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 N6-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 Clofar. The process for manufacturing clofarabine1 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 KOt-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.

The chloroarabinose 8 was synthesized by conversion of 6 to the triflate 7 followed by treatment with LiCl.5 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 SNAr reaction of sodium methoxide.

* Reagents, conditions, and yields: a) HBr/HOAc, CH2Cl2, rt, 19 h, 89% yield; b) KOR-Bu, MeCN, CICH2CH2Cl, 67% n-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).

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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 KOt-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. It was found that the best conditions for formation of 18 were simultaneous addition of 3 and KOt-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 debenzyolation 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 N6 to reversibly afford the imine 19c (eq 2).

NMR (1H, 13C, COSY, NOESY) data for 19a and 19b support the assigned structures. The stereochemical assignment at the anomeric carbons (C1′ and C1′′) is somewhat more tenuous. On the basis of the mechanism, one would not anticipate loss of anomeric stereochemistry at C1′. Indeed, this is supported by observed NOEs between H8 and H3′ in both 19a and 19b. Compound 19a has an additional NOE between H8 and H3′, which supports an assignment where these substituents are on the same face of the sugar ring. Interestingly, the H1′ resonance in all the bis-sugar compounds appears broad, while H1′′ 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 degradants.

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

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 SNAr 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 chloride 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 the production of individual degradants for isolation. 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 D2O 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 H5′ protons to be indistinguishable from each other. One explanation for this phenomenon is restricted rotation about the C6′–C7′ bond, which could be accomplished by the oxatane ring found in the structure.

The formation of 24 might have occurred via intramolecular attack of the 5′ alkoxide to C4′ of a C5′–C7′ epoxide. These nucleoside epoxides are known12 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

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

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<th>cmpd</th>
<th>RRT</th>
<th>UVmax. nm</th>
<th>% area</th>
<th>mass (Da)</th>
<th>MS isotopic Cl pattern?</th>
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<tr>
<td>degradant A (29)</td>
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<td>219, 277</td>
<td>2.64</td>
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<td>no</td>
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<tr>
<td>degradant B (20)</td>
<td>0.489</td>
<td>248, 292</td>
<td>5.34</td>
<td>285</td>
<td>no</td>
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<tr>
<td>degradant C (23)</td>
<td>0.572</td>
<td>252</td>
<td>6.04</td>
<td>285</td>
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<tr>
<td>degradant D (28)</td>
<td>0.872</td>
<td>264</td>
<td>5.02</td>
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<td>yes</td>
</tr>
<tr>
<td>degradant E (24)</td>
<td>0.920</td>
<td>264</td>
<td>6.15</td>
<td>283</td>
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<tr>
<td>clofarabine (1)</td>
<td>1.000</td>
<td>264</td>
<td>53.58</td>
<td>303</td>
<td>yes</td>
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<tr>
<td>degradant F (31)</td>
<td>1.450</td>
<td>264</td>
<td>6.87</td>
<td>570</td>
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</tr>
</tbody>
</table>

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

![Scheme 4](image)

* Reagents and conditions: a) KOt-Bu, CH3OH, CH2Cl2, tert-amyl-OH, rt, 20 h; b) NaOH, H2O, rt, 72 h.

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11

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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 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 SNAr 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.

The structure shown for compound 31 corresponds to one 3′,5′-di-tert-OH’s, which would give evidence for the assignment of structure 31. The Hs and NH2 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, 13C, and 19F 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 H2O/MeCN or H2O/MeOH. HPLC data were collected using photodiode array detectors on dual pump systems. Conditions for HPLC are given in the Supporting Information.

**Arabinose Trilate 7.** Triflic anhydride (2.5 mL, 14.9 mmol) was added to a solution of 6 (5.63 g, 12.2 mmol), CH2Cl2 (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, UV254, Rf of 0.50, Rf of 7 = 0.62). A solution of NaHCO3 (50 mL, 5 wt %) was added (off-gassing). The layers were separated, and the aqueous layer was extracted with CH2Cl2 (25 mL). The organic portions were dried (MgSO4) and concentrated. Purification by chromatography (silica gel, CH2Cl2/MeOH 25:1, Rf of 7 = 0.62, trace) revealed a significant amount of degradant A with a small amount of degradant E. Peak assignments Numbers 2, 3, and 4 were also observed in the compound 31.1H NMR (CDCl3) δ 8.15−8.01 (m, 4H, 7.50−7.39 (m, 6H, 3), 5.55 (dd, 1H, J = 6, 3), 4.86 (g, 1H, J = 3), 4.75 (dd, 1H, J = 12, 3), 4.63 (dd, 1H, J = 12, 3).13C NMR (CDCl3) 165.8, 165.5, 164.7, 134.0, 133.9, 133.5, 130.0, 129.6, 129.1, 128.6, 128.5, 128.4, 128.3, 102.4, 84.3, 79.4, 69.9, 63.4 ppm. UV (H2O/MeCN) λmax 230 nm, λmax 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) was stirred at ambient temperature for 17.5 h. The reaction was judged complete by TLC (silica gel GHLF, 50% EtOAc/50% hexanes, UV254, Rf of 0.62, Rf of 8 = 0.65). H2O (100 mL) was added, and the mixture was extracted with TBME (3 × 75 mL). The organic portions were washed with H2O (100 mL), dried (MgSO4), 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 (CDCl3) δ 8.12−8.03 (m, 6H, 7.61−7.53 (m, 3H, 7.44−7.37 (m, 6H, 6.68 (s, 1H, J = 3), 5.62 (d, 1H, J = 3), 4.85−4.74 (m, 3H, 4.62 (s, 1H, 13C NMR (CDCl3) 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,
The crude solid was used in the coupling step as is. 1H NMR (CDCl3) δ 7.41 (m, 2H), 6.61 (s, 1H), 5.58 (d, 1H, J = 4.72), 4.44 (ddd, 1H, J = 6), 3.78 (m, 5H), 3.19 (br s, 3H). UV (H2O/MeOH) λmax1 232 nm, λmax2 276 nm. MS m/z [M + H]+ = 198.

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), CaH2 (0.64 g, 15.2 mmol), MeCN (5 mL), and KOrBu (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 (97.4 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-d6) 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), 4.89 (dm, 1H, J = 19), 5.77 (dm, 1H, J = 50), 4.78–4.78 (m, 3H), 3.68 (br s, 1H), 3.18 (br s, 1H). 13C NMR (DMSO-d6) 165.4, 161.8, 154.5, 152.9, 150.9, 138.7 (d, JCF = 6), 133.9, 135.5, 129.7, 129.2, 128.7, 128.6, 117.7, 92.9 (d, JCF = 18), 92.1 (d, JCF = 18), 78.4, 76.3 (d, JCF = 29), 63.7, 37.3 ppm. 19F NMR (DMSO-d6) = 198.3 (dt, J = 51, 19) ppm. IR (KBr) 3496, 2939, 1726, 1602, 1452, 1314, 1270, 1109, 711 cm−1. UV (H2O/MeOH) λmax1 223 nm, λmax2 276 nm. MS m/z [M + H]+ = 540. Anal. Calcd for C10H16F2Cl2N3O2: C, 57.84; H, 4.49; 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 mL, 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-d6) δ 8.30 (d, 1H, J = 2), 6.36 (dd, 1H, J = 14), 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), 3.87 (dd, 1H, J = 10), 3.73–3.62 (m, 5H), 3.19 (br s, 3H). 13C NMR (DMSO-d6) 154.4, 152.7, 150.9, 138.7, 117.8, 95.2 (d, JCF = 192), 83.5 (d, JCF = 42), 81.5 (d, JCF = 17), 72.5 (d, JCF = 23), 60.3, 37.4 ppm. 19F NMR (DMSO-d6) = 198.9 (dt, J = 53, 18) ppm. IR (KBr) 3374, 3132, 2925, 1723, 1606, 1344, 1035, 793 cm−1. UV (H2O/MeOH) λmax1 218 nm, λmax2 276 nm. MS m/z [M + H]+ = 332. Anal. Calcd for C13H16F2Cl2N3O2: C, 43.45; H, 4.56; Cl, 5.73; F, 3.57; N, 21.11. Found: C, 43.50; H, 4.42; Cl, 10.69; 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), CaH2 (0.21 g, 4.9 mmol), MeCN (10 mL), and KOrBu (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-d6) δ (mix of anomers). 13C NMR (DMSO-d6) (mix of anomers). 19F NMR (DMSO-d6) minor anomer = 190.5 (dt, J = 50, 16) ppm, major anomer = 199.7 (dt, J = 50, 20) ppm. IR (KBr) 3460, 3329, 3189, 1724, 1604, 1271, 1109, 711 cm−1. UV (H2O/MeOH) λmax1 220 nm, λmax2 279 nm. MS m/z [M + H]+ = 493. Anal. Calcd for C15H18F2Cl2N3O2: 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/CHCl₃, 2/3) gave 14 as a white solid (0.12 g, 99.0% purity, 19% yield). Mp = 175–178 °C (dec.). 1H NMR (DMSO-d₆) δ 8.71 (d, 1H, J = 3), 6.81 (s, 2H), 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). 13C NMR (DMSO-d₆) 160.4, 156.2, 151.5, 136.0, 112.4, 95.3 (d, JCF = 192), 83.5, 81.1 (d, JCF = 17), 72.9 (d, JCF = 23), 60.3 ppm. 19F NMR (DMSO-d₆) −198.2 (d, JCF = 218) ppm. IR (KBr) 3337, 3204, 2929, 1605, 1478, 1415, 1286, 1222, 1040, 792 cm⁻¹. UV (H₂O/MeOH) λmax 215 nm, MS m/z [M + H]⁺ = 285. Anal. Calc'd for C₁₀H₁₄FN₅O₄: C, 44.15; H, 4.72; F, 6.35; N, 25.70. Found: C, 42.10; H, 4.88; F, 6.38 N, 25.90.

**Compound 15.** A solution of 3 (6.61 g, 15.6 mmol) in MeOH (45 mL) was added to a suspension of 12 (67.5% purity, 4.50 g, 14.2 mmol), KOr-Bu (1.67 g, 14.9 mmol), CaH₂ (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.). 1H NMR (DMSO-d₆) 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, 27), 3.80 (dq, 1H, J = 51, 2), 4.82–4.68 (m, 3H). 13C NMR (DMSO-d₆) 165.4, 164.8, 156.6, 150.0, 144.6, 139.8 (d, JCF = 6), 133.9, 133.5, 129.7, 129.2, 128.7, 128.6, 118.0, 92.9 (d, JCF = 189), 82.0 (d, JCF = 17), 78.3, 76.3 (d, JCF = 28), 63.7 ppm. 19F NMR (DMSO-d₆) −198.2 (d, JCF = 51, 18) ppm. IR (KBr) 3393, 3358, 3167, 2359, 1726, 1642, 1586, 1452, 1349, 1272, 1096, 711 cm⁻¹. UV (H₂O/MeCN) λmax 215 nm, λmax 231 nm, λmax 264 nm. MS m/z [M + H]⁺ = 285. Anal. Calc'd for C₁₀H₁₄FN₅O₄: C, 44.15; H, 4.72; F, 6.35; N, 25.70. Found: C, 42.10; H, 4.88; F, 6.38; N, 25.90.

**Compound 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), KOr-Bu (1.06 g, 9.45 mmol), CaH₂ (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 slurring 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. 1H NMR (DMSO-d₆) 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). 13C NMR (DMSO-d₆) 165.4, 164.8, 156.8, 153.2, 149.8, 140.1, 133.9, 135.5, 129.6, 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, 1432, 1307, 1271, 1094, 1027, 710 cm⁻¹. UV (H₂O/MeCN) λmax 212 nm, λmax 231 nm, λmax 264 nm. MS m/z [M + H]⁺ = 528. Anal. Calc'd for C₁₅H₁₂BrFN₅O₃: C, 34.56; H, 3.62; Cl, 13.42; Br, 21.26. Found: C, 34.62; H, 3.32; Cl, 13.39; Br, 24.82.
Protected Bis-sugars (18a,b). A suspension of 5 (11.38 g, 22.2 mmol), MeCN (80 mL), and CaH₂ (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 KOr-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 KOr-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 KOr-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 CH₂Cl₂ (700 mL). The solution was washed with H₂O (2 × 250 mL), dried (MgSO₄), and concentrated. Purification by chromatography (silica gel, hexanes/10–45% EtOAc) gave a yellow oil (0.536 g, 75% purity).

The oil was further purified by preparative scale HPLC (Nova-Pak silica, hexanes/EtOAc, 69/31 w/o 2% Ne2) to give the protected bis-sugars: 18a (101 mg, 94% purity). Mp = 87–90 °C. 1H NMR (DMSO-d₆) 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). 13C NMR (DMSO-d₆) δ 154.1, 152.9, 150.4, 141.0, 95.8 (d, JCF = 192), 95.3 (d, JCF = 192), 83.5 (d, JCF = 6), 82.8, 81.5 (d, JCF = 17), 78.8 (br s), 73.1 (d, JCF = 23), 72.4 (d, JCF = 22), 61.4, 60.3 ppm. IR (KBr) 3401, 2935, 1622, 1467, 1426, 1343, 1238, 1038, 952 cm⁻¹. UV (H₂O/MeOH) λmax₁ 212 nm, λmax₂ 266 nm. MS m/z [M + H]+ = 438. Anal. Calcd for C₁₅H₁₈ClF₂N₅O₆: 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. 1H NMR (DMSO-d₆) 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). 13C NMR (DMSO-d₆) δ 155.3, 142.5 (d, JCF = 4), 100.9 (d, JCF = 60), 96.8 (d, JCF = 194), 85.6 (br d), 84.3 (d, JCF = 17), 75.3 (d, JCF = 24), 74.7 (d, JCF = 25), 62.6, 62.1 ppm. IR (KBr) 3355, 2933, 1619, 1466, 1344, 1307, 1238, 1042, 680 cm⁻¹. UV (H₂O/MeOH) λmax₁ 210 nm, λmax₂ 268 nm. MS m/z [M + H]+ = 438. Anal. Calcd for C₁₅H₁₈ClF₂N₅O₆C: 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, 8.76; N, 15.72.

Degradant B, Nucleoside 20. A solution of clofarabine (1, 0.352 g, 1.16 mmol), H₂O (6 mL), LiOH (0.083 g, 3.47 mmol), and HOAc (0.24 mL, 30 wt %, 2.35 mmol) was stirred at 60 °C for 24 h. HPLC analysis showed 88% conversion. A solution of N₃-Or in H₂O 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, C₁₈, H₂O–O⁻100% MeOH) gave 20 as a white solid (0.16 g, 99.3% purity, 48% yield). Mp = 262–282 °C (dec). 1H NMR (DMSO-d₆) 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). 13C NMR (DMSO-d₆) δ 156.1, 151.8 (br), 137.7 (br m), 104.4 (br), 95.4 (d, JCF = 192), 83.5 (d, JCF = 5), 81.0 (br), 72.8 (d, JCF = 24), 60.4 (IR (KBr) 3371, 3171, 1675, 1643, 1606, 1379, 1040 cm⁻¹, UV (H₂O/MeOH) λmax₁ 247 nm, λmax₂ 292 nm. MS m/z [M + H]+ = 285. Anal. Calcd for C₁₂H₁₂F₂N₂O₂: 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, 32.77 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 KOr-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 CH₂Cl₂ (100 mL). The mixture was filtered, and the flask and solids were washed with CH₂Cl₂ (2 × 50 mL). The mixture was concentrated, and the residue was recrystallized from boiling MeOH to give 22 as a pale yellow solid (7.2 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. 1H NMR
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. H2O (250 mL) was added, and the mixture was extracted with TBME (2 x 250 mL). The combined organic portions were washed with H2O (250 mL), dried (MgSO4), and concentrated to give 25 as a pale yellow oil (17.12 g, 98.6% purity, 96% yield). 1H NMR (DMSO-d6) 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), 4.35 (s, 1H, J = 5), 3.92 (m, 1H, J = 6). 13C NMR (DMSO-d6) δ 198.3, 181.9, 176.4, 167.9, 152.5, 144.8, 131.9, 129.8, 129.6, 129.5, 129.0, 128.9, 128.6, 128.4, 128.2, 128.0, 127.6, 127.5, 127.4, 127.0, 126.9, 126.8, 114.6, 102.4, 102.3, 83.4, 79.3, 63.8, 50.4 ppm. IR (KBr) 3434, 1725, 1270, 1095, 710 cm⁻¹. MS m/z [M + Na]+ = 547. Anal. Calcd for C10H10ClN5O3: C, 42.36; H, 3.55; Cl, 13.68. Found: C, 42.36; H, 3.55; Cl, 13.68. 

Compound 26. HBr/HOAc (10.7 mL, 33 wt %, 62 mmol) was added to a solution of 25 (16.71 g, 318 mmol) in CH2Cl2 (125 mL), and the mixture was stirred at ambient temperature for 23 h. The mixture was poured into saturated NaHCO3 (300 mL) with stirring (off-gassing). The layers were separated, and the aqueous layer was extracted with CH2Cl2 (100 mL). The organic portions were washed with saturated NaHCO3 (100 mL), dried (MgSO4), 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 solution of 4 (4.52 g, 26.7 mmol), TAA (25 mL), MeCN (22.5 mL), and KOt-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. 1H NMR (DMSO-d6) δ 8.40 (s, 1H), 8.09 (d, 2H, J = 7), 7.97 (br s, 1H), 7.93 (d, 2H, J = 7), 7.74 (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). 13C NMR (DMSO-d6) δ 165.4, 164.8, 156.9, 153.2, 149.7, 139.9, 134.0, 133.5, 129.6, 129.1, 128.8, 128.6, 128.5, 83.6, 79.0, 77.7, 64.1, 50.2 ppm. IR (KBr) 3325, 3177, 1717, 1699, 1598, 1317, 1272, 1094, 710 cm⁻¹. UV (H2O/O2MeOH) λmax1 227 nm, λmax2 245 nm, λmax3 276 nm, MS m/z [M + Na]+ = 594. Anal. Calcd for C23H23BrO2N4: C, 39.58; H, 3.04; Br, 15.11. Found: C, 39.58; H, 3.04; Br, 15.11.
4.20–4.18 (m, 2H), 3.58–3.51 (m, 2H). 13C NMR (DMSO-d6) 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 (H2O/MeOH) λ max1 211 nm, λ max2 264 nm. MS m/z [M + H]⁺ = 284. Anal. Calcd for C10H10ClN5O3: 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 H2O (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, H2O/5–95% MeCN, 6 min) gave 29 as a white solid (0.107 g, 99% purity, 29% yield). Mp 290 °C (dec). 1H NMR (DMSO-d6) 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). 13C NMR (DMSO-d6) 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, 1675, 1596, 1045, 844, 782 cm⁻¹. MS m/z [M + H]⁺ = 266. Anal. Calcd for C10H11N5O4: C, 45.28; H, 4.18; N, 26.41. Found: C, 44.99; H, 4.18; N, 24.38.

**Degradant F, Compound 31.** A solution of 1 (9.73 g, 32.0 mmol), NaOH (2.88 g, 72 mmol), and H2O (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 MeOH, and the mixture was filtered. The solid was triturated with MeCN, and the mixture was filtered. Purification by chromatography (2 x, silica gel, hexanes/0–100% EtOH) gave 31 (116 mg, 77% purity). Final purification by preparative HPLC (C-18, 79% H2O/MeCN/MeOH, 79/14/7) gave 31 as a white solid (74 mg, 99.0% purity, 0.8% yield). Mp = 211–213 °C. 1H NMR (DMSO-d6) δ 8.33 (d, 1H, J = 3), 8.12 (d, 1H, J = 3), 7.93 (br s, 2H), 7.53 (br s, 2H), 6.32 (dd, 1H, J = 20, 4), 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). 13C NMR (DMSO-d6) 159.9, 156.8, 153.4, 150.7, 150.2, 141.0 (d, JCF = 6), 138.6 (d, JCF = 4), 117.3, 115.1, 95.4 (d, JCF = 193), 93.0 (d, JCF = 191), 83.5 (d, JCF = 5), 82.9, 82.6 (d, JCF = 17), 81.4 (d, JCF = 17), 77.6 (d, JCF = 28), 72.7 (d, JCF = 24), 60.8, 60.4 ppm. 19F NMR (DMSO-d6) –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 (H2O/MeOH) λ max 211 nm, λ max2 264 nm. MS m/z [M + H]⁺ = 571. Anal. Calcd for C20H21ClF2N10O6: 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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