Preparations and applications of synthetic linked azamacrocycle ligands and complexes

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Abstract

Covalently linked azamacrocycles have been known for several decades, but only a modest number of these ligands and their complexes had been described prior to 2000. Since that time, a number of new synthetic methods for their preparation have been discovered, yielding a growing collection of these interesting ligands. Additionally, the number of uses to which these ligands and their metal complexes have been applied has expanded. In this review, the important synthetic methods yielding linked azamacrocycles will be outlined, and their applications will be discussed.

1. Introduction

Inspired by the natural porphyrin and corrin rings (Fig. 1), chemists have produced synthetic azamacrocycles intentionally and in high yields for only about 50 years. Predated only by the phthalocyanines (Fig. 1) [1], an important yet limited early family of synthetic macrocycles with a strong resemblance to the natural sys-
This review will focus on linked synthetic azamacrocycles (where all donor atoms are nitrogen), concentrating on systems published since the year 2000. Significant progress has been made concerning this subset of linked macrocycles since that time, with no major reviews appearing that we are aware of. Several previous reviews on linked macrocycles were published about that time [6], with two specifically targeting the linked azamacrocycles [7]. Our goal is to update the progress in linked azamacrocycles since then. We will first focus on new synthetic methods (and new examples of old methods) used to produce linked azamacrocycles. We will then turn our attention to the application of linked azamacrocycles to specific problems, beginning with applications of the free ligands and then moving to the major applications of their metal complexes. Due to space constraints, the complexation of metal ions to linked azamacrocycles and the subsequent physical and chemical characterization of those complexes will not be considered a separate “application” for the purposes of this review. The literature since 2000 includes a large number of such studies [8] and would be an excellent topic for review. However, we have chosen to focus this part of the review on instances where these complexes are applied to other chemical problems, as a way to highlight the importance of linked azamacrocycles and their complexes to the broader field of chemistry.

2. Synthetic methods for linking azamacrocycles

2.1. N–N linking

Classically, the nitrogen–nitrogen (N–N) linking of azamacrocycles has proved to be the most common and practical form of linkage due to the reactivity of the nitrogen functionality. However, controlling the mono N–N linkage has often posed a challenge in the presence of the multiple reactive nitrogens. In recent years, several innovative methods for the synthesis of these linked azamacrocycles have been described. These methodologies have most commonly exploited the nucleophilicity of the amine functionality, either as a donor in displacement reactions or as a Schiff base coupling partner.

2.1.1. Bis-aminal strategies

One such broad-scoped methodology was reported by Handel and co-workers in 2001 [9]. The authors described the reactivity of bis-aminals formed by the condensation of tetraazamacrocycles (e.g. cyclen, cyclam, homocyclam) with glyoxal [10]. The alkylation of these bis-aminals to form the corresponding ammonium salts was found to be highly dependent on proper solvent selection. When dihalides were employed as electrophiles, exclusive mono-alkylation was observed in either THF or benzene. The success of this selectivity can be attributed to the low solubility and resulting precipitation of the initially formed ammonium salt in the stated reaction media. However, these mono-ammonium salts were found to be sufficiently soluble in acetonitrile to allow for subsequent reactions. Alkylation with a second equivalent of bis-aminal resulted in the formation of the corresponding N–N linked bis-macrocyclic ligand, thus allowing for the formation of either symmetrical or unsymmetrical bis-macrocyclic ligands (Scheme 1). Alternatively, the direct formation of symmetrical bis-macrocycles was achieved in a one-pot process using acetonitrile as the solvent and with proper choice of stoichiometry. The authors found that the bis-ammonium macrocycles could be neutralized by transamination using hydroxylamine.

It was also found that the synthesis of N–N linked macrocycles with pendant arms was possible with the bis-aminal methodology. Exploiting a Gabriel-type synthesis, the authors alkylated cyclen with bromoalkyl phthalimide derivatives (Scheme 2). By control-
ling stoichiometry and with the use of acetonitrile as a solvent, the authors realized selective mono-addition. Subsequent treatment with p-xyllyl dibromide in DMF affected the desired linkage to afford the bis-macroyclic aminal derivative. Final treatment with hydrazine liberated the fully free decaaza bis-macrocycle 1.

The above synthesis illustrates a key defining feature of the bis-aminal strategy. Notably, the alkylation steps occur in a selective N1–N3 regiochemistry. The authors explored this high selectivity by variable temperature NMR studies and by performing calculations at the AM1 level of theory. It was found that bis-aminals of this nature exhibit a cis configuration about the two-carbon bridge (Fig. 1). Arising from this relatively rigid structural feature is the necessity for a bowl-like folded geometry with convex and concave faces. The lone pairs on N1 and N3 point towards the convex face of the macrocycle, making them more nucleophilic than the lone pairs on N2 and N4, which point towards the interior concave face. Thus alkylation preferentially occurs at N1 and N3 (Fig. 3).

Interestingly, the authors investigated relatively few different functionalities as pendant arms. They did report the synthesis of macrocycles 2 and 3 possessing ester and alcohol-functionalized side-arms [11]. Alkylation of the cyclen/glyoxal-derived bis-aminal compound with ethyl bromoacetate, followed by linkage with m- or p-xyllyl dibromide readily afforded the polyammonium salt as above. In lieu of transamination with hydroxylamine or hydrazine, the authors neutralized the products via sodium borohydride reduction, thus forming the corresponding cross-bridged bis-cyclen derivatives [12]. Depending on reaction conditions (e.g. solvent selection), the products were either alcohols or the unreduced esters (Scheme 3).

Archibald and Hubin have shown that glyoxal-derived mono-N-alkylated aza bis-macrocycles undergo reductive ring cleavage to form the side-bridged derivatives [13a]. In particular, the gly-
oxal derivative of cyclam was linked with \( p \)-xylyl dibromide, then reduced with sodium borohydride to afford \( 4a \) (Scheme 4). Exhaustive methylation of the linked bromide salt, followed by sodium borohydride reduction gives the cross-bridged cyclam analogue \( 4b \) (Scheme 4) [13b].

Averin, Beletskaya, and co-workers exploited the bis-aminal methodology in their synthesis of bi- and polycyclic cryptand-type compounds (Scheme 5) [14]. Treatment of the cyclen/glyoxal-derived bis-aminal with 2 equivalents of \( \text{H}_2\text{N}_2\text{C}_6\text{Cl}_2 \) in acetonitrile, followed by aminal deprotection with aqueous base resulted in the desired dialkylated macrocycle. Buchwald amination[15] using a variety of terminal diamines led to mixtures of the expected cryptands as well as tricyclic cryptands resulting from \([2+2]\) cyclocondensation.

A similar strategy was employed for the preparation of xylylene bridged hexaacetate bis-macrocycles (Scheme 6) [16]. Initially, the linkage was performed on the glyoxal derivative of cyclen to afford the standard xylylene-linked bis-macrocycles \( 5 \) and \( 6 \). Exhaustive treatment with ethyl bromoacetate afforded the hexaesters, which were readily hydrolyzed with HCl.

### 2.1.2. Protecting group manipulation

In addition to the bis-aminal strategy brought forth by Handel and others, a variety of amine alkylation strategies have been reported for the construction of the N–N linkage of various azamacrocycles. A strategy of particular importance has been the simple linkage of tri-functionalized/tri-protected cyclen or cyclam derivatives. These methods can provide the means for an initial introduction of desired pendant arms. Additionally, differentially protected azamacrocycles allow for the possibility of selective deprotection/refunctionalization methods for the selective formation more complex structures than are commonly available via other techniques. The obvious disadvantage of any of these techniques, however, involves the initial selective functionalization of some reactive nitrogens while leaving one or more of the nitrogens free.

The tri-functionalized tetraazamacrocycle strategy has been extensively employed by Gunnlaugsson and co-workers in their syntheses of macrocyclic cyclen ligands. They initially reported the synthesis of tri-\( N \)-substituted cyclen derivative \( 7 \) in greater than 50% yield by direct alkylation of cyclen with three equivalents of the requisite \( \alpha \)-chloroamide [17]. When performed at elevated temperature and for extended periods of time in acetonitrile, the formation of mono-, di-, and tetrasubstituted derivatives was minimized. They found that treatment of \( 7 \) with \( p \)-xylyl dichloride in the presence of CsCO\(_3\) in methanol under Finkelstein conditions resulted in the formation of the expected bis-macrocycle \( 8 \), albeit in a poor 10% yield [18]. An explanation for the poor yield was not presented, although under the reaction conditions a competing etherification might seem plausible (Scheme 7).

The same group also reported attempts to alkylate crypten derivatives of the form \( 7 \) with \( 9 \) [19]. The reaction, however, was retarded by the poor solubility of \( 9 \) in the organic solvents explored and a maximum 16% yield of \( 10 \) was obtained. The poor yield and tedious workup required were overcome by performing the initial coupling reaction of \( 9 \) with a molybdenum carbonyl protected variant of cyclen [20]. This strategy allowed for the selective functionalization of a single nitrogen atom of the macrocycle. Deprotection was readily accomplished by treatment with dilute acid. Finally, amide pendant arms were introduced by treatment with the requisite \( \alpha \)-chloroamides (Scheme 8).

Faulkner arrived at \( 6 \) by utilizing a tri-functionalized cyclen derivative [21]. Starting with the known tri-ester \( 11 \), standard linkage with \( p \)-xylyl dibromide readily produced \( 12 \). The use of the \( t \)-butyl esters allowed for ready cleavage by simple treatment with TFA (Scheme 9).

![Scheme 3](image-url)

![Scheme 4](image-url)
In addition, he prepared a similar ligand 13 with a phenolic linker that allowed for differentiated metal binding [22].

Wang and co-workers also reported the synthesis of a bis-cyclen scaffold functionalized with six carboxylic acid moieties (Scheme 10, 14), albeit with a diamine linker as opposed to the more common aromatic spacer [23]. The preparation involved the coupling of the dimethyl ester of ethylenediamine diacetic acid with the tri-functionalized cyclen system. This procedure possesses the advantage of bypassing the ester hydrolysis step required in the aforementioned systems. Remarkably, the reaction was found to proceed in 88% yield, even in the presence of the amino groups of the β-amino ester starting material.

Mochizuki and Numata used N-benzyl protecting groups in their synthesis of an unsymmetrical bis-macrocycle ligand based on the 1,4,7-triazacyclononane (tacn) scaffold [24]. The known dibenzylated tetraazamacrocycle 15 [25] was treated with epichlorohydrin, followed by tacn to afford diprotected bis-macrocycle. This intermediate was easily debenzylated under catalytic hydrogenation conditions to afford 16. The use of epichlorohydrin (instead of the more common dihalide moiety) in this synthesis is notable in that it allowed for a linker functionalized with an alcohol moiety capable of coordinating a metal center. Furthermore, the use of such an electrophile allows for differentiated reactivity and the ability to easily control selectivity of nucleophilic addition (Scheme 11).

Desreux and co-workers also employed an epoxide ring opening reaction in the synthesis of an alcohol-functionalized ligand based on the cyclen and 1,10-phenanthroline scaffolds [26]. The authors reported two different routes towards 17, both originating...
Scheme 8.

Scheme 9.

Scheme 10.
with the epoxide formed from treatment of 1,10-phenanthroline with bleach. The first route commenced with an epoxide ring opening with tetraazamacrocycle 18 followed by alkylation with bromoacetic acid to afford 17. In the second route, tri-t-buty ester 19 was utilized as the nucleophile for a ring opening of the epoxide. The reaction was found to be quite sensitive to reaction conditions, with any unreacted 19 resulting in a quite tedious chromatographic separation of esters. Best results were obtained using a Yb(OTf)3 catalyst and acetonitrile as the solvent. Subsequent treatment with TFA liberated free acid 17 (Scheme 12).

Peters and co-workers prepared a bis-cyclam ligand with an alcohol-functionalized linker by treatment of tri-Boc-protected cyclam with 1,3-dibromo-2-propanol [27]. This approach mirrored previous syntheses reported by the groups of Sessler [28] and Morrow [29] in their respective syntheses of 1,3-bis(tacn)-2-propanol and represents a satisfactory method for the preparation of symmetrical bis-macrocycles. Exhaustive Boc-deprotection using HCl, followed by neutralization generated the target molecule 20 (Scheme 13).

Tri(N-Boc)cyclam 21 has been found to be a suitable precursor for the synthesis of unsymmetrical heteroditopic ligands [30]. Protection of cyclam with substoichiometric quantities of di-t-butyl dicarbonate (Boc₂O) led to a chromatographically separable mixture of Boc-protected derivatives [31]. The tri-protected derivative was carried forward in a reaction with 4-(chloromethyl)benzoyl chloride 22 to afford the expected amide. The use of this acid halide allowed for a selective stepwise coupling of heterocycles and minimization of homocoupling due to the greater propensity for N-acylation when in competition with N-alkylation. Coupling of the second heterocycle 23 under Finkelstein conditions led to the bis-macrocycle. Boc-deprotection using TFA, followed by borane-mediated carbonyl reduction led to the fully deprotected N–N linked macrocycle 24. This general strategy was found to be useful for the synthesis of a number of bis-macrocycles of various ring types (Scheme 14).

The same group employed a similar strategy for the synthesis of a similar series of heterotritopic and cofacial ligands of the type 25 and 26 [32]. These syntheses required quantities of orthogonally di-N-protected cyclam. Such a requirement typically poses a challenge with regard to selectivity of protection, as well as isolation of the desired protected macrocycle. The researchers avoided the problems associated with low selectivity of protection by employing as starting material the orthogonally dibenzylated cyclam, a selective synthesis of which had previously been reported [33]. Boc-protection with the requisite anhydride was followed by debenzylation via catalytic hydrogenation to afford the di-Boc-protected species in quantitative yield over two steps. Treatment of this heterocycle with a substoichiometric quantity of TrocCl afforded a mixture of the mono- and di-Troc protected, with the desired mono-protected compound being formed in a 46% yield. Substoichiometric quantities (0.8 equiv.) of TrocCl were required for maximum yield; the use of greater quantities led to lower yields due to competitive diacylation (Scheme 15).
For the synthesis of heterotritopic ligand 25, the amidation was carried out as before with 4-(chloromethyl)benzoyl chloride, followed by treatment with the Boc-protected N$_2$S$_2$-macrocycle 23. Troc-deprotection was readily performed by treatment with Zn/HOAc and the resulting product was subsequently again amidated with 4-(chloromethyl)benzoyl chloride. Treatment of this with aza-crown ether 27 afforded 25 after standard Boc-deprotection and carbonyl reduction (Scheme 16).

Synthesis of cofacial ligand 26 required the use of di-Troc protected compound 28. Troc-deprotection of 28 was followed by cyclization in the presence of terephthaloyl dichloride. The cyclization protocol required the use of highly dilute conditions in order to minimize polymerization. Boc-deprotection and carbonyl reduction as before resulted in the formation of 26 (Scheme 17).

Lindoy, Smith, and co-workers also utilized 4-(chloromethyl)benzoyl chloride to affect the bis linkage of 29 with two equivalents of tri(N-Boc)cyclam in their preparation of three-ring macrocycles, such as 30 [34]. Amidation of 29 with the acid chloride occurred selectively on the amine nitrogens, thus setting the stage for displacement with tri(N-Boc)cyclam (Scheme 18).

Beer and Gao exploited the latent functionality of the 3-nitrobenzyl protecting group in the synthesis of N–N linked macrocyclic dendrimers [35]. This work represents the first example of a dendrimer containing the tacn subunit. The core was prepared by treatment of 1,4,7-triazacyclononanone with 3-nitrobenzyl chloride, followed by reduction of the nitro groups with H$_2$/Raney Ni. The resulting triamino compound was subjected to standard amide coupling to afford the first generation dendrimer 31 in 90% yield (Scheme 19). A subsequent Raney Ni reduction/amide coupling sequence was utilized to form the second generation dendrimer in 60% yield.

2.1.3. Template cyclization

Whereas the previous syntheses have primarily focused on the construction of bis-macrocycles by the coupling of two or more macrocycles with an appropriate tether, a powerful alternative involves the coupling of acyclic precursors for the construction of the macrocyclic ring systems. For N–N linked macrocycles, this has very commonly involved an aldehyde/diamine condensation to form an imine. In such circumstances, assembly of the macrocycle is quite often controlled by the presence of an N-ligated metal (e.g. Cu or Ni) used to bring the ends of the acyclic molecules into the proximity required for cyclization. These methods typically have the advantage of being direct and brief; however, they commonly suffer from poor yields and the inability to selectively produce unsymmetrical ligands.

An illustrative example of this type of reaction was reported by Kim and co-workers [36]. They reported the treatment of 32 with CuCl$_2$, followed by reaction with ethylene diamine and formaldehyde in the presence of triethylamine to accomplish the synthesis of 33 in 19% yield (Scheme 20). Although the yield was unremarkable, the brevity of the synthesis of the ligand/macrocycle complex is noteworthy.

Mochizuki reported a synthesis of 35–Cu complex by reacting hexaamine 34 with 2,6-diformylpyridine in the presence of Cu(NO$_3$)$_2$ [37]. The initially formed imine was then reduced with NaBH$_4$ to give 35 as the Cu complex. The authors found that the metal-free ligand 35 could be obtained by reaction of this complex with NaCN (Scheme 21).

Salavati-Niasari and Amiri reported a one-pot multi-component construction of a bis-macrocycle using Ni$^{2+}$ coordination as a key ring-formation strategy [38]. An appropriate aromatic diamine was treated with formaldehyde, followed by 1,3-diaminopropane, Ni$^{2+}$ salt, and 2,4-pentanedione to afford the N–N linked cycle 36–Ni (Scheme 22, illustrated with diamine = p-phenylenediamine).

2.2. C–C linking

Although N–N linkage of bis-azamacrocycles is a popular technique due to the nucleophilicity of the nitrogens involved, carbon–carbon linkages are also not uncommon. Inspired in no small part by the seminal work of Busch and co-workers [39], a number of reports of macrocyclic bis(cyclidene) ligands and their corresponding transition metal complexes have appeared in...
recent years. A common theme found in a number of these syntheses involves the reaction of bis enol ethers with diamines to afford C–C linked macrocycles tethered by diaminoalkyl linkers of various chain lengths. Such methodology typically allows for the initial complexation of various metals into the macrocycles and, as a result, either homo- or heterodinuclear complexes are readily attainable.

Korybut-Daszkiewicz and co-workers reported several homodinuclear cyclidine complexes of Cu and Ni [40]. Enamine/aldehyde complex 37–M (M = Cu or Ni) was readily O-methylated by treatment with methyl trifluoromethanesulfonate to afford enol ether 38–M. Treatment with terminal diamines accomplished cyclization to afford the metal-complexed macrocycles 39–M (Scheme 23).

It is noteworthy that when the reaction was carried out under certain conditions, specifically when employing 1,7-diaminohexane and excess dibenzo-24-crown-8 under highly dilute conditions, a novel catenation process resulted, affording 40–M. Unlike “normal” catenanes formed by direct coordination of the metals, the authors reported that π–π interactions between the aromatic and the cyclidine rings were the primary attractions controlling cyclization (Scheme 24).

Another example of the heterodinuclear methodology was recently reported by the same group [41]. Copper complex 38–Cu was treated with various terminal diamines to affect an exchange of the alkoxide groups of the enol ethers for the amines. Treatment of this with nickel complex 38–Ni afforded the heterodinuclear Cu/Ni complex 41–Cu2+Ni2+. The final coupling step proceeded in a somewhat low yield (15%). This is in part attributable to the competitive formation of both the Cu2+/Cu2+ (15%) and the Ni2+/Ni2+ (10%) homodinuclear complexes under the reaction conditions. The authors later found that the same type of synthesis was possible using N,N-dimethylated diamines, thus producing tetra-N-methylated bis-macrocycles [42] (Scheme 25).

By a quite different strategy, Wang and Lönnberg created a small library of di- and tri(azacrown) complexes based on the 1,5,9-triazacyclododecane scaffold [43]. The synthesis commenced with the formation of the benzaldehyde/glycerol acetal. Benzylation of the remaining free hydroxyl group, followed by acetal cleavage and tosylation under standard conditions afforded 42. Displacement with 1,5,7-triazacyclodecane was followed by acetal cleavage and tosylation under standard conditions afforded 42. Displacement with 1,5,7-triazacyclodecane afforded the corresponding orthoamide. This was fully deprotected to liberate the free alcohol and amines by reac-
tion with aqueous HCl and the free amines were again protected as their Boc derivatives to produce 43 (Scheme 26).

Coupling of the free alcohol 43 with a variety of benzylic di- or trihalides resulted in the formation of the corresponding di- and tri(azacrown) compounds which could be exhaustively deprotected again by treatment with aqueous HCl (Scheme 27, shown with benzylic dihalide = 1,4-di(bromomethyl)benzene).

3. Applications of linked azamacrocycle ligands

3.1. CXCR4 antagonists

Clearly, the most famous application of linked azamacrocycles to date has been their use as medicinal compounds, utilizing their ability to bind to the CXCR4 chemokine receptor [44]. Interestingly,
the discovery of this biological activity intersects with one of the earliest linked azamacrocycles. Virologist De Clercq and co-workers discovered that an impurity in a commercial sample of cyclam was responsible for that sample’s anti-HIV properties [45, 44b]. The impurity turned out to be a C2–C2′ linked bicyclam (44) initially identified and published by Barefield in 1981 [46]. Subsequent work determined that an aromatic linking group greatly enhanced the activity of the compounds, and the previously synthesized compound now well known as AMD3100 (45) was launched [47]. This compound has recently been approved as a commercial drug (Plerixafor; Mozobil), although for stem cell mobilization rather than as an antiviral.

Receptor mutagenesis studies where Asp residues were mutated to Asn followed by AMD3100 binding experiments determined that Asp171 and Asp262 of CXCR4 were the probable binding sites for bicyclams [49]. It is likely that each cyclam ring is protonated at physiological pH and can hydrogen bond with the Asp carboxylate groups. In addition to the potential for hydrogen bonding to CXCR4, complexation of Zn²⁺ to the aspartate carboxylates may actually provide the interaction with CXCR4 in vivo. In this case, AMD3100 would be acting as a prodrug, with the metal complex the active drug.

A large catalog of AMD3100 analogues has been prepared and evaluated for their HIV activity [51]. The systematic studies included changing the macrocycle ring size (cyclam, cyclen, homocyclen, 15[ane]N4, 16[ane]N4, iso-cyclam) and the linker (m-xylyl, p-xylyl, all of the dimethylene pyridyl and pyrazyl linkers, and various monosubstituted meta and para xylenes). AMD3100 (45) has continued to be the lead compound for CXCR4 interaction [48]. Transition metal complexes of AMD3100 and of numerous analogues as improved AMD3100 analogues will be discussed in Section 4.4.

CXCR4 antagonists based on linked azamacrocycles will likely continue to garner interest by medicinal and coordination chemists. CXCR4 chemokine receptors are found on the surface of immune, and other, cells, and together with the specific natural ligand, stromal cell-derived factor-1 (SDF-1α, also known as CXCL12), play a role in a number of disease states other than HIV and stem cell transplantation. For example, the CXCR4–CXCL12 system is involved in a number of inflammatory diseases, and the development of rheumatoid arthritis [52]. CXCR4 expression has also been reported in at least 23 different epithelial, mesenchymal and hematopoietic cancers [53]. CXCL12 stimulation of tumor growth, angiogenesis, and metastasis of breast cancer cells has been described [54]. Target organs for breast metastases such as liver, lung, and bone have high levels of CXCL12, triggering the specific migration of breast tumor cells that express the CXCR4 receptor [55]. Undoubtedly, further work utilizing linked tetraazamacrocycle drug candidates to combat these CXCR4-related diseases is warranted.

3.2. Anion and small molecule receptors

An early example of anion binding to linked azamacrocycles was published in 1990 [56]. Protonated pentaaza- and hexaazamacrocycles had demonstrated an ability to bind anions [57]. Kimura and co-workers postulated that linking two such molecules might improve anion binding, and thus produced polyether and propylene linked pentaazamacrocycles 46–47. These ligands were evaluated titrmetrically and polarographically for anion binding and were found to make stable 1:1 complexes with citrate³⁻, AMP²⁻, ATP⁴⁻, HPO₄²⁻, [Fe(CN)₆]⁴⁻ and [Fe(CN)₆]³⁻. In each case, two linked azamacrocycles always resulted in stronger anion binding than for a single azamacrocycle. This result seems logical, as it is typically easier to hold onto a macroscopic object with two hands rather than one. Chemically speaking, something akin to the chelate effect [58] is likely at work.
More recently, Handel and co-workers have investigated the binding of various phosphate anions to polyprotonated linked tetraazamacrocycles [59]. Aromatic-linked cyclens 48–51 were studied initially [59a] by potentiometric methods and NMR spectroscopy for binding to orthophosphate, PO$_4^{3-}$, pyrophosphate, P$_2$O$_7^{4-}$, and triphosphate, P$_3$O$_{10}^{5-}$. Conclusions from the work included: (1) The strength of the ternary (anion, ligand, H) complexes generally increases PO$_4^{3-}$$<$$P_2O_7^{4-}$$<$$P_3O_{10}^{5-}$, which was attributed to charge–charge interactions. (2) 50, with its pyridine linker able to contribute to the anion binding below pH 2, was the most efficient phosphate anion binder. Interestingly, at high pH 9–12, repulsion from the pyridine lone pair causes the complete release of triphosphate, making 50 a potentially useful analytical reagent.

Linking the same cyclen azamacrocycles a second time to produce macrotricyclic ligands (52–56) was achieved [59a] and their phosphate anion binding ability examined in a similar fashion [59b,59c]. In a result that is reminiscent of the relationship of the macrocycle effect [60] compared to the chelate effect [58], the twice-linked systems exhibited higher phosphate binding constants and selectivity compared to the once-linked analogues, which was attributed to the more rigid cavity of the macrotricyclic ligands [59b].

Size effects were more evident in the twice-linked systems as well, especially at acidic pH’s, where an increase in cavity size clearly resulted in an increase in triphosphate binding [59b]. Surprisingly, 54, containing two pyridyl linkers that clearly increased binding efficiency compared to the xylyl linkers in the once-linked systems, is actually less efficient at triphosphate binding than 56, with one pyridyl and one m-xylyl linker [59c]. A proposed explanation is that protonation of both pyridine nitrogens does not occur under the studied pH range. If one pyridine is protonated, but the other is not, the increased binding offered by the protonated pyridine is defeated by the lone pair repulsion (as observed at high pH for 50) of the other.

Handel et al. turned to an examination of triphosphate binding of linked cyclam 45 in comparison to similarly p-xylyl linked linear tetraamines (57) [59d]. Over the entire pH range studied, the linear system was a more efficient binder of triphosphate than 45. The key factor in this result was identified as the ability of the linear system to more easily form polyprotonated species than linked azamacrocycle 45. In addition, the flexibility of 57 allows it to more optimally fit around the triphosphate anion. The advantages macrocycles hold over linear polydentate chelates are manifold when it comes to metal ion complexation [5], but this study shows that these advantages may not be as evident when coordinating multi-atom anions such as triphosphate.

In 2008, this study was extended to AMP, ADP, and ATP complexation to 49 and 50 [61]. 1:1 complexes between all of these nucleotides and the two linked azamacrocycles were formed. As was seen in the previous phosphate anion binding studies, 50 proved to be the most efficient host molecule over the entire pH = 2–12 range. Again, the pyridine linker provides an additional point of interaction with the guest that is beneficial in terms of binding ability. A general trend observed was that binding increased as protonation of the linked azamacrocycle increased. AMP was the best guest for 49, while ATP was particularly strongly bound to 50, until around pH = 9. NMR studies also indicated that π–π stacking between the aromatic linker and the adenine group in ATP is likely playing a role in stabilizing the complexes. This effect appears to be pH dependent for 50, coinciding with the pyridine linker protonation (around pH 4) and its change in role from H-bonding to the phosphate groups at low pH [58].
4. Applications of linked azamacrocyclc metal complexes

4.1. Medical imaging

4.1.1. Magnetic resonance imaging

One key improvement in MRI diagnostics has been the use of “contrast agents,” paramagnetic metal containing compounds administered prior to the MRI scan that enhance the resolution of the image by accelerating the nuclear relaxation of tissue water protons. Contrast agents are now used in about 30% of all MRI exams [62]. Gadolinium(III) is the preferred metal ion to use, since its seven unpaired electrons result in the largest magnetic moment for any metal ion, and thus provides the largest resolution gains. Gd(III) alone is quite toxic, so clinically useful contrast agents must bind the metal ion in an organic ligand that will shepherd the metal ions safely through the patient. Currently used macrocyclic contrast agents typically contain a DOTA [63] type ligand (59) bound to only one Gd(III) ion per molecule (60), and usually have relaxivities (a measure of effectiveness) of about 3–5 mM$^{-1}$ s$^{-1}$.

Improving the relaxivity (R$_1$) of contrast agents has been a long-standing goal of inorganic chemists. The Solomon–Bloembergen–Morgan theory predicts that a relaxivity over 100 mM$^{-1}$ s$^{-1}$ could be achieved by the manipulation of four key factors: the number of open coordination sites available to interact with water, electron spin relaxation rates, water exchange rates, and molecular reorientation [64]. The electron spin relaxation rate has not yet lent itself to rational optimization. However, each of the other areas has been individually improved through rational design of new ligands to bind Gd(III). The factor most relevant to linked azamacrocycles is the rigidity of the xylene core, a property confirmed in xylyl NMRD data for a series of known Gd(III) mononuclear MRI contrast agents, including the three commercially used contrast agents at that time [67]. Interestingly, it was found from the EPR data that the dimeric complexes had transverse electronic relaxation rates much greater than the monomeric species and that the temperature dependence of these rates changed in the opposite direction compared to the monomers. The conclusion was that a previously undocumented additional contribution to electronic relaxation due to intramolecular dipole–dipole interactions between the Gd(III) ions was the cause of this unusual behavior. Unfortunately, the enhancement of the overall relaxivity due to this effect is relatively minor compared to other factors, such as the water exchange rate.

An enhancement of relaxivity due to slower tumbling as measured by $\tau_k$ was also observed for the dimeric complexes. This relaxivity enhancement was larger for the 61 complex (171 ps) than for the complex of 62 (106 ps) vs. $\tau_k$ = 77 ps for 60 [67]. The data suggests that the more flexible linker of 62 allows the linked complexes to rotate independently of each other mitigating the desired effect of linking the complexes together. The authors point out that the rigidity of the linker in effective dimeric contrast agents must be such that the entire complex rotates as a whole in order to take advantage of an enhanced $\tau_k$.

Finally, as in Gd$_2$(62), sacrificing carboxylate ligands in order to link the azamacrocycles results in less crowding, and slower dissociative water exchange rates [1.5×10$^6$ s$^{-1}$ for Gd$_2$(61) and 1.4×10$^5$ s$^{-1}$ for Gd$_2$(62)] vs. 4.1×10$^5$ s$^{-1}$ for Gd(DOTA)$\cdot$60]. The authors recommend screening any potential polymeric MRI contrast agents by studying their dimeric analogues in order to ensure that the linker rigidity and the water exchange rates are optimized prior to synthesizing the polymeric versions. Overall, the increases in relaxivity at 40 °C due to enhanced electronic relaxation and molecular reorientation are a modest [68] [5.8 mM$^{-1}$ s$^{-1}$ per Gd for Gd$_2$(61) and 4.9 mM$^{-1}$ s$^{-1}$ per Gd for Gd$_2$(62)] vs. the value of 3.4 mM$^{-1}$ s$^{-1}$ for Gd(DOTA)$\cdot$60].

Linked azamacrocycles prepared for this purpose have largely contained DOTA (59) derivatives where one of the acetate pendant arms has been replaced with a linker (61–67). The Gd$_2$(63) complex [66] was found to have a 3-fold increase in $\tau_k$ compared to 60 as expected for the larger molecule, but the overall increase in relaxivity was only modest: 4.61 mM$^{-1}$ s$^{-1}$ per Gd compared to 3.4 mM$^{-1}$ s$^{-1}$, respectively. The less than expected enhancement of R$_1$ reflected an unanticipated reduction in the water exchange rate by a factor of 5 compared to 60. Apparently, the smaller, more weakly donating hydroxo group is not as effective as another carboxylate donor in forcing water dissociation.

The novel linked azamacrocycles 61 and 62 Gd$_2$ complexes were included in the first integrated analysis of $^{17}$O NMR, EPR, and NMRD data for a series of known Gd(III) mononuclear MRI contrast agents, including the three commercially used contrast agents at that time [67]. Interestingly, it was found from the EPR data that the dimeric complexes had transverse electronic relaxation rates much greater than the monomeric species and that the temperature dependence of these rates changed in the opposite direction compared to the monomers. The conclusion was that a previously undocumented additional contribution to electronic relaxation due to intramolecular dipole–dipole interactions between the Gd(III) ions was the cause of this unusual behavior. Unfortunately, the enhancement of the overall relaxivity due to this effect is relatively minor compared to other factors, such as the water exchange rate.

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linked non-cyclic systems [62]. An additional change in these ligands is that no coordinating function has been added to the linker, leaving the ligands heptadentate, and potentially allowing for more water molecule coordination. Measurements, however, proved that each Gd complex with these ligands only had one water molecule per Gd. Interestingly, the aromatic linkers appear to promote aggregation of the neutral Gd complexes in water. The large aggregates lead to slow rotation. From NMRD studies, \( \tau_r = 889, 570, \) and 974 ps for the complexes of 65–67, respectively. An additional advantage these complexes demonstrate is a water exchange rate much higher than the previously discussed linked azamacrocycle systems. The values presented are 7.5 \( \times 10^6 \), 11.0 \( \times 10^6 \), and 12.0 \( \times 10^6 \) s\(^{-1}\) for the complexes of 65–67, respectively. Relaxivities characterized [16] as “remarkably high” and “twice as high as for nonaggregated complexes of similar size” were observed for these aggregates. Unfortunately, exhaustive attempts at disaggregating the particles in order to study the individual complex properties, including their relaxivities, failed.

An MRI contrast agent taking advantage of coordination chemistry for linking multiple rings was published in 1999 with more details being recently provided [26,69]. Complex 68 links three DOTA-like macrocycles through coordination of a phenanthroline pendant arm to a central Fe ion. Compared to the monomer, formation of the Fe\(^{3+}\) complex results in a 4-fold increase in the rotational correlation time, owing to the large size of the trimer and its rigid architecture around the Fe\(^{3+}\). The resulting effect on the relaxivity is an increase of 90% compared to the monomer: 12.2 mM\(^{-1}\) s\(^{-1}\) for 68.

A final MRI contrast agent utilizing linked azamacrocycles was aimed at producing a calcium-sensitive probe [70]. This “smart” contrast agent uses a physiological trigger, Ca\(^{2+}\) concentration, to expose the Gd\(^{3+}\) to bulk water, while it is shielded from bulk water in the “silent” state. Ca\(^{2+}\) plays an important role in the nervous system, with fluctuations in concentration associated with normal and abnormal brain activity [71]. The Gd complexes of 69–71 represents an effort to translate the Ca\(^{2+}\) concentrations into changes in MRI contrast.

At low Ca\(^{2+}\) concentrations, some of the linker donor atoms would be bound to Gd, partially shielding Gd from interacting with water. Ca\(^{2+}\) binding would occupy the linker donor atoms, thus freeing Gd for more water interaction, and higher relaxivity.

The proton relaxivities of the Gd complexes with these ligands were studied at various Ca\(^{2+}\) ion concentrations. The relaxivities in the absence of Ca\(^{2+}\) were 6.22 mM\(^{-1}\) s\(^{-1}\) with 69, 8.19 mM\(^{-1}\) s\(^{-1}\) with 70, and 6.05 mM\(^{-1}\) s\(^{-1}\) for 71. While the complexes of 69 and 71 were sensitive to Ca\(^{2+}\) concentration, with the relaxivity increasing 15% and 32%, respectively, the change for the 70 complex only changed 6%. This result was attributed to the longer, more rigid separation of the Gd ions from the Ca\(^{2+}\) binding central group in 70. Such rigidity is reflected in the larger relaxivity of this complex in the absence of Ca\(^{2+}\), but isolates the Gd centers from the effects of Ca\(^{2+}\) binding.

4.1.2. Luminescence

Several of the lanthanide ions having luminescence properties useful to the fields of microscopy and bioassay are emissive in the near-infrared frequencies [72]. They either absorb and emit light outside of the usual range of biomolecules (Nd\(^{3+}\), Er\(^{3+}\), and Yb\(^{3+}\)), or have particularly long luminescent lifetimes (Eu\(^{3+}\) and Tb\(^{3+}\)). Faulkner et al., have studied these properties utilizing various linked azamacrocycle as ligands [21,22,73]. Ligand 72 was complexed to Yb\(^{3+}\). Excitation at 337 nm represented energy transfer from the ligand to the Yb\(^{3+}\), which resulted in a typical ytterbium-centered time-resolved emission spectrum [22]. Since the relative weighting of the short and long components of the decay were the same in both H\(_2\)O and D\(_2\)O, it was concluded that there must be two distinct environments for Yb\(^{3+}\). Values of \( q \) (the number of bound water molecules) were determined to be 0.3 and 1.0 for these two environments. Apparently, the linking phenolate is shared unequally, as might be expected due to the bulky pendant-armed macrocycles preventing the close approach of both metal ions required for bridging by the phenolate oxygen.

An interesting hetero-lanthanide complex was synthesized from 70, in order to study Tb\(^{3+}\)-sensitized Yb\(^{3+}\) luminescence [73]. Tb\(^{3+}\) was complexed to the DOTA macrocycles, and the resulting complexes showed classic Tb\(^{3+}\) emission behavior and indicated that the two Tb\(^{3+}\) binding sites were identical. Subsequent complexation of Yb\(^{3+}\) to the DTPA-like linker gave the mixed lanthanide complex, where the kinetically inert macrocycles maintain their bound Tb\(^{3+}\). The Yb\(^{3+}\) ion appears to have a coordination number of eight, all coming from the DTPA-like linker; \( q \) was calculated at 0.2 demonstrating little water interaction. Excitation of

\[ \begin{align*}
 & 68 \\
 & 69 \quad X = \cdot H_2 \\
 & 70 \quad X = \cdot CaH_2 \\
 & 71 \\
 & 72
\end{align*} \]
the Tb³⁺ excitation band at 488 nm resulted in Yb³⁺ emission at 980 nm, the first use of one lanthanide as an antenna to excite another.

Finally, ligand 65 was complexes to Eu³⁺, Tb³⁺, and Yb³⁺ in order to study their luminescent properties [21]. The aromatic linker, demonstrating similar absorptivity in the free ligand and in the complexes, was used as an antenna to increase the absorption properties of the complexes. The Eu³⁺ emission was clearly sensitized by the aromatic linker, although it was relatively weak due to non-radiative quenching of the LMCT state. Tb³⁺ likewise demonstrated efficient energy transfer, as emission was much stronger when the linker was excited (260 nm) than when the Tb³⁺ itself (366 nm) was excited. The Yb³⁺ complex, unlike the Yb³⁺ complex, demonstrated only one type of coordination environment. In fact, for all three lanthanides, both metal ions appeared to have the same coordination environment in both macrocycles. Interestingly, the lanthanide contraction was observed in the calculated value of q, which decreased from 2 to 1.4 to 0.4 for Eu³⁺, Tb³⁺, and Yb³⁺, respectively.

A structure similar to, but even more complex than 68 was used as a luminescence sensor for the important intracellular signal messenger Inositol 1,4,5-triphosphate (IP₃, 73) [74]. Initially, 75 binding of achiral cis-1,3,5-cyclohexanetriol triphosphate (CTP₃, 74) was studied prior to the more complicated system. Because the Zn²⁺ ions in 75 [74] and the phosphate groups in 74 should both be around 10 Å apart, a C₃-symmetric complex was envisioned. Potentiometric pH titrations revealed that a complex did form with an apparent complexation constant of logK= 8.0. Based on this result, a new 2,2′-bipyridine linked azamacrocycle dimer 76 was synthesized [74] and used to assemble a sensor for IP₃ and CTP₃ (G5) based on the known Ru(bpy)₃ luminescent properties [75].

1:2 complex formation between 77 and 74 was observed by NMR titration and UV spectroscopic methods. Luminescence titrations revealed that addition of 74 to solutions of 77 results in a large increase (4.2-fold) in emission with a 26 nm blue shift. The enhancement is ascribed to conformational restriction of 77 upon 74 binding. Apparent binding constants calculated from potentiometric pH titrations were found to be logK=30.6 for the first CTP₃ to bind, and logK=19.0 for the second. Chiral IP₃ (73) addition (3 equiv.) studied by luminescence titration yielded a 2-fold increase in 77 emission with a similar blue shift. Achiral CTP₃ (74) added to this same solution resulted in further emission enhancement, suggesting that the chiral IP₃ cannot bind all of the stereoisomers of 77 present in solution. This indicates a high degree of enantioselectivity in 77/73 binding.

Another application of luminescent linked azamacrocycle complexes targets the medicinally important dicarboxylates [76]. The dinuclear Tb³⁺ complex of linked azamacrocycle 78 was designed to bind dicarboxylates, one functional group to each Tb, and use the xylene linker as an antenna to excite the Tb³⁺ ions [18]. In water, the Tb₂ complex was characterized as having two bound water molecules per Tb³⁺ ion, potentially labile sites to be replaced by chelating carboxylates.
Excitation of the xylene linker resulted in emission from the Tb\(^{3+}\) ions, although quenching from the bound water molecules rendered the Tb\(^{3+}\) emission weak. Addition of monocarboxylate 79 resulted in concentration dependent enhancement of Tb\(^{3+}\) emission, as 79 replaces the quenching water ligands, and acts as an antenna for Tb\(^{3+}\) excitation. Analysis of the fluorescent changes indicated that the Tb\(^{3+}\) ions were not communicating. Aromatic dicarboxylate 80 increased the emission of the complex 26-fold, with a maximum at 1:1 stoichiometry, indicating a bridging of the two Tb\(^{3+}\) ions by the dicarboxylate.

A range of aliphatic dicarboxylates (81–83) were also tested for binding and luminescence enhancement. The luminescence enhancement for these dicarboxylates was much less than for aromatic 79 and 80, because they cannot act as antenna, leaving only the xylene linker to play this role. 81 formed a 2:1 complex, and is thus apparently too short to bridge both lanthanides. 83 formed a 1:1 complex, having the appropriate length to span both metal ions. Interestingly, 82 caused no changes to the luminescence, indicating the Tb\(_2\) complex is somewhat selective toward dicarboxylate binding.

**4.1.3. Radiolabelling**

Research and clinical applications for macrocycles bound to radioactive isotopes, typically conjugated to antibodies or some other biomolecule, are becoming quite common [77]. Linked azamacrocycles, however, have only contributed to this application in small numbers. Two-ring (84) and three-ring (85) systems specifically designed for bioconjugation for radiolabelling studies have been reported [34,78]. In the initial report, only the syntheses of these linked azamacrocycles was reported. Very recently, [78] details regarding mAbs conjugation, \(^{64}\)Cu interaction, and animal studies have appeared. 2:1 \(^{64}\)Cu\(^{2+}\) complexation of 84a > 95% was achieved at pH 5.0 after heating to 50 °C for 1 h. Even at pH 8.0, 85a could bind only 83% of the possible \(^{64}\)Cu\(^{2+}\) for 3:1 complexation. Once formed the 84a 2:1 complex is quite stable in human serum, although the 85a complex is less so, with loss of 20% of its \(^{64}\)Cu\(^{2+}\) after 24 h incubation. This loss was attributed to the more weakly Cu-binding dioxocyclam central ring. 2:1 complexation of 85a resulted in a much more stable complex, with only 2% loss of the radioisotope.

Conjugation of the 2:1 \(^{64}\)Cu:ligand complex of 85b to B72.3 mAb, which targets the Tag-72 antigen of colorectal and ovarian cancers, was carried out. Studies showed 2.7 ligands (or \(5.5 \times 10^3\) \(^{64}\)Cu\(^{2+}\)) per mAb for this conjugate and the immunoactivity was >80%. Finally, the tris-azamacrocycle 85b mAb conjugate showed levels of accumulated radioactivity at the tumor similar to a mono-macrocycle analogue, but was able to reach this level with only about 70% of the antibody conjugate required, due to the presence of the second \(^{64}\)Cu\(^{2+}\) binding site.

**4.2. Binding DNA and its constituents**

Kimura et al., have produced a number of papers describing linked [Zn(cyclen)]\(_2\)\(^{2+}\) complexes that can efficiently bind biological molecules. Barbital was bound in a 1:1 complex with \(\log K = 5.8\) at pH = 8 to a para-xylyl linked bis Zn(cyclen)\(_2\)\(^{2+}\) complex 88 [81]. The 2:2 complex obtained at higher barbital concentration was actually crystallized and an X-ray crystal structure showed two barbital anions sandwiched between face-to-face 88 complexes. Molecular mechanics calculations confirmed that the 2:2 complex is 73.9 kJ/mol more stable than the 1:1 complex.
The meta analogue 89 was published shortly thereafter, having a barbital binding constant of log $K = 5.6$ at pH $= 8$ [82]. In this paper, it was noted that monomeric versions of 89 bind barbital only in a 1:1 fashion, whereas 89 has two macrocycle complexes (linked) per barbital. This “strong chelation” [82] effect increases the binding constant from log $K = 4.9$ in the monomeric Zn(cyclen)$^{2+}$ analogue and allows for double deprotonation of barbital. Additionally, 4-nitrophenyl phosphate dianion binding was probe using 89 as a potential model of alkaline phosphatase [83], an enzyme having two Zn$^{2+}$ cations at the catalytic center. A 1:1 affinity constant of log $K = 4.0$ was determined, but no phosphatase activity was found.

Inspired by a crystal structure where three Zn(cyclen)$^{2+}$ complexes interact equivalently with the three unsubstituted oxygens of 4-nitrophenyl phosphate, Kimura et al., linked three Zn(cyclen)$^{2+}$ complexes through a mesitylene core (75) [84]. The likely C$_3$-symmetric linked azamacrocycle metal complex host, and its binding to a range of phosphate dianions was probed. Phenyl phosphate (log $K = 6.6$), 4-nitrophenyl phosphate (log $K = 5.8$), α-d-glucose-1-phosphate (log $K = 7.0$), and phenyl phosphonate (log $K = 7.9$) were all found to bind 75. Use of the complex as a model for phosphatases that have three Zn$^{2+}$ ions was also proposed.

Zn(cyclen)$^{2+}$ binds thymidine highly efficiently (log $K = 3.1$) [85], through zinc coordination to the deprotonated imide and cyclen NH to thymidine C$\equiv$O hydrogen bonds (90). This observation has led to a number of studies involving linked azamacrocycle complexes. In an effort to prevent the UV photodamage of nucleic acids, 88 and 89 were studied for their effect on the photo[2 + 2]cycloaddition of thymidyl(3′-5′)thymidine 91 and the photosplitting of the cis-syn cyclobutane thymine dimer 92 [86].

The affinity constants of 88 and 89 towards 91 were found to be identical within experimental error, at log $K = 6.4$ for the 1:1 complexes. This value is about 1000 times greater than the affinity of the monomeric Zn(cyclen)$^{2+}$ 2:1 complex.

Compared to control reactions, 88 and 89 reduced the initial rates of the photodimerization reaction by 70% and 85%, respectively, with both complexes allowing ∼90% of 91 to remain intact at equilibrium. Beginning with pure 92, the reverse reaction was carried out in the presence of 89 and monomeric Zn(cyclen)$^{2+}$, independently. The dimeric complex was more efficient at returning to an equilibrium mixture with 91 very similar to the one achieved in the forward reaction experiments. Finally, poly(dT) was photodimerized in the presence and absence of 89. Not only did 89 reduce the equilibrium amount of photodamage (35% compared to 50% for the control), but addition of 89 to a control reaction at equilibrium returned the sample to lower levels of photodamage, indicating that 89 is actually capable of promoting photodamage repair.

The thymidine dimer (TpT) interaction with 88 was examined in the same publication in which a linearly arranged trimer of Zn(cyclen)$^{2+}$ complexes linked by para-xylyl groups (93) was synthesized and interacted with the thymidine trimer (TpTpT) [87]. UV titration confirmed that 1:1 complexes of TpT with 88 and TpTpT with 93 were formed in aqueous solution. The TpT dimer 88 interaction, from NMR measurements, appears to use the same binding motif as in 90, with log $K = 5.5$ obtained from potentiometric pH titrations. An even more impressive log $K = 6.4$ was determined for the TpTpT 93 complex. Overall 1:1 affinities for 90 ($K_d = 0.079$ nM), TpT 88 ($K_d = 0.63$ μM), and TpTpT 93 ($K_d = 0.80$ nM) species are perhaps most useful for comparison.

Because several anti-HIV reverse transcriptase inhibitors (AZT = 3′-azido-3′-deoxythymidine, ddC = 2′,3′-dideoxyadenosine) [88] must undergo phosphorylation to triphosphate derivatives to be activated inside the cell, lipophilic carrier molecules for the nucleotide phosphates would be medically useful [89]. Combining their knowledge of the binding ability for nucleotides already discussed with the evidence that Zn(cyclen)$^{2+}$ binds dianionic phosphates [84], Kimura et al., decided to evaluate their linked azamacrocycle systems 88 and 89 with respect to a number of thymidine, uridine, and AZT phosphorylated nucleotides [90]. Due to the additional interaction of the phosphate group with the second Zn(cyclen)$^{2+}$ moiety, apparent binding constants with 88 and 89 were up to 1000 times higher than for the same phosphorylated nucleotides with monomeric Zn(cyclen)$^{2+}$, with the para isomer typically slightly better than the meta isomer. The selectivity of these linked azamacrocycle complexes for d(U) nucleotides and the possibility of lipophilic functionality attachment to produce novel drug transport agents was noted [90].
25 °C at concentrations of 4 and 2.5 μM, respectively. Complete denaturation of poly(dA-dT)$_2$ under the same conditions by 88 and 93 occurred at concentrations of 7 μM and 4 μM, respectively. Selective binding of 88, 89, 75, and 93 to poly(dT) was examined using a micrococcal nuclease footprinting assay of a 150-bp DNA fragment. The poly(dT) regions were particularly well protected from hydrolysis, while poly(dA) regions on the complementary strand were made more susceptible to hydrolysis. IC$_{50}$ (inhibitory concentration) values against the nucleasen were found to be much lower (0.5–6 μM) than for the monomeric Zn(cyclen)$_{2}^{2+}$ complex (>100 μM). The most effective compound in this assay (0.5 μM) was 93. Binding constants for 93 were determined as $K = 2.1 \times 10^{10}$ M$^{-1}$ with poly(dA) poly(dT) and $K = 4.7 \times 10^{10}$ M$^{-1}$ with poly(dA-dT)$_2$. These values suggest that 93 binds to the poly(dT) sequence in double-stranded DNA as strongly as the known minor groove binder Distamycin A [92].

4.3. Catalysis

4.3.1. Ester cleavage

Tren-based linked azamacrocycle complex trimers [93] have been used to mimic the active site of multinuclear metalloenzymes, such as P1 nucleases, which use three metal ions to cleave nucleotide phosphate esters [94]. The ligands 94 and 95 are C$_3$-symmetric, like 75, but are more flexible due to the tren-based linker compared to the aromatic core of 75. The Zn$^{2+}$ complexes of 94 and 95 were examined as hydrolytic agents using the activated ester 96 and the activated phosphate ester 97 [93a]. Hydrolysis of ester 96 was pH dependent, with little activity at low pH due to hydroxido ligands bridging between Zn$^{2+}$ ions. At higher pH's when bridging is not observed, hydrolysis increases. The 94 complex exhibits a second order rate constant of $k' = 4.2 M^{-1} s^{-1}$ compared to the 95 complex value of $k' = 3.7 M^{-1} s^{-1}$. Both of the values are much higher than the mononuclear Zn(cyclen)$^{2+}$ rate constant $k' = 0.11 M^{-1} s^{-1}$ [95]. The authors observe that “…the rate constants increase with the pK$_a$ value, i.e. with the nucleophilicity of the Zn-OH functions” [93a]. The Zn$^{2+}$ ions do not appear to cooperate, and the typical bimolecular mechanism is observed.

Phosphate ester hydrolysis using 97 follows similar pH dependence, but the rate is much enhanced. Five- and 14-fold rate enhancements are observed for the zinc complexes of 94 ($k = 1.1 \times 10^{4} M^{-1} s^{-1}$) and 95 ($k = 3.1 \times 10^{4} M^{-1} s^{-1}$), respectively, vs. the mononuclear Zn(cyclen)$^{2+}$ rate constant $k' = 0.21 \times 10^{4} M^{-1} s^{-1}$ [95]. This result suggest the metal centers are cooperating in this case. A mechanism is proposed by which the substrate phosphate bridges two of the Zn$^{2+}$ cations prior to hydrolytic attack from one of the bound hydroxido groups. Evidence for this mechanism is found in that the hydrolytic ability decreases as the pK$_a$ of the complex increases. This is the opposite behavior as found for the ester case, suggesting an associative cleavage mechanism. The trinuclear Cu$^{2+}$ complex of 94 was prepared and tested for its effect on 97 hydrolysis, as well [93b]. A pseudo-first order rate constant for the Cu$_{2}$ 94 complex of $k = 3.5 \times 10^{6} s^{-1}$ was determined, which was declared “not particularly efficient” [93b]. Apparently, this complex does not form the desired hydroxo complexes, which are often the active species in 97 hydrolysis.

![Diagram of 98](image)

Cleavage of phosphodiesterse and other RNA analogues by the di-zinc complex of 98 has been thoroughly studied in recent years [29,96]. Initial studies [96a] with 99 as the substrate resulted in the calculation of an observed $k_{cat} = 4.1 \times 10^{-3} s^{-1}$ at pH = 7.6 which makes this complex one of the most active dinuclear metal ion phosphodiester cleavage catalysts identified. Mechanistic insight gained by this study included the likely direct proton transfer from the C2-hydroxyl of the substrate to the catalyst, and the cooperative role of the two metal ions in stabilizing the transition state for the catalyzed reaction. Cleavage by Zn$_{2}$ 98 of an oligoribonucleotide made of six adenosines resulted in 90% cleavage after 24 h [29], proving that the catalyst is not effective only on the activated 99 ester.

Comparison of the cleavage of “minimal” substrate 99 to a larger nucleoside substrate 100 showed that the catalyst was 50 times faster at catalyzing the hydrolysis of smaller 99 than of larger 100 [96b]. Hydroxide ion catalyzed cleavage of 100 is 10,000 faster than for 99, due to several cumulative structural effects. The surprising decrease in activity for Zn$_{2}$ 98 was ascribed to steric effects that potentially restrict access to the catalytic core of Zn$_{2}$ 98.

Changing the metal ions complexed to 98 from Zn$^{2+}$ to Cu$^{2+}$ and Cd$^{2+}$ was carried out, along with an analysis of how this affected the catalytic nature of the resulting complexes [96c]. Cd$^{2+}$ appears to prefer to make a mononuclear complex with 98 until the pH is raised to above pH = 10, with only the dinuclear complex having significant activity towards 99 cleavage. However, once formed at high pH, the Cd$_{2}$ 98 complex is about 5 times more active than the Zn$_{2}$ 98 complex, possibly due only to the higher basicity of the Cd$^{2+}$ bound hydroxide ion. Another possible explanation is that the larger Cd$^{2+}$ ions are more sterically available and, thus more reactive, than in the Zn complex. The dinuclear Cu$_{2}$ 98 complex is a poor catalyst (165 times slower than Zn$_{2}$ 98), most likely due to a lack of coordination sites for the substrate and the required hydroxide ligand, as Cu$^{2+}$ is five coordinate in the complex according to X-ray crystal structure data [97].

Solvent deuterium isotope effects were explored for Zn$_{2}$ 98 catalysis of 100 [96d], since a concerted movement of a proton at the rate-determining transition state is signaled by faster cleavage of phosphodiesterse in H$_{2}$O than in D$_{2}$O [98]. The possible kinetic primary solvent deuterium isotope effect was not observed. Con-
clusions from the study were that there is no movement of the proton at the rate-determining transition state, and therefore, no significant transition state stabilization due to concerted proton transfer. The major implication for the catalyst mechanism is that the observed rate acceleration must be due primarily to interaction of the dianionic transition state with the two metal dications. The authors conclude: “These results illustrate the power of simple electrostatics in small molecule catalysis of phosphate diester cleavage...”

Extension of the study of Zn₉₈ phosphate diester cleavage was finally extended to a ribonucleotide substrate 101 that would have the same leaving group as RNA [96e]. It was conjectured that an enhanced rate acceleration might be observed, due to a likely stabilization of the strongly basic anion leaving group by the densely charged catalyst. In previously studied 99 and 100, the leaving group is a weakly basic 4-nitrophenoxide anion. In fact, this altered electrostatic interaction was observed, and was characterized as a 2.1 kcal/mol greater stabilization of the transition state for 101 cleavage, than for 100 cleavage. However, the desired rate enhancement was not realized, as the stronger transition state interaction apparently slows the overall catalytic rate.

A number or linked azamacrocycle zinc complexes using 1,5,9-triazacyclododecane have been evaluated as phosphate diester catalysts [43]. Monomer Zn[1,5,9-triazacyclododecane]²⁺ cleavage of 101 is only enhanced slightly by the dimer complexes 102–107. However, the trimer complex 107 accelerates this cleavage rate to 50 times that of its dimeric analogue 103. The proposed explanation for this observation is that the dimeric complexes coordinate their Zn²⁺ ions to each of the uracil bases of 101 in a similar manner to thymidine binding to Zn(cyclen)²⁺ 90. The central phosphate ester is not, therefore, directly engaged by either metal ion for cleavage. However, the trimer complex 107 can bind the two uracil bases and still interact with the phosphate ester for cleavage with the third Zn²⁺ complex. If either of the uracil bases of 101 is replaced by adenine, which cannot bind efficiently to the Zn[1,5,9-triazacyclododecane]²⁺ complex, cleavage is up to 100 times faster with the dimer complexes of 102–106 than cleavage of 101. Now, strongly binding one Zn²⁺ complex to the lone uracil helps place the other linked Zn²⁺ complex in position to effectively cleave the phosphate ester.

The effect of reduced solvent polarity on the cleavage rate of 99 by the zinc complex of a linked azamacrocyclic has recently been probed [99]. The catalyst examined uses the same macrocycle as 102–107, but only a propylene linker (108). Enzyme active sites may be considered “non-aqueous” [100]. Thus, enhancing electrostatic interactions between substrate and catalyst by studying the reaction in a solvent like methanol may lead to enhanced reaction rates. Indeed, acceleration of the reaction rate in methanol for the monomer Zn[1,5,9-triazacyclododecane]²⁺ by 1000 times the analogous aqueous rate was observed, which is also 6500 faster than methoxide alone. A truly remarkable rate enhancement is observed with the dimer complex of 108. The rate is now 1.1 × 10⁸ times faster than methoxide alone and several orders of magnitude faster than any previously reported catalytic cleavage of 99 [99b]. Strikingly, the reactivity of Zn[108] in water is not any greater than monomeric Zn[1,5,9-triazacyclododecane]²⁺ [101], demonstrating that solvent effects are indeed important.

Recent experiments with the same catalyst cleaving DNA model methyl p-nitrophenyl phosphate in aqueous ethanol [102] shows rate acceleration of this reaction (a factor 1.6 × 10⁷ relative to background OH⁻ promoted reaction). This work demonstrates that control of solvent in addition to the use of linked azamacrocycle catalysts can produce enzyme-like catalytic rates.

Another linked azamacrocycle used to produce hydrolytic catalyst complexes is 109 [103]. Cleavage of substrate 97 to produce 4-nitrophenolphosphate was observed by NMR for the Zn²⁺ complex. Copper(II), nickel(II), and zinc(II) complexes were all then evaluated spectrophotometrically for 97 cleavage kinetics. Interestingly, the Ni²⁺ complex was about 50 times more active than Zn²⁺, and the Cu²⁺ complex did not show any rate enhancement of the reaction. It was speculated that perhaps not enough coordination sites are available in the dicopper complex for catalysis to occur. The larger rate enhancement for the Ni²⁺ complex was explained by the fact that a OH⁻ coordinated to Ni²⁺ is more basic than when bound to Zn²⁺, a fact that agreed with the proposed catalytic mechanism.
exhibits a structure where the two Zn^{2+} ions are coordinated to only the three secondary nitrogens of each cyclen and are bridged by a OH− ion, which may be an active participant in ester hydrolysis. All Zn^{2+} complexes enhance the hydrolysis of carboxylic ester under physiological conditions [104a]. Under the studied conditions, Zn(cyclen)^{2+} exhibited a rate constant for 96 hydrolysis of kcat = 106 M−1 s−1. The monohydroxo species of di- and tri-pyridyl linked complexes of 113 and 114 gave kcat values of 7170 and 7860 M−1 s−1, respectively. The shorter linked ligands 110–112 performed somewhat better, with kcat values of 41,000, 31,000, and 44,000 M−1 s−1, respectively. These latter values are very similar to the activity of tren-based Zn(II), which had a kcat value of 42,000 M−1 s−1 [93]. However, it was pointed out that these complexes perform somewhat better at physiological pH than does Zn(II). The crown-ether functionality of 111 appears to impart some steric crowding, which may explain the somewhat smaller activity.

Phosphodiester cleavage of these complexes was evaluated using 97 as the substrate [104b]. At pH = 8, the second order rate constants for the Zn2+ complexes of 110, 111, and 113 were found to be 1.28 × 10^{-3}, 2.45 × 10^{-3}, and 0.244 × 10^{-3} M−1 s−1, respectively. These values are 100–1000 times faster than the reactions catalyzed by the monomeric analogues [104b]. The longer linker of 113 appears to be disadvantageous to phosphate ester cleavage, as it was in carboxylic ester cleavage (vide supra). The 110 and 111 catalysts are 1–2 orders of magnitude more active than the previously discussed catalyst Zn(II) at pH = 8 [105], making these complexes better suited for phosphate ester hydrolysis under physiological conditions.

4.3.2. Other catalytic reactions

Catalytic disproportionation of H2O2 was the goal behind the synthesis of a series of linked triazacyclononanes having 2-pyridylmethyl pendant arms [106]. A range of linking groups was utilized to produce ligands 115–119 for Mn2+ complexation. These ligands increase the denticity of the triazacyclononanes for better complex stability, and the pyridine donors might be expected to help stabilize higher oxidation states of Mn. X-ray crystal structures of the Mn2+ complexes of 115–117, as well as the monomeric ligand analog, were obtained, and all showed pentacoordination of the pendant-armed macrocycle plus a single chloride ligand. Addition of excess H2O2 to DMF solutions of these complexes resulted in color changes, as well as the evolution of dioxygen. Coordinative saturation was expected to preclude this type of reactivity, as most Mn catalysts of this reaction were known to be μ-oxo dimers of tridentate ligands [107]. Electrochemical and EPR experiments suggested that the complex of ligand 119 likely forms an endogenous propoxide bridge, which makes its electrochemical behavior unique. The 115 complex alone exhibited EPR behavior most likely due to dissociation of some N-donors and bridging by exogenous O donors. Unfortunately, precipitation of MnO2 during the H2O2 reaction with all complexes indicated that the complexes decompose during the catalytic process.

A linked cyclam ligand (120) using the same 2-hydroxypropane linker as 98 and 119 has recently been used to make manganese and iron complexes also studied as hydrogen peroxide disproportionation catalysts [27]. X-ray crystal structures with both Mn and Fe show the propoxide oxygen acting as a bridging ligand between cyclam bound metal ions. EPR measurements in an acetonitrile water solvent mixture confirm that the Mn2+ ions are still bridged in an aqueous environment. A turnover number in the reaction of the Mn2+ complex with H2O2 in water of 20,000 was reported, and consistent catalase activity is reported for over an hour after H2O2 addition. The cyclam macrocycles appear better able than 115–119 to stabilize the likely high oxidation state manganese ions produced during the catalytic cycle without decomposition. The iron complex of 120 was not an active catalyst for H2O2 decomposition. Efforts to isolate higher oxidation state complexes of both metal ions are ongoing.

Ni(cyclam)^{2+}’s success in the catalytic reduction of CO2 to CO [108] spurred the study of a number of linked azamacrocycle systems (121–129) for similar reactivity [109]. The presence of two nickel ions was hoped to result in the reduction of CO2 beyond CO. The catalytic reduction of CO2 with the surface-confined dinickel complexes of ligands 121–129 was evaluated using a mercury-coated silver electrode under CO2 gas. Interestingly, the dinuclear pentaza complexes’ catalytic currents were only about half of the value for a mononuclear analogue. The conclusion from this result is that only one nickel site per dimer is adsorbing on the electrode, and is thus able to maintain catalytic activity. The more rigid, planar 125 complex had the largest catalytic current, likely because its structure most favored both nickel centers achieving planarity on the electrode.

The catalytic current for the complexes of 121–124 increased with the length of the linker, which was attributed to greater flexibility allowing both nickel sites better interaction with the electrode surface. Bulk CO2 electrolysis and product analysis was carried out only on the complex of 123. CO2 and H2 were the major products detected. After about 10% of the available CO2 was reduced to CO, changes in current and product distribution corresponded with deactivation of the catalyst due to nickel deposition.

The dinickel complex of 126 was a poor catalyst for CO2 reduction having a catalytic current about 15% of the pentaaza complexes, perhaps due to the additional steric hindrance. The tetraazaazacyclononane fragment of ligands 127–129 occupies the...
same space around Ni$^{2+}$ as ethylenediamine. Therefore, these ligands can be thought of as acting as 13-membered macrocycles around Ni$^{2+}$. These sterically hindered 127–129 complexes were also relatively poor catalysts, with catalytic currents only about 10% of the pentaaza complexes. As was the case for 121–124, the catalytic current did increase with chain length (i.e. flexibility) for the complexes of 127–129.

### 4.4. CXCR4 antagonists

Due to the important place CXCR4 antagonist linked azamacrocycles and their metal complexes hold in the medicinal chemistry of several diseases [44–55] (see Section 3.1 above) comprehensive reviews devoted to these medicinal applications are periodically published [44]. Reviews detailing the early development of the organic molecules [44b, 110], discovery and exploration of the effect of metal complexation [111], and recent state of the art [44], cover this broad area of active chemical and biological research so well as not to need replication here. Instead, we have chosen to focus on only the most recent findings regarding linked azamacrocyclic metal complexes and their interaction with the CXCR4 receptor. In particular, the recent work on linked bridged azamacrocycle complexes published by one of us in collaboration with Archibald and De Clercq will be highlighted.

Bridge, Sadler, and De Clercq were instrumental in defining the importance of metal ion binding to linked azamacrocycles like AMD3100 (45). Metal complexes of 45 were first tested against HIV in 1999, when an order of activity of Zn$^{2+}$ > Ni$^{2+}$ > Cu$^{2+}$ > Co$^{2+}$ > Pd$^{2+}$ was identified [51c]. Zn(cyclam)$^{2+}$ and Zn$_2$45 complexes studied by X-ray crystallography and NMR have revealed that multiple configurations of the macrocycle around Zn$^{2+}$ are possible, and likely in equilibrium in aqueous solution [112]. Acetate binding [112], as a model for aspartate in CXCR4 [49] (see Section 3.1) results in the increase in concentration of the cis-V configuration, as chelation of the carboxylate group likely occurs. An X-ray crystal structure of Zn$_2$45(OAc)$_n$ shows this configuration for both metal centers [112a].

There is evidence that interaction through only one metal ion to Asp262 for 45 complexes is sufficient to explain the enhanced binding over 45 alone [113]. The nature of the binding interaction with Asp171 is not as clear, although nothing has ruled out carboxylate coordination by the second metal ion of 45 complexes. CXCR4 docking studies employing configurations for the two Zn-cyclam complexes of Zn$_2$45 of cis-V/trans-I [112a] and trans-III/trans-III [114] have been independently performed, both of which strongly suggest metal coordination of both metal ions is important.

Our recent work in this area has centered on the synthesis and evaluation of the CXCR4 binding properties of a series of configurationally restricted analogues of 45. If metal ion coordination to the aspartate residues is important, the configuration of the macrocycle around the metal ion is critical as well (Fig. 4). Controlling the configuration could easily improve the binding efficiency of potential drug molecules, by pre-organizing the complex into the preferred binding geometry prior to interaction with the aspartate carboxylates. A strategy to select for specific configurations is to add an additional ethylene bridge between nitrogen atoms of the cyclam rings of 45, as in 130 (side-bridged) and 131 (cross-bridged). The side-bridged cyclam complexes adopts only the trans-II configuration when the macrocycle is mono-alkylated [13a, 115]. The cross-bridged cyclam is topologically constrained to the cis-V configuration in its transition metal complexes [13b, 116]. Linked bridged azamacrocycles 132 and 133 have been prepared, and their Cu$^{2+}$ and Zn$^{2+}$ complexes tested for CXCR4 binding ability and anti-HIV activity [13, 179, 115].

Our initial report [13a] focused on Zn$_2$132, its crystal structure with bound acetate anions, and its anti-HIV activity. In the solid state, both side-bridged cyclams are in trans-II configurations around Zn$^{2+}$ with the acetate ligands bound to each Zn$^{2+}$ in an anisobidentate fashion. High-field NMR studies showed only one configuration in aqueous solution, as well, which differs from the Zn$_2$45 complex, which shows multiple configurations in equilibrium [112a]. The Zn$_2$132 complex was shown to bind CXCR4 by flow cytometry displacement studies with an anti-CXCR4 antibody. In identical studies for infection of HIV-1(IIIb) in MT4 cells, the EC$_{50}$ (average effective concentration) of Zn$_2$132, 45, and Zn$_2$45 were determined as 0.0025, 0.011, and 0.008 μM, respectively. Clearly, the trans-II restricted Zn$_2$132 is at least as active as the configurationally unrestricted Zn$_2$45 complex, reflecting the relevance of configurational restriction to the optimization of CXCR4 binding. The Cu$_2$132 complex was found to be likewise more active than its Cu$_2$45 counterpart, with EC$_{50}$ values measured as 0.026 and 0.047 μM, respectively [115a]. As previously observed for 45 complexes [51c], the Cu$^{2+}$ was not as active against HIV as the Zn$^{2+}$ complex, in this case by about an order of magnitude.

In our most recent work, we have demonstrated that Cu$^{2+}$ complexes can, in fact, be as active against HIV as Zn$^{2+}$ complexes [13b]. Cu$_2$133 did not yield usable crystals, but a monomeric analogue using 131 having one methyl and one benzyl R group, did. The complex Cu$_2$131(OAc)$_2$ has a distorted square pyramidal geometry around a Cu$^{2+}$ ion bound to all four nitrogens of the macrocycle and...
only one of the acetate oxygens. The azamacrocycle is forced into a cis-V configuration by the ethylene bridge. Importantly, the bound acetate oxygen is forming a shorter equatorial bond rather than the longer axial bond that would be available in the most common configuration for Cu(cyclam) complexes: trans-III. If the same structure is present for the 133 Cu(II) complex, binding to aspartate in CXCR4 (modeled by acetate here) would be expected to be shorter and stronger than in an unbridged ligand complex, such as 45.

![Diagram](image)

Evidence of this stronger binding is found in the EC_{50} value of the Cu_{6} complex: 0.0042 μM. This value is nearly as good as the Zn_{6} complex (0.0025 μM) and better than the Zn_{6} complex (0.008 μM). Additional evidence of strong binding to CXCR4 was provided by flow cytometry displacement studies with an anti-CXCR4 antibody over the period of 4 days, after only one dose of the CXCR4 antagonist. Cu_{6} retained a significant amount of antibody binding inhibition 48 h after dosing, whereas 45 and Cu_{6} showed no inhibition after 48 h.

5. Conclusions

This review of recent syntheses and applications demonstrates that the future of linked azamacrocycles is bright. New methods to make these interesting molecules have been found, leading to a large number of new systems. Important chemical, biological, and medicinal problems have been assaulted using linked azamacrocycles and their metal complexes as useful tools, with significant advances made in a number of these areas. It is our perspective that these trends will continue and even intensify, with linked azamacrocycles assuming an ever more important role in chemistry.

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