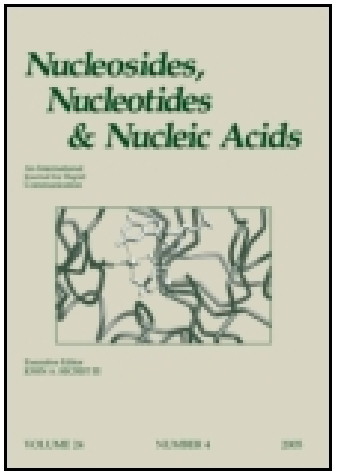

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mt-tRNA Components: Synthesis of (2-Thio)Uridines Modified with Blocked Glycine/Taurine Moieties at C-5,1

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mt-tRNA COMPONENTS: SYNTHESIS OF (2-THIO)URIDINES MODIFIED WITH BLOCKED GLYCINE/TAURINE MOIETIES AT C-5,1

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□ *In this paper, we discuss the usefulness of reductive amination of 5-formyl-2′,3′-O-isopropylidene(-2-thio)uridine with glycine or taurine esters in the presence of sodium triacetoxyborohydride ($NaBH(OAc)_3$) for the synthesis of the native mitochondrial (mt) tRNA components 5-carboxymethylaminomethyl(-2-thio)uridine ($cmnm^5(s^2)U$) and 5-taurinomethyl(-2-thio)uridine ($\tau m^5(s^2)U$) with a blocked amino acid function. 2-(Trimethylsilyl)ethyl and 2-(p-nitrophenyl)ethyl esters of glycine and 2-(2,4,5-trifluorophenyl)ethyl ester of taurine were selected as protection of carboxylic and sulfonic acid residues, respectively. The first synthesis of 5-formyl-2′,3′-O-isopropylidene-2-thiouridine is also reported.*

Keywords Modified nucleosides; reductive amination; 5-formyluridine (f^5U); 5-formyl-2-thiouridine (f^5s^2U); 5-carboxymethylaminomethyl(-2-thio)uridine ($cmnm^5(s^2)U$); 5-taurinomethyl(-2-thio)uridine ($\tau m^5(s^2)U$)

INTRODUCTION

5-Carboxymethylaminomethyluridine ($cmnm^5U$ **1**, Figure 1) and its 2-thioanalogue ($cmnm^5s^2U$ **2**, Figure 1) have been found in the wobble position (the first anticodon letter) of *Saccharomyces cerevisiae* mitochondrial (mt) tRNAs specific for Leu and Lys, respectively.[1, 2] A structurally similar pair of wobble uridines, 5-taurinomethyluridine (τm^5U **3**, Figure 1) and 5-taurinomethyl-2-thiouridine (τm^5s^2U **4**, Figure 1), have been isolated from mammalian mt-tRNAs specific for Leu(UUR) and Lys, respectively.[1, 3] Recently, taurine-modified nucleosides have been also identified in the mt-$tRNA^{Leu,Lys}$ of a squid, *Loligo bleekeri*.[4]

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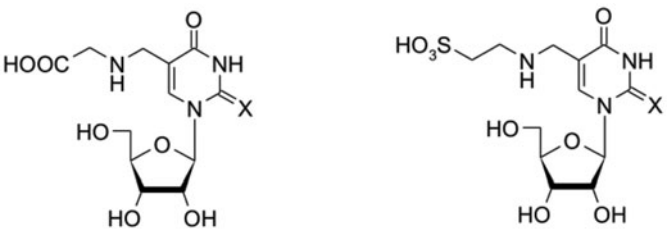


FIGURE 1 Chemical structures of 5-carboxymethylaminomethyluridine (cmnm^{5}U, **1**), 5-carboxymethylaminomethyl-2-thiouridine (cmnm^{5}s^{2}U, **2**), 5-taurinomethyluridine (τm^{5}U, **3**), and 5-taurinomethyl-2-thiouridine (τm^{5}s^{2}U, **4**).

Modified nucleosides **1–4** are crucial structural elements of mt-tRNA that determines the biopolymer decoding capacity.[2, 4, 5] Site-specific mutations in yeast mt-DNA induce the loss of modifications **1** and **2** leading to a significant failure of mitochondrial protein biosynthesis.[6, 7] A similar mechanism has been proposed to explain the absence of modifications **3** and **4** in human mt-tRNA specific for Leu(UUR) and Lys, respectively, and this phenomenon results in decoding disorders responsible for the incurable mitochondrial diseases MELAS and MERRF.[8, 9] Recently, yeast cells have been proposed as a useful model for studies of molecular and cellular effects related to the above-mentioned mitochondrial pathologies.[7] For a long time, modified tRNA fragments have served as key components of oligomer synthesis by the semienzymatic "RNA fragment recombination" methodology.[5, 10] Using this method, 3′,5′-*O*-bisphosphates of cmnm^{5}U and τm^{5}U have been inserted into position 34 of *Escherichia coli* tRNA^{Leu}(UUR) and the same technology has been applied in constructing an analogue of the human mt-tRNA^{Leu}(UUR) anticodon arm domain modified with τm^{5}U at the wobble position.[5] The incorporation of the hypermodified nucleosides **1–4** into oligoribonucleotide sequences by phosphoramidite chemistry on solid support requires appropriate protection of their amino acid residues. The variously substituted phenols have been tested for the protection of the sulfonic acid residue of nucleosides **3**,**4**, and the synthesis of respective, fully blocked 3′-*O*-phosphoramidites of τm^{5}U and τm^{5}s^{2}U has also been reported.[11] Their application to the synthesis of modified RNAs was, however, limited to the dimer level, and final oligomers, in particular those modified with τm^{5}s^{2}U, were contaminated with considerable amounts of side products.[12]

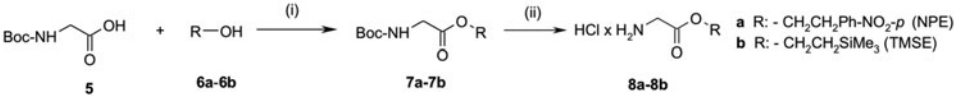
In the literature, only one paper describes the chemical synthesis of RNA anticodon triplets modified with cmnm^{5}U and cmnm^{5}s^{2}U (a triester approach in solution).[13]

Reductive amination of 5-formyl-2′-deoxyuridine (f^5dU) has found wide application in the synthesis of 5-alkylamino derivatives of pyrimidine nucleosides[14] and nucleic acid conjugates.[15] To date, no reports have been published concerning the utilization of 5-formyluridine (f^5U) for reductive amination with amino acids or the synthesis of 5-formyl-2-thiouridine (f^5s^2U).

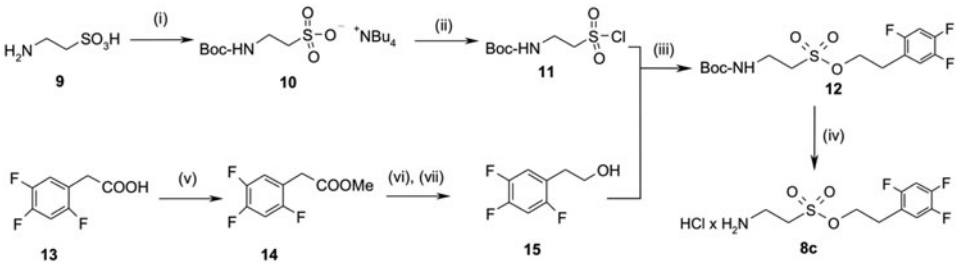
In this paper, we present a practical approach for synthesis of 5-methyleneaminoacid(-2-thio)uridines utilizing a two-step procedure of reductive amination of 5-formyl(-2-thio)uridine with glycine and taurine esters in the presence of sodium triacetoxyborohydride ($NaBH(OAc)_3$) as a reducing agent. Based on literature data, alternative procedures that utilize 5-chloromethyl(-2-thio)uridine[16] or a quaternary ammonium salt of 5-pyrrolidinomethyluridine[17] as the starting material have also been experimentally examined.

RESULTS AND DISCUSSION

Two groups, 2-(*p*-nitrophenyl)ethyl (NPE, **8a**, Scheme 1) and 2-(trimethylsilyl)ethyl (TMSE, **8b**, Scheme 1), were tested for the protection of the glycine carboxyl function. The usefulness of the above-mentioned protecting groups was previously verified by incorporation of N^6-threonylcarbamoyladenosine (t^6A) and its 2-methylthio analogue (ms^2t^6A) into the anticodon arm sequence of tRNA by phosphoramidite chemistry.[18] *N*-Boc glycine esters **7a** and **7b** (Scheme 1) were obtained by condensation of the *N*-Boc-protected glycine **5** with 2-(*p*-nitrophenyl)ethyl or 2-(trimethylsilyl)ethyl alcohol, (**6a**) or (**6b**), respectively, in the presence of DCC. We found that both masking groups in compounds **7a** and **7b** were stable in 8 M ethanolic ammonia and easily removed under standard conditions for their deprotection during oligoribonucleotide synthesis, 10% DBU in MeCN, 40 min, 40°C for NPE ester and 1 M TBAF in NMP for TMSE ester.[18] The stability of the 2-(trimethylsilyl)ethyl ester protection in 8 M ethanolic ammonia offers a simple way for simultaneous deblocking of alkaline labile protecting groups, e.g., 2-cyanoethyl, -tac (or -pac) and cleavage oligomer from CPG-support without risk of amide formation. Subsequent removal of TMSE group can be performed together with TBDMS protection of sugar residues. In contrary, the 2-(*p*-nitrophenyl)ethyl group is removed by DBU in β-elimination process before deprotection of base-labile groups



SCHEME 1 *Reagents and conditions:* (i) DCC, py, MeCN, 12 h, 0°C → rt; (ii) 4 M HCl/dioxane, 1.5 h, rt.



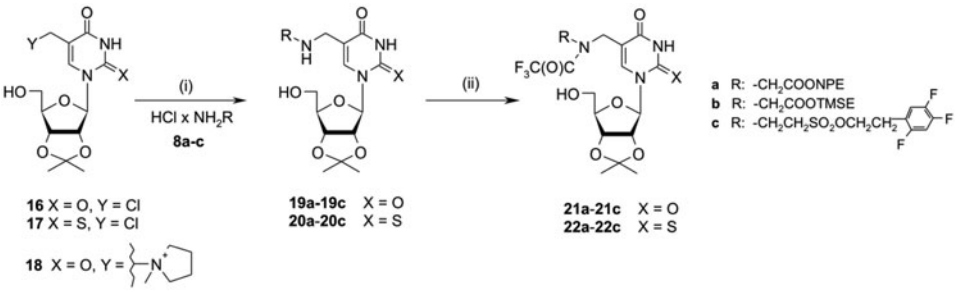
SCHEME 2 *Reagents and conditions:* (i) Boc_2O, Bu_4NOH, H_2O, acetone, 12 h, rt; (ii) triphosgene, $DMF_{(cat.)}$, CH_2Cl_2, 30 min, rt; (iii) Et_3N, CH_2Cl_2, 2 h, 0°C → rt; (iv) 4 M HCl/dioxane, 1 h, rt; (v) TMSCl, 2,2-dimethoxypropane, MeOH, 24 h, rt; (vi) $NaBH_4$, THF, 15 min, 65°C; (vii) MeOH, 2.5 h, reflux.

as well as cleavage oligomer from the solid support. This strategy also eliminates the risk of aminolysis. Despite numerous attempts we were not able to protect the taurine acidic function with the TMSE group, while the NPE blockage was found too labile to be used in oligoribonucleotide synthesis. The list of known masking groups for the protection of the sulfonic acid residue is short, in particular for taurine-modified nucleosides applied in oligomer syntheses via phosphoramidite chemistry.[11, 12]

We synthesized several 2-arylethyl esters of *N*-Boc taurine (*p*-trifluoromethyl-, 2,4-difluoro-, 2,4,5-trifluoro-, 2,4-dichloro-, *p*-fluoro-, *p*-nitrophenylethyl esters) by conversion of *N*-Boc taurine tetrabutylammonium salt[19] (**10**) (Scheme 2) to sulfonyl chloride **11**,[20] which was subsequently reacted with an appropriate alcohol using a modified procedure of sulfonyl chloride esterification.[21] To synthesize alcohols, commercially available variously substituted phenylacetic acids were almost quantitatively converted to methyl esters,[22] which were then reduced with $NaBH_4$/MeOH/THF to desired products.[23] Nevertheless, only *N*-Boc taurine 2-(2,4,5-trifluorophenyl)ethyl ester (**12**) revealed the stability under alkaline conditions, e.g., triethylamine, isopropyldiethylamine, necessary for the synthesis of suitable monomer unit. We found that the 2-(2,4,5-trifluorophenyl)ethyl ester protection can be easily removed by the β-elimination process under relatively mild conditions (10% DBU in MeCN, 40 min, 40°C).

N-Boc glycine and taurine esters, **7a**, **7b,** and **12**, respectively, were transformed to hydrochlorides **8a–8c** with very good yields by treatment with 4 M hydrochloric acid in dioxane (Schemes 1 and 2).

In the literature, 5-chloromethyl-2′,3′-*O*-isopropylidene(-2-thio)uridine or quaternary ammonium salt of 2′,3′-*O*-isopropylidene-5-pyrrolidinomethyl(-2-thio)uridine were reacted with methyl, ethyl or *tert*-butyl glycine esters to give products of nucleophilic substitution at the activated 5-methylene group, albeit with moderate yields.[16, 17] Using these procedures, we tried to synthesize $cmnm^5U$, τm^5U, and their

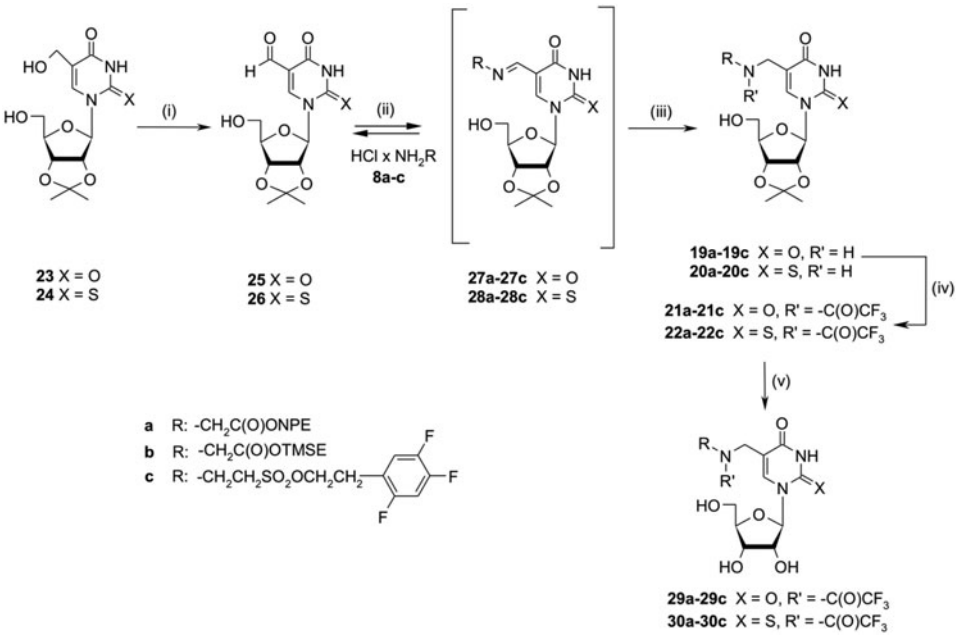


SCHEME 3 *Reagents and conditions:* (i) Et_3N, DMF or MeCN, rt; (ii) TFAA, py, 1 h, 0°C → rt.

2-thio analogues protected with NPE/TMSE (for glycine residue) and 2-(2,4,5-trifluorophenyl)ethyl (for taurine residue; Scheme 3). The resulting nucleosides **19a–19c/20a–20c** underwent partial degradation under conditions of silica gel column chromatography. Thus, the crude products **19a–19c/20a–20c** were treated with trifluoroacetic anhydride (TFAA) in pyridine to obtain amides **21a–21c/22a–22c**, which are stable during silica gel column purification. The trifluoroacetyl group has been used as standard protection of the aliphatic, secondary amine function in the synthesis of RNA fragments modified with uridines containing such a group.[16, 24] The final yields of the isolated products **21a–21c/22a–22c** (Scheme 3) were poor (15–25%; experimental data not shown).

As an alternative, we tested reductive amination of 5-formyl(-2-thio)uridine with amino acid esters in the presence of $NaBH(OAc)_3$ (Scheme 4). We found that oxidation of 5-hydroxymethyl-2′,3′-*O*-isopropylideneuridine (**23**) to 5-formyl-2′,3′-*O*-isopropylideneuridine (**25**) with activated MnO_2 in acetone/CH_2Cl_2 (4:1, v/v) solution at elevated temperature (55°C, 2–7 h) gives better yield than the previously reported procedure[25] (55% versus 37%). Surprisingly, the same protocol may be used for the oxidation of 5-hydroxymethyl-2′,3′-*O*-isopropylidene-2-thiouridine (**24**) to the appropriate 5-formyl-2-thiouridine **26** without the formation of side-products of oxidation/oxidative desulphurization of the 2-thiocarbonyl function.[26]

The 5-formyluridine **25** and its 2-thio analogue **26** were subjected to reductive amination with amino acid esters **8a–8c** (Scheme 4) according to a two-step procedure that involves the formation of imines **27a–27c/28a–28c** and subsequently their reduction with $NaBH(OAc)_3$. The crude secondary amines **19a–19c** and their 2-thio analogues **20a–20c** were acylated with TFAA in pyridine to respective amides **21a-21c/22a-22c**. According to NMR data, resulting uridines exist as a mixture of rotamers about the $-NC(O)CF_3$ amide bond. Glycine derivatives **21a** and **21b** were isolated in 66% and 81% yields, respectively (the yields calculated refer to aldehydes **25** and **26**, respectively). For the 2-thio analogues **22a** and **22b**, the yields were



SCHEME 4 *Reagents and conditions*: (i) activated MnO_2, acetone : CH_2Cl_2 (4:1, v/v), 2–7 h, 55°C, (ii) Et_3N, CH_2Cl_2/DMF, 1–1.5 h, rt; (iii) $NaBH(OAc)_3$, 40 min–2 h, rt; (iv) TFAA, py, 1 h, 0°C → rt; (v) 25% aq. AcOH, 1 h, 90°C.

slightly lower, 56% and 78%, respectively. However, reductive amination of aldehydes **25** and **26** with taurine 2-(2,4,5-trifluorophenyl)ethyl ester **8c** gave a notably lower yield (~35%). The significant decrease in the yield of reductive amination involving taurine ester **8c** may be explained as a result of unfavorable equilibrium position of the reversible reaction of Schiff bases formation (**27c**, **28c**) and/or their less susceptibility to reduction with sodium triacetoxyborohydride. In this specific case, a Michael-type addition of phenyl vinylsulfate with the 5-aminomethyl(-2-thio)uridine derivative is the most effective methodology and should be considered the method of choice.[11]

It is worth to notice that presented strategy of the reductive amination allowed to increase the yield of C-N-C linkage formation by 40%–60% in the case of glycine esters and 20% in the case of taurine ester in comparison with methods described in the literature.[16, 17]

The acetal protection of the sugar moieties in compounds **21a–21c** and **22a–22c** was removed with 25% acetic acid (90°C, 1h) and the title compounds **29a–29c** and **30a–30c** were isolated with 80% yield.

In conclusion, we report facile synthesis of (2-thio)uridines substituted at C-5,1 with protected glycine or taurine residues (**29a–29c**/**30a–30c**) as useful intermediates in the chemical synthesis of oligomers modified with

5-carboxymethylaminomethyl(-2-thio)uridine (**1**,**2**) and 5-taurinomethyl(-2-thio)uridine (**3**,**4**).

EXPERIMENTAL

General Procedure for the Synthesis of Hydrochlorides of Glycine Esters 8a and 8b

N-Boc glycine **5** (1.1 g, 6.0 mmol, 1.0 equiv) was dissolved in anhydrous MeCN (6.0 mL); alcohol **6a**/**6b** (6.6 mmol, 1.1 equiv) and anhydrous pyridine (1.5 mL) were added. The mixture was cooled in an ice bath and DCC (1.4 g, 6.6 mmol, 1.1 equiv) was added. The cooling bath was removed and the mixture was stirred overnight at room temperature (rt). The reaction was quenched by 1 M aqueous solution of oxalic acid (1.1 mL) and stirred at rt for 30 min. The precipitated DCU was filtered and washed with EtOAc. The filtrate was extracted with 1 M aqueous solution of $NaHCO_3$ (3 mL), water (3 mL), and then dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was co-evaporated with toluene to give *N*-Boc glycine ester **7a**/**7b**. The crude compound **7a**/**7b** (5.0 mmol) was treated with 4 M solution of HCl/dioxane (50 mL) and stirred at rt for 1.5 h. The mixture was concentrated under reduced pressure and precipitated with hexane affording hydrochloride **8a**/**8b**.

Hydrochloride of glycine 2-(*p*-nitrophenyl)ethyl ester (8a)

Compound **7a** was isolated in 95% yield. TLC $R_f = 0.57$ ($CHCl_3$/MeOH 98:2, v/v); ^{1}H NMR (250 MHz, CD_3OD) δ 1.45 (s, 9H), 3.08 (t, 2H, J = 6.50 Hz), 3.88 (bs, 2H), 4.41 (t, 2H, J = 6. 50 Hz), 4.98 (bs, 1H), 7.37–7.41 (m, 2H), 8.16–8.20 (m, 2H). Compound **8a** was obtained in 95% yield as a white powder. TLC $R_f = 0.15$ ($CHCl_3$/MeOH 98:2 v/v); ^{1}H NMR (250 MHz, CD_3OD) δ 3.15 (t, 2H, J = 6.50 Hz), 3.81 (s, 2H), 4.52 (t, 2H, J = 6.50 Hz), 7.52 (d, 2H, J = 8.75 Hz), 8.17 (d, 2H, J = 8.75 Hz); ^{13}C NMR (62.9 MHz, CD_3OD) δ 34.10, 39.58, 65.51, 123.24, 129.78, 145.72, 146.92, 167.07; HRMS *m/z* calcd for $C_{10}H_{13}N_2O_4$ $[M+H]^+$ 225.0875, found 225.0873.

Hydrochloride of glycine 2-trimethylsilylethyl ester (8b)

Compound **7b** was isolated in 85% yield. TLC $R_f = 0.66$ ($CHCl_3$/MeOH 98:2, v/v); ^{1}H NMR (250 MHz, CD_3OD) δ 0.05 (s, 9H), 1.04–1.11 (m, 2H), 1.32 (s, 9H,), 3.79 (s, 2H), 4.30–4.37 (m, 2H). Compound **8b** was obtained in 95% yield as a white powder. TLC R_f = 0.25 ($CHCl_3$/MeOH 98:2 v/v); ^{1}H NMR (250 MHz, CD_3OD) δ 0.06 (s, 9H,), 1.04–1.11 (m, 2H), 3.80 (s, 2H), 4.30–4.37 (m, 2H); ^{13}C NMR (62.9 MHz, CD_3OD) δ –2.94, 16.86, 39.78, 64.34, 167.15; HRMS *m/z* calcd for $C_7H_{18}NO_2Si$ $[M+H]^+$ 176.1107, found 176.1111.

Hydrochloride of taurine 2-(2,4,5-trifluorophenyl)ethyl ester (8c)

Taurine **9** (1.25 g, 10 mmol, 1 equiv) was dissolved in water (10 mL), and then 40% aqueous solution of *n*-Bu_4NOH (6.5 mL, 10 mmol, 1 equiv) was added. Next, Boc_2O (2.18 g, 10 mmol, 1 equiv) in acetone (30 mL) was added dropwise. The mixture was stirred overnight at rt. Acetone was evaporated under reduced pressure and the aqueous phase was extracted with CH_2Cl_2 (3 × 12 mL). Organic layers were combined and dried over $MgSO_4$. The solvent was evaporated affording 4.4 g (95%) of tetrabutylammonium salt of *N*-Boc taurine (**10**) as a yellowish oil. $R_f = 0.6$ ($CHCl_3$/MeOH 9:1, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.00 (t, 12H, $J = 7.50$ Hz), 1.36–1.51 (m, 17H), 1.58–1.71 (m, 8H), 2.92–2.97 (m, 2H), 3.25–3.32 (m, 8H), 3.52–3.57 (m, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 13.4, 19.49, 23.75, 28.26, 36.83, 50.51, 58.44, 78.17, 155.88. The crude material **10** (2 g, 4.29 mmol, 1.0 equiv) was dissolved in anhydrous CH_2Cl_2 (15 mL), DMF (0.032 mL, 0.43 mmol, 0.43 equiv), and then triphosgene (0.51 g, 1.72 mmol, 0.4 equiv) was added. The mixture was stirred at rt for 30 min and then concentrated under reduced pressure, dissolved in EtOAc/hexane (1:1, v/v, 20 mL) and filtered over a small amount of silica gel with EtOAc/hexane (1:1, v/v) as eluent. Evaporation under reduced pressure afforded 0.85 g (81%) of *N*-Boc taurine chloride **11** as a white powder. $R_f = 0.53$ (EtOAc/hexane 1:1, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.99−3.04 (m, 2H), 3.07–3.14 (m, 2H), 4.34 (bs, 1H). 2-(2,4,5-Trifluorophenyl)ethanol **15** (0.54 g, 3.07 mmol, 1.0 equiv) and triethylamine (0.47 mL, 3.38 mmol, 1.1 equiv) were dissolved in CH_2Cl_2 (1.2 mL) and cooled in an ice bath. *N*-Boc taurine chloride **11** (1.12 g, 4.61 mmol, 1.5 equiv) in CH_2Cl_2 (8 mL) was then added dropwise. After the addition, the cooling bath was removed and the mixture was stirred at rt for 2 h. Then, the mixture was diluted with CH_2Cl_2 (8 mL), washed with saturated aqueous NH_4Cl (12.5 mL), H_2O (12.5 mL) and brine (12.5 mL), and then dried and evaporated. The crude product was purified by column chromatography using hexane/EtOAc (1:1, v/v) as eluent to give 0.92 g (78%) of *N*-Boc taurine 2-(2,4,5-trifluorophenyl)ethyl ester (**12**). R_f = 0.72 ($CHCl_3$/MeOH 98:2, v/v), 0.84 (EtOAc/hexane 1:1, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.44 (s, 9H), 3.05 (t, 2H, $J = 7.50$ Hz), 3.27 (t, 2H, $J = 7.50$ Hz), 3.56 (q, 2H, $J = 7.50$ Hz), 4.40 (t, 2H, $J = 7.50$ Hz), 5.05 (bs, 1H), 6.89–6.99 (m, 1H), 7.05–7.15 (m, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 26.47, 26.98, 33.50, 48.45, 66.34, 78.33, 103.49–104.28 (m), 116.89–117.28 (m), 153.78. Compound **12** (0.9 g, 2.35 mmol) was treated with a solution of 4 M HCl/dioxane (47 mL) and stirred at rt for 1 h. The mixture was concentrated under reduced pressure, washed with petroleum ether (20 mL) and filtered, affording 0.68 g (91%) of hydrochloride of taurine 2-(2,4,5-trifluorophenyl)ethyl ester (**8c**) as a white powder. ^{1}H NMR (250 MHz, CD_3OD) δ 3.02 (bs, 2H), 3.28 (bs, 2H), 3.58 (bs, 2H), 4.43 (bs, 2H), 7.00–7.10 (m, 1H), 7.17–7.30 (m, 1H); ^{13}C NMR (62.9 MHz,

CD_3OD) δ 29.27; 35.52, 47.21, 71.16, 106.48 (dd, $J = 21.30$ Hz, $J = 28.90$ Hz), 120.20 (dd, $J = 5.30$ Hz, $J = 19.60$ Hz), 121.71 (dt, $J = 4.80$ Hz, $J = 17.90$ Hz), 148.25 (dd, $J = 12.20$ Hz, $J = 242.30$ Hz), 150.54 (dt, $J = 13.30$ Hz, $J = 248.60$ Hz), 157.51 (dd, $J = 9.70$ Hz, $J = 244.40$ Hz); HRMS *m/z* calcd for $C_{10}H_{13}NO_3F_3S$ $[M+H]^+$ 284.0568, found 284.0566.

2-(2,4,5-trifluorophenyl)ethanol (15)

To a suspension of 2,4,5-trifluorophenylacetic acid (**13**, 5 g, 26.3 mmol, 1 equiv) in anhydrous methanol (11 mL), 2,2-dimethoxypropane (42 mL) and TMS-Cl (0.33 mL, 2.6 mmol, 0.1 equiv) were added. After stirring at rt for 24 h, the mixture was concentrated under reduced pressure and then purified by gravity column chromatography on silica gel using $CHCl_3$ as eluent. 5.26 g (98%) of 2,4,5-trifluorophenylacetic acid methyl ester (**14**) was obtained as a transparent liquid. TLC $R_f = 0.68$ (hexane/AcOEt, 3:1 v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.62 (s, 2H), 3.72 (s, 3H), 6.88–6.99 (m, 1H), 7.07–7.17 (m, 1H). Compound **14** (5.0 g, 24.5 mmol, 1 equiv) was dissolved in THF (88 mL) and sodium borohydride powder (5.5 g, 0.15 mol, 6 equiv) was added. The suspension was stirred at 65°C for 15 min and then methanol (88 mL) was added dropwise. The mixture was refluxed for 2.5 h. After cooling to rt, the reaction was quenched by 2 N aq. HCl (4.9 mL). The mixture was concentrated under reduced pressure and extracted with EtOAc (3 × 100 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by gravity column chromatography ($CHCl_3$), affording 3.7 g (86%) of 2-(2,4,5-trifluorophenyl)ethanol (**15**) as a brownish viscous liquid. TLC $R_f = 0.38$ (hexane/AcOEt, 2:1 v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.85 (t, $J = 7.50$ Hz, 2H), 3.85 (t, $J = 7.50$ Hz, 2H), 6.85–6.96 (m, 1H), 7.04–7.14 (m, 1H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 31.75, 61.99, 105.34 (dd, $J = 20.60$ Hz, $J = 28.30$ Hz), 118.74 (dd, $J = 6.20$ Hz, $J = 19.00$ Hz), 121.93 (dt, $J = 4.80$ Hz, $J = 18.70$ Hz), 146.63 (ddd, $J = 3.20$ Hz, $J = 11.80$ Hz, $J = 244.30$ Hz), 148.70 (dt, $J = 13.70$ Hz, $J = 249.20$ Hz), 156.11 (ddd, $J = 2.50$ Hz, $J = 8.90$ Hz, $J = 243.90$ Hz).

General Procedure for the Synthesis of 5-formyl-2′,3′-*O*-isopropylidene(-2-thio)uridine (25/26)

Nucleoside **23**[27]/**24**[28] (9.5 mmol, 1 equiv) was dissolved in acetone/CH_2Cl_2 (4:1, v/v; 80 mL), then activated MnO_2 (14.8 g, 0.17 mol, 17.5 equiv) was added. After being stirred for 2 h (**23**)/7 h (**24**) at 55°C, the mixture was cooled to rt, and MnO_2 was centrifuged. The solution was transferred to a fresh tube and the residual MnO_2 was resuspended in a mixture of acetone/CH_2Cl_2 (1:1, v/v; 3 × 40 mL), shaken vigorously, and

centrifuged. The combined solutions were filtered through Celit. The filtrate was concentrated under reduced pressure and the solid residue was purified by column chromatography using MeOH in $CHCl_3$.

5-formyl-2′,3′-*O*-isopropylideneuridine (25)

Compound **25** was isolated in 55% yield using 6% MeOH in $CHCl_3$ as eluent. TLC $R_f = 0.45$ ($CHCl_3$/MeOH, 9:1 v/v); ^{1}H NMR (700 MHz, DMSO-d_6) δ 1.29 (s, 3H), 1.48 (s, 3H), 3.56–3.64 (m, 2H), 4.23–4.25 (m, 1H), 4.75 (dd, 1H, $J = 6.25$ Hz, $J = 3.00$ Hz), 4.94 (dd, 1H, $J = 6.25$ Hz, $J = 2.25$ Hz), 5.16 (t, 1H, $J = 5.00$ Hz), 5.85 (d, 1H, $J = 2.25$ Hz), 8.61 (s, 1H), 9.76 (s, 1H), 11.80 (s, 1H); ^{13}C NMR (176 MHz, DMSO-d_6) δ 24.61, 26.44, 60.63, 80.11, 84.14, 87.28, 92.55, 109.96, 112.11, 147.73, 149.13, 161.21, 185.68; HRMS (FAB$^+$) calcd for $C_{13}H_{17}N_2O_7$ $[M+H]^+$ 313.1036, found 313.1042.

5-formyl-2′,3′-*O*-isopropylidene-2-thiouridine (26)

Compound **26** was isolated in 65% yield using 2% MeOH in $CHCl_3$ as eluent. TLC $R_f = 0.50$ ($CHCl_3$/MeOH, 9:1 v/v); ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.34 (s, 3H), 1.56 (s, 3H), 3.70 (dd, 1H, $J = 3.50$ Hz, $J = 11.90$ Hz), 3.77 (dd, 1H, $J = 2.80$ Hz, $J = 11.90$ Hz), 4.34–4.35 (m,1H), 4.83 (dd, 1H, $J = 3.50$ Hz, $J = 6.30$ Hz), 4.92 (dd, 1H, $J = 2.10$ Hz, $J = 6.30$ Hz), 6.74 (d, 1H, $J = 2.10$ Hz), 8.79 (s, 1H), 9.83 (s, 1H); ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 25.69, 27.45, 60.86, 79.78, 85.64, 87.79, 94.71, 113.28, 114.42, 146.87, 158.55, 177.33, 186.71; HRMS (FAB$^+$) calcd for $C_{13}H_{17}N_2O_6S$ $[M+H]^+$ 329.0807, found 329.0802.

General Procedure for the Synthesis of Compounds 21a-21c/22a-22c

Nucleoside **25**/**26** (4.2 mmol, 1.0 equiv) was suspended in CH_2Cl_2 (22 mL) and a few drops of DMF was added to complete substrate dissolution. Then, hydrochloride of amino acid ester **8a-8c** (5.0 mmol, 1.2 equiv) and Et_3N (702 μL, 5.0 mmol, 1.2 equiv) were added and the mixture was stirred at rt for 1–1.5 h. TLC analysis ($CHCl_3$/MeOH 95:5 or 90:10, v/v) showed full conversion of aldehyde **25**/**26** into suitable imine **27a-27c**/**28a-28c.** The reaction mixture was treated with $NaBH(OAc)_3$ (1.1 g, 5.0 mmol, 1.2 equiv) and stirring continued for another 40 min for compounds **27a**, **27c**, **28a** or for 2 h for **27b**, **28b**, **28c**. The reaction was quenched with 5% aq. $NaHCO_3$ (8 mL) and then extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material **19a-19c**/**20a-20c** (3.5 mmol, 1 equiv) was co-evaporated with anhydrous pyridine, dissolved in the same solvent (59 mL), cooled in ice bath and trifluoroacetic anhydride (2.5 mL, 17.5 mmol, 5.0 equiv) was added dropwise. The reaction mixture was stirred

at 0°C for 1 h, quenched with 5% aq. $NaHCO_3$ (25 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residual pyridine was removed by co-evaporation with anhydrous toluene, and the resulting foam was purified by column chromatography affording product **21a-21c/22a-22c** as rotamers about $-NC(O)CF_3$ amide bond (two chemical shifts were observed for some of the ^1H and ^{13}C NMR resonances; the secondary shifts in ^{13}C NMR spectra are given in parentheses). The yields calculated for **21a-21c/22a-22c** refer to substrate **25** or **26**.

2′,3′-*O*-isopropylidene-*N*-[(1-β-D-ribofuranosyl-1*H*-pyrimidin-5-yl)methyl]-*N*-trifluoroacetylglycine 2-(*p*-nitrophenyl)ethyl ester (21a)

Compound **21a** was purified by column chromatography with $CHCl_3$ as eluent to obtain a white foam in 66% yield. TLC $R_f = 0.52$ ($CHCl_3$/MeOH 95:5, v/v); ^1H NMR (250 MHz, $CDCl_3$) δ 1.36 (s, 3H), 1.58 (s, 3H), 3.08 (t, 2H, $J = 6.50$ Hz), 3.80 (dd, 1H, $J = 2.50$ Hz, $J = 12.00$ Hz), 4.00 (dd, 1H, $J = 2.00$ Hz, $J = 12.00$ Hz), 4.09 (d, 1H, $J = 14.25$ Hz), 4.24 (d, 1H, $J = 14.25$ Hz), 4.40–4.63 (m, 5H), 4.77–4.94 (m, 2H), 5.80 (d, 0.9H, $J = 2.75$ Hz), 5.87 (d, 0.1H, $J = 3.00$ Hz), 7.38 (d, 2H, $J = 8.75$ Hz), 7.80 (s, 0.1H), 8.01 (s, 0.9H), 8.19 (d, 2H, $J = 8.75$ Hz), 8.79 (s, 0.9H), 8.89 (s, 0.1H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 24.94 (25.11), 26.93 (27.04), 34.58, 46.50, 50.45 (49.12), 62.57, 65.00, 80.69 (80.16), 85.11 (84.36), 87.33 (86.27), 94.59 (93.24), 107.44 (107.70), 113.71, 114.53 (q, $J = 104.01$ Hz), 123.72, 129.53, 143.19, 144.65, 146.92, 149.37, 157.92 (q, $J = 36.43$ Hz), 163.93, 167.93; HRMS calcd for $C_{25}H_{26}N_4O_{11}F_3$ $[M\text{-}H]^-$ 615.1550, found 615.1559.

2′,3′-*O*-isopropylidene-*N*-[(1-β-D-ribofuranosyl-1*H*-pyrimidin-5-yl)methyl]-*N*-trifluoroacetylglycine 2-(trimethylsilyl)ethyl ester (21b)

Compound **21b** was purified by column chromatography using 2% MeOH in $CHCl_3$ as eluent to obtain a white foam in 81% yield. TLC $R_f = 0.46$ ($CHCl_3$/MeOH 95:5, v/v); ^1H NMR (250 MHz; $CDCl_3$): δ 0.04–0.06 (m, 9H), 0.98–1.05 (m, 2H), 1.36 (s, 3H), 1.58 (s, 3H), 3.80 (dd, 1H, $J = 2.75$ Hz, $J = 12.25$ Hz), 3.99 (dd, 1H, $J = 2.00$ Hz, $J = 12.25$ Hz), 4.09–4.35 (m, 4H), 4.44–4.62 (m, 3H), 4.86 (dd, 1H, $J = 2.50$ Hz, $J = 6.00$ Hz), 4.92 (dd, 1H, $J = 1.75$ Hz, $J = 6.00$ Hz), 5.79 (d, 0.9H, $J = 2.50$ Hz), 5.91 (d, 0.1H, $J = 2.75$ Hz), 7.85 (s, 0.1H), 8.01 (s, 0.9H), 8.71 (s, 0.9H), 8.80 (s, 0.1H); ^{13}C NMR (176 MHz, $CDCl_3$): δ -1.82 (-1.76), 17.14 (17.07), 24.91 (25.07), 26.89 (27.02), 46.49 (44.59), 50.62 (49.28), 62.46 (62.17), 64.27 (63.76), 80.63 (80.12), 84.93 (84.31), 87.29 (86.25), 94.55 (92.84), 107.68 (107.83), 113.66 (114.12), 115.68 (q, $J = 287.76$ Hz), 143.18, 149.73

(149.64), 157.90 (q, $J = 36.78$ Hz), 163.75 (162.65), 168.21 (167.73); HRMS calcd for $C_{22}H_{33}N_3O_9F_3Si$ $[M+H]^+$ 568.1938, found 568.1937.

2′,3′-*O*-isopropylidene-*N*-[(1-β-D-ribofuranosyl-1*H*-pyrimidin-5-yl)methyl]-*N*-trifluoroacetyltaurine 2-(2,4,5-trifluorophenyl)ethyl ester (21c)

Compound **21c** was purified by column chromatography using 2% of MeOH in $CHCl_3$ as eluent to obtain a white foam in 36% yield. TLC $R_f =$ 0.42 ($CHCl_3$/MeOH 95:5, v/v); ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.35 (s, 3H), 1.58 (s, 3H); 3.03–3.08 (m, 2H), 3.41–3.57 (m, 2H), 3.75–3.83 (m, 1H), 3.91–3.98 (m, 1H), 4.01–4.24 (m, 3H), 4.35–4.47 (m, 4H), 4.77 (dd, 0.27H, $J = 2.80$ Hz, $J = 5.60$ Hz), 4.85 (dd, 0.7H, $J = 2.80$ Hz, $J = 6.30$ Hz), 4.87 (dd, 0.3H, $J = 2.80$ Hz, $J = 5.60$ Hz), 4.91 (dd, 0.7H, $J = 2.80$ Hz, $J = 6.30$ Hz), 5.80 (d, 0.7H, $J = 2.80$ Hz), 5.93 (d, 0.3H, $J = 2.80$ Hz), 6.92–6.96 (m, 1H), 7.07–7.14 (m, 1H), 7.78 (s, 0.3H), 8.04 (s, 0.7H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 25.13 (25.28), 27.12 (27.23), 28.72 (29.68), 43.67 (42.04), 44.91 (45.01), 46.66 (48.67), 62.67 (62.48), 68.56 (68.83), 80.79 (80.34), 85.08 (84.85), 87.38 (86.53), 93.09 (94.63), 105.53–105.68 (m), 107.11 (107.73), 113.98 (114.32), 115.90 (q, $J = 287.16$ Hz), 118.87–119.01 (m), 119.43–119.58 (m), 144.33, 146.74 (ddd, $J = 3.70$ Hz, $J = 12.80$ Hz, $J = 245.21$ Hz), 149.29 (dt, $J = 13.52$ Hz, $J = 251.00$ Hz), 149.89 (149.65), 156.16 (ddd, $J = 2.10$ Hz, $J = 8.90$ Hz, $J = 244.50$ Hz), 158.0 (q, $J = 36.16$ Hz), 163.54 (162.35); HRMS calcd for $C_{25}H_{26}N_3O_{10}F_6S$ $[M-H]^-$ 674.1243, found 674.1250.

2′,3′-*O*-isopropylidene-*N*-[(1-β-D-ribofuranosyl-1*H*-2-thiopyrimidin-5-yl)methyl]-*N*-trifluoroacetylglycine 2-(*p*-nitrophenyl)ethyl ester (22a)

Compound **22a** was purified by column chromatography with $CHCl_3$ to give a light yellow foam in 56% yield. TLC $R_f = 0.64$ ($CHCl_3$/MeOH 95:5, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.37 (s, 3H), 1.61 (s, 3H), 3.07 (t, 2H, $J =$ 5.00 Hz), 3.84–4.26 (m, 4H), 4.41–4.67 (m, 5H), 4.80 (dd, 1H, $J = 2.25$ Hz, $J = 6.00$ Hz), 4.92 (dd, 1H, $J = 2.50$ Hz, $J = 6.00$ Hz), 6.65 (d, 0.9H, $J =$ 2.25 Hz), 6.87 (d, 0.1H, $J = 2.25$ Hz), 7.38 (d, 2H, $J = 8.50$ Hz), 8.12 (d, 2H J $= 8.50$ Hz), 8.33 (s, 1H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 25.33 (25.55), 27.15 (27.35), 34.77 (34.67), 46.66, 50.67 (49.30), 62.25 (61.75), 65.24 (65.30), 80.16 (79.89), 86.10 (85.64), 87.57 (86.31), 96.01 (94.01), 112.05 (112.57), 113.89 (114.57), 115.79 (q, $J = 288.00$ Hz), 123.90, 129.75, 142.91, 144.87, 147.15, 158.10 (q, $J = 37.00$ Hz), 160.83, 168.09 (167.95), 175.06 (174.97); HRMS calcd for $C_{25}H_{26}N_4O_{10}F_3S$ $[M-H]^-$ 631.1322, found 631.1316.

2′,3′-*O*-isopropylidene-*N*-[(1-β-D-ribofuranosyl-1*H*-2-thiopyrimidin-5-yl)methyl]-*N*-trifluoroacetylglycine 2-(trimethylsilyl)ethyl ester (22b)

Compound **22b** was purified by column chromatography using 10% MeOH in $CHCl_3$ to obtain a light yellow foam in 78% yield. TLC R_f = 0.58 ($CHCl_3$/MeOH 95:5, v/v); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.04 (s, 9H), 0.96–1.05 (m, 2H), 1.38 (s, 3H), 1.62 (s, 3H), 3.86 (dd, 1H, J = 2.25 Hz, J = 12.25 Hz), 4.05–4.11 (m, 1H), 4.20–4.30 (m, 4H), 4.42–4.50 (m, 2H), 4.52–4.62 (m, 1H), 4.81 (dd, 1H, J = 6.00 Hz, J = 2.50 Hz), 4.91 (dd, 1H, J = 6.00 Hz, J = 2.50 Hz), 6.65 (d, 0.9H, J = 2.50 Hz), 6.89 (d, 0.1H, J = 2.75 Hz), 8.16 (s, 0.1H), 8.33 (s, 0.9H), 9.56 (bs, 1H); ^{13}C NMR (176 MHz, $CDCl_3$) δ -1.56, 17.40, 25.31 (25.54), 27.16 (27.35), 46.73, 50.88, 62.28, 64.63 (64.71), 80.23 (79.18), 86.13 (85.61), 87.62 (86.32), 96.19, 112.21 (112.66), 113.83 (114.57), 115.87 (q, J = 286.88 Hz), 142.70, 158.31 (q, J = 36.00 Hz), 160.22, 168.27, 168.68, 175.03; HRMS calcd for $C_{22}H_{33}N_3O_8F_3SiS$ 584.1709, found 584.1709.

2′,3′-*O*-isopropylidene-*N*-[(1-β-D-ribofuranosyl-1*H*-2-thiopyrimidin-5-yl)methyl]-*N*-trifluoroacetyltaurine 2-(2,4,5-trifluorophenyl)ethyl ester (22c)

Compound **22c** was purified by column chromatography using 2% MeOH in $CHCl_3$ as eluent to obtain a light yellow foam in 32% yield. TLC R_f = 0.66 ($CHCl_3$/MeOH 95:5, v/v); ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.61 (s, 3H), 1.85 (s, 3H), 3.15–3.20 (m, 2H), 3.50–4.21 (m, 4H), 4.00–4.58 (m, 7H), 4.74 (dd, 0.4H, J = 2.80 Hz, J = 5.60 Hz), 4.86 (dd, 0.6H, J = 2.80 Hz, J = 6.30), 4.92 (dd, 0.4H, J = 2.80 Hz, J = 5.60 Hz), 4.98 (dd, 0.6H, J = 2.80 Hz, J = 6.30 Hz), 6.80 (d, 0.6H, J = 2.80 Hz), 6.95 (d, 0.4H, J = 2.80 Hz), 7.00–7.03 (m, 1H), 7.16–7.21 (m, 1H), 8.22 (s, 0.4H); 8.41 (s, 0.6H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 25.36 (25.53), 27.18 (27.35), 28.71 (29.69), 42.53 (43.58), 44.69 (45.32), 48.59 (46.59), 62.10 (61.70), 68.62 (68.92), 79.99 (79.19), 85.82 (85.70), 87.24 (86.47), 95.44 (94.07), 105.56–105.87 (m), 111.71 (112.68), 114.06 (114.43), 115.92 (q, J = 287.00 Hz), 118.83–119.01 (m), 138.91, 143.48, 145.97–150.09 (m), 155.38–155.55 (m), 156.77–156.96 (m), 158.01 (q, J = 36.00 Hz), 160.65 (159.28), 175.72 (175.10); HRMS calcd for $C_{25}H_{27}N_3O_9F_6NaS_2$ $[M+Na]^+$ 714.0991, found 714.0985.

General Procedure for the Synthesis of Compounds 29a-29c/30a-30c

Nucleoside **21a-21c**/**22a-22c** (1 mmol) was dissolved in 25% aq. acetic acid (20 mL). After being stirred for 1 h at 90°C the mixture was cooled to rt and then concentrated under reduced pressure. The solid residue was

co-evaporated with anhydrous toluene and purified by column chromatography using MeOH in $CHCl_3$. The resulting product **29a-29c/30a-30c** exist as rotamers about $-NC(O)CF_3$ amide bond (two chemical shifts were observed for some of the ^1H and ^{13}C NMR resonances; the secondary shifts in ^{13}C NMR spectra are given in parentheses).

N-[(1-β-D-ribofuranosyl-1*H*-pyrimidin-5-yl)methyl]-*N*-trifluoroacetylglycine 2-(*p*-nitrophenyl)ethyl ester (29a)

Compound **29a** was purified by column chromatography using 10% MeOH in $CHCl_3$ as eluent to obtain a white foam in 85% yield. TLC R_f = 0.33 ($CHCl_3$/MeOH 90:10, v/v); ^1H NMR (700 MHz, C_6D_5N) δ 2.97 (t, 1H, J = 7.00 Hz), 3.03 (t, 1H, J = 6.30 Hz), 4.23–4.52 (m, 4H), 4.61–4.99 (m, 9H), 6.84 (d, 0.5H, J = 4.20 Hz), 6.86 (d, 0.5H, J = 2.80 Hz), 7.13 (bs, 1H), 7.36 (d, 1H, J = 8.40 Hz), 7.40 (d, 1H, J = 8.40 Hz), 8.17 (d, 1H, J = 8.40 Hz), 8.21 (d, 1H, J = 8.40 Hz), 8.86 (s, 0.5H), 8.97 (s, 0.5H); ^{13}C NMR (176 MHz, C_6D_5N) δ 34.48 (34.45), 46.42 (45.57), 48.89 (50.12), 60.99 (61.60), 64.93 (65.21), 71.09 (70.59), 76.39 (76.02), 86.22 (86.01), 90.30, 108.31 (107.99), 116.45 (q, J = 288.82 Hz) [116.65 (q, J = 288.80 Hz)], 129.95, 130.00, 140.08, 142.26, 145.80 (145.88), 146.97 (146.91), 151.45 (151.49), 156.95–157.78 (m), (164.06) 164.72, (168.19) 168.79; HRMS calcd for $C_{22}H_{23}N_4O_{11}F_3Na$ $[M+Na]^+$ 599.1213, found 599.1215.

N-[(1-β-D-ribofuranosyl-1*H*-pyrimidin-5-yl)methyl]-*N*-trifluoroacetylglycine 2-(trimethylsilyl)ethyl Ester (29b)

Compound **29b** was purified by column chromatography using 6% MeOH in $CHCl_3$ as eluent to obtain a white foam in 81% yield. TLC R_f = 0.38 ($CHCl_3$/MeOH 90:10, v/v); ^1H NMR (250 MHz, $CDCl_3$) δ 0,03 (s, 2H), 0.04 (s, 7H), 0.97–1.04 (m, 2H), 3.47 (bs, 1H), 3.64–4.55 (m, 12H), 4.80 (bs, 1H), 5.79 (d, 0.8H, J = 3.75 Hz), 5.84 (bs, 0.2H), 8.03 (s, 0.2H), 8.17 (s, 0.8H), 9.91 (bs, 0.9H); ^{13}C NMR (176 MHz, $CDCl_3$) δ -0.66, 18.28 (19.27), 47.36 (45.99), 51.56 (50.17), 62.61 (62.13), 65.54 (65.47), 70.84 (71.49), 75.96 (76.16), 86.36 (85.95), 91.95 (91.23), 109.69, 116.89 (q, J = 287.80 Hz), 143.51, 152.08 (151.95), 158.87 (q, J = 36.08 Hz), 164.97 (164.06), 169.57 (169.03); HRMS calcd for $C_{19}H_{28}N_3O_9F_3NaSi$ $[M+Na]^+$ 550.1445, found 550.1446.

N-[(1-β-D-ribofuranosyl-1*H*-pyrimidin-5-yl)methyl]-*N*-trifluoroacetyltaurine 2-(2,4,5-trifluorophenyl)ethyl ester (29c)

Compound **29c** was purified by column chromatography using 8% MeOH in $CHCl_3$ as eluent to obtain a white foam in 75% yield. TLC $R_f = 0.30$ ($CHCl_3$/MeOH 90:10, v/v); ^{1}H NMR (700 MHz, CD_3OD) δ 3.14–3.18 (m, 2H), 3.54–3.93 (m, 5H), 4.02–4.14 (m, 2H), 4.21–4.26 (m, 2H), 4.32–4.39 (m, 1H), 4.46–4.56 (m, 3H), 5.98 (d, 0.5H, J = 4.90 Hz), 6.00 (d, 0.5H, J = 4.20 Hz), 7.19–7.24 (m, 1H), 7.37–7.41 (m, 1H), 8.19 (s, 0.5H), 8.25 (s, 0.5H); ^{13}C NMR (176 MHz, CD_3OD) δ 27.95, 41.86 (42.52), 43.80 (44.70), 45.73, 60.54 (61.11), 69.05 (68.95), 69.86 (70.07), 74.91 (74.48), 85.08 (85.13), 89.42 (89.45), 104.98–105.29 (m), 107.81 (107.89), 113.74–120.53 (m), 139.67, 142.37, 145.89–149.76 (m), 150.73, 156.83–157.47 (m), 163.45 (164.02); HRMS calcd for $C_{22}H_{23}N_3O_{10}F_6NaS$ $[M+Na]^+$ 658.0906, found 658.0901.

N-[(1-β-D-ribofuranosyl-1*H*-2-thiopyrimidin-5-yl)methyl]-*N*-trifluoroacetylglycine 2-(*p*-nitrophenyl)ethyl ester (30a)

Compound **30a** was purified by column chromatography using 5% MeOH in $CHCl_3$ as eluent to obtain a yellow foam in 80% yield. TLC R_f = 0.41 ($CHCl_3$/MeOH 90:10, v/v); ^{1}H NMR (700 MHz, C_6D_5N) δ 2.90–2.99 (m, 2H), 4.16–4.81 (m, 11H), 7.27 (bs, 1H), 7.33 (d, 1H, J = 8.40 Hz), 7.37 (d,1H, J = 8.40 Hz), 8.04 (d, 1H, J = 8.40 Hz), 8.15 (d, 1H, J = 8.40 Hz), 8.90 (s, 0.5H), 9.07 (s, 0.5H); ^{13}C NMR (176 MHz, C_6D_5N) δ 34.50 (34.46), 46.64 (45.86), 49.15 (50.24), 60.56 (59.95), 65.22 (65.52), 69.48 (69.87), 69.48 (69.87), 76.32 (76.44), 85.66 (85.85), 94.65 (94.73), 112.97 (113.02), 116.52 (q, J = 279.65 Hz) [116.82 (q, J = 288.11 Hz)], 130.14, 130.20, 139.91, 142.06, 146.06 (146.11), 146.86 (146.91), 149.16, 157.40 (q, J = 31.15 Hz) [157.95 (q, J = 36.12 Hz)], 161.59 (160.96), 168.92 (168.34), 176.58 (176.44); HRMS calcd for $C_{22}H_{23}N_4O_{10}F_3NaS$ $[M+Na]^+$ 615.0985, found 615.0978.

N-[(1-β-D-ribofuranosyl-1*H*-2-thiopyrimidin-5-yl)methyl]-*N*-trifluoroacetylglycine 2-(trimethylsilyl)ethyl ester (30b)

Compound **30b** was purified by column chromatography using 5% MeOH in $CHCl_3$ as eluent to obtain a light yellow foam in 85% yield. TLC R_f = 0.24 ($CHCl_3$/MeOH 95:5, v/v); ^{1}H NMR (700 MHz, CD_3OD) δ 0,05 (s, 3.3H), 0.06 (s, 5.7H), 0.99–1.02 (m, 2H), 3.48–4.27 (m, 9H), 4.39–4.70 (m, 3H), 6.58 (d, 0.4H, J = 2.10 Hz), 6.59 (d, 0.6H, J = 2.10 Hz), 8.38 (s, 0.4H),

8.46 (s, 0.6H); ^{13}C NMR (176 MHz, CD_3OD) δ -1.56, 18.22, 47.31, 51.38, 61.51 (60.82), 65.37 (65.06), 70.28 (69.86), 76.73, 86.02, 95.16, 113.86 (113.76), 115.12–120.02 (m), 143.108 (142.78), 158.48–159.20 (m), 162.40 (161.74), 170.24 (169.68), 177.28 (177.16); HRMS calcd for $C_{19}H_{28}N_3O_8F_3NaSiS$ $[M+Na]^+$ 566.1216, found 566.1213.

N-[(1-β-D-ribofuranosyl-1*H*-2-thiopyrimidin-5-yl)methyl]-*N*-trifluoroacetyltaurine 2-(2,4,5-trifluorophenyl)ethyl ester (30c)

Compound **30c** was purified by column chromatography using 10% MeOH in $CHCl_3$ as eluent to obtain a light yellow foam in 80% yield. TLC $R_f = 0.46$ ($CHCl_3$/MeOH 90:10, v/v); ^{1}H NMR (700 MHz, CD_3OD) δ 3.14–3.17 (m, 2H), 3.55–3.59 (m, 2H), 3.82–4.16 (m, 5H), 4.19–4.22 (m, 1H), 4.27–4.29 (m, 1H), 4.33–4.58 (m, 4H), 6.66 (d, 0.6H, $J = 2.10$ Hz), 6.68 (d, 0.4H, $J = 2.80$ Hz), 7.19–7.24 (m, 1H), 7.36–7.41 (m, 1H), 8.44 (s, 0.6H), 8.52 (s, 0.4H); ^{13}C NMR (176 MHz, CD_3OD) δ 27.96, 42.15 (42.67), 44.12, 45.73 (44.95), 59.33 (60.16), 68.43 (68.91), 69.06 (68.95), 75.39 (75.25), 84.58 (84.64), 93.77 (93.72), 104.98–105.27 (m), 112.18 (111.88), 116.02 (q, $J = 287.00$ Hz), 118.61–120.55 (m), 139.17, 142.04, 145.87–149.84 (m), 155.58–157.57 (m), 160.16 (160.78), 175.72 (175.89); HRMS calcd for $C_{22}H_{23}N_3O_9F_6NaS_2$ $[M+Na]^+$ 674.0678, found 674.0681.

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