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Synthesis and Structure of Dinucleotides Featuring Canonical and Non-canonical A-Type Duplex α, β and δ Torsion Angle Combinations (LNA/α,β-D-CNA)

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The synthesis of diastereoisomers of LNA/α,β-D-CNA dinucleotide building units of nucleic acids in which one δ torsional angle is constrained by an LNA structure and the α and β torsional angles are stereocontrolled by a dioxaphosphorinane ring structure (D-CNA family) is described. NMR structural analysis showed that the δ_a, α_b and β_b torsional angles of the ($R_{C5'}$,R_P)-configured LNA/α,β-D-CNA TT dimer are restricted to the canonical {δ_a,α_b,β_b} = {*gauche*(+)/*anticlinal*(+), *gauche*(–), *trans*} conformation typically observed in A-type duplexes.

Introduction

In the chemical search for improved antisense molecules, the conformational restriction of the sugar moiety of nucleosides has been widely explored. Early studies showed that the most efficient carbohydrate modification was to lock the sugar-puckering into a C3′-*endo* conformation in order to create stable RNA mimics.[1–3]

Undoubtedly the most prominent examples of this class of compound are locked nucleic acids (LNAs).[4–6] The preorganised conformation of the LNA nucleoside was predicted to be an N-type sugar-puckering, characteristic of A-type double helices such as RNA–RNA duplexes. The LNA oligonucleotide conformational structure was determined by NMR analysis by examining both the sugar-puckering and the sugar-phosphate backbone and confirmed this initial assumption. Further studies also revealed that LNA monomers are able to twist the neighbouring, unmodified nucleotides from an S-type towards an N-type conformation in DNA–LNA mixmer oligonucleotides and LNA-containing duplexes.[7,8]

A new and highly interesting application of analogues that retain the A-type geometry of RNA emerged with the discovery of RNAi. In this context, LNAs are relatively well tolerated when incorporated into selected positions of RNAi, particularly when located near the 3′ or 5′ ends of the siRNA.[9] Mainly driven by the need to improve their stability towards nucleases, the properties of RNA mimics with modified sugar-phosphate backbones were reinvestigated in the new context of RNA-mediated inhibition of gene expression.[10]

Also based on the concept of preorganisation,[11] we have explored the chemistry of DNA- and RNA-constrained analogues (termed D-CNAs) with a backbone modified by the introduction of a neutral 1,3,2-dioxaphosphorinane ring structure at key positions along the sugar-phosphate backbone.[12,13] We first reported on α,β-D-CNA featuring non-canonical torsional angles which frequently occur in secondary nucleic acid structures such as bulges or hairpin loops.[14,15] On the other hand, D-CNA dinucleotides with α and β locked at canonical values within a duplex exhibited a high level of stabilisation.[16] Interestingly, whereas the level of stabilisation reached up to 6 °C/modification when forming B-type duplexes (DNA–DNA), this level was much lower (1 to 3 °C/modification) in an A-type duplex (DNA–RNA). Although, the A- and B-type duplexes are characterised by the same torsional angles α [*gauche*(–)] and β (*trans*), the observed difference could be explained by the fact that the neutral dioxaphosphorinane modification can shift the North/South equilibrium of the deoxysugar-puckering in such a way that these α,β-D-CNAs fit better the B-type rather than with the A-type double-helix structure.[17,18] Consequently, the question that arises is which level of contribution to the observed stabilisation could be attributed to the preorganisation of the torsional angles α and β relative to the South orientation of the sugar-puckering?

With respect to these points, we decided to prepare D-CNA dinucleotides in which the sugar-puckering of the upper nucleoside would be constrained in the North configu-

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ration by a LNA structure (Figure 1). These new building units should, on one hand, allow us to discriminate between the impact of each constraint on duplex formation ability with regards to the known properties of LNA and α,β-D-CNA, and on the other hand, bring high enzymatic stability due to the phosphotriester nature of the internucleotidic junction.

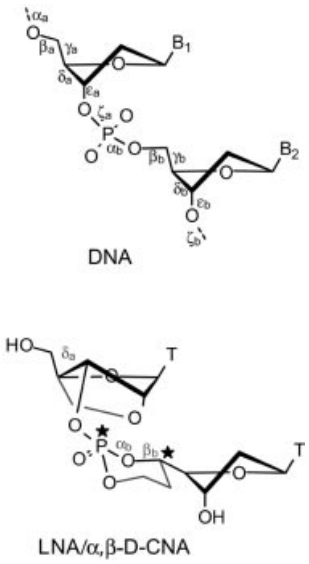


Figure 1. Left: the six backbone torsion angles (labeled α_a to ζ_a for the upper nucleotide and α_b to ζ_b for the lower nucleotide) of nucleic acids. Right: the LNA/α,β-D-CNA unit is a dinucleotide in which δ_a is locked by a LNA structure and α_b and β_b are stereocontrolled to canonical or non-canonical values by a dioxaphosphorinane ring structure exhibiting two new asymmetric centers.

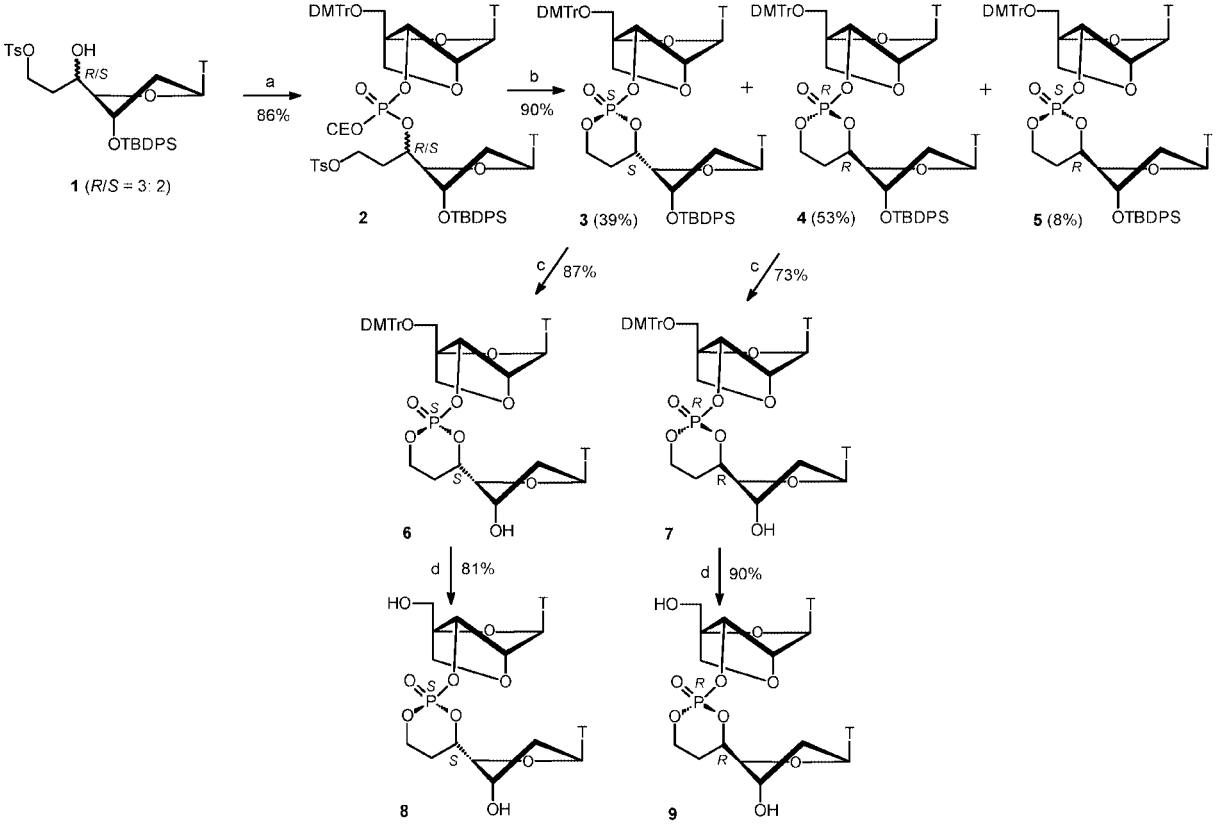
In the present work, we report on the diastereoselective synthesis and conformational behaviour of ($S_{C5'}$,S_P) and ($R_{C5'}$,R_P) isomers of this novel class of constrained dinucleotide building units, termed LNA/α,β-D-CNA.

Results and Discussion

Synthesis of LNA/α,β-D-CNA and T^{L}T Dinucleotides

The dioxaphosphorinane ring structure was constructed as described previously,[14] that is, from the cyclisation reaction of a dinucleotide precursor in which the phosphate oxyanion can attack an activated carbon atom. In the present work, a diastereoisomeric 5′-substituted and 2′-*O*-4′-*C*-locked-dithymidine **2** represents the appropriate acyclic precursor of this process. It was prepared by coupling already published 5′-*C*-tosyloxyethylthymidine **1** (5′-C-epimeric mixture of *R*/*S* in a 3:2 ratio)[12] with the commercially available LNA-thymidine phosphoramidite by using standard phosphoramidite technology[19] (Scheme 1).

Under mild conditions, K_2CO_3 in anhydrous dimethylformamide at room temperature, **2** cyclised in good yield (90%) into three out of the four possible isomers of the



Scheme 1. Reagents and conditions: a) TLNA phosphoramidite (2 equiv.), tetrazole (10 equiv.), CH_3CN then collidine, I_2/H_2O, room temp, 45 min; b) K_2CO_3 (16 equiv.), DMF, room temp, 3 h; c) nBu$_4$NF, THF, room temp, 1 h; d) 3% TFA in CH_2Cl_2, room temp, 15 min.

fully protected LNA/α,β-D-CNA **3**, **4** and **5**, as shown by ^{31}P NMR spectroscopy (δ_P = –9.7, –9.9 and –8.2 ppm, respectively). At this stage the isomers are easily separable by means of silica gel chromatography. As previously observed, the cyclisation of the 5′-C-(*S*) isomer is highly diastereoselective as the ($S_{C5'}$,R_P) isomer is not detected.[14] Note that the in the Cahn–Ingold–Prelog system, the same dioxaphosphorinane geometry is obtained for the α,β-D-CNA ($S_{C5'}$,S_P) and the LNA/α,β-D-CNA ($S_{C5'}$,R_P) isomers due to the change of priority brought about by the LNA structure. Interestingly, the cyclisation of the 5′-C-(*R*) isomer of **2** occurred with a higher diastereoselectivity (*de* = 87%) than was observed for the corresponding α,β-D-CNA (*de* = 70%).[12] This improvement in selectivity may be attributed to a higher steric hindrance of the LNA structure of the upper nucleoside which could be much more favourable for the formation of the appropriately substituted dioxaphosphorinane ring, that is, with the upper nucleoside in the anomerically favourable axial position and the lower in the preferred equatorial position for steric reasons.[20]

The two major compounds **3** and **4** were therefore submitted to the action of tetrabutylammonium fluoride to remove the 3′-*O*-silyl protecting group, providing LNA/α,β-D-CNA **6** and **7** in 87 and 73% yields, respectively. Finally, the 5′-*O*-dimethoxytrityl group was removed from **6** and **7** under acidic conditions to obtain **8** and **9** (81 and 90% yields, respectively) for solution-state structure determination in the absence of protecting groups.

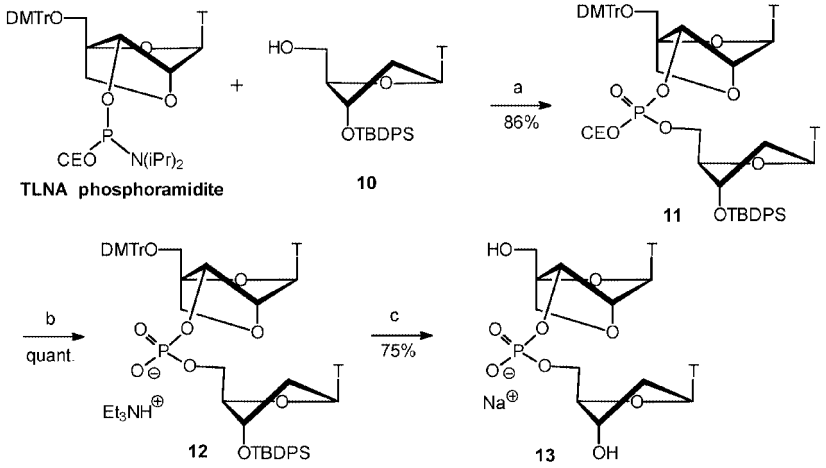
As a reference for the conformational NMR study of the dinucleotides, we prepared the dinucleotide T^LT **13** (Scheme 2). Under standard phosphoramidite coupling conditions, the commercially available T-LNA phosphoramidite was added to the readily available 3′-*O*-(*tert*-butyldiphenylsilyl)thymidine (**10**) to provide the fully protected dinucleotide isomers **11** (mixture of isomers, δ_P = –2.5 and –3.0 ppm) in a good yield (86% based on the expensive T-LNA phosphoramidite). The cyanoethyl phosphate protecting group was first removed with triethylamine in acetonitrile to give quantitatively the triethylammonium phosphate salt of the dinucleotide **12** as a single isomer (δ_P = –2.4 ppm). Finally, the T^LT **13** was obtained after sequential removal of the hydroxy protecting groups with fluoride ion and trifluoroacetic acid. Replacement of the tetrabutylammonium counter-ion of the phosphate by sodium was performed with a Dowex 50WX8 ion-exchange resin, giving the sodium salt of **13** (δ_P = –1.4 ppm) in 75% overall yield from **12**.

Structural Assigment of LNA/α,β-D-CNA TT and T^LT Dimers

To determine the behaviour of the two thymine moieties in LNA/α,β-D-CNA **8** and **9**, the CD spectra were measured in phosphate buffer (pH 7.0) at 20, 40, 60 and 80 °C and compared with those of the T^LT dinucleotide **13** (Figure 2). The most striking features are the lower intensity and the very small temperature-dependence of the positive Cotton effect at around 280 nm for ($S_{C5'}$,S_P) LNA/α,β-D-CNA **8**. Whereas T^LT **13** and ($R_{C5'}$,R_P)-LNA/α,β-D-CNA **9** showed a decrease in the stacking of the thymine bases as the temperature increased, very little variation was observed for ($S_{C5'}$,S_P)-LNA/α,β-D-CNA **8** ($\Delta\Delta\varepsilon_{269}$ = 0.38 $M^{-1}cm^{-1}$). These results could undoubtedly be explained in terms of the relative conformational rigidity of the dioxaphosphorinane structure which does not allow any base-stacking in this particular conformation of the torsion angles α and β.

Interestingly, the variation is $\Delta\varepsilon$ is lower for ($R_{C5'}$,R_P) LNA/α,β-D-CNA **9** than for T^LT **13**, $\Delta\Delta\varepsilon_{276}$ = 1.76 and $\Delta\Delta\varepsilon_{278}$ = 3.10 $M^{-1}cm^{-1}$, respectively. This suggests a conformation of **9** close to the natural one but with increased rigidity.

On the other hand, the solution-state structures were established by NMR spectroscopy in order to obtain the relative spatial arrangement of the nucleotides in the various synthesised dimers; ^1H, $^1H\{^{31}P\}$, 2D COSY $^1H/^1H$, 2D TOCSY and 2D NOESY NMR spectra were recorded at 500 MHz in deuteriated chloroform, deuteriated methanol or deuterium oxide.



Scheme 2. Reagents and conditions: a) tetrazole (5 equiv.), CH_3CN then collidine, I_2/H_2O, room temp, 45 min; b) Et_3N (4 equiv.), CH_3CN, 60 °C, 1 h; c) (i) nBu_4NF, THF, room temp, 1 h; (ii) 3% TFA in CH_2Cl_2, room temp, 15 min; (iii) Dowex 50WX8 sodium form.

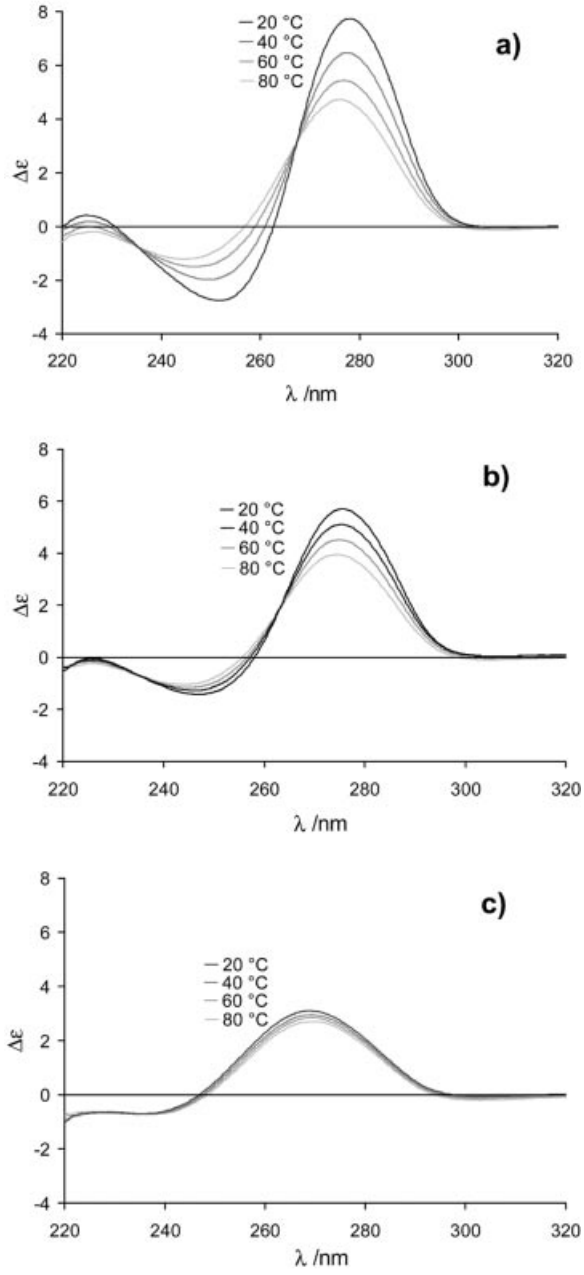


Figure 2. CD spectra in 10 mM sodium phosphate solution (pH 7.0) at 80, 60, 40 and 20 °C of a) T^{L}T **13**, b) ($R_{C5'}$,R_P)-LNA/α,β-D-CNA TT **9** and c) ($S_{C5'}$,S_P)-LNA/α,β-D-CNA TT **8**.

The puckering of the 2′-deoxyribose moieties of the fully protected LNA/α,β-D-CNA TT dimers **3** and **4** and of the deprotected dinucleotides **8**, **9** and T^{L}T **13** was assigned by examination of the sugar ring H/H coupling constants (Table 1). Whereas all the upper nucleosides are in a North conformation dictated by the LNA structure [J(1′,2′) = J(2′,3′) = 0 Hz], the lower nucleosides exhibit coupling constants similar in each case to those found in the standard C2′-*endo* (South) conformation of natural 2′-deoxyribose.[21] The conformational equilibrium between North and South of the 2′-deoxysugar moieties was estimated by use of the approximation of Altona and Sundaralingam: South (%) = $[^3J_{H1',H2'}/(^3J_{H1',H2'} + {}^3J_{H3',H4'})] \times 100$.[22] Whereas the protected LNA/α,β-D-CNA TT dimers **3** and **4** exhibit a pronounced C2′-*endo* conformation (90 and 82%, respectively), the proportion of the corresponding deprotected dinucleotides **8** and **9**, like T^{L}T **13**, that adopt the South-type conformation is 59%. These values are similar to those found for the unmodified TT dimer (63% of the C2′-*endo* conformation for the lower thymidine).[21] It is therefore evidenced that neither the LNA structure nor the dioxaphosphorinane ring have influence on the level of the C2′-*endo* conformation of the lower nucleoside at the dinucleotide stage.

Table 1. H/H coupling constants (Hz) in the ^{1}H-NMR spectra (500 MHz) of ($S_{C5'}$,S_P) and ($R_{C5'}$,R_P) LNA/α,β-D-CNA diastereoisomers (**3** and **4** in $CDCl_3$, **8** and **9** in CD_3OD) and of T^{L}T (**13**) in D_2O.

Nucleoside		Coupling constants, ^3J [Hz]				
		J(1′,2′)		J(2′,3′)		J(3′,4′)
3	upper	0	–	0	–	–
	lower	9.3	5.2	5.5	<1	<1
4	upper	0	–	0	–	–
	lower	8.5	6.0	5.7	2.2	1.9
8	upper	0	–	0	–	–
	lower	6.5	6.5	8.0	3.5	4.5
9	upper	0	–	0	–	–
	lower	6.5	6.5	8.1	4.5	4.5
13	upper	0	–	0	–	–
	lower	6.5	6.5	5.9	5.9	4.8

The chair conformation of the dioxaphosphorinane structure of the fully protected LNA/α,β-D-CNAs **3** and **4** is, like in the previously described α,β-D-CNA diastereoisomers,[12,14] clearly established from ^{1}H and ^{1}H{^{31}P} NMR spectra (Table 3), with no detectable coupling constant between the 5′b-H involved in the dioxaphosphorinane system and the phosphorus atom ($^3J_{H5'b/P} \approx 0$), which is characteristic of an axial position of this proton.[23] The observation of small (<1 Hz) and large (>20 Hz) $^3J_{H/P}$ coupling constants between the dioxaphosphorinane 7′b-H protons and phosphorus are also indicative of an axial and an equatorial position of these protons, respectively. Analysis of the deprotected LNA/α,β-D-CNAs **8** and **9** was complicated by the overlapping of the 7′b-H and 3′b-H signals. Whereas the $^3J_{H5'b/P}$ coupling constants were readily accessible (0 and 2.3 Hz, respectively), in accordance with a chair conformation of the dioxaphosphorinane ring, the $^3J_{H7'b/P}$ values were not accessible. Even broad band irradiation of the 2′b-H and 6′b-H protons (δ = 2.42–1.95 and 2.43–2.00 ppm for **8** and **9**, respectively) together with ^{31}P decoupling did not provide an analysable pattern (Figure 3). The only clear information provided by these experiments is that the 7′b-H atom does not exhibit a measurable coupling constant with phosphorus and therefore can be identified as the 7′b-H atom in the axial position, in accord with the chair conformation of the dioxaphosphorinane structure of **9**.

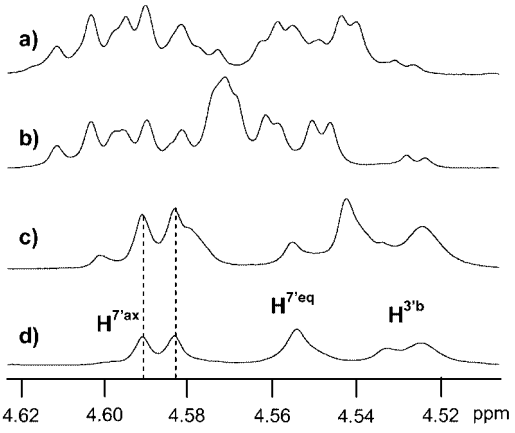


Figure 3. NMR spectroscopic data for 7′b-H and 3′b-H of the ($R_{C5'}$,R_P) LNA/α,β-D-CNA TT **9**. a) ^{1}H NMR; b) ^{1}H{^{31}P} NMR; c) ^{1}H NMR with broad band irradiation at 2.42–1.95 ppm; d) ^{1}H{^{31}P} NMR with broad band irradiation at 2.42–1.95 ppm.

Nevertheless, we can reasonably assume a chair conformation of the dioxaphosphorinane ring, allowing us to assign the conformation of the backbone torsion angles α and β as (α, β) = (g^+, *t*) for **8** and (α, β) = (g^-, *t*) for **9**.

In contrast, a higher value of 3.6 Hz was observed for the $^3J_{H/P}$ coupling constants involving the 5′b-H proton of the minor isomer **5**, thus suggesting that the dioxaphosphorinane structure of this isomer is in a twist-chair conformation, as already observed for the ($R_{C5'}$,R_P) or ($S_{C5'}$,S_P) minor isomers of α,β-D-CNA.[12,24]

Another important piece of information provided by the $J_{H/P}$ coupling constant analysis, is the observation of a long-range coupling constant between 4′b-H of the lower nucleoside and the phosphorus in all the examined dinucleotide structures (Table 2). This is indicative of a more or less pronounced W-shaped P–O5′–C5′–C4′–H4′ junction that corresponds to the canonical values of β (*trans*) and γ [*gauche*(+)] observed in unmodified dinucleotide units (the measured coupling constant for the unmodified thymidine dimer was $J_{H4'b/P}$ = 1.6 Hz).[21]

Table 2. H/P coupling constants in the ^{1}H NMR spectra (500 MHz) of the ($S_{C5'}$,S_P) and ($R_{C5'}$,R_P) LNA/α,β-D-CNA diastereoisomers (**3** and **4** in $CDCl_3$; **8** and **9** in CD_3OD) and T^{L}T (**13**) in D_2O.

Dinucleotide	Coupling constants, *J* [Hz]				
	^3J(3′a,P)	^4J(4′b,P)	^3J(5′b,P)	^3J(7b′,P)	
3	5.5	3.5	0.0	<1	26.8
4	3.8	3.3	0.0	<1	23.8
8	4.5	2.9	0.0	n.d.	n.d.
9	4.0	2.4	2.3	<1	n.d.
13	6.1	2.0	3.8, 4.0	–	–

In addition, the empirical equation established by Lankhorst et al.[25] allowed us to determine, from the coupling constant $J_{H3'a/P}$ (4.5 Hz for **8**, 4.0 Hz for **9** and 6.1 Hz for **13**), the values of the torsional angle ε (–178° for **8**, –171° for **9** and –159° for **13**) and therefore the relative position of the upper nucleosides with respect to the dioxaphosphorinane ring. Interestingly the value observed for **13** is clearly related to the A-type double-helix structure.[26] In contrast, **8** exhibits a value in accordance with the B_I-type structure whereas the ε torsional angle of **9** is located in between the A- and B-type canonical values.

The overall conformations of the dinucleotides ($S_{C5'}$,S_P) LNA/α,β-D-CNA **8**, ($R_{C5'}$,R_P) LNA/α,β-D-CNA **9** and T^{L}T **13** were further investigated by examination of the 2D NOESY NMR spectra. Although many cross peaks are present for **13**, they are all derived from intraresidual H/H interactions indicative of sugar-puckering (North-type for the upper nucleoside and South-type for the lower) and the relative positions of the thymine bases. These results suggest a conformation in which the distance between the two nucleotides is relatively long and therefore corresponds to the relative conformation of unmodified nucleotides.[27]

Except for the NOESY cross-peaks observed between 4′b-H and both of the 6′b-H protons and between 3′b-H and 6′ax-H, indicative of the relative position of the lower sugar unit of **9** with respect to the dioxaphosphorinane ring, all the other detected interactions are issued from intraresidual H/H interactions. As for the dinucleotide **13**, we can therefore conclude that the ($R_{C5'}$,R_P) LNA/α,β-D-CNA **9** has a conformation that is close to the one adopted by unmodified nucleotides.

The typical North conformation of the upper sugar unit is corroborated for the ($S_{C5'}$,S_P) LNA/α,β-D-CNA **8**, as for the other dinucleotides **9** and **13**, by the cross peaks exhibited between 1′a-H and 2′a-H and one of the protons of the methylene bridge between 4′-C and 2′-O. An important piece of information provided by the NOESY spectrum of **8** is that 3′a-H exhibits weak but still significant cross peaks with 5′b-H and 3′b-H, indicating that it is located in between these two protons. Therefore we can determine the relative position of the upper nucleoside with respect to the dioxaphosphorinane ring and the lower nucleoside (Figure 4). Another clear piece of information provided by this 2D NMR spectrum is the interaction between 6b-H and both 6′bax-H and 3′b-H corroborating the relative position of the thymine moiety in the lower nucleoside with respect to the dioxaphosphorinane and the sugar rings. The characteristic position of 4′b-H (W-shape with phosphorus) is confirmed by the cross peak observed between this proton and 6′bax-H and, with a lower intensity, 6′beq-H.

All together the data deduced from NMR spectroscopy helped us to propose the ranges of the torsional angles α–ε for each LNA/α,β-D-CNA diastereoisomer and the T^{L}T dimer (Table 3). The torsional angles of a dinucleotide with a natural A-type duplex conformation are given as reference.[26]

When compared with the backbone conformations summarised in Table 3, we can expect that DNA oligonucleotides incorporating the ($R_{C5'}$,R_P) dinucleotide element would form A-type duplexes that are conformationally fixed. If this structural feature is confirmed this compound might find important applications in determining the role played by the control of torsional angles to A- or B-type canonical values in their duplex or triplex formation ability. On the other hand, the ($S_{C5'}$,S_P) stereoisomer exhibits a

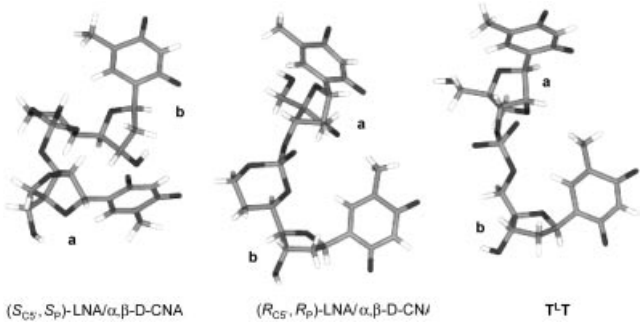


Figure 4. Tentative model of the conformation of the ($S_{C5'}$,S_P) and ($R_{C5'}$,R_P) LNA/α,β-D-CNA diastereoisomers **8** and **9** and of T^{L}T (**13**) derived from NMR spectroscopic data. The upper nucleosides are denoted as **a** and the lower as **b**.

Table 3. Summary of the backbone torsion angles α-ε of A-type dinucleotide, of the LNA/α,β-D-CNA diastereoisomers (**8** and **9**) and of a T^{L}T (**13**) dinucleotide.[a]

Structure	Torsion angle					
	δ_a	ε_a	α_b	β_b	γ_b	δ_b
A-type	g$^+$/a$^+$	a$^-$/t	g$^-$	t	g$^+$	g$^+$/a$^+$
8 ($S_{C5'}$,S_P)	g$^+$/a$^+$	t	g$^+$	t	g$^+$	a$^+$
9 ($R_{C5'}$,R_P)	g$^+$/a$^+$	a$^-$/t	g$^-$	t	g$^+$	a$^+$
13 (T^{L}T)	g$^+$/a$^+$	a$^-$/t	g$^-$	t	g$^+$	a$^+$

[a] The torsion angle ranges are indicated in parentheses for α–ε: *gauche*(+) = 60 ± 30° (g$^+$), *anticlinal*(+) = 120 ± 30° (a$^+$), *trans* = 180 ± 30° (t), *anticlinal*(–) = 240 ± 30° (a$^-$) and *gauche*(–) = 300 ± 30° (g$^-$).

more unusual $\delta_a/\alpha_b/\beta_b$ combination [*gauche*(+)/*anticlinal*(+), *gauche*(+), *trans*] which could represent an important structural element stabilising unpaired structures of DNA or RNA such as bulges or hairpins.

Conclusions

The combination of an LNA structure and a D-CNA structure within a dinucleotide has allowed control, on the one hand, of the δ torsional angle of the upper nucleoside and, on the other, control of the α and β torsional angles of the lower nucleoside. Two major diastereoisomers (out of four possible) were therefore prepared and their structures investigated by means of NMR spectroscopy and circular dichroism. The ($R_{C5'}$,R_P) LNA/α,β-D-CNA isomer exhibits torsional angles (α–ε) close to those encountered in the natural A-type double-helix conformation.

The introduction of such preformed LNA/α,β-D-CNA building blocks into preselected positions of the native backbone of DNA is therefore expected to result in preorganisation of the single-stranded nucleic acid in such a way as to highly stabilise the A-type duplex. In comparison with the properties of the LNA and α,β-D-CNA, this should provide new insights into the impact of the preorganisation of the sugar-puckering relative to the sugar-phosphate backbone, thus making this class of dinucleotide an interesting tool for learning more about the intrinsic structural and functional properties of DNA structures.

Experimental Section

General Remarks: Products were purified by medium-pressure liquid chromatography with a Jobin et Yvon Modoluprep apparatus by using Amicon 6-35 mm or Merck 15 mm silica. NMR spectra were recorded with a Bruker AC-250, Avance-300 or Avance 500 spectrometers (250, 300 or 500 MHz for ^{1}H and 63, 75 or 125 MHz 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.

Cyanoethyl 3′-*O*-[(5′-*O*-Dimethoxytrityl)-(2′-*O*-4′-*C*)-methylenethymidinyl] 5′-*C*-Tosyloxyethyl-3′-(*tert*-butyldiphenylsilyl)thymidinyl Phosphoric Ester (Mixture of Diastereoisomers) (2): Compound **1** (733 mg, 1.08 mmol), thymidine-LNA O$^{3'}$-phosphoramidite (1.25 g, 1.62 mmol) were dissolved with an anhydrous acetonitrile solution of tetrazole (0.45 M, 8 mL) and stirred for 45 min at room temperature. After the addition of collidine (0.64 mL, 4.86 mmol), the phosphite was oxidised with iodine [0.1 M solution in THF(2)/H_2O(1)] until a dark brown colour persisted. The reaction mixture was diluted with AcOEt and washed with an aqueous solution of sodium thiosulfate (15%) to remove excess iodine. The organic layer was washed with water, brine and dried with $MgSO_4$ and the solvent was removed in vacuo. The crude material was purified by chromatography on silica gel with AcOEt as eluent. After evaporation of the solvent compound **2** (mixture of four diastereoisomers) was recovered as a white foam (1.27 g, yield 86%). TLC: R_f (AcOEt) = 0.54. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –2.8, –2.9, –4.1, –4.2 ppm. MS (ESI): *m*/*z* = 1389.1 [M + Na]$^+$.

($S_{C5'}$,S_P) 5′-*O*-Dimethoxytrityl-3′-(*tert*-butyldiphenylsilyl)-LNA/α,β-D-CNA TT (3), ($R_{C5'}$,R_P) 5′-*O*-Dimethoxytrityl-3′-(*tert*-butyldiphenylsilyl)-LNA/α,β-D-CNA TT (4) and ($R_{C5'}$,S_P) 5′-*O*-Dimethoxytrityl-3′-(*tert*-butyldiphenylsilyl)-LNA/α,β-D-CNA TT (5): Potassium carbonate (2.05 g, 14.9 mmol) was added to dinucleotides **2** (1.27 g, 0.93 mmol) in anhydrous DMF (20 mL). After 4 h of stirring at 25 °C, the excess base was filtered off and the reaction mixture was diluted with AcOEt (200 mL) and washed three times with water (3 × 20 mL) and once with brine. The organic layer was dried with $MgSO_4$ and the solvent removed in vacuo. Compounds **3** (370 mg), **4** (500 mg) and **5** (80 mg) were separated by silica gel chromatography with AcOEt/petroleum ether (9:1) as eluent (90% yield). TLC: R_f (AcOEt) = 0.68 (**4**), 0.45 (**3**) and 0.23 (**5**).

Data for 3: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.74 (s, 1 H, NH), 8.50 (s, 1 H, NH), 7.59–7.76 (m, 4 H, Ph), 7.47 (d, *J* = 1.2 Hz, 1 H, 6-H), 7.42–7.19 (m, 16 H, Ph and 6-H), 6.81 (m, 4 H, DMTr), 6.64 (dd, *J* = 5.1 and 9.3 Hz, 1 H, 1′b-H), 5.65 (s, 1 H, 1′a-H), 4.76 (d, $J_{H/P}$ = 5.5 Hz, 1 H, 3′a-H), 4.61 (s, 1 H, 2′a-H), 4.34–4.21 (m, 1 H, 7′b-H), 4.26 (br. d, *J* = 5.1 Hz, 1 H, 3′b-H), 4.11 (m, *J* = 4.0 and 11.7 Hz, $J_{H/P}$ = 26.8 Hz, 1 H, 7′b-H), 3.91 (A part of an AB system, *J* = 8.1 Hz, 1 H, 2′-*O*-4′-*C*-methylene), 3.75 (s, 6 H, MeO), 3.63 (B part of an AB system, *J* = 8.1 Hz, 1 H, 2′-*O*-4′-*C*-methylene), 3.70 (m, $J_{H/P}$ = 3.5 Hz, 1 H, 4′b-H), 3.48 (br. s, 2 H, 5′a-H), 3.35 (br. d, *J* = 10.8 Hz, 1 H, 5′b-H), 2.36 [A part of an ABX(Y) system, *J* = 5.4 and 13.5 Hz, 1 H, 2′b-H], 2.26–2.11 (m, 1 H, 6′b-H), 2.00 [B part of an ABX(Y) system, *J* = 5.5, 9.5 and 13.5 Hz, 1 H, 2′b-H], 1.92 (s, 3 H, Me), 1.64 (s, 3 H, Me$_a$), 1.30–1.23 (m, 1 H, 6′b-H), 1.07 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz,

$CDCl_3$): δ = 164.2, 164.1, 158.7, 150.9, 150.3, 143.8, 135.7, 135.6, 135.3, 134.9, 133.7, 133.2, 132.6, 130.1, 129.8, 128.2, 128.0, 127.13, 113.3, 112.3, 111.1, 87.7, 87.6, 87.5, 87.4, 87.0, 86.9, 85.1, 80.5, 77.9, 74.7, 73.3, 71.6, 67.7, 60.4, 57.8, 55.2, 39.7, 36.5, 31.4, 26.8, 21.0, 19.0, 14.2, 12.6, 12.3 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –9.7 ppm. MS (ESI): *m*/*z* = 1163.9 [M + Na]$^+$, 1179.8 [M + K]$^+$.

Data for 4: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.83 (s, 1 H, NH), 8.77 (s, 1 H, NH), 7.66–7.63 (m, 4 H, Ph), 7.51 (d, *J* = 1.0 Hz, 1 H, 6-H), 7.46–7.25 (m, 15 H, Ph), 7.13 (d, *J* = 1.0 Hz, 1 H, 6-H), 6.84 (m, 4 H, DMTr), 6.30 (dd, *J* = 6.0 and 8.5 Hz, 1 H, 1′b-H), 5.62 (s, 1 H, 1′a-H), 4.78 (d, $J_{H/P}$ = 3.8 Hz, 1 H, 3′a-H), 4.57 (s, 1 H, 2′a-H), 4.56 (m, 1 H, 3′b-H), 4.32 (ddd, *J* = 1.9, 3.7 and 12.0 Hz, 1 H, 5′b-H), 4.00 (A part of an ABX system, *J* = 4.0 and 11.0, $J_{H/P}$ = 23.8 Hz, 1 H, 7′b-H), 3.88 (ddd, *J* = 1.9 and 3.8, $J_{H/P}$ = 3.3 Hz, 1 H, 4′b-H), 3.86 (m, *J* = 2 and 11.0, $J_{H/P}$ < 1.0 Hz, 1 H, 7′b-H), 3.81 and 3.75 (AB system, *J* = 8.0 Hz, 2 H, 2′-*O*-4′-*C*-methylene), 3.79 (s, 6 H, MeO), 3.60 and 3.37 (AB system, *J* = 11.0 Hz, 2 H, 5′a-H), 2.17 [A part of an ABX(Y) system, *J* = 3.2, 5.9 and 13.9 Hz, 1 H, 2′b-H], 2.14–2.08 (m, 1 H, 2′b-H), 1.84 (s, 3 H, Me), 1.64 (s, 3 H, Me_a), 1.42 (m, *J* = 2.0, 4.0, 12.5 and 15.5 Hz, 1 H, 6′b-H), 1.16 (B part of an AB system, *J* = 15.5 Hz, 1 H, 6′b-H), 1.07 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.1, 163.9, 158.8, 150.4, 149.9, 143.9, 135.9, 135.8, 134.9, 134.8, 132.8, 132.7, 130.2, 129.2, 128.1, 127.8, 127.3, 113.4, 113.1, 112.6, 111.6, 87.7, 87.6, 87.3, 87.1, 85.9, 80.2, 80.1, 73.3, 72.1, 71.7, 68.6, 57.6, 55.3, 46.0, 39.4, 29.7, 26.9, 19.1, 12.7, 12.3 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –9.9 ppm. MS (ESI): *m*/*z* = 1163.9 [M + Na]$^+$, 1179.8 [M + K]$^+$.

Data for 5: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.72 (s, 1 H, NH), 8.67 (s, 1 H, NH), 7.65–7.53 (m, 4 H, Ph), 7.50 (d, *J* = 1.5 Hz, 1 H, 6-H), 7.44–7.13 (m, 15 H, Ph), 7.13 (d, *J* = 1.5 Hz, 1 H, 6-H), 6.81 (m, 4 H, DMTr), 6.29 (dd, *J* = 6.0 and 8.2 Hz, 1 H, 1′b-H), 5.60 (s, 1 H, 1′a-H), 4.80 (d, $J_{H/P}$ = 4.5 Hz, 1 H, 3′a-H), 4.56 (s, 1 H, 2′a-H), 4.46 (ddd, *J* = 2.5 and 12.0, $J_{H/P}$ = 3.6 Hz, 1 H, 5′b-H), 4.30 (m, 1 H, 3′b-H), 4.19–4.03 (m, 2 H, 7′b-H), 3.84 (q, *J* = 2.4 Hz, 1 H, 4′b-H), 3.77 and 3.76 (2s, 6 H, MeO), 3.61 (A part of an AB system, *J* = 8.4 Hz, 1 H, 2′-*O*-4′-*C*-methylene), 3.48 (A part of an AB system, *J* = 11.1 Hz, 1 H, 5′a-H), 3.40 (B part of an AB system, *J* = 8.4 Hz, 1 H, 2′-*O*-4′-*C*-methylene), 3.34 (B part of an AB system, *J* = 11.1 Hz, 1 H, 5′a-H), 2.14 [A part of an ABX(Y) system, *J* = 2.1, 5.9 and 12.0 Hz, 1 H, 2′b-H], 1.88–1.79 (m, 1 H, 2′b-H), 1.86 (s, 3 H, Me), 1.57 (s, 3 H, Me_a), 1.48–1.33 (m, 1 H, 6′b-H), 1.08–0.98 (m, 1 H, 6′b-H), 1.05 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.9, 158.8, 150.4, 149.9, 136.0, 135.7, 135.1, 135.0, 134.0, 132.7, 132.6, 130.4, 130.2, 130.1, 128.1, 127.2, 113.3, 111.7, 111.1, 87.8, 87.3, 87.2, 87.1, 86.9, 85.7, 80.3, 77.9, 74.0, 72.5, 71.5, 67.7,60.4, 57.3, 55.2, 53.7, 39.6, 26.8, 24.7, 19.0, 14.2, 12.6, 12.5 ppm.^{31}P NMR (121 MHz, $CDCl_3$): δ = –8.2 ppm. MS (ESI): *m*/*z* = 1163.8 [M + Na]$^+$.

($S_{C5'}$,S_P) 5′-*O*-Dimethoxytrityl-LNA/α,β-D-CNA TT (6) and ($R_{C5'}$,R_P) 5′-*O*-Dimethoxytrityl-LNA/α,β-D-CNA TT (7): Tetrabutylammonium fluoride (190 μL, 0.19 mmol for **3** or 360 μL, 0.36 mmol for **4**) was added under argon at 0 °C to the dinucleotide **3** (200 mg, 0.18 mmol) (or **4**, 380 mg, 0.33 mmol) in anhydrous THF (2 mL for **3** or 3.8 mL for **4**). Stirring was maintained for 1 h. After removal of the solvent under reduced pressure the crude product was purified on a silica gel column with AcOEt/methanol (9:1) as eluent. Compound **6** (152 mg) (242 mg of **7**) was recovered as a white foam in 87% yield (73% yield for **7**).

Data for 6: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.79 (s, 1 H, NH), 7.59 (d, *J* = 1.1 Hz, 1 H, 6-H), 7.37–7.12 (m, 10 H, Ph and 6-H), 6.80 (m, 4 H, Ph), 6.22 (t, *J* = 6.6 Hz, 1 H, 1′b-H), 5.54 (s, 1 H, 1′a-H), 4.90 (d, $J_{H/P}$ = 3.9 Hz, 1 H, 3′a-H), 4.66 (dt, *J* = 2.4 Hz and 11.7, 1 H), 4.58 (s, 1 H, 2′a-H), 4.40–4.33 (m, 2 H), 4.24–4.20 (m, 1 H), 3.90 (A part of an AB system, *J* = 8.4 Hz, 1 H, 2′-*O*-4′-*C*-methylene), 3.82–3.79 (m, 1 H), 3.77 (A part of an AB system, *J* = 8.4 Hz, 1 H, 2′-*O*-4′-*C*-methylene), 3.67 and 3.66 (2s, 6 H, Me), 3.50 and 3.43 (AB system, *J* = 11.1 Hz, 2 H, 5′a-H), 2.26–2.12 (m, 3 H), 1.79 (m, 1 H), 1.73 and 1.49 (br. s, 6 H, Me) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.4, 158.6, 150.9, 150.2, 144.0, 135.6, 135.1, 134.0, 130.1, 128.0, 127.1, 113.3, 112.0, 111.0, 87.6, 87.4, 87.1, 86.8, 86.7, 86.6, 84.7, 80.9, 77.9, 73.5, 71.8, 62.2, 57.7, 55.2, 39.6, 12.5, 12.2 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –9.3 ppm. MS (ESI): *m*/*z* = 925.3 [M + Na]$^+$, 941.7 [M + K]$^+$.

Data for 7: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.81 (s, 1 H, NH), 7.63 (d, *J* = 1.0 Hz, 1 H, 6-H), 7.39–7.16 (m, 10 H, Ph and 6-H), 6.80 (m, 4 H, Ph), 6.13 (t, *J* = 6.6 Hz, 1 H, 1′b-H), 5.53 (s, 1 H, 1′a-H), 4.95 (d, $J_{H/P}$ = 5.4 Hz, 1 H, 3′a-H), 4.58 (s, 1 H, 2′a-H), 4.46–4.31 (m, 2 H), 4.29–4.17 (m, 1 H), 3.81–3.76 (m, 2 H), 3.73 (br. s, 1 H), 3.69 (m, 7 H), 3.56 and 3.41 (AB system, *J* = 11.1 Hz, 2 H, 5′a-H), 2.21–2.07 (m, 3 H), 1.71 (br. s, 3 H, Me), 1.49 (br. s, 3 H, Me), 1.01–0.93 (m, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.6, 168.4, 162.7, 154.5, 154.2, 147.9, 140.5, 138.9, 137.9, 134.0, 132.0, 131.2, 117.3, 115.2, 114.9, 91.6, 91.5, 91.1, 90.9, 90.3, 89.7, 84.2, 77.4, 75.7, 73.2, 61.5, 59.1, 49.2, 16.4, 16.0 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –8.2 ppm. MS (ESI): *m*/*z* = 925.3 [M + Na]$^+$.

($S_{C5'}$,S_P) LNA/α,β-D-CNA TT (8) and ($R_{C5'}$,R_P) LNA/α,β-D-CNA TT (9): Compound **6** (or **7**) (20 mg, 0.021 mmol) was dissolved in a solution of trifluoroacetic acid (2%) in dichloromethane (1 mL) at room temperature. After 15 min the red solution was evaporated to dryness. The crude material was dissolved in THF and purified by silica gel chromatography. It was first eluted with AcOEt to remove the dimethoxytrityl residue and then with AcOEt/20% methanol to collect the LNA/α,β-D-CNA ($S_{C5'}$,S_P) **8** [or the LNA/α,β-D-CNA ($R_{C5'}$,R_P) **9**] as a white foam after evaporation of the solvent (**8**: 10.2 mg, 81% yield; **9**: 11.3 mg, 90% yield).

Data for 8: ^{1}H NMR (500 MHz, CD_3OD): δ = 7.74 (d, *J* = 1.0 Hz, 1 H, 6a-H), 7.59 (d, *J* = 1.0 Hz, 1 H, 6b-H), 6.43 (t, *J* = 6.5 Hz, 1 H, 1′b-H), 5.67 (s, 1 H, 1′a-H), 4.90 (td, *J* = 1.5 and 11.5 Hz, 1 H, 5′b-H), 4.71 (d, $J_{H/P}$ = 4.5 Hz, 1 H, 3′a-H), 4.66 (s, 1 H, 2′a-H), 4.59–4.50 (m, 3 H, 3′b-H and 7′b-H) 4.07 (A part of an AB system, *J* = 8.5 Hz, 1 H, 2′-*O*-4′-*C*-methylene), 4.04 and 3.98 (AB system, *J* = 13.4 Hz, 2 H, 5′a-H), 3.97 (m, *J* = 2.0 and 4.5, $J_{H/P}$ = 2.9 Hz, 1 H, 4′b-H), 3.93 (B part of an AB system, *J* = 8.5 Hz, 1 H, 2′-*O*-4′-*C*-methylene), 2.41–2.34 (m, 1 H, 6′b-H), 2.25 and 2.21 [ABX(Y) system, *J* = 3.5, 6.2, 8.0 and 13.5 Hz, 2 H, 2′b-H], 1.95 (m, 1 H, 6′b-H), 1.92 and 1.91 (br. s, 6 H, Me) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 165.0, 164.8, 151.0, 150.5, 129.9, 129.0, 111.0, 109.9, 88.7, 88.6, 86.9, 84.6, 81.0, 78.1, 73.2, 73.1, 71.3, 71.1, 69.0, 55.5, 39.1, 27.6, 11.3, 11.0 ppm. ^{31}P NMR (202 MHz, CD_3OD): δ = –7.9 ppm. MS (ESI): *m*/*z* = 623.3 [M + Na]$^+$. $C_{23}H_{29}N_4O_{13}P$ (600.47): calcd. C 46.01, H 4.87, N 9.33; found C 46.52, H 4.83, N 9.66.

Data for 9: ^{1}H NMR (500 MHz, CD_3OD): δ = 7.72 (br. s, 1 H, 6a-H), 7.50 (br. s, 1 H, 6b-H), 6.22 (t, *J* = 6.5 Hz, 1 H, 1′b-H), 5.65 (s, 1 H, 1′a-H), 4.88 (dddd, *J* = 2.5, 4.5 and 10.7, $J_{H/P}$ = 2.3 Hz, 1 H, 5′b-H), 4.69 (d, $J_{H/P}$ = 4.0 Hz, 1 H, 3′a-H), 4.68 (s, 1 H, 2′a-H), 4.61–4.54 (m, 3 H, 3′b-H and 7′b-H), 4.08 (A part of an AB system, *J* = 8.5 Hz, 1 H, 2′-*O*-4′-*C*-methylene), 4.02 and 3.99 (AB system, *J* = 13.2 Hz, 2 H, 5′a-H), 3.93 (B part of an AB system, *J* = 8.5 Hz, 1 H, 2′-*O*-4′-*C*-methylene), 3.90 (m, *J* = 4.5 and 4.5, $J_{H/P}$ = 2.4 Hz, 1 H, 4′b-H), 2.25 and 2.21 [ABX(Y) system, *J* = 4.5, 6.5, 6.7, 8.1 and 13.5 Hz, 2 H, 2′b-H], 2.24–2.18 (m, 1 H, 6′b-H),

2.03 (m, J = 2.5, 2.5 and 15.5 Hz, 1 H, 6′b-H), 1.91 (br. s, 6 H, Me-a), 1.85 (br. s, 6 H, Me-b) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 164.9, 164.8, 150.8, 150.4, 136.8, 134.7, 110.5, 109.9, 88.8, 87.0, 86.9, 86.8, 86.0, 80.5, 78.0, 73.2, 73.1, 71.1, 69.6, 69.5, 55.6, 38.8, 27.5, 11.2, 11.0 ppm. ^{31}P NMR (202 MHz, CD_3OD): δ = –8.2 ppm. MS (ESI): m/z = 623.3 $[M + Na]^+$. $C_{23}H_{29}N_4O_{13}P$ (600.47): calcd. C 46.01, H 4.87, N 9.33; found C 45.85, H 4.74, N 9.36.

Cyanoethyl 3′-*O*-[(5′-*O*-dimethoxytrityl)-(2′-*O*-4′-*C*)-methylenethymidinyl] 3′-(*tert*-Butyldiphenyl silyl)thymidinyl Phosphoric Ester (Mixture of Diastereoisomers) (11): 3′-*O*-(*tert*-Butyldiphenylsilyl)-thymidine (denoted as **10** in Scheme 2) (310 mg, 0.64 mmol) and thymidine-LNA O$^{3'}$-phosphoramidite (0.25 g, 0.32 mmol) were dissolved with an anhydrous acetonitrile solution of tetrazole (0.45 M, 1.6 mL) and the mixture was stirred for 45 min at room temperature. After addition of collidine (0.13 mL, 0.95 mmol), the phosphite was oxidised with iodine [0.1 M solution in THF(2)/H_2O(1)] until a dark brown colour persisted. The reaction mixture was diluted with AcOEt and washed with an aqueous solution of sodium thiosulfate (15%) to remove excess iodine. The organic layer was washed with water, brine and dried with $MgSO_4$ and the solvent was removed in vacuo. The crude material was purified by chromatography on silica gel with AcOEt as eluent. After evaporation of the solvent compound **11** (mixture of diastereoisomers) was recovered as a white foam (0.32 g, yield 86%). TLC: R_f (AcOEt) = 0.48. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.57–7.18 (m, 38 H, Ph), 7.14 (br. d, J = 1.2 Hz, 1 H, 6-H), 7.03 (br. d, J = 1.2 Hz, 1 H, 6-H), 6.82–6.76 (m, 8 H, Ph), 6.32–6.24 (m, 2 H, 1′b-H), 5.59 and 5.57 (s, 2 H, 1′a-H), 4.85 (m, 2 H, 3′a-H), 4.66 and 4.63 (s, 2 H, 2′a-H), 4.26 (m, 1 H, 3′b-H), 3.98 (m, 2 H), 3.82 (m, 2 H), 3.78–3.60 (m, 21 H), 3.54 (A part of an AB system, J = 11.1 Hz, 1 H, 5′a-H), 3.35 and 3.33 (B parts of AB systems, J = 11.1 Hz, 2 H, 5′a-H), 2.54 (t, J = 6.0 Hz, 2 H), 2.37–2.17 (m, 4 H), 1.89–1.79 (m, 2 H), 1.76, 1.73, 1.54 and 1.49 (br. s, 12 H, Me), 1.01 (s, 18 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.1, 164.0, 158.9, 158.8, 150.5, 150.2, 150.1, 144.1, 143.9, 135.7, 135.1, 135.0, 132.9, 132.8, 132.7, 130.3, 130.2, 130.1, 128.1, 128.0, 121.3, 116.4, 116.1, 113.4, 111.4, 111.3, 111.0, 110.9, 87.5, 87.4, 87.3, 87.2, 84.9, 84.8, 84.7, 78.0, 74.8, 74.7, 72.8, 72.5, 71.7, 71.6, 62.7, 62.6, 62.3 60.4, 57.8, 55.3, 55.2, 40.4, 40.2, 26.9, 19.5, 19.4, 19.3, 12.6, 12.5, 12.4 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –2.5, –3.0 ppm. MS (ESI): m/z = 1190.9 $[M + Na]^+$, 1206.8 $[M + K]^+$.

3′-*O*-[(5′-*O*-Dimethoxytrityl)-(2′-*O*-4′-*C*)-methylenethymidinyl] 3′-(*tert*-Butyldiphenylsilyl)thymidinyl Phosphoric Ester Triethylammonium Salt (12): Triethylamine (48 μL, 0.34 mmol) was added at room temperature to compound **11** (200 mg, 0.17 mmol) in anhydrous acetonitrile (5 mL). After 2 h at 60 °C, all the solvent was removed under high vacuum. Phosphoric ester **12** was recovered as a white foam (207 mg, 100% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.60 (br. s, 1 H, 6-H), 7.52–7.48 (m, 5 H, Ph), 7.38–7.08 (m, 15 H, Ph), 6.74–6.69 (m, 4 H, Ph), 6.34 (dd, J = 5.4 and 8.4 Hz, 1 H, 1′b-H), 5.51 (s, 1 H, 1′a-H), 4.63 (s, 1 H, 2′a-H), 4.60 (d, $J_{H/P}$ = 3.6 Hz, 1 H, 3′a-H), 4.34 (br. d, J = 4.8 Hz, 1 H, 3′b-H), 3.87 (br. s, 1 H, 4′b-H), 3.77–3.65 (m, 10 H, 5′b-H, 2′-*O*-4′-*C*-methylene and OMe), 3.39 (s, 2 H, 5′a-H), 2.83 (q, J = 7.2 Hz, 6 H, NCH_2), 2.04 [A part of an ABX(Y) system, J = 5.7 and 13.5 Hz, 1 H, 2′b-H], 1.77 (m, 1 H, 2′a-H), 1.75 (s, 3 H, Me), 1.58 (s, 3 H, Me), 1.07 (t, J = 7.2 Hz, 9 H, Me), 0.99 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.5, 164.4, 158.6, 150.8, 150.4, 144.5, 135.7, 135.5, 133.4, 133.2, 130.2, 130.1, 129.9, 128.2, 127.9, 127.8, 127.0, 113.2, 110.8, 109.9, 87.5, 87.4, 86.5, 85.1, 78.5, 74.4, 73.0, 60.3, 55.2, 45.3, 40.8, 26.9, 21.0, 19.0, 14.2, 12.6, 12.2, 8.4 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = –2.4 ppm. MS (ESI): m/z = 1114.0 $[M]^-$.

3′-*O*-[(2′-*O*-4′-*C*)-Methylenethymidinyl]thymidinyl Thymidinyl Phosphoric Ester Sodium Salt (13): Tetrabutylammonium fluoride (190 μL, 0.19 mmol) was added under argon at 0 °C to the dinucleotide **12** (205 mg, 0.17 mmol.) in anhydrous THF (5 mL). After 15 min, THF was removed under vacuum. The crude material was treated with 3% TFA/CH_2Cl_2 (5 mL) for 5 min and dried under high vacuum. The crude material was purified by chromatography on silica gel with AcOEt/methanol (0 to 20%) as eluent. R_f (AcOEt/20% MeOH) = 0.18. The tetrabutylammonium counter-ion was then replaced by a sodium cation by passing the dinucleotide in aqueous solution through a column filled with Dowex 50WX8 resin in its sodium form. After removal of water, **13** was recovered as a white solid (76 mg, 75% yield). ^{1}H NMR (500 MHz, D_2O): δ = 7.68 (d, J = 1.0 Hz, 1 H, 6b-H), 7.49 (d, J = 1.0 Hz, 1 H, 6a-H), 6.11 (t, J = 6.5 Hz, 1 H, 1′b-H), 5.46 (s, 1 H, 1′a-H), 4.61 (s, 1 H, 2′a-H), 4.37 (q, J = 5.3 Hz, 1 H, 3′b-H), 4.28 (d, $J_{H/P}$ = 6.1 Hz, 1 H, 3′a-H), 4.06 [A part of an ABX(Y) system, J = 2.6, and 11.5, $J_{H/P}$ = 3.8 Hz, 1 H, 5′b-H], 3.98 (m, J = 2.0 and 2.7, $J_{H/P}$ = 2.0 Hz, 1 H, 4′b-H), 3.94 [B part of an ABX(Y), J = 2.9 and 11.6, $J_{H/P}$ = 4.0 Hz, 1 H, 5′b-H], 3.95 (A part of an AB system, J = 8.3 Hz, 1 H, 2′-*O*-4′-*C*-methylene), 3.93 and 3.89 (AB system, J = 13.5 Hz, 2 H, 5′a-H), 3.85 (B part of an AB system, J = 8.3 Hz, 1 H, 2′-*O*-4′-*C*-methylene), 2.26 [A part of an ABX(Y) system, J = 5.9, 6.4 and 13.4 Hz, 1 H, 2′b-H], 2.14 [B part of an ABX(Y) system, J = 5.9, 6.3 and 13.4 Hz, 1 H, 2′b-H], 1.72 (br. s, 3 H, Me_b), 1.69 (br. s, 3 H, Me_a) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 166.3, 166.2, 151.5, 151.0, 136.5, 135.5, 111.2, 110.6, 88.6, 88.6, 86.9, 85.1, 84.8, 84.7, 77.8, 71.9, 71.5, 69.2, 64.0, 63.9, 55.9, 39.2, 23.2, 11.8, 11.3 ppm. ^{31}P NMR (202 MHz, D_2O): δ = –1.4 ppm. MS (ESI): m/z = 573.4 $[M]^-$. $C_{21}H_{26}N_4NaO_{13}P$ (596.41): calcd. C 42.29, H 4.39, N 9.39; found C 42.85, H 4.43, N 9.22.

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

[1] C. J. Leumann, *Bioorg. Med. Chem.* **2002**, *10*, 841–854.
[2] J. Wengel, *Acc. Chem. Res.* **1999**, *32*, 301–310.
[3] P. Herdewijn, *Biochim. Biophys. Acta* **1999**, *1489*, 167–179.
[4] S. Obika, D. Nanbu, Y. Hari, K.-I. Morio, Y. In, T. Ishida, T. Imanishi, *Tetrahedron Lett.* **1997**, *38*, 8735–8738.
[5] S. Obika, D. Nanbu, Y. Hari, J.-I. Andoh, K.-I. Morio, T. Doi, T. Imanishi, *Tetrahedron Lett.* **1998**, *39*, 5401–5404.
[6] S. K. Singh, P. Nielsen, A. A. Koshkin, J. Wengel, *Chem. Commun.* **1998**, 455–456.
[7] K. E. Nielsen, J. Rasmussen, R. Kumar, J. Wengel, J. P. Jacobsen, M. Petersen, *Bioconjugate Chem.* **2004**, *15*, 449–457.
[8] M. Petersen, K. Bondensgaard, J. P. Jacobsen, J. Wengel, *J. Am. Chem. Soc.* **2002**, *124*, 5974–5982.
[9] M. Manoharan, *Curr. Opin. Chem. Biol.* **2004**, *8*, 570–579.
[10] E. Rozners, *Curr. Org. Chem.* **2006**, *10*, 675–692.
[11] E. T. Kool, *Chem. Rev.* **1997**, *97*, 1473–1487.
[12] C. Dupouy, I. Le Clezio, P. Lavedan, H. Gornitzka, J.-M. Escudier, A. Vigroux, *Eur. J. Org. Chem.* **2006**, 5515–5525.

[13] I. Le Clezio, H. Gornitzka, J.-M. Escudier, A. Vigroux, *J. Org. Chem.* **2005**, *70*, 1620–1629.
[14] I. Le Clezio, J.-M. Escudier, A. Vigroux, *Org. Lett.* **2003**, *5*, 161–164.
[15] I. Le Clezio, C. Dupouy, P. Lavedan, J.-M. Escudier, *Eur. J. Org. Chem.* **2007**, 3894–3900.
[16] C. Dupouy, N. Iché-Tarrat, M.-P. Durrieu, F. Rodriguez, J.-M. Escudier, A. Vigroux, *Angew. Chem.* **2006**, *118*, 3705–3709; *Angew. Chem. Int. Ed.* **2006**, *45*, 3623–3627.
[17] M. Ikehara, *Heterocycles* **1984**, *21*, 75–90.
[18] M. Sekine, O. Kurasawa, K. Shohda, K. Seio, T. Wada, *J. Org. Chem.* **2000**, *65*, 6515–6524.
[19] M. H. Caruthers, *Acc. Chem. Res.* **1991**, *24*, 278–284.
[20] D. G. Gorestein, *Chem. Rev.* **1987**, *87*, 1047–1077.
[21] a) D. J. Wood, F. E. Hruska, K. K. Ogilvie, *Can. J. Chem.* **1974**, *52*, 3353–3366; b) D. J. Wood, K. K. Ogilvie, F. E. Hruska, *Can. J. Chem.* **1975**, *53*, 2781–2790.
[22] C. Altona, M. Sundaralingam, *J. Am. Chem. Soc.* **1973**, *95*, 2333–2344.
[23] Typical upper and lower limits observed for the $^3J_{H/P}$ coupling constants for dioxaphosphorinane structures in the chair conformation are $^3J_{Hax/P} \leq 3$ Hz and $^3J_{Heq/P} \geq 20$ Hz, respectively. See D. G. Gorenstein, R. Rowell, J. Findlay, *J. Am. Chem. Soc.* **1980**, *102*, 5077–5081.
[24] I. Le Clezio, A. Vigroux, J.-M. Escudier, *Eur. J. Org. Chem.* **2007**, 1935–1941.
[25] P. P. Lankhorst, C. A. G. Haasnoot, C. Erkelens, C. J. Altona, *J. Biomol. Struct. Dyn.* **1984**, *1*, 1387–1405. The torsional angle ε was determined by using the following relation: $\varepsilon = -\theta - 120°$ and θ was calculated with $J_{H3'/P} = 15.3\cos^2(\theta) - 6.1\cos\theta + 1.6$.
[26] B. Schneider, S. Neidle, H. M. Berman, *Biopolymers* **1997**, *42*, 113–124.
[27] N. Camerman, J. K. Fawcett, A. Camerman, *J. Mol. Biol.* **1976**, *107*, 601–621.

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