Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 11235–11243

Synthesis of spiro ε,ζ-D-CNA in *xylo* configuration featuring noncanonical δ/ε/ζ torsion angle combination

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

[a]*Laboratoire de Synthèse et Physico-Chimie de Molécules d'Intérêt Biologique, UMR 5068 CNRS, Université Paul Sabatier, 31062 Toulouse Cedex 9, France*
[b]*Service Commun de RMN, Université Paul Sabatier, 31062 Toulouse Cedex 9, France*

Received 20 July 2007; revised 22 August 2007; accepted 29 August 2007
Available online 1 September 2007

Abstract—The synthesis of diastereoisomers of spiro *xylo*-ε,ζ-D-CNA dinucleotide building units of nucleic acids, in which the ε and ζ torsional angles are stereocontrolled by a dioxaphosphorinane ring structure (D-CNA family), is described from uridine with a Mukaïyama's aldol condensation as key reaction. The NMR structure analysis showed that δ, ε and ζ torsional angles of the ($S_{C3'}$,R_P)- and ($S_{C3'}$,S_P)-configured *xylo*-ε,ζ-D-CNA TT dimer are restricted to the {δ, ε, ζ}={cis, trans, *gauche*(+)} or {cis, trans, *gauche*(−)} conformation, respectively, that are significantly different from those typically observed in A- or B-type duplexes.

1. Introduction

The construction of conformationally restricted nucleosides analogues has been mainly devoted to enhance oligonucleotides properties in the field of antisense applications.[1–3] However, to date only few reports deal with the design of conformationally restricted nucleotides for the special purpose of mimicking biologically relevant helical distortions of B-DNA or important non-helical secondary structures of functional RNA.[4]

Bulges, hairpins, branched junctions or U-turn are alternative structures frequently adopted by nucleic acids in addition to the double-stranded helical conformation.[5] Non-Watson–Crick pairs or unpaired nucleotides are remarkable elements of these secondary structures and they are characterized by a variety of backbone conformations that markedly differ from the regular conformational states of double-stranded helices. The important role in fundamental biological processes such as protein–nucleic acid interactions, nucleic acids folding or catalytic activity displayed by these disparate structures is associated with a significant local conformational heterogeneity in the sugar–phosphate backbone.[6]

It has been showed that the α and γ torsional angles (Fig. 1) can adopt noncanonical conformations with *gauche*(+) or trans and *gauche*(−) or trans values, respectively, in protein-bound B-DNA oligomers in contrast to free B-DNA structures.[7] 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.

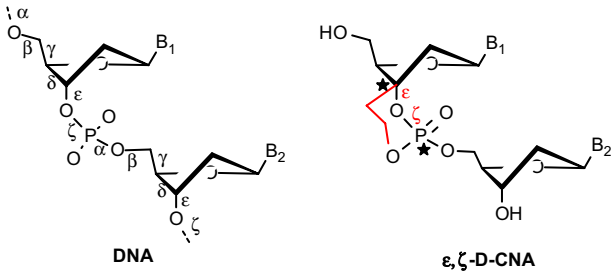


Figure 1. Left: the six backbone torsional angles (labelled α to ζ) of nucleic acids. Right: ε,ζ-D-CNA unit are dinucleotide in which ε and ζ are stereocontrolled to canonical or noncanonical values by a dioxaphosphorinane ring structure exhibiting an asymmetric phosphorus centre.

Protein–DNA recognition processes seems to be also very sensitive to the DNA intrinsic structure and flexibility that can originate from backbone equilibria between canonical BI and BII states associated with helical changes.[8,9] These two backbone conformations differ in the torsional angles ε and ζ which are, respectively, trans/*gauche*(−) in BI and *gauche*(−)/trans in BII. On the other hand, it has been showed that more unusual conformations of ε and ζ

Keywords: Strained molecules; Nucleotides; DNA structures; Spiro compounds; Conformation analysis.
* Corresponding author. E-mail: escudier@chimie.ups-tlse.fr

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.08.098

participate in specific structural distortions within nucleic acids involved in particular biologically relevant processes.[10]

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.

In order to take into account these elements and in accordance with the preorganization concept,[11] we began the development of constrained dinucleotide building units in which the backbone torsional angles are covalently locked within a dioxaphosphorinane ring and can adopt predefined values. We first reported on the stereoselective synthesis of an α,β-D-CNA (Dioxaphosphorinane-Constrained Nucleic Acid) featuring noncanonical values of the torsional angle α and β,[12] and later on, on its properties within oligonucleotides.[13,14] The methodology was then extended to complete the α,β-D-CNA family,[15–17] and to prepare α,β,γ-D-CNA or δ,ε,ζ-D-CNA.[18] The latter exhibit, in the case of one isomer a BII like conformation with ε and ζ in a {*gauche*(−)/*anticlinal*(+),trans} conformation and for the other isomer an unusual {*gauche*(−)/*gauche*(−)} ε/ζ combination. In both cases the sugar puckering was not altered and the 2′-deoxyriboses were in a C2′-*endo* conformation (δ=a$^+$/t).

In order to enlarge the ε,ζ combination set, and therefore to have access to an increase variety of structure, we chose to introduce the dioxaphosphorinane ring by connecting the 3′-C to the oxygen atom of the phosphate with an ethylene bridge (Fig. 1). The resulting spiro structure can be represented by four diastereoisomers. In the present work, we report on synthesis from uridine and conformational behaviour of ($S_{C3'}$,S_P) and ($S_{C3'}$,R_P) isomers of this novel class of constrained dinucleotide building units termed as *xylo*-ε,ζ-D-CNA.

2. Results and discussion

2.1. Synthetic plan

Our retrosynthetic analysis for the synthesis of the four possible stereoisomers of a given ε,ζ-D-CNA dinucleotide step is summarized in Figure 2. It is based on the very simple strategy that consists of using both steric and anomeric effects to stereocontrol the cyclization reaction of a dinucleotide precursor in which the *pro*-(*R*) and *pro*-(*S*)-phosphate oxyanions can attack an electrophilic tosyloxy-substituted carbon atom.[12] Among the four possible ε,ζ-D-CNA diastereoisomers, we anticipated that the ($S_{C3'}$,R_P)- and ($R_{C3'}$,S_P)-isomers with the alkoxy group ON_2 axial (equatorial P═O) and the more hindered 4′-C carbon equatorial would be formed preferentially due to the sterically and anomerically favourable trans relationship between ON_2 and 4′-C in the corresponding six-membered chair conformations of the dioxaphosphorinane ring. As shown in Figure 2, the key starting material for the preparation of these diastereoisomers are the diastereopure 3′(*S*)- or 3′(*R*)-*C*-tosyloxyethyl-substituted nucleosides. A brief survey of the literature showed that whereas the 3′(*S*)-C configured nucleoside could be obtained from *ribo* nucleosides as starting material,[19–21] its (*R*) epimer should be prepared from D-glucose.[22–24]

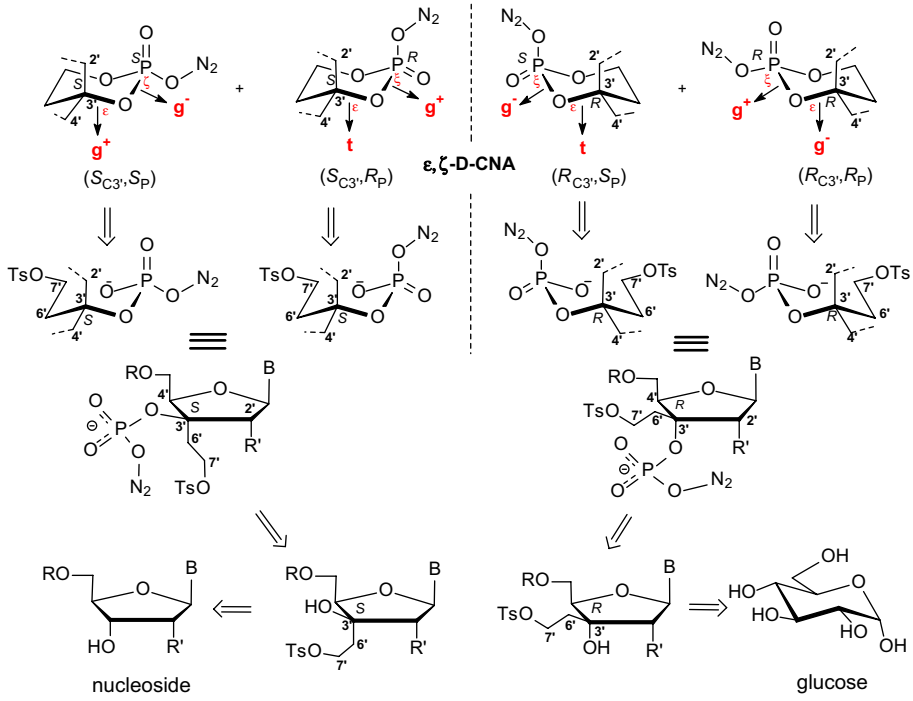


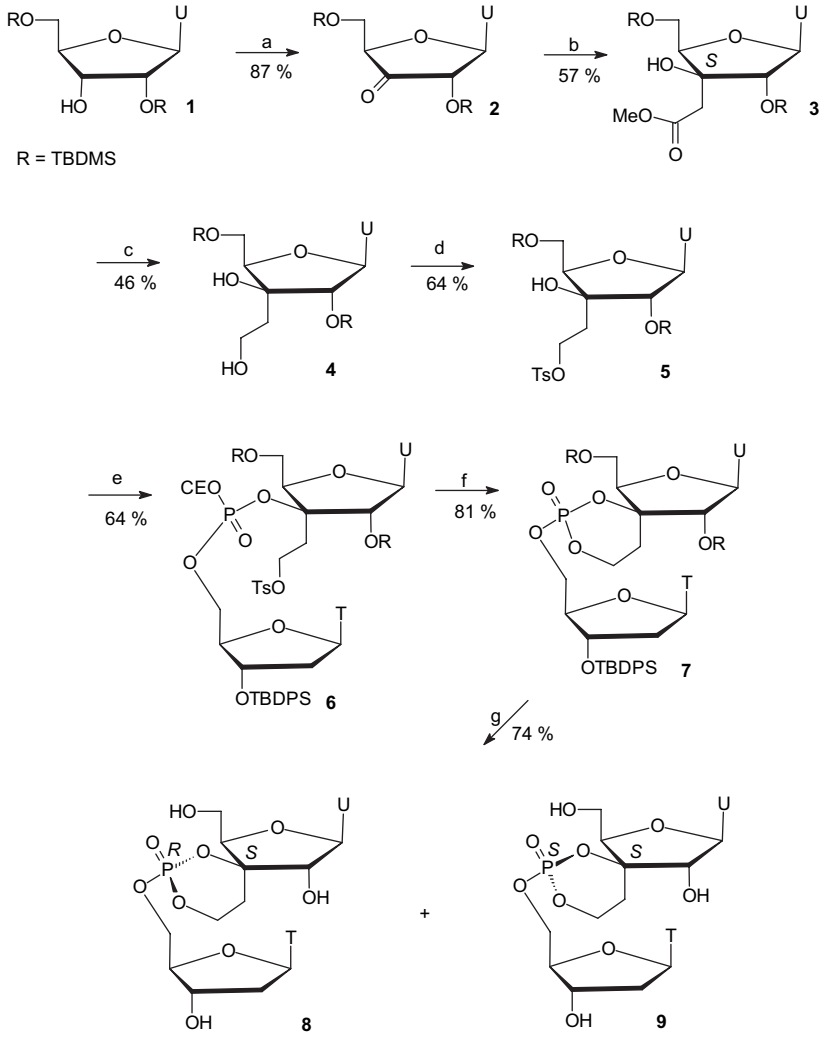
Figure 2. Retrosynthetic pathway for the diastereoselective synthesis of the four possible stereoisomers of ε,ζ-D-CNA dimers. N_2 stand for the remaining atomic fragment that define the lower nucleoside unit. The expected *gauche*(+), *gauche*(−), or trans conformations of ε (C3′–O3′) and ζ (O3′–P) are indicated for the hypothetical true chair conformations associated with each diastereoisomer.

2.2. Synthesis

The synthesis of the key intermediate 3′-*C*-(2-tosyloxyethyl)xylouridine **5** involves the selective tosylation of the primary hydroxyl function of the diastereopure 3′-*C*-(2-hydroxyethyl)xylouridine **4** (Scheme 1) achieved by treatment with tosyl chloride in the presence of pyridine in 64% yield.[25] The latter compound was obtained by the diisobutylaluminium hydride mediated reduction of the methyl ester group of **3** in a moderate 46% yield. The 3′-*C*-(methoxycarbonylmethyl)xylouridine **3** was obtained via a diastereoselective Mukaïyama's reaction with the *tert*-butyldimethylsilyl-methyl-ketene acetal[26] catalyzed by the trifluoroboron/etherate complex on the keto-uridine **2** in 57% yield.[27] Dess–Martin oxidation procedure[28] provided **2** from the 3′,5′-di-*O*-*tert*-butyldimethylsilyl uridine **1**[29] in high yield (87%).[30] We chose to start from the keto-uridine instead of the obvious keto-thymidine analogue because the latter is prone to base elimination in basic and in acidic conditions due to the high acidic character of the 2′ protons.

The absolute configuration of the substituted 3′-C carbon of **3** was determined by examination of the NOESY spectrum (Fig. 3). The 6′-H and 6″-H protons exhibit an AB pattern (2.85 and 2.74 ppm, ^2J=17.0 Hz) with no cross peak with the 5′-H and 5″-H protons. On the other hand, strong NOE cross peaks are observed between 4′-H and both of 6′-H and 6″-H and 2′-H with 6″-H. With respect to the C3′-*endo* conformation (North) of the sugar ($^3J_{1',2'}$=0 Hz), these detected interactions clearly demonstrate that the 3′-C carbon has a '*S*' absolute configuration.

3′-*C*-(2-Tosyloxyethyl)xylouridine **5** was then coupled with the readily available 3′-*O*-*tert*-butyldiphenylsilyl-5′-*O*-phosphoramidite thymidine according to standard phosphoramidite procedure[31] to give two diastereoisomeric dinucleotides **6** in an equimolar ratio. *xylo*-ε,ζ-D-CNA **7** were obtained as a mixture of diastereoisomers (1:1 ratio as depicted by ^{31}P NMR: δ_P= −6.4 and −8.9 ppm), in good yield (81%) by treatment of **6** with K_2CO_3 in anhydrous dimethylformamide at room temperature. In this particular case, no diastereoselectivity was observed certainly because



Scheme 1. Reagents and conditions: (a) Dess–Martin periodinane, Pyr, CH_2Cl_2, room temperature 24 h; (b) *tert*-butyldimethylsilyl-methyl-ketene, $BF_3\cdot Et_2O$, CH_2Cl_2, −78 °C, 2 h; (c) DIBAH, CH_2Cl_2, 0 °C, 2 h then room temperature 12 h; (d) tosylchloride, Pyr, $CHCl_3$, room temperature 16 h; (e) 3′-*O*-*tert*-butyldiphenylsilylthymidine-5′-*O*-phosphoramidite (2 equiv), tetrazole, CH_3CN then collidine, I_2/H_2O; (f) K_2CO_3, DMF, room temperature, 4 h; (g) *n*-Bu_4NF (3.3 equiv), THF, room temperature, 30 min.

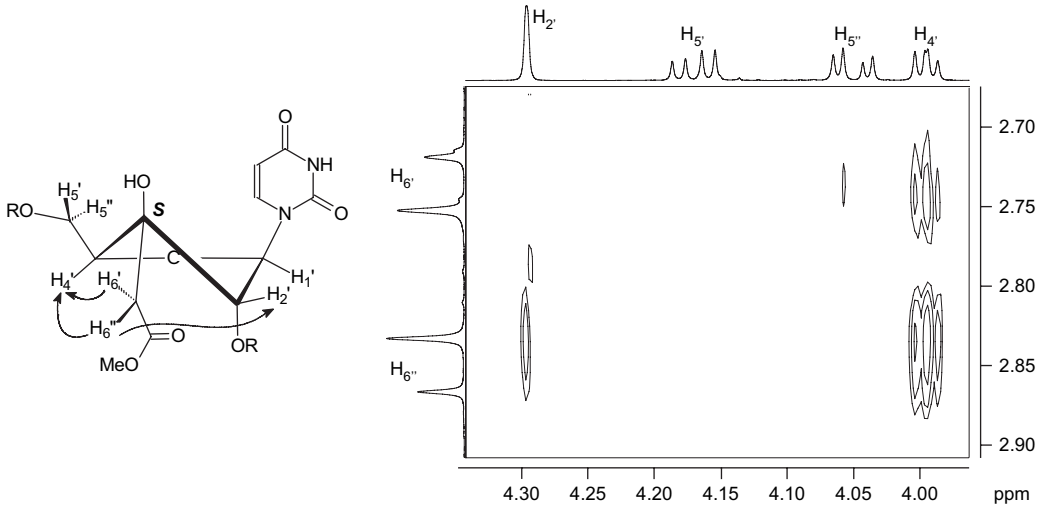


Figure 3. Schematic drawings and NOE spectrum of 3′-C substituted uridine **3** showing the main NOE effects observed given evidence for the absolute configuration of 3′-C.

the substitution on 4′-C and 2′-C are sterically equivalent and therefore there is no discrimination in the transition state to favour one dioxaphosphorinane diastereoisomer.

A diastereoisomeric mixture **7** was treated with TBAF to remove the silyl protecting groups, and the formed unprotected diastereoisomers ($S_{C3'}$,R_P) *xylo*-ε,ζ-D-CNA **8** and ($S_{C3'}$,S_P) *xylo*-ε,ζ-D-CNA **9** were isolated by reversed-phase HPLC. Because of the spiro structure with an '*S*' configuration of 3′-C between the upper sugar ring and the dioxaphosphorinane cycle, the relative position of the 2′-hydroxyl function with respect to the phosphorus atom does not allow any trans-esterification process since the favourable 'on line' conformation cannot be reached.[32] This particular conformation provide a stable phosphotriester function regardless of the presence of the usually reactive free 2′-hydroxyl function.

2.3. Structural assignment of the ($S_{C3'}$,S_P) and ($S_{C3'}$,R_P)-*xylo*-ε,ζ-D-CNA

To determine the behaviour of the uracyl and thymine moieties in the *xylo*-ε,ζ-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 an unmodified T_pT dinucleotide (Fig. 4). The most striking features are the very little temperature dependence observed for the ($S_{C3'}$,S_P)-*xylo*-ε,ζ-D-CNA **9** of the positive Cotton effect around 260 nm and the absence of a negative band. These results could be undoubtedly explained in terms of the relative conformational rigidity of the dioxaphosphorinane structure that does not allow any bases stacking in this particular conformation of the torsional angle ε and ζ. The CD spectrum of this dinucleotide is therefore highly comparable with the CD spectrum of the corresponding monomer.[33] Whereas T_pT and ($S_{C3'}$,R_P)-*xylo*-ε,ζ-D-CNA **8** showed a decrease of the stacking of the bases as the temperature increased, the nearly mirror-image spectra demonstrate that the constrained dinucleotide must have a stacked conformation that is quite different from the linear dinucleotide. Although these spectra showed a crossover near the normal absorption maximum (λ=263 and 266 nm for **8** and T_pT, respectively), when the temperature reached 80 °C, the negative band of the CD spectrum of **8** (λ=278 nm) disappeared and the resulting spectrum closely look like to the CD spectrum of its diastereoisomer **9**. This suggest a quite flexible relative conformation of the bases in the ($S_{C3'}$,R_P)-*xylo*-ε,ζ-D-CNA **8**.

In order to determine the relative spatial arrangement of the two nucleotides, ^{1}H, ^{1}H–{^{31}P}, 2D COSY ^{1}H/^{1}H, 2D COSY ^{1}H/^{31}P and 2D ROESY NMR spectra were recorded at 300 and 500 MHz in deuterium oxide for ($S_{C3'}$,R_P)-*xylo*-ε,ζ-D-CNA **8** and ($S_{C3'}$,S_P)-*xylo*-ε,ζ-D-CNA **9**.

The puckering of the xylose and 2′-deoxyribose moieties of **8** and **9** was assigned by examination of the sugar ring H/H coupling constants (Table 1). Whereas neither the $H_{3'}/H_{4'}$ nor the $H_{2'}/H_{3'}$ coupling constant can be used to clearly

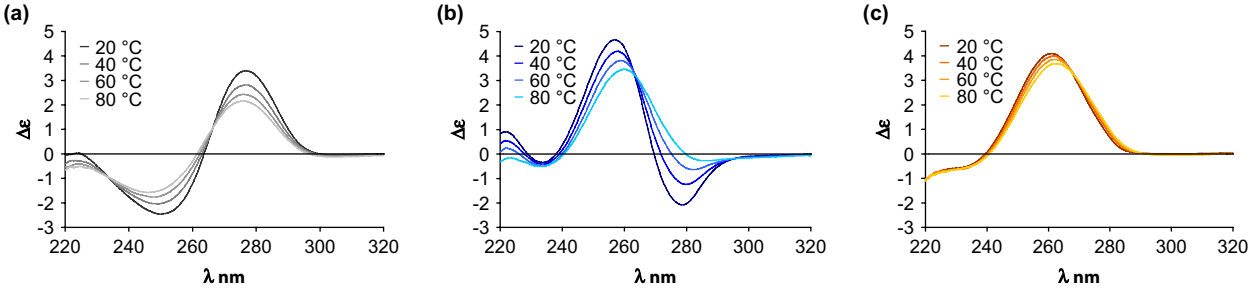


Figure 4. CD spectra in 10 mM sodium phosphate (pH 7.0) at 80, 60, 40 and 20 °C of (a) T_pT (b) ($S_{C3'}$,R_P)-*xylo*-ε,ζ-D-CNA **8** and (c) ($S_{C3'}$,S_P)-*xylo*-ε,ζ-D-CNA **9**.

Table 1. H/H coupling constants (Hz) in the ^{1}H NMR spectra (500 MHz) of ($S_{C3'}$,R_P) and ($S_{C3'}$,S_P) *xylo*-ε,ζ-D-CNA diastereoisomers **8** and **9** in D_2O

Nucleoside		Coupling constant ^3J (Hz)				
		$J(1',2')$		$J(2',3')$	$J(3',4')$	
8	Upper	0	—	—	—	—
	Lower	6.9	6.9	7.2	3.2	2.9
9	Upper	0	—	—	—	—
	Lower	6.6	6.6	6.6	4.7	4.1

establish the puckering of the upper nucleosides due to the spiro junction at 3′-C, the C3′-*endo* conformation can be assumed with a undetectable coupling constant between 1′-H and 2′-H. For the lower sugar, the relatively small $J_{H3'/H4'}$ measured for the lower nucleosides and the values of $J_{H2'/H3'}$ and $J_{H1'/H2'}$ are close in each case to those found in the standard C2′-*endo* conformation of the natural 2′-deoxyribose.[34,35]

The chair conformation of the dioxaphosphorinane structure of **8** is clearly established from the ^{1}H NMR spectra, with the observation of small ($^3J_{H7'/P}\approx 3$ Hz) and large ($^3J_{H7''/P}\approx 22$ Hz) $^3J_{H/P}$ coupling constants between the 7′-H and 7″-H protons and phosphorus, which is characteristic of an axial position ($0\leq{}^3J_{Hax/P}\leq 3$ Hz) and an equatorial position ($20\leq{}^3J_{Heq/P}\leq 30$ Hz) of these protons (Table 2).[36] The determination of these coupling constants involved a combined record of ^{1}H–{^{31}P} spectrum with irradiation at 2.32 ppm (6′-H) and simulation with the WIN-DAISY software[37] in order to first determine the $^2J_{H7'/H7''}$ coupling constant (12.0 Hz) and then the $^3J_{H7'/P}$ by simulation of the ^{1}H spectrum irradiated at 2.32 ppm (Fig. 5).

Table 2. H/P coupling constants (Hz) in the ^{1}H NMR spectra (500 MHz) of ($S_{C3'}$,R_P) and ($S_{C3'}$,S_P) *xylo*-ε,ζ-D-CNA diastereoisomers **8** and **9** in D_2O

Dinucleotide	Coupling constant J (Hz)					
	$^4J(4'_a,P)$	$^4J(4'_b,P)$	$^3J(5'_b,P)$		$^3J(7'_a,P)$	
8	4.2	0	4.0	6.5	3.0	22.3
9	5.4	3.0	5.9	6.7	4.5	10.5

In contrast, average values of 4.5 and 10.5 Hz were observed for the $^3J_{H/P}$ coupling constants involving the 7′-H and 7″-H protons of ($S_{C3'}$,S_P) *xylo*-ε,ζ-D-CNA **9**, thus suggesting that the dioxaphosphorinane structure of this isomer is in a twist-chair conformation. However, in both diastereoisomers a long range coupling constant was observed between 4′a-H and the phosphorus, indicative of a typical W-shaped conformation between these two atoms. Therefore the ε torsional angle adopts, in these structures, a trans conformation. Interestingly, only the isomer **9** exhibits a $^4J_{H4'b/P}$ (3.0 Hz), which is frequently observed in nucleotide as a consequence of the canonical *gauche*(+) conformation of γ.[35]

The overall conformations of dinucleotides ($S_{C3'}$,R_P) *xylo*-ε,ζ-D-CNA **8** and ($S_{C3'}$,S_P) *xylo*-ε,ζ-D-CNA **9** were further investigated by examination of 2D NOESY NMR spectra. Although many cross peaks are present for **8** and **9**, most of them are derived from intraresidual H/H interactions indicative of the sugar puckering (North-type of the upper nucleoside and South-type for the lower) and the relative position of the thymine bases. The NOESY cross peaks

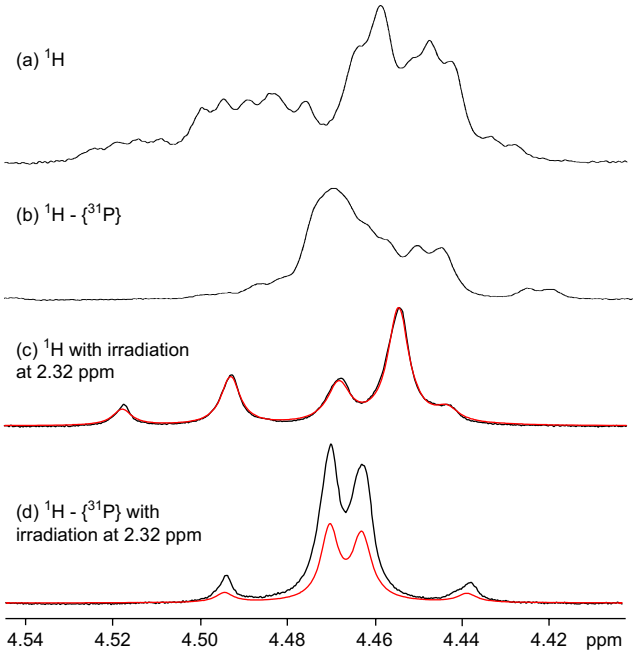


Figure 5. ^{1}H NMR data for 6′a-H of the ($S_{C3'}$,R_P) *xylo*-ε,ζ-D-CNA **8**. Superimposition with the simulated pattern by the WIN-DAISY software are provided in (c) and (d).

observed for **8**, between 2′a-H and 4′a-H with the equatorial and axial 6′a-H protons, respectively, give evidence for the stereochemistry of the spiro junction, and is indicative of the relative position of the upper sugar unit of **8** with respect of the dioxaphosphorinane ring (Fig. 6).

The data collected from NMR spectroscopy led us to propose the conformational ranges of δ, ε and ζ torsional angles for each *xylo*-ε,ζ-D-CNA diastereoisomer (Table 3). The canonical values for these torsional angles observed in A-, BI- or BII-type duplexes are given as reference.[38]

When compared with the backbone conformations summarized in Table 3, we can expect that DNA oligonucleotides incorporating one of these new D-CNA elements will display an original pattern that could represent an important structural feature in order to stabilize unpaired structure of DNA or RNA occurring in relevant biological processes.

3. Conclusion

We have synthesized two new members of the D-CNA family in which the backbone torsional angles (ε,ζ) of nucleic acids are simultaneously locked in a dioxaphosphorinane ring structure. Structural analysis indicates that ($S_{C3'}$,R_P) *xylo*-ε,ζ-D-CNA **8** and ($S_{C3'}$,S_P) *xylo*-ε,ζ-D-CNA **9** have δ,ε, and ζ torsions locked in the (c, t, g^+) and (c, t, g^-) conformations, respectively. 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 non-helical secondary structures of functional RNA and we are currently examining this possibility with *xylo*-ε,ζ-D-CNA.

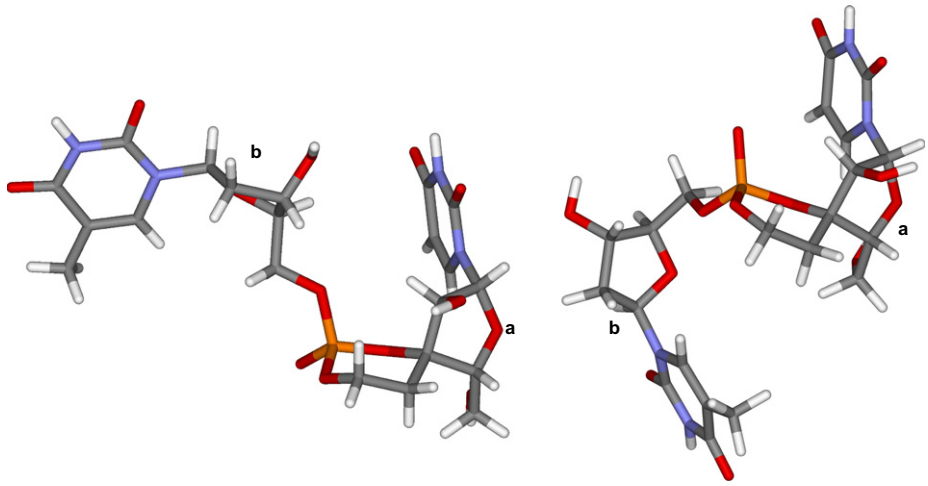


Figure 6. Tentative model of the conformation of the ($S_{C3'}$,R_P) *xylo*-ε,ζ-D-CNA and ($S_{C3'}$,S_P) *xylo*-ε,ζ-D-CNA derived from NMR data. The upper nucleoside is denoted as **a** and the lower as **b**.

Table 3. Summary of the backbone torsional angles δ,ε and ζ derived from the canonical A- and B-DNA duplex structures and of *xylo*-ε,ζ-D-CNA dimeric units **8** and **9**[a]

Structure	δ	ε	ζ	ε−ζ
A-type	g^+/a^+	a^-/t	g^-	<0°
BI-type	a^+	t	g^-/a^-	<0°
BII-type	a^+/t	g^-/a^-	t	>0°
($S_{C3'}$,R_P) **8**	c	t	g^+	>0°
($S_{C3'}$,S_P) **9**	c	t	g^-	<0°

[a] The following six-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.

4. Experimental section

4.1. General

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

4.1.1. 2′,5′-Di-*O*-*tert*-butyldimethylsilyl-3′-*C*-(methoxycarbonylmethyl)xylouridine 3. To a solution of 3′-ketoneuridine **2** (1.0 g, 2.12 mmol) in anhydrous dichloromethane (10 mL) under an inert atmosphere, were added, at −78 °C, $BF_3 \cdot Et_2O$ (1.35 mL, 10.6 mmol, 5 equiv) and a solution of *tert*-butyldimethylsilyl-methyl-ketene acetal (2.40 mL, 12.75 mmol, 6 equiv) in anhydrous dichloromethane (2 mL). After 2 h of stirring, the reaction was stopped by addition of a saturated aqueous solution of NH_4Cl (20 mL) and extracted with ethyl acetate. The organic layer was washed with water and brine and dried over $MgSO_4$. After removal of the solvent under reduced pressure, the crude material was chromatographed on silica gel with ethyl acetate/petroleum ether (7:3) as eluent. After evaporation of the solvent, compound **3** was recovered as white foam (m=0.72 g, yield: 57%). TLC: R_f [petroleum ether/(CH_2Cl_2/AcOEt 4:1) 1:4]=0.37. ^{1}H NMR (500 MHz, $CDCl_3$): δ_{ppm}=7.89 (d, J=8.0 Hz, 1H, 6-H), 5.74 (s, 1H, 1′-H), 5.67 (dd, J=8.0 and 2.0 Hz, 1H, 5-H), 4.30 (s, 1H, 2′-H), 4.17 and 4.05 (AB part of an ABX syst., J=5.0, 3.7 and 11.1 Hz, 2H, 5′-H), 3.99 (dd, J=3.8 and 4 Hz, 1H, 4′-H), 3.73 (s, 3H, MeO), 2.85 and 2.74 (AB syst., J=17.0 Hz, 1H, 6′-H), 0.92 and 0.90 (s, 18H, *t*-Bu), 0.24, 0.19, 0.16 and 0.11 (s, 12H, Me). ^{13}C NMR (125 MHz, $CDCl_3$): δ_{ppm}=172.3, 163.5, 150.3, 141.2, 100.9, 91.4, 83.8, 82.3, 79.5, 61.0, 51.9, 36.0, 25.8, 25.7, 18.2, 17.9, −4.3, −5.5, −5.6, −5.8. MS (DCI): 545.6 $(M+H)^+$, 562.6 $(M+NH_4)^+$. $C_{24}H_{44}N_2O_8Si_2$ (544.79): calcd C 52.91, H 8.14, N 5.14; found C 53.12, H 8.01, N 5.38.

4.1.2. 2′,5′-Di-*O*-*tert*-butyldimethylsilyl-3′-C-(2-hydroxyethyl)xylouridine 4. To a solution of 3′-methylethanoateuridine **3** (0.52 g, 0.96 mmol) in anhydrous dichloromethane (12 mL) under an inert atmosphere, was added, at 0 °C, a solution of diisobutyl aluminum hydride (DIBAH) in hexane (4.8 mL, sol 1 M, 4.8 mmol, 5 equiv). After 2 h of stirring at 0 °C and 12 h at room temperature the reaction was stopped by addition of methanol and water and extracted with ethyl acetate. The organic layer was washed with HCl (0.5 N), water and brine and dried over $MgSO_4$. Compound **4** (270 mg, 46% yield) was isolated as a white foam after chromatography on silica gel eluted with ethyl acetate/dichloromethane 2:3. TLC: R_f (CH_2Cl_2/AcOEt 3:2)=0.35. ^{1}H NMR (250 MHz, CD_3OD): δ_{ppm}=7.93 (d, J=8.0 Hz, 1H, 6-H), 5.63 (s, 1H, 1′-H), 5.60 (d, J=8.0 Hz, 1H, 5-H), 4.17–3.98 (m, 3H), 3.85–3.68 (m, 1H), 3.30 (br s, 1H, 4′-H), 2.06–1.81 (m, 2H, 6′-H), 0.87 and 0.86 (s, 18H, *t*-Bu), 0.10, 0.07 and −0.09 (s, 12H, Me). ^{13}C NMR (63 MHz, CD_3OD): δ_{ppm}=163.6, 149.2, 140.4, 97.9, 89.9, 84.8, 80.5, 78.7, 60.1, 56.3, 31.9, 23.5, 16.3, 16.0, −6.7, −8.2. MS (DCI): 517.0 $(M+H)^+$. $C_{23}H_{44}N_2O_7Si_2$ (516.78): calcd C 53.46, H 8.58, N 5.42; found C 52.98, H 8.13, N 5.66.

4.1.3. 2′,5′-Di-*O*-*tert*-butyldimethylsilyl-3′-C-(2-tosyloxyethyl)xylouridine 5. To compound **4** (0.3 g, 0.58 mmol)

dissolved in anhydrous chloroform were added at 0 °C pyridine (450 μL) and tosyl chloride (166 mg, 0.87 mmol, 1.5 equiv). 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, brine and dried over $MgSO_4$. After removal of the solvent under reduced pressure the crude product was deposited on a silica gel column and eluted first with ethyl acetate/dichloromethane 1:9 and then with 1:4. Compound **5** was recovered as white foam (390 mg, yield: 64%). TLC: R_f (CH_2Cl_2/AcOEt 2:3)=0.66. ^{1}H NMR (250 MHz, $CDCl_3$): δ_{ppm}=7.91 (d, J=8.0 Hz, 1H, 6-H), 7.77 (A part of an AB syst., J=8.1 Hz, 2H, ph), 7.33 (B part of an AB syst., J=8.1 Hz, 2H, ph), 5.59 (s, 1H, 1′-H), 5.58 (dd, J=8.0 and 2.0 Hz, 1H, 5-H), 4.35–3.89 (m, 4H, 5′-H and 7′-H), 3.39 (m, 2H, 2′-H and 4′-H), 2.44 (s, 3H, Me), 2.21–1.82 (m, 2H, 6′-H), 0.91 and 0.83 (s, 18H, *t*-Bu), 0.19, 0.16, 0.15 and 0.00 (s, 12H, MeSi). ^{13}C NMR (63 MHz, $CDCl_3$): δ_{ppm}=164.1, 150.7, 145.1, 132.8, 130.1, 128.3, 100.9, 91.6, 82.3, 81.5, 81.5, 80.9, 66.7, 62.3, 31.4, 25.9, 25.8, 21.8, 18.2, 18.1, −3.8, −5.5, −5.6, −5.7. MS (DCI): 671.0 $(M+H)^+$, 688 $(M+NH_4)^+$. $C_{30}H_{50}N_2O_9SSi_2$ (670.96): calcdC 53.70, H 7.51, N 4.18; found C 53.23, H 7.11, N 4.31.

4.1.4. (3′-*O*-*tert*-Butyldiphenylsilyl-5′-thymidinyl)-(2-cyanoethyl)-2′,5′-di-*O*-*tert*-butyldimethylsilyl-3′-*C*-(2-tosyloxyethyl)xylouridine-3′-phosphate (two diastereoisomers) 6. Compound **5** (110 mg, 0.16 mmol), 3′-*O*-*tert*-butyldiphenylsilylthymidine-5′-*O*-phosphoramidite (447 mg, 0.66 mmol) and freshly sublimed tetrazole (78 mg, 1.12 mmol) were diluted into anhydrous acetonitrile (1.5 mL) and stirred for 3 h at room temperature. After addition of collidine (130 μL), the phosphite was oxidized with a solution of iodine [0.1 M in THF(2)/H_2O(1)] until the dark brown colour persist. 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 **6** (130 mg, 64% yield) was isolated (two 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)=0.57 and 0.28. Data for the fast eluted diastereoisomer: ^{1}H NMR (250 MHz, $CDCl_3$): δ_{ppm}=7.78 (A part of an AB syst., J=8.2 Hz, 2H, ph), 7.66–7.64 (m, 4H, ph), 7.50–7.42 (m, 8H, ph), 7.33 (B part of an AB syst., J=8.2 Hz, 2H, ph), 6.38 (dd, J=5.7 and 8.2 Hz, 1H, 1′b-H), 5.51 (s, 1H, 1′a-H), 5.44 (d, J=8.2 Hz, 1H, 5a-H), 4.90 (m, 1H, 4′a-H), 4.41–3.62 (m, 12H), 2.63 (m, 2H), 2.47–2.27 (m, 3H), 2.44 (s, 3H, Me), 1.88 (s, 3H, Me), 1.09, 0.90 and 0.83 (3s, 21H, *t*-Bu), 0.09 (br s, 12H, $SiMe_2$). ^{13}C NMR (63 MHz, $CDCl_3$): δ_{ppm}=164.4, 164.3, 150.3, 150.2, 145.1, 139.7, 136.2, 135.7, 133.0, 132.8, 132.6, 130.2, 130.0, 128.1, 127.9, 116.4, 111.5, 101.1, 91.3, 90.6, 90.5, 86.6, 86.5, 85.8, 85.1, 85.0, 80.7, 73.0, 68.7, 65.3, 62.6, 62.5, 60.7, 39.6, 31.7, 26.9, 21.6, 19.4, 19.3, 19.0, 18.4, 17.9, 12.0, −4.3, −5.4. ^{31}P NMR (81 MHz, $CDCl_3$): δ_{ppm}= −4.9. MS (DCI): 1266.7 $(M+H)^+$. Data for the slow eluted diastereoisomer: ^{1}H NMR (250 MHz, $CDCl_3$): δ_{ppm}=7.75 (A part of an AB syst., J=8.2 Hz, 2H, ph), 7.65–7.62 (m, 4H, ph), 7.56 (d, J=8.0 Hz, 1H, 6a-H), 7.46–7.44 (m, 7H, ph), 7.33 (B part of an AB syst., J=8.2 Hz, 2H, ph), 7.13 (s, 1H, 6-H), 6.27 (t, J=6.7 Hz, 1H, 1′-H), 5.64 (d, J=8.2 Hz, 1H, 5a-H), 5.52 (d, J=2.1 Hz, 1H, 1′a-H), 4.72 (ls, 1H, 4′a-H), 4.28–3.85 (m, 12H), 2.60 (m, 3H), 2.44 (s, 3H, Me), 2.38–2.28 (m, 2H), 1.85 (s, 3H, Me), 1.08, 0.89 and 0.81 (3s, 21H, *t*-Bu), 0.08 (br s, 12H, $SiMe_2$). ^{13}C NMR (63 MHz, $CDCl_3$): δ_{ppm}=163.8, 163.7, 150.4, 150.2, 145.1, 140.1, 135.9, 135.7, 132.7, 132.6, 130.3, 130.0, 128.1, 127.9, 116.2, 111.3, 101.6, 91.1, 90.9, 90.8, 86.0, 84.8, 84.7, 81.2, 72.7, 68.1, 68.0, 65.3, 62.5, 62.4, 61.0, 60.4, 39.9, 31.5, 26.8, 25.9, 25.7, 25.6, 21.6, 19.4, 19.3, 19.0, 18.4, 17.9, 14.2, 12.3, −4.5, −5.2, −5.3, −5.4. ^{31}P NMR (81 MHz, $CDCl_3$): δ_{ppm}= −5.5. MS (DCI): 1266.7 $(M+H)^+$.

4.1.5. (2′,5′-Di-*O*-*tert*-butyldimethylsilyl)-(3″-O-*tert*-butyldiphenylsilyl)-*xylo*-ε,ζ-D-CNA (mixture of diastereoisomers) 7. To dinucleotide **6** (120 mg, 0.095 mmol) in anhydrous DMF (2.0 mL), was added potassium carbonate (0.21 g, 1.51 mmol). After 4 h of stirring at 25 °C, the excess of base was filtered off and the reaction mixture was diluted with ethyl acetate (50 mL), washed three times with water (3×20 mL) and once with brine. The organic layer was dried over $MgSO_4$, and the solvent removed in vacuo. The *xylo*-ε,ζ-D-CNA UT **7** (mixture of two isomers in a 1:1 ratio) was purified by silica gel chromatography with ethyl acetate as eluent and recovered as a white foam (80 mg, 81% yield). TLC: R_f (AcOEt)=0.40. ^{1}H NMR (300 MHz, $CDCl_3$): δ_{ppm}=7.65–7.59 (m, 8H, ph), 7.52–7.39 (m, 14H, ph), 7.32 (d, J=1.0 Hz, 1H, 6b-H), 7.10 (d, J=1.0 Hz, 1H, 6b-H), 6.44 (dd, J=5.7 and 8.7 Hz, 1H, 1′b-H), 6.25 (t, J=6.3 Hz, 1H, 1′b-H), 5.70 (d, J=1.5 Hz, 1H, 1′a-H), 5.61–5.53 (m, 3H, 5-H and 1′-H), 4.93 (d, J=1.5 Hz, 1H, 2′a-H), 4.75 (s, 1H, 2′a-H), 4.39–3.00 (m, 12H), 3.92 (m, 2H), 3.77–3.72 (m, 2H), 3.65–3.56 (m, 1H), 2.36–2.18 (m, 6H), 1.85–1.78 (m, 8H), 1.08 and 1.07 (2s, 18H, *t*-Bu), 0.92, 0.91, 0.86 and 0.85 (4s, 36H, *t*-Bu), 0.25, 0.23, 0.18, 0.12, 0.11, 0.10 and 0.09 (7s, 24H, $SiMe_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ_{ppm}=163.9, 163.7, 163.6, 150.4, 150.3, 150.2, 139.8–128.0, 111.3, 111.2, 101.3, 101.1, 91.3, 91.2, 91.0, 90.6, 90.3, 90.2, 85.8, 85.3, 85.2, 85.1, 84.9, 84.2, 84.1, 79.0, 78.3, 73.0, 72.7, 68.1, 67.5, 65.1, 60.2, 60.0, 40.2, 40.1, 29.7, 26.8, 25.9, 25.8, 25.7, 19.0, 18.3, 18.0, 17.9, 12.4, 12.2, −4.1, −4.2, −5.3, −5.4, −5.5, −5.7. ^{31}P NMR (81 MHz, $CDCl_3$): δ_{ppm}= −6.4 and −8.9. MS (APCI/MeOH): 1041.7 $(M+H)^+$.

4.1.6. ($S_{C3'}$,R_P)-*xylo*-ε,ζ-D-CNA (8) and ($S_{C3'}$,S_P)-*xylo*-ε,ζ-D-CNA 9. Tetrabutylammonium fluoride (240 μL, 0.24 mmol, 1 M solution in THF) was added under inert atmosphere of argon at room temperature to the dinucleotide **7** (75 mg, 0.072 mmol) in anhydrous THF (1 mL). Stirring was maintained for 0.5 h. After removal of the solvent under reduced pressure the crude product was purified on a reverse phase column (Kromasil C18, 250×20 mm) with a water/acetonitrile gradient from 9:1 to 8:2 in 40 min as eluent. Compound **8** (15 mg) and **9** (16 mg) were recovered as a white solid after removal of the solvent (74% yield). HPLC (Kromasil C18, 7 μm, 250×4.6 mm; water/acetonitrile gradient from 9:1 to 8:2 in 40 min), t_R=5.70 min for **8** and 7.45 min for **9**. Data for **8**: ^{1}H NMR (500 MHz, D_2O): δ_{ppm}=7.74 (d, J=8.1 Hz, 1H, 6a-H), 7.30 (s, 1H, 6b-H), 6.09 (t, J=6.9 Hz, 1H, 1′b-H), 5.66 (s, 1H, 1′a-H), 5.58 (d, J=8.1 Hz, 1H, 5a-H), 4.83 (s, 1H, 2′a-H), 4.51–4.42 (m, 2H, 7′a-H), 4.33 (ddd, J=7.1 and 5.6 Hz, $J_{H/P}$=4.2 Hz,

1H, 4′a-H), 4.24 (m, J=6.3, 3.1 and 2.9 Hz 1H, 3′b-H), 4.21 (A part of an ABX(Y) syst., J=11.1 and 2.6 Hz, $J_{H/P}$=4.0 Hz, 1H, 5′b-H), 4.06 (td, J=8.7, 2.9 and 2.9 Hz 1H, 4′b-H), 3.98 (A part of an ABX syst., J=12.5 and 4.2 Hz, 1H, 5′a-H), 3.95 (B part of an ABX syst., J=12.5 and 7.2 Hz, 1H, 5′a-H), 3.94 (B part of an ABX(Y) syst., J=11.1 and 8.7 Hz, $J_{H/P}$=6.5 Hz, 1H, 5′b-H), 2.34 (A part of an ABX(Y) syst., J=14.0, 6.9 and 3.2 Hz, 1H, 2′b-H), 2.33–2.25 (m, J=15.6 Hz, $J_{H/P}$= 2.2 Hz, 2H, 6′a-H), 2.10 (B part of an ABX(Y) syst., J=14.0, 7.2 and 6.8 Hz, 1H, 2′b-H), 1.79 (s, 3H, Me). ^{13}C NMR (125 MHz, D_2O): δ_{ppm}=166.5, 166.1, 151.4, 140.8, 136.9, 111.1, 100.6, 92.8, 92.4, 86.4, 85.5, 84.3, 76.4, 70.4, 67.6, 66.8, 58.8, 38.9, 24.2, 11.5. ^{31}P NMR (81 MHz, D_2O): δ_{ppm}= −7.1. MS (ESI, MeOH): 597.3 $(M+Na)^+$, 619.3 $(M+K)^+$. $C_{21}H_{27}N_4O_{13}P$ (574.43): calcd C 43.91, H 4.74, N 9.75; found C 43.82, H 4.89, N 9.67. Data for **9**: ^{1}H NMR (500 MHz, D_2O): δ_{ppm}=7.75 (d, J=8.1 Hz, 1H, 6a-H), 7.39 (s, 1H, 6b-H), 6.21 (t, J=6.6 Hz, 1H, 1′b-H), 5.73 (d, J=8.1 Hz, 1H, 5a-H), 5.71 (s, 1H, 1′a-H), 4.75 (s, 1H, 2′a-H), 4.53–4.46 (m, J=11.7, 11.6, 4.8, 3.4 and 2.7 Hz, $J_{H/P}$=10.5 and 4.5 Hz, 2H, 7′a-H), 4.41 (m, J=6.4, 4.7 and 4.1 Hz 1H, 3′b-H), 4.32 (ddd, J=6.8 and 4.6 Hz, $J_{H/P}$=5.4 Hz, 1H, 4′a-H), 4.21 and 4.18 (AB part of an ABX(Y) syst., J=11.5, 3.8 and 2.5 Hz, $J_{H/P}$=6.7 and 5.9 Hz, 2H, 5′b-H), 4.06 (m, J=4.1, 3.7 and 2.4 Hz, $J_{H/P}$=3.0 Hz, 1H, 4′b-H), 3.95 and 3.92 (A part of an ABX syst., J=12.1, 6.8 and 4.6 Hz, 2H, 5′a-H), 2.37–2.24 (m, J=14.0, 6.6 and 4.7 Hz, 2H, 2′b-H), 2.36–2.27 (m, 2H, 6′a-H), 1.82 (s, 3H, Me). ^{13}C NMR (125 MHz, D_2O): δ_{ppm}=166.4, 151.6, 151.3, 141.3, 136.9, 111.5, 100.4, 92.1, 91.3, 85.5, 85.2, 83.9, 77.3, 69.9, 68.0, 67.9, 66.8, 66.5, 58.8, 38.4, 30.2, 24.3, 11.5. ^{31}P NMR (81 MHz, D_2O): δ_{ppm}= −7.7. MS (ESI, MeOH): 597.3 $(M+Na)^+$, 619.3 $(M+K)^+$. $C_{21}H_{27}N_4O_{13}P$ (574.43): calcd C 43.91, H 4.74, N 9.75; found C 44.02, H 4.84, N 9.66.

4.2. 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 range of 0.1 mM in a 10 mM Na_2HPO_4, 100 mM NaCl, 0.1 mM Na_2EDTA, buffer, pH 7.00±0.02. Molar extinction coefficients were calculated from those of dinucleotides using the nearest-neighbour approximation method assuming that *xylo*-ε,ζ-D-CNA UT has the same molar extinction coefficient as U_pT. Dinucleotide concentration was determined from UV absorbance at high temperature (90 °C). All CD spectra were recorded after stabilization of the temperature for 10 min and were normalized by substraction of the background scan with buffer. Taking the known dinucleotide concentration into account, the normalized spectra were converted to variation of molar extinction coefficient ($\Delta\varepsilon$).

References and notes

1. Leumann, C. J. *Bioorg. Med. Chem.* **2002**, *10*, 841.
2. Wengel, J. *Acc. Chem. Res.* **1999**, *32*, 301.
3. Herdewijn, P. *Biochim. Biophys. Acta* **1999**, *1489*, 167.
4. (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; (f) Sharma, P. K.; Mikkelsen, B. H.; Christensen, M. S.; Nielsen, K. E.; Kirchhoff, C.; Pedersen, S. L.; Sørensen, A. M.; Østergaard, K.; Petersen, M.; Nielsen, P. *Org. Biomol. Chem.* **2006**, *4*, 2433.
5. Belmont, P.; Constant, J.-F.; Demeunynck, M. *Chem. Soc. Rev.* **2001**, *30*, 70.
6. Antson, A. A. *Curr. Opin. Struct. Biol.* **2000**, *10*, 87.
7. Várnai, P.; Djuranovic, D.; Lavery, R.; Hartmann, B. *Nucleic Acids Res.* **2002**, *30*, 5398.
8. Djuranovic, D.; Hartmann, B. *Biopolymers* **2004**, *73*, 356.
9. Heddi, B.; Foloppe, N.; Bouchemal, N.; Hantz, E.; Hartmann, B. *J. Am. Chem. Soc.* **2006**, *128*, 9170.
10. Duarte, C. M.; Pyle, A. M. *J. Mol. Biol.* **1998**, *284*, 1465.
11. Kool, E. T. *Chem. Rev.* **1997**, *97*, 1473.
12. Le Clezio, I.; Escudier, J.-M.; Vigroux, A. *Org. Lett.* **2003**, *5*, 161.
13. Dupouy, C.; Iché-Tarrat, N.; Durrieu, M.-P.; Rodriguez, F.; Escudier, J.-M.; Vigroux, A. *Angew. Chem.* **2006**, *118*, 3705; Dupouy, C.; Iché-Tarrat, N.; Durrieu, M.-P.; Rodriguez, F.; Escudier, J.-M.; Vigroux, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 3623.
14. Le Clezio, I.; Dupouy, C.; Lavedan, P.; Escudier, J.-M. *Eur. J. Org. Chem.* **2007**, 3894.
15. Dupouy, C.; Le Clezio, I.; Lavedan, P.; Gornitzka, H.; Escudier, J.-M.; Vigroux, A. *Eur. J. Org. Chem.* **2006**, 5515.
16. Le Clezio, I.; Vigroux, A.; Escudier, J.-M. *Eur. J. Org. Chem.* **2007**, 1935.
17. Dupouy, C.; Lavedan, P.; Escudier, J.-M. *Eur. J. Org. Chem.* **2007**. doi:10.1002/ejoc.200700535
18. Le Clezio, I.; Gornitzka, H.; Escudier, J.-M.; Vigroux, A. *J. Org. Chem.* **2005**, *70*, 1620.
19. Robins, M. J.; Sarker, S.; Samano, V.; Wnuk, S. F. *Tetrahedron* **1997**, *53*, 447.
20. Hansske, F.; Robins, M. J. *Tetrahedron Lett.* **1983**, *24*, 1589.
21. Huss, S.; De Las Heras, F. G.; Camarasa, M. J. *Tetrahedron* **1991**, *47*, 1727.
22. Nielsen, P.; Pfundheller, H. M.; Wengel, J. *Chem. Commun.* **1997**, 825.
23. Nielsen, P.; Pfundheller, H. M.; Olsen, C. E.; Wengel, J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3423.
24. Nielsen, P.; Petersen, M.; Jacobsen, J. P. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3706.
25. Kabalka, G. W.; Varma, M.; Varma, R. S.; Srivastava, P. C.; Knapp, F. F. *J. Org. Chem.* **1986**, *51*, 2386.
26. Mukaïyama, T. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 817.
27. (a) Banuls, V.; Escudier, J.-M. *Tetrahedron* **1999**, *55*, 5831; (b) Escudier, J.-M.; Tworkowski, I.; Bouziani, I.; Gorrichon, L. *Tetrahedron Lett.* **1996**, *50*, 4689.
28. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.
29. Hakimelahi, G. H.; Proba, Z. A.; Ogilvie, K. K. *Tetrahedron Lett.* **1981**, *22*, 5243.
30. Samano, V.; Robins, M. J. *J. Org. Chem.* **1990**, *55*, 5186.
31. Caruthers, M. H. *Acc. Chem. Res.* **1991**, *24*, 278.

32. Torres, R. A.; Bruice, T. C. *J. Am. Chem. Soc.* **2000**, *122*, 781.
33. Johnson, W. C. *Circular Dichroism, Principles and Applications*; Berova, N., Nakanishi, K., Woody, R. W., Eds.; John Wiley & Sons: New York, NY, 2000; pp 703–718.
34. Wood, D. J.; Hruska, F. E.; Ogilvie, K. K. *Can. J. Chem.* **1974**, *52*, 3353.
35. Wood, D. J.; Ogilvie, K. K.; Hruska, F. E. *Can. J. Chem.* **1975**, *53*, 2781.
36. Gorenstein, D. G.; Rowell, R.; Findlay, J. *J. Am. Chem. Soc.* **1980**, *102*, 5077.
37. WIN-DAISY 4.0©. Brüker-Franzen Analytik GmbH, 1997.
38. Schneider, B.; Neidle, S.; Berman, H. M. *Biopolymers* **1997**, *42*, 113.