Synthesis of (±)-Emtricitabine and (±)-Lamivudine by Chlorotrimethylsilane-Sodium Iodide Promoted Vorbrüggen Glycosylation

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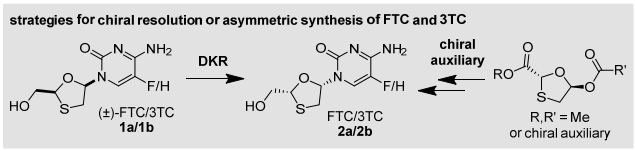
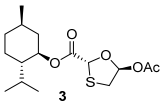
ABSTRACT: By simply adding water and sodium iodide (NaI) to chlorotrimethylsilane (TMSCl), promotion of a Vorbrüggen glycosylation en route to essential HIV drugs emtricitabine (FTC) and lamivudine (3TC) is achieved. TMSCl-NaI in wet solvent (0.1 M water) activates a 1,3-oxathiolanyl acetate donor for *N*-glycosylation of silylated cytosine derivatives, leading to *cis* oxathiolane products with up to 95% yield and >20:1 dr. This telescoped sequence is followed by recrystallization and borohydride reduction, resulting in rapid synthesis of (±)-FTC/3TC from an achiral tartrate ester.

Introduction

Emtricitabine (FTC) and lamivudine (3TC) comprise key components of most combination therapies used for treatment of HIV infection.[1] These active ingredients contain subtle structural complexities, most notably the *cis* configuration about the 2′,3′-dideoxy framework and the epimerizable thioacetal of the oxathiolane. Both FTC and 3TC display opposite geometry relative to naturally occurring nucleosides and are dosed in enantiopure form due to the higher toxicity of their enantiomers.[1] Merely two approaches prepare these targets from chiral building blocks while the majority of reported synthetic strategies employ either chemical or enzymatic kinetic resolution of (±)-FTC and (±)-3TC for isolation of the desired enantiomer (Figure 1).[2] Resolution strategies which are enacted prior to *N*-glycosylation include a longstanding route invented by GlaxoSmithKline (GSK) and a recent report by Medicines for All (M4All).[3,4] Successful strategies for enzymatic resolution (DKR) of early intermediates include ester cleavage or acetylation of intermediates, yet these approaches achieve poor diastereoselectivity in subsequent *N*-glycosylation.[5,6] Resolution after *N*-glycosylation constitutes another viable strategy.[7–15] Herein we report a method for chlorotrimethylsilane-sodium iodide promoted Vorbrüggen glycosylation en route to FTC and 3TC, and apply the optimized method to a synthesis of diastereomerically pure (±)-FTC and (±)-3TC suitable for chiral resolution.

Formation of an anomeric iodide to react with a silylated pyrimidine by a Vorbrüggen glycosylation has emerged as an effective strategy for achieving selective formation of desired *cis* oxathiolane with chiral glycosyl donor **3** (Figure 1).[3,10,16] Precedented methods include the use of iodotrimethylsilane (TMSI) or I_2-triethylsilane (*in situ* generation of HI) to access the iodide. These reagents are effective yet exhibit disadvantages including cost and instability. The more inexpensive polymethylhydrosiloxane (PMHS) replaces triethylsilane for a cost-effective option, although separation of the product from polymeric siloxanes remains problematic. We sought to develop a promoter system for this desirable Vorbrüggen glycosylation which would provide improved ease of handling and use economical reagents.



Starting Material	Author	Iodide Source	Nuc	Yield *cis*	dr
	**This work**	**TMSCl-NaI-H_2O**	**5a, 5c**	**81-90%**	**>20:1**
	Whitehead, 2005	TMSI	**5b**	70%	10:1
	Tse, 1995	TMSI	**5d**	72%	>20:1
	D'Alonzo, 2015	I_2-Silane	**5a-c**	80-98%	>20:1

3

Figure 1. Strategies for synthesis of FTC/3TC and contextualization of TMSCl-NaI method with precedent. PG = protecting group.[3,4,10,16]

Results and Discussion

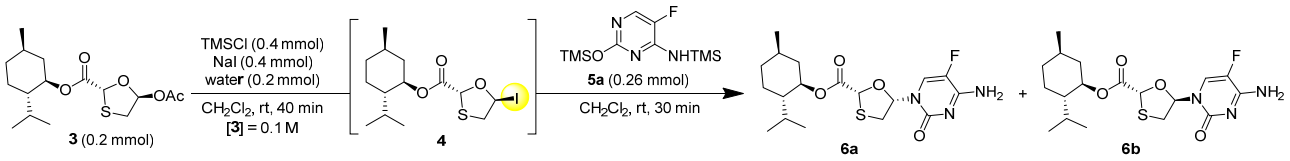
Considering the precedented use of chlorotrimethylsilane (TMSCl) in combination with sodium iodide in acetonitrile (MeCN) for the dealkylation of ethers and esters, we hypothesized that treatment of **3** with TMSCl and NaI would lead directly to iodide **4**.[17] We assayed the formation of **4** by ^{1}H NMR after treatment of **3** with TMSCl-NaI, monitoring the anomeric proton of **4** which possesses a diagnostic chemical shift of 7.2 ppm and coupling constant of 4.2 Hz (see Supporting Information). Preliminary experiments encouragingly showed formation of **4** using TMSCl-NaI both in MeCN and CH_2Cl_2. In a

control experiment no conversion was observed in absence of NaI, but formation of **4** (87% conversion) was obtained upon late addition of the salt to the mixture (Supporting Information, Figure S1).

The formation of **4** via activation of **3** with TMSCl-NaI was telescoped into a two-step sequence to assay the glycosylation of silylated 5-fluorocytosine (**5a**). It was found that water content and solvent have remarkable effects on yield and diastereoselectivity, respectively (Table 1). An initial test of TMSCl-NaI in MeCN showed conversion of intermediate **4** to desired product **6a**, with moderate diastereoselectivity (Table 1, entry 1). Suspecting that adventitious water was introduced into the reaction by NaI, extra care was taken to exclude water in a second trial. Surprisingly, lower yield and lower conversion were observed, indicating that the desired transformation is promoted by water (Table 1, entry 2). By doping explicit quantities of water into the reaction, we observed complete conversion to product with similar dr (Table 1, entry 3). Improvement of diastereoselectivity was achieved by switching from MeCN to CH_2Cl_2 as solvent, albeit with drastic loss in conversion (Table 1, entry 5). Other solvents were evaluated and deemed ineffective due to either poor solubility of the nucleobase or incompatibility with the reaction conditions (Table 1, entry 4). Introduction of water by pre-saturation of CH_2Cl_2 with water (wet CH_2Cl_2) gave nearly quantitative yield with dramatic improvement in dr (Table 1, entry 6). Wet CH_2Cl_2 was prepared by shaking CH_2Cl_2 and water in a separatory funnel, and Karl-Fischer titration indicated 0.10-0.12 M water. Altering the stoichiometric ratio of TMSCl and NaI led to no observable change, and lower yield was observed with decreased stoichiometry of TMSCl and NaI relative to **3** (Table 1, entries 7-10). In a control experiment where NaI was excluded no product was observed, with high recovery of starting material (Table 1, entry 11).

Upon closer investigation of the effect of water stoichiometry on yield of the glycosylation reaction we observed that yield was proportional to the stoichiometry of water added (Figure 2), and at least one equivalent of water relative to **3** was required to achieve high conversion. We reason that water acts as a proton donor for the *in situ* generation of HI. Therefore, maximum conversion of **3** is achieved by maintaining a reaction concentration below the maximum solubility of water in CH_2Cl_2, or approximately 0.1 M.

Table 1. Reaction optimization for TMSCl-NaI promoted Vorbrüggen glycosylation.



Entry	TMSCl/NaI (mmol)	Solvent	Water (mmol)	3 (%)[a]	6a + 6b (%)[a]	dr (6a:6b)
1	0.4/0.4	MeCN	-	51	44	8.4:1
2[b]	0.4/0.5	MeCN	<5 ppm	50	14	8.6:1
3[c]	0.4/0.4	MeCN	0.1	5	92	8.2:1
4[d]	0.4/0.4	2-MeTHF	0.2	3	75	>20:1
5	0.4/0.4	CH_2Cl_2	<5 ppm	93	5	>20:1
6	0.4/0.4	wet CH_2Cl_2	0.2	<1	>95(81)	>20:1
7	0.4/0.8	wet CH_2Cl_2	0.2	<1	>95	>20:1
8	0.8/0.4	wet CH_2Cl_2	0.2	0	>95	>20:1
9	0.26/0.26	wet CH_2Cl_2	0.2	2	92	>20:1
10	0.24/0.24	wet CH_2Cl_2	0.2	3	84(71)	>20:1
11	0.4/0	wet CH_2Cl_2	0.2	87	0	-

[a]Yield determined by ^{1}H NMR analysis using 1,3,5-trimethoxybenzene as internal standard, isolated yields in parentheses. [b][**3**] = 0.04 M. [c]Water (1.8 µL, 0.1 mmol) added by microliter syringe. [d]α,α,α-trifluorotoluene was also evaluated but deemed ineffective due to insolubility of **5a**.

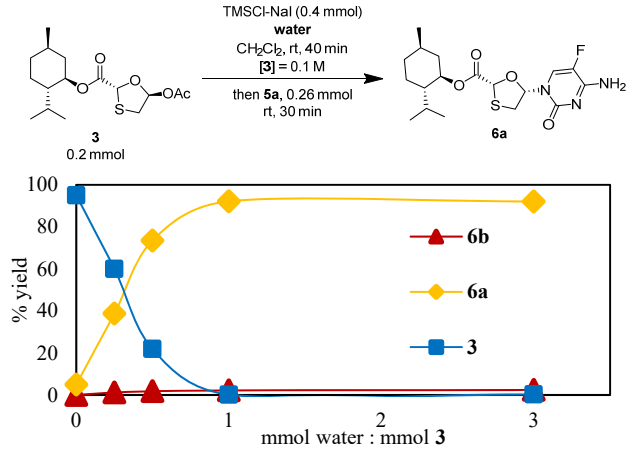
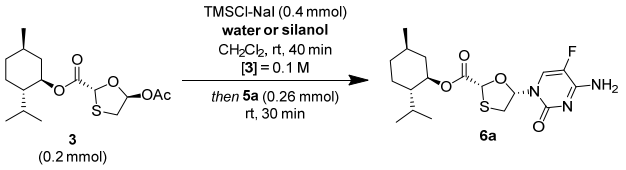


Figure 2. Effect of water stoichiometry in the activation of **3** with TMSCl-NaI-H_2O for *N*-glycosylation. Yield determined by ^{1}H NMR.

We envisioned that a more nonpolar proton donor such as an alcohol or silanol could replace water in the TMSCl-NaI-H_2O reagent system to allow for higher reaction concentrations. Isopropanol afforded slow conversion of **3** and poor mass balance, which was rationalized with the undesired reaction of iodide **4** with isopropanol (Table 2, entry 2). Methanol gave rapid and higher conversion, but the mass balance remained poor (Table 2, entry 3). Trimethysilanol (TMSOH, entry 4) showed rapid conversion of **3** and improved mass balance, while triisopropylsilanol (iPr$_3$SiOH, entry 5) showing the highest selectivity for the desired *N*-glycosylated product.

Table 2. Assay for TMSCl-NaI-ROH promoted Vorbrüggen glycosylation.



Entry	ROH, mmol	t_{rxn} (min)	3 (%)[a]	6a + 6b (dr) (%)[a]
1	H_2O, 0.2	40	1	>99 (44:1)
2	iPrOH, 0.4	165	46	22 (21:1)
3	MeOH, 0.4	90	1	35 (34:1)
4	TMSOH, 0.4	40	2	90 (44:1)
5	iPr$_3$SiOH, 0.4	90	2	96 (31:1)

[a]Determined by ^{1}H NMR analysis using 1,3,5-trimethoxybenzene as internal standard, dr in parentheses as a ratio of **6a**:**6b**.

Despite the suitability of TMSOH or iPr$_3$SiOH as proton donors in this TMSCl-NaI-ROH reagent system, we proceeded to scale-up and isolation with the TMSCl-NaI-H_2O combination which afforded the highest conversion and yield. Accordingly, we found that *N*-glycosylation of pyrimidines 5-fluorocytosine and *N*-acyl cytosine with **3** is achievable on 1 mmol scale, yielding FTC and 3TC precursors **6** and **7** with high yield and diastereoselectivity (Figure 3). The use of unprotected cytosine in place of *N*-acyl cytosine gave low yields due to the insolubility of **5b**. These findings are directly applicable to the prominent chiral auxiliary-based manufacturing routes to FTC and 3TC which proceed via chiral intermediate **3**.[18] Interestingly, thymine derivative **8** was also synthesized without issue (Figure 3).

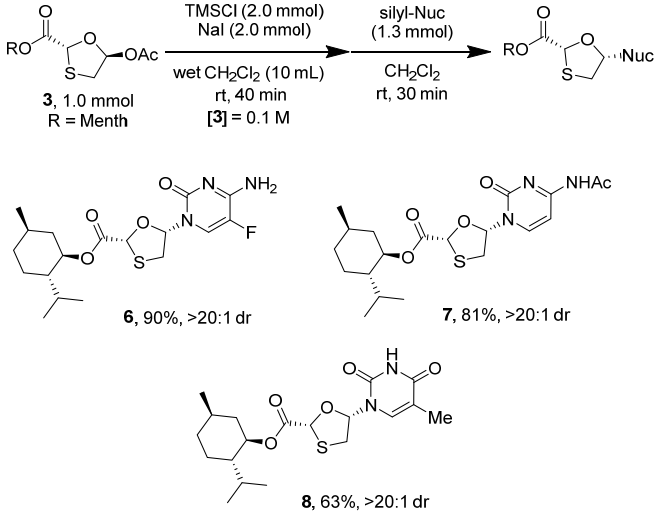


Figure 3. Synthesis of nucleoside analogs on 1 mmol scale by TMSCl-NaI promoted Vorbrüggen glycosylation.

In addition to the relevance of this method for synthesis of FTC and 3TC by a chiral auxiliary strategy, we envisioned that this method could bolster an improved route to (±)-FTC/3TC. Numerous strategies exist for resolution to the enantiopure active pharmaceutical ingredients (Supporting Information, Figure S2). We find that a key inefficiency in the existing routes is the glycosylation step. Commonly, a protected primary alcohol intermediate similar to **12** is accessed, followed by glycosylation using TMSOTf, TMSI, or $SnCl_4$, which yields glycosylated products such as **14** with approximately 1:1 dr.[5,6,8,19] We proposed a different synthetic strategy to access (±)-FTC/3TC via an achiral ester (Figure 4, top), by analogy to the precedented HI-promoted glycosylation using menthyl ester **3**.[3,16]

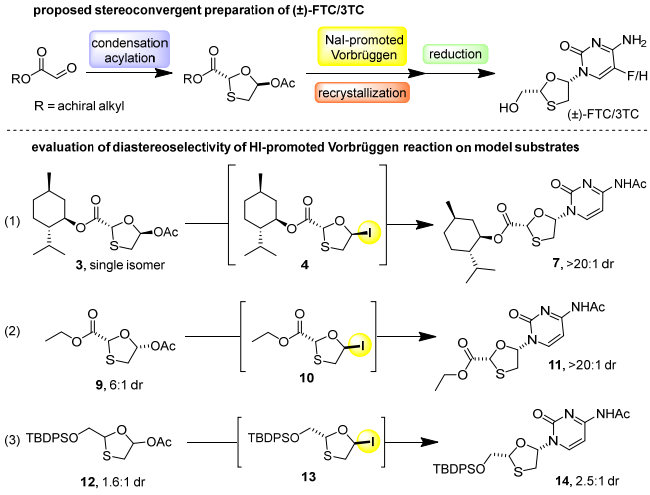


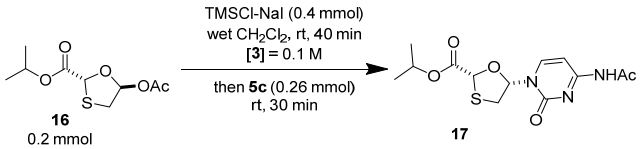
Figure 4. (top) Proposed route to (±)-FTC/3TC. (bottom) Mechanistic investigation of HI-promoted Vorbrüggen glycosylation using I_2-triethylsilane.

To elucidate the role of the alcohol protecting group in determining the diastereoselectivity of an HI-promoted *N*-glycosylation event, we designed a series of three model substrates (Figure 4, compounds **3**, **9**, **12**). Ethyl ester **9** was prepared analogously to the GSK manufacturing route using commercially

available ethyl glyoxylate. Meanwhile, model substrate **12** was prepared from ethylene glycol via a 5-step sequence. After exposing these model substrates to reported conditions for HI-promoted glycosylation with I_2-triethylsilane, we observed high diastereoselectivity with esters **3** and **9** and low selectivity with silyl ether **12** (Figure 4). These results support our proposal that a simple achiral ester is sufficient to promote a highly diastereoselective glycosylation event, and provides improved access to the *cis* oxathiolane product when compared with other alcohol protecting groups.

Following this mechanistic observation, we sought to develop the proposed route to (±)-FTC/3TC. Optimally, the glyoxylate ester was prepared by diol cleavage of diisopropyl tartrate and the resulting mixture was telescoped directly to oxathiolane formation with dithiane diol to yield hydroxyoxathiolane **15** (Figure 5).[20] The isopropyl group improved the solubility profile of the substrate in subsequent steps relative to the corresponding ethyl or methyl ester. Crude hydroxyoxathiolane **15** was directly acetylated and delivered glycosyl donor **16** in 3 steps from tartrate without intermediate purification. The results of the reaction optimization with **3** were validated using **16** and similar trends were observed (Table 3). Scale-up of the optimized Vorbrüggen reaction to 1 mmol scale proceeded without issue, and glycosylated products **17** and **18** were isolated in 72% and 95% yield, respectively.

Table 3. Optimization of Vorbrüggen glycosylation with *N*-acetyl cytosine and achiral ester.



Entry	TMSCl/ NaI (mmol)	Solvent	16 (%)[a]	17 (%)[a]	dr (cis:trans)
1[b]	0.4/0.5	MeCN[b]	41	60	10:1
2	0.4/0.4	MeCN[c]	13	77	10:1
3	0.4/0.4	CH_2Cl_2[b]	59	24	>20:1
4	0.4/0.4	CH_2Cl_2[d]	<5	83	>20:1
5	0.26/0.26	CH_2Cl_2[d]	<5	67	>20:1
6	0.26/0.22	CH_2Cl_2[d]	6	48	>20:1
7	0.22/0.26	CH_2Cl_2[d]	2	58	>20:1
8	0.22/0.22	CH_2Cl_2[d]	<5	68	>20:1
9	0.26/0	CH_2Cl_2[d]	80	0	ND
10	0/0.26	CH_2Cl_2[d]	87	0	ND

[a]Determined by ^{1}H NMR analysis using 1,3,5-trimethoxybenzene as internal standard. [b]Anhydrous solvent. [c]Water (1.8 µL, 0.1 mmol) added by microliter syringe. [d]CH_2Cl_2 pre-saturated with water (0.2 mmol).

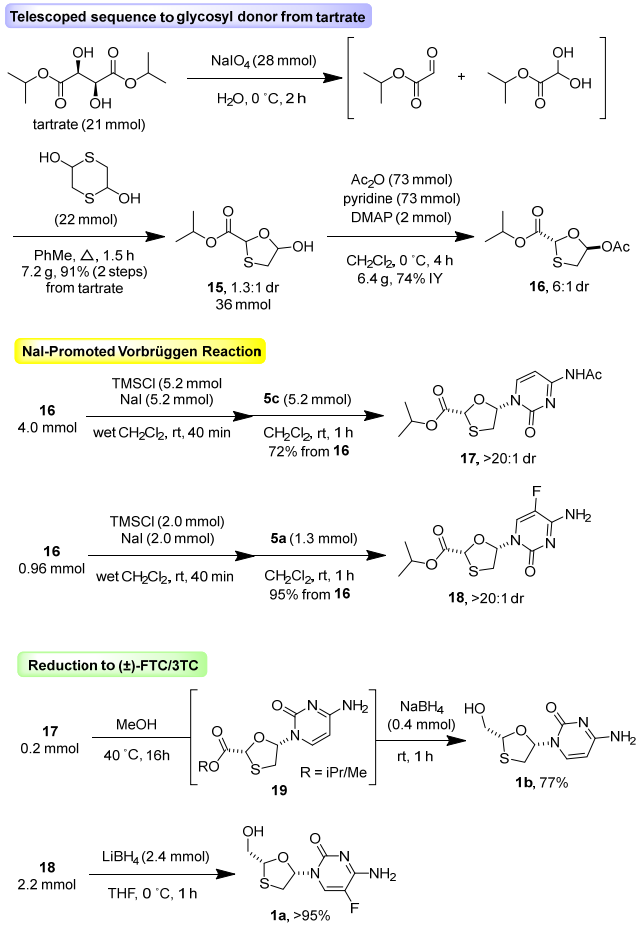
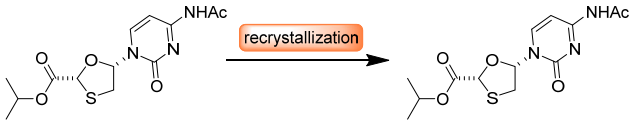


Figure 5. Complete route to penultimate FTC/3TC intermediates.

The precedented $NaBH_4/K_2HPO_4/NaOH$ reduction used for removal of the menthyl ester protecting group did not translate well to reduction of esters **17** and **18**. We observed ester hydrolysis with no conversion to the primary alcohol. Instead, we found that **18** is cleanly reduced to (±)-FTC with 1.1 equiv $LiBH_4$. Higher stoichiometry of the reductant led to undesired product formation. For *N*-acyl derivative **17**, hydrolysis of the amide was required to achieve clean reduction to **1b** with $NaBH_4$ (Supporting Information, Figure S3). To overcome the challenge of isolating the polar nucleoside product from the reaction mixture, the reduction was quenched with sodium sulfate decahydrate (Glauber's salt), followed by filtration through Celite. The resulting filtrate was concentrated to yield the desired API.

Although the isolation of 5-fluorocytosine glycosylation product **18** by precipitation is precedented,[3] purification of *N*-acyl cytosine product **17** is not known. Therefore, we saw value in the development of recrystallization conditions for the isolation of **17** (Table 4). Gentle heating of the crude reaction mixture in a 1:2 mixture of ethyl acetate and hexanes, followed by cooling to room temperature and filtration provided a 53% yield of the desired *cis* product with >600:1 dr.

Table 2. Screening of recrystallization conditions for glycosylation product 17.



17, 20:1 dr (5% *trans*) **17**, >600:1 dr (<0.1% *trans*)

Solvent*	soluble at rt?	soluble with heating?	crystallization observed?
MeCN/hex 1:1	Yes	Yes	No
IPA/hex 1:1	No	Yes	No
IPA/hex 2:1	No	Yes	No
IPA/hex 1:2	No	Yes	at -20˚C
EA/hex 1:1	No	Yes	at -20˚C
EA/hex 1:2	No	Yes	at rt

*MeCN = acetonitrile, IPA = isopropanol, EA = ethyl acetate, hex = hexanes. **17** (10 mg, 0.03 mmol) was added to a vial and solvent (1 mL) was added. The resulting mixture was heated with a heat gun until dissolution was observed and monitored for 24 h at room temperature, then moved to -20 ˚C for 24 h.

Conclusion

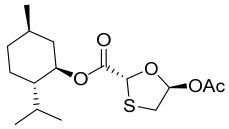
In conclusion, we have detailed the development of an improved synthetic route to (±)-FTC/3TC starting from a commercially available and inexpensive tartrate ester and employing a TMSCl-NaI-H_2O promoted Vorbrüggen glycosylation. The diastereoselectivity of the glycosylation step is crucial for the preparation of material suitable to access emtricitabine (FTC) and lamivudine (3TC) via chiral resolution.

EXPERIMENTAL SECTION

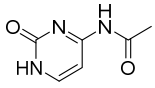
General Section

Reagents were used as supplied commercially without further purification. Solvents were dried and sparged with Argon using a solvent purification system prior to use unless otherwise noted. Reactions were run under Argon atmosphere unless otherwise noted. Thin-layer chromatography (TLC) was performed using 0.2 mm coated glass silica gel plates and visualized using either ultraviolet light or staining with $KMnO_4$ solution. Purification by column chromatography over silica gel was performed on a Biotage Selekt flash chromatography system using Isco RediSep Rf Gold silica gel columns. All NMR spectra were collected on Bruker instruments. Spectra reported with field strength 400 MHz were collecting using a two-channel Bruker Avance-III HD Nanobay spectrometer operating at 400.09 MHz. Spectra reported with field strength 500 MHz were collected using a three-channel Bruker Avance Neo spectrometer operating at 500.34 MHz. Both spectrometers were equipped with a 5 mm liquid-nitrogen cooled Prodigy broad band observe (BBO) cryoprobe. Chemical shifts (δ) are reported in units of ppm, relative to the residual solvent peak, which was adjusted to match reported values. Individual peaks are assigned multiplicity with the definitions: s = singlet, d = doublet, t = triplet, q = quartet, hept = heptet, m = multiplet. Reported NMR data follow the general format: Nuclei NMR (resonance frequency, reference solvent) chemical shift (multiplicity, coupling constants, integration). An asterisk (*) denotes signals corresponding to the minor diastereomer in a mixture of diastereomers. High-resolution mass spectrometry data was recorded using a JEOL AccuTOF 4G LC-plus equipped with a Direct Analysis in Real Time (DART) source. Infrared (IR) resonances were observed using an Agilent Cary 630 FTIR spectrometer. IR samples were prepared as solutions in dichloromethane then loaded onto a diamond surface.

Synthesis of Starting Materials



(1R,2S,5R)-2-Isopropyl-5-methylcyclohexyl (2R,5R)-5-acetoxy-1,3-oxathiolane2-carboxylate, (**3**).[16] By modification of a reported procedure, (2*R*)-5-hydroxy-1,3-oxathiolane-2-carboxylic acid (1*R*,2*S*,5*R*)-5-methyl-2-(1-methylethyl)cyclohexyl ester (8.65 g, 30.0 mmol, Combi-Blocks) was dissolved in CH_2Cl_2 (200 mL) and acetic anhydride (5.7 mL, 60 mmol) was added. The solution was cooled in an ice-water bath, then pyridine (4.8 mL, 60 mmol) was added dropwise with stirring. 4-dimethylaminopyridine (730 mg, 6.0 mmol) was added in one portion. The reaction mixture was warmed to room temperature and stirred for 4 h. Reaction progress was monitored by TLC (EtOAc/hexanes). The reaction mixture was cooled in an ice-water bath, then quenched by addition of water and transferred to a separatory funnel. The organic layer was washed twice with 1 M HCl (aq), then twice with 1 M $NaHCO_3$ (aq). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to yield an orange oil. The crude residue was purified by column chromatography (7-60% EtOAc/hexanes). The resulting material was dissolved in 400 mL *n*-hexane with 2 mL triethylamine. The solution was heated to boiling, then filtered hot by gravity filtration. The filtrate was collected in an Erlenmeyer flask and cooled to room temperature, then cooled at -20 ˚C for 72 h. The crystals were collected by filtration using a medium porosity sintered glass funnel, washing with hexanes. The filtrate was collected and filtered a second time to collect a second crop (3.82 g, 39%, white needles).



N-*Acyl cytosine*, (**19**).[21] A mixture of cytosine (2.22g, 20.0 mmol), acetic anhydride (9.5 mL, 100 mmol), and pyridine (11.3 mL, 140 mmol) was heated to reflux (125 ˚C) and stirred for 1.5 h. The mixture was cooled to room temperature. EtOAc (10 mL) was added and the mixture was stirred for 30 min. The white solid was filtered and washed with EtOAc then dried under vacuum to yield a grey-pink amorphous solid (2.98g, 97%).

General Procedure for Optimization of TMSCl-NaI Glycosylation Leading to 6, 7, 8, 17, and 18.

5-Fluorocytosine (33.6 mg, 0.26 mmol) was added to an oven-dried 20 mL vial. CH_2Cl_2 (5 mL) was added, followed by *N,O*-bis(trimethylsilyl)acetamide (180 µL, 0.7 mmol). The mixture was capped tightly with a septum screwcap and heated to 47 ˚C in a heat block until a clear solution was obtained, indicating formation of **5a**. The solution was then cooled to room temperature. To prepare wet CH_2Cl_2, anhydrous CH_2Cl_2 (10 mL) and

DI water (10 mL) were added to a separatory funnel. The biphasic mixture was shaken vigorously, then allowed to separate. The organic layer was collected (wet CH_2Cl_2). Concurrently, NaI (60 mg, 0.40 mmol) was weighed into a separate oven-dried 20 mL vial. Wet CH_2Cl_2 (2 mL) was directly added to the vial containing NaI which was then capped with a septum screwcap. Chlorotrimethylsilane (51 μL, 0.4 mmol) was added and the heterogeneous mixture was stirred vigorously for 5 min, followed by addition of **3** (66 mg, 0.20 mmol) in one portion. This mixture was stirred vigorously for 40 min leading to the formation of **4**. The prepared silylated nucleobase solution was added rapidly by syringe to the stirring solution of **4** and the resulting mixture was stirred at rt for 30 min. The reaction mixture was transferred to a separatory funnel, diluting with CH_2Cl_2 (20 mL). The organic layer was washed with a 5:1 mixture of 1 N $Na_2S_2O_3$ / saturated $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (20 mL). The combined organic layers were washed with brine. The organic layer was dried over Na_2SO_4. 1,3,5-Trimethoxybenzene was added and the solution was swirled vigorously to dissolve. An aliquot was removed and concentrated under reduced pressure, then analyzed by ^{1}H NMR in $CDCl_3$ with a relaxation delay of 25 s. Yield and conversion was determined by integration of the sp^2 ^{1}H signal on the 5-fluorocytosine ring of **6** (**6a** δ 8.53 ppm, **6b** δ 7.51 ppm) and the anomeric proton of **3** (δ 6.80 ppm) versus the sp^2 proton signal of TMB (δ 6.11 ppm). If the sp^2 protons of **6** were obscured by the N-H ^{1}H NMR signal, the anomeric proton was used instead (**6a** δ 6.44 ppm, **6b** δ 6.69 ppm).

Modifications to General Procedure: When anhydrous solvent was used (Table 1, entries 1-4), the vial was capped with a septum screwcap after addition of NaI, then flame-dried under vacuum. The indicated solvent was then added followed by the indicated amount of water or silanol using a microliter syringe. When MeCN was used for preparation of **5a** or **5c**, the mixture was heated to 87 ˚C. For screening results in Table 3: **16** (47 mg, 0.20 mmol) was used and was added as a solution in CH_2Cl_2 (1 mL). For screening with **19** to prepare **5c**, the following amount of material was used: **19** (40 mg, 0.26 mmol) and *N,O*-bis(trimethylsilyl)acetamide (98 μL, 0.40 mmol) in CH_2Cl_2 (5 mL).

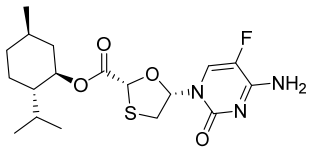
Representative Examples of TMSCl-NaI Glycosylation Screening (Table 1)

Entry 6: *(1R,2S,5R)-2-Isopropyl-5-methylcyclohexyl (2R,5S)-5-(4-amino-5-fluoro-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylate*, (**6**).[16] From **3** (66 mg, 0.20 mmol) as described in the General Procedure. Purified by column chromatography (3-10% MeOH/CH_2Cl_2) to yield a crystalline white solid (65 mg, 81%, >20:1 dr).

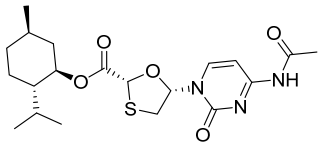
Entry 10: *(1R,2S,5R)-2-Isopropyl-5-methylcyclohexyl (2R,5S)-5-(4-amino-5-fluoro-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylate*, (**6**).[16] From **3** (66 mg, 0.20 mmol) using chlorotrimethylsilane (31 μL, 0.24 mmol) and NaI (36 mg, 0.24 mmol). Purified by column chromatography (3-10% MeOH/CH_2Cl_2) to yield a crystalline white solid (60 mg, 71%, >20:1 dr).

(1R,2S,5R)-2-isopropyl-5-methylcyclohexyl (2R,5S)-5-(4-acetamido-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylate, (**7**).[16] From **3** (66 mg, 0.20 mmol), **19** (40 mg, 0.26 mmol), *N,O*-bis(trimethylsilyl)acetamide (159 μL, 0.65 mmol) isolated by column chromatography (2-12% MeOH/CH_2Cl_2) as a white solid (69 mg, 81%).

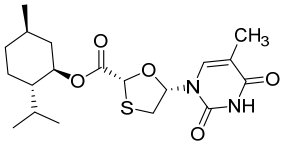
Synthesis of Nucleoside Analogs on 1.0 mmol Scale



(1R,2S,5R)-2-Isopropyl-5-methylcyclohexyl (2R,5S)-5-(4-amino-5-fluoro-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylate, (**6**).[16] 5-fluorocytosine (168 mg, 1.3 mmol) was weighed into a flame-dried 100 mL round-bottom flask. CH_2Cl_2 (25 mL) was added, followed by *N,O*-bis(trimethylsilyl)acetamide (905 μL, 3.7 mmol). The mixture was sonicated to disperse the solids, then heated to reflux temperature (47 ˚C in an oil bath) until dissolution was observed, about 1 h. The solution was cooled to room temperature (**5a)**. Separately, NaI (300 mg, 2.0 mmol) was added into a flame-dried 100 mL round-bottom flask. Wet CH_2Cl_2 (10 mL) was added, followed by chlorotrimethylsilane (254 μL, 2.0 mmol). The mixture was stirred for 5 min, followed by the addition of **3** (330 mg, 1.0 mmol). The mixture was stirred vigorously for 1 h and 40 min (formation of **4**). The solution of **5a** was added rapidly to the stirring solution of **4** and the resulting mixture was stirred for 30 min at room temperature. The reaction mixture was transferred to a separatory funnel, diluting with CH_2Cl_2 (100 mL). The mixture was washed with a 5:1 mixture of 1 N $Na_2S_2O_3$ and saturated Na_2HCO_3. After separation of the layers, the aqueous layer was back-extracted with CH_2Cl_2. The organic layers were combined and washed with brine, then dried over Na_2SO_4 and filtered through a sintered glass funnel. The filtrate was concentrated and purified by column chromatography (2-20% MeOH/CH_2Cl_2) by dry-loading onto silica gel to yield a white solid (361 mg, 90%, >20:1 dr).



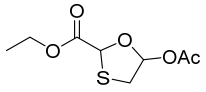
(1R,2S,5R)-2-isopropyl-5-methylcyclohexyl (2R,5S)-5-(4-acetamido-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylate, (**7**).[16] From **3** (330 mg, 1.0 mmol), chlorotrimethylsilane (254 μL, 2.0 mmol), and NaI (300 mg, 2.0 mmol) in wet CH_2Cl_2 (10 mL); combined with **19** (199 mg, 1.3 mmol), and *N,O*-bis(trimethylsilyl)acetamide (905 μL, 3.7 mmol) in CH_2Cl_2 (25 mL). Purified by column chromatography (2-20% MeOH/CH_2Cl_2) after dry-loading onto silica gel to yield a white solid (403 mg, 95%, >20:1 dr).



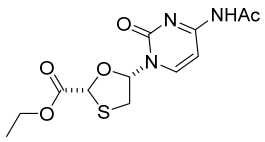
(1R,2S,5R)-2-Isopropyl-5-methylcyclohexyl (2R,5S)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylate, (**8**). From **3** (300 mg, 1.0 mmol), chlorotrimethylsilane (254 μL, 2.0 mmol), and NaI (300 mg, 2.0 mmol) in wet CH_2Cl_2 (10 mL); combined with thymine (164 mg, 1.3 mmol) and *N,O*-bis(trimethylsilyl)acetamide (905 μL, 3.7 mmol) in CH_2Cl_2 (25 mL). The product was purified by column chromatography (2-20% MeOH/CH_2Cl_2) to yield a colorless foam (248 mg, 63%, >20:1 dr). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.26 (s, NH), 8.10 (d, J = 1.4 Hz, 1H), 6.45 (dd, J = 7.7, 4.7 Hz, 1H), 5.41 (s, 1H), 4.77 (td, J = 11.0, 4.4 Hz, 1H),

3.37 (dd, J = 11.8, 4.8 Hz, 1H), 3.14 (dd, J = 11.9, 7.8 Hz, 1H), 2.08 – 2.03 (m, 1H), 1.97 (d, J = 1.3 Hz, 3H), 1.95-1.89 (m, 1H), 1.85 – 1.76 (m, 1H), 1.70 (dt, J = 12.7, 2.8 Hz, 2H), 1.55-1.48 (s, 1H), 1.47 – 1.39 (m, 1H), 1.03 (q, J = 11.6 Hz, 2H), 0.93 – 0.89 (m, 6H), 0.77 (d, J = 6.9 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ 167.0, 163.7, 150.4, 136.0, 111.6, 89.0, 77.6, 47.3, 40.9, 35.0, 34.2, 31.6, 26.2, 23.4, 22.1, 20.9, 16.3, 12.8. HRMS (DART/AccuTOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{29}N_2O_5S$ 397.1792; Found 397.1809. IR 2956, 2930, 2870, 2359, 2337, 1733, 1700, 1465, 1372, 1279, 1238, 1189, 1077. Specific Rotation $[\alpha]_D^{20}$ -55.1 (c 0.66, $CHCl_3$).

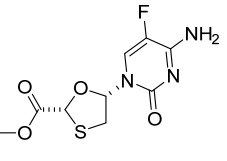
Synthesis of Derivatives for Mechanistic Investigation



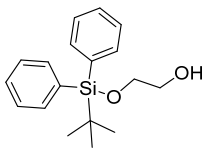
Ethyl 5-acetoxy-1,3-oxathiolane-2-carboxylate, (**9**). Ethyl glyoxylate (50% solution in toluene, 1.5 mL, 7.5 mmol) was added to a 20 mL vial equipped with a magnetic stirrer. 1,4-dithiane-2,5-diol (460 mg, 3.0 mmol) was added. The heterogeneous mixture was heated to reflux until a clear solution was obtained. The crude residue was directly purified by flash column chromatography (45% EtOAc/hexanes). The resulting clear oil was diluted in CH_2Cl_2 (40 mL). Acetic anhydride (1.1 mL, 12 mmol) and 4-dimethylaminopyridine (150 mg, 1.2 mmol) were added, and the solution was cooled in an ice-water bath. Pyridine (970 μL, 12 mmol) was added. The mixture was warmed to room temperature and stirred for 4 h, then quenched by addition of water. The reaction mixture was diluted with CH_2Cl_2 and transferred to a separatory funnel. The organic layer was washed with saturated $NaHCO_3$ (aq) and washed with 1 M HCl (aq), then washed with brine and dried over $MgSO_4$. The crude mixture was purified by flash column chromatography (7-60% EtOAc/hexanes) to yield a clear oil (852 mg, 65%, 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.74 (d, *J* = 4.1 Hz, 1H), 6.61 (m, 1H*), 5.62 (s, 1H*), 5.59 (s, 1H), 4.34 – 4.07 (m, 2H+2H*), 3.39 (dd, *J* = 11.8, 4.1 Hz, 1H), 3.25 (dd, *J* = 11.4, 4.1 Hz, 1H*), 3.18 (dd, *J* = 11.3, 1.2 Hz, 1H*), 3.13 (d, *J* = 11.7 Hz, 1H), 2.06 (s, 3H*), 2.06 (s, 3H), 1.24-1.29 (t, *J* = 7.1 Hz, 3H + 3H*). $^{13}C\{^{1}H\}$ NMR (126 MHz, $CDCl_3$) δ 170.1*, 169.6, 169.2*, 169.0, 99.8, 99.3*, 80.3*, 79.8, 62.0, 61.9*, 37.7*, 37.2, 21.2*, 21.1, 14.11*, 14.05. HRMS (DART/AccuTOF) m/z: $[M+NH_4]^+$ Calcd for $C_8H_{16}NO_5S$ 238.0749; Found 238.0747. IR 3485, 2974, 2162, 1741, 1431, 1372, 1230, 1185, 1096, 1036, 962, 857.



Ethyl 5-(4-acetamido-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylate, (**11**). Iodine (457 mg, 1.8 mmol) was suspended in CH_2Cl_2 (20 mL) and triethylsilane (575 μL, 3.6 mmol) was added. The solution was mixed until a light pink color was obtained. The resulting solution was cooled in an ice-water bath and a solution of **9** (330 mg, 1.5 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The reaction became yellow-orange in color. CH_2Cl_2 (5 mL) was used to complete the transfer. The reaction was stirred for 1 h. Concurrently, *N,O*-bis(trimethylsilyl)acetamide (744 μL, 3.0 mmol) was added to a suspension of **19** (306 mg, 2.0 mmol) in anhydrous CH_2Cl_2 (15 mL). The mixture was warmed to 40 ˚C until a clear solution was observed. The mixture was cooled to room temperature, then transferred into the stirring solution of **5c**, using addition CH_2Cl_2 (5 mL) for rinsing. The reaction was warmed to room temperature and stirred for 1 h. The reaction was diluted with CH_2Cl_2, and quenched with saturated $NaHCO_3$ (aq). The emulsion was washed with 1 N $Na_2S_2O_3$, then with brine. The aqueous layer was back-extracted with 20% methanol in CH_2Cl_2 (2 x 20 mL). The organic layer was dried with magnesium sulfate, then concentrated under reduced pressure. The product was isolated by column chromatography (0-15% MeOH/CH_2Cl_2) by dry loading onto silica gel to yield an amorphous solid (305 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.15 (s, 1H), 8.66 (d, *J* = 7.6 Hz, 1H), 7.49 (d, *J* = 7.5 Hz, 1H), 6.43 (t, *J* = 5.3 Hz, 1H), 5.55 (s, 1H), 4.30 (q, *J* = 7.1 Hz, 2H), 3.67 (dd, *J* = 12.3, 4.8 Hz, 1H), 3.20 (dd, *J* = 12.3, 5.9 Hz, 1H), 2.29 (s, 3H), 1.34 (t, *J* = 7.1 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ 171.2, 169.4, 163.3, 155.0, 145.3, 96.9, 90.6, 79.5, 62.5, 37.1, 24.9, 14.0. HRMS (DART/AccuTOF) m/z: $[M+H]^+$ Calcd for $C_{12}H_{16}N_3O_5S$ 314.0819; Found 314.0821. IR 3444, 3004, 2971, 2359, 1741, 1439, 1368, 1215.

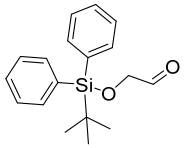


Ethyl (2R,5S)-5-(4-amino-5-fluoro-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylate, (**20**). Following the same general procedure as for **11** from **9** (220 mg, 1.0 mmol), iodine (300 mg, 1.2 mmol) and triethylsilane (480 μL, 3 mmol), combined with 5-fluorocytosine (168 mg, 1.3 mmol) and *N,O*-bis(trimethylsilyl)acetamide (1.04 mL, 4.2 mmol). Solid formation was observed during aqueous workup, complicating the separation of layers. Purification by column chromatography (0-10% MeOH/EtOAc) after dry-loading onto silica gel yielded the title compound as a brown solid (129 mg, 45%). ^{1}H NMR (400 MHz, DMSO) δ 8.19 (d, J = 7.2 Hz, 2H), 7.92 (s, 1H), 7.68 (s, 1H), 6.29 (td, J = 4.9, 2.5 Hz, 2H), 5.71 (s, 2H), 4.23 (q, J = 7.1 Hz, 4H), 4.09 (q, J = 5.2 Hz, 1H), 3.54 (dd, J = 12.1, 5.0 Hz, 2H), 3.22 (dd, J = 12.1, 6.3 Hz, 2H), 1.24 (t, J = 7.1 Hz, 6H). $^{13}C\{^{1}H\}$ NMR (101 MHz, DMSO) δ 169.8, 157.8, 157.6, 153.1, 137.3, 134.9, 125.3, 125.0, 89.2, 77.7, 62.0, 35.3, 13.9. HRMS (DART/AccuTOF) m/z: $[M+H]^+$ Calcd for $C_{10}H_{13}N_3O_4SF$ 290.0605; Found 290.0638. IR 3370, 3042, 1741, 1689, 1625, 1502, 1178, 1066, 1021.

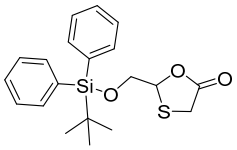


2-((Tert-butyldiphenylsilyl)oxy)ethan-1-ol, (**21**).[22,23] Sodium hydride, 60% in mineral oil (4.0g, 100 mmol) was added to a flame-dried 300 mL round-bottom flask equipped with a magnetic stirrer. Hexanes (100 mL) was added and the slurry was swirled gently, then the solvent was removed by cannula. This washing process was repeated once more, then vacuum was pulled on the flask to remove excess hexanes. THF (160 mL) was added, and the mixture was cooled to 0 ˚C. Ethylene glycol (6.21 g, 100 mmol) was dissolved in THF (10 mL), then added

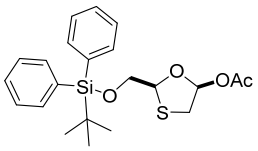
to the stirring suspension of sodium hydride. The resulting mixture was stirred for 45 min. Tert-butyldimethylsilyl chloride (26 mL, 100 mmol) was added slowly over 5 min. The mixture was warmed to rt and stirred for 2.5 h. The reaction mixture was diluted with diethyl ether (150 mL) and transferred to a separatory funnel and washed twice with a saturated $NaHCO_3$ (2 x 300 mL), followed by brine (150 mL). The organic layer was dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure. The resulting residue was purified by column chromatography (2-20% EtOAc/hexanes), resulting in the target compound as a clear oil. (13.6 g, 53%).



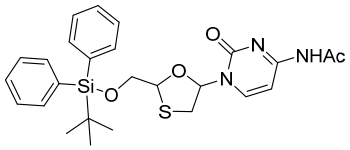
2-((Tert-butyldiphenylsilyl)oxy)acetaldehyde, (**22**).[22] A solution of oxalyl chloride (472 µL, 5.5 mmol) in 20 mL CH_2Cl_2 was stirred at -78 ˚C. DMSO (781 µL, 11 mmol) was added, followed by **21** (1.5 g, 5.0 mmol) as a solution in CH_2Cl_2 (3 mL). The mixture was stirred for 15 min, then triethylamine (3.5 mL) was added. The reaction was warmed to rt. The solvent was removed under reduced pressure to afford a white solid, which was triturated with a 1:4 mixture of EtOAc/hexanes and filtered through a plug of silica gel, washing with the same solvent (100 mL). The solvent was removed under reduced pressure to yield an oil which was telescoped to the next step (1.36 g with 83% purity, 75% yield).



2-(((Tert-butyldiphenylsilyl)oxy)methyl)-1,3-oxathiolan-5-one, (**23**).[24] **22** (708 mg with 66% purity, 1.57 mmol) was dissolved in toluene (20 mL) in a flame-dried 100 mL round-bottom flask. Thioglycolic acid (130 µL, 1.9 mmol) was added, and the mixture was refluxed for 4 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography (3-30% EtOAc/hexanes) to yield a white solid (483 mg, 83% yield).

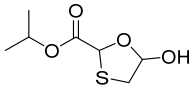


2-(((Tert-butyldiphenylsilyl)oxy)methyl)-1,3-oxathiolan-5-yl acetate, (**12**).[25] **23** (670 mg, 1.8 mmol) was dissolved in CH_2Cl_2 (25 mL) in a 250 mL round-bottom flask. The solution was cooled to -78 ˚C and DIBAL-H was added (2.0 mL of 1M solution in toluene, 2.0 mmol) over 10 min. The resulting mixture was stirred for 1 h at -78 ˚C. Reaction monitoring by TLC (30% EtOAc/hexanes) showed low conversion of **23**, thus additional DIBAL-H (2.0 mL of 1M solution in toluene, 2.0 mmol) was added and the mixture was stirred at -78 ˚C for an additional 2.5 h. The reaction was quenched with 5% H_2O/MeOH (10 mL), warmed to room temperature, and stirred for 30 min resulting in a clear solution. Saturated potassium sodium tartrate solution (50 mL) was added, resulting in a slurry. This was stirred at rt until separation of layers was observed, about 30 min. The biphasic mixture was transferred to a separatory funnel and washed with water, then washed with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude residue was suspended in CH_2Cl_2. Acetic anhydride (260 µL, 2.7 mmol) was added and the mixture was cooled to 0 ˚C. N,N-dimethylaminopyridine (66 mg, 0.54 mmol) was added, followed by pyridine (290 µL, 3.6 mmol). The mixture was stirred for 1.5 h until full conversion was observed by TLC (15% EtOAc/hexanes). The reaction was quenched with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The resulting crude residue was purified by column chromatography (3-30% EtOAc/hexanes) to yield the title compound as a clear oil (231 mg, 31% yield, 1.6:1 dr).



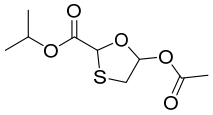
N-(1-(2-(((tert-butyldiphenylsilyl)oxy)methyl)-1,3-oxathiolan-5-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)acetamide, (**13**).[11] In a 2-dram vial, *N,O*-bis(trimethylsilyl)acetamide (34 µL, 0.14 mmol) was added to a suspension of **19** (14 mg, 0.088 mmol) in CH_2Cl_2 (2 mL). The mixture was warmed to 40 ℃ in a heat block until a clear solution was observed (**5c**). The solution was cooled to room temperature. Concurrently, iodine (21 mg, 0.082 mmol) was dissolved in CH_2Cl_2 (2 mL) and triethylsilane (26 µL, 0.16 mmol) was added. The solution was mixed until a light pink color was achieved. After 15 min, the resulting mixture was cooled to 0 ℃ and a solution of **11** (28.4 mg, 0.068 mmol) in CH_2Cl_2 (1 mL) was added dropwise with stirring. The reaction became yellow in color. The reaction was then stirred for 10 min. The cooled solution of **5c** was added rapidly using additional CH_2Cl_2 (5 mL) for rinsing. The reaction was warmed to room temperature and stirred for 1 h. The reaction was quenched with a few drops of saturated $NaHCO_3$. The emulsion was washed with 1 N $Na_2S_2O_3$, then with brine. The aqueous layer was back-extracted with CH_2Cl_2. The organic layers were dried with Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (5-25% MeOH/CH_2Cl_2) to yield the product as a clear oil (21 mg, 59%, 2.5:1 dr).

Synthesis of (±)-FTC/3TC

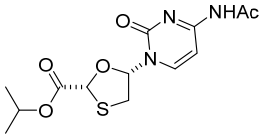


Isopropyl 5-hydroxy-1,3-oxathiolane-2-carboxylate (**15**). Diisopropyl-L-tartrate (5.05 g, 20.8 mmol) was dissolved in water (10 mL) in a 100 mL round-bottom flask. The solution was cooled in an ice/water bath. A solution of sodium periodate (5.9 g, 28 mmol) in water (40 mL) was added dropwise with vigorous stirring over 20 min. After completion of addition, the resulting suspension was stirred at 0 ˚C for 2 h. The reaction mixture was warmed to room temperature and extracted with EtOAc (5 x 30 mL). The combined extracts were dried over Na_2SO_4 and concentrated. The resulting crude residue was dissolved in toluene (5 mL) and transferred to a 100 mL round-bottom flask. 1,4-Dithian-2,5-diol (3.4 g, 22 mmol) was added and the flask was equipped with a reflux condenser. The mixture was heated to reflux for 1 h until the solution turned from yellow to colorless. The mixture was cooled for 5 min, then concentrated under reduced pressure with heating at 45 ˚C (7.2 g, 91%, 1.1:1 dr). The crude material was carried forward to the

next step without purification. For characterization, the crude material was purified by column chromatography (10-70% EtOAc/hexanes) to yield the title compound as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.01 (d, J = 3.9 Hz, 1H), 5.94 – 5.88 (m, 1H), 5.54 (d, J = 5.6 Hz, 1H), 5.15 – 5.00 (m, J = 6.3, 5.8 Hz, 1H), 3.28 (dd, J = 11.2, 4.4 Hz, 1H), 3.14 (dd, J = 11.1, 2.1 Hz, 1H), 1.28 (q, J = 5.3 Hz, 6H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 172.0, 169.5, 103.0, 101.3, 80.0, 77.80, 70.7, 69.7, 40.1, 38.3, 21.7, 21.7, 21.5, 21.4. HRMS (DART/AccuTOF) m/z: $[M+H]^+$ Calcd for $C_7H_{13}O_4S$ 193.0529; Found 193.0534. IR 3023, 2967, 1737, 1439, 1364, 1230, 1215, 1204.

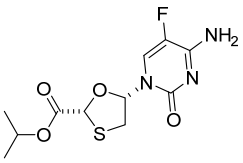


Isopropyl 5-acetoxy-1,3-oxathiolane-2-carboxylate (**16**). **15** (7.00 g, 36.4 mmol) was dissolved in CH_2Cl_2 in a 250 mL round-bottom flask. The resulting solution was cooled in an ice-water bath. Acetic anhydride (6.9 mL, 73 mmol) was added, followed by pyridine (5.9 mL, 73 mmol) and 4-dimethylaminopyridine (220 mg, 1.8 mmol). The mixture was warmed to room temperature and stirred for 4 h. The reaction mixture was diluted with CH_2Cl_2 and transferred to a separatory funnel. The organic layer was washed with water, saturated $NaHCO_3$, 1 M HCl (aq), then brine. The organic layer was dried with $MgSO_4$ then concentrated under reduced pressure. The resulting residue was purified by column chromatography (7-60% EtOAc/hexanes) to yield the title compound as a clear oil (6.35 g, 9:1 dr with 94% purity, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.78 (d, *J* = 4.1 Hz, 1H), 6.64 (d, *J* = 4.0 Hz, 1H*), 5.62 (s, 1H*), 5.60 (s, 1H), 5.07 (hept, *J* = 6.3 Hz, 1H+1H*), 3.43 (dd, *J* = 11.7, 4.1 Hz, 1H), 3.28 (dd, *J* = 11.3, 4.0 Hz, 1H*), 3.21 (dd, *J* = 11.2, 1.2 Hz, 1H*), 3.15 (d, *J* = 11.7 Hz, 1H), 2.11 (s, 3H*), 2.10 (s, 3H), 1.27 (d, *J* = 6.3 Hz, 6H+6H*). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.9*, 169.4, 168.5*, 168.3, 99.7, 99.1*, 80.2*, 79.8, 69.5, 69.4*, 37.5*, 37.0, 21.5*, 21.5, 21.4*, 21.3, 21.0*, 20.9. HRMS (DART/AccuTOF) m/z: $[M+NH_4]^+$ Calcd for $C_9H_{18}NO_5S$ 252.0900; Found 252.0901. IR 3507, 2981, 2937, 2356, 1748, 1467, 1375, 1230, 1181, 1148, 1100, 1018, 965.



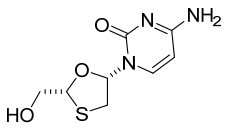
Isopropyl (RS,SR)-5-(4-acetamido-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylate, by recrystallization (**17**). **19** (800 mg, 5.2 mmol) was weighed into an oven-dried 200 mL roundbottom flask. CH_2Cl_2 (100 mL) was added. *N,O*-bis(trimethylsilyl)acetamide (2.0 mL, 8.0 mmol) was added and the mixture was heated to reflux in an oil bath until complete dissolution was observed, approximately 30 min. Concurrently, NaI (780 mg, 5.2 mmol) was added to a 250 mL round-bottom flask followed by wet CH_2Cl_2 (25 mL, water content 0.1 M). With stirring, chlorotrimethylsilane (660 µL, 5.2 mmol) was added, and heterogeneous mixture was stirred at room temperature vigorously for 5 min. **16** (936.24 mg, 4 mmol) was dissolved in dichloromethane (4 mL), then add to the TMSCl-NaI mixture, using additional CH_2Cl_2 (12 mL) to complete the transfer. The resulting mixture was stirred for 15 min at room temperature, resulting in a dark brown solution. The solution of **5c** was added by cannulation, using CH_2Cl_2 (10 mL) to complete the transfer. The resulting mixture was stirred at room temperature for 40 min. The reaction was quenched by addition of saturated $NaHCO_3$ (10 mL) with rapid stirring. The slurry was filtered through a pad of Celite, washing thoroughly with CH_2Cl_2 to yield an orange-yellow filtrate. The filtrate was transferred to a separatory funnel and washed with a 1:1 mixture of 1 N $Na_2S_2O_3$ and saturated $NaHCO_3$, then with brine. The organic layer was dried with $MgSO_4$ and filtered, then concentrated under reduced pressure. ^{1}H NMR analysis showed full conversion of **16** to **17** with 20:1 dr. The crude product was dissolved in a minimal amount of EtOAc and held at -20 ˚C overnight to yield a precipitate which was collected by vacuum filtration over a sintered glass funnel with fine porosity (697 mg, 53%, >150:1 dr). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.70 (d, J = 7.5 Hz, 1H), 7.47 (d, J = 7.6 Hz, 1H), 6.42 (dd, J = 5.8, 4.8 Hz, 1H), 5.51 (s, 1H), 5.13 (hept, J = 6.2 Hz, 1H), 3.66 (dd, J = 12.3, 4.8 Hz, 1H), 3.19 (dd, J = 12.3, 5.9 Hz, 1H), 2.27 (s, 3H), 1.31 (dd, J = 8.0, 6.2 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7, 169.1, 163.1, 155.1, 145.6, 96.8, 90.9, 79.87, 70.7, 37.2, 25.1, 21.8, 21.6. HRMS (DART/AccuTOF) m/z: $[M+H]^+$ Calcd for $C_{13}H_{18}N_3O_5S$ 328.0962; Found 328.0967. IR 3194, 2359, 2333, 1659, 1614, 1562, 1498, 1390, 1320, 1279, 1245, 1185, 1074.

Isopropyl (RS,SR)-5-(4-acetamido-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylate (**17**). As above, isolation by column chromatography. Using **19** (800 mg, 5.2 mmol), *N,O*-bis(trimethylsilyl)acetamide (2.0 mL, 8.0 mmol), and CH_2Cl_2 (100 mL) to prepare **5c**. Using chlorotrimethylsilane (660 µL, 5.2 mmol), NaI (780 mg, 5.2 mmol), and wet CH_2Cl_2 (25 mL), followed by **16** (936 mg, 4.0 mmol) in a solution of CH_2Cl_2 (4 mL), using additional CH_2Cl_2 (12 mL) to complete the transfer, resulting in a dark brown solution. The solution of **5c** was transferred by cannulation, using CH_2Cl_2 (12 mL) to complete the transfer. The reaction was quenched by aqueous workup, transferring the unquenched reaction mixture to a separatory funnel, then washing with a 1:1 mixture of saturated $Na_2S_2O_3$ and saturated $NaHCO_3$. A pink solid was observed in the aqueous layer which interfered with the separation (presumably excess **20**). The crude residue was purified by column chromatography (0-7% MeOH/EtOAc) resulting in a pearly white foam (1.05 g, 72%, >20:1 dr).

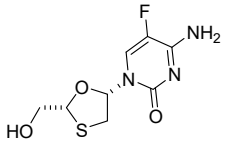


Isopropyl (RS,SR)-5-(4-amino-5-fluoro-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolane-2-carboxylate, (**18**). Using 5-fluorocytosine (168 mg, 1.3 mmol) and *N,O*-bis(trimethylsilyl)acetamide (1.1 mL, 3.4 mmol) in CH_2Cl_2 (25 mL) to prepare **5a**. Using **16** (243 mg, 92% purity, 0.96 mmol), chlorotrimethylsilane (254 µL, 2.0 mmol), and NaI (300 mg, 2.0 mmol), in wet CH_2Cl_2 (10 mL). The reaction was quenched by aqueous workup, transferring the unquenched reaction mixture to a separatory funnel, then washing with a 1:1 mixture of saturated $Na_2S_2O_3$ / saturated $NaHCO_3$ and extracting with CH_2Cl_2. The product was isolated by column chromatography (0-20% MeOH/CH_2Cl_2) by dry-loading onto silica gel, yielding the title compound as a white solid (288 mg, >95%). ^{1}H NMR (400 MHz, $CDCl_3$ plus MeOD) δ 8.39 (d, J = 6.6 Hz, 1H), 6.32 (ddd, J = 6.7, 4.8, 1.8 Hz, 1H), 5.38 (s, 1H), 5.06 (hept, J = 6.3 Hz, 1H), 3.46 (dd, J = 12.1, 4.7 Hz, 1H), 3.20 (s, 2H), 3.06 (dd, J = 12.1, 6.6 Hz, 1H),

1.25 (t, J = 5.9 Hz, 6H). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$ plus MeOD) δ 169.5, 154.3, 153.9, 138.0, 135.5, 125.9, 125.6, 90.4, 78.7, 70.6, 49.6, 49.4, 49.2, 48.9, 48.7, 36.2, 21.6, 21.3. HRMS (DART/AccuTOF) m/z: $[M+H]^+$ Calcd for $C_{11}H_{15}N_3O_4FS$ 304.0762; Found 304.0766. IR 3276, 3086, 2986, 2359, 2341, 1737, 1681, 1610, 1498, 1346, 1290, 1238, 1189, 1159, 1077, 932, 787.



4-amino-1-((2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl)pyrimidin-2(1H)-one, (**1b**).[16] **17** (67 mg, 0.20 mmol) was dissolved in methanol (5 mL) in a 20 mL vial and was heated in a heat block to 40 ˚C for 16 h until cleavage of the acetyl group was observed by HPLC. Sodium borohydride (15 mg, 0.40 mmol) was added and the solution was stirred for 1 h. Glauber's salt (sodium sulfate decahydrate) was added. The mixture was filtered through Celite, washing with methanol. Quantitative NMR analysis with 1,3,5-trimethoxybenzene showed an assay yield of 77% (see Supporting Information). The NMR sample was recovered, dissolved in water, and transferred to a separatory funnel. The aqueous layer was extracted with diethyl ether (3 x 10 mL). The aqueous layer was concentrated to yield a white solid (80 mg, with ca. 44% purity, 77%).



4-amino-5-fluoro-1-((2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl)pyrimidin-2(1H)-one, (**1a**).[16] **18** (44 mg, 0.15 mmol) was suspended in THF (5 mL) in a 25 mL round-bottom flask. The suspension was sonicated to disperse the material, which is sparingly soluble. Lithium borohydride (83 µL of 2.0 M solution in THF, 0.17 mmol) was added to the suspension at 0 ℃. The solution was warmed to room temperature and stirred until complete conversion was observed by TLC (5% MeOH/CH_2Cl_2), 1 h. The solution was quenched by addition of MeOH (0.5 mL), followed by addition of silica gel (1 g). The slurry was stirred for 10 min, then transferred to a sintered glass funnel containing a 1 g pad of silica gel. The pad of silica was washed with 20% MeOH/CH_2Cl_2 (25 mL) and the filtrate was evaporated to dryness to yield a white solid (43 mg, >95%, >20:1 dr).

4-amino-5-fluoro-1-((2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl)pyrimidin-2(1H)-one, (**1a**).[16] **20** (638 mg, 2.21 mmol) was suspended in 30 mL dry THF in a dry 100 mL round-bottom flask. The suspension was sonicated to disperse material, which is sparingly soluble. Lithium borohydride solution (1.22 mL 2 M in THF, 2.43 mmol) was added dropwise to the suspension at 0 ℃. The solution was warmed to room temperature and stirred for 30 min. The reaction was quenched with MeOH (2 mL) followed by slow addition of silica gel (4 g). Gas evolution was observed on addition of silica gel. The slurry was stirred for 30 min, then transferred to a short column and eluted with CH_2Cl_2/MeOH (496 mg, 91%, 14:1 dr).

SUPPORTING INFORMATION

Experimental methods for NMR assay, supplementary reaction schemes, HPLC traces, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

A previous version of this manuscript is available on ChemRxiv at doi: 10.33774/chemrxiv-2021-fk5c0.[26]

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Funding Sources

The authors thank the Bill and Melinda Gates Foundation (Medicines For All Institute, OPP1176590) for generous support of the research in our laboratory. Additionally, this material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. (1745302). Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation or the Bill and Melinda Gates Foundation.

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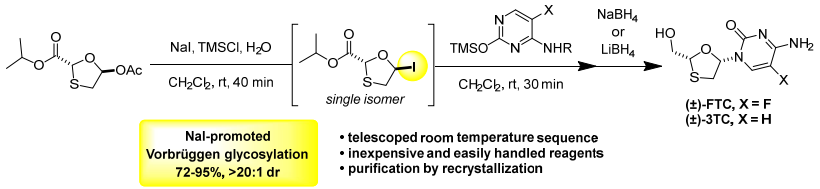

NaI-promoted Vorbrüggen glycosylation 72-95%, >20:1 dr

- telescoped room temperature sequence
- inexpensive and easily handled reagents
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