Dramatic selectivity differences in the association of DNA and RNA models with new ethylene- and propylene diamine derivatives and their copper complexes†

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The affinities of polyamines consisting of ethylenediamine units equipped with either one or two terminal naphthyl-, anthryl-, or acridyl units towards PolyA.PolyU as an RNA model, and Poly(dA).Poly(dT) as a DNA model are screened by measuring the melting point changes (ΔT_m) of the double strands, and also partially by a fluorimetric binding assay using ethidium bromide. The larger aromatic moieties with long spacers between them allow bisintercalation; this leads to an increased preference for DNA in comparison to RNA, where ion pairing of the ammonium centers with the major RNA groove phosphates dominates. Allosteric affinity control by metalation is achieved *e.g.* with Cu^{2+} ions, which induce conformational distortions within the chains. With anthryl- in contrast to naphthyl derivatives intercalation can be so strong that distortion of the ethylenediamine chain by metalation is not powerful enough. A particularly high concentration of positive charges is accessible with tripodal derivatives built up from ethylenediamine and propylenediamine units; in the absence of aryl parts, which interfere with the RNA groove preference, one observes the highest affinity difference known until today, reflected in a melting point ratio of $\Delta T_{m(RNA)}/\Delta T_{m(DNA)} = 40$, whereas other synthetic ligands reach only a $\Delta T_{m(RNA)}/\Delta T_{m(DNA)}$ ratio of about 3.

Introduction

In view of the increasing demand for targeting retroviruses, RNA-selective ligands, which will interact less with DNA, are of particular interest.[1–3] Earlier investigations have already shown that considerable affinity and also selectivity of cationic ligand binding to nucleic acids can be achieved with relatively simple organic compounds. The minor groove of DNA has been found to be the binding site of *e.g.* many cationic amidine or aromatic diamidine derivatives.[4] Systematic studies of polyamines and their comparison to natural antibiotics help to understand the relevant interaction mechanisms and thus the rational design of new drugs. The combination of ethylene- or propylenediamine chains and aromatic residues at the terminal positions (Scheme 1) offers several new features with respect to interactions with nucleic acids.[5] The high charge density at the closely positioned protonated nitrogen centers leads to high affinities, and the possible intercalation of the terminal aryl moieties can lead to mono- and eventually to bisintercalation.

This, and in addition the complexation of the ene chelating units with *e.g.* Cu^{2+} ions can lead to promising affinities and selectivities towards RNA and DNA.

Association of polyamines with nucleic acids is primarily due to salt bridges with the groove phosphates; for series of structurally related compounds differing essentially in the number of positively charged nitrogen centers, the affinities correlate directly with that number (Fig. B2 in ref. 6). With more structural variety the correlation is less linear, but still shows a similar sensitivity (slope, Fig. 2 in ref. 7). That ion pairing and not the frequently quoted hydrogen bonding dominate the binding is evident from the affinities measured with peralkylated polyamine derivatives.[8] For most ligands, including aminoglycosides, both the affinity and the distinct selectivity for RNA increases with the number of positive charges.[1] The preference for RNA is in line with binding to the deep major groove of the RNA double helix, which is also the location for several aminoglycosides.[1–3,9]

Results and discussion

As a measure of the affinity and selectivity we use the difference ΔT in melting points induced by the ligands with double stranded PolyA.PolyU as an RNA model, and with Poly(dA).Poly(dT) as a DNA model.[10] In some cases a spectroscopic affinity assay[11] was also used as a value C_{50}, which is the concentration necessary for lowering the ethidium bromide EB fluorescence emission to 50% of the original value. Fig. 1 (and Fig. S1 in ESI†) shows that as long as the underlying ligand structures are similar the two affinity measures indeed correlate well, with quite similar sensitivities (correlation line slopes and abscissas) for the RNA and DNA models as well as for another series of ligands with calf-thymus DNA. The correlations break down if there are large structural differences between the ligands, as seen for the deviations using

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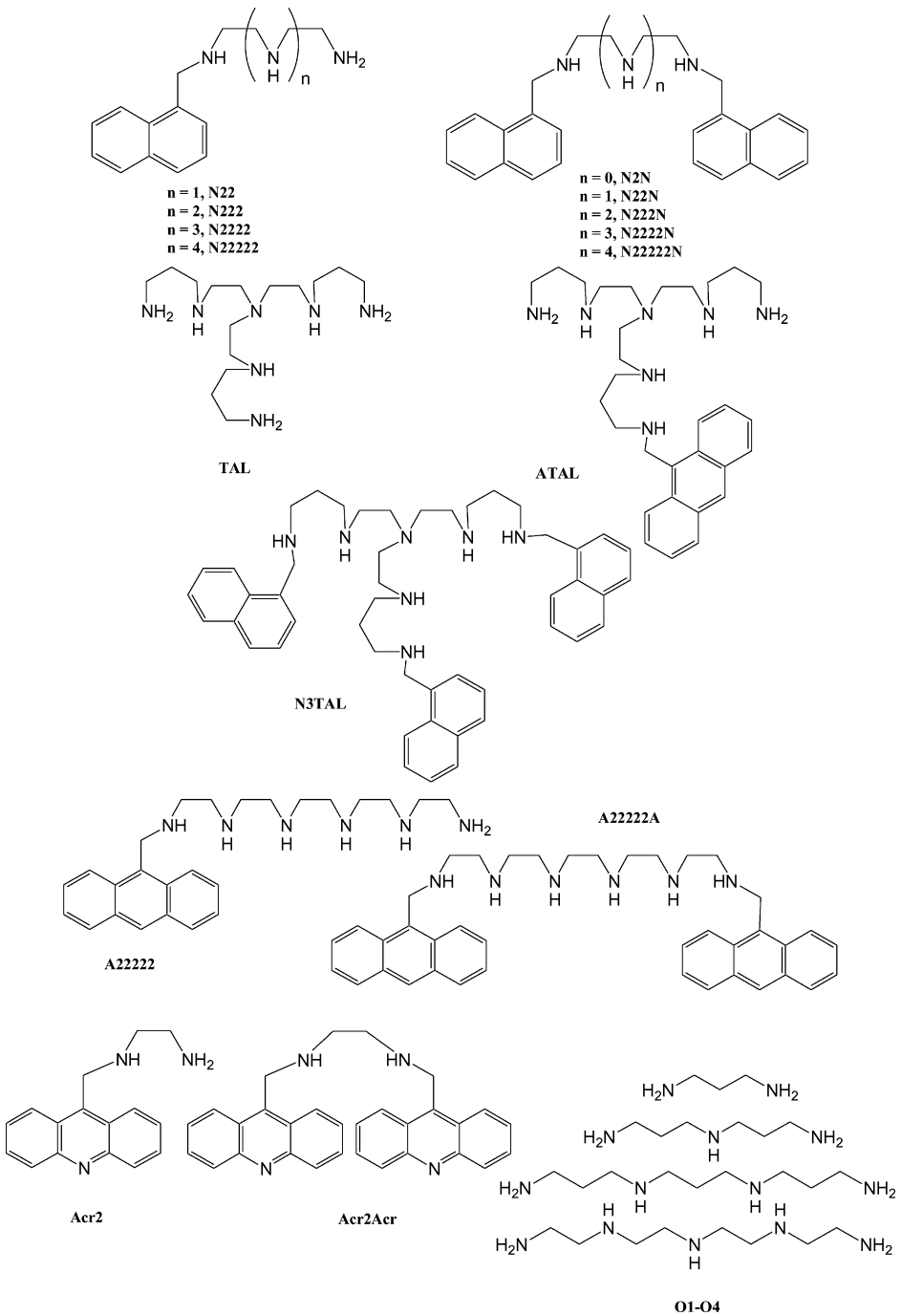


Scheme 1 Polyamine structures used in the present investigation.

tripodal compounds (**TAL**, **ATAL** or **N3TAL**). New methods such as Surface Plasmon Resonance (SPR) could provide a more direct affinity measure;[12] the ΔT values, however, generally do reflect the affinity differences. Biphasic melting profiles sometimes disturb the evaluation, particularly at lower molar ratios r of ligand to nucleic acid where there is still melting of unoccupied double strands.[10] Potentiometric measurements[5] have shown that at the physiological conditions used for the nucleic acid studies the linear polyamines **N*n*N** (**N2N**–**N22222N** in Scheme 1) bear between 2.0 and 3.8 protons, with the tripodal compounds (**TAL**, **ATAL** and **N3TAL**) bearing over 5 positive charges.

The presence of organic, in particular of aromatic, linkers between the charge centers lowers or even inverts the selectivity between RNA and DNA models due to less favorable interactions with the rather narrow and hydrophilic RNA groove.[1] With aromatic macrocyclic amines large variations have been observed usually favoring RNA.[7,10,13] Introduction of bulky substituents favors binding to the wider DNA groove.[14] The terminally substituted bis(naphthylmethyl) polyamines (**N*n*N** compounds) discussed in the present paper have offered for the first time a method of allosteric control of the affinity and selectivity towards nucleic acids by the metalation of ligands containing

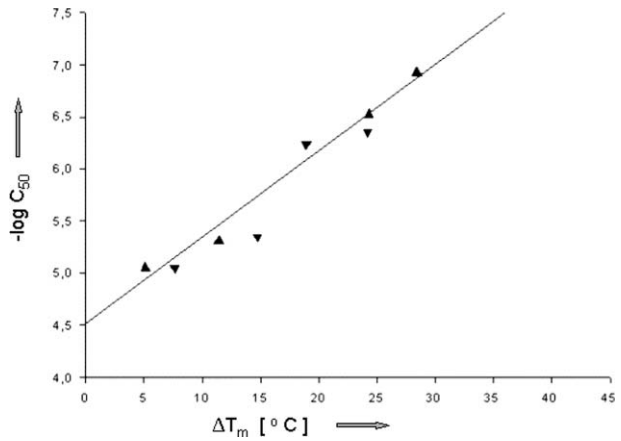


Fig. 1 Correlation between affinity assay ($-\log C_{50}$) and melting points from **N22222N**, **N22222N**, **N222N**, **N222N** with Poly(dA).Poly(dT) (▼) and PolyA.PolyU (▲). Slope $a = 0.0845 \pm 10\%$; abscissa $y_0 = 4.43 \pm 3.5\%$; linear correlation coefficient $r = 0.9711$.

diamine units.[15] Only those polyamines in which two intercalative aromatic moieties such as the α,ω-dinaphthyl units in the **N*n*N** derivatives are separated by at least 12 atoms can undergo bisintercalation into double strands.[16] The bisintercalation can be disrupted by the conformational distortion of the spacer which is induced by the addition of *e.g.* Cu^{2+} ions.[15] This allosteric effect is seen in the large differences in the melting points, which is visible at different ligand to nucleobase ratios *r*.

That the Cu^{2+} ions interact not only with the amine ligands but also with the nucleic acid phosphates is in line with the formation of binuclear complexes.[5] Also, that the allosteric effect reaches a maximum if two Cu^{2+} ions are present per ligand instead of one [15] is in gratifying accord with the observation of ternary Cu_2H_nL(AMP) complexes above pH 6 with **N22222N**.[5] In contrast, although binuclear Cu_2H_nL(AMP) complexes are also found for tripodal ligands such as **TAL**, **ATAL** or **N3TAL** (see ref. 5), the allosteric effect with these ligands is nearly the same with one or two Cu^{2+} ions per ligand. This is not unexpected as coordination of the second Cu^{2+} metal ion can occur almost independently in a different arm of the tripodal ligand, and as, in view of the increased number of positive charges, the affinity of the tripodal ligands is so high that conformational distortion by metal complexation will play a lesser role. On the other hand, with the tripodal amines there are quite distinct differences in the effect of added metal ions between RNA and DNA: with Poly(dA).Poly(dT) Cu^{2+} addition leads to a stabilization of about $\Delta T_m = 4$ °C, whereas with PolyA.PolyU a destabilization with $\Delta T_m =$ about -10 °C is observed (Table 1). The presence of an additional anthryl unit A in **ATAL** leads to even larger effects: with the RNA model the copper salt initiates a $\Delta T_m = -18$ °C destabilization (with Poly(dA).Poly(dT) as a DNA model and **ATAL** alone there are two transitions which makes the identification of the Cu^{2+} effects less clear).

As a preliminary check for intercalation one can use the NMR signals of the aromatic ligand, which due to the anisotropy effects exerted by stacking nucleobases show typical shielding effects and line broadening effects.[17,18] Addition of the ligands to calf-thymus DNA in the concentration range needed for NMR measurements lead in most cases to precipitation, but with **N22** upfield shifts of up to 0.15 ppm, and line broadening by up to 15 Hz could be measured, which indicated intercalation. Earlier analyses with many aryl derivatives of differing sizes has shown that starting with indole-shaped systems weak intercalation is possible, provided that association to the double strands is supported by additional positive charges in the side chain.[19] Intercalation by the naphthyl derivatives is therefore expected; the measurements with **N22**, however, had to be done with rather high concentrations of the ligand. In consequence the shielding and line width effects are diminished by the presence of unbound ligand, which exchanges rapidly on the NMR time-scale.

The attachment of aromatic moieties at the terminal positions of polyamines is also a way of altering the RNA/DNA selectivity, as apparent from the results in Table 2. The affinity can dramatically increase by intercalation of the additional aryl units, but interestingly this is only so with DNA. The explanation is again the high negative charge density in the deep major RNA groove; here the presence of aryl groups is more of a disadvantage. The tight contact between the RNA groove phosphates and the ammonium centers limits flexibility and draws the ligand more into the groove; in consequence the opportunity for intercalation is diminished. The

Table 1 Melting point changes ΔT_m and C_{50} values of Poly(dA).Poly(dT) and PolyA.PolyU with the amines **TAL**, **ATAL**, **N3TAL** and their copper complexes (L–Cu^{2+} 1 : 1 and 1 : 2)[a]

		ΔT_m/°C Poly(dA).Poly(dT)			ΔT_m/°C PolyA.PolyU		
Ligand	r^b and C_{50}	L	L–Cu^{2+} 1 : 1[c]	L–Cu^{2+} 1 : 2	L	L–Cu^{2+} 1 : 1[c]	L–Cu^{2+} 1 : 2
TAL $n \approx 5$–6[d]	0.1	0.9	3.0	4.4	30.9	21.7	22.5
	0.2	1.4	4.8	4.9	38.9	30.4	31.0
	0.3	1.4	6.8	7.3	43.2	39.7	38.6
	C_{50}	1.6×10^{-6}			5.3×10^{-7}		
ATAL $n \approx 5$[d]	0.1	6.8/26.6	18.0	16.5	30.3	12.5	13.1
	0.2	28.5	21.6	19.0	36.5	18.2	18.3
	0.3	33.8	27.8	28.2	38.5	19.4	18.9
	C_{50}	1.3×10^{-7}			1.4×10^{-7}		
N3TAL[e] $n \approx 5$[d]	0.1	1.8/6.5	—[f]	—[f]	1.5/15.3	—[f]	—[f]
	0.2	9.2	—	—	22.5	—	—
	0.3	11.5	—	—	26.1	—	—

[a] Conditions: 0.01 M MES buffer, pH 6.25; error in $\Delta T_m = \pm 0.5$ °C; biphasic profiles where two ΔT_m values are given. [b] r = molar ratio ligand/nucleic acid phosphate. [c] Effect of Cu^{2+} alone: 1) $r = 0.1$, $\Delta T_m = 3.5$; 2) $r = 0.2$, $\Delta T_m = 4.8$; 3) $r = 0.3$, $\Delta T_m = 6.8$. [d] Number of positive charges LH_n. [e] Measured in 3% DMSO. [f] Precipitation.

Table 2 Melting point studies[a] using Poly(dA).Poly(dT), PolyA.PolyU, amine ligands and their copper complexes

		ΔT_m/°C Poly(dA).Poly(dT)			ΔT_m/°C PolyA.PolyU		
Ligand	r[b]	L	L–Cu^{2+} 1 : 2[c]	Parent amine **O1**–**O4**	L	L–Cu^{2+} 1 : 2[c]	Parent amine **O1**–**O4**
N22222N	0.1	24.2	12.8	—	24.3	7.0	—
	0.2	23.4[d]	10.8	—	27.1	10.4	—
	0.3	22.4[d]	8.4	—	26.8[d]	9.3[d]	—
N2222N	0.1	26.7	8.1	2.9	15.5	−0.2	29.9
	0.2	27.0[d]	6.7	10.7	18.3	1.3	36.7
	0.3	27.4[d]	6.4	13.1	17.2[d]	1.5	39.5
N222N	0.1	16.7	10.4	17.3	5.1	2.7	39.8
	0.2	19.6	16.4	>50	8.1	7.5	>55
	0.3	20.0	18.6	>50	7.3[d]	7.9	[f]
N22N	0.1	10.7	7.7	8.4	1.0	0.4	26.4
	0.2	13.2	10.4	14.9	1.5	0.8	35.8
	0.3	16.7	13.2	17.4	3.3	1.5 & 3.4	41.1
N2N	0.1	3.8[e]	[f]	0.4	0.1[e]	[f]	1.5
	0.2	4.8[e]	[f]	1.1	0.1[e]	[f]	2.2
	0.3	5.3[e]	[f]	1.5	0.2[e]	[f]	3.1
N22	0.1	9.1	3.5 & 5.2	—	1.3	−0.5	—
	0.2	11.2	7.0	—	1.4	−0.2	—
	0.3	12.3	8.3	—	2.1	1.5	—

[a] Parent amines **O1** to **O4**: structures without aryl substituents at terminal nitrogen atoms; conditions: 0.01 M MES buffer, pH 6.25; error in $\Delta T_m = \pm$ 0.5 °C; ΔT_m only approximate. [b] r = molar ratio ligand/nucleic acid phosphate. [c] Effect of Cu^{2+} alone: 1) $r = 0.1$, $\Delta T_m = 3.5$; 2) $r = 0.2$, $\Delta T_m = 4.8$; 3) $r = 0.3$, $\Delta T_m = 6.8$. [d] Broad phase transition. [e] Measured with 3% DMSO. [f] Precipitation.

ΔT_m difference between RNA and DNA becomes smaller with longer distance between the naphthyl units, as bisintercalation will increase this way, and stacking with the nucleobases itself is known to contribute little to selectivity. Mononaphthyl derivatives show higher ΔT_m values with RNA by 4 to 6 °C in comparison to the parent bisnaphthyl compounds, with DNA ΔT_m values lower by 4 to 6 °C.

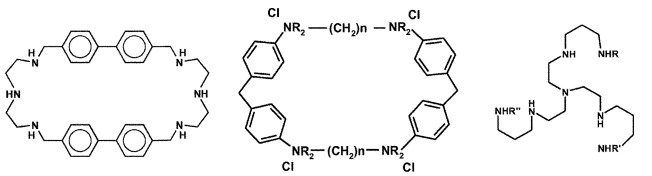
Extension of the aromatic units in the new anthryl- and acridyl moieties (Table 3) is expected to produce more pronounced intercalation. Indeed, the anthryl derivatives have a distinctly larger affinity towards DNA, whereas the corresponding naphthyl ligands (Table 2) with similar melting points with RNA and DNA overcome the preference of polyamines for RNA only partially. Again, the largest DNA over RNA preference is seen with the bisintercalating bisanthryl derivative (**A22222A**). The intercalation here dominates so strongly, that in contrast to the less effective naphthyl derivatives metalation with Cu^{2+} cannot induce strong conformational changes, as evident from the invariable ΔT_m values in the presence of the metal salts. The short linker in the diacridyl derivative prohibits bisintercalation; therefore one observes only small affinities, selectivities and metalation effects here (Table 3). It should be noted, that Cu^{2+} ions alone only lead to small melting changes.[7,15]

Table 3 Melting points of Poly(dA).Poly(dT) and PolyA.PolyU with anthryl compounds (**A22222**, **A22222A**), acridinyl derivatives (**Acr2**, **Acr2Acr**), and their copper complexes[a]

		ΔT_m/°C Poly(dA).Poly(dT)		ΔT_m/°C PolyA.PolyU	
Ligand	r[b]	L	L–Cu^{2+} 1 : 2[c]	L	L–Cu^{2+} 1 : 2[c]
Acr2	0.1	5.2	6.0	5.2	7.3
	0.2	8.6	9.4	6.8	7.6
	0.3	10.0	13.2	8.1	7.8
Acr2Acr	0.1	2.9	4.4	2.1	3.2
	0.2	4.4	5.6	3.7	4.3
	0.3	5.9	7.0	5.0	4.7
A22222	0.1	42.5	40.1	35.1	38.1
	0.2	44.6	42.2	38.2[d]	40.2[d]
	0.3	>45	43.6	44.2[d]	40.6[d]
A22222A	0.1	41.8	42.0	20.5	17.3
	0.2	>45	47.3	21.6[d]	21.0[d]
	0.3	>45	47.5	23.5[d]	21.2[d]

[a] Conditions: 0.01 M MES buffer, pH 6.25; error in $\Delta T_m = \pm 0.5$ °C; ΔT_m only approximate. [b] r = molar ratio ligand/nucleic acid phosphate. [c] Effect of Cu^{2+} alone: 1) $r = 0.1$, $\Delta T_m = 3.5$; 2) $r = 0.2$, $\Delta T_m = 4.8$; 3) $r = 0.3$, $\Delta T_m = 6.8$. [d] Broad phase transition.

Scheme 2 illustrates with some examples that the affinity and RNA/DNA selectivity of rather simple synthetic polyamines compare well with those of natural groove binders such as aminoglycosides, which of course are not directed towards the simple nucleic acid models used in the present study. Nevertheless, the ΔT_m values of the synthetic ligands often exceed those observed with aminoglycosides bearing the same charge. The selectivity of



Scheme 2 Examples of particularly RNA/DNA-selective polyamines. Melting point changes ΔT_m with PolyA.PolyU as an RNA model, and with Poly(dA).Poly(dT) as a DNA model. Measured at ligand to nucleic acid ratio $r = 0.3$, experimental conditions; charges omitted. A: unpublished measurements, conditions as in ref. 14 and ref. 1; B: ref. 14; C: Table 1. *In ref. 1 a destabilization with $\Delta T_m = -5.4$ °C was reported.

macrocyclic polyamines such as those in Scheme 2 reflects either the unfavorable placement of lipophilic aromatic moieties into the highly charged and thus hydrophilic RNA major groove,[1] or alternatively a DNA destabilization with cyclophanes of a certain size fitting better into the wider DNA groove, with subsequent flipping out of the nucleobase which can form an intracavity complex with the macrocycle.[20]

From all hitherto known polyamines the tripodal derivative **TAL** shows by far the strongest preference for RNA with ΔT_m = 40 °C; the melting point ratio $\Delta T_{m(RNA)}/\Delta T_{m(DNA)}$ here reaches 40, whereas other synthetic ligands reach only $\Delta T_{m(RNA)}/\Delta T_{m(DNA)}$ = *ca.* 3[1] (Table 1). This can be attributed to the combination of a high charge density and significant flexibility which allows optimal contact with as many phosphate groups as possible in the deep RNA groove. Attachment of terminal naphthyl units leads, as observed with the diaryl derivatives (Tables 2 and 3), to lower preferences, for the reasons discussed above. The presence of three naphthyl units leads to even lower affinities in comparison to the derivative with one aryl group, presumably due to steric hindrance for the still dominating ion pairing, in line with the always preferred binding to RNA. The effect of added Cu^{2+} ions is as expected negligible for the aryl-free derivative **TAL**, but visible with the mononaphthyl compound; the triaryl podand leads to precipitation with Cu^{2+} salts.

Conclusions and outlook

The results demonstrate how high selectivity for binding RNA in comparison to DNA can be achieved with rather simple compounds, where a high positive charge density coupled with a high ligand flexibility allows particularly deep and undistorted groove binding. It can be expected that the RNA preference is retained if a group such as **TAL** is attached with a flexible spacer to another unit (*e.g.* an oligonucleotide), which can then recognize specific sequences. The presence of highly charged groups such as in **TAL** will also help to overcome the problem of low affinity with sequence-selective ligands such as oligonucleotides alone. Inversion to preferred binding to DNA is observed if larger aromatic units are introduced which provide intercalation. Allosteric control of the binding to nucleic acids can be achieved by the introduction of flexible metal-binding spacers between the aromatic units, which can only bisintercalate in the absence of suitable metal salts. Furthermore, the unfolding effects of Cu^{2+} in particular with the tripodal ligands holds promise for the potential use of such complexes for RNA cleavage.

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