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6-Aza-2′-deoxyisocytidine: Synthesis, Properties of Oligonucleotides, and Base-Pair Stability Adjustment of DNA with Parallel Strand Orientation

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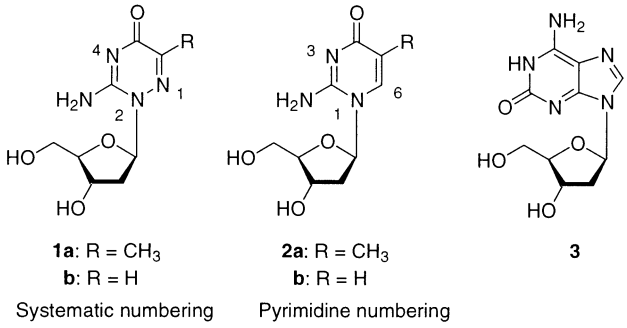
6-Aza-5-methyl-2′-deoxyisocytidine (**1a**) and 6-aza-2′-deoxyisocytidine (**1b**) have been synthesized, converted into phosphoramidite building blocks, and incorporated into oligodeoxynucleotides. The glycosylic bond stability of **1a**,**b** under acidic conditions increases compared to that of 5-methyl-2′-deoxyisocytidine (**2a**) and 2′-deoxyisocytidine (**2b**). Oligonucleotides incorporating **1a** or **1b** show an enhanced stability against the 3′-exonuclease snake-venom phosphodiesterase. The duplexes containing 6-azapyrimidine nucleosides **1a** or **1b** have lower T_m values than duplexes containing **2a** or **2b** with either antiparallel or parallel chain orientation. This was used to adjust the stability of the tridentate m^5iC_d-dG base pair to the level of the bidentate reverse Watson−Crick dA-dT pair.

Introduction

The hybridization of nucleic acids is commonly performed under the formation of duplexes with antiparallel chain orientation. The reversal of the strand orientation—from antiparallel (aps) to parallel (ps)—was originally accomplished by the selection of particular sequences of oligonucleotides but was limited to oligonucleotides with dA-dT base pairs.[1,2] A general way which allows the formation of parallel-stranded hybrids incorporating the four canonical DNA constituents in one strand requires the structural manipulations of the monomeric nucleic acid components of the second one. The configurational change at the anomeric center from β-D to α-D[3] or the transposition of the nucleobase substituents leading from dG to iG_d (**3**) (Scheme 1) or from dC to m^5iC_d[4−6] are established protocols to accomplish this objective. Recently, the structure of such a ps-DNA containing iG_d-dC as well as m^5iC_d-dG base pairs was studied by NMR spectroscopy.[7] Such a parallel hybridization offers advantages over the naturally occurring antiparallel-stranded hybrids: Oligonucleotides become resistant against various enzymes, and reporter groups used for diagnostic purposes can be located in positions which are otherwise difficult to access.

SCHEME 1



In the case of aps-DNA the tridentate dG-dC base pair is significantly more stable than the bidentate dA-dT pair. Thus, duplexes being rich in dG-dC pairs show increased stability. As a consequence oligonucleotides containing base mismatches can be more stable than perfectly matching duplexes containing mainly dA-dT pairs. This problem obscures sequence data obtained from hybridization experiments with oligonucleotide libraries which are performed in solution or on a solid support (biochips). Thus, it is desirable to destabilize dG-dC-rich duplexes and/or to increase the stability of dA-dT-rich duplexes while maintaining the sequence specificity of the hybridization. This is accomplished by replacing one or more of the native nucleosides in the

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probe (or the target) with modified, nonstandard nucleosides. The substitution of guanine residues by 7-deazaguanine, for example, will generally destabilize duplexes, whereas substituting adenine residues with 7-halogenated 8-aza-7-deazapurine-2,6-diamines will enhance duplex stability.[8,9] The incorporation of 6-azapyrimidine nucleoside analogues into oligonucleotide probes generally decreases their binding affinity for complementary nucleic acids.[10]

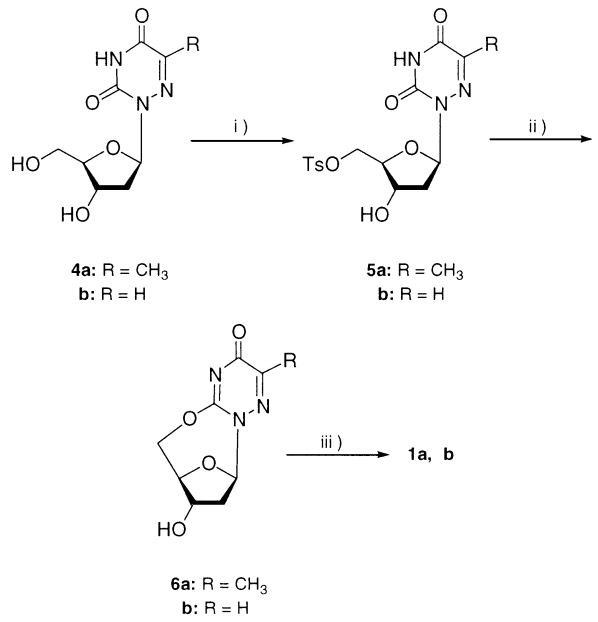
The same problem appears in parallel-stranded DNA. For instance, the PM3 semiempirical calculation in the gas phase suggested that the tridentate reverse Watson−Crick base pairs of both m^5iC_d-dG ($\Delta G° = -12.0$ kcal/mol) and iG_d-dC ($\Delta G° = -13.7$ kcal/mol) are more stable than the bidentate reverse Watson−Crick dA-dT base pair ($\Delta G° = -4.6$ kcal/mol) in parallel DNA. This was underlined experimentally in various DNA duplexes showing this parallel strand orientation.[5,6] When parallel hybridization is employed with oligonucleotide libraries incorporating iG_d-dC or m^5iC_d-dG base pairs, it is desirable to search for the stabilization of the dA-dT pair or to destabilize the iG_d-dC (or m^5iC_d-dG) pair to the same level of free energy. Thus, efforts were undertaken to harmonize the tridentate and bidentate base pair stabilities in these cases.

The stabilization of the reverse Watson−Crick dA-dT base pairs in ps-DNA has already been investigated by using base-modified analogues which are able to retain the base pair recognition selectivity of the dA residue. The stability of the parallel-stranded DNA duplex is increased by incorporation of the 7-substituted 7-deaza-2′-deoxyadenosine derivatives instead of dA.[11] Studies on the iG_d-dC base pair stability have also been performed using 7-deaza-2′-deoxyisoguanosine instead of 2′-deoxyisoguanosine.[12] However, a significant destabilization of the tridentate base pair was not accomplished. In this paper we investigate the adjustment of the stability of the tridentate m^5iC_d-dG base pair to that of the reverse Watson−Crick dA-dT pair. For this purpose the pyrimidine nucleoside m^5iC_d (**2a**) or iC_d (**2b**) is replaced by 6-aza-5-methyl-2′-deoxyisocytidine (**1a**) or 6-aza-2′-deoxyisocytidine (**1b**) (Scheme 1). Likewise, the destabilization of the iG_d-m^5iC_d pair formed in antiparallel DNA will be studied for comparison. The resistance of oligonucleotides containing **1a** and **1b** against degradation by exonucleases, e.g., snake-venom phosphodiesterase, will be reported as well.

Results and Discussion

1. Monomers. Both **1a** ($m^5z^6iC_d$) and **1b** (z^6iC_d) (pyrimidine numbering is used throughout the text; the systematic numbering is used in the Experimental Section) were synthesized from 6-aza-2′-deoxythymidine (**4a**) or 6-aza-2′-deoxyuridine (**4b**) by chemical transformation of their nucleobases (Scheme 2). Similar work has already been performed on 5-methyl-2′-deoxyisocytidine (**2a**) and its desmethyl derivative **2b**.[13−15] The starting materials,

SCHEME 2[a]



[a] Reagents and conditions: (i) TsCl, pyridine, rt, 70% for **5a**, 43% for **5b**; (ii) DBU, MeCN, reflux, 47% for **6a**, 50% for **6b**; (iii) NH_3/MeOH, rt, 80% for **1a**, 75% for **1b**.

compounds **4a**,**b**, were prepared by the stereoselective glycosylation of silylated 6-azathymine or 6-azauracil with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride in the presence of CuI.[16,17] The toluoylated β-D-nucleosides were isolated as major components. The β-D-configuration was assigned according to small ^{1}H NMR chemical shift differences between the 4′ and 5′ proton signals.[18] After detoluoylation, compounds **4a** and **4b** were obtained in 77% and 83% yield, respectively. For these nucleosides, the assignment of the glycosylation position was made according to Hall[19] on the basis of UV data. Both nucleosides show a hypsochromic shift of the UV maxima in alkaline medium compared to a neutral molecule. We have also performed ^{1}H NMR NOE experiments for an unambiguous assignment of the anomeric configuration.[20] Irradiation of H−C(1′) resulting in NOEs on H−C(4′) of 3.0% (**4a**) and 1.3% (**4b**) confirms the configurational assignment.

Next, the tosylation of **4a** and **4b** was performed to yield the 5′-*O*-*p*-toluoylsulfonyl derivatives **5a** and **5b** selectively in 70% and 43% yield. The 2,5′-anhydronucleosides **6a** and **6b** are then formed by refluxing **5a** and **5b** with DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) in anhydrous acetonitrile. Ammonolysis of **6a** and **6b** furnished **1a** (80% yield) and **1b** (75% yield). This reaction

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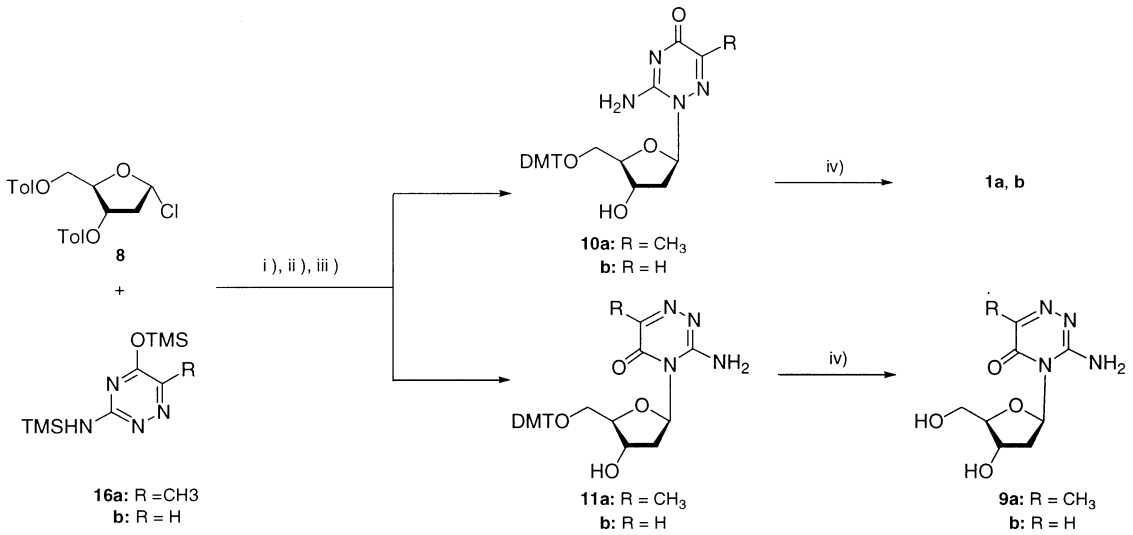
SCHEME 3[a]



[a] Reagents and conditions: (i) CuI, $CHCl_3$, rt; (ii) 0.2 M NaOMe, rt; (iii) DMTCl, pyridine, rt; (iv) 2.5% DCA in CH_2Cl_2, rt.

TABLE 1. Half-Life Values of 1a and Derivatives Determined in Acidic and Alkaline Media

	half-life (τ, min)			half-life (τ, min)	
compd	0.1 M HCl[a]	25% aqueous NH_3[b]	compd	0.1 M HCl[a]	25% aqueous NH_3[b]
1a	250	stable within 16 h at 25 °C	**12a**	>440	4 min at 25 °C
1b	75	stable within 16 h at 25 °C	**12b**	210	3 min at 25 °C
2a[5]	17	stable within 16 h at 60 °C	dmf^2iC_d[23]	16	5 min at 25 °C
2b[23]	5	stable within 16 h at 25 °C	$dmf^2m^5iC_d$[5]	28	7 min at 40 °C

[a] Hydrolysis was followed UV-spectrophotometrically (235 nm) at 40 °C for **1a**,**b**, at 275 nm for **12a**, and at 280 nm for **12b**; the half-life values refer to the glycosylic bond cleavage. [b] Hydrolysis was followed at 275 nm for **12a** and 270 nm for **12b**; the half-life values refer to the cleavage of the protecting groups.

takes place much faster than in the case of the 2,5′-anhydro derivatives of 2′-deoxy-5-methylisocytidine or its 5-desmethyl derivative.

Compounds **1a** and **1b** can also be synthesized by a convergent synthetic approach. The nucleobases 6-aza-5-methylisocytosine (**7a**) and 6-azaisocytosine (**7b**) were prepared from pyruvic acid and chloral, respectively, via cyclization of the corresponding guanylhydrazones.[21] Silylation of **7a** or **7b** according to Langer[22] with HMDS/TMSCl furnished **16a** or **16b**, which were used for the condensation with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride (**8**) in $CHCl_3$ with CuI as a catalyst (Scheme 3). Unlike the synthesis of **4a** and **4b**, where only the N(1) regioisomer is formed, the glycosylation of **16a** and **16b** furnished two regioisomers—the N(1) (**1a**,**b**) and N(3) (**9a**,**b**) compounds in nearly the same yield. The ratio of isomers was determined from the signal intensities of the anomeric protons in the ^{1}H NMR spectra. The formation of regioisomeric reaction products in this CuI-catalyzed reaction was also observed earlier upon the glycosylation of 5,6-dimethyluracil.[16]

As both regioisomers show almost identical R_f values, it was impossible to separate them chromatographically on the stage of the toluoylated or the free nucleosides (**1a**,**b** or **9a**,**b**) (Scheme 3). However, when they were derivatized by the DMT (4,4′-dimethoxytriphenylmethyl) residue on 5′-OH, the N(1) isomers **10a**,**b** were separated from the N(3) isomers **11a**,**b** by column chromatography. Removal of the DMT groups with 2.5% dichloroacetic acid (in CH_2Cl_2) afforded compounds **1a**,**b** or **9a**,**b**. The N(1) isomers **1a**,**b** were found to be identical with those obtained from the above-mentioned nucleoside transformation. For the other isomers, glycosylation at N(6) is unlikely, since it would lead to either a positively charged quaternary nitrogen atom or a nonaromatic ring system which should give rather different spectroscopic characteristics. Therefore, the structures of compounds **9a** and **9b** were suggested to be the N(3) isomers and were confirmed by NMR spectroscopy. The β-D-configuration of the anomeric centers of **9a**,**b** was established by ^{1}H NMR NOE experiments. Irradiation of H−C(1′) resulted in NOEs on H−C(4′) (η = 1.5% for **9a** and η = 1.3% for **9b**).

Then, the stability of the nucleosides **1a**,**b** was studied under acidic and alkaline conditions. This study was undertaken as it was found that the parent 2′-deoxyisocytidine nucleosides **2a** and **2b** are very labile in acidic and alkaline media, which are used in oligonucleotide synthesis and during the workup conditions.[23−25] At first, the hydrolysis was performed in 0.1 M HCl (40 °C). The reaction was followed by RP18-HPLC as well as UV-

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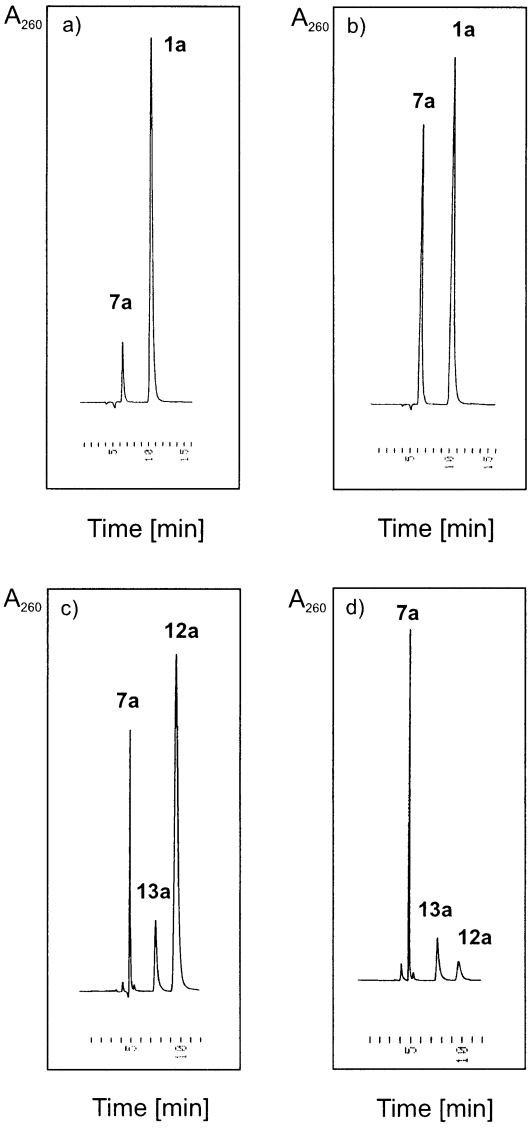

FIGURE 1. Reversed-phase HPLC (250 × 4 mm RP-18 column) profiles of the hydrolysis products of **1a** and **12a** in 0.1 M HCl at 40 °C. Samples were taken at the times indicated. Hydrolysis of **1a** for (a) 90 min and (b) for 4 h. Buffer A: 5% MeCN in 0.1 M (Et_3NH)OAc, pH 7.0, 0.6 mL/min. Hydrolysis of **12a** (c) for 4 h and (d) for 20 h. Buffer: 10% MeCN in buffer A, 0.6 mL/min.

spectrophotometrically. According to Figure 1a, compound **1a** is not significantly hydrolyzed within 90 min. After 4 h (Figure 1b), half of the educt is degraded by liberation of the base **7a**. Compound **1b** gave **7b** with a faster rate. According to the half-life values (Table 1), the presence of a nitrogen in position 6 of the pyrimidine ring increases the glycosylic bond stability toward acid by a factor of 15 compared to that of the parent compounds and the methyl group increases the stability 3 times further.

Next, the stability of **1a** and **1b** under alkaline conditions was studied. As shown in Figure 2a compound **1a** was deaminated to give **4a** under standard oligonucleotide deprotection conditions (25% aqueous NH_3, 60 °C, 16 h); the same reaction gave **4b** from **1b** with a faster rate. This shows that the 6-pyrimidine nitrogen (**1a**,**b**) increases the deamination velocity under alkaline conditions. However, when mild deprotection conditions (room temperature, 16 h) were applied, no deamination took place either on **1a** or on **1b** (Figure 2b).

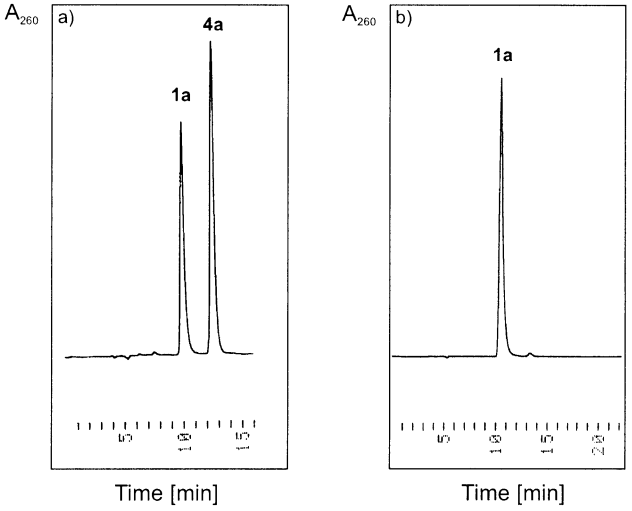

FIGURE 2. Reversed-phase HPLC (250 × 4 mm RP-18 column) profiles of the hydrolysis products of **1a** and **12a** in 25% aqueous NH_3. **1a** (a) at 60 °C for 16 h (for the buffer see Figure 1a) and (b) at room temperature for 16 h (for the buffer see Figure 1a).

In light of this instability problem the *N*,*N*-dimethylformamidine (dmf) protecting group was chosen for the amino group protection of **1a** or **1b**.[26a,b] This protecting group has already been used for the protection of other isocytidine derivatives[27] and 2′-deoxyisocytidine derivatives.[23] Treatment of **1a** and **1b** with dimethylformamide dimethyl acetal in methanol furnished the amidines **12a** and **12b** in almost quantitative yield (Scheme 4). The applicability of the amidines **12a** and **12b** was also investigated with regard to their stability under acidic and basic conditions. As shown in Figure 1c,d, the free base **7a** is the final product of the acidic hydrolysis of **12a**, and a small amount of dmf-protected 6-aza-5-methylisocytosine (**13a**) can be detected as an intermediate. Compound **12b** underwent the same reaction but with a faster rate. The acidic removal of the dmf group has been described previously.[5] According to Table 1, the dmf group increases the stability of the glycosylic bond of **1a**,**b** as described for other 2′-deoxyisocytidine derivatives, such as the dmf-protected 5-methyl-2′-deoxyisocytidine ($dmf^2m^5iC_d$) or dmf-protected 2′-deoxyisocytidine (dmf^2iC_d). Under the mild deprotection conditions the dmf group of **12a** or **12b** was removed to give **1a** or **1b** via an intermediate, which is expected to be the formyl derivative.[28] The half-lives of **12a** and **12b** were determined UV-spectrophotometrically to be 4 and 3 min (Table 1).

Compounds **12a** and **12b** were then converted into the DMT derivatives **14a** and **14b** (68%, 70%) by means of the standard conditions[29] (Scheme 4). The phosphor-

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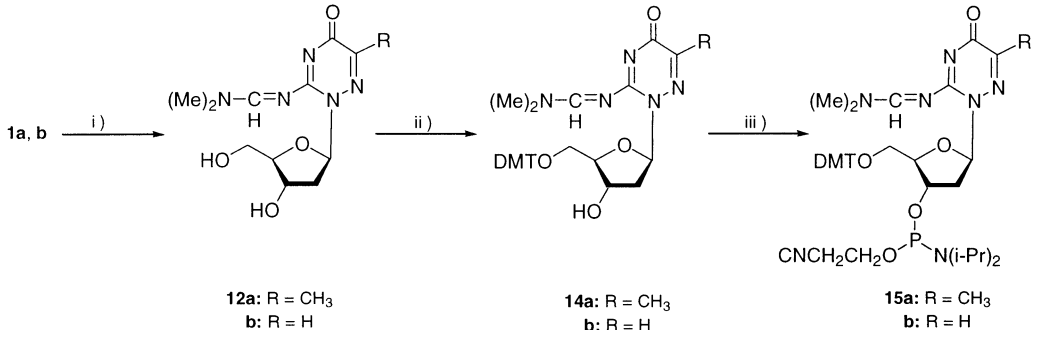
SCHEME 4[a]



[a] Reagents and conditions: (i) $Me_2NCH(OMe)_2$, MeOH, rt, 98% for **12a**, 97% for **12b**; (ii) DMTCl, pyridine, rt, 68% for **14a**, 70% for **14b**; (iii) $CN(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2EtN$, CH_2Cl_2, rt, 87% for **15a**, 85% for **15b**.

amidites **15a**,**b** were obtained after chromatographic workup. All monomeric compounds were characterized by ^1H, ^{13}C, and ^{31}P NMR spectroscopy as well as by UV spectroscopy and elemental analyses (see the Experimental Section). The ^{13}C NMR chemical shifts were assigned form the gated-decoupled ^{13}C NMR spectra.

2. Oligonucleotides. 2.1. Synthesis and Analysis by MALDI-TOF Mass Spectrometry. The oligonucleotide synthesis was performed on a solid phase employing standard phosphoramidite chemistry. The phosphoramidites **15a** and **15b** (from **1a** or **1b**) and also the others gave coupling yields higher than 95%. After the synthesis, the oligonucleotides **17**, **18**, **22**, **23**, **24**, and **31**−**37** (Tables 3 and 4) were deprotected in concd aqueous ammonia at 60 °C for 16 h. The oligonucleotides incorporating **1a**, **1b**, or **2b** (Tables 3 and 4) were deprotected in concd aqueous ammonia solution at room temperature (16 h). In these cases, the dA and dG phosphoramidites with labile 4-(*tert*-butylphenoxy)acetyl (tac) protecting groups were used. The 5′-DMT oligonucleotides were purified by RP18-HPLC (see the Experimental Section), and the DMT groups were removed with 2.5% $CHCl_2$-COOH in CH_2Cl_2 (5 min, room temperature). The detritylated oligomers were purified again, desalted by RP18-HPLC, and lyophilized. The homogeneity of the oligonucleotides was proven by both ion-exchange chromatography and reversed-phase HPLC, as well as by MALDI-TOF mass spectrometry (data see the Supporting Information). According to the increased stability of **1a**,**b** compared to **2a**,**b** toward acid, only the oligonucleotides containing **1a**,**b** gave the expected MH^+ peaks without forming degradation products. Oligonucleotides incorporating either compound **2a** or **2b** were partially degraded by the acidic matrix. Apart from the MH^+ peaks additional peaks which resulted from the stepwise "depyrimidination" were present as the result of the low N-glycosylic bond stability.

The base composition of oligonucleotides containing **1a** or **1b** was determined by complete enzymatic hydrolysis using snake-venom phosphodiesterase followed by alkaline phosphatase and subsequent reversed-phase HPLC chromatography. No deamination to 6-aza-2′-deoxythymidine or 6-aza-2′-deoxyuridine was observed. However, treatment under the regular conditions did not lead to a complete cleavage. Only an extended incubation (48 h instead of 45 min) led to complete digestion. Thus, the phosphodiester bond of the base-modified nucleotide residue is partially resistant against hydrolysis by the exonuclease. Also other cases were reported where other base-modified nucleosides retard the phosphodiester hydrolysis of oligonucleotides by exonucleases.[30] Therefore, the partial enzymatic hydrolysis of oligonucleotides containing **1a** or **1b** by 3′ → 5′ snake-venom phosphodiesterase was investigated by MALDI-TOF spectrometry. The digestion pattern of 5′-d(T **1a**ATAA**1a** T**1a 1a**TA) (**20**) (1.4 A_{260} units, SVP, 0.1 unit, in H_2O (25 μL) at 37 °C) is shown in Figure 3. From the reaction mixtures aliquots (1 μL) were taken after different time intervals for MALDI-TOF mass spectrometric analysis. Within 60 min the hydrolysis of **20** gave only three peaks corresponding to the full-length oligonucleotide (3626.7 Da), as well as the $n-1$ and $n-2$ oligomers (Figure 3a and Table 2). Upon excessive treatment (20 h), the first two peaks disappeared, and only the peak corresponding to 5′-d(T **1a**ATAA**1a** T**1a 1a**) (3006.6 Da) was detected (Figure 3b). After 25 h, the modified nucleotides were split off and a ladder pattern was obtained with peaks being in accordance with the sequence (Table 2). As the truncated oligonucleotides containing **1a** at the 3′-terminus (3006.6, 2703.0, and 2094.8 Da) have much higher intensities than the oligonucleotides with regular residues at the 3′-terminus, the resistance toward phosphodiester cleavage induced by the modified base is apparent (Figure 3c). It is not clear whether the change of the electronic properties of the base or their high-anti conformation affects the binding and the processing of the oligonucleotide. The phenomenon might be exploited

TABLE 2. MALDI-TOF Mass Spectrometric Data (Positive Mode) of an Oligonucleotide Digest Cleaved by Snake-Venom Phosphodiesterase[a]

Oligonucleotide	MH^+(Da) Calcd.	MH^+(Da) Found	ΔM(Da) Calcd.	ΔM(Da) Found
5′-d(T **1a**ATAA**1a** T**1a 1a**TA) (**20**)	3625.4	3626.7		
5′-d(T **1a**ATAA**1a** T**1a 1a**T)	3312.2	3314.2	313.2	312.5
5′-d(T **1a**ATAA**1a** T**1a 1a**)	3008.0	3006.6	304.2	307.6
5′-d(T **1a**ATAA**1a** T**1a**)	2703.8	2703.0	304.2	303.6
5′-d(T **1a**ATAA**1a** T)	2399.6	2399.0	304.2	304.0
5′-d(T **1a**ATAA**1a**)	2095.4	2094.8	304.2	304.2
5′-d(T **1a**ATAA)	1791.2	1791.3	304.2	303.5

[a] For details see Figure 3.

(30) Rosemeyer, H.; Ramzaeva, N.; Becker, E. M.; Feiling, E.; Seela, F. *Bioconjugate Chem.* **2002**, *13*, 1274−1285.

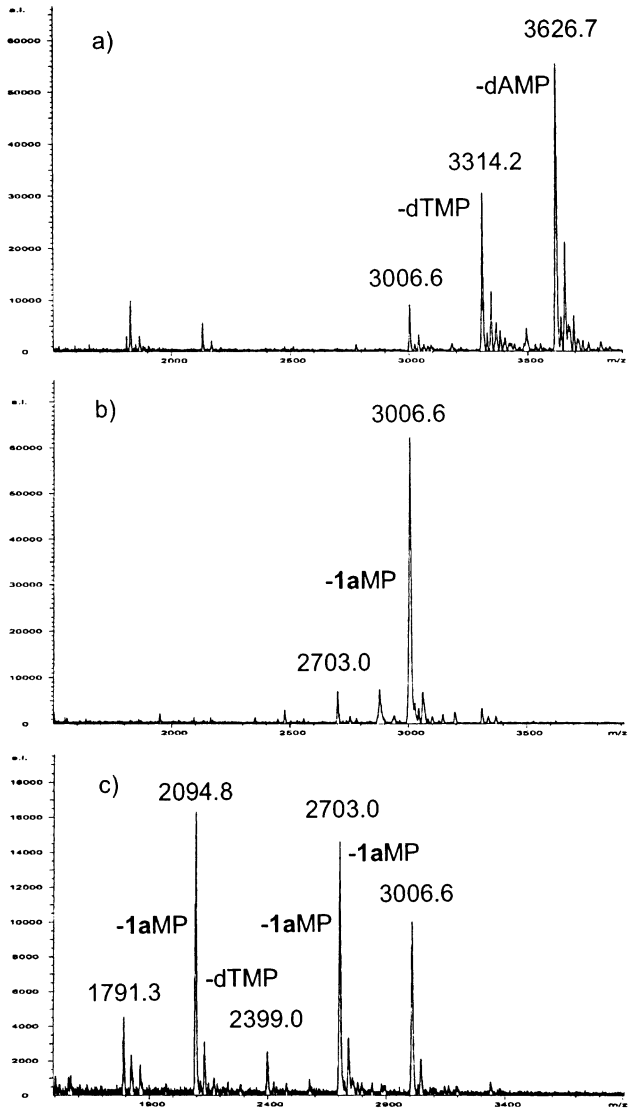
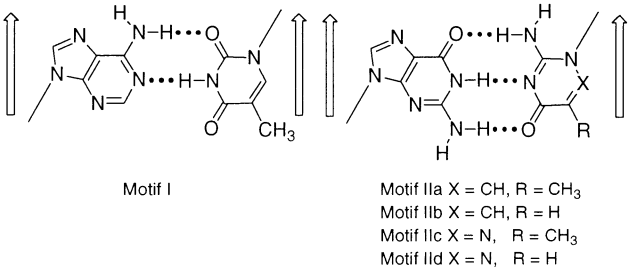


FIGURE 3. MALDI-TOF mass spectra (positive mode) taken from a partial enzymatic digest of the oligonucleotide **20** by snake-venom phosphodiesterase: (a) 60 min treatment, (b) 20 h treatment, and (c) 25 h treatment. 3-Hydroxypicolinic acid was used as matrix. For details see the Experimental Section.

to increase the stability of ps-DNA or aps-DNA in antisense technology.

2.2. Stability of the Duplexes. Very little has been reported on the influence of base-modified nucleosides on the base pair stability of parallel-stranded DNA.[31] As we have accomplished the harmonization of stability of a bidentate dA-dT base pair and the tridentate dG-dC pair,[8,9] we have now focused our interest to adjust the base pair stability of the reverse Watson−Crick dA-dT base pairs (motif I) to the m^5iC_d-dG pair (motif IIa) in parallel DNA. The stabilization of motif I was already studied with modified dA analogues incorporated in ps-duplexes. On the other hand, the destabilization of the tridentate iG_d-dC base pairs failed when iG_d analogues were used.[12] The observed destabilization of 6-azapyrimidine nucleosides in aps-DNA[16] prompted us to investigate this modification in ps-DNA with the final aim to harmonize the m^5iC_d-dG base pair (motif IIa) stability to the level of a reverse Watson−Crick dA-dT pair (motif I). For this purpose, oligonucleotides were studied carrying the 6-azapyrimidine nucleoside analogue **1a** or **1b** instead of m^5iC_d or iC_d. They were hybridized in a parallel way with those containing dG opposite 6-azapyrimidine residues. The base pair of dG-$m^5z^6iC_d$ is shown in motif IIc.



Our studies were focused toward oligonucleotides containing m^5iC_d-dG base pairs apart from dA-dT pairs but not incorporating iG_d-dC pairs (Table 3). In one set of experiments only one strand was modified. As reference, the parallel duplex **36·37** was chosen incorporating only dA-dT pairs. The T_m difference between **36·37** and **22·18** is 24 °C, which corresponds to 6 °C for one **2a**-dG/dA-dT base pair replacement. The usage of the desmethylated residue **2b**, which is rather sensitive to acidic and alkaline conditions, reduces this difference to 20 °C (5 °C for one **2b**-dG/dA-dT pair substitution). A strong destabilization of the tridentate base pair is observed when 6-aza-5-methyl-2′-deoxyisocytidine (see **22·20**) replaces 5-methyl-2′-deoxyisocytidine (**22·18**). The T_m difference between duplex **22·20** and duplex **36·37** is now only 15 °C corresponding to about 3.5 °C for one **1a**-dG/dA-dT substitution and 10 °C in the case of the desmethylated duplexes (**22·21** vs **36·37**), which equals 2.5 °C per **1b**-dG/dA-dT base pair replacement. For comparison, the modified base residues were also incorporated into both strands. In such cases, the parallel duplex **31·33** was used as a reference. The ΔT_m is 19 °C between **23·24** and **31·33**, corresponding to 4.8 °C for the presence of one **2a**-dG base pair. In such cases, the replacement of **2a** by **1a** led to a $\Delta T_m = 10$ °C between duplexes **25·26** and **31·33**, which corresponds to 2.5 °C for one **1a**-dG/dA-dT base pair replacement. By removing the methyl groups, a further destabilization is observed ($\Delta T_m = 5$ °C between duplexes **27·28** and **31·33**), which equals only around 1 °C per **1b**-dG/dA-dT base pair replacement. The methyl effect is also shown by comparing the T_m values of the duplexes **25·26** with those of **25·28** or **27·26**.

The influence of the 6-azapyrimidine nucleosides was also studied in aps-DNA. Earlier, it was shown that the base pairs of iG_d-m^5iC_d or iG_d-iC_d (motif IVa,b) are more stable than the dG-dC pair (motif III).[5,25,32] Moreover, enzymatic incorporation of the base pair was accomplished.[33,34] The stability of the corresponding isoguanine-

(31) Seela, F.; He, Y. Parallel DNA: The application of base-modified nucleosides to control chain orientation. In *Modified Nucleosides, Synthesis and Applications*; Loakes, D., Ed.; Transworld Research Network: Trivandrum, Kerala, India, 2002; pp 57−85.

(32) Horn, T.; Chang, C.; Collins, M. L. *Tetrahedron Lett.* **1995**, *36*, 2033−2036.

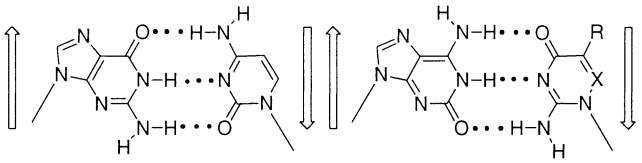
(33) Switzer, C. Y.; Moroney, S. E.; Benner, S. A. *Biochemistry* **1993**, *32*, 10489−10496.

TABLE 3. T_m Values and Thermodynamic Data of Parallel Duplexes[a]

Duplexes	T_m [°C]	ΔH° [kcal/mol]	ΔS° [cal/mol K]	$\Delta G°_{310}$ [kcal/mol]	ΔT_m [b] [°C]
5′-d(A A TATT A A A A AT) **36** 5′-d(T **T** ATAA **T** T **T** TTA) **37**	22	-45	-127	-5.6	
5′-d(A G TATT G A G G AT) **22** 5′-d(T **2a**ATAA**2a** T**2a** **2a**TA) **18**	46	-100	-287	-10.3	24
5′-d(A G TATT G A G G AT) **22** 5′-d(T **2b**ATAA**2b** T**2b** **2b**TA) **19**	42	-77	-219	-9.0	20
5′-d(A G TATT G A G G AT) **22** 5′-d(T **1a**ATAA**1a** T**1a** **1a**TA) **20**	37	-70	-200	-7.8	15
5′-d(A G TATT G A G G AT) **22** 5′-d(T **1b**ATAA**1b** T**1b** **1b**TA) **21**	32	-67	-195	-6.7	10
5′-d(TA A AT **T** A ATA**T**T) **31** 5′-d(AT **T T**A A T T ATAA)**33**	20	-53	-155	-4.8	
5′-d(TAG G T**2a**AATA**2a**T) **23** 5′-d(AT**2a2a**AG TTAT GA) **24**	39	-69	-195	-8.4	19
5′-d(TAG GT**1a**AATA**1a**T) **25** 5′-d(AT**1a1a**AGTT AT GA) **26**	30	-62	-179	-6.5	10
5′-d(TAG G T**1b**AATA**1b**T) **27** 5′-d(AT**1b1b**AGTTAT GA) **28**	25	-52	-149	-5.7	5
5′-d(TAG G T**1a**AATA**1a**T) **25** 5′-d(AT**1b1b**AGTTA T GA) **28**	28	-52	-148	-6.4	
5′-d(TAG G T**1b**AATA**1b**T) **27** 5′-d(AT**1a1a**AGTT AT GA) **26**	28	-61	-177	-6.0	

[a] Measured UV-spectrophotometrically at 260 nm in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM sodium cacodylate (pH 7.0). The oligonucleotide concentration is 5 μM + 5 μM. [b] Compared with corresponding duplex **31·33** or **36·37**.

isocytosine base pair incorporated in RNA fragments was investigated recently.[35] The pairing properties of pyranosyl RNA containing isoguanine or 5-methylisocytosine residues was investigated.[36]



Motif III

Motif IVa X = CH, R = CH_3
Motif IVb X = CH, R = H
Motif IVc X = N, R = CH_3
Motif IVd X = N, R = H

According to Table 4, a ΔT_m of −16 °C was observed between a duplex containing 4 dG-dC and 8 dA-dT base pairs (**29·30**) and that incorporating 12 dA-dT pairs (**31·32**). This corresponds to a 4 °C stability increase of the tridentate vs bidentate base pair. The related duplexes containing four iG_d-m^5iC_d pairs (motif IVa) instead of four dA-dT pairs (**17·18**) amounts to a ΔT_m = −23 °C in relation to the duplex **34·35**. For the related substitution of m^5iC_d with the 6-azapyrimidine nucleoside **1a** or **1b**,

(34) Piccirilli, J. A.; Krauch, T.; Moroney, S. E.; Benner, S. A. *Nature* **1990**, *343*, 33−37.
(35) Chen, X.; Kierzek, R.; Turner, D. H. *J. Am. Chem. Soc.* **2001**, *123*, 1267.
(36) Krishnamurthy, R.; Pitsch, S.; Minton, M.; Miculka, C.; Windhab, N.; Eschenmoser, A. *Angew. Chem.* **1996**, *108*, 1619−1623.

TABLE 4. T_m Values and Thermodynamic Data of Antiparallel Duplexes[a]

Duplexes	T_m [°C]	ΔH° [kcal/mol]	ΔS° [cal/mol K]	$\Delta G°_{310}$ [kcal/mol]	ΔT_m [b] [°C]
5′-d(TA**AATT**AATA**TT**) **31** 3′-d(AT**TT**A**A**TT AT**AA**)**32**	35	-67	-192	-7.4	
5′-d(TA**GGTC**AATA**CT**) **29** 3′-d(AT**CC**A**G**TTAT**G**A) **30**	51	-91	-256	-11.4	16
5′-d(TA **A A** A **A** TT AT **A** A)**34** 3′-d(AT **T T** T **T** AA TA **T** T) **35**	38	-80	-230	-8.2	
5′-d(TA **3 3** A **3** TTAT **3** A) **17** 3′-d(AT **2a 2a**T**2a**AATA**2a**T) **18**	61	-104	-286	-15.6	23
5′-d(TA **3 3** A **3** TTAT **3** A) **17** 3′-d(AT**2b 2b**T**2b**AATA**2b**T) **19**	57	-88	-241	-13.2	19
5′-d(TA **3 3** A **3** TTAT **3** A) **17** 3′-d(AT **1a 1a**T**1a**AATA**1a**T) **20**	50	-89	-251	-11.5	12
5′-d(TA **3 3** A **3** TTAT **3** A) **17** 3′-d(AT **1b 1b**T**1b**AATA**1b**T) **21**	45	-90	-257	-10.1	7

[a,b] See Table 3.

the T_m difference between tridentate- and bidentate-pair-rich duplexes can be reduced to 12 °C (motif IVa → motif IVc) and 7 °C (motif IVa → motif IVd), respectively. Thus, the 6-azapyrimidine nucleosides **1a** and **1b** reduce the stability of base pairs in ps- and aps-DNA. While an adjustment of the tridentate vs bidentate base pair stability was achieved by the 6-azapyrimidine nucleosides, a complete harmonization of the stability of the m^5iC_d-dG base pair to that of the reverse Watson−Crick base pair of dA-dT, however, was not fully accomplished. Further investigations with other modified nucleoside residues are in progress.

Conclusion

A nitrogen present in the 6-position of pyrimidine nucleosides such as in **1b** and its methyl derivative **1a** stabilizes the glycosylic bond against acidic degradation but leads to a faster deamination rate in alkaline medium. Oligonucleotides containing multiple incorporations of **1a** and **1b** residues can be synthesized when the 2-amino group is blocked with a (dimethylamino)methylidene residue and mild deprotection conditions are chosen. These oligonucleotides show an enhanced stability toward exonuclease, e.g., snake-venom phosphodiesterase. The 6-azapyrimidine nucleosides destabilize both aps- and ps-DNA. This allows the adjustment of the stability of oligonucleotides incorporating m^5iC_d-dG base pairs to that of oligonucleotides with reverse Watson−Crick dA-dT pairs. The adjustment is of particular importance for the parallel hybridization performed with oligonucleotide libraries in solution or on polymeric surfaces (biochips). Although the harmonization of the stability of the two base pairs formed in ps-DNA was not completely achieved, the use of the 6-azapyrimidine nucleosides **1a** and **1b** reduces the stability between the tridentate and bidentate pairs significantly.

Experimental Section

General Procedures. See ref 5. The solid-phase synthesis of oligonucleotides was carried out on an automated DNA

TABLE 5. ^{13}C NMR Chemical Shifts of Nucleosides and Derivatives[a]

	C(2)	C(4)	C(5)	C=N	Me	Me_2N	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	MeO
1a	155.6	162.5	146.9		16.9		88.2	37.2	70.5	87.6	62.0	
1b	155.2	162.3	139.4				88.3	37.1	70.3	87.6	61.8	
9a	155.4	162.5	147.0		17.0		87.4	37.0	69.7	86.1	60.9	
9b	155.1	162.3	139.5				87.6	37.0	69.7	86.1	60.8	
7a	156.5	163.6	146.1		16.7							
7b	156.1	163.4	139.0									
13a	160.3	164.1	148.1	158.9	16.9	34.7/40.8						
13b	160.2	164.1	140.5	159.1		34.8/40.9						
12a	159.0	162.9	148.5	158.8	17.2	35.1/41.0	86.3	37.6	70.9	87.4	62.5	
12b	159.0	162.8	140.7	158.9		35.1/41.0	86.3	37.4	70.7	87.3	62.3	
14a	158.9	162.8	148.3	157.9	16.8	35.1/41.0	86.8	b	71.4	85.7	65.0	54.8
14b	159.0	162.8	140.5	157.9		35.2/41.1	86.6	b	70.6	85.2	64.3	54.9
4a	148.8	156.6	143.4		16.3		87.2	36.9	70.6	84.5	62.2	
4b	148.2	156.5	136.0				87.1	36.8	70.4	84.6	62.0	
5a	148.4[c]	156.5[c]	144.9		16.1		85.0	37.4	70.7	83.3	70.3	
5b	147.8	156.4	136.0				85.1	37.1	70.5	83.2	70.0	
6a	157.8	163.0	148.2		16.7		87.6	37.0	70.3	86.6	62.0	
6b	157.1	163.0	140.3				87.6	36.9	70.1	87.0	61.8	
11a	158.0	162.5	144.8		17.0		87.3	37.1	70.4	84.2	63.5	54.9
10a	157.9	162.5	144.9		16.7		87.4	37.7	70.8	85.7	64.6	54.9
11b	158.0	162.3	139.7				87.5	37.3	70.4	84.2	63.5	55.0
10b	157.9	162.3	139.3				87.4	37.5	70.0	85.4	64.0	54.9

[a] Measured in d_6-DMSO at 25 °C. [b] Superimposed by d_6-DMSO. [c] Tentative.

synthesizer for phosphoramidite chemistry. All reagents are commercially available and were used as received. The solvents were purified and dried according to the standard procedures. Thin-layer chromatography (TLC) was performed on aluminum sheets of silica gel 60 F_{254} (0.2 mm). Flash chromatography (FC) was performed at 0.4 bar on silica gel 60 H. Reversed-phase HPLC was performed on a 4 × 250 mm RP-18 (10 μm) column with an HPLC pump, a variable-wavelength monitor, a controller, and an integrator. For UV spectra, λ_{max} is in nanometers and ϵ is in M^{-1} cm^{-1}; half-life values (τ) were measured with a UV spectrophotometer, connected with a temperature controller. Melting curves were determined using a UV/vis spectrophotometer equipped with a thermoelectrical controller. The calculation of thermodynamic data was performed with the program MeltWin (version 3.1)[37] using the curve fitting of the melting profiles according to a two-state model. The standard errors of thermodynamic data for $\Delta H°$ and $\Delta S°$ obtained from the curve fitting are within an error of ±15%. For NMR spectra, δ values are in parts per million downfield from the chemical shift of internal $SiMe_4$ (^{1}H, ^{13}C (Table 5)) or external 85% H_3PO_4 (^{31}P). MALDI-TOF mass spectra were recorded in the reflector mode. For details see ref 30.

Composition Analysis of Oligonucleotides. The oligonucleotides (0.2 A_{260} unit) were dissolved in 0.1 M Tris−HCl buffer (pH 8.3, 200 μL) and treated with snake-venom phosphodiesterase (EC 3.1.4.1, *Crotalus adamanteus*, 3 μL) at 37 °C for 45 min and then with alkaline phosphatase (EC 3.1.3.1, *E. coli*, 3 μL) at 37 °C for 30 min for the unmodified oligonucleotides. For oligonucleotides containing **1a**,**b**, the incubation time was extended to 48 h. The mixture was analyzed on reversed-phase HPLC (RP-18, gradient III, at 260 nm). Quantification of the constituents was made on the basis of the peak areas, which were divided by the extinction coefficients of the nucleosides (ϵ_{260}: dG, 11700; dT, 8800; dA, 15400; **1a**, 6100; **1b**, 5800).

MALDI-TOF Analysis of the Oligonucleotide Digests. The oligonucleotides (1.4 A_{260} units) were dissolved in H_2O to a total volume of 25 μL and treated with snake-venom phosphodiesterase (EC 3.1.4.1, *C. adamanteus*, 1 μL, 0.1 unit) at 37 °C. Aliquots of 1 μL of the enzymatic digests were analyzed by MALDI-TOF mass spectrometry.

(37) McDowell, J. A.; Turner, D. H. *Biochemistry* **1996**, *35*, 14077−14089.

Silylation of 6-Azauracil, 6-Azathymine, 6-Aza-5-methylisocytosine (7a), and 6-Azaisocytosine (7b). This general method described for the silylation of 6-azauracil was used for all the other heterocycles. A suspension of 6-azauracil (5.0 g, 44 mmol), HMDS (90 mL), and TMSCl (10 mL) was heated to reflux (170 °C) under anhydrous conditions for 3 h. The NH_4-Cl sublimed nearly quantitatively into the reflux condenser and was thus removed from the reaction mixture. The excess of HMDS/TMSCl was removed by distillation in vacuo. The residual oil solidified upon storage in vacuo to furnish a powder (11.0 g). The product was used directly without further purification in the following glycosylation reactions.

Glycosylation of Silylated 6-Aza-5-methylisocytosine (16a) with the Halogenose 8. To a stirred solution of compound **8** (13.1 g, 33.7 mmol) in dry $CHCl_3$ (400 mL) was added CuI (6.8 g, 35.7 mmol), and stirring was continued for 5 min at rt under exclusion of moisture. The silylated base **16a** (7.5 g, 27.7 mmol) was added to the solution, and the mixture was stirred for 3 h at rt. The reaction was quenched with saturated aqueous $NaHCO_3$ (400 mL). Stirring was continued for 15 min, and the two-phase mixture was filtered through a pad of Celite. The $CHCl_3$ layer was separated, washed with saturated aqueous NaCl (200 mL), dried (Na_2-SO_4), concentrated, and applied to an FC column (silica gel; column 5.5 × 10 cm; CH_2Cl_2/MeOH, 95:5). Isolation of the main zone afforded a colorless solid, which was a mixture of the N(1)/N(3)-isomers in a 1:1 ratio (determined from the anomeric proton signal). This regioisomeric mixture was deprotected with 0.2 M NaOMe (100 mL) at rt for 1 h. The solution was neutralized (Dowex-50, H^+-form) and filtered. The resin was washed with MeOH, and the combined filtrate was concentrated and applied to an FC column (silica gel; column 4.5 × 11 cm; CH_2Cl_2/ MeOH, 95:5 → 3:1). A colorless powder (2.9 g, 43%) of a **1a**/**9a** mixture was isolated which proved to be nonseparable.

3-Amino-4-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-6-methyl-1,2,4-triazin-5(4*H*)-one (11a) and 3-Amino-2-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-6-methyl-1,2,4-triazin-5(2*H*)-one (10a). The mixture of **1a**/**9a** (1.7 g, 7.0 mmol) was dried by coevaporation with anhydrous pyridine (3 × 10 mL) and dissolved in anhydrous pyridine (10 mL). Then, 4,4′-dimethoxytrityl chloride (4.1 g, 12.1 mmol) was added in two portions (every 10 min) at rt under stirring, and stirring was continued for 2 h. MeOH (5 mL) was added, and after 10 min the solution

was evaporated to dryness. The residue was dissolved in CH_2Cl_2 (35 mL), washed with 5% aqueous $NaHCO_3$ (2 × 15 mL) and H_2O (20 mL), and dried (Na_2SO_4). After evaporation a foam was formed which was redissolved in a small volume of CH_2Cl_2 and applied to an FC column (silica gel; column 4 × 12 cm; CH_2Cl_2/MeOH, 95:5).

From the faster migrating main zone compound **11a** was isolated. Colorless foam (880 mg, 23%). TLC (CH_2Cl_2/MeOH/Et_3N, 95:5:0.2): R_f 0.25. UV (MeOH): 234 (27300). ^{1}H NMR (d_6-DMSO): δ 2.08 (s, 3H); 2.34−2.51 (m, 2H); 3.00−3.19 (m, 2H); 3.74 (s, 6H); 4.07 (m, 2H); 5.33 (d, 1H); 6.05 (t, 1H); 6.88−7.40 (m, 15H). Anal. Calcd for $C_{30}H_{32}N_4O_6$ (544.6): C, 66.16; H, 5.92; N, 10.29. Found: C, 66.21; H, 5.80; N, 9.93.

The slower migrating zone furnished compound **10a**. Colorless foam (1.3 g, 34%). TLC (CH_2Cl_2/MeOH/Et_3N, 95:5:0.2): R_f 0.18. UV (MeOH): 234 (27900). ^{1}H NMR (d_6-DMSO): δ 1.79 (s, 3H); 2.11−2.57 (m, 2H); 3.02 (m, 2H); 3.72 (s, 6H); 3.94 (m, 1H); 4.32 (m, 1H); 5.28 (d, 1H); 6.14 (dd, 1H); 6.80−7.33 (m, 13H). 7.37(s, 2H). Anal. Calcd for $C_{30}H_{32}N_4O_6$ (544.6): C, 66.16; H, 5.92; N, 10.29. Found: C, 66.09; H, 5.86; N, 10.25.

Glycosylation of Silylated 6-Azaisocytosine (16b) with the Halogenose 8. Compound **16b** (10.6 g, 41.3 mmol) was treated as described for **16a** with **8** (13.0 g, 33.4 mmol) and CuI (7.6 g, 39.9 mmol). FC (silica gel; column 5.5 × 10 cm; CH_2Cl_2/MeOH, 95:5) furnished the toluoylated N(1)/N(3) mixture. It was detoluoylated as described above for **16a**. FC (silica gel; column 4.5 × 11 cm; CH_2Cl_2/MeOH, 9:1 → 3:1) furnished a mixture of **1b**/**9b** (4.3 g, 56%).

3-Amino-4-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-1,2,4-triazin-5(4*H*)-one (11b) and 3-amino-2-[2-deoxy-5-*O*-(4,4′-dimethoxytrityl)-*β*-D-*erythro*-pentofuranosyl]-1,2,4-triazin-5(2*H*)-one (10b). As described for **1a**/**9a**, the mixture of **1b**/**9b** (1.0 g, 4.2 mmol) was treated with 4,4′-dimethoxytrityl chloride (2.8 g, 8.3 mmol) in anhydrous pyridine (55 mL). It was worked up by FC (silica gel; column 4 × 12 cm; CH_2Cl_2/MeOH/Et_3N, 9:1:0.2).

From the faster migrating main zone compound **11b** was isolated. Colorless foam (500 mg, 22%). TLC (CH_2Cl_2/MeOH/Et_3N, 9:1:0.2): R_f 0.39. UV (MeOH): 234 (26500). ^{1}H NMR (d_6-DMSO): δ 2.34−2.58 (m, 2H); 2.94−3.19 (m, 2H); 3.74 (s, 6H); 4.04 (m, 2H); 5.33 (br s, 1H); 6.09 (t, 1H); 6.88−7.40 (m, 14H); 7.48 (s, 2H). Anal. Calcd for $C_{29}H_{30}N_4O_6$ (530.6): C, 65.65; H, 5.70; N, 10.56. Found: C, 65.41; H, 5.82; N, 10.55.

The slower migrating zone gave compound **10b**. Colorless foam (800 mg, 34%). TLC (CH_2Cl_2/MeOH/Et_3N, 95:5:0.2): R_f 0.35. UV (MeOH): 234 (25000). ^{1}H NMR (d_6-DMSO): δ 2.11−2.61 (m, 2H); 3.01 (m, 2H); 3.72 (s, 6H); 3.89 (m, 1H); 4.32 (m, 1H); 5.30 (d, 1H); 6.14 (dd, 1H); 6.80−7.37 (m, 13H); 7.56 (2H). Anal. Calcd for $C_{29}H_{30}N_4O_6$ (530.6): C, 65.65; H, 5.70; N, 10.56. Found: C, 65.25; H, 5.86; N, 10.25.

3-Amino-2-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-methyl-1,2,4-triazin-5(4*H*)-one (9a). A solution of **11a** (450 mg, 0.8 mmol) in CH_2Cl_2 (15 mL) was treated with 2.5% DCA in CH_2Cl_2 (5 mL) for 5 min at room temperature. When the DMT group was removed (TLC monitoring), it was neutralized with Et_3N. The solvent was evaporated to give an oily residue which was applied to an FC column (silica gel; column 4 × 7.5 cm; CH_2Cl_2/MeOH, 95:5 → 3:1). Compound **9a** was obtained as a colorless solid (200 mg, nearly quantative yield). TLC (CH_2Cl_2/MeOH, 3:1): R_f 0.55. UV (MeOH): 247 (7700). ^{1}H NMR (d_6-DMSO): δ 2.06 (s, 3H); 2.46 (m, 2H); 3.55 (m, 2H); 3.90 (m, 1H); 4.09 (m, 1H); 4.74 (t, 1H); 5.32 (d, 1H); 5.94 (t, 1H−C); 7.28 (s, 2H). Anal. Calcd for $C_9H_{14}N_4O_4$ (242.2): C, 44.63; H, 5.83; N, 23.13. Found: C, 44.56; H, 5.75; N, 23.20.

3-Amino-2-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-1,2,4-triazin-5(4*H*)-one (9b). Compound **11b** (500 mg, 0.9 mmol) in CH_2Cl_2 (15 mL) was treated with 2.5% DCA in CH_2Cl_2 as described for **11a**. FC (silica gel; column 2.2 × 10.5 cm; CH_2Cl_2/MeOH, 95:5 → 3:1) furnished **9b** as a colorless solid (140 mg, 65%). TLC (CH_2Cl_2/MeOH, 8:1): R_f 0.15. UV (MeOH): 254 (6200). ^{1}H NMR (d_6-DMSO): δ 2.04−2.56 (m, 2H); 3.34−3.43 (m, 2H); 3.86 (m, 1H); 4.09 (m, 1H); 4.74 (t, 1H); 5.30 (d, 1H); 5.98 (t, 1H); 7.40 (s, 1H); 7.43 (s, 2H). Anal. Calcd for $C_8H_{12}N_4O_4$ (228.2): C, 42.10; H, 5.30; N, 24.55. Found: C, 42.11; H, 5.35; N, 24.36.

2-(2-Deoxy-5-*O*-tosyl-*β*-D-*erythro*-pentofuranosyl)-6-methyl-1,2,4-triazin-3,5(2*H*,4*H*)-dione (5a). 6-Azathymidine (**4a**) (2.64 g, 10.9 mmol) was coevaporated with anhydrous pyridine (3 × 10 mL) and then dissolved in anhydrous pyridine (30 mL). To the solution was added *p*-toluenesulfonyl chloride (2.3 g, 12.1 mmol) at 0 °C, and the resulting solution was stirred at rt overnight. After being quenched with H_2O (25 mL), the solution was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 60 mL) and H_2O (2 × 60 mL), dried (Na_2SO_4), concentrated, and then applied to an FC column (silica gel; column 4.5 × 11 cm; CH_2Cl_2/MeOH, 9:1). Collection of the main fraction and evaporation gave compound **5a** as a colorless foam (3.0 g, 70%). TLC (CH_2Cl_2/MeOH, 9:1): R_f 0.42. UV (MeOH): 223 (14100), 264 (6000). ^{1}H NMR (d_6-DMSO): δ 1.98 (s, 3H); 2.09−2.30 (m, 2H); 2.38 (s, 3H); 3.88 (m, 2H); 4.16 (m, 1H); 4.28 (m, 1H); 5.38 (d, 1H); 6.27 (dd, 1H); 7.38−7.77(dd, 4H); 12.09 (s, 1H). Anal. Calcd for $C_{16}H_{19}N_3O_7S$ (397.4): C, 48.36; H, 4.82; N, 10.57. Found: C, 48.42; H, 4.62; N, 10.47.

2-(2-Deoxy-5-*O*-tosyl-*β*-D-*erythro*-pentofuranosyl)-1,2,4-triazin-3,5(2*H*,4*H*)-dione (5b). As described for **5a,** 6-aza-2′-deoxyuridine (**4b**) (2.8 g, 12.2 mmol) in anhydrous pyridine (35 mL) was treated with *p*-toluenesulfonyl chloride (3.1 g, 16.3 mmol). FC (silica gel; column 4.5 × 7 cm; CH_2Cl_2/MeOH, 8:1) yielded compound **5b** as a colorless foam (2.0 g, 43%). TLC (CH_2Cl_2/MeOH, 8:1): R_f 0.56. UV (MeOH): 224 (14100), 262 (6000). ^{1}H NMR (d_6-DMSO): δ 2.07−2.28 (m, 2H); 2.40 (s, 3H); 3.86 (m, 2H); 4.16 (m, 1H); 4.20 (m, 1H); 5.39 (d, 1H); 6.26 (dd, 1H); 7.38−7.77(dd, 5H); 12.20 (s, 1H). Anal. Calcd for $C_{15}H_{17}N_3O_7S$ (383.4): C, 46.99; H, 4.47; N, 10.96. Found: C, 47.00; H, 4.59; N, 11.02.

2,5′-Anhydro-6-azathymidine (6a). Compound **5a** (2.8 g, 7.0 mmol) was dissolved in dry MeCN (dried over 4 Å molecular sieves). DBU (1.9 mL, 12.9 mmol) was added, and the reaction mixture was refluxed at 120 °C for 1.5 h. Evaporation of the mixture gave an oil which was applied to an FC column (silica gel; column 2.5 × 12 cm; CH_2Cl_2/MeOH, 9:1). Trituration with acetone yielded **6a** as a colorless powder (760 mg, 47%). TLC (CH_2Cl_2/MeOH, 9:1): R_f 0.69. UV (MeOH): 228 (6700), 255 (6800). ^{1}H NMR (d_6-DMSO): δ 2.10−2.56 (m, 2H); 2.12 (s, 3H); 3.47−3.71(m, 2H); 4.28−4.67 (m, 2H); 5.25 (d, 1H); 6.25 (dd, 1H).

2,5′-Anhydro-6-aza-2′-deoxyuridine (6b). As described for **6a**, compound **5b** (1.8 g, 4.7 mmol) dissolved in dry MeCN was treated with DBU (1.3 mL, 8.5 mmol) for 1 h. FC (silica gel; column 4.3 × 9.5 cm; CH_2Cl_2/MeOH, 8:1) followed by precipitation from acetone afforded **6b** as a colorless powder (496 mg, 50%). TLC (CH_2Cl_2/MeOH, 8:1): R_f 0.33. UV (MeOH): 223 (8200), 257 (5600). ^{1}H NMR (d_6-DMSO): δ 2.10−2.52 (m, 2H); 3.32−3.45(m, 2H); 3.72 (m, 1H); 4.27 (m, 1H); 5.25 (d, 1H); 6.25 (dd, 1H); 7.66 (s, 1H).

3-Amino-2-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-6-methyl-1,2,4-triazin-5(2*H*)-one (1a). Method A. 6a (700 mg, 3.1 mmol) was dissolved in MeOH (100 mL) which was presaturated with NH_3 at 0°C. The solution was stirred in a sealed bottle at rt until the starting material had disappeared (20 h; TLC monitoring with CH_2Cl_2/MeOH, 3:1). The solvent was evaporated to dryness, and the residue was applied to an FC column (silica gel; column 2.5 × 12 cm; CH_2Cl_2/MeOH, 3:1). Evaporation of the main fraction gave compound **1a** as a colorless powder (600 mg, 80%). TLC (CH_2Cl_2/MeOH, 3:1): R_f 0.55. UV (MeOH): 247 (7300). ^{1}H NMR (d_6-DMSO): δ 2.03 (s, 3H); 2.04−2.56 (m, 2H); 3.48 (m, 2H); 3.74 (m, 1H); 4.29 (m, 1H); 4.85 (t, 1H); 5.24 (d, 1H); 6.05 (t, 1H); 7.29 (s, 2H). Anal. Calcd for $C_9H_{14}N_4O_4$ (242.2): C, 44.63; H, 5.83; N, 23.13. Found: C, 44.82; H, 5.99; N, 22.89.

Method B. Compound **10a** (600 mg, 1.1 mmol) was treated as described for **11a**. After purification by FC (silica gel; column 2.5 × 12 cm; CH_2Cl_2/MeOH, 9:1 → 3:1) compound **1a**

was obtained as a colorless powder (250 mg, 94%) showing the same analytical data as described above.

3-Amino-2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,2,4-triazin-5(2*H*)-one (1b). Method A. Compound **1b** was prepared as described for **1a** from **6b** (900 mg, 4.2 mmol) in MeOH (100 mL) which was presaturated with NH_3 at 0 °C. Colorless powder (719 mg, 75%). TLC (CH_2Cl_2/MeOH, 8:1): R_f 0.15. UV (MeOH): 253 (6300). ^{1}H NMR (d_6-DMSO): δ 2.04−2.56 (m, 2H); 3.45 (m, 2H); 3.74 (m, 1H); 4.26 (m, 1H); 4.86 (t, 1H); 5.26 (d, 1H); 6.06 (t, 1H); 7.34 (s, 1H); 7.46 (s, 2H). Anal. Calcd for $C_8H_{12}N_4O_4$ (228.2): C, 42.11; H, 5.30; N, 24.55. Found: C, 42.21; H, 5.40; N, 24.25.

Method B. Compound **10b** (500 mg, 0.9 mmol) was treated as described for **11b**. Purification by FC (silica gel; column 2.5 × 12 cm; CH_2Cl_2/MeOH, 9:1 → 3:1) afforded **1b** as a colorless powder (140 mg, 65%) showing analytical data identical to that described above.

3-[(*N*,*N*-Dimethylamino)methylidene]amino-6-methyl-1,2,4-triazin-5(2*H*)-one (13a). To a suspension of 6-aza-5-methylisocytosine[21] (126 mg, 1.0 mmol) in MeOH (20 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (0.3 mL, 2.3 mmol). The reaction mixture was stirred at 50 °C. After the starting material had disappeared (∼2 h; TLC monitoring with CH_2Cl_2/MeOH, 9:1), the mixture was evaporated to dryness. The resulting white powder was dissolved in CH_2Cl_2 and applied to an FC column (silica gel; column 2.5 × 12 cm; CH_2Cl_2/MeOH, 4:1). Compound **13a** was obtained as a colorless powder (180 mg, nearly quantitative yield). TLC (CH_2Cl_2/MeOH, 9:1): R_f 0.33. UV (MeOH): 272 (32000). ^{1}H NMR (d_6-DMSO): δ 2.05 (s, 3H), 3.04 (s, 3H); 3.17 (s, 3H); 8.65 (s, 1H); 12.61 (s, 1H). Anal. Calcd for $C_7H_{11}N_5O$ (181.2): C, 46.40; H, 6.12; N, 38.65. Found: C, 46.67; H, 6.25; N, 38.26.

3-[(*N*,*N*-Dimethylamino)methylidene]amino-1,2,4-triazin-5(2*H*)-one (13b). Compound **13b** was prepared as described for **13a** from 6-azaisocytosine (112 mg, 1.0 mmol) with *N*,*N*-dimethylformamide dimethyl acetal (0.3 mL, 2.3 mmol). Evaporation of the main fraction gave **13b** as a colorless powder (160 mg, 96%). TLC (CH_2Cl_2/MeOH, 9:1): R_f 0.21. UV (MeOH): 272 (29500). ^{1}H NMR (d_6-DMSO): δ 3.05 (s, 3H); 3.18 (s, 3H); 7.36 (s, 1H); 8.66 (s, 1H); 12.86 (s, 1H). Anal. Calcd for $C_6H_9N_5O$ (167.2): C, 43.11; H, 5.43; N, 41.89. Found: C, 43.25; H, 5.33; N, 41.72.

2-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-[(*N*,*N*-dimethylamino)methylidene]amino-6-methyl-1,2,4-triazin-5(2*H*)-one (12a). To a suspension of compound **1a** (400 mg, 1.7 mmol) in MeOH (35 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (0.6 mL, 4.5 mmol). The reaction mixture was stirred at rt for ∼2 h (TLC monitoring with CH_2Cl_2/MeOH, 9:1). The reaction mixture was evaporated to dryness and the residue applied to an FC column (silica gel; column 4.5 × 5 cm; CH_2Cl_2/MeOH, 4:1). Evaporation of the main fraction gave compound **12a** as a colorless powder (483 mg, 98%). TLC (CH_2Cl_2/MeOH, 9:1): R_f 0.33. UV (MeOH): 276 (31200). ^{1}H NMR (d_6-DMSO): δ 2.05−2.43 (m, 2H); 2.07 (s, 3H); 3.08 (s, 3H); 3.20 (s, 3H); 3.50 (m, 2H); 3.71 (m, 1H); 4.31 (m, 1H); 4.68 (t, 1H); 5.19 (d, 1H); 6.86 (t, 1H); 8.61 (s, 1H). Anal. Calcd for $C_{12}H_{19}N_5O_4$ (297.3): C, 48.48; H, 6.44; N, 23.56. Found: C, 48.31; H, 6.39; N, 23.37.

2-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-3-[(*N*,*N*-dimethylamino)methylidene]amino-1,2,4- triazin-5(2*H*)-one (12b). As described for **12a**, compound **12b** was obtained from **1b** (100 mg, 0.4 mmol) by reaction with *N*,*N*-dimethylformamide dimethyl acetal (0.16 mL, 1.2 mmol). Colorless powder (120 mg, 97%). TLC (CH_2Cl_2/MeOH, 9:1): R_f 0.23. UV (MeOH): 276 (27000). ^{1}H NMR (d_6-DMSO): δ 2.08−2.47 (m, 2H); 3.09 (s, 3H); 3.22 (s, 3H); 3.47 (m, 2H); 3.70 (m, 1H); 4.27 (m, 1H); 4.69 (t, 1H); 5.21 (d, 1H); 6.86 (t, 1H); 7.51 (s, 1H); 8.62 (s, 1H). Anal. Calcd for $C_{11}H_{17}N_5O_4$ (283.3): C, 46.64; H, 6.05; N, 24.72. Found: C, 46.65; H, 6.04; N, 24.64.

2-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-3-[(*N*,*N*-dimethylamino)methylidene]amino-6-methyl-1,2,4-triazin-5(2*H*)-one (14a). Compound **12a** (422 mg, 1.42 mmol) was dried by coevaporation with anhydrous pyridine (3 × 10 mL) and then dissolved in anhydrous pyridine (10 mL). 4,4′-Dimethoxytrityl chloride (673 mg, 2.0 mmol) was added in two portions (every 10 min) at rt under stirring. The mixture was stirred for a further 2 h. After addition of MeOH (5 mL), the reaction mixture was evaporated and the residue dissolved in CH_2Cl_2 (35 mL). The solution was washed with 5% aqueous $NaHCO_3$ (2 × 15 mL) and H_2O (20 mL) and dried (Na_2SO_4), and the organic layer was evaporated to give a colorless foam. This was applied to an FC column (silica gel, column 4 × 12 cm). After the column was washed with CH_2Cl_2/acetone/Et_3N (70:30:0.5), compound **14a** was eluted with CH_2Cl_2/MeOH/Et_3N (90:10:0.1) to give a colorless foam (580 mg, 68%). TLC (CH_2Cl_2/MeOH/Et_3N, 90:10:0.1): R_f 0.46. UV (MeOH): 236 (33600), 275 (36300). ^{1}H NMR (d_6-DMSO): δ 1.78 (s, 3H); 2.12−2.40 (m, 2H); 3.11 (s, 3H); 3.21 (s, 3H); 3.02 (m, 2H); 3.72 (s, 6H); 3.94 (m, 1H); 4.32 (m, 1H); 5.25 (d, 1H); 6.94 (t, 1H); 6.82−7.59 (m, 14H); 8.68 (s, 1H). Anal. Calcd for $C_{33}H_{37}N_5O_6$ (599.7): C, 66.09; H, 6.22; N, 11.68. Found: C, 66.36; H, 6.31; N, 11.64.

2-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-3-[(*N*,*N*-dimethylamino)methylidene]amino-1,2,4-triazin-5(2*H*)-one (14b). Compound **14b** was prepared as described for **14a** with compound **12b** (330 mg, 1.2 mmol) and 4,4′-dimethoxytrityl chloride (673 mg, 2.0 mmol). This compound was obtained as a colorless foam after FC (silica gel; column 4 × 12 cm; CH_2Cl_2/acetone/Et_3N, 70:30:0.5 → CH_2Cl_2/MeOH/Et_3N, 90:10:0.1) (480 mg, 70%). TLC (CH_2Cl_2/MeOH/Et_3N, 90:10:0.1): R_f 0.46. UV (MeOH): 235 (34900), 276 (34900). ^{1}H NMR (d_6-DMSO): δ 2.14−2.42 (m, 2H); 3.13 (s, 3H); 3.23 (s, 3H); 3.02 (m, 2H); 3.72 (s, 6H); 3.85 (m, 1H); 4.33 (m, 1H); 5.25 (d, 1H); 6.91 (t, 1H); 6.82−7.37 (m, 13H); 8.68 (s, 1H). Anal. Calcd for $C_{32}H_{35}N_5O_6$ (585.7): C, 65.63; H, 6.02; N, 11.96. Found: C, 65.56; H, 6.10; N, 11.89.

2-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-3-[(*N*,*N*-dimethylamino)methylidene]amino-6-methyl-1,2,4-triazin-5(2*H*)-one 3′-*O*-(2-Cyanoethyl diisopropylphosphoramidite) (15a). To a solution of compound **14a** (590 mg, 1.0 mmol) in dry CH_2Cl_2 (15 mL) was added 2-cyanoethyl diisopropylphosphoramidochloridite (0.3 mL 1.3 mmol) together with *N*-ethyldiisopropylamine (0.3 mL, 1.7 mmol) under argon at rt. The mixture was stirred for 20 min at room temperature. Then, 5% aqueous $NaHCO_3$ (15 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 15 mL). The extract was dried (Na_2SO_4) and evaporated to an oil. This residue was applied to an FC column (silica gel; column 4 × 7.5 cm; CH_2Cl_2/MeOH/Et_3N, 95:5:0.2), giving compound **15a** as a colorless foam (680 mg, 87%). TLC (CH_2Cl_2/MeOH/Et_3N, 95:5:0.2): R_f 0.54. UV (MeOH): 235 (30800), 276 (30700). ^{31}P NMR ($CDCl_3$): δ 149.3, 149.7.

2-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-3-[(*N*,N-dimethylamino)methylidene]amino-1,2,4-triazin-5(2*H*)-one 3′-*O*-(2-Cyanoethyl diisopropylphosphoramidite) (15b). Compound **14b** (350 mg, 0.6 mmol) in dry CH_2Cl_2 (15 mL) was treated with 2-cyanoethyl diisopropylphosphoramidochloridite (0.15 mL, 0.7 mmol) together with *N*-ethyldiisopropylamine (0.15 mL, 0.86 mmol) as described for **14a**. FC furnished the title compound as a foam (400 mg, 85%). TLC (CH_2Cl_2/MeOH/Et_3N, 95:5:0.2): R_f 0.34. UV (MeOH): 236 (29800), 275 (32600). ^{31}P NMR ($CDCl_3$): δ 149.6, 149.9.

Oligonucleotides. The oligonucleotide synthesis was carried out on a 1 μmol scale using the phosphoramidites of **1a**,**b** and the phosphoramidite of iG_d (**3**) on a synthesizer. After the synthesis the oligonucleotides **17**, **18**, **22**, **23**, **24**, and **31**−**37** were deprotected in 25% aqueous NH_3 at 60 °C for 16 h. The oligonucleotides containing **1a**,**b** and **2b** were deprotected in 25% aqueous NH_3 at room temperature for 16 h. Accordingly, the dA and dG residues were protected with the labile 4-(*tert*-butylphenoxy)acetyl (tac) group which can be removed within 2 h at room temperature with 25% aqueous NH_3. Oligonucleotides were purified by reversed-phase HPLC using the follow-

ing solvent systems: 0.1 M aqueous (Et_3NH)OAc (pH 7.0) (A), MeCN (B), and 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN, 95:5 (C). They were used in the following order: gradient I, 3 min 15% B in C, 12 min 15−40% B in C, 5 min 40−15% B in C with a flow rate of 1.0 mL/min; gradient II, 20 min 0−20% B in C with a flow rate of 1.0 mL/min; gradient III, 30 min 100% C with a flow rate of 0.6 mL/min. The oligomers carrying 5′-DMT residues were purified by HPLC (250 × 4 mm, RP-18 column) using gradient I. The DMT residues were removed by treating the oligomers with 2.5% $Cl_2CHCOOH/CH_2Cl_2$ for 5 min at room temperature. The detritylated oligomers were purified by HPLC (250 × 4 mm RP-18 column) using gradient II. The oligomers separated by RP18-HPLC were desalted on a 4 cm column (RP-18, silica gel) with H_2O to elute the salt, while the oligomers were eluted by $MeOH/H_2O$ (3:2). The purified oligonucleotides were lyophilized on an evaporator to yield colorless solids which were dissolved in 100 μL of H_2O and stored frozen at −18 °C. The synthesized oligonucleotides were characterized by enzymatic hydrolysis as well as by MALDI-TOF mass spectrometry.

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Supporting Information Available: ^{13}C and ^{1}H NMR coupling constants (Hz) of **1a**,**b** and **9a**,**b** and MALDI-TOF mass spectral data (MH^+) of selected oligonucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

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