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Kinetics and mechanism of the defluorination of 8-fluoropurine nucleosides in basic and acidic media

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Abstract

For investigating the stability of C(8)–fluorine bond in 8-fluoropurine nucleosides some protected 8-fluoroguanosine, 8-fluoroinosine and 8-fluoroadenosine derivatives were prepared by direct fluorination of acetyl-protected purine nucleosides with elemental fluorine in solvents such as chloroform, acetonitrile and nitromethane. Fluorination reactions conducted in chloroform medium gave better yields of 8-fluoropurines. The fluorination yields were slightly lower when acetonitrile or nitromethane was used as solvent, but the product purification was found to be much easier. When the synthesized, protected fluoronucleosides were subjected to standard basic (NH_3 in methanol or 2-propanol) and acidic (HCl in methanol) deprotection conditions relevant to nucleoside chemistry, an efficient defluorination reaction took place. The kinetics of these defluorination reactions were conveniently followed, under pseudo-first-order reaction conditions, using ^{19}F NMR spectroscopy. ^{1}H NMR, LC–MS and mass spectroscopy identified the products of the kinetic reaction mixtures. The defluorination reaction rate constants (k_{obs}) in basic media depended upon the electron density at C(8) while the k_{obs} data in acidic medium were determined by the pK_a of N^7. An addition–elimination based mechanism (S_NAr) has been proposed for the defluorination reactions of these 8-fluoropurine nucleosides.

Keywords: Fluorination of purine nucleosides; Defluorination of 8-fluoropurine nucleoside; Stability of 8-fluoropurine nucleoside; Addition–elimination mechanism and 8-fluoropurine nucleosides

1. Introduction

Purine nucleosides with C(8) substituents have been known to exhibit significant biological activities [1]. During the past 25 years, reports have appeared on the pharmacological properties of 8-chloro- and 8-bromopurine nucleosides [2]. By contrast, little data are available on the behavior, in biological systems, of 8-fluoropurine nucleosides, mainly because of difficulties associated with their synthesis [3]. A simple procedure for the synthesis of 8-fluoropurine nucleosides with elemental fluorine has previously been developed in our laboratory [4]. Using this direct fluorination technique with F_2 purine nucleosides, both protected as well as unprotected, are selectively fluorinated at C(8) position. We have extended this convenient synthesis to the preparation of several ^{18}F-labeled 8-fluoroguanosine nucleosides [5] and have utilized them successfully in non-invasive *in vivo* determination of HSV1-tk mediated gene expression in mice using positron emission tomography (PET) [6].

Significant defluorination was observed when the protected 8-fluoropurine nucleosides were subjected to the standard deprotection conditions in basic media [1]. For example, deacetylation of N^2-acetyl-2′,3′,5′-tri-*O*-acetyl-8-fluoroguanosine with methanolic ammonia yielded 8-fluoroguanosine (50%) along with a defluorinated derivative, namely 8-methoxyguanosine (50%) [4b]. An analogous deprotection of 2′,3′,5′-tri-*O*-acetyl-8-fluoroadenosine suffered a complete defluorination [7,8]. Further, this 8-fluoroadenosine analog is reported to be unstable in acidic media [8]. In fact, deprotection of tri-*O*-acetyl-8-fluoroadenosine without concomitant defluorination could be achieved only via an enzymatic procedure [7]. In view of the potential importance of 8-fluoropurine nucleosides in the field of medicinal chemistry, it would be useful to understand the limitations of the general deprotection conditions employed for their preparation. In this investigation, we have synthesized certain protected 8-fluoro analogs of guanosine, inosine and adenosine, treated them under basic

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and acidic deprotection conditions relevant to nucleoside chemistry, and have evaluated the rates of defluorination and the factors influencing the stability of the C(8)–fluorine bond, the results of which are reported herein.

2. Results and discussion

2.1. Chemistry

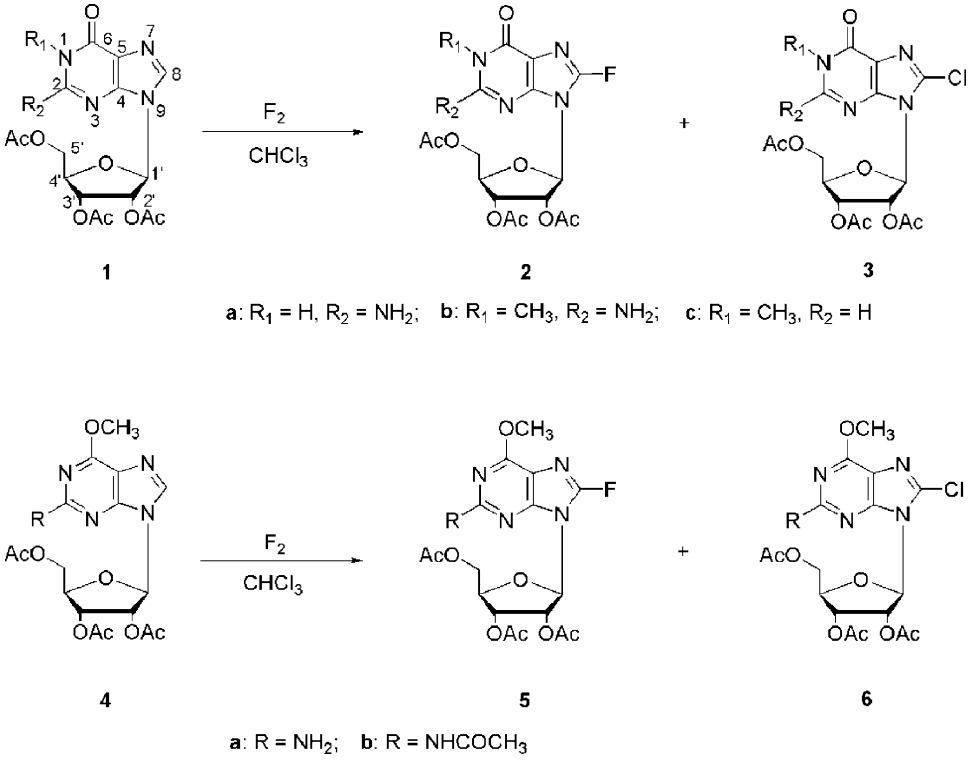
The C(8)–fluoropurine nucleosides used in this study **2a–c** and **5a**,**b** were synthesized (Scheme 1) by following reported procedures with some modifications [4]. The acetylated guanosine or inosine derivatives **1a–c** and **4a**,**b** [9–12] were reacted at room temperature with elemental fluorine (1% in helium). Previously, it was recognized that organic solvents such as chloroform, acetonitrile or nitromethane, which act also as radical scavengers, would facilitate electrophilic fluorination by reducing non-selective radical side reactions [13]. In our hands, the fluorination reactions using chloroform as the solvent led to the formation of 8-chloropurine nucleosides as side products in amounts of 16–30% of the total halogenated products, as a result probably of purinyl radical abstraction of chlorine from the solvent [14]. The C(8)-chlorinated products **3c**, **6a** and **6b** were separated from their corresponding 8-fluoro counterparts **2c**, **5a** and **5b** by semi-preparative HPLC. Fluorination of **1b** in $CHCl_3$ gave an inseparable mixture of **2b** and **3b** even by several different semi-preparative HPLC conditions. However, treatment of the product mixture with HCl in methanol selectively converted the 8-fluoro product **2b** into the corresponding 8-methoxy analog **9b** leaving the 8-chloro derivative **3b** intact. This new product mixture was then conveniently separated by preparative HPLC. Similarly, the 8-chloro derivative **3a** was also isolated from fluorination of **1a** in chloroform.

As expected, the formation of the 8-chloropurine side products in the fluorination reactions could be avoided by using solvents such as acetonitrile or nitromethane. For example, when the purine derivative **1b** in acetonitrile was reacted with F_2, the product **2b** was obtained free from any contamination of the chlorinated analog **3b**. In general, conducting the fluorination reactions in acetonitrile or nitromethane yielded the 8-fluoro products in lower yields, in comparison with those obtained when chloroform was used as the reaction medium, but the overall purification process was found to be easier. The fluorination of triacetylguanosine analog **1a** could not be conducted in acetonitrile because of its poor solubility.

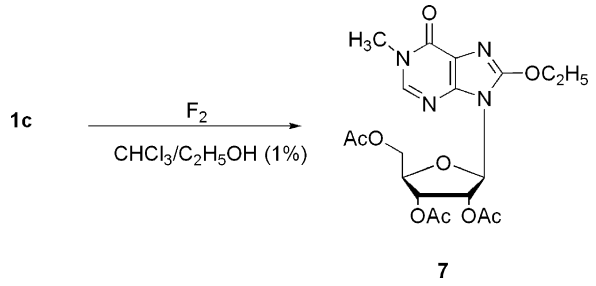
Interestingly, when chloroform stabilized with 1% EtOH (Aldrich) was used as the solvent in the fluorination reaction of 2′,3′,5′-tri-*O*-acetyl-N^1-methylinosine (**1c**), 2′,3′,5′-tri-*O*-acetyl-8-ethoxy-N^1-methylinosine (**7**) was produced as the major product (Scheme 2), as determined by UV, ^{1}H NMR and mass spectroscopy. The fact that the isolated 8-fluoro product **2c** remained stable in EtOH for several hours suggests that the 8-ethoxy product **7** was formed directly from the starting material **1c** during fluorination reaction. Using chloroform stabilized with amylene (Aldrich) as the solvent for fluorination avoided the formation of this side product.

2.2. Defluorination studies

The stability of the C(8)–fluorine bond in nucleosides **2a–c**, **5a**,**b** and **12** [4] was assessed by monitoring the reaction rates of



Scheme 1. Preparation of 8-fluoronucleosides.



Scheme 2. Fluorination of nucleoside in chloroform/ethanol solution.

their defluorination at 28 ± 0.1 °C in basic (NH_3 in methanol or 2-propanol) and acidic (HCl in methanol) media using ^{19}F NMR spectroscopy. The ^{19}F NMR signal of C(8)–fluorine in these fluoronucleosides (δ values between −99 and −110 ppm, upfield from $CFCl_3$ standard) was conveniently used to follow the progress of the reaction. The decrease in the concentration of the fluoronucleosides with time was determined using the conventional external standard (4-fluorotoluene in DMSO-d_6, $\delta_F = -117.0$ ppm) technique. It has been reported that the reaction of 8-chloro-9-methylpurine with piperidine or sodium ethoxide follows a second-order kinetics (first-order with respect to each reactant) [15]. The kinetics of base catalyzed defluorination reaction of 8-fluoropurines is likely to be analogous to the kinetics of dechlorination of 8-chloro-9-methylpurine with bases. The reaction would obey a second-order kinetics provided the concentrations of the reagents are maintained at appropriate stoichiometric levels [16]. To mimic the general conditions utilized for the deprotection of acetyl groups in nucleoside chemistry [1], the defluorination kinetic reactions were conducted with >100 molar excess of ammonia in methanol or 2-propanol. Under such conditions, the defluorination reaction would follow a pseudo-first-order kinetics. The 8-fluoropurines utilized in this investigation were all found to be stable even in boiling methanol and 2-propanol. A concentration of 0.5 M NH_3 in methanol was chosen for the kinetic studies because at this concentration a convenient measurement of the reaction rates by ^{19}F NMR could be followed. The pseudo-first-order reaction rate constants (k_{obs}) and the corresponding half-life ($t_{1/2}$) values for the defluorination of 8-fluoropurines in methanolic ammonia are provided in Table 1.

It was deemed probable that a partial or complete deacetylation of the protected nucleosides **2a–c**, **5a**,**b** and **12** would also concurrently occur during the defluorination reactions under basic conditions. This was found to be the case and supported by liquid chromatography–mass spectrometry (LC–MS) analysis of selected dedicated kinetic reaction mixtures at various time points of the reaction. The data summarized in Table 2 clearly show that partial deacetylation is a relatively fast process in methanolic ammonia. Early samples showed the presence of di- and monoacetylated products but no attempt was made to distinguish which of the acetyl groups got cleaved first. The ^{19}F NMR data from the kinetic reactions also supported the LC–MS results and accordingly exhibited closely spaced C(8)–fluorine signals corresponding to various acetylated forms of a given fluoronucleoside. The k_{obs} data given in Table 1, therefore, constitute an overall defluorination rate as assessed by the integration of all the C(8)–fluorine signals of different acetylated forms in the reaction mixture. An overall linear dependence of defluorination rates with time ($R^2 > 0.92$) for all the fluoropurines was observed. This finding strongly suggests that the relative rates of defluorination of all possible acetylated forms of a given nucleoside are of the same order of magnitude.

The defluorination reaction mixture, after three to four half-lives of kinetic data collection, was allowed to undergo complete deacetylation by letting it sit for at least 24 h or by treatment with 2 M NH_3 in methanol. The fully deacetylated mixture was then subjected to the semi-preparative HPLC separation and the products (Scheme 3) were isolated and identified by UV, ^1H NMR and HRMS. The reaction of 8-fluoropurines with 0.5 M NH_3 in methanol gave the corresponding 8-methoxy and 8-aminopurines as the major and minor products, respectively. The ratios of these two products formed in the kinetic reaction mixtures were also determined after complete deacetylation by either ^1H NMR or analytical HPLC and the data are given in Table 1.

Using the fluoronucleosides **2** as a typical example, a probable mechanism for the formation of 8-methoxy- and 8-aminopurines is shown in Scheme 4. The base catalyzed defluorination of the fluoropurines is presumed to follow a two-step addition–elimination (S_NAr) mechanism similar to the general mechanism of activated aromatic nucleophilic substitution reactions involving good leaving groups [17]. It is well known that C(8) in purine nucleosides is a reactive site [18] and

Table 1
Kinetic data for defluorination of 8-fluoropurines in 0.5 M NH_3/CH_3OH, along with ^{19}F and ^{13}C NMR chemical shifts of C(8)–F

Compound	k_{obs} (min^{-1})	$t_{1/2}$	Product ratio (**9/8**, **11/10** and **14/13**)	δ_F[a,b] (ppm)	δ_F[a,c] (ppm)	$\delta_{C(8)}$[d] (ppm)
2a	$(3.26 \pm 0.1) \times 10^{-4}$	35.4 h	100/0	−107.7	−110.4	148.8
2b	$(8.62 \pm 0.38) \times 10^{-4}$	13.4 h	100/0	−107.5	−108.2	147.8
2c	$(2.00 \pm 0.1) \times 10^{-2}$	34.7 min	76/24	−103.9	−104.2	149.8
5a	$(4.34 \pm 0.46) \times 10^{-3}$	2.8 h	86/14	−106.7	−107.1	149.6
5b	$(7.53 \pm 0.28) \times 10^{-2}$	9.2 min	65/35	−102.9	−103.3	150.9
12	$(4.06 \pm 0.17) \times 10^{-2}$	17 min	77/23	−103.7	−103.3	150.8

[a] External reference: 4-fluorotoluene/DMSO-d_6 (−117.0 ppm).
[b] In CH_3OH.
[c] In 0.5 M NH_3/CH_3OH.
[d] In $CDCl_3$.

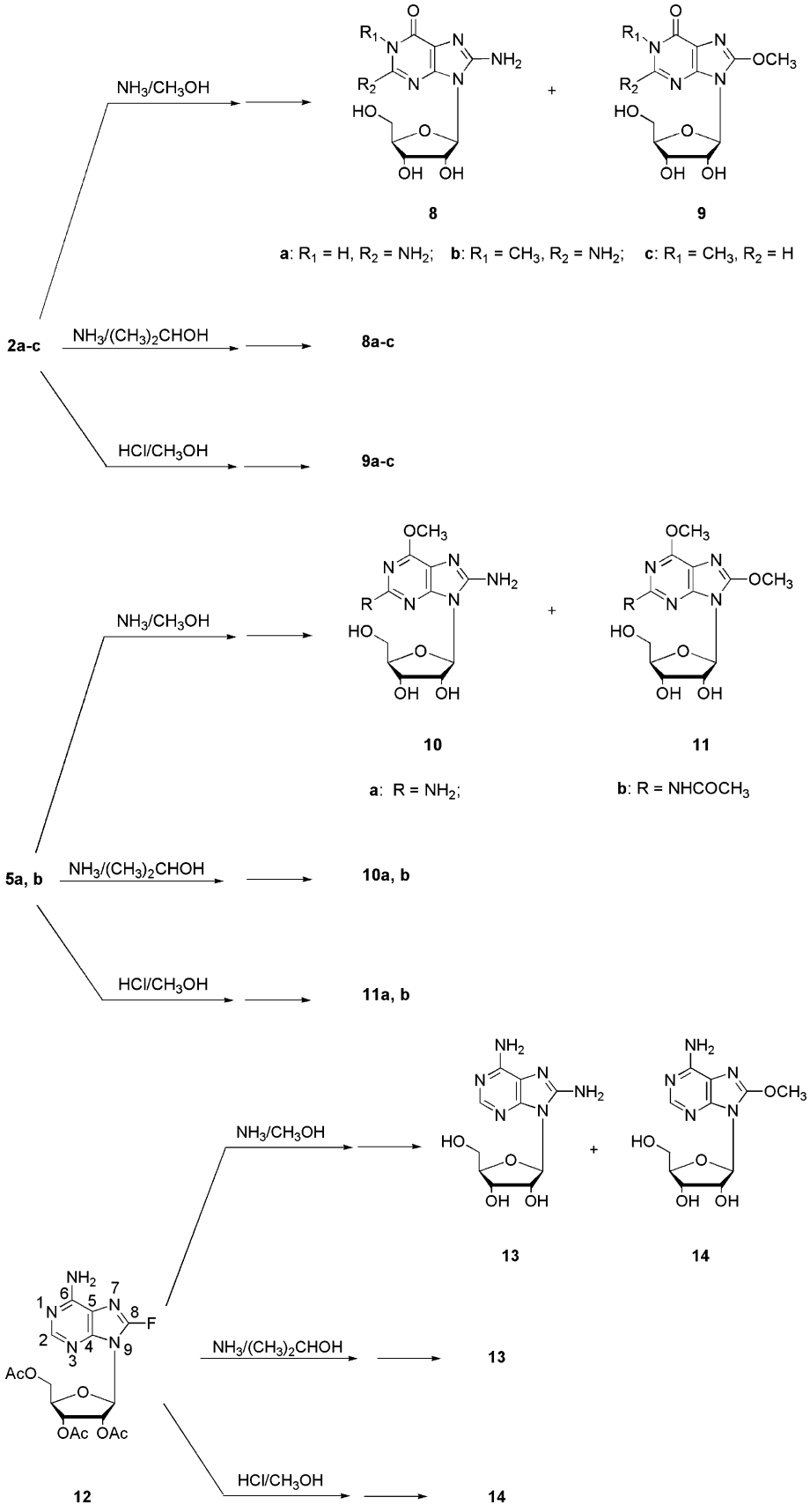
Table 2
Deacetylation of selected 8-fluoropurine nucleosides during the kinetic reactions as analyzed by LC–MS

Compound	Time (min)	Acetylated form (%)			
		Tri-Ac	Di-Ac	Mono-Ac	Fully deacetylated
(a) In 0.5 M NH_3 in methanol					
2a	0	84.5	806	6.7	
	31	17.9	2.4	69.9	9.9
	98	0.6		67.1	32.3
	376			21.6	78.4
	737			9.8	90.2
	2177				100.0
5a	0	60.8	11.0	21.8	
	31	1.0	4.8	65.8	27.4
	63			55.3	44.7
	94			35.6	64.4
	126			32.6	67.4
	290			13.6	86.4
12	0	77.2	11.9	2.7	
	31	12.7	4.9	73.5	9.0
	63	3.8		77.5	18.7
(b) In 0.5 M NH_3 in 2-propanol					
2c	0	100.0			
	15	100.0			
	56	100.0			
	84	95.7	4.3		
	177	92.0	8.0		
	236	88.6	11.4		
12	0	100.0			
	14	100.0			
	41	100.0			
	69	95.6	4.4		
	192	92.4	7.6		
(c) In 0.01 M HCl in methanol					
2a	0	100.0			
	18	100.0			
	35	100.0			
	70	100.0			
2c	0	100.0			
	18	100.0			
	35	100.0			
12	0	100.0			
	129	95.7	4.3		
	606	89.4	10.6		
	745	88.2	11.8		
	884	87.0	13.0		
	1385	86.0	14.0		
	3071	80.3	19.7		

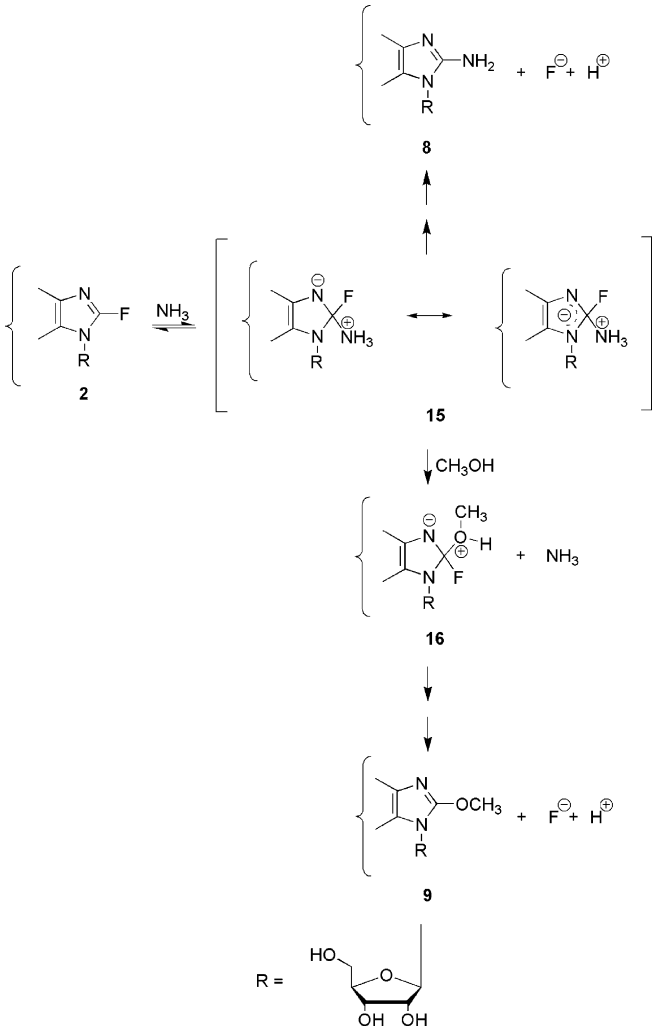
fluorine because of its high electronegativity can induce a positive charge on C(8) making it amenable to S_NAr type reactions [19]. This is supported by the observation that reactions of 2-fluoroimidazole, a model analog of 8-fluoropurines with nucleophiles, follow an addition–elimination mechanism [20]. Interestingly, reactions of certain substituted chloropurines have also been reasoned to occur via S_NAr mechanisms [21]. Thus, in the S_NAr reaction shown in Scheme 4 the addition of NH_3 to the 8-fluoropurines **2**, a likely rate-determining step [22], would lead to the formation of the Meisenheimer complex [23] **15** which could collapse to the corresponding 8-amino derivatives **8**. The highly solvated tetrahedral intermediates [24] **15** can also undergo methanolysis to yield the 8-methodoxy analogs **9**. The proportion of the 8-amino product formed from a given 8-fluoropurine depended upon its observed rate of reaction (Table 1). Faster reacting purines, e.g. **5b**, **12**, **2c** and **5a**, provided higher amounts of 8-amino products than the slower reacting derivatives like **2b** and **2a**. Overall, there was preponderance for the formation of the 8-methoxy analogs over the 8-amino derivatives, suggesting an efficient solvation of the Meisenheimer complex by methanol [24].

Defluorination rates of the 8-fluoropurines were also investigated with 0.5 M NH_3 in 2-propanol and the data are provided in Table 3. The rates of defluorination in 2-propanol were uniformly lower than the corresponding values in methanol. Both the polarity of the reaction medium and the extent of solvation of the Meisenheimer complex **15** can have a major effect on S_NAr rate constants. It is known in S_NAr reactions that amine nucleophiles tend to react faster in more polar solvents [17b]. Thus, in the relatively less polar 2-propanol [$E_T(30)$ value: 48.4] the 8-fluoropurines would react slower than in methanol [$E_T(30)$ value: 55.4] [25]. Additionally, the Meisenheimer complex **15** may be more efficiently solvated by methanol than 2-propanol due to lesser steric demands, leading to a faster rate of reaction. Similar solvent effects in S_NAr mechanism based reactions have previously been reported [26]. It is interesting to note an exclusive formation of 8-aminopurines as the product of defluorination with ammonia in 2-propanol (Table 3) in contrast to the 8-alkoxypurines as the major product in the case of ammonia in methanol. This observation also strongly suggests the solvation of the tetrahedral intermediate **15** by 2-propanol is quite restricted due to its steric size preventing the formation of 8-isopropoxypurine derivatives. The role of steric hindrance in S_NAr reactions is also known [15b,17b]. Under the condition of 0.5 M ammonia in 2-propanol, the competing deacetylation reactions also became relatively slower. The selected LC–MS data (Table 2) suggest that the defluorination reaction happened essentially in the triacetyl form under these conditions.

The k_{obs} data presented in Tables 1 and 3 show some interesting correlations. For example, the 8-fluoroadenosine derivative **12** was defluorinated, both in NH_3-methanol and 2-propanol media, faster than the guanosine analogs **2a,b**. In S_NAr reactions, the attack by the nucleophile is enhanced at the reaction site with decreased electron density [19]. It would be intriguing to compare the k_{obs} data for the fluoropurines with their electron density at the site of reaction, namely C(8), but unfortunately the electron density data for these fluoropurines are not known yet. However, such data for C(8) in adenine (0.728) and adenosine (0.928) are known to be lower than those of the corresponding carbons in guanine (0.991) and guanosine (0.991) [27,28]. The higher k_{obs} value for 8-fluoroadenosine analog **12** relative to those of 8-fluoroguanosine derivatives **2a,b** (Table 1) thus suggests the trend of electron density at C(8) in adenosine and guanosine may also be extended to their



Scheme 3. Defluorination reactions of 8-fluoronucleosides in basic and acidic media.



Scheme 4. Mechanism of base catalyzed defluorination of 8-fluoronucleosides.

respective 8-fluoro analogs. More direct evidence in this regard was obtained from ^{13}C and ^{19}F NMR data. Both the ^{13}C chemical shift for C(8) and the ^{19}F chemical shift in the adenosine derivative **12** were shifted downfield when compared with the corresponding values for the guanosine analogs **2a**,**b** (Table 1), attesting to the electron density correlations. In general, substrates with more deshielded carbon-8 and fluorine tend to react faster because of the facilitated attack of the

Table 3
Defluorination kinetic data for 8-fluoropurines in 0.5 M NH_3/2-PrOH and ^{19}F NMR chemical shifts

Compound	k_{obs} (min^{-1})	$t_{1/2}$	δ_F [a,b] (ppm)
2a	Too slow to measure		
2b	$(1.65 \pm 0.00) \times 10^{-5}$	29.2 d	−108.2
2c	$(6.27 \pm 0.11) \times 10^{-3}$	1.8 h	−104.1
5a	$(5.73 \pm 0.16) \times 10^{-4}$	20.2 h	−107.0
5b	$(4.04 \pm 0.16) \times 10^{-2}$	17.0 min	−103.4
12	$(1.61 \pm 0.04) \times 10^{-2}$	43.0 min	−104.0

[a] External reference: 4-fluorotoluene/DMSO-d_6 (−117.0 ppm).
[b] In 0.5 M NH_3/2-PrOH.

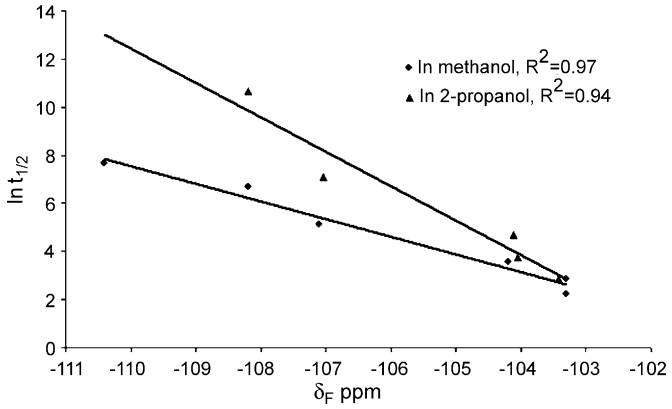


Fig. 1. Correlations between ln $t_{1/2}$ values of 8-fluoropurine nucleosides (**2a**-**b**, **5a**,**b** and **12**) and the ^{19}F NMR chemical shifts in 0.5 M NH_3 solutions.

nucleophile at C(8) (Scheme 4), a concept consistent with a S_NAr mechanism [19].

A closer look into the data given in Tables 1 and 3 also reveals that a significant correlation exists between the rate constants and ^{19}F and C(8) NMR chemical shifts. Fig. 1, for example, illustrates the relationship between $t_{1/2}$ values and the ^{19}F NMR chemical shifts in 0.5 M NH_3/CH_3OH and 0.5 M NH_3/2-PrOH. Fig. 2 represents the correlation of defluorination half-lives with the ^{13}C NMR chemical shifts for C(8) in $CDCl_3$. Unlike the ^{19}F NMR data, the ^{13}C NMR spectra could not be obtained in 0.5 M NH_3/CH_3OH or 2-propanol due to the short half-lives of some of the fluoropurines in basic media (Table 1) and the relatively long acquisition times required for NMR data collection. As expected, substitution of $CDCl_3$ as the solvent for ^{13}C NMR data collection had a profound effect on the C(8) chemical shift correlation of the fluoroguanosine analog **2a** with its k_{obs} value in 0.5 M NH_3/CH_3OH, most likely due to the deprotonation of N^1-hydrogen [29] under the basic defluorination reaction condition [30]. This deprotonation is also supported by ^{19}F NMR data. In methanolic ammonia the ^{19}F NMR chemical shift for the derivative **2a** moved upfield by 2.7 ppm in comparison with that in methanol (Table 1),

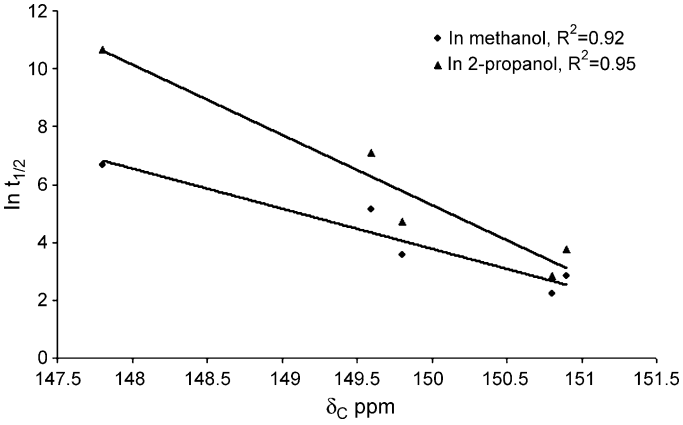
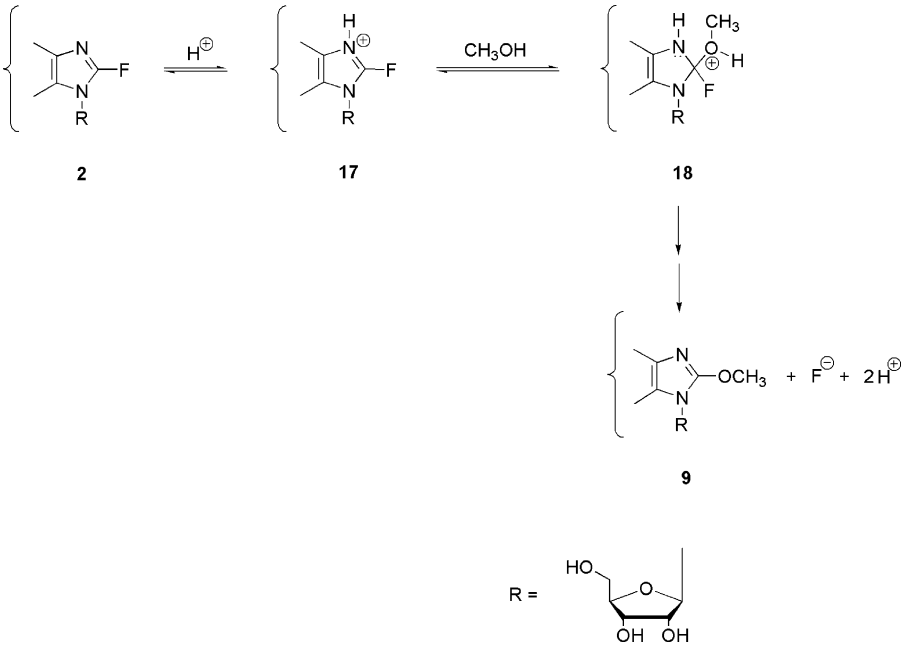


Fig. 2. Correlations between ln $t_{1/2}$ values of 8-fluoropurines nucleosides (**2b**,**c**, **5a**,**b** and **12**) in 0.5 M NH_3 solutions and ^{13}C NMR the chemical shifts of C(8) in $CDCl_3$. Data for the guanosine analog **2a** is not included because of deprotonation of N^1 in basic media which profoundly affects the ^{13}C chemical shift of carbon-8 as described in Section 2.2 (Discussion).



Scheme 5. Mechanism of acid catalyzed defluorination of 8-fluoronucleosides.

presumably due to the conjugative distribution of the resultant negative charge into the imidazole ring. In contrast, the corresponding ^{19}F NMR signal for the N^1-blocked analog **2b** in methanolic ammonia moved upfield by only 0.7 ppm. Thus, in basic medium the guanosine derivative **2a** had the most shielded fluorine and the negative charge built up in the imidazole ring would induce an electrostatic repulsion between the approaching nucleophile (NH_3) and the reaction center C(8) (Scheme 4). In agreement with this rationalization, the rate of defluorination of the guanosine analog **2a** in methanolic ammonia was found to be the slowest among all the fluoropurines studied (Table 1). More dramatically, its defluorination rate in 0.5 M NH_3 in 2-propanol was too slow to measure by ^{19}F NMR (Table 3).

As expected, other structural characteristics of the fluoropurine derivatives also had considerable effects on the defluorination rates in 0.5 M NH_3/CH_3OH or 2-propanol. Chief among them is the presence of an electron donating N^2-amino group [31,32] in the fluoropurines **2a**,**b** and **5a**. The ^{19}F NMR signals in these derivatives were shifted upfield when compared with that of the other analogs; a similar trend was also observed for C(8) ^{13}C NMR chemical shifts (Table 1). In methanolic ammonia the inosine analog **2c** ($\delta_F = -104.2$ ppm; $\delta_{C(8)} = 149.8$ ppm) reacted more than twenty times faster than the guanosine derivative **2b** ($\delta_F = -108.2$ ppm; $\delta_{C(8)} = 147.8$ ppm). Similarly, the rate of defluorination of the O^6-methyl analog **5b** ($\delta_F = -103.3$ ppm; $\delta_{C(8)} = 150.9$ ppm) with a deactivated N^2-amino function was nearly twenty times faster than its counterpart **5a** ($\delta_F = -107.1$ ppm; $\delta_{C(8)} = 149.6$ ppm) with a free amino group (Table 1).

In general, the fully aromatic fluoropurines **5a**,**b** and **12** underwent defluorination faster than the non-aromatic analogs, most likely due to some delocalization of the electron density from the π-electron rich imidazole ring into the π-electron deficient pyrimidine ring [33] enabling a facile approach of the nucleophile to C(8) (Scheme 4). Overall, the defluorination rates in methanol and 2-propanol media exhibited similar trends.

The acid catalyzed defluorination rates of 8-fluoropurine nucleosides **2a–c**, **5a**,**b** and **12** were also examined under pseudo-first-order reaction conditions in 0.01 M dry HCl in methanol at 28 ± 0.1 °C (Schemes 3 and 5). The rate constants (k_{obs}), the half-life ($t_{1/2}$) data and the ^{19}F NMR chemical shifts in 0.01 M methanolic HCl are given in Table 4. Product analysis by LC–MS as well as ^{1}H NMR spectroscopy showed that all 8-fluoropurines exclusively yielded their corresponding 8-methoxy derivatives **9a–c**, **11a**,**b** and **14**. Further, during all the kinetic runs no deacetylation was observed for the guanosine and inosine derivatives while the adenosine analog **12** defluorinated mainly in the triacetyl form along with a smaller contribution from its diacetyl derivative (Table 2). LC–MS also indicated that no deglycosylation of any of 8-fluoropurine nucleosides took place under the kinetic reaction condition.

Several interesting trends in the defluorination rates of 8-fluoropurines in methanolic HCl (Table 4) were observed when

Table 4
Data for kinetics of defluorination of 8-fluoropurines in 0.01 M HCl/CH_3OH and ^{19}F NMR chemical shifts

Compound	k_{obs} (min^{-1})	$t_{1/2}$	δ_F[a,b] (ppm)
2a	$(1.03 \pm 0.02) \times 10^{-1}$	6.7 min	−107.5
2b	$(1.84 \pm 0.04) \times 10^{-1}$	3.8 min	−107.7
2c	$(7.37 \pm 0.04) \times 10^{-2}$	9.4 min	−104.0
5a	$(1.67 \pm 0.02) \times 10^{-1}$	4.1 min	−104.3
5b	$(2.45 \pm 0.03) \times 10^{-1}$	2.8 min	−103.0
12	$(3.43 \pm 0.08) \times 10^{-4}$	33.7 h	−99.2

[a] External reference: 4-fluorotoluene/DMSO-d_6 (−117.0 ppm).
[b] In 0.01 M HCl/CH_3OH.

compared with the corresponding rates in basic media (Tables 1 and 3). For instance, the 8-fluororadenosine analog **12**, one of the fastest reacting substrates in methanolic ammonia, reacted more than a hundred times slower in methanolic HCl. In sharp contrast, the guanosine derivative **2a,** with a half-life of 35.4 h in methanolic ammonia and whose defluorination rate in 2-propanolic ammonia was too slow to measure, had a $t_{1/2}$ value of only 6.7 min in 0.01 M HCl in methanol. In methanolic HCl all the guanosine and inosine derivatives reacted 10^2 to 10^3 times faster than the adenosine analog **12**. Unlike in methanolic ammonia the ^{19}F NMR chemical shifts of the fluoropurines in methanolic HCl did not show a linear correlation with the $t_{1/2}$ data. For example, derivatives **2b** and **5a** exhibited close k_{obs} values in spite of a difference of 3.4 ppm in their ^{19}F NMR chemical shifts (Table 4). All these observations imply that, in acid medium, defluorination rates may not be as closely related to the electron densities at C(8) as found in basic media.

Based on these data, a defensible reaction mechanism for the defluorination reaction in acid medium using the guanosine analog **2** as a typical example is provided in Scheme 5. The first step of the reaction is the equilibrium protonation of the purine nucleoside. The principal site of protonation in purine nucleosides has been well documented [31,32,34,35]. For example, using ^{15}N NMR, it has been shown that N^7 in guanosine and inosine is the primary site of protonation while N^1 is the corresponding site in adenosine [31,32,34b]. Similarly, an analogous trend of protonation has been observed in the case of several 8-substituted guanosine, inosine and adenosine derivatives [32,34d,36]. Interestingly, the pK_a measurement of the fluoroadenosine analog **12** also indicates N^1 as the primary site of protonation [7]. Thus, it is quite probable the primary sites of protonation in the 8-fluoropurines discussed herein parallel those of the corresponding non-fluorinated parent nucleosides (i.e., guanosine, inosine, and adenosine). The inductive effect due to the fluorine atom in 8-fluoropurines will, however, decrease the basicity of N^7 and hence its protonation will not be very facile. In methanolic HCl a solvated, protonated derivative **17** (Scheme 5) for the guanosine and inosine cases would lead to the formation of the intermediate **18** which would then eliminate fluoride ion forming the 8-methoxy product **9**. Elimination of fluoride ion from a tetrahedral intermediate such as **18** is a fast step in S_NAr reactions [19] and hence could not be the rate-determining step. Thus, the equilibrium protonation of N^7 in the fluoropurines followed by a rate-limiting addition of methanol to the protonated substrate **17** would determine their acid catalyzed

Table 5
Basicities of purine nucleosides

Compound	pK_a for N^7	Reference
Guanosine	2.11 ± 0.04	[37]
N^1-Methylguanosine	2.6	[38]
N^1-Methylinosine	1.15 ± 0.03	[39]
O^6-Methylguanosine	2.4	[38]
N^2-Acetyl-O^6-methylguanosine	2.69 ± 0.02	This work[a]
Adenosine	−1.50 ± 0.15	[36]

[a] The pK_a was measured by standard pH titration method.

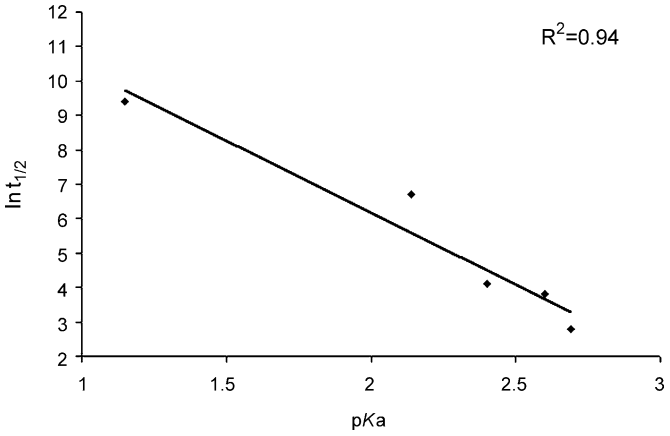


Fig. 3. Correlation between ln $t_{1/2}$ values of 8-fluoropurine nucleosides (**2a–c**, **5a**,**b**) in 0.01 M HCl/CH_3OH solution and N^7 pK_a values of their corresponding parent nucleosides (Table 5). Data for 8-fluoroadenosine analog **12** is not included in this plot since it has been reported [7] that 8-fluoroadenosine (pK_a = 2.95) is protonated at N^1 in 0.01 M HCl and hence a second protonation at N^7 is more difficult. An analogous trend of protonation has previously been observed with adenosine [31,32,34b].

defluorination rate constants (Scheme 5) and hence the pK_a of N^7 in the 8-fluoropurines would be a suitable parameter to reflect the propensity of its protonation. Unfortunately, the short $t_{1/2}$ values of the other 8-fluoropurines in acid medium (2.8–9.4 min, Table 4) precluded the measurement of their basicities with the facilities described. Hence, for an explanation of the kinetic data presented in Table 4, it was reasoned that the N^7 pK_a values of the readily available non-fluorinated parent guanosine, inosine and adenosine derivatives (Table 5) could logically be utilized because the effect of fluorine substitution would be relatively similar when inter-comparisons of k_{obs} data are made. This rationalization is indeed shown to be valid when the pK_a data shown in Table 5 were plotted against ln$t_{1/2}$ values provided in Table 4 and yielded a straight line correlation (Fig. 3, R^2 = 0.94).

Among the parent nucleosides (Table 5) the compound with a higher pK_a would be more readily protonated. The higher basicity of N^1-methylguanosine (pK_a = 2.6) than that of N^1-methylinosine (pK_a = 1.15) is well reflected in the k_{obs} data of the corresponding 8-fluoroanalogs. The acid catalyzed defluorination rate constant for the guanosine analog **2b** is thus 2.5 times higher than that for the inosine derivative **2c** (Table 4). Similarly, the relatively high stability of the adenosine analog **12** in acid medium ($t_{1/2}$ = 33.7 h) amongst all the 8-fluoronucleosides can clearly be correlated to the low N^7 basicity (pK_a = −1.50, Table 5) of adenosine, resulting from its facile N^1 protonation (pK_a = 3.2) [7].

3. Conclusions

^{19}F NMR spectroscopy is a convenient tool for assessing the defluorination rates of 8-fluoropurine nucleosides. In general, the electron density at C(8), as determined by ^{19}F and ^{13}C NMR, is identified as the underlying factor that determines their stability in basic media. In acid medium the stability of 8-fluoropurines is related to their N^7 pK_a values. More

importantly, the results of this investigation indicate acidic conditions are incompatible with 8-fluoroguanosine and 8-fluoroinosine analogs while basic conditions should be avoided with 8-fluoroadenosine derivatives.

4. Experimental

4.1. General

The ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded at 360, 125 and 338.9 MHz, respectively. The chemical shifts (δ) are expressed in parts per million (ppm) downfield from internal tetramethylsilane (TMS) for ^{1}H and ^{13}C and from external trichlorofluoromethane for ^{19}F NMR. Ultraviolet spectra were recorded in methanol solutions. The on-line HPLC-UV spectra were recorded with a diode array detector. High resolution mass spectra were obtained using either fast atom bombardment ionization technique (FAB HRMS) or electron impact ionization technique (HRMS). Column chromatography was run on silica gel columns (0.063–0.200 mm), which employed a stepwise solvent polarity gradient that was correlated with TLC mobility. TLC analyses were performed on silica gel plates and the spots were visualized with UV light (254 nm).

4.2. Synthesis of starting materials (*1a–c, 4a,b*)

Guanosine was acetylated with acetic anhydride in pyridine-DMF at room temperature for 50 h to give 2′,3′,5′-tri-*O*-acetylguanosine (**1a**) [9]. Guanosine and inosine were methylated with methyl iodide or methyl 4-toluenesulfonate in the presence of potassium carbonate [11] followed by acetylation of the sugar moiety to give 2′,3′,5′-tri-*O*-acetyl-N^1-methylguanosine (**1b**) and 2′,3′,5′-tri-*O*-acetyl-N^1-methylinosine (**1c**), respectively. For the synthesis of N^2-acetyl-2′,3′,5′-tri-*O*-acetyl-O^6-methylguanosine (**4b**), guanosine was first acetylated with acetic anhydride in pyridine at 50 °C in the presence of DMAP and triethylamine [10] to give N^2-acetyl-2′,3′,5′-tri-*O*-acetylguanosine, which upon O^6-methylation via its O^6-(*N*-methylpyrrolidinium) derivative [12] afforded **4b**. Compound **4b** dissolved in methanol was completely deacetylated with 25–28% NH_4OH at room temperature [12] and evaporated to dryness. The residue was dried overnight over P_2O_5 under vacuum and reacetylation of the sugar moiety yielded 2′,3′,5′-tri-*O*-acetyl-O^6-methylguanosine (**4a**).

4.3. Fluorination

4.3.1. General procedure [4]

To a solution of the starting material (**1a–c** or **4a**,**b**) in anhydrous solvents (e.g., CH_3CN, CH_3NO_2 or $CHCl_3$) (0.1 mmol/1.5 mL) was bubbled with 1% F_2 in argon or helium (1.5 mol equiv.) at room temperature over a period of 2–5 h. The reaction mixture was evaporated to dryness under vacuum. The product mixture was chromatographed on 200–400 times its weight of silica gel and the isolated products were further purified by semi-preparative HPLC (Whatman Partisil Silica column, 10 μm, 9 mm × 500 mm; flow rate 6.0 mL/min). Specific solvents for fluorination and conditions for silica gel column chromatography as well as analytical HPLC are given below for each compound. Analytical and semi-preparative HPLC eluents were monitored with UV detectors set at a wavelength of 254 nm.

4.3.2. 8-Fluoro-2′,3′,5′-tri-O-acetylguanosine (*2a*)

Method A—Reaction solvent: CH_3NO_2. Product R_f 0.37 (TLC, CH_2Cl_2/CH_3OH 95/5). Silica gel column chromatography eluent: EtOAc/EtOH (98/2 and 95/5). Semi-preparative HPLC eluent: CH_2Cl_2/CH_3OH (97.5/2.5), t_R = 75 min. Yield: 5.2%, semi-solid. UV(CH_3OH) λ_{max} = 242 and 276 nm; ^{1}H NMR (CD_3CN) δ (ppm) 2.01 (s, 3H), 2.07 (s, 3H), 2.10 (s, 3H), 4.21 (dd, J = 12.1 and 5.1 Hz, 1H), 4.35 (m, 1H), 4.48 (m, dd, J = 12.1 and 3.6 Hz, 1H), 5.61 (broad s, exchangeable, 2H), 5.69 (pseudo-t, 1H), 5.87 (d, J = 5.2 Hz, 1H), 5.82 (pseudo-t, 1H), 9.48 (s, exchangeable, 1H); ^{13}C NMR (CD_3CN) δ (ppm) 20.4, 20.5, 20.7, 63.0, 70.5, 71.9, 80.0, 85.1, 110.3 ($J_{C,F}$ = 15.2 Hz), 148.8 ($J_{C,F}$ = 246.3 Hz), 150.1, 154.2, 158.1, 169.4, 169.6 and 170.7; ^{19}F NMR (CD_3OD) δ = −107.7 ppm; HRMS calcd. for $C_{16}H_{18}N_5O_8F$ 427.1139, found 427.1120.

Method B—Reaction solvent: $CHCl_3$ (stabilized with amylene). Product **2a** was contaminated with 15% of **3a** as shown by analytical HPLC [Alltech Partisil Silica column, 5 μm, 4.6 mm × 250 mm; eluent: CH_2Cl_2/CH_3OH (95/5); flow rate: 1.0 mL/min]. The product mixture was reacted with 0.01 M HCl/CH_3OH at room temperature for 2 h. Semi-preparative HPLC purification [eluent: CH_2Cl_2/CH_3OH (95/5)] gave 8-chloro-2′,3′,5′-tri-*O*-acetylguanosine (**3a**), t_R = 11 min. Yield: 2%, colorless film. UV(CH_3OH) λ_{max} = 260 and 275 (sh) nm; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.04 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 4.36 (m, 2H), 4.50 (dd, J = 10.6 and 2.5 Hz, 1H), 5.98 (d, J = 6.1 Hz, 1H), 6.00 (pseudo-t, 1H), 6.29 (pseudo-t, 1H), 6.39 (broad s, exchangeable, 2H, NH_2), 11.93 (s, 1H, NH). FAB HRMS calcd. for $C_{16}H_{18}N_5O_8ClNa$ (M + Na) 466.0744, found 466.0736.

4.3.3. 8-Fluoro-2′,3′,5′-tri-O-acetyl-N^1-methylguanosine (*2b*)

Method A—Reaction solvent: CH_3CN. Product R_f 0.45 (TLC, CH_2Cl_2/CH_3OH 95/5). Silica gel column chromatography eluent: EtOAc/CH_3OH (from 98/2 to 90/10). Semi-preparative HPLC eluent: CH_2Cl_2/CH_3OH (97.5/2.5), t_R = 24 min. Yield: 10%, semi-solid. UV(CH_3OH) λ_{max} = 244 and 276 nm; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.06 (s, 3H), 2.12 (s, 3H), 2.14 (s, 3H), 3.51 (s, 3H), 4.32 (dd, J = 11.0 and 6.1 Hz, 1H), 4.39 (m, 1H), 4.50 (dd, J = 11.0 and 3.6 Hz, 1H), 5.73 (broad s, exchangeable, 2H), 5.82 (m, 2H), 6.05 (pseudo-t, 1H); ^{13}C NMR ($CDCl_3$): δ (ppm) 20.5, 20.6, 20.7, 28.3, 62.9, 70.2, 71.8, 79.5, 84.6, 110.3 ($J_{C,F}$ = 12.8 Hz), 146.8 ($J_{C,F}$ = 3.7 Hz), 147.8 ($J_{C,F}$ = 249.3 Hz), 153.8 ($J_{C,F}$ = 2.6 Hz), 156.3 ($J_{C,F}$ = 2.4 Hz), 169.6, 169.8 and 170.9; ^{19}F NMR(CD_3OD) δ = −107.5 ppm; FAB HRMS calcd. for $C_{17}H_{21}N_5O_8F$ (M + 1) 442.1374, found 442.1371.

Method B—Reaction solvent: CH_3NO_2. Yield: 5.7%. Physical data were identical to the product **2b** obtained from the reaction in CH_3CN.

Method C—Reaction solvent: $CHCl_3$ (stabilized with amylene). Product **2b** was found to be contaminated with 20% of **3b** as shown by ^{1}H NMR and analytical HPLC [Alltech Econosil Silica column, 5 μm, 4.6 mm × 250 mm; linear gradient elution with CH_2Cl_2/CH_3OH (98.2/1.5 to 97.5/2.5) during 120 min; flow rate: 1.0 mL/min]. The product mixture was treated with 0.01 M HCl/CH_3OH at room temperature for 2 h. Semi-preparative HPLC purification [eluent CH_2Cl_2/CH_3OH (97.4/2.6)] gave 8-chloro-2′,3′,5′-tri-*O*-acetyl-N^1-methylguanosine (**3b**), t_R = 25 min. Yield: 3.9%, colorless film. UV(CH_3OH) λ_{max} = 262 and 280 nm (sh); ^{1}H NMR ($CDCl_3$) δ (ppm) 2.04 (s, 3H), 2.13 (s, 3H), 2.14 (s, 3H), 3.50 (s, 3H), 4.33 (dd, J = 11.8 nd 6.1 Hz, 1H), 4.40 (m, 1H), 4.50 (dd, J = 11.8 and 3.8 Hz, 1H), 5.17 (broad s, exchangeable, 2H), 6.01 (d, J = 3.8 Hz, 1H), 6.05 (pseudo-t, 1H), 6.20 (pseudo-t, 1H); HRMS calcd. for $C_{17}H_{20}N_5O_8Cl$ 457.1000, found 457.0999.

4.3.4. 8-Fluoro-2′,3′,5′-tri-O-acetyl-N^1-methylinosine (**2c**)

Method A—Reaction solvent: CH_3CN. Product R_f 0.24 (TLC, EtOAc). Silica gel column chromatography eluent: CH_2Cl_2/CH_3OH (99/1 followed by 95/5). Semi-preparative HPLC eluent: CH_2Cl_2/CH_3OH (98.3/1.7); t_R = 27 min. Yield: 16.5%, white foam. UV(CH_3OH) λ_{max} = 238 and 268 nm; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.09 (s, 2× 3H), 2.14 (s, 3H), 3.63 (s, 3H), 4.27 (dd, J = 11.7 and 5.3 Hz, 1H), 4.38 (m, 1H), 4.44 (dd, J = 11.7 and 3.6 Hz, 1H), 5.64 (pseudo-t, 1H), 5.97 (d, J = 5.2 Hz, 1H), 6.03 (pseudo-t, 1H), 8.02 (s, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 20.3, 20.5, 20.6, 34.6, 62.9, 70.3, 71.8, 80.2, 85.1, 119.2 ($J_{C,F}$ = 13.4 Hz), 145.6 ($J_{C,F}$ = 3.3 Hz), 147.8 ($J_{C,F}$ = 3.3 Hz), 149.8 ($J_{C,F}$ = 252.1 Hz), 155.9 ($J_{C,F}$ = 2.2 Hz), 169.3, 169.5, 170.4; ^{19}F NMR (CD_3OD) δ = −103.9 ppm; FAB HRMS calcd. for $C_{17}H_{20}N_4O_8F$ (M + 1) 427.1265, found 427.1257.

Method B—Reaction solvent: $CHCl_3$ (stabilized with amylene). Semi-preparative HPLC eluent: CH_2Cl_2/CH_3OH (99/1). **2c**, yield: 16.2%, t_R = 64 min. 8-Chloro-2′,3′,5′-tri-*O*-acetyl-N^1-methylinosine (**3c**), t_R = 74 min. Yield: 7.2%, colorless film. UV(CH_3OH) λ_{max} = 248 and 270 (sh) nm; ^{1}H NMR($CDCl_3$) δ (ppm) 2.08 (s, 3H), 2.11 (s, 3H), 2.16 (s, 3H), 3.64 (s, 3H), 4.31 (dd, J = 11.0 and 6.1 Hz, 1H), 4.39 (m, 1H), 4.46 (dd, J = 11.0 and 3.7 Hz, 1H), 5.76 (pseudo-t, 1H), 6.09 (d, J = 5.9 Hz, 1H), 6.21 (pseudo-t, 1H), 8.02 (s, 1H, H-2); FAB HRMS calcd. for $C_{17}H_{20}N_4O_8Cl$ (M + 1) 443.0970, found 443.0975.

Method C—Reaction solvent: $CHCl_3$ (stabilized with 1% EtOH). Silica gel column chromatography eluent: CH_2Cl_2/CH_3OH (98/2 followed by 95/5). 8-Ethoxy-2′,3′,5′-tri-*O*-acetyl-N^1-methylinosine (**7**), R_f 0.13 (TLC, EtOAc). Yield: 2.8%, semi-solid. UV(CH_3OH) λ_{max} = 250 and 276 nm; ^{1}H NMR ($CDCl_3$) δ (ppm) 1.49 (t, J = 7.2 Hz, 3H), 2.07 (s, 3H), 2.10 (s, 3H), 2.13 (s, 3H), 3.62 (s, 3H), 4.22 (dd, J = 12.2 and 6.1 Hz, 1H), 4.32 (m, 1H), 4.43 (dd, J = 12.2 and 3.6 Hz, 1H), 4.65 (q, J = 7.2 Hz, 2H), 5.69 (pseudo-t, 1H), 6.05 (m, 2H), 7.92 (s, 1H); FAB HRMS calcd. for $C_{19}H_{25}N_4O_9$ (M + 1) 253.1630, found 253.1622.

4.3.5. 8-Fluoro-2′,3′,5′-tri-O-acetyl-O^6-methylguanosine (**5a**)

Reaction solvent: $CHCl_3$ (stabilized with amylene). Product R_f 0.65 (TLC, EtOAc/CH_3OH 95/5). Silica gel column chromatography eluent: CH_2Cl_2/CH_3OH (99/1 followed by 95/5). Semi-preparative HPLC eluent: CH_2Cl_2/CH_3OH (99.3/0.7), t_R = 55 min. Yield: 3.2%, colorless film. UV(CH_3OH) λ_{max} = 244 and 278 nm; ^{1}H NMR(CD_3CN) δ (ppm) 2.06 (s, 3H), 2.09 (s, 3H), 2.18 (s, 3H), 3.98 (s, 3H), 4.20 (dd, J = 12.1 and 5.9 Hz, 1H), 4.33 (m, 1H), 4.47 (dd, J = 12.1 and 3.6 Hz, 1H), 5.40 (broad s, exchangeable, 2H), 5.76 (pseudo-t, 1H), 5.91 (d, J = 4.6 Hz, 1H), 5.98 (pseudo-t, 1H); ^{13}C NMR ($CDCl_3$) δ (ppm) 20.4, 20.5, 20.6, 54.2, 62.8, 70.4, 72.0, 79.8, 85.0, 109.2 ($J_{C,F}$ = 15.4 Hz), 149.6 ($J_{C,F}$ = 250.9 Hz), 151.7 ($J_{C,F}$ = 3.5 Hz), 158.8 ($J_{C,F}$ = 3.3 Hz), 160.9 ($J_{C,F}$ = 3.6 Hz), 169.3, 169.5, 170.6; ^{19}F NMR (CD_3OD) δ = −106.7 ppm; FAB HRMS calcd. for $C_{17}H_{21}N_5O_8F$ (M + 1) 442.1374, found 442.1379. 8-Chloro-2′,3′,5′-tri-*O*-acetyl-O^6-methylguanosine (**6a**), t_R = 66 min. Yield: 1.3%, colorless film. UV(CH_3OH) λ_{max} = 254 and 284 nm; ^{1}H NMR($CDCl_3$) δ (ppm) 2.02 (s, 3H), 2.12 (s, 3H), 2.15 (s, 3H), 4.04 (s, 3H), 4.37 (m, 2H), 4.49 (dd, J = 10.9 and 2.4 Hz, 1H), 5.01 (broad s, exchangeable, 2H), 6.03 (d, J = 4.1 Hz, 1H), 6.09 (pseudo-t, 1H), 6.26 (pseudo-t, 1H); HRMS calcd. for $C_{17}H_{20}N_5O_8Cl$ 457.1000, found 457.0987.

4.3.6. 8-Fluoro-N^2-acetyl-2′,3′,5′-tri-O-acetyl-O^6-methylguanosine (**5b**)

Reaction solvent: $CHCl_3$ (stabilized with amylene). Product R_f 0.41 (TLC, EtOAc). Silica gel column chromatography eluent: EtOAc/Hexane (10/90) followed by EtOAc. Semi-preparative HPLC eluent: CH_2Cl_2/CH_3OH (99/1). **5b**, t_R = 30 min. Yield: 29.5%, colorless film. UV(CH_3OH) λ_{max} = 261 nm; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.10 (s, 3H), 2.12 (s, 3H), 2.16 (s, 3H), 2.51 (s, 3H), 4.12 (s, 3H), 4.32 (dd, J = 11.2 and 5.7 Hz, 1H), 4.41 (m, 1H), 4.51 (dd, J = 11.2 and 3.3 Hz, 1H), 5.83 (pseudo-t 1H), 5.95 (d, J = 4.7 Hz, 1H), 6.04 (pseudo-t, 1H), 8.05 (s, 1H, exchangeable); ^{13}C NMR ($CDCl_3$) δ (ppm) 20.4, 20.5, 20.6, 25.2, 54.7, 63.0, 70.4, 72.2, 80.0, 85.4, 112.3 ($J_{C,F}$ = 13.5 Hz), 150.8, 150.9 ($J_{C,F}$ = 253.1 Hz), 151.9, 160.6, 169.4, 169.5, 170.5, 170.7; ^{19}F NMR (CD_3OD) δ = −102.9 ppm; HRMS calcd. for $C_{19}H_{22}N_5O_9F$ 484.1480, found 484.1475. 8-Chloro-N^2-acetyl-2′,3′,5′-tri-*O*-acetyl-O^6-methylguanosine (**6b**), t_R = 39 min. Yield: 1.8%, white foam. UV (CH_3OH) λ_{max} = 268 nm. ^{1}H NMR ($CDCl_3$) δ (ppm) 2.01 (s, 3H), 2.11 (s, 3H), 2.16 (s, 3H), 2.49 (s, 3H), 4.14 (s, 3H), 4.34 (dd, J = 11.2 and 6.1 Hz, 1H), 4.41 (m, 1H), 4.54 (dd, J = 11.2 and 3.6 Hz, 1H), 6.01 (pseudo-t, 1H), 6.06 (d, J = 3.6 Hz, 1H), 6.21 (pseudo-t, 1H), 8.12 (s, exchangeable, 1H); HRMS calcd. for $C_{19}H_{22}N_5O_9Cl$ 499.1106, found 499.1113.

4.4. Basic and acidic solutions for kinetic determinations

Solutions were prepared as follows: (A) 0.5 M NH_3/CH_3OH by diluting commercial 2 M NH_3/CH_3OH (Aldrich) with anhydrous methanol (Aldrich); (B) 0.5 M NH_3/2-propanol by diluting commercial 2 M NH_3/2-propanol (Aldrich) with anhydrous 2-propanol (Aldrich); (C) 0.01 M HCl/CH_3OH by diluting commercial 10% dry HCl/CH_3OH solution (TCI America) with anhydrous methanol (Aldrich). The exact

concentrations of all the prepared solutions were determined by standard titration methods. All the standardized solutions were protected from moisture and kept refrigerated in individual vials sealed with Teflon septa.

4.5. Kinetic measurements

The defluorination reactions were followed by ^{19}F NMR spectroscopy at 28 ± 0.1 °C. All kinetic determinations were conducted under pseudo-first-order reaction conditions. The samples were prepared by dissolving 0.5 mg of 8-fluoropurine nucleosides (**2a–c**, **5a**,**b** and **12**) in 0.3 mL of 0.5 M NH_3/CH_3OH, NH_3/2-propanol or 0.01 M HCl/CH_3OH solution and quickly transferred by syringe to a NMR tube equipped with a capillary tube for external reference and tightly capped with a screw-cap to prevent evaporation of volatile reagents or solvents during data collection. The capillary tube was filled with a solution of 4-fluorotoluene in DMSO-d_6 (25 μL/0.5 mL) as the external reference ($\delta = -117.0$ ppm from $CFCl_3$). The ^{19}F NMR data were collected at a sweep width of 27,800 Hz and with a relaxation delay of 2.0 s. Typically 20 scans were collected for each time point and the Fourier transformed signals yielded spectra with excellent S/N ratio. From the relative intensity of ^{19}F signals (−100 to −110 ppm) due to 8-fluoronucleoside and the reference, the concentration of the fluoronucleoside could be determined at any time point. For each sample, more than 12 data points were collected at intervals until more than 70% of the product was defluorinated. For slower reacting compounds (i.e., $t_{1/2} > 5$ h) the samples in NMR tubes were kept at 28 ± 0.1 °C in a thermostatic bath in-between successive time points for data collection. The kinetic data were analyzed using the pseudo-first-order rate equation: $\ln C_t/C_0 = -k_{obs}t$ where C_0 is the initial concentration of the 8-fluoronucleoside and C_t is the concentration at time t. A plot of the logarithmic concentration of the 8-fluoronucleosides versus time yielded straight line graphs with correlation coefficients (R^2) ranging between 0.94 and 0.99. The observed rate constants (k_{obs}) were calculated from the slopes of the straight lines. The half-life values were calculated using the equation $t_{1/2} = 0.693/k_{obs}$. At least two or three samples were examined for each nucleoside and the averaged k_{obs} values are provided in Tables 1, 3 and 4.

Defluorination/deacetylation reactions monitored and $t_{1/2}$ LC–MS were carried out in capped autosampler vials at 24.0 ± 0.1 °C and the analyses were performed on an Agilent 1100 LC–MS instrument. The system was coupled to a diode array detector (detection wavelength: 254 nm) and the mass spectrometer was operated under API-ES ionization mode (capillary voltage 4 kV and chamber temperature 350 °C). The HPLC for LC–MS were run on an Alltech Adsorbsphere C18 column (5 μm, 4.6 mm × 250 mm), eluted at 1 mL/min with the following mobile phases—for compound **2a** (kinetic reaction condition: 0.5 M NH_3/CH_3OH) CH_3OH/H_2O linear gradient: 2:8 to 4:6 over a period of 20 min; for compound **5a** (kinetic reaction condition: 0.5 M NH_3/CH_3OH) CH_3CN/0.02 M NH_4OAc linear gradient: 1:9 to 6:4 over a period of 25 min; for compound **12** (kinetic reaction condition: 0.5 M NH_3/CH_3OH) CH_3CN/0.02 M NH_4OAc linear gradient: from 1:9 to 2:8 over a period of 8 min, to 3:7 in 20 min and held at 3:7 to 28 min; for compound **2a** (kinetic reaction condition: 0.01 M HCl/CH_3OH) CH_3OH/H_2O (2:8 isocratic); and for compounds **5a** (kinetic reaction condition: 0.5 M NH_3/2-PrOH), **2c** (kinetic reaction conditions: 0.5 M NH_3/2-PrOH and 0.01 M HCl/CH_3OH) and **12** (kinetic reaction conditions: 0.5 M NH_3/2-PrOH and 0.01 M HCl/CH_3OH) CH_3CN/0.02 M NH_4OAc (3:7, isocratic).

4.6. Product analysis of kinetic reaction mixtures

4.6.1. Products from 0.5 M NH_3/CH_3OH

Samples were all left at room temperature for 24–48 h in the NMR tubes, after kinetic data collection, to allow for a complete *O*-deacetylation. The reaction mixtures were evaporated under vacuum and the residues were then analyzed by ^1H NMR, mass spectroscopy and reverse phase analytical HPLC [(Alltech Econosil C18 column, 5 μm, 4.6 mm × 250 mm); eluent: 10–20% 0.02 M NH_4OAc/CH_3CN; flow rate: 0.5 mL/min]. On-line UV spectra were recorded for each eluting peak. The ratios of 8-methoxy to 8-amino products, namely **9a/8a**, **9b/8b** and **9c/8c** were judged by the peak areas in analytical HPLC, while those of the others (**11a/10a**, **14/13** and **11b/10b**) were evaluated by ^1H NMR by the integrated ratios of the proton signals indicated below. These product ratios are provided in Table 1.

*4.6.1.1. 8-Methoxyguanosine (**9a**) [40].* HPLC eluent: CH_3CN/0.02 M NH_4OAc (1/9). $t_R = 4.0$ min. $\lambda_{max} = 244.5$ and 285 nm; FAB MS ($M + 1$) 314. Unreacted 8-fluoroguanosine (less than 10%): $t_R = 3.2$ min. $\lambda_{max} = 242.5$ and 277.5 nm.

*4.6.1.2. 8-Methoxy-N^1-methylguanosine (**9b**).* HPLC eluent: CH_3CN/0.02 M NH_4OAc (1/9). $t_R = 5.9$ min. $\lambda_{max} = 251$ and 283 nm; ^1H NMR (CD_3CN) of the residue δ (ppm) 3.35 (s, 3H), 3.60–3.75 (m, 3H), 4.02 (m, 1H), 4.04 (s, 3H), 4.27 (m, 1H), 4.82 (dd, $J = 6.3$ and 5.7 Hz, 1H), 4.88 (broad s, 1H, exchangeable), 5.41 (s, 2H, exchangeable), 5.63 (d, $J = 6.3$ Hz, 1H); FAB HRMS calcd. for $C_{12}H_{18}N_5O_6$ ($M + 1$) 328.1257, found 328.1257.

*4.6.1.3. 8-Methoxy-N^1-methylinosine (**9c**) and 8-amino-N^1-methylinosine (**8c**).* HPLC eluent: CH_3CN/0.02 M NH_4OAc (15/85). **9c**: $t_R = 2.8$ min. $\lambda_{max} = 253$ and 275 nm (sh). **8c**: $t_R = 2.3$ min. $\lambda_{max} = 264$ and 292 nm (sh). **9c/8c** = 76:24. Product **9c** and **8c** were also isolated by semi-preparative C18 HPLC [(Alltech Econosil column, 10 μm, 10 mm × 500 mm); eluent: CH_3OH/H_2O (15/85) at 4 mL/min]. **9c**: UV (CH_3OH) $\lambda_{max} = 250$ nm ($\varepsilon = 9.200$) and 278 nm ($\varepsilon = 5.200$); ^1H NMR (CD_3CN) δ (ppm) 3.49 (s, 3H), 3.60 (s, exchangeable, 1H), 3.63 (dd, $J = 12.3$ and 3.4 Hz, 1H), 3.74 (dd, $J = 12.3$ and 2.8 Hz, 1H), 3.87 (broad s, exchangeable, 1H), 4.05 (m, 1H), 4.11 (s, 3H), 4.27 (pseudo-t, 1H), 4.40 (broad s, 1H, exchangeable), 4.79 (pseudo-t, 1H), 5.73 (d, $J = 7.2$ Hz, 1H), 8.02 (s, 1H); FAB HRMS calcd for $C_{12}H_{17}N_4O_6$ ($M + 1$) 313.1148, found 313.1149. **8c**: UV

(CH_3OH): λ_{max} = 262 nm (ε = 7.600) and 292 nm (ε = 3.800); ^1H NMR (CD_3CN) δ (ppm) 3.48 (s, 3H), 3.75 (m, 2H), 4.07 (m, 1H), 4.10 (broad s, exchangeable, 1H), 4.25 (m, 1H), 4.45 (broad s, 1H, exchangeable), 4.65 (pseudo-t, 1H), 5.55 (s, 2H, exchangeable), 5.83 (d, J = 7.3 Hz, 1H), 7.91 (s, 1H); HRMS calcd. for $C_{11}H_{16}N_5O_5$ 298.1151, found 298.1144.

*4.6.1.4. 8-Methoxy-O^6-methylguanosine (**11a**) and 8-amino-O^6-methylguanosine (**10a**).* HPLC eluent: CH_3CN/0.02 M NH_4OAc (2/8). **11a**: t_R = 3.0 min. UV (CH_3OH) λ_{max} = 247.5 and 283 nm; FAB HRMS calcd. for $C_{12}H_{18}N_5O_6$ (M + 1) 328.1257, found 328.1248. **10a**: t_R = 2.2 min. λ_{max} = 256 and 293 nm; FAB HRMS calcd. for $C_{11}H_{17}N_6O_5$ (M + 1) 313.1260, found 313.1265. **11a**:**10a** = 86:14 ($\delta_{H\text{-}2}$ = 4.86 ppm/$\delta_{H\text{-}2}$ = 4.78 ppm).

*4.6.1.5. N^2-Acetyl-8-methoxy-O^6-methylguanosine (**11b**) and N^2-acetyl-8-amino-O^6-methylguanosine (**10b**).* HPLC eluent: CH_3CN/0.02 M NH_4OAc (15/85). **11b**: t_R = 5.3 min. λ_{max} = 268 nm. **10b**: t_R = 3.5 min. λ_{max} = 283 nm; FAB HRMS calcd. for $C_{13}H_{19}N_6O_6$ (M + 1) 355.1366, found 355.1351. **11b**:**10b** = 65:35 ($\delta_{H\text{-}2}$ = 4.90 ppm/$\delta_{H\text{-}2}$ = 4.76 ppm). Some **11b** was also isolated by reverse phase semi-preparative HPLC (Alltech Econosil C18 10 μm, 10 mm × 500 mm), eluent: CH_3OH/H_2O (12/88) at 4 mL/min. UV (CH_3OH) λ_{max} = 268 nm (ε = 19.100); ^1H NMR (CD_3CN) δ (ppm) 2.29 (s, 3H), 3.63 (m, 1H), 3.73 (dd, J = 12.2 and J = 3.5 Hz, 1H), 3.89 (broad s, exchangeable, 1H), 3.98 (m, 1H), 4.05 (s, 3H), 4.43 (pseudo-t, 1H), 4.91 (d, pseudo-t, 1H), 5.74 (d, J = 5.9 Hz, 1H), 8.46 (broad s, 1H, exchangeable); FAB HRMS calcd. for $C_{14}H_{20}N_5O_7$ (M + 1) 370.1362, found 370.1348.

*4.6.1.6. 8-Methoxyadenosine (**14**) [41] and 8-aminoadenosine (**13**) [41].* HPLC eluent: CH_3CN/0.02 M NH_4OAc (15/85). **14**: t_R = 3.9 min. λ_{max} = 260 nm; FAB MS (M + 1) 298. **13**: t_R = 2.5 min. λ_{max} = 273 nm; FAB MS (M + 1) 283. **14**:**13** = 81:19 ($\delta_{H\text{-}2}$ = 8.08 ppm/$\delta_{H\text{-}2}$ = 8.00 ppm).

4.6.2. Products from 0.5 M NH_3/2-propanol

After completion of the defluorination (6 weeks for compound **2b**, 26 h for product **5a**, 8 h for the purine **2c**, 6 h for the derivative **12** and 4 h for the compound **5b**), the products were treated with 2 M NH_3/CH_3OH at room temperature for 24 h for a complete deacetylation. The samples were then evaporated and the residues were analyzed by HPLC (the same conditions as above) and high resolution mass spectroscopy. On-line UV spectra were also recorded for each peak. All the products (**8b**,**c**, **10a**,**b** and **13**) were identical to the 8-amino derivatives obtained in experiment A. In all cases only the 8-amino products were observed.

4.6.3. Products from 0.01 M HCl/CH_3OH

When defluorination was completed (2 h for guanosine and inosine derivatives **2a–c**, **5a**,**b** and 5 days for adenosine derivative **12**), the reaction mixtures were treated with 2 M NH_3/CH_3OH at room temperature for 7 h for complete deacetylation. After evaporation, the residues were analyzed by HPLC (the same conditions as in experiment A) and high resolution mass spectroscopy. In all cases only the 8-methoxy derivatives were observed.

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