CRITICAL REVIEW

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Cyclam complexes and their applications in medicine

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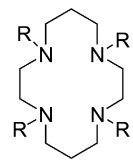
Cyclams are 14-membered tetraamine macrocycles which bind strongly to a wide range of metal ions. Medical interest has centred on clinical trials of a bicyclam for the treatment of AIDS and for stem cell mobilization, and on adducts with Tc and Cu radionuclides for diagnosis and therapy. Other potential applications particularly for Cr, Mn, Zn, and Ru cyclams are also emerging. We discuss in this *critical review* the mechanism of metal complexation, stability of metal cyclams and their structures, with particular emphasis on the variety of configurational states which metal cyclams can adopt. Understanding the factors which control the thermodynamics and kinetics of the interconversion of configurational states of metallo-cyclams may be a key factor in designing novel cyclam derivatives for future use in medicine. (136 References.)

1 Introduction

A large number of synthetic biofunctional macrocyclic compounds has been prepared and investigated since there are many examples of metal complexes of naturally occurring macrocyclic ligands, *e.g.* porphyrin, corrin ring derivatives.[1] Macrocyclic polyamines generate continuous interest because of their biological properties and their importance in coordination chemistry.[2] Saturated macrocyclic polyamines exhibit new properties beyond those anticipated from mere assemblies of amines or linear polyamines.[3] They show a pronounced ability to bind a wide variety of metals and in many cases to undergo marked conformational changes during binding.[1] The effect of increased stability of a metal coordination complex of a tetraamine macrocyclic ligand over that of similar noncyclic tetraamine ligands has been called the macrocyclic effect.[4] Macrocyclic structures are extremely favorable for metal complexation. The strong affinity shown by polyamines and their selective binding of certain metals result in their use (1) as models for carrier molecules in studies of the selective uptake and transport of metal ions,[5] and oxygen[6] in biological systems, (2) in metal recovery, which depends on selective extraction by macrocyclic polyamines,[7] (3) as metal catalysts[8] and active site mimics of metalloenzymes,[9] (4) as agents which cleave phosphate esters,[10] including DNA[11] and RNA,[12] (5) as MRI[13] contrast agents, (6) for radioactive diagnosis[14] and treatment,[15] and (7) as anti-HIV agents.[16]



R = -H, **1**; $-CH_3$, **2**; $-CH_2COOH$, **3**;

$-CH_2CH_2OH$, **4**; $-CH_2(CH_2)_2OH$, **5**;

$-CH_2PO_3H_2$, **6**; $-CH_2P(O)(C_6H_5)OH$, **7**

Cyclam (1,4,8,11-tetraazacyclotetradecane) **1** is one of the most used macrocyclic polyamines. It can complex various cations including transition metals, often with very high thermodynamic and kinetic stability with respect to metal ion dissociation. However, cyclams are of little use as selective metal chelating agents. Whilst the majority of studies have focused on fully *N*-substituted derivatives[17], *e.g.* **2–7**, with four additional coordinating arms, the number of reported selective syntheses of mono-, di- and tri-substituted derivatives is increasing. Macrocycles bearing additional coordinating groups have been of

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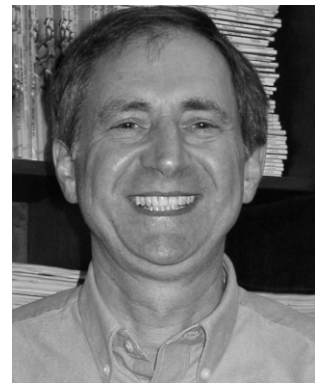

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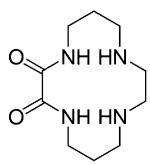
particular interest, as their properties may be quite different from those of the unsubstituted parent macrocycles.



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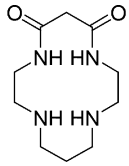

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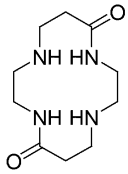

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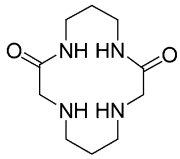

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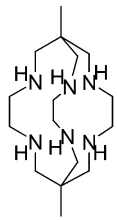
Dioxocyclams **8–11**, which contain two amino nitrogens and two amides, have also received attention.[18] They are able to bind to metals such as copper(II) and nickel(II) with simultaneous dissociation of the two amide protons, and consequently metal binding is highly pH-sensitive. The cross-bridged cyclams **12–15** are also important and exhibit new properties.[19] Upon addition of the constraining bridge, metal-ligand binding can be strengthened further.



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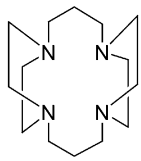

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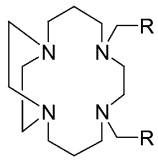

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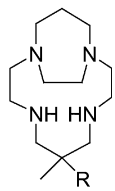

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2 Metal cyclam complexes

Macrocyclic ligand complexes play important and fundamental roles in biological systems. Extensive biological applications have also stimulated development of the chemistry of macrocyclic complexes, ranging from the synthesis of new macrocyclic ligands and studies on the properties and function of macrocyclic complexes to their applications in industry, medicine, and other fields. Cyclam is flexible enough to complex a wide variety metal ions. In particular, cyclam has been the subject of numerous investigations of the chemistry and structure of complexes with transition metals.

2.1 Mechanism of formation

Kaden first studied the kinetics of formation of Ni(II) cyclam in aqueous solution.[20] In contrast to oxygen-donor and sulfur-donor macrocyclic ligands, macrocyclic polyamines are much more basic and subject to protonation. The monoprotonated and diprotonated ligand species exhibit formation rate constants which are 3×10^4 times slower than those observed for the corresponding open-chain species. In order to avoid ligand protonation in the study of the formation kinetics of cyclam complexes, Lin and co-workers employed strongly basic aqueous media.[21] Under such conditions, Cu(II) is present as $Cu(OH)_3^-$ and $Cu(OH)_4^{2-}$, with $Cu(OH)_3^-$ being more reactive. The comparative kinetic behavior of cyclam and 3,6,10,13-tetraazapentadecane (Et_2-2,3,2-tet) indicates that ligand cyclization itself has only a relatively small influence upon the complex formation rate constants. Instead, the more significant kinetic effects arise from substitution at the nitrogen donor atoms of the open-chain ligands or from the alkyl backbone of the cyclic compounds. Jahn–Teller (tetragonal) inversion following the first-bond formation was proposed as the rate-determining step with $Cu(OH)_3^-$. Second-bond formation was proposed as the rate-determining step for the reaction of $Cu(OH)_4^{2-}$ with cyclam. However, highly substituted cyclam shifted the rate-determining step of the formation reaction to second-bond formation for both $Cu(OH)_3^-$ and $Cu(OH)_4^{2-}$ species.[22] In order to relieve the steric congestion attributable to the methyl groups and to avoid multiple desolvation occurring prior to the rate-determining step, the highly substituted cyclam folds or twists in the complexation reaction with $Cu(OH)_x^{2-x}$.[22a] The proposed mechanism of stepwise complexation of Cu(II) by cyclam is shown in Fig. 1.

Investigations of the rates of incorporation of metal ions carried out in aqueous solution have led to problems of interpretation due to either protonation and solvation of the ligand or formation of hydroxo species such as $Cu(OH)_3^-$ and $Cu(OH)_4^{2-}$ in the case of copper. To avoid these problems, dipolar aprotic solvents such as acetonitrile[23], DMF[24] and DMSO[25] have been employed for studies of the formation of cyclam complexes. The process of metal coordination by cyclic ligands is more complex and not necessarily metal-controlled, as it is in the case of the Eigen–Wilkins mechanism. There are two steps in complex formation in most systems studied as described below by eqns. (1) and (2).[26] The steric constraints of the cyclic system lead to ligand-control in that the second (or a later) coordination step becomes rate-limiting.

$$M + L \underset{\text{fast}}{\overset{k_1\left(M^{-1}s^{-1}\right)}{\rightleftharpoons}} [ML]_{int} \quad (1)$$

$$[ML]_{int} \underset{\text{slow}}{\overset{k_2\left(s^{-1}\right)}{\rightleftharpoons}} [ML] \quad (2)$$

(L = cyclam; M = transition metal; $[ML]_{int}$ = intermediate)

The initial second-order formation of the intermediate is followed by slow first-order product formation. The second-order rate constant k_1 depends mostly on the properties of the substituents on cyclam, such as their positions and numbers. In contrast to k_1, the first-order rate constant k_2 is much less affected by the substituents on cyclam. The intermediate $[ML]_{int}$ adopts a planar geometry. The formation of the second M–N bond controls the rate of the intermediate formation of the first stage. Fig. 2 shows a mechanism for the formation of the intermediate. There are six steps as follows: (a) rapid outer-sphere complexation and equilibration between the solvated metal cation and cyclam; (b) formation of the first M–N bond; (c) formation of the second M–N bond with cyclam in a folded conformation (with more *N*-methylation on cyclam, this step could be the rate-determining step); (d) further loss of a solvent molecule leading to a probable meridional N_3 coordination; (e) formation of the intermediate $[ML]_{int}$ with cyclam being N_4-coordinated in a square-planar arrangement; (f) the intermediate more or less solvated and equilibrated amongst three species $[ML]_{int}$, $[MLS]_{int}$ and $[MLS_2]_{int}$.

The consecutive first-order step (rate constant k_2) is a stereochemical rearrangement process in which the intermediate $[ML]_{int}$, rapidly formed in the initial second-order intermediate formation step with planar coordination geometry, is converted to a thermodynamically more stable [ML] as product. During the course of the rather fast process for formation of the intermediate, there is not enough time for the macrocyclic ligand to accommodate the metal in the thermodynamically most stable configuration. The latter therefore forms in the subsequent first-order step, which is

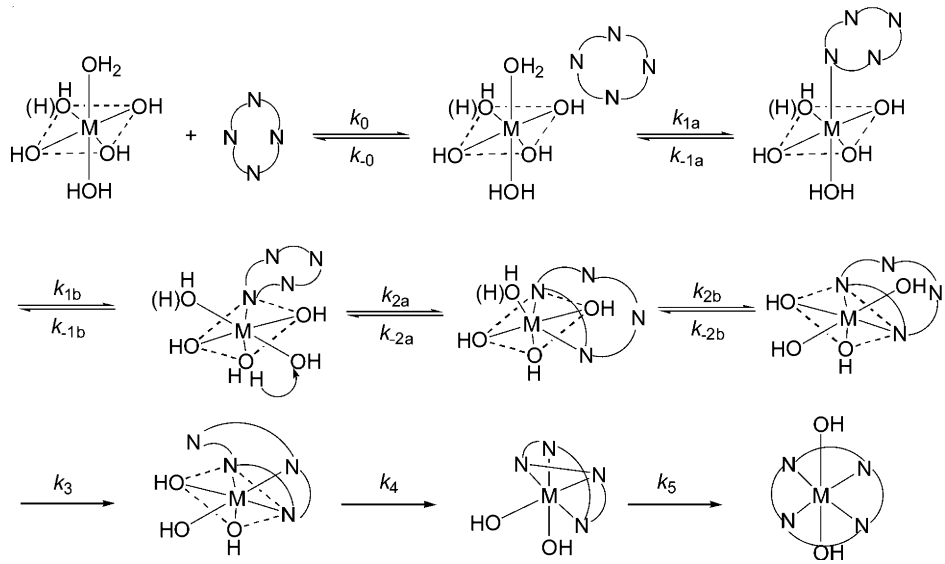


Fig. 1 Stepwise complexation of a metal M (*e.g.* Cu(II)) by cyclam (adapted from ref. 22b). The first Cu(II)–N bond is formed by replacement of an axial solvent molecule (k_{1a}) followed by Jahn–Teller inversion (k_{1b}) to bring the coordinated nitrogen into an equatorial position. Second-bond formation is proposed to occur by a similar two-step sequence (k_{2a} and k_{2b}). The coordination number of species with two or more nitrogens bound may be less than six.

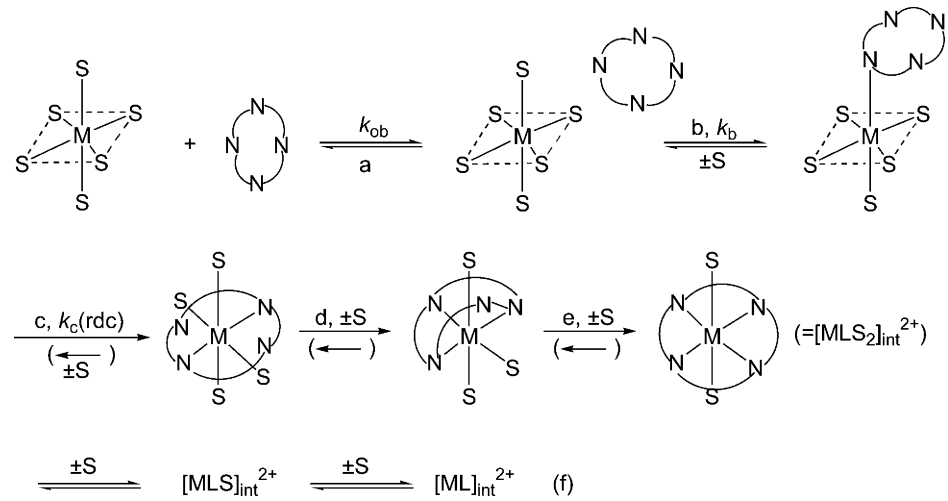


Fig. 2 Mechanism of the initial second-order intermediate formation of cyclam complexes (S = solvent) (adapted from ref. 26a).

slow because N-inversions are involved. In these steps, rearrangement of the carbon skeleton and M–N bond inversion occurs, which finally leads to a product in the *trans*-I or *trans*-III configuration. In the presence of bases, the first-order step, during which the arrangement occurs, can be subject to base catalysis. Analysis of NMR data has revealed that the first-order reaction of the intermediate $[Ni(TMC)]_{int}$ (TMC = 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane) corresponds to isomerization, in which the intermediate $[Ni(TMC)]_{int}$ in the *trans*-II configuration isomerizes to the final product $[Ni(TMC)(DMF)]^{2+}$ in the *trans*-I configuration, with the four methyl groups and one coordinated DMF molecule on the same side of the cyclam ring plane (Fig. 3).

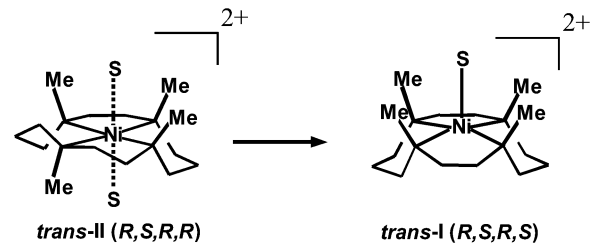


Fig. 3 The stereochemical rearrangement of *trans*-II $[Ni(TMC)]_{int}^{2+}$ to *trans*-I $[Ni(TMC)(DMF)]^{2+}$ in the final step for reaction of Ni(II) with tetra-*N*-methylcyclam (TMC) in DMF (adapted from ref. 26b).

2.2 Structure

The importance of configurational isomers, which differ in the chirality of coordinated nitrogens, in the chemistry of metal complexes of tetraaza macrocyclic ligands is becoming more and more apparent.[27] Upon metal coordination, there are five possible configurations of metal cyclam complexes depending on the spatial alignment of the NH protons: *RSRS*, *RSRR*, *SSRR*, *RSSR*, and *RRRR*, designated *trans*-I to *trans*-V, respectively, in Fig. 4.[28] Each of the four metal-coordinated N atoms is chiral, and the N–H bonds lie above or below the mean ligand plane.

The energies of the different configurations have been estimated on the basis of molecular models and calculated using molecular mechanics, semi-empirical methods and local density functional theory. Semiquantitative estimates of the relative strain energies of the five isomers of cyclam by Bosnich and co-workers[28] and later by Whimp and co-workers[29] have indicated that the *trans*-III form is the most stable in octahedral coordination and is the lowest energy configuration available for Ni(II) cyclam. Molecular mechanics calculations have shown that the *trans*-I configuration becomes more stable relative to the *trans*-III configuration on going

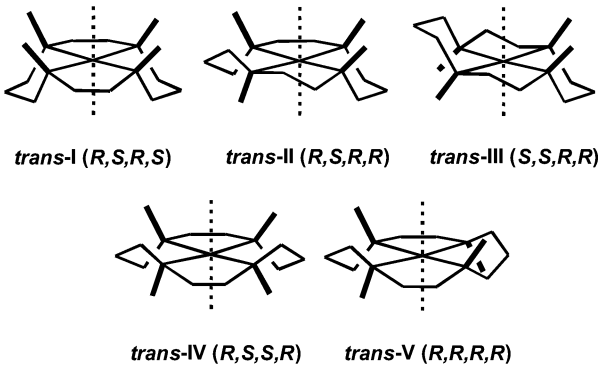


Fig. 4 Configurations of metal cyclam complexes which differ in the chirality of the coordinated N atoms.

from octahedral Ni(II) cyclam complexes to square-planar, square-pyramidal, and trigonal-bipyramidal complexes.[30] The occurrence of *trans*-I macrocycles was higher for square-planar complexes than for octahedral complexes. This is due to the non-bonded interactions between the atoms/substituents at positions 1,4,8,11 and the nonmacrocyclic coordinating ligands (Fig. 5).[31] From

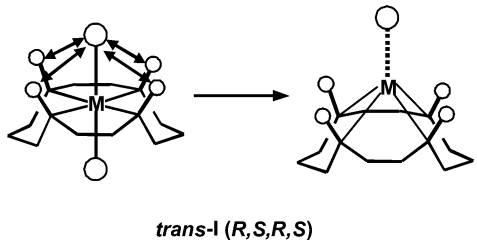


Fig. 5 Cyclam complexes with the *trans*-I configuration are more stable for square-planar, square-pyramidal, and trigonal-bipyramidal complexes than for octahedral complexes due to non-bonded interactions in the latter.

analysis of all the nickel complexes containing the cyclam backbone in the Cambridge Structural Database (CSD), Donnelly and Zimmer concluded that the most commonly found configuration is the most stable configuration, *trans*-III (Table 1).[31] The majority (77.8%) of octahedral nickel(II) complexes with a 1,4,8,11-substituted 1,4,8,11-tetraazacyclotetradecane backbone adopted a *trans*-III configuration, while only 22.3% were *trans*-I. For the square-planar, square-pyramidal, and trigonal-bipyramidal molecules, the situation was reversed. Only 26.7% adopted the *trans*-III configuration, while 73.3% had the preferred *trans*-I configuration. This confirms the molecular mechanical calculations which show that the *trans*-I configuration becomes more stable relative to the *trans*-III configuration in going from octahedral nickel(II) cyclam complexes to the square-planar, square-pyramidal, and trigonal-bipyramidal complexes (Fig. 5). A similar result was also obtained for Cu(II) complexes with a cyclam backbone.[32]

More recently, complicated equilibria for Zn(II) cyclam complexes in aqueous solution, in which there are three configurations present, *trans*-I, *trans*-III and *cis*-V, were analyzed with the aid of 2D NMR spectroscopy in our laboratory. Like most other metal cyclam complexes, all Zn(II) cyclam complexes adopt the *trans*-III configuration in crystal structures found in the Cambridge Structural Database. In the *trans*-III configuration, the two six-membered chelate rings adopt chair conformations and the two five-membered rings have gauche conformations, as found in $[Zn(cyclam)(ClO_4)_2]$[33] and $[Zn(cyclam)(NCS)_2]$.[34] After dissolution of the crystalline complex $[Zn(cyclam)(H_2O)_2](OAc)_2$ in aqueous solution, the stable *trans*-III configuration found in the solid-state equilibrates slowly with *trans*-I (*R,S,R,S*) and *cis*-V (*R,R,R,R*) configurations (hours at 298 K). The distribution of the three different configurations, *trans*-I, *trans*-III and *cis*-V present in aqueous solution at equilibrium is 11.9:45.3:42.8, respectively. However, only two configurations, *cis*-V and *trans*-I were detected in aqueous solutions of Cd(II) cyclam complexes. The difference between Zn(II) and Cd(II) cyclam complexes is caused by the metal ion sizes. The best-fit M–N bond length for metal coordination to cyclam is 2.06 Å.[35] Such a distance corresponds to a metal with an ionic radius of *ca.* 0.65–0.7 Å.[35] Because of the flexibility of the cyclam ring, smaller metal ions (ionic radius < 0.75 Å, mainly those of the first row transition metal series) ensure that the cyclam ring adopts the most stable configuration, *trans*-III.[36] With increasing ionic radius, metal ions no longer fit the cyclam cavity well. For example, Hg(II) (1.10 Å) cyclam exists in the *trans*-I configuration in which the metal lies above the cyclam plane,[35] and Pb(II) (1.21 Å) cyclam adopts a *cis*-V configuration.[37] In addition, extensive ligand *N*- or *C*-substitution also favours thermodynamically unstable configurational isomers.[27] On this basis, it is likely that Cd(II) cyclam adopts the *trans*-I rather than *trans*-III configuration in aqueous solution. Therefore, there are only two configurations, *trans*-I and *cis*-V for Cd(II) cyclam complexes instead of three configurations for Zn(II) cyclam complexes. The favorable NMR properties of cadmium ($I = \frac{1}{2}$ for ^{111}Cd and ^{113}Cd), compared to zinc, provide an insight into the *trans*-I configuration.[38] The five-membered rings of the *trans*-I configuration adopt the eclipsed conformation, and the six-membered rings adopt chair conformations.

In aqueous solution, the distribution of the three configurations of Zn(II) cyclam complexes is strongly dependent on the counter-ion. Aqueous solutions of the perchlorate and chloride complexes of Zn(II) cyclam consist mainly of the *trans*-III configuration, whereas for carboxylato complexes, the *cis*-V form is predominant. Titration of the chloride complex, $Zn(cyclam)Cl_2$ with acetate also led to an increase in the proportion of the *cis*-V configuration at the expense of *trans*-III. It appears that the carboxylate group of acetate can act as a bidentate ligand for Zn(II) and also form hydrogen bonds to the two amine NH protons on the opposite face of the macrocycle, thus stabilizing the *cis*-V configuration in aqueous solution. We have recently found that the acetate salt of a Zn(II)

Table 1 Distribution of the configurations of 139 Ni(II) and 89 Cu(II) complexes containing the cyclam backbone structure in the CSD. Data are from refs. 31 and 32

			Oct[a]		Spl		Spy		Tbp	
	% Total		%		%		%		%	
	Ni	Cu	Ni	Cu	Ni	Cu	Ni	Cu	Ni	Cu
% Total			54.7	44.6	40.0	20.7	2.67	24.9	0.7	10.9
trans-I	20.3	20.7	9.3	4.9	29.3	10.5	75.0	50.0	100	40.0
trans-II	0.0	1.1	0.0	0.0	0.0	0.0	0.0	4.5	0.0	0.0
trans-III	57.2	68.5	52.0	90.2	67.2	89.5	25.0	40.9	0.0	0.0
trans-V	1.5	0	0.0	0.0	3.5	0.0	0.0	0.0	0.0	0.0
cis-V	21.0	8.7	38.7	0.0	0.0	0.0	0.0	4.5	0.0	60.0

[a] Oct = octahedral; Spl = square-planar; Spy = square-pyramidal; Tbp = trigonal bipyramidal.

bicyclam, [Zn(xylyl-bicyclam)$(OAc)_2$]$(OAc)_2$ (a Zn(II) complex of the anti-HIV drug **18**, *vide infra*), crystallizes in such a *cis*-V configuration (Fig. 6a).[39] In aqueous solution, some of the latter complex converts to *trans*-I and *trans*-III configurations.

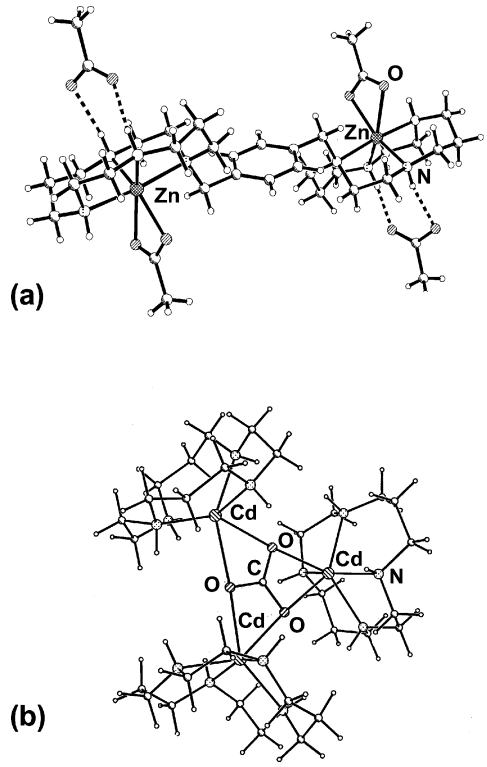


Fig. 6 (a) X-ray crystal structure of the Zn(II) acetate complex of the antiviral bicyclam **18**. The cyclam units adopt the folded *cis*-V configuration with a double H-bond between cyclam N–H protons and acetate on the opposite face to the coordinated acetate (adapted from ref 39). (b) X-ray structure of the cation in $[Cd_3(cyclam)_3(CO_3)](ClO_4)_4{\cdot}3H_2O$ which adopts the unusual folded *cis*-I configuration (all N–H bonds oriented up). The complex contains triply-bridging carbonate fixed from atmospheric CO_2. (Adapted from ref. 38.)

2.3 Stability

Macrocyclic polyamines can form complexes with virtually all transition and some other metal ions (Table 2).[3] However, these complexes are generally so stable that macrocyclic polyamines are of little practical use as selective metal chelating agents.

Table 2 Stability constants (log *K*) for cyclam and tetra-*N*-methylcyclam (TMC) complexes and ionic radii

		log *K*	
M^{2+}	*r* (Å)	Cyclam	TMC
Cu^{2+}	0.72	27.2[46]	18.3[42]
Ni^{2+}	0.69	22.2[47]	8.6[40]
Zn^{2+}	0.74	15.0[48] or 15.5[49]	10.4[42]
Co^{2+}	0.75	12.7[50]	7.58[42]
Cd^{2+}	0.97	11.7[51]	9.0[42]
Hg^{2+}	1.10	23.0[52]	20.3[42]
Pb^{2+}	1.18	11.3[40]	~7.5[40]

The stability of macrocyclic complexes is enhanced by coordination to a tetramine macrocyclic ligand compared to similar noncyclic tetramine ligands due to the macrocyclic effect.[4] There are both enthalpy and entropy contributions to the macrocyclic effect in going from the complex formed by the open-chain 1,4,8,11-tetraazaundecane (2,3,2-tet) to the analogous macrocyclic complex, as shown in Table 3.[40] The important contributions to the increased stability of the complex formed by the macrocyclic ligand

Table 3 Enthalpy and entropy contributions to the "macrocyclic effect". Data are from ref. 40

		Cu(II)	Ni(II)	Zn(II)
log K_1	cyclam	27.2	22.2	15.5
	2,3,2-tet	23.2	16.1	12.7
	Δ(log *K*)	4.0	6.1	2.8
ΔH^a	cyclam	−32.4	−24.1	−14.8
	2,3,2-tet	−26.5	−17.9	−11.6
	$\Delta(\Delta H)$	−5.9	−6.2	−3.2
ΔS^b	cyclam	12	21	21
	2,3,2-tet	16	15	19
	$\Delta(\Delta S)$	−4	6	2

[a] kcal mol^{-1}. [b] cal K^{-1} mol^{-1}.

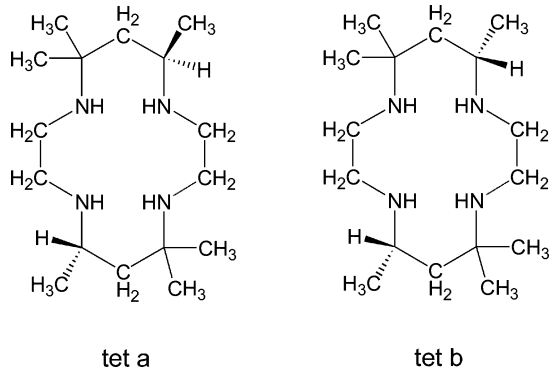
over that of the open-chain ligand mainly come from the enthalpy of complex formation. The main contribution to the favorable $\Delta H°$ of macrocyclic complex formation is the preorganization of the ligand and the increased basicity of the donor nitrogens due to increased alkylation.[41]However, turning the nitrogens on cyclams from secondary into tertiary by adding *N*-methyl groups decreases the complexing ability, in that log *K* drops from 27.2 for Cu(II) cyclam to 18.3 for tetra-*N*-methyl-cyclam (TMC).[42,43] The reason for this is almost certainly the large increase in steric strain found when complexes have *N*-alkyl groups added.

Factors such as pH[38] and oxidation state of the metal[44] can affect the stability of cyclam complexes, and may lead to their demetallation. At low pH, free cyclam is easily protonated, which results in a shift of the equilibrium towards dissociation. The hole size provided by the macrocyclic ligand has a major influence on the stability of cyclam complexes with the metal in various oxidation states. Changes in the oxidation state increase or decrease the metal size, and the cyclam ring size may then be a bad match for the metal, leading to a decrease in the stability of the cyclam complex.

Addition of Au(III) to solutions of Ni(II) macrocyclic polyamines can lead to metal exchange.[45] The replacement reaction depends on not only the stability of the complexes, but also the conformation of the starting complex. The four- and six-coordinate Ni(II) species, $[Ni^{II}(L)]^{2+}$ and $[Ni^{II}(L)Cl_2]$ can react with $AuCl_4^-$, but five-coordinate Ni(II) species, $[Ni^{II}(L)Cl]^+$ do not react. Since both $[Ni^{II}(L)]^{2+}$ and $[Ni^{II}(L)Cl_2]$ adopt the *trans*-III configuration, in which two adjacent NH protons are oriented on one side and the other two are oriented on the opposite side, the approach of $AuCl_4^-$ to the Ni(II) complex is facile. However, the five-coordinate $[Ni^{II}(L)Cl]^+$ adopts the *trans*-I configuration, in which all four NH protons are oriented on the same side with the metal, Ni(II). Access of $AuCl_4^-$ to the deprotonated secondary amine is then difficult due to the steric hindrance.

2.4 Dynamics

2.4.1 Basic catalysis. There are two isomers, tet a and tet b of the macrocyclic ligand 5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane. In neutral or slightly acidic solution, each of these isomers reacts with copper(II) to form initially a blue complex which is readily converted into the more thermodynamically stable red isomer at high pH.[4,53] Due to the different colours, insight has been gained into the kinetics of blue-to-red interconversion of the copper(II) complexes with tet a[54] and tet b[55]. In crystals, the blue isomer of $[Cu(tet\ a)]^{2+}$ adopts the *trans*-II configuration and the red isomer of $[Cu(tet\ a)]^{2+}$ adopts *trans*-III.[56] Each five-membered ring has an eclipsed conformation and each six-membered ring has a skew-boat conformation for the blue isomer of $[Cu(tet\ a)]^{2+}$. The blue isomer of $[Cu(tet\ a)]^{2+}$ with high energy is only stable in dilute acidic solution, but in neutral or slightly acidic solution it converts very slowly to the stable red isomer. The blue isomer of $[Cu(tet\ a)]^{2+}$ differs from the red isomer only in the configuration of a



tet a tet b

single chiral nitrogen centre. Addition of anions, L^-, to aqueous solutions of $[Cu(tet\ a)(blue)]^{2+}$ results in the formation of $[Cu(tet\ a)(red)]^{2+}$. In the presence of Cl^-, Br^-, I^-, NCS^- and N_3^-, the rate of the blue-to-red conversion is significantly slower than that in the absence of these anions.[54] This result can be explained by the binding of these halide or pseudohalide anions to the metal centres, blocking hydroxide coordination which is thought to catalyze the interconversion reaction. These coordinated anions are either weak bases, such as Cl^-, Br^-, and I^-, or lack another lone pair in the vicinity of the N–H group, such as N_3^- and NCS^-. Although NCS^- contains lone pairs, these are too far away to react with the N–H group that must be inverted during the blue-to-red reaction. In the presence of SH^-, NO_2^-, or OAc^-, the rate constants are significantly larger than those in the presence of halide or pseudohalide anions. In the case of the coordinated anions that contain another lone pair in the vicinity of the nitrogen hydrogen, the rate constants increase as the basicity of the anionic ligands increases. It seems that the hydrogen is partially removed from the nitrogen to the coordinated base in the activated complex as shown in Fig. 7. The hydrogen-bonded ring structure may be important in

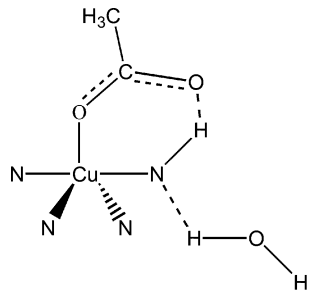
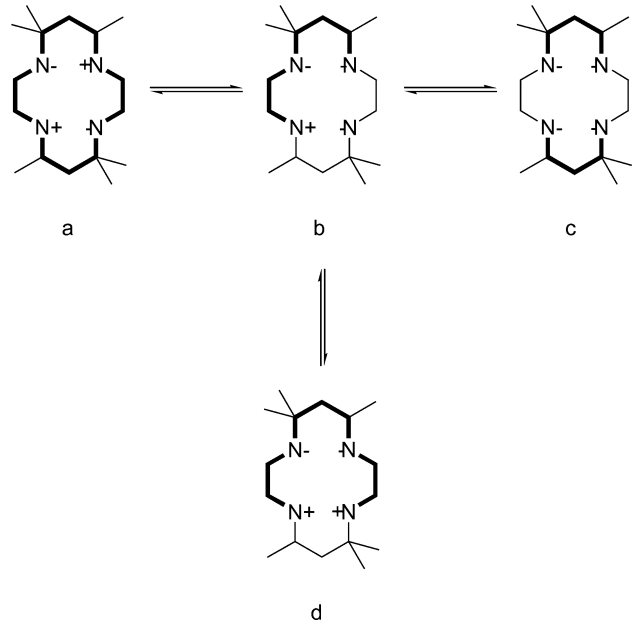


Fig. 7 H-bonding of cyclam NH to coordinated acetate enhances the rate of N inversion in $[Cu(tet\ a)(OAc)(blue)]^+$ (adapted from ref. 54).

helping to maintain an activated species long enough to allow the five-membered and six-membered rings to twist and the nitrogen to attract a proton from a solvent molecule on the opposite site of the coordinated base and lead to the inversion.

The reactivity order for the blue-to-red interconversion of $[Cu(tet\ a)L(blue)]^{2+}$ is $L = OH^- > SH^- > OAc^- > NO_2^- >> Cl^-$, Br^-, I^-, SCN^-, and N_3^-. $[Cu(tet\ a)(OH)(blue)]^+$ has the fastest rate of interconversion, showing that OH^- has the strongest ability to catalyze the interconversion of cyclam complexes. Hydroxide-induced catalytic isomerization is a common phenomenon for cyclam complexes in aqueous solution.

Chung and co-workers also performed studies of the kinetics of the blue-to-red interconversion of $[Cu(tet\ b)]^{2+}$ through an intermediate with coordinated hydroxide.[55] The crystalline, blue complex $\{[Cu(tet\ b)]_2Cl\}(ClO_4)_3$ adopts a trigonal-bi-pyramidal geometry with the folded *cis*-V configuration and five-membered rings in the gauche conformation and six-membered rings in the chair conformation. The product of the isomerization, $[Cu(tet\ b)(red)](ClO_4)_2$ adopts a slightly distorted square-planar geometry with the *trans*-I configuration with the five-membered rings in the partially eclipsed conformation and six-membered rings in the chair conformation. The structures in Fig. 8 indicate the relative positions of the hydrogen atoms on the four nitrogens. It can be seen that two of the four nitrogens must be inverted during the blue-to-red



a b c d

Fig. 8 During the blue-to-red isomerization of $[Cu(tet\ b)]^{2+}$, two of the four nitrogens are inverted, where (a) is $[Cu(tet\ b)(blue)]^{2+}$, (b) is an unstable intermediate, (c) $[Cu(tet\ b)(red)]^{2+}$, and (d) is a second red product present in small amounts in solution (+ indicates NH proton above the cyclam plane, – below. H-atoms are not shown. Gauche conformations in the five-membered rings and chair conformations in the six-membered rings are indicated by bold lines) (adapted from ref. 55).

isomerization. The blue isomer of $[Cu(tet\ b)]^{2+}$ is stable in dilute acidic solution, but in slightly basic solutions it converts very slowly to the red isomer. The rate of the blue-to-red interconversion increases as the pH increases up to pH 12, after which the rate becomes nearly constant with pH. The mechanism proposed for the blue-to-red interconversion based on the kinetic studies for $[Cu(tet\ b)]^{2+}$ is *via* $[Cu(tet\ b)(OH)(blue)]^+$, and the coordinated hydroxide anion assists the inversion of the two nitrogen atoms which the isomerization requires. The positions of the amine hydrogens in $[Cu(tet\ b)(OH)(blue)]^+$ are shown in Fig. 9. Two water molecules

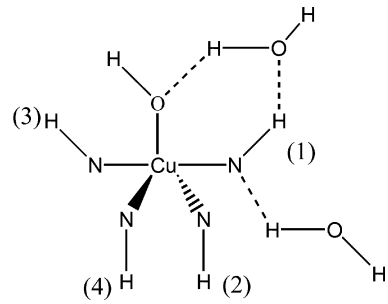


Fig. 9 Role of coordinated hydroxide and second-coordination-sphere water molecules in N inversion (adapted from ref. 55). Cyclam NH groups are labelled (1)–(4).

are included in the vicinity of H(1) to indicate how they might assist in the proton transfer and inversion reactions. H(1) and H(3) will have a much greater chance of reacting with the coordinated OH^- (*via* a water molecule) than H(2) and H(4). Fig. 8 shows the reaction pathway in terms of the required ring configurational changes.

The mechanism discussed above is helpful for understanding the complicated system for Zn(II) cyclam complexes, for which three configurations co-exist in aqueous solution.[57] On increasing the pH of aqueous solutions of $[Zn(cyclam)(H_2O)_2](OAc)_2$, the proportion of the *cis*-V form decreases and *trans*-I form increases, whereas the

proportion of the *trans*-III form remains almost constant. The situation is analogous to the Cu tet case described above. The isomerization occurs from *cis*-V to *trans*-I configurations. Such inversion is very common for cyclam complexes and has been studied extensively.[58] For Zn(cyclam)$(ClO_4)_2$, the proportion of the *trans*-III form decreased with increase in pH, and that of *trans*-I increased, whereas the proportion of the *cis*-V configuration was almost constant. In the latter case, the isomerization occurs from the *trans*-III to *trans*-I configuration. Thus the *trans*-I configuration is more favoured at high pH for both complexes. Normally, at alkaline pH, Zn(II) macrocycles can form hydroxo complexes and hydroxide-catalysed isomerization can occur. Therefore a similar mechanism of intramolecular proton-transfer and inversion can explain the isomerization of Zn(II) complexes. The hydroxo complexes also have a high affinity for atmospheric CO_2 giving rise to a hydrogen carbonate complex, which can react further to form a trinuclear complex.[59] We have recently isolated such a trinuclear carbonate complex of Cd(II) cyclam.[38] In such carbonate complexes, all NH protons are situated on the same side of the ring as CO_3^{2-} with the macrocycle in the *trans*-I configuration, and H-bonds can form readily between CO_3^{2-} and the NH protons of cyclam, Fig. 6b.

2.4.2 Acidic catalysis. The isomerization process can also be catalyzed by acid. However, the acid-catalyzed dissociation of cyclam complexes occurs concurrently. Chung and co-workers investigated isomerization of $[Cu(tet\ a)(red)]^{2+}$ and $[Cu(cyclam)]^{2+}$, and their dissociation in strongly acidic media.[60] For the dissociation reactions, the cleavage of the first Cu–N bond is predominantly *via* the protonation pathway, in which Cu–N bond-breaking and direct protonation of a nitrogen donor occur first, with rapid metal ion solvation occurring in a second step. The cleavage of the second Cu–N bond of cyclam complexes is mainly *via* the intramolecular H-bonding pathway, in which Cu–N bond-breaking and intramolecular H-bonding formation occur first, with rapid protonation of a nitrogen donor and metal ion solvation occurring in a second step.

3 Anti-HIV activity

3.1 Introduction

Virus entry into target cells is the key step of virus replication.[61] The entry of HIV into the cells requires the sequential interaction of the viral glycoprotein gp120 with a receptor CD4 and a co-receptor protein CCR5 (receptor number 5 for a chemotactic protein containing a Cys–Cys or CC motif in its amino acid sequence) or CXCR4 (receptor number 4 for cytokines containing a Cys–X–Cys sequence) on the host cell surface, whereas virus–cell membrane fusion is mediated by the transmembrane protein gp41 (Fig. 10).[62] The initial step in infection is the binding of the viral glycoprotein gp120 to the receptor CD4 on the target cell membrane. This binding leads to important conformational changes that result in the formation of a new recognition site on gp120 for the co-receptor.[63] Proteins gp120 and gp41 are non-covalently associated and form a trimer on the surface of the virus particle.[64] The interaction between gp120 and the co-receptor induces additional conformational changes within the gp120/gp41 trimer that trigger the insertion of a fusion peptide at the tip of gp41 into the cell membrane of the target T cells or macrophages.[65] After fusion, the viral core containing the HIV genome enters the cytoplasm of the cell. Molecular details of the initial stage of the virus infection are important because they may provide a basis for anti-HIV therapy.

CXCR4, a G-protein-coupled, 7-helix transmembrane domain receptor, is a co-receptor for T-cell tropic strains of HIV-1 and allows fusion and entry of the virus into human white blood cells.[66] The physiological ligand for CXCR4 is the stromal cell-derived factor-1 (SDF-1), a chemokine. Therefore CXCR4 can serve as a target for fusion inhibitors – a new class of drugs also referred to as entry inhibitors which specifically block HIV-1 entry and membrane fusion through the CXCR4 co-receptor. The discovery of this new target for therapeutic intervention is considered to be a breakthrough.

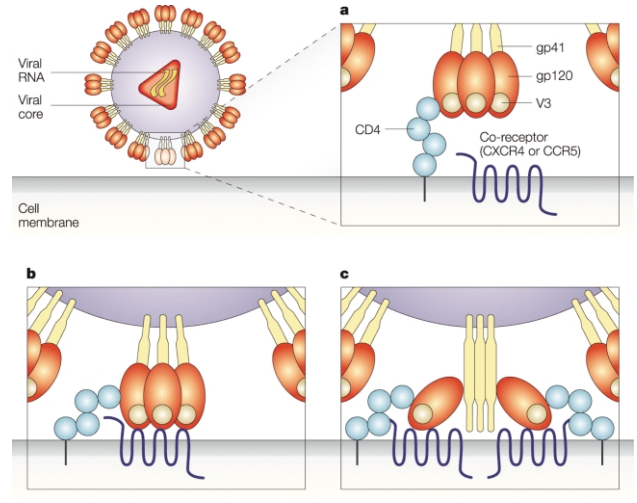


Fig. 10 Recognition of transmembrane co-receptor proteins CXCR4 and CCR5 by viral protein gp120. (a) The viral envelope glycoprotein gp120 interacts with the CD4 receptor at the host cell membrane; (b) subsequently, gp120 interacts with the co-receptor CXCR4 or CCR5; (c) the viral gp 41 anchors into the cell membrane. Reprinted by permission from Nature Reviews Drug Discovery (vol 2, p. 583, ref. 62) copyright (2003) Macmillan Magazines Ltd.

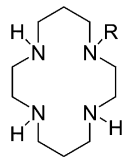
Pandemic HIV-1 infection has led to an intensive search for antiviral agents to control this disease. Theoretically, any stage in the viral replication cycle could be a target for anti-viral therapy.[67] In reality, only a few virus-specific events could function as targets for chemotherapeutic intervention. Limited classes of drugs have already been demonstrated to be effective in the treatment of HIV infection. The current clinical drugs act either at the substrate binding site (Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine) or at a non-substrate binding site of the reverse transcriptase (Nevirapine, Delavirdine), or the viral protease (Saquinavir, Ritonavir, Indinavir, Nelfinavir).[68] A few serious drawbacks[69] have highlighted the urgent need to develop new classes of anti-HIV drugs which attack the other targets in the viral replication cycle.

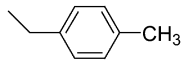
3.2 Anti-HIV cyclams

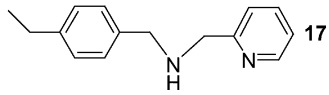
The unmetallated cyclams exhibit strong anti-HIV activities and are discussed first in this section.

3.2.1 Monocyclam. Monocyclam has slight activity against HIV-1 (IC_{50}, 399 μM) and HIV-2 (IC_{50}, 150 μM).[70] Upon addition of substituents, the new cyclam derivatives possess greatly increased anti-HIV activity, such as **16** (*N*-(4-methylbenzyl)cyclam, HIV-1 IC_{50}, 1.4 μM and HIV-2 IC_{50}, 1.1 μM).[70] The substituted mono-cyclam **17** (AMD3465), with a pyridine moiety linked *via* a phenyl bridge, is a more potent inhibitor of X4 strains, with IC_{50} values of 2 nM–0.02 μM, and with no inhibitory effect on R5 HIV strains that utilize the CCR5 co-receptor.[71]

3.2.2 Bicyclam. Bicyclams are a new class of highly potent and selective HIV inhibitors, which interact with the receptor CXCR4, the main co-receptor used by T-cell tropic strains of HIV.[72] Bicyclam activity originated from the serendipitous discovery of anti-HIV activity in products from the synthesis of cyclam which

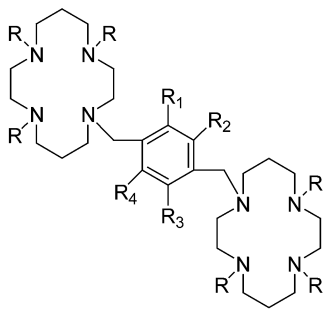


R =  **16**

 **17**

contained the bicyclam, **40** (AMD1657, referred to as JM1657 in earlier literature) as a contaminant.[73] Compound **40** was found to be active against HIV-1 and HIV-2 at concentrations as low as 0.14 μM, with a selectivity index of > 5000.[74] Starting from this lead compound, a large number of bicyclams, represented by **32** (AMD2763, JM2763) and **18** (AMD3100, JM3100), were prepared that showed increased anti-HIV activity. These molecules consist of two cyclam rings linked either by an aliphatic bridge as in **32**, or an aromatic bridge such as in **18**. Time-of-addition experiments revealed that bicyclams interact with a process following virus adsorption but preceding reverse transcription, suggesting virus-cell fusion and/or uncoating as a likely target for mechanistic intervention.[74] Unlike the non-nucleoside type of HIV-1-specific reverse transcriptase inhibitors, which are inhibitory to HIV-1 but not HIV-2, the bicyclams are active against both HIV-1 and HIV-2.[72] Some analogues, such as compound **32**, which have aliphatic linkers, inhibit both HIV-1 and HIV-2 replication but fail to inhibit the formation of giant cells in direct syncytium formation. In contrast, analogues which contain an aromatic linker, such as compound **18**, inhibit both HIV-1 and HIV-2 replication as well as giant cell formation.[75]

Amongst this series, the bicyclam **18**[76] is the most active and extensively studied. It inhibits the replication of HIV-1(III_B) and HIV-2(ROD) with EC_{50} values of 4.2 and 5.9 nM, respectively,



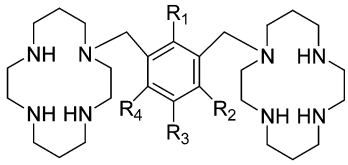
R = H R_1, R_2, R_3, R_4 = H **18**

R_1, R_2, R_3 = H R_4 = -NO_2, **19**

R_2, R_4 = H R_1, R_3 = -Cl, **20**; -CH_3, **21**; -OCH_3, **22**

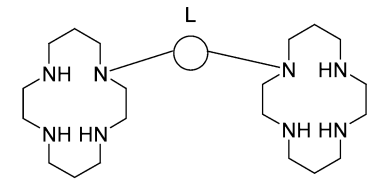
R_1, R_2, R_3, R_4 = -F, **23**

R = -Et R_1, R_2, R_3, R_4 = H **24**

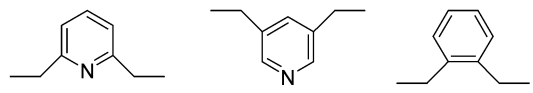


R_1, R_2, R_3, R_4 = H **25**

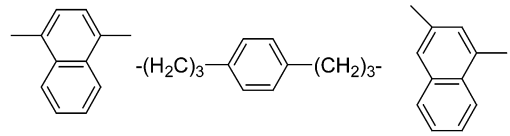
R_1, R_2, R_4 = H R_3 = -NO_2, **26**; -Ph, **27**; -Br, **28**
R_2, R_3, R_4 = H R_1 = -Br, **29**
R_1, R_2, R_3, R_4 = -Cl **30**



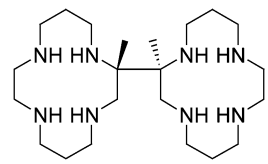
L = -$(CH_2)_m$- m = 2 **31**
3 **32**
6 **33**



34 **35** **36**



37 **38** **39**



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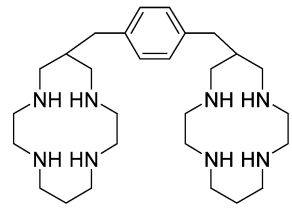

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while not being toxic to MT-4 cells at concentrations exceeding 421 μM, thus achieving a selectivity index of 100,000 or higher (Table 4). It is active against both laboratory strains and clinical isolates of HIV in T4 lymphocytes and monocytes. Finally, it acts additively when combined with dideoxynucleoside analogues such as AZT or

Table 4 HIV-1 and HIV-2 activities and cellular cytotoxicity data for cyclams and bicyclams. Data are from refs. 70,71,73, and 79

	EC_{50}(μM)		Cytotoxicity
Compound	HIV-1 (III_B)	HIV-2 (ROD)	CC_{50} (μM)
1	399	150	> 1248
16	1.42	1.15	> 324
17	0.024	0.024	> 609
18	0.0042	0.0059	> 421
19	0.065	0.073	> 203
20	0.011	0.0025	> 58
21	0.0064	0.0011	> 208
22	0.0058	0.0066	> 206
23	0.0079	0.0079	> 47
24	7.76	14.45	342
25	0.034	0.044	> 421
26	0.041	0.057	44
27	0.21	0.025	> 198
28	0.084	0.054	> 192
29	0.14	0.25	> 144
30	0.52	1.96	9
31	18.6	124.6	349
32	0.25	1.00	> 622
33	0.62	0.62	290
34	0.024	0.065	> 409
35	0.032	0.071	> 395
36	1.36	3.13	> 168
37	0.055	0.039	55
38	0.40	14.49	283
39	0.16	0.079	207
40	0.14	1.01	319
41	0.50	0.68	406

DDI. Compound **18** did not lead to development of resistance (as measured by viral cytopathicity) following at least 14 to 30 passages (49 to 105 days) of HIV-1(III_B) in CEM cells in the presence of various drug concentrations. However, full resistance to the bicyclam **32** was observed.[72] Compound **18** was selected as the clinical candidate, which, after initial Phase I studies, proceeded to Phase II trials, but, unfortunately, significant cardiac side-effects led to its withdrawal from further development as an anti-HIV agent.[77] However, there is continued clinical interest in the compound on account of its ability to mobilise stem cells.[62]

All bicyclam analogs that show activity against HIV-1 NL4–3 are also active against an X4 HIV-1 clinical isolate AOM and the HIV-1 RF and 168.10 strains.[78] There is a close correlation between the antiviral activity of the different compounds against these three HIV-1 strains. The interaction of bicyclams with the CXCR4 co-receptor (monitored by interaction of 12G5 mAb binding) follows a similar structure–activity relationship as described above for inhibition of HIV-1 replication. In addition, the anti-HIV activity of bicyclams also parallels their capacity to inhibit the intracellular Ca^{2+} signal induced by SDF-1α.[78]

De Clercq and co-workers have generalized the structure–activity relationship for phenylenebis(methylene) linked bis-tetraazamacrocycles.[70] Potent anti-HIV activity and low cytotoxicity to MT-4 cells are highly dependent upon the linker and the macrocycles. In general, increasing the size of the macrocyclic ring from 12 to 14 resulted in increases in both the anti-HIV-1 and HIV-2 activity while the cytotoxicity decreased. However, once the size of the macrocyclic ring exceeded 14 ring members, a substantial reduction in anti-HIV potency was observed. Furthermore, the constrained macrocyclic structure is essential for potent activity, while identical macrocyclic rings are not required. Finally, the activity of bicyclam analogs appears to be insensitive to the electron-withdrawing or -donating properties of substituents introduced onto the linker, but sterically-hindering groups such as phenyl markedly reduced the activity.

Joao and co-workers studied the quantitative structure–activity relationship (QSAR) for bicyclams which correlates their structures and anti-HIV activity, resulting in a model which has a high predictive capacity (predictive $r^2 = 0.79$).[79] The model will aid the design of more effective inhibitors. The descriptors from the partial least-squares (PLS) analysis include structural parameters, macrocyclic ring size, and metal chelating ability. The most important features for anti-HIV activity of bicyclams are as follows. First, the optimal distance between the two metal-binding centres in the bicyclams is 9.5–11.5 Å. All compounds which are unable to take up a conformation fulfilling this requirement are observed to have significantly reduced activities. Second, active molecules take up conformations in which the angle between the planes of the cyclam rings are in the range of 40–70° and 110–140°, while conformations in which angles of 0–35° and 160–180° occur lead to low activity. Third, the metal affinity for each cyclam ring is also important for the anti-HIV activity. Bicyclams with reduced affinity for metal binding also have significantly low anti-HIV activities. Thus, decreases in affinity for metal ions arising from substitution of N in the cyclam ring with S and O lead to a drop in anti-HIV activity. Finally, the presence of two chelating macrocyclic rings with optimum ring sizes of 14 atoms achieves high activity.

3.2.3 Conjugates. Among the current clinical drugs for the treatment of AIDS, AZT is the most extensively studied and was the first to be approved by the US Food and Drug Administration.[80] A large number of attempts to improve this drug has been made.[81] Kraus and co-workers have synthesized covalently-coupled monocyclam and bicyclam conjugates with AZT, which might provide a combination-drug effect, and further tested their anti-HIV activity against MT4 cells (Table 5).[82] Monocyclam-AZT conjugates

Table 5 Antiviral evaluation of cyclam/bicyclam-AZT conjugates. Data from ref. 82

	$EC_{50}{}^a$ (μM)	$CC_{50}{}^b$ (μM)	SI[c]	log P^d
Compound	Syncytium formation	12G5 binding	CC_{50}/EC_{50}	Partition coefficients
42	0.01	30	3000	−0.21
43	0.1	30	300	2.34
44	0.01	100	10000	0.14
45	0.05	50	1000	2.68
46	0.05	75	1500	1.81
47	0.05	5	100	5.85
48	0.005	75	15000	1.65
49	0.005	5	1000	5.70
50	0.01	75	7500	−1.35
51	0.03	0.5	17	2.69
AZT	0.05–0.01	50	> 1000	−0.88

[a] EC_{50}: concentration in μM required to inhibit *syncytia* formation by 50% on MT4 cells. [b] CC_{50}: concentration in μM required to cause 50% death of uninfected MT4 cells. [c] SI: selective index = CC_{50}/EC_{50}. [d] log *P* determinations were performed using ACD (Advanced Chemistry Development, Inc.)/log *P* 1.0 base calculations.

showed slightly less anti-HIV activity than the corresponding bicyclam-AZT conjugates. Compounds **48** and **49** appeared to be one order of magnitude more active than the parent drug AZT, while compounds **50** and **51** were equipotent to AZT. The lowering of the EC_{50} values for **48** and **49** compared to that of the parent AZT was explained as an increase in cellular uptake due to increased lipophilicity followed by intracellular release of AZT. The most active anti-HIV conjugates **48**, **49**, **50**, and **51** were also tested for their ability to bind to the co-receptor CXCR4. Among them, **48** was the most effective in competing with 12G5 mAb binding and decreased the mAb binding by up to 69% and 47% at concentrations of 5.88 μM and 58.8 nM, respectively, whereas compound **49** did not inhibit binding to CXCR4. It seems that conjugate **48** targets

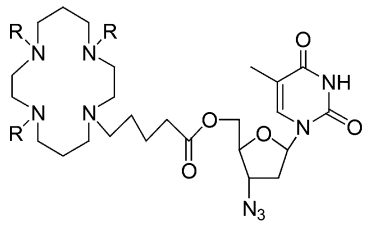
CXCR4 on the cell surface and selectively delivers AZT into cells, increasing its efficiency of action.

3.3 Anti-HIV complexes

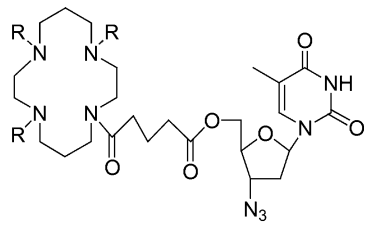
The initial events (virus adsorption and cell fusion) in the replicative cycle of human immunodeficiency virus (HIV) can serve as targets for the antiviral action of metal-binding compounds such as polyanionic compounds, bicyclams and G-octet-forming oligonucleotides.[83]

Zinc may play a key role in the anti-HIV activity of the bicyclams, as is evident from Table 6. The zinc and nickel complexes with the bicyclam **18** (*i.e*. AMD3479, and AMD3462, respectively) are slightly more active than **18** itself, whereas other metal bicyclam complexes (AMD3469, AMD3461, and AMD3158) containing Cu(II), Co(III), and Pd(II), are less active, the Pd(II) complex being inactive as an anti-HIV compound.[83a] Furthermore, the early events in HIV infection are known, *i.e*. virus adsorption to the cell and virus-cell fusion, and have been shown to be the points of attack for Zn(II) complexes of bicyclam.

For metal-**18** complexes, a close correlation is found between the activity and the interaction with the co-receptor CXCR4 as monitored by inhibition of 12G5 mAb binding and the intracellular Ca^{2+} signal induced by SDF-1a, the order of decreasing activity being Zn(II) ~ Ni(II) > Cu(II) > Co(III) > Pd(II).[78] The Zn(II) complex (AMD3479) and the Ni(II) complex (AMD3462) are slightly more active than the bicyclam **18** itself in their capacity to inhibit 12G5 binding (Table 6). The Cu(II) (AMD3469) and Co(III) (AMD3461) complexes are less active than **18**. The Pd(II) complex (AMD3158) is virtually inactive. The IC_{50} for 12G5 binding to SUP-T1 cells closely paralleled the EC_{50} for anti-HIV activity. Similarly, the EC_{50} for anti-viral activity of metal complexes correlated with the IC_{50} for inhibition of the Ca^{2+} flux induced by



R = -H, **42**; -Boc, **43**



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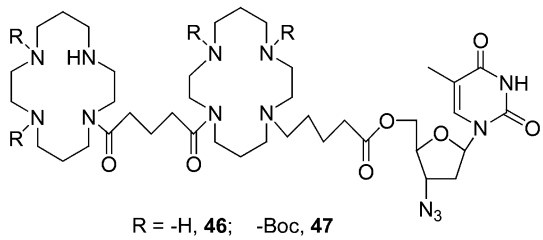
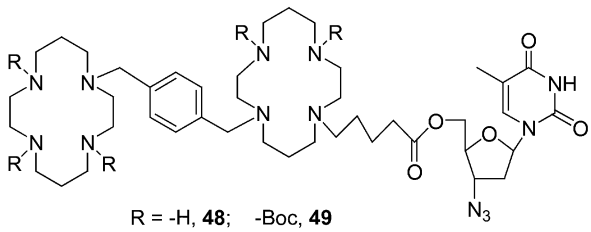
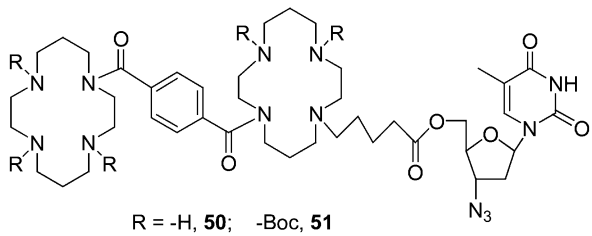


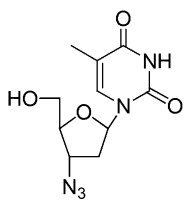
R = -H, **46**; -Boc, **47**



R = -H, **48**; -Boc, **49**



R = -H, **50**; -Boc, **51**



AZT

Table 6 Anti-HIV activity, inhibition of 12G5 mAb binding and inhibition of $[Ca^{2+}]_i$ flux for AMD3100 and its transition metal complexes. Data from ref. 78 and 83a

	EC_{50} (μM)					
	Viral cytopathicity	Syncytium formation	12G5[a]	$[Ca^{2+}]_i$[b]	IC_{50} (μM)	
Compound	HIV-1(III_B)	HIV-2(ROD)	HIV-1(III_B)	HIV-2(ROD)		
AMD3100	0.018	0.042	0.20	3.58	0.020	0.0099
Zn_2-AMD3100	0.010	0.033	0.13	0.26	0.0013	0.0039
Ni_2-AMD3100	0.011	0.037	0.17	0.39	0.021	0.0026
Cu_2-AMD3100	0.076	0.33	2.37	3.79	0.32	0.079
Co_2-AMD3100	0.97	23.8	81.67	163	0.65	0.78
Pd_2-AMD3100	84.9	> 309	> 309	> 309	15.5	86.6

[a] IC_{50}: 50% inhibitory concentration, or concentration of the compound required to inhibit by 50% the binding of 12G5 mAb to CXCR4+ SUP-T1 cells. [b] IC_{50} $[Ca^{2+}]_i$: concentration of the compound required to inhibit $[Ca^{2+}]_i$ increase by 50% induced by SDF-1α in SUP-T1 cells.

SDF-1α, indicating the dependence of anti-HIV activity on the interaction of metal-complexed bicyclams with CXCR4.

3.4 Mechanism of action

The HIV antagonist **18** inhibits virus entry through the co-receptor CXCR4 and completely blocks HIV-1 infection mediated by a mutant CXCR4 bearing a deletion of most of the amino-terminal extracellular domain.[84] Relative resistance to **18** is conferred by different single amino acid substitutions in the second extracellular loop (ECL2), or in the adjacent membrane-spanning domain, TM4. Only substitutions of a neutral residue for aspartic acid (Asp) and of a non-aromatic residue for phenylalanine (Phe) are associated with drug resistance.[84] This suggests a direct interaction of **18** with these amino acids rather than indirect effects of their mutation on CXCR4 structure.

Based on the known strong propensity of the cyclam rings to bind carboxylate groups and of metal ions to bind to His residues, Schwartz and co-workers mutated individually all His residues of CXCR4 located in the extracellular loops or in the transmembrane domains (*i.e.* His113, His203, His281, and His294) to Ala residues, and four Asp residues (Asp171 located in TM-IV, Asp182 and Asp193 located in extracellular loop 2, and Asp262 located in TM4) to Asn residues.[85] Among the acidic residues located in the main ligand-binding crevice of the CXCR4 receptor, Asp171 was identified as the binding site for cyclam in competition with SDF-1. Substitution of the other Asp residues in this main pocket including Asp262 in TM4, did not affect binding of cyclam, while **18** interacted with the CXCR4 co-receptor through binding to both Asp171 in TM-IV and Asp262 in TM4 with each of its cyclam rings. It is possible that cyclam also binds to Asp262, but that its affinity for Asp262 is relatively low compared to Asp171 (K_i, 13 μM). However, binding to Asp262 was not detected in the competition experiments. When the supposedly main binding site for cyclam is eliminated by the D171N mutation, the affinity of the monocyclam was determined to be ~400 μM, perhaps an indication of the affinity of cyclam for the Asp262 site. Another possibility is that although cyclam binds to Asp262, this binding could be silent with respect to the effect on SDF-1 binding. Interaction with Asp262 in TM4 appears to be an important feature for this series of compounds with respect to their function as anti-HIV agents. The binding of all of the bicyclam compounds was highly dependent on interaction with Asp171, but their dependence on Asp262 varied. Importantly, a strong correlation was demonstrated between the antiviral potency of the compounds and the apparent loss of their binding affinity observed when Asp262 is substituted with a non-charged Asn residue.

The anti-HIV bicyclams which target the co-receptor CXCR4 can form strong complexes with transition metals, including Zn(II). Mallick and co-workers[86] proposed that bis-transition metal complexes of **18** can provide complementary coordinating sites for the amino acid residues on a protein surface. The conditional dissociation constant (K_d) for cyclam at blood plasma pH (7.4) is *ca.* 0.1 pM. Since the level of free Zn(II) in plasma is *ca.* 1 nM and the affinity of alkyl-substituted cyclams is likely to be even higher than for cyclam itself, it is reasonable to expect that bicyclams can exist as a Zn(II) complex *in vivo*.[39] In this manner, **18** could be viewed as a prodrug for a transition metal complex in a similar manner to the anti-tumour agent bleomycin.[87]

The affinity of **18** for the co-receptor CXCR4 is enhanced by incorporation of the transition metal ions: Cu^{2+}, Zn^{2+} and Ni^{2+} (Fig. 11).[88] Density functional calculations indicate that the order of the

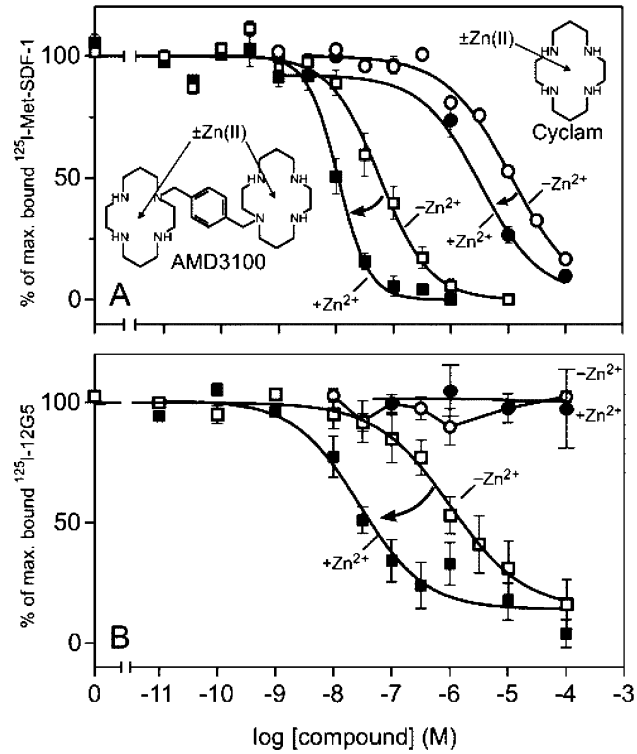


Fig. 11 Zn^{2+} increases the affinity of AMD3100 and cyclam for the CXCR4 co-receptor, as determined using either ^{125}I-SDF-1 (A) or ^{125}I-12G5 monoclonal receptor antibody (B) as radiotracer (AMD3100 (□), Zn_2-AMD3100 (■), cyclam (○), and Zn-cyclam (●)). Whole cell competition binding was performed on wild-type CXCR4 receptor expressed in COS-7 cells. Reprinted with permission from ref. 88. Copyright (2003) American Chemical Society.

affinity for the transition metal complexes correlates with the calculated energy for binding of acetate to the metal ions coordinated in a cyclam ring. Incorporation of Zn^{2+} into cyclam increased its apparent affinity for the CXCR4 co-receptor in a similar manner as for **18**.

Sadler and co-workers reported that Zn(II) cyclam complexes adopt three configurations in aqueous solution, *trans*-I, *trans*-III

and *cis*-V, in contrast to the most stable configuration in the solid state, *trans*-III.[57] Titration of Zn(cyclam)Cl_2 with acetate indicated that acetate can induce a configurational change to the unusual *cis*-V configuration. It appears that acetate can stabilize the *cis*-V configuration in solution *via* coordination and H-bonding. Carboxylates in general can exert a strong influence over the population of the configurational substates of Zn(II) cyclam. In Zn(II)-**18** complexes, in which the two cyclam rings are connected *via* a 1,4-phenylenebis(methylene) linker, each cyclam unit behaves in a similar manner to the monocyclam complex, adopting three configurations in aqueous solution.[39] Acetate can also induce the same configurational changes, giving rise to the *cis*-V configuration. In Zn(II)-**18** complexes, the aromatic linker is close to the two flat monocyclam rings if they are in *trans* configurations, which can result in the folding of each monocyclam and adoption of the *cis*-V configuration. Acetate can further stabilize the *cis*-V configuration by bidentate coordination and formation of hydrogen bonds (Fig. 6a).[39] Therefore, the Zn(II) bicyclam complex with acetate, in contrast to the Zn(II) cyclam complex with acetate, adopts the *cis*-V configuration in the solid state.[39] Such a configurational change may contribute to the increased anti-HIV activity of Zn(II) bicyclam complexes, and the aromatic linker may also play an important role in the anti-HIV activity (Fig. 12). Inactive Pd(II)-cyclams do not

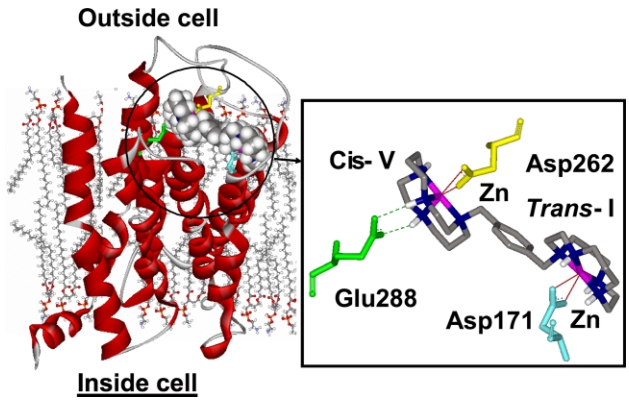


Fig. 12 Model of the zinc complex of the anti-HIV drug **18** (Zn_2-**18**) bound to the CXCR4 co-receptor. One of the Zn(II)-cyclams has axial coordination to the oxygens of Asp262 and double H-bonds between two of its NH groups and the oxygens of Glu288 on the opposite cyclam face and is in the *cis*-V configuration. The second Zn(II)-cyclam has the *trans*-I configuration with axial coordination of Zn(II) to Asp171.

bind axial carboxylates, and therefore cannot adopt the *cis*-V configuration. The lack of direct coordination of Pd(II)-cyclam to carboxylates[39,57b] may be a major factor in determining its anti-HIV inactivity through lack of strong binding to the CXCR4 receptor. However, since the free ligand **18** does bind to the receptor, stereospecific H-bond interactions are also likely to play a role. Furthermore, the kinetics of configurational interconversion is of importance. Although, Co(III)-cyclams bind strongly to carboxylates and, moreover, are capable of undergoing isomerization from *trans* to *cis* configurations,[89] their low anti-HIV activity may be attributable to their kinetic inertness. Configurational interconversions may also be relatively slow for Cu(II)- and Ni(II)-cyclam complexes, which may contribute to their lower activity in comparison to Zn(II)-cyclams.

De Clercq and co-workers have studied in detail the effect of aromatic linkers on the activity of bicyclams.[90] They found that the activity depends highly on the substitution of the aromatic linker connecting the cyclam rings. For example, 2,6- and 3,5-pyridine-linked bicyclams are potent inhibitors of HIV-1 and HIV-2 replication, whereas the 2,5- and 2,4-substituted pyridine-linked compounds exhibit substantially reduced activity and, in addition, are highly toxic to MT-4 cells. A model has been proposed to explain the deactivating effects of 2,5- and 2,4-substituted pyridine-linked bicyclams based on the ability of the pyridine nitrogen to participate in pendant complexation along with four nitrogen atoms of the adjacent cyclam ring, which may involve coordination to a transition metal (Fig. 13). The coordination of the pendant arm

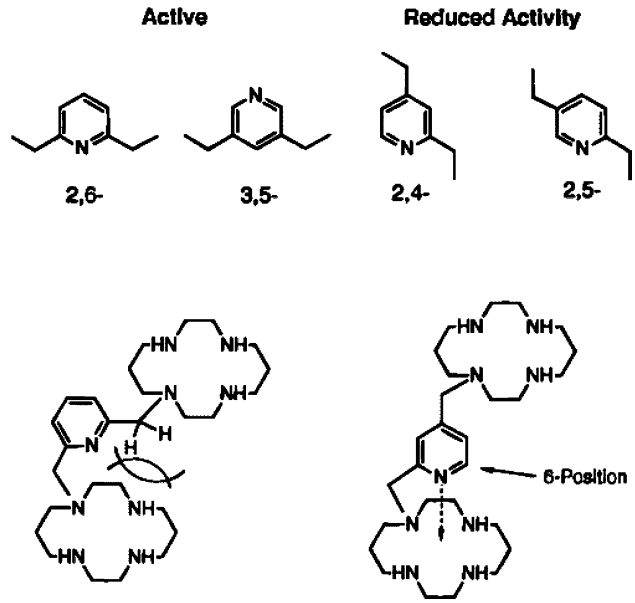


Fig. 13 Models of pyridine-linked bicyclams in which pyridine nitrogen participates in pendant complexation along with four nitrogen atoms of the adjacent cyclam ring. Reprinted with permission from ref 90. Copyright (1996) American Chemical Society.

gives rise to the wrong molecular shape for binding of the bicyclam to the target. The introduction of a sterically-hindering group such as phenyl at the 6-position of the 2,4-substituted pyridine-linked bicyclam appears to prevent coordination of the pendant arm, providing an analog with anti-HIV-1 and anti-HIV-2 activities comparable to the parent *m*-phenylenebis(methylene)-linked bicyclam. If the binding of bicyclams to the molecular target is not transition metal-mediated, then the pendant heteroatom of the linker may hydrogen-bond to the protons shared between the nitrogen groups of the macrocyclic ring thereby inducing an unfavorable conformation with respect to anti-HIV activity, and possibly competing for the azamacrocyclic binding site.

The proton affinities of cyclam (pK_1, 11.5; pK_2, 10.3) are higher than those of the corresponding linear secondary amines, and cyclam is readily protonated to form $[H_2cyclam]^{2+}$ under physiological conditions (pH 7.4).[3] The relatively high basicity of cyclam together with a potential to act as a strong hydrogen-bond donor has been utilized in selective anion recognition, anion transport, catalysis and enzyme models.[3] Both H-bonding and electrostatic interactions can be involved in the binding of $[H_2cyclam]^{2+}$ to anions. Therefore, it is reasonable to assume that protonated cyclam rings of the bicyclam drug **18** could bind directly to side-chain carboxylate groups, *e.g.* aspartate residues, of the negatively-charged domain of CXCR4, in particular extracellular loop-2 (ECL2; 5 acidic residues; net charge, −3). This may block cell entry of HIV-1 by preventing electrostatic interactions between CXCR4 and the HIV-1 envelope protein gp120.[84]

4 Radiopharmaceuticals

4.1 Introduction

Metalloradiopharmaceuticals containing a metallic radionuclide (*e.g.*, ^{67}Cu, ^{90}Y, ^{99m}Tc, ^{111}In, ^{186}Re) are used for the diagnosis or therapy of various diseases, including cancer, infection, thrombosis, kidney and liver abnormalities, and cardiological and neurological disorders.[91] Radiopharmaceuticals can be divided into two primary classes: non target-specific and target-specific, depending on the biodistribution.[92] Targeting can be achieved by receptor binding or other biological interactions. A target-specific metalloradiopharmaceutical can be coupled to targeting biomolecules,

such as antibodies or peptides, through a bifunctional chelator which covalently links to the targeting molecule and coordinates to the metallic radionuclide (Fig. 14). The radionuclides in metal-

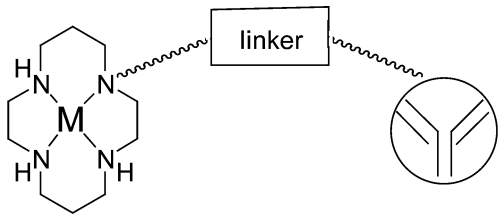


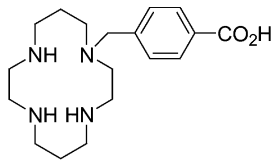
Fig. 14 Schematic structure of a radiopharmaceutical. Cyclam binds to a radionuclide (M) and is linked to a biological targeting molecule, such as an antibody or a peptide.

loradiopharmaceuticals must be bound to the bifunctional chelator tightly, thus achieving high stability *in vivo*, and should preferably also be kinetically inert. Cyclam is one of the most commonly used bifunctional chelators due to the high stabilities of its complexes with radionuclides and ease of substitution on the nitrogen atoms of the cyclam ring.

4.2 $^{64/67}$Cu

The radionuclides of copper offer a selection of diagnostic (^{60}Cu, ^{61}Cu, ^{62}Cu, and ^{64}Cu) and therapeutic (^{64}Cu and ^{67}Cu) isotopes.[91] The positron-emitting diagnostic nuclides have a wide range of half-lives (10 min to 12.7 h) and are cyclotron- or generator-produced.[91] Shorter-lived copper radionuclides are currently used in lipophilic copper complexes for measuring blood flow and hypoxia.[91] The availability of longer-lived copper radionuclides, such as ^{64}Cu and ^{67}Cu, has led to the development of copper-labelled biological molecules for tumour targeting using monoclonal antibodies and peptides.[91]

Most of the bifunctional chelators used for $^{64/67}$Cu are based on cyclam.[91] Copper(II) complexes with macrocyclic ligands have high stabilities and also much greater kinetic inertness, combining rapid metalation, and aqueous solubility with resistance to exchange of Cu(II) *in vivo*.[93] Two well-studied bifunctional chelators, 4-[(1,4,8,11-tetraazacyclotetradecane-1-yl)methyl]benzoic acid (**52**, CPTA) and 6-[*p*-(bromoacetamido)benzyl]-1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (**53**, BAT), have been employed in the formulation of $^{64/67}$Cu(II)-labelled conjugates for human clinical trials.[94] The two copper complexes, Cu(II)-cyclam (log K = 28.09)[95] and Cu(II)-TETA (log K = 21.9)[96] (the macrocyclic chelate portions of the bifunctional chelators, **52** and **53**) have high stability constants. They also exhibit high stabilities *in vitro* and *in vivo.*



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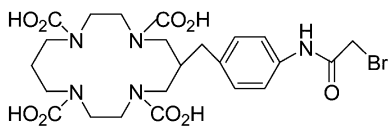


53

The chelating agent **52** has been conjugated to AB35, a monoclonal antibody directed against carcinoembryonic antigen (CEA), without a significant loss of immunoreactivity.[97] Under optimal conditions, the conjugate can be labelled with ^{67}Cu in acetate buffer with a full occupancy of ligands within 20 minutes. The stability of Cu-**52** has been studied spectrophotometrically at pH 7.3 and 37 °C in the presence of excess EDTA. Within 7 days, no spectral change was observed, indicating the high kinetic stability of the Cu(II) complex of **52** at physiological pH. The radiolabelled conjugate was also extremely stable in human serum for 8 days. Biodistribution studies on the ^{67}Cu-labelled antibody in tumour-bearing mice showed that tumour uptake was high with 15 ± 3% ID (injected dose) g^{-1} after 24 h and 32 ± 7% ID g^{-1} after 96 h, whereas radioactivity in normal organs decreased with time after 24 h. Residence times in the tumour of up to 4 days were found for ^{67}Cu-labelled AB35 (Fig. 15).

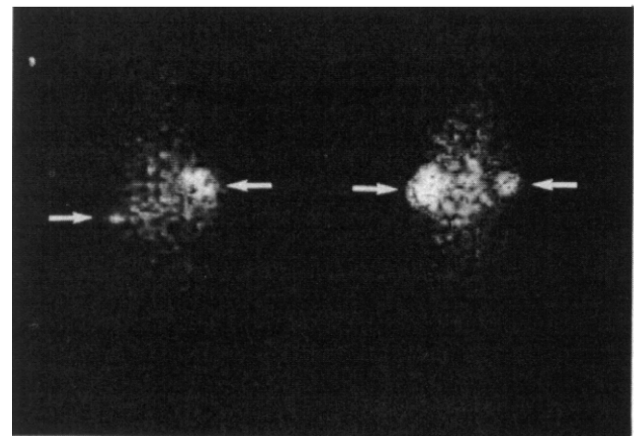


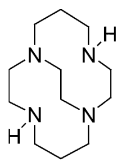
Fig. 15 Anterior γ-camera images of two nude mice bearing LoVo tumours 4 days post-injection of ^{67}Cu-CTPA-AB35 with two tumours each (arrows) ranging between 34 and 215 mg. Mice were injected with 20 μg of ^{67}Cu-labelled AB35 (500 μCi mg^{-1}). Reprinted with permission from ref. 97. Copyright (1991) American Chemical Society.

The chelating agent **53** has been conjugated with 1A3, an anti-colorectal carcinoma monoclonal antibody, and its fragments 1A3-F(ab′)$_2$.[98] The labelling efficiency of ^{64}Cu for intact 1A3 and 1A3 fragments ranged from 60% to 75% using spin column purification techniques. The isotope dilution method using ^{64}Cu and cold $CuCl_2$ indicated that 1.8 chelates were attached to intact 1A3, and 1.4 chelates were attached to 1A3-F(ab′)$_2$. The immunoreactivity values for ^{64}Cu-benzyl-TETA-1A3 ranged from 85% to 95%, whereas the immunoreactivity value for the ^{64}Cu-labelled fragments was 66%. ^{64}Cu-benzyl-TETA-1A3 was stable both *in vitro* and *in vivo* as determined by FPLC of rat blood samples. ^{64}Cu-labelled intact 1A3 and 1A3-F(ab′)$_2$ have been evaluated as potential imaging agents for PET. Biodistribution studies of ^{64}Cu-benzyl-TETA-1A3 and ^{64}Cu-benzyl-TETA-1A3-F(ab′)$_2$ in tumour-bearing hamsters have been compared with those of ^{111}In-Br phi HBED-1A3, ^{111}In-Br phi HBED-1A3-F(ab′)$_2$, and ^{125}I-labelled intact 1A3 and 1A3-F(ab′)$_2$. Tumour uptake of ^{64}Cu-labelled intact 1A3 and fragments in the hamster model was superior to both ^{111}In- and ^{125}I-labelled intact 1A3 and fragments. Human dosimetry data for ^{64}Cu- and ^{123}I-labelled 1A3 and 1A3-F(ab′)$_2$ have been calculated from biodistribution data in rats. High kidney uptake of ^{64}Cu-benzyl-TETA-1A3-F(ab′)$_2$ has precluded clinical studies at present; however, the data show that ^{64}Cu-benzyl-TETA-1A3 would be suitable for positron tomography imaging of colorectal cancer in patients.

Anderson and co-workers have compared the biodistribution and metabolism, in animal models, of **52** and **53** conjugated to the anti-colorectal monoclonal antibody 1A3 and antibody fragments 1A3-F(ab′)$_2$ labelled with ^{64}Cu.[99] The biodistributions of the conjugates were evaluated in normal rats and in the tumour-bearing hamsters. Tumour uptake for all conjugates was high in hamsters at 24 h post-injection: *ca.* 18% and 13% ID g^{-1} for ^{64}Cu-BAT-2IT-1A3 and ^{64}Cu-CPTA-1A3, respectively, and *ca.*14% and 8% for ^{64}Cu-BAT-2IT-1A3-F(ab′)$_2$ and ^{64}Cu-CPTA-1A3-F(ab′)$_2$, respectively. The distributions of both ^{64}Cu-BAT-2IT-1A3 and ^{64}Cu-CPTA-1A3 were significantly low in non-target organs for both rats and tumour-bearing hamsters. The distributions of both ^{64}Cu-BAT-2IT-

1A3-F(ab′)$_2$ and ^{64}Cu-CPTA-1A3-F(ab′)$_2$ were also significantly low in non-target organs, except kidney, for both rats and tumour-bearing hamsters. In hamsters, kidney uptake of ^{64}Cu-BAT-2IT-1A3-F(ab′)$_2$ *ca.* 8% ID g^{-1} was 3-fold lower than for ^{64}Cu-CPTA-1A3-F(ab′)$_2$ (*ca.* 24%), while rats injected with ^{64}Cu-CPTA-1A3-F(ab′)$_2$ had nearly twice the uptake of ^{64}Cu-BAT-2IT-1A3-F(ab′)$_2$. The *in vivo* metabolism was investigated by excising the livers and kidneys of normal rats from 1–5 days post-injection of the radiolabelled conjugates. The ^{67}Cu-labelled 1A3-F(ab′)$_2$ conjugates were >85% degraded in the kidneys to low-molecular-weight metabolites by 1-day post-injection. In contrast, in the liver at 1-day post-injection, greater than 70% of the ^{67}Cu-labelled 1A3 conjugates were unmetabolized. It appears that the chelate charge and lipophilicity have significant effects on the *in vivo* behavior of the copper-radiolabelled antibodies, while trans-chelation of the copper radiolabel to proteins such as superoxide dismutase (SOD) appeared to be a significant factor for accumulation in the liver. Welch and co-workers further confirmed that the positively-charged complexes exhibited high accumulation in the kidneys and liver out to 24 h post-injection, while neutral and negatively-charged complexes similarly showed lower liver uptake and rapid clearance through the kidneys.[93]

Based on the above findings, some new neutral and negatively-charged bifunctional chelators have been developed, such as **9** and **11**. In contrast to cyclam which forms complexes of the type ML^{2+}, the potentially bifunctional chelator **11** can coordinate to Cu(II) with loss of a nitrogen-bound proton to form the neutral MH$_{-2}$L complex.[18a] ^{64}Cu-labelled **11** has been analyzed by electrophoresis confirming that the complex is neutral. The octanol–water partition coefficient (*P*) of ^{64}Cu-labelled **11** was determined to be log *P* = −1.02 ± 0.13.[100] ^{64}Cu-labelled **11** is stable in *vitro*, remaining *ca.* 100% intact after 2 h in rat serum. ^{64}Cu-labelled **11** initially showed high blood uptake, which decreased rapidly after 2 h with no further clearance after 24 h. The rapid blood clearance of ^{64}Cu-labelled **11** indicates that the complex does not dissociate in the blood and is likely to be stable *in vivo*, consistent with the stability in serum *in vitro*. Relatively low liver uptake was observed initially and decreased over time. No significant brain or heart uptake was observed. The uptake in the clearance organs shows that ^{64}Cu-labelled **11** is cleared fairly rapidly through the kidneys and into the bladder with a small amount of clearance *via* the liver into the intestines. By 2 h, > 90% of the activity was excreted. The Cu(I) complex is not stable, and therefore it is likely that reduction of Cu(II) to Cu(I) would cause Cu(I) to dissociate from the chelate and bind to proteins. The *in vivo* thermodynamic and kinetic stability of the Cu(II) complex with this dioxocyclam may result from the high reduction potential which prevents reduction of Cu(II). Compound **11** is a potential alternative to currently-used macrocyclic bifunctional chelators.



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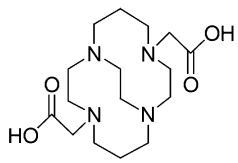

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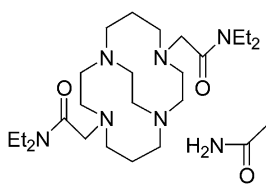

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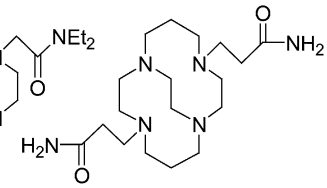

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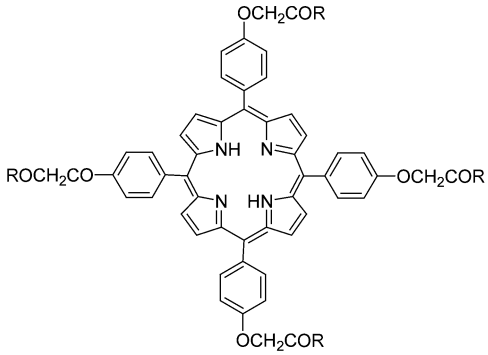
A new class of highly constrained cyclams, the ″cross-bridged″ cyclam derivatives, form highly stable complexes with Cu(II) that are resistant to dissociation in strong acid.[101] ^{64}Cu complexes of **54–57** have been prepared in high yields. Only deprotonated ^{64}Cu-**55** is neutral, and the others are positively-charged. All complexes are highly stable *in vitro* with no decomposition observed in rat serum for 24 h. Biodistribution experiments in Sprague-Dawley rats have indicated that ^{64}Cu-**54–56**, and -**57** are taken up by the liver and kidney and cleared slowly over 24 h, whereas ^{64}Cu-**55** is cleared rapidly from all tissues. The rapid clearance of ^{64}Cu-**55** from the blood and liver, as well as liver metabolism experiments in rats, suggest that it is also highly stable *in vivo*. The bifunctional chelator **55** is a potential candidate for labelling biological molecules with copper radionuclides for diagnostic imaging and targeted radiotherapy.

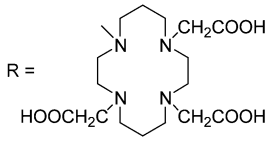
4.3 ^{99}Tc

Many ^{99m}Tc-based radiopharmaceuticals have been successfully developed for determining organ function or assessing disease status by imaging methods. They have been extensively used clinically for imaging bone, cerebral blood flow, renal function, myocardial perfusion and breast cancer.[102] The metastable radionuclide ^{99m}Tc has become the mainstay of diagnostic nuclear medicine.[102] It is produced by the decay of ^{99}Mo.[103] The radioactive half-life of ^{99m}Tc is 6.02 h and when it decays it releases a gamma ray of 140 keV. This half-life is optimum for most nuclear medical scanners. Technetium can exist in any oxidation state from VII to I. The chemistry is relatively straightforward with the final oxidation state of the technetium being determined to a large extent by the ligand environment.[104] Starting with ligands that use simple σ donation from nitrogen, oxygen and sulfur leads almost exclusively to Tc(V) complexes. When the overall charge on the Tc(V) complex is positive, the geometry is often octahedral with a *trans* O=Tc=O core and four equatorial neutral donors.

4.3.1 Non-peptide pharmaceuticals. Murugesan and co-workers have synthesized, characterized and labelled a water-soluble cyclam acid porphyrin (CAP, **58**) with ^{99m}Tc.[105] Chromatographic assay of ^{99m}Tc-CAP showed a radiochemical yield of greater than 95% and the labelled product was found to be stable (*in vitro*) for 4 h at room temperature. *In vivo* distribution studies have been performed in C_6-gliomas and *N*-nitroso-*N*-methylurea (NMU)-induced mammary tumour bearing rats and scintiimages were obtained 5 h post-administration of the labelled ligand. Tumour-to-muscle (*T/M*) ratios were determined and compared with currently available tumour-seeking radiopharmaceuticals such as ^{99m}Tc(V)-DMSA, ^{99m}Tc-citrate and ^{201}TlCl. In the case of NMU-induced mammary tumours in rats, the *T/M* ratios were 6.93, 1.97, 5.30 and 3.29; while in the case of C_6-gliomas the ratios were 5.58, 2.18, 3.96 and 3.02 for ^{99m}Tc-CAP, ^{99m}Tc(V)-DMSA, ^{99m}Tc-citrate and ^{201}TlCl, respectively. An ideal agent should localize in the target tissue with a target to non-target ratio greater than 3.0.[106] The tumour-to-muscle ratio obtained for ^{99m}Tc-CAP for mammary tumour-bearing rats was 6.9, and for C_6-glioma was 5.6. These observations suggest that the ^{99m}Tc-CAP has potential for detection of cancer. In addition to the use of radiolabelled CAP for detection of tumours, this agent could be employed to monitor the progression or regression of tumours following treatment before, during, and after chemotherapy or radiation therapy.

Technetium-99m labelled cyclam *N*-2′-methoxyethyl-2-(3′-nitro-1′-triazole) acetamide (cyclam AK 2123, **59**) has been synthesized and has undergone preclinical testing as an hypoxic tumour imaging agent.[107] Compound **59** has been found to concentrate in hypoxic cells and is currently undergoing Phase I clinical trials for the treatment of advanced head and neck cancers.[108] Chromatographic assays of ^{99m}Tc-cyclam **59** showed a radiochemical purity of greater than 95% and the labelled product was relatively stable for more than 3 h at room temperature. Biodistribution studies carried out in Wistar rats at different time intervals after administration of radiolabelled cyclam **59** indicated





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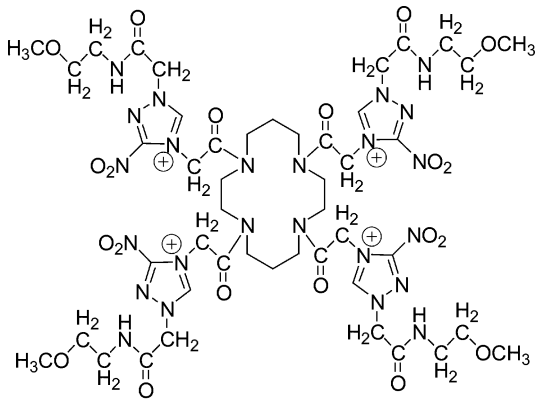
that the compound is excreted through both hepatobiliary and renal systems. The thyroid uptake was very low (0.12% at 5 min; 0.04% at 4 h), indicating that there was negligible free pertechnetate present in the formulated product. The stomach uptake remained relatively constant over the 4 h post injection at ~ 1.1%, suggesting that ^{99m}Tc uptake was not due to free pertechnetate.



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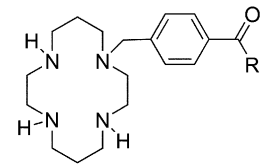
In vivo biodistribution studies have also been performed on mammary tumour-bearing rats at 5 h postinjection using a gamma camera system. The tumour-to-muscle ratio (*T*/*M*) of ^{99m}Tc-cyclam **59** was 8.5, which was compared with other tumour seeking radiopharmaceuticals, viz. ^{99m}Tc-(V) DMSA (3.07), ^{99m}Tc-citrate (5.29) and ^{201}T1C1 (3.29). *T*/*M* ratios were also evaluated in comparison with radioiodinated iodoazomycin galactopyranoside (^{125}I-IAZG). The ratio obtained was 18 for ^{99m}Tc-cyclam **59** and 20 for ^{125}I-IAZG, respectively.

Moreover, an ideal nuclear medicine hypoxia marker should have a partition coefficient (lipid/water solubility) which promotes rapid and nearly equal distribution to body tissues. The partition coefficient of ^{99m}Tc-cyclam **59** obtained in this study was 0.1, which fell in the optimum range for an ideal hypoxic marker. This value is less than that of ^{125}I-IAZG (0.63), ^{99m}Tc-BMS 18321 (40), ^{99m}Tc-BMS 194796 (12) and ^{99m}Tc-HL91 (0.8). *T*/*M* ratio of ^{99m}Tc-cyclam **59** obtained in this study is comparable to those of ^{125}I-IAZG and ^{125}I-IAZXP, and much superior to ^{125}I-IAZA.

4.3.2 Peptide pharmaceuticals. Some specifically-targeted technetium radiopharmaceuticals, in which the targeting moiety (*e.g.*, antibody,[109] hormone[110]) has been labelled with ^{99m}Tc by a bifunctional chelating cyclam, have been reported. However, the radiolabelling efficiency of the cyclam-based bifunctional chelators is very low. Here we discuss two examples which involve the cyclam-based bifunctional chelating agent, CPTA (**52**) which was synthesized at high pH (*ca.* 10) with a high labelling yield.

D-Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr-NH_2 ([D-Ala^1]TNH_2), an analog of the ligand for the CD4/T4 receptor involved in human immunodeficiency virus infection, has been combined with cyclam as a site for metal incorporation to afford the bifuctional ligand cyc-([D-Ala^1]TNH_2) (**60**), in which the N-terminus of the peptide has been used as the site for the attachment of the cyclam ring.[111] Due to high affinity and selectivity, it has been regarded as a candidate for the development of a CD4/T4 receptor imaging agent. Cyc-([D-Ala^1]TNH_2) was reacted with $[^{99m}TcO_4]^-$ and Sn^{2+} to yield the monocationic complex $[^{99m}Tc(O)_2$(cyc-([D-Ala^1]TNH_2)$]^+$. A high yield of formation (> 95%) was achieved at pH 10 after 15 min of reaction.



R = -D-Ala-Ser-Thr-Thr-Thr-Asn-Tyr-Thr-NH_2 **60**

-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-NH_2 **61**

The biological activities of both the cyclam-peptide conjugate and the resulting ^{99m}Tc-complex have been evaluated by measuring their chemotactic indexes. The cyclam-peptide was tolerated (maximal activity at 10^{-8} M) in the chemotactic assay and $[^{99m}Tc(O)_2$(cyc-[D-Ala^1]TNH_2)$]^+$ was active as a chemo-attractant at concentrations within the range of 10^{-9}–10^{-15} M. Both cyc-[D-Ala^1]TNH_2 and $[^{99m}Tc(O)_2$(cyc-[D-Ala^1]TNH_2)$]^+$ retain the high chemotactic capacity of the original octapeptide. Thus coordination of the metal to cyc-[D-Ala^1]TNH_2 does not appear to affect its biological properties. Biodistribution studies in rats of $[^{99m}Tc(O-)_2$(cyc-[D-Ala^1]TNH_2)$]^+$ revealed that this complex was rapidly eliminated through the kidneys and with no significant accumulation in other organs. The facile preparation, retention of chemotactic activity, and lack of non-specific organ enrichment with the complex $[^{99m}Tc(O)_2$(cyc-[D-Ala^1]TNH_2)$]^+$ suggest that this radiopharmaceutical could be of potential interest as a marker for CD_4/T_4 receptors.

Bradykinin is a potent vasodilator and inflammatory nonapeptide that produces its effects through a specific receptor. Due to its high affinity and selectivity, HOE 140, a decapeptide (D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg), has been regarded as a candidate for the development of a bradykinin receptor imaging agent which can be used for the diagnosis of diseases where bradykinin and the bradykinin receptor are involved. HOE 140 has been labelled with ^{99m}Tc using CPTA (4-[(1,4,8,11-tetraazacyclotetradecane-1-yl)methyl]benzoic acid) as a bifunctional chelating agent (**61**).[112] At pH 10, greater than 95% complexation was achieved after 10 minutes using tin(II) citrate and sodium [^{99m}Tc]pertechnetate(VII). The biodistribution data showed no enrichment of any specific organ except liver and kidney, which are important for the excretion of ^{99m}Tc-labelled CPTA-HOE 140. It is also rapidly cleared from the blood. ^{99m}Tc-labelled CPTA-HOE 140 therefore has the potential to become a scintigraphic imaging agent for bradykinin receptors.

4.4 ^{186}Re

^{186}Re-HEDP and ^{153}Sm-EDTMP are phosphonate complexes labelled with radionuclides emitting β^- particles. These complexes have been used as palliative agents for the treatment of skeletal metastases.[113] Pillai and co-workers have developed two new phosphonate derivatives, including cyclam substituted with aminomethylphosphonate groups on the nitrogen atoms (**6**) and further

labelled with ^{186}Re or ^{177}Lu.[17g,114] However, the complexation yield of ^{177}Lu-**6** was low and no *in vivo* experimentation was carried out.

The reactor-produced[115] radionuclide ^{186}Re emits a 1.076 MeV beta and a 136 keV gamma photon (9% abundance) and has a 89.25 h half-life.[116] The ^{186}Re complex of **6** has been prepared under optimized conditions with a yield of 98% and is stable for 6 days, remaining >97% pure.[17g] Biodistribution studies performed in male Wistar rats revealed that skeletal uptake was 23 ± 3.6% (% ID per organ) at 3 h post injection and remained almost constant up to 48 h. The activity in blood was <0.8% and the complex showed major renal clearance. The uptake was low in soft tissues, *viz*. liver, kidney, muscles and intestine. Scintigraphic images in rabbits after injection of 70–100 MBq of ^{186}Re-**6** were acquired at 3, 24 and 48

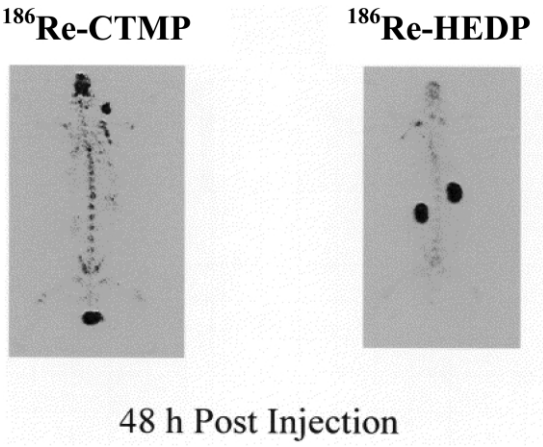


Fig. 16 Comparison of scintigraphic images of ^{186}Re-CTMP and current clinical radiopharmaceutical ^{186}Re-HEDP for bone pain palliation in rabbits at 48 h post injection. Reprinted with permission from ref. 17g. Copyright (2001) Elsevier.

h (Fig. 16). The uptake of ^{186}Re-**6** in the skeleton was quite significant, while activity was also observed in the kidneys, but no activity was observed in any other soft tissues. The skeletal activity was retained for more than 48 h post injection for ^{186}Re-**6** with insignificant activity observable in the kidneys. ^{186}Re-**6** may have advantages over ^{186}Re-HEDP which is in current clinical use.

5 NO donor/scavenger

Nitric oxide is a physiologically important molecule, being synthesized and secreted by a number of mammalian cells. It plays a key role in many bioregulatory systems including the control of cardiovascular function, signalling between nerves in both the peripheral and central nervous system, and defence against micro-organisms and tumours.[117] Excessive production of NO has been implicated at least partly in hypotension, inflammation-associated tissue damage, rheumatoid arthritis, and insulin-dependent diabetes mellitus. On the other hand, diminished NO production has been implicated in pulmonary hypertension, arteriosclerosis, and re-perfusion injury.[118] There has been much interest in the use of metallonitrosyl complexes which are able to scavenge or release nitric oxide as pharmaceuticals.[119] Some of them, such as those of Fe and Ru, are capable of regulating the level of NO in biological systems without affecting NO-synthase action, by the release or binding of nitric oxide in the situation of its under- or over-production, respectively.[117]

The vasodilating effects of nitrovasodilators, such as, sodium nitroprusside (SNP) and nitroglycerine which are widely used in treatment of various cardiovascular disorders and for reducing blood pressure, are mediated by generation of NO.[120] The biological actions of NO are attributed to its stimulation of soluble guanylate cyclase that acts on GTP to produce cGMP, leading to vascular relaxation.[121] There is a need to develop new drugs which control NO levels due to the drawbacks and undesirable side-effects of current clinical medicines.[122] The synthesis, structural characterization and chemical reactivity of the controlled NO-releasing complex $[Ru(cyclam)Cl(NO)]^{2+}$, a potential nitrovasodilator, have been studied.[123] Reduction of $[Ru(cyclam)Cl(NO)]^{2+}$ results in the rapid loss of Cl^- (k = 1.5 s^{-1}) followed by the slower release of NO ($k = 6.1 \times 10^{-4}$ s^{-1}). Due to the slow dissociation of NO, this complex does not exhibit biological activity either in terms of cancer cell toxicity or in affecting hippocampal neuronal firing. However, it can deliver NO in a controlled manner, acting as a long-lasting, although softer, vasodilator. The ability of $[Ru(cyclam)Cl(NO)]^{2+}$ to release NO has been tested *in vivo*, in conscious Wistar rats. For normotensive rats, the complex produced a sustained 10% blood pressure reduction of basal mean arterial pressure for 7 to 11 min.[124] In acute hypertensive rats, the complex produced blood pressure reduction 3-fold larger than in normotensive rats and similar to that of SNP. However, the duration of the effect of $[Ru(cyclam)Cl(NO)]^{2+}$ was 13- to 21-fold longer than that of SNP. The Ru complex is particularly non-toxic (IC_{50} > 3.0 mM), especially when compared with SNP (IC_{50} = 0.06 mM).

De Leo and Ford have reported a new strategy of NO generation from an air-stable, water-soluble Cr(III) complex via the photolytic cleavage of coordinated nitrite.[125]

$$trans\text{-}\left[\mathrm{Cr(cyclam)(ONO)_2}\right]^+ \xrightarrow{h\nu} trans\text{-}\{\mathrm{Cr(cyclam)(O)(ONO)}\}^+ + \mathrm{NO} \quad (5)$$

The photolysis of *trans*-$[Cr(cyclam)(ONO)_2]^+$ in aqueous solution leads to the formation of an intermediate complex, *trans*-$[Cr^{IV}(cyclam)(O)(ONO)]^+$ with concurrent production of NO. In the presence of O_2, the putative Cr(IV) species is trapped to give a more stable Cr(V) complex. *Trans*-$[Cr(III)(cyclam)(ONO)]^{2+}$ is thermally stable in aerated aqueous solution and undergoes a high quantum yield photoreaction that leads to NO formation. This reaction is rapidly reversible in anaerobic media.

6 Superoxide dismutase mimics

Superoxide dismutase (SOD) enzymes are a class of oxidoreductases which contain either Cu/Zn, Fe, or Mn at the active site and catalyze dismutation of the free radical superoxide, the one-electron reduction product of molecular oxygen, to nonradical products.[126] The SOD enzymes have a protective effect in injuries, inflammation, Parkinson's disease, cancer, AIDS, and pulmonary disorders, such as asthma, chronic-obstructive pulmonary diseases and respiratory syncytial virus infections.[127] Low-molecular-weight mimics of the enzyme SOD have been proposed for the treatment of a wide variety of diseases. Most of them are copper complexes[128] as (Cu-Zn)SOD mimics, and some iron[129] and manganese[130]-containing molecules as FeSOD or MnSOD models. Macrocyclic ligand complexes can exhibit high SOD catalytic activity.[131]

Peroxynitrite ($ONOO^-$) may lead to the death of glucose-deprived immunostimulated astrocytes.[132] The increased vulnerability of immunostimulated astrocytes to glucose deprivation is mainly caused by accumulation of peroxynitrite secondary to complete depletion of intracellular antioxidants, including reduced glutathione, a well-known peroxynitrite scavenger. The cell membrane-permeable synthetic superoxide dismutase mimetic, Mn(III)-cyclam can completely scavenge the peroxynitrite produced in glucose-deprived immunostimulated astrocytes, and significantly block the depolarization of mitochondrial transmembrane potential in those cells, and therefore completely inhibit the death of glucose-deprived immunostimulated astrocytes.[133] The injury or death of astrocytes has been assessed by measurement of the activity of the lactate dehydrogenase (LDH) released into the medium. A significant release of LDH was observed in IFN-γ/LPS-treated astrocytes after 4 h incubation with glucose-free DMEM. This LDH release was completely blocked by 10 μM Mn(III)-cyclam and partially by Ni(II)-cyclam (Fig. 17). The enhanced

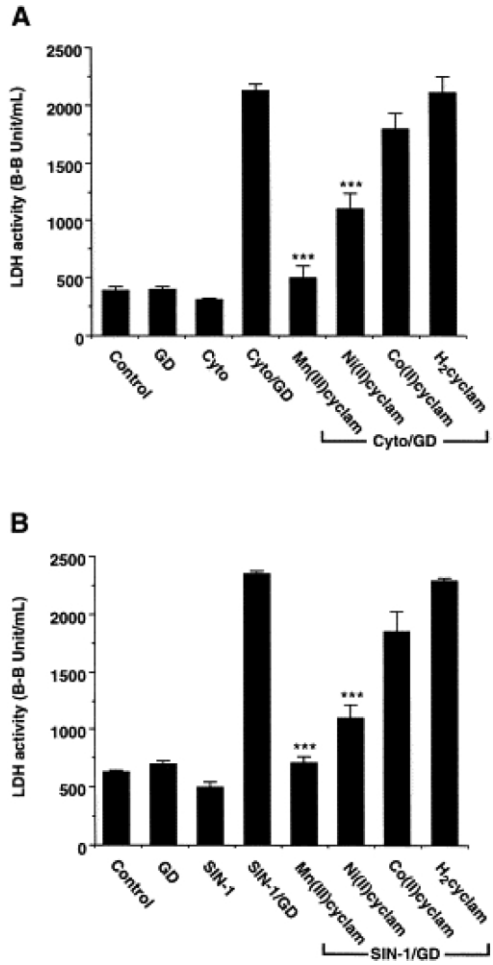


Fig. 17 Mn(III)-cyclam decreases LDH release in glucose-deprived immunostimulated or SIN-1-treated astrocytes. (A) Cells were pre-treated for 24–48 h with IFN-γ (100 U ml^{-1}) and LPS (1 μg ml^{-1}) (*i.e.*, Cyto). Cells were then deprived of glucose (*i.e.*, GD) in the absence and presence of various kinds of cyclams (10 μM for each). (B) Astrocytes were simultaneously exposed for 3 h to glucose deprivation and SIN-1 (200 μM) in the absence and presence of various kinds of cyclams (10 μM for each). LDH levels were determined at 4 h (A) or 3 h (B) after starting the glucose deprivation. Reprinted with permission from ref. 133. Copyright (2003) Elsevier.

release of LDH in glucose-deprived 3-morpholinosydnonimine (SIN-1)-treated astrocytes was also completely blocked by Mn(III)-cyclam, but only partially by Ni(II)-cyclam. Maximal cytoprotective effects of Mn(III)- and Ni(II)-cyclams were obtained at 10 μM concentration. Compared with Mn(III)-cyclam, Ni(II)-cyclam was less efficacious for preventing peroxynitrite-evoked cell death and scavenging peroxynitrite. Depolarization of MTP has been established as one of the crucial signs of cell death. Obvious blockade of MTP depolarization was observed only for Mn(III)-cyclam, but not for Ni(II)-cyclam. Compared to natural cell-impermeable proteins such as SOD and catalase, Mn(III)-cyclam was found to enter immunostimulated astrocytes rapidly and effectively scavenge the $ONOO^-$ produced endogenously in those cells. In several respects, cyclam compounds are thought to be better agents for clinical use than porphyrins. Cyclams are lower molecular-weight and may permeate better through the blood–brain-barrier. In the future, these cell membrane-permeable synthetic SOD mimetics might serve as therapeutic agents for inflammatory reaction-associated ischemic injury.

The peroxynitrite anion ($ONOO^-$) is relatively unreactive, but upon protonation or addition of Lewis acids it oxidizes susceptible molecules by one- and/or two-electron processes. In the absence of reacting partners, it rapidly decomposes to form NO_3^- as the stable product in acidic media.[134] The oxidative chemistry of peroxynitrite is therefore highly pH dependent. Peroxynitrite can directly oxidize a substrate.[135] The mechanism of oxidation of Ni(II) cyclam by peroxynitrite is given by reactions 6 and 7

$$ONOOH + Ni(II)cyclam \rightarrow Ni(III)cyclam + NO_2\cdot + OH^- \quad 2k_2 = 6.5 \times 10^4\ M^{-1}s^{-1} \quad (6)$$

$$Ni(II)cyclam + NO_2\cdot \rightarrow Ni(III)cyclam + NO_2^- \quad (7)$$

The rate constant for reaction (7) is unknown.

7 Summary and perspective

Cyclams and their metal complexes are finding widespread biological applications. Such applications greatly promote the development of macrocyclic chemistry. The enhancement of the thermodynamic stability of cyclam complexes compared to similar noncyclic tetramine ligands (the "macrocyclic effect"), together with their potential kinetic inertness, are important for applications in medicine.

Although the mechanisms of formation of metal-cyclam complexes have been widely studied, the interpretation of data for aqueous solutions is complicated by protonation and solvation effects and further investigations in this area are warranted. Metal cyclams can adopt a variety of configurations and the relative energies of these configurations are dependent of the nature of the substituents on the cyclam ring, size of the metal ion, and interactions between the metal and cyclam ring (especially NH groups), additional ligands, including solvent and counterions, and pH.

The structures of metal cyclam complexes can potentially have a major influence on their biological activity. For Zn(II) cyclam, three configurations, *trans*-I, *trans*-III and *cis*-V are readily detectable in aqueous solutions by NMR, although Zn(II) cyclam usually adopts the most stable *trans*-III configuration in the crystalline state.[57a] The stable *trans*-III configuration converts slowly to *trans*-I and *cis*-V configurations and equilibrates with the other two configurations.[57a] This interconversion can be catalyzed by both acids and bases. Under basic conditions, coordinated hydroxide can assist the inversion of the nitrogen atoms, which the isomerization requires.[55] Acid-catalyzed configurational interchange can involve concurrent dissociation of the bound metal ion.[60] Stability is an important concern for cyclam complexes.

The bicyclam **18** is the most active and selective HIV inhibitor among bicyclams which interact with the co-receptor CXCR4 and block HIV entry into the host cells, and was selected as the clinical candidate.[62,76,77,84] The anti-HIV activity exhibited by bicyclams is particularly intriguing due to the possibility that binding to the molecular target at the HIV-inhibitory step involves acquisition of Zn(II).[39] Recognition of Zn_2-**18** by the CXCR4 co-receptor may involve binding to the carboxylate groups of both Asp171 and Asp262 of the co-receptor protein. Carboxylates can exert a strong influence over selective recognition of the various configurations of Zn_2(II)-**18**.[39] For example acetate can induce a configurational change to the *cis*-V configuration for each cyclam unit of Zn_2(II)-**18**, and stabilize the *cis*-V configuration by bidentate coordination to Zn(II) and formation of hydrogen bonds on the opposite face of the cyclam ring. Analogous interactions with the CXCR4 co-receptor may involve the carboxylates of Asp171 and Asp262. Configurational changes of Zn(II) cyclam can be very slow (minutes to hours).[57a] Further work on the kinetics of configurational interconversions is warranted and may be important for elucidating structure–activity relationships of metal cyclam complexes. Although clinical trials of bicyclam **18** have been halted because of side-effects, there is continued clinical interest in the compound on account of its ability to mobilize stem cells in the body and its use in transplant therapy.[62] There are also potential applications for the treatment of a range of other conditions.

Cu(II) cyclam complexes are highly stable and ^{64}Cu/^{67}Cu radiopharmaceuticals are in use. Most of the bifunctional chelators used for $^{64/67}$Cu are based on cyclam. The cyclam derivative **52** conjugated to AB35, a monoclonal antibody directed against

carcinoembryonic antigen and derivative **53** conjugated to 1A3, an anticolorectal carcinoma monoclonal antibody, and its fragment 1A3-F(ab′)$_2$, exhibit high tumour uptake.[94,97,98] Comparison of the biodistribution and metabolism in animal models of **52**- and **53**-conjugates has shown that the chelate charge and lipophilicity have significant effects on the *in vivo* behavior of the copper radiolabelled antibodies.[99] The neutral and negatively charged complexes show lower liver uptake and rapid clearance through the kidneys. Interesting new developments include the use of structurally-reinforced macrocycles, "cross-bridged" cyclams, which can form highly stable complexes with Cu(II) that are resistant to dissociation in strong acid.[101]

There is interest in the use of metallonitrosyl complexes which are able to scavenge or release nitric oxide as pharmaceuticals. The controlled NO-releasing agent, [Ru(cyclam)Cl(NO)]$^{2+}$ has recently been studied as a potential nitrovasodilator.[123] It can deliver NO in a controlled manner, acting as a long-lasting vasodilator. The complex produces blood pressure reduction similar to that of the clinical drug sodium nitroprusside; however, the duration of the effect of [Ru(cyclam)Cl(NO)]$^{2+}$ is 13- to 21-fold longer than that of latter.[124] A new strategy of NO generation from an air-stable, water-soluble complex *via* the photolytic cleavage of coordinated nitrite has also been reported.[125] The photolysis of *trans*-[Cr(cyclam)(ONO)$_2$]$^+$ in aqueous solution leads to the formation of an intermediate complex, *trans*-[Cr(IV)(cyclam)(O)(ONO)]$^+$, with concurrent production of NO. In the presence of O_2, the putative Cr(IV) species is trapped to give a more stable Cr(V) complex. *Trans*-[Cr(III)(cyclam)(ONO)]$^{2+}$ is indeed thermally stable in aerated aqueous solution and undergoes a high quantum yield photoreaction that leads to NO formation.

The synthetic SOD mimetic, Mn(III)-cyclam completely scavenges the peroxynitrite produced in glucose-deprived immunostimulated astrocytes and significantly blocks the depolarization of the mitochondrial transmembrane potential in those cells, and therefore inhibits the death of glucose-deprived immunostimulated astrocytes.[133] Compared to natural cell-impermeable proteins such as SOD and catalase, Mn(III)-cyclam was found to rapidly enter the immunostimulated astrocytes and effectively scavenge the $ONOO^-$ produced endogenously in those cells.[133]

Zinc is an essential metal in the body, being necessary for the active sites of many enzymes and proteins. In addition, there is a variety of other biomolecules which will compete with cyclam for the binding of Zn(II), *e.g.* amino acids. All these factors may affect uptake and release rates of Zn and other metals by cyclams and therefore influence their biological activities. Measurement of the uptake and release rates of Zn and other metals under physiological conditions will aid understanding of the mechanism of action of anti-HIV cyclam complexes and provide evidence for the role of the metals in the anti-HIV activities. Recent work[136] using high frequency (800 MHz) ^{1}H NMR spectroscopy has shown that Zn(II) binds to cyclam relatively rapidly at pH 7 and at physiologically relevant concentrations (15 μM). Surprisingly, at pH 7, under the conditions used, Zn(II) appears to bind more rapidly to cyclam than Cu(II). Although carboxylates exert a strong influence over selective recognition of the configurational substates of Zn_2(II)-**18**, experimental structural studies of the interaction between the co-receptor CXCR4 and anti-HIV cyclam complexes are lacking and needed. The binding studies will provide important structural insights for improved drug design. Recognition occurs when molecules match the binding surface of the target protein. Designing molecules that recognize specific binding sites of the co-receptor protein in the desirable configuration and trigger subsequent signalling events is an important topic for future research.

The successful development of radiopharmaceuticals mostly depends on the synthesis of bifunctional chelators. Considerations of high labelling efficiency and formation of stable complexes make substituted cyclams with additional conjugation sites potentially important in future work. Since the lipophilicity and charge of chelators greatly influence accumulation and retention of radiolabelled complexes in the body, the choice of substituents is important.

Abbreviations

1A3	anticolorectal carcinoma monoclonal antibody
AB35	monoclonal antibody against carcinoembryonic antigen
1A3-F(ab′)$_2$	fragment of anticolorectal carcinoma monoclonal antibody
AIDS	acquired immune deficiency syndrome
AK 2123	*N*-2′-methoxyethyl-2-(3′-nitro-1′-triazole) acetamide
AZT	3′-azido-3′-deoxythymidine
BAT	6-[*p*-(bromoacetamido)benzyl]-1,4,8,11-tetra-azacyclotetradecane-1,4,8,11-tetraacetic acid
BFCA	bifunctional chelating agent
BMS	5-oxa-amine-oxime nitroimadazole
CCR5	G-protein-coupled, 7-transmembrane domain receptor
CD4	a receptor protein of T-cell
CEA	carcinoembryonic antigen
*c*GMP	3′,5′-cyclic guanosine monophosphate
CPTA	4-[(1,4,8,11-tetraazacyclotetradecane-1-yl)methyl]benzoic acid
CSD	Cambridge Structural Database
CXCR4	G-protein-coupled, 7-transmembrane domain receptor
cyclam	1,4,8,11-tetraazacyclotetradecane
DDI	2′,3′-dideoxyinosine
DMEM	Dulbeccos′ modified Eagle's media
DMF	dimethylformamide
DMSA	dimercaptosuccinic acid
DMSO	dimethyl sulfoxide
EC_{50}	molar concentration of an agonist, which produces 50% of the maximum possible response
ECL2	the second extracellular loop of CXCR4 protein
EDTA	ethylenediaminetetraacetate
EDTMP	ethylenediaminetetramethylene phosphonate
Et_2-2,3,2-tet	3,6,10,13-tetraazapentadecane
FPLC	fast protein liquid chromatography
gp41	41 kDa virus envelope glycoprotein
gp120	120 kDa virus envelope glycoprotein
GTP	guanosine 5′-triphosphate
HEDP	1-hydroxyethylidene-1,1-diphosphonate
HIV	human immunodeficiency virus
HL91	4,9-diaza-3,3,10,10-tetramethyldodecan-2,11-dione dioxime
HOE 140	D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg
HPLC	high performance liquid chromatography
I	spin quantum number
^{125}I-IAZG	radioiodinated iodoazomycin galactoside
IC_{50}	concentration which inhibits growth of 50% of the cells
ID	injected dose
IFN-γ	interferon-γ
HBED	*N*,*N*′-bis(2-hydroxybenzyl)-1-(4-bromo-acetamidobenzyl)1,2-ethylenediamine-*N*,*N*′-diacetic acid
IAZXP	radioiodinated iodoazomycin galactopyranoside
K	complex formation constant
k_d	dissociation rate constant
k_f	formation rate constant
LDH	lactate dehydrogenase
LPS	lipopolysaccharide

mAb	monoclonal antibody
MRI	magnetic resonance imaging
MTP	mitochondrial transmembrane potential
NMU	*N*-nitroso-*N*-methylurea
P	octanol–water partition coefficient
PLS	partial least-squares
QSAR	quantitative structure-activity relationship
SDF-1	stromal cell-derived factor-1 chemokine
SIN-1	3-morpholinosydnoimine
SNP	sodium nitroprusside
SOD	superoxide dismutase
tet a	*C-meso*- 5,5,7,12,12,14-hexamethyl-1,4,8,12-tetraazacyclotetradecane
TETA	1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetra-acetic acid
tet b	*C-rac*-5,5,7,12,12,14-hexamethyl-1,4,8,12-tetra-azacyclotetradecane
2,3,2-tet	1,4,8,11-tetraazaundecane
T/*M*	tumour-to-muscle ratio
TMC	1,4,8,11-tetramethyl-1,4,8,11-tetraaza-cyclo-tetradecane
TM4	the fourth transmembrane domain of CXCR4 protein
Xyl-bicyclam	xylylbicyclam

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