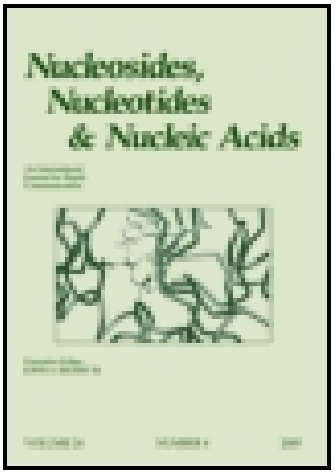

Nucleosides, Nucleotides and Nucleic Acids

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Conformationally Locked Nucleosides. Synthesis of Oligodeoxynucleotides Containing 3′-Amino-3′-deoxy-3′-N,5′(R)-C-ethylenethymidine

Guangyi Wang [a] & Vesna Stoisavljevic [a]

[a] Chemistry Laboratory, ICN Pharmaceuticals, Inc. , 3300 Hyland Avenue Costa Mesa, California, 92626, U.S.A.

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NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(9), 1413–1425 (2000)

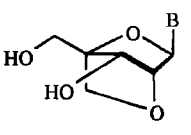
Conformationally Locked Nucleosides. Synthesis of Oligodeoxynucleotides Containing 3'-Amino-3'-deoxy-3'-*N*,5'(*R*)-*C*-ethylenethymidine

Guangyi Wang* and Vesna Stoisavljevic

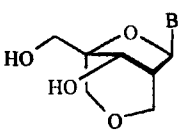
Chemistry Laboratory, ICN Pharmaceuticals, Inc., 3300 Hyland Avenue
Costa Mesa, California 92626, U.S.A.

Abstract: 3'-Amino-3'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-3'-*N*,5'(*R*)-*C*-ethylenethymidine (**6**) was synthesized starting from 3'-azido-3'-deoxythymidine. Condensation of **6** with 5'-*O*-(*H*-phosphonyl)thymidine and 5'-*O*-(*p*-nitrophenoxycarbonyl)thymidine derivatives gave dinucleotide and dinucleoside derivatives, respectively, which were incorporated into oligodeoxynucleotides (ODNs). Tm data of the modified ODNs are also presented.

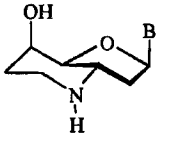
Oligonucleotides (ONs) containing conformationally restricted nucleosides have recently drawn considerable attention.[1-5] It was reported that 2',4'-bridged nucleosides (**a**, **b**) having a locked, C3'-endo sugar pucker dramatically increased hybridization of the modified ONs to complementary RNA.[3-5] It was well established that the C3'-endo sugar pucker is predominant in DNA/RNA duplexes.[6] One can expect that the modified ONs having a locked, C3'-endo sugar pucker have advantages over natural ONs in entropy contribution[7] to the duplex stability as the preorganized, conformationally favorable sugar pucker already exists. In addition to the favorable sugar modifications, a number of modified backbones such as phosphoramidate[8] and MMI (methylene(methylimino))[9] also substantially increase DNA/RNA duplex stability. One may be curious to know what effects can be observed from a combination of a favorable, locked C3'-endo sugar pucker and a favorable backbone. In order to test such a combination, the 5'-*O*-(4,4'-



a

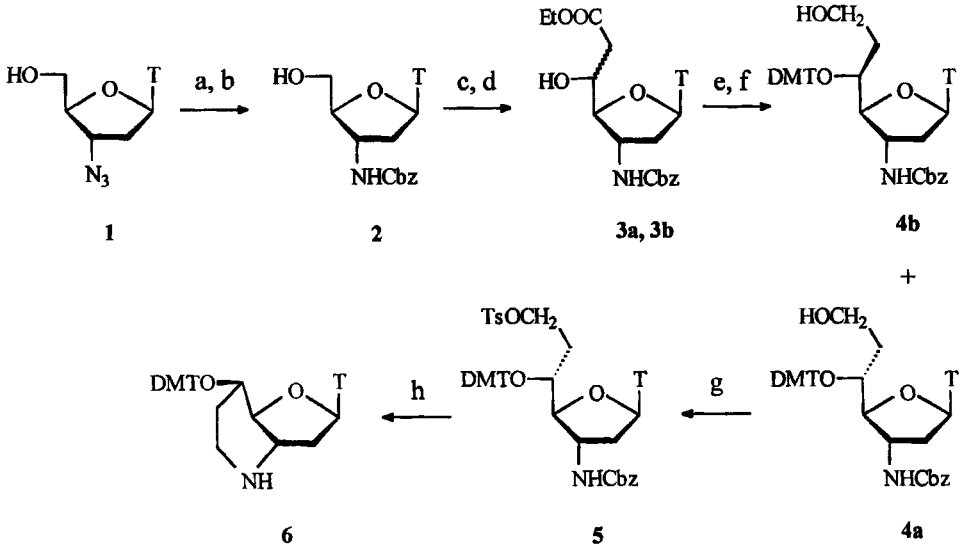


b



c

Scheme 1.

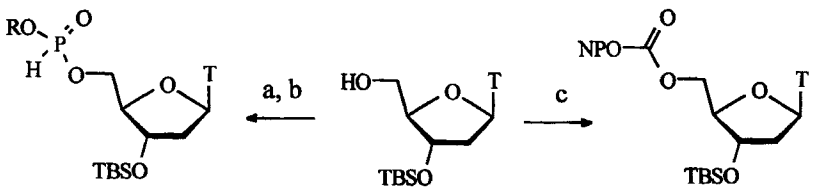


a) H_2, 10% Pd/C, r.t., 6 h; b) BnOCOCl, Na_2CO_3, r.t., 1 h, 97% (two steps); c) DMSO, DCC, TFA, pyridine, r.t., 4 h, 88%; d) Zn, $BrCH_2COOEt$, THF, 45 °C, 20 h, 62%; e) DMT-Cl, $AgNO_3$, pyridine, 50 °C, 24 h, 91%; f) $LiAlH_4$, THF, 0 °C, 2 h, 84%; g) TsCl, pyridine, r.t., 4 h; h) H_2, 10% Pd/C, r.t., 14 h, 67% (two steps).

dimethoxytrityl) derivative **6** of 3'-amino-3'-deoxy-3'-*N*,5'(*R*)-*C*-ethylenethymidine (**c**)[10] was synthesized as a monomer for construction of ONs having a locked, C3'-endo sugar pucker and a favorable N-backbone. In this article we present synthesis of dinucleotides, dinucleosides, and ODNs containing **c** and unmodified thymidine as well as Tm data of the modified ODNs.

Synthesis of 3'-amino-3'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-3'-*N*,5'(*R*)-*C*-ethylene-thymidine (**6**)[10] is shown in Scheme 1. 3'-Azido-3'-deoxythymidine (**1**)[11] was hydrogenolyzed to a 3'-amino derivative, which was protected with carbobenzyloxy, to give **2**. A mild oxidation with DMSO converted **2** to an aldehyde, which was subjected to a Reformatsky reaction to give **3a** and **3b** (the 5'(*R*)- and 5'(*S*)-isomer). Tritylation of **3a** and **3b**, followed by a reduction, gave a mixture of **4a** and **4b** (the 5'(*R*)- and 5'(*S*)-isomer), which could be separated by chromatography. Compound **4a** was converted to the tosylate **5**, which was subjected to a controlled catalytic hydrogenolysis over Pd/C and a subsequent cyclization to give **6** in good yield. It seems that the cyclization occurred immediately following the release of the amino group as the 3'-amino

Scheme 2.



8 R = Me
9 R = CE

7

10

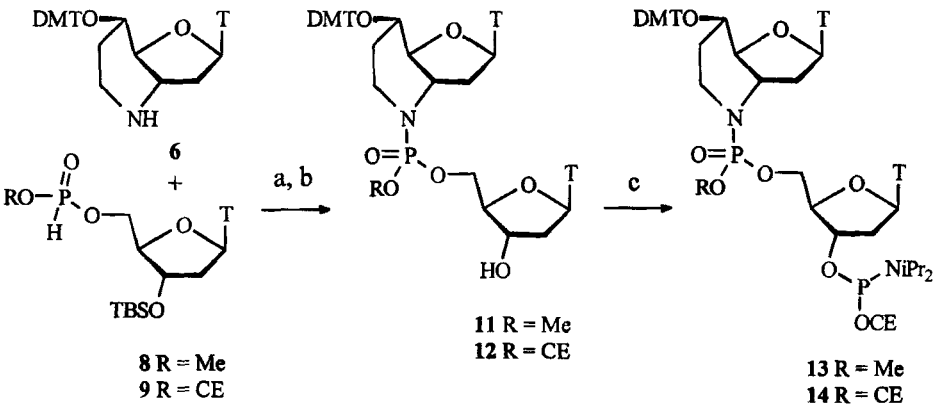
a) ROP(NiPr$_2$)Cl, iPr$_2$NEt, rt, 0.5 h, 89% (R = Me), 85% (R = CE); b) 1*H*-tetrazole, CH_3CN/H_2O (9:1), rt, 0.5 h, 96% (R = Me), 97% (R = CE); c) $O_2NC_6H_4OCOCl$, pyridine, CH_2Cl_2, rt, 15 h, 95%.

intermediate was not observed. The configurational assignment of **6** was reported in our previous communication.[10]

Reaction of 3'-*O*-(*t*-butyldimethylsilyl)thymidine (**7**)[12] with methyl *N*,*N*-diisopropylchlorophosphoramidite yielded a 5'-phosphoramidite, which was treated with 1*H*-tetrazole in water/acetonitrile to give the 5'-*O*-(*H*-phosphonyl)thymidine **8**. Compound **9** was prepared in the same way except that the reagent was 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite. Reaction of **7** with *p*-nitrophenyl chloroformate gave the 5'-*O*-(*p*-nitrophenoxycarbonyl)thymidine **10** in very good yield.

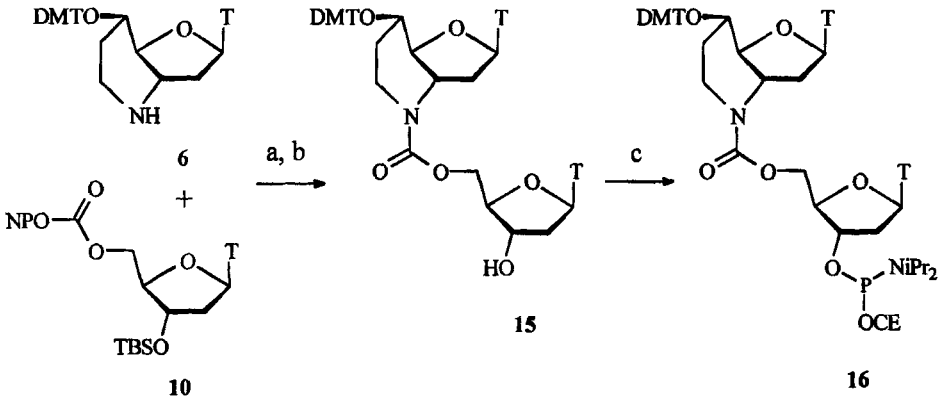
Condensations of **6** with **8** and **9** were conducted, separately, in the presence of tetrachloromethane and triethylamine by a reported procedure for synthesis of phosphoramidate oligonucleotides.[8] The resulting coupling products were treated with TBAF to give **11** and **12**, respectively. When commercially available 1.0 M TBAF in THF (pH >9) was used, the cyanoethyl group of **12** was also partially removed. However, AcOH-neutralized TBAF solution in THF (pH ~5.5) worked well with minimal removal of the cyanoethyl. Compound **11** was converted to the phosphoramidite **13** in good yield whereas the phosphoramidite **14** was obtained in a moderate yield. Although **14** was formed almost quantitatively, it could not well survive during a flash chromatography (silica gel, 5% triethylamine and 25% acetone in methylene chloride). A possible explanation is that triethylamine abstracted an α-hydrogen of the cyanoethyl of the phosphoramidate linkage in **14**, leading to the removal of the cyanoethyl. Each of the dinucleotide phosphoramidites **13** and **14** comprises four diastereoisomers resulting from the two phosphorus chiral centers, clearly indicated by ^{31}P NMR.

Scheme 3.



a) Et_3N, $CCl_4/CH_3CN/CH_2Cl_2$ (1:1:1), rt, 0.5 h; b) AcOH/TBAF, THF, rt, 24 h, 74% for **11** (two steps), 66% for **12** (two steps); c) same as a in Scheme 1, 87% for **13**, 45% for **14**.

Scheme 4.



a) DMAP, CH_2Cl_2, rt, 20 h; b) TBAF, THF, rt, 1 h, 91% (two steps); c) same as a in Scheme 1, 84%.

Condensation of **6** with **10** proceeded smoothly, and the coupling product was treated with TBAF to give **15** in very good yield. Compound **16** was obtained from **15** in good yield.

Modified ODNs containing 3'-amino-3'-deoxy-3'-*N*,5'(*R*)-*C*-ethylenethymidine (**c**) were synthesized using the phosphoramidites **13, 14,** and **16**. A standard procedure[13] in

Table 1. Tm data of ODNs containing 3'-amino-3'-deoxy-3'-*N*,5'(*R*)-*C*-ethylenethymidine

Sequence	Tm °C DNA	ΔTm °C/Mod.	Tm °C RNA	ΔTm °C/Mod.
1. 5'-d(TTTTTTTTTTTTTTT)-3'	49.3		42.2	
2. 5'-d(TTTTTT$\mathbf{t_1}$T$\mathbf{t_1}$TTTTTT)-3'	46.6	–1.4	40.4	–0.9
3. 5'-d(TTTTTT$\mathbf{t_2}$T$\mathbf{t_2}$TTTTTT)-3'	41.5	–3.9	35.1	–3.6
4. 5'-d(TTTTTT$\mathbf{t_3}$T$\mathbf{t_3}$TTTTTT)-3'	36.7	–6.3	32.1	–5.1

$\mathbf{t_1}$ = 3'-amino-3'-deoxy-3'-*N*,5'(*R*)-*C*-ethylenethymidine having a phosphoramidate linkage at the 3'-amino; $\mathbf{t_2}$ = 3'-amino-3'-deoxy-3'-*N*,5'(*R*)-*C*-ethylenethymidine having a phosphoramidate methyl ester linkage at the 3'-amino; $\mathbf{t_3}$ = 3'-amino-3'-deoxy-3'-*N*,5'(*R*)-*C*-ethylenethymidine having a carbamate linkage at the 3'-amino. The samples for Tm measurements contain 2.0 μM of modified ODNs and 2.0 μM of either complementary DNA or RNA in a buffer (10 mM sodium phosphate, 0.1 mM EDTA, and 1.0 M sodium chloride, pH 7.0).

the phosphoramidite approach was used except for a more concentrated solution (0.11M) and a longer reaction time (5 min) for the couplings using **13**, **14**, and **16** and the couplings next to the modified. The coupling yields for **13**, **14**, and **16** (97-99%) are comparable to those for the unmodified phosphoramidites. For Sequence 4 containing two carbamate linkages with DMT at the 5'-end, deprotection and purification followed the usual procedure[14] in the reverse-phase HPLC approach with 0.1 M TEAA and acetonitrile as mobile phase except that the ammonia treatment was conducted at rt for 3 h. Sequence 3 having two phosphoramidate methyl ester linkages with no DMT at the 5'-end was synthesized using **13**. The aqueous ammonia was evaporated immediately after the ODNs were cleaved from solid supports. The crude was purified on an ion-exchange column (Dionex OmniPac NA-100, 9 x 250 mm) with a mobile phase of 1.5 M NaCl and 10 mM NaOH (pH 12). The purified ODNs were desalted twice with Applied Biosystems' OPC columns. Sequence 2 having two phosphoramidate linkages with no DMT at the 5'-end was synthesized using both **13** and **14**. When **13** was used, the aqueous ammonia solution was heated at 55 °C for 24 h (the condition at which methyl was removed was verified at the dinucleotide level), followed by the same ion-exchange HLPC purification as Sequence 3. When **14** was used, the ammonia treatment was conducted at rt for 3 h only. It may be worth while mentioning that Sequence 2 was not very stable at even weakly acidic condition.

Hybridization of the modified ODNs to DNA and RNA was studied through melting measurements.[14] As can be seen in Table 1, the modifications decrease hybridization of

the ODNs to both DNA and RNA. The bicyclothymidine $\mathbf{t_3}$ (carbamate linkage) in Sequence 4 decreased hybridization with both DNA and RNA dramatically. The bicyclothymidine $\mathbf{t_2}$ (phosphoramidate methyl ester linkage) in Sequence 3 also severely destabilized the duplexes with both DNA and RNA. The bicyclothymidine $\mathbf{t_1}$ (phosphoramidate linkage) in Sequence 2 destabilized the duplexes with RNA only moderately. These ODNs containing $\mathbf{t_1}$, $\mathbf{t_2}$, or $\mathbf{t_3}$ in which the C5'-O5' bond has an orientation similar to that in the natural ODNs were expected to have at least a satisfactory Tm. The decrease in Tm implicated an unfavorable deformation of the sugar moiety.

In summary, we have reported synthesis of dinucleosides, dinucleotides, and oligodeoxynucleotides containing 3'-amino-3'-deoxy-3'-*N*,5'(*R*)-*C*-ethylenethymidine. The bicyclothymidine destabilized duplexes of the modified ODNs with both DNA and RNA, to a varied degree. For further studies on ONs containing favorable, conformationally locked nucleosides and a favorable backbone, 2',4'-bridged nucleosides (**a** or **b**) and phosphoramidate linkage may be a good combination.

EXPERIMENTAL SECTION

Proton NMR spectra were recorded on a 300 MHz or 500 MHz spectrometer and chemical shifts are reported in δ values (parts per million) with tetramethylsilane (TMS) as the internal standard. ^{31}P NMR spectra are reported in δ values (ppm) with phosphoric acid as an external standard. Mass spectra were obtained on an electrospray mass spectrometer using both positive and negative ionization modes from Mass Consortium, Corp, San Diego. Elemental analysis data were obtained from NuMega Resonance Labs, San Diego. Anhydrous solvents containing <0.005% water were purchased from Fluka or Aldrich and used directly without further treatment unless as indicated. Thin layer chromatography plates and silica gel for column chromatography were supplied by ICN Biomedicals.

A usual work-up procedure: a quenched reaction mixture was diluted with ethyl acetate (or methylene chloride), washed successively with water (or brine), dilute sodium bicarbonate, water (or brine), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to dryness. The residue was subjected to chromatographic purification on a silica gel column.

3'-*C*-Carbobenzyloxyamino-3'-deoxythymidine (2). A mixture of **1**[11] (10.0 g, 37.5 mmol) and 10% Pd/C (1.0 g) in methanol (200 mL) was shaken in a hydrogenation

apparatus (hydrogen: 55 psi) at rt for 4 h. The catalyst was filtered and washed with methanol. The filtrate was concentrated to dryness and dried overnight under vacuum. The same scale reaction was repeated once. The combined crude product was dissolved in water (400 mL), mixed with an aqueous sodium carbonate (7.95 g, 75 mmol, 200 mL), and then cooled to 0 °C. To this solution was added benzylchloroformate (16.1 mL, 112.7 mmol), and the resulting reaction mixture was stirred at 0 °C for 1 h and then at rt for an additional hour. Precipitate was filtered, washed with water, and dried in a vacuum oven at 70 °C to give 27.3 g (97.2%) of **2** as a colorless solid; ^{1}H NMR (DMSO-d_6) δ 1.76 (s, 3 H, 5-Me), 2.06-2.26 (m, 2 H, H-2'), 3.50-3.67 (m, 2 H, H-5'), 3.76 (m, 1 H, H-4'), 4.14 (m, 1 H, H-3'), 5.02 (s, 2 H, benzyl), 5.12 (t, br, 1 H, OH), 6.13 (t, J = 6.6 Hz, H-1'), 7.34 (m, 5 H, benzyl), 7.75 (s, 1 H, H-6), 7.76 (s, 1 H, NHCbz), 11.29 (s, br, 1 H, NH); MS: 398 ($M+Na^+$), 374 $(M-H)^-$. Anal. Calcd for $C_{18}H_{21}N_3O_6$: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.40; H, 5.45; N, 11.14.

3'-*C*-Carbobenzyloxyamino-3'-deoxy-5'(*R,S*)-*C*-ethoxycarbonylmethyl-thymidines (3a and 3b). Compound **2** (27.1 g, 72.26 mmol) and DCC (30.7 g, 148.8 mmol) were dissolved in anhydrous DMSO (200 mL) and ether (100 mL) and the resulting solution was cooled to 0 °C. A solution of TFA (2.9 mL, 37.2 mmol) and pyridine (6.0 mL, 74.4 mmol) in DMSO (20 mL) was added. Then, the reaction mixture was stirred at ambient temperature for 5 h, cooled to 0 °C, and quenched by adding oxalic acid (13.5g, 150 mmol) in methanol (60 mL). The mixture was stirred at rt for 1 h, and the precipitate was filtered and washed with ethyl acetate. The filtrate was further diluted with ethyl acetate, washed thoroughly with brine, dried over anhydrous sodium sulfate, and concentrated. Chromatography (1.2 % methanol in methylene chloride/ethyl acetate, 1:1) gave 23.6 g (88%) of a formyl derivative as a colorless solid.

To a stirred zinc powder (freshly washed with dilute hydrochloric acid and dried, 8.24 g, 126 mmol) in anhydrous THF (freshly distilled over calcium hydride, 120 mL) under argon was added one-third portion of ethyl bromoacetate (13.3 mL, 120 mmol). After stirring at ambient temperature for 5 min, a reaction started and a yellow color occurred. The rest of the reagent was added in portions to keep a gentle reaction. After the addition, the mixture was stirred at 45 °C for 30 min, and a yellow, cloudy mixture was formed. A solution of the formyl derivative (7.46 g, 20.0 mmol) in THF (60 mL) was added, and the reaction mixture was stirred at 42-45 °C for 20 h, then cooled to 0 °C, diluted with ethyl acetate, and acidified with dilute acetic acid. After the usual work-up and chromatography (ethyl acetate/hexanes, 3:1), a mixture of **3a** and **3b** (~1:1, 5.71 g, 61.9%) was obtained as a colorless foam; ^{1}H NMR (two isomers in acetone-d_6) δ 1.06,

1.10 (2 t, J = 7.2 Hz, 3 H, CH_2CH_3), 1.60, 1.73 (2 s, 3 H, 5-Me), 2.12-2.72 (m, 4 H, 5'-*C*-CH_2, H-2'), 3.76 (s, 6 H, 2 OMe), 3.86, 3.89 (2 q, J = 7.2 Hz, 2 H, CH_2CH_3), 3.93-4.09 (m, 2 H, H-4' and H-5'), 4.40-4.65 (m 1 H, H-3'), 5.08 (m, 2 H, benzyl), 5.12 (t, br, 1 H, OH), 6.18, 6.29 (2 t, J = 6.9 Hz, H-1'), 6.78 (t, J = 8.4 Hz, NHCbz), 6.81-6.90 (m, 4 H, DMT), 7.17-7.70 (m, 15 H, 6-H, benzyl, DMT), 10.0 (br, 1 H, NH); MS: 484 ($M+Na^+$), 460 $(M-H)^-$. Anal. Calcd for $C_{22}H_{27}N_3O_8$: C, 57.26; H, 5.90; N, 9.11. Found: C, 56.90; H, 5.78; N, 8.96.

3'-*C*-Carbobenzyloxyamino-3'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-5'(*R,S*)-*C*-hydroxyethylthymidines (4a and 4b). To a stirred solution of the mixture of **3a** and **3b** (14.1 g, 30.58 mmol) and 4,4'-dimethoxytrityl chloride (31.09 g, 91.75 mmol) in anhydrous pyridine (90 mL) was added in portions silver nitrate (15.59 g, 91.75 mmol). The resulting mixture was stirred at 55 °C for 14 h. An additional portion of DMT-Cl (10.36 g, 30.58 mmol) and silver nitrate (5.20 g, 30.58 mmol) was added. The mixture was stirred at 55 °C for additional 20 h, then cooled to 0 °C, diluted with ethyl acetate, and filtered. The usual work-up and chromatography (ethyl acetate/hexanes, 1:1 to 2:1) gave a mixture of the 5'-*O*-DMT derivatives of **3a** and **3b** (21.28 g, 91%) as a yellow foam (the yellow color resulted from a small amount of impurity).

To a stirred suspension of lithium aluminum hydride (2.98 g, 78.38 mmol) in anhydrous THF (160 mL) at 0 °C under argon was added slowly a solution of **3a** and **3b** (11.93 g, 15.67 mmol) in THF (60 mL). The mixture was stirred at 0 °C for 1.5 h, quenched by slowly adding 10% sodium bicarbonate (10 mL), and diluted with ethyl acetate. The usual work-up and a subsequent chromatography (4% ethanol in methylene chloride) gave 4.83 g of the 5'(*R*)-isomer **4a** and 4.61 g of the 5'(*S*)-isomer **4b**, both as a colorless foam. The total yield was 84%. ^{1}H NMR (**4a** in acetone-d_6) δ 1.73 (s, 3 H, 5-Me), 1.68-1.92 (m, 2 H, CH_2CH_2OH), 2.24-2.34 (m, 1 H, H-2'), 2.46-2.56 (m, 1 H, H-2'), 3.23 (m, 2 H, CH_2CH_2OH), 3.32 (m, 1 H, OH), 3.45-3.52 (m, 1 H, H-5'), 3.75, 3.78 (2s, 6 H, 2 x OMe), 3.99 (dd, J = 6.9, 2.7 Hz, 1 H, H-4'), 4.55 (m 1 H, H-3'), 5.04 (m, 2 H, benzyl), 6.23 (t, J = 6.3 Hz, 1 H, H-1'), 6.67 (d, J = 7.5 Hz, 1 H, NHCbz), 6.85 (m, 4 H, DMT), 7.17-7.57 (m, 14 H, benzyl, DMT), 7.96 (s, 1 H, H-6), 10.2 (br, 1 H, NH). ^{1}H NMR (**4b** in acetone-d_6) δ 1.62 (s, 3 H, 5-Me), 1.78-1.99 (m, 2 H, 5'-*C*-CH_2), 2.12-2.21 (m, 1 H, H-2'), 2.28-2.39 (m, 1 H, H-2'), 3.25-3.37 (m, 2 H, H-5', OH), 3.49 (m, 2 H, CH_2OH), 3.75 (s, 6 H, 2 x OMe), 3.99 (dd, J = 6.9, 1.8 Hz, 1 H, H-4'), 4.43 (m 1 H, H-3'), 4.99, 5.11 (AB, J = 12.6 Hz, 2 H, benzyl), 6.13 (t, J = 7.2 Hz, H-1'), 6.71 (d, J = 8.1 Hz, 1 H, NHCbz), 6.82-6.88 (m, 4 H, DMT), 7.15-7.58 (m, 15 H, H-6, benzyl, DMT), 10.1 (br, 1 H, NH). MS (a mixture of **4a** and **4b**): 744 ($M+Na^+$), 720 $(M-H)^-$.

3'-*C*-Amino-3'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-3-*N*,5'(*R*)-*C*-ethylene-thymidine (6). A solution of **4a** (2.85 g, 3.95 mmol), DMAP (443 mg, 3.95 mmol), and *p*-tosyl chloride (2.26 g, 11.85 mmol) in anhydrous pyridine (15 mL) was stirred at rt for 5 h, cooled to 0 °C, quenched with water (1.5 mL), and stirred at rt for 30 min. The mixture was diluted with ethyl acetate, washed successively with brine, 10% acetic acid, brine, and 10% sodium bicarbonate, dried over anhydrous sodium sulfate, and concentrated to dryness to give 3.28 g of the crude **5** as a slightly pink foam.

A mixture of the crude **5** (1.08 g) and 10% Pd/C (240 mg) in 180 mL of ethanol and 3 mL of triethylamine was shaken in a hydrogenation apparatus (hydrogen: 50 psi) at rt for 22 h. The same scale reaction was repeated for another two times. The catalyst was filtered and washed with ethanol. The combined filtrate was concentrated to dryness and the residue was subjected to a chromatography (5% triethylamine and 4% ethanol in methylene chloride) to give **6** (1.51 g, 67%, 2 steps) as a colorless foam; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.27-0.32 (m, 1 H, H-5"), 0.92-0.99 (m, 1 H, H-5"), 1.24 (s, 3 H, 5-Me), 2.18-2.30 (m, 2 H, H-2'), 2.59-2.65 (m, 1 H, NCH_2), 2.85-2.92 (m, 1 H, NCH_2), 3.41 (dd, J = 9.9, 2.0 Hz, 1 H, H-4'), 3.65 (dd, J = 18.7, 10.2 Hz, 1 H, H-3'), 3.79 (s, 6 H, 2 x OMe), 4.52 (s, 1 H, H-5'), 6.24 (m, 1 H, H-1'), 6.82 (m, 4 H, DMT), 7.22-7.56 (m, 9 H, DMT), 7.74 (s, 1 H, H-6); MS: 592 ($M+Na^+$), 568 $(M-H)^-$. Anal. Calcd for $C_{33}H_{35}N_3O_6$: C, 69.58; H, 6.19; N, 7.38. Found: C, 69.20; H, 5.99; N, 7.38.

3'-*O*-(*t*-Butyldimethylsilyl)-5'-*O*-(*H*-methylphosphonyl)thymidine (8). To a solution of **7**[12] (1.07 g, 3.0 mmol) and diisopropylethylamine (2.1 mL, 12.0 mmol) at 0 °C under argon was added *N*,*N*-diisopropylmethylchlorophosphoramidite (1.17 mL, 6.0 mmol). The resulting solution was stirred at rt for 30 min, cooled to 0 °C, then diluted with ethyl acetate, washed with cold 10% sodium bicarbonate, dried over anhydrous sodium sulfate, and concentrated in vacuo at rt to dryness. Chromatography on silica gel (5% triethylamine in ethyl acetate/hexanes, 1:2) gave 1.39 g (89.5%) of an amidite as a colorless foam.

To a solution of the amidite (1.0 g, 1.93 mmol) in acetonitrile (8 mL) was added a 0.45 M 1*H*-tetrazole in acetonitrile/water (9:1, 12.9 mL, 5.8 mmol). The solution stood at rt for 30 min, then cooled to 0 °C, diluted with ethyl acetate, washed successively with cold water, cold 5% sodium bicarbonate, cold water, dried over anhydrous sodium sulfate, concentrated to dryness in vacuo at rt, and dried under vacuum to give 806 mg (96%) of **8** as a colorless foam; ^{1}H NMR (two diastereoisomers in acetone-d_6) δ 0.13 (s, 6 H, TBS), 0.91 (s, 9 H, TBS), 1.83 (d, J = 1.2 Hz, 3 H, 5-Me), 2.19-2.38 (m, 2 H, H-2'), 4.06 (m, 1 H, H-4'), 4.29 (m, 2 H, H-5'), 4.60 (m, 1 H, H-3'), 6.33 (m, 1 H, H-1'), 6.845

(d, J = 702 Hz, 0.5 H, P-H), 6.851 (d, J = 701 Hz, 0.5 H, P-H), 7.58 (m, 1 H, H-6), 10.04 (s, 1 H, NH).

3'-*O*-(*t*-Butyldimethylsilyl)-5'-*O*-[*H*-(2-cyanoethyl)phosphonyl]thymidine (9) as a viscous foam was prepared in 82% yield (2 steps) from **7**[12] and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite by the same procedure as described for **8**. ^{1}H NMR (two diastereoisomers in acetone-d_6) δ 0.133, 0.137 (2 s, 6 H, TBS), 0.91 (s, 9 H, TBS), 1.83 (s, 3 H, 5-Me), 2.19-2.40 (m, 2 H, H-2'), 2.946, 2.954 (2 t, J = 6.0 Hz, 2 H, CH_2CN), 4.08 (m, 1 H, H-4'), 4.34 (m, 4 H, H-5', OCH_2), 4.61 (m, 1 H, H-3'), 6.977 (d, J = 713 Hz, 0.5 H, P-H), 6.985 (d, J = 716 Hz, 0.5 H, P-H), 6.33 (m, 1 H, H-1'), 7.57 (m, 1 H, H-6), 10.04 (s, 1 H, NH).

3'-*O*-(*t*-Butyldimethylsilyl)-5'-*O*-(*p*-nitrophenoxycarbonyl)]thymidine (10). A solution of **7**[12] (1.07 g, 3.0 mmol) and *p*-nitrophenyl chloroformate (726 mg, 3.6 mmol) in a mixture of anhydrous dichloromethane (15 mL) and pyridine (1.5 mL) was stirred at rt overnight, then cooled to 0 °C, and quenched with water. The mixture was stirred at rt for 30 min, diluted with methylene chloride, washed successively with 10% acetic acid, water, 5% sodium bicarbonate, dried over anhydrous sodium sulfate, and concentrated. Chromatography (ethyl acetate/hexanes, 3:2) gave 1.49 g (95%) of **10** as a colorless foam; ^{1}H NMR ($CDCl_3$) δ 0.11 (s, 6 H, TBS), 0.90 (s, 9 H, TBS), 1.91 (d, J = 1.2 Hz, 3 H, 5-Me), 2.17-2.39 (m, 2 H, H-2'), 4.12 (dd, J = 7.5, 3.9 Hz, 1 H, H-4'), 4.41-4.56 (m, 3 H, 3'-H, H-5'), 6.29 (t, J = 6.3 Hz, 1 H, H-1'), 7.33 (d, J = 1.2 Hz, 1 H, H-6), 7.38, 8.31 (2 m, AA'BB', 4 H, np), 8.49 (s, 1 H, NH). MS: 544 ($M+Na^+$), 520 $(M-H)^-$.

Preparation of the dinucleotide phosphoramidite 13. A solution of **6** (276 mg, 0.48 mmol) in a mixture of acetonitrile/dichloromethane/tetrachloromethane (1:1:1, 3 mL) and triethylamine (90 μL) was added to a sealed flask containing **8** (274 mg, 063 mmol). The resulting solution stood at rt for 30 min and concentrated to dryness. Chromatography (4-6% ethanol in methylene chloride) gave 430 mg of the coupling product, which was dissolved in THF (5 mL). An acetic acid-neutralized TBAF (0.9 M, pH 5.5, 1.73 mL) was added, and the resulting solution stood at rt for 24 h, then concentrated to dryness in vacuo. Chromatography (10% ethanol in methylene chloride) gave 283 mg (74%) of **11** (two diastereoisomers) as a colorless foam.

^{1}H NMR (the higher R_f isomer in $CDCl_3$) δ 0.20-0.30 (m, 1 H, H-5''), 0.88-1.03 (m, 1 H, H-5''), 1.24 (s, 3 H, 5-Me), 1.88 (d, J = 1.2 Hz, 3 H, 5-Me), 2.14-2.26 (m, 1 H, H-2'), 2.37-2.50 (m, 2 H, H-2'), 2.61-2.75 (m, 1 H, H-2'), 2.9-3.2 (m, 2 H, NCH_2), 3.48 (m, 1 H,

H-4'), 3.65-3.8 (m, 1 H, H-4'), 3.71, 3.75 (2 s, 3 H, OMe), 3.79 (s, 6 H, 2 x OMe), 4.06 (m, 1 H), 4.21 (m, 2 H), 4.39 (d, J = 4.2 Hz, OH), 4.51 (m, 2 H), 6.18 (d, J = 7.8 Hz, 1 H, H-1'), 6.28 (t, J = 6.3 Hz, 1 H, H-1'), 6.83 (m, 4 H, DMT), 7.20-7.57 (m, 10 H, H-6, DMT), 7.70 (s, 1 H, H-6), 9.46 (s, 1 H, NH), 9.75 (s, 1 H, NH); ^{31}P NMR (acetone-d_6) δ 10.75; MS: 910 ($M+Na^+$), 886 $(M-H)^-$. Anal. Calcd for $C_{22}H_{27}N_3O_8$: C, 59.52; H, 5.68; N, 7.89. Found: C, 59.34; H, 5.50; N, 7.86.

^{1}H NMR (the lower R_f isomer in acetone-d_6) δ 0.12-0.22 (m, 1 H, H-5"), 0.98-1.11 (m, 1 H, H-5"), 1.25 (d, J = 1.0 Hz, 3 H, 5-Me), 1.80 (d, J = 1.2 Hz, 3 H, 5-Me), 2.14-2.31 (m, 2 H, H-2'), 2.47-2.54 (m, 1 H, H-2'), 2.66-3.1 (m, 3 H, H-2', NCH_2), 3.54 (m, J = 9.9, 2.4 Hz, 1 H, H-4'), 3.66, 3.70 (2 s, 3 H, OMe), 3.77. 3.78 (2 s, 6 H, 2 x OMe), 3.89-4.02 (m, 1 H), 4.09 (m, 1 H), 4.20 (m, 2 H), 4.40-4.48 (m, 2 H), 4.68 (br, OH), 6.23 (d, J = 6.9 Hz, 1 H, H-1'), 6.33 (dd, J = 7.8, 6.3 Hz, 1 H, H-1'), 6.87 (m, 4 H, DMT), 7.20-7.61 (m, 9 H, DMT), 7.63 (d, J = 1.5 Hz, 1 H, H-6), 7.70 (d, J = 1.2 Hz, 1 H, H-6), 10.10 (s, 1 H, NH), 10.14 (s, 1 H, NH). ^{31}P NMR (acetone-d_6) δ 11.05.

To a solution of **11** (290 mg, 0.32 mmol) and diisopropylethylamine (227 µL, 1.3 mmol) at 0 °C under argon was added 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (146 µL, 0.65mmol). The resulting solution was stirred at rt for 30 min. After a similar work-up as described for **8**, the residue was subjected to a chromatography (5% triethylamine and 20% acetone in methylene chloride) to give 309 mg (87%) of **13** as a colorless foam; ^{1}H NMR (four diastereoisomers in acetone-d_6) δ 0.12-0.28 (m, 1 H, H-5"), 0.86-1.12 (m, 1 H, H-5"), 1.18, 1.20 (2 s, 12 H, $NiPr_2$), 1.37 (m, 3 H, 5-Me), 1.79 (m, 3 H, 5-Me), 2.24-2.82 (m, 6 H, H-2', CH_2CN), 2.9-3.2 (m, 2 H, NCH_2), 3.52-3.72 (m, 6 H, POMe, OCH_2), 3.77. 3.78 (2 s, 6 H, 2 x OMe), 3.80-4.02 (m, 3 H, 2 NiPr), 4.16-4.3 (m, 3 H), 4.40-4.47 (m, 1 H), 4.58-4.72 (m, 1 H), 6.24 (d, 1 H, H-1'), 6.33 (m, 1 H, H-1'), 6.87 (m, 4 H, DMT), 7.20-7.75 (m, 11 H, DMT, 2 x H-6), 10.1 (s, 2 H, 2 NH); ^{31}P NMR (four diastereoisomers in acetone-d_6) δ 150.27 (2 P), 149.77, 149.65, 10.91, 10.81, 10.67, 10.56; MS 1110 ($M+Na^+$), 1122 ($M+Cl^-$).

Preparation of the dinucleotide phosphoramidite 14. The condensation of **6** with **9** and a subsequent removal of the *t*-butyldimethylsilyl group was conducted according to the same procedure as described above to give **12** (66%, 2 steps) as a colorless foam; MS: 949 ($M+Na^+$), 925 (M-H).

Compound **12** was subjected to the same conversion as **11**. The crude was purified by chromatography (5% triethylamine and 25% acetone in methylene chloride) to give **14** in 45% yield as a colorless foam; ^{1}H NMR (four diastereoisomers in acetone-d_6) δ 0.12-

0.31 (m, 1 H, H-5"), 0.84-1.1 (m, 1 H, H-5"), 1.18, 1.20 (2 s, 12 H, 2 x iPr), 1.38, 1.38, 1.42 (3 d, J = ~1.0 Hz, 3 H, 5-Me), 1.79 (d, J = ~1.0 Hz, 3 H, 5-Me), 2.24-2.9 (m, 4 H, H-2'), 2.9-3.4 (m, 10 H, 2 x CH_2CN, NCH_2), 3.4-4.1 (m, 5 H), 3.77. 3.78 (2 s, 6 H, 2 x OMe), 3.80-4.02 (m, 3 H, $NiPr_2$), 4.16-4.32 (m, 4 H), 4.38-4.46 (m, 1 H), 4.58-4.8 (m, 1 H), 6.17-6.46 (m, 2 H, 2 x H-1'), 6.86 (m, 4 H, DMT), 7.20-7.78 (m, 11 H, DMT, 2 x H-6), 10.1 (br, 2 H, 2 x NH); ^{31}P NMR (four diastereoisomers in acetone-d_6) δ 150.29, 150.27, 149.76, 149.67, 9.59, 9.49, 9.47, 9.37; MS 1149 ($M+Na^+$).

Preparation of the dinucleoside phosphoramidite 16. To a flask containing **6** (285 mg, 0.5 mmol) and DMAP (62 mg, 0.5 mmol) at 10 °C was added a solution of **10** (313 mg, 0.6 mmol) in anhydrous dichloromethane (5 mL). The resulting solution stood at rt for 20 h, then diluted with ethyl acetate, washed successively with 5% acetic acid, water, 10% sodium bicarbonate, dried over anhydrous sodium sulfate, and concentrated to dryness. The residue was dissolved in THF (6 mL) and 1.0 M TBAF in THF (2 mL) was added. The solution stood at rt for 1 h and concentrated to dryness. Chromatography (8% ethanol in methylene chloride) gave 380 mg (90.8%, two steps) of **15** as a colorless foam; ^{1}H NMR (acetone-d_6) δ 0.27-0.37 (m, 1 H, H-5"), 1.04-1.16 (m, 1 H, H-5"-H), 1.46 (d, J = 0.9 Hz, 3 H, 5-Me), 1.88 (d, J = 1.2 Hz, 3 H, 5-Me), 2.14-2.30 (m, 2 H, H-2'), 2.78-2.85 (m, 2 H, H-2'), 3.0-3.3 (m, 2 H, NCH_2), 3.52-3.64 (m, 2 H, 2 x H-4'), 3.76, 3.77 (2 s, 6 H, 2 x OMe), 4.0-4.1 (m, 1 H), 4.26 (d, J = 4.8 Hz, 2 H), 4.41 (m, 2 H), 4.58 (d, J = 4.2 Hz, 1 H), 6.26 (m, 1 H, H-1'), 6.28 (t, J = 6.3 Hz, 1 H, H-1'), 6.86 (m, 4 H, DMT), 7.20-7.65 (m, 10 H, H-6, DMT), 7.76 (s, 1 H, H-6), 10.05 (s, 1 H, NH), 10.10 (s, 1 H, NH); MS: 860 ($M+Na^+$), 836 $(M-H)^-$. Anal. Calcd for $C_{44}H_{47}N_5O_{12}$: C, 63.07; H, 5.65; N, 8.36. Found: C, 62.83; H, 5.53; N, 8.33.

Compound **15** was converted to **16** as a colorless foam in 84% yield by the same procedure as described for **13**. ^{1}H NMR (acetone-d_6) δ 0.27-0.37 (m, 1 H, H-5"), 1.03-1.15 (m, 1 H, H-5"), 1.19 (m, 12 H, $NiPr_2$), 1.47 (m, 3 H, 5-Me), 1.78 (m, 3 H, 5-Me), 2.29-2.50 (m, 2 H, H-2'), 2.78-3.12 (m, 4 H, H-2', CH_2CN), 3.44-3.72 (m, 4 H), 3.77, 3.78 (2 s, 6 H, 2 x OMe), 3.74-3.94 (m, 2 H), 4.09-4.43 (m, 5 H), 4.60 (m, 1 H), 6.27 (m, 2H, H-1'), 6.86 (m, 4 H, DMT), 7.20-7.93 (m, 11 H, DMT, 2 x H-6), 10.05 (s, 2 H, 2 x NH); ^{31}P NMR (two diastereoisomers in acetone-d_6) δ 149.93 149.82; MS 1060 ($M+Na^+$), 1036 $(M-H)^-$.

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