Bioorganic & Medicinal Chemistry 20 (2012) 4098–4102

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Hybridizing ability and nuclease resistance profile of backbone modified cationic phosphorothioate oligonucleotides

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ARTICLE INFO

Article history:
Received 21 March 2012
Revised 1 May 2012
Accepted 2 May 2012
Available online 12 May 2012

Keywords:
Phosphorothioate
Backbone modification
Aminoalkyl moiety
UV melting experiment
Nuclease resistance

ABSTRACT

Various stereochemically pure cationic phosphorothioate oligonucleotides bearing aminoalkyl moieties were synthesized, and their duplex-forming ability against single-stranded DNA (ssDNA), single-stranded RNA (ssRNA) and triplex-forming ability against double-stranded DNA (dsDNA) were evaluated by UV melting experiments. The cationic *Rp* stereoisomers showed improved duplex-forming ability against ssDNA, triplex-forming ability against dsDNA and nuclease stability.

1. Introduction

Chemically modified oligonucleotides are increasingly used in antisense,[1] antigene,[2] RNA interference (RNAi)[3] and other genomic technologies such as nucleic acid nanotechnology and drug target validation.[4,5] One of the simplest chemical modifications of oligonucleotides is the phosphorothioate (PS) modification in which a non-bridging oxygen on phosphorus is replaced by a sulfur atom.[6] PS oligodeoxynucleotides (ODN) showed great promises in antisense technology because of their ease of synthesis and high resistance to nuclease degradation.[7] The first antisense drug, Vitravene™ (Formivirsen), containing PS-ODN was approved by the FDA in 1998 for the treatment of cytomegalovirus retinitis,[8] and several others are in clinical trials.[9] Derivatives of PS-ODNs have recently been applied in sequence specific DNA cleavage[10] and construction of DNA nanostructures.[11] However, one of the major problems encountered in the use of PS-ODNs is their low hybridizing ability against complementary strands. Duplex-forming ability against ssDNA or ssRNA is reduced gradually[12,13] and triplex-forming ability is greatly diminished or totally lost upon PS modification.[14,15] To overcome these problems, modifications such as introduction of cationic residues, polyamines and stacking enforcers on the nucleobase have been accomplished.[16–21] These strategies improved the duplex-forming ability in many cases; however, triplex-forming ability was either reduced or not determined. Alternatively, mixing of natural and synthetic polyamines of various sizes has also been investigated for improving hybrid stability.[22,23]

Introduction of an additional functional moiety on the backbone to improve the hybridizing ability of ODNs and their nuclease resistance was also investigated.[24] Grafting of cationic functional moieties on the backbone of natural β-ODNs was found to destabilize duplexes/triplexes, whereas grafting with the non-natural α-ODNs was shown to improve the duplex- and triplex-forming abilities.[25] The stabilization of hybrids by cationic moieties lies in their ability to neutralize the repulsive negative charges on the phosphate backbones and generate an electrostatic interaction with the anionic phosphate backbone. Cationic moieties conjugated to ODNs are also known to improve the uptake of ODNs into cells.[26] Among the various modifications engineered on the backbone, most have been performed on the usual phosphodiester linkage and only very few attempts were undertaken to functionalize the sulfur atom of PS-ODN.[10,11,27,28] Labeling of the sulfur atom of PS-ODN was achieved by some groups using alkylating agents containing haloacetamides, aziridine sulfonamides, γ-bromo-α,β-unsaturated carbonyl compounds[27] or malemidyl derivatives.[28] However, incorporation of such groups on the sulfur atom resulted in destabilization of the duplex and no data related to triplex formation has been reported.

The high nuclease resistance of PS-ODN and the lack of sufficient examples of backbone modification prompted us to investigate the conjugation of cationic amino groups on the backbone of PS-ODN. Therefore, we planned to introduce several cationic amino

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http://dx.doi.org/10.1016/j.bmc.2012.05.009

linkers of different chain lengths to the PS-ODN based on a post-synthetic modification protocol (Scheme 1).[28] Herein, we describe the introduction of various monoamines and diamines of variable lengths onto the PS backbone and their effects on hybridization and nuclease resistance profiles.

2. Results and discussion

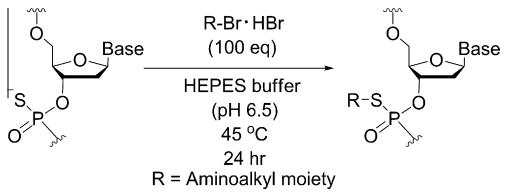
2.1. Conjugation of aminoethyl moiety to oligonucleotide containing purine bases

Initially, a 12 mer PS-ODN, 5′-d(GCGTTTsTTTGCT)-3′ (**S1**) containing one phosphorothioate modification at the middle (labeled as s), was used for introducing the 2-aminoethyl group on the sulfur atom according to the reported procedure.[28] The corresponding aminoethyl PS-ODN, 5′-d(GCGTTTs-$_R$TTTGCT)-3′, R = $-CH_2CH_2NH_2$ (**S2**) was obtained, together with a number of cleavage products resulting from alkylation of the guanine residues.[29] The purified diastereoisomeric mixtures of aminoethyl PS-ODN were hybridized with 12 mer complementary ssDNA and ssRNA and the thermal stability or UV melting temperature (T_m) was determined. The thermal stability of the duplex formed with aminoethyl PS-ODN against ssDNA is equal, and that against ssRNA was slightly lower, compared to the T_ms obtained for non-alkylated PS-ODN. The unmodified natural phosphodiester ODN, 5′-d(GCGTTTTTGCT)-3′ (**S3**) possessed higher T_m than the PS-ODN and aminoethyl PS-ODN.[30]

2.2. Conjugation of aminoalkyl moieties to the polypyrimidine oligonucleotide

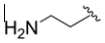
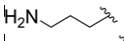
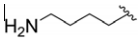
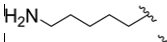
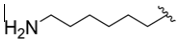
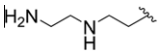
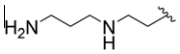
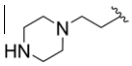
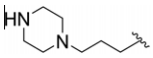
Because of the partial formation of cleavage products due to guanine-alkylation and subsequent purification problems, we decided to use a sequence without guanine residue and ultimately selected two separate stereochemically pure *Sp* and *Rp* PS-ODNs 5′-d(CCCTTTsTTTCCT)-3′ (**1*Sp***, **1*Rp***) bearing a polypyrimidine tract.[31] Stereochemistry of the PS-ODNs **1** were determined by comparing the nuclease stability against snake venom phosphodiesterase which cleaves *Rp* isomer faster than *Sp* isomer.[32] A number of monoamines and diamines with variable alkyl chains were conjugated with the stereochemically pure PS-ODNs using the reported protocol[28] and the corresponding aminoalkyl PS derivatives **2*Sp*–10*Sp*** and **2*Rp*–10*Rp*** were obtained in 24–55% yields (Table 1). The aminoalkyl PS-ODNs were purified by RP-HPLC and characterized by MALDI-TOF mass spectrometry (for purification, yield and MALDI-TOF mass data see the Supplementary data).

The synthesized cationic PS-ODNs **2*Sp*–10*Sp*** and **2*Rp*–10*Rp*** were then investigated for their duplex-forming ability against complementary ssDNA and ssRNA targets and the results are compared to the parent PS-ODNs **1*Sp*** and **1*Rp***, respectively (Tables 2 and 3). The T_m of ODNs **1*Sp*** and **1*Rp*** against ssDNA under specified conditions (Table 2) was found to be identical (42 °C). Alkylation by the 2-aminoethyl moiety to the *Sp*- isomer **1*Sp*** (ODN **2*Sp***) decreased the T_m slightly, while that of the *Rp*-isomer (**2*Rp***) increased the T_m by 3 °C. Increasing the aminoalkyl chain length of the *Sp*-isomers, that is introduction of 3-aminopropyl, 4-aminobutyl, 5-aminopentyl and 6-aminohexyl groups (**3*Sp*–6*Sp***) destabilized the duplex to a larger extent than that observed for the short 2-aminoethyl chain (**2*Sp***).



Scheme 1. Conjugation of aminoalkyl groups to phosphorothioate triester.

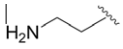
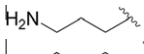
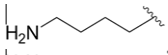
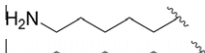
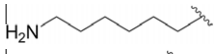
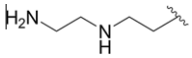
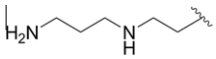
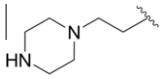
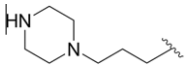
Table 1
Yields of aminoalkylated phosphorothioate oligonucleotides

Oligonucleotide		Yield (%)	
		Sp	*Rp*
5′-d(CCCTTT$_{s\text{-}R}$TTTCCC)-3′			
R= 	(**2**)	39	49
	(**3**)	41	34
	(**4**)	49	42
	(**5**)	53	43
	(**6**)	44	49
	(**7**)	24	39
	(**8**)	28	41
	(**9**)	55	55
	(**10**)	33	53

Cationization of **1*Sp*** by acyclic and cyclic diamino moieties (**7*Sp*–10*Sp***) also destabilized the duplex and the corresponding *Rp*-ODNs (**7*Rp*–10*Rp***) exhibited enhanced duplex stability (T_m increased by 3 °C). For most of the *Rp* conjugates, thermal stability was equal to or slightly improved than that of the natural phosphodiester ODN **11**. Melting temperatures in the absence of salt (NaCl) were also recorded for **2*Sp***, **2*Rp*** and **9*Sp***, **9*Rp*** and the results were compared to those obtained for **1*Sp*** and **1*Rp***. The ΔT_m of both **2*Rp*** and **9*Rp*** compared to **1*Rp*** was found to be +3 °C and that for the *Sp*-isomers **2*Sp*** and **9*Sp*** was −2 °C compared to **1*Sp*** (data not shown). Therefore, the increase and the decrease of T_m in the absence or presence of NaCl were similar, indicating that these changes of T_ms are related to the structure of the ODNs. The increase of T_m against ssDNA using *Rp*-ODNs might be very interesting for constructing recently reported protein based nanostructures[11] or for designing amino modified ODN probes for DNA microarrays.[33] The difference of T_m between *Sp*- and *Rp*-isomers is significant; for example for **7*Rp*** and **7*Sp*** the difference is 5 °C by a single modification. Anionic sulfur atoms of *Sp*- and *Rp*-ODNs are faced to the inside and the outside of the duplex, respectively. Inward orientation is expected to cause a negative charge repulsion between anionic sulfur atom and negative charge present in complementary strand.[34] Aminoalkylation neutralizes negative charge at the sulfur atom and decreases the unfavorable repulsion. This neutralization effect stabilized duplexes formed with the *Rp*-ODNs whereas the stabilization effect might be weak in case of outward-oriented *Sp*-ODNs.

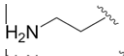
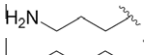
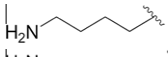
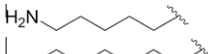
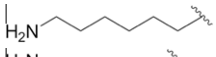
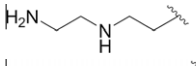
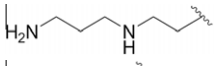
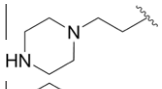
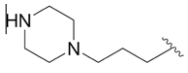
Against the complementary ssRNA (Table 3), all aminoalkyl functional *Sp* PS-ODNs (**2*Sp*–10*Sp***) showed decreased T_m compared to the parent ODN **1*Sp***. *Rp*-isomers (**2*Rp*–10*Rp***) also afforded slightly decreased or equal T_m. These results show that irrespective of the stereoisomers, modification of a sulfur atom on a phosphorothioate linkage by an aminoalkyl moiety usually is detrimental for A-form DNA/RNA duplex stability and destabilization is more pronounced in duplexes formed by *Sp*-conformers. This destabilization effect may result from an unfavorable steric interaction or disturbance of the hydration pattern found in A-form duplexes.[35]

Table 2
Thermal stability of duplexes formed by cationic *S*p and *R*p isomers with complementary ssDNA[a,b]

Oligonucleotide		*S*p		*R*p	
		T_m[c]	ΔT_m	T_m[c]	ΔT_m
5′-d(CCCTTT$_s$TTTCCC)-3′ 5′-d(CCCTTT$_{s\text{-}R}$TTTCCC)-3′	(**1**)	42	—	42	—
R= 	(**2**)	41	−1	45	+3
	(**3**)	40	−2	43	+1
	(**4**)	40	−2	44	+2
	(**5**)	39	−3	44	+2
	(**6**)	37	−5	42	0
	(**7**)	40	−2	45	+3
	(**8**)	40	−2	45	+3
	(**9**)	40	−2	45	+3
	(**10**)	41	−1	45	+3

[a] Target ssDNA: 5′-d(AGGAAAAAAGGG)-3′.
[b] T_m values of the natural oligonucleotide 5′-d(CCCTTTTTTCCT)-3′ (**11**) for ssDNA is 44 °C.
[c] T_m condition: 10 mM sodium phosphate buffer (pH 7.2), 100 mM NaCl, 4 μM each oligonucleotide.

Table 3
Thermal stability of duplexes formed by cationic *S*p and *R*p isomers with complementary ssRNA[a,b]

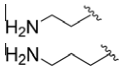
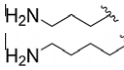
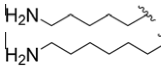
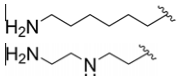
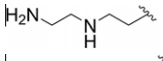
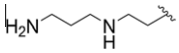
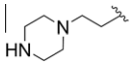
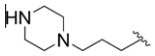
Oligonucleotide		*S*p		*R*p	
		T_m[c]	ΔT_m	T_m[c]	ΔT_m
5′-d(CCCTTT$_s$TTTCCC)-3′ 5′-d(CCCTTT$_{s\text{-}R}$TTTCCC)-3′	(**1**)	46	—	46	—
R= 	(**2**)	43	−3	45	−1
	(**3**)	43	−3	46	0
	(**4**)	43	−3	45	−1
	(**5**)	42	−4	44	−2
	(**6**)	41	−5	43	−3
	(**7**)	44	−2	45	−1
	(**8**)	44	−2	46	0
	(**9**)	44	−2	46	0
	(**10**)	43	−3	45	−1

[a] Target ssRNA: 5′-r(AGGAAAAAAGGG)-3′.
[b] T_m values of the natural oligonucleotide 5′-d(CCCTTTTTTCCT)-3′ (**11**) for ssRNA is 48 °C.
[c] T_m condition: 10 mM sodium phosphate buffer (pH 7.2), 100 mM NaCl, 4 μM each oligonucleotide.

It is possible that the cationic *R*p-isomers enhanced the stability of B-form DNA/DNA duplexes while destabilizing the A-form DNA/RNA duplex due to the fact that in the case of the A-form duplex, long lived hydration patterns are present in the deep major groove and involve a sequence independent water bridge between the *pro*Rp oxygen atom and the adjacent phosphate group,[36] whereas such interactions are absent in B-form duplexes because the corresponding distance is too long.[37]

Next, we investigated the triplex formation at pH 5.5 towards 22-bp dsDNA target bearing a homopurine–homopyrimidine tract. The same ODNs used in duplex studies were employed for the triplex study and the results are summarized in Table 4. All the aminoalkyl conjugates of the *S*p-isomer caused destabilization of the triplex as shown by the ΔT_m values. In remarkable contrast, all aminoalkyl *R*p-conformers increased the T_m values with respect to that obtained for **1*R*p**. The increase of T_m varies depending on the size and structural features of the aminoalkyl substituents and the highest increase of T_m (ΔT_m = +6 °C) was noted for ODN **8*R*p** consisting of a 2-(3-aminopropyl)aminoethyl moiety. Another notable increase of T_m was found for the diamine derivative **7*R*p** (ΔT_m = +5 °C) bearing the 2-(2-aminoethyl)aminoethyl moiety. The increase of T_ms using **7*R*p** and **8*R*p** is also greater than that

Table 4
Thermal stability of triplexes formed by cationic *S*p and *R*p isomers with complementary dsDNA[a,b]

Oligonucleotide		*S*p		*R*p	
		T_m[c]	ΔT_m	T_m[c]	ΔT_m
5′-d(CCCTTT$_s$TTTCCC)-3′ 5′-d(CCCTTT$_{s\text{-}R}$TTTCCC)-3′	(**1**)	26	—	25	—
R= 	(**2**)	24	−2	29	+4
	(**3**)	24	−2	29	+4
	(**4**)	24	−2	27	+2
	(**5**)	24	−2	27	+2
	(**6**)	23	−3	27	+2
	(**7**)	25	−1	30	+5
	(**8**)	25	−1	31	+6
	(**9**)	24	−2	28	+3
	(**10**)	23	−3	27	+2

[a] Target dsDNA: 5′-d(GCAGCGGGAAAAAAGGAGCAGC)-3′/3′- d(CGTCGCCCTTTTTTCCTCGTCG)-5′.
[b] T_m values of the natural oligonucleotide 5′-d(CCCTTTTTTCCT)-3′ (**11**) for dsDNA is 29 °C.
[c] T_m condition: 7 mM sodium phosphate buffer (pH 5.5), 140 mM KCl, 10 mM $MgCl_2$, 1.5 μM Oligonucleotide.

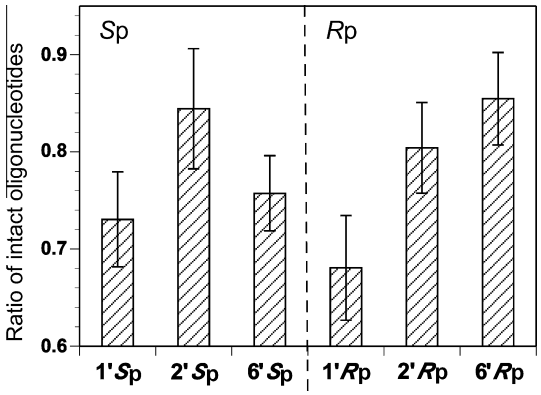


Figure 1. Enzymatic stability of 5′-d(CCCTTTsT)-3′ (**1′*Sp*, 1′*Rp***) and 5′-d(CCCTTTs-$_R$T)-3′ (**2′*Sp*, 2′*Rp*** and **6′*Sp*, 6′*Rp***) against *Crotalus Admanteus* Venom Phosphodiesterase (CAVP). Experiments were carried out at 37 °C for 15 min in buffer containing 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, each ODNs and CAVP.

observed for natural unmodified phosphodiester ODN **11**. The T_m observed for **8*Rp*** was 2 °C higher than that obtained for the natural phosphodiester ODN **11**. This increase of T_m by the *R*p congeners might arise from neutralization of the repulsive forces in the phosphate backbone via cationization of the third strand or via favorable intra or interstrand interaction between positively charged amino groups and phosphate oxygens, similar to that obtained using polyamines.[38]

Triplex formation using phosphorothioate ODN is inefficient in most cases and usually a large decrease of T_m is observed upon PS modification, which restricts the use of PS-ODN in antigene technology.[14,15] However, the incremental increase of T_m by 4 or 5 °C by single 3-(2-aminoethyl)aminoethyl or 2-(2-aminopropyl)aminoethyl linked PS-ODN implies that cationic PS-ODNs might be interesting candidates for antigene technology. This property along with the increased T_m over natural unmodified ODN might be very interesting for designing triplex-forming oligonucleotides (TFO) for antigene technologies and recently reported site specific DNA cleavage using PS-TFO.[10]

2.3. Evaluation of nuclease stability of the conjugated oligonucleotides

Conjugation of a functional moiety to the sulfur atom of PS-ODN is effective in improving the nuclease resistance property.[10] We evaluated the nuclease stability of aminoalkylated PS-ODN using 3′-exo nuclease [*Crotalus Admanteus* Venom Phosphodiesterase (CAVP)]. First, we attempted to investigate the stability of ODN **8*Rp***, which showed the highest triplex-forming ability, and its diastereomer **8*Sp***. However, unexpectedly, **8*Rp*** and **8*Sp*** were heat-labile. Therefore, **8*Sp***, **8*Rp*** were substituted for **2*Sp***, **2*Rp*** and **6*Sp***, **6*Rp*** bearing amino moieties at the same position as **8*Sp***, **8*Rp***. For easier comparison of the nuclease stability of aminoalkylated PS-ODNs, 12 mer ODNs were digested from the 3′-position in advance to give 7 mer ODNs, 5′-d(CCCTTTsT)-3′ (**1′*Sp***, **1′*Rp***), 5′-d(CCCTTTs-$_R$T)-3′ (R = $-(CH_2)_2NH_2$, **2′*Sp***, **2′*Rp***), (R = $-(CH_2)_6NH_2$, **6′*Sp***, **6′*Rp***). The resulting ODNs were incubated with CAVP once again and the ratio of the remaining ODNs at 15 min was evaluated (Fig. 1). The *S*p PS-ODN **1′*Sp*** showed slightly higher stability than *R*p PS-ODN **1′*Rp***. The aminoalkylated *S*p PS-ODNs (**2′*Sp*, 6′*Sp***) showed comparable or higher nuclease stability than **1′*Sp***. In contrast, aminoalkylated *R*p PS-ODN (**2′*Rp*, 6′*Rp***) showed further enhanced nuclease stability compared to **1′*Rp***. Interestingly, the stability of the *R*p and *S*p isomers was reversed by conjugation of an aminohexyl moiety. Aminoalkylation on the sulfur atom is effective to improve the nuclease stability of PS-ODN, notably *R*p-isomers.

3. Conclusion

In conclusion, we have successfully introduced various aminoalkyl moieties to the sulfur group of stereodefined PS-ODNs. The aminoalkyl conjugated *R*p-isomers of phosphorothioate exhibited increased stabilization of DNA duplexes while the *S*p-conformers destabilized the duplexes. Both the cationic *R*p- and *S*p-isomers showed decreased affinity towards RNA. Triplex formation was enhanced by all the aminoalkyl functionalized *R*p-isomers. The most significant triplex stabilization was observed with 2-(3-aminopropyl)aminoethyl and 2-(2-aminoethyl)aminoethyl linked *R*p-PS-ODNs. We also revealed that the alkylation of PS-ODNs is effective to enhance the nuclease stability and increase of nuclease stability

is more pronounced for *R*p isomers. Considering all the results we assume that cationic *R*p-PS-ODNs might be an interesting candidate for DNA based technologies such as DNA microarray, DNA nanostructures and antigene technologies.

4. Experimental section

4.1. General information

Melting points are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a JEOL JNM-ECS-400 spectrometer. Chemical shifts are reported in parts per million downfield from residual solvent of D_2O (4.79 ppm) for ^{1}H NMR or methanol-d_4 (49.0 ppm) for ^{13}C NMR. Mass spectra were measured on JEOL JMS-700 mass spectrometers. MALDI-TOF mass spectra were recorded on a Bruker Daltonics Autoflex II TOF/TOF mass spectrometer. For high performance liquid chromatography (HPLC), Shimadzu LC-10AT$_{VP}$, SPD-10A$_{VP}$ and CTO-10$_{VP}$ were used. Thermal denaturation experiments were carried out on Shimadzu UV-1650 and UV-1800 spectrophotometers equipped with a T_m analysis accessory. Oligonucleotide **1** was purchased from GeneDesign Inc. Aminoalkylating reagents were purchased (ethyl and propyl) or synthesized (others except for 6-bromohexylammonium bromide)[39–41] as the respective ammonium bromides.

4.2. Synthesis of 6-bromohexylammonium bromide

6-Aminohexanol (500 mg, 4.27 mmol) was slowly added to a stirring 48% hydrogen bromide solution (5.1 mL) at 0 °C and the resulting mixture was stirred at 80 °C for 20 h. The mixture was concentrated and crystallized from toluene/ethanol = 50/1 to give 6-bromohexylammonium bromide (674 mg, 2.6 mmol, 61%) as a white solid.

Mp 130–133 °C (toluene/ethanol = 50/1). ^{1}H NMR (400 MHz, D_2O): δ 1.37–1.48 (4H, m), 1.61–1.68 (2H, m), 1.82–1.87 (2H, m), 2.96 (2H, t, J = 8 Hz), 3.48 (2H, t, J = 7 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 26.6, 28.4, 28.6, 33.6, 34.2, 40.6. MS (FAB) *m/z* 180 (M–Br$^-$). HRMS (FAB): Calcd for $C_6H_{15}NBr$ (M–Br$^-$): 180.0382. Found: 180.0395.

4.3. Synthesis of aminoalkylated PS-ODNs

Each aminoalkylation reagent (1.0 M, 2 μL, 2 μmol) in DMF (for ODN **2**–**6**) or H_2O (for ODN **7**–**10**) was added to a solution of ODN **1** (10 nmol) in 22 mM HEPES buffer (18 μL, pH 6.5) and the reaction mixture was incubated at 45 °C for 24 h. After completing the reaction, ODN was precipitated by adding ethanol (100 μL). The mixture was kept at 0 °C for 15 min, centrifuged at 13,200 rpm for 15 min at 4 °C, and the resulting supernatant solution was removed. The obtained ODN was purified by RP-HPLC and characterized by MALDI-TOF mass spectrometry.

4.4. Preparation of enzymatically digested ODNs

Crotalus Admanteus Venom Phosphodiesterase (CAVP, Pharmacia Biotech) (1.3 μg for **1**′ and **6**′***R*p**, 4.5 μg for **2**′***S*p**, 4.1 μg for **2**′***R*p**, 1.3 μg for **6**′***S*p**) was added to a solution of 3.3 μM ODN (5.0 nmol for **1**′ and **6**′***R***, 4.5 nmol for **2**′***S*p**, 4.1 nmol for **2**′***R*p**, 5.1 nmol for **6**′***S*p**) in 25 mM Tris–HCl buffer (pH 8.5 for **1**′, **6**′, pH 7.5 for **2**′) containing 4.0 mM $MgCl_2$. The reaction mixture was incubated at 37 °C for 30 min and heated to 90 °C for 30 min to inactivate the nuclease. The mixture was concentrated and purified by RP-HPLC.

4.5. Evaluation of nuclease stability

Crotalus Admanteus Venom Phosphodiesterase (CAVP, Pharmacia Biotech) (0.40 μg) was added to a solution of 3.3 μM ODN (0.40 nmol) in 50 mM Tris–HCl buffer (pH 7.5) containing 10 mM $MgCl_2$. The reaction mixture was incubated at 37 °C for 15 min and heated to 90 °C for 5 min to inactivate the nuclease. The amount of intact ODN was quantified by HPLC.

Acknowledgments

A part of this work was supported by the bilateral joint projects conducted by Japan Society of Promotion of Science (JSPS) and University Grants Commission of Bangladesh (UGC)

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.05.009.

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