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Synthesis and Structure of Dinucleotides with S-Type Sugar Puckering and Noncanonical ε and ζ Torsion Angle Combination (ν_2,ε,ζ-D-CNA)

Christelle Dupouy,[a] Pierre Lavedan,[b] and Jean-Marc Escudier*[a]

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The synthesis of the diastereoisomers of ν_2,ε,ζ-D-CNA dinucleotide building units of nucleic acids and their (2′,5′)-ν_2,ε′,ζ′-D-CNA analogues, in which the ε and ζ torsional angles are stereocontrolled by a dioxaphosphorinane ring structure (D-CNA family) is described from a common 3-deoxy-3-(hydroxymethyl)-D-allofuranose intermediate. NMR spectroscopic and circular dichroism structure analysis of ν_2,ε,ζ-D-CNA shows that the sugar puckering is fixed in the C-2′-*endo* conformation and that these D-CNA structural elements allow stabilization of the ε/ζ torsion angle combinations, which are significantly different from the typical combinations observed in canonical A- or B-form duplexes.

Introduction

The synthesis and evaluation of oligonucleotide analogues for the construction of tools in DNA diagnostics, in therapy, and in bio- or nanotechnology have recently been the topics of intense research interest.[1–3] As part of these analogues, conformationally constrained nucleosides such as LNA or di- and tricyclo-DNA are a useful class of analogues.[4–8] They were especially designed to mimic the conformation adopted by the sugar moiety of nucleotides in duplexes in order to enhance, once incorporated in a single-stranded DNA or RNA, the duplex formation ability.[9] In contrast, mimicking important biologically relevant nonhelical secondary structures of functional DNA or RNA by designing conformationally restricted nucleotides has received less attention.[10,11]

Together with the double-helix structure, nucleic acids can adopt alternative conformations such as bulges, hairpins, branched junctions, or U-turns.[12] These secondary structures are often characterized by non-Watson–Crick pairs or unpaired nucleotides and remarkable sugar–phosphate backbone distortions with torsion-angle conformations that are significantly different from those observed in regular double-stranded helices.

Sequence-specific protein–DNA recognition processes very often require fine adjustment of the sugar–phosphate backbone conformation to form stable duplexes. In some cases, the α and γ torsion angles (Figure 1) can exhibit noncanonical conformations with *gauche*(+) or *trans* and *gauche*(–) or *trans* values, respectively, in protein-bound B-DNA oligomers.[13] In contrast, it was shown that ε and ζ conformations need to be shifted from the canonical BI state to the BII state to achieve the helical changes that are necessary so that DNA can properly interact with the proteins.[14] Interestingly, many biologically relevant nucleic acid structures exhibit more unusual conformations of the α to ζ torsional angles that participate in structural distortions specifically involved in particular macromolecular processes.[15] Unfortunately, experimental studies aimed at determining the structural and functional implications of such helical deformations are somewhat complicated by the intrinsically transient nature of the corresponding backbone states. Stable structural analogues of these distorted backbone geometries would be very useful in the elucidation of the role that helical deformations play in nucleic acid interactions.

We are interested in the development of conformationally constrained dinucleotide building units, in which the backbone α to ζ torsion angles of nucleic acids can have predefined values that closely match, or on the contrary significantly deviate from, the typical values observed in DNA and RNA duplexes. Our general strategy used to lock the phosphate backbone torsional angles is based on the introduction of the 1,3,2-dioxaphosphorinane structure at key positions along the phosphate backbone (D-CNA family).

We previously reported the synthesis of α,β-D-CNA (dioxaphosphorinane-constrained nucleic acid) featuring canonical or noncanonical values of the α and β torsional angles,[16,17] and subsequently, on their properties within

[a] Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique, UMR 5068 CNRS, Université Paul Sabatier, 31062 Toulouse Cedex 9, France
E-mail: escudier@chimie.ups-tlse.fr
[b] Service Commun de RMN, Université Paul Sabatier, 31062 Toulouse Cedex 9, France

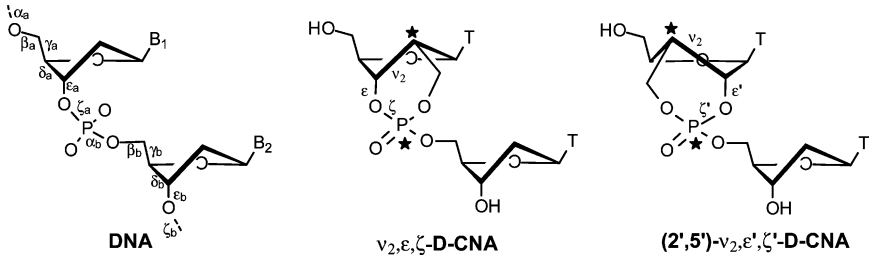


Figure 1. Left: the six backbone torsion angles (labeled α_a to ζ_a for the upper nucleotide and α_b to ζ_b for the lower nucleotide) of nucleic acids; middle: ν_2,ε,ζ-D-CNA dinucleotide, in which ν_{2a}, ε_a, and ζ_a are stereocontrolled to canonical or noncanonical values by a dioxaphosphorinane ring structure exhibiting two new asymmetric centers; right: (2′,5′)-ν_2,ε′,ζ′-D-CNA dinucleotide, in which ν_{2a}, ε′, and ζ′ are stereocontrolled by a dioxaphosphorinane ring structure exhibiting an asymmetric phosphorus center.

oligonucleotides.[18,19] The methodology was then extended to enlarge the α,β-D-CNA family[20,21] and to prepare α,β,γ-D-CNA, δ,ε,ζ-D-CNA, or *xylo*-ε,ζ-D-CNA.[22,23] The ε/ζ combinations reached to date were in most cases noncanonical forms of the B-type duplex, which were excepted with one isomer of δ,ε,ζ-D-CNA featuring a BII-like conformation with ε and ζ in a {*gauche*(–)/*anticlinal*(+),*trans*} conformation. In most of these constrained dinucleotides, the sugar puckering was not altered and the 2′-deoxyribose units were in a C-2′-*endo* conformation.

To complete the ε,ζ combination set and therefore to dispose of new members of the D-CNA family, we fused the dioxaphosphorinane ring to the sugar ring by connecting an oxygen atom of the phosphate group to the 3′- or 2′-C with a methylene bridge. In these new D-CNAs, termed ν_2,ε,ζ-D-CNA or (2′,5′)-ν_2,ε′,ζ′-D-CNA, the inner sugar ν_2 torsional angle of the sugar is constrained together with ε and ζ or newly defined ε′ and ζ′, respectively. In the present work, we report the synthesis, from 3-deoxy-3-(hydroxymethyl)-D-allofuranose, and conformational behavior of the ($R_{C3'}$,S_P) and ($R_{C3'}$,R_P) isomers of (2′,5′)-ν_2,ε′,ζ′-D-CNA and the ($R_{C2'}$,R_P) and ($R_{C2'}$,S_P) isomers of ν_2,ε,ζ-D-CNA, which are two novel classes of constrained dinucleotide building units.

Results and Discussion

Our retrosynthetic analysis for the preparation of the two diastereoisomers of the ν_2,ε,ζ-D-CNA unit is described in Figure 2 (Path A). The dioxaphosphorinane structure will be formed by the cyclization process already reported for the synthesis of other D-CNA, that is, ring closure reaction of a 2′-C-substituted dinucleotide precursor in which the pro-(*R*) and pro-(*S*)-phosphate oxyanions can attack an electrophilic carbon atom.[16] The acyclic dinucleotide precursor can be prepared from protected 2-deoxy-2-hydroxymethyl-D-ribofuranosyl nucleoside **II** by standard phosphoramidite procedures.[24] The base will be introduced by Vorbrüggen's reaction[25] on protected 2-deoxy-2-hydroxymethyl-D-ribofuranose **I**.[26] By following the procedure described by Marquez and Driscoll, this substituted ribofuranose will be synthesized by oxidative cleavage of the C-1/C-2 bond of 3-deoxy-3-(hydroxymethyl)-D-allofuranose

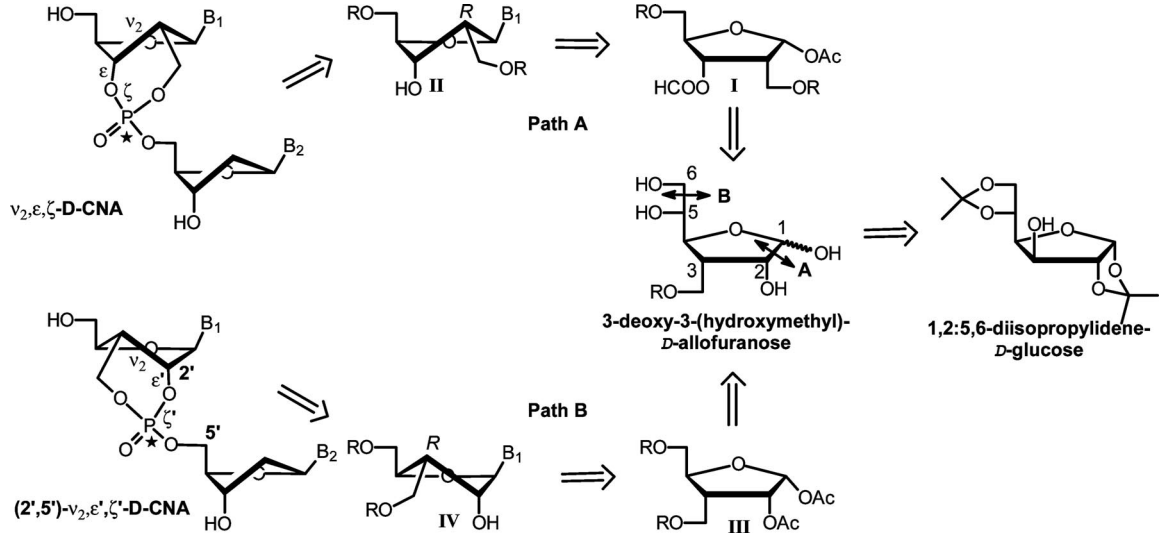
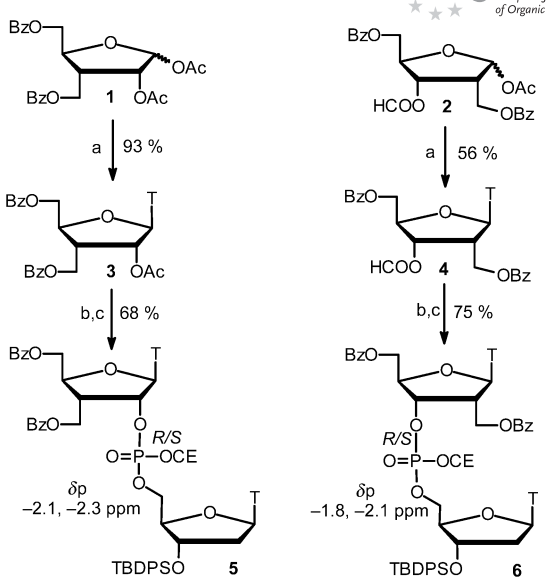
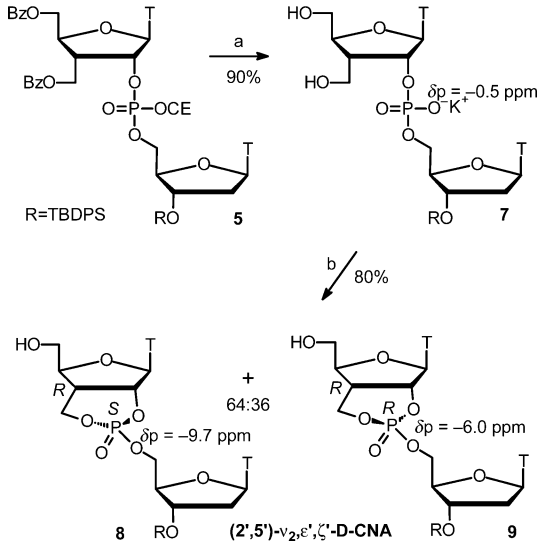


Figure 2. Retrosynthetic pathway for the synthesis of ν_2,ε,ζ-D-CNA dimers and their (2′-5′)-ν_2,ε′,ζ′-D-CNA analogues from common intermediate 3-deoxy-3-(hydroxymethyl)-D-allofuranose.

prepared from commercially available 1,2:5,6-diisopropylidene-D-glucose. We chose to use 3-deoxy-3-(hydroxymethyl)-D-allofuranose as the precursor instead of the more obvious 3-deoxy-3-(hydroxymethyl)-D-ribofuranose[27] or 2′-C-functionalized nucleosides[28,29] because it can undergo additional oxidative cleavage of the C-5/C-6 bond (Figure 2, Path B) to provide protected 3-deoxy-3-hydroxymethyl-D-ribofuranose **III**. Compound **III** is the starting material for the synthesis of the (2′,5′)-ν_2,ε′,ζ′-D-CNA analogues of ν_2,ε,ζ-D-CNA. Protected 3-deoxy-3-hydroxymethyl-D-ribofuranosyl nucleoside **IV** will be coupled to a 5′-*O*-phosphoramidite nucleotide to provide the acyclic precursor that will be cyclized to form the dioxaphosphorinane structure. In (2′,5′)-ν_2,ε′,ζ′-D-CNA, we introduced the new torsional angles ε′ and ζ′ defined as follows: ε′ (P–O2′–C2′–C3′) and ζ′ (O5′–P–O2′–C2′).



Scheme 1. Reagents and conditions: (a) TMSOTf, bis(trimethylsilyl)thymine, CH_3CN, reflux, 2 h; (b) concentrated NH_4OH, room temp., 2 h; (c) 3′-*O*-*tert*-butyldiphenylsilylthymidine-5′-*O*- phosphoramidite (2 equiv.), tetrazole (10 equiv.), CH_3CN then collidine, I_2/H_2O, room temp., 3 h.



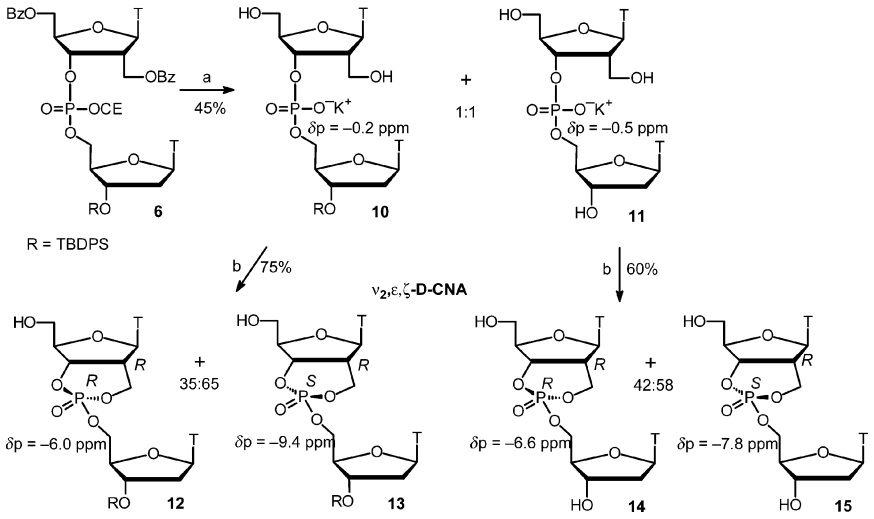
Scheme 2. Reagents and conditions: (a) K_2CO_3, $MeOH/H_2O$ (4:1), room temp., 6 h; (b) 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT), pyridine, room temp. for 1 h, then 80 °C for 30 min, and 90 °C for 30 min.

Synthesis of ν_2,ε,ζ-D-CNA and (2′,5′)-ν_2,ε′,ζ′-D-CNA Dinucleotides

Starting 2′- or 3′-C-hydroxymethyl-substituted ribofuranose **1** and **2** were obtained after oxidative cleavage of the suitably protected 3-deoxy-3-(hydroxymethyl)-D-allofuranose as described by Marquez and Driscoll.[26] Introduction of the thymine base by the Vorbrüggen procedure proceeded in high yield with a high selectivity in favor of the β-anomer, as expected for 2-*O*-acetylribose **1** (Scheme 1). In contrast, a lower yield (56%) and a lower selectivity (β/α = 9:1 as determined by ^{1}H NMR spectroscopy) was obtained from 3-*O*-formylribose **2**. This could be explained by the fact that the 2′-benzoyloxymethyl group is less efficient in the stabilization of the intermediate carbocation, which therefore allows a small level of undesirable α-face attack. The 2′- or 3′-hydroxy functionalities were released by treatment of nucleosides **3** or **4** with ammonia. The resulting nucleosides were then coupled with the readily available 3′-*O*-*tert*-butyldiphenylsilyl-5′-*O*-phosphoramidite thymidine according to a standard phosphoramidite procedure[24] to give dinucleotides **5** and **6** in each case as a diastereoisomeric mixture (1:1 ratio as determined by ^{31}P NMR spectroscopy: δ_P = –2.1 and –2.3 ppm for **5**; δ_P = –1.8 and –2.1 ppm for **6**).

We applied the well-known phosphotriester methodology in the presence of 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) as an activating agent[30] to prepare the ($R_{C3'}$, S_P) and ($R_{C3'}$, R_P) (2′,5′)-ν_2,ε′,ζ′-D-CNA diastereoisomers **8** and **9**, respectively, in good yield (80%). They were obtained as a 1.8:1 mixture of diastereoisomers as determined by ^{31}P NMR spectroscopy: δ_P = –9.7 and –6.0 ppm. In this particular case, the diastereoselectivity observed was certainly due to a more favorable axial position of the lower nucleoside in the transition state that favored the ($R_{C3'}$,S_P)-configured dioxaphosphorinane diastereoisomer. Acyclic phosphodiester **7** was generated in high yield from **5** by removal of the benzoyl and cyanoethyl groups with K_2CO_3 (90%, Scheme 2).

Surprisingly, when applied to the synthesis of ν_2,ε,ζ-D-CNA, the removal of the benzoyl and cyanoethyl protecting groups only proceeded in moderate yield (45%), and we noticed concomitant cleavage of the *tert*-butyldiphenylsilyl protecting group that provided acyclic dinucleotides **10** and **11** in a 1:1 ratio (Scheme 3). Nevertheless, when treated with 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole, **10** underwent diastereoselective cyclization to provide 3′-*O*-protected ν_2,ε,ζ-D-CNA **12** and **13** in a 1:1.8 ratio (^{31}P NMR: δ_P = –6.0 and –9.4 ppm, respectively) in 75% yield. This result is highly comparable with that obtained for the



Scheme 3. Reagents and conditions: (a) K_2CO_3, MeOH/H_2O (4:1), room temp., 6 h; (b) 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT), pyridine, room temp. for 1 h, then 80 °C for 30 min, and 90 °C for 30 min.

formation of (2′,5′)-ν$_2$,ε′,ζ′-D-CNA. In contrast, the dioxaphosphorinane rings were formed with a lower diastereoselectivity from fully deprotected dinucleotide **11** to give ν$_2$,ε,ζ-D-CNA **14** and **15** in a 1:1.4 ratio (^{31}P NMR: δ_P = –6.6 and –7.8 ppm, respectively) in 60% yield.

Structural Assignment of the (2′,5′)-ν$_2$,ε′,ζ′-D-CNA TT Dimers

To obtain insight into the structural behavior of each constrained dinucleotide, ^{1}H, ^{1}H–{^{31}P}, 2D COSY ^{1}H–^{1}H, 2D COSY ^{1}H–^{31}P, and 2D NOESY NMR spectra were recorded at 300 and 500 MHz in deuterated chloroform for ($R_{C3'}$,S_P) (2′,5′)-ν$_2$,ε′,ζ′-D-CNA **8** and ($R_{C3'}$,R_P) (2′,5′)-ν$_2$,ε′,ζ′-D-CNA **9**.

The puckering of the 2′- and 3′-deoxyribose moieties of **8** and **9** was assigned by examination of the sugar ring H,H coupling constants (Table 1). The C3′-*endo* conformation of the upper nucleosides was clearly established with an undetectable coupling constant between 1′-H and 2′-H and a large coupling constant between 3′-H and 4′-H. For the lower sugar, the relatively small $J_{H3',H4'}$ measured for the lower nucleosides and the values of $J_{H2',H3'}$ and $J_{H1',H2'}$ are close in each case to those found in the standard C2′-*endo* conformation of the natural 2′-deoxyribose.[31,32] Therefore, as a consequence of the introduction of the dioxaphosphorinane ring, these dinucleotides display a constrained North/South junction.

Table 1. H,H coupling constants in the ^{1}H NMR spectra (500 MHz, $CDCl_3$) of ($R_{C3'}$,S_P) and ($R_{C3'}$,R_P) (2′,5′)-ν$_2$,ε′,ζ′-D-CNA diastereoisomers (n.d. = not determined).

Nucleoside		Coupling constant ^3J [Hz]				
		$J_{1',2'}$		$J_{2',3'}$		$J_{3',4'}$
8	upper	0	–	4.5	–	11.0
	lower	7.0	7.0	5.5	4.0	n.d.
9	upper	0	–	6.0	–	9.7
	lower	8.0	5.5	2.5	n.d.	2.5

The chair conformation of the dioxaphosphorinane structure of **8** is clearly established from the ^{1}H NMR spectra, and small ($^3J_{H6',P} < 1$ Hz) and large ($^3J_{H6',P} \approx 22$ Hz) $^3J_{H,P}$ coupling constants between the 6′a-H and 6"a-H protons and phosphorus are observed, which is characteristic of an axial position ($0 \leq {}^3J_{Hax,P} \leq 3$ Hz) and an equatorial position ($20 \leq {}^3J_{Heq,P} \leq 30$ Hz) of these protons, respectively (Table 2).[33] The undetectable coupling constant between the 2′a-H proton and the phosphorus atom also provided evidence for the axial position of this proton and for the chair conformation of the dioxaphosphorinane ring.

Table 2. H,P coupling constants in the ^{1}H NMR spectra (500 MHz, $CDCl_3$) of ($R_{C3'}$,S_P) and ($R_{C3'}$,R_P) (2′,5′)-ν$_2$,ε′,ζ′-D-CNA diastereoisomers.

Dinucleotide	Coupling constant J [Hz]				
	$^3J_{2'a,P}$	$^4J_{4'b,P}$	$^3J_{5'b,P}$		$^3J_{6'a,P}$
8	0	<1	8.0; 3.0	22.0	<1
9	4.3	0	6.7; 2.5	20.3	5.6

In contrast, average values of 4.3, 5.6, and 20.3 Hz were observed for the $^3J_{H,P}$ coupling constants involving the 2′a-H, 6′a-H, and 6′′a-H protons, respectively of ($R_{C3'}$,R_P) (2′,5′)-ν$_2$,ε′,ζ′-D-CNA **9**, which thus suggests that the dioxaphosphorinane structure of this isomer is in a twist-chair conformation. In both diastereoisomers, there is no observable coupling constant between 4′b-H and phosphorus; this is indicative of the fact that these compounds do not adopt the canonical *gauche*(+) γ conformation frequently observed in nucleotides.[31,32] Thus, on the basis of careful examination of the $^3J_{H,H}$ and $^3J_{H,P}$ coupling constants for the P-coupled CH and CH_2 signals of **8** and **9**, we were able to establish conclusively the conformation of the backbone torsional angles ε′ (P–O2′–C2′–C3′) and ζ′ (O5′–P–O2′–C2′)as(c^+,g^+/a^+)and(c^-,a^-)forthe($R_{C3'}$,S_P)and ($R_{C3'}$, R_P) diastereoisomers, respectively.

Structural Assignment of the ν_2,ε,ζ-D-CNA TT Dimers

CD spectra were recorded in phosphate buffer (pH 7.0) at 20, 40, 60, and 80 °C and compared with those of an unmodified T_PT dinucleotide (Figure 3) in order to have information about the behavior of the two thymine moieties in ν_2,ε,ζ-D-CNA **14** and **15**.

The most striking features are the low intensity and the very little temperature dependence of the positive Cotton effect observed around 270 nm for ν_2,ε,ζ-D-CNA **14** and **15** relative to that of the unstrained T_PT. These results can be undoubtedly explained in terms of the relative conformational rigidity of the dinucleotide structure, which does not allow base stacking in this particular conformation of the ε and ζ torsion angles. The CD spectra of these dinucleotides are therefore highly comparable to the CD spectrum of the corresponding monomer.[34] Surprisingly, the minimum intensity of the positive Cotton effect ($\Delta\varepsilon$ = 0.66 $M^{-1}cm^{-1}$) was observed at 20 °C for ($R_{C2'}$,R_P) ν_2,ε,ζ-D-CNA **14**, whereas $\Delta\varepsilon$ increased when the temperature reached 80 °C ($\Delta\varepsilon$ = 0.94 $M^{-1}cm^{-1}$); this was accompanied by a shift in the absorption maximum ($\Delta\lambda$ = 7 nm). This result suggests that the dioxaphosphorinane structure could be affected by increasing temperature and, therefore, should not be in a rigid chair conformation.

To determine the relative spatial arrangement of the two nucleotides, ^1H, ^1H–{^{31}P}, 2D COSY ^1H–^1H, 2D COSY ^1H–^{31}P, and 2D NOESY NMR spectra were recorded at 300 and 500 MHz in deuterated chloroform for 3′-*O*-protected ν_2,ε,ζ-D-CNA **12** and **13** and in deuterated methanol for ν_2,ε,ζ-D-CNA **14** and **15**.

The puckering of the 2′-deoxyribose moieties in **12**, **13**, **14**, and **15** was assigned by examination of the sugar ring H,H coupling constants (Table 3). In all cases, according to the approximation of Altona and Sundaralingam: South (%) = $[^3J_{H1',H2'}/(^3J_{H1',H2'} + {}^3J_{H3',H4'})] \times 100$.[35] The sugar puckering of the upper nucleoside of ν_2,ε,ζ-D-CNA was determined to be in a constrained South (C2′-*endo*) conformation (100%). With smaller $^3J_{H1',H2'}$ and larger $^3J_{H3',H4'}$ and $^3J_{H2',H3'}$ values, the lower sugar moieties of ν_2,ε,ζ-D-CNA exhibit coupling constants close to those found for the unmodified TT dimer (63% of C2′-endo conformation for the lower thymidine).[31]

Table 3. H,H coupling constants in the ^1H NMR spectra (500 MHz) of ($R_{C2'}$,R_P) and ($R_{C2'}$,S_P) ν_2,ε,ζ-D-CNA diastereoisomers (**12** and **13** in $CDCl_3$, **14** and **15** in CD_3OD, n.d. = not determined).

Nucleoside		Coupling constant ^3J [Hz]				
		$J_{1',2'}$		$J_{2',3'}$		$J_{3',4'}$
12	upper	9.0	–	4.5	–	0
	lower	8.0	6.0	6.0	2.0	<1
13	upper	9.5	–	4.5	–	<1
	lower	6.5	6.5	6.5	7.0	n.d.
14	upper	9.0	–	5.0	–	<1
	lower	7.0	7.0	6.5	6.5	3.6
15	upper	9.5	–	4.5	–	<1
	lower	7.0	7.0	6.5	6.5	3.3

The chair conformation of the dioxaphosphorinane structure of 3′-*O*-protected ($R_{C2'}$,S_P) ν_2,ε,ζ-D-CNA **13** and ($R_{C2'}$,S_P) ν_2,ε,ζ-D-CNA **15** is clearly established from ^1H and ^1H–{^{31}P} NMR spectra (Table 4), and no detectable coupling constant between the 3′a-H involved in the dioxaphosphorinane system and phosphorus ($^3J_{H3'a,P} \approx 0$) was observed, which is characteristic of an axial position of this proton.[33] The observation of small (<1 Hz) and large (>24 Hz) $^3J_{H,P}$ coupling constants between the dioxaphosphorinane 6′a-H protons and the phosphorus atom are also indicative of an axial and an equatorial position of these protons, respectively.

Table 4. H,P coupling constants in the ^1H NMR spectra (500 MHz) of ($R_{C2'}$,R_P) and ($R_{C2'}$,S_P) ν_2,ε,ζ-D-CNA diastereoisomers (**12** and **13** in $CDCl_3$, **14** and **15** in CD_3OD, n.d. = not determined).

Dinucleotide	Coupling constant J [Hz]				
	$^3J_{3'a,P}$	$^4J_{4'b,P}$	$^3J_{5'b,P}$		$^3J_{6'a,P}$
12	<1	<1	5.5; 3.5	20.0	2.4
13	<1	<1	6.0; <1	26.0	<1
14	2.5	2.2	n.d.	20.1	6.9
15	0	0	7.7; 3.3	24.0	1.5

The analysis of minor isomer ($R_{C2'}$,R_P) ν_2,ε,ζ-D-CNA **14** was complicated by the overlapping of the 6′a-H, 3′b-H, and 5′b-H signals (Figure 4). Whereas the $^3J_{H6''a,P}$ coupling constants were readily accessible (6.9 Hz) from the ^1H and ^1H–{^{31}P} NMR spectra, the $^3J_{H6'a,P}$ was not. We recorded a ^1H COSY spectrum performed with a selective irradiation

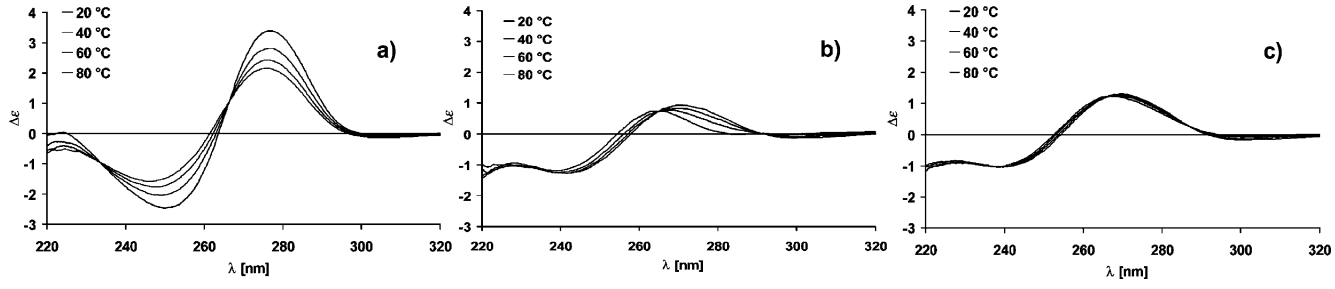


Figure 3. CD spectra in 10 mM sodium phosphate (pH 7.0) at 80, 60, 40, and 20 °C of: (a) TpT; (b) ($R_{C2'}$,R_P) ν_2,ε,ζ-D-CNA TT **14**; and (c) ($R_{C2'}$,S_P) ν_2,ε,ζ-D-CNA TT **15**.

at δ = 2.89 ppm corresponding to saturation of the 2′a-H signal. The resulting pattern obtained around 4.46 ppm only exhibited a signal from the 6′a-H proton, which has an effective scalar coupling with 2′a-H. We were, therefore, able to determine the value of $^3J_{H6'a,P}$ (20.1 Hz), together with values of $^3J_{H6'a,H2'a}$ and $^3J_{H6'a,H6"a}$ (2.1 and 12.2 Hz, respectively).

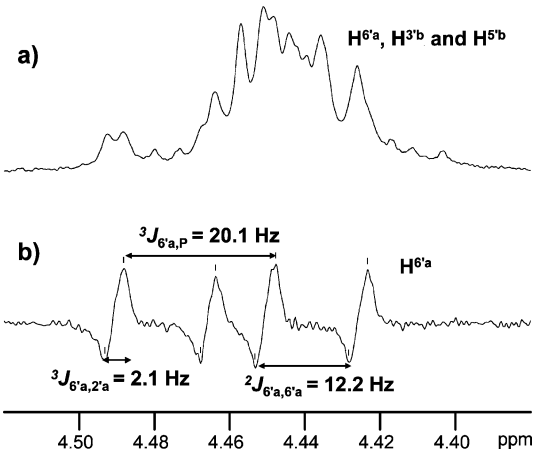


Figure 4. NMR spectroscopic data for 6′b-H of ($R_{C2'}$,R_P) ν_2,ε,ζ-D-CNA TT **14**: (a) ^{1}H NMR and (b) ^{1}H COSY with selective irradiation at δ = 2.89 ppm.

The average values of 2.4, 6.9, 20.0, and 20.1 Hz observed for the $^3J_{H,P}$ coupling constants involving the 6′a-H and 6"a-H protons of **12** and **14**, respectively, suggest that the dioxaphosphorinane structure of these ($R_{C2'}$,R_P)-configured isomers are in a twist-chair conformation. This "in-between" conformation probably results from the involvement of conflicting steric and anomeric effects in the chair conformations of **12** and **14** as a result of the preferred C2′-*endo* puckering of the sugar of the upper nucleoside and the equatorial positioning of the dioxaphosphorinane ring in the lower nucleoside substituent. Interestingly, only isomer **14** exhibits a $^4J_{H4'b,P}$ (2.2 Hz) frequently observed in nucleotides as a consequence of the canonical *gauche*(+) γ conformation.[32]

The overall conformations of dinucleotides ($R_{C2'}$,R_P) ν_2,ε,ζ-D-CNA **14** and ($R_{C2'}$,S_P) ν_2,ε,ζ-D-CNA **15** were further investigated by examination of the 2D NOESY NMR spectra and their comparison with an unmodified dinucleotide (Figure 5). Although many cross peaks are present for **14** and **15**, all of them are derived from intraresidual H,H interactions that are indicative of the sugar puckering (South-type) and the relative position of the thymine bases towards theirs corresponding 2′-deoxyribose rings.

All together, the data collected from NMR spectroscopy helped us to propose the conformational ranges of the δ–ζ torsional angles for each ν_2,ε,ζ-D-CNA diastereoisomer (Table 5). Those of a dinucleotide involved in a natural A-, BI-, or BII-type duplex are given as reference.[37]

Table 5. Summary of the backbone δ, ε, and ζ torsion angles derived from the canonical A-DNA duplex structure and that of ν_2-ε,ζ-D-CNA dimeric units **14** and **15**.[a]

Structure	δ	ε	ζ	ε–ζ
A-type	g^+/a^+	$^-$/t	g^-	<0
B_I-type	a^+	t	g^-/a^-	0
B_{II}-type	a^+/t	g^-/a^-	t	>0
($R_{C2'}$,R_P)-**14**	a^+/t	a^+/t	a^-	<0
($R_{C2'}$,S_P)-**15**	a^+/t	a^+/t	g^+	0

[a] The following sixfold staggered pattern of the torsional angles is used: *cis* = 0 ± 30° (c), *gauche*(+) = 60 ± 30° (g^+), *anticlinal*(+) = 120 ± 30° (a^+), *trans* = 180 ± 30° (t), *anticlinal*(–) = 240 ± 30° (a^-), *gauche*(–) = 300 ± 30° (g^-). The notation a^-/t is used to designate a torsion angle on the border of *anticlinal*(–) and *trans*.

When compared with the backbone conformations summarized in Table 5, we can expect that DNA oligonucleotides incorporating the ($R_{C2'}$, R_P) dinucleotide element would help to better understand the relative contribution of a fixed C2′-*endo* sugar puckering with respect to a slightly distorted B_I-like substrate (ε–ζ < 0°, centered around –90°). In contrast, stereoisomer ($R_{C2'}$,S_P)-**15** exhibits a more unusual δ/ε/ζ combination [*anticlinal*(+)/*trans*, *anticlinal*(+)/*trans*, *gauche*(+)], which could represent an important structural element that can be used to stabilize unpaired structures in DNA or RNA, such as bulges or hairpins, that could require a distorted conformation of the backbone (ε–ζ > 0°, centered around +90°) while maintaining a C2′-*endo* conformation of the sugar moiety.

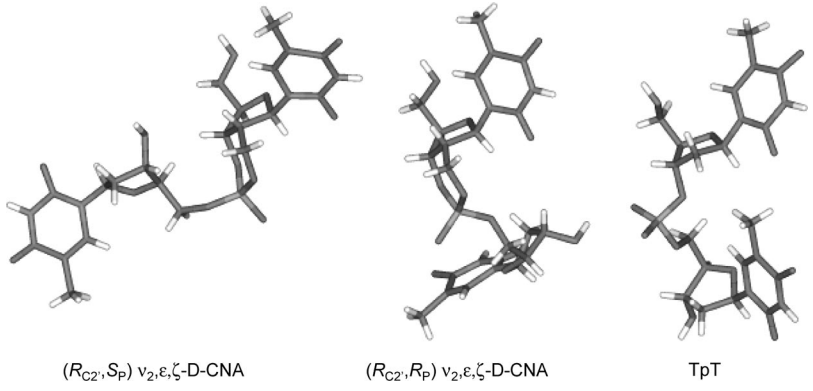


Figure 5. Tentative model of the conformation of the ($R_{C2'}$,S_P) and ($R_{C2'}$,R_P) ν_2,ε,ζ-D-CNA diastereoisomers derived from the NMR spectroscopic data and that of TpT.[36]

Conclusions

From a common 3-deoxy-3-(hydroxymethyl)-D-allofuranose intermediate, we synthesized two new members of the D-CNA family, in which the backbone torsional angles (ε, ζ or ε′, ζ′) of nucleic acids are simultaneously locked in a dioxaphosphorinane ring structure. Structural analysis indicated that the sugar rings of ν_2-ε,ζ-D-CNA dinucleotides are locked in a C2′-*endo* (South) conformation, whereas their (2′,5′)-ν_2,ε′,ζ′-D-CNA analogues exhibit a C3′-*endo* (North) conformation for the upper nucleoside sugar moiety. The dioxaphosphorinane ring structure introduced as an internucleotidic linkage in the ($R_{C2'}$,S_P) and ($R_{C2'}$,R_P) ν_2,ε,ζ-D-CNA diastereoisomers provide a (δ, ε, ζ) torsional angle set locked in the (a^+/t, a^+/t, g^+) and (a^+/t, a^+/t, a^-) conformations, respectively. In contrast, the two ($R_{C3'}$, R_P) and ($R_{C3'}$, S_P) diastereoisomers of (2′,5′)-ν_2,ε′,ζ′-D-CNA exhibit very unusual backbone conformations due to the transposition of the O3′–O5′ internucleotidic linkage to O2′–O5′. As can be seen, all of these structures are associated with very different backbone conformations and could provide interesting tools to discriminate between the impact of sugar puckering and backbone constrains on duplex formation ability. The incorporation of D-CNA building blocks at preselected positions in an oligonucleotide is expected to create a remarkable variety of different shapes including helical distortions of B-DNA or nonhelical secondary structures of functional RNA.

Experimental Section

Products were purified by medium-pressure liquid chromatography with a Jobin et Yvon Modoluprep apparatus by using Amicon 6–35 μm or Merck 15 μm silica. NMR spectra were recorded with a Bruker AC-250, Avance-300, or Avance 500 spectrometer (250, 300, or 500 MHz, respectively, for ^{1}H and 63, 75, or 125 MHz, respectively, for ^{13}C). Chemical shifts were referenced to tetramethylsilane. Mass spectra were recorded with a Nermag R10–10 or a Perkin–Elmer API 365. All solvents were distilled and dried before use.

5′-*O*-Benzoyl-3′-deoxy-3′-[(benzoyloxy)methyl]-2′-*O*-acetyl-5-methyluridine (3): A mixture of thymine (1 g, 7.9 mmol), hexamethyldisilazane (15 mL), and trimethylsilyl chloride (0.3 mL) were heated at reflux in anhydrous pyridine (10 mL) until the solution became clear. After removal of the excess amounts of reactants under vacuum, the bis(silylated) thymine was distilled under reduced pressure and stored under an inert atmosphere of argon. At room temperature, under an inert atmosphere of argon, to 5-*O*-benzoyl-3-deoxy-3-[(benzoyloxy)methyl]-1,2-di-*O*-acetylribose (**1**; 210 mg, 0.46 mmol) dissolved in anhydrous acetonitrile (1.8 mL) was added silylated thymine (249 mg, 0.92 mmol) and a solution of trimethylsilyltrifluoromethanesulfonate in anhydrous toluene (1 mL, 0.55 M, 0.55 mmol). The mixture was heated at 85 °C for 2 h. The reaction mixture was diluted with ethyl acetate and washed with a saturated aqueous solution of $NaHCO_3$. The organic layer was collected and washed with water and brine and dried with $MgSO_4$. After removal of the solvent under reduced pressure, the crude product was deposited on a silica-gel column and eluted with ethyl acetate/dichloromethane, 1:4. Compound **3** was recovered as a white foam in 93% yield (224 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.48 (br. s, 1 H, NH), 8.05–7.96 (m, 4 H, H, ph), 7.62–7.39 (m, 6 H, ph), 7.15 (d, J = 1.2 Hz, 1 H, 6-H), 5.85 (d, J = 3.0 Hz, 1 H, 1′-H), 5.65 (dd, J = 2.7 and 6.9 Hz, 1 H, 2′-H), 4.83 (m, 1 H, 6′-H), 4.60 (A part of an ABX syst., J = 6.3 and 11.7 Hz, 1 H, 5′-H), 4.57–4.50 (m, 2 H, 4′-H and 6′-H), 4.45 (B part of an ABX syst., J = 6.6 and 11.7 Hz, 1 H, 5′-H), 3.21–3.11 (m, 1 H, 3′-H), 2.13 (s, 3 H, CH_3), 1.67 (d, J = 1.2 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.1, 166.1, 164.8, 150.3, 137.7, 137.0, 136.7, 133.5, 133.1, 129.6–128.2, 110.4, 90.9, 80.1, 76.8, 66.5, 64.1, 64.4, 60.7, 40.7, 32.9, 27.4, 23.0, 19.2, 10.9 ppm. MS (electrospray): m/z = 545.5.0 $[M + Na]^+$, 523.2 $[M + H]^+$. $C_{27}H_{26}N_2O_9$ (522.51): calcd. C 62.07, H 5.02, N 5.36; found C 61.89, H 5.12, N 5.32.

5′-*O*-Benzoyl-2′-deoxy-2′-[(benzoyloxy)methyl]-3′-*O*-formyl-5-methyluridine (4): Compound **4** was prepared following the same procedure as that described for **3**. Starting from 5-*O*-benzoyl-3-*O*-formyl-2-deoxy-2-[(benzoyloxy)methyl]-1-*O*-acetylribose (**2**; 380 mg, 0.86 mmol), **4** (240 mg, 55%) was recovered as an oil after silica gel chromatography (ethyl acetate/dichloromethane, 1:4). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.30 (br. s, 1 H, NH), 8.17 (s, 1 H, HCOO), 8.06–8.03 (m, 2 H, ph), 7.90–7.87 (m, 2 H, ph), 7.66–7.38 (m, 6 H, ph), 7.10 (d, J = 1.5 Hz, 1 H, 6-H), 6.40 (d, J = 9.0 Hz, 1 H, 1′-H), 5.69 (br. d, J = 6.0 Hz, 1 H, 3′-H), 4.75–4.66 (m, 2 H, 5′-H and 6′-H), 4.61 (B part of an ABX syst., J = 3.0 and 12.0 Hz, 1 H, 5′-H), 4.51 (dd, J = 9.0 and 12.0 Hz, 1 H, 6′-H), 4.42 (m, J = 3.0 Hz, 1 H, 4′-H), 3.13–3.04 (m, 1 H, 2′-H), 2.04 (s, 3 H, CH_3), 1.29 (d, J = 1.5 Hz, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.0, 165.9, 163.3, 159.9, 150.6, 134.1–128.5, 112.2, 86.4, 82.0, 74.1, 64.1, 59.9, 45.6, 12.0 ppm. MS (DCI): m/z = 526.3 $[M + NH_4]^+$. $C_{26}H_{24}N_2O_9$ (508.48): calcd. C 61.42, H 4.76, N 5.51; found C 61.58, H 4.79, N 5.36.

(3′-*O*-*tert*-Butyldiphenylsilyl-5′-thymidinyl)-(2-cyanoethyl)-5′-*O*-benzoyl-3′-deoxy-3′-[(benzoyloxy)methyl]-5-methyluridine-2′-phosphate (5): Compound **3** (0.1 g, 0.19 mmol) dissolved in methanol (0.5 mL) was treated with a concentrated ammonia solution (28% in water, 2.5 mL), at room temperature for 120 min. After complete removal of the solvent, the crude material was dried under high vacuum and used without further purification. To this compound was added 3′-*O*-*tert*-butyldiphenylsilylthymidine-5′-*O*- phosphoramidite (388 mg, 0.57 mmol) and freshly sublimed tetrazole (93 mg, 1.33 mmol), and the mixture was diluted with anhydrous acetonitrile (7.8 mL) and stirred for 3 h at room temperature under an inert atmosphere of argon. After the addition of collidine (150 μL), the phosphite was oxidized with a solution of iodine [0.1 M in THF/H_2O, 2:1] until the dark-brown color persisted. After extraction with ethyl acetate, the organic layer was washed with an aqueous solution of sodium thiosulfate (15%), water, and brine and dried with $MgSO_4$ before removal of the solvent. Compound **5** (140 mg, 68%) was isolated (two diastereoisomers) as a white foam after chromatography on silica gel (CH_2Cl_2/AcOEt, 1:1, then AcOEt). R_f (CH_2Cl_2/AcOEt, 1:1) = 0.19. ^{31}P NMR (81 MHz, $CDCl_3$): δ = –2.1, –2.3 ppm. MS (ESI): m/z = 1098.54 $[M + Na]^+$. $C_{54}H_{58}N_5O_{15}PSi$ (1076.13): calcd. C 60.27, H 5.43, N 6.51; found C 60.07, H 5.23, N 6.65.

(3′-*O*-tert-Butyldiphenylsilyl-5′-thymidinyl)-(2-cyanoethyl)-5′-*O*-benzoyl-2′-deoxy-2′-[(benzoyloxy)methyl]-5-methyluridine-3′-phosphate (6): Compound **6** was prepared following the same procedure as that described for **5**. Starting from **4** (190 mg, 0.37 mmol), compound **6** (two diastereoisomers) was recovered as a white foam (290 mg, 75%) after silica-gel chromatography (ethyl acetate/dichloromethane, 1:1, then ethyl acetate. ^{31}P NMR (121 MHz, CD_3OD): δ = –1.8 and –2.1 ppm. MS (ESI): m/z = 1098.50 $[M + Na]^+$. $C_{54}H_{58}N_5O_{15}PSi$ (1076.13): calcd. C 60.27, H 5.43, N 6.51; found C 59.85, H 5.61, N 6.42.

(3′-*O*-*tert*-Butyldiphenylsilyl-5′-thymidinyl)-3′-deoxy-3′-[(hydroxy)-methyl]-5-methyluridine-2′-phosphate (7): To compound **5** (140 mg, 0.13 mmol) in a solution of methanol/water (4:1, 2.5 mL) was added potassium carbonate (36 mg, 0.26 mmol), and the mixture was stirred for 6 h. After removal of the solvents, **7** was isolated (95 mg, 90%) by HPLC (Kromasil C18, 7 μm, 250 × 4.6 mm; water/acetonitrile, gradient 95:5→50:50 in 30 min), t_R = 16.80 min. ^{1}H NMR (300 MHz, CD_3OD): δ = 7.72–7.58 (m, 6 H, ph), 7.48–7.42 (m, 6 H, ph, 6a-H and 6b-H), 6.49 (dd, J = 6.3 and 8.4 Hz, 1 H, 1′b-H), 5.94 (d, J = 1.2 Hz, 1 H, 1′a-H), 4.70 (dd, J = 6.0 and 9.3 Hz, 1 H), 4.58 (m, 1 H), 4.11 (s, 1 H), 4.01–3.87 (m, 4 H), 3.78 (dt, J = 3.6 and 11.0 Hz, 1 H), 3.67 (A part of an ABX syst., J = 3.0 and 12.7 Hz, 1 H), 3.59 (B part of an ABX syst., J = 5.1 and 12.7 Hz, 1 H), 2.67–2.57 (m, 1 H, 2′a-H), 2.14–2.10 (m, 2 H, 2′b-H), 1.89 (s, 3 H, Me), 1.81 (s, 3 H, Me), 1.12 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 165.3, 165.1, 151.3, 150.7, 136.9, 136.4, 135.5, 133.0, 132.9, 129.8,129.7, 127.7, 127.6, 110.6, 109.8, 90.8, 86.4, 84.5, 82.1, 79.9, 74.7, 64.7, 60.8, 57.0, 44.9, 39.9, 26.0, 18.4, 11.4, 11.1 ppm. ^{31}P NMR (121 MHz, CD_3OD): δ = –0.5 ppm. MS (ESI): m/z = 813.24 $[M]^+$. $C_{37}H_{46}KN_4O_{13}PSi$ (852.94): calcd. C 52.10, H 5.44, N 6.57; found C 51.87, H 5.55, N 6.32.

3′-*O*-(*tert*-Butyldiphenylsilyl)-(2′,5′)-ν_2,ε′,ζ′-D-CNA ($R_{C3'}$,S_P) (8) and ($R_{C3'}$,R_P) (9): To compound **7** (30 mg, 0.038mmol) in anhydrous pyridine (1 mL) was added, under an inert atmosphere of argon, 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (22 mg, 0.075 mmol), and the mixture was stirred for 1 h at room temperature, 1 h at 80 °C, and then 30 min at 90 °C. After addition of ethyl acetate/methanol (10%, 5 mL), the solvent were removed under high vacuum and compounds **8** (16 mg) and **9** (9 mg) were isolated (80%) by HPLC (Kromasil C18, 7 μm, 250 × 4.6 mm; water/acetonitrile, 1:1), t_R = 13.33 (for **8**), 15.03 (for **9**) min. Data for **8**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.68–7.65 (m, 4 H, ph), 7.55 (s, 1 H, 6-H), 7.49–7.41 (m, 6 H, ph), 7.07 (s, 1 H, 6-H), 6.23 (t, J = 7.0 Hz, 1 H, 1′b-H), 5.61 (s, 1 H, 1′a-H), 5.09 (d, J = 4.5 Hz, 1 H, 2′a-H), 4.48 (br. d, J = 11.0 Hz, 1 H, 4′a-H), 4.43–4.37 (m, 2 H, 6′a-H and 3′b-H), 4.21–4.14 (m, J = 12.0, $J_{H/P}$ = 22.0 Hz, 1 H, 6′a-H), 4.18 (A part of an AB syst., J = 12.0 Hz, 1 H, 5′a-H), 4.11 (br. s, 1 H, 4′b-H), 4.10–4.07 (m, J = 11.5 and 3.0, $J_{H,P}$ = 2.0 Hz, 1 H, 5′b-H), 3.82 (B part of an AB syst., J = 12.0 Hz, 1 H, 5′a-H), 3.82–3.77 (m, J = 11.5 and 6.0, $J_{H,P}$ = 8.0 Hz, 1 H, 5′b-H), 2.82 (m, J = 5.5 Hz, 1 H, 3′a-H), 2.40 (A part of an ABXY syst., J = 4.0, 6.8, and 13.4 Hz, 2′b-H), 2.06 (B part of an ABXY syst., J = 5.5, 7.0, and 13.4 Hz, 2′b-H), 1.91 (s, 3 H, Me), 1.88 (s, 3 H, Me), 1.10 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 164.0, 150.3, 150.2, 137.3, 136.3, 135.8, 135.7, 132.8, 132.7, 130.3, 130.2, 128.1, 128.0, 111.3, 110.5, 86.0, 85.1, 84.4, 80.4, 71.9, 66.1, 64.7, 59.6, 39.7, 26.8, 21.1, 19.0, 12.4 ppm. ^{31}P NMR (202 MHz, $CDCl_3$): δ = –9.7 ppm. MS (ESI): m/z = 797.8 $[M + H]^+$, 819.5 $[M + Na]^+$, 835.8 $[M + K]^+$. $C_{37}H_{45}N_4O_{12}PSi$ (796.84): calcd. C 55.77, H 5.69, N 7.03; found C 56.01, H 5.57, N 6.99. Data for **9**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.68–7.65 (m, 4 H, Ph), 7.51–7.43 (m, 6 H, Ph), 7.40 (s, 1 H, 6b-H), 7.26 (s, 1 H, 6a-H), 6.44 (dd, J = 5.5 and 8.0 Hz, 1 H, 1′b-H), 5.67 (s, 1 H, 1′a-H), 5.29 (t, J = 4.3 and 6.0 Hz, 1 H, 2′a-H), 4.73 (m, J = 3.5, 5.6 and 12.5 Hz, 1 H, 6′a-H), 4.42 (m, J = 2.5 and 6.1 Hz, 1 H, 3′b-H), 4.29 (dt, J = 2.5 and 9.7 Hz, 1 H, 4′a-H), 4.20 (ddd, J = 2.0, 12.5, and 20.3 Hz, 1 H, 6′a-H), 4.11 (A part of an ABX syst., J = 2.5 and 12.4 Hz, 1 H, 5′a-H), 4.08 (br. s, 1 H, 4′b-H), 4.09–4.05 (m, J = 2.5, 6.7 and 16.0 Hz, 1 H, 5′b-H), 3.81 (B part of an ABX syst., J = 2.5 and 12.4 Hz, 1 H, 5′a-H), 3.73–3.69 (m, J = 2.5 Hz, 1 H, 5′b-H), 3.00 (m, 1 H, 3′a-H), 2.39 (A part of an ABXY syst., J = 2.5, 5.5, and 13.5 Hz, 1 H, 2′b-H), 1.95–1.90 (m, 1 H, 2′b-H), 1.92 (s, 3 H, Me), 1.89 (s, 3 H, Me), 1.11 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.4, 163.1, 150.1, 149.8, 136.9, 135.7, 135.5, 132.9, 132.7, 130.3, 130.2, 128.1, 111.3, 85.3, 85.2, 85.1, 85.0, 83.9, 83.8, 80.6, 72.9, 68.2, 68.1, 64.3, 60.8, 40.4, 30.1, 29.7, 26.8, 19.0, 12.5, 12.4 ppm. ^{31}P NMR (202 MHz, $CDCl_3$): δ = –6.0 ppm. MS (ESI): m/z = 819.5 $[M + Na]^+$, 836.0 $[M + K]^+$. $C_{37}H_{45}N_4O_{12}PSi$ (796.84): calcd. C 55.77, H 5.69, N 7.03; found C 55.53, H 5.78, N 7.10.

(3′-*O*-*tert*-Butyldiphenylsilyl-5′-thymidinyl)-2′-deoxy-2′-[(hydroxy)-methyl]-5-methyluridine-2′-phosphate (10) and (5′-Thymidinyl)-2′-deoxy-2′-[(hydroxy)methyl]-5-methyluridine-2′-phosphate (11): To compound **6** (290 mg, 0.27 mmol) in a solution of methanol/water (4:1, 5 mL) was added potassium carbonate (224 mg, 1.62 mmol), and the mixture was stirred for 6 h. After removal of the solvents, **10** and **11** were isolated (40 mg each, 45%) by HPLC [Kromasil C18, 7 μm, 250 × 4.6 mm; water/acetonitrile, gradient 95:5 (t = 0 to 12 min) to 50:50 in 42 min], t_R = 25.07 (for **10**), 3.75 (for **11**) min. Data for **10**: ^{1}H NMR (300 MHz, CD_3OD): δ = 7.82 (d, J = 1.2 Hz, 1 H, 6a-H), 7.67 (d, J = 1.2 Hz, 1 H, 6b-H), 7.63–7.60 (m, 4 H, ph), 7.42–7.39 (m, 6 H, ph), 6.43 (dd, J = 5.4 and 9.0 Hz, 1 H, 1′b-H), 5.97 (d, J = 9.0 Hz, 1 H, 1′a-H), 4.76–4.70 (m, 2 H), 4.47 (d, J = 4.8 Hz, 1 H, 3′a-H), 4.02 (m, 1 H), 3.98 (m, 1 H), 3.87–3.81 (m, 2 H), 3.57 (d, J = 2.7 Hz, 1 H), 3.50–3.44 (m, 2 H), 2.53 (m, 1 H, 2′a-H), 2.16 (A part of an ABXY syst., J = 1.5, 5.8, and 13.3 Hz, 1 H, 2′b-H), 2.06 (m, J = 5.1, 9.0 and 13.8 Hz, 1 H, 2′b-H), 1.85 (d, J = 1.2 Hz, 3 H, Me), 1.82 (d, J = 1.2 Hz, 3 H, Me), 1.04 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 165.1, 165.0, 151.3, 151.2, 136.8, 136.6, 135.5, 133.0, 132.9, 130.0, 129.9, 127.8, 127.7, 110.9, 110.5, 86.7, 86.6, 86.3, 86.2, 85.0, 76.5, 76.4, 74.8, 65.4, 65.3, 61.8, 56.9, 51.2, 51.2, 40.0, 26.0, 18.4, 11.2, 11.1 ppm. ^{31}P NMR (121 MHz, CD_3OD): δ = –0.2 ppm. MS (ESI): m/z = 813.5 $[M]^-$. $C_{37}H_{46}KN_4O_{13}PSi$ (852.94): calcd. C 52.10, H 5.44, N 6.57; found C 51.92, H 5.66, N 6.61. Data for **11**: ^{1}H NMR (300 MHz, D_2O): δ = 7.54 (d, J = 1.2 Hz, 1 H, 6a-H), 7.53 (d, J = 1.2 Hz, 1 H, 6b-H), 6.17 (t, J = 6.9 Hz, 1 H, 1′b-H), 5.95 (d, J = 8.7 Hz, 1 H, 1′a-H), 4.68 (m, 1 H, 3′a-H), 4.42 (m, 1 H, 3′b-H), 4.15 (m, 1 H, 4′a-H), 4.01 (m, 2 H, 4′b-H and 5′b-H), 3.96 (m, 1 H, 5′b-H), 3.80 (A part of an ABX syst., J = 6.6 and 11.4 Hz, 1 H, 6′a-H), 3.65 (m, 2 H, 5′a-H), 3.57 (B part of an ABX syst., J = 6.6 and 11.4 Hz, 1 H, 6′a-H), 2.57 (m, 1 H, 2′a-H), 2.25 (dd, J = 5.4 and 6.9 Hz, 1 H, 2′b-H), 1.77 (d, J = 1.2 Hz, 3 H, Me), 1.75 (d, J = 1.2 Hz, 3 H, Me) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 166.4, 166.3, 151.7, 151.6, 137.3, 137.2, 111.8, 111.5, 86.8, 85.3, 85.1, 75.9, 70.7, 65.3, 61.2, 57.0, 48.9, 38.5, 11.6, 11.5 ppm. ^{31}P NMR (121 MHz, D_2O): δ = –0.5 ppm. MS (ESI): m/z = 575.3 $[M]^-$. $C_{21}H_{28}KN_4O_{13}P$ (614.54.25): calcd. C 41.04, H 4.59, N 6.36; found C 39.78, H 4.73, N6.63.

3′-*O*-(*tert*-Butyldiphenylsilyl)-ν_2,ε,ζ_5-D-CNA ($R_{C3'}$,R_P) (12) and ($R_{C3'}$,S_P) (13): Compounds **12** and **13** were prepared following the same procedure as that described for **8** and **9**. Starting from **10** (40 mg, 0.049 mmol), compounds **12** (10 mg) and **13** (19 mg) were isolated (75%) by HPLC (Kromasil C18, 7 μm, 250 × 4.6 mm; water/acetonitrile, 1:1), t_R = 14.82 (for **12**), 17.00 (for **13**) min. Data for **12**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.67–7.61 (m, 5 H, ph), 7.50–7.41 (m, 6 H, ph), 7.28 (s, 1 H, 6a-H), 6.41 (dd, J = 6.0 and 8.0 Hz, 1 H, 1′b-H), 6.22 (d, J = 9.0 Hz, 1 H, 1′a-H), 5.26 (d, J = 4.5 Hz, 1 H, 3′a-H), 4.69 (m, J = 3.0 and 12.0, $J_{H,P}$ = 2.4 Hz, 1 H, 6′a-H), 4.40 (m, J = 2.5 and 5.5 Hz, 1 H, 3′b-H), 4.29 (dd, J = 11.5, $J_{H,P}$ = 20.0 Hz, 1 H, 6′a-H), 4.10–4.07 (m, 3 H, 4′a-H, 4′b-H and 5′b-H), 3.85 (AB syst., J = 10.8 Hz, 2 H, 5′a-H), 3.66 (m, J = 3.0 and 11.0, $J_{H,P}$ = 3.5 Hz, 1 H, 5′b-H), 2.82 (m, 1 H, 2′a-H), 2.39 (A part of an ABXY syst., J = 2.0, 5.5, and 13.5 Hz, 1 H, 2′b-H), 1.98 (m, J = 6.0, 7.5, and 14.0 Hz, 1 H, 2′b-H), 1.88 (s, 3 H, Me), 1.86 (s, 3 H, Me), 1.10 (s, 9 H, *t*Bu) ppm. ^{13}C NMR

(125 MHz, $CDCl_3$): δ = 164.2, 163.8, 150.9, 150.4, 136.0, 135.9, 135.7, 132.9, 132.7, 130.4, 130.2, 128.1, 128.0, 111.9, 111.1, 86.1, 85.9, 85.8, 85.4, 85.3, 85.2, 81.8, 73.2, 68.5, 68.4, 63.5, 62.3, 60.4, 43.8, 40.4, 26.9, 12.6, 12.4 ppm. ^{31}P NMR (202 MHz, $CDCl_3$): δ = –6.0 ppm. MS (ESI): *m/z* = 819.5 $[M + Na]^+$, 836.0 $[M + K]^+$. $C_{37}H_{45}N_4O_{12}PSi$ (796.84): calcd. C 55.77, H 5.69, N 7.03; found C 55.89, H 5.78, N 7.14. Data for **13**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.68–7.65 (m, 4 H, ph), 7.52–7.42 (m, 6 H, ph), 7.40 (s, 1 H, 6a-H), 7.10 (s, 1 H, 6b-H), 6.16 (t, *J* = 6.5 Hz, 1 H, 1′b-H), 6.02 (d, *J* = 9.5 Hz, 1 H, 1′a-H), 5.10 (d, *J* = 4.5 Hz, 1 H, 3′a-H), 4.43–4.39 (m, 2 H, 3′b-H and 6′a-H), 4.26 (m, *J* = 8.5, $J_{H,P}$ = 26.0 Hz, 1 H, 6′a-H), 4.25 (s, 1 H, 4′a-H), 4.11 (m, *J* = 3.0, and 6.0 Hz, 1 H, 4′b-H), 3.98 (A part of an ABXY syst., *J* = 3.0, 6.0, and 11.2 Hz, 1 H, 5′b-H), 3.89 (A part of an AB syst., *J* = 11.0 Hz, 1 H, 5′a-H), 3.84 (B part of an ABX syst., *J* = 11.0, $J_{H,P}$ = 6.0 Hz, 1 H, 5′b-H), 3.72 (B part of an AB syst., *J* = 11.5 Hz, 1 H, 5′a-H), 3.03 (m, 1 H, 2′a-H), 2.37 (A part of an ABXY syst., *J* = 3.5, 6.5, and 13.6 Hz, 1 H, 2′b-H), 2.14 (B part of an ABXY syst., *J* = 7.0 and 13.5 Hz, 1 H, 2′b-H), 1.90 (s, 3 H, Me), 1.88 (s, 3 H, Me), 1.10 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 164.4, 164.2, 135.7, 135.6, 132.8, 132.7, 130.4, 130.3, 128.1, 128.0, 111.6, 111.1, 86.7, 86.2, 86.1, 86.0, 84.9, 84.8, 83.1, 83.0, 72.3, 66.2, 64.4, 62.3, 42.2, 39.5, 26.9, 19.0, 12.4, 12.3 ppm. ^{31}P NMR (202 MHz, $CDCl_3$): δ = –9.4 ppm. MS (ESI): *m/z* = 819.5 $[M + Na]^+$, 836.0 $[M + K]^+$. $C_{37}H_{45}N_4O_{12}PSi$ (796.84): calcd. C 55.77, H 5.69, N 7.03; found C 55.81, H 5.55, N 7.16.

ν_2,ε,ζ-D-CNA ($R_{C3'}$,R_P) (14) and ($R_{C3'}$,S_P) (15): Compounds **14** and **15** were prepared following the same procedure as that described for **8** and **9**. Starting from **11** (40 mg, 0.049 mmol), compounds **14** (10 mg) and **15** (13 mg) were isolated (60%) by HPLC (Kromasil C18, 7 μm, 250 × 4.6 mm; water/acetonitrile, gradient 95:5→60:40 in 30 min), t_R = 27.91 (for **14**), 27.12 (for **15**) min. Data for **14**: ^{1}H NMR (500 MHz, CD_3OD): δ = 7.89 (d, *J* = 1.0 Hz, 1 H, 6a-H), 7.53 (d, *J* = 1.0 Hz, 1 H, 6b-H), 6.47 (d, *J* = 9.0 Hz, 1 H, 1′a-H), 6.31 (t, *J* = 7.0 Hz, 1 H, 1′b-H), 5.31 (m, *J* = 5.0, $J_{H,P}$ = 2.5 Hz, 1 H, 3′a-H), 4.76 (A part of an ABXY syst., *J* = 3.7 and 12.5 Hz, $J_{H,P}$ = 6.9 Hz, 1 H, 6′a-H), 4.49–4.39 (m, 4 H, 6′a-H, 5′b-H, and 3′b-H), 4.30 (br. s, 1 H, 4′a-H), 4.09 (m, *J* = 3.6, $J_{H,P}$ = 2.2 Hz, 1 H, 4′b-H), 3.84 (d, *J* = 3.0 Hz, 2 H, 5′a-H), 2.89 (m, 1 H, 2′a-H), 2.33–2.24 (m, *J* = 6.5, 6.5, and 7.0 Hz, 2 H, 2′b-H), 1.90 (d, *J* = 1.0 Hz, 3 H, Me), 1.89 (d, *J* = 1.0 Hz, 3 H, Me) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 164.9, 164.8, 151.5, 150.9, 136.1, 135.9, 111.0,110.5, 86.0, 85.9, 84.9, 84.7, 84.6, 84.4, 82.7, 70.5, 68.9, 68.8, 63.9, 63.8, 61.4, 61.3, 43.8, 43.7, 39.0, 11.1 ppm. ^{31}P NMR (202 MHz, CD_3OD): δ = –6.6 ppm. MS (ESI): *m/z* = 559.3 $[M + H]^+$, 581.2 $[M + Na]^+$, 597.5 $[M + K]^+$. $C_{21}H_{27}N_4O_{12}P$ (558.43): calcd. C 45.17, H 4.87, N 10.03; found C 45.23, H 4.78, N 9.92. Data for **15**: ^{1}H NMR (500 MHz, CD_3OD): δ = 7.92 (d, *J* = 1.0 Hz, 1 H, 6a-H), 7.55 (d, *J* = 1.0 Hz, 1 H, 6b-H), 6.51 (d, *J* = 9.5 Hz, 1 H, 1′a-H), 6.28 (t, *J* = 7.0 Hz, 1 H, 1′b-H), 5.27 (d, *J* = 4.5 Hz, 1 H, 3′a-H), 4.69 (A part of an ABXY syst., *J* = 3.2 and 12.5 Hz, $J_{H,P}$ = 1.5 Hz, 1 H, 6′a-H), 4.46 (m, 1 H, 3′b-H), 4.42 (B part of an ABXY syst., *J* = 12.5 Hz, $J_{H,P}$ = 24.0 Hz, 1 H, 6′a-H), 4.40 (A part of an ABXY syst., *J* = 11.3 Hz, $J_{H,P}$ = 3.3 Hz, 1 H, 5′b-H), 4.36 (B part of an ABXY syst., *J* = 5.8 and 11.3 Hz, $J_{H,P}$ = 7.7 Hz, 1 H, 5′b-H), 4.27 (br. s, 1 H, 4′a-H), 4.11 (m, *J* = 3.3, 3.4, and 5.8 Hz, 1 H, 4′b-H), 3.81 (ABX syst., *J* = 2.5, 3.0, and 12.0 Hz, 2 H, 5′a-H), 2.84 (m, 1 H, 2′a-H), 2.32 (m, *J* = 6.5 and 7.0 Hz, 2 H, 2′b-H), 1.92 (d, *J* = 1.0 Hz, 3 H, Me), 1.90 (d, *J* = 1.0 Hz, 3 H, Me) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 164.9, 164.8, 151.5, 150.8, 136.6, 136.1, 110.9, 110.5, 86.2, 86.1, 85.6, 84.7, 84.6, 84.1, 83.8, 83.7, 70.3, 67.1, 67.0, 64.7, 64.6, 61.5, 43.1, 43.0, 38.9, 11.1, 11.0 ppm. ^{31}P NMR (202 MHz, CD_3OD): δ = –7.8 ppm. MS (ESI): *m/z* = 559.3 $[M + H]^+$, 581.2 $[M + Na]^+$, 597.5 $[M + K]^+$. $C_{21}H_{27}N_4O_{12}P$ (558.43): calcd. C 45.17, H 4.87, N 10.03; found C 44.97, H 4.92, N 10.14.

Circular Dichroism Studies: These experiments were carried out with a Jasco J-815 CD spectrometer equipped with a Peltier controller Jasco PTC-4235/15 at a dinucleotide concentration range of 0.1 mM in a 10 mM Na_2HPO_4, 100 mM NaCl, 0.1 mM Na_2EDTA, buffer, pH 7.00 ± 0.02. Molar extinction coefficients were calculated from those of dinucleotides by using the nearest-neighbor approximation method by assuming that ν_2,ε,ζ-D-CNA TT has the same molar extinction coefficient as DNA. Dinucleotide concentration was determined from UV absorbance at high temperature (90 °C). All CD spectra were recorded after stabilization of the temperature for 10 min and were normalized by subtraction of the background scan with buffer. By taking the known dinucleotide concentration into account, the normalized spectra were converted into a variation of the molar extinction coefficient ($\Delta\varepsilon$).

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