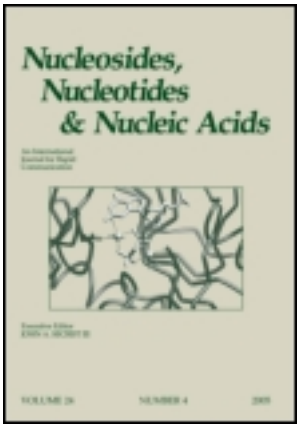

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DESIGN AND SYNTHESIS OF LNA-BASED MERCAPTOACETAMIDO-LINKED NUCLEOSIDE DIMERS

Vivek K. Sharma,[1] Sunil K. Singh,[1] Kapil Bohra,[1] Chandra Shekhar Reddy L.,[1] Vinod Khatri,[1] Carl E. Olsen,[2] and Ashok K. Prasad[1]

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□ *Three LNA-based mercaptoacetamido-linked nonionic nucleoside dimers* $\boldsymbol{T^L}$***-S-T***, ***T-S-*** $\boldsymbol{T^L}$, *and* $\boldsymbol{T^L}$***-S-*** $\boldsymbol{T^L}$ *have been synthesized by HOBT and HBTU catalyzed condensation of silyl-protected 2-S-(thymidin-5′-yl)mercaptoacetic acid or 2-S-(2′-O,4′-C-methylenethymidin-5′-yl)mercaptoacetic acid with 3′-amino-3′-deoxy-5′-O-DMT-2′-O,4′-C-methylenethymidine or with 3′-amino-3′-deoxy-5′-O-DMT-β-thymidine followed by desilylation of the protected dimers. The 3′-O-phosphoramidite derivative of one of the nucleoside dimers was successfully prepared by condensation with [P(-Cl)(-*OCH_2CH_2CN*){-*$N(iPr)_2$*}] in DCM in the presence of* N,N*-diisopropylethylamine (DIPEA), which is a building block for the preparation of mercaptoacetamido-linked oligonucleotides of therapeutic applications.*

[Supplementary materials are available for this article. Go to the publisher's online edition of *Nucleosides, Nucleotides & Nucleic Acids* for the following free supplemental resource: supplementary information.doc.]

Keywords Locked nucleic acid; phosphate backbone modification; mercaptoacetamido-linkage; phosphoramidite-derivative

INTRODUCTION

The recent development in antisense, antigene, and RNA interference technologies by using chemically modified oligonucleotides (ONs) has attracted a great deal of both chemists and biologists.[1–5] The modified

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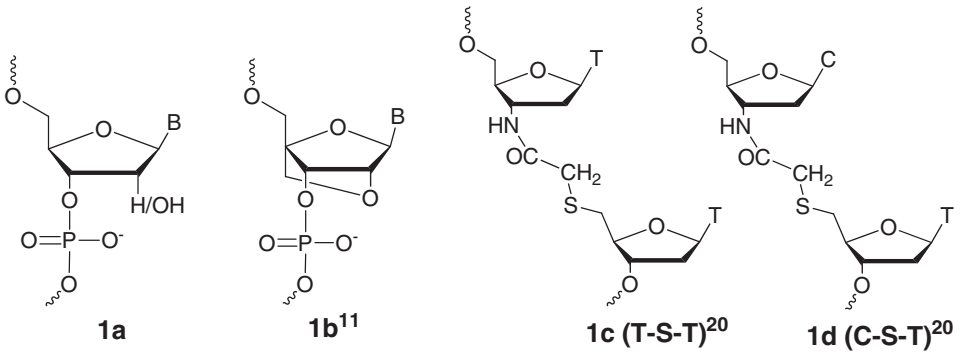


FIGURE 1 Structure of DNA/RNA **1a**; LNA **1b**[11]; and Mercaptoacetamido-linked dimers **1c** and **1d**[20]. B = Nucleobases; T = Thymine; C = Cytosine.

oligonucleotides are also being increasingly utilized in nucleic acid nanotechnology, oligonucleotide-based diagnostics, gene-function determination, and drug target validation.[6–8] The novel utility of these agents resides in their ability to selectively prevent the expression of a particular disease-associated gene in a sequence specific manner. The ongoing synthetic studies have been focused on chemical modifications of backbone,[9] base,[10] and sugar[11] functionalities of the natural DNA/RNA (**1a**, Figure 1) and have resulted in significant progress toward establishing oligonucleotides as viable therapeutic agents. One such modification in the sugar moiety has resulted in locked nucleic acid (LNA **1b**, Figure 1), where the furanose conformation is locked in an *N*-type (C3′-*endo*) form by the introduction of a 2′-*O*,4′-*C* methylene-linkage.[11–13] LNA has been found to be very useful for antisense applications, since incorporation of one or more LNA monomer unit(s) into an ON shows extraordinary thermal stability when hybridized with either DNA, RNA, or LNA itself.[11,12,14]

Further, oligonucleotide analogues with differently modified backbone such as thioformacetal,[15] 5′-*N*-carbamate,[16] methylene(methy limino),[17] amide-,[18] triazole,[19] etc. have been designed and synthesized to circumvent the physical and biological limitations of natural phosphodiester linkage. Recently, Kumar et al.[20] have synthesized two 5-atom mercaptoacetamido-linked dimers, T-S-T **1c** and C-S-T **1d** (Figure 1), as compared to the 4-atom-linked natural DNA. The UV-T_m experiments for binding affinity of these mixed backbone modified ONs with complementary DNA and RNA sequences revealed important results such as significantly higher RNA-binding selectivity (ΔT_m/mod upto + 13°C) as compared to complementary DNA. Replacement of phosphate backbone with mercaptoacetamido linkage in an ONs have several distinct advantages in terms of its antisense properties, i.e., (i) it has strong binding affinity toward both natural DNA and RNA, which may be due to the neutral backbone that does not have any repulsive interactions with the anionic phosphate backbone;

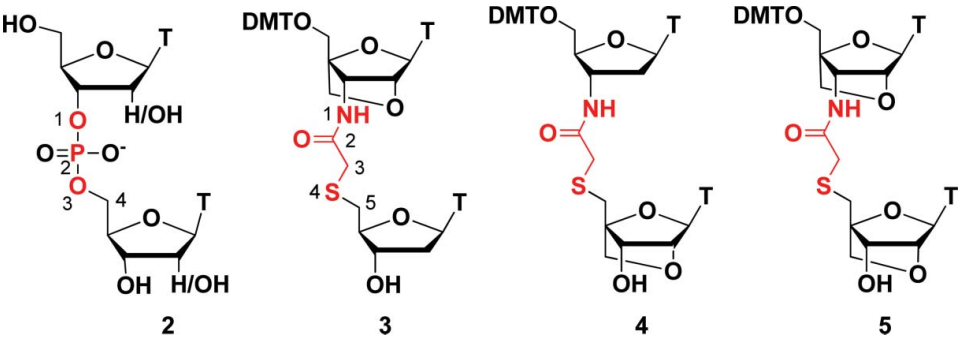


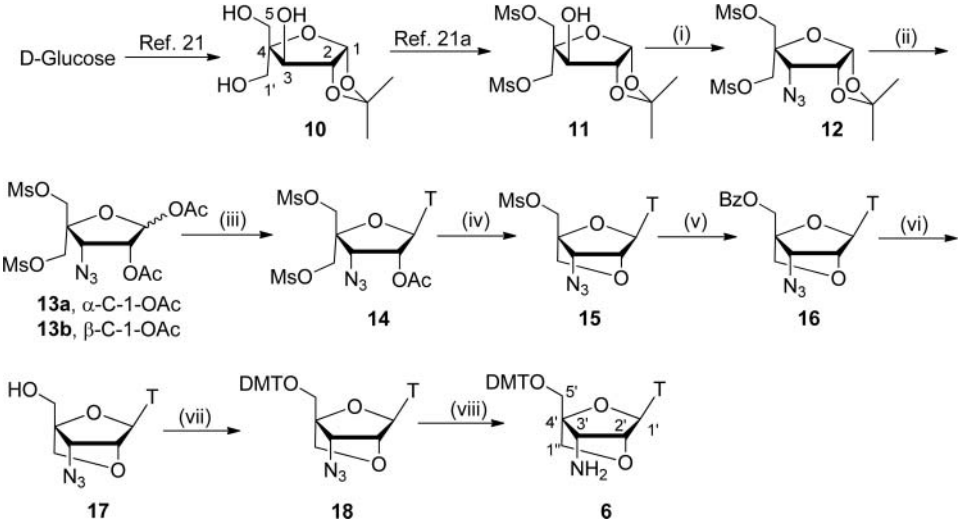
FIGURE 2 Structures of natural dinucleotide **2**; designed LNA-based mercaptoacetamido-linked nonionic nucleoside dimers **3**, **4**, and **5**. The natural phosphate linkage O-P-O in **2** has been replaced by mercaptoacetamido-linkage S-CH_2-CO-NH in **3**, **4**, and **5**, as shown in red (Color figure available online).

(ii) it provides a very high degree of nuclease resistance; and (iii) it enhances membrane permeability due to elimination of negative charge from the backbone.

In this article we have designed and synthesized three LNA-based mercaptoacetamido-linked nonionic nucleoside dimers i.e., 1-(3′-deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidin-3′-yl)-4-(5″-deoxy-β-thymidin-5″-yl) mercaptoacetamide (T^L-S-T, **3**); 1-(3′-deoxy-5′-*O*-DMT-β-thymidin-3′-yl)-4-(5″-deoxy-2″-*O*,4″-*C*-methylenethymidin-5″-yl)mercaptoacetamide (T-S-T^L, **4**); and 1-(3′-deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidin-3′-yl)-4-(5″-deoxy-2″-*O*,4″-*C*-methylenethymidin-5″-yl)mercaptoacetamide (T^L-S-T^L, **5**) containing the features of both, LNA- and mercaptoacetamido-linked nucleoside dimers (Figure 2) and 3′-*O*-phosphoramidite derivative **27** of one nucleoside dimer, which is a building block for the preparation of mercaptoacetamido-linked oligonucleotides.

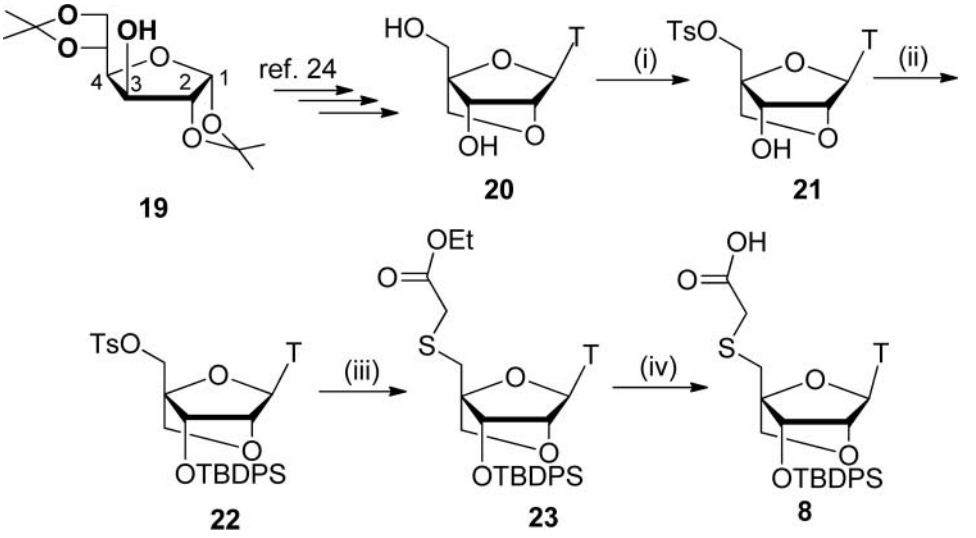
It was envisaged to synthesize the nucleoside dimer T^L-S-T **3** by the condensation of 3′-amino-3′-deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidine (**6**) with 2-*S*-(β-thymidin-5′-yl)mercaptoacetic acid (**9**); nucleoside dimers T-S-T^L **4** and T^L-S-T^L **5** by condensing 3′-amino-3′-deoxy-5′-*O*-DMT-β-thymidine (**7**); and nucleoside **6** with 2-*S*-(2′-*O*,4′-*C*-methylenethymidin-5′-yl)mercaptoacetic acid (**8**). The aminonucleoside **6** has been synthesized from 1,2-*O*-isopropylidene-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-β-L-*threo*furanose **11**, which was obtained from D-glucose through trihydroxyfuranoside **10** following the procedure of Matin[21] in an overall yields of 39%. The dimesylated compound **11** was converted into 3-azido-3-deoxy-1,2-*O*-isopropylidene-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxy methyl-α-D-*ribo*furanose **12** in two steps, i.e., triflation of the lone hydroxyl group using triflic anhydride in DCM-pyridine followed by nucleophilic displacement of the triflate with sodium azide in DMF in combined yields of 50%. Acetolysis of compound **12** with acetic acid-acetic anhydride-conc. H_2SO_4 yielded mixture of anomers **13a–13b** in 84% yield, which on

Vorbrüggen coupling[22] with thymine resulted into the formation of monoacetylated nucleoside **14** in 75% yield. The deacetylation followed by cyclization was affected on monoacetylated nucleoside **14** using 2M NaOH solution in water:dioxane to afford bicyclic nucleoside **15** in 90% yield. The nucleoside **15** was demesylated to obtain 3′-azido-3′-deoxy-2′-*O*,4′-*C*-methylenethymidine (**17**) in an overall yield of 72% through benzoylation with sodium benzoate in dry DMF followed by basic hydrolysis of the resulted benzoate **16** using 2M NaOH in THF:water (1:1) (Scheme 1). The azidobicyclic nucleoside **17** was converted into DMT-protected aminonucleoside **6** by reaction with DMT-Cl in pyridine followed by reduction of the resulted compound **18** with PPh_3-pyridine and aq. ammonia solution in overall yields of 68%. The synthesis of 3′-amino-3′-deoxy-5′-*O*-DMT-β-thymidine (**7**) was accomplished from β-thymidine through DMT-protection, C2-*O*-C3′-anhydro formation, azidation followed by reduction of the azide group with PPh_3-pyridine/aq. ammonia solution in overall yields of 49% following literature procedure.[23]



SCHEME 1 Synthesis of 3′-amino-3′-deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidine (**6**). *Reagents & conditions:* (i) (a) Trifluoromethanesulfonic anhydride, DCM:pyridine (10:1), –10°C, (b) NaN_3, DMF, 100°C; (ii) AcOH, Ac_2O, H_2SO_4 (100:10:0.1), 0°C; (iii) T = Thymine, *N*,*O*-*bis*-trimethylsilyl acetamide, trimethylsilyltrifluoromethane sulfonate, acetonitrile, 80°C; (iv) 2M NaOH, dioxane:water (1:1), 0°C; (v) Sodium benzoate, DMF, 100°C; (vi) 2M NaOH, water:THF (1:1), 0°C; (vii) 4,4′-Dimethoxytrityl chloride, pyridine, 25°C; (viii) PPh_3, pyridine, 28% aqueous ammonia solution, 25°C.

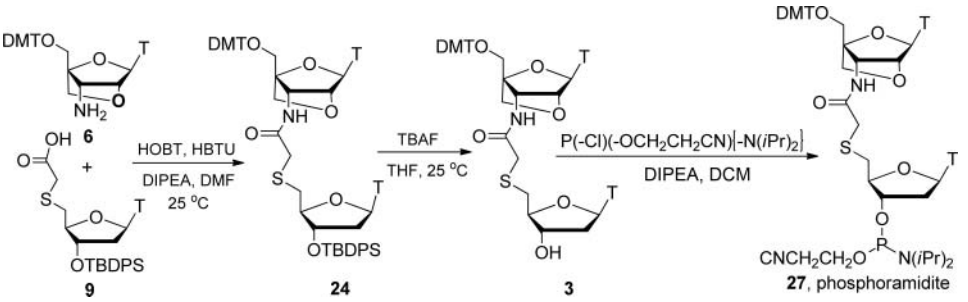
The synthesis of 2-*S*-(2′-*O*,4′-*C*-methylenethymidin-5′-yl)mercaptoacetic acid (**8**) was accomplished from 2′-*O*,4′-*C*-methylenethymidine (LNA T, **20**), which was synthesized from diacetone-D-glucose **19** following multistep synthetic procedure as reported in the literature (Scheme 2).[24] The primary OH group in bicyclic nucleoside **20** was tosylated using *p*-toluenesulfonyl



SCHEME 2 Synthesis of 2-*S*-(5′-deoxy-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*,4′-*C*-methylenethymidine-5′-yl)mercaptoacetic acid (**8**). *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; and (iv) 2M NaOH, methanol.

chloride in pyridine to afford nucleoside **21** in 65% 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*,4′-C-methylenethymidine (**22**) in 90% yield. The targeted compound **8** was synthesized in overall yields of 77% in two steps from compound **22**, i.e., substitution of tosyl group with ethyl mercaptoacetate using sodium hydride in DMF followed by de-esterification of the resulted compound **23** using 2M NaOH in methanol (Scheme 2). The synthesis of 2-*S*-(β-thymidin-5′-yl)mercaptoacetic acid (**9**) was accomplished from β-thymidine through tosylation, silylation, ethoxycarbonylmethylthionation followed by de-esterification in an overall yields of 51% using similar method applied for the synthesis of nucleoside **8**. The structure of compounds **6–23** was established on the basis of its spectral data (IR, ^{1}H-, ^{13}C NMR, and HRMS) analysis. Further, the structure of known compounds **6**, **7**, **10–13**, and **17–20** was confirmed by comparing its physical and spectral data with those reported in the literature.[23–28]

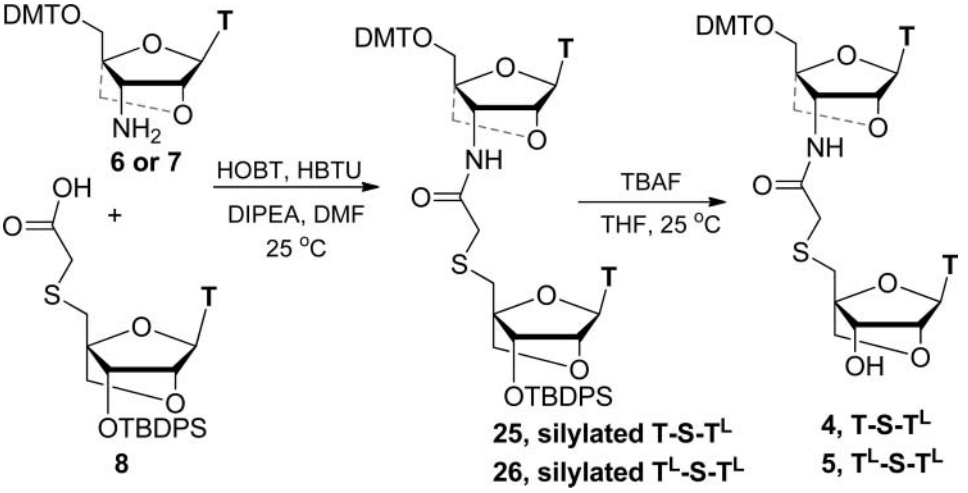
Finally, the synthesis of LNA-based mercaptoacetamido-linked nonionic nucleoside dimer T^{L}-S-T **3** was accomplished by HOBT/HBTU catalyzed coupling of 3′-amino-nucleoside **6** and mercaptoacetic acid **9** in DMF to afford 1-(3′-deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidin-3′-yl)-4-(5″-deoxy-3″-*O*-TBDPS-β-thymidin-5″-yl)mercaptoacetamide **24** in 65% yield. The mercaptoacetamide **24** was desilylated using tetrabutylammoniun fluoride to obtain the desired mercaptoacatamido-linked nonionic nucleoside dimer **3** in 75% yield (Scheme 3).



SCHEME 3 Synthesis of mercaptoacetamido-linked nucleoside dimer T^L-S-T **3** and its 3′-*O*-phosphoramidite.

Further, nucleoside dimers T-S-T^L **4** and T^L-S-T^L **5** were synthesized in two steps, i.e. first by HOBT/HBTU catalyzed coupling of nucleoside **8** with 3′-aminonucleosides **7** and **6** to afford the silylated nucleoside dimers **25** and **26** in 62% yield in each cases. In the second step, desilylation of protected mercaptoacetamides **25** and **26** using tetrabutylammoniun fluoride afforded the desired mercaptoacatamido-linked nucleoside dimers **4** and **5** in 80% and 70% yields, respectively (Scheme 4).

One of the LNA-based mercaptoacetamido-linked nonionic nucleoside dimer T^L-S-T **3** was condensed with [P(-Cl)(-OCH_2CH_2CN){-N(iPr)$_2$}] in DCM in the presence of *N*,*N*-diisopropylethylamine (DIPEA) and converted into its corresponding 3′-*O*-phosphoramidite **27**, a building block for the synthesis of mercaptoacetamido-linked oligonucleotide of therapeutic application (Scheme 3). The structure of protected nucleoside dimers **24**, **25**, and **26**; hydroxyl dimers T^L-S-T **3**, T-S-T^L **4**, and T^L-S-T^L **5**; and phosphoramidite



SCHEME 4 Synthesis of mercaptoacetamido-linked nucleoside dimers T-S-T^L **4** and T^L-S-T^L **5**.

27 was unambiguously established on the basis of their spectral (IR, ^{1}H-, ^{13}C-, ^{31}P NMR spectra, and HRMS) data analysis.

Synthesis of three LNA-based mercaptoacetamido-linked nonionic nucleoside dimers T^{L}-S-T **3**, T-S-T^{L} **4**, and T^{L}-S-T^{L} **5** has been achieved using commonly available starting compound, i.e., D-glucose or thymidine. Phosphoramidite derivative of one of the nucleoside dimers, T^{L}-S-T **3** has been prepared, which can be used for the synthesis of LNA-based mercaptoacetamido-linked nonionic oligonucleotides of potential antisense application.

EXPERIMENTAL SECTION

Melting points (m.p.) were determined on Buchi M-560 instrument and are uncorrected. The IR spectra were recorded on a Perkin–Elmer model 2000 FTIR spectrometer by making KBr disc for solid samples and thin film for oils. The ^{1}H- and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer at 300 MHz and 75.5 MHz or at Jeol alpha-400 spectrometer at 400 MHz and 100.5 MHz, 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-β-thymidine (7).[23] It was synthesized from β-thymidine according to the literature procedure as light yellow foam solid in 49% yield. m.p.: 110–112°C; $[\alpha]_D^{24} = +1.9$ ($c = 0.1$, $CHCl_3$); HR-ESI-TOF-MS: *m/z* 566.2241 ($[M+Na]^+$), calcd. for $[C_{31}H_{33}N_3O_6+Na]^+$ 566.2262.

4-*C*-Hydroxymethyl-1,2-*O*-isopropylidene-β-L-*threo*-pento-furanose (10).[21] It was synthesized according to the literature procedure as white solid in 65% yield. m.p.: 99–100°C (lit[21a] mp: 98–99°C); $[\alpha]_D^{24} = -5.69$ ($c = 0.34$, MeOH); {lit[21a] $[\alpha]_D = -5.73$ (c 0.34, MeOH)}; HR-ESI-TOF-MS *m/z* 243.0840 ($[M+Na]^+$), calcd. for $[C_9H_{16}O_6+Na]^+$ 243.0839.

1,2-*O*-Isopropylidene-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-β-L-*threo*-pentofuranose (11).[21a] It was synthesized from **10** according to the literature procedure as white solid in 60% yield. m.p.: 131–132°C (Lit.[21a] m.p.: 132–133°C), $[\alpha]_D^{25} = -8.4$ (c 0.25, $CHCl_3$) {Lit.[21a] $[\alpha]_D^{25} = -8.0$ (c 0.25, $CHCl_3$)}; HR-ESI-TOF-MS *m/z* 399.0387 ($[M+Na]^+$), calcd. for $[C_{11}H_{20}O_{10}S_2+Na]^+$ 399.0390.

3-Azido-3-deoxy-1,2-*O*-isopropylidene-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-β-D-*ribo*furanose (12).[25b] To a stirred solution of compound **11** (5.0 g, 13.30 mmol) in dichloromethane:anhydrous pyridine (10:1, 45 mL) was added dropwise the solution of trifluoromethanesulfonyl

chloride (3.35 mL, 19.95 mmol). After stirring the mixture for 1.5 hours at −10°C, 50 mL ice-cold water was added and the mixture was extracted with chloroform (3 × 100 mL). The chloroform layer was washed with saturated bicarbonate solution (3 × 50 mL), and dried over anhydrous sodium sulfate. Excess of solvent was evaporated at reduced pressure and the residue thus obtained was heated with NaN_3 (0.95 g, 14.62 mmol) in DMF (70 mL) at 100°C to afford compound **12** (2.70 g) in an overall yield of 50% as sticky colorless solid. $R_f = 0.5$ (5% methanol in chloroform). $[\alpha]_D^{35} = +78.7$ (*c* 0.1, $CHCl_3$). HR-ESI-TOF-MS *m/z* 424.0449 ($[M+Na]^+$), calcd. for $[C_{11}H_{19}N_3O_9S_2+Na]^+$ 424.0455.

1,2-Di-*O*-acetyl-3-azido-3-deoxy-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-α,β-D-*ribo*furanose (13a–13b).[27] It was synthesized from **12** according to the literature procedure as colorless viscous oil **13a–13b** (α:β = *ca.* 2:8, 4.22 g, 84% yield). HR-ESI-TOF-MS *m/z* 468.0352 ($[M+Na]^+$), calcd. for $[C_{12}H_{19}N_3O_{11}S_2+Na]^+$ 468.0353.

2′-*O*-Acetyl-3′-azido-3′-deoxy-5′-*O*-methanesulfonyl-4′-*C*-methanesulfonyloxymethyl-thymidine (14). *N*,*O*-*bis*(Trimethysilyl)acetamide (BSA) (12.5 mL, 50.29 mmol) was added to an anomeric mixture of compound **13a–13b** (5.60 g, 12.57 mmol) and thymine (2.38 g, 18.86 mmol) in dry acetonitrile (120 mL), under nitrogen atmosphere. The reaction mixture was refluxed for 1 hour to get a clear solution. Reaction mixture was brought to room temperature (r.t.) and then trimethylsilyl triflate (TMSOTf) (4.0 mL, 21.37 mmol) was added, and refluxing of the reaction was continued further for 14 hours. The solution was cooled to r.t., extracted with chloroform (3 × 100 ml), and washed with saturated aqueous bicarbonate solution. The organic layer was further washed with water and brine, dried over sodium sulfate, concentrated under reduced pressure and the residue thus obtained was purified on silica gel column chromatography using methanol in chloroform as a gradient solvent system to give **14** (4.82 g, 75%) as light yellow foam solid. $R_f = 0.5$ (10% methanol in chloroform). m.p.: 70–72°C. $[\alpha]_D^{35} = -9.9$ (*c* 0.1, $CHCl_3$). IR (thin film) νmax: 3029, 2942, 2123, 1753, 1695, 1358, 1216, 1176, 1085, 1003, 966, 823, and 756 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.52 (1H, brs), 7.08 (1H, s), 5.73–5.65 (2H, m), 4.94 (1H, $J = 6.6$ Hz, d), 4.49–4.33 (4H, m), 3.14 (3H, s), 3.11 (3H, s), 2.20 (3H, s), 1.92 (3H, s); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 169.97, 163.64, 150.31, 137.99, 112.05, 92.55, 83.52, 74.35, 68.13, 67.15, 62.86, 37.86, 37.76, 20.49, 12.23; HR-ESI-TOF-MS *m/z* 534.0563 ($[M+Na]^+$), calcd. for $[C_{15}H_{21}N_5O_{11}S_2+Na]^+$ 534.0571.

3′-Azido-3′-deoxy-5′-*O*-methanesulfonyl-2′-*O*,4′-*C*-methylenethymidine (15). To a stirred solution of compound **14** (4.70 g, 9.19 mmol) in dioxane:water (22 mL, 1:1) was added 2M NaOH (21.4 mL) and reaction mixture was stirred at r.t. for 1 hour. The reaction mixture was extracted with ethyl acetate (3 × 100 ml), washed with bicarbonate solution and brine,

dried over sodium sulfate, and concentrated under reduced pressure. The residue thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to give **15** (2.88 g, 90%) as off white solid. $R_f = 0.5$ (10% methanol in chloroform). m.p.: 77–79°C. $[\alpha]_D^{35} = +64.4$ (*c* 0.1, $CHCl_3$); IR (thin film) νmax: 3179, 3026, 2121, 1691, 1464, 1355, 1276, 1175, 1111, 1059, 1024, 998, 966, 812, and 757 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 11.39 (1H, brs), 7.45 (1H, s), 5.55 (1H, s), 4.79 (1H, $J = 12.0$ Hz, d), 4.64 (1H, $J = 12.0$ Hz, d), 4.60 (1H, s), 4.20 (1H, s), 3.88–3.80 (2H, m), 3.32 (3H, s), 1.79 (3H, s); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 163.85, 149.93, 134.35, 108.67, 86.59, 85.84, 78.52, 70.89, 65.09, 60.60, 36.96,12.27; HR-ESI-TOF-MS *m/z* 396.0579 ($[M+Na]^+$), calcd. for $[C_{12}H_{15}N_5O_7S+Na]^+$ 396.0584.

3′-Azido-5′-*O*-benzoyl-3′-deoxy-2′-*O*-4′-*C*-methylenethymidine (16). A mixture of compound **15** (3.5 g, 10.02 mmol) and sodium benzoate (2.89 g, 20.05 mmol) was suspended in anhydrous DMF (170 mL) and was stirred for 4 hours at 100°C. The mixture was cooled to r.t., filtered, and concentrated under reduced pressure. The residue thus obtained was redissolved in ethyl acetate (200 mL) and washed with saturated $NaHCO_3$ (2 × 200 mL) and H_2O (4 × 200 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system to afford the nucleoside **16** (3.19 g, 80%) as white solid. $R_f = 0.5$ (8% methanol in chloroform). m.p.: 168–170°C; $[\alpha]_D^{25} = +89.2$ (*c* 0.1, $CHCl_3$); IR (thin film) νmax: 3178, 3041, 2959, 2827, 2130, 1709, 1686, 1467, 1270, 1111, 1060, 1026, 940, 907, 871, and 719 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 11.40 (1H, brs), 8.05 (2H, $J = 8.1$ Hz, d), 7.74–7.57 (3H, m), 7.34 (1H, s), 5.56 (1H, s), 4.79 (2H, s), 4.61 (1H, s), 4.37 (1H, s), 3.94 (2H, $J = 16.8$ and 8.7 Hz, dd), 1.59 (3H, s); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 165.16, 163.80, 149.90, 134.11, 133.81, 129.33, 128.92, 108.53, 86.59, 86.45, 78.51, 71.14, 66.32, 60.65, 12.04; HR-ESI-TOF-MS *m/z* 422.1062 ($[M+Na]^+$), calcd. for $[C_{18}H_{17}N_5O_6+Na]^+$ 422.1071.

3′-Azido-3′-deoxy-2′-*O*,4′-*C*-methylenethymidine (17).[28] To a stirred solution of compound **16** (3.11 g, 7.78 mmol) in THF:water (1:1, 50 mL) was added 2M NaOH (11.8 mL), and the mixture was stirred at 0°C for 1 hour. After completion of reaction acetic acid (3.0 mL) was added, and the solution was concentrated under reduced pressure. Purification was performed on silica gel column using methanol in chloroform as gradient solvent system to yield azido-LNA **17** (2.07 g, 90%) as a white powder. m.p.: 94–95°C (Lit.[28] m.p.: 94–96°C); $[\alpha]_D^{35} = +87.1$ (*c* 0.1, MeOH); HR-ESI-TOF-MS *m/z* 318.0816 ($[M+Na]^+$), calcd. for $[C_{11}H_{13}N_5O_5+Na]^+$ 318.0809.

3′-Azido-3′-deoxy-5′-*O*-DMT-2′-*O*-4′-*C*-methylenethymidine (18).[25a] It was synthesized from **17** according to the literature procedure to afford **18** (0.45 g, 75%) as white foam solid. m.p.: 125–126°C, (Lit.[25a] m.p.:

125–128°C); $[\alpha]_D^{22}$ = +19.1 (*c* 0.75, acetone); HR-ESI-TOF-MS: *m/z* 620.2107 ($[M+Na]^+$), calcd. for $[C_{32}H_{31}N_5O_7+Na]^+$ 620.2116.

3′-Amino-3′-deoxy-5′-*O*-DMT-2′-*O*-4′-*C*-methylenethymidine (6).[25a] It was synthesized from **18** according to the literature procedure to afford **6** (0.10 g, 90% yield) as light yellow foam solid. m.p.: 130–132°C, (Lit.[25a] m.p.: 131–134°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*,4′-*C*-methylenethymidine (20).[24] Compound **20** was synthesized from D-glucose according to the literature procedure as a white solid material. m.p.: 196–197°C (Lit.[24] m.p.: 196–198°C); $[\alpha]_D^{23}$ = + 57.9 (*c* = 1, EtOH); HR-ESI-TOF-MS: *m/z* 293.0752 ($[M+Na]^+$), calcd. for $[C_{11}H_{14}N_2O_6+Na]^+$ 293.0744.

5′-*O*-*p*-Toluenesulfonyl-2′-*O*,4′-*C*-methylenethymidine (21). *p*-Toluene sulfonyl chloride (12 mmol) dissolved in 10 mL of dry pyridine was added drop wise during 4 hours to the solution of **20** (10 mmol) in 30 mL of dry pyridine at 0°C. The reaction mixture was stirred for additional 4 hours at r.t.. 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 × 50 mL) 10% aq. $NaHCO_3$ solution followed by cold water (2 × 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 **21** as white solid (2.75 g, 65% yield). R_f = 0.4 (10% methanol in chloroform); m.p.: 100–102°C; $[\alpha]_D^{22}$ = + 71.7 (*c* = 0.1, MeOH); IR (KBr) ν_{max}: 3401, 3198, 3070, 2952, 1690, 1597, 1466, 1363, 1275, 1176, 1054, 983, and 810 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 9.72 (1H, brs), 7.82 (2H, *J* = 8.2 Hz, d), 7.46 (1H, s), 7.39 (2H, *J* = 8.2 Hz, d), 5.52 (1H, s), 4.60 (1H, s), 4.41 (2H, s), 4.23 (1H, s), 4.00 (1H, brs), 3.99 (1H, *J* = 8.0 Hz, d), 3.79 (1H, *J* = 7.9 Hz, d), 2.46 (3H, s), 1.85 (3H, s); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 164.35, 150.20, 149.55, 145.64, 134.36, 132.30, 130.21, 110.56, 87.18, 86.22, 79.15, 77.01, 69.94, 64.27, 21.70, 12.45; HR-ESI-TOF-MS: *m/z* 447.0838 ($[M+Na]^+$), calcd. for $[C_{18}H_{20}N_2O_8S+Na]^+$ 447.0833.

5′-*O*-*p*-Toluenesulfonyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*,4′-*C*-methylene thymidine (22). A solution of compound **21** (6.0 mmol), *tert*-butyldiphe nylsilyl chloride (7.2 mmol), imidazole (14.8 mmol) in 20 mL of anhydrous DMF was stirred for 6 hours under nitrogen atmosphere at r.t.. Excess of DMF was removed in vacuo and the residue was dissolved in 200 mL of ethyl acetate, washed with water (3 × 50 mL) followed by brine (2 × 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 **22** as light yellow crystalline solid (3.51 g, 90% yield). R_f = 0.5 (50% ethyl acetate in petroleum ether); m.p.: 95–96°C;

$[\alpha]_D^{23}$ = + 6.2 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3181, 3048, 2955, 2859, 1689, 1597, 1466, 1370, 1274, 1178, 1104, 1054, 823, 744, and 705 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.71 (1H, brs), 7.81–7.34 (15H, m), 5.47 (1H, s), 4.84–4.35 (2H, m), 4.14 (1H, J = 7.2 Hz, d), 4.02–3.79 (3H, m), 2.48 (3H, s), 1.74 (3H, s), 1.03 (9H, s); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 163.48, 149.13, 145.60, 135.61, 135.33, 133.71, 132.35, 131.98, 131.39, 130.45, 130.37, 130.15, 128.06, 127.95, 110.20, 86.86, 85.81, 78.47, 71.50, 63.70, 26.90, 21.72, 19.04, 12.37; HR-ESI-TOF-MS: *m/z* 663.2162 ($[M+H]^+$), calcd. for $[C_{34}H_{38}N_2O_8SSi+H]^+$ 663.2191.

Ethyl 2-*S*-(3′-*O*-*tert*-butyldiphenylsilyl-5′-deoxy-2′-*O*,4′-*C*-methylenethymidin-5′-*yl*)mercaptoacetate (23). A solution of NaH (0.29 g, 60% in hexane, 7.25 mmol.) and ethyl mercaptoacetate (5.5 mmol) in anhydrous DMF (5 mL) was stirred for 30 minutes. Compound **22** (4.50 mmol) dissolved in DMF (5 mL) was then added slowly at 0°C. The reaction mixture was stirred for 1 hour at r.t.. 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 × 50 mL) and with brine (2 × 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 **23** as viscous oil (2.26 g, 82% yield). R_f = 0.5 (50% ethyl acetate in petroleum ether); $[\alpha]_D^{23}$ = + 41.2 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3183, 3069, 2931, 2858, 1690, 1459, 1275, 1155, 1113, 1051, 943, 856 and 744 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 9.20 (1H, brs), 7.63–7.31 (11H, m), 5.51 (1H, s), 4.22–4.14 (3H, m), 3.93 (2H, s), 3.91 (1H, s), 3.70 (1H, s), 3.33–3.11 (3H, m), 1.78 (3H, J = 1.0 Hz, d), 1.28 (3H, J = 7.2 Hz, t), 1.09 (9H, s); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 169.80, 163.62, 149.25, 145.90, 135.66, 135.57, 135.43, 134.24, 132.16, 131.80, 130.39, 130.34, 130.14, 127.98, 127.93, 109.85, 88.39, 86.96, 78.75, 73.61, 72.87, 61.60, 34.79, 28.88, 26.75, 21.07, 19.10, 12.18; HR-ESI-TOF-MS: *m/z* 633.2028 ($[M+Na]^+$), calcd. for $[C_{31}H_{38}N_2O_7SSi+Na]^+$ 633.2061.

2-*S*-(5′-Deoxy-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*,4′-*C*-methylenethymidin-5′-yl)mercaptoacetic acid (8). Sodium hydroxide (2M, 5 mL) solution was added to a solution of **23** (5.30 mmol) in 10 mL methanol. The reaction mixture was stirred for 30 minutes. 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 **8** as white solid (2.32 g, 94% yield). R_f = 0.5 (50% methanol in chloroform); m.p.: 208–210°C; $[\alpha]_D^{23}$ = +34.5 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3449, 3064, 2931, 2858, 1689, 1580, 1469, 1427, 1389, 1273, 1156, 1110, 1052, 855 and 704 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 11.26 (1H, brs), 7.58–7.32 (11H, m), 5.30 (1H, s), 4.04 (1H, J = 8.1 Hz, d), 3.82 (1H, J = 8.1 Hz, d), 3.74 (1H, s),

3.61 (1H, s), 3.28 (2H, s), 3.12 (2H, s), 1.64 (3H, s), 1.02 (9H, s); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 172.60, 163.58, 149.39, 135.13, 134.95, 133.72, 132.17, 131.44, 130.17, 127.87, 127.77, 108.69, 88.34, 86.03, 78.10, 73.14, 72.46, 38.58, 27.69, 26.47, 18.63, 12.22; HR-ESI-TOF-MS: *m/z* 605.1731 ([M+Na]$^+$), calcd. for [$C_{29}H_{34}N_2O_7SSi$+Na]$^+$ 605.1748.

2-*S*-(5′-Deoxy-3′-*O*-*tert*-butyldiphenylsilyl-β-thymidin-5′-yl)mercaptoacetic acid (9). It was synthesized from β-thymidine following the same procedure used for the synthesis of mercaptoacetic acid **8** from **20** as white solid (2.70 g, 92% yield). m.p.: 69–70°C; $[\alpha]_D^{27} = +29.13$ (*c* 0.1, MeOH); IR (thin film) νmax: 3180, 3071, 2932, 2859, 1702, 1474, 1428, 1364, 1276, 1197, 1112, 998, 924, 823, 756, 703, and 669 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.48 (1H, brs), 7.65–7.61 (4H, m), 7.45–7.40 (6H, m), 7.21 (1H, J = 1.2 Hz, d), 6.27 (1H, J = 6.8 Hz, t), 4.24–4.20 (1H, m), 4.12–4.08 (1H, m), 3.12 (2H, J = 33.2 Hz and J = 14.8 Hz, dd), 2.63–2.62 (1H, m), 2.58–2.53 (1H, m), 2.37–2.31 (1H, m), 1.95–1.88 (1H, m), 1.83 (3H, s), 1.07 (9H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.46, 164.60, 150.34, 136.02, 135.70, 132.86, 130.20, 127.97, 111.22, 86.15, 84.58, 74.52, 40.17, 34.29, 34.20, 26.81, 18.95, 12.29; HR-ESI-TOF-MS *m/z* 577.1788 ([M+Na]$^+$), calcd. for [$C_{28}H_{34}N_2O_6SSi$+Na]$^+$ 577.1799.

General procedure for the synthesis of silylated dimers 24–26. To a solution of mercaptoacetic acid **8**/**9** (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 **6**/**7** (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 hours. The reaction mixture was concentrated to dryness, dissolved in ethyl acetate (30 mL) and washed with aqueous 5% $NaHCO_3$ solution (2 × 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 **24** and **25**/**26** in 65% and 62% yields, respectively.

1-(3′-Deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidin-3′-yl)-4-(5″-deoxy-3″-*O*-*tert*-butyldiphenylsilyl-β-thymidin-5″-yl)mercaptoacetamide (24). It was obtained by the HOBT/HBTU catalyzed coupling reaction of acid **9** with amine **6** as white solid (1.24 g, 65% yield). m.p.: 104–105°C. $[\alpha]_D^{27}$ = +26.71 (*c* 0.1, MeOH); IR (thin film) νmax: 3199, 3069, 2932, 2859, 1691, 1509, 1466, 1384, 1275, 1252, 1178, 1112, 1055, 1035, 978, 916, 830, 792, 756, 704, 665, and 613 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.90 (1H, brs), 8.58 (1H, brs), 8.01 (1H, brs), 7.62–7.60 (4H, m), 7.45–7.25 (18H, m), 6.82 (4H, J = 8.4 Hz, d), 6.17–5.82 (1H, m), 5.30 (1H, J = 5.2 Hz, d), 4.51 (1H, J = 8.0 Hz, d), 4.47 (1H, J = 3.6 Hz, d), 4.23–4.21 (1H, m), 3.97–3.91 (1H, m), 3.78–3.76 (6H, m), 3.43 (2H, m), 3.41 (1H, J = 7.2 Hz, d), 2.88–2.87

(2H, m), 2.46–2.41 (1H, m), 2.24–2.22 (3H, m), 1.84 (3H, s), 1.63 (3H, s), 1.06 (9H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 169.71, 163.77, 163.62, 158.13, 150.43, 149.91, 144.51, 135.75, 135.38, 134.97, 133.87, 132.82, 132.59, 130.07, 129.76, 128.00, 127.72, 126.75, 113.24, 109.81, 108.84, 87.63, 86.30, 85.88, 85.43, 84.05, 79.18, 78.54, 75.36, 71.15, 57.97, 55.00, 51.18, 38.42, 33.65, 26.66, 18.59, 12.41, 12.00; HR-ESI-TOF-MS *m/z* 1130.4031 ([M+Na]$^+$), calcd. for [$C_{60}H_{65}N_5O_{12}SSi$+Na]$^+$ 1130.4012.

1-(3′-Deoxy-5′-*O*-DMT-β-thymidin-3′-yl)-4-(5″-deoxy-3″-*O*-*tert*-butyldiphenylsilyl-2″-*O*,4″-*C*-methylenethymidin-5″-yl)mercaptoacetamide (25). It was obtained by the HOBT/HBTU catalyzed coupling reaction of acid **8** with amine **7** as white solid (1.17 g, 62% yield). R_f = 0.7 (10% methaol in chloroform); m.p.: 138–140°C; $[\alpha]_D^{24}$ = +18.3 (*c* 0.05, $CHCl_3$); IR (KBr) ν_{max}: 3338, 3064, 2930, 2857, 1686, 1507, 1459, 1252, 1176, 1112, 1051, 829, and 703 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.47 (2H, 2 x brs), 7.63–7.56 (5H, m), 7.41–7.19 (17H, m), 6.83 (4H, J = 8.4 Hz, d), 6.34 (1H, brs), 5.50 (1H, s), 4.66 (1H, s), 4.11 (1H, J = 7.8 Hz, d), 4.03 (1H, s), 3.87–3.82 (2H, m), 3.71 (7H, s), 3.46 (2H, s), 3.29 (2H, s), 3.06–2.88 (2H, m), 2.40–2.30 (2H, m), 1.74 (3H, s), 1.37 (3H, s), 1.06 (9H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 168.56, 163.70, 163.56, 158.14, 150.38, 149.43, 144.71, 135.71, 135.45, 135.19, 135.03, 133.58, 132.12, 131.47, 130.26, 129.77, 127.92, 127.85, 127.72, 126.79, 113.23, 109.67, 108.73, 87.85, 86.17, 85.88, 83.66, 83.15, 78.23, 73.03, 72.69, 63.66, 55.05, 49.61, 36.74, 35.84, 28.30, 26.49, 22.51, 18.65, 12.26, 11.72; HR-ESI-TOF-MS: *m/z* 1130.3980 ([M+Na]$^+$), calcd. for [$C_{60}H_{65}N_5O_{12}SSi$+Na]$^+$ 1130.4012.

1-(3′-Deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidin-3′-yl)-4-(5″-deoxy-3″-*O*-*tert*-butyldiphenylsilyl-2″-*O*,4″-*C*-methylene-thymidin-5″-yl)mercaptoacetamide (26). It was obtained by the HOBT/HBTU catalyzed coupling reaction of acid **8** with amine **6** as white solid (1.20 g, 62% yield). R_f = 0.7 (10% methaol in chloroform); m.p.: 118–120°C; $[\alpha]_D^{25}$ = + 4.2 (*c* 0.05, $CHCl_3$); IR (KBr) ν_{max}: 3199, 3069, 2931, 1691, 1608, 1509, 1465, 1252, 1177, 1112, 1053, 980, 831, and 703 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.62–7.22 (22H, m), 6.84 (4H, J = 8.7 Hz, d), 5.64 (1H, s), 5.48 (1H, s), 4.50 (1H, s), 4.45 (1H, J = 8.1 Hz, d), 4.09 (1H, J = 7.8 Hz, d), 3.92 (1H, s), 3.85 (1H, s), 3.78 (7H, s), 3.73 (2H, m), 3.48 (2H, s), 3.26 (2H, J = 14.4 Hz, q), 2.95 (1H, J = 15.0 Hz, d), 2.81 (1H, J = 15.0 Hz, d), 1.69 (6H, s), 1.06 (9H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 169.73, 163.75, 163.56, 158.12, 150.39, 149.89, 144.49, 135.79, 135.44, 135.28, 134.95, 133.11, 132.73, 110.01, 108.81, 87.59, 86.27, 85.87, 83.21, 80.68, 78.51, 72.88, 71.06, 57.88, 57.23, 54.99, 51.10, 45.67, 38.12, 33.58, 33.22, 26.68, 18.88, 12.39, 11.93;HR-ESI-TOF: *m/z* 1136.3997 ([M+H]$^+$), calcd. for [$C_{61}H_{65}N_5O_{13}SSi$+H]$^+$ 1136.4101.

General procedure for the synthesis of mercaptoacetamido-linked dimers 3, 4, and 5. A solution of **24**/**25** or **26** (0.90 mmol) and TBAF (1.20 mL, 1M in THF) in anhydrous tetrahydrofuran (15 mL) was stirred at

r.t. for 1 hour. Excess of tetrahydrofuran was evaporated in vacuo and residue was redissolved in dichloromethane (50 mL). The solution was washed with water (2 × 20 mL) followed by brine (2 × 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 dimers **3**/**4**/**5** in 75%, 80%, and 70% yields, respectively.

1-(3′-Deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidin-3′-yl)-4-(5″-deoxy-β-thymidin-5″-yl)mercaptoacetamide (3). It was obtained as white solid powder (0.59 g, 75% yield). m.p.: 173–175°C (decompose); $[\alpha]_D^{30} = +29.1$ (*c* 0.05, MeOH); IR (KBr) *ν*max: 3448, 3067, 2927, 1690, 1508, 1466, 1252, 1178, 1054, 977, 895, 823, 760, 727, and 705 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): *δ* 10.36 (1H, brs), 9.87 (1H, brs), 7.61 (1H, brs), 7.47 (2H, *J* = 7.2 Hz, d), 7.36–7.21(10H, m), 7.14 (1H, brs), 6.84 (4H, *J* = 9.2 Hz and *J* = 2.8 Hz, dd), 5.98 (1H, s), 5.59 (1H, s), 5.04 (1H, *J* = 16.0 Hz, d), 4.59 (1H, *J* = 7.6 Hz, d), 4.25 (2H, *J* = 20.0 Hz, d), 3.96 (2H, *J* = 8.4 Hz, d), 3.78 (6H, s), 3.48 (2H, *J* = 23.2 Hz and *J* = 11.2 Hz, dd), 3.23 (2H, s), 2.79–2.71 (2H, m), 2.28–2.25 (1H, *J* = 12.0 Hz, d), 1.84 (3H, s), 1.68 (3H, s); ^{13}C NMR (DMSO-d_6, 100 MHz): *δ* 169.88, 163.80, 158.15, 150.45, 149.91, 144.60, 135.95, 135.25, 134.98, 133.92, 129.86, 127.96, 127.72, 126.79, 113.27, 109.77, 108.82, 87.65, 86.30, 85.89, 85.18, 83.81, 79.19, 78.53, 72.41, 71.16, 57.99, 55.05, 51.23, 39.18, 34.01, 12.40, 12.05; HR-ESI-TOF-MS *m/z* 892.2771 ($[M+Na]^+$), calcd. for $[C_{44}H_{47}N_5O_{12}S+Na]^+$ 892.2834.

1-(3′-Deoxy-5′-*O*-DMT-β-thymidin-3′-yl)-4-(5″-deoxy-2″-*O*,4″-*C*-methylene thymidin-5″-yl)mercaptoacetamide (4). It was obtained as white solid powder (0.61 g, 80% yield). $R_f = 0.5$ (10% methaol in chloroform); m.p.: 158–160°C; $[\alpha]_D^{24} = +102.2$ (c 0.1, $CHCl_3$); IR (KBr) ν_{max}: 3353, 3064, 2930, 1694, 1509, 1466, 1252, 1178, 1105, 1042, 830, and 584 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): *δ* 11.35 (2H, brs), 8.54 (1H, *J* = 7.2 Hz, d), 7.53 (1H, s), 7.66 (1H, s), 7.37 (2H, *J* = 7.2 Hz, d), 7.30–7.20 (8H, m), 6.88 (4H, *J* = 8.8 Hz and *J* = 2.0 Hz, dd), 6.20 (1H, *J* = 6.4 Hz, t), 5.85 (1H, *J* = 4.4 Hz, d), 5.43 (1H, s), 4.50–4.43 (1H, m), 4.13 (1H, s), 3.87 (2H, *J* = 4.4 Hz, d), 3.81 (1H, *J* = 8.0 Hz, d), 3.70–3.66 (7H, m), 3.32–3.25 (2H, m), 3.18–3.11 (2H, m), 3.00–2.90 (2H, m), 2.37–2.32 (1H, m), 2.18–2.12 (1H, m), 1.75 (3H, s), 1.42 (3H, s); ^{13}C NMR (DMSO-d_6, 100 MHz): *δ* 168.87, 163.35, 158.14, 150.40, 149.95, 144.60, 135.80, 135.45, 135.27, 134.64, 129.80, 127.94, 127.74, 126.82, 113.25, 109.77, 108.65, 88.00, 86.51, 85.72, 83.67, 83.09, 79.09, 72.57, 70.68, 63.51, 55.07, 49.56, 45.74, 36.76, 35.98, 28.34, 12.40, 11.76; HR-ESI-TOF-MS: *m/z* 892.2851 ($[M+Na]^+$), calcd. for $[C_{44}H_{47}N_5O_{12}S+Na]^+$ 892.2834.

1-(3′-Deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidin-3′-yl)-4-(5″-deoxy-2″-*O*,4″-*C*-methylenethymidin-5″-yl)mercaptoacetamide (5). It was obtained as white solid powder (0.55 g, 70% yield). $R_f = 0.5$ (10% methaol in chloroform); m.p.: 158–160°C; $[\alpha]_D^{24} = +47.1$ (*c* 0.05, $CHCl_3$); IR (KBr)

ν_{max}: 3195, 2924, 2853, 1694, 1609, 1509, 1463, 1251, 1178, 1053, 978, and 832 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.45 (2H, brs), 8.16 (1H, J = 8.4 Hz, d), 7.70 (1H, s), 7.58 (1H, s), 7.44 (2H, J = 7.6 Hz, d), 7.33–7.23 (8H, m), 6.89 (4H, J = 8.8 Hz and J = 2.0 Hz, dd), 4.85 (1H, s), 5.52 (2H, J = 16.0 Hz, d), 4.44 (2H, J = 7.2 Hz, d), 4.32 (1H, s), 3.85–3.84 (2H, m), 3.73 (8H, s), 3.34–3.31 (2H, m), 3.26–3.19 (2H, m), 3.04 (2H, J = 14.8 Hz, d), 1.78 (3H, s), 1.54 (3H, s); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 169.68, 163.69, 158.14, 149.90, 144.60, 134.97, 134.31, 133.86, 129.83, 129.78, 127.92, 127.66, 126.77, 113.25, 108.77, 87.56, 87.10, 86.47, 86.28, 85.84, 78.53, 77.48, 72.65, 72.00, 71.13, 57.93, 55.02, 50.88, 45.64, 34.59, 28.14, 12.21; HR-ESI-TOF-MS: m/z 898.2925 ($[M+H]^+$), calcd. for $[C_{45}H_{47}N_5O_{13}S+H]^+$ 898.2911.

1-(3′-Deoxy-5′-*O*-DMT-2′-*O*,4′-*C*-methylenethymidin-3′-yl)-4-(3″-*O*-β-cyanoethyl-*N*,*N*-diisopropylphosphoramidite-5″-deoxy-β-thymidin-5″-yl)mercaptoacetamide (27). To a stirred solution of compound 3 (0.1g, 0.115 mmol) in dry DCM (2 mL), *N*,*N*-Diisopropylethylamine(25 μL, 0.283 mmol) and 2-cyanoethyl *N*,*N*-diisopropylamino chlorophosphoramidite (64 μL, 0.283 mmol) were added under argon atmosphere at 0°C. The reaction mixture was stirred at r.t. for 30 minutes. After completion of reaction on analytical TLC examination, the reaction mixture was diluted with dry DCM (20 mL) and washed with 5% $NaHCO_3$ solution (2 × 10 mL). The organic phase was dried and concentrated under reduced pressure. Purification was done by flash column chromatography using hexane: ethyl acetate (1:8) solvent gradient. The phosphoramidite 27 was dried overnight over P_2O_5 and KOH in a desiccator before characterization. It was obtained as white solid, R_f = 0.5 (ethyl acetate); ^{31}P NMR ($CDCl_3$): δ 148.96, 148.70; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.59 (1H, s), 7.49 (1H, J = 4.0 Hz, d), 7.38–7.10 (11H, m), 6.86 (4H, J = 8.0 Hz, d), 6.01–5.99 (1H, m), 5.84–5.83 (1H, m), 5.68 (1H, s), 5.04–4.93 (3H, m), 4.56–4.46 (3H, m), 3.95–3.60 (11H, m), 3.50–3.48 (2H, m), 3.24–3.21 (2H, m), 2.88–2.87 (3H, m), 2.66–2.64 (2H, m), 1.72 (3H, s), 1.49 (3H, s), 1.30 (6H, J = 8.0 Hz, d), 1.20 (6H, J = 8.0 Hz, d); HR-ESI-TOF-MS: m/z 1092 ($[M+Na]^+$), calcd. for $[C_{53}H_{64}N_7O_{13}PS+Na]^+$ 1092.

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