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Stereoselective facile synthesis of 2′-spiro pyrimidine pyranonucleosides via their key intermediate 2′-*C*-cyano analogues. Evaluation of their bioactivity

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ABSTRACT

A novel series of 2′-spiro pyrimidine pyranonucleosides has been designed and synthesized. Their precursors, 2′-*C*-cyano nucleosides **5a**,**b** and **6a**,**b**, were obtained by subjecting **1a**,**b** to the sequence of selective protection of the primary hydroxyl group, acetalation, oxidation, and finally treatment with sodium cyanide. Deoxygenation at the 2′-position of cyanohydrins **5a**,**b** or **6a**,**b** led to the 2′-deoxy derivatives **9a**,**b**. Fully deprotection of **5a**,**b**, **6a**,**b**, and **9a**,**b** gave the desired 2′-*C*-cyano **7a**,**b**, **8a**,**b,** and 2′-*C*-cyano-2′-deoxy pyranonucleosides **10a**,**b**, respectively. Mesylation of the corresponding cyanohydrins **5a**,**b** and **6a**,**b** afforded compounds **11a**,**b** and **12a**,**b** which after base treatment and subsequent deprotection furnished the spiro nucleosides **15a**,**b** and **16a**. The new analogues were evaluated for their potential cytostatic activities in cell culture.

Nucleoside analogues have long played a pivotal role in the treatment of viral infections and cancer.[1–4] These therapeutic compounds mimic physiological nucleosides in terms of uptake and metabolism and are incorporated into newly synthesized DNA, resulting in synthesis inhibition and DNA chain termination.[5] However, their clinical use is often limited by important side-effects and natural or acquired drug resistance, thus there is an ongoing medical need for more effective, selective, and non-toxic derivatives.

Nucleosides containing sugar-ring modifications[6,7] are recognized as an important class of biologically active molecules. Particularly, branched-chain cyano furanonucleosides have drawn much attention from medicinal chemists due to their antiviral and anticancer properties[8,9] and their potential to provide an access to the synthesis of branched-chain spiro derivatives endowed with potent anti-HIV properties.[10,11] Spirobicyclic cores display an important role in the development of new bioactive substances,[12] while a number of spiro nucleosides, as conformationally restricted analogues, exhibited interesting antiviral activities.[13–15]

Pyranosyl nucleosides are undoubtedly one of the most important modifications of natural nucleosides, offering promising avenues in the development of potential antitumor,[16,17] antiviral,[18,19] antioxidant,[20] and antimicrobial[21] agents. In our previous investigations, we demonstrated that insertion of the cyano functional group at the 3′-*C*- or 4′-*C*-position of the pyranose ring, leads to derivatives endowed with significant cytostatic activity against different cancer cell lines.[22,23]

With the above applications in mind and in order to: (i) better understand the cyano substituent position effect on the properties of this class of analogues and (ii) promote an efficient way to pharmacologically active spiro branched-chain nucleosides, we found it of particular interest to further extend our studies toward the preparation of 2′-*C*-cyano pyrimidine pyranonucleosides as potential useful synthons for 2′-spiro pyranonucleosides bearing a 4-amino-1,2-oxathiole-2,2-dioxide ring. The chemical synthesis and biological activity of these compounds are presented herein.

Our synthetic approach was focused on the preparation of 2′-*C*-cyano pyranonucleosides of uracil **5a**,**6a** and 5-fluorouracil **5b**,**6b** as key intermediates (Scheme 1). Commercially available 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose was condensed with the proper pyrimidine base to give, after deacetylation, the β-galactopyranonucleosides of uracil **1a**[24] and 5-fluorouracil **1b**.[25] Selective protection of the primary hydroxyl group of **1a** and **1b** with *t*-butyldimethylsilyl chloride (TBDMSCl) followed by acetalation,

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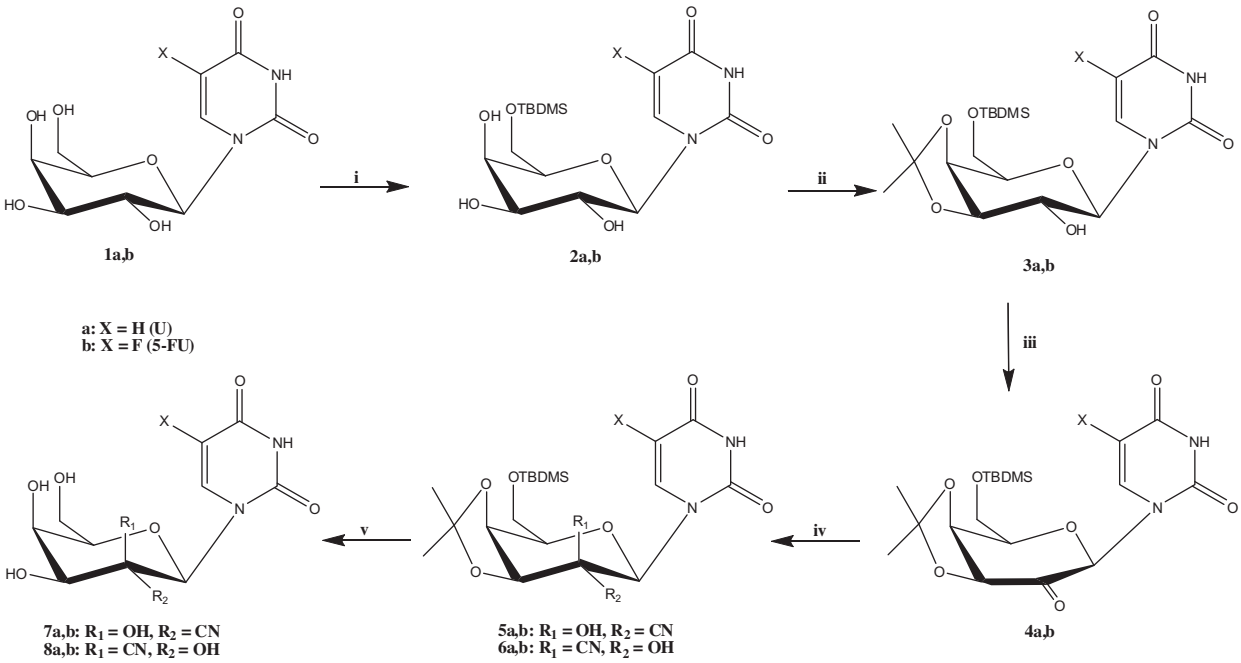


Scheme 1. Reagents and conditions: (i) TBDMSCl, pyridine, DMAP, **2a**: 85%, **2b**: 83%; (ii) acetone, 2,2-dimethoxypropane, *p*-toluenesulfonic acid, **3a**: 86%, **3b**: 85%; (iii) PDC, Ac_2O, dry CH_2Cl_2, **4a**: 80%, **4b**: 83%; (iv) H_2O, Et_2O, NaCN, $NaHCO_3$, **5a**: 28% and **6a**: 52%, **5b**: 25% and **6b**: 56%; (v) TFA 90% in MeOH, **7a**: 74%, **7b**: 74%, **8a**: 79%, **8b**: 78%.

furnished the partially protected derivatives **3a**,**b**, which upon oxidation[26] gave the 2′-ketonucleosides **4a**,**b**. Reaction of **4a**,**b** with sodium cyanide (NaCN),[23] afforded a chromatographically separable mixture of the two possible 2′-cyanohydrin epimers **5a**,**b** and **6a**,**b** in an approximate 1:2 ratio, respectively, contrary to previously published stereospecific preparation of 3′-*C*- and 4′-*C*-cyano pyranonucleosides.[22,23] The proposed structures of compounds **5a**,**b** and **6a**,**b** were established by comparison of their ^{1}H NMR spectra and were assigned as talopyranosyl and galactopyranosyl analogues, respectively, based on the deshielding effect of the adjacent cyano group[27–29] over H-1′ which appeared at lower field in **5a**,**b** than in **6a**,**b** ($\Delta\delta$ = 0.42 and 0.31 ppm, respectively). This suggests that H-1′ and the cyano group in **5a**,**b** are on the same side of the plane. The stereochemistry of C-2′ was further confirmed by NOE measurements performed on pyranosyl analogue **6b**, as depicted in Figure 1. The mutual NOE enhancements observed between the free OH group of galactopyranosyl derivative **6b** and H-1′, show that these protons are on the same side of the ring system. In contrast, no effect was observed between the 2′-OH hydroxyl proton and the H-1′ on talopyranosyl analogue **5b**, when either 2′-OH group or H-1′ was irradiated. Finally, deprotection of analogues **5a**,**b** and **6a**,**b** using trifluoroacetic acid (TFA) gave the desired nucleosides **7a**,**b** and **8a**,**b**, in good yields (74–79%).

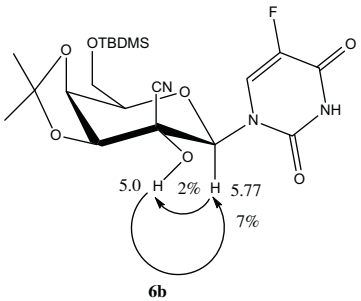
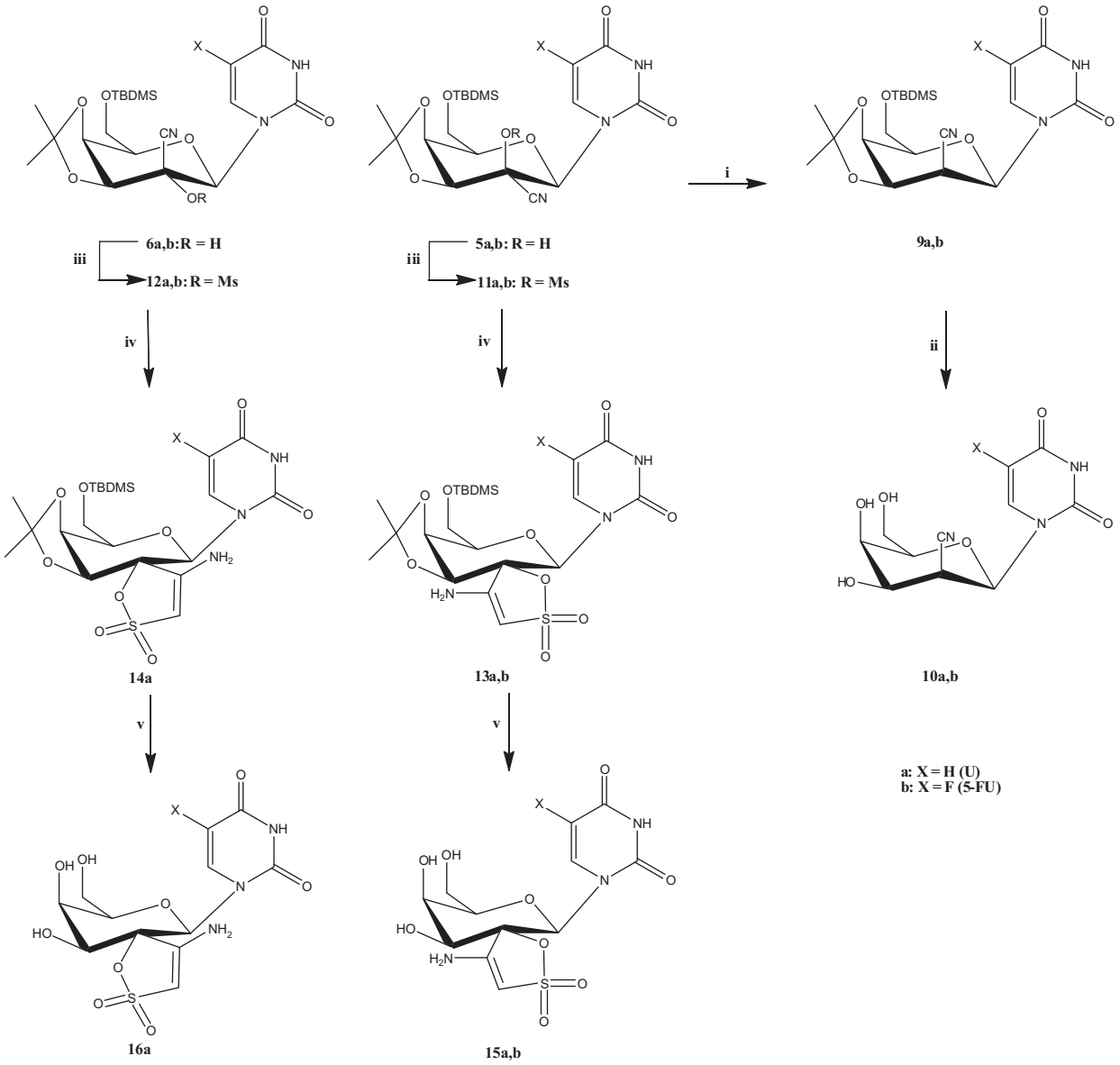


Figure 1. NOEs observed for compound **6b**.

In an effort to further explore structure–activity relationships of this series of compounds, the synthesis of the 2′-*C*-cyano-2′-deoxy derivatives **10a**,**b** was also accomplished (Scheme 2). Phenoxythiocarbonylation of **5a**,**b** or **6a**,**b**, followed by deoxygenation with tris(trimethylsilyl)silane [$(Me_3Si)_3SiH$][30] in the presence of 2,2′-azobis(isobutyronitrile) (AIBN), and subsequent deprotection with TFA led to the formation of the 2′-*C*-cyano-2′-deoxy derivatives **10a**,**b**. ^{1}H NMR data obtained for the newly synthesized nucleosides **10a**,**b** ($J_{1',2'}$ = 2.6 and 1.7 Hz, respectively) revealed an axial/equatorial relationship between H-1′ and H-2′, and thus an axial-oriented cyano group. This demonstrated that deoxygenation of 2′-*C*-cyanohydrins occurs in a stereospecific manner, in accordance with the approach of the hydrogen atom from the less hindered side of the molecule.[22,23]

Finally, the cyanohydrins **5a**,**b** and **6a**,**b** were used as starting materials for the synthesis of the desired 2′-spiro pyrimidine pyranonucleosides **13**–**16**, as outlined in Scheme 2. Their reaction with mesyl chloride (MsCl) in pyridine furnished the corresponding 2′-*C*-cyano-2′-*O*-mesyl derivatives **11a**,**b** and **12a**,**b**. Subsequent treatment of the cyanomesyl nucleosides **11a**,**b** and **12a** with 8-diazabicycloundec-7-ene (DBU)[31] in the presence of acetonitrile (CH_3CN) furnished the 2′-spiro derivatives **13a**,**b** and **14a**, respectively, in fair to good yields (60–74%), while only decomposition products were recovered upon treatment of 5-fluorouracil analogue **12b** either with DBU or cesium carbonate. The cyclization step of spiro derivatives **13a**,**b** and **14a** most likely involves abstraction of the acidic proton from the mesylate group, followed by nucleophilic attack of the resulting carbanion at the nitrile carbon atom.[31,32] The formation of the spiroaminooxathiole dioxide ring in the aforementioned compounds was evident by the disappearance in their ^{1}H NMR spectra of the characteristic signal at 3.28–3.24 ppm assigned to the mesyl group and the presence of two new singlets at 6.02–5.35 ppm corresponding to NH_2-4″ and at 6.04–5.43 ppm assigned to H-3″. In agreement, the IR spectra of the newly synthesised compounds **13a**,**b** and **14a**, exhibited characteristic absorption bands at 3320–3455 cm^{-1} assigned to the stretching vibrations of the NH_2 group. In the final step, deprotection of **13a**,**b** and **14a** under mild acidic conditions,



Scheme 2. Reagents and conditions: (i) phenyl chlorothionoformate, Et_3N, DMAP, CH_3CN, 0 °C; then $(Me_3Si)_3SiH$, AIBN, toluene, 100 °C, **9a**: 70% from **5a** or 67% from **6a**, **9b**: 64% from **5b** or 67% from **6b**, (two steps); (ii) TFA 90% in MeOH, **10a**: 74%, **10b**: 79%; (iii) MsCl, pyridine, **11a**: 69%, **11b**: 68%, **12a**: 69%, **12b**: 59%; (iv) DBU, CH_3CN, **13a**: 60%, **13b**: 74%, **14a**: 70%; (v) CH_2Cl_2, methanolic hydrogen chloride, **15a**: 58%, **15b**: 59%, **16a**: 67%.

performed by methanolic hydrogen chloride,[33,34] resulted in removal of both silyl and isopropylidene groups to deliver the target nucleosides **15a**,**b** and **16a**, respectively.

The cytostatic activity of 2′-*C*-cyano **7a**,**b**, **8a**,**b**, 2′-*C*-cyano-2′-deoxy **10a**,**b**, 2′-spiro pyranonucleosides **13a**,**b**, **14a** and their unprotected derivatives **15a**,**b**, **16a** was determined against murine leukemia L1210, human $CD4^+$ T-lymphocyte (CEM) and human cervix carcinoma HeLa cell cultures (Table 1). Whereas the uracil derivatives (a-series of compounds) showed rather poor if any cytostatic activity, several 5-fluorouracil derivatives (b-series) showed pronounced antiproliferative activity. In particular, the 2′-*C*-cyano (up or down)-2′-hydroxy derivatives **7b** and **8b** showed a cytostatic activity profile identical to that of the free base 5-fluorouracil (5-FU). The three other 5-FU derivatives, that is, the 2′-*C*-cyano(up)-2′-deoxynucleoside analogue **10b** and the 2′-spironucleoside analogues **13b** and **15b** were devoid of significant cytostatic activity (Table 1). The pronounced cytostatic activity of **7b** and **8b** can be due to intrinsic instability of the compounds (spontaneously releasing 5-FU upon cell culture exposure)

Table 1
Cytostatic activity of test compounds against tumor cell proliferation in cell culture

Compound	IC_{50}[a] (μM)		
	L1210	CEM	HeLa
7a	>250	>250	>250
8a	>250	>250	>250
10a	>250	>250	>250
13a	30 ± 10	22 ± 1	28 ± 12
14a	111 ± 3	110 ± 2	87 ± 2
15a	>250	>250	>250
16a	>250	>250	⩾250
7b	0.79 ± 0.45	16 ± 4	0.60 ± 0.04
8b	0.68 ± 0.11	20 ± 6	0.65 ± 0.23
10b	34 ± 1	112 ± 6	22 ± 2
13b	23 ± 2	24 ± 1	16 ± 5
15b	88 ± 13	>250	49 ± 6
F-Uracil	0.33 ± 0.17	18 ± 5	0.54 ± 0.12

[a] 50% inhibitory concentration.

and/or to enzymatic cleavage by serum proteins and/or intracellular enzymes. The presence of a TBDMS group at the 6′-position of the molecules (i.e., **13a**, **13b**) seems to provoke an increased cytostatic activity of the compounds.

In conclusion, we have accomplished the stereoselective synthesis of 2′-*C*-cyano pyrananonucleosides bearing 5-fluorouracil and uracil, along with the preparation of their 2′-deoxy derivatives. We further utilized the cyanohydrins as excellent templates for the synthesis of novel 2′-spiro nucleosides, via a convenient strategy, involving the intramolecular cyclocondensation of their corresponding cyanomesylates. The target nucleosides were tested for their inhibitory effects on the proliferation of murine leukemia (L1210), human lymphocyte (CEM), and human cervix carcinoma (HeLa) cell cultures. Several 5-fluorouracil derivatives showed a pronounced cytostatic activity, comparable to the parent compound, 5-FU.

1. Experimental

1.1. General procedure

Melting points were recorded in a Mel-Temp apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on Merck precoated $60F_{254}$ plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by charring with sulfuric acid. Flash chromatography was performed using silica gel (240–400 mesh, Merck). Microanalyses were performed on a Perkin–Elmer 2400-II Analyzer. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker AVANCE^{III} 300 spectrometer and ^{13}C NMR spectra at 75.5 MHz on the same spectrometer, using chloroform-*d* ($CDCl_3$), dimethyl sulfoxide-d_6 (DMSO-d_6). Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (TMS) as internal standard. UV–Vis spectra were recorded on a PG T70 UV–Vis spectrometer and mass spectra were obtained on a Thermo Quest Finnigan AQA Mass Spectrometer (electrospray ionization). Optical rotations were measured using an Autopol I polarimeter. Infrared spectra were obtained with a Thermo Scientific Nicolet IR100 FT-IR spectrometer. Dichloromethane (CH_2Cl_2) was distilled from phosphorus pentoxide and stored over 4 Å molecular sieves. CH_3CN and toluene were distilled from calcium hydride and stored over 3 Å molecular sieves. Pyridine was stored over potassium hydroxide pellets. All reactions sensitive to oxygen or moisture were carried out under nitrogen atmosphere using oven-dried glassware.

1.2. Synthesis of 1-(2′-*C*-cyano-β-D-talopyranosyl)uracil (7a) and 1-(2′-*C*-cyano-β-D-galactopyranosyl)uracil (8a)

1.2.1. 1-(6′-*O*-*t*-Butyldimethylsilyl-β-D-galactopyranosyl)uracil (2a)

To a stirred solution of **1a** (4.11 g, 15.0 mmol) in pyridine (75.0 mL) were added successively TBDMSCl (3.39 g, 22.5 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction mixture was stirred for 30 min at 0 °C under nitrogen and then at room temperature for 5 h. After, the reaction mixture was quenched with MeOH and evaporated under reduced pressure. The resulting residue was purified by flash chromatography (EtOAc–MeOH, 9:1), to give **2a** (4.97 g, 85%) as a colorless oil. $[\alpha]_D^{22}$ +2 (*c* 0.2, MeOH); R_f = 0.52 (EtOAc–MeOH, 8:2); UV (MeOH): λ_{max} 258 nm (ε 7286); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.07 (br s, 1H, NH), 7.63 (d, 1H, $J_{5,6}$ = 8.1 Hz, H-6), 5.68 (d, 1H, H-5), 5.34 (d, 1H, $J_{1',2'}$ = 9.1 Hz, H-1′), 5.27 (d, 1H, *J* = 4.5 Hz, OH), 5.01 (s, 1H, OH), 4.58 (d, 1H, *J* = 6.1 Hz, OH), 3.76–3.49 (m, 6H, H-2′, H-3′, H-4′, H-5′, H-6a′,b′), 0.83 (s, 9H, *t*-Bu), 0.02, 0.01 (2s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 163.3, 151.1, 141.4, 102.2, 83.2, 78.2, 75.9, 74.0, 68.7, 62.7, 26.1, 18.3, −4.9. Anal. Calcd for $C_{16}H_{28}N_2O_7Si$: C, 49.47; H, 7.26; N, 7.21. Found: C, 49.71; H, 7.43; N, 7.02. ESIMS *m*/*z* 389.18 [M+H$^+$].

1.2.2. 1-(6′-*O*-*t*-Butyldimethylsilyl-3′,4′-*O*-isopropylidene-β-D-galactopyranosyl)uracil (3a)

To a stirred suspension of **2a** (4.97 g, 12.8 mmol) in anhydrous acetone (226.0 mL) and 2,2-dimethoxypropane (12.7 mL) was added *p*-toluenesulfonic acid monohydrate (0.46 g, 2.4 mmol). After 3 h the resulting solution was neutralized with triethylamine (Et_3N) so pH did not exceed 7. The solution was concentrated and the residue was purified by flash chromatography (CH_2Cl_2–MeOH, 9.5:0.5), to give **3a** (4.72 g, 86%) as a white solid, mp 205–207 °C. $[\alpha]_D^{22}$ +42 (*c* 0.6, $CDCl_3$); R_f = 0.47 (CH_2Cl_2–MeOH, 9:1); UV ($CDCl_3$): λ_{max} 259 nm (ε 4831); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.43 (br s, 1H, NH), 7.41 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 5.74 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.57 (d, 1H, H-5), 4.32–4.26 (m, 2H, H-3′, H-4′), 4.07 (dt, 1H, $J_{4',5'}$ = 1.4 Hz, $J_{5',6a'} = J_{5',6b'}$ = 6.4 Hz, H-5′), 3.89–3.78 (m, 2H, H-6a′,b′), 3.72 (dd, 1H, $J_{2',3'}$ = 5.8 Hz, H-2′), 1.53, 1.37 (2s, 6H, 2CH_3), 0.88 (s, 9H, *t*-Bu), 0.06, 0.05 (2s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 163.1, 151.0, 139.9, 110.1, 102.9, 82.9, 78.9, 76.3, 72.9, 71.9, 61.8, 28.0, 25.8, 18.3, −5.3, −5.4. Anal. Calcd for $C_{19}H_{32}N_2O_7Si$: C, 53.25; H, 7.53; N, 6.54. Found: C, 53.32; H, 7.43; N, 6.72. ESIMS *m*/*z* 429.22 [M+H$^+$].

1.2.3. 1-(6′-*O*-*t*-Butyldimethylsilyl-3′,4′-*O*-isopropylidene-β-D-lyxo-hexopyranosyl-2′-ulose)uracil (4a)

A mixture of **3a** (4.72 g, 11.0 mmol), pyridinium dichromate (PDC) (4.93 g, 13.1 mmol) and Ac_2O (3.10 mL, 32.7 mmol) was stirred in dry CH_2Cl_2 (110.0 mL) for 6 h, under nitrogen at room temperature. Purification by flash chromatography (EtOAc–hexane, 5:5) gave **4a** (3.75 g, 80%) as a white solid, mp 153–155 °C. $[\alpha]_D^{22}$ −80 (*c* 1.0, $CDCl_3$); R_f = 0.45 (EtOAc–hexane, 6:4); UV ($CDCl_3$): λ_{max} 257 nm (ε 11296); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.72 (br s, 1H, NH), 7.10 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 6.21 (s, 1H, H-1′), 5.77 (d, 1H, H-5), 4.71–4.67 (m, 2H, H-3′, H-4′), 4.32 (dt, 1H, $J_{4',5'}$ = 0.7 Hz, $J_{5',6a'} = J_{5',6b'}$ = 6.7 Hz, H-5′), 3.89 (d, 2H, H-6a′,b′), 1.45, 1.41 (2s, 6H, 2CH_3), 0.89 (s, 9H, *t*-Bu), 0.08, 0.07 (2s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 198.5, 162.5, 150.2, 141.0, 111.2, 103.0, 82.2, 77.8, 77.1, 75.9, 61.3, 27.4, 25.8, 25.7, 18.3, −5.4, −5.5. Anal. Calcd for $C_{19}H_{30}N_2O_7Si$: C, 53.50; H, 7.09; N, 6.57. Found: C, 53.69; H, 7.13; N, 6.41. ESIMS *m*/*z* 427.21 [M+H$^+$].

1.2.4. 1-(6′-*O*-*t*-Butyldimethylsilyl-2′-*C*-cyano-3′,4′-*O*-isopropylidene-β-D-talopyranosyl)uracil (5a) and 1-(6′-*O*-*t*-butyldimethylsilyl-2′-*C*-cyano-3′,4′-*O*-isopropylidene-β-D-galactopyranosyl)uracil (6a)

A mixture of **4a** (3.75 g, 8.80 mmol), H_2O (130.0 mL), Et_2O (260.0 mL), $NaHCO_3$ (1.48 g, 17.6 mmol) and NaCN (430 mg, 8.80 mmol) was stirred vigorously at room temperature for 72 h. The organic phase was separated, and the aqueous phase was washed with Et_2O. The combined ether phases were dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by flash chromatography (CH_2Cl_2–EtOAc, 7:3).

The fastest moving fractions afforded **5a** (1.12 g, 28%) as a white foam. $[\alpha]_D^{22}$ −18 (*c* 0.6, $CDCl_3$); R_f = 0.30 (CH_2Cl_2–EtOAc, 7:3); UV ($CDCl_3$): λ_{max} 259 nm (ε 9500); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.13 (br s, 1H, NH), 7.52 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 6.54 (br s, 1H, 2′-OH), 6.15 (s, 1H, H-1′), 5.72 (d, 1H, H-5), 4.57–4.53 (m, 2H, H-3′, H-4′), 4.42 (t, 1H, $J_{5',6a'} = J_{5',6b'}$ = 6.5 Hz, H-5′), 3.78 (d, 2H, H-6a′,b′), 1.59, 1.38 (2s, 6H, 2CH_3), 0.89 (s, 9H, *t*-Bu), 0.07, 0.06 (2s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 162.6, 150.1, 141.5, 116.4, 110.7, 102.2, 79.4, 76.1, 74.0, 71.6, 71.2, 62.6, 26.1, 25.7, 24.8, 18.1, −5.4, −5.6. Anal. Calcd for $C_{20}H_{31}N_3O_7Si$: C, 52.96; H, 6.89; N, 9.26. Found: C, 52.88; H, 6.93; N, 9.17. ESIMS *m*/*z* 454.20 [M+H$^+$].

The slowest moving fractions afforded **6a** (2.08 g, 52%) as a white foam. $[\alpha]_D^{22}$ +24 (*c* 0.5, $CDCl_3$); R_f = 0.18 (CH_2Cl_2–EtOAc, 7:3); UV ($CDCl_3$): λ_{max} 258 nm (ε 3883); ^1H NMR ($CDCl_3$, 300 MHz): δ 10.06 (br s, 1H, NH), 7.45 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 6.15 (br s, 1H, 2′-OH), 5.84 (d, 1H, H-5), 5.73 (s, 1H, H-1′), 4.52 (d, 1H, $J_{3',4'}$ = 5.9 Hz, H-3′), 4.43 (dd, 1H, $J_{4',5'}$ = 2.8 Hz, H-4′), 4.28–4.24 (m, 1H, H-5′), 4.01–3.89 (m, 2H, H-6a′,b′), 1.74, 1.39 (2s, 6H, $2CH_3$), 0.89 (s, 9H, *t*-Bu), 0.08, 0.07 (2s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 162.0, 150.7, 141.6, 116.8, 111.2, 102.4, 80.1, 75.7, 73.7, 71.9, 69.8, 62.1, 26.4, 25.9, 24.3, 18.2, −5.4, −5.6. Anal. Calcd for $C_{20}H_{31}N_3O_7Si$: C, 52.96; H, 6.89; N, 9.26. Found: C, 52.80; H, 6.96; N, 9.32. ESIMS *m*/*z* 454.21 [M+H$^+$].

1.2.5. 1-(2′-*C*-Cyano-β-D-talopyranosyl)uracil (7a)

The protected nucleoside **5a** (0.37 g, 0.82 mmol) was dissolved in 4.1 mL of 90% TFA in MeOH. The solution was stirred for 10 min at room temperature and then concentrated under reduced pressure, in order to remove traces of TFA. The residue was purified by flash chromatography (CH_2Cl_2–MeOH, 9:1), to give **7a** (0.18 g, 74%) as a white foam. $[\alpha]_D^{22}$ −6 (*c* 0.1, MeOH); R_f = 0.24 (CH_2Cl_2–MeOH, 8.5:1.5); UV (MeOH): λ_{max} 257 nm (ε 5362); IR (KBr, cm^{-1}): 2225 (CN); ^1H NMR (DMSO-d_6, 300 MHz): δ 11.40 (br s, 1H, NH), 7.66 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 7.58 (s, 1H, H-1′), 6.34 (d, 1H, *J* = 5.2 Hz, OH), 6.24 (s, 1H, OH), 5.63 (d, 1H, H-5), 5.26 (d, 1H, *J* = 4.3 Hz, OH), 4.71 (t, 1H, *J* = 4.3 Hz, OH), 4.18–4.93 (m, 4H, H-3′, H-4′, H-5′, H-6a′), 4.42 (dd, 1H, $J_{5',6b'}$ = 5.3 Hz, $J_{6a',6b'}$ = 11.3 Hz, H-6b′); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 163.1, 150.6, 142.9, 118.9, 101.2, 80.6, 73.6, 73.3, 72.4, 63.4, 56.9. Anal. Calcd for $C_{11}H_{13}N_3O_7$: C, 44.15; H, 4.38; N, 14.04. Found: C, 44.29; H, 4.31; N, 14.20. ESIMS *m*/*z* 300.09 [M+H$^+$].

1.2.6. 1-(2′-*C*-Cyano-β-D-galactopyranosyl)uracil (8a)

Uracil derivative **8a** was synthesized from **6a** by the similar procedure as described for **7a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 9:1), to give **8a** (0.36 g, 79%) as a white foam. $[\alpha]_D^{22}$ +14 (*c* 0.3, MeOH); R_f = 0.23 (CH_2Cl_2–MeOH, 8.5:1.5); UV (MeOH): λ_{max} 257 nm (ε 4032); IR (KBr, cm^{-1}): 2250 (CN); ^1H NMR (DMSO-d_6, 300 MHz): δ 11.19 (br s, 1H, NH), 7.69 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 7.08 (s, 1H, H-1′), 5.82 (d, 1H, *J* = 2.7 Hz, OH), 5.75 (d, 1H, H-5), 5.59 (s, 1H, OH), 4.93 (s, 1H, OH), 4.71 (t, 1H, *J* = 4.7 Hz, OH), 3.87–3.58 (m, 5H, H-3′, H-4′, H-5′, H-6a′,b′); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 163.2, 149.9, 140.2, 117.6, 101.5, 80.1, 79.6, 67.3, 66.2, 60.9, 36.8. Anal. Calcd for $C_{11}H_{13}N_3O_7$: C, 44.15; H, 4.38; N, 14.04. Found: C, 44.49; H, 4.56; N, 13.96. ESIMS *m*/*z* 300.10 [M+H$^+$].

1.3. Synthesis of 1-(2′-*C*-cyano-2′-deoxy-β-D-talopyranosyl) uracil (10a)

1.3.1. 1-(6′-*O*-*t*-Butyldimethylsilyl-2′-*C*-cyano-2′-deoxy-3′,4′-*O*-isopropylidene-β-D-talopyranosyl)uracil (9a)

Phenyl chlorothionoformate (0.17 mL, 1.2 mmol) was added to a solution of **5a** or **6a** (0.37 g, 0.82 mmol), DMAP (0.04 g, 0.3 mmol), and Et_3N (0.17 mL, 1.2 mmol) in CH_3CN (12.0 mL) under nitrogen at 0 °C. The mixture was stirred for 1 h and then diluted with AcOEt. The whole was washed with H_2O and the separated organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was coevaporated two times with toluene and then was dissolved in toluene (12.0 mL). $(Me_3Si)_3SiH$ (0.34 mL, 1.1 mmol) was added to the above solution containing AIBN (0.08 g, 0.5 mmol) at 90 °C under nitrogen. After being heated for 50 min, the solvent was removed under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2–MeOH, 9.8:0.2) to give **9a** (0.25 g, 70% from **5a** or 0.24 g, 67% from **6a**) as a colorless oil. $[\alpha]_D^{22}$ +54 (*c* 0.3, $CDCl_3$); R_f = 0.33 (CH_2Cl_2–MeOH, 9.8:0.2); UV ($CDCl_3$): λ_{max} 257 nm (ε 4773); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.78 (br s, 1H, NH), 7.23 (d, 1H, $J_{5,6}$ = 8.3 Hz, H-6), 5.79 (d, 1H, H-5), 5.55 (d, 1H, $J_{1',2'}$ = 2.7 Hz, H-1′), 4.57 (dd, 1H, $J_{2',3'}$ = 7.1 Hz, $J_{3',4'}$ = 5.7 Hz, H-3′), 4.29 (dd, 1H, $J_{4',5'}$ = 2.9 Hz, H-4′), 4.11–3.89 (m, 3H, H-5′,H-6a′,b′), 3.65 (dd, 1H, H-2′), 1.74, 1.39 (2s, 6H, $2CH_3$), 0.89 (s, 9H, *t*-Bu), 0.10, 0.09 (2s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 161.6, 150.7, 141.0, 116.8, 110.9, 102.7, 80.1, 75.7, 73.4, 71.3, 62.6, 36.8, 26.1, 25.6, 24.8, 18.2, −5.4, −5.6. Anal. Calcd for $C_{20}H_{31}N_3O_6Si$: C, 54.90; H, 7.14; N, 9.60. Found: C, 54.78; H, 7.11; N, 9.82. ESIMS *m*/*z* 438.22 [M+H$^+$].

1.3.2. 1-(2′-*C*-Cyano-2′-deoxy-β-D-talopyranosyl)uracil (10a)

Uracil derivative **10a** was synthesized from **9a** by the similar procedure as described for **7a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 9:1), to give **10a** (0.12 g, 74%) as a white foam. $[\alpha]_D^{22}$ +6 (*c* 0.2, MeOH); R_f = 0.27 (CH_2Cl_2–MeOH, 8.5:1.5); UV (MeOH): λ_{max} 258 nm (ε 6229); IR (KBr, cm^{-1}): 2250 (CN); ^1H NMR (DMSO-d_6, 300 MHz): δ 11.48 (br s, 1H, NH), 7.70 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 5.74–5.69 (m, 2H, H-5, OH), 5.66 (d, 1H, $J_{1',2'}$ = 2.6 Hz, H-1′), 4.86 (d, 1H, *J* = 2.8 Hz, OH), 4.76 (t, 1H, *J* = 5.3 Hz, OH), 4.01–3.65 (m, 5H, H-3′, H-4′, H-5′, H-6a′,b′), 3.47 (dd, 1H, $J_{2',3'}$ = 5.3 Hz, H-2′); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 163.2, 149.9, 140.2, 117.6, 101.5, 80.0, 79.6, 67.3, 66.2, 60.9, 36.9. Anal. Calcd for $C_{11}H_{13}N_3O_6$: C, 46.65; H, 4.63; N, 14.84. Found: C, 46.78; H, 4.58; N, 14.76. ESIMS *m*/*z* 284.09 [M+H$^+$].

1.4. Synthesis of [1-(β-D-talopyranosyl)uracil]-2′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (15a) and [1-(β-D-galactopyranosyl)uracil]-2′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (16a)

1.4.1. 1-(6′-*O*-*t*-Butyldimethylsilyl-2′-*C*-cyano-3′,4′-*O*-isopropylidene-2′-*O*-mesyl-β-D-talopyranosyl)uracil (11a)

The cyanohydrin **5a** (0.37 g, 0.82 mmol) was dissolved in dry pyridine (4.0 mL) and mesyl chloride (0.32 mL, 4.1 mmol) was added. The mixture was stirred at 5–8 °C for 48 h, poured into ice and water, and extracted with chloroform (2 × 15 mL). The combined extracts were washed with 1 N HCl (15 mL), aqueous sodium hydrogen carbonate (15 mL), and brine (15 mL), dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue was purified by flash chromatography (CH_2Cl_2–MeOH, 9.8:0.2) to give **11a** (0.30 g, 69%) as a colorless oil. $[\alpha]_D^{22}$ −3 (*c* 0.2, $CDCl_3$); R_f = 0.52 (CH_2Cl_2–MeOH, 9.5:0.5); UV ($CDCl_3$): λ_{max} 256 nm (ε 9053); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.47 (br s, 1H, NH), 7.49 (d, 1H, $J_{5,6}$ = 8.3 Hz, H-6), 6.65 (s, 1H, H-1′), 5.80 (d, 1H, H-5), 5.03 (d, 1H, $J_{3',4'}$ = 6.9 Hz, H-3′), 4.65 (dd, 1H, $J_{4',5'}$ = 1.4 Hz, H-4′), 4.33 (dt, 1H, $J_{5',6a'} = J_{5',6b'}$ = 6.5 Hz, H-5′), 3.83–3.79 (m, 2H, H-6a′,b′), 3.26 (s, 3H, CH_3SO_2), 1.69, 1.41 (2s, 6H, $2CH_3$), 0.89 (s, 9H, *t*-Bu), 0.07, 0.06 (2s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 161.6, 150.6, 138.6, 112.4, 112.3, 103.1, 81.5, 79.8, 79.0, 78.4, 72.6, 61.4, 40.3, 25.8, 25.7, 25.1, 18.3, −5.3, −5.4. Anal. Calcd for $C_{21}H_{33}N_3O_9SSi$: C, 47.44; H, 6.26; N, 7.90. Found: C, 47.32; H, 6.30; N, 7.97. ESIMS *m*/*z* 532.16 [M+H$^+$].

1.4.2. 1-(6′-*O*-*t*-Butyldimethylsilyl-2′-*C*-cyano-3′,4′-*O*-isopropylidene-2′-*O*-mesyl-β-D-galactopyranosyl)uracil (12a)

The cyanomesylate **12a** was synthesized from **6a** by the similar procedure as described for **11a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 9.8:0.2) to give **12a** (0.56 g, 69%) as a colorless oil. $[\alpha]_D^{22}$ +26 (*c* 0.5, $CDCl_3$); R_f = 0.45 (CH_2Cl_2–MeOH, 9.5:0.5); UV ($CDCl_3$): λ_{max} 256 nm (ε 9647); ^1H NMR ($CDCl_3$, 300 MHz): δ 8.64 (br s, 1H, NH), 7.68 (d, 1H, $J_{5,6}$ = 8.3 Hz, H-6), 5.92 (s, 1H, H-1′), 5.83 (d, 1H, H-5), 4.79 (d, 1H, $J_{3',4'}$ = 6.1 Hz, H-3′), 4.50 (dd, 1H, $J_{4',5'}$ = 2.7 Hz, H-4′), 4.24 (dt, 1H, $J_{5',6a'} = J_{5',6b'}$ = 6.4 Hz, H-5′), 4.01–3.89 (m, 2H, H-6a′,b′), 3.24 (s, 3H, CH_3SO_2), 1.77, 1.42 (2s,

6H, $2CH_3$), 0.89 (s, 9H, *t*-Bu), 0.08, 0.07 (2s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 161.7, 150.3, 138.9, 112.9, 112.4, 103.4, 81.8, 79.9, 78.7, 78.6, 72.5, 61.3, 40.2, 25.9, 25.8, 25.0, 18.3, −5.3, −5.4. Anal. Calcd for $C_{21}H_{33}N_3O_9SSi$: C, 47.44; H, 6.26; N, 7.90. Found: C, 47.51; H, 6.19; N, 7.82. ESIMS *m*/*z* 532.18 [M+H$^+$].

1.4.3. [1-(6′-*O*-*t*-Butyldimethylsilyl-3′,4′-*O*-isopropylidene-β-D-talopyranosyl)uracil]-2′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (13a)

A solution of **11a** (0.3 g, 0.6 mmol) in dry acetonitrile (6.0 mL) was treated with DBU (0.09 mL, 0.6 mmol), and the mixture was stirred at room temperature for 36 h. The solution was neutralized with acetic acid and the solvent was evaporated to dryness. The residue was purified by flash chromatography (CH_2Cl_2–MeOH, 9.5:0.5) to give **13a** (0.18 g, 60%) as a white foam. $[\alpha]_D^{22}$ −6 (*c* 0.4, $CDCl_3$); R_f = 0.38 (CH_2Cl_2–MeOH, 9.5:0.5); UV ($CDCl_3$): λ_{max} 253 nm (ε 3429); IR (KBr, cm^{-1}): 3420, 3320 (NH_2), 1665 (C=C–N); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.29 (br s, 1H, NH), 7.56 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 6.49 (s, 1H, H-1′), 6.02 (br s, 2H, NH_2-4″), 5.79 (d, 1H, H-5), 5.43 (s, 1H, H-3″), 4.65 (d, 1H, $J_{3',4'}$ = 7.5 Hz, H-3′), 4.48 (d, 1H, H-4′), 4.35 (t, 1H, $J_{5',6a'} = J_{5',6b'}$ = 4.4 Hz, H-5′), 3.84 (d, 2H, H-6a′,b′), 1.57, 1.37 (2s, 6H, $2CH_3$), 0.92 (s, 9H, *t*-Bu), 0.10, 0.08 (2s, 6H, 2Si-CH_3); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 163.9, 153.4, 151.8, 140.4, 112.5, 103.5, 89.1, 83.0, 79.9, 77.3, 75.7, 73.1, 62.5, 25.9, 25.6, 24.6, 17.9, −5.5, −5.8. Anal. Calcd for $C_{21}H_{33}N_3O_9SSi$: C, 47.44; H, 6.26; N, 7.90. Found: C, 47.62; H, 6.34; N, 7.84. ESIMS *m*/*z* 532.20 [M+H$^+$].

1.4.4. [1-(6′-*O*-*t*-Butyldimethylsilyl-3′,4′-*O*-isopropylidene-β-D-galactopyranosyl)uracil]-2′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (14a)

Spiro derivative **14a** was synthesized from **12a** by the similar procedure as described for **13a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 9.5:0.5) to give **14a** (0.39 g, 70%) as a white foam. $[\alpha]_D^{22}$ +6 (*c* 0.5, $CDCl_3$); R_f = 0.37 (CH_2Cl_2–MeOH, 9.5:0.5); UV ($CDCl_3$): λ_{max} 257 nm (ε 8611); IR (KBr, cm^{-1}): 3455, 3360 (NH_2), 1650 (C=C–N); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.94 (br s, 1H, NH), 7.88 (d, 1H, $J_{5,6}$ = 8.1 Hz, H-6), 6.27 (s, 1H, H-1′), 6.04 (s, 1H, H-3″), 5.43 (d, 1H, H-5), 5.35 (br s, 2H, NH_2-4″), 4.61–4.52 (m, 2H, H-3′,H-4′), 4.43 (t, 1H, $J_{5',6a'} = J_{5',6b'}$ = 6.1 Hz, H-5′), 3.91–3.81 (m, 2H, H-6a′,b′), 1.59, 1.41 (2s, 6H, $2CH_3$), 0.91 (s, 9H, *t*-Bu), 0.09 (s, 6H, 2Si-CH_3); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 164.7, 151.6, 150.3, 141.4, 110.6, 100.5, 92.9, 83.2, 79.3, 75.7, 73.3, 71.4, 61.8, 26.2, 25.7, 24.9, 18.1, −5.5, −5.6. Anal. Calcd for $C_{21}H_{33}N_3O_9SSi$: C, 47.44; H, 6.26; N, 7.90. Found: C, 47.52; H, 6.39; N, 7.78. ESIMS *m*/*z* 532.19 [M+H$^+$].

1.4.5. [1-(β-D-Talopyranosyl)uracil]-2′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (15a)

To a solution of **13a** (0.17 g, 0.32 mmol) in dry CH_2Cl_2 (1.0 mL) was added methanolic hydrogen chloride (2.5 mL), which was obtained from acetyl chloride (0.2 mL) in dry MeOH (5.0 mL) at 0 °C. After completion of the reaction (6 h), the mixture was diluted with MeOH, and treated with an excess of $NaHCO_3$. The resulting mixture was filtered through Celite and the solvent was evaporated to dryness. The residue was purified by flash chromatography (CH_2Cl_2–MeOH, 8:2) to give **15a** (0.07 g, 58%) as a white foam. $[\alpha]_D^{22}$ −26 (*c* 0.1, DMSO); R_f = 0.22 (CH_2Cl_2–MeOH, 8:2); UV (DMSO): λ_{max} 260 nm (ε 10206); IR (KBr, cm^{-1}): 3420, 3330 (NH_2), 1655 (C=C–N); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.23 (br s, 1H, NH), 7.50 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 6.67 (br s, 2H, NH_2-4″), 6.34 (s, 1H, H-1′), 6.18 (br s, 2H, 2OH), 5.58 (d, 1H, H-5), 5.46 (s, 1H, H-3″), 4.72 (br s, 1H, OH), 4.13–3.59 (m, 5H, H-3′, H-4′, H-5′, H-6a′,b′); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 163.2, 154.4, 150.5, 142.4, 101.6, 91.3, 86.4, 79.5, 75.4, 73.3, 66.1, 58.4. Anal. Calcd for $C_{12}H_{15}N_3O_9S$: C, 38.20; H, 4.01; N, 11.14. Found: C, 38.29; H, 3.92; N, 11.19. ESIMS *m*/*z* 378.08 [M+H$^+$].

1.4.6. [1-(β-D-Galactopyranosyl)uracil]-2′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (16a)

Spiro derivative **16a** was synthesized from **14a** by the similar procedure as described for **15a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 8:2) to give **16a** (0.18 g, 67%) as a white foam. $[\alpha]_D^{22}$ +36 (*c* 0.1, DMSO); R_f = 0.21 (CH_2Cl_2–MeOH, 8:2); UV (DMSO): λ_{max} 260 nm (ε 8426); IR (KBr, cm^{-1}): 3400, 3380 (NH_2), 1620 (C=C–N); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.19 (br s, 1H, NH), 7.65 (d, 1H, $J_{5,6}$ = 8.2 Hz, H-6), 7.26 (br s, 2H, NH_2-4″), 6.53 (br s, 1H, OH), 5.97 (s, 1H, H-1′), 5.92 (br s, 1H, OH), 5.58 (m, 2H, H-5, H-3″), 4.73 (br s, 1H, OH), 4.21–3.61 (m, 5H, H-3′, H-4′, H-5′, H-6a′,b′); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 162.9, 152.8, 150.7, 140.8, 101.8, 92.2, 91.4, 82.2, 81.2, 72.3, 68.7, 60.6. Anal. Calcd for $C_{12}H_{15}N_3O_9S$: C, 38.20; H, 4.01; N, 11.14. Found: C, 38.46; H, 4.11; N, 11.25. ESIMS *m*/*z* 378.06 [M+H$^+$].

1.5. Synthesis of 1-(2′-*C*-cyano-β-D-talopyranosyl)-5-fluorouracil (7b) and 1-(2′-*C*-cyano-β-D-galactopyranosyl)-5-fluorouracil (8b)

1.5.1. 1-(6′-*O*-*t*-Butyldimethylsilyl-β-D-galactopyranosyl)-5-fluorouracil (2b)

5-Fluorouracil derivative **2b** was synthesized from **1b** by the similar procedure as described for **1a**. It was purified by flash chromatography (EtOAc–MeOH, 9:1) to give **2b** (5.06 g, 83%) as a white solid, mp 136–138 °C. $[\alpha]_D^{22}$ +7 (*c* 0.5, MeOH); R_f = 0.60 (EtOAc–MeOH, 8:2); UV (MeOH): λ_{max} 267 nm (ε 4579); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.75 (br s, 1H, NH), 8.01 (d, 1H, $J_{6,F5}$ = 7.1 Hz, H-6), 5.33 (d, 1H, $J_{1',2'}$ = 9.0 Hz, H-1′), 5.18 (d, 1H, *J* = 2.9 Hz, OH), 4.79 (s, 1H, OH), 4.35 (d, 1H, *J* = 7.4 Hz, OH), 3.82–3.51 (m, 6H, H-2′, H-3′, H-4′, H-5′, H-6a′,b′), 0.86 (s, 9H, *t*-Bu), 0.03, 0.02 (2s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 157.4, 149.9, 140.5, 126.0, 83.6, 78.3, 73.7, 68.9, 68.8, 62.6, 26.2, 18.5, −4.8. Anal. Calcd for $C_{16}H_{27}FN_2O_7Si$: C, 47.28; H, 6.70; N, 6.89. Found: C, 47.34; H, 6.82; N, 6.96. ESIMS *m*/*z* 407.15 [M+H$^+$].

1.5.2. 1-(6′-*O*-*t*-Butyldimethylsilyl-3′,4′-*O*-isopropylidene-β-D-galactopyranosyl)-5-fluorouracil (3b)

5-Fluorouracil derivative **3b** was synthesized from **2b** by the similar procedure as described for **2a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 9.5:0.5) to give **3b** (4.70 g, 85%) as a white solid, mp 94–96 °C. $[\alpha]_D^{22}$ +20 (*c* 0.5, $CDCl_3$); R_f = 0.50 (CH_2Cl_2–MeOH, 9:1); UV ($CDCl_3$): λ_{max} 267 nm (ε 4452); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.15 (br s, 1H, NH), 7.51 (d, 1H, $J_{6,F5}$ = 5.9 Hz, H-6), 5.57 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1′), 4.33–4.26 (m, 2H, H-3′, H-4′), 4.07 (dt, 1H, $J_{4',5'}$ = 2.1 Hz, $J_{5',6a'} = J_{5',6b'}$ = 6.3 Hz, H-5′), 3.92–3.80 (m, 2H, H-6a′,b′), 3.74 (dd, 1H, $J_{2',3'}$ = 6.1 Hz, H-2′), 1.56, 1.38 (2s, 6H, $2CH_3$), 0.90 (s, 9H, *t*-Bu), 0.08, 0.07 (2s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 156.9, 149.7, 140.7, 124.3, 110.2, 83.2, 78.5, 76.2, 72.8, 71.8, 61.8, 27.9, 25.8, 18.3, −5.3, −5.4. Anal. Calcd for $C_{19}H_{31}FN_2O_7Si$: C, 51.10; H, 7.00; N, 6.27. Found: C, 50.92; H, 7.17; N, 6.21. ESIMS *m*/*z* 447.21 [M+H$^+$].

1.5.3. 1-(6′-*O*-*t*-Butyldimethylsilyl-3′,4′-*O*-isopropylidene-β-D-lyxo-hexopyranosyl-2′-ulose)-5-fluorouracil (4b)

5-Fluorouracil derivative **4b** was synthesized from **3b** by the similar procedure as described for **4a**. It was purified by flash chromatography (EtOAc–hexane, 5:5) to give **4b** (3.87 g, 83%) as a white solid, mp 155–157 °C. $[\alpha]_D^{22}$ −2 (*c* 0.3, $CDCl_3$); R_f = 0.50 (EtOAc–hexane, 6:4); UV ($CDCl_3$): λ_{max} 264 nm (ε 13813); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.46 (br s, 1H, NH), 7.19 (d, 1H,

$J_{6,F5}$ = 5.7 Hz, H-6), 6.15 (s, 1H, H-1′), 4.71–4.68 (m, 2H, H-3′, H-4′), 4.31 (t, 1H, $J_{5',6a'} = J_{5',6b'}$ = 6.7 Hz, H-5′), 3.89 (d, 2H, H-6a′,b′), 1.48, 1.42 (2s, 6H, $2CH_3$), 0.91 (s, 9H, *t*-Bu), 0.09 (s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 198.0, 156.2, 148.9, 140.6, 128.6, 111.4, 82.7, 77.7, 76.4, 76.3, 61.4, 27.3, 26.0, 25.8, 18.3, −5.4, −5.5. Anal. Calcd for $C_{19}H_{29}FN_2O_7Si$: C, 51.34; H, 6.58; N, 6.30. Found: C, 51.42; H, 6.72; N, 6.22. ESIMS *m*/*z* 445.20 [M+H$^+$].

1.5.4. 1-(6′-*O*-*t*-Butyldimethylsilyl-2′-*C*-cyano-3′,4′-*O*-isopropylidene-β-D-talopyranosyl)-5-fluorouracil (5b) and 1-(6′-*O*-*t*-butyldimethylsilyl-2′-*C*-cyano-3′,4′-*O*-isopropylidene-β-D-galactopyranosyl)-5-fluorouracil (6b)

The cyanohydrins **5b** and **6b** were synthesized from **4b** by the similar procedure as described for **5a** and **6a**. The residue was purified by flash chromatography (CH_2Cl_2–EtOAc, 7:3).

The fastest moving fractions afforded **5b** (1.03 g, 25%) as a white foam. $[\alpha]_D^{22}$ +2 (*c* 0.6, $CDCl_3$); R_f = 0.41 (CH_2Cl_2–EtOAc, 7:3); UV ($CDCl_3$): λ_{max} 261 nm (ε 7775); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.72 (br s, 1H, NH), 7.55 (d, 1H, $J_{6,F5}$ = 5.3 Hz, H-6), 6.08 (s, 1H, H-1′), 5.78 (br s, 1H, 2′-OH), 4.56–4.48 (m, 2H, H-3′, H-4′), 4.42 (dt, 1H, $J_{4',5'}$ = 1.9 Hz, $J_{5',6a'} = J_{5',6b'}$ = 6.5 Hz, H-5′), 3.81–3.79 (m, 2H, H-6a′,b′), 1.62, 1.39 (2s, 6H, $2CH_3$), 0.90 (s, 9H, *t*-Bu), 0.08, 0.07 (2s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 157.4, 148.7, 139.8, 127.0, 116.5, 111.5, 79.2, 75.6, 73.6, 71.5, 70.6, 62.1, 26.4, 25.8, 24.1, 18.3, −5.4, −5.6. Anal. Calcd for $C_{20}H_{30}FN_3O_7Si$: C, 50.94; H, 6.41; N, 8.91. Found: C, 50.81; H, 6.52; N, 8.85. ESIMS *m*/*z* 472.19 [M+H$^+$].

The slowest moving fractions afforded **6b** (2.29 g, 56%) as a white foam. $[\alpha]_D^{22}$ +7 (*c* 0.2, $CDCl_3$); R_f = 0.27 (CH_2Cl_2–EtOAc, 7:3); UV ($CDCl_3$): λ_{max} 263 nm (ε 13813); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.47 (br s, 1H, NH), 7.85 (d, 1H, $J_{6,F5}$ = 6.1 Hz, H-6), 5.77 (s, 1H, H-1′), 5.00 (br s, 1H, 2′-OH), 4.52 (d, 1H, $J_{3',4'}$ = 6.3 Hz, H-3′), 4.43 (dd, 1H, $J_{4',5'}$ = 2.6 Hz, H-4′), 4.22–4.18 (m, 1H, H-5′), 4.02–3.88 (m, 2H, H-6a′,b′), 1.73, 1.41 (2s, 6H, $2CH_3$), 0.91 (s, 9H, *t*-Bu), 0.10, 0.09 (2s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 157.3, 149.0, 139.9, 126.3, 116.7, 111.6, 79.3, 75.3, 73.7, 71.2, 70.8, 62.3, 26.5, 25.4, 24.6, 18.2, −5.4, −5.6. Anal. Calcd for $C_{20}H_{30}FN_3O_7Si$: C, 50.94; H, 6.41; N, 8.91. Found: C, 51.03; H, 6.44; N, 8.97. ESIMS *m*/*z* 472.21 [M+H$^+$].

1.5.5. 1-(2′-*C*-Cyano-β-D-talopyranosyl)-5-fluorouracil (7b)

5-Fluorouracil derivative **7b** was synthesized from **5b** by the similar procedure as described for **7a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 9:1) to give **7b** (0.17 g, 74%) as a white foam. $[\alpha]_D^{22}$ −2 (*c* 0.4, MeOH); R_f = 0.29 (CH_2Cl_2–MeOH, 8.5:1.5); UV (MeOH): λ_{max} 266 nm (ε 3192); IR (KBr, cm^{-1}): 2240 (CN); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.98 (br s, 1H, NH), 7.80 (d, 1H, $J_{6,F5}$ = 7.2 Hz, H-6), 7.62 (s, 1H, H-1′), 6.36 (d, 1H, *J* = 5.6 Hz, OH), 6.21 (s, 1H, OH), 5.28 (d, 1H, *J* = 4.8 Hz, OH), 4.72 (t, 1H, *J* = 5.2 Hz, OH), 4.07–3.92 (m, 4H, H-3′, H-4′, H-5′, H-6a′), 3.64 (dd, 1H, $J_{5',6b'}$ = 5.7 Hz, $J_{6a',6b'}$ = 12.8 Hz, H-6b′); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 157.3, 149.6, 140.8, 124.7, 118.6, 82.7, 80.4, 73.9, 72.6, 68.2, 60.6. Anal. Calcd for $C_{11}H_{12}FN_3O_7$: C, 41.65; H, 3.81; N, 13.25. Found: C, 41.54; H, 3.76; N, 13.35. ESIMS *m*/*z* 318.08 [M+H$^+$].

1.5.6. 1-(2′-*C*-Cyano-β-D-galactopyranosyl)-5-fluorouracil (8b)

5-Fluorouracil derivative **8b** was synthesized from **6b** by the similar procedure as described for **7a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 9:1) to give **8b** (0.40 g, 78%) as a white foam. $[\alpha]_D^{22}$ +8 (*c* 0.2, MeOH); R_f = 0.29 (CH_2Cl_2–MeOH, 8.5:1.5); UV (MeOH): λ_{max} 266 nm (ε 6595); IR (KBr, cm^{-1}): 2235 (CN); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.89 (br s, 1H, NH), 7.81 (d, 1H, $J_{6,F5}$ = 7.0 Hz, H-6), 7.04 (s, 1H, H-1′), 5.74 (s, 1H, OH), 5.60 (s, 1H, OH), 4.85 (s, 1H, OH), 4.66 (s, 1H, OH), 3.91–3.61 (m, 5H, H-3′, H-4′, H-5′, H-6a′,b′); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 159.3, 150.4, 140.7, 128.8, 117.9, 101.2, 81.1, 76.4, 68.3, 66.8, 61.2. Anal. Calcd for $C_{11}H_{12}FN_3O_7$: C, 41.65; H, 3.81; N, 13.25. Found: C, 41.62; H, 3.90; N, 13.39. ESIMS *m*/*z* 318.10 [M+H$^+$].

1.6. Synthesis of 1-(2′-*C*-cyano-2′-deoxy-β-D-talopyranosyl)-5-fluorouracil (10b)

1.6.1. 1-(6′-*O*-*t*-Butyldimethylsilyl-2′-*C*-cyano-2′-deoxy-3′,4′-*O*-isopropylidene-β-D-talopyranosyl)-5-fluorouracil (9b)

5-Fluorouracil derivative **9b** was synthesized from **5b** or **6b** by the similar procedure as described for **9a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 9.8:0.2) to give **9b** (0.21 g, 64% from **5b** or 0.22 g, 67% from **6b**) as a colorless oil. $[\alpha]_D^{22}$ −2 (*c* 0.2, $CDCl_3$); R_f = 0.36 (CH_2Cl_2–MeOH, 9.8:0.2); UV ($CDCl_3$): λ_{max} 264 nm (ε 4259); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.01 (br s, 1H, NH), 7.83 (d, 1H, $J_{6,F5}$ = 5.9 Hz, H-6), 5.59 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1′), 4.59 (t, 1H, $J_{2',3'} = J_{3',4'}$ = 6.5 Hz, H-3′), 4.30 (dd, 1H, $J_{4',5'}$ = 2.4 - Hz, H-4′), 4.10–3.89 (m, 3H, H-5′,H-6a′,b′), 3.67 (dd, 1H, H-2′), 1.74, 1.39 (2s, 6H, $2CH_3$), 0.90 (s, 9H, *t*-Bu), 0.10 (s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 157.3, 148.9, 141.0, 127.2, 116.5, 111.3, 79.4, 75.8, 73.5, 71.6, 62.1, 36.9, 26.2, 25.3, 24.9, 18.2, −5.4, −5.6. Anal. Calcd for $C_{20}H_{30}FN_3O_6Si$: C, 52.73; H, 6.64; N, 9.22. Found: C, 52.60; H, 6.72; N, 9.33. ESIMS *m*/*z* 456.22 [M+H$^+$].

1.6.2. 1-(2′-*C*-Cyano-2′-deoxy-β-D-talopyranosyl)-5-fluorouracil (10b)

5-Fluorouracil derivative **10b** was synthesized from **9b** by the similar procedure as described for **10a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 9:1) to give **10b** (0.11 g, 79%) as a white foam. $[\alpha]_D^{22}$ −40 (*c* 0.2, MeOH); R_f = 0.33 (CH_2Cl_2–MeOH, 8.5:1.5); UV (MeOH): λ_{max} 264 nm (ε 7366); IR (KBr, cm^{-1}): 2220 (CN); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.15 (br s, 1H, NH), 7.92 (d, 1H, $J_{6,F5}$ = 7.0 Hz, H-6), 5.77 (br s, 1H, OH), 5.66 (d, 1H, $J_{1',2'}$ = 1.7 Hz, H-1′), 4.96 (d, 1H, *J* = 3.9 Hz, OH), 4.85 (t, 1H, *J* = 5.5 Hz, OH), 3.99–3.61 (m, 5H, H-3′, H-4′, H-5′, H-6a′,b′), 3.49 (dd, 1H, $J_{2',3'}$ = 5.3 Hz, H-2′); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 158.4, 149.8, 140.3, 127.9, 118.9, 81.6, 76.7, 75.0, 73.2, 65.1, 37.8. Anal. Calcd for $C_{11}H_{12}FN_3O_6$: C, 43.86; H, 4.02; N, 13.95. Found: C, 43.94; H, 4.13; N, 13.73. ESIMS *m*/*z* 302.10 [M+H$^+$].

1.7. Synthesis of [1-(β-D-talopyranosyl)-5-fluorouracil]-2′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (15b)

1.7.1. 1-(6′-*O*-*t*-Butyldimethylsilyl-2′-*C*-cyano-3′,4′-*O*-isopropylidene-2′-*O*-mesyl-β-D-talopyranosyl)-5-fluorouracil (11b)

The cyanomesylate **11b** was synthesized from **5b** by the similar procedure as described for **11a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 9.8:0.2) to give **11b** (0.27 g, 68%) as a colorless oil. $[\alpha]_D^{22}$ +2 (*c* 0.2, $CDCl_3$); R_f = 0.54 (CH_2Cl_2–MeOH, 9.5:0.5); UV ($CDCl_3$): λ_{max} 263 nm (ε 8012); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.16 (br s, 1H, NH), 7.53 (d, 1H, $J_{6,F5}$ = 6.2 Hz, H-6), 6.62 (s, 1H, H-1′), 5.05 (d, 1H, $J_{3',4'}$ = 6.8 Hz, H-3′), 4.66 (dd, 1H, $J_{4',5'}$ = 1.4 Hz, H-4′), 4.34 (dt, 1H, $J_{5',6a'} = J_{5',6b'}$ = 6.2 Hz, H-5′), 3.84–3.82 (m, 2H, H-6a′,b′), 3.28 (s, 3H, CH_3SO_2), 1.69, 1.42 (2s, 6H, $2CH_3$), 0.91 (s, 9H, *t*-Bu), 0.09 (s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 156.4, 149.2, 140.7, 123.6, 112.9, 112.3, 81.1, 80.2, 78.8, 78.4, 72.7, 61.4, 39.9, 25.8, 25.7, 24.9, 18.4, −5.3, −5.4. Anal. Calcd for $C_{21}H_{32}FN_3O_9SSi$: C, 45.89; H, 5.87; N, 7.64. Found: C, 45.71; H, 5.96; N, 7.54. ESIMS *m*/*z* 550.17 [M+H$^+$].

1.7.2. 1-(6′-*O*-*t*-Butyldimethylsilyl-2′-*C*-cyano-3′,4′-*O*-isopropylidene-2′-*O*-mesyl-β-D-galactopyranosyl)-5-fluorouracil (12b)

The cyanomesylate **12b** was synthesized from **6b** by the similar procedure as described for **11a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 9.8:0.2) to give **12b** (0.52 g, 59%) as a colorless oil. $[\alpha]_D^{22}$ +12 (*c* 0.3, $CDCl_3$); R_f = 0.48 (CH_2Cl_2–MeOH, 9.5:0.5); UV ($CDCl_3$): λ_{max} 262 nm (ε 3678); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.59 (br s, 1H, NH), 7.75 (d, 1H, $J_{6,F5}$ = 5.9 Hz, H-6), 5.93 (s, 1H, H-1′), 4.81 (d, 1H, $J_{3',4'}$ = 6.1 Hz, H-3′), 4.50 (dd, 1H, $J_{4',5'}$ = 2.3 Hz, H-4′), 4.23–4.18 (m, 1H, H-5′), 4.02–3.89 (m, 2H, H-6a′,b′), 3.26 (s, 3H, CH_3SO_2), 1.77, 1.43 (2s, 6H, $2CH_3$), 0.90 (s, 9H, *t*-Bu), 0.09 (s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 156.5, 149.2, 140.8, 123.6, 112.9, 112.4, 81.8, 79.8, 78.6, 78.5, 72.5, 61.3, 40.2, 25.9, 25.8, 24.9, 18.3, −5.4, −5.5. Anal. Calcd for $C_{21}H_{32}FN_3O_9SSi$: C, 45.89; H, 5.87; N, 7.64. Found: C, 45.82; H, 5.94; N, 7.73. ESIMS *m*/*z* 550.15 [M+H$^+$].

1.7.3. [1-(6′-*O*-*t*-Butyldimethylsilyl-3′,4′-*O*-isopropylidene-β-D-talopyranosyl)-5-fluorouracil]-2′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (13b)

Spiro derivative **13b** was synthesized from **11b** by the similar procedure as described for **13a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 9.5:0.5) to give **13b** (0.20 g, 74%) as a white foam. $[\alpha]_D^{22}$ −22 (*c* 0.5, $CDCl_3$); R_f = 0.40 (CH_2Cl_2–MeOH, 9.5:0.5); UV ($CDCl_3$): λ_{max} 265 nm (ε 7399); IR (KBr, cm^{-1}): 3410, 3360 (NH_2), 1620 (C=C–N); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.61 (br s, 1H, NH), 7.68 (d, 1H, $J_{6,F5}$ = 6.0 Hz, H-6), 6.47 (s, 1H, H-1′), 5.86 (br s, 2H, NH_2-4″), 5.45 (s, 1H, H-3″), 4.65 (d, 1H, $J_{3',4'}$ = 7.5 Hz, H-3′), 4.48 (d, 1H, H-4′), 4.36 (t, 1H, $J_{5',6a'} = J_{5',6b'}$ = 4.6 Hz, H-5′), 3.86 (d, 2H, H-6a′,b′), 1.58, 1.38 (2s, 6H, $2CH_3$), 0.93 (s, 9H, *t*-Bu), 0.12, 0.10 (2s, 6H, 2Si-CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 157.9, 153.2, 150.5, 141.3, 124.8, 112.6, 89.4, 82.9, 80.4, 77.3, 75.7, 72.9, 62.6, 25.9, 25.6, 24.6, 18.7, −5.6, −5.7. Anal. Calcd for $C_{21}H_{32}FN_3O_9SSi$: C, 45.89; H, 5.87; N, 7.64. Found: C, 45.99; H, 5.81; N, 7.70. ESIMS *m*/*z* 550.19 [M+H$^+$].

1.7.4. [1-(β-D-Talopyranosyl)-5-fluorouracil]-2′-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) (15b)

Spiro derivative **15b** was synthesized from **13b** by the similar procedure as described for **15a**. It was purified by flash chromatography (CH_2Cl_2–MeOH, 8:2) to give **15b** (0.08 g, 59%) as a white foam. $[\alpha]_D^{22}$ −12 (*c* 0.1, DMSO); R_f = 0.24 (CH_2Cl_2–MeOH, 8:2); UV (DMSO): λ_{max} 263 nm (ε 2532); IR (KBr, cm^{-1}): 3450, 3320 (NH_2), 1655 (C=C–N); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.61 (br s, 1H, NH), 7.69 (d, 1H, $J_{6,F5}$ = 7.3 Hz, H-6), 6.69 (s, 1H, H-1′), 6.32 (br s, 2H, NH_2-4″), 6.19 and 6.15 (2 br s, 2H, 2OH), 5.47 (s, 1H, H-3″), 4.66 (br s, 1H, OH), 4.16–3.59 (m, 5H, H-3′, H-4′, H-5′, 6-a′,b′); ^{13}C NMR (DMSO-d_6, 75.5 MHz): δ 162.3, 158.7, 150.4, 140.8, 126.5, 90.8, 86.2, 79.1, 75.2, 70.4, 66.5, 62.5. Anal. Calcd for $C_{12}H_{14}FN_3O_9S$: C, 36.46; H, 3.57; N, 10.63. Found: C, 36.52; H, 3.68; N, 10.78. ESIMS *m*/*z* 396.05 [M+H$^+$].

1.8. Antiproliferative assays

The cytostatic effects of the test compounds on murine leukemia cells (L1210), human T-lymphocyte cells (CEM), and human cervix carcinoma cells (HeLa) were evaluated as follows: an appropriate number of cells suspended in growth medium were allowed to proliferate in 200 μL-wells of 96-well-microtiter plates in the presence of variable amounts of test compounds at 37 °C in a humidified CO_2-controlled atmosphere. After 48 h (L1210), 72 h (CEM), or 96 h (HeLa), the number of cells was counted in a Coulter counter. The IC_{50} value was defined as the compound concentration required to inhibit cell proliferation by 50%.

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