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Constrained Nucleic Acids (CNA). Part 2. Synthesis of Conformationally Restricted Dinucleotide Units Featuring Noncanonical α/β/γ or δ/ϵ/ζ Torsion Angle Combinations

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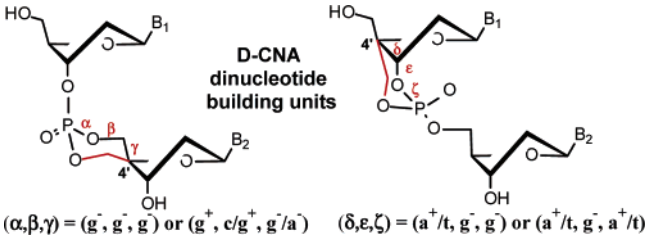

Four dinucleotide building units of nucleic acids in which three out of six backbone torsion angles are included in a dioxaphosphorinane ring structure (D-CNA family) have been synthesized: two diastereoisomeric α,β,γ-D-CNA {cis and trans} and two diastereoisomeric δ,ϵ,ζ-D-CNA {($S_{C4'}$,R_P) and ($S_{C4'}$,S_P)}. The structural analysis of these conformationally restricted dinucleotides, using NMR spectroscopy and X-ray crystallography, shows that these D-CNA structural elements allow the stabilization of torsion angle combinations, α/β/γ and δ/ϵ/ζ, that are significantly different from those typically observed in canonical A- or B-form duplexes.

Introduction

The development of conformationally restricted nucleosides has attracted a lot of attention mainly due to important potential applications of antisense oligonucleotides.[1] However, much less attention has been devoted to the design of conformationally restricted nucleotides for the special purpose of mimicking biologically relevant helical distortions of B-DNA or important nonhelical secondary structures of functional RNA.[2]

In addition to the double-stranded helical conformation, nucleic acids may adopt many other alternative structures such as bulges, hairpins, U-turns, or branched junctions.[3] These secondary structures always contain unpaired nucleotides or non-Watson−Crick pairs and are

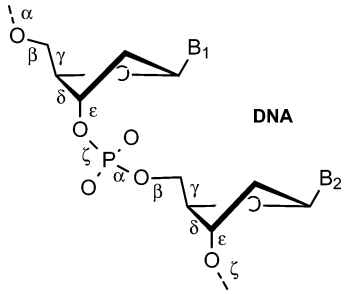

FIGURE 1. The six backbone torsion angles (labeled α to ζ) of nucleic acids.

characterized by a variety of backbone conformations that markedly differ from the regular conformational states of double-stranded helices (Figure 1 and Table 1). It is now well established that these disparate structures, which are prone to promote a significant local conformational heterogeneity in the sugar−phosphate backbone, play a crucial role in fundamental biological processes where protein−nucleic acid interactions, RNA folding, or RNA catalytic activity are involved.[4]

The determination of the precise biological role played by nonstandard *helical* conformations during biochemically important processes (e.g., protein−DNA complex-

† Current address: Laboratoire Hétérochimie Fondamentale et Appliquée, Université Paul Sabatier.

(1) For reviews, see: (a) Leumann, C. J. *Bioorg. Med. Chem.* **2002**, *10*, 841. (b) Wengel, J. A. *Acc. Chem. Res.* **1999**, *32*, 301.

(2) See for example: (a) Seio, K.; Wada, T.; Sakamoto, K.; Yokoyama, S.; Sekine, M. *J. Org. Chem.* **1998**, *63*, 1429. (b) Sekine, M.; Kurasawa, O.; Shohda, K.; Seio, K.; Wada, T. *J. Org. Chem.* **2000**, *65*, 6515. (c) Kirchhoff, C.; Nielsen, P. *Tetrahedron Lett.* **2003**, *44*, 6475. (d) Børsting, P.; Sørensen, A. M.; Nielsen, P. *Synthesis* **2002**, 797. (e) Sørensen, A. M.; Nielsen, K. E.; Vogg, B.; Jacobsen, J. P.; Nielsen, P. *Tetrahedron* **2001**, *57*, 10191.

(3) Belmont, P.; Constant, J.-F.; Demeunynck, M. *Chem. Soc. Rev.* **2001**, *30*, 70 and references therein.

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TABLE 1. Summary of the Backbone Torsion Angles Derived from the Canonical B- and A-DNA Duplex Structures[a]

duplex conformation		torsion angle					
		α	β	γ	δ	ϵ	ζ
B	B_I	g^-	t	g^+	a^+	t	g^-/a^-
	B_{II}	g^-	t	g^+	a^+/t	g^-/a^-	t
A		g^-	t	g^+	g^+/a^+	a^-/t	g^-

[a] The following 6-fold 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.

ation, DNA processing, and DNA packaging) is also an area of intense study.[5] Recently, an important study based on an analysis of available high-resolution crystallographic data and molecular simulation techniques has shown that, in contrast to free B-DNA structures, protein-bound B-DNA oligomers regularly involve noncanonical backbone geometries.[6] It was found that in the crystal structures of protein−DNA complexes, the α and γ torsion angles can adopt noncanonical combinations where α populates the gauche(+) or trans conformation while γ populates the gauche(−) or trans conformation. These unusual backbone states are believed to contribute to the specific recognition of DNA by proteins in assisting, at some stage, the fine structural adjustments that are required between DNA and proteins to form stable complexes. 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.

With this in mind, we became interested in the development of covalently constrained dinucleotide building units in which the backbone torsional angles of nucleic acids can have predefined values that are significantly different from the typical values observed in A- or B-type duplexes (Table 1). Work in our laboratory on the development of constrained nucleic acids (CNA) has led to an interest in the 1,3,2-dioxaphosphorinane ring structure. Specifically, we are interested in the structural properties that this system may impart to the DNA double helix depending on its position along the sugar−phosphate backbone.

We have already reported on the diastereoselective synthesis of a dioxaphosphorinane-CNA (D-CNA) dinucleotide building unit {referred to as ($S_{C5'}$,R_P)-α,β-D-CNA} in which the α and β torsion angles are locked in a (g^+, t) conformation that frequently occurs in protein−DNA complexes and in bulged regions of nucleic acids.[7] In this paper, we report the synthesis of two additional

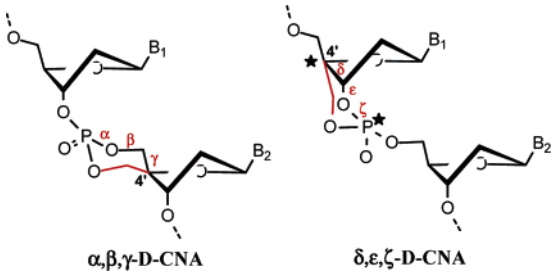


FIGURE 2. α,β,γ-D-CNA and δ,ϵ,ζ-D-CNA are constrained nucleic acids in which the backbone torsion angles (α, β, γ) and (δ, ϵ, ζ), respectively, are stereocontrolled by a dioxaphosphorinane ring structure.

D-CNA members, α,β,γ-D-CNA and δ,ϵ,ζ-D-CNA, in which the torsion angles α, β, γ and δ, ϵ, ζ, respectively, are constrained to noncanonical values that depend on the spatial arrangement of the dioxaphosphorinane ring structure (Figure 2).

Results and Discussion

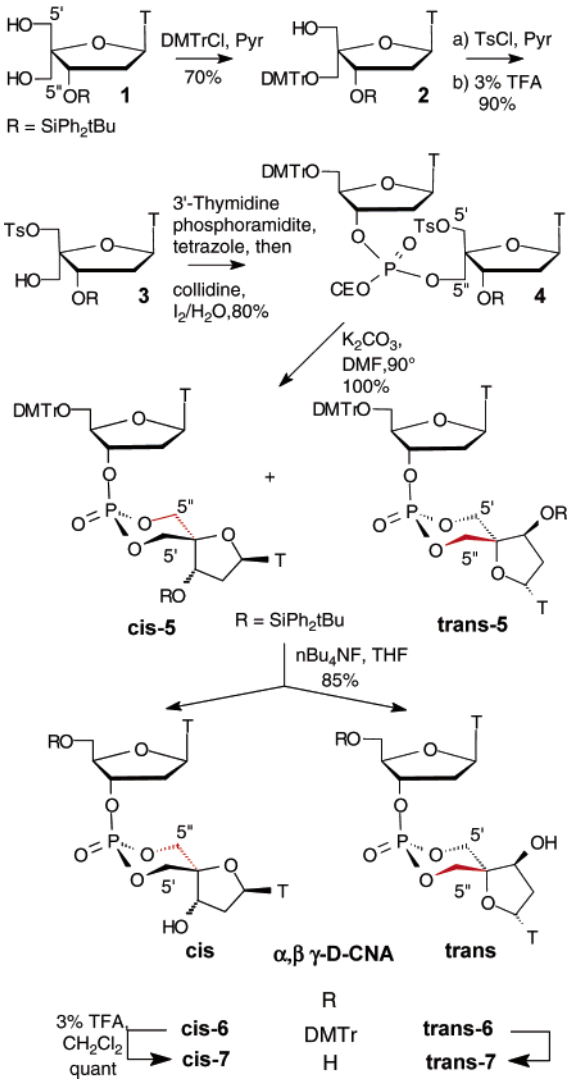
Synthesis of Cis and Trans α,β,γ-D-CNA. The dioxaphosphorinane ring structure was introduced as previously described,[7] i.e., from the cyclization reaction of a dinucleotide precursor in which the phosphate oxyanions can attack an electrophilic tosyloxy-substituted carbon atom. The corresponding acyclic precursor involved in the present work is the diastereoisomeric 4′-substituted dithymidine **4** prepared by coupling 5′-*O*-tosyl-4′-*C*-hydroxymethylthymidine **3** with the commercially available thymidine phosphoramidite by using standard phosphoramidite technology[8] (Scheme 1). Modified thymidine **3** has been obtained by treatment of **2** with tosyl chloride in the presence of pyridine followed by the removal (with trifluoroacetic acid) of the dimethoxytrityl group selectively introduced on the 5″-hydroxyl function of starting 4′-*C*-hydroxymethylthymidine **1**.[9]

By treatment with K_2CO_3 in dry dimethylformamide at 90 °C for 2 h, **4** quantitatively cyclized into the cis and trans isomers of phosphotriester **5** in a 2:1 ratio as observed by ^{31}P NMR (δ_P −9.6 and −7.8, respectively). Removal of the silyl protective group with fluoride ion provided the corresponding mixture of *cis*-**6** and *trans*-**6** isomers, which were separated at this stage (these two compounds will be subsequently converted into their corresponding phosphoramidites for incorporation into oligonucleotides). Finally, α,β,γ-D-CNA *cis*-**7** and *trans*-**7** were obtained upon removal of the remaining dimethoxytrityl protective group in acidic conditions.

Structural Assigment of Cis and Trans α,β,γ-D-CNA. The dinucleotide analogues, *cis*-**7** and *trans*-**7,** were fully characterized by mass spectroscopy and by ^1H, ^{13}C, and ^{31}P NMR. The chair conformation of the dioxaphosphorinane structure of *cis*-**7** is clearly established from the ^1H NMR spectra, with the observation of small and large $^3J_{H/P}$ coupling constants between the 5′- and 5″-H protons and phosphorus, which is characteristic of an axial position ($^3J_{Hax/P} \approx 2$ Hz) and an equatorial position

(4) See for example: Antson, A. A. *Curr. Opin. Struct. Biol.* **2000**, *10*, 87 and references therein.
(5) (a) Lu, X.-J.; Shakked, Z.; Olson, W. K. *J. Mol. Biol*. **2000**, *300*, 819−840. (b) Olson, W. K.; Gorin, A. A.; Lu, X.-J.; Hock, L. M.; Zhurkin, V. B. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 11163−11168.
(6) Várnai, P.; Djuranovic, D.; Lavery, R.; Hartmann, B. *Nucleic Acids Res*. **2002**, *30*, 5398.
(7) Le Clézio, I.; Escudier, J.-M.; Vigroux, A. *Org. Lett*. **2003**, *5*, 161.
(8) Caruthers, M. H. *Acc. Chem. Res*. **1991**, *24*, 278.
(9) (a) Youssefyeh, R. D.; Verheyden, J. P. H.; Moffatt, J. G. *J. Org. Chem*. **1979**, *44*, 1301. (b) Jones, G. H.; Taniguchi, M.; Tegg, D.; Moffatt, J. G. *J. Org. Chem*. **1979**, *44*, 1309.

SCHEME 1. Synthesis of Cis and Trans α,β,γ-D-CNA Dinucleotides



($^3J_{Heq/P}$ = 22 Hz) of these protons. The observation of a long-range coupling constant between the 5′- and 5″-H protons ($^4J_{H/H}$ = 2.9 Hz) is also consistent with the typical W-shaped $H_{eq}-C-C-C-H_{eq}$ junction involved in chair conformations. In contrast, average values of 11.5 and 13.2 Hz were observed for the $^3J_{H/P}$ coupling constants involving the 5′- and 5″-H protons of *trans*-**7**, thus suggesting that the dioxaphosphorinane structure of this isomer is in a twist-chair conformation. By flipping from the chair conformation to the twist-chair conformation, the trans isomer should minimize the repulsive electrostatic interaction between the lone pairs of the endocyclic phosphate oxygens and the O4′ sugar atom (Figure 3).

The puckering of the 2′-deoxyribose moieties of *cis*-**7** and *trans*-**7** was assigned by examination of the sugar ring H/H coupling constants (Table 2). Whereas the $H_{3'}/H_{4'}$ coupling constant cannot be used to clearly establish the puckering of the lower nucleosides, the small $J_{H3'/H4'}$ (~2 Hz) measured for the upper 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.[10]

To further investigate the overall conformation of dinucleotides *cis*-**7** and *trans*-**7**, we carried out a 2D

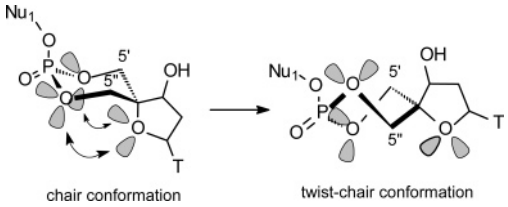


FIGURE 3. Schematic drawings showing electronic interactions favoring the flipping of *trans*-**7** from the chair conformation to the twist-chair conformation.

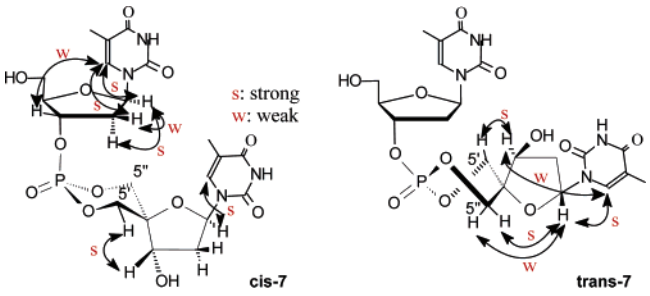


FIGURE 4. Schematic drawings of *cis*-**7** and *trans*-**7** showing the main NOE effects observed between the sugar, the base, and the dioxaphosphorinane subunits of α,β,γ-D-CNA.

TABLE 2. H/H Coupling Constants (Hz) in the ^{1}H NMR Spectra (400 MHz) of *cis*-7 and *trans*-7 α,β,γ-D-CNA

		coupling constant J (Hz)				
		J(1′,2′)		J(2′,3′)		J(3′,4′)
cis-**7**	upper nucleoside	5.8	8.4	6.0	2.1	2.0
	lower nucleoside	6.9	6.9	6.8	3.8	
trans-**7**	upper nucleoside	5.8	5.8	5.8	1.8	1.9
	lower nucleoside	5.9	5.9	5.5	2.1	

NOESY NMR study. NMR spectra of *cis*-**7** and *trans*-**7** were acquired at 400 MHz. Schematic views of both diastereoisomers showing the observed proton proximity relationships are given in Figure 4. The chair conformation of the dioxaphosphorinane ring of *cis*-**7** is corroborated by a single strong NOESY cross-peak observed between the H3′ of the lower nucleoside and the H5′ equatorial proton of the six-membered ring. The twist-chair conformation of *trans*-**7** is corroborated by the cross-peak exhibited by H1′ (of the lower nucleoside) with both of the H5″ protons. The strong cross-peaks displayed by each thymine H-6 proton with their corresponding sugar H1′ and H2′ protons are indicative of the relative position of the thymine bases toward the sugar rings. The empirical equation established by Lankhorst et al.[11] allowed us to determine, from the coupling constant $J_{H3'/P}$ (6.2 Hz for *cis*-**7** and 6.8 Hz for *trans*-**7**), the value of the torsional angle ϵ (−158° for *cis*-**7** and −155° for *trans*-**7**) and therefore the relative position of the upper nucleosides toward the dioxaphosphorinane ring. Although many cross-peaks are present for *cis*-**7** and *trans*-**7**, all of them are derived from intraresidual H/H interactions. This suggests that these dinucleotides have conforma-

(10) Wood, D. J.; Ogilvie, K. K.; Hruska, F. E. *Can. J. Chem.* **1975**, *53*, 2781.

(11) Lankhorst, P. P.; Haasnoot, C. A. G.; Erkelens, C.; Altona, C. J. *J. Biomol. Struct. Dynam.* **1984**, *1*, 1387. The torsional angle ϵ was determined by using the relation $\epsilon° = -\theta - 120°$ and θ was calculated with $J_{H3'/P} = 15.3\cos^2(\theta) - 6.1\cos(\theta) + 1.6$.

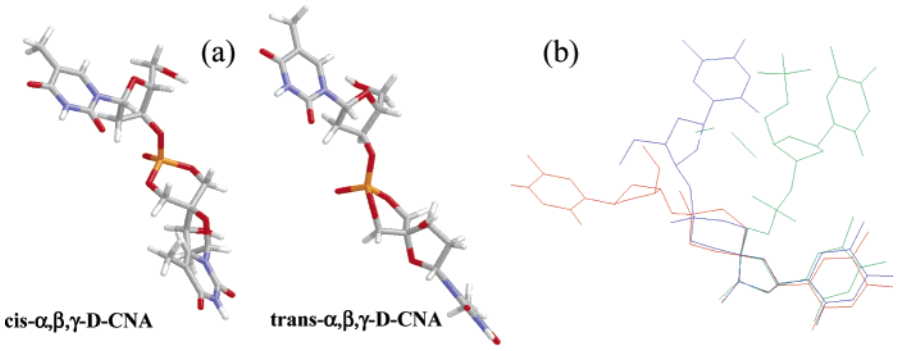


FIGURE 5. (a) Tentative model of the conformation of *cis*-**7** and *trans*-**7** α,β,γ-D-CNA derived from NMR data. (b) Superimposition of the structures of *cis*-**7** (blue) and *trans*-**7** (red) α,β,γ-D-CNA with an X-ray structure of unmodified pTpT[12] (green).

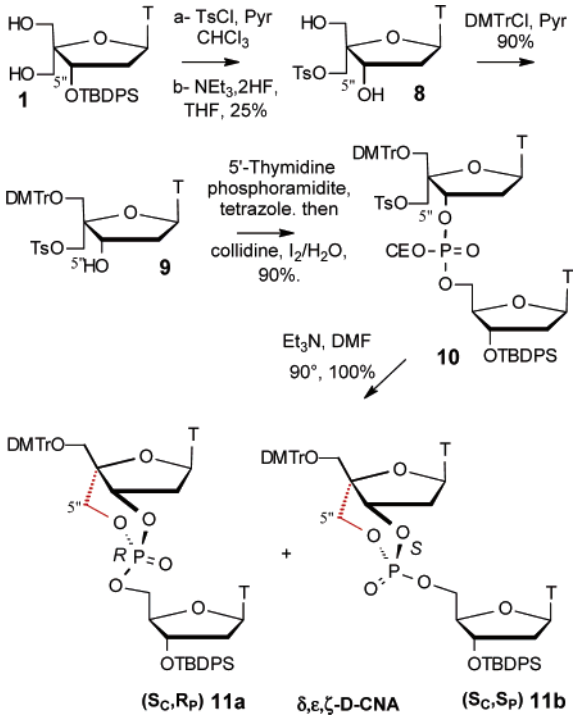
tions in which the distance between the protons of the upper and the lower nucleoside are greater than 4 Å. Thus, the observed NOESY spectra corroborate the $^3J_{H/P}$ evidence discussed above for the chair and twist-chair conformations of *cis*-**7** and *trans*-**7**. A hand-built model of both compounds constructed to fit the observed proximity relationships is shown in Figure 5a.

The chair and twist-chair conformations of cis and trans α,β,γ-D-CNA allow the stabilization of unusual conformational states {(g^-, g^-, g^-) and (g^+, c/g^+, g^-/a^-)}, which greatly differ from the canonical (g^-, t, g^+) backbone conformation adopted by the B- and A-forms of the DNA double helix (Table 1 and Figure 5b). Although a thorough analysis is required to precisely determine what structural consequences can be expected from these alternative backbone conformations, it is likely that once incorporated into DNA or RNA oligomers,[13] cis and trans α,β,γ-D-CNA dinucleotides will either favor the formation of unpaired secondary motifs or induce a significant conformational distortion from the ideal B- or A-form helical geometry.

Synthesis of ($S_{C4'}$,R_P) and ($S_{C4'}$,S_P) δ,ϵ,ζ-D-CNA. The 1/1 diastereoisomeric mixture of **11a** and **11b** was quantitatively obtained from the expected base-induced cyclization of the *O*-5″-tosylated acyclic precursor **10** (Scheme 2). The 1/1 mixture of P epimers of **10** was generated (following the standard procedure) from the coupling reaction of the diastereopure (4′*S*)-*C*-tosyloxymethylthymidine **9** with 5′-thymidine phosphoramidite. Diastereomerically pure nucleoside **9** was prepared through the selective tosylation of the 5″-OH function of **1**, followed by removal of the *tert*-butyldiphenylsilyl group (producing **8** in 25% combined yield) and dimethoxytritylation of the remaining primary 5″-hydroxyl function.

Although the synthetic pathway of Scheme 2 gives access to the easily separable, fully protected, diastereoisomers **11a** and **11b**, the overall yield from **1** is low (18%). This is due to the selective tosylation of **1** which, under standard conditions, proceeds only with limited success. In an attempt to increase the overall yield of **11**, we decided to take advantage of the greater reactivity of the 5″-hydroxyl function later in the synthetic pathway, i.e., right at the cyclization step producing the dioxaphosphorinane structure (dinucleotide **14** in Scheme 3). Thus, we applied the well-known phosphotriester methodology[14] in the presence of the 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) as an activating agent[15] to quantitatively prepare the ($S_{C4'}$,R_P) and ($S_{C4'}$,S_P) diastereoisomers **15a** and **15b**, respectively. The acyclic phosphodiester **14** was obtained in a good overall yield (80%) from starting nucleoside **1** in a five-step sequence (Scheme 3). Dimethoxytritylation of both primary hydroxyl functions of **1** followed by desilylation of the O3′ oxygen atom (with tetrabutylamonium fluoride) gave nucleoside **12** in a high combined yield.[16] After coupling **12** with a thymidine bearing a phosphoramidite function at the 5′ position, both dimethoxytrityl groups were removed under acidic conditions to give the expected 1/1 diastereoisomeric

SCHEME 2. Synthesis of ($S_{C4'}$,R_P) and ($S_{C4'}$,S_P) δ,ϵ,ζ-D-CNA



(12) Camerman, N.; Fawcett, J. K.; Camerman, A. *J. Mol. Biol*. **1976**, *107*, 601.

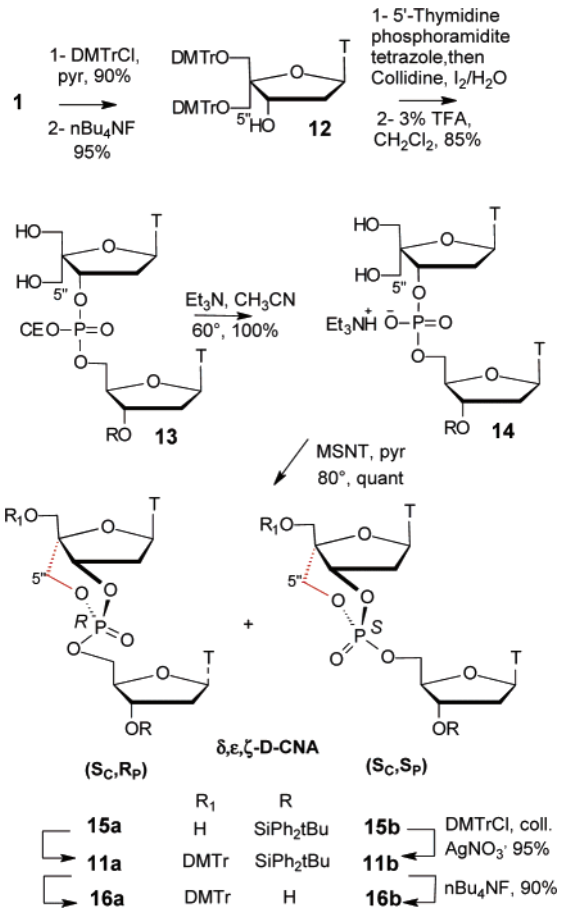
(13) In progress. Note that in contrast to acyclic or macrocyclic phosphotriesters, the dioxaphosphorinane structure is very stable in concentrated aqueous ammonia and is therefore perfectly compatible with automated synthetic procedures.

(14) Sonveaux, E. *Bioorg. Chem*. **1986**, *14*, 274.

(15) (a) Reese, C. B.; Titmas, R. C.; Yan, L. *Tetrahedron Lett*. **1978**, 2727. (b) Jones, S. S.; Rayner, B.; Reese, C. B.; Ubasawa, A.; Ubasawa, M. *Tetrahedron* **1980**, *36*, 3075.

(16) Thrane, H.; Fensholdt, J.; Regner, M.; Wengel, J. *Tetrahedron* **1995**, *51*, 10389.

SCHEME 3. Improved Synthesis of ($S_{C4'}$,R_P) and ($S_{C4'}$,S_P) δ,ϵ,ζ-D-CNA



mixture of **13**. The negatively charged phosphodiester was released by treatment of **13** with triethylamine, producing the acyclic precursor **14** which, in the presence of MSNT in hot pyridine, quantitatively cyclized into the 1/1 diastereoisomeric mixture of **15a** and **15b** as observed by ^{31}P NMR (δp −2.3 and −3.8).

That the dioxaphosphorinane ring was actually formed by reaction of the 5″-hydroxyl group was shown by recovering the 1/1 diastereoisomeric mixture of **11a** and **11b** upon dimethoxytritylation of **15**. Finally, removal of the *tert*-butyldiphenylsilyl protective group with floride ion provided the ($S_{C4'}$,R_P) and ($S_{C4'}$,S_P) diastereoisomers **16a** and **16b**, respectively, which are ready to be converted into their corresponding phosphoramidite building blocks for oligonucleotide synthesis purposes. This synthetic pathway allows for the preparation of **16a** and **16b** in 55% overall yield from **1** compared with 18% for the first approach described in Scheme 2.

Structural Assignment of δ,ϵ,ζ-D-CNA. The determination of the absolute configuration at the newly created asymmetric centers 4′-C and P of δ,ϵ,ζ-D-CNA **11**, **15**, and **16** was greatly simplified by the X-ray structure solved for **15b** identified as the ($S_{C4'}$,S_P) diastereoisomer of **15** (Figure 6). This crystal structure gives further evidence that the dioxaphosphorinane ring system was formed via the reaction of the 5″-hydroxyl function of **14**. The major information revealed by the X-ray structure of **15b** is the twist-chair conformation of the six-membered cyclic phosphotriester with dihedral

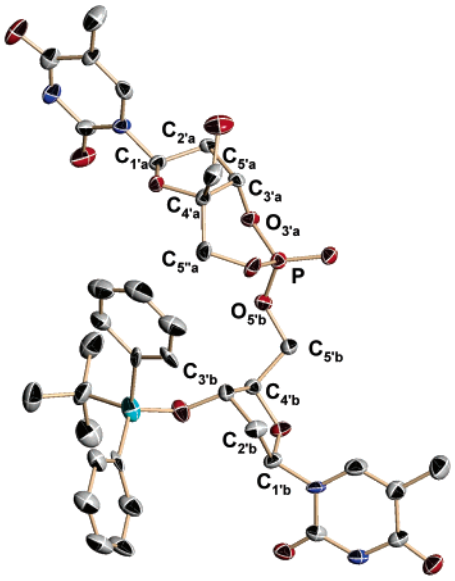


FIGURE 6. X-ray molecular structure of ($S_{C4'}$,S_P) δ,ϵ,ζ-D-CNA **15b** with the adopted numbering scheme, displaying the twist-chair and C2′-endo conformations of the dioxaphosphorinane and sugar subunits, respectively. For clarity, hydrogen atoms and cocrystallized solvent molecules have been omitted and thermal ellipsoids are shown at 40% probability.

TABLE 3. H/H Coupling Constants (Hz) in the ^{1}H NMR Spectra (400 MHz) of 15a and 15b

		coupling constant J (Hz)				
		J(1′,2′)		J(2′,3′)		J(3′,4′)
15a	upper nucleoside	5.6	8.9	5.4	0	
	lower nucleoside	6.2	7.5	5.4	2	<1
15b	upper nucleoside	5.5	9.2	6.2	0	
	lower nucleoside	6.1	8.0	6.5	2.7	2.5

angle values δ = 140.7°, ϵ = −62.4°, and ζ = 152.5°. Both sugar rings are in the C2′-endo conformation, which is typical of B-DNA, with δ = 140.7° for the fused dioxaphosphorinane/sugar system and δ = 149.9° for the unmodified sugar moiety. The C2′-endo conformation is also observed for the 2′-deoxyribose units of **15a** as outlined by the analysis of the coupling constants of the sugar ring protons of **15a** and **15b** (Table 3).

The solid-state structure of **15b** agrees with the solution-state structure of **11** and **15** obtained by NMR spectroscopy.[17] The $^3J_{H/P}$ coupling constants measured for the H3′ and H5″ protons of the dioxaphosphorinane unit in all compounds have intermediate values ranging between 6.3 and 10.7 Hz (H3′) and between 11.6 and 16.8 Hz (H5″), indicating that neither the ($S_{C4'}$,S_P) nor the ($S_{C4'}$,R_P) stereoisomers of **11** and **15** are in a true chair conformation. From the inspection of hand-built models, it appears that the ($S_{C4'}$,R_P) stereoisomers of **11**, **15**, and **16** (i.e., **11a**, **15a**, and **16a**) might well adopt a chair conformation with the favorable 1,2-trans diequatorial orientation of the C2′ and C5′ sugar atoms, and with both sugar rings in a C2′-endo conformation. However, as already mentioned in the case of *trans*-**7**, this arrangement probably leads to important repulsive electrostatic interactions between the lone pairs of the axially oriented

(17) Sartillo-Piscil, F.; Cruz, S.; Sánchez, M.; Höpfl, H.; Anaya de Parrodi, C.; Quintero, L. *Tetrahedron* **2003**, *59*, 4077.

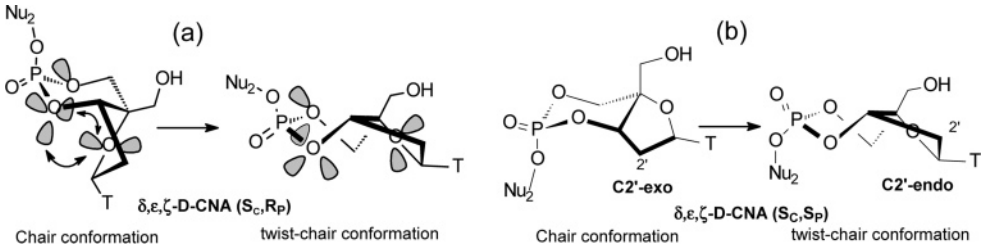


FIGURE 7. Conformation within the dioxaphosphorinane ring of ($S_{C4'}$,R_P) δ,ϵ,ζ-D-CNA (a) and ($S_{C4'}$,S_P) δ,ϵ,ζ-D-CNA (b).

O4′ sugar atom and the two endocyclic phosphate oxygens (Figure 7a). By flipping from the chair to the twist-chair conformation, the ($S_{C4'}$,R_P) stereoisomers **11a**, **15a**, and **16a** should minimize these interactions. On the other hand, the ($S_{C4'}$,S_P) stereoisomers **11b**, **15b**, and **16b** are unlikely to adopt a chair conformation based on the unfavorable 1,2-trans diaxial orientation of the C2′ and C5′ sugar atoms and the required 2′-exo conformation of the fused sugar unit (Figure 7b).

Thus, based on the X-ray structure of **15b** and the careful examination of the $^3J_{H/P}$ coupling constants for the P-coupled CH and CH_2 signals of **11**, **15**, and **16**, we were able to establish conclusively the conformation of the backbone torsional angles δ (C5′−C4′−C3′−O3′), ϵ (C4′−C3′−O3′−P), ζ (C3′−O3′−P−O5′) as (a^+/t, g^-, a^+/t) and (a^+/t, g^-, g^-) for the ($S_{C4'}$,S_P) and ($S_{C4'}$,R_P) diastereoisomers, respectively. When compared with the backbone conformations summarized in Table 1, we can expect that DNA oligonucleotides incorporating the ($S_{C4'}$,S_P) dinucleotide element would form B-type duplexes with unique B_{II}-like substates conformationally fixed. If this structural feature is confirmed this compound might find important applications in determining the role played by the unusual B_{II} substate in DNA curvature as well as in structures of protein-bound DNA.[18] On the other hand, the ($S_{C4'}$,R_P) stereoisomer exhibits an even more unusual ϵ/ζ combination (ϵ − ζ = 0°), which could represent an important transient substate between the B_I (ϵ − ζ < 0°, centered around −90°) and B_{II} (ϵ − ζ > 0°, centered around +90°) conformations of the DNA backbone.

Conclusion

We have synthesized two new members of the D-CNA family in which the backbone torsional angles (α, β, γ) or (δ, ϵ, ζ) of nucleic acids are simultaneously locked in a dioxaphosphorinane ring structure. Structural analysis indicates that cis and trans α,β,γ-D-CNA have α, β, and γ torsions locked in the (g^-, g^-, g^-) and (g^+, c/g^+, g^-/a^-) conformations, respectively, while the two diastereoisomers ($S_{C4'}$,R_P) and ($S_{C4'}$,S_P) of δ,ϵ,ζ-D-CNA have δ, ϵ, and ζ torsions locked in the (a^+/t, g^-, g^-) and (a^+/t, g^-, a^+/t) conformations. As can be seen, these structures are associated with very different backbone conformations. 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 and we are currently examining this possibility with α,β,γ- and δ,ϵ,ζ-D-CNA.

(18) Djuranovic, D.; Hartmann, B. *Biopolymers* **2004**, *73*, 356.

Experimental Section

3′-*O*-*tert*-Butyldiphenylsilyl-4′-*C*-(hydroxymethyl)thymidine (1) was prepared (45% yield) according to the procedure described by Jones and co-workers[9b] (see compound **28a**) and purified by silica gel chromatography with use of a 1/1 mixture of ethyl acetate/ dichloromethane as eluent. TLC, R_f (CH_2Cl_2/AcOEt 1/1) = 0.3. ^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm} 9.67 (s, 1H, NH); 7.66−7.63 (m, 4H, ph); 7.46−7.35 (m, 6H, ph); 6.86 (s, 1H, H_6); 6.39 (dd, 1H, J = 7.6, 4.3 Hz, $H_{1'}$); 4.71 (t, 1H, J = 7.5 Hz, $H_{3'}$); 3.94, 3.92, 3.62 and 3.42 (A and B part of AB systems, 4H, J = 12.5 Hz, $H_{5'}$ and $H_{5''}$); 2.33 and 1.77 (m, 2H, $H_{2'}$); 1.66 (s, 3H, Me_7); 1.08 (s, 9H, tBu). ^{13}C NMR (63 MHz, $CDCl_3$) δ_{ppm} 164.2; 150.9; 135.8; 133.0; 132.4; 130.3; 127.9; 111.1; 88.8; 83.4; 72.7; 62.6; 62.2; 39.7; 26.9; 19.1; 12.3. Anal. Calcd (found): C 63.51 (63.71), H 6.71 (6.74), N 5.49 (5.22).

4′-*C*(*S*)-(4,4′-Dimethoxytrityloxymethyl)-3′-*O*-*tert*-butyldiphenylsilylthymidine (2). To a solution of 3′-*O*-*tert*-butyldiphenylsilyl-4′-*C*-(hydroxymethyl)thymidine (**1**) (1.0 g, 1.96 mmol) in anhydrous pyridine (10 mL) is added dimethoxytrityl chloride (0.9 g, 2.65 mmol). After 12 h of stirring, the reaction is stopped by addition of a saturated aqueous solution of NH_4Cl (20 mL) and extracted with ethyl acetate. The organic layer is washed with water and brine and dried over $MgSO_4$. After removal of the solvent under reduced pressure, the crude product was chromatographed on silica gel with ethyl acetate as solvent. Compound **2** is obtained as a white foam (m = 1.11 g, 70% yield). Its isomer (m = 223 mg, 20%) bearing the dimethoxytrityl group on the 5′-O position was also collected (R_f = 0.16). TLC, R_f (CH_2Cl_2/AcOEt 4/1) = 0.23. ^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm} 8.60 (s, 1H, NH); 7.47−7.21 (m, 20H, ph and H_6); 6.84−6.79 (m, 4H, ph); 6.32 (t, 1H, J = 6.3 Hz, $H_{1'}$); 4.49 (t, 1H, J = 6.6 Hz, $H_{3'}$); 3.84 (A part of an ABX system, 1H, J = 4.6, 11.9 Hz, $H_{5'}$); 3.77 (s, 6H, OMe); 3.59 (A part of an AB system, 1H, J = 10.4 Hz, $H_{5''}$); 3.26 (B part of an ABX system, 1H, J = 7.3, 11.9 Hz, $H_{5'}$); 3.09 (B part of an AB system, 1H, J = 10.4 Hz, $H_{5''}$); 2.39 (m, 1H, $H_{2'}$); 1.98 (m, 1H, $H_{2'}$); 1.84 (s, 3H, Me_7); 0.91 (s, 9H, tBu). ^{13}C NMR (63 MHz, $CDCl_3$) δ_{ppm} 164.4; 158.5; 150.5; 144.9; 136.6; 135.8; 135.7; 132.9; 132.8; 130.1; 128.2; 128.0; 127.8; 126.8;113.3; 111.8; 88.7; 86.6; 84.2; 72.7; 64.7; 63.6; 55.2; 40.3; 26.9; 19.1; 12.5. Anal. Calcd (found): C 70.91 (71.26), H 6.45 (6.74), N 3.45 (3.14).

5′-*O*-Tosyl-4′-*C*(*R*)-hydroxymethyl-3′-*O*-*tert*-butyldiphenylsilylthymidine (3). To 4′-*C*(*S*)-(4,4′-dimethoxytrityloxymethyl)-3′-*O*-*tert*-butyldiphenylsilylthymidine (**2**) (560 mg, 0.69 mmol) dissolved in anhydrous pyridine is added at 0 °C the tosyl chloride (264 mg, 1.38 mmol). Stirring was maintained for 12 h and the reaction mixture was diluted with ethyl acetate and washed with a saturated aqueous solution of NH_4Cl. The organic layer is collected and washed with water and brine and dried over $MgSO_4$. After removal of the solvent and pyridine under reduced pressure the crude product was diluted with a solution of trifluoroacetic acid (3%) in dichloromethane (50 mL) and stirred for 6 h. After evaporation of the solvent and trifluoroacetic acid under high vacuum, the crude material is diluted with ethyl acetate (150 mL) and washed twice with a saturated aqueous solution of $NaHCO_3$ (50 mL), and with water and brine. After silica gel chromatography eluted with ethyl acetate/dichloromethane 1/1, compound **3** was recovered

as a white foam (458 mg, 90% yield). TLC, R_f (CH_2Cl_2/AcOEt 1/1) = 0.44. ^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm} 9.35 (s, 1H; NH); 7.65 (A part of an AB system, 2H, J = 8.6 Hz, ph Ts); 7.61−7.57 (m, 4H, ph); 7.46−7.35 (m, 6H; ph); 7.27 (B part of an AB system, 2H, J = 8.6 Hz, ph Ts); 7.13 (s, 1H, H_6); 6.36 (t, 1H, J = 6.7; $H_{1'}$); 4.59 (dd, 1H; J = 4.3; 7.0; $H_{3'}$); 4.14 (A part of an AB system, 1H, J = 12.4 Hz, $H_{5'}$); 3.89 (A part of an AB system, 1H, J = 12.5 Hz, $H_{5''}$); 3.87 (B part of an AB system, 1H, J = 12.4 Hz, $H_{5'}$); 3.58 (B part of an AB system, 1H, J = 12.5 Hz, $H_{5''}$); 2.42 (s, 3H, Me Ts); 2.28 (A part of an ABX system, 1H, J = 4.3, 6.7, 13.3 Hz; $H_{2'}$); 1.89 (m, 1H, $H_{2'}$); 1.85 (s, 3H; Me); 1.06 (s, 9H; tBu). ^{13}C NMR (63 MHz, $CDCl_3$) δ_{ppm} 163.8; 150.4; 145.3; 135.7; 135.2; 132.4; 131.9; 130.5; 130.0; 128.1; 127.8; 111.8; 86.6; 83.3; 73.4; 70.1; 62.7; 40.5; 30.9; 26.9; 21.7; 19.1; 12.3. Anal. Calcd (found): C 61.42 (61.22), H 6.06 (6.01), N 4.21 (4.24).

Cyanoethyl 3′-*O*-(5′-*O*-Dimethoxytrityl)thymidinyl-4′-*C*(*R*)-oxymethyl(3′-*tert*-butyldiphenylsilyl)-5′-*O*-tosylthymidinyl Phosphoric Ester (mixture of diastereoisomers) (4). 5′-*O*-tosyl-4′-*C*(*R*)-hydroxymethyl-3′-*O*-*tert*-butyldiphenylsilylthymidine **(3)** (500 mg, 0.75 mmol), 3′-*O*-thymidine phosphoramidite (1.12 g, 1.5 mmol), and freshly sublimed tetrazole (525 mg, 7.5 mmol) were dissolved with anhydrous acetonitrile (5 mL) and stirred for 20 min at room temperature. After addition of collidine (454 μL, 3.0 mmol), the phosphite was oxidized with iodine [0.1 M solution in THF(2)/H_2O(1)] until the dark brown color persists. The reaction mixture was diluted with ethyl acetate and washed with an aqueous solution of sodium thiosulfate (15%) to remove an excess of iodine. The organic layer was washed with water and brine and the solvent was removed in vacuo. The crude material was chromatographed on silica gel with ethyl acetate as solvent. After evaporation of the solvent, compound **4** (mixture of diastereoisomers) was recovered as a white foam (0.79 g, 80% yield). TLC, R_f (AcOEt/CH_2Cl_2 1/1) = 0.25. ^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm} 9.46, 9.41, 9.35, 9.31 (4s, 4H, NH); 7.64−6.81 (m, 58H, ph and H_6); 6.44−6.29 (m, 4H, $H_{1'}$); 5.18 (m, 2H, $H_{3'a}$); 4.60−4.55 (m, 2H, $H_{3'b}$); 4.54−4.37 and 4.27−4.00 (m, 8H, $H_{5'b}$ and $H_{5''b}$); 4.26 (br s, 2H, $H_{4'}$); 3.86 (m, 4H, CH_2OP); 3.77 (s, 12H, OCH_3); 3.48−3.33 (m, 4H, $H_{5'a}$); 2.75 and 2.58 (2t, 4H, NCCH_2); 2.76−2.30 (m, 4H, $H_{2'}$); 2.42 (br s, 6H, CH_3 Ts); 2.04−1.86 (m, 2H, $H_{2'}$); 1.80 and 1.37 (s, 12H, Me_7); 1.29−1.19 (m, 2H, $H_{2'}$); 1.08, 1.06 (2s, 18H, tBu). ^{13}C NMR (63 MHz, $CDCl_3$) δ_{ppm} 163.7; 158.8; 150.5; 149.9; 145.3; 144.0; 136.6; 135.8; 134.9; 132.6; 130.4; 131.9; 130.4; 130.2; 130.0; 128.1; 127.3; 116.3; 113.3; 111.8; 111.2; 87.3; 86.9; 85.8; 84.3; 76.6; 74.3; 68.6; 62.3; 55.3; 39.3; 30.9; 26.8; 21.7; 19.5; 19.1; 14.2; 12.3; 11.6. ^{31}P NMR (81 MHz, $CDCl_3$) δ_{ppm} −2.06; −3.05; MS (FAB) 1362 (MK^+, 5%); 1346 (MNa^+, 84%); 1323 (M^+, 13%).

5′-*O*-Dimethoxytrityl-3′-*O*-*tert*-butyldiphenylsilyl-α,*β*,*γ*-CNA *cis*-(5) and *trans*-(5). To a solution of **4** (150 mg, 0.11 mmol) in anhydrous DMF (4 mL) was added K_2CO_3 (62 mg, 0.45 mmol). After 2 h of heating at 90 °C the reaction mixture is cooled and diluted with ethyl acetate (50 mL) and washed three times with water (3 × 10 mL) and once with brine (10 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed in vacuo. The cyclic phosphotriesters **5** and **6** (2/1 ratio) were recovered as a white foam (123 mg, 100% yield). TLC, R_f (AcOEt/CH_2Cl_2 1/1) = 0.2. ^{31}P NMR (81 MHz, $CDCl_3$) δ_{ppm} −7.8; −9.6. ^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm} 9.19, 9.16, 9.07 and 9.06 (4s, 4H, NH); 7.74−7.26 (m, 40H, ph and H_6); 7.00 (d, 1H, H_6); 6.87−6.84 (m, 8H, ph); 6.82 (d, 1H, H_6); 6.51−6.46 (m, 2H, $H_{1'}$); 6.11−6.07 (m, 2H, $H_{1'}$); 5.29 (m, 1H, $H_{3'a}$); 5.20 (m, 1H, $H_{3'a}$); 4.85 (m, 1H, $H_{3'b}$); 4.66 (m, 1H, $H_{4'a}$); 4.59−4.41 (m, 4H, $H_{5'b}$ and $H_{5''b}$); 4.38 (q, 1H, J = 4.8; 7.2 Hz, $H_{3'b}$); 4.33−4.18 (m, 2H, $H_{5'b}$ or $H_{5''b}$); 3.98−3.84 (m, 2H, $H_{5'b}$ or $H_{5''b}$); 3.81 and 3.80 (2s, 12H, OMe); 3.55−3.41 (m, 4H, $H_{5'a}$); 2.73−2.41 (m, 6H, $H_{2'}$); 2.31−2.15 (m, 2H, $H_{2'}$); 1.88, 1.83, 1.43 and 1.40 (4d, 12H, H_7); 1.12 and 1.11 (2s, 18H, tBu). ^{13}C NMR (63 MHz, $CDCl_3$) δ_{ppm} 164.2; 164.0; 159.0; 158.8; 150.8; 150.2; 150.0; 144.2; 139.7; 136.1; 136.0; 135.9; 135.2; 133.1. 133.0; 132.3; 132.1; 131.0; 130.8; 130.6; 130.3. 128.5; 128.0; 127.5; 127.3; 113.6; 113.4; 112.0; 111.8. 111.4; 111.1. 88.0; 87.6; 87.5; 86.0. 85.5; 84.9; 84.5, 81.3; 81.1. 78.3; 74.2; 73.9; 72.1; 63.6; 62.1; 55.5; 40.2; 39.9; 37.9; 27.1; 19.4; 19.3; 15.5; 12.7; 12.6; 12.5; 11.9. MS (electrospray) 1121 (100%, $[M + Na]^+$), 1137 (10%, $[M + K]^+$).

5′-*O*-Dimethoxytrityl-α,*β*,*γ*-CNA *cis*-(6) and *trans*-(6). To compounds **5a** and **5b** (110 mg; 0.1 mmol) dissolved in anhydrous THF (2 mL) is added at room temperature the tetrabutylammonium fluoride (150 μL, 0.15 mmol). Stirring was maintained for 1 h. After removal of the solvent under reduce pressure the crude product was deposed on a silica gel column and eluted with ethyl acetate 3% methanol. Compounds α,*β*,*γ*-CNA *cis*-**6** and *trans*-**6** were recovered as a white foam (73 mg, 85% yield). *cis*-**6** was separated from *trans*-**6** by means of reverse-phase HPLC (Kromasil C18, ϕ 20 mm, 250 mm) with CH_3CN/H_2O 1/1 as solvent. Data for *cis*-**6**: HPLC: Tr = 4.22 min. Probably due to the formation of aggregates in solution, the NMR spectra were obtained with a very poor resolution for ^{1}H and without detection of the aliphatic carbon for ^{13}C. This phenomenon has already been observed on the corresponding derivative of α,*β*-CNA (see Supporting Information of ref 7). ^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm} 7.56 (m, 1H, H_6); 7.31−7.22 (m, 10H, ph and H_6); 6.83−6.79 (m, 4H, ph); 6.44 (m, 1H, $H_{1'}$); 6.13 (m, 1H, $H_{1'}$); 5.19 (m, 1H, $H_{3'}$); 4.70−4.20 (m, 6H); 3.76 (s, 6H, OMe); 3.46 (m, 1H, $H_{5'a}$); 2.63−2.25 (m, 4H, $H_{2'}$); 1.86 and 1.37 (2s, 6H, H_7). ^{13}C NMR (63 MHz, $CDCl_3$) δ_{ppm} 164.1; 163.2; 150.9; 150.7; 144.1; 135.0; 129.9; 128.3; 113.3; 111.9; 111.5; 88.1; 80.4; 55.3. ^{31}P NMR (81 MHz, $CDCl_3$) δ_{ppm} −9.4. MS (electrospray) 883.1 ($[M + Na]^+$); 899.1 ($[M + K]^+$). Data for *trans*-**6**: HPLC: Tr = 3.17 min. Same remark as for *cis*-**6** (above) concerning the lack of resolution of the ^{1}H and ^{13}C NMR spectra. ^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm} 7.58 (m, 1H, H_6); 7.32−7.21 (m, 9H, ph); 7.00 (m, 1H, H_6); 6.84−6.80 (m, 4H, ph); 6.42 (m, 1H, $H_{1'}$); 6.06 (m, 1H, $H_{1'}$); 5.20 (m, 1H, $H_{3'}$); 4.69−4.20 (m, 6H); 3.76 (s, 6H, OMe); 3.43 (m, 1H, $H_{5'a}$); 2.65 and 2.44 (m, 4H, $H_{2'}$); 1.81 and 1.38 (2s, 6H, H_7). ^{13}C NMR (63 MHz, $CDCl_3$) δ_{ppm} 164.2; 163.2; 151.1; 150.8; 144.1; 135.1; 130.1; 128.1; 113.4; 111.9; 111.4; 87.3; 80.7; 55.3. ^{31}P NMR (81 MHz, $CDCl_3$) δ_{ppm} −7.4. MS (electrospray) 883.1 ($[M + Na]^+$); 899.2 ($[M + K + Na]^+$).

α,*β*,*γ*-CNA *cis*-7 and α,*β*,*γ*-CNA *trans*-7. Compound *cis*-**6** (45 mg, 0.052 mmol) (or *trans*-**6**, 32 mg, 0.037 mmol) is dissolved in a solution of trifluoroacetic acid (3%) in dichloromethane (2 mL) at room temperature. After 15 min the red solution is evaporated to dryness. The crude material is dissolved with THF and submitted to silica gel chromatography. It was first eluted with ethyl acetate to remove the dimethoxytrityl residue and then with ethyl acetate/methanol (20%) to collect the α,*β*,*γ*-CNA *cis*-**7** (29 mg, yield: 99%) or α,*β*,*γ*-CNA *trans*-**7** (20 mg, 98% yield) obtained as a white foam after evaporation of the solvent. Data for *cis*-**7**: TLC, R_f (AcOEt, 20% MeOH) = 0.25. ^{31}P NMR (200 MHz, CD_3OD) δ_{ppm} −6.05. ^{1}H NMR (400 MHz, CD_3OD) δ_{ppm} 7.83 (d, 1H, J = 1.2 Hz, H_{6a}); 7.51 (d, 1H, J = 1.2 Hz, H_{6b}); 6.36 (dd, 1H, J = 5.8; 8.4 Hz, $H_{1'a}$); 6.31 (t, 1H, J = 6.9 Hz, $H_{1'b}$); 5.13 (tt, 1H, J = 2.0; 6.0; Hz, $J_{3'a/P}$ = 6.2 Hz, $H_{3'a}$); 4.68 (A part of an ABX, 1H, J = 11.9 Hz, $J_{5'bax/P}$ = 2.1 Hz, $H_{5'bax}$); 4.66 (A part of an ABX, 1H, J = 12.3 Hz, $J_{5''bax/P}$ = 1.9 Hz $H_{5''bax}$); 4.50 (B part of an ABX(Y), 1H, J = 12.3; 2.9 Hz, $J_{5''beq/P}$ = 22.0 Hz, $H_{5''beq}$); 4.46 (B part of an ABX(Y), 1H, J = 11.9; 2.9 Hz, $J_{5'beq/P}$ = 22.0 Hz, $H_{5'beq}$); 4.27 (∼q, 1H, J = 2.0; 3.1 Hz, $H_{4'a}$); 4.12 (X part of an ABX, 1H, J = 3.8; 6.6 Hz $H_{3'b}$); 3.84 (d, 1H, J = 3.2 Hz, $H_{5'a}$); 2.59 (A part of an ABX(Y), 1H, J = 14.1; 5.8; 2.1 Hz, $H_{2'a}$); 2.51 (B part of an ABX(Y), 1H, J = 14.0, 6.9; 6.8 Hz, $H_{2'b}$); 2.44 (B part of an ABX(YZ), 1H, J = 14.2; 8.4; 6.1 Hz, $J_{2'a/P}$ = 1.1 Hz, $H_{2'a}$); 2.39 (A part of an ABX(Y), 1H, $J_{2'b1/2'b2}$ = 14.0; 6.7; 3.8 Hz, $H_{2'b}$); 1.92 (d, 3H, J = 1.1 Hz, H_{7b}); 1.90 (d, 3H, J = 1.2 Hz, H_{7a}). ^{13}C NMR (100 MHz, CD_3OD) δ_{ppm} 165.3 (C_{4a}, C_{4b}); 151.4 (C_{2a}); 151.1 (C_{2b}); 137.5 (C_{6b}); 136.9 (C_{6a}); 110.9 (C_{5a}, C_{5b}); 86.8 ($C_{1'b}$); 86.1 ($C_{4'a}$); 85.0 ($C_{1'a}$); 81.3 ($C_{4'b}$); 79.0 ($C_{3'a}$); 74.0 ($C_{5'b}$); 72.9 ($C_{5''b}$); 71.9 ($C_{3'b}$); 61.6 ($C_{5'a}$); 39.1 ($C_{2'b}$); 38.5 ($C_{2'a}$); 11.4 (C_{7a}, C_{7b}). Anal. Calcd (found): N 10.03 (9.98); C

45.17 (45.01); H 4.87 (4.92). Data for *trans*-**7**: TLC, R_f (AcOEt, 20% MeOH) = 0.25. ^{31}P NMR (200 MHz, CD_3OD) δ_{ppm} −4.77. ^{1}H NMR (400 MHz, CD_3OD) δ_{ppm} 7.80 (d, 1H, J = 1.2 Hz, H_{6a}); 7.44 (d, 1H, J = 1.2 Hz, H_{6b}); 6.34 (t, 1H, J = 5.9 Hz, $H_{1'b}$); 6.32 (t, 1H, J = 5.8 Hz, $H_{1'a}$); 5.14 (tt, 1H, J = 1.8; 5.8 Hz, $J_{3'a/P}$ = 6.8 Hz, $H_{3'a}$); 4.74 (B part of an ABXY, 1H, J = 11.5; 13.2; 1.0 Hz, $H_{5''beq}$); 4.58 (B part of an ABXY, 1H, J = 11.7 Hz, $J_{5'beq/P}$ = 13.1 Hz, $J_{5'beq/5''beq}$ = 1.0 Hz, $H_{5'beq}$); 4.55 (X part of an ABX, 1H, J = 2.1; 5.5 Hz, $H_{3'b}$); 4.47 (A part of an ABX, 1H, J = 11.7; 11.5 Hz, $H_{5'bax}$); 4.42 (A part of an ABX, 1H, J = 11.5 Hz, $J_{5''bax/P}$ = 11.2 Hz, $H_{5''bax}$); 4.24 (∼q, 1H, J = 1.9; 3.1 Hz, $H_{4'a}$); 3.78 (A part of an ABX, 1H, J = 12.1; 3.0 Hz, $H_{5'a}$); 3.75 (B part of an ABX, 1H, J = 12.1; 3.1 Hz, $H_{5'a}$); 2.59 (A part of an ABX(Y), 1H, J = 13.8; 5.7; 5.5 Hz, $H_{2'b}$); 2.56 (A part of an ABX(Y), 1H, J = 14.2; 5.7; 1.7 Hz $H_{2'a}$); 2.39 (B part of an ABX(YZ), 1H, J = 14.2; 5.9 Hz, $J_{2'a/P}$ = 1.4 Hz, $H_{2'a}$); 2.33 (B part of an ABX(Y), 1H, J = 13.8; 5.7; 2.1 Hz, $H_{2'b}$); 1.91 (d, 1H, J = 1.1 Hz, H_{7b}); 1.89 (d, 1H, J = 1.2 Hz, H_{7a}). ^{13}C NMR (100 MHz, CD_3OD) δ_{ppm} 165.3 (C_{4a}, C_{4b}); 151.3 (C_{2a}, C_{2b}); 137.1 (C_{6b}); 136.9 (C_{6a}); 111.2 (C_{5b}); 110.9 (C_{5a}); 86.4 ($C_{1'b}$); 86.1 ($C_{4'a}$); 85.1 ($C_{1'a}$); 80.6 ($C_{4'b}$); 80.1 ($C_{3'a}$); 70.6 ($C_{5'b}$); 72.6 ($C_{5''b}$); 71.5 ($C_{3'b}$); 61.6 ($C_{5'a}$); 38.2 ($C_{2'b}$); 38.1 ($C_{2'a}$); 11.5 (C_{7b}); 11.4 (C_{7a}). Anal. Calcd (found): N 10.03 (10.21); C 45.17 (45.42); H 4.87 (4.65).

4′-*C*(*S*)-Tosyloxymethylthymidine (8). Compound **1** (500 mg, 0.98 mmol) was diluted in anhydrous pyridine (1.7 mL) and chloroform (3 mL), then tosyl chloride was added at 0 °C (187 mg, 0.98 mmol). After 12 h of stirring the reaction mixture was diluted with ethyl acetate and washed with a saturated aqueous solution of NH_4Cl. The organic layer is collected and washed with water and brine and dried over $MgSO_4$. After removal of the solvent and pyridine under reduced pressure the crude material is submitted to NEt_3·2HF (386 μL, 2.48 mmol) in THF (7 mL) for 15 h at 60 °C. The reaction medium was cooled to 0 °C and the reaction was stopped by addition of a saturated aqueous solution of $NaHCO_3$ (20 mL) and extracted with ethyl acetate. The organic layer was washed with water and brine and dried over $MgSO_4$. After evaporation of the solvent the crude material was chromatographed on silica gel with a first elution with ethyl acetate/dichloromethane (1/1) as solvent and then with ethyl acetate to collect compound **8** (104 mg, 25% yield) as a white foam after evaporation of the solvent. TLC, R_f (AcOEt) = 0.20. ^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm} 9.05 (s, 1H; NH); 7.75 (A part of an AB system, 2H, J = 8.3 Hz; Ts); 7.58 (s, 1H, H_6); 7.33 (B part of an AB, 2H, J = 8.3 Hz; Ts); 6.11 (t, 1H, J = 6.8 Hz; $H_{1'}$); 4.46 (t, 1H; J = 4.9 Hz; $H_{3'}$); 4.15 (s, 2H, $H_{5''}$ or $H_{5'}$); 3.64 (s, 2H, $H_{5''}$ or $H_{5'}$); 2.40 (s, 3H, Me Ts); 2.26 (dd, 2H, J = 5.3; 6.7 Hz; $H_{2'}$); 1.84 (s, 3H; Me_7). ^{13}C NMR (63 MHz, $CDCl_3$) δ_{ppm} 166.4; 152.2; 146.6; 138.2; 133.7; 131.3; 129.4; 112.2; 88.5; 86.4; 73.1; 71.4; 64.1; 62.0; 41.8; 22.7; 15.2; 13.4. Anal. Calcd (found): N 6.57 (6.80); C 50.70 (50.32); H 5.20 (5.25).

5′-*O*-Dimethoxytrityl-4′-*C*(*S*)-tosyloxymethylthymidine (9). To a solution of compound **8** (100 mg, 0.234 mmol) in anhydrous dichloromethane (5 mL) was added silver nitrate (40 mg, 0.234 mmol), dimethoxytrityl chloride (85 mg, 0.257 mmol), and collidine (39 μL, 0.292 mmol). After 12 h of stirring at room temperature, the reaction was stopped by addition of a saturated aqueous solution of $NaHCO_3$ (10 mL) at 0 °C and extracted with ethyl acetate. The organic layer was washed with water and brine and dried over $MgSO_4$. After removal of the solvent compound **9** (153 mg, 90% yield) was isolated as a white foam after chromatography on silica gel eluted first with CH_2Cl_2/AcOEt 4/1 and with 1/1. TLC, R_f (CH_2Cl_2/AcOEt 1/1) = 0.4. ^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm} 8.69 (s, 1H; NH); 7.72 (A part of an AB, 2H, J = 8.2 Hz; ph Ts); 7.31−6.78 (m, 16H, ph and H_6); 6.25 (t, 1H, J = 7.0 Hz; $H_{1'}$); 4.52 (t, 1H; J = 6.1 Hz; $H_{3'}$); 4.28 (A part of an AB system, 1H, J = 10.4 Hz; $H_{5'}$); 4.12 (B part of an AB, 1H, J = 10.4 Hz; $H_{5'}$); 3.79 (s, 6H, Me DMTr); 3.38 (A part of an AB, 1H, J = 10.0 Hz; $H_{5''}$); 3.19 (B part of an AB, 1H, J = 10.0 Hz; $H_{5''}$); 2.40 (s, 3H, Me Ts); 2.30 (m, 2H, $H_{2'}$); 2.25 (s, 3H; Me_7). ^{13}C NMR (63 MHz, $CDCl_3$) δ $_{ppm}$ 164.2; 158.9; 157.4; 150.6; 148.0; 145.2; 144.3; 135.9; 135.3; 132.7; 130.3; 130.0; 129.4; 128.4; 128.2; 128.0; 127.4; 121.6; 113.5; 113.4; 113.2; 111.4; 87.4; 86.9; 73.0; 70.0; 64.9; 55.5; 40.9; 24.2; 15.6; 12.1. Anal. Calcd (found): N 3.84 (3.92); C 64.27 (64.56); H 5.53 (5.61).

Cyanoethyl 3′-*O*-(5′-*O*-dimethoxytrityl-4′-*C*-tosyloxymethyl)thymidinyl-5′-*O*-(3′-*O*-*tert*-butyldiphenylsilyl)thymidinyl Phosphoric Ester (mixture of diastereoisomers) (10). Compound **9** (83 mg, 0.114 mmol), 5′-phosphoramidite-3′-*tert*-butyldiphenylsilylthymidine (155 mg, 0.228 mmol), and freshly sublimed tetrazole (80 mg, 1.14 mmol) were diluted into anhydrous acetonitrile (1.5 mL) and stirred for 45 min at room temperature. After addition of collidine (70 μL), the phosphite was oxydized with a solution of iodine [0.1 M in THF(2)/H_2O(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 over $MgSO_4$ before removal of the solvent. Compound **10** (140 mg, 93% yield) was isolated (mixture of diastereoisomers) as a white foam after chromatography on silica gel eluted first with CH_2Cl_2/AcOEt 1/1 and with AcOEt. TLC, R_f (CH_2Cl_2/AcOEt 1/1) = 0.14. ^{31}P NMR (81 MHz, $CDCl_3$) δ_{ppm} −2.8 and −3.1 (dias). ^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm} 8.89; 8.82 and 8.79 (3s, 4H; NH); 7.68−7.13 (m, 58H; ph and H_6); 6.38−6.32 (m, 2H, $H_{1'}$); 6.18−6.13 (m, 1H, $H_{1'}$); 6.10−6.04 (m, 1H, $H_{1'}$); 5.09 (m, 2H, $H_{3'a}$), 4.34 (m, 2H); 4.16−3.79 (m, 10H); 3.77 (s, 12H, Me DMTr); 3.38−3.12 (m, 4H, CH_2); 2.47 and 2.46 (2s, 6H, Me Ts); 2.63−2.29 (m, 8H, CH_2), 1.82; 1.79; 1.50 and 1.49 (4s, 12H, Me_7); 1.92; 1.90; 1.32 and 1.27 (4m, 8H, $H_{2'}$); 1.08 and 1.07 (s, 18H, tBu). ^{13}C NMR (63 MHz, $CDCl_3$) δ_{ppm} 164.2; 164.0; 159.0; 158.9; 150.8; 150.7; 150.4; 145.5; 145.4; 144.0; 136.2; 135.9; 134.9; 134.8; 133.1; 133.0; 132.9; 130.5; 130.4; 130.3; 130.2; 130.1; 128.3; 128.2; 128.0; 127.5; 127.3; 121.6; 116.7; 116.5; 113.5; 111.8; 111.7; 111.6; 87.7; 87.6; 86.2; 86.0; 85.8; 85.5; 85.4; 85.3; 85.1; 85.0; 78.9; 78.7; 73.3; 73.0; 69.0; 68.9; 67.9; 64.8; 64.7; 62.7; 62.6; 55.5; 40.2; 40.0; 38.9; 38.7; 27.0; 21.9; 19.7; 19.2; 12.5; 12.1. Anal. Calcd (found): N 5.29 (5.42); C 61.67 (62.02); H 5.63 (5.57).

5′-*O*-Dimethoxytrityl-3′-*O*-*tert*-butyldiphenylsilyl-(S_C,R_P)-δ,ϵ,ζ-CNA (11a) and 5′-*O*-Dimethoxytrityl-3′-*O*-*tert*-butyldiphenylsilyl-(S_C,S_P)-δ,ϵ,ζ-CNA (11b). Starting from **10** (140 mg, 0.1 mmol), the fully protected δ,ϵ,ζ-CNA **11a** and **11b** (116 mg, 100% yield) were obtained following the procedure described for the α,β,γ-CNA **5**. Starting from a mixture of **15a** and **15b** (200 mg, 0.25 mmol), **11a** and **11b** are prepared by adding dimetoxytrityl chloride (0.63 mmol, 214 mg), silver nitrate (0.63 mmol, 107 mg), and collidine (2.5 mmol, 0.33 mL) in anhydrous DMF (6 mL). The reaction mixture was stirred for 5 h at 90 °C. The reaction was stopped by addition of a saturated aqueous solution of $NaHCO_3$ (10 mL) at 0 °C and extracted with ethyl acetate. The organic layer was washed with water (twice) and brine and dried over $MgSO_4$. After removal of the solvent **11a** and **11b** were separated by chromatography on silica gel with ethyl acetate/dichloromethane 1/1 as eluent. Data for **11a**: TLC, R_f (CH_2Cl_2/AcOEt 1/1) = 0.2. ^{31}P NMR (81 MHz, $CDCl_3$) δ_{ppm} −1.4. ^{1}H NMR (250 MHz, $CDCl_3$) δ_{ppm} 9.59 and 9.35 (2s, 2H; NH); 7.67−6.83 (m, 25H; ph, H_{6a} and H_{6b}); 6.57 (dd, 1H, J = 8.8, 5.7 Hz, $H_{1'a}$); 6.45 (dd, 1H, J = 8.4, 6.0 Hz, $H_{1'b}$); 5.01 (br t, 1H, J = 6.0 Hz, $J_{3'a/P}$= 6.4 Hz, $H_{3'a}$); 4.30 (m, 1H, $H_{3'b}$); 4.25 (A part of an ABX, 1H, J = 12.0 Hz, $J_{5''a/P}$ = 11.6 Hz, $H_{5''a}$); 4.07 (m, 1H, $H_{5'b}$); 4.01 (B part of an ABX, 1H, J = 12.0 Hz, $J_{5''a/P}$ = 16.8 Hz, $H_{5''a}$); 3.83 (m, 1H, $H_{5'b}$); 3.79 (s, 6H, Me DMTr); 3.71 (m, 1H, J = 6.1, 11.2 Hz, $J_{4'b/P}$ = 8.2 Hz, $H_{4'b}$); 3.46 (A part of an AB, 1H, J = 10.4 Hz, $H_{5'a}$); 3.31 (B part of an AB, 1H, J = 10.0 Hz, $H_{5'a}$); 2.75 (dd, 1H, J = 14.0, 5.6 Hz, $H_{2'a}$); 2.58 (m, 1H, $J_{2'a/P}$ < 1 Hz, $H_{2'a}$); 2.34 (m, 1H, J = 12.0, 5.6, <1 Hz, $H_{2'b}$); 1.83 (m, 1H, $H_{2'b}$); 1.80 and 1.51 (2s, 6H, Me_7); 1.11 (s, 9H, tBu). ^{13}C NMR (63 MHz, $CDCl_3$) δ_{ppm} 164.5; 164.4; 151.0; 150.8; 144.4; 136.4−128.1; 114.1; 112.5; 112.0; 111.8; 88.2; 86.3; 85.8; 85.0; 84.9; 82.5; 73.6; 70.0; 68.5; 65.8; 56.0; 41.0; 40.4; 39.8; 30.4; 27.5; 19.7; 13.0; 12.5. Anal. Calcd (found): N 5.10 (5.12);

C 63.38 (63.23); H 5.78 (5.82). Data for **11b**: TLC R_f (CH_2Cl_2/AcOEt 1/1) = 0.3. ^{31}P NMR (81 MHz, $CDCl_3$) δ_{ppm} −2.8. ^1H NMR (250 MHz, $CDCl_3$) δ_{ppm} 9.49 and 9.40 (2s, 2H; NH); 7.68−6.85 (m, 25H; ph, H_{6a} and H_{6b}); 6.57 (dd, 1H, J = 9.0, 5.7 Hz, $H_{1'a}$); 6.38 (dd, 1H, J = 8.0, 5.7 Hz, $H_{1'b}$); 5.19 (t, 1H, J = 5.3 Hz, $J_{3'a/P}$ = 6.3 Hz, $H_{3'a}$); 4.38 (m, 1H, $H_{3'b}$); 4.09 (m, 2H; $H_{4'b}$ and $H_{5'b}$); 3.96 (m, 2H, $J_{5''a/P}$ = 13.2 Hz, $H_{5''a}$); 3.81 (s, 6H, Me DMTr); 3.65 (m, 1H, Hz, $H_{5'b}$); 3.54 (A part of an AB, 1H, J = 10.1 Hz, $H_{5'a}$); 3.37 (B part of an AB, 1H, J = 10.2 Hz, $H_{5'a}$); 2.55 (m, 2H, $H_{2'a}$); 2.43 (m, 1H, J = 13.4, 5.7, 2.2 Hz; $H_{2'b}$); 1.91 (m, 1H, J = 14.0, 8.0, 2.0 Hz, $H_{2'b}$); 1.85 and 1.36 (2s, 6H, Me_7); 1.09 (s, 9H, tBu). ^{13}C NMR (63 MHz, $CDCl_3$) δ_{ppm} 164.5; 164.1; 159.6; 150.9; 144.2; 136.4−128.2; 114.1; 112.9; 111.7; 88.5; 86.1; 85.8; 85.5; 84.9; 81.7; 73.4; 70.0; 68.6; 65.9; 56.0; 41.3; 39.6; 30.4; 27.5; 19.6; 13.0; 12.3. Anal. Calcd (found): N 5.10 (5.22); C 63.38 (63.05); H 5.78 (5.67).

Cyanoethyl 3′-*O*-(4′-*C*-Hydroxymethyl)thymidinyl-5′-*O*-(3′-*O*-*tert*-butyldiphenylsilyl)thymidinyl Phosphoric Ester (13). Compound **12** (1.86 g; 2.12 mmol) was coupled with 5′-*O*-(cyanoethyldiisopropylphosphoramidite)-3′-*O*-*tert*-butyldiphenylsilylthymidine (2.89 g, 4.24 mmol) in the presence of tetrazole (1.48 g, 21.2 mmol) according to protocol described for **10**. The crude material obtained was dissolved in 20 mL of a solution of dichloromethane with 3% of TFA, at room temperature. After 15 min, the red solution was evaporated to dryness. The dinucleotide **13** (1.56 g, 85% yield) was obtained as a white foam by purification on silica gel with CH_2Cl_2/AcOEt 1/1 then AcOEt and finally AcOEt/5% MeOH as eluent. TLC, R_f (AcOEt/5%MeOH) = 0.22. ^{31}P NMR (81 MHz, $CDCl_3$) δ_{ppm} −2.9 and −2.7 (diastereoisomers). ^1H NMR (250 MHz, $CDCl_3$) δ_{ppm} 7.61−7.13 (m, 24H, ph and H_6); 6.32−6.08 (m, 4H, $H_{1'}$); 5.07 (m, 2H, $H_{3'a}$); 4.29 (m, 2H); 4.11 (m, 8H); 3.83 (m, 4H); 3.68 (m, 6H); 2.94 and 2.66 (2m, 8H); 2.43 and 2.25 (2m, 8H, $H_{2'}$); 1.80 and 1.79 (2s, 12H, Me_7); 1,03 (s. 18H, tBu). ^{13}C NMR (63 MHz, $CDCl_3$) δ_{ppm} 164.5; 150.7; 144.8; 135.6; 132.7; 132.5; 130.2; 128.7; 128.1; 128.0; 127.9; 125.6; 116.7; 111.3; 110.9; 88.7; 84.9; 79.0; 72.7; 62.8; 62.6; 61.5; 39.7; 39.0; 26.6; 21.1; 19.4; 19.3; 19.2; 18.8; 12.2; 12.0. MS (FAB) 890 ($M + Na^+$). Anal. Calcd (found): N 5.81 (5.67); C 55.36 (54.92); H 5.81 (5.67).

3′-*O*-(4′-*C*-Hydroxymethyl)thymidinyl-5′-*O*-(3′-*O*-*tert*-butyldiphenylsilyl)thymidinyl Phosphoric Acid Triethylammonium Salt (14). Compound **13** (1.6 g; 1.84 mmol) was treated with triethylamine (530 μL, 3.68 mmol) in 15 mL of acetonitrile at 60 °C for 3 h. After removal of the solvent and excess of base under vacum, the dinucleotide **14** was obtained as a white foam (1.7 g, 100% yield). TLC R_f (AcOEt/20%MeOH) = 0.25. ^{31}P NMR (81 MHz, CD_3OD) δ_{ppm} −0.25. ^1H NMR (400 MHz, CD_3OD) δ_{ppm} 7.89 (d, 1H, J = 1.5 Hz, H_6); 7.69−7.67 (m, 5H, ph and H_6); 7.46−7.44 (m, 6H, ph); 6.47 (dd, 1H, J = 5.5; 8.9 Hz, $H_{1'b}$); 6.25 (t, 1H, J = 7.0 Hz, $H_{1'a}$); 4.91 (m, 1H, $H_{3'a}$); 4.56 (d, 1H, J = 5.3; 1.5 Hz, $H_{3'b}$); 4.12 (m, 1H, J = 1.5 Hz; $J_{4'b/P}$ = 2.3 Hz, $H_{4'b}$); 3.92 (A part of an ABX, 1H, J = 2.9; 11.2 Hz, $J_{5'b/P}$ = 4.3 Hz, $H_{5'b}$); 3.75 (m, 2H, J = 6.0; 12.1 Hz, $H_{5'a}$); 3.68 (B part of an ABX, 1H, J = 11.4 Hz; $J_{5''b/P}$ < 1 Hz, $H_{5''b}$); 3.62 (m, 2H, J = 11.9 Hz, $H_{5''a}$); 3.18 (q, 6H, CH_2 Et_3N); 2.41 (A part of an ABX(Y), 1H, J = 3.6; 6.3; 13.7 Hz, $H_{2'a}$); 2.30 (B part of an ABX(Y), 1H, J = 6.6; 6.7; 13.7 Hz, $H_{2''a}$); 2.19 (A part of an ABX(Y), 1H, J = 1.6; 5.6; 13.5 Hz, $H_{2'b}$); 2.08 (B part of an ABX(Y), 1H, J = 5.4; 9.0; 13.4 Hz, $H_{2'b}$); 1.91 and 1.90 (2s, 6H, Me_7); 1.28 (q, 9H, CH_3 Et_3N); 1.03 (s, 9H, tBu). ^{13}C NMR (63 MHz, CD_3OD) δ_{ppm} 165.3; 165.2; 151.3; 151.7; 137.1; 136.7; 135.7; 133.2; 133.1; 130.0; 127.9; 111.3; 111.0; 89.1; 89.0; 86.6; 86.5; 85.0; 84.5; 75.7; 74.6; 65.4; 63.2; 61.7; 40.0; 39.6; 26.2; 18.6; 11.5; 11.4; 8.1. MS (electrospray) 813.3 (M^+).

3′-*O*-*tert*-Butyldiphenylsilyl-(S_C,R_P)-δ,ϵ,ζ-CNA (15a) and 3′-*O*-*tert*-Butyldiphenylsilyl-(S_C,S_P)-δ,ϵ,ζ-CNA (15b). To compound **14** (500 mg, 0.55 mmol) dissolved in 5 mL of anhydrous pyridine was added 2,4,6-trimethylphenyl-3-nitro-1,2,4-triazol-1-yl (MSNT) (380 mg, 1.25 mmol). After 2 h of stirring at 90 °C, the reaction mixture was diluted with ethyl acetate and washed with a saturated aqueous solution of $NaHCO_3$ (10 mL). The organic layer was washed with water and brine and dried over $MgSO_4$. After removal of the solvent the two δ,ϵ,ζ-CNA **15a** (210 mg) and **15b** (208 mg) (1/1 ratio, 98% yield) were separated by silica gel chromatography with CH_2Cl_2/5% MeOH as eluent. Data for **15a**: TLC, R_f (CH_2Cl_2/5% MeOH) = 0.10. ^{31}P NMR (162 MHz, $CDCl_3$) δ_{ppm} −2.3. ^1H NMR (400 MHz, $CDCl_3$) δ_{ppm} 10.11 and 9.97 (s, 1H, NH); 7.67−7.64 (m, 4H, ph); 7.49−7.40 (m, 7H, ph and H_6); 7.22 (s, 1H, H_6); 6.29 (dd, 1H, J = 6.2, 7.5 Hz, $H_{1'b}$); 6.18 (dd, 1H, J = 5.6, 8.9 Hz, $H_{1'a}$); 5.01 (dd, 1H, J = 5.4 Hz, $J_{3'a/P}$ = 7.8 Hz, $H_{3'a}$); 4.41 (m, 1H, OH); 4.38 (m, 1H, $H_{3'b}$); 4.20 and 4.11 (AB part of an ABX, 2H, J = 12.1 Hz, $J_{5''a/P}$ = 15.1 Hz, $H_{5''a}$); 4.02 (A part of an ABX(Y), 1H, J = 10.0; 2.2 Hz, $J_{5'b/P}$ = 5.0 Hz, $H_{5'b}$); 4.09 (br s, 1H, $H_{4'b}$); 3.76 (B part of an ABX(Y), 1H, J = 10.0; 4.1 Hz, $J_{5'b/P}$ = 5.5 Hz, $H_{5'b}$); 3.68 (br s, 2H, $H_{5'a}$); 2.86 (m, 1H, J = 14.2, 8.9, 5.4 Hz, $H_{2'a}$); 2.56 (m, 1H, J = 14.2, 5.6 Hz, $H_{2'a}$); 2.36 (m, 1H, J = 13.4, 6.0, 2.0 Hz, $H_{2'b}$); 2.00 (m, 1H, J = 13.4, 7.4, 5.4 Hz, $H_{2'b}$); 1.85 (s, 6H, Me_7); 1.09 (s, 9H, tBu). ^{13}C NMR (63 MHz, $CDCl_3$) δ_{ppm} 165.1; 165.0; 151.3; 151.1; 136.2; 133.4; 133.3; 130.8; 128.6; 111.8; 111.6; 88.9; 86.4; 85.7; 85.4; 85.3; 83.3; 73.4; 64.2; 63.2; 61.7; 46.4; 40.7; 39.6; 27.4; 26.4; 19.6; 12.9; 9.1. MS (electrospray) 819.2 ($M + Na^+$). Anal. Calcd (found): N 7.03 (7.12); C 55.77 (55.85); H 5.69 (5.73). Data for **15b**: TLC, R_f (CH_2Cl_2/5% MeOH) = 0.05. ^{31}P NMR (202 MHz, CD_3OD) δ_{ppm} −3.8. ^1H NMR (500 MHz, CD_3OD) δ_{ppm} 7.74−7.70 (m, 5H, ph and H_6); 7.50−7.43 (m, 7H, ph and H_6); 6.44 (dd, 1H, J = 5.5; 9.2 Hz, $H_{1'a}$); 6.38 (dd, 1H, J = 6.1, 8.0 Hz, $H_{1'b}$); 5.14 (dd, 1H, J = 6.2 Hz, $J_{3'a/P}$ = 10.7 Hz, $H_{3'a}$); 4.51 (m, 1H, J = 2.5 Hz, $H_{3'b}$); 4.34 (A part of an ABX, 1H, J = 12.0 Hz, $J_{5''a/P}$ = 14.1 Hz, $H_{5''a}$); 4.17−4.09 (m, 3H, $H_{4'b}$, $H_{5''a}$, $H_{5'b}$); 3.86 (B part of an ABX(Y), 1H, J = 11.2; 4.1 Hz, $J_{5'b/P}$ = 6.8 Hz, $H_{5'b}$); 3.77 and 3.68 (AB, 2H, J = 11.7 Hz, $H_{5'a}$); 2.51 (A part of an ABX(YZ), 1H, J = 14.5; 6.2; 9.2 Hz, $J_{2'a/P}$ = 1.5 Hz, $H_{2'a}$); 2.41 (B part of an ABX(Y), 1H, J = 14.5; 5.5 Hz, $H_{2'a}$); 2.37 (A part of an ABX(Y), 1H, J = 13.5; 2.7; 6.0 Hz, $H_{2'b}$); 2.13 (B part of an ABX(Y), 1H, J = 13.5; 6.5; 8.0 Hz, $H_{2'b}$); 1.91 and 1.85 (s, 3H, Me_7); 1.11 (s, 9H, tBu). ^{13}C NMR (126 MHz, $CDCl_3$) δ_{ppm} 164.8; 150.9; 150.7; 136.1; 136.0; 135.6; 132.9; 132.7; 130.0; 129.9; 127.8; 127.7; 110.7; 110.5; 85.1; 85.0; 84.9; 82.4; 729.4; 69.2; 67.6; 62.9; 39.7; 38.4; 38.3; 26.0; 18.4; 11.1. MS (electrospray) 819.2 ($M + Na^+$). Anal. Calcd (found): N 7.03 (7.02); C 55.77 (55.65); H 5.69 (5.66).

5′-*O*-Dimethoxytrityl-(S_C,R_P)-δ,ϵ,ζ-CNA (16a) and 5′-*O*-Dimethoxytrityl-(S_C,S_P)-δ,ϵ,ζ-CNA (16b). Compound **11a** (or **11b**) was dissolved in anhydrous THF (5 mL/0.10 mmol) and nBu_4NF (110 μL, sol 1 M in THF) was added at 0 °C (120 μL, 0.11 mmol). After 1 h of stirring, the solvent was removed and the crude material was chromatographed on silica gel with first AcOEt as eluent and then AcOEt 10% MeOH to recover **16a** (or **16b**) (80 mg, 90% yield) as a white foam after removal of the solvent. Data for **16a**: ^{31}P NMR (81 MHz, CD_3OD) δ_{ppm} −3.5. ^1H NMR (250 MHz, $CDCl_3$) δ_{ppm} 9.76 (m, 2H, NH); 7.35−6.80 (m, 15H, ph and H_6); 6.55 (t, 1H, $H_{1'b}$); 6.26 (t, 1H, $H_{1'a}$); 5.15 (m, 1H, $H_{3'a}$); 4.35−4.06 (m, 6H); 3.74 (s, 6H, DMTr); 3.45 (m, 1H); 3.26 (m, 1H); 2.88 (m, 1H, $H_{2'}$); 2.75 (m, 1H, $H_{2'}$); 2.54 (m, 1H, $H_{2'}$); 2.28 (m, 1H, $H_{2'}$); 1.72 and 1.48 (2s, 6H, Me_7). ^{13}C NMR (75 MHz, CD_3OD) δ_{ppm} 164.1; 163.9; 158.9; 150.6; 150.5; 143.8; 135.8; 135.3;134.6; 130.0; 129.9; 128.2; 127.9; 127.4; 113.5; 111.9; 111.1; 87.5; 85.4; 85.2; 84.5; 84.2; 81.9; 70.3; 69.3; 67.8; 64.9; 60.4; 55.3; 51.9; 39.8; 39.2; 29.7; 29.5; 29.3; 25.7; 20.3; 12.4; 11.9. MS (electrospray) 883.1 ($[M + Na^+]^+$); 899.0 ($[M + K^+]^+$). Anal. Calcd (found): N 6.51 (6.44); C 58.60 (58.47); H 5.27 (5.21). Data for **16b**: ^{31}P NMR (81 MHz, CD_3OD) δ_{ppm} −4.4. ^1H NMR (250 MHz, $CDCl_3$) δ_{ppm} 10.50 and 9.82 (s, 2H, NH); 7.48 and 7.39 (s, 2H, H_6); 7.31−7.19 (m, 9H, ph); 6.86 (m, 4H, ph); 6.69 (dd, 1H, J = 9.1, 5.5 Hz, $H_{1'b}$); 6.27 (t, 1H, J = 6.7 Hz, $H_{1'a}$); 5.23 (dd, 1H, J = 16.5, 6.1 Hz, $H_{3'a}$); 4.55−4.37 (m, 4H); 4.25−4.10 (m, 4H); 3.79 (s, 6H, DMTr); 3.37 (s, 2H, $H_{5'a}$); 2.76 (m, 1H, $H_{2'}$); 2.46 (m, 3H, $H_{2'}$); 2.14 (m, 1H, $H_{2'}$); 1.88 and 1.34 (s, 6H, Me_7). ^{13}C NMR (63 MHz, CD_3OD) δ_{ppm} 163.4; 159.7; 151.1; 150.3; 143.4; 134.3; 130.1; 130.0. 128.3;

128.0; 127.6; 113.6; 112.8; 111.1; 87.9; 85.5; 84.8; 84.6; 82.4; 70.6; 68.2; 55.3; 52.2; 40.1; 29.8; 20.4; 12.4; 11.6. MS (electrospray) 883.1 ($[M + Na^+]^+$); 899.0 ($[M + K^+]^+$). Anal. Calcd (found): N 6.51 (6.39); C 58.60 (57.99); H 5.27 (5.34).

Crystal data for 15b: $C_{39}H_{47.25}Cl_{5.75}N_4O_{12}PSi$, $M = 1026.96$, monoclinic, $P2_1$, $a = 10.670(1)$ Å, $b = 18.748(1)$ Å, $c = 23.941(2)$ Å, $\beta = 98.397(2)°$, $V = 4737.6(6)$ Å^3, $Z = 4$, $T = 193(2)$ K. 21327 reflections (13121 independent, $R_{int} = 0.0507$) were collected at low temperatures, using an oil-coated shock-cooled crystal on a Bruker-AXS CCD 1000 diffractometer with Mo Kα radiation ($\lambda = 0.71073$ Å). The structure was solved by direct methods (SHELXS-97)[19] and all non-hydrogen atoms were refined anisotropically using the least-squares method on F^2.[20] Largest electron density residue: 0.483 e Å^{-3}, R_1 (for $I > 2\sigma(I)$) = 0.0614 and $wR_2 = 0.1454$ (all data) with $R_1 = \sum||F_o| - |F_c||/\sum|F_o|$ and $wR_2 = (\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2)^{0.5}$.

(19) Sheldrick, G. M. *Acta Crystallogr.* **1990**, *A46*, 467−473.

(20) SHELXL-97, Program for Crystal Structure Refinement; G. M. Sheldrick, University of Göttingen, 1997.

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Supporting Information Available: General methods; dinucleotide numbering; dioxaphosphorinane protons region of ^{1}H and ^{1}H−{^{31}P},^{1}H-COSY, 2D-NOESY, and 1D-NOESY NMR spectra of α,β,γ-CNA *cis*-**7** and *trans*-**7**; dioxaphosphorinane protons region of ^{1}H and ^{1}H−{^{31}P} NMR spectra of (S_C,R_P) δ,ϵ,ζ-CNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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