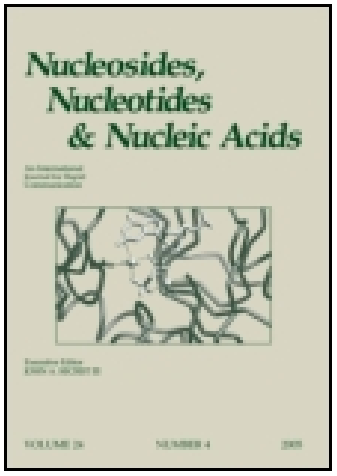

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Design and Synthesis of Dephosphono DNA Analogues Containing 1,2,3-Triazole Linker and Their UV-Melting Studies with DNA/RNA

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DESIGN AND SYNTHESIS OF DEPHOSPHONO DNA ANALOGUES CONTAINING 1,2,3-TRIAZOLE LINKER AND THEIR UV-MELTING STUDIES WITH DNA/RNA

Vangala Madhuri and Vaijayanti A. Kumar
Division of Organic Chemistry, National Chemical Laboratory, Pune, Maharashtra, India

□ *This article describes the synthesis of 3′/5′ linked 1,2,3-triazolyl dithymidine derivatives, their incorporation into oligonucleotides, and evaluation of their thermal stabilities toward complementary DNA/RNA.*

Keywords Oligonucleotide analogues; isostere; triazole; UV melting

INTRODUCTION

Chemical modification of oligonucleotides came to prominence in the early 1990s with the advent of the antisense approach to control gene expression.[1] Among several nucleic acid analogues that have been studied over the last two decades, only a very few are backbones with linkers that are either shorter[2] or longer[3] than the four-atom linker O3′-P-O5′-C5′ connecting sugar ring moieties in DNA and RNA. The four-atom amide substitution for four-atom phosphodiester linkage led to chimeric oligonucleotides with amide-phosphodiester groups. Such chimeric oligonucleotides formed complexes with complementary nucleic acids with a moderate RNA-binding selectivity and with moderately diminished DNA affinity.[4] Amide linker is known to be shorter than the phosphate linker, a five-atom amide linker, provided the requisite flexibility in the linker and thus stabilized the duplexes with RNA.[5] The amide linkers in 2′-*O*-methyl substituted nucleosides also led to the stabilization of RNA duplexes. The replacement of 3′-O with a methylene group and the addition of a methylene group in either *R* or *S* configuration also led to the stabilization of duplexes[6] (Figure 1). Some amide-linked oligonucleotides were also reported by our group where a

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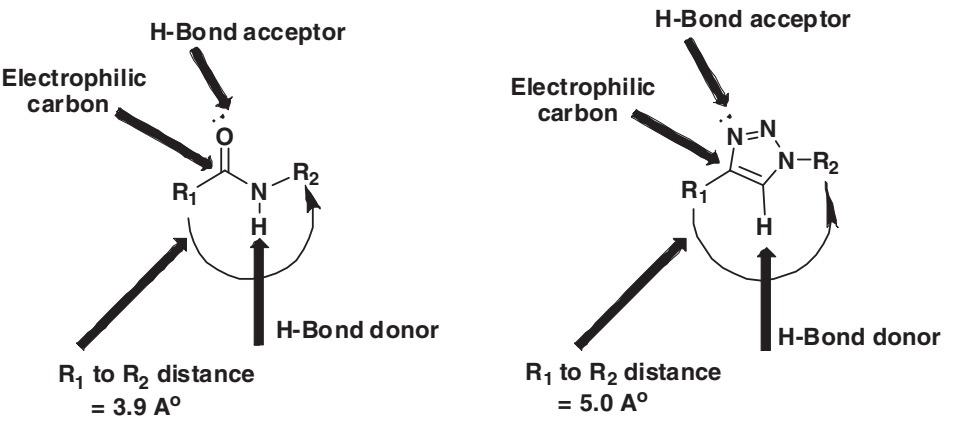


FIGURE 1 Topological and electronic similarities of amides and 1,2,3-triazoles.

novel class of thioacetamido backbone[7] and an amino acid backbone extended DNA modification[8] were found to improve the binding affinity and selectivity toward DNA/RNA recognition.

As triazole is known to be the nonclassical isostere of amide (Figure 1), we envisaged the replacement of the amide linkage in the DNA dimer block with a six-atom 1,2,3-triazole linkage (Figure 2). The planar aromatic cyclic structure of triazole in the linker might require extended backbone flexibility

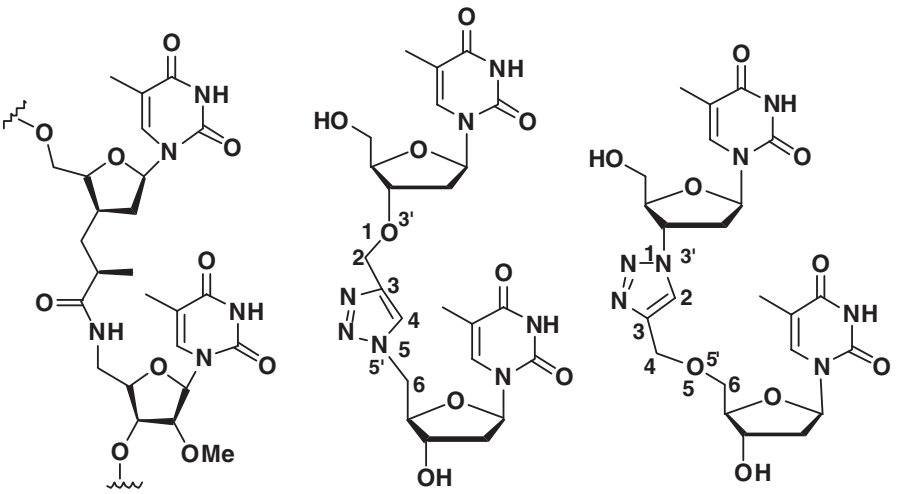


FIGURE 2 Chemical and structural representation of (A) amide-linked dimer, (B) 3′-O-alkynyl-5′-azide triazolyl **tt** dimer, and (C) 5′-O-alkynyl-3′-azide triazolyl **tt** dimer.

as compared with the amide linker. A recent article appeared where one-atom extended 1,2,3-triazole linker was found to be suitable in acyclic amide-linked PNA backbone.[9]

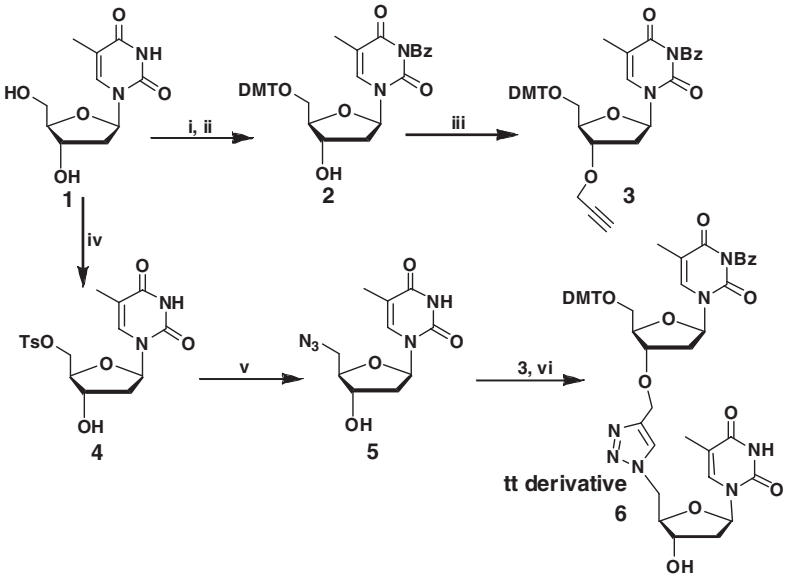
The Huisgen 1,3-dipolar cycloaddition between azides and alkynes, performed by the recently discovered copper(I) catalysis[10], appears to be a promising way to generate oligomers and particularly nonnatural triazole-linked oligodeoxynucleotides. Previous studies, introducing a four-atom triazole linkage in oligonucleosides backbone, have been realized using thymidine as nucleoside (homothymidines) in both solution-phase and solid-phase synthesis.[11] We propose an extended six-atom, 1,4-disubstituted-1,2,3-triazole as the linker that would conserve the directional character of DNA strands. While this work was under revision, a very interesting application of the same six-atom triazolide linker group has appeared in the literature.[12] Owing to the extended six-atom backbone, there is a possibility of cross pairing with both DNA and RNA. This modification may also impart stability to the oligomers due to its neutral nature that will not have any repulsive interactions with the anionic phosphate backbone of natural DNA/RNA. The rigid planar, π-rich backbone and possibility of metal coordination of the triazole rings may prove to be important in the structural chemistry and biology of oligonucleotides. We report in this article, the two triazole-linked dimers (Figure 2), when the triazole ring is formed at the chiral C-3′ carbon center and also when it is present at the achiral C-5′ carbon of the sugar by synthesizing two different dimer units. Synthesis of the two dimer blocks, triazolyl **tt** dimer from 3′-*O*-alkynyl and 5′-azido monomers (Figure 2B) and triazolyl **tt** dimer from 5′-*O*-alkynyl and 3′-azido monomers (Figure 2C), their incorporation into oligonucleotides, and UV-T_m studies with complementary DNA and RNA are presented.

RESULTS AND DISCUSSIONS

Synthesis of Triazolyl Dithymidine tt Dimer Block (6) from 3′-*O*-Alkynyl Thymidine and 5′-Azido Thymidine

The free OH groups of thymidine were protected as trimethylsilyl (TMS) ethers using TMS-Cl in pyridine followed by the addition of benzoyl chloride to get the *N*3-benzoylated compound. The protection of the N3 atom of thymine was necessary because if left unprotected, the latter alkylation reaction with propargyl bromide gave exclusively *N*-alkylated product rather than the required *O*-alkylated compound. The **5**′-hydroxyl group of *N*3-benzoyl-thymidine was converted to its dimethoxttrityl (DMT) derivative by treatment with DMTr-Cl in pyridine to get **2**, which was alkylated using propargyl bromide and NaH in dimethylformamide (DMF) to get the 3′-*O*-alkyne derivative **3** in 55% yield (Scheme 1). Some amount of debenzoylation

was observed under these reaction conditions as the thin layer chromatography (TLC) of the reaction mixture showed some lower moving impurities which could be a mixture of *N*- or *O*-alkylated products arising from the debenzoylated compound. The yield of N-3 Bz-3′-*O*-alkylated product was therefore only 55%.



Reagents: i. (a) TMS-Cl/pyridine **(b)** Bz-Cl **(c)** aq NH_3 sol **ii.** DMTr-Cl/pyridine 85% **iii.** propargyl bromide/NaH, DMF 55% **iv.** Ts-Cl/pyridine 85% **v.** NaN_3/DMF 87% **vi.** 0.2% $CuSO_4$, 0.5% Na-ascorbate, t-BuOH:H_2O 80%

SCHEME 1 Synthesis of 3′-*O*-alkynyl-5′-azido triazolyl **tt** dimer unit (**6**).

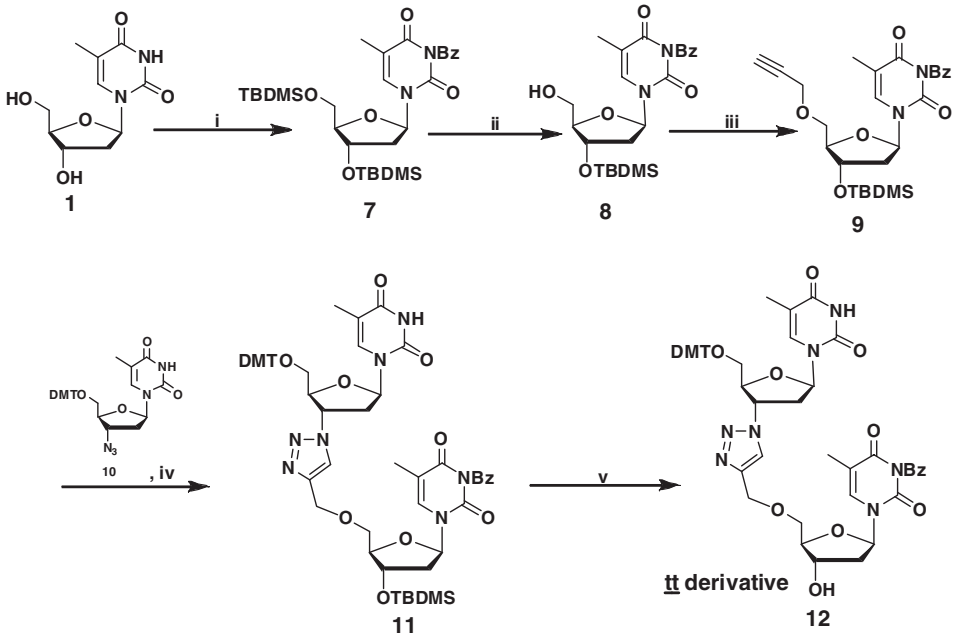
Monomer **5** was synthesized from thymidine through monotosylation at the 5′ position using tosyl chloride in pyridine under dilute conditions to get compound **4**. Compound **4** was then treated with excess of sodium azide in dry DMF to get compound **5**, which was confirmed from the IR spectrum showing characteristic absorption for azide group at 2100 cm^{-1}. Derivatives **3** and **5** were then coupled using copper (I)-catalyzed cycloaddition in order to obtain 1,4-dithymidine-substituted-1,2,3-triazole **6**. This click reaction was performed using $CuSO_4$ and sodium ascorbate.[13]

Synthesis of Triazolyl Dithymidine tt Dimer Unit (12) from 5′-*O*-Alkynyl Thymidine and 3′-Azido Thymidine

The two free hydroxyl groups of thymidine were protected as *t*-butyldimethylsilyl (TBDMS) ethers using TBDMS-Cl in DMF, and the N3 position of thymine was protected as its benzoyl derivative using benzoyl chloride in pyridine to get compound **7**. In the next step, the primary 5′ hydroxyl group was deprotected selectively, keeping intact the secondary

3′ TBDMS ether using I_2/MeOH[14] to get compound **8**. This reaction was carried out at room temperature for a period of 3–4 hours and was monitored by TLC. It was observed that when the reaction was left for a longer time, both primary and secondary TBDMS ethers were deprotected and N3-benzoyl thymidine was obtained. The free 5′ hydroxyl group in **8** was then alkylated using propargyl bromide and NaH in DMF to get the alkylated compound **9** in good yield.

The 3′-azido thymidine derivative was obtained from a known procedure.[15] The alkyne monomer unit **9** and azide **10** were subjected to click reaction using $CuSO_4$ and sodium ascorbate. The reaction was carried out in 1:1 ratio of *t*-butanol and H_2O to get the 1,4-dithymidine substituted 1,2,3-triazole dimer **11** regioselectively in good yields. The TBDMS group was deprotected using 1 M tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) to get the desilylated product **12** (Scheme 2).

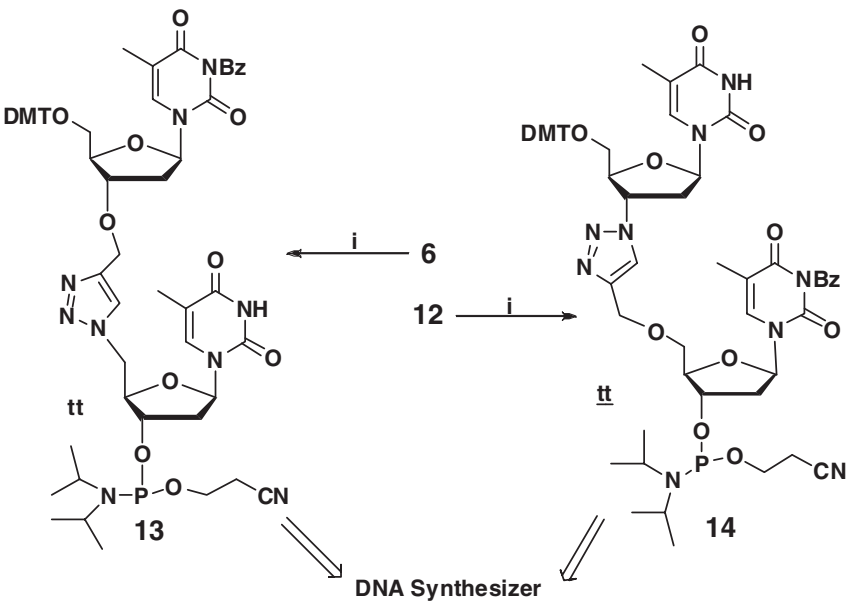


Reagents: i. (a) TBDMS-Cl/DMF 95% **(b)** Bz-Cl/pyridine 90% **ii.** I2/MeOH 70% **iii.** propargyl bromide, NaH/DMF 80%, **iv. 10**, 0.2eq $CuSO_4$, 0.5eq Na-ascorbate *t*-BuOH:H_2O 80% **v.** 1M TBAF/THF, 87%

SCHEME 2 Synthesis of 5′-*O*-alkynyl 3′-azide triazolyl dithymidine dimer unit (**12**).

Synthesis of tt and tt Phosphoramidite Building Blocks

The 3′ hydroxyl group in the dimers **6** and **12** was phosphitylated by treating with chloro(cyanoethoxy-*N*,*N*′-diisopropylamino)phosphine to give



Reagents: i. Chloro (cyanoethoxy-N, N'-diisopropylamino)phosphine, DIEA, DCM 87%

SCHEME 3 Synthesis of phosphoroamidites.

the phosphoramidites **13** and **14**, ready for the use in an automated DNA synthesizer for their incorporation into oligomers (Scheme 3).

Oligomers Synthesized by Incorporation of tt/tt Dimers, Purification, and MALDI-TOF Analysis

A series of chimeric oligonucleotides (Table 1) containing one or two dimer blocks incorporated at the predetermined positions were synthesized by automated solid-phase synthesis using the phosphoramidite approach. Control 18mer oligonucleotide (DNA-a) chosen was specific for the splice correction of an aberrant β-globin intron (705 site).[16] The control 19mer oligonucleotide (DNA-b) is the antisense sequence for bcr/abl b2a2 gene, which is responsible for chronic myeloid leukemia.[17] The 10mer sequence (DNA-c) is again the antisense sequence for bcr/abl b3a2 gene, which is responsible for chronic myeloid leukemia.[17] The modified sequences were synthesized by the incorporation of a single **tt** or **tt** blocks, or two modified **tt** or **tt** blocks were incorporated in the above-mentioned unmodified DNA sequences. Oligonucleotides were synthesized on a 40 nmol scale in an ABI 3900 High Throughput DNA synthesizer using standard β-cyanoethyl phophoramidate chemistry on a controlled pore glass (CPG) resin. Final deprotection was done by giving ammonia treatment at 55°C for 5 hours. The crude yield of the oligomers based on the scale of synthesis was found

TABLE 1 Oligomers synthesized, their HPLC retention times, and MALDI-TOF mass

Code	Sequences	HPLC (rt) (minutes)	Experimental mass	Observed mass
DNA-a	5′ CCT CTT ACC TCA GTT ACA 3′		5369.6	
DNA-a-**tt-1**	5′ CCT CTT ACC TCA G**tt** ACA 3′	7.9	5371.35	5370.76
DNA-a-**tt-2**	5′ CCT C**tt** ACC TCA G**tt** ACA 3′	8.3	5373.10	5370.91
DNA-a-**tt-3**	5′ CCT C**tt** ACC TCA GTT ACA 3’	8.1	5371.35	5369.95
DNA-a-**tt-1**	5′ CCT CTT ACC TCA G**tt** ACA 3′	7.8	5371.35	5375.66
DNA-a-**tt-2**	5′ CCT C**tt** ACC TCA G**tt** ACA 3′	8.4	5373.10	5381.60
DNA-a-**tt-3**	5′ CCT C**tt** ACC TCA GTT ACA 3′	8.2	5371.35	5381.30
DNA-b	5′ GAA GGG CTT TTG AAC TCT T 3′		5833.90	
DNA-b-**tt-1**	5′ GAA GGG C**tt** TTG AAC TCT T 3′	7.3	5835.65	5838.67
DNA-b-**tt-2**	5′ GAA GGG C**tt tt**G AAC TCT T 3′	8.4	5837.40	5847.82
DNA-b-**tt-3**	5′ GAA GGG CTT **tt**G AAC TCT T 3′	7.4	5835.65	5832.62
DNA-b-**tt-1**	5′ GAA GGG C**tt** TTG AAC TCT T 3′	7.9	5835.65	5837.73
DNA-b-**tt-2**	5′ GAA GGG C**tt tt**G AAC TCT T 3′	8.1	5837.40	5845.82
DNA-b-**tt-3**	5′ GAA GGG CTT **tt**G AAC TCT T 3′	7.8	5835.65	5835.69

to be 90% and the yield of the high-performance liquid chromatography (HPLC) purified oligomers was found to be in the range of 60%–70%. The sequences synthesized were deprotected and cleaved from the solid support. The purification was carried out by reversed-phase HPLC (RP-HPLC) and the purified oligomers were characterized by MALDI-TOF (matrix-assisted laser desorption/ionization [MALDI] time-of-flight [TOF]) mass spectrometry.

UV-T_m of tt/tt Incorporated Oligomers Incorporated into 18mer (DNA4)

The UV-T_m experiments of duplexes (1 μM) of each strand were prepared in 10 mM sodium phosphate buffer, pH 7.2, containing NaCl (100 mM) and ethylenediaminetetraacetic acid (EDTA; 0.1 mM) and were annealed by keeping the samples at 90°C for 5 minutes followed by slow cooling to room temperature. Absorbance versus temperature profiles were obtained by monitoring at 260 nm with CARY 300 BIO UV-Visible spectrophotometer scanning from 5°C to 85°C at a ramp rate of 0.2°C per minute. The data were processed using Microcal Origin 5.0 software and T_m values derived from the derivative curves. Introduction of a single **tt** dimer unit toward the 3′ end in 18mer oligomer DNA-a-**tt**-1 showed the destabilization of 13°C and 12.3°C with complementary DNA and RNA. The amount of destabilization was same when the **tt** dimer was incorporated toward the 5′ end as in DNA-a-**tt**-3. A further destabilization was observed for the complex DNA-a-**tt**-2:DNA1/RNA1 when two units of modified **tt** dimer were incorporated. The binding of triazolyl-substituted oligonucleotides containing single

TABLE 2 UV-T_m (°C) of DNA-a-**tt** /DNA-a-**tt** oligomers with complementary DNA/RNA

	UV-T_m (°C)	
Code	DNA1	RNA1
DNA-a	53.8	58.1
DNA-a-**tt-1**/**DNA**-a-**tt-1**	40.8/41.8	45.8/36.0
DNA-a-**tt-2**/**DNA**-a-**tt-2**	36.3/30.8	35.4/32.8
DNA-a-**tt-3**/**DNA**-a-**tt-3**	43.0/37.5	44.6/39.3

mismatch at the middle position of the sequence was studied with complementary RNA1. For oligonucleotides DNA-a-**tt**-1, DNA-a-**tt**-3, and DNA-a-**tt**-2, the decrease of binding affinity was 12.3°C, 10.7°C, and 9.2°C, respectively, in comparison with RNA1 when they were hybridized with one single-base mismatch RNA4. The results are tabulated in Table 2. The destabilization of **tt** dimer incorporated triazolyl oligomers DNA-a-**tt**-1, DNA-a-**tt**-2, and DNA-a-**tt**-3 was found to be more when compared with **tt** triazolyl oligomers. DNA-a-**tt**-1 and DNA-a-**tt**-3 triazolyl oligomers with a single **tt** dimer unit toward the 3′ and 5′ end showed a binding affinity of 41.8°C and 36.0°C, respectively. The ΔT_m between DNA-a-**tt**-1:RNA1 and DNA-a-**tt**-1:RNA1 and that of DNA-a-**tt**-3:RNA1 and DNA-a-**tt**-3:RNA1 was found to be −9.8°C and −5.3°C, respectively. The cumulative destabilization effect was observed when two **tt** dimer units were incorporated. The UV-T_m derivative curves and their results are shown in Figure 3.

UV-T_m of tt/tt Oligomers Incorporated into 19mer (DNA5)

The **tt** dimer building block when incorporated into the 19mer (DNA5) in the middle of the sequence DNA-b-**tt**-1 decreased the T_m by 15.5°C when compared with the unmodified sequence. The binding of DNA-b-**tt**-1 with RNA2 showed a decrease in T_m of 7.4°C. The DNA-b-**tt**-2 triazolyl oligomer with two **tt** dimer units incorporated together showed a T_m of 40.8°C and 42.5°C, respectively, with complementary DNA and RNA (Table 3). The thermal stability of DNA-b-**tt**-3 sequence with DNA2/RNA2 was found to be 46.5°C and 48.2°C, respectively. The difference in T_m with single and double

TABLE 3 UV-T_m (°C) of DNA-b-**tt**/DNA-b-**tt** oligomers with complementary DNA/RNA

	UV-T_m (°C)	
Code	DNA2	RNA2
DNA-b	56.8	54.3
DNA-b-**tt-1**/**DNA**-b-**tt-1**	41.3/38.2	46.9/39.2
DNA-b-**tt-2**/**DNA**-b-**tt-2**	40.8/35.3	42.5/35.0
DNA-b-**tt-3**/**DNA**-b-**tt-3**	46.5/39.2	48.2/38.1

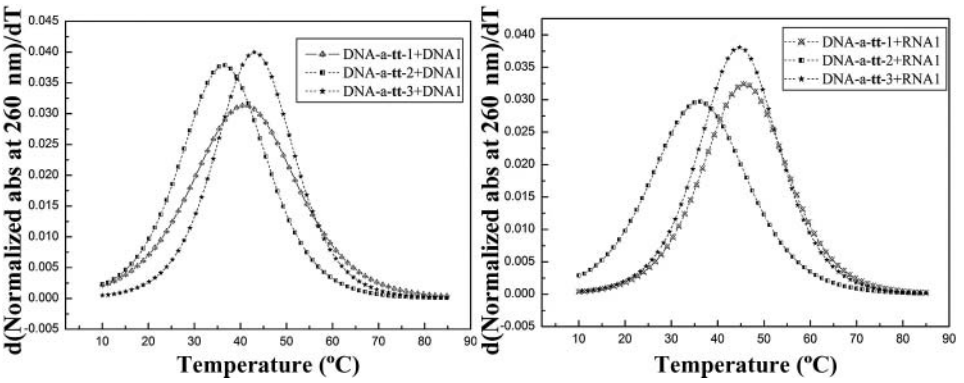


FIGURE 3 UV derivative curves for DNA-a-**tt** oligomers with complementary DNA/RNA (10 mM sodium phosphate buffer, pH 7.2, 100 mM NaCl, 0.1 mM EDTA). The values reported here are an average of three independent experiments and are accurate to ±0.5°C.

modification is not very large, which means when the modified units are together, the degree of destabilization is not high as compared with oligomers when the modified units are apart. The stabilization of **tt** dimer incorporated DNA-b-**tt**-1 and DNA-b-**tt**-3 oligomers showed a T_m of 38.2°C and 39.2°C with complementary DNA2 and 39.2°C and 38.1°C with complementary RNA2. In comparison with DNA-b-**tt**-1 and DNA-b-**tt**-3 **tt** based triazolyl oligomers, the decrease in T_m observed for DNA-b-**tt**-1, DNA-b-**tt**-3 with DNA2/RNA2 was 3.1°C, 7.3°C and 7.7°C, 10.1°C, respectively. The thermal stability of DNA-b-**tt**-2 with two modifications showed a T_m of 35.3°C and 35.0°C with DNA2 and RNA2, respectively. The UV-T_m derivative curves and their results are shown in Figure 4.

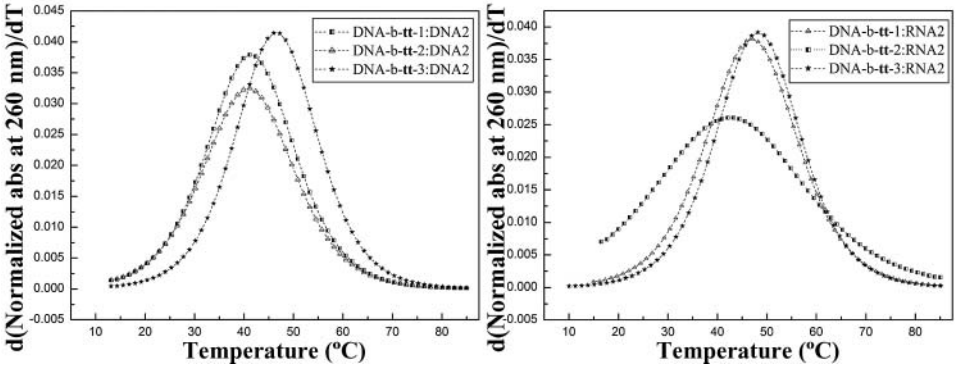


FIGURE 4 UV derivative curves for DNA-b-**tt** oligomers with complementary DNA/RNA (10 mM sodium phosphate buffer, pH 7.2, 100 mM NaCl, 0.1 mM EDTA). The values reported here are an average of three independent experiments and are accurate to ±0.5°C.

CONCLUSION

The 3′-*O*-alkynyl-5′-azido triazolyl dithymidine dimer unit (**tt**) and 5′-*O*-alkynyl 3′-azide triazolyl dithymidine dimer unit (**tt**) have been synthesized and incorporated into the oligomers by phosphoramidite method using an automated DNA synthesizer. The **tt** oligomers showed 10°C–13°C of destabilization when a single dimer unit was incorporated and destabilization increased further when two dimer units are incorporated. The **tt** oligomers also showed 10°C–15°C of destabilization when a single dimer unit was incorporated and destabilization increased further when two dimer units were incorporated.

EXPERIMENTAL

General

All the reagents were purchased from Sigma-Aldrich and used without purification. DMF and pyridine were dried over KOH and 4 Å molecular sieves. THF was passed over basic alumina and dried by distillation over sodium. TLCs were run on Merck 5554 silica 60 aluminum sheets. Column chromatography was performed for purification of compounds on silica gel (100–200 mesh, LOBA Chemie). TLCs were performed using dichloromethane–methanol or petroleum ether–ethyl acetate solvent systems for most compounds. Compounds were visualized with UV light and/or by spraying with perchloric acid solution and heating. ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR spectra were recorded on a Bruker ACF 200 spectrometer fitted with an Aspect 3000 computer, and all the chemical shifts (δ/ppm) are referred to internal TMS for ^{1}H NMR and chloroform-*d* and DMSO-d_6 for ^{13}C NMR. Mass spectra were recorded on a Finnigan-Matt mass spectrometer, while MALDI-TOF spectra were obtained from a KRATOS PCKompact instrument. UV experiments were performed on a Perkin Elmer λ35 UV-VIS spectrophotometer fitted with a Peltier temperature programmer and a Julabo water circulator. The DNA oligomers were synthesized on CPG solid support using an ABI 3900 High Throughput DNA synthesizer by β-cyanoethyl phosphoramidite chemistry followed by ammonia treatment and their purities checked by HPLC prior to use. RNA was purchased from Sigma-Aldrich.

CHARACTERIZATIONS: SPECTROSCOPIC DATA OF SELECTED NEW COMPOUNDS

5′-*O*-Dimethoxytrityl 3′-Propynyl N^3-Benzoyl Thymidine (3)

Compound **2** (1 g, 1.54 mmol) was taken in 10:1 dry DMF and acetonitrile and cooled to 0°C. NaH 60% in hexane (92 mg, 2.31 mmol) was

added in portions followed by the addition of propargyl bromide (0.48 mL, 3.08 mmol) and the reaction was stirred for 2–3 hours with continuous monitoring by TLC. Leaving the reaction for a long time led to the deprotection of benzoyl group and the yield of the reaction also decreased. The reaction mixture was quenched by adding few milliliters of MeOH to it. DMF was removed under vacuum and the mixture was extracted into EtOAc (50 mL × 2), washed with water, brine, and dried over Na_2SO_4. The crude mixture was purified by column chromatography using 25% EtOAc in petroleum ether and concentrated under vacuum to get compound **3** as a colorless foam. Yield: 0.57 g (55%); IR ($CHCl_3$) ν/cm^{-1} 3307, 3020, 1652, 1215; ^1H NMR (200 MHz, $CDCl_3$) δ_H 1.46 (s, 3H), 2.29–2.57 (m, 2H), 2.40 (t, 1H), 3.35–3.58 (m, 2H), 3.79 (s, 6H), 4.16 (m, 2H), 4.55 (m, 1H), 4.72 (m, 1H), 6.39 (dd, 1H, $\Delta J_1 = 6.0$ Hz, $\Delta J_2 = 7.88$ Hz), 6.81–6.87 (d, 4H, $\Delta J = 8.17$ Hz), 7.28–7.75 (m, Ar), 7.91–7.95 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C 12.3, 36.5, 55.2, 60.4, 63.5, 70.6, 72.0, 76.5, 85.5, 86.3, 86.8, 110.3, 113.0, 127.1, 127.7, 127.9, 128.1, 129.1, 130.0, 134.3, 135.4, 139.5, 144.3, 150.2, 158.4, 158.6, 162.4; MS (EI) *m/z* 686.75 (calc.), found 709.73 $(M+Na)^+$.

3′-*O*-Alkynyl 5′-Azide Dithymidine tt Dimer (6)

Individual monomer units **3** (1 g, 1.45 mmol) and **5** (0.31 g, 1.16 mmol) were taken in 10 mL of 1:1 *t*-BuOH and water. $CuSO_4$ (72.6 mg, 0.29 mmol) and sodium ascorbate (144 mg, 0.72 mmol) were added to the reaction. The reaction mixture was left overnight. *t*-BuOH was removed under reduced pressure and the mixture was extracted into EtOAc (100 mL × 2), washed with water, brine, and dried over Na_2SO_4. The crude mixture was purified by column chromatography using 90% EtOAc/petroleum ether to 3% MeOH in EtOAc and concentrated under vacuum to get compound **6** as a colorless foam. Yield: 80%; IR ($CHCl_3$) ν/cm^{-1} 3017, 2929, 1702, 1508; ^1H NMR (200 MHz, $CDCl_3$) δ_H 1.36 (s, 3H), 1.76 (s, 3H), 2.17–2.36 (m, 5H), 3.32–3.52 (m, 2H), 3.81 (s, 6H), 4.03–4.18 (m, 2H), 4.26–4.56 (m, 4H), 5.22–5.25 (m, 1H), 6.0–6.24 (m, 2H), 6.72 (s, 1H), 6.84–6.89 (d, 4H, $\Delta J = 8.77$ Hz), 7.26–8.04 (m, Ar), 9.8 (br, 1H); MS (EI) *m/z* 953.99 (calc.), found 976.33 $(M+Na)^+$.

N^3-Benzoyl-3′-*O*-*tert*-Butyldimethylsilyl Thymidine (8)

To compound **7** (2 g, 4.34 mmol) in 20 mL MeOH, I_2 (~300 mg) was added and the reaction mixture was stirred for 3–4 hours at room temperature with continuous monitoring by TLC. It was observed that if the reaction was left for a long time, 3′-OTBDMS group also gets deprotected and benzoyl thymidine was obtained. When the slightest formation of benzoyl thymidine was observed, the reaction mixture was quenched immediately by adding $Na_2S_2O_3$ till the solution, which is dark brown, becomes colorless. MeOH was removed under reduced pressure and the mixture was extracted

into organic layer, washed with water, brine, and dried over Na_2SO_4. The crude mixture was purified by column chromatography using 40% EtOAc in petroleum ether and concentrated under vacuum to get compound **8** as a colorless solid. Yield: 1.12 g (70%); ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 0.07 (s, 6H), 0.087 (s, 9H), 1.88 (s, 3H), 2.09–2.26 (m, 3H), 3.65–3.87 (m, 3H), 4.37–4.44 (m, 1H), 6.14–6.21 (t, 1H, $\Delta J = 6.71$ Hz), 7.44–7.64 (m, 3H), 7.90–7.93 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C −4.7, 12.6, 25.7, 40.9, 61.9, 71.8, 86.4, 87.8, 110.8, 129.1, 130.4, 131.5, 135.1, 136.7, 149.4, 163.0, 169.0; MS (EI) *m/z* 460.60 (calc.), found 462.03 $(M+1)^+$, 484.09 $(M+Na)^+$.

N^3-Benzoyl-5′-O-Propynyl 3′-*O*-*tert*-Butyldimethylsilyl Thymidine (9)

Compound **8** (1 g, 2.17 mmol) was taken in dry DMF (20 mL) and acetonitrile (2 mL) and cooled to 0°C. Propargyl bromide (0.28 mL, 3.26 mmol) was added and NaH (0.104 g, 2.60 mmol) was added in portions, and the reaction mixture was stirred for 2–3 hours with continuous monitoring by TLC. The reaction mixture was quenched by adding few milliliters of MeOH to it. DMF was removed under vacuum and the mixture was extracted into EtOAc (50 mL × 2), washed with water, brine, and dried over Na_2SO_4. The crude mixture was purified by column chromatography using 30% EtOAc in petroleum ether and concentrated under vacuum to get compound **9** as a colorless foam. Yield: 0.86 g (80%); IR ($CHCl_3$) ν/cm^{-1} 3308, 3019, 2955, 2930, 1707, 1671; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 0.00 (s, 6H), 0.80 (s, 9H), 1.60 (s, 3H), 2.00–2.39 (m, 3H), 3.55–3.76 (m, 1H), 4.13 (m, 1H), 4.34–4.71 (m, 4H), 6.23–6.29 (dd, 1H, $\Delta J_1 = 6.37$ Hz, $\Delta J_2 = 7.01$ Hz), 7.34–7.54 (m, 3H), 7.90–7.95 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C −4.6, 12.9, 25.68, 30.3, 41.2, 58.5, 63.7, 70.4, 70.6, 78.1, 85.8, 86.2, 110.4, 128.7, 129.5, 133.6, 150.0, 162.3; MS (EI) *m/z* 498.64 (calc.), found 499.57 $(M+1)^+$, 521.58 $(M+Na)^+$.

5′-*O*-Dimethoxytrityl 3′-*O*-*tert*-Butyldimethylsilyl 1,4-disubstituted 1,2,3-Triazolyl Dithymidine Dimer (11)

Individual monomer units **9** (1 g, 2.0 mmol) and **10** (0.91 g, 1.6 mmol) were taken in 10 mL of 1:1 *t*-BuOH and water. $CuSO_4$ (0.1 g, 0.401 mmol) and sodium ascorbate (0.198 g, 1.0 mmol) were added to the reaction. The reaction mixture was left overnight at room temperature. *t*-BuOH was removed under reduced pressure and the mixture was extracted into EtOAc (100 mL × 2), washed with water, brine, and dried over Na_2SO_4. The crude mixture was purified by column chromatography using 85% EtOAc in petroleum ether and concentrated under vacuum to get compound **11** as a colorless foam. Yield: 1.7 g (80%); IR ($CHCl_3$) ν/cm^{-1} 3017, 2929, 1702, 1508; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 0.00 (s, 6H), 0.87 (s, 9H), 1.24

(s, 3H), 1.53 (s, 3H), 2.03–2.46 (m, 4H), 2.59–2.73 (m, 1H), 3.27–3.3 (m, 1H), 3.59–3.68 (m, 2H), 3.77 (s, 6H), 4.19–4.21 (m, 1H), 4.41–4.49 (m, 2H), 4.56–4.67 (m, 1H), 5.0–5.11 (m, 1H), 6.31–6.49 (m, 2H), 6.80–6.84 (d, 4H, ΔJ = 8.74 Hz), 7.25–8.02 (m, Ar); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C −4.8, 12.0, 12.9, 17.9, 25.6, 29.6, 38.7, 41.4, 55.2, 62.2, 63.7, 71.9, 75.3, 78.9, 83.5, 85.7, 87.0, 110.3, 111.4, 113.3, 127.2, 128.0, 128.7, 129.4, 130.0, 133.6, 135.0, 144.1, 150.1, 150.5, 158.7, 166.1; MS (EI) *m/z* 1068.25 (calc.), found 1091.30 $(M+Na)^+$.

5′-*O*-Alkynyl 3′-Azide Triazolyl Dithymidine tt Dimer Unit (12)

Compound **11** (1 g, 0.93 mmol) was taken in dry THF (10 mL) and cooled to 0°C. Then 1 M TBAF in THF (0.48 mL, 1.87 mmol) was added to it at 0°C. The reaction mixture was stirred for a period of 2 hours. THF was removed and the reaction mixture was extracted into EtOAc (100 mL × 2), washed with brine, and dried over Na_2SO_4. The crude mixture was purified by column chromatography using EtOAc and concentrated under vacuum to get compound **12** as a colorless foam. Yield: 0.77 g (87%): ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.49 (s, 3H), 1.64 (s, 3H), 2.1–2.21 (m, 1H), 2.46–2.73 (m, 2H), 2.88–3.01 (m, 1H), 3.28–3.61 (m, 4H), 3.76 (s, 6H), 4.27–4.64 (m, 5H), 5.01 (s, 1H), 6.40–6.53 (m, 2H), 6.79–6.83 (d, 4H, ΔJ = 8.98 Hz), 7.23–7.64 (m, Ar), 7.87 (s, 1H), 7.97–8.0 (d, 2H), 9.6 (hump, 1H) NH; ^{13}C NMR (50 MHz, $CDCl_3$) δ_C 11.9, 12.9, 14.2, 35.8, 40.7, 55.2, 60.4, 62.4, 64.3, 71.3, 83.5, 84.9, 87.1, 110.4, 111.1, 113.1, 123.5, 128.0, 128.6, 129.5, 130.0, 133.5, 135.0, 143.4, 144.1, 150.5, 150.6, 158.7, 162.9, 164.2, 166.3; MS (EI) *m/z* 953.99 (calc.), found 976.33 $(M+Na)^+$.

Synthesis of 5′-*O*-(4,4′-Dimethoxy) Trityl-tt/tt-3′-*O*-(2-Cyanoethyl-N,N′-Diisopropyl Phosphoramidite) Dimer (13, 14)

Compound **6/12** (0.35 g, 0.366 mmol) was dissolved in dry dichloromethane (DCM; 10 mL) followed by the addition of diisopropyl ethyl amine (0.25 mL, 1.46 mmol) and chloro(2-cyanoethoxy-*N,N′*-diisopropylamino)-phosphine (0.16 mL, 0.73 mmol), and the reaction mixture was stirred at room temperature for 2 hours. The contents were then diluted with dry DCM and washed with 5% $NaHCO_3$ solution. The organic phase was dried over anhydrous Na_2SO_4 and concentrated to foam. The residue was dissolved in DCM and precipitated with hexane to obtain **13/14**. The phosphoramidite **13/14** were dried overnight over P_2O_5 and KOH in a desiccator before applying on a DNA synthesizer. TLC shows two close moving spots for two diastereomers. Rf = 0.5, 2% methanol-dichloromethane; Yield: 0.37 g (87%); ^{31}P NMR ($CDCl_3$) δ 149.0, 149.08.

Synthesis of Complementary Oligonucleotides

The DNA oligonucleotides were synthesized on an Applied Biosystems ABI 3900 High Throughput DNA synthesizer using standard β-cyanoethyl phosphoramidate chemistry. The oligomers were synthesized in the 3′ to 5′ direction on a polystyrene solid support, followed by ammonia treatment. The oligonucleotides were desalted by gel filtration, their purity ascertained by RP-HPLC on a C18 column was found to be more than 95%, and were used without further purification. The RNA oligonucleotides were obtained commercially.

HPLC Purification of Control/Triazolyl Oligomers

The purity of the oligomers was checked by RP-HPLC (C18 column, 0.1 N TEAA Buffer-ACN). The purity was again ascertained by analytical RP-HPLC and integrity was confirmed by MALDI-TOF using THAP (2,4,6-trihydroxyacetophenone) matrix with diammonium citrate as additive.

UV-T_m Studies of Triazolyl Oligomers

The binding affinity of triazolyl oligonucleotides with complementary DNA/RNA was studied by a temperature-dependent UV absorbance experiment. The T_m experiments of duplexes were carried out in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl and 0.1 mM EDTA. The triazolyl DNA:DNA/RNA chimeric oligonucleotides were individually hybridized with the complementary DNA and RNA strands to obtain duplexes.

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