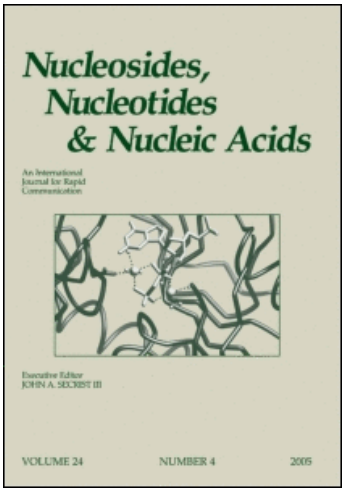

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An Extended Phosphate Linkage: Synthesis, Hybridization and Modeling Studies of Modified Oligonucleotides

Becky Haly[a]; Laurent Bellon[a]; Venkatraman Mohan[a]; Yogesh Sanghvi[a]
[a] Medicinal Chemistry Department, Isis Pharmaceuticals, Carlsbad, CA

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AN EXTENDED PHOSPHATE LINKAGE: SYNTHESIS, HYBRIDIZATION AND MODELING STUDIES OF MODIFIED OLIGONUCLEOTIDES

Becky Haly, Laurent Bellon†, Venkatraman Mohan and Yogesh Sanghvi*

Medicinal Chemistry Department, Isis Pharmaceuticals, 2292 Faraday Avenue
Carlsbad, CA 92008

Abstract: Novel stretched oligonucleotides (**A**-**D**) containing a 3′-α-*C*-methylene phosphodiester bridge (5-atoms long) have been synthesized on an automated synthesizer utilizing phosphoramidite chemistry. The key building-block 1-[3′′-*O*-β-cyanoethyldiisopropylaminophosphiryl-2,3-dideoxy-5-*O*-dimethoxytriphenylmethyl-3-*C*-(hydroxymethyl)-β-D-*erythro*-pentofuranosyl]thymine (**21**) was prepared in a stereoselective manner from thymidine. Hybridization studies indicated a drop (1.8-3.0 °C/mod.) in affinity for the complementary RNA and DNA targets. Molecular modeling results indicated that the 5-atom modified backbone had a different geometry around the phosphodiester linkage compared to the natural phosphodiester linkage. The stretched backbone may not be useful for antisense or triplex constructs, however it may find applications in biochemical/enzyme studies.

INTRODUCTION

Antisense therapeutics is a rapidly growing area of research which is based on a rational drug design approach towards inhibition of gene expression.[1] In principle, short synthetic oligonucleotides can interact in a sequence-specific manner with the messenger-RNA of a disease-related protein, thereby blocking the translation of messenger-RNA into the protein. The antisense strategy, though conceptually simple, presents some major challenges. These challenges include designing and synthesizing nuclease resistant oligonucleotides that can enter cells readily, hybridize to the target messenger-RNA with high affinity and specificity, and support terminating events, such as RNase H mediated cleavage. Among various strategies employed to address these issues, modification of the 4-atom phosphate backbone has been the most successful and widely used.[2]

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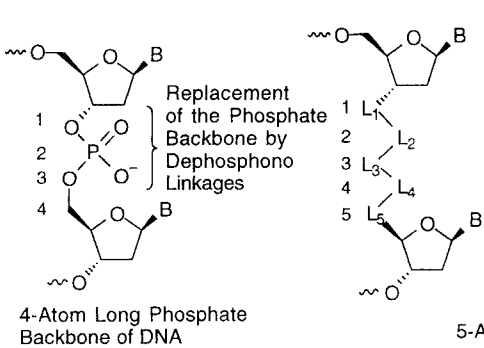
† Present address: Ribozymes Pharmaceuticals Inc., 2950 Wilderness Place, Boulder, CO 80301.



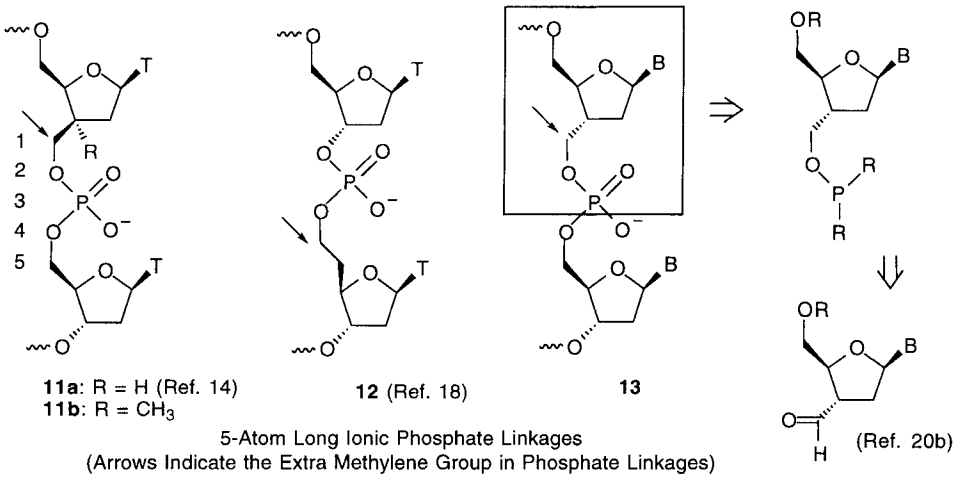
	L_1	L_2	L_3	L_4	L_5	Ref.
1	O	CH_2	CO	O	CH_2	5
2	O	CH_2	CH_2	NH	CO	6
3	O	CH_2	CO	NH	CH_2	7
4	O	$CHCH_3$	CO	NH	CH_2	8
5	O	CH_2	CH_2	NH	CH_2	9
6	NR	CH_2	CH_2	NR	CH_2, CO	10
7		– Piperazine –			CH_2, CO	10
8	NH	CO	CH_2	NH	CO	11
9	CH_2	CH_2	CH_2	NH	CH_2	12
10	CH_2	CH_2	CO	NH	CH_2	12

FIGURE 1

The backbone modifications can be broadly divided into two categories: (i) ionic phosphate modifications, and (ii) non-ionic dephosphono linkages.[3] Additionally, examples of compressed[4] (3-atom linkage) and stretched [5-12] (5-atom linkage) backbones have been reported in both groups. Interestingly, the very first 5-atom dephosphono linker **1** was reported a quarter of a century ago.[5] Since then, several other stretched linkers **2-10** have been synthesized (Figure 1). Some of these modifications, when incorporated into oligonucleotides, show interesting hybridization properties and enhanced stability towards nucleolytic digestion. For example, the amide modification **4** had higher affinity for a complementary RNA target compared to the unmodified oligonucleotide.[8] Recently, Stork *et al.*[12] have studied the effects of 4 vs. 5-atom linkers on duplex stability and pointed out that in certain cases the 5-atom connectors (**9**, **10**) were better than the 4-atom linkers.

On the other hand, 5-atom ionic phosphate linkages have not been explored fully in antisense constructs.[13] In a recent report[14] Wengel *et al.* have synthesized the extended phosphate linkers **11a**, **b**. Incorporation of **11** into oligonucleotides resulted in an overall destabilization (1-3 °C/mod.) of DNA:DNA duplexes. However, 3′-end capping of oligonucleotides with **11** provided extra stability towards degradation by snake venom phosphodiesterase, compared to uncapped oligonucleotides. We noticed that the stereochemistry of this linkage at the 3′-carbon was *threo* (β) and not *erythro* (α), a common feature of other extended backbones.

In view of the interesting properties of the stretched backbones and the ease[15] of their synthesis, we decided to prepare *erythro* (α) linked oligonucleotides (as shown in **13** of Figure 2) and evaluate their potential for antisense applications. Additionally, our NMR and modeling studies[16] have indicated that the 3′-methylene group in a backbone modification



5-Atom Long Ionic Phosphate Linkages
(Arrows Indicate the Extra Methylene Group in Phosphate Linkages)

FIGURE 2

shifts the sugar conformation to a desired 3′-*endo* pucker, thus providing a preferred A-type geometry for duplex formation. Replacement of a 3′-oxygen atom with a methylene group also reduces the ring gauche effects and may enhance the conformational stability.[17] In addition, alterations in the position of the negative charge of the phosphate backbone in oligonucleotides may change the necessary geometrical and conformational parameters required for binding of RNase H and other cellular nucleases. This may improve our understanding of the enzyme to substrate binding and provide insight into designing better antisense molecules. In a recent meeting[18a] Pedersen *et al.* reported the synthesis of oligonucleotides containing stretched backbone linkages **12** and **13** for triplex studies. Herein we report the first synthesis[18b] of a new thymidine phosphoramidite **21** and its incorporation into oligonucleotides. The modified oligonucleotides were characterized by HPLC, CGE and electrospray MS analysis, and studied for their hybridization properties with complementary RNA and DNA. The hybridization results are explained by molecular modeling of the stretched backbone **13**.

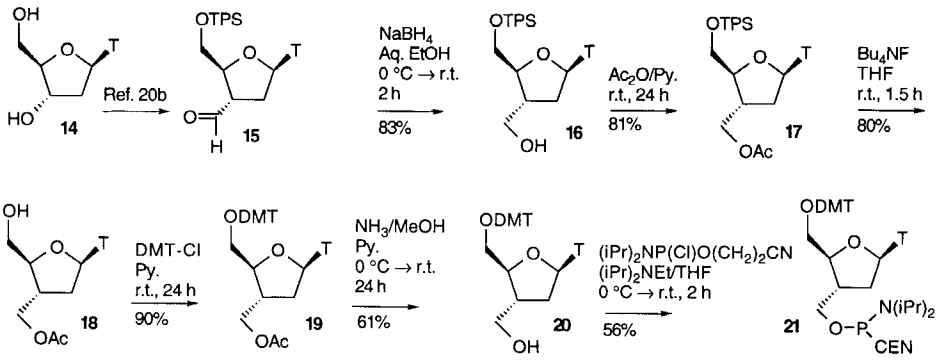
RESULTS AND DISCUSSION

Incorporation of a stretched backbone **13** required an α-*C*-*C* bond forming reaction. There are several reports in the literature[19] describing the synthesis of α-*C*-branched nucleosides, however, we believe that our methodology[20] of introducing an α-*C*-*C* unit onto 2′-deoxynucleosides is much more efficient and stereoselective. The reaction involved

regioselective addition of a *C*-centered radical (*C*-3′) to β-tributylstannylstyrene followed by β-elimination of a $Bu_3Sn^•$ species. The *C*-3′-styryl nucleoside was then transformed to the *C*-3′-formyl nucleoside under oxidative-cleavage conditions. Therefore, synthesis of phosphoramidite building-block **21** was carried out in the following manner (Scheme 1). Thymidine (**14**) was conveniently transformed into 1-[5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-3-*C*-formyl-β-D-*erythro*-pentofuranosyl]thymine (**15**) using the radical reaction.[20] Selection of the *tert*-butyldiphenylsilyl group for protection of the 5′-hydroxyl group was based on the fact that it can be easily removed under mild conditions without affecting the other protecting group. Reduction of **15** using $NaBH_4$ in aq. EtOH afforded the 3′-*C*-hydroxymethyl derivative **16** in 83% yield. Acylation of **16** with Ac_2O in pyridine gave protected **17** (81%). Selective deprotection of **17** with Bu_4NF in THF furnished **18** (80%). Dimethoxytritylation of **18** in a standard manner[21] provided **19** (90%) which on ammonolysis gave **20** (61%). Phosphitylation[21] of **20** using 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite and *N*,*N*-diisopropylethylamine in anhydrous THF furnished the desired phosphoramidite **21** (56%) after purification.

The modified stretched oligonucleotides **A-D** (Table 1) were prepared on a 1 μmole scale following standard phosphoramidite chemistry using a Millipore Expedite DNA synthesizer employing **21** and commercial 2′-deoxynucleoside phosphoramidites. The average yield and coupling cycle times are presented in Table 1. Introduction of an extended wait step (30 sec to 120 sec) during the coupling had little effect on the overall yield. The modified oligonucleotides **A-D** were cleaved off the CPG support by NH_4OH treatment[21] and purified by reverse phase HPLC. Subsequent detritylation and precipitation provided pure oligonucleotides **A-D** in good quantities. The structural identity and purity of oligonucleotides **A-D** was confirmed by electrospray mass spectrometry (ES-MS) and capillary gel electrophoresis (CGE) studies. The consistant mass range (~2 units) between observed and calculated values of the ES-MS provided unambiguous structural proof of the chemically modified oligonucleotides.

The results[22] of the T_m studies with oligomers **A-D** containing a 5-atom bridge at various positions are summarized in Table 2. The study indicated that the modified oligomers **A-D** had a lower affinity for duplex formation with their complementary RNA sequences (average ΔT_m/mod. = –1.8 °C), whereas a greater destabilization effect was observed with complementary DNA (average ΔT_m/mod. = –3.0 °C). Hybridization with uniformly modifed oligonucleotide **D** was found to be more destabilizing possibly due to an overall elongation of the backbone compared to **A-C**. The better affinity of the 3′-stretched oligonucleotides for RNA targets rather than the DNA targets can be attributed to the 3′-endo pucker of the modified sugar residue which favors A-type (RNA: RNA) duplex formation. In addition, the phosphate-phosphate distance[23] in RNA is slightly longer (~ 1



Abbreviations: T = thymine; TPS = *t*-butyldiphenysilyl; DMT = 4, 4'-dimethoxytriphenylmethyl; Py. = pyridine; CEN = $O(CH_2)_2CN$

Scheme 1

TABLE 1. Properties of the Stretched Oligonucleotides (**T** = modified nucleoside)

A: 5′-CTCGTACCTTTCCGGTCC-3′; **B**: 5′-CTCGTACTTTTCCGGTCC-3′
C: 5′-GCGTTTTTTTTTTGCG-3′; **D**: 5′-GCG**TTTTTTTTTT**GCG-3′

Oligo	Coupl Step[1]	Avg. Yield[2]	HPLC R.T.[3]	Purity % HPLC[3]/CGE[4]	Final OD[5]	MS Calculated; Observed[6]
A	30	97.7	19.6	98.2/95.0	65	5391.5; 5389.6
B	120	97.0	20.1	98.3/100	34	5420.5; 5418.5
C	30	95.2	19.8	97.2/98.2	45	4945.2; 4943.3
D	30	91.4	19.7	98.0/92.3	35	5015.2; 5013.4

[1]Wait step in sec.; [2]%Yield calculated by trityl cation monitoring; [3]Column Supelco LC18, 4.6 mm x 15 cm, 5μ, 5% to 18% CH_3CN in TEAA 0.05M, pH 7.0, over 20 min. then isocratic 18%, 1 ml/min.; UV detector 260 nm; [4]12% Non crosslinked polyacrylamide (40 cm total/20 cm effective, 100 μm I.D.), buffer-100 mM Bis-Tris Borate, 7 M urea; [5]Optical density units at 260 nm; [6]See ref. 22 for experimental details.

TABLE 2. Hybridization Data[1] (T_m °C) of the Stretched Oligonucleotides **A-D**

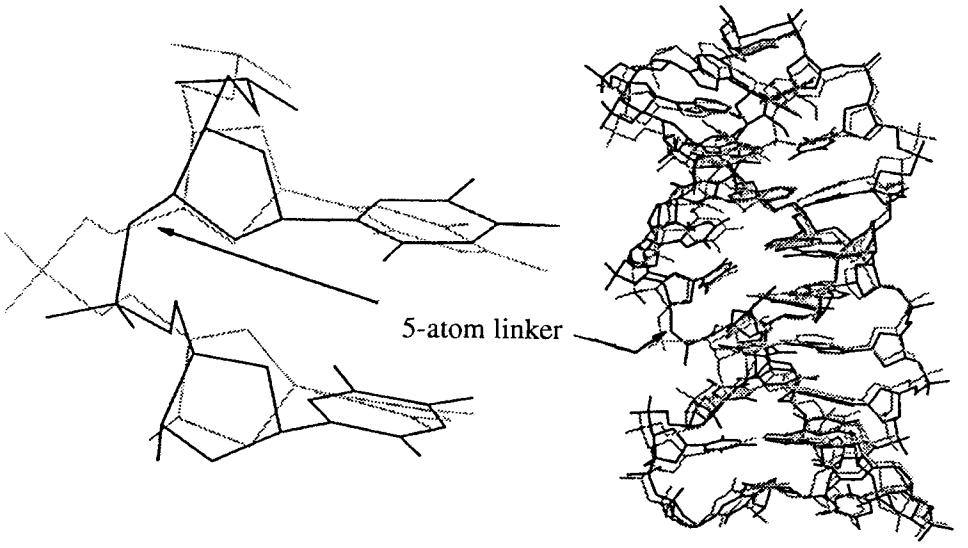
Oligo	Compl. Target	T_m	T_m Wild Type	ΔT_m	No. of Mod.	ΔT_m/mod.	Average ΔT_m/mod.
A	RNA	63.4	65.2	-1.8	1	-1.8	
B	RNA	58.5	61.5	-3.0	2	-1.5	-1.8
C	RNA	39.7	48.5	-8.9	5	-1.7	
D	RNA	26.0	48.5	-22.6	10	-2.2	
C	DNA	39.8	53.5	-13.7	5	-2.7	-3.0
D	DNA	22.0	53.5	-31.5	10	-3.2	

[1] Oligonucleotides were hybridized with complementary RNA or DNA of the same length and absorbance vs. temperature profiles were measured at 4 mM concentration of each strand in 100 mM Na^+, 10 mM phosphate, 0.1 mM EDTA at pH 7.0. See reference 22 for experimental details.

Å) compared to DNA which could account for a better fit for the stretched backbone linkage.

In order to understand and explain the lower binding affinity of the stretched oligonucleotides towards both RNA and DNA targets, we performed energy minimization calculations employing an all atom force field.[24] An unmodified hexamer duplex $(AT)_6$ was constructed in the canonical B-form conformation. Another duplex with a single point modification containing the 5-atom phosphate bridge was also constructed. The modified duplex was obtained by inserting an extra methylene group in the natural phosphodiester linkage. Both of these duplexes were subjected to energy minimization studies using the following protocol. A distance dependent dielectric constant was used to mimic the effect of solvent.[25] In addition, the charges on the phosphate groups were scaled down to account for the absence of counterions in our calculations. A total of 1000 steps of energy minimization was carried out using the conjugate gradient algorithm. Figure 3a shows superimposed energy minimized structures of the portions of duplexes containing unmodified phosphodiester (4-atom linkage) and modified 5-atom phosphate linkages.
We believe that the inability of the 5-atom linked phosphate backbone to assume a geometry similar to the unmodified phosphate linkage may be responsible for the observed lower binding to the target DNA and RNA.

Additionally, we have explored the utility of the extended phosphate linker in the context of triple helix formation. An unmodified decamer triplex ($T_{10}A_{10}T_{10}$) was constructed. Another triplex with a single point modification (at the center of the triplex on the third



3a 3b

FIGURE 3a: Superimposition of a portion of the natural DNA phosphate (bold lines, 3′-O-PO_2-O-CH_2-4′) over extended linkage (thin lines; 3′-CH_2-O-PO_2-O-CH_2-4′). Hydrogen atoms are omitted for clarity. See references 24 and 25 for experimental details. **3b**: Superimposed energy minimized structures of unmodified $T_{10}A_{10}T_{10}$ triplex (thin lines) over modified triplex (bold lines) indicating distortion around the 5-atom linker.

strand) containing the 5-atom phosphate linkage was also constructed. The two triplexes were subjected to energy minimization studies following the protocol described above. In Figure 3b, superimposed energy minimized structures of the triplexes are shown. The fact that the 5-atom linked backbone in the third strand does not have the optimal length was borne out in the rms deviation (1.9 Å) of the two superimposed structures. This may result in a destabilization of the modifed triplex compared to the unmodified triplex. Pedersen *et al.* have reported[6c] similar results with a 5-atom linker **2** (Figure 1).

In summary, a stereoselective synthesis of 3′-*C*-hydroxymethyl derivative **21** and its incorporation into oligonucleotides has been accomplished. These stretched oligonucleotides had lower affinity for single stranded RNA and DNA targets due to differences in backbone geometry. The poor binding affinity of this modification discouraged us from pursuing the RNase H activity and nuclease stability studies with the above oligonucleotides. It is evident that these modifications may not have utility in antisense or triplex constructs. In addition, we have reported[20] the synthesis of 3′-*C*-*C*

bond formation in purine nucleosides, these may be useful precursors for the synthesis of stretched oligonucleotides containing mixed bases. Pedersen *et al.* had shown[6b] that the non-ionic 5-atom linker **2** causes complete blockage of DNA polymerization and was found to be resistant to degradation by exonuclease III despite a small destabilization (1.5-2.5 °C/mod.) in duplex formation. In view of these and other[13] results the 5-atom ionic bridge **13** described herein may find use in biochemical assays and enzyme studies.

Experimental Section

Details of the general experimental procedures have been published elsewhere.[20b] The procedures for ES-MS[26a], CGE[26b] and T_m measurements[22] have been described before.

1-[5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-3-*C*-(hydroxymethyl)-β-D-*erythro*-pentofuranosyl]thymine (16). To a stirred solution of **15**[20b] (4.0 g, 8.12 mmol) in aq. EtOH (8: 2, v/v, 100 mL) was added portion wise $NaBH_4$ (1.08 g, 28.6 mmol) at 0 °C. After addition, the mixture was allowed to warm up to r.t. After 2 h, the reaction mixture was poured into ice-water (150 mL), extracted (EtOAc), dried ($MgSO_4$) and the solvent evaporated. The residue was purified (silica gel, EtOAc/CH_2Cl_2; 2:8-9:1, v/v) to afford **16** (3.33 g, 83%). TLC (R_f 0.52; EtOAc); ^1H NMR ($CDCl_3$): δ 8.88 (br, 1 H, NH), 7.69-7.66 (m, 4 H, Ph), 7.47-7.39 (m, 6 H, Ph), 7.37 (s, 1 H, H6), 6.14 (dd, 1 H, J = 5.7 Hz, J = 6.7 Hz, H1′), 4.02 (dd, 1 H, J = 11.1 Hz, J = 3.0 Hz, H5′), 3.99-3.91 (m, 1 H, H4′), 3.84 (dd, 1H, J = 11.1 Hz, J = 3.0 Hz, H5′′), 3.81-3.64 (m, 2 H, CH_2OH), 2.61-2.59 (m, 1 H, H3′), 2.38-2.29 (m, 1 H, H2′), 2.16 -2.14 (m, 1 H, H2′′), 1.64 (s, 3 H, CH_3), 1.10 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 163.7 (C4), 150.3 (C2), 135.5-135.4, 132.9, 132.4, 130.1 (C6), 127.9, 110.8 (C5), 84.8 (C1′), 83.2 (C4′), 64.9 (CH_2OH), 63.3 (C5′), 41.6 (C3′), 35.5 (C2′), 26.9, 19.3, 12.2. FAB MS m/z 495 (MH^+). Anal. Calcd for $C_{27}H_{34}N_2O_5Si$ (494.662): C, 65.56; H, 6.93; N, 5.66; Si, 5.68. Found: C, 65.06; H 7.01; N, 5.60; Si, 5.82.

1-[3-*C*-(Acetoxymethyl)-5-*O*-(*tert*-butyldiphenylsilyl)-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (17). To a stirred solution of **16** (0.5 g, 1.01 mmol) in pyridine (10 mL) was added Ac_2O (0.20 mL, 2.12 mmol) under an argon atmosphere at r.t. After 24 h, the solution was concentrated, the residue extracted (CH_2Cl_2), washed (H_2O and $NaHCO_3$) and dried ($MgSO_4$). The solvent was evaporated and the residue purified (silica gel, EtOAc/Hexane, 6:4, v/v) to yield **17** (0.44 g, 81%) as a white foam. TLC (R_f 0.80; EtOAc); ^1H NMR ($CDCl_3$): δ 8.93 (br, 1 H, NH), 7.69-7.66 (m, 4 H, Ph), 7.48 (d, 1 H, J = 1.3 Hz, H6), 7.46-7.37 (m, 6 H, Ph), 6.17 (dd, 1 H, J = 5.7 Hz, J = 6.7 Hz, H1′), 4.09-4.05 (m, 3 H, H5′ & CH_2OAc), 3.93-3.91 (m, 1 H,

H5′), 3.79 (dd, 1 H, J = 11.5, J = 3.2 Hz, H5′′), 2.80-2.78 (m, 1 H, H3′), 2.33-2.21 (m, 1 H, H2′), 2.19-2.16 (m, 1 H, H2′′), 2.03 (s, 3 H, $COCH_3$), 1.62 (s, 3 H, CH_3), 1.10 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 170.7 (C=O), 163.7 (C4), 150.3 (C2), 135.5-135.2 (d), 133.0, 132.5, 130.1 (C6), 127.9-127.8 (d), 110.9 (C5), 84.7 (C1′), 82.9 (C4′), 64.4 (CH_2OAc), 64.2 (C5′), 37.3 (C3′), 35.8 (C2′), 26.9, 20.8, 19.4, 12.1. Anal. Calcd for $C_{29}H_{36}N_2O_6Si$ (536.699): C, 64.90; H, 6.76; N, 5.22. Found: C, 64.57; H 6.79; N, 5.12.

1-[3-*C*-(Acetoxymethyl)-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (18). Bu_4NF (1.0 M sol. in THF, 8.8 mL, 8.8 mmol) was added to **17** (2.00 g, 3.73 mmol) in dry THF (36 mL) at r.t. under argon. After 1.5 h, the solvent was removed in vacuo. The residual oil was purified (silica gel, EtOAc/Hexane, 4:6 - 9:1, v/v) to afford **18** (0.88 g, 80%); ^1H NMR ($CDCl_3$): δ 8.89 (br, 1 H, NH), 7.55 (d, 1 H, J = 1.1 Hz, H6), 6.13 (dd, 1 H, J = 4.9 Hz, J = 6.6 Hz, H1′), 4.17 & 4.09 (m, 2 H, CH_2OAc), 4.07 (d, 1 H, J = 11.7 Hz, H5′), 3.94-3.90 (m, 1 H, H4′), 3.76 (d, 1 H, J = 11.7 Hz, H5′′), 2.80-2.76 (m, 1 H, H3′), 2.60 (br, 1 H, OH), 2.29-2.25 (m, 2 H, H2′ & H2′′), 2.09 (s, 3 H, $COCH_3$), 1.91 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): 170.8 (C=O), 163.8 (C4), 150.3 (C2), 136.3 (C6), 110.8 (C5), 85.4 (C1′), 83.5 (C4′), 64.2 (C5′), 36.8 (C3′), 35.6 (C2′), 20.8, 12.5. Anal. Calcd for $C_{13}H_{18}N_2O_6$. 0.3 H_2O (303.70): C, 51.41; H, 6.17; N, 9.22. Found: C, 51.37; H 6.14; N, 9.18.

1-[3-*C*-(Acetoxymethyl)-2,3-dideoxy-5-*O*-dimethoxytriphenylmethyl-β-D-*erythro*-pentofuranosyl]thymine (19). 4, 4′-Dimethoxytrityl chloride (1.14 g, 3.36 mmol) was added to **18** (0.50 g, 1.68 mmol) in pyridine (10 mL) under argon. After 24 h, the reaction was quenched (MeOH, 5 mL) and concentrated. The residue was extracted (CH_2Cl_2), washed ($NaHCO_3$ and brine), dried (Na_2SO_4) and concentrated. The residue was purified (silica gel, CH_2Cl_2/EtOAc/Et_3N 95:5:0.3-80:20:0.3, v/v/v) to give **19** (0.91 g, 90%). ^1H NMR ($CDCl_3$): δ 8.63 (br, 1 H, NH), 7.71 (s, 1 H, H6), 7.41, 7.32-7.25 (2m, 9 H, Tr), 6.82 (m, 4 H, Tr), 6.15 (dd, 1 H, J = 4.7 Hz, J = 6.7 Hz, H1′), 4.06 & 4.01 (m, 2 H, CH_2OAc), 3.99-3.96 (m, 1 H, H4′), 3.79 (s, 6 H, OCH_3), 3.61 (dd, 1 H, J = 3.6 Hz, J = 10.7 Hz, H5′), 3.30 (dd, 1 H, J = 3.6 Hz, J = 10.7 Hz, H5′′), 2.85-2.79 (m, 1 H, H3′), 2.30 - 2.25 (m, 2 H, H2′ & H2′′), 1.93 (s, 3 H, $COCH_3$), 1.44 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): 170.7, 163.6 (C4), 158.7, 150.1 (C2), 144.4, 135.5 (C6), 130.1, 128.2 & 127.9 (d), 127.1, 123.8, 113.2, 110.7 (C5), 86.7, 85.0 (C1′), 82.7 (C4′), 64.3 (C5′), 55.2, 37.3, 36.3, 20.7, 11.8. Anal. Calcd for $C_{34}H_{36}N_2O_8Si$ (628.753): C, 67.99; H, 6.04; N, 4.66. Found: C, 67.85; H 6.11; N, 4.80.

1-[2,3-Dideoxy-5-*O*-dimethoxytriphenylmethyl-3-*C*-(hydroxymethyl)-β-D-*erythro*-pentofuranosyl]thymine (20). To a stirred solution of **19** (0.70 g, 1.17 mmol) in MeOH (5 mL) was added methanolic NH_3 (1.5 mL, sat. at 0 °C) at 0 °C. The

reaction mixture was stirred at r.t. for 24 h, concentrated to dryness, and the residue purified (silica gel, EtOAc/Hexane; 6:4-8:2) to furnish **20** (0.40 g, 61%). ^{1}H NMR ($CDCl_3$): δ 8.68 (br, 1 H, NH), 7.61 (m, 1 H, H6), 7.43, 7.33-7.28 (2m, 9 H, Tr), 6.82 (m, 4 H, Tr), 6.14 (dd, 1 H, J = 4.7 Hz, J = 6.7 Hz, H1′), 3.99-3.97 (m, 1 H, H4′), 3.79 (s, 6 H, OCH_3), 3.64-3.61 (t, 2 H, CH_2OH), 3.51 (dd, 1 H, J = 3.6 Hz, J = 10.5 Hz, H5′), 3.36 (dd, 1 H, J = 3.6 Hz, J = 10.5 Hz, H5′′), 2.62 - 2.60 (m, 1 H, H3′), 2.35-2.31 (m, 1 H, H2′), 2.24-2.20 (m, 1 H, H2′′), 2.12 (m, 1 H, OH), 1.52 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): 163.7 (C4), 158.6, 150.3 (C2), 144.3, 135.7 (C6), 135.4, 130.1, 128.0 & 127.9 (d), 127.1, 113.2, 110.7 (C5), 86.9, 85.1 (C1′), 82.3 (C4′), 64.1 (C5′), 63.1, 55.2, 41.5 (C3′), 32.8 (C2′), 12.0.

1-[3-*O*-β-Cyanoethyldiisopropylaminophosphiryl-2,3-dideoxy-5-*O*-dimethoxytriphenylmethyl-3-*C*-(hydroxymethyl)-β-D-*erythro*-pentofuranosyl]thymine (21). To a stirred solution of *N*, *N*-Diisopropylethylamine (0.50 mL, 2.87 mmol) and **20** (0.51 g, 0.91 mmol) in THF (14 mL) was added 2-cyanoethyl *N*, *N*-diisopropylchlorophosphoramidite (0.50 mL, 2.11 mmole) at 0 °C. After 2 h, the solution was poured into cold saturated $NaHCO_3$ (30 mL), extracted (EtOAc), dried (Na_2SO_4) and concentrated. The residue was purified (silica gel, CH_2Cl_2:MeOH:Et_3N, 98:1:1, v/v/v) to yield **21** (0.39 g, 56%). ^{1}H NMR ($CDCl_3$): δ 8.90 (br, 1 H, NH), 7.49-7.42, 7.43-7.28 (2m, 14 H, Tr and H6), 6.10 (dd, 1 H, J = 5.1 Hz, J = 6.7 Hz, H1′), 4.00 (m, 1 H, H4′), 3.75 (s, 6 H, OCH_3), 3.71-3.60 (m, 2 H, CH_2OP), 3.57-3.50 (m, 2 H, $CNCH_2CH_2O$), 3.39-3.35 (m, 1 H, H5′), 3.37-3.33 (m, 1 H, H5′′), 2.64-2.53 (m, 3 H, H3′ & $CNCH_2CH_2O$), 2.30-2.14 (m, 2 H, H2′ & H2′′), 1.91 (s, 3 H, CH_3), 1.16-1.08 (m, 12 H, $N(i\text{-}Pr)_2$); ^{31}P NMR ($CDCl_3$): δ 148.1, 147.9 (d).

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