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A New Process for Antineoplastic Agent Clofarabine‖

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Abstract:

Clofarabine is a promising DNA polymerase inhibitor currently in clinical trials for a variety of liquid and solid tumor indications. The efforts for development of a new manufacturing process for clofarabine are presented. This new process allows for the reliable and efficient production of drug substance in high anomeric excess and high overall purity, without using chromatography. The high anomeric selectivity is achieved by reacting 2-chloroadenine with 1-bromo-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α-D-ribofuranose (4) and potassium *tert*-butoxide in a mixture of three solvents. Following crystallization, anomeric ratios exceeding 50 (*β*/α) are achieved. Deprotection and additional crystallization afford a clofarabine drug substance containing less than 0.1% of the α-anomer.

Introduction

Adenosine-related antimetabolites, such as cladribine (2-CDA) **1** and fludarabine (2-F-araA) **2** have proven to be useful chemotherapeutic agents for the treatment of various leukemias, including those which have become resistant to alkylating agents (Figure 1).[1] Clofarabine **3**, a "second generation" drug in this class, is protected against adenosine deaminase-catalyzed deamination by the 2-chloro substituent on the adenine ring and rendered more hydrolytically stable at the anomeric position by the 2′-fluoro substituent.[2] Inhibition of DNA repair by clofarabine in leukemic lymphocytes has also recently been reported.[3] Clinical trials for clofarabine against a variety of cancers are currently ongoing. The original synthesis of clofarabine,[2b−c] now over 10 years old, involved chromatography and was not sufficiently cost-effective and scalable to meet our needs. Therefore, we sought to develop a new process, which would avoid chromatography, produce drug substance of high purity based on modern analytical methods, and provide clear cost advantages over the published synthesis.

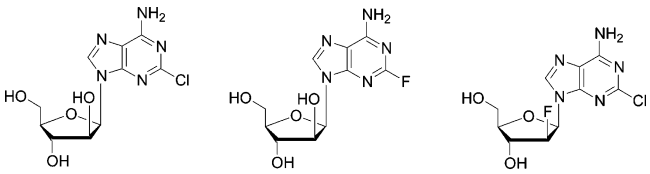

Figure 1.

Results and Discussion

Bromosugar **4**, which is readily prepared as the 1-α anomer with HBr from commercially available 2-deoxy-2-*β*-fluoro-1,3,5-tri-*O*-benzoyl-1-α-D-arabinofuranose,[4] has been used to synthesize various nucleoside analogues.[5] The anomeric selectivity observed in these reactions is dependent upon solvent polarity. Solvents with lower dielectric constants favor the formation of the *β*-anomer in the condensation products. This is probably due to the suppression of dissociative (S_N1-type) mechanisms, which can lead to both *β*- or α-anomeric products (Scheme 1). The effect of solvent polarity on the anomeric outcome of alkylations with **4** has been studied by Howell and co-workers.[6] In the Howell study, where halogenated solvents were compared, it was found that higher anomeric beta-selectivity was associated with solvents of lower dielectric constant, which would disfavor the less selective S_N1 mechanism. Notably, this strategy obviates the use of Lewis acids.[7]

In the earlier literature synthesis of clofarabine by Montgomery and co-workers, 2,6-dichloropurine was used

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‖ Dedicated to Dr. Arthur F. Lewis on the occasion of his birthday.

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Scheme 1

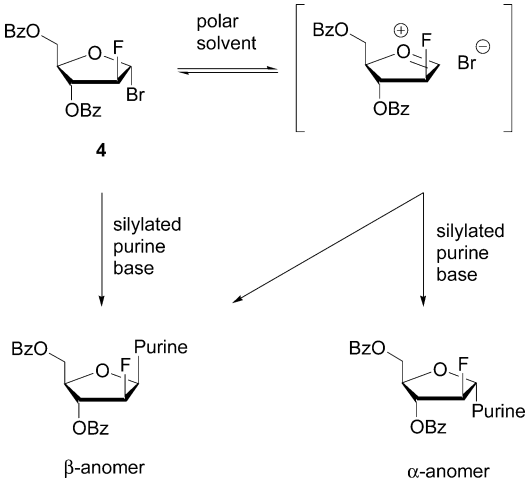


Table 1. **Base and additive screening reactions of bromosugar 4 with 2-chloroadenine in MeCN[a,c]**

entry	base	additive	conv[b] (%)	ratio **5**/**6**
1	NaH	none	86[d]	1.3
2	DBU	none	66	0.9
3	TMG[e]	none	52	0.9
4	K_2CO_3	none	52	1.2
5	Cs_2CO_3	none	70	2.0
6	NaO*t*-Bu	none	89	1.3
7	KO*t*-Bu	none	68	2.5
8	KO*t*-Bu	CaH_2	90	4.2
9	KO*t*-Bu	$CsBr_2$	88	2.0
10	KO*t*-Bu	Cs_2CO_3	64	2.8
11	KO*t*-Bu	TBAB[f]	84	1.8
12	NaO*t*-Bu	TBAB	49	1.3
13	NaO*t*-Bu	CaH_2	89	3.8

[a] Reactions run in MeCN for 18 h, except entry 12, which was run for 16 h. 2-Chloroadenine (1 equiv) was used. [b] Conversion = (area **5** + **6**)/(area 2-Cl-adenine + **5** + **6**)*100. [c] MeCN dielectric constant = 35.94.[12] [d] The percent conversion could not be determined because 2-chloroadenine could not be integrated properly. [e] *N*,*N*,*N*′,*N*′-Tetramethylguanidine. [f] Tetrabutylammonium bromide.

as the nucleophile in the condensation step, and the chlorine at the 6-position was selectively displaced with ammonia under pressure.[2b,5a] For safety and practicality reasons, we chose to have the amino group incorporated as part of the purine nucleophile. Examination of the literature revealed that 2-chloroadenine has been selectively N-alkylated at N_9 with an allylic tosylate.[8] Therefore, we concluded that the amino group at C_6 did not require a protecting group.

An array of parallel reactions between 2-chloroadenine and bromosugar **4**, affording anomeric benzoylated nucleosides **5** and **6**,[9] was undertaken to evaluate various solvent, additive, and base candidates (Scheme 2). We reasoned that all but the most polar solvents would result in heterogeneous reaction mixtures due to the limited solubility of 2-chloroadenine. However, the use of highly polar solvents would

Table 2. **Screen of lower polarity solvents**

entry	base	solvent	additive[a]	time	conv (%)	ratio **5**/**6**
1	KO*t*-Bu	THF[b]	none	18 hours	54	5.3
2	KO*t*-Bu	*t*-BuOH[c]	none	9 days	62	8.8
3	Cs_2CO_3	*t*-BuOH	none	9 days	14	8.7
4	NaO*t*-Bu	*t*-BuOH	none	9 days	13	0.8
5	KO*t*-Bu	*t*-BuOH	TBAB[d]	9 days	83	1.0
6	KO*t*-Bu	*t*-BuOH	CaH_2	9 days	57	9.6
7	NaO*t*-Bu	*t*-BuOH	TBAB	9 days	78	0.8

[a] Additive (1 equiv) was used. [b] Dielectric constant = 7.58.[12] [c] Dielectric constant = 12.47.[12] [d] Tetrabutylammonium bromide.

be counterproductive to our goal of achieving high anomeric selectivity, as outlined in Scheme 1. Therefore, we concluded that heterogeneous mixtures would have to be used in order to attain both reactivity and anomeric selectivity.

The reactions were run in 5 mL vials at ambient temperature. We analyzed each reaction by HPLC to determine the overall conversion and anomeric ratio. In all cases, the reactions were stirred as heterogeneous mixtures. The variables studied were solvent, base, and additives. Table 1 summarizes the effectiveness of various bases and additives in acetonitrile.

Acetonitrile (MeCN) is a solvent commonly used, in conjunction with bases such as sodium hydride, in the coupling steps of nucleoside syntheses.[7] Sodium hydride gave only a modest preference for the beta anomer **5** (**5**/**6** = 1.3), whereas the amidine and guanidine bases (DBU and *N*,*N*,*N*′,*N*′-tetramethylguanidine (TMG)) afforded the alpha anomer **6** in preference to **5** (entries 1−3, Table 1).[2c] Potassium carbonate[10] gave an anomeric ratio similar to sodium hydride (**5**/**6** = 1.2), while cesium carbonate[10b] gave a slightly improved ratio (2) and higher overall conversion (70%) (entries 4−5, Table 1). A marked increase in the conversion was observed with sodium *tert*-butoxide (89%), although the anomeric ratio remained similar to sodium hydride (1.3). A higher ratio (2.5) was observed with potassium *tert*-butoxide (entry 7, Table 1).[11]

A number of additives were also explored in acetonitrile (Table 1, entries 8−13). The best results were observed with a mixture of calcium hydride (we previously found that calcium hydride alone was a poor base for the reaction) and potassium *tert*-butoxide (**5**/**6** = 4.2, entry 7, Table 2). The calcium hydride had a beneficial effect by removing trace amounts of water from the solvent, as demonstrated in

(8) Obara, T.; Shuto, S.; Yasuyoshi, S.; Snoeck, R.; Andrei, G. et al. *J. Med. Chem.* **1996**, *39*, 3847−3852.

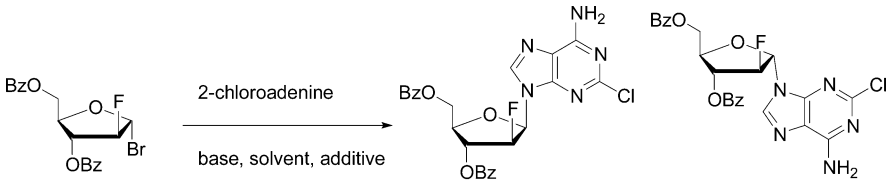
(9) Structural assignments for **5** and **6** were confirmed by 2D ^{1}H NMR COSY and NOESY experiments and by the conversion of **5** to authentic clofarabine. See Supporting Information for details. The authors acknowledge Professor Judith Walmsley (University of Texas, San Antonio) for 2D NMR experiments.

(10) Potassium carbonate and cesium carbonate have been used in various nucleoside syntheses with variable anomeric selectivities. See: (a) Seela, F.; Gumbiowski, R. *Helv. Chim. Acta* **1991**, *74*, 1048. (b) Reitz, A. B.; Rebarchak, M. C. *Nucleosides Nucleotides* **1992**, *11*, 1115. (c) Seela, F.; Bourgeois, W. *Synthesis* **1989**, 912. (d) Seela, F.; Bindig, U.; Driller, H.; Kaiser, K.; Kehne, A.; Rosemeyer, H.; Steker, H. *Nucleosides Nucleotides* **1987**, *6*, 11. (e) Rosemeyer, H.; Seela, F. *J. Org. Chem.* **1987**, *52*, 5136. (f) Wang, Y.; Fleet, G. W. J.; Wilson, F. X.; Storer, R.; Myers, P. L.; Wallis, C. J.; Doherty, O.; Watkin, D. J.; Vogt, K.; Witty, D. R.; Peach, J. M. *Tetrahedron Lett.* **1991**, *32*, 1675. (g) Seela, F.; Gumbiowski, R. *Liebigs Ann. Chem.* **1992**, 679.

(11) Potassium *tert*-butoxide was reported to be an effective base for the condensation of purines with 2-deoxy-2,2-difluoro-D-ribofuranosyl-3,5-dibenzoate. See: Chou, T.-S.; Jones, C. D. U.S. Patent 5,821,357, 1993.

(12) Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*, 2nd ed.; VCH: Weinheim, 1988.

Scheme 2



4 5 6

Table 3. **Screen of binary solvent mixtures**[a]

entry	solvent	solvent ratio	additive	time (h)	conv (%)	ratio **5**/**6**
1	MeCN/*t*-BuOH[b]	1:1	none	9 days	83	10.3
2	MeCN/*t*-BuOH	1:1	CaH_2	18	83	13.2
3	MeCN/*t*-BuOH	9:1	CaH_2	60	89	5.5
4	MeCN/*t*-BuOH	7:3	CaH_2	60	88	9.5
5	MeCN/*t*-BuOH	3:7	CaH_2	60	77	11.6
6	MeCN/*t*-BuOH	1:9	CaH_2	60	69	12.7
7[c]	MeCN/*t*-BuOH	1:1	CaH_2	16	32	1.0
8	MeCN/DCM[d]	1:1	CaH_2	18	8	15.2
9	MeCN/PhMe[f]	1:1	CaH_2	18	NR[g]	NR
10	MeCN/*tert*-amylOH	1:1	CaH_2	60	81	11.5
11	THF/*t*-BuOH	19:9	CaH_2	60	71	8.7
12	THF/*t*-BuOH	2:5	CaH_2	60	67	8.2
13	THF/*t*-BuOH	1:1	CaH_2	60	69	8.1
14	THF/*t*-BuOH	2:5	CaH_2	60	67	8.1
15	MeCN/DCE[h]	1:2	CaH_2	14	81	9.1
16	DCE/*t*-BuOH	1:2	CaH_2	14	53	16.6
17	DCE/*tert*-amylOH	1:2	CaH_2	14	60	18.0
18	DCE/*tert*-amylOH	1:4	CaH_2	14	58	18.9
19	MeCN/*tert*-amylOH	1:2	CaH_2	24	83	12.5
20	MeCN/*tert*-amylOH	1:2	KBr	24	81	12.7
21	MeCN/*tert*-amylOH	1:2	KCl	24	81	12.5
22	MeCN/*tert*-amylOH	1:2	CuI	24	3	1.2
23	MeCN/*tert*-amylOH	1:2	$ZnBr_2$	24	NR[e]	NA
24	MeCN/*tert*-amylOH	1:2	LiBr	24	21	1.4
25	MeCN/*tert*-amylOH	1:2	NaI	24	75	0.7
26	MeCN/*tert*-amylOH	1:2	CsI	24	85	1.7
27	MeCN/*tert*-amylOH	1:2	$MgBr_2$	24	0	NA
28	MeCN/*tert*-amylOH	1:2	$Cu(OAc)_2$	24	1	**5** only

[a] Reactions were run with KO*t*-Bu unless otherwise indicated. [b] *t*-BuOH dielectric constant = 12.47, MeCN dielectric constant = 35.94.[12] [c] NaO*t*-Bu was used. [d] Dichloromethane dielectric constant = 8.93.[12] [e] None of the alpha anomer was detected. [f] Toluene dielectric constant = 2.38.[12] [g] No reaction. [h] 1,2-Dichloroethane dielectric constant = 10.37.[12]

Table 4. **Screen of hydroxide bases**

entry	base	solvent	solvent ratio	additive	time (h)	conv (%)	ratio **5**/**6**
1	NaOH	MeCN/*t*-BuOH	1:1	none	16	60	1.2
2	KOH	MeCN/*t*-BuOH	1:1	none	42	68	11.0
3	KOH	MeCN/*t*-BuOH	1:1	CaH_2 (1 equiv)	42	46	14.7
4	KOH	MeCN/ *tert*-amylOH	1:2	CaH_2 (2 equiv)	25	79	14.2

experiments where water was deliberately added to reactions with and without CaH_2.[13] Not surprisingly, calcium hydride increased the anomeric ratio when combined with both sodium and potassium *tert*-butoxide, *although* the results for potassium were still superior. The conversions in reactions with calcium hydride were also significantly improved.

In keeping with the idea that solvents of lower polarity would suppress the α-anomer formation, we investigated some reactions in THF and *tert*-butanol. The results are summarized in Table 2. Despite the higher dielectric constant of *tert*-butanol compared to THF, the former solvent gave better anomeric selectivity. As observed in acetonitrile, sodium *tert*-butoxide and the addition of TBAB in combination with either sodium or potassium *tert*-butoxide were deleterious to anomeric selectivity. However, the selectivity observed with cesium carbonate was better in *tert*-butanol than in acetonitrile.

The preliminary data strongly indicated that potassium *tert*-butoxide was the preferred base and that a move to solvents of lower polarity improved anomeric ratio, while sacrificing percent conversion. We also observed that potassium cation had a beneficial effect on anomeric stereoselectivity, while sodium and amine-based cations significantly decreased anomeric stereoselectivity.

To reconcile the conflicting requirements for both conversion and anomeric ratio, we next examined *tert*-butoxide base in mixtures of two solvents (Table 3). The solvents tested were acetonitrile, *tert*-butyl alcohol, *tert*-amyl alcohol, dichloromethane (DCM), 1,2-dichloroethane (DCE), THF, and toluene (PhMe).

In entries 1−6 (Table 3), we see the expected trend in anomeric ratio and conversion as the reaction is run with potassium *tert*-butoxide in varying ratios of acetonitrile and *tert*-butyl alcohol. Dichloromethane and toluene both gave exceedingly poor conversion with acetonitrile and potassium *tert*-butoxide (entries 8 and 9, Table 3). It was also not surprising that sodium *tert*-butoxide (entry 7, Table 3) gave essentially no selectivity in a mixture of acetonitrile and *tert*-butanol (entry 6, Table 3). Of the other solvent mixtures, the highest anomeric ratios (in some cases exceeding 20) were observed with potassium *tert*-butoxide in DCE and either *tert*-butanol or *tert*-amyl alcohol (entries 16−18, Table 3). Conversions in these cases were in the 50−60% range. Various other additives were also studied (entries 19−28, Table 3). Both KBr and KCl had a surprisingly beneficial

(13) In parallel experiments where 1% (v/v) water had been deliberately added to the solvent, the anomeric ratio increased by 36% when CaH_2 was used. In a comparable reaction with CaH_2 and no added water, the anomeric ratio increased by 113%.

(14) An anomeric ratio of 10 (β/α) has been reported in the reaction of **4** with N_6-benzoyl adenine has been reported in $MeCN/CH_2Cl_2$ using sodium hydride. See: Vemishetti, P.; Howell, H. G.; Walker, D. G.; Brodfuehrer, P. R.; Shih, K.-M. Eur. Pat. EP0428109, 1991.

(15) (a) Piskala, A.; Masojidkova, M.; Saman, D. *Collect. Czech Chem. Commun.* **1996**, *61*, S23−S25. (b) Seela, F.; Bourgeois, W. *Synthesis* **1990**, *10*, 945−950. (c) Dziewiszek, K.; Schinazi, R. F.; Chao, T. C.; Su, T. L.; Dzik, J. M.; Rode, W.; Watanabe, K. A. *Nucleosides Nucleotides* **1994**, *13*, 77−94. (d) Harayama, T.; Yanada, R.; Taga, T.; Machida, K.; Cadet, J.; Yoneda, F. *J. Chem. Soc., Chem. Commun*. **1986**, *19*, 1469−1471. (e) Chavis, C.; Dumont, F.; Wightman, R. H.; Ziegler, J. C.; Imbach, J. L. *J. Org. Chem.* **1982**, *47*, 202−206.

Table 5. **Screen of ternary solvent mixtures with calcium hydride additive**

entry	base	solvent	solvent ratio	time (h)	conv (%)	ratio **5**/**6**
1	KO*t*-Bu	MeCN/*t*-BuOH/DCE	1:9:5	60	55	16.9
2	KO*t*-Bu	MeCN/*t*-BuOH/DCE	1:11:2	60	65	14.9
3	KO*t*-Bu	MeCN/*t*-BuOH/DCE	6:6:5	60	66	11.7
4	KO*t*-Bu	MeCN/*t*-BuOH/DCE	11:1:3	60	32	4.4
5	KO*t*-Bu	MeCN/*tert*-amylOH/DCM	1:2:3	10	63	8.5
6	KO*t*-Bu	MeCN/*tert*-amylOH/DCM	1:3:3	10	69	13.2
7	KO*t*-Bu	MeCN/*tert*-amylOH/DCM	1:2:1	10	83	17.7
8	KO*t*-Bu	MeCN/*t*-BuOH/*tert*-amylOH	15:2:13	60	84	11.2
9	KO*t*-Bu	MeCN/*t*-BuOH/*tert*-amylOH	9:2:19	60	80	14.0
10	KO*t*-Bu	MeCN/DCE/*tert*-amylOH	1:2:2	14	74	21.4
11	KO*t*-Bu	MeCN/*t*-BuOH/*tert*-amylOH	1:3:2	20	67	18.6
12	KO*t*-Bu	MeCN/*t*-BuOH/*tert*-amylOH	1:2:2	20	72	17.8
13	KO*tert*-amyl	MeCN/*tert*-amylOH/PhMe	1:2:2	10	68	12.7

effect on anomeric selectivity, somewhat comparable in magnitude to calcium hydride (compare entries 20−21 with entry 10, Table 3). However, some metal salts (Cu, Na, Li, Zn, and Mg) had detrimental effects on the reaction (entries 22−28, Table 3). The reaction did not occur at all with magnesium or zinc bromide and only to a very slight extent in the presence of copper salts. Under the same solvent conditions, cesium iodide gave high conversion but only a 1.7 anomeric ratio. The benefits of potassium as a counterion are borne out by these data.[12]

Another interesting observation is that sodium and potassium hydroxide can be used as bases in acetonitrile/*tert*-butyl alcohol or acetonitrile/*tert*-amyl alcohol (Table 4). The use of hydroxide bases in nucleoside syntheses is precedented[7] and has been accomplished with phase transfer catalysis in polar solvents.

Potassium hydroxide was superior to sodium hydroxide in promoting selectivity (entries 1−2, Table 4). The addition of the drying agent calcium hydride was also beneficial (entries 3−4, Table 4). These results are interesting in part because the base generates at least 1 equiv of water during the course of the reaction. Both *tert*-butanol and *tert*-amyl alcohol were suitable cosolvents.

Mixtures of three solvents were explored next, all in the presence of CaH_2 (Table 5). As expected, higher proportions of acetonitrile generally led to higher conversions at the expense of anomeric ratio (for example, compare entries 2 and 4). Smaller proportions of acetonitrile gave generally better results in combination with tertiary alcohols (entries 8, 9, 11, and 12) or, better yet, with a combination of tertiary alcohol and a halogenated solvent (entries 7 and 10).

Through a series of gram-scale experiments, the reaction parameters were further defined (Table 6). The solvent volume ratios were slightly adjusted to 1:2:1 MeCN/*tert*-amyl alcohol/DCE and 50 °C was chosen as the reaction temperature. Good conversions were observed at 20 g scale after 5 h but the reaction could also be run for longer times without affecting the purity profile or yield.

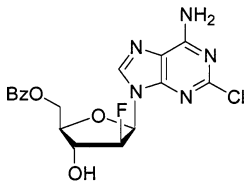
The isolation and purification of benzoylated clofarabine **5** from the heterogeneous reaction mixture presented a number of challenges. First, the rather insoluble 2-chloroadenine had to be removed along with inorganic byproducts, then intermediate **5** had to be isolated and its anomeric ratio (**5**/**6**) improved without resorting to chromatography. The problem was solved by filtering most of the 2-chloroadenine; then the solvent was exchanged for *n*-butyl acetate, which effectively dissolved the product mixture. Addition of heptane precipitated crude **5**, giving a slight increase in anomeric ratio and allowed for ready manipulation of the solid. We were unable to find satisfactory recrystallization conditions for **5** and eventually opted for a slurry procedure in methanol, which could be conducted either at reflux or at room temperature (see below). Anomeric ratios of >30:1 (β/α) were routinely achieved for isolated **5**. Anomeric ratio could be further improved by secondary methanol slurry, after which anomeric ratios >50:1 (β/α) were routinely observed.

Table 6. **Bench scale reactions between 4 and 2-chloroadenine to afford 5 and 6**

entry	scale (g of 2-chloroadenine)	time (h)	slurry (°C)	yield (%)	ratio **5**/**6** crude[a]	ratio **5**/**6** final[b]	HPLC assay area (%)
1	22.9	6.0	reflux	53	27.9	81.5	93.8
2	28.8	5.0	reflux	55	26.3	58.8	95.9
3	28.8	17.5	reflux	54	21.6	37.1	98.6
4	29.2	5.0	reflux	13[c]	25.9	163.6	98.1
5	29.0	21.0	reflux	32[c]	28.3	94.9	96.8
6	20.0	21.5	22.5	48	22.9	53.1	97.5
7	20.0	20.5	25.2	52	19.3	39.6	96.1
8	20.0	19.0	23.9	49	17.4	51.4	96.7
9	20.0	19.0	27.1	50	14.6	80.8	97.3
10	20.0	19.2	22.3	59	21.3	39.3	96.6
11	20.0	19	reflux	41	21.2	116.5	97.9
12	20.0	19	reflux	49	21.9	102.5	97.9
13	18.0	22	26	45	19.0	87.5	97.2
14	18.6	17.5	23.2	46	23.6	55.2	96.5
15	18.4	19	21.9	48	20.4	39.0	95.3

[a] Represents ratio after the *n*-BuOAc/heptane precipitation. [b] Represents ratio after one MeOH slurry. [c] Partial deprotection observed during reslurry.

An important consideration in performing the methanol slurry procedure was the presence of adventitious base. For example, in entry 4 (Table 6), only 13% yield of protected clofarabine was isolated. The remaining mass balance was clofarabine and the corresponding $O_{5'}$-benzoate **7** (Figure 2). To avoid the formation of **7**, the pH of reaction mixtures was routinely checked and adjusted to pH 5−7 with acetic acid prior to the precipitation step. Compound **7** was purified by column chromatography and characterized. The structure



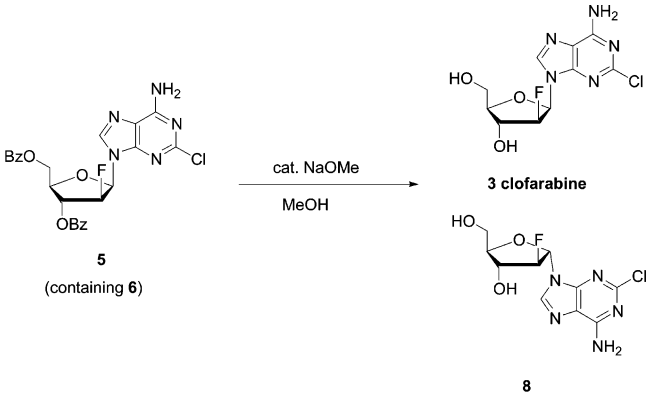
7

Figure 2.

Table 7. **Comparison of OH resonances for 7 and clofarabine (DMSO-d_6)**

compound	3′-OH ppm (DMSO-d_6)	5′-OH ppm (DMSO-d_6)
clofarabine	5.95 (d, 1H, $J = 5$)	5.08 (t, 1H, $J = 5$)
7	6.19 (d, 1H, $J = 5$)	not observed

Scheme 3



was assigned by NMR on the basis of the 3′-hydroxyl doublet (see Table 7). The relative chemical shift and coupling constant for this resonance correlate well with the corresponding clofarabine resonance. As expected, the 5′-hydroxyl triplet is absent.

Montgomery and co-workers also reported the formation of **7** as a byproduct during the aminolysis of 2,6-dichloro-9-(3′-acetyl-5′-*O*-benzoyl-2′-deoxy-2′-fluoro-α-D-arabinofuranosyl)-9*H*-purine.[2b] Based on our work, it cannot be determined whether **7** was formed as a kinetic or thermodynamic product. The selective formation of **7** might be attributed to induction by the vicinal fluorine atom, but additional work would be required to resolve this question.

The deprotection reaction of **5** to afford clofarabine was accomplished with catalytic sodium methoxide in methanol (Scheme 3). The product was crystallized directly from the reaction mixture by cooling and then recrystallized from methanol to afford clofarabine. The product thus obtained contained <0.1% of the corresponding anomer **8** (Table 8). An authentic sample of clofarabine α-anomer **8** was obtained by deprotection of **6** under similar conditions. Stereochemical assignments were confirmed by the COSY and NOESY spectra (see Supporting Information).

As can be seen from Table 8, methoxide charges of as little as 4 mol % can be used to carry out the reaction. However, later work revealed that 20−25 mol % was preferable on a larger scale, since faster rates and lower

Table 8. **Reactions of 5 with NaOMe to afford 3**

entry	scale (g of **5**)	anomer ratio (**5**/**6**)	NaOMe (mol %)	yield **3** (%)	HPLC[a] (%)	weight assay[b] (%)
1	20.0	49	4	61	99.3	98.5
2	25.7	38.5	5	64	99.6	ND[c]
3	10.0	87	27	61	99.7	98.9
4	23.4	80	11	60	99.2	ND
5	8.0	40	25	68	99.7	98.7

[a] This is the area assay for **3**. [b] This is the HPLC weight assay for **3** based on a reference standard. The result is not corrected for water or solvent content. [c] Not determined.

reaction temperatures are possible under these conditions. It is also noteworthy that the anomeric ratio (**5**/**6**) can be as low as 30 and still produce anomerically pure clofarabine (**3**).

Conclusions

We have demonstrated the use of 2-chloroadenine and bromosugar **4**, in conjunction with potassium tertiary alkoxide base and a three solvent system, as a selective route to protected clofarabine (**5**) and hence clofarabine (**3**), after debenzoylation. The superiority of potassium as a counterion in this chemistry was clear from the screening experiments. Significantly, the coupling reaction was run in a heterogeneous manner. It was also concluded that the addition of calcium hydride as a drying agent was beneficial to selectivity.

Experimental Section

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 a Brucker 250, 400, or 500 MHz Varian instrument. IR spectra were obtained using KBr plates on a Matson Infinity Gold FTIR instrument. UV spectra were obtained using a Beckman DU640 spectrophotometer. HPLC data were collected on either of two Waters instruments: Waters System 600 Dual Pump Controller or Waters 515 HPLC pump with Waters 996 Photodiode Array detector running Millenium software. HPLC methods are described in the Supporting Information.

2-Deoxy-1-α-bromo-2-*β*-fluoro-3,5-di-*O*-benzoyl-d-ribofuranose (4). Procedure A. A three-neck round-bottom flask (3 L) was equipped with a stir bar and nitrogen inlet adapter and charged with dichloromethane (1.44 L) and commercially available 2-deoxy-2-*β*-fluoro-1,3,5-tri-*O*-benzoyl-1-α-D-ribofuranose (354.3 g, 0.763 mol) at room temperature to give a solution. HBr (33% in acetic acid) (279 mL, 1.62 mol) was charged (the solution changed from colorless to golden yellow to orange), the nitrogen line was removed to ensure that HBr was not lost, and the resultant mixture was stirred at ambient temperature overnight. The acid was neutralized with saturated sodium bicarbonate solution (aq.) (5 × 800 mL portions) using a separatory funnel, whereupon the pH of the aqueous layer was 7−8. The organic phase was separated, dried over $MgSO_4$ (150 g), and filtered. An HPLC chromatogram revealed that all the benzoic acid byproduct had been removed. Rotary

evaporation of the solvents, followed by pumping under high vacuum, afforded **4** (313.4 g, 0.740 mol) as a viscous yellow gum. This material was stored under nitrogen and used without further purification. The NMR spectrum was consistent with that reported in the literature.[4] Although **4** crystallizes slowly from concentrated dichloromethane solution, it is possible to obtain the compound as a solid through seeding.

Procedure B. A 1000-mL flask was charged with 2-deoxy-2-β-fluoro-1,3,5-tri-*O*-benzoyl-1-α-D-ribofuranose (45.3, g, 97.55 mmol), HBr (33 wt % in acetic acid, 35 mL, 204.9 mmol), and CH_2Cl_2 (200 mL). The flask was capped with a rubber septum to prevent escape of HBr. The reaction mixture was stirred at ambient temperature for 18.5 h. HPLC analysis showed no starting material remaining. The reaction mixture was poured into saturated $NaHCO_3$ (600 mL) with stirring. Stirring was stopped, and the layers were allowed to separate. The top aqueous layer was discarded. The bottom organic layer was washed with saturated $NaHCO_3$ (aq.) (100 mL). The aqueous layer pH was 8−8.5 (pH paper). The organic layer was dried with $MgSO_4$ and $NaHCO_3$. The organic layer was filtered, and the filtrate was combined with heptane (350 mL). The volume was reduced by rotary evaporation to remove CH_2Cl_2. The resulting emulsion was seeded with authentic crystals of **4** while stirring. Crystals started to form within 1 min. Solids stuck to the sides of the flask. The solids were scraped from the sides of the flask and the suspension was stirred at ambient temperature for 45 min. The mixture was filtered, and the flask and filter cake were washed with the mother liquor. The wet solid was placed in a vacuum oven (40 °C, 27 inHg) for 3.5 h. The white solid weighed 36.5 g (88.5% yield).

Mp = 73 °C. ^{1}H NMR 400 MHz ($CDCl_3$) δ 4.69−4.86 (m, 3H, $H_{4'}$, $H_{5'}$), 5.54 (dm, 1H, J = 18, H_3), 5.60 (d, 1H, J = 48, H_2), 6.64 (d, 1H, J = 10, H_1), 7.04−7.63 (m, 6H, aromatic H), 8.04−8.13 (m, 4H, aromatic H) ppm. ^{13}C NMR 100 MHz ($CDCl_3$) 166.0 (CO), 165.5 (CO), 133.9 (para), 133.2 (para), 130.0 (ortho), 129.8 (ortho), 129.4 (ipso), 128.6 (meta), 128.5 (ipso), 128.4 (meta), 100.6 (d, J_{CF} = 191, C_2), 87.5 (d, J_{CF} = 30, C_1), 84.7 (C_4), 76.2 (d, J_{CF} = 33, C_3), 62.5 (C_5) ppm. ^{19}F NMR ($CDCl_3$) −167.2 (ddd, J = 50, 22, 12 Hz) ppm. COSY and NOESY spectra were consistent with the assigned structure.

Representative Experimental Procedure for a Parallel Reaction Experiment (See Tables 1−5). A set of 5-mL screw cap vials with stir bars was charged with 50 mg (0.29 mmol) of 2-chloroadenine, followed by the indicated base and anhydrous MeCN (1.0 mL). A stock solution of **4** was prepared by dissolving 1.25 g in 5.0 mL of anhydrous MeCN. The stock solution of **4** (600 μL, 0.28 mmol) was added to each vial at room temperature, and the mixture was stirred for 2 days. The reactions were analyzed by HPLC.

6-Amino-2-chloro-9-(2′-deoxy-2′-fluoro-3′,5′-di-*O*-benzoyl-β-d-arabinofuranosyl)-9*H*-purine (5). A jacketed glass 1-L reactor, equipped with mechanical stirrer, reflux condenser, and temperature probe under stirring (189 rpm) was charged with 2-chloroadenine (20 g, 0.12 mol) and acetonitrile (100 mL) to give a suspension. *tert*-Amyl alcohol (200 mL) was added, followed by potassium *tert*-butoxide (13.9 g, 0.12 mmol) and CaH_2 (5.0 g, 0.12 mol). The mixture was heated to 48 to 53 °C for 40 min. A 1.1 M solution of bromosugar (**4**) (110 mL, 0.12 mol) in 1,2-dichloroethane (DCE) was added, followed by DCE (24 mL), and the mixture was heated at 48 to 53 °C for 19 h. In-process analysis revealed an anomeric ratio of 15 (β/α). After cooling to 27 °C, DCE (300 mL) was charged, the resultant mixture was suction filtered through a pad of Celite, and the filter cake was rinsed with DCE (80 mL). The pH of the filtrate was measured with wet pH paper and found to be 6. Rotary evaporation of the filtrate afforded 79.7 g of crude solid. This was dissolved in butyl acetate (150 mL). Heptane (750 mL) was added to the butyl acetate solution over 1 h, and the resultant suspension was cooled to 5 to −5 °C for 1 h and filtered and the filter cake was washed with a mixture of 5:1 (v:v) heptane/butyl acetate (160 mL) and then with heptane (100 mL) to afford 40.7 g of crude **5** with an anomeric ratio of 15 (β/α). A portion (40 g) of this material was mixed with methanol (400 mL), and the resultant slurry was stirred (approximately 215 rpm) at 20 to 30 °C for 16 h. The mixture was cooled to 0 to −10 °C, stirred for 60 min, and filtered. The filter cake was washed with methanol (160 mL) and heptane (164 mL). After vacuum-drying, **5** (30.4 g, 0.059 mol) was obtained in 50% yield with an anomeric ratio of 80.1 (β/α). This material could be used in the subsequent step without further purification.

Chromatographic Preparation of a Reference Standard for 5. Benzoylated clofarabine **5** (54.5 g, 0.11 mol) was mixed with EtOAc (230 mL) and stirred overnight. The undissolved solid (18.2 g) was removed by filtration, and the filtrate was chromatographed on a silica gel column (13 × 51 cm^2) with 2:1 EtOAc/heptane eluent. The TLC on silica gel (1:1 EtOAc/heptane) gave R_f values of 0.21 and 0.10 for **5** and **6**, respectively. Fractions (500 mL) were collected and analyzed by HPLC. Fractions containing >99.9% **5** were pooled and evaporated to afford pure **5** (22 g, 0.043 mol).

Mp = 159−162 °C. ^{1}H NMR 250 MHz ($CDCl_3$) δ 4.53−4.57 (m, 1H, $H_{4'}$), 4.80 (d, 1H, J = 4, $H_{5'}$), 5.60 (dd, 1H, J = 50, J = 3, $H_{2'}$), 5.74 (dd, 1H, J = 15, J = 3, $H_{3'}$), 6.48 (s, 2H, NH_2), 6.56 (dd, 1H, J = 23, J = 3, $H_{1'}$), 7.40−7.67 (m, 6H, aromatic H), 8.05−8.09 (m, 5H, aromatic H and H_8) ppm. ^{13}C NMR ($CDCl_3$) 63 MHz δ 63.27 ($C_{5'}$), 81.12 ($C_{3'}$, $C_{4'}$), 83.42 (d, J_{C-F} = 17, $C_{1'}$), 92.62 (d, J_{C-F} = 192, $C_{2'}$), 117.72 (C_5), 128.04 (Ph), 128.68 (Ph), 129.28 (Ph), 129.69 (Ph), 129.95 (Ph), 133.36 (Ph), 134.14 (Ph), 140.17 (d, J_{C-F} = 7, C_8), 150.47 (C_6), 154.32 (C_4), 156.33 (C_2), 165.16 (CO), 166.12 (CO) ppm. IR (KBr) 3327m, 1725s, 1643s, 1594s, 1452m, 1352m, 1311m, 1270s, 1178m, 1109s, 1096s, 1070m, 1027m, 923w, 711m, 684w cm^{-1}. UV (MeOH) λmax$_1$ 212 nm (ϵ = 33 608), λmax$_2$ 232 nm (ϵ = 30 876), λmax$_3$ 262 nm (ϵ = 17 253). MS *m*/*z* (% rel. abundance) 512 (MH^+) (100), 513 (24), 514 (32), 515 (5) (characteristic 100:32:5 Cl isotope pattern observed for parent ion). Anal. Calcd for $C_{24}H_{19}ClFN_5O_5$: C, 56.31; H, 3.74; N, 13.68; F, 3.71; Cl, 6.93. Found: C, 56.26; H, 3.78; N, 13.08; F, 3.57; Cl, 6.72. COSY and NOESY spectra support the assigned structure.

Chromatographic Preparation of a Reference Standard for 6-Amino-2-chloro-9-(2′-deoxy-2′-fluoro-3′,5′-di-*O*-benzoyl-α-D-arabinofuranosyl)-9*H*-purine (6). A sample of crude **5** (43.9 g, 0.086 mol) was stirred with EtOAc (250 mL) and filtered to remove a small amount of insoluble material. The filtrate was chromatographed on a silica gel column (13 × 54 cm), eluting with 1:1 EtOAc/heptane (R_f (**6**) = 0.10). Fractions (500 mL) were analyzed by HPLC, and those containing >98% pure **6** were pooled and evaporated to afford **6** (8.27 g, 0.016 mol). Combined samples from several columns were pooled and further purified by preparatory HPLC (Waters Nova-Pak Silica column, 6 μm, 19 × 300 mm^2), eluting with EtOAc/MTBE/heptane (1:1:1) containing 1 mL of Et_3N per 250 mL of EtOAc. Pure fractions were pooled, evaporated, and dried under high vacuum to afford **6** (3.28 g, 6.4 mmol).

Mp = 101 to 104 °C. ^{1}H NMR 250 MHz ($CDCl_3$) δ 4.63−4.75 (m, 2H, $H_{5'}$), 4.93−4.96 (m, 1H, $H_{4'}$), 5.79 (dm, J = 17, $H_{3'}$), 6.16 (d, 1H, J = 49, $H_{2'}$), 6.43 (d, 1H, J = 14, $H_{1'}$), 6.73 (s br, 2H, NH_2), 7.25−7.77 (m, 8H, aromatic H), 8.00−8.09 (m, 3H, aromatic H and H_8) ppm. ^{13}C NMR 63 MHz ($CDCl_3$) δ 63.31 ($C_{5'}$), 77.20 ($C_{1'}$), 83.97 ($C_{4'}$), 89.24 (d, J_{C-F} = 36, $C_{3'}$), 96.50 (d, J_{C-F} = 188, $C_{2'}$), 118.88 (C_5), 128.04 (Ph), 128.42 (Ph), 128.53 (Ph), 129.28 (Ph), 129.63 (Ph), 129.76 (Ph), 133.31 (Ph), 133.90 (Ph), 138.92 (C_8), 150.15 (C_6), 154.36 (C_4), 156.40 (C_2), 164.97 (CO), 166.10 (CO) ppm. IR (KBr) 3343m, 1726s, 1642s, 1593s, 1453m, 1345m, 1315m, 1269s, 1179m, 1096s, 1028w, 710s cm^{-1}. UV (MeOH) λmax$_1$ 213 nm (ϵ = 32 399), λmax$_2$ 231 nm (ϵ = 28 187), λmax$_3$ 263 nm (ϵ = 15 108). MS *m*/*z* (% rel. abundance) 217 (51), 279 (30), 512 (MH^+) (100), 514 (42), 515 (6), 534 (4).

6-Amino-2-chloro-9-(2′-deoxy-2′-fluoro-5′-*O*-benzoyl-β-D-arabinofuranosyl)-9*H*-purine (7). A three-neck 1-L flask was charged with 2-chloroadenine (29.2 g, 172 mmol), MeCN/*tert*-amyl alcohol (2:1) (v/v) (440 mL), potassium *tert*-butoxide (20.3 g, 181 mmol), and CaH_2 (7.24 g, 172 mmol). The mixture was heated for 30 min at 48−58 °C. Bromosugar (**4**) (72.96 g, 172 mmol) was added as a solution in DCE (146 mL). Stirring was continued for 25 h at approximately 50 °C. The reaction was allowed to cool to ambient temperature, and DCE (300 mL) was added. The mixture was cooled to 0−5 °C for 2.5 h and filtered through Celite, which was washed with DCE (2 × 20 mL). Rotary evaporation of the filtrate gave a solid, which was dissolved in butyl acetate (221 mL). Heptane (1550 mL) was then added to the butyl acetate solution with vigorous stirring over 1 h. The resultant suspension was cooled in an ice bath for 1 h and filtered, and the filter cake was washed with butyl acetate/heptane (1:7) (v/v). Drying under vacuum afforded 67.5 g of white solid. A portion of the solid (57.4 g) was suspended in MeOH (570 mL) and heated to reflux for 30 min. The resultant slurry was stirred at ambient temperature for 14 h. Solids were filtered and washed with MeOH (15 mL) and dried under vacuum to afford **6** (11.4 g, 22 mmol) with an anomeric ratio of 164:1 (β/α). Concentration of the combined methanol filtrates afforded 44.7 g of white solid. This solid was dissolved in butyl acetate (550 mL), and then heptane (784 mL) was added to generate a solid (33.0 g), which was filtered and dried. The HPLC analysis of this material revealed a mixture of clofarabine (**3**) and **7** (1:2.1). The mixture was purified by flash chromatography on a silica gel (2.08 kg) column, eluting with 3:1 (EtOAc/heptane) (silica gel TLC R_f (**7**) = 0.48 in 4:1 EtOAc/heptane). Fractions were collected and analyzed by TLC and HPLC. The combined pure fractions afforded 12.64 g of **7**.

Mp = 196−198 °C. ^{1}H NMR 250 MHz ($CDCl_3$) δ 4.17−4.23 (m, 1H, $H_{4'}$), 4.53−4.67 (m, 3H, $H_{3'}$, $H_{5'}$), 5.29 (dt, 1H, J = 53, J = 5, $H_{2'}$), 6.37 (dd, 1H, J = 15, $H_{1'}$), 6.19 (d, 1H, J = 5, $OH_{3'}$), 6.39 (dd, 1H, J = 15, 4, $H_{1'}$), 7.48−7.55 (m, 2H, aromatic H), 7.63−7.69 (m, 1H, aromatic H), 7.91 (br s, 1H, NH_2), 7.96−8.00 (m, 2H, aromatic H), 8.17 (d, 1H, J = 2, H_8) ppm. ^{13}C NMR 63 MHz (DMSO) δ 64.1 ($C_{5'}$), 73.7 (d, J_{C-F} = 23, $C_{3'}$), 80.6 (d, J = 6, $C_{4'}$), 81.6 (d, J_{C-F} = 19, $C_{1'}$), 95.0 (d, J = 195, $C_{2'}$), 114.68 (C_5), 128.79 (Ph), 129.27 (Ph), 129.36 (Ph), 133.52 (Ph), 140.1 (C_8), 150.19 (C_6), 153.40 (C_4), 156.85 (C_2), 161.98 (CO) ppm. UV (H_2O/MeOH) λmax$_1$ 212 nm, λmax$_2$ 233 nm, λmax$_3$ 263 nm.

6-Amino-2-chloro-9-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-9*H*-purine (3). Clofarabine. A 1-L reactor equipped with a condenser and overhead stirrer was charged with protected clofarabine (**5**) (25.7 g, 84.8 mmol) and methanol (154 mL) to give a slurry. Sodium methoxide solution (30% w/w in MeOH) (0.20 mL, 1.05 mmol) was added, and the reaction was stirred and heated to 33 °C for 7 h, at which point a clear yellow solution resulted. The reaction was complete by HPLC analysis. The solution was cooled to room temperature and neutralized with glacial acetic acid (0.05 mL). The mixture was cooled to −10 °C for 1 h, and the resultant white solid was suction filtered and washed with −15 °C methanol (77 mL) to afford 15.2 g of wet product. A loss-on-drying analysis performed on a small aliquot revealed 25.3% solvent content. The wet cake was mixed with methanol (310 mL) and heated with stirring to 63 °C, whereupon the solid dissolved. The solution was then cooled to −8 to −12 °C and stirred at this temperature for 1 h, and then the resultant crystals were vacuum filtered. The cake was washed with cold (−15 °C) methanol (22 mL) and dried under vacuum to afford clofarabine (**3**) (9.7 g, 32.01 mmol) in 64% yield.

Mp = 237 °C. ^{1}H NMR 500 MHz (DMSO-d_6) δ 8.27 (d, 1H, J = 5, H_8), 7.87 (br s, 2H, NH_2), 6.32 (dd, J = 14, J = 5, $H_{1'}$), 5.95 (d, 1H, J = 5, $OH_{3'}$), 5.22 (dt, 1H, J = 53, J = 5, $H_{2'}$), 5.08 (t, 1H, J = 6, $OH_{5'}$), 4.43 (dm, 1H, J = 20, $H_{3'}$), 3.85 (m, 1H, $H_{4'}$), 3.60−3.72 (m, 2H, $H_{5'}$) ppm. ^{13}C NMR 126 MHz (DMSO-d_6) δ 60.34 ($C_{5'}$), 72.56 (d, J = 24, $C_{3'}$), 81.44 (d, J = 17, $C_{1'}$), 83.50 (d, J = 6, $C_{4'}$), 95.33 (d, J = 194, $C_{2'}$), 117.35 (C_5), 140.00 (C_8), 150.16 (C_6), 153.26 (C_4), 156.80 (C_2) ppm. IR (KBr) 3330s, 1646s, 1595s, 1507w, 1466w, 1351m, 1307m, 1248w, 1215w, 1038m, 708w cm^{-1}. UV (H_2O) λmax$_1$ 212 nm (ϵ 22 500), λmax$_2$ 263 nm (ϵ 15 989). Mass spec. (electrospray) *m*/*e* (% rel. abundance) 170 (32) (−2-chloroadenine), 300 (26), 302 (39), 303 (M^+), 304 (100) (MH^+), 305 (21), 306 (41). Elem. anal. calcd for $C_{10}H_{11}ClFN_5O_3$: C, 39.55; H, 3.65; N, 23.06; F,

6.26; Cl, 11.67. Found: C, 39.24; H, 3.58; N, 22.98; F, 5.93; Cl, 11.38. Opt. rot. $[\alpha]_D = 39.93°$ ($c = 5$ mg/mL in DMF).

6-Amino-2-chloro-9-(2′-deoxy-2′-fluoro-α-d-arabino-furanosyl)-9*H*-purine (8). A 100-mL three-neck round-bottom flask was charged with **6** (5.5 g, 11 mmol), followed by methanol (44 mL) and then NaOMe (30 wt. % in MeOH) (0.20 mL, 1.1 mmol). The stirred slurry became essentially clear within 10 min. The reaction was complete by 2.5 h, at which point acetic acid (62 μL, 1.1 mmol) was added to adjust the pH to 9. Heptane (44 mL) was added, and the resultant slurry was stirred for 20 h. The layers were separated, and the methanol phase was evaporated. To the residue was added heptane (26 mL) with stirring, followed by additional heptane (26 mL) after 25 min. The white solid was vacuum filtered and washed twice with heptane (10 mL). The resultant solid (3.75 g) was slurried in heptane (50 mL) for 20 min and filtered to give 3.57 g of solid. This was dissolved in methanol (50 mL), and heptane (30 mL) was added. The solvent was evaporated to roughly half of the original volume, and the product was vacuum filtered and washed with heptane (2 $\times$10 mL). Drying under high vacuum afforded **8** (2.87 g, 8.98 mole) in 82% yield, contaminated with 5% (HPLC area) of **3**.

Mp = 237 °C. ^{1}H NMR 250 MHz (DMSO) δ 8.32 (s, 1H, H_8), 7.90 (s br, 2H, NH_2), 6.20 (dd, 1H, $J = 15$, $J = 3$, $H_{1'}$), 6.02 (d, 1H, $J = 4$, $OH_{3'}$), 5.62 (dt, 1H, $J = 52$, $J = 3$, $H_{2'}$), 5.01 (t, 1H, $J = 6$, $OH_{5'}$), 4.36 (dm, 1H, $J = 20$, $H_{3'}$), 4.20−4.27 (m, 2H, $H_{4'}$), 3.48−3.63 (m, 2H, $H_{5'}$) ppm. ^{13}C NMR ($CDCl_3$) δ 60.6 (C5′), 73.3 (d, $J_{C-F} = 23$, $C_{3'}$), 85.9 (d, $J_{C-F} = 20$, $C_{1'}$), 86.21 (d, $J_{C-F} = 10$, $C_{4'}$), 99.41 (d, $J_{C-F} = 184$, $C_{2'}$), 118.08 (C_5), 139.87 (C_8), 150.03 (C_6), 153.22 (C_4), 156.84 (C_2) ppm. UV (H_2O) λmax$_1$ 210 nm (ϵ 23 406), λmax$_2$ 263 nm (ϵ 13 765). IR (KBr) 3321s, 1661s, 1599s, 1507w, 1466w, 1354m, 1262m, 1211w, 1102w, 1042m, 940m, 840w, 769w cm^{-1}. Mass spec. (electrospray) *m*/*e* (% rel. abundance) 170 (21) (−2-chloroadenine), 300 (24), 303 (64) (M^+), 304 (100) (MH^+), 305 (14), 306 (27).

Supporting Information Available

General experimental methods; chromatograms for Tables 1−3, 5, 6, and 8; and characterization data for compounds 3−8. This material is available free of charge via the Internet at http://pubs.acs.org.

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