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Synthesis and NMR-studies of dinucleotides with conformationally restricted cyclic phosphotriester linkages

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Abstract—Four diastereomeric dinucleotides in which the phosphodiester linkages are conformationally restricted in cyclic phosphotriester structures are synthesised. From the epimeric 5′-*C*-vinyl thymidine derivatives, dinucleotides containing two terminal alkene moieties are constructed via standard phosphoramidite chemistry, and applied as substrates in ring-closing metathesis (RCM) reactions. Hereby, four diastereomeric dinucleotides with seven membered phosphepine rings in the inter-nucleoside linkages are obtained and separated, and their configurations elucidated by advanced NMR-studies in combination with restrained molecular dynamics (rMD) simulations. The seven membered rings are found to give some degree of conformational restriction in the natural nucleic acid backbone, and one of the four dinucleotides is found to favour stacking between the two adjacent thymine moieties. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

The introduction of conformational restriction in nucleic acid structures has been intensively investigated and approached at several levels.[1,2] Thus, oligonucleotides displaying high-affinity nucleic acid recognition have been obtained by, e.g. the incorporation of nucleoside analogues with bi- and tricyclic carbohydrate moieties.[3] One of the most successful of these artificial nucleic acid analogues is locked nucleic acid (LNA),[4–7] which has also been intensively studied by advanced NMR techniques.[8–12] Conformational restriction has also been introduced in the inter-nucleoside linkages[13] by, e.g. constrained heterocycles as non-ionic alternatives to the phosphodiester moiety.[14–16] Furthermore, larger ring structures for the construction of compounds mimicking nucleic acid secondary structures have been introduced by Sekine and co-workers.[17–20] Thus, dinucleotides have been designed in which linkages have been established between the 2′-position in the upper nucleoside and the nucleobase in the lower.[17,18] Other dinucleotides have linkages between the lower nucleobase and the phosphate moiety, which is hereby converted to an uncharged phosphotriester moiety.[19,20] These conformationally restricted mimics of nucleic acid structural motifs have all been obtained through conventional synthetic methods, where the phosphodi(tri)ester functionalities were established in the final steps by standard phosphoramidite chemistry.[17–20]

We have recently recognised the ring-closing metathesis (RCM) method as being of great potential in the construction of conformationally restricted nucleosides[21] as well as dinucleotide structures.[22] The RCM synthetic method has recently been intensively investigated for its convenient and very general applications in the synthesis of medium and large ring systems.[23–25] For example, RCM reactions have been used in peptide chemistry for the synthesis of cyclic di- and oligopeptides[26,27] as well as for the construction of a short conformationally restricted α-helix model.[28,29] Applying the very convenient RCM methodology, we have recently introduced the synthesis of a conformationally restricted cyclophosphate dinucleotide as a mixture of four diastereomers.[22] This represented the first introduction of isolated conformational restriction to the intact phosphodiester linkage, and furthermore, a very simple and general chemical methodology for the preparation of conformationally restricted nucleic acid analogues. Very recently, also the related cross metathesis method has been applied in the synthesis of other types of modified dinucleotides.[30,31] Herein, we report in full, the details of the synthesis of the four pure diastereomeric compounds **8**–**11** and the total stereochemical elucidation of these using advanced NMR-techniques including NOESY- and *J*-scaled ^{1}H–^{31}P HMBC-spectroscopy in combination with restrained molecular dynamics (rMD) simulations.

2. Results and discussion

2.1. Chemical synthesis

The easily available protected thymidine derivative **1** was oxidised using the Dess–Martin periodinane[32] and used in a

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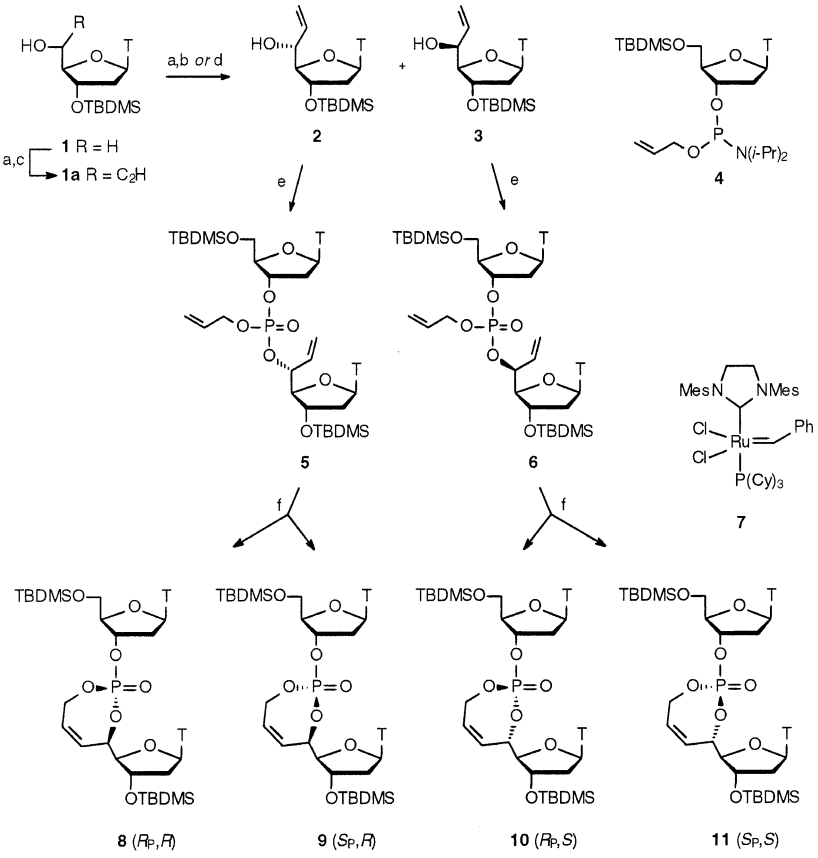
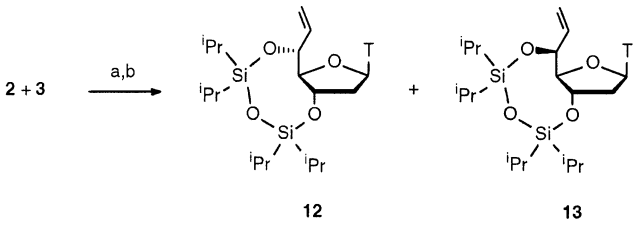


Scheme 1. *Reagents and conditions*: (a) Dess–Martin periodinane, CH_2Cl_2; (b) vinylMgBr, THF, 33% (2 steps); (c) ethynylMgBr, THF, 52% (2 steps); (d) H_2, Lindlar catalyst, EtOH, 98%; (e) (i) **4**, 1*H*-tetrazole, $(i\text{-Pr})_2NH$, CH_3CN; (ii) *t*-BuOOH, toluene, CH_2Cl_2, 53–87%; (f) **7**, CH_2Cl_2, 59–91%. T=thymin-1-yl. Mes=2,4,6-trimethylphenyl. Cy=cyclohexyl. TBDMS=*t*-butyldimethylsilyl.

Grignard reaction applying a commercial solution of vinyl magnesium bromide (Scheme 1).[33] This afforded the isomers **2** and **3** in an equimolar ratio but in a relatively low 41% yield (33% over the two steps).[22] Other Grignard reactions performed on the same aldehyde have been reported to give similar low yields especially due to a reductive side-reaction giving the starting material **1**.[34] Higher yields, however, have been reported with additives such as Cu(I) salts[35,36] or $CeCl_3$[35] but in our case, no improvements were observed,[22] even though the vinyl Grignard reagent has been successfully combined with $CeCl_3$ in connection to another nucleoside substrate.[37] As an alternative to the vinyl Grignard reaction, the use of an acetylenic Grignard reagent followed by a reduction using the Lindlar catalyst was applied. Thus, the Grignard addition afforded the epimeric mixture **1a** in 52% yield followed by a reduction in 98% yield to give the mixture of **2** and **3** in 50% overall yield from **1**. In this case, however, a 1.6:1 ratio of **2** and **3**, respectively, was obtained. The two products **2** and **3** were separated using HPLC. Nevertheless, the assignment of the two diastereomeric compounds could not be performed at this stage (vide infra).



Scheme 2. *Reagents and conditions*: (a) TBAF, THF; (b) $(i\text{-Pr})_2Si(Cl)O\text{-}Si(Cl)(i\text{-Pr})_2$, pyridine, 61% (2 steps). T=thymin-1-yl.

The equimolar mixture of **2** and **3** obtained through the first method as well as the two pure compounds were reacted with the allyl phosphoramidite **4**[38] in standard phosphoramidite coupling reactions[39] using 1*H*-tetrazole as the activator (Scheme 1). After oxidation, the dinucleotides **5** and **6** were obtained in medium to high yields either as one mixture of four diastereomers[22] or as two separate mixtures each containing two diastereomers in equimolar ratios differing only on the geometry at the phosphorous atom. In our first investigation, the mixture of **5** and **6** containing four diastereomers in an equimolar ratio was used as the substrate in RCM reactions using different conditions.[22] As Grubbs' improved catalyst **7**[40–42] with refluxing dichloromethane as the solvent was proven to be superior in this case affording an equimolar mixture of the four isomers **8**–**11** in 97% yield,[22] both **5** and **6** were reacted separately using these reaction conditions. Hence, the cyclophosphate dinucleotides **8**–**11** were obtained as four pure compounds after column chromatographic separations (Scheme 1). We have earlier proven that the mixture of **8**–**11** could be readily deprotected using trifluoroacetic acid for the hydrolysis of the silyl ethers.[22] However, we decided not to deprotect each of the four isomers separately

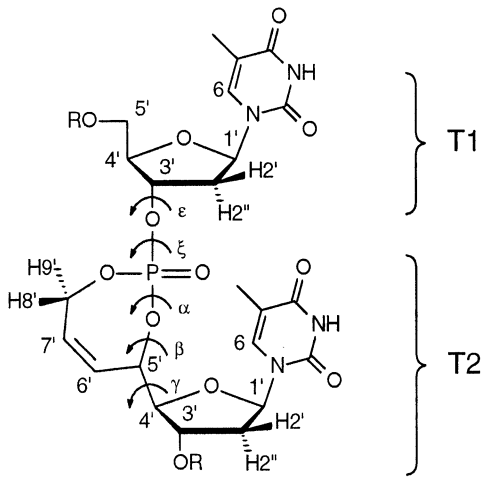


Figure 1. A general structure of **8**–**11** displaying the assignment of T1/T2, all atom numbering and standard torsional angles.[43]

at this stage as this is predictably unproblematic but unnecessary for the stereochemical determinations.

In order to elucidate the configurations of all compounds, the configurations of the two separated 5′-*C*-vinyl thymidine derivatives **2** and **3** were solved. Thus, the original equimolar mixture of **2** and **3** was deprotected using TBAF and reprotected in 61% overall yield with the disiloxane bidentate protecting group (Scheme 2). This group has been used before on similar compounds for the same purpose of elucidating the configurations of 5′-epimers.[34,36] The two cyclic silyl ethers **12** and **13** were separated using column chromatography and evaluated using NMR-spectroscopy. Thus, in an NOE-difference experiment mutual contacts between H-5′ and H-3′ as well as between H-5′ and H-6 confirm the 5′(*R*)-configuration of **12**. On the other hand, in the corresponding spectra of **13**, much larger mutual contacts between H-5′ and H-4′ in combination with the lack of contacts between H-5′ and H-3′/H-6 confirm the 5′(*S*)-configuration. Subsequently, the separated compounds **2** and **3** were treated in a similar way to give (in analytical experiments) compounds identical to **12** and **13**, respectively. Hence, **2** and subsequently the dinucleotides **5**, **8** and **9** were determined to have 5′(*R*)-configuration, whereas **3**, **6**, **10** and **11** were determined to have 5′(*S*)-configuration (Scheme 1). On the other hand, the configurations corresponding to the chiral phosphorous atoms should also be elucidated, and for this purpose, advanced NMR-techniques were applied as described in Section 2.2.

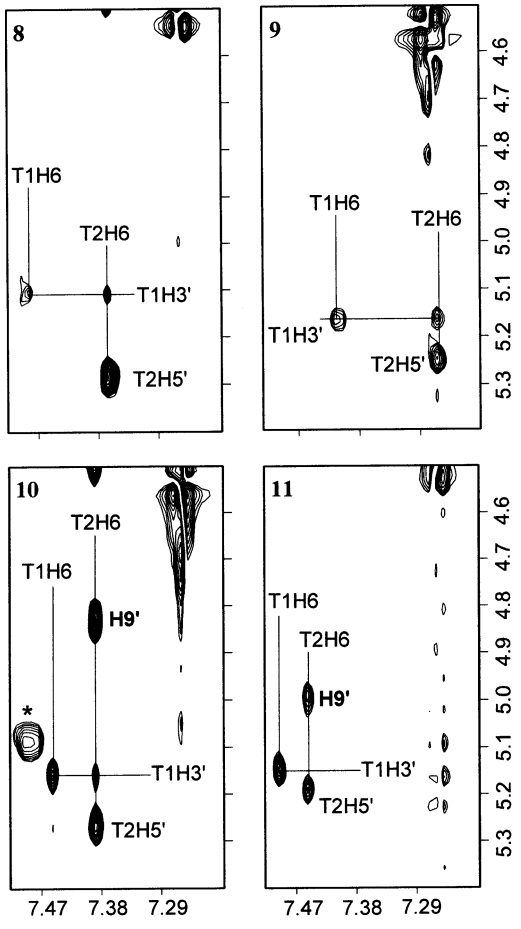


Figure 2. A region of the NOESY spectra of **8**–**11** displaying the aromatic to H-8′ and H-9′ protons. The large signals at 7.26 ppm are assigned to $CHCl_3$. * Impurity.

2.2. NMR-analysis of compounds 8–11. Spectral assignments and determination of NOE distances

All protons in the four compounds **8**–**11** were assigned with combined use of DQF-COSY spectra and NOESY spectra. The cross peaks in the 400 ms NOESY spectra have negative signs and the diagonal peaks have positive signs indicating rapid rotational motion of the molecules. In the following, all atom labelling follows the numbering shown in Fig. 1 with T1 being the 'upper' 5′-nucleotide and T2 the 'lower' 3′-nucleotide.[43]

The H-8′ and H-9′ protons on the methylene group in the seven membered ring were defined as the pro-*R* and pro-*S* protons, respectively, and were assigned by their cross peaks to T2 H-6 and T2 H-5′. Thus, as shown in Fig. 2, a connectivity between T2 H-6 and H-9′ was present in the two dimers known to have *S* configuration at T2 C-5′ (**10** and **11**) and absent in the two dimers having *R* configuration around T2 C-5′ (**8** and **9**). For compounds **8** and **9**, having 5′(*R*) configuration, H-8′ and H-9′ were assigned by their cross peaks with T2 H-5′. The strongest, albeit still weak cross peak was displayed by H-9′. The $^{1}H-^{1}H$ and $^{1}H-^{31}P$ *J* coupling constants were derived from the 1D ^{1}H NMR spectra and the *J* scaled $^{1}H-^{31}P$ HMBC spectra by measuring the splitting of doublet components.[44] In Fig. 3, the *J* scaled $^{1}H-^{31}P$ HMBC spectrum for **10** is shown. Furthermore, the $^{13}C-^{31}P$ *J* coupling constants were determined for compound **11** by measuring the doublet splitting of the lines in a ^{1}H–decoupled 1D ^{13}C spectrum (75.5 MHz). Assignment of the ^{13}C resonances was accomplished by use of an HSQC spectrum. For **8**–**10**, the $^{13}C-^{31}P$ *J* coupling constants were obtained from ^{1}H–decoupled 1D ^{13}C spectra (62.5 MHz) assuming similar assignments of the ^{13}C resonances in all the four isomers. In Table 1, all *J* coupling constants obtained for the four compounds are listed. From these, torsional angles were evaluated using Karplus equations.[45]

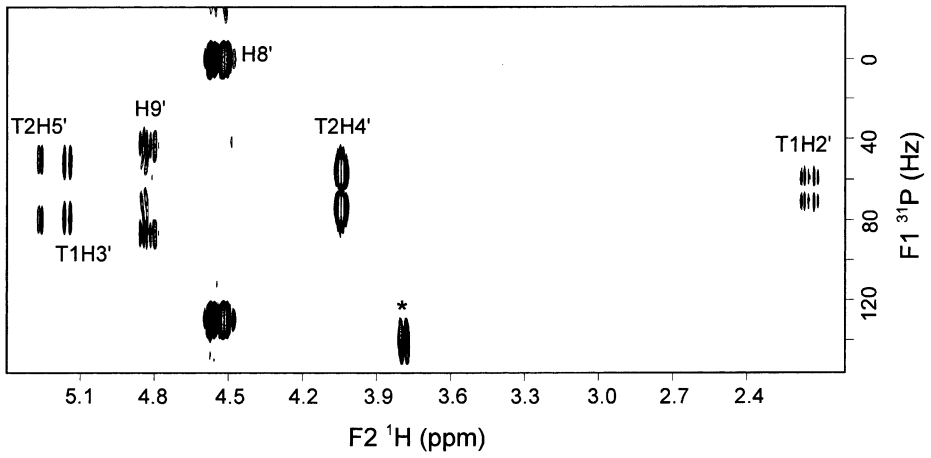


Figure 3. A *J*-scaled $^{1}H-^{31}P$ HMBC spectrum for **10**. * Impurity.

The NOESY spectra with a mixing time of 400 ms were used to derive NOE distance restraints for use in the rMD protocol. The isolated spin pair approximation (ISPA) approach was utilised and a number of non-covalently fixed distances in the four compounds were obtained. For **8**, **9** and **10**, 34 distance restraints were obtained, and 29 distance restraints were found for compound **11**.

As the $^{3}J_{T1\ C\text{-}4'-P}$, $^{3}J_{T1\ C\text{-}2'-P}$ and $^{3}J_{T1\ H\text{-}3'-P}$ coupling constants, all defining the ε-angle (Fig. 1),[43] are in agreement with a dynamic situation, in which the ε-angle can assume equal populations of *gauche*(+)-, *gauche*(−)- and *trans*-conformations, no torsional restraints were included for this angle. Due to these indications of dynamic behaviour, only three torsional restraints were used in total for all rMD calculations. These were the T2 H-4′–H-5′, the T2 C-4′–P as well as the H-8′–P torsions as these are all indicated by the corresponding coupling constants to be restrained in single conformations.

Table 1. *J*-coupling constants (in Hz) obtained for **8**–**11**

	8		**9**		**10**		**11**	
	T1[a]	T2[b]	T1[a]	T2[b]	T1[a]	T2[b]	T1[a]	T2[b]
$J_{1'2'}$	9.5	8.9	ND	ND	9.4	6.7	9.1	6.7
$J_{1'2''}$	5.0	5.4	4.9	ND	5.2	6.7	5.0	6.7
$J_{2'3'}$	5.5	5.5	5.5	ND	5.5	6.5	5.5	6.4
$J_{2''3'}$	0–1	1.7	0–1	ND	0–1	3.4	0.4	3.2
$J_{2'2''}$	−13.9	−13.0	−13.4	ND	−13.9	−13.4	−13.9	−13.5
$J_{2'P}$	2.0	–	–	–	1.5	–	2.0	–
$J_{3'4'}$	0–1	1.7	0–1	3.7	0–2	3.4	0–1	3.3
$J_{3'P}$	5.5	–	5.5	–	5.5	–	5.7	–
$J_{4'5'}$	0–2	4.5	0–2	3.7	0–2	3.4	0–2	3.3
$J_{4'P}$	–	3.4	–	3.7	–	3.4	–	3.3
$J_{5'P}$	–	5.4	–	ND	–	5.9	–	6.0
$J_{C4'\text{-}P}$[c]	5.2	10.5	4.7	10.6	6.0	10.0	5.5	9.9
$J_{C2'\text{-}P}$[c]	5.0	–	5.8	–	4.0	–	4.6	–
$J_{8'P}$	–	27.1	–	26.8	–	25.8	–	26.6
$J_{8'9'}$	–	16.4	–	16.9	–	16.0	–	16.6
$J_{8'7'}$	–	5.0	–	5.0	–	5.4	–	ND
$J_{9'P}$	–	7.8	–	7.4	–	8.7	–	7.7
$J_{9'7'}$	–	2.3	–	2.3	–	2.5	–	3.0
$J_{6'7'}$	–	12.4	–	12.0	–	12.0	–	ND

Obtained by the use of 1D ^{1}H-spectra and *J*-scaled $^{1}H-^{31}P$ HMBC spectra in $CDCl_3$.

[a] T1 refers to the 'upper' 5′-nucleoside.

[b] T2 refers to the 'lower' 3′-nucleotide including the phosphepine ring. ND denotes a situation where the *J*-coupling constant value could not be obtained due to spectral overlap.

[c] For **11**, $^{31}P-^{13}C$ *J*-coupling constants were obtained by the use of a proton-decoupled 75.5 MHz ^{13}C spectrum in combination with a HRQC spectrum. For **8**–**10**, $^{31}P-^{13}C$ *J*-coupling constants were obtained via analogous assignments in 62.5 MHz ^{13}C spectra.

2.3. Determination of the configuration at P

The determination of whether the phosphotriester has R_P or S_P configuration on the four diastereomers **8**–**11** was carried out using rMD calculations. The simulated annealing technique was employed in order to achieve the structures that most accurately reproduced the experimental restraints. As the configuration at the T2 C-5′ atom had previously been determined (using NOE difference spectra of **12** and **13**, vide supra), two starting model structures were used for each compound, these differing only by the stereochemistry around the phosphorous atom. Identical restraints were used in the two calculations, except for a chirality restraint keeping the phosphorous in the proper configuration depending on the starting model. The calculated structures were used to back calculate the NOESY spectra by utilising the *intense* and *spectrum* modules in the AMBER 5.0 program package.[46] By carefully evaluating the experimental and back-calculated spectra, a number of repulsive distance bounds were subsequently added to the rMD calculation, in cases where cross-peaks not observed in the experimental

Table 2. Results from the rMD calculations. The values given (in kcal/mol) are average values from all the calculated 20 structures in each run

	Configuration[a]	Constraint energy[b]	Largest violations[c]
8	R_PR	11.1	No violations >0.3 Å
	S_PR	21.8	1.0 Å on T1 H-3′-H-9′
	R_PR	23.8	1.2 Å on T1 H-3′-T2 CH_3
9			1.2 Å on T2 H-5′-T1 H-2″
	S_PR	5.5	No violations >0.3 Å
10	R_PS	2.9	No violations >0.25 Å
	S_PS	17.1	1.1 Å on T1 H-4′-T2 CH_3
11	R_PS	6.3[d]	0.7 Å on T2 H-5′-T1 H-2″
	S_PS	1.9[d]	No violations >0.25 Å

Performed using the AMBER 5.0 program package.[46]

[a] Starting structures referring to configurations at the phosphorous atom and at T2 C-5′, respectively.

[b] Average constraint energies for the starting structures. In each calculation, 34 distance bounds were obtained.

[c] Violated distance bounds compared to experimental NOESY distances.

[d] For **11**, only 29 distance bounds were obtained.

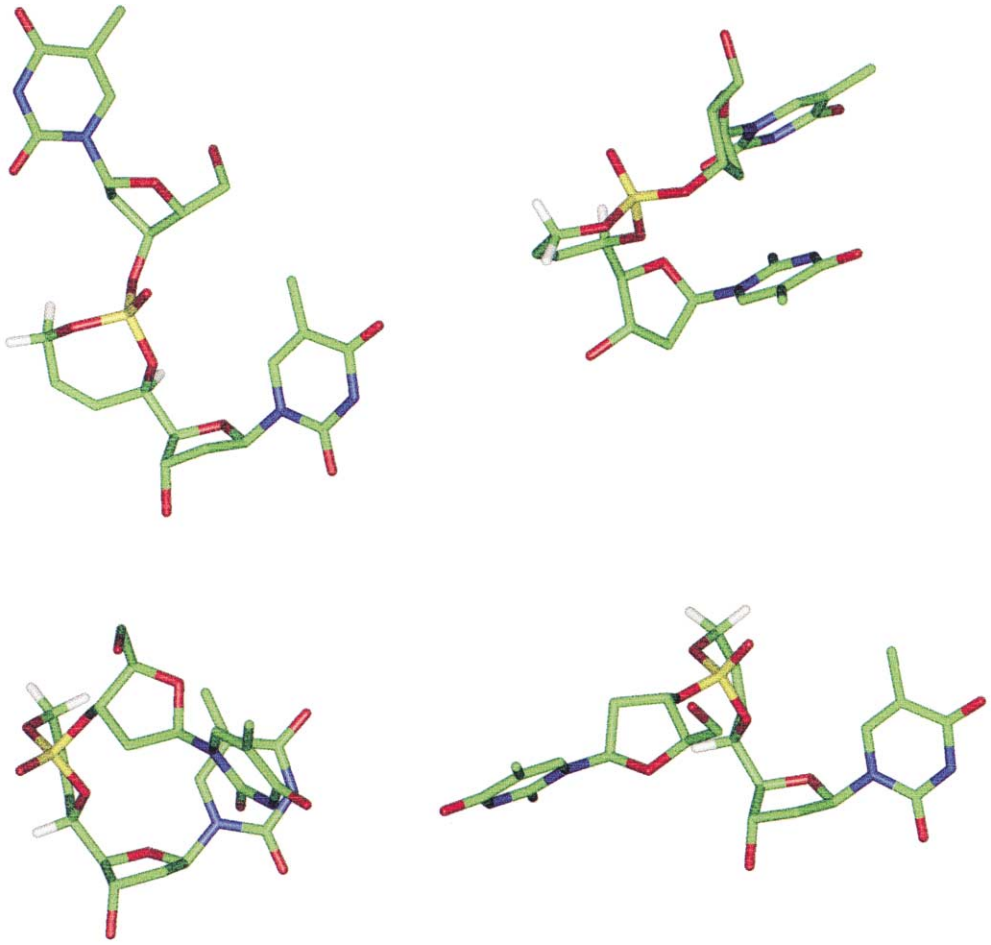


Figure 4. Representations of the four calculated average structures for **8**–**11**. Upper left, **8**; upper right, **9**; lower left, **10** and lower right, **11**. All TBDMS protecting groups excluded. All hydrogen's except H-5′, H-8′ and H-9′ excluded.

spectra were seen in the theoretical spectra of the obtained energy minimised structures. Twenty structures were generated for each starting model in each calculation and all calculated structures converged towards one final structure. The average RMSD values in each run were between 0.25 and 1.0 Å. Subsequently, the two final structures for each compound were evaluated by their total energy, divided into the contributions from constraint energy (energy penalty from distances violated) and force field energy (all other contributions). The contributions of the force field energies to the total energy did not differ significantly between the two starting structures, meaning that no gross violations of the covalent structure had occurred. Thus, the violations of the restraints employed were used in the refinement. Table 2 gives the results in the form of average constraint energies and violated distance bounds for the two starting structures of each compound. It must be noted that the constraint energies should not be compared between two different compounds, as the number of constraints differ from compound to compound. Thus, compound **11**, which had the lowest number of constraints, also achieves the lowest constraint energies for its two starting structures. By inspection of Table 2, it is observed that for each compound, only one starting structure could achieve a low force field energy without violating one or more of the distance bounds given. Consequently, the configuration at the phosphorous in that starting structure must correspond to the actual configuration of this compound giving the final assignments of the compounds **8**–**11** shown in Scheme 1. Representations of the four calculated structures are shown in Fig. 4. The backbone torsion angles α, β and γ (Fig. 1) measured on the optimised structures of the four compounds are shown in Table 3.

Table 3. Backbone angles measured in the four dinucleotides **8**–**11**. All values are given as the average values as measured on all 20 calculated structures ± a standard deviation

	α (°)	β (°)	γ (°)
8	−51.2±2.1	181.8±0.8	53.0±1.8
9	−167.0±0.9	170.2±3.8	68.3±4.4
10	−147.3±0.7	197.5±0.4	42.2±0.4
11	72.2±11	188.1±2.0	57.6±2.7

3. Discussion

The four isomers **8**, **9**, **10** and **11** have been synthesised, separated and obtained in reasonable yields. One of the original purposes was the introduction of conformational restriction by a simple and convenient methodology, i.e. relatively few synthetic steps. Using the experience from the deprotection step on the diastereomeric mixture,[22] the

deprotected dinucleotides could be obtained in only five steps from **1** or, preferably, six steps through **1a**. Even though tedious separation of isomers was applied, this RCM strategy does in fact display a very fast and simple introduction of a conformationally restrictive cyclic structure into the natural nucleic acid structure. The synthesis might be optimised by an alternative introduction of the vinyl group affording **2** and **3**, and stereoselective methods might be developed. Hereby, the potentially more interesting of the two alternative 5′-configurations could be favoured. Thus, the completely stereoselective introduction of an allyl group on the 5′-C position of the 3′-*O*-TBDPS protected analogue of **1** has recently been demonstrated.[47]

The compounds **8–11** were investigated using NMR-spectroscopy including NOESY spectra and rMD calculations, and hence their configurations have been determined. In summary, **8** and **9** have been determined to have 5′(*R*)- and **10** and **11** 5′(*S*)-configuration, whereas **8** and **10** have been determined to have R_P- and **9** and **11** S_P-configuration, respectively. The validity of the stereo specific assignments of the phosphotriesters was further evaluated by examining the chemical shift differences of the H-9′ protons. For the H-8′ protons, only small differences between the four isomers (≤0.08 ppm) are observed, whereas for the H-9′ protons, larger differences, up to 0.23 ppm, were seen (Fig. 2). These differences group the compounds together in pairs according to the chemical shift of H-9′, as **9** and **11** have δ-values of 5.06 and 5.00 ppm, respectively, and **8** and **10** have δ-values of 4.87 and 4.83 ppm, respectively. **9** and **11** both have S_P configuration bringing H-9′ and the double-bonded oxygen on P into close proximity and thereby deshielding the proton more than for **8** and **10**, where H-9′ and the oxygen are far apart. Examination of the phosphorous chemical shifts reveals an identical trend. In **8** and **10**, the chemical shift values of the phosphorous atoms are 2.42 and 2.70 ppm, respectively, and in **9** and **11**, the values are 1.90 and 2.00 ppm, respectively.

The structures determined (Fig. 4) are average structures and do not represent the actual dynamics of the structures in solution. This is also indicated by the values of the coupling constants (vide supra). Especially, the coupling constants at the link between nucleotide T1 and the phosphotriester group indicate a highly dynamic structure, with free rotation around the C-3′–O-3′ and O-3′–P bonds described by the ε- and ξ-torsion angles, respectively (Fig. 1). Furthermore, the coupling constants, particularly $J_{1'2'}$, $J_{2''3'}$ and $J_{3'4'}$, in the T2 deoxyribose ring in compounds **9**, **10** and **11** indicate a dynamic equilibrium between N- and S-type[43] sugar conformations. Due to the corresponding coupling constants in the T2 ring in **8** as well as in the T1 ring of all the four isomers, the equilibrium of these deoxynucleotides seems to be shifted towards S-type conformations. The coupling constants around the β- and γ-angles (Fig. 1) indicate a more conformationally restricted system. Thus, the presence of a $^4J_{4'P}$ coupling constant indicates a W-conformation of this (P–O–C–C–H-4′) moiety, and the large $^3J_{C4'P}$ coupling constants dictate the β-torsion angle to be approximately 180°. The restriction around γ is probably due to steric interactions between the seven membered ring and the remaining part of the T2 nucleotide.

When evaluating the backbone torsion angles, some deviations of the values from the normal ranges for right-handed duplex structures are seen (Table 3). In right-handed duplex structures, the α-, β- and γ-angles normally have *gauche*(−)-, *trans*- and *gauche*(+)-conformations, respectively.[43] All four compounds have the β- and γ-angles in a normal (*trans*, *gauche*(+))-conformation, whereas the α-angle differs among the compounds. In **9** and **10** the α-angle adopts a *trans*-conformation, in **11** the α-angle is in a *gauche*(+)-conformation but in **8**, the α-angle adopts a more regular *gauche*(−)-conformation. Thus, this compound might after deprotection and incorporation into a nucleic acid sequence favour the thermal stability of a duplex structure.

By examination of the achieved low-energy structures in Fig. 4, it is seen that the calculated structure of compound **9** exhibits some degree of stacking between the two nucleobases. An increased stacking of the nucleobases is often reflected in the chemical shift values of the aromatic protons, and by inspection of the 1D ^{1}H NMR spectra of the four compounds, it is found that **9** has the lowest chemical shift values of its aromatic protons, H-6, on both nucleobases (Fig. 2). This stacking was also suggested by some of the NOESY cross peaks, especially the relatively large cross peak between T1 H-1′ and T2 H-6. Therefore, also this compound after incorporation into oligonucleotide sequences, might favour the hybridisation with complementary DNA or RNA sequences. On the other hand, the four dinucleotides might be useful for the introduction of bends in the nucleic acid structure or for inducing conformational shifts in the structure as approached before using other cyclic dinucleotide structures.[17–20] The conformational analysis in the present work, however, has been performed on the TBDMS-protected compounds **8–11**, dissolved in chloroform. Changes in these conformations on deprotection or change of solvent cannot be excluded, although there may be no reason to expect such a change. Nevertheless, in our opinion, we find it safe to use the observed conformational shifts between the compounds **8–11** to predict the different behaviour that can be expected when these dinucleotides are incorporated into oligonucleotides. For the synthesis of these oligonucleotides, the allylic nature of the cyclic phosphotriester moieties might turn out to be a very unstable structure which can not be combined by the conventional solid phase methodology[39] for nucleic acid synthesis. Thus, alternative methods might be needed for the incorporation of the deprotected dinucleotides of **8–11** into oligonucleotide sequences. Furthermore, the olefinic moiety should be easily derivatised or hydrogenated, hereby, however, releasing some of the conformational restriction.

4. Conclusions

Four diastereomeric dinucleotides conformationally restricted around their internucleotide linkages by seven membered phosphotriester containing rings have been synthesised and their configurations determined. The RCM method has proven very convenient for the introduction of conformational restriction in nucleic acid structures. Furthermore, the complete stereochemical elucidation of the

four isomers can be applied also for potential analogues of **8**–**11**. The conformational behaviour of **8**–**11** has also been described. From this knowledge, any of the four protected compounds, or analogues thereof, can be deprotected[22] and applied in the construction of oligonucleotides with a conformational restriction determined by the four possible cyclophosphate ring configurations. Thus, in **9** and **10**, the nucleobases are apparently in a close proximity, whereas in **8** and **11**, a large increase in the distance between two adjacent nucleobases is introduced. As suggested from the preferred torsion angles, the dinucleotide **8** might fit reasonably well into duplex structures. Due to the apparent tendency for stacking, however, **9** might also form a favourable conformational restriction in oligonucleotides with high affinity for complementary nucleic acids. Further work on the incorporation of the deprotected, and/or saturated, analogues of **8**–**11** in oligonucleotides as well as further application of the very simple RCM-methodology for the construction of conformationally restricted nucleic acid structural analogues is in progress in our laboratory.

5. Experimental

All commercial reagents were used as supplied. All reactions were performed under an atmosphere of nitrogen and Column chromatography was carried out on glass columns using Silica gel 60 (0.040–0.063 mm). NMR spectra were obtained on a Bruker AC 250 FT, a Varian Gemini 2000 or a Varian Unity 500 spectrometer. ^{1}H NMR spectra were recorded at 250, 300 or 500 MHz, ^{13}C NMR spectra were recorded at 62.5 or 75.5 MHz and ^{31}P NMR spectra were recorded at 121.5 MHz. Values for δ are in ppm, relative to tetramethylsilane as internal standard or 85% H_3PO_4 as external standard. FAB mass spectra were recorded in positive ion mode on a Kratos ms50TC spectrometer. Accurate mass determinations were performed an Ionspec Ultima Fourier Transform mass spectrometer. Microanalyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen. Analytic and preparative HPLC was performed on a Waters 600E, System Controller and a Waters 484, Tunable Absorbance Detector. The eluent was 35% CH_3CN, 65% Phosphate-buffer (0.02 M, pH 7). For analytical HPLC, a C-18 column (300×3.9 mm^2) was used and for preparative HPLC a C-18 column (300×57 mm^2) with Waters Delta Pak 15μ C-18.

5.1. NMR spectroscopy of 8–11

All NMR experiments were performed on a Varian UNITY 500 spectrometer at 25°C in $CDCl_3$. 1D ^{1}H NMR spectra of **8**–**11** were recorded with a spectral width of 5500 Hz. A ^{1}H, ^{13}C–HSQC spectrum of **11** was recorded using WURST ^{13}C-decoupling during data acquisition. This spectrum was acquired with spectral widths of 5500 and 25,000 Hz in the $F2$ and $F1$ dimensions, respectively, collecting 96 transients for each of the 640 t_1-experiments sampled with 4K points. Phase sensitive NOESY spectra with a mixing time of 400 ms were recorded for **8**–**11** with 2K points in the t_2-dimension using spectral widths of 5500 Hz in both dimensions and 56 transients for each of the 512 t_1-experiments. Phase sensitive DQF-COSY spectra were recorded with 4K points sampled in $F2$, 5500 Hz spectral widths in both dimensions and 1024 t_1-experiments, each recorded with 32 transients. J scaled ^{1}H–^{31}P HMBC spectra[44] were recorded with a scaling factor of κ=5, using a spectral width of 5500 Hz in the t_2-dimension and 500 Hz in the t_1-dimension. 4K points were sampled in $F2$ and 112 t_1-experiments were recorded each with 96 transients. The $F1$ dimension was subsequently linear predicted from 56 to 112 points. Prior to Fourier transformation, the FIDs from all 2D experiments were apodised by a skewed sinebell squared window function in both dimensions.

5.2. Restrained MD simulations of 8–11

All MD calculations were performed using the AMBER 5.0 program package[46] on an SGI/O2 workstation. Due to the lack of suitable angular parameters in the AMBER 5.0 forcefield to describe the modifications at the phosphorous atom, a series of ab initio calculations were performed using the Gaussian 98 program[48] and the 3-21G and 6-31G(d,p) basis sets on a model system including the four stereoisomers of 1-methoxy-3-methyl-1-oxo-3*H*,6*H*-2,7-dioxaphosphepine. These ab initio energy optimised structures were used to measure ideal angles to be included in the force field calculations. Atomic charges around the modified phosphorous were calculated using the restrained electrostatic potential (RESP) procedure.[49]

Distance restraints were derived from the NOESY spectra using the ISPA approach. After carefully evaluating back-calculated NOESY spectra based on the energy minimized structures, additional restraints were later included in form of broad repulsive restraints. All distance restraints were incorporated into the refinement as distance bounds with bounds of the calculated ISPA value ±0.2 Å. Torsional restraints were derived from J coupling constants and were included only in cases where the values of the J coupling constants excluded more than one conformation. In addition, six chirality restraints at the chiral atoms in the two deoxyribose sugar rings were included in each calculation.

A simulated annealing protocol was utilized to obtain average structures of the four compounds. For each compound, two different starting structures, differing only in the configuration at the phosphorous atom, were used. For compounds **8** and **9** starting structures with *R* configuration at T2 C-5′ were used, and for **10** and **11** the starting structures had *S* configuration at T2 C-5′. For each starting structure, additional chiral restraints at C-5′ and P were added. Each structure was then initially energy minimized before being subjected to 28 ps of molecular dynamics in time-steps of 1 fs:4 ps at 900 K followed by cooling to 200 K over 24 ps. Finally, a restrained energy minimization was performed for each structure. For all restraints the force constant used was K=20 kcal/mol Å^2. A distance dependent dielectric constant, $\varepsilon = 4r$, was used and the non-bonded cut-off was 16 Å.

5.3. Chemical preparations

5.3.1. Preparation of 3′-*O*-(*tert*-butyldimethylsilyl)-5′(*R*)-*C*-vinylthymidine (2) and 3′-*O*-(*tert*-butyldimethylsilyl)-5′(*S*)-*C*-vinylthymidine (3). Compound **1** (2.50 g,

7.01 mmol) was dissolved in anhydrous CH_2Cl_2 (25 mL) and the Dess–Martin periodinane (3.47 g, 8.18 mmol) was added. The reaction mixture was stirred at rt for 3 h. The mixture was added CH_2Cl_2 (50 mL) and filtered through a layer of celite. The filtrate was washed with a 10% aqueous solution of $Na_2S_2O_3$ (2×75 mL), H_2O (75 mL), a saturated aqueous solution of $NaHCO_3$ (2×75 mL) and brine (75 mL). The organic phase was dried with Na_2SO_4 and concentrated in vacuo. The product was purified by column chromatography (30–70% EtOAc in hexanes) and the aldehyde intermediate isolated as an off-white solid (yield 1.99 g, ~80%), which was used without further purification. The crude aldehyde (1.40 g, 3.95 mmol) was dissolved in anhydrous THF (60 mL) and a 1.0 M solution of vinylmagnesium bromide in THF (11.9 mL, 11.9 mmol) was added dropwise during 10 min. The reaction mixture was stirred at rt for 20 h. The reaction was quenched with H_2O (125 mL) and neutralised to pH~6 with 4 M acetic acid. The mixture was extracted with CH_2Cl_2 (400 mL), and the organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (125 mL), dried with Na_2SO_4, and concentrated in vacuo. The product was purified with column chromatography (30–50% EtOAc in hexanes) and isolated as a clear oil and as a ~1:1 mixture of epimers. Yield 620 mg, 41%; R_f 0.45 (75% EtOAc in hexanes); FAB MS *m/z*: 383 (MH^+, 32%). Subsequently, the mixture was separated on HPLC giving the two pure epimers **2** and **3**. **2**: ^{1}H NMR ($CDCl_3$) δ 0.04–0.07 (6H, m, $Si(CH_3)_2$), 0.88 (9H, s, $C(CH_3)_3$), 1.91 (3H, s, CH_3), 2.12 (^{1}H, ddd, J=12.9, 5.8, 1.6 Hz, H-2′), 2.34 (1H, ddd, J=12.9, 8.8, 5.9 Hz, H-2′), 2.98 (1H, br s, OH), 3.95 (1H, m, H-5′), 4.42–4.52 (2H, m, H-4′ and H-3′), 5.31 (1H, d, J=10.7 Hz CH=CH_2), 5.45 (1H, d, J=17.2 Hz, CH=CH_2), 5.92 (1H, ddd, J=17.2, 10.7, 4.9 Hz, CH=CH_2), 6.16 (1H, dd, J=8.8, 5.7 Hz, H-1′), 7.47 (1H, br s, H-6), 8.74 (1H, br s, NH); ^{13}C NMR ($CDCl_3$) δ −4.73, −4.53 ($Si(CH_3)_3$), 12.47 (CH_3), 17.80 ($C(CH_3)_3$), 25.68 (C(CH_3)$_3$), 40.48 (C-2′), 70.81, 72.70 (C-5′ and C-3′), 87.49, 90.17 (C-1′ and C-4′), 110.95 (C-5), 116.73 (CH=CH_2), 136.34, 137.40 (CH=CH_2 and C-6), 150.36 (C-2), 163.66 (C-4). **3**: ^{1}H NMR ($CDCl_3$) δ 0.09 (6H, s, $Si(CH_3)_2$), 0.90 (9H, s, $C(CH_3)_3$), 1.91 (3H, s, CH_3), 2.18 (1H, m, H-2′), 2.36 (1H, m, H-2′), 3.89 (1H, m, H-5′), 4.27 (1H, m, H-4′), 4.52 (1H, m, H-3′), 5.27 (1H, d, J=10.5 Hz, CH=CH_2), 5.38 (1H, d, J=17.0 Hz, CH=CH_2), 5.99 (1H, ddd, J=17.0, 10.5, 5.8 Hz, CH=CH_2), 6.14 (1H, t, J=6.9 Hz, H-1′), 7.45 (1H, br s, H-6), 8.84 (1H, br s, NH); ^{13}C NMR ($CDCl_3$) δ −4.80, −4.64 ($Si(CH_3)_3$), 12.47, (CH_3), 17.91 ($C(CH_3)_3$), 25.70 (C(CH_3)$_3$), 40.10 (C-2′), 72.53, 72.60 (C-5′ and C-3′), 87.23, 89.51 (C-1′ and C-4′), 110.88 (C-5), 116.69 (CH=CH_2), 137.20, 137.53 (CH=CH_2 and C-6), 150.26 (C-2), 163.72 (C-4).

5.3.2. Preparation of 3′-*O*-(*tert*-butyldimethylsilyl)-5′-*C*-ethynylthymidine (1a). To a solution of the Dess–Martin periodinane (2.0 g, 4.7 mmol) in anhydrous CH_2Cl_2 (45 mL), a solution of **1** (1.202 g, 3.36 mmol) dissolved in anhydrous CH_2Cl_2 (15 mL) was added at rt. The reaction mixture was stirred for 1 h at rt. The mixture was poured into a mixture of a saturated aqueous solution of $NaHCO_3$ (50 mL) and a saturated aqueous solution of $Na_2S_2O_3$ (50 mL) and extracted with CH_2Cl_2 (2×60 mL). The organic phase was dried with Na_2SO_4 and concentrated in vacuo to give the crude aldehyde (1.18 g, ~98%) as a white solid. The crude aldehyde was dissolved in anhydrous THF (30 mL) and stirred at 0°C. A 0.5 M solution of ethynylmagnesium bromide (13.2 mL, 6.6 mmol) was added dropwise. The reaction mixture was stirred at rt for 18 h. The reaction was quenched with H_2O (42 mL) and neutralised to pH~6 with 4 M acetic acid. The mixture was extracted with CH_2Cl_2 (4×30 mL), and the organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (50 mL), dried with Na_2SO_4 and concentrated in vacuo. The product was purified with column chromatography (30–50% EtOAc in hexanes) and isolated as a white solid and as a ~1.6:1 mixture of epimers, which was used without further purification in the next step. Yield 648 mg, 52%; R_f 0.45 (75% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$) δ 0.10 (s, $Si(CH_3)_2$), 0.12 (s, $Si(CH_3)_2$), 0.90 (s, $C(CH_3)_3$), 0.90 (s, $C(CH_3)_3$), 1.89–1,92 (m, CH_3), 2.10–2.24 (m, H-2′), 2.30–2.46 (m, H-2′), 2.55 (d, J=2.1 Hz, C≡CH), 2.57 (d, J=2.1 Hz, C≡CH), 3.35–3.43 (m, OH), 4.02 (m, H-4′), 4.07 (m, H-4′), 4.51–4.64 (m, H-3′, H-5′), 6.14 (dd, J=8.1, 6.2 Hz, H-1′), 6.22 (dd, J=9.0, 5.7 Hz, H-1′), 7.38 (br s, H-6), 7.51 (br s, H-6), 8.67 (br s, NH), 8.71 (br s, NH). ^{13}C NMR ($CDCl_3$) δ −4.79, −4.71, −4.67, −4.64 ($Si(CH_3)_2$), 12.46, 12.50 (CH_3), 17.85, 17.90 ($C(CH_3)_3$), 25.69 (C(CH_3)$_3$), 39.87, 40.28 (C-2′), 62.11, 62.86 (C-5′), 72.28 (C-3′), 74.28, 75.33 (C≡CH), 81.41 (C≡CH), 87.50, 87.99 (C-1′), 89.16, 89.63 (C-4′), 111.04, 111.06 (C-5), 137.20, 137.31 (C-6), 150.31 (C-2), 163.57, 163.58 (C-4); ESI MS *m/z*: 381 (MH^+, 100%).

5.3.3. Alternative preparation of 3′-*O*-(*tert*-butyldimethylsilyl)-5′-*C*-vinylthymidine (mixture of 2 and 3). To a stirred solution of **1a** (0.629 g, 1.65 mmol) in EtOAc (20 mL) was added Lindlar's catalyst (0.6 g). The mixture was degassed with Ar, saturated with H_2 for 5 min. and stirred at rt under a H_2 atmosphere for 24 h. The mixture was filtered through a layer of celite and concentrated in vacuo to give the product as an ~1.6:1 mixture of the epimers **2** and **3**, respectively. Yield 0.623 g, 98%.

5.3.4. Preparation of allyl 3′-*O*-(5′-*O*-*tert*-butyldimethylsilyl)thymidinyl 5′-*O*-(3′-*O*-*tert*-butyldimethylsilyl)-5′-*C*-vinylthymidinyl phosphoric ester (mixture of 5 and 6). The ~1:1 mixture of compounds **2** and **3** (235 mg, 0.614 mmol) was mixed with compound **4** (835 mg unpurified material ~1.0 mmol), coevaporated twice with anhydrous CH_3CN and redissolved in anhydrous CH_3CN (40 mL). A 0.45 M solution of 1*H*-tetrazole in CH_3CN (4.10 mL, 1.85 mmol) was added. The reaction mixture was stirred at rt for 1 h and the reaction was quenched with anhydrous MeOH (1.0 mL). The residue was concentrated in vacuo, coevaporated twice with CH_2Cl_2, and redissolved in anhydrous CH_2Cl_2 (25 mL). The solution was cooled to 0°C and a 3.0 M solution of *tert*-butylhydrogenperoxide in toluene (1.25 mL, 3.75 mmol) was added. The reaction mixture was stirred at 0°C for 2.5 h, diluted with MeOH (2.0 mL) and concentrated in vacuo. The product was purified twice by column chromatography (0–6% MeOH in CH_2Cl_2 and afterwards 10% EtOAc in hexanes) and isolated as a white solid and an equimolar mixture of four diastereomers (**5**+**6**). Yield 449 mg, 87%; R_f 0.30 (5% MeOH in $CHCl_3$); ^{31}P NMR ($CDCl_3$) δ −1.97, −1.46, −1.25, −0.85; FAB MS *m/z*: 841 (MH^+, 1%);

HiRes MALDI FT-MS *m/z*: [MNa^+] found/calcd 863.3462/863.3454.

5.3.5. Preparation of allyl 3′-*O*-(5′-*O*-*tert*-butyldimethylsilyl)thymidinyl 5′-*O*-(3′-*O*-*tert*-butyldimethylsilyl)-5′(*R*)-*C*-vinylthymidinyl phosphoric ester (5). The same procedure as described for the mixture of compounds **5** and **6** was applied using **2** (88 mg, 0.23 mmol), **4** (417 mg unpurified material ~0.5 mmol), 0.45 M solution of 1*H*-tetrazole in anhydrous CH_3CN (1.75 mL, 0.79 mmol), anhydrous CH_3CN (20 mL) and 1 h reaction time for the phosphitylation and 3.0 M solution of *tert*-butylhydrogenperoxide in anhydrous toluene (485 μL, 1.50 mmol), anhydrous CH_2Cl_2 (15 mL) and 2 h reaction time for the oxidation. The product was purified by column chromatography (2×0–6% MeOH in CH_2Cl_2, 30–80% EtOAc in petrol ether and 10% EtOAc in petrol ether), and the product was isolated as a white solid and an equimolar mixture of two diastereomers. Yield 106 mg, 55%; R_f 0.66 (1% MeOH in EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.06–0.15 (12H, m, 2×$Si(CH_3)_2$), 0.89 (9H, s, $C(CH_3)_3$), 0.92 (9H, s, $C(CH_3)_3$), 1.90–1.98 (6H, m, 2×CH_3), 2.01–2.28 (3H, m, 3×H-2′), 2.48–2.55 (1H, m, H-2′), 3.77–3.99 (3H, m, 2×H-5′ and H-4′), 4.23–4.28 (1H, m, H-4′), 4.45–4.63 (3H, m, H-3′ and $CH_2CH{=}CH_2$), 4.88–5.04 (2H, m, H-3′ and H-5′), 5.24–5.57 (4H, m, 2×$CH{=}CH_2$), 5.83–6.00 (2H, m, 2×$CH{=}CH_2$), 6.24–6.39 (2H, m, 2×H-1′), 7.41–7.51 (2H, m, 2×H-6), 8.53–8.68 (2H, m, 2×NH); ^{31}P NMR ($CDCl_3$) δ −1.92, −1.22; FAB MS *m/z*: 863 (MNa^+, 11%).

5.3.6. Preparation of allyl 3′-*O*-(5′-*O*-*tert*-butyldimethylsilyl)thymidinyl 5′-*O*-(3′-*O*-*tert*-butyldimethylsilyl)-5′(*S*)-*C*-vinylthymidinyl phosphoric ester (6). The same procedure as described for the mixture of **5** and **6** was applied using **3** (88 mg, 0.23 mmol), **4** (401 mg unpurified material ~0.5 mmol), 0.45 M solution of 1*H*-tetrazole in anhydrous CH_3CN (1.65 mL, 0.74 mmol), anhydrous CH_3CN (20 mL) and 5 h reaction time for the phosphitylation and 3.0 M solution of *tert*-butylhydrogenperoxide in anhydrous toluene (500 μL, 1.50 mmol), anhydrous CH_2Cl_2 (20 mL) and 2 h reaction time for the oxidation. The product was purified by column chromatography (2×0–6% MeOH in CH_2Cl_2, 30–80% EtOAc in petrol ether and 10% EtOAc in petrol ether), and the product was isolated as a white solid and an equimolar mixture of two diastereomers. Yield 102 mg, 53%; R_f 0.66 (1% MeOH in EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.06–0.14 (12H, m, 2×$Si(CH_3)_2$), 0.86–0.94 (18H, m, 2×$C(CH_3)_3$), 1.92 (3H, s, CH_3), 1.95 (3H, s, CH_3), 1.98–2.14 (2H, m, 2×H-2′), 2.22–2.30 (1H, m, H-2′), 2.49–2.57 (1H, m, H-2′), 3.76–3.99 (3H, m, 2×H-5′ and H-4′), 4.23–4.27 (1H, m, H-4′), 4.37–4.42 (1H, m, H-3′), 4.50–4.58 (2H, m, $CH_2CH{=}CH_2$), 4.80–5.00 (2H, m, H-3′ and H-5′), 5.24–5.56 (4H, m, 2×$CH{=}CH_2$), 5.83–6.05 (2H, m, 2×$CH{=}CH_2$), 6.26–6.40 (2H, m, 2×H-1′), 7.44–7.59 (2H, m, 2×H-6), 8.16–8.34 (2H, m, 2×NH); ^{31}P NMR ($CDCl_3$) δ −1.50, −0.87; FAB MS *m/z*: 841 (MH^+, 8%).

5.3.7. Preparation of 1-(2(*R*)-(*tert*-butyldimethylsilyl)-oxymethyl-5(*R*)-(thymin-1-yl)tetrahydrofuran-3(*S*)-oxyl)-3-(3(*S*)-(*tert*-butyldimethylsilyl)oxy-5(*R*)-(thymin-1-yl)-tetrahydrofuran-2(*S*)-oxyl)-1-oxo-3,6-dihydro-2,7-dioxaphosphepine (mixture of 8–11). An equimolar mixture of compounds **5** and **6** (38 mg, 0.045 mmol) was coevaporated with anhydrous CH_2Cl_2 and redissolved in anhydrous CH_2Cl_2 (2.2 mL). Catalyst **7** (1.9 mg, 0.0023 mmol) was added and the reaction mixture was stirred at 40°C for 2 h. The solution was concentrated in vacuo and the product was purified by column chromatography (45–80% EtOAc in hexanes) and isolated as a clear off-white oil and as an equimolar mixture of four diastereomers. Yield 33.4 mg, 91%. R_f 0.36 and 0.24 (25% EtOAc in hexanes); ^{31}P NMR ($CDCl_3$) δ 1.80, 2.00, 2.42, 2.71; HiRes MALDI FT-MS *m/z*: [MNa^+] found/calcd 835.3141/835.3141.

5.3.8. Preparation of 1(R_P and S_P)-(2(*R*)-(*tert*-butyldimethylsilyl)oxymethyl-5(*R*)-(thymin-1-yl)tetrahydrofuran-3(*S*)-oxyl)-3(*R*)-(3(*S*)-(*tert*-butyldimethylsilyl)oxy-5(*R*)-(thymin-1-yl)tetrahydrofuran-2(*S*)-yl)-1-oxo-3,6-dihydro-2,7-dioxaphosphepine (8) and (9). The same procedure as described for the mixture **8–11** was applied using the diastereomeric mixture **5** (48 mg, 0.057 mmol), anhydrous CH_2Cl_2 (3.0 mL), catalyst **7** (2.4 mg, 0.0028 mmol) and 1 h reaction time. The product was purified by column chromatography (50% EtOAc in petrol ether) and preparative TLC (75%EtOAc in petrol ether and subsequently 5% MeOH in CH_2Cl_2) affording two compounds as white solids; **8** (12.9 mg, 28%) and **9** (14.4 mg, 31%). **8**: R_f 0.22 (75% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$) δ 0.09–0.14 (12H, m, 2×$Si(CH_3)_2$), 0.87–0.94 (18H, m, 2×$C(CH_3)_3$), 1.92 (3H, s, T1 CH_3), 1.94 (3H, s, T2 CH_3), 2.00 (1H, ddd, *J*=13.0, 8.9, 5.5 Hz, T2 H-2′), 2.14 (1H, dddd, *J*=13.9, 9.5, 5.5, 2.0 Hz, T1 H-2′), 2.26 (1H, ddd, *J*=13.0, 5.4, 1.7 Hz, T2 H-2″), 2.53 (1H, dd, *J*=13.9, 5.0 Hz, T1 H-2″), 3.83–3.93 (2H, m, T1 H-5′ and T1 H-5″), 4.03 (1H, ddd, *J*=4.5, 3.4, 1.7 Hz, T2 H-4′), 4.27 (1H, s, T1 H-4′), 4.49 (1H, ddd, *J*=5.5, 1.7, 1.7 Hz, T2 H-3′), 4.61 (1H, ddd, *J*=27.1, 16.4, 5.0 Hz, H-8′), 4.87 (1H, ddd, *J*=16.4, 7.8, 2.3 Hz, H-9′), 5.11 (1H, dd, *J*=5.5, 5.5 Hz, T1 H-3′) 5.29 (1H, m, T2 H-5′), 5.78 (1H, d, *J*=12.4 Hz, H-6′), 5.88 (1H, dddd, *J*=12.4, 5.0, 2.3, 2.3 Hz, H-7′), 6.32 (1H, dd, *J*=8.9, 5.4 Hz, T2 H-1′), 6.39 (1H, dd, *J*=9.5, 5.0 Hz, T1 H-1′), 7.37 (1H, s, T2 H-6), 7.49 (1H, s, T1 H-6), 8.62 (1H, s, NH) 8.79 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ −5.5, −5.4, −4.8, −4.5, 12.3, 12.5, 17.8, 18.3, 25.7, 25.9, 39.3 (d, *J*=5.0 Hz, T1 C-2′), 40.8, 63.4, 65.0 (d, *J*=5.5 Hz), 71.9, 74.4 (d, *J*=5.4 Hz), 80.0 (d, *J*=3.7 Hz), 84.6, 85.8, 86.1 (d, *J*=5.2 Hz, T1 C-4′), 88.1 (d, *J*=10.5 Hz, T2 C-4′), 111.3, 111.4, 126.2, 128.8, 134.7, 135.2, 150.1, 150.3, 163.4, 163.5; ^{31}P NMR ($CDCl_3$) δ 2.42; FAB MS *m/z*: 813 (MH^+, 12%), 339 ([3′-deoxy-5′-*O*-TBDMS-thymidine]$^+$, 100%). **9**: R_f 0.34 (75% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$) δ 0.07–0.15 (12H, m, 2×$Si(CH_3)_2$), 0.87–0.94 (18H, m, 2×$C(CH_3)_3$), 1.92 (3H, s, T1 CH_3), 1.95 (3H, s, T2 CH_3), 2.07 (1H, m, T2 H-2′), 2.24 (2H, m, T2 H-2′ and T2 H-2″), 2.47 (1H, dd, *J*=13.4, 4.9 Hz, T1 H-2″), 3.82–3.93 (2H, m, T1 H-5′ and T1 H-5″), 4.00 (1H, ddd, *J*=3.7, 3.7, 3.7 Hz, T2 H-4′), 4.27 (1H, s, T1 H-4′), 4.56 (1H, m, H-8′), 4.57 (1H, m, T2 H-3′), 5.06 (1H, ddd, *J*=16.9, 7.4, 2.3 Hz, H-9′), 5.16 (1H, dd, *J*=5.5, 5.5 Hz, T1 H-3′), 5.25 (1H, m, T2 H-5′), 5.77 (1H, d, *J*=12.0 Hz, H-6′), 5.87 (1H, dddd, *J*=12.0, 5.0, 2.3, 2.3 Hz, H-7′), 6.35 (1H, m, T2 H-1′), 6.36 (1H, m, T1 H-1′), 7.26 (1H, s, T2 H-6), 7.41 (1H, s, T1 H-6), 9.28 (1H, s, NH), 9.31 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ −5.5, −5.4, −4.8, −4.4, 12.5, 12.6, 17.8, 18.3, 25.7, 25.9, 39.0 (d, *J*=5.8 Hz, T1 C-2′), 40.7, 63.3, 64.3 (d, *J*=5.5 Hz), 70.6, 74.8 (d, *J*=7.7 Hz), 79.8 (d, *J*=4.2 Hz), 84.4, 84.7, 85.5

(d, J=4.7 Hz, T1 C-4′), 87.1 (d, J=10.6 Hz, T2 C-4′), 111.4, 111.5, 126.6, 128.2, 134.6, 135.6, 150.4, 150.5, 163.6, 163.6; ^{31}P NMR ($CDCl_3$) δ 1.90; FAB MS m/z: 835 (MNa^+, 34%), 339 ([3′-deoxy-5′-*O*-TBDMS-thymidine]$^+$, 100%).

5.3.9. Preparation of 1(R_P and S_P)-(2(*R*)-(*tert*-butyldimethylsilyl)oxymethyl-5(*R*)-(thymin-1-yl)tetrahydrofuran-3(*S*)-oxyl)-3(*S*)-(3(*S*)-(*tert*-butyldimethylsilyl)oxy-5(*R*)-(thymin-1-yl)tetra-hydro-furan-2(*S*)-yl)-1-oxo-3,6-dihydro-2,7-dioxaphosphepine (10) and (11). The same procedure as described for the mixture **8**–**11** was applied using the diastereomeric mixture **6** (47 mg, 0.056 mmol), anhydrous CH_2Cl_2 (3.0 mL), catalyst **7** (4.9 mg, 0.0058 mmol) and 20 h reaction time. The product was purified by column chromatography (50% EtOAc in petrol ether) and preparative TLC (75%EtOAc in petrol ether and subsequently 5% MeOH in CH_2Cl_2) affording two compounds as white solids; **10** (14.5 mg, 32%) and **11** (15.0 mg, 33%). **10**: R_f 0.37 (75% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$) δ ^{1}H NMR ($CDCl_3$) δ 0.10–0.14 (12H, m, 2×Si(CH_3)$_2$), 0.87–0.95 (18H, m, 2×C(CH_3)$_3$), 1.91 (3H, s, T1 CH_3), 1.93 (3H, s, T2 CH_3), 2.15 (1H, dddd, J=13.9, 9.4, 5.5, 1.5 Hz, T1 H-2′), 2.19 (1H, m, T2 H-2′), 2.31 (1H, ddd, J=13.4, 6.7, 3.4 Hz, T2 H-2″), 2.58 (1H, dd, J=13.9, 5.2 Hz, T1 H–2″), 3.84–3.94 (2H, m, T1 H-5′ and T1 H-5″), 4.04 (1H, ddd, J=3.4, 3.4, 3.4 Hz, T2 H-4′), 4.29 (1H, s, T1 H-4′), 4.49 (1H, ddd, J=6.5, 3.4, 3.4 Hz, T2 H-3′), 4.54 (1H, ddd, J=25.8, 16.0, 5.4 Hz, H-8′), 4.83 (1H, m, H-9′), 5.16 (1H, dd, J=5.5, 5.5 Hz, H-3′ in T1), 5.25 (1H, m, T2 H-5′), 5.81 (1H, d, J=12.0 Hz, H-6′), 5.90 (1H, dddd, J=12.0, 5.4, 2.5, 2.5 Hz, H-7′), 6.27 (1H, dd, J=6.7, 6.7 Hz, T2 H-1′), 6.31 (1H, dd, J=9.4, 5.2 Hz, T1 H-1′), 7.39 (1H, s, T2 H-6), 7.45 (1H, s, T1 H-6), 8.93 (1H, s, NH), 8.94 (1H, br s, NH); ^{13}C NMR ($CDCl_3$) δ −5.5, −5.4, −4.8, −4.6, 12.5, 12.7, 17.8, 18.3, 25.7, 25.9, 39.5 (d, J=4.0 Hz, T1 C-2′), 40.7, 63.3, 64.6 (d, J=6.3 Hz), 72.1, 74.1 (d, J=5.0 Hz), 80.2 (d, J=4.4 Hz), 84.6, 85.6, 85.7 (d, J=6.0 Hz, T1 C-4′), 87.9 (d, J=10.0 Hz, T2 C-4′), 111.0, 111.3, 128.0, 128.3, 134.6, 135.7, 150.1, 150.2, 163.5, 163.7; ^{31}P NMR ($CDCl_3$) δ 2.70; FAB MS m/z: 813 (MH^+, 11%), 339 ([3′-deoxy-5′-*O*-TBDMS-thymidine]$^+$, 100%). **11**: R_f 0.24 (75% EtOAc in hexanes); ^{1}H NMR ($CDCl_3$) δ 0.09–0.14 (12H, m, 2×Si(CH_3)$_2$), 0.88–0.94 (18H, m, 2×C(CH_3)$_3$), 1.92 (3H, s, T1 CH_3), 1.93 (3H, s, T2 CH_3), 2.13 (1H, dddd, J=13.9, 9.1, 5.5, 2.0 Hz, T1 H-2′), 2.22 (2H, m, T2 H-2′ and T2 H-2″), 2.60 (1H, dd, J=13.9 Hz, 5.0, T1 H-2″), 3.83–3.93 (2H, m, T1 H-5′ and T1 H-5″), 4.02 (1H, ddd, J=3.3, 3.3, 3.3 Hz, T2 H-4′), 4.28 (1H, s, T1 H-4′), 4.44 (1H, ddd, J=6.4, 3.3, 3.2 Hz, T2 H-3′), 4.62 (1H, dd, J=26.6, 16.6 Hz, H-8′), 5.00 (1H, ddd, J=16.6, 7.7, 3.0 Hz, H-9′), 5.15 (1H, dd, J=5.7, 5.5 Hz, T1 H-3′), 5.19 (1H, m, T2 H-5′), 5.84–5.90 (2H, m, H-6′ and H-7′), 6.33 (1H, dd, J=6.7, 6.7 Hz, T2 H-1′), 6.36 (1H, dd, J=9.1, 5.0 Hz, T1 H-1′), 7.45 (1H, s, T2 H-6), 7.50 (1H, s, T1 H-6), 8.50 (1H, s, NH), 8.51 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ −5.5, −5.4, −4.8, −4.6 (Si(CH_3)$_2$), 12.5, 12.5 (CH_3), 17.9, 18.3 (*C*(CH_3)$_3$), 25.7, 25.9 (C(*C*H_3)$_3$), 39.2 (d, J=4.6 Hz, T1 C-2′), 40.8 (T2 C-2′), 63.4 (T1 C-5′), 65.0 (d, J=5.4 Hz, C-8′), 72.5 (T2 C-3′), 74.9 (d, J=7.7 Hz, T2 C-5′), 80.2 (d, J=4.0 Hz, T1 C-3′), 84.7 (T1 C-1′), 85.3 (T2 C-1′), 86.1 (d, J=5.5 Hz, T1 C-4′), 88.2 (d, J=9.9 Hz, T2 C-4′), 111.3, 111.5 (2×C-5), 127.2, 129.0 (C-6′, C-7′), 134.7, 135.8 (2×C-6), 150.2, 150.2 (2×C-4), 163.6, 163.5 (2×C-2); ^{31}P NMR ($CDCl_3$) δ 2.00; FAB MS m/z: 835 (MNa^+, 7%), 339 ([3′-deoxy-5′-*O*-TBDMS-thymidine]$^+$, 100%).

5.3.10. Preparation of 3′,5′-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxandiyl)-5′(*R*)-*C*-vinylthymidine (12) and 3′,5′-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxandiyl)-5′(*S*)-*C*-vinylthymidine (13). A solution of the ~1:1 mixture of **2** and **3** (47 mg, 0.12 mmol) in anhydrous THF (1.5 mL) was added a 1.0 M solution of TBAF in THF (190 μL, 0.190 mmol). The solution was stirred at rt for 1 h and concentrated in vacuo. The product was purified by column chromatography (5% MeOH in CH_2Cl_2) affording 5′-*C*-vinylthymidine (24 mg, 0.090 mmol); R_f 0.22 (10% MeOH in CH_2Cl_2). The product was coevaporated with anhydrous pyridine and redissolved in anhydrous pyridine (600 μL) and 1,2-dichlorethane (50 μL). The reaction mixture was stirred at 0°C, and 1,3-dichlor-1,1,3,3-tetraisopropyl-disiloxane (72 μL, 0.23 mmol) was added. The mixture was stirred at rt for 16 h and diluted with CH_2Cl_2 (30 mL). The mixture was washed with a saturated aqueous solution of $NaHCO_3$ (2×15 mL), H_2O (15 mL) and brine (15 mL), dried (Na_2SO_4), concentrated in vacuo and coevaporated with toluene. The residue was purified by column chromatography (10–20% EtOAc in petrol ether and subsequently, 12% EtOAc in petrol ether), to give three fractions as white solids; pure **12** (18 mg), pure **13** (10 mg) and a mixture of **12** and **13** (10 mg). Combined yield 38 mg, 61%. **12**: R_f 0.52 (75% EtOAc in petrol ether); ^{1}H NMR ($CDCl_3$) δ 0.96–1.19 (28H, m, 4×CH(CH_3)$_2$, 2×Si(CH)$_2$), 1.94 (3H, s, CH_3), 2.30–2.54 (2H, m, 2×H-2′), 3.54 (1H, dd, J=9.0, 5.9 Hz, H-4′), 4.38 (1H, m, H-5′), 4.61 (1H, m, H-3′), 5.19 (1H, dt, J=10.6, 1.7 Hz, CH=CH_2), 5.42 (1H, dt, J=17.0, 1.7 Hz, CH=CH_2), 5.96–6.05 (2H, m, H-1′ and C*H*=CH_2), 7.05 (1H, d, J=1.0 Hz, H-6), 8.29 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ 12.70, 13.02, 13.19, 13.47 (2×Si(CH)$_2$), 16.88, 16.95, 17.17, 17.30, 17.40, 17.44, 17.52, 17.66 (4×CH_2(*C*H_3)$_2$), 40.99 (C-2′), 74.34, 75.50 (C-3′ and C-5′), 85.00, 87.80 (C-1′ and C-4′), 110.97, 114.95 (C-5 and CH=*C*H_2), 135.88, 137.66 (C-6 and *C*H=CH_2), 149.69 (C-2), 163.32 (C-4); FAB MS m/z: 511 (MH^+, 7%); HiRes MALDI FT-MS m/z: [MNa^+] found/calcd 533.2474/533.2474. **13**: R_f 0.48 (75% EtOAc in petrol ether); ^{1}H NMR ($CDCl_3$) δ 0.98–1.20 (28H, m, 4×CH(CH_3)$_2$ and 2×Si(CH)$_2$), 1.92 (3H, s, CH_3), 2.20 (1H, ddd, J=13.8, 7.2, 2.3 Hz, H-2′), 2.46 (1H, m, H-2′), 3.82 (1H, dd, J=7.8, 3.1 Hz, H-4′), 4.46 (1H, m, H-3′), 4.61 (1H, m, H-5′), 5.30 (1H, d, J=10.5 Hz, CH=CH_2), 5.47 (1H, d, J=17.1 Hz, CH=CH_2), 5.95–6.07 (2H, m, H-1′ and C*H*=CH_2), 7.47 (1H, s, H-6), 8.37 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ 12.71, 12.90, 13.20, 13.52 (4×Si(CH)$_2$), 16.86, 17.00, 17.10, 17.29, 17.33, 17.47 (4×CH(*C*H_3)$_2$), 39.60 (C-2′), 67.57, 69.57 (C-3′ and C-5′), 83.90, 86.65 (C-1′ and C-4′), 110.23, 115.55 (C-5 and CH=*C*H_2), 135.16, 135.79 (C-6 and *C*H=CH_2), 149.87 (C-2), 163.54 (C-4); FAB MS m/z: 511 (MH^+, 8%); HiRes MALDI FT-MS m/z: [MNa^+] found/calcd 533.2457/533.2474.

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