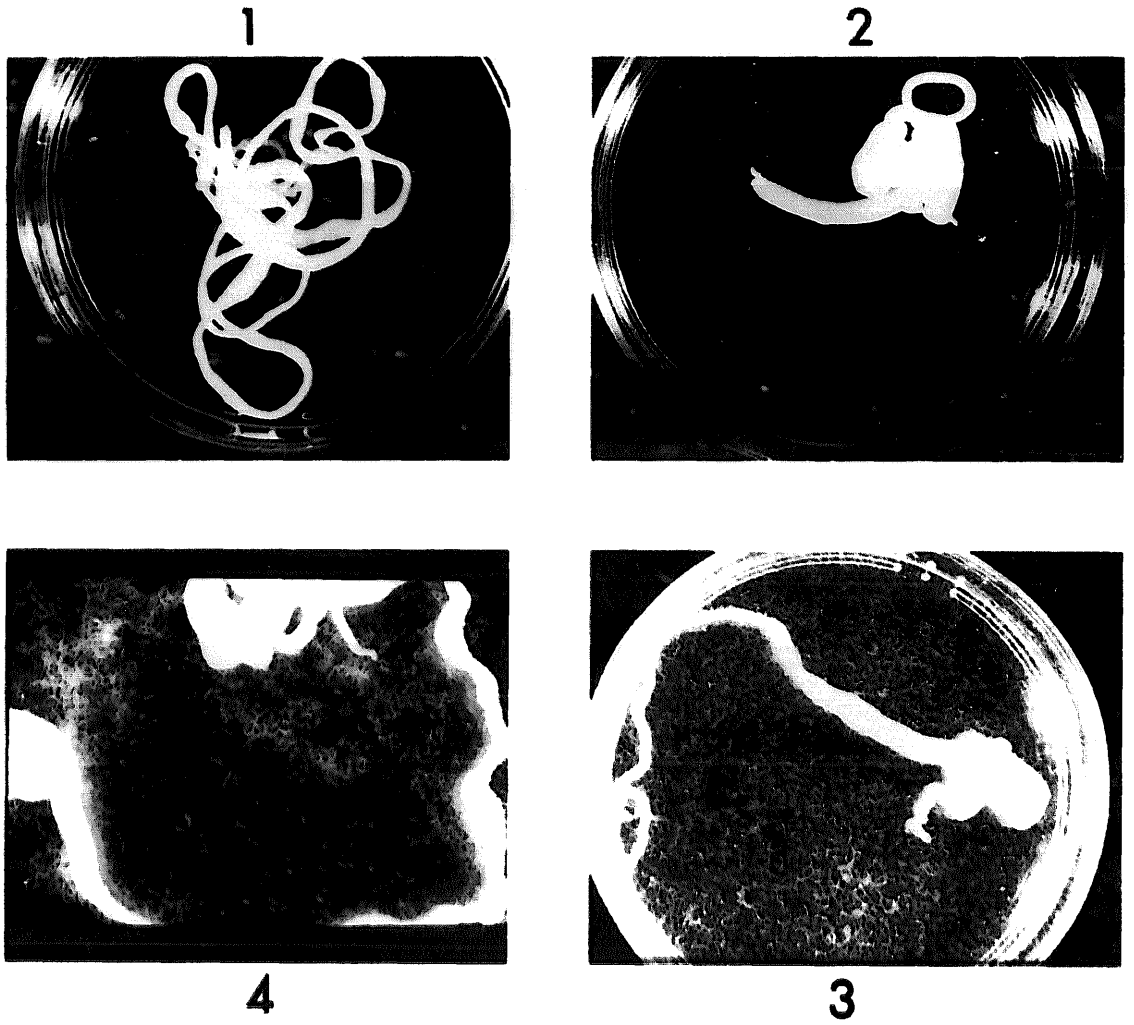


Fig. 8. Lethal effects of compound **5** (10^{-3} M) on the cestode *Hymenolepis diminuta* in buffered physiological saline solution: Plate 1, prior to addition of complex; Plate 2, after 30 s; Plate 3, after 8 min; Plate 4, magnified (× 2) view of plate 3.

cage stayed together and the organism has no way of dismembering the cage complex. There is evidence, however, that the tail can be lost in the passage through the gastrointestinal tract but the cage itself is unaffected. Presumably this occurs by the normal lipid metabolism pathway. The cobalt complex minus the paraffin tail was also injected intraperitoneally. In this instance practically all the complex was excreted in the urine; see Table 4. Very little appeared in the faeces. The studies show that the cage head group inhibits absorption or desorption of the complex detergent through the wall of the alimentary tract. This displays an important regiospecificity for the molecule. In contrast, the cationic detergent cetylpyridiumchloride, given orally, is known to be absorbed systemically. It is also known to depress the immune system in animals and man [14]. Another fact of relevance here is that the immune system is one of the natural ways that the animal has of controlling the parasite [15], at least in a modest way. Given this understanding, from different sources, it

Table 1
In vitro test results[a]

Compound	Conc. (mM)	*H. polygyrus*	*H. diminuta*	*F. hepatica*
4	1.0	−		
5	1.6	+	++++	++++
	0.8	+	+++	+++
	0.16	−	−	+
6	1.0	−	+++	
	0.5	−	+++	
	0.1	−	−	
8	1.0	−	−	
12	1.0		++	
	0.5		++	
	0.1		+	
14	1.7		−	
15	1.0	−	+++	
16	1.0	+	++++	++++
	0.5	+	+++	++++
	0.1	+	+++	+++
17	1.0	−	−	
19	1.0	−	++++	
21	1.0		++++	
	0.5		+++	
	0.1		++	
CPC	1.0	++	++++	++++
	0.1	+	+++	++++
	0.01	+	−	−

[a] Time to kill all parasites at concentration stated: ++++, <15 min; +++, <1 h; ++, <3 h; +, <24 h; −, no lethal effect.

becomes clear why the tapeworms grew larger in the animals treated with cetylpyridiumchloride. It is evident then that since the complex detergent is not absorbed systemically, it does not influence the immune system. The only apparent problem is that the cobalt complex spends too long a time in the stomach, presumably bound to lipid membrane in the food. This problem could be circumvented by delivering the complex in a capsule which was pH sensitive. If the capsule were stable to acid but not to alkali, it would travel through the acidic stomach unaffected, but at the top of the small intestine where the pH is now alkaline and where the worms live waiting for the food from the stomach, the capsule would hydrolyse and the drug would be delivered in a more effective way. Such systems are available for drug delivery and this aspect is currently being explored.

Not all membranous surfaces are affected in the same way by these complex detergents. Host-dependent nematodes, cestodes and trematodes are influenced, but it appears that free-living nematodes are not so affected. The rats are clearly not stressed by the treatment. In the sacrificed animals it can be seen that some of it is absorbed on the surface of the alimentary tract but it is all excreted eventually.

Blood cells are very sensitive to the complex detergent and rupture instantly [16].

Table 2
Biodistribution (% injected dose) of [^{57}Co]-[Co(NH_2sar$NHC_{10}H_{21}$)]Cl_3 in rats over 48 h after oral administration[a]

Tissue	Time (h)			
	4	8	12	48
	Number of animals			
	6	4	4	3
Liver	1.1 (0.5)	1.0 (0.8)	1.5 (0.5)	1.2 (0.1)
Spleen	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Kidneys	0.2 (0.1)	0.1 (0.1)	0.2 (0.2)	0.2 (0.0)
Muscle	2.4 (2.0)	1.4 (1.0)	0.9 (0.6)	0.2 (0.2)
Skin	1.1 (0.8)	0.6 (0.7)	0.6 (0.8)	0.1 (0.0)
Bone	0.7 (0.6)	0.4 (0.5)	0.3 (0.1)	0.1 (0.1)
Lungs	0.1 (0.1)	0.2 (0.2)	0.1 (0.1)	0.0 (0.0)
Heart	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Blood	0.5 (0.4)	0.5 (0.1)	0.3 (0.1)	0.0 (0.0)
Urine + bladder[b]	2.0 (1.8)	2.8 (1.0)	1.0 (0.4)	1.2 (0.6)
Stomach	71.2 (16.8)	56.5 (18.9)	52.7 (19.6)	0.1 (0.1)
Gut[c]	15.2 (12.2)	29.3 (14.7)	34.2 (21.1)	14.8 (10.9)
Faeces	0.0 (0.0)	0.3 (0.5)	0.5 (0.9)	73.6 (11.3)
Total	94.6 (4.1)	93.1 (9.4)	92.2 (9.1)	91.4 (5.1)

[a] Mean (standard deviation) of 3–6 animals; 0.0 indicates less than 0.05%.
[b] Urine + bladder indicates sum of activities found in total urine plus bladder.
[c] Gut indicates sum of activity found in all gastrointestinal tract except stomach.

Table 3
[^{3}H]-[Co($(NH_2)_2$-sar)]$^{3+}$ biodistribution results (% recovered dose) after oral administration[a]

Tissue	Time (h)		
	24	48	72
Liver	0.0	0.1	0.0
Spleen	0.0	0.1	0.0
Kidneys	0.0	0.3	0.1
Muscle	0.1	0.3	0.3
Skin	0.1	0.5	0.1
Bone	0.0	0.1	0.0
Blood	0.0	0.0	0.0
Urine + bladder[b]	1.0	3.1	3.6
Stomach	0.2	0.0	0.0
Gut[c]	94.8	66.9	68.5
Faeces	3.7	28.8	27.4

[a] Mean of 3 animals in each group; 0.0 indicates less than 0.05%.
[b] Urine + bladder indicates sum of activities found in total urine plus bladder.
[c] Gut indicates sum of activity found in all gastrointestinal tract except stomach.

Table 4
[^{57}Co]-[Co($(NH_2)_2$-sar)]Cl_3 biodistribution results (% recovered dose) after intraperitoneal injection[a]

Tissue	Time (h)	
	2	24
Liver	1.87	1.24
Spleen	0.12	0.04
Kidney	1.47	0.89
Muscle	8.41	1.52
Skin	9.47	2.21
Bone	4.89	0.56
Lungs	0.25	0.10
Heart	0.14	0.00
Blood	2.10	0.07
Urine + bladder[b]	67.01	90.14
Gut[c]	3.67	1.94
Stomach	0.55	0.04
Gut 1	0.30	0.07
Gut 2	1.09	0.12
Gut 3	1.86	0.09
Cae 1	0.14	0.80
Cae 2	0.10	0.32
Rectum	0.17	0.54
Faeces	0.07	1.25

[a] Mean of 2 animals in each group; 0.0 indicates less than 0.05%.
[b] Urine + bladder indicates sum of activities found in total urine plus bladder.
[c] Gut indicates sum of activity found in all gastrointestinal tract except stomach.

Such molecules would therefore be very toxic if injected intravenously. Other organisms are also affected by these molecules. *Giardia intestinalis*, for example, is destroyed at about 10^{-6} M of the complex detergent **6** *in vitro*; see Table 5 [12]. This is a parasite which pervades South East Asia and both the disease and the current treatment are very unpleasant. Benign molecules of this type could be a very attractive

Table 5
In vitro effect of compounds against *Giardia intestinalis*

Compound	ID_{50} (M)[a]
4	2.3×10^{-5}
5	1.4×10^{-6}
6	7×10^{-7}
15	$>3 \times 10^{-4}$
16	1.7×10^{-5}
17	$>3 \times 10^{-4}$
CPC	4×10^{-6}

[a] ID_{50} is the concentration required to inhibit uptake of ^{3}H-thymidine by 50%. For methods see Ref. [12].

treatment of such parasites. It is also apparent that their effect on different types of membrane is quite variable and that more exploration of their properties is required.

3. Metal ion capture and toxicity treatment

The ability of the cages to capture transition metal ions and retain them points to their potential for use in detoxifying biological systems. A problem which immediately comes to mind is Wilson's disease, where children have a congenital inability to eliminate copper ion. This leads to an accumulation of the metal to toxic levels in the liver and brain and to premature death. Earlier tests have shown how readily the metal complexes of the cages are eliminated in the urine [12]. The available evidence also indicates that the cage ligands themselves are not toxic. They do not bind alkali or alkaline earth elements in water and therefore do not influence sodium or potassium metabolism, nor do they influence calcium metabolism for example, unlike the polyoxa-cryptand ligands, some of which are very toxic [10]. The metal complexes once formed should therefore be eliminated rather rapidly through the urinary tract.

Equilibria are not established in months with these hexamine systems. Once the metal ion is captured, it remains in the cage [8,9]. The critical feature is therefore how effectively the ligands compete for the transition metal ions that are accessible in the biological systems.

The ability of the cages diamsar and aminocapten to compete for different metals is displayed in Tables 6–8 [17] and some interesting facts emerge. When the ratio of metal:copper:ligand is 1:1:1, copper is taken in preference, marginally; see Table 6. However, when the ratio of metal:copper:ligand is 10:10:1, then copper is preferred almost completely; see Table 7. This is the situation for the diamsar ligand. Using aminocapten, however, the pattern is considerably different. Even for a 1:1:1 ratio of metal:copper:ligand, copper is taken in preference to the extent of 93% or more; see Table 8. Clearly this is a more selective ligand than diamsar for Cu^{2+} but is also less kinetically inert.

It may seem strange that the ratio of metal ion to ligand is important in this competition process. The results imply that initially more than one copper ion is

Table 6
Amount of diamsar reacted with Cu^{2+} from the competition experiment, where the ratio of the mixture was $M^{2+}:Cu^{2+}:L = 1:1:1$ (2.5×10^{-3} M), measured 10–15 min after mixing, at 25 °C

M^{2+}	Relative amount of ligand reacted with Cu^{2+} (%)
Mg^{2+}	100 ± 3
Mn^{2+}	93
Ni^{2+}	82
Zn^{2+}	62
Cd^{2+}	66
Hg^{2+}	65

Table 7
Amount of diamsar and aminocapten reacted with Cu^{2+} in the presence of excess metal ions relative to the ligand, in which the ratio of the mixture was $M^{2+}:Cu^{2+}:L=10:10:1$, measured 10–15 min after mixing, at 25 °C

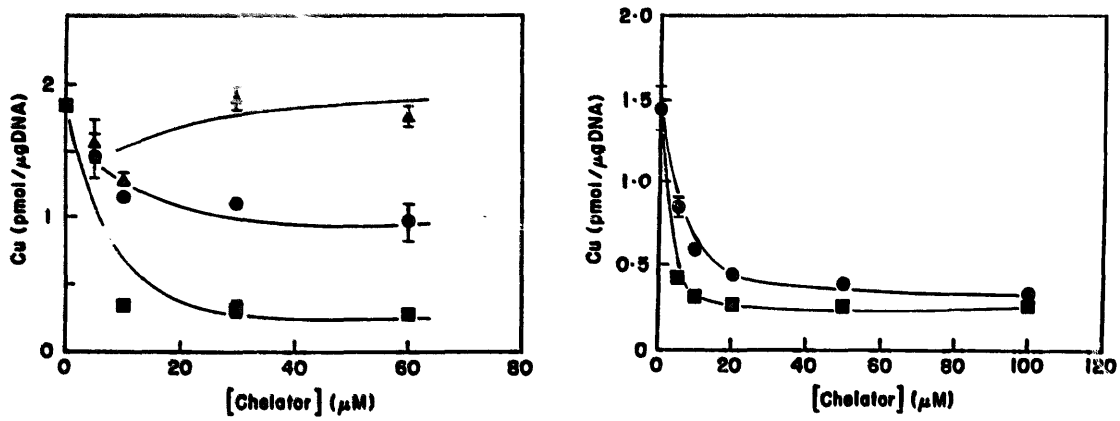
Ligand	Metal ion Mg^{2+}	Mn^{2+}	Fe^{2+}	Co^{2+}	Ni^{2+}	Zn^{2+}	Cd^{2+}	Hg^{2+}
Diamsar (%)	100	100	—	—	100	100	100	—
Aminocapten (%)	96	100	93	95	98	99	99	95

Table 8
Amount of NH_2 capten reacted with Cu^{2+} from the competition experiment, where the ratio of the mixture was $M^{2+}:Cu^{2+}:L=1:1:1$, measured 10–15 min after mixing, at 25 °C

M^{2+}	Relative amount of aminocapten reacted with Cu^{2+} (%)
Mg^{2+}	92
Mn^{2+}	93
Fe^{2+}	99
Co^{2+}	90
Ni^{2+}	92
Zn^{2+}	89
Cd^{2+}	92
Hg^{2+}	88

bound per ligand when the metal is in excess. There is evidence for this also in the kinetics of formation of the metal complexes [18]. Finally, of course, one of the metal ions must come off to complete the encapsulation. The overall implication of these studies is that copper could be taken preferentially to the other possible metal ions provided that such competition conditions could be basically duplicated in the biological system. The ability of the free cages to remove copper from hepatocites was therefore examined with cultured hepatocites [19]. The cages were also compared with penicillamine, which is the current reagent of preference for detoxifying Wilson's disease patients. Fig. 9 displays (a) the ability of penicillamine, sar and diamsar to prevent the uptake of copper by the cells and (b) the ability of the reagents to remove copper from the cells. It is apparent immediately that diamsar is very effective at both removing copper and preventing copper being taken up by the cells. The sar ligand is somewhat less effective in this respect and, interestingly, penicillamine is not effective in either path. The last result is important because it shows that the role of penicillamine in treating Wilson's disease patients is not to remove copper from the cells but to prevent it from getting into the hepatocites and is probably only related to the ability of penicillamine to bind copper and assist its elimination through the urine. These *in vitro* results therefore looked very promising from the point of view of treating the Wilson's disease problem.

In vivo tests were then carried out on a mouse model which displayed the character-



The effect of different concentrations of sar (●), diamsar (■) and penicillamine (▲) on copper uptake by the cells. The cells were incubated for 30 min with the chelators and 1μCi/ml ^{64}Cu. The results are expressed in absolute units and are the mean ±SEM of four observations.

a

The effect of increasing sar or diamsar concentration on the release of ^{64}Cu taken up by hepatocytes during a 30 minute incubation. The cells were incubated with ^{64}Cu for 30 min, washed and incubated with increasing diamsar (■) or sar (●) concentrations overnight. They were then washed and treated. The results are the mean ±SEM of at least twelve replicate observations.

b

Fig. 9. Influence of chelating agents on copper levels in hepatocytes.

istics of Wilson's disease. These mice were treated with the ligands diamsar and aminocapten. The significant result of these studies was that both zinc and copper were preferentially removed from the animal [20]. A similar study with sheep yielded a similar result and diamsar was more effective than aminocapten in both instances [21]. Presumably the levels of zinc are too great in the bloodstream of these animals and the discrimination for Cu^{2+} over Zn^{2+} by the ligands is not large enough. It follows that these reagents will not be useful in treating Wilson's disease, since the removal of the zinc would compound the patients' problems of the excess copper. However, they have been useful in determining aspects of copper metabolism [22] and they appear to have interesting properties in inhibiting cell division [23].

They could be useful, however, in another context. The diamsar ligand has been condensed with adipic acid to form a nylon polymer [24]; see Fig. 10. This polymeric species is not soluble; it swells in aqueous solution and forms a nice column material to capture metal ions. If the assertions about the stability of these complexes are correct, the cages should capture the metal ions and reduce them to very low levels in aqueous solution. In order to test this proposal, a 2 cm column of the material was treated with a 0.1 M solution of Analytical Reagent sodium nitrate. Anodic stripping voltammetry (ASV) showed that this solution had approximately 60 ppb of Cu^{2+} ion. The ASV spike is displayed in Fig. 11. After passage of the solution through the column, it can be seen that the spike is no longer evident. The resulting solution was then spiked with 60 ppb of Cu^{2+} to largely restore the original concentration. More extensive tests have shown that the polymer will reduce the levels of

CAGE POLYMER

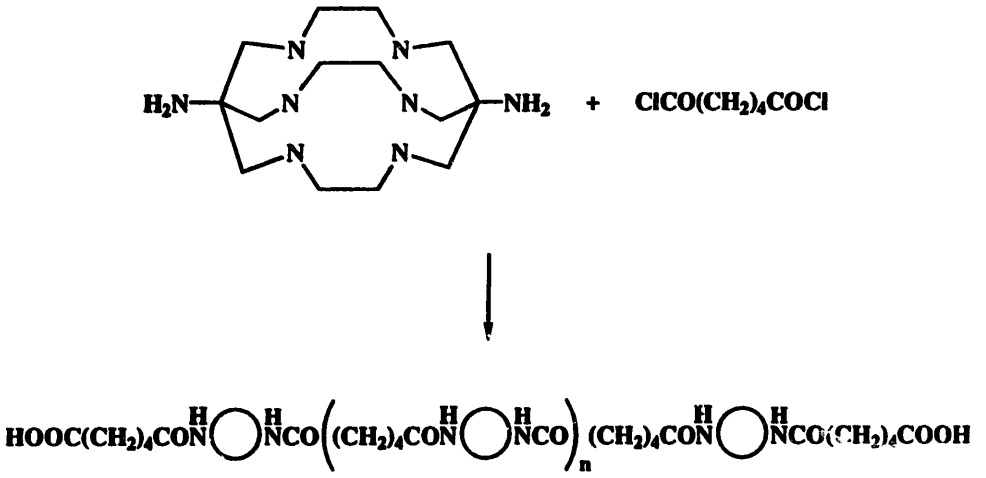


Fig. 10. Cage polymer.

metal concentration to at least 10^{-12} M for Cd^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+} and Pb^{2+}. This makes it probably the best reagent of its type available.

There are at least two possible uses for this material. One is the last link in a purifying chain to generate ultrapure water free of transition metal ions for use in the manufacture of pure silicon. The other use is in molecular biology to titrate media to exceptionally low levels of transition metal ion without removing essential Na^+, K^+, Ca^{2+} and Mg^{2+} ions.

4. Imaging and therapeutic agents

The cages also offer interesting potential for imaging agents of either the paramagnetic or the radioactive kind. The high spin d^5 $[Mn(diamsar)]^{2+}$ ion alone is a modest relaxation agent for water protons, but tied to a protein it becomes very much more effective [25] with a relaxivity of $16000\ M^{-1}\ s^{-1}$. Increases in the rotational and translational correlation times for the Mn ion are responsible for the effect. The ability that these molecules have to retain the metal ion also makes them interesting possibilities for therapeutic agents. Using short-lived radio tracers which bind rapidly to the empty cage and by tying the product quickly to a monoclonal antibody, specific tumours could be targeted [26]. Both of these strategies are being pursued at the present time.

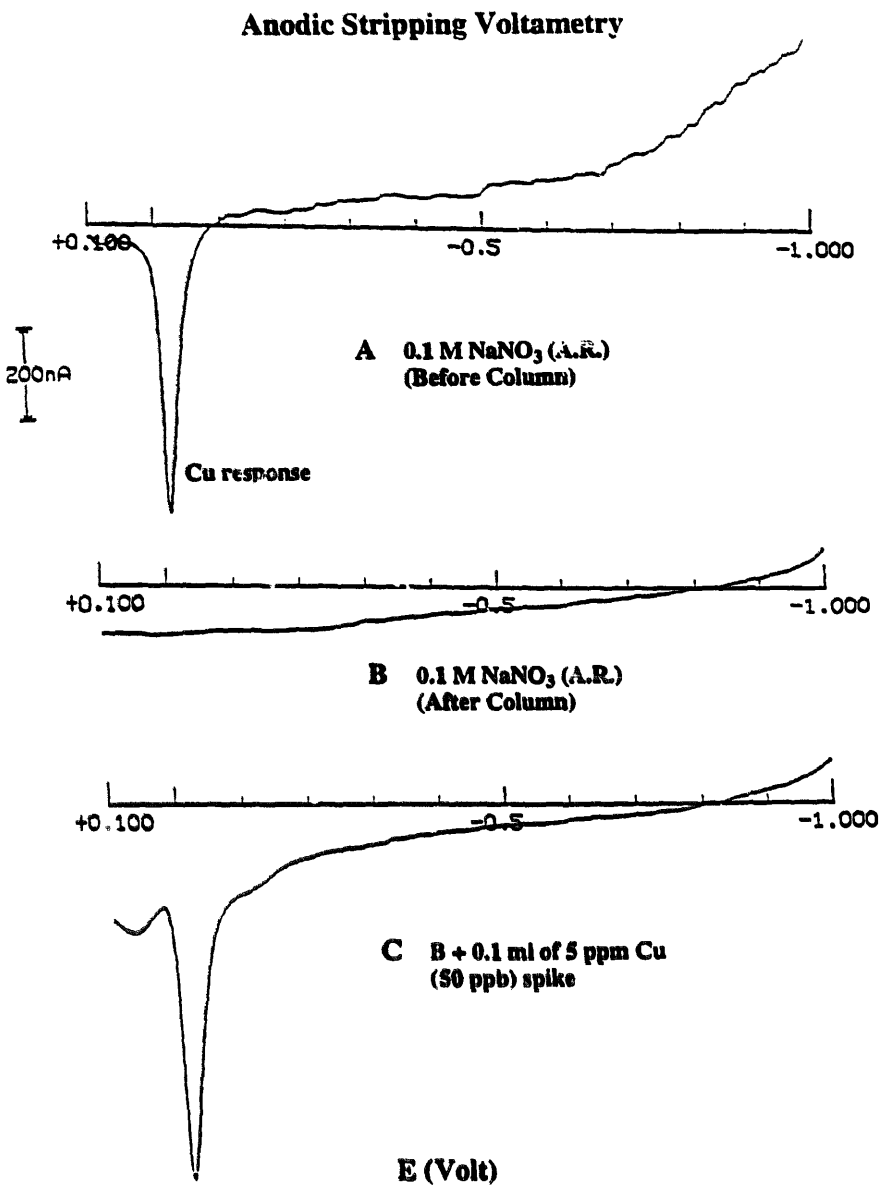


Fig. 11. Cage polymer removal of Cu^{2+} from 0.1 M $NaNO_3$ solution.

5. Aromatic intercalation and electrostatic binding to DNA

The final segment of this paper addresses the properties of aromatic molecules attached to the cage. Such molecules can be made simply by a reductive alkylation using an aromatic aldehyde condensed with an amine group on the cage [27], as shown in Scheme 2, or by building the aromatic group directly into the bridgehead; see Fig. 12 [28]. Examples of the former kind are shown in Fig. 13. Binding the hydrophilic cobalt(III) complex to the anthracene moiety profoundly changes its properties. Anthracene, which is normally insoluble in water, becomes quite soluble as the 3+ cationic species. As a result, the planar aromatic molecule now has the

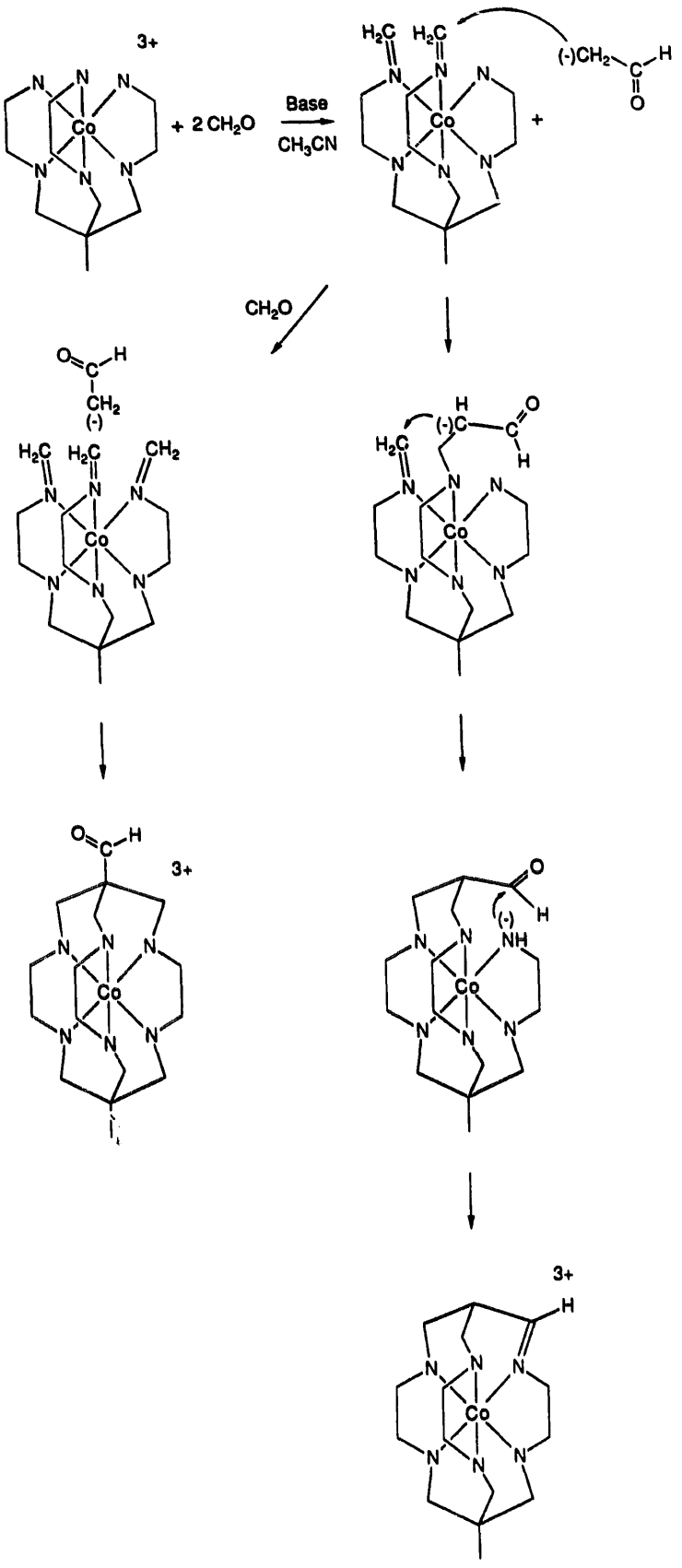


Fig. 12. Template synthesis of formyl and imine cage complexes.

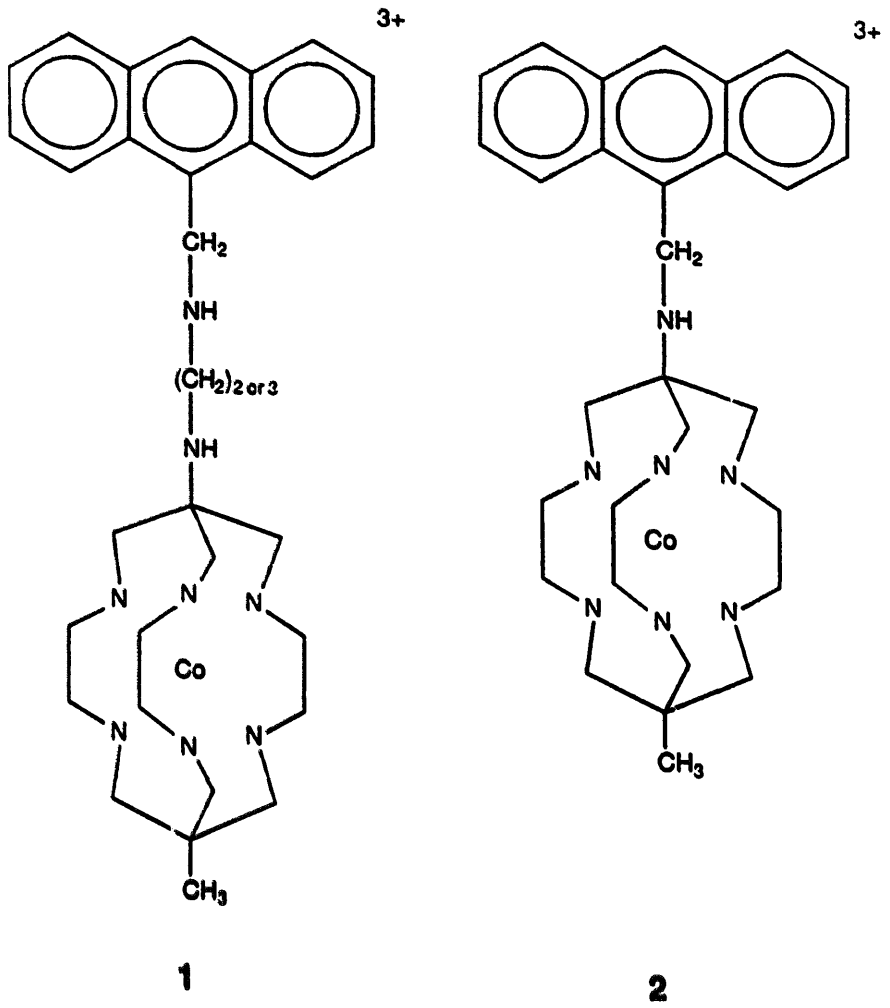


Fig. 13. Anthracenyl cage complexes from reductive alkylations.

potential to undergo intercalative binding with DNA· see Fig. 14. Moreover, this binding should be enhanced by the electrostatic binding between the 3+ cation and the negatively charged phosphate backbone of DNA. An experiment showing this binding [29] is displayed in Fig. 15. As the anthracene complex is titrated with DNA

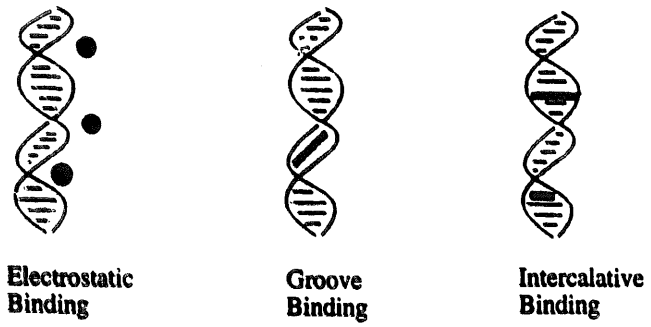


Fig. 14. Binding modes for cations with DNA.

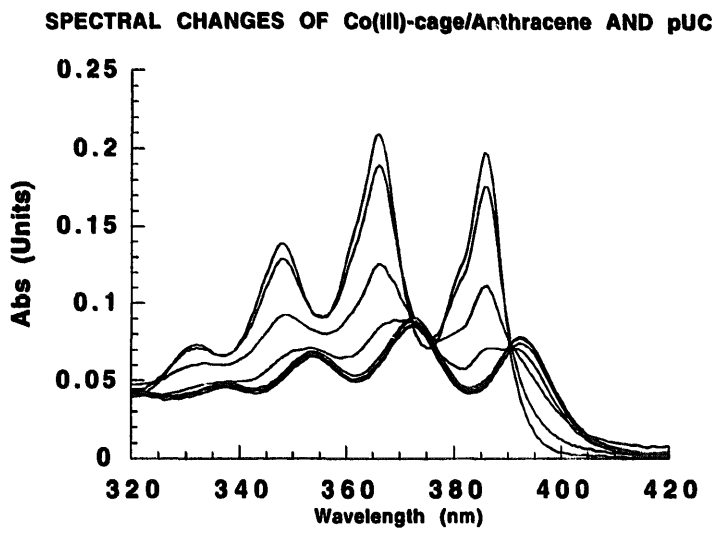


Fig. 15. Spectral changes for titration of Co(II)anthracenyl cage (**2**, 20 μM) with varying DNA (10–200 μM in 30 mM Tris–HCl buffer, pH 7.6, 25 °C).

plasmid pUC9, the spectrum due to the π–π* transitions is modified profoundly until at a concentration of about 70 μM a limiting condition is reached. Not only are the band intensities reduced but the spectrum is also shifted towards the red. Clearly there is an electronic interaction between the base pairs of DNA and the anthracene moiety.

This intercalative binding has also been established by the ability of the anthracene complex to relax the supercoiled form of the pUC9 plasmid (form I); see Fig. 16 [29]. The relaxed plasmid (form II) is electrophoretically less mobile than form I

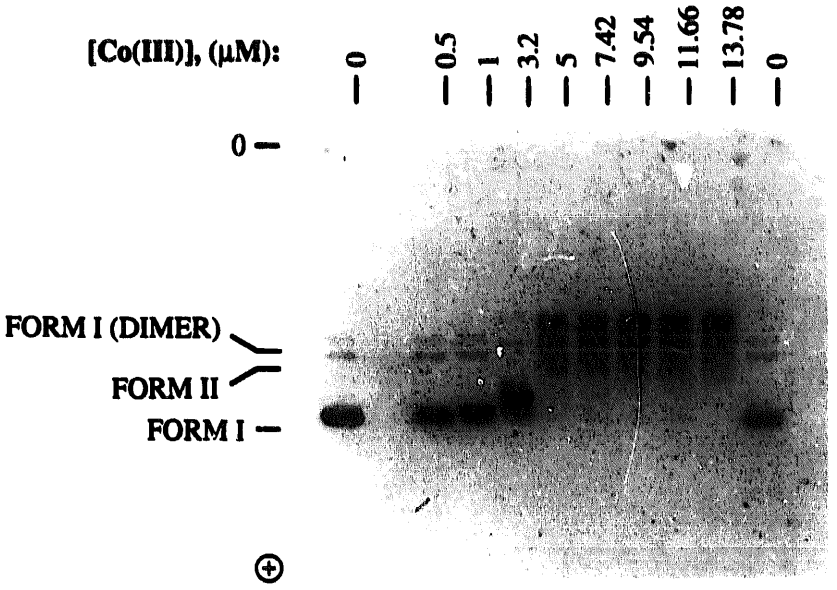


Fig. 16. Effect of [Co(III)anthracenyl cage]$^{3+}$ (**2**) on electrophoretic mobility of negatively supercoiled plasmid DNA (pUC-9, 32.6 μM): form I, supercoiled DNA; form II, released circular DNA.

and this is readily detected in the experiment displayed in Fig. 15. The binding constant for this complex is approximately 10^5 M^{-1}. In more recent times other complexes of this type have been synthesized by the method shown in Fig. 12. In this way, anthracene, phenanthrene and anthraquinone complexes of the kind shown in Fig. 17 have been made [30]. They have also been shown to bind to DNA in this intercalative manner [29].

In addition to these molecules, the formyl-substituted cage shown in Fig. 12 and diamsar have been linked by the reductive alkylation strategy to give a 9+ trimer, Fig. 18 [31], which also binds tightly to DNA. One of the reasons for making these molecules was to generate new reagents able to bind DNA by intercalative or electrostatic modes and thereby influence its behaviour. It was also thought that these molecules may be able to cleave DNA photochemically. This has proven to be the case, although not in the manner expected. Irradiation at 254 nm has led to cleavage of the supercoiled plasmid in the presence of the bound reagent but not in its absence; see Fig. 19 [29]. Relaxation of the plasmid follows its cleavage and its

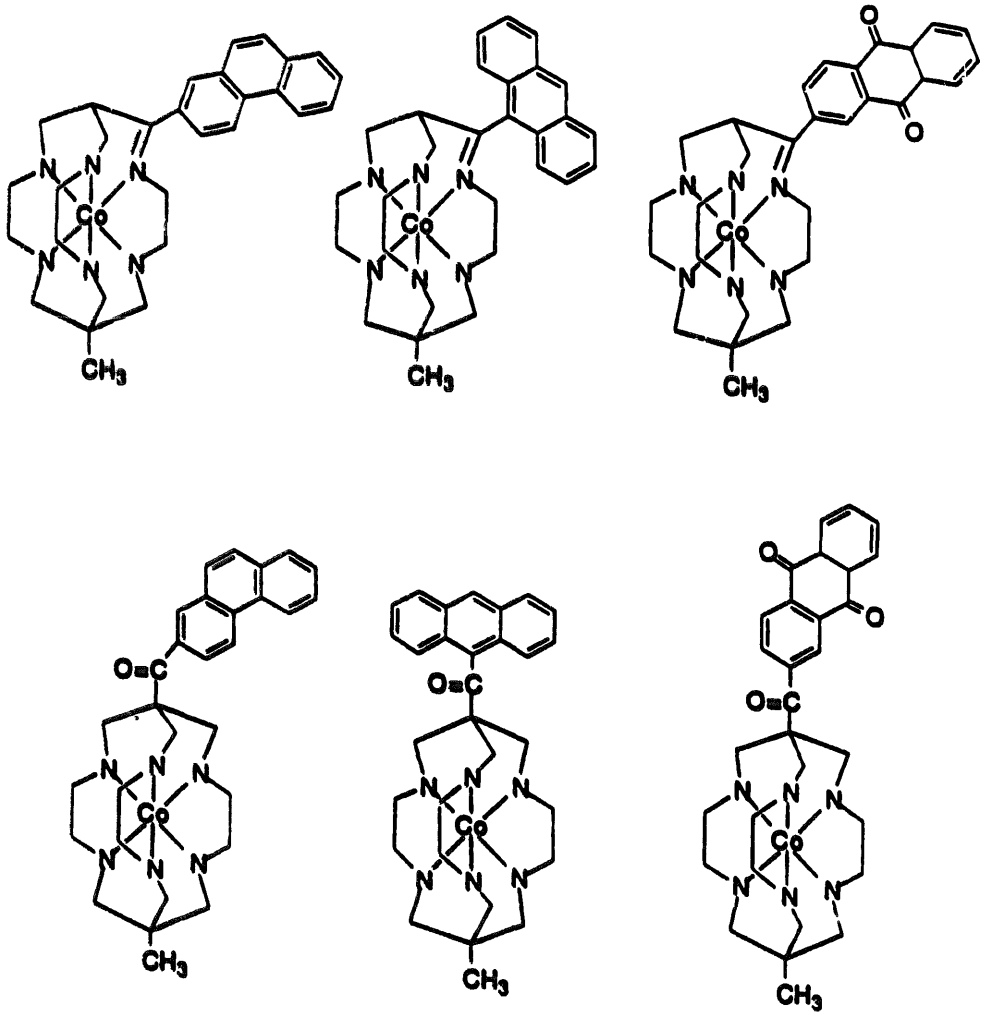


Fig. 17. Aromatic substituents attached to cage by template methods.

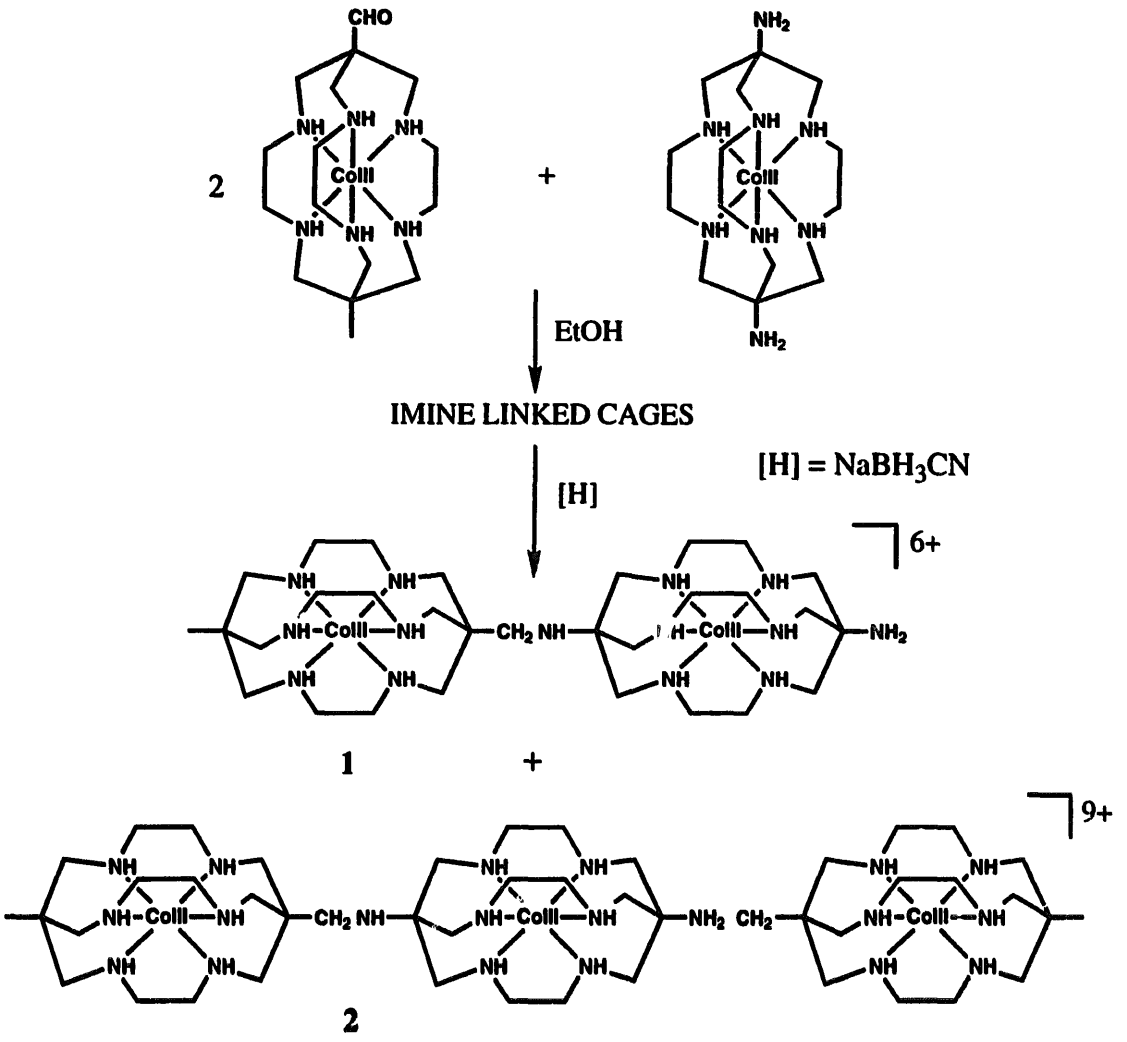


Fig. 18. Coupling of cage complexes by reductive alkylation.

mobility decreases. The mechanism for this process appears to arise from the ligand-to-metal charge transfer which is induced at this wavelength, similar to that proposed for $[Co(NH_3)_6]^{3+}$ cleaving DNA [32]. This generates a cobalt(II) ion and a ligand radical cation, which is a powerful oxidant. Presumably the latter cleaves DNA by oxidizing the ribose moiety in a manner similar to that described in previous publications by H atom abstraction [33].

The 9+ trimeric cation, however, cannot bind in an intercalative manner. Its interaction with DNA must be simply electrostatic and/or aligned along the major groove but it also undergoes the charge transfer reaction. Because of the facile reduction of the Co(III) ion by the excited singlet state of anthracene [27], it was expected that the resultant anthracinium cation would be a good oxidant in this context. The results show that this is not the case, since Fig. 19 indicates little or no cleavage of DNA at 365 or 302 nm. Perhaps it is not so surprising given the substantial effect that intercalation has on the π–π^* absorption bands of the anthra-

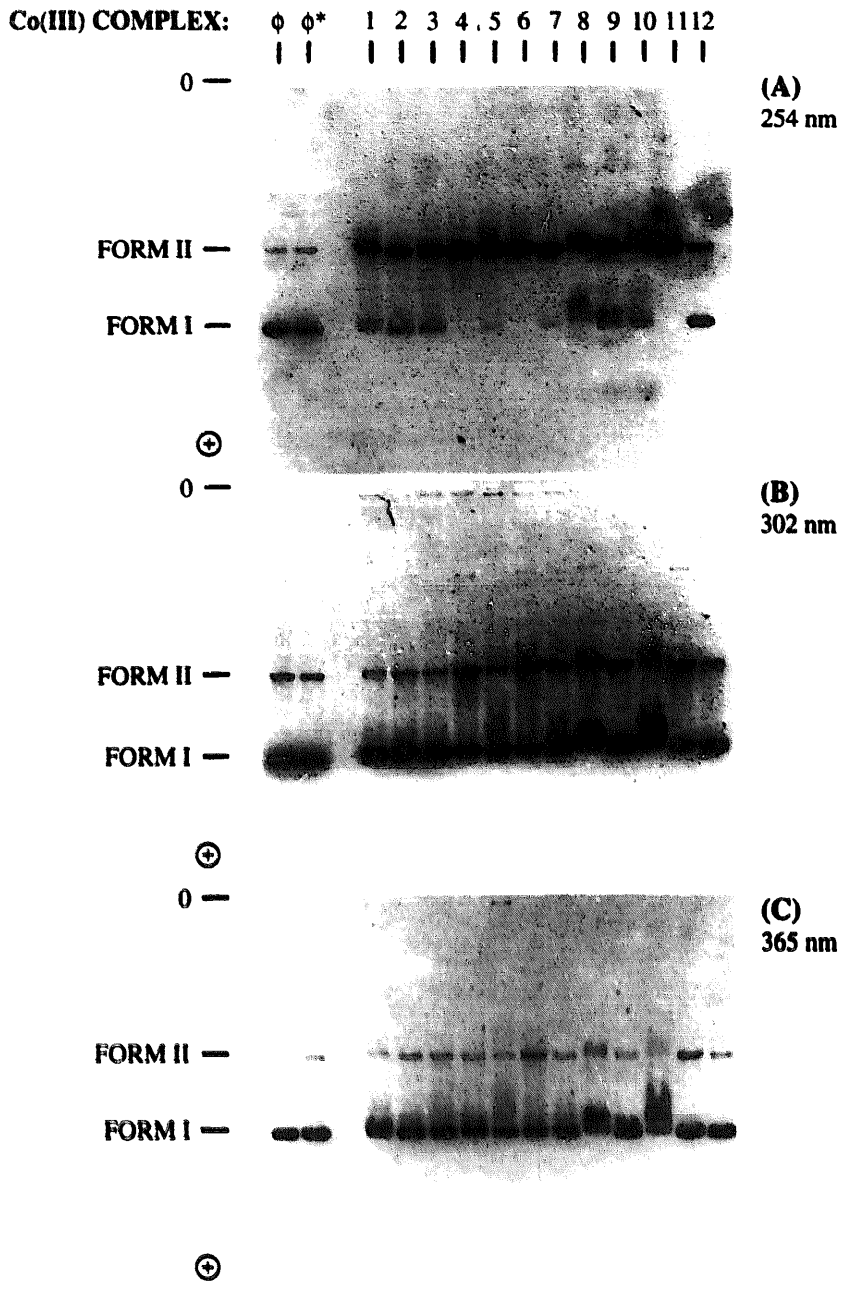


Fig. 19. Photochemical cleavage of DNA by complexes **1**–**12** (20 μM in 30 mM Tris-HCl buffer, pH 7.6). Lanes ϕ and ϕ* are controls with plasmid but without complex.

cene moiety. These bands which give rise to the singlet state are clearly less allowed in the intercalative mode and moved to lower energy. The formation of the active singlet state is thereby impeded.

It is not likely that these reagents alone will be regiospecific. To get specificity in the cleavage, it will probably be necessary to tie the cage to a small peptide which binds as a footprint to DNA, or to an oligonucleotide or polyamine which does the same thing, or to another molecule such as daunomycin which has a specific binding

site on the DNA. Clearly the various strategies which have been developed to elaborate the cage allow many opportunities for attaching such molecules and achieving the required specificity.

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References

[1] R.J. Geue, T.W. Hambley, J.McB. Harrowfield, A.M. Sargeson and M.R. Snow, J. Am. Chem. Soc., 106 (1984) 5478.
[2] L.R. Gahan, T.W. Hambley, A.M. Sargeson and M.R. Snow, Inorg. Chem., 21 (1982) 2699.
[3] G.A. Bottomley, I.J. Clark, I.I. Creaser, L.M. Engelhardt, R.J. Geue, K.S. Hagen, J.M. Harrowfield, G.A. Lawrance, P.A. Lay, A.M. Sargeson, A.J. See, B.W. Skelton, A.H. White and F.R. Wilner, Aust. J. Chem., 47 (1994) 143.
[4] P. Bernhard, D.J. Bull, W.T. Robinson and A.M. Sargeson, Aust. J. Chem., 45 (1992) 1241.
[5] I.J. Clark, I.I. Creaser, L.M. Engelhardt, J.M. Harrowfield, E.R. Krausz, G.M. Moran, A.M. Sargeson and A.H. White, Aust. J. Chem., 46 (1993) 111.
[6] P.A. Anderson, I.I. Creaser, C. Dean, J.M. Harrowfield, E. Horn, L.L. Martin, A.M. Sargeson, M.R. Snow and E.R.T. Tiekink, Aust. J. Chem., 46 (1993) 449.
[7] I.I. Creaser, L.M. Engelhardt, J.M. Harrowfield, A.M. Sargeson, B.W. Skelton and A.H. [illegible] e, Aust. J. Chem., 46 (1993) 465.
[8] L. Grøndahl, Ph.D. Thesis, University of Copenhagen, 1994.
[9] A.M. Sargeson, Pure Appl. Chem., 58 (1986) 1511.
[10] J.-M. Lehn, Pure Appl. Chem., 49 (1977) 857.
[11] B. Korybut-Daszkiewicz, R.M. Hartshorn and A.M. Sargeson, J. Chem. Soc., Chem. Commun., (1989) 1375.
[12] C.A. Behm, P.F.L. Boreham, I.I. Creaser, B. Korybut-Daskiewicz, D.J. Maddalena, A.M. Sargeson and G.M. Snowden, Aust. J. Chem., 48 (1995) 1009.
[13] C.A. Behm, I.I. Creaser, B. Korybut-Daszkiewicz, R.J. Geue, A.M. Sargeson and G.W. Walker, J. Chem. Soc., Chem. Commun., (1993) 1844.
[14] R.B. Ashman, R.V. Blanden, B.W. Ninham and D.F. Evans, Immunol. Today, 7 (1986) 278, and references cited therein.
[15] E.M. Bennet, C.A. Behm and C. Bryant, Int. J. Parasitol., 20 (1990) 841.
[16] A. Neil, B.Sc. Hons. Thesis, Australian National University, Canberra, 1990.
[17] R. Anggraini, M.Sc. Thesis, Australian National University, Canberra, 1990.
[18] I.J. Clark, Ph.D. Thesis, University of Western Australia, Perth, 1991.
[19] H.J. McArdle, S.M. Gross, I.I. Creaser, A.M. Sargeson and D.M. Danks, Am. J. Physiol., 256 (1989) G667.
[20] J. Mercer, unpublished results.
[21] J. Mason, personal communication.
[22] H.J. McArdle, J.F.B. Mercer, A.M. Sargeson and D.M. Danks, J. Nutrition, 120 (1991) 1370.
[23] H.J. McArdle, personal communication.
[24] D. Baldwin, G. Batley and A.M. Sargeson, in preparation.

[25] L.S. Szczepaniak, A.M. Sargeson, I.I. Creaser, R.J. Geue, M.F. Tweedle and R.G. Bryant, Bioconjugate Chem., 3 (1993) 27.
[26] N. Di Bartolo, A.M. Sargeson and S. Smith, in preparation.
[27] A.W.-H. Mau, W.H.F. Sasse, I.I. Creaser and A.M. Sargeson, New J. Chem., 10 (1986) 589.
[28] A. Höhn, R.J. Geue and A.M. Sargeson, J. Chem. Soc. Chem. Commun., (1990) 1473.
[29] P. Hendry, S. Moghaddas, N.E. Dixon, A. Bygott, C. Qin, R.J. Geue and A.M. Sargeson, Int. Conf. on Macrocyclic Chemistry, Townsville, 1989, in preparation.
[30] A. Bygott, Ph.D. Thesis, Australian National University, Canberra, 1995.
[31] C. Qin, R.J. Geue and A.M. Sargeson, unpublished results.
[32] M.B. Fleisher, K.C. Waterman, N.J. Turro and J.K. Barton, Inorg. Chem., 25 (1986) 3549.
[33] B. Giese, P. Erdmann, L. Giraud, T. Göbel, M. Petretta, M.V. Raumer and T. Schäffer, Tetrahedron Lett., 35 (1994) 2683, and references cited therein.