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Diastereoselective and Regioselective Synthesis of Conformationally Restricted Thio-dioxa- and Oxo-oxathiaphosphorinane Dinucleotides Featuring Noncanonical α/β Torsion Angle Combinations (α,β-CNAs)

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Control of the regioselective cyclisation of a stereodefined 5′-C-tosyloxy phosphorothioate dinucleotide unit to provide conformationally restricted dinucleotides of either thio-dioxa- or oxo-oxathiaphosphorinane types is described. NMR structural analysis of the newly synthesized building units showed the α and β torsional angles to be constrained to noncanonical values {*gauche*(+), *trans*} or {*anticlinal*(–), *trans*}.

Introduction

Conformational restrictions in nucleic acid structures have been introduced and studied mainly with the goal of improving the abilities of these analogues to form stable Watson–Crick (WC) duplexes with complementary nucleic acid targets.[1–4] It is therefore desirable that chemical modifications made on natural nucleic acids should preserve, as much as possible, the two major interactions that stabilize the double-helical structures of nucleic acid duplexes: namely the WC hydrogen bonds and base stacking interactions. In structural terms, the most promising DNA/RNA analogues are usually expected to be those in which the backbone torsion angles closely match the geometries of torsion angles α–ζ in standard A- or B-type duplexes.

The preparation of well defined structures in nucleic acids requires control over the backbone torsion angles, still a particularly challenging task in the field of oligonucleotide chemistry.[5–6] The capacity to control the α–ζ dihedrals along the sugar-phosphate backbone would help structural chemists better understand the complex interrelationship that underlies these torsions and the overall solution structures of DNA and RNA, and would also allow the physical, biochemical and biological properties of various backbone geometries to be studied, irrespective of the base sequence.

From the concept of structural preorganization,[7] it could be anticipated that if the conformational states of an oligonucleotide single strand could be locally reduced to those that match the geometry of this strand in the target structure, then an entropic benefit could be expected, and that this should result in an overall stabilization of the structure. One possible approach through which to preorganize single-stranded oligonucleotides is to include one or more backbone bonds (α–ζ) within a well-defined ring structure.

We have undertaken an experimental program to explore the chemistry of DNA and RNA analogues containing modified backbones that might, at least in principle, favour unusual helical conformations as well as the formation of structurally well defined non-base-pair states. As an initial step in this direction, we have already reported on the synthesis of a covalently constrained dinucleotide building unit in which α and β torsions are locked in a noncanonical (g^+, *t*) conformation that frequently occurs in protein–DNA complexes and in bulged regions of nucleic acids.[8] Our approach is based on the introduction of the neutral 1,3,2-dioxaphosphorinane ring structure at key positions along the sugar-phosphate backbone (Figure 1). We reasoned that the resulting constrained nucleic acids (D-CNAs) could locally adopt either helical or nonhelical conformations, depending on the spatial arrangements of the dioxaphosphorinane systems and on which backbone bonds were included

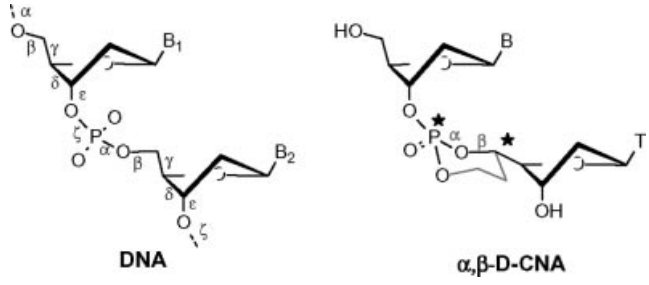

Figure 1. Left: the six backbone torsion angles (labelled α to ζ) in nucleic acids. Right: α,β-D-CNA units are dinucleotides in which α and β are stereocontrolled by dioxaphosphorinane ring structures featuring two new asymmetric centres.

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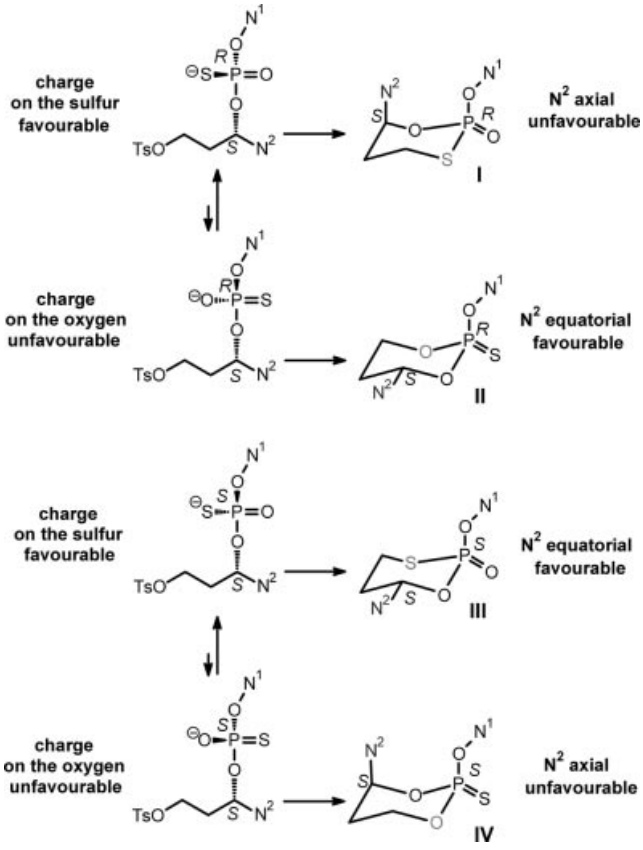
in the ring structures. This approach was usefully applied to prepare α,β-D-CNAs with exceptional binding properties associated with the introduced constraint, regardless of the charge reduction[9] and dinucleotide units, with α,β,γ and δ,ε,ζ possessing noncanonical values (α,β,γ-D-CNA and δ,ε,ζ-D-CNA).[10]

However, the synthetic route developed for α,β-D-CNA dinucleotides allowed us to prepare only three out of the four possible diastereoisomers efficiently.[11] In order to extend the α,β-torsional angle value set and therefore to provide access to an additional α,β-CNA dinucleotide unit exhibiting new noncanonical values, we explored a novel synthetic pathway through the cyclisation of stereodefined phosphorothioate esters.

Results and Discussion

Our previous retrosynthetic approach towards dioxaphosphorinane-constrained dinucleotide building blocks had been based on steric and electronic control in the cyclisation of the 5′C-tosyloxy dinucleotide phosphate precursor. Because of the high selectivity of the *pro-R* oxygen attack for the cyclisation of the 5′-C(*S*)-configured dinucleotide precursor, the (S_C,S_P) isomer of the α,β-D-CNA cannot be prepared in a reasonable yield for further investigation.[11] We therefore anticipated that replacement of one of the oxygen atoms in the phosphate group by a sulfur might help to induce the formation of a geometrical analogue of the previously inaccessible α,β-D-CNA isomer. Note that in the Cahn–Ingold–Prelog system the same geometries are designated by (S_C,S_P) for the α,β-dioxaphosphorinane-CNA and by (S_C,R_P) for the α,β-oxo-oxathiaphosphorinane-CNA isomers.

Whereas the attack of the oxygen atom (O-cyclisation) could give rise to the thio-dioxaphosphorinane (denoted as **II** in Scheme 1) with all the substituents in the correct positions, we expected that the preferred charge localization on the sulfur atom, despite the unfavourable conformation of the six-member transition state cycle with the N_2 substituent in the axial position, could be a determining element (S-cyclisation) for the formation of the desired isomer (denoted as **I** in Scheme 1), if starting from the (S_C,R_P) isomer of the acyclic thiophosphate precursor. In contrast, if starting from the (S_C,S_P) isomer of the acyclic thiophosphate precursor, the regiochemical outcome of the cyclisation reaction should be only in favour of the formation of the oxo-oxathiaphosphorinane (denoted as **III** in Scheme 1) because of the nucleophile behaviour of the sulfur atom together with the favoured transition state cycle substituted with N_2 in an equatorial position and ON_1 in the axial one.



Scheme 1. Elements for regioselective S- and O-cyclisation. N^1 and N^2 stand for the remaining atomic fragments that define the upper and lower nucleoside units, respectively.

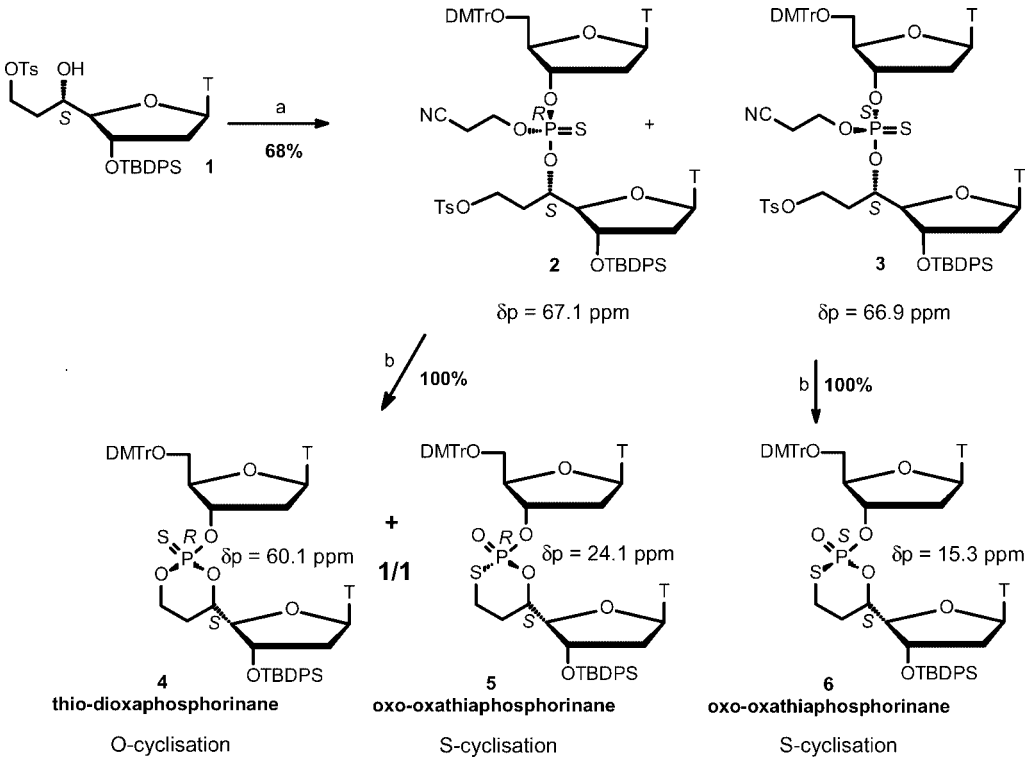
Synthesis of the ($S_{C5'}$,R_P)-Thio-dioxa- and ($S_{C5'}$,R_P)- or ($S_{C5'}$,S_P)-Oxo-oxathiaphosphorinane Diastereoisomers of the α,β-CNA TT Dimer

A diastereoisomeric mixture of the thiophosphate dinucleotides **2** and **3** was prepared in an equimolar ratio by standard procedures from the diastereopure 5′-C(*S*)-tosyloxyethyl thymidine **1** (Scheme 2).[12] They were easily separated by silica gel chromatography. The more rapidly eluted compound was further identified (by its ability to cyclise, see Scheme 1) as the (S_C,R_P) isomer **2** (δ_P = 67.1 ppm) and so the more slowly eluted compound was the (S_C,S_P) isomer **3** (δ_P = 66.9 ppm).

We first removed the cyanoethyl phosphate protective groups of **2/3** under our previously described conditions (Et_3N, DMF, 90 °C), therefore promoting the formation of the phosphorinane ring. As planned, **3** underwent cyclisation into a single isomer characterized by ^{31}P NMR spectroscopy as the oxo-oxathiaphosphorinane **6** by a chemical shift of 15.3 ppm. From **2**, on the other hand, we observed the formation of two compounds through the O- and S-cyclisation pathways in a 1:1 ratio.

The O-cyclisation provided the thio-dioxaphosphorinane **4** (δ_P = 60.1 ppm), while the attack of the sulfur afforded the oxo-oxathiaphosphorinane **5** (δ_P = 24.1 ppm), the geometrical analogue of the inaccessible α,β-D-CNA (S_C,S_P).[11] In order to modify the ratio of O- to S-cyclisation, we investigated various sets of reaction conditions (base, temperature, counterion). The results are summarized in Table 1.

When the temperature was increased to 150 °C, the S-cyclisation pathway became predominant, showing that



Scheme 2. Reagents and conditions. a) (i) thymidine phosphoramidite (3 equiv.), tetrazole (15 equiv.), CH_3CN, room temp., 45 min. (ii) toluene, Pyr, S_8 (4 equiv.). b) NEt_3 (4 equiv.), DMF, 90 °C, 2 h.

Table 1. Reaction conditions parameters for the regioselective cyclisation of **2** into the thio-dioxaphosphorinane **4** (O-cycl) or into the oxo-oxathiaphosphorinane **5** (S-cycl).

	Base	*T* [°C]	Salt	O-cycl vs. S-cycl [%][a]
1	Et_3N	90	–	50:50
2	Et_3N	150	–	15:85
3	Et_3N	90	$CdCl_2$	50:50
4	Et_3N	90	$MgCl_2$	0:100
5	Cs_2CO_3	25	–	57:43
6	K_2CO_3	25	–	57:43
7[b]	K_2CO_3	25	$MgCl_2$	10:90
8[c]	K_2CO_3	25	$CdCl_2$	100:0

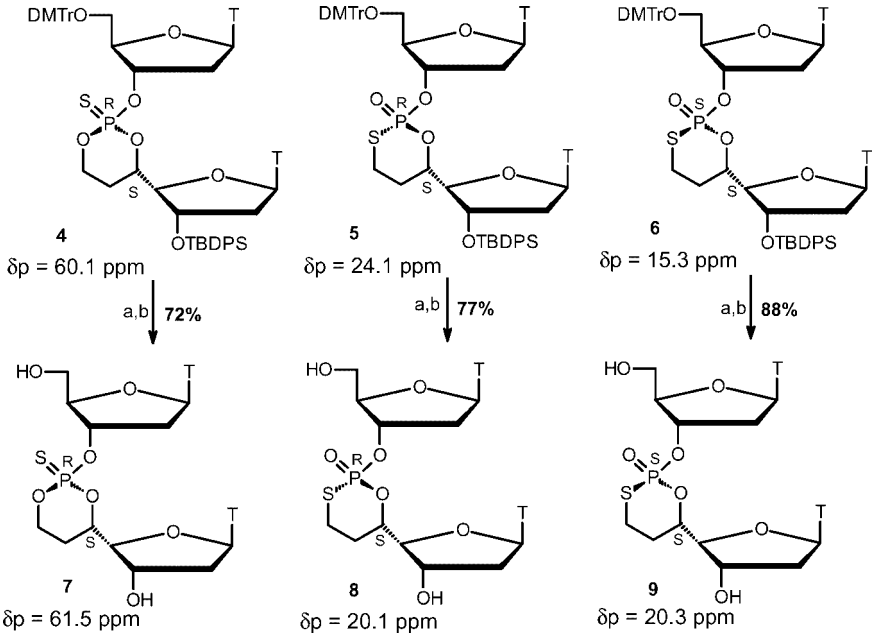
[a] Relative percentages were determined by ^{31}P NMR after quantitative conversion of **2**. [b] Reaction time was extended to 15 d. [c] Reaction time was extended to 18 d.

lowering of the ionic pair strength can increase the selectivity of the cyclisation process (Table 1, Entry 2). We therefore considered changing the counterion of the phosphorothiate from triethylammonium to cadmium or magnesium. Whereas addition of a cadmium salt did not change the ratio of the O- to S-cyclisation (Entry 3), the low affinity of magnesium towards sulfur anion provided, as expected,[13] a loose ionic pair very favourable to the selective formation of the oxo-oxathiaphosphorinane **5** (**4/5** = 0:100, Entry 4). In a same way, the use of Cs_2CO_3 or K_2CO_3 as base slightly increased the ratio in favour of the O-cyclisation (Entries 5 and 6). In these cases, the potassium cation can be effectively replaced by cadmium or magnesium with dramatic effects on the cyclisation process. The reaction became very slow and highly selective in both cases (O-cycl to S-cycl from 10:90 to 100:0, Entries 7 and 8). To obtain evidence on the cation effect, we measured ^{31}P chemical shifts at 54.2, 55.0 and 51.9 ppm, corresponding to the reaction conditions with K_2CO_3, $K_2CO_3/MgCl_2$ and $K_2CO_3/CdCl_2$, respectively.[14] The displacement from 51.9 to 55.0 ppm is indicative of a change in the strength of the ionic pair between the phosphorothioate and cadmium or magnesium. The lower chemical shift observed for cadmium shows a tight ionic pair favourable for the O-cyclisation, whereas the weak ionic pair between sulfur and magnesium is characterised by a higher chemical shift showing a more dissociated ionic pair resulting in the formation of the oxo-oxathiaphosphorinane as the major compound.

These favourable reaction conditions found for the selective O-cyclisation of **2**, when applied to **3**, failed to provide any thio-dioxophosphorinane formation because of the highly favourable nature of the S-cyclisation process (Scheme 1).

Structural Assignment of Thio-dioxa- and Oxo-oxathiaphosphorinane α,β-CNA TT Dimers

In order to obtain solution-state structure determinations of both isomers, independently of protective groups, by NMR spectroscopy, we removed the 5′-*O*-dimethoxytrityl and the 3′-*O*-*tert*-butyldiphenylsilyl groups by standard procedures to provide the dinucleotides **7**, **8** and **9** from the corresponding thio-dioxaphosphorinane **4** or oxo-oxathiaphosphorinane **5** and **6**, respectively (Scheme 3).



Scheme 3. Reagents and conditions. a) nBu$_4$NF (1.1 equiv.), THF, room temp., 1 h. b) 2% TFA/CH_2Cl_2, room temp., 15 min.

Like that of the dioxaphosphorinane ($S_{C5'}$,R_P) isomer,[8] the chair conformation of **7** is clearly established from the ^{1}H and ^{1}H-{^{31}P} NMR spectra, with no detectable coupling constant between the 5′-H involved in the thio-dioxaphosphorinane system and phosphorus ($^3J_{H5',P} < 1$ Hz), which is characteristic of an axial position of this proton.[15] The observation of small (1 Hz) and large (24 Hz) $^3J_{H,P}$ coupling constants between the dioxaphosphorinane 7′-H protons and phosphorus are also indicative of axial and equatorial positions, respectively, for these protons,.

In contrast, average values of 15.3 and 23.0 Hz were observed for the $^3J_{H,P}$ coupling constants involving the two 7′-H protons of **8**, thus suggesting that the oxo-oxathiaphosphorinane structure of this ($S_{C5'}$,R_P)-configured isomer (Scheme 1) is in a twist-chair conformation. This "in-between" conformation is probably the result of the involvement of conflicting steric and anomeric effects in either chair conformation of **8** due to the *cis* relationship between the ON_1 and N_2 groups.

Other important structural information relating to compounds **7**, **8** and **9** can be found in the observation of long-range coupling constants between the 4′-H protons of the lower sugar units and phosphorus ($^4J_{H4',P}$ = 4.6, 5.0 and 5.7 Hz for **7**, **8** and **9**, respectively). These couplings are indicative of typical W-shaped P–O5′–C5′–C4′–H4′ junctions, which is consistent with *gauche*(+) conformations of γ in **7** and **9** and *anticlinal*(–) conformations of γ in **8**. The W-shaped P–O5′–C5′–C4′–H4′ junction deduced in the case of **8** also suggests that the backbone torsion angles α and β are only slightly affected by the conformational change involved between the true chair and twist-chair forms of **8**. Overall, these NMR spectroscopic data allow us to assign the conformations of the backbone torsion angles α, β and γ as (α, β, γ) = (g^+, t, g^+) for **7** and **9** and (α, β, γ) = (a^-, a^+/t, a^-) for **8**. In addition, the empirical equation established by Lankhorst et al.[16] can be used to determine – from the coupling constants $J_{H3',P}$ (5.9 Hz for **7**, 5.8 Hz for **8** and 6.4 Hz for **9**) – the values of the torsional angles ε (around –160° for all) and therefore the relative positions of the upper nucleosides in relation to the phosphorus triester linkages.

The puckerings of the 2′-deoxyribose moieties of **7**, **8** and **9** in solution were assigned by examination of the sugar ring H/H coupling constants (Table 2). The small $J_{H3',H4'}$ coupling constants measured for **7**, **8** and **9** and the values of $J_{H2',H3'}$ and $J_{H1',H2'}$ are consistent with the standard C2′-*endo* conformations previously observed in natural 2′-deoxyribose units.[17]

Table 2. H,H coupling constants (Hz) in the ^{1}H NMR spectra (400 MHz) of α,β-CNA TT dimers **7**, **8** and **9** (n.d. = not determined).

		Coupling constant J (Hz)				
	Nucleoside	J(1′,2′)		J(2′,3′)		J(3′,4′)
7	upper	5.6	8.7	5.2	1.7	1.7
	lower	6.2	8.0	n.d.	n.d.	2.6
8	upper	5.7	8.5	5.8	1.9	1.7
	lower	6.2	7.6	6.3	3.3	2.9
9	upper	5.7	8.6	6.0	1.9	1.9
	lower	7.4	7.4	6.0	3.0	2.9

Conclusions

Regioselective cyclisations of a stereodefined 5′-C-tosyloxy phosphorothioate dinucleotide unit to provide conformationally restricted dinucleotides (α,β-CNAs) of either thio-dioxa- or oxo-oxathiaphosphorinane types has been described. We have shown that the regiochemical outcome

of the cyclisation process can be controlled by addition of suitable cations (Mg^{2+} or Cd^{2+}) to the reaction medium.

Structural analysis by means of NMR spectroscopy indicates that the ($S_{C5'}$,S_P) diastereoisomer of the oxo-oxathiaphosphorinane and the ($S_{C5'}$,R_P)-thio-dioxaphosphorinane have their internucleotide linkages in true chair conformations with α and β locked in the atypical (g^+, *t*) conformations frequently found in B-type duplexes in complexation with proteins or in bulged or loop region of nucleic acids (Table 3). The remaining ($S_{C5'}$,R_P)-oxo-oxathiaphosphorinane diastereoisomer is characterized by the very unusual *anticlinal*(–) conformation of α and the twist-chair conformation of its phosphorinane linkage. The puckering mode of the 2′-deoxyribose units, however, is clearly C2′-*endo* (S-type), which is characteristic of B-type duplexes.

Table 3. Summary of the backbone torsion angles α, β and γ in the oxo-oxathia- and thio-dioxaphosphorinane α,β-CNA dimeric units.[a]

Diastereoisomer	*α*	*β*	*γ*
thio-dioxa ($R_{C5'}$,S_P)	g^+	t	g^+
oxo-oxathia ($S_{C5'}$,R_P)	a^-	a^+/t	a^-
oxo-oxathia ($S_{C5'}$,S_P)	g^+	t	g^+

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

We therefore have available an increasing number of building units featuring noncanonical values of the α torsional angles, providing unique opportunities to explore their capabilities to stabilize unpaired structures of nucleic acids such as bulges or hairpin loops.

Experimental Section

Cyanoethyl 3′-*O*-(5′-*O*-Dimethoxytrityl)thymidinyl-5′-C(*S*)-tosyloxyethyl-(3′-*tert*-butyldiphenyl silyl)thymidinyl Thiophosphoric Esters (S_C,R_P)-2 and (S_C,S_P)-3: 3′-*O*-*tert*-Butyldiphenylsilyl-5′-C(*S*)-(tosyloxyethyl)thymidine (**1**, 500 mg, 0.736 mmol), thymidine $O_{3'}$-phosphoramidite (1.1 g, 1.47 mmol) and freshly sublimed tetrazole (515 mg, 7.36 mmol) were dissolved in anhydrous acetonitrile (10 mL) and the mixture was stirred for 45 min at room temperature. After addition of pyridine (7.7 mL, 95.3 mmol), the phosphite was oxidized with S_8 (752 mg, 2.94 mmol) and toluene (7 mL). The reaction mixture was stirred for 1 h and diluted with ethyl acetate and the organic layer was washed with an aqueous solution saturated with $NaHCO_3$, with water and with brine. The organic layer was then dried with $MgSO_4$, the solvent was removed in vacuo, and the crude material was chromatographed on silica gel with ethyl acetate/petroleum ether (7:3) as eluent. After evaporation of the solvent, compound **2** and **3** were recovered as white foams (320 mg of **2** and 325 mg of **3**, 68% yield).

Data for 2: TLC, R_f (AcOEt/petroleum ether, 8:2) = 0.44. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 9.36 and 9.20 (2 × s, 2 H, NH), 7.77–6.82 (m, 29 H, Ph and 6-H), 6.50 and 6.24 (2 × m, 2 H, 1′a-H and 1′b-H), 5.16–5.12 (m, 1 H, 3′a-H), 4.39–3.90 (m, 8 H, 3′b-H, 4′-H, 5′-H and 2 × CH_2), 3.79 (s, 6 H, Me DMTr), 3.49–3.33 (m, 2 H, CH_2), 2.44 (s, 3 H, Me Ts), 2.30–2.10 (m, 4 H, 2 × CH_2), 1.87 (s, 6 H, 2 Me), 1.47 and 1.28 (2 × m, 4 H, 2 × CH_2), 1.06 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 163.7, 158.8, 150.5, 150.4, 144.9, 144.1, 135.8–111.5, 87.3, 87.2, 84.8, 84.3, 79.8, 74.7, 69.6, 63.1, 62.5, 55.3, 40.0, 38.7, 26.8, 24.5, 21.6, 19.1, 19.0, 12.6, 11.8 ppm. ^{31}P NMR (81 MHz, $CDCl_3$): δ = 67.1 ppm. MS (FAB, MNBA): 1376 $[M + Na]^+$, 1353 $[M + H]^+$.

Data for 3: TLC, R_f (AcOEt/petroleum ether, 8:2) = 0.30. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 9.33 and 9.11 (2 × s, 2 H, NH), 7.73–6.82 (m, 29 H, Ph and 6-H), 6.52 and 6.32 (2 × m, 2 H, 1′a-H and 1′b-H), 5.29–5.27 (m, 1 H, 3′a-H), 4.36–3.82 (m, 8 H, 3′b-H, 4′-H, 5′-H and 2 × CH_2), 3.78 (s, 6 H, Me DMTr), 3.35–3.31 (m, 2 H, CH_2), 2.59–2.52 (m, 2 H, CH_2), 2.41(s, 3 H, Me Ts), 2.29–2.22 (m, 2 H, CH_2), 1.89 and 1.84 (2 × s, 6 H, 2 Me), 1.47 and 1.25 (2 × m, 4 H, 2 × CH_2), 1.06 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (63 MHz, $CDCl_3$): δ = 163.8, 158.8, 150.6, 144.9, 144.0, 135.8–111.7, 87.3, 84.5, 79.8, 74.5, 69.4, 63.2, 62.8, 55.3, 39.8, 39.0, 26.9, 24.5, 21.6, 19.2, 19.0, 12.6, 11.8 ppm. ^{31}P NMR (81 MHz, $CDCl_3$): δ = 66.9 ppm. MS (FAB, MNBA): 1376 $[M + Na]^+$, 1353 $[M + H]^+$.

Thio-dioxaphosphorinane Derivative (S_C,R_P)-4: K_2CO_3 (34 mg, 0.250 mmol) and $CdCl_2$ (20 mg, 0.103 mmol) were added under argon to compound **2** (20 mg, 0.014 mmol) dissolved in anhydrous DMF (2 mL). After 18 d of stirring at room temperature, the reaction mixture was diluted with ethyl acetate and washed with water and brine. The organic layer was then dried with $MgSO_4$ and the solvent was removed in vacuo to provide compound **4** (15 mg, 95% yield). TLC, R_f (AcOEt) = 0.60. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.63 and 9.37 (2 × s, 2 H, NH), 7.61–6.80 (m, 25 H, Ph, 6a-H and 6b-H), 6.58 (dd, *J* = 5.6 and 9.1 Hz, 1 H, 1′b-H), 6.42 (dd, *J* = 5.2 and 9.6 Hz, 1 H, 1′a-H), 5.35 (dd, *J* = 4.6 and $J_{H,P}$ = 9.6 Hz, 1 H, 3′a-H), 4.28 (m, 1 H, 7′b-H), 4.24 (d, *J* = 5.5 Hz, 1 H, 3′b-H), 4.12 (m, 1 H, 7′b-H), 4.01 (m, 1 H, 4′a-H), 3.80 (m, 1 H, 5′b-H), 3.75 (s, 6 H, MeO), 3.66 (d, *J* = 5.3 HZ, 1 H, 4′b-H), 3.35 (m, *J* = 1.0 and 11.0 Hz, 1 H, 5′a-H), 3.33 (s, 1 H, 5′a-H), 2.41 (m, 2 H, 2′a-H), 2.23 (m, 2 H, 2′b-H and 6′b-H), 1.95 (s, 3 H, Me), 1.84 (m, 1 H, 2′b-H), 1.31 (m, 1 H, 6′b-H), 1.42 (s, 3 H, Me), 1.03 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.0, 158.9, 150.9, 150.8, 144.2, 135.9, 135.7, 135.3, 135.2, 135.1, 133.4, 132.6, 130.6, 130.4, 130.2, 128.3, 128.2, 127.4, 113.6, 112.5, 112.2, 88.0, 87.6, 85.1, 84.7, 84.6, 80.2, 78.9, 74.7, 67.5, 63.7, 55.5, 40.0, 39.2, 27.6, 27.0, 19.1, 12.7, 11.9 ppm. ^{31}P NMR (81 MHz, $CDCl_3$): δ = 60.1 ppm. MS (ESI): 1151.6 $[M + Na^+]^+$, 1167.5 $[M + K]^+$. $C_{59}H_{65}N_4O_{13}PSSi$ (1129.30): calcd. C 60.86, H 5.53, N 4.78; found: C 61.35, H 5.61, N 4.86.

Oxo-oxathiaphosphorinane Derivative (S_C,R_P)-5: Triethylamine (8 μL, 0.058 mmol) and $MgCl_2$ (5 mg, 0.053 mmol) were added under argon to compound **2** (20 mg, 0.014 mmol) dissolved in anhydrous DMF (2 mL). After 2 h of stirring at 95 °C, the reaction mixture was diluted with ethyl acetate and washed with water and brine. The organic layer was then dried with $MgSO_4$ and the solvent was removed in vacuo to provide compound **5** (15 mg, 95% yield). TLC, R_f (AcOEt) = 0.44. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.65 and 8.75 (2 × s, 2 H, NH), 7.60–6.79 (m, 25 H, Ph, 6a-H and 6b-H), 6.49 (dd, *J* = 5.5 and 8.7 Hz, 1 H, 1′b-H), 6.22 (t, *J* = 7.1 Hz, 1 H, 1′a-H), 5.30 (m, 1 H, 3′a-H), 3.71 (d, *J* = 5.7 Hz, 1 H, 3′b-H), 4.12 (s, 1 H, 4′a-H), 3.76 (s, 6 H, MeO), 3.75 (m, 1 H, 5′b-H), 3.71 (m, $J_{H,P}$ = 5.5 Hz, 1 H, 4′b-H), 3.45 and 3.36 (2 × m, *J* = 10.8, 3.0 and 2.3 Hz, 2 H, 5′a-H), 2.98 (m, $J_{H,P}$ = 15.3 Hz, 1 H, 7′b-H), 2.82 (m, $J_{H,P}$ = 23 Hz, 1 H, 7′b-H), 2.38 (m, 2 H, 2′a-H), 2.33 (m, 1 H, 2′b-H), 2.01 (m, 1 H, 6′b-H), 1.87 (m, 1 H, 2′b-H), 1.83 (s, 3 H, Me), 1.60 (m, 1 H, 6′b-H), 1.32 (s, 3 H, Me), 1.04 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.6, 158.8, 150.5, 150.1, 143.9, 135.8–127.3, 113.4, 111.6, 111.4, 88.3, 87.4, 84.3, 84.2, 79.7, 79.3, 74.4, 63.1, 55.2, 40.4, 39.7, 29.7, 27.7, 26.9, 19.0, 12.6, 1.6 ppm. ^{31}P NMR (81 MHz, $CDCl_3$): δ = 24.1 ppm.

MS (ESI) 1167.4 $[M + K^+]^+$, 1151.5 $[M + Na]^+$. $C_{59}H_{65}N_4O_{13}PSSi$ (1129.30): calcd. C 60.86, H 5.53, N 4.78; found: C 60.75, H 5.53, N 4.66.

Oxo-oxathiaphosphorinane Derivative (S_C,S_P)-6: Triethylamine (80 μL, 0.58 mmol) was added under argon to compound **3** (200 mg, 0.14 mmol) dissolved in anhydrous DMF (20 mL). After 2 h of stirring at 95 °C, the reaction mixture was diluted with ethyl acetate and washed with water and brine. The organic layer was then dried with $MgSO_4$ and the solvent was removed in vacuo to provide compound **6** (160 mg, 100% yield). TLC, R_f (AcOEt) = 0.50. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.85 and 9.43 (2×s, 2 H, NH), 7.62–6.80 (m, 25 H, Ph, 6a-H and 6b-H), 6.61 (dd, J = 5.6 and 9.1 Hz, 1 H, 1′b-H), 6.46 (dd, J = 5.0 and 9.4 Hz, 1 H, 1′a-H), 5.20 (dd, J = 5.0 and $J_{H,P}$ = 8.4 Hz, 1 H, 3′a-H), 4.30 (d, J = 5.7 Hz, 1 H, 3′b-H), 4.16 (m, 1 H, 4′a-H), 3.75 (s, 6 H, MeO), 3.68 (d, J = 6.7 Hz, 1 H, 4′b-H), 3.45 (m, J = 2.0 and 10.3 Hz, 1 H, 5′a-H), 3.37 (m, 2 H, 5′a-H and 5′b-H), 2.89 (m, 1 H, 7′b-H), 2.76 (m, 1 H, 7′b-H), 2.68 (m, 1 H, 2′a-H), 2.40 (m, 1 H, 2′a-H), 2.31 (m, 1 H, 2′b-H), 2.00 (m, 1 H, 6′b-H), 1.95 (m, 1 H, 2′b-H), 1.88 (s, 3 H, Me), 1.59 (m, 1 H, 6′b-H), 1.36 (s, 3 H, Me), 1.04 (s, 9 H, *t*Bu) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.2, 159.0, 151.1, 150.9, 144.2, 135.9, 135.8, 135.3, 133.4, 132.6, 130.5, 130.3, 130.2, 128.7, 127.5, 113.6, 112.3, 112.1, 88.9, 87.6, 85.2, 84.6, 83.3, 79.4, 74.9, 63.6, 55.4, 40.2, 38.8, 28.2, 27.4, 27.0, 19.2, 12.6, 11.6.) ppm. ^{31}P NMR (81 MHz, $CDCl_3$): δ = 15.4 ppm. MS (ESI): 1167.5 $[M + K]^+$, 1151.6 $[M + Na]^+$. $C_{59}H_{65}N_4O_{13}PSSi$ (1129.30): calcd. C 60.86, H 5.53, N 4.78; found: C 60.71, H 5.61, N 4.92.

Thio-dioxaphosphorinane, Oxo-oxathiaphosphorinane, and Oxo-oxathiaphosphorinane Derivatives (S_C,R_P)-7, (S_C,R_P)-8, (S_C,S_P)-9: Tetrabutylammonium fluoride (93 μL, 0.039 mmol) was added under argon at room temperature to the dinucleotide **4** (or **5/6**) (30 mg, 0.026 mmol) in anhydrous THF (2 mL). After 15 min, THF was removed under vacuum. The crude material was treated with TFA 2%/CH_2Cl_2 for 5 min, dried under high vacuum. and chromatographed on silica gel with ethyl acetate/methanol (0 to 20%) as eluent. Compounds **7** (or **8/9**) were recovered in 72, 77 and 88% yields, respectively.

Data for 7: TLC, R_f (AcOEt/MeOH, 8:2) = 0.4. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.84 (m, J = 1.1 Hz, 1 H, 6b-H), 7.68 (m, J = 1.1 Hz, 1 H, 6a-H), 6.46 (dd, J = 6.2 and 8.0 Hz, 1 H, 1′b-H), 6.38 (dd, J = 5.6 and 8.7 Hz, 1 H, 1′a-H), 5.22 (m, J = 1.7, 1.7, 5.3, and $J_{H,P}$ = 5.9 Hz, 1 H, 3′a-H), 4.88 (m, 1 H, 5′b-H), 4.60 (m, 1 H, $J_{H,P}$ = 1.0 Hz, 7′b-H), 4.50 (m, 1 H, 3′b-H), 4.45 (m, $J_{H,P}$ = 24 Hz, 1 H, 7′b-H), 4.32 (m, 1 H, 4′a-H), 3.93 (m, J = 1.8, 2.6, and $J_{H,P}$ = 4.6 Hz, 1 H, 4′b-H), 3.85 (d, J = 3.2 Hz, 2 H, 5′a-H), 2.60 (A part of an ABX system, J = 5.2, 5.6 and 14.1 Hz, 1 H, 2′a-H), 2.45 (B part of an ABX system, J = 1.7, 8.7, 14.1 and $J_{H,P}$ = 1.0 Hz, 1 H, 2′a-H), 2.39 (m, 1 H, 6′b-H), 2.24–2.20 (m, 2 H, 2′b-H), 1.98 (br d, 3 H, a-Me), 1.91 (br d, 3 H, b-Me), 1.88 (m, 1 H, 6′b-H) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 165.2, 151.3, 136.6, 136.0, 111.4, 110.7, 87.3, 87.4, 85.0, 84.7, 79.6, 79.3, 71.8, 68.0, 61.6, 39.4, 38.1, 27.7, 12.8, 11.5 ppm. ^{31}P NMR (81 MHz, CD_3OD): δ = 61.5 ppm. MS (DCI/NH_3): 606.0 $[M + NH_4]^+$, 589.0 $[M + H]^+$. $C_{22}H_{29}N_4O_{11}PS$ (588.52): calcd. C 44.90, H 4.97, N 9.52; found: C 45.23, H 4.85, N 9.34.

Data for 8: TLC, R_f (AcOEt/MeOH, 8:2) = 0.4. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.85 (m, J = 1.2 Hz, 1 H, 6b-H), 7.67 (m, J = 1.2 Hz, 1 H, 6a-H), 6.42 (dd, J = 6.2 and 7.6 Hz, 1 H, 1′b-H), 6.38 (dd, J = 5.7 and 8.5 Hz, 1 H, 1′a-H), 5.22 (m, J = 1.7, 1.9, and 5.8 Hz, 1 H, 3′a-H), 4.89 (m, 1 H, 5′b-H), 4.52 (m, 1 H, 3′b-H), 4.33 (dd, J = 3.0 and 5.4 Hz, 1 H, 4′a-H), 3.95 (m, J = 1.9, 2.8, and $J_{H,P}$ = 5.0 Hz, 1 H, 4′b-H), 3.84 (d, J = 3.0 Hz, 2 H, 5′a-H), 2.98 and 2.82 (m, $J_{H,P}$ = 15.3 and 23.0 Hz, 2 H, 7′b-H), 2.64 (A part of an ABX system, J = 8.5, 1.8 and 14.2 Hz, 1 H, 2′a-H), 2.47 (B part of an ABX system, J = 5.7, 14.2 and $J_{H,P}$ = 1.4 Hz, 1 H, 2′a-H), 2.28 (m, 1 H, 2′b-H), 2.01 (m, 1 H, 6′b-H), 1.92 (br d, 6 H, Me), 1.87 (m, J = 3.3, 7.6 and 14.0 Hz, 1 H, 2′b-H), 1.60 (m, 1 H, 6′b-H) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 166.3, 152.4, 137.8, 137.4, 112.2, 112.0, 89.2, 87.2, 86.2, 86.1, 85.4, 85.3, 79.8, 72.7, 62.5, 40.8, 39.4, 29.5, 28.5, 12.6, 12.5 ppm. ^{31}P NMR (81 MHz, CD_3OD): δ = 20.1 ppm. MS (DCI/NH_3): 606.2 $[M + NH_4]^+$, 589.2 $[M + H]^+$.

Data for 9: TLC, R_f (AcOEt/MeOH, 8:2) = 0.4. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.84 (m, J = 1.2 Hz, 1 H, 6a-H), 7.65 (m, J = 1.2 Hz, 1 H, 6a-H), 6.40 (dd, J = 7.4 and 7.5 Hz, 1 H, 1′b-H), 6.37 (dd, J = 5.7 and 8.6 Hz, 1 H, 1′a-H), 5.20 (m, J = 1.9, 1.9, and 6.0 Hz, 1 H, 3′a-H), 4.88 (m, J = 1.8, 6.0, and 7.8 Hz, $J_{H,P}$ < 1 Hz, 1 H, 5′b-H), 4.49 (m, J = 2.9, 3.0, and 6.0 Hz, 1 H, 3′b-H), 4.31 (dd, J = 2.0 and 3.0 Hz, 1 H, 4′a-H), 3.93 (m, J = 1.9, 2.9, and $J_{H,P}$ = 5.7 Hz, 1 H, 4′b-H), 3.83 (d, J = 3.0 Hz, 2 H, 5′a-H), 3.24–3.10 (m, 2 H, 7′b-H), 2.62 (A part of an ABX system, J = 1.9, 5.7 and 14.1 Hz, 1 H, 2′a-H), 2.45 (B part of an ABX system, J = 6.0, 8.6, 14.2 and $J_{H,P}$ = 1.5 Hz, 1 H, 2′a-H), 2.24 (m, 2 H, 2′b-H), 2.18 (m, 2 H, 6′b-H), 1.90 and 1.89 (br d, 6 H, Me) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 166.1, 153.1, 137.8, 137.2, 111.9, 111.7, 90.0, 86.9, 86.5, 86.0, 85.7, 83.1, 79.3, 73.2, 62.1, 40.5, 40.0, 29.5, 28.4, 12.5 ppm. ^{31}P NMR (81 MHz, CD_3OD): δ = 20.3 ppm. MS (DCI/NH_3) 606.0 $[M + NH_4]^+$, 589.0 $[M + H]^+$. $C_{22}H_{29}N_4O_{11}PS$ (588.52): calcd. C 44.90, H 4.97, N 9.52; found C 44.20, H 5.12, N 8.99.

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