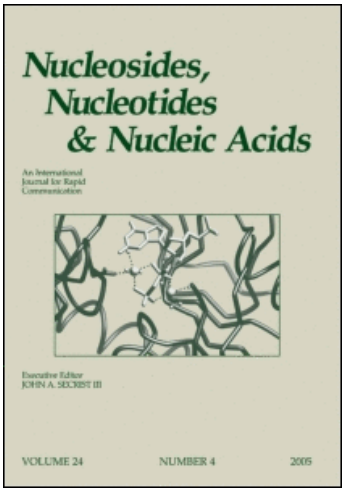

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Oligonucleotides Containing 9-(2-Deoxy-2-Fluoro-β-D-Arabinofuranosyl)-Adenine and -Guanine: Synthesis, Hybridization and Antisense Properties

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NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(10–12), 1861–1884 (2000)

OLIGONUCLEOTIDES CONTAINING 9-(2-DEOXY-2-FLUORO-β-D-ARABINOFURANOSYL)-ADENINE AND -GUANINE: SYNTHESIS, HYBRIDIZATION AND ANTISENSE PROPERTIES*

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ABSTRACT: Synthesis of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-adenine (**7**, ara-$A^{2'F}$) and –guanine (**12**, ara-$G^{2'F}$) was accomplished *via* the condensation of 2,6-dichloropurine (**1**) with 2-deoxy-2-fluoro-1,3,5-tri-*O*-benzoyl-α-D-arabinofuranose (**2**) as a key chemical step. Condensation of silylated N^6-benzoyladenine (**6**) with **2** gave, after deblocking and chromatographic separation, ara-$A^{2'F}$ (**7**) (14%), it's α-anomer **8** (14%) and N^7-α-isomer **9** (25%). The PSEUROT analysis of N^9-β-D-arabinosides **7** and **12** manifested slight preference for the *S* rotamer (64%) for the former, and an equal population of the *N* and *S* rotamers for the latter. The arabinosides **7** and **12** were used for the preparation of the respective phosphoamidite building blocks **13** and **14** for automated oligonucleotide synthesis. Four 15-mer oligonucleotides (ONs) complementary to the initiation codon region of firefly luciferase mRNA were prepared: unmodified 2'-deoxy-ON (AS1) and containing (*i*) ara-$A^{2'F}$ instead of the only ***A*** (AS2), (*ii*) ara-$G^{2'F}$ *vs.* 3-***G*** from the 5'-terminus (AS3), and (*iii*) both arabinosides at the same positions (AS4). All these ONs display practically the same (*i*) affinity to both complementary DNA and RNA, and (*ii*) ability to inhibit a luciferase gene expression in a cell-free transcription-translation system.

INTRODUCTION

Oligonucleotides (ONs) have been shown in both *in vitro* and *in vivo* systems to be promising antiviral and anticancer agents based on the antisense concept.[1-4] Antisense ONs bind specifically to mRNA thereby selectively blocking gene translation or,

* This paper is dedicated to the memory of Prof. Alexander A. Krayevsky.

otherwise, activating ribonuclease H (RNase H) catalyzed cleavage of the mRNA part of an RNA:DNA duplex. This latter process is generally considered as a major effector of antisense activity. Therefore, one of the continuing challenges in the development of oligonucleotide-based pharmaceuticals is the affinity and specificity of the ON for the target RNA sequence and the subsequent ability of the hybrid to be a substrate of RNase H.

Unfortunately, regular 2'-deoxyoligonucleotides (2'dONs) are subject to rapid exo- and endonuclease digestion in serum, precluding their application as antisense. The search for ONs with enhanced resistance against nucleases, improved hybridization characteristics and an ability to support RNase H activity has led to many structural modifications. Derivatisation of the 2'-position of antisense ONs is recognized as a useful modification site.[1,2,5-7] Results of earlier studies on the effects of 2'-deoxy-2'-fluoro thymine nucleosides on the stability of model 2'-deoxyoligonucleotide duplexes demonstrated their stabilization in the case of both the β-D-*ribo* and β-D-*arabino* configuration of the fluorine atom.[8-10] A detailed study of the influence of the 2'-fluoro-*ribo*-substitution revealed greatly increased binding affinity of such uniformly modified ONs, 2'F-rONs, for the complementary RNA *vs*. the parent RNA-DNA duplex.[11] The CD spectra of the 2'-deoxy-2'-fluoro-*ribo*-oligonucleotides hybridized with RNA exhibit the characteristic A-form pattern, which is in accord with the predominant *N* (C3'-*endo*) conformation of the sugar ring of 2'-deoxy-2'-fluoro-β-D-ribofuranosyl nucleosides. The uniformly modified ONs containing 2'-deoxy-2'-fluoro-*ribo*-nucleosides (2'F-rONs) are, however, not able to activate the RNase H mediated cleavage of the targeted RNA in the 2'F-rONs:RNA heteroduplex.[11,12] This result points to a critical importance of the spatial arrangement of a duplex like RNA:DNA to be recognizable by RNase H. Furthermore, it might be thus suggested that the factors governing the high affinity of ONs to the targeted RNA, on the one hand, and facilitating an activation of RNase H, on the other, are counteractive.

These data[11,12] along with the previously observed enhancement of DNA:DNA duplex stability by incorporation of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-thymine (FMAU) or –5-iodouracil (FIAU)[9,10] prompted us to synthesize the 2'dONs containing 2'-deoxy-2'-fluoro-β-D-*arabino*-nucleosides (2'F-aONs) and investigate the influence of point substitution of purine 2'-deoxynucleosides with the corresponding purine 2'-

fluoroarabinonucleosides on the binding affinity to the target nucleic acids and on their antisense properties. During the course of this work, the contribution of FMAU on the stability of the canonical B-DNA conformation of the Dickerson-Drew dodecamer duplex {[d(CGCGAATTCGCG)]$_2$; DD-duplex} was investigated and it was found that incorporation of two FMAUs greatly stabilizes the double helix.[13] The crystal structures of two DD-duplexes with incorporated FMAU revealed that the FMAU residues adopt an ^{o}E-conformation (O4'-*endo*) compatible with an overall B-form duplex geometry.[14] An inspection of the crystal structures of the DD-duplexes led authors to the conclusion that incorporation of 2-deoxy-2-fluoro-β-D-arabinofuranosyl purine nucleosides into DNA oligonucleotides would result in a destabilization of duplex formation.[14]

More recently, uniformly modified model 2'F-aONs have been shown to produce the duplexes with complementary RNA with higher thermal stability *vs.* the parent DNA:RNA hybrids.[15] The most striking was the finding that the susceptibility of the 2'F-aON:RNA hibrids to RNase H cleavage is similar to that observed for the parent DNA:RNA hybrid.[15] A computational study of the 2'F-aON:RNA and DNA:RNA hibrids revealed a close similarity in spatial arrangement, which is intermediate between A and B forms.[16]

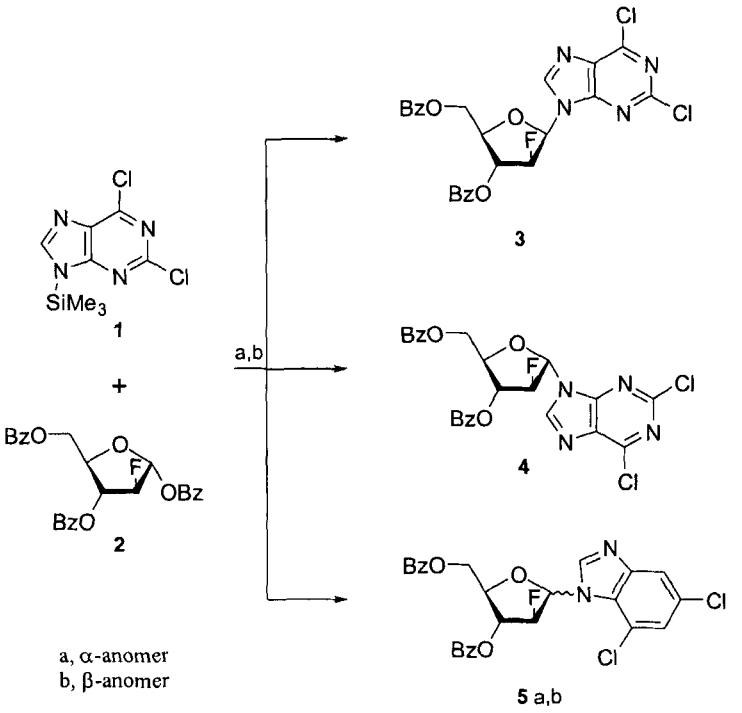
In this paper, we report on the synthesis of 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine (**7**, ara-A$^{2'F}$), 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)guanine (**12**, ara-G$^{2'F}$), antisense ONs, containing ara-A$^{2'F}$ or/and ara-G$^{2'F}$, investigation of hybridization thermodynamics of modified ONs with the DNA and RNA and inhibition of luciferase gene expression in a cell-free transcription-translation system.

RESULTS AND DISCUSSION

Synthesis.- Different routes to synthesize arabinosides of adenine **7**[17-21] and guanine **12**[20-23] have been developed. Most of these studies have dealt with the coupling of purine bases with 3,5-di-*O*-acyl-2-deoxy-2-fluoroarabinofuranosyl bromide, albeit the glycosylation process suffers from low chemical efficiency. An alternative approach for the synthesis of arabinosides **7** and **12** consists in the direct fluorination ("direct approach"[20]) of the selectively blocked ribonucleosides with DAST. However, preparation of the selectively blocked natural purine nucleosides is the main drawback of this approach. In the present work, we have decided in favor of the convergent synthesis

taking into account that the versatile intermediate N^9-β-glycoside **3** may be transformed into both arabinosides **7** and **12** by conventional methods.

Scheme 1

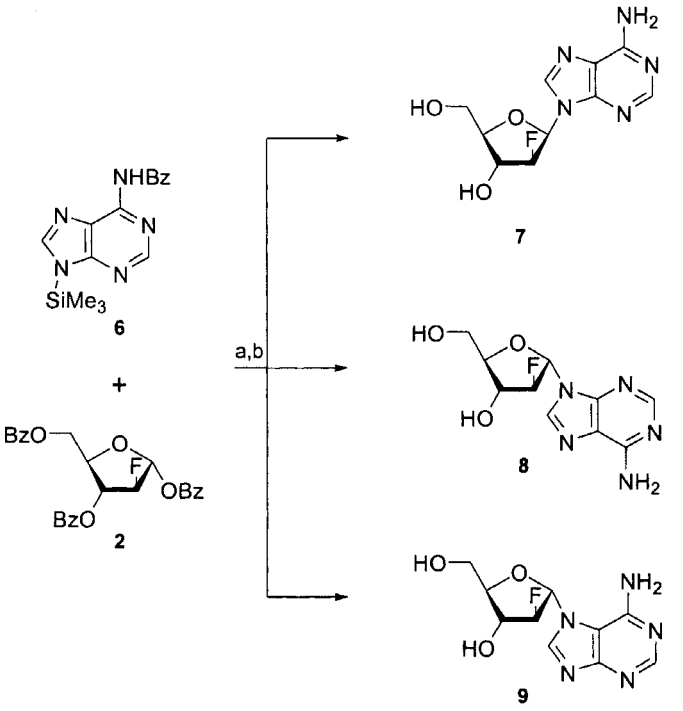


(a) **1**/**2**/TMS-Tfl (2.0:1.0:3.0, mol), MeCN, reflux, 20 min (HPLC: **3**, 35-45%; **4**, 30-35%; **5**b, 7-12%; **5**a, 3-5%); (b) silica gel column chromatography.

We have previously demonstrated that the coupling of trimetylsilylated 2,6-dichloropurine **1** with 1-*O*-acetyl-3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-ribofuranose or 1,5-di-*O*-benzoyl-2,3-dideoxy-3-fluoro-β-D-*erythro*-pentofuranose in the presence of an excess trimethylsilyl triflate (TMS-Tfl) in anhydrous acetonitrile or dichloromethane under reflux led to predominant formation of N^9-glycosides in a high yield.[24,25] Similar reaction conditions were employed for the preparation of the key N^9-β-glycoside **3**. The condensation of trimethylsilylated 2,6-dichloropurine **1** with commercially available benzoate **2** led to the formation of a mixture of the N^9-β-glycoside **3** as principal product along with the N^9-α-anomer **4** and the N^7-β- and -α-isomers **5**,b,a (the **3**:**4**:**5**b:**5**a ratio *ca.* 10:8:2.5:1.0 according to the HPLC data; 90%, combined) (Scheme 1). Chromatographic

separation of this mixture on a column of silica gel gave the corresponding individual compounds **3**, **4** and **5**b; the N^7-α-isomer **5**a was not isolated in an individual form.

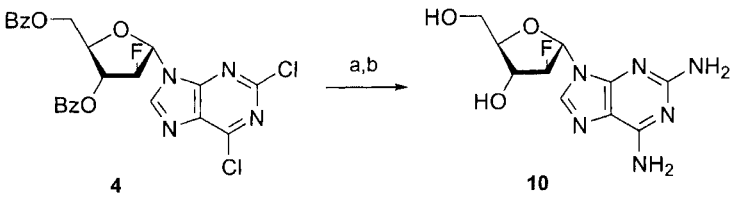
Scheme 2



(a) **6**/**2**/TMS-Tfl (2.0:1.0:4.0, mol), MeCN, reflux, 4 h; silica gel column chromatography, 1.05 g of a mixture of three blocked nucleosides (84%); (b) NH_3/MeOH, room temperature, 48 h; silica gel column chromatography (**7**, 14%; **8**, 14%; **9**, 25%).

We have also studied an alternative synthetic route to adenine nucleoside **7**, which employs the same glycosylating sugar. Condensation of trimethylsilylated N^6-benzoyladenine **6** with benzoate **2** in the presence of an excess TMS-Tfl in anhydrous acetonitrile under reflux led to a mixture of isomeric benzoylated nucleosides that could not be clearly separated by silica gel column chromatography. Debenzoylation of this mixture followed by double silica gel column chromatography permitted isolation of pure nucleosides **7**, **8** and **9** in yields of 14, 14 and 25%, respectively (Scheme 2). This method proved to be less successful than the procedure described above in terms of both yield of the desired N^9-β-arabinoside **7** and tedious chromatographic separation. Among the adenine nucleosides synthesized, only the β-anomer **7** was found to be a substrate of adenosine deaminase (ADase) demonstrated by UV monitoring of the enzymatic reaction.

Scheme 3



(a) (1) LiN_3, EtOH, reflux, 2 h; (2) BzCl, pyridine, room temperature, 18 h; (3) $SnCl_2$, CH_2Cl_2/MeOH (6:1, vol); (b) saturated at 0°C methanolic ammonia, 20°C, 24 h (**10**, a + b, 72%).

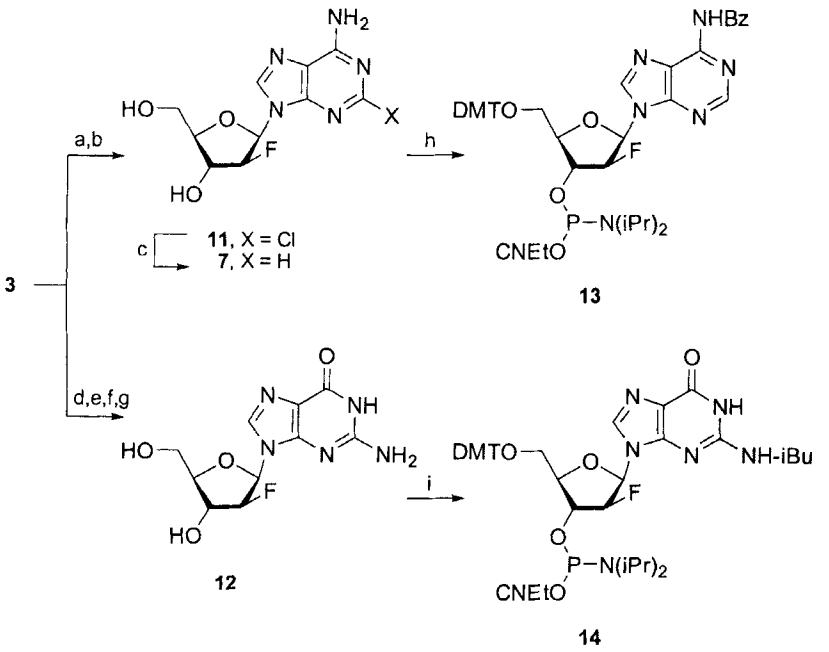
Treatment of N^9-α-nucleoside **4** with LiN_3 in EtOH at reflux is accompanied by partial debenzoylation of intermediary 2,6-diazido derivative. Therefore, in order to prevent the loss of nucleoside material, the reaction mixture was evaporated, the residue was re-benzoylated and then subjected to reduction with $SnCl_2$ in a mixture of dichloromethane-methanol.[26] Standard debenzoylation gave, after silica gel column chromatography, the N^9-α-nucleoside **10** in a 72% combined yield. The latter compound remains unchanged upon treatment with ADase.

Transformation of the N^9-β-nucleoside **3** to adenine arabinoside **11** was effected *via* the successive action of a saturated solution of ammonia in 1,2-dimethoxyethane at room temperature for 24 h[24,25,27] followed by treatment with methanolic ammonia. After silica gel column chromatography, nucleoside **11** was obtained in a 72% combined yield. Compound **11** was then catalytically hydrogenated in the presence of 10% Pd/C according to the data[28] to afford adenine nucleoside **7** in an 82% yield (Scheme 4).

Alternatively, the universal precursor N^9-β-nucleoside **3** was transformed to the intermediary 2,6-diaminopurine derivative, which was treated with ADase to give guanine nucleoside **12** in a 80% combined yield (Scheme 4).

NMR Spectroscopic Studies.- The structures of the compounds **3**, **4**, **5**b, **7**-**9**, **10** and **12** were confirmed by the ^1H and ^{13}C NMR (TABLES 1-4) and UV spectroscopy (EXPERIMENTAL). The correctness of assignments was substantiated by means of the 2D ^1H-^1H and ^1H-^{13}C NMR correlation spectra, and, in some cases, by homonuclear decoupling experiments. The site of glycosylation of 2,6-dichloropurine and anomeric configuration of compounds **3**, **4** and **5**b were determined by comparison of their ^1H

Scheme 4



(a) saturated at 20°C ammonia in 1,2-dimethoxyethane, 20°C, 24 h; (b) saturated at 0°C methanolic ammonia, 20°C, 24 h (**11**, a + b, 72%); (c) H_2, 10% Pd/C, EtOH + 25% aq. ammonia, room temperature, 18 h (**7**, 82%); (d) (1) LiN_3, EtOH, reflux, 2 h; (2) BzCl, pyridine, room temperature, 18 h; (e) $SnCl_2$, CH_2Cl_2/MeOH (6:1, vol); (f) NH_3/MeOH, room temperature, 24 h; (g) ADase, H_2O, 2 h (**12**, d + e + f + g, Σ80%); (h) (1) transient protection/BzCl, pyridine, room temperature, 18 h; (2) DMT-Cl, pyridine, room temperature, 18 h; (3) phosphitylation (**13**, Σ72%); (i) (1) transient protection/iBuCl, pyridine, room temperature, 18 h; (2) DMT-Cl, pyridine, room temperature, 18 h; (3) phosphitylation (**14**, Σ67%).

NMR spectra (TABLE 1) with those of the related purine N^7- and N^9-glycosides.[24,25,29,30] Diagnostic of the β-anomeric configuration of nucleosides **3** and **5b** is the $^5J_{H,F}$ long-range coupling of H-8 to fluorine of 3.04 and 2.15 Hz, respectively, exhibited in their ^{1}H NMR spectra. This coupling is generally indicative of physical proximity of the nuclei involved and is not observed in the α-anomer **4**. Similarly, the presence of the long-range coupling of H-8 to fluorine proved the N^9-β-structure of **7** and **12**.

The most informative feature of the ^{1}H NMR spectra of the α-anomers **4**, **8** and **10** *vs.* the corresponding β-anomers **3**, **7** and **12** is the shift of the H-2' and H-4' resonances to a lower field (*cf.* with the data in Ref.[31] for related anomeric pairs). The $N^9 \rightarrow N^7$ change of the site of glycosylation as with the **3** and **5b** resulted in the displacement of

TABLE 1. ^{1}H NMR Data for the Benzoylated 2-Deoxy-2-fluoro-D-arabinofuranosides of 2,6-Dichloropurine **3**, **4** and **5b** in $CDCl_3$.

Com-pound	Chemical shifts, δ_{TMS}, ppm							Coupling constants, Hz										
	base	*sugar*						^3J(H,H)					$^2J_{gem}$	^5J (F,H)	^3J(F,H)		$^2J_{gem}$	Others
	H-8	H-1'	H-2'	H-3'	H-4'	H-5'	H-5"	1',2'	2',3'	3',4'	4',5'	4',5"	5',5"	8,F	1',F	3',F	2',F	
3	8.39 d	6.65 dd	5.41 ddd	5.78 m	4.63 m	4.85 dd	4.83 dd	2.77	0.77	2.76	5.45	3.68	minus 12.26	3.04	21.80	16.93	minus 49.87	$^4J_{F,4'}$ -0.66
4	8.36 s	6.56 ddd	6.12 dt	5.82 m	4.99 m	4.74 dd	4.73 dd	1.38	1.55	3.14	6.20	6.20	-	-	14.12	16.99	minus 48.52	$^4J_{F,4'}$ -0.71 $^4J_{1',3'}$ -0.64
5b	8.71 d	6.96 dd	5.43 ddd	5.74 m	4.69 m	4.87 m	4.84 m	2.79	0.97	2.93	5.32	3.57	minus 12.32	2.15	19.20	16.55	minus 49.99	$^4J_{1',8}$ -0.30 $^5J_{F,5'}$ 0.68 $^5J_{F,5"}$ 0.46 $^4J_{F,4'}$ -0.66

TABLE 2. ^{1}H NMR Data for the 9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)adenine (**7**), it's α-anomer **8** and N^7-α-isomer **9** in D_2O, and for the 2,6-Diamino-9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)purine (**10**) and 9-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)guanine (**12**) in DMSO-d_6.

Compound	Chemical shifts, δ_{TMS}, ppm								Coupling constants, Hz										
	base		*sugar*						^3J(H,H)					$^2J_{gem}$ (H,H)	^5J (F,H)	^3J(F,H)		$^2J_{gem}$ (F,H)	Others
	H-2	H-8	H-1'	H-2'	H-3'	H-4'	H-5'	H-5"	1',2'	2',3'	3',4'	4',5'	4',5"	5',5"	8,F	1',F	3',F	2',F	
7	7.94 s	8.10 d	6.21 dd	5.12 ddd	≈4.46 m	4.03 m	3.85 ddd	≈3.76 ddd	3.86	2.98	4.90	3.63	n.d.	minus 12.52	2.5	17.31	18.0	minus 51.25	$^5J_{F,5'}$≈1.03 $^5J_{F,5"}$≈0.7
8	7.88 s	8.09 s	6.17 ddd	5.44 dt	≈4.46 m	4.37 m	≈3.78 ddd	≈3.71 dd	2.28	2.60	3.81	5.22	5.68	minus 12.46	-	15.71	18.0	minus 51.25	$^5J_{F,5'}$0.84
9	8.25 s	8.16 s	6.37 ddd	5.60 dt	4.54 dddd	4.47 m	3.84 ddd	3.78 ddd	2.19	2.20	5.04	3.68	5.62	minus 12.58	-	15.92	19.80	minus 50.08	$^4J_{1',3'}$-0.55 $^5J_{F,5'}$ 0.95 $^5J_{F,5"}$ 0.77
10 a)	-	7.90 s	6.08 dd	5.61 ddd	4.33 ddd	4.21 m	3.53 ddd	3.58 ddd	3.20	3.80	5.98	5.16	4.13	minus 11.98	-	16.64	21.24	minus 52.43	$^4J_{1',8}$-0.10 $^4J_{2',4'}$-0.22 $^5J_{F,5'}$ 1.03 $^5J_{F,5"}$ 1.33
12 b)	-	7.79 d	6.13 dd	5.09 ddd	4.37 ddd	3.81 m	3.60 ddd	3.64 ddd	4.22	3.37	4.76	5.21	4.52	minus 11.82	2.32	16.28	17.76	minus 52.39	$^4J_{2',4'}$- 0.18 $^5J_{F,5'}$ 1.41 $^5J_{F,5"}$ 1.58 $^4J_{F,4'}$- 0.88

a) 6.80 br.s., 2-NH_2; 5.90 br.s., 6-NH_2; 6.08 d, $^3J_{OH,3'}$ = 5.46 Hz, 3'-OH; 4.98 t, $^3J_{OH,5'}$ = 5.77, $^3J_{OH,5"}$ = 5.61, 5'-OH.
b) 6.55 br.s., 2-NH_2; 5.92 br.s., 5'-OH; 5.05 br.s., 3'-OH.

TABLE 3. ^{13}C NMR Data for the 2-Deoxy-2-fluoro-D-arabinofuranosides **7**-**10** and **12** in DMSO-d_6. (Chemical shifts)

Compound	^{13}C Chemical shifts, δ_{TMS}, ppm ($^1J_{13C,H}$, Hz)									
	base					*sugar*				
	C-6	C-4	C-2	C-8	C-5	C-1'	C-2'	C-3'	C-4'	C-5'
7	157.67	150.87	154.90 [a]	143.70 d (203.0)	121.15	85.47 d (165.3)	97.65 d (166.1)	76.25 d (151.3)	86.50 d (150.2)	63.56 (143.8)
8	157.58	150.58	154.86 [a]	143.19 (203.8)	120.38	90.35 d (169.1)	102.20 d (159.3)	76.88 d (152.4)	89.62 d (149.9)	63.56 (143.8)
9	151.08	158.34	155.43 (203.5)	143.44 (216.3)	110.97	90.57 d (169.7)	102.18 d (164.8)	76.95 d (150.7)	89.71 d (150.8)	63.56 (143.9)
10	160.24	156.17	151.17 [a]	135.85 (213.0)	113.16	85.42 d (164.5)	99.69 d (166.1)	73.63 d (150.4)	85.71 d (148.2)	60.58 (140.5)
12	156.61	153.83 d	150.80 [a]	135.71 d (216.0)	115.87	81.20 d (162.8)	95.05 d (164.7)	72.61 d (150.0)	83.64 d (147.5)	60.36 (141.2)

[a] Resonances of C-2 displayed in the proton-coupled ^{13}C NMR spectrum additional couplings of 3.1 Hz (**7**), 2.3 Hz (**8**), 3.2 Hz (**10**), and 4.3 Hz (**12**) (tentatively $^5J_{C2,H8}$).

TABLE 4. ^{13}C NMR Data for the 2-Deoxy-2-fluoro-D-arabinofuranosides **7-10** and **12** in DMSO-d_6. (Coupling constants)

Compound	Coupling constants, Hz [a]										
	$J_{13C,H}$					$^1J_{F,13C}$	$^2J_{F,13C}$		$^3J_{F,13C}$	$^4J_{F,13C}$	
	C8,H1'	C4,H1'	C4,H8	C5,H8	Others	F,C2'	F,C1'	F,C3'	F,C4'	F,C8	F,C4
7	4.55	~2.0	5.45	10.9	$^3J_{C6,H2}$ 10.2 $^3J_{C4,H2}$ 11.6	192.37	16.96	25.39	3.39	5.33	<2.0
8	3.75	~1.8	~5.0	10.7	$^3J_{C6,H2}$ 10.2 $^3J_{C4,H2}$ 13.2	186.44	36.88	25.72	3.58	<2.0	<2.0
9	3.5	<2.0	10.9	n.d. [c]	n.d [b]	186.14	37.03	25.86	3.32	<2.0	<2.0
10	4.00	<2.0	4.40	11.2	$^3J_{C5,6NH2}$ 4.2	184.63	35.71	23.44	5.33	<2.0	<2.0
12	4.55	<2.0	4.30	11.4		191.77	16.84	23.35	3.97	4.73	4.43

[a] Resonance of C-5' displayed no $^4J_{F,13C}$ couplings in the ^{13}C NMR of all spectra.

[b] The value of $^3J_{C6,H2}$ was not determined owing to an overlap of the resonance by the intense upfield line of the C-2 doublet in the proton-coupled ^{13}C NMR spectrum.

[c] The value was not determined as the C-5 resonance was not observed in the proton-coupled ^{13}C NMR spectrum under the conditions used.

the H-8 and H-1' resonances to a lower field, which is in good agreement with data, previously reported for closely related pairs of isomers.[24,30,31] A similar trend is observed in the case of the α-anomers **8** and **9**.

Further support for the above structural consideration was found in an examination of the ^{13}C NMR data (TABLES 3 and 4). Previous empirical correlations of the effect of configuration of vicinal substituents in the furanose ring on the δ ^{13}C values of atoms, bearing these groups,[29,32-34] were taken into account. Thus, in the N^9-β-arabinoside **7**, as compared with N^9- and N^7-α-arabinosides **8** and **9**, 1,2-eclipsing interaction exists between the *cis* 1'-base/2'-fluorine atom which shifts the signals of C-1' and C-2' upfield by *ca.* 5.0 and 4.5 ppm, respectively. Similarly, when passing from the β-arabinoside **12** to the α-arabinoside **10** the C-1' and C-2' resonances are shifted downfield by *ca.* 4.5 ppm resulting from a removal of eclipsing interaction of the base and a fluorine atom. It is noteworthy that by going from the β-arabinosides **7** and **12** to the α-anomers **8**, **9** and **10** the C-4' resonance is shifted downfield by 2-3 ppm.

Of interest are some other observations. Thus, when passing from the β-arabinosides **7** and **12** to the α-anomers **8**, **9** and **10** (*i*) the $^1J_{F,C}$ couplings are decreased by 6-7 Hz, and (*ii*) the $^2J_{F,C-1'}$ couplings are increased. At the same time, the $^2J_{F,C-3'}$ couplings remain conservative. In the case of guanine β-arabinoside **12** two long-range $^4J_{F,C}$ couplings between fluorine atom and both C-8 and C-4 are observed, whereas in the case of adenine β-arabinoside **7** only C-8 displays this coupling. The vicinal $^3J_{H-1',C-8}$ and $^3J_{H-1',C-4}$ couplings unambiguously prove the predominant *anti*-conformation about the glycosyl bond.[32,35]

The conformational analysis of the furanose rings of nucleosides **7-10** and **12** was performed by the PSEUROT (version 6.2) program, which calculates the best fits of three experimental ^3J(H,H) coupling constants ($^3J_{H-1',H-2'}$, $^3J_{H-2',H-3'}$ and $^3J_{H-3',H-4'}$) to the five conformational parameters (P and ψ_m for both N- and S-type conformers and corresponding mole fractions).[36] In the PSEUROT program, a minimization of the differences between the experimental and calculated couplings is accomplished by a non-linear Newton-Raphson minimization. This procedure is enhanced if the ratio of the number of data points *vs.* the number of optimized parameters increases. The conformational behavior of nucleosides **7-10** and **12** was evaluated by the PSEUROT

analysis of the ^3J(H,H) values only, essentially as it was described previously.[37] The resulting optimized geometries of *N*- and *S*-pseudorotamers are presented in TABLE 5.

The factors affecting the conformation of the pentofuranose rings of nucleosides in solution have been extensively investigated during last years by Chattopadhyaya and coworkers (for a recent review, see Ref.[39]). The sugar moieties of nucleosides are involved in a two-state *N*↔*S* pseudorotational equilibrium, which is driven by the relative strength of various *gauche* and anomeric stereoelectronic effects. It was shown that the strong *gauche* effect of a highly electronegative fluorine substituent governs the overall conformation of the pentofuranose rings.[40-45] Based on this assessment, the extreme *S*-conformation of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC) was predicted.[41] In fact, an early conformational analysis by ^{1}H NMR spectroscopy revealed an equal mixture of the *S*- and *N*-conformers.[46] Moreover, an X-ray analysis of FIAC showed that in the solid state the sugar ring adopts the *N*-conformation (C-3'-*endo*/C-2'-*exo*; $^3T_2$).[47] An equal population of the *N*- and *S*-type puckered conformers of FIAC results from the competing *gauche* interactions of two F-C2'-C1'-O4' and HO-C3'-C4'-O4' fragments for the *S*-type, and the only F-C2'-C3'-OH fragment for the *N*-type. This implies the predominant population of the *S*-type in solution. However, the *N*-type conformation is energetically favoured over the *S*-type in terms of the anomeric effect of the heterocyclic base[39], leading in the case of FIAC to an equal population of the *N*- and *S*-type puckered conformers.

Similar to FIAC, the sugar moieties of the β-D-arabinosides **7** and **12** are in a two-state *N*↔*S* pseudorotational equilibrium in the ratio of 36:64 and 50:50, respectively (TABLE 5). It is noteworthy that 9-(2-deoxy-2-fluoro-β-L-arabinofuranosyl)purine, similarly to FIAC, was found to be in the solid state in the *N*-conformation ($^1T_2$, C1'-*endo*/C2'-*exo*; *P* –29.5°).[21] It is remarkable that the β→α change of the anomeric configuration as with **7**→**8** does not lead to any essential shift of the pseudorotational parameters of a furanose ring. On the contrary, by going from **12** to **10** a twofold decrease in the mole fraction of the *S*-conformer is observed.

Unfortunately, three ^3J(H,H) values are of limited value for conformational analysis of a pentofuranose ring especially if the equilibrium under consideration represents a mixture of conformations present in comparable proportions. With reference to fluorodeoxy nucleosides, Chattopadhyaya and coworkers have very recently

TABLE 5. Pseudorotational Parameters of Some Selected Compounds.[a)]

Compound	P_N	$\psi_{m(N)}$	P_S	$\psi_{m(S)}$	rms	$\lvert\Delta J_{max}\rvert$	%S
7	26.4	40[b)]	133.1	40[b)]	0.009	0.01	64
2'd,2'F-Ado	29.8	44[b)]	180.1	44[b)]	0.000	0.00	26[c)]
	−2.6	40[b)]	167.9	40[b)]	0.001	0.00	29[c)]
8	22.5	32[b)]	148.3	35[b)]	0.000	0.00	66
	30.5	40[b)]	141.3	40[b)]	0.000	0.00	73
2'd,2'F-α-Ado	-17.9	41.8	174[b)]	40[b)]	0.000	0.00	19
9	17.7	30	124.1	30	0.001	0.00	64
10	29.2	29[b)]	196.6	30[b)]	0.000	0.00	25
2'd,2'F-α-Guo	7.0	40[b)]	180.0	40[b)]	0.005	0.00	14
12	54.5	41[b)]	181.0	41[b)]	0.000	0.00	50
2'd,2'F-Guo	7.0	38[b)]	168.0	40[b)]	0.000	0.00	27[c)]

[a)] The data for the α- and β-anomers of 2'-deoxy-2'-fluoro-adenosine (2'd,2'F-Ado and -α-Ado) and –guanosine[38] (2'd,2'F-Guo and -α-Guo) are included for comparison.

[b)] The values indicated were fixed during the final calculations.

[c)] These results are somewhat conflicting with those published for the same compounds,[6] whereas our data[38] as well as reported[6,45] for 2'-deoxy-2'-fluorocytidine are in fair agreement.

developed a new Karplus-type relation between vicinal proton-fluorine coupling constants and the corresponding H-C-C-F torsion angles.[45] The use of temperature-dependent ^3J(F,H) coupling constants in combination with ^3J(H,H) greatly facilitates the conformational analysis of pentofuranose rings because of the overwhelming increase of the number of experimental data points over the puckering parameters P and ψ_m. In the present communication, we have qualitatively examined the $^3J_{F,C\text{-}4'}$ spin-couplings as an additional conformational probe of furanosyl rings in solution (*cf.* with the data in Ref.[34]). In the ^{13}C NMR spectra of 2'-deoxy-2'-fluoro-β-D-ribofuranosyl nucleosides we have never observed the $^3J_{F,C\text{-}4'}$ couplings, which is in good agreement with the predominant population of the *N*-conformation.[38] On the contrary, the ^{13}C NMR spectra of arabinosides **7**, **8**, **9**, **10** and **12** display these $^3J_{F,C\text{-}4'}$ couplings, thus strongly reflecting the considerable population of the *N*-conformation of the furanose rings. Close similarity of the $N \leftrightarrow S$ pseudorotational equilibrium of furanose rings of adenine nucleosides **7**, **8**, and **9** is in good agreement with similar values of the $^3J_{F,C\text{-}4'}$ couplings. Considerable differences between the $N \leftrightarrow S$ pseudorotational equilibrium of the β-anomer **12** and the

α-anomer **10** is well represented by the $^3J_{F,C\text{-}4'}$ values (TABLE 4). More definitive conclusions may, however, be drawn after detailed conformational analysis using both ^3J(H,H) and ^3J(F,H) coupling constants.[45]

Comparison of the $N \leftrightarrow S$ pseudorotational equilibrium for the β-D-arabinosides **7** and **12** with that of the related 2'-deoxy-2'-fluoro-adenosine and –guanosine[38] shows a close stereochemical resemblance of the former to the 2'-deoxynucleosides. This conclusion gives an insight into the important role of the $N \leftrightarrow S$ conformational mobility of the β-D-arabinosides in combination with their stereochemical similarity to the 2'-deoxynucleosides in the ability of the 2'F-aON:RNA hibrids to support the RNase H activity similar to that observed for the parent DNA:RNA hybrid.

Oligonucleotide Synthesis.- The arabinosides **7** and **12** were used for the preparation of the respective phosphoamidite building blocks **13** and **14** for automated oligonucleotide synthesis (Scheme 4). Transient protection of the bases,[48] followed by standard protocol of tritylation and phosphytilation,[49] afforded compounds **13** and **14** in a yield of 72 and 67%, respectively. Four 15-mer oligonucleotides (ONs) were prepared (FIG. 1): unmodified 2'-deoxy-ON (**AS1**) and three ONs, containing (*i*) ara-$A^{2'F}$ substituting for **A** (**A-12** from the 5'-terminus, **AS2**), (*ii*) ara-$G^{2'F}$ substituting for **G** (**G-3** from the 5'-terminus, **AS3**), and (*iii*) ara-$G^{2'F}$ and ara-$A^{2'F}$ substituting for both **G** and **A** (**G-3** and **A-12** from the 5'-terminus, **AS4**). The oligonucleotides were assembled according to a slightly modified recommended protocol (2 min condensation time was employed to introduce ara-$A^{2'F}$ and/or ara-$G^{2'F}$ units). No difference in coupling yields (>98 % as determined by the trityl assay) was detected between monomers **13**, **14** and commercial phosphoramidites of unmodified nucleosides.

After purification by HPLC and desalting[50], polyacrylamide gel electrophoresis revealed the reasonable purity of the oligomers synthesized. The final characterization of **AS1**- **AS4** was achieved by electrospray ionization mass spectrometry[51]. The difference between the measured and calculated average molecular masses of the oligonucleotides was always less than 0.02% (TABLE 6).

Thermodynamic analysis.-The thermodynamic parameters for the dissociation melting curves of the oligonucleotides **AS1**-**AS4** and a single-stranded complementary

AS1 3′-TTTACCTTCTGCGGT-5′

AS2 3′-TTTÂCCTTCTGCGGT-5′ Â = ara-$A^{2'F}$

AS3 3′-TTTACCTTCTGC GT-5′ = ara-$G^{2'F}$

AS4 3′-TTTÂCCTTCTGC GT-5′

D: 5′-TACTGTTGGTA**AAATGGAAGACGCCA**AAAACATA-3′

M: 5′-TACTGTTGGTA**AAATGGAAC**ACGCCAAAAACATA-3′

R: 5′-UACUGUUGGUA**AAAUGGAAGACGCCA**AAAACAUA-3′

FIG. 1. Structures of Oligonucleotides.

TABLE 6. Measured and Theoretically Calculated Average Molecular Masses of Oligonucleotides Synthesized.

Oligonucleotide	Theoretical mass	Measured mass	Error (%)
AS1	4 525,0	4 524,3	0,015
AS2	4 543,0	4 542,3	0,015
AS3	4 543,0	4 542,9	0,002
AS4	4 561,0	4 560,3	0,015

DNA sequence (**D**), a DNA stretch, containing a single mismatch in the middle of the sequence (**M**) or a complementary RNA sequence (initiation codon region of the firefly luciferase mRNA, **R**) were calculated by applying a two-state model[52] and the MeltWin software, which employs a non-linear least-squares method – the Marquardt-Levenberg fitting routine, A *vs.* T. Melting temperatures and thermodynamic parameters of association for the fluoro-oligonucleotides (**AS2 – AS4**) and unmodified sequence **AS1** with **D** and **M** are given in TABLE 7 and those for oligonucleotides (**AS1–AS4**) with **R** in TABLE 8.

One can clearly see that oligonucleotides **A2-A4** appear to display practically the same affinity and selectivity to both complementary DNA (**D**) and RNA (**R**) sequences as their parent oligonucleotide **AS1**. These data demonstrate that in contrast to ONs containing pyrimidine 2'-fluoroarabinonucleosides,[13] the introduction of purine 2'-fluoroarabinonucleosides does not influence the complex formation of oligonucleotide with both DNA and RNA.

TABLE 7. Melting Temperatures T_m (°C) and Thermodynamic Parameters of Association for the Oligonucleotides (**AS1–AS4**) with the DNA Sequences (**D** and **M**).

Oligonuc -leotide	Target	T_m (°C)	ΔT_m [a] (°C)	-ΔH° (kcal/mol)	-ΔS° (cal/K×mol)	$-\Delta G°_{37°}$ (kcal/mol)	Sel_{37} [b] (kcal/mol)
AS2	D	59.8		89.0	237.1	15.5	
	M	44.3	-15.5	84.6	236.3	11.3	-4.2
AS3	D	59.5		92.8	248.6	15.6	
	M	44.3	-15.2	83.3	232.3	11.3	-4.3
AS4	D	58.7		90.8	243.2	15.3	
	M	43.5	-15.2	80.7	224.6	11.0	-4.3
AS1	D	60.1		96.5	259.3	16.1	
	M	45.0	-15.1	84.9	236.5	11.5	-4.6

[a] The ΔT_m = **Tm (ASn – D** complex) – **Tm (ASn – M** complex)
[b] The Sel_{37} = $\Delta G°_{37°}$ **(ASn – D** complex) - $\Delta G°_{37°}$ **(ASn – M** complex)

TABLE 8. Melting Temperatures T_m (°C) and Thermodynamic Parameters of Association for the Oligonucleotides (**AS1–AS4**) with the RNA Sequence (**R**).

Oligonucleotide	T_m (°C)	-ΔH° (kcal/mol)	-ΔS° (cal/K×mol)	$-\Delta G°_{37°}$ (kcal/mol)
AS2	64.6	76.4	196.0	15.6
AS3	63.8	78.8	203.5	15.6
AS4	63.7	76.3	196.4	15.4
AS1	63.9	75.5	193.7	15.4

Inhibition of luciferase gene expression in an in vitro *transcription-translation system.*-Oligonucleotides **AS1-AS4** contained sequences complementary to the initiation codon region of the firefly luciferase mRNA, and therefore it was possible to test their antisense properties in a cell free luciferase gene expression system which proved to be a useful tool in related studies.[53,54] This type of experiment would mimic the testing with living cells and help to elucidate the potency of our conjugates as antisense agents. The FIG. 2 shows that modified oligonucleotides **AS2-AS4**, as well as oligomer **AS1,** all containing the antisense sequence, were able to decrease the luciferase activity in the same manner (by more than 80 % at 2 μM concentration), while the control oligonucleotide 5'-TTTACCTTCTGCGGT-3' (**C**), containing a reversed sequence,

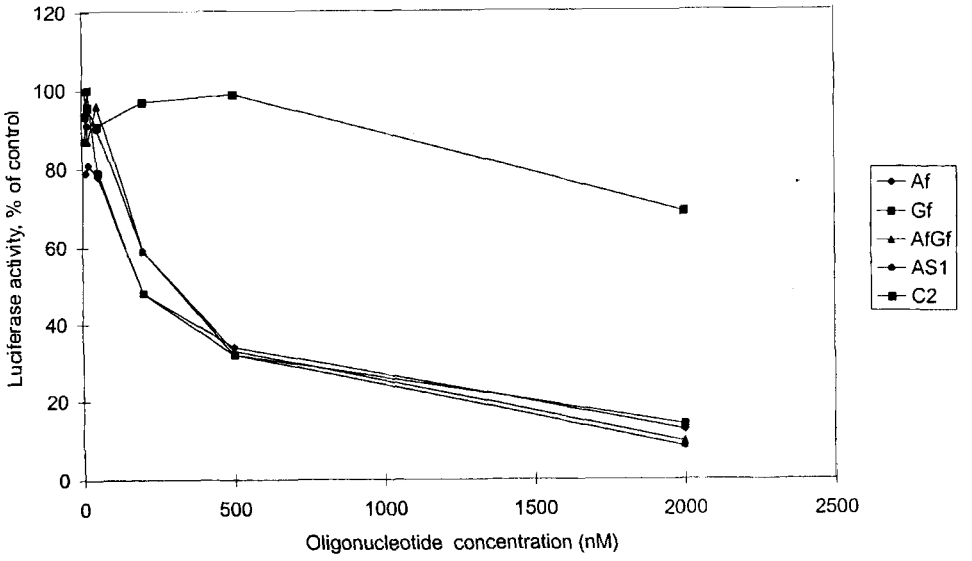


FIG. 2. Influence of the antisense (**AS1-AS4**) and control (**C**) oligonucleotides on the luciferase reporter gene expression in cell-free system.

showed inhibition of only about 25 % under the same conditions. Thus, in an *in vitro* transcription-translation assay, the RNase H mediated antisense action[53] of modified oligomers **AS2-AS4** appears to be as pronounced as that of oligodeoxyribonucleotide **AS1**.

In conclusion, we have synthesized 2-deoxy-2-fluoro-β-D-arabinofuranosides, ara-$A^{2'F}$ and ara-$G^{2'F}$, and have studied their conformational properties by means of the PSEUROT program. It was found that adenine N^9-β-D-arabinoside manifests a slight preference for the *S*-rotamer, while ara-$G^{2'F}$ displays an equal population of the *N*- and *S*-conformers. Furthermore, we have investigated the influence of point substitution of purine 2'-deoxynucleosides with the corresponding fluorides ara-$A^{2'F}$ and ara-$G^{2'F}$ in the 15-mer 2'-deoxyoligonucleotides on the binding affinity to the complimentary DNA and RNA sequences. It was found that 2'-deoxyoligonucleotides containing and ara-$A^{2'F}$ or/and ara-$G^{2'F}$ display practically the same affinity and selectivity to both complimentary DNA and RNA sequences as their parent unmodified 2'-deoxyoligonucleotide. This finding contradicts the theoretically predicted destabilization

of duplex formation upon substitution of purine 2'-deoxynucleosides with the related 2-deoxy-2-fluoro-β-D-arabinofuranosides[14] and is in accord with the experimental data by Damha *et al.*[15] In addition, we have demonstrated that in an *in vitro* transcription-translation assay, the RNase H mediated antisense action of modified oligomers is identical to that of unmodified oligodeoxyribonucleotide.

EXPERIMENTAL

General.- The melting points values reported are uncorrected. ^{1}H and ^{13}C NMR spectra were measured at 400.13 and 100.614 MHz, respectively, at 23 °C on an AM-400WB spectrometer (*Bruker*, Germany) with tetramethylsilane (TMS) as an internal standard (s = singlet; d = doublet; t = triplet; m = multiplet; br.s = broad signal); assignments of proton resonances were confirmed, when possible, by selective homonuclear decoupling experiments and correlation spectra. ^{1}H NMR spectra were simulated and iterated by means of PERCH (PEak ResearCH) program for 1D-NMR spectra and the data are presented in TABLES 1 and 2.[56] Adenosine deaminase (E.C. 3.5.4.4; from calf intestine mucosa; specific activity 200 units/mg protein, solution in 50% glycerol) was purchased from *Boehringer* (Mannheim, Germany). The solvent employed for recording the spectra was $CDCl_3$, unless otherwise stated. Standard Kieselgel 60 F_{254} (*E.Merck*, Germany) plates were used for thin layer chromatography (TLC). Flash silica gel column chromatography of nucleosides was performed on 230-400 mesh 60 silica gel Merck (*E. Merck*, Germany). The 2-deoxy-2-fluoro-1,3,5-tri-*O*-benzoyl-α-D-arabinofuranose (F-7770) was purchased from *Sigma* (USA). In all condensation reactions, freshly distilled trimetylsilyl trifluoromethanesulfonate (TMS-Tfl) (*Fluka*, Switzerland) was used. The solutions of compounds in organic solvents were dried with anhydrous sodium sulfate for 4 h. Except otherwise indicated, the reactions were carried out at 20 °C. Oligonucleotides were prepared on a PE Applied Biosystems 392 DNA synthesizer (1 μmol scale), employing reagents of Glen Research Corporation. Mass spectra were acquired and molecular weights were reconstructed as reported earlier.[51,53] UV spectra were recorded and melting experiments were performed on a Varian Cary 300 Bio UV-Visible Spectrophotometer, equipped with Cary Temperature Controller and multicell Peltier block. Cary Win UV Thermal Application Software was employed to increase temperature at a rate of 0.5 °C/min and record melting curves at

260 nm. The oligomer concentrations were in the micromolar range in a buffer, containing 10 mM Tris-HCl, pH 6.8; 0.1 M NaCl; 10 mM $MgCl_2$. Extinction coefficients of oligonucleotides were calculated by the nearest-neighbour method.[55] The thermodynamic parameters for dissociation melting curves of complexes were extracted by employing the MeltWin software (University of Rochester, USA). Uncertainty in the $\Delta G^{\circ}_{37^{\circ}}$, ΔH° and ΔS° values was estimated to be less than ±10% and that in the Tm values less than ±0.3 °C, based on triple repetition of each measurement. The influence of the oligonucleotides on the luciferase expression *in vitro* in the cell-free system was studied using TNT Coupled Wheat Germ Extract Systems kit (*Promega*) as described earlier.[53,54]

9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-adenine (7) and -guanine (12).- Chlorotrimethylsilane (0.6 mL) was added to a suspension of 2,6-dichloropurine (0.7 g, 3.68 mmol) in hexamethyldisilazane (10 mL), the mixture was refluxed for 3 h and evaporated to dryness. The residue was co-evaporated with anh CH_3CN (2x25 mL) and toluene (25 mL), and dissolved in anh CH_3CN (60 mL). Arabinoside **2** (1.0 g, 2.15 mmol) and then TMS-Tfl (1.0 mL, 5.5 mmol) were added to the solution and the mixture was refluxed for 20 min. The mixture was poured into 5% aq solution of $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (3x25 mL). The organic extracts were combined, dried, and evaporated to afford a mixture of isomers (1.10 g, 96%). The HPLC analysis [(column: Genesis C18, 4μ; linear gradient (50→100%) of buffer B (0.05 M TEAA in 80% aq CH_3CN) in buffer A (0.05 M TEAA) at a flow rate of 1mL/min (time of analysis 25 min)], retention time: N^9-β-isomer **3** – 16.86 min (37.2); N^9-α-isomer **4** – 16.06 min (35.8); N^7-β-isomer **5a** – 15.06 min (10.5); N^7-α-isomer **5b** – 14.58 min (5.0).

The residue was applied to a chromatographic column (5x40 cm) and eluted with a mixture of toluene-EtOAc (3:1). The fractions containing the N^9-β-isomer **3** (0.39 g, 35%; mp 159-160 °C, from MeOH; Lit.[21] mp 153-154 °C), N^9-α-isomer **4** (0.36 g, 33%; white foam), N^7-β-isomer **5a** (55 mg, 5%; mp 166-167 °C, from MeOH) and the mixture of **5a** and **5b** (55 mg, 5%; white foam).

The N^9-α-isomer **4** (0.53 g, 1.0 mmol) was treated with LiN_3 (0.255 g, 5.0 mmol) in EtOH (50 mL) under reflux for 2 h.[21] The reaction mixture was evaporated to dryness the residue was benzoylated in pyridine to afford, after standard work-up, syrupy product. Treatment of the latter with $SnCl_2$ (0.44 g, 2.31 mmol) in a mixture of CH_2Cl_2

(20 mL) and MeOH (2 mL)[26], followed by standard debenzoylation and silica gel column (5x20 cm) chromatography gave compound **10** (0.2 g, 72%; mp 203-205 °C, from MeOH with few drops 35% aq ammonia) UV (H_2O), λ_{max}, nm ($\varepsilon x10^{-3}$): 279.1 (7.30), 255.9 (7.09), 215 (18.62). Anal.: ($C_{10}H_{13}FN_6O_3$; 284.25), C, H, N.

In a similar way, starting from the N^9-β-isomer **3** (1.06 g, 2.0 mmol), N^9-β-analogue of **10** was obtained (0.47 g, 83%, white solid). The latter was dissolved in water (5 mL) and treated with ADase (100 μL).[26] Upon standing at room temperature overnight, crystalline nucleoside **12** deposited. The product was filtered off, washed with water and EtOH to afford pure **12** (0.45 g, 80%; mp 248-250 °C; Lit.[21] mp 249-251 °C). UV (H_2O), λ_{max}, nm ($\varepsilon x10^{-3}$): 253.5 (13.7), ~270 sh.

The solution of the N^9-β-isomer **3** (1.06 g, 2.0 mmol) in anh 1,2-dimethoxyethane (25 mL), saturated with dry ammonia gas at room temperature, was kept for 72 h and evaporated to dryness.[27] The residue was dissolved in MeOH (20 mL), saturated with dry ammonia gas at 0 °C, the mixture was allowed to stand for 48 h and evaporated. The residue was triturated with a mixture of EtOH-ether (9:1, 10 mL), the precipitate was filtered off and washed with the same mixture to give nucleoside **11** (0.44 g, 72%; amorphous). UV (H_2O), λ_{max}, nm ($\varepsilon x10^{-3}$): 265.0 (13.7), 213.4 (25.1). Compound **11** was catalytically hydrogenated[28] in the presence of 5% Pd/C to give adenine nucleoside **7** (0.32 g, 82%; mp 233-234 °C, from EtOH; Lit.[18] 231-234 °C). UV (H_2O), λ_{max}, nm ($\varepsilon x10^{-3}$): 259.7 (13.8), 210.2 (18.1).

Condensation of 2-deoxy-2-fluoro-1,3,5-tri-*O*-benzoyl-α-D-arabinofuranose (2) with silylated N^6-benzoyladenine.- A solution of arabinoside **2** (1.0 g, 2.15 mmol), trimethylsilyl derivative of N^6-benzoyladenine (**6**) [obtained from N^6-benzoyladenine (1.03 g, 4.30 mmol) by refluxing in HMDS in the presence of $(NH_4)_2SO_4$] and TMS-Tfl (1.56 mL, 8.60 mmol) in anh CH_3CN (25 mL) was refluxed for 4 h. After standard workup, the residue (1.05 g, 84%) was treated with MeOH (25 mL), saturated with dry ammonia gas at 0 °C, for 48 h and evaporated. The residue was chromatographed [silica gel column, 5x40 cm; elution with a linear EtOAc-hexane (1:1; 2.0 L) gradient in EtOAc-hexane (95:5; 2.0 L)] to yield the N^9-β-isomer **7** (70 mg, 14%), the N^9-α-isomer **8** (70 mg, 14%; amorphous) and the N^7-α-isomer **9** [0.12 g, 25%; amorphous; UV, λ_{max}, nm ($\varepsilon x10^{-3}$): (H_2O) 211.1 (17.30), 272.2 (7.90); (H_2O, pH 1.0) 206.3 (10.60), 219.1 (10.72), 273.6 (11.4).

Phosphoamidites 13 and 14 were prepared by transient protection of the bases,[48] followed by standard protocol of tritylation and phosphytilation[49] in yields of 72 and 67%, respectively. Spectral data: **13**, ^{31}P NMR ($CDCl_3$), δ_{H3PO4}, ppm: 148.82 (0.5P) and 148.60 (0.5P); ESI mass spectrum: calc. mass – 875.36; measured mass 876.24; **14**, ^{31}P NMR ($CDCl_3$), δ_{H3PO4}, ppm: 149.12 (0.5P) and 149.01 (0.5P); ESI mass spectrum: calc. mass – 871.38; measured mass 870.07.

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