2′-Ethynyl-DNA: Synthesis and Pairing Properties

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2-Ethynyl-DNA was developed as a potential DNA-selective oligonucleotide analog. The synthesis of 2′-*arabino*-ethynyl-modified nucleosides was achieved starting from properly protected 2′-ketonucleosides by addition of lithium (trimethylsilyl)acetylide followed by reduction of the tertiary alcohol. After a series of protecting-group manipulations, phosphoramidite building blocks suitable for solid-phase synthesis were obtained. The synthesis of oligonucleotides from these building blocks was successful when a fast deprotection scheme was used. The pairing properties of 2′-*arabino*-ethynyl-modified oligonucleotides can be summarized as follows: *1*) The 2′-*arabino*-ethynyl modification of pyrimidine nucleosides leads to a strong destabilization in duplexes with DNA as well as with RNA. The likely reason is that the ethynyl group sterically influences the torsional preferences around the glycosidic bond leading to a conformation not suitable for duplex formation. *2*) If the modification is introduced in purine nucleosides, no such influence is observed. The pairing properties are not or only slightly changed, and, in some cases (deoxyadenosine homo-polymers), the desired stabilization of the pairing with a DNA complementary strand and destabilization with an RNA complement is observed. *3*) In oligonucleotides of alternating deoxycytidine-deoxyguanosine sequence, the incorporation of 2′-*arabino*-ethynyl deoxyguanosine surprisingly leads to the formation of a left-handed double helix, irrespective of salt concentration. The rationalization for this behavior is that the ethynyl group locks such duplexes in a left-handed conformation through steric blockade.

Introduction. – In general, modified oligonucleotides, like regular oligodeoxynucleotides, form only slightly stronger hybrids with complementary RNA than with DNA [1]. Notable exceptions are anhydrohexitol oligonucleotides (*O*-4′-homo-DNA), 2′,5′-linked DNA, and 2′,3′-bridged DNA, which, in certain sequence contexts, bind much more strongly to RNA [2–4]. However, rarely have attempts been made to design oligonucleotides that exclusively bind a DNA but not an RNA complement [5][6]. DNA Specificity would be highly desirable to improve the efficiency of oligonucleotide drugs. There is only one active copy of a disease-related gene within a cell but many more RNA transcripts. This, however, requires a DNA-selective oligonucleotide to be capable of strand invasion or, alternatively, to interfere at a replication fork or the site of transcription. Other possible applications include oligonucleotide-based DNA sensors in probes where both nucleic acids might be present or DNA-selective priming.

The design of such oligonucleotide analogs relies on the premise that DNA should be an easier target than RNA, since DNA double helices are capable of adopting several different conformations, whereas RNA is confined to A-form duplexes [7]. Selectivity for a DNA complement should thus be possible if an A-form conformation of the resulting double helix is not accessible (*Fig. 1*). This should be feasible by modifying individual nucleosides since the overall duplex conformation is dictated by the sugar-phosphate backbone conformation. Preorganization of the (deoxy)ribose moiety towards a C(2′)-*endo* (B-form) conformation, should additionally, lead to more-

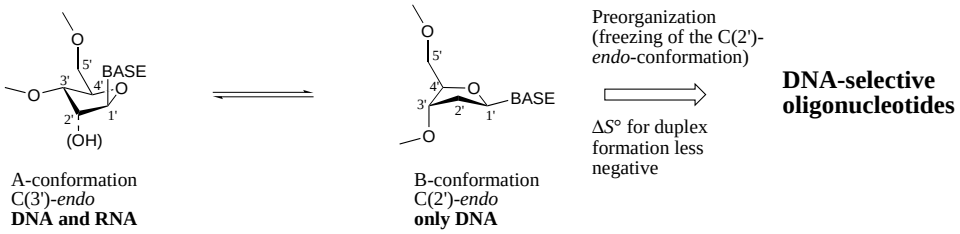

Fig. 1. *Design of oligonucleotide analogs that selectively form duplexes with complementary DNA strands.* RNA Duplexes are confined to the A-form conformation; thus, selectivity for a DNA complement might be possible if an A-form conformation of the resulting double helix is not accessible. This could be realized by preorganizing the (deoxy)ribose moiety in a C(2′)-*endo*-conformation, which, additionally, should lead to more-stable duplexes. The freezing of internal degrees of freedom will render the loss in entropy upon duplexation less negative.

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Preorganizing the sugar moiety has been realized in several ways [8]. The flexible furanose ring can be rigidified by extending it to a pyranose ring or by bridging two of the ring positions. The most striking example of the effects of conformational preorganization so far has been LNA (locked nucleic acid) [9]. The additional bridge between C(4′) and O(2′) of a ribonucleoside leads to oligonucleotides with greatly enhanced thermal stabilities. But, as expected, only a slight preference for RNA complements could be observed.

To restrict the conformational freedom of individual nucleosides in a B-form double helix, we sought to introduce an *arabino*-configured ethynyl substituent at the C(2′) position of deoxynucleosides (*Figs. 2* and **1**–**5** in *Fig. 3*). We assumed that, in such oligodeoxynucleotides, the individual nucleosides are adopting a C(2′)-*endo* conformation typical of B-form DNA double helices because the alternative C(3′)-*endo* (A-form RNA or DNA) conformation would lead to sterically unfavorable interactions of the ethynyl group with the phosphodiester residue or the nucleobase of the 3′-neighboring nucleotide (*Fig. 2*). The same reasoning would imply that the inverse configuration of the ethynyl residue should favor an RNA-like conformation. While several modifications are known to preorganize an oligonucleotide on a mononucleo-

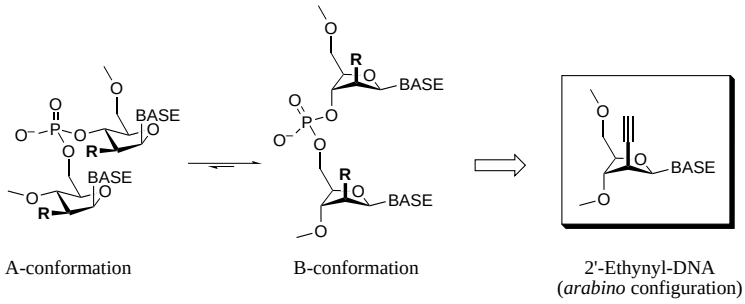
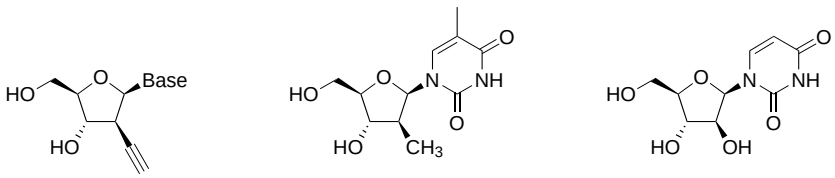


Fig. 2. *Design rationale for 2′-ethynyl-DNA*



1 Base = Uracil **6** **7**
2 Thymine
3 Cytosine
4 Adenine
5 Guanine

Fig. 3. *Structures of 2′-deoxy-2′-ethynyl*-arabino-*nucleosides used in this study and of related arabinonucleosides*

tide level [8], this modification is expected to maintain a certain duplex conformation through steric interactions in a dinucleotide step within the duplex.

The ethynyl residue which should act as a conformational stopper was chosen for two reasons. First, the residue should be as small as possible, and, second, it must not form H-bonds. Studies on oligonucleotides containing 2′-methyl-*arabino*-thymidine (**6**) had shown that the binding to complementary RNA strands is greatly diminished [10]. Unfortunately, no data on the corresponding duplex with complementary DNA was given. If the Me group is *ribo*-configured, the pairing of the corresponding oligonucleotides to an RNA complement is also weaker, although, not quite as much as in the former case. This contrasts with our predictions that 2′-methyl-*ribo*-thymidine would restrict a duplex in the A-form. The Me group might simply be sterically too demanding – an ethynyl residue, the smallest possible alkyl substituent, was, therefore, chosen for this study.

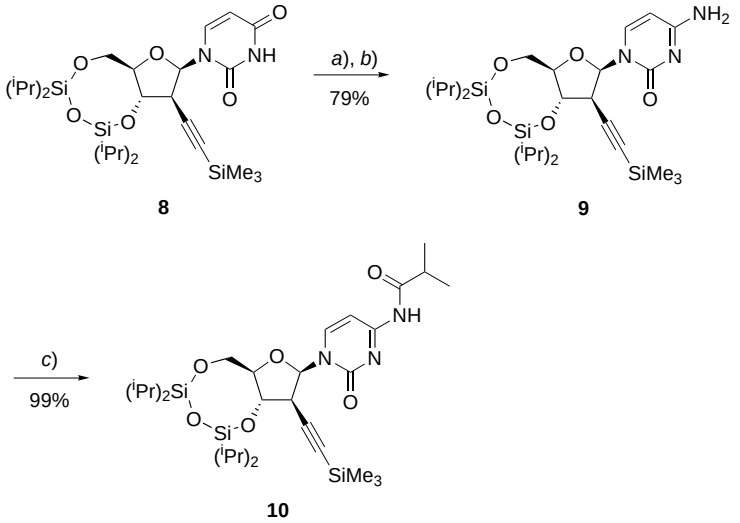
A configurationally similar modification is present in oligoarabinonucleotides [11]. The thermal stability of duplexes containing arabinonucleosides, such as **7**, is greatly diminished compared to the DNA or RNA references, however. The reason may be the fact that an *arabino*-configured electronegative substituent at the 2′-position is in an unfavorable antiplanar arrangement of the 3′-OH group when the nucleoside adopts a C(2′)-*endo* conformation. Arabinonucleosides, therefore, likely prefer a O(4′)-*endo* conformation [12], which may be incompatible with both A- and B-form duplexes.

Results and Discussion. – *Nucleoside Synthesis.* The pyrimidine nucleosides **1**–**3**, *i.e.* 2′-deoxy-2′-ethynyl-*arabino*-uridine, 2′-ethynyl-*arabino*-thymidine, and 2′-deoxy-2′-ethynyl-*arabino*-cytidine, have previously been synthesized as potential antiviral agents by *Matsuda* and co-workers [13][1]). The exocyclic NH_2 group of silyl-protected cytidine derivative **9** (*Scheme 1*) – obtained from the known uridine derivative **8** [13] – was protected as isobutyramide (→**10**), a protecting group compatible with automated oligonucleotide synthesis and more readily removed than the traditional benzamide.

For the synthesis of the corresponding 2′-ethynylpurine nucleosides **4** and **5**, we pursued a strategy analogous to the synthesis of **8** (*Scheme 2*). Oxidation of **13**

[1]) Preparation of the 2′-deoxy-2′-ethynyl-*arabino*-adenosine (**4**) and -guanosine (**5**) has been claimed before as well [14]. However, we were not successful in reproducing this procedure.

Scheme 1. *Synthesis of Protected 2′-Deoxy-2′-ethynylcytidine* **10** *from the Corresponding Uridine Derivative*



a) $^{i}Pr_3C_6H_2SO_2Cl$, *N*,*N*-dimethylpyridin-4-amine (DMAP), Et_3N, MeCN, r.t. 20 h. *b*) aq. NH_3 soln., r.t. 3 h. *c*) $(^{i}PrCO)_2O$, pyridine, r.t., 16 h.

(obtained from **11** *via* **12**) [15], followed by lithium acetylide addition to **14** and radical deoxygenation of the intermediate tertiary alcohol **15** led to **16** in a stereoselective manner (*vide infra*). Phenoxyacetyl protection of the exocyclic NH_2 group[2]) then gave 2′-deoxy-2′-ethynyl-*arabino*-adenosine derivative **17**.

The synthesis of protected 2′-deoxy-2′-ethynylguanosine **22** was almost identical (*Scheme 3*). Guanosine (**18**) was converted in 76% yield to the 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS)-protected derivative **19** in two steps [17]. Oxidation of the unprotected OH group in **19** with *Dess-Martin* periodinane resulted in the isolation of a mixture of ketone **20** and its hydrate in a 1:1 ratio[3]). This mixture was treated with lithium (trimethylsilyl)acetylide at $-78°$ to give **21**. The addition proceeded with very high selectivity, and only one diastereoisomer could be isolated in 80% yield. Removal of the tertiary alcohol function was achieved by radical reduction. Compound **21** was esterified with methoxalyl chloride (=chlorooxoacetic acid methyl ester) to the corresponding mixed oxalic acid diester, which was then reduced with Bu_3SnH to give **22** in 65% yield.

The configuration of **22** was deduced from ^{1}H-NMR coupling constants in the sugar moiety. These were the same as for the corresponding adenosine derivative **17** for which an X-ray crystal structure could be obtained (*Fig. 4*) [18]. NOE Experiments

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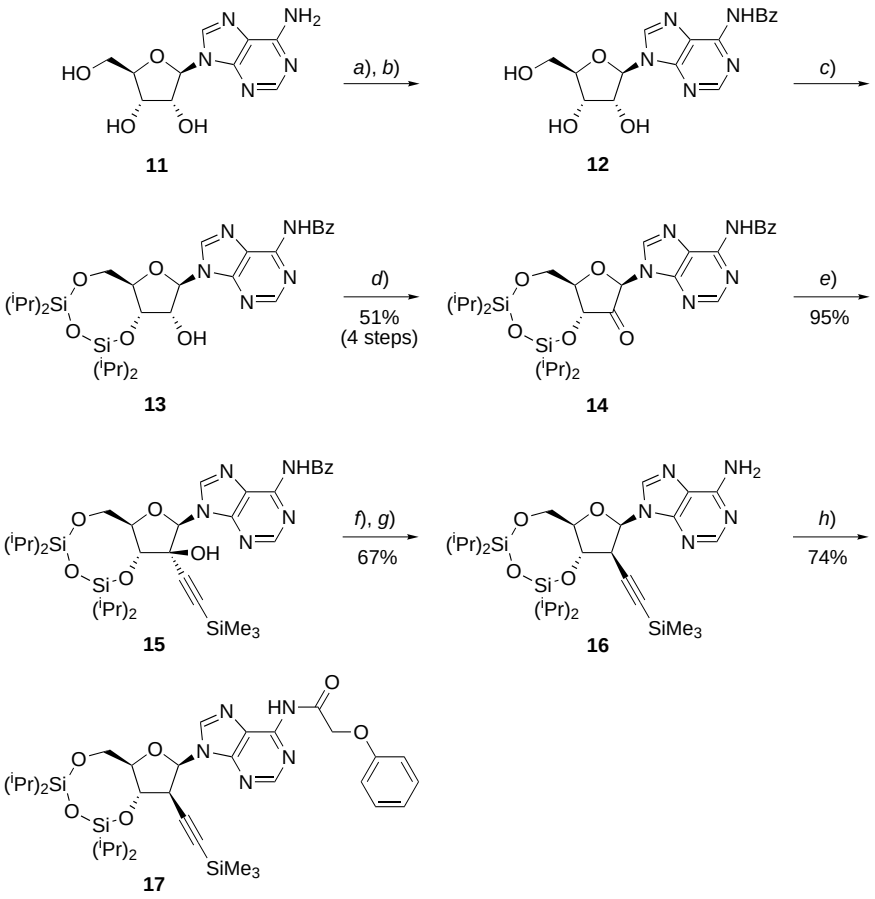
[2]) Phenoxyacetyl protection was performed analogously to the (benzyloxy)carbonyl protection of a sugar-protected adenosine derivative described in [16]. The initially chosen benzoyl protecting group required prolonged ammonia deprotection of the adenine-containing oligonucleotides, leading to β-elimination of the 3′-phosphodiester due to the electron-withdrawing nature of the ethynyl residue.

[3]) Attempts to isolate the pure ketone by chromatography or the addition of drying agents failed. Therefore, the 1:1 mixture was directly used in the next step without further purification.

Scheme 2. *Synthesis of 2′-Deoxy-2′-ethynyladenosine* **17**



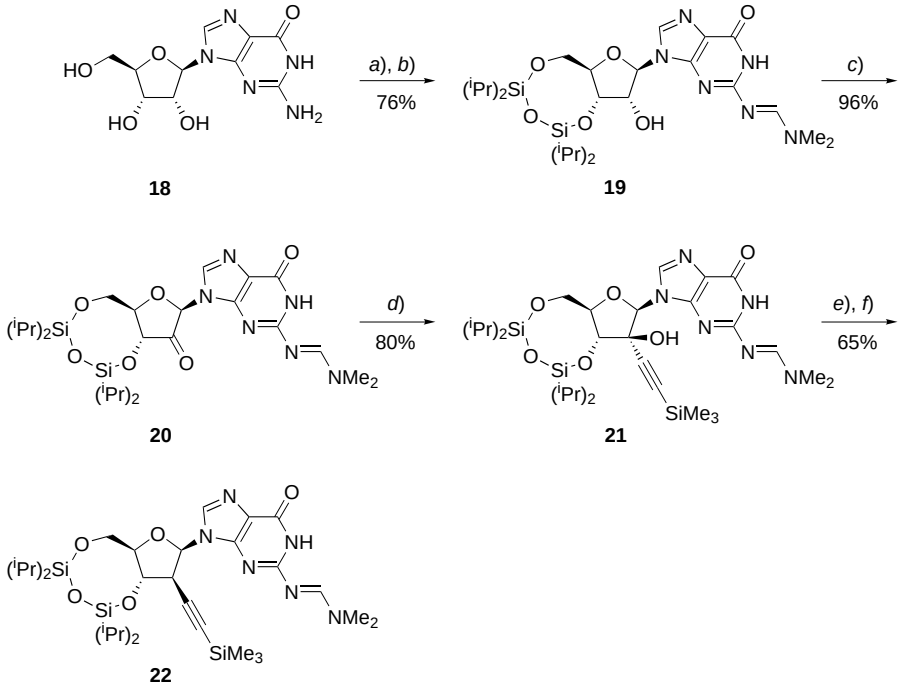
a) BzCl, pyridine. *b*) NaOMe, MeOH. *c*) $(^iPr_2SiCl)_2O$, pyridine, r.t., 16 h. *d*) *Dess-Martin* periodinane, CH_2Cl_2, r.t., 24 h. *e*) $Me_3SiC{\equiv}CH$, BuLi, THF, $-78°$, 3 h. *f*) MeO_2CCOCl, DMAP, CH_2Cl_2, r.t., 10 h. *g*) Bu_3SnH, 2,2′-dimethyl-2,2′-azobis[propanenitrile] (AIBN),toluene, 95°, 3 h. *h*) 1-(phenoxyacetyl)-1*H*-imidazole, $(Et_3O)BF_4$, CH_2Cl_2, r.t., 19 h.

with compound **25**, obtained from **22** after treatment with Bu_4NF (*Scheme 4*) further supported the configurational assignment.

The sugar moiety of 2′-ethynyl-nucleosides **8**, **10**, **17**, **22**, and **23** was deprotected by reaction with Bu_4NF and AcOH yielding **7**, **26**, **24**, **25**, and **6**, respectively (*Scheme 4*)[4]). Fluoride treatment simultaneously removed the silyl-ether moiety as well as the C-bound Me_3Si group. The (partially) deprotected nucleosides were then converted to their 5′-*O*-dimethoxytrityl derivatives **27**–**31** and finally to the corresponding

[4]) The addition of AcOH to the reaction mixture was crucial, otherwise considerable degradation of the free nucleosides took place. The ethynyl residue presumably enhances β-elimination of the 3′-OH group when the reaction mixture is not buffered.

Scheme 3. *Synthesis of TIPDS-Protected-2′-Deoxy-2′-ethynylguanosine* **22**



a) $Me_2NCH(OMe)_2$, MeOH, r.t. *b*) $(^iPr_2SiCl)_2O$, pyridine, r.t., 16 h. *c*) *Dess-Martin* periodinane, CH_2Cl_2, r.t., 15 h. *d*) $Me_3SiC{\equiv}CH$, BuLi, THF, $-78°$, 90 min. *e*) MeO_2CCOCl, DMAP, CH_2Cl_2, r.t., 4 h. *f*) Bu_3SnH, AIBN, toluene, 95°, 2 h.

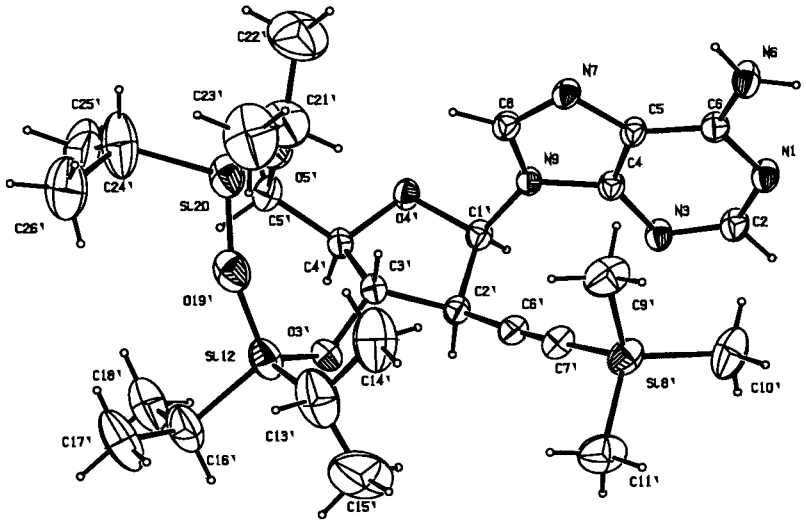
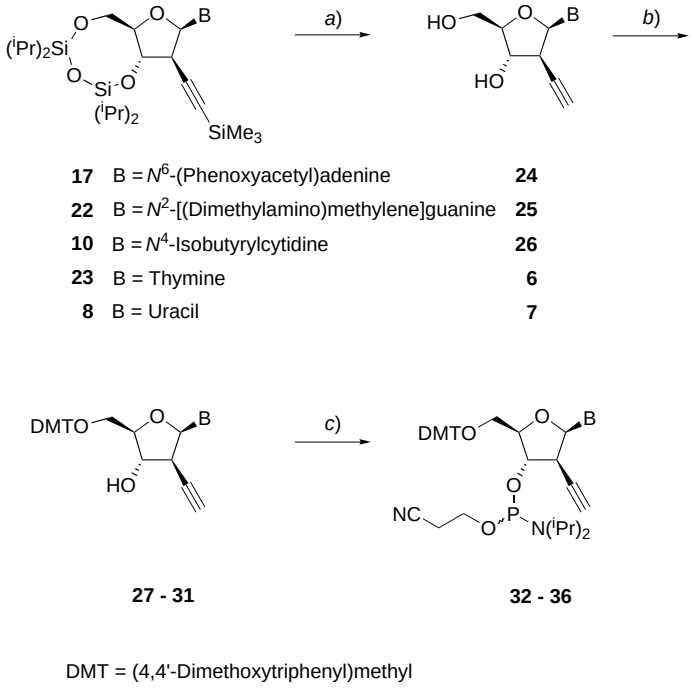


Fig. 4. *Single-crystal X-ray structure of* arabino-*adenosine derivative* **16** *establishing the configuration of the ethynyl residue at C(2′)*

Scheme 4. *Synthesis of 2′-Deoxy-2′-ethynylnucleoside Phosphoramidites* **32**–**36**



a) Bu_4NF, AcOH, THF, r.t., 30 min – 3 h. *b*) $(MeO)_2TrCl$, DMAP, pyridine, r.t., 1 – 7 h. *c*) $(^iPr_2N)(NCCH_2CH_2O)PCl$, $^iPr_2EtN$, THF, r.t., 1 h.

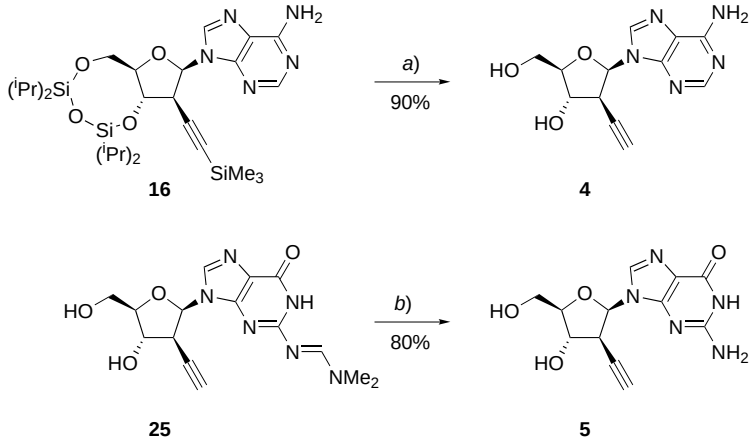
phosphoramidites **32**–**36** suitable for oligonucleotide synthesis according to standard procedures [19].

The newly synthesized adenosine and guanosine derivatives **16** and **25** were fully deprotected to give 2′-deoxy-2′-ethynyl-*arabino*-adenosine (**4**) and -guanosine (**5**), respectively (*Scheme 5*). The evaluation of their antiviral and cytotoxic profile is currently under way.

Oligonucleotide Synthesis. Phosphoramidites **32**–**36** were used for the preparation of oligonucleotides **37**–**58** (see *Tables 6*–*8* in the *Exper. Part*) by automated solid-phase synthesis. With 1*H*-tetrazole as activator, average coupling yields for ethynyl-modified nucleoside phosphoramidites were considerably lower (< 90%) than for unmodified ones. However, the use of 5-(benzylthio)-1*H*-tetrazole [20] increased the coupling efficiencies to over 97%. Complete deprotection was achieved by treatment with concentrated ammonia at 55° for 30 min. Crude oligonucleotides were purified by ion-exchange and reversed-phase HPLC. The integrity of the isolated oligonucleotides was subsequently confirmed by mass spectrometry (see *Tables 6*–*8* in the *Exper. Part*).

The use of the more labile amine-protecting groups phenoxyacetyl, (dimethylamino)methylene (dimethylformamidine), and isobutyryl for the adenosine, guanosine, and cytidine phosphoramidites **32**–**34** was mandated by the fact that the ethynyl group considerably weakened the internucleotidic linkage. The sequence d(TTTTT^{Et}TTTTT)

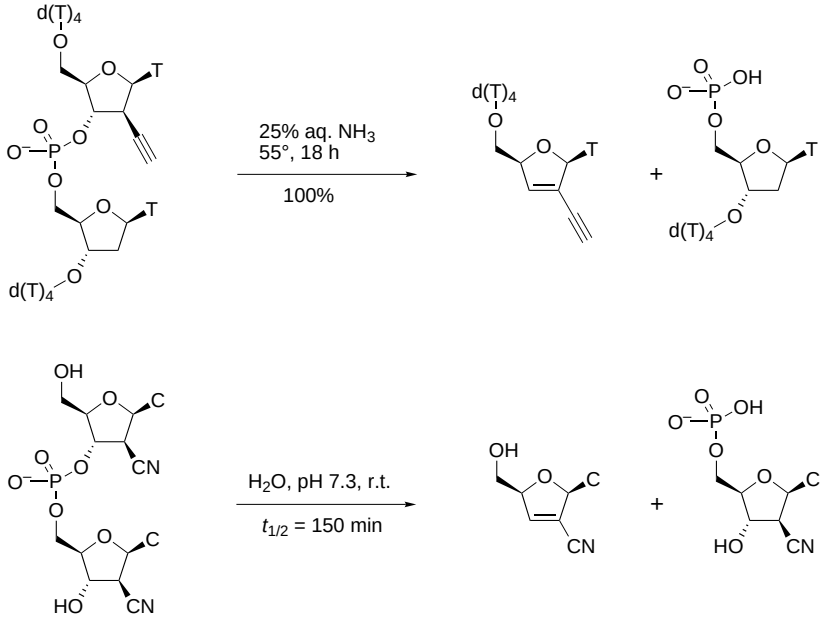
Scheme 5. *Deprotection of 2′-Deoxy-2′-ethynyladenosine and -guanosine Derivatives to* **4** *and* **5**



a) Bu_4NF, AcOH, THF, r.t., 1 h. *b*) aq. NH_3 soln., r.t., 1 h.

(**37**; T_d^{Et} = 2′-ethynyl-*arabino*-thymidine (**2**)) was partially cleaved between the modified nucleotide and the thymidine residue in 3′-direction (*Scheme 6*) under standard deprotection conditions (conc. ammonia, 55°, overnight). The two fragments could be identified by MALDI-TOF mass spectrometry [21]. This β-elimination is

Scheme 6. *Comparison of the Behavior of an Oligonucleotide Containing 2′-Ethynyl-Modified Nucleoside with that of the Similarly Substituted 2′-Cyano-dinucleotide Described by* Hayakawa *and* Matsuda [22] *on Prolonged Base Treatment*



precedented by the similar reactivity of a dinucleotide consisting of 2′-cyano-substituted deoxycytidine [22]. The configuration of the ethynyl residue is not inverted by the ammonia treatment as could be shown for 2′-ethynyl-*arabino*-deoxyadenosine (**4**).

Pairing Properties of Homobase Sequences. Pairing properties of oligonucleotides **37**–**44** were obtained from analysis of UV/melting curves in buffer solutions containing 1M NaCl at pH 7.0. T_m data are summarized in *Table 1*. Replacing one thymidine residue with 2′-ethynyl-*arabino*-thymidine in the middle of the reference duplex d(T_{10})·d(A_{10}) (**59**·**60**) led to a dramatic decrease in thermal stability (**37**·**60**, $\Delta T_m = -13°$). Contrary to our expectations, a somewhat smaller decrease was observed in the corresponding DNA·RNA heteroduplex **37**·poly(A) ($\Delta T_m = -8°$). A rationale for this destabilization might be that the ethynyl substituent unfavorably interacts with the thymine methyl group of the neighboring nucleoside, thus forcing the base to adopt a conformation not suitable for pairing. This could be tested by replacing uracil for thymine. Besides the expected overall decrease in stability due to this modification – d(U_{10})·d(A_{10}) (**62**·**60**) melts 17° lower than d(T_{10})·d(A_{10}) – the ethynyl substitution still negatively affected duplex stability (**38**·**60**, $\Delta T_m = -4°$; **38**·poly(A), $\Delta T_m = -4°$). This effect was somewhat diminished in a completely modified sequence **39**, but, again, relative to the reference systems, the RNA complement formed a more stable duplex than the DNA one.

Table 1. *Melting Temperatures* (T_m) *of 2′-Ethynyloligonucleotides* **37**–**44**[a])

	T_m [°][b])	ΔT_m/mod [°]
d(T_{10})·d(A_{10}) (**59**·**60**)	33	–
d(TTTTT^{Et}TTTTT)·d(A_{10}) (**37**·**60**)	20	−13
d(T_{10})·poly(A) (**59**·**61**)	37	–
d(TTTTT^{Et}TTTTT)·poly(A) (**37**·**61**)	29	−8
d(U_{10})·d(A_{10}) (**62**·**60**)	16	–
d(UUUUU^{Et}UUUUU)·d(A_{10}) (**38**·**60**)	12	−4
d(U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U)·d(A_{10}) (**39**·**60**)	<5	<−1.2
d(U_{10})·poly(A) (**62**·**61**)	28	–
d(UUUUU^{Et}UUUUU)·poly(A) (**38**·**61**)	24	−4
d(U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U)·poly(A) (**39**·**61**)	26	−0.2
d(AAAAAA^{Et}AAAA)·d(T_{10}) (**40**·**59**)	33	0
d(AAAAAA^{Et}AAAA)·d(U_{10}) (**40**·**62**)	19	+3
d(A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A)·d(T_{10}) (**41**·**59**)	38	+0.6
d(A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A)·d(U_{10}) (**41**·**62**)	25	+1
d(A_{10})·poly(U) (**60**·**63**)	56[c])	–
d(AAAAAA^{Et}AAAA)·poly(U) (**40**·**63**)	54[c])	−2
d(A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A)·poly(U) (**31**·63)	35[c])	−2.3
d(C_6)·d(G_6) (**64**·**65**)	30	–
d(CCC^{Et}CCC)·d(G_6) (**42**·**65**)	19	−11
d(C^{Et}C^{Et}C^{Et}C^{Et}C^{Et}C)·d(G_6) (**43**·**65**)	10	−4
d(G^{Et}G^{Et}G^{Et}G^{Et}G^{Et}G)·d(C_6) (**44**·**64**)	30	0
d(G_6)·poly(C) (**65**·**66**)	53	–
d(G^{Et}G^{Et}G^{Et}G^{Et}G^{Et}G)·poly(C) (**44**·**66**)	53	0

[a]) Oligonucleotide concentration: 4 μM in 10 mM NaH_2PO_4, 1M NaCl, pH 7.0. [b]) Absorbance detected at 260 nm. Melting temperature values represent the mean of three melting curves. Heating rate: 0.5°/min. [c]) **40**, **41**, and **60** are forming triplexes with poly(U) as was shown by UV-titration experiments [23].

A completely different picture was observed in the case of the ethynyl-modification of deoxyadenosine. Whereas monosubstitution in $d(T_{10}) \cdot d(A_{10})$ (**59** · **60**) did not affect thermal stability (**40** · **59**, $\Delta T_m = 0°$), the respective DNA · RNA complex **40** · poly(U) ($\Delta T_m = -2°$) was destabilized. The same substitution in $d(U_{10}) \cdot d(A_{10})$ (**62** · **60**) led to a stabilization (**40** · **62**, $\Delta T_m = +3°$). The fully modified oligonucleotide **41** clearly showed enhanced pairing to $d(T_{10})$ and $d(U_{10})$ as compared to the reference system but decreased affinity for the corresponding RNA complement.

The same trends were found for the corresponding 2′-ethynyldeoxyguanosine and -cytidine oligonucleotides. For the latter, a strong destabilization was found. Replacing five deoxyguanosines in the duplex $d(G_6) \cdot d(C_6)$ (T_m 30°) by 9-(2′-deoxy-2′-*C*-ethynyl-β-D-*arabino*-pentofuranosyl)guanine did not alter the pairing affinity at all (*Table 1*). The same was true when the hexaguanylates were paired to poly(C). From CD spectra, it was concluded that the duplex $d(G^{Et}G^{Et}G^{Et}G^{Et}G^{Et}G) \cdot d(C_6)$ (**44** · **64**) adopts a regular B-type conformation (data not shown).

These results were only partially consistent with our assumptions on the conformation-driving effect of the *arabino*-configured 2′-ethynyl group. The failure of this concept in an oligopyrimidine context might be due to the interference of the ethynyl group with H–C(6) of the pyrimidine bases. The sterically less-demanding five-membered imidazole rings of the purine bases may leave more space for the ethynyl residue such that the desired preference for a DNA complement is indeed observed. The ethynyl-DNA · DNA complex **41** · **62** is even more stable than the unmodified reference system.

This stabilization is governed by enthalpy, as can be seen from the thermodynamic parameters (*Table 2*) obtained by the concentration-variation method [24]. The pairing

Table 2. *Thermodynamic Parameters for Duplex Formation of 2′-Ethynyl-Modified d(A₁₀)* **40** *and* **41** *with d(T₁₀)* (**59**) *and r(U₁₀)* (**67**)[a])

	$\Delta H°$ [kcal/mol]	$\Delta S°$ [cal/K · mol]	$\delta G°$ (25°) [kcal/mol]
$d(A_{10}) \cdot d(T_{10})$ (**60** · **59**)	– 69.7	– 203	– 9.2
$d(A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A) \cdot d(T_{10})$ (**41** · **59**)	– 87.9	– 256	– 11.7
$d(A_{10}) \cdot r(U_{10})$ (**60** · **67**)	– 37.5	– 104	– 6.5
$d(AAAAAA^{Et}AAAA) \cdot r(U_{10})$ (**40** · **67**)	– 39.9	– 113	– 6.1

[a]) Oligonucleotide concentration: 4–45 μM in 10 mM NaH_2PO_4, 1M NaCl, pH 7.0.

enthalpy in the modified system **41** · $d(T_{10})$ is more than 25% higher than in the unmodified one ($d(A_{10}) \cdot d(T_{10})$). This is counterbalanced somewhat by the less-favorable pairing entropy. Both trends were found in the pairing of ethynyl-modified $d(A_{10})$ to an RNA complement as well. However, the increase in pairing enthalpy is just 6%, which results in an overall destabilization.

Surprisingly, the ethynyl modification in the $d(A_{10}) \cdot d(T_{10})$ and $d(A_{10}) \cdot r(U_{10})$ systems has little effect on the structures of the respective duplexes, as can be judged from CD spectroscopy (*Fig. 5*). In both cases, the characteristic shape of the curves, indicative of a B-form conformation in DNA · DNA duplexes and of an A-form duplex structure in the DNA · RNA case, remain intact. No wavelength shift was observed for the typical bands. They even are more intense in the 2′-ethynyl-modified case which may be a result of the increased thermodynamic stability.

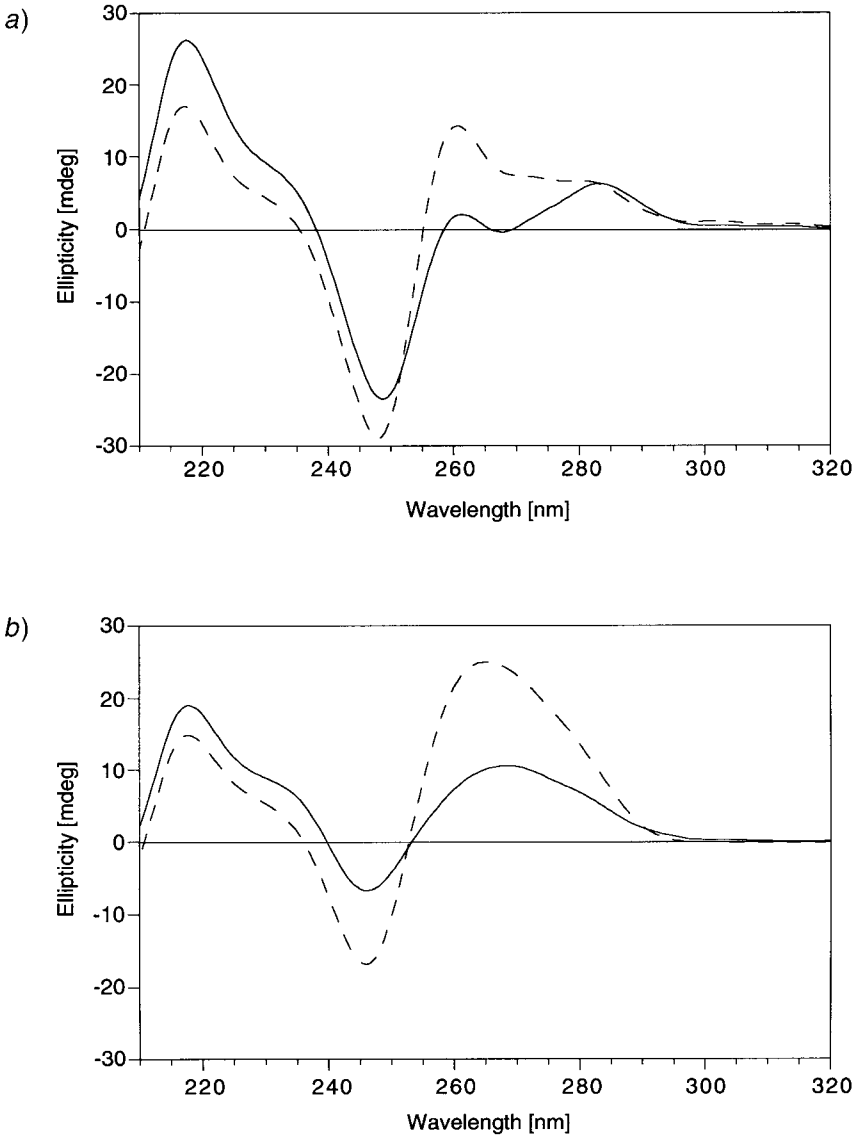


Fig. 5. a) *CD Spectra of* $d(A_{10})\cdot d(T_{10})$ (**60**·**59**; —) *and* $d(A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et})\cdot d(T_{10})$ (**41**·**59**; – – –), *both being indicative of a B-form duplex*. b) *CD Spectra of duplexes* $d(A_{10})\cdot r(U_{10})$ (**60**·**67**; —) *and* $d(A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et})\cdot r(U_{10})$ (**41**·**67**; – – –); *both being characteristic for A-form duplexes.* Oligonucleotide concentration: 4 μM in 10 mM NaH_2PO_4, 1M NaCl, pH 7.0, 4°.

Pairing Properties of Mixed Sequences. The increase in thermodynamic stability and the selectivity for complementary DNA strands in homopurine 2′-ethynyl-DNA vanishes in oligonucleotides of mixed or alternating base composition (*Table 3*). The 2′-ethynyl-*arabino* modification of deoxyadenosine in the alternating sequence $d(AU)_5$

(**68**; T_m 17°) leads to a decrease of 2.5° per modification. Obviously, the ethynyl residue not only interferes with the purine base of the residue carrying the modification but also with the base of the 3′-neighboring nucleoside.

Even when the modified deoxyadenosine or deoxyguanosine nucleosides are part of a short homopurine tract, as in d(CTGA^{Et}ATCGAC)·d(GTCGATTCAG) (**47**·**70**), the ethynyl residue depresses thermal stability, although not by much. A little surprising, however, was the finding that it had almost no effect on duplex stability when the complementary strand was RNA. Consequently, it was to be expected that an ethynyl modification of the *Dickerson-Drew* dodecamer d(CGCGAATTCGCG) (**74**) – the prototype of a B-form duplex [25] – leads to an even stronger destabilization.

Table 3. *Melting Temperatures* (T_m) *of 2′-Ethynyloligonucleotides with Mixed-Base Sequence*[a])

	T_m [°][b])	ΔT_m/mod [°]
d(AUAUAUAUAU)$_2$ ((**68**)$_2$)	17	–
d(A^{Et}UA^{Et}UA^{Et}UA^{Et}UA^{Et}U)$_2$) ((**45**)$_2$)	< 5	< – 2.4
d(CTGAATCGAC)·d(GTCGATTCAG) (**69**·**70**)	51	–
d(CTGA^{Et}ATCGAC)·d(GTCGATTCAG) (**47**·**70**)	49	– 2
d(CTGA^{Et}A^{Et}TCGAC)·d(GTCGATTCAG) (**48**·**70**)	47	– 2
d(CTGAATCGAC)·r(GUCGAUUCAG) (**69**·**71**)	41	–
d(CTGA^{Et}ATCGAC)·r(GUCGAUUCAG) (**47**·**71**)	41	0
d(CTGA^{Et}A^{Et}TCGAC)·r(GUCGAUUCAG) (**48**·**71**)	40	– 0.5
d(GAGAAAG)·d(CTTTCTC) (**72**·**73**)	19	–
d(G^{Et}AG^{Et}AAAG)·d(CTTTCTC) (**46**·**73**)	17	– 1
d(CGCGAATTCGCG)$_2$ ((**74**)$_2$)	69	–
d(CGCG^{Et}A^{Et}ATTCGCG)$_2$ ((**49**)$_2$)	62	– 1.8
d(CG^{Et}CGAATTCG^{Et}CG)$_2$ ((**50**)$_2$)	60 (36)[c])	– 2.3

[a]) Oligonucleotide concentration: 4 μM in 10 mM NaH_2PO_4, 1M NaCl, pH 7.0. [b]) Absorbance detected at 260 nm. Melting temperatures represent the mean value of three melting curves. Heating rate: 0.5°/min. [c]) The *Dickerson-Drew* dodecamer sequence is known to form a hairpin complex in addition to the duplex [25b]. The value in brackets indicates T_m for the hairpin.

The decrease in pairing strength for the 2′-ethynyl modification in a mixed sequence context becomes more obvious when the corresponding thermodynamic parameters are extracted (*Table 4*). In contrast to the modified d(A$_{10}$)·d(T$_{10}$) system, the pairing enthalpy in the duplexes d(CTGA^{Et}ATCGAC)·d(GTCGATTCAG) (**47**·**70**) and d(CTGA^{Et}A^{Et}TCGAC)·d(GTCGATTCAG) (**48**·**70**) is decreased by *ca.* 8%. At the same time, pairing entropy is decreased as well, but not to an extent sufficient to compensate for the loss in enthalpy.

Table 4. *Thermodynamic Parameters for Duplex Formation of Oligonucleotides d(CTGAATCGAC)* (**69**), *d(CTGA^{Et}ATCGAC)* (**47**), *and d(CTGA^{Et}A^{Et}TCGAC)* (**48**) *with d(GTCGATTCAG)* (**70**)[a])

	ΔH^0 [kcal/mol]	ΔS^0 [cal/K·mol]	ΔG^0 (25°) [kcal/mol]
d(CTGAATCGAC)·d(GTCGATTCAG) (**69**·**70**)	– 77.6	– 213	– 13.8
d(CTGA^{Et}ATCGAC)·d(GTCGATTCAG) (**47**·**70**)	– 71.7	– 198	– 12.8
d(CTGA^{Et}A^{Et}TCGAC)·d(GTCGATTCAG) (**48**·**70**)	– 67.1	– 184	– 12.4

[a]) Oligonucleotide concentration: 4 – 48 μM in 10 mM NaH_2PO_4, 1M NaCl, pH 7.0.

The results from the melting experiments of random sequences clearly showed that an *arabino*-configured 2′-ethynyl group does not prevent pairing to complementary RNA. CD Spectra of the duplexes d(CTGA^{Et}A^{Et}TCGAC)·d(GTCGATTCAG) (**48**·**70**) and d(CTGA^{Et}A^{Et}TCGAC)·r(GUCGAUUCAG) (**48**·**71**, *Fig. 6*) further support-

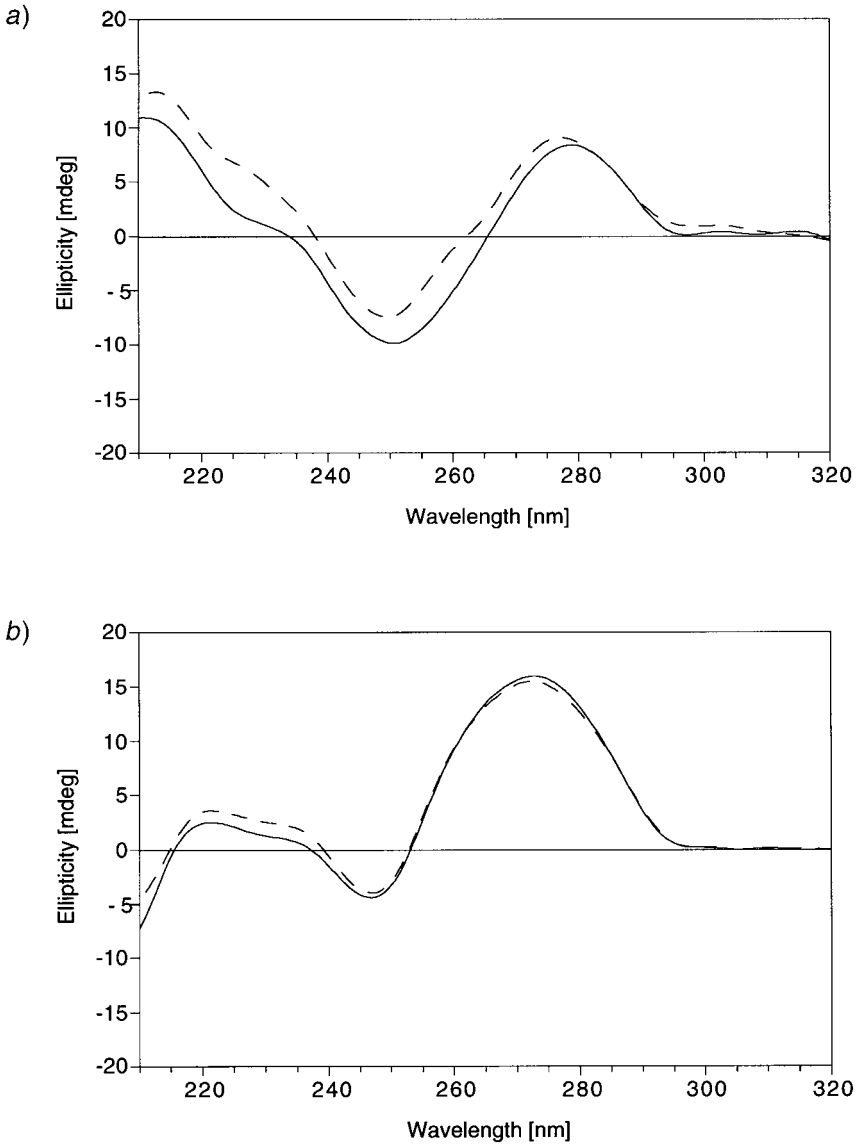


Fig. 6. a) *CD Spectra of DNA duplexes d(CTGAATCGAC)·d(GTCGATTCAG)* (**69**·**70**, —) *and d(CTGA^{Et}-A^{Et}TCGAC)·d(GTCGATTCAG)* (**48**·**70**, ---), *both being characteristic of a B-form duplex*. b) *CD Spectra of DNA·RNA heteroduplexes d(CTGAATCGAC)·r(GUCGAUUCAG)* (**69**·**71**, —) *and d(CTGA^{Et}A^{Et}TCGA-C)·r(GUCGAUUCAG)* (**48**·**71**, ---), *displaying the typical bands of an A-form duplex*. Oligonucleotide concentration: 4 μM in 10 mM NaH_2PO_4, 1M NaCl, pH 7.0, 4°.

ed this observation. In both cases, the duplex structure was only marginally disturbed. The modified DNA · RNA duplex still adopts an A-form conformation. The rod-like substituent thus must enforce a sugar pucker compatible with an overall A-form conformation.

Molecular-Dynamics Simulation of Mixed-Sequence Duplexes. The modified duplex d(CTGA^{Et}A^{Et}TCGAC) · d(GTCGATTCAG) (**48** · **70**) was studied by molecular modelling to rationalize the failure of the concept of conformational restriction within a dinucleotide step by a 2′-ethynyl residue in mixed sequences. In these simulations, the parent DNA duplex d(CTGAATCGAC) · d(GTCGATTCAG) (**69** · **70**) is found to adopt an overall B-form conformation. The base pairs are perpendicular to the helix axis. The pyrimidine nucleosides in the center of the duplex are characterized by an O(4′)-*endo* conformation of the corresponding deoxyribose residues. The sugars of the purine nucleosides adopt a C(2′)-*endo* conformation, as expected, or a closely related conformation intermediate between C(2′)-*endo* and C(1′)-*exo*. The simulation also predicts a very narrow minor groove along the purine tract d(GAA). This is indeed observed in X-ray crystal structures of homopurine stretches [7].

In the modified duplex d(CTGA^{Et}A^{Et}TCGAC) · d(GTCGATTCAG) (**48** · **70**; *Fig. 7*), a larger portion of the central region is now in the C(2′)-*endo*/C(1′)-*exo* conformation, including the modified dA^{Et} residues. Overall, the duplex closely resembles the structure of the unmodified one. The conformation of the two dA^{Et}, half-way between C(2′)-*endo* and C(1′)-*exo*, seems to best accommodate the ethynyl residue. The pseudo-equatorially oriented bases are in a high-*anti* conformation, as can be seen in an overlay of the dA^{Et} residues in **48** · **70** with the corresponding unmodified one (*Fig. 7*). The plane of the aromatic base is tilted towards the C(1′) – O(4′) bond. This arrangement is usually associated with an A-form duplex and may explain the failure of the concept.

Pairing Properties of Alternating d(CG) Sequences. The ethynyl-modification of alternating d(CG) sequences leads to an interesting and completely unexpected behavior [26]. The self-complementary oligonucleotide d(CG^{Et}CG^{Et}CG) (**51**) generally is more stable than the natural reference **76** (*Table 5*). This is in contrast to corresponding AU sequences where the ethynyl-substituent considerably decreased pairing affinity (*vide supra*). As expected, the T_m of **51** increases with increasing ionic strength. However, the T_m of its natural counterpart decreases when more NaCl is added.

Table 5. *Melting Temperatures* (T_m) *of 2′-Ethynyl Oligonucleotides with Mixed-Base Sequence*[a])

	T_m [°][b]) (ΔT_m/mod)		
	0.15M NaCl	1M NaCl	4M NaCl
d(CGCGCG)$_2$ (**(76)**$_2$)	47	47	40
d(CG^{Et}CG^{Et}CG)$_2$ (**(51)**$_2$)	50 (+ 0.8)	58 (+ 2.8)	59 (4.8)
d(CG^{Et}CGCG)$_2$ (**(52)**$_2$)	36 (– 5.5)	40 (– 3.5)	41 (+ 0.5)
d(CGCG^{Et}CG)$_2$ (**(53)**$_2$)	33 (– 7.0)	37 (– 5.0)	40 (0)
d(GCGCGC)$_2$ (**(77)**$_2$)	47	48	38
d(G^{Et}CG^{Et}CG^{Et}C)$_2$ (**(56)**$_2$)	41 (– 1)	48 (0)	49 (+ 1.8)
d(C^{Et}GC^{Et}GC^{Et}G)$_2$ (**(54)**$_2$)	< 5	< 5	< 5

[a]) Oligonucleotide concentration: 6.7 μM in 10 mM NaH_2PO_4, pH 7.0, and the indicated amount of NaCl. [b]) Absorbance detected at 280 nm. Melting temperature values represent the mean of three melting curves. Heating rate: 0.5°/min.

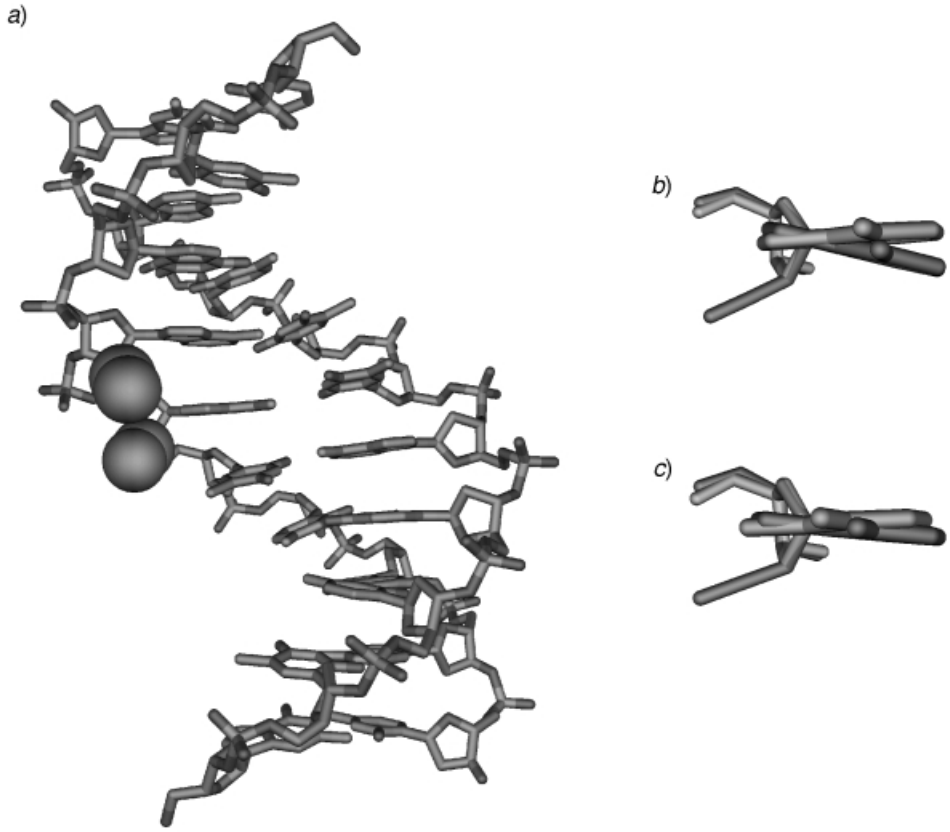


Fig. 7. a) *Structure of the duplex d(CTGA^{Et}A^{Et}TCGAC)·d(GTCGATTCAG)* (**48**·**70**) *in a 200-ps molecular dynamics simulation* (average over last 50 ps; ethynyl residues in space-filling representation; overall structure very similar to the unmodified DNA duplex (not shown)). b) *Top view of an overlay of the deoxyadenosine residues* (fourth nucleoside from 5′-end in **48** and **69**, resp.) *of the DNA and ethynyl-DNA duplexes* **69**·**70** *and* **48**·**70** (ethynyl-modified nucleoside in dark, the unmodified one in light grey; both display a conformation intermediate between C(2′)-*endo* and C(1′)-*exo* with the bases in a pseudo-equatorial position allowing for an optimal distance between the ethynyl residue and the adenine in **48**; adenine base of dA^{Et} in a high-*anti* conformation usually associated with A-form duplexes). c) *Overlay of the fifth* (deoxyadenosine) *residues in* **69**·**70** *and* **48**·**70**.

This different behavior of oligonucleotides **76** and **51** towards salt concentration is also reflected in their CD spectra (*Fig. 8*). While at 150 mM NaCl concentration, unmodified **76** displays a CD spectrum typical for a B-form (2′-*endo*) conformation, the CD spectrum of **51** has largely opposite ellipticities indicative of a left-handed helical structure [7]. The unmodified oligonucleotide **76** shows a similar spectrum only at much higher ionic strength, while the spectrum of **51** is not changed at 4M NaCl.

Even at 10 mM buffer concentration (no added NaCl), **51** displays this typical CD spectrum. To induce such a left-handed helical structure at lower ionic strength, only a single ethynyl-modification is necessary within d(CG)$_3$ (**76**). Oligonucleotides **52** and **53** show spectra similar to that of **76** at 150 mM NaCl concentration but behave like **51**

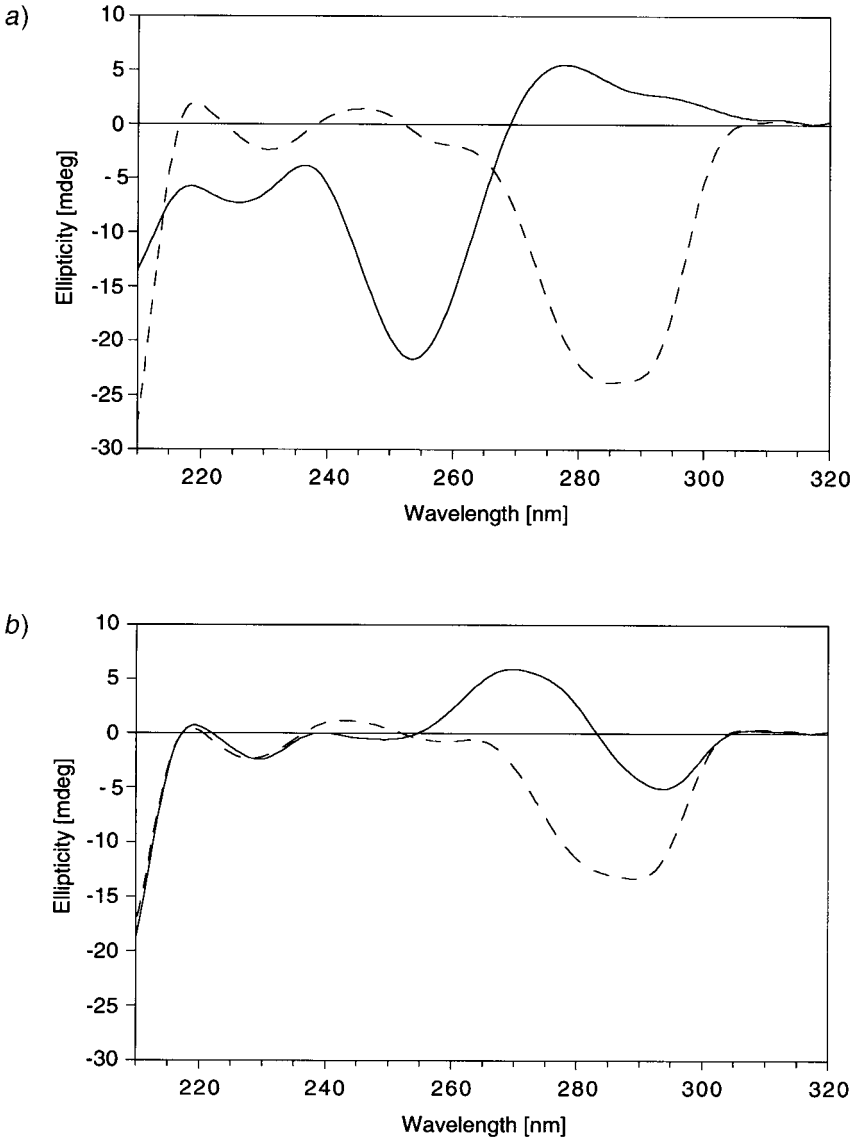


Fig. 8. a) *CD Spectra of self-complementary oligonucleotides d(CG)$_3$* (**76**; ——) *and D(CG^{Et}CG^{Et}CG)* (**51**; - - -) (oligonucleotide duplex concentration: 6.7 μM in 10 mM NaH_2PO_4, 0.15M NaCl, pH 7.0, 4°). b) *CD Spectra of Z-DNA forming self-complementary oligonucleotides d(CG)$_3$* (**76**; ——) *and d(CG^{Et}CG^{Et}CG)* (**51**; - - -) *at high salt concentration* (oligonucleotide duplex concentration: 6.7 μM in 10 mM NaH_2PO_4, 4M NaCl, pH 7.0, 4°).

at 1M NaCl. Surprisingly, for the triply modified oligonucleotide d(G^{Et}CG^{Et}CG^{Et}C) (**56**), the same CD characteristics as for **51** are found from low to high salt conditions. This is in contrast to the natural counterpart **77**, which, even at 4M NaCl, does not undergo a conformational change.

These results indicate that the 2′-ethynyl modification of deoxyguanosine within alternating CG sequences induces a left-handed helical structure. This structural change becomes independent of ionic strength when two or more substitutions are present. The effect is limited to the modification of deoxyguanosine residues – d($C^{Et}GC^{Et}GC^{Et}G$) (**54**), for instance, does not form a duplex at all[5]).

Molecular-Dynamics Simulation and NMR-Spectroscopic Characterization of d($CG^{Et}CG^{Et}CG$)$_2$ (**(51)**$_2$). A molecular-dynamics simulation of d($CG^{Et}CG^{Et}CG$)$_2$ (**(51)**$_2$) was carried out to shed some light on the factors that force this ethynyl-modified duplex into a left-handed helix with the guanine bases presumably in a *syn* conformation. During the simulation, the duplex did not deviate the slightest from the starting Z-DNA conformation (*Fig. 9*)[6]). The averaged structure of the last 50 ps of the run shows some interesting features, which might indeed explain the unexpected stability of this duplex (*Fig. 10*).

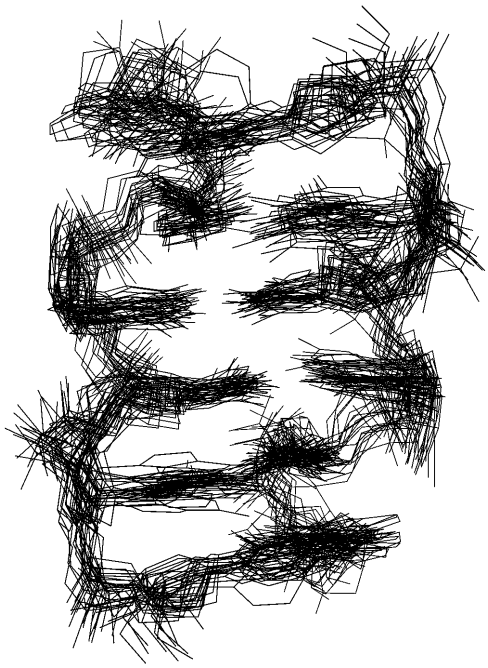


Fig. 9. *Overlay of 20 frames* (every 10 ps) *of the duplex d($CG^{Et}CG^{Et}CG$)$_2$* (**(51)**$_2$) *in a 200-ps molecular-dynamics simulation* (no constraints applied). Only small deviations from the starting left-handed Z-DNA structure are observed.

The ethynyl moieties are oriented perpendicular to the major groove. The modified deoxyguanosines display a C(2′)-*exo* conformation closely related to the C(3′)-*endo* conformation of the deoxyguanosines in the X-ray crystal structure of d(CGCGCG)$_2$

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[5]) The fully modified oligonucleotide d($C^{Et}G^{Et}C^{Et}G^{Et}C^{Et}G$) exclusively adopts a B-form conformation irrespective of salt concentration.

[6]) When the simulation was performed on a regular right-handed B-conformation duplex, the left-handed Z-form was not observed.

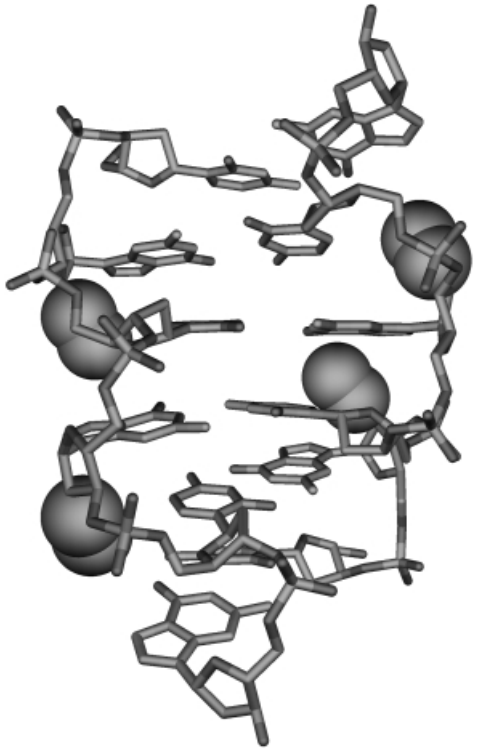

Fig. 10. *Structure of the duplex d(CG^{Et}CG^{Et}CG)$_2$ ((**51**)$_2$) in the molecular-dynamics simulation* (average over last 50 ps). Ethynyl residues in space-filling representation.

((**76**)$_2$) [27]. In this C(2′)-*exo* conformation, the ethynyl groups are in a pseudo-equatorial position, which probably is an energetically very favorable arrangement that could already be present in the corresponding monomer. Furthermore, the ethynyl groups are aligned perpendicularly to the plane of the 3′-neighboring cytosine base (*Fig. 11*). This interaction is reminiscent of the herringbone motif in the crystal structure of benzene. The contact of the positively polarized H–C(5) and H–C(6) in cytosine with the π-electron face of the acetylene group might be favorable *per se*. Additionally, this arrangement might lock the cytosine in a base pair with the opposite guanine. The sum of the factors described could very well explain the tendency of d(CG^{Et}CG^{Et}CG) (**51**) to form a Z-DNA-like structure even at low ionic strength.

Unequivocal proof for the Z-DNA conformation of d(CG^{Et}CG^{Et}CG) (**51**) could not be obtained so far[7]). A 2D-NMR study on this duplex showed at least that the bases of the modified deoxyguanosine residues are in a *syn*-conformation. A detailed sequential assignment of sugar and base resonances was not possible due to the very

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[7]) Efforts towards the crystallization of the complex were not successful so far. The material from a 10-μmol synthesis of d(CG^{Et}CG^{Et}CG) (**51**) was subjected to a commercially available DNA crystallization screen according to the hanging-drop method. In most cases, the oligonucleotide was simply precipitated.

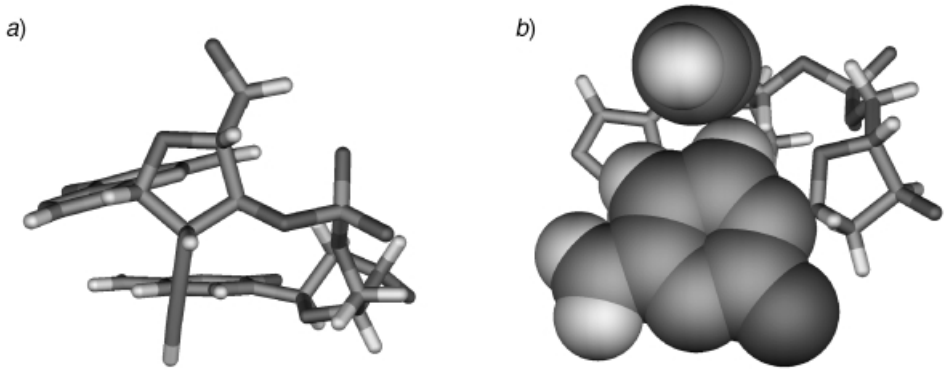

Fig. 11. a) *Side and* b) *bottom views of a d(G^{Et}C) dinucleotide step from the structure of d(CG^{Et}CG^{Et}CG)$_2$* ((**51**)$_2$) *shown in* Fig. 12. *a*) Steric blockade of the cytosine base in the 3′-direction by the ethynyl residue preventing an unpairing. *b*) Electrostatically favorable interaction of the π-face of the ethynyl group with H–C(5) and H–C(6) of cytosine, this interaction being further corroborated by an upfield ^{1}H-NMR chemical shift of these two protons.

small chemical-shift differences between nucleosides at different positions in the duplex.

A comparison between the limited data extractable from NOESY spectra of d(CG^{Et}CG^{Et}CG) (**51**) and the fully characterized d(CGCme^{8}GCG) (**78**) [28], which forms a Z-DNA duplex, shows that the base-proton resonances are very similar. In both duplexes, the H–C(5) signals of the cytosine bases are shifted upfield by almost 2 ppm with respect to 2′-deoxycytidine. This is most likely due to the shielding effect of the imidazole ring of a 5′-neighboring deoxyguanosine, an arrangement uniquely found in Z-DNA (*Fig. 11*). Shielding is also found for H–C(6), although not quite as dramatic. The fact that the H–C(6) resonances are shifted further upfield in d(CG^{Et}CG^{Et}CG) (**51**) than in d(CGCme^{8}GCG) (**78**) could indicate the vicinity of the ethynyl residue (*Fig. 11*). The chemical shifts of H–C(8) of the guanine bases are again very similar in both complexes.

Conclusions. – The results described above demonstrate that the original intention to restrict the duplex conformation to a B-form – and thus achieve selectivity for complementary DNA – by an *arabino*-configured 2′-ethynyl substitution did not work for random sequences. The desired selectivity was observed only for dA^{Et} homopolymers. Poly(dA)·poly(dT) duplexes are distinctly different from DNAs with alternating or random distributions of bases. They are characterized by an unusual stiffness and the resistance to transform into other helical forms. In this situation, the ethynyl modification might contribute to pairing affinity through additional π-stacking or other favorable *Van der Waals* interactions, as indicated by the increased enthalpy of duplex formation. The entropy change upon pairing is increased as well in this case, counteracting the stabilizing influence somewhat. In random sequences, the opposite is true. The pairing enthalpy as well as the entropy – although not as much – are diminished. Thus, the concept of enforcing a certain duplex conformation by steric blockade probably failed because the introduction of an additional substituent does not effectively reduce the internal degrees of freedom. The ethynyl group may even

severely distort the arrangement of the bases in the same or a neighboring nucleoside, as in the case of homopyrimidine stretches.

The study also showed that such a sugar modification can have a beneficial effect in a different context. The stabilization of a left-handed helical structure was entirely unexpected. Alternating d(CG) sequences usually undergo B- to Z-DNA transformation only under very high cation concentrations or in the presence of dehydrating agents. If the deoxyguanosines are 2′-ethynyl-modified, this change does not require salt at all. The *syn*-orientation of the guanine bases, as required for Z-DNA formation, may be more favorable here due to π-π interactions between the ethynyl group and guanine. The rod-like substituent likely also restricts the flexibility of the adjacent cytosine base and thus stabilizes the duplex. Additional structural investigations will be needed to clarify this behavior.

Experimental Part

General. All reactions were carried out under Ar. Solvents for extraction: technical grade, distilled. Solvents for reactions: reagent grade, distilled from CaH_2 (MeCN, pyridine) or Na (THF). All reagents were purchased from *Fluka AG*, highest quality available, except 2-cyanoethyl diisopropylphosphoramidochloridite which was purchased from *Aldrich Co*. Flash-chromatography (FC): silica gel (40–63 μm; *Fluka*). TLC: *Macherey-Nagel SIL-G-25-UV$_{254}$* plates. Optical rotation: *Perkin-Elmer 241* polarimeter; 10-cm cell. UV Spectra: *Perkin-Elmer Lambda Bio*, λ_{max} in nm (ε). IR Spectra: *Perkin-Elmer FTIR 1600*; $\tilde{\nu}$ in cm^{-1}. NMR: *Bruker AC-300*, *DRX-400*, or *DRX-500*; δ in ppm, calibration to residual solvent peak, *J* in Hz, ^{13}C multiplicities derived from DEPT spectra; ^{31}P calibration to external H_3PO_4 (=0 ppm). MS: *Micromass Autospec Q*, Cs^+ beam, 25 keV; matrix dithiothreitol/dithioerythritol (DTT/DTE) 5:1. HR-MS: LSI-MS peak-matching with *PEG-600* or *PEG-900* as internal standard.

*1-{2′-Deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-*C*-[(trimethylsilyl)ethynyl]-β-*D*-arabino-furanosyl}-*N^4*-isobutyrylcytosine* (**10**). Isobutyric anhydride (0.781 ml, 0.774 g, 4.71 mmol) was added to a soln. of **9** [13] (2.22 g, 3.92 mmol) in pyridine (22 ml). After stirring the mixture for 16 h at r.t., MeOH (2 ml) was added and stirring continued for 30 min. Then the mixture was concentrated and the residual oil co-evaporated with toluene (2 × 20 ml). The residue was dried under h.v. for several h and then submitted to FC (80 g of silica gel, hexane/AcOEt 1:1): **10** (2.47 g, 99%). Colorless foam. R_f 0.22 (hexane/AcOEt 1:1). $[\alpha]_D^{25} = +77.9$ ($c = 1.05$, $CHCl_3$). UV (EtOH): 250 (16240), 302 (7570). IR (KBr): 3448*m* (br.), 3234*m*, 3142*m*, 3080*m*, 2946*s*, 2896*m*, 2869*s*, 2177*w*, 1724*s*, 1674*s*, 1627*s*, 1558*s*, 1494*s*, 1468*s*, 1436*m*, 1397*s*, 1316*s*, 1266*m*, 1250*s*, 1221*m*, 1183*m*, 1150*s*, 1105*s*, 1076*s*, 1035*s*, 993*m*, 954*m*, 920*w*, 885*s*, 855*s*, 802*m*, 775*m*, 761*m*, 701*s*, 615*w*, 605*w*, 595*w*, 557*w*, 520*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.78 (br. *s*, H–N(4)); 7.98 (*d*, *J* = 7.4, H–C(6)); 7.42 (*d*, *J* = 7.7, H–C(5)); 6.44 (*d*, *J* = 7.7, H–C(1′)); 4.40 (*t*, *J* = 8.6, H–C(3′)); 4.14 (*dd*, *J* = 2.2, 13.2, 1 H–C(5′)); 4.02 (*dd*, *J* = 2.6, 13.2, 1 H–C(5′)); 3.78 (*ddd*, *J* = 2.2, 2.6, 8.2, H–C(4′)); 3.51 (*dd*, *J* = 7.5, 9.0, H–C(2′)); 2.63 (*sept.*, *J* = 7.0 Hz, Me_2CHCO); 1.23 (*d*, *J* = 7.0, Me_2CHCO); 1.02–1.14 (*m*, 28 H, ^{i}Pr); −0.01 (*s*, Me_3Si). ^{13}C-NMR (75 MHz, $CDCl_3$): 177.01 (*s*, Me_2CHCO); 162.15 (*s*, C(4)); 154.82 (*s*, C(2)); 144.80 (*d*, C(6)); 100.29 (*s*, Si–*C*≡C); 95.72 (*d*, C(5)); 90.73 (*s*, *C*≡C–Si); 84.33, 83.58 (2*d*, C(1′), C(4′)); 74.17 (*d*, C(3′)); 59.88 (*t*, C(5′)); 43.62 (*d*, C(2′)); 36.74 (*d*, Me_2CHCO); 19.13, 18.87 (2 *q*, Me_2CHCO); 17.39, 17.27, 17.24, 17.19, 17.02, 16.98, 16.94 (7 *q*, Me_2CHSi); 13.70, 13.03, 12.63, 12.28 (4 *d*, Me_2CHSi); −0.59 (*q*, Me_3Si). LSI-MS (DTT/DTE): 637 (14), 636 (28, $[M+H]^+$), 289 (8), 261 (7), 183 (10), *182* (100), 177 (6), 175 (4), 147 (5), 119 (4), 112 (15). Anal. calc. for $C_{30}H_{53}N_3O_6Si_3$ (636.03): C 56.65, H 8.40, N 6.61; found: C 56.72, H 8.35, N 6.52.

*N^6-Benzoyl-9-{3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-*C*-[(trimethylsilyl)ethynyl]-β-*D*-arabino-furanosyl}adenine* (**15**). (Trimethylsilyl)acetylene (2.80 ml, 20.2 mmol) was added dropwise to a soln. of 1.6M BuLi in hexane (12.0 ml, 19.2 mmol) in THF (60 ml) at −17°. After stirring for 30 min, the soln. was cooled to −78°, and a soln. of **14** [15] (4.10 g, 6.70 mmol) in THF (30 ml) was added dropwise over 10 min. The mixture was stirred for 3 h at −78° and then quenched by addition of sat. aq. NH_4Cl soln. (100 ml). The mixture was extracted with AcOEt (3 × 100 ml). The org. layers were washed with brine (80 ml), dried ($MgSO_4$), and evaporated. The residue was recrystallized from ^{t}BuOMe/hexane 2:1 to afford colorless needles. The mother liquor was purified by FC (150 g of silica gel, AcOEt) to give additional **15** (total 4.50 g, 95%). Colorless solid. M.p. 172–174°. R_f 0.46 (AcOEt). $[\alpha]_D^{25} = -61.1$ ($c = 1.17$, $CHCl_3$). UV (EtOH): 279 (21400). IR (KBr): 3256*m*,

3066*m*, 2947*s*, 2896*s*, 2868*s*, 2361*w*, 2171*w*, 1898*w*, 1709*s*, 1616*s*, 1586*s*, 1550*w*, 1515*s*, 1456*s*, 1406*m*, 1388*m*, 1337*s*, 1297*s*, 1251*s*, 1166*s*, 1069*s*, 1033*s*, 957*m*, 919*m*, 886*s*, 844*s*, 794*m*, 762*s*, 703*s*, 642*m*, 625*m*, 607*m*, 560*m*, 517*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.14 (*s*, H–N(6)); 8.70, 8.30 (2*s*, H–C(2), H–C(8)); 8.04–8.07 (*m*, 2 arom. H); 7.49–7.63 (*m*, 3 arom. H); 6.34 (*s*, H–C(1′)); 4.45 (*d*, $J = 7.0$, H–C(3′)); 4.01–4.17 (*m*, H–C(4), 2 H–C(5′), OH–C(2′)); 0.96–1.11 (*m*, 28 H, ^{i}Pr); 0.18 (*s*, Me_3Si). ^{13}C-NMR (75 MHz, $CDCl_3$): 164.66 (*s*, Ph*C*O); 152.45 (*d*, C(2)); 151.79 (*s*, C(6)); 149.30 (*s*, C(4)); 142.43 (*d*, C(8)); 133.65 (*s*, arom. C); 132.67, 128.78, 127.95 (*d*, arom. C); 122.90 (*s*, C(5)); 100.86 (*s*, C≡C); 94.98 (*s*, C(2′)); 88.37 (*d*, C(1′)); 82.48 (*d*, C(4′)); 76.73 (*d*, C(3′)); 61.69 (*t*, C(5′)); 17.49, 17.38, 17.34, 17.31, 17.03, 17.01, 16.83 (7*q*, *Me*$_2$CH); 13.46, 13.11, 12.95, 12.65 (4*d*, Me$_2$*C*H); –0.36 (*q*, MeSi). LSI-MS: 710 (11, $[M+H]^+$), 241 (18), *240* (100), 105 (20). Anal. calc. for $C_{34}H_{51}N_5O_6Si_3$: C 57.51, H 7.24, N 9.86; found: C 57.26, H 7.51, N 9.80.

*9-[2′-Deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-*C*-[(trimethylsilyl)ethynyl]-β-*D*-arabino-furanosyl]adenine* (**16**). Methoxalyl chloride ((=chlorooxoacetic acid methyl ester; 0.94 ml, 10.2 mmol) was added to a soln. of **15** (1.20 g, 1.70 mmol) and DMAP (1.24 g,10.2 mmol) in CH_2Cl_2 (10 ml) at –17°. After 2 h, the cooling bath was removed and the mixture allowed to react for 10 h at r.t. The mixture was washed with H_2O (3 × 20 ml) and brine (20 ml), the org. layer dried ($MgSO_4$), evaporated, and co-evaporated with toluene (3 × 20 ml). Then a soln. of the residue, AIBN (10 mg), and Bu_3SnH (3.00 ml, 10.9 mmol) in toluene (15 ml) was heated for 2 h at 95°. Additional AIBN (7 mg) and Bu_3SnH (1.00 ml, 3.62 mmol) were added to the mixture, and heating was continued for another 60 min. After evaporation, the residue was purified by FC (200 g of silica gel, CH_2Cl_2/MeOH 20:1): **16** (0.68 g, 67%; crystallized from MeCN). Colorless needles. M.p. 184°. R_f 0.28 (CH_2Cl_2/MeOH 20:1). $[\alpha]_D^{25} = -44.1$ ($c = 0.74$, $CHCl_3$). UV (EtOH): 261 (12500). IR (KBr): 3482*w*, 3333*m*, 3174*m*, 2947*s*, 2869*s*, 2362*w*, 2181*w*, 1649*s*, 1597*s*, 1575*m*, 1470*m*, 1417*m*, 1367*w*, 1331*m*, 1296*m*, 1250*s*, 1210*m*, 1152*s*, 1118*m*, 1096*m*, 1065*m*, 1030*s*, 955*m*, 918*w*, 886*m*, 846*s*, 780*m*, 760*w*, 697*m*, 665*m*, 648*w*, 614*w*, 594*w*, 534*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.34, 8.13 (2*s*, H–C(2), H–C(8)); 6.46 (*d*, $J = 7.4$, H–C(1′)); 5.77 (*s*, NH_2–C(6)); 4.79 (*dd*, $J = 8.5, 9.6$, H–C(3′)); 4.23 (*dd*, $J = 2.9, 13.2$, 1 H–C(5′)); 4.05 (*dd*, $J = 2.9, 13.2$, 1 H–C(5′)); 3.85 (*dt*, $J = 2.9, 8.2$, H–C(4′)); 3.58 (*dd*, $J = 7.4, 9.9$, H–C(2′)); 0.99–1.19 (*m*, 28 H, ^{i}Pr); –0.22 (*s*, Me_3Si). ^{13}C-NMR (75 MHz, $CDCl_3$): 155.39 (*s*, C(6)); 152.74 (*d*, C(2)); 149.97 (*s*, C(4)); 139.06 (*d*, C(8)); 119.58 (*s*, C(5)); 99.44 (*s*, *C*≡C); 90.88 (*s*, C≡*C*); 83.91 (*d*, C(4′)); 83.30 (*d*, C(1′)); 74.03 (*d*, C(3′)); 60.55 (*t*, C(5′)); 44.67 (*d*, C(2′)); 17.47, 17.35, 17.30, 17.19, 17.02, 17.00, 16.94 (7*q*, *Me*$_2$CH); 13.56, 13.05, 12.84, 12.36 (4*d*, Me$_2$*C*H); –0.91 (*q*, MeSi). LSI-MS (DTT/DTE): 593 (12), 592 (33), 591 (51), *590* (100, $[M+4]^+$), 136 (81). Anal. calc. for $C_{27}H_{47}N_5O_4Si_3$: C 54.97, H 8.03, N 11.87; found: C 54.74, H 8.17, N 11.70.

*9-[2′-Deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-*C*-[(trimethylsilyl)ethynyl]-β-*D*-arabino-furanosyl]-*N^6*-(phenoxyacetyl)adenine* (**17**). Triethyloxonium tetrafluoroborate (4.00 g, 21.0 mmol) was added to a soln. of 1-(phenoxyacetyl)-1*H*-imidazole (4.60 g, 22.7 mmol) in CH_2Cl_2 (30 ml) at 0°. After 10 min, the cooling bath was removed and the mixture stirred for 3 h at r.t. A soln. of **16** (0.88 g, 1.50 mmol) in CH_2Cl_2 (10 ml) was added and stirring was continued for 19 h at r.t. Then the reaction was quenched by addition of sat. aq. $NaHCO_3$ soln. (30 ml). The mixture was extracted with CH_2Cl_2 (3 × 100 ml), and the org. layers were washed with sat. aq. $NaHCO_3$ soln. (3 × 50 ml), dried ($MgSO_4$), and evaporated. The remaining oil was purified by FC (170 g of silica gel, CH_2Cl_2/^{t}BuOMe 10:1): **17** (0.80 g, 74%; crystallized from hexane). Colorless needles. M.p. 108°. R_f 0.27 (CH_2Cl_2/^{t}BuOMe 10:1). $[\alpha]_D^{25} = -33.1$ ($c = 0.72$, $CHCl_3$). UV (EtOH): 263 (14700). IR (KBr): 3380*w*, 3134*m*, 2946*s*, 2895*m*, 2868*s*, 2179*w*, 1736*m*, 1720*m*, 1702*m*, 1648*w*, 1612*s*, 1586*s*, 1560*w*, 1522*w*, 1496*m*, 1466*s*, 1406*m*, 1388*w*, 1350*w*, 1329*w*, 1304*m*, 1249*s*, 1219*s*, 1152*s*, 1101*s*, 1076*s*, 1034*s*, 955*w*, 886*s*, 846*s*, 788*w*, 758*m*, 692*m*, 670*w*, 645*w*, 613*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.41 (*s*, H–N(6)); 8.80, 8.32 (2*s*, H–C(2), H–C(8)); 7.32–7.39 (*m*, 2 arom. H); 7.05–7.09 (*m*, 3 arom. H); 6.51 (*d*, $J = 7.4$, H–C(1′)); 4.89 (*s*, PhOC*H*$_2$-CO); 4.85 (*dd*, $J = 8.3, 9.7$, H–C(3′)); 4.24 (*dd*, $J = 3.1, 13.1$, 1 H–C(5′)); 4.06 (*dd*, $J = 2.9, 12.9$, 1 H–C(5′)); 3.89 (*dt*, $J = 3.0, 8.2$, H–C(4′)); 3.63 (*dd*, $J = 7.4, 9.6$, H–C(2′)); 0.99–1.21 (*m*, 28 H, ^{i}Pr); –0.25 (*s*, Me_3Si). ^{13}C-NMR (75 MHz, $CDCl_3$): 166.78 (*s*, PhOCH_2*C*O); 156.99 (*s*, arom. C); 152.03 (*d*, C(2)); 151.76, 148.07 (2*s*, C(4), C(6)); 142.00 (*d*, C(8)); 129.85 (*d*, arom. C); 122.52 (*d*, arom. C); 122.28 (*s*, C(5)); 115.00 (*d*, arom. C); 99.18 (*s*, *C*≡C); 91.27 (*s*, C≡*C*); 84.14 (*d*, C(4′)); 83.61 (*d*, C(1′)); 74.06 (*d*, C(3′)); 68.14 (*t*, PhO*C*H$_2$CO); 60.53 (*t*, C(5′)); 44.67 (*d*, C(2′)); 17.46, 17.35, 17.30, 17.17, 17.01, 16.97, 16.92 (7*q*, *Me*$_2$CH); 13.55, 13.03, 12.87, 12.37 (4*d*, Me$_2$*C*H); –0.91 (*q*, MeSi). LSI-MS: 725 (13), 724 (23, $[M+H]^+$), 271 (17), *270* (100), 177 (11). HR-MS: 724.3366 ($C_{35}H_{54}N_5O_6Si_3^+$; calc. 724.3382).

*N^2-[(Dimethylamino)methylene]-9-[3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-*D*-*erythro*-pent-2′-ulofuranosyl]guanine* (**20**). *Dess-Martin* periodinane (0.25 g, 0.58 mmol) was added to a soln. of **19** [17] (0.16 g, 0.28 mmol) in CH_2Cl_2 (10 ml). After stirring for 15 h at r.t., Et_2O (100 ml) was added, and the reaction was quenched by addition of sat. aq. $NaHCO_3$ soln. (2 × 100 ml) containing sodium thiosulfate (5 g). The aq. layers were extracted again with Et_2O (100 ml), and the org. layers were dried ($MgSO_4$) and evaporated: crude **20**

(0.16 g, 96%; keto and hydrate ratio 1:1 by ^{1}H-NMR). The yellowish solid was used in the next step without further purification. R_f 0.41 (CH_2Cl_2/MeOH 10:1). ^{1}H-NMR (300 MHz, $CDCl_3$): 10.12, 9.73 (2*s*, 1 H, H–N(1)); 8.31 (*s*, 1 H, H–C(8)); 7.72, 7.45 (2*s*, 1 H, Me_2NCH); 6.41 (br. *s*, 0.5 H, OH–C(2′)); 6.03, 5.90 (2*s*, 1 H, H–C(1′)); 5.64 (br. *s*, 0.5 H, OH–C(2′)); 4.80 (*d*, *J* = 9.2, 0.5 H, H–C(3′)); 4.28 (*d*, *J* = 8.8, 0.5 H, H–C(3′)); 4.17 (*dd*, *J* = 2.8, 7.5, 1 H, H–C(5′)); 4.02–4.10 (*m*, 1.5 H, H–C(4′), H–C(5′)); 3.86 (*dt*, *J* = 2.9, 8.7, 0.5 Hz, H–C(4′)); 3.12, 3.07, 2.99, 2.85 (4*s*, 6 H, Me_2N); 1.00–1.12 (*m*, 28 H, ^{i}Pr). ^{13}C-NMR (75 MHz, $CDCl_3$): 205.38 (*s*, C(2′)); 158.17, 157.87 (2*d*, Me_2NC); 157.24, 156.73 (2*s*, C(6)); 150.48, 150.41 (2*s*, C(4)); 137.16, 135.93 (2*d*, C(8)); 120.01, 119.02 (2*s*, C(5)); 99.17 (*s*, C(2′) (hydrate)); 87.35 (*d*, C(1′)); 79.86 (*d*, C(4′)); 79.56 (*d*, C(1′)); 78.51 (*d*, C(4′)); 74.01, 72.73 (2*d*, C(3′)); 61.21, 61.05 (2*t*, C(5′)); 41.56, 41.37, 35.27, 34.99 (4*q*, Me_2N); 17.41, 17.28, 17.16, 17.07, 16.99, 16.85, 16.83, 16.78 (8*q*, Me_2CH); 13.66, 13.42, 13.02, 12.93, 12.76, 12.67, 12.50, 12.44 (8*d*, Me_2CH). LSI-MS: 597 (14, $[M+H+H_2O]^+$), 579 (2, $[M+H]^+$), 235 (14), 208 (12), *207* (100), 206 (10).

N^2-*[(Dimethylamino)methylene]-9-{3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-*C*-[(trimethylsiliyl)-ethynyl]-β-*D*-arabinofuranosyl]guanine* (**21**). (Trimethylsilyl)acetylene (4.00 ml, 28.5 mmol) was added dropwise to a soln. of 1.6M BuLi in hexane (17.3 ml, 27.7 mmol) in THF (100 ml) at −17°. After stirring for 30 min, the soln. was cooled to −78°. A soln. of **20** (5.30 g, 9.20 mmol) in THF (130 ml) was added dropwise over 10 min to the above soln. at −78°. The mixture was stirred for 90 min at −78° and then quenched by addition of sat. aq. NH_4Cl soln. (150 ml). The mixture was extracted with AcOEt (3 × 100 ml), the org. layer washed with brine (100 ml), dried ($MgSO_4$), and evaporated, and the residue recrystallized from hexane/acetone 2:1 to afford colorless needles. The mother liquor was purified by FC (10 g of silica gel, CH_2Cl_2/MeOH 10:1): **21** (5.00 g, 80%). Colorless solid. M.p. 132–135°. R_f 0.38 (CH_2Cl_2/MeOH 10:1). $[\alpha]_D^{25} = -23.9$ (*c* = 1.03, $CHCl_3$). UV (EtOH): 236 (14600), 303 (19100). IR (KBr): 3132*m*, 2947*s*, 2896*s*, 2869*s*, 2172*w*, 1744*w*, 1696*s*, 1633*s*, 1542*s*, 1465*s*, 1426*s*, 1389*m*, 1349*s*, 1319*m*, 1249*s*, 1165*s*, 1117*s*, 1064*s*, 1035*s*, 956*m*, 919*w*, 884*s*, 846*s*, 818*m*, 780*m*, 764*m*, 701*s*, 624*m*, 602*w*, 557*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.26 (br. *s*, H–N(1)); 8.51 (*s*, H–C(8)); 7.90 (*s*, Me_2NCH); 6.05 (*s*, H–C(1′)); 4.48 (br. *s*, OH–C(2′)); 4.37 (*d*, *J* = 6.6, H–C(3′)); 4.06–4.10 (*m*, 2 H–C(5′)); 3.98–4.04 (*m*, H–C(4′)); 3.19, 3.08 (2*s*, Me_2N); 0.95–1.13 (*m*, 28 H, ^{i}Pr); 0.16 (*s*, Me_3Si). ^{13}C-NMR (75 MHz, $CDCl_3$): 158.11 (*d*, Me_2NC); 157.99 (*s*, C(6)); 156.70 (*s*, C(2)); 149.84 (*s*, C(4)); 137.28 (*d*, C(8)); 120.03 (*s*, C(5)); 101.72, 101.56 (2*s*, C≡C); 93.77 (*s*, C(2′)); 88.20 (*d*, C(1′)); 82.20 (*d*, C(4′)); 76.61 (*d*, C(3′)); 61.80 (*t*, C(5′)); 41.40, 35.13 (2*q*, Me_2N); 17.42, 17.27, 17.25, 17.17, 16.95, 16.92, 16.72 (7*q*, Me_2CH); 13.35, 13.04, 12.88, 12.56 (4*d*, Me_2CH); −0.33 (*q*, MeSi). LSI-MS (DTT/DTE): 678 (18), 677 (28, $[M+H]^+$), 208 (12), *207* (100). HR-MS: 677.3299 ($C_{30}H_{53}N_6O_6Si_3^+$; calc. 677.3334).

*9-{2′-Deoxy-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-*C*-[(trimethylsilyl)ethynyl]-β-*D*-arabino-furanosyl}-*N^2*-[(dimethylamino)methylene]guanine* (**22**). Methoxalyl chloride (3.40 ml, 36.9 mmol) was added to a soln. of **21** (5.00 g, 7.40 mmol) and DMAP (4.95 g, 40.6 mmol) in CH_2Cl_2 (80 ml) at −17°. After 90 min, the mixture was warmed to r.t. and stirring continued for 4 h. The mixture was then washed with H_2O (3 × 60 ml) and brine (40 ml), the org. phase dried ($MgSO_4$) and evaporated, and the residue co-evaporated with toluene (3 × 20 ml). A soln. of the residue, AIBN (40 mg), and Bu_3SnH (10.0 ml, 37.7 mmol) in toluene (30 ml) was heated for 60 min at 95°. Additional AIBN (7 mg) and Bu_3SnH (2.00 ml, 7.24 mmol) were added, and the mixture was heated for another 60 min at 95°. Evaporation and purification of the residue by FC (500 g of silica gel, CH_2Cl_2/MeOH 10:1) gave **22** (3.20 g, 65%; crystallized from MeCN). Colorless solid. M.p. 175–178°. R_f 0.47 (CH_2Cl_2/MeOH 10:1). $[\alpha]_D^{25} = +80.5$ (*c* = 0.86, $CHCl_3$). UV (EtOH): 236 (14700), 303 (19800). IR (KBr): 3412*w*, 3121*m*, 2946*s*, 2896*m*, 2869*s*, 2362*w*, 2176*w*, 1682*s*, 1633*s*, 1540*s*, 1465*m*, 1424*s*, 1388*m*, 1345*s*, 1318*m*, 1249*s*, 1201*w*, 1152*s*, 1116*s*, 1066*s*, 1033*s*, 955*m*, 918*w*, 885*m*, 846*s*, 779*m*, 700*s*, 600*w*, 558*m*, 524*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.84 (br. *s*, H–N(1)); 8.60 (*s*, Me_2NCH); 7.91 (*s*, H–C(8)); 6.34 (*d*, *J* = 7.6, H–C(1′)); 4.63 (*dd*, *J* = 8.4, 9.6, H–C(3′)); 4.11 (*dd*, *J* = 2.9, 13.0, 1 H–C(5′)); 4.04 (*dd*, *J* = 2.9, 13.0, 1 H–C(5′)); 3.79 (*dt*, *J* = 2.9, 8.3, H–C(4′)); 3.52 (*dd*, *J* = 7.5, 9.6, H–C(2′)); 3.20, 3.11 (2*s*, Me_2N); 0.95–1.19 (*m*, 28 H, ^{i}Pr); −0.09 (*s*, Me_3Si). ^{13}C-NMR (75 MHz, $CDCl_3$): 157.88 (*s*, Me_2NC); 157.64 (*s*, C(6)); 156.30 (*s*, C(2)); 150.30 (*s*, C(4)); 136.35 (*d*, C(8)); 120.01 (*s*, C(5)); 99.67, 91.31 (2*s*, C≡C); 83.71 (*d*, C(4′)); 82.31 (*d*, C(1′)); 74.31 (*d*, C(3′)); 60.63 (*t*, C(5′)); 44.55 (*d*, C(2′)); 41.32, 35.20 (2*q*, Me_2N); 17.48, 17.32, 17.29, 17.23, 17.04, 17.02, 16.97 (7*q*, Me_2CH); 13.60, 13.06, 12.88, 12.39 (4*d*, Me_2CH); −0.68 (*q*, MeSi). LSI-MS: 663 (10), 662 (18), 661 (33, $[M+H]^+$), 208 (11), *207* (100), 206 (11). Anal. calc. for $C_{30}H_{52}N_6O_5Si_3$: C 54.51, H 7.93, N 12.71; found: C 54.45, H 7.72, N 12.68.

*9-(2′-Deoxy-2′-*C*-ethynyl-β-*D*-arabinofuranosyl)-*N^6*-(phenoxyacetyl)adenine* (**24**). A 1.1M Bu_4NF soln. in THF (4.00 ml, 4.40 mmol) was added to **17** (0.80 g, 1.10 mmol) and AcOH (0.55 ml, 9.20 mmol) in THF (20 ml) at r.t. The mixture was stirred for 1 h at r.t. and then evaporated. The remaining oil was purified by FC (100 g of silica gel; $CHCl_3$/MeOH 10:1): **24** (0.43 g, 95%; crystallized from EtOH/H_2O 10:1). Colorless needles. M.p. 190° (dec.) R_f = 0.34 (CH_3Cl/MeOH 10:1). $[\alpha]_D^{25} = -9.9$ (*c* = 0.70, DMSO). UV (EtOH): 262 (15300). IR (KBr): 3392*s*, 3295*s*, 3113*m*, 3026*w*, 2930*m*, 2884*m*, 2120*w*, 1918*w*, 1846*w*, 1720*s*, 1608*s*, 1582*s*, 1522*m*, 1500*s*,

1492*s*, 1456*s*, 1431*s*, 1400*s*, 1336*m*, 1324*m*, 1303*s*, 1221*s*, 1196*s*, 1174*s*, 1143*s*, 1126*m*, 1098*s*, 1076*s*, 1056*s*, 1041*s*, 1029*s*, 1013*s*, 964*w*, 943*w*, 923*w*, 912*m*, 901*w*, 891*m*, 858*m*, 846*m*, 801*m*, 780*m*, 757*s*, 726*m*, 693*s*, 677*m*, 656*s*, 640*s*, 596*m*, 580*w*, 551*m*, 542*m*, 516*m*, 510*m*. ^{1}H-NMR (300 MHz, (D_6)DMSO): 10.86 (*s*, H–N(6)); 8.71, 8.67 (2*s*, H–C(2), H–C(8)); 7.27–7.34 (*m*, 2 arom. H); 6.94–6.98 (*m*, 3 arom. H); 6.57 (*d*, $J = 7.4$, H–C(1′)); 5.95 (*d*, $J = 5.5$, OH–C(3′)); 5.18 (br. *t*, OH–C(5′)); 5.03 (*s*, PhOCH_2CO); 4.53 (*dd*, $J = 8.3, 13.4$, H–C(3′)); 3.59–3.83 (*m*, H–C(2′), H–C(4′), 2 H–C(5′)); 2.81 (*d*, $J = 2.6$, H–C≡C). ^{13}C-NMR (75 MHz, (D_6)DMSO): 167.52 (*s*, PhOCH$_2$*C*O); 158.01 (*s*, arom. C); 151.81 (*d*, C(2)); 151.72 (*s*, C(6)); 149.05 (*s*, C(4)); 142.86 (*d*, C(8)); 129.68 (*d*, arom. C); 122.72 (*s*, C(5)); 121.27 (*d*, arom. C); 114.76 (*d*, arom. C); 85.59, 83.11 (2*d*, C(1′), C(4′)); 79.27, 76.04 (*s*, *d*, C≡C); 73.25 (*d*, C(3′)); 67.42 (*t*, PhO*C*H$_2$CO); 59.90 (*t*, C(5′)); 43.67 (*d*, C(2′)). LSI-MS (DTT/DTE): 410 (10, $[M+H]^+$), 309 (30), 155 (71), 154 (22), 153 (15), 137 (10), 135 (25), *119* (100), 103 (41). Anal. calc. for $C_{20}H_{19}N_5O_5 \cdot 1/3\ H_2O$: C 57.83, H 4.77, N 16.86; found: C 57.82, H 4.67, N 16.90.

*9-(2′-Deoxy-2′-*C*-ethynyl-β-*D*-arabinofuranosyl)-*N^2*-[(dimethylamino)methylene]guanine* (**25**). A soln. of 1.0M Bu_4NF in THF (14.5 ml, 14.5 mmol) was added to **22** (3.20 g, 4.80 mmol) and AcOH (0.83 ml, 14.5 mmol) in THF (60 ml) at r.t. This soln. was stirred for 3 h at r.t. and then evaporated. The remaining oil was purified by FC (300 g of silica gel, $CHCl_3$/MeOH 10:1): **25** (1.30 g, 78%; crystallized from EtOH/H_2O 5:1). Colorless needles. M.p. 220° (dec.). R_f 0.23 (CH_3Cl/MeOH 5:1). $[\alpha]_D^{25} = +29.0$ ($c = 0.70$, DMSO). UV (EtOH): 236 (15800), 304 (20400). IR (KBr): 3377*s*, 3301*s*, 3107*m*, 3095*s*, 2913*m*, 2872*m*, 2348*w*, 2282*w*, 1674*s*, 1636*s*, 1540*s*, 1464*m*, 1430*s*, 1390*s*, 1346*s*, 1319*s*, 1288*m*, 1239*s*, 1223*m*, 1204*m*, 1160*s*, 1122*s*, 1101*s*, 1083*s*, 1071*s*, 1054*s*, 1030*m*, 1014*s*, 913*w*, 874*w*, 853*m*, 794*m*, 785*m*, 739*m*, 716*m*, 706*m*, 666*m*, 652*m*, 595*m*, 558*m*, 516*w*. ^{1}H-NMR (300 MHz, (D_6)DMSO): 11.31 (*s*, H–N(1)); 8.58 (*s*, Me_2NCH); 7.99 (*s*, H–C(8)); 6.32 (*d*, $J = 7.4$, H–C(1′)); 5.87 (*d*, $J = 5.8$, OH–C(3′)); 5.04 (*t*, $J = 5.3$, OH–C(5′)); 4.46 (*dd*, $J = 7.7$, 14.0, H–C(3′)); 3.68–3.73 (*m*, H–C(4′), 1 H–C(5′)); 3.59–3.65 (*m*, 1 H–C(5′)); 3.51 (*ddd*, $J = 2.5, 7.5, 8.8$, H–C(2′)); 3.16, 3.03 (2*s*, Me_2N); 2.91 (*d*, $J = 2.5$, H–C≡C). Difference-NOE (500 MHz,(D_6)DMSO): 6.32 (H–C(1′)) → 7.99 (H–C(8)), 3.51 (H–C(2′)); 3.51 (H–C(2′)) → 6.32 (H–C(1′)). ^{13}C-NMR (75 MHz, (D_6)DMSO): 158.20 (*d*, Me_2NC); 157.81 (*s*, C(6)); 157.36 (*s*, C(2)); 149.88 (*s*, C(4)); 137.00 (*d*, C(8)); 119.10 (*s*, C(5)); 85.26, 82.15 (2*d*, C(1′), C(4′)); 79.56, 76.02 (*s*, *d*, C≡C); 73.46 (*d*, C(3′)); 60.00 (*t*, C(5′)); 43.59 (*d*, C(2′)); 40.82, 34.81 (2*q*, Me_2N). LSI-MS: 347 (33, $[M+H]^+$), 309 (39), 242 (16), 207 (18), 155 (82), 154 (23), 153 (15), 137 (10), 135 (21), 121 (15), *119* (100), 103 (40), 102 (10). Anal. calc. for $C_{15}H_{18}N_6O_4$: C 52.02, H 5.24, N 24.26; found: C 52.09, H 5.51, N 24.01.

*1-(2′-Deoxy-2′-*C*-ethynyl-β-*D*-arabinofuranosyl)-*N^4*-isobutyrylcytosine* (**26**). To a soln. of **11** (2.13 g, 3.35 mmol) and AcOH (0.575 ml, 0.604 g, 10.1 mmol) in THF (34 ml), Bu_4NF (3.17 g, 10.1 mmol) was added. The soln. was stirred for 30 min at r.t. and then evaporated and the remaining oil submitted to FC (75 g of silica gel, CH_2Cl_2/MeOH 19:1): **26** (0.974 g, 90%). Colorless foam. R_f 0.30 (CH_2Cl_2/MeOH 9:1). $[\alpha]_D^{25} = +148.0$ ($c = 1.00$, EtOH). UV (EtOH): 213 (17100), 249 (15200), 299 (7630). IR (KBr): 3289*m* (br.), 2972*m*, 2934*m*, 2876*m*, 2120*w*, 1724*m*, 1654*s*, 1560*s*, 1491*s*, 1431*m*, 1396*s*, 1315*s*, 1273*m*, 1222*m*, 1184*m*, 1137*m*, 1099*s*, 1026*m*, 965*w*, 932*w*, 876*w*, 798*m*, 744*m*, 670*m*, 660*m*, 561*m*. ^{1}H-NMR (300 MHz, (D_6)DMSO): 10.83 (*s*, H–N(4)); 8.24 (*d*, $J = 7.7$, H–C(6)); 7.25 (*d*, $J = 7.4$, H–C(5)); 6.17 (*d*, $J = 6.6$, H–C(1′)); 5.85 (*d*, $J = 5.5$, OH–C(3′)); 5.12 (*d*, $J = 5.5$, OH–C(5′)); 4.18 (*q*, $J = 5.5$, H–C(3′)); 3.76–3.81 (*m*, H–C(4′)); 3.59–3.73 (*m*, 2 H–C(5′)); 3.41 (*ddd*, $J = 2.6, 5.9, 6.6$, H–C(2′)); 3.07 (*d*, $J = 2.6$, H–C≡C); 2.70 (*sept.*, $J = 7.0$, Me_2HCO); 1.05 (*d*, $J = 7.0$, Me_2CHCO). ^{13}C-NMR (75 MHz, (D_6)DMSO): 178.09 (*s*, Me_2CH*C*O); 162.78 (*s*, C(4)); 154.49 (*s*, C(2)); 145.64 (*d*, C(6)); 95.09 (*d*, C(5)); 86.16 (*d*, C(1′)); 85.31 (*d*, C(4′)); 79.53 (*s*, *C*≡CH); 76.90 (*d*, H*C*≡C); 74.66 (*d*, C(3′)); 60.26 (*t*, C(5′)); 43.47 (*d*, C(2′)); 35.15 (*d*, Me_2*C*HCO); 19.23, 19.21 (2*q*, *Me*$_2$CHCO). LSI-MS: 322 (25, $[M+H]^+$), 243 (13), 242 (73), 184 (8), 183 (10), *182* (100), 142 (9), 112 (34).

*9-[2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-*C*-ethynyl-β-*D*-arabinofuranosyl]-*N^6*-(phenoxyacetyl)adenine* (**27**). The 4,4′-dimethyltrityl chloride ($(MeO)_2$TrCl, 0.68 g, 2.00 mmol) was added under vigorous stirring to a soln. of **24** (0.50 g, 1.20 mmol) in pyridine (6 ml). After 3 h, additional $(MeO)_2$TrCl (0.41 g, 1.20 mmol) was added, and stirring was continued for 2 h. The reaction was quenched by addition of sat. aq. $NaHCO_3$ soln. (100 ml), and the mixture was extracted with CH_2Cl_2 (3 × 50 ml). The combined org. layers were dried (Na_2SO_4), and evaporated. The remaining oil was purified by FC (150 g of silica gel, CH_2Cl_2/MeOH 20:1 + 2‰ NEt_3): **27** (0.50 g, 61%; crystallized from MeCN). Colorless needles. M.p. 201°. R_f 0.29 (CH_2Cl_2/MeOH 20:1 + 2‰ Et_3N). $[\alpha]_D^{25} = -17.7$ ($c = 0.84$, $CHCl_3$). M.p. 201°. UV (EtOH): 236 (22600), 271 (20000). IR (KBr): 3390*m*, 3257*m*, 3105*w*, 3059*w*, 2951*w*, 2836*w*, 1736*m*, 1674*w*, 1609*s*, 1587*s*, 1551*w*, 1512*s*, 1460*s*, 1399*m*, 1356*m*, 1323*m*, 1300*s*, 1250*s*, 1177*s*, 1154*m*, 1118*m*, 1087*s*, 1064*m*, 1034*s*, 990*m*, 903*w*, 835*m*, 791*m*, 756*m*, 728*m*, 707*m*, 648*m*, 592*m*, 562*w*, 516*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.55 (*s*, H–N(6)); 8.73, 8.35 (*s*, H–C(2), H–C(8)); 7.45–7.48 (*m*, 2 arom. H); 7.19–7.37 (*m*, 9 arom. H); 7.03–7.08 (*m*, 3 arom. H); 6.81–6.84 (*m*, 4 arom. H); 6.60 (*d*, $J = 7.0$, H–C(1′)); 4.84 (*s*, PhOCH_2CO); 4.76 (*t*, $J = 6.4$, H–C(3′)); 4.15 (*dd*, $J = 4.8, 11.0$, H–C(4′)); 3.79 (*s*, 2 MeO); 3.73–3.81 (*m*, H–C(2′)); 3.53–3.59 (*m*, 2 H–C(5′)); 1.86 (*d*, $J = 2.7$, H–C≡C). ^{13}C-NMR (75 MHz, $CDCl_3$):

167.45 (*s*, $PhOCH_2CO$); 158.21, 158.17, 158.00 (3*s*, arom. C); 151.68 (*d*, C(2)); 151.49 (*s*, C(6)); 149.04 (*s*, C(4)); 144.91 (*s*, arom. C); 143.12 (*d*, C(8)); 135.71, 135.57 (2*s*, arom. C); 129.86, 129.65, 127.89, 126.80 (4*d*, arom. C); 122.94 (*s*, C(5)); 121.25, 114.73, 113.26, 113.21 (4*d*, arom. C); 85.71 (*s*, *C*−O−C(5′)); 83.75, 83.39 (2*d*, C(1′), C(4′)); 79.17, 76.17 (*s*, *d*, C≡C); 74.38 (*d*, C(3′)); 67.40 (*t*, $PhOCH_2CO$); 63.45 (*t*, C(5′)); 55.15 (*q*, MeO); 43.30 (*d*, C(2′)). LSI-MS: 712 (5, $[M+H]^+$), 309 (33), 307 (10), 155 (72), 154 (23), 153 (15), 137 (10), 135 (24), 121 (15), *119* (100), 103 (41). Anal. calc. for $C_{41}H_{37}N_5O_7$: C 69.19, H 5.24, N 9.84; found: C 68.92, H 5.13, N 9.88.

9-[2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)-2′*-C-*ethynyl)-β-*D-*arabinofuranosyl]*-N^2-*[(dimethylamino)methylene]guanine* (**28**). A soln. of $(MeO)_2TrCl$ (1.63 g, 4.80 mmol) and **25** (3.12 g, 4.80 mmol) in pyridine (100 ml) was stirred at r.t. After 3 h, more $(MeO)_2TrCl$ (0.73 g, 0.45 mmol) was added. After a total of 7 h, the reaction was quenched by addition of sat. aq. $NaHCO_3$ soln. (150 ml), and the mixture was extracted with CH_2Cl_2 (3 × 100 ml). The org. phase was dried (Na_2SO_4) and evaporated and the residue purified by FC (320 g of silica gel, CH_2Cl_2/MeOH 20:1 + 2‰ Et_3N): **28** (2.10 g, 67%; crystallized from MeCN). Colorless needles. M.p. 189–191°. R_f 0.40 (CH_2Cl_2/MeOH 10:1). $[\alpha]_D^{25} = -38.0$ (*c* = 0.78, DMSO). UV (EtOH): 235 (33400), 305 (19200). IR (KBr): 3285*m*, 3117*m*, 2999*m*, 2930*m*, 2836*m*, 2046*w*, 1686*s*, 1682*s*, 1632*s*, 1608*m*, 1540*s*, 1463*m*, 1428*s*, 1391*m*, 1350*s*, 1318*m*, 1249*s*, 1177*s*, 1154*m*, 1115*s*, 1091*s*, 1032*s*, 913*w*, 830*m*, 785*m*, 756*m*, 742*m*, 727*ms*, 705*m*, 656*m*, 584*m*, 556*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.60 (*s*, H−N(1)); 8.50 (*s*, Me_2NCH); 7.88 (*s*, H−C(8)); 7.14–7.47 (*m*, 9 arom. H); 6.75–6.81 (*m*, 4 arom. H); 6.45 (*d*, *J* = 6.6, H−C(1′)); 4.91 (br. *s*, OH−C(3′)); 4.72 (*t*, *J* = 5.9, H−C(3′)); 4.17 (*q*, *J* = 5.4, H−C(4′)); 3.75 (*s*, 2 MeO); 3.43–3.62 (*m*, H−C(2′), 2 H−C(5′)); 3.02, 2.97 (2*s*, Me_2N); 1.89 (*d*, *J* = 2.6, H−C≡C). ^{13}C-NMR (75 MHz, $CDCl_3$): 158.43, 158.08 (2*s*, *d*, Me_2NC, C(6), arom. C); 156.65 (*s*, C(2)); 150.08 (*s*, C(4)); 144.56 (*s*, arom. C); 137.20 (*d*, C(8)); 135.83, 135.73 (2*s*, arom. C); 130.01, 128.15, 127.81, 126.79 (4*d*, arom. C); 119.52 (*s*, C(5)); 113.11 (*d*, arom. C); 86.37 (*s*, *C*−O−C(5′)); 84.00, 83.23 (2*d*, C(1′), C(4′)); 78.51, 75.07 (*s*, *d*, C≡C); 75.07 (*d*, C(3′)); 63.60 (*t*, C(5′)); 55.19 (*q*, MeO); 44.13 (*d*, C(2′)); 41.31, 35.07 (2*q*, Me_2N). LSI-MS (DTT/DTE): 649 (8, $[M+H]^+$), 304 (23), *303* (100), 207 (41). Anal. calc. for $C_{36}H_{36}N_6O_6$: C 66.65, H 5.59, N 12.95; found: C 66.48, H 5.83, N 12.92.

1-[2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)-2′*-C-*ethynyl-β-*D-*arabinofuranosyl]*-N^4-*isobutyrylcytosine* (**29**). To a soln. of **26** (0.660 g, 2.05 mmol) and DMAP (0.025 g, 0.205 mmol) in pyridine (20 ml), $(MeO)_2TrCl$ (0.835 g, 2.47 mmol) was added. After stirring for 3 h at r.t., the soln. was diluted with CH_2Cl_2 and washed with sat. $NaHCO_3$ soln. (2 × 50 ml). The aq. layers were re-extracted with 50 ml of CH_2Cl_2. The combined org. phase was dried (Na_2SO_4) and evaporated and the residue submitted to FC (80 g of silica gel, CH_2Cl_2/MeOH 24:1): **29** (1.07 g, 83%). Yellow foam. R_f 0.34 (CH_2Cl_2/MeOH 19:1). $[\alpha]_D^{25} = +13.6$ (*c* = 1.15, $CHCl_3$). UV (EtOH): 237 (27100), 284 (6880), 301 (7620). IR (KBr): 3289*m* (br.), 3068*m*, 3034*m*, 2969*m*, 2933*m*, 2875*m*, 2836*m*, 2046*w*, 1724*m*, 1652*s*, 1609*s*, 1558*s*, 1509*s*, 1488*s*, 1446*m*, 1393*s*, 1312*s*, 1251*s*, 1177*s*, 1134*s*, 1090*s*, 1033*s*, 990*m*, 954*w*, 932*w*, 914*w*, 829*m*, 792*m*, 772*m*, 756*m*, 727*m*, 702*m*, 656*m*, 585*m*, 516*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.59 (br. *s*, H−N(4)); 8.08 (*d*, *J* = 7.5, H−C(6)); 7.45–7.48 (*m*, 2 arom. H); 7.22–7.37 (*m*, 8 arom. H, H−C(5)); 6.84–6.87 (*m*, 4 arom. H); 6.41 (*d*, *J* = 6.1, H−C(1′)); 4.45 (*t*, *J* = 4.4, H−C(3′)); 4.20 (*dt*, *J* = 4.4, 5.6, H−C(4′)); 4.05 (br. *s*, OH−C(3′)); 3.81 (*s*, 2 MeO); 3.79–3.82 (*m*, H−C(2′)); 3.52 (*dd*, *J* = 4.3, 10.4, 1 H−C(5′)); 3.47 (*dd*, *J* = 5.7, 10.4, 1 H−C(5′)); 2.64 (*sept.*, *J* = 6.9, Me_2CHCO); 1.98 (*d*, *J* = 2.6, H−C≡C); 1.21 (*d*, *J* = 7.0, Me_2CHCO). ^{13}C-NMR (75 MHz, $CDCl_3$): 177.16 (*s*, Me_2CHCO); 162.53 (*s*, C(4)); 158.60 (*s*, arom. C); 155.50 (*s*, C(2)); 145.18 (*d*, C(6)); 144.35 (*s*, arom. C); 135.73, 135.66 (2*s*, arom. C); 130.10, 130.06, 128.25, 127.93, 127.01, 113.23 (6*d*, arom. C); 96.10 (*d*, C(5)); 86.65 (*d*, C(1′)); 86.52 (*s*, *C*−O−C(5′)); 85.25 (*d*, C(4′)); 78.38 (*s*, *C*≡CH); 76.91 (*d*, C(3′)); 75.34 (*d*, H*C*≡C); 62.69 (*t*, C(5′)); 55.24 (*q*, MeO); 43.71 (*d*, C(2′)); 36.56 (*d*, Me_2CHCO); 19.12, 19.08 (2*q*, Me_2CHCO). LSI-MS: 624 (6, $[M+H]^+$), 304 (22), *303* (100), 182 (27), 112 (10).

1-[2′-Deoxy-5′-O-*(4,4′-dimethoxytrityl)-2′*-C-*ethynyl-β-*D-*arabinofuranosyl]thymine* (**30**). To a soln. of **6** [13] (0.439 g, 1.65 mmol), DMAP (0.020 g, 0.165 mmol), DMAP (0.020 g, 0.165 mmol), and Et_3N (0.345 ml, 0.250 g, 2.47 mmol) in pyridine (16.5 ml), $(MeO)_2TrCl$ (0.670 g, 1.98 mmol) was added. After stirring for 1 h at r.t., the soln. was diluted with CH_2Cl_2 (100 ml) and extracted with H_2O (50 ml) and sat. $NaHCO_3$ soln. (50 ml). The aq. solns. were washed with CH_2Cl_2 (50 ml) and the combined org. layers dried (Na_2SO_4) and evaporated. The remaining oil was submitted to FC (70 g of silica gel, CH_2Cl_2/MeOH 24:1): **30** (0.737 g, 79%). Yellow foam. R_f 0.12 (2.5% MeOH/CH_2Cl_2). $[\alpha]_D^{25} = +13.2$ (*c* = 1.18, $CHCl_3$). UV (EtOH): 233 (21400), 270 (10700). IR ($CHCl_3$): 3602*w*, 3394*m*, 3306*m*, 3063*w*, 3005*w*, 2935*m*, 2839*w*, 2052*w*, 1689*s*, 1608*m*, 1510*m*, 1467*m*, 1410*w*, 1364*w*, 1297*m*, 1248*s*, 1183*m*, 1127*m*, 1083*m*, 1037*m*, 912*m*, 829*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.20 (*s*, H−N(3)); 7.50 (*d*, *J* = 1.1, H−C(6)); 7.43–7.47 (*m*, 2 arom. H); 7.21–7.37 (*m*, 7 arom. H); 6.81–6.86 (*m*, 4 arom. H); 6.31 (*d*, *J* = 7.0, H−C(1′)); 4.53 (*t*, *J* = 6.4, H−C(3′)); 3.98 (*dt*, *J* = 3.9, 6.6, H−C(4′)); 3.81 (br. *s*, OH−C(3′)); 3.79 (*s*, 2 MeO); 3.43–3.56 (*m*, H−C(2′), 2 H−C(5′)); 2.18 (*d*, *J* = 2.6, H−C≡C); 1.64 (*d*, *J* = 1.1, Me−C(5)). ^{13}C-NMR (75 MHz, $CDCl_3$): 163.85 (*s*, C(4)); 158.64 (*s*, arom. C); 150.25 (*s*, C(2)); 144.43 (*s*, arom. C); 135.98 (*d*, C(6)); 135.58, 135.53 (2*s*, arom. C); 130.09, 130.05, 128.17, 127.92, 127.03, 113.22

(6*d*, arom. C); 110.01 (*s*, C(5)); 86.59 (*s*, *C*–O–C(5′)); 84.25, 83.64 (2*d*, C(1′), C(4′)); 78.50 (*d*, H*C*≡C)); 75.90 (*d*, C(3′)); 75.02 (*s*, *C*≡CH); 61.96 (*t*, C(5′)); 55.21 (*q*, MeO); 43.83 (*d*, C(2′)); 12.14 (*q*, *Me*–C(5)). LSI-MS: 569 (12, $[M+H]^+$), 304 (21), *303* (100), 155 (9), 135 (7), 119 (17), 103 (6).

*1-[2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-*C*-ethynyl-β-*D*-arabinofuranosyl]uracil* (**31**). $(MeO)_2TrCl$ (0.932 g, 2.75 mmol) was added to a soln. of **7** [13] (0.578 g, 2.29 mmol) and DMAP (0.028 g, 0.229 mmol) in pyridine (23 ml). After stirring for 2.5 h at r.t., the mixture was diluted with CH_2Cl_2 (100 ml) and then washed with sat. aq. $NaHCO_3$ soln. (2 × 50 ml). The aq. phases were re-extracted with CH_2Cl_2 (50 ml). The combined org. layer was dried (Na_2SO_4) and evaporated and the remaining oil submitted to FC (75 g of silica gel, CH_2Cl_2/MeOH 97:3): **31** (1.03 g, 81%). Yellow foam. R_f 0.29 (CH_2Cl_2/MeOH 19:1). $[\alpha]_D^{25} = +21.7$ ($c = 0.98$, $CHCl_3$). UV (EtOH): 235 (20500), 257 (11700), 263 (11400). IR (KBr): 3400*m* (br.), 3281*m*, 3060*m*, 2933*m*, 2836*m*, 2046*w*, 1694*s*, 1608*s*, 1582*m*, 1510*s*, 1464*s*, 1441*m*, 1393*m*, 1299*m*, 1252*s*, 1177*s*, 1153*m*, 1109*m*, 1090*s*, 1032*s*, 1000*m*, 913*w*, 870*w*, 829*m*, 791*m*, 773*w*, 756*m*, 727*w*, 704*m*, 657*m*, 634*m*, 585*m*, 554*m*, 533*w*, 510*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.27 (br. *s*, H–N(3)); 7.86 (*d*, *J* = 8.1, H–C(6)); 7.41–7.44 (*m*, 2 arom. H); 7.24–7.33 (*m*, 7 arom. H); 6.83–6.88 (*m*, 4 arom. H); 6.32 (*d*, *J* = 7.0, H–C(1′)); 5.45 (*d*, *J* = 8.1, H–C(5)); 4.52 (*t*, *J* = 7.0, H–C(3′)); 3.96 (*dt*, *J* = 3.5, 7.0, H–C(4′)); 3.79 (*s*, 2 MeO); 3.59 (*dd*, *J* = 3.3, 10.7, 1 H–C(5′)); 3.49 (*dd*, *J* = 4.0, 11.1, 1 H–C(5′)); 3.44 (*dt*, *J* = 2.6, 7.0, H–C(2′)); 3.27 (br. *s*, OH–C(3′)); 2.19 (*d*, *J* = 2.6, H–C≡C). ^{13}C-NMR (75 MHz, $CDCl_3$): 163.33 (*s*, C(4)); 158.69 (*s*, arom. C); 150.26 (*s*, C(2)); 144.35 (*s*, arom. C); 140.59 (*d*, C(6)); 135.33, 135.40 (2*s*, arom. C); 130.12, 128.18, 127.99, 127.14, 113.29 (5*d*, arom. C); 101.75 (*d*, C(5)); 86.94 (*s*, *C*–O–C(5′)); 84.33, 83.84 (2*d*, C(1′), C(4′)); 78.04 (*d*, H*C*≡C); 77.23 (*s*, *C*≡CH); 75.15 (*d*, C(3′)); 61.33 (*t*, C(5′)); 55.26 (*q*, MeO); 43.92 (*d*, C(2′)). LSI-MS: 555 (1.2, $[M+H]^+$), 304 (29), *303* (100), 135 (3), 119 (3), 113 (2). HR-MS: 555.2119 ($C_{32}H_{31}N_2O_7^+$, $[M+H]^+$; calc. 555.2131).

Phosphoramidites **32**–**36**: *General Procedure.* To a soln. of tritylated nucleosides **27**–**31** (1 mmol) and ^{i}Pr$_2$NEt (3.00 mmol) in 10 ml of THF (for **28**, CH_2Cl_2), 2-cyanoethyl diisopropylphosphoramidochloridite (1.50 mmol) was added. After stirring for 1 h (6 h for **28**) at r.t., the reaction was quenched by addition of sat. aq. $NaHCO_3$ soln., and the mixture was extracted twice with CH_2Cl_2. The org. phase was dried (Na_2SO_4) and evaporated and the remaining crude product purified by FC for prep. chromatography, 1‰ Et_3N was added to the solvent to prevent detritylation and hydrolysis of the phosphoramidite). Phosphoramidite products were obtained as brittle colorless foams.

*9-[2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-*C*-ethynyl-β-*D*-arabinofuranosyl]-*N*^{6}-(phenoxyacetyl)adenine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**32**). Yield 80%. R_f 0.29, 0.22 (AcOEt/hexane 2:1). UV (EtOH): 235 (23300), 272 (21700). IR (KBr): 3381*m*, 3283*m*, 3059*w*, 2966*m*, 2932*m*, 2837*w*, 2252*w*, 2051*w*, 1721*m*, 1674*w*, 1609*s*, 1587*s*, 1550*w*, 1510*s*, 1460*s*, 1397*m*, 1365*m*, 1330*m*, 1300*m*, 1250*s*, 1219*s*, 1178*s*, 1154*m*, 1121*m*, 1082*s*, 1032*s*, 979*m*, 898*m*, 831*m*, 793*m*, 756*m*, 728*m*, 702*m*, 644*m*, 583*m*, 562*m*, 529*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.45 (*s*, H–N(6)); 8.78 (*s*, H–C(2)); 8.37, 8.34 (2*s*, H–C(8)); 7.46–7.50 (*m*, 2 arom. H); 7.22–7.39 (*m*, 9 arom. H); 7.05–7.09 (*m*, 3 arom. H); 6.80–6.86 (*m*, 4 arom. H); 6.61 (*d*, *J* = 6.6, H–C(1′)); 4.87 (*s*, PhOC*H*$_2$CO); 4.80–4.85 (*m*, H–C(3′)); 4.22–4.29 (*m*, H–C(4′)); 3.80, 3.79 (2*s*, 2 MeO); 3.49–3.86 (*m*, H–C(2′), 2 H–C(5′), CH_2CH_2O, 2 Me$_2$C*H*); 2.63 (*t*, *J* = 6.3, 1 H, CH_2CN); 2.42 (*t*, *J* = 6.3, 1 H, CH_2CN); 1.98, 1.93 (2*d*, *J* = 2.9, H–C≡C)); 1.17–1.21 (*m*, 9 H, *Me*$_2$CH); 1.08 (*d*, *J* = 6.6, 3 H, *Me*$_2$CH). ^{13}C-NMR (75 MHz, $CDCl_3$): 166.45 (*s*, PhOCH$_2$*C*O); 158.63, 158.59, 157.07 (3*s*, arom. C); 152.51 (*d*, C(2)); 151.34 (*s*, C(6)); 148.16 (*s*, C(4)); 144.42 (*s*, arom. C); 142.06, 142.02 (2*d*, C(8)); 135.65 (*s*, arom. C); 130.19, 130.11, 129.84, 128.32, 128.23, 127.86, 126.98, 126.91 (8*d*, arom. C); 123.56 (*s*, C(5)); 122.45 (*d*, arom. C); 117.28, 117.24 (2*s*, CN); 115.00, 113.16 (2*d*, arom. C); 86.52, 86.46 (2*s*, *C*–O–C(5′)); 84.61, 84.56, 84.41, 84.23 (4*d*, C(1′), C(4′)); 78.53 (*dd*, *J*(C,P) = 17.1, C(3′)); 77.82 (*dd*, *J*(C,P) = 15.9, C(3′)); 77.66, 77.20, 75.90, 75.86 (2*s*, 2*d*, C≡C); 68.15 (*t*, PhO*C*H$_2$COC); 62.71 (*dt*, *J*(P,C) = 17.7, C(5′)); 58.49 (*dt*, *J*(C,P) = 19.5, CH_2CH_2O); 55.25, 55.21 (2*q*, MeO); 43.85 (*dd*, *J*(C,P) = 4.9, C(2′)); 43.80 (*dd*, *J*(C,P) = 4.2, C(2′)); 43.47, 43.44 (2*dd*, *J*(C,P) = 12.2, Me$_2$*C*); 24.68, 24.58, 24.49, 24.47 (4*q*, *Me*$_2$CH); 20.29, 20.11 (*dt*, *J*(C,P) = 7.4, *C*H$_2$CN). ^{31}P-NMR (162 MHz, $CDCl_3$): 156.72; 156.30. LSI-MS: 912 (6, $[M+H]^+$), 270 (17), 304 (23), *303* (100). Anal. calc. for $C_{50}H_{54}N_7O_8P$: C 65.85, H 5.97, N 10.75; found: C 65.57, H 6.20, N 10.65.

*9-[2′-Deoxy-(4,4′-dimethoxytrityl)-2′-*C*-ethynyl-5′-*O*-β-*D*-arabinofuranosyl]-*N*^{2}-[(dimethylamino)methylene]guanine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**33**). Yield 95%. R_f 0.30, 0.17 (CH_2Cl_2/acetone 1:1). UV (EtOH): 233 (24600), 305 (14100). IR (KBr): 3410*w*, 3286*m*, 2966*m*, 2931*m*, 2837*w*, 2252*w*, 2048*w*, 1759*w*, 1744*w*, 1696*s*, 1632*s*, 1539*s*, 1513*s*, 1464*m*, 1424*s*, 1396*m*, 1348*s*, 1312*m*, 1250*s*, 1202*m*, 1179*s*, 1153*m*, 1115*m*, 1080*m*, 1032*s*, 979*m*, 900*m*, 880*m*, 831*m*, 787*m*, 756*m*, 726*m*, 704*m*, 647*w*, 586*m*, 556*w*, 524*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.70, 9.68 (2*s*, H–N(1)); 8.57, 8.56 (2*s*, Me$_2$NC*H*); 7.91, 7.89 (2*s*, H–C(8)); 7.44–7.51 (*m*, 2 arom. H); 7.14–7.37 (*m*, 7 arom. H); 6.77–6.85 (*m*, 4 arom. H); 6.42 (*d*, *J* = 6.6, H–C(1′)); 6.41 (*d*, *J* = 6.2, H–C(1′)); 4.62–4.79 (*m*, H–C(3′)); 4.17 (*q*, *J* = 5.0, H–C(4′)); 3.78, 377 (2*s*, 2 MeO); 3.41–3.88 (*m*, 2 Me$_2$C*H*, CH_2O, H–C(2′), 2 H–C(5′)); 3.15, 3.09 (2*s*, Me$_2$N); 2.61, 2.60, 2.42 (3*t*, *J* = 6.3, CH_2CN); 2.04,

2.01 (2*d*, *J* = 2.9, H–C≡C); 1.12–1.21 (*m*, 9 H, *Me*$_2$CH); 1.01–1.06 (*m*, 3 H, *Me*$_2$CH). ^{13}C-NMR (75 MHz, $CDCl_3$): 158.64, 158.57 (2*s*, C(6)); 158.54 (*s*, arom. C); 157.99, 157.88 (2*d*, Me$_2$N*C*); 157.79 (*s*, arom. C); 156.66 (*s*, C(2)); 149.80 (*s*, C(4)); 144.48, 144.45 (2*s*, arom. C); 136.81 (*s*, arom. C); 135.74, 135.71, 135.63, 135.57 (4*s*, arom. C); 130.13, 130.06, 128.27, 128.18, 127.80, 126.90, 126.84 (7*d*, arom. C); 120.09, 119.94 (2*s*, C(5)); 117.40, 117.23 (2*s*, CN); 113.13 (*d*, arom. C); 86.41, 86.36 (2*s*, *C*–O–C(5′)); 83.89, 83.81, 83.71, 83.57 (4*d*, C(1′), C(4′)); 78.95 (*dd*, *J*(C,P) = 17.7, C(3′)); 78.28 (*dd*, *J*(C,P) = 17.6, C(3′)); 78.16, 77.86, 75.88, 75.74 (2*s*, 2*d*, C≡C); 63.09, 62.89 (2*t*, C(5′)); 58.56 (*dt*, *J*(C,P) = 19.5, CH_2O); 58.46 (*dt*, *J*(C,P) = 20.1, CH_2O); 55.24, 55.21 (2*q*, MeO); 43.71 (*dd*, *J*(C,P) = 7.3, Me$_2$*C*H); 43.66 (*dd*, *J*(C,P) = 9.2, Me$_2$*C*H); 43.52, 43.36 (2*d*, C(2′)); 41.25, 35.14 (2*q*, Me$_2$N); 24.66, 24.57, 24.48, 24.45 (4*q*, *Me*$_2$CH); 20.27, 20.18 (2*dt*, *J*(C,P) = 13.4, *C*H_2CN). ^{31}P-NMR (162 MHz, $CDCl_3$): 156.56; 156.32. LSI-MS: 849 (15, $[M+H]^+$), 304 (23), *303* (100), 207 (19). HR-MS: 849.3847 ($C_{45}H_{54}N_8O_7P^+$; calc. 849.3853).

1-[2′-Deoxy-5′-O*-(4,4′-dimethoxytrityl)-2′*-C*-ethynyl-β*-D*-arabinofuranosyl]-*N^4*-isobutyrylcytosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite*) (**34**). Yield 96%. R_f 0.49, 0.40 (AcOEt). UV (EtOH): 237 (27600), 284 (7400), 301 (8050). IR (KBr): 3285*m*, 3072*m*, 2968*m*, 2933*m*, 2837*m*, 2252*w*, 2046*w*, 1721*s*, 1673*s*, 1609*s*, 1557*s*, 1509*s*, 1489*s*, 1446*m*, 1395*s*, 1365*m*, 1312*s*, 1252*s*, 1221*m*, 1201*m*, 1179*s*, 1155*m*, 1130*s*, 1077*s*, 1031*s*, 979*s*, 934*m*, 902*m*, 879*m*, 829*s*, 792*s*, 755*m*, 727*m*, 703*m*, 643*m*, 585*m*, 524*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 8.58, 8.52 (2 br. *s*, 1 H, H–N(4)); 8.05, 7.99 (2*d*, *J* = 7.5, 1 H, H–C(6)); 7.45–7.48 (*m*, 2 arom. H); 7.22–7.37 (*m*, 8 H, arom. H, H–C(5)); 6.82–6.88 (*m*, 4 H, arom. H); 6.33, 6.31 (2*d*, *J* = 6.0, 1 H, H–C(1′)); 4.62 (*dt*, *J* = 4.1, 10.6, 0.5 H, H–C(3′)); 4.53 (*dt*, *J* = 3.6, 9.4, 0.5 H, H–C(3′)); 4.21–4.26 (*m*, 1 H, H–C(4′)); 3.815, 3.814, 3.806, 3.804 (4*s*, 6 H, MeO); 3.72–3.91 (*m*, 1 H, CH_2O); 3.42–3.68 (*m*, 6 H, Me$_2$*CH*, CH_2O, H–C(2′), H–C(5′)); 2.58–2.66 (*m*, 2 H, CH_2CN, Me$_2$C*H*CO); 2.44 (*t*, *J* = 6.4, 1 H, CH_2CN); 2.03, 2.02 (2*d*, *J* = 2.8, 1 H, H–C≡C); 1.17–1.23 (*m*, 15 H, *Me*$_2$CH, *Me*$_2$CHCO); 1.09 (*d*, *J* = 6.8, 3 H, *Me*$_2$CH). ^{13}C-NMR (100 MHz, $CDCl_3$): 176.69 (*s*, Me$_2$CH*C*O); 162.35 (*s*, C(4)); 158.73, 158.70 (2*s*, arom. C); 154.79 (*s*, C(2)); 145.07 (*d*, C(6)); 144.33 (*s*, arom. C); 135.64, 135.60 (2*s*, arom. C); 130.20, 130.17, 130.14, 128.37, 128.29, 127.88, 127.06, 127.00 (8*d*, arom. C); 117.30, 117.20 (2*s*, CN); 113.22 (*d*, arom. C); 95.47, 95.40 (2*d*, C(5)); 86.61 (*s*, *C*–O–C(5′)); 86.55, 86.39 (2*d*, C(1′)); 84.88, 84.82 (2*d*, C(4′)); 78.83 (*dd*, *J*(C,P) = 17.1, C(3′)); 78.28 (*dd*, *J*(C,P) = 17.7, C(3′)); 78.13, 78.00 (2*s*, *C*≡CH); 75.84, 75.80 (2*d*, H*C*≡C); 62.66, 62.37 (2*t*, C(5′)); 58.61 (*dt*, *J*(C,P) = 19.6, CH_2O); 58.54 (*dt*, *J*(C,P) = 19.3, CH_2O); 55.23 (*q*, MeO); 43.54, 43.48 (2*dd*, *J*(C,P) = 12.6, Me$_2$*C*H); 43.00 (*d*, C(2′)); 36.78, 36.74 (2*d*, Me$_2$*C*HCO); 24.66, 24.59, 24.52, 24.49, 24.45 (5*q*, *Me*$_2$CH); 20.24, 20.12 (2*dt*, *J*(C,P) = 7.4, *C*H_2CN); 19.07, 19.00 (2*q*, *Me*$_2$CHCO). ^{31}P-NMR (162 MHz, $CDCl_3$): 151.79; 151.29. LSI-MS: 824 (0.4, $[M+H]^+$), 606 (0.6), 304 (24), *303* (100), 182 (24). Anal. calc. for $C_{45}H_{54}N_5O_8P$: C 65.60, H 6.61, N 8.50; found: C 65.52, H 6.74, N 8.26.

1-[2′-Deoxy-5′-O*-(4,4′-dimethoxytrityl)-2′*-C*-ethynyl-β*-D*-arabinofuranosyl]thymine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**35**). Yield 87%. R_f 0.48, 0.40 (hexane/AcOEt 1:2). UV (EtOH): 233 (21900), 268 (11400). IR (KBr): 3412*w* (br.), 3279*m*, 3059*m*, 2967*m*, 2932*m*, 2836*m*, 2252*w*, 2046*w*, 1690*s*, 1608*m*, 1582*m*, 1509*s*, 1464*s*, 1397*m*, 1365*m*, 1298*m*, 1252*s*, 1201*m*, 1179*s*, 1155*m*, 1128*m*, 1082*s*, 1032*s*, 979*s*, 901*m*, 879*m*, 829*m*, 792*m*, 770*m*, 755*m*, 727*m*, 704*m*, 643*m*, 585*m*, 550*m*, 526*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.87, 8.80 (2br. *s*, 1 H, H–N(3)); 7.55, 7.52 (2*s*, 1 H, H–C(6)); 7.45–7.47 (*m*, 2 H, arom. H); 7.21–7.36 (*m*, 7 arom. H); 6.81–6.87 (*m*, 4 arom. H); 6.30 (*d*, *J* = 7.0, 0.5 H, H–C(1′)); 6.29 (*d*, *J* = 6.6, 0.5 H, H–C(1′)); 4.73 (*dt*, *J* = 5.9, 10.3, 0.5 H, H–C(3′)); 4.61 (*dt*, *J* = 5.6, 8.8, 0.5 H, H–C(3′)); 4.04–4.08 (*m*, 1 H, H–C(4′)); 3.80, 3.79 (2*s*, 6 H, MeO); 3.74–3.90 (*m*, 1 H, CH_2O); 3.50–3.64, 3.36–3.43 (2*m*, 6 H, Me$_2$*CH*, CH_2O, H–C(2′), H–C(5′)); 2.64, 2.38 (2*t*, *J* = 6.4, 2 H, CH_2CN); 2.20, 2.19 (2*d*, *J* = 2.6, 1 H, H–C≡C); 1.67, 1.60 (2*s*, 3 H, Me–C(5)); 1.14–1.21 (*m*, 9 H, *Me*$_2$CH); 1.02 (*d*, *J* = 6.6, 3 H, *Me*$_2$CH). ^{13}C-NMR (75 MHz, $CDCl_3$): 163.70 (*s*, C(4)); 158.68, 158.63 (2*s*, arom. C); 150.06 (*s*, C(2)); 144.39, 144.33 (2*s*, arom. C); 136.14, 136.02 (2*d*, C(6)); 135.56, 135.47, 135.46 (3*s*, arom. C); 130.27, 130.21, 130.15, 130.10, 128.37, 128.24, 127.86, 127.08, 126.99 (9*d*, arom. C); 117.36, 117.25 (2*s*, CN); 113.14, 113.12 (2*d*, arom. C); 109.86, 109.68 (2*s*, C(5)); 86.52, 86.45 (2*s*, *C*–O–C(5′)); 84.63, 84.40, 83.76, 83.71 (4*d*, C(1′), C(4′)); 78.45 (*dd*, *J*(C,P) = 23.2, H–C(3′)); 78.20 (*dd*, *J*(C,P) = 22.0, C(3′)); 77.63 (*d*, HC≡C)); 75.55 (*s*, C≡CH); 62.03, 61.66 (2*t*, C(5′)); 58.41 (*dt*, *J*(C,P) = 20.1, CH_2O); 58.37 (*dt*, *J*(C,P) = 19.5, CH_2O); 55.24, 55.19 (2*q*, MeO); 43.43, 43.35 (2*dd*, *J*(C,P) = 12.2, Me$_2$*C*H); 43.04, 42.98 (2*d*, C(2′)); 24.65, 24.56, 24.51, 24.41 (4*q*, *Me*$_2$CH); 20.23, 20.03 (2*dt*, *J*(C,P) = 7.9, *C*H_2CN); 12.09, 11.95 (2*q*, *Me*–C(5)). ^{31}P-NMR (81 MHz, $CDCl_3$): 150.4; 149.6. LSI-MS: 769 (1, $[M+H]^+$), 551 (2), 304 (23), *303* (100), 201 (5).

1-[2′-Deoxy-5′-O*-(4,4′-dimethoxytrityl)-2′*-C*-ethynyl-β*-D*-arabinofuranosyl]uracil 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**36**). Yield 92%. R_f 0.48, 0.40 (hexane/AcOEt 1:2). UV (EtOH): 234 (22300), 264 (11300). IR (KBr): 3279*m*, 3061*m*, 2967*m*, 2933*m*, 2837*w*, 2253*w*, 2046*w*, 1694*s*, 1608*m*, 1582*w*, 1508*s*, 1460*s*, 1395*m*, 1380*m*, 1365*m*, 1297*m*, 1272*m*, 1252*s*, 1221*m*, 1201*m*, 1179*s*, 1155*m*, 1124*m*, 1080*s*, 1032*s*, 979*m*, 902*m*, 879*m*, 829*m*, 811*m*, 791*m*, 756*m*, 727*m*, 704*m*, 644*w*, 584*m*, 552*w*, 526*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.12 (br. *s*,

1 H, H–N(3)); 7.83, 7.75 (2*d*, *J* = 8.1, 1 H, H–C(6)); 7.40–7.45 (*m*, 2 arom. H); 7.24–7.35 (*m*, 7 arom. H); 6.82–6.87 (*m*, 4 arom. H); 6.32 (*d*, *J* = 7.0, 0.5 H, H–C(1′)); 6.31 (*d*, *J* = 7.0, 0.5 H, H–C(1′)); 5.43, 5.39 (2*d*, *J* = 8.1, 1 H, H–C(5)); 4.75 (*dt*, *J* = 5.9, 10.3, 0.5 H, H–C(3′)); 4.63 (*dt*, *J* = 5.6, 9.2, 0.5 H, H–C(3′)); 4.04–4.10 (*m*, 1 H, H–C(4′)); 3.73–3.94 (*m*, 1 H, CH_2O); 3.81, 3.80 (2*s*, 6 H, MeO); 3.41–3.63 (*m*, 6 H, Me_2CH, CH_2O, H–C(2′), H–C(5′)); 2.64, 2.40 (2*t*, *J* = 6.4, 2 H, CH_2CN); 2.21, 2.20 (2*d*, *J* = 2.6, 1 H, H–C≡C); 1.16–1.21 (*m*, 9 H, Me_2CH); 1.04 (*d*, *J* = 6.6, 3 H, Me_2CH). ^{13}C-NMR (75 MHz, $CDCl_3$): 163.18 (*s*, C(4)); 158.71, 158.67 (2*s*, arom. C); 150.13 (*s*, C(2)); 144.29, 144.27 (2*s*, arom. C); 140.56 (*d*, C(6)); 135.40, 135.34, 135.32, 135.27 (4*s*, arom. C); 130.27, 130.18, 128.37, 128.28, 127.89, 127.14, 127.06 (7*d*, arom. C); 117.24 (*s*, CN); 113.18 (*d*, arom. C); 101.53, 101.43 (2*s*, C(5)); 86.76, 86.70 (2*s*, *C*–O–C(5′)); 84.63, 84.40, 83.76, 83.71 (4*d*, C(1′), C(4′)); 78.06 (*dd*, *J*(C,P) = 16.5, C(3′)); 77.24 (*d*, HC≡C)); 75.81, 75.76 (2*s*, *C*≡CH); 61.71, 61.34 (2*t*, C(5′)); 58.44 (*dt*, *J*(C,P) = 19.5, CH_2O); 55.26, 55.23 (2*q*, MeO); 43.47, 43.40 (2*dd*, *J*(C,P) = 12.8, Me_2CH); 43.08 (*d*, C(2′)); 24.67, 24.57, 24.54, 24.44 (4*q*, Me_2CH); 20.26 (*dt*, *J*(C,P) = 7.9, CH_2CN); 20.08 (*dt*, *J*(C,P) = 7.3, CH_2CN). ^{31}P-NMR (162 MHz, $CDCl_3$): 152.32, 151.55. LSI-MS: 755 (0.9, $[M+H]^+$), 537 (3), 304 (24), *303* (100), 201 (7).

2′-Deoxy-2′-C-*ethynyl-9-β*-D-*arabinofuranosyladenine* (**4**). A soln. of 1.1M Bu_4NF in THF (3.00 ml, 3.30 mmol) was added to **16** (0.48 g, 0.81 mmol) and AcOH (0.34 ml, 5.70 mmol) in THF (10 ml). The mixture was stirred for 1 h at r.t. and then evaporated. The remaining oil was purified by FC (100 g of silica gel, CH_2Cl_2/MeOH 7:1): **4** (0.20 g, 90%). White foam. M.p. 180° (dec.). R_f 0.27 (CH_2Cl_2/MeOH 7:1). $[\alpha]_D^{25} = -20.9$ (*c* = 0.67, DMSO). UV (EtOH): 260 (15000). IR (KBr): 3344*s*, 3198*s*, 2960*m*, 2920*s*, 2843*s*, 2681*m*, 2360*w*, 2122*w*, 1896*w*, 1734*w*, 1694*m*, 1670*s*, 1605*s*, 1567*s*, 1508*m*, 1479*s*, 1461*m*, 1436*m*, 1419*s*, 1376*m*, 1338*s*, 1306*s*, 1248*m*, 1233*m*, 1208*s*, 1194*s*, 1176*m*, 1150*s*, 1108*s*, 1050*s*, 1042*s*, 1021*s*, 996*m*, 952*w*, 930*m*, 856*w*, 850*m*, 795*m*, 784*m*, 760*m*, 741*m*, 725*m*, 708*s*, 684*m*, 652*s*, 624*m*, 613*m*, 587*m*, 568*w*, 538*m*, 520*w*. ^{1}H-NMR (500 MHz, CD_3OD): 8.50 (*s*, H–C(2)); 8.23 (*s*, H–C(8)); 6.54 (*d*, *J* = 7.3, H–C(1′)); 4.68 (*dd*, *J* = 7.0, 8.1, H–C(3′)); 3.87–4.02 (*m*, H–C(4′), 2 H–C(5′)); 3.59–3.69 (*m*, H–C(2′)); 2.39 (*d*, *J* = 2.5, H–C≡C). Difference-NOE (500 MHz,

Table 6. *Purification Conditions and MALDI-TOF-MS Analysis of Oligonucleotides* **37**–**44**

	HPLC	MALDI-TOF-MS ($[M-H]^-$)	
		m/z (calc.)	*m/z* (found)
d(TTTTT^{Et}TTTTT) (**37**)	RP[a]): 0–40% *B* in 25 min; t_R 20 IE[b]): 0–40% *B* in 25 min; t_R 16	3003.0	3002.1
d(UUUUU^{Et}UUUUU) (**38**)	RP[c]): 0–30 % *B* in 30 min; t_R 19 IE[d]): 15–45% *B* in 30 min; t_R 22	2862.8	2863.1
d($U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U^{Et}U$) (**39**)	RP[c]): 0–30% *B* in 30 min; t_R 20 IE[d]): 15–45% *B* in 30 min; t_R 21	3055.0	3054.9
d(AAAAAA^{Et}AAAA) (**40**)	RP[c]): 0–30% *B* in 30 min; t_R 24 IE[d]): 30–55% *B* in 30 min; t_R 22	3093.2	3095.4
d($A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A^{Et}A$) (**41**)	RP[c]): 0–30% *B* in 30 min; t_R 20 IE[d]): 30–55% *B* in 30 min; t_R 26	3285.4	3289.0
d(CCC^{Et}CCC) (**42**)	RP[a]): 0–20% *B* in 16 min; t_R 13 IE[b]): 0–25% *B* in 15 min; t_R 14	1696.2	1700.3
d($C^{Et}C^{Et}C^{Et}C^{Et}C^{Et}C$) (**43**)	RP[a]): 0–20% *B* in 16 min; t_R 14 IE[b]): 0–30% *B* in 15 min; t_R 14	1792.3	1798.8
d($G^{Et}G^{Et}G^{Et}G^{Et}G^{Et}G$) (**44**)	RP[a]): not successful IE[e]): 25–40% *B* in 15 min; t_R 15	2032.4	2032.4

[a]) *Pharmacia PepRPC HR 10/10*; solvent *A:* 0.1M (Et_3NH)OAc in H_2O, pH 7.0; solvent *B:* 0.1M (Et_3NH)OAc in H_2O/MeCN 1:4, pH 7.0; flow 2.5 ml/min; detection at 254 nm. [b]) *Pharmacia MonoQ HR 5/5*; solvent *A:* 10 mM Na_2HPO_4 in H_2O/MeCN 4:1, pH 6.0; solvent *B:* 10 mM Na_2HPO_4, 1M NaCl in H_2O/MeCN 4:1, pH 6.0; flow 1.0 ml/min; detection at 254 nm. [c]) *Brownlee Aquapore RP-300*, 7 μ, 4.6 × 220 nm; solvent *A:* 0.1M (Et_3NH)OAc in H_2O, pH 7.0; solvent *B:* 0.1M (Et_3NH)OAc in H_2O/MeCN 1:4, pH 7.0; flow 1.0 ml/min; detection at 260 nm. [d]) *Macherey-Nagel Nucleogen DEAE 60-7 125/4*; solvent *A:* 10 mM Na_2HPO_4 in H_2O/MeCN 4:1, pH 7.0; solvent *B:* 10 mM Na_2HPO_4, 1M NaCl in H_2O/MeCN 4:1, pH 7.0; flow 1.0 ml/min; detection at 260 nm. [e]) *Pharmacia MonoQ HR 5/5*; solvent *A:* 10 mM Na_2HPO_4 in H_2O/MeCN 4:1, pH 10.0; solvent *B:* 10 mM Na_2HPO_4, 1M NaCl in H_2O/MeCN 4:1, pH 10.0; flow 1.0 ml/min; detection at 254 nm.

CD_3OD): 6.54 (H–C(1′)) → 8.23 (H–C(8)); 3.59–3.69 (H–C(2′)); 3.59–369 (H–C(2′)) → 6.54 (H–C(1′)); 4.68 (H–C(3′)) → 8.23 (H–C(8)). ^{13}C-NMR (75 MHz, (D_6)DMSO): 148.04 (*s*, C(6)); 144.34 (*d*, C(2)); 141.07 (*s*, C(4)); 132.00 (*d*, C(8)); 124.73 (*s*, C(5)); 77.52, 76.15, 69.96, 66.15 (*s*, 3*d*, C(1′), C(4′), C≡C); 65.77 (*d*, C(3′)); 51.92 (*t*, C(5′)); 36.54 (*d*, C(2′)). LSI-MS (DTT/DTE): 277 (11), *276* (100, $[M+H]^+$), 155 (43), 149 (10), 147 (13), 136 (90), 135 (24), 119 (91), 103 (53). Anal. calc. for $C_{12}H_{13}N_5O_3$: C 52.36, H 4.76, N 25.44; found: C 52.49, H 4.90, N 25.34.

*9-(2′-Deoxy-2′-*C*-ethynyl-β-*D*-arabinofuranosyl)guanine* (**5**). Compound **25** (0.31 g, 0.61 mmol) was treated with aq. ammonia (5 ml) for 1 h at r.t. The solvent was evaporated and the residue purified by FC (15 g of silica gel, $CHCl_3$/MeOH 2:1): **5** (0.14 g, 80%). White solid. M.p. 185° (dec.). R_f 0.35 ($CHCl_3$/MeOH 2:1). $[\alpha]_D^{25} = +32.6$ ($c = 0.63$, DMSO). UV (10 mM Na_2HPO_4 buffer pH 7.0): 254 (9900). IR (KBr): 3289*w*, 3134*s*, 2939*m*, 2762*m*, 2361*w*, 2117*w*, 1686*s*, 1605*s*, 1572*s*, 1535*s*, 1414*s*, 1358*m*, 1290*m*, 1253*m*, 1222*m*, 1180*m*, 1090*m*, 1049*m*, 910*w*, 848*w*, 784*w*, 686*m*, 672*m*, 619*m*. ^{1}H-NMR (300 MHz, (D_6)DMSO): 10.59 (br. *s*, H–N(1)); 7.89 (*s*, H–C(8)); 6.47 (br. *s*, H–N(2)); 6.13 (*d*, $J = 7.4$, H–C(1′)); 5.86 (*d*, $J = 5.9$, OH–C(3′)); 5.09 (*t*, $J = 5.2$, OH–C(5′)); 4.32–4.41 (*m*, H–C(3′)); 3.58–3.74 (*m*, H–C(4′), 2 H–C(5′)); 3.41–3.49 (*m*, H–C(2′)); 2.91 (*d*, $J = 2.6$, H–C≡C). ^{13}C-NMR (75 MHz, (D_6)DMSO): 157.05, 153.83, 151.12 (3*s*, C(6), C(2), C(4)); 135.70 (*d*, C(8)); 116.16 (*s*, C(5)); 85.26, 82.29 (2*d*, C(1′), C(4′)); 79.49, 76.02 (*s*, *d*, C≡C); 73.39 (*d*, C(3′)); 59.96 (*t*, C(5′)); 43.65 (*d*, C(2′)). LSI-MS (DTT/DTE): 292 (11, $[M+H]^+$), 243 (17), *242* (100), 240 (10), 155 (38), 147 (13), 142 (10), 135 (18), 119 (69), 103 (32). HR-MS: 292.1046 ($C_{12}H_{14}N_5O_4^+$; calc. 292.1046).

Oligonucleotide Synthesis, Purification, and Characterization. Oligodeoxynucleotides **37**–**58** (*Tables 6–8*) were prepared from phosphoramidites **32**–**36** and commercially available dA, dC, dG, and T phosphoramidites (*Glen Research*) and deoxynucleoside-CPG (1.3 μmol, *Glen Research*) with *Pharmacia Gene-Assembler-Special* automated DNA synthesizer by standard solid-phase phosphoramidite chemistry [19] with slight modifications. As coupling catalyst, 5-(benzylthio)-1*H*-tetrazole [20] was used instead of 1*H*-tetrazole, and the coupling time for phosphoramidites **32**–**36** was prolonged to 6 min. Coupling efficiencies were > 97%. After chain elongation and final detritylation, the oligonucleotides were cleaved from the resin and deprotected by treatment with 1 ml of conc. aqueous ammonia at 55° overnight. The crude oligonucleotides were purified by reversed phase HPLC

Table 7. *Purification Conditions and MALDI-TOF-MS Analysis of Oligonucleotides* **45**–**50**

	HPLC	MALDI-TOF-MS ($[M-H]^-$)	
		m/z (calc.)	*m/z* (found)
d($A^{Et}UA^{Et}UA^{Et}UA^{Et}UA^{Et}U$) (**45**)	RP[a]): 0–30% *B* in 30 min; t_R 22 IE[b]): 25–50% *B* in 30 min; t_R 30	3074.1	3074.4
d($U^{Et}AU^{Et}AU^{Et}AU^{Et}AU^{Et}A$) (**79**)	RP[c]): 0–25 *B* in 18 min; t_R 15 IE[d]): 10–30% *B* in 25 min; t_R 17	3074.1	3075.0
d($G^{Et}AG^{Et}AAG$) (**46**)	RP[c]): 10–20% *B* in 10 min; t_R 7 IE[e]): 11–30% *B* in 16 min; t_R 15	2225.6	2225.9
d(CTGA^{Et}ATCGAC) (**47**)	RP[c]): 5–20% *B* in 13 min; t_R 12 IE[e]): 20–35% *B* in 16 min; t_R 15	3035.1	3037.6
d(CTG$A^{Et}A^{Et}$TCGAC) (**48**)	RP[c]): 7–20% *B* in 13 min; t_R 12 IE[e]): 20–40% *B* in 16 min; t_R 12	3059.1	3060.7
d(CGCG$^{Et}A^{Et}$ATTCGCG) (**49**)	RP[c]): 10–20% *B* in 16 min; t_R 15 IE[d]): 30–50% *B* in 15 min; t_R 15	3693.5	3694.2
d(CG^{Et}CGAATTCG^{Et}CG) (**50**)	RP[c]): 10–20% *B* in 16 min; t_R 15 IE[e]): 20–60% *B* in 20 min; t_R 16	3693.5	3696.7

[a]) *Brownlee Aquapore RP-300* 7 μ, 4.6 × 220 mm; solvent *A:* 0.1M (Et_3NH)OAc in H_2O, pH 7.0; solvent *B:* 0.1M (Et_3NH)OAc in H_2O/MeCN 1:4, pH 7.0; flow 1.0 ml/min; detection at 260 nm. [b]) *Macherey-Nagel Nucleogen DEAE 60-7 125/10*; solvent *A:* 10 mM Na_2HPO_4 in H_2O/MeCN 4:1, pH 6.0; solvent *B:* 10 mM Na_2HPO_4, 1M NaCl in H_2O/MeCN 4:1, pH 6.0; flow 3.0 ml/min; detection at 260 nm. [c]) *Pharmacia PepRPC HR 10/10*; solvent *A:* 0.1M (Et_3NH)OAc in H_2O, pH 7.0; solvent *B:* 0.1M (Et_3NH)OAc in H_2O/MeCN 1:4, pH 7.0; flow 2.5 ml/min; detection at 254 nm. [d]) *Pharmacia MonoQ HR 5/5*; solvent *A:* 10 mM Na_2HPO_4 in H_2O/MeCN 4:1, pH 6.0; solvent *B:* 10 mM Na_2HPO_4, 1M NaCl in H_2O/MeCN 4:1, pH 6.0; flow 1.0 ml/min; detection at 254 nm. [e]) *Pharmacia MonoQ HR 10/10*; solvent *A:* 10 mM Na_2HPO_4 in H_2O/MeCN 4:1, pH 6.0; solvent *B:* 10 mM Na_2HPO_4, 1M NaCl in H_2O/MeCN 4:1, pH 6.0; flow 2.5 ml/min; detection at 254 nm.

Table 8. *Purification Conditions and MALDI-TOF-MS Analysis of Oligonucleotides* **51**–**58**

	HPLC	MALDI-TOF-MS ($[M-H]^-$)	
		m/z (calc.)	m/z (found)
d($CG^{Et}CG^{Et}CG$) (**51**)	RP[a]): 0–40% *B* in 18 min; t_R 16 IE[c]): 10–30% *B* in 16 min; t_R 14	1840.3	1840.6
d($CG^{Et}CGCG$) (**52**)	RP[a]): 0–40% *B* in 18 min; t_R 14 IE[b]): 20–60% *B* in 30 min; t_R 20	1817.3	1822.7
d($CGCG^{Et}CG$) (**53**)	RP[a]): 0–40% *B* in 18 min; t_R 14 IE[b]): 20–60% *B* in 30 min; t_R 20	1817.3	1818.7
d($C^{Et}GC^{Et}GC^{Et}G$) (**54**)	RP[a]): 0–40% *B* in 18 min; t_R 16 IE[d]): 20–60% *B* in 30 min; t_R 19	1864.3	1866.6
d($C^{Et}G^{Et}C^{Et}G^{Et}C^{Et}G$) (**55**)	RP[a]): 0–40% *B* in 18 min; t_R 17 IE[d]): 10–30% *B* in 16 min; t_R 15	1912.3	1913.1
d($G^{Et}CG^{Et}CG^{Et}C$) (**56**)	RP[a]): 0–20% *B* in 16 min; t_R 14 IE[a]): 20–50% *B* in 15 min; t_R 14	1864.3	1864.1
d($GC^{Et}GC^{Et}GC$) (**57**)	RP[a]): 0–20% *B* in 16 min; t_R 14 IE[d]): 20–50% *B* in 15 min; t_R 16	1840.3	1840.0
d($TG^{Et}TG^{Et}TG^{Et}TG^{Et}TG$) (**58**)	RP[a]): 0–30% *B* in 20 min; t_R 20 IE[b]): 20–60% *B* in 30 min; t_R 21	3200.2	3206.5

[a]) *Pharmacia PepRPC HR 10/10:* solvent *A:* 0.1M (Et_3NH)OAc in H_2O, pH 7.0; solvent *B:* 0.1M (Et_3NH)OAc in H_2O/MeCN 1 : 4, pH 7.0; flow 2.5 ml/min; detection at 254 nm. [b]) *Macherey-Nagel Nucleogen DEAE 60-7 125/10*; solvent *A:* 10 mM Na_2HPO_4 in H_2O/MeCN 4 : 1, pH 6.0; solvent *B:* 10 mM Na_2HPO_4, 1M NaCl in H_2O/MeCN 4 : 1, pH 6.0; flow 3.0 ml/min; detection at 260 nm. [c]) *Pharmacia MonoQ HR 10/10*; solvent *A:* 10 mM Na_2HPO_4 in H_2O/MeCN 4 : 1, pH 6.0; solvent *B:* 10 mM Na_2HPO_4, 1M NaCl in H_2O/MeCN 4 : 1, pH 6.0; flow 2.5 ml/min; detection at 254 nm. [d]) *Pharmacia MonoQ HR 5/5*; solvent *A:* 10 mM Na_2HPO_4 in H_2O/MeCN 4 : 1, pH 6.0; solvent B: 10 mM Na_2HPO_4, 1M NaCl in H_2O/MeCN 4 : 1, pH 6.0; flow 1.0 ml/min; detection at 254 nm.

(RP) followed by ion-exchange HPLC (IE) with the media indicated in *Tables 6–8* by means of a *Pharmacia FPLC* system or a *Pharmacia LKB-2249* HPLC pump equipped with an *ABI-Kratos Spectroflow-757-UV* detector and a *Tarkan-W + W-600* recorder. The isolated oligonucleotides were desalted over *SEP-PAK-C-18* cartridges (*Waters*) following the manufacturer's protocol. Incorporation of intact 2′-ethynyl nucleosides and integrity of oligodeoxynucleotides **37**–**58** were confirmed by MALDI-TOF mass spectometry (linear MALDI-TOF-MS, 20 keV, N_2 laser 337 nm; matrix conditions as described previously [29]. The observed single-product ions were all within 0.1% of the calculated mass (*Tables 6–8*).

UV Melting Experiments. They were performed on a *Cary 3E-UV/VIS* spectrophotometer (*Varian*) equipped with a temperature controller. Data collection was performed with a generic *Pentium II*™ PC with the *Cary* WinUV Thermal software. Melting curves were recorded at 260 and 284 nm in a consecutive heating-cooling-heating cycle (0–90°) with a temp. gradient of 0.5°/min. All measurements were conducted in a buffer consisting of 10 mM NaH_2PO_4 and NaCl (as indicated), pH 7.0, at the oligonucleotide concentrations indicated. T_m Values are determined from the first derivative of the melting curve by means of the software package *Origin*™ V5.0. Thermodynamic data were calculated from *Van't Hoff* plots according to [24].

CD Spectra. They were recorded with a *Jasco J-715* spectropolarimeter equipped with a *Jasco PFO-3505* temp. controller and monitored by a 166-MHz *Pentium-MMX* PC. The temp. was measured directly in the cuvette (1-cm square quartz cuvettes from *Halma*, probe volume 1 ml). From the raw CD spectra, the background spectrum (buffer soln.) was subtracted with the function 'Arithmetics *vs.* Spectrum' in the *Jasco* Software. The resulting spectra were smoothed with the function 'Noise Reduction' (filter positions, top, 7; bottom, 17).

Molecular-Dynamics Calculations. They were performed with the software package InsightII from *Molecular Simulations Inc.*, San Diego, running on an *Octane* workstation from *SGI*. Starting duplex structures for the simulations were built from the canonical B-DNA templates from the 'Biopolymer' module of InsightII. All calculations were preformed by means of the AMBER forcefield [30][31] as implemented in the Discover3 of InsightII without the explicit inclusion of water molecules or counterions. A distance-dependent dielectric

constant of $4 \cdot r$ was used instead as a screening function [32]. The 1,4 nonbonded interactions were scaled by 0.5 [30]. No cut-offs on nonbonded interactions were applied. Prior to molecular-dynamics calculations, duplex structures were energy-minimized. First, a steepest-decent algorithm was used until the energy gradient dropped below 10 kcal/mol · Å. Then the conjugate-gradient method was used until the energy gradient reached 0.05 kcal/mol · Å. For molecular dynamics, a timestep of 1 fs was used during all simulations. The energy-minimized structures were first heated stepwise from 0 to 300 K (velocity scaling method): 1 ps at 50 K, 1 ps at 100 K, 2 ps at 150 K, 2 ps at 200 K, 4 ps at 250 K, and 10 ps at 300 K. The system was then kept at 300 K for 200 ps (coupling to an external bath [33]). Coordinates and energy terms were stored every 0.5 ps. The trajectories of the molecular-dynamics runs were analyzed with the 'Analysis and Decipher' modules of InsightII. For structure representation, the trajectories were averaged over the last 50 ps of the simulation and the corresponding data was visualized by *MSI* WebLab ViewerPro 3.5.

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