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Sugar-Modified Mercaptoacetamido-linked Nonionic Nucleoside Dimer

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Abstract

Sugar-modified mercaptoacetamido-linked nonionic nucleoside dimer 1-(3′-deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidin-3′-yl)-4-(5"-deoxy-2′′-*O*-methyl-*ribo*-thymidin-5"-yl)mercaptoacetamide, **T^{L}-S-T^{OMe}** has been synthesized by HOBT and HBTU-catalyzed condensation of 2-*S*-(3′-*O*-*tert*-butyldiphenylsilyl-5′-deoxy-2′-*O*-methyl-*ribo*-thymidin-5'-yl)mercaptoacetic acid with 3′-amino-3′-deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidine followed by desilylation of the resultant protected dimer. Introduction of sugar modifications, *i.e.* 2′-*O*-methyl *and* 2′-*O*,4′-*C*-methylene modifications restrict the sugar puckering into C3′-*endo* conformation and hence the synthesized neutral and dephosphono-linked **T^{L}-S-T^{OMe}** dimer can be a potential antisense candidate.

Key words: Locked nucleic acid, 2′-modified nucleosides, locked nucleoside, phosphate backbone modification, mercaptoacetamido-linkage

Introduction

The demand for antisense oligonucleotides (AONs) is increasing due to their use as a tool for gene validation in drug discovery and their potential as a new class of drugs for the treatment of a variety of diseases. The first oligonucleotide to enter the clinic was Vitravene[1] for the treatment of cytomegalovirus infection followed by Kynamro[2] which has recently been approved by FDA for the treatment of a type of hypercholesterolemia (HoFH). Nucleic acids composed of naturally occurring DNA or RNA nucleotides pose some limitations which directly affect the versatility because of their poor binding affinity and low degree of nuclease resistance. To overcome these limitations, the ongoing synthetic studies have been focused on chemical modifications of backbone,[3] base[4] and sugar[5] functionalities of the natural DNA/RNA and have resulted in significant progress towards establishing oligonucleotides as viable therapeutic agents. Among the numerous modifications known, enhancement of conformational rigidity of sugar moiety in nucleosides has shown broad usefulness within chemical biology.[5,6]

The major structural difference between DNA and RNA is the 2′-substitution on the furanose of RNA apart from the variation of the pyrimidine bases.[7] Hence in order to improve the RNA binding behavior of antisense oligonucleotides, mimicking RNA structures by 2′-modified nucleosides had shown excellent results[8] (Figure 1).

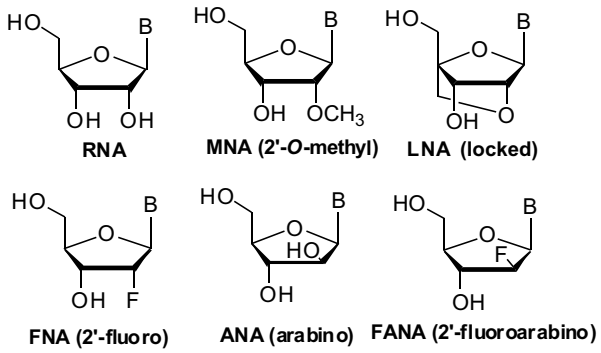

Figure 1. Structure of RNA-like 2′-substituted nucleosides; B = nucleobases

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Substituents like fluorine and oxygen, which have high electronegativity influence the furanose sugar C3′-*endo* conformation.[9] This conformational rigidity may be due to preferred *gauche* orientation of the 2′-substituent and the ring oxygen (Figure 2). As a result RNA and 2′-modified nucleosides are found predominantly in the C3′-*endo* conformation that is exclusively present in A-type duplexes.[10] 2′-*O*-Methyl-RNA, a second-generation of modified oligonucleotides which binds complimentary sequence with high affinity relative to analogous DNA/RNA oligonucleotides,[11] has several distinct features including rapid hybridisation kinetics and resistence to RNase H and other nucleases that make it an attractive antisense reagent. The first-generation phosphorothioate oligodeoxynucleotides (PS-ODNs) revealed limitations with regard to their use as antisense therapeutics because of their relatively low binding affinity to RNA (ca. 1°C < DNA per modified nucleotide), and their non-specific binding to proteins. Moreover, PS-ODNs do not penetrate the blood-brain barrier and have poor oral bioavailability.[12] However 2′-*O*-methyl-phosphorothioate oligonucleotides (Me-PS-ONs) showed improved specific binding affinity over PS-ODNs.[8]

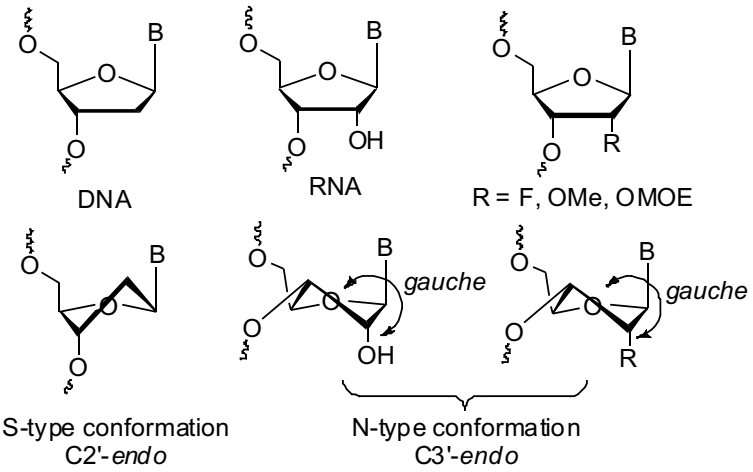


Figure 2. Conformational preference of 2′-substituted pentofuranose sugar in modified nucleosides; B = nucleobases

Locked nucleic acid (LNA, Figure 1), contains a ribose ring which is locked in an N-type (C3′-*endo*) sugar puckering by the introduction of a 2′-*O*,4′-*C* methylene-linkage.[5,13] Incorporation of one or more LNA monomer unit(s) into an ONs shows extraordinary thermal stability when hybridised with either DNA, RNA or with LNA itself.[14,15] LNA offers key properties needed for successful therapeutic exploitation of oligonucleotides, including (1) unprecedented binding affinity towards RNA (and DNA), (2) excellent base pairing specificity, (3) high bio-stability (resistance towards nucleolytic degradation, (4) low toxicity (at least for many LNA oligonucleotides) in animals and (5) convenient chemistry for manufacturing and modification. LNAs are widely used in the field of nucleic acids research, covering a broad range of applications. For example, LNA building blocks are employed to improve targeting, specificity and stability of aptamers, utilized in DNAzymes to increase targeting and cleavage efficiency, applied to enhance RNA in situ hybridization, employed for transfectant-independent delivery of oligonucleotides and used in antisense and siRNA approaches.[16-22] Structural investigation of LNA oligonucleotides by NMR spectroscopy and X-ray crystallography revealed their similarities with natural nucleic acid duplexes, and confirmed the RNA mimicking structures adopted by LNA.[23]

Further, oligonucleotide analogues with differently modified backbone, such as 5′-*N*-carbamate,[24] methylene(methylimino),[25] amide,[26] triazole,[27] phosphorothioate,[28] phosphorodithioate,[29] thioether,[30] thioformacetal[31] and mercaptoacetamido[32,33] *etc.* has been designed and synthesized to circumvent the physical and biological limitations of natural phosphodiester linkage (Figure 3). Among the various modifications, sulphur-based backbone modification has been utilised in antisense oligonucleotide drug

Vitravene[1] which involves the first-generation phosphorothioate oligodeoxynucleotides (PS-ODNs). However 2′-*O*-substituted-phosphorothioate oligonucleotides showed improved specific binding affinitya over PS-ODNs[8] and hence are utilised in Kynamro[2] which is the second antisense oligonucleotide based drug recently approved by FDA.

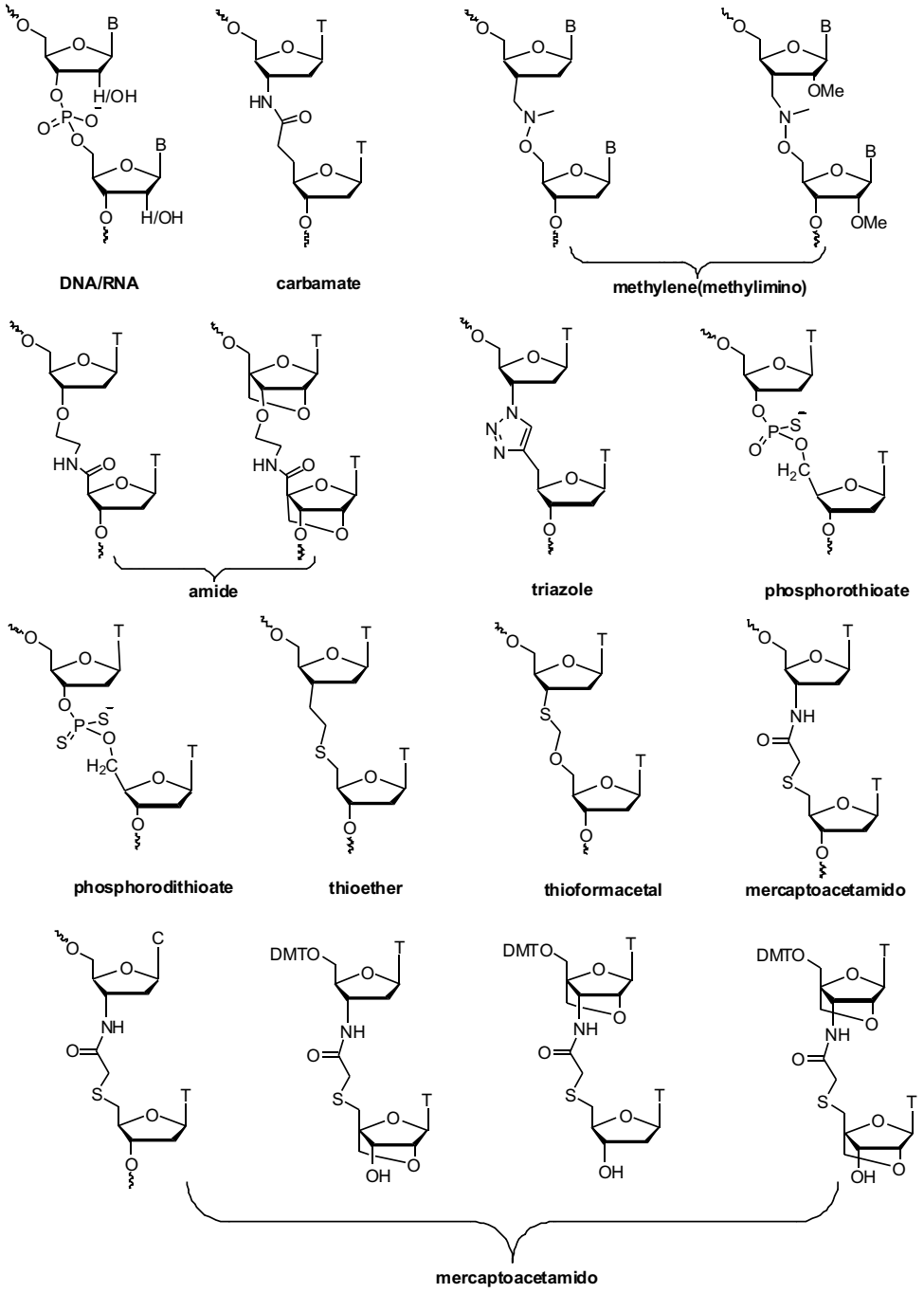


Figure 3. Structure of phosphate backbone modified internucleoside residues. B = nucleobases; T = thymin-1-yl, C = cytosin-l-yl.

Results and Discussion

In continuation to our recent report of the synthesis of LNA-based mercaptoacetamido-linked nucleoside dimers[33] (Figure 3), we herein report the synthesis of 2′-OMe modified LNA-based mercaptoacetamido-linked nucleoside dimer, *i.e.* 1-(3′-deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidin-3′-yl)-4-(5"-deoxy-2′′-*O*-methyl-*ribo*-thymidin-5"-yl)mercaptoacetamide, **T^{L}-S-T^{OMe}** containing the features of both, LNA and 2′-OMe-substitution (Figure 4).

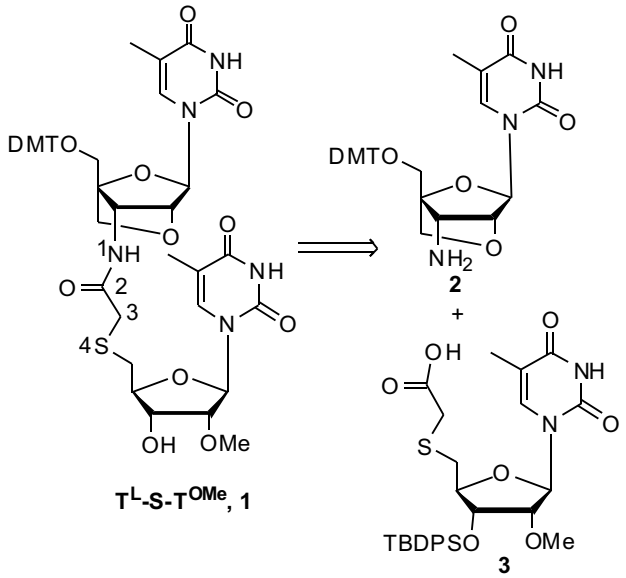
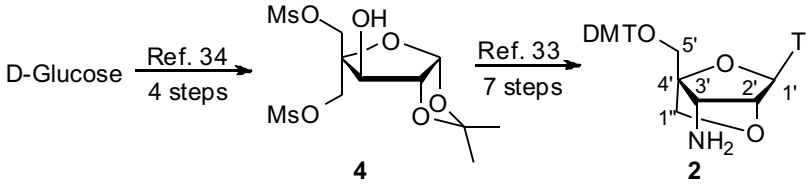


Figure 4. Retro-approach for the synthesis of sugar-modified mercaptoacetamido-linked dimer **T^{L}-S-T^{OMe}**

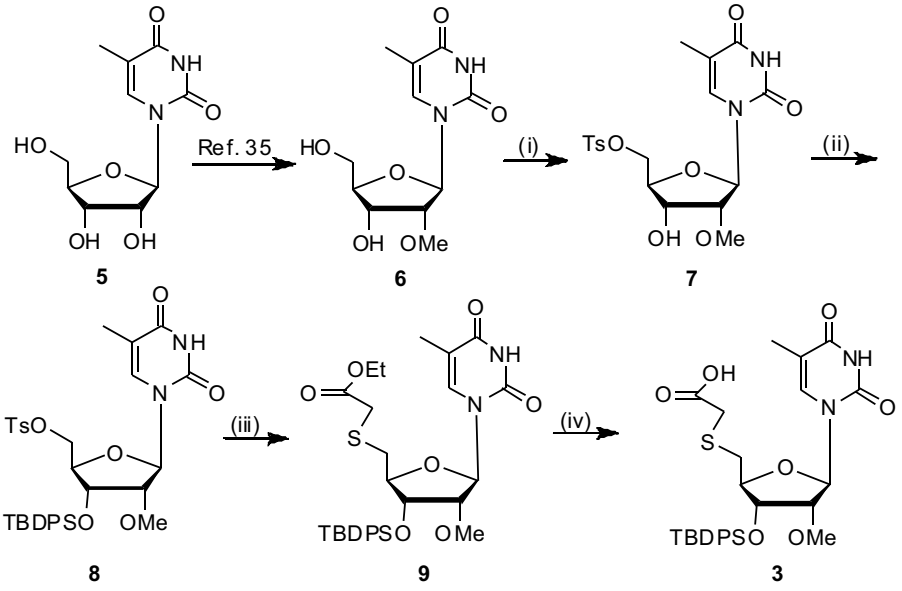
It was envisaged to synthesize the nucleoside dimer **T^{L}-S-T^{OMe} 1** by the condensation of 3′-amino-3′-deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidine (**2**) with 2-*S*-(3′-*O*-*tert*-butyldiphenylsilyl-5′-deoxy-2′-*O*-methyl-ribo-thymidin-5'-yl)mercaptoacetic acid (**3**), (Figure 4). The aminonucleoside **2** has been synthesized by following literature procedure[33] starting from 1,2-*O*-isopropylidene-5-*O*-methanesulfonyl-4-C-methanesulfonyloxymethyl-*β*-L-*threo*furanose (**4**), which has, in turn been obtained from D-glucose[34] in an overall yields of 39% (Scheme 1*)*.

The synthesis of 2-S-(3′-*O*-tert-butyldiphenylsilyl-5′-deoxy-2′-*O*-methyl-*ribo*-thymidin-5'-yl)mercaptoacetic acid (**3**) was accomplished from 2′-*O*-methyl-*ribo*-thymidine (**6**), which was synthesized from *ribo*-thymidine (**5**) following multi-step synthetic procedure as reported in the literature[35] (Scheme 2). The primary OH group in nucleoside **6** was tosylated using p-toluenesulfonyl chloride in pyridine to afford nucleoside **7** in 66 % yield, which on silylation with *tert*-butyldiphenylsilyl chloride in DMF in the presence of imidazole afforded 5′-*O*-p-toluenesulfonyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*-methyl-*ribo*-thymidine (**8**) in 92 % yield. The targeted compound 3 was synthesized



Scheme 1: Synthesis of 3′-amino-3′-deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidine (**2**)

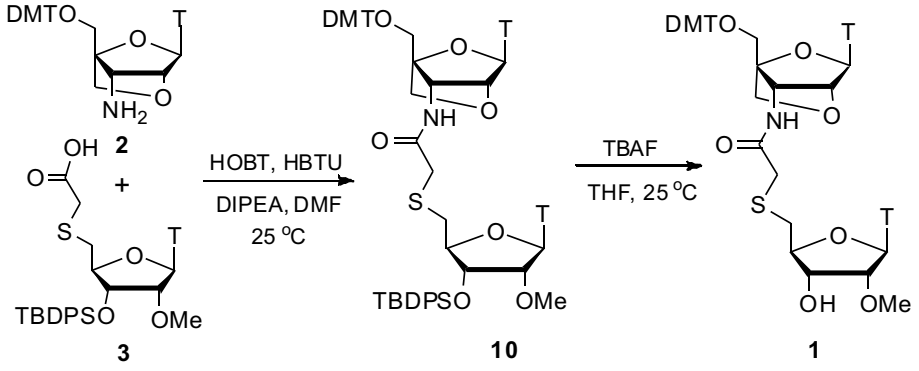
in overall yields of 77 % in two steps from compound **8**, *i.e.* substitution of tosyl group with ethyl mercaptoacetate using sodium hydride in DMF followed by de-esterification of the resulted compound **9** using 2M NaOH in methanol (Scheme 2).



Scheme 2: Synthesis of 2-S-(3′-*O*-*tert*-butyldiphenylsilyl-5′-deoxy-2′-*O*-methyl-*ribo*-thymidin-5'-yl) mercaptoacetic acid (**3**)

Reagents & conditions: (i) *p*-Toluenesulfonyl chloride, pyridine, 0 °C; (ii) *tert*-butyl diphenylsilyl chloride, imidazole, DMF, 25 °C; (iii) NaH, DMF, ethyl mercaptoacetate, 0 °C; (iv) 2M NaOH, methanol, 25 °C.

Finally, the synthesis of mercaptoacetamido-linked nonionic nucleoside dimer $\mathbf{T^{L}\text{-}S\text{-}T^{OMe}}$ **1** was accomplished by HOBT/HBTU-catalyzed coupling of 3′-amino-nucleoside **2** and mercaptoacetic acid **3** in DMF to afford 1-(3′-deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidin-3′-yl)-4-(3′′-*O*-*tert*-butyldiphenylsilyl-5"-deoxy-2′′-*O*-methyl-*ribo*-thymidin-5"-yl)mercaptoacetamide (**10**) in 67 % yield. The mercaptoacetamide **10** was desilylated using tetrabutylammoniun fluoride to obtain the desired mercaptoacetamido-linked non-ionic nucleoside dimer **1** in 74 % yield (Scheme 3). The structures of compounds **1**-**4** and **6**-**10** was unambiguously established on the basis of their spectral data (IR, ^{1}H-, ^{13}C NMR and HRMS) analysis. Further, the structure of known compounds **2**, **4** and **6** was confirmed by comparing their physical and spectral data with those reported in the literature.[33,35]



Scheme 3: Synthesis of sugar-modified mercaptoacetamido-linked nucleoside dimer $\mathbf{T^{L}\text{-}S\text{-}T^{OMe}}$ **1** ; T = thymin-1-yl.

Synthesis of LNA-based mercaptoacetamido-linked nonionic nucleoside dimer **T^{L}-S-T^{OMe} 1** has been achieved using commonly available starting compound, *i.e.* D-glucose or *ribo*-thymidine. All the synthesized compounds were well characterized on the basis of their spectral (IR, ^{1}H-, ^{13}C-NMR spectra and HRMS) data analysis. The enhancement of conformational inflexibity of sugar moiety along with neutral and dephosphono-linker have been of great interest in the designing of antisense oligonucleotides, hence the synthesized **T^{L}-S-T^{OMe}** dimer can be a potential antisense oligonucleotide monomer.

Experimental Section

Melting points were determined on Buchi M-560 instrument and are uncorrected. The IR spectra were recorded on a Perkin-Elmer model 2000 FT-IR spectrometer by making KBr disc for solid samples and thin film for oils. The ^{1}H- and ^{13}C NMR spectra were recorded at Jeol alpha-400 spectrometer at 400 and 100.5 MHz or at Bruker AC-300 at at 300 and 75.5 MHz, respectively, using TMS as internal standard. HR-ESI-TOF-MS analyses were carried out on a microTOF-Q instrument from Bruker Daltonics, Bremen. The optical rotations were measured with Rudolph autopol II automatic polarimeter using light of 546 nm wavelength. Analytical TLCs were performed on precoated Merck silica-gel 60F_{254} plates; the spots were detected either under UV light or by charring with 4 % alcoholic H_2SO_4. Silica gel (100-200 mesh) was used for column chromatography.

3′-Amino-3′-deoxy-5′-*O*-DMT-2′-*O*-4′-*C*-methylenethymidine (2).[33] It was synthesized following literature procedure as light yellow foam in 90 % yield; mp: 130-132 °C, (Lit.[33] mp: 130-132°C). $[\alpha]_D^{22}$ = + 22.9 (*c* 1, acetone). HR-ESI-TOF-MS: *m/z* 594.2214 ([M+Na]$^+$), calcd. for $[C_{32}H_{33}N_3O_7+Na]^+$ 594.2211.

2′-*O*-Methyl-*ribo*-thymidine (6).[35] Compound **6** was synthesized from *ribo*-thymidine according to the literature procedure as a white solid material; mp: 193-195 °C (Lit.[35] mp: 192-195 °C); $[\alpha]_D^{30}$ = + 23.9 (*c* = 0.05, MeOH); HR-ESI-TOF-MS: *m/z* 295.0901 ([M+Na]$^+$), calcd. for $[C_{11}H_{16}N_2O_6+Na]^+$ 295.0901.

5′-*O*-p-Toluenesulfonyl-2′-*O*-methyl-*ribo*-thymidine (7). *p*-Toluenesulfonyl chloride (12 mmol) dissolved in 10 mL of dry pyridine was added drop wise during 4 h to the solution of **6** (10 mmol) in 30 mL of dry pyridine at 0 °C. The reaction mixture was stirred for additional 4 h at RT. Pyridine was removed under reduced pressure and residue thus obtained was dissolved in 100 mL ethyl acetate and the organic layer was washed with (2 x 50 mL) 10 % aq. $NaHCO_3$ solution followed by cold water (2 x 50 mL). The organic layer was dried over anhydrous Na_2SO_4 and was evaporated at reduced pressure. The crude product so obtained was purified over silica gel column using methanol in chloroform as gradient solvent system to afford **7** as white solid (2.75 g, 66 % yield). R_f = 0.4 (10 % methanol in chloroform); mp: 64-66 °C; $[\alpha]_D^{32}$ = + 14.69 (*c* = 0.1, MeOH); IR (thin film) ν_{max}: 3395, 3199, 3067, 2929, 2853, 1773, 1691, 1466, 1363, 1269, 1212, 1190, 1176, 1122, 1092, 1011, 978, 948, 916, 875, 815, 787, 754, 711, 683 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ1.93 (3H, s), 2.44 (3H, s), 2.78 (1H, brs), 3.56 (3H, s), 3.74-3.76 (1H, m), 4.02-4.05 (1H, m), 4.16-4.21 (1H, m), 4.23 (1H, *J* = 2.0 Hz, d), 4.38 (1H, *J* = 2.0 Hz and *J* = 9.2 Hz, dd), 5.94 (1H, *J* = 2.4 Hz, d), 7.36 (2H, *J* = 8.0 Hz, d), 7.45 (1H, *J* = 1.6 Hz, d), 7.77 (2H, *J* = 8.8 Hz, d), 8.96 (1H, brs); ^{13}C NMR ($CDCl_3$, 100 MHz): δ12.30, 21.63, 58.64, 67.81, 68.35, 81.23, 82.85, 87.20, 111.50, 127.83, 130.11, 132.09, 135.03, 145.51, 150.38, 164.03; HR-ESI-TOF-MS: *m/z* 449.0988 ([M+Na]$^+$), calcd. for $[C_{18}H_{22}N_2O_8S+Na]^+$ 449.0989.

5′-*O*-*p*-Toluenesulfonyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*-methyl-*ribo*-thymidine (8). A solution of compound **7** (6.0 mmol), *tert*-butyldiphenylsilyl chloride (7.2 mmol), imidazole (14.8 mmol) in 20 mL of anhydrous DMF was stirred for 6 h under nitrogen atmosphere at RT. Excess of DMF was removed in *vacuo* and the residue was dissolved in 200 mL of ethyl acetate, washed with water (3 x 50 mL) followed by brine (2 x 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and solvent was then removed in *vacuo*. The residue thus obtained was purified by silica gel column chromatography using ethyl acetate in petroleum ether as gradient solvent system to afford **8** as light yellow crystalline solid (3.51 g, 92 % yield). R_f = 0.5 (50 % ethyl acetate in petroleum ether); mp: 74-75 °C; $[\alpha]_D^{32}$ = + 39.82 (*c* 0.1, MeOH); IR (thin film) ν_{max}: 3179, 3050, 3014, 2932, 2859, 1681, 1598, 1464, 1428, 1367, 1307, 1265, 1232, 1212, 1190, 1178, 1114, 1095, 1058, 1020, 986, 951, 884, 841, 821, 751, 704, 666 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.02 (9H, s), 1.84 (3H, s), 2.43 (3H, s), 3.14 (1H, *J* = 2.8 Hz, q), 3.25 (3H, s), 3.90 (1H, *J* = 2.0 Hz and *J* = 9.2 Hz, dd), 4.04 (1H, *J* = 5.2 Hz and *J* = 6.4 Hz, dd), 4.14-4.20 (2H, m), 5.88 (1H, *J* = 3.2 Hz, d), 7.17 (1H, *J* = 1.6 Hz, d), 7.30-7.43 (8H, m), 7.57 (2H, *J* = 1.2 Hz and *J* = 8.0 Hz, dd), 7.65-7.68 (4H, m), 9.05 (1H, brs); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 8.80, 15.73, 18.23, 23.35, 54.34, 63.71, 66.43, 77.00, 79.24, 83.44, 107.71, 124.28, 124.43, 124.53, 126.62, 126.78, 128.63, 128.96,

129.12, 131.29, 132.23, 132.30, 141.96, 146.52, 160.35; HR-ESI-TOF-MS: *m/z* 687.2161 ([M+Na]$^+$), calcd. for [$C_{34}H_{40}N_2O_8SSi$+Na]$^+$ 687.2167.

Ethyl 2-*S*-(3′-*O*-*tert*-butyldiphenylsilyl-5′-deoxy-2′-*O*-methyl-*ribo*-thymidin-5′-yl)mercaptoacetate (9). A solution of NaH (0.29 g, 60 % in hexane, 7.25 mmol.) and ethyl mercapto-acetate (5.5 mmol) in anhydrous DMF (5 mL) was stirred for 30 min. Compound **8** (4.50 mmol) dissolved in DMF (5 mL) was then added slowly at 0 °C. The reaction mixture was stirred for 1 h at RT. Excess of DMF was removed under reduced pressure, and the residue was dissolved in 200 mL ethyl acetate. The organic layer was washed with water (3 x 50 mL) and with brine (2 x 30 mL). The organic layer was dried over anhydrous Na_2SO_4 and solvent was removed in *vacuo*. The crude thus obtained was purified on silica gel column using methanol in chloroform as gradient solvent system to afford **9** as viscous oil (2.26 g, 82 % yield). R_f = 0.5 (50 % ethyl acetate in petroleum ether); $[\alpha]_D^{32}$ = + 5.19 (*c* 0.05, MeOH); IR (thin film) ν_{max}: 3189, 3070, 2932, 2858, 1694, 1508, 1464, 1428, 1390, 1268, 1232, 1156, 1113, 1066, 1030, 895, 840, 822, 743, 704, 667, 622, 613 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.07 (9H, s), 1.23 (3H, *J* = 7.2 Hz, t), 1.81 (3H, s), 2.70-2.75 (1H, m), 2.95 (1H, *J* = 2.8 Hz and *J* = 14.8 Hz, dd), 3.10-3.20 (3H, m), 3.25 (3H, s), 3.92 (1H, *J* = 6.0 Hz and *J* = 7.2 Hz, dd),), 4.12 (2H, *J* = 7.2 Hz, q), 4.25-4.29 (1H, m), 5.86 (1H, *J* = 2.4 Hz, d), 7.13 (1H, *J* = 1.2 Hz, d), 7.34-7.44 (6H, m), 7.61 (2H, *J* = 1.6 Hz and *J* = 8.0 Hz, dd), 7.68 (2H, *J* = 1.6 Hz and *J* = 8.0 Hz, dd), 8.76 (1H, brs); ^{13}C NMR ($CDCl_3$, 100 MHz): δ16.45, 18.25, 23.37, 31.00, 37.54, 38.46, 61.96, 65.54, 77.00, 81.34, 86.66, 86.92, 91.89, 115.11, 131.85, 132.05, 134.21, 134.34, 136.94, 139.47, 139.96, 153.85, 167.67, 174.17; HR-ESI-TOF-MS**:** *m/z* 635.2216 ([M+Na]$^+$), calcd. for **[**$C_{31}H_{40}N_2O_7SSi$+Na]$^+$ 635.2218**.**

2-*S*-(3′-*O*-*tert*-butyldiphenylsilyl-5′-deoxy-2′-*O*-methyl-*ribo*-thymidin-5'-yl)mercaptoacetic acid (3). Sodium hydroxide (2M, 5 mL) solution was added to a solution of **9** (5.30 mmol) in 10 mL methanol. The reaction mixture was stirred for 30 min. After completion of reaction on analytical TLC examination, the sodium salt of the acid and excess NaOH present in the solution was neutralized by DOWEX-50H$^+$ resin. The resin was filtered and washed with 2:1 mixture of methanol:water. The filtrate was concentrated and dried under vacuum to afford **3** as white solid **(**2.32 g, 94 % yield). R_f = 0.5 (50 % methanol in chloroform); mp: 76-77 °C; $[\alpha]_D^{32}$ = -5.47 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3426, 2929, 2857, 1702, 1458, 1262, 1112, 821, 741, 703, 611, 504 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ1.06 (9H, s), 1.77 (3H, s), 2.79 (1H, *J* = 6.0 Hz and *J* = 14.8 Hz, dd), 2.93 (1H, *J* = 2.8 Hz and *J* = 14.8 Hz, dd), 3.14-3.17 (2H, m), 3.23 (3H, s), 3.44 (1H, s), 3.89-3.92 (1H, m), 4.24-4.28 (1H, m), 5.83 (1H, *J* = 1.6 Hz, d), 7.15 (1H, s), 7.34-7.42 (6H, m), 7.60 (2H, *J* = 6.8 Hz, d), 7.67 (2H, *J* = 6.8 Hz, d), 10.03(1H, brs); ^{13}C NMR (DMSO-d_6, 100 MHz): δ16.48, 23.46, 31.11, 37.83, 38.76, 62.09, 77.00, 81.52, 86.71, 86.94, 92.16, 115.29, 131.99, 132.20, 134.35, 134.49, 136.95, 137.01, 140.06, 140.38, 154.24, 168.98, 178.57; HR-ESI-TOF-MS**:** *m/z* 607.1893 ([M+Na]$^+$), calcd. for **[**$C_{29}H_{36}N_2O_7SSi$+Na]$^+$ 607.1905**.**

1-(3′-Deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidin-3′-yl)-4-(3′′-*O*-*tert*-butyldiphenylsilyl-5"-deoxy-2′′-*O*-methyl-*ribo*-thymidin-5"-yl)mercaptoacetamide (10). To a solution of mercaptoacetic acid **3** (1.72 mmol) in dry DMF (5 mL), HBTU (2.06 mmol), DIPEA (5.20 mmol) and HOBt (0.1 mmol) were added and stirred for 15 minutes at 25 °C. Amine **2** (1.70 mmol) was dissolved in 5 mL DMF and was then added into the reaction mixture and the reaction mixture was further stirred at 25 °C for 4 hrs. The reaction mixture was concentrated to dryness, dissolved in ethyl acetate (30 mL) and washed with aqueous 5 % $NaHCO_3$ solution (2 x 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product so obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to afford mercaptoacetamido-linked dimer **10** as white solid (1.24 g, 67 % yield). mp: 137-138 °C. $[\alpha]_D^{30}$ = +15.96 (*c* 0.05, MeOH); IR (thin film) ν_{max}: 3198, 3069, 2931, 2857, 1690, 1508, 1465, 1252, 1177, 1112, 1058, 1035, 977, 898, 832, 756, 703, 664 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.99 (9H, s), 1.52 (3H, s), 1.71 (3H, s), 2.69-2.81 (3H, m), 3.00 (4H, *J* = 12.4 Hz, t), 3.10-3.14 (1H, m), 3.71-3.74 (10H, m), 4.09-4.17 (2H, m), 4.32 (1H, s), 4.44 (1H, *J* = 8.8 Hz, d), 5.49 (1H, s), 5.92 (1H, *J* = 5.2 Hz, d), 6.87 (4H, *J* = 8.8 Hz, d), 7.28-7.32 (6H, m), 7.38-7.46 (10H, m), 7.59 (3H, *J* = 8.0 Hz, d), 7.68 (2H, *J* = 6.4 Hz, d), 8.14 (1H, *J* = 8.0 Hz, d), 11.40 (1H, s), 11.43 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz): δ169.71, 163.77, 163.62, 158.13, 150.43, 149.91, 144.51, 135.75, 11.93, 12.39, 18.88, 26.68, 33.22, 33.58, 38.25, 45.67, 54.99, 57.23, 57.88, 71.14, 72.88, 78.51, 80.68, 83.21, 85.87, 86.27, 87.59, 108.81, 110.01, 113.23, 126.76, 129.74, 129.84, 129.97, 132.73, 133.86, 134.95, 135.28, 135.44, 135.79, 144.49, 149.89, 150.39, 158.12, 163.56, 163.75, 169.73; HR-ESI-TOF-MS *m/z* 1160.4081

([M+Na]$^+$), calcd. for [$C_{61}H_{67}N_5O_{13}SSi$+Na]$^+$ 1160.4118. **1-(3′-Deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidin-3′-yl)-4-(5"-deoxy-2′′-*O*-methyl-*ribo*-thymidin-5"-yl)mercaptoacetamide (1)**. A solution of **10** (0.90 mmol) and TBAF (1.20 mL, 1M in THF) in anhydrous tetrahydrofuran (15 mL) was stirred at RT for 1h. Excess of tetrahydrofuran was evaporated under *vacuo* and residue was re-dissolved in dichloromethane (50 mL). The solution was washed with water (2 x 20 mL) followed by brine (2 x 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to afford mercaptoacetamido-linked nucleosiode dimer **1** as white solid (1.4 g, 74 % yield). mp: 90-92 °C. $[\alpha]_D^{30}$ = +47.21 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3214, 2931, 2492, 1700, 1508, 1466, 1384, 1251, 1177, 1056, 1033, 977, 897, 831, 758, 703 and 582 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.69 (3H, s), 1.89 (3H, s), 2.80-2.88 (3H, m), 3.03 (2H, *J* = 7.2 Hz, q), 3.22 (2H, s), 3.47 (1H, s), 3.55 (3H, s), 3.71 (1H, *J* = 9.6 Hz, d), 3.78 (6H, s), 3.95 (3H, *J* = 8.8 Hz, d), 4.09 (1H, *J* = 9.6 Hz, t), 4.48 (1H, *J* = 8.0 Hz, d), 4.55 (1H, s), 5.64 (2H, *J* = 12.4 Hz, d), 6.84 (4H, *J* = 8.4 Hz, d), 7.18-7.35 (9H, m), 7.46 (1H, *J* = 8.0 Hz, d), 7.58 (1H, s), 9.39 (2H, s); ^{13}C NMR (DMSO-d_6, 100 MHz): δ12.04, 12.46, 33.73, 33.98, 38.31, 51.25, 55.09, 57.59, 57.98, 62.86, 70.86, 71.22, 78.56, 81.31, 83.34, 85.95, 86.34, 86.46, 87.69, 108.88, 110.02. 113.32, 126.86, 127.77, 128.02, 129.85, 133.98, 134.55, 135.02, 135.31, 136.17, 144.65, 149.96, 150.57, 158.20, 158.36, 163.78, 163.87, 170.00; HR-ESI-TOF-MS *m/z* 922.2889 ([M+Na]$^+$), calcd. for [$C_{45}H_{49}N_5O_{13}S$+Na]$^+$ 922.2940.

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