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Synthesis of Phostone-Constrained Nucleic Acid (P-CNA) Dinucleotides Through Intramolecular Arbuzov's Reaction

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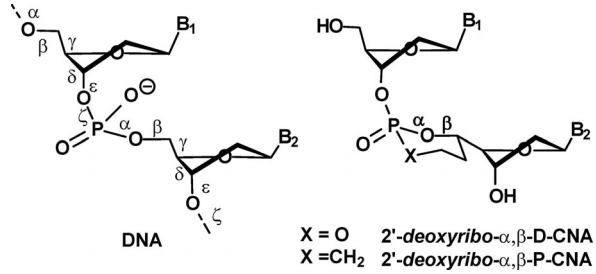
P-CNAs are dinucleotide building blocks in which the torsional angles α and β of the sugar/phosphate backbone are constrained to non-canonical values within a cyclic phosphonate structure (phostone) synthesised by diastereoselective intramolecular Arbuzov reaction. The reaction has been improved through the use of microwave activation and addition of lithium bromide.

Introduction

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

In addition to the double-stranded helical conformation, nucleic acids may adopt many other alternative structures such as bulges, hairpins, U-turns, or branched junctions.[3] These secondary structures always contain unpaired nucleotides or non-Watson–Crick pairs and are characterized by a variety of backbone conformations that differ markedly from the regular conformational states of double-stranded helices.[4] It is now well-established that these disparate structures play a crucial role in fundamental biological processes where protein–nucleic acid interactions, DNA/RNA folding, or RNA catalytic activity are involved. The determination of the precise biological role of a particular backbone conformation is currently an area of intense study. Unfortunately, structural and functional studies are somewhat complicated by the fragile and flexible nature of single-stranded fragments. With stable structural analogues of these secondary structures, we could expect to learn more about their role in vivo, the information they carry, and the key elements of their recognition by other macromolecules.

With this in mind, we became interested in the development of covalently constrained dinucleotide building units in which the backbone torsion angles of nucleic acids (Scheme 1) can have predefined values that are significantly different from the typical values observed in DNA and RNA duplexes.[5] Our general strategy is based on the introduction of the 1,3,2-dioxaphosphorinane ring structure at key positions along the phosphate backbone. We have already reported on the diastereoselective synthesis of a dioxaphosphorinane-CNA (D-CNA) dinucleotide building unit in which the α and β torsion angles are locked in a (g^+,t) conformation (Scheme 1).[6]



Scheme 1. Left: the six backbone torsion angles (labeled α to ζ) of nucleic acids. Right: D- or P-CNA dinucleotides in which α and β are stereocontrolled by a dioxaphosphorinane or a phostone ring structure, respectively.

However, the high level of diastereoselectivity observed for its formation led us to investigate the synthesis of phostone-constrained nucleic acids (P-CNA) in which the dioxaphosphorinane structure was replaced by a cyclic phosphonate to provide access to all possible diastereoisomers of the cyclic structure (Scheme 1).

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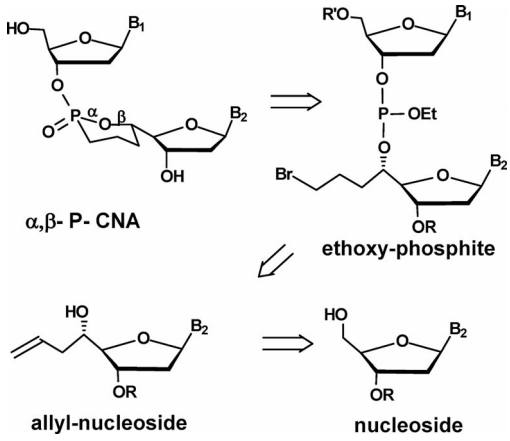
Similar compounds, featuring a seven-membered cyclic phosphonate ring have been obtained through a ring closing metathesis (RCM) reaction between a vinyl phosphonate and the 2′-*C*-allyl moiety of a dinucleotide.[7] Seven-membered phostone rings can also be prepared by cyclic acetal ring enlargement,[8] and smaller ring sizes can be achieved by esterification or cyclization of the formed phosphonate.[9] Because, in our case, the 5′-oxygen of the lower nucleoside must be involved in a six-membered cyclic phosphonate structure as an internucleotidic linkage, we speculated that an intramolecular Arbuzov reaction could be suitable for this task.[10]

Results and Discussion

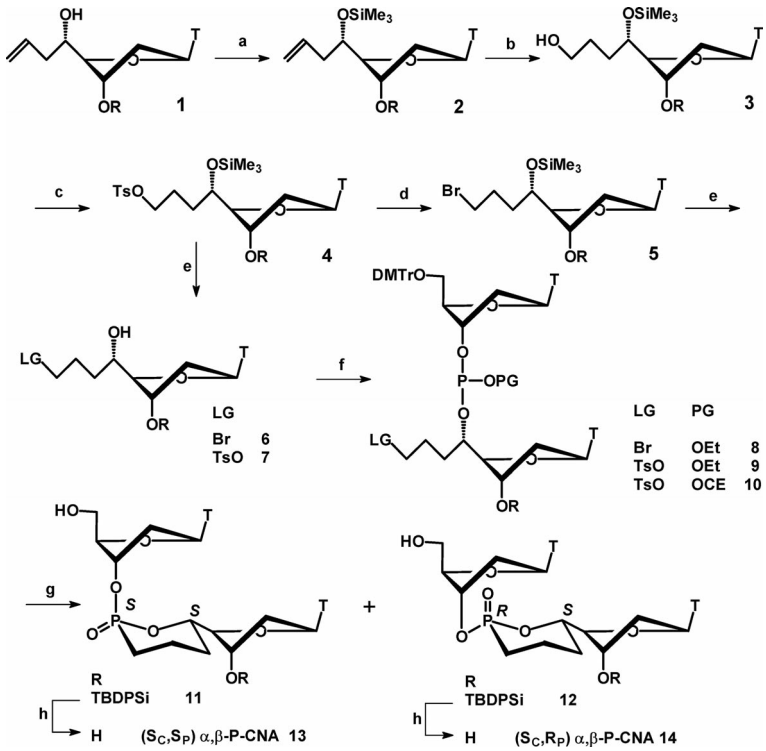
In an initial analysis, the key intermediate in the retrosynthetic analysis would be a dinucleotide composed of an ethoxy phosphite and a 5′-*C*-bromopropyl-substituted lower nucleoside (Scheme 2). The latter would be prepared from 5′-*C*-allyl nucleoside, which can be synthesized from natural nucleoside through a Sakuraï addition on the corresponding 5′-aldehyde.[11] The (5′*S*)-*C*-tosyloxy- or -(bromopropyl)thymidine (**7** and **6**, respectively Scheme 3) were prepared from protected (5′*S*)-*C*-tosyloxypropylthymidine (**4**); the former by direct removal of the silyl group under acidic conditions to prevent the formation of the furan ring, and the latter through displacement of the tosyl group with lithium bromide followed by a desilylation step.



Scheme 2. Retrosynthesis of phostone-constrained nucleic acid (P-CNA).

5′-*O*-Protection by trimethylsilane of diastereomerically pure 5′-*C*-allylthymidine (**1**) and subsequent hydroboration/oxidation afforded **3**, which was converted into **4** by treatment with tosyl chloride in pyridine (Scheme 3).

Dinucleoside phosphites **8**–**10** were formed as a 1:1 diastereoisomeric mixture (^{31}P NMR analysis) by coupling 5′-*C*-functionalized nucleosides **6** or **7** with commercially available thymidine ethyl or cyanoethyl phosphoramidites using standard phosphoramidite technology, without an oxidation step.[12]



Scheme 3. Synthesis of α,β-P-CNA. *Reagents and conditions:* (a) Me_3SiCl, pyr, room temp., 3 h, 95%. (b) BH_3/Me_2S, THF, room temp., 2 h then $NaOH/H_2O_2$, room temp., 0.5 h, 90%. (c) TsCl, $CHCl_3$, pyr, room temp., 16 h, 85%. (d) LiBr, Me_2CO/DMF (4:1), 60 °C, 0.75 h, quant. (e) PTSA, MeOH, room temp., 1 h, 70–90%. (f) thymidine phosphoramidite, tetrazole, room temp., 60–85%. (g) (i) see Table 1 for Arbuzov reaction conditions. (ii) TFA, CH_2Cl_2, quant. (h) TBAF, THF, room temp., 1 h, 90%.

Initially, we attempted to conduct the Arbuzov reaction with dinucleotide 5′-*C*-bromopropyl-ethoxyphosphite (**8**) under standard conditions (i.e., high temperature in dioxane or toluene; Table 1, entries 1 and 2), however, partial loss of the dimethoxytrityl protective group occurred. The crude material was submitted to acidic conditions (trifluoroacetic acid, TFA) to provide phostones **11** and **12**, as revealed by ^{31}P NMR analysis (δ_P = 28.2 and 32.1 ppm, respectively). As described for the intermolecular Arbuzov-type reaction involving nucleoside phosphites,[13] the reaction was difficult to reproduce, and yields of phostones ranged from 15 to 70% in a heterogeneous mixture of compounds that consisted of mainly alkylphosphonates or phosphates (^{31}P NMR analysis). Changing the solvent to dioxane or pyridine also failed to provide any of the desired phostone. We then investigate the use of microwave-assisted reaction conditions in various solvents and found that, as well as reducing the required time and temperature, the use of acetonitrile proved to be significantly more effective (Table 1, entries 7 and 8 vs. 5 and 6).

Table 1. Optimization of the Arbuzov reaction conditions with **8**.

Entry	Conditions Solvent	Activation[a]	*t* [h]	*T* [°C]	Phostone S_P/R_P *d.r.*[b]	Yield [%]
1	dioxane	–	10.0	130	1.6:1	20–65
2	toluene	–	20.0	130	1.7:1	15–70
3	DMSO	–	14.0	130	–	0
4	pyridine	–	8.0	140	–	0
5	THF	MW	0.5	110	–	0
6	CH_2Cl_2	MW	0.5	110	–	0
7	MeCN	MW	0.5	110	1.8:1	35
8	MeCN	MW	1.5	105	2.2:1	55
9	MeCN	MW, LiBr	1.0	105	2.7:1	40
10	MeCN	MW, LiBr	3.0	90	1.9:1	85

[a] MW: microwave, 40–60 W. [b] Determined by ^{31}P NMR spectroscopic analysis.

The first step in the Arbuzov reaction, i.e., attack of the phosphorus electronic lone pair, is actually intramolecular but the following halide-mediated dealkylation is not. Therefore, we focused on the possibility of improving the Arbuzov reaction by adding an external halide source such as LiBr. Indeed, in combination with microwave activation and a longer time period with a lower temperature, the yield of phostone **11/12** reached 85%, with a diastereoisomeric ratio of approximately 2:1. Interestingly, under these new conditions, ethoxy or standard cyanoethoxy phosphites **8** and **9** with a tosylate as leaving group, proved to be good substrates for the intramolecular Arbuzov reaction (Scheme 3 and the Supporting Information).[14]

Although surprising, with an initial equimolecular mixture of phosphite, the observed diastereoselectivity can be explained by phosphite epimerization[15] and equilibrium in the Arbuzov reaction (see the Supporting Information).

The diastereoisomeric P-CNAs were separated by reverse phase HPLC, and the remaining silyl protective group was removed in good yield by treatment with fluoride ions, providing (S_C,S_P)-α,β-P-CNA (**13**) as the major isomer and (S_C,R_P)-α,β-P-CNA (**14**) as the minor isomer.

The two isomers **13** and **14** were first characterized by their chemical shifts in their respective ^{31}P NMR spectra (recorded in CD_3OD), which were measured at δ = +26.9 and +31.3 ppm, respectively. In the CNA dinucleotide series, this difference in chemical shift was always observed between phosphorus epimers, and the isomer featuring downfield chemical shift was always assigned as the isomer with all the substituents in the most stabilizing position on the internucleotidic six-membered ring.[5,6]

The phostone chair conformation is evident from the ^{1}H NMR spectra (Table 2), with a very small coupling constant between the 5′-H involved in the oxaphosphorinane system and the phosphorus atom, which is characteristic of an axial position of the 5′-H proton.[16] The observation of a long-range coupling between the 4′-H of the lower sugar unit and the phosphorus atom is indicative of a typical W-shaped P–O5′–C5′–C4′-H4′ junction, which is consistent with a *gauche*(+) conformation of γ in both P-CNA moieties. The puckering of the 2′-deoxyribose moieties were assigned by examination of the sugar ring H/H coupling constants (Table 2). The small $J_{H3'/H4'}$ (ca. 2 Hz) value measured for the upper nucleosides and the values of $J_{H2'/H3'}$ and $J_{H1'/H2'}$ are close in each case to those found in the standard C2′-*endo* conformation of the natural 2′-deoxyribose. However, the south conformation of the upper nucleoside is enforced by the neutral phostone internucleotidic linkage.

Table 2. H/H coupling constants [Hz] in the ^{1}H NMR spectra (500 MHz) of (S_C,S_P)-α,β-P-CNA **13** and (S_C,R_P)-α,β-P-CNA **14**.

		Coupling constant *J* /Hz						
		J(1′,2′)		*J*(2′,3′)		*J*(3′,4′)	*J*(3′,P)	*J*(5′,P)
13	upper nucleoside	8.0	6.0	6.0	2.0	2.0	6.5	–
	lower nucleoside	7.0	7.0	6.0	4.0	3.0	–	< 1
14	upper nucleoside	8.5	5.5	5.5	1.5	1.5	4.5	–
	lower nucleoside	8.0	6.0	6.0	2.5	2.5	–	2.0

The structural analysis was corroborated by geometry optimization procedures (see the Supporting Information and Figure 1), showing that the difference in energy was ΔE = 0.7 kcal (water and MeOH) between the two phostones in chair conformations in favor of (S_C,S_P)-α,β-P-CNA (**13**), which presents all the substituents in less sterically hindered positions (apical upper nucleoside and equatorial lower nucleoside).

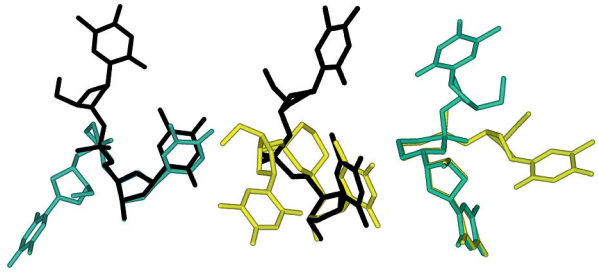


Figure 1. Superimposition of α,β-P-CNA minimized structures. Left and middle: (S_C,S_P)-**13** and (S_C,R_P)-**14** α,β-P-CNA with X-ray structure of unmodified TpT,[17] respectively. Right: (S_C,S_P)-**13** (blue) with (S_C,R_P)-**14** (yellow) α,β-P-CNA.

Overall, these elements allow us to assign the conformation of the backbone torsion angles α, β, and γ as (α,β,γ) = (g^+, t, g^+) for (S_C,S_P)-α,β-P-CNA (**13**) and (α,β,γ) = (a^-/t, t, g^+) for (S_C,R_P)-α,β-P-CNA (**14**).

Conclusions

An improvement to the classical Arbuzov reaction by applying microwave activation and addition of a salt has allowed the synthesis of six-membered cyclic-phosphonate (phostone) as an internucleotidic linkage with defined restrain on the α and β torsion angles. The dioxaphosphorinane-constrained nucleic acid (D-CNA) approach has already provide us with constrained dinucleotides exhibiting promising properties,[18] the phostone-constrained nucleic acid (P-CNA) provides access to new constrained dinucleotides featuring atypical α/β values (a^-/t, t).

With the need to develop functional DNA and RNA molecules,[19] new nucleotide analogue building blocks featuring preorganized structures should be useful for the elaboration of synthetic, distorted nucleic acids with improved stability and folding ability. The extended CNA family offers interesting elements that can be used to elaborate and study unusually shaped nucleic acids. Incorporation of these new P-CNA conformationally constrained building units into selected unpaired nucleic acid secondary structures will be published in due course.

Experimental Section

General: Products were purified by medium pressure liquid chromatography with a Jobin et Yvon Modoluprep apparatus by using Amicon 6–35 μm or Merck 15 μm silica. NMR spectra were recorded with a Bruker AC-250, Avance-300, or Avance 500 spectrometer (250, 300, or 500 MHz for ^{1}H and 63, 75, or 125 MHz for ^{13}C). Chemical shifts are referenced to tetramethylsilane. Mass spectra were recorded with a Nermag R10-10 or with a Perkin–Elmer API 365. Microwave-assisted reactions were performed with a CEM Discover apparatus. All solvents were distilled and dried before use. Compounds **1**–**3** have been described previously.[11]

3′-*O*-(*tert*-Butyldiphenylsilyl)-5′-*O*-(trimethylsilyl)-(5′*S*)-*C*-(tosyloxypropyl)thymidine (4): To a solution of **3** (1.47 g, 2.41 mmol) at 0 °C under argon in freshly distilled chloroform (8 mL), pyridine (5 mL) and tosyl chloride (688 mg, 3.61 mmol) were added. The reaction was stirred at room temp. for 16 h then quenched with saturated aqueous NH_4Cl (40 mL). The mixture was diluted with ethyl ether (100 mL) and washed with brine. The organic layer was dried with $MgSO_4$ and concentrated in vacuo. The crude product was deposited on a silica gel column and eluted with diethyl ether/petroleum ether (7:3) to give **4** (1.60 g, 87%) as a white foam after removal of the solvent. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.07 (s, 1 H, NH), 7.77 (A of an AB, ^3J = 8.4 Hz, 2 H, Ts), 7.71 (br. d, $^4J_{6-7}$ = 1.2 Hz, 1 H, H^6), 7.64–7.60 (m, 4 H, Ph), 7.46–7.39 (m, 6 H, Ph), 7.34 (B of an AB, ^3J = 8.4 Hz, 2 H, Ts), 6.55 (dd, $^3J_{1′-2″}$ = 9.3, $^3J_{1′-2′}$ = 5.4 Hz, 1 H, H$^{1′}$), 4.18 (d, $^3J_{3′-2″}$ = 5.1 Hz, 1 H, H$^{3′}$), 3.91 (t, $^3J_{8′-7′}$ = 6.0 Hz, 2 H, H$^{8′}$), 3.77 (s, 1 H, H$^{4′}$), 2.96 (m, 2 H, H$^{5′}$), 2.44 (s, 3 H, Me$_{Ts}$), 2.28 [A of an ABX(Y), $^2J_{2′-2″}$ = 12.9, $^3J_{2′-1′}$ = 5.4 Hz, 1 H, H$^{2′}$], 1.84 (d, $^4J_{7-6}$ = 1.2 Hz, 3 H, H^7), 1.83 [B of an ABX(Y), $^2J_{2″-2′}$ = 12.9, $^3J_{2″-1′}$ = 9.3, $^3J_{2″-3′}$ = 5.1 Hz, 1 H, H$^{2″}$], 1.51–1.29 (m, 4 H, H$^{6′}$ and H$^{7′}$), 1.08 (s, 9 H, *t*Bu), –0.09 (s, 9 H, $SiMe_3$) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 163.5 (C^4), 150.3 (C^2), 144.9 (C^{q}S, Ts), 136.1 (C^6), 135.8 and 135.7 (4 × CH, Ph), 133.4 and 133.0 (2 × C^q, Ph), 133.1 (*C*^{q}CH$_3$, Ts), 130.2 and 130.1 (2 × CH, Ph), 129.9 (2 × CH, Ts), 128.0 (4 × CH, Ph), 127.9 (2 × CH, Ts), 110.9 (C^5), 88.9 (C$^{4′}$), 85.0 (C$^{1′}$), 76.1 (C$^{3′}$), 72.4 (C$^{5′}$), 70.1 (C$^{8′}$), 40.7 (C$^{2′}$), 30.0 (C$^{7′}$), 26.9 (C*Me*$_3$), 24.9 (C$^{6′}$), 21.7 (CH$_3$, Ts), 19.1 (*C*Me$_3$), 12.5 (C^7), 0.2 (SiMe$_3$) ppm. $C_{39}H_{52}N_2O_8SSi_2$ (765.08): calcd. C 61.23, H 6.85, N 3.66; found C 59.99, H 6.96, N 3.77.

(5′*S*)-*C*-(Bromopropyl)-3′-*O*-(*tert*-butyldiphenylsilyl)-5′-*O*-(trimethylsilyl)thymidine (5): To a solution of dried LiBr (360 mg, 4.15 mmol) at room temp. under argon in a mixture of anhydrous acetone/dimethylformamide (4:1; 28 mL, 0.15 M), was added a solution of **4** (2.12 g, 2.77 mmol) in the same solvent mixture (7 mL, 0.4 M). The reaction mixture was heated to reflux for 45 min, then the acetone was evaporated and saturated $NaHCO_3$ (50 mL) was added. The aqueous phase was extracted with EtOAc, then the organic phase was washed three times with water and brine, dried with $MgSO_4$, and the solvent was evaporated under vacuum to give **5** (1.85 g, quant.) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 9.71 (s, 1 H, NH), 7.76 (d, $^4J_{6-7}$ = 1.2 Hz, 1 H, H^6), 7.65–7.60 (m, 4 H, Ph), 7.47–7.36 (m, 6 H, Ph), 6.60 (dd, $^3J_{1′-2″}$ = 9.0, $^3J_{1′-2′}$ = 5.1 Hz, 1 H, H$^{1′}$), 4.21 (d, ^3J = 5.1 Hz, 1 H, H$^{3′}$), 3.81 (s, 1 H, H$^{4′}$), 3.24 (t, $^3J_{8′-7′}$ = 6.0 Hz, 2 H, H$^{8′}$), 2.99 (m, 1 H, H$^{5′}$), 2.31 [A of an ABX(Y), $^2J_{2′-2″}$ = 13.2, $^3J_{2′-1′}$ = 5.4 Hz, 1 H, H$^{2′}$], 1.90–1.81 (m, 4 H, H$^{2″}$ and H^7), 1.72–1.41 (m, 4 H, H$^{6′}$ and H$^{7′}$), 1.07 (s, 9 H, *t*Bu), 0.08 (s, 9 H, $SiMe_3$) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 164.1 (C^4), 150.7 (C^2), 136.2 (C^6), 135.8 and 135.7 (4 × CH, Ph), 133.5 and 133.0 (2 × C^q, Ph), 130.2 and 130.1 (2 × CH, Ph), 128.0 (4 × CH, Ph), 110.0 (C^5), 88.9 (C$^{4′}$), 85.1 (C$^{1′}$), 76.1 (C$^{3′}$), 72.0 (C$^{5′}$), 40.8 (C$^{2′}$), 33.3 (C$^{6′}$), 32.6 (C$^{7′}$), 28.6 (C$^{8′}$), 27.0 (C*Me*$_3$), 19.1 (*C*Me$_3$), 12.6 (C^7), 0.2 (SiMe$_3$) ppm. $C_{32}H_{45}BrN_2O_5Si_2$ (673.79): calcd. C 57.04, H 6.73, N 4.16; found C 56.82, H 6.77, N 4.21.

(5′*S*)-*C*-(Bromopropyl)-3′-*O*-(*tert*-butyldiphenylsilyl)thymidine (6): To a solution of **5** (1.69 g, 2.51 mmol) in methanol (12 mL, 0.2 M), *p*-toluenesulfonic acid (95 mg, 0.50 mmol) was added. After 1 h stirring at room temp., $NaHCO_3$ (42 mg, 0.50 mmol) was added and the reaction mixture was diluted with EtOAc (150 mL). The organic phase was washed twice with water and brine, dried with $MgSO_4$, and the solvent was evaporated to give **6** (1.05 g, 70%) after isolation by silica gel chromatography using dichloromethane/ethyl acetate (4:1) as eluents. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.24 (br. s, 1 H, NH), 7.67–7.61 (m, 4 H, Ph), 7.48–7.38 (m, 6 H, Ph), 7.25 (d, $^4J_{6-7}$ = 1.2 Hz, 1 H, H^6), 6.11 (dd, $^3J_{1′-2″}$ = 8.4, $^3J_{1′-2′}$ = 6.0 Hz, 1 H, H$^{1′}$), 4.45 [X of an ABX(Y), $^3J_{3′-2″}$ = 4.8, $^3J_{3′-2′}$ = 2.4, $^3J_{3′-4′}$ = 2.1 Hz, 1 H, H$^{3′}$], 3.77 (t, $^3J_{4′-3′}$ = $^3J_{4′-5′}$ = 2.1 Hz, 1 H, H$^{4′}$), 3.32 (t, $^3J_{8′-7′}$ = 6.6 Hz, 2 H, H$^{8′}$), 3.03 (br. s, 1 H, H$^{5′}$), 2.35 [A of an ABX(Y), $^2J_{2″-2′}$ = 13.5, $^3J_{2″-1′}$ = 8.4, $^3J_{2″-3′}$ = 4.8 Hz, 1 H, H$^{2″}$], 2.23 [B of an ABX(Y), $^2J_{2′-2″}$ = 13.5, $^3J_{2′-1′}$ = 6.0, $^3J_{2′-3′}$ = 2.4 Hz, 1 H, H$^{2′}$], 1.88 (d, $^4J_{7-6}$ = 1.2 Hz, 3 H, H^7), 1.84–1.68 (m, 2 H, H$^{7′}$), 1.51–1.42 (m, 2 H, H$^{6′}$), 1.09 (s, 9 H, *t*Bu) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 164.1 (C^4), 150.5 (C^2), 137.8 (C^6), 135.8 and 135.7 (4 × CH, Ph), 133.4 and 133.1 (2 × C^q, Ph), 130.2 and 130.1 (2 × CH, Ph), 128.0 and 127.9 (4 × CH, Ph), 110.0 (C^5), 89.7 (C$^{4′}$), 88.1 (C$^{1′}$), 74.4 (C$^{3′}$), 70.1 (C$^{5′}$), 39.5 (C$^{2′}$), 35.6 (C$^{6′}$), 32.7 (C$^{7′}$), 29.0 (C$^{8′}$), 27.0 (C*Me*$_3$), 19.1 (*C*Me$_3$), 12.5 (C^7) ppm. $C_{29}H_{37}BrN_2O_5Si$ (601.61): calcd. C 57.90, H 6.20, N 4.66; found C 57.63, H 6.27, N 4.70.

3′-*O*-(*tert*-Butyldiphenylsilyl)-(5′*S*)-*C*-(tosyloxypropyl)thymidine (7): To a solution of **4** (1.2 g, 1.62 mmol) in methanol (8 mL, 0.2 M), *p*-toluenesulfonic acid (27 mg) was added. After 1 h stirring at room temp., an aqueous solution of $NaHCO_3$ (10 mL) was added and the methanol was removed under vacuum before the reaction mixture was diluted with EtOAc (120 mL). The organic phase was washed twice with water and brine, dried with $MgSO_4$, and the solvent evaporated. Compound **7** (1.01 g, 90%) was isolated by silica gel chromatography using dichloromethane/ethyl acetate (4:1) as eluents. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.14 (br. s, 1 H, NH), 7.76 (A of an AB, ^3J = 8.4 Hz, 2 H, Ts), 7.65–7.60 (m, 4 H, Ph), 7.46–7.37 (m, 6 H, Ph), 7.33 (B of an AB, ^3J = 8.4 Hz, 2 H, Ts), 7.24 (d, $^4J_{6-7}$ = 1.2 Hz, 1 H, H^6), 6.09 (dd, $^3J_{1'-2''}$ = 8.4, $^3J_{1'-2'}$ = 6.0 Hz, 1 H, H$^{1'}$), 4.43 [X of an ABX(Y), $^3J_{3'-2''}$ = 5.4, $^3J_{3'-4'}$ = 2.4, $^3J_{3'-2'}$ = 2.1 Hz, 1 H, H$^{3'}$], 3.97 (A of an ABX$_2$, $^2J_{8''-8'}$ = 9.6, $^3J_{8''-7'}$ = 6.0 Hz, 1 H, H$^{8'}$), 3.92 (B of an ABX$_2$, $^2J_{8''-8'}$ = 9.6, $^3J_{8''-7'}$ = 6.0 Hz, 1 H, H$^{8''}$), 3.72 (t, $^3J_{4'-3'}$ = $^3J_{4'-5'}$ = 2.4 Hz, 1 H, H$^{4'}$), 2.98 [X of an ABX(Y), $^3J_{5'-6'}$ = 8.7, $^3J_{5'-6''}$ = 5.1, $^3J_{5'-4'}$ = 2.4 Hz, 1 H, H$^{5'}$], 2.44 (s, 3 H, Me_{Ts}), 2.33 [A of an ABX(Y), $^2J_{2''-2'}$ = 13.2, $^3J_{2''-1'}$ = 8.4, $^3J_{2''-3'}$ = 5.4 Hz, 1 H, H$^{2''}$], 2.20 [B of an ABX(Y), $^2J_{2'-2''}$ = 13.2, $^3J_{2'-1'}$ = 6.0, $^3J_{2'-3'}$ = 2.1 Hz, 1 H, H$^{2'}$], 1.87 (d, $^4J_{7-6}$ = 1.2 Hz, 3 H, H^7), 1.64–1.29 (m, 4 H, H$^{6'}$ and H$^{7'}$), 1.00 (s, 9 H, *t*Bu) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 164.1 (C^4), 150.5 (C^2), 144.8 (C^{q}S, Ts), 137.9 (C^6), 135.7 (4 × CH, Ph), 133.3 (2 × C^q, Ph), 133.0 (*C*^{q}CH$_3$, Ts), 130.2 (2 × CH, Ph), 130.1 (2 × CH, Ts), 128.0 (4 × CH, Ph), 127.9 (2 × CH, Ts), 110.0 (C^5), 89.7 (C$^{4'}$), 88.0 (C$^{1'}$), 74.4 (C$^{3'}$), 70.4 (C$^{5'}$), 70.3 (C$^{8'}$), 39.4 (C$^{2'}$), 30.0 (C$^{7'}$), 26.9 (C*Me*$_3$), 25.3 (C$^{6'}$), 21.7 (CH_3, Ts), 19.0 (*C*Me$_3$), 12.4 (C^7) ppm. $C_{36}H_{44}N_2O_8SSi$ (692.89): calcd. C 62.40, H 6.40, N 4.04; found C 61.97, H 6.51, N 4.14.

Ethyl [(5′*S*)-5′-*C*-(3-Bromopropyl)-3′-*O*-(*tert*-butyldiphenylsilyloxy)thymidine-5′-*O*-yl][(5′-*O*-dimethoxytrityl)thymidine-3′*O*-yl] Phosphate (8): At room temp., under argon, **6** (200 mg, 0.33 mmol), thymidine ethyl phosphoramidite (250 mg, 0.35 mmol), and the activation reagent (7.6 mL of 5-ethylthiotetrazole in acetonitrile; 0.25 M) were added to a flask. The mixture was stirred for 45 min, then degassed EtOAc (50 mL) was added. The organic phase was washed with cooled 10% aqueous Na_2CO_3 (20 mL) and dried with $MgSO_4$. The solvent was evaporated under vacuum and the solid residue was isolated by silica gel chromatography using petroleum ether/ethyl acetate (7:3 + triethylamine) as eluents. Compound **8** (373 mg, 92%, 1:1 mixture of isomers) was obtained as a white foam. ^{1}H and ^{13}C NMR spectra were highly complex due to the diastereoisomeric mixture of thymidine dinucleotide analogues. ^{31}P NMR (C_6D_6, 60 MHz): δ = 142.5, 141.6 ppm. MS: *m*/*z* = 1243.4 [M + Na$^+$].

Ethyl [(5′*S*)-5′-*C*-(Tosyloxypropyl)-3′-*O*-(*tert*-butyldiphenylsilyloxy)thymidine-5′-*O*-yl][(5′-*O*-dimethoxytrityl)thymidine-3′*O*-yl] Phosphite (9): Compound **9** (1:1 mixture of diastereoisomers, 250 mg, 66%) was obtained by using an identical procedure to that described for **8**, starting from **7** (200 mg, 0.29 mmol), thymidine ethylphosphoramidite (250 mg, 0.35 mmol), and activation reagent (7.6 mL of 5-ethylthiotetrazol in acetonitrile 0.25 M). ^{1}H and ^{13}C NMR spectra were highly complex due to the diastereoisomeric mixture of thymidine dinucleotide analogues. ^{31}P NMR (C_6D_6, 60 MHz): δ = 143.1, 142.2 ppm. MS: *m*/*z* = 1349.6 [M + K$^+$].

Cyanoethyl [(5′S)-3′-*O*-(*tert*-Butyldiphenylsilyloxy)-5′-*C*-(tosyloxypropyl)thymidine-5′-*O*-yl][(5′-*O*-dimethoxytrityl)thymidine-3′*O*-yl] Phosphate (10): Compound **10** (1:1 mixture of diastereoisomers, 340 mg, 60%) was obtained by using an identical procedure to that described for **8**, starting from **7** (290 mg, 0.42 mmol), thymidine cyanoethylphosphoramidite (330 mg, 0.44 mmol), and activation reagent (10.0 mL of 5-ethylthiotetrazol in acetonitrile; 0.25 M). ^{1}H and ^{13}C NMR spectra were highly complex due to the diastereoisomeric mixture of thymidine dinucleotide analogues. ^{31}P NMR (C_6D_6, 100 MHz): δ = 142.4, 140.8 ppm. MS: *m*/*z* = 1374.4 [M + K$^+$].

3′-*O*-(*tert*-Butyldiphenylsilyloxy)-(S_C,S_P) (α,β-P-CNA; 11): Dried LiBr (10 equiv.) and phosphites **8**, **9**, or **10** (1 equiv.) were dissolved under argon in degassed anhydrous acetonitrile (30 mg/mL) in a closed microwave flask and the mixture was stirred at 90 °C under microwave irradiation (30–50 W) for 4 h (phosphites **8** and **9**) or 8 h (phosphite **10**). The solution was then diluted with EtOAc, washed with water and brine, dried with $MgSO_4$, and the solvent was evaporated. The crude product was treated with 3% trifluoroacetic acid in dichloromethane for 1–4 h (the reaction may be followed on RP-HPLC). The acid was subsequently neutralized with saturated $NaHCO_3$, and the organic phase was washed with brine, dried with $MgSO_4$ and the solvent was evaporated to yield a mixture of the two diastereoisomers **11** and **12** (80–90%) in a diastereoisomeric ratio of approximately 2:1. The two diastereoisomers were then separated using reverse phase HPLC (80% MeOH in water). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.55–7.45 (m, 8 H, H^{6a}, H^{6b}, Ar), 6.43 (A of an AX$_2$, $^3J_{1'a-2'a}$ = 7.5, $^3J_{1'a-2''a}$ = 6.0 Hz, 1 H, H$^{1'a}$), 6.37 (A of an AX$_2$, $^3J_{1'b-2'b}$ = 9.5, $^3J_{1'b-2''b}$ = 5.5 Hz, 1 H, H$^{1'b}$), 5.09 [A of an AX$_2$(Y), $^3J_{3'a-P}$ = 7.5, $^3J_{3'a-2''a}$ = 6.0, $^3J_{3'a-2'a}$ = $^3J_{3'a-4'a}$ = 3.0 Hz, 1 H, H$^{3'a}$], 4.36 (d, $^3J_{3'b-2'b}$ = 5.5 Hz, 1 H, H$^{1'b}$), 3.82 (m, $^4J_{4'b-P}$ = 5.0 Hz, 1 H, H$^{4'b}$), 3.78 (q, $^3J_{4'a-3'a}$ = $^3J_{4'a-5'a}$ = $^3J_{4'a-5''a}$ = 3.0 Hz, 1 H, H$^{4'a}$), 3.59 (A of an ABX, $^2J_{5'a-5''a}$ = 12.0, $^3J_{5'a-4'a}$ = 3.0 Hz, 1 H, H$^{5'a}$), 3.54 (B of an ABX, $^2J_{5''a-5'a}$ = 12.0, $^3J_{5''a-4'a}$ = 3.0 Hz, 1 H, H$^{5''a}$), 3.51–3.49 (m, 2 H, H$^{5'b}$), 2.44 [A of an ABX(Y), $^2J_{2''a-2'a}$ = 14.0, $^3J_{2''a-1'a}$ = 6.0, $^3J_{2''a-3'a}$ = 3.5 Hz, 1 H, H$^{2''a}$], 2.32 (m, 2 H, H$^{2'a}$, H$^{2'b}$), 2.07 (m, 2 H, H$^{2''b}$, H$^{8'b}$), 1.96 (d, $^4J_{7a-6a}$ = 1.0 Hz, 3 H, H^{7a}), 1.95 (d, $^4J_{7b-6b}$ = 1.0 Hz, 3 H, H^{7b}), 1.73–1.57 (m, 3 H, H$^{6'b}$, H$^{8'b}$), 1.42–1.39 (m, 2 H, H$^{7'b}$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 163.9, 163.8, 150.7 and 150.5 (C^{2a}, C^{4a}, C^{2b} and C^{4b}), 135.8 and 135.7 (C^{6a} and C^{6b}), 133.4 and 132.5 (C^q$_{Ph}$), 130.4, 130.3, 128.1 (Ph), 112.0 and 111.5 (C^{5a} and C^{5b}), 88.5 (C$^{4'b}$), 85.5 (C$^{4'a}$), 85.7 (C$^{1'a}$), 85.2 (C$^{1'b}$), 81.3 (C$^{5'b}$), 75.2 (C$^{3'a}$), 75.1 (C$^{3'b}$), 61.5 (C$^{5'a}$), 39.9 (C$^{2'b}$), 39.0 (C$^{2'a}$), 27.7 (C$^{6'b}$), 26.8 [C(*C*H_3)$_3$], 23.0 (C$^{8'b}$), 20.6 (C$^{7'b}$), 12.7 and 12.4 (C^{7a} and C^{7b}), 1.0 [*C*(CH$_3$)$_3$] ppm. ^{31}P NMR (MeOD, 100 MHz): δ = 25.1 ppm. MS (Maldi-Tof): *m*/*z* = 809.2 [M + H$^+$], 831.1 [M + Na$^+$], 847.1 [M + K$^+$].

3′-*O*-(*tert*-Butyldiphenylsilyloxy)-(S_C,R_P) (α,β-P-CNA; 12): ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.67–7.61 (m, 4 H, Ar), 7.47–7.36 (m, 8 H, H^{6a}, H^{6b}, Ar), 6.47 (A of an AX$_2$, $^3J_{1'b-2'b}$ = $^3J_{1'b-2''b}$ = 5.5 Hz, 1 H, H$^{1'b}$), 6.09 (A of an AX$_2$, $^3J_{1'a-2'a}$ = 7.0, $^3J_{1'a-2''a}$ = 6.5 Hz, 1 H, H$^{1'a}$), 5.22 (m, 1 H, H$^{3'a}$), 4.42 (br. d, $^3J_{3'b-4'b}$ = 5.5 Hz, 1 H, H$^{3'b}$), 4.11 [A of a AX(Y), $^3J_{4'b-3'b}$ = 5.5, $^3J_{4'b-5'b}$ = 2.5 Hz, 1 H, H$^{4'b}$], 3.88 (A of an ABX, $^2J_{5'a-5''a}$ = 12.0 Hz, 1 H, H$^{5'a}$), 3.82 (B of an ABX, $^2J_{5''a-5'a}$ = 12.0 Hz, 1 H, H$^{5''a}$), 3.76 (m, 1 H, H$^{4'a}$), 3.71 (m, 1 H, H$^{5'b}$), 2.34–1.34 (m, 10 H, H$^{2'a}$, H$^{2'b}$, H$^{6'b}$, H$^{7'b}$, H$^{8'b}$), 1.90 (m, 6 H, H^{7a}, H^{7b}) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 164.0, 163.9, 150.4 and 150.3 (C^{2a}, C^{4a}, C^{2b} and C^{4b}), 135.8 and 135.7 (C^{6a} and C^{6b}), 133.2 and 132.7 (C^q$_{Ph}$), 130.2, 128.1, 128.0 (Ph), 111.2 and 111.0 (C^{5a} and C^{5b}), 88.6 (C$^{4'b}$), 85.7 (C$^{4'a}$), 85.2 (C$^{1'a}$), 84.8 (C$^{1'b}$), 79.6 (C$^{5'b}$), 76.5 (C$^{3'b}$), 74.6 (C$^{3'a}$), 61.9 (C$^{5'a}$), 40.4 (C$^{2'b}$), 39.1 (C$^{2'a}$), 28.1 (C$^{6'b}$), 26.9 [C(*C*H_3)$_3$], 23.9 (C$^{8'b}$), 20.5 (C$^{7'b}$), 12.8 and 12.6 (C^{7a} and C^{7b}), 1.0 [*C*(CH$_3$)$_3$] ppm. ^{31}P NMR (MeOD, 100 MHz): δ = 29.8 ppm. MS (MALDI-TOF): *m*/*z* = 831.1 [M + Na$^+$], 847.1 [M + K$^+$].

(S_C,S_P) α,β-P-CNA (13): To a solution of **10** (22.6 mg, 28.0 μmol) in distilled THF (1 mL), cooled to 0 °C, a solution of TBAF (1 M

in THF, 40 µL, 40 µmol) was added. The mixture was stirred for 1 h at room temp., then the THF was evaporated and the crude product was filtered through silica gel using EtOAc/MeOH (80:20) as solvent. The solvent was evaporated to yield **13** (15.3 mg, 93%) as a white foam. ^{1}H NMR (MeOD, 500 MHz): δ = 7.80 (d, $^4J_{6b–7b}$ = 1.0 Hz, 1 H, H^{6b}), 7.69 (d, $^4J_{6a–7a}$ = 1.5 Hz, 1 H, H^{6a}), 6.39 (dd, $^3J_{1'b–2''b}$ = $^3J_{1'b–2'b}$ = 7.0 Hz, 1 H, H$^{1'b}$), 6.36 (dd, $^3J_{1'a–2''a}$ = 8.0, $^3J_{1'a–2'a}$ = 6.0 Hz, 1 H, H$^{1'a}$), 5.18 [X of an ABX(Y), $^3J_{3'a–P}$ = 7.0, $^3J_{3'a–2'a}$ = 6.0, $^3J_{3'a–4'a}$ = $^3J_{3'a–2''a}$ = 2.0 Hz, 1 H, H$^{3'a}$], 4.59 (m, $^3J_{5'b–P}$ < 0.5 Hz, 1 H, H$^{5'b}$), 4.51 [X of an ABX(Y), $^3J_{3'b–2''b}$ = 6.0, $^3J_{3'b–2'b}$ = 4.0, $^3J_{3'b–4'b}$ = 3.0 Hz, 1 H, H$^{3'b}$], 4.25 (dt, $^3J_{4'a–5'a}$ = $^3J_{4'a–5''a}$ = 3.0, $^3J_{4'a–3'a}$ = 2.0 Hz 1 H, H$^{4'a}$), 3.91 (dd, $^4J_{4'b–P}$ = 4.0, $^3J_{4'b–3'b}$ = 3.0, $^3J_{4'b–5'b}$ = 2.5 Hz, 1 H, H$^{4'b}$),3.84 (A of an ABX, $^2J_{5'a–5''a}$ = 15.5, $^3J_{5'a–4'a}$ = 3.0 Hz, 1 H, H$^{5'a}$), 3.81 (B of an ABX, $^2J_{5''a–5'a}$ = 15.5, $^3J_{5''a–4'a}$ = 3.0 Hz, 1 H, H$^{5''a}$), 2.56 [A of an ABX(Y), $^2J_{2''a–2'a}$ = 14.0, $^3J_{2''a–1'a}$ = 8.0, $^3J_{2''a–3'a}$ = 2.0 Hz, 1 H, H$^{2''a}$], 2.46 [B of an ABX(Y), $^2J_{2'a–2''a}$ = 14.0, $^3J_{2'a–1'a}$ = 6.0, $^3J_{2'a–3'a}$ = 6.0 Hz, 1 H, H$^{2'a}$], 2.29 [A of an ABX(Y), $^2J_{2''b–2'b}$ = 19.5, $^3J_{2''b–1'b}$ = 7.0, $^3J_{2''b–3'b}$ = 6.0 Hz, 1 H, H$^{2''b}$], 2.26 [B of an ABX(Y), $^2J_{2'b–2''b}$ = 19.5, $^3J_{2'b–1'b}$ = 7.0, $^3J_{2'b–3'b}$ = 4.0 Hz, 1 H, H$^{2'b}$], 2.24 (m, 1 H, H$^{7'b}$), 2.19 (m, 1 H, H$^{8'b}$), 1.96 (m, 1 H, H$^{7'b}$), 1.93 (d, $^4J_{7a–6a}$ = 1.5 Hz, 3 H, H^{7a}), 1.91 (d, $^4J_{7b–6b}$ = 1.0 Hz, 3 H, H^{7b}), 1.87 (m, 2 H, H$^{6'b}$), 1.80 (m, 1 H, H$^{8'b}$) ppm. ^{13}C NMR (MeOD, 125 MHz): δ = 165.0, 164.9, 151.0 and 150.9 (C^{2a}, C^{4a}, C^{2b} and C^{4b}), 136.5 and 136.2 (C^{6a} and C^{6b}), 110.8 and 110.5 (C^{5a} and C^{5b}), 87.6 (C$^{4'b}$), 85.7 (C$^{4'a}$), 84.7 (C$^{1'a}$), 84.6 (C$^{1'b}$), 81.7 (C$^{5'b}$), 76.0 (C$^{3'a}$), 71.3 (C$^{3'b}$), 61.0 (C$^{5'a}$), 39.3 (C$^{2'b}$), 38.4 (C$^{2'a}$), 27.6 (C$^{6'b}$), 22.0 (C$^{8'b}$), 20.5 (C$^{7'b}$), 11.2 and 11.1 (C^{7a} and C^{7b}) ppm. ^{31}P NMR (MeOD, 100 MHz): δ = 26.9 ppm. $C_{23}H_{31}N_4O_{11}P$ (570.49): calcd. C 48.42, H 5.48, N 9.82; found C 46.87, H 5.70, N 9.49 (+ H_2O).

(S_C,R_P) α,β-P-CNA (14): Compound **14** (13 mg, 90%) was obtained by using an identical procedure as described for **13**, starting from **12** (20 mg, 24.7 µmol), and TBAF solution (40 µL, 40 µmol). ^{1}H NMR (MeOD, 500 MHz): δ = 7.82 (d, $^4J_{6b–7b}$ = 1.0 Hz, 1 H, H^{6b}), 7.68 (d, $^4J_{6a–7a}$ = 1.0 Hz, 1 H, H^{6a}), 6.37 (dd, $^3J_{1'b–2''b}$ = 8.0, $^3J_{1'b–2'b}$ = 6.0 Hz, 1 H, H$^{1'b}$), 6.29 (dd, $^3J_{1'a–2''a}$ = 8.5, $^3J_{1'a–2'a}$ = 5.5 Hz, 1 H, H$^{1'a}$), 5.27 [X of an ABX(Y), $^3J_{3'a–2''a}$ = 5.5, $^3J_{3'a–P}$ = 4.5, $^3J_{3'a–4'a}$ = $^3J_{3'a–2'a}$ = 1.5 Hz, 1 H, H$^{3'a}$], 4.67 [A of an AX$_2$(Y), $^3J_{5'b–6'b,ax}$ = 11.0, $^3J_{5'b–6'b,ec}$ = $^3J_{5'b–4'b}$ = 2.5, $^3J_{5'b–P}$ < 2.0 Hz, 1 H, H$^{5'b}$], 4.46 [X of an ABX(Y), $^3J_{3'b–2''b}$ = 6.0, $^3J_{3'b–4'b}$ = $^3J_{3'b–2'b}$ = 2.5 Hz, 1 H, H$^{3'b}$], 4.22 (td, $^3J_{4'a–5'a}$ = $^3J_{4'a–5''a}$ = 3.0, $^3J_{4'a–3'a}$ = 1.5 Hz, 1 H, H$^{4'a}$), 3.97 (td, $^4J_{4'b–P}$ = 4.0, $^3J_{4'b–3'b}$ = $^3J_{4'b–5'b}$ = 2.5 Hz, 1 H, H$^{4'b}$), 3.83 (A of an ABX, $^2J_{5'a–5''a}$ = 13.5, $^3J_{5'a–4'a}$ = 3.0 Hz, 1 H, H$^{5'a}$), 3.80 (B of an ABX, $^2J_{5''a–5'a}$ = 13.5, $^3J_{5''a–4'a}$ = 3.0 Hz, 1 H, H$^{5''a}$), 2.46 [A of an ABX(Y), $^2J_{2'a–2''a}$ = 14.5, $^3J_{2'a–1'a}$ = 5.5, $^3J_{2'a–3'a}$ = 1.5 Hz, 1 H, H$^{2'a}$], 2.39 [B of an ABX(Y), $^2J_{2''a–2'a}$ = 14.5, $^3J_{2''a–1'a}$ = 8.5, $^3J_{2''a–3'a}$ = 5.5 Hz, 1 H, H$^{2''a}$], 2.29 (m, 1 H, H$^{7'b}$), 2.27 [A of an ABX(Y), $^2J_{2'b–2''b}$ = 14.0, $^3J_{2'b–1'b}$ = 6.0, $^3J_{2'b–3'b}$ = 2.5 Hz, 1 H, H$^{2'b}$], 2.18 [B of an ABX(Y), $^2J_{2''b–2'b}$ = 14.0, $^3J_{2''b–1'b}$ = 8.0, $^3J_{2''b–3'b}$ = 6.0 Hz, 1 H, H$^{2''b}$], 2.12 (m, 1 H, H$^{8'b}$), 2.07 (m, 1 H, H$^{7'b}$), 1.95 (d, $^4J_{7a–6a}$ = 1.0 Hz, 3 H, H^{7a}), 1.93 (m, 2 H, H$^{6'b}$), 1.89 (m, 1 H, H$^{8'b}$), 1.88 (d, $^4J_{7b–6b}$ = 1.0 Hz, 3 H, H^{7b}) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 165.3, 165.2, 151.2 and 150.9 (C^{2a}, C^{4a}, C^{2b} and C^{4b}), 136.2 and 136.0 (C^{6a} and C^{6b}), 110.8 and 110.5 (C^{5a} abd C^{5b}), 87.8 (C$^{4'b}$), 86.0 (C$^{4'a}$), 85.1 (C$^{1'b}$), 84.4 (C$^{1'a}$), 80.8 (C$^{5'b}$), 78.3 (C$^{3'a}$), 71.7 (C$^{3'b}$), 61.3 (C$^{5'a}$), 39.7 (C$^{2'b}$), 39.1 (C$^{2'a}$), 27.7 (C$^{6'b}$), 22.8 (C$^{8'b}$), 20.1 (C$^{7'b}$), 11.6 and 11.1 (C^{7a} and C^{7b}) ppm. ^{31}P NMR (MeOD, 100 MHz): δ = 31.3 ppm. $C_{23}H_{31}N_4O_{11}P$ (570.49): calcd. C 48.42, H 5.48, N 9.82; found C 47.01, H 5.62, N 9.49 (+H_2O).

Supporting Information (see footnote on the first page of this article): Calculation procedure, putative mechanism for phosphite isomerization, ^{31}P NMR spectra obtained during the Arbuzov reaction.

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