Synthesis of Readily Accessible Triazole-Linked Dimer Deoxynucleoside Phosphoramidite for Solid-Phase Oligonucleotide Synthesis

Srivari Chandrasekhar,*[a] Pabbaraja Srihari,[a] Chidalla Nagesh,[a] Nayani Kiranmai,[a] Narayana Nagesh,[b] M. Mohammed Idris*[b]

[a] Organic Chemistry Division – I, Indian Institute of Chemical Technology, Hyderabad 500 007, India
Fax +91(40)27160512; E-mail: srivaric@iict.res.in

[b] Centre for Cellular and Molecular Biology, Hyderabad 500 007, India
E-mail: idris@ccmb.res.in.

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Abstract: A concise preparation of triazole-linked deoxynucleoside phosphoramidite for its direct use in solid-phase oligonucleotide synthesis is reported. This dimer has successfully been utilized in solid-phase synthesis to make the 10- and 12-mer oligomers.

Key words: nucleotides, triazole, click, oligomer, solid-phase synthesis

Modified nucleosides/nucleotides continue to attract significant focus in medical applications for gene silencing such as, antisense, antigene, and RNA interference.[1] As naturally occurring oligonucleotides suffer from degradation due to endo- and exonucleases, modifications have become necessary to enhance the stability and also improve pharmacokinetic properties from a therapeutic point of view. Since the introduction of antisense agents, diverse analogues have been developed that include, modifications in the phosphate backbone (see Figure 1); phosphate analogues such as phosphorothioate **A**, phosphoramidate, methyl phosphonate,[2] and nucleotide borano phosphates **B**;[3] complete replacement of the phosphodiester linkage by more stable functionalities like amide **C**,[4] amine[5] and heterocycles **D**, **E**,[6] **F**;[7] modifications in nucleoside bases;[8] derivatization/substitution of the sugar moiety[9] and complete replacement of furano phosphate moiety to result in PNA;[10] and replacement of the sugar moiety with morpholine etc.[11] In parallel, the latest, innovative reactions such as the click reaction, have been well utilized to derive modified nucleosides.[12] Our own interest in the click reaction has encouraged us to utilize it for the synthesis of new scaffolds from Baylis-Hillman adducts to result in triazoles[13] and tetrazoles[14] that displayed potency when screened against TNF-α inhibition studies towards anti-arthritis.

Recently, Isobe et al. have accomplished the synthesis of a 10-mer ^{TL}DNA oligomer chain with click chemistry wherein the phosphate group has been completely replaced with by a triazole.[7] This new analogue was found to form a stable double strand with the complementary strand of natural DNA. The linkage of sugars through the triazole moieties throughout the chain makes it a limiting factor for further exploration. To overcome this, we initiated synthesis of a dimer nucleoside phosphoramidite with a triazole linkage that can be utilized directly for oligonucleotide synthesis on a solid support and substituted/linked up at the requisite site(s) in the chain. In continuation to our research focused on click chemistry,[15] we herein wish to report the synthesis of a dimer nucleoside phosphoramidite **1**, wherein the two monomer nucleoside units are interlinked through a triazole moiety rather than the regular phosphate ester linkage 1′ (Figure 2).

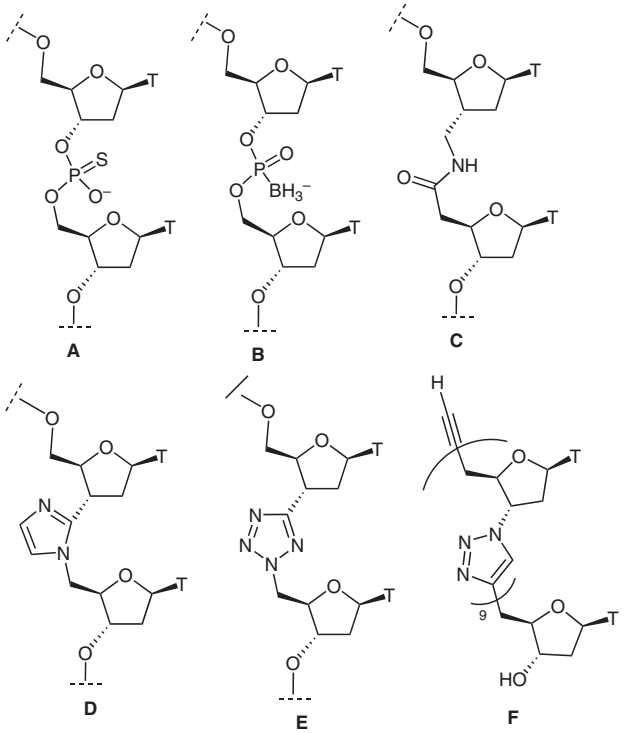

Figure 1 Some modified thymidine dinucleotides

Even though the replacement of the phosphodiester moiety with heterocycles, such as imidazole and tetrazoles, is known,[6] replacement with the triazole moiety has not been well explored.[12,16] We started to design a new dinucleoside phosphoramidite **1** with the sugar part of the nucleotide unaltered. Retrosynthetic analysis for **1** revealed two key fragments, azide **2** (AZT) and acetylene **3**. Com-

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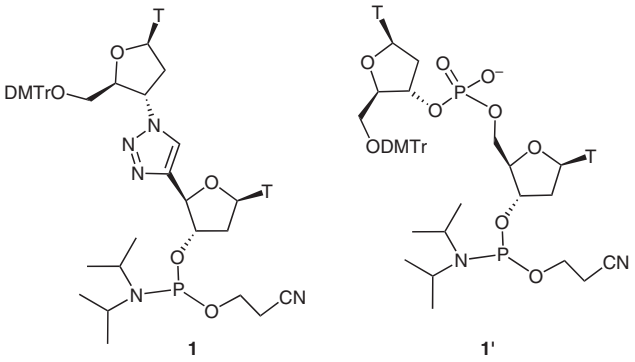
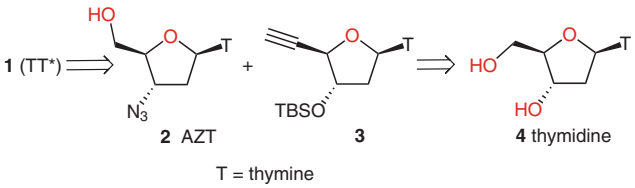


Figure 2 Thymidine dimers

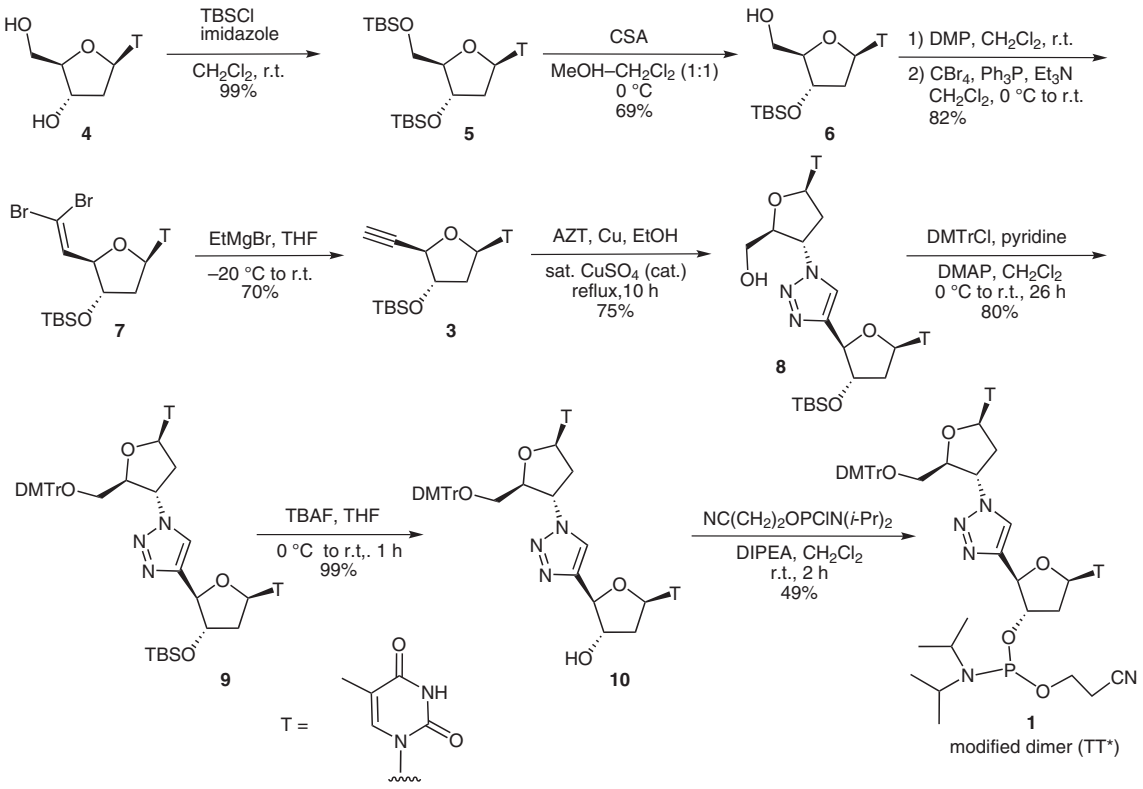


Scheme 1 Retrosynthesis for modified dimer nucleoside phosphoramidite **1**

pound **3** was easily accessible from commercially available thymidine **4** in five steps (Scheme 1).

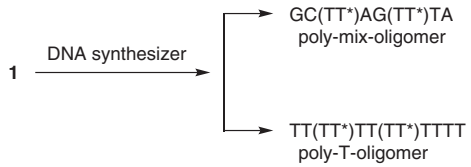
For the synthesis, we started with thymidine **4** which was protected as its bis(silyl ether) **5** at the 3′- and 5′-positions. Selective primary silyl deprotection was achieved with 10-camphorsulfonic acid[17] to give the free primary alcohol **6**. The alcohol **6** was oxidized with Dess–Martin periodinane and was subjected to Corey–Fuchs protocol[18] to give dibromomethylene **7**. Compound **7** was treated with freshly generated ethylmagnesium bromide to give the monomer **3** with a terminal acetylene moiety.[19] Compound **3** was subjected to cycloaddition with commercially available AZT **2** in the presence of copper metal in ethanol at reflux temperature to give the nucleoside dimer **8** in 75% yield.[15a] Our next goal was to protect the free primary alcohol (5′-OH) with 4,4′-dimethoxytrityl chloride and convert the protected hydroxy group at the 3′-position to a phosphoramidite to obtain the readily accessible dimer nucleoside phosphoramidite. Thus, we proceeded with the protection of free 5′-OH group in **8** as the corresponding dimethoxytrityl ether **9** with 4,4′-dimethoxytrityl chloride in the presence of pyridine and 4-(dimethylamino)pyridine. Tetrabutylammonium fluoride mediated deprotection of silyl ether **9** afforded **10** which was converted into the target phosphoramidite **1** using 2-cyanoethyl diisopropylchlorophosphoramidite in the presence of *N*,*N*-diisopropylethylamine in anhydrous dichloromethane at room temperature.[16f] Thus, we have accomplished the synthesis of the target dimer deoxynucleoside phosphoramidite **1** (Scheme 2).

The synthesized dinucleoside **1** was a suitably protected dimer that can be utilized directly for solid-phase oligonucleotide synthesis. We have successfully demonstrated the utilization of compound **1** for the synthesis of two oligomers of length 10-mer poly mix GC(TT*)AG(TT*)TA and 12-mer (poly T, TT(TT*)TT(TT*)TTTT which were synthesized in the oligo synthesizer (Scheme 3). These oligos were characterized by MALDI analysis with *m/z*



Scheme 2 Synthesis of modified dinucleotide **1** (triazole-linked dimer)

2975.697 for 10-mer poly mix, GC(TT*)AG(TT*)TA and *m*/*z* 3530.752 for 12-mer poly T, TT(TT*)TT(TT*)TTTT.



Scheme 3 Application of modified dimer in oligomer synthesis

In conclusion, a triazole-linked modified dinucleoside phosphoramidite synthesis and its incorporation into long-chain oligonucleotides on solid phase were demonstrated. Further investigations on the properties of this dimer nucleoside phosphoramidite and its application in long-chain oligomer synthesis and also the synthesis of other dinucleotide combinations are in progress.

All the reagents employed were obtained commercially from M/s. Aldrich and used without further purifications unless otherwise stated. For anhydrous reactions, solvents were dried by literature procedures; removal of solvent was performed under reduced pressure using a rotary evaporator. All reactions requiring anhydrous conditions were carried out in oven-dried glassware under N_2. All reactions and fractions from column chromatography were monitored by TLC using plates with a UV fluorescent indicator (normal silica gel, Merck 60 F254). ^{1}H NMR spectra were recorded at 300 or 400 MHz [relative to the solvent residual signal in $CDCl_3$ (δ = 7.26) or to TMS], and ^{13}C NMR spectra were recorded at 75 MHz/100 MHz at r.t. [relative to the solvent signal $CDCl_3$ (δ = 77.00) unless otherwise noted]. One or more of the following methods were used for visualization: UV absorption by fluorescence quenching; I_2 staining; phosphomolybdic acid/$Ce(SO_4)_2$/H_2SO_4/H_2O (10 g:1.25 g:12 mL:238 mL) spray; anisaldehyde spray (EtOH–AcOH–H_2SO_4–anisaldehyde; 200 mL:2 mL:6.35 mL:3 mL). Column chromatography was performed using 60–120 mesh silica gel.

Oligonucleotide Synthesis

The solid phase oligonucleotide synthesis was performed on an ABI-394 DNA synthesizer (USA) following standard procedures. Detritylation was achieved using 2–3% trichloroacetic acid in CH_2Cl_2. The incoming base was first allowed to react with weak acid tetrazole to form an active tetrazolyl phosphoramidite intermediate which reacts with the 5′-hydroxy group of the recipient. Unreacted free hydroxy groups were capped by Ac_2O and *N*-methylimidazole in MeCN. Stabilization of the phosphate linkage between two bases was achieved by treating the resin-supported oligomer with I_2 in THF–H_2O. After the completion of the chain synthesis, the mixture was incubated at 55 °C for 16 h to cleave the resin as well as remove the protection groups. The modified base phosphoramidite as well as $DMTr^+$ on oligonucleotides were purified by passing through the $DMTr^+$ Glen-Pak Cartridges (Glen Research Inc, USA) and they were characterized by mass spectroscopy and gel electrophoresis.

1-{(2*R*,4*S*,5*R*)-4-(*tert*-Butyldimethylsilyloxy)-5-[(*tert*-butyldimethylsilyloxy)methyl]tetrahydrofuran-2-yl}-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (5)

To the soln of thymidine **4** (3.0 g, 12.38 mmol) in CH_2Cl_2 (20 mL) at 0 °C under N_2 was added imidazole (2.52 g, 37.15 mmol) and the mixture was stirred for 20 min. TBSCl (4.65 g, 30.96 mmol) in CH_2Cl_2 (10 mL) was added dropwise and the mixture was stirred at r.t. for 1 h. After completion of the reaction, the mixture was quenched with H_2O (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine (30 mL), dried (anhyd Na_2SO_4), and concentrated in vacuo. The residue was subjected to column chromatography (silica gel, EtOAc–hexane, 1:5) to yield **5** (5.8 g, 99%) as a white solid; mp 141–142 °C; R_f = 0.80 (EtOAc–hexane, 1:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.66 (s, 1 H), 7.4 (d, *J* = 0.9 Hz, 1 H), 6.25 (dd, *J* = 7.3, 6.0 Hz, 1 H), 4.43–4.50 (m, 1 H), 3.72–3.98 (m, 3 H), 2.24 (ddd, *J* = 13.1, 5.89, 3.03 Hz, 1 H), 1.98 (ddd, *J* = 13.1, 7.5, 6.5 Hz, 1 H), 1.91 (d, *J* = 0.9 Hz, 3 H), 0.94 (s, 9 H), 0.91 (s, 9 H), 0.11 (d, *J* = 0.9 Hz, 6 H), 0.08 (d, *J* = 3.4 Hz, 6 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.5, 150.1, 135.4, 110.7, 87.8, 84.8, 72.2, 62.9, 41.3, 25.9, 25.7, 18.3, 17.9, 12.5, –4.6, –4.8, –5.3.

HRMS: *m*/*z* calcd [M + Na]$^+$ for $C_{22}H_{42}N_2O_5NaSi_2$: 493.2516; found: 493.2529.

1-[(2*R*,4*S*,5*R*)-4-(*tert*-Butyldimethylsilyloxy)-5-(hydroxymethyl)tetrahydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (6)

CSA (0.812 g, 3.49 mmol) was added portionwise to a stirred soln of **5** (2.78 g, 5.91 mmol) in CH_2Cl_2–MeOH (1:1, 20 mL) at 0 °C under N_2 and the mixture was stirred at 0 °C for 16 h. The mixture was quenched with aq $NaHCO_3$ (20 mL) at 0 °C and stirred for 30 min. The solvents were evaporated in vacuo and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried (anhyd Na_2SO_4), and concentrated in vacuo. The residue was subjected to column chromatography (silica gel, EtOAc–hexane, 2:3) to yield **6** (1.45 g, 69%) as a white solid; mp 89–91 °C; R_f = 0.50 (EtOAc).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.80 (br s, 1 H), 7.36 (s, 1 H), 6.07 (t, *J* = 6.6 Hz, 1 H), 4.45–4.54 (m, 1 H), 3.82–3.94 (m, 2 H), 3.64–3.76 (m, 1 H), 3.10 (br s, 1 H), 2.27–2.42 (m, 1 H), 2.21–2.25 (m, 1 H), 1.88 (s, 3 H), 0.90 (s, 9 H), 0.09 (s, 6 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.2, 150.6, 137.2, 111.1, 87.7, 86.9, 71.7, 62.1, 40.7, 25.9, 18.1, 12.6, –4.5, –4.6.

HRMS: *m*/*z* calcd [M]$^+$ for $C_{16}H_{28}N_2O_5Si$: 356.1776; found: 356.1767.

1-[(2*R*,4*S*,5*R*)-4-(*tert*-Butyldimethylsilyloxy)-5-(2,2-dibromovinyl)tetrahydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (7)

To a stirred soln of **6** (1.38 g, 3.87 mmol) in CH_2Cl_2 (30 mL) was added Dess–Martin periodinane (1.78 g, 4.21 mmol) and the mixture was stirred at r.t. for 30 min. The reaction was quenched with sat. aq $Na_2S_2O_3$ (10 mL) at 0 °C. The mixture was diluted with CH_2Cl_2 (10 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were washed with sat. aq $NaHCO_3$ (10 mL) and brine (10 mL), dried (anhyd Na_2SO_4), and concentrated in vacuo to give crude aldehyde (1.30 g) that was utilized directly in the next reaction without further purification. To the stirred soln of Ph_3P (3.7 g, 14.12 mmol) in CH_2Cl_2 (10 mL) at 0 °C under N_2 was added CBr_4 (2.34 g, 7.06 mmol) dissolved in CH_2Cl_2 (10 mL) slowly and the reaction was stirred at this temperature for 30 min and then warmed to r.t. The mixture was cooled to 0 °C and Et_3N (1.07 g, 10.59 mmol) was added and the mixture was allowed to warm to r.t. for 15 min. The mixture was recooled to 0 °C and to this was added the above aldehyde (1.38 g, 3.53 mmol) dissolved in CH_2Cl_2 (10 mL). When the reaction was complete, the mixture was quenched with H_2O (30 mL) and stirred for 30 min, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried (anhyd Na_2SO_4), and concentrated in vacuo. The residue was subjected to column chromatography (silica gel, EtOAc–hexane, 1:4) to yield **7** (1.62 g, 82%) as a light brown solid; mp 149–151 °C; R_f = 0.50 (EtOAc–hexane, 4:6).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.44 (br s, 1 H), 7.06 (d, *J* = 1.1 Hz, 1 H), 6.50 (d, *J* = 8.5 Hz, 1 H), 6.01 (t, *J* = 6.6 Hz, 1 H), 4.52 (dd, *J* = 8.5, 3.9 Hz, 1 H), 4.29–4.36 (m, 1 H), 2.19–2.40 (m, 2 H), 1.95 (d, *J* = 1.1 Hz, 3 H), 0.91 (s, 9 H), 0.12 (s, 3 H), 0.10 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.9, 150.4, 136.1, 135.5, 111.4, 87.1, 86.7, 75.5, 73.4, 40.4, 25.8, 18.1, 12.8, –4.4, –4.6.

HRMS: *m*/*z* calcd [M + Na]$^+$ for $C_{17}H_{26}Br_2N_2O_4NaSi$: 530.9897; found: 530.9926.

1-[(2*R*,4*S*,5*R*)-4-(*tert*-Butyldimethylsilyloxy)-5-ethynyltetrahydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (3)

EtBr (3.04 g, 27.93 mmol) was added to a suspension of Mg (0.50 g, 18.62 mmol) in anhyd THF (20 mL) dropwise. After generation of EtMgBr, the mixture was cooled to –20 °C and to this was added compound **7** (0.95 g, 1.86 mmol) in THF (5 mL). After consumption of the starting material, the mixture was quenched with sat. aq NH_4Cl (10 mL) at 0 °C, the mixture was diluted with H_2O (10 mL), and the aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with brine (10 mL), dried (anhyd Na_2SO_4), and concentrated in vacuo. The residue was subjected to column chromatography (silica gel, EtOAc–hexane, 1:4) to yield **3** (0.45 g, 70%) as a white solid; mp 158–160 °C; R_f = 0.50 (EtOAc–hexane, 1:1).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.98 (br s, 1 H), 7.52 (s, 1 H), 6.42 (dd, *J* = 5.8, 8.1 Hz, 1 H), 4.58 (s, 1 H), 4.51 (d, *J* = 3.66, 4.3 Hz, 1 H), 2.75 (d, *J* = 1.5 Hz, 1 H), 2.43 (ddd, *J* = 4.4, 5.8, 13.1 Hz, 1 H), 2.16 (ddd, *J* = 4.4, 8.1, 13.1 Hz, 1 H), 1.94 (s, 3 H), 1.91 (s, 9 H), 0.12 (s, 6 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.7, 150.3, 135.5, 111.1, 86.8, 80.5, 77.6, 76.9, 40.5, 25.6, 17.9, 12.7, –4.8, –4.9.

HRMS: *m*/*z* calcd [M + Na]$^+$ for $C_{17}H_{26}N_2O_4NaSi$: 373.1556; found: 373.1559.

1-[(2*R*,4*S*,5*R*)-4-(*tert*-Butyldimethylsilyloxy)-5-(1-{(2*S*,3*S*,5*R*)-2-(hydroxymethyl)-5-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl}-1*H*-1,2,3-triazol-4-yl)tetrahydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (8)

Cu(0) (20 mg) was added to a stirred soln of AZT **2** (0.431 g, 1.61 mmol) and **3** (0.565 g, 1.61 mmol) in EtOH (20 mL). Sat. aq $CuSO_4$ (0.5 mL) was added to the mixture and it was refluxed for 12 h. After the completion of the reaction, the mixture was filtered through Celite and washed with $CHCl_3$, and the filtrate was concentrated in vacuo. The residue was subjected to column chromatography (silica gel, MeOH–$CHCl_3$, 2:98) to yield **8** (0.74 g, 75%) as a white solid; mp 139–141 °C; R_f = 0.30 (EtOAc).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.25 (s, 1 H), 9.21 (s, 1 H), 7.89 (s, 1 H), 7.59 (d, *J* = 1.1 Hz, 1 H), 7.49 (d, *J* = 0.95 Hz, 1 H), 6.28 (t, *J* = 6.8 Hz, 1 H), 6.22 (t, *J* = 6.2 Hz, 1 H), 5.42–5.51 (m, 1 H), 4.98 (d, *J* = 3.2 Hz, 1 H), 4.70–4.77 (m, 1 H), 4.42–4.48 (m, 1 H), 3.99–4.08 (m, 1 H), 3.74–3.85 (m, 1 H), 3.41–3.50 (m, 1 H), 2.91–3.08 (m, 2 H), 2.64–2.72 (m, 1 H), 2.36 (ddd, *J* = 3.7, 6.2, 13.4 Hz, 1 H), 1.94 (d, *J* = 0.75 Hz, 3 H), 1.91 (d, *J* = 0.75 Hz, 3 H), 0.86 (s, 9 H), 0.03 (s, 3 H), 0.01 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.0, 150.8, 150.5, 146.1, 137.7, 137.6, 123.2, 111.1, 88.3, 87.5, 85.3, 81.0, 76.1, 61.1, 58.9, 39.8, 37.6, 29.6, 25.6, 17.9, 12.5, 12.4, –4.8.

HRMS: *m*/*z* calcd [M]$^+$ for $C_{27}H_{39}N_7O_8Si$: 617.2629; found: 617.2638.

1-[(2*R*,4*S*,5*S*)-5-{[Bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-(4-{(2*R*,3*S*,5*R*)-3-(*tert*-butyldimethylsilyloxy)-5-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-2-yl}-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9)

Nucleoside **8** (0.500 g, 0.809 mmol) was co-evaporated with dry pyridine (2 × 10 mL) and dried under vacuum for 30 min. To a soln of dried **8** (0.500 g, 0.809 mmol) in dry pyridine (40 mL) was added DMAP (0.217 g, 1.78 mmol) and 4,4′-dimethoxytrityl chloride (0.329 g, 0.971 mmol) at r.t. under argon. The mixture was stirred at r.t. for 20 h, at which time TLC indicated that the reaction was not complete (only 20% conversion). To the mixture was added additional DMAP (0.217 g, 1.78 mmol) and 4,4′-dimethoxytrityl chloride (0.329 g, 0.971 mmol). The mixture was stirred at r.t. until the reaction was complete (~6 h). The solvent was removed, and the residue was purified by chromatography (silica gel, MeOH–$CHCl_3$, 2:98) to give **9** (0.591g, 80%) as a light yellow foam; mp 162–163 °C; R_f = 0.45 (MeOH–$CHCl_3$, 1:9).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.94 (br s, 1 H), 8.75 (br s, 1 H), 7.69 (d, *J* = 1.1Hz, 1 H), 7.66 (d, *J* = 0.7 Hz, 1 H), 7.45 (s, 1 H,), 7.37–7.43 (m, 2 H), 7.24–7.35 (m, 7 H), 6.84 (dd, *J* = 8.8, 9.0 Hz, 4 H), 6.42–6.53 (m, 2 H), 5.32–5.41 (m, 1 H), 4.88 (d, *J* = 2.8 Hz, 1 H), 4.63–4.69 (m, 1 H), 4.39–4.46 (m, 1 H), 3.79 (s, 6 H), 3.70 (dd, *J* = 11.4, 10.9 Hz, 1 H), 3.35 (dd, *J* = 10.9, 10.7 Hz, 1 H), 3.03–3.13 (m, 1 H), 2.58–2.82 (m, 2 H), 2.31–2.41 (m, 1 H), 1.87 (s, 3 H), 1.60 (s, 3 H), 0.87 (s, 9 H), 0.07 (s, 6 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.3, 158.8, 150.4, 150.1, 146.2, 143.9, 136.6, 135.3, 134.9, 134.8, 130.0, 128.1, 127.9, 127.3, 122.2, 113.3, 111.6, 111.2, 87.1, 86.2, 85.2, 83.6, 80.6, 77.1, 76.1, 62.0, 59.7, 55.2, 40.0, 38.3, 25.6, 17.9, 12.5, 12.0, 0.9. –4.8, –4.8.

HRMS: *m*/*z* calcd [M + H]$^+$ for $C_{48}H_{58}N_7O_{10}Si$: 920.4009; found: 920.4003.

1-[(2*R*,4*S*,5*S*)-5-{[Bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-4-(4-{(2*R*,3*S*,5*R*)-3-hydroxy-5-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-2-yl}-1*H*-1,2,3-triazol-1-yl)tetrahydrofuran-2-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (10)

A 1 M soln of TBAF (0.11 g, 0.43 mmol) was added to a stirred soln of **9** (0.40 g, 0.43 mmol) in THF (5 mL) at 0 °C under N_2 and the mixture was stirred for 1 h. The reaction was quenched with sat. $NaHCO_3$ and stirred for 10 min. The aqueous layer was extracted with EtOAc (3 × 5 mL), the combined organic layers were washed with brine (5 mL), dried (anhyd Na_2SO_4), and concentrated in vacuo. The residue was subjected to column chromatography (silica gel, MeOH–$CHCl_3$, 5:95) to yield **7** (0.35 g, 99%) as a light foamy white solid; mp 123–125 °C; R_f = 0.40 (MeOH–$CHCl_3$, 1:9).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.22 (s, 1 H), 7.68 (s, 1 H), 7.62 (s, 2 H), 7.19–7.43 (m, 9 H), 6.82 (d, *J* = 7.7 Hz, 4 H), 6.42–6.58 (m, 2 H), 5.32–5.45 (m, 1 H), 5.04 (d, *J* = 5.0 Hz, 1 H), 4.68–4.81 (m, 1 H), 4.38–4.46 (m, 1 H), 3.77 (s, 6 H), 3.60–3.72 (m, 1 H), 3.32–3.43 (m, 1 H), 2.92–2.99 (m, 1 H), 2.59–2.83 (m, 2 H), 2.42–2.53 (m, 1 H), 1.83 (s, 3 H), 1.55 (s, 3 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.6, 163.4, 158.8, 150.5, 150.3, 146.2, 144.0, 136.7, 135.3, 135.0, 134.8, 130.0, 128.1, 127.9, 127.3, 122.3, 113.3, 111.7, 111.3, 87.2, 86.0, 85.3, 83.7, 80.1, 75.2, 62.5, 60.2, 55.2, 39.3, 38.3, 12.5, 12.0.

HRMS: *m*/*z* calcd [M + Na]$^+$ for $C_{42}H_{43}N_7O_{10}Na$: 828.2970; found: 828.2969.

(2*R*,3*S*,5*R*)-2-({1-[(2*S*,3*S*,5*R*)-2-{[Bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-5-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl}-1*H*-1,2,3-triazol-4-yl)-5-[5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]tetrahydrofuran-3-yl 2-Cyanoethyl diisopropylphosphoramidite (1)

To a soln of alcohol **10** (0.1 g, 0.12 mmol) in anhyd CH_2Cl_2 (8 mL) at r.t. was added DIPEA (0.8 mL, 0.49 mmol) and 2-cyanoethyl diisopropylchlorophosphoramidite (0.053 g, 0.22 mmol) in CH_2Cl_2 (0.5 mL) under N_2. After completion of the reaction (2 h), the mixture was cooled to 0 °C and diluted with H_2O (4 mL) and CH_2Cl_2 (8 mL). The organic layer was separated and washed with sat. aq $NaHCO_3$ (10 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 8 mL). The combined organic layers were dried (anhyd Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified via flash chromatography (silica gel 100–200 mesh, $CHCl_3$–MeOH–Et_3N, 99:0.5:0.5) to obtain **1** (0.06 g, 49%) as a creamy powder; R_f = 0.50 (MeOH–$CHCl_3$, 1:9).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.81 (d, *J* = 0.9 Hz, 1 H), 7.73 (d, *J* = 0.9 Hz, 1 H), 7.69 (br s, 1 H), 7.63–7.67 (m, 2 H), 7.48 (br s, 2 H), 7.36–7.42 (m, 4 H), 7.22–7.35 (m, 12 H), 6.80–6.89 (m, 8 H), 6.47–6.59 (m, 4 H), 5.28–5.40 (m, 2 H), 5.21 (br s, 1 H), 5.12 (br s, 1 H), 4.68–4.82 (m, 2 H), 4.41–4.54 (m, 2 H), 3.51–3.94 (m, 18 H), 3.30–3.39 (m, 2 H), 2.94–3.09 (m, 4 H), 2.45–2.77 (m, 8 H), 2.64 (t, *J* = 6.4 Hz, 4 H), 1.86 (s, 6 H), 1.56 (d, *J* = 5.8 Hz, 6 H), 1.24–1.10 (m, 24 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.4, 163.3, 158.8, 150.3, 150.0, 146.1, 144.0, 136.6, 136.5, 135.3, 130.0, 128.1, 128.0, 127.3, 122.7, 122.2, 113.3, 111.5, 111.3, 87.2, 86.0, 85.8, 85.2, 85.0, 83.6, 83.5, 79.4, 79.1, 62.3, 62.2, 60.0, 55.2, 43.4, 43.2, 38.5, 38.4, 24.6, 24.5, 24.4, 24.4, 22.9, 12.6, 12.0.

^{31}P NMR (161.91 MHz, $CDCl_3$): δ = 150.8, 149.1.

HRMS: *m/z* calcd [M + Na]$^+$ for $C_{51}H_{60}N_9O_{11}PNa$: 1028.4042; found: 1028.4048.

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