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Halogenated 7-deazapurine nucleosides: stereoselective synthesis and conformation of 2′-deoxy-2′-fluoro-β-D-arabinonucleosides

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The stereoselective syntheses of 5-halogenated 7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]-pyrimidine nucleosides **3b**–**d**, **4a**–**c** as well as 7-deaza-2′-deoxyisoguanosine **2** are described. Nucleobase anion glycosylation of 2-amino-4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (**5**) with 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide (**6**) exclusively gave the β-D-anomer **7**, which was deblocked (→ **8**), aminated at C(4) (→ **3a**) and selectively deaminated at C(2) to yield 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl 7-deazaisoguanine (**2**). Condensation of the 5-halogenated 4-chloro-2-pivaloylamino-7*H*-pyrrolo[2,3-*d*]pyrimidines **9a**–**c** with **6** furnished the N^7-nucleosides **10a**–**c** together with N^2,N^7-bisglycosylated compounds **11a**–**c**. The former was converted to the corresponding 2,4-diamino-compounds **3b**–**d**, and the latter was deblocked by NaOMe/MeOH to yield the 4-methoxy-nucleosides **4a**–**c**. Conformational analysis of the sugar moiety of the nucleosides **2** and **3a**–**d** was performed on the basis of vicinal [^{1}H,^{1}H] coupling constants. The fluorine atom in the sugar moiety shifts the sugar conformation from *S* towards *N* by about 10%, while the halogen substituents in the base moiety increase the hydrophobicity and polarizability of the nucleobases.

Introduction

Halogenated nucleosides are widely used in biochemistry, medicinal chemistry, and pharmacology.[1–3] Much effort has been devoted to the synthesis of various halogenated nucleoside and nucleotide analogues,[3,4] which are widely employed as experimental antitumor and antiviral agents.[5–11] They are also useful for probing the structure of protein–RNA, protein–DNA and DNA–RNA complexes in crosslinking experiments.[12] Beyond this, halogenated 7-deazapurine (pyrrolo[2,3-*d*]pyrimidine) nucleosides have gained extensive attention since some of them, such as 7-iodotubercidin **1a**,[13] 2′-deoxy-2′-fluoroarabino-tubercidin **1b**[14] and 2-amino-2′-deoxy-2′-fluoroarabino-tubercidin **3a**,[15] exhibit a broad spectrum of biological activity (purine numbering is used throughout the general section). Furthermore, 7-halogenated 7-deazapurine nucleosides can stabilize the DNA duplex structure[16–20] and are useful for antisense purposes.[20]

Recently, it has been shown that sugar modifications, in particular the addition of a fluorine atom 'up' in the 2′-position can enhance the biological activity, while increasing chemical stability.[9,10,21] Apart from the effects of the fluorine substituent on the biological activity, the introduction of a fluorine atom strongly influences the *N*/*S*-conformational equilibrium of the pentofuranose moiety. The electronegative character of the fluorine substituent shifts the conformation of the sugar moiety from *S* to *N*. This phenomenon is observed for substituents introduced near the anomeric centre, *e.g.* in the 7-position of the nucleobase as well as the 2′-position of the sugar moiety. This was confirmed by a recent observation made on 7-fluoro-2′-deoxytubercidin **1c** synthesized in our laboratory as well as on other 2′-deoxy-2′-fluoroarabinonucleosides.[22,23a] Steric as well as stereoelectronic effects are responsible for this behaviour.

Previously, several 7-deazapurine nucleosides containing fluoro-sugar moieties have been synthesized by glycosylation of 6-chloro-7-deazapurines with 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-β-D-arabinofuranosyl bromide (**6**) using sodium hydride for the generation of the nucleobase anion.[14,15] However, 7-halogenated 7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-7-deazapurine nucleosides are still unknown. In continuation of our studies performed on 7-deazapurine nucleosides[16–19,24–28] and on fluoronucleosides,[22,23] we report herein the synthesis and conformational properties of the halogenated 7-deazapurine nucleosides **2**, **3a**–**d**, **4a**–**c** with a F substituent in the 2′-*arabino*-orientation. They are the key intermediates for later studies on oligonucleotides.

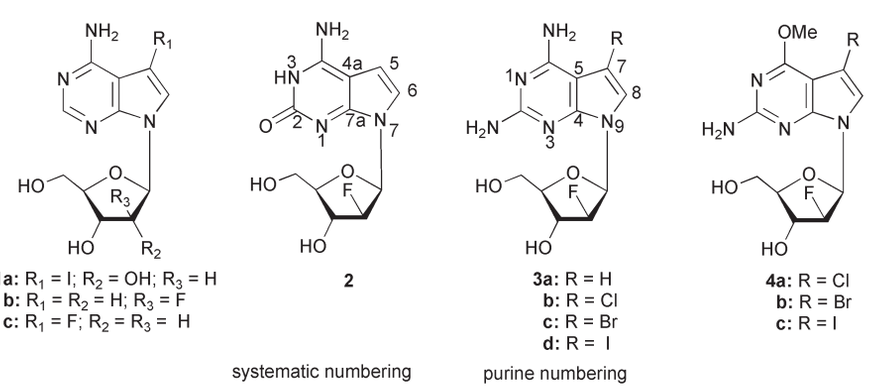

Fig. 1 Structures of nucleosides **1**–**4**.

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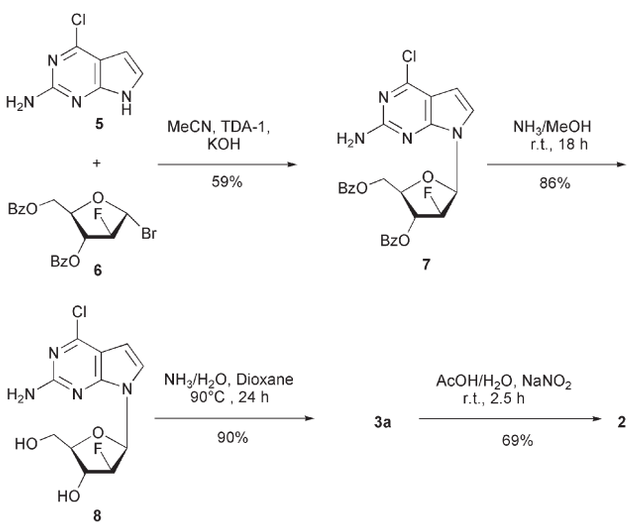
Results and discussion

The synthesis of 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl 7-deazaisoguanine (**2**) was accomplished by nucleobase-anion glycosylation[25,29] of 2-amino-6-chloro-7-deazapurine (**5**)[26b] with 3,5-di-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranosyl bromide (**6**)[30] (Scheme 1). The reaction of **5** with bromide **6** in MeCN in the presence of powdered KOH and TDA-1 {tris[2-(2-methoxyethoxy)ethyl]amine} exclusively gave the β-D-nucleoside **7** (59%). The protected nucleoside **7** was deblocked with methanolic ammonia (saturated at 0 °C) at room temperature affording compound **8**, which on amination with 25% aqueous ammonia at 90 °C gave the 2-amino-7-deazaadenosine **3a**. Selective deamination of compound **3a** with sodium nitrite in AcOH/H_2O (v/v, 1 : 5) furnished compound **2** in 69% yield.



Scheme 1 The synthesis of nucleoside **2** by nucleobase-anion glycosylation.

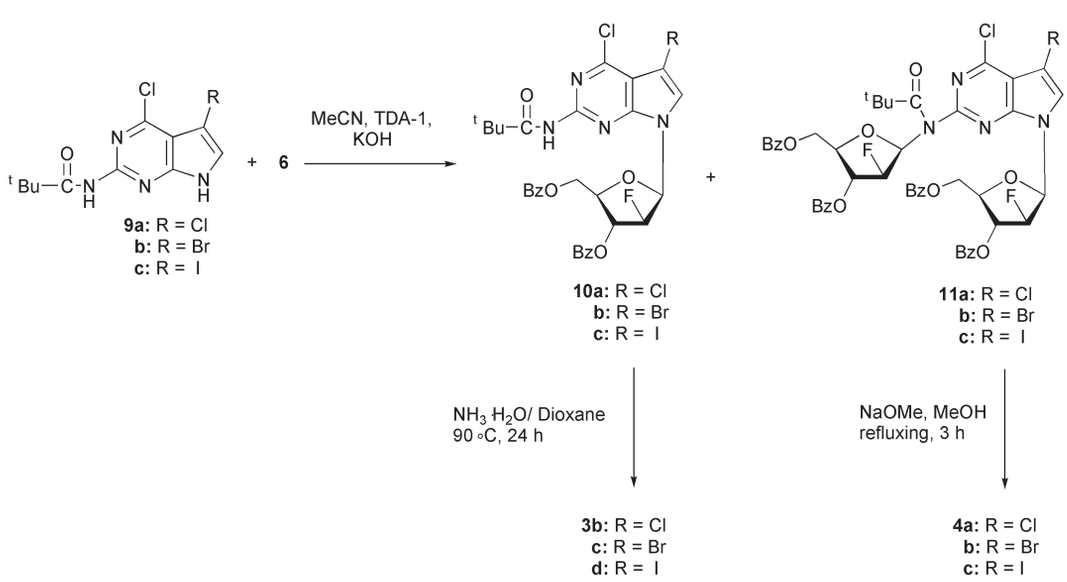
The synthesis of the 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl 7-halogenated 7-deazapurine nucleosides **3b**–**d**, **4a**–**c** was accomplished according to Scheme 2. In order to introduce 7-halogen atoms, the 7-halogenated bases **9a**–**c**[28] were employed for the glycosylation reaction because of their good solubility. The condensation of **9a**–**c** with the halogenose **6** was performed in MeCN in the presence of powdered KOH and TDA-1. This reaction resulted in the formation of the desired β-D-nucleosides **10a**–**c** (44–45%) along with the N^2,N^9-bisglycosylated compounds **11a**–**c** (11–12%), which was not found in the closely related glycosylation of the same bases **9a**–**c** with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride.[28] It was also not observed in the glycosylation reaction of the 2-amino-unprotected base **5** with the fluorosugar **6**. The most likely explanation for the formation of **11a**–**c** is that on one hand, the pivaloyl group increases the acidity of N-H(2), which is easily deprotonated by KOH, affording a nitrogen anion, on the other hand the presence of the F-atom in the 2 position and the Br-atom in the 1 position of compound **6** make it more reactive than deoxyribose. Compounds **11a**–**c** were assigned as N^2,N^9-bisglycosylated compounds on the basis of ^{1}H- and ^{13}C-NMR as well as elemental analysis studies. Both ^{1}H- and ^{13}C-NMR spectra of **11a**–**c** show two sets of the sugar signals, while only one set of the base moiety appears. In addition, the proton signal of the 2-amino group in the base moiety has disappeared from the ^{1}H-NMR spectra. The sugar moiety attached to the 2-amino group is very labile and was lost when being kept in MeOH at room temperature for several days.

The removal of the benzoyl protecting groups and displacement of the 6-chloro group of **10a**–**c** were performed by treatment with 25% aqueous ammonia in a steel bomb at 90 °C without affecting the 7-halogen substituents, furnishing compounds **3b**–**d**. The displacement of the 6-chloro group as well as the deprotection of the benzoyl and pivaloyl groups of N^2,N^9-bisglycosylated compounds **11a**–**c** was accomplished by refluxing in 0.5 N NaOMe/MeOH, in the meantime the sugar moiety attached to the 2-amino group was removed, which afforded the corresponding nucleosides **4a**–**c**. Without separation, the mixtures of compounds **10a**–**c** and **11a**–**c** can directly be converted to the corresponding **3b**–**d** or **4a**–**c** by the procedures mentioned above.

All new compounds were characterized by ^{1}H- and ^{13}C-NMR spectroscopy (Tables 1 and 2) as well as by elemental analysis. Compounds **2**, **3a**–**d** and **4a**–**c** are assigned as having β-D-configuration from the ^{1}H- and ^{13}C-NMR spectra, referring to reference 23*a*. The assignments of the carbon resonances of the bases are made according to reference 28.

Conformation of sugar moiety in solution

A conformational analysis of the sugar moiety in solution was performed with the aid of the PSEUROT (version 6.3) program employing updated values for the substituent electronegativity constants[31a,b] (Due to the lack of accurate electronegativity data of the modified bases, the default electronegativity values for the normal purine bases were used). In the PSEUROT program a minimization of the differences between the experimental and calculated couplings is accomplished by a nonlinear Newton–Raphson minimization. This procedure presupposes the existence of a two-state *N*/*S* equilibrium (Fig. 2).[31c] The



Scheme 2 The synthesis of 7-halogenated nucleosides.

Table 1 ^{1}H NMR data of nucleosides **2**–**8** in DMSO-d_6

	Chemical shifts, δ_{TMS} (ppm)						Coupling constants/Hz						
	Base	Sugar					^{3}J(H,H)			^{3}J(H,F)		^{5}J(H,F)	
	H-6	H-1′	H-2′	H-3′	H-4′	H-5′	1′,2′	2′,3′	3′,4′	1′,F	3′,F	6,F	Others
2	6.84	6.26 dd	5.00 dt	4.27 dm	3.73–3.75 m	3.51–3.59 m	4.14	3.30	5.06	17.00	19.51	3.31	5.10 (1 H, t, *J* 5.5, 5′-OH); 5.91 (1 H, d, *J* 4.4, 3′-OH); 6.42 (1 H, d, *J* 3.6, 5-H); 7.62 (2 H, br s, NH_2); 10.86 (1 H, br s NH).
3a	6.82 d	6.34 dd	4.96 dt	4.29 dq	3.73–3.75 m	3.56–3.60 m	4.20	3.31	5.20	17.22	19.30	3.52	5.07 (1 H, t, *J* 5.4, 5′-OH); 5.63, 6.57 (4 H, 2 s, 2 × NH_2); 5.85 (1 H, d, *J* 4.1, 3′-OH); 6.38 (1 H, d, *J* 3.6, 5-H)
3b	6.96 d	6.36 dd	5.00 dt	4.30 dq	3.71–3.77 m	3.54–3.62 m	3.96	3.42	5.01	17.05	19.04	2.25	5.04 (1 H, t, *J* 5.6, 5′-OH); 5.86 (1 H, d, *J* 4.7, 3′-OH); 5.92, 6.37 (4 H, 2s, 2 × NH_2)
3c	7.03 d	6.35 dd	5.02 dt	4.32 dt	3.76 q	3.56–3.60 m	3.90	3.40	5.02	17.12	18.98	2.42	5.04–5.06 (1 H, m, 5′-OH); 5.86 (1 H, d, *J* 4.7, 3′-OH); 5.94, 6.33 (4 H, 2s, 2 × NH_2)
3d	7.05 d	6.32 dd	4.98 dt	4.30 dq	3.71–3.76 m	3.56–3.60 m	4.21	3.26	5.20	16.72	19.08	2.36	5.04 (1 H, t, *J* 5.4, 5′-OH); 5.86 (1 H, d, *J* 4.7, 3′-OH); 5.90, 6.21 (4 H, 2s, 2 × NH_2)
4a	7.11 d	6.39 dd	5.06 dt	4.31 dq	3.73–3.77 m	3.55–3.62 m	4.21	3.82	4.64	16.02	18.30	1.82	3.94 (3 H, s, OMe); 5.06 (1 H, t, *J* 5.6, 5′-OH); 5.89 (1 H, d, *J* 4.6, 3′-OH); 6.52 (2 H, s, NH_2)
4b	7.15 d	6.38 dd	5.06 dt	4.32 dq	3.75 q	3.57–3.63 m	4.23	3.80	4.04	16.15	18.26	2.00	3.94 (3 H, s, OMe); 5.07 (1 H, t, *J* 5.6, 5′-OH); 5.90 (1 H, d, *J* 4.6, 3′-OH); 6.52 (2 H, s, NH_2)
4c	7.16 d	6.36 dd	5.03 dt	4.32 dq	3.75–3.79 m	3.54–3.60 m	4.16	3.68	4.50	16.90	18.12	1.93	3.94 (3 H, s, OMe); 5.05 (1 H, t, *J* 5.3, 5′-OH); 5.88–5.90 (1 H, m, 3′-OH); 6.49 (2 H, s, NH_2)
7	7.20 d	6.57 dd	5.69 dd	5.76 dd	4.64–4.77 m	4.64–4.77 m	3.22	1.65	4.43	20.90	19.04		6.37 (1 H, d, *J* 3.7, 5-H); 6.84 (2 H, br s, NH_2); 7.50–8.10 (10 H, m, arom)
8	7.28 d	6.41 dd	5.10 dt	4.35 dq	3.76–3.80 m	3.59–3.63 m	4.21	3.70	4.50	15.66	18.45	2.42	5.07 (1 H, t, *J* 5.5, 5′-OH); 5.92 (1 H, d, *J* 4.6, 3′-OH); 6.37 (1 H, d, *J* 3.6, 5-H); 6.78 (2 H, s, NH_2)

Table 2 ^{13}C-NMR chemical shifts (δ) of 7-deazapurine-2′-deoxy-2′-fluroarabinonucleosides[a]

	Chemical shifts, δ_{TMS} (ppm)											
	Base						Sugar					Others
Compd [b,c]	C(2)[d]C(2)	C(4) [d]C(6)	C(4a)C(5)	C(5)C(7)	C(6)C(8) ($^4J_{F,C}$)	C(7a) [d]C(4)	C-1′ ($^2J_{F,C}$)	C-2′ ($^1J_{F,C}$)	C-3′ ($^2J_{F,C}$)	C-4′ ($^3J_{F,C}$)	C-5′	OMe
2	154.0	156.1	91.5	100.7	120.2	152.6	81.0 (16.5)	95.9 (191.0)	73.2 (23.7)	83.0 (4.2)	60.6	
3a	159.3	157.1	94.8	99.4	118.0	152.1	80.0 (16.6)	95.0 (190.6)	72.3 (23.7)	82.2	60.1	
3b	160.4	157.1	92.8	103.3	115.8 (4.3)	151.9	80.4 (16.6)	95.5 (191.0)	72.6 (23.3)	83.0 (4.5)	60.4	
3c	160.2	157.3	94.0	87.6	118.4 (3.9)	152.3	80.5 (16.5)	95.6 (189.9)	72.7 (23.4)	83.0 (4.1)	60.5	
3d	159.9	157.5	95.9	52.5	123.8	152.7	80.6 (16.3)	95.5 (190.7)	72.8 (23.5)	83.0	60.5	
4a	159.9	162.7	94.6	103.2	117.7 (3.9)	153.1	80.6 (16.7)	95.5 (191.3)	72.5 (23.3)	83.0 (4.6)	60.3	53.2
4b	159.8	162.8	95.9	87.2	120.1	153.5	80.7 (16.7)	95.5 (191.2)	72.5 (23.4)	83.0 (4.7)	60.3	53.2
4c	159.5	162.8	98.3	51.7	125.5	154.1	80.9 (16.5)	95.6 (191.3)	72.7 (23.5)	83.1 (4.3)	60.4	53.1
7	159.5	153.9	108.3	100.4	122.9	151.4	81.6 (16.5)	93.1 (190.6)	76.4 (29.4)	78.0	63.6	
8	159.4	153.7	108.4	99.6	124.4	151.2	80.9 (16.9)	95.5 (191.3)	72.7 (23.2)	83.1 (4.4)	60.4	
10a	152.6	151.0	109.4	103.8	125.7	150.5	81.6 (16.2)	93.3 (192.8)	76.3 (27.9)	77.6	63.8	
11a	158.6	151.1	109.4	103.8	125.2	150.6	81.9 (16.4), 94.4 (16.6)	92.3 (197.1), 93.3 (191.5)	75.5 (24.8), 76.2 (28.4)	77.9, 78.3	63.7, 64.6	
11b	158.4	151.5	109.8	88.1	127.6	151.0	81.9 (16.8), 94.5 (16.4)	92.2 (196.3), 93.6 (191.8)	75.5 (24.9), 76.1 (28.9)	77.8, 78.2	63.6, 64.5	
11c	158.0	151.9	111.8	54.4	132.8	151.5	81.8 (16.9), 94.5 (16.5)	92.2 (199.3); 93.2 (191.6)	75.5 (25.5), 76.3 (28.7)	77.8, 78.3	63.6, 64.5	

[a] Measured in (d_6)-DMSO. [b] Systematic numbering. [c] Purine numbering. [d] Tentative.

Table 3 Pseudorotational parameters of compounds **2** and **3a**–**d**[a]

	Pseudorotational parameters						
Compd	%*N*	%*S*	P_N/deg	Ψ_N [b]/deg	P_S/deg	Ψ_S [b]/deg	rms/Hz
2	35	65	−1.9	41.0	129.0	41.0	0.116
3a	34	66	10.4	41.0	130.2	41.0	0.082
3b	36	64	−3.6	41.0	130.8	41.0	0.012
3c	37	63	−2.1	41.0	131.0	41.0	0.016
3d	36	64	5.1	41.0	129.8	41.0	0.154

[a] Measured in D_2O. [b] Kept fixed during the final minimization.

program calculates the best fits of three $^3J_{H,H}$ and two $^3J_{H,F}$ experimental coupling constants to the five conformational parameters: the phase angles (P_S and P_N) and puckering amplitudes (Ψ_S and Ψ_N) of the *S*- and *N*-conformers, and the population of the *S*-type conformer (X_S; $X_S + X_N = 1$).

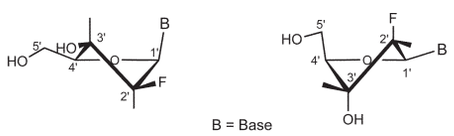


Fig. 2 *N* and *S* conformations of sugar rings of fluoroarabinonucleosides.

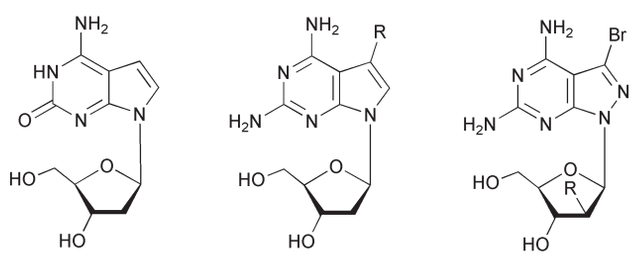
The input contained the following coupling constants: ^3J(H1′,H2′), J(H2′,H3′), J(H3′,H4′), J(H1′,F), J(H3′,F). In our cases, the PSEUROT calculation was started with a 'free trial' run without fixing any parameters, which shows that the *N* conformer is the minor one. In following runs, the puckering amplitude of the minor form *N* was fixed and the fixed value was varied stepwise (27, 30, 33,...45 degrees) in order to meet two targets: a) the lowest rms; b) the puckering amplitude of the two forms should not differ too much. It was found that fixing the puckering amplitude at 41 degrees resulted in the lowest rms value. Therefore, the value of 41 degrees was chosen for the puckering amplitude of both conformers (Ψ_S and Ψ_N) and fixed in the final PSEUROT calculation. For detailed procedures of the PSEUROT calculation refer to references 31*a*, 32*a*. The calculated pseudorotational parameters are shown in Table 3.

From the data given in Table 4 it can be seen that the presence of the fluorine atom in the sugar moiety drives the $N \rightleftarrows S$ equilibrium of 7-deaza-2′-deoxy-2′-fluoroisoguanosine **2** towards the *N*-conformation (35% *N*) in comparison with the corresponding 2′-deoxyribonucleoside **12** (27% *N*).[28] The same observation can be also made in the case of 7-deazapurin-2,6-diamine 2′-deoxy-2′-fluoroarabinonucleosides **3a**–**d** (34–37% *N*) and their corresponding 2′-deoxyribonucleosides **13a**–**d** (26–29% *N*).[28] This means that a fluorine atom 'up' in the 2-position enhances the population of the *N* conformers by 8% in the cases of both the 7-deazaisoguanine nucleoside and the 2-amino-7-deazaadenine nucleosides (similar results for adenosine analogs were reported by Marquez and co-workers[32*b*]). This enhancement is more pronounced in the case of 8-aza-7-deazapurine nucleosides. The *N*-conformer population of 3-bromo-1-(2-deoxy-2-fluoro-β-arabinofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4,6-diamine (systematic numbering) (**14**)[22] increases to about 100% which is 63% higher than that of the corresponding 2′-deoxyribonucleoside **15** (37% *N*).[33] This is due to the replacement of the 2′-ara-proton by an electronegative fluorine atom creates the repulsive Coulomb interactions between the N8 and the F2′driving compound **14** to adopt an *N* conformation, while the corresponding effect does not exist in the 2′-deoxynucleoside **15** and 7-deazapurine 2′-arabinofluoronucleosides.

Similar to the 2′-deoxyribonucleosides,[28] the 7-halogen substituents have a minor influence on the $N \rightleftarrows S$ equilibrium of the 7-halogenated nucleosides **3b**–**d**. The non-halogenated nucleoside **3a** shows a population of 34% *N*, while the conformations

Table 4 Sugar conformations of the fluoro-nucleosides **2**, **3a**–**d**, **14**[22] and the 2′-deoxyribonucleosides **12**,[28] **13a**–**d**,[28] **15**[33] (Scheme 3)

	Conformation		Conformation
2	35% *N*	**12**	27% *N*
3a	34% *N*	**13a**	26% *N*
3b	36% *N*	**13b**	28% *N*
3c	37% *N*	**13c**	29% *N*
3d	36% *N*	**13d**	28% *N*
14	100% *N*	**15**	37% *N*



Scheme 3 The structures of nucleosides **12**–**15**.

of the nucleosides **3b**–**d** with 7-halogen substituents are shifted only a little towards the *N*-population (36–37% *N*).[34,35]

Normally, the *N*/*S* pseudorotational equilibrium in the nucleosides is controlled by steric interactions and stereoelectronic factors known as the anomeric effect (AE) and *gauche effect* (GE).[31*c*,32*a*] Due to the AE the heterocyclic base at C1′ is driven to adopt the pseudoaxial position (*gauche* C4′–O4′–C1′–B). Preference for a pseudoaxial position at C1′ always results in a drive towards the *N* form in the β-D-series nucleosides. Therefore, the difference of the $N \rightleftarrows S$ equilibrium between 2′-deoxy-2′-fluoroarabinonucleosides and 2′-deoxyribonucleosides is primarily induced by GE and steric effects.

The term GE means that a vicinal fragment such as X–C–C–Y prefers to adopt a *gauche* conformation along the central C–C bond in cases where X and Y represent highly electronegative ligands (or electron pairs). For 2′-deoxy-2′-fluoroarabinonucleosides, let us look along the C(1′)–C(2′) and C(2′)–C(3′) bonds of the sugar ring, concentrating on the effect involving the C(2′)–F bond (Fig. 2). In the *N*-form the strongly polar C(2′)–F bond is *gauche* oriented with respect to C(3′)–O(3′) and C(1′)–N, simultaneously, it is *trans* to C(1′)–O(4′) and to C(3′)–C(4′). Therefore, compared to 2′-deoxyribonucleosides, fluoronucleosides show a larger *N* conformer population because of the preferred *gauche* orientation of the strongly polar C(2′)–F bond to C(3′)–O(3′) and C(1′)–N. This is also found in the case of 8-aza-7-deazapurine[22] and 5-aza-7-deazaguanine nucleosides.[23*a*]

pK_a Values, hydrophobicity and polarizability of fluoronucleosides

Although the introduction of halogen substituents on the base moiety can not change the $N \rightleftarrows S$ equilibrium of nucleosides

so evidently, they greatly influence other properties of nucleosides, such as pK_a values, hydrophobicity and polarizability. This might lead to significant changes of the biological properties of oligonucleotides containing these nucleosides.[36] The halogen substituents alter the mobility of the nucleosides on a reversed-phase HPLC column with the iodinated nucleoside **3d** as the slowest migrating compound (Fig. 3), which shows that the hydrophobic properties of the 7-halogenated nucleosides are enhanced. The introduction of 7-halogen substituents also increases the polarizabilities ($\alpha_m/10^{-24}$ cm^3) of the nucleobases (15.77 for **3a**, 17.70 for **3b**, 18.40 for **3c** and 20.80 for **3d** calculated by Hyperchem 7.0). These data suggest that the stacking interactions of **3b–d** can be strengthened compared to that of non-halogenated nucleoside **3a**, therefore, it is reasonable that the incorporation of compounds **3b–d** into oligonucleotides will stabilize the DNA duplex.[36]

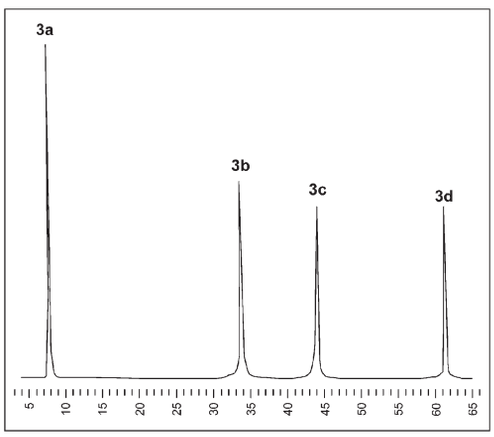


Fig. 3 HPLC profiles of the nucleosides **3a–d** (the nucleoside mixtures were analyzed by reversed-phase HPLC at 260 nm on a RP-18 column (200 × 10 cm). Gradient: 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN 90 : 10, flow rate 1.0 mL min^{-1}).

Furthermore, the pK_a values of the halogenated compounds **3b–d** were measured by spectrophotometric titration[37] (pH 1.5–12.5) at 220–350 nm and compared to that of **3a**. Compounds **3b–d** show very similar pK_a values (4.72 for **3b**, 4.77 for **3c** and 4.82 for **3d**), which are lower than that of **3a** (5.67) (Fig. 4). Obviously, the electron-withdrawing halogen substituents reduce the basicity of the 7-deazaadenine moieties; in the meantime, the 6-amino group can become a better proton donor. With regard to these properties, **3b–d** might form more stable base pairs with thymine than compound **3a**. Also, 7-deazaisoguanosine **2** was found to give two pK_a values (4.47 and 10.38) (Fig. 4(d)); the lower one corresponds to protonation, the higher one to deprotonation of the base.

Finally, the fluoronucleosides **2**, **3a–d** and **4b** were evaluated *in vitro* for their cytotoxity and activity against five human viruses, namely human immunodeficiency virus-1 (HIV-1), bovine viral diarrhea virus (BVDV), yellow fever virus (YFV), dengue virus 2 (DENV-2) and west Nile virus (WNV).[38] Compound **3a** shows activity against HIV-1 and nucleoside **3d** is active against all viruses, but develops also cytotoxicity.

Conclusions

It was reported that nucleobase anion glycosylation[25,29] of 2-amino-6-chloro-7-deazapurine (**5**) or its 7-halogenated derivatives **9a–c** with the fluoro-sugar bromide **6** proceeds in a stereoselective way with almost exclusive formation of the β-D-anomers leading to the 2′-deoxy-2′-fluoroarabinonucleosides **2**, **3a–d** and **4a–c**. From the conformational analyses of the sugar moieties of these nucleosides, it is obvious that compared to the 2′-deoxynucleosides, a 2′-fluoro substituent in *arabino* configuration shifts the $N \rightleftarrows S$ equilibrium of the 7-deazapurine 2′-deoxy-2′-fluoronucleosides towards *N*. The 7-halogenated nucleosides **3b–d** show higher polarizabilities and hydrophobicities and lower pK_a values compared to the non-halogenated compound **3a**. These properties would contribute to the stabilization of oligoribonucleotide duplexes. Currently,

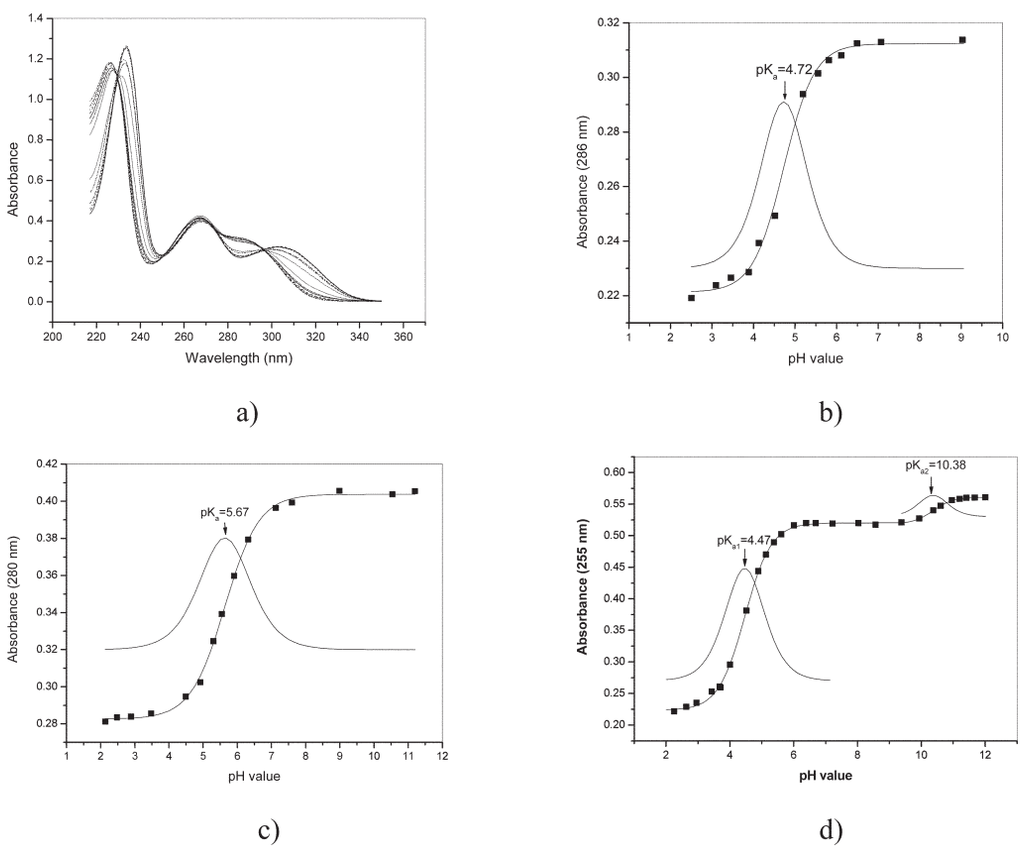


Fig. 4 a) UV-spectra of compound **3b** in phosphate buffer solution (7.8 g $NaH_2PO_4 \cdot H_2O$ in 500 mL H_2O) from pH 1.5 to 12.5. b) Absorbance of compound **3b** as a function of pH values measured at 286 nm. c) Absorbance of compound **3a** as a function of pH values measured at 280 nm. d) Absorbance of compound **2** as a function of pH values measured at 255 nm.

biochemical and biophysical experiments of the incorporation of nucleosides **2** and **3a**–**d** into nucleic acids are in progress.

Experimental

General

All chemicals were purchased from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). 1,3,5-Tri-*O*-benzoyl-2-deoxy-2-fluoro-α-D-arabinofuranose was a commercial product. 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. Solvent systems of FC and TLC: CH_2Cl_2 (A), CH_2Cl_2/MeOH 98 : 2 (B), CH_2Cl_2/MeOH 9 : 1 (C), CH_2Cl_2/MeOH 4 : 1 (D); Sample collection with a Multi-Rac fractions collector (LKB Instruments Sweden). UV-Spectra were recorded on a U-3200 spectrophotometer (Hitachi, Japan), λ_{max} in nm, ε in dm^3 mol^{-1} cm^{-1}. NMR spectra were measured on an Avance DPX 250 or an AMX-500 spectrometer (Bruker, Rheinstetten, Germany); chemical shifts (δ) are in ppm downfield from internal TMS (^{1}H, ^{13}C). The *J*-values are given in Hz. Melting points were determined with a Linström apparatus and are not corrected. Elemental analyses were performed by the Mikroanalytisches Laboratorium Beller, Göttingen, Germany.

1-Bromo-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-arabinofuranose 6[30]

Into a solution of 1,3,5-tri-*O*-benzoyl-2-deoxy-2-fluro-α-D-arabinofuranose (1.0 g, 2.15 mmol) in CH_2Cl_2 (6.0 mL), a 30% solution of HBr in acetic acid (1.2 mL) was added, and the mixture was stirred at room temperature for 16 h and evaporated to dryness. The syrup was redissolved in CH_2Cl_2 (20.0 mL), then washed with an aqueous saturated $NaHCO_3$ solution (10.0 mL) and water (10.0 mL), dried over anhydrous Na_2SO_4, and concentrated to a viscous syrup which was further dried under high vacuum for 24 h. The colorless syrup **6** (839 mg, 1.98 mmol, 92%) was used in the next step without purification.

2-Amino-4-chloro-7-(2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 7

To a stirred suspension of powdered KOH (528 mg, 85%, 8.0 mmol) in MeCN (35 mL), TDA-1 (0.1 mL, 0.3 mmol) was added at room temperature. After 10 min of stirring, compound **5** (334 mg, 1.98 mmol) was added, and the stirring was continued for another 10 min. Then, the solution of **6** (839 mg, 1.98 mmol) in MeCN (5.0 mL) was added in two portions. The reaction was continued for 30 min, after which it was filtered, condensed, and loaded onto a silica gel column. Flash chromatography (FC) with CH_2Cl_2–MeOH (99 : 1) gave compound **7** as a colorless foam (596 mg, 59%) (Found: C, 58.99; H, 4.01; N, 11.01%. $C_{25}H_{20}ClFN_4O_5$ requires C, 58.77; H, 3.95; N, 10.97%); TLC (silical ge, CH_2Cl_2–MeOH, 98 : 2) R_f 0.20; λ_{max} (MeOH)/nm 232 (ε/dm^3 mol^{-1} cm^{-1} 55 900), 316 (7 200); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −199.31 (dt, $^2J_{F,\,H2'}$ = 51.9, $^3J_{F,\,H3'}$ = 19.0, $^3J_{F,\,H1'}$ = 20.9 Hz).

4-Chloro-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 8

A suspension of **7** (556 mg, 1.09 mmol) in ammonia-saturated MeOH (20.0 mL) was stirred in a sealed vessel for 18 h at room temperature. Concentration and purification by FC with CH_2Cl_2–MeOH (95 : 5) as eluent furnished compound **8** as a colorless foam, which was crystallized from MeOH, yielding colorless crystals (284 mg, 86%) (Found: C, 43.39; H, 4.02; N, 18.33%. $C_{11}H_{12}ClFN_4O_3$ requires C, 43.65; H, 4.00; N 18.51%); mp 164 °C (from MeOH); TLC (silica gel, CH_2Cl_2–MeOH, 95 : 5) R_f 0.35; λ_{max} (MeOH)/nm 234 (ε/dm^3 mol^{-1} cm^{-1} 45 800), 262 (7 400) and 318 (8 900).

7-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2,4-diamine 3a

A suspension of **8** (260 mg, 0.86 mmol) in a mixture of 25% aqueous ammonia (40 mL) and dioxane (20 mL) was stirred in a sealed vessel for 24 h at 90 °C. The clear solution was evaporated under reduced pressure to leave a yellow oil, which was purified by column chromatography on silica gel using CH_2Cl_2–MeOH (95 : 5) as eluent to give **3a** as a white solid (220 mg, 90%). Crystallization from MeOH yielded colorless crystals (Found: C, 46.45; H, 5.01; N, 24.53%. $C_{11}H_{14}FN_5O_3$ requires C, 46.64; H, 4.98; N 24.72%); mp 188 °C (from MeOH); TLC (silica gel, CH_2Cl_2–MeOH, 9 : 1) R_f 0.21; λ_{max} (MeOH)/nm 220 (ε/dm^3 mol^{-1} cm^{-1} 31 900), 263 (12 100) and 284sh (9 300); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −198.69 (dt, $^2J_{F,\,H2'}$ = 52.3, $^3J_{F,\,H3'}$ = 19.3, $^3J_{F,\,H1'}$ = 17.2 Hz).

4-Amino-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-one 2

To a solution of **3a** (200 mg, 0.71 mmol) in 15% AcOH–H_2O (v/v 1 : 5; 5.0 mL), a solution of $NaNO_2$ (150 mg) in H_2O (3 mL) was added dropwise at room temperature under stirring. The stirring was continued for 2.5 h, and the pH of the dark solution was adjusted to 5.5 with 25% aqueous ammonia. The mixture was purified by column chromatography on Serdolit AD04 (resin 0.1–0.2; Serva, Germany) using H_2O–^{i}PrOH (100 : 0 → 95 : 5) as eluent. Compound **2** was directly crystallized from the solvent as yellowish crystals (138 mg, 69%) (Found: C, 46.70; H, 4.59; N, 19.42%. $C_{11}H_{13}FN_4O_4$ requires C, 46.48; H, 4.61; N 19.71%); mp 197 °C (from H_2O); TLC [silica gel, NH_3 (25%)–^{i}PrOH–H_2O, 1 : 7 : 2] R_f 0.90; λ_{max} (MeOH)/nm 225 (ε/dm^3 mol^{-1} cm^{-1} 26 300), 256 (7 900) and 304 (6 900); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −198.67 (dt, $^2J_{F,\,H2'}$ = 52.7, $^3J_{F,\,H3'}$ = 19.5, $^3J_{F,\,H1'}$ = 17.0 Hz).

7-(2-Deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-4,5-dichloro-2-pivaloylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine 10a and 4,5-dichloro-2,7-di-(2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-2-pivaloylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine 11a

To a stirred suspension of powdered KOH (528 mg, 85%, 8.0 mmol) in MeCN (40 mL), TDA-1 (0.1 mL, 0.3 mmol) was added at room temperature. After 10 min of stirring, 4,5-dichloro-2-pivaloylamino-7*H*–pyrrolo[2,3-*d*]pyrimidine[28] (**9a**: 568 mg, 1.98 mmol) was added, and the stirring was continued for another 10 min. Then, the solution of **6** (839 mg, 1.98 mmol) in MeCN (5.0 mL) was added in two portions. The reaction was continued for 30 min, after which it was filtered, condensed, and loaded onto a silica gel column. Flash chromatography (FC) with CH_2Cl_2–MeOH (99 : 1) gave two compounds in the following order.

10a. Slower migrating zone (562 mg, 45%) (Found: C, 57.30; H, 4.35; N, 9.00%. $C_{30}H_{27}Cl_2FN_4O_6$ requires C, 57.24; H, 4.32; N, 8.90%); TLC (silica gel, CH_2Cl_2–MeOH, 98 : 2) R_f 0.31; λ_{max} (MeOH)/nm 232 (ε/dm^3 mol^{-1} cm^{-1} 34 900), 252 (35 900) and 341 (2 100); δ_H (250.13 MHz; [d_6]DMSO; Me_4Si) 1.23 (9 H, s, 3 × Me), 4.51–4.77 (3 H, m, 4′-H, 5′-H), 5.80 (1 H, dm, $J_{2',F}$ 52.0, 2′-H), 5.98 (1 H, dm, $J_{3',F}$ 19.9, 3′-H), 6.78 (1 H, dd, $J_{1',F}$ 16.5, $J_{1',2'}$ 4.2, 1′-H), 7.82 (1 H, d, $J_{6,F}$ 2.4, 6-H), 7.47–8.25 (10 H, m, 2 × Ph), 10.40 (1 H, s, NH).

11a. Faster migrating zone (206 mg, 11%) (Found: C, 60.92; H, 4.36; N, 5.85%. $C_{49}H_{42}Cl_2F_2N_4O_{11}$ requires C, 60.56; H, 4.36; N, 5.77%); TLC (silica gel, CH_2Cl_2–MeOH, 98 : 2) R_f 0.61; λ_{max} (MeOH)/nm 233 (ε/dm^3 mol^{-1} cm^{-1} 64 200), 310 (4 900) and 342 (2 100); δ_H (250.13 MHz; [d_6]DMSO; Me_4Si) 1.05 (9 H, s, 3 × Me), 4.56–4.76 (6 H, m, 4′-H, 5′-H), 5.60–5.86 (4 H, m, 2′-H, 3′-H), 6.60–6.62 (1 H, m, 1′-H), 6.76 (1 H, dd, $J_{1',F}$ 16.6, $J_{1',2'}$ 4.1, 1′-H), 7.70 (1 H, s, 6-H), 7.45–8.09 (20 H, m, 4 × Ph).

5-Bromo-4-chloro-7-(2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-2-pivaloylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine 10b and 5-bromo-4-chloro-2,7-di-(2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-2-pivaloylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine 11b

As described for **10a** and **11a**, with **9b** (656 mg, 1.98 mmol), KOH (528 mg, 85%, 8.0 mmol), TDA-1 (0.1 mL, 0.3 mmol), MeCN (40 mL) and **6** (839 mg, 1.98 mmol).

10b. Slower migrating zone (600 mg, 45%) TLC (silical gel, CH_2Cl_2–MeOH, 98 : 2) R_f 0.31. Directly used for the next step without further analysis.

11b. Faster migrating zone (203 mg, 10%) (Found: C, 57.58; H, 4.25; N, 5.36%. $C_{49}H_{42}BrClF_2N_4O_{11}$ requires C, 57.91; H, 4.17; N, 5.51%); TLC (silica gel, CH_2Cl_2–MeOH, 98 : 2) R_f 0.61; λ_{max} (MeOH)/nm 233 (ε/dm^3 mol^{-1} cm^{-1} 64 300), 310 (5 000) and 343 (2 100); δ_H (250 MHz; [d_6]DMSO; Me_4Si) 1.06 (9 H, s, 3 × Me); 4.56–4.74 (6 H, m, 4′-H, 5′-H); 5.60–5.82 (4 H, m, 2′-H, 3′-H); 6.60–6.62 (1 H, m, 1′-H); 6.78 (1 H, dd, $J_{1',F}$ 16.5, $J_{1',2'}$ 4.2, 1′-H); 7.71 (1 H, s, 6-H); 7.48–8.07 (20 H, m, 4 × Ph).

4-Chloro-7-(2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-5-iodo-2-pivaloylamino-7*H*-pyrrolo[2,3-*d*]pyrimidine 10c and 4-chloro-2,7-di-(2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)-5-iodo-2-pivaloylamino-7*H*-pyrrolo[2,3-*d*]-pyrimidine 11c

As described for **10a** and **11a**, with **9c** (750 mg, 1.98 mmol), KOH (528 mg, 85%, 8.0 mmol), TDA-1 (0.1 mL, 0.3 mmol), MeCN (40 mL) and **6** (839 mg, 1.98 mmol).

10c. Slower migrating zone (628 mg, 44%) (Found: C, 50.11; H, 3.83; N, 7.71%. $C_{30}H_{27}ClFIN_4O_6$ requires C, 49.98; H, 3.77; N, 7.77%); TLC (silica gel, CH_2Cl_2–MeOH, 98 : 2) R_f 0.31; λ_{max} (MeOH)/nm 232 (ε/dm^3 mol^{-1} cm^{-1} 34 800), 252 (35 900) and 341 (2 000); δ_H (500 MHz; [d_6]DMSO; Me_4Si) 1.24 (s, 9 H, 3 × CH_3); 4.66–4.81 (3 H, m, 4′-H, 5′- H); 5.78 (1 H, dm, $J_{2',F}$ 51.2, 2′-H); 5.99 (1 H, dm, $J_{3',F}$ 19.6, 3′-H); 6.76 (1 H, dd, $J_{1',F}$ 16.5, $J_{1',2'}$ 4.3, H-1′); 7.83 (1 H, d, $J_{6,F}$ 2.2, 6-H); 7.52–8.10 (10 H, m, 2 × Ph); 10.34 (1 H, s, NH).

11c. Faster migrating zone (245 mg, 12%) (Found: C, 55.10; H, 3.92; N, 5.34%. $C_{49}H_{42}ClF_2IN_4O_{11}$ requires C, 55.35; H, 3.98; N, 5.27%); TLC (silical gel, CH_2Cl_2–MeOH, 98 : 2) R_f 0.61; λ_{max} (MeOH)/nm 232 (ε/dm^3 mol^{-1} cm^{-1} 64 500), 309 (5 000) and 341 (2 200); δ_H (250 MHz; [d_6]DMSO; Me_4Si) 1.05 (9 H, s, 3 × Me); 4.50–4.76 (6 H, 3 m, 4′-H, 5′-H); 5.61–5.89 (4 H, m, 2′-H, 3′-H); 6.60–6.62 (1 H, m, 1′-H); 6.75 (1 H, dd, $J_{1',F}$ 16.6, $J_{1',2'}$ 4.1, 1′-H); 7.73 (1 H, d, $J_{6,F}$ 2.1, 6-H); 7.45–8.07 (20 H, m, 4 × Ph).

5-Chloro-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2,4-diamine 3b

As described for **3a**, with **10a** (400 mg, 0.64 mmol), dioxane (20 mL) and 25% aqueous ammonia (40 mL). FC with CH_2Cl_2–MeOH (95 : 5) furnished compound **3b** as white solid (176 mg, 87%), which was crystallized from MeOH, yielding colorless crystals (Found: C, 41.53; H, 4.07; N, 21.92%. $C_{11}H_{13}ClFN_5O_3$ requires C, 41.59; H, 4.12; N, 22.04%); mp 198 °C (from MeOH); TLC (silica gel, CH_2Cl_2–MeOH, 9 : 1) R_f 0.25; λ_{max} (MeOH)/nm 226 (ε/dm^3 mol^{-1} cm^{-1} 26 100), 267 (10 100) and 287 (7 100); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −198.70 (dt, $^2J_{F,H2'}$ = 52.4, $^3J_{F,H3'}$ = 18.9, $^3J_{F,H1'}$ = 16.9 Hz).

5-Bromo-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2,4-diamine 3c

As described for **3a**, with **10b** (400 mg, 0.59 mmol), dioxane (20 mL) and 25% aqueous ammonia (40 mL). Compound **3c** was obtained after FC with CH_2Cl_2–MeOH (95 : 5) followed by crystallization from MeOH, yielding colorless crystals (194 mg, 90%) (Found: C 36.69, H 3.61, N 19.25%. $C_{11}H_{13}BrFN_5O_3$ requires C 36.48, H 3.62, N 19.34%); mp 197 °C (from MeOH); TLC (silica gel, CH_2Cl_2–MeOH, 9 : 1) R_f 0.25; λ_{max} (MeOH)/nm 227 (ε/dm^3 mol^{-1} cm^{-1} 27 800), 267 (9 600) and 287 (7 200); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −198.89 (dt, $^2J_{F,H2'}$ = 52.4, $^3J_{F,H3'}$ = 19.0, $^3J_{F,H1'}$ = 17.1 Hz).

7-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-2,4-diamine 3d

As described for **3a**, with **10c** (400 mg, 0.55 mmol), dioxane (20 mL) and 25% aqueous ammonia (40 mL). Compound **3d** (196 mg, 86%), colorless crystals (Found: C, 32.26; H, 3.24; N, 17.30%. $C_{11}H_{13}FIN_5O_3$ requires C, 32.29; H, 3.20; N, 17.12%); mp 198 °C (from MeOH); TLC (silica gel, CH_2Cl_2–MeOH, 9 : 1) R_f 0.25; λ_{max} (MeOH)/nm 230 (ε/dm^3 mol^{-1} cm^{-1} 26 800), 269 (8 700), 286sh (7 000); δ_F (250 MHz; [d_6]DMSO; Me_4Si) −198.91 (dt, $^2J_{F,H2'}$ = 52.4, $^3J_{F,H3'}$ = 19.0, $^3J_{F,H1'}$ = 16.7 Hz).

2-Amino-5-chloro-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine 4a

A solution of compound **11a** (180 mg, 0.19 mmol) in 0.5 M MeONa/MeOH (20 mL) was heated under reflux for 3 h. The solution was neutralized with glacial acetic acid, concentrated and adsorbed on silica gel for FC with CH_2Cl_2–MeOH (95 : 5) to give compound **4a** as a colorless solid (55 mg, 89%) (Found: C, 43.20; H, 4.22; N, 16.80%. $C_{12}H_{14}ClFN_4O_4$ requires C, 43.32; H, 4.24; N, 16.84%); mp 160 °C (from MeOH); TLC (silica gel, CH_2Cl_2–MeOH, 9 : 1) R_f 0.38; λ_{max} (MeOH)/nm 228 (ε/dm^3 mol^{-1} cm^{-1} 27 100), 263 (9 700) and 287 (7 200).

2-Amino-5-bromo-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine 4b

As described for **4a**, with **11b** (180 mg, 0.18 mmol) and 0.5 M MeONa/MeOH (20 mL). FC gave **4b** as colorless solid (60 mg, 90%) (Found: C, 38.42; H, 3.75; N, 15.05%. $C_{12}H_{14}BrFN_4O_4$ requires C, 38.21; H, 3.74; N, 14.85%); mp 159 °C (from MeOH); TLC (silica gel, CH_2Cl_2–MeOH, 9 : 1) R_f 0.38; λ_{max} (MeOH)/nm 229 (ε/dm^3 mol^{-1} cm^{-1} 27 000), 264 (8 900) and 287 (7 000).

2-Amino-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodo-4-methoxy-7*H*-pyrrolo[2,3-*d*]pyrimidine 4c

As described for **4a**, with **11c** (220 mg, 0.21 mmol) and 0.5 M MeONa/MeOH (20 mL). FC gave **4c** as colorless solid (72 mg, 82%) (Found: C, 33.80; H, 3.30; N, 13.11%. $C_{12}H_{14}FIN_4O_4$ requires C, 33.98; H, 3.33; N, 13.21%); mp 157 °C (from MeOH); TLC (silica gel, CH_2Cl_2–MeOH, 9 : 1); R_f 0.38; λ_{max} (MeOH)/nm 227 (ε/dm^3 mol^{-1} cm^{-1} 26 100), 262 (8 800) and 287 (7 000).

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