8-Azaadenosine and Its 2′-Deoxyribonucleoside: Synthesis and Oligonucleotide Base-Pair Stability

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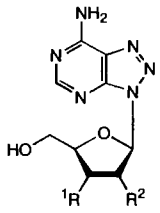
The synthesis of 8-azaadenosine (**1a**; z^8A) has been performed by $SnCl_4$-catalyzed glycosylation of 8-azaadenine (**4**) with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**5**), followed by the separation of the regioisomers **6** and **7** and subsequent deacetylation. The ribonucleoside **1a** as well as its 2′-deoxy derivative **1b** (z^8A_d) were converted into oligonucleotide building blocks – the phosphonate **2** as well as the phosphoramidites **3** and **19**. They were used to prepare the oligoribonucleotide $(z^8A\text{-}U)_6$ and oligodeoxyribonucleotides. The T_m values and the thermodynamic data of duplex formation of the modified duplexes showed no significant changes compared to those containing A_d or A residues. This indicates that the stereoelectronic effect of the 8-azaadenine base which was found for the monomeric nucleoside has only a minor influence on the duplex stability.

Introduction. Among the aza and deaza derivatives of purines, the 8-azapurines (3*H*-1,2,3-triazolo[4,5-*d*]pyrimidines; purine numbering is used throughout the *General Part*) have attracted particular interest [1], because their antifungal, antiviral, and anticancer activities have been recognized quite early [2]. Furthermore, 8-azaguanine (pathocidin) has been isolated from the culture filtrates of *Streptomyces albus* [3]. A number of 8-azapurine ribonucleosides [4], 2′-deoxyribonucleosides [5] as well as 2′,3′-dideoxyribonucleosides [6] have been prepared chemically. However, their synthesis is still fraught with difficulties because of the additional N(8)-atom which enlarges the number of glycosylation products and causes problems regarding regioselectivity and stereoselectivity.

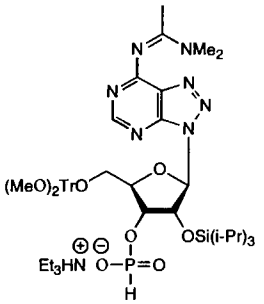
Both the ribofuranosyl and the 2′-deoxyribofuranosyl derivatives of 8-azaguanine were prepared enzymatically using either ribose 1-phosphate or 2′-deoxyribose 1-phosphate [7]. Also polynucleotides containing 8-azaguanosine were synthesized enzymatically [8]. Moreover, the template-directed incorporation of 8-aza-2′-deoxyguanosine in DNA using *E. coli* DNA polymerase I has been described [9]. However, the chemical and enzymatic incorporation of 8-azaadenosine or its 2′-deoxy derivative into 3′,5′-linked oligonucleotides has not been reported. Only the enzymatic synthesis of 2′,5′-oligoadenylates (dimers to pentamers) containing 8-azaadenosine has been performed [10].

In an earlier investigation, our laboratory reported on the solid-phase synthesis of 8-azaguanine-containing oligonucleotides derived from the sequences $d(C\text{—}G)_3$, and $d(TG_4T)$ [11]. We now describe the synthesis of 8-azaadenosine as well as of oligonucleotide building blocks of 8-azaadenosine and 8-aza-2′-deoxyadenosine. Oligonucleotides containing these nucleosides are prepared, and their properties investigated and compared with those containing adenosine or 2′-deoxyadenosine. The idea for this study results from recent findings that an 8-azapurine ring exerts a significant stereoelectronic effect on the 2′-deoxy-β-D-ribofuranose moiety, thereby driving the sugar puckering

towards the *N*-conformation ($^{3'}T_{2'}$) which is usually occupied by a ribonucleoside [12]. Therefore, it was anticipated that an oligodeoxynucleotide containing 8-azapurine 2′-deoxynucleotide units is merged towards an A-type secondary structure and can be effectively hybridized with a complementary RNA target which would improve its applicability as antisense oligonucleotide [13].



1a $R^1 = R^2 = OH$
b $R^1 = OH$; $R^2 = H$
c $R^1 = R^2 = H$



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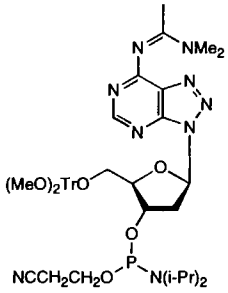

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Results and Discussion. – *Syntheses of 8-Azaadenosine* (**1a**) *and 8-Aza-2′-deoxyadenosine* (**1b**). Several 8-azaadenosine syntheses have been reported, but they are all associated with difficulties. This results mainly from the number of regioisomers formed during the glycosylation reaction. A synthesis of 8-azaadenosine using the nonanoylated base in a glycosylation reaction has been reported by *Montgomery*; however, the amount of isolated nucleoside was low [4b]. Therefore, the $SnCl_4$-catalyzed glycosylation of unprotected 8-azaadenine (**4**) with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**5**) [14] was investigated (*Scheme 1*). When the reaction was performed in MeCN solution at room temperature (24 h), the tri-*O*-acetyl-protected N^9-isomer **6** was isolated in 34% yield together with the N^8-isomer **7** (47%), both, as pure β-D-anomers. TLC Monitoring (silica gel, CH_2Cl_2/MeOH 9:1) of the glycosylation mixture showed that at the beginning of the reaction, the N^8-isomer **7** was formed (kinetic control) and that the N^9-isomer **6** appeared later. An increase of the reaction temperature shifted the ratio of isomers towards the N^9-nucleoside **6** but with a penalty of a lower total yield (*Table 1*).

The chromatographic separation of the isomers **6** and **7** was performed by flash chromatography. Because the reaction mixture obtained at elevated temperature contained more by-products which were difficult to separate, the room-temperature conditions were used for further experiments. The regioisomers **6** and **7** were deprotected (conc. aq. NH_3 solution, room temperature), and the ribonucleosides **1a** and **8** were obtained crystalline in 75 and 59% yield, respectively. The synthesis of the corresponding 8-aza-2′-deoxyadenosine (**1b**; z^8A_d) was performed as described earlier [5a].

The ^{13}C-NMR data of the 8-azaadenine nucleosides as well as of precursors are listed in *Table 2* [5a]. For comparison, the data of the corresponding 2′,3′-dideoxy nucleosides are enclosed which have been published earlier by our laboratory [6a]. A selection of revelant *J*(H,C) coupling constants are given in *Table 3*. Because of discrepancies in the

Scheme 1

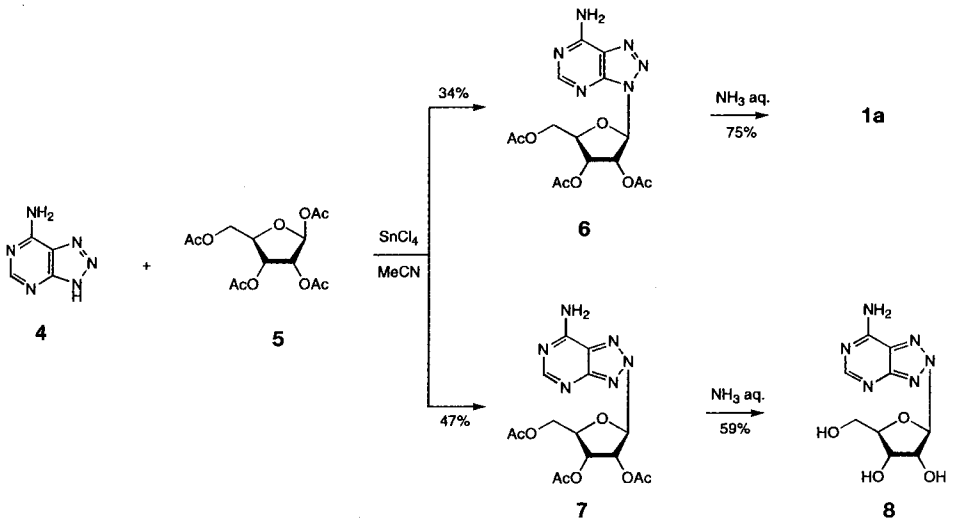


Table 1. *Distribution of Regioisomers* **6** *and* **7** *Formed upon the Glycosylation of 8-Azaadenine* (**4**) *as a Function of the Reaction Temperature*

Ratio of N^9/N^8-isomers **6**/**7**	Total yield [%]	Temp. [°]	Reaction time [h]
3:4	81	20	24
1:1	69	40	24
4:3	65	95	6

^{13}C-NMR assignments of 8-azaadenine derivatives in the literature [15][16], a more detailed NMR study was undertaken.

The C(2) ^{13}C-NMR signals of the nucleosides can be assigned unequivocally, because C(2) is the only one which shows a ^1J(C,H) coupling. The assignment of C(4) and C(6), however, remains tentative. The C(5) signal exhibits in all spectra the smallest coupling constant and resonates at highest field compared with the other ^{13}C-NMR signals; it exhibits a typical *t* due to a ^3J coupling with the protons of the NH_2 group. Analogous assignments made by *Dea et al.* [15], which also rely on gated-decoupled ^{13}C-NMR spectra, are consistent with our results while the assignments of *Purnell* and *Hodgson* differ from ours [16]. A change of the glycosylation position from N(9) to N(8) is indicated by a significant downfield shift of the fusion-site C(4) signals as well as of the C(1′) signals. The assignment of ^{13}C-NMR resonances of the glycosyl moiety was made on the basis of heteronuclear correlation spectra as well as gated-decoupled ^{13}C-NMR spectra. The anomeric configuration of the N^9- and N^8-regioisomers **1a** and **8** was confirmed by ^{1}H-NOE difference spectroscopy according to [17].

Nucleoside Conformation in Solution and Fluorescence Data. From the vicinal ^3J(H,H) coupling constants of the sugar protons, the conformation of 8-azaadenine ribo- (**1a**) and 2′-deoxyribonucleoside (**1b**) were determined. Information on the preferred sugar puckering ($^{3'}T_{2'}$ (*N*) ⇔ $_{3'}T^{2'}$ (*S*)) and the rotation about the C(4′)–C(5′) bond ($\gamma^{+(g)} \Leftrightarrow \gamma^{t} \Leftrightarrow \gamma^{-(g)}$) was obtained using the PSEUROT 6.2 program [18] as well as the method of *Westhof et al.* [19]. In the case of the deoxynucleosides, the H,H

Table 2. *^{13}C-NMR Data of 8-Azaadenine Nucleosides*[a])

	C(4)[b]) C(3a)[c])	C(2)[b]) C(5)[c])	C(6)[b]) C(7)[c])	C(5)[b]) C(7a)[c])	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
zA	151.4[a])	155.9	156.1[d])	123.1					
z^8A (N^9) (**1a**)	148.9	157.0	156.4	124.3	89.9	73.1	70.9	86.4	62.0
z^8A_d (N^9) (**1b**)	148.7	157.0	156.3	124.3	85.5	38.1	70.9	89.0	62.2
z^8A_{dd} (N^9) (**1c**)	148.5	156.8	156.2	123.9	85.9	30.6	27.0	82.7	63.8
N^8z^8A (**8**)	156.9	157.3	157.6	125.9	97.6	74.8	70.7	86.5	62.0
$N^8z^8A_d$	157.4	157.6	157.1	125.8	93.8	39.5	70.7	89.0	62.2
$N^8z^8A_{dd}$	157.5	157.1	156.9	125.5	94.4	31.9	26.5	83.8	63.9
6	149.0	157.4	156.2	123.9	86.7	72.5	70.2	79.8	62.3
7	157.1	158.0	157.8	126.5	94.8	73.8	70.4	80.5	65.5

[a]) Measured in (D_6)DMSO at 303 K. [b]) Purine numbering. [c]) Systematic numbering. [d]) Assignment tentative.

Table 3. *$^1H,^{13}C$ Coupling Constants* [Hz] *of 8-Azapurine Ribonucleosides*[a])[b])

	1a	**8**	**6**	**7**
J(C(4),*H*–C(2))	13.0	11.2	13.6	10.0
J(C(2),*H*–C(2))	201	199	201	200
J(C(6),*H*–C(2))	14.0	12.8	11.3	13.0
J(C(5),NH_2)	4.4	4.5	4.6	n.d.
J(C(1′),*H*–C(1′))	166	171	171	175
J(C(2′),*H*–C(2′))	149	148	160	156
J(C(3′),*H*–C(3′))	149	149	151	153
J(C(4′),*H*–C(4′))	149	149	151	153
J(C(5′),*H*–C(5′))	140	140	149	150

[a]) Measured in $(D)_6$DMSO at 303 K. [b]) Systematic numbering.

coupling constants $^3J(1',2'_A)$,$^3J(1',2'_B)$,$^3J(2'_A, 3')$,$^3J(2'_B, 3')$, and $^3J(3',4')$, and in the case of the ribonucleosides, the H,H couplings constants $^3J(1',2')$, $^3J(2',3')$, and $^3J(3',4')$ were used.

For the 8-aza-2′-deoxyadenosine (**1b**), populations of 50% *N* and 50% *S* are calculated (*Table 4*). The parent 2′-deoxyadenosine shows populations of 28% *N* and 72% *S* [12]. The 8-azadenosine (**1a**) exhibits the same *N*/*S* conformer population ratio (50:50) as the 8-aza-2′-deoxyadenosine (**1b**). On the other hand, adenosine (21% *N*, 79% *S*) shows a much lower population of the *N*-conformer than 8-azaadenosine (**1a**). This means that the introduction of an additional N-atom at position 8 of 2′-deoxyadenosine or adenosine leads to a significant stereoelectronic effect on the *N*/*S* equilibrium, thereby biasing the conformer populations towards a conformation which is usually occupied by a ribonucleoside [13].

Moreover, the conformation about the C(4′)–C(5′) bond of **1b** ($\gamma^{+(g)}$ 39%, γ^t 42%, $\gamma^{-(g)}$ 19%) is strikingly shifted compared to that of A_d ($\gamma^{+(g)}$ 59%, γ^t 25%, $\gamma^{-(g)}$ 16%). As can be seen, the $-sc$ (*t*) conformation of **1b** is increased at the expense of the $+sc$ ($(+)g$) conformation, while the *ap* ($(-)g$) conformation of both compounds is similar

Table 4. *$^3J(H,H)$ Coupling Constants of the Sugar Moieties and Conformer Populations of Nucleosides* **1a** *and* **1b** *and of Their Parents A and A_d, Respectively, at 303 K*[a])

	^3J(H,H) [Hz]							Conformation				
	1',2' (or 1',2_A)	1',$2'_B$	2',3' (or $2'_A$,3')	$2'_B$,3'	3',4'	4',$5'_A$	4',$5'_B$	% *N*	% *S*	% $\gamma^{+(g)}$	% γ^{t}	% $\gamma^{-(g)}$
z^8A (**1a**)	4.45	–	4.85	–	4.85	3.00	4.80	50	50	58	31	11
A [20]	6.20	–	5.30	–	3.40	2.80	3.60	21	79	72	24	6
z^8A_d (**1b**)	6.45	6.60	5.65	5.15	5.20	3.70	5.80	50	50	39	42	19
A_d [18]	7.20	6.50	6.50	3.30	3.20	3.45	4.30	28	72	59	25	16

[a]) Solvent, D_2O; r.m.s. ≤ 0.4 Hz; $|\Delta J_{max}| \leq 0.5$ Hz.

(*Table 4*). These results are almost identical to those obtained for 8-aza-2'-deoxyguanosine [12], for 8-aza-2'-deoxyinosine [21], as well as for the corresponding ribonucleosides [22].

8-Azaadenine exhibits fluorescence under neutral conditions [23][24]. We observed that also 8-aza-2'-deoxyadenosine (**1b**) exhibits significant fluorescence at neutral pH with an emission maximum at 348 nm (*Fig. 1, a*). The same has been reported for ribonucleoside **1a** [24] (*Fig. 1, b*). The purine base adenine as well as the nucleoside adenosine and 2'-deoxyadenosine are almost nonfluorescent under these conditions. Also guanine and its nucleosides do not show significant fluorescence. On the other hand, 8-aza-2'-deoxyguanosine is highly fluorescent in alkaline medium [24]; its fluorescence at pH 7 is a residual emission from the nucleoside anion [24].

Building-Block Synthesis and Properties of Oligonucleotides. For the preparation of oligonucleotide buildings blocks, standard protecting groups were chosen. At first, 8-azaadenosine (**1a**) was benzoylated using the protocol of transient protection (→ **9**) [25] (*Scheme 2*). UV Spectrophotometric determination (285 nm) of the half-life value (τ) of the debenzoylation of **9** (25% aq. NH_3 solution, 40°) showed that this protecting group is rather stable with a τ of 140 min (*cf.* $^{bz}A_d$, 175 min [26]) which is quite long for oligoribonucleotide synthesis. Therefore, the (dimethylamino)methylidene group was introduced [27]. The resulting formamidine moiety proved to be very labile and difficult to handle so that we switched to the more stable acetamidine residue [26]. For this purpose, compound **1a** was treated with *N*,*N*-dimethylacetamidine dimethyl acetal in MeOH to give compound **10** in 76% yield. UV-Spectrophotometric monitoring (324 nm) of the basic hydrolysis (25% aq. NH_3 solution, 40°) revealed a τ value of 12 min (*cf.* acetamidine derivative of A_d, 9 min [26]). Compound **11** (81%) was obtained from **10** upon dimethoxytritylation. Compound **11** was treated with triisopropylsilyl chloride in pyridine with $AgNO_3$ as catalyst [28]. This furnished the 2'-*O*-silylated isomer **12** as the main reaction product (71%) together with the 3'-*O*-isomer **13** as by-product (18%). Reaction of compound **12** with *in-situ* prepared tris(1*H*-1,2,4-triazol-1-yl)phosphine in CH_2Cl_2 and subsequent hydrolysis in $(Et_3NH)HCO_3$ gave the phosphonate **2** [29] which was characterized by ^{1}H-, ^{13}C-, and ^{31}P-NMR spectroscopy (*Table 5* and *Exper. Part*).

Next, the phosphoramidite of the 2'-deoxynucleoside **1b** was prepared. Transient protection of **1b** [25] followed by isobutyrylation furnished compound **14** (24%; τ

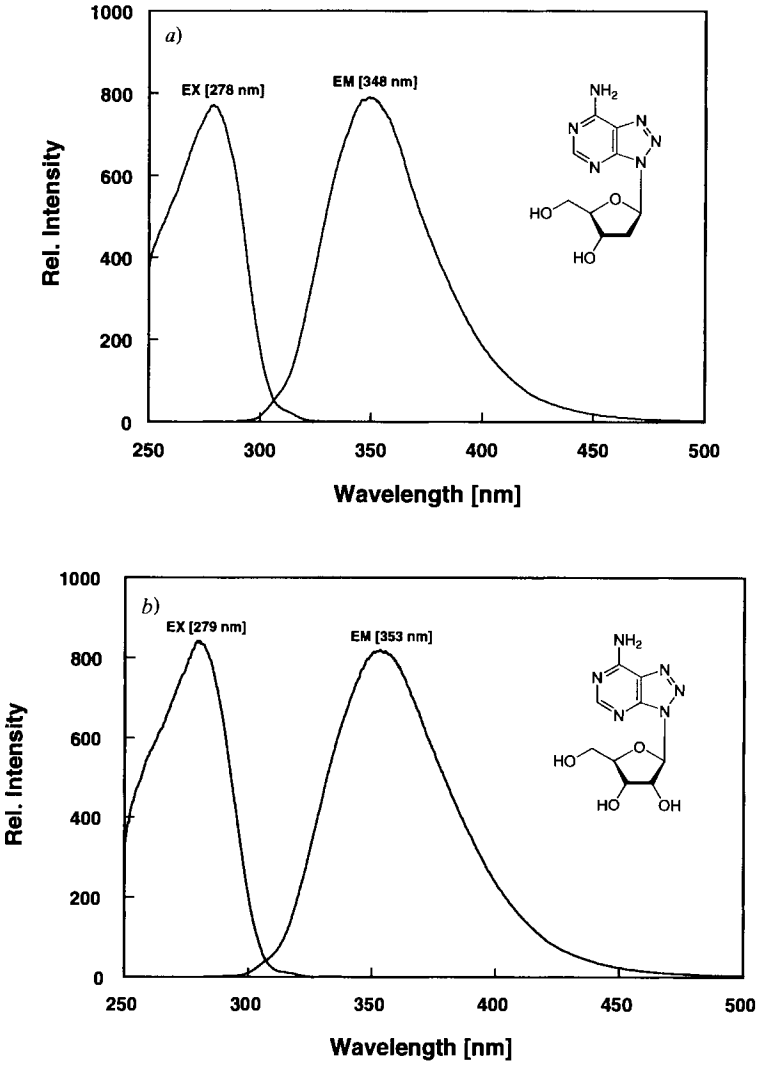


Fig. 1. *Fluorescence spectrum of* a) *8-aza-2′-deoxyadenosine* (**1b**; 10^{-5} M) *and* b) *8-azaadenosine* (**1a**; 10^{-5} M) *at pH 7*

(25% aq. NH_3 solution, room temperature, 279 nm): 13 min) (*Scheme 3*). Subsequent 4,4′-dimethoxytritylation failed due to a loss of the isobutyryl group. Therefore, this route was rejected and the dimethylacetamidine **15** (91%) was prepared. The latter compound showed a τ of 5 min (25% aq. NH_3 solution, room temperature, 324 nm). Subsequent dimethoxytritylation of **15**, however, was tedious. This problem was overcome when compound **1b** was first converted into the $(MeO)_2Tr$ derivative **16** and the NH_2-protecting groups were introduced thereafter (→ **17**, **18**). Subsequently, the phosphoramidites **3** and **19** were prepared under standard conditions [30].

Scheme 2

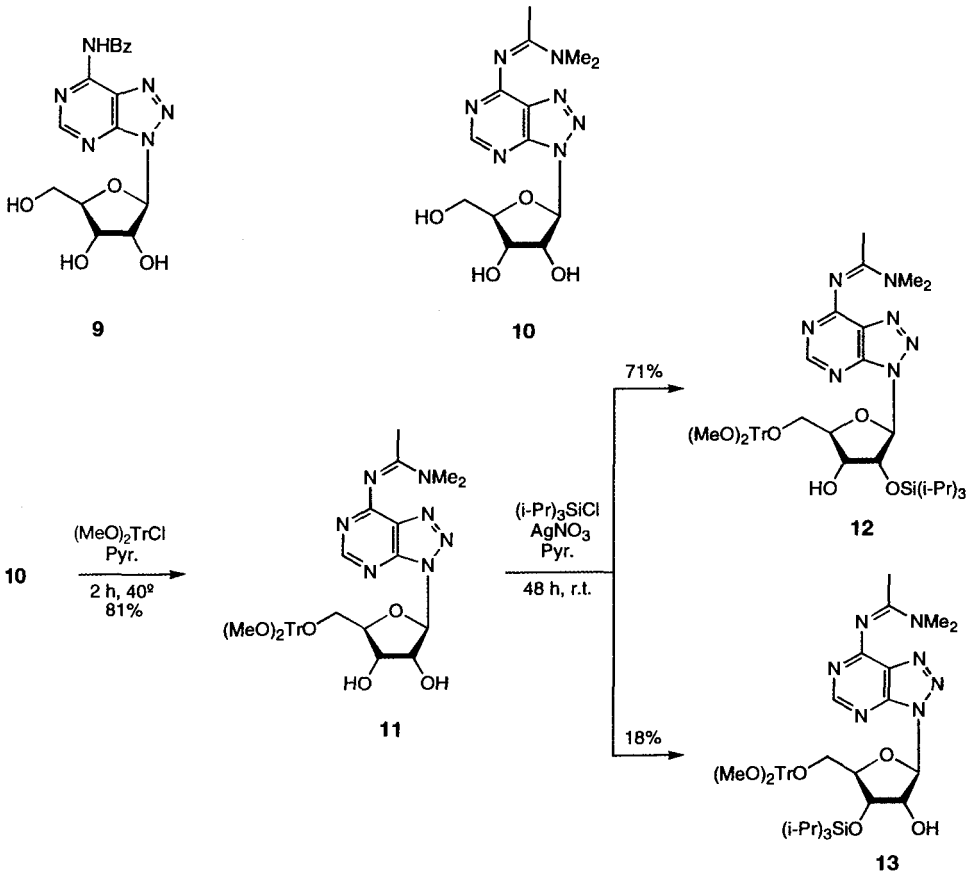
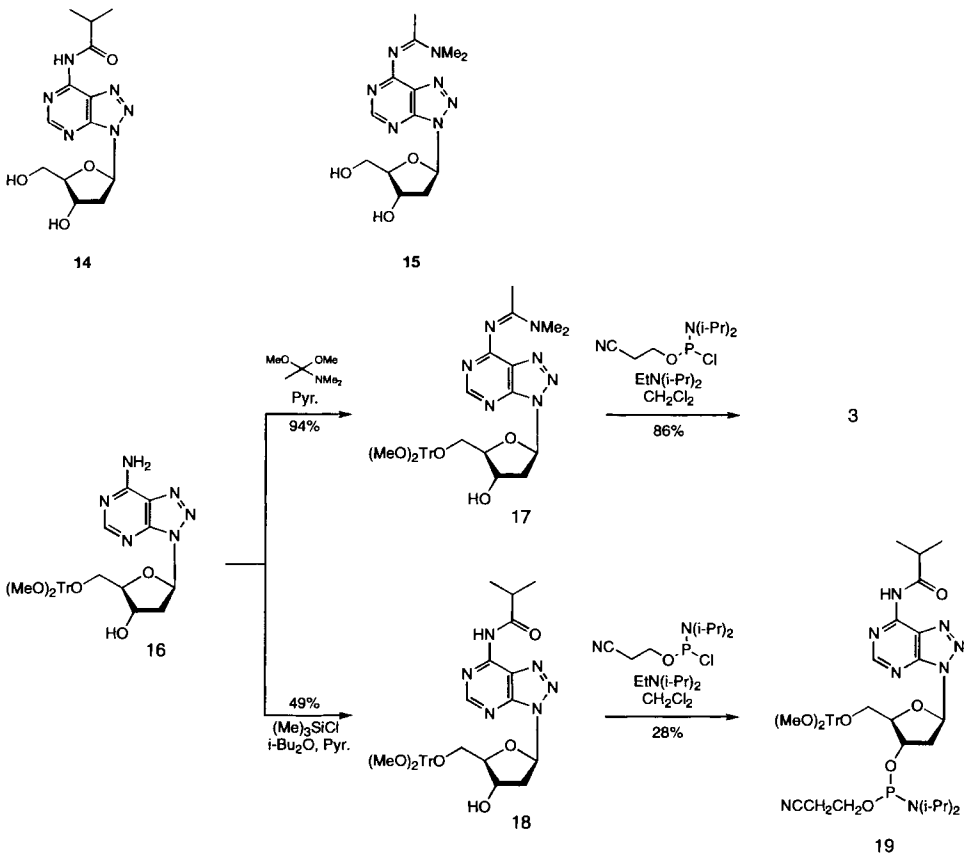


Table 5. *^{13}C-NMR Data of 8-Azaadenine β-D-2′-Deoxyribonucleoside Derivatives*[a])

	C(4)[b]) C(3a)[c])	C(2)[b]) C(5)[c])	C(6)[b]) C(7)[c])	C(5)[b]) C(7a)[c])	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C=O	N=C	CH	Me	SiMe
9	155.8	152.2	150.2	n.d.	89.6	73.1	70.6	86.2	61.7	166.9				
10	149.6	156.6	159.7	129.8	89.9	73.0	70.9	86.3	62.1		164.8		17.7	
11	149.7	156.7	159.7	129.8	89.5	73.2	70.8	83.5	63.9		164.7		17.6	
12	150.0	156.8	159.7	129.8	89.3	74.8	71.0	85.5	63.4		164.6		17.6	17.6, 11.6
13	149.7	156.7	159.7	129.8	89.6	73.0	72.0	83.5	63.1		164.6		17.6	17.6, 11.8
2	150.2	156.6	159.7	129.8	88.3	73.6	72.9	84.2	63.5		164.3		17.6	17.6 11.5
15	149.3	156.5	159.6	129.9	85.4	38.0	70.8	88.4	62.0		164.6		17.6	
14	150.1	156.2	151.5	126.6	85.3	38.0	70.6	88.4	61.8	175.7		34.6	19.1	
16	148.7	156.8	156.2	124.1	85.1	38.0	70.4	86.0	64.0					
17	149.3	156.4	159.6	129.8	85.1	38.0	70.5	86.0	64.1		169.5		17.4	
18	149.9	156.0	151.3[e])	[d])	85.3	38.1	70.4	86.1	63.9	175.5		34.6	19.0	

[a]) Measured in (D_6)DMSO; 303 K. [b]) Purine numbering. [c]) Systematic numbering. [d]) Superimposed by DMSO. [e]) Superimposed by 4,4′-dimethoxytrityl.

Scheme 3



Oligonucleotides Containing 8-Azaadenine. There are only a few reports in the literature describing oligonucleotides with 8-azapurines [11][31]. Oligonucleotides with 8-azapurine bases – in contrast to those containing purines – do not form *Hoogsteen* base pairs [11]. This is the result of the reduced basicity of N(7) in an 8-azapurine system compared to the purine heterocycle. The additional N-atom at the 8-position makes the triazole ring system more π-electron-deficient [32]. The decreased electron density which affects the whole system makes the protonation of an 8-azapurine base more difficult than that of a purine base. The pK of protonation of adenosine is 3.5, whereas 8-azaadenosine exhibits a value of 2.4 [5a]. With regard to the proton-acceptor properties at position 7, the 8-azapurines behave similarly as 7-deazapurines or 8-aza-7-deazapurines. It was found that consecutively arranged 8-azaguanine residues are not able to form G quartets [11] – a behavior which was also reported for oligonucleotides containing 7-deazaguanine or 8-aza-7-deazaguanine [28c]. Furthermore, oligonucleotides containing 8-azaguanine · cytosine base pairs form more stable duplex structures than those containing pairs of guanine · cytosine [11].

The oligonucleotides containing 8-aza-2′-deoxyadenosine **22**–**25**, **30**, and **33** (*Table 6*) were synthesized according to standard procedures of solid-phase DNA or RNA synthesis [33]. The nucleoside composition of all oligodeoxyribonucleotides **22**–**33** was confirmed by MALDI-TOF spectra (*Table 7*, *Exper. Part*) as well as by enzymatic hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase. The resulting nucleoside mixtures were analyzed by reversed-phase HPLC (*RP-18*; *Table 8*, *Fig. 2, a* and *b*).

To study the influence of the thermodynamic stability of 8-azaadenine-containing oligonucleotides, the non-selfcomplementary sequences **20** and **21** were selected, and the

Table 6. T_m *Values and Thermodynamic Data of Oligonucleotide Duplexes*[a])[b])

		T_m [°C][c])	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/K mol]	$\Delta G°_{298}$ [kcal/mol]
5′-d(T-A-G-G-T-C-A-A-T-A-C-T)-3′	(**20**)	47	−81.8	−230.3	−10.4
3′-d(A-T-C-C-A-G-T-T-A-T-G-A)-5′	(**21**)				
5′-d(T-A-G-G-T-C-zA-zA-T-A-C-T)-3′	(**22**)	45	−72.6	−203.0	−9.6
3′-d(A-T-C-C-zA-G-T-T-zA-T-G-A)-5′	(**23**)				
5′-d(T-zA-G-G-T-C-zA-zA-T-zA-C-T)-3′	(**24**)	46	−86.9	−247.5	−10.2
3′-d(zA-T-C-C-zA-G-T-T-zA-T-G-zA)-5′	(**25**)				
5′-d(T-A*-G-G-T-C-A*-A*-T-A*-C-T)-3′	(**26**)	47	−87.0	−230.0	−10.9
3′-d(A-T-C-C-A*-G-T-T-A*-T-G-A)-5′	(**27**)				
5′-d(T-A-G-G-T-C-A-A-T-A-C-T)-3′	(**20**)	45	−92.1	−264.0	−10.2
3′-r(A-U-C-C-A-G-U-U-A-U-G-A)-5′	(**28**)				
5′-d(T-A-G-G-T-C-zA-zA-T-A-C-T)-3′	(**22**)	42	−68.7	−191.9	−9.2
3′-r(A-U-C-C-A-G-U-U-A-U-G-A)-5′	(**28**)				
5′-d(T-zA-G-G-T-C-zA-zA-T-zA-C-T)-3′	(**24**)	42	−80.6	−230.0	−9.3
3′-r(A-U-C-C-A-G-U-U-A-U-G-A)-5′	(**28**)				
5′-d(G-T-A-G-A-A-T-T-C-T-A-C)-3′	(**29**)	43	−84.0	−225.0	−8.6
3′-d(C-A-T-C-T-T-A-A-G-A-T-G)-5′	(**29**)				
5′-d(G-T-zA-G-zA-zA-T-T-C-T-zA-C)-3′	(**30**)	43	−77.6	−220.6	−9.2
3′-d(C-zA-T-C-T-T-zA-zA-G-zA-T-G)-5′	(**30**)				
$d(A)_{12} \cdot d(T)_{12}$ (**31** · **32**)		37	−91.0	−267.0	−7.9
5′-d(A-A-A-zA-A-zA-A-zA-A-A-A-A)-3′	(**33**)	35	−76.3	−221.6	−7.5
3′-d(T-T-T-T-T-T-T-T-T-T-T-T)-5′	(**32**)				

[a]) $zA_d = z^8A_d$ = 8-Aza-2′-deoxyadenosine (**1b**); $A^*_d = c^7z^8A_d$ = 8-aza-7-deaza-2′-deoxyadenosine. [b]) Single-strand concentration was 10 μM. [c]) 10 mM Na-cacodylate, 10 mM $MgCl_2$, 100 mM NaCl, pH 7.0.

Table 7. *MALDI-TOF Mass Data of Oligonucleotides*

Oligomer	**22**	**23**	**24**	**25**	**30**	**33**
M^+(calc.)	3647.7	3647.4	3649.4	3649.4	3649.4	3700.5
m/z	3647.4	3647.1	3650.1	3650.3	3649.2	3701.5

Table 8. *Nucleoside Composition of Oligonucleotides after Enzymatic Hydrolysis with Snake-Venom Phosphodiesterase and Alkaline Phosphatase*[a])

Oligomer	Calculated (found in parenthesis)				
	z^8A_d (z^8A)	A_d	T_d (U)	G_d	C_d
22	1.0 (1.0)	1.0 (1.0)	2.0 (3.3)	1.0 (1.2)	1.0 (1.3)
23	1.0 (1.0)	1.0 (0.7)	2.0 (2.3)	1.0 (0.9)	1.0 (1.0)
24	2.0 (2.0)	–	2.0 (2.2)	1.0 (1.1)	1.0 (1.1)
25	2.0 (2.0)	–	2.0 (2.3)	1.0 (0.9)	1.0 (0.9)
30	2.0 (2.0)	–	2.0 (2.4)	1.0 (1.0)	1.0 (1.2)
34	1.0 (1.0)	–	1.0 (1.0)	–	–

[a]) For details, see *Exper. Part.*

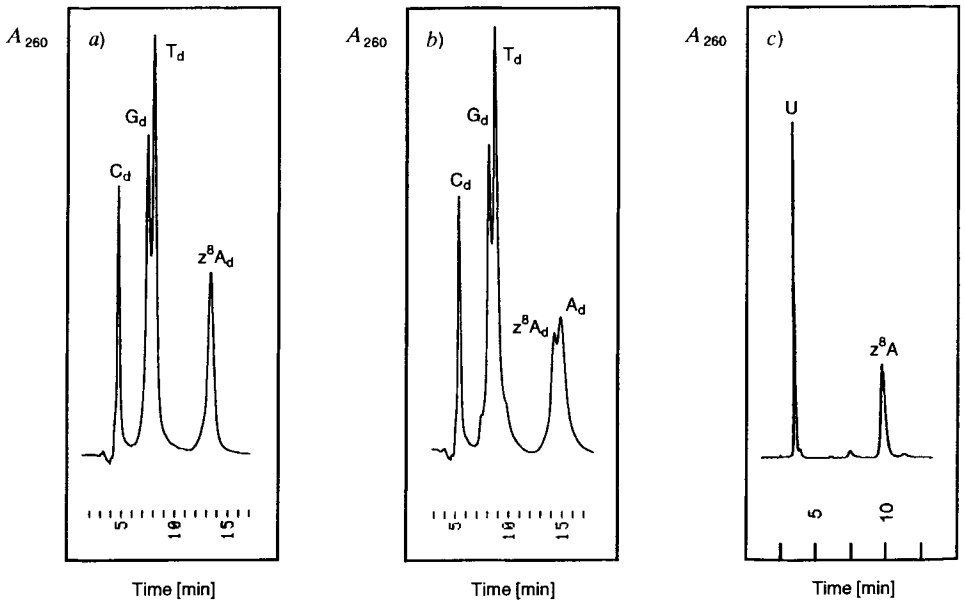


Fig. 2. *Reversed-phase HPLC (RP-18) of the reaction products obtained during enzymatic hydrolysis of the oligomers* a) **24**, b) **22**, *and* c) **34** *by snake-venom phosphodiesterase at 37°, after subsequent addition of alkaline phosphatase.* For details, see *Exper. Part.*

UV melting profiles were measured at 260 nm in 10 mM Na-cacodylate buffer (0.1M NaCl, 10 mM $MgCl_2$, pH 7). The duplex **20** · **21** has become a standard sequence in our laboratory to study the influence of base modification on the thermodynamics of duplex formation. The resulting T_m values were compared with those of the corresponding unmodified random duplex [34] (*Table 6*). In the duplex **22** · **23**, only 50% of the adenine bases were replaced by 8-azaadenine, whereas the duplex **24** · **25** represents a 100% replacement. In both cases, the duplex stability was only marginally affected. The same result has been found for the corresponding oligomer duplex containing 8-aza-7-deaza-2′-deoxyadenosine ($c^7z^8A_d = A_d^*$; **16** · **28**, *Table 6*) [35].

In the case of a chimeric DNA · RNA duplex, containing one oligoribo- and one oligodeoxyribonucleotide strand, the T_m-decreasing effect of an 8-azaadenine base is similar to that of oligomers containing regular adenine residues. The synthesis of chimeric RNA · DNA or RNA · RNA duplexes containing 8-azaadenine in the ribo strand can be achieved using the phosphonate **11**. We tested the feasibility of this building block and obtained the alternating oligoribonucleotide $(z^8A\text{-}U)_6$ (**34**). The latter was characterized by enzymatic tandem hydrolysis using snake-venom phosphodiesterase and alkaline phosphatase (*Fig. 2, c*). Apart from non-selfcomplementary duplexes, the influence of an 8-azaadenine residue was tested in the self-complementary duplex of **29**. This duplex contains the same number of A-T and G-C base pairs as the non-selfcomplementary duplex **20** · **21**. In this case, the T_m values of the modified (**30** · **30**) and the non-modified duplex (**29** · **29**) were identical.

It is highly surprising that the replacement of adenine (see A_d) by 8-azaadenine (see z^8A_d) as well as by 8-aza-7-deazaadenine (see $c^7z^8A_d$) has almost no influence on the oligonucleotide duplex stability within a random sequence. Even oligonucleotides with consecutive 8-aza-2′-deoxyadenosine residues showed a similar stability as those of the parent A_d-containing oligomers. This leads us to the conclusion that despite of the fact that the π-electron system as well as the dipole moment of an 8-azaadenine base is different from that of adenine [32], the duplex stability depends almost exclusively on the strength of the H-bonds between the bases. Also, the stereoelectronic effect of the 8-azaadenine analogue which is observed on the monomeric nucleosides does not exert a significant change of the duplex stability. If this would be the case, the T_m values of the chimeric DNA · RNA duplexes (*Table 6*) should increase when z^8A_d is introduced. However, this was not the case.

To investigate secondary-structure changes induced by 8-azaadenine, the CD spectra of the DNA · DNA duplexes **20** · **21** and **24** · **25** as well as of the DNA · RNA duplexes **20** · **28**, **22** · **28**, and **24** · **28** (*Fig. 3, a* and *b*) were measured at 15°. It can be seen that the modified duplex **24** · **25** still represents a B-DNA secondary structure, whereas the chimeric DNA · RNA duplexes exhibit the typical shape of an A-type structure [36]. Bathochromic shifts of the positive CD bands can be attributed to differences of the UV spectra of 8-aza-2′-deoxyadenosine (278 nm, ε 11300) compared to 2′-deoxyadenosine (259 nm, ε 15000).

Conclusion. It has been generally accepted in nucleic-acid chemistry that apart from the helical structure, both, the strength of H-bonds as well as the π-overlap of the bases and their dipole moment, determine the stability of a duplex. Usually, the incorporation of modified bases having another donor-acceptor pattern as the parent ones leads to a strong decrease of the duplex stability [37]. Therefore, it is surprising that the altered π-electron system of the 8-azaadenine system as compared to adenine [32] does not exert a strong influence on the stability of a duplex. Similar results have been observed for 7-deazaadenine and 8-aza-7-deazaadenine-containing oligonucleotides [34][38]. This leads to the question whether π-interactions and dipole moments of the bases are decisive for the duplex stability. The results discussed above imply that in cases in which regular bases are substituted by modified ones, the spatial requirements as well as the strength of the donor-acceptor pattern of the base are important. Obviously, the stacking interactions within the duplex or the sugar conformation of the monomeric nucleoside seems to be of minor importance. Nevertheless, modified bases

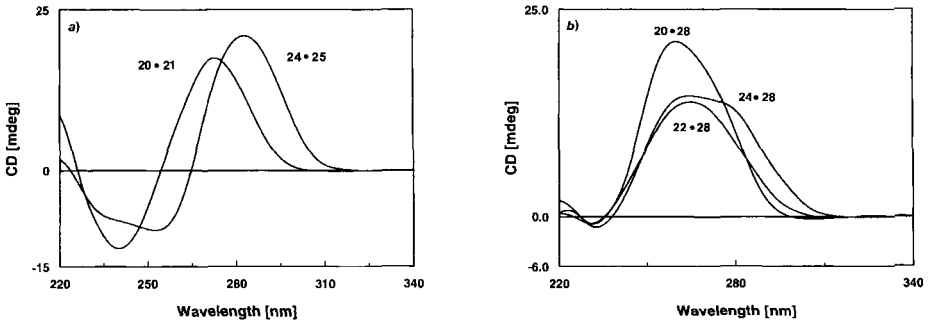


Fig. 3. *CD Spectra of* a) *DNA · DNA duplexes and* b) *DNA · RNA duplexes concentration in 10 mM Na-cacodylate buffer* (10 mM $MgCl_2$, 100 mM NaCl, pH 7) *at 15°*

being isosteric to the parent ones can change the local geometry of a helix as well as its solvation.

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Experimental Part

1. *General.* See [38]. Flash chromatography (FC): at 0.5 bar on silica gel *60H* (*Merck*, Germany); solvent systems for FC and TLC: CH_2Cl_2/MeOH 98:2 (*A*), CH_2Cl_2/MeOH 95:5 (*B*), CH_2Cl_2/MeOH 9:1 (*C*), CH_2Cl_2/MeOH 8:2 (*D*), CH_2Cl_2/MeOH/Et_3N 88:10:2 (*E*) CH_2Cl_2/acetone/Et_3N 88:10:2 (*F*), AcOEt/petroleum ether 9:1 (*G*), AcOEt/petroleum ether 8:2 (*H*), AcOEt/petroleum ether 1:1 (*I*). UV/VIS Spectra: λ_{max} (ε) in nm. CD Spectra: *Jasco-600* spectropolarimeter, thermostatically controlled 1-cm cuvettes with a *Lauda-RCS-6* bath. Melting curves: *Cary-1E-UV/VIS* spectrophotometer (*Varian*, Australia) equipped with a thermoelectrical controller; the actual temp. was measured in the reference cell with a Pt-*100* resistor; evaluation of thermodynamic data from the melting curves according to a two-state model was performed using the program 'Meltwin' (version 3.1 [39]). NMR Spectra: *AMX-500* and *AC-250* spectrometers (*Bruker*, Germany); δ in ppm rel. to int. Me_4Si (^1H, ^{13}C) and to external 85% H_3PO_4 soln. (^{31}P). MALDI-TOF Mass spectra of the oligonucleotides were measured by Mrs. *Julia Gross* (Institute of Medical Physics and Biophysics, Westfälische Wilhelms-Universität Münster) on a home-built apparatus.

2. *Monomers. 2-(2',3',5'-Tri-O-acetyl-β-*D*-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine* (**6**) *and 2-(2',3',5'-Tri-O-acetyl-β-*D*-ribofuranosyl)-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine* (**7**). To a suspension of 1*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine (**4**; 340 mg, 2.5 mmol) and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**5**; 800 mg, 2.5 mmol) in MeCN (10 ml), $SnCl_4$ (0.88 ml, 7.5 mmol) is added within 5 min, and the mixture is stirred under Ar for 24 h at r.t. Then, the soln. is poured into sat. aq. $NaHCO_3$ soln. (32 ml) and the precipitate filtered off and washed with H_2O (2 × 10 ml). The combined filtrate and washings are extracted with CH_2Cl_2 (4 × 15 ml). After drying (Na_2SO_4) and evaporation, a yellowish foam (0.87 g) is obtained which is submitted to FC (5.5 × 20 cm, gradient *A* → *C*): 0.34 g (34%) of **6**, followed by 0.46 g (47%) of **7**.

Data of **6**: Colorless foam. TLC (silica gel, *C*): R_f 0.45. UV (MeOH): 280 (10.900). ^{1}H-NMR ((D$_6$)DMSO): 1.89, 2.10, 2.11 (3*s*, 3 Ac); 4.20 (*m*, 2 H–C(5')); 4.48 (*m*, H–C(4')); 5.74 (*t*, *J* = 5.5, H–C(3')); 6.07 (*t*, *J* = 3.7, H–C(2')); 6.48 (*d*, *J* = 2.8, H–C(1')); 8.25, 8.60 (2*s*, NH_2); 8.34 (*s*, H–C(5)). Anal. calc. for $C_{15}H_{18}N_6O_7$ (394.3): C 45.69, H 4.60, N 21.31; found: C 45.98, H 4.72, N 21.40.

Data of **7**: Colorless foam. TLC (silica gel, *C*): R_f 0.35. UV (MeOH): 253 (3.900), 300 nm (10400). ^{1}H-NMR ((D$_6$)DMSO): 1.94, 2.07, 2.11 (3*s*, 3 Ac); 4.25 (*m*, 2 H–C(5')); 4.52 (*m*, H–C(4')); 5.74 (*t*, *J* = 6.0, H–C(3')); 5.91 (*d*, *J* = 3.4, H–C(2')); 6.53 (*s*, H–C(1')); 8.3, 8.45 (2*s*, NH_2); 8.34 (*s*, H–C(5)). Anal. calc. for $C_{15}H_{18}N_6O_7$ (394.3): C 45.69, H 4.60, N 21.31; found: C 45.86, H 4.71, N 21.22.

*3-(β-*D*-Ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine* (= *8-Azaadenosine*; **1a**). Compound **6** (2.04 g, 5.17 mmol) is stirred for 2 h in MeOH/25% aq. NH_3 soln. 1:1 (10 ml) at r.t. Evaporation and crystallization from H_2O (3 ml) give 1.04 g (75%) of colorless crystals. M.p. 217° (dec.) ([40]: 227° (dec.)). TLC (silica

gel, *D*): R_f 0.45. UV (pH 7): 279 (11600). ^{1}H-NMR ((D$_6$)DMSO): 3.56 (*m*, 2 H–C(5')); 4.01 (*m*, H–C(4')); 4.29 (*m*, H–C(3')); 4.85 (*m*, H–C(2')); 5.01 (*t*, *J* = 5.9, OH–C(5')); 5.28 (*d*, *J* = 5.7, OH–C(3')); 5.56 (*d*, *J* = 6.0, OH–C(2')); 6.15 (*d*, *J* = 5.2, H–C(1')); 8.31 (*s*, H–C(5)); 8.53, 8.19 (2*s*, NH_2).

*2-(β-*D*-Ribofuranosyl)-2*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine* (**8**). Compound **7** (0.81 g, 2.05 mmol) is deprotected as described for **6**: 0.32 g (58%) of colorless crystals (H_2O). M.p. 209° (dec.) ([40]: M.p. 218–219° (dec.)). TLC (silica gel, *D*): R_f 0.2. UV (MeOH): 255 (4400), 263 (4100), 297 (10400). ^{1}H-NMR ((D$_6$)DMSO): 3.59 (*m*, 2 H–C(5')); 4.06 (*m*, H–C(4')); 4.33 (*m*, H–C(3')); 4.61 (*m*, H–C(2')); 4.76 (*t*, *J* = 5.3, OH–C(5')); 5.27 (*d*, *J* = 5.8, OH–C(3')); 5.68 (*d*, *J* = 5.5, OH–C(2')); 6.08 (*s*, *J* = 3.4, H–C(1')); 8.31 (*s*, H–C(5)); 8.12 (*s*, NH_2).

N-*[3-(β-*D*-Ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-yl]benzenamide* (**9**). To a soln. of **1a** (100 mg, 0.37 mmol) in anh. pyridine, Me_3SiCl (0.47 ml, 3.7 mmol) is added under Ar. After stirring for 30 min at r.t., benzoyl chloride (0.25 ml, 2.0 mmol) is added, and stirring is continued for another 4 h. After cooling to 0–5°, H_2O (1 ml), and 5 min later, 25% aq. NH_3 soln. (2 ml) are added. After 30 min, the solvent is evaporated, and the residue co-evaporated with toluene. After takeup in sat. aq. $NaHCO_3$ soln. (15 ml), the soln. is extracted once with CH_2Cl_2 and several times with AcOEt. The combined org. layer is dried (Na_2SO_4) and evaporated: 50 mg (36%) of colorless crystalline **9** (MeOH). M.p. 188° (dec.). TLC (silica gel, *C*): R_f 0.25. UV (MeOH): 242 (9400), 282 (20300). ^{1}H-NMR ((D$_6$)DMSO): 3.56 (*m*, 2 H–C(5')); 4.04 (*m*, H–C(4')); 4.36 (*m*, H–C(3')); 4.84 (*t*, OH–C(5')); 4.92 (*m*, H–C(2')); 5.33 (*d*, *J* = 5.2, OH–C(3')); 5.66 (*d*, *J* = 5.4, OH–C(2')); 6.30 (*d*, *J* = 4.3, H–C(1')); 7.54–8.11 (*m*, arom. H); 8.94 (*s*, H–C(5)); 11.99 (br. *s*, NH). Anal. calc. for $C_{16}H_{16}N_6O_5$ (372.3): C 51.61, H 4.33, N 22.57; found: 51.49, H 4.43, N 22.74.

N,N-*Dimethyl*-N'-*[3-(β-*D*-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-yl]ethanimidamide* (**10**). To a soln. of **1a** (500 mg, 1.86 mmol) in MeOH (10 ml), *N,N*-dimethylacetamide dimethyl acetal (0.91 ml, 5.59 mmol) is added. After stirring for 14 h at r.t., the soln. is evaporated to an oil which is then co-evaporated with toluene. The residue is taken up in MeOH and again stirred for 2 h at r.t. After evaporation, the residue is submitted to FC (4 × 20 cm, gradient *A* → *C*): 0.48 g (76%) of colorless foam. TLC (silica gel, *C*): R_f 0.35. UV (MeOH): 233 (9300), 270 (3600), 324 (26100). ^{1}H-NMR ((D$_6$)DMSO): 2.28 (MeC=N); 3.20 (*s*, Me_2N); 3.55 (*m*, 2 H–C(5')); 4.00 (*m*, H–C(4')); 4.30 (*m*, H–C(3')); 4.86 (*m*, H–C(2')); 4.98 (*t*, OH–C(5')); 5.29 (*d*, *J* = 5.2, OH–C(3')); 5.57 (*d*, *J* = 5.9, OH–C(2')); 6.18 (*d*, *J* = 5.2, H–C(1')); 8.54 (*s*, H–C(5)). Anal. calc. for $C_{13}H_{19}N_7O_4$ (337.3): C 46.29, H 5.68, N 29.06; found: C 46.45, H 5.63, N 28.97.

N'-*{3-[5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-β-*D*-ribofuranosyl]-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-yl}*-N,N-*dimethylethanimidamide* (**11**). Compound **10** (0.34 g, 1.0 mmol) is dried by co-evaporation with anh. pyridine and then dissolved in anh. pyridine (4 ml). Thereupon, 0.41 g (1.2 mmol) of 4,4'-dimethoxytriphenylmethyl chloride is added, and the mixture is stirred for 2 h at 40°. After cooling to r.t., MeOH (5 ml) is added, and stirring is continued for another 30 min. Then, the mixture is reduced to ½ of its volume, and a sat. aq. $NaHCO_3$ soln. (15 ml) is added. The mixture is extracted with CH_2Cl_2 (4 × 10 ml) and the combined org. extract washed with brine (15 ml) and dried (Na_2SO_4). Evaporation gives 0.73 g of a pale yellow foam which is submitted to FC (2 × 20 cm, *B*): 0.52 g (81%) of colorless foam. TLC (silica gel, *C*): R_f 0.45. UV (MeOH): 234 (29900), 275 (13800), 324 (24800). ^{1}H-NMR ((D$_6$)DMSO): 2.24 (*s*, MeC=N); 3.10 (*m*, 2 H–C(5')); 3.19 (*s*, Me_2N); 3.69 (*s*, MeO); 4.14 (*m*, H–C(4')); 4.51 (*m*, H–C(3')); 4.86 (*m*, H–C(2')); 5.28 (*d*, *J* = 6.2, OH–C(3')); 5.68 (*d*, *J* = 5.1, OH–C(2')); 6.25 (*d*, H–C(1')); 6.71–7.26 (*m*, 13 arom. H); 8.54 (*s*, H–C(5)). Anal. calc. for $C_{34}H_{37}N_7O_6$ (639.7): C 63.84, H 5.83, N 15.33; found: C 63.64, H 5.84, N 15.31.

N'-*{3-[5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-2'-*O*-[tris(1-methylethyl)silyl]-β-*D*-ribofuranosyl}-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-yl}*-N,N-*dimethylethanimidamide* (**12**) *and* N'-*{3-[5'-*O*-(4,4'-Dimethoxytriphenylmethyl)-3'-*O*-[tris(1-methylethyl)silyl]-β-*D*-ribofuranosyl}-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-yl}*-N,N-*dimethylethanimidamide* (**13**). To a soln. of **11** (0.35 g, 0.55 mmol) in anh. pyridine (4 ml), $AgNO_3$ (140 mg, 0.82 mmol) and (i-Pr)$_3$SiCl (145 µl, 0.69 mmol) – dissolved in THF (5 ml) – are added under Ar. The mixture is stirred in the dark at r.t. After 24 h, another portion of (i-Pr)$_3$SiCl (120 µl, 0.55 mmol) is added, and stirring is continued for another 48 h at r.t. Precipitated AgCl is filtered off and washed with a small amount of THF. To the filtrate, a sat. aq. $NaHCO_3$ soln. (10 ml) is added, and the mixture is extracted with CH_2Cl_2 (4 × 10 ml). Drying (Na_2SO_4) of the org. phase and evaporation give 0.60 g of a slightly yellowish oil which is purified by FC (3 × 20 cm, *H*): 0.31 g (71%) of **12**, followed by 80 mg (18%) of **13**.

Data of **12**: Colorless foam. TLC (silica gel, *G*): R_f 0.3. UV (MeOH): 234 (29600), 274 (6800), 325 (25900). ^{1}H-NMR ((D$_6$)DMSO): 0.83–0.97 (*m*, 3 Me_2CH); 2.24 (*s*, MeC=N); 3.10 (*m*, 2 H–C(5')); 3.19 (*s*, Me_2N); 3.70 (*s*, 2 MeO); 4.19 (*m*, H–C(4')); 4.43 (*m*, H–C(3')); 5.21 (*t*, *J* = 4.4, H–C(2')); 5.27 (*d*, *J* = 6.3, OH–C(3')); 6.30 (*d*, *J* = 4.3, H–C(1')); 6.75–7.35 (*m*, 13 arom. H); 8.53 (*s*, H–C(5)). Anal. calc. for $C_{43}H_{57}N_7O_6Si$ (796.0): C 64.83, H 7.23, N 12.32; found: C 64.94, H 7.37, N 12.13.

Data of **13**: Colorless foam. TLC (silica gel, *G*): R_f 0.15. UV (MeOH): 234 (29800), 274 (7400), 327 (24700). ^{1}H-NMR ((D$_6$)DMSO): 0.98 (*s*, 3 Me$_3$CH); 2.19 (*s*, MeC=N); 3.10 (*m*, 2 H–C(5')); 3.18 (*s*, Me$_2$N); 3.68 (*s*, 2 MeO); 4.17 (*m*, H–C(4')); 4.92 (*m*, H–C(3'), H–C(2')); 5.64 (*d*, *J* = 5.1, OH–C(2')); 6.27 (*d*, *J* = 6.0, H–C(1')); 6.70–7.20 (*m*, 13 arom. H); 8.55 (*s*, H–C(5)).

*7-{[1-(Dimethylamino)ethylidene]amino}-3-{5-O'-(4,4'-dimethoxytriphenylmethyl)-2'-O-[tris(1-methylethyl)silyl]-β-*D*-ribofuranosyl}-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine 3'-[Triethylammonium phosphonate]* (**2**). To a soln. of PCl_3 (114 µl, 1.3 mmol) and *N*-methylmorpholine (1.43 ml, 13 mmol) in anh. CH_2Cl_2, 1*H*-1,2,4-triazole (0.67 g, 9.62 mmol) is added under Ar. After stirring for 30 min at r.t., the mixture is cooled to 0°, and **12** (210 mg, 0.26 mmol) – dissolved in anh. CH_2Cl_2 (2.5 ml) – is added dropwise. The soln. is stirred for another 20 min at 0° and then hydrolyzed by addition of 1M $(Et_3NH)HCO_3$ buffer (pH 7–8). The aq. layer is extracted with CH_2Cl_2 (3 × 20 ml), the combined org. phase dried (Na_2SO_4) and evaporated, and the residue submitted to FC (silica gel *60*, 3 × 10 cm, *E*). The main zone is pooled and evaporated, the residue dissolved in CH_2Cl_2 (20 ml), and the soln. extracted with 0.1M $(Et_3NH)HCO_3$ buffer (pH 7–8) (4 × 5 ml). The org. layer is dried (Na_2SO_4) and evaporated: 0.21 g (84%) of colorless foam. TLC (silica gel, *E*): R_f 0.6. UV (MeOH): 234 (27300), 274 (11700), 325 (16400). ^{1}H-NMR ((D$_6$)DMSO): 0.75–0.95 (*m*, 3 Me$_3$CH); 1.15, 2.99 (2*m*, *MeCH*$_2$N); 2.24 (*s*, MeC=N); 3.20 (*s*, Me$_2$N); 3.69 (*s*, 2 Me); 4.40 (*m*, H–C(4')); 4.79 (*m*, H–C(3')); 5.44 (*m*, H–C(2')); 5.50, 7.91 (*d*, *J*(P,H) = 602, PH); 6.27 (*d*, *J* = 6.0, H–C(1')); 6.76–7.40 (*m*, 13 arom. H); 8.50 (*s*, H–C(5)); 10.90 (br. *s*, NH). ^{31}P-NMR ((D)$_6$DMSO): 2.55 (*dd*, ^1J(P,H) = 602, ^3J(P,H) = 9.5).

*N'-[3-(2'-Deoxy-β-*D*-*erythro*-pentofuranosyl)-3H-1,2,3-triazolo[4,5-*d*]pyrimidin-7-yl]-*N,N*-dimethylethanimidamide* (**15**). A suspension of 8-aza-2'-deoxyadenosine (**1b**; 100 mg, 0.40 mmol) in abs. MeOH (2 ml) is treated with *N,N*-dimethylacetamide dimethyl acetal (90% purity; 0.17 ml, 1.05 mmol). After stirring for 16 h at r.t. the clear yellowish soln. is evaporated and the resulting oil submitted to FC (silica gel *60*, 9 × 13 cm, *B*): colorless foam (117 mg, 91%). TLC (silica gel, *C*): R_f 0.25. UV (MeOH): 236 (10500), 324 (24700). ^{1}H-NMR ((D$_6$)DMSO): 2.26 (MeC=N); 2.41 (*m*, H$_\beta$–C(2')); 3.05 (*m*, H$_\alpha$–C(2')); 3.18 (*s*, Me$_2$N); 3.39, 3.56 (2*m*, 2 H–C(5')); 3.89 (*m*, H–C(4')); 4.55 (*m*, H–C(3')); 4.86 (*t*, *J* = 5.5, OH–C(5')); 5.40 (*d*, *J* = 4.2, OH–C(3')); 6.64 (*d*, *J* = 6.2, H–C(1')); 8.53 (*s*, H–C(5)). Anal. calc. for $C_{13}H_{19}N_7O_3$ (321.34): C 48.59, H 5.96, N 30.51; found: C 48.75, H 5.98, N 30.26.

*3-[2'-Deoxy-5-O'-(4,4'-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-3H-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine* (**16**). A suspension of 8-aza-2'-deoxyadenosine (**1b**; 978 mg, 3.88 mmol) in anh. pyridine (3 ml) is treated with 4,4'-dimethoxytriphenylmethyl chloride (1.95 g, 5.75 mmol). After stirring at r.t. for 1 h, MeOH is added, and after additional stirring for 10 min, the mixture is cooled in an ice bath and hydrolyzed with 5% aq. $NaHCO_3$ soln. (5 ml). The mixture is extracted with AcOEt (2 × 20 ml), the combined org. phase washed with H_2O (10 ml) and brine (3 × 10 ml), dried ($MgSO_4$), and evaporated, and the residue submitted to FC (silica gel *60*, 6 × 20 cm, *I*): colorless foam (1.58 g, 73%). TLC (silica gel, *C*): R_f 0.43. UV (MeOH): 235 (21000), 276 (11800). ^{1}H-NMR ((D$_6$)DMSO): 2.49 (*m*, H$_\beta$–C(2')); 3.08 (*m*, H$_\alpha$–C(2')), 2 H–C(5')); 3.71 (*s*, 2 MeO); 4.04 (*m*, H–C(4')); 4.68 (*m*, H–C(3')); 5.42 (*d*, *J* = 4.6, OH–C(3')); 6.67 (*m*, H–C(1')); 6.70–7.27 (*m*, 13 arom. H); 8.12, 8.44 (2*s*, NH$_2$); 8.31 (*s*, H–C(5)). Anal. calc. for $C_{30}H_{30}N_6O_5$ (554.61): C 64.97, H 5.45, N 15.15; found: C 65.25, H 5.70, N 14.89.

*N'-{3-[2'-Deoxy-5-O'-(4,4'-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-3H-1,2,3-triazolo-[4,5-*d*]pyrimidin-7-yl}-N,N-dimethylethanimidamide* (**17**). To a soln. of **16** (1.58 g, 2.85 mmol) in MeOH (16 ml) is added *N,N*-dimethylacetamide dimethyl acetal (1.25 ml, 8.55 mmol), and the mixture is allowed to stir at r.t. for 16 h. Then, the mixture is evaporated and the residue submitted to FC (silica gel *60*, 6 × 10 cm, *B*): 1.66 g (93%) of colorless foam. TLC (silica gel, *B*): R_f 0.21. UV (MeOH): 235 (22200), 325 (19400). ^{1}H-NMR ((D$_6$)DMSO): 2.24 (*s*, MeC=N); 2.50 (*m*, H$_\beta$–C(2')); 3.08 (*m*, H$_\alpha$–C(2'), 2 H–C(5')); 3.21 (*s*, Me$_2$N); 3.70, 3.71 (2*s*, 2 MeO); 4.05 (*m*, H–C(4')); 4.69 (*m*, H–C(3')); 5.44 (*d*, *J* = 5.0, OH–C(3')); 6.71 (*m*, H–C(1')); 6.69–6.77 (*m*, arom. H); 7.13–7.27 (*m*, arom. H); 8.55 (*s*, H–C(5)). Anal. calc. for $C_{34}H_{37}N_7O_5$ (623.72): C 65.47, H 5.98, N 15.72; found: C 65.45, H 6.20, N 15.69.

*3-[2'-Deoxy-5'-O-(4,4'-dimethoxytriphenylmethyl)-β-*D*-*erythro*-pentofuranosyl]-7-[(1-dimethylamino)ethylidene]amino-3H-1,2,3-triazolo[4,5-*d*]pyrimidine 3'-[(2-Cyanoethyl) Diisopropylphosphoramidite]* (**3**). A soln. of **17** (397 mg, 0.64 mmol) and (i-Pr)$_2$EtN (0.33 ml, 1.9 mmol) in anh. CH_2Cl_2 (15 ml) is treated dropwise with (2-cyanoethyl diisopropylphosphoramidochloridite (0.43 ml, 1.9 mmol). After stirring at r.t. for 2 h, the reaction is quenched by adding 5% aq. $NaHCO_3$ soln. (10 ml). Then, the aq. layer is extracted with CH_2Cl_2 (2 × 10 ml), the combined org. phase washed with brine (10 ml), dried ($MgSO_4$), and evaporated, and the resulting oil applied to FC (silica gel, 9 × 3 cm, *F*): **3** (444 mg, 92%). Colorless foam. TLC (silica gel, *C*): R_f 0.5. ^{1}H-NMR ($CDCl_3$): 1.06–1.27 (*m*, *Me*$_2$CH); 2.43 (*m*, H$_\beta$–C(2')); 2.53–2.76 (*m*, *CH*$_2$CH$_2$CN); 3.15–3.40 (*m*, Me$_2$N, H$_\alpha$–C(2'), CH$_2$*CH*$_2$CN); 3.48–3.88 (*m*, 2 H–C(5')); 3.74 (*s*, MeO); 4.29 (*m*, H–C(4')); 4.92 (*m*, H–C(3')); 6.65–6.82, 7.10–7.33 (*m*, H–C(1'), arom. H); 8.59 (*s*, H–C(5)). ^{31}P-NMR ($CDCl_3$): 149.4, 149.2.

N-*[3-(2'-Deoxy-β-*D-erythro-*pentofuranosyl)-3*H-*1,2,3-triazolo[4,5-*d*]pyrimidin-7-yl]-2-methylpropanamide* (**14**). 8-Aza-2'-deoxyadenosine (**1b**; 100 mg, 0.40 mmol) is dried 3 times by co-evaporation with anh. pyridine and then suspended in anh. pyridine (2 ml). Me_3SiCl (0.25 ml, 2.0 mmol) is added at r.t. After stirring for 15 min, the soln. is treated with isobutyric anhydride (0.33 ml, 2.0 mmol) and maintained at r.t. for 3 h. The mixture is then cooled in an ice bath, and H_2O (0.4 ml) is added. After 5 min, 25% aq. NH_3 soln. (0.4 ml) is added, and stirring is continued for 15 min. The mixture is diluted with H_2O (5 ml) and extracted with AcOEt (3 × 10 ml), the combined org. phase washed with H_2O (2 × 5 ml) and brine (5 ml), dried ($MgSO_4$), and evaporated and the crude product purified by FC (silica gel, 3 × 10 cm, *B*): 34 mg (26%) of **14**. Colorless foam. TLC (silica gel, *C*): R_f 0.25. UV (MeOH): 279 (11900). ^{1}H-NMR ((D$_6$)DMSO): 1.16 (*d*, *J* = 6.8, Me); 2.47 (*m*, H_β–C(2')); 2.98 (*m*, H_α–C(2')); 3.09 (*m*, CH); 3.35, 3.55 (2*m*, 2 H–C(5')); 3.91 (*m*, H–C(4')); 4.59 (*m*, H–C(3')); 4.77 (br. *s*, OH–C(5')); 5.44 (*s*, OH–C(3')); 6.73 (*t*, *J* = 6.1, H–C(1')); 8.86 (*s*, H–C(5)); 11.41 (br. *s*, NH). Anal. calc. for $C_{13}H_{18}N_6O_4$ (322.3): C 48.44, H 5.63, N 26.07; found: C 48.56, H 5.62, N 25.95.

N-*{3-[2'-Deoxy-5'-*O*-(4,4'-dimethoxytriphenylmethyl)-β-*D*-erythro-pentofuranosyl]-3*H*-1,2,3-triazolo-[4,5-*d*]pyrimidin-7-yl}-2-methylpropanamide* (**18**). As described for **14**, but with **16** (233 mg, 0.42 mmol), Me_3SiCl (0.27 ml, 2.10 mmol), and isobutyric anhydride (0.35 ml, 2.10 mmol). FC (silica gel *60*, 6 × 10 cm, *A*) gave 129 mg (49%) of **8**. Colorless foam. TLC (silica gel, *B*): R_f 0.17. UV (MeOH): 235 (17400), 275 (14400). ^{1}H-NMR ((D$_6$)DMSO): 1.16 (*d*, *J* = 6.4, Me); 2.51 (*m*, H_β–C(2')); 3.07 (*m*, H_α–C(2'), 2 H–C(5')); 3.70, 3.71 (2*s*, 2 MeO); 4.08 (*m*, H–C(4')); 4.68 (*m*, H–C(3')); 5.48 (br. *s*, OH–C(3')); 6.69–6.81 (*m*, H–C(1'), arom. H); 7.11–7.25 (*m*, arom. H); 8.85 (*s*, H–C(5)); 11.42 (br. *s*, NH). Anal. calc. for $C_{34}H_{36}N_6O_6$ (624.7): C 65.37, H 5.81, N 13.45; found: C 65.48, H 5.85, N 13.44.

*7-[(2-Methyl-1-oxopropyl)amino]-3-[2'-deoxy-5'-*O*-(4,4'-dimethoxytriphenylmethyl)-β-*D*-erythro-pentofuranosyl]-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine 3'-[(2-Cyanoethyl) Diisopropylphosphoramidite]* (**19**). The phosphoramidite **19** was prepared as described for **3**, but with **18** (250 mg, 0.40 mmol), (i-Pr)$_2$EtN (*Hünig*'s base; 210 µl, 1.20 mmol), and 2-cyanoethyl diisopropylphosphoramidochloridite (270 µl, 1.20 mmol): Colorless foam (127 mg, 38%). TLC (silica gel, *C*): R_f 0.5. ^{1}H-NMR ($CDCl_3$): 1.16 (*m*, Me); 1.31 (*d*, *J* = 6.8, Me); 2.44 (*m*, H_β–C(2')); 2.66 (*m*, CH_2CH_2CN); 3.16–3.37 (*m*, CH, H_α–C(2'), CH_2CH_2CN); 3.52–3.88 (*m*, 2 H–C(5')); 3.74 (*s*, MeO); 4.32 (*m*, H–C(4')); 4.92 (*m*, H–C(3')); 6.65–6.72, 7.12–7.31 (*m*, arom. H); 6.81 (*m*, H–C(1')); 8.76 (*s*, H–C(5)). ^{31}P-NMR ($CDCl_3$): 149.6, 149.4.

3. *Oligonucleotide Synthesis and Purification.* 3.1. *Oligoribonucleotides.* The oligoribonucleotide synthesis was performed on a 1-µmol scale using the phosphonate **2** as well as that of uridine (*ABI-381-A* synthesizer, *Applied Biosystems*, Weiterstadt, Germany) applying the 'trityl-off' mode. Cleavage of the oligonucleotides from the solid support was made using 25% aq. NH_3 soln./EtOH 3:1 (16 h). For hydrolysis of the base-protecting groups of (A-U)$_6$, the ammoniacal soln. was heated for 16 h to 55°; in case of (z^{8}A-U)$_6$ (**36**), the soln. was heated to 40° for 3 h. Subsequently, both solns. were evaporated at r.t. and then co-evaporated with abs. EtOH. Cleavage of the silyl groups was performed by 1M Bu_4NF/THF within 16 h (r.t.).

Pre-desalting. A *Quiagen-tip-500* anion-exchange column was equilibrated with 0.1M (Et_3NH)HCO_3 (5 ml) and then loaded with the corresponding oligomer soln. After washing with the same buffer (5 ml), the RNA was eluted with 1M (Et_3NH)HCO_3. The eluate was fractionated (TLC plates with fluorescence indicator), and appropriated fractions were pooled and evaporated *in vacuo* (*Speed Vac*, *Savant Instruments*, Farmingdale, USA).

Preparative HPLC. Next, the oligoribonucleotides were dissolved in 1% aq. diethyl pyrocarbonate (DEPC) soln. (400 µl), heated to 95° for 2 min, and then quickly cooled to 0°. Thereupon, the oligomer soln. was purified by prep. HPLC (20 × 1 cm, *RP-18*, *LiChrosorb*) in portions of 50–100 µl. Solvent systems: *A*, 0.1M (Et_3NH)OAc (steril, pH 7.5)/MeCN 95:5; *B*, MeCN. System I: linear gradient, 20 min 0–20% *B* in *A*. System II: 30 min 0–20% *B* in *A*. Retention times: (A-U)$_6$, 15.5 min with system I, (z^{8}A-U)$_6$, 28.6 min with system II (flow rate 1 ml/min).

Desalting. RP-18 Cartridges (*Oligopak*, *Millipore*, Eschborn, Germany) were sterilized and equilibrated with MeCN (5 ml) and 0.05M (Et_3NH)OAc/MeCN 1:1 (5 ml), followed by 0.05M (Et_3NH)OAc (5 ml). Thereupon, the corresponding oligomer soln. was loaded onto the column and washed with 0.05M (Et_3NH)OAc (5 ml). The oligoribonucleotides were eluted with MeOH/MeCN/H_2O 1:1:1 in 1-ml fractions. The oligomer-containing fractions (anal. HPLC (*RP-18*) monitoring) were pooled and evaporated as described above. After lyophilization, they were stored at –25°. Yields: (A-U)$_6$, 1.8 A_{260} units (λ_{max}, 258 nm); (z^{8}A-U)$_6$, 2.2 A_{260} units (λ_{max}, 265 nm).

3.2. *Enzymatic Hydrolysis of the Oligoribonucleotides.* The oligonucleotides (0.2 A_{260} units, each) were dissolved in *Tris* · HCl buffer (0.1M, pH 8.3, 200 µl), and snake-venom phosphodiesterase (4 µg) was added. After incubation for 30 min at 37°, alkaline phosphatase (3 µg) was added, and the soln. was kept at 37° for another 15 min. The resulting nucleosides were analyzed and quantified by anal. HPLC (*RP-18*, 20 × 0.5 cm; mobile phase, 0.1M (Et_3NH)HCO_3/MeCN 95:5; flow rate 1 ml/min). Retention times of the nucleosides: A, 11.4 min; z^{8}A,

10.0 min; I, 4.8 min; U, 3.6 min. For quantification, the corresponding peak areas of the elution profiles were compared using the following nucleoside extinction coefficients: A, 15300; z^8A, 7100; I, 7400; U, 10200 (260 nm) (*Table 8*).

3.3. *Oligodeoxyribonucleotides.* The syntheses were carried out on a DNA synthesizer, model *392* (*Applied Biosystems*, Weiterstadt, Germany) using the corresponding phosphoramidites on a 1-μmol scale. Purification of the oligonucleotides was performed using oligonucleotide purification cartridges (*OPC*, *Applied Biosystems*). Desalting was made on reversed-phase columns (*RP-18*, 5 × 20 mm). Occasionally, some of the oligodeoxynucleotides were further purified on a reversed-phase column (*RP-18*, 5 × 20 mm; linear gradient: 0–30 min, 20–80% of 0.1M *Tris* · HCl/MeCN 95:5 (pH 7) in MeCN; flow rate, 0.75 ml/min). The following extinction coefficients (ε_{260}) were used to calculate the ε_{260} values of the oligonucleotides: A_d, 15200; T_d, 8800; C_d, 7300; G_d, 11700; z^8A and z^8A_d, 7100. For the enzymatic oligodeoxynucleotide hydrolysis, see above.

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