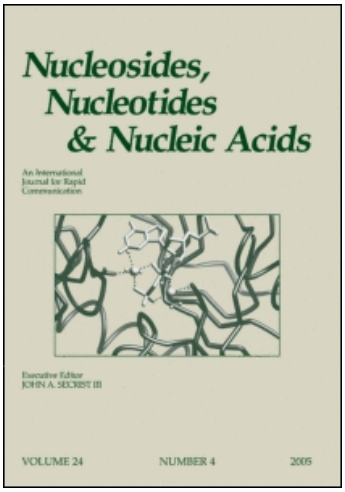

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PREPARATION OF A FLUDARABINE INTERMEDIATE VIA SELECTIVE ALKYLATION OF 2-FLUOROADENINE

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□ *The reaction between 2-fluoroadenine (**3**) and 1,3,5-tri-*O*-benzyl-1-α-*D*-chloroarabinofuranose (**4**) with potassium* t*-amylate was evaluated in various solvents to afford 9-β-*D*-(2,3,5-tri-*O*-benzyl-arabinofuranosyl)-2-fluoroadenine (**5**) and the corresponding α-anomer (**6**). In addition, 7-β-*D*-(2,3,5-tri-*O*-benzyl-arabinofuranosyl)-2-fluoroadenine (**7**) and an unusual "*bis*-fluoroadenine" nucleoside (**8**) were isolated as by-products. The highest anomeric ratio (β/α > 10) and conversion (>80%) were observed with the highly polar solvent sulfolane. This reaction was demonstrated on gram scale as a practical laboratory synthesis of **5**, a known intermediate in the synthesis of fludarabine.*

Keywords Solvent effects; Anomeric selectivity

INTRODUCTION

Fludarabine phosphate (**1**) is the active pharmaceutical ingredient in the commercial oncolytic drug Fludara, which is used to treat chronic lymphocytic leukemia (CLL).[1] In vivo, the $O_{5'}$-phosphate group, which serves to increase solubility, is cleaved to give fludarabine (**2**), which is sequentially rephosphorylated in the cell to the corresponding $O_{5'}$-triphosphate and functions by inhibiting multiple enzymes involved in DNA synthesis: DNA polymerase alpha, ribonucleotide reductase, and DNA primase. Hence, fludarabine phosphate is a prodrug form of fludarabine.

Several syntheses of fludarabine (**2**) have been reported.[2–5] The original syntheses by Montgomery and coworkers involved 2,6-diaminopurine nucleosides, which were selectively diazotized and fluorinated at the 2 position with $HBF_4/NaNO_2$ (Balz-Schiemann reaction)[6] in 36% yield.[2a,b] The preparation of the requisite 2,6-diaminopurine nucleosides was less

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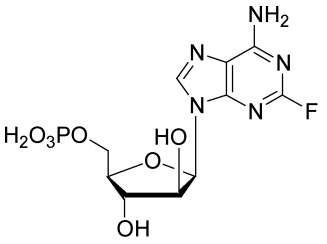

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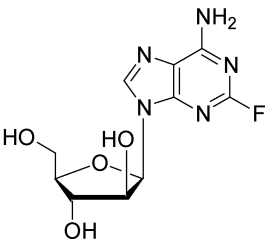

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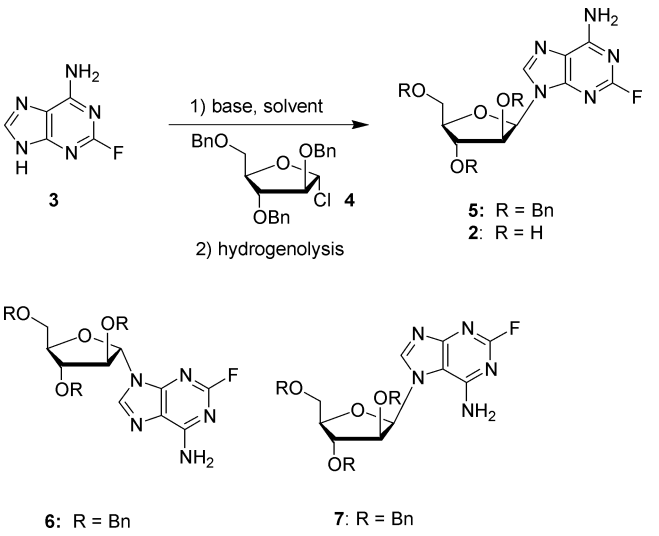
straightforward. For example, the condensation of 2,6-dichloropurine with 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride in the presence of mercuric cyanide in nitromethane afforded the desired β-anomer of the 2,6-dichloropurine nucleoside in 11% yield and the α-anomer in 25% yield.[2a] Conversion to the 2,6-diamino nucleoside was then accomplished by azide displacement and hydrogenation. An improved route to 2-amino-9-(2,3,5-tri-*O*-benzyl-β-D-arabinofuranosyl)adenine from 2,6-diacetamidopurine, via direct chlorosugar condensation in refluxing 1,2-dichloroethane in 40% yield was later reported by Montgomery and coworkers.[3] More recently, Markovac and coworkers described the reaction of 2-fluoro-9-trimethysilyladenine and 2-fluoro-*bis*-(6,9-trimethylsilyl)-adenine, both prepared by silylation of 2-fluoroadenine, with the aforementioned chloroarabinose to afford the corresponding benzylated nucleosides in 39% and 52% yield, respectively.[4] In addition, the same authors reported the condensation reaction of 2-fluoro-6-methoxyacetyl adenine with the chloroarabinose and diisopropylamine in 44% yield. An alternative approach reported by Baumann and coworkers starts with guanosine and sequentially converts the functionality at purine carbons 2 and 6 to fluorine in order to achieve selective displacement at position 6 by ammonia.[5] While this approach is elegant, and the raw material is readily available, nine transformations are required from guanosine.

We present here a synthesis of fludarabine that has, as a key step, the diastereoselective condensation of 2-fluoroadenine (**3**)[7] with the known chloroarabinose (**4**) to afford the previously described[2] nucleoside intermediate (**5**).

RESULTS AND DISCUSSION

Our approach to fludarabine is shown in Scheme 1. We anticipated that 2-fluoroadenine (**3**) could be reacted under suitable solvent and base conditions with the known chloroarabinose (**4**) to stereoselectively afford 9-β-D-(2,3,5-tri-*O*-benzyl-arabinofuranosyl)-2-fluoroadenine (**5**), which could then be converted to fludarabine (**2**) by known methods.[2a,b] Key to our approach is the avoidance of a silylation step or protecting groups on the



SCHEME 1

purine. In order for the condensation to be successful, reaction conditions were needed that would sufficiently dissolve 2-fluoroadenine (**3**) and also provide anomeric selectivity. This in turn would depend on the degree to which the stereospecific S_N2 pathway could be optimized relative to the competitive S_N1 pathway, while maintaining an adequate rate of conversion. In the case of halo sugars such as **4**, anomeric stereoselectivity in very polar solvents would be anticipated to be poor, owing to the competitive S_N1 pathway. Based on our previous experience with clofarabine, we anticipated that a heterogeneous reaction would be indicated, due to the exceedingly low solubility of the purine.[8]

We are not aware of any examples where 2-fluoroadenine (**3**) has been reacted, in un-protected form, with sugar derivatives to prepare nucleosides, although alkylation at N_9 with S_N2-reactive electrophiles in DMF has been documented.[9] This might in part be explained by the very low solubility of 2-fluoroadenine in all but the most polar solvents.

By analogy to our previous study with 2-chloroadenine, we explored the heterogeneous reaction of 2-fluoroadenine and chlorosugar **4** with potassium *tert*-amylate in a variety of solvents, seeking a solvent that would give both acceptable conversion and anomeric ratio (**5**/**6**). Potassium *tert*-amylate was chosen since our previous study had demonstrated that tertiary alkoxide bases led to improved anomeric selectivity.[8] Calcium hydride was added as a drying agent, consistent with our previous results, where the presence of water was found to be detrimental to anomeric selectivity. Our results are shown in Table 1. The screening was done in magnetically

TABLE 1 Reaction of 2-Fluoroadenine (3) with Chlorosugar 4 and Potassium *t*-Amylate in Various Solvents

Entry	Solvent	Temp (°C)	Time (h)	Dielectric constant ε_r[a]	Conv. (%)[b]	Anomer ratio (β/α) **5/6**	Ratio (N_9/N_7) **5/7**
1	DCE[c]	70	17	10.37	0.81	5 only	5 only
2	DME	70	17	7.2	21.1	2.6	5.6
3	Dioxane	70	17	2.21	17.7	6.4	8.8
4	*n*-BuOAc	70	17	NA[d]	36.1	>99	1.5
5	3-Pentanone	55	23	17.00	43.2	4.7	5.9
6	MEK[e]	55	23	18.51	64.7	3.6	6.9
7	Cyclohexanone	55	23	16.10	46.8	3.1	4.2
8	MeCN	70	17	35.94	62.5	5.0	8.7
9	Benzonitrile	55	23	NA	70.5	5.8	5.7
10	*t*-Butyl alcohol	70	17	12.47	34.7	6.7	3.5
11	Pyridine	70	17	12.91	2.5	0.1	ND[f]
12	NMP[g]	55	24	32.2	75.0	1.0	1.0
13	DMEU[h]	55	23	37.60	61.5	1.8	1.5
14	DMPU[i]	70	17	36.12	59.0	7.7	0.8
15	Propylene carbonate	55	23	64.92	56.9	4.2	2.4
16	Sulfolane	70	17	43.3	84.4	9.4	7.9
17	Sulfolane	45	22	43.3	89.4	10.4	14.7
18	Di-n-Propyl sulfone	55	23	NA	51.5	3.6	1.9
19	2,4-Dimethyl sulfolane	55	23	NA	46.7	4.0	2.8

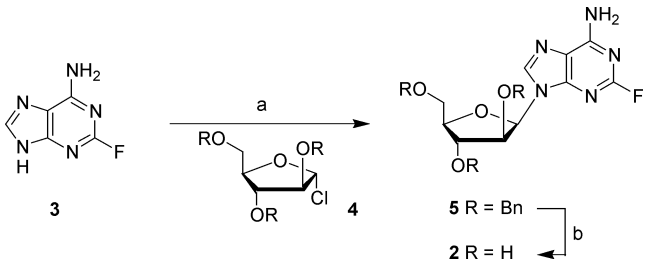
[a]Ref. 7.
[b]Conversion = (area $\mathbf{5} + \mathbf{6} + \mathbf{7}$)/($\mathbf{3} + \mathbf{5} + \mathbf{6} + \mathbf{7}$) $*$ 100.
[c]DCE = 1,2-dichloroethane.
[d]NA = not available.
[e]MEK = 2-butanone.
[f]ND = N_7 isomer not detected.
[g]NMP = 1-methyl-2-pyrrolidinone.
[h]DMEU = 1,3-dimethyl-imidazolidinone.
[i]DMPU = 1,3-dimethyl-3,4,5,6-tetrahydro-2(*1 H*)-pyrimidinone.

stirred tubes under nitrogen and the reaction conversion and selectivity were assayed by HPLC.

Representative solvents were chosen from the protic, halogenated hydrocarbon, ether, amine, and polar aprotic classes. It would generally be expected that more polar solvents, those more capable of solvating both 2-fluoroadenine and its conjugate base, would lead to greater conversion. By the same token, those same polar solvents should cause a decrease in anomeric selectivity due to the competitive S_N1 pathway, as was found in our previous study. The polarities of these solvents, as reflected in their dielectric constants, are given in Table 1. However, it is understood that this is by no means the sole parameter responsible for the observed differences in conversion and selectivity, which will be affected by subtle differences in the solvents' donor and acceptor properties and their ability to solvate the uncharged substrates, anions, and cations involved in this reaction.

In addition to the expected β-nucleoside **5**, two by-products were routinely observed. One of these was the α-anomeric nucleoside **6** and the other was assigned as the N_7 nucleoside **7** (Scheme 1). These three compounds were isomeric based on their mass spectra. Compounds **5** and **6** had very similar UV spectra (**5**, $\lambda_{max} = 261$ nm; **6**, $\lambda_{max} = 262.2$ nm). The β-anomeric stereochemistry of **5** was supported by a positive NOE between H_8 and $H_{3'}$ and other analytical data, which were consistent with the structural assignment and reported data.[2c] Compound **6** displayed NOEs between H_8 and $H_{4'}$, and $H_{2'}$ and $H_{4'}$, consistent with the assigned structure. Nucleoside **7** had positive NOEs between $H_{1'}$ and $H_{4'}$, as well as between H_8 and $H_{3'}$, supporting a β-anomeric stereochemistry, and, significantly, a distinctive UV spectrum ($\lambda_{max_1} = 238.7$ nm, $\lambda_{max_2} = 269.3$) characteristic of N_7 glycosidic attachment.[10]

In general, less polar solvents, in which the solubility of 2-fluoroadenine would be very low and the formation of charged intermediates unfavorable, gave high anomeric selectivity (**5**/**6**) but low reaction conversion. For example, 1,2-dichloroethane (DCE) gave almost complete selectivity for the beta anomer but only slight conversion (Table 1, entry 1). Ethereal solvents resulted in better conversion but decreased anomeric ratios (entries 2 and 3). Notably, DCE has a higher dielectric constant than either DME or dioxane, but very different donor–acceptor properties.[11] Of the solvents with ketone or ester groups (entries 4–7), n-butyl acetate gave the best anomeric ratio (>99), but conversions were less than 50% in all cases. The two nitrile solvents, MeCN and BnCN, gave conversions greater than 50% but anomeric ratios were modest (entries 8 and 9). *t*-Butanol gave modest selectivity and conversion (entry 10), whereas pyridine favored formation of the α-anomer (**6**) (entry 11). The strongly polar aprotic solvents gave variable results. *N*-Methyl pyrrolidinone (NMP) gave no selectivity but good conversion (entry 12). The cyclic ureas 1,3-dimethyl-imidazolidinone (DMEU) (entry 13) and DMPU (entry 14) gave conversions over 50% but DMPU was significantly more selective. Propylene carbonate (entry 15), with the highest dielectric constant of the solvents studied, nevertheless resulted in an anomeric ratio (4.2) comparable to the keto solvents. Surprisingly, sulfolane was found to give an anomeric ratio in the 9–10 range and conversions greater than 80% (entries 16 and 17). By contrast, the acyclic di-n-propyl sulfone (entry 18) gave an anomeric ratio of 3.6 and 2,4-dimethylsulfolane gave a ratio of 4 (entry 19). The reason for the enhanced anomeric selectivity in sulfolane is unclear. The rate of S_N1 heterolysis for 2-bromo-2-phenyl adamantane in sulfolane was reported to be less than in acetonitrile and γ-butyrolactone, possibly due to the lower electrophilicity of sulfolane compared to the other solvents.[12] Interestingly, sulfolane is only slightly better than acetonitrile in its cation and anion solvating power.[13]

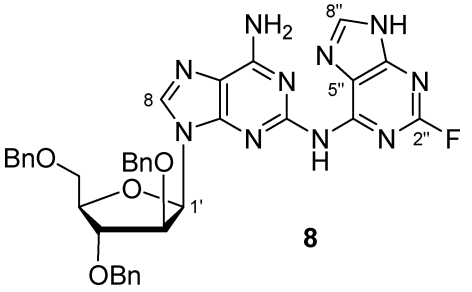


SCHEME 2 Reagents and conditions: (a) KO*t*-amyl, CaH_2, sulfolane, 50°C (44%). (b) Montgomery and Hewson,[2a] Montgomery.[2b]

The relationship between solvent polarity and formation of the N_7 isomer is not clear-cut. Solvents of lower polarity generally gave less of the N_7 isomer relative to the N_9 isomer. However, some polar solvents also gave high selectivity for N_9. For example, the ketone solvents (entries 6 and 7) and the nitrile solvents (entries 8 and 9) gave N_9/N_7 ratios greater than 5 and sulfolane (entries 16 and 17) gave ratios greater than 7.

The sulfolane reaction was performed on gram scale (Scheme 2). HPLC analysis of the reaction mixture revealed a crude anomeric ratio (**5**/**6**) of 10.4 and the ratio of **5**/**7** was 14.7. A yield of 44% with an anomer ratio greater than 300 was obtained after workup and crystallization.

Closer scrutiny of this and similar gram-scale reactions revealed an impurity eluting close to **5**, whose mass spectrum was consistent with the condensation of **5** with another 2-fluoroadenine molecule and concomitant loss of HF. In a separate experiment, the crude reaction mixture was chromatographed to obtain samples of nucleosides **6** and **7**. Deliberate reaction of **5** with 2-fluoroadenine provided a source of compound **8** for characterization (see Experimental).



The structure was assigned based on HMQC and HMBC NMR spectroscopy. A correlation was established between $H_{1'}$ and C_8. The C_8 and $C_{8''}$ resonances were differentiated by correlation to the respective protons (H_8 and $H_{8''}$). The resonance that was assigned to $H_{8''}$ correlated to the $C_{5''}$ resonance, which was a doublet split by the fluorine in the 2″ position. The

presence of fluorine on only one of the purine rings, combined with C_8-$H_{1'}$ coupling, allowed for ready differentiation of the rings. Structure **8** appears to be a previously unreported nucleoside analog.

In conclusion, we have demonstrated that the known fludarabine intermediate (**5**) can be prepared from 2-fluoroadenine (**3**) and chlorosugar **4** in high anomeric ratio using potassium *t*-butoxide and sulfolane. Of the solvents tested, sulfolane exhibits unique properties that allow for high conversion and anomeric selectivity in the reaction between 2-fluoroadenine and sugar **4**.

EXPERIMENTAL

Reactions were run under N_2 atmosphere. Melting points were obtained on a Buchi B545 melting point apparatus and are uncorrected. NMR spectra were obtained on either a Brucker 250 or 400 MHz or Varian 500 MHz instrument. IR spectra were obtained using KBr pellets on a Matson Infinity Gold® FTIR instrument. UV spectra were obtained using a Beckman DU640 spectrophotometer. HPLC data was collected on either of two Waters instruments: Waters System 600 Dual Pump Controller with Waters 996 Photodiode Array detector or Waters Alliance® system.

*2,3,5-Tri-O-benzyl-1-α-D-Arabinofuranosyl Chloride (**4**)*[14]. HCl (g) (7.7 g, 214 mmol) was bubbled over 5 min through a solution of 2,3,5-tri-*O*-benzyl-1-(4-nitrobenzoyloxy)-D-arabinofuranose (as a mixture of anomers) (15.1 g, 26.5 mmol) in CH_2Cl_2 (290 mL) at room temperature. The mixture became cloudy and was stirred for 17 h at room temperature. Thin-layer chromatography (silica gel plates, eluted with 1:1 heptane: Et_2O, starting material $R_f = 0.65$ and 0.51, **4** $R_f = 0.23$) revealed the reaction to be complete. The mixture was flushed with dry nitrogen and filtered through Celite. The filter cake was washed with CH_2Cl_2 (25 mL) and the filtrate was evaporated to an oil (11.8 g, 102% of theoretical yield). This material was used without purification.

Solvent Screening Experiments

The following procedure is representative. A set of screw cap vials with magnetic stir bars were each charged with 2-fluoroadenine (**3**) (98 mg, 0.64 mmol). Calcium hydride (27 mg, 0.64 mmol) was added, followed by the appropriate solvent (4.0 mL) and then potassium *tert*-amylate (20% wt soln in *tert*-amyl alcohol) (0.47 mL, 0.64 mmol). The resultant mixture was stirred at 55°C for approximately 5 min before chlorosugar (**4**) was added. The progress of reactions was monitored by periodic sampling and HPLC analysis.

HPLC method A (Table 1, entries 1, 3, 4, 8, 10, 16): Column, Waters XTerra RP18, 3.5 μm, 4.6 × 150 mm; mobile phase, MeCN: H_2O (0.15% TFA), 1:1 v/v; flow rate, 1 mL/min; temp. 30°C; detection 263 nM.

HPLC method B (Table 1, entries 2, 5, 6, 7, 9, 12, 13, 14, 15, 16, 18, 19): Column, Symmetry C18, 3.5 μm, 4.6 × 150 mm; mobile phase, MeCN: H_2O, 2:1 v/v; flow rate, 1 mL/min; temp. 30°C; detection 263 nM.

*Preparation of 9-β-D-(2,3,5-tri-O-benzyl-arabinofuranosyl)-2-fluoroadenine (**5**) from 2-fluoroadenine (**3**) and 2,3,5-tri-O-benzyl-1-α-chloroarabinofuranose (**4**).* A 40-mL cylindrical flask was equipped with a magnetic stirrer, temperature controller, and heating block. The flask was flushed with nitrogen before 2-fluoroadenine (**3**) (0.5 g, 3.3 mmol) and calcium hydride (0.14 g, 3.3 mmol) was charged. Anhydrous sulfolane (15 mL) was charged. A solution of **4** (1.9 g, 4.2 mmol) in sulfolane (5 mL) was prepared in a separate vessel. Potassium *tert*-amylate (1.4 M in *t*-amyl alcohol, 2.4 mL, 3.3 mmol) was charged, and the reactor was heated to 45°C and held for 15 min before the solution of **4** was charged. After 22 h, an in-process HPLC assay showed 89% conversion to **5**, with an anomeric ratio (β/α) of 10. The reaction was cooled to room temperature and then diluted with ethyl acetate (25 mL). The mixture was vacuum filtered over Celite, washed with ethyl acetate (15 mL), and the pH of the filtrate was determined to be 6 by pH paper. The filtrate was diluted with additional ethyl acetate (60 mL) and then extracted with portions of water (4 × 100 mL), adding NaCl brine (40 mL) as required to break emulsions. The organic layer was then dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. In-process HPLC analysis showed this crude product to be 88% pure with an anomeric ratio of 6.7:1. The residue was dissolved in dichloromethane (13 mL) and crystallized by dripping in heptane (65 mL) over a period of 15 min. The crystals were stirred overnight at room temperature and then cooled in an ice bath for one hour before being suction filtered and dried under vacuum at 50°C. HPLC analysis showed the crude product to be 88% pure with an anomeric ratio of 10.2:1. The yield at this point was 1.4 g (77.8%). The crystals were stirred as a slurry in methanol (14 mL) under reflux for 30 min. The mixture was cooled to room temperature before being cooled for one hour in an ice bath. After vacuum filtration and washing with methanol, the product was dried in a vacuum oven to afford **5** (0.8 g, 1.44 mmol) as a white solid in 44% yield. This material had an anomeric ratio of 310 and was 99.5% pure by HPLC area. ^{1}H NMR (DMSO-d_6) δ 8.12 (s, 1 H, H_8), 7.87 (br s, 2 H, NH_2), 7.38–7.19 (m, 13 H, phenyl), 6.96–6.94 (m, 2 H, phenyl), 6.33, (d, 1 H, J = 5.4 Hz, H_1'), 4.69 (d, 1 H, J = 12.1 Hz, benzyl), 4.65 (d, 1 H, J = 11.8 Hz, benzyl), 4.52–4.49 (m, 3 H, benzyl, H_2'), 4.44 (d, 1 H, J = 11.6 Hz, benzyl), 4.39 (t, 1 H, J = 5.4 Hz, H_3'), 4.23 (d, 1 H, J = 11.7 Hz, benzyl), 4.14 (q, 1 H, J = 5.1 Hz, H_4'), 3.74–3.69 (m, 2 H, H_5'). ^{13}C NMR (DMSO-d_6) 158.7 (d, J_{CF} = 204 Hz, C_2), 157.6 (d, J_{CF} = 22 Hz, C_4), 150.6 (d, J_{CF} = 22 Hz, C_6), 140.1 (C_8), [138.0, 137.9, 137.0, 128.2, 128.1,

127.7, 127.5, 127.4, (phenyl)] 116.6, (C_5), 82.1, 81.6, 80.6, 79.8, 72.3, 71.8, 71.3, 69.3 ppm. ^{19}F NMR (DMSO-d_6) −52.8 ppm.

Preparation of bis-*Purine* ***8***. A 40-mL cylindrical flask, equipped with a magnetic stirrer, temperature controller, and heating block was charged with **5** (0.51 g, 0.918 mmol), 2-fluoroadenine (**3**) (0.21 g, 1.37 mmol), and sulfolane (6 mL). A solution of potassium *t*-butoxide (1.0 M in *t*-butanol, 1.3 mL) was added and the mixture was heated to 75°C for 5 days. Reaction progress was checked by HPLC and was found to be >97% complete. The reaction mixture was diluted with EtOAc (35 mL) and was neutralized with HOAc to pH = 7. The organic layer was washed with water (5×), dried ($MgSO_4$) and reduced by rotary evaporation to give crude **8** (0.54 g). Purification by flash chromatography (4 g of silica gel, hexanes/EtOAc, gradient, 0 to 100% EtOAc over 15 min) gave **8** as a white solid. mp = 145–147°C. ^{1}H NMR (DMSO-d_6) δ 9.20 (br s, 1 H), 9.16 (s, 1 H), 8.23 (s, 1 H), 8.18 (br s, 2 H), 7.97 (br s, 1 H), 7.38–7.28 (m, 10 H), 7.14–7.12 (m, 3 H), 6.95–6.93 (m, 2 H), 6.46 (d, 1 H, J = 5.6 Hz), 4.69 (apparent q, 2 H, J = 13.0 Hz), 4.57–4.48 (m, 4 H), 4.42 (t, 1 H, J = 5.5 Hz), 4.28 (d, 1 H, J = 11.8 Hz), 4.19 (apparent q, 1 H, J = 5.1 Hz), 3.73 (apparent septet, 2 H, J = 5.3 Hz). ^{13}C NMR (DMSO-d_6) 162.8 (d, J_{CF} = 22 Hz), 159.1 (d, J_{CF} = 202 Hz), 156.1, 154.1 (d, J_{CF} = 21 Hz), 149.4, 149.2, 145.9, 140.5, 138.0, 137.8, 137.0, 128.3, 128.2, 128.0, 127.7, 127.5, 127.4, 116.8, 107.6 (d, J_{CF} = 6.5 Hz), 82.4, 81.8, 80.7, 80.0, 72.3, 71.9, 71.4, 69.2 ppm. ^{19}F NMR (DMSO-d_6) −53.3 ppm. IR (KBr) 3321, 3169, 2923, 2866, 1639, 1401, 1096, 698 cm^{-1}. UV (H_2O/MeOH) λ_{max_1} 240 nm (0.40 AU), λ_{max_2} 269 nm (0.31 AU) λ_{max_3} 301 nm (0.28 AU). Mass spec. (electrospray, positive) m/e $[M + H]^+$ = 689. Anal. Calcd for $C_{36}H_{33}FN_{10}O_4$: C, 62.78; H, 4.83; F, 2.76; N, 20.34. Found: C, 62.51; H, 4.92; F, 2.68; N, 20.37.

Isolation of By-Products 6 and 7

The filtrate from a typical recrystallization of **5** was reduced by rotary evaporation and eluted on a flash column (silica gel 60, 230–400 mesh, 5 cm diameter × 15 cm height, 295 cc volume). A step gradient was used according to Table 2.

Fractions were analyzed by TLC and HPLC according to Table 3.

TABLE 2 Step Gradient Chart for Isolation of **6** and **7**

Fractions	EtOAc (%)	Hexanes (%)	Volume (mL)
1–38	30	70	1000
39–88	40	60	1000
89–120	50	50	640
121–160	60	40	800
161–240	100	0	1600

TABLE 3 HPLC Analysis of Fractions

Fractions	Comment
1–14, 24–40	Very little material (~0.006 AU).
15–23	Small amounts of all components carried by sulfolane along solvent front.
41–71, 75–78	89% **5**, 6.9% impurity (5.22 min)
72–74	Mostly **5**
79–95	92.2% **5**
96–104, 111–120	27.2% **5**, 70.8% anomer (**6**)
105–106	11.7% **5**, 86.4% anomer (**6**)
107–120	3.6% **5**, 94.1% anomer (**6**)
187–200	98.5% impurity (4.49 min); N_7 isomer (**7**); re-purified
201–206	89.7% N_7 isomer (**7**)
207–230	74% *bis*-purine nucleoside (**8**). 13.2% N_7 isomer (**7**)

Fractions 187–200 were re-purified (silica gel 60, 230–400 mesh, 4 g, gradient hexanes/EtOAc, 0 to 100% EtOAc over 11 min). Fractions 12 and 13 were combined and reduced to give **7** as a pale yellow foam (0.20 g). ^{1}H NMR (DMSO-d_6) δ 8.49 (s, 1 H), 7.51 (br s, 2 H), 7.39–7.18 (m, 13 H), 6.88 (dd, 1 H, J = 7.6, 1.8 Hz), 6.48 (d, 1 H, J = 4.6 Hz), 4.60 (dd, 2 H, J = 12.1, 2.9 Hz), 4.52 (dd, 2 H, J = 12.2, 4.6 Hz), 4.34–4.31 (m, 2 H), 4.27–4.25 (m, 1 H), 4.13–4.10 (m, 1 H), 4.01 (d, 1 H, J = 11.5 Hz), 3.75 (dd, 1 H, J = 11.1, 2.9 Hz), 3.62 (dd, 1 H, J = 11.2, 3.8 Hz). ^{13}C NMR (DMSO-d_6) 161.8 (d, J_{CF} = 20 Hz), 158.5 (d, J_{CF} = 199 Hz), 153.4 (d, J_{CF} = 21 Hz), 145.8, 137.6, 137.5, 136.9, 128.3, 128.0, 127.8, 127.6, 127.5, 127.2, 109.7 (d, J_{CF} = 6 Hz), 86.3, 82.5, 81.0, 80.2, 72.2, 71.5, 71.3, 67.5 ppm. ^{19}F NMR (DMSO-d_6) −54.2 ppm. IR (KBr) 3425, 3359, 3189, 2866, 1637, 1387, 1101, 698 cm^{-1}. UV (H_2O/MeOH) λ_{max_1} 235.2 nm (0.17 AU), λ_{max_2} 277.5 nm (0.28 AU). Mass spec. (electrospray, positive) m/e $[M + H]^+$ = 556. Anal. Calcd for $C_{31}H_{30}FN_5O_4$: C, 67.01; H, 5.44; F, 3.42; N, 12.61. Found: C, 66.18; H, 5.26; F, 3.06; N, 12.44.

Fractions 96–120 were re-purified (silica gel 60, 230–400 mesh, gradient hexanes/EtOAc, 25% to 100% EtOAc over 17 min). Fractions 18–25 were combined and reduced to give crude **6** as an oil. The oil crystallized upon standing. The solid was triturated with 1/1 EtOAc/heptane (8 mL). The suspension was filtered to give pure **6** (38 mg). mp 155–157°C. ^{1}H NMR (DMSO-d_6) δ 8.24 (s, 1 H, H_8), 7.87 (br s, 2 H, NH_2), 7.37–7.19 (m, 15 H, phenyl), 6.12 (d, 1 H, J = 3.3, H_1'), 4.78 (t, 1 H, J = 3.6, H_2'), 4.61 (q, 1 H, J = 5.0, H_4'), 4.58–4.54 (m, 6 H, benzyl), 4.21 (dd, 1 H, J = 4.9, 3.8, $H_{3'}$), 3.63 (d, 2 H, J = 5.0, H_5'). ^{13}C NMR (DMSO-d_6) 158.5 (d, $J_{C\text{-}F}$ = 205), 157.6 (d, $J_{C\text{-}F}$ = 23), 150.1 (d, $J_{C\text{-}F}$ = 20), 139.5, 138.1, 137.6, 137.3, 128.21, 128.17, 127.7, 127.6, 127.5, 117.587.4, 84.9, 82.7, 82.3, 72.3, 71.4, 71.3, 69.6 ppm. ^{19}F NMR (DMSO-d_6) −52.5 ppm. IR (KBr) 3290, 3145, 2361, 1678, 1615, 1363, 1101, 734 cm^{-1}. UV (H_2O/MeCN) λ_{max_1} 206.2 nm (0.10 AU), λ_{max_2} 261.5 nm (0.037 AU). Mass spec. (electrospray, positive) m/e $[M + H]^+$ = 556.

Anal. Calcd for $C_{31}H_{30}FN_5O_4$: C, 67.01; H, 5.44; F, 3.42; N, 12.61. Found: C, 67.11; H, 5.69; F, 3.42; N, 12.60.

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