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Thio- and Seleno-Dioxaphosphorinane-Constrained Dinucleotides (D-CNA), Synthesis and Conformational Study

Béatrice Gerland,*[a] Claudia Addamiano,[a] Brice-Loïc Renard,[a] Corinne Payrastre,[a] Deshmukh Gopaul[b] and Jean-Marc Escudier*[a]

Abstract: Thio- and seleno-α,β- and α,β,γ-CNA (Constrained Nucleic Acid) dinucleotides in which two or three torsional angles of the sugar/phosphate backbone are controlled within a dioxaphosphorinane structure have been prepared. Their structural determination have been carried out by means of NMR to show only slight variation on the torsional angle control in comparison with their oxo analogues. Unexpected selenium migration has been pointed out from seleno-phosphotriester moiety to phosphoramidite group, but this drawback can be overcome using H-phosphonate chemistry.

Introduction

Conformationnaly restricted nucleotides have been investigated for therapeutic purposes to enhance duplex formation and stability with the target strand once incorporated into synthetic oligonucleotides.[1] The main success in this approach relies on bicyclic analogues with furanose ring conformational equilibrium locked in the C3'-*endo* form (North).[2] This conformation can mainly be reached by introduction of a 2'OH-C4' methylene bridge within the ribose for LNA (Locked Nucleic Acid), by constrain applied to the C4'-C5' exocyclic bond or by combining both.[3] Tricylic analogues (tc-DNA) modified with a C3'-C5' ethylene bridge further rigidified by an additional fused cyclopropane unit have also been designed.[4] Another approach by-passes the need of a bicyclic scaffold to lock the sugar conformation. 2'-Fluorination of the furanose ring induces a North pucker preference due to strong stereoelectronic effects,[5] and the introduction of a second fluorine atom definitely rigidifies the 2',4'-difluoro nucleotides in a pure North conformation.[6]

We recently showed that restricting the sugar phosphate backbone rotational freedom by means of dioxaphosphorinane rings can be favorable not only to stabilize duplex[7] but also to preorganize loops and bulges when those structural elements feature unusual torsion angles values sets and, as a consequence, become able to stabilize secondary structures (Figure 1).[8]

Interestingly, oligodeoxynucleotides (ONs) modified with dioxaphosphorinane constrained nucleic acid dinucleotides (D-CNA) displaying either the canonical *gauche*(-) or the non-canonical *gauche*(+) value of α (Figure 1), exhibited biological properties such as supporting RNase H cleavage[9] or acting as chain terminators of DNA polymerases.[10] However during the automated chemical synthesis of the highly modified ONs involved in these experiments, it appeared that the dioxaphosphorinane ring could be partially cleaved by the final and harsh ammonia treatment. Therefore, in order to increase the stability of the ring structure towards hydrolysis while maintaining the geometry constrain on the sugar/phosphate backbone, we have undertaken the synthesis of thio- and seleno-analogues of D-CNA by replacing the exo and non-bridging oxygen of the dioxaphosphorinane ring by a sulfur or selenium atom (Figure 1). Our previous attempts towards thio-α,β-D-CNA analogues, based on a stereoselective thio-phosphate ring closure suffered from a competition between O- and S-cyclisation and a low yield of the target compound with the sulfur at the desired exocyclic location.[11]

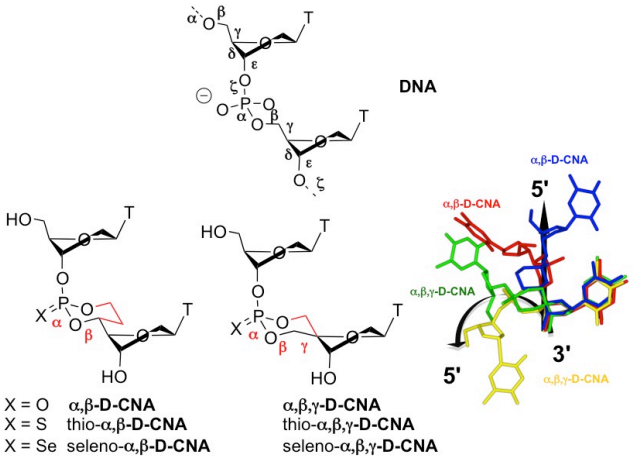

Figure 1. Top: the DNA backbone torsion angles (labelled α to ζ). Down left and middle: Dioxaphosphorinane-Constrained Nucleic Acid dinucleotides in which either α and β or α, β and γ are stereocontrolled within the ring structure. Right: structure superimposition of α,β-D-CNA {blue: alpha = *gauche*(-), beta = *trans*, red: alpha = *gauche*(+), beta = *trans*} and α,β,γ-D-CNA {green: alpha = *gauche*(-), beta = *gauche*(-), gamma = *gauche*(-), yellow: alpha = *gauche*(+), beta = *cis*/*gauche*(+), gamma = *gauche*(-)/*anti*(-)} showing the change in directionality from 3'-end to 5'-end.

Herein, we describe the efficient synthesis of all the possible isomers of two classes of CNA namely thio- and seleno-α,β-D-CNA and thio- and seleno-α,β,γ-D-CNA. The structural impact of replacement of the exocyclic oxygen of the dioxaphosphorinane ring by a sulfur or selenium atom is evaluated by means of NMR spectroscopy. These rigid dinucleotides analogues are expected to be able to describe a large conformational space (Figure 1) and to act within artificial oligonucleotides as mimics of distorted transient DNA structures.

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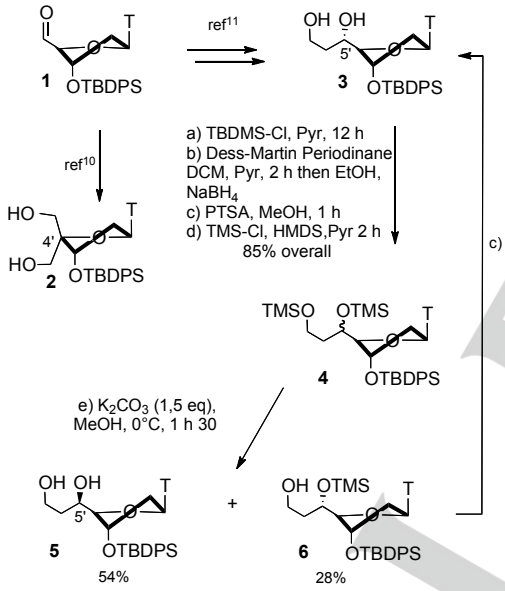
Results and Discussion

Our seminal approach of D-CNA synthesis was based on the steric and electronic control of an intramolecular cyclisation by attack of a phosphate thio or oxyanion of an activated 4' or 5'-carbon to displace a tosylate group.[12] In order to install a sulfur or selenium atom on a common phosphite intermediate for each D-CNA, we chose to condense a thymidine phosphorodiamidite on 4'-*C*-hydroxymethylthymidine **2** (for α,β,γ-D-CNA) and on 5'-*C*-hydroxyethylthymidine **3** and **5** (for α,β-D-CNA) (Scheme 1). Therefore oxidation of the resulting phosphite either by S_8 or KSeCN would provide the target compounds with the hetero element as a non-bridging atom (Scheme 2 and 3).

Synthesis of thymidine diol precursors

4'-*C*-Hydroxymethylthymidine **2** can be obtained in a moderate yield (45%) from thymine aldehyde **1** and formaldehyde in basic medium following a previously described method.[13] 5'-*C*(*S*)-Hydroxyethylthymidine **3** was easily accessible in pure form from thymidine aldehyde **1** in a two steps procedure in good yield (75%) and high quantity.[14] On the other hand, synthesis of the 5'-*C*(*R*) isomer **5** was previously achieved through a fastidious multi-steps procedure in a modest yield.[15] Therefore, we engaged a methodology of racemization/separation from **3** to gain a better access to **5** (Scheme 1).

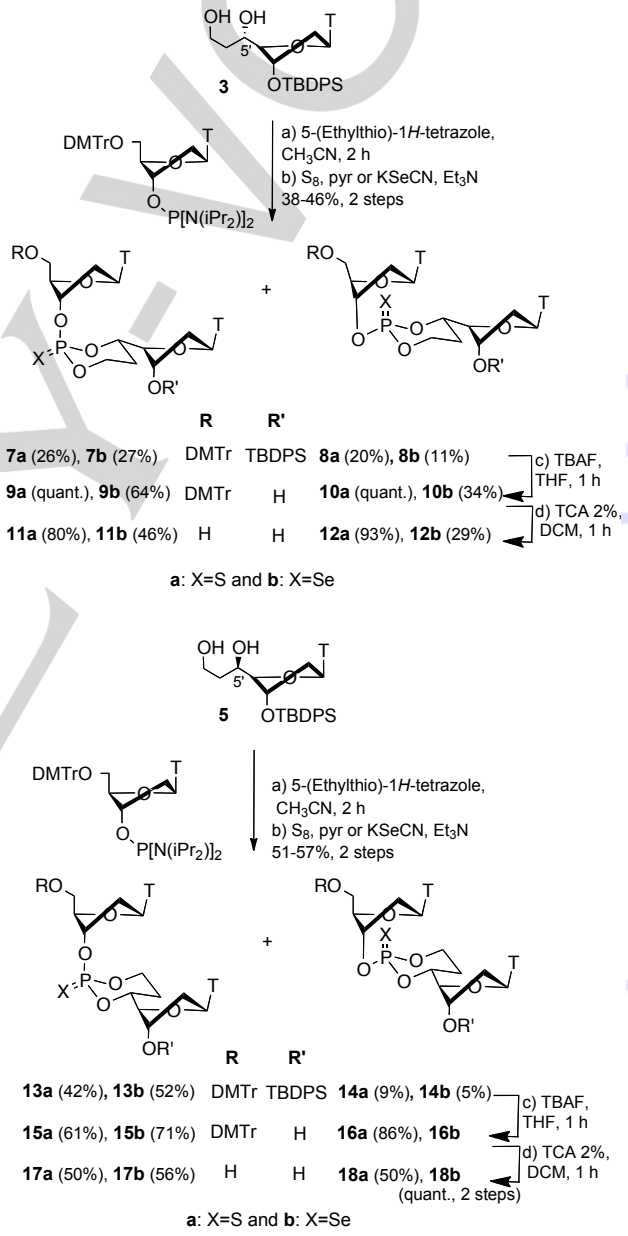


Scheme 1. Synthesis of diastereoisomeric 4'-*C*-hydroxymethylthymidine **2** and 5'-*C*-hydroxylethylthymidine **3** and **5** precursors.

The primary hydroxyl group of **3** was selectively protected by a *tert*-butyldimethylsilyl ether in anhydrous pyridine in quantitative yield. Oxidation of the remaining secondary hydroxyl group was performed with Dess-Martin periodinane[16] and the resulting ketone was reduced *in situ* with sodium borohydride. Acidic removal of the *tert*-butyldimethylsilyl ether followed by *per*-silylation with trimethylsilyl chloride afforded **4** as a 2/1 inseparable diastereoisomeric mixture in favor of the 5'-*C*(*R*) isomer as depicted by ^{1}H NMR. At this stage, a kinetic removal of the silyl ether on the secondary hydroxyl group of the 5'-*C*-(*R*) isomer of **4** can be achieved with potassium carbonate in methanol at low temperature with a special care in the reaction time, while the silyl ether on the primary hydroxyl group of epimeric **4** was removed. Then, 5'-*C*-(*R*)-hydroxyethylthymidine **5** was easily separated from the remaining 5'-*C*-(*S*)-hydroxyethyl thymidine **6** still bearing the silyl ether on the 5'-hydroxyl group. Eventually, **6** can be converted back to **3** in acidic medium quantitatively.

Synthesis of thio- and seleno-α,β-D-CNA

The two 5'-*C* isomers hydroxyethylthymidine **3** and **5** were independently engaged in a coupling with the freshly prepared *bis*(di*iso*propylamino)phosphodiamidite of thymidine and activated with 5-(ethylthio)-1*H*-tetrazole (Scheme 2).[17]



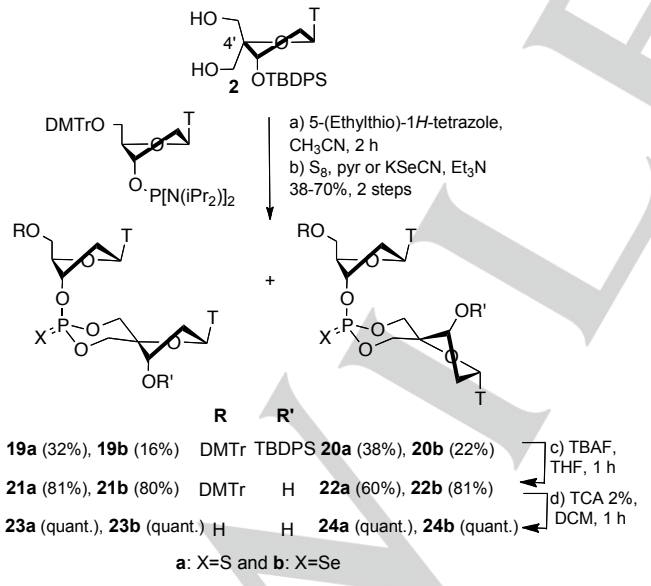
Scheme 2. Synthesis of thio- and seleno-α,β-CNA dinucleotides.

The intermediate phosphite can be oxidized either by S_8 to provide **7a**/**8a** or **13a**/**14a** or by KSeCN to install the selenium atom to obtain **7b**/**8b** or **13b**/**14b** respectively from **3** and **5** in moderate to good yield. Interestingly, the ratio of the diastereoisomeric couple **7**/**8** or **13**/**14** was 1.6/1 as depicted by ^{31}P NMR indicative of an unexpected diastereoselection when compare with the 1/1 ratio during formation of larger cycle (13-membered) following the same methodology with a starting nucleoside bearing two primary hydroxyl groups.[17a] In the present case, it could be explained, first by the difference in the nucleophile involved that exhibits a primary and a secondary hydroxyl group and secondly by the more favorable formation of the 6-membered ring phosphite intermediate with all properly positioned substituents with the upper nucleoside in the apical position and with the lower nucleoside and the sulfur or selenium atom in the equatorial position.

A relative instability appeared for the minor isomers **8b** and **14b** (P=Se) that were slowly converted into their phosphoryl counterparts with formation of elemental selenium upon standing.[18] Eventually, removal of the *tert*-butyldiphenylsilyl protective group by treatment with fluoride ion provided **9a**,**b**/**10a**,**b** and **15a**,**b**/**16a**,**b** in moderate to good yield. It is noteworthy that due to its poor stability, **16b** was not isolated at this stage and was directly involved in the next deprotection step. Acidic conditions allowed dimethoxytrityl group removal in good yield, giving access to four diastereoisomers of thio-α,β-D-CNA, **11a**, **12a**, **17a**, **18a** and four diastereoisomers of seleno-α,β-D-CNA, **11b**, **12b**, **17b**, **18b**.

Synthesis of thio- and seleno-α,β,γ-D-CNA

The synthetic pathway towards thio- and seleno-α,β,γ-D-CNA followed the same steps applied for the thio- and seleno-α,β-D-CNA approach, and started with the activation by 5-(ethylthio)-1*H*-tetrazole of the *bis*(di*iso*propylamino)phosphoramidite of thymidine with 4'-C-hydroxymethyl thymidine **2**, in 38-70% yield (Scheme 3).



Scheme 3. Synthesis of thio- and seleno-α,β,γ-CNA dinucleotides.

Here, because of a precursor with two primary hydroxyl groups, the ratio of the formed dioxaphosphorinane isomers *cis* (3'-O of the upper nucleoside and 4'-O of the lower nucleoside on the same side of the average plan of the dioxaphosphorinanne ring) and *trans* (3'-O of the upper nucleoside and 4'-O of the lower nucleoside on the opposite sides of the average plan of the dioxaphosphorinanne ring) was 1/1 for both **19a**/**20a** (P=S) and **19b**/**20b** (P=Se). However, *cis* and *trans* isomers **19a**/**20a** and **19b**/**20b** were easily separable by means of silica gel chromatography whereas theirs D-CNA oxo-analogues required a tedious reverse phase HPLC separation. Successive removals of the *tert*-butyldiphenylsilyl and the dimethoxytrityl protective groups were accomplished by means of fluoride ion and trichloroacetic acid respectively in good yields leading to fully deprotected thio-α,β,γ-D-CNA **23a**/**24a** and seleno-α,β,γ-D-CNA **23b**/**24b** for structural study purpose.

NMR structural assignment of CNA

Sugar puckering

The overall conformation analysis of the newly synthesized thio- and seleno-α,β- or α,β,γ-CNA was carried out by means of ^{1}H, ^{13}C, ^{31}P and 2D NMR study.

The sugar puckering of the upper and lower deoxyribose moieties within each dinucleotide (**11a**,**b**, **12a**,**b**, **17a**,**b**, **18a**,**b**, **23a**,**b** and **24a**,**b**) was estimated by the approximation of Altona and Sundaralingam: % South = $[J_{H1',H2'}/(J_{H1',H2'} + J_{H3',H4'})]$ x 100 (Table 1).[19]

Table 1. H/H coupling constants (Hz) of 2'-deoxyribose moieties in the ^{1}H NMR spectra of α,β-CNA dinucleotides **11a**,**b**, **12a**,**b**, **17b**, **18a** (500 MHz) and **17a** (400 MHz) and of α,β,γ-CNA dinucleotides **23a**,**b** and **24a**,**b** (500 MHz).

	sugar	Coupling constant *J* (Hz)					%
		J(1',2')		*J*(2',3')		*J*(3',4')	South
11a	upper	5.8	8.8	1.8	5.7	1.9	82
	lower	6.1	8.1	3.1	5.9	2.5	76
11b	upper	5.7	8.8	1.7	5.6	1.6	84
	lower	6.3	8.2	2.9	5.6	2.7	75
12a	upper	5.5	9.1	1.2	5.5	1.4	87
	lower	6.0	8.2	2.4	6.0	2.3	78
12b	upper	5.5	9.1	1.2	5.5	1.3	87
	lower	5.9	8.4	2.2	6.0	2.6	76
17a	upper	5.7	8.7	1.8	6.0	2.0	81
	lower	6.6	7.5	nd	nd	3.0	71
17b	upper	5.8	8.6	1.7	5.8	2.2	80
	lower	6.6	7.3	3.2	6.3	3.1	70
18a	upper	5.5	8.4	1.8	5.8	1.8	82
	lower	5.8	8.4	1.9	5.9	2.0	81
18b	upper	6.1	9.1	2.1	5.6	2.0	82
	lower	5.9	8.4	2.0	5.7	2.0	81
23a	upper	5.9	8.4	nd	5.8	1.9	82
	lower	6.6	7.3	nd	3.9	-	nd
23b	upper	6.0	9.4	1.8	6.1	1.9	83
	lower	7.0	7.0	6.4	3.4	-	nd
24a	upper	5.8	8.6	nd	5.2	1.7	83
	lower	5.9	8.6	2.6	6.0	-	nd
24b	upper	6.0	8.5	1.5	6.0	1.5	85
	lower	6.1	8.0	6.0	2.8	-	nd

With a neutral internucleotidic linkage and with an increasing atomic radius size coupled with a lower electronegativity of the non-bridging heteroatom on the phosphorus from oxygen[15, 20] to sulfur and then to selenium atom, neither the 5'-sugar nor the 3'-sugar puckering of all CNA dinucleotides exhibited a change in their South conformation as depicted by the analysis values of H/H coupling constants (Table 1). The upper nucleoside sugar puckering was still in all cases, strongly pushed in South (C2'-*endo*) conformation due to the loss of the negative charge of the internucleotidic linkage.[21]

The downstream nucleosides of α,β-CNAs exhibited H/H coupling constants and sugar puckering (South, C2'-*endo*) in accordance with unmodified 2'-deoxyribonucleosides.[22] Because of the spiro-junction between the sugar and the dioxaphosphorinane moieties, the Sundaralingam's rule was not applicable to the lower nucleosides of α,β,γ-CNAs for determining the puckering. However, examination of the remaining $J_{1'/2'}$ and $J_{2'/3'}$ coupling constants in comparison with those of unmodified thymidine, suggested that the sugar was also in South conformation.

Dioxaphosphorinane ring conformation

In the early 80's Gorenstein showed that dioxaphosphorinane structure in chair conformation could be characterized by examination of the coupling data in ^{1}H NMR spectra. Typically, axial protons should exhibit a small H/P coupling constant ($^3J_{Hax/P}$ < ca. 3 Hz) together with large coupling constant for equatorial protons ($^3J_{Heq/P}$ > ca. 20 Hz).[23] $^3J_{H/P}$ coupling constants values depicted for the newly synthesized CNA are reported in Table 2 for α,β-CNA dinucleotides and in Table 3 for α,β,γ-CNA dinucleotides. The coupling constants observed for dinucleotides **11a**,**b** and **17a**,**b** were very similar and clearly established that these α,β-CNAs were in chair conformation with all the substituents around the dioxaphosphorinane ring in favorable position (apical for the upper nucleoside, equatorial for the lower nucleoside and for the sulfur or selenium atom).

In contrast, for the minor isomers **12a**,**b** and **18a**,**b** average values around 3, 4 or 5 Hz were observed for the $^3J_{H/P}$ coupling constants involving the 5'b- and 7'b-H respectively, thus suggesting that the dioxaphosphorinane structure of these isomers was in a slightly twisted chair conformation.

Interestingly, Stec reported that the coupling constant $^1J_{P/Se}$ between ^{31}P directly bonded with ^{77}Se (the NMR active isotope with a natural abundance of 7%) could be used for structural determination on the basis that $^1J_{P/Se}$(ax) was always lower than $^1J_{P/Se}$(eq) for diastereoisomeric cyclic phosphoroselenoate compounds with an exocyclic selenium atom.[24] Indeed, we observed for **11b** and **17b** a $^1J_{P/Se}$ coupling constant of 1006 and 1008 Hz respectively, whereas coupling constants of 954 and 974 Hz were depicted for **12b** and **18b** respectively, which was indicative of the equatorial position of the selenium atom within the dioxaphosphorinane structure for **11b** and **17b** and axial for **12b** and **18b**. As a consequence, it can be assumed that in all the α,β-CNAs regardless of the stereochemistry, the lower nucleoside adopts an equatorial or pseudo-equatorial position on the dioxaphosphorinane ring.

This was confirmed by the observation in all cases of a long range coupling (3.5 Hz < $^4J_{H/P}$ < 4.5 Hz) between the 4'-H of the down-stream nucleoside and the phosphorus atom, that was indicative of a W-shaped P-O5'-C5'-C4'-H4' junction, only observable if the nucleoside adopts the equatorial position.

Therefore, all these observations let us to conclude that the backbone torsional angle values of α and β associated with these conformations remain unchanged with respect to their oxo-analogues[15] and are in the (g^+,t) and (g^-,t) range for **11a**,**b** and **17a**,**b**, respectively and (t,t) for **12a**,**b** and **18a**,**b**.

Table 2. H/P coupling constants (Hz) within dioxaphosphorinane moieties in the ^{1}H NMR spectra (400 or 500 MHz) and estimated α,β torsional angle values of α,β-CNA dinucleotides (**11a**,**b**, **12a**,**b**, **17a**,**b** and **18a**,**b**).

	Coupling constant *J* (Hz)			Torsional angle[a]	
	J(5'/P)	*J*(7'/P)	*J*(7''/P)	α	β
11a	1.4	1.7	23.0	g^+	t
11b	1.4	2.0	24.3	g^+	t
12a	3.1	4.1	24.6	t	t
12b	2.5	4.0	25.0	t	t
17a	1.6	2.4	24.3	g^-	t
17b	1.4	2.6	23.0	g^-	t
18a	2.8	5.0	23.3	t	t
18b	2.8	4.9	>20	t	t

[a] The following 6-fold staggered pattern of the torsional angles is used: *cis*= ±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 i used to designate a torsion angle on the border of *anticlinal*(+) and *trans*.

The dioxaphosphorinane conformation within α,β,γ-CNA can b readily assigned by examination of the two couples of couplin constants $^3J_{H/P}$ exhibited by the methylene protons 5'-H and 5''-H with the phosphorus atom (Table 3). The chair conformation c the structure of the *cis*-thio isomer **23a** is clearly established wit a small ($^3J_{Hax/P}$ ~ 2 Hz) and a large coupling constant ($^3J_{Heq/P}$ ~ 2 Hz) for each methylene proton both characteristic of a neat axia and equatorial position for each of them. In contrast, the *tran* isomer **24a** adopted a twist-chair conformation suggested by th average values 11.5 Hz < $^3J_{H/P}$ < 14.0 Hz observed for th coupling constants. On the other hand, none of the selen derivatives **23b** or **24b** were found to be in chair conformation a denoted by the average values 6.2 Hz < $^3J_{H/P}$ < 9.5 Hz of th coupling constants observed for the protons involved in th dioxaphosphorinane ring. But as expected the minor isomer **24l** showed a more pronounced twist-chair conformation than **23l** that sill exhibited large coupling constant for two protons ($^3J_{H/P}$ 20 Hz) indicative of a pseudo-equatorial position.

Moreover, examination of the $^1J_{P/Se}$ coupling constant showed only a small difference between the *cis* and *trans* isomers with 1009 and 992 Hz, respectively. This could suggest that in both cases the exocyclic selenium atom was close to the equatorial position. In contrast with the *cis*-oxo- or thio-α,β,γ-CNA, the dioxaphosphorinane structure within *cis*-seleno-α,β,γ-CNA **23b** adopting a slightly twisted-chair conformation induced a deviation of the associated torsional angles from those proposed for the oxo- or thio-analogues with β shifting to c/g^- and γ to g^-/a^- while α remained unchanged in g^- conformation (Table 3).

Table 3. H/P coupling constants (Hz) within dioxaphosphorinane moieties in the ^{1}H NMR spectra (500 MHz) and estimated α,β,γ torsional angle values of α,β,γ-CNA dinucleotides (**23a,b** and **24a,b**).

	Coupling constant *J* (Hz)				Torsional angle[a]		
	J(5'/P)	*J*(5'/P)	*J*(5''/P)	*J*(5''/P)	α	β	γ
23a	2.5	25	2.0	25	g$^-$	g$^-$	g$^-$
23b	6.2	20.0	6.7	19.0	g$^-$	c/ g$^-$	g$^-$/ a$^-$
24a	11.5	14.0	12.5	11.5	a$^+$/g$^+$	c/ g$^-$	g$^-$/a$^-$
24b	8.5	11.5	9.5	14.0	a$^+$/g$^+$	c/ g$^-$	g$^-$/a$^-$

[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$^+$/g$^+$ is used to designate a torsion angle on the border of *anticlinal*(+) and *gauche*(+).

The *trans* thio- and seleno isomers geometry was conserved with respect of the previously described oxo-*trans* α,β,γ-CNA conformation with a (α,β,γ) value set of (a$^+$/g$^+$, c/g$^-$, g$^-$/a$^-$).
Finally, an another structural information can be obtained from the measure of the coupling constant $^3J_{H3'/P}$ between the 3'-H of the upper nucleoside and the phosphorus atom shown to be correlated with the torsional angle ε (C4'-C3'-O3'-P) following the rule of Altona: $\varepsilon = -\theta - 120°$ and θ was calculated with $^3J_{H3'/P} = 15.3\cos^2(\theta) - 6.1\cos(\theta) + 1.6$.[25] The value of ε provides good information about the position of the upper nucleoside relatively to the dioxaphosphorinane ring within CNA. Depicted $^3J_{H3'/P}$ coupling constants and calculated values for ε torsional angle for CNAs dinucleotides **11a**,**b**, **12a**,**b**, **17a**,**b**, **18a**,**b**, **23a**,**b** and **24a**,**b** are given in Table 4 with those of their oxo-analogues (P=O) previously synthesized as reference.

Table 4. 3'-H/P coupling constants (Hz) value in the ^{1}H NMR spectra (500 MHz) and calculated ε torsional angle values (°) of α,β-CNA and α,β,γ-CNA dinucleotides.

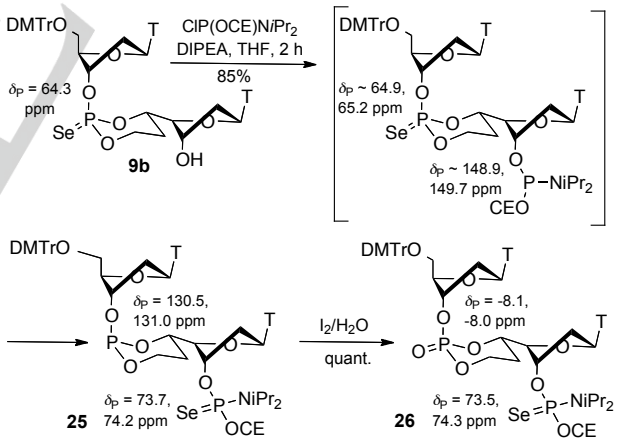
CNA	α,β-CNA 5'-*C*(S)					
	R_P P=O[a]	**11a** P=S	**11b** P=Se	S_P P=O[a]	**12a** P=S	**12b** P=Se
$^3J_{3'-H/P}$	6.6	9.1	9.9	nd	8.4	9.0
ε	-156	-142	-136	nd	-146	-142
CNA	α,β-CNA 5'-*C*(R)					
	R_P P=O[b]	**17a** P=S	**17b** P=Se	S_P P=O[b]	**18a** P=S	**18b** P=Se
$^3J_{3'-H/P}$	5.8	9.0	9.8	6.4	8.8	9.7
ε	-160	-142	-136	-157	-144	-137
CNA	α,β,γ-CNA					
	cis P=O[c]	**23a** P=S	**23b** P=Se	*trans* P=O[c]	**24a** P=S	**24b** P=Se
$^3J_{3'-H/P}$	6.2	8.9	10.0	6.8	8.7	10.0
ε	-158	-143	-135	-155	-145	-135

[a] The values are taken from ref [14]. [b] The values are taken from ref [15] .[c] The values are taken from ref [20].

For each CNA structure, it clearly appeared that increasing the radius of the atom connected at the phosphorus atom resulted in an increase of the value of the coupling constant (from around 6 Hz for P=O to around 10 Hz for P=Se) and therefore a strong variation of ε close to 20°.
These observations evidenced that the upper nucleoside rotates above the dioxaphosphorinane ring when the exocyclic hetero element on the phosphorus atom switched from oxygen (radius of 0.48 Å) to sulfur (0.88 Å) and then to selenium atom (1.03 Å), in order to accommodate the increasing steric hindrance.

The selenium trip around phosphorus atoms

In order to use CNA for the synthesis of conformationaly constrained ONs by automated supported synthesis according to the phosphoramidite technology,[26] the thio- and seleno-CNA dinucleotides must be converted into their corresponding O3'-phosphoramidite by treatment with *N,N*-di*iso*propylamino-cyanoethyl-phosphonamidic chloride. Whereas the thio-α,β- or α,β,γ-CNA derivatives can be readily converted into the appropriate phosphoramidites (data not shown) the seleno-D-CNA exhibited an unexpected behaviour. As a typical exemple, seleno-α,β-CNA **9b** (^{31}P NMR δ = 64.9 ppm) was treated with the phosphitylating agent and provided in good yield (85%) a clean product **25** after purification on silica gel chromatography (Scheme 4). Surprisingly, while two peaks with ^{31}P NMR chemical shift around 150 ppm were expected for the phosphoramidite moiety and two others around 65 ppm for the seleno-dioxaphosphorinane, **25** exhibited two couples of peaks at 131.0/130.5 ppm and 74.2/73.7 ppm respectively. It appeared that **25** was a regioisomer of the desired seleno-α,β-CNA phosphoramidite, corresponding to a migration of the selenium atom from the dioxaphosphorinane ring to the newly introduced phosphoramidate leading to a phosphite triester (131.0/130.5 ppm) and a seleno-phosphoramidate (74.2/73.7 ppm).



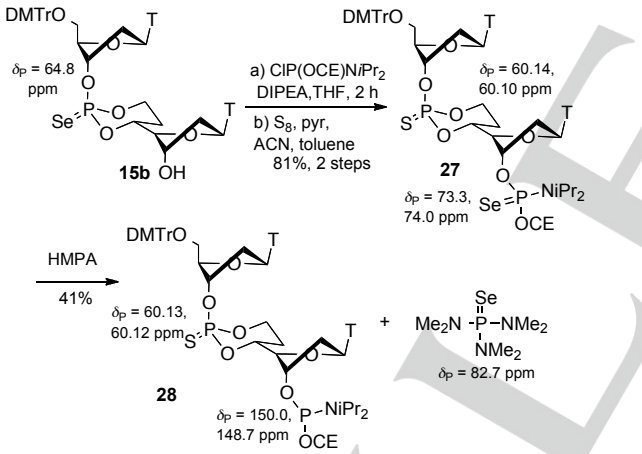
Scheme 4. Selenium migration during phosphoramidite synthesis.

The proposed structure for **25** was confirmed by its conversion by iodine/water oxidation into the α,β-D-CNA derivative **26** with two ^{31}P NMR chemical shifts at -8.0/8.1 ppm which are characteristic for the dioxaphosphorinane moiety in chair conformation[14] and the seleno-phosphoramidate part remained

around 74 ppm. Interestingly, this also demonstrated that the selenium migration/oxidation process occurred with a retention of the phosphorus configuration as outlined by ^{31}P NMR while the other isomer should be depicted around -3 ppm.[15]

It seemed that seleno-D-CNA (in between brackets, Scheme 4) was a source of eletrophilic selenium. The reaction was fast and occurred readily under mild conditions but only after formation of the phosphoramidite at 3'-O position. A selenium transfer to the *N*,*N*-di*iso*propylamino-cyanoethyl-phosphonamidic chloride prior to the reaction with the free hydroxyl group can be rejected because two equivalents of the phosphonamidic chloride derivative were used and therefore **25** would not have been isolated in a 85% yield. Moreover, the putative seleno-phosphonamidic chloride would present a lower reactivity and an extra dinucleotide featuring both the phosphite in the ring and the phosphoramidite moieties should have been detected in ^{31}P NMR.

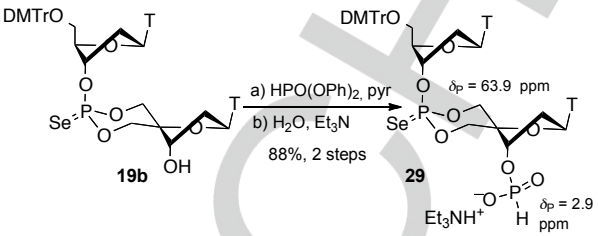
We reproduced a similar synthetic sequence with the major isomer **15b** of seleno-α,β-CNA (Scheme 5). After phosphitylation and selenium transfer, the intermediate was readily oxidized by means of S_8 in pyridine to produce **27** that exhibited the expected couples of peaks in ^{31}P NMR around 74 ppm and 60 ppm. Since we observed that the selenium atom migrates from the phosphorus atom involved in seleno-phosphorinane to the phosphorus atom of the phosphoramidite group, and similarly to reported phosphorus-based selenium-transferring reagent,[27] we postulate that **27** could be a source of electrophilic selenium for an oxidative migration to hexamethylphosphoramide (HMPA). Indeed, after treatment with two equivalents of HMPA at room temperature in acetonitrile, **27** readily transferred its selenium atom and Se-HMPA was formed as observed in ^{31}P NMR with a chemical shift of 82.7 ppm.[28]



Scheme 5. Recovering process from seleno-α,β-CNA to thio-α,β-CNA phosphoramidite by selenium migration over phosphorus.

Thio-α,β-CNA phosphoramidite **28** was isolated and characterized by ^{31}P NMR with typical chemical shifts of 60.1 ppm for the thio-dioxaphosphorinane and 150.0/148.7 ppm representative of the recovered phosphoramidite group. On the other hand, **28** can be straightforwardly obtained from **15a** upon reaction with *N*,*N*-di*iso*propylamino-cyanoethyl- phosphonamidic chloride in standard conditions.

Eventually, while trying to circumvent the issue of selenium migration that prevents the use of seleno-CNA derivatives in building oligonucleotide through phosphoramidite technology, we explored the compatibility of seleno-dioxaphosphorinane with H-phosphonate chemistry. As an example, seleno-α,β,γ-CNA **19b** was converted into its H-phosphonate triethylammonium salt **29** by reacting diphenyl phosphonate on the 3'-hydroxyl group of **19b** (Scheme 6). **29** exhibited the expected ^{31}P NMR spectrum with a characteristic signal at 63.9 ppm corresponding to the seleno-dioxaphosphorinane and another at 2.9 ppm belonging to the H-phosphonate.



Scheme 6. Synthesis of seleno-α,β,γ-CNA H-phosphonate **29**.

Interestingly, the seleno-dioxaphosphorinane group within **19b** did not behave as a selenium-transferring reagent neither on the diphenyl phosphonate reactant nor on the H-phosphonate moiety of **29** whereas this was observed with triphenyl phosphine selenide.[27] Therefore H-phosphonate chemistry may be better adapted for the construction of ONs featuring seleno-phosphorinane-modified nucleotides.

Conclusions

Four diastereoisomers of each thio- and seleno-α,β-CNA and two isomers of each thio- and seleno-α,β,γ-CNA dinucleotides in which the α,β or α,β,γ torsional angles of the sugar/phosphate backbone are controlled within a dioxaphosphorinane structure with a sulfur or selenium as exocyclic hetero element have been synthesized and their conformation determined.

It appeared that replacing the exocyclic oxygen of the dioxaphosphorinane ring either by a sulfur or a selenium atom did not modified notably the constrain applied to the torsional angle involved in the cycle. The main 5'-*C*(*R*) or 5'-*C*(*S*) isomers of thio- and seleno-α,β-CNA displayed (g^-, t) and (g^+, t) set of values for (α, β) respectively. The same trend was observed for the thio- and seleno-*trans*-α,β,γ-CNA and for thio-*cis*-α,β,γ-CNA as they behave the same as their oxo-counterparts. A slight variation was observed for the *cis*-seleno-α,β,γ-CNA in which while α remained in g^- conformation, β moved to (cis/g^-) and γ angle to (g^-/a^-) conformation due to the selenium atom that distorted the dioxaphosphorinane chair conformation. Eventually, in all D-CNA because of the increasing atom size from oxygen to sulfur and then to selenium atom, ε torsional angle was impacted resulting in a rotation of the upper nucleoside above the dioxaphosphorinane ring that reached 20° in seleno-CNA with respect to the oxo-analogues.

We observed that selenium atom within seleno-CNA behaves as an electrophilic specie able to migrate from dioxaphosphorinane group to oxidize a phosphoramidite moiety that ablates the synthesis of the corresponding dinucleotide phosphoramidite. This issue can be overcome by using H-phosphonate chemistry

that allows for the preparation of H-phosphonate derivatives of seleno-D-CNA.

These newly synthesized CNAs complete our set of constrained dinucleotides featuring non-canonical conformations, their behaviour within biologically relevant nucleic acids secondary structures are under evaluation and will be reported in due course.

Experimental Section

Products were purified by medium pressure liquid chromatography on a Waters 2545 apparatus by using Amicon 6-35 mm or Merck 15 mm silica unless stated otherwise. Alternatively, they were purified by high performance liquid chromatography (HPLC and RP-HPLC) using a Waters 2545 Binary Gradient Module (BGM, high-pressure mixing binary gradient pump) combined to a Waters 2489 UV/Vis detector. HPLC instrument control, data collection and analysis were performed on a PC equipped with Microsoft Windows XP, and Acquity software.

NMR spectra were recorded on a Bruker Avance-300, Avance-400 or Avance 500 spectrometers (300, 400 or 500 MHz for ^{1}H; 75, 100 or 125 MHz for ^{13}C and 121 or 202 MHz for ^{31}P). Chemical shifts for ^{1}H and ^{13}C are reported in ppm (δ) relative to an internal standard (solvent residual peak). Mass spectra were recorded on a Nermag R10-10, on a Perkin-Elmer API 365 or on a MALDI-TOF Waters Micro MX. All solvents were distilled and dried before use.

Compounds **1**, **2, 7a, 9a** and **11a** have been previously described,[20] as compounds **3**[14] and **5**.[15]

3'-*O*-*tert*-Butyldiphenylsilyl-5'-*O*-trimethylsilyl-5'-(trimethylsilyloxy ethyl) thymidine (4): To a solution of **3** (1 g, 1.91 mmol) in anhydrous pyridine (6 mL) was added *tert*-butyltrimethylsilyl chloride (0.316 g, 2.1 mmol) at room temperature. After 12 h of stirring, the reaction was stopped by addition of a saturated aqueous solution of NH_4Cl. The silylated intermediate was extracted with EtOAc and dried over $MgSO_4$. The crude white foam obtained (1.25 g) was directly subjected to the oxidation/reduction process by addition of Dess-Martin periodinane (1.62 g) in 10 mL of anhydrous dichloromethane and pyridine (80 mL). After 2 h of stirring at room temperature the reaction mixture was diluted with ethanol (20 mL). After 30 min, $NaBH_4$ was added (432 mg, 11.4 mmol) and stirring was maintained for 2 h. As TLC control (EP/CH_2Cl_2/EtOAc, 3:5.6:1.4) showed a single spot corresponding to the starting material, the reaction was stopped by addition of a saturated aqueous solution of NH_4Cl and the epimeric mixture was extracted with EtOAc and the organic layer dried over magnesium sulphate. The *tert*-butyldimethylsilyl ether was then removed by dilution of the crude product in dry methanol (20 mL) and *p*-toluenesulfonic acid (250 mg) was added. After 1 h, the reaction was stopped by addition of a saturated aqueous solution of $NaHCO_3$. The methanol was evaporated and the resulting diol was extracted with ethyl acetate, washed with brine and the organic layer dried over $MgSO_4$. The crude material was then submitted to persilylation with hexamethyldisilazane (0.808 mL, 0.381 mmol) and trimethylsilyl chloride (0.970 mL, 0.381 mmol) in anhydrous pyridine (6 mL). After 2 h of stirring at room temperature, the reaction was stopped by addition of a saturated aqueous solution of NH_4Cl and compound **4** was extracted with diethyl ether, the organic layer was washed with water and brine, and dried over magnesium sulphate. After removal of the solvent, compound **4** (1.08 g, 85% overall yield from **3**) was isolated after silica gel chromatography (CH_2Cl_2/EtOAc, 4:1) as a 1/2 mixture of 5'-C(*S*) and 5'-C(*R*) diastereoisomers. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.20 (m, 3 H, NH), 7.69-7.66 (m, 12 H, Ph), 7.45-7.41 (m, 19 H, Ph and H_{6S}), 7.12 (d, *J* = 1.2 Hz, 2 H, H_{6R}), 6.59 (dd, *J* = 5.4, 9.3 Hz, 1 H, $H_{1'S}$), 6.35 (dd, *J* = 5.7, 8.7 Hz, 2 H, $H_{1'R}$), 4.43 (m, 2 H, $H_{3'R}$), 4.22 (m, 1 H, $H_{3'S}$), 3.96 (m, 3 H, $H_{4'S}$ and $H_{4'R}$), 3.88 (m, 2 H, $H_{5'R}$), 3.49-3.43 (m, 7 H, $H_{7'}$ and $H_{5'S}$), 2.24-2.11 (m, 3 H, $H_{2'}$), 1.84 (m, 9 H, H_7), 1.71-1.37 (m, 9 H, $H_{2''}$ and $H_{6'}$), 1.06 (s, 27 H, *t*Bu), 0.13, 0.07, 0.02 and -0.11 (s, 60 H, $SiMe_3$) ppm.

3'-*O*-*tert*-Butyldiphenylsilyl-5'-*O*-trimethylsilyl-5'-oxyethylthymidine (6): Diastereoisomers **4** (1 g, 1.5 mmol) were submitted to K_2CO_3 (310 mg, 2.24 mmol) in anhydrous methanol at 0°C for 1.5 h. The reaction was stopped by addition of a saturated aqueous solution of NH_4Cl and compounds **5** and **6** were extracted with EtOAc. After drying, the organic layer was concentrated *in vacuo* and the crude material was submitted to silica gel chromatography with a mixture of CH_2Cl_2/EtOAc (1:1) as eluent. 424 mg (54%) of **5** and 250 mg (28%) of **6** (and 161 mg (18%) of a 1/2 mixture of **6** and its 5'-*C* epimer) were isolated. Data for **5**.[15] Data for **6**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.77 (bq, *J* = 1.2 Hz, 1 H, H_6), 7.64-7.60 (m, 4 H, Ph), 7.44-7.36 (m, 6 H, Ph), 6.58 (dd, *J* = 5.4, 9.3 Hz, 1 H, $H_{1'}$), 4.20 (d, *J* = 5.4 Hz, 1 H, $H_{3'}$), 3.92 (s, 1 H, $H_{4'}$), 3.50 (m, 2 H, $H_{7'}$), 3.27 (ddd, *J* = 1.2, 5.2, 7.6 Hz, 1 H, $H_{5'}$), 2.29 (A part of an ABX syst, *J* = 5.5, 13.1 Hz, 1 H, $H_{2''}$), 1.89-1.82 (m, 4 H, H_7 and $H_{2'}$), 1.73-1.57 (m, 2 H, $H_{6'}$), -0.08 (s, 9 H, $SiMe_3$) ppm.^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.0, 150.5, 136.0, 135.6, 133.2, 132.9, 129.9, 127.8, 127.7, 110.8, 89.5, 84.5, 84.9, 76.0, 70.0, 58.3, 40.7, 37.0, 26.8, 18.9, 14.4, 0.0 ppm.

General procedure A for the preparation of protected α,β-thio-D-CNA and α,β,γ-thio-D-CNA dinucleotides: 4'-*C*-Hydroxymethyl thymidine **2** or 5'-*C*-hydroxyethylthymidine **3** or **5** (1 eq.) and 5-(ethylthio)-1*H*-tetrazole (5 eq.) were dissolved in dry acetonitrile (C = 0.5 M) at r.t. under an argon atmosphere. A solution of 5'-*O*-dimethoxytrityl thymidine phosphorodiamidite[17b] (1.2 eq.) in dry acetonitrile (C = 0.5 M) was added dropwise to the mixture over a period of 15 min. The resulting mixture was stirred at r.t. until TLC analysis indicated the completion of the reaction (ca. 2 h). To the reaction mixture were added dry pyridine (24 eq.) and S_8 (5 eq.). The yellow suspension was stirred and heated at 60°C for 2 h. The reaction mixture was then allowed to cool down to r.t. The suspension was filtered through a sintered funnel to remove elemental sulfur and the solvents were evaporated. The residue was then dissolved in H_2O (10 mL) and the resultant solution extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL), dried over $MgSO_4$ and then concentrated *in vacuo*. The crude product was purified by silica gel column chromatography by eluting either with a mixture of EtOAc/DCM/Et_3N (5:5:0.05) to yield protected α,β-thio-D-CNA dinucleotides as white foams or with a mixture of EtOAc/petroleum ether/Et_3N (1:1:0.05) to EtOAc/MeOH/Et_3N (95:5:0.05) to yield α,β,γ-thio-D-CNA dinucleotides as white foams.

General procedure B for the preparation of protected α,β-seleno-D-CNA and α,β,γ-seleno-D-CNA dinucleotides: 4'-*C*-Hydroxymethyl thymidine **2** or 5'-*C*-hydroxyethylthymidine **3** or **5** and 5-(ethylthio)-1*H*-tetrazole (5 eq.) were dissolved in dry acetonitrile (C = 0.5 M) at r.t. under an argon atmosphere. A solution of 5'-*O*-dimethoxytritylthymidine phosphorodiamidite (1.2 eq.)[17b] in dry acetonitrile (2 mL) was added dropwise to the mixture over a period of 10 min. The resulting mixture was stirred at r.t. for 2 h until TLC analysis indicated the completion of the reaction. To the reaction mixture were added dry Et_3N (10 eq.) and potassium selenocyanate (5 eq.). The pink suspension was stirred and heated at 60°C for 1 h. The reaction mixture was then allowed to cool down to r.t. over night. After 15 h, the suspension was filtered through a silica plug and washed with EtOAc to remove toxic KSeCN. The solvents

were evaporated and co-evaporated with toluene. The residue was then dissolved in H_2O (10 mL) and the resultant solution extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with H_2O (20 mL) and brine (20 mL), dried over $MgSO_4$ and then concentrated *in vacuo*. The crude product was purified by silica gel column chromatography eluting with a mixture of EtOAc/petroleum ether/Et_3N (5:5:0.05 to 9:1:0.05) to yield protected α,β-seleno-D-CNA and α,β,γ-seleno-D-CNA as pink solids.

General procedure C for the fluoride ion deprotection of α,β-D-CNA and α,β,γ-D-CNA dinucleotides: To fully protected D-CNA (1 eq.) in solution in dry THF (C = 0.1 M) was added TBAF (1 M solution in THF, 1 eq.). The solution was stirred under an argon atmosphere at 0°C. After TLC analysis showed complete conversion of starting material (40 min – 1 h), the solvent was concentrated under reduced pressure controlling the temperature of the rotavapor bath (T < 25°C) and the crude was then purified by silica gel column chromatography eluting with a mixture of EtOAc/MeOH/Et_3N (10:0:0.05 to 9:1:0.05) to give 5'-DMTr dinucleotides D-CNA as white solids.

General procedure D for the detritylation of α,β-D-CNA and α,β,γ-D-CNA dinucleotides. The 5'-DMTr dinucleotide D-CNA (1 equiv.) was dissolved in a TCA solution (2% in CH_2Cl_2, 35 mL/mmol) and stirred at r.t. and after 30 min, the solution was concentrated and the residue was re-dissolved in the same volume of the 2% TCA solution for another 30 min. One drop of a saturated solution of $NaHCO_3$ was added and the solution concentrated under reduced pressure. The crude was dissolved in the minimum of eluant EtOAc/MeOH (9:1) and was purified by silica gel column chromatography eluting with a mixture of EtOAc/MeOH (95:5 to 90:10) to yield to fully deprotected thio-D-CNA dinucleotides as white solids and fully deprotected seleno-D-CNA dinucleotides as pink solids.

5'-*O*-Dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-α,β-thio-D-CNA (S_C,S_P) (7a) and 5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-α,β-thio-D-CNA (S_C,R_P) (8a): Following the general procedure A starting from **3** gave the products **7a** (26%, 550 mg)[11] and **8a** (20%, 410 mg). Data for **8a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.10 (s, 1 H, NH), 9.07 (s, 1 H, NH), 7.65-7.60 (m, 5 H, Ph), 7.53 (d, $^4J_{6\text{-}7}$ = 1.3 Hz, 1 H, H_6), 7.49-7.13 (m, 15 H, Ph, H_6), 6.83-6.79 (m, 4 H, Ph), 6.51 (dd, $^3J_{1'b\text{-}2'b}$ = 5.7, $^3J_{1'b\text{-}2'b}$ = 8.9 Hz, 1 H, $H_{1'b}$), 6.28 (dd, $^3J_{1'b\text{-}2'b}$ = 6.1, $^3J_{1'b\text{-}2'b}$ = 8.3 Hz, 1 H, $H_{1'a}$), 5.57 (m, 1 H, $H_{3'a}$), 4.26-4.10 (m, 3 H, $H_{3'b}$, 2 × $H_{7'b}$), 4.02 (br. s, 1 H, $H_{4'a}$), 3.78 (br. s, 1 H, $H_{3'b}$), 3.77 (s, 6 H, OMe), 3.71 (d, ^3J = 5.4 Hz, 1 H, $H_{4'b}$), 3.40 (m, 2 H, 2 × $H_{5'a}$), 2.42-2.36 (m, 2 H, 2 × $H_{2'a}$), 2.28-2.19 (m, 3 H, $H_{6'b}$, 2 × $H_{2'b}$), 1.89 (d, $^4J_{7\text{-}6}$ = 1.1 Hz, 3 H, H_7), 1.90-1.80 (m, 1 H, $H_{6'b}$), 1.44-1.14 (m, 1 H, $H_{6'b}$), 1.38 (d, $^4J_{7\text{-}6}$ = 1.0 Hz, 3 H, H_7), 1.07 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.1, 164.0, 158.9, 158.8, 150.6, 150.3, 144.3, 136.0, 135.9, 135.4, 135.24, 135.20, 133.4, 132.9, 130.4, 130.2, 129.3, 128.5, 128.3, 128.2, 128.0, 127.9, 127.3, 113.6, 113.4, 111.8, 111.7, 88.2, 88.0, 87.5, 86.0, 85.6, 81.1, 77.5, 74.4, 66.6, 63.6, 55.5, 40.5, 39.7, 28.5, 27.1, 19.2, 13.0, 11.9 ppm.^{31}P NMR (121 MHz, $CDCl_3$): δ = 67.6 ppm. MS (Malfi Tof): m/z = 1151.8 $[M+Na]^+$.

5'-*O*-Dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-α,β-seleno-D-CNA (S_C,S_P) (7b) and 5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-α,β-seleno-D-CNA (S_C,R_P) (8b): Following the general procedure B starting from **3** gave the products **7b** (27%, 780 mg) and **8b** (11%, 310 mg). Data for **7b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.53 and 9.10 (bs, 2 H, NH), 7.61-7.46 (m, 6 H, Ph), 7.40-7.21 (m, 15 H, Ph), 6.81 (bd, 4 H, Ph), 6.59 (dd, ^3J = 5.1, 9.6 Hz, 1 H, $H_{1'}$), 6.45 (dd, ^3J = 6.0, 9.0 Hz, 1 H, $H_{1'}$), 5.43 (dd, ^3J = 4.2, 10.8 Hz, 1 H, $H_{3'a}$), 4.25-4.18 (m, 3 H, 2 × $H_{7'b}$ and $H_{4'a}$), 4.02 (bs, 1 H, $H_{4'b}$), 3.76 (s, 6 H, OMe), 3.65 (d, ^3J = 5.1 Hz, 1 H, $H_{5'b}$), 3.41-3.29 (m, 3 H, $H_{3'a}$ and 2 × $H_{5'a}$), 2.45-2.21 (m, 4 H, $H_{2'}$), 1.98 (d, $^4J_{7\text{-}6}$ = 0.9 Hz, 3 H, H_7), 1.90-1.81 (m, 1 H, $H_{6'b}$), 1.43 (d, $^4J_{7\text{-}6}$ = 0.9 Hz, 3 H, H_7), 1.40-1.34 (m, 1 H, $H_{6'b}$), 1.03 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.0, 163.9, 158.9, 158.8, 150.9, 150.8, 144.1, 135.8, 135.7, 135.3, 135.2, 135.1, 133.3, 132.5, 130.6, 130.3, 130.2, 130.1, 130.0, 128.2, 128.0, 113.5, 113.4, 112.4, 112.1, 87.8, 87.7, 87.5, 87.2, 85.5, 84.5, 80.9, 78.5, 75.9, 74.5, 63.6, 55.4, 45.6, 45.4, 39.8, 39.1, 23.6, 22.3, 19.0, 12.7, 11.8 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 64.6 ppm. Data for **8b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.95 and 8.92 (bs, 2 H, NH), 7.72-7.64 (m, 5 H, Ph), 7.57 (bs, 1 H, H_6), 7.50-7.23 (m, 15 H, Ph), 6.85 (bd, 4 H, Ph), 6.54 (dd, ^3J = 5.4, 8.4 Hz, 1 H, $H_{1'}$), 6.32 (t, ^3J = 7.2 Hz, 1 H, $H_{1'}$), 5.71 (dd, ^3J = 3.3, 10.2 Hz, 1 H, $H_{3'a}$), 4.41-4.35 (m, 3 H, $H_{3'b}$ and $H_{7'b}$), 4.25 (bs, 1 H, $H_{4'b}$), 4.21-4.09 (m, 1 H, $H_{7'b}$), 3.89 (bdd, ^3J = 2.1 and 11.4 Hz, 1 H, $H_{5'b}$), 3.81 (s, 6 H, OMe), 3.76 (m, 1 H, $H_{4'a}$), 3.50 and 3.41 (AB part of an ABX syst, ^3J = 2.1, 2.4, 11.1 Hz, 2 H, $H_{5'a}$, $H_{5''a}$), 2.49-2.39 (m, 3 H, $H_{2'}$), 2.27-2.14 (m, 1 H, $H_{2'}$), 1.94 (d, $^4J_{7\text{-}6}$ = 0.9 Hz, 3 H, H_7), 1.91-1.81 (m, 1 H, $H_{6'b}$), 1.42 (d, $^4J_{7\text{-}6}$ = 0.9 Hz, 3 H, H_7), 1.33-1.23 (m, 1 H, $H_{6'b}$), 1.11 (s, 9 H, *t*Bu) ppm.^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.7, 163.6, 158.8, 150.4, 150.1, 144.1, 135.9, 135.7, 135.2, 135.0, 134.9, 133.2, 132.7, 130.2, 130.1, 130.0, 128.3, 128.2, 128.1, 128.0, 128.0, 127.2, 113.4, 111.6, 111.5, 87,8, 87.6, 87,4, 85.4, 84.6, 84.2, 81.8, 74.3, 71.4, 67.0, 63.4, 55.3, 40.3, 39.5, 26.9, 25.7, 19.0, 12.9, 11.7 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 71.9 ppm.

5'-*O*-Dimethoxytrityl-α,β-thio-D-CNA (S_C,S_P) (9a) Following the general procedure C starting from **7a** gave product **9a** (quantitative, 435 mg).[20]

5'-*O*-Dimethoxytrityl-α,β-thio-D-CNA (S_C,R_P) (10a): Following the general procedure C starting from **8a** gave product **10a** (quantitative, 356 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.02 (br. s, 2 H, 2 × NH), 7.59 (s, 1 H, H_{6a}), 7.45 (s, 1 H, H_{6b}), 7.35-7.19 (m, 9 H, Ph), 6.81 (d, ^3J = 9.0 Hz, 4 H, Ph), 6.38 (dd, $^3J_{1'b\text{-}2'b}$ = 6.1, $^3J_{1'b\text{-}2''b}$ = 8.0 Hz, 1 H, $H_{1'b}$), 6.22 (dd, $^3J_{1'a\text{-}2'a}$ = 5.4, $^3J_{1'a\text{-}2''a}$ = 9.0 Hz, 1 H, $H_{1'a}$), 5.86 (m, $^3J_{3'a\text{-}P}$ = 1.6 Hz, 1 H, $H_{3'a}$), 4.86 (m, $^3J_{5'b\text{-}P}$ = 1.8 Hz, 1 H, $H_{5'b}$), 4.60 (m, $^3J_{7'b\text{-}P}$ = 0.5 Hz, 1 H, $H_{7'b}$), 4.49 (m, 1 H, $H_{3'b}$), 4.39-4.32 (m, $^3J_{7''b\text{-}6''b}$ = 3.2, $^3J_{7''b\text{-}6'b}$ = 5.3, $^3J_{7''b\text{-}7'b}$ = 11.5, $^3J_{7''b\text{-}P}$ = 25.6 Hz, 1 H, $H_{7''b}$), 4.27 (br. s, 1 H, $H_{4'a}$), 4.07 (m, 1 H, $H_{4'b}$) 3.76 (s, 6 H, OMe), 3.41 (br. s, 2 H, 2 × $H_{5'a}$), 2.65 (dd, $^3J_{2'a\text{-}1'a}$ = 5.2, $^3J_{2'a\text{-}2''a}$ = 13.9 Hz, 1 H, $H_{2'a}$), 2.44-2.39 (m, 3 H, $H_{2'a}$, $H_{2'b}$, $H_{6'b}$), 2.13-2.07 (m, 1 H, $H_{2'b}$), 1.89 (d, $^4J_{7\text{-}6}$ = 0.5 Hz, 3 H, H_{7b}), 1.83 (m, 1 H, $H_{6'b}$), 1.40 (d, $^4J_{7\text{-}6}$ = 0.5 Hz, 3 H, H_{7a}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.5 (C_{4b}), 164.5 (C_{4a}), 158.9 (CH_{Ph}), 151.1 (C_{2b}), 151.0 (C_{2a}), 144.3 ($C_{q,Ph}$), 135.6 (C_{6b}), 135.3 (C_{6a}), 135.2, 135.1 ($C_{q,Ph}$), 130.22, 130.20, 128.3, 128.2, 127.4 (CH_{Ph}), 113.6 (CH_{Ph}), 111.84 (C_{5b}), 111.82 (C_{5a}), 87.6 ($C_{q,Ph}$), 87.0 ($^3J_{4'b\text{-}P}$ = 8.2 Hz, $C_{4'b}$), 85.7 ($C_{1'b}$), 84.9 ($C_{1'a}$), 84.8 ($^3J_{4'a\text{-}P}$ = 8.6 Hz, $C_{4'a}$), 81.7 ($C_{3'a}$), 78.7 ($^3J_{5'b\text{-}P}$ = 4.6 Hz, $C_{5'b}$), 72.6 ($C_{3'b}$), 67.0 ($C_{7'}$), 63.7 ($C_{5'a}$), 55.5 (OMe), 40.7 ($C_{2'b}$), 40.7 ($C_{2'a}$), 28.3 ($C_{6'}$), 13.0 (C_{7b}), 12.0 (C_{7a}) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 66.6 ppm. MS (Malfi Tof): *m/z* = 913.4 $[M+Na]^+$, 929.4 $[M+K]^+$.

5'-*O*-Dimethoxytrityl-α,β-seleno-D-CNA (S_C,S_P) (9b): Following the general procedure C starting from **7b** gave the products **9b** (64%, 400 mg). As already been observed on previous protected D-CNA, the NMR spectra were obtained with a very poor resolution for ^{1}H spectra and without detection of the aliphatic carbons for the ^{13}C spectrum.[20] ^{1}H NMR (300 MHz, CD_3OD): δ = 7.45 (bd, $^4J_{6\text{-}7}$ = 1.2 Hz, H_6), 7.34 (bd, $^4J_{6\text{-}7}$ = 1.2 Hz, H_6), 7.23-7.05 (m, 9 H, Ph), 6.67 (m, 4 H, Ph), 6.26 (m, 2 H, 2 × $H_{1'}$), 5.30 (dd, ^3J = 4.8, 10.2 Hz, $H_{3'a}$), 4.67 (bd, ^3J = 11.4 Hz, $H_{3'b}$), 4.43-4.21 (m, 2 H, $H_{7'b}$), 4.17 (m, 2 H, $H_{4'a,}$ $H_{4'b}$), 3.61 (s, 6 H, OMe), 3.70 (m, 1

H, $H_{5'b}$), 3.39 and 3.25 (AB part of an ABX syst, ^3J = 2.7, 3.0, 10.8 Hz, 2 H, 2 × $H_{5'a}$), 2.46-2.26 (m, 2 H, $H_{2'}$ and $H_{6'b}$), 2.01 and 1.88 (AB part of an ABX(Y) syst, ^3J = 2.4, 5.4, 5.7, 9.0, 13.2 Hz, 2 H, $H_{2'}$), 1.83 (d, $^4J_{7\text{-}6}$ = 1.2 Hz, 3 H, H_7), 1.70 (bd, ^3J = 14.4 Hz, 1 H, $H_{6''b}$), 1.23 (d, $^4J_{7\text{-}6}$ = 1.2 Hz, 3 H, H_7) ppm. ^{31}P NMR (121 MHz, CD_3OD): δ = 64.3 ppm. MS (Malfi Tof): *m/z* = 961.6 $[M+Na]^+$. HRMS (ESI): calcd for $C_{43}H_{47}N_4O_{13}PNaSe$, 961.1945 $[M+Na]^+$; found 961.1931.

5'-*O*-Dimethoxytrityl-α,β-seleno-D-CNA (S_C,R_P) (10b): Following the general procedure C starting from **8b** gave the product **10b** (34%, 84 mg) and was engaged immediately in the next detritylation step. As for **9b**, NMR spectra were recorded with a very low resolution. ^{1}H NMR (300 MHz, CD_3OD): δ = 7.59 (bd, $^4J_{6\text{-}7}$ = 1.2 Hz, H_6), 7.42 (bd, $^4J_{6\text{-}7}$ = 1.2 Hz, H_6), 7.39-7.23 (m, 9 H, Ph), 6.82 (m, 4 H, Ph), 6.38 (m, ^3J = 5.7, 7.5 Hz, 1 H, $H_{1'}$), 6.24 (m, ^3J = 5.4, 9.0 Hz, 1 H, $H_{1'}$), 5.64 (dd, ^3J = 5.4, 8.4 Hz, $H_{3'a}$), 4.88 (bd, ^3J = 10.8 Hz, 1 H, $H_{3'b}$), 4.62 (m, 1 H, $H_{7'b}$), 4.48 (m, 2 H, $H_{4'}$), 4.37-4.20 (m, 2 H, $H_{7'b}$ and $H_{4'}$), 4.05 (m, 1 H, $H_{5'b}$), 3.76 (s, 6 H, OMe), 3.43 (m, 2 H, 2 × $H_{5'a}$), 2.67 (A part of an ABX syst, ^3J = 5.4, 14.1 Hz, 1 H, $H_{2'}$), 2.49-2.35 (m, 1 H, $H_{2'}$), 2.11 (m, 1 H, $H_{6'b}$), 1.92 (d, $^4J_{7\text{-}6}$ = 1.0 Hz, 3 H, H_7), 1.83 (bd, ^3J = 14.4 Hz, $H_{6''b}$), 1.42 (d, $^4J_{7\text{-}6}$ = 1.0 Hz, 3 H, H_7). ^{31}P NMR (121 MHz, CD_3OD): δ = 70.9 ppm. MS (Malfi Tof): m/z = 961.4 $[M+Na]^+$.

α,β-thio-D-CNA (S_C,S_P) (11a): Following the general procedure D starting from **9a** gave the product **11a** (80%, 16 mg). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.80 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6a}), 7.65 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6b}), 6.43 (dd, $^3J_{1'b\text{-}2'b}$ = 6.1, $^3J_{1'b\text{-}2''b}$ = 8.1 Hz, 1 H, $H_{1'b}$), 6.36 (dd, $^3J_{1'a\text{-}2'a}$ = 5.8, $^3J_{1'a\text{-}2''a}$ = 8.8 Hz, 1 H, $H_{1'a}$), 5.21 (ddt, $^3J_{3'a\text{-}4'a}$ = 1.9, $^3J_{3'a\text{-}2'a}$ = 1.6, $^3J_{3'a\text{-}2''a}$ = 5.7, $^3J_{3'a\text{-}P}$ = 9.1 Hz, 1 H, $H_{3'a}$), 4.86 (dddd, $^3J_{5'b\text{-}4'b}$ = 1.9, $^3J_{5'b\text{-}6''b}$ = 1.9, $^3J_{5'b\text{-}6'b}$ = 11.6, $^3J_{5'b\text{-}P}$ = 1.4 Hz, 1 H, $H_{5'b}$), 4.58 (dddd, $^3J_{7'b\text{-}6''b}$ = 2.1, $^3J_{7'b\text{-}7''b}$= 10.8, $^3J_{7'b\text{-}6'b}$ = 13.0, $^3J_{7'b\text{-}P}$ = 1.7 Hz, 1 H, $H_{7'b}$), 4.48 (ddd, $^3J_{3'b\text{-}2'b}$ = 3.1, $^3J_{3'b\text{-}4'b}$ = 2.5, $^3J_{3'b\text{-}2''b}$ = 5.8 Hz, 1 H, $H_{3'b}$), 4.43 (dddd, $^3J_{7''b\text{-}6''b}$ = 1.5, $^3J_{7''b\text{-}6'b}$ = 4.9, $^3J_{7''b\text{-}7'b}$ = 11.1, $^3J_{7''b\text{-}P}$ = 23.0 Hz, 1 H, $H_{7''b}$), 4.30 (td, $^3J_{4'a\text{-}3'a}$ = 1.9, $^3J_{4'a\text{-}5'a}$ = 3.2, $^3J_{4'a\text{-}5''a}$ = 3.2 Hz, 1 H, $H_{4'a}$), 3.93 (ddd, $^3J_{4'b\text{-}5'b}$ = 1.8, $^3J_{4'b\text{-}3'b}$ = 2.5, $^3J_{4'b\text{-}P}$ = 4.5 Hz, 1 H, $H_{4'b}$), 3.83 (ABX syst, $^3J_{5'a\text{-}4'a}$ = 3.1, $^3J_{5''a\text{-}4'a}$ = 3.1, $^3J_{5'a\text{-}5''a}$ = 12.2 Hz, 2 H, $H_{5'a}$, $H_{5''a}$), 2.58 (ddd, $^3J_{2'a\text{-}3'a}$ = 1.8, $^3J_{2'a\text{-}1'a}$ = 5.8, $^3J_{2'a\text{-}2''a}$ = 14.1 Hz, 1 H, $H_{2'a}$), 2.42 (ddd, $^3J_{2''a\text{-}3'a}$ = 5.7, $^3J_{2''a\text{-}1'a}$ = 8.8, $^3J_{2''a\text{-}2'a}$ = 13.9 Hz, 1 H, $H_{2''a}$), 2.39 (dddd, $^3J_{6'b\text{-}7''b}$ = 5.0, $^3J_{6'b\text{-}5'b}$ = 11.7, $^3J_{6'b\text{-}7'b}$= 13.0 Hz, $^3J_{6'b\text{-}6''b}$ = 14.3 Hz, 1 H, $H_{6'b}$), 2.23 (ddd, $^3J_{2'b\text{-}3'b}$ = 3.1, $^3J_{2''b\text{-}1'b}$ = 6.1, $^3J_{2'b\text{-}2''b}$ = 13.3 Hz, 1 H, $H_{2'b}$), 2.18 (ddd, $^3J_{2''b\text{-}3'b}$ = 5.9, $^3J_{2''b\text{-}1'b}$ = 8.0, $^3J_{2''b\text{-}2'b}$ = 13.3 Hz, 1 H, $H_{2''b}$), 1.94 (d, $^4J_{7\text{-}6}$ = 1.1 Hz, 3 H, H_{7b}), 1.89 (d, $^4J_{7\text{-}6}$ = 1.1 Hz, 3 H, H_{7a}), 1.87 (dddd, $^3J_{6''b\text{-}7''b}$ = 1.6, $^3J_{6''b\text{-}5'b}$ = 1.9, $^3J_{6''b\text{-}7'b}$ = 2.2, $^3J_{6''b\text{-}6'b}$ = 14.4 Hz, 1 H, $H_{6''b}$) ppm.^{13}C NMR (125 MHz, CD_3OD): δ = 166.5 (C_{4a}), 166.4 (C_{4b}), 152.6 (C_2), 152.5 (C_2), 137.9 (C_{6a}), 137.4 (C_{6b}), 112.7 (C_{5b}), 112.1 (C_{5a}), 88.7 ($^3J_{4'b\text{-}P}$ = 7.7 Hz, $C_{4'b}$), 87.3 ($^3J_{4'a\text{-}P}$ = 5.5 Hz, $C_{4'a}$), 86.5 ($C_{1'a}$), 86.1 ($C_{1'b}$), 80.9 ($^2J_{5'b\text{-}P}$ = 9.1 Hz, $C_{5'b}$), 80.7 ($^2J_{3'a\text{-}P}$ = 2.9 Hz, $C_{3'a}$), 73.1 ($C_{3'b}$), 69.4 ($^2J_{7'b\text{-}P}$ = 9.1 Hz, $C_{7'}$), 63.0 ($C_{5'a}$), 40.8 ($C_{2'b}$), 39.5 ($^3J_{2'a\text{-}P}$ = 4.8 Hz, $C_{2'a}$), 29.1 ($^3J_{6'b\text{-}P}$ = 4.0 Hz, $C_{6'b}$), 12.8 (C_{7b}), 12.6 (C_{7a}). ^{31}P NMR (202 MHz, CD_3OD): δ = 60.3 ppm. HRMS (ESI): calcd for $C_{22}H_{30}N_4O_{11}PS$ 589.1369 $[M+H]^+$; found 589.1379.

α,β-thio-D-CNA (S_C,R_P) (12a): Following the general procedure D starting from **10a** gave the product **12a** (93%, 16 mg). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.81 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6a}), 7.63 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6b}), 6.38 (dd, $^3J_{1'b\text{-}2'b}$ = 6.0, $^3J_{1'b\text{-}2''b}$ = 8.3 Hz, 1 H, $H_{1'b}$), 6.27 (dd, $^3J_{1'a\text{-}2'a}$ = 5.5, $^3J_{1'a\text{-}2''a}$ = 9.1 Hz, 1 H, $H_{1'a}$), 5.44 (ddd $^3J_{3'a\text{-}2'a}$ = 1.2, $^3J_{3'a\text{-}4'a}$ = 1.6, $^3J_{3'a\text{-}2''a}$ = 5.3, $^3J_{3'a\text{-}P}$ = 8.4 Hz, 1 H, $H_{3'a}$), 4.90 (dddd, $^3J_{5'b\text{-}4'b}$ = 2.3, $^3J_{5'b\text{-}6'b}$ = 2.6, $^3J_{5'b\text{-}6'b}$ = 11.6, $^3J_{5'b\text{-}P}$ = 3.1 Hz, 1 H, $H_{5'b}$), 4.66 (dddd, $^3J_{7'b\text{-}6''b}$ = 2.6 Hz, $^3J_{7'b\text{-}7''b}$ = 11.7 Hz, $^3J_{7'b\text{-}6'b}$ = 12.4, $^3J_{7'b\text{-}P}$ = 4.1 Hz, 1 H, $H_{7'b}$), 4.45 (dddt, $^3J_{7''b\text{-}6'b}$ = 1.9, $^3J_{7''b\text{-}6'b}$ = 5.0, $^3J_{7''b\text{-}7'b}$ = 11.7 Hz, $^3J_{7''b\text{-}P}$ = 24.6 Hz, 1 H, $H_{7'b}$), 4.40 (dt, $^3J_{3'b\text{-}4'b}$ = 2.2, $^3J_{3'b\text{-}2'b}$ = 2.4, $^3J_{3'b\text{-}2''b}$ = 5.9 Hz, 1 H, $H_{3'b}$), 4.24 (td, $^3J_{4'a\text{-}3'a}$ = 1.4, $^3J_{4'a\text{-}5'a}$ = 2.9, $^3J_{4'a\text{-}5''a}$ = 2.9 Hz, 1 H, $H_{4'a}$), 4.00 (ddd, $^3J_{4'b\text{-}3'b}$ = 2.3, $^3J_{4'b\text{-}5'b}$ = 2.6, $^3J_{4'b\text{-}P}$ = 4.4 Hz, 1 H, $H_{4'b}$), 3.83 and 3.77 (AB part of an ABX syst, $^3J_{5'a\text{-}4'a}$ = 2.9, $^3J_{5''a\text{-}4'a}$ = 2.9, $^3J_{5'a\text{-}5''a}$ = 12.0 Hz, 2 H, $H_{5'a}$, $H_{5''a}$), 2.39 (ddd, $^3J_{2'a\text{-}3'a}$ = 1.2, $^3J_{2'a\text{-}1'a}$ = 5.5, $^3J_{2'a\text{-}2''a}$ = 14.1 Hz, 1 H, $H_{2'a}$), 2.36 (dddd, $^3J_{6'b\text{-}7''b}$ = 5.0, $^3J_{6'b\text{-}5'b}$ = 11.4, $^3J_{6'b\text{-}7'b}$ = 12.6, $^3J_{6'b\text{-}6''b}$ = 14.5 Hz, 1 H, $H_{6'b}$), 2.44 (ddd, $^3J_{2''a\text{-}3'a}$ = 5.5, $^3J_{2''a\text{-}1'a}$ = 8.9, $^3J_{2''a\text{-}2'a}$ = 14.1 Hz, 1 H, $H_{2''a}$), 2.26 (ddd, $^3J_{2'b\text{-}3'b}$ = 2.4, $^3J_{2'b\text{-}1'b}$ = 6.0, $^3J_{2'b\text{-}2''b}$ = 13.8 Hz, 1 H, $H_{2'b}$), 2.12 (ddd, $^3J_{2''b\text{-}3'b}$ = 6.0, $^3J_{2''b\text{-}1'b}$ = 8.3, $^3J_{2''b\text{-}2'b}$ = 13.8 Hz, 1 H, $H_{2''b}$), 1.94 (d, $^4J_{7\text{-}6}$ = 1.0 Hz, 3 H, H_{7b}), 1.90 (dddd, $^3J_{6''b\text{-}7''b}$ = 1.9, $^3J_{6''b\text{-}7'b}$ = 2.6, $^3J_{6''b\text{-}5'b}$ = 2.6, $^3J_{6''b\text{-}6'b}$ = 14.4 Hz, 1 H, $H_{6''b}$), 1.87 (d, $^4J_{7\text{-}6}$ = 1.1 Hz, 3 H, H_{7a}) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 166.51 (C_{4b}), 166.48 (C_{4a}), 152.5 (C_{2b}), 152.2 (C_{2a}), 137.8 (C_{6a}), 137.4 (C_{6b}), 112.3 (C_{5b}), 111.9 (C_{5a}), 88.8 ($^3J_{4'b\text{-}P}$ = 8.7 Hz, $C_{4'b}$), 87.3 ($^3J_{4'a\text{-}P}$ = 7.4 Hz, $C_{4'a}$), 86.8 ($C_{1'b}$), 86.1 ($C_{1'a}$), 81.7 ($^2J_{3'a\text{-}P}$ = 2.9 Hz, $C_{3'a}$), 80.2 ($^2J_{5'b\text{-}P}$ = 5.0 Hz, $C_{5'b}$), 73.3 ($C_{3'b}$), 68.4 ($^2J_{7'b\text{-}P}$ = 4.8 Hz, $C_{7'}$), 63.0 ($C_{5'a}$), 41.2 ($C_{2'b}$), 40.3 ($^3J_{2'a\text{-}P}$ = 4.4, $C_{2'a}$), 29.6 ($^3J_{6'b\text{-}P}$ = 5.5 Hz, $C_{6'b}$), 13.1 (C_{7b}), 12.6 (C_{7a}) ppm. ^{31}P NMR (202 MHz, CD_3OD): δ = 66.9 ppm. HRMS (ESI): calcd for $C_{22}H_{30}N_4O_{11}PS$ 589.1369 $[M+H]^+$; found 589.1379.

α,β-seleno-D-CNA (S_C,S_P) (11b): Following the general procedure D starting from **9b** gave the product **11b** (46%, 11 mg). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.83 (d, $^4J_{6\text{-}7}$ = 1.3 Hz, 1 H, H_{6a}), 7.65 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6b}), 6.44 (dd, $^3J_{1'b\text{-}2'b}$ = 6.3, $^3J_{1'b\text{-}2''b}$ = 8.2 Hz, 1 H, $H_{1'b}$), 6.37 (dd, $^3J_{1'a\text{-}2'a}$ = 5.7, $^3J_{1'a\text{-}2''a}$ = 8.8 Hz, 1 H, $H_{1'a}$), 5.24 (ddt, $^3J_{3'a\text{-}4'a}$ = 1.6, $^3J_{3'a\text{-}2'a}$ = 1.6, $^3J_{3'a\text{-}2''a}$ = 5.8, $^3J_{3'a\text{-}P}$ = 9.9 Hz, 1 H, $H_{3'a}$), 4.86 (dddd, $^3J_{5'b\text{-}4'b}$ = 2.0, $^3J_{5'b\text{-}6''b}$ = 2.0, $^3J_{5'b\text{-}6'b}$ = 11.8, $^3J_{5'b\text{-}P}$ = 1.4 Hz, 1 H, $H_{5'b}$), 4.65 (ddt, $^3J_{7'b\text{-}6''b}$ = 2.6, $^3J_{7'b\text{-}7''b}$ = 11.3, $^3J_{7'b\text{-}6'b}$ = 12.7, $^3J_{7'b\text{-}P}$ = 2.0 Hz, 1 H, $H_{7'b}$), 4.48 (dt, $^3J_{3'b\text{-}2'b}$ = 2.7, $^3J_{3'b\text{-}4'b}$ = 2.7, $^3J_{3'b\text{-}2''b}$ = 5.6 Hz, 1 H, $H_{3'b}$), 4.43 (dddd, $^3J_{7''b\text{-}6'b}$ = 1.7, $^3J_{7''b\text{-}6'b}$ = 4.7 $^3J_{7''b\text{-}7'b}$ = 11.1, $^3J_{7''b\text{-}P}$ = 24.3 Hz, 1 H, $H_{7''b}$), 4.33 (td, $^3J_{4'a\text{-}3'a}$ = 1.8, $^3J_{4'a\text{-}5'a}$ = 3.1, $^3J_{4'a\text{-}5''a}$ = 3.1 Hz, 1 H, $H_{4'a}$), 3.93 (ddd, $^3J_{4'b\text{-}5'b}$ = 1.8, $^3J_{4'a\text{-}3'b}$ = 2.4, $^3J_{4'b\text{-}P}$ = 4.5 Hz, 1 H, $H_{4'b}$), 3.83 (ABX, $^3J_{5'a\text{-}4'a}$ = 3.2, $^3J_{5''a\text{-}4'a}$ = 3.2, $^3J_{5'a\text{-}5''a}$ = 12.1 Hz, 2 H, $H_{5'a}$, $H_{5''a}$), 2.60 (ddd, $^3J_{2'a\text{-}3'a}$ = 1.7, $^3J_{2'a\text{-}1'a}$ = 5.7, $^3J_{2'a\text{-}2''a}$ = 14.1 Hz, 1 H, $H_{2'a}$), 2.44 (dddd, $^3J_{6'b\text{-}7''b}$ = 4.8 Hz, $^3J_{6'b\text{-}5'b}$= 11.8, $^3J_{6'b\text{-}7'b}$= 12.6, $^3J_{6'b\text{-}6''b}$ = 14.7 Hz, 1 H, $H_{6'b}$), 2.43 (ddd, $^3J_{2''a\text{-}3'a}$ = 5.6, $^3J_{2''a\text{-}1'a}$ = 8.7, $^3J_{2''a\text{-}2'a}$ = 14.1 Hz, 1 H, $H_{2''a}$), 2.22 (ddd, $^3J_{2'b\text{-}3'b}$ = 2.9, $^3J_{2'b\text{-}1'b}$ = 6.0, $^3J_{2'b\text{-}2''b}$ = 13.5 Hz, 1 H, $H_{2'b}$), 2.17 (ddd, $^3J_{2''b\text{-}3'b}$ = 5.6, $^3J_{2''b\text{-}1'b}$ = 8.0, $^3J_{2'b\text{-}2''b}$ = 13.6, 1 H, $H_{2''b}$), 1.99 (d, $^4J_{7\text{-}6}$ = 1.2 Hz, 3 H, H_{7b}), 1.95 (dddd, $^3J_{6''b\text{-}7''b}$ = 1.7, $^3J_{6''b\text{-}7'b}$ = 1.9, $^3J_{6''b\text{-}5'b}$ = 1.9, $^3J_{6''b\text{-}6'b}$ = 14.8, $^3J_{6''b\text{-}P}$ = 0.6 Hz, 1 H, $H_{6''b}$), 1.89 (d, $^4J_{7\text{-}6}$ = 1.2 Hz, 3 H, H_{7a}) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 166.5 (C_4), 166.4 (C_4), 152.60 (C_2), 152.56 (C_2), 137.9 (C_{6a}), 137.3 (C_{6b}), 112.7 (C_{5b}) 112.1 (C_{5a}), 88.7 ($^3J_{4'b\text{-}P}$ = 7.5 Hz, $C_{4'b}$), 87.3 ($^3J_{4'a\text{-}P}$ = 5.3 Hz, $C_{4'a}$), 86.4 ($C_{1'a}$), 86.0 ($C_{1'b}$), 81.5 ($^2J_{3'a\text{-}P}$ = 4.0 Hz, $C_{3'a}$), 80.7 ($^2J_{5'b\text{-}P}$ = 9.8 Hz, $C_{5'b}$), 73.1 ($C_{3'b}$), 69.2 ($^2J_{7'b\text{-}P}$ = 9.8 Hz, $C_{7'}$), 63.0 ($C_{5'a}$), 40.7 ($C_{2'b}$), 39.4 ($^3J_{2'a\text{-}P}$ = 4.8 Hz, $C_{2'a}$), 29.2 ($^3J_{6'b\text{-}P}$ = 6.3 Hz, $C_{6'b}$), 13.0 (C_{7b}), 12.6 (C_{7a}) ppm. ^{31}P NMR (202 MHz, CD_3OD): δ = 63.7 ($J_{P\text{-}Se}$ = 1006.2 Hz) ppm. MS (ESI) *m/z* = 637.1 $[M+H]^+$, 659.1 $[M+Na]^+$. HRMS (ESI): calcd for $C_{22}H_{30}N_4O_{11}PSe$ 636.0856 $[M+H]^+$; found 636.0862.

α,β-seleno-D-CNA (S_C,R_P) (12b): Following the general procedure D starting from **10b** gave the product **12b** (29%, 10 mg) which underwent degradation during purification. ^{1}H NMR (500 MHz, CD_3OD): δ = 7.81 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6a}), 7.63 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6b}), 6.38 (dd, $^3J_{1'b\text{-}2'b}$ = 5.9, $^3J_{1'b\text{-}2''b}$ = 8.4 Hz, 1 H, $H_{1'b}$), 6.27 (dd, $^3J_{1'a\text{-}2'a}$ = 5.5, $^3J_{1'a\text{-}2''a}$ = 9.1 Hz, 1 H, $H_{1'a}$), 5.54 (dddd, $^3J_{3'a\text{-}4'a}$ = 1.3, $^3J_{3'a\text{-}2'a}$ = 1.3, $^3J_{3'a\text{-}2''a}$ = 5.2, $^3J_{3'a\text{-}P}$ = 9.0 Hz, 1 H, $H_{3'a}$), 4.94 (dddd, $^3J_{5'b\text{-}4'b}$ = 2.3, $^3J_{5'b\text{-}6''b}$ = 2.3, $^3J_{5'b\text{-}6'b}$ = 11.6, $^3J_{5'b\text{-}P}$ = 2.5 Hz, 1 H, $H_{5'b}$), 4.69 (dddd, $^3J_{7'b\text{-}6''b}$ = 2.5, $^3J_{7'b\text{-}7''b}$ = 11.5, $^3J_{7'b\text{-}6'b}$ = 12.6, $^3J_{7'b\text{-}P}$ = 4.0 Hz, 1 H, $H_{7'b}$), 4.41 (dddd, $^3J_{7''b\text{-}6'b}$ = 1.6, $^3J_{7''b\text{-}6'b}$ = 5.0, $^3J_{7''b\text{-}7'b}$ = 11.5, $^3J_{7''b\text{-}P}$ > 25 Hz, 1 H, $H_{7'b}$), 4.38 (dddd, $^3J_{3'b\text{-}2'b}$ = 2.2, $^3J_{3'b\text{-}4'b}$ = 2.6, $^3J_{3'b\text{-}2''b}$ = 5.9 Hz, 1 H, $H_{3'b}$), 4.26 (td, $^3J_{4'a\text{-}3'a}$ = 1.3, $^3J_{4'a\text{-}5'a}$ = 2.9, $^3J_{4'a\text{-}}$

$_{5''a}$ = 2.9 Hz, 1 H, $H_{4'a}$), 4.00 (ddd, $^3J_{4'b-5'b}$ = 2.2, $^3J_{4'a-3'b}$ = 2.6, $^3J_{4'b-P}$ = 4.5 Hz, 1 H, $H_{4'b}$), 3.82 (ABX syst, $^3J_{5'a-4'a}$ = 2.9, $^3J_{5''a-4'a}$ = 2.9, $^3J_{5'a-5''a}$ = 12.0 Hz, 2 H, $H_{5'a}$, $H_{5''a}$), 2.45 (m, $^3J_{2'a-3'a}$ = 1.2, $^3J_{2'a-2''a}$ = 14.3, $^3J_{2'a-1'a}$ = 5.4 Hz, 1 H, $H_{2'a}$), 2.42 (dddd, $^3J_{6'b-7''b}$ = 5.0, $^3J_{6'b-5'b}$ = 11.5, $^3J_{6'b-7'b}$ = 12.8, $^3J_{6'b-6''b}$ = 15.8 Hz, 1 H, $H_{6'b}$), 2.38 (ddd, $^3J_{2''a-3'a}$ = 5.5, $^3J_{2''a-1'a}$ = 9.1, $^3J_{2''a-2'a}$ = 14.3 Hz, 1 H, $H_{2''a}$), 2.26 (ddd, $^3J_{2'b-3'b}$ = 2.2, $^3J_{2'b-1'b}$ = 6.0, $^3J_{2'b-2''b}$ = 13.8 Hz, 1 H, $H_{2'b}$), 2.11 (ddd, $^3J_{2''b-3'b}$ = 6.0, $^3J_{2''b-1'b}$ = 8.4, $^3J_{2''b-2'b}$ = 13.9 Hz, 1 H, $H_{2''b}$), 1.95 (d, $^4J_{7-6}$ = 1.1 Hz, 3 H, H_{7b}), 1.95 (dddd, $^3J_{6''b-7''b}$ = 1.6, $^3J_{6''b-7'b}$ = 2.4, $^3J_{6''b-5'b}$ = 2.4, $^3J_{6''b-6'b}$ = 15.8 Hz, 1 H, $H_{6''b}$), 1.87 (d, $^4J_{7-6}$ = 1.2 Hz, 3 H, H_{7a}) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 166.5 (C_4), 166.4 (C_4), 152.5 (C_{2b}), 152.2 (C_{2a}), 137.7 (C_{6a}), 137.3 (C_{6b}), 112.3 (C_{5b}), 111.9 (C_{5a}), 88.7 ($^3J_{4'b-P}$ = 9.1 Hz, $C_{4'b}$), 87.2 ($^3J_{4'a-P}$ = 7.4 Hz, $C_{4'a}$), 86.8 ($C_{1'b}$), 86.1 ($C_{1'a}$), 83.5 ($C_{3'a}$), 80.3 ($^2J_{5'b-P}$ = 5.1 Hz, $C_{5'b}$), 73.4 ($C_{3'b}$), 68.5 ($^2J_{7'b-P}$ = 4.6 Hz, $C_{7'}$), 63.0 ($C_{5'a}$), 41.2 ($C_{2'b}$), 40.3 ($^3J_{2'a-P}$ = 4.3 Hz, $C_{2'a}$), 29.7 ($^3J_{6'b-P}$ = 5.5 Hz, $C_{6'b}$), 13.1 (C_{7b}), 12.6 (C_{7a}) ppm. ^{31}P NMR (202 MHz, CD_3OD): δ = 70.7 ($J_{P\text{-}Se}$ = 954 Hz) ppm. MS (ESI) *m/z* (%) = 637.08 $[M+H]^+$. HRMS (ESI): calcd for $C_{22}H_{30}N_4O_{11}PSe$ 636.0856 $[M+H]^+$; found 636.0873.

5'-*O*-Dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-α,β-thio-D-CNA (R_C,R_P) (13a) and 5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-α,β-thio-D-CNA (R_C,S_P) (14a): Following the general procedure A starting from **5** gave the product **13a** (42%, 457 mg) and **14a** (9%, 94 mg). Data for **13a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.95 (s, 1 H, NH), 8.87 (s, 1 H, NH), 7.68-7.62 (m, 4 H, Ph), 7.50 (bd, $^4J_{6-7}$ = 0.9 Hz, 1 H, H_6), 7.43-7.22 (m, 16 H, Ph and H_6), 6.82 (bd, 4 H, Ph), 6.37 (dd, ^3J = 6.3, 8.1 Hz, 1 H, $H_{1'}$), 6.34 (dd, ^3J = 5.4, 9.0 Hz, 1 H, $H_{1'}$), 5.50 (dd, ^3J = 5.1, 9.3 Hz, 1 H, $H_{3'a}$), 4.58 (m, 1 H, $H_{3'b}$), 4.52 (ddd, ^3J = 2.1, 4.2, 12.3 Hz, 1 H, $H_{5'b}$), 4.24 (m, 1 H, $H_{7'b}$), 4.16 (bq, ^3J = 3.0 Hz, 1 H, $H_{4'a}$), 4.09-3.97 (m, 1 H, $H_{7'b}$), 3.94 (ddd, ^3J = 1.5, 4.2, 9.0 Hz, 1 H, $H_{4'b}$), 3.41 and 3.35 (AB part of an ABX syst, J = 2.4, 2.7, 10.8 Hz, 2 H, $H_{5'a}$), 2.38 (A part of an ABX syst, ^3J = 5.1, 13.8 Hz, 1 H, $H_{2'}$), 2.30-2.10 (m, 3 H, $H_{2'}$), 1.86 (d, $^4J_{7-6}$ = 0.9 Hz, 3 H, H_7), 1.42 (m, 4 H, H_7 and $H_{6'b}$), 1.20 (m, 1 H, $H_{6'b}$), 1.06 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.1, 158.8, 150.9, 144.1, 136.0, 135.8, 135.2, 135.0, 134.9, 132.9, 132.8, 130.2, 130.0, 128.1, 128.0, 127.3, 113.4, 112.1, 111.5, 88.2, 87.4, 85.6, 84.7, 84.5, 79.7, 79.2, 72.8, 67.7, 63.5, 55.3, 39.6, 38.7, 26.9, 19.1, 12.6, 11.8 ppm. ^{31}P NMR (120 MHz, $CDCl_3$): δ = 59.9 ppm. HRMS (ESI): calcd for $C_{59}H_{65}N_4O_{13}PNaSSi$ 1151.3680 $[M+Na]^+$; found 1151.3698. Data for **14a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.18 (s, 1 H, NH), 9.04 (s, 1 H, NH), 7.67-7.58 (m, 6 H, Ph and H_6), 7.43-7.19 (m, 15 H, Ph and H_6), 6.81 (bd, 4 H, Ph), 6.42 (t, ^3J = 4.7 Hz, 1 H, $H_{1'}$), 6.39 (t, ^3J = 4.8 Hz, 1 H, $H_{1'}$), 5.50 (dd, ^3J = 5.7, 9.9 Hz, 1 H, $H_{3'a}$), 4.60 (qd, ^3J = 2.1, 5.7, 11.7 Hz, 1 H, $H_{5'b}$), 4.47 (d, ^3J = 5.4 Hz, 1 H, $H_{3'b}$), 4.29 (m, ^3J = 3.0, 11.4, $J_{7'b-P}$ = 7.5 Hz, 1 H, $H_{7'b}$), 4.03-3.95 (m, 1 H, $H_{7'b}$), 4.03 (bs, 1 H, $H_{4'a}$), 3.90 (m, ^3J = 0.8, 2.4, $^3J_{4'b-P}$ = 4.5 Hz, 1 H, $H_{4'b}$), 3.76 (s, 6H, OMe), 3.33 (bs, 2 H, $H_{5'a}$), 2.51 (A part of an ABX syst, ^3J = 5.4, 14.1 Hz, 1 H, $H_{2'}$), 2.41-2.29 (m, 2 H, $H_{2'}$), 1.92-1.81 (m, 2 H, $H_{2'}$ and $H_{6'b}$), 1.71 (d, $^4J_{7-6}$ = 1.2 Hz, 3 H, H_7), 1.40 (d, $^4J_{7-6}$ = 1.2 Hz, 3 H, H_7), 1.18 (m, 1 H, $H_{6'}$), 1.05 (s, 9 H, *t*Bu) ppm.^{13}C NMR (75 MHz, $CDCl_3$): δ = 158.8, 150.7, 150.5, 144.1, 135.9, 135.8, 135.1, 134.9, 130.3, 130.0, 129.2, 128.1, 128.0, 127.8, 111.9, 111, 5, 88.3, 87.4, 86.1, 85.7, 85.1, 84.5, 80.7, 79.1, 72.9, 66.8, 63.5, 55.3, 40.5, 39.3, 26.9, 19.1, 12.5, 11.7 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 65.2 ppm. HRMS (ESI): calcd for $C_{59}H_{65}N_4O_{13}PNaSSi$ 1151.3680 $[M+Na]^+$; found 1151.3673.

5'-*O*-Dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-α,β-seleno-D-CNA (R_C,R_P) (13b) and 5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-α,β-seleno-D-CNA (R_C,S_P) (14b): Following the general procedure B starting from **5** gave the product **13b** (52%, 813 mg) and **14b** (5%, 79 mg) after a second purification by direct phase HPLC using a Sunfire C_{18} column (5μ, 30 mm × 150 mm) eluting with a mixture of EtOAc/petroleum ether (6:4). **14b** is unstable and was converted partially to its α,β-oxo-D-CNA counterpart (3%, 38 mg). Data for **13b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.23 (s, 1 H, NH), 9.10 (s, 1 H, NH), 7.68-7.62 (m, 5 H, Ph), 7.51 (d, $^4J_{6-7}$ = 1.2 Hz, 1 H, H_6), 7.44-7.23 (m, 16 H, H_6, Ph), 6.83-6.80 (m, 4 H, Ph), 6.39 (dd, $^3J_{1'b-2'b}$ = 6.5, $^3J_{1'b-2''b}$ = 8.1 Hz, 1 H, $H_{1'b}$), 6.35 (dd, $^3J_{1'a-2'a}$ = 5.4, $^3J_{1'a-2''a}$ = 9.1 Hz, 1 H, $H_{1'a}$), 5.46 (dddd, $^3J_{3'a-2'a}$ = 1.4, $^3J_{3'a-4'a}$ = 1.6, $^3J_{3'a-2''a}$ = 5.2, $^3J_{3'a-P}$ = 10.1 Hz, 1 H, $H_{3'a}$), 4.58 (m, 2 H, $H_{5'b}$, $H_{3'b}$), 4.31 (dddd, $^3J_{7'b-6''b}$ = 1.9, $^3J_{7'b-7''b}$ = 10.9, $^3J_{7'b-6'b}$ = 12.8, $^3J_{7'b-P}$ = 2.6 Hz, 1 H, $H_{7'b}$), 4.18 (td, $^3J_{4'a-3'a}$ = 1.5, $^3J_{4'a-5'a}$ = 2.5, $^3J_{4'a-5''a}$ = 2.5 Hz, 1 H, $H_{4'a}$), 4.05-3.98 (dddd, $^3J_{7''b-6''b}$ = 1.9, $^3J_{7''b-6'b}$ = 4.4, $^3J_{7''b-7'b}$ = 10.9, $^3J_{7''b-P}$ > 20 Hz, 1 H, $H_{7''b}$), 3.90 (ddd, $^3J_{4'b-3'b}$ = 1.6, $^3J_{4'b-5'b}$ = 4.2, $^3J_{4'b-P}$ = 3.0 Hz, 1 H, $H_{4'b}$), 3.77 (s, 6 H, OMe), 3.44 (A of an ABX syst, $^3J_{5'a-4'a}$ = 2.5, $^3J_{5'a-5''a}$ = 10.8 Hz, 1 H $H_{5'a}$), 3.35 (B of an ABX syst, $^3J_{5''a-4'a}$ = 2.5, $^3J_{5''a-5'a}$ = 10.8 Hz, 1 H, $H_{5''a}$), 2.39 (dd app, $^3J_{2'a-3'a}$ = 1.4, $^3J_{2'a-1'a}$ = 5.4, $^3J_{2'a-2''a}$ = 13.8 Hz, 1 H, $H_{2'a}$), 2.28 (ddd, $^3J_{2''a-3'a}$ = 5.4, $^3J_{2''a-1'a}$ = 9.2, $^3J_{2''a-2'a}$ = 13.9 Hz, 1 H, $H_{2''a}$), 2.20 (ddd, $^3J_{2'b-3'b}$ = 2.3, $^3J_{2'b-1'b}$ = 6.6, $^3J_{2'b-2''b}$ = 14.0 Hz, 1 H, $H_{2'b}$), 2.16 (ddd, $^3J_{2''b-3'b}$ = 5.2, $^3J_{2''b-1'b}$ = 8.0, $^3J_{2''b-2'b}$ = 13.6 Hz, 1 H, $H_{2''b}$), 1.87 (d, $^4J_{7-6}$ = 0.9 Hz, 3 H, H_7), 1.53-1.41 (m, 2 H, 2 × $H_{6'b}$), 1.41 (d, $^4J_{7-6}$ = 1.1 Hz, 3 H, H_7), 1.06 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.0 (C_4), 164.9 (C_4), 158.9 (C_{Ph}), 150.9 (C_2), 150.8 (C_2), 144.2 (C_{Ph}), 136.1, 136.0 (C_{Ph}), 135.3 135.1, 135.0 (C_{Ph}, C_{6a}, C_{6b}), 133.0, 132.9, 130.32, 130.30, 130.2, 128.3, 128.2, 128.4, 127.4, 113.6 (C_{Ph}), 112.2 (C_5), 111.6 (C_5), 88.3 ($^3J_{4'b-P}$ = 7.8 Hz, $C_{4'b}$), 87.5 (C_{Ph}), 85.8 ($C_{1'b}$), 84.7 ($^3J_{4'a-P}$ = 5.8 Hz, $C_{4'a}$), 84.5 ($C_{1'a}$), 80.8 ($^2J_{3'a-P}$ = 2.4 Hz, $C_{3'a}$), 79.0 ($^2J_{5'b-P}$ = 9.7 Hz, $C_{5'b}$), 72.9 ($C_{3'b}$), 67.5 ($^2J_{7'b-P}$ = 10.5 Hz, $C_{7'}$), 63.6 ($C_{5'a}$), 55.4 (OMe), 39.6 ($C_{2'b}$), 38.7 ($^3J_{2'a-P}$ = 3.2 Hz, $C_{2'a}$), 27.07 ($C\underline{Me}_3$), 27.01 ($C_{6'b}$), 19.3 ($\underline{C}Me_3$) 12.7 (C_{7b}), 11.9 (C_{7a}) ppm. ^{31}P NMR (120 MHz, $CDCl_3$): δ = 64.5 ($J_{P\text{-}Se}$ = 1015.3 Hz) ppm. HRMS (ESI): calcd for $C_{59}H_{65}N_4O_{13}PNaSeSi$ 1198.3164 $[M+Na]^+$; found 1198.3181. Data for **14b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.49 (s, 1 H, NH), 8.39 (s, 1 H, NH), 7.65-7.61 (m, 5 H, Ph), 7.58 (d, $^4J_{6-7}$ = 0.9 Hz, 1 H H_6), 7.41-7.17 (m, 19 H, H_6, Ph), 6.83-6.80 (m, 4 H, Ph), 6.40 (m, 2 H, $H_{1'a}$, $H_{1'b}$), 6.41 (dd, $^3J_{1'a-2'a}$ = 5.4, $^3J_{1'a-2''a}$ = 8.4 Hz, 1 H, $H_{1'a}$), 6.39 (dd, $^3J_{1'b-2'b}$ = 5.3, $^3J_{1'b-2''b}$ = 8.6 Hz, 1 H, $H_{1'b}$), 5.64 (ddd, ^3J = 1.4, 2.5, 5.0, $^3J_{3'a-P}$ = 10.6 Hz, 1 H, $H_{3'a}$), 4.65 (dq, ^3J = 2.7, 2.7, 11.4, $^2J_{5'b-P}$ = 2.7 Hz, 1 H, $H_{5'b}$), 4.47 (dd app, ^3J = 1.2, 1.5 Hz, 4.9 Hz, 1 H, $H_{3'b}$), 4.32 (dddd, $^3J_{7'b-6''b}$ = 3.2, $^3J_{7'b-P}$ = 5.2, $^3J_{7'b-7''b}$ = 10.9, $^3J_{7'b-6'b}$ = 12.4 Hz, 1 H, $H_{7'b}$), 4.05 (br dd, $^3J_{4'a-5'a}$ = 1.7, $^3J_{4'a-5''a}$ = 1.7, $^3J_{4'a-3'a}$ = 2.4 Hz, 1 H, $H_{4'a}$), 4.02-3.92 (m, 1 H, $H_{7''b}$), 3.90 (ddd, $^3J_{4'a-3'b}$ = 1.5, $^3J_{4'b-5'b}$ = 2.9, $^3J_{4'b-P}$ = 4.0 Hz, 1 H, $H_{4'b}$), 3.76 (s, 6 H, OMe), 3.35 (ABX syst, $^3J_{5'a-4'a}$ = 2.1, $^3J_{5''a-4'a}$ = 2.1, $^3J_{5'a-5''a}$ = 10.6 Hz, 2 H, $H_{5'a}$, $H_{5''a}$), 2.52 (dd app, $^3J_{2'a-3'a}$ = 1.3, $^3J_{2'a-1'a}$ = 5.4, $^3J_{2'a-2''a}$ = 13.9 Hz, 1 H, $H_{2'a}$), 2.40 (ddd, $^3J_{2''a-3'a}$ = 5.3, $^3J_{2''a-1'a}$ = 8.4, $^3J_{2''a-2'a}$ = 14.0 Hz, 1 H, $H_{2''a}$), 2.33 (ddd, $^3J_{2'b-3'b}$ = 1.3, $^3J_{2'b-1'b}$ = 5.3, $^3J_{2'b-2''b}$ = 13.1 Hz, 1 H, $H_{2'b}$) 1.85 (ddd, $^3J_{2''b-3'b}$ = 4.4, $^3J_{2''b-1'b}$ = 8.6, $^3J_{2''b-2'b}$ = 13.1 Hz, 1 H, $H_{2''b}$), 1.39 (d, $^4J_{7-6}$ = 0.9 Hz, 3 H, H_7), 1.18-1.10 (m, 2 H, 2 × $H_{6'b}$), 1.44 (d, $^4J_{7-6}$ = 0.9 Hz, 3 H, H_7), 1.04 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.59 (C_4), 166.57 (C_4), 159.03, 159.02 (C_{Ph}), 150.4 (C_2), 152.3 (C_2), 144.2 (C_{Ph}), 136.1, 136.0 (C_{Ph}), 135.5 (C_6), 135.4 (C_6), 135.2, 135.1, 133.1, 132.8, 130.5, 130.2, 128.34, 128.3, 128.2, 127.5, 113.7 (C_{Ph}), 112.0 (C_5), 111.6 (C_5), 88.3 ($^3J_{4'b-P}$ = 8.8 Hz, $C_{4'b}$), 85.9 ($C_{1'b}$), 85.3 ($^3J_{4'a-P}$ = 6.2 Hz, $C_{4'a}$), 84.6 ($C_{1'a}$), 81.7 ($^2J_{3'a-P}$ = 1.2 Hz, $C_{3'a}$), 79.2 ($^2J_{5'b-P}$ = 4.7 Hz, $C_{5'b}$), 73.0 ($C_{3'b}$), 66.9 ($^2J_{7'b-P}$ = 5.6 Hz, $C_{7'}$), 63.7 ($C_{5'a}$), 55.5 (OMe), 40.7 ($C_{2'b}$), 39.4 ($^3J_{2'a-P}$ = 4.7 Hz, $C_{2'a}$), 27.1 ($C\underline{Me}_3$, $C_{6'b}$), 19.3 ($\underline{C}Me_3$) 12.7 (C_{7b}), 11.8 (C_{7a}) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 69.6 ($J_{P\text{-}Se}$ = 972.2 Hz) ppm. HRMS (ESI): calcd for $C_{59}H_{65}N_4O_{13}PNaSeSi$, 1198.3164 $[M+Na]^+$; found 1198.3172.

5'-*O*-Dimethoxytrityl-α,β-thio-D-CNA (R_C,R_P) (15a): Following the general procedure C starting from **13a** gave the product **15a** (61%, 93 mg). ^{1}H NMR (300 MHz, MeOD): δ = 7.63 (d, J = 1.2 Hz, 1 H, H_{6a}), 7.58

(d, J = 1.2 Hz, 1 H, H_{6b}), 7.44-7.27 (m, 9 H, Ph), 6.87 (d, ^3J = 9.0 Hz, 4 H, Ph), 6.38-6.31 (m, 2 H, $H_{1'b}$, $H_{1'a}$), 5.48 (m, ^3J = 2.7, 2.7, 6.2, $^3J_{3'a\text{-}P}$ = 8.7 Hz, 1 H, $H_{3'a}$), 4.89 (m, 1 H, $H_{5'b}$), 4.60 (ddd, ^3J = 3.1, 4.0, 5.9 Hz, 1 H, $H_{3'b}$), 4.49 (ddd, $^3J_{7'b\text{-}6''b}$ = 2.2, ^3J = 12.1, 13.3 Hz, 1 H, $H_{7'b}$), 4.42-4.34 (m, 1 H, $H_{7''b}$), 4.30 (dd, ^3J = 2.9, 5.5 Hz, 1 H, $H_{4'a}$), 3.96 (ddd, ^3J = 3.2, 3.2, $^3J_{4'b\text{-}P}$ = 3.4 Hz, 1 H, $H_{4'b}$), 3.78 (s, 6 H, OMe), 3.45 (br. t, ^3J = 2.4 Hz, 2 H, 2 × $H_{5'a}$), 2.23 (ddd, $^3J_{2'a\text{-}3'a}$ = 2.6, $^3J_{2'a\text{-}1'a}$ = 6.0, $^3J_{2'a\text{-}2''a}$ = 14.2 Hz, 1 H, $H_{2'a}$), 2.06 (ddd, $^3J_{2''a\text{-}3'a}$ = 6.2, $^3J_{2''a\text{-}1'a}$ = 8.0, $^3J_{2''a\text{-}2'a}$ = 14.3 Hz, 1 H, $H_{2''a}$), 2.32-2.19 (m, 3 H, 2 × $H_{2'b}$, $H_{6'b}$), 1.90 (d, $^4J_{7\text{-}6}$ = 1.1 Hz, 3 H, H_{7b}), 1.88 (m, 1 H, $H_{6''b}$), 1.41 (d, $^4J_{7\text{-}6}$ = 1.1 Hz, 3 H, H_{7a}) ppm. ^{13}C NMR (75 MHz, MeOD): δ = 166.3 (C_{4a}), 166.2 (C_{4b}), 160.5 (C_{Ph}), 152.5 (C_{2b}), 152.3 (C_{2a}), 145.9 (C_{Ph}), 137.7 (C_{6b}), 137.3 (C_{6a}), 136.8, 136.7 (C_{Ph}), 131.5, 129.5, 129.2, 128.4, (CH_{Ph}), 114.5 (CH_{Ph}), 112.22 (C_5), 112.19 (C_5), 88.9 ($^3J_{4'b\text{-}P}$ = 8.3 Hz, $C_{4'b}$), 88.6 (C_{Ph}), 86.0 ($C_{1'b}$, $C_{4'a}$, $C_{1'a}$), 81.0 ($^2J_{5'b\text{-}P}$ = 8.7 Hz, $C_{5'b}$), 80.1 ($^2J_{3'a\text{-}P}$ = 4.3 Hz, $C_{3'a}$), 71.0 ($C_{3'b}$), 69.6 ($^2J_{7'b\text{-}P}$ = 8.5 Hz, $C_{7'}$), 64.5 ($C_{5'a}$), 55.9 (OMe), 40.8 ($C_{2'b}$), 39.7 ($^3J_{2'a\text{-}P}$ = 4.0 Hz, $C_{2'a}$), 28.7 ($^3J_{6'b\text{-}P}$ = 7.1 Hz, $C_{6'b}$), 13.1 (C_{7b}), 12.3 (C_{7a}) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 60.7 ppm. MS (Malfi Tof) m/z = 913.5 $[M+Na]^+$.

5'-*O*-Dimethoxytrityl-α,β-thio-D-CNA (R_C,S_P) (16a): Following the general procedure C starting from **14a** gave the product **16a** (86%, 63 mg). ^{1}H NMR (300 MHz, MeOD): δ = 7.65 (d, J = 1.2 Hz, 1 H, H_{6a}), 7.49 (d, J = 1.2 Hz, 1 H, H_{6b}), 7.43-7.24 (m, 9 H, Ph), 6.88 (d, ^3J = 8.6 Hz, 4 H, Ph), 6.33 (dd, $^3J_{1'b\text{-}2'b}$ = 6.0, $^3J_{1'b\text{-}2'b}$ = 8.1 Hz, 1 H, $H_{1'b}$), 6.27 (dd, $^3J_{1'a\text{-}2'a}$ = 5.8, $^3J_{1'a\text{-}2''a}$ = 8.4 Hz, 1 H, $H_{1'a}$), 5.48 (m, 1 H, $H_{3'a}$), 4.91 (m, ^3J = 2.6, 2.9, 11.6 Hz, 1 H, $H_{5'b}$), 4.59 (m, $^3J_{7'b\text{-}6''b}$ = 3.9, $^3J_{7'b\text{-}7''b}$ = 11.5, $^3J_{7'b\text{-}6'b}$ = 11.5, $^3J_{7'b\text{-}P}$ = 5.1 Hz, 1 H, $H_{7'b}$), 4.47 (ddd, ^3J = 1.8, 2.1, 5.9 Hz, 1 H, $H_{3'b}$), 4.39-4.32 (m, $^3J_{7''b\text{-}6'b}$ = 2.1, $^3J_{7''b\text{-}6'b}$ = 4.7, $^3J_{7''b\text{-}7'b}$ = 11.2, $^3J_{7''b\text{-}P}$ = 23.7 Hz, 1 H, $H_{7''b}$), 4.25 (q, ^3J = 2.3 Hz, 1 H, $H_{4'a}$), 3.96 (dd, ^3J = 2.1, 3.2 Hz, 1 H, $H_{4'b}$), 3.78 (s, 6 H, OMe), 3.43 (br. t, ^3J = 2.6 Hz, 2 H, 2 × $H_{5'a}$), 2.23 (ddd, $^3J_{2'a\text{-}3'a}$ = 2.0, $^3J_{2'a\text{-}1'a}$ = 5.9, $^3J_{2'a\text{-}2''a}$ = 13.6 Hz, 1 H, $H_{2'a}$), 2.06 (ddd, $^3J_{2''a\text{-}3'a}$ = 6.0, $^3J_{2''a\text{-}1'a}$ = 8.4, $^3J_{2''a\text{-}2'a}$ = 13.6 Hz, 1 H, $H_{2''a}$), 2.15-1.90 (m, 4 H, 2 × $H_{2'b}$, 2 × $H_{6'b}$), 1.78 (d, $^4J_{7\text{-}6}$ = 1.1 Hz, 3 H, H_{7b}), 1.42 (d, $^4J_{7\text{-}6}$ = 1.1 Hz, 3 H, H_{7a}) ppm. ^{13}C NMR (75 MHz, MeOD): δ = 164.1 (C_4), 163.9 (C_4), 159.0 (C_{Ph}), 151.1 (C_2), 150.7 (C_2), 144.3 ($C_{q,Ph}$), 135.4 (C_6), 135.4 (C_6), 135.3, 135.2 ($C_{q,Ph}$), 130.22, 130.19, 128.3, 128.2, 127.5 (CH_{Ph}), 113.6 (CH_{Ph}), 112.3 (C_5), 111.7 (C_5), 87.6 (C_{Ph}), 87.5 ($C_{4'b}$), 85.3 ($C_{1'a}$), 87.4 ($C_{1'b}$), 87.4 ($C_{4'a}$), 80.9 ($C_{3'a}$), 79.5 ($C_{5'b}$), 71.3 ($C_{3'b}$), 66.9 ($C_{7'}$), 63.8 ($C_{5'a}$), 55.5 (OMe), 40.5 ($C_{2'b}$), 39.1 ($C_{2'a}$), 28.1 ($C_{6'}$), 14.4 (C_{7b}), 12.8 (C_{7a}) ppm. ^{31}P NMR (100 MHz, $CDCl_3$): δ = 62.8 ppm. MS (Malfi Tof) m/z = 913.4 $[M+Na]^+$, 929.4 $[M+K]^+$.

5'-*O*-Dimethoxytrityl-α,β-seleno-D-CNA (R_C,R_P) (15b): Following the general procedure C starting from **13b** gave the product **15b** (71%, 463 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.56 (d, $^4J_{6\text{-}7}$ = 0.9 Hz, 1 H, H_6), 7.39-7.20 (m, 10 H, H_6, Ph), 6.82 (d, ^3J = 8.9 Hz, 4 H, Ph), 6.42 (dd, $^3J_{1'b\text{-}2'b}$ = 5.3, $^3J_{1'b\text{-}2''b}$ = 9.1 Hz, 1 H, $H_{1'b}$), 6.14 (t, $^3J_{1'a\text{-}2'a}$ = 6.8, $^3J_{1'a\text{-}2''a}$ = 6.8 Hz, 1 H, $H_{1'a}$), 5.51 (ddd, ^3J = 0.8, 5.3, $^3J_{3'a\text{-}P}$ = 10.2 Hz, 1 H, $H_{3'a}$), 4.86 (ddd, $^3J_{5'b\text{-}6''b}$ = 1.6, $^3J_{5'b\text{-}4'b}$ = 4.9, $^3J_{5'b\text{-}6'b}$ = 11.6, $^3J_{5'b\text{-}P}$ < 1 Hz, 1 H, $H_{5'b}$), 4.72 (ddd, ^3J = 3.3, 4.4, 7.1 Hz, 1 H, $H_{3'b}$), 4.55 (dddd, $^3J_{7'b\text{-}6''b}$ = 2.1, $^3J_{7'b\text{-}7''b}$ = 11.7, $^3J_{7'b\text{-}6'b}$ = 11.7, $^3J_{7'b\text{-}P}$ < 1 Hz, 1 H, $H_{7'b}$), 4.33 (m, $^3J_{7''b\text{-}6''b}$ = 1.5, $^3J_{7''b\text{-}6'b}$ = 4.1, $J_{7''b\text{-}7'b}$ = 11.3, $^3J_{7''b\text{-}P}$ > 20 Hz, 1 H, $H_{7''b}$), 4.26 (td, $^3J_{4'a\text{-}3'a}$= 1.5, $^3J_{4'a\text{-}5'a}$ = 2.5, $^3J_{4'a\text{-}5''a}$ = 2.5 Hz, 1 H, $H_{4'a}$), 3.88 (ddd, $^3J_{4'b\text{-}3'b}$ = 3.6, $^3J_{4'b\text{-}5'b}$ = 4.6, $^3J_{4'b\text{-}P}$ < 1 Hz, 1 H, $H_{4'b}$), 3.76 (s, 6 H, OMe), 3.46 (A of an ABX syst, $^3J_{5'a\text{-}4'a}$ = 2.6, $^3J_{5'a\text{-}5''a}$ = 10.4 Hz, 1 H, $H_{5'a}$), 3.38 (B of an ABX syst, $^3J_{5''a\text{-}4'a}$ = 2.5, $^3J_{5''a\text{-}4'a}$ = 10.4 Hz, 1 H, $H_{5''a}$), 2.71-2.60 (m, 2 H, 2 × $H_{2'a}$), 2.51-2.18 (m, 4 H, 2 × $H_{2'a}$, 2 × $H_{6'b}$), 1.89 (d, $^4J_{7\text{-}6}$ = 0.9 Hz, 3 H, H_7), 1.42 (d, $^4J_{7\text{-}6}$ = 0.8 Hz, 3 H, H_7) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.3 (C_4), 164.2 (C_4), 158.9 (C_{Ph}), 151.2 (C_2), 151.1 (C_2), 144.3 (C_{Ph}), 135.3, 135.2 (C_{Ph}), 135.3, 135.1 (C_6, C_6), 130.2, 128.3, 128.2, 127.4, 113.6 (C_{Ph}), 112.1 (C_5), 111.6 (C_5), 87.6 ($C_{4'b}$), 87.5 (C_{Ph}), 85.7 ($C_{1'b}$), 84.7 ($C_{4'a}$), 84.6 ($C_{1'a}$), 80.6 ($C_{3'a}$), 79.4 ($C_{5'b}$), 70.8 ($C_{3'b}$), 63.6 ($C_{7'}$), 60.6 ($C_{5'a}$), 55.5 (OMe), 39.5 ($C_{2'b}$), 38.9 ($C_{2'a}$), 27.8 ($C_{6'b}$), 12.8 (C_{7b}), 11.9 (C_{7a}) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 64.8 ($J_{P\text{-}Se}$= 1007.2 Hz) ppm. HRMS (ESI): calcd for $C_{43}H_{47}N_4O_{13}PNaSe$, 961.1945 $[M+Na]^+$; found 961.1934.

5'-*O*-Dimethoxytrityl-α,β-seleno-D-CNA (R_C,S_P) (16b): Following the general procedure C starting from **14b** (40 mg, 0.034 mmol) gave the product **16b** as a yellow oil. The crude was immediatly engaged in the next step.

α,β-thio-D-CNA (R_C,R_P) (17a): Following the general procedure D starting from **15a** gave the product **17a** (50%, 11 mg). ^{1}H NMR (400 MHz CD_3OD, 40°C): δ = 7.78 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6a}), 7.55 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6b}), 6.35 (m, $^3J_{1'a\text{-}2'a}$= 5.7, $^3J_{1'b\text{-}2'b}$ = 6.6, $^3J_{1'b\text{-}2''b}$ = 7.5 Hz, $^3J_{1'a\text{-}2''a}$ = 8.7 Hz, 2 H, $H_{1'b}$, $H_{1'a}$), 5.20 (dddd, $^3J_{3'a\text{-}4'a}$ = 2.0, $^3J_{3'a\text{-}2'a}$ = 2.0, $^3J_{3'a\text{-}2''a}$ = 6.0, $^3J_{3'a\text{-}P}$ = 9.0 Hz, 1 H, $H_{3'a}$), 4.88 (dddd, $^3J_{5'b\text{-}6''b}$ = 2.2, $^3J_{5'b\text{-}4'b}$ = 3.4, $^3J_{5'b\text{-}6'b}$ = 12.0, $^3J_{5'b\text{-}P}$ = 1.6 Hz, 1 H, $H_{5'b}$), 4.61-4.55 (A of ABX(Y) syst, $^3J_{7''b\text{-}6'b}$ = 2.6, $^3J_{7'b\text{-}7''b}$ = 11.7, $^3J_{7'b\text{-}6'b}$ = 12.4, $^3J_{7'b\text{-}P}$ = 2.4 Hz, 1 H, $H_{7'b}$), 4.48 (A of AMX(Y) syst, $^3J_{3'b\text{-}2'b}$ = 2.3, $^3J_{3'b\text{-}4'b}$ = 3.1, $^3J_{3'b\text{-}2''b}$ = 6.0 Hz, 1 H, $H_{3'b}$), 4.44 (dddd, $^3J_{7''b\text{-}6''b}$ = 1.6, $^3J_{7''b\text{-}6'b}$ = 4.8, $^3J_{7''b\text{-}7'b}$ = 11.3, $^3J_{7''b\text{-}P}$ = 24.3 Hz, 1 H, $H_{7''b}$), 4.27 (td, $^3J_{4'a\text{-}3'a}$ = 2.1, $^3J_{4'a\text{-}5'a}$ = 3.1, $^3J_{4'a\text{-}5''a}$ = 3.1 Hz, 1 H, $H_{4'a}$), 3.92 (ddd, $^3J_{4'b\text{-}3'b}$ = 3.0, $^3J_{4'b\text{-}5'b}$ = 3.5, $^3J_{4'b\text{-}P}$ = 3.5 Hz, 1 H, $H_{4'b}$), 3.82 (ABX, $^3J_{5'a\text{-}4'a}$ = 3.0, $^3J_{5''a\text{-}4'a}$ = 3.0, $^3J_{5'a\text{-}5''a}$ = 11.9 Hz, 2 H, $H_{5'a}$, $H_{5''a}$), 2.56 (ddd, $^3J_{2'a\text{-}3'a}$ = 1.8, $^3J_{2'a\text{-}1'a}$ = 5.7, $^3J_{2'a\text{-}2''a}$ = 14.1 Hz, 1 H, $H_{2'a}$), 2.35 (ddd, $^3J_{2''a\text{-}3'a}$ = 6.0, $^3J_{2''a\text{-}1'a}$ = 8.7, $^3J_{2''a\text{-}2'b}$ = 14.2 Hz, 1 H, $H_{2''a}$), 2.31 (m, 1 H, $H_{2'b}$), 2.25 (m, 1 H, $H_{2''b}$), 2.23 (dddd, $^3J_{6'b\text{-}7''b}$ = 4.9, $^3J_{6'b\text{-}7'b}$ = 11.8, $^3J_{6'b\text{-}5'b}$ = 11.8, $^3J_{6'b\text{-}6''b}$ = 14.4 Hz, 1 H, $H_{6'b}$), 1.96 (m, $^3J_{6''b\text{-}7''b}$ = 1.7, $^3J_{6''b\text{-}6'b}$= 14.5 Hz, 1 H, $H_{6''b}$), 1.93 (d, $^4J_{7\text{-}6}$ = 1.2 Hz, 3 H, H_{7b}), 1.88 (d, $^4J_{7\text{-}6}$ = 1.2 Hz, 3 H, H_{7a}) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 166.43 (C_{4a}), 166.37 (C_{4b}), 152.51 (C_2), 152.46 (C_2), 138.0 (C_{6a}), 137.7 (C_{6b}), 112.3 (C_{5b}), 112.1 (C_{5a}), 89.1 ($^3J_{4'b\text{-}P}$ = 8.3 Hz, $C_{4'b}$), 87.4 ($^3J_{4'a\text{-}P}$ = 6.3 Hz, $C_{4'a}$), 86.6 ($C_{1'a}$), 86.1 ($C_{1'b}$), 81.1 ($^2J_{5'b\text{-}P}$ = 9.7 Hz, $C_{5'b}$), 80.8 ($^2J_{3'a\text{-}P}$ = 4.4 Hz, $C_{3'a}$), 71.3 ($C_{3'b}$), 69.8 ($^2J_{7'b\text{-}P}$ = 9.2 Hz, $C_{7'}$), 62.8 ($C_{5'a}$), 40.7 ($C_{2'b}$), 39.5 ($^3J_{2'a\text{-}P}$ = 4.1 Hz, $C_{2'a}$), 28.7 ($^3J_{6'b\text{-}P}$ = 6.0 Hz, $C_{6'b}$), 13.0 (C_{7b}), 12.7 (C_{7a}) ppm. ^{31}P NMR (202 MHz, CD_3OD): δ = 60.4 ppm. HRMS (ESI): calcd for $C_{22}H_{30}N_4O_{11}PS$ 589.1369 $[M+H]^+$; found 589.1374.

α,β-thio-D-CNA (R_C,S_P) (18a): Following the general procedure D starting from **16a** gave the product **18a** (50%, 13 mg). ^{1}H NMR (500 MHz CD_3OD): δ = 7.79 (d, $^4J_{6\text{-}7}$ = 1.0 Hz, 1 H, H_{6a}), 7.57 (d, $^4J_{6\text{-}7}$ = 1.0 Hz, 1 H, H_{6b}), 6.31-6.27 (m, $^3J_{1'a\text{-}2'a}$ = 5.5, $^3J_{1'a\text{-}2''a}$ = 8.4, $^3J_{1'b\text{-}2'b}$ = 5.8, $^3J_{1'b\text{-}2''b}$ = 8.4 Hz, 2 H, $H_{1'a}$, $H_{1'b}$), 5.34 (ddt, $^3J_{3'a\text{-}4'a}$ = 1.8, $^3J_{3'a\text{-}2'a}$ = 1.8, $^3J_{3'a\text{-}2''a}$ = 5.7, $^3J_{3'a\text{-}P}$ = 8.8 Hz, 1 H, $H_{3'a}$), 4.94 (dddd, $^3J_{5'b\text{-}4'b}$ = 2.8, $^3J_{5'b\text{-}6'b}$ = 2.8, $^3J_{5'b\text{-}6'b}$ = 11.8 $^3J_{5'b\text{-}P}$ = 2.8 Hz, 1 H, $H_{5'b}$), 4.62 (ddt, $^3J_{7'b\text{-}6'b}$ = 2.8, $^3J_{7'b\text{-}6'b}$ = 11.8, $^3J_{7'b\text{-}7''b}$ = 11.8, $^3J_{7'b\text{-}P}$ = 5.0 Hz, 1 H, $H_{7'b}$), 4.54 (ddt, $^3J_{3'b\text{-}2'b}$ = 2.0, $^3J_{3'b\text{-}4'b}$ = 2.0, $^3J_{3'b\text{-}2''b}$ = 5.7 Hz, 1 H, $H_{3'b}$), 4.49 (dddd, $^3J_{7''b\text{-}6''b}$ = 2.0, $^3J_{7''b\text{-}6'b}$ = 4.9, $^3J_{7''b\text{-}7'b}$ = 11.5, $^3J_{7''b\text{-}P}$ = 23.3 Hz, 1 H, $H_{7''b}$), 4.19 (td, $^3J_{4'a\text{-}3'a}$ = 1.8, $^3J_{4'a\text{-}5'a}$ = 2.9, $^3J_{4'a\text{-}5''a}$ = 2.9 Hz, 1 H, $H_{4'a}$), 4.00 (ddd, $^3J_{4'b\text{-}3'b}$ = 2.0, $^3J_{4'b\text{-}5'b}$ = 3.0, $^3J_{4'b\text{-}P}$ = 3.5 Hz, 1 H, $H_{4'b}$), 3.77 (ABX syst, $^3J_{5'a\text{-}4'a}$ = 3.1, $^3J_{5''a\text{-}4'a}$ = 3.1, $^3J_{5'a\text{-}5''a}$ = 12.3 Hz, 2 H, $H_{5'a}$, $H_{5''a}$), 2.48 (ddd, $^3J_{2'a\text{-}3'a}$ = 1.8, $^3J_{2'a\text{-}1'a}$ = 5.7, $^3J_{2'a\text{-}2''a}$ = 14.1 Hz, 1 H, $H_{2'a}$), 2.39 (ddd, $^3J_{2''a\text{-}3'a}$ = 5.8, $^3J_{2''a\text{-}1'a}$ = 8.6, $^3J_{2''a\text{-}2'a}$ = 14.3 Hz, 1 H, $H_{2''a}$), 2.29 (ddd, $^3J_{2'b\text{-}3'b}$ = 1.9, $^3J_{2'b\text{-}1'b}$ = 5.8, $^3J_{2'b\text{-}2''b}$ = 13.6 Hz, 1 H, $H_{2'b}$), 2.23 (dddd, $^3J_{6'b\text{-}7''b}$ = 4.9, $^3J_{6'b\text{-}7'b}$ = 11.8, $^3J_{6'b\text{-}5'b}$ = 11.8, $^3J_{6'b\text{-}6''b}$ = 14.4 Hz, 1 H, $H_{6'b}$), 2.14 (ddd, $^3J_{2''b\text{-}3'b}$ = 5.9, $^3J_{2''b\text{-}1'b}$ = 8.4, $^3J_{2''b\text{-}2'b}$ = 13.6 Hz, 1 H, $H_{2''b}$), 2.01 (dddd, $^3J_{6''b\text{-}7''b}$ = 2.0, $^3J_{6''b\text{-}7'b}$ = 3.0, $^3J_{6''b\text{-}5'b}$ = 3.0, $^3J_{6''b\text{-}6'b}$ = 14.4 Hz, 1 H, $H_{6''b}$), 1.93 (bs, 3 H, H_{7b}), 1.88 (bs, 3 H, H_{7a}) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 166.7 (C_4), 166.6 (C_4), 152.6 (C_{2a}), 152.5 (C_{2b}), 138.0 (C_{6b}), 137.5 (C_{6a}), 112.1 (C_5), 112.0 (C_5), 89.5 ($^3J_{4'b\text{-}P}$ = 8.3 Hz, $C_{4'b}$), 87.4 ($^3J_{4'a\text{-}P}$ = 6.1 Hz, $C_{4'a}$), 86.9 ($C_{1'a}$), 86.4 ($C_{1'b}$), 81.6 ($^2J_{3'a\text{-}P}$ = 5.5 Hz, $C_{3'a}$),

80.9 ($^2J_{5'b\text{-}P}$ = 5.5 Hz, $C_{5'b}$), 71.9 ($C_{3'b}$), 68.5 ($^2J_{7'b\text{-}P}$ = 5.8 Hz, $C_{7'}$), 62.8 ($C_{5'a}$), 41.3 ($C_{2'b}$), 39.8 ($^3J_{2'a\text{-}P}$ = 4.7 Hz, $C_{2'a}$), 29.2 ($^3J_{6'b\text{-}P}$ = 6.5 Hz, $C_{6'b}$), 12.9 (C_{7b}), 12.6 (C_{7a}). ^{31}P NMR (202 MHz, CD_3OD): δ = 64.2 ppm. HRMS (ESI): calcd for $C_{22}H_{30}N_4O_{11}PS$ 589.1369 $[M+H]^+$; found 589.1379.

α,β-seleno-D-CNA (R_C,R_P) (17b): Following the general procedure D starting from **15b** gave the product **17b** (56%, 13 mg). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.81 (d, $^4J_{6\text{-}7}$ = 1.1 Hz, 1 H, H_{6a}), 7.56 (d, $^4J_{6\text{-}7}$ = 1.1 Hz, 1 H, H_{6b}), 6.36 (dd, $^3J_{1'b\text{-}2'b}$ = 6.6, $^3J_{1'b\text{-}2''b}$ = 7.3 Hz, 1 H, $H_{1'b}$), 6.33 (dd, $^3J_{1'a\text{-}2'a}$ = 5.8, $^3J_{1'a\text{-}2''a}$ = 8.6 Hz, 1 H, $H_{1'a}$), 5.25 (dddt, $^3J_{3'a\text{-}2'a}$ = 1.8, $^3J_{3'a\text{-}4'a}$ = 2.2, $^3J_{3'a\text{-}2''a}$ = 5.8, $^3J_{3'a\text{-}P}$ = 9.8 Hz, 1 H, $H_{3'a}$), 4.95 (dddd, $^3J_{5'b\text{-}6''b}$ = 2.3, $^3J_{5'b\text{-}4'b}$ = 3.3, $^3J_{5'b\text{-}6'b}$ = 12.1, $^3J_{5'b\text{-}P}$ = 1.4 Hz, 1 H, $H_{5'b}$), 4.66 (dddd, $^3J_{7'b\text{-}6''b}$ = 2.1, $^3J_{7b'\text{-}7b''}$ = 11.4, $^3J_{7'b\text{-}6'b}$ = 12.0, $^3J_{7'b\text{-}P}$ = 2.6 Hz, 1 H, $H_{7'b}$), 4.60 (td, $^3J_{3'b\text{-}4'b}$ = 3.2, $^3J_{3'b\text{-}2'b}$ = 3.2, $^3J_{3'b\text{-}2''b}$ = 6.3 Hz, 1 H, $H_{3'b}$), 4.43 (dddd, $^3J_{7''b\text{-}6'b}$ = 1.4, $^3J_{7''b\text{-}6'b}$ = 4.9, $^3J_{7''b\text{-}7'b}$= 11.4 Hz, $^3J_{7''b\text{-}P}$ = 23.0 Hz, 1 H, $H_{7'b}$), 4.29 (q, $^3J_{4'a\text{-}3'a}$ = 2.1, $^3J_{4'a\text{-}5'a}$ = 2.7, $^3J_{4'a\text{-}5''a}$ = 2.7 Hz, 1 H, $H_{4'a}$), 3.91 (ddd, $^3J_{4'b\text{-}5'b}$ = 3.1, $^3J_{4'b\text{-}3'b}$ = 3.1, $^3J_{4'b\text{-}P}$ = 3.5 Hz, 1 H, $H_{4'b}$), 3.83 (d, ^3J = 3.0 Hz, 2 H, $H_{5'a}$, $H_{5''a}$), 2.57 (m, $^3J_{2'a\text{-}3'a}$ = 1.7, $^3J_{2'a\text{-}1'a}$ = 5.9, $^3J_{2'a\text{-}2''a}$ = 14.1 Hz, 1 H, $H_{2'a}$), 2.37 (ddd, $^3J_{2''a\text{-}3'a}$ = 5.8, $^3J_{2''a\text{-}1'a}$ = 8.6, $^3J_{2''a\text{-}2'a}$ = 14.0 Hz, 1 H, $H_{2''a}$), 2.35-2.19 (m, 3 H, $H_{2'b}$, $H_{2''b}$, $H_{6'b}$), 2.05 (dddd, $^3J_{6''b\text{-}7''b}$ = 1.6, $^3J_{6''b\text{-}7'b}$ = 2.4, $^3J_{6''b\text{-}5'b}$ = 2.4, $^3J_{6''b\text{-}6'b}$ = 14.8 Hz, 1 H, $H_{6''b}$), 1.95 (d, $^4J_{7\text{-}6}$ = 1.1 Hz, 3 H, H_{7b}), 1.88 (d, $^4J_{7\text{-}6}$ = 1.1 Hz, 3 H, H_{7a}) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 166.42 (C_{4a}), 166.36 (C_{4b}), 152.52 (C_2), 152.48 (C_2), 137.9 (C_{6a}), 137.6 (C_{6b}), 112.4 (C_{5b}), 112.1 (C_{5a}), 88.9 ($^3J_{4'b\text{-}P}$ = 8.0 Hz, $C_{4'b}$), 87.1 ($^3J_{4'a\text{-}P}$ = 6.2 Hz, $C_{4'a}$), 86.4 ($C_{1'a}$), 86.0 ($C_{1'b}$), 81.3 ($^2J_{3'a\text{-}P}$ = 3.9 Hz, $C_{3'a}$), 80.7 ($^2J_{5'b\text{-}P}$ = 9.9 Hz, $C_{5'b}$), 71.1 ($C_{3'b}$), 69.3 ($^2J_{7'b\text{-}P}$ = 10.0 Hz, $C_{7'}$), 62.8 ($C_{5'a}$), 40.7 ($C_{2'b}$), 39.4 ($^3J_{2'a\text{-}P}$ = 4.1 Hz, $C_{2'a}$), 28.8 ($^3J_{6'b\text{-}P}$ = 6.7 Hz, $C_{6'b}$), 13.1 (C_{7b}), 12.6 (C_{7a}) ppm. ^{31}P NMR (202 MHz, CD_3OD): δ = 63.9 ($J_{P\text{-}Se}$ = 1008.2 Hz) ppm. HRMS (ESI): calcd for $C_{22}H_{30}N_4O_{11}PSe$ 636.0856 $[M+H]^+$; found 636.0861.

α,β-seleno-D-CNA (R_C,S_P) (18b): Following the general procedure D starting from **16b** gave the product **18b** (quant., 21 mg) over two steps. ^{1}H NMR (500 MHz, CD_3OD): δ = 7.81 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6a}), 7.56 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6b}), 6.38 (dd, $^3J_{1'b\text{-}2'b}$ = 5.9, $^3J_{1'b\text{-}2''b}$ = 8.4 Hz, 1 H, $H_{1'b}$), 6.27 (dd, $^3J_{1'a\text{-}2'a}$ = 6.1, $^3J_{1'a\text{-}2''a}$ = 9.1 Hz, 1 H, $H_{1'a}$), 5.44 (dddt, $^3J_{3'a\text{-}4'a}$ = 2.0, $^3J_{3'a\text{-}2'a}$ = 2.1, $^3J_{3'a\text{-}2''a}$ = 5.3, $^3J_{3'a\text{-}P}$ = 9.7 Hz, 1 H, $H_{3'a}$), 4.97 (dq, $^3J_{5'b\text{-}4'b}$ = 2.8, $^3J_{5'b\text{-}6''b}$ = 2.8, $^3J_{5'b\text{-}6'b}$ = 11.8, $^3J_{5'b\text{-}P}$ = 2.8 Hz, 1 H, $H_{5'b}$), 4.65 (dddd, $^3J_{7'b\text{-}6''b}$ = 2.7, $^3J_{7'b\text{-}7''b}$ = 11.4, $^3J_{7'b\text{-}6'b}$ = 12.0, $^3J_{7'b\text{-}P}$ = 4.9 Hz, 1 H, $H_{7'b}$), 4.54 (dt, $^3J_{3'b\text{-}4'b}$ = 2.1, $^3J_{3'b\text{-}2'b}$ = 2.1, $^3J_{3'b\text{-}2''b}$ = 5.8 Hz, 1 H, $H_{3'b}$), 4.43 (dddd, $^3J_{7''b\text{-}6'b}$ = 1.6, $^3J_{7''b\text{-}6'b}$ = 4.9, $^3J_{7''b\text{-}7'b}$ = 11.4, $^3J_{7''b\text{-}P}$ > 20 Hz, 1 H, $H_{7''b}$), 4.21 (dt, $^3J_{4'a\text{-}3'a}$ = 1.8, $^3J_{4'a\text{-}5'a}$ = 3.0, $^3J_{4'a\text{-}5''a}$ = 3.0 Hz, 1 H, $H_{4'a}$), 4.01 (ddd, $^3J_{4'b\text{-}3'b}$ = 2.0, $^3J_{4'b\text{-}5'b}$ = 2.6, $^3J_{4'b\text{-}P}$ = 3.9 Hz, 1 H, $H_{4'b}$), 3.78 (d, ^3J = 3.0 Hz, 2 H, $H_{5'a}$, $H_{5''a}$), 2.49 (m, $^3J_{2'a\text{-}3'a}$ = 2.1, $^3J_{2'a\text{-}1'a}$ = 6.1, $^3J_{2'a\text{-}2''a}$ = 14.2 Hz, 1 H, $H_{2'a}$), 2.41 (ddd, $^3J_{2''a\text{-}3'a}$ = 5.6, $^3J_{2''a\text{-}1'a}$ = 8.6, $^3J_{2''a\text{-}2'a}$ = 14.1 Hz, 1 H, $H_{2''a}$), 2.29 (ddd, $^3J_{2'b\text{-}3'b}$ = 2.0, $^3J_{2'b\text{-}1'b}$ = 5.9, $^3J_{2'b\text{-}2''b}$ = 13.7, 1 H, $H_{2'b}$), 2.26 (dddd, $^3J_{6'b\text{-}7''b}$ = 4.8, $^3J_{6'b\text{-}5'b}$ = 10.9, $^3J_{6'b\text{-}7'b}$ = 12.0, $^3J_{6'b\text{-}6''b}$ = 14.4, 1 H, $H_{6'b}$), 2.11 (ddd, $^3J_{2''b\text{-}3'b}$ = 5.7, $^3J_{2''b\text{-}1'b}$ = 8.4, $^3J_{2''b\text{-}2'b}$ = 13.7 Hz, 1 H, $H_{2''b}$), 1.95 (dddd, $^3J_{6''b\text{-}7''b}$ = 1.6, $^3J_{6''b\text{-}7'b}$ = 2.4, $^3J_{6''b\text{-}5'b}$ = 2.4, $^3J_{6''b\text{-}6'b}$ = 15.8 Hz, 1 H, $H_{6''b}$), 1.93 (d, $^4J_{7\text{-}6}$ = 1.2 Hz, 3 H, H_{7b}), 1.88 (d, $^4J_{7\text{-}6}$ = 1.2 Hz, 3 H, H_{7a}) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ = 166.5 (C_{4a}), 166.4 (C_{4b}), 152.5 (C_{2a}), 152.4 (C_{2b}), 138.0 (C_{6a}), 137.4 (C_{6b}), 112.1 (C_{5b}), 112.1 (C_{5a}), 89.4 ($^3J_{4'b\text{-}P}$ = 8.3 Hz, $C_{4'b}$), 87.4 ($^3J_{4'a\text{-}P}$ = 6.2 Hz, $C_{4'a}$), 86.9 ($C_{1'b}$), 86.4 ($C_{1'a}$), 82.1 ($^2J_{3'a\text{-}P}$ = 5.3 Hz, $C_{3'a}$), 81.1 ($^2J_{5'b\text{-}P}$ = 5.4 Hz, $C_{5'b}$), 71.8 ($C_{3'b}$), 68.6 ($^2J_{7'b\text{-}P}$ = 5.5 Hz, $C_{7'}$), 62.7 ($C_{5'a}$), 41.3 ($C_{2'b}$), 39.8 ($^3J_{2'a\text{-}P}$ = 4.7 Hz, $C_{2'a}$), 29.4 ($^3J_{6'b\text{-}P}$ = 6.4 Hz, $C_{6'b}$), 13.0 (C_{7b}), 12.6 (C_{7a}) ppm. ^{31}P NMR (202 MHz, CD_3OD): δ = 67.3 ($J_{P\text{-}Se}$= 973.7) ppm. HRMS (ESI): calcd for $C_{22}H_{30}N_4O_{11}PSe$ 636.0856 $[M+H]^+$; found 636.0872.

5'-*O*-Dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-α,β,γ-thio-D-CNA *cis* (19a) and 5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-α,β,γ-thio-D-CNA trans (20a): Following the general procedure A starting from **2** gave the product **19a** (32%, 345 mg) and product **20a** (38%, 410 mg). Data for **19a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.51 (bs, 2 H, NH), 7.71-7.60 (m, 5 H, Ph and H_{6a}), 7.50-7.42 (m, 8 H, Ph), 7.37-7.32 (m, 7 H, Ph), 7.05 (s, 1 H, H_{6b}), 6.90-6.87 (d, ^3J = 9.0 Hz, 4 H, Ph), 6.53-6.49 (dd, ^3J = 5.7, 8.7 Hz, 1 H, $H_{1'a}$), 6.08-6.04 (m, 1 H, $H_{1'b}$), 5.48-5.43 (m, 1 H, $H_{3'a}$), 4.69-4.63 (m, 1 H, $H_{5''b}$), 4.58-4.47 (m, 2 H, $H_{3'b}$ and $H_{5''b}$), 4.28 (m, 1 H, $H_{4'a}$), 4.05-3.94 (m, 2 H, $H_{5'b}$), 3.82 (s, 6 H, OMe), 3.57-3.47 (m, 2 H, $H_{5'a}$), 2.67-2.61 (m, 1 H, $H_{2'a}$), 2.57-2.45 (m, 2 H, $H_{2'a}$ and $H_{2'b}$), 2.43-2.32 (m, 1 H, $H_{2'b}$), 1.84 (s, 3 H, H_{7b}), 1.50 (s, 3 H, H_{7a}), 1.14 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.2 (C_{4a}), 164.0 (C_{4b}), 158.8 (C_{Ph}), 150.5 (C_{2a}), 149.7 (C_{2b}), 144.2 (C_{Ph}), 138.3 (C_{6b}), 135.9 (C_{6a}), 135.8, 135.3, 135.2, 135.1, 132.4, 132.0, 130.7, 130.5, 130.1, 128.3, 128.2, 128.1, 127.3, 113.5, 113.3 (C_{Ph}), 111.7 (C_{5a}), 110.3 (C_{5b}), 88.2 ($C_{1'b}$), 87.4 (C_{Ph}), 84.9 ($C_{4'a}$), 84.5 ($C_{1'a}$), 81.1 ($C_{4'b}$), 79.7 ($C_{3'a}$), 74.0 ($C_{3'b}$), 72.3 ($C_{5''b}$), 70.8 ($C_{5''b}$), 63.5 ($C_{5'a}$), 55.3 (OMe), 39.5 ($C_{2'a}$), 39.3 ($C_{2'b}$), 26.9 ($C\underline{Me}_3$), 19.2 ($\underline{C}Me_3$), 12.3 (C_{7b}), 11.8 (C_{7a}) ppm. ^{31}P NMR (121 MHz, $CDCl_3$) δ = 60.0 ppm. HRMS (ESI): calcd for $C_{58}H_{62}N_4O_{13}PSSi$ 1113.3541 $[M-H]^-$; found 1113.3544. Data for **20a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ = 9.61 (bs, 2 H, NH), 7.77-7.71 (m, 4 H, Ph), 7.62 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6a}), 7.52-7.42 (m, 8 H, Ph), 7.36-7.27 (m, 7 H, Ph), 6.90-6.87 (m, 5 H, Ph and H_{6b}), 6.53-6.48 (dd, ^3J = 5.4, 9.0 Hz, 1 H, $H_{1'a}$), 6.15-6.10 (m, 1 H, $H_{1'b}$), 5.59-5.54 (m, 1 H, $H_{3'a}$), 4.92-4.84 (dd, ^3J = 11.7, 14.7 Hz, 1 H, $H_{5''b}$), 4.65-4.63 (m, 1 H, $H_{3'b}$), 4.43-4.19 (m, 4 H, $H_{5''b}$, 2 × $H_{5'b}$, $H_{4'a}$), 3.83 (s, 6 H, OMe), 3.57-3.43 (m, 2 H, $H_{5'a}$), 2.74-2.68 (m, ^3J = 5.4, 11.7 Hz, 1 H, $H_{2'a}$), 2.51-2.41 (m, 1 H, $H_{2'a}$), 2.24-2.06 (m, 2 H, $H_{2'b}$), 1.84 (s, 3 H, H_{7b}), 1.47 (s, 3 H, H_{7a}), 1.15 (s, 9 H, *t*Bu) ppm.^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.0 (C_{4a}), 163.8 (C_{4b}), 158.8 (C_{Ph}), 150.8 (C_{2a}), 150.2 (C_{2b}), 144.3 (C_{Ph}), 136.0 (C_{6b}), 135.8 (C_{6a}), 135.3 (C_{Ph}), 135.2 (C_{Ph}), 135.1 (C_{Ph}), 132.9 (C_{Ph}), 131.5 (C_{Ph}), 130.5 (C_{Ph}), 130.4 (C_{Ph}), 130.1 (C_{Ph}), 128.4 (C_{Ph}), 128.2 (C_{Ph}), 128.1 (C_{Ph}), 127.3 (C_{Ph}), 113.5 (C_{Ph}), 111.8 (C_{5a}), 111.5 (C_{5b}), 87.3 (C_{Ph}), 87.0 ($C_{1'b}$), 84.8 ($C_{4'a}$), 84.5 ($C_{1'a}$), 81.3 ($C_{4'b}$), 80.3 ($C_{3'a}$) 74.0 ($C_{3'b}$), 71.4 ($C_{5'b}$), 69.0 ($C_{5''b}$), 63.5 ($C_{5'a}$), 55.3 (OMe), 39.3 ($C_{2'a}$), 38.1 ($C_{2'b}$), 27.0 ($C\underline{Me}_3$), 19.3 ($\underline{C}Me_3$), 12.5 (C_{7b}), 11.8 (C_{7a}) ppm. ^{31}P NMR (121 MHz, $CDCl_3$) δ = 62.2 ppm. HRMS (ESI): calcd for $C_{58}H_{62}N_4O_{13}PSSi$ 1113.3541 $[M-H]^-$; found 1113.3546.

5'-*O*-Dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-α,β,γ-seleno-D-CNA *cis* (19b) and 5'-*O*-dimethoxytrityl-3'-*O*-*tert*-butyldiphenylsilyl-α,β,γ-seleno-D-CNA *trans* (20b): Following the general procedure B starting from **2** gave the product **19b** (16%, 454 mg) and product **20b** (22%, 624 mg). Data for **19b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.78 and 8.69 (s, 2 H, NH), 7.65-7.56 (m, 5 H, Ph), 7.44-7.22 (m, 15 H, H_6, Ph), 6.94 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_6), 6.82 (m, 4 H, Ph), 6.44 (dd, ^3J = 5.7, 9.0 Hz, 1 H, $H_{1'a}$), 6.02 (t, ^3J = 6.6 Hz, 1 H, $H_{1'b}$), 5.50 (dd, ^3J = 5.4, 11.5 Hz, 1 H, $H_{3'a}$), 4.62 (A part of an AB syst, ^3J = 9.0, 11.7 Hz, 1 H, $H_{5'b}$), 4.49-4.39 (m, 2 H, $H_{5''b}$ and $H_{3'b}$), 4.24 (m, 1 H, $H_{4'a}$), 4.03-3.90 (m, 2 H, $H_{5'b}$ and $H_{5''b}$), 3.77 (s, 6 H, OMe), 3.52 and 3.41 (AB part of an ABX syst, ^3J = 2.4, 10.5 Hz, 2 H, $H_{5'a}$), 2.55 (A part of an ABX syst, J = 6.4, 15.0 Hz, 1 H, $H_{2'}$), 2.49-2.36 (m, 2 H, $H_{2'}$), 2.22 (m, 1 H, $H_{2'}$), 1.82 (d, $^4J_{7\text{-}6}$ = 0.9 Hz, 3 H, H_7), 1.43 (d, $^4J_{7\text{-}6}$ = 0.9 Hz, 3 H, H_7), 1.08 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ = 164.3, 164.2, 158.8, 150.5, 149.7, 144.1, 138.8, 135.8, 135.7, 135.2, 130.7, 130.5, 130.1, 128.2, 128.1, 127.3, 113.5, 111.7, 110.1, 88.7, 87.4, 84.7, 84.5, 81.1, 80.5, 74.2, 71.9, 70.4, 63.5, 55.3, 39.2, 33.9, 29.7, 26.9, 25.7, 25.0, 19.2, 12.3, 11.8 ppm. ^{31}P NMR (121 MHz, $CDCl_3$) δ = 65.5 ($J_{P\text{-}Se}$ = 1006.8 Hz) ppm. HRMS (ESI): calcd for $C_{58}H_{62}N_4O_{13}PSeSi$ 1161.2985 $[M-H]^-$; found 1161.2989. Data for **20b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.21 and 9.14 (s, 2 H, NH), 7.70-7.63 (m, 5

H, Ph), 7.56 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_6), 7.48-7.25 (m, 15 H, H_6, Ph), 6.80 (m, 4 H, Ph), 6.44 (dd, ^3J = 5.4, 9.0 Hz, 1 H, $H_{1'a}$), 6.01 (t, ^3J = 6.9 Hz, 1 H, $H_{1'b}$), 5.63 (dd, ^3J = 5.7, 10.2 Hz, 1 H, $H_{3'a}$), 4.64 (t, ^3J = 11.9 Hz, 1 H, $H_{5'b}$), 4.51 (m, 1 H, $H_{3'b}$), 4.35-4.12 (m, 4 H, $H_{5'b}$, 2 × $H_{5''b}$ and $H_{4'a}$), 3.77 (s, 6 H, OMe), 3.51 and 3.36 (AB part of an ABX syst, ^3J = 2.7, 10.8 Hz, 2 H, 2 × $H_{5'a}$), 2.64 (A part of an ABX syst, ^3J = 5.1, 14.1 Hz, 1 H, $H_{2'}$), 2.42 (m, 1 H, $H_{2'}$), 2.16-2.10 (m, 2 H, $H_{2'}$), 1.79 (d, $^4J_{7\text{-}6}$ = 0.9 Hz, 3 H, H_7), 1.40 (d, $^4J_{7\text{-}6}$ = 0.9 Hz, 3 H, H_7), 1.09 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ = 164.0, 163.9, 158.8, 150.8, 150.2, 114.2, 136.4, 135.9, 135.8, 135.3, 135.1, 132.8, 131.5, 130.5, 130.1, 128.4, 128.2, 128.1, 127.3, 113.5, 111.8, 111.4, 87.3, 87.1, 84.7, 84.5, 81.2, 81.1, 74.1, 71.5, 69.4, 67.0, 63.5, 39.2, 38.4, 27.0, 19.3, 12.5, 11.8 ppm. ^{31}P NMR (100 MHz, $CDCl_3$): δ = 67.4 ($J_{P\text{-}Se}$ = 993.5 Hz) ppm. HRMS (ESI): calcd for $C_{58}H_{62}N_4O_{13}PSeSi$: 1161.2985 $[M\text{-}H]^-$; found 1161.2993.

5'-*O*-Dimethoxytrityl-α,β,γ-thio-D-CNA *cis* (21a): Following the general procedure C starting from **19a** gave the product **21a** (81%, 200 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.59 (s, 1 H, H_{6a}), 7.39-7.37 (m, 2 H, Ph), 7.32-7.20 (m, 10 H, Ph and H_{6b}), 6.86-6.83 (d, 4 H, Ph), 6.47-6.43 (m, 1 H, $H_{1'a}$), 6.13-6.08 (m, 1 H, $H_{1'b}$), 5.40-5.36 (m, 1 H, $H_{3'a}$), 4.82-4.23 (m, 6 H, 2 × $H_{5''b}$, $H_{3'b}$, 2 × $H_{5'b}$, $H_{4'a}$), 3.78 (s, 6 H, OMe), 3.54-3.39 (m, 2 H, 2 × $H_{5'a}$), 2.70-2.35 (m, 4 H, $H_{2'}$), 1.89 (s, 3 H, H_{7b}), 1.51 (s, 3 H, H_{7a}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.5 (C_{4a}), 163.9 (C_{4b}), 158.9 (C_{Ph}), 151.8 (C_{2a}), 150.9 (C_{2b}), 144.2 (C_{Ph}), 136.0 (C_{6a}), 135.2 (C_{6b}), 135.1, 130.1, 128.2, 128.1, 127.3 (C_{Ph}), 113.5 (C_{Ph}), 112.6 (C_{5a}), 111.3 (C_{5b}), 87.8 ($C_{1'b}$), 87.5 (C_{Ph}), 84.6 ($C_{4'a}$), 84.5 ($C_{1'a}$), 81.2 ($C_{4'b}$), 80.3 ($C_{3'a}$), 72.7 ($C_{5'b}$), 71.8 ($C_{5''b}$), 70.5 ($C_{3'b}$), 55.4 (OMe), 39.4 ($C_{2'a}$), 37.4 ($C_{2'b}$), 12.5 (C_{7b}), 12.0 (C_{7a}) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 59.4 ppm. HRMS (ESI): calcd for $C_{42}H_{44}N_4O_{13}PS$ 875.2363 $[M\text{-}H]^-$; found 875.2333.

5'-*O*-Dimethoxytrityl-α,β,γ-thio-D-CNA *trans* (22a): Following the general procedure C starting from **20a** gave the product **22a** (60%, 195 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.60 (s, 1 H, H_{6a}), 7.40-7.37 (m, 2 H, Ph), 7.32-7.25 (m, 7 H, Ph), 7.09 (s, 1 H, H_{6b}), 6.86-6.83 (d, ^3J = 9.0 Hz, 4 H, Ph), 6.47-6.42 (m, 1 H, $H_{1'a}$), 6.15-6.10 (m, 1 H, $H_{1'b}$), 5.50-5.46 (m, 1 H, $H_{3'a}$), 4.73-4.64 (m, 2 H, $H_{5''b}$, $H_{3'b}$), 4.50-4.26 (m, 4 H, $H_{5''b}$, 2 × $H_{5'b}$, $H_{4'a}$), 3.78 (s, 6 H, OMe), 3.53-3.40 (m, 2 H, $H_{5'a}$), 2.71-2.46 (m, 2 H, $H_{2'a}$), 2.47-2.43 (m, 2 H, $H_{2'b}$), 1.84 (s, 3 H, H_{7b}), 1.45 (s, 3 H, H_{7a}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.3 (C_{4a}), 164.2 (C_{4b}), 158.8 (C_{Ph}), 151.0 (C_{2a}), 150.8 (C_{2b}), 144.2 (C_{Ph}), 136.9 (C_{6a}), 135.3 (C_{6b}), 135.1, 130.1, 128.2, 128.0, 127.3 (C_{Ph}), 113.5 (C_{Ph}), 112.0 (C_{5a}), 111.4 (C_{5b}), 87.7 ($C_{1'b}$), 87.3 (C_{Ph}), 84.7 ($C_{4'a}$), 84.5 ($C_{1'a}$), 81.2 ($C_{4'b}$), 80.5 ($C_{3'a}$), 72.2 ($C_{3'b}$), 71.7 ($C_{5'b}$), 69.3 ($C_{5''b}$), 63.5 ($C_{5'a}$), 55.3 (OMe), 39.2 ($C_{2'a}$), 38.3 ($C_{2'b}$), 12.5 (C_{7b}), 11.8 (C_{7a}) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 62.1 ppm. HRMS (ESI): calcd for $C_{42}H_{44}N_4O_{13}PS$ 875.2363 $[M\text{-}H]^-$; found 875.2341.

5'-*O*-Dimethoxytrityl-α,β,γ-seleno-D-CNA *cis* (21b): Following the general procedure C starting from **19b** gave the product **21b** (80%, 290 mg). Probably due to aggregates formation[20], the NMR specra were recorded with a very poor resolution for ^{1}H and with a low detection level of the aliphatic carbon for ^{13}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.46 (bs, 2 H, NH), 7.60 (s, 1 H, H_6), 7.40-7.19 (m, 10 H, H_6 and Ph), 6.87 (d, 4 H, Ph), 6.47 (dd, ^3J = 5.1, 8.4 Hz, 1 H, $H_{1'a}$), 6.08 (t, ^3J = 6.3 Hz, 1 H, $H_{1'b}$), 5.47 (dd, ^3J = 3.6, 9.0 Hz, 1 H, $H_{3'a}$), 4.83-4.70 (m, 3 H), 4.61 (bs, 1 H, $H_{4'a}$), 4.48-4.28 (3 H), 3.79 (s, 6 H, OMe), 3.52 and 3.45 (AB syst, J = 9.9 Hz, 2 H, 2 × $H_{5'a}$), 2.67-2.41 (m, 4 H, $H_{2'}$), 1.87 (s, 3 H, H_7), 1.50 (s, 3 H, H_7) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.1, 165.9, 160.1, 152.4, 151.8, 145.5, 139.4, 136.8, 136.6, 136.5, 131.5, 129.5, 128.6, 114.8, 113.3, 112.0, 89.4, 88.8, 86.2, 85.8, 82.7, 81.9, 73.7, 73.5, 72.2, 72.0, 64.9, 62.0, 56.6, 40.4, 22.3, 15.4, 13.5, 12.9 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 63.9 ppm. HRMS (ESI): calcd for $C_{42}H_{45}N_4O_{13}PNaSe$ 947.1784 $[M+Na]^+$; found 947.1798.

5'-*O*-Dimethoxytrityl-α,β,γ-seleno-D-CNA *trans* (22b): Following the general procedure C starting from **20b** gave the product **22b** (81%, 400 mg). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.06 (bs, 2 H, NH), 7.61 (s, 1 H, H_6), 7.41-7.20 (m, 9 H, Ph), 7.10 (s, 1 H, H_6), 6.87 (d, 4 H, Ph), 6.45 (dd, ^3J = 6.3, 8.4 Hz, 1 H, $H_{1'a}$), 6.10 (t, ^3J = 6.9 Hz, 1 H, $H_{1'b}$), 5.60 (dd, ^3J = 4.5, 8.4 Hz, 1 H, $H_{3'a}$), 4.75-4.67 (m, 2 H), 4.51 (t, J = 13.0 Hz, 1 H), 4.42-4.26 (m, 3 H), 3.78 (s, 6 H, OMe), 3.53 and 3.40 (AB syst, ^3J = 9.0 Hz, 2 H, 2 × $H_{5'a}$), 2.71-2.43 (m, 4 H, $H_{2'}$), 1.85 (s, 3 H, H_7), 1.44 (s, 3 H, H_7) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.4, 164.3, 158.7, 151.0, 150.8, 144.2, 135.3, 135.0, 130.0, 128.2, 128.1, 128.0, 127.3, 113.5, 111.9, 111.3, 87.3, 84.7, 81.5, 81.3, 72.3, 63.8, 60.5, 55.3, 21.1, 14.2, 12.5, 11.8 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 66.8 ppm. HRMS (ESI): calcd for $C_{42}H_{45}N_4O_{13}PNaSe$ 947.1784 $[M+Na]^+$; found 947.1788.

α,β,γ-thio-D-CNA *cis* (23a): Following the general procedure D starting from **21a** gave the product **23a** (quant., 65 mg). ^{1}H NMR (500 MHz, DMSO): δ = 11.36 (s, 2 H, NH), 7.73 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6a}), 7.57 (d $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6b}), 6.35 (dd, $^3J_{1'b\text{-}2'b}$ = 6.6, $^3J_{1'b\text{-}2''b}$ = 7.3 Hz, 1 H, $H_{1'b}$), 6.25 (dd, $^3J_{1'a\text{-}2'a}$ = 5.9, $^3J_{1'a\text{-}2''a}$ = 8.4 Hz, 1 H, $H_{1'a}$), 5.85 (d, ^3J = 4.5 Hz, 1 H, OH_b), 5.36 (t, ^3J = 5.1 Hz, 1 H, OH_a), 5.05 (m, ^3J = 1.8, 5.8, J_P = 8.9 Hz, 1 H, $H_{3'a}$), 4.65 (A part of an ABX syst, ^3J = 11.7, J_P = 2.5 Hz, 1 H, $H_{5'b}$), 4.54 (A part of an ABX syst, ^3J = 12.0, J_P = 2.0 Hz, 1 H, $H_{5''b}$), 4.36 (m, ^3J = 12.0, J_P = 25.0 Hz, 2 H, $H_{5'b}$ and $H_{5''b}$), 4.30 (m, 1 H, $H_{3'b}$), 4.18 (dd, ^3J = 1.9, 3.6 Hz, 1 H, $H_{4'a}$), 3.65 (t, J = 4.4 Hz, 2 H, $H_{5'a}$), 2.50 (m, 1 H, $H_{2'a}$), 2.40 (m, 2 H, $H_{2'a}$ and $H_{2'b}$), 2.18 (B part of an ABX syst, ^3J = 3.9, 6.6, 13.7 Hz, 1 H, $H_{2'b}$), 1.81 (d, $^4J_{7\text{-}6}$ = 0.9 Hz, 3 H, H_7), 1.78 (d, $^4J_{7\text{-}6}$ = 0.9 Hz, 3 H, H_7) ppm. ^{13}C NMR (125 MHz, DMSO): δ = 164.13, 164.11, 150.82, 150.78, 136.8, 136.3, 110.5, 110.1, 85.3, 84.2, 84.1, 80.3, 78.9, 73.2, 71.8, 71.3, 61.3, 38.1, 37.6, 12.4, 12.2. ^{31}P NMR (200 MHz, DMSO): δ = 59.6 ppm. HRMS (ESI): calcd for $C_{21}H_{26}N_4O_{11}PS$ 573.1056 $[M\text{-}H]^-$; found 573.1057.

α,β,γ-thio-D-CNA *trans* (24a): Following the general procedure D starting from **22a** gave the product **24a** (quant., 65 mg). ^{1}H NMR (500 MHz, DMSO): δ = 11.33 (s, 2 H, NH), 7.71 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6a}), 7.52 (d, $^4J_{6\text{-}7}$ = 1.2 Hz, 1 H, H_{6b}), 6.32 (dd, $^3J_{1'b\text{-}2''b}$ = 5.9, $^3J_{1'b\text{-}2'b}$ = 8.6 Hz, 1 H, $H_{1'b}$), 6.21 (dd, $^3J_{1'a\text{-}2'a}$ = 5.8, $^3J_{1'a\text{-}2''a}$ = 8.6 Hz, 1 H, $H_{1'a}$), 5.97 (d, ^3J = 4.8 Hz, 1 H, OH_b), 5.30 (t, ^3J = 5.1 Hz, 1 H, OH_a), 5.14 (m, ^3J = 1.7, 5.2, $^3J_{3'a\text{-}P}$ = 8.7 Hz, 1 H, $H_{3'a}$), 4.57 (A part of an ABX syst, ^3J = 11.7, J_P = 11.5 Hz, 1 H, $H_{5'b}$), 4.55 (A part of an ABX syst, ^3J = 11.7, J_P= 12.5 Hz, 1 H, $H_{5''b}$), 4.37 (dd, ^3J = 2.2, 5.6 Hz, 1 H, $H_{3'b}$), 4.36 (m, ^3J = 11.7, J_P = 14.0 Hz, 2 H, $H_{5'b}$ and $H_{5''b}$), 4.12 (dd, ^3J = 1.7, 3.3 Hz, 1 H, $H_{4'a}$), 3.60 (m, 2 H, 2 × $H_{5'a}$), 2.48 (m, 1 H, $H_{2'a}$), 2.37 (m, 1 H, $H_{2'a}$), 2.27 (m, 1 H, $H_{2'b}$), 2.16 (B part of an ABX syst, ^3J = 2.6, 6.0, 13.5 Hz, 1 H, $H_{2'b}$), 1.80 (d, 3 H, $^4J_{7\text{-}6}$ = 0.9 Hz, H_7), 1.76 (d, 3 H, $^4J_{7\text{-}6}$ = 0.9 Hz, H_7) ppm. ^{13}C NMR (125 MHz, DMSO): δ = 164.06 (C_4), 164.05 (C_4), 150.8 (C_2), 150.7 (C_2), 136.4 (C_6), 136.2 (C_6), 110.5 (C_5), 110.1 (C_5), 85.4 ($^3J_{4'a\text{-}P}$ = 4.9 Hz, $C_{4'a}$), 84.4 ($C_{1'}$), 84.0 ($C_{1'}$), 80.41 ($C_{3'a}$), 80.35 ($C_{4'b}$), 71.9 ($^2J_{5'b\text{-}P}$ = 6.8 Hz, $C_{5'b}$), 70.9 ($C_{3b'}$), 69.9 ($^2J_{5''b\text{-}P}$ = 6.9 Hz, $C_{5'b}$), 61.2 ($C_{5'a}$), 37.8 ($C_{2'b}$), 37.7 ($^3J_{2'a\text{-}P}$ = 3.5 Hz, $C_{2'a}$), 12.4 (C_7), 12.2 (C_7) ppm. ^{31}P NMR (202 MHz, DMSO): δ = 61.9 ppm. HRMS (ESI): calcd for $C_{21}H_{26}N_4O_{11}PS$ 573.1056 $[M\text{-}H]^-$; found 573.1050.

α,β,γ-seleno-D-CNA *cis* (23b): Following the general procedure D starting from **21b** gave the product **23b** (quant., 9 mg). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.84 (d, $^4J_{6\text{-}7}$ = 0.9 Hz, 1 H, H_{6a}), 7.51 (d, $^4J_{6\text{-}7}$ = 0.9 Hz, 1 H, H_{6b}), 6.37 (dd, $^3J_{1'a\text{-}2'a}$ = 6.0, $^3J_{1'a\text{-}2''a}$ = 9.4 Hz, 1 H, $H_{1'a}$), 6.28 (dd, $^3J_{1'b\text{-}2''b}$ = 7.0, $^3J_{1'b\text{-}2'b}$ = 7.0 Hz, 1 H, $H_{1'b}$), 5.29 (dddd, $^3J_{3'a\text{-}4'a}$ = 1.9, $^3J_{3'a\text{-}2'a}$

= 1.9, $^3J_{3'a-2''a}$ = 6.1, $^3J_{3'a-P}$ = 10.0 Hz, 1 H, $H_{3'a}$), 4.70 (A part of an ABX syst, ^3J = 12.0, J_P = 6.2 Hz, 1 H, $H_{5'b}$), 4.66 (A part of an ABX syst, ^3J = 11.8, ^3J_P = 6.7 Hz, 1 H, $H_{5''b}$), 4.42 (dd, $^3J_{3'b-2''b}$ = 3.4, $^3J_{3'b-2'b}$ = 6.4 Hz, 1 H, $H_{3'b}$), 4.39 (B part of an ABX syst, ^3J = 2.5, 12.0 Hz, J_P = 20 Hz, 1 H, $H_{5'b}$), 4.35 (B part of an ABX syst, ^3J = 2.5, 11.8, J_P = 19 Hz, 1 H, $H_{5''b}$), 4.30 (m, 1 H, $H_{4'a}$), 3.85 and 3.84 (ABX syst, ^3J = 3.0, 3.5, 12.5 Hz, 2 H, 2 × $H_{5'a}$), 2.58 (A part of an ABX syst, $^3J_{2'a-3'a}$ = 1.8, $^3J_{2'a-1'a}$ = 5.8, $^3J_{2'a-2''a}$ = 14.2 Hz, 1 H, $H_{2'a}$), 2.52 (A part of an ABX syst, $^3J_{2'b-3'b}$ = 6.4, $^3J_{2'b-1'b}$ = 7.2, $^3J_{2'b-2'b}$ = 14.1 Hz, 1 H, $H_{2'b}$), 2.42 (B part of an ABX syst, $^3J_{2''a-3'a}$ = 6.4, $^3J_{2''a-1'a}$ = 8.7, $^3J_{2''a-2'a}$ = 14.3 Hz, 1 H, $H_{2''a}$), 2.38 (B part of an ABX syst, $^3J_{2''b-3'b}$ = 3.4, $^3J_{2''b-1'b}$ = 6.7, $^3J_{2''b-2'b}$ = 14.0 Hz, 1 H, $H_{2''b}$), 1.91 (d, $^4J_{7-6}$ = 0.9 Hz, 3 H, H_7), 1.89 (d, $^4J_{7-6}$ = 0.9 Hz, 3 H, H_7) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 166.3 (C_4), 166.3 (C_4), 152.5 (C_2), 152.3 (C_2), 138.7 (C_6), 138.1 (C_6), 112.1 (C_5), 111.9 (C_5), 87.9 ($C_{1'a}$), 87.1 ($^3J_{4'a-P}$ = 5.2 Hz, $C_{4'a}$), 86.4 ($C_{1'b}$), 82.7 ($^3J_{4'b-P}$ = 6.3 Hz, $C_{4'b}$), 81.5 ($^2J_{3'a-P}$ = 4.0 Hz, $C_{3'a}$), 73.4 ($C_{3'b}$), 73.4 ($C_{5'b}$), 72.1 ($^2J_{5''b-P}$ = 8.6 Hz, $C_{5''b}$), 62.9 ($C_{5'a}$), 40.1 ($C_{2'b}$), 39.5 ($^3J_{2'a-P}$ = 4.5 Hz, $C_{2'a}$), 12.6 (C_7), 12.5 (C_7) ppm. ^{31}P NMR (202 MHz, CD_3OD): δ = 63.8 ($J_{P\text{-}Se}$ = 1009.3 Hz) ppm. HRMS (ESI): calcd for $C_{21}H_{26}N_4O_{11}PSe$ 621.0502 $[M\text{-}H]^-$; found 621.0499.

α,β,γ-seleno-D-CNA *trans* (24b): Following the general procedure D starting from **22b** gave the product **24b** (quant., 8 mg). ^{1}H NMR (500 MHz, CD_3OD): δ = 7.81 (d, $^4J_{6-7}$ = 0.9 Hz, 1 H, H_{6a}), 7.47 (d, $^4J_{6-7}$ = 0.9 Hz, 1 H, H_{6b}), 6.33 (dd, $^3J_{1'b-2''b}$ = 6.1, $^3J_{1'b-2'b}$ = 8.0 Hz, 1 H, $H_{1'b}$), 6.31 (dd, $^3J_{1'a-2'a}$ = 6.0, $^3J_{1'a-2''a}$ = 8.5 Hz, 1 H, $H_{1'a}$), 5.36 (dddd, 1 H, $^3J_{3'a-4'a}$ = 1.5, $^3J_{3'a-2'a}$ = 1.5, $^3J_{3'a-2''a}$ = 6.0, $^3J_{3'a-P}$ = 10.0 Hz, $H_{3'a}$), 4.70 (A part of an ABX syst, ^3J = 12.0, J_P = 8.5 Hz, 1 H, $H_{5'b}$), 4.60 (A part of an ABX syst, ^3J = 12.0, J_P = 9.5 Hz, 1 H, $H_{5''b}$), 4.47 (dd, $^3J_{3'b-2''b}$ = 3.0, $^3J_{3'b-2'b}$ = 6.0 Hz, 1 H, $H_{3'b}$), 4.38 (B part of an ABX syst, ^3J = 1.5, 12.0, J_P = 11.5 Hz, 1 H, $H_{5'b}$), 4.34 (B part of an ABX syst, ^3J = 2.0, 12.0, J_P = 14.0 Hz, 1 H, $H_{5''b}$), 4.26 (m, 1 H, $H_{4'a}$), 3.77 (m, 2 H, $H_{5'a}$), 2.54 (A part of an ABX syst, $^3J_{2'b-3'b}$ = 6.0, $^3J_{2'b-1'b}$ = 8.0, $^3J_{2'b-2'b}$ = 14.1 Hz, 1 H, $H_{2'b}$), 2.52 (A part of an ABX syst, $^3J_{2'a-3'a}$ = 1.1, $^3J_{2'a-1'a}$ = 5.9, $^3J_{2'a-2''a}$ = 13.9 Hz, 1 H, $H_{2'a}$), 2.39 (B part of an ABX syst, $^3J_{2''a-3'a}$ = 6.4, $^3J_{2''a-1'a}$ = 8.0, $^3J_{2''a-2'a}$ = 14.2 Hz, 1 H, $H_{2''a}$), 2.32 (B part of an ABX syst, $^3J_{2''b-3'b}$ = 2.8, $^3J_{2''b-1'b}$ = 6.0, $^3J_{2''b-2'b}$ = 13.8 Hz, 1 H, $H_{2''b}$), 1.91 (d, $^4J_{7-6}$ = 0.9 Hz, 3 H, H_7), 1.88 (d, $^4J_{7-6}$ = 0.9 Hz, 3 H, H_7) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 166.5 (C_4), 166.4 (C_4), 152.6 (C_2), 152.4 (C_2), 138.7 (C_{6b}), 138.1 (C_{6a}), 112.3 (C_5), 112.0 (C_5), 87.4 ($C_{1'b}$), 87.3 ($^3J_{4'a-P}$ = 4.6 Hz, $C_{4'a}$), 86.4 ($C_{1'a}$), 82.7 ($^3J_{4'b-P}$ = 6.0 Hz, $C_{4'b}$), 82.2 ($^2J_{3'a-P}$ = 6.3 Hz, $C_{3'a}$), 73.2 ($^2J_{5'b-P}$ = 6.8 Hz, $C_{5'b}$), 72.9 ($C_{3'b}$), 71.4 ($^2J_{5''b-P}$ = 6.9 Hz, $C_{5''b}$), 62.8 ($C_{5'a}$), 39.6 ($C_{2'b}$, $C_{2'a}$), 12.6 (C_7), 12.5 (C_7) ppm. ^{31}P NMR (202 MHz, CD_3OD): δ = 65.5 ($J_{P\text{-}Se}$= 992.3 Hz) ppm. HRMS (ESI): calcd for $C_{21}H_{26}N_4O_{11}PSe$ 621.0502 $[M\text{-}H]^-$; found 621.0500.

5'-*O*-Dimethoxytrityl-3'-*O*-[(cyanoethyl)(diisopropylamino)-selenophosphamide]-α,β-D-CNA (S_C) phosphite (25): Compound **9b** (432 mg, 0.46 mmol) was dissolved under argon in dry THF (3 mL) at room temperature. *N*-Ethyldi*iso*propylamine (0.32 mL, 1.84 mmol) and then 2-cyanoethyl-*N*,*N*-di*iso*propylchlorophosphoramidite (242 mg, 0.92 mmol) were added. The reaction mixture was stirred for 2 h, the white precipitate was filtered and the filtrate diluted in EtOAc saturated with argon. The organic layer was washed with a cold aqueous solution of potassium carbonate (10%), dried over $MgSO_4$, filtered and concentrated with care under reduced pressure. The crude was then purified by silica gel column chromatography eluting with a mixture of EtOAc/Et_3N (10:0.02) to give **25** (85%, 443 mg) contaminated with inseparable residual *H*-phosphonate) as a white foam. ^{31}P NMR (121 MHz, CD_3OD): δ = 131.0, 130.5, 74.2, 73.7 ppm. MS (ESI): *m/z* = 1161.3 $[M+Na]^+$.

5'-*O*-Dimethoxytrityl-3'-*O*-[(cyanoethyl)(diisopropylamino)-selenophosphamide]-α,β-D-CNA (S_C,S_P) (26): To **9b** (5 mg) in a NMR tube was added a commercially available solution of iodine I_2 (0.75 ml, 0.02 M, THF/pyridine/water 70:20:10) used during oligonucleotide synthesis. ^{31}P NMR spectrum was recorded 5 min after addition. ^{31}P NMR (121 MHz, no lock): δ = 74.3, 73.5, - 8.00, -8.12 ppm.

5'-*O*-Dimethoxytrityl-3'-*O*-[(cyanoethyl)(diisopropylamino)-selenophosphamide]-thio-α,β-D-CNA (S_C,S_P) (27): Compound **15b** (328 mg, 0.31 mmol) was dissolved under an argon atmosphere in dry THF (2 mL) at room temperature. *N*-Ethyldi*iso*propylamine (0.21 mL, 1.24 mmol) and then 2-cyanoethyl-*N*,*N*-di*iso*propylchlorophosphoramidite (146 mg, 0.62 mmol) were added. The reaction mixture was stirred for 2 h, the white precipitate was filtered and the filtrate diluted in EtOAc saturated with argon. The organic layer was washed with a cold aqueous solution of potassium carbonate (10%), dried over $MgSO_4$, filtered and concentrated with care under reduced pressure. The crude was then dissolved in a mixture of acetonitrile/toluene (1:1, 4 mL) and pyridine (2 mL) and elemental sulfur S_8 (290 mg, 1.13 mmol) were added. The reaction mixture was heated at 60°C for 1 h and was allowed to cool down to r.t. before the filtration of the residual S_8. The filtrate was then diluted in EtOAc and the organic layer washed with water, dried over $MgSO_4$, filtered and concentrated to give **27** as a yellow foam (81%, 286 mg, contaminated with inseparable residual *H*-phosphonate). ^{31}P NMR (121 MHz, $CDCl_3$): δ = 74.0, 73.3, 60.14, 60.10 ppm.

5'-*O*-Dimethoxytrityl-3'-*O*-[(cyanoethyl)(diisopropylamino)-phosphoramidite]-thio-α,β-D-CNA (S_C,S_P) (28): Compound **27** (286 mg, 0.25 mmol) was then dissolved in anhydrous acetonitrile (2 mL) and a solution of $P(NMe_2)_3$ (C = 0.5 M, 1 mL, 0.50 mmol) was added. The reaction mixture was stirred at r.t. for 3 h under an argon atmosphere. The solution was hydrolyzed with a cold aqueous solution of potassium carbonate (10%) then diluted in EtOAc saturated with argon. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated with care under reduced pressure. ^{31}P NMR analysis of the crude indicated the presence of a signal at 82.7 ppm caracteristic of $SeP(NMe_2)_3$.[28] The crude was then purified by silica gel column chromatography eluting with a mixture of ether EtOAc/petroleum ether (7:3) with 10% of Et_3N to give **28** (41%, 110 mg) as a white foam. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 150.0, 148.7, 60.13, 60.12 ppm. HRMS (ESI): calcd for $C_{52}H_{65}N_6O_{14}P_2S$ 1091.3755 $[M+H]^+$; found 1091.3730.

Triethylammonium salt of 5'-*O*-dimethoxytrityl-3'-*O*-*H*-phosphonate-α,β,γ-seleno-D-CNA *cis* (29): To a solution of **19b** (111 mg, 0,12 mmol) in anhydrous pyridine (600 μL) was added diphenylphosphonate (115 μL, 0.60 mmol). The reaction mixture was stirred at r.t. for 45 min then cooled to 0°C and H_2O (50 μL) and Et_3N (50 μL) were added. After 15 min, the reaction mixture was poured into a saturated solution of $NaHCO_3$ (5 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over $MgSO_4$ and then concentrated *in vacuo*. Addition of the minimum volume of EtOAc led to the precipitatation of the product. After filtration, the solid was further purified by silica gel chromatography, eluting with a mixture of EtOAc/MeOH/Et_3N (90:10:0.05) to yield **29** as a white solid (114 mg, 88%). As already observed for **19b**, the NMR spectra were recorded with a very poor resolution for ^{1}H and with a low detection level of the aliphatic carbon for ^{13}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.56 (s, 1 H, H_6), 7.38-7.16 (m, 10 H, H_6 and Ph), 6.83 (d, $^1J_{H\text{-}P}$ = 629.8 Hz, 1 H, H-P), 6.81 (d, ^3J = 8.7 Hz, 4 H, Ph), 6.39 (dd, ^3J = 5.6, 8.6 Hz, 1 H, $H_{1'a}$), 6.11 (t, ^3J = 6.2 Hz, 1 H, $H_{1'b}$), 5.48-5.43 (m, J_P = 10.7 Hz, 1 H, $H_{3'a}$), 4.92 (m, 1 H, $H_{3'b}$), 4.77 (m, 2 H, 2

× $H_{5'b}$), 4.40-4.13 (m, 3 H, 2 × $H_{5''b}$, $H_{4'a}$), 3.75 (s, 6 H, OMe), 3.52 and 3.37 (m, 2 H, 2 × $H_{5'a}$), 2.97 (m, 6 H), 2.68-2.38 (m, 4 H, $H_{2'}$), 1.86 (s, 3 H, H_7), 1.36 (s, 3 H, H_7), 1.25 (t, 9 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.2, 164.1, 158.9, 150.8, 150.4, 144.4, 135.4, 135.3, 130.29, 130.26, 128.8, 127.4, 113.6, 111.8, 111.1, 87.5, 86.9, 84.8, 84.6, 80.5, 73.8, 71.2, 63.7, 55.5, 47.1, 39.2, 38.8, 29.9, 23.3, 22.9, 12.1, 11.9, 9.2 ppm. ^{31}P NMR (121 MHz, $CDCl_3$): δ = 63.9, 2.9 ppm. HRMS (ESI): calcd for $C_{42}H_{45}N_4O_{15}P_2Se$ 987.1527 $[M]^-$; found 987.1541.

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Keywords: Nucleotide • oligonucleotide • structure • dioxaphosphorinane • selenium

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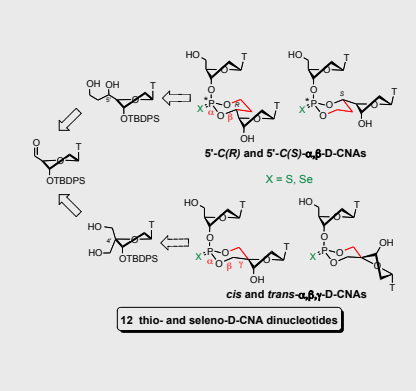
FULL PAPER

We report the synthesis and structural determination of thio- and seleno-α,β- and α,β,γ-Constrained Nucleic Acid dinucleotides (D-CNA) in which a dioxaphosphorinane ring locks two or three torsional angles of the sugar/phosphate backbone. For solid phase synthesis, selono-CNA H-phoshonates were prepared to address selenium migration occurring during the synthesis of seleno-CNA phosphoramidites.



Nucleotides

Béatrice Gerland, Claudia Addamiano, Brice-Loïc Renard, Corinne Payrastre, Deshmukh Gopaul and Jean-Marc Escudier**

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Thio and seleno-Dioxaphosphorinane -Constrained dinucleotides (D-CNA), synthesis and conformational study