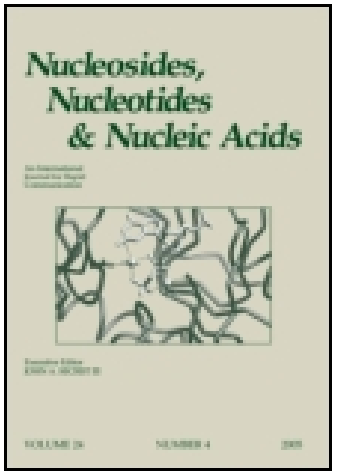

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The Zn^{2+} Complex of 1,4,7,10-Tetraazacyclododecane as an Artificial Nucleobase

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THE Zn^{2+} COMPLEX OF 1,4,7,10-TETRAAZACYCLODODECANE AS AN ARTIFICIAL NUCLEOBASE

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□ *{2-Deoxy-3-O-[2-cyanoethoxy(diisopropylamino)phosphino]-5-O-(4,4′-dimethoxytrityl)-α-D-erythro-pentofuranosyl}-N-{2-[4,7,10-tris(2,2,2-trifluoroacetyl)-1,4,7,10-tetraazacyclododecan-1-yl]ethyl}acetamide (**1**) was prepared and incorporated into a 2′-O-methyl oligoribonucleotide. The hybridization of this oligonucleotide with complementary 2′-O-methyl oligoribonucleotides incorporating one to five uracil bases opposite to the azacrown structure was studied in the absence and presence of Zn^{2+}. Introduction of Zn^{2+} moderately stabilized the duplex with U-bulged targets.*

Keywords Oligonucleotide; azacrown; Zn^{2+} complex; conjugate

INTRODUCTION

Zn^{2+} complexes of azacrown-conjugated oligonucleotides have been shown to serve as artificial ribonucleases that cleave oligoribonucleotides in a sequence-selective manner.[1] In addition, the Zn^{2+} azacrown chelates have another interesting property; they recognize uracil and thymine bases.[2] This binding is so tight that a trinuclear Zn^{2+} complex of 1,3-tris[(1,3,5-triazacyclododecan-3-yloxy)methyl]benzene, for example, cleaves at submillimolar concentrations of oligoribonucleotides selectively at UpU sites.[3] Two of the Zn^{2+} azacrown moieties anchor the cleaving agent to the uracil bases and the third one cleaves the intervening phosphodiester linkage. For the same reason, oligonucleotide conjugates bearing a single Zn^{2+} azacrown moiety are unable to cleave a U_5-bulge opposite to the cleaving agent, although an A_5-bulge is cleaved; the azacrown chelate is engaged in uracil binding and this prevents its catalytic action.[1b] The latter finding raises the question of the applicability of Zn^{2+} chelates as adenine base surrogates that recognize uracil and thymine bases. For this purpose, {2-deoxy-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphino]-5-*O*-

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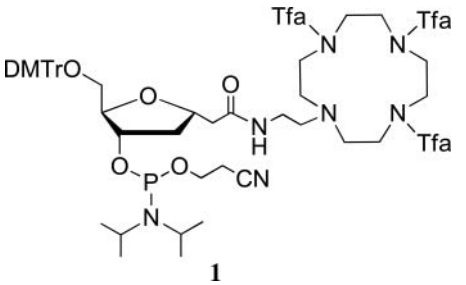

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FIGURE 1 Structure of {2-deoxy-3-*O*-[2-cyanoethoxy (diisopropylamino)phosphino]-5-*O*-(4,4′-dimethoxytrityl)-α-D-*erythro*-pentofuranosyl}-*N*-{2-[4,7,10-tris(2,2,2-trifluoroacetyl)-1,4,7,10-tetraazacyclododecan-1-yl]ethyl}acetamide (**1**).

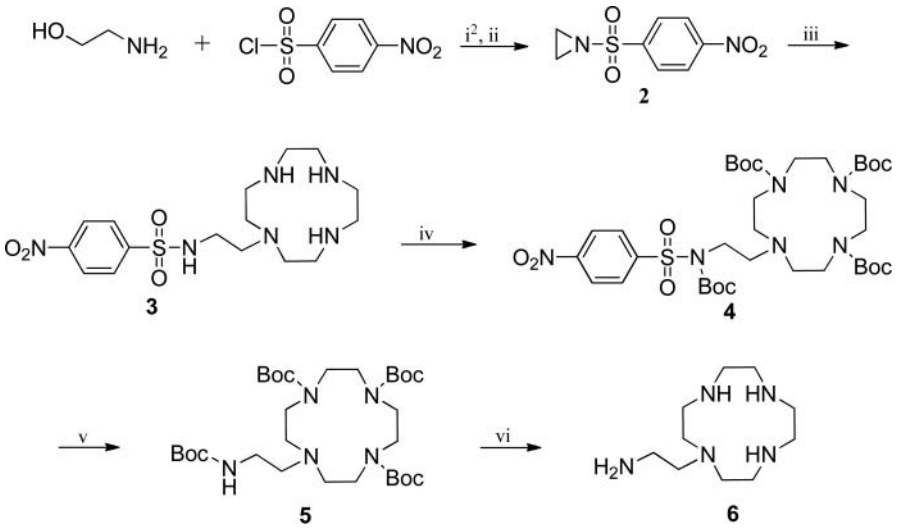
(4,4′-dimethoxytrityl)-α-*D*-*erythro*-pentofuranosyl}-*N*-{2-[4,7,10-tris(2,2,2-trifluoroacetyl)-1,4,7,10-tetraazacyclododecan-1-yl]ethyl}acetamide (**1**) has been prepared and incorporated into a 2′-*O*-methyl oligoribonucleotide (Figure 1). The hybridization of this oligonucleotide with complementary 2′-*O*-methyl oligoribonucleotides incorporating one to five uracil bases opposite to the azacrown moiety has been studied in the absence and presence of Zn^{2+}.

RESULTS AND DISCUSSION

Preparation of {2-deoxy-3-*O*-[2-cyanoethoxy(diisopropylamino) phosphino]-5-*O*-(4,4′-dimethoxytrityl)-α-D-erythro-pentofuranosyl}-*N*-{2-[4,7,10-tris(2,2,2-trifluoroacetyl)-1,4,7,10-tetraazacyclododecan-1-yl]ethyl}acetamide (1)

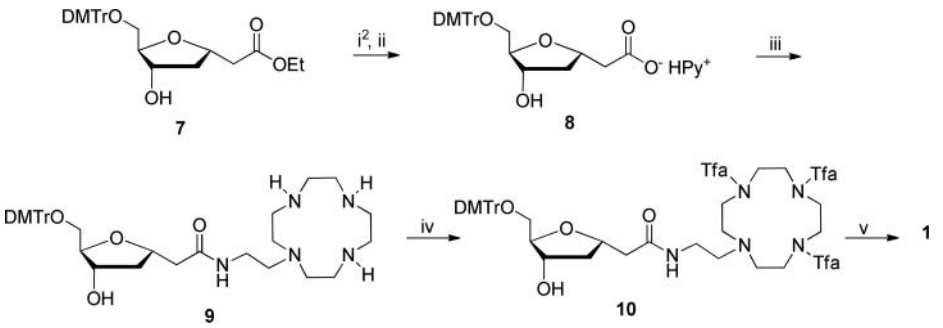
Commercially available 1,4,7,10-tetraazacyclododecane (cyclen) was first converted to its *N*-(2-aminoethyl) derivative (**6**) to allow tethering to previously[1a] prepared 5-*O*-(4,4′-dimethoxytrityl)-1*C*-(2-ethoxy-2-oxoethyl)-1,2-dideoxy-α-D-*erythro*-pentofuranose (**7**). For this purpose, 2-ethanolamine was converted to *N*-(4-nitrophenylsulfonyl)aziridine[4] (**2**), with which cyclen was alkylated in MeCN to afford *N*-[2-(1,4,7,10-tetraazacyclododecan-1-yl)ethyl]-4-nitrobenzenesufonamide (**3**) (Scheme 1). To allow purification by silica gel chromatography, all amino functions were protected with *tert*-butoxycarbonyl (BOC) groups, giving *N*-{2-[4,7,10-tris(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecan-1-yl]ethyl}-*N*-(*tert*-butoxycarbonyl)-4-nitrobenzenesulfonamide (**4**). Removal of the 4-nitrosulfonyl group yielded BOC-protected 1-(2-aminoethyl)-1,4,7,10-tetraazacyclododecane (**5**), which by acidolytic deprotection was converted to **6**.

The preparation of ethyl [2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-α-*D*-*erythro*-pentofuranosyl]acetate (**7**) has been described earlier.[1a] The ester function was hydrolyzed in aqueous alkali and the resulting potassium carboxylate was converted to pyridinium salt (**8**) and coupled to *N*-(2-aminoethyl)cyclen



SCHEME 1 Synthesis of *N*-(2-aminoethyl)cyclen. Reagents: i: DCM-Py (2:1, *v/v*); ii: KOH, DCM, H_2O; iii: Cyclen, MeCN; iv: Boc_2O, Et_3N, Py; v: $HSCH_2COOH$, DBU, DMF; vi: CF_3COOH, DCM.

(**6**) by O-(1Hbenzotriazol-1-yl)-1,1,3,3-tetramethyluronium haxafluorophosphate/*N*,*N*-diisopropylethylamine (HBTU/DIEA) activation in 1,4-dioxan to obtain amide **9** (Scheme 2). The amino groups of the cyclen moiety were protected with trifluoroacetyl groups giving **10**, and finally, the 3-OH group of the sugar moiety was phosphitylated with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite to obtain [2-deoxy-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphino]-5-*O*-(4,4′-dimethoxytrityl)-α-*D*-*erythro*-pentofuranosyl]-*N*-{2-[4,7,10-tris(2,2,2-trifluoroacetyl)-1,4,7,10-tetraazacyclododecan-1-yl]ethyl}acetamide (**1**).



SCHEME 2 Synthesis of the cyclen-derived building block. Reagents: i: KOH, H_2O, 1,4-dioxan; ii: Dowex (50WX8-200) resin in HPy^+ form; iii: **5**, DIEA, HBTU, 1,4-dioxan; iv: CF_3COOMe, Et_3N, MeOH; v: $NCCH_2CH_2OP(Cl)N(iPr)_2$, Et_3N, DCM.

TABLE 1 HPLC retention times and ESI-MS for the 2′-*O*-methyl oligoribonucleotides prepared

Sequence		t_R/minute[a]	*m*/*z* obsd.	*m*/*z* calcd.
11	5′-CGC GCX GGC CC-3′[b]	30.5	3723.9	3723.9
12	5′-CGC GCA GGC CC-3′	23.8	3632.7	3631.7
13	5′-GGG CCU GCG CG-3′	25.6	3689.7	3688.7
14	5′-AAG CCU GCG AA-3′	26.6	3665.8	3664.7
15	5′-GCC UGC GC-3′	23.4	2611.5	2611.5
16	5′-GGG CCU UUG CGC G-3′	22.0	4329.9	4328.8
17	5′-GGG CCU UUU UGC GCG-3′	23.2	4971.0	4968.9

[a]For the chromatographic conditions, see the Experimental section. [b]X refers to (2-deoxy-α-D-*erythro*-pentofuranosyl)-*N*-[2-(1,4,7,10-tetraazacyclododecan-1yl)ethyl]acetamide (*cf.* compound **9**).

Synthesis of Oligonucleotides

The 11-mer 2′-*O*-methyl oligoribonucleotide **11** incorporating the azacrown-functionalized non-nucleosidic unit and the unmodified 2′-*O*-methyl oligoribonucleotides **12–17** were assembled by the standard phosphoramidite protocol of solid-phase synthesis. The coupling yield for the non-nucleosidic block **1** phosphoramidite was 94%. The crude oligonucleotides were purified by reversed-phase high-performance liquid chromatography (RP-HPLC) and their identity was verified by electrospray ionization mass spectrometry (ESI-MS) analysis. Table 1 records the oligonucleotides prepared.

Melting Temperature Measurements

Melting temperatures (T_m) for the duplexes of the azacrown containing oligonucleotide **11** with unmodified targets **13–17** were measured at various concentrations of $ZnCl_2$ in a 20-mmol L^{-1} cacodylate buffer at pH 7.4, the ionic strength being adjusted to 1.0 or 0.1 mol L^{-1} with NaCl. The concentration of the oligonucleotides was 2 μmol L^{-1}. For comparison, the hybridization efficiency of oligonucleotide **12**, an analog of **11** containing 2′-*O*-methyladenosine instead of the azacrown block, was quantified. Table 2 summarizes the results.

Belousoff et al.[5] have previously attempted to increase the affinity of peptide nucleic acid (PNA) to the HIV-1 trans-activation response (TAR) mRNA by conjugating cyclen to the amino terminus of the peptide chain. The base sequence of the conjugate was designed in such a manner that the azacrown moiety could interact with the uracil base within the hairpin hexaloop. Introduction of the azacrown actually slightly stabilized the duplex formation, but contrary to expectations, addition of Zn^{2+} did not result in extra stabilization. The results of the present study are rather different. Replacement of 2′-*O*-methyladenosine within a 2′-*O*-methyl oligoribonucleotide with the cyclen-derived 2′-deoxyriboside (**10**) dramatically destabilizes the

TABLE 2 Melting points for the duplexes of the azacrown functionalized oligonucleotide **11** and its adenine analog **12** with 2′-*O*-methyl oligoribonucleotide targets in 20 mmol L^{-1} cacodylate buffer (pH 7.4) at various concentrations of Zn^{2+}

Duplex	$[Zn^{2+}]/\mu$mol L^{-1} [a]	I/mol L^{-1} [b]	T_m/°C
5′-CGC GCA GGC CC-3′ (**12**)			
3′-CG CGU CCG-5′ (**15**)	0	1.0	81.8
	20	1.0	81.9
	50	1.0	81.0
	100	1.0	81.2
5′-CGC GC**X** GGC CC-3′ (**11**) [c]			
3′-CG CGU CCG-5′ (**15**)	0	1.0	51.6
	20	1.0	51.6
	50	1.0	50.7
	100	1.0	51.6
5′-CGC GC**X** GGC CC-3′ (**11**)			
3′-GCG CGU CCG GG-5′ (**13**)	0	1.0	75.6
	20	1.0	75.7
	50	1.0	75.2
	100	1.0	75.7
5′-CGC GC**X** GGC CC-3′ (**11**)			
3′-GCG CGU_3CCG GG-5′ (**16**)	0	1.0	75.3
	20	1.0	76.5
	50	1.0	76.2
	100	1.0	77.6
5′-CGC GC**X** GGC CC-3′ (**11**)			
3′-GCG CGU_5CCG GG-5′ (**17**)	0	1.0	72.9
	20	1.0	74.8
	50	1.0	74.3
	100	1.0	76.0
5′-CGC GCA GGC CC-3′ (**12**)			
3′-GCG CGU CCG GG-5′ (**13**)	0	0.1	83.7
	50	0.1	84.6
5′-CGC GC**X** GGC CC-3′ (**11**)			
3′-GCG CGU CCG GG-5′ (**13**)	0	0.1	65.2
	50	0.1	67.9
5′-CGC GC**X** GGC CC-3′ (**11**)			
3′-AAG CGU CCG AA-5′ (**14**)	0	0.1	30.6
	50	0.1	33.7
5′-CGC GC**X** GGC CC-3′ (**11**)			
3′-CG CGU CCG-5′ (**15**)	0	0.1	45.8
	50	0.1	47.6

[a] Introduced as $ZnCl_2$. [b] Adjusted with NaCl. [c] X refers to (2-deoxy-α-D-*erythro*-pentofuranosyl)-*N*-[2-(1,4,7,10-tetraazacyclododecan-1yl)ethyl]acetamide (*cf.* compound **9**).

hybridization to 2′-*O*-methyl oligoribonucleotide targets containing uridine opposite to the non-nucleosidic block. The melting temperature for a duplex with an 8-mer (**15**) and 11-mer (**13**) sequence dropped by 30°C ($I = 1$ mol L^{-1}) and 20°C ($I = 0.1$ mol L^{-1}), respectively. Addition of Zn^{2+} up to 100 μmol L^{-1} did not change the situation at high ionic strength: the difference between the T_m values of duplex **11**/**15** and **12**/**15** remained unchanged. At

low ionic strength, the addition of Zn^{2+} slightly decreased the melting temperature difference between **12**/**13** and **11**/**13**. Interestingly, hybridization of **11** to targets that formed a U_3- or U_5-bulge opposite to the cyclen-derived unit was modestly enhanced upon increasing concentration of Zn^{2+} from 0 to 100 μmol L^{-1}: the T_m was increased by 2°C–3°C. Evidently, formation of a ternary complex between uracil base, cyclen, and Zn^{2+} ion is not possible without disruption of the normal double helical structure, and hence, even a modest stabilization is observed only when interaction with a bulged uracil base is possible.

EXPERIMENTAL

General Methods

The ^1H, ^{13}C, and ^{31}P NMR spectra were recorded by a Bruker 400 or 500 NMR spectrometer and the chemical shifts are given in ppm. The mass spectra were recorded on a Bruker micrOTOF-Q ESI-MS system. Oligonucleotides were prepared by using ABI-392 DNA/RNA synthesizer. Oligonucleotides were purified by RP-HPLC on a Hypersil ODS column C18 (250 × 4.6 mm or 250 × 4 mm, 5 μm; flow rate 1 mL $minute^{-1}$; buffer A = 0.1 mol L^{-1} TEAA and B = 0.1 mol L^{-1} TEAA containing 50% MeCN; a linear gradient from 0% to 50% buffer B in buffer A in 25 minutes, followed by 50% buffer B in buffer A for 5 minutes).

N-{2-[4,7,10-Tris(tert-butoxycarbonyl)-1,4,7,10-tetraazacyclododecan-1-yl]ethyl}-N-(tert-butoxycarbonyl)-4-nitrobenzenesulfonamide (4)

4-Nitrobenzenesulfonyl chloride (24.01 g, 108 mmol) was dissolved in a mixture of dichloromethane (DCM) and pyridine (2:1). Ethanolamine (2.18 mL, 36 mmol) was slowly added on an ice bath. The ice bath was removed and the reaction mixture was stirred for 3 hours at room temperature, diluted with DCM (300 mL), and washed with 2 mol L^{-1} aqueous HCl (3 × 100 mL) and 2 mol L^{-1} aqueous KOH (5 × 200 mL). The organic phase was dried with Na_2SO_4 and the solvent was removed under reduced pressure affording *N*-(4-nitrophenylsulfonyl)aziridine (**2**). Compound **2** in dry MeCN (82 mL) was added dropwise into the solution of cyclen (6.29 g, 36.5 mmol) in dry MeCN during 4 hours at 0°C. The reaction mixture was allowed to warm up to room temperature, stirred overnight, and evaporated to dryness affording *N*-[2-(1,4,7,10-tetraazacyclododecan-1-yl)ethyl]-4-nitrobenzenesulfonamide (**3**). Di-*tert*-butyl dicarbonate (42 mL, 183 mmol) and Et_3N (51 mL, 365 mmol) were added into the solution of compound **3** in dry pyridine and the mixture was stirred overnight at room temperature. To ensure the completeness of the reaction, another portion of di-*tert*-butyl dicarbonate (17 mL, 74 mmol) and Et_3N (51 mL, 365 mmol) was added. The mixture was stirred for another 72 hours and evaporated to dryness. The

residue was subjected to DCM/aqueous sodium bicarbonate workup and the organic phase was dried with Na_2SO_4. After evaporation, the residue was purified by silica gel chromatography (3%–5% MeOH in CH_2Cl_2 and a mixture of petroleum ether and EtOAc, 7:3, *v*/*v*). The yield was 4.09 g (14%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_{ppm} 8.41–8.35 (m, 2H), 8.21 (br. s, 2H), 3.95 (dd, 2H, J = 7.6 Hz, 8.4 Hz), 3.54–3.35 (m, 12H), 2.95 (dd, 2H, J = 7.6 Hz, 8.4 Hz), 2.79 (br. s, 4H), 1.47–1.38 (m, 36H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 171.1, 155.8, 150.3, 145.4, 129.6, 124.4, 123.9, 85.6, 80.5, 79.5, 77.0, 60.4, 54.7, 53.7, 50.5, 50.0, 48.0, 42.4, 28.5, 21.0, 14.18. HRMS(ESI): obsd. 801.4040 $[M+H]^+$, calcd. 801.4069 $[M+H]^+$.

N-(2-Aminoethyl)cyclen (6)

Compound **4** (1.23 g, 1.5 mmol) was dissolved in *N*,*N*-dimethylformamide (DMF) (5 mL). Thioglycolic acid (250 μL, 14.7 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (2.2 mL, 14.7 mmol) were added and the reaction mixture was stirred for 2.5 hours at room temperature. The mixture was diluted with EtOAc (30 mL) and washed with saturated aqueous solution of $NaHCO_3$ (3 × 15 mL). The organic phase was dried with Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel chromatography (petroleum ether/EtOAc, 1:1, *v*/*v*) affording compound **5**. The yield was 0.44 g (46%). To a solution of compound **5** (1.22 g, 2.0 mmol) in DCM, trifluoroacetic acid (5 mL) was added and the mixture was stirred overnight at room temperature. The volatiles were removed under reduced pressure and the residue was coevaporated with H_2O (5 mL) and MeOH (5 mL). Yield 40%. HRMS(ESI): calcd. 216.2188 $[M+H]^+$, obsd. 216.2011 $[M+H]^+$.

[2-Deoxy-5-O-(4,4′-dimethoxytrityl)-α-D-erythro-pentofuranosyl]-N-{2-[4,7,10-tris(2,2,2-trifluoroacetyl)-1,4,7,10-tetraazacyclododecan-1-yl]ethyl}acetamide (10)

Previously[1a] prepared ethyl [2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-α-*D*-*erythro*-pentofuranosyl]acetate (**7**) (0.49 g, 0.9 mmol) was dissolved in 1,4-dioxane (20 mL). Aqueous KOH (0.29 g in 5 mL water) was added dropwise, and the reaction mixture was stirred overnight and evaporated to dryness under reduced pressure. The residue was dissolved in pyridine (20 mL), Dowex resin in HPy^+ form was added, and the resulting mixture stirred for 2 hours at room temperature. The resin was collected by filtration and washed with pyridine (4 × 10 mL), and the filtrate was evaporated to dryness to obtain pyridinium [2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-α-*D*-*erythro*-pentofuranosyl]acetate **8** (0.66 g). *N*-(2-Aminoethyl)cyclen (**6**, 0.25 g, 1.15 mmol) was dissolved in 1,4-dioxan (4 mL) and DIEA (1.2 mL, 6.89 mmol) was added. To a solution of compound **8** in 1,4-dioxan (2 mL), DIEA (0.34 mL, 1.95 mmol) and HBTU (0.55 g, 1.45 mmol) were added and the mixture was

stirred for 15 minutes. The solution of compound **8** was added dropwise to *N*-(2-aminoethyl)cyclen (**6**) in 1,4-dioxane during 1 hour. The reaction mixture was stirred overnight at room temperature and evaporated to dryness to give [2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-*α*-*D*-*erythro*-pentofuranosyl]-*N*-[2-(1,4,7,10-tetraazacyclododecan-1-yl)ethyl]acetamide **9**. Trifluoroacetic acid (20 mL) and methanol (10.5 mL) were stirred at room temperature and the methyl ester formed was isolated by distillation. Et_3N (2 mL, 14.42 mmol) and CF_3COOMe (1.17 mL, 11.63 mmol) were added into the solution of compound **9** in MeOH (2 mL) and the reaction mixture was stirred at room temperature. After 72 hours, another portion of Et_3N (1 mL, 7.21 mmol) and CF_3COOMe (0.58 mL, 5.76 mmol) was added and the mixture was stirred for 3 hours. Pyridine (5 mL) was added and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc (100 mL) and washed with saturated aqueous solutions of $NaHCO_3$ (100 mL) and NaCl (100 mL). The organic phase was dried with Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel chromatography ($MeOH/CH_2Cl_2$, 5:95, *v*/*v* containing 0.1% Et_3N and MeOH/EtOAc, 95:5, *v*/*v* containing 0.1% Et_3N). The yield of **10** was 0.34 g (36%). ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.45–6.75 (m, 13H), 4.46–4.41 (m, 1H), 4.29 (s, 1H), 4.15–4.10 (m, 1H), 3.87–3.32 (m, 12H), 3.80 (s, 6H), 3.25–3.20 (m, 2H), 3.10–3.07 (m, 1H), 2.95–2.71 (m, 4H), 2.70–2.46 (m, 4H), 1.77–1.72 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 171.95, 158.56, 144.78, 135.85, 130.00, 128.05, 127.92, 126.94, 113.15, 86.20, 85.30, 77.38, 77.05, 76.79, 75.05, 74.19, 74.01, 64.00, 60.42, 56.80, 55.23, 53.45, 49.35, 49.08, 49.06, 49.04, 49.00, 48.98, 47.29, 46.89, 46.86, 46.83, 46.00, 45.96, 45.89, 45.86, 45.84, 42.77, 42.74, 42.54, 42.52, 40.30, 39.97, 38.62. HRMS(ESI): obsd. 986.3321 $[M+Na]^+$, calcd. 986.3363 $[M+Na]^+$.

{2-Deoxy-3-O-[2-cyanoethoxy(diisopropylamino)phosphino]-5-O-(4,4′-dimethoxy-trityl)-α-D-erythro-pentofuranosyl}-N-{2-[4,7,10-tris(2,2,2-trifluoroacetyl)-1,4,7,10-tetraazacyclododecan-1-yl]ethyl}acetamide (1)

Compound **10** (0.34 g, 0.35 mmol) was dissolved in dry MeCN (4 mL). Dry Et_3N (250 μL, 1.77 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (102 μL, 0.46 mmol) were added under argon. The reaction mixture was shaken for 1 hour and purified by silica gel chromatography (Et_3N/EtOAc, 5:95, *v*/*v*). The product was coevaporated with MeCN. The yield was 0.31 g (75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm} 7.49–6.88 (m, 13H), 4.52–4.49 (m, 1H), 4.46–4.40 (m, 1H), 4.11–4.06 (m, 1H), 3.93 (m, 1H), 3.78 (s, 6H), 3.76–3.47 (m, 13H), 3.46–3.38 (m, 1H), 3.38–3.29 (m, 2H), 3.17–3.04 (m, 2H), 2.95–2.77 (m, 4H), 2.64 (t, 1H, J = 6.0, 6.0 Hz), 2.56–2.53 (m, 1H), 2.52–2.50 (t, 1H, J = 6.0, 6.0 Hz), 2.48–2.38 (m, 2H), 2.01–1.96 (m, 1H), 1.85–1.75 (m, 1H), 1.18–1.13 (m, 12H), 1.09–1.05 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_{ppm} 170.60, 158.64, 145.26,

136.12, 130.05, 128.10–126.78, 118.50, 117.20, 113.06, 85.83, 83.79, 77.26, 75.50, 64.00, 58.48–57.82, 65.11, 54.90, 50.78, 49.20, 48.55–48.40, 47.00, 45.33, 44.06, 42.96–42.84, 42.67–42.62, 41.30, 39.89, 39.53–39.40, 37.88, 35.88, 33.41–33.11, 30.03, 28.69, 25.75, 23.93–23.84, 20.10–19.94, 14.95, 0.84–(−0.15). ^{31}P NMR (200 MHz, $CDCl_3$) δ_{ppm} 147.7, 147.6. HRMS(ESI): obsd. 1186.4451 $[M+Na]^+$, calcd. 1186.4441 $[M+Na]^+$.

Synthesis of Oligonucleotides

Oligonucleotides **11–17** were synthesized by conventional phosphoramidite strategy from 2′-*O*-methyl-protected phosphoramidite building blocks (Glenn Research) on a 1-μmol L^{-1} scale, following the standard RNA-coupling protocol of ABI-392 DNA/RNA synthesizer. The products were released from support and protecting groups of base moiety were removed by concentrated aqueous ammonia treatment (33% aq. NH_3, 2 hours at room temperature and 4–10 hours at 55°C). The crude oligonucleotides were purified and desalted by RP-HPLC. The identity of the products was verified by ESI-MS analysis (Table 1).

Determination of the Duplex Melting Temperatures

The melting curves (absorbance versus temperature) were measured using a Perkin Elmer Lambda 35 ultraviolet/visible (UV/VIS) spectrometer equipped with a Peltier temperature programmer. The melting curves were collected by using a 0.5°C/minute temperature ramp from 10°C to 90°C (for both forward and reverse temperature ramps) measuring the absorbance at 260 nm every half minute. The measurements were performed in 20 mmol L^{-1} cacodylate buffer (pH 7.4) containing 1.0 mol L^{-1} (or $I = 0.1$ mol L^{-1}) NaCl. The oligonucleotide concentration was 2 μmol L^{-1} and $ZnCl_2$ (dissolved in water from the anhydrous salt) was used at the concentrations of 20, 50, and 100 μmol L^{-1}. The measurements were also performed without $ZnCl_2$. All aqueous solutions were prepared in sterilized water and sterilized equipment was used for their handling. At the beginning of each melting temperature experiment, all samples were held at room temperature for 1 hour and after that at 10°C for 5 minutes. The resulting absorbance versus temperature profiles were analyzed by using a UV WinLab Data Processor & Wiewer program. The T_m values were determined as the maximum of the first derivate of the melting curve.

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