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# Probing Binding Preferences of DNA and RNA: Backbone Chirality of Thioacetamido-Linked Nucleic Acids and *iso*-Thioacetamido-Linked Nucleic Acids to Differentiate DNA versus RNA Selective Binding

Sachin S. Gokhale, Khirud Gogoi, and Vaijayanti A. Kumar*

*Division Of Organic Chemistry, National Chemical Laboratory, Dr. Homi Bhabha Road, Pune-411008, India*

*va.kumar@ncl.res.in*

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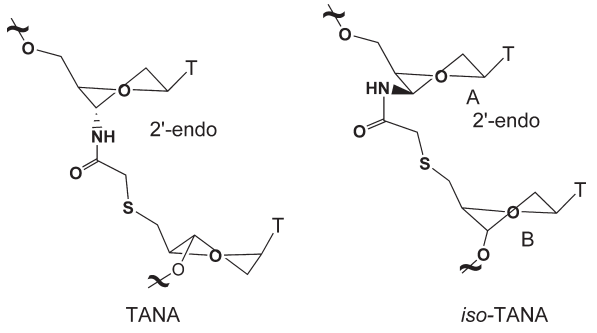


Subtle differences in RNA and DNA duplex geometry could be sensed by the changed stereochemistry at 3′-amino function in the 5-atom thioacetamido linker of thioacetamido-linked nucleic acids and *iso*-thioacetamido-linked nucleic acids modified oligomers. In contrast to the preferred N-type sugar conformations for either 3′- ribo- or xylo amino nucleosides, predominant S-type sugar conformations were found in the dimers. Although the CD spectral differences for the dimer blocks were found to be identical for those found in phosphodiester linked ribo/xylo dimers, the 5-atom thioactamido linker could reverse the RNA binding selectivity to DNA binding selectivity by the change in configuration at the 3′-amino-substituted sugar.

Native DNA and RNA prefer to be in S- or N-type sugar conformations, giving rise to either B- or A-form structures in equilibrium.[1] The electronegativity of the 3′-substituents in ribo-3′-deoxy-3′-amino nucleosides[2] as well as in -*N*-phosphoramidates[3] is known to set the sugars in preferred N-type conformation and they show preferential binding to RNA over DNA. The thioacetamido linked nucleic acid (TANA) dimer blocks (**TsT** and **CsT**) as well as the homogeneous thioacetamido linked nucleoside backbone, where the 3′-amino functionality was in ribo-configuration, also exhibited preferential binding to RNA over DNA.[4] In this particular case, however, the $^1$H NMR studies at the dimer level pointed out the conformational equilibration in 3′-acetamido sugars to be similar to the native 3′-5′phosphate linked DNA. The RNA selectivity of binding was therefore presumed to be arising from the extended backbone linker that is probably inherently folded to be competent to bind to RNA over DNA as was found with other reported five-atom amide linked ON analogues.[4,5] Thus, the **TsT** and **CsT** TANA dimer blocks were found to be compatible in the DNA backbone to selectively form DNA:RNA complexes. The change from phosphodiester linker in the backbone to TANA linker might have adopted an overall conformation that eventually resulted in better RNA binding over DNA binding. The 3′-xylose-nucleosides are known to adopt 3′-endo geometry[6] possibly due to the favorable O4′−C4−C3−O3′ gauche effect in 3′-endo conformation.[7] In spite of this, incorporation of a single xylo-configured nucleotide unit destabilized duplexes and incorporation of a few xylo-configured nucleotides had a further negative effect on the stability of modified DNA:DNA duplexes.[6] Wengel et al. showed that although the binding of xylo-thyminyl 14-mer DNA with complementary RNA sequence is much stronger than that with cDNA, in the mixed purine−pyrimidine sequences, the duplexes with either DNA or RNA were destabilized or were not detected.[8] In a more recent work, a phosphodiester linked oligomer modified with three units of N-type restricted $\beta$-D-xylo analogue also was found to completely destabilize the complexes with both DNA and RNA ($T_m = < 10$ °C).[9] We thought of using the xylo-configured amino nucleosides that prefer 3′-endo conformations in our approach to replace phosphodiester linker by five-atom amide linker and study its consequences on DNA/RNA binding ability. The xylo-configured amino sugar could possibly affect the internucleoside linker length, folding geometry of the linker, and also the preference for 3′-endo or 2′-endo conformation. In this note we report the studies that address

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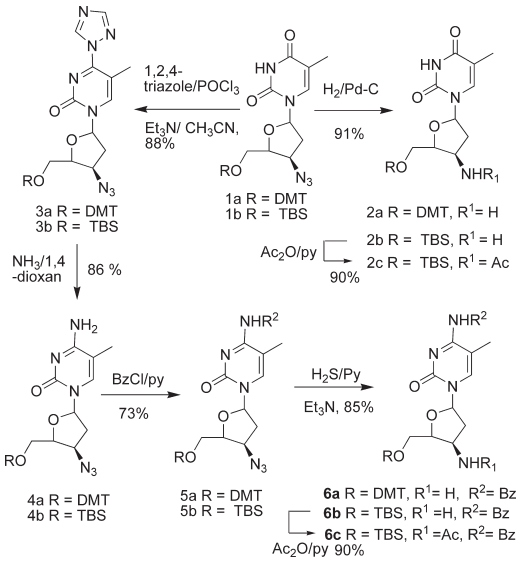
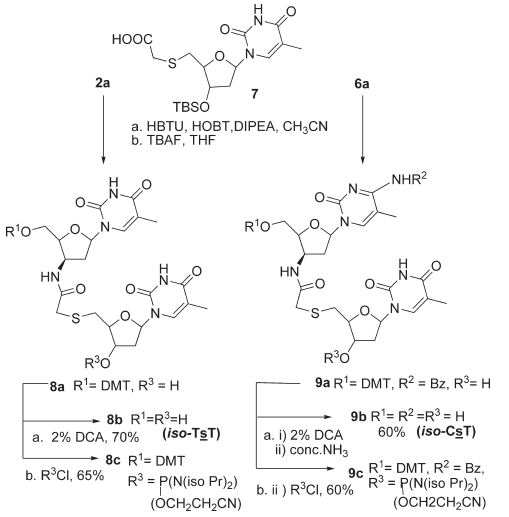
**SCHEME 1**




the synthesis of 1′-(3′-amino-2′,3′-dideoxy-β-D-*threo*-pentofuranosyl)thymine and 1′-(3′-amino-2′,3′-dideoxy-β-D-*threo*-pentofuranosyl)-5-methylcytosine derivatives to obtain *iso*-TANA dimers which would be epimeric with respect to TANA dimer due to changed configuration at the 3′-amino center in the nucleoside. The studies toward the synthesis, NMR, as well as CD analysis of the 1′-(3′-amino-2′,3′-dideoxy-β-D-*threo*-pentofuranosyl)thymine and 1′-(3′-amino-2′,3′-dideoxy-β-D-*threo*-pentofuranosyl)-5-methylcytosine, and *iso*-TANA dimers in comparison with the corresponding TANA dimers are reported here.[4a] Incorporation of these dimer blocks into mixed purine−pyrimidine oligonucleotides and their binding with cDNA/RNA is also reported in this note.

The 1′-(3′-amino-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-β-D-*threo*-pentofuranosyl)thymine **2a** was synthesized according to the reported procedure from 1′-(3′-azido-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-β-D-*threo*-pentofuranosyl)thymine **1a**.[10] The predominant 3′-endo conformation (% S = 36) of the amino nucleoside was derived by using the sum rule (see the SI).[11] The 1′-(3′-amino-2′,3′-dideoxy-5′-*O*-dimethoxytrityl-β-D-*threo*-pentofuranosyl)-$N^4$-benzyol-5-methylcytosine **6a** was also synthesized from **1a** via $C^4$-activation of the nucleobase by the formation of 1,2,4 triazolide **3a**, followed by ammonia treatment to obtain the cytosine nucleoside **4a**.[12,4] The exocyclic amino group was then benzoyl protected to obtain **5a**. The azide functionality in **5a** could be reduced to the amino nucleoside **6a** with $H_2S$ in pyridine as shown in Scheme 1.[4] Compounds **2b** and **6b** were synthesized from 5′-*O*-*tert*-butyl dimethylsilyl ether of 1′-(3′-azido-2′,3′-dideoxy-β-D-*threo*-pentofuranosyl)thymine **1b** followinga similar set of reactions to obtain **2b** and **6b**, respectively. Compounds **2b** and **6b** were *N*-acetylated with pyridine and acetic anhydride to obtain **2c** and **6c**, respectively. The 5′-silyl-protected 3′-amino compounds **2b** and **6b** and acylamino derivatives **2c** and **6c** were used for the conformational studies by NMR

**SCHEME 2**




spectroscopy. Compounds **2a** and **6a** were then condensed with 3′-*O*-TBS-5′-*S*-thyminylmercapto acetic acid[4] **7** to obtain the protected dimer blocks which on deprotection with TBAF in THF gave the dimer blocks **8a** and **9a**, respectively (Scheme 2). The removal of the 5′-DMT group in **8a** and **9a** was effected with 2% DCA in DCM to obtain **8b** (*iso*-**TsT**) and $N^4$-benzoyl-protected **9b**, respectively. The $N^4$-benzoyl-protected compound was then subjected to the treatment with concd $NH_3$ to obtain fully deprotected dimer block **9b** (*iso*-**CsT**). The dimers **8b** and **9b** were fully characterized by 2D COSY $^1$H NMR, and HRMS analysis. The scalar couplings for individual sugar H1′ protons were obtained from 1D $^1$H spectra and were used for conformational analysis. The free secondary hydroxy group of **8a** and **9a** was then phosphitylated to obtain the phosphoramidites **8c** and **9c**, respectively (Scheme 2).

The sugar ring conformations of 1′-(3′-amino-2′,3′-dideoxy-β-D-*threo*-pentofuranosyl) nucleosides **2a** and **6a**, of acetamido derivatives **2c** and **6c**, and of the individual sugars in the dimer blocks **8b** and **9b** were elucidated with an empirical method of analysis by using the vicinal coupling constants $^3J_{1'2'}$ and $^3J_{1'2''}$.[11] The populations of the 2′-endo sugar conformations (% S) were calculated from the equation used in this analysis (see the SI). The predominant N-type sugar conformations (**2a**, % S = 36.2; **6a**, % S = 34.4) were observed for 1′-(3′-amino-2′,3′-dideoxy-β-D-*threo*-pentofuranosyl) nucleosides similar to the reported 1′-(3′-amino-2′,3′-dideoxy-β-D-*threo*-pentofuranosyl)thymine.[10] The acetylated amino nucleosides **2c** and **6c**, however, adopted predominant S-type sugar conformations (**2c**, %S = 67.2; **6c**, %S = 64.4). Also, in the dimer compounds *iso*-**TsT** and *iso*-**CsT**, for the acetylated xylo-configured amino sugars in **8b** and **9b**, a shift toward S-type conformations (**8b**, % S = 57.8; **9b**; % S = 46.4) was observed compared to **2a** and **6a**, respectively. Similarly, in TANA derivatives, the acetylated ribo-configured amino sugars in **TsT** (% S = 64.4) and **CsT** (% S = 71.4) dimers adopt predominant S-type sugar conformations, comparable to their *N*-acetyl derivatives.[4] In the case of TANA (**TsT**/**CsT**) amino sugar, the gauche effect and steric effects augment each other in S-type geometry. The comparatively less percent of S-type geometry in 3′-amino sugar in *iso*-TANA dimers (**8b**/**9b**) than that in TANA dimers (**TsT**/**CsT**) could be due to the unfavorable gauche effect in the O4−C4−C3−NH fragment of acetylated amino substituent in *iso*-TANA (**8b**/**9b**) in S-type

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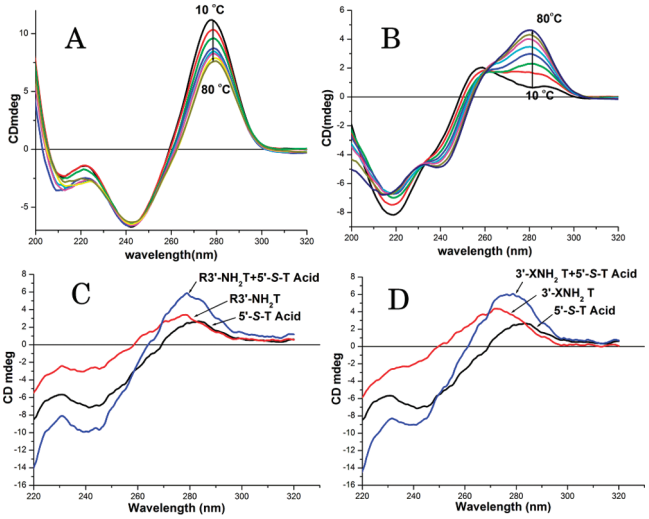


**FIGURE 1.** Temperature-dependent CD profiles of (A) TANA **TsT**,[4a] (B) *iso*-TANA *iso*-**TsT**, **8b**, (C) CD profiles of 2′,3′-dideoxy-3′-amino thymidine, *S*-5′-thymidinyl mercapto acetic acid and their addition spectrum, and (D) CD profiles of 1′-(3′-amino-2′,3′-dideoxy-5′- β-D-*threo*-pentofuranosyl)thymine, *S*-5′-thymidinylmercapto acetic acid and their addition spectrum.

conformation as well as the steric effects. Thus in TANA dimers (**TsT**/**CsT**), the sugars were found to be predominantly in S-type geometry and in the epimeric *iso*-TANA derivatives (**8b**/**9b**), a shift toward N-type conformation was observed and the 3′-amino sugar exhibited almost equal proportion of S/N conformational preference.

The CD spectra of the derived *iso*-**TsT** dimer block **8b** were then investigated in comparison with the TANA **TsT** dimer block reported earlier by us,[4] to see the effect of their epimeric nature on the base stacking interactions. For the TANA **TsT** dimer block, a strong positive CD band was observed at 280 nm. The amplitude in the CD spectrum at 280 nm of the TANA **TsT** dimer block (Figure 1A) was much larger as compared to the additive CD of the two individual monomers (Figure 1C). In contrast, in the case of *iso*-**TsT**, an opposite CD profile was observed, i.e., a weak band with minima at 275−280. (Figure 1B), although the additive CD of the individual monomers exhibited a maxima at 280 nm (Figure 1D). The resulting CD pattern in the dimers thus could be due to the different stacking interactions due to the adopted conformations of the backbone in **TsT** and *iso*-**TsT**. Temperature-dependent CD changes were observed in the case of both the dimers. For TANA **TsT**, the amplitude of positive CD band gradually reduced on heating (Figure 1A) while for *iso*-TANA **TsT** dimer the minima found at 280 nm slowly changed to a maximum at 280 nm (Figure 1B) with increasing temperature. At 80 °C the CD profiles in both cases were the same as the addition CD spectra of the corresponding monomer units. This observation entails us to believe that the CD maximum for **TsT** dimer and CD minimum for *iso*-**TsT** dimer are arising due to the stacking interactions which are different in two epimeric dimers.[2] The dimer blocks containing xylo-cytosine unit in *iso*-**CsT** also exhibited opposite temperature effect on the amplitude of CD band at 280 nm as compared to TANA **CsT**[4] (see the SI) although the change observed was not as significant as in the case of **TsT** and *iso*-**TsT** dimers. Very similar patterns of CD differences were observed for TpT and xylo-TpT dimer blocks reported earlier.[13] Conformational analysis of xylo-TpT dimer block was carried out earlier by Seela et al.[13a] using the Eschenmoser and Dobler method,[14] assuming a 2′-endo (S-type) sugar pucker for xylo sugar residue. In that study, the 3′-endo sugar pucker (N-type) was ruled out considering the steric repulsions between the heterocyclic base and the 3′ phosphate residues on the β-side of the glyconic ring and on the basis of molecular modeling a left-handed single-stranded helix was proposed with a 2′-endo sugar pucker. Very recently a model has been proposed for xylose DNA assuming a 3′-endo sugar pucker (N-type) that also led to a left-handed Z-DNA duplex.[15] Thus the molecular modeling studies that considered either S-type or N-type sugar conformations led singularly to the formation of left-handed structures in xylo-configured sugar−phosphate linked dimers. In the present study, NMR studies point out toward the preferred S-type sugar conformations for either ribo or xylo sugars in TANA or *iso*-TANA containing dimers, but the stacking interactions as studied by CD spectroscopy are very similar to the corresponding ribo or xylo sugar−phosphate thymidine dimer blocks. We also observed such results in our recent work of the amide-linked thymine dimers in which the sugar conformations were S-type but the linker group comprised either L-pro or D-pro amino acids.[16]

**TABLE 1. DNA Sequences Modified with TANA and *iso*-TANA Dimer Blocks**

| no. | sequences | mass calcd/obsd |
|---|---|---|
| **DNA-1** | 5′CCTCTTACCTCAGTTACA | |
| **DNA-3** | 5′CCTC**TsT**ACCTCAGTTACA | 5366.7/5370.4[a] |
| **DNA-4** | 5′CCTC**TsT**ACCTCAG**TsT**ACA | 5356.9/5353.9[a] |
| **DNA-5** | 5′GAAGGGCTTTTGAACTCTT | |
| **DNA-7** | 5′GAAGGG**CsT**TT**TsT**GAACT**CsT**T | 5841.0/5837.8[a]<br>5841.0/5840.3[b] |
| **DNA-8** | 5′GAAGGG**CsT**TT**TsT**GAA**CsT**C**sT**T | 5849.0/5849.3[a]<br>5849.0/5844.0[b] |

[a]MALDI-TOF mass values for sequences containing *iso*-TANA dimers. [b]MALDI-TOF mass values for sequences containing TANA dimers.[4a] **TsT** being thymidine-thioacetamido-thymidine and **CsT** being cytidine-thioacetamido-thymidine.

In our earlier work,[4] the oligonucleotides containing TANA linked dimers were shown to form stable complexes with RNA as compared to DNA although in either case, the complexes were less stable as compared to the unmodified duplexes. We now synthesized a series of chimeric ONs (Table 1) containing one to four **TsT**[4a] and **CsT**[4a] and *iso*-**TsT** and *iso*-**CsT** dimer building blocks. The building blocks **8c** and **9c** were incorporated into DNA sequences **DNA-1** and **DNA-5** at appropriate positions as shown in Table 1 (**TsT** being thymidine-thioacetamido-thymidine and **CsT** being cytidine-thioacetamido-thymidine) to obtain the modified oligomers by automated solid phase synthesis using the phosphoramidite approach with DNA Synthesizer. After cleavage from the support, the oligomers were purified by gel filtration and reverse phase HPLC. The purity of the oligomers was checked by reverse phase HPLC analysis on a C18 column and the oligomers were characterized by mass spectrometry (Table 1).

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**TABLE 2. UV $T_m$ (°C) Data for TANA and *iso*-TANA Modified DNA Sequences with Complementary DNA and RNA[a]**

| | UV $T_m$ (°C) for *iso*-TANA | | UV $T_m$ (°C) for TANA | |
|---|---|---|---|---|
| | **DNA-2** | **RNA-2** | **DNA-2** | **RNA-2** |
| **DNA-1** | 54.6 | 53.8 | 54.7 | 54.7 |
| **DNA-3** | 49.3 | 46.7 | 39.8[4a] | 47.5[4a] |
| **DNA-4** | 42.6 | 40.9 | 43.5[4a] | 52.8[4a] |
| | UV $T_m$ (°C) for *iso*-TANA | | UV $T_m$ (°C) for TANA | |
| | **DNA-6** | **RNA-6** | **DNA-6** | **RNA-6** |
| **DNA-5** | 55.3 | 55.5 | 54.6 | 55.1 |
| **DNA-7** | 43.3 | 38.7 | 43.2 | 54.5 |
| **DNA-8** | 44.2 | 33.2 | 41.6 | 43.6 |

[a]The complexes were prepared in 10 mM sodium phosphate buffer, pH 7.2 containing NaCl (100 mM) and EDTA (0.1 mM). All values are an average of at least three experiments and accurate to within ±1.0°C. DNA-2:5′ TGTAACTGAGGTAAGAGG 3′, RNA-2:5′ UGU AACU-GAGGUAAGAGG 3′, DNA-6:5′ AAGAGTTCAAAAGCC CTTC 3′, RNA-6:5′ AAGAGUUCAAAAGCCCUUC 3′.

These oligonucleotides containing either TANA or *iso*-TANA dimer units (**DNA-3**/**4** and **DNA-7**/**8**) were subjected to thermal denaturation−UV measurement experiments for testing their binding affinity to complementary sequences **DNA-2**/**RNA-2** or **DNA-6**/**RNA-6**, respectively, and the results are summarized in Table 2. The unmodified **DNA-1** and **DNA-5** bind to complementary (**DNA-2** and **RNA-2**) and (**DNA-6** and **RNA-6**), respectively, with almost equal strength as noted by the UV−$T_m$ values. Incorporation of one (**DNA-3**) or two (**DNA-4**) TANA dimer units in **DNA-1** seems to stabilize the duplexes with **RNA-2** more effectively as compared to their duplexes with complementary **DNA-2**. In another DNA sequence, **DNA-5**, three (**DNA-7**), or four (**DNA-8**) modified TANA units were incorporated. The duplex of **DNA-7** with **RNA-6** was found to be almost as stable as unmodified duplex **DNA-5**:**RNA-6** but the complex of **DNA-7** with **DNA-6** was much more destabilized ($\Delta T_m = -11.3$ °C). These result are similar to the other TANA-DNA chimera that we reported earlier.[4] Unexpectedly, the modified sequence with four modified TANA units, **DNA-8**, in which the second consecutive TANA **CsT** unit was incorporated in addition to the three TANA blocks in **DNA-7**, formed the duplex with complementary **RNA-6** with lower thermal stability ($\Delta T_m = -10.9$ °C). In contrast, with incorporation of *iso*-**TsT** and *iso*-**CsT** dimer blocks in the same sequences, i.e., in **DNA-1** (**DNA-3**, **DNA-4**) and in **DNA-5** (**DNA-7**, **DNA-8**), the complexes with cDNA (**DNA-3**/**4**:**DNA-2** or **DNA-7**/**8**:**DNA-6**) were found to be more stable compared to complexes with complementary RNA (**DNA-3**/**4**:**RNA-2**, **DNA-7**/**8**:**RNA-6**) with $\Delta T_m = -2$ to 4 °C and −5 to 11 °C, respectively.

Our results from NMR studies point out the shift toward S-type sugar conformations in 5-atom thioacetamido linked dimer blocks in either ribo- or xylo (*iso*)-configured amino sugars. CD studies point out the differences in the geometries of the stacked forms in the two ribo/xylo (*iso*)-configured dimers, which could arise from differences in the folding geometry of the amide linker in two isomeric forms. Incorporation of these TANA/*iso*-TANA dimers in the mixed purine-pyrimidine oligomers allows them to differentiate between DNA and RNA backbones, TANA modifications favoring RNA binding and *iso*-TANA favoring DNA binding. Thus, on the basis of temperature-dependent CD and UV melting experiments, it could be concluded, in the context of the sequences studied, that (1) the internucleoside distances due to the extended 5-atom thioacetamido linker are more compatible for RNA binding when in ribo configuration and (2) the different linker folding geometry in the xylo configured amino sugar in the 5-atom thioacetamido linked dimers (*iso*-TANA) seems to be more favorable for DNA binding over that for RNA. The backbone chirality in thioacetamido linker thus is able to sense the subtle structural differences in DNA and RNA. The RNA selectivity of binding of TANA-modified oligomers could be reversed to DNA binding selectivity by the change in configuration at the 3′-amino-substituted sugar. The significance of the thioacetamido linker in either ribo- or xylo-sugar configurations is thus evident, and is in contrast to the 3-xylo-configured phosphodiester linked oligonucleotides, where no co-operative transition was observed in the mixed purine−pyrimidine duplexes with either RNA or DNA.

## Experimental Section

**Synthesis of *iso*-TANA Dimers.** *S*-5′-(3′-*O*-TBS-thymidinyl)-mercapto acetic acid **7** (1 g, 2.32 mmol), TBTU (0.89 g, 2.784 mmol), and HOBt (0.15 g 1.16 mmol) were taken in dry $CH_3CN$:dry DMF (5:1) (5 mL), diisopropylethyl amine (1.00 mL, 5.8 mmol) was added, and the solution was stirred for 15 min. Then corresponding amino compound **2a**/**6a** (2.32 mmol) dissolved in 4 mL of $CH_3CN$ was added into the reaction mixture with a further 2 h of stirring. Then the reaction mixture was concentrated and the product was extracted by the usual workup procedure. 3′-*O*-TBS protection of dimers (**8a**/**9a**) was removed with 1 N TBAF in THF and the product was purified by column chromatography. These dimers were then converted into their phosphoramidite derivatives (**8c**/**9c**). The protecting groups in the dimers (**8a**/**9a**) were removed appropriately to give dimers (**8b**/**9b**). All the nucleoside sugar protons were assigned by 2D COSY experiments These dimers were used for sugar conformational analysis by NMR and for CD analysis.

**NMR and HRMS Analysis for Dimer 8b.** $^1$H NMR (200 MHz, $D_2O$) $\delta$ 1.75 (s, 6H, $T^A$, $T^B$, C*H*$_3$), 2.04−2.08 (m, 1H, $T^A$, *H2*′), 2.21−2.31 (m, 2H, $T^B$, *H2*′, *H2*′), 2.66−2.73 (m, 2H, $T^A$, *H2*″, $T^B$, *H5*′), 2.86−2.89 (m, 1H, $T^B$, *H5*″), 3.20−3.28 (dd, 2H, SC*H*$_2$CO, $J$ = 15.65 Hz), 3.63−3.75 (m, 2H, $T^A$, *H5*′, *H5*″), 3.86−3.90 (m, 1H, $T^B$, *H4*′), 4.08−4.11 (m, 1H, $T^A$, *H4*′), 4.23−4.27 (m, 1H, $T^B$, *H3*′), 4.49−4.53 (m, 1H, $T^A$, *H3*′), 5.86−5.89 (m, 1H, $T^A$, *H1*′, $J$ = 5.87 Hz, 7.34 Hz), 6.06−6.09 (m, 1H, $T^B$, *H1*′, $J$ = 6.85 Hz, 6.84 Hz), 7.35, 7.59 (s, 2H, $T^A$, $T^B$, *H6*). Mass calcd. 562.15, obsd 562.21. HRMS calcd for ($C_{22}H_{29}N_5O_9NaS$) 562.1583, obsd 562.1572

**NMR and HRMS Analysis for Dimer 9b.** $^1$H NMR (200 MHz, $D_2O$) $\delta$ $\delta$ 1.84, 1.92 (s, 6H, $C^A$, $T^B$, C*H*$_3$), 2.10−2.14 (m, 1H, $C^A$, *H2*′), 2.31−2.40 (m, 2H, $T^B$, *H2*′, *H2*′), 2.77−2.83 (m, 2H, $C^A$, *H2*″, $T^B$ *H5*′), 2.91−2.95 (m, 1H, $T^B$, *H5*″), 3.31−3.38 (m, 2H, SC*H*$_2$CO), 3.75−3.88 (m, 2H, $C^A$, *H5*′, *H5*″), 3.95−3.99 (m, 1H, $T^B$, *H4*′), 4.19−4.22 (m, 1H, $C^A$, *H4*′), 4.23−4.35 (m, 1H, $T^B$, *H3*′), 4.56−4.60 (m, 1H, $C^A$, *H3*′), 5.91−5.94 (m, 1H, $C^A$, *H1*′, $J$ = 5.8 Hz, 6.8 Hz), 6.14−6.18 (m, 1H, $T^B$, *H1*′, $J$ = 6.7 Hz, 6.7 Hz), 7.44, 7.68 (s, 2H, $C^A$, $T^B$, *H6*). Mass calcd 561.17, obsd 561.20, 585.57. HRMS calcd for ($C_{22}H_{30}N_6O_8NaS$) 561.1743, obsd 562.1747, calcd for ($C_{22}H_{30}N_6O_8KS$) 577.1692, obsd 577.1679

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**Supporting Information Available:** Synthetic procedures, $^1$H, $^{13}$C, and mass spectra of selected compounds in Scheme 1, HRMS and 2D COSY spectra of compounds **8b** and **9b**, HPLC and MALDI-TOF analysis of modified sequences, and UV−$T_m$ graphs for the derived complex. This material is available free of charge via the Internet at http://pubs.acs.org.