Zn^{2+} Complexes of 3,5-Bis[(1,5,9-triazacyclododecan-3-yloxy)methyl]phenyl Conjugates of Oligonucleotides as Artificial RNases: The Effect of Oligonucleotide Conjugation on Uridine Selectivity of the Cleaving Agent

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2-(3,5-Bis{[1,5,9-tris(trifluoroacetyl)-1,5,9-triazacyclododecan-3-yloxy]methyl}phenoxy)ethanol was synthesized and converted to a *O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite building block, **12**. 2′-*O*-Methyl oligoribonucleotides incorporating a 2-[(2*S*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)ethyl 4-oxopentanoate or a 2-{2-[2-({[(2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl]acetyl}amino)ethoxy]ethoxy}ethyl 4-oxopentanoate non-nucleosidic unit close to the 3′-terminus were assembled on a solid support, the 4-oxopentanoyl protecting groups were removed by treatment with hydrazinium acetate on-support, and **12** was coupled to the exposed OH function. The deprotected conjugates were purified by HPLC, and their ability to cleave a complementary RNA containing either uridine or some other nucleoside at the potential cleaving site was compared. Somewhat unexpectedly, conjugation to an oligonucleotide did not enhance the catalytic activity of the Zn^{2+}–bis(azacrown) complex and virtually abolished its selectivity towards the uridine sites.

Introduction. – Metal ion chelates conjugated to either an oligonucleotide or a peptide nucleic acid (PNA) probe have received considerable interest as man-made restriction enzymes, with which large RNA molecules could be tailored in a sequence-selective manner [1]. Upon hybridization of the probe with its complementary target sequence, the concentration of the cleaving agent in the vicinity of one particular phosphodiester linkage is increased, resulting in accelerated chain cleavage. Since metal ion chelates are able to cleave phosphodiester bonds only within single-stranded regions [2], the cleaving agent is usually incorporated either into a terminal position of the probe, or the base sequence of the probe is designed to form upon hybridization a bulge opposite to an intrachain-cleaving agent. Owing to the proximity effect, the cleaving activity of the conjugated chelate typically is 100-fold compared to the chelate monomer [3], although, in special cases, more marked accelerations have been reported. For example, a 2′-*O*-methyl oligoribonucleotide bearing two 3-(3-hydroxypropyl)-1,5,9-triazacyclododecane ligands on a 2-hydroxyethyl 3′-*O*-(2-hydroxyethyl)-β-D-ribofuranoside branching unit (**1**; *Fig. 1*) exhibits, as a Zn^{2+} complex, 1000-fold cleaving activity compared to monomeric Zn^{2+} chelate of 1,5,9-triazacyclododecane [4], and a Cu^{2+} complex of 2,9-dimethyl-1,10-phenanthroline (**2**) experiences an even greater increase in catalytic activity when tethered into an intrachain position within a PNA probe creating a tetranucleotide bulge [5].

Interestingly, a base-moiety-selective cleavage may also be achieved by dinucleating ligands. (1,5,9-Triazacyclododecan-3-yl)oxy groups, when attached to an aromatic

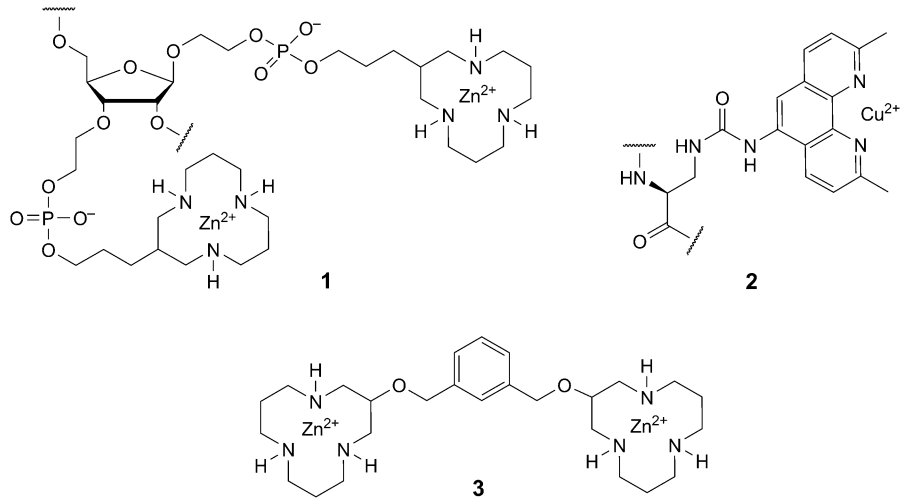


Fig. 1. *Structures of metal ion-based cleaving agents of RNA phosphodiester linkages*

scaffold, **3**, cleave RNA at the 5′-side, and, to a somewhat lesser extent, at the 3′-side, of uridines [6]. Evidently, one of the Zn^{2+}–azacrown moieties anchors the cleaving agent to the uracil base, while the other azacrown moiety serves as a catalyst for the phosphodiester transesterification. Owing to this proximity effect, the cleavage is up to two orders of magnitude faster at a uridine site than at an adenosine or a cytidine site. While this difference in reaction rate is sufficient for uridine-selective cleavage of short oligonucleotides, random background cleavage still is too fast to allow controlled manipulation of sequences longer than 20–30 nucleotides [6c]. Bearing in mind that conjugation of a metal ion chelate to an oligonucleotide increases, owing to the proximity effect, its cleaving activity, one might expect this to be the case also with **3**, leading to increasingly selective cleavage at a single uridine site. The present study is aimed at elucidating whether exploitation of the uracil-anchoring ability of **3** in synergy with sequence recognition by a 2′-*O*-methyl oligoribonucleotide probe really enhances the catalytic efficiency and, hence, ensures cleavage precisely at a single phosphodiester bond. Accordingly, oligonucleotide conjugates depicted in *Fig. 2* have been prepared, and their ability to cleave various oligoribonucleotide targets has been studied.

Results. – *Preparation of 2-{3,5-Bis[(1,5,9-triazacyclododecan-3-yloxy)methyl]-phenoxy}ethanol and Its Conversion to a Phosphoramidite Building Block.* Commercially available dimethyl 5-hydroxyisophthalate was converted to its allyl ether **4**, and the COOMe groups were reduced to CH_2OH functions with $LiAlH_4$ in Et_2O (*Scheme 1*). The dimethanol **5** obtained was then tosylated in dioxane to **6**, and the Ts groups were replaced by 1,5,9-tris[(*tert*-butoxy)carbonyl]-1,5,9-triazacyclododecan-3-ol (**7**), prepared as described in [6a]. The allyloxy group of the bis(azacrown) conjugate **8** was converted to 2-oxoethoxy group by OsO_4 hydroxylation and subsequent $NaIO_4$ oxidation, and the resulted aldehyde **9** was reduced with $NaBH_4$ to **10**. Finally, the Boc protections were replaced with CF_3CO (Tfa) groups (→**11**), and

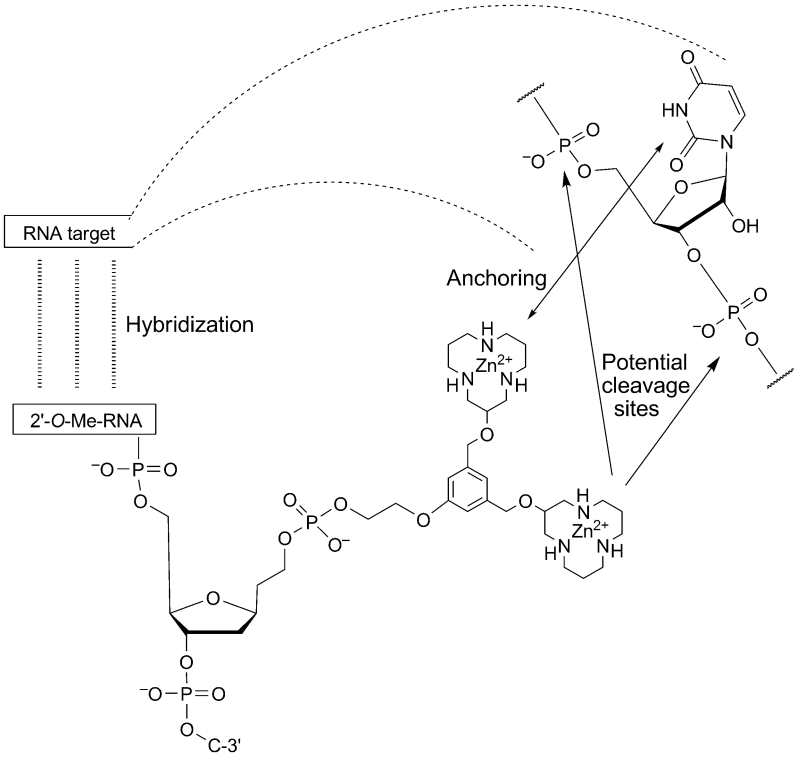


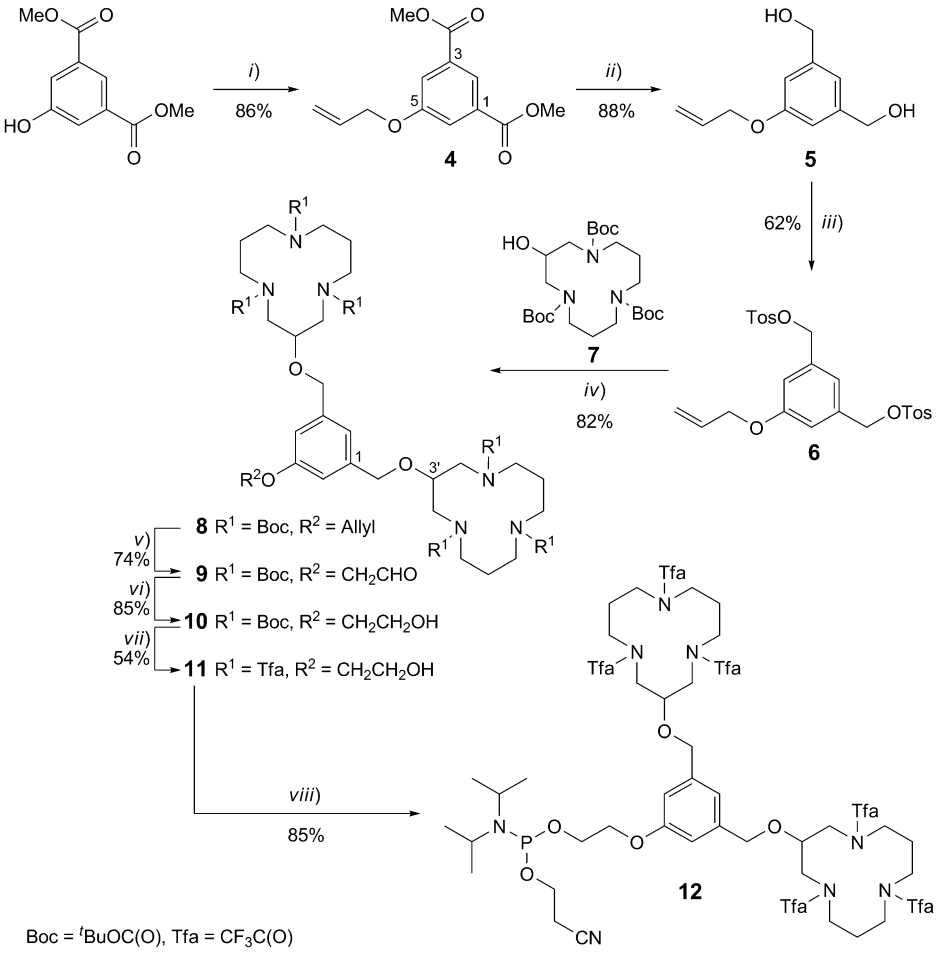
Fig. 2. *The underlying principle of action of the artificial RNases prepared*

the OH function was phosphitylated to 2-cyanoethyl *N*,*N*-diisopropylphosphoramidite **12**.

Preparation of Non-Nucleosidic Phosphoramidite Building Blocks. Non-nucleosidic building blocks allowing attachment of the bis(azacrown) conjugate as a phosphoramidite reagent, **12**, to the oligonucleotide chain were prepared as follows. 2-[(2*S*,4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-hydroxytetrahydrofuran-2-yl]ethyl 4-oxopentanoate (**13**) was obtained as described in [7]. Its analog **17**, with a longer side chain at C(2), was synthesized by subjecting the earlier prepared [7] ethyl [(2*R*,4*S*,5*R*)-5-{[bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-{[(*tert*-butyl)(dimethyl)silyl]oxy}tetrahydrofuran-2-yl]acetate (**14**) to aminolysis with 2-[2-(2-aminoethoxy)ethoxy]ethanol to obtain **15**, acylating the OH function with $(Lev)_2O$ (→**16**), and removing the TBDMS group with Bu_4NF in THF (→**17**; *Scheme 2*). Compounds **13** and **17** were then converted to phosphoramidite building blocks **18** and **19**, respectively, by conventional methods.

Synthesis of Oligonucleotide Bis(azacrown) Conjugates **20** *and* **21**. Conjugates of 2′-*O*-methyl oligoribonucleotides bearing the bis(azacrown) cleaving agent at the penultimate site from the 3′-terminus were assembled by the conventional phosphoramidite chemistry on a commercially available support with N^4-benzoyl-5′-*O*-[bis(4-methoxyphenyl)(phenyl)methyl]-2′-*O*-methylcytidine as the 3′-terminal nucleoside

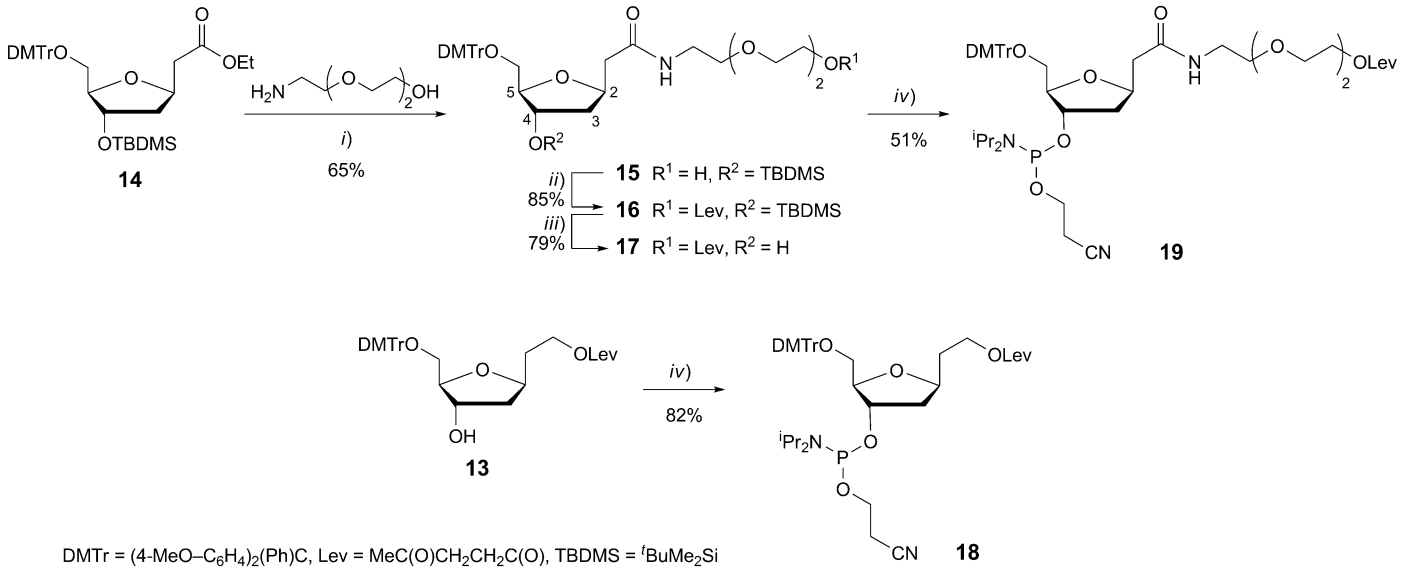
Scheme 1. *Synthesis of 2-[3,5-Bis({[1,5,9-tris(trifluoroacetyl)-1,5,9-triazacyclododecan-3-yl]oxy}methyl)phenoxy]ethyl 2-Cyanoethyl N,N-Diisopropylphosphoramidite* (**12**)



i) Allyl bromide, EtN^iPr_2, DMF. *ii*) $LiAlH_4$, Et_2O. *iii*) TosCl, NaOH, H_2O, dioxane. *iv*) **7**, NaH, DMF. *v*) OsO_4, $NaIO_4$, H_2O, dioxane. *vi*) $NaBH_4$, EtOH. *vii*) 1. TfaOH, CH_2Cl_2; 2. TfaOMe, Et_3N, MeOH; 3. $(Tfa)_2O$, pyridine, CH_2Cl_2; 4. Et_3N, MeOH. *viii*) 2-Cyanoethyl *N,N*-diisopropylphosphonamidic chloride, Et_3N, CH_2Cl_2.

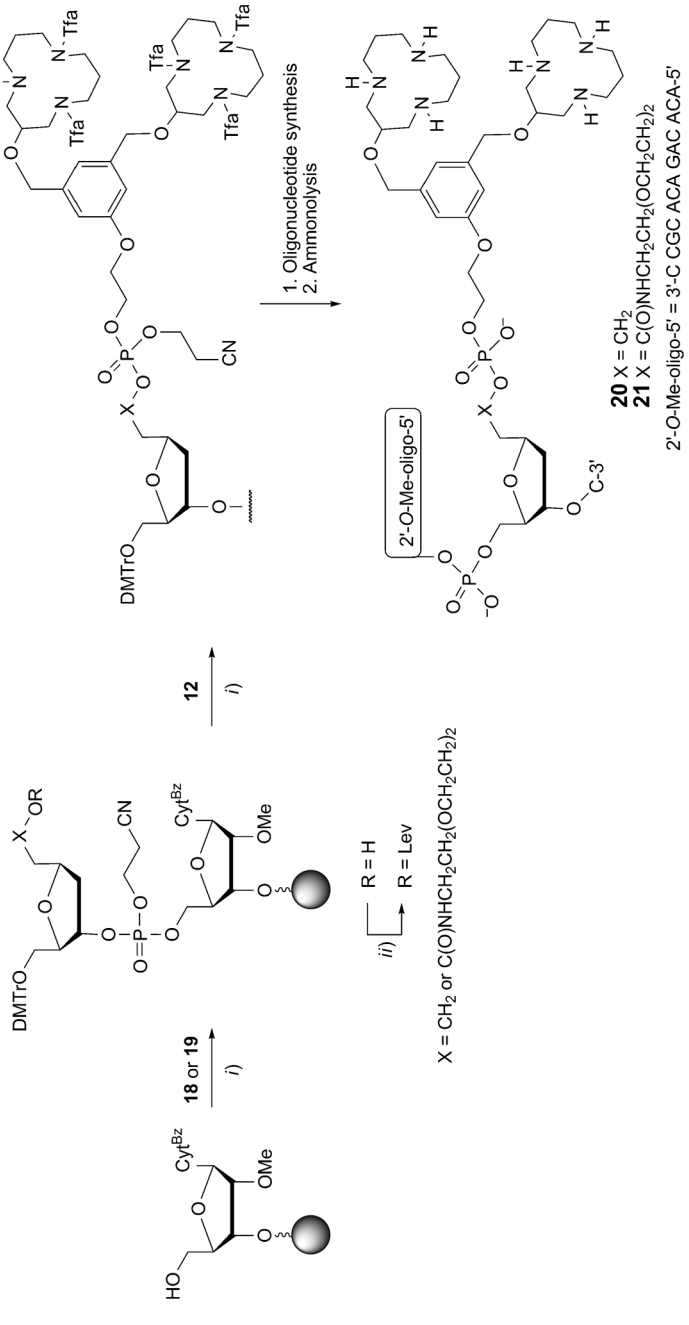
(*Scheme 3*). The 5′-*O*-protection was removed, and the non-nucleosidic building block, either **18** or **19**, was coupled manually using a prolonged coupling time. The Lev group was removed from the non-nucleosidic block by H_2NNH_3OAc treatment in pyridine, and the bis(azacrown) block **12** was coupled manually, using again a prolonged coupling time. The support was then loaded on the synthesizer, and the 2′-*O*-methyl oligoribonucleotide sequence 5′-ACA CAG ACA CGC CZC-3′ (Z stands for the non-nucleosidic block) was assembled, and the support was subjected to ammonolysis to obtain the fully deprotected conjugate **20** or **21**.

Scheme 2. *Synthesis of Non-Nucleosidic Building Blocks Compatible with Oligonucleotide Synthesis by the Phosphoramidite Strategy*



i) 1. 1,4-Dioxane, aq. KOH; 2. *Dowex-50* (pyridinium form), pyridine; 3. HBTU (= *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate), EtN^iPr_2, DMF. *ii*) $(Lev)_2O$ (= levulinic anhydride), pyridine. *iii*) Bu_4NF, THF. *iv*) 2-Cyanoethyl *N*,*N*-diisopropylphosphonamidic chloride, Et_3N, CH_2Cl_2.

Scheme 3. *Synthesis of Oligonucleotide Bis(azacrown) Conjugates*



i) 5-Benzylthiotetrazole, MeCN. *ii*) H_2NNH_3OAc, AcOH, pyridine.

Hybridization of Oligonucleotide Bis(azacrown) Conjugate **21** *with Chimeric Oligoribonucleotide/2′-*O*-Methyl Oligoribonucleotide Targets. Table 1* records the melting temperatures for duplexes of conjugate **21** with several chimeric oligoribonucleotide/2′-*O*-methyl oligoribonucleotide targets, **22**–**27**. All the targets are fully complementary to **21** over the 3′-terminal sequence, 3′-UGU GUC UGU GCG C-5′, but contain different 5′-terminal ribonucleotide overhangs. For comparative purposes, the melting temperatures of the duplexes of unconjugated 2′-*O*-methyl oligoribonucleotide 5′-ACA CAG ACA CGC C-3′ (**28**) with the same targets are also included.

Table 1. *Melting Temperatures* (T_m [°]) *of the Duplexes of Oligonucleotide Conjugate* **21** *and Reference Oligonucleotide* **28** *with Chimeric Targets* **22**–**27** *in the Absence and Presence of* Zn^{2+} *Ion at pH 7.0.* The data refers to [oligomer] = 2 μM and [Zn^{2+}] = 10 μM at the ionic strength of 0.1M, adjusted with NaCl. pH was adjusted with 10 mM phosphate buffer. Bold letters refer to ribonucleotides, normal letters to 2′-*O*-methyl ribonucleotides.

Target	**21**		**28**	
	with Zn^{2+}	without Zn^{2+}	with Zn^{2+}	without Zn^{2+}
3′-UGU GUC UGU **GCG GAC AAC AA**-5′ (**22**)	76.1	78.3	73.8	74.9
3′-UGU GUC UGU **GCG GUA AAC AA**-5′ (**23**)	76.7	82.0	73.3	75.0
3′-UGU GUC UGU **GCG GAU AAC AA**-5′ (**24**)	76.7	79.7	73.5	76.1
3′-UGU GUC UGU **GCG GAA UAC AA**-5′ (**25**)	77.5	79.8	75.0	74.6
3′-UGU GUC UGU **GCG GAA AUC AA**-5′ (**26**)	78.2	78.5	74.5	74.4
3′-UGU GUC UGU **GCG GAA AAU AA**-5′ (**27**)	75.9	77.9	75.5	74.4

The data in *Table 1* clearly show that conjugate **21** hybridizes with all the targets even slightly better than its unconjugated counterpart **28**. The duplexes formed are stable at 35°, the temperature at which the kinetic measurements were carried out. Addition of Zn^{2+} still enhanced the hybridization of **21**, the influence being most prominent with target **23**. In this case, the melting temperature, T_m, is increased by 5.3°, while with the unconjugated reference oligonucleotide **28** the increment is 1.7°, and with target **22**, containing no uridine within the overhang, addition of Zn^{2+} increases the T_m value of the duplex with conjugate **21** by 2.2°. Evidently, the cleaving agent really recognizes the uracil base within the overhang of **23**.

Cleaving Activity of the Zn^{2+} *Complexes of Oligonucleotide Bis(azacrown) Conjugates* **20** *and* **21**. It has been shown previously [6c] that the dinuclear Zn^{2+} complex of bis(azacrown) monomer **3** cleaves oligoribonucleotide 5′-CAAUAC-3′ 32 times faster than oligoribonucleotide 5′-CAACAC-3′, the cleavage taking place predominantly on both sides of the uridine residue [6c]. The rate constants referring to 100 μM concentration of **3**(Zn^{2+})$_2$ at pH 7.5 and 35° (I = 0.1M) have been reported to be $(7.70 \pm 0.05) \cdot 10^{-6}\ s^{-1}$ and $(0.24 \pm 0.03) \cdot 10^{-6}\ s^{-1}$, respectively. As discussed in more detail in [6], the marked rate acceleration accompanying the replacement of the intrachain cytidine with uridine may in all likelihood be attributed to anchoring of **3**(Zn^{2+})$_2$ through one of the azacrown chelates to the uracil base, the other chelate serving as the cleaving agent. Since oligonucleotide conjugate **21** in the presence of Zn^{2+} clearly recognizes the uracil base within the overhang of target **23**, one might expect this anchoring to lead to enhanced, highly specific cleavage of the overhang at the site of anchoring. However, this is not the case. *Table 2* contains the rate constants

for the cleavage of targets **22**–**27** by conjugates **20** and **21** in the presence of Zn^{2+}. On the contrary, target **22** containing no uridine in the overhang was cleaved even more efficiently than the uridine-containing targets. In other words, tethering of **3** to a sequence recognizing oligonucleotide does not enhance but rather retards its uridine-selective cleaving ability. Tentatively, one might speculate that the tethering of **3** results in constrain that prevents **3** to interact simultaneously with the uracil base and the adjacent phosphodiester linkage. This assumption is supported by the finding that even slightly higher cleavage rates were observed when, instead of conjugate **20** of **21**, a mixture of unconjugated complementary oligonucleotide **28** and monomeric **3** was used at $[Zn^{2+}]$ of 90 μM to promote the reaction (*Table 2*). Accordingly, the situation appears to be similar to that reported in [3c] for mono(azacrown) conjugates of oligonucleotides: uracil base in the target sequence retards the cleavage by binding to the cleaving agent. Consistent with this, the slower cleavage of target **23** compared to target **24** probably results from the presence of guanosine next to uridine. It is known [6b] that **3**$(Zn^{2+})_2$ tends to interact with both bases in 3′-GpU-5′, although a monomeric Zn^{2+} chelate binds to guanine base less firmly than to uracil base. Hence, both azacrown groups on **20** or **21** may be largely involved in anchoring to **23**.

Table 2. *Rate Constants* (k $[10^{-6}\ s^{-1}]$) *for the Cleavage of Chimeric Oligonucleotides* **22**–**27** *by Complementary Oligonucleotide Conjugates* **20** *and* **21** *at pH 7.3 and 35°* ($I = 0.1$M). The data refers to [oligomer] = [**3**] = 18 μM and $[Zn^{2+}] = 90$ μM at the ionic strength of 0.1M. pH was adjusted with a *HEPES* buffer. Bold letters refer to ribonucleotides, the rest to 2′-*O*-methyl ribonucleotides.

Target	**20**	**21**	**28** + **3**
3′-UGU GUC UGU **GCG GAC AAC AA**-5′ (**22**)	2.44 ± 0.07	1.66 ± 0.08	0.8 ± 0.1
3′-UGU GUC UGU **GCG GUA AAC AA**-5′ (**23**)	1.09 ± 0.04	0.19 ± 0.03	0.9 ± 0.1
3′-UGU GUC UGU **GCG GAU AAC AA**-5′ (**24**)	2.70 ± 0.10	0.44 ± 0.03	4 ± 1
3′-UGU GUC UGU **GCG GAA UAC AA**-5′ (**25**)	0.39 ± 0.04	0.25 ± 0.02	0.5 ± 0.1
3′-UGU GUC UGU **GCG GAA AUC AA**-5′ (**26**)		0.82 ± 0.05	0.8 ± 0.1
3′-UGU GUC UGU **GCG GAA AAU AA**-5′ (**27**)		0.79 ± 0.08	1.5 ± 0.2

Experimental Part

General. DMF and THF were dried over 3-Å, and CH_2Cl_2, MeCN, and pyridine over 4-Å molecular sieves. Et_3N was dried by distillation and storage over CaH_2. NMR Spectra: *Bruker Avance* at 500 MHz. The chemical shifts, δ, in ppm from internal Me_4Si and the coupling constants J in Hz; appropriate 2D-NMR methods (COSY, HSQC, and HMBC) used for peak assignment. ESI-MS: in m/z.

Dimethyl 5-(Allyloxy)isophthalate (= *Dimethyl 5-(Prop-2-en-1-yloxy)benzene-1,3-dicarboxylate*; **4**). Dimethyl 5-hydroxyisophthalate (= dimethyl 5-hydroxybenzene-1,3-dicarboxylate; 4.9 g, 23 mmol), EtN^iPr_2 (8.2 ml, 46 mmol), and allyl bromide (5.6 g, 46 mmol) were dissolved in DMF (5.0 ml), and the mixture was stirred at r.t. for 48 h. H_2O (50 ml) was added, and the product was extracted with Et_2O (3 × 30 ml). The combined org. layers were washed with sat. $NaHCO_3$, dried (Na_2SO_4), and evaporated to dryness. The crude product was purified by silica-gel column chromatography (CC) to yield **4** (5.0 g, 86%). Colorless solid flakes. ^{1}H-NMR (500 MHz, $CDCl_3$): 8.29 (*t*, $J = 1.2$, H–C(2)); 7.77 (*d*, $J = 1.3$, H–C(4), H–C(6)); 6.06 (*m*, $CH_2CH{=}CH_2$); 5.46 (*m*, 1 H, $CH_2CH{=}CH_2$); 5.34 (*m*, 1 H, $CH_2CH{=}CH_2$); 4.64 (*m*, $CH_2CH{=}CH_2$); 3.95 (*s*, 2 MeO). ^{13}C-NMR (125 MHz, $CDCl_3$): 166.1 (*C*OOMe); 158.6 (C(5)); 132.4 ($CH_2CH{=}CH_2$); 131.7 (C(1), C(3)); 123.1 (C(2)); 120.1 (C(4), C(6)); 118.2 ($CH_2CH{=}CH_2$); 69.2 ($CH_2CH{=}CH_2$); 52.4 (MeO).

(5-Allyloxy-1,3-phenylene)dimethanol (= *[5-(Prop-2-en-1-yloxy)benzene-1,3-diyl]dimethanol*; **5**). $LiAlH_4$ (0.93 g, 25 mmol) was slowly added to a mixture of **4** (2.1g, 8.3 mmol) in Et_2O (25 ml) at 0°. The mixture was allowed to warm up and stirred for 3 h at r.t. The reaction was quenched by addition of H_2O (100 ml), and the product was extracted with AcOEt (3 × 50 ml). The combined org. layers were washed with sat. aq. NaCl, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by CC (5% MeOH in CH_2Cl_2) to afford **5** (1.4 g, 88%). Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 6.87 (*s*, H–C(2)); 6.78 (*s*, H–C(4), H–C(6)); 6.05 (*m*, CH_2CH=CH_2); 5.41 (*m*, 1 H, CH_2CH=CH_2); 5.29 (*m*, 1 H, CH_2CH=CH_2); 4.55 (*d*, *J* = 5.0, 2 CH_2OH); 4.51 (*m*, CH_2CH=CH_2); 3.08 (*t*, *J* = 5.0, CH_2OH). ^{13}C-NMR (125 MHz, $CDCl_3$): 158.9 (C(5)); 142.8 (C(1), C(3)); 133.2 (CH_2CH=CH_2); 117.74 (CH_2CH=CH_2); 117.71 (C(2)); 112.3 (C(4), C(6)); 68.8 (CH_2CH=CH_2); 64.8 (CH_2OH).

[(5-Allyloxy-1,3-phenylene)bis(methylene)]bis(4-methylbenzenesulfonate) (= *[5-(Prop-2-en-1-yloxy)-benzene-1,3-diyl]dimethanediyl Bis(4-methylbenzenesulfonate)*; **6**). TosCl (6.2 g, 33 mmol) in Et_2O (6.0 ml) was slowly added under stirring to a mixture of **5** (2.1 g, 11 mmol), NaOH (1.7 g, 43 mmol), H_2O (9.0 ml), and THF (5.0 ml) at 0°. Stirring was continued for 4 h at 0°, sat. aq. $NaHCO_3$ was added, and then the crude product was extracted with AcOEt (3 × 50 ml). The combined org. layers were dried (Na_2SO_4) and evaporated to dryness. CC (3% MeOH in CH_2Cl_2) of the residue yielded 3.4 g (62%) of **6** as colorless oil and 0.76 g of incompletely tosylated diol (*i.e.*, monotosylated **5** as colorless oil).

Data for **6**. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.79 (*m*, 4 arom. H, Tos); 7.34 (*m*, 4 arom. H, Tos); 6.74 (*m*, H–C(4), H–C(6)); 6.69 (*m*, H–C(2)); 5.99 (*m*, CH_2CH=CH_2); 5.39 (*m*, 1 H, CH_2CH=CH_2); 5.30 (*m*, 1 H, CH_2CH=CH_2); 4.96 (*s*, 2 CH_2OH); 4.44 (*m*, CH_2CH=CH_2); 2.46 (*s*, 2 Me). ^{13}C-NMR (125 MHz, $CDCl_3$): 158.9 (C(5)); 145.1 (C(1), C(3)); 135.3 (Tos)); 133.1 (Tos)); 132.6 (CH_2CH=CH_2); 129.9 (Tos)); 128.0 (Tos)); 120.4 (CH_2CH=CH_2); 118.0 (C(2)); 115.1 (C(4), C(6)); 71.2 (CH_2CH=CH_2); 68.9 (CH_2OH); 21.7 (Me).

*Hexa(*tert*-butyl) 3,3′-{{[5-(2-Oxoethoxy)-1,3-phenylene]bis(methylene)}bis(oxy)}bis(1,5,9-triazacyclododecane-1,5,9-tricarboxylate)* (= *Hexa(*tert*-butyl) 3,3′-{[5-(2-Oxoethoxy)benzene-1,3-diyl]bis(methanediyloxy)}bis(1,5,9-triazacyclododecane-1,5,9-tricarboxylate)*; **9**). NaH (39 mg of 60% dispersion in mineral oil, 0.97 mmol) was added to a mixture of **6** (0.15 g, 0.30 mmol), *tri(*tert*-butyl) 3-hydroxy-1,5,9-triazacyclododecane-1,5,9-tricarboxylate* (**7**; 0.31 g, 0.64 mmol) and DMF. The mixture was stirred for 1 h at r.t., the reaction was quenched by slow addition of MeOH (0.5 ml) and H_2O (20 ml), and the product was extracted with Et_2O (6 × 15 ml). The org. fractions were combined, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by CC (40% AcOEt in CH_2Cl_2) to yield 0.28 g (82%) of *hexa(*tert*-butyl) 3,3′-{{[5-(allyl)oxy-1,3-phenylene]bis(methylene)}bis(oxy)}bis(1,5,9-triazacyclododecane-1,5,9-tricarboxylate)* (= *hexa(*tert*-butyl) 3,3′-{[5-(prop-2-en-1-yloxy)benzene-1,3-diyl]bis(methanediyloxy)}bis(1,5,9-triazacyclododecane-1,5,9-tricarboxylate)*; **8**) as colorless oil.

Compound **8** (0.40 g, 0.35 mmol) was dissolved in a mixture of 1,4-dioxane (4.0 ml) and H_2O (1.0 ml), and a soln. of OsO_4 in *t*BuOH (1:39 (*w*/*w*); 56 µl, 4.4 µmol) was added. The mixture was stirred for 30 min at r.t., cooled to 0°, a soln. of $NaIO_4$ (0.17 g, 0.80 mmol) in H_2O (1.0 ml) was added, and stirring was continued for 2 h. Sat. aq. $NaHCO_3$ (10 ml) was added to the mixture, and the product was extracted with AcOEt (3 × 5 ml). The combined org. layers were evaporated to dryness, and the residue was subjected to a CC (3% MeOH in CH_2Cl_2) to yield **9** (0.30 g, 74%). White foam. ^{1}H-NMR (500 MHz, $CDCl_3$): 9.85 (*s*, CH_2CH=O); 6.85 (*m*, H–C(2), H–C(4), H–C(6)); 4.62 (*s*, CH_2CH=O); 4.58 (*s*, 2 CH_2OH); 4.20–2.90 (*m*, 26 H, azacrown); 2.02 (*m*, 4 H, azacrown); 1.77 (*m*, 4 H, azacrown); 1.55 (*s*, 18 Me). ^{13}C-NMR (125 MHz, $CDCl_3$): 199.1 (CH_2CH=O); 158.1 (C(5)); 156.3 (Boc); 156.0 (Boc); 140.5 (C(1), C(3)); 119.7 (C(2)); 112.8 (C(4), C(6)); 80.0 (CH_2CH=O); 79.7 (Boc); 75.6 (azacrown); 72.7 (CH_2O); 71.3, 53.4, 49.3, 47.0, 44.9, 29.5 (azacrown); 28.4 (Boc).

3,3′-{{[5-(2-Hydroxyethoxy)-1,3-phenylene]bis(methylene)}bis(oxy)}bis(1,5,9-triazacyclododecane-1,5,9-triyl)tris(2,2,2-trifluoroethanone) (= *1,1′,1″,1‴,1⁗,1⁗′-{[5-(2-Hydroxyethoxy)benzene-1,3-diyl]-bis(methanediyloxy-1,5,9-triazacyclododecane-3,1,5,9-tetrayl)}hexakis(trifluoroethanone)*; **11**). Aldehyde **9** 0.30 g (0.26 mmol) was dissolved in a mixture of CH_2Cl_2 (3.0 ml) and EtOH (4.0 ml), and $NaBH_4$ (27 mg, 0.71 mmol) was added to the mixture. The mixture was stirred for 4 h at r.t., the reaction was quenched by addition of sat. aq. NH_4Cl (10 ml), and the mixture was extracted with CH_2Cl_2 (4 × 10 ml). The combined org. fractions were dried (Na_2SO_4), evaporated to dryness, and the residue was subjected to CC (5% MeOH in CH_2Cl_2) to yield 0.25 g (85%) of *hexa(*tert*-butyl) 3,3′-{{[5-(2-hydroxyethoxy)-1,3-*

phenylene]bis(methylene)}bis(oxy)}bis(1,5,9-triazacyclododecane-1,5,9-tricarboxylate) (= *hexa(*tert-*butyl) 3,3′-{[5-(2-hydroxyethoxy)benzene-1,3-diyl]bis(methanediyloxy)}bis(1,5,9-triazacyclododecane-1,5,9-tricarboxylate)*; **10**). HR-ESI-MS: 1137.7235 ($[M+H]^+$, $C_{58}H_{101}N_6O_{16}^+$; calc. 1137.7274).

TfaOH (1.0 ml) was added to **10** (0.25 g) in CH_2Cl_2 (1.0 ml), and the mixture was stirred overnight at r.t. and evaporated to dryness. The residue was co-evaporated with H_2O, MeOH, and pyridine, dissolved in MeOH (1.0 ml), and Et_3N (0.33 ml, 2.4 mmol) and TfaOMe (0.24 ml, 2.4 mmol) were added. The mixture was stirred overnight at r.t. and evaporated to dryness. Sat. aq. $NaHCO_3$ (10 ml) was added, and the incompletely *N*-trifluoroacetylated products were extracted with AcOEt (3 × 5 ml). The combined org. layers were dried (Na_2SO_4), evaporated to dryness, and the residue was dissolved in a mixture of CH_2Cl_2 (1.0 ml) and pyridine (0.1 ml). $(Tfa)_2O$ (0.17 ml, 1.2 mmol) was added, and the mixture was stirred overnight at r.t. and evaporated to dryness. To expose the trifluoroacetylated OH group, the residue was dissolved in MeOH (1.0 ml) containing Et_3N (50 µl), mixed for 5 h, and evaporated to dryness. $NaHCO_3$ (10 ml) was added to the residue, and the product was extracted with AcOEt (3 × 5 ml). The org. layers were combined, dried (Na_2SO_4), and evaporated to dryness. The crude product was purified by CC (40% AcOEt in CH_2Cl_2) to yield **11** (0.16 g, 54%). White foam. ^{1}H-NMR (500 MHz, CDCl): 6.67–6.70 (*m*, H–C(2), H–C(4), H–C(6)); 4.49–4.54 (*m*, 2 Ar-CH_2O); 3.86–4.16 (*m*, CH_2CH_2OH, 2 H–C(3′)); 2.45–3.86 (*m*, 24 H, azacrown); 2.04–2.44 (*m*, 4 H, azacrown); 1.54–2.01 (*m*, 4 H, azacrown). HR-ESI-MS: 1135.2849 ($[M+Na]^+$, $C_{40}H_{46}F_{18}N_6NaO_{10}^+$; calc. 1135.2886.

3,3′-{{{5-(2-[(2-Cyanoethoxy)(diisopropylamino)phosphinooxy]ethoxy}-1,3-phenylene}bis(methylene)}bis(oxy)}bis(1,5,9-triazacyclododecane-1,5,9-triyl)tris(2,2,2-trifluoroethanone) (= *2-[3,5-Bis({[1,5,9-tris(trifluoroacetyl)-1,5,9-triazacyclododecan-3-yl]oxy}methyl)phenoxy]ethyl 2-Cyanoethyl Dipropan-2-ylphosphoramidoite*; **12**). 2-Cyanoethyl *N,N*-diisopropylaminophosphoro chloridite (= 3-[chloro(diisopropylamino)phosphinooxy]propanenitrile = 2-cyanoethyl dipropan-2-ylphosphoramidochloridoite; 21 µl, 94 µmol) was added to a mixture of **11** (80 mg, 72 µmol), Et_3N (50 µl, 0.36 mmol), and CH_2Cl_2 (1.0 ml) under N_2. The mixture was stirred for 1 h at r.t. and then directly subjected to CC. Elution with Et_3N/AcOEt/petroleum ether 5:70:25 yielded **12** (80 mg, 85%). White foam. ^{1}H-NMR (500 MHz, CD_3CN:) 6.82 (*m*, H–C(2), H–C(4), H–C(6)); 4.53–4.61 (*m*, 2 Ar–CH_2O); 3.89–4.29 (*m*, OCH_2CH_2O, OCH_2CH$_2$CN, 2 H–C(3′)); 2,97–3.89 (*m*, 26 H, azacrown, 2 NC*H*Me$_2$); 2.67–2.78 (*m*, OCH$_2$CH_2CN); 1.76–2.46 (*m*, 8 H, azacrown); 1.19–1.26 (*m*, 4 Me). ^{31}P-NMR (200 MHz, CD_3CN): 148.5. HR-ESI-MS: 1335.4007 ($[M+Na]^+$, $C_{49}H_{63}F_{18}N_8NaO_{11}P^+$; calc. 1335.3964).

*2-[(2*R*,4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-{[(*tert-*butyl)(dimethyl)silyl]oxy}tetrahydrofuran-2-yl]-*N*-{2-[2-(2-hydroxyethoxy)ethoxy]ethyl}acetamide* (**15**). *Ethyl [(2*R*,4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-{[(*tert-*butyl)(dimethyl)silyl]oxy}tetrahydrofuran-2-yl]acetate* (**14**; 0.44 g, 0.71 mmol) [7] was dissolved in 1,4-dioxane (15 ml) and aq. KOH (0.20 g, 3.5 mmol in 5 ml of H_2O) was added. The mixture was stirred overnight at r.t. Solvent was removed *in vacuo*, the residue was dissolved in pyridine (15 ml), and the soln. was stirred with a *Dowex-50* resin (pyridinium form) for 5 h. The resin was filtered off, pyridine was evaporated, and, after co-evaporation with dry pyridine, the residue was dissolved in dry DMF (2 ml). EtN^{i}Pr$_2$ (0.15 ml, 0.85 mmol), HBTU (0.32 g, 0.85 mmol), and 2-[2-(2-aminoethoxy)ethoxy]ethanol (0.15 g, 0.85 mmol) were added, and the mixture was strirred for 2.5 h at r.t. DMF was removed by evaporation, the residue was dissolved in Et_2O, washed with sat. $NaHCO_3$, dried (Na_2SO_4), and evaporated to dryness. Purification by CC (0.1% Et_3N and 3% MeOH in CH_2Cl_2) yielded **15** (0.33 g, 65%). Yellowish oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.23–7.46 (*m*, 9 H, DMTr); 6.85 (*d*, *J* = 8.8, 4 H, DMTr); 6.78 (*t*, *J* = 5.5, NH); 4.46–4.49 (*m*, H–C(5)); 4.25–4.26 (*m*, H–C(2)); 3.95–3.97 (*m*, H–C(4)); 3.81 (*s*, 6 H, DMTr); 3.42–3.69 (*m*, NHCH_2CH_2(OCH_2-CH_2)$_2$OH); 3.14 (*dd*, *J* = 9.9, 4.5, 1 H, C(5)–CH_2O); 3.09 (*dd*, *J* = 9.9, 5.3, 1 H, C(5)–CH_2O); 2.53 (*dd*, *J* = 15.1, 3.8, 1 H, C(2)–CH_2C=O); 2.46 (*dd*, *J* = 15.1, 8.1, 1 H, C(2)–CH_2C=O); 1.91 (*ddd*, *J* = 12.8, 5.2, 1.3, 1 H, CH$_2$(3)); 1.71 (*m*, 1 H, CH$_2$(3)); 0.86 (*s*, ^{t}Bu); 0.03 (*s*, MeSi); 0.01 (*s*, MeSi). ^{13}C-NMR (125 MHz, $CDCl_3$): 171.0 (C=O); 158.5, 144.8, 136.1, 130.1, 128.2, 127.8, 126.8, 113.1, 86.9 (DMTr); 86.1 (C(5)); 75.1 (C(4)); 74.1 (C(2)); 72.5, 70.3, 70.2, 69.9 (NHCH$_2$*C*H$_2$O*C*H$_2$*C*H$_2$O*C*H$_2$CH$_2$OH); 64.1 (C(5)–*C*H$_2$O); 61.7 (OCH$_2$*C*H$_2$OH); 55.2 (DMTr); 42.5 (C(2)); 41.1 (C(2)–*C*H$_2$C=O); 39.1 (N*C*H$_2$CH$_2$O); 25.8 (TBDMS); 18.0 (TBDMS); −4.7 (TBDMS); −4.8 (TBDMS). HR-ESI-MS: 746.3642 ($[M+Na]^+$, $C_{40}H_{57}NNaO_9Si^+$; calc. 746.3695).

*2-{2-[2-({[(2*R*,4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-{[(*tert*-butyl)(dimethyl)silyl]oxy}tetrahydrofuran-2-yl]acetyl}amino)ethoxy]ethoxy}ethyl 4-Oxopentanoate* (**16**). Levulinic anhydride ($(Lev)_2O$; 0.29 g, 1.4 mmol) was added to a soln. of **15** (0.33 g, 0.46 mmol) in pyridine. The mixture was stirred overnight at r.t. and evaporated to dryness. The residue was dissolved in CH_2Cl_2 and washed with sat. aq. $NaHCO_3$. The org. phase was dried (Na_2SO_4) and evaporated to dryness. The crude product was purified by CC (0.1% Et_3N and 3% MeOH in CH_2Cl_2) to yield **16** (0.32 g, 85%). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.21–7.45 (*m*, 9 H, DMTr); 6.84 (*d*, *J* = 8.9, 4 H, DMTr); 6.69 (*t*, *J* = 5.5, NH); 4.43–4.50 (*m*, H–C(5)); 4.25–4.26 (*m*, H–C(2)); 4.21 (*t*, *J* = 4.8, OCH_2CH_2OLev); 3.94–3.96 (*m*, H–C(4)); 3.81 (*s*, 2 MeO); 3.43–3.64 (*m*, $NHCH_2CH_2OCH_2CH_2OCH_2CH_2OLev$); 3.12 (*dd*, *J* = 9.9, 4.5, 1 H, C(5)–CH_2O); 3.07 (*dd*, *J* = 9.9, 5.3, 1 H, C(5)–CH_2O); 2.75 (*t*, *J* = 6.5, 2 H, Lev); 2.60 (*t*, *J* = 6.5, 2 H, Lev); 2.52 (*dd*, *J* = 15.1, 4.0, 1 H, C(2)–CH_2C=O); 2.45 (*dd*, *J* = 15.1, 8.1, 1 H, C(2)–CH_2C=O); 2.20 (*s*, 3 H, Lev); 1.89–1.93 (*m*, 1 H, $CH_2(3)$); 1.67–1.73 (*m*, 1 H, $CH_2(3)$); 0.86 (*s*, ^{t}Bu); 0.02 (*s*, MeSi); 0.01 (*s*, MeSi). ^{13}C-NMR (125 MHz, $CDCl_3$): 206.6 (Lev); 172.7 (Lev); 170.9 (CH_2C(O)NH); 158.5, 144.8, 136.0, 130.1, 128.2, 127.8, 126.8, 113.1, 86.9 (DMTr); 86.0 (C(5)); 75.1 (C(4)); 74.1 (C(2)); 70.5, 70.2, 69.9, 69.0 ($NHCH_2CH_2OCH_2CH_2OCH_2CH_2OLev$); 64.1 ($OCH_2CH_2OLev$); 63.7 (C(5)–$CH_2O$); 55.2 (DMTr); 42.6 (C(3)); 41.3 (C(2)–CH_2C=O); 39.2 ($NHCH_2CH_2O$); 37.9 (Lev); 29.9 (Lev); 27.9 (Lev); 25.8 (TBDMS); 18.0 (TBDMS); −4.6 (TBDMS); −4.8 (TBDMS). HR-ESI-MS: 844.4068 ($[M+Na]^+$, $C_{45}H_{63}NNaO_{11}Si^+$; calc. 844.4063).

*2-{2-[2-({[(2*R*,4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-hydroxytetrahydrofuran-2-yl]acetyl}amino)ethoxy]ethoxy}ethyl 4-Oxopentanoate* (**17**). $Bu_4NF \cdot H_2O$ (0.30 g, 1.1 mmol) was added to a soln. of **16** (0.31 g, 0.38 mmol) in THF (3 ml). The mixture was stirred for 2 h at r.t., and then volatiles were removed. The residue was dissolved in CH_2Cl_2 and washed with H_2O. The org. phase was dried (Na_2SO_4) and evaporated to dryness. The crude product was purified by CC (0.1% Et_3N and 3% MeOH in CH_2Cl_2) to yield **17** (0.21 g, 78%). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.23–7.46 (*m*, 9 H, DMTr); 6.84 (*d*, *J* = 8.9, 4 H, DMTr); 6.68 (*t*, *J* = 5.5, NH); 4.46–4.53 (*m*, H–C(5)); 4.33–4.34 (*m*, H–C(2)); 4.21 (*t*, *J* = 4.8, OCH_2CH_2OLev); 3.98–4.00 (*m*, H–C(4)); 3.81 (*s*, 6 H, DMTr); 3.44–3.65 (*m*, $NHCH_2CH_2OCH_2CH_2OCH_2CH_2OLev$); 3.24 (*dd*, *J* = 9.7, 4.7, 1 H, C(5)–CH_2O); 3.11 (*dd*, *J* = 9.7, 5.7, 1 H, C(5)–CH_2O); 2.75 (*t*, *J* = 6.5, 2 H, Lev); 2.60 (*t*, *J* = 6.5, 2 H, Lev); 2.41–2.54 (*m*, C(2)–CH_2C=O); 2.19 (*s*, 3 H, Lev); 2.04 (*ddd*, *J* = 15.1, 5.6, 2.0, 1 H, $CH_2(3)$); 1.81–1.87 (*m*, 1 H, $CH_2(3)$). ^{13}C-NMR (125 MHz, $CDCl_3$): 206.8 (Lev); 172.8 (Lev); 170.7 (CH_2C(O)NH); 158.5, 144.8, 136.0, 130.1, 128.2, 127.8, 126.8, 113.1, 86.2 (DMTr); 86.2 (C(5)); 75.0 (C(4)); 74.2 (C(2)); 70.5, 70.3, 69.8, 69.0 ($NHCH_2CH_2OCH_2CH_2OCH_2CH_2OLev$); 64.5 ($OCH_2CH_2OLev$); 63.7 (C(5)–$CH_2O$); 55.2 (DMTr); 42.6 (C(3)); 40.8 (C(2)–CH_2C=O); 39.2 ($NHCH_2CH_2O$); 37.9 (Lev); 29.9 (Lev); 27.9 (Lev). HR-ESI-MS: 730.3355 ($[M+Na]^+$, $C_{39}H_{49}NNaO_{11}^+$; calc. 730.3198).

*2-{2-[2-({[(2*R*,4*S*,5*R*)-5-{[Bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-({(2-cyanoethoxy)[di(propan-2-yl)amino]phosphanyl}oxy)tetrahydrofuran-2-yl]acetyl}amino)ethoxy]ethoxy}ethyl 4-Oxopentanoate* (**19**). 2-Cyanoethyl *N*,*N*-diisopropylaminophosphorochloridite (30 µl, 0.14 mmol) was added to a mixture of **17** (74 mg, 0.10 mmol) and Et_3N (73 µl, 0.52 mmol) in CH_2Cl_2 (0.5 ml) under N_2. The mixture was stirred for 1 h at r.t. and then subjected directly to CC (5% Et_3N in AcOEt) to give **19** (48 mg, 51%). Colorless oil. ^{1}H-NMR (500 MHz, CD_3CN): 7.23–7.48 (*m*, 9 H, DMTr); 6.86–6.89 (*m*, 4 H, DMTr); 6.62 (*t*, *J* = 4.4, NH); 4.37–4.41 (*m*, H–C(2), H–C(5)); 4.12 (*t*, *J* = 4.7, OCH_2CH_2OLev); 3.95–4.02 (*m*, H–C(4)); 3.77 (*s*, 6 H, DMTr); 3.28–3.76 (*m*, $NHCH_2CH_2OCH_2CH_2OCH_2CH_2OLev$, OCH_2CH_2CN); 3.10–3.16 (*m*, 1 H, C(5)–CH_2O); 3.02–3.05 (*m*, 1 H, C(5)–CH_2O); 2.70 (*t*, *J* = 6.4, 2 H, Lev); 2.62 (*m*, 2 $NCHMe_2$); 2.52 (*t*, *J* = 6.4, 2 H, Lev); 2.48 (*m*, OCH_2CH_2CN); 2.42–2.40 (*m*, 1 H, C(2)–CH_2C=O); 2.15–2.04 (*m*, 1 H, C(2)–CH_2C=O); 2.10 (*s*, 3 H, Lev); 1.98–1.94 (*m*, 1 H, $CH_2(3)$); 1.78–1.85 (*m*, 1 H, $CH_2(3)$); 1.05–1.25 (*m*, 2 $NCHMe_2$). ^{13}C-NMR (125 MHz, CD_3CN) 206.8 (Lev); 172.6 (Lev); 170.1 (CH_2C(O)NH); 158.7, 145.3, 136.1, 130.1, 128.1, 127.8, 126.8, 113.1, 85.9 (DMTr); 85.5 (C(5)); 75.6 (C(4)); 75.1 (C(2)); 70.2, 69.9, 69.3, 68.7 ($NHCH_2CH_2OCH_2CH_2OCH_2CH_2OLev$); 64.2 ($OCH_2CH_2OLev$); 63.5 (C(5)–$CH_2O$); 60.0 ($OCH_2CH_2CN$); 54.9 (MeO); 43.0 ($NCHMe_2$); 42.9 (C(3)); 41.9 (C(2)–CH_2C=O); 39.6 ($NHCH_2CH_2O$); 38.8 (Lev); 29.0 (Lev); 27.7 (Lev); 23.9 ($CHMe_2$); 20.1 (OCH_2CH_2CN). ^{31}P-NMR (200 MHz, CD_3CN) 147.3, 147.2. HR-ESI-MS: 930.4545 ($[M+Na]^+$, $C_{48}H_{66}N_3NaO_{12}P^+$; calc. 930.4276).

Synthesis of Oligonucleotide Conjugates **20** *and* **21**. Oligonucleotide conjugates **20** and **21** were assembled by the phosphoramidite chemistry on a commercially available support bearing DMTr-

protected N^4-benzoyl-2′-*O*-methylcytidine, **8** (1.0 μmol) as the 3′-terminal nucleoside. The DMTr group was manually removed with 3% $Cl_2CHCOOH$ in CH_2Cl_2, and the deprotected support was washed with CH_2Cl_2 and MeCN and dried. The non-nucleosidic building block, either **18** or **19** (10 μmol in 100 μl of MeCN) was coupled manually using 5-(benzylthio)tetrazole (50 μl, 0.25M in MeCN) as activator and 0.5 h coupling time. Another portion of activator was added, and the reaction was allowed to proceed for another 0.5 h. The support was washed with MeCN, CH_2Cl_2, and THF, and then subjected to the capping and oxidation steps according to the standard RNA coupling protocol. The Lev protecting groups of the support-bound non-nucleosidic block were removed with 0.5M H_2NNH_3OAc in pyridine ($NH_2NH_2 \cdot H_2O$/pyridine/AcOH 0.124 : 4 : 1, 0.5 h). The support was washed with pyridine and MeCN, and dried. The azacrown building block **12** (11 mg, 8.6 μmol in 33 μl of MeCN) was then coupled manually using 5-(benzylthio)tetrazole (50 μl, 0.25M in MeCN) as activator. The mixture was kept 2 h at r.t., and then another portion of activator was added. After 1 h, the support was washed with MeCN, CH_2Cl_2, and THF, and subjected to the capping and oxidation steps according to the standard RNA coupling protocol. The 2′-*O*-methyl oligonucleotide sequence was then assembled on an *Applied Biosystems 3400* DNA synthesizer in 1.0-μmol scale applying the conventional phosphoramidite chemistry and the standard RNA coupling protocol. The fully protected 2′-*O*-methyl oligoribonucleotide conjugates **20** and **21** were released from the support and deprotected with conc. aq. NH_3 (5 h at 55°). The crude product mixture was filtered, and the filtrate was evaporated to dryness, dissolved in H_2O, and then subjected to RP-HPLC purification. The authenticity of the conjugate was verified by ESI-MS spectroscopy. **20**: ESI-HR-MS: 1808.0987 ($[M-3\,H]^{3-}$), giving 5426.31 ($[M-H]^-$; calc. 5426.30). **21**: ESI-HR-MS: 1857.2 ($[M-3\,H]^{3-}$), 1392.6 ($[M-4\,H]^{4-}$), 1113.9 ($[M-5\,H]^{5-}$), giving 5573.5 ($[M-H]^-$; calc. 5573.4).

Chimeric 2′-*O*-methyl oligoribonulceotides/oligoribonucleotides **22**–**27**, used as targets for the artificial ribonucleases **20** and **21**, and 2′-*O*-methyl oligoribonucleotide **28**, used as a reference material for the melting-temperature (T_m) studies, were assembled from commercially available 2′-*O*-methyl and 2′-*O*-[(triisopropylsilyloxy)methyl]-protected 2-cyanoethyl *N,N*-diisopropylphosphoramidite building blocks (*Glen Research*) by the conventional phosphoramidite strategy using a 1.0-μmol scale and applying the standard RNA coupling protocol of *Applied Biosystems 392* or *3400* DNA synthesizer. All buffer solns. were prepared in sterilized H_2O, and sterilized equipment was used for their handling.

T_m *Studies.* The melting curves (absorbance *vs.* temp.) were recorded at 260 nm on a *Perkin-Elmer Lambda 35* UV/VIS spectrometer equipped with a multiple cell holder and a *Peltier* temp. controller. The temp. was changed at a rate of 0.5°/min (from 15 to 90°). The recordings were performed in 10 mM potassium phosphate buffer (pH 7) containing 0.1M NaCl. The oligonucleotide conjugates **20** and **21**, reference oligonucleotide **28**, and their targets **22**–**27** were used at a concentration of 2 μM. The T_m values were determined as the maximum of the first derivative of the melting curve.

Kinetic Measurements. The reactions were carried out in *Eppendorf* tubes immersed in a water bath, the temp. of which was kept at 35.0 ± 0.1°. The pH was adjusted to 7.3 with a *HEPES* buffer (0.1M), and the Zn^{2+} ion was added as a nitrate salt to give the total metal ion concentration of 90 μM. The ionic strength was adjusted to 0.1M with $NaNO_3$. The concentrations of the azacrown-functionalized oligonucleotides and their targets were 18 μM. 4-Nitrobenzenesulfonate ion was used as an internal standard. The total volume of the reaction mixture was 200 μl in each kinetic run. Aliquots of 20 μl were withdrawn at suitable intervals, and the reaction was quenched by adding aq. HCl (1.0 μl of 1.0M soln.). The samples were analyzed immediately by cap. zone electrophoresis (*Beckman Coulter P/ACE MDQ CE* System) using a fused silica capillary (inner diameter 50 μm, effective length 50 cm). The inverted polarity, citrate buffer (0.2M, pH 3.1), and −30 kV voltage were used. The temp. of the capillary was kept at 25°. The samples were injected using hydrodynamic injection with 2 psi for 8 s. The capillary was flushed for 3 min with H_2O, 10 mM aq. HCl, and the background electrolyte buffer between every anal. run. The quantification of the target and product oligonucleotides was based on comparison of their UV absorption at 254 nm to that of the internal standard. The peak area was first normalized by dividing it by the migration time and then by the similarly normalized peak area of the internal standard. First-order rate constants for the cleavage of the target oligonucleotides were calculated by applying the integrated first-order rate law to the disappearance of the starting material.

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