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Cleavage of short oligoribonucleotides by a Zn^{2+} binding multi-nucleating azacrown conjugate

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ABSTRACT

A multi-nucleating azacrown conjugate (**5a**) consisting of two 3,5-bis(1,5,9-triazacyclododecan-3-yloxymethyl)benzyl groups attached to 1 and 7 sites of cyclen was prepared and tested as an artificial ribonuclease. The conjugate in the presence of five equivalents of zinc nitrate expectedly showed uridine selectivity comparable to that 1,3,5-tris(1,5,9-triazacyclododecan-3-yl)benzene (**2**), a compound known to bind to two adjacent uridine residues and cleave the intervening phosphodiester bond. **5a** was, however, unable to discriminate between two and three adjacent uridine residues, but cleaved oligonucleotides containing a UpU and UpUpU site at a comparable rate, even when present at sub-saturating concentrations.

1. Introduction

Metal ions and their complexes promote the cleavage of RNA phosphodiester bonds. Most likely, the dianionic oxyphosphorane intermediate, obtained in a rapid pre-equilibrium step by an intramolecular attack of the 2′-hydroxy function on the phosphorus atom, is stabilized by coordination of a metal aqua ion to a non-bridging oxygen and the rate-limiting departure of the 5′-linked nucleoside is facilitated by transfer of a proton from the aqua ligand to the departing 5′-oxygen (Scheme 1) [1]. Exploitation of this reaction for tailoring of RNA *in vitro* has been the subject of numerous studies during the past two decades [2]. The cleavage has usually been made sequence selective by tethering the metal ion complex to a sequence recognizing oligomer, either an oligonucleotide or peptide nucleic acid (PNA). The most successful example is offered by a PNA conjugate of the Cu^{2+} chelate of 2,9-dimethylphenantroline that has been shown to cleave the target RNA at a bulge site, the half-life being 30 min at 37 °C [3].

Base moiety selectivity has, in turn, been achieved by simple di- or tri-nuclear complexes bearing no additional conjugate group for recognition. The dinuclear Zn^{2+} complex of 1,3-bis[(1,5,9-triazacyclododecan-3-yl)oxymethyl]benzene (**1**), for example, cleaves the phosphodiester bonds at both sides of uridine from 30 to 40 times as fast as those neighboring adenosine or cytidine [4]. One of the Zn^{2+} azacrown moieties anchors the complex to uracil base, as shown by Kimura et al. [5], while the other one serves as the actual cleaving agent. In the presence of two neighboring uridines, the cleaving activity disappears, since both Zn^{2+} azacrowns become engaged in uracil binding. By contrast, the trinuclear complex of tris[(1,5,9-triazacyclododecan-3-yl)oxymethyl]benzene (**2**) is still active, cleaving the phosphodiester bond between the uridines. Guanine base also is a potential anchoring site, but binding to deprotonated N1 of guanine is considerably weaker than binding to deprotonated N3 of uracil [6]. At low catalyst concentrations, the cleavage at a uridine site still is almost one order of magnitude faster than the cleavage at a guanosine site. Trinuclear Zn^{2+} complexes of cone calix[4]arenes bearing three nucleating 2,6-bis(dimethylaminomethyl)pyridine residues at the upper rim (**3**) constitute another family of base moiety selective cleaving agents [7]. Among dinucleoside-3′,5′-monophosphates, GpG was cleaved 8 times as fast as UpU, 12 times as fast as CpC and 160 times as fast as ApA. The corresponding trinuclear Cu^{2+} complex bearing (1,5,9-triazacyclononan-1-yl)methyl groups as nucleating ligands cleaved UpG and UpU more than one order of magnitude faster than other dimers [8].

Although **2** selectively cleaves short oligonucleotides at a UpU site [4b], the selectivity is not sufficient for tailoring of long oligonucleotides. The present study was undertaken to find out whether the base moiety selectivity could be improved by increasing the number of Zn^{2+} azacrown chelates on the cleaving agent. This expectation was based on the previous report of Kimura et al. [9], according to which the dissociation constant for the 1:1 complex of the trinuclear Zn^{2+} complex of 1,7-bis

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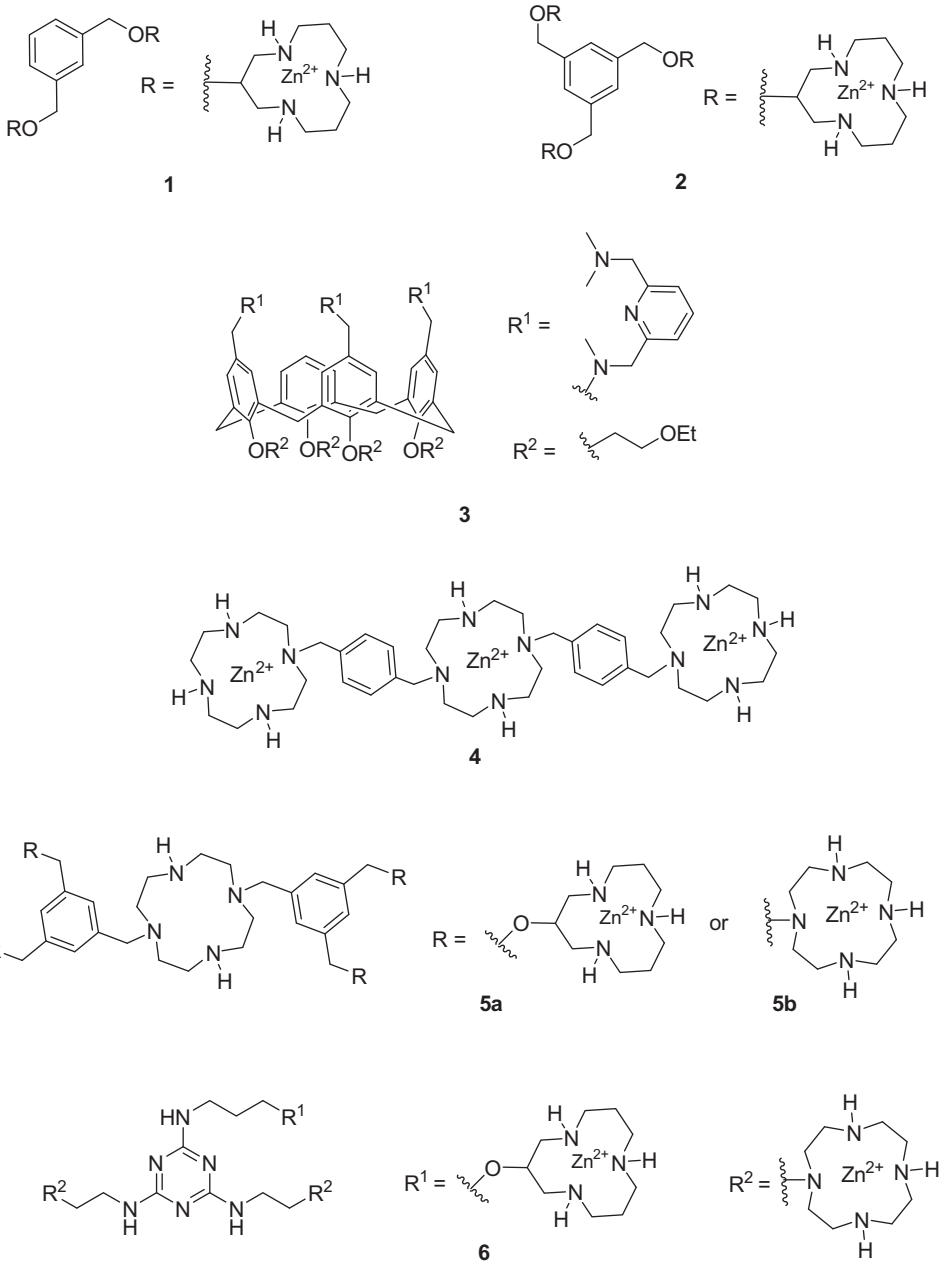
[4-(1,4,7,10-tetraazacyclododecan-1-ylmethyl)benzyl]-1,4,7,10-tetraazacyclododecane (**4**) with TpTpT was as low as 0.8 nM.

As a construct that could recognize three consecutive 2,4-dioxopyrimidine bases, but additionally has Zn^{2+}(azacrown) chelates to serve as cleaving agents, pentaazacrown conjugates **5a**,**b** were considered as feasible alternatives. In **5a** and **5b**, two azacrown moieties are connected by a 6 and 5 atom long linker, respectively, while **4** contains 6 atom linkers. To decide which one of these is a more attractive candidate, N^2,N^4-bis(1,4,7,10-tetraazacyclododecan-1-ylethyl)-N^6-(1,5,9-triazacyclododecan-3-ylpropyl)-1,3,5-triazine-2,4,6-triamine (**6**) was synthesized and the concentration dependence of its cleaving activity was compared with that reported [4a] for **2**. We have shown previously that Zn^{2+}(1,5,9-triazacyclododecane) is a more efficient cleaving agent than Zn^{2+}(1,4,7,10-tetraazacyclododecane) [10], but the high affinity of **4** to TpTpT suggests that the tetraazacyclododecane complex might be superior from the point of view of anchoring. On the basis of the results obtained, only compound **5a** was prepared to be used as the multinucleating ligand in the cleavage studies of short oligonucleotides.



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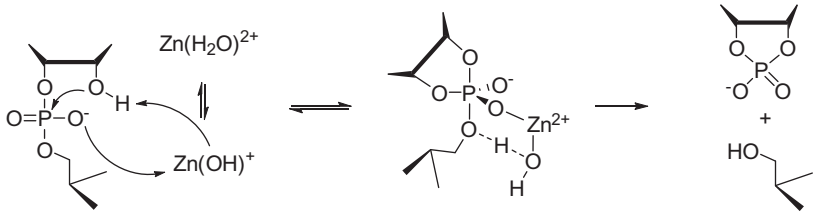


Scheme 1. Mechanism for Zn^{2+} promoted cleavage of RNA phosphodiester bonds.

2. Results and discussion

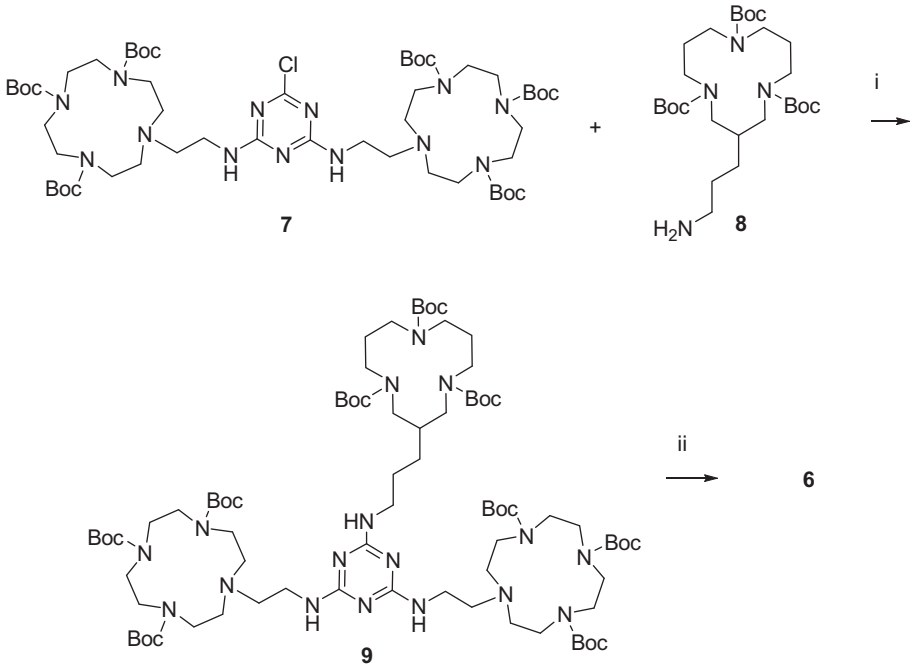
2.1. Synthesis of **6** and the cleaving activity of its trinuclear Zn^{2+} complex

Compound **6** was prepared as depicted in Scheme 2. In other words, *tert*-butoxycarbonyl (Boc) protected N^2,N^4-bis(1,4,7,10-tetraazacyclododecan-1-ylethyl)-6-chloro-1,3,5-triazine-2,4,6-triamine (**7**) [11] was subjected to aromatic nucleophilic displacement with 3-aminopropyl-1,5,9-tri(*tert*-butoxycarbonyl)-1,5,9-triazacyclododecane (**8**) [12] in MeCN in the presence of *N*,*N*-diisopropylethylamine to obtain a fully protected tri(azacrown) conjugate **9**. Removal of the Boc protecting groups with TFA in a mixture of MeOH and CH_2Cl_2, followed by conversion to free base with the aid of a strong anion exchange resin, gave the desired product (**6**).

The pseudo first-order rate constants obtained for the cleavage of UpU, ApU and UpA in the presence of **6** and $Zn(NO_3)_2$ are plotted in Fig. 1 against the concentration of cleaving agent **6**. The concentration of $Zn(NO_3)$ was always threefold compared to the concentration of **6** and the initial concentration of the dinucleoside-3′,5′-monophosphates was 0.05 mM. As seen from Fig. 1, the affinity of **6** to UpU in the presence of Zn^{2+} is high; the cleavage rate levels off to a constant value already on passing 0.1 mM concentration of **6**. With ApU and UpA, the saturation is achieved only at one order of magnitude higher concentration, but the rate achieved at 1.0 mM concentration of **6** is 3-fold compared to the cleavage rate of UpU. In fact, ApU and UpA are cleaved with **6** approximately as readily as with **2**, but the saturation takes place at a somewhat higher concentration. The cleavage rate of UpU is, in turn, only 37% of that obtained with **2**. Accordingly, partial replacement of the 1,5,9-triazacyclododecanyl groups with 1,4,7,10-tetraazacyclododecanyl groups neither improved the cleaving activity compared to **2** nor afforded a higher affinity to uridine. Accordingly, **5a** was used as the multinucleating ligand in the kinetic studies with short oligonucleotides.

2.2. Preparation of multinucleating cleaving agent ***5a***

The synthesis of **5a** is outlined in Scheme 3. Commercial 1,4,7,10-tetraazacyclododecane (cyclen) was first converted 1,7-di(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane (**10**) by treating with 2 equiv. of *N*-(*tert*-butoxycarbonyloxy)succinimide in $CHCl_3$, as described previously by Piersanti et al. [13]. Alkylation of the product with 1,3,5-tri(bromomethyl)benzene in the presence of Na_2CO_3 in $CHCl_3$ gave 4,10-bis[3,5-di(bromomethyl)benzyl]-1,7-di(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane (**11**). The bromo substituents where then displaced with 3-hydoxy-1,5,9-tri(*tert*-butoxycarbonyl)-1,5,9-triazacyclododecane (**12**) in DMF in the presence of NaH to obtain **13** that was finally subjected to removal of the *tert*-butoxycarbonyl groups in methanolic hydrogen chloride and conversion to free amine (**5a**) with a strong anion exchange resin in water.



Scheme 2. (i) MeCN, iPr_2NEt; (ii) 1. TFA, MeOH:CH_2Cl_2 (17:83, v/v), 2. Dowex 1X2, OH^--form, water.

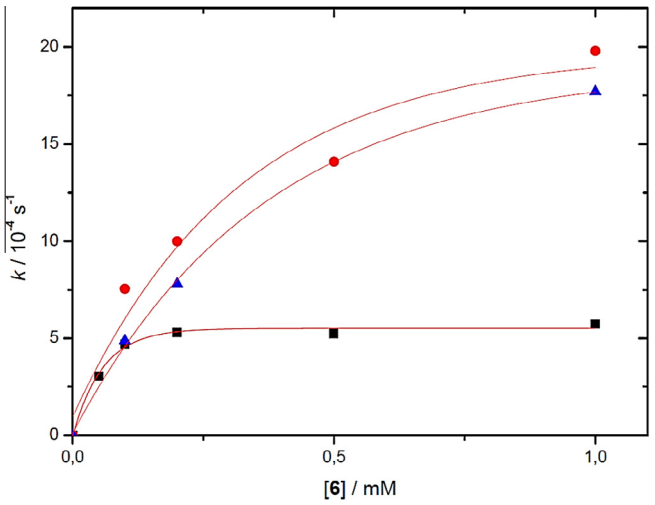


Fig. 1. First-order rate constants for the cleavage of UpU (■), ApU (●) and UpA (▲) at different concentrations of cleaving agent **6** and Zn^{2+} ($[Zn^{2+}] = 3 \times [\mathbf{6}]$) in 0.1 M HEPES buffer at pH 6.84 (T = 90 °C, I = 0.1 M).

Table 1
First-order rate constants for the cleavage of short oligoribonucleotides by cleaving agent **5a** and Zn^{2+} in 0.10 M HEPES buffer at pH 7.5 (T = 35.0 °C, I = 0.1 M).

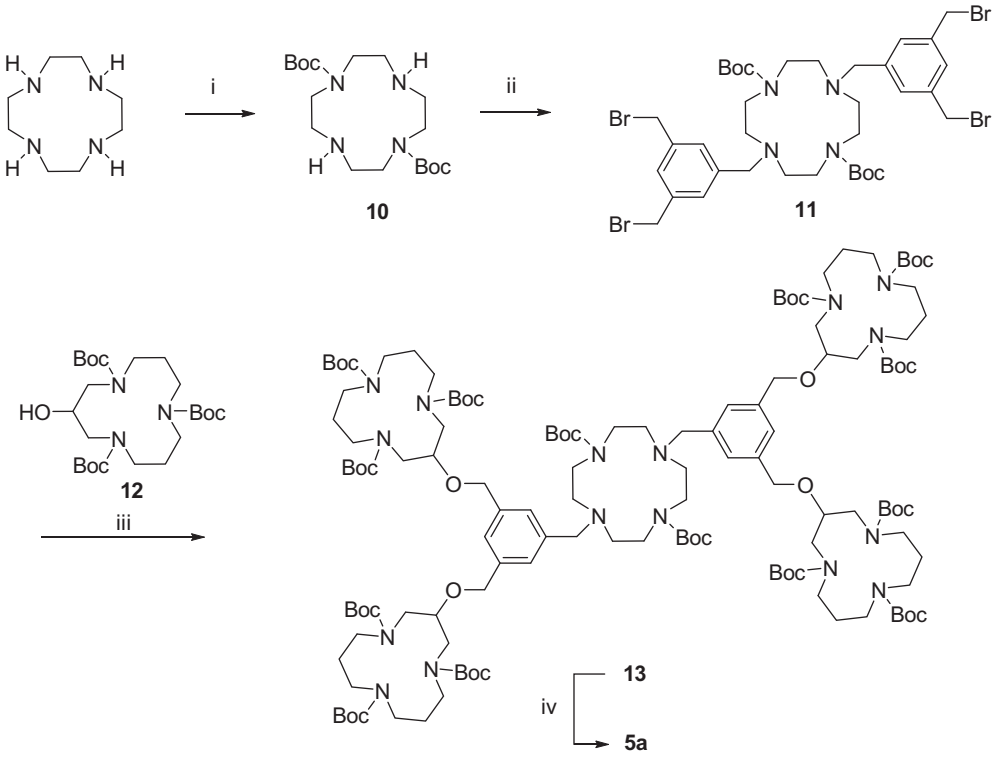
Oligonucleotide	[**5a**]/mM	$[Zn^{2+}]$/mM	$k/10^{-5}$ s^{-1}
5′-CUUUC-3′	0.5	2.5	2.03 ± 0.09
	0.2	1.0	1.58 ± 0.12
	0.1	0.5	0.99 ± 0.08
	0.05	0.25	0.40 ± 0.02
5′-CUCUC-3′	0.5	2.5	2.07 ± 0.13
	0.2	1.0	1.40 ± 0.17
	0.1	0.5	1.30 ± 0.04
	0.05	0.25	0.99 ± 0.04
5′-CCUUC-3′	0.5	2.5	1.97 ± 0.08
	0.2	1.0	1.47 ± 0.15
	0.1	0.5	1.77 ± 0.04
	0.05	0.25	0.79 ± 0.03
5′-CUUCC-3′	0.5	2.5	1.22 ± 0.10
	0.2	1.0	1.23 ± 0.12
	0.1	0.5	1.04 ± 0.04
	0.05	0.25	0.62 ± 0.01
5′-ACUUUAC-3′	0.5	2.5	3.10 ± 0.08
	0.2	1.0	1.76 ± 0.11
	0.1	0.5	1.08 ± 0.05
	0.05	0.25	0.63 ± 0.03

Oligoribonucleotides, used as targets in the cleaving reactions, were assembled from commercially available 5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-triisopropylsilyloxymethyl protected phosphoramidite building blocks by conventional phosphoramidite strategy. Their identity and homogeneity was verified by ESI-MS and RP-HPLC.

2.3. Cleavage experiments with ***5a***

Table 1 lists the short oligoribonucleotides used as target sequences in the cleavage experiments. The reactions were carried out in 0.10 M HEPES buffer at pH 7.5 and 35.0 ± 0.1 °C. The concentration of **5a** was varied from 0.050 to 0.50 mM and the concentration of $Zn(NO_3)_2$ was always 5-fold compared to that of **5**. The concentration of the target oligonucleotide was in all experiments 0.050 mM. 4-Nitrobenzenesulfonate (0.10 mM) was used as an internal standard. The progress of reactions was followed by withdrawing aliquots at suitable intervals and analyzing their composition by capillary electrophoresis.

Table 1 records the kinetic data obtained. Our previous studies with tris[(1,5,9-triazacyclododecan-3-yl)oxymethyl]benzene (**2**) have shown that the first-order rate constant for the disappearance



Scheme 3. Reagents: (i) *N*-(*tert*-Butoxycarbonyloxy)succinimide, $CHCl_3$; (ii) 1,3,5-tri(bromomethyl)benzene, Na_2CO_3, $CHCl_3$; (iii) NaH, DMF; (iv) 1. HCl, MeOH, 2. Dowex 1X2, OH^- form, water.

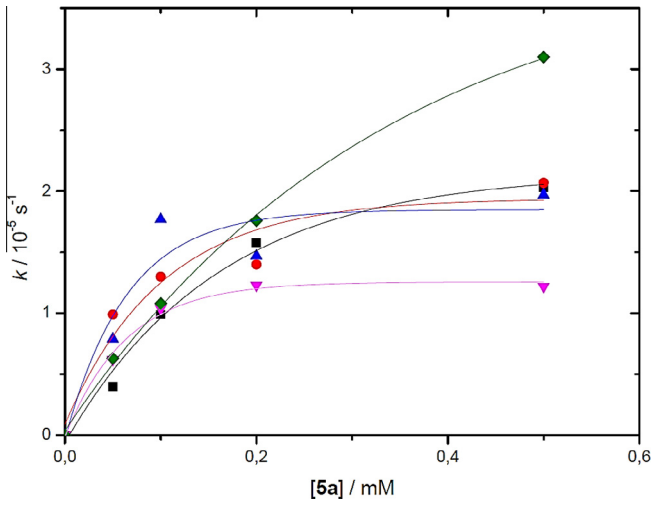


Fig. 2. First-order rate constants for the cleavage of 5′-CUUUC-3′ (■), 5′-CUCUC-3′ (●), 5′-CCUUC-3′ (▲), 5′-CUUCC-3′ (▼) and 5′-ACUUUAC-3′ (◆) at different concentrations of cleaving agent **5a** and Zn^{2+} ($[Zn^{2+}] = 5 \times [\mathbf{6}]$) in 0.1 M HEPES buffer at pH 7.50 (T = 35 °C, I = 0.1 M).

of 6-mer oligoribonucleotides containing only adenosine and cytidine is around 4×10^{-7} s^{-1} when the concentration of the cleaving agent is 0.10 mM ($[Zn(NO_3)_2]$ = 0.30 mM) and the other experimental conditions were those used in the present study. Incorporation of a UpU site within such an oligonucleotide has resulted in a 50-fold rate acceleration ($k = 2.0 \times 10^{-5}$ s^{-1}), the cleavage taking place entirely between the phosphorus and 5′-linked uridine [4b]. As seen from Table 1, rather similar rates were obtained with **5a** in the presence of Zn^{2+}. In other words, increasing the number of potential nucleating sites from 3 to 5 did not enhance the cleaving activity. Half of the maximal rate was usually achieved at $[\mathbf{5a}] \approx 0.1$ mM, *i. e.* when the total concentration of **5a** is double compared to the target. Unexpectedly, oligonucleotides containing three adjacent uridines (5′-CUUUC-3′ and 5′-ACUUUAC-3′) were recognized slightly less efficiently than those containing only two adjacent uridines (5′-CCUUC-3′ and 5′-CUUCC3′) (see Fig. 2). Even 5′-CUCUC-3′ was bound at least as efficiently as 5′-CUUUC-3′. The original aim of high affinity binding was not realized with **5a**. Evidently only two of the azacrown moieties are simultaneously engaged in Zn^{2+} mediated binding to uracil bases. Which ones among the five azacrown participate in anchoring, may depend on the target sequence and, hence, the selectivity may be even lower than with the trinucleating **2**. It is worth noting that there is no direct evidence of **5a** being predominantly present as a pentanuclear complex under the experimental conditions. The minimum requirement for selective cleavage is that four out of the five azacrown moieties of **5a** bear Zn^{2+}. To warrant high affinity binding to a UUU-site, the central 1,4,7,10-tetraazacyclododecane unit should bind Zn^{+2} and both of the 1,5,9-triazacyclododecane units one should bind at least one Zn^{2+}. Additionally, one of the 1,5,9-triazacyclododecane units should bear another Zn^{2+} for catalysis of the bond cleavage. Evidently, this kind of tetranuclear species is not formed in sufficient amount.

3. Conclusions

1,7-Bis{[3′,5′-bis[(1,5,9-triazacyclododecan-3-yloxy)methyl]benzyl}-1,4,7,10-tetraazacyclododecane (**5a**) was prepared as a multinucleating ligand that was aimed at selectively recognizing and cleaving, in the presence of Zn^{2+}, oligoribonucleotides containing three consecutive uridine residues. Unfortunately, the affinity to such oligonucleotides was not higher than to their counterparts containing only two adjacent uridines.

4. Experimental

4.1. General methods

The NMR spectra were recorded on a Bruker Avance 400 or 500 NMR spectrometer. The chemical shifts are given in ppm. The mass spectra were recorded on a Bruker micrOTOF-Q ESI-MS system. Oligonucleotides were prepared on an ABI-3400 DNA/RNA synthesizer and purified by RP- HPLC on a Hypersil ODS column (250 × 4.6 mm, particle size 5 μm; flow rate 1 mL min^{-1}, buffer A = 50 mmol L^{-1} aq NH_4OAc and B = 1:1 mixture of buffer A and MeCN, a linear gradient from 0 to 25% buffer B in buffer A in 25 min, followed by a linear gradient from 25% to 40% buffer B in buffer A in 5 min). Aliquots withdrawn from reaction solutions were analyzed by capillary electrophoresis on a Beckman-Coulter P/ACE-MDQ System equipped with a sample-cooling unit. A fused silica capillary (75 μm inner diameter, 50 cm effective length), −20 kV voltage, citrate buffer (0.2 mol L^{-1}, pH 3.1) was used and the detection wavelength was 254 nm. All solutions used in the kinetic experiments were prepared in sterilized water and sterilized equipment was used for their handling.

4.2. N^2,N^4-Bis{2-[1,4,7,10-tetraazacyclododecan-1-yl]ethyl}-N^6-{3-[1,5,9-triazacyclododecan-3-yl]propyl}-1,3,5-triazine-2,4,6-triamine (**6**)

Previously prepared N^2,N^4-bis{2-[4,7,10-tri(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecan-1-yl]ethyl}-6-chloro-1,3,5-triazine-2,4-diamine (**7**) [11] (0.175 mmol, 200 mg) and 3-aminopropyl-1,5,9-tris(*tert*-butoxycarbonyl)-1,5,9-triazacyclododecane (**8**) [12] (0.350 mmol, 185 mg) were dissolved in MeCN (3 mL) and 20 equiv. of *N*,*N*-diisopropylethylamine (600 μL) was added. The mixture was stirred at 50 °C for 10 days, after which 1 equiv. (0.175 mmol, 93 mg) of **8** and 10 equiv. of *N*,*N*-diisopropylethylamine (300 μL) were added. The stirring was continued for 5 days, volatiles were removed under reduced pressure and the product was isolated by silica gel chromatography eluting with a 95:5 (v/v) mixture of CH_2Cl_2 and MeOH containing 1% triethylamine. The desired N^2,N^4-bis{2-[4,7,10-tri(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecan-1-yl]ethyl}-N^6-{3-[1,5,9-tri(*tert*-butoxycarbonyl)-1,5,9-triazacyclododecan-3-yl]propyl}-1,3,5-triazine-2,4,6-triamine (**9**) was obtained in 64% yield (184 mg). The *tert*-butoxycarbonyl protections were removed by treatment with a mixture containing trifluoroacetic acid (4.0 mL), CH_2Cl_2 (3.5 mL) and MeOH (0.75 mL). After completion of the reaction (4.5 h), the mixture was evaporated to dryness and the residue was passed through a Dowex 1X2 ion exchange resin (mesh 200, OH^- form). The yield was 93% (74 mg). ^{1}H NMR (500 MHz, D_2O, pD 7.5) δ 1.13–1.22 (m, 2H), 1.49 (br.s 2H), 1.59–1.63 (m, 4H), 1.69 (br.s, 1H), 2.46-2.83 (m, 48H), 3.25 (br.s, 6H); ^{13}C NMR (126 MHz, D_2O) 24.7, 26.3, 27.9, 35.1, 38.5, 40.2, 43.3, 44.0, 44.8, 46.8, 47.2, 51.0, 52.2, 53.5, 165.2, 167.6; HRMS(ESI): obsd. 734.6630 $[M+H]^+$, Calc. 734.6470 $[M+H]^+$.

4.3. 1,7-Bis[3,5-di(bromomethyl)benzyl]-4,10-di(tert-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane (**11**)

N-(*tert*-Butoxycarbonyloxy)succinimide (2.50 g, 11.62 mmol) in chloroform (30 mL) was added dropwise into the solution of 1,4,7,10-tetraazacyclododecane (1.00 g, 5.80 mmol) in $CHCl_3$ (50 mL) during 7 h. The reaction mixture was stirred 24 h at room temperature and the solvent was removed under reduced pressure.

The residue was suspended in aqueous NaOH (3 M, 50 mL) and the aqueous phase was extracted with $CHCl_3$ (3 × 50 mL). The combined extracts were dried with K_2CO_3 and evaporated to dryness to give 1,7-bis(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane (**10**) in quantitative yield.

1,3,5-Tris(bromomethyl)benzene (7.65 g, 21.62 mmol) was dissolved in $CHCl_3$ (150 mL) and Na_2CO_3 (1.71 g, 16.15 mmol) was added. Compound **10** (1.08 g, 2.90 mmol) in $CHCl_3$ (50 mL) was added dropwise into the reaction mixture during 11 hours at 52 °C. The reaction mixture was refluxed for 3 days at 62 °C, filtrated, and evaporated to dryness. The residue was purified by silica gel chromatography (40–70% EtOAc in hexane), giving **11** in 27% yield (0.722 g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (s, 6H), 4.45 (s, 8H), 3.71 (s, 4H), 3.21–3.57 (m, 8H), 2.55–2.68 (m, 8H), 1.27 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_{ppm} 155.8, 140.8, 138.5, 129.9, 128.3, 79.3, 59.5, 55.2, 46.1, 32.9, 28.4. HRMS(ESI): obsd. 921.0840 $[M+H]^+$, Calcd. 921.0795 $[M+H]^+$.

*4.4. 1,7-Bis{3,5-bis[1,5,9-tri(tert-butoxycarbonyl)-1,5,9-triazacyclododecan-3-yloxymethyl]benzyl}-4,10-di(tert-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane (**13**)*

Preparation of 3-hydroxy-1,5,9-tri(*tert*-butoxycarbonyl)-1,5,9-triazacyclododecane (**12**) has been reported previously [4a]. This compound (1.47 g, 3.02 mmol) was dissolved in dry DMF (10 mL) and added into a solution of **11** (0.56 g, 0.61 mmol) in DMF (10 mL). Sodium hydride (76.2 mg, 3.18 mmol) was added and the mixture was stirred for 3 h at room temperature under nitrogen. MeOH (3 mL) was added and the volatiles were removed under reduced pressure. The residue was dissolved in water (60 mL) and extracted with CH_2Cl_2 (1 × 60 mL, 5 × 30 mL). The combined extracts were dried with Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel chromatography (EtOAc/CH_2Cl_2, 25:75, v/v, containing 1% Et_3N). The yield was 0.570 g (36%). ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.13 (s, 6H), 4.57 (s, 8H), 3.89 (br. s, 4H), 3.65–3.05 (m, 60H), 2.61 (br.s, 8H), 2.09–1.99 (m, 8H), 1.83-1.73 (m, 8H), 1.45 (s, 126H). ^{13}C NMR (126 MHz, $CDCl_3$) δ_{ppm} 156.3, 156.0, 155.6, 139.2, 138.6, 127.3, 125.0, 79.8, 79.6, 79.0, 75.9, 72.0, 58.9, 54.6, 49.5, 47.1, 46.0, 44.7, 29.5, 28.5, 28.3. HRMS(ESI): obsd. 2550.6926 $[M+H]^+$, Calcd. 2550.6779 $[M+H]^+$.

*4.5. 1,7-Bis{[3′,5′-bis[(1,5,9-triazacyclododecan-3-yloxy)methyl]benzyl}-1,4,7,10-tetraazacyclododecane (**5a**)*

Compound **13** (0.18 g, 0.07 mmol) was dissolved in MeOH (8 mL). Aqueous HCl (6 M, 1.6 mL) was added and the mixture was stirred for 5 h at 42 °C. The volatiles were removed under reduced pressure and the residue was coevaporated with H_2O (5 × 10 mL). The residue was dissolved in water (40 mL) and Dowex 1X2 resin (100–200 mesh, OH^- form) was added and the resulting mixture was stirred for 3 h. The resin was collected by filtration and washed with H_2O (3 × 15 mL) and the filtrate was evaporated to dryness. The yield was 78 mg (98%). ^{1}H NMR (500 MHz, D_2O/MeOD, 3:2, v/v) δ_{ppm} 7.21 (s, 4H), 7.17 (s, 2H), 4.42 (s, 8H), 3.55 (s, 4H), 3.50–3.45 (m, 4H), 2.81–2.73 (m, 16H), 2.66–2.56 (m, 32H), 2.46 (br.s, 16H), 1.55–1.48 (m, 16H). ^{13}C NMR (126 MHz, D_2O/MeOD, 3:2, v/v) δ_{ppm} 139.8, 138.3, 128.2, 126.8, 76.1, 70.8, 58.3, 50.7, 49.5, 48.0, 47.8, 43.3, 24.6. HRMS (ESI): obsd. 1149.9405 $[M+H]^+$, Calcd. 1149.9438 $[M+H]^+$. UV: λ_{max} 265 nm and 275 nm.

4.6. Synthesis of oligonucleotides

Oligoribonucleotides were synthesized from commercially available 2′-*O*-triisopropylsilyloxymethyl (2′-*O*-TOM) protected phosphoramidite building blocks (Glenn Research) by conventional phosphoramidite strategy using the standard RNA-coupling protocol of ABI-3400 DNA/RNA synthesizer and a 1.0 μmol L^{-1} scale. The oligonucleotides were released from support by treating with concentrated aqueous ammonia (1.5 mL) for 2 h at RT and then 4 hours at 55 °C. 2′-*O*-TOM protecting groups were removed with triethylamine trihydrofluoride (160 μL) in DMSO (600 μL) by heating the mixture for 30 min at 65 °C. Aqueous sodium acetate (0.1 M, 600 μL) was added and the heating was continued for 30 min at 65 °C to complete the removal of the hydroxymethyl remnants of the TOM groups. The reaction mixture was cooled on an ice bath to RT and the crude oligoribonucleotides were purified by RP-HPLC and desalted. The identity of the oligonucleotide sequences was verified by MS analysis. 5′-CUUUC-3: *m*/*z* obsd. (Calcd.) 732.1 (732.4) $[M-2H]^{2-}$, 487.7 (488.0) $[M-3H]^{3-}$, 365.5 (365.7) $[M-4H]^{4-}$. 5′-CUCUC-3′: *m*/*z* obsd. (Calcd.) 731.6 (732.0) $[M-2H]^{2-}$, 487.4 (487.6) $[M-3H]^{3-}$, 365.3 (365.5) $[M-4H]^{4-}$. 5′-CCUUC-3′: *m*/*z* obsd. (Calcd.) 731.6 (732.0) $[M-2H]^{2-}$, 487.4 (487.6) $[M-3H]^{3-}$, 365.3 (365.5) $[M-4H]^{4-}$. 5′-CUUCC-3′: *m*/*z* obsd. (Calcd.) 731.6 (732.0) $[M-2H]^{2-}$, 487.4 (487.6) $[M-3H]^{3-}$, 365.3 (365.5) $[M-4H]^{4-}$. 5′-ACUUUAC-3′: *m*/*z* obsd. (Calcd.) 707.1 (707.4) $[M-3H]^{3-}$, 530.1 (530.3) $[M-4H]^{4-}$, 423.8 (424.1) $[M-5H]^{5-}$.

4.7. Kinetic experiments

The reactions were carried out in Eppendorf tubes immersed in a water bath, the temperature of which was kept at 35 ± 0.1 °C. The pH was adjusted to 7.5 with a HEPES buffer (0.1 M). The concentration of the target oligonucleotide was 50 μM, potassium 4-nitrobenzenesulfonate (0.1 mM) was used as an internal standard and the ionic strength was adjusted to 0.1 M with sodium nitrate. The solution of the Zn^{2+} complex was prepared by mixing stoichiometric amounts of compound **5a** and $Zn(NO_3)_2$. In other words, the Zn^{2+} ion concentration was 0.25 mM, 0.5 mM, 1.0 mM or 2.5 mM and the concentration of **5a** 0.05 mM, 0.1 mM, 0.2 mM or 0.5 mM, respectively. The total volume of the reaction mixture was 200 μL and aliquots of 20 μL were withdrawn at suitable time intervals. The reaction was quenched by adding aqueous hydrogen chloride (1 μL of 1.0 M solution). The aliquots were then cooled to 0 °C and analyzed immediately by capillary zone electrophoresis using a fused silica capillary. The temperature of the capillary was kept at 25 °C. The samples were injected using hydrodynamic injection with 2 psi for 8 s. The capillary was flushed for 3 min with water, 10 mM aqueous hydrogen chloride and the background electrolyte buffer (0.2 M citrate buffer, pH = 3.1) between every analytical run.

First-order rate constants were calculated by applying the integrated first-order rate law to the diminution of the signal of the starting material. The peak area was first normalized by dividing the area by the migration time and then by the similarly normalized area of the internal standard.

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