6-Azauracil or 8-aza-7-deazaadenine nucleosides and oligonucleotides: the effect of 2′-fluoro substituents and nucleobase nitrogens on conformation and base pairing

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The stereoselective syntheses of 6-azauracil- and 8-aza-7-deazaadenine 2′-deoxy-2′-fluoro-β-D-arabinofuranosides **1c** and **2c** employing nucleobase anion glycosylation with 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide **6** as the sugar component are described; the 6-azauracil 2′-deoxy-2′-fluoro-β-D-ribofuranoside **1d** was prepared from 6-azauridine **8** *via* the 2,2′-anhydro intermediate **9** and transformation of the sugar with DAST. Compounds show a preferred *N*-conformer population (100% *N* for **1c**, **1d** and 78% *N* for **2c**) being rather different from nucleosides not containing the combination of a fluorine atom at the 2′-position and a nitrogen next to the glycosylation site. Oligonucleotides incorporating **1c** and **2c** were synthesized using the phosphoramidites **3b** and **4**. Although the *N*-conformation is favoured in the series of 6-azauracil- and 8-aza-7-deazaadenine 2′-deoxy-2′-fluoroarabinonucleosides only the pyrimidine compound **1c** shows an unfavourable effect on duplex stability, while oligonucleotide duplexes containing the 8-aza-7-deazaadenine-2′-deoxy-2′-fluoroarabinonucleoside **2c** were as stable as those incorporating dA or 8-aza-7-deaza-2′-deoxyadenosine **2a**.

Introduction

The introduction of a fluorine atom to an organic compound does not result in a considerable change in the size or the shape of the molecule. However, the fluorine substituent can alter the physico-chemical properties and biological activity. As the C–F bond is stronger than the C–H bond, fluorinated compounds are chemically more stable and more resistant against metabolic degradation.[1–3] Thus fluorinated analogues of biological molecules provide useful tools for probing or modifying the functions of biologically active compounds.

The ability of a fluorine atom to mimic the hydroxyl group makes it suitable for introduction into the sugar moiety of nucleosides. The potent effect of introducing fluorine has already led to useful therapeutic agents. Among these fluorinated nucleosides are compounds like 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)thymine (FMAU),[4] 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU) and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine,[5,6] which are potent antiviral agents, while 2′,2′-difluorocytidine (gemcitabine) shows anticancer activity.[7] Corresponding "purine" nucleosides such as 4-amino-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1-*H*-pyrazolo[3,4-*d*]-pyrimidine **2c** display significant activity against HBV (Hepatitis B virus).[8] The polymerase catalyzed synthesis of the 5′-triphosphates of 2′-deoxy-2′-fluoroarabinonucleosides (2′F-araNTPs) has been reported and these compounds are accepted as substrates by various DNA polymerases or have found application in *in vitro* selection studies of DNA aptamers.[9] The 2′F-araNA modification is well suited to tune the physico-chemical and biological properties of G-quartets.[10]

The higher electronegativity of the fluorine atom can alter the conformational properties of the sugar residue by shifting the conformational equilibrium of the pentofuranose moiety to either the north or the south conformation as a function of its position on the (sugar) ring, the stereochemistry, and interactions with the neighboring atoms (substituents).[11a,b] Nevertheless, the contribution of the electronic structure of the heterocyclic base in conjunction with the electronegative substituent of the sugar moiety attracts attention. It was recognized that 3-bromo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1-*H*-pyrazolo[3,4-*d*]pyrimidine-4,6-diamine[12a] having a nitrogen atom placed next to the glycosylation site, shows rather unique conformational properties when compared to the corresponding purine 2′-deoxyribonucleoside[12b] with a 100% population of the *N*-conformer.

Recently, we reported on the pH-dependent duplex stability of oligonucleotides containing 6-aza-2′-deoxyuridine **1a**.[13] Because of the pK_a value of the 6-azauracil moiety (6.5), the strength of the base pair and the resulting oligonucleotide duplex stability are pH-dependent. At neutral pH value the duplexes are already partially deprotonated at the modified base leading to destabilization of the "dA–dT" base pair. The related 8-aza-7-deaza-2′-deoxy-2′-fluoroarabinoadenosine **2c** does not show such properties although it favours the *N*-conformation. This manuscript reports on the stereoselective synthesis of the 6-azapyrimidine 2′-deoxy-2′-fluoronucleosides **1c**, **1d** as well as the related pyrazolo[3,4-*d*]pyrimidine 2′-deoxy-2′-fluoroarabinonucleoside **2c** (2′F-ara-$c^7z^8A_d$),

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all with a fluorine atom at the 2′-position and a nitrogen placed next to the glycosylation site. Oligonucleotides containing **1c** and **2c** were synthesized. For this the phosphoramidite building blocks **3b** and **4** were prepared and employed in solid-phase oligonucleotide synthesis (Scheme 1). The effect of the 2′-fluoro substituent on the conformational properties and on the duplex stability was determined and correlated with the structurally related purine and pyrimidine 2′-deoxyribonucleosides **2a** and **1a**.

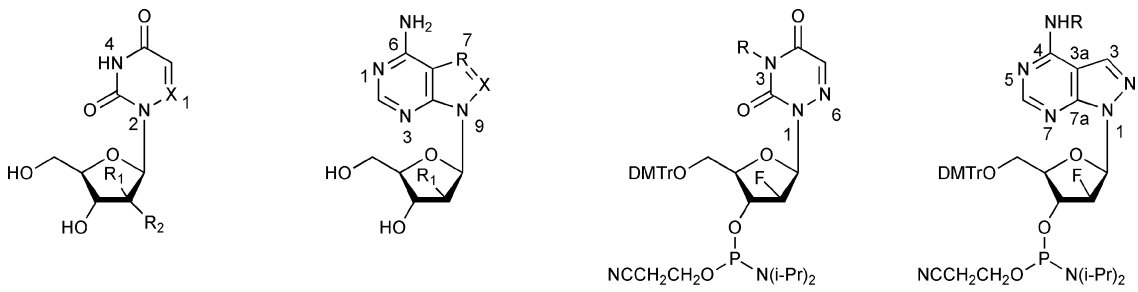
Results and discussion

1. Synthesis and properties of monomers

The convergent syntheses of a number of 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl nucleosides have already been reported.[14,15] The preparation of the 6-aza-2′-deoxy-2′-fluoro-arabinofuranosyl uracil **1c** (6-aza-2′ F-ara-U; pyrimidine numbering is used throughout the text; the systematic numbering is used in the experimental section) started with the known 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide **6** which was obtained from the commercially available 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose by a three-step procedure.[16–18] This was directly coupled to the persilylated 6-azauracil **5** in the presence of CuI following the protocol of Freskos.[19] This reaction protocol led exclusively to the formation of the protected β-D-nucleoside **7**. Apparently, the stereochemical outcome of 2′-deoxy-2′-fluoroarabinonucleoside formation follows a similar mechanism to that of the 2′-deoxyribonucleoside.[19,20] The protected compound **7** was deprotected with 0.2 M NaOMe in MeOH to afford nucleoside **1c** (Scheme 2).

Next, the corresponding 2′-deoxy-2′-fluororibonucleoside **1d** was prepared. Generally, 2′-deoxy-2′-fluoro-β-D-ribonucleosides can be obtained by various routes: (i) by the fluorination of an appropriate arabinonucleoside or anhydronucleoside;[21] (ii) by condensation of a 2-fluororibosugar halide with the heterocyclic base;[22] and (iii) by transglycosylation of 2′-deoxy-2′-fluororibonucleosides with another nucleobase.[23] We selected route (i) by fluorinating the 6-azauracil arabinonucleoside **10**. The fluorination of arabinonucleosides undergoes S_N2 displacement with inversion of configuration when treated with diethylaminosulfur trifluoride (DAST) or with tetra-*n*-butylammonium fluoride (TBAF).[24,25]

The arabinonucleoside **10** was obtained from the ribonucleoside **8** *via* the intermediate 2,2′-anhydro-6-azauridine **9**. The 3′,5′-hydroxyl groups of **8** were protected by using the Markiewicz clamp (1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane; TIPDS) and further treatment with methane sulfonyl chloride followed by TBAF yielded compound **9**. Next, the 3′,5′-hydroxyl groups were selectively protected with 3,4-dihydropyran (DHP) in DMF, followed by the opening of the anhydro ring with the help of mild basic hydrolysis to form the protected 6-azauridine arabinonucleoside **10**. Compound **10** was then treated with DAST



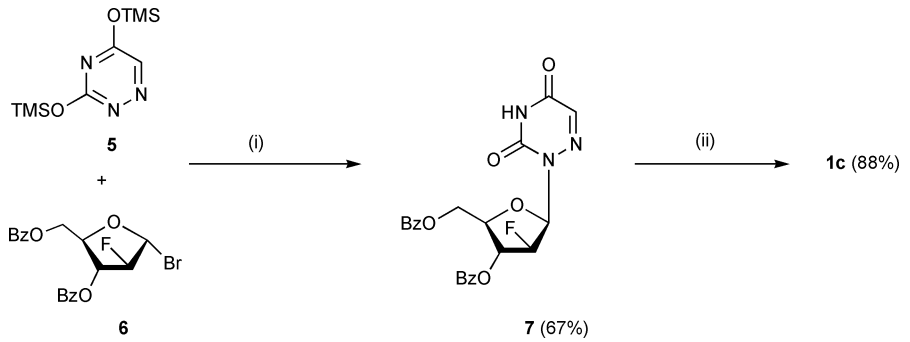
1a: X = N; R_1 = R_2 = H
b: X = CH; R_1 = F; R_2 = H
c: X = N; R_1 = F; R_2 = H
d: X = N; R_1 = H; R_2 = F

2a: R = CH; X = N; R_1 = H
b: R = N; X = CH; R_1 = F
c: R = CH; X = N; R_1 = F

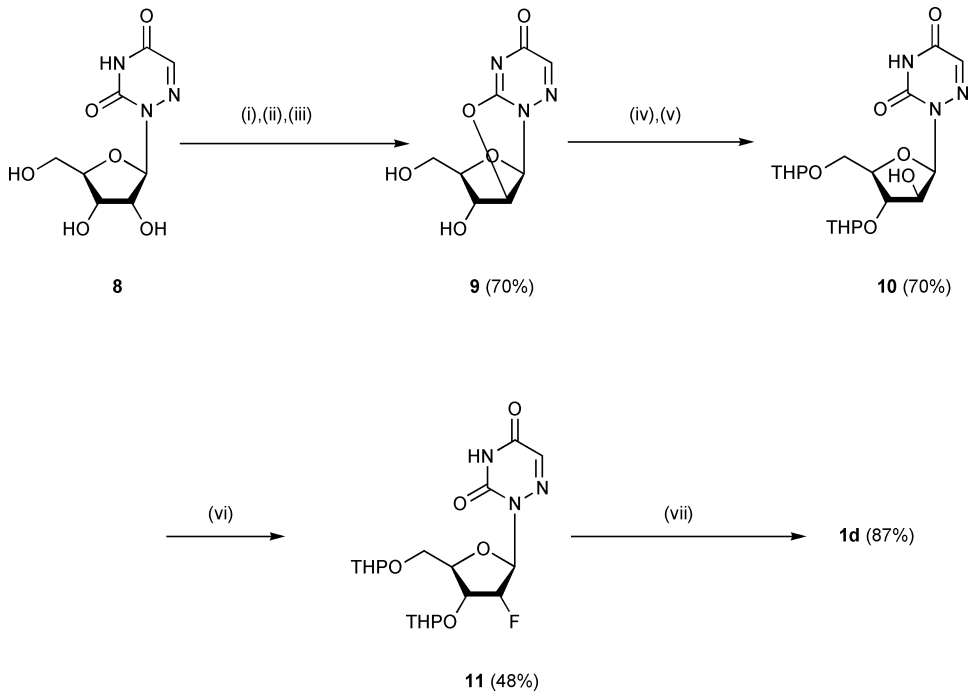
3a: R = H
3b: R = *o*-anisoyl

4: R = Me_2CHCO

Scheme 1 Structures of nucleosides and the corresponding phosphoramidites.



Scheme 2 *Reagents and conditions*: (i) CuI, $CHCl_3$, overnight, r.t.; (ii) 0.2 M NaOMe–MeOH, 2 h, r.t.



8 **9** (70%) **10** (70%)

11 (48%)

Scheme 3 *Reagents and conditions*: (i) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS), pyridine, 5 h, r.t.; (ii) CH_3SO_2Cl, pyridine, 16 h, r.t.; (iii) 1 M TBAF in THF, 2 h, r.t.; (iv) 3,4-dihydropyran, DMF, *p*-toluenesulfonic acid, 4 h, 0 °C; (v) 1 N NaOH, MeOH, 2 h, r.t.; (vi) DAST, pyridine, CH_2Cl_2, −60 °C; (vii) pyridinium *p*-toluenesulfonate, EtOH, 3 h, 60 °C.

to give the intermediate **11** in 48% yield. Finally, the removal of the THP groups from **11** with pyridinium *p*-toluenesulfonate gave the desired nucleoside **1d** (Scheme 3).

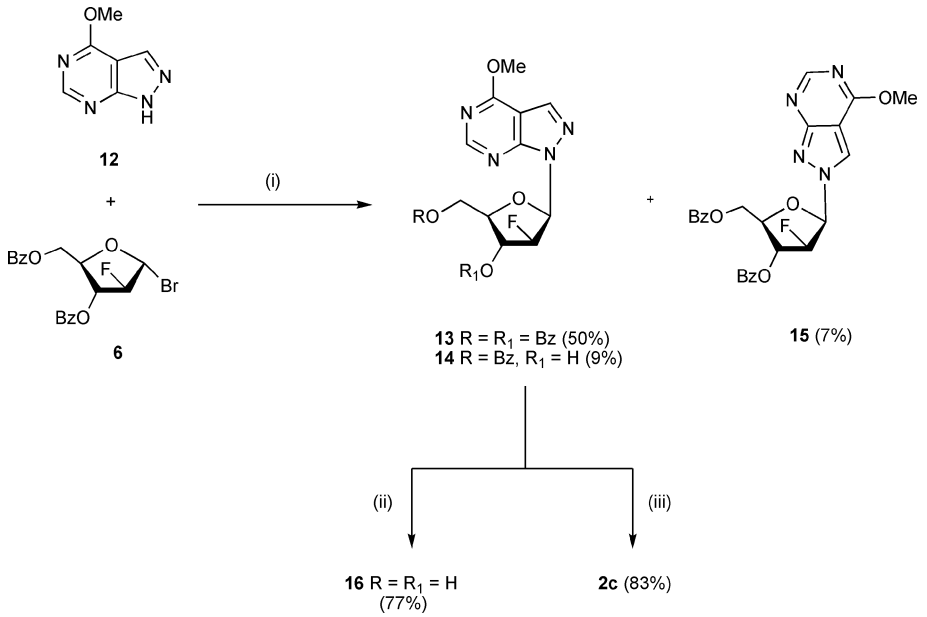
More recently, we have reported on the synthesis of 4-amino-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-1-*H*-pyrazolo[3,4-*d*]-pyrimidine **2c**[8] (systematic numbering is used). In this method the glycosylation of 4-methoxypyrazolo[3,4-*d*]pyrimidine **12** with 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide (**6**) in the presence of DBU resulted in the formation of a mixture of the α-D and β-D-anomers of N^1- and N^2-glycosides (94%, overall yield) with 45% of the desired N^1-β-D-anomer. Because of the unsatisfactory stereochemical outcome of the above method, here, we used an improved protocol. The condensation of 4-methoxypyrazolo[3,4-*d*]pyrimidine **12**, which was performed with the halogenose **6** in MeCN in the presence of powdered KOH and TDA-1 (tris[2-(2-methoxyethoxy)ethyl]amine) at r.t., afforded the desired N^1-β-D-nucleoside **13** (50%) along with the 3′-debenzoylated derivative **14** (9%) and a trace amount of the N^2-β-D-regioisomer **15** (7%). Deprotection of compounds **13** and **14** with 25% aqueous ammonia resulted in the removal of the benzoyl groups and a simultaneous conversion of the 4-methoxy substituent to an amino group yielding **2c**. When the deprotection was performed in NaOMe–MeOH, it gave the methoxy nucleoside **16** (Scheme 4).

2. Spectroscopic data

The structures of all synthesized compounds were characterized by ^{1}H, ^{13}C and ^{19}F NMR spectra (Table 1 and 2) as well as by elemental analysis. In the case of **1c** the configuration of the fluorine atom was assigned as β-D, as it was proven by single crystal X-ray analysis[26] and was in accordance with the fixed stereogenic centers of the sugar halide not involved in the glycosylation reaction. To confirm the configuration of the fluorine atom at the 2′-position in **1d**, a ^{1}H-NMR NOE spectrum was measured. Irradiation of H-1′ resulted in NOE effects at H-4′ (1%) and OH-3′ (4.7%); irradiation of H-3′ gave NOE at H-(2′-β) (3.2%). Based on the spatial relationships of the H-atoms the position of fluorine is assigned as α-D (2′-F down). The assignment of the ^{13}C NMR chemical shifts of the base moieties in nucleosides **1c**, **1d** and **2c** was made according to the previous reports of related compounds.[13,27] The ^{19}F NMR spectra of 2′-deoxy-2′-fluoroarabino *ortho*-aza nucleosides differ substantially from those of other 2′-deoxy-2′-fluoroarabinonucleosides. The fluorine signal in the ^{19}F NMR spectra of **1c**, **2c** and its derivatives forms a doublet of doublets (geminal H2′–F and vicinal H3′–F couplings) and not a doublet of two doublets as a result of an additional vicinal H1′–F coupling. It implies that there is only one vicinal H–F coupling observed. Similar behaviour was also found in other *ortho*-aza nucleosides such as 3-bromopyrazolo[3,4-*d*]pyrimidine 2′-deoxy-2′-fluoro-β-D-arabinonucleosides.[12a]

3. Nucleoside conformation

It has been noticed previously, that the *N*–*S* pseudorotational equilibrium of the sugar moieties of nucleosides is driven by various gauche and anomeric effects. The anomeric effect depends on the electronic nature of the heterocyclic base and can drive the sugar moiety of β-D-nucleosides toward the *N*-type conformers.[28–30] We have already studied the conformational properties of the azapyrimidine nucleosides **1c**, **1d**[31a,b] and the azapurine nucleoside **2c** in solution by applying the PSEUROT program (version 6.3).[32] The use of both $^3J_{H,H}$, $^3J_{H,F}$ coupling constants and semi-empirical calculations using the HyperChem 7.0 program (Hypercube Inc.,



Scheme 4 *Reagents and conditions*: (i) MeCN, TDA-1 (= tris[2-(2-methoxyethoxy)ethyl]amine), KOH, 30 min, r.t.; (ii) 0.2 M NaOMe–MeOH, 2 h, r.t.; (iii) NH_3 in H_2O–dioxane (4 : 1), 24 h, 90 °C.

Table 1 ^{1}H NMR data of 2′-deoxy-2′-fluoro nucleosides measured in DMSO-d_6 at 298 K

	Chemical shifts, δ_{TMS}/ppm					Coupling constants/Hz					
	Sugar					^3J(H,H)			^3J(H,F)		
	H-1′	H-2′	H-3′	H-4′	H-5′	1′,2′	2′,3′	3′,4′	1′,F	3′,F	Others
7	6.58 d	5.82 dt	6.06 m	4.65–4.68 m	4.48–4.52 m	6.7	7.5	7.8	<1.0	18.8	7.43–8.04 (s, 1 H, 5-H; m, 10 H, arom.); 12.43 (s, 1 H, NH).
1c	6.40 d	5.28 dt	4.33 m	3.62–3.69 m	3.52–3.54 m	7.0	7.0	7.3	<1.0	18.3	4.76 (t, 1 H, 5′-OH); 5.79 (br d, 1 H, 3′-OH); 7.6 (s, 1 H, 5-H); 12.33 (s, 1 H, NH).
11	6.16 d	5.20 dd	4.27 m	4.07–4.15 m	3.69 m	<1.0	—	—	20.65	24.1	4.55, 4.1, 1.5 (m, 18 H, THP); 7.58 (s, 1 H, 5-H); 12.33 (s, 1 H, NH).
1d	6.07 d	5.08 dd	4.23 m	3.80–3.65 m	3.60 m	<1.0	3.9	7.8	20.73	23.5	7.55 (s, 1 H, 5-H); 12.13 (s, 1 H, NH).
2c	6.60 d	5.40 dt	4.74 m	3.80 m	3.78 m	6.3	6.97	6.8	<1.0	18.0	4.84 (br t, 5′-OH); 5.86 (br d, 3′-OH); 7.80 (br dd, NH_2); 8.20 (s, H-3, H-6).
17	6.50 d	5.28 dt	4.38 m	4.01 m	3.20 m	6.5	7.0	7.2	<1.0	18.9	3.72 (s, 6 H, OMe); 5.77 (br d, 3′-OH); 6.81–7.39 (s, 1 H, 5-H; m, 13 H, arom.).
18	6.42 d	5.34 dt	4.40 m	3.74 m	3.53–3.69 m	6.6	7.1	7.0	<1.0	19.2	3.71 (s, 3 H, OMe); 4.78 (t, J = 5.75, 5′-OH); 5.83 (br d, J = 5.45, 3′-OH); 7.13–8.00 (s, 1H, 5-H; m, 4 H, arom.).
19	6.47 d	5.27 dt	4.40 m	3.92 m	3.16–3.26 m	6.6	7.0	6.9	<1.0	19.3	3.73 (s, 9 H, OMe); 5.89 (br d, J = 5.55, 3′-OH); 6.86–8.00 (s, 1 H, 5-H; m, 17 H, arom.).
20	6.76 d	5.42 dt	4.68 m	3.79 m	3.60–3.67 m	6.3	6.6	6.4	<1.0	19.7	1.05–1.17 (d, 6 H, 2Me); 2.86–2.94 (m, 1 H, CH); 4.79 (t, 1 H, J = 5.30, 5′-OH); 5.85 (d, 1 H, J = 5.72, 3′-OH); 8.57 (s, 1 H, 3-H); 8.69 (s, 1 H, 6-H); 1.29 (s, 1 H, NH).
21	6.71 d	5.46 dt	4.85 m	3.99 m	3.15 m	—	6.9	6.5	<1.0	18.0	1.06–1.17 (d, 6 H, 2Me); 2.88–2.98 (m, 1 H, CH); 5.88 (d, 1 H, J = 5.50, 3′-OH); 6.75–7.31 (m, 13 H, arom.); 8.46 (s, 1 H, 3-H); 8.71 (s, 1 H, 6-H); 11.32 (s, 1 H, NH).

Gainesville, FL, USA, 2001) permits[31a] a detailed conformational analysis of the pentofuranose ring. The input contained the following coupling constants: ^3J(H1′,H2′), J(H2′,H3′), J(H3′,H4′), J(H1′,F), J(H3′,F). They were taken from the well resolved ^{1}H NMR spectra measured in D_2O. The results of semi empirical calculations (PM3)[31a] are in good agreement with the X-ray data. Recently, Chattopadhyaya and co-workers have developed a new Karplus-type relationship between vicinal proton–fluorine coupling constants and the corresponding H–C–C–F torsion angles. Correction terms for substituent electronegativity and for the H–C–C (α_{HCC}) and F–C–C (α_{FCC}) bond angle deviations from tetrahedral values[33] are also implemented. This protocol is not used in the present study.

In order to understand the effect of the 2′-fluoro substituent as well as the heterocyclic base on the conformational properties, a series of related purine and pyrimidine nucleosides were compared

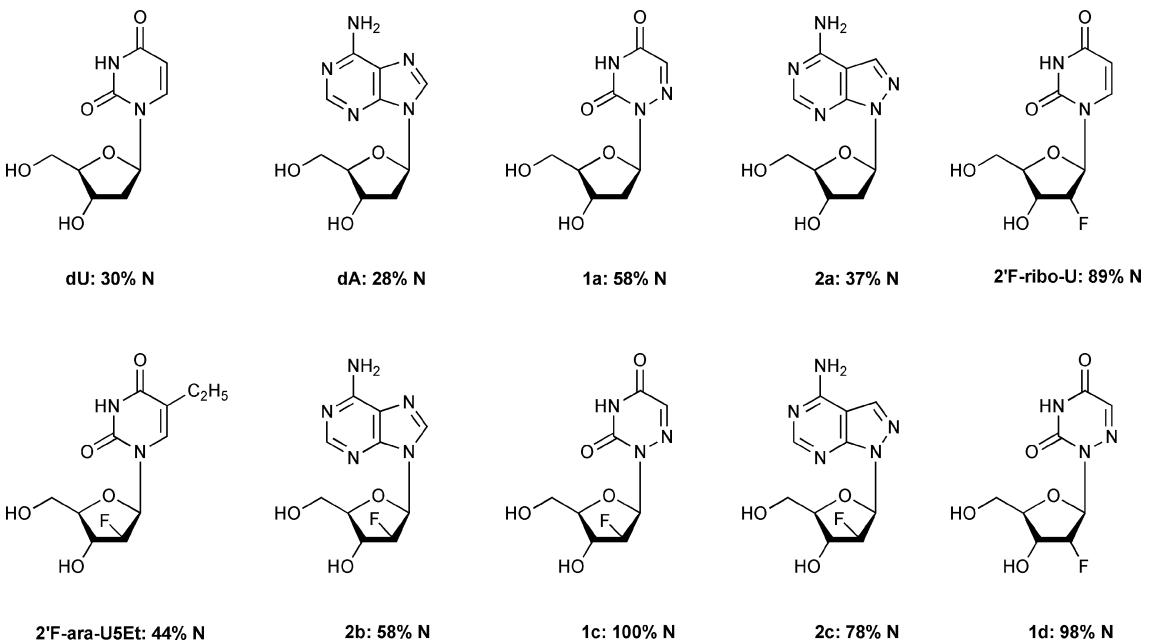
Table 2 ^{13}C NMR data of nucleosides in DMSO-d_6

	Chemical shifts, δ_{TMS}/ppm										
	Base					Sugar					
	C(2)[a] (C-6)[b]	C(4)[a] (C-7a)[b]	C(5)[a] (C-3a)[b]	C(6)[a] (C-4)[b]	C(7)[a] (C-3)[b]	C-1′ ($^2J_{F,C}$)	C-2′ ($^1J_{F,C}$)	C-3′ ($^2J_{F,C}$)	C-4′ ($^3J_{F,C}$)	C-5′	Others
7	148.1	156.0	136.8	—	—	81.4 (17.2)	92.1 (198.7)	74.9 (22.2)	76.4 (9.7)	64.2	
1c	148.3	156.2	136.2	—	—	81.1 (17.0)	95.1 (196.2)	72.3 (20.1)	82.1 (10.1)	62.3	
9	160.0	164.5	142.2	—	—	90.3[c]	89.9[c]	75.2	89.3[c]	60.9	
10	148.4	156.3	135.5	—	—	84.3	80.7	74.7	80.3	61.3	98.1, 68.5, 61.2, 30.3, 24.9, 18.9 -THP
11	148.6	157.4	137.6	—	—	88.8 (34.6)	94.1 (184.0)	76.8 (16.2)	80.3 (10.1)	62.3	98.7, 66.7, 62.0, 30.8, 25.7, 19.7 -THP
1d	148.0	156.6	136.6	—	—	87.5 (34.6)	93.8 (182.2)	68.9 (16.3)	83.1	61.1	
18	146.7	154.1	136.1	—	—	81.7 (17.04)	95.1 (196.5)	72.3 (19.81)	82.34 (11.0)	62.3	56.42 -OCH_3
19	146.6	154.0	135.9	—	—	81.77 (17.4)	95.0 (195.9)	72.4 (20.18)	80.1 (11.3)	64.1	56.42, 55.01 -OCH_3
2c	156.3	154.5	99.9	158.1	133.7	80.4 (18.0)	95.1 (196.3)	72.26 (20.06)	82.25 (10.7)	62.5	
20	155.1	152.3	103.3	155.5	137.5	80.33 (17.7)	95.05 (196.34)	72.28 (19.87)	82.35 (10.7)	62.5	34.4 -CH; 19.1, 19.07 -$(CH_3)_2$
21	155.1	152.3	103.3	155.6	137.4	80.2 (18.0)	94.84 (197.2)	72.35 (19.9)	85.23	64.3	54.9 -OCH_3, 34.4 -CH; 19.1, 19.07 -$(CH_3)_2$

[a] Pyrimidine numbering or purine numbering. [b] Systematic numbering of the purine base. [c] Tentative.

which are compiled in Scheme 5. The top row formulas show a series of 2′-deoxyribonucleosides (dU,[34] dA,[35] **1a**[13] and **2a**[35]) and their conformer populations; the lower row shows the corresponding 2′-fluoroarabinonucleosides (2′F-ara-U5Et,[36] **2b**,[37,38] **1c** and **2c**[31a,b]).

The introduction of a fluorine atom in the 2′-arabino configuration of the nucleoside moiety enhances the population of the *N*-conformers (dU = 30% → 2′F-ara-U5Et = 44%), (dA = 28% → 2′F-ara-A, (**2b**) = 58%). From Scheme 5, it can be concluded that this effect is more predominant in the case of nucleosides with an extra ring nitrogen atom placed next to the glycosylation site. The *N*–*S* equilibrium in the case of the 2′-deoxyribonucleoside of 6-azapyrimidine **1a** shows an *N*-conformer population of 58%, while the 2′-fluoro derivative **1c** (2′F-ara "up") shows a 100% *N*-conformer population (6-aza-dU (**1a**) = 58% → 6-aza-2′F-ara-U (**1c**) = 100%). A similar situation is observed for the azapurine nucleoside **2c** ($c^7z^8A_d$, (**2a**) = 37% *N* → 2′F-ara-$c^7z^8A_d$, (**2c**) = 78% *N*; purine numbering is used). It was suggested that these differences are connected with the stronger anomeric effect caused by the 6-azauracil base compared to that of the uracil moiety. The same effect is observed in the pyrazolo[3,4-*d*]pyrimidine base compared to that of the purine base. The 6-aza-2′-fluororibonucleoside **1d** with the fluoro substituent in the ribo configuration shows a 98% *N*-conformer population[31b] being similar to that of 2′F-ribo-U with 89% *N*-conformer (Scheme 5).



Scheme 5 Conformer population of 2′-deoxy and 2′-fluoro nucleosides.

The gauche effect of the 2′-F "up" and the 3′-OH is considered to be a contributing factor in driving the conformation towards *N*; it is not the case for the ribo analogue (2′-F "down").

The conformational properties of **1c** were also studied by X-ray analysis. In the single-crystal of the nucleoside **1c**[26] the sugar moiety adopts the *N* conformation with a torsion angle of the glycosylic bond in the anti range ($\chi = -125.37$ (13)°; ($^3T_2$) with $P = 359.2°$ and $\tau_m = 31.4$) similar to that found in solution. The gauche effect between the O4′ and the extra nitrogen in the heterocyclic ring is variable and controlled by the gycosylic bond torsional angle.

This places the O4′ and N6 in a perfect gauche position in both the *N* and the *S* conformations. However, only in the *N* conformation is a clash between the 2′-F "up" and N6 avoided, while in the *S* conformation the 2-oxo group of the base clashes with the 2′-F "up" substituent.

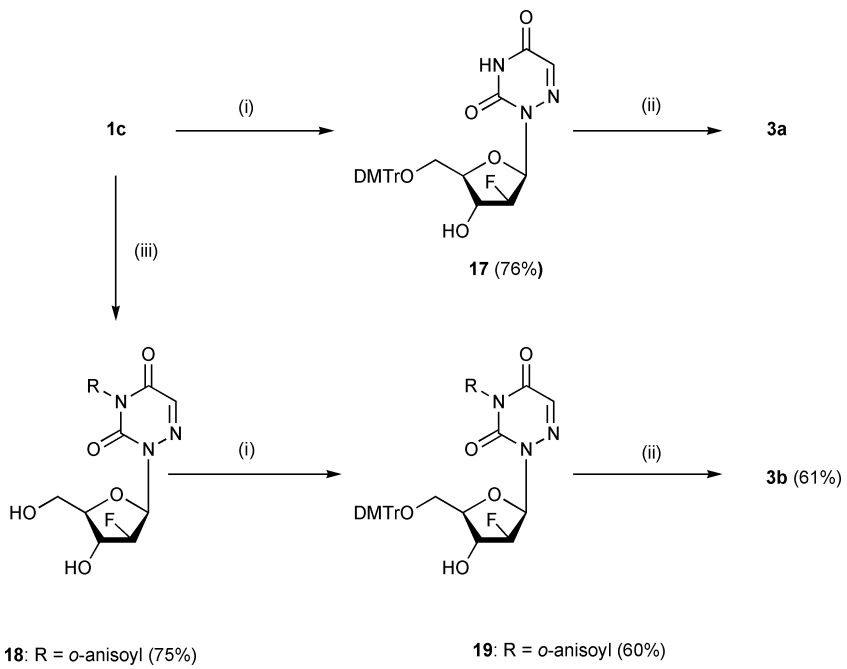
4. Nucleobase protection and phosphoramidite synthesis

In order to study the influence of the 2′-fluoro substituent as well as an additional ring nitrogen atom in the nucleobase, oligonucleotides containing **1c** and **2c** were prepared. For that the phosphoramidite building blocks **3b** and **4** were synthesized and employed in solid-phase oligonucleotide synthesis. Initial experiments using the unprotected nucleoside **1c** for phosphoramidite synthesis failed (Scheme 6). The phosphitylation of **17** with chloro-(2-cyanoethoxy)-*N*,*N*-diisopropylaminophosphine in CH_2Cl_2 at r.t. afforded the phosphoramidite **3a** (TLC-monitoring). However, its chromatographical purification was cumbersome due to its degradation into unresolved products (Scheme 6). We also observed this phenomenon for the related 6-aza-2′-deoxyuridine phosphoramidite building block.[13] As we reasoned that this behaviour results from the lactam moiety, the pK_a value of **1c** was determined by spectrophotometric titration[39] and was found to be the same as that of **1a** (p$K_a = 6.8$). These values are significantly lower when compared to that of 2′-deoxyuridine (p$K_a = 9.3$). Consequently, the protection of the lactam moiety was performed employing the *o*-anisoyl group by using the protocol of transient protection.[40] For that Et_3SiCl was employed in the presence of pyridine to protect the sugar hydroxyls. Then, the acylating reagent was added in pyridine solution. The silyl groups were removed by treatment with 5% trifluoroacetic acid in dichloromethane–methanol (7 : 3) to yield **18**. The *N*-3 anisoyl protected nucleoside **18** was further converted into the DMT derivative **19**, later it was transformed into the phosphoramidite building block **3b** (Scheme 6) which was used for the oligonucleotide synthesis.

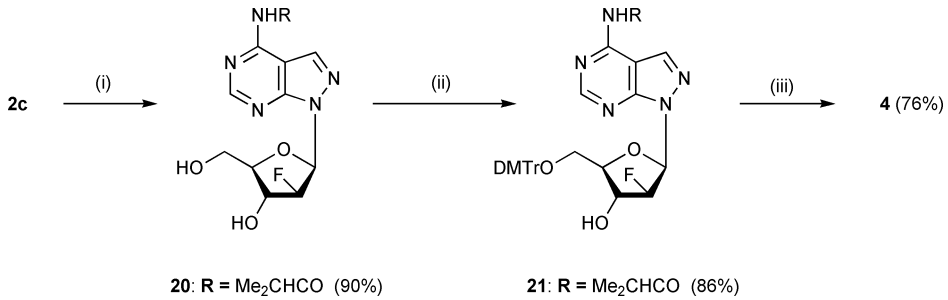
Next, the phosphoramidite of the pyrazolo[3,4-*d*]pyrimidine nucleoside **2c** was synthesized. The amino group of **2c** was protected with an isobutyryl residue using the protocol of transient protection.[41] The protected **20** was then converted into the DMT derivative **21** and further transformed into the phosphoramidite **4** under standard conditions (Scheme 7). The structures of all protected compounds were characterized by ^1H, ^{13}C and ^{19}F NMR spectroscopy as well as by elemental analysis. The ^{31}P NMR spectra of **3b** and **4** show a four-bond coupling between the P-3′ and F-2′.

5. Synthesis, base pairing and duplex stability of oligonucleotides

Oligonucleotide synthesis was carried out on solid phase with an ABI 392-08 synthesizer at a 1 μmol scale employing the synthesized phosphoramidites **3b**, **4** as well as standard building blocks. The coupling yields were always higher than 95%. The synthesis of oligonucleotides was performed by employing the DMT-on mode. After cleavage from the solid support, the oligomers were deprotected in 25% aqueous ammonia solution for 14–16 h at 60 °C. The purification of the 5′-dimethoxytritylated oligomers was carried out by reversed-phase HPLC (see experimental part).



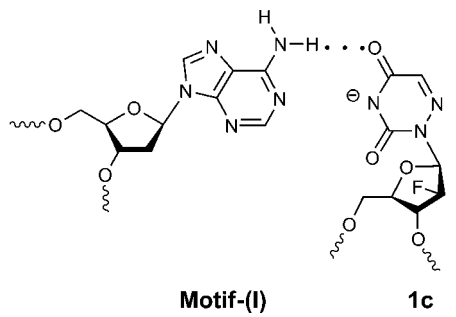
Scheme 6 *Reagents and conditions*: (i) DMTr-Cl, pyridine, r.t.; (ii) $^iPr_2NP(Cl)OCH_2CH_2CN$, $^iPr_2EtN$, CH_2Cl_2, 30 min, r.t.; (iii) Et_3SiCl, pyridine, *o*-anisoyl chloride, 5% CF_3COOH (CH_2Cl_2–MeOH 70 : 30) r.t.



Scheme 7 *Reagents and conditions*: (i) Me_3SiCl, isobutyric anhydride, pyridine, 4 h, r.t.; (ii) DMTr-Cl, pyridine, r.t.; (iii) $^{i}Pr_2NP(Cl)OCH_2CH_2CN$, $^{i}Pr_2EtN$, CH_2Cl_2, 30 min, r.t.

The molecular masses of the oligonucleotides were determined by MALDI-TOF Biflex-III mass spectrometry (Bruker Saxonia, Leipzig, Germany) with 3-hydroxypicolinic acid (3-HPA) as a matrix. The detected masses were identical with the calculated values (see experimental part, Table 5).

To evaluate the combined effect of 2′-fluoro substitution and the presence of a ring nitrogen placed next to the glycosylation site of the nucleosides **1c** and **2c** on the duplex stability, hybridization experiments were performed using the oligonucleotide duplex 5′-d(TAGGTCAATACT)-3′ **22** and 3′-d(ATCCAGTTATGA) **23** as a reference. The replacement of dT in one strand of the duplex by the 6-aza-2′-deoxy-2′-fluoroarabinouracil **1c** results in a ΔT_m of −13 °C (**22**·**25**) when paired with dA (Scheme 8), while its 2′-deoxyribonucleoside analogue **1a** shows less of a decrease with a ΔT_m of only −5 °C (**22**·**26**). This effect is substantial when all the dT residues in one strand are replaced by **1c** (**22**·**24**) (Table 3).



Scheme 8 Face to face base pair motif of dA–**1c**.

Earlier, we anticipated that the low pK_a value of **1a** (pK_a = 6.8) has an unfavourable effect on the base pair stability. This is now also suggested for the nucleoside **1c**. At neutral pH the 6-azapyrimidine nucleoside molecule predominantly exists as a negatively charged species. As this withstands base pair formation the anion is generating a mismatch. At pH values below 7.0 a proton is delivered from the solution leading partially or totally to the expected base pair thereby enhancing the T_m value of duplex melting. In order to confirm this, the duplex stability of the modified oligonucleotides containing **1c** and **1a** was determined at two different pH values (Table 3). At pH 6.0 an increase in the T_m of 5 °C for one incorporation of **1c** (**22**·**25**) and 10 °C for four incorporations of **1c** (from 20 °C to 30 °C) was observed, when compared with T_m values measured at pH 7.0. Duplexes containing 6-aza-2′-deoxyuridine **1a** instead of the fluoro analogue

Table 3 T_m Values of oligonucleotide duplexes containing regular and base-modified nucleosides at different pH values[a]

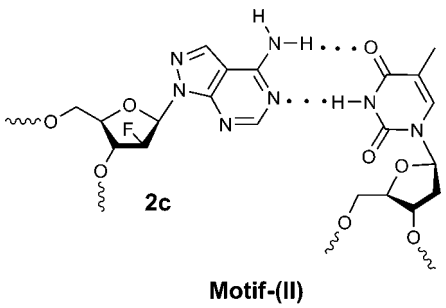
	pH 7.0		pH 6.0	
Duplex	T_m/°C	ΔT_m/°C	T_m/°C	ΔT_m/°C
5′-d(TAG GTC AAT ACT)-3′ (**22**) 3′-d(ATC CAG TTA TGA)-5′ (**23**)	50	—	51	—
5′-d(TAG GTC AAT ACT)-3′ (**22**) 3′-d(A**1c**C CAG **1c1c**A **1c**GA)-5′ (**24**)	20	−30	30	−21
5′-d(TAG GTC AAT ACT)-3′ (**22**) 3′-d(ATC CAG **1c**TA TGA)-5′ (**25**)	37	−13	42	−9
5′-d(TAG GTC AAT ACT)-3′ (**22**) 3′-d(ATC CAG **1a**TA TGA)-5′ (**26**)	45	−5	48	−3
5′-d(TAG G**1a**C AA**1a** ACT)-3′ (**27**) 3′-d(**A1a**C CAG TTA **1a**GA)-5′ (**28**)	28	−22	38	−13
5′-d(TAG GTC **2c**AT ACT)-3′ (**29**) 3′-d(ATC CAG TTA TGA)-5′ (**23**)	49	−1	49	−2

[a] Measured at 260 nm in 1M NaCl, 100 mM $MgCl_2$ and 60 mM Na-cacodylate (pH 7.0 and 6.0) with a 5 μM single-strand concentration.

1c show higher T_m values and smaller pH-dependent changes. Although the pK_a values of lactam deprotonation are the same for **1a** (6.8) and **1c** (6.8) the pH-dependent duplex stability is different. Other factors such as stacking interactions and changes in the populations of protonated and non-protonated bases within the duplex might be of importance.

The results obtained from the fluorinated 6-azapyrimidine nucleoside prompted us to study the above effects on "purine" arabinonucleoside **2c** with a 2′-fluoro substituent in the "up" position. From previous reports, it was known from oligonucleotides containing 2′F-ara-A; **2b** and 9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) guanine (2′F-ara-G) that they do not have much influence on the stability of DNA and RNA.[37] Here,

we studied the effect of 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl pyrazolo[3,4-*d*]pyrimidine (**2c**). For that purpose oligonucleotides containing **2c** were prepared by replacing dA in the standard sequences (Table 4). One incorporation of **2c** leads to a stable duplex (**29**·**23**) with a T_m of 49 °C, and the replacement of four dA residues by **2c** (**30**·**23**) does not change this value (Table 4). These results clearly demonstrate that **2c** has no significant influence on the duplex stability as almost similar results were found for the 2′-deoxyribonucleoside **2a** indicating that the presence of a 2′-fluorine atom within the 8-azapurine nucleoside **2c** does not change the base pair and duplex stability (Scheme 9). Next, the effect of such a modification on the stability of DNA–RNA hybrids was studied. The standard DNA–RNA hybrid (**22**·**34**) shows a T_m = 48 °C, whereas four modifications with **2c** result in a ΔT_m value of −3 °C (**30**·**34**) which is lower than that for the DNA–DNA duplex. Even though nucleoside **2c** has a high *N*-conformer population similar to that of **1c**, surprisingly we observed stable DNA–DNA duplexes and less stable DNA–RNA hybrids for oligonucleotides incorporating **2c**. Also a dependence of the duplex stability on pH value is not observed for **2c** (**29**·**23**; Table 3) as was found for the corresponding pyrimidine nucleosides.



Scheme 9 Face to face base pair motif of **2c**–dT.

Conclusions

Oligonucleotides containing 6-azapyrimidine- or 8-aza-7-deazapurine 2′-deoxy-2′-fluoroarabinonucleosides **1c**, **2c** were synthesized using the phosphoramidite building blocks **3b** and **4** by employing solid-phase synthesis. The nucleosides **1c**, **2c** as well as the 2′-deoxy-2′-fluoro ribofuranosyl nucleoside **1d** were either prepared by convergent stereoselective synthesis or by nucleoside transformation. A fluoro substituent in the 2′-'up" position drives the sugar conformation towards a higher *N*-population (dU = 30% → 2′F-ara-U5Et = 44%; dA = 28% → 2′F-ara-A, **2b** = 58%). A combination of a 2′-fluoroarabino substituent and a nitrogen placed next to the glycosylation site drives the *N*-conformation near to 100% (**1c** = 100% and **2c** = 78%). The destabilization found for duplexes incorporating **1c** is caused by the deprotonation of the nucleobase as well as the fluoro substitution, which is clearly evidenced from the higher T_m values of the 2′-deoxyribo counterpart **1a**. In the case of purine nucleoside **2c** we observed no negative influence of the 2′-F substituent and the 8-aza-modification on the duplex stability of oligonucleotides. Even though nucleoside **2c** has a high *N*-conformer population similar to that of **1c**, surprisingly more stable DNA–DNA duplexes were formed than DNA–RNA hybrids.

Table 4 T_m Values of DNA–DNA and DNA–RNA duplexes containing regular and base-modified nucleosides **2c**, **2a** and **1c**[a]

Duplex	T_m/°C	ΔG°_{310}/ kcal mol^{-1}	ΔT_m/°C
5′-d(TAG GTC AAT ACT)-3′ (**22**) 3′-d(ATC CAG TTA TGA)-5′ (**23**)	50	−11.8	—
5′-d(TAG GTC **2c**AT ACT)-3′ (**29**) 3′-d(ATC CAG TTA TGA)-5′ (**23**)	49	−11.0	−1
5′-d(T**2c**G GTC **2c2c**T **2c**CT)-3′ (**30**) 3′-d(ATC CAG TTA TGA)-5′ (**23**)	49	−11.0	−1
5′-d(TAG GTC A**2a**T ACT)-3′ (**31**) 3′-d(ATC CAG TTA TGA)-5′ (**23**)	51	−12.1	+1
3′-d(ATC C**2a**G TT**2a** TGA)-5′ (**32**) 5′-d(T**2a**G GTC AAT **2a**CT)-3′ (**33**)	50	−11.6	0
5′-d(TAG GTC **2c**AT ACT)-3′ (**29**) 3′-d(ATC CAG **1c**TA TGA)-5′ (**23**)	37	−8.1	−13
5′-d(TAG GTC AAT ACT)-3′ (**22**) 3′-r(AUC CAG UUA UGA)-5′ (**34**)	48	−10.8	—
5′-d(T**2c**G GTC **2c2c**T **2c**CT)-3′ (**30**) 3′-r(AUC CAG UUA UGA)-5′ (**34**)	45	−10.4	−3

[a] Measured at 260 nm in 1M NaCl, 100 mM $MgCl_2$ and 60 mM Na-cacodylate at pH 7.0 with a 5 μM single-strand concentration.

Experimental

General

All chemicals were purchased from Acros, Aldrich, Sigma, or Fluka (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). Solvents were of laboratory grade. Thin layer chromatography (TLC): aluminium sheets, silica gel 60 F_{254}, 0.2 mm layer (VWR International, Darmstadt, Germany). Column flash chromatography (FC): silica gel 60 (VWR International, Darmstadt, Germany) at 0.4 bar; sample collection with an UltroRac II fraction collector (LKB Instruments, Sweden). UV spectra: U-3200 spectrometer (Hitachi, Tokyo, Japan); λ_{max} (ε) in nm. NMR spectra: Avance-250 or AMX-500 spectrometers (Bruker, Karlsruhe, Germany), at 250.13 MHz for ^{1}H and ^{13}C; δ in ppm relative to Me_4Si as internal standard, or external standard $CFCl_3$ for ^{19}F, or external standard 85% H_3PO_4 for ^{31}P. The *J* values are in Hz. Elemental analyses were performed by Mikroanalytisches Laboratorium Beller (Göttingen, Germany). The melting curves were measured with a Cary-1/3 UV–vis spectrophotometer (Varian, Australia) equipped with a Cary thermoelectrical controller. The temperature was measured continuously in the reference cell with a Pt-100 resistor, and the thermodynamic data of duplex formation were calculated by the Meltwin 3.0 program.[42a,b]

Synthesis, purification and characterization of oligonucleotides

The oligonucleotide synthesis was performed on a DNA synthesizer, model ABI 392–08 (Applied Biosystems, Weiterstadt, Germany) at a 1μmol scale using the phosphoramidites and following the synthesis protocol for 3′-cyanoethyl phosphoramidites (users' manual for the 392 DNA sythesizer, Applied Biosystems, Weiterstadt, Germany). The coupling efficiency was always higher

Table 5 Molecular masses $[M + H]^+$ of oligonucleotides measured by MALDI-TOF mass spectrometry

Oligonucleotide	$[M + H]^+$ (calc.)	$[M + H]^+$ (found)
5′-d(TAG GTC AAT ACT)-3′ (**22**)	3645.4	3645
5′-d(AGT ATT GAC CTA)-3′ (**23**)	3645.4	3645
5′-d(AG**1c** A**1c1c** GAC C**1c**A)-3′ (**24**)	3665.3	3665
5′-d(AGT AT**1a** GAC CTA)-3′ (**26**)	3631.7	3631
5′-d(TAG G**1a**C AA**1a** ACT)-3′ (**27**)	3619.3	3619
5′-d(AG**1a** ATT GAC C**1a**A)-3′ (**28**)	3619.3	3619
5′-d(TAG GTC **2c**AT ACT)-3′ (**29**)	3661.4	3662
5′-d(T**2c**G GTC **2c2c**T **2c**CT)-3′ (**30**)	3716.4	3716

than 95%. After cleavage from the solid support the oligonucleotides were deprotected with 25% aqueous NH_3 for 14–16 h at 60 °C. The purification of the 5′-*O*-(dimethoxytrityl)oligomers was carried out on reversed-phase HPLC (Merck–Hitachi-HPLC: 250 × 4 mm RP-18 column) with the following gradient system [A: 0.1M (Et_3NH)OAc (pH 7.0)–MeCN 95 : 5 and B: MeCN]; gradient: 3 min 20% B in A, 12 min 20–50% B in A and 25 min 20% B in A with a flow rate of 1 ml min^{-1}. The purified 'trityl-on' oligonucleotides were treated with 2.5% dichloroacetic acid in CH_2Cl_2 for 5 min at 0 °C to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified again by reversed-phase HPLC (gradient: 0–20 min 0–20% B in A, flow rate 1 ml min^{-1}). The oligomers were desalted on a short column (RP-18, silica gel) and lyophilized on a Speed-Vac evaporator to yield colorless solids which were frozen at −24 °C.

The molecular masses of the oligonucleotides were determined by MALDI-TOF Biflex-III mass spectrometry (Bruker Saxonia, Leipzig, Germany) with 3-hydroxypicolinic acid (3-HPA) as a matrix. The detected masses were identical with the calculated values (Table 5).

1-Bromo-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranose (6)[16–18]

To a solution of 1,3,5-tri-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranose (1.0 g, 2.15 mmol) in CH_2Cl_2 (6.0 ml), 30% solution of HBr in acetic acid (1.2 ml) was added, and the reaction mixture was stirred at r.t. for 16 h, and evaporated to dryness. The oily residue was redissolved in CH_2Cl_2 (20 ml), then washed with water (10 ml) and then with aqueous saturated $NaHCO_3$ solution (10 ml), dried over Na_2SO_4, and concentrated to a viscous syrup which was further dried under high vacuum for 18 h at r.t. The colourless syrup **6** (0.86 g, 95%) was used in the next step without purification.

2-(2-Deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-1,2,4-triazin-3,5(2*H*,4*H*)-dione (7)

A suspension of 6-azauracil (0.35 g, 3.09 mmol), hexamethyldisilazane (HMDS) (1.2 ml) and chlorotrimethylsilane (TMSCl) (0.11 ml) was heated to reflux (170 °C) under anhydrous conditions for 3 h. The excess amount of HMDS–TMSCl was removed by distillation *in vacuo*. The residual oil solidified upon storage *in vacuo* to furnish **5** (0.77 g, 2.98 mmol). A mixture of **6** (0.93 g, 2.01 mmol) and **5** (0.61 g, 2.4 mmol) in dry chloroform (9 ml) was stirred at r.t. under argon, CuI (0.5 g, 2.6 mmol) was added to the solution and stirred for 24 h at r.t. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ (15 ml), stirring was continued for 15 min and the reaction mixture was filtered through a pad of celite. The $CHCl_3$ layer was separated, washed with saturated aqueous NaCl (50 ml), dried (Na_2SO_4), concentrated, and then applied to FC (silica gel, column 10 × 3 cm, eluted with petroleum ether–EtOAc, 2 : 1) to yield the title compound **7** as a colorless foam (0.72 g, 67%) (found: C, 58.22; H, 3.84; N, 9.21%. $C_{22}H_{18}FN_3O_7$ requires C, 58.02; H, 3.98; N, 9.23%); TLC (silica gel, petroleum ether–EtOAc, 2 : 1): R_f 0.33; λ_{max}(MeOH)/nm 263 (ε/dm^3 mol^{-1} cm^{-1} 7 000).

2-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-1,2,4-triazin-3,5(2*H*,4*H*)-dione (1c)

Compound **7** (0.21 g, 0.46 mmol) was suspended in NaOMe–MeOH (0.2 N, 20 ml), and the solution was stirred at r.t. for 2 h. The solution was neutralized (Dowex-50 H^+-form) and filtered. The resin was washed with MeOH, and the combined filtrate was concentrated and applied to FC (silica gel, column 10 × 3 cm, eluted with CH_2Cl_2–MeOH, 9 : 1) furnishing **1c** as a colorless solid (0.10 g, 88%) (found: C, 38.68; H, 4.06; N, 16.76%. $C_8H_{10}FN_3O_5$ requires C, 38.87; H, 4.08; N, 17.00%); TLC (silica gel, CH_2Cl_2–MeOH, 9 : 1): R_f 0.42; λ_{max}(MeOH)/nm 263 (ε/dm^3 mol^{-1} cm^{-1} 5 900); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −205.18 (dd, $^2J_{F,H2'}$ = 53, $^3J_{F,H3'}$ = 20 Hz).

2,2′-*O*-Anhydro(β-D-arabinofuranosyl)-1,2,4-triazin-3,5(2*H*,4*H*)-dione (9)

To a solution of compound **8** (2.02 g, 8.24 mmol) in pyridine (36.8 ml) was added 1,3-dichloro-1,1,3,3-tetraisopropyl-disiloxane (2.5 ml) and the mixture stirred at r.t. for 5 h. The solution was then evaporated and the residue dissolved in EtOAc. This was successively washed with cold aqueous $NaHCO_3$, saturated aqueous NaCl, then dried over Na_2SO_4, and evaporated to dryness. The resulting foam was dissolved in pyridine (34 ml), and to this solution methane sulfonyl chloride was added (0.8 ml) and the mixture stirred for 16 h at r.t. After that H_2O (2.6 ml) was added to the reaction mixture, the solvents were removed by evaporation, and the residue was dissolved in toluene. The organic phase was extracted twice with 5% aqueous $NaHCO_3$, dried over Na_2SO_4 and evaporated to dryness. Residual pyridine was co-evaporated with toluene. The above residue was dissolved in THF (35 ml), and to this solution 1M tetra-*n*-butylammonium fluoride in THF (2.2 ml) was added and stirred at r.t. for about 2 h. The solvent was removed by evaporation and the residue dissolved in H_2O and extracted with EtOAc. The aqueous phase was evaporated and residual water was removed by co-evaporation with ethanol. The residue was applied to FC (silica gel, column 15 × 3 cm, eluted with CH_2Cl_2–MeOH, 4 : 1) furnishing **9** as a colorless solid (1.3 g, 70%) (found: C, 42.48; H, 3.90; N, 18.39%. $C_8H_9N_3O_5$ requires C, 42.30; H, 3.99; N, 18.50%); TLC (silica gel, CH_2Cl_2–MeOH, 8 : 2): R_f 0.7; λ_{max} (MeOH)/nm 256 (ε/dm^3 mol^{-1} cm^{-1} 6 300) and 221 (7 100). δ_H (250.13 MHz; [d_6]DMSO; Me_4Si) 3.22–3.42 (2 H, m, 5′-H), 4.14 (1 H, m, 4′-H), 4.44 (1 H, m, 3′-H), 4.98 (1 H, 't', *J* 4.9, 5′-OH), 5.28 (1 H, d, *J* 5.9, 2′-H), 5.92 (1 H, 't', *J* 4.1, 3′-OH), 6.33 (1 H, d, *J* 5.9, 1′-H), 7.63 (1 H, s, 5-H).

1-(3,5-Di-*O*-tetrahydropyran-2-yl-β-D-arabinofuranosyl)-1,2,4-triazin-3,5(2*H*,4*H*)-dione (10)

To a suspension of **9** (0.25 g, 1.10 mmol) in DMF (4.4 ml) and 3,4-dihydropyran (2.64 ml) was added *p*-toluenesulfonic acid (0.2 g) at 0 °C for 4 h, after which time a clear solution was obtained. This was neutralized with Et_3N and then evaporated to dryness. The residue was redissolved in EtOAc, washed with saturated $NaHCO_3$ and the organic phase was dried over Na_2SO_4. Removal of the solvent gave a residue which was triturated with hexanes and filtered. The filter cake was washed with hexanes and dried to give the protected compound as a white solid, which was suspended in MeOH (4.5 ml) and 1 N NaOH (1.5 ml) and stirred at r.t. for 2 h, then neutralized with dilute acetic acid. The mixture was evaporated to dryness and the residue was applied to FC (silica gel, 15 × 3 cm, eluted with CH_2Cl_2–MeOH, 98 : 2) to give the title compound **10** as a colorless foam (0.32 g, 70%) (found: C, 52.72; H, 6.59; N, 10.30%. $C_{18}H_{27}N_3O_8$ requires C, 52.29; H, 6.58; N, 10.16%); TLC (silica gel, CH_2Cl_2–MeOH, 95 : 5): R_f 0.6; λ_{max}(MeOH)/nm 266 (ε/dm^3 mol^{-1} cm^{-1} 5 600); δ_H (250.13 MHz; [d_6]DMSO; Me_4Si) 1.45–1.61 (12 H, m, H of THP), 3.36–3.92 (7 H, m, 5′-H, H of THP and 4′-H), 4.19 (1 H, m, 3′-H), 4.44 (m, 1 H, 2-′H), 4.57–4.74 (2 H, m, H of THP), 5.49 (1 H, d, *J* 5.9, 2′-OH), 6.18 (1 H, d, *J* 7.0, 1′-H), 7.59 (1 H, s, 5-H), 12.21 (1 H, s, NH).

1-(2-Deoxy-2-fluoro-3,5-di-*O*-tetrahydropyran-2-yl-β-D-ribofuranosyl)-1,2,4-triazin-3,5(2*H*,4*H*)-dione (11)

To a stirred mixture of **10** (0.25 g, 0.60 mmol) in CH_2Cl_2 (3.6 ml) and pyridine (0.6 ml) was added diethylaminosulfur trifluoride (DAST) (0.30 g, 1.86 mmol) at −60 °C under N_2. The resulting mixture was slowly warmed to room temperature and then refluxed for 4 h. The reaction was quenched with saturated $NaHCO_3$ and ice-water, then extracted with CH_2Cl_2, washed with saturated $NaHCO_3$ and dried over Na_2SO_4. Removal of the solvent gave a dark-brown syrup. The residue was applied to FC (silica gel column, 15 × 3 cm, eluted with CH_2Cl_2–MeOH, 99 : 1) to give **11** as a colorless foam (0.12 g, 48%) (found: C, 52.20; H, 6.24; N, 10.06%. $C_{18}H_{26}FN_3O_7$ requires C, 52.04; H, 6.31; N, 10.12%); TLC (silica gel, CH_2Cl_2–MeOH, 9 : 1): R_f 0.77; λ_{max} (MeOH)/nm 261 (ε/dm^3 mol^{-1} cm^{-1} 5 700); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −199.16 (dt, $^2J_{F,H2'} = 52$, $^3J_{F,H3'} = 24$, $^3J_{F,H1'} = 21$).

1-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)-1,2,4-triazin-3,5(2*H*,4*H*)-dione (1d)

A solution of compound **11** (0.14 g, 0.34 mmol) in EtOH (8.5 ml) was stirred with pyridinium *p*-toluene sulfonate (0.17 g, 0.67 mmol) at 60 °C for 3 h. The mixture was then evaporated and the residue was applied to FC (silica gel column, 15 × 3 cm, eluted with CH_2Cl_2–MeOH, 9 : 1) to give **1d** as a colorless foam (72 mg, 87%) (found: C, 38.62; H, 4.24; N, 16.64%. $C_8H_{10}FN_3O_5$ requires C, 38.87; H, 4.08; N, 17.00%); TLC (silica gel, CH_2Cl_2–MeOH, 9 : 1): R_f 0.37; λ_{max}(MeOH)/nm 263 (ε/dm^3 mol^{-1} cm^{-1} 5 300); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −202.50 (dt, $^2J_{F,H2'} = 52$, $^3J_{F,H3'} = 24$, $^3J_{F,H1'} = 21$).

Glycosylation of 4-methoxy-1*H*-pyrazolo-[3,4-*d*]pyrimidine (12) with the sugar bromide 6

4-Methoxy-1*H*-pyrazolo-[3,4-*d*]pyrimidine (**12**)[43] (0.2 g, 1.3 mmol) and KOH (0.36 g, 6.4 mmol) were suspended in MeCN (20 ml) and stirred for 10 min at r.t. Then, the phase-transfer catalyst TDA-1 (0.1 ml) was added, and stirring was continued for another 10 min. A solution of bromide **6** (0.6 g, 1.42 mmol) in MeCN was added in 3 portions to the reaction mixture during 10 min, and it was stirred for another 10 min and filtered. The filtrate was evaporated to dryness, and the residue was applied to FC (silica gel, column 15 × 3 cm, eluted with petroleum ether–EtOAc). The fractions containing individual compounds were combined to give the three compounds below in the order of their elution.

1-(2-Deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (13)

Colorless solid (0.33 g, 50.3%); TLC (silica gel, petroleum ether–ethyl acetate, 2 : 1): R_f 0.63; λ_{max}(MeOH)/nm 231 (ε/dm^3 mol^{-1} cm^{-1} 31 000), which is identical to the compound previously synthesized.[8]

2-(2-Deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (15)

Colorless solid (45 mg, 6.8%); TLC (silica gel, petroleum ether–ethyl acetate, 2 : 1): R_f 0.52; λ_{max}(MeOH)/nm 222 (ε/dm^3 mol^{-1} cm^{-1} 32 800), which is identical to that of a previous report.[8]

1-(5-*O*-Benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl)-4-methoxy-1*H*-pyrazolo[3,4-*d*]pyrimidine (14)

Colorless solid (45 mg, 8.7%) (found: C, 56.01; H, 4.69; N, 14.64%. $C_{18}H_{17}FN_4O_5$ requires C, 55.67; H, 4.41; N, 14.43%); TLC (silica gel, petroleum ether–ethyl acetate, 2 : 1): R_f 0.36; λ_{max}(MeOH)/nm 230 (ε/dm^3 mol^{-1} cm^{-1} 30 800); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −205.69 (dd, $^2J_{F,H2'} = 53$, $^3J_{F,H3'} = 18$).

2-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-1,2,4-triazin-3,5(2*H*,4*H*)-dione (17)

Compound **1c** (0.37 g, 1.50 mmol) was suspended in anhydrous pyridine (5 ml), 4,4′-dimethoxytrityl chloride (0.59 g, 1.78 mmol) was added in portions and the mixture was stirred at r.t. for 3 h. The reaction was quenched by the addition of MeOH and the mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2, washed with H_2O and dried over Na_2SO_4. The organic layer was co-evaporated with toluene and the residue was applied to FC (silica gel, column 12 × 3 cm, eluted with CH_2Cl_2–MeOH, 9 : 1) furnishing **17** as a colorless foam (0.63 g, 76.5%). TLC (silica gel, CH_2Cl_2–MeOH, 9 : 1): R_f 0.74; λ_{max} (MeOH)/nm 233 (ε/dm^3 mol^{-1} cm^{-1} 28 700), 267 (8 900).

2-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-N^4-(2-methoxybenzoyl)-1,2,4-triazin-3,5(2*H*,4*H*)-dione (18)

Compound **1c** (0.25 g, 1.01 mmol) was treated with triethylsilyl chloride (0.44 ml, 2.62 mmol) in pyridine (1.6 ml) and stirred at

r.t. for 30 min and then *o*-anisoyl chloride (0.3 ml, 2.01 mmol) was added. After being stirred for 5 h, the mixture was evaporated then co-evaporated with toluene to remove pyridine completely. The oily residue was further treated with 5% CF_3COOH in CH_2Cl_2–CH_3OH (7 : 3, 10 ml), stirred at r.t. for 1 h and evaporated. To this residue 5% aqueous $NaHCO_3$ was added and the mixture extracted with CH_2Cl_2, the extract was dried over Na_2SO_4, evaporated and the residue was applied to FC (silica gel, column 15 × 3 cm, eluted with CH_2Cl_2–MeOH, 98 : 2). Evaporation of the main zone afforded a colorless foam, which was recrystallized from MeOH to give **18** as colorless crystals (0.29 g, 75%) (found: C, 50.10; H, 4.20; N, 10.85; F, 4.60%. $C_{16}H_{16}FN_3O_7$ requires C, 50.40; H, 4.23; N, 11.02; F, 4.98%); TLC (silica gel, CH_2Cl_2–MeOH, 95 : 5): R_f 0.29; λ_{max}(MeOH)/nm 259 (ε/dm^3 mol^{-1} cm^{-1} 15 200) and 324 (4 200); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −205.18 (dd, $^2J_{F,H2'} = 53$, $^3J_{F,H3'} = 20$).

2-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-*N*^{4}-(2-methoxybenzoyl)-1,2,4-triazin-3,5(2*H*,4*H*)-dione (19)

Compound **18** (0.25 g, 0.65 mmol) was suspended in anhydrous pyridine (3.1 ml), 4,4′-dimethoxytrityl chloride (0.26 g, 0.78 mmol) was added in portions and the mixture was stirred at room temperature for 3 h. The reaction was quenched by the addition of MeOH and the mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2, washed with H_2O and dried over Na_2SO_4. The organic layer was co-evaporated with toluene and the residue was applied to FC (silica gel, column 12 × 3 cm, eluted with CH_2Cl_2–acetone, 9 : 1) furnishing **19** as a colorless foam (0.27 g, 60%) (found: C, 65.32; H, 5.21; N, 5.97; F, 2.60%. $C_{37}H_{34}FN_3O_9$ requires C, 65.00; H, 5.01; N, 6.15; F, 2.78%); TLC (silica gel, CH_2Cl_2–acetone, 95 : 5): R_f 0.78; λ_{max}(MeOH)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 17 800) and 323 (3 400); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −205.53 (dd, $^2J_{F,H2'} = 54$, $^3J_{F,H3'} = 19$).

2-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-*N*^{4}-(2-methoxybenzoyl)-1,2,4-triazin-3,5(2*H*,4*H*)-dione) 3′-(2-cyanoethyl diisopropylphosphoramidite) (3b)

A stirred solution of **19** (0.24 g, 0.35 mmol) in anhydrous CH_2Cl_2 (6 ml) was pre-flushed with argon and treated with (*i*-Pr)$_2$EtN (0.11 ml, 0.63 mmol) followed by 2-cyanoethyl diisopropyl phosphoramidochloridite (0.09 ml, 0.40 mmol) at r.t.. After 30 min, the mixture was diluted with CH_2Cl_2 (20 ml), washed with 5% aqueous $NaHCO_3$ (10 ml), dried over Na_2SO_4, and evaporated to an oil. The residue was applied to FC (silica gel, column 10 × 3 cm, eluted with CH_2Cl_2–acetone–Et_3N, 95 : 5: 0.2) to give compound **3b** as a colorless foam (0.19 g, 61%). TLC (silica gel, CH_2Cl_2–acetone, 95 : 5): R_f 0.85. δ_P ($CDCl_3$) 152.59, 152.27.

2-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-4-(isobutyrylamino)-2*H*-pyrazolo[3,4-*d*]pyrimidine (20)

Compound **2c** (0.15 g, 0.56 mmol) was repeatedly evaporated with pyridine and suspended in pyridine (2.5 ml). Me_3SiCl (0.34 ml) was added and stirred for 15 min, followed by the addition of isobutyric anhydride (1.45 ml) and stirring was continued for 4 h at r.t. The reaction mixture was cooled in an ice-bath, and 0.5 ml H_2O was added. After 15 min, 0.5 ml 25% aqueous NH_3 solution was added, and the solution stirred for another 15 min. The solution was evaporated to dryness, dissolved in 10 ml H_2O, and extracted with CH_2Cl_2 (3 × 15 ml). The organic layer was dried over Na_2SO_4, evaporated and the residue was applied to FC (silica gel, column 15 × 3 cm, eluted with CH_2Cl_2–MeOH, 95 : 5). Compound **20** was obtained as a colorless foam (0.17 g, 90%) (found: C, 49.69; H, 5.51; N, 20.7%. $C_{14}H_{18}FN_5O_4$ requires C, 49.55; H, 5.35; N, 20.64%); TLC (CH_2Cl_2–MeOH 90 : 10): R_f 0.65; λ_{max}(MeOH)/nm 267 (ε/dm^3 mol^{-1} cm^{-1} 10 400) and 232 (9 400); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −205.14 (dd, $^2J_{F,H2'} = 54$, $^3J_{F,H3'} = 19$).

2-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-4-(isobutyrylamino)-2*H*-pyrazolo[3,4-*d*]pyrimidine (21)

Compound **20** (0.3 g, 0.88 mmol) was suspended in anhydrous pyridine (3.5 ml), 4,4′-dimethoxytrityl chloride (0.35 g, 1.03 mmol) was added in portions and the mixture was stirred at r.t. for 3 h. The reaction was quenched by the addition of MeOH and the mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2, washed with H_2O and dried over Na_2SO_4. The organic layer was co-evaporated with toluene and the residue was applied to FC (silica gel, column 12 × 3 cm, eluted with CH_2Cl_2–MeOH, 90 : 10) furnishing **21** as a colorless foam (0.49 g, 86%) (found: C, 65.45; H, 5.80; N, 10.80%. $C_{35}H_{36}FN_5O_6$ requires C, 65.51; H, 5.65; N, 10.91%). TLC (CH_2Cl_2–acetone 9 : 1): R_f: 0.83; λ_{max} (MeOH)/nm 267 (ε/dm^3 mol^{-1} cm^{-1} 12 400), 233 (30 200); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −205.77 (dd, $^2J_{F,H2'} = 54$, $^3J_{F,H3'} = 19$).

2-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-fluoro-β-D-arabinofuranosyl]-4-(isobutyrylamino)-2*H*-pyrazolo[3,4-*d*]-pyrimidine 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (4)

A stirred solution of **21** (0.31 g, 0.48 mmol) in anhydrous CH_2Cl_2 (8 ml) was pre-flushed with argon and treated with (*i*-Pr)$_2$EtN (0.14 ml, 0.80 mmol) followed by 2-cyanoethyl diisopropyl phosphoramidochloridite (0.12 ml, 0.53 mmol) at r.t. After 30 min, the mixture was diluted with CH_2Cl_2 (20 ml), and quenched by adding a 5% aqueous $NaHCO_3$ solution (20 ml). Then, the aqueous layer was extracted with CH_2Cl_2 (3 × 15 ml), the combined organic layers dried over Na_2SO_4 and evaporated to an oil. The residue was applied to FC (silica gel, column 10 × 3 cm, eluted with CH_2Cl_2–acetone–Et_3N, 95 : 5: 0.2) to give compound **4** as a colorless foam (0.31 g, 76%). TLC (silica gel, CH_2Cl_2–acetone, 95 : 5): R_f 0.87. δ_P ($CDCl_3$) 152.55, 152.27.

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