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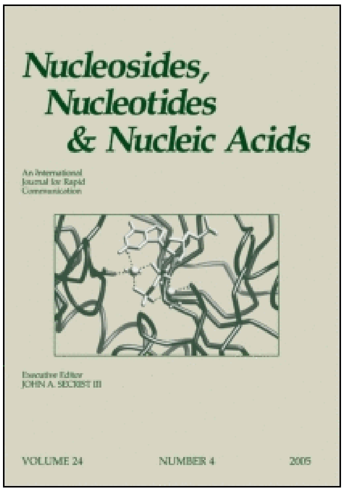

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SYNTHETIC STUDIES ON FORMAMIDOPYRIMIDINES RELATED TO CLOFARABINE

William R. Cantrell, Jr.,[1] Dennis Lovett,[1] Tracy Engles,[1] Bruce Anderson,[1] William E. Bauta,[1] and Paul C. Wolstenholme-Hogg[2]

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□ *Degradation of clofarabine (**3**) in 0.9% saline solution at 100°C afforded three degradation products which were determined to be formamidopyrimidines **4–6**. Compounds **4** and **5** were assigned as $C_{1'}$ anomers on the basis of one-dimensional and two-dimensional NMR experiments, whereas **6** was found to be the formamidopyrimidine lacking the sugar moiety. An improved procedure for the synthesis of formamidopyrimidines was developed, wherein benzoylated clofarabine (**11**) was treated with allyl chloroformate, followed by deprotection of the alloc group with catalytic $Pd(PPh_3)_4$ and dimedone. A synthesis of compound **6** from **4** is also described.*

Keywords Purine degradation; NMR characterization

INTRODUCTION

The degradation of the purine ring to a formamidopyrimidine is well known to occur when DNA is attacked by reactive oxygen species such as hydroxyl radicals. This type of damage has been implicated in the development of cancer, and the study of DNA containing such structural changes is an area of active research.[1] Formamidopyrimidines are also formed when certain nucleosides are exposed to alkaline conditions (Scheme 1). Concomitant isomerization of the sugar moiety from a furanose to a pyranose, deformylation, as well as detachment of the sugar from the formamidopyrimidine, are commonly observed and the mechanism of these transformations has been studied.[2] As synthetic targets, formamidopyrimidine nucleoside derivatives can present some significant synthetic challenges. Among these, the instability of the formamido function, which

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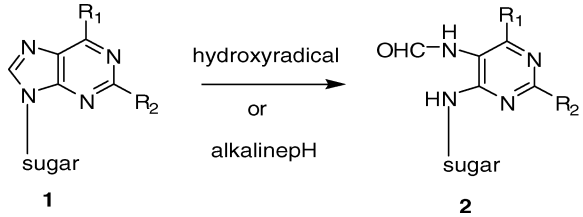

SCHEME 1 Degradation of a purine ring to a formamidopyrimidine.

can revert to the purine or be hydrolytically removed, limits the options for protecting groups. In addition, the sugar moiety is prone to both isomerization and detachment from the heterocycle.

During the course of our alkaline degradation studies on clofarabine (**3**), we observed a variety of byproducts but no significant formamidopyrimidine formation. In contrast, we did observe two degradation products, whose molecular weights corresponded to isomeric formamidopyrimidine nucleosides, when clofarabine was heated in dilute sodium chloride or sodium bicarbonate solution. In addition, a third degradant was observed whose molecular weight corresponded to the formamidopyrimidine lacking the sugar moiety. The degradation was cleanest under pH 7 buffered conditions.

We present here the results of our studies on the degradation of clofarabine (**3**) into two degradation products (**4** and **5**), and our synthetic studies on the preparation of both furanosyl and pyranosyl formamidopyrimidine nucleosides.

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RESULTS AND DISCUSSION

Total synthesis provided sufficient material for complete spectral characterization (Tables 1 and 2). The major product from the degradation was assigned the structure **4** (Figure 1). The mass spectrum was consistent with addition of a molecule of water to clofarabine and an IR absorption at 1638 cm^{-1} suggested a formamide had been formed. The ^{13}C spectrum showed a resonance for the formamide carbon at 162 ppm (CH in the DEPT spectrum) as well as a smaller resonance at 165 ppm corresponding to an amide rotamer. A combination of one-dimensional and two-dimensional NMR spectra completed the structural elucidation. H_9 occurs as a singlet at 8.90 ppm and the formamide H_7 at 8.19 ppm, which is a doublet (J = 9.0 Hz). Both peaks exchange with D_2O. The NH_2 group at C_6 shows as a broad singlet at 6.76 ppm. A D_2O-exchangeable doublet for the $C_{3'}$ hydroxyl group is seen at 5.44 ppm (J = 5.4 Hz). The resonance for $H_{1'}$ has an apparent *td* pattern at 5.30 ppm that can be further simplified by D_2O exchange of H_7, revealing couplings to $H_{2'}$ (J = 8.2 Hz) and F

TABLE 1 ^{1}H NMR data for formamidopyrimidines 4 and 5 (DMSO)

		4				**5**			
Entry	Assign	δ_H (ppm)	Mult.	Int.	J (Hz)	δ_H (ppm)	Mult.	Int.	J (Hz)
1	H_9	8.89	s	1	NA[a]	8.95	s br	1	NA
2	H_9 rotamer	8.45	d			8.53	d		
3	H_8	8.19	s	1	NA	8.12	s	1	NA
4	H_8 rotamer	7.84	d		10.5	7.78	s		
5	NH_2 rotamer	6.97	s br			6.93	s		
6	NH_2	6.76	s	2	NA	6.74	s br	2	NA
7	H_7	7.50	d	1	9.0	6.19	d	1	9.6
8	$H_{1'}$	5.30	ddd	1	4.2, 8.2, 9.0	5.59	ddd	1	23.2, 1.6, 9.6
9	$OH_{3'}$	5.44	d	1	5.5	5.49	s br	1	NA
10	$OH_{3'}$ rotamer	5.46	d		5.5	NA	NA	NA	NA
11	$OH_{4'}$	4.98	d	1	3.3	4.88	d	1	5.3
12	$OH_{4'}$ rotamer	4.94	d		3.3	NA	NA	NA	NA
13	$H_{2'}$ rotamer	4.77	dt		8.0, 50.7	4.61	ddd		1.6, 4.9, 47.4
14	$H_{2'}$	4.70	dt	1	8.2, 50.7	4.57	ddd	1	1.6, 4.9, 47.4
15	$H_{3'}$[b]	3.85	ddd	1	3.6, 8.7, 20.1	4.02	ddd	1	6, 7.5, 16
16	$H_{4'}$[b]	3.85	d	1	3.6	3.70	m	1	5, 7, 9
17	$H_{5'}$ (eq)	3.76	d	1	2.4	3.53	d	1	7.1
18	$H_{5'}$ (axial)	3.63	d	1	12.1	NA	NA	NA	NA

[a] NA = not applicable.

[b] The apparent multiplet containing the $H_{3'}$ and $H_{4'}$ resonances was further analyzed using DFQ-COSY and TOCSY to give the chemical shifts and couplings shown for each of these hydrogen atoms. The ddd designation for $H_{3'}$ is based primarily on DFQ-COSY.

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TABLE 2 ^{13}C NMR data for formamidopyrimidines 4 and 5 (DMSO)

		4			**5**		
Entry	Assign	δ_C (ppm)	DEPT	J (Hz)	δ_C (ppm)	DEPT	J (Hz)
1	C_8 rotamer	NA[a]	NA	NA	165.35	CH	NA
2	C_8	161.61	CH	NA	161.27	CH	NA
3	C_6	161.43	Cq	NA	161.08	Cq	NA
4	C_4	159.14	Cq	NA	157.81	Cq	NA
5	C_2	156.66	Cq	NA	156.40	Cq	NA
6	C_5	93.31	Cq	NA	94.06	Cq	NA
7	$H_{2'}$	90.41	CH	178.1	92.76	CH	181.4
8	$H_{1'}$	78.74	CH	23.9	74.70	CH	16.7
9	$H_{3'}$	71.26	CH	17.7	67.29	CH	23.5
10	$H_{4'}$	68.76	CH	8.1	64.43	CH	NA
11	$H_{5'}$	66.87	CH_2	NA	64.06	CH_2	NA

[a] NA = not applicable.

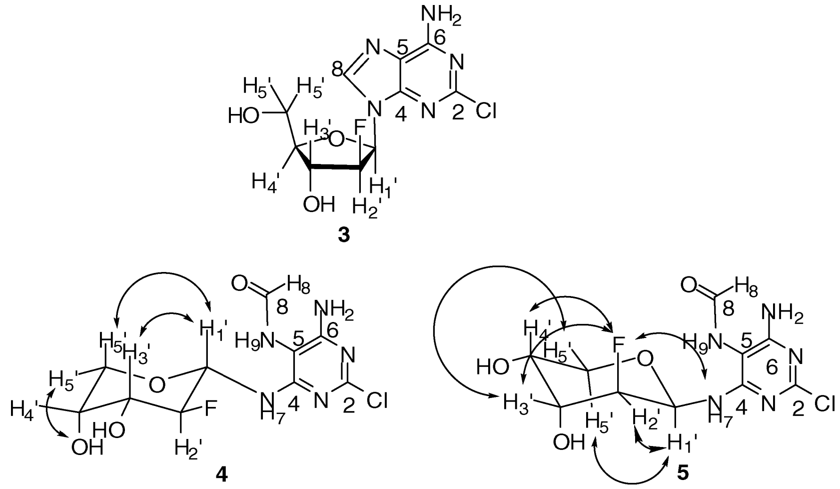

FIGURE 1 Structures of clofarabine (**3**) and degradation products **4** and **5**. (*Note.* Arrows indicate NOEs.).

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($J = 4.2$ Hz); the H_7 coupling being 9.0 Hz. The remaining hydroxyl ($C_{4'}$) appears as a doublet at 4.98 ppm ($J = 3.1$ Hz). $H_{2'}$ is a *dt* pattern showing the characteristic geminal fluorine coupling ($J = 50.7$ Hz) as well as vicinal couplings of 8.1 Hz. $H_{3'}$ and $H_{4'}$ show an overlapping multiplet at 3.91–3.81 ppm, which was further resolved by examination of the DQF-COSY and TOCSY spectra (individual resonances for $H_{3'}$ 3.85 ppm (ddd, $J \approx 3.6$, 8.7, and 20.1 Hz) and for $H_{4'}$ at 3.85 ppm (d, $J \approx 3.6$ Hz) could be estimated from the DQF-COSY or by applying a Gaussian window function). Consistent with the pyranose assignment, distinct resonances were observed for $H_{5'}$ (axial) and $H_{5'}$ (equatorial). The resonance for $H_{5'}$ (equatorial) occurs as a doublet at 3.76 ppm ($J = 12.1$ Hz), whereas $H_{5'}$ (axial) appears at 3.63 ppm ($J = 12.1$ Hz). A smaller coupling between $H_{5'}$ (equatorial) and $H_{4'}$ ($J \approx 2.4$ Hz) could be observed upon application of the Gaussian window function and also in the DQF-COSY spectrum. These observations suggest a dihedral angle of 40–60° between $H_{5'}$ (equatorial) and $H_{4'}$ and 60–90° between $H_{5'}$ (axial) and $H_{4'}$. Finally, the anomeric stereochemical assignment for **4** is supported by the ROESY spectrum, where the supportive ROEs between $H_{3'}$, $H_{5'}$ (axial) and $H_{1'}$, respectively, are indicated by arrows in Scheme 2. The pyranose chair conformation is further supported by an ROE between $H_{5'}$ (equatorial) and $H_{4'}$.

Compound **5** showed many of the same spectroscopic features as **4** with some key differences. As with **4**, there was evidence of rotamers in the spectra; this was confirmed by variable temperature NMR experiments. The ratios of individual resonances to their minor rotamers, based on integration, varied from 7.7:1 to 10:1 at 298 K. Coalescence was observed as the temperature was raised to 348 K. The transience of certain NOEs (e.g.,

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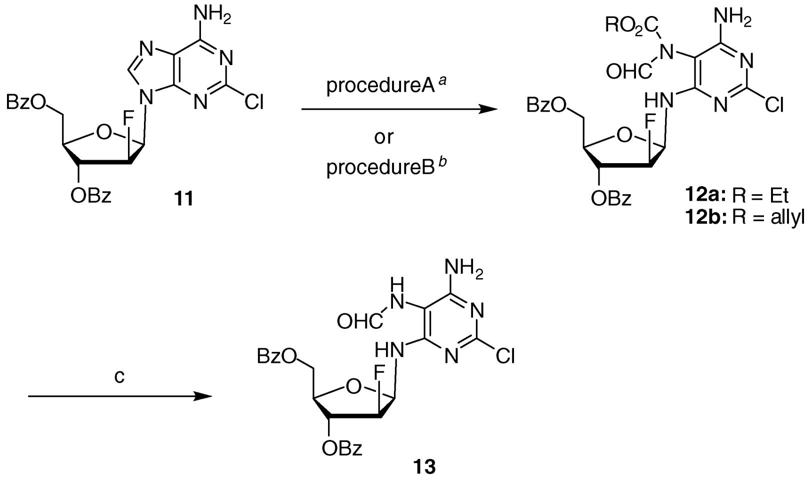
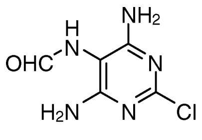


SCHEME 2 Conversion of the 2-chloroadenine ring to a formamidopyrimidine. (*Key*: [a]Procedure A: diethyl pyrocarbonate. [b]Procedure B: allyl chloroformate (1.5 eq), $NaHCO_3$ (6 eq), H_2O (7 eq), THF, room temperature, 2 days. [c]$Pd(PPh_3)_4$ (1 mol%), dimedone (6 eq), THF, room temperature 3.5 hours (30% from **11**).)

$H_{2'}$ NOEs) complicated the interpretation of this data and an optimized mixing time had to be used. However, useful NOEs were observed between the axial F, $H_{3'}$, $H_{4'}$, $H_{2'}$, and H_7, respectively. Significantly, the coupling constant between $H_{1'}$ and F was markedly larger for **5** ($J = 23.2$ Hz) compared with **4** ($J = 4.2$ Hz), consistent with the more favorable 180° dihedral angle in **5**. The coupling constant between $H_{1'}$ and $H_{2'}$ decreased by 6.6 Hz, and similarly the decrease in coupling constant between $H_{2'}$ and $H_{3'}$ of 3.3 Hz, both support the 60° dihedral angles between these hydrogen atoms, as shown in structure **5**. Further evidence for the anomeric assignment and chair conformation comes from NOEs, indicated by arrows (Scheme 2).

As was mentioned, a third degradation product was isolated whose mass was consistent with a formamidopyrimidine lacking the sugar moiety. This was also synthesized and characterized spectroscopically. The structure was assigned as formamidopyrimidine **6** (Figure 2). The UV and IR spectra bore similarity to those of compounds **4** and **5**. Consistent with the proposed structure, the ^1H NMR spectrum (Table 3) showed three kinds of hydrogen atoms, and their associated rotamers. This data is substantiated by the



6

FIGURE 2 Structure of formamido pyrimidine **6**.

TABLE 3 ^{1}H NMR data for formamidopyrimidine 6

Entry	Assign	δ (ppm)	Int.	D_2O exchangeable
1	5-NH	8.734	1 H	Yes
2	CHO	8.07	0.9 H	No
3	CHO rotamer	7.76	0.1 H	No
4	NH_2 rotamer	6.63	0.6 H	Yes
5	NH_2	6.45	3.4 H	Yes

^{13}C spectrum (Table 4). Variable temperature 1H NMR spectra showed coalescence of the rotamer peaks at 363 K.

The synthesis of formamidopyrimidines **4–6** will now be described. The ring-opening of adenine and related purine nucleosides and nucleotides at C_8 to afford formamidopyrimidines has precedent in the literature and has been studied mechanistically.[2] This type of degradation has most often been observed in the presence of aqueous base, but other conditions, including gamma irradiation,[3] hot formamide,[4] potassium amide in liquid ammonia,[5] and diethyl pyrocarbonate,[6] result in similar structural changes. Under basic conditions, the furanose portion of the nucleoside often isomerizes to the corresponding pyranose. The base-sensitivity of clofarabine leads to a variety of byproducts that do not include the formamidopyrimidines. Attempts to prepare **4** via diaminonitropyrimidines in a manner similar to Greenberg were also unsuccessful.[7]

Reports by Leonard and coworkers detail the use of diethyl pyrocarbonate, in the presence of water, to promote ring-opening on purine nucleosides.[6] Thus, adenosine reacted with diethyl pyrocarbonate and water to give a mixture of pyrimidines **7** and **8** as the major products. In contrast, 9-propyladenine afforded the formamides **9** and **10** (Figure 3). We, therefore, decided to investigate the reactions of benzoylated clofarabine derivative **11** with pyrocarbonates.

Our initial effort focused on the reaction of **11** with excess diethyl pyrocarbonate. Under conditions analogous to Leonard,[6] we were able to isolate the formylated adduct **12a** from a mixture of components that were not fully characterized. However, attempted cleavage of ethyl carbamate

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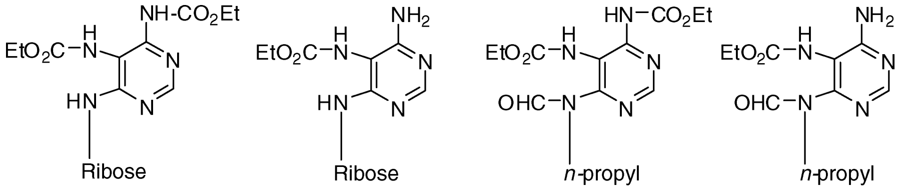

7 **8** **9** **10**

FIGURE 3 Compounds resulting from the diethylpyrocarbonate ring opening of purines.

TABLE 4 ^{13}C NMR data for formamidopyrimidine 6

Entry	Assign	δ (ppm)	DEPT	Relative height
1	Formamide rotamer	165.6	CH	9.0
2	C_4, C_6 rotamer	162.2	C	12.7
3	Formamide	161.1	CH	61.3
4	C_4, C_6	160.9	C	87.5
5	C_2	156.6	C	2.5
6	C_2	156.4	C	23.9
7	C_5 rotamer	92.4	C	5.7
8	C_5	91.9	C	32.2

group under acidic or basic conditions led to loss of the formamide group and various byproducts. In order to derive an intermediate with a more readily cleaved carbamate function, namely the allyl carbamate (Alloc), we investigated the reactions of allyl chloroformate with benzoylated clofarabine (**11**) (Scheme 2).

The reaction of **11** with allyl chloroformate and sodium bicarbonate was carried out in THF containing water at room temperature. Under these conditions, crude **12b** was formed (78% a/a by HPLC) upon stirring for 2 days. The purification of **12b** was complicated by its sensitivity to silica gel, loss of the formamide group being a major byproduct. Nevertheless, purified **12b** could be isolated in 19% yield. Alternatively, the crude reaction mixture was deprotected using catalytic $Pd(PPh_3)_4$ and dimedone[8] to afford formamide **13** in 30% overall yield after chromatography; some cyclization of **13** back to **11** was also observed during chromatography.

Deprotection of **13** was readily accomplished by heating with catalytic sodium methoxide in methanol, which resulted in concomitant removal of the benzoate esters and furanose to pyranose rearrangement to give **4**. Upon simple evaporation of the solvent and washing the resultant solid with hexane to remove methyl benzoate, **4** was isolated in 52% yield. The deprotection reaction also produced the same isomeric formamidopyrimidine (**5**) that was observed during degradation. Purification of the latter by preparative HPLC chromatography provided sufficient sample for characterization. Thus the crude reaction mixture was purified by chromatography to afford **4** (35%) and **5** (12%). In addition, isomerization of **4** to **5** could also be accomplished with DBU in DMF to afford a more favorable mixture (**5**/**4** = 4) mixture of products **5**:**4** (Scheme 3). The mechanism of the anomerization is believed to involve reversible base promoted opening of the aminal to an imine.

Removal of the sugar moiety from **4** was accomplished in two steps. It was observed that the formamide was cleaved when **4** was treated with methanolic HCl at room temperature. If the same reaction was

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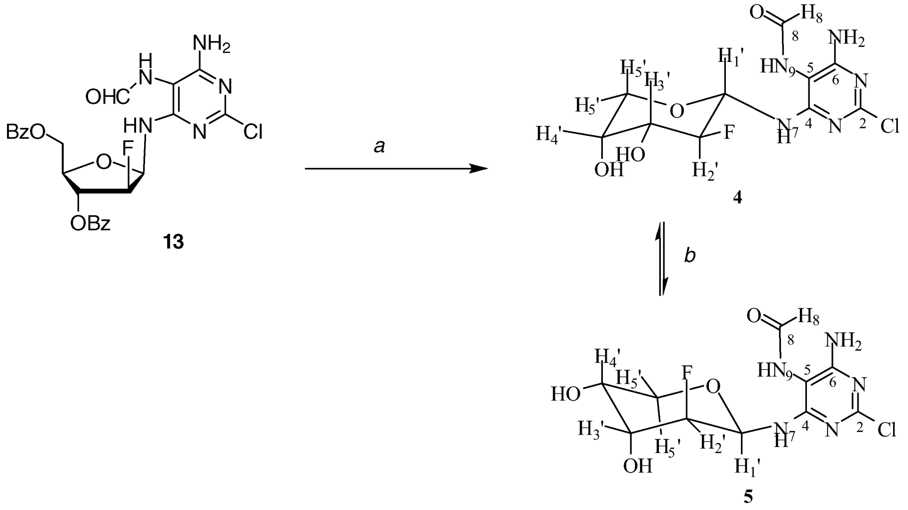

SCHEME 3 Formation and isomerization of formamidopyrimidines **4** and **5**. (*Key*: [a]NaOMe (10 mol%), MeOH, 40°C, 21 hours, **4** (52%). [b] DBU, DMF, room temperature.)

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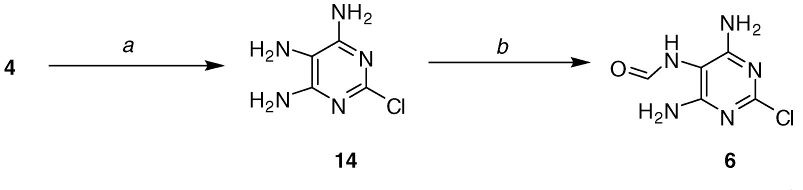
run at 40°C, both the formamide and the sugar were cleaved to afford 2–chloro–4,5,6–triaminopyrimidine (**14**). The formamide was reintroduced by reaction of **14** with formic-pivalic anhydride and triethylamine, affording the previously observed degradant **6** (Scheme 4).

In conclusion, we demonstrated a practical procedure for the synthesis of formamidopyrimidines related to clofarabine. The use of allyl chloroformate in slight stoichiometric excess and subsequent cleavage of the Alloc group gave access to these sensitive compounds without affecting the sugar moiety.

EXPERIMENTAL

Reactions were run under N_2 atmosphere. Melting points were obtained on a Buchi B545 melting point apparatus and are uncorrected. NMR spectra were conducted on a 400 MHz Bruker Avance II system unless otherwise stated. IR spectra were obtained using KBr pellets on a Matson Infinity Gold FTIR instrument. UV spectra were obtained using a Beckman



SCHEME 4 Synthesis of formamidopyrimidine **6**. (*Key*: [a]1M HCl, MeOH, 40°C, 24 hours. [b]HCO_2H, *t*-BuCOCl, Et_3N, room temperature, 24 hours.)

DU640 spectrophotometer. HPLC data was collected on either of two Waters instruments: Waters System 600 Dual Pump Controller with Waters 996 Photodiode Array detector or Waters Alliance system. HPLC methods are described herein.

Preparation of (1R)-N-(4-Amino-2-chloro-6-((2S,3S,4R,5R)-3-fluoro-4,5-dihydroxytetrahydro-2H-pyran-2-ylamino)pyrimidin-5-yl)formamide (4) by Forced Degradation of Clofarabine (3)

To a 2 L glass round-bottomed flask was charged 5.00 g of clofarabine (**3**) and 500 mL of 50 mM sodium phosphate buffer (8.6 g NaH_2PO_4 and 11.25 g NaCl in 500 mL, buffered to pH 7 with NaOH). The reaction was heated overnight at reflux for a total of 19 hours. Reaction was checked by HPLC and it was determined to contain 0.31 area% of **4**. Upon cooling the reaction, solid clofarabine precipitated from the reaction mixture. This solid was examined by HPLC and found to contain no **4**. The aqueous filtrate was washed twice with 500 mL of MeCN to further remove clofarabine (MeCN and saline solution are not miscible). The aqueous layer was evaporated to dryness in vacuo to give a mixture of **4**, **3**, and NaCl. This mixture was stirred in ~200 mL of MeCN until the solids were free flowing and no clumps remained. Brief sonication assisted in breaking the clumps apart. The salt was subsequently filtered out of the solution, the filtrate evaporated and the residue purified by reverse phase preparative chromatography to yield 24.9 mg of **4**.

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(1*R*,2*R*,3*R*,4*S*,5*R*)-5-(5-(*N*-(Allyloxycarbonyl)formamido)-6-amino-2-chloropyrimidin-4-ylamino)-2-(benzoyloxymethyl)-4-fluorotetrahydrofuran-3-yl benzoate (12b)

A 200 mL flask was charged with protected clofarabine **11** (9.36 g, 18.3 mmol), sodium bicarbonate (10.75 g, 128 mmol), THF (50 mL), and water (2.0 mL, 110 mmol). Allyl chloroformate (2.9 ml, 27.4 mmol) was added and the mixture was stirred at ambient temperature for 43 hours, after which HPLC analysis showed the reaction to be complete. The reaction mixture was filtered to remove inorganic salts, the flask and solids were washed with THF (50 mL) and the filtrate was reduced via rotary evaporation. A portion of the residue containing **12b** (11.06 g, 78% a/a HPLC) was used in the next step without further purification. A portion of crude **12b** (1.05 g) was purified twice by silica gel chromatography (hexanes/EtOAc, 0 to 100% EtOAc) to give **12b** suitable for characterization (200 mg) in 19% yield. m.p. 106°C (dec). ^1H NMR (mixture of rotamers) δ 9.12 (s, 0.4H, 5-NCHO), 9.06 (s, 0.6H, 5-NCHO), 8.05 (dm, 2H, $J = 8.3$, *o*-Ph), 7.95 (dm, 2H, $J = 8.2$, *o*-Ph), 7.73 (d, 1H, *J* = 9.4, 4-NH), 7.72, (tm, 1H, *J* = 7, *p*-Ph), 7.65 (t, 1H, *J* = 7.4, *p*-Ph), 7.57 (tm, 2H, *J* = 7.0, *m*-Ph), 7.48 (t, 2H, *J* = 7.8, *m*-Ph), 7.12 (s, 2H, 6-NH_2), 6.24–6.17 (m, 1H, $H_{1'}$), 5.95–5.83 (m, 1H, $H_{2''}$), 5.77–5.68 (m, 1H, $H_{3'}$), 5.44 (dm, 1H, *J* = 48, $H_{2'}$), 5.35–5.12 (m, 2H, $H_{3''}$, $H_{4''}$), 4.75–4.66 (m, 2H, $H_{1"}$), 4.61–4.42 (m, 2H, $H_{5'}$), 4.36–4.30 (m, 1H, $H_{4'}$). ^{13}C NMR 165.4, 165.0, 162.8, 162.6, 161.54, 161.49, 158.4, 158.3, 157.9, 152.2, 133.8, 133.4, 131.9, 129.6, 129.3, 129.2, 128.8, 128.6, 117.6, 93.9, 92.5, 92.4, 92.0, 80.5, 80.4, 80.2, 76.9, 76.7, 76.6, 76.5, 76.0, 75.8, 66.7, 64.6, 64.4, 59.7 ppm. IR (KBr) 3367, 1723, 1597, 1577, 1327, 1275, 1112, 959, 712 cm^{-1}. UV (MeOH/H_2O) λmax$_1$ = 222 nm, λmax$_2$ = 261 nm. Mass spec.(electrospray, positive) m/e [M$^+$ H] = 614. Anal. Calcd for $C_{28}H_{25}ClFN_5O_8$: C, 54.77; H, 4.10; Cl, 5.77; F, 3.09; N, 11.41. Found: C, 54.61; H, 4.25 Cl, 5.58; F, 2.94; N, 11.14.

(1R,2R,3R,4S,5R)-5-(6-Amino-2-chloro-5-formamidopyrimidin-4-ylamino)-2-(benzoyloxymethyl)-4-fluorotetrahydrofuran-3-yl benzoate (13)

A 200 mL flask was charged with crude **12b** (7.84 g, 12.8 mmol, based on 100% purity), dimedone (10.74 g, 76.6 mmol), Pd(PPh_3)$_4$ (0.171 g, 0.148 mmol), and THF (100 mL). The system was inerted with nitrogen and the mixture was stirred at ambient temperature for 3 hours after which HPLC analysis showed the reaction to be complete. The solvent was removed under vacuo, and the residue was triturated with EtOAc (100 mL). The suspension was filtered to remove excess dimedone and the filtrate washed with sat. $NaHCO_3$ (3 × 50 mL). The filtrate was treated with charcoal and $MgSO_4$ and then filtered. Heptane (50 mL) was added and EtOAc was removed *via* azeotropic distillation. The resulting suspension was filtered and the flask and solids were further washed with heptane (25 mL). The solids were dried (ambient temp, 50 mm Hg) for 16 hours to give 5.91 g of the crude **13**. Purification by silica gel chromatography (2 ×, hexanes/EtOAc, 0 to 100% EtOAc) gave pure **13** as an off-white solid (2.06 g) in 30% yield from **11**. m.p. 203–205°C. ^{1}H NMR δ 9.01 (d, 1H, *J* = 0.8, 5-NH), 8.16 (d, 1H, *J* = 1.1, 5-NCHO), 8.06 (dm, 2H, *J* = 7.1, *o*-Ph), 7.98 (dm, 2H, *J* = 7.1, *o*-Ph), 7.72, tm, 1H, *J* = 10.6, *p*-Ph), 7.66 (tm, 1H, *J* = 8.8, *p*-Ph), 7.57 (tm, 2H, *J* = 7.6, *m*-Ph), 7.51 (tm, 2H, *J* = 7.8, *m*-Ph), 6.86 (br s, 2H, 6-NH_2), 6.51 (d, 1H, *J* = 9.6, 4-NH), 6.13 (ddd, 1H, *J* = 3.6, 9.9, 20.6, $H_{1'}$), 5.61 (ddd, 1H, *J* = 3.6, 4.4, 19.9, $H_{3'}$), 5.45 (ddd, 1H, *J* = 1.7, 3.6, 50.5, $H_{2'}$), 4.61–4.49 (m, 2H, $H_{5'}$), 4.41–4.37 (m, 1H, $H_{4'}$). ^{13}C NMR 165.4, 164.9, 161.2, 157.4, 156.4, 133.8, 133.5, 129.6, 129.2, 128.8, 128.7, 94.7, 94.0, 92.8, 80.9, 80.7, 76.8, 76.5, 64.2 ppm. IR (KBr) 3364, 1726, 1599, 1576, 1501, 1452, 1315, 1114, 1070, 959, 713 cm^{-1}. UV (MeOH/H_2O) λmax$_1$ = 220 nm, λmax$_2$ = 266 nm. Mass spec.(electrospray, positive) m/e [M$^+$H] = 530. Anal. Calcd

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for $C_{24}H_{21}ClFN_5O_6$: C, 54.40; H, 3.99; Cl, 6.69; F, 3.59; N, 13.22. Found: C, 54.41; H, 3.74; Cl, 6.46; F, 3.37; N, 13.04.

(1R)-*N*-(4-Amino-2-chloro-6-((2S,3S,4R,5R)-3-fluoro-4,5-dihydroxytetrahydro-2H-pyran-2-ylamino)pyrimidin-5-yl)formamide (4)

A 200 mL flask was charged with **13** (2.05 g, 3.87 mmol), methanol (23 mL), and sodium methoxide (88.5 μL, 0.387 mmol). The mixture was stirred at 40°C for 20.5 hours. The resulting suspension was filtered and the flask and solids were washed with hexanes to remove methyl benzoate. The solid was dried (25°C, 5 torr) to give **4** as a white solid (0.59 g, 2.01 mmol) in 52% yield. m.p. 212°C (dec). ^{19}F NMR -200.4 (d, $J = 44$). IR (KBr) 3419, 1638, 1601, 1508, 1473, 1435, 1385, 1316, 1079, 1038, 772 cm^{-1}. UV (MeOH/H_2O) $\lambda max_1 = 219$ nm, $\lambda max_2 = 264$ nm. Mass spec. (electrospray, positive) m/e [M^+H] = 322. Anal. Calcd for $C_{10}H_{13}ClFN_5O_4$: C, 37.34; H, 4.07; Cl, 11.02; F, 5.91; N, 21.77. Found: C, 37.34; H, 3.74; Cl, 11.12; F, 5.82; N, 21.51.

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Isolation of (1R)-*N*-(4-Amino-2-chloro-6-((2S,3S,4R,5R)-3-fluoro-4,5-dihydroxytetrahydro-2H-pyran-2-ylamino)pyrimidin-5-yl)formamide (4) and (1S)-*N*-(4-Amino-2-chloro-6-((2S,3S,4R,5R)-3-fluoro-4,5-dihydroxytetrahydro-2H-pyran-2-ylamino)pyrimidin-5-yl)formamide (5) by Preparative HPLC

A 50 mL reaction vial was charged with **13** (0.142 g, 0.268 mmol) and MeOH (4 mL). NaOMe (25wt%, 10 μL, 0.044 mmol) was added and the mixture was stirred at room temperature for 3 days, then at 40°C for 22 hours. Water (0.5 mL) was added followed by AcOH (10 μL, pH = 4). The reaction mixture was washed with hexanes to remove MeOBz and the aqueous layer reduced by rotary evaporation to give the crude residue (75 mg), which was purified by preparative HPLC. Preparative HPLC conditions: column; Waters Atlantis dC_{18}, 5 μm, 19 × 100 mm; flow; 15.0 mL/min; solvent A = H_2O; solvent B = MeCN; Isocratic (6% B, 94% A) from 0 to 4 min; gradient (6% B to 100% B) from 4 min to 8 min. Fractions were collected to afford (**5**) in 12% yield and (**4**) in 34% yield. IR (KBr) 3429, 2965, 2875, 1631, 1449, 1315, 1201, 1096, 1053 cm^{-1}. UV (MeOH/H_2O) $\lambda max_1 = 219$ nm, $\lambda max_2 = 264$ nm. Mass spec. (electrospray, positive) m/e [M+H] = 322.

Base Promoted Equilibration of 4 and 5

Compound **4** (14 mg) was dissolved in DMF (1.0 mL). Triethylamine (9 μL) was added and the mixture was heated to 50°C. No change was observed in the absence of DBU. DBU (10 μL) was added and heating continued. After 3 days, HPLC area ratio of **4** to **5** was 4:1.

2-Chloropyrimidine-4,5,6-triamine (14)

A 500 mL flask was charged with **4** (10.36 g, 19.55 mmol), MeOH (350 mL), and 1 M HCl (29 mL). The mixture was heated to 40°C and stirred for 24 hours after which HPLC analysis showed no more **4** remaining and the reaction was subsequently worked up. The volatiles were removed under vacuum and the residue triturated with EtOAc (250 mL). The suspension was filtered and the flask and solids were washed with EtOAc (50 mL). The solid was dried under vacuum to give crude **14** (3.02 g, 97% recovery). The material was used in the next step without further purification.

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N-(4,6-diamino-2-chloro-pyrimidin-5-yl)-formamide (6)

A 200 mL flask was charged with formic acid (25 mL). The flask was cooled in an ice/water bath and NEt_3 (7.3 mL, 52.5 mmol) was added followed by pivaloyl chloride (1.6 mL, 12.9 mmol). The cooling bath was removed and **14** was added in one portion. The mixture was stirred at ambient temperature for 24 hours where after ethyl acetate (100 mL) was added and the resultant suspension filtered. The flask and solids were washed with EtOAc (50 mL) and the solid dried under vacuum to give crude **6** (0.95 g, 76% a/a HPLC purity).

Several lots of crude **6** were combined for purification. A 200 mL flask was charged with **6** (3.73 g) and saturated $NaHCO_3$ (25 mL) was added slowly to the solid; gas evolution was observed. The suspension was filtered and the flask and solids were washed with water (10 mL). The solid was dried under vacuum to give pure **6** (2.41 g, 13 mmol) in 64% overall yield. mp = 275°C. ^{1}H NMR δ (mixture of rotamers) 8.73 (br s, 1 H, 5-NH), 8.07 (s, 0.9H, 5-NCHO), 7.76 (s, 0.1H, 5-NCHO), 6.63 (s, 0.6 H, NH_2), 6.45 (br s, 3.4H, NH_2). ^{13}C NMR 165.6 (CHO minor rotamer), 162.2 (C_4, C_6 minor rotamer), 161.1 (CHO major rotamer), 160.9 (C_4, C_6, major rotamer), 156.6 (C_2 minor rotamer), 156.4 (C_2 major rotamer), 92.4 (C_5 minor rotamer), 91.9 (C_5 major rotamer) ppm. IR (KBr) 3422, 3145, 1632, 1570, 1477, 1314, 1184, 1049, 963, 769 cm^{-1}. UV (MeCN/H_2O) $\lambda max_1 = 217$ nm, $\lambda max_2 = 262$ nm. Mass spec.(electrospray, positive) m/e [M^+H] = .188. Anal. Calcd for $CHClN_5O_6$: C, 32.01; H, 3.22; Cl, 18.90; N, 37.33. Found: C, 31.99; H, 3.35; Cl, 18.99; N, 37.16.

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