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Synthesis of modified oligonucleotides containing 4-guanidino-2-pyrimidinone nucleobases

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Abstract—Oligonucleotides incorporating 4-guanidino-2-pyrimidinone nucleobases have been prepared. These nucleobase analogues were designed to mimic the double hydrogen bond donor pattern of protonated cytosines in parallel triple helices. Guanidine-, *N*-methyl-, *N*,*N*-dimethyl-, and *N*,*N*′-dimethylguanidine-containing nucleoside *H*-phosphonates were used for the synthesis of oligonucleotide analogues with minor modifications in standard solid-phase procedures.

1. Introduction

Oligonucleotides are currently used in molecular biology for the detection, sequencing, amplification and mutation of gene segments.[1] More recently, therapeutic applications have also been designed based on their hybridization potential, which would permit the recognition of cellular targets such as RNA or DNA.[2] Interaction with proteins has also been devised. The formation of specific complexes could either block, enhance or simply regulate the function of the corresponding gene or protein, thus correcting the genetic disorder previously produced.

In particular, segments of nuclear double stranded (ds) DNA could be targeted by the formation of triple helices.[3] In such complexes, the third oligonucleotide chain binds to DNA polypurine tracks establishing Hoogsteen-type hydrogen bonds in the major groove. Different triple helix motifs[4] may be formed depending on the sequence of the third chain and its relative orientation with respect to the polypurine-containing strand of the duplex, which becomes the central strand of the triplex. Pyrimidine-containing oligonucleotides form T(A·T) and C^+(G·C) triads[5] and are oriented parallel to the polypurine strand, while in the purine motif the alignment of the third chain is antiparallel and A(A·T) and G(G·C) triplets are formed.

Although triple helix-forming oligonucleotides produce regulatory effects in biological media,[6] their possible pharmacological application is hampered by several limitations.[7] First, they only recognize polypurine tracks. Second, triple helices are less stable than double helices, because of the increased electrostatic repulsion between negatively charged chains, and their stability depends on the pH and ion concentration (Mg^{2+}, K^+) of the medium. Finally, unmodified oligonucleotides are not stable to nucleases, and their permeation across cellular membranes is quite limited.

In order to increase the affinity of the third strand for dsDNA, several options have been studied. On the one hand, triple-helix formation can be facilitated by the addition of DNA ligands[8] or polyamines.[9] On the other hand, chemical modification of the third strand either at the internucleoside phosphate groups,[10] or at the sugar[11] or nucleobase moieties[12] often increases the stability to

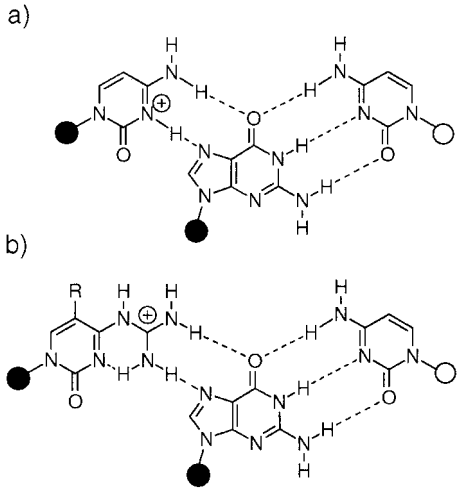

Figure 1. (a) Canonical C^+(G·C) triad in triple helices (relative orientation of strands is represented by white and black circles, respectively); (b) 4-Guanidino-2-pyrimidinone nucleobase as an analogue of protonated cytosine in the C^+(G·C) triplet.

Keywords: guanidine; *H*-phosphonate; nucleobase; triple helix; oligonucleotide analogues.

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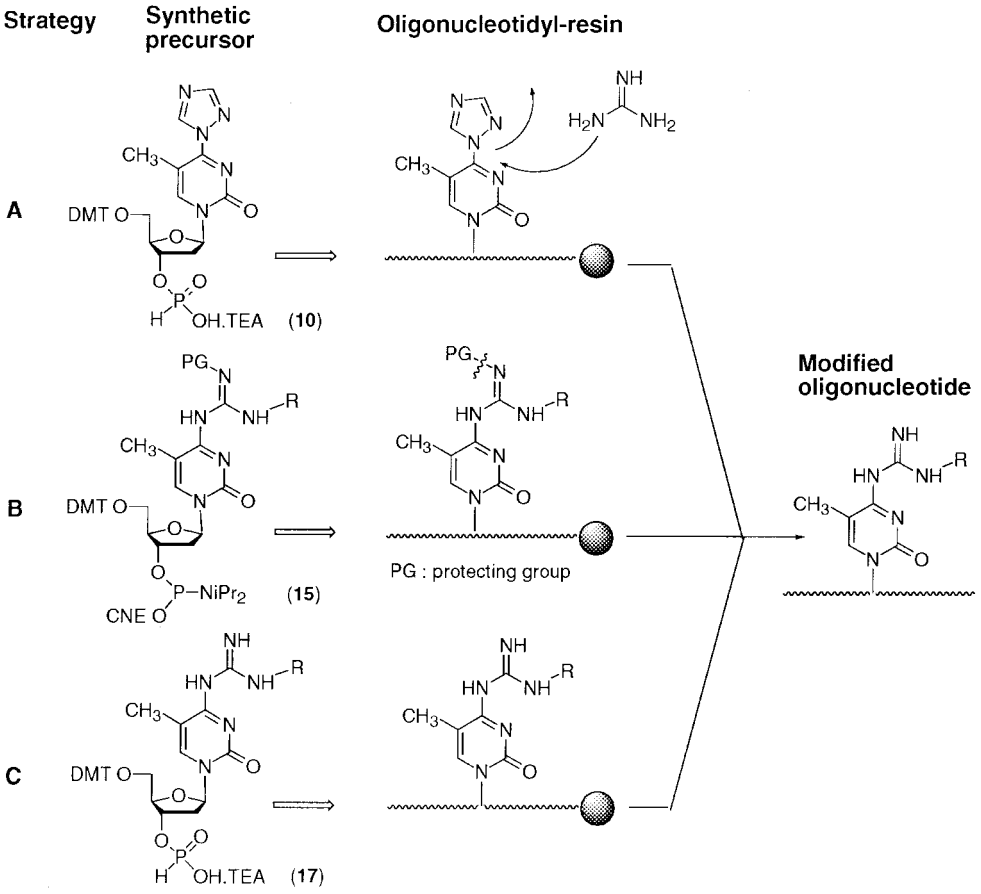


Figure 2. Alternatives for the preparation of modified oligonucleotides containing 4-guanidino-2-pyrimidinone nucleobases.

nucleases, reduces the electrostatic repulsion between negatively charged chains and provides additional interactions that favour triplex formation.

Much more specific problems have been also addressed. The most important is the pH dependence for parallel triple helix formation, which often precludes the formation of this type of complex in physiological media.[13] The reason is that cytosine needs to be protonated to form two Hoogsteen hydrogen bonds with the central guanine in the C^+(G·C) triad (Fig. 1). Although several studies indicate that the pK_a of cytosine (pK_a=4.5) increases when it is immersed in a polyanionic oligonucleotide strand,[14] triplex stability decreases as the pH increases.[15] In order to overcome this pH dependence, several analogues have been designed. Some are neutral analogues that reproduce the hydrogen bond scheme of protonated cytosine,[16–23] others are either more basic than cytosine or have better stacking properties.[24–26] Most stabilize the triple helix at neutral pH when introduced in the third strand, and even suppress the pH dependence, but they are difficult to synthesize. Only 8-oxoadenine and 5-methylcytosine derivatives are commercially available.

In order to retain the hybridization properties of protonated cytosine, we reasoned that an amidinium group could act as the double hydrogen bond donor. Pyrimidine nucleobases modified at the 4-position are easy to obtain,[27] so we considered the 4-guanidino-2-pyrimidinone analogue shown in Fig. 1 a good candidate. Moreover, since the guanidine group (pK_a=13) is more basic than the imino nitrogen of cytosine, the guanidine-modified nucleobase would be protonated at neutral pH.

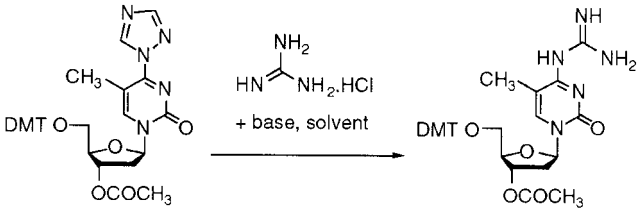
In comparison with protonated cytosine, the guanidine group introduces additional bonds between the third and the central strands, which probably displaces the backbone of the third strand and leads to non-isomorphic triplets (Fig. 1). However, lack of isomorphism with standard triplets does not seem to significantly lower the affinity of the third strand, since protonated cytosine analogues in which the sugar was also displaced away from the major groove do form triple helices.[16–18] Behr and cowokers had also proposed the use of oligonucleotides containing guanidine nucleobases analogues for triple helix formation,[28] and they reported on the synthesis of a 4-guanidino-2-pyrimidinone nucleoside.[29] They also described the synthesis of closely-modified oligonucleotides[30,31] but, to our knowledge, the introduction of 4-guanidino-2-pyrimidinone nucleoside in oligonucleotides has not been accomplished yet.

Here we report on the development of a synthetic methodology to obtain oligonucleotides containing this protonated cytosine analogue.

2. Results

We assayed three strategies to obtain modified oligonucleotides incorporating 4-guanidino-2-pyrimidinone nucleobases

Table 1. Conversion yields for the transformation of 4-triazolyl- and 4-phenoxy-2-pyrimidinone nucleosides into 4-guanidino derivatives (specific details for each treatment are shown in the Experimental)



Z	Base[a]			
	K_2CO_3	DBU	$N(C_2H_5)_3$	$N(C_2H_5)_3$/NMI
Triazolyl (**1**)	****	**	***	***
3-Nitrotriazolyl (**2**)	****	***	***	***
3-Bromotriazolyl (**3**)	***		***	
4-Nitrophenoxy (**4**)	***		*	*
2-Nitrophenoxy (**5**)	***	nr	*	
2,4-Dichlorophenoxy (**6**)	***	nr	*	
2,4,5-Trichlorophenoxy (**7**)	**	nr	nr	
2,3,4,5,6-Pentachlorophenoxy (**8**)	**	nr	nr	

[a] For conversion yields, the following convention is used: ****, 100% after 6–8 h; ***, 100% after 8–16 h; **, 50–75% after 16 h; *, 25–50% after 16 h; nr, no reaction.

by solid phase synthesis (Fig. 2). The first alternative (A) uses a convertible nucleobase derivative,[32] which can be transformed into the desired guanidine derivative in a single step before the final deprotection and cleavage of the oligonucleotide from the resin. In both the second and third strategies (B and C), guanidino-derivatized nucleosides are the synthons used for oligonucleotide assembly. The main difference between B and C is the protection of the guanidine group, and different methods for the formation of internucleoside phosphate linkages have also been used.

2.1. Synthesis using strategy A

Strategy A required reaction conditions to replace a leaving group by a guanidine moiety without damaging the oligonucleotide chain. Several triazolyl[33,34] and phenoxy-based[34,35] leaving groups were assayed, since they are accessible from pyrimidine nucleosides and labile to oxygen and nitrogen nucleophiles to yield the corresponding substitution products. In particular, we synthesized several 3′-*O*-acetyl-5′-*O*-dimethoxytrityl-5-methyl-2-pyrimidinone derivatives (see below) as model nucleosides to test the reaction conditions for their conversion into guanidine-containing derivatives by reaction with guanidine hydrochloride in the presence of a base. The results of these experiments are summarized in Table 1.

As can be inferred from Table 1, the best combination was using triazolyl or 3-nitrotriazolyl convertible groups, and K_2CO_3 as the base for their transformation into guanidine derivatives. Poorer results were always obtained with phenoxy derivatives, even those activated towards nucleophile attack by electron-withdrawing groups. No improvements (data not shown) were made by changing the solvent (acetonitrile, tetrahydrofuran, dioxane or water). Since we anticipated that the low solubility of K_2CO_3 in DMF would not facilitate the reaction on a solid matrix, addition of a crown ether or use of Li_2CO_3, which would procure a more homogeneous reaction medium, were also evaluated, but in no case did results improve.

These results were applied to preliminary experiments on a solid support. The synthetic precursor of the modified nucleoside was the 3′-*H*-phosphonate derivative of 5′-*O*-DMT-5-methyl-4-triazolyl-2-pyrimidinon-1-yl-2′-deoxyriboside (Fig. 2, strategy A), which was obtained from 5′-*O*-DMT-thymidine in a single step.[36] In our hands, this reaction gave much better results than substitution of guanidine for the triazolyl group on the phosphoramidite derivative. Trimer TXT (where X represents the triazolyl-modified nucleoside) was assembled using standard *H*-phosphonate chemistry,[37] and tuning of the modification and cleavage/deprotection conditions was assayed. Best results were achieved when a 4 h-treatment of the oligonucleotide-resin with guanidine (60°C) was followed by reaction with conc. aq. ammonia at room temperature. Analysis of the crude by reversed phase HPLC and MALDI-TOF mass spectrometry showed TT^gT (where T^g is the guanidine-modified nucleoside) as the major product (86%). Two minor side-products, TC^mT (where C^m accounts for 5-methylcytosine) and TTT, were also observed, and their relative amount increased significantly when the treatment with aqueous ammonia was carried out at 50°C.

We then proceeded with the synthesis of a longer chain, $^{5'}TTTTT^gTTT^gTT^gTTTTT^{3'}$. The oligomer was solid-phase assembled using the same methodology as above, and the resin was treated with the guanidine·HCl/K_2CO_3 mixture in DMF at 60°C for 6 h to introduce the guanidine groups. The modified oligonucleotide was then cleaved from the resin by treatment with ammonia at room temperature for 2 h, and the resulting crude was purified by reversed phase HPLC. Detailed HPLC and PAGE analysis of the purified strand under more stringent conditions showed that it was a mixture of products. Analysis by MALDI-TOF mass spectrometry confirmed that the mixture was constituted by at least three products, containing one, two or three guanidino-modified nucleobases, respectively. Assuming that a longer oligomer might require more time for the substitution of triazole by guanidine, increased times for the modification step were also assayed (8, 12 or 48 h), but results did not improve. Further attempts to isolate the desired product from the mixture also failed. Consequently, preparation of oligonucleotides modified in several positions using convertible nucleosides appeared not to be the method of choice. Even with the relatively strong conditions used for the substitution of triazolyl groups by guanidine, mixtures of products were obtained from which the target oligonucleotide could not be isolated.

2.2. Synthesis using strategy B

A second approach used a preformed guanidino-modified nucleoside for the synthesis of the oligonucleotide (Fig. 2, strategy B), which could thus be obtained avoiding the reaction with guanidine on the solid support. We protected the guanidine group for the solid-phase assembly of the oligonucleotide by the phosphite triester approach. As stated below, this is unnecessary if the synthesis is performed

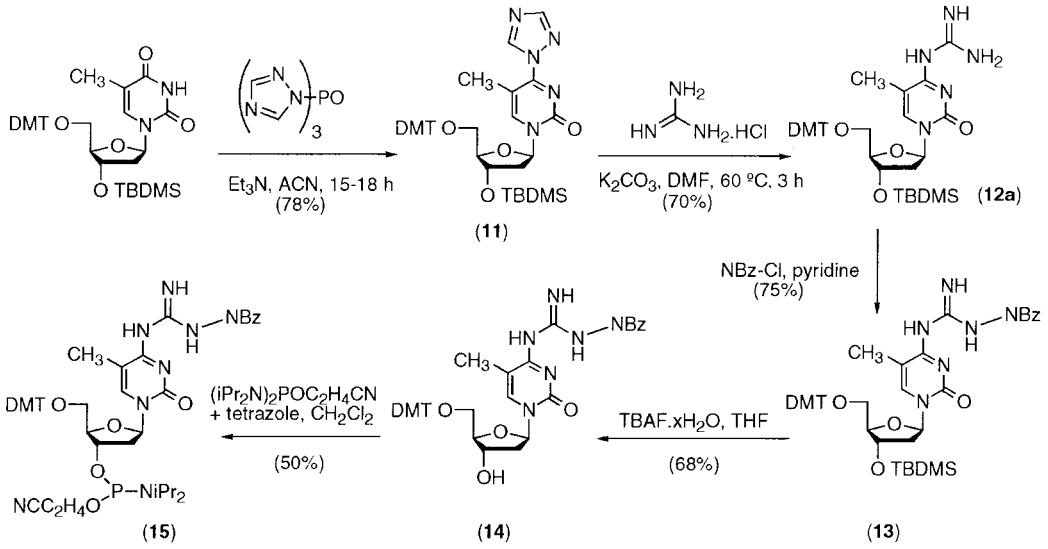


Figure 3. Synthesis of the protected guanidine nucleoside synthon.

using *H*-phosphonate chemistry. Thus, our first goal was to find a suitable protection for the guanidine moiety. Since the permanent protection scheme in oligonucleotide synthesis is based on groups labile to bases, we chose the same type of protection for guanidines to allow all protecting groups to be removed in a single step.

The first protecting group that we assayed was 9-fluorenylmethoxycarbonyl (Fmoc), which is suitable for the protection of the arginine side chain.[38] However, the nucleobase-linked guanidine reacted poorly with the protecting chloroformate reagent, even in harsh conditions. The 4-nitrobenzoyl group was found to be a good alternative, since it could be easily introduced onto guanidine and was labile enough to aqueous ammonia at room temperature. The protected intermediate for oligonucleotide synthesis was obtained as shown in Fig. 3.

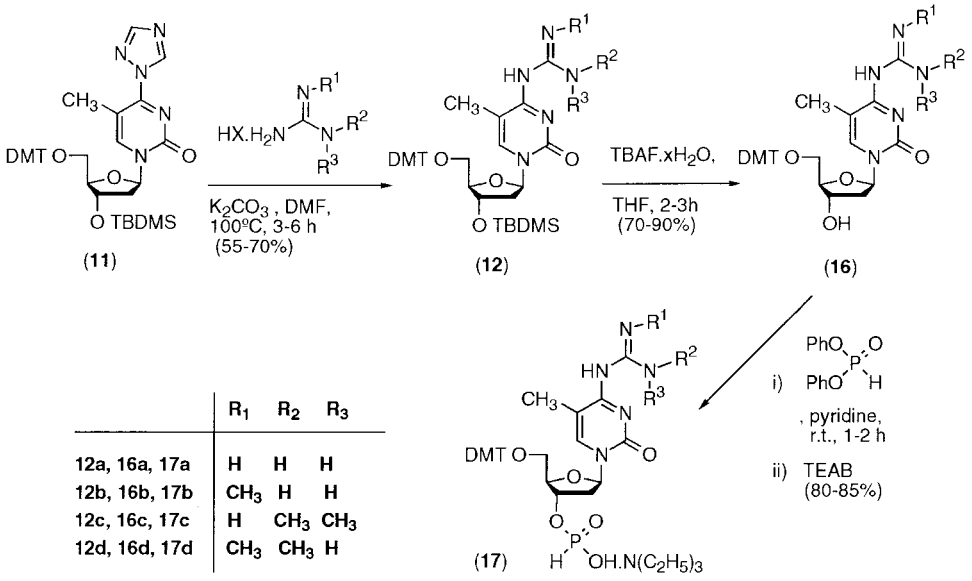
First, triazolyl-modified nucleoside **11** was prepared from 5′-*O*-DMT-3′-*O*-TBDMS-thymidine by reaction with phosphotriazolide.[39] Subsequently, the guanidine group was introduced using the optimized conditions shown in Table 1 (guanidine·HCl+K_2CO_3 in DMF, 60°C) to yield modified nucleoside **12a**. Guanidine protection was then performed by reaction with excess 4-nitrobenzoyl chloride in pyridine at room temperature, yielding the acylated derivative **13** as inferred from ^{1}H NMR and FAB-MS analysis. In the following step, the secondary hydroxyl group was deprotected by treatment with tetrabutylammonium fluoride. Finally, nucleoside **14** was functionalized as the phosphoramidite derivative **15** required for oligonucleotide synthesis.

Trimer TYT (where Y represents the guanidino-protected nucleoside) was assembled on the resin using standard phosphoramidite chemistry. During the synthesis, the coupling efficiency of the modified nucleoside phosphoramidite was similar to that of thymidine phosphoramidite. Two reagents to cleave and deprotect the trimer were assayed: concentrated aqueous ammonia, and a solution of LiOH in methanol, which affords mild deprotection conditions.[40] Unfortunately, HPLC analysis of the crude showed that in neither case was a major product obtained, even after long reaction times (ca. 16 h). The crude from the ammonia treatment was formed by a complex mixture, in which the protected trimer was the main component, and a second, longer treatment with ammonia at 50°C was not effective either. The crude obtained after the treatment with LiOH contained TTT as the major product. As we had checked in a separate experiment that the guanidino-modified nucleobase was stable to the LiOH treatment at room temperature, transformation into thymine was attributed to partial hydrolysis of the protected guanidine nucleobase, which is probably more labile than the unprotected one because of the withdrawing effect of the nitrobenzoyl protecting group.

2.3. Synthesis using strategy C

From the two previous strategies assayed, three conclusions could be drawn. First, because of the difficulty of introducing guanidine at the pyrimidinone ring of a resin-linked oligonucleotide, it seemed advisable to use a preformed guanidino-nucleoside to assemble the modified oligomer. Second, the nucleophilicity of guanidine was diminished by the electron-withdrawing character of the pyrimidinone ring, since the nucleobase-linked guanidine reacted only with strongly electrophilic reagents such as nitrobenzoyl chloride. Third, the electron-withdrawing character of the nitrobenzoyl protecting group also rendered the pyrimidinone-guanidine bond more labile to the nucleophile reagents commonly used for the oligonucleotide deprotection. Thus, a third alternative was designed (Fig. 2, strategy C) that relied on the use of unprotected guanidine derivatives for the synthesis of oligonucleotides.

We then turned our attention into the *H*-phosphonate chemistry. Because of the rather specific reactivity with *O*-nucleophiles of the intermediate generated in the coupling step, a mixed acyl-phosphonyl anhydride, oligonucleotides can be synthesized with unprotected nucleobases without interference.[41] In our case, *N*-reaction of



	R1	R2	R3
12a, 16a, 17a	H	H	H
12b, 16b, 17b	CH_3	H	H
12c, 16c, 17c	H	CH_3	CH_3
12d, 16d, 17d	CH_3	CH_3	H

Figure 4. Synthesis of the guanidine nucleosides 3′-*H*-phosphonate derivatives.

guanidine groups during oligonucleotide synthesis should also be disfavoured because of their lower nucleophilicity (see above). Nevertheless, in order to assess that secondary reactions would not take place during the oligonucleotide assembly, the guanidine unprotected nucleoside **12a** was treated with adamantoyl chloride in the presence of pyridine. No acylation of the nucleoside was observed even after 6 h-reaction at room temperature.

Hence, the guanidino-nucleoside *H*-phosphonate building blocks were obtained as summarized in Fig. 4. 5′-*O*-DMT-3′-*O*-TBDMS-4-guanidino-3-methyl-2-pyrimidinone derivative (**12a**) was first obtained as described above (see Fig. 3), and the same reaction was used to synthesize other derivatives such as *N*-methylguanidine (**12b**), *N*,*N*-dimethylguanidine (**12c**) and *N*,*N*′-dimethylguanidine nucleosides (**12d**). Treatment of nucleosides **12** with tetrabutylammonium fluoride rendered the 3′-hydroxyl-deprotected derivatives **16**, from which the desired *H*-phosphonate derivatives **17** were obtained by selective *O*-phosphonylation with diphenylphosphite in pyridine[42] and basic hydrolysis of the intermediate phosphite diester. All products were characterized by NMR and mass spectrometry.

In order to evaluate the synthetic suitability of the new analogues, a series of trimers TZT (where Z represents the modified nucleosides, namely guanidino-, *N*-methylguanidino-, *N*,*N*′-dimethylguanidino- and *N*,*N*-dimethylguanidino-derivatives) were synthesized and characterized. Standard *H*-phosphonate methodology was used, with the only exception that the amount of pyridine in the coupling reaction mixture was increased because of the low solubility of the *H*-phosphonate derivatives **17** in 1:1 acetonitrile/pyridine mixtures. As a result, pyridine solutions of both these nucleosides and the thymidine *H*-phosphonate

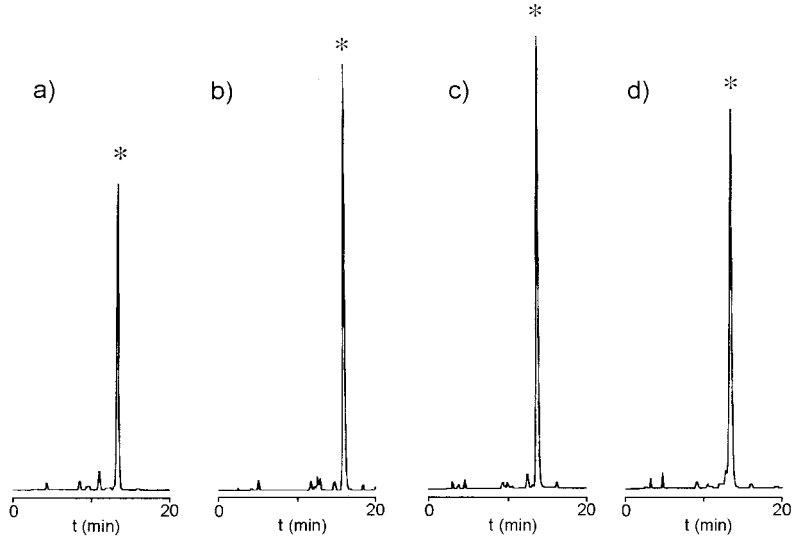


Figure 5. Reversed phase HPLC traces of TZT crudes: (a) Z: guanidine derivative; (b) Z: *N*-methylguanidine derivative; (c) Z: *N*,*N*′-dimethylguanidine derivative; (d) Z: *N*,*N*-dimethylguanidine derivative.

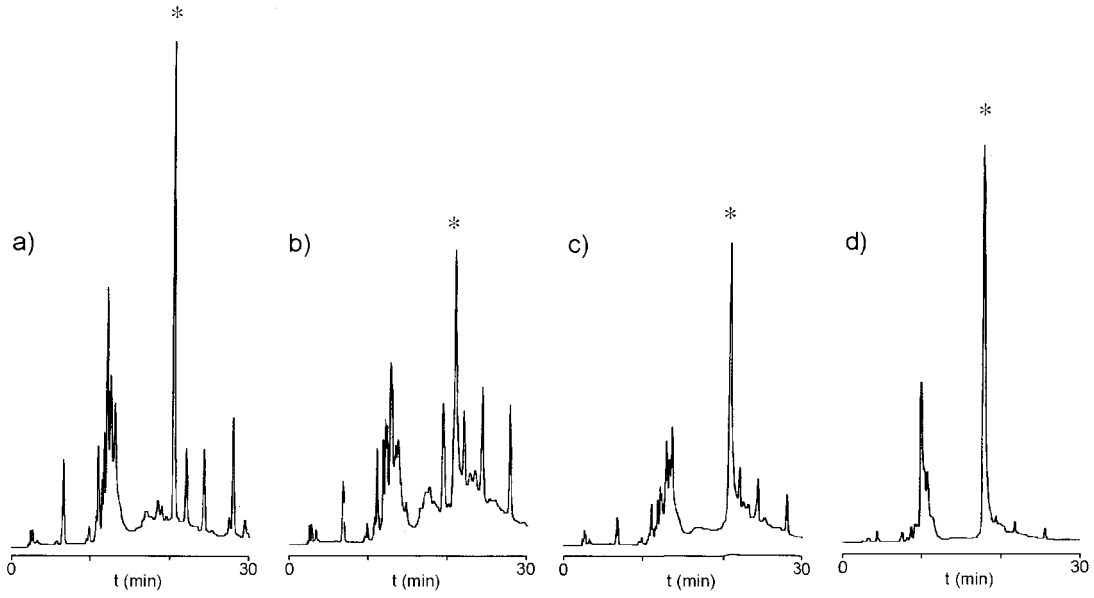


Figure 6. Reversed phase HPLC traces of crudes $^{5'}$TTTTZTTZTZTTTTT$^{3'}$ (**19**), where Z is: (a) guanidine derivative; (b) *N*-methylguanidine derivative; (c) *N*,*N*′-dimethylguanidine derivative; (d) *N*,*N*-dimethylguanidine derivative.

building blocks, and an acetonitrile solution of adamantoyl chloride were used. In these experiments, the coupling yields of the modified *H*-phosphonate derivatives **17** were identical to that of thymidine (90–95%), except for the guanidine derivative **17a** which coupled less efficiently (87%). Once assembled, trimers were cleaved from the resin by treatment with either conc. aqueous ammonia or 0.05 M LiOH in methanol at room temperature for 3 h, and the corresponding crudes were analysed by reversed phase HPLC (Fig. 5). HPLC profiles showed, for all of the four derivatives and any cleavage treatment, a single major product, which was identified by MALDI-TOF mass spectrometry as the target oligonucleotide. Yields after synthesis and purification were also comparable to those obtained with unmodifed nucleosides (45–60%).

Since no side products due to either *N*-acylation of guanidine or partial substitution of guanidine by water or ammonia were found, we undertook the synthesis of longer strands. In particular, we synthesized the modified oligonucleotides $^{5'}$TTTTCTTZTCTTTTT$^{3'}$ (**18**) and $^{5'}$TTTTZTTZTZTTTTT$^{3'}$ (**19**), where Z represents either the guanidino-, *N*-methylguanidino-, *N*,*N*′-dimethylguanidino- or *N*,*N*-dimethylguanidino-modified nucleoside, respectively, by the same procedure described for the assembly of trimers TZT. The 3′-*H*-phosphonate of 4-*N*-benzoyl-5′-*O*-DMT-2′-deoxycytidine was used to introduce cytidine in 15-mer **18**. Again, no significant differences were observed in the coupling efficiency of the modified nucleosides. The corresponding 5′-*O*-DMT-oligonucleotide-resins were then treated with conc. aqueous ammonia at room temperature to produce the deprotection and cleavage from the resins, for 3 h in the case of 15-mers **19**, and for 15 h to completely deblock cytidines in 15-mers **18**. Reversed phase HPLC analysis of the crudes showed that in all cases major products had been obtained, although the degree of homogeneity was different between the various oligonucleotides. Independently of the number of modified nucleosides present, *N*,*N*′-dimethylguanidine- and *N*,*N*-dimethylguanidine-containing oligonucleotides were purer than those incorporating guanidine or *N*-methylguanidine (Fig. 6). The major products were purified and obtained homogeneous, as shown by reversed phase HPLC or PAGE analysis, and unambiguously identified as the desired oligonucleotides by MALDI-TOF mass spectrometry, except the guanidine-containing oligomers. These particularly modified oligonucleotides were identified by MALDI-TOF and electrospray mass spectrometry to be mixtures of products with different numbers of guanidine groups, resulting from the cleavage of the guanidine-nucleoside linkage and formation of either thymine or 5-methylcytosine. Consequently, only short chains with a single guanidine modification can be obtained with this method.

3. Discussion

Three strategies have been explored for the solid phase synthesis of modified oligonucleotides containing 4-guanidino-2-pyrimidinone nucleobases, which could act as new analogues of protonated cytosine and increase the stability of triple helices at neutral pH. This is an important goal to allow oligonucleotides to be used in antigene strategies for the control of gene expression.

Our first choice was to use the ‘convertible-nucleoside’ approach. Setting up the best conditions for the substitution reaction required testing different compounds as well as different bases to liberate guanidine from its hydrochloride (Table 1). Of the different leaving groups tested, triazolyl and 3-nitrotriazolyl were more easily substituted by guanidine than phenoxy-functionalized 2-pyrimidinones, and best results were achieved when K_2CO_3 was added to the reaction mixture.

Oligonucleotides were synthesized using the *H*-phosphonate method and the 4-triazolyl-2-pyrimidinone nucleoside derivative. Two conclusions were inferred from these

experiments. In the first place, complete conversion of triazolyl- into guanidino-nucleobases is much more difficult to achieve on a solid support than in a homogeneous reaction medium. $TC^{m}T$ and TTT were found to accompany the target trinucleotide $TT^{g}T$, which could nevertheless be isolated, and the deprotection crude of $^{5'}TTTTT^{g}TTT^{g}TT^{g}TTTTT^{3'}$ contained, among others, mono-, di-, and trisubstituted guanidine-containing products. Thus, incomplete replacement of triazolyl groups by guanidine was followed by reaction with aqueous ammonia to yield either thymine or 5-methylcytosine.

Secondly, when the guanidine-treated oligonucleotide-resin was deprotected with ammonia at a higher temperature, the relative proportion of $TC^{m}T$ and TTT with respect to $TT^{g}T$ increased. This indicated a certain lability of the guanidine-pyrimidinone linkage to nucleophiles, which increased with temperature.

As a result, we decided to evaluate a different alternative using nucleoside synthons containing the guanidine group, which was protected to prevent side reactions during oligonucleotide elongation.

Guanidine protection was found not to be straightforward, since its nucleophilicity was reduced by covalent linkage to the 2-pyrimidinone nucleobase. It reacted only with strongly electrophilic carbonyl derivatives such as 4-nitrobenzoyl chloride, but not with 9-fluorenylmethyl chloroformate. The 4-nitrobenzoyl group was labile to final deprotecting reagents such as concentrated aqueous ammonia or methanolic LiOH solutions, using conditions in which the guanidine-pyrimidinone linkage was stable.

Our first synthetic target was, again, modified trimer $TT^{g}T$. Oligonucleotide assembly by the phosphite triester approach proceeded smoothly, but the crude obtained after ammonia deprotection was a complex mixture of products containing the guanidine-protected trinucleotide. Deprotection with LiOH afforded TTT as the main product. Two conclusions were deduced from these findings. On the one hand, reactions on resin-linked oligonucleotides may be more difficult than on nucleosides in solution. Quantitative removal of the 4-nitrobenzoyl group from the guanidine-modified nucleoside had been achieved, whereas not all protecting groups could be eliminated from the insolubilized trimer. On the other hand, the guanidine-pyrimidinone linkage was labile to nucleophiles, especially to hydroxide anions. This lability was most probably enhanced because of the additional electron-withdrawing effect of the nitrobenzoyl group.

Since it was difficult to find a suitable protecting group for the guanidine moiety, we worked with guanidine-containing nucleoside analogues without protecting the guanidine group. In order to minimize side reactions at this function, *H*-phosphonate derivatives were used for the oligonucleotide elongation, but the combination of solvents for the nucleoside synthons and the coupling reagent had to be optimized.

We prepared four guanidine-modified nucleosides, containing either the naked guanidine moiety or *N*-methyl-, *N*,*N*-dimethyl-, or *N*,*N*′-dimethylguanidine. They were all readily obtained by reaction of a protected triazolyl-derivatized nucleoside with the corresponding guanidine derivative, using essentially the same conditions previously optimized for the 'convertible-nucleoside' approach.

The syntheses of the four different trinucleotides TZT (Z=guanidino-nucleoside) proceeded smoothly, and homogeneous crudes were obtained after the deprotection step. However, the syntheses of more complex oligonucleotides, such as $^{5'}TTTTCTTZTCTTTTT^{3'}$ (**18**) and $^{5'}TTTTZTTZTZTTTTT^{3'}$ (**19**), indicated that dimethylguanidine-containing oligonucleotides are more easily accessible than the others. The *N*-methylguanidine-containing oligomers could also be isolated and characterized as the target products, but not the guanidine-containing oligonucleotides.

Even though it is generally accepted that guanidine groups are difficult to acylate because they generally remain protonated, we cannot rule out that the byproducts accompanying the target oligonucleotides were branched oligomers formed by incorporation of nucleotide synthons onto the unprotected guanidine group (or, to a lesser extent, onto the *N*-methyl substituted derivative). Nevertheless, the lability of the guanidine-pyrimidinone linkage to the oligonucleotide deprotection conditions, which is enhanced when the guanidine moiety is linked to electron-withdrawing groups, may also explain the low homogeneity of guanidine- and *N*-methylguanidine-containing oligonucleotides. An increase in the number of methyl substituents, either by steric hindrance or by electronic factors, yields more homogeneous crudes and thus allows the target products to be obtained more easily.

In summary, oligonucleotide analogues incorporating 4-alkylguanidino-2-pyrimidinones have been prepared using the *H*-phosphonate methodology. We are currently studying their hybridization to single- or double-stranded DNA fragments.

4. Experimental

Unless otherwise indicated, all chemicals were purchased from commercial suppliers (reagent grade) and used without purification. Dry CH_3CN was obtained by distillation over CaH_2 and storage over CaH_2 lumps. CH_2Cl_2 was neutralized and dried by passing through basic Al_2O_3 and storage over CaH_2. Dry tetrahydrofuran was obtained by distillation over sodium metal in the presence of benzophenone. *N*,*N*-dimethylformamide was bubbled with nitrogen to remove volatile contaminants and dried by storage over CaH_2. Amine-free pyridine was obtained by distillation over ninhydrin and further dried by storage over CaH_2 lumps.

NMR spectra were recorded with either a Varian Gemini or a Varian Unity operating at 200 and 300 MHz, respectively. (δ values are relative to internal $Si(CH_3)_4$ (^{1}H, ^{13}C) or external H_3PO_4 (^{31}P). IR-FT spectra were recorded in a Nicolet 510 apparatus. UV analyses were performed using a Varian Cary 5E spectrophotometer. Low resolution mass spectra of nucleosides were recorded in a Hewlett–Packard

5988 A (FAB) apparatus and oligonucleotides were analysed using VG-Quattro (ES) or Perseptive Biosystems Voyager DE™-RP (MALDI-TOF) instruments. High resolution mass analyses were carried out in the facilities of the University of Vigo (Spain). Calculated values for neutral compounds (M) are indicated in all cases. Oligonucleotides were quantified by their UV absorption at 260 nm in OD_{260} units.

4.1. 3′-*O*-Acetyl-5′-*O*-dimethoxytrityl-1-[5-methyl-4-triazolylpyrimidin-2(1*H*)-onyl]-β-D-2′-deoxyriboside (1)

Triazole was suspended in dry CH_3CN (10 mL/mmol azole) under argon atmosphere, and the mixture was cooled in an ice bath. $POCl_3$ (2 equiv.) and dry triethylamine (15 equiv.) were subsequently added, and left to react for 30 min. Then, a solution of 3′-*O*-acetyl-5′-*O*-dimethoxytritylthymidine in CH_3CN (10 mL/mmol azole) was added dropwise through a cannula. When the addition was finished, the ice bath was removed and the mixture left to react overnight at room temperature. The dark mixture was cooled in an ice bath, and treated with 1 M aq. triethylammonium hydrogencarbonate (5 mL). After 10 min, the suspension was concentrated by partial elimination of the solvent (approx. 50%). AcOEt (100 mL) was added, and the organic phase washed twice with both 1 M aq. triethylammonium hydrogencarbonate and brine. The organic solution was dried over Na_2SO_4, filtered to remove the dessicant and evaporated to dryness. Triazolyl derivative **1** was obtained as a brown solid (quantitative yield, 5–8 mmol scale). No further purification was needed.

Mp 103–105°C; TLC (CH_2Cl_2/MeOH 10:1): R_f=0.75 (blue fluorescent spot); ^{1}H NMR (200 MHz, $CDCl_3$): δ=9.30 (s, 1H; H-triazolyl), 8.32 (2, 1H; H-6), 8.09 (s, 1H; H-triazolyl); 7.40–7.20 (m, 9H; H-phenyl), 6.83 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.42 (m, 1H; H–C1′), 5.41 (m, 1H; H–C3′), 4.15 (m, 1H; H–C4′), 3.78 (s, 6H; CH_3O), 3.47 (m, 2H; H–C5′), 2.92 (m, 1H; H–C2′), 2.43 (m, 1H; H′–C2′), 2.10 (s, 3H; CH_3CO), 2.00 (s, 3H, CH_3–C5); ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.2 (O*C*OCH_3), 158.6 (Cphenyl), 158.1 (C4), 153.6 (C2), 153.3 (Ctriazolyl), 146.4 (Ctriazolyl), 144.9 (Cphenyl), 144.0 (C6), 135.0, 134.9 (Cphenyl), 129.9, 127.9, 127.8, 127.1, 113.2 (Cphenyl–H), 106.0 (C5), 87.3, 87.1, 84.9 (Cq, C1′, C4′), 74.9 (C3′), 63.6 (C5′), 55.2 (CH_3O), 39.6 (C2′), 21.0 (*C*H_3CO), 16.5 (CH_3–C5); LRMS (FAB, magic bullet, Xe, positive mode): *m/z*: 661.2, 660.2 $[M+Na]^+$, 639.2, 638.2 $[M+H]^+$; calcd for $C_{35}H_{35}O_7N_5$ [M]: 637.6819 (average).

4.2. 3′-*O*-Acetyl-5′-*O*-dimethoxytrityl-1-[4-azolyl-5-methylpyrimidin-2(1*H*)-onyl]-β-D-2′-deoxyribosides (3-nitrotriazolyl derivative 2, 3-bromotriazolyl derivative 3)

A similar procedure was used to obtain derivative **1**, except for carrying out the reaction with 2.5 equiv. of azole (3-nitrotriazole[43] or 3-bromotriazole[44]), 5 equiv. of triethylamine, and diphenylphosphochloridate (2 equiv.) instead of $POCl_3$. 3-Nitrotriazolyl derivative **1** was obtained as dark brown solid (85% yield, 3 mmol scale), and 3-bromotriazole derivative **2** as a light brown solid (65% yield, 3 mmol scale). No further purification was necessary.

4.2.1. 3-Nitrotriazolyl derivative 2. Mp 102–104°C; TLC (AcOEt/hexane 2:1): R_f=0.25; ^{1}H NMR (200 MHz, $CDCl_3$): δ=9.38 (s, 1H; H-triazolyl), 8.44 (s, 1H; H–C6), 7.40–7.20 (m, 9H; H-phenyl), 6.85 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.38 (m, 1H; H–C1′), 5.45 (m, 1H; H–C3′), 4.17 (m, 1H; H–C4′), 3.79 (s, 6H; CH_3O), 3.46 (m, 2H; H–C5′), 2.92 (m, 1H; H–C2′), 2.45 (m, 1H; H′–C2′), 2.10 (s, 3H; CH_3CO), 2.00 (s, 3H, CH_3–C5); ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.2 (O*C*OCH_3), 158.7 (Cphenyl), 156.9 (C4), 153.1 (C2) 148.2 (Ctriazolyl), 146.2 (Ctriazolyl), 144.2 (C6), 143.9 (Cphenyl), 135.0, 134.8 (Cphenyl), 129.9, 128.0, 127.9, 127.3, 113.2 (Cphenyl–H), 105.6 (C5), 87.8, 87.3, 85.3 (Cq, C1′, C4′), 74.8 (C3′), 63.3 (C5′), 55.3 (CH_3O), 39.7 (C2′), 21.0 (*C*H_3CO), 16.0 (CH_3–C5); LRMS (FAB, magic bullet, Xe, positive mode): *m/z*: 683.1 $[M+H]^+$; calcd for $C_{35}H_{34}O_9N_6$ [M]: 682.6795 (average).

4.2.2. 3-Bromotriazolyl derivative 3. Mp 94–96°C; TLC (CH_2Cl_2/MeOH 10:1): R_f=0.75; ^{1}H NMR (200 MHz, $CDCl_3$): δ=9.18 (s, 1H; H-triazolyl), 8.37 (s, 1H; H–C6), 7.40–7.20 (m, 9H; H-phenyl), 6.83 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.39 (m, 1H; H–C1′), 5.44 (m, 1H; H–C3′), 4.18 (m, 1H; H–C4′), 3.79 (s, 6H; CH_3O), 3.44 (m, 2H; H–C5′), 2.90 (m, 1H; H–C2′), 2.42 (m, 1H; H′–C2′), 2.10 (s, 3H; CH_3CO), 2.00 (s, 3H; CH_3–C5); ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.3 (O*C*OCH_3), 158.7 (Cphenyl), 157.1 (C4), 153.6 (C2), 146.9 (Ctriazolyl), 146.1 (Ctriazolyl), 143.9 (Cphenyl), 142.8 (C6), 135.0, 134.7 (Cphenyl), 129.9, 128.0, 127.9, 127.2, 113.3 (Cphenyl–H), 105.8 (C5), 87.5, 87.2, 85.1 (Cq, C1′, C4′), 74.9 (C3′), 63.4 (C5′), 55.3 (CH_3O), 39.6 (C2′), 21.0 (*C*H_3CO), 16.4 (CH_3–C5); LRMS (FAB, magic bullet, positive mode): *m/z*: 850.4, 848.4 $[M+Xe]^+$, 756.4 $[M+K]^+$, 740.4, 738.4 $[M+H]^+$, 718.5, 716.4, 715.4 $[M+H]^+$; calcd for $C_{35}H_{34}O_7N_5Br$ [M]: 716.5780 (average).

4.3. 3′-*O*-Acetyl-5′-*O*-dimethoxytrityl-1-[5-methyl-4-phenoxy-pyrimidin-2(1*H*)-onyl]-β-D-2′-deoxyribosides (4-nitrophenoxy derivative 4, 2-nitrophenoxy derivative 5, 2,4-dichlorophenoxy derivative 6, 2,4,5-trichlorophenoxy derivative 7 and 2,3,4,5,6-pentachlorophenoxy derivative 8)

4.3.1. General procedure. 3′-*O*-Acetyl-5′-*O*-dimethoxytritylthymidine (0.6 g, 1.0 mmol) was dissolved in dry CH_2Cl_2 (10 mL) under inert atmosphere, and the flask was cooled in an ice bath. Triethylamine (0.7 mL, 5.0 mmol), mesitylene chloride (0.3 g, 1.5 mmol) and dimethylaminopyridine (60 mg, 0.5 mmol) was added. After 10 min, the bath was removed and the mixture left to react for 6 h at room temperature. The reaction flask was cooled again in an ice bath, and triethylamine (0.85 mL, 6.0 mmol), DBU (0.1 mL, 0.8 mmol) and the corresponding phenol (4-nitrophenol, 2-nitrophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol or 2,3,4,5,6-pentachlorophenol, 5 mmol) was added. After 5 min, the ice bath was removed and the mixture left to react for 3 h, after which time the reaction flask was cooled in an ice bath and 10% aqueous $NaHCO_3$ was added to quench the reaction. After 10 min, the solvents were removed by evaporation under vacuum, and the corresponding crude purified by silica gel column chromatography using AcOEt/hexane/triethylamine 50:50:1 as

eluent. The desired fractions were pooled and the solvent removed under vacuum to yield the target product as a solid.

4.3.2. 4-Nitrophenoxy derivative 4. Yellow solid, 60% yield; mp 105–107°C; TLC (AcOEt/hexane 2:1): R_f=0.45; ^{1}H NMR (200 MHz, $CDCl_3$): δ=8.28 (d, ^3J(H, H)=9 Hz; H-nitrophenyl), 8.11 (s, 1H; H–C6), 7.40–7.20 (m, 11H; H-phenyl, H-nitrophenyl), 6.85 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.40 (m, 1H; H–C1′), 5.42 (m, 1H; H–C3′), 4.11 (m, 1H; H–C4′), 3.81 (s, 6H; CH_3O), 3.43 (m, 2H; H–C5′), 2.70 (m, 1H; H–C2′), 2.34 (m, 1H; H′–C2′), 2.08 (s, 3H; CH_3CO), 1.67 (s, 3H, CH_3–C5); ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.3 (O*C*OCH$_3$), 169.4 (C4), 158.6 (Cphenyl), 156.4 (Cnitrophenyl), 154.8 (C2), 145.3 (C6), 144.1 (Cphenyl), 142.0 (Cnitrophenyl), 135.2, 135.1 (Cphenyl), 130.0, 128.0, 127.9, 127.1, 113.3 (Cphenyl–H), 125.3, 122.7 (Cnitrophenyl–H), 104.4 (C5), 87.1, 85.5, 84.2 (Cq, C1′, C4′), 75.0 (C3′), 63.5 (C5′), 55.3 (CH_3O), 39.3 (C2′), 21.0 (*C*H$_3$CO), 11.8 (CH_3–C5); LRMS (FAB, magic bullet, Xe, positive mode): *m/z*: 841.4, 840.4 $[M+Xe]^+$, 731.5, 730.5 $[M+Na]^+$, 707.5, 708.6 $[M+H]^+$, calcd for $C_{39}H_{37}O_{10}N_3$ [M]: 707.7253 (average).

4.3.3. 2-Nitrophenoxy derivative 5. Yellow solid, 40% yield; mp 98–100°C; TLC (AcOEt/hexane 2:1): R_f=0.30; ^{1}H NMR (200 MHz, $CDCl_3$): δ=8.18 (dd, ^3J(H, H)=7 Hz, 1 Hz; H-nitrophenyl), 8.12 (s, 1H; H–C6), 7.65 (m, 1H; nitrophenyl), 7.40–7.20 (m, 11H; H-phenyl, H-nitrophenyl), 6.87 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.40 (m, 1H; H–C1′), 5.40 (m, 1H; H–C3′), 4.20 (m, 1H; H–C4′), 3.81 (s, 6H; CH_3O), 3.46 (m, 2H; H–C5′), 2.70 (m, 1H; H–C2′), 2.37 (m, 1H; H′–C2′), 2.10 (s, 3H; CH_3CO), 1.70 (s, 3H; CH_3–C5); ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.3 (O*C*OCH$_3$), 169.8 (C4), 158.6 (Cphenyl), 154.8 (C2), 145.3 (C6), 144.1 (Cphenyl), 141.7 (Cnitrophenyl), 135.2, 134.9 (Cphenyl, Cnitrophenyl), 130.0, 128.0, 127.9, 127.1, 113.3 (Cphenyl–H), 126.5, 126.1, 125.7 (Cnitrophenyl–H), 104.6 (C5), 87.1, 86.5, 84.4 (Cq, C1′, C4′), 75.2 (C3′), 63.5 (C5′), 55.3 (CH_3O), 39.2 (C2′), 21.0 (*C*H$_3$CO), 11.8 (CH_3–C5); LRMS (FAB, magic bullet, positive mode): *m/z*: 841.4, 840.4 $[M+Xe]^+$, 731.6, 730.5 $[M+Na]^+$, 708.7, 707.5 $[M+H]^+$, calcd for $C_{39}H_{37}O_{10}N_3$ [M]: 707.7253 (average).

4.3.4. Dichlorophenoxy derivative 6. White solid, 65% yield; mp 88–90°C; TLC (AcOEt/hexane 2:1): R_f=0.45; ^{1}H NMR (200 MHz, $CDCl_3$): δ=8.12 (s, 1H; H–C6), 7.42 (dd, ^3J(H, H)=7 Hz, 1 Hz; H-chlorophenyl), 7.40–7.20 (m, 11H; H-phenyl, H-chlorophenyl), 6.85 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.42 (m, 1H; H–C1′), 5.42 (m, 1H; H–C3′), 4.11 (m, 1H; H–C4′), 3.81 (s, 6H; CH_3O), 3.44 (m, 2H; H–C5′), 2.70 (m, 1H; H–C2′), 2.37 (m, 1H; H′–C2′), 2.08 (s, 3H; CH_3CO), 1.71 (s, 3H; CH_3–C5); ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.3 (O*C*OCH$_3$), 169.3 (C4), 158.6 (Cphenyl), 155.0 (C2), 149.6 (C6), 144.1 (Cphenyl), 141.5 (Cchlorophenyl), 135.2, 135.1 (Cphenyl), 131.7, 129.9, 128.0, 124.8, 113.3 (Cphenyl–H, Cchlorophenyl–H), 104.3 (C5), 87.1, 85.4, 84.4 (Cq, C1′, C4′), 75.1 (C3′), 63.5 (C5′), 55.3 (CH_3O), 39.2 (C2′), 21.0 (*C*H$_3$CO), 11.8 (CH_3–C5); LRMS (FAB, magic bullet, positive mode): *m/z*: 865.4, 863.4 $[M+Xe]^+$, 755.4, 753.4, $[M+Na]^+$, 732.8, 731.5 $[M+H]^+$, calcd for $C_{39}H_{36}O_8N_2Cl_2$ [M]: 731.6172 (average).

4.3.5. Trichlorophenoxy derivative 7. White solid, 70% yield; mp 112–114°C; TLC (AcOEt/hexane 2:1): R_f= 0.50; ^{1}H NMR (200 MHz, $CDCl_3$): δ=8.10 (s, 1H; H–C6), 7.50, 7.08 (s, s, 2H; H-chlorophenyl), 7.40–7.20 (m, 9H; H-phenyl), 6.85 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.40 (m, 1H; H–C1′), 5.40 (m, 1H; H–C3′), 4.10 (m, 1H; H–C4′), 3.81 (s, 6H; CH_3O), 3.46 (m, 2H; H–C5′), 2.70 (m, 1H; H–C2′), 2.33 (m, 1H; H′–C2′), 2.08 (s, 3H; CH_3CO), 1.68 (s, 3H; CH_3–C5); ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.4 (O*C*OCH$_3$), 169.3 (C4), 158.6 (Cphenyl), 155.0 (C2), 146.7 (C6), 144.1 (Cphenyl), 142.0 (Cchlorophenyl), 135.2, 135.1 (Cphenyl), 131.0, 129.9, 128.0, 127.2, 125.5, 117.8, 113.3 (Cphenyl–H, Cchlorophenyl–H), 104.3 (C5), 87.1, 86.5, 84.8 (Cq, C1′, C4′), 75.0 (C3′), 63.5 (C5′), 55.3 (CH_3O), 39.3 (C2′), 21.0 (*C*H$_3$CO), 11.7 (CH_3–C5); LRMS (FAB, magic bullet, positive mode): *m/z*: 899.2, 897.3 $[M+Xe]^+$, 791.3, 789.4, 787.4 $[M+Na]^+$, 766.3, 764.2 $[M+H]^+$, calcd for $C_{39}H_{35}O_8N_2Cl_3$ [M]: 766.0620 (average).

4.3.6. Pentachlorophenoxy derivative 8. White solid, 55% yield; mp 87–89°C; TLC (AcOEt/hexane 2:1): R_f=0.60; ^{1}H NMR (200 MHz, $CDCl_3$): δ=8.13 (s, 1H; H–C6), 7.40–7.20 (m, 9H, H-phenyl), 6.85 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.40 (m, 1H; H–C1′), 5.42 (m, 1H; H–C3′), 4.10 (m, 1H; H–C4′), 3.81 (s, 6H; CH_3O), 3.46 (m, 2H; H–C5′), 2.75 (m, 1H; H–C2′), 2.38 (m, 1H; H′–C2′), 2.08 (s, 3H; CH_3CO), 1.71 (s, 3H; CH_3–C5); ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.3 (O*C*OCH$_3$), 168.9 (C4), 158.6 (Cphenyl), 154.6 (C2), 144.1 (C2), 142.4 (Cchlorophenyl), 135.2, 135.1 (Cphenyl), 129.9, 128.0, 127.2, 113.3 (Cphenyl–H), 103.7 (C5), 87.1, 85.6, 84.5 (Cq, C1′, C4′), 75.0 (C3′), 63.5 (C5′), 55.3 (CH_3O), 39.3 (C2′), 21.0 (*C*H$_3$CO), 11.6 (CH_3–C5); LRMS (FAB, magic bullet, positive mode): *m/z*: 969.0, 967.1, 965.2 $[M+Xe]^+$, 861.3, 859.3, 857.3, 855.2 $[M+Na]^+$, 834.3 $[M+H]^+$, calcd for $C_{39}H_{33}O_8N_2Cl_5$ [M]: 834.9515 (average).

4.4. 3′-*O*-Acetyl-5′-*O*-dimethoxytrityl-1-[4-guanidine-5-methyl-pyrimidin-2(1*H*)-onyl]-β-D-2′-deoxyriboside (9)

4.4.1. Experiments of Table 1. Approx. 0.03 mmol of derivatives **1**–**8** were dissolved in a small vial in 300 μL of dry dimethylformamide (or dimethylformamide/triethylamine) and treated with guanidine hydrochloride (15 mg) and the corresponding mixture of reagents (see Table 1 for details). The reaction was monitored by TLC analysis (CH_2Cl_2/MeOH 10:1).

4.4.2. From nucleoside 1. Nucleoside (0.50 g, 0.8 mmol) was dissolved in dry dimethylformamide (10 mL). K_2CO_3 (0.32 g, 2.4 mmol) and guanidine hydrochloride (0.15 g, 1.6 mmol) were added, and the corresponding mixture was heated in an external bath at 100°C. The reaction was completed in 3 h according to TLC analysis. The mixture was left to cool at ambient temperature and filtered to remove the insoluble residue. The solid was further washed with $CHCl_3$ and all the filtrates were pooled. The solvent was removed by evaporation in vacuo to obtain a residue which was purified by silica gel column chromatography eluting with CH_2Cl_2/MeOH/Et_3N (100:5:1). The desired fractions were pooled and evaporated to dryness to yield a white solid (0.35 g, 70% yield). Mp 180°C (decomp.); TLC (CH_2Cl_2/MeOH 100:10): R_f=0.3 (blue fluorescent spot); ^{1}H

NMR (200 MHz, $CDCl_3$): δ=7.60 (s, 1H; H–C6), 7.40–7.20 (m, 9H; H-phenyl), 6.82 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.46 (m, 1H; H–C1′), 5.38 (m, 1H; H–C3′), 4.17 (m, 1H; H–C4′), 3.78 (s, 6H; CH_3O), 3.45 (m, 2H; H–C5′), 2.45 (m, 2H; H–C2′), 2.07 (s, 3H; CH_3CO), 1.55 (s, 3H; CH_3–C5); ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.3 ($OCOCH_3$), 169.0 (Cguanid), 160.3 (C4), 158.5 (Cphenyl), 156.0 (C2), 144.2 (Cphenyl), 135.5 (C6), 134.3 (Cphenyl), 130.0, 128.0, 127.9 127.0, 113.4 (Cphenyl–H), 110.5 (C5), 86.9, 85.5, 83.8 (Cq, C1′, C4′), 75.3 (C3′), 63.6 (C5′), 55.2 (CH_3O), 38.6 (C2′), 21.0 (CH_3CO), 13.0 (CH_3–C5); IR (KBr; only most significant bands): ($\bar{\nu}$=3500–3200 (NH st), 1730 (OC=O st), 1675 (C=O st), 1605 (C–NH guan. st) cm^{-1}; HRMS (FAB, magic bullet, positive mode): *m/z* (%): calcd for $C_{34}H_{38}O_7N_5$ $[M+H]^+$: 628.2771, found 628.2753 (100).

4.5. Triethylammonium 5′-*O*-dimethoxytrityl-1-[5-methyl-4-triazolylpyrimidin-2(1*H*)-onyl]-β-D-2′-deoxyriboside 3′-*H*-phosphonate (10)

Triazole (2.0 g, 29.3 mmol) was suspended in dry CH_3CN (30 mL) under argon atmosphere, and the mixture was cooled in an ice bath. PCl_3 (0.80 mL, 9.0 mmol) and dry triethylamine (6.3 mL, 45.0 mmol) were subsequently added, and the mixture left to react for 30 min. Then, a solution of 5′-dimethoxytritylthymidine (1.0 g, 1.8 mmol) in CH_2Cl_2/CH_3CN (2:1, 30 mL) was added dropwise through a canula. When the addition was finished, the ice bath was removed and the mixture left to react overnight at room temperature. The reaction flask was cooled in an ice bath and 1 M aq. triethylammonium hydrogencarbonate was added (5 mL). After 10 min, the suspension was concentrated by partial elimination of solvent (approx. 50%). AcOEt (100 mL) was added, and the corresponding organic phase was washed twice with 1 M aq. triethylammonium hydrogencarbonate (100 mL). The organic solution was dried over Na_2SO_4 and the solvent was removed in vacuo. The residue was purified by column chromatography (SiO_2) using CH_2Cl_2/MeOH/Et_3N 100:5:1 as eluent. The desired fractions were pooled, and after evaporation of the solvent and coevaporation with dry CH_2Cl_2, a white solid was obtained (0.87 g, 62% yield). Mp 168–169°C (decomp.); TLC (CH_2Cl_2/MeOH/Et_3N 100:10:1): R_f=0.45 (blue fluorescent spot); ^{1}H NMR (200 MHz, $CDCl_3$): δ=9.30 (s, 1H; H-triazolyl), 8.38 (s, 1H; H–C6), 8.07 (s, 1H, H-triazolyl), 7.45–7.20 (m, 9H; H-phenyl), 6.88 (d, ^2J(H, P)=620 Hz, 1H; H–P), 6.84 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.39 (m, 1H; H–C1′), 5.05 (m, 1H; H–C3′), 4.18 (m, 1H; H–C4′), 3.78 (s, 6H; CH_3O), 3.45 (m, 2H; H–C5′), 3.03 (q, ^3J(H, H)=7 Hz, 6H; CH_2 ammonium), 2.43 (m, 1H; H–C2′), 1.92 (s, 3H; CH_3–C5), 1.33 (t, ^3J(H, H)=7 Hz, 9H; CH_3 ammonium); ^{13}C NMR (50 MHz, $CDCl_3$) δ=158.5 (Cphenyl), 158.0 (C4), 153.9 (C2), 153.2 (Ctriazolyl), 146.7 (Ctriazolyl), 144.9 (Cphenyl), 144.1 (C6), 135.2 (Cphenyl), 130.0, 128.0, 127.9 127.0, 113.2 (Cphenyl–H), 105.7 (C5), 87.3, 86.9, 85.9 (Cq, C1′, C4′), 72.8 (d, ^3J(C, P)=4 Hz; C3′), 62.7 (C5′), 55.2 (CH_3O), 45.6 (CH_2 ammonium), 41.1 (C2′), 16.4 (CH_3–C5), 8.8 (CH_3 ammonium); ^{31}P NMR (121 MHz, $CDCl_3$) δ=3.54; IR (KBr; only most significant bands): $\bar{\nu}$=2630–2600, 2500 (PO–H st), 1065, 1030 (P=O st) cm^{-1}; HRMS (FAB, magic bullet, positive mode): *m/z* (%): calcd for $C_{33}H_{35}O_8N_5P$ $[M+H]^+$: 660.2223, found 660.2217 (47).

4.6. 3′-*O*-*t*-Butyldimethylsilyl-5′-*O*-dimethoxytrityl-1-[5-methyl-4-triazolylpyrimidin-2(1*H*)-onyl]-β-D-2′-deoxyriboside (11)

5′-Dimethoxytritylthymidine (5.7 g, 10.4 mmol) was dissolved in dry pyridine (50 mL) under inert atmosphere. To this solution, imidazole (2.4 g, 15.6 mmol) and *t*-butyldimethylsilyl chloride (1.1 g, 15.6 g) was added. When the initial nucleoside was completely consumed according to TLC analysis (approx. 6 h), the excess of silylating agent was hydrolysed by addition of water (5 mL). After 10 min, the solvents were removed by evaporation. The residue was partitioned between AcOEt (200 mL) and 10% aq. tartaric acid (200 mL). The organic phase was separated and washed with 10% aq. tartaric acid (200 mL), 10% aq. $NaHCO_3$ and brine. The organic solution was dried over Na_2SO_4 and the solvent was removed by evaporation in vacuo to yield a white solid (6.4 g, 97% yield), which was further used without purification. Mp 101–102°C; TLC (CH_2Cl_2/MeOH 10:1): R_f=0.65; ^{1}H NMR (200 MHz, $CDCl_3$): δ=8.85 (s, 1H; H–N3), 7.64 (s, 1H; H–C6), 7.45–7.25 (m, 9H; H-phenyl), 6.80 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.33 (m, 1H; H–C1′), 4.54 (m, 1H; H–C3′), 3.93 (m, 1H; H–C4′), 3.76 (s, 6H; CH_3O), 3.32 (m, 2H; H–C5′), 2.24 (m, 2H; H–C2′), 1.45 (s, 3H, CH_3–C5), 0.80 (s, 9H, CH_3–CSi), 0.04, 0.06 (ss, 6H, CH_3–Si); ^{13}C NMR (50 MHz, $CDCl_3$) δ=163.8 (C4), 158.6 (Cphenyl), 150.2 (C2), 144.2 (Cphenyl), 135.6, 135.4, 135.3 (C6, Cphenyl), 130.0, 128.0, 127.9 127.0, 115.2 (Cphenyl–H), 110.0 (C5), 86.7, 84.8 (Cq, C1′, C4′), 72.0 (C3′), 62.8 (C5′), 55.2 (CH_3O), 41.6 (C2′), 25.7 (CH_3–CSi), 18.0 (CqSi), 11.9 (CH_3–C5), −4.6, −4.8 (CH_3Si).

For the following reaction, the same procedure described for the preparation of derivative **1** was used. After the reaction and the corresponding work-up, the product was redissolved in CH_2Cl_2 and precipitated with diethylether/hexane (1:1, 200 mL). A light brown solid was obtained after filtration and drying (5.4 g, 76% overall yield). TLC and NMR analyses showed that the product was pure enough not to be further purified. Mp 96–98°C; TLC (CH_2Cl_2/MeOH 10:1): R_f=0.40 (blue fluorescent spot); ^{1}H NMR (200 MHz, $CDCl_3$): δ=9.38 (s, 1H; H-triazolyl), 8.48 (s, 1H; H–C6), 8.23 (s, 1H; H-triazolyl), 7.45–7.22 (m, 9H; H-phenyl), 6.85 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.37 (m, 1H; H–C1′), 4.58 (m, 1H; H–C3′), 4.15 (m, 1H; H–C4′), 3.85 (s, 6H; CH_3O), 3.48 (m, 2H; H–C5′), 2.65 (m, 1H; H–C2′), 2.38 (m, 1H; H′-C2′), 2.00 (s, 3H; H_3C–C5), 0.87 (s, 9H, CH_3–CSi), 0.15, 0.13 (ss, 6H, CH_3–Si); ^{13}C NMR (50 MHz, $CDCl_3$) δ=158.6 (Cphenyl), 158.0 (C4), 154.0 (C2), 153.2 (Ctriazolyl), 145.5 (Ctriazolyl), 145.0 (Cphenyl), 144.6 (C6), 135.1 (Cphenyl), 130.0, 128.0, 127.9 127.1, 113.2 (Cphenyl–H), 105.7 (C5), 87.5, 87.1, 86.8 (Cq, C1′, C4′), 70.6 (C3′), 62.0 (C5′), 55.2 (CH_3O), 42.3 (C2′), 26.7 (CH_3–CSi), 17.9 (CqSi), 16.5 (CH_3–C5), −4.6, −4.9 (CH_3Si); LRMS (FAB, magic bullet, Xe, negative mode): *m/z*: 847.4 $[M-2H+Xe]^-$, 788.1 $[M-3H+2K]^-$, 747.9, 747.1 $[M-2H+K]^-$; calcd for $C_{39}H_{47}O_6N_5Si$ [M]: 709.9061 (average).

4.7. 3′-*O*-*t*-Butyldimetylsilyl-5′-*O*-dimethoxytrityl-1-[4-guanidine-5-methylpyrimidin-2(1*H*)-onyl]-β-D-2′-deoxyribosides (12a, 12b, 12c, 12d)

Derivatives **12** were obtained using the same general procedure described for derivative **9**. Triazolyl compound **11** (2–5 mmol) was dissolved in dry dimethylformamide (10 mL/mmol nucleoside) under inert atmosphere. K_2CO_3 (3 equiv.) and the guanidine derivative (2 equiv.; guanidine·HCl for **12a**, methylguanidine·HCl for **12b**, 1,1-dimethylguanidine·H_2SO_4 for **12c** and 1,2-dimethylguanidine·HBr[45] for **12d**) was then added. The mixture was stirred in a bath at 100°C until the initial nucleoside was completely consumed according to TLC analysis (6–12 h depending on the analogue). The mixture was left to cool, and filtered to remove the insoluble residue. The solid was further washed with $CHCl_3$ and all the filtrates were pooled. The solvents were finally removed by evaporation in vacuo to obtain a residue which was purified by column chromatography (SiO_2) using CH_2Cl_2/MeOH/Et_3N as eluent: **12a** and **12d** eluted with a 100:10:1 mixture, **12b** with 100:4:1 and **12c** with 100:3.1. The corresponding product was obtained by evaporation of solvents.

4.7.1. Guanidine derivative 12a. White solid, 70% yield; mp 128–130°C; TLC (CH_2Cl_2/MeOH 10:1): R_f=0.25; ^{1}H NMR (200 MHz, $CDCl_3$): δ=7.61 (s, 1H; H–C6), 7.40–7.15 (m, 9H; H-phenyl), 6.75 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.31 (m, 1H; H–C1′), 4.28 (m, 1H; H–C3′), 3.90 (m, 1H; H–C4′), 3.71 (s, 6H; CH_3O), 3.32 (m, 2H; H–C5′), 2.32, 2.15 (m, 2H; H–C2′), 1.55 (s, 3H, CH_3–C5), 0.75 (s, 9H, CH_3–CSi), −0.07, −0.13 (ss, 6H, CH_3–Si); ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.4 (Cguanid), 161.0 (C4), 158.6 (Cphenyl), 155.6 (C2), 144.5 (Cphenyl), 136.0 (C6), 135.7 (Cphenyl), 130.1, 128.2, 127.8, 126.9, 113.2 (Cphenyl–H), 112.1 (C5), 86.6, 86.3, 85.6 (Cq, C1′, C4′), 71.9 (C3′), 62.8 (C5′), 55.3 (CH_3O), 41.9 (C2′), 25.7 (CH_3–CSi), 18.0 (CqSi), 11.6 (CH_3–C5), −4.7, −4.9 (CH_3Si); HRMS (FAB, magic bullet, positive mode): *m/z* (%): calcd for $C_{38}H_{50}O_6N_5Si$ $[M+H]^+$: 700.3530, found 700.3554 (100).

4.7.2. *N*-Methylguanidine derivative 12b. White and hygroscopic solid, 60% yield; mp 125–127°C; TLC (CH_2Cl_2/MeOH 10:1): R_f=0.30; ^{1}H NMR (200 MHz, $CDCl_3$): δ=7.68 (s, 1H; H–C6), 7.46–7.25 (m, 9H; H-phenyl), 6.80 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.43 (m, 1H; H–C1′), 4.45 (m, 1H; H–C3′), 4.12 (m, 1H; H–C4′), 3.85 (s, 6H; CH_3O), 3.42 (m, 2H; H–C5′), 2.98 (bs, 3H; CH_3–N), 2.45, 2.13 (m, 2H; H–C2′), 1.70 (s, 3H; CH_3–C5), 0.88 (s, 9H, CH_3–CSi), 0.07 (bs, 6H, CH_3–Si); ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.0 (Cguanid), 160.5 (C4), 158.3 (Cphenyl), 156.2 (C2), 143.6 (Cphenyl), 135.5 (C6, Cphenyl), 129.9, 128.0, 127.7, 127.0, 113.0 (Cphenyl–H), 112.0 (C5), 86.4, 86.1, 85.6 (Cq, C1′, C4′), 71.5 (C3′), 62.8 (C5′), 55.1 (CH_3O), 42.0 (C2′), 28.2 (CH_3–N), 25.6 (CH_3–CSi), 18.1 (CqSi), 11.4 (CH_3–C5), −4.5, −4.8 (CH_3Si); LRMS (FAB, 3-amino-1,2-propanodiol, Xe, negative mode): *m/z*: 712.6 $[M-H]^-$; calcd for $C_{39}H_{51}O_6N_5Si$ [M]: 713.9378 (average).

4.7.3. *N,N*-Dimethylguanidine derivative 12c. White solid, 55% yield; mp 126–128°C; TLC (CH_2Cl_2/MeOH 10:1): R_f=0.60; ^{1}H NMR (200 MHz, $CDCl_3$): δ=8.08 (s, 1H; H–N3), 7.65 (s, 1H; H–C6), 7.48–7.25 (m, 9H; H-phenyl), 6.84 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.44 (m, 1H; H–C1′), 4.52 (m, 1H; H–C3′), 4.02 (m, 1H; H–C4′), 3.86 (s, 6H; CH_3O), 3.40 (m, 2H; H–C5′), 3.17 (bs, 6H, CH_3–N), 2.40, 2.25 (m, 2H; H–C2′), 1.73 (s, 3H; CH_3–C5), 0.88 (s, 9H, CH_3–CSi), 0.07, 0.06 (bs, 6H, CH_3–Si); ^{13}C NMR (50 MHz, $CDCl_3$) δ=169.1 (Cguanid), 159.0 (C4), 158.4 (Cphenyl), 156.1 (C2), 144.5 (Cphenyl), 137.7 (C6), 137.1 (Cphenyl), 130.0, 128.1, 127.8, 126.8, 113.1 (Cphenyl–H), 112.0 (C5), 86.4, 86.0, 85.5 (Cq, C1′, C4′), 71.6 (C3′), 62.6 (C5′), 55.2 (CH_3O), 41.9 (C2′), 29.7 (CH_3–N), 25.7 (CH_3–CSi), 18.0 (CqSi), 14.5 (CH_3–C5), −4.7, −4.9 (CH_3Si); LRMS (FAB, magic bullet, Xe, negative mode): *m/z*: 728.1, 727.0 $[M-H]^-$; calcd for $C_{40}H_{53}O_6N_5Si$ [M]: 727.9644 (average).

4.7.4. *N,N′*-Dimethylguanidine derivative 12d. White solid, 70% yield; mp 85–88°C; TLC (CH_2Cl_2/MeOH 10:1): R_f=0.60; ^{1}H NMR (200 MHz, $CDCl_3$): δ=8.30 (s, 1H; H–N3), 7.75 (s, 1H; H–C6), 7.50–7.32 (m, 9H; H-phenyl), 6.85 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.41 (m, 1H; H–C1′), 4.55 (m, 1H; H–C3′), 4.07 (m, 1H; H–C4′), 3.83 (s, 6H; CH_3O), 3.43 (m, 2H; H–C5′), 2.93 (bs, 6H; CH_3–N), 2.42, 2.25 (m, 2H; H–C2′), 1.65 (s, 3H; CH_3–C5), 0.87 (s, 9H, CH_3–CSi), 0.06, 0.05 (bs, 6H, CH_3–Si); ^{13}C NMR (50 MHz, $CDCl_3$) δ=169.1 (Cguanid), 158.4 (Cphenyl), 147.0 (C2), 142.2 (Cphenyl), 135.3 (C6, Cphenyl), 129.9, 127.9, 127.8, 126.8, 113.0 (Cphenyl–H), 112.0 (C5), 86.5, 86.4, 85.3 (Cq, C1′, C4′), 71.5 (C3′), 62.6 (C5′), 55.1 (CH_3O), 41.7 (C2′), 28.2 (CH_3–N), 25.6 (CH_3–CSi), 16.4 (CqSi), 14.5 (CH_3–C5), −4.7, −5.0 (CH_3Si); LRMS (FAB, magic bullet, Xe, negative mode): *m/z*: 808.5, 806.5 $[M-3H+2K]^-$, 728.1, 727.0 $[M-H]^-$; calcd for $C_{40}H_{53}O_6N_5Si$ [M]: 727.9644 (average).

4.8. 3′-*O*-*t*-Butyldimetylsilyl-5′-*O*-dimethoxytrityl-1-[5-methyl-4-(4-nitrobenzoyl)guanidine-pyrimidin-2(1*H*)-onyl]-β-D-2′-deoxyriboside (13)

Guanidine derivative **12a** (0.7 g, 1 mmol), previously dried by coevaporation with dry CH_3CN, was dissolved in dry pyridine (10 mL) under inert atmosphere. 4-Nitrobenzoyl chloride (0.4 g, 2.2 equiv.) was added to the solution. The initial nucleoside was completely consumed in 2 h according to TLC analysis. The solution was cooled in an ice bath, and 10% aq. $NaHCO_3$ (2 mL) was added to hydrolyse the excess of acid chloride. After 10 min, solvent was removed by evaporation. The residue was partitioned between AcOEt (100 mL) and 10% aq. $NaHCO_3$ (100 mL). The organic phase was separated and washed again with 10% aq. $NaHCO_3$ (100 mL), 10% aq. tartaric acid (100 mL) and brine. The organic solution was dried over Na_2SO_4 and the solvent removed. The resulting residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH/Et_3N 100:1:1 as eluent. Solvent elimination rendered the product as a bright yellow solid (0.45 g, 75% yield). Mp 126–128°C; TLC (CH_2Cl_2/MeOH 100:10): R_f=0.80; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.45–8.20 (m, 5H; H-nitrophenyl, H–C6), 7.50–7.28 (m, 9H; H-phenyl), 6.90 (d, ^2J(H, H)=9 Hz, 4H; H-phenyl), 6.38 (m, 1H; H–C1′), 4.60 (m, 1H; H–C3′), 4.05 (m, 1H; H–C4′), 3.85 (s, 6H; CH_3O), 3.45 (m, 2H; H–C5′), 2.60, 2.36 (m, 2H; H–C2′), 1.67 (s, 3H, CH_3–C5), 0.87 (s, 9H, CH_3–CSi), 0.07, 0.05 (bs, 6H, CH_3–Si); ^{13}C NMR (75 MHz, $CDCl_3$) δ=158.8 (Cphenyl), 148.9

(C6), 144.3 (Cphenyl), 135.3 (Cphenyl), 130.1, 128.2, 128.0, 127.2, 113.3 (Cphenyl–H), 129.7, 123.7, 123.2 (Cnitrophenyl–H), 86.8, 86.7 (Cq, C1′, C4′), 71.8 (C3′), 63.2 (C5′), 55.2 (CH_3O), 42.2 (C2′), 25.6 (CH_3–CSi), 17.9 (CqSi), 11.6 (CH_3–C5), −4.7, −5.0 (CH_3Si); LRMS (FAB, magic bullet, Xe, negative mode): *m/z*: 848.6, 847.6 $[M-H]^-$; calcd for $C_{45}H_{52}O_9N_6Si$ [M]: 849.0149 (average).

4.9. 5′-*O*-Dimethoxytrityl-1-[5-methyl-4-(4-nitrobenzoyl) guanidine-pyrimidin-2(1*H*)-onyl]-β-D-2′-deoxyriboside (14)

Nucleoside **13** (0.55 g, 0.6 mmol) was dissolved in dry tetrahydrofuran (10 mL) under inert atmosphere. Solid tetrabutylammonium fluoride trihydrate (0.37 g, 1.2 mmol) was added. When the initial nucleoside was completely consumed according to TLC analysis (approx. 6 h), MeOH was added (2 mL), and 10 min later the solvent was removed by evaporation. The crude was directly purified by column chromatography (SiO_2) using CH_2Cl_2/MeOH/Et_3N 100:1:1 as eluent. After evaporation of the solvent, a bright yellow solid was obtained (0.30 g, 68% yield). Mp 146–148°C; TLC (CH_2Cl_2/MeOH 100:10): R_f=0.45; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.35–8.05 (m, 5H; H-nitrophenyl, H–C6), 7.40–7.25 (m, 9H; H-phenyl), 6.85 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.38 (m, 1H; H–C1′), 4.60 (m, 1H; H–C3′), 4.18 (m, 1H; H–C4′), 3.70 (s, 6H; CH_3O), 3.42 (m, 2H; H–C5′), 2.70, 2.55 (m, 2H; H–C2′), 1.68 (s, 3H; CH_3–C5); ^{13}C NMR (75 MHz, $CDCl_3$) δ=159.3 (Cnitrophenyl), 158.7 (Cphenyl), 149.8 (C6), 144.3 (Cphenyl), 135.3 (Cphenyl), 130.0, 128.1, 128.0, 127.2, 113.3 (Cphenyl–H), 123.2 (Cnitrophenyl–H), 86.9, 86.7 (Cq, C1′, C4′), 71.8 (C3′), 63.3 (C5′), 55.2 (CH_3O), 42.1 (C2′), 12.5 (CH_3–C5); HRMS (FAB, magic bullet, positive mode): *m/z* (%): calcd for $C_{39}H_{39}O_9N_6$ $[M+H]^+$: 735.2778, found 735.2772 (72).

4.10. 5′-*O*-Dimethoxytrityl-1-[5-methyl-4-(4-nitrobenzoyl) guanidine-pyrimidin-2(1*H*)-onyl]-β-D-2′-deoxyriboside 3′-*O*-cyanoethyl-*N*,*N*-diisopropylphosphoramidite (15)

Nucleoside **14** (0.20 g, 0.27 mmol), previously dried by coevaporation with dry CH_3CN, was dissolved in dry CH_2Cl_2 (5 mL) under inert atmosphere. Then, 2-cyanoethoxy-bis(*N*,*N*-diisopropylamino)phosphine (100 μL, 0.32 mmol) and tetrazole (10 mg, 0.13 mmol) were added. When the initial nucleoside (R_f=0.15) was completely consumed, as shown by TLC analysis (approx. 1 h), MeOH (1 mL) was added to destroy the excess of phosphine. After 10 min, the solvent was removed by evaporation. The residue was dissolved in CH_2Cl_2 (50 mL), and washed with 10% aq. $NaHCO_3$ (2×50 mL) and brine. The solution was dried over Na_2SO_4, and the solvent removed by evaporation. The product was purified by column chromatography (SiO_2) using CH_2Cl_2/hexane/Et_3N (47.5:47.5:5) as eluent. Evaporation of solvents and precipitation with cold hexane rendered the product (mixture of two diastereomers) as a yellowish solid (125 mg, 50% yield). Mp 129–130°C (decomp.); TLC (CH_2Cl_2/AcOEt/triethylamine 45:45:10): R_f=0.45, 0.40; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.35–8.05 (m, 5H; H-nitrophenyl, H–C6), 7.40–7.20 (m, 9H; H-phenyl), 6.82 (m, 4H; H-phenyl), 6.35 (m, 1H; H–C1′), 4.63 (m, 1H; H–C3′), 4.20, 4.17 (m, 1H; H–C4′), 3.76, 3.75 (s, s, 6H; CH_3O), 3.60–3.45, 3.35 (m, 5H; H–C5′, H–C *i*Pr, CH_2OP), 2.70 (m, 1H; H–C2′), 2.59 (m, 1H; H–CHCN), 2.42–2.25 (m, 2H; H–CHCN, H–C2′), 1.58 (bs, 3H; H_3C–C5), 1.15–1.10 (m, 12H; CH_3 *i*Pr); ^{31}P NMR (121 MHz, $CDCl_3$): δ=149.3, 148.7; HRMS (FAB, magic bullet, positive mode): *m/z* (%): calcd for $C_{48}H_{56}O_{10}N_8P$ $[M+H]^+$: 935.3857, found 935.3813 (100).

4.11. 5′-*O*-Dimethoxytrityl-1-[4-guanidine-5-methyl-pyrimidin-2(1*H*)-onyl]-β-D-2′-deoxyribosides (16a, 16b, 16c, 16d).

4.11.1. General procedure to obtain derivatives 16.

Nucleoside **12** (1–4 mmol) was dissolved in dry tetrahydrofuran (10 mL/mmol nucleoside) under inert atmosphere, and solid tetrabutylammonium fluoride trihydrate was added (2 equiv.). When the initial nucleoside was completely consumed (TLC analysis, normally after 4–6 h), MeOH was added (2 mL) and, after 10 min, the solvent was removed by evaporation. The crude was directly purified by silica gel column chromatography using CH_2Cl_2/MeOH/Et_3N as eluent: **16a** eluted with a 100:10:1 mixture, **16b** and **16c** with 100:5:1, and **16d** with 100:7.1. The product was obtained after elimination of the solvent.

4.11.2. Guanidine derivative 16a. White solid, 85% yield; mp 160–162°C; TLC (CH_2Cl_2/MeOH 10:1): R_f=0.20; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.65 (s, 1H; H–C6), 7.47–7.20 (m, 9H; H-phenyl), 6.83 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.38 (m, 1H; H–C1′), 4.46 (m, 1H; H–C3′), 4.18 (m, 1H; H–C4′), 3.79 (s, 6H; CH_3O), 3.40 (m, 2H; H–C5′), 2.46 (m, 1H; H–C2′), 2.25 (m, 1H; H–C2′), 1.59 (s, 3H; CH_3–C5); ^{13}C NMR (50 MHz, $CDCl_3$) δ=170.6 (Cguanid), 160.9 (C4), 158.3 (Cphenyl), 156.6 (C2), 144.3 (Cphenyl), 136.2, (C6), 135.5, 135.4 (Cphenyl), 129.9, 127.9, 127.8, 126.6, 112.9 (Cphenyl–H), 112.1 (C5), 86.6, 86.1, 85.7 (Cq, C1′, C4′), 71.8 (C3′), 63.4 (C5′), 55.1 (CH_3O), 41.6 (C2′), 14.0 (CH_3–C5); IR (KBr; only most significant bands): $\bar{\nu}$=3500–3200 (NH, OH st), 1685 (C=O st), 1610 (C–NH guan. st) cm^{-1}; HRMS (FAB, magic bullet, positive mode): *m/z* (%): calcd for $C_{32}H_{36}O_6N_5$ $[M+H]^+$: 586.2665, found 586.2692 (100).

4.11.3. *N*-Methylguanidine derivative 16b. White solid, 90% yield; mp 121–122°C; TLC (CH_2Cl_2/MeOH 10:1): R_f=0.20; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.60 (s, 1H; H–C6), 7.45–7.20 (m, 9H; H-phenyl), 6.82 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.44 (m, 1H; H–C1′), 4.55 (m, 1H; H–C3′), 4.08 (m, 1H; H–C4′), 3.77 (s, 6H; CH_3O), 3.40 (m, 2H; H–C5′), 2.87 (bs, 3H; CH_3–N), 2.44 (m, 1H; H–C2′), 2.23 (m, 1H; H–C2′), 1.61 (bs, 3H; CH_3–C5); ^{13}C NMR (75 MHz, $CDCl_3$) δ=169.8 (Cguanid), 160.3 (C4), 158.6 (Cphenyl), 156.6 (C2), 144.6 (Cphenyl), 135.8, 135.7 (C6, Cphenyl), 130.1, 128.2, 127.9, 126.9, 113.3 (Cphenyl–H), 113.2 (C5), 86.7, 85.8, 85.6 (Cq, C1′, C4′), 72.3 (C3′), 63.9 (C5′), 55.2 (CH_3O), 41.8 (C2′), 29.8 (CH_3–N), 14.1 (CH_3–C5); IR (KBr; only most significant bands): $\bar{\nu}$=3500–3200 (NH, OH st), 1680 (C=O st), 1610 (C–NH guan. st) cm^{-1}; HRMS (FAB, magic bullet, positive mode): *m/z* (%): calcd for $C_{33}H_{38}O_6N_5$ $[M+H]^+$: 600.2822, found 600.2850 (45).

4.11.4. *N,N*-Dimethylguanidine derivative 16c. Yellowish solid, 70% yield; mp 137–139°C; TLC (CH_2Cl_2/MeOH 10:1): R_f=0.30; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.60 (s, 1H; H–C6), 7.45–7.20 (m, 9H; H-phenyl), 6.82 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.56 (m, 1H; H–C1′), 4.56 (m, 1H; H–C3′), 4.10 (m, 1H; H–C4′), 3.78 (s, 6H; CH_3O), 3.38 (m, 2H; H–C5′), 3.07 (bs, 6H; CH_3–N), 2.52 (m, 1H; H–C2′), 2.25 (m, 1H; H–C2′), 1.61 (bs, 3H; CH_3–C5); ^{13}C NMR (75 MHz, $CDCl_3$) δ=168.4 (Cguanid), 159.3 (C4), 158.4 (Cphenyl), 156.2 (C2), 144.6 (Cphenyl), 135.6 (Cphenyl), 135.3 (C6), 130.0, 128.1, 127.9, 126.8, 113.1 (Cphenyl–H), 113.0 (C5), 86.8, 85.6, 85.5 (Cq, C1′, C4′), 72.4 (C3′), 64.1 (C5′), 55.2 (CH_3O), 41.8 (C2′), 26.9 (CH_3–N), 14.2 (CH_3–C5); IR (KBr; only most significant bands): $\bar{\nu}$=3500–3200 (NH, OH st), 1700 (C=O st), 1665, 1610 (C–NH guan. st) cm^{-1}; HRMS (FAB, magic bullet, positive mode): *m/z* (%): calcd for $C_{34}H_{40}O_6N_5$ $[M+H]^+$: 614.2978, found 614.2950 (100).

4.11.5. *N,N′*-Dimethylguanidine derivative 16d. White solid, 70% yield; mp 149–150°C; TLC (CH_2Cl_2/MeOH 10:1): R_f=0.25; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.60 (s, 1H; H–C6), 7.45–7.20 (m, 9H; H-phenyl), 6.82 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.43 (m, 1H; H–C1′), 4.51 (m, 1H; H–C3′), 4.13 (m, 1H; H–C4′), 3.78 (s, 6H; CH_3O), 3.42 (m, 2H; H–C5′), 2.92, 2.90 (s, s, 6H; CH_3–N), 2.52 (m, 1H; H–C2′), 2.20 (m, 1H; H–C2′), 1.65 (bs, 3H; CH_3–C5); ^{13}C NMR (75 MHz, $CDCl_3$) δ=169.1 (Cguanid), 159.1 (C4), 158.3 (Cphenyl), 156.4 (C2), 144.5 (Cphenyl), 135.6 (Cphenyl), 135.3 (C6), 129.9, 128.0, 127.8, 126.8, 113.0 (Cphenyl–H), 112.6 (C5), 86.6, 85.7, 85.6 (Cq, C1′, C4′), 72.0 (C3′), 63.4 (C5′), 55.2 (CH_3O), 41.6 (C2′), 27.8 (CH_3–N), 14.2 (CH_3–C5); IR (KBr; only most significant bands): $\bar{\nu}$=3500–3200 (NH, OH st), 1680 (C=O st), 1620 (C–NH guan. st) cm^{-1}; HRMS (FAB, magic bullet, positive mode): *m/z* (%): calcd for $C_{34}H_{40}O_6N_5$ $[M+H]^+$: 614.2978, found 614.2983 (100).

4.12. Triethylammonium 5′-*O*-dimethoxytrityl-1-[4-guanidine-5-methylpyrimidin-2(1*H*)-onyl]-β-D-2′-deoxyriboside 3′-*H*-phosphonate (17a, 17b, 17c, 17d)

4.12.1. General procedure to obtain derivatives 17. Nucleoside **16** (0.5–1 mmol), previously dried by coevaporation with dry CH_3CN, was dissolved in dry pyridine (10 mL/mmol) under inert atmosphere. Diphenylphosphite (10 equiv.) was then added. The reaction, according to TLC analysis, was normally completed in 45–60 min. The reaction mixture was then cooled in an ice bath, and 1 M aq. triethylammonium hydrogencarbonate was added (2–5 mL). After 30 min, the solvents were removed by evaporation. The residue was redissolved in $CHCl_3$ (50 mL) and washed with 1 M aq. triethylammonium hydrogencarbonate (50 mL). The organic phase was set apart, and the aqueous phase was reextracted with $CHCl_3$ (50 mL) until TLC analysis showed no nucleoside in the aqueous solution. The organic phases were pooled, dried over Na_2SO_4 and the solvent was removed by evaporation. The crude was purified by column chromatography (SiO_2) using CH_2Cl_2/MeOH/triethylamine 100:20:2 as eluent. The desired products were obtained after evaporation of the solvent and precipitation with diethylether/hexane 1:1.

4.12.2. Guanidine derivative 17a. White solid, 80% yield; mp 118–120°C (decomp.); TLC (CH_2Cl_2/MeOH/Et_3N 100:10:1): R_f=0.20; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.71 (s, 1H; H–C6), 7.45–7.20 (m, 9H; H-phenyl), 6.88 (d, ^2J(H, P)=620 Hz, 1H; H–P), 6.83 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.42 (m, 1H; H–C1′), 4.93 (m, 1H; H–C3′), 4.25 (m, 1H; H–C4′), 3.78 (s, 6H; CH_3O), 3.41 (m, 2H; H–C5′), 2.83 (q, ^3J(H, H)=7 Hz, 6H; CH_2 ammonium), 2.63 (m, 1H; H–C2′), 2.35 (m, 1H; H–C2′), 1.54 (s, 3H; CH_3–C5), 1.05 (t, ^3J(H, H)=7 Hz, 9H; CH_3 ammonium); ^{13}C NMR (75 MHz, $CDCl_3$) δ=167.0 (Cguanid), 159.1 (C4), 158.4 (Cphenyl), 155.2 (C2), 144.2 (Cphenyl), 138.1, (C6), 135.3 (Cphenyl), 129.8, 127.9, 127.7, 126.7, 112.7 (Cphenyl–H), 109.1 (C5), 86.6, 85.7, 85.1 (Cq, C1′, C4′), 73.4 (C3′), 63.1 (C5′), 55.0 (CH_3O), 45.3 (CH_2 ammonium), 40.3 (C2′), 19.4 (CH_3–C5), 9.8 (CH_3 ammonium); ^{31}P NMR (121 MHz, $CDCl_3$) δ=3.3; IR (KBr; only most significant bands): $\bar{\nu}$=3400–3100 (NH, OH st), 2340 (PO–H st), 1680 (C=O st), 1630 (C–NH guan. st), 1065 (P=O st) cm^{-1}; HRMS (FAB, magic bullet, positive mode): *m/z* (%): calcd for $C_{32}H_{37}O_8N_5P$ $[M+H]^+$: 650.2380, found 650.2385 (56).

4.12.3. *N*-Methylguanidine derivative 17b. White solid, 85% yield; mp 160–162°C (decomp.); TLC (CH_2Cl_2/MeOH/Et_3N 100:10:1): R_f=0.20; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.85 (s, 1H; H–C6), 7.40–7.20 (m, 9H; H-phenyl), 6.84 (d, ^2J(H, P)=620 Hz, 1H; H–P), 6.80 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.28 (m, 1H; H–C1′), 4.95 (m, 1H; H–C3′), 4.23 (m, 1H; H–C4′), 3.75 (s, 6H; CH_3O), 3.42 (m, 2H; H–C5′), 3.01 (q, ^3J(H, H)=7 Hz, 6H; CH_2 ammonium), 2.95 (bs, 3H; CH_3–N), 2.66 (m, 1H; H–C2′), 2.37 (m, 1H; H–C2′), 1.60 (s, 3H; CH_3–C5), 1.27 (t, ^3J(H, H)= 7 Hz, 9H; CH_3 ammonium); ^{13}C NMR (75 MHz, $CDCl_3$) δ=163.7 (Cguanid), 158.6 (Cphenyl), 156.5 (C2), 144.4 (Cphenyl), 135.8, 135.4 (C6, Cphenyl), 130.1, 128.2, 127.9, 127.1, 113.2 (Cphenyl–H), 111.1 (C5), 86.7, 85.6, 85.0 (Cq, C1′, C4′), 73.9 (C3′), 63.2 (C5′), 55.2 (CH_3O), 45.7 (CH_2 ammonium), 40.5 (C2′), 28.3 (CH_3–N), 12.3 (CH_3–C5), 9.0 (CH_3 ammonium); ^{31}P NMR (121 MHz, $CDCl_3$) δ=3.2; IR (KBr; only most significant bands): $\bar{\nu}$=3500–2900 (NH, OH st), 2365 (PO–H st), 1700 (C=O st), 1620 (C–NH guan. st), 1080 (P=O st) cm^{-1}; HRMS (FAB, magic bullet, positive mode): *m/z* (%): calcd for $C_{33}H_{39}O_8N_5P$ $[M+H]^+$: 664.2536, found 664.2507 (100).

4.12.4. *N,N*-Dimethylguanidine derivative 17c. White solid, 85% yield; mp 150–152°C (decomp.); TLC (CH_2Cl_2/MeOH/Et_3N 100:10:1): R_f=0.25; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.55 (s, 1H; H–C6), 7.55–7.25 (m, 9H; H-phenyl), 6.83 (d, ^2J(H, P)=620 Hz, 1H; H–P), 6.82 (d, ^3J(H, H)= 9 Hz, 4H; H-phenyl), 6.28 (m, 1H; H–C1′), 4.92 (m, 1H; H–C3′), 4.22 (m, 1H; H–C4′), 3.78 (s, 6H; CH_3O), 3.38 (m, 2H; H–C5′), 3.09 (bs, 6H; CH_3-N), 3.04 (q, ^3J(H, H)=7 Hz, 6H; CH_2 ammonium), 2.63 (m, 1H; H–C2′), 2.35 (m, 1H; H–C2′), 1.50 (s, 3H; CH_3–C5), 1.32 (t, ^3J(H, H)=7 Hz, 9H; CH_3 ammonium); ^{13}C NMR (75 MHz, $CDCl_3$) δ=168.7 (Cguanid), 158.6 (C4), 158.4 (Cphenyl), 156.5 (C2), 144.4 (Cphenyl), 135.6, 135.4 (C6, Cphenyl), 130.1, 128.1, 127.8, 126.8, 113.1 (Cphenyl–H), 111.8 (C5), 86.7, 85.2, 85.1 (Cq, C1′, C4′), 73.8 (C3′), 63.6 (C5′), 55.2 (CH_3O), 45.5 (CH_2 ammonium), 40.3 (C2′), 27.0 (CH_3–N), 14.2 (CH_3–C5), 8.6 (CH_3 ammonium); ^{31}P NMR (121 MHz, $CDCl_3$) δ=3.7; IR (KBr; only most significant bands): $\bar{\nu}$=3500–

3000 (NH, OH st), 2380 (PO–H st), 1675 (C═O st), 1605 (C–NH guan. st), 1060 (P═O st) cm^{-1}; HRMS (FAB, magic bullet, positive mode): *m/z* (%): calcd for $C_{34}H_{41}O_8N_5P$ $[M+H]^+$: 678.2693, found 678.2660 (100).

4.12.5. *N,N′*-Dimethylguanidine derivative 17d. White solid, 76% yield; mp 118–120°C (decomp.); TLC (CH_2Cl_2/MeOH/Et_3N 100:10:1): R_f=0.25; ^1H NMR (300 MHz, $CDCl_3$): δ=7.58 (s, 1H; H–C6), 7.55–7.20 (m, 9H; H-phenyl), 6.89 (d, ^2J(H, P)=620 Hz, 1H; H–P), 6.82 (d, ^3J(H, H)=9 Hz, 4H; H-phenyl), 6.48 (m, 1H; H–C1′), 4.92 (m, 1H; H–C3′), 4.27 (m, 1H; H–C4′), 3.78 (s, 6H; CH_3O), 3.40 (m, 2H; H–C5′), 2.97 (q, ^3J(H, H)=7 Hz, 6H; CH_2 ammonium), 2.90 (bs, 6H; CH_3–N), 2.65 (m, 1H; H–C2′), 2.30 (m, 1H; H–C2′), 1.58 (s, 3H; CH_3–C5), 1.28 (t, ^3J(H, H)=7 Hz, 9H; CH_3 ammonium); ^{13}C NMR (75 MHz, $CDCl_3$) δ=166.4 (Cguanid), 159.8 (C4), 158.4 (Cphenyl), 144.4 (Cphenyl), 135.6 (Cphenyl), 135.2 (C6), 130.1, 128.1, 127.8, 126.8, 113.1 (Cphenyl–H), 111.8 (C5), 86.6, 85.4, 85.1 (Cq, C1′, C4′), 74.0 (C3′), 63.5 (C5′), 55.2 (CH_3O), 45.6 (CH_2 ammonium), 40.2 (C2′), 27.9 (CH_3–N), 14.0 (CH_3–C5), 9.0 (CH_3 ammonium); ^{31}P NMR (121 MHz, $CDCl_3$) δ=3.6; IR (KBr; only most significant bands): $\bar{\nu}$=3500–3000 (NH, OH st), 2340 (PO–H st), 1700 (C═O st), 1615 (C–NH guan. st), 1070 (P═O st) cm^{-1}; HRMS (FAB, magic bullet, positive mode): *m/z* (%): calcd for $C_{34}H_{41}O_8N_5P$ $[M+H]^+$: 678.2693, found 678.2665 (100).

4.13. Oligonucleotide synthesis

Solid-phase syntheses were performed using a 380B Applied Biosystems synthesizer. All reagents, nucleoside derivatives or resins not described in this Experimental Section or prepared according to published procedures were of commercial origin (Glen Research). Standard procedures were used for the assembly of oligonucleotides using phosphite triester chemistry[46] (DMT-on).

4.14. Oligonucleotide synthesis using *H*-phosphonate chemistry

Oligonucleotide syntheses were performed on DMT-T-CPG at the 2–3 μmol scale. Steps of the synthesis cycle (adapted from standard *H*-phosphonate chemistry[37]): (1) deprotection: 3% trichloroacetic acid in dichloromethane; (2) coupling (5×): 0.035 M nucleoside 3′-*H*-phosphonate+0.17 M adamantoyl chloride, both in either pyridine/CH_3CN 1:3 (synthesis with triazolyl nucleoside (**10**) or pyridine/CH_3CN 1:1 (syntheses of oligonucleotides containing the guanidine analogues (**17**), 1 min; (3) capping: 0.1 M isopropyl phosphite+0.17 M adamantoyl chloride in pyridine/CH_3CN 1:3, 2 min. To simplify the purification step, the dimethoxytrityl group was kept on the 5′-hydroxyl group of the last nucleoside. After the chain assembly, oxidation of *H*-phosphonate diester bonds to phosphates was carried out by reaction with 3% iodine in lutidine/tetrahydrofuran/water 75:23:2 for 30 min, followed by a second treatment with 1.5% iodine in lutidine/triethylamine/tetrahydrofuran/water 38:25:36:2 for an additional 30 min.

4.15. Deprotection and cleavage

Oligonucleotide-resins (10–25 mg) were placed in a vial and treated with the corresponding solution (2 mL was generally used) for 2 h. The mixture was filtered through glass wool to remove the glass beads, which were washed with water (2 mL). The filtrates were then concentrated by evaporation in vacuo to 1–2 mL. If these solutions were not clear, filtering through 0.45 μm nylon filters was carried out.

4.16. Analysis, purification and characterization

Reversed-phase HPLC analyses of oligonucleotides were carried out on Nucleosil C18 columns (25×0.5 cm, 10 μm), using linear gradients of 0.01 M aq. triethylammonium acetate (solvent A) and 1:1 CH_3CN/H_2O (solvent B), (flow: 1 mL/min, detection wavelength: 260 nm). Trimers TXT were analysed using a linear gradient from 15 to 35% of B in 20 min (gradient A), and 15-mers with either gradient A or a gradient from 15 to 75% of B in 30 min (gradient B). Analyses of 15-mers by gel electrophoresis were run under denaturing conditions (7 M urea), on 20% polyacrylamide gels, at 500–750 V for 3–4 h). Reaction with 'stain-all' dye (Sigma) was used to detect the products in the gel.

Oligonucleotides (protected with the DMT group at the 5′-hydroxyl) were purified on a semipreparative PRP-1 column (Hamilton), using the same gradient as for the analysis of the crude (see above), but with 0.05 M aq. triethylammonium acetate as eluent A (flow: 2 mL/min, detection Wavelength: 280 nm). The desired fractions were pooled and lyophilized to dryness. Oligonucleotides were dissolved in water/HAcO 3:1 and left to react for 30 min at 4°C in order to eliminate the DMT group, and lyophilized again. Oligonucleotides were redissolved in water and eluted through a Sephadex G-10 exclusion column to remove organic impurities, and the oligonucleotide-containing fractions pooled and lyophilized.

Oligonucleotides were analysed MALDI-TOF MS (negative mode), using a trihydroxyacetophenone/ammonium citrate matrix mixture, and either a reflector (trimers) or a linear detector (15-mers). The following convention for the nucleoside analogues is used: T^g=4-guanidine-, T^{mg}=4-*N*-methylguanidine-, T^{dmg}=4-*N*,*N*-dimethylguanidine- and T^{dmg2}=4-*N*,*N′*-dimethylguanidine-2-pyrimidinone nucleoside.

4.16.1. $^{5'}$TXT$^{3'}$. X=T^g: 45% yield (synthesis+purification); reversed-phase HPLC t_R (gradient A): 13.4 min; MS (MALDI-TOF, negative mode): 890.8 $[M-H]^-$; calcd for $C_{31}H_{43}O_{18}N_9P_2$ [M]: 891.67 (average); X=T^{mg}: 55% yield (synthesis+purification); reversed-phase HPLC t_R (gradient A): 13.3 min; MS (MALDI-TOF, negative mode): 903.74 $[M-H]^-$; calcd for $C_{32}H_{45}O_{18}N_9P_2$ [M]: 905.70 (average); X=T^{dmg}: 55% yield (synthesis+purification); reversed-phase HPLC t_R (gradient A): 13.8 min; MS (MALDI-TOF, negative mode): 917.65 $[M-H]^-$; calcd for $C_{33}H_{47}O_{18}N_9P_2$ [M]: 919.72 (average); X=T^{dmg2}: 60% yield (synthesis+purification); reversed-phase HPLC t_R (gradient A): 13.5 min; MS (MALDI-TOF, negative mode): 917.89 $[M-H]^-$; calcd for $C_{33}H_{47}O_{18}N_9P_2$ [M]: 919.72 (average).

4.16.2. $^{5'}$TTTTCTTXTCTTTTT$^{3'}$ (18). X=T^{g}: after purification by HPLC, mixture of two products; MS (MALDI-TOF, negative mode): 4507.98 $[M-H]^-$, 4466.11 $[M-CN_2H_3]^-$, 2554.67 $[M-2H]^{2-}$; calcd for $C_{149}H_{197}O_{100}N_{35}P_{14}$ [M]: 4511.97 (average); X=T^{mg}: 12% yield (synthesis+purification); reversed-phase HPLC t_R (gradient A): 12.9 min; MS (MALDI-TOF, negative mode): 4523.41 $[M-H]^-$, 2261.99 $[M-2H]^{2-}$; calcd for $C_{150}H_{199}O_{100}N_{35}P_{14}$ [M]: 4525.99, (average); X=T^{dmg}: 25% yield (synthesis+purification); reversed-phase HPLC t_R (gradient A): 12.9 min; MS (MALDI-TOF, negative mode): 4537.18 $[M-H]^-$, 2269.20 $[M-2H]^{2-}$; calcd for $C_{151}H_{201}O_{100}N_{35}P_{14}$ [M]: 4540.02 (average); X=T^{dmg2}: 22% yield (synthesis+purification); reversed-phase HPLC t_R (gradient A): 13.0 min; MS (MALDI-TOF, negative mode): 4536.43 $[M-H]^-$ 2269.09 $[M-2H]^{2-}$; calcd for $C_{151}H_{201}O_{100}N_{35}P_{14}$ [M]: 4540.02, (average).

4.16.3. $^{5'}$TTTTXTTXTXTTTTT$^{3'}$ (19). X=T^{g}: after purification by HPLC, mixture of products; MS (MALDI-TOF, negative mode): 4618.97 $[M-H]^-$, 4576.94 $[M-CN_2H_3]^-$, 4536.76 $[M-C_2N_4H_5]^-$, 4492.95 $[M-C_3N_6H_{14}]^-$; calcd for $C_{153}H_{205}O_{100}N_{39}P_{14}$ [M]: 4624.10 (average); X=T^{mg}: 8% yield (synthesis+purification); reversed-phase HPLC t_R (gradient A): 12.8 min; MS (MALDI-TOF, negative mode): 4661.98 $[M-H]^-$ 2334.13 $[M-2H]^{2-}$; calcd for $C_{156}H_{211}O_{100}N_{39}P_{14}$ [M]: 4666.18 (average); X=T^{dmg}: 16% yield (synthesis+purification); reversed-phase HPLC t_R (gradient A): 13.2 min; MS (MALDI-TOF, negative mode): 4705.14 $[M-H]^-$, 2354.38 $[M-2H]^{2-}$; calcd for $C_{159}H_{217}O_{100}N_{39}P_{14}$ [M]: 4708.26 (average); X=T^{dmg2}: 14% yield (synthesis+purification); reversed-phase HPLC t_R (gradient A): 13.1 min; MS (MALDI-TOF, negative mode): 4703.93 $[M-H]^-$, 2352.78 $[M-2H]^{2-}$; calcd for $C_{159}H_{217}O_{100}N_{39}P_{14}$ [M]: 4708.26 (average).

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References

1. Caruthers, M. H. *Science* **1985**, *230*, 281–285.
2. Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543–580.
3. Soyfer, V. N.; Potaman, V. N. *Triple-Helical Nucleic Acids*; Springer: New York, 1996.
4. (a) Soyfer, V. N.; Potaman, V.N. *Triple-Helical Nucleic Acids*; Springer: New York, 1996; pp 104–109. (b) Thuong, N. T.; Hélène, C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 666–690.
5. Here, triades are represented by Watson–Crick bound nucleobases into parenthesis and the third strand nucleobase on the left. C^+ refers to protonated cytosine (see Fig. 1).
6. Giovannangeli, C.; Hélène, C. *Antisense Nucleic Acids Drug Dev.* **1997**, *7*, 413–421.
7. Chan, P. P.; Glazer, P. M. *J. Mol. Med.* **1997**, *75*, 267–282.
8. Mergny, J. L.; Duval-Valentin, G.; Nguyen, C. H.; Perrouault, L.; Faucon, B.; Rougée, M.; Montenay-Garestier, T.; Nisagni, E.; Hélène, C. *Science* **1992**, *256*, 1681–1684.
9. Musso, M.; Thomas, T.; Shirahata, A.; Sigal, L. H.; van Dyke, M. W.; Thomas, T. J. *Biochemistry* **1997**, *36*, 1441–1449.
10. Nielsen, P. E. *Ann. Rev. Biophys. Biomol. Struct.* **1995**, *24*, 167–183.
11. Freier, S. M.; Altmann, K.-H. *Nucleic Acids Res.* **1997**, *25*, 4429–4443.
12. Luyten, I.; Herdewijn, P.; Eur *J. Med. Chem.* **1998**, *33*, 515–576.
13. Plum, G. E.; Park, Y. W.; Singleton, S. F.; Dervan, P. B.; Breslauer, K. *J. Proc. Natl. Acad. Sci. USA* **1990**, *87*, 9436–9440.
14. Asensio, J. L.; Lane, A. N.; Dhesi, J.; Bergqvist, S.; Brown, T. *J. Mol. Biol.* **1998**, *275*, 811–822.
15. Plum, G. E.; Breslauer, K. J. *J. Mol. Biol.* **1995**, *248*, 679–695.
16. (a) Krawczyk, S. H.; Milligan, J. F.; Wadwani, S.; Moulds, C.; Froehler, B. C.; Matteucci, M. D. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 3761–3764. (b) Miller, P. S.; Bhan, P.; Cushman, C. D.; Trapane, T. L. *Biochemistry* **1992**, *31*, 6788–6793.
17. Koh, J. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 1470–1478.
18. Koshlap, K. M.; Schultze, P.; Brunar, H.; Dervan, P. B.; Feigon, J. *Biochemistry* **1997**, *36*, 2659–2668.
19. Marfurt, J.; Parel, S. P.; Leumann, C. J. *Nucleic Acids Res.* **1997**, *25*, 1875–1882.
20. Ono, A.; Ts'o, P. O. P.; Kan, L.-S. *J. Org. Chem.* **1992**, *57*, 3225–3230.
21. Krosigk, U.; Benner, S.; A *J. Am. Chem. Soc.* **1995**, *117*, 5361–5362.
22. (a) Xiang, G.; Bogacki, R.; McLaughlin, L. W. *Nucleic Acids Res.* **1996**, *24*, 1963–1970. (b) Prasch, U.; Engels, J. W. *Chem. Eur. J.* **2000**, *6*, 2409–2424.
23. Bédu, E.; Benhida, R.; Devys, M.; Fourrey, J.-L. *Tetrahedron Lett.* **1999**, *40*, 835–838.
24. Lee, J. S.; Woodsworth, M. L.; Latimer, L. J. P.; Morgan, A. R. *Nucleic Acids Res.* **1984**, *12*, 6603–6614.
25. (a) Bates, P. J.; Laughton, C. A.; Jenkins, T. C.; Capaldi, D. C.; Roselt, P. D.; Reese, C. B.; Neidle, S. *Nucleic Acids Res.* **1996**, *24*, 4176–4184. (b) Cassidy, S. A.; Slickers, P.; Trent, J. O.; Capaldi, D. C.; Roselt, P. D.; Reese, C. B.; Neidle, S.; Fox, K. R. *Nucleic Acids Res.* **1997**, *25*, 4891–4898.
26. Rajeev, K. G.; Jadhav, V. R.; Ganesh, K. N. *Nucleic Acids Res.* **1997**, *25*, 4187–4193.
27. Ueda, T. In *Chemistry of Nucleosides and Nucleotides*, Townsend, T., Ed.; Plenum: New York, 1988; Vol. 1, pp 41–46.
28. Doronina, S. O.; Behr, J.-P. *Chem. Soc. Rev.* **1997**, 63–71.
29. Doronina, S. O.; Behr, J.-P. *Tetrahedron Lett.* **1998**, *39*, 547–550.
30. (a) Blanalt-Feidt, S.; Doronina, S. O.; Behr, J. P. *Nucleosides Nucleotides* **1999**, *18*, 605–606. (b) Doronina, S. O.; Blanalt-Feidt, S.; Behr, J. P. *Nucleosides Nucleotides* **1999**, *18*, 1617–1618.
31. Blanalt-Feidt, S.; Doronina, S. O.; Behr, J.-P. *Tetrahedron Lett.* **1999**, *40*, 6229–6232.
32. MacMillan, A. M.; Verdine, G. L. *Tetrahedron* **1991**, *47*, 2603–2616.

33. Xu, Y.-Z.; Zheng, Q.; Swann, P. F. *J. Org. Chem.* **1992**, *57*, 3839–3845.
34. Miah, A.; Reese, C. B.; Song, Q. *Nucleosides Nucleotides* **1997**, *16*, 53–65.
35. Allerson, C. R.; Chen, S. L.; Verdine, G. L. *J. Am. Chem. Soc.* **1997**, *119*, 7423–7433.
36. Shaw, J.-P.; Milligan, J. F.; Krawczyk, S. H.; Matteucci, M. *J. Am. Chem. Soc.* **1991**, *113*, 7765–7766.
37. Froehler, B. C. Methods in Molecular Biology. In *Protocols for Oligonucleotides and Analogs—Synthesis and Properties*, Agrawal, S., Ed.; Humana: Totowa, 1993; Vol. 26, pp 63–80.
38. Robles, J.; Beltrán, M.; Marchán, V.; Pérez, Y.; Travesset, I.; Pedroso, E.; Grandas, A. *Tetrahedron* **1999**, *55*, 13251–13264.
39. Reese, C.; Skone, P. A. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1263–1271.
40. Robles, J.; Pedroso, E.; Grandas, A. *Nucleic Acids Res.* **1995**, *23*, 4151–4161.
41. (a) Kung, P.-P.; Jones, R. A. *Tetrahedron Lett.* **1992**, *33*, 5869–5872. (b) Wada, T.; Sato, Y.; Honda, F.; Kawahara, S.-I.; Sekine, M. *J. Am. Chem. Soc.* **1997**, *119*, 12710–12721. (c) Wada, T.; Honda, F.; Sato, Y.; Sekine, M. *Tetrahedron Lett.* **1999**, *40*, 915–918.
42. Jankowska, J.; Sobkowski, M.; Stawinski, J.; Kraszewski, A. *Tetrahedron Lett.* **1994**, *35*, 3355–3358.
43. Browne, E. J. *Aust. J. Chem.* **1969**, *22*, 2251–2255.
44. Kröger, C. F. *Chem. Ber.* **1967**, *100*, 2250–2257.
45. Mold, J. D.; Ladino, J. M.; Schantz, E.; J *J. Am. Chem. Soc.* **1953**, *75*, 6321–6322.
46. Beaucage, S. L. Methods in Molecular Biology. In *Protocols for Oligonucleotides and Analogs—Synthesis and Properties*, Agrawal, S., Ed.; Humana: Totowa, 1993; Vol. 26, pp 33–61.