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Isolation, Synthesis, and Characterization of Impurities and Degradants from the Clofarabine Process

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Abstract:

The identification of clofarabine process impurities and their subsequent isolation, synthesis, and characterization is described. Two isomeric process impurities resulting from N_6-attachment of a fluoroarabinose to clofarabine were found. Clofarabine's base degradation products, which were different from the process impurities, were also synthesized and characterized. These compounds resulted from modifications to the sugar moiety, the purine ring, or both. A mechanistic rationale for the formation of the various process impurities and degradation products is provided.

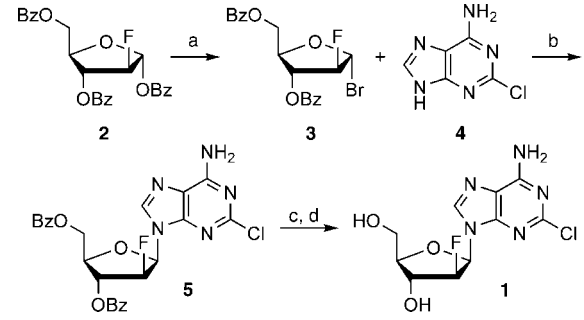
Introduction

Clofarabine (**1**) is the active ingredient in the recently approved pediatric antileukemia drug Clolar. The process for manufacturing clofarabine[1] resulted in some impurities at ≥0.10% (HPLC area). Consequently, it was a regulatory requirement to isolate and characterize these substances. Six major degradation products resulted from heating clofarabine in aqueous sodium hydroxide. This report describes the identification, synthesis (or isolation), and characterization of the clofarabine impurities and degradants.

Process Impurities. The clofarabine process is shown in Scheme 1.[1] Fluoroarabinose **2** is converted to the corresponding bromosugar **3** using HBr/HOAc in dichloromethane. Bromosugar **3** is next condensed with 2-chloroadenine (**4**) using KO*t*-Bu. The resulting nucleoside **5** is precipitated from *n*-butyl acetate using heptane, then purified by slurrying in hot methanol. Nucleoside **5** is deprotected using catalytic sodium methoxide in methanol, followed by recrystallization from methanol to give clofarabine (**1**).

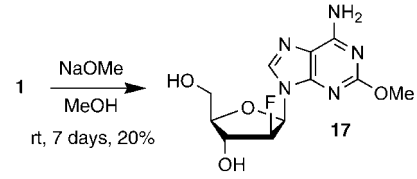
Although this process is more efficient than earlier ones,[2] it resulted in a number of process impurities routinely observed in the API in levels greater than 0.10% (HPLC area). Accordingly,[3] these impurities as well as several potential impurities, were identified and characterized.

Scheme 1. **Clofarabine manufacturing process**[a]



[a] Reagents, conditions, and yields: a) HBr/HOAc, CH_2Cl_2, rt, 19 h, 89% yield; b) KO*t*-Bu, MeCN, $ClCH_2CH_2Cl$, *tert*-amyl-OH, 50 °C, 19 h, 50% yield; c) MeOH, NaOMe, rt, 5 h; d) recrystallization, MeOH, 64 °C to rt, 64% yield (2 steps).

Purine Impurities. Six potential and observed nucleoside impurities were prepared. Some of these were anticipated to arise from related impurities in the starting materials. For example, purines **10** and **11** were observed as impurities in 2-chloroadenine and would react to form nucleosides **13** and **14**, respectively. Nucleoside **15** was detected as a minor component in the API, resulting from displacement of the 2-chloro substituent of **4** by bromide during the condensation step. Nucleoside **16** was also detected in a manufacturing batch and determined to arise from the 2-chlorosugar impurity **8**. The syntheses of these impurities are shown in Scheme 2 and eq 1.



(1)

The chloroarabinose **8** was synthesized by conversion of **6** to the triflate **7** followed by treatment with LiCl.[4] Treatment of **8** with HBr/HOAc gave the bromosugar **9**. Purine **10** was made by reacting dimethylamine with 2,6-dichloropurine.[5] Purine **11** is commercially available. Diazotization of 2,6-diaminopurine in the presence of antimony tribromide afforded purine **12**.[6] The coupling reactions to produce the nucleosides **13**−**16** employed conditions similar to those of the coupling step of the clofarabine process. Nucleoside **17** was thought to have originated from the formal S_NAr reaction of sodium methoxide

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(1) Bauta, W. E.; Schulmeier, B. E.; Burke, B.; Puente, J. F.; Cantrell, W. R., Jr.; Lovett, D.; Goebel, J.; Anderson, B.; Ionescu, D.; Gou, R. *Org. Process Res. Dev.* **2004**, *8*, 889–896.

(2) Montgomery, J. A.; Shortnacy-Fowler, A. T.; Clayton, S. D.; Riordan, J. M.; Secrist, J. A., III. *J. Med. Chem.* **1992**, *35*, 399–401. Montgomery, J. A.; Fowler, A. T.; Secrist, J. A., III. Patent WO 01/60383 A, 2001.

(3) The following are the ICH documents and FDA Guidelines which cover the topic of impurities and degradants for control of drugs during development to ensure patient safety: ICH guidelines Q6A, Q3A, Q3B, Q2A, Q2B, Q1A(R2), Q1E; FDA Guidelines: "Analytical Procedures and Methods Validation", "INDs - Approaches to Complying with CGMP During Phase 1", "INDs for Phase 2 and Phase 3 Studies Chemistry, Manufacturing, and Controls Information".

(4) Tann, C. H.; Brodfuehrer, P. R.; Brundidge, S. P.; Sapino, C., Jr.; Howell, H. G. *J. Org. Chem.* **1985**, *50*, 3644–3647.

(5) Montgomery, J. A.; Holum, L. B. *J. Am. Chem. Soc.* **1958**, *80*, 404–408.

(6) Sampath, U.-S.; Bartlett, L. U.S. Patent 6,252,061 B1, 2001.

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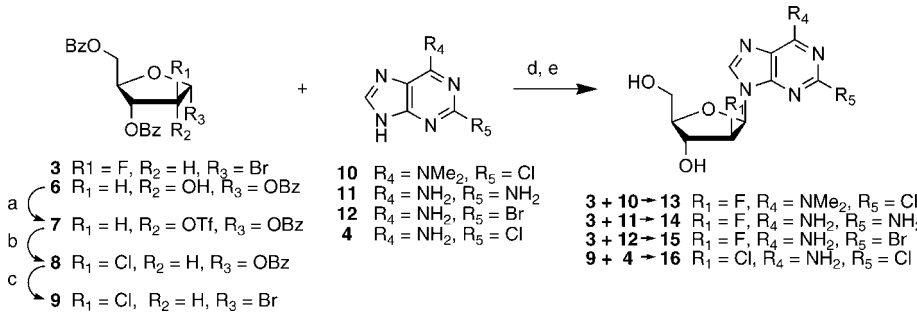
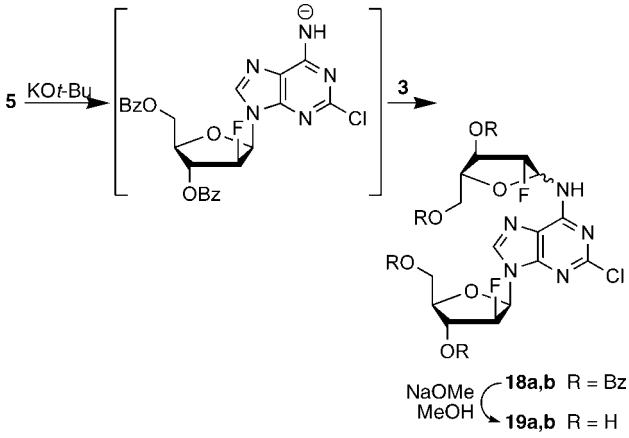
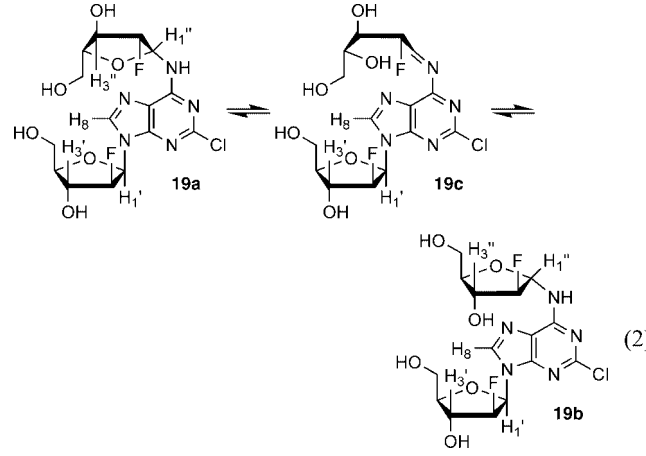
Scheme 2. **Synthesis of purine impurities**[a]



[a] Reagents and conditions: a) Tf_2O, CH_2Cl_2, −30 to −4 °C, 3 h; b) LiCl, NMP, rt, 18 h; c) HBr/HOAc, CH_2Cl_2, rt, 17 h; d) KO*t*-Bu, CaH_2, MeCN, $ClCH_2CH_2Cl$, 50 °C, 1 to 16 h; e) MeOH, NaOMe, rt, 2 to 28 h.

Scheme 3. **Possible pathway to the bis-sugar impurities**





with clofarabine in the deprotection step and was formed under the deprotection conditions (eq 1).

Bis-sugar Nucleosides. Two isomeric process impurities were detected by LC/MS with a mass of 437. The structures **19a**,**b** were proposed for these compounds. They were thought to arise via deprotonation of the exocyclic purine nitrogen and subsequent condensation with **3** to afford **18a**,**b**, which then gave rise to **19a**,**b** upon deprotection (Scheme 3).[7]

Attempts to form **18a**,**b** by addition of excess KO*t*-Bu and excess bromosugars **3** to **5** failed to significantly increase the amount of **18a**,**b** in the mixture, even when zinc chloride was used as a promoter.[6] It was found that the best conditions for formation of **18** were simultaneous addition of **3** and KO*t*-Bu to a 65−70 °C solution of protected clofarabine **5** in MeCN, whereby the isomers constituted up to 34% of the crude mixture by HPLC and were isolable by silica chromatography. However, when the individual purified isomers (**18a** or **18b**) were exposed to the debenzoylation conditions (NaOMe, MeOH), rapid isomerization to a 55:45 mixture of the unprotected bis-sugar nucleosides (**19a**,**b**) occurred. The resulting isomeric mixture could then be separated by preparative HPLC. The mechanism for this isomerization is unclear, but may involve deprotonation of the exocyclic N_6 to reversibly afford the imine **19c** (eq 2).

NMR (^1H, ^{13}C, COSY, NOESY) data for **19a** and **19b** support the assigned structures. The stereochemical assignment at the anomeric carbons ($C_{1'}$ and $C_{1''}$) is somewhat more tenuous. On the basis of the mechanism, one would not anticipate loss of anomeric stereochemistry at $C_{1'}$. Indeed, this is supported by observed NOEs between H_8 and $H_{3'}$ in both **19a** and **19b**. Compound **19a** has an additional NOE between H_8 and $H_{3''}$, which supports an assignment where these substituents are on the same face of the sugar ring. Interestingly, the $H_{1''}$ resonanace in all the bis-sugar compounds appears broad, while $H_{1'}$ is a sharp doublet of doublets.

Degradants. ICH guidelines require that drug substances and drug products be stressed to aid in the development of stability-indicating analytical methods.[8] Clofarabine is a relatively stable compound; very little degradation was observed upon exposure to acid, peroxide, and light. However, several degradants were observed when clofarabine was dissolved in aqueous 1 M NaOH and heated to 80 °C for 1 h. The reaction mixture was sampled, and the resulting HPLC chromatogram revealed six major degradants. The degradants were labeled A through F according to their relative retention times (RRT). Table 1 shows the results of the UV and mass spectral analysis.

The UV spectra of degradants A, B, and C are noticeably different from that of clofarabine as well as different from each other. Assuming that there is no contribution by the sugar moiety to the UV spectra, we thought that degradants A, B, and C had undergone some modification of the purine. Comparison of the UV spectra of degradant C and guanosine showed a remarkable similarity. The UV spectra of degradants D, E, and F are very similar to clofarabine, suggesting that little or no modification of the purine had occurred in these

(7) Related structures have been reported: Jain, P.; Anand, N. *Indian J. Chem.* **1968**, *6*, 616–618.

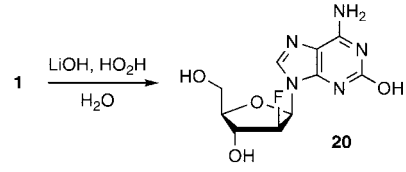
(8) Reynolds, D. W.; Facchine, K. L.; Mullaney, J. F.; Alsante, K. M.; Hatajik, T. D.; Motto, M. G. *Pharm. Technol.* **2002**, (February), 48–56.

Table 1. **Results from LC/MS analysis of the degradant mixture**

cmpd	RRT	UV_{max}, nm	% area	mass	MS isotopic Cl pattern?
degradant A (**29**)	0.322	219, 277	2.64	265	no
degradant B (**20**)	0.489	248, 292	5.34	285	no
degradant C (**23**)	0.572	252	6.04	285	no
degradant D (**28**)	0.872	264	5.02	283	yes
degradant E (**24**)	0.920	264	6.15	283	yes
clofarabine (**1**)	1.000	264	53.58	303	yes
degradant F (**31**)	1.450	264	6.87	570	yes

degradants. Degradants A, B, and C also showed loss of the signature isotopic chlorine pattern in the mass spectrum. Assuming replacement of chloride by hydroxyl via formal S_NAr reaction, then the expected mass of the isomeric guanidines would be 285. Both degradants B and C have a mass of 285. Degradants D and E had a molecular weight of 283, which could result from a base-induced HF elimination. Degradant A has a mass of 265 which could result from the loss of HF in addition to replacement of chlorine by hydroxyl. NMR data later confirmed the loss of fluorine by lack of distinctive coupling patterns in both the proton and carbon spectra of degradants A, D, and E. Degradant F has a mass of 570, which was assumed to be a dimer of clofarabine minus HCl.

The alkaline degradation was repeated under various conditions via design of experiments (DoE) in an attempt to optimize the production of individual degradants for isolation. Unfortunately, the enhancements in individual degradant levels was insufficient for preparative purposes in most cases. The best approach to characterizing the degradants was to synthesize them. The degradants with a mass of 285 were our first synthetic targets. It is known that peroxide anion is more nucleophilic than hydroxide.[9] Therefore, clofarabine was reacted with hydrogen peroxide in aqueous lithium hydroxide to cleanly give degradant B (**20**).[10] After purification and characterization, **20** was assigned the structure shown in eq 3.



(3)

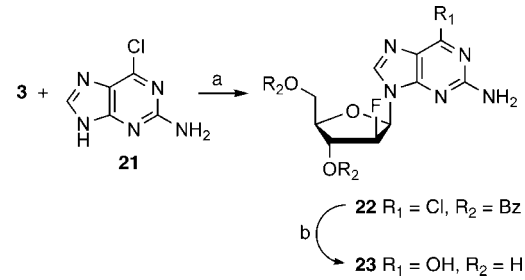
We assumed that the other degradant with a mass of 285 was derived by a base-induced rearrangement as was reported in the hydroxide degradation of cladribine.[11] We reasoned that a nucleoside such as **22** would react with hydroxide to give the isomeric degradant C (**23**). Synthesis of the isomeric nucleoside **22** was accomplished under clofarabine coupling conditions, and the subsequent S_NAr reaction with hydroxide cleanly produced **23** (see Scheme 4).

(9) Smith, M. B.; March, J. *March's Advanced Organic Chemistry*, 5th ed.; Wiley Inter-Science: New York, 2001; p 445.

(10) (a) Pewkupec, S.; Svedružić-Gazivoda, T.; Mrvoš-Sermek, D.; Nagl, A.; Grdiša, M.; Pavelić, K.; Balzarini, J.; De Clerq, E.; Folkers, G.; Scapozza, L.; Mintas, M.; Raić-Malić, S. *J. Med. Chem.* **2003**, *46*, 5763–5772. (b) Cantrell, W. R., Jr.; Bauta, W. E.; Engles, T. *Tetrahedron Lett.* **2006**, *47*, 4249.

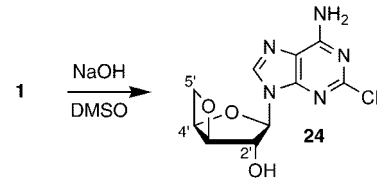
(11) Andrzejewska, M.; Dzierzgowska-Szmidt, A.; Kazimierczuk, Z. *Pharmazie* **2003**, *58*, 122–124.

Scheme 4. **Independent synthesis of degradant C (23)**[a]



[a] Reagents and conditions: a) KO*t*-Bu, CaH_2, MeCN, $ClCH_2CH_2Cl$, *tert*-amyl-OH, rt, 20 h; b) NaOH, H_2O, rt, 72 h.

Degradant E was successfully synthesized and isolated by using nonaqueous degradation conditions (eq 4). When clofarabine was heated with solid NaOH in DMSO, clean formation of degradant E resulted along with the starting material. Unfortunately, further heating resulted in decomposition of the desired degradant. Isolation of degradant E (**24**) was accomplished by chromatography, and sufficient material was acquired for characterization. Extensive NMR experiments indicated that the structure was **24** as shown in eq 4. Proton NMR showed seven resonances attributed to the sugar moiety (compared to eight for clofarabine). The D_2O exchange experiment showed the peak at δ 5.97 as the only peak that was diminishing, which indicated that only one hydroxyl group remained in the molecule. DEPT and HETCOR indicated one methylene and four methine carbon resonances on the sugar moiety. As stated previously, both proton and carbon spectra showed the absence of fluorine. The COSY spectrum indicated that the hydroxyl group was in the 2′ position. The other salient feature of the proton spectrum was separation of the 2 $H_{5'}$ resonances by 0.78 ppm. The spectra of most of the other nucleosides we studied showed the $H_{5'}$ protons to be indistinguishable from each other. One explanation for this phenomenon is restricted rotation about the $C_{4'}-C_{5'}$ bond, which could be accomplished by the oxatane ring found in the structure.



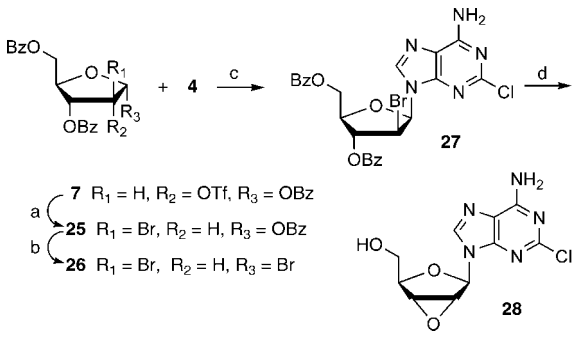
(4)

The formation of **24** might have occurred via intramolecular attack of the 5′ alkoxide to $C_{4'}$ of a $C_{3'}$-$C_{4'}$ epoxide. These nucleoside epoxides are known[12] and are usually formed by base-induced internal nucleophilic displacement of halide with alkoxide.

Coincidently, a nucleoside epoxide of structure **28** fit the data that was obtained for degradant D (Scheme 5). We set out to independently synthesize degradant D from the corresponding 2′-bromonucleoside **27**. The triflate **7** was treated with KBr to give the bromide **25**. Compound **25** was converted to the 1′,2′-dibromo sugar **26** using the clofarabine process conditions. The subsequent coupling reaction with 2-chloroadenine (**4**) gave the

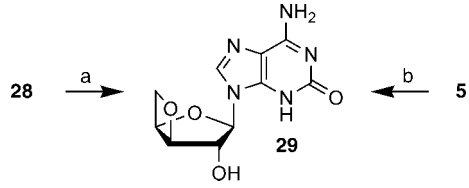
(12) Robles, R.; Rodríguez, C.; Izquierdo, I.; Plaza, M. T.; Mota, A.; de Cienfuegos, L. A. *Tetrahedron: Asymmetry* **2000**, *11*, 3069–3077.

Scheme 5. **Independent synthesis of degradant D (28[a]**



[a] Reagents and conditions: a) LiBr, NMP, rt, 20 h; b) HBr/HOAc, CH_2Cl_2, rt, 23 h; c) KO*t*-Bu, CaH_2, MeCN, $ClCH_2CH_2Cl$, *tert*-amyl-OH, rt, 18 h; d) MeOH, NaOMe, rt, 16 days.

Scheme 6. **Synthetic pathways towards degradant A (29)[a]**



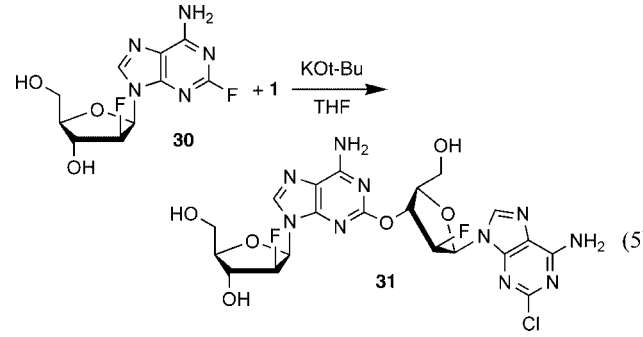
[a] Reagents and conditions: a) H_2O, 100 °C, 5 h; b) H_2O_2, LiOH, H_20, 60 °C, 4 h, trace.

protected nucleoside **27**. Treatment with a slight excess of sodium methoxide cleanly produced degradant D (**28**).

Purification of **28** was accomplished by recrystallization in boiling water. Examination of the mother liquors by HPLC revealed a significant amount of degradant A with a small amount of degradant E (**24**). Therefore, degradant A (**29**) was easily prepared when crude **28** was heated in water at 100 °C (Scheme 6). Interestingly, traces of **29** were also observed in the S_NAr reaction of clofarabine with alkaline peroxide. Further evidence for the assignment of structure **29** for degradant A is the similarity to the proton NMR of **24**.

Attempts made to prepare degradant F by reacting clofarabine (**1**) with the analogous difluoronucleoside **30** were successful on small scale (eq 5). However, when this reaction was scaled up, none of the desired dimers could be isolated. Therefore, degradant F was isolated from a degradation mixture using the DoE optimized conditions. The structure of **31** was confirmed by comparing a proton spectrum with the corresponding D_2O wash spectrum. Three peaks were observed to diminish upon addition of D_2O: a doublet at 5.96 ppm and two triplets at 5.15 and 5.09 ppm, respectively. These peaks corresponded to one 3′-OH and two 5′-OH's, which would give the structure shown for compound **31** in eq 5. Peak assignments were based mostly on the COSY data. The H_8 and NH_2 protons were assigned by analogy to compounds **1** and **17**. Surprisingly, none of the isomer derived from attachment of 5′-OH was detected by LC/MS in the degradation mixtures.

In summary, all significant impurities in the clofarabine process over 0.1% were either synthesized or isolated, and were characterized. In addition, all of the six major compounds from the aqueous sodium hydroxide degradation of clofarabine were isolated and characterized. Confirmation of structure by independent syntheses was achieved for all compounds except for compound **31**.



Experimental Section

Reactions were run under nitrogen. ^1H, ^{13}C, and ^{19}F NMR spectra were obtained at 400, 100, and 376 MHz, respectively. IR spectra were obtained as KBr pellets. UV spectra were obtained as solutions in H_2O/MeCN or H_2O/MeOH. HPLC data were collected using photodiode array detectors on dual pump systems. Conditions for HPLC are given in the Supporting Information.

Arabinose Triflate 7. Triflic anhydride (2.5 mL, 14.9 mmol) was added to a solution of **6** (5.63 g, 12.2 mmol), CH_2Cl_2 (65 mL), and pyridine (4.9 mL, 60.6 mmol) over 2 min at −30 °C during which time the temperature rose to −25 °C. The reaction was allowed to warm to −4 °C over 2.75 h. Reaction progress was judged complete by TLC (silica gel GHLF, 50% EtOAc/50% hexanes, UV_{254}, R_f of **6** = 0.50, R_f of **7** = 0.62). A solution of $NaHCO_3$ (50 mL, 5 wt %) was added (off-gassing). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (25 mL). The organic portions were dried ($MgSO_4$) and concentrated. Heptane was added to the residue, and the mixture was concentrated. The residue was dissolved in *tert*-butyl methyl ether (TBME, 100 mL), and the solution was filtered through silica gel. The silica was washed with TBME (2 × 100 mL), and the combined filtrates were concentrated to a pale yellow oil which was used in the next step as is. ^1H NMR ($CDCl_3$) δ 8.15−8.01 (m, 6H), 7.65−7.58 (m, 3H), 7.50−7.39 (m, 6H), 6.87 (d, 1H, J = 4), 5.79 (dd, 1H, J = 6, 3), 5.55 (dd, 1H, J = 6, 4), 4.86 (q, 1H, J = 3), 4.75 (dd, 1H, J = 12, 3), 4.63 (dd, 1H, J = 12, 3). ^{13}C NMR ($CDCl_3$) 165.8, 165.5, 164.7, 134.0, 133.9, 133.5, 130.09, 130.0, 129.6, 129.1, 128.8, 128.6, 128.5, 128.4, 118.4 (q, J_{CF} = 320), 93.1, 82.0, 79.4, 69.9, 63.4 ppm. UV (H_2O/MeCN) λmax_1 230 nm, λmax_2 274 nm.

Chloroarabinose 8. A suspension of **7** (7.24 g, 12.2 mmol, assuming 100% yield), LiCl (2.58 g, 60.9 mmol), and *N*-methylpyrrolidinone (NMP, 25 mL) were stirred at ambient temperature for 17.5 h. The reaction was judged complete by TLC (silica gel GHLF, 50% EtOAc/50% hexanes, UV_{254}, R_f of **7** = 0.62, R_f of **8** = 0.65). H_2O (100 mL) was added, and the mixture was extracted with TBME (3 × 75 mL). The organic portions were washed with H_2O (100 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography (silica gel, EtOAc/hexanes, 3/17) gave **8** as a clear oil (5.11 g, 98.1% purity, 86% yield). ^1H NMR ($CDCl_3$) δ 8.12−8.03 (m, 6H), 7.61−7.53 (m, 3H), 7.44−7.37 (m, 6H), 6.68 (s, 1H), 5.62 (d, 1H, J = 3), 4.85−4.74 (m, 3H), 4.62 (s, 1H). ^{13}C NMR ($CDCl_3$) 166.1, 165.2, 164.5, 133.8, 133.7, 133.1, 129.8, 129.7, 129.4, 129.0, 128.6, 128.5, 128.4, 128.3, 102.4, 84.3, 79.9, 63.9,

61.8 ppm. IR (KBr) 3436, 3063, 1725, 1601, 1452, 1316, 1270, 1108, 1067, 1025, 937, 708 cm^{-1}. UV (H_2O/MeCN) λmax_1 230 nm, λmax_2 274 nm. MS *m/z* $[M + Na]^+ = 503$. Anal. Calcd for $C_{26}H_{21}ClO_7$: C, 64.90; H, 4.40; Cl, 7.37. Found: C, 64.47; H, 4.26; Cl, 7.45.

Chlorobromosugar 9. HBr/HOAc (3.7 mL, 33 wt %, 21.4 mmol) was added to a solution of **3** (4.88 g, 10.1 mmol) in CH_2Cl_2 (25 mL). The mixture was stirred at ambient temperature for 17 h. The reaction was judged complete by TLC (silica gel GHLF, 15% EtOAc/85% hexanes, UV_{254}, R_f of **8** = 0.23, R_f of **9** = 0.40). Saturated $NaHCO_3$ (100 mL) was added with stirring (off-gassing). The mixture was extracted with CH_2Cl_2 (60 mL). The organics were washed with saturated $NaHCO_3$ (75 mL), dried ($MgSO_4$), and concentrated to give **9** as a yellow oil (4.36 g, 98% recovery), which was used in the next step as is. ^1H NMR ($CDCl_3$) δ 8.12−8.09 (m, 4H), 7.64−7.54 (m, 2H), 7.50−7.41 (m, 4H), 6.61 (s, 1H), 5.58 (d, 1H, $J = 4$), 4.93 (s, 1H), 4.90−4.72 (m, 3H). ^{13}C NMR ($CDCl_3$) 166.1, 165.5, 133.9, 133.3, 130.0, 129.9, 129.4, 128.6, 128.5, 128.4, 90.4, 85.2, 79.6, 66.5, 62.6 ppm. IR (neat) 3063, 3033, 2955, 2926, 2870, 1726, 1602, 1451, 1266, 1098, 710 cm^{-1}. MS *m/z* $[M + Cl]^- = 473$.

2-Chloro-*N*,*N*-dimethyl-9H-purin-6-amine 10. Triethylamine (4.6 mL, 33 mmol) was added to a suspension of 2,6-dichloropurine (3.10 g, 16.4 mmol), dimethylamine hydrochloride (2.01 g, 24.6 mmol), and DMF (10 mL) over 1 min. A solid mass formed with accompanying exotherm. More DMF (4 mL) was added, and the mixture was stirred for 45 min. Saturated $NaHCO_3$ (50 mL) was added, and the mixture was stirred for 10 min and filtered. The wet cake was dried (50 °C, 27 Torr) to give **10** as a white solid (3.00 g, 97.2% purity, 90% yield). Mp = 296−297 °C (dec). ^1H NMR (DMSO-d_6) δ 13.06 (br s, 1H), 8.09 (s, 1H), 3.41 (br s, 6H). ^{13}C NMR (DMSO-d_6) 154.4, 152.3, 152.0, 138.2, 117.9, 37.8 ppm. IR (KBr) 3099, 2955, 2826, 2700, 1593, 1359, 1316, 1277, 964 cm^{-1}. UV (H_2O/MeOH) λmax_1 223 nm, λmax_2 276 nm. MS *m/z* $[M + H]^+ = 198$. Anal. Calcd for $C_7H_8ClN_5$: C, 42.54; H, 4.08; Cl, 17.94; N, 35.44. Found: C, 42.60; H, 3.89; Cl, 18.16; N, 35.14.

2-Bromo-9H-purin-6-amine 12. Dibromomethane (150 mL) and *tert*-butyl nitrite (90 wt %, 9.8 mL, 74.3 mmol) were added to a suspension of 1,2-diaminopurine (10.08 g, 67.2 mmol), antimony(III) bromide (36.40 g, 101 mmol), and DMSO (100 mL) over 20 min, and the mixture was stirred at ambient temperature for 16 h. The reaction was neutralized with saturated $NaHCO_3$ until pH = 7. The mixture was filtered, the crude solid (60% purity) was dried (50 °C, 27 Torr) and suspended in 1.0 M NaOH (100 mL). The mixture was filtered, and the solids were washed with H_2O (5 × 10 mL) and then discarded. The filtrate was neutralized with 12 M HCl until the pH = 6. The resulting suspension was filtered, and the solid was dried (50 °C, 27 Torr). The solid (8.68 g) was suspended in H_2O (100 mL), and the mixture was heated at 85 °C for 1 h. The mixture was cooled to ambient temperature and filtered. The solid was washed with H_2O (3 × 10 mL) and dried (45 °C, 24 Torr) to give **12** as a brown solid (5.29 g, 68% purity). The crude solid was used in the coupling step as is. ^1H NMR (DMSO-d_6) δ 13.0 (br s, 1H), 8.11 (s, 1H), 7.64 (s, 2H). UV (H_2O/MeOH) λmax_1 212 nm, λmax_2 265 nm. MS *m/z* $[M + H]^+ = 214$.

Nucleoside 13. A solution of **3** (7.71 g, 18.2 mmol) in 1,2-dichloroethane (DCE, 6.5 mL) was added to a suspension of **10** (3.00 g, 15.2 mmol), CaH_2 (0.64 g, 15.2 mmol), MeCN (5 mL), and KO*t*-Bu (15.9 mL, 1.0 M, 15.9 mmol) at 50 °C over 10 min. The reaction was stirred at 50 °C for 16 h. The mixture was filtered, and the filtrate was concentrated to give a brown tar (9.74 g). Purification by chromatography (silica gel, EtOAc/heptane, 3/7) gave the protected nucleoside (4.83 g, 95.3% purity, 56% yield). Mp = 84−86 °C. ^1H NMR (DMSO-d_6) δ 8.20 (d, 1H, $J = 3$), 8.10−8.08 (m, 2H), 7.99−7.97 (m, 2H), 7.74−7.70 (m, 1H), 7.67−7.63 (m, 1H), 7.60−7.52 (m, 2H), 7.50−7.48 (m, 2H), 6.57 (dd, 1H, $J = 19, 4$), 5.89 (dm, 1H, $J = 19$), 5.77 (dm, 1H, $J = 50$), 4.78−4.67 (m, 3H), 3.68 (br s, 1H), 3.18 (br s, 1H). ^{13}C NMR (DMSO-d_6) 165.4, 164.8, 154.5, 152.9, 150.9, 138.7 (d, $J_{CF} = 6$), 133.9, 133.5, 129.7, 129.2, 128.7, 128.6, 117.7, 92.9 (d, $J_{CF} = 193$), 82.1 (d, $J_{CF} = 18$), 78.4, 76.3 (d, $J_{CF} = 29$), 63.7, 37.3 ppm. ^{19}F NMR (DMSO-d_6) −198.3 (dt, $J = 51, 19$) ppm. IR (KBr) 3446, 2933, 1726, 1602, 1452, 1314, 1271, 1109, 711 cm^{-1}. UV (H_2O/MeOH) λmax_1 223 nm, λmax_2 276 nm. MS *m/z* $[M + H]^+ = 540$. Anal. Calcd for $C_{26}H_{23}ClFN_5O_5$: C, 57.84; H, 4.29; Cl, 6.57; F, 3.52; N, 12.97. Found: C, 58.44; H, 4.32; Cl, 6.34; F, 3.45; N, 12.39. NaOMe (0.16 mL, 25 wt %, 0.70 mmol) was added to a suspension of the protected nucleoside (1.91 g, 3.54 mmol) in MeOH (25 mL). The mixture was stirred for 2 h and HOAc (40 μL, 0.7 mmol) was added. The filtrate was concentrated and the residue was purified by chromatography (silica gel, EtOAc) to give **13** as a white solid (0.91 g, 95.9% purity, 75% yield). Mp = 169−170 °C. ^1H NMR (DMSO-d_6) δ 8.30 (d, 1H, $J = 2$), 6.36 (dd, 1H, $J = 14, 5$), 5.97 (d, 1H, $J = 5$), 5.25 (dt, 1H, $J = 53, 4$), 5.10 (t, 1H, $J = 6$), 4.44 (ddd, 1H, $J = 19, 9, 5$), 3.87 (dd, 1H, $J = 10, 5$), 3.73−3.62 (m, 5H), 3.19 (br s, 3H). ^{13}C NMR (DMSO-d_6) 154.4, 152.7, 150.9, 138.7, 117.8, 95.2 (d, $J_{CF} = 192$), 83.5 (d, $J_{CF} = 4.2$), 81.5 (d, $J_{CF} = 17$), 72.5 (d, $J_{CF} = 23$), 60.3, 37.4 ppm. ^{19}F NMR (DMSO-d_6) −198.9 (dt, $J = 53, 18$) ppm. IR (KBr) 3374, 3132, 2925, 1723, 1606, 1354, 1311, 1035, 793 cm^{-1}. UV (H_2O/MeOH) λmax_1 218 nm, λmax_2 276 nm. MS *m/z* $[M + H]^+ = 332$. Anal. Calcd for $C_{12}H_{15}ClFN_5O_3$: C, 43.45; H, 4.56; Cl, 10.69; F, 5.73; N, 21.11. Found: C, 43.50; H, 4.42; Cl, 10.66; F, 5.94; N, 21.13.

Nucleoside 14. A solution of **3** (2.48 g, 5.86 mmol) in DCE (10 mL) was added to a suspension of **11** (0.82 g, 4.9 mmol), CaH_2 (0.21 g, 4.9 mmol), MeCN (10 mL), and KO*t*-Bu (5.4 mL, 1.0 M, 5.4 mmol) at 50 °C over 30 min. The reaction was stirred at 50 °C for 16 h and filtered, and the filtrate was concentrated to give a brown tar. Purification by chromatography (silica gel, acetone/EtOAc, 3/7) gave the protected nucleoside as a white solid (0.50 g, 97.6% purity, 20% yield). Mp = 127−128 °C. ^1H NMR (DMSO-d_6) δ (mixture of anomers). ^{13}C NMR (DMSO-d_6) (mixture of anomers). ^{19}F NMR (DMSO-d_6) minor anomer −190.5 (dt, $J = 50, 16$) ppm, major anomer −198.7 (dt, $J = 50, 20$) ppm. IR (KBr) 3460, 3329, 3189, 1724, 1604, 1271, 1109, 711 cm^{-1}. UV (H_2O/MeOH) λmax_1 220 nm, λmax_2 279 nm. MS *m/z* $[M + H]^+ = 493$. Anal. Calcd for $C_{24}H_{21}FN_6O_5$: C, 58.53; H, 4.30; F, 3.86;

N, 17.07. Found: C, 58.99; H, 4.31; F, 3.96; N, 16.19. NaOMe (0.10 mL, 25 wt %, 0.44 mmol) was added to a suspension of the protected nucleoside (1.09 g, 2.21 mmol) in MeOH (23 mL). The mixture was stirred for 17 h and HOAc (0.2 mL) was added. The filtrate was concentrated and triturated with heptane. Purification by chromatography (silica gel, EtOAc/CH_2Cl_2, 2/3) gave **14** as a white solid (0.12 g, 99.0% purity, 19% yield). Mp = 175−178 °C (dec). ^{1}H NMR (DMSO-d_6) δ 7.81 (d, 1H, J = 3), 6.81 (s, 2 H), 6.20 (dd, 1H, J = 17, 4), 5.94 (d, 1H, J = 4), 5.91 (s, 2H), 5.11 (dt, 1H, J = 53, 4), 5.10 (br t, 1H, J = 5), 4.43−4.36 (m, 1H), 3.83 (dd, 1H, J = 10, 5), 3.68−3.59 (m, 2H). ^{13}C NMR (DMSO-d_6) 160.4, 156.2, 151.5, 136.0, 112.4, 95.3 (d, J_{CF} = 192), 83.5, 81.1 (d, J_{CF} = 17), 72.9 (d, J_{CF} = 23), 60.6 ppm. ^{19}F NMR (DMSO-d_6) −198.5 (dt, J = 52, 17) ppm. IR (KBr) 3337, 3204, 2929, 1605, 1478, 1415, 1344, 1279, 1222, 1040, 792 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 215 nm, λmax$_2$ 255 nm, λmax$_3$ 279 nm. MS m/z $[M + H]^+$ = 285. Anal. Calcd for $C_{10}H_{13}FN_6O_3$: C, 42.26; H, 4.61; F, 6.68; N, 29.57. Found: C, 42.05; H, 4.88; F, 6.38 N, 25.90.

Compound 15. A solution of **3** (6.61 g, 15.6 mmol) in DCE (45 mL) was added to a suspension of **12** (67.5% purity, 4.50 g, 14.2 mmol), KO*t*-Bu (1.67 g, 14.9 mmol), CaH_2 (0.60 g, 14.3 mmol), MeCN (25 mL), and *tert*-amyl alcohol (TAA, 25 mL) at 50 °C over 38 min. The mixture was stirred at 50 °C for 16 h and filtered, and the filtrate was concentrated. Purification by chromatography (silica gel, EtOAc/hexanes, 3/2) gave a solid (3.00 g, 92% purity). Further purification by trituration in MeOH (25 mL) gave the protected nucleoside (1.82 g, 98% purity, 23% yield). Mp = 121−122 °C dec. ^{1}H NMR (DMSO-d_6) δ 8.19 (d, 1H, J = 3), 8.19−7.98 (m, 4H), 7.76−7.50 (m, 6H), 6.57 (dd, 1H, J = 18, 4), 5.94 (dq, 1H, J = 19, 2), 5.80 (dq, 1H, J = 51, 2), 4.82−4.68 (m, 3H). ^{13}C NMR (DMSO-d_6) 165.4, 164.8, 156.6, 150.0, 144.6, 139.8 (d, J_{CF} = 6), 133.9, 133.5, 129.7, 129.2, 128.7, 128.6, 118.0, 92.9 (d, J_{CF} = 189), 82.0 (d, J_{CF} = 17), 78.3, 76.3 (d, J_{CF} = 28), 63.7 ppm. ^{19}F NMR (DMSO-d_6) −198.2 (dt, J = 51, 18) ppm. IR (KBr) 3463, 3358, 3167, 2359, 1726, 1642, 1586, 1452, 1349, 1272, 1096, 711 cm^{-1}. UV (H_2O/MeCN) λmax$_1$ 214 nm, λmax$_2$ 231 nm, λmax$_3$ 264 nm. MS m/z $[M + H]^+$ = 556. Anal. Calcd for $C_{24}H_{19}BrFN_5O_5$: C, 51.81; H, 3.44; Br, 14.36; F, 3.41; N, 12.59. Found: C, 53.01; H, 3.14; Br, 12.06; F, 3.55; N, 11.57. NaOMe (0.14 mL, 25 wt %, 0.65 mmol) was added to a suspension of the protected nucleoside (1.72 g, 3.09 mmol) in MeOH (35 mL),and the mixture was stirred at ambient temperature for 4.7 h. HOAc (0.5 mL) was added,and the reaction mixture was washed with hexanes (3 × 30 mL). The bottom MeOH layer was concentrated and the residue was triturated with IPA/hexane (15 mL/2 mL). The suspension was filtered, and the solid was washed with IPA/hexane (2 mL/2 mL). The solid was suspended in acetone (50 mL) and filtered through silica gel. The filtrate was concentrated to give **15** as a white solid (0.42 g, 98% purity, 39% yield). Mp = 220−221 °C. ^{1}H NMR (DMSO-d_6) δ 8.27 (s, 1H), 7.90 (s, 2H), 6.34 (dd, 1H, J = 14, 4), 5.98 (br s, 1H), 5.25 (d, 1H, J = 53), 5.10 (br s, 1H), 4.45 (d, 1H, J = 19), 3.87 (br s, 1H), 3.70−3.67 (m, 2H). ^{13}C NMR (DMSO-d_6) 156.6, 150.0, 144.5, 139.9, 117.7, 95.3 (d, J_{CF} = 192), 83.5 (d, J_{CF} = 4), 81.4 (d, J_{CF} = 15), 72.6 (d, J_{CF} = 23), 60.4 ppm. ^{19}F NMR (DMSO-d_6) −198.8 (br dd, J = 52, 15) ppm. IR (KBr) 3323, 3160, 2927, 1666, 1594, 1426, 1355, 1298, 1178, 1035, 915, 804, 668, 547 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 213 nm, λmax$_2$ 263 nm. MS m/z $[M + H]^+$ = 348. Anal. Calcd for $C_{10}H_{12}BrFN_5O_3$: C, 34.50; H, 3.18; Br, 22.95; F, 5.46; N, 20.12. Found: C, 34.79; H, 2.88; Br, 23.54; F, 5.63; N, 19.91.

Nucleoside 16. A solution of **9** (4.35 g, 9.89 mmol) in DCE (8 mL) was added to a suspension of **4** (1.53 g, 9.02 mmol), KO*t*-Bu (1.06 g, 9.45 mmol), CaH_2 (0.38 g, 9.03 mmol), MeCN (8 mL), and TAA (8 mL) at 50 °C over 30 min, and the mixture was stirred at 50 °C for 1 h. The reaction mixture was filtered, the filtrate was concentrated, and the residue was purified by slurrying in MeOH (3 × 44 mL) to give the protected nucleoside as an off-white solid (1.78 g, 97% purity, 36% yield). Mp = 172−174 °C. ^{1}H NMR (DMSO-d_6) δ 8.38 (s, 1H), 7.98 (br s, 2H), 8.11−7.94, (m, 4H), 7.76−7.45 (m, 6H), 6.68 (d, 1H, J = 6), 6.24 (t, 1H, J = 6), 5.44 (t, 1H, J = 6), 4.88−4.80 (m, 2H), 4.67 (q, 1H, J = 6). ^{13}C NMR (DMSO-d_6) 165.4, 164.8, 156.8, 153.2, 149.8, 140.1, 133.9, 133.5, 129.6, 129.2, 128.8, 128.7, 128.6, 117.9, 83.6, 78.6, 77.8, 64.0, 60.3 ppm. IR (KBr) 3452, 3331, 3177, 1724, 1639, 1593, 1452, 1307, 1271, 1094, 1027, 710 cm^{-1}. UV (H_2O/MeCN) λmax$_1$ 212 nm, λmax$_2$ 231 nm, λmax$_3$ 264 nm. MS m/z $[M + H]^+$ = 528. Anal. Calcd for $C_{24}H_{19}Cl_2N_5O_5$: C, 54.56; H, 3.62; Cl, 13.42; N, 13.26. Found: C, 54.26; H, 3.32; Cl, 13.39; N, 13.25. NaOMe (0.14 mL, 25 wt %, 0.61 mmol) was added to a suspension of the protected nucleoside (1.58 g, 2.99 mmol) in MeOH (31 mL) and the mixture was stirred at ambient temperature for 28 h. HOAc (0.4 mL) was added, the solvent was concentrated, and the residue was triturated with hexanes (50 mL). The suspension was filtered, and the crude solid was purified by crystallization (acetone/MeOH) to give **16** as a white solid (0.46 g, 99% purity, 48% yield). Mp = 237−241 °C (dec). ^{1}H NMR (DMSO-d_6) δ 8.41 (s, 1H), 7.88 (br s, 1H), 6.42 (d, 1H, J = 6), 6.12 (br d, 1H, J = 4), 5.22 (t, 1H, J = 5), 4.80 (dd, 1H, J = 8, 7), 4.45 (br q, 1H, J = 4), 3.86−3.72 (m, 3H). ^{13}C NMR (DMSO-d_6) 156.8, 153.2, 150.3, 139.7, 117.5, 83.6, 82.6, 73.8, 63.6, 59.8 ppm. IR (KBr) 3389, 3216, 3113, 2906, 2360, 1649, 1600, 1461, 1305, 1083, 708 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 211 nm, λmax$_2$ 265 nm. MS m/z $[M + H]^+$ = 320. Anal. Calcd for $C_{10}H_{11}Cl_2N_5O_3$: C, 37.52; H, 3.46; Cl, 22.15; N, 21.88. Found: C, 37.66; H, 3.23; Cl, 22.01; N, 21.84.

Compound 17. A suspension of clofarabine (**1**, 1.04 g, 3.42 mmol), MeOH (20 mL), and NaOMe (1.56 mL, 25 wt %, 6.84 mmol) was heated at 60 °C for 7 days. The reaction was quenched with 5 M HCl until pH = 6 and was filtered, and the filtrate was concentrated. Purification by chromatography (silica gel, MeOH/CH_2Cl_2, 1/9) gave **17** as a white solid (200 mg, 99% purity, 20% yield). Mp = 216 °C. ^{1}H NMR ($CDCl_3$) δ 8.04 (s, 1H), 7.35 (s, 2H), 6.29 (dd, 1H, J = 15, 4), 5.97 (s, 1H), 5.19 (dm, 1H, J = 53), 5.07 (s br, 1H), 4.47 (d, 1H, J = 19), 3.83 (s, 4H), 3.70−3.62 (m, 2H). ^{13}C NMR (DMSO-d_6) 161.9, 156.7, 150.9, 138.2, 114.7, 95.3 (d, J_{CF} = 193), 83.4 (d, J_{CF} = 3), 81.2 (d, J_{CF} = 17), 72.8 (d, J_{CF} = 24), 60.6, 54.0 ppm. IR (KBr) 3470, 3312, 2922, 1639, 1507, 1475, 1369, 1262, 1205, 1119, 1059, 953, 870, 703 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 210 nm, λmax$_2$ 267 nm. MS m/z $[M + Na]^+$ = 322. Anal. Calcd for $C_{11}H_{14}FN_5O_4$: C, 44.15; H, 4.72; F, 6.35; N, 23.40. Found: C, 43.74; H, 4.28; F, 6.09; N, 22.61.

Protected Bis-sugars (18a,b). A suspension of **5** (11.38 g, 22.2 mmol), MeCN (80 mL), and CaH_2 (0.479 g, 11.4 mmol) was heated to 70 °C. Solutions of bromosugar **3** (2.35 g, 5.55 mmol in MeCN (5.6 mL)), and KO*t*-Bu (1.0 M, 5.6 mL, 5.6 mmol) were added simultaneously via syringe pump (0.2 mL/min). Simultaneous addition was reiterated over 5 days until no further reaction progress by HPLC was observed. Eight additions were made, totaling 2 equiv each of **3** and KO*t*-Bu. The mixture was filtered, and the solvent was concentrated to give a dark brown oil (27.6 g), which was dissolved in MeCN (70 mL) and resubjected to the same reaction conditions. Seven additions of **3** and KO*t*-Bu (0.25 equiv each per addition) were performed over 3 days. HPLC analysis showed a level of 14% and 19%, respectively, of **18a**,**b**. The reaction mixture was filtered and diluted with CH_2Cl_2 (700 mL). The solution was washed with H_2O (2 × 250 mL), dried ($MgSO_4$), and concentrated. Purification by chromatography (silica gel, hexanes/10−45% EtOAc) gave a yellow oil (0.530 g, 75% purity). The oil was further purified by preparative scale HPLC (Nova-Pak silica, hexanes/EtOAc, 69/31 w/0.2% NEt_3) to give the protected bis-sugars: **18a** (101 mg, 94% purity). Mp = 87−90 °C. ^{1}H NMR ($CDCl_3$) δ 8.11−8.07 (m, 10H), 7.41−7.69 (m, 12H), 6.78 (br m, 1H), 6.58 (br dd, 2H, J = 22, 3), 5.75 (dd, 1H, J = 18, 3), 5.64 (dd, 1 H, J = 18, 3), 5.36 (dm, 1H, J = 52), 5.24 (dm, 1H, J = 52), 4.80 (m, 2H), 4.66 (m, 2H), 4.53 (m, 2H). ^{13}C NMR ($CDCl_3$) 166.2, 166.1, 165.2, 165.2, 154.4, 154.0, 140.9 (d, J_{CF} = 8), 134.2, 133.9, 133.4, 133.1, 129.8 (m), 128.6 (m), 94.0 (d, J_{CF} = 188), 92.7 (d, J_{CF} = 192), 83.6, 83.4, 81.3, 79.5, 63.8, 63.3 ppm. IR (KBr) 3421, 3064, 1727, 1617, 1586, 1531, 1452, 1316, 1272, 1178, 1110, 1027, 918, 805, 710 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 218 nm, λmax$_2$ 230 nm, λmax$_3$ 266 nm. MS m/z $[M + H]^+$ = 854. Anal. Calcd for $C_{43}H_{34}ClF_2N_5O_{10}$: C, 60.46; H, 4.01; Cl, 4.15; F, 4.45; N, 8.20. Found: C, 60.81; H, 4.03; Cl, 3.68; F, 4.38; N, 7.35. **18b** (141 mg, 85% purity). Mp = 87−90 °C. ^{1}H NMR ($CDCl_3$) δ 8.11−8.08 (m, 9H), 7.66−7.41 (m, 12H), 6.70 (br s, 2H), 6.58 (dd, 1H, J = 23, 3), 5.77−6.69 (m, 2H), 5.41 (d, 1H, J = 49), 5.35 (dd, 1H, J = 49, 2), 4.80−4.79 (m, 2H), 4.75−4.68 (m, 1H), 4.66−4.60 (m, 2H), 4.58−4.55 (m, 1H). ^{13}C NMR ($CDCl_3$) 166.2, 166.1, 165.2, 165.1, 154.4, 153.7, 140.8 (d, J_{CF} = 5), 134.2, 134.0, 133.4, 133.2, 130.0, 129.83, 129.76, 129.6, 129.4, 128.7, 128.5, 128.4, 128.1, 118.5, 97.0 (d, J_{CF} = 188), 92.7 (d, J_{CF} = 193), 86.0 (br), 83.5 (d, J_{CF} = 17), 83.5, 81.2, 63.9, 63.3 ppm. ^{19}F NMR (DMSO-d_6) −189.1 (br d), −198.8 (dt, J = 30, 19) ppm. IR (KBr) 3415, 3333, 3064, 1726, 1616, 1452, 1380, 1272, 1178, 1096, 1027, 805, 710 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 218 nm, λmax$_2$ 230 nm, λmax$_3$ 268 nm. MS m/z $[M + H]^+$ = 854. Anal. Calcd for $C_{43}H_{34}ClF_2N_5O_{10}$: C, 60.46; H, 4.01; Cl, 4.15; F, 4.45; N, 8.20. Found: C, 60.74; H, 3.62; Cl, 3.81; F, 4.41; N, 7.96.

Bis-sugars (19a,b). A mixture of protected bis-sugar isomers (**18a**,**b** 834 mg, 0.976 mmol, 86% purity) was combined with MeOH (8 mL) and NaOMe (90 μL, 25 wt %, 0.39 mmol) and stirred at ambient temperature for 4.5 h. HOAc (30 μL) was added, and the reaction mixture was concentrated. Purification by preparative HPLC (Atlantis reverse-phase, 5 μm, 19 mm × 100 mm, H_2O/MeCN, 41/9, 14 mL/min) gave the bis-sugars as white solids: **19a** (127 mg, 95% purity). Mp = 146−152 °C. ^{1}H NMR (DMSO-d_6) δ 8.41 (d, 1H, J = 2), 8.18 (br s, 1H), 6.38 (dd, 1H, J = 13, 5), 6.22 (br s, 1H), 5.98 (d, 1H, J = 5), 5.73 (d, 1H, J = 5), 5.26 (dt, 1H, J = 52, 4), 5.12−4.90 (m, 3H), 4.44 (dm, 1H J = 9), 4.30 (m, 1H), 3.87 (m, 1H), 3.72−3.63 (m, 3H), 3.55−3.51 (m, 2H). ^{13}C NMR (DMSO-d_6) 154.1, 152.9, 150.4, 141.0, 95.8 (d, J_{CF} = 192), 95.3 (d, J_{CF} = 192), 83.5 (d, J_{CF} = 6), 82.8, 81.5 (d, J_{CF} = 17), 78.8 (br s), 73.1 (d, J_{CF} = 23), 72.4 (d, J_{CF} = 22), 61.4, 60.3 ppm. IR (KBr) 3401, 2935, 1622, 1536, 1467, 1426, 1343, 1238, 1038, 952 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 212 nm, λmax$_2$ 266 nm. MS m/z $[M + H]^+$ = 438. Anal. Calcd for $C_{15}H_{18}ClF_2N_5O_6$: C: 41.15; H, 4.14; Cl, 8.10; F, 8.68; N, 16.00. Found: C, 41.34; H, 4.17; Cl, 8.30; F, 8.50; N, 15.75. **19b** (50 mg, 99% purity). Mp = 134−135 °C. ^{1}H NMR (DMSO-d_6) δ 8.30 (d, 1H, J = 4), 8.19 (br s, 1H), 6.41 (dd, 1H, J = 16, 4), 6.22 (br s, 1H), 5.15 (dm, 1H, J = 52), 5.09 (dt, 1H, J = 52, 3), 4.51 (dm, 1H, J = 18), 4.34 (dm, 1H, J = 18), 4.11 (br q, 1H, J = 3), 3.98 (q, 1H, J = 3), 3.87−3.78 (m, 2H), 3.72−3.63 (m, 2H). ^{13}C NMR (DMSO-d_6) 155.3, 142.5 (d, J_{CF} = 4), 100.9 (d, J_{CF} = 60), 96.8 (d, J_{CF} = 194), 85.6 (br d), 84.3 (d, J_{CF} = 17), 75.3 (d, J_{CF} = 24), 74.7 (d, J_{CF} = 25), 62.6, 62.1 ppm. IR (KBr) 3355, 2933, 1662, 1619, 1466, 1344, 1307, 1238, 1042, 680 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 210 nm, λmax$_2$ 268 nm. MS m/z $[M + H]^+$ = 438. Anal. Calcd for $C_{15}H_{18}ClF_2N_5O_6$: C: 41.15; H, 4.14; Cl, 8.10; F, 8.68; N, 16.00. Found: C, 40.55; H, 4.39; Cl, 8.15; F, 7.67; N, 15.72.

Degradant B, Nucleoside 20. A solution of clofarabine (**1**, 0.352 g, 1.16 mmol), H_2O (6 mL), LiOH (0.083 g, 3.47 mmol), and H_2O_2 (0.24 mL, 30 wt %, 2.35 mmol) was stirred at 60 °C for 24 h. HPLC analysis showed 88% conversion. A solution of $Na_2S_2O_3$ in H_2O was added until the peroxide test (starch-iodide paper) was negative. HOAc was added until the pH was 4−5, and the mixture was concentrated. Purification by chromatography (reverse phase, C-18, H_2O/0−100% MeOH) gave **20** as a white solid (0.16 g, 99.3% purity, 48% yield). Mp = 262−282 °C (dec). ^{1}H NMR (DMSO-d_6) δ 10.74 (br s, 1H), 7.84 (1H, d, J = 2), 7.80 (br s, 2H), 6.13 (1H, dd, J = 16, 4), 5.93 (1H, d, J = 5), 5.09 (1H, dt, J = 53, 4), 5.13 (1H, br s), 4.35 (1H, ddd, J = 19, 8, 5), 3.79 (1H, q, J = 5), 3.80−3.56 (2H, m). ^{13}C NMR (DMSO-d_6) 156.1, 151.8 (br), 137.7 (br m), 104.4 (br), 95.4 (d, J_{CF} = 192), 83.5 (d, J_{CF} = 5), 81.0 (br), 72.8 (d, J_{CF} = 24), 60.4. IR (KBr) 3371, 3171, 1675, 1643, 1606, 1379, 1040 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 247 nm, λmax$_2$ 292 nm. MS m/z $[M + H]^+$ = 285. Anal. Calcd for $C_{10}H_{12}FN_5O_4$: C, 42.11; H, 4.24; F, 6.66; N, 24.55. Found: C, 41.96; H, 4.00; F, 6.43; N, 24.57.

Protected Nucleoside 22. A solution of **3** (13.83 g, 32.7 mmol) in DCE (25 mL) was added to a suspension of **21** (5.04 g, 29.7 mmol), TAA (28 mL), MeCN (25 mL), and KO*t*-Bu (36 mL, 36 mmol) over 5 min. The reaction was stirred at ambient temperature for 20 h. HOAc (0.3 mL, pH = 6−7) was added followed by CH_2Cl_2 (100 mL). The mixture was filtered, and the flask and solids were washed with CH_2Cl_2 (2 × 50 mL). The mixture was concentrated, and the residue was recrystallized from boiling MeOH to give **22** as a pale yellow solid (7.72 g, 93% purity, 47% yield). A portion of this material was purified by chromatography (silica gel, hexanes/0−100% EtOAc) for characterization. Mp = 87−89 °C. ^{1}H NMR

(DMSO-d_6) δ 8.19 (d, 1H, $J = 3$), 8.10 (dm, 2H, $J = 8$), 8.01 (dm, 2H, $J = 8$), 7.76−7.66 (m, 2H), 7.62−7.11 (m, 4H), 7.11 (s, 2H), 6.50 (dd, 1H, $J = 20, 4$), 5.90 (dm, 1H, $J = 20$), 5.77 (dm, $J = 52$), 4.82−4.70 (m, 3H). ^{13}C NMR (DMSO- d_6) 165.5, 164.7, 160.0, 153.6, 149.8, 141.1 (d, $J_{CF} = 6$), 134.0, 133.5, 129.6, 129.2, 128.8, 128.7, 128.6, 92.8 (d, $J_{CF} = 192$), 82.0 (d, $J_{CF} = 15$), 78.6, 76.7 (d, $J_{CF} = 28$), 63.7 ppm. ^{19}F NMR (DMSO-d_6) −198.4 (dt, $J = 51, 19$) ppm. IR (KBr) 3383, 1725, 1615, 1566, 1468, 1272, 1110, 908, 711 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 223 nm, λmax$_2$ 308 nm. MS *m*/ *z* $[M + H]^+ =$ 512. Anal. Calcd for $C_{24}H_{19}ClFN_5O_5$: C, 56.31; H, 3.74; Cl, 6.93; F, 3.71; N, 13.68. Found: C, 56.10; H, 3.63; Cl, 6.81; F, 3.59; N, 13.44.

Degradant C, Nucleoside 23. A solution of **22** (3.48 g, 6.8 mmol), THF (45 mL), H_2O (25 mL), and NaOH (0.85 g, 21.3 mmol) was stirred at ambient temperature for 7 h. HCl (12 M, 2 mL) was added, and THF was removed by rotary evaporation. The remaining aqueous layer was washed with CH_2Cl_2 (2 × 100 mL). The aqueous layer was stirred with NaOH (0.54 g, 13.6 mmol) and H_2O_2 (0.7 mL, 30 wt %, 6.8 mmol) at ambient temperature for 30 min. A solution of $Na_2S_2O_3$ in H_2O was added until the peroxide test (starch-iodide paper) was negative. HC1 (12 M, 1.4 mL) was added until the pH was 5−6. The suspension was filtered, and the flask and solids were washed with THF (100 mL). Purification by chromatography (C-18, H_2O/5 to 100% MeOH) gave **23** as a white solid (0.36 g, 18% yield). Mp = 275−279 °C. ^{1}H NMR (DMSO-d_6) δ 10.68 (s, 1H), 7.79 (d, 1H, $J = 2$), 6.54 (br s, 2H), 6.11 (dd, 1H, $J = 16$, 4), 5.94 (d, 1H, $J = 5$), 5.09 (dt, $J = 53$, 4), 5.08 (t, 1H, $J =$ 6), 4.35 (ddd, 1H, $J = 18$, 8, 5), 3.79 (q, 1H, $J = 5$), 3.60 (apparent octet 2H, $J = 6$). ^{13}C NMR (DMSO-d_6) 157.2, 154.1, 151.4, 136.6, 116.1, 95.5 (d, $J_{CF} = 192$), 83.8 (d, $J_{CF} = 5$), 81.6 (d, $J_{CF} = 17$), 72.9 (d, $J_{CF} = 23$), 60.7 ppm. IR (KBr) 3396, 3132, 1691, 1535, 1376, 1045 cm^{-1}.UV (H_2O/MeOH) λmax$_1$ 252 nm. MS *m*/*z* $[M + H]^+ = 285$. Anal. Calcd for $C_{10}H_{12}FN_5O_4$: C, 42.11; H, 4.24; F, 6.66; N, 24.55. Found: C, 40.06; H, 4.23; F, 5.84; N, 21.73.

Degradant E compound 24. A suspension of clofarabine (**1**, 1.59 g, 5.24 mmol), NaOH (1.26 g, 31.5 mmol), and DMSO (110 mL) was heated to 54 °C over 30 min and held at that temperature for 3 h. HPLC analysis showed formation of the product (38% purity). The mixture was decanted, HOAc (2 mL) was added, and the mixture was concentrated. The residue was triturated with MeOH (200 mL), the suspension was filtered, and the filtrate was concentrated. Purification by chromatography (silica gel, acetone/EtOAc, 3/7) and crystallization from a boiling mixture of IPA/H_2O (8 mL/1.3 mL) gave **24** as a white solid (0.38 g, 97.3% purity, 25% yield). Mp = 239−242 °C. ^{1}H NMR (DMSO-d_6) δ 8.42 (s, 1H), 7.88 (br s, 2H), 6.27 (s, 1H), 5.98 (d, 1H, $J = 2$), 5.24 (d, 1H, $J = 4$), 5.17 (ddd, 1H, $J = 6$, 4, 2), 5.03 (s, 1H), 4.72 (dd, 1H, $J = 4$, 8), 3.94 (dd, 1H, $J = 8$, 2). ^{13}C NMR (DMSO-d_6) 156.8, 153.3, 150.9, 139.3, 117.7, 92.6, 90.6, 80.2, 77.0, 76.6 ppm. IR (KBr) 3412, 3331, 3218, 3126, 2887, 1652, 1597, 1576, 1460 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 211 nm, λmax$_2$ 265 nm. MS *m*/*z* $[M + H]^+ =$ 284. Anal. Calcd for $C_{10}H_{10}ClN_5O_3$: C, 42.34; H, 3.55; Cl, 12.50; N, 24.69. Found: C, 42.28; H, 3.42; Cl, 12.65; N, 24.65.

Compound 25. LiBr (14.56 g, 168 mmol) was added to a solution of **7** (19.94 g, 33.5 mmol) in NMP (50 mL), and the mixture was stirred at ambient temperature for 20 h. H_2O (250 mL) was added, and the mixture was extracted with TBME (2 × 250 mL). The combined organic portions were washed with H_2O (250 mL), dried ($MgSO_4$), and concentrated to give **25** as a pale yellow oil (17.12 g, 98.6% purity, 96% yield). ^{1}H NMR (DMSO-d_6) δ 8.08−8.01 (m, 6H), 7.74−7.67 (m, 3H), 7.58−7.48 (m, 6H), 6.64 (s, 1H), 5.72 (d, 1H, $J = 3$), 5.02 (s, 1H), 5.02−4.99 (m, 1H), 4.79 (dd, 1H, $J = 12$, 4), 4.68 (dd, 1H, $J = 12$, 6). ^{13}C NMR (DMSO-d_6) 165.4, 164.8, 163.9, 133.93, 133.87, 133.5, 129.5, 129.4, 129.2, 128.8, 128.7, 102.3, 83.4, 79.3, 63.8, 50.4 ppm. IR (KBr) 3434, 1725, 1270, 1095, 1067, 929, 709 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 229 nm, λmax$_2$ 274 nm. MS *m*/*z* $[M + Na]^+ = 547$. Anal. Calcd for $C_{26}H_{21}BrO_7$: C, 59.44; H, 4.03; Br, 15.21. Found: C, 59.62; H, 4.18; Br, 14.88.

Compound 26. HBr/HOAc (10.7 mL, 33 wt %, 62 mmol) was added to a solution of **25** (16.71 g, 31.8 mmol) in CH_2Cl_2 (125 mL), and the mixture was stirred at ambient temperature for 23 h. The mixture was poured into saturated $NaHCO_3$ (300 mL) with stirring (off-gassing). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (100 mL). The organic portions were washed with saturated $NaHCO_3$ (100 mL), dried ($MgSO_4$), and concentrated to give **26** as an oil (14.80 g, 96% recovery). The crude material was used in the next step as is.

Nucleoside 27. A solution of **26** (14.19 g, 29.3 mmol) in DCE (22.5 mL) was added to a suspension of **4** (4.52 g, 26.7 mmol), TAA (25 mL), MeCN (22.5 mL), and KO*t*-Bu (32 mL, 32 mmol) over 8 min. After stirring at ambient temperature for 18 h, the reaction mixture was filtered, and the filtrate was concentrated. MeOH (100 mL) was added, and the mixture was heated to reflux. The suspension was cooled and filtered, and the solid was dried (50 °C, 50−100 Torr). Purification by chromatography (silica gel, EtOAc/hexanes, 3/2) gave **27** as a white solid (2.85 g, 94.4% purity, 18% yield). Mp 172−175 °C. ^{1}H NMR (DMSO-d_6) δ 8.40 (s, 1H), 8.09 (d, 2H, $J = 7$), 7.97 (br s, 1H), 7.93 (d, 2H, $J = 7$), 7.74 (apparent t, 1H, $J =$ 7), 7.66−7.58 (m, 3H), 7.45 (apparent t, 2H, $J = 8$), 6.61 (d, 1H, $J = 8$), 6.37 (t, 1H, $J = 7$), 5.43 (t, 1H, $J = 7$), 4.84 (d, 2H, $J = 5$), 4.63 (q, 1H, $J = 6$). ^{13}C NMR (DMSO-d_6) 165.4, 164.8, 156.9, 153.2, 149.7, 139.9, 134.0, 133.5, 129.6, 129.1, 128.8, 128.64, 128.57, 83.6, 79.0, 77.7, 64.1, 50.2 ppm. IR (KBr) 3325, 3177, 1717, 1669, 1598, 1312, 1272, 1094, 710 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 213 nm, λmax$_2$ 231 nm, λmax$_3$ 264 nm. MS *m*/*z* $[M + Na]^+ = 594$. Anal. Calcd for $C_{24}H_{19}BrClN_5O_5$: C, 50.32; H, 3.34; Br, 13.95; Cl, 6.19; N, 12.23. Found: C, 50.59; H, 3.09; Br, 13.56; Cl, 6.43, N, 11.98.

Degradant D, Compound 28. A suspension of **27** (1.33 g, 2.33 mmol), MeOH (3.6 mL), and NaOMe (0.59 mL, 25 wt %, 2.6 mmol) was stirred at ambient temperature for 16 days. HOAc (0.5 mL) was added, the mixture was concentrated, and the residue was triturated with hexanes. Purification by chromatography (silica gel, hexanes/0−100% EtOH, 15 min) gave **28** as a white solid (0.347 g, 99.1% purity, 52% yield). Mp 207−209 °C. ^{1}H NMR (DMSO-d_6) δ 8.35 (s, 1H), 7.84 (br s, 2H), 6.13 (s, 1H), 5.03 (br t, 1H, $J = 5$), 4.46 (d, 1H, $J = 3$),

4.20−4.18 (m, 2H), 3.58−3.51 (m, 2H). ^{13}C NMR (DMSO-d_6) 156.8, 153.1, 150.2, 139.9, 117.8, 81.9, 81.3, 60.8, 58.5, 57.6 ppm. IR (KBr) 3414, 3328, 1644, 1315, 1065, 1018, 585 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 211 nm, λmax$_2$ 264 nm. MS m/z $[M + H]^+ = 284$. Anal. Calcd for $C_{10}H_{10}ClN_5O_3$: C, 42.34; H, 3.55; Cl, 12.50; N, 24.69. Found: C, 42.23; H, 3.44 Cl, 12.38, N, 24.54.

Degradant A, Compound 29. A solution of **28** (0.393 g, 1.39 mmol) and H_2O (11 mL) was heated at 100 °C for 6.2 h. The volatiles were concentrated to give crude **29** (278 mg). Purification by preparative HPLC (C-18, H_2O/5−95% MeCN, 6 min) gave **29** as a white solid (0.107 g, 99% purity, 29% yield). Mp = 290 °C (dec). ^{1}H NMR (DMSO-d_6) δ 7.82 (s, 1H), 6.03 (s, 1H), 4.80 (d, 1H, J = 3), 4.75 (s, 1H), 4.65 (apparent quintet, 1H, J = 4), 3.48 (dd, 1H, J = 12, 4), 3.24 (dd, 1H, J = 11, 7). ^{13}C NMR (DMSO-d_6) 157.8, 153.1, 140.2, 132.8, 109.9, 86.3, 85.3, 74.0, 60.6, 56.1 ppm. IR (KBr) 3404, 3330, 3204, 1675, 1596, 1581, 1045, 844, 782 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 220 nm, λmax$_2$ 239 nm, λmax$_3$ 276 nm. MS m/z $[M + H]^+ = 266$. Anal. Calcd for $C_{10}H_{11}N_5O_4$: C, 45.28; H, 4.18; N, 26.41. Found: C, 44.99; H, 4.18; N, 26.22.

Degradant F, Compound 31. A solution of **1** (9.73 g, 32.0 mmol), NaOH (2.88 g, 72 mmol), and H_2O (145 mL) was stirred at 80 °C for 1.9 h. HPLC analysis showed the reaction mixture contained 11.9% **31**. HOAc (2.5 mL) was added, and the suspension was cooled to 5 °C and filtered. The solid was triturated with H_2O, and the mixture was filtered. The solid was triturated with MeOH, and the mixture was filtered. Purification by chromatography (2×, silica gel, hexanes/0−100% EtOH) gave **31** (116 mg, 77% purity). Final purification by preparative HPLC (C-18, 79% H_2O/MeCN/MeOH, 79/14/7) gave **31** as a white solid (74 mg, 99.0% purity, 0.8% yield). Mp = 211−213 °C. ^{1}H NMR (DMSO-d_6) δ 8.33 (d, 1H, J = 3), 8.12 (d, 1H, J = 3), 7.93 (br s, 2H), 7.53 (br s, 2H), 6.38 (dd, 1H, J = 20, 4), 6.32 (dd, 1H, J = 15, 5), 5.96 (d, 1H, J = 5), 5.66 (dm, 1H, J = 17), 5.50 (dt, 1H, J = 50, 2), 5.20 (dt, 1H, J = 50, 4), 5.15 (t, 1H, J = 6), 5.09 (t, 1H, J = 6), 4.42 (ddd, 1H, J = 14, 9, 5), 4.21 (dd, 1H, J = 9, 5), 3.87−3.76 (m, 3H), 3.71−3.62 (m, 2H). ^{13}C NMR (DMSO-d_6) 159.9, 156.8, 153.4, 150.7, 150.2, 140.1 (d, J_{CF} = 6), 138.6 (d, J_{CF} = 4), 117.3, 115.1, 95.4 (d, J_{CF} = 193), 93.0 (d, J_{CF} = 191), 83.5 (d, J_{CF} = 5), 82.9, 82.6 (d, J_{CF} = 17), 81.4 (d, J_{CF} = 17), 77.6 (d, J_{CF} = 28), 72.7 (d, J_{CF} = 24), 60.8, 60.4 ppm. ^{19}F NMR (DMSO-d_6) −198.3 (dt, J = 53, 17 Hz), −199.0 (dt, J = 48, 18 Hz) ppm. IR (KBr) 3429, 1649, 1595, 1475, 1346, 1215, 1044 cm^{-1}. UV (H_2O/MeOH) λmax$_1$ 211 nm, λmax$_2$ 264 nm. MS m/z $[M + H]^+ = 571$. Anal. Calcd for $C_{20}H_{21}ClF_2N_{10}O_6$: C, 42.08; H, 3.71; Cl, 6.21; F, 6.66; N, 24.53. Found: C, 41.98; H, 3.78; Cl, 6.10; F, 6.63; N, 24.38.

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Supporting Information Available

HPLC conditions and spectral data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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