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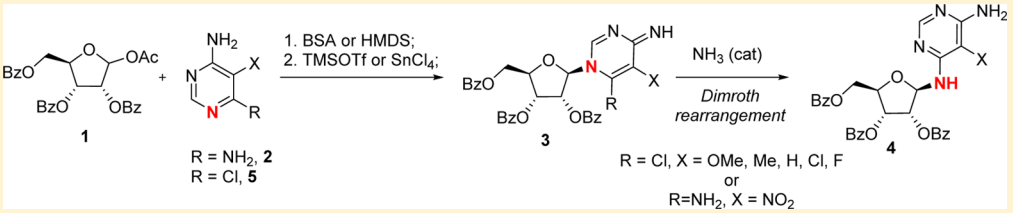
Unexpected Observation of the Dimroth Rearrangement in the Ribosylation of 4-Aminopyrimidines

Ramil Y. Baiazitov,* Nadiya Sydorenko, Hongyu Ren, and Young-Choon Moon

PTC Therapeutics, Inc., 100 Corporate Ct., South Plainfield, New Jersey 07080, United States

Supporting Information



ABSTRACT: A method for the preparation of 1-(*N*-ribofuranosyl)-6-imino-1,6-dihydropyrimidin-4-amines **3** or 4-(*N*-ribofuranosyl)-6-aminopyrimidines **4** via glycosylation of 4-aminopyrimidines **2** or **5** is described. Silylated 4-aminopyrimidines **2** or **5** upon ribosylation with **1** provide products **3**. When intermediates **3** contain a strongly electron-withdrawing group, such as C(4)-Cl or C(5)-NO_2, they rearrange to products **4** in the presence of aqueous ammonia. A mechanism is proposed that involves a ring-opening/ring-closing (Dimroth) rearrangement.

INTRODUCTION

Clitocine (Figure 1) was first isolated from the mushroom *Clitocybe inversa* in 1986.[1] The structure of this adenosine analog has been confirmed by the synthesis of the molecule and X-ray crystal structure spectroscopy.[2] Clitocine attracted significant attention from the scientific community because in addition to strong insecticidal activity,[1] this compound also exhibits antitumor[3] and read-through[4] activities. These pharmacological properties prompted several research groups to optimize the synthesis of clitocine and its analogs.[3−5]

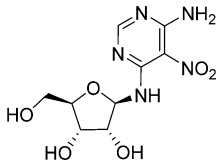


Figure 1. Structure of clitocine.

Clitocine and its analogs have been prepared following two major routes (Scheme 1). In the first route, the ribofuranosylamine **i** can react as the nucleophile with a 4-chloropyrimidine **ii** as the electrophile by an SN_{Ar} mechanism with formation of the nucleoside core.[5] Unfortunately, nucleophile **i** is not thermally stable and is known to decompose via extrusion of ammonia and formation of the bisglycosylamine.[6] This instability severely limits the reaction scope such that only highly activated electrophiles, such as 5-nitro-, 5-sulfonyl, or 5-alkoxycarbonyl-4-chloropyrimidines, are suitable substrates. An additional drawback of this approach is that **i** consists of a mixture of anomers at C(1) resulting in the formation of mixtures of α- and β-anomeric products **iii**.

In the second route, condensation of a ribofuranosyl electrophile (such as **iv**) with a 4-aminopyrimidine nucleophile, **v**, can produce the desired analog by a Vorbrüggen reaction[7] in the presence of a Lewis acid (Scheme 1). In this route, aminopyrimidine **v** is usually silylated prior to reaction to increase both solubility and reactivity. Surprisingly, this method has been used to prepare only a limited number of 4-(*N*-ribofuranosyl)-6-amino- (or 6-hydroxy-) pyrimidines.[8] Moreover, it has been demonstrated that the 4-(*N*-ribofuranosyl)-6-aminopyrimidine **vii** is not the kinetic product of this reaction but rather the dearomatized 1-(*N*-ribofuranosyl)-6-imino-1,6-dihydropyrimidin-4-amine (**vi**, Y = NH, or the tautomer), which isomerizes to product **vii** upon exposure to silica gel or in acetic acid.[8b] No mechanism for this rearrangement has been proposed.

Isomerization such as from **vi** to **vii** is not common. There are numerous examples of the Vorbrüggen reaction with 4-aminopyrimidines where the endocyclic nitrogen atom, usually opposite to the exocyclic amino group, formed the glycosylic bond with the sugar residue and no isomerization followed.[9] Apparently, the endocyclic nitrogen atom is the most nucleophilic one. Products in which the exocyclic amino group forms the glycosylic bond are rare and only a few such products could be found in literature. Moreover, it is not clear if these products were formed directly or as a result of a similar isomerization.[8a,d] The phenomenon of a secondary isomerization requires an explanation.

In this report, we demonstrate that silylated 4,6-diaminopyrimidines during Vorbrüggen reaction attack the ribofuranoside donor by the endocyclic nitrogen atom opposite to the

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Scheme 1

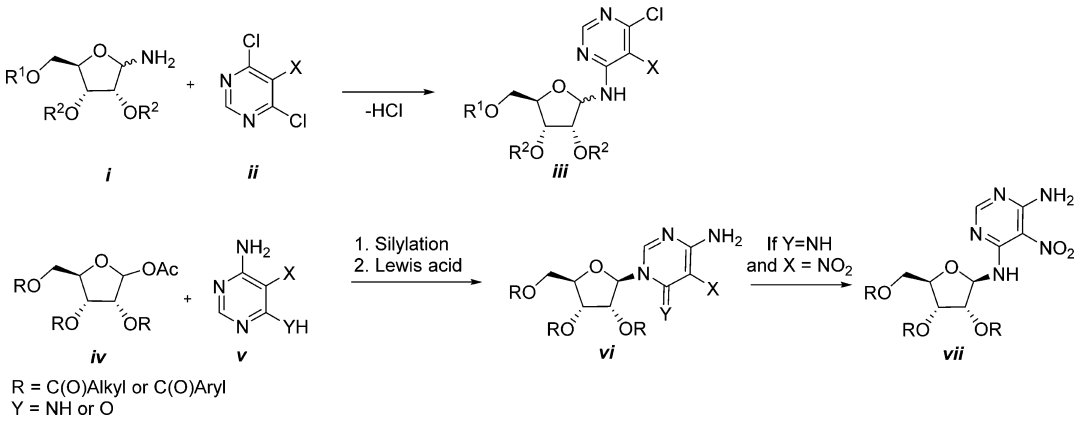
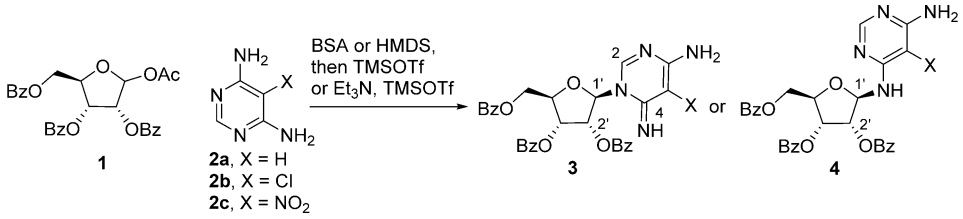


Table 1. Reactions between 4,6-Diaminopyrimidines 2 and Ribofuranoside 1



entry	X	conditions	temp, time[a]	product	yield, %[b]
1	H	BSA/TMSOTf	rt, 8 h	**3a**	39
2	H	BSA/TMSOTf	60 °C, 50 min	**3a**	85
3	Cl	TEA/TMSOTf	rt, 1.5 h	**3b**	86
4[c]	NO_2	HMDS/TMSOTf	rt, 18 h	**4c**	72
5	NO_2	TEA/TMSOTf	rt, 16 h	**4c**	92

[a]Temperature and time in the presence of TMSOTf. [b]Isolated yield. [c]Taken from ref 8c.

exocyclic amino group to form 1-(*N*-ribofuranosyl)-6-imino-1,6-dihydropyrimidin-4-amines **vi**. Depending on the nucleophile substitution, a rearrangement of **vi** can take place via the pyrimidine ring opening/closure (i. e., Dimroth rearrangement[10]) catalized by a nucleophile, such as ammonia. This rearrangement is facile only when the intermediate is activated by an electron-withdrawing substituent on the pyrimidine, such as a nitro-group at C(5). This limitation helps to explain the scarcity of the literature examples of such reactions. However, we found that even the intermediates without C(5) activation can isomerize when one of the NH_2-groups in the nucleophile is replaced by an electron-withdrawing Cl-group, which provides the required activation. This electron-withdrawing Cl-group overcomes this limitation and enables preparation of 4-(N-ribofuranosyl)-6-aminopyrimidines via Vorbrüggen reaction using 4-chloro-6-aminopyrimidines as the nucleophiles, followed by reaction with ammonia and in situ Dimroth-like rearrangement to provide products **vii**.

RESULTS AND DISCUSSION

Reactions between Silylated 4,6-Diaminopyrimidines 2 and Ribofuranoside 1. Only a very limited number of clitocine analogs were prepared by Vorbrüggen reaction. The analogs of clitocine in which the C(5)-nitro group is replaced by hydrogen or chlorine atoms (**4a**, X = H; **4b**, X = Cl; Table 1) were targeted first. It was expected that using conditions similar to the previously published clitocine preparation procedure (Table 1, entry 4), 4,6-diaminopyrimidine **2a** and **2b** will be converted to **4a** (X = H) and **4b** (X = Cl). The procedure[8c] involved silylation of the 4,6-diaminopyrimidine **2c** (Table 1, X = NO_2) with HMDS, followed by Vorbrüggen reaction and optional isomerization on silica gel.[8b]

However, in our hands, the control reaction between **1** and **2c** was not reproducible and the product of ribosylation often could not be isolated in pure form after chromatography on silica gel as judged by HPLC and NMR analyses. Moreover, the reaction was difficult to monitor by TLC or HPLC, perhaps, because of the relatively fast conversion of **3c** into **4c** during the analysis. Serendipitously, it was found that when the sample for the HPLC-MS analysis was dissolved in acetonitrile containing ammonium hydroxide, the HPLC-peak corresponding to the product **4c** could be clearly seen and no peak corresponding to **3c** could be observed, suggesting very fast isomerization from **3c** to **4c**. This fast isomerization was further confirmed when ammonium hydroxide was used for the reaction quench and upon isolation of **4c**.

In the optimized procedure, 4,6-diaminopyrimidine **2c** (Table 1, X = NO_2) was combined with ribofuranoside **1** in the presence of TMSOTf (2 equiv) and triethylamine (1 equiv) to form **4c** in 92% yield upon quench with ice-cold acetonitrile-ammonium hydroxide mixture and column chromatography (Table 1, entry 5). This improved procedure for the synthesis of **4c** was very reproducible in our hands.

Next, similar reactions with other 4,6-diaminopyrimidines were attempted. Unexpectedly, silylated 4,6-diaminopyrimidine **2a** (Table 1, X = H) combined with ribofuranoside **1** in the presence of TMSOTf to form **3a** as a single β-isomer (Table 1,

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entries 1 and 2) instead of the anticipated **4a**. Heating to 60 °C accelerated the reaction and improved the isolated yield of **3a** to 85%.

The structure of **3a** was established using 1D and 2D-NMR spectroscopy (Figure 2). For example, the $^{1}H-^{13}C$-HMBC

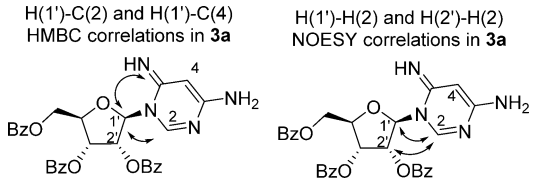

Figure 2. Structural assignment of **3a**.

spectrum (see Supporting Information, page S11) clearly shows coupling (the cross-peaks) that is comparable in magnitude between H(1′) of the furanose (δ = 6.43, d, J = 5.0 Hz, 1H) and both C(2) and C(4) of pyrimidine (δ = 154.0 (C(4)), 148.7 (C(2))). Additionally, 2D-NOESY experiment (see Supporting Information, page S13) demonstrated through-space correlation between H(2) of the pyrimidine ring with both H(1′) and H(2′) of the furanose. The signal of H(1′) in **3a** is a doublet, as opposed to the broad singlet (doublet of doublets in several other analogs) observed in **4a** (c.f. Figure 3).

Reaction of the 5-chloropyrimidine **2b** proceeded in an analogous fashion to provide **3b** in 86% yield (Table 1, entry 3).[11] In neither case could the product of isomerization **4** be observed by HPLC or NMR analysis. Longer treatment of **3a** with ammonium hydroxide led to removal of the benzoate group at C(2′) and C(3′) without causing isomerization of the pyrimidine ring.[12]

Dimroth Rearrangement from 3 to 4. The described above failure to prepare **4a** and **4b** was unexpected. It was demonstrated previously[8b] that a similar analog **3c** could be isolated and isomerized to **4c** in the presence of silica gel or acetic acid, Scheme 2. The reaction mechanism was not proposed, although the acidic conditions suggest that it may involve breaking of the C(1′)-N^b bond in the intermediate **3c** and making of the C(1′)-N^a bond instead in $\mathbf{N^a}$**-4c**. This may proceed, for example, via ionization at C(1′). The lability of C(1′)-N^b in an aminal, such as **3c**, especially under acidic conditions (such as in the presence of acetic acid or silica gel as in ref 8b) supports this mechanism.

On the other hand, it is possible, that isomerization of **3c** to **4c** (Scheme 3) is initiated by a nucleophilic attack onto the pyrimidine ring (NuH may be HOH or H_2NH, etc.) with a concominant ring opening to form a short-lived intermediate **xii**. Rotation of the bond in **xii** indicated on the scheme and extrusion of the nucleophilic catalyst regenerates the pyrimidine ring in $\mathbf{N^b}$**-4c**. The difference between $\mathbf{N^a}$**-4c** and $\mathbf{N^b}$**-4c** is in the position of the nitrogen atoms: atoms $\mathbf{N^a}$ and $\mathbf{N^b}$ exchange positions within the pyrimidine ring.

To establish which mechanism is operative, ribosylation with ^{15}N-labeled pyrimidine **8** was conducted (Scheme 4). This intermediate was prepared by treatment of 4,6-dichloro-5-nitropyrimidine **7** with ^{15}N-labeled ammonium hydroxide. The pyrimidine **8** was silylated and glycosylated with **1** in the presence of tin(IV) chloride. After quenching with NH_4OH, product **10** was isolated in 47% yield.

The structure of **10** was established by inspection of the magnitude of the $^{1}H-^{15}N$ coupling constants from ^{1}H NMR spectroscopy.[13] Thus, the absolute values for the ^{1}J ($^{1}H-^{15}N$) couplings in arylamines (Aryl-^{15}NH) are expected to be around 90 Hz. Typical values for the endocyclic ^{2}J ($^{1}H-^{15}N$) couplings are between 0 and 20 Hz; and in pyridines it is close to 17 Hz.[13] By comparison, for the ^{3}J ($^{1}H-^{15}N$) couplings the absolute values are typically between 0 and 5 Hz.[14,15] ^{1}H NMR analysis of **10** revealed that each of the NH_2 protons is a doublet with the characteristically large ^{1}J ($^{1}H-^{15}N$) coupling constants of 90 and 95 Hz. However, the other NH signal shows a characteristically smaller ^{3}J ($^{1}H-^{15}N$) coupling constant value of 3.9 Hz. This suggests that the N atom attached to the anomeric center is no longer ^{15}N-labeled. Instead, the ring N(1)-atom is labeled, which is further supported by the characteristic ^{2}J ($^{1}H(2)$-$^{15}N(1)$) coupling constant value of 16 Hz. By comparison, in **8** this H(2) is a singlet (^{4}J ($^{1}H-^{15}N$) ~ 0 Hz).

The position of the labeled ^{15}N atoms in structure of **10** supports the mechanism presented in Scheme 3. This result proves that the isomerization of a kinetic product **9** (or **3c**) into the final product **10** (or **4c**) is possible through the pyrimidine ring opening when the ring is activated enough. This attack by a nucleophilic catalyst onto **3c** is faster than onto **3a** and **3b**, which contain the less electrophilic pyrimidine rings. As a consequence, **3a** and **3b** do not isomerize under these conditions. This result does not preclude the possibility of an alternative mechanism that may be operative when the rearrangement is conducted in the presence of silica gel or acetic acid.

Reactions between Silylated 4-Chloro-6-Aminopyrimidines and Ribofuranoside 1. Another serendipitous discovery, which also involves Dimroth rearrangement, enabled the preparation of the desired **4a** as well as several other analogs. Silylation (BSA/TMSOTf) of 4-chloro-6-aminopyrimidine (**5a**, X= H, Table 2, entry 1) followed by treatment with **1** afforded exocyclic product **4a** (X = H) as a single β-isomer after quenching the mixture with ammonium hydroxide. This ribosylation is much faster than the formation of the endocyclic product **3a** during the reaction with **2a.** Complete conversion is observed after only 1 h at room temperature in the presence of TMSOTf (2 equiv). Upon quenching into an

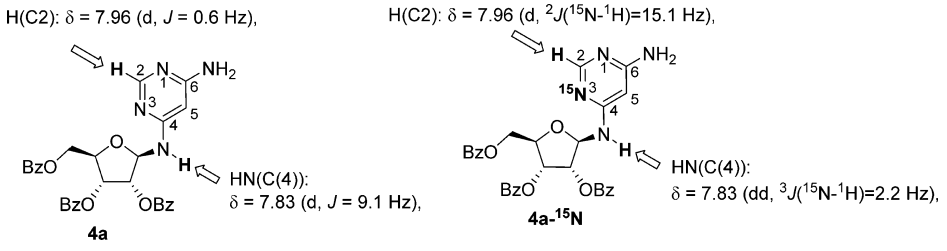

Figure 3. ^{1}H NMR spectra of **4a** and **4a-^{15}N** confirming the position of the ^{15}N atom.

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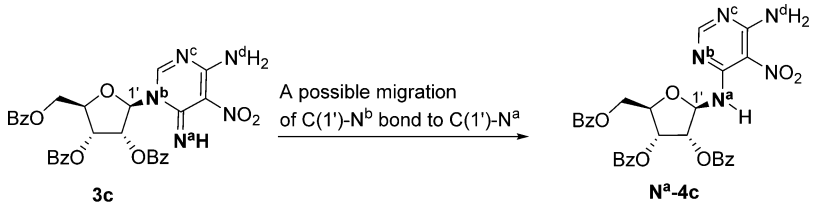
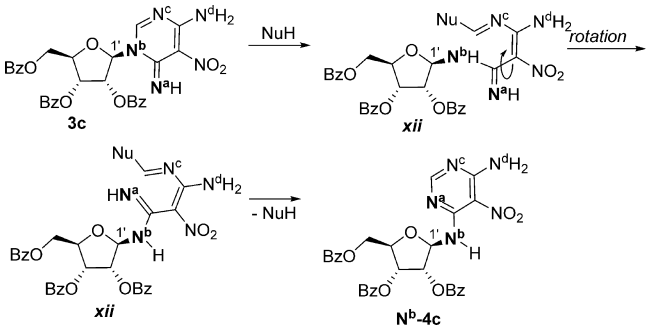
Scheme 2



Scheme 3



ice-cold mixture of acetonitrile/ammonium hydroxide (approximately 10/1) **4a** was isolated in 89% yield.

The structure of **4a** was established via 1D and 2D-NMR spectroscopy. For example, the COSY spectrum (see Supporting Information, pages S32, S33) shows a coupling between the N(H) linking the two rings and H(1′) of the furanose. A similar cross-peak in the COSY spectrum of **3a** is absent (see Supporting Information, page S10). Unlike the HMBC-spectrum of **3a**, there is no coupling in HMBC spectrum of **4a** between H(1′) and any of the aromatic carbon atoms (see Supporting Information, page S34). The N(H) signal is a doublet in DMSO-d_6 and the H(1′) signal is a doublet of doublets in some analogs (a broad signal in others).

The reaction scope is quite broad as illustrated by the examples in Table 2. Substrates bearing electron donating methyl- and methoxy-groups at C(5) of the pyrimidine are viable (**2d**, entry 3 and **2e**, entry 4). As entry 4 in Table 2 demonstrates, HMDS can be used for silylation instead of BSA, and $SnCl_4$ can replace TMSOTf as the Lewis acid.[17,18]

Substrates bearing electron-withdrawing groups at C(5) are less viable. For example, while the 5-chloropyrimidine (entry 2) provided product **4b** in 65% yield, a trace amount of, presumably, product **3b** could be observed by LC-MS analysis of the crude reaction mixture. Reaction of the 5-fluoropyrimidine was even more complex (Table 2, entries 5 and 6). Thus, silylation of pyrimidine **5f** with BSA followed by ribosylation using TMSOTf as the Lewis acid (entry 6) afforded **4f** in 34% yield as a mixture of two epimers at C(1′) together with a 45% of **3f**. Use of the HMDS/$SnCl_4$ combination (Table 2, entry 5) afforded the pure β-isomer of **4f** albeit in only 26% yield. The α-isomer was not observed in this case; **3f** was also formed, but could not be isolated in pure form.

Proposed Mechanism of the Reaction between the Silylated Aminopyrimidines with Ribofuranoside 1. Formation of the different products upon a small change (**3** from **2** vs **4** from **5**) of the nucleophile structures requires an explanation. It may seem that in silylated 6-chloro-4-aminopyrimidines **5** the most nucleophilic nitrogen atom is the exocyclic one, while in the 4,6-diaminopyrimidine **2** the most nucleophilic nitrogen atom is the endocyclic one. However, another explanation is possible, which involves the initial formation of the same product of the endocyclic attack in both cases, followed by isomerization, which is possible only when the chlorinated nucleophile is used.

For example, the reaction can start with the attack of the N(3) atom of the silylated pyrimidine **ix** (Scheme 5), onto the cationic intermediate **viii** from the convex face with formation of intermediate **x**. If the intermediate **x** contains the electron-withdrawing chloro-group at C(4) (Z = Cl), then the pyrimidine ring in **x** may be electronically activated enough to be opened with ammonia during the subsequent reaction quench providing intermediate **xi** by the attack at C(2). Upon Cl-atom displacement in **xi** with ammonia and C=C bond isomerization, intermediate **xii** forms, which cyclizes with extrusion of ammonia and regeneration of the aromatic pyrimidine **4**. This process is very similar to the isomerization from **3c** to **4c**, Scheme 3. In each case the dearomatized intermediate is electronically activated toward attack by ammonia followed by Dimroth rearrangement and aromatization. The difference is in the nature and position of the activating group: C(4)-Cl in **x** vs C(5)-NO_2 in **3c**. Formation of **3f** (Table 2, entries 5 and 6) may be explained by the alternative attack with ammonia at C(4) of the chlorinated intermediate **x** (Z = Cl). Similar to **3a** or **3b** (but unlike **3c**), pyrimidine **3f** cannot be opened with ammonia at the conditions studied because it is not activated enough.

Scheme 4

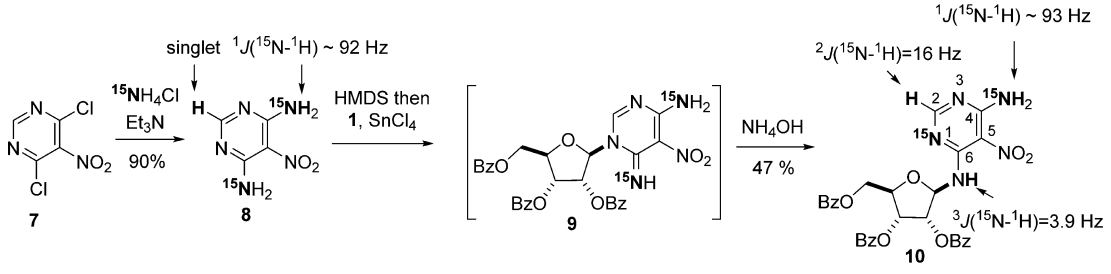


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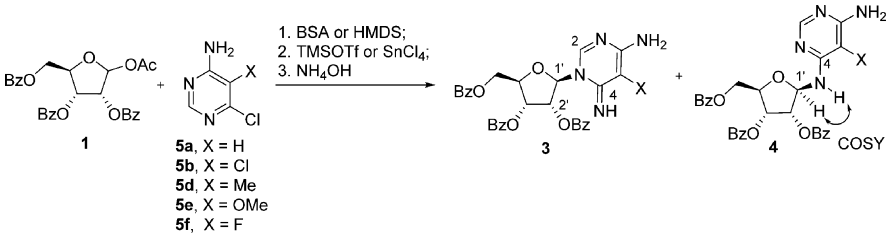
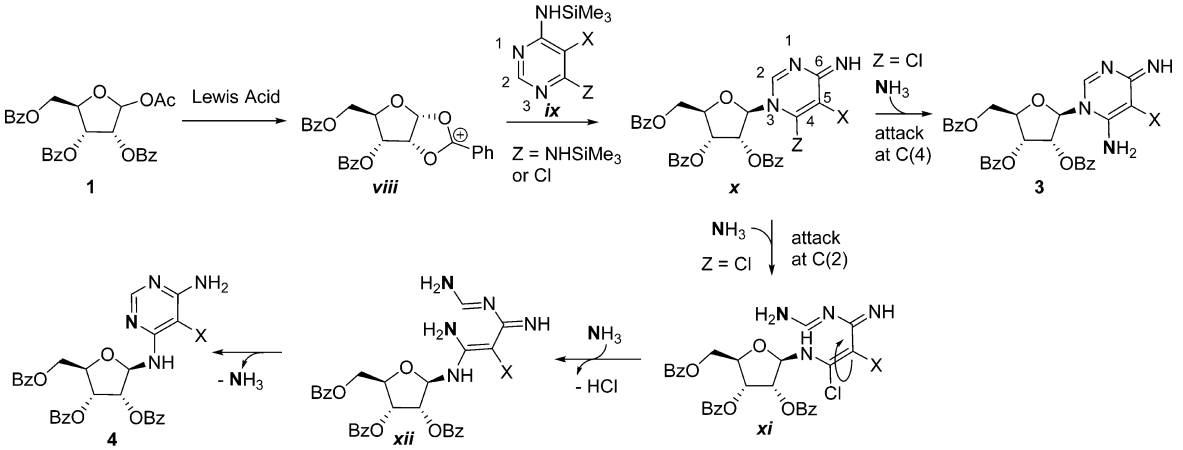
Table 2. Preparation of 4 by Ribosylation of 4-Chloro-6-Aminopyrimidines 5



entry	X	conditions	time, temp	product	yield, %
1	H	BSA/TMSOTf	rt, 1 h	**4a**	89
2	Cl	BSA/TMSOTf	0 °C, 1 h	**4b**[a]	65
3	Me	BSA/TMSOTf	0 °C, 1 h	**4d**	100
4	OMe	HMDS/$SnCl_4$	0 °C, 1h	**4e**	73
5	F	HMDS/$SnCl_4$	0 °C, 2.5 h	**4f**-β[b]	26
6	F	BSA/TMSOTf	rt, 1 h	**4f**-β/ **4f**-α ~ 2.7	34 (+ 45% of **3f**)

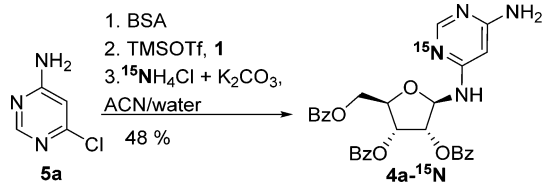
[a]*Endo*-product **3b** was observed by reversed-phase LC-MS[16] but not isolated (~10% relative to the exoproduct, UV-254 nm). *Exo*-product **4b**: M+H^+ = 589, 1.41 min; *endo*-product **3b**: M+H^+ = 589, 1.17 min. [b]*Endo*-product **3f** was observed by LC-MS but not isolated (~30% peak area relative to the exoproduct, UV-254 nm). *Exo*-product **4f**: M+H^+ = 573, 1.34 min; *endo*-product **3f**: M+H^+ = 573, 1.16 min.

Scheme 5



If the mechanism described in Scheme 5 is operative, then the bold nitrogen atom in **4** was not originally there but comes from the ammonia used in the quench. To confirm the proposed mechanism, ^{15}N-labeled ammonia was used for the reaction quench, Scheme 6. As would be expected from the mechanistic proposal, the product **4a**-^{15}**N** (which was isolated in 48% yield) incorporated the label at the endocyclic N(2) position.

Scheme 6



Structure Elucidation of 4a-^{15}N. The position of the ^{15}N-atom in **4a**-^{15}**N** was determined by ^1H NMR analysis (Figure 3). ^{15}N NMR spectra of several pyrimidines **4** in CD_3CN also were recorded. The analysis of the ^{15}N NMR spectra is complicated because we were unable to reference them according to IUPAC recommendations. The ^1H NMR spectrum (DMSO-d_6) of **4a**-^{15}**N** features a doublet for H(C(2)) due to the ^2J ($^1H-^{15}N$) coupling to the neighboring ^{15}N atom. The magnitude of this coupling (15.1 Hz) is within the range for the absolute values typical for the endocyclic ^2J ($^1H-^{15}N$) couplings (between 0 and 20 Hz; and in pyridines it is close to 17 Hz).[13] In contrast, in the ^{14}N-analog **4a** this signal is a barely resolved doublet (J = 0.6 Hz) at 500 MHz. The magnitude (15.1 Hz) of this $^1H-^{15}N$ coupling in **4a**-^{15}**N** is consistent with the structure **4a**-^{15}**N**. This value (15.1 Hz) is matched by the ^{15}N NMR data[19] of **4a**-^{15}**N**, which exhibits a doublet at 223 ppm (J = 15.6 Hz). Additionally, the signal for H−N connected to C(4) is a doublet of doublets (J = 2.2, 9.5 Hz, in the unlabeled analog **4a** this signal is a doublet, J = 9.1 Hz). The magnitude of the smaller coupling constant (2.2 Hz) is consistent with ^3J ($^1H-^{15}N$), further confirming the structure. Unfortunately, the signal for H(C(5)) could not be analyzed due to an overlap; H_2N(C(6)) is a broad singlet.

CONCLUSIONS

A method of preparation of 1-(*N*-ribofuranosyl)-6-imino-1,6-dihydropyrimidin-4-amines **3** and 4-(*N*-ribofuranosyl)-6-aminopyrimidines **4** via glycosylation of 4-aminopyrimidines is presented. Upon Vorbrüggen reaction with a ribofuranosyl donor **1** silylated 4-aminopyrimidines attack by the endocyclic

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nitrogen atom, opposite to the exocyclic amino group. When sufficiently activated and in the presence of ammonium hydroxide, the kinetic product **3** can isomerize to **4**. The activation can be provided by C(4)-Cl or C(5)-NO_2 groups. The formation of **4** from **3** involves ring-opening of the intermediate. Extension to other substrates is possible.[20]

EXPERIMENTAL SECTION

^{1}H and ^{13}C NMR spectra were recorded on a Bruker 500 MHz NMR spectrometer (500 MHz ^{1}H, 126 MHz ^{13}C) in methanol-d_4, acetone-d_6, or DMSO-d_6. Data are reported in the following order: chemical shift in ppm (δ); multiplicities are indicated (br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet)); coupling constants, J, are reported in hertz (Hz); integration is provided and assignment when possible. ^{1}H and ^{13}C NMR assignments are corroborated by 2D experiments (COSY, HMQC, HMBC, and NOESY) when relevant. Spectra are available in the SI. HRMS analysis (ESI positive) was performed on an LTQ Orbitrap Discovery mass spectrometer. UPLC-MS analysis was conducted using a column Acquity UPLC HSS C18 1.8 μM; Solvent A: 0.1% aqueous formic acid, Solvent B: acetonitrile; gradient from A:B = 95:5 to A:B = 5:95 over 2 min or similar. Analytical thin-layer chromatography was performed on silica gel plates with F-254 indicator. Visualization was accomplished by UV light. Column chromatography was performed with silica gel. Anhydrous solvents were purchased from Acros. All commercially available reagents were purchased and used without further purification. All temperatures refer to the external aluminum heat block temperature unless otherwise noted, all reactions were conducted in test tubes equipped with a stir bar, a septum, and a nitrogen inlet through a needle.

(2*R*,3*R*,4*R*,5*R*)-2-((6-Amino-5-nitropyrimidin-4-yl)amino)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl Dibenzoate (4c). To 5-nitro-2,6-diaminopyrimidine (**2c**, 465 mg, 3 mmol) in a test tube cooled in a room temperature water bath was added acetonitrile (6 mL) and triethylamine (0.42 mL, 3 mmol), followed by dropwise addition of TMSOTf (1.08 mL, 6 mmol). The mixture was stirred at room temperature for 1 h, then cooled in ice bath and **1** (1.51 g, 3 mmol) was added in one portion. The reaction mixture was stirred at room temperature for 16 h, then was quenched with an ice-cold mixture of acetonitrile and ammonium hydroxide (10/1, 20 mL), partitioned between water and ethyl acetate, washed with brine, dried with $MgSO_4$, concentrated, and purified by chromatography on silica gel (gradient hexanes/ethyl acetate from 1/0 to 60/40 to provide the title compound **4c** as a white solid material (1.65 g, 92%). The ^{1}H NMR spectrum of this material matched previously reported data.[8c]

(2*R*,3*R*,4*R*,5*R*)-2-(4-Amino-6-iminopyrimidin-1(6H)-yl)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl Dibenzoate (3a). To 4,6-diaminopyrimidine (**2a**, 110 mg, 1 mmol) was added (3R,4R,5R)-2-acetoxy-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl dibenzoate **1** (504 mg, 1 mmol, 1 equiv), the tube was flushed with nitrogen, and acetonitrile (3 mL) was added, followed by bis(trimethylsilyl)acetamide (BSA, 0.5 mL, 2 mmol). The reaction mixture was heated to 90 °C under nitrogen for 10 min, then cooled in an ice bath. TMSOTf (0.36 mL, 2 mmol) was added and the contents were heated to 60 °C for 1 h, when the UPLC/MS analysis showed complete consumption of **1**. The reaction mixture was quenched into an ice-cold mixture of acetonitrile (20 mL) and ammonium hydroxide (2 mL), extracted with toluene, washed with water, brine, dried with $MgSO_4$, concentrated, and purified by chromatography on silica gel (gradient dichloromethane/ethyl acetate from 1/0 to 0/1, then ethyl acetate/trimethylamine (50/1, then 20/1), then ethyl acetate/methanol ~10/1 to provide an oily residue. The material was triturated with ether while cooling in an ice bath, diluted with equal volume of pentane, filtered, and washed with pentane, then dried under vacuum to provide the title compound **3a** as a white solid material (473 mg, 85%): TLC (streaking in EtOAc/MeOH ~ 10/1) Rf ~ 0.44–0.72; ^{1}H NMR (500 MHz, methanol-d_4) δ = 8.58 (s, 1H, H(2)), 8.09 (dd, J = 1.3, 8.2 Hz, 2H), 8.04 (dd, J = 1.1, 8.4 Hz, 2H), 7.97 (dd, J = 1.3, 8.5 Hz, 2H), 7.68–7.60 (m, 3H), 7.52 (t, J = 7.9 Hz, 2H), 7.48–7.40 (m, 4H), 6.36 (d, J = 5.0 Hz, 1H, H(1′)), 6.05 (t, J = 6.0 Hz, 1H, H(2′)), 5.94 (t, J = 5.8 Hz, 1H, H(3′)), 5.78 (s, 1H, H(5)), 4.97 (td, J = 3.6, 5.4 Hz, 1H, H(4′)), 4.86 (t, J = 4.4 Hz, 2H, H(5′)); ^{1}H NMR (500 MHz, DMSO-d_6)[21] δ = 8.72 (s, 1H, H(2)), 8.22–8.04 (m, 6H, includes NH and NH_2), 7.99 (dd, J = 7.6, 15.4 Hz, 4H), 7.79–7.64 (m, 3H), 7.58 (t, J = 7.6 Hz, 2H), 7.52 (q, J = 7.8 Hz, 4H), 6.47 (d, J = 5.0 Hz, 1H, H(1′)), 6.08 (t, J = 5.7 Hz, 1H, H(2′)), 5.98 (t, J = 6.0 Hz, 1H, H(3′)), 5.73 (s, 1H, H(5)), 4.97–4.76 (m, 3H) ^{13}C NMR (126 MHz, DMSO-d_6) δ = 165.5, 164.56, 164.54, 162.2 (C(6)), 154.0 (C(4)), 148.7 (C(2)), 134.1, 134.00, 133.7, 129.49, 129.45, 129.3, 129.1, 128.84, 128.7, 128.5, 128.3, 87.7, 80.9 (C(5)), 80.1 (C(4′)), 73.4 (C(2′)), 69.9 (C(3′)), 63.5 (C(5′)). One signal is missing, probably, due to overlap. UPLC: 1.05 min, 555 $[M+H]^+$, also 445, 341, 201; HRMS (ESI) $[M+H]^+$ calcd for $C_{30}H_{27}N_4O_7^+$ 555.1874, found 555.1857.

(2*R*,3*R*,4*R*,5*R*)-2-(4-(l4-Azanyl)-5-chloro-6-iminopyrimidin-1(6H)-yl)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl Dibenzoate (3b). 5-chloropyrimidine-4,6-diamine **2b** (144 mg, 1.0 mmol) and trimethylamine (0.14 mL, 1.0 mmol) were mixed in dry acetonitrile (2 mL) under N_2. The mixture was cooled to 0 °C in an ice–water bath and trimethylsilyl trifluoromethanesulfonate (0.4 mL, 2.2 mmol) was added slowly. The reaction was warmed up to room temperature and allowed to stir for 1 h at room temperature. It was then cooled to 0 °C in an ice–water bath and **1** (504 mg, 1.0 mmol) was added after which the reaction mixture was stirred at room temperature for 1.5 h until UPLC showed complete consumption of **1**. It was then quenched with 20 mL 10% NH_4OH/acetonitrile solution at 0 °C, concentrated and purified using silica gel column chromatography (eluent 20% ethyl acetate/hexane to 100% ethyl acetate) to yield the title compound **3b** as a white solid material (505 mg, 86%). ^{1}H NMR (500 MHz, methanol-d_4) δ = 8.64 (s, 1H, H(2)), 8.11–8.07 (m, 2H), 8.05–7.98 (m, 4H), 7.68–7.62 (m, 3H), 7.54–7.49 (m, 2H), 7.48–7.42 (m, 4H), 6.46 (d, J = 4.1 Hz, 1H, H(1′)), 6.07 (dd, J = 3.8, 5.7 Hz, 1H, H(2′)), 5.95 (t, J = 6.0 Hz, 1H, H(3′)), 5.01 (td, J = 3.8, 6.0 Hz, 1H, H(4′)), 4.87 (dd, J = 1.7, 3.8 Hz, 2H, H(5′)); ^{1}H NMR (500 MHz, acetone) δ = 8.95 (s, 1H, H(2)), 8.24–8.06 (m, 6H, includes NH_2), 8.06–7.99 (m, 2H), 7.87 (br. s, 1H), 7.72–7.67 (m, 2H), 7.65 (tt, J = 1.3, 7.6 Hz, 1H), 7.60–7.37 (m, 6H), 6.81 (d, J = 3.8 Hz, 1H, H(1′)), 6.27 (dd, J = 3.8, 5.4 Hz, 1H, H(2′)), 6.13 (dd, J = 5.7, 6.6 Hz, 1H, H(3′)), 5.24 (ddd, J = 2.8, 4.0, 6.7 Hz, 1H, H(4′)), 5.03 (dd, J = 4.1, 12.9 Hz, 1H, *H*H(5′)), 4.94 (dd, J = 2.8, 12.9 Hz, 1H, H*H*(5′)); ^{13}C NMR (126 MHz, Acetone) δ = 166.69, 166.65, 165.8, 160.1 C(6), 152.4 C(4), 146.9 (C(2)), 135.2, 134.8, 134.5, 130.9, 130.7, 130.6, 129.8, 129.72, 129.71, 129.6, 123.4, 120.9, 91.29 (C5), 91.25 (C(1′)), 82.5 (C(4′)), 75.5 (C(2′)), 70.6 (C(3′)), 63.9 (C5′)); UPLC: 1.39 min, 589 $[M+H]^+$, also 297, 282; HRMS (ESI) $[M+H]^+$ calcd for $C_{30}H_{26}ClN_4O_7^+$ 589.1485, found 589.1461.

*Alternative Procedure for (2R,3R,4R,5R)-2-(4-(l4-Azanyl)-5-chloro-6-iminopyrimidin-1(6H)-yl)-5-((benzoyloxy)methyl)-tetrahydrofuran-3,4-diyl Dibenzoate (**3b**).* To **1** (504 mg, 1.0 mmol), 5-chloropyrimidine-4,6-diamine **2b** (144 mg, 1.0 mmol, 1 equiv) and N,O-bis(trimethylsilyl)acetamide (0.5 mL, 2.0 mmol, 2 equiv) in dry acetonitrile (3 mL) were heated to 90 °C under N_2 for 10 min after which clear solution was formed. The reaction mixture was then cooled in an ice–water bath and trimethylsilyl trifluoromethanesulfonate (0.36 mL, 2.0 mmol) was added slowly. The reaction mixture was stirred for 1.5 h at room temperature and then heated to 60 °C for 2.5 h until UPLC analysis showed complete consumption of **1**. The reaction was then quenched with 20 mL 10% NH_4OH/acetonitrile solution at 0 °C, concentrated and purified using silica gel column chromatography (eluent 20% ethyl acetate/hexane to 100% ethyl acetate) to yield the title compound **3b** as an off-white solid material (124 mg, 21%). The ^{1}H NMR and ^{13}C NMR spectra of this material match the one obtained via a different procedure (see above).

(2*R*,3*R*,4*R*,5*R*)-2-((6-Aminopyrimidin-4-yl)amino)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl Dibenzoate (4a). To 4-chloro-6-aminopyrimidine (**5a**, 130 mg, 1 mmol) was added **1** (504 mg, 1 mmol, 1 equiv), the tube was flushed with nitrogen, and acetonitrile (3 mL) was added, followed by bis-(trimethylsilyl)acetamide (BSA, 0.25 mL, 1 mmol, 1 equiv). The

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reaction mixture was heated to 90 °C under nitrogen for 10 min, then cooled in an ice bath. TMSOTf (0.36 mL, 2 mmol, 2 equiv) was added and the contents were stirred at room temperature for 1 h, when the UPLC/MS analysis showed almost complete consumption of both starting materials. The reaction mixture was quenched into an ice-cold mixture of acetonitrile (20 mL) and ammonium hydroxide (2 mL), extracted with toluene/ethyl acetate ~1/1, washed with water, brine, dried with $MgSO_4$, concentrated and purified by chromatography on silica gel (gradient dichloromethane/ethyl acetate from 1/0 to 0/1, then ethyl acetate/methanol ~20/1 to 10/1 to provide the title compound **4a** as a white solid material (495 mg, 89%): ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.05−8.02 (m, 2H), 7.96 (d, J = 0.6 Hz, 1H, H(2)), 7.91−7.88 (m, 2H), 7.87−7.84 (m, 2H), 7.83 (d, J = 9.1 Hz, 1H, NH), 7.70−7.60 (m, 3H), 7.54−7.50 (m, 2H), 7.49−7.41 (m, 4H), 6.33 (s, 2H, NH_2), 6.04 (br. s., 1H, H(1′)), 5.80 (dd, J = 4.4, 5.7 Hz, 1H, H(3′)), 5.62−5.56 (m, 2H, H(2′) and H(5)), 4.59−4.49 (m, 3H, H(4′ and 5′)); ^{13}C NMR (126 MHz, DMSO-d_6) δ = 165.5, 164.8, 164.7, 163.9 (C(4) or C(6)), 161.5 (C(4) or C(6)), 157.7 (C(2)), 133.8, 133.7, 133.5, 129.34, 129.28, 129.23, 129.20, 128.78, 128.71, 128.67, 128.65, 128.58, 83.9 (C(5)), 83.4 (C(1′)), 77.3 (C(4′)), 73.7 (C(2′)), 71.1 (C(3′)), 64.3 (C(5′)); UPLC/MS: 1.16 min, 555 [M+H]$^+$; HRMS (ESI) [M+H]$^+$ calcd for $C_{30}H_{27}N_4O_7^+$ 555.1874, found 555.1849.

(2*R*,3*R*,4*R*,5*R*)-2-((6-Amino-5-methylpyrimidin-4-yl)amino)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl Dibenzoate (4d). **4d** was prepared following the same procedure as for **4a**. Yield: 574 mg (100%) of white solid material. TLC R_f ~ 0.39 (EtOAc); ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.11 (s, 1H, H(2)), 8.03−7.99 (m, 2H), 7.93−7.89 (m, 2H), 7.85−7.82 (m, 2H), 7.79 (d, J = 8.8 Hz, 1H, NH), 7.69−7.59 (m, 3H), 7.53−7.45 (m, 4H), 7.44−7.39 (m, 2H), 6.88 (br. s., 2H, NH_2), 6.26 (dd, J = 5.0, 9.1 Hz, 1H, H(1′)), 5.88 (t, J = 5.7 Hz, 1H, H(3′)), 5.84 (dd, J = 4.7, 6.0 Hz, 1H, H(2′)), 4.63−4.55 (m, 2H, H(4′+5a′)), 4.51 (dd, J = 4.3, 11.2 Hz, 1H, H(5b′)), 1.89 (s, 3H, Me); ^{13}C NMR (126 MHz, DMSO-d_6) δ = 165.5, 164.8, 164.7, 158.7, 157.7, 150.9 (C(2)), 133.9, 133.8, 133.5, 129.32, 129.30, 129.28, 129.22, 128.8, 128.71, 128.68, 128.63, 128.59, 91.3 (C(5)), 84.0 (C(1′)), 77.3 (C(4′)), 73.8 (C(2′)), 70.8 (C(3′)), 63.9 (C(5′)), 8.9 (Me). UPLC: 1.18 min, 569 [M+H]$^+$; HRMS (ESI) [M+H]$^+$ calcd for $C_{31}H_{29}N_4O_7^+$ 569.2031, found 569.2005.

(2*R*,3*R*,4*R*,5*R*)-2-((6-Amino-5-methoxypyrimidin-4-yl)-amino)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl Dibenzoate (4e). Mixture of 6-chloro-5-methoxypyrimidin-4-amine **5e** (159 mg, 1.0 mmol), $(NH_4)_2SO_4$ (159 mg, 1.2 mmol) and pyridine (0.6 mL) in hexamethyldisilazane (5 mL) under inert atmosphere (N_2) was heated to 140 °C in a sealed tube over a period of 1 h. The reaction mixture was cooled and concentrated under reduced pressure. To the crude silylated pyrimidine was added **1** (504 mg, 1.0 mmol) and dry acetonitrile (3 mL). The resulting mixture was cooled in an ice−water bath and $SnCl_4$ (1.0 M solution in CH_2Cl_2, 2 mL, 2 mmol) was added dropwise. After 1 h at 0 °C the UPLC analysis showed complete consumption of **1**. The mixture was then quenched with 20 mL of 10% NH_4OH/acetonitrile solution at 0 °C. The white precipitate was removed by filtration, the filtrate was concentrated and purified using silica-gel column chromatography (eluent 10% ethyl acetate/hexane to 100% ethyl acetate) to yield the title compound **4e** as a white solid material (428 mg, 73%). ^{1}H NMR (500 MHz, acetone-d_6) δ = 8.11 (d, J = 7.88 Hz, 2 H), 7.99 (dd, J = 14.03, 8.04 Hz, 4 H), 7.86 (s, 1 H, H(2)), 7.57−7.68 (m, 3 H), 7.50 (t, J = 7.57 Hz, 2 H), 7.45 (q, J = 7.88 Hz, 4 H), 6.99 (d, J = 9.60 Hz, 1 H, NH), 6.43 (dd, J = 9.60, 6.00 Hz, 1 H, H(1′)), 5.96 (dd, J = 5.99, 4.41 Hz, 1 H, H(3′)) 5.92 (dd, J = 6.30, 6.00 Hz, 1 H, H(2′)), 5.75 (br. s., 2 H, NH_2), 4.71 (dd, J = 11.03, 3.47 Hz, 1 H, H(5′a)), 4.65 (q, J = 4.40 Hz, 1 H, H(4′)), 4.62 (dd, J = 11.00, 4.40 Hz, 1 H, H(5′b)), 3.59 (s, 3 H, OMe) . ^{13}C NMR (126 MHz, Acetone) δ = 166.6, 166.0, 165.9, 157.5 (C(4) or C(6)), 154.9 (C(4) or C(6)), 153.7 (C2)), 134.40, 134.35, 134.1, 130.9, 130.5, 130.42, 130.41, 130.35, 130.29, 129.5, 129.4 (may be two overlapping signals), 123.6 (C(5)), 84.9 (C(1′)), 79.1 (C(4′)), 75.2 (C(2′)), 72.5 (C(3′)), 65.2 (C(5′)), 58.9 (OMe); UPLC/MS: 1.25 min, 585 [M+H]$^+$; HRMS (ESI) [M+H]$^+$ calcd for $C_{31}H_{29}N_4O_8^+$ 585.1980, found 585.1984.

(2*R*,3*R*,4*R*,5*R*)-2-((6-Amino-5-chloropyrimidin-4-yl)amino)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl Dibenzoate (4b). **4b** was prepared following the same procedure as for **4a**. Yield: 378 mg (65%), a white solid material. TLC Rf ~ 0.38 (EtOAc/hexanes ~1/1); ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.03 (dd, J = 1.3, 8.2 Hz, 2H, H(2)), 7.94 (s, 1H), 7.90 (dd, J = 1.3, 8.2 Hz, 2H), 7.85−7.81 (m, 3H, contains NH), 7.69−7.59 (m, 3H), 7.51 (t, J = 7.7 Hz, 2H), 7.47 (t, J = 7.9 Hz, 2H), 7.41 (t, J = 7.9 Hz, 2H), 6.82 (br. s., 2H, NH_2), 6.25 (dd, J = 4.7, 9.1 Hz, 1H, H(1′)), 5.91−5.86 (m, 2H, H(2′+3′)), 4.62−4.48 (m, 3H, H(4′+5′)); ^{13}C NMR (126 MHz, DMSO-d_6) δ = 165.5, 164.8, 164.7, 159.4 (C(6) or C(4)), 156.4 (C(6) or C(4)), 154.8 (C(2)), 133.8, 133.7, 133.5, 129.4, 129.30, 129.27, 129.22, 128.8, 128.71, 128.68, 128.66, 128.62, 91.8 (C(5)), 83.7 (C(1′)), 77.3 (C(4′)), 73.7 (C(2′) or C(3′)), 70.8 (C(2′) or C(3′)), 64.0 (C(5′)); UPLC/MS: 1.39 min, 589 [M+H]$^+$; HRMS (ESI) [M+H]$^+$ calcd for $C_{30}H_{26}ClN_4O_7^+$ 589.1485, found 589.1457.

(3*R*,4*R*,5*R*)-2-((6-Amino-5-fluoropyrimidin-4-yl)amino)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl Dibenzoate (4f, mixture α/β) and (2*R*,3*R*,4*R*,5*R*)-2-(4-Amino-5-fluoro-6-imino-pyrimidin-1(6H)-yl)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl Dibenzoate (3f). **4f** and **3f** were prepared following the same procedure as for **4a**. Purification by chromatography (gradient ethyl acetate/hexanes ~1/4 to 1/0, then to ethyl acetate/methanol 5/1) provided the less polar material, which was dissolved in ether and precipitated by adding slowly the same volume of hexanes, then filtered, and washed with hexanes to provide a white solid material containing **4f** (mixture α/β ~ 1/2.7). Yield: 194 mg (34%). ^{1}H NMR (500 MHz, Acetone-d_6, integration of the mixture is normalized, signals of the minor components are set as 1H, see the Supporting Information for the spectrum) δ = 8.13−8.06 (m, 7H), 8.04−7.92 (m, 15H), 7.85 (d, J = 1.3 Hz, 2.7H), 7.81 (d, J = 1.6 Hz, 1H), 7.68−7.56 (m, 11H), 7.54−7.39 (m, 23H), 7.24 (d, J = 9.1 Hz, 2.7H), 6.74 (dd, J = 5.0, 10.1 Hz, 1H), 6.41 (dd, J = 6.0, 9.5 Hz, 2.7H), 6.28 (d, J = 8.8 Hz, 1H), 6.01 (t, J = 5.0 Hz, 1H),5.99−5.92 (m, 10H), 5.88 (t, J = 5.7 Hz, 2.7H). The more polar material (**3f**, 370 mg, 64%) was dissolved in 2 mL of ethyl acetate, diluted with 20 mL of ether, cooled in an ice bath, diluted with 20 mL of hexanes, stirred, then filtered and dried under vacuum to provide **3f** as a white solid material. Yield 255 mg (45%). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.61 (s, 1H), 8.48−8.15 (m, 4H, NH_2+NH+extra H), 8.02 (dd, J = 1.3, 8.5 Hz, 2H), 7.95 (dt, J = 1.1, 8.1 Hz, 4H), 7.72−7.64 (m, 3H), 7.54 (t, J = 7.7 Hz, 2H), 7.48 (q, J = 7.6 Hz, 4H), 6.49 (d, J = 4.7 Hz, 1H, H(1′)), 6.05 (dd, J = 5.0, 6.0 Hz, 1H, H(2′)), 5.95 (t, J = 5.8 Hz, 1H, H(3′)), 4.92−4.87 (m, 1H, H(4′)), 4.85−4.75 (m, 2H, CH_2(5′)); ^{13}C NMR (126 MHz, DMSO-d_6) δ = 165.5, 164.51, 164.49, 150.7 (d, J = 9.1 Hz), 144.9 (d, J = 20.0 Hz), 144.2 (d, J = 6.4 Hz, C(2)), 134.1, 134.0, 133.7, 129.5, 129.42, 129.32, 129.0, 128.8, 128.7 (two overlapping signals), 128.42, 128.37, 126.4 (d, J = 237.1 Hz, C(5)), 88.1 (C1′)), 80.4 (C(4′)), 73.9 (C(2′)), 69.8 (C(3′)), 63.4 (C(5′)), (four extra signals observed between 116 and 125 ppm. Cannot explain); UPLC/MS: 1.14 min, 573 [M+H]$^+$ also 445, 201; HRMS (ESI) [M+H]$^+$ calcd for $C_{30}H_{26}FN_4O_7^+$ 573.1780, found 573.1750.

(2*R*,3*R*,4*R*,5*R*)-2-((6-Amino-5-fluoropyrimidin-4-yl)amino)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl Dibenzoate (4f, β−isomer). To 2-chloro-3-fluoro-4-amino-pyrimidine (**2f**, 148 mg, 1 mmol) was added ammonium sulfate (7 mg, 5 mol%), HMDS (3 mL), and the mixture was heated under nitrogen at 135 °C (external temperature) overnight (15 h). A clear solution was not formed. Pyridine (1 mL) was added and a clear solution was formed immediately. After 30 min the mixture was concentrated under vacuum. To the crude silylated pyrimidine was added **1** (504 mg, 1 mmol), acetonitrile (3 mL), the reaction mixture was cooled in an ice bath and treated with $SnCl_4$ (1 M solution in DCM, 2 mL), which led to instantaneous dissolution. After 3 h at this temperature, the reaction mixture was quenched into ice-cold mixture of acetonitrile (20 mL) and ammonium hydroxide (2 mL). The reaction mixture was diluted with toluene and filtered through Celite, concentrated and purified by chromatography twice. First purification used a gradient ethyl acetate/hexanes ~1/4 to 1/0, then to ethyl acetate/methanol ~5/1. Second purification used a gradient ethyl acetate/dichloromethane ~1/4 to 1/0, then to ethyl acetate/methanol ~5/1. The product (352 mg, 0.61

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mmol) was dissolved in ether and precipitated with pentane, then washed with pentane to provide the title compound as a white solid material (147 mg, 26%, single beta-isomer). The endoisomer **3f** was also present in the more polar fractions; however, it could not be isolated clean. Data for **4f**: ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.07 (d, J = 9.1 Hz, 1H, NH), 8.05−8.01 (m, 2H), 7.93−7.88 (m, 2H), 7.87−7.82 (m, 2H), 7.81 (d, J = 1.6 Hz, 1H), 7.69−7.58 (m, 3H), 7.55−7.49 (m, 2H), 7.49−7.44 (m, 2H), 7.44−7.39 (m, 2H), 6.67 (br.s, 2H, NH_2), 6.19 (dd, J = 5.4, 9.5 Hz, 1H, H(1′)), 5.89−5.84 (m, 1H, H(3′)), 5.78 (t, J = 5.7 Hz, 1H, H(2′)), 4.62−4.48 (m, 3H, H(4′ and 5′)); ^{13}C NMR (126 MHz, DMSO-d_6) δ = 165.5, 164.8, 164.7, 152.2 (d, J = 10.0 Hz, C(2)), 151.7 (d, J = 8.2 Hz, C(4) or C(6)), 148.3 (d, J = 7.3 Hz, C(4) or C(6)), 133.8, 133.7, 133.5, 129.4, 129.3, 129.24, 129.20, 130.1 (d, J = 246.1 Hz, C(5)), 128.8, 128.7 (broad, three signals may overlap here), 128.6, 83.1 (C(1′)), 77.3 (C(4′)), 73.7 (C(2′)), 70.9 (C(3′)), 64.1 (C(5′)); UPLC/MS: 1.36 min, 573 $[M+H]^+$; HRMS (ESI) $[M+H]^+$ calcd for $C_{30}H_{26}FN_4O_7^+$ 573.1780, found 573.1750.

(2*R*,3*R*,4*R*,5*R*)-2-((6-Aminopyrimidin-4-yl-3−15N)amino)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl Dibenzoate (4a-^{15}N). To **2a** (130 mg, 1 mmol) was added **1** (504 mg, 1 mmol), acetonitrile (3 mL), BSA (0.25 mL), and the mixture was heated to 90 °C for 10 min, then cooled in an ice bath. TMSOTf was added (0.36 mL, 2 mmol) and the reaction was continued at room temperature for 25 min, then quenched into stirred ice-cold mixture of acetonitrile (20 mL), water (5 mL), $^{15}NH_4Cl$ (98% ^{15}N, 550 mg, 10 mmol), and K_2CO_3 (1,380 mg, 10 mmol). The mixture was diluted with toluene, washed with water, brine, dried ($MgSO_4$), concentrated, and purified by chromatography (gradient ethyl acetate/dichloromethane ~0/1 to 1/0). The isolated material was dissolved in ether, cooled in an ice bath, and precipitated by addition of hexanes. The solid material was washed with pentane and dried under vacuum to provide the title compound as a white solid material (159 mg, 0.29 mmol, 29%). The mother liquor was partially concentrated in a flow of nitrogen at rt to provide additional amount of the title compound (105 mg, 0.19 mmol). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.05−8.00 (m, 2H), 7.96 (d, J = 15.1 Hz, 1H, H(2)), 7.92−7.88 (m, 2H), 7.87−7.84 (m, 2H), 7.83 (dd, J = 2.2, 9.5 Hz, 1H, NH), 7.70−7.60 (m, 3H), 7.55−7.50 (m, 2H), 7.49−7.41 (m, 4H), 6.34 (s, 2H, NH_2), 6.04 (br. s., 1H, H(1′)), 5.80 (dd, J = 4.4, 5.7 Hz, 1H, H(3′)), 5.62−5.55 (m, 2H, H(2′) and H(5)), 4.59−4.47 (m, 3H, H(4′) and H(5′)); ^{13}C NMR (126 MHz, DMSO-d_6) δ = 165.5, 164.8, 164.7, 163.9 (d, J = 1.8 Hz, C(4 or 6)), 161.5 (d, J = 4.5 Hz, C(4 or 6)), 157.8, 133.83, 133.76, 133.5, 129.4, 129.28, 129.25, 129.23, 128.8, 128.73, 128.71, 128.66, 128.6, 83.9 (C(5)), 83.4 (C(1′)), 77.3 (C(4′)), 73.7 (C(2′)), 71.1 (C(3′)), 64.3 (C(5′)); UPLC/MS: 1.15 min, 556 $[M+H]^+$; HRMS (ESI) $[M+H]^+$ calcd for $C_{30}H_{27}N_3{}^{15}NO_7^+$ 556.1845, found 556.1820.

5-Nitropyrimidine-4,6-diamine-$^{15}N_2$ (8). To $^{15}NH_4Cl$ (460 mg, 8.4 mmol) was added acetonitrile and the mixture was cooled in an ice bath. Triethylamine (2.1 mL, 15 mmol) was added, followed by 4,6-dichloro-5-nitropyrimidine (**7**, 386 mg, 2.0 mmol) and the mixture was stirred in a sealed tube at room temperature for 6 h, then diluted with water and filtered, washed the solid material with water and acetonitrile, then dried to provide **8** as a light brown colored material (281 mg, 1.8 mmol, 90%). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.49 (d, J = 92.4 Hz, 2H, 2 × NH^aH^b), 8.42 (d, J = 91.4 Hz, 2H, 2 × NH^aH^b), 7.87 (s, 1H, H(2)). Satisfactory ^{13}C NMR spectrum could not be obtained due to poor solubility of the material. See HSQC spectrum in the Supporting Information, page S102) for the approximate location of the C(2) signal (158.5 ppm); HRMS (ESI) [M+H]+ calcd for $C_4H_5N_3{}^{15}N_2O_2^+$ 158.0457, found 158.0448.

(2*R*,3*R*,4*R*,5*R*)-2-((4-(Amino-^{15}N)-5-nitropyrimidin-6-yl-1-^{15}N)amino)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl Dibenzoate (10). To **8** (159 mg, 1 mmol) was added ammonium sulfate (7 mg, 0.05 equiv), HMDS (3 mL), pyridine (1 mL), and the mixture was heated under nitrogen in a 130 °C heat block. After 5 h a clear solution was formed and the reaction mixture was concentrated under vacuum using rotovap, then under high vacuum. To this crude silylated aminopyrimidine was added **1** (454 mg, 0.9 mmol), acetonitrile (3 mL), TMSOTf (0.36 mL, 2 mmol) and the mixture was left at room temperature overnight (18 h) and then quenched with ammonium hydroxide (0.5 mL), partitioned between EtOAc and brine, the organic layer was dried with $MgSO_4$, concentrated, and purified by chromatography (gradient ethyl acetate/dichloromethane ~0/1 to 1/3). The isolated material was recrystallized from isopropanol to provide the title product **10** (281 mg, 47%). Additional recrystallization from methanol provided analytically pure **10** (147 mg) as a lightly colored yellow solid material. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.63 (dd, J = 3.9, 8.4 Hz, 1H, HN), 8.63 (d, J = 91.1 Hz, 1H, NH_aH_b), 8.56 (d, J = 93.6 Hz, 1H, NH_aH_b), 8.04 (d, J = 15.8 Hz, 1H, H(2)), 8.02−8.00 (m, 2H), 7.92−7.88 (m, 2H), 7.87−7.83 (m, 2H), 7.69−7.60 (m, 3H), 7.53−7.40 (m, 7H), 6.37 (dd, J = 4.9, 8.4 Hz, 1H, H(1′)), 6.00 (dd, J = 5.0, 6.0 Hz, 1H, H(2′)), 5.91 (t, J = 5.4 Hz, 1H, H(3′)), 4.67−4.61 (m, 2H, H(4′+5′a)), 4.60−4.54 (m, 1H, H(5′b)). ^{13}C NMR (126 MHz, DMSO-d_6) δ = 165.5, 164.7, 164.6, 159.1 (d, J = 2.7 Hz, (C(2))), 158.5 (dd, J = 1.8, 21.8 Hz, (C(6))), 156.5 (d, J = 3.6 Hz, (C(4))), 133.8, 133.7, 133.5, 129.29, 129.27, 129.22, 128.8, 128.68, 128.67, 128.63, 128.5, 112.5 (C(5)), 83.9 (C(1′)), 78.0, 74.0 (C(2′)), 71.0 (C(3′)), 63.8 (C(5′)). One Bz signal is missing, probably due to overlap. UPLC: 1.43 min, 602 $[M+H]^+$, also 445, 201; HRMS (ESI) $[M+H]^+$ calcd for $C_{30}H_{25}N_3{}^{15}N_2O_9^+$ 602.1666, found 602.1637.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b00780.

Spectral data for all new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*rbaiazitov@ptcbio.com

ORCID

Ramil Y. Baiazitov: 0000-0001-9559-7337

Notes

The authors declare the following competing financial interest(s): All authors are employees of PTC Therapeutics.

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(11) BSA/TMSOTf combination also worked; however, the yield was lower (21%).
(12) The product could not be isolated sufficiently pure.
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(14) Although there may be a trend that the absolute value of ^{3}J (^{1}H-^{15}N) is lower in heterocycles than that of ^{2}J (^{1}H-^{15}N), this is not always true and cautious analysis is required.
(15) Unfortunately, there is no clear trend for the ^{13}C-^{15}N couplings. For example, the magnitude of ^{3}J (^{13}C-^{15}N) in pyridine is larger (−3.85 Hz) than ^{2}J (^{13}C-^{15}N) coupling (+2.53 Hz) or ^{1}J (^{13}C-^{15}N) coupling constant (+0.62 Hz). As a consequence, the ^{13}C NMR coupling constants could not be used for the structure confirmation.
(16) See the Experimental Section for details.
(17) Various combinations of silylating agents (HMDS, BSA, BSTFA, TMSOTf/triethylamine) and Lewis acids (TMSOTf or $SnCl_4$) in dichloromethane, acetonitrile or 1,2-dichloroethane can often be used. Moreover, 2,3,5-triacetate analog of **1** can be used (86% yield, not shown).
(18) Attempted debenzoylation of several analogs **4** with ammonia or NaOMe in methanol led to formation of a material that was a complex mixture of products according to NMR analysis. The mass spectrometry analysis suggested that a compound with the expected mass is present. TLC analysis shows a single spot with the retention different from either animopyrimidine or ribose. We believe that upon deprotection the product undergoes isomerization via mutarotation and, like many other reducing furanosides, may exist as a mixture of several interconverting forms: α/β-furanose, α/β-pyranose, as well as in the open form (imine).
(19) The sample was referenced to the ^{15}N signal at natural abundance of CD_3CN, which was set as 244 ppm. See Supporting Information, page S113
(20) Preliminary data (not shown) suggest that 2-chloro-3-nitro-4-aminopyridine reacts similarly to provide the deazo-analog of **3**. Use of 6-chloro-N-methylpyrimidin-4-amine and ammonium hydroxide quench may provide the N(6)-Me analog of **4a**. When 5-H was used for the reaction and NH2Me used for the quench, the Me could be introduced to the N(4) of **3a**.
(21) ^{1}H NMR spectra of this compound in DMSO or acetone show an extra proton between 7.9 and 8.2 ppm. This proton is absent in the spectrum taken in methanol.

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