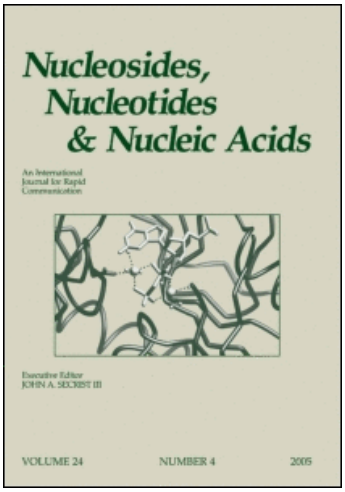

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TT DINUCLEOTIDES CONTAINING AN ISOXAZOLINE MOIETY: SYNTHESIS AND BINDING AFFINITY STUDY

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TT DINUCLEOTIDES CONTAINING AN ISOXAZOLINE MOIETY: SYNTHESIS AND BINDING AFFINITY STUDY

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ABSTRACT

We have prepared four diastereomers of TT dinucleotides containing an isoxazoline moiety and four dodecanucleotides incorporating these TT dinucleotides in the middle of the DNA sequence. We also have determined the melting temperatures of these modified oligonucleotides (Tm values) by measuring change in UV absorbance.

INTRODUCTION

Synthesis of modified oligonucleotides is very important especially in the field of antisense and antigene based drug discovery[1]. We have been interested in the replacement of the phosphodiester linkages of the natural oligonucleotides with novel, neutral, and heterocyclic linkages based on their stability and rigidity. Recently we have reported the design and synthesis of the isoxazoline linkage for replacement of nucleotide phosphodiester[2]. We further developed and elaborated the idea for the modification of the natural

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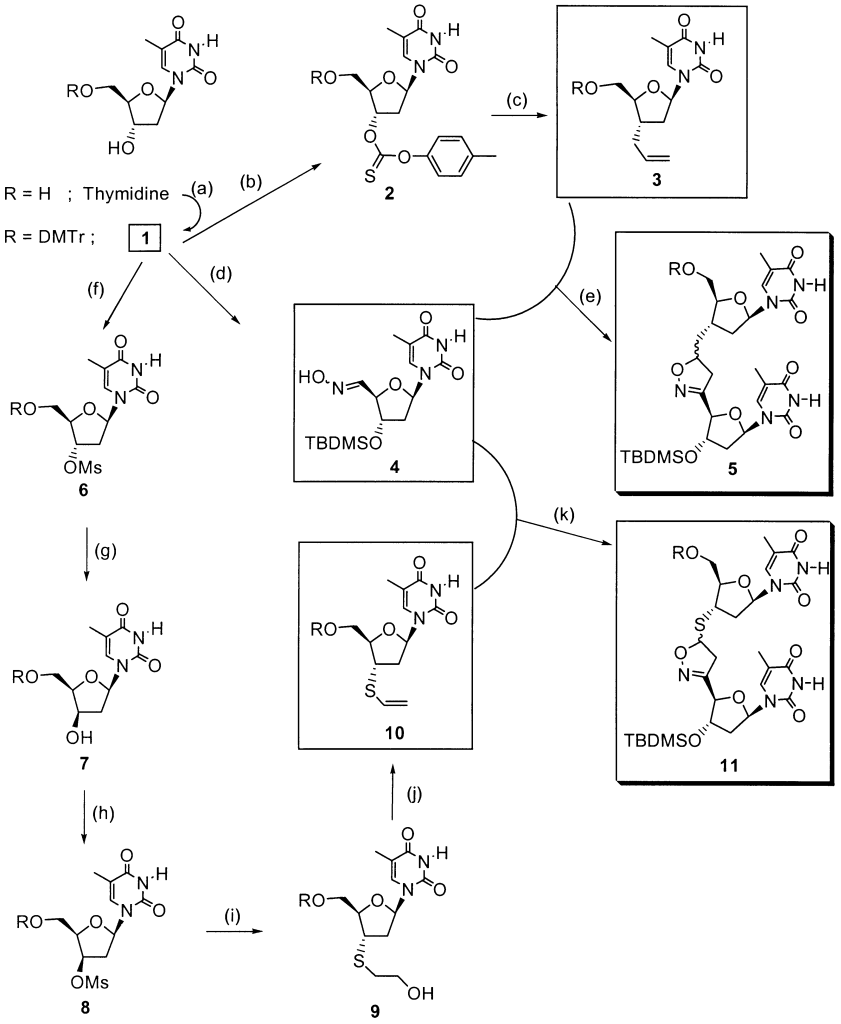
phosphodiester linkage and report here a facile synthesis of TT dinucleotides with modified linkages and determination of the Tm values of DNA dodecanucleotides with modified dinucleotides linkages.

RESULTS AND DISCUSSION

Synthetic routes for four diastereomers are summarized in Scheme 1. Selective protection of 5′-hydroxyl group of thymidine with 4,4′-dimethoxy-



Reagents and conditions: (a) DMTrCl, Py, rt, 88%; (b) *p*-tolylchlorothionoformate, DMAP, CH_2Cl_2, 84%; (c) Al tributyltin, AIBN, Toluene, 80°C, 65%; (d) ref 2; (e) NaOCl (4% aqeous), CH_2Cl_2, 61%; (f) MsCl, Py, 0°C, 94%; $LiOH{\cdot}H_2O$, $EtOH/H_2O$, 84%; (h) MsCl, Py, 0°C, 92%; (i) Mercaptoethanol, DBU, DMF, 70°C, 74%; (j) 1. MsCl, 0°C; 2. *t*-BuOK, THF, 0°C, 83%; (k) NaOCl (4% aqueous), CH_2Cl_2, 74%.

Scheme 1.

trityl (DMTr) group followed by *p*-tolylthionocarbonation of 3′-hydroxyl group of thymidine with *p*-tolylchlorothionoformate provided compound **2** in good yield. Radical allylation with allyltributyltin and AIBN afforded the desired dipolarophiles **3**[3]. Nitrile oxide cycloaddition between the precursor **4**[2] and the dipolarophile **3** gave a 1:1 diastereomeric mixture of TT dinucleotide **5**. We isolated the less polar diastereomer **5a** ($R_f = 0.30$ in the eluent of ethyl acetate:hexane = 2:1) in 31% yield and the more polar one **5b** ($R_f = 0.26$ in the eluent of ethyl acetate:hexane = 2:1) in 30% yield. At this moment we cannot determine the absolute configuration of the isoxazoline stereogenic center in each diastereomer decisively and tentatively assign both diastereomers based on their polarity.

TT dinucleotide diastereomers with sulfur heteroatom were synthesized by using a cycloaddition with the sulfur containing dipolarophile **10**. DMTr protection of 5′-hydroxyl group of thymidine followed by mesylation of 3′-hydroxyl group provided compound **6**. Treatment of compound **6** with $LiOH \cdot H_2O$ and another mesylation afforded compound **8** in 77% overall yield. Three-step sequence (displacement with mercaptoethanol, mesylation, and elimination) of **8** gave the desired dipolarophile **10**. Nitrile oxide cycloaddition between the precursor **4**[2] and the dipolarophile **10** provided a 3:1 diastereomeric mixture of TT dinucleotide **11**. We isolated by column chromatography the less polar compound **11a** ($R_f = 0.25$ in the eluent of ethyl acetate:hexane = 2:1) as the major product in 59% yield and the more polar one **11b** ($R_f = 0.23$ in the eluent of ethyl acetate:hexane = 2:1) in 20% yield.

With four diastereomers (**5a**, **5b**, **11a**, and **11b**) in hand, we next carried out measurement of the Tm values of the DNA dodecanucleotides containing these dinucleotidic fragments. We prepared 2-cyanoethyl phosphoramidite building blocks of TT dinucleosides and directly applied them to solid phase oligonucleotide synthesis protocols[4] on the ABI 391 DNA synthesizer. The sequences and Tm values of DNA dodecanucleotides are described in Table 1, and Fig. 1 shows the typical curves of relative absorbance for Tm value determination.

Compared to unmodified duplex, incorporation of one isoxazoline backbone (**5a**) little decreased the duplex stability by a ΔTm of $-3.3\,^{\circ}C$, and another type of duplex with **5b** more decreased ($-16.5\,^{\circ}C$) duplex stability. On the other hand, the duplexes containing both the higher and lower R_f's sulfur-isoxazoline backbone slightly more decreased ($-5.5\,^{\circ}C$ and $-5.0\,^{\circ}C$) than the duplex containing higher R_f's isoxazoline backbone (**5a**). Substitution of a phosphodiester linkage with isoxazoline or sulfur-isoxazoline backbone reduced the contribution of the base hydrogen bonding of the oligonucleotides slightly. These results suggest that the isoxazoline (**5a** and **5b**) and sulfur-isoxazoline (**11a**) backbone modified nucleotides can be useful antisense oligonucleotides candidates for antiviral agents. Furthermore more substitutions with these TT dinucleotides containing an isoxazoline moiety

Table 1. Tm Values of Dodecanucleotides Containing One Isoxazoline and Sulfur-isoxazoline Backbone

Oligo name	Sequence	Tm (°C)[a]	ΔTm (°C)
A12	5′–AAAAAAAAAAAA–3′		
T12[b]	5′–TTTTTTTTTTTT–3′	38.1	0
T12-(5a)[b]	5′–TTTTT**TT**TTTTT–3′	34.8	−3.3
T12-(5b)[b]	5′–TTTTT**TT**TTTTT–3′	21.6	−16.5
T12[c]	5′–TTTTTTTTTTTT–3′	38.5	0
T12-(11a)[c]	5′–TTTTT**TT**TTTTT–3′	33.0	−5.5
T12-(11b)[c]	5′–TTTTT**TT**TTTTT–3′	33.5	−5.0

[a] Melting temperatures were determined by measuring change in absorbance at 260 nm (cuvette, 1 cm path length) as a function of temperature in Tris–HCl buffer (10 mM, pH 7.0) containing 100 mM NaCl and 20 mM $MgCl_2$. Temperature was raised 0.5 °C/min. All the values are averaged from at least three experiments.
[b] Total strands concentration was 2.5 μM.
[c] Total strands concentration was 3.0 μM.

may provide positive hybridization data and RNA hybridization may afford positive Tm changes.

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were obtained on FT-300 Mhz Bruker Apect 3000 spectrometer. The molecular mass was determined by using Jeol JMS-AX505WA (FAB). The IR spectra were obtained on Bruker FT-IR

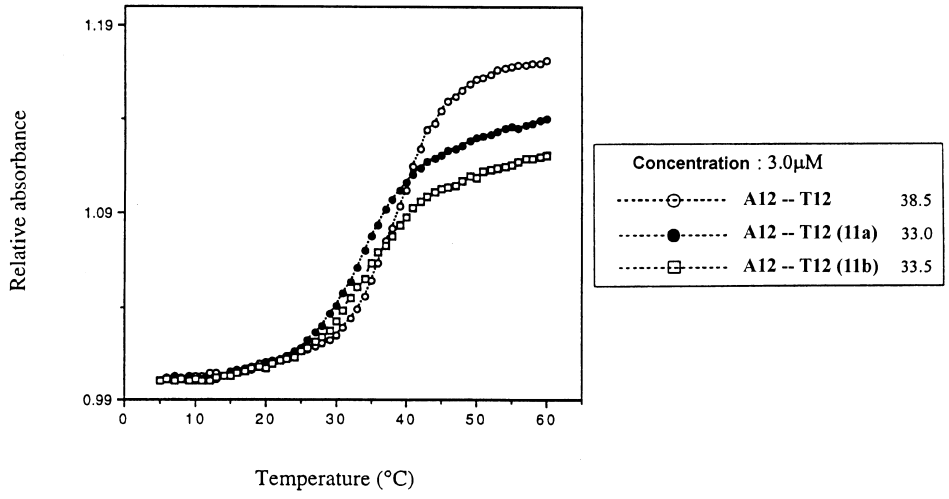


Figure 1. Thermal denaturation curves of duplex oligonucleotides.

PS55+ and melting point was determined by Electrothermal 1A9000 instrument. Silica gel column chromatography was performed on Merck, Kiesel gel 60 (70–230 mesh).

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-(4-methylphenoxythiocarbonyl)thymidine (2)

A solution of **1** (1.1 g, 1.95 mmol) in dichloromethane (20 mL) was cooled in an ice-water bath and *p*-tolylchlorothionoformate (318 μL, 2.11 mmol) was added. After the reaction mixture was stirred for 5 min, triethylamine (326 μL, 2.34 mmol) and 4-dimethylaminopyridine (286 mg, 2.34 mmol) were added. The resulting reaction mixture was allowed to warm to room temperature and then stirred for 2 h. Distilled water (30 mL) was added and the organic layer was separated off. The aqueous layer was extracted two times with dichloromethane (20 ml). The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography to give **2** as a white solid (1.14 g, 1.64 mmol, 84%): $R_f = 0.85$ (EA:MC = 1:2); IR, ν($CHCl_3$, cm^{-1}) 3182, 3059, 2931, 2836, 1694, 1508, 1252, 1224, 1193; ^1H NMR($CDCl_3$) 10.03(br, 1H, NH), 7.69(d, 1H, J = 0.8 Hz, C6-H), 7.43(d, 2H, J = 7.2 Hz, phH), 7.34–7.18(m, 10H, phH), 6.97(d, 2H, J = 8.5 Hz, phH), 6.84(d, 4H, J = 8.2 Hz, phH), 6.55(m, 1H, H1′), 6.01(m, 1H, H3′), 4.44(s, 1H, H4′), 3.75(s, 6H, OMe), 3.68–3.48(2 m, H5′), 2.35(m, 2H, H2′), 2.34(s, 3H, phMe), 1.49(s, 3H, C5-Me); ^{13}C NMR($CDCl_3$) 195.0, 164.7, 159.2, 159.2, 151.7, 151.2, 144.7, 137.0, 135.7, 135.6, 130.6, 130.5, 128.5, 127.6, 121.8, 113.8, 112.2, 87.8, 85.0, 84.8, 84.3, 78.1, 77.6, 77.3, 55.7, 21.5, 21.4, 14.6, 12.2; MS(FAB^+, m/z) 694.2(M^+); $[\alpha]_D^{20} = -3.43$(c 1.0, CH_2Cl_2); mp 101.5–103.9 °C.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*C*-allyl-3′-deoxy-thymidine (3)

A mixture of **2** (2.21 g, 3.21 mmole), allyltributyltin (5 mL, 16.0 mmole), and AIBN (102 mg, 62 mmole) in toluene (32 mL) was refluxed for 15 h. The reaction mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give **3** as a white solid (1.19 g, 2.09 mmol, 65%): $R_f = 0.80$ (Hex:EA = 1:2); IR, ν($CHCl_3$, cm^{-1}) 3182.5, 3058.7, 2930.6, 2835.9, 1693.8, 1507.9, 1251.6, 1224.0, 1192.6; ^1H NMR($CDCl_3$) 10.19(br, 1H, NH), 7.76(s, 1H, C6-H), 7.47–7.21(m, 9H, phH), 6.84(d, 4H, J = 8.46 Hz, phH), 6.09(t, 1H, J = 3.45 Hz, H1′), 5.73(m, 1H, C<u>H</u> = CH_2), 5.06(dd, 2H, J = 35 Hz, 13.1 Hz, CH = C<u>H</u>$_2$), 3.86(m, 1H, H4′), 3.75(s, 6H, OMe), 3.59(m, 1H, H5′), 3.26(m, 1H, H5′), 2.55–2.47(m, 1H, H2′), 2.33–2.25(m, 1H, H2′), 2.20–2.03(m, 2H, CH_2), 1.51(s, 3H, C5-Me); ^{13}C NMR($CDCl_3$) 1164.74, 158.08, 150.99, 144.85, 136.22, 135.98, 135.67, 130.74, 130.54, 128.94, 128.62, 128.34, 127.45, 117.44, 113.63, 110.76, 86.95, 85.58, 85.50, 55.63, 37.60, 36.32, 21.41, 14.60, 12.45; MS(FAB^+, m/z) 568.43(M^+); $[\alpha]_D^{23} = +8.7$ (c 1.0, CH_2Cl_2); mp 78.4–79.0 °C; Anal. Calcd. for $C_{34}H_{36}O_6N_2$: C, 71.81; H, 6.38; N, 4.93. Found: C, 72.18; H, 6.53; N, 4.69.

TT Dinucleotide 5

To a solution of **3** (370 mg, 0.67 mmol) and **4** (490 mg, 1.33 mmol) in dichloromethane (12 mL) was added NaOCl (1.8 mL, 4% aqueous solution) over a period of 48 hours. Methyl sulfide (200 μL) was added and then the reaction mixture was stirred for 1 hours. Distilled water (30 mL) was added and the organic layer was separated off. The organic residue was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography to give **5a** and **5b** as a white solid (**5a** = 31%; **5b** = 30%); **5a**: $R_f = 0.30$ (EA:Hex = 2:1); IR, ν(neat, cm^{-1}) 3188, 3059, 2953, 2930, 2856, 1691, 1509, 1469, 1253, 835; ^{1}H NMR (300 MHz, Acetone-d_6) δ 10.28–10.24 (2s, 2H, NH), 7.72 (s, 1H, C6-H), 7.59 (s, 1H, C6-H), 7.52–7.23 (m, 9H, phH), 6.89 (d, 4H, J = 7.15 Hz, phH), 6.32 (d, 1H, H1′), 6.11 (d, 1H, H1′), 4.82 (s, 1H, isoxazoline-H), 4.59 (s, 2H, isoxazoline-CH_2), 3.90 (d, 1H, J = 8.0 Hz, H4′), 3.77 (s, 6H, OMe), 3.56 (d, 1H, J = 9.75 Hz, H4′), 3.33 (m, 1H, H3′), 3.21 (m, 1H, H5′), 2.46 (m, 2H, H2′), 2.32 (2 m, 2H, H2′), 1.80 (s, 3H, C5-Me), 1.70 (m, 2H, CH_2), 1.54 (s, 3H, C5-Me), 0.91 (s, 9H, *tert*-Bu), 0.12 (d, 6H, J = 5.55 Hz, 2Me; ^{13}C NMR (75.5 MHz, acetone-d_6) δ 164.3, 164.0, 159.2, 157.7, 150.9, 145.5, 137.2, 136.3, 136.2, 136.1, 130.6, 128.6, 128.3, 127.3, 113.5, 110.7, 110.0, 86.7, 85.3, 82.7, 80.3, 74.1, 63.5, 55.1, 40.6, 39.6, 39.5, 37.7, 36.1, 30.3, 30.0, 29.7, 29.5, 29.2, 29.0, 28.7, 25.7, 18.0, 12.3, 12.1, −5.0; MS (FAB, m/z) 958.29 (M^++Na); $[\alpha]_D^{16} = -3.1$ (c = 0.96, CH_2Cl_2); mp: 123.6–124.2 °C; **5b**: $R_f = 0.26$ (EA:Hex = 2:1); IR, ν(neat, cm^{-1}) 3201, 3061, 2953, 2930, 2857, 1690, 1509, 1469, 1253, 836; ^{1}H NMR (300 MHz, acetone-d_6) δ 10.22, 10.17 (2s, 2H, NH), 7.69 (s, 1H, C6-H), 7.50–7.19 (m, 10H, phH + C6-H), 6.86 (d, 4H, J = 8.5 Hz, phH), 6.29 (m, 1H, H1′), 6.06 (d, 1H, J = 4.6 Hz, H1′), 4.80 (m, 1H, H4′), 4.60 (m, 1H, isoxazoline-H), 4.59 (m, 1H, H4′), 3.74 (m, 1H, H3′), 3.34 (m, 1H, H5′), 3.31 (m, 2H, H5′+H3′), 2.74 (m, 2H, isoxazoline CH_2), 2.43 (m, 2H, H2′), 2.35–2.21 (2 m, 2H, H2′), 1.76 (s, 3H, C5-Me), 1.51 (s, 3H, C5-Me), 0.88 (s, 9H, *tert*-Bu), 0.08 (d, 6H, 2Me); ^{13}C NMR (75.7 MHz, acetone-d_6) δ 164.3, 164.1, 159.2, 159.2, 157.8, 151.9, 145.45, 137.1, 136.2, 136.1, 136.0, 130.5, 128.7, 128.3, 127.2, 126.3, 113.6, 110.8, 110.5, 109.9, 109.1, 86.8, 86.4, 85.4, 85.3, 82.5, 79.9, 78.8, 78.5, 78.1, 63.1, 55.1, 40.2, 39.6, 40.0, 37.7, 35.2, 28.9, 28.7, 22.9, 12.1, 12.1, −5.1; MS (FAB, m/z) 958.20 (M^++Na); $[\alpha]_D^{16} = +52.6$ (c = 1.01, CH_2Cl_2); mp: 114.1–114.6 °C.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-mesyl-3′-deoxy-thymidine (6)

A solution of **1** (3.0 g, 5.31 mmol) in pyridine (70 mL) was cooled in an ice-water bath and methane sulfonylchloride (1.23 mL, 15.9 mmol) was added. After the reaction mixture was stirred for 5 h at 0°, 5% $NaHCO_3$ aqueous solution (100 ml) was added and extracted with dichloromethane. The organic residue was dried over $MgSO_4$ and evaporated *in vacuo*. The

residue was purified by silica gel column chromatography to give **6** as a white solid (3.10 g, 4.99 mmol, 94%): $R_f = 0.55$ (EA:MC = 1:1); IR, ν($CHCl_3$, cm^{-1}) 3186, 3058, 2934, 2837, 1692, 1509, 1252, 1110; ^1H NMR(aceton-d_6) 10.24(br, 1H, NH), 7.60(s, 1H, C6-H), 7.51–7.25(2 m, 9H, phH), 6.90(d, 4H, J = 8.5 Hz, phH), 6.37(m, 1H, H1′), 5.51(s, 1H, H3′), 4.34(s, 1H, H4′), 3.77(s, 6H, OMe), 3.49(m, 2H, H5′), 3.16(s, 3H, SO_2CH_3), 2.69(m, 2H, H2′), 1.49(s, 9H, C5-Me); ^{13}C NMR(Aceton-d_6) 163.92, 159.31, 150.87, 145.20, 135.89, 130.56, 128.36, 127.41, 113.61, 110.10, 87.36, 84.75, 83.99, 81.17, 63.52, 55.13, 38.13, 37.10, 30.26, 30.00, 29.74, 29.49, 29.23, 28.98, 28.72, 11.72; MS(FAB^+, m/z) 622.0(M^+); $[\alpha]_D^{20} = +15.0$ (c 1.0, CH_2Cl_2); mp 81.8–82.2 °C.

1-(2′-Deoxy-5′-*O*-4,4′-dimethoxytrityl-β-*D*-threo-penosyl)thymidine (7)

To a solution of **6** (2.0 g, 3.23 mmol) in ethanol (20 mL) and distilled water (12 mL) was added $LiOH \cdot H_2O$ (406 mg, 9.69 mmole). After the reaction mixture was refluxed for 3 h, the ethanol was evaporated and extracted with dichloromethane. The organic residue was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography to give **7** as a white solid (1.47 g, 2.71 mmol, 84%): $R_f = 0.45$ (EA:MC = 1:1); IR, ν($CHCl_3$, cm^{-1}) 3600–3300, 3165, 3036, 2933, 2836, 1697, 1509, 1251, 1069; ^1H NMR(aceton-d_6) 10.36(br, 1H, NH), 7.75(s, 1H, C6-H), 7.55–7.19(3 m, 9H, phH), 6.86(d, 4H, J = 8.7 Hz, phH), 6.20(d, J = 6.7 Hz, 1H, H1′), 4.47(s, 1H, OH), 4.41(m, 1H, H3′), 4.17(m, 1H, H4′), 3.73(s, 6H, OMe), 3.62(t, 1H, J = 8.7 Hz, H5′), 3.42(m, 1H, H5′), 2.62(m, 1H, H2′), 2.08(m, 1H, H2′), 1.72(s, 3H, C5-Me); ^{13}C NMR(aceton-d_6) 164.7, 160.0, 151.2, 145.8, 137.98, 136.4, 136.4, 130.6, 128.6, 128.2, 121.1, 113.5, 109.4, 86.1, 85.6, 84.3, 70.5, 63.3, 55.2, 41.6, 12.5; MS(FAB^+, m/z) 544.1(M^+); $[\alpha]_D^{20} = -22.6$ (c 1.0, CH_2Cl_2); mp 82.2–82.6 °C.

1-(2′-Deoxy-3′-*O*-mesyl-5′-*O*-4,4′-dimethoxytrityl-β-*D*-lyxosyl)thymidine (8)

A solution of **7** (9.0 g, 15.9 mmol) in pyridine (160 mL) was cooled in an ice-water bath and methane sulfonylchloride (3.7 mL, 47.9 mmol) was added. After the reaction mixture was stirred for 8 h at 0°, 5% $NaHCO_3$ aqueous solution was added and extracted with dichloromethane. The organic residue was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography to give **6** as a white solid (9.10 g, 14.6 mmol, 92%): $R_f = 0.55$ (EA:MC = 1:1); IR, ν($CHCl_3$, cm^{-1}) 3181, 3054, 3034, 2934, 2837, 2551, 1698, 1508, 1250, 1142; ^1H NMR(aceton-d_6) 10.07(br, 1H, NH), 8.54(t, 1H, J = 2.7 Hz, C6-H), 7.39–7.22(m, 9H, phH), 6.87(d, 4H, J = 8.9 Hz, phH), 6.27(t, 1H, J = 4.0 Hz, H1′), 5.40(d, 1H, J = 3.8 Hz, H3′), 4.42(t, 1H, J = 1.7 Hz, H4′), 3.76(s, 6H, OMe), 3.58(m, 1H,

H5′), 3.37(m, 1H, H5′), 3.00(s, 3H, SO_2Me), 2.99(m, 1H, H2′), 2.45(m, 1H, H2′), 1.70(s, 3H, C5-Me); ^{13}C NMR(Aceton-d_6) 163.4, 163.4, 158.9, 150.6, 150.5, 149.8, 135.8, 135.7, 135.4, 130.2, 128.2, 127.9, 113.2, 113.2, 110.0, 86.7, 83.8, 81.3, 79.6, 61.7, 54.7, 39.2, 11.9; MS(FAB^+, m/z) 622.0(M+); $[\alpha]_D^{23} = -31.5$ (c 1.0, CH_2Cl_2); mp 85.4–86.7 °C.

3′-Deoxy-3′-*S*-(2-hydroxyethylthio)-5′-*O*-(4,4′-dimethoxytrityl)thymidine (9)

A solution of **8** (9.08 g, 14.6 mmole) in DMF (40 mL) was heated at 700 °C and a solution of DBU (3.5 mL, 25.4 mmole) and mercaptoethanol (7.2 mL, 101.8 mmole) in DMF (100 mL) was added. After the reaction mixture was stirred for 3 h at 70 °C, distilled water (100 mL) was added and the reaction mixture was extracted with dichloromethane. The organic residue was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography to give **10** as a white solid (6.53 g, 10.8 mmol, 74%): $R_f = 0.30$ (EA:MC = 1:1); IR, ν($CHCl_3$, cm^{-1}) 3750–3300, 3182, 3056, 2931, 1688, 1252, 1035; 1H NMR(aceton-d_6) 10.31(br, 1H, NH), 7.78(s, 1H, C6-H), 7.56–7.22(2 m, 9H, phH), 6.91(d, 4H, J = 8.7 Hz, phH), 6.20(dd, 1H, J = 6.9 Hz, 6.9 Hz, H1′), 3.99(m, 2H, H3′+H4′), 3.70(s, 6H, OMe), 3.58(m, 2H, CH_2), 3.56(m, 1H, H5′), 3.41(m, 1H, H5′), 2.70(m, 2H+1H, CH_2+H2′), 2.44(m, 1H, H2′), 1.29(s, 3H, C6-Me); ^{13}C NMR(aceton-d_6) 164.2, 159.3, 150.9, 145.4, 136.3, 136.1, 130.6, 128.6, 128.3, 127.3, 113.6, 110.2, 86.8, 85.5, 84.9, 63.0, 62.2, 55.1, 41.8, 40.6, 34.3, 31.8, 12.0; MS(FAB^+, m/z) 604.09(M^+); $[\alpha]_D^{20} = +22.7$ (c 1.0, CH_2Cl_2); mp 77.4–78.1 °C; Anal. Calcd. for $C_{33}H_{36}O_7N_2S$: C, 65.55; H, 6.00; N, 4.63. Found: C, 65.50; H, 6.12; N, 4.52.

2′-Deoxy-3′-*S*-(1-vinylsulfinyl)-5′-*O*-(4,4′-dimethoxytrityl)thymidine (10)

A solution of **9** (840 mg, 1.38 mmol) in pyridine (26 mL) was cooled in an ice-water bath and methane sulfonylchloride (322 μL, 4.16 mmol) was added. After the reaction mixture was stirred for 8 h at 0 °C, 5% $NaHCO_3$ aqueous solution was added and extracted with dichloromethane. The organic residue was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was dissolved in dry THF (26 mL) and cooled at 0 °C. ^{t}BuOK (467 mg, 4.16 mmol) was added and stirred for 10 min at 0 °C. Distilled water (30 mL) was added and extracted with dichloromethane. The organic residue was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography to give **10** as a white solid (679 mg, 1.16 mmol, 84%): $R_f = 0.70$ (EA:MC = 1:1); IR, ν($CHCl_3$, cm^{-1}) 3182, 3056, 2930, 2885, 1689, 1509, 1001; 1H NMR(aceton-d_6) 10.17(br, 1H, NH), 7.77(s, 1H, C6-H), 7.77–7.24(2 m, 9H, phH), 6.89(d, 4H, J = 9.0 Hz, phH), 6.39(m, 1H, $\underline{CH}$=CH_2), 6.22(m, 1H, H1′), 5.18(m, 2H, CH=$\underline{CH_2}$), 4.00(m, 2H, H3′+H4′),

3.78(s, 6H, OMe), 3.56(m, 1H, H5′), 3.36(d, 1H, J = 10.8 Hz, H5′), 2.70–2.44(2 m, 2H, H2′), 1.54(s, 3H, C5-Me); ^{13}C NMR(aceton-d_6) 174.8, 159.2, 151.0, 144.6, 135.9, 135.7, 135.7, 130.2, 128.6, 128.4, 127.6, 115.6, 113.7, 113.7, 113.4, 87.2, 86.0, 85.1, 78.0, 77.8, 77.6, 77.2, 62.5, 55.7, 41.5, 40.0, 14.6, 12.4; MS(FAB$^+$, m/z) 586.06(M^+); $[\alpha]_D^{23} = +8.7$ (c 0.1, CH_2Cl_2); mp 73.2–73.7 °C; Anal. Calcd. for $C_{33}H_{34}O_6N_2S$: C, 67.56; H, 5.84; N, 4.77. Found: C, 67.25; H, 6.04; N, 4.61.

TT Dinucleotide 11

To a solution of **10** (1.00 g, 11.7 mmol) and 4 (945 mg, 2.56 mmol) in dichloromethane (34 mL) was added NaOCl (4.7 mL, 4% aqueous solution) over a period of 48 h. Methyl sulfide (200 μL) was added and then the reaction mixture was stirred for 1 h. Distilled water (50 mL) was added and the organic layer was separated off. The organic residue was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography to give **11a** and **11b** as a white solid (**11a** = 59%; **11b** = 20%); **11a** $R_f = 0.25$ (EA:Hex = 2:1); IR, ν(neat, cm^{-1}) 3186, 3062, 2930, 2856, 1695, 1509, 1470, 1253, 834; ^{1}H NMR (300 MHz, acetone-d_6) δ 10.30 (s, 2H, NH), 7.76 (s, 1H, C6-), 7.52-.76-.22 (2 m, 10H, C60-+phH), 6.88 (m, 4H, phH), 6.36 (m, 1H, H1′), 6.19 (d, 1H, J = 3.57 Hz, isoxazoline-), 5.86 (d, 1H, J = 5.6 Hz, H1′), 4.88 (m, 1H, H3′), 4.63 (d, 1H, J = 3.4 Hz, H4′), 4.04-3.98 (m, 2H, H4′+H3′), 3.75 (s, 6H, OMe), 3.60 (m,1H, H5′), 3.41 (m, 2H, isoxazoline-CH_2), 2.95 (m, 1H, H5′), 2.65–2.30 (3 m, 4H, H2′), 1.49 (s, 3H, C5-e), 1.09 (s, 3H, C5-Me), 0.89 (s, 9H, *tert*-Bu), 0.12 (d, 6H, 2Me); ^{13}C NMR (75.5 MHz, acetone-d_6) δ 164.2, 164.1, 159.3, 159.0, 150.9, 145.2, 136.8, 136.2, 135.9, 135.8, 130.6, 128.6, 128.3, 127.4, 113.6, 111.0, 110.3, 86.9, 86.4, 85.5, 84.7, 84.6, 82.1, 74.2, 62.5, 55.1, 41.9, 41.2, 40.3, 39.6, 18.1, 12.3, 12.0, −5.0, −5.0; MS (FAB, m/z) 976.27 (M^++Na) ; $[\alpha]_D^{23} = -133$ (c = 1.0, CH_2Cl_2); mp: 123.6–124.2 °C. **11b**: $R_f = 0.23$ (EA:Hex = 2:1); IR, ν (neat, cm^{-1}) 3194, 3062, 2953, 2930, 2856, 1693, 1447, 1363, 1252, 779; ^{1}H NMR (300 MHz, acetone-d_6) δ 10.40, 10.38 (2 s, 2H, NH), 7.74 (s, 1H, C6-H), 7.74–7.24 (2 m, 10H, phH+ C6-H), 6.90 (d, 4H, J = 8.40 Hz, phH), 6.31 (t, 1H, J = 6.6 Hz, H1′), 6.22 (d, 1H, J = 6.3 Hz, H1′), 6.14 (dd, 1H, J = 9.6 Hz, 9.9 Hz, isoxazoline-H), 4.87 (dd, 1H, J = 3.9 Hz, J = 2.4 Hz, H3′), 4.70 (d, 1H, J = 3.9 Hz, H4′), 4.07 (d, 2H, H3′+H4′), 3.78–3.71 (s+m, 7H, OMe+H5′), 3.51 (s, 3H, isoxazoline-CH_2), 3.03 (m, 1H, H5′), 2.85–2.41 (3 m, 4H, H2′), 1.78 (s, 3H, C6-Me), 1.52 (s, 3H, C6-Me), 0.92 (s, 9H, *tert*-Bu), 0.14 (d, 6H, 2Me); ^{13}C NMR(75.5 MHz, acetone-d_6) δ 164.2, 164.1, 159.2, 159.2, 159.1, 151.0, 145.5, 137.4, 136.3, 136.1, 136.1, 130.6, 128.6, 128.3, 127.2, 126.3, 113.6, 110.8, 110.4, 87.0, 85.1, 84.6, 84.4, 82.1, 74.2, 62.9, 55.1, 42.1, 41.1, 40.6, 39.5, 25.7, 18.1, 12.1, 11.9, −5.0; MS (FAB, m/z) 976.23 (M^++Na); $[\alpha]_D^{16} = +174$ (c = 0.96, CH_2Cl_2); mp: 113.5–114.6 °C.

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