DOI: 10.1002/cbic.200800095

Zn^{2+} Complexes of Di- and Tri-nucleating Azacrown Ligands as Base-Moiety-Selective Cleaving Agents of RNA 3′,5′-Phosphodiester Bonds: Binding to Guanine Base

Qi Wang,[a] Ewelina Leino,[a] Attila Jancsó,[b] István Szilágyi,[b] Tamás Gajda,*[b] Emilia Hietamäki,[a] and Harri Lönnberg*[a]

*The ability of the dinuclear Zn^{2+} complex of 1,4-bis[(1,5,9-triazacyclododecan-3-yloxy)methyl]benzene (**L¹**) to promote the cleavage of the phosphodiester bond of dinucleoside-3′,5′-monophosphates that contain a guanine base has been studied over a narrow pH range from pH 5.8 to 7.2 at 90 °C. Comparative measurements have been carried out by using the trinuclear Zn^{2+} complex of 1,3,5-tris[(1,5,9-triazacyclododecan-3-yloxy)methyl]-benzene (**L²**) as a cleaving agent and guanylyl-3′,5′-guanosine (5′-GpG-3′) as a substrate. The strength of the interaction between the cleaving agent and the starting material has been elucidated by UV spectrophotometric titrations. The speciation and binding mode have been clarified by potentiometric titrations with hydrolytically stable 2′-O-methylguanylyl-3′,5′-guanosine and ^{1}H NMR spectroscopic measurements with guanylyl-3′,5′-guanosine. The results show that the guanine base is able to serve as a site for anchoring for the Zn^{2+}–azacrown moieties of the cleaving agents **L¹** and **L²**, analogously to uracil base. The interaction is, however, weaker than with the uracil base and, hence, only the 5′-GpG-3′ site (in addition to 5′-GpU-3′ and 5′-UpG-3′ sites) is able to markedly modulate the phosphodiester cleavage by the Zn^{2+} complexes of di- and trinucleating azacrown ligands containing an ether oxygen as a potential H-bond-acceptor site.*

Introduction

Dinuclear metal ion complexes have received considerable attention as chemical models of phosphoesterases that contain two metal ions in their catalytic center.[1] The common aim of these studies has been the design of a ligand structure that allows a synergistic action of the two metal ions and, hence, results in rapid phosphoester hydrolysis. Marked rate accelerations, which are attributed to double Lewis acid activation have been achieved with both substitution-inert Co^{3+} complexes[2] and substitution-labile Cu^{2+} and Zn^{2+} complexes.[3,4] The fact that metal ion complexes not only cleave phosphoester bonds, but might also recognize base moiety structures, affords another type of application for dinuclear metal complexes. Azacrown complexes of Zn^{2+} are particularly interesting in this respect. These complexes are known to recognize uracil and thymine bases,[5] and they exhibit marked cleaving activity towards RNA phosphodiester linkages.[6] In principle, they allow the design of base-selective cleaving agents. We have shown previously that di- and trinuclear Zn^{2+} complexes of di- and trinucleating azacrown ligands can be used to modulate the phosphodiester cleavage of dinucleoside-3′,5′-monophosphates by simultaneous interaction with the base and phosphate moieties.[7,8] Among the four possible dinucleotide 3′,5′-phosphates that are derived from adenosine (A) and uridine (U), viz. 5′-ApA-3′, 5′-ApU-3′, 5′UpA-3′ and 5′-UpU-3′, the heterodimers (5′-ApU-3′ and 5′-UpA-3′) are cleaved by the dinuclear Zn^{2+} complexes of 1,4-bis[(1,5,9-triazacyclododecan-3-yl)oxymethyl]benzene (**L¹**) and its congeners up to two orders of magnitude more readily than 5′-UpU-3′ or 5′-ApA-3′.[7] The corresponding trinuclear complex of 1,3,5-tris[(1,5,9-triazacyclododecan-3-yl)oxymethyl]benzene (**L²**), however, cleaves 5′-UpU-3′ as readily as 5′-ApU-3′ and 5′-UpA-3′, whereas the cleavage of 5′-ApA-3′ remains slow. UV spectrophotometric and ^{1}H NMR spectroscopic studies have verified the binding of the cleaving agent to the uracil-containing substrates at less than millimolar concentrations, but no interaction with the base moieties of 5′-ApA-3′ is observed. With 5′-ApU-3′ and 5′-UpA-3′, one of the Zn^{2+}–azacrown moieties in all likelihood anchors the cleaving agent to the deprotonated N3 site of the uracil base of the substrate, while the other azacrown moiety serves as a catalyst for the phosphodiester transesterification. With 5′-UpU-3′, two azacrown moieties are engaged in the uracil binding. The catalytic activity of $(Zn^{2+})_2\mathbf{L^1}$ is, hence, lost, but it can be restored by the addition of a third azacrown group on the cleaving agent, as with $(Zn^{2+})_3\mathbf{L^2}$. A similar, although weaker, the tendency for preferential cleavage of uracil-containing dimers has been reported for di- and trinuclear Cu^{2+} complexes of calix[4]arens that are functionalized at the upper rim with 1,5,9-triazacyclododecan-1-ylmethyl groups.[4b] With oligonucleotide substrates, cleavage of 5′-CpA-3′ bonds has, however, been reported to predominate.[4a] An-

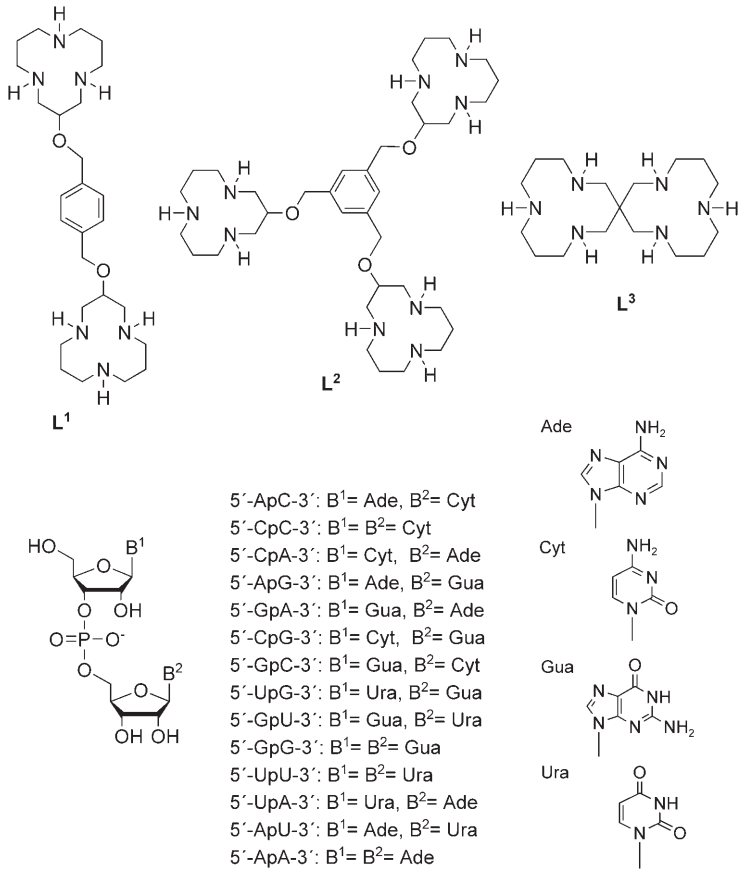
[a] *Dr. Q. Wang, E. Leino, E. Hietamäki, Prof. H. Lönnberg*
Department of Chemistry
University of Turku, 20014 Turku (Finland)
E-mail: harlon@utu.fi

[b] *Dr. A. Jancsó, Dr. I. Szilágyi, Prof. T. Gajda*
Department of Inorganic and Analytical Chemistry
University of Szeged, P.O. Box 440, 6720 Szeged (Hungary)
E-mail: tamas.gajda@chem.u-szeged.hu

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

other example of base–moiety selectivity is offered by the heterodinuclear Ni^{2+},Zn^{2+} complex of a spiro di(azacrown) ligand, 2,6,10,14,18,22-hexaazaspiro[11.11]tricosane (**L**^{3}), which cleaves uracil-containing dinucleoside-3′,5′-monophosphates considerably more rapidly than ApA.[8]

This paper is aimed at clarifying whether interaction with guanine base affects the catalytic activity of the Zn^{2+} complexes of **L**^{1} and **L**^{2}. In other words, the previous studies that were carried out with dinucleoside-3′,5′-monophosphates that were derived from A and U are extended to dimers that additionally contain guanosine (G) and cytidine (C). According to the pioneering studies of Kimura, the Zn^{2+} complex of 1-(acridin-9-ylmethyl)-1,4,7,10-tetraazacyclododecane recognizes guanine base, although the interaction with monoanionic guanine base is two orders of magnitude weaker than with monoanionic uracil or thymine base.[5] The extra oxygen atom that is present in **L**^{1} and **L**^{2} might, however, strengthen the binding. While the central ion of the complex is coordinated to the deprotonated N1 site, and one of the secondary amino groups becomes hydrogen bonded to O^6 of the guanine base, the 2-NH_2 group of the guanine base might hydrogen bond to the ether oxygen of the ligand. The strength of the interaction is evaluated by potentiometric and UV spectrophotometric titrations, and ^{1}H NMR spectroscopic studies. The effect on the cleavage rate is then determined by kinetic measurements with 5′-ApC-3′, 5′-CpA-3′, 5′-CpC-3′, 5′-ApG-3′, 5′-GpA-3′, 5′-CpG-3′, 5′-GpC-3′, 5′-UpG-3′, 5′-GpU-3′ and 5′-GpG-3′.



Results and Discussion

UV spectrophotometric titrations

The stability of the ternary complexes that uracil-containing dinucleoside-3′,5′-monophosphates form with Zn^{2+} and ligand **L**^{1} has previously been evaluated by UV-spectrophotometric titrations.[7] For comparative purposes, similar measurements have now been performed with guanine-containing dinucleoside-3′,5′-monophosphates. Accordingly, 50 μmol L^{-1} solutions of 5′-GpG-3′, 5′-GpA-3′ and 5′-GpU-3′ were titrated at three different constant pH values (pH 7.0, 7.5, and 8.0; $T = 25\,^{\circ}C$, $I =$ 0.1 mol L^{-1}) with a solution that contained Zn^{2+} and **L**^{1} in a 2:1 ratio ($[\mathbf{L}^1]_{tot} = 7.5$ mmol L^{-1}). Figure 1 shows the data that was obtained with 5′-GpG-3′ and 5′-GpA-3′ at pH 8.0. Figure 2, in turn, shows the changes that were observed in the UV absorbance of 5′-GpG-3′, 5′-GpU-3′, and 5′-GpA-3′ solutions as a function of the concentration of Zn^{2+} and **L**^{1}. The latter data refers to pH 7.5 to allow comparison with the data that were reported previously for 5′-UpU-3′, 5′-ApU-3′ and 5′-ApA-3′.

The decrease in the UV absorbance of 5′-GpG-3′ and 5′-GpU-3′ at 255 nm that was observed upon increasing the concentration of Zn^{2+} and **L**^{1} undoubtedly results from the binding of Zn^{2+} complexes of **L**^{1} to the base moieties of these dinucleoside monophosphates. With 5′-GpA-3′, the UV spectrum experiences only minor changes upon addition of Zn^{2+} and **L**^{1} at pH 8.0 (shown in Figure 1), and at pH 7.5 practically no changes were observed. Evidently the interaction with a single guanine base is quite weak. The data in Figure 2 do not allow an accurate determination of any particular stability constant. As discussed below, upon analyzing the results of potentiometric studies, different binding modes between 5′-GpG-3′, Zn^{2+}, and **L**^{1} overlap, and because the molar absorptivities of these species are not known, their contribution to the overall change in UV absorption cannot be accurately analyzed. The strength of the interaction between Zn^{2+}, **L**^{1}, and 5′-NpN-3′ appears, however, to decrease in the order: 5′-UpU-3′ > 5′-UpA-3′ ≈ 5′-GpU-3′ ≈ 5′-GpG > 5′-GpA-3′ ≈ 5′-ApA-3′, which is consistent with the well-known high affinity of Zn^{2+}–azacrown complexes to uracil base,[5] and the observation that the Zn^{2+} complexes of dinucleating azacrown ligands, such as **L**^{1}, bind to 5′-TpT-3′ much more efficiently than to the heterodimers, such as 5′-dGpT-3′, 5′-dApT-3′ or 5′-dCpT-3′.[9] It is noteworthy that, not only with the uracil-containing dimers, but also with 5′-GpG-3′, the complexation appears to be quantitative already at millimolar concentrations of Zn^{2+} and **L**^{1} at pH 7.5. Because the base–moiety binding is largely based on competition between the Zn^{2+} ion and a proton for the deprotonated N3 of the uracil base and the deprotonated N1 of guanine base, the stability of the ternary complex is quite sensitive to pH. The titration curves that were obtained with 5′-GpU-3′ offer an illustrative example: half-saturation of

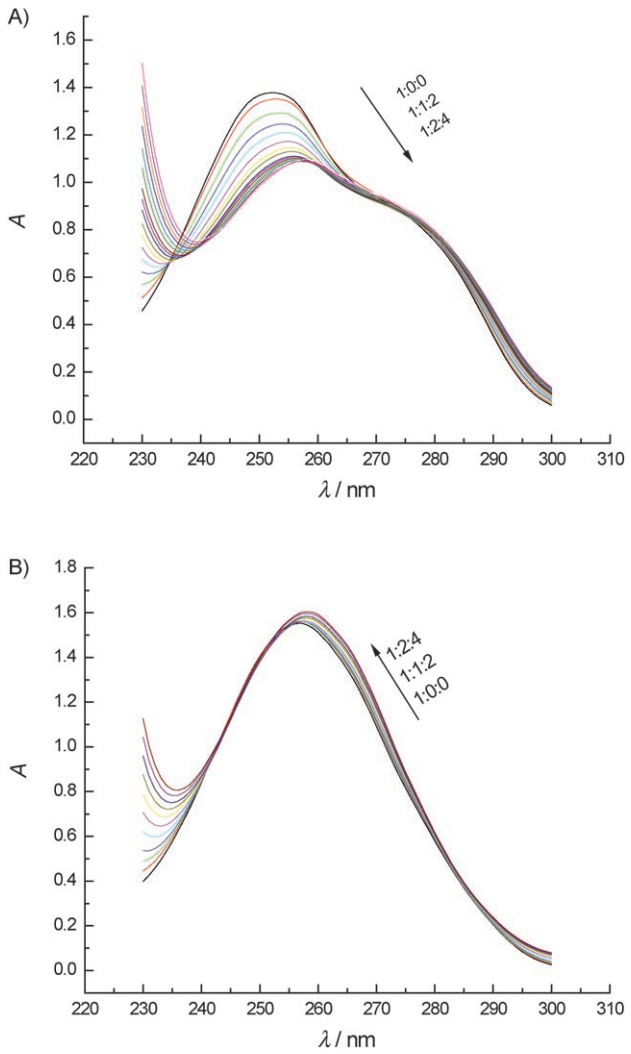


Figure 1. UV-absorption spectrum of A) 5′-GpG-3′ B) and 5′-GpA-3′ (50 μmol L^{-1} aqueous solution) titrated with a solution that contained Zn^{2+} and **L**^{1} in a 2:1 ratio ($[\mathbf{L}^1]_{tot}$ = 7.5 mmol L^{-1}) at pH 8.0 (25 °C; I = 0.1 mol L^{-1} with $NaNO_3$). Notation: the molar ratio of 5′-GpG-3′, **L**^{1} and Zn^{2+} that is indicated by the arrow refers to the curves from the top to the bottom.

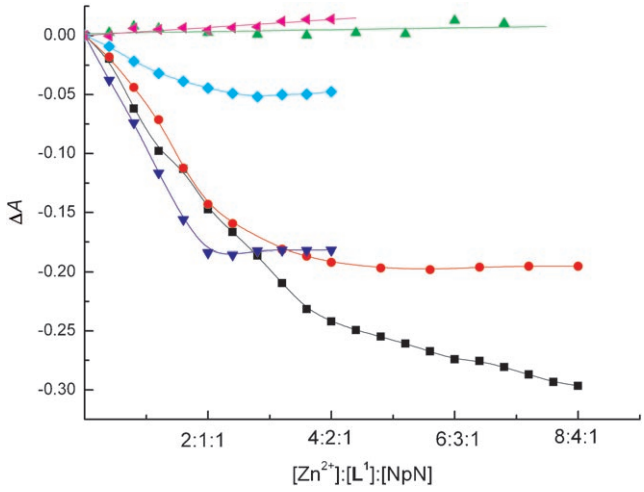


Figure 2. UV spectrophotometric titration of dinucleoside-3′,5′-monophosphates with a solution that contained Zn^{2+} and **L**^{1} in a 2:1 ratio ($[\mathbf{L}^1]_{tot}$ = 7.5 mmol L^{-1}) at pH 7.5 (T = 25 °C, I = 0.1 mol L^{-1}). The changes that were observed in the absorbance of 50 μmol L^{-1} solutions of 5′-GpG-3′ (■, 255 nm), 5′-GpU-3′ (●, 255 nm), 5′-GpA-3′ (▲, 257 nm), 5′-UpU-3′ (▼, 262 nm),[7] 5′-ApU-3′ (◆, 260 nm)[7] and 5′-ApA-3′ (◀, 258 nm)[7] as a function of the molar ratio of Zn^{2+}, **L**^{1} and NpN. The contribution of the absorbance of **L**^{1} has been subtracted.

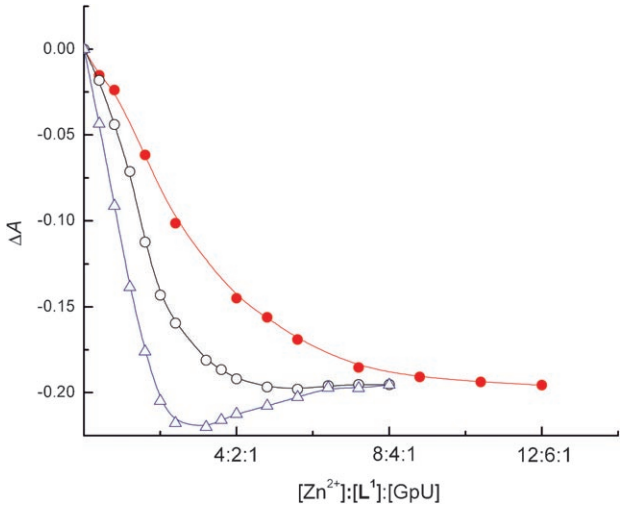


Figure 3. UV spectrophotometric titration (255 nm) of 5′-GpU-3′ with a solution that contained Zn^{2+} and **L**^{1} in a 2:1 ratio at pH 7.0 (●), pH 7.5 (○) and pH 8.0 (Δ) (T = 25 °C, I = 0.1 mol L^{-1}). The changes observed in the absorbance of a 50 μM solution of 5′-GpU-3′ as a function of the molar ratio of Zn^{2+}, **L**^{1} and 5′-GpU-3′. The contribution of the absorbance of **L**^{1} has been subtracted.

5′-GpU-3′ (at the concentration of 50 μmol L^{-1}) is achieved by the addition of 0.5, 0.7 and 1.3 equivalents of $(Zn^{2+})_2\mathbf{L}^1$ at pH 8.0, 7.5 and 7.0, respectively (Figure 3).

^{1}H NMR spectroscopic measurements

To verify the ternary complex formation with 5′-GpG-3′, as suggested by the UV spectrophotometric data, the interaction of 5′-GpG-3′ with Zn^{2+} and **L**^{1} was studied by ^{1}H NMR spectroscopy in D_2O at pD 7.1 and 25 °C. Figure 4 shows the partial NMR spectra of a 0.1 mmol L^{-1} solution of 5′-GpG-3′ (a), 5′-GpG-3′ + 2 equivalents of $Zn(NO_3)_2$ (b), 5′-GpG-3′ + 1 equivalent of **L**^{1} (c), and 5′-GpG-3′ + 2 equivalents of $Zn(NO_3)_2$ + 1 equivalent of **L**^{1} (d). In addition to the H8 resonances of the guanine bases and H1′ resonances of the sugar moieties, small signals from impurities in the commercial product were detected (marked with *). The addition of $Zn(NO_3)_2$ only slightly broadens the H8 signals at 7.8 and 7.9 ppm, and the addition of **L**^{1} has virtually no effect. By contrast, the addition of both $Zn(NO_3)_2$ and **L**^{1} results in marked broadening of both the H8 and H1′ resonances. The signals that refers to the impurities remain sharp, which strongly suggests that the broadening is not a consequence of lowered resolution, but should in all likelihood be attributed to the formation of a ternary complex between 5′-GpG-3′, Zn^{2+}, and **L**^{1}. However, no marked shift of the Zn^{2+}–**L**^{1} complex proton resonances to a higher field takes place, as was observed with 5′-UpU-3′ upon complex formation.

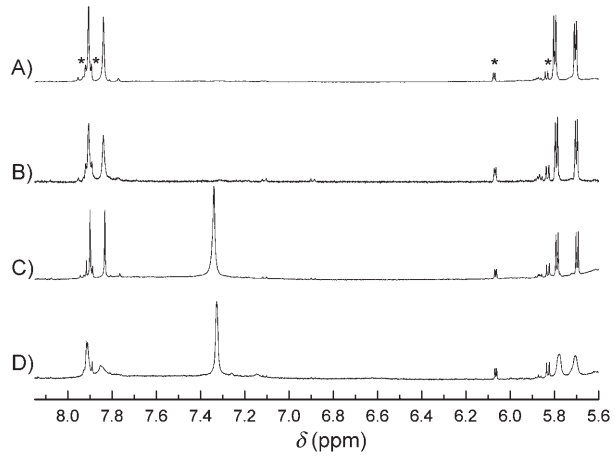


Figure 4. The H8 and H1′ NMR signals of 5′-GpG-3′ (0.1 mmol L^{-1}) in the absence and presence of Zn^{2+} and **L^1** at pD 7.1 ($T = 25\,^{\circ}C$). Notation: A) 5′-GpG-3′ alone, B) 5′-GpG-3′ + 2 equiv $Zn(NO_3)_2$, C) 5′-GpG-3′ + 1 equiv **L^1**, D) 5′-GpG-3′ + 2 equiv $Zn(NO_3)_2$ + 1 equiv **L^1**.

Table 1. Some derived equilibrium data that are related to the main species that is formed in the Zn^{2+}–**L^1** and Zn^{2+}–**L^2** binary, as well as the Zn^{2+}–5′-$G^{2'm}$pG-3′–**L^1** and Zn^{2+}–5′-$G^{2'm}$pG-3′–**L^2** ternary systems ($I = 0.1$ mol L^{-1} with $NaNO_3$, $T = 298$ K). H_2**G** refers to 5′-$G^{2'm}$pG-3′ monoanion that has the N1 sites protonated, and the phosphodiester bond deprotonated. The pK_a values of the ligands are: pK(G) = 1.49,[b] 2.51,[b] 9.08, and 9.86; pK(**L^1**) = 6.11, 6.82; pK(**L^2**) = 5.82, 6.33, 6.91. The complete sets of equilibrium data are given in the Supporting Information.

Process	pK[a]
$(Zn^{2+})^2\mathbf{L^1} = (Zn^{2+})_2(OH^-)\mathbf{L^1} + H^+$	8.29
$(Zn^{2+})_2(OH^-)\mathbf{L^1} = (Zn^{2+})_2(OH^-)_2\mathbf{L^1} + H^+$	7.17
$(Zn^{2+})_3\mathbf{L^2} = (Zn^{2+})_3(OH^-)\mathbf{L^2} + H^+$	7.52
$(Zn^{2+})_3(OH^-)\mathbf{L^2} = (Zn^{2+})_3(OH^-)_2\mathbf{L^2} + H^+$	7.26
$(Zn^{2+})_3(OH^-)_2\mathbf{L^2} = (Zn^{2+})_3(OH^-)_3\mathbf{L^2} + H^+$	8.77
$(Zn^{2+})_2(H_2G)\mathbf{L^1} = (Zn^{2+})_2(HG)\mathbf{L^1} + H^+$	7.79
$(Zn^{2+})_2(HG)\mathbf{L^1} = (Zn^{2+})_2(G)\mathbf{L^1} + H^+$	8.52
$(Zn^{2+})_2(G)\mathbf{L^1} = (Zn^{2+})_2(OH^-)(G)\mathbf{L^1} + H^+$	10.29
$(Zn^{2+})_3(H_2G)\mathbf{L^2} = (Zn^{2+})_3(HG)\mathbf{L^2} + H^+$	6.62
$(Zn^{2+})_3(HG)\mathbf{L^2} = (Zn^{2+})_3(G)\mathbf{L^2} + H^+$	7.49
$(Zn^{2+})_3(G)\mathbf{L^2} = (Zn^{2+})_3(OH^-)(G)\mathbf{L^2} + H^+$	9.15
$(Zn^{2+})_3(OH^-)(G)\mathbf{L^2} = (Zn^{2+})_3(OH^-)_2(G)\mathbf{L^2} + H^+$	10.28
	log K
$(Zn^{2+})_2\mathbf{L^1} + H_2G = (Zn^{2+})_2(H_2G)\mathbf{L^1}$	5.19
$(Zn^{2+})_2\mathbf{L^1} + HG = (Zn^{2+})_2(HG)\mathbf{L^1}$	6.48
$(Zn^{2+})_2\mathbf{L^1} + G = (Zn^{2+})_2G\mathbf{L^1}$	7.82
$(Zn^{2+})_3\mathbf{L^2} + H_2G = (Zn^{2+})_3(H_2G)\mathbf{L^2}$	5.06
$(Zn^{2+})_3\mathbf{L^2} + HG = (Zn^{2+})_3(HG)\mathbf{L^2}$	7.54
$(Zn^{2+})_3\mathbf{L^2} + G = (Zn^{2+})_3G\mathbf{L^2}$	9.91

[a] $pK_{pqrs} = \log\beta_{pqrs} - \log\beta_{p(q-1)rs}$. [b] Taken from ref. [10]

Potentiometric titrations

To obtain more quantitative data on the interaction of the Zn^{2+} complexes of **L^1** and **L^2** with 5′-GpG-3′, and to clarify the stoichiometry of the ternary complexes, systems that consisted of 2′-*O*-methylguanylyl-3′,5′-guanosine (5′-$G^{2'm}$pG-3′), $Zn(NO_3)_2$ and either **L^1** or **L^2** were subjected to a potentiometric study.

Titration of 5′-$G^{2'm}$pG-3′ solution ($T = 25\,^{\circ}C$, $I = 0.1$ mol L^{-1}) with 0.1 mol L^{-1} aqueous sodium hydroxide between pH 3–11 showed a three-step deprotonation process that exhibited pK_a values of ~2.7, 9.08 ± 0.04 and 9.86 ± 0.04. The accuracy of the first pK_a value, which corresponds to the deprotonation at one of the N7H$^+$ sites, is relatively low, due to the low concentration of 5′-$G^{2'm}$pG-3′ (≈ 0.8 mmol L^{-1}) that was used. The other N7H$^+$ site is deprotonated well below pH 2 and, hence, it is undetectable under the experimental conditions. Therefore the pK_a values that are reported for the deprotonations at the N7H$^+$ sites of 5′-GpG-3′ (pK_a = 1.49 and 2.51, $I = 0.5$ mol L^{-1})[10] have been used for the evaluation of the potentiometric data that were obtained in the Zn^{2+}-containing systems. The two higher pK_a values can be assigned to deprotonation of the N1H sites of the two nucleobase moieties. They are compatible with the values that were reported for 5′-GpG-3′ ($T = 25\,^{\circ}C$, $I = 0.1$ mol L^{-1}).[11]

The pK_a values of 1,5,9-triazacyclododecane ([12]aneN_3) have been reported to be 2.41, 7.57, and 12.60 ($T = 25\,^{\circ}C$, $I = 0.1$ mol L^{-1}).[12] With di- and triazacrown ligands, **L^1** and **L^2**, the difference between the highest and lowest pK_a values is most likely even larger. It is, hence, understandable that only the intermediary pK_a values of these ligands could accurately be determined by potentiometric titration under the conditions of the present study. The values that were obtained with **L^1** are given in Table 1.

Potentiometric titrations that were performed at 1:1 and 2:1 Zn^{2+}-to-**L^1** ratios indicated the formation of differently protonated mono- and dinuclear Zn^{2+} complexes. Deprotonation constants that are related to the dinuclear complexes are collected in Table 1. The detailed description of the equilibrium data is presented in the Supporting Information. Figure 5A shows the species distribution that was obtained for the Zn^{2+}/**L^1** system when Zn^{2+} was applied in twofold excess over **L^1**. Under these conditions, the Zn^{2+} aqua ion equilibrates, depending on the pH, with the dinuclear diaqua complex, $(Zn^{2+})_2$·**L^1**, dinuclear monohydroxo complex, $(Zn^{2+})_2(OH^-)$·**L^1** and dinuclear dihydroxo complex, $(Zn^{2+})_2(OH^-)_2$·**L^1**, beside the small amounts of mononuclear species that are present in larger quantities in equimolar solutions. As seen from Figure 5A, the hydroxo complexes predominate at pH > 7.7, and the most dominant species is the dihydroxo complex.

The proton releases from the two Zn^{2+}-bound water ligands take place in a cooperative manner that is reflected by the small fraction of the $(Zn^{2+})_2(OH^-)$·**L^1** monohydroxo species. Similar cooperation was observed by McCue and Morrow[13] for the dinuclear Cu^{2+} complexes of bis(triazacyclononane) ligands that are attached to *m*- or *p*-xylyl scaffolds. The authors suggested the formation of intra- and intermolecular μ-dihydroxo bridges for the *m*- and *p*-xylyl linked compounds, respectively. In ligand **L^1**, the linker between the two azacrown moieties is longer and more flexible, consequently, both types of μ-dihydroxo species may be formed.

Equilibrium studies on the Zn^{2+}–**L^2** system at different metal-ion-to-ligand ratios showed the formation of mono-, di- and trinuclear complexes, though the multinuclear species predominated. The complexation processes are rather similar to those that were described for the binucleating ligand above. The species distribution curves that were obtained for the Zn^{2+}/**L^2**, 3:1 system are presented on Figure 5B. In threefold

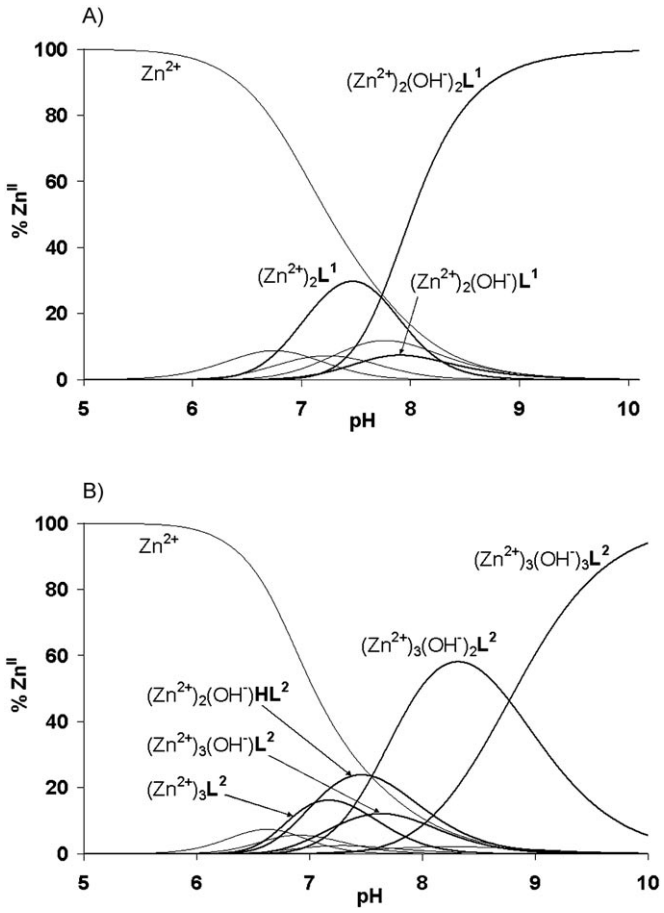


Figure 5. Species distribution curves for the A) Zn^{2+}/$\mathbf{L^1}$ (2:1), and B) Zn^{2+}/$\mathbf{L^2}$ (3:1) systems; $T = 25\,^{\circ}C$, $I = 0.1\ mol\,L^{-1}$ ($NaNO_3$), $[Zn^{2+}]_{tot} = 1.4\ mmol\,L^{-1}$ (A) and $2.1\ mmol\,L^{-1}$ (B).

excess of Zn^{2+} over L^2, the majority of the complexed Zn^{2+} ions are distributed between the trinuclear aqua complex, $(Zn^{2+})_3\mathbf{L^2}$, and the respective mono-, di- and tri-hydroxo complexes (viz. $(Zn^{2+})_3(OH^-)\cdot\mathbf{L^2}$, $(Zn^{2+})_3(OH^-)_2\cdot\mathbf{L^2}$, and $(Zn^{2+})_3(OH^-)_3\cdot\mathbf{L^2}$), respectively. In addition, a dinuclear monohydroxo complex, $(Zn^{2+})_2(OH^-)H\cdot\mathbf{L^2}$, that has one of the azacrowns protonated, is also present in a significant amount. The hydroxo complexes start to predominate at a slightly lower pH, (viz., pH 7.3) than with the Zn^{2+}–$\mathbf{L^1}$ system. The dihydroxo complex, $(Zn^{2+})_3(OH^-)_2\mathbf{L^2}$, is again formed by cooperative deprotonation processes from the trinuclear aqua complex. In the alkaline pH region (above pH 8.8), the trinuclear complex with a hydroxo ligand bound to each Zn^{2+} predominates.

Previous studies have shown that Zn^{2+} interacts only weakly with guanosine and its 2-deamino analogue inosine; the $\log K$ values for the formation of the N7 complex with neutral guanosine and N1 complex with inosine N1 monoanion are 0.8 and 2.4, respectively.[14] The stabilities that are reported for the N7-bound Cd^{2+} complex of 2′-deoxyguanosine ($\log K = 1.53$) and 5′-GpG-3′ ($\log K = 1.75$) also show weak monodentate binding.[11] Consistent with these results, only a very modest interaction between Zn^{2+} and $G^{2'm}$pG was potentiometrically observed at pH < 6.5; this refers in all likelihood to the formation of a mononuclear N7 complex [$Zn^{2+} + H_2G = (Zn^{2+})H_2G$, $\log K = 1.76$]. At higher pH, precipitation prevented reliable measurements. By contrast, the ternary complexes of 5′-$G^{2'm}$pG-3′ turned out to be quite stable. Figure 6 shows the species distribution curves for these two systems, when 5′-$G^{2'm}$pG-3′, $\mathbf{L^1}$, and Zn^{2+} are present in a 1:1:2 ratio, and 5′-$G^{2'm}$pG-3′, $\mathbf{L^2}$ and Zn^{2+} are in a 1:1:3 concentration ratio. Comparison of the titration curves of $\mathbf{L^1}$, the $\mathbf{L^1}$–Zn^{2+} 1:2 binary, and the 5′-$G^{2'm}$pG-3′–$\mathbf{L^1}$–Zn^{2+} 1:1:2 ternary systems clearly shows the formation of ternary complexes (Figure S1). As seen, the ternary complexes clearly predominate at pH > 7. 5′-$G^{2'm}$pG-3′ has five metal-binding sites with different Zn^{2+}-binding affinities: the phosphodiester unit, and the N1 and N7 nitrogens of both guanine bases. In the ternary complexes, $(Zn^{2+})_2(H_2G)\mathbf{L^1}$ and $(Zn^{2+})_3(H_2G)\mathbf{L^2}$ form at low pH, and both N1 nitrogens of 5′-$G^{2'm}$pG-3′ appear to be protonated (H_2G refers to 5′-$G^{2'm}$pG-3′ monoanion, having N1-sites protonated and phosphodiester bond deprotonated). Although, the monodentate coordination of N7 to Zn^{2+} is rather weak, the binding strength of $(Zn^{2+})_2\mathbf{L^1}$ or $(Zn^{2+})_3\mathbf{L^2}$ to 5′-$G^{2'm}$pG-3′ can be significantly enhanced by the formation of a hydrogen bond between O^6 of the guanine base and the azacrown ring,[5a] as well as by the possible additional coordination of the phosphodiester unit to one of Zn^{2+} ions. This might explain the unexpectedly high stability of the

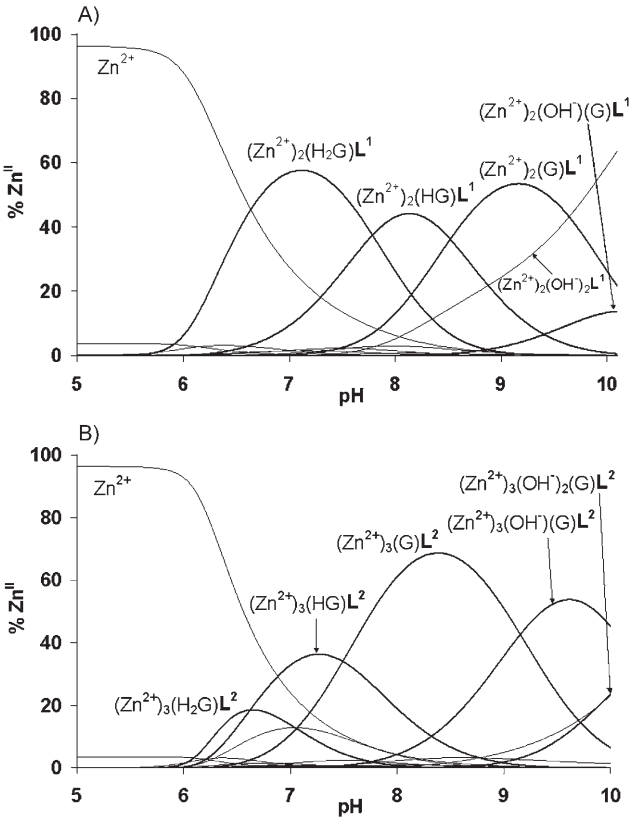


Figure 6. Species distribution curves of the A) Zn^{2+}–$G^{2'm}$pG–$\mathbf{L^1}$ 2:1:1 and B) Zn^{2+}–$G^{2'm}$pG–$\mathbf{L^2}$ 3:1:1 systems {$T = 25\,^{\circ}C$, $I = 0.1\ mol\,L^{-1}$ ($NaNO_3$), $[Zn^{2+}]_{tot} = 1.4\ mmol\,L^{-1}$ (A) and $2.1\ mmol\,L^{-1}$ (B)}. H_2G in the labels of the curves refers to 5′-$G^{2'm}$pG-3′ monoanion that has the N1 sites protonated, and the phosphodiester bond deprotonated.

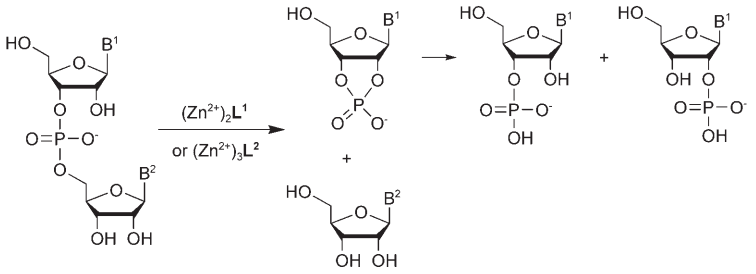
diprotonated ternary complexes ($\log K_{Zn_2L^1+H_2G} = 5.19$ and $\log K_{Zn_3L^2+H_2G} = 5.06$, Table 1). Their existence is strongly supported by the erroneous fitting of the experimental titration curves when such species are not involved in the equilibrium model (see Figure S2). The next two successive deprotonations of the above-mentioned ternary complexes (Figure 6) are related to the Zn^{2+}-promoted proton loss of the two N1H sites of 5′-G$^{2'm}$pG-3′. The ternary complexes $(Zn^{2+})_2(HG)\mathbf{L^1}$ and $(Zn^{2+})_3(HG)\mathbf{L^2}$, which have one deprotonated N1H site are significantly more stable than the $(Zn^{2+})_2(H_2G)\mathbf{L^1}$ and $(Zn^{2+})_3(H_2G)\mathbf{L^2}$ complexes that were discussed above (Table 1). The increased stability might be attributed to high basicity of the metal-bound N1 nitrogen, as well as increased electron density at O^6, which serves as the H-bond acceptor. Binding of the fully deprotonated 5′-G$^{2'm}$pG-3′ to the binary aqua species is even more favored, as indicated by the high stabilities of the $(Zn^{2+})_2G\mathbf{L^1}$ and $(Zn^{2+})_3G\mathbf{L^2}$ species (Table 1). It is also worth noting that the ternary complexes are considerably more stable in the case of $\mathbf{L^2}$ compared to $\mathbf{L^1}$ ($\log K_{Zn_2L^1+G} = 7.82$, $\log K_{Zn_3L^2+G} = 9.91$, Table 1). This suggests that the position of the azacrown rings on the xylyl spacer plays an important role in determining the stability of the ternary species. The *m*-xylyl linked $\mathbf{L^2}$ accommodates better than the *p*-substituted $\mathbf{L^1}$ to the steric requirements of binding to 5′-G$^{2'm}$pG-3′, which increases the stability of the ternary complex by two orders of magnitude.

The ternary species $(Zn^{2+})_3G\mathbf{L^2}$ undergoes two further deprotonations, with p*K* values 9.25 and 10.28. The first one is related to the deprotonation of the water molecule coordinated to the Zn^{2+} ion of the third azacrown ligand, not engaged in the base–moiety binding. Similar processes have been already described for the binary system. The second deprotonation can probably be attributed to loss of proton from an aqua ligand of one of the N1-bound Zn^{2+} ions. The analogous deprotonation of $(Zn^{2+})_2G\mathbf{L^1}$ exhibits a very similar p*K* (10.29).

One has to bear in mind that the ternary complexes that are described above might appear as protonation isomers. For example, the major species for $(Zn^{2+})_2G\mathbf{L^1}$ is probably that with two Zn^{2+}-bound guanine bases, but its protonation isomer with the formula $(Zn^{2+})_2(HG)\mathbf{L^1}(OH^-)$, that is, with one Zn^{2+}-bound hydroxide ion, might also exist. The presence of these protonation isomers might have a notable impact on the phosphodiester cleavage by the complexes described below.

Cleavage of dinucleoside-3′,5′-monophosphates by Zn^{2+} complexes of $\mathbf{L^1}$ and $\mathbf{L^2}$

The cleavage of dinucleoside-3′,5′-monophosphates that was promoted by the Zn^{2+} complexes of $\mathbf{L^1}$ was followed by analyzing the composition of aliquots that were withdrawn at appropriate intervals by RP-HPLC. The products were identified by spiking with authentic samples. The compounds that were studied were 5′-ApC-3′, 5′-CpA-3′, 5′-CpC-3′, 5′-ApG-3′, 5′-GpA-3′, 5′-CpG-3′, 5′-GpC-3′, 5′-UpG-3′, 5′-GpU-3′ and 5′-GpG-3′. The reactions were carried out with an excess of the cleaving agent; the concentration of the substrate, Zn^{2+} and $\mathbf{L^1}$ were 0.05, 1.00 and 0.50 mmol L^{-1}, respectively. In each case, the cleavage led to release the 2′,3′-cyclic phosphate and the 5′-linked nucleoside. The 2′,3′-cyclic phosphate was subsequently hydrolyzed to a mixture of 2′- and 3′-phosphates (Scheme 1).



Scheme 1. Cleavage of dinucleoside-3′,5′-monophosphates.

The pseudo-first-order rate constants for the disappearance of the starting dinucleoside-3′,5′-monophosphates were determined over a pH range of 5.90–7.15 ($T = 90\,^\circ$C, $I = 0.1$ mol L^{-1} with $NaNO_3$). The cleavage of 5′-ApG-3′, 5′-GpA-3′, 5′-GpC-3′, 5′-UpG-3′ and 5′-GpU-3′ was additionally studied at a higher concentration of Zn^{2+} and $\mathbf{L^1}$ in the pH range of 5.9–6.9: $[Zn^{2+}] = 4.0$ mmol L^{-1}, $[\mathbf{L^1}] = 2.0$ mmol L^{-1} ($T = 90\,^\circ$C, $I = 0.1$ mol L^{-1} with $NaNO_3$). At a higher pH, precipitation took place. When the kinetic data is compared to the potentiometric data, one should bear in mind that the pH values at 25 °C correspond to 0.8 unit lower values at 90 °C, because the ionic product of water is 1.6 log units lower than at 25 °C.[15]

Figure 7 shows the pH–rate profiles that were obtained for the cleavage of dinucleoside 3′,5′-monophosphates at $[Zn^{2+}] = 1.00$ mmol L^{-1} and $[\mathbf{L^1}] = 0.50$ mmol L^{-1} at 90 °C. At pH 6.9, the half-lives range from 7.5 min to 8.4 h, depending on the identity of the base moieties. Consistent with the various base moiety interactions that are discussed above, the compounds fall into five different categories. The cleavage of dimers that contain only adenine and cytosine bases is not markedly accelerated compared to the cleavage that is promoted by mononuclear Zn^{2+}[12]aneN$_3$ (Table 2). In fact, the rate enhancement is only 10–20% when the catalytic activity of 1 mmol L^{-1} Zn^{2+} solution in the presence of 0.5 mmol L^{-1} $\mathbf{L^1}$ is compared to that observed in the presence of 1.0 mmol L^{-1} [12]aneN$_3$. According to the UV spectrophotometric titrations, there is no interaction with the base moiety, and evidently no cooperation between the two azacrown moieties of $(Zn^{2+})_2\mathbf{L^1}$. Consistent with the absence of marked pre-equilibrium binding to the base moiety, the cleavage rate of 5′-ApA-3′ has been shown[7] to be increased with increasing concentration of Zn^{2+} and $\mathbf{L^1}$, although not strictly proportionally to their concentration (Table 2).

Dimers that contain a guanine base in addition to either adenine or cytosine form the second category. They are cleaved two to fourfold more readily than the dimers that contain only A or C, but still considerably less readily than 5′-ApU-3′ or 5′-UpA-3′. UV spectrophotometric data do not lend much support to formation of a ternary complex between of Zn^{2+}, $\mathbf{L^1}$,

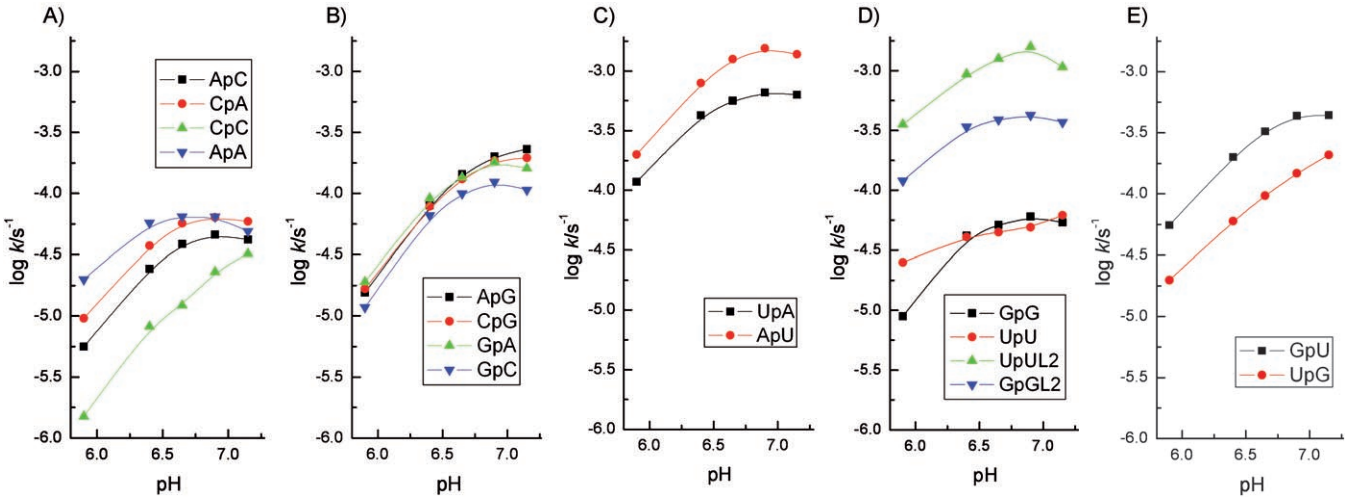


Figure 7. pH–rate profiles (A–E) for the cleavage of dinucleoside-3′,5′-monophosphates in the presence of Zn^{2+} (1.0 mM) and $\mathbf{L^1}$ (0.5 mM) in the pH range 5.9–7.2 at 90 °C (I = 0.1 M adjusted with $NaNO_3$). The figure that refers to cleavage of 5′-GpG-3′ and 5′-UpU-3′ additionally includes the pH-rate profiles that were obtained with the trinuclear complex $(Zn^{2+})_2\mathbf{L^2}$ (curves UpUL2 and GpGL2 in D). The data that refer to 5′-ApA-3′, 5′-ApU-3′, 5′-UpA-3′ and 5′-UpU-3′ are taken from ref. [7].

Table 2. Pseudo-first-order rate constants for the cleavage of dinucleoside-3′,5′-monophosphates promoted by the Zn^{2+} complexes of $\mathbf{L^1}$, $\mathbf{L^2}$ and [12]aneN$_3$ at pH 6.9 and 90 °C (I = 0.1 M with $NaNO_3$).

	$[Zn^{2+}] = 1.0$ mmol L^{-1} $[\mathbf{L^1}] = 0.5$ mmol L^{-1}	$[Zn^{2+}] = 4.0$ mmol L^{-1} $[\mathbf{L^1}] = 2$ mmol L^{-1}	$[Zn^{2+}] = 1.5$ mmol L^{-1} $[\mathbf{L^2}] = 0.5$ mmol L^{-1}	$[Zn^{2+}] = 1.0$ mmol L^{-1} [12]aneN$_3$] = 1.0 mmol L^{-1}
5′-ApC-3′	0.46 ± 0.01			0.42 ± 0.02
5′-CpA-3′	0.64 ± 0.01			0.59 ± 0.03
5′-CpC-3′	0.23 ± 0.01			
5′-ApA-3′[a]	0.65 ± 0.02	1.27 ± 0.04	1.43 ± 0.03	0.54 ± 0.01
5′-ApG-3′	2.01 ± 0.03	4.54 ± 0.09		1.47 ± 0.05
5′-CpG-3′	1.85 ± 0.02	2.44 ± 0.02		1.48 ± 0.03
5′-GpA-3′	1.82 ± 0.04	3.16 ± 0.06		
5′-GpC-3′	1.24 ± 0.03	2.80 ± 0.01		0.45 ± 0.03
5′-UpA-3′[a]	6.6 ± 0.5	7.6 ± 0.1	13.3 ± 0.5	0.60 ± 0.01
5′-ApU-3′[a]	15.6 ± 0.2	13.5 ± 0.2	20.3 ± 0.6	0.27 ± 0.01
5′-GpG-3′	0.60 ± 0.02		4.3 ± 0.2	
5′-UpU-3′[a]	0.49 ± 0.02	1.58 ± 0.02	15.8 ± 0.3	0.26 ± 0.01
5′-GpU-3′	4.36 ± 0.03	3.04 ± 0.10		0.32 ± 0.02
5′-UpG-3′	1.47 ± 0.01	2.86 ± 0.07		1.18 ± 0.07

[a] Taken from ref. [7].

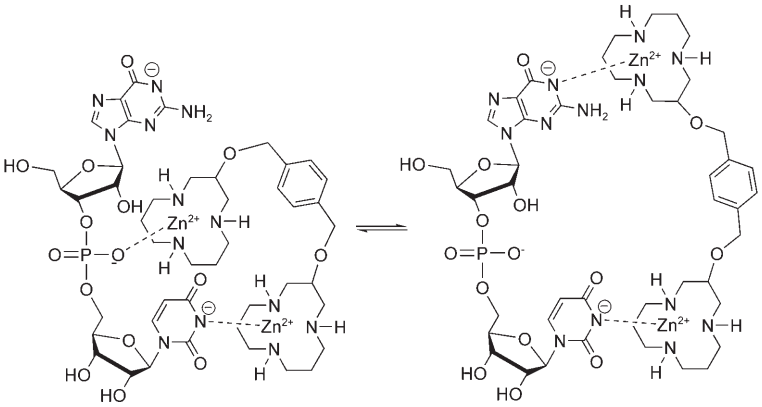
and 5′-GpA-3′, but evidently a weak interaction exists, and some pre-equilibrium binding of the cleaving agent to the substrate takes place. The starting material is not, however, saturated with the Zn^{2+} complex of $\mathbf{L^1}$, but the cleavage rate depends on the catalyst concentration (see the data for 5′-ApG-3′, 5′-CpG-3′, 5′-GpA-3′, and 5′-GpC-3′ in Table 2).

With 5′-ApU-3′ and 5′-UpA-3′, which form the third category, pre-equilibrium anchoring of the catalyst has been shown to increase the cleavage rate from one to two orders of magnitude.[7] As discussed previously, one of the Zn^{2+}–azacrown moieties is evidently bound to the uracil base, whereas the other azacrown moiety might interact with the phosphodiester bond and facilitate the departure of the 5′-linked nucleoside by intracomplex general acid catalysis; the Zn^{2+}-bound aqua ligand serves as the proton donor (Figure 8).[7,16] The cleavage rate is independent of the catalyst concentration at $[(Zn^{2+})_2\mathbf{L^1}] > 0.5$ mmol L^{-1} (Table 2).

5′-GpG-3′ and 5′-UpU-3′ constitute the fourth category. Their cleavage in not markedly accelerated on going from a Zn^{2+}–[12]aneN$_3$ complex to the dinuclear Zn^{2+} complex of $\mathbf{L^1}$, but the introduction of a third azacrown ligand to obtain a trinuclear Zn^{2+} complex of $\mathbf{L^2}$ accelerates the hydrolysis by a factor of 7 and 32 with 5′-GpG-3′ and 5′-UpU-3′, respectively. Evidently the dinuclear cleaving agent is largely anchored to both base moieties of the dinucleoside-3′,5′-monophosphate and it is, hence, unable to serve as a catalyst. In fact, under the conditions that were used in the kinetic experiments, such ternary complexes predominate more clearly than indicated in Figure 6A due to the tenfold excess of Zn^{2+}–$\mathbf{L^1}$ complexes over the dinucleoside-3′,5′-monophosphate. The speciation diagram

that refers to the concentrations of kinetic experiments (but 25 °C) is given in the Supporting Information. In spite of this macrochelate formation, the cleavage is not inhibited, and it shows almost first-order dependence of the rate on the concentration of Zn^{2+} and **L**[1] (see the data for 5′-UpU-3′ in Table 2). In other words, the phosphodiester bond is cleaved by the excess of the cleaving agent, in spite of the fact that both of the base moieties are engaged in formation of a mixed-ligand Zn^{2+} complex together with **L**[1].

Finally, 5′-UpG-3′ and 5′-GpU-3′ exhibit features of the formation of both productive and nonproductive complexes (Scheme 2). The uracil base anchors the dinuclear Zn^{2+} complex of **L**[1] through one of its azacrown moieties, whereas the other azacrown moiety might interact either with the guanine base or the phosphodiester linkage. Accordingly, the rate acceleration is more marked than with 5′-UpU-3′, but less marked than with 5′-UpA-3′ or 5′-ApU-3′.



Scheme 2. Structures of the mixed-ligand Zn^{2+} complex of 5′-GpU-3′ and the binucleating ligand **L**[1].

In summary, the results of the present paper indicate that, in addition to uracil,[7] guanine base might serve as a site of anchoring for the Zn^{2+} complexes of 1,5,9-triazacyclododecan-3-yloxy-derived ligands, **L**[1] and **L**[2], that is, azacrown-derived ligands that contain an ether oxygen as a potential H-bond acceptor site. Binding to N1-deprotonated guanine is, however, considerably weaker than binding to N3-deprotonatyed uracil and, hence, dinucleoside-3′,5′-monophosphates that bear one guanine base in addition to adenine or cytosine base are cleaved by the dinuclear Zn^{2+} complex of **L**[1] approximately one order of magnitude less readily than their uracil counterparts, but still three times as fast as the dimers that contain only adenine and/or cytosine bases. 5′-GpG-3′ forms a stable ternary complex with Zn^{2+} and **L**[1], where both of the Zn^{2+} ions bridge the N1 sites of the base moieties to **L**[1]. In excess of Zn^{2+} and **L**[1], the cleavage is as fast as with dinucleoside phosphates that are derived from adenosine and cytosine, which indicates that a macrochelate formation through the N1 sites does not inhibit the phosphodiester cleavage by free Zn^{2+} complexes in solution. The trinuclear complex that forms between Zn^{2+}, **L**[2] and 5′-GpG-3′ is even more stable. Because only two of the azacrown ligands are engaged in the base–moiety binding, the third Zn^{2+}-azacrown moiety might serve as an intracomplex cleaving agent. For this reason, 5′-GpG-3′ is cleaved by the Zn^{2+} complex of **L**[2] seven times as fast as by the Zn^{2+} complex of **L**[1]. Compared to 5′-UpU-3′,[7] the cleavage still is three times slower.

Experimental Section

Materials: Dinucleoside-3′,5′-monophosphates, nucleosides, nucleoside 2′,3′-cyclic monophosphates, nucleoside 2′-monophosphates, nucleoside 3′-monophosphates and 1,5,9-triazacyclododecane were products of Sigma and they were used as received after checking the purity by HPLC. The syntheses of the di- and triazacrown ligands, 1,4-bis[(1,5,9-triazacyclododecan-3-yloxy)methyl]-benzene (**L**[1]) and 1,3,5-tris[(1,5,9-triazacyclododecan-3-yloxy)methyl]benzene (**L**[2]), have been reported previously.[7] The buffer solutions that were used in kinetic studies and UV spectrophotometric titrations were prepared in quartz distilled water. All the reagents that were used for the preparation of the reaction solutions were of reagent grade.

2′-*O*-Methylguanylyl-3′,5′-(2′,3′-*O*-isopropylideneguanosine): N^2-(Dimetylaminomethylene)-2′,3′-*O*-isopropylideneguanosine (0.53 mmol, 200 mg) was phosphitylated with N^2-(dimetylaminomethylene)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-methylguanosine 3′-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) (0.53 mmol, 453 mg) in MeCN by using tetrazole as an activator. The phosphite triester that was obtained was oxidized to the corresponding phosphate triester with I_2 in THF/H_2O/2,6-lutidine (4:2:1). The product was purified by silica gel chromatography by using CH_2Cl_2 that contained MeOH (10%) and Et_3N (1%) as an eluent. The volatiles were removed by evaporation and the residue (455 mg) was subjected to ammonolysis in concentrated aq. ammonia (10 mL) for 16 h. The solution was evaporated to dryness, and the residue was dissolved in aq. AcOH (80%, 50 mL). After 50 min, the solution was evaporated to dryness and the residue was purified on a silica gel column by using MeCN that contained water (20%) as an eluent. Yield 38% (0.18 mmol, 120 mg as ammonium salt). ^{1}H NMR (500 MHz, [D_6]DMSO + D_2O, 25 °C, TMS): δ = 7.95 (s, 1H), 7.93 (s, 1H), 5.92 (d, J = 3.0 Hz, 1H), 5.75 (d, J = 7.0 Hz, 1H), 5.18 (dd, J_1 = 3.0, J_2 = 6.0 Hz, 1H), 5.04 (dd, J_1 = 2.5, J_2 = 6.0 Hz, 1H), 4.67 (ddd, J_1 = 2.5, J_2 = 5.0, J_3 = 7.5 Hz, 1H), 4.28 (m, 1H), 4.23 (m, 1H), 4.11 (m, 1H), 3.96 (dd, J_1 = 5.5, J_2 = 11.0 Hz, 1H), 3.86 (dd, J_1 = 5.5, J_2 = 11.0 Hz, 1H), 3.56 (dd, J_1 = 3.5, J_2 = 12.5 Hz, 1H), 3.50 (dd, J_1 = 3.0, J_2 = 12.5 Hz, 1H), 3.20 (s, 3H), 1.48 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (125.8 MHz, [D_6]DMSO + MeOD, 25 °C, TMS): δ = 157.4, 157.3, 154.2, 154.1, 151.9, 151.2, 136.8, 135.9, 117.0, 116.8, 113.5, 89.5, 85.7, 85.6, 84.9, 84.0, 82.3, 82.1, 72.7, 65.0, 64.9, 57.7, 27.1, 25.2; HRMS (ESI$^+$): calcd for $C_{24}H_{32}N_{10}O_{12}P$: 683.1933 $[M+H]^+$, found: 683.1982; calcd for $C_{24}H_{31}N_{10}O_{12}PNa$: 705.1753 $[M+Na]^+$, found 705.1799; calcd for $C_{24}H_{31}N_{10}O_{12}PK$: 721.1492 $[M+K]^+$, found: 721.1451.

2′-*O*-Methylguanylyl-3′,5′-guanosine: 2′-*O*-Methylguanylyl-3′,5′-(2′,3′-*O*-isopropylideneguanosine (0.13 mmol, 90 mg) was dissolved in THF/H_2O (30 mL, 1:1, *v*/*v*). The pH was adjusted to 1.5 with aq. 1 M HCl and this mixture was stirred at 35 °C for 48 h. The removal of the 2′,3′-*O*-isopropylidene group was monitored by taking aliquots from the reaction mixture at appropriate intervals, and analyzing their composition by HPLC on a RP column. After the reaction was completed, the solution was neutralized with 1.0 mol L^{-1} NaOH, and lyophilized to dryness. The crude compound was puri-

fied by HPLC by using aq. MeCN as eluent. The Na salt of the purified compound was lyophilized again. The purity was checked by HPLC, ESI-MS, ^{1}H NMR and potentiometric titrations; yield 60%. Analytical data: *m/z* 643.1 $[M+H]^+$ and 641.1 $[M-H]^+$ (ESI-MS, positive and negative mode, respectively). The calculated monoisotopic molecular mass is: 642.15.

UV spectrophotometric titrations: A 50 μmol L^{-1} solution (3 mL) of either 5′-GpA-3′, 5′-GpU-3′ or 5′-GpG-3′, the pH of which had been adjusted to the desired value (7.0, 7.5 or 8.0), was placed in a quartz cell that had an optical path of 1 cm. The temperature of the cell housing block was adjusted to 25.0 ± 0.1 °C, and the UV spectrum was measured in the wavelength range of 230–300 nm. A much more concentrated solution of the ligand (7.19 mmol L^{-1}) and Zn^{2+} (14.4 mmol L^{-1}) was added portionwise, and after readjusting the pH to the desired value, the UV spectrum was measured after each addition.

Potentiometric measurements: The protonation and coordination equilibria were investigated by potentiometric titrations in aqueous solution (I = 0.1 M $NaNO_3$, and T = 298.0 ± 0.1 K) under argon atmosphere by using an automatic titration set that included a PC-controlled Dosimat 665 (Metrohm, Herisau, Switzerland) autoburette and an Orion 710A (Beverly, MA, USA) precision digital pH meter. The Metrohm Micro pH glass electrode (125 mm) was calibrated with the modified Nernst equation [Eq. (1)]:

$$E = E_0 + K \times \log [H^+] + J_H \times [H^+] + J_{OH} \times (K_w/[H^+]) \quad (1)$$

where J_H and J_{OH} are fitting parameters in acidic and alkaline media for the correction of experimental errors, mainly due to the liquid junction and to the alkaline and acidic errors of the glass electrode;[17] the value of $10^{-13.75}$ M^2 was used for the autoprotolysis constant, K_w, of water.[18] The parameters were calculated by the nonlinear least-squares method. The protonation and complex formation constants were calculated by using the PSEQUAD computer program.[19] The highest pK_a values of the azacrown rings (pK_a > 11) could not be determined at the concentrations that were used in the present study. However, their Zn^{2+}-promoted deprotonation takes place below pH 8–9, which should be taken into account with negative stoichiometric numbers (for example, Zn_2H_{-2}**L^1** denotes the species where a metal ion is bound to both azacrown rings of **L^1**). This kind of denotation is the correct way of data handling, but would be confusing in the body text of manuscript. Therefore, the full details of the evaluation of the potentiometric data are described in the Supporting Information. Consequently, in the text body **L^1** and **L^2** denote the neutral ligand molecules, and the charges of the complexes are omitted for the sake of clarity.

The protonation constants were determined from 3–4 independent titrations (60–80 data points per titration). The complex formation constants were evaluated from 5–10 independent titrations (50–100 data points per titration), depending on the complexity of the system. The metal-to-ligand ratios varied between 4:1–1:1, 2:1–1:1 and 3:1–1:1 in the binary Zn^{2+}–5′-G$^{2'm}$pG-3′, Zn^{2+}–**L^1** and Zn^{2+}–**L^2** systems, respectively, the Zn^{2+} concentration fell in the range of 7.0×10^{-4}–2.5×10^{-3} M. In the Zn^{2+}–5′-G$^{2'm}$pG-3′–**L^1**(**L^2**) ternary system the concentration ratios were 2:1:1 and 2:2:1 (3:1:1, 2:1:1 and 3:2:1) with the Zn^{2+} concentration in the range of 7.0×10^{-4}–2.0×10^{-3} M.

Kinetics measurements: The pH of the reaction solutions was adjusted with 0.1 M HEPES [*N*-(2-hydroxyethyl)piperazine-*N*′-(ethanesulfonic acid)] buffer. The pH that was measured at room temperature was extrapolated to the temperature of the kinetic measurements with the aid of the known temperature dependence of the pK_a value of HEPES.[20] The ionic strength was adjusted to 0.1 M with $NaNO_3$. The final concentration of the Zn^{2+} complex, 0.5 or 2.0 mmol L^{-1}, was obtained by adding appropriate amounts of the ligand and $Zn(NO_3)_2$.

The reactions were carried out in sealed tubes that were immersed in a water bath, the temperature of which was adjusted to 90.0 ± 0.1 °C. Aliquots (ten for each reaction) were withdrawn at suitable intervals to cover at least one half-life (usually 2–3) of the cleavage and immediately cooled in an ice-water bath. A 0.5 M aq. AcOH solution (10 μL) was added to every sample to quench the reaction and the samples were stored in a freezer before analysis.

The composition of the aliquots was analyzed by RP-HPLC by using a Waters Atlantis column dC18 (Milford, MA, USA; 250× 4.6 mm, particle size 5 μm). For the analysis of the reaction products, a 0.1 M KH_2PO_4/KOH buffer (pH 6.0, that contained 2.0 mM EDTA) and a gradient elution from 1% to 21% of MeCN was employed. The flow rate was 1.0 mL min^{-1} and the UV detection wavelength was 260 nm throughout the work. The products (nucleosides, nucleoside 2′,3′-cyclic monophosphates, nucleoside 2′-monophosphates, and nucleoside 3′-monophosphates) were identified by spiking with authentic samples.

The pseudo-first-order rate constants (k_{obs}) for the cleavage of dinucleoside-3′,5′-monophosphates were calculated by applying the integrated first-order rate law to the area of the peak of the starting material that was obtained upon using a 10 μL loop injection.

Acknowledgements

This work was partly supported by the Hungarian Scientific Research Found (OTKA K63606). A.J. wishes to thank the Hungarian Academy of Sciences for the János Bolyai Research Grant.

Keywords: artificial nucleases · azacrown · hydrolysis · kinetics · RNA

[1] For recent reviews see: a) F. Mancin, P. Tecilla, *New J. Chem.* **2007**, *31*, 800–817; b) N. Mitic, S. J. Smith, A. Neves, L. W. Guddat, L. R. Gahan, G. Schenk, *Chem. Rev.* **2006**, *106*, 3338–3363; c) F. Mancin, P. Scrimin, P. Tecilla, U. Tonellato, *Chem. Commun. (Cambridge)* **2005**, 2540–2548; d) J. Weston, *Chem. Rev.* **2005**, *105*, 2151–2174; e) J. R. Morrow, O. Iranzo, *Curr. Opin. Chem. Biol.* **2004**, *8*, 192–200; f) J. A. Cowan, *Curr. Opin. Chem. Biol.* **2001**, *5*, 634–642.

[2] a) N. H. Williams, B. Takasaki, M. Wall, J. Chin, *Acc. Chem. Res.* **1999**, *32*, 485–493; b) N. H. Williams, W. Cheung, J. Chin, *J. Am. Chem. Soc.* **1998**, *120*, 8079–8087; c) N. H. Williams, J. Chin, *Chem. Commun. (Cambridge)* **1996**, 131–132.

[3] a) A. Scarso, G. Zaupa, F. B. Houillon, L. J. Prins, P. Scrimin, *J. Org. Chem.* **2007**, *72*, 376–385; b) M.-Y. Yang, J. R. Morrow, J. P. Richard, *Bioorg. Chem.* **2007**, *35*, 366–374; c) G. Feng, D. Natale, R. Prabaharan, J. C. Marque-Rivas, N. H. Williams, *Angew. Chem.* **2006**, *118*, 7214–7217; *Angew. Chem. Int. Ed.* **2006**, *45*, 7056–7059; d) A. O'Donoghue, S. Y. Pyun, M.-Y. Yang, J. R. Morrow, J. P. Richard, *J. Am. Chem. Soc.* **2006**, *128*, 1615–1621; e) K. Selmeczi, M. Giorgi, G. Speier, E. Farkas, M. Reglier, *Eur. J. Inorg. Chem.* **2006**, 1022–1031; f) B. Bauer-Siebenlist, F. Meyer, E. Farkas, D. Vidovic, S. Dechert, *Chem. Eur. J.* **2005**, *11*, 4349–4360; g) B. Bauer-Siebenlist, F. Meyer, E. Farkas, D. Vidovic, J. A. Cuesta-Seijo, R. Herbst-Irmer, H. Pritzkow, *Inorg. Chem.* **2004**, *43*, 4189–4202; h) O. Iranzo, A. Y. Kovalevsky, J. R. Morrow, J. P. Richard, *J. Am. Chem. Soc.* **2003**, *125*, 1988–1993; i) O. Iranzo, T. Elmer, J. P. Richard, J. R. Morrow, *Inorg. Chem.* **2003**, *42*, 7737–7746; j) A. Jancsó, S. Mikkola, H. Lönnberg, K. Hegetschweiler, T. Gajda, *Chem. Eur. J.* **2003**, *9*, 5404–5415; k) S. Albedyhl, D. Schnieders, A. Jancsó, T. Gajda, B. Krebs, *Eur. J. Inorg. Chem.* **2002**, 1400–1409; l) T. Gajda, A. Jancsó, S. Mikkola, H. Lönnberg, H.

Sirges, *J. Chem. Soc. Dalton Trans.* **2002**, 1757–1763; m) T. Gajda, R. Krämer, A. Jancsó, *Eur. J. Inorg. Chem.* **2000**, 1635–1644; n) P. Molenveld, W. M. G. Stikvoort, H. Kooijman, A. L. Spek, J. F. J. Engbersen, D. N. Reinhoudt, *J. Org. Chem.* **1999**, *64*, 3896–3906; o) P. Molenveld, S. Kapsabelis, J. F. J. Engbersen, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1997**, *119*, 2948–2949; p) M. Wall, R. C. Hynes, J. Chin, *Angew. Chem.* **1993**, *105*, 1696–1697; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1633–1635.

[4] a) R. Cacciapaglia, A. Casnati, L. Mandolini, A. Perracchi, D. N. Reinhoudt, R. Salvio, A. Sartori, R. Ungaro, *J. Am. Chem. Soc.* **2007**, *129*, 12512–12520; b) R. Cacciapaglia, A. Casnati, L. Mandolini, D. N. Reinhoudt, R. Salvio, A. Sartori, R. Ungaro, *J. Am. Chem. Soc.* **2006**, *128*, 12322–12330.

[5] a) S. Aoki, E. Kimura, *Chem. Rev.* **2004**, *104*, 769–787; b) S. Aoki, E. Kimura, *Rev. Mol. Biotechnol.* **2002**, *90*, 129–155; c) E. Kimura, M. Kikuchi, H. Kitamura, T. Koike, *Chem. Eur. J.* **1999**, *5*, 3113–3123; d) M. Shionoya, T. Ikeda, E. Kimura, M. Shiro, *J. Am. Chem. Soc.* **1994**, *116*, 3848–3859; e) E. Kimura, T. Shiota, M. Koike, M. Shiro, M. Kodama, *J. Am. Chem. Soc.* **1990**, *112*, 5805–5811.

[6] a) U. Kaukinen, L. Bielecki, S. Mikkola, R. W. Adamiak, H. Lönnberg, *J. Chem. Soc. Perkin Trans. 2* **2001**, 1024–1031; b) I. Zagorowska, S. Kuusela, H. Lönnberg, *Nucleic Acids Res.* **1998**, *26*, 3392–3396; c) S. Kuusela, H. Lönnberg, *J. Chem. Soc. Perkin Trans. 2* **1994**, 2301–2306; d) S. Kuusela, H. Lönnberg, *J. Phys. Org. Chem.* **1993**, *6*, 347–356.

[7] Q. Wang, H. Lönnberg, *J. Am. Chem. Soc.* **2006**, *128*, 10716–10728.

[8] Q. Wang, S. Mikkola, H. Lönnberg, *Chem. Biodiv.* **2004**, *1*, 1316–1326.

[9] a) E. Kimura, H. Kitamura, K. Ohtani, T. Koike, *J. Am. Chem. Soc.* **2000**, *122*, 4668–4677; b) E. Kimura, M. Kikuchi, H. Kitamura, T. Koike, *Chem. Eur. J.* **1999**, *5*, 3113–3123; c) T. Koike, M. Takashige, E. Kimura, H. Fujioka, M. Shiro, *Chem. Eur. J.* **1996**, *2*, 617–623; d) H. Fujioka, T. Koike, N. Yamada, E. Kimura, *Heterocycles* **1996**, *42*, 775–787.

[10] N. Ogasawara, Y. Watanabe, Y. Inoue, *J. Am. Chem. Soc.* **1975**, *97*, 6571–6576.

[11] C. P. Da Costa, H. Sigel, *Inorg. Chem.* **2003**, *42*, 3475–3482.

[12] L. J. Zompa, *Inorg. Chem.* **1978**, *17*, 2531–2536.

[13] K. P. McCue, J. M. Morrow, *Inorg. Chem.* **1999**, *38*, 6136–6142.

[14] H. Lönnberg, P. Vihanto, *Inorg. Chim. Acta* **1981**, *56*, 157–161.

[15] W. L. Marshall, E. U. Franck, *J. Phys. Chem. Ref. Data* **1981**, *10*, 295–304.

[16] a) T. Lönnberg, H. Lönnberg, *Curr. Opin. Chem. Biol.* **2005**, *9*, 665–673; b) S. Mikkola, E. Stenman, K. Nurmi, E. Yousefi-Salakdeh, R. Strömberg, H. Lönnberg, *J. Chem. Soc. Perkin Trans. 2* **1999**, 1619–1625.

[17] F. J. C. Rosotti, H. Rosotti, *The Determination of Stability Constants*, McGraw–Hill, New York, **1962**, p. 149.

[18] E. Högfeldt, *Stability Constants of Metal–Ion Complexes, Part A. Inorganic Ligands*, Pergamon, New York, **1982**, p. 32.

[19] L. Zékány, I. Nagypál, G. Peintler, *PSEQUAD for Chemical Equilibria*, Technical Software Distributors: Baltimore, **1991**.

[20] N. E. Good, G. D. Winget, W. Winter, T. N. Connolly, S. Izawa, R. M. M. Singh, *Biochemistry* **1966**, *5*, 467–477.

Received: February 13, 2008
Published online on June 23, 2008

