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Visibly Emitting Thiazolyl-uridine Analogues as promising fluorescent probes

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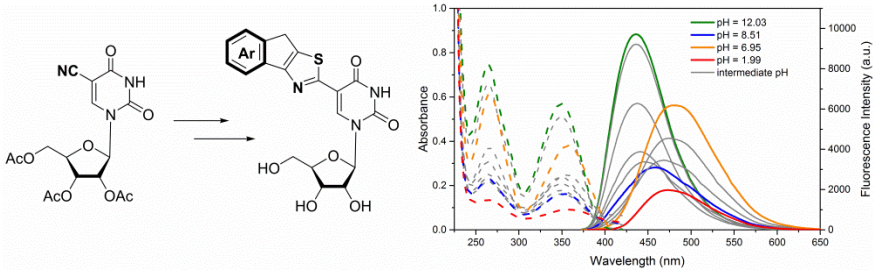


Abstract: Microenvironment sensitive fluorescent (ESF) nucleosides are powerful tools for nucleic acids research. The new 5-substituted uridine analogues are synthesized, which comprise a 4*H*-cyclopenta[*d*]thiazole ring obtained by Hantzsch synthesis reaction of 5-thioamide-uridine with aromatic α-bromocarbonyl compounds. The emission maximum of new compounds is in the visible region. They exhibit strong solvent and pH dependent fluorescent properties, indicating their promising ability to be fluorescent probes.

Introduction

RNA displays a wide range of functions, including gene regulation,[1] protein synthesis[2] and reaction catalysts.[3] The functional diversity of RNAs originates from their ability to fold into a number of secondary and tertiary structure elements, which are crucial for a variety of binding events with other biomolecules .[4-7] Several biophysical methods, such as NMR, electron paramagnetic resonance (EPR) and fluorescence combined with circular dichroism (CD) have been developed to understand structures and functions of RNAs.[8-10] Among them, Fluorescent spectroscopy are widely utilized in nucleic acids research with many advantages, such as high sensitivity, low cost, easy detection, etc.[11-13] As nature nucleic acids are almost nonemissive, numerous fluorescent nucleoside derivatives are used to explore nucleic acid conformation, recognition, sequence and interactions of nucleic acid and protein.[14-17] An attractive feature of emissive nucleobase analogues with similar shape to canonical nucleobases is that they do not perturb DNA structure and retain nucleobase recognition. In particular, the microenvironment sensitive fluorescent (ESF) nucleosides are powerful tools for nucleic acids research.[17] Among the monomeric uridine analogues are 5-(furan-2-yl)-uridine, 5-(thiophen-2-yl)-uridine, 5-(benzo[*b*]thiophen-2-yl)-uridine and 5-(benzofuran-2-yl)-uridine[18] (Figure 1). The research has shown that 5-benzo[*b*]thiophen-2-yl-uridine **2b** displays remarkable solvent dependent fluorescence with emission maximum in the visible region. T7 RNA polymerase can incorporate its corresponding triphosphate into RNA oligonucleotides. The fluorescence quantum yield of **2b** is not remarkably quenched when placed within a complementary duplex or an ssRNA.[18c] However, its quantum yield is not satisfied. We try to synthesize new fluorescent ribonucleoside analogues whose expected favorable photophysical properties would be useful for promising fluorescence probe.

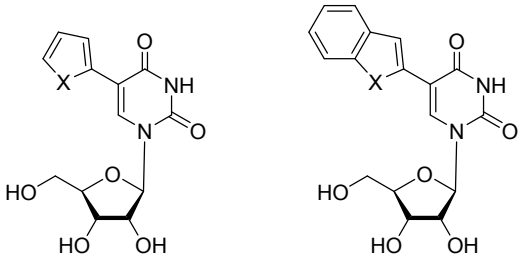


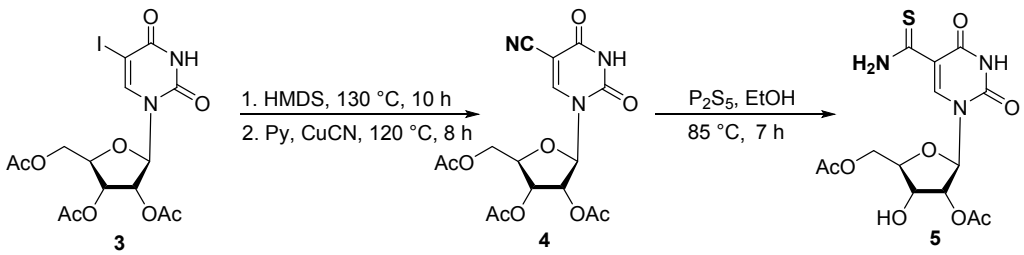
Figure 1. The emissive 5-conjugated uridine analogues.

There are three categories for fluorescent pyrimidine analogues: extended fluorescent scaffolds by use of linkers; modified pyrimidine substituents and ring-fused pyrimidine ring systems.[19] Among the three approaches, linking a fluorophore to the pyrimidine framework is an important approach to generate a fluorescent pyrimidine nucleoside. To date, most extensions on the pyrimidine ring take place at the 5 position to make use of the conjugation with the N1 nitrogen and 5, 6 double bond and to avoid perturbing base pairing.[19] Previous researches have shown that the heterocycle moiety attached to the 5 position of uridine may enhance the π conjugation and impart better photophysical properties to the nucleobase.[18b,19-21] This manuscript reports on Hantzsch synthesis reaction of 5-thioamide-uridine with aromatic α-bromocarbonyl compounds to generate thiazol-conjugated fluorescent uridine analogues.

Results and discussion.

The first two thiazol-conjugated 2'-deoxyuridine analogues have been synthesized via palladium-catalyzed coupling reaction of 2-tributylstannylthiazoles with 3',5'-di-*O*-p-toluyl-IdU.[22] Later, Matsuda and co-workers have synthesized substituted 5-(benzothiazol-2-yl)-2'-deoxyuridine by 2-aminobenzenethiol and 5-furyl-2'-deoxyuridine.[23] To avoid the toxic heteroarylstannanes and expand the diversity of uridine analogues, we report a new method to synthesize thiazol-conjugated uridine analogues with 5-thioamide-uridine and aromatic α-bromocarbonyl compounds. 5-

thioamide-uridine can be achieved from 5-cyano-uridine. Bleackl group has firstly reported the synthesis of 5-cyano-2'-deoxyuridine from 5-iodo-2'-deoxyuridine, using hexamethyldisilazane and CuCN, with 5% yield.[24] Later, Terrence and coworkers have used protected 5-bromo-uridine and NaCN to get protected 5-cyano-uridine, with yield of 35%.[25] Because CuCN is safer and easier to be accessible than NaCN, we use CuCN to prepare 5-cyano-uridine. As 5-cyano-uridine is a high polar molecule and difficult to be purified, the 2',3',5'-tri-actyl-5-iodo-uridine (**3**) is used as starting material and is silylated with hexamethyldisilazane at 130 °C for 10 hours. The silylated intermediate is reacted with CuCN in pyridine at 120 °C for 8 h under nitrogen, providing protected 5-cyano-uridine (**4**) in improved yield (83%) over two steps (Scheme 1).



Scheme 1. The synthesis route of key intermediate **5**.

The first protocol for conversion of cyano function to a thioamide group has been reported by Herdewijn and coworkers.[26] They have saturated the solution of protected 5-cyano-2'-deoxyuridine in DMF with H_2S, which is stored at room temperature for 24 h. Here, we report a new protocol of the conversion of non-fluorescent compound **4** to the intermediate 5-thioamide-uridine analogue (**5**) by treatment with phosphorus pentasulfide. The compound **4** and P_2S_5 are reacted at 85 °C for 7 h to afford compound **5** which was partially deprotected at 3'-OH as major product (65%) (Scheme 1). The mechanism of partial deprotection is not clear. The reaction product is purified by flash column chromatography and identified by using ESI-TOF mass spectra and NMR spectra. The reaction enables mild and safe conditions to convert the cyano function to a thioamide group compared to using hydrogen sulfide.

For the desirable fluorescent nucleobases, they exhibit emission wavelength in the

visible region. Incorporating heteroatoms and extending π-systems can make optical profiles redshifted.[19] Here, we incorporate N and S heteroatoms and extended π-systems by classic Hantzsch synthesis reaction.[27] The non-fluorescent 5-thioamide-uridine (**5**) is allowed to react with a series of non-fluorescent aromatic α-haloketones (**6a-m**) in EtOH (**6k** in DMF) and deprotected in 0.05 M K_2CO_3 in MeOH to afford the target uridine analogues **7a-m**, in yields 40%-80% (Scheme 2). The reaction mechanism of fluorescent uridine analogues is shown in Figure 2.

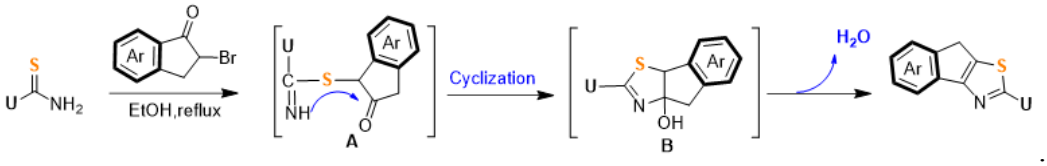
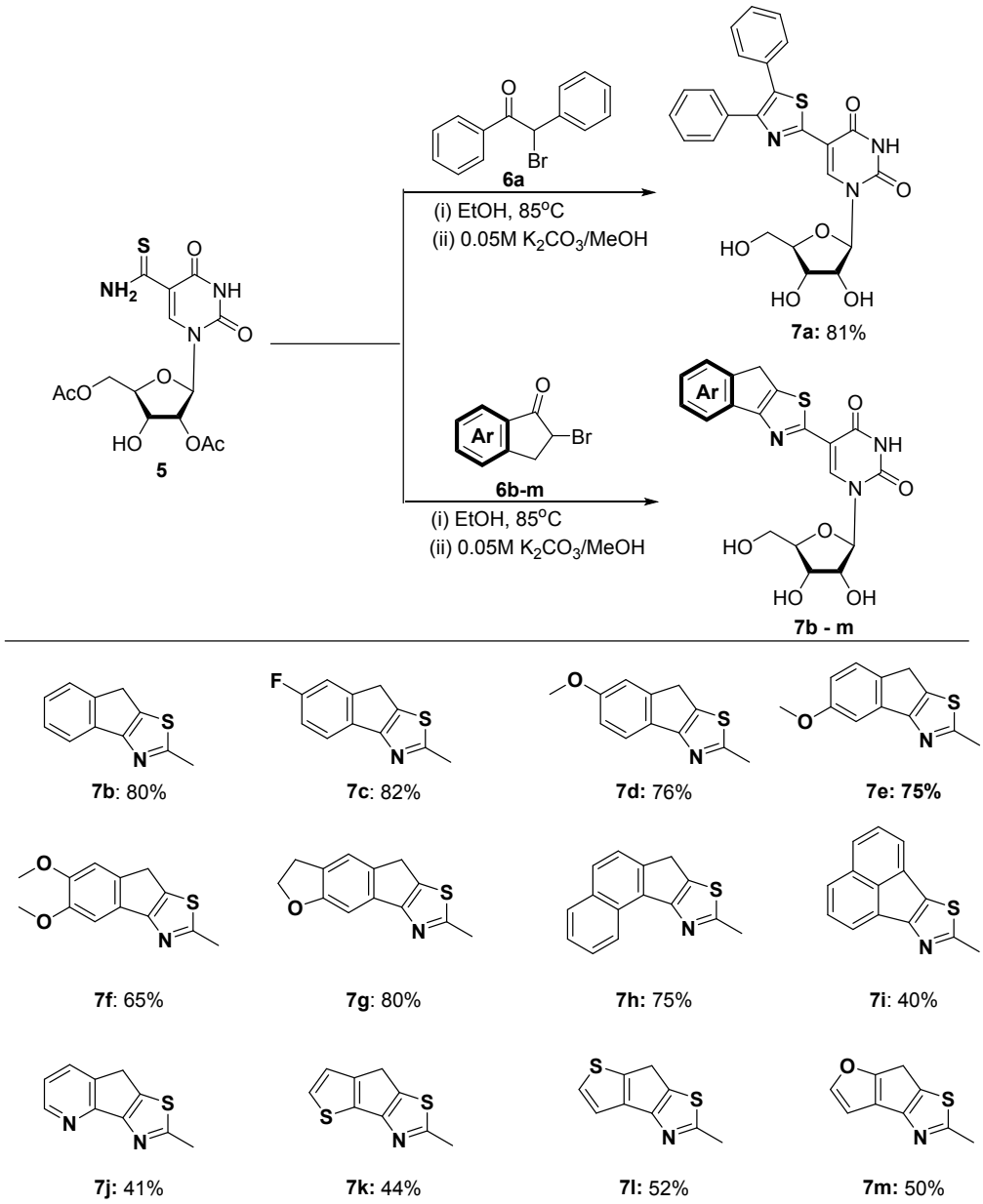


Figure 2. Reaction mechanism of fluorescent uridine analogues



Scheme 2. Transformation of the intermediate **5** to the nucleosides **7a-m**.

The photophysical properties of new pyrimidine analogues **7a–m** are investigated and shown in table 1. All thiazol-conjugated uridine analogues are fluorescent. The absorbance maxima are in 350-400 nm and the emission maxima rang from ca. 450 to 560 nm, in the visible region. Especially for **7i**, its emission max is 557 nm in water. Compared to the known fluorescent 5-modified uridines **2** and **3**,[18] the newly synthesized compounds show red-shifted absorption and emission spectra. All derivatives display rather large Stokes shifts (>5000 cm^{-1}), especially for compound **7i** (>10000 cm^{-1}).

Increasing the electron-rich character of the moiety, such as methoxyphenyl,

dimethoxyphenyl, thienyl, furanyl and so on, combination with a 4*H*-cyclopenta[*d*]thiazole ring leads to a red shift for both absorption and emission maxima. This illustrates the impact of the combined moiety on both the ground and excited state. For most of fluorescent nucleosides, their highest quantum yield is in organic solvent[28]. But interestingly, for nucleosides **7a** and **7j**, they have highest relative quantum yields in water.

Next, the photophysical properties of **7a–m** in various solvents are examined. We observed that the absorption and emission properties of **7a–m** have been significantly affected by the solvent polarity (Figure 3 and S1-13). A red shift of $\lambda_{em\ max}$ is seen for all the new compounds when solvent polarity is increased. The plot of the Stokes shift determined in solvents vs Reichardts' microscopic solvent polarity parameter $E_T(30)$[29] shows a very good linear correlation, which quantifies this effect.

The observed emission intensity in various solvents is in accordance with the relative quantum yield in different solvents (Table 1), which further ascertains the responsiveness of the fluorescent nucleosides **7a-m** to microenvironmental changes (Figure S1-13).

Table 1. Photophysical Properties of Fluorescent Nucleosides **7a-m**[a]

Compound	solvent	$\lambda_{abs,max}$ (nm)	ε^b	λ_{em} (nm)	Φ^c	Brightness $\varepsilon \times \Phi \times 10^3$	Stokes shift λ_{abs}-λ_{em} cm^{-1} (nm)
7a	dioxane	350	21.6	422	0.026	0.56	5946 (72)
	methanol	345	21.2	452	0.031	0.66	6862 (107)
	water	345	16.6	472	0.073	1.21	7572 (127)
7b	dioxane	359	24.3	439	0.165	4.01	5076 (80)
	methanol	355	22.9	460	0.317	6.43	6430 (105)
	water	355	13.0	484	0.117	1.52	7550 (129)
7c	dioxane	363	13.8	442	0.123	1.70	4924 (79)
	methanol	358	13.7	456	0.294	4.03	6003 (98)
	water	359	10.2	489	0.072	0.74	7405 (130)
7d	dioxane	373	14.2	464	0.331	4.70	5257 (91)
	methanol	370	13.5	502	0.04	0.59	7107 (132)
	water	371	10.7	507	0.014	0.11	7230 (136)
7e	dioxane	355	15.5	442	0.082	1.27	5544 (87)
	methanol	358	9.7	468	0.153	1.68	6565 (113)
	water	360	9.0	499	0.014	0.13	7737 (139)
7f	dioxane	373	12.1	493	0.276	3.23	6526 (120)
	methanol	371	12.1	530	0.001	0.01	8080 (159)
	water	370	7.5	539	$<10^{-3}$	<0.01	8508 (169)
7g	dioxane	365	15.0	455	0.097	1.46	5419 (90)
	methanol	364	11.2	491	0.019	0.21	7106 (127)
	water	374	8.6	508	0.002	0.02	7787 (134)
7h	dioxane	372	13.1	461	0.071	0.93	5190 (90)
	methanol	370	12.9	495	0.033	0.43	6825 (125)
	water	412	8.0	522	0.003	0.02	5115 (110)
7i	dioxane	350	30.0	549	0.004	0.12	10356 (199)
	methanol	348	29.3	549	0.004	0.12	10521 (201)
	water	350	22.4	557	0.001	0.02	10628 (207)

7j	dioxane	354	15.6	420	0.082	1.28	4439 (66)
	methanol	347	13.1	428	0.112	1.47	5454 (81)
	water	347	14.8	447	0.161	2.38	6447 (100)
7k	dioxane	369	12.4	468	0.039	0.48	5733 (99)
	methanol	371	11.4	502	0.040	0.46	7034 (131)
	water	372	9.6	535	0.002	0.02	8190 (163)
7l	dioxane	363	10.5	457	0.091	0.96	5666 (90)
	methanol	363	10.0	486	0.175	1.75	6972 (123)
	water	364	8.6	513	0.005	0.04	7979 (149)
7m	dioxane	370	10.4	464	0.126	1.45	5475 (94)
	methanol	367	11.5	497	0.069	0.79	7127 (130)
	water	356	10.4	485	0.005	0.05	7471 (129)

[a]Absorption and steady-state emission spectroscopy were measured using samples from a concentrated DMSO stock solution. [b]ε In [10^3 M^{-1} cm^{-1}]. [c]The fluorescence quantum yields (ϕ) were calculated using quinine sulfate in 0.5 M H_2SO_4 (ϕ = 0.55) as the standard .

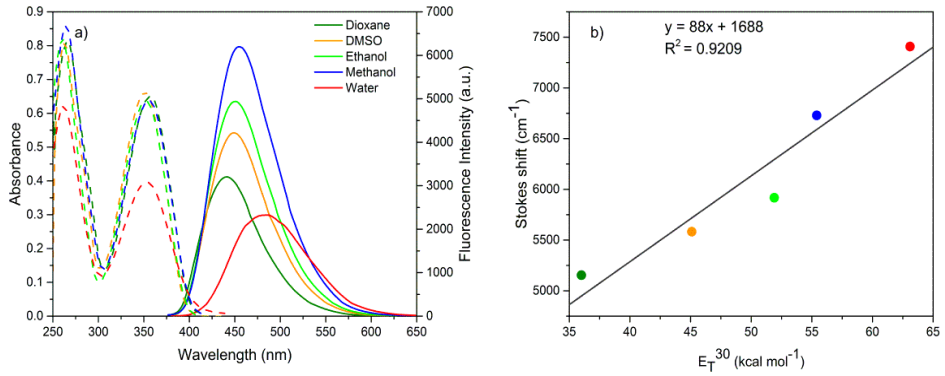


Figure 3. (a) Absorption (30 μM, dash) and emission (5.0 μM, solid) spectra of compound **7b** in different solvents. (b) Correlating Stokes shift vs $E_T(30)$ values obtained from respective solvents for **7b**.

Since uridine is a pH sensitive molecule and its N3 can be deprotonated at basic condition, the properties of **7a-m** under a variety of pH conditions are invesigated. As the thiazole system is prone to degradation, the stability of the compounds is examined in harsh condition. The compounds are treated at pH 1 and pH 13 at room temperature for 3 h separately and the reaction is examined by RP18-HPLC. After 3h treatment, almost no change is observed (S14-17). We observe that the fluorescence of newly synthesized nucleosides exhibit a high pH-dependency (Figure 4). In basic conditions (from pH 7.0-10.0), absorption and emission spectra of most compounds reveal a strong influence of pH on the intensity and maximum. The electron properties of the combined moiety to a 4*H*-cyclopenta[*d*]thiazole impact the changes of absorption and emission properties in basic conditions. The electron-donating character of the combined moiety to a 4*H*-cyclopenta[*d*]thiazole, such as methoxyphenyl, thienyl, furanyl and so on, results in

increase of both absorption and emission intensity, while the electron-withdrawing character of the combined moiety, such as Pyridyl (**7j**), decreases the intensity of emission and increases the intensity of absorption. The pH-dependent fluorescence (except **7f**, it is difficult to dissolve in buffer) is used to determine the pK_a value of deprotonation (Figure 4 and S18-28). As shown in Table 2, compared to uridine, the pK_a values of the compounds changes result from the electron-withdrawing character of the thiozole groups making deprotonation more favourable. It is almost identical to the pK_a value obtained by using UV spectrophotometry [30] (Figure 4 and S18-28). The possible pyrimidine nucleoside tautomers and suggested p*K*a values corresponding to possible deprotonation sites are shown in Figure 5.

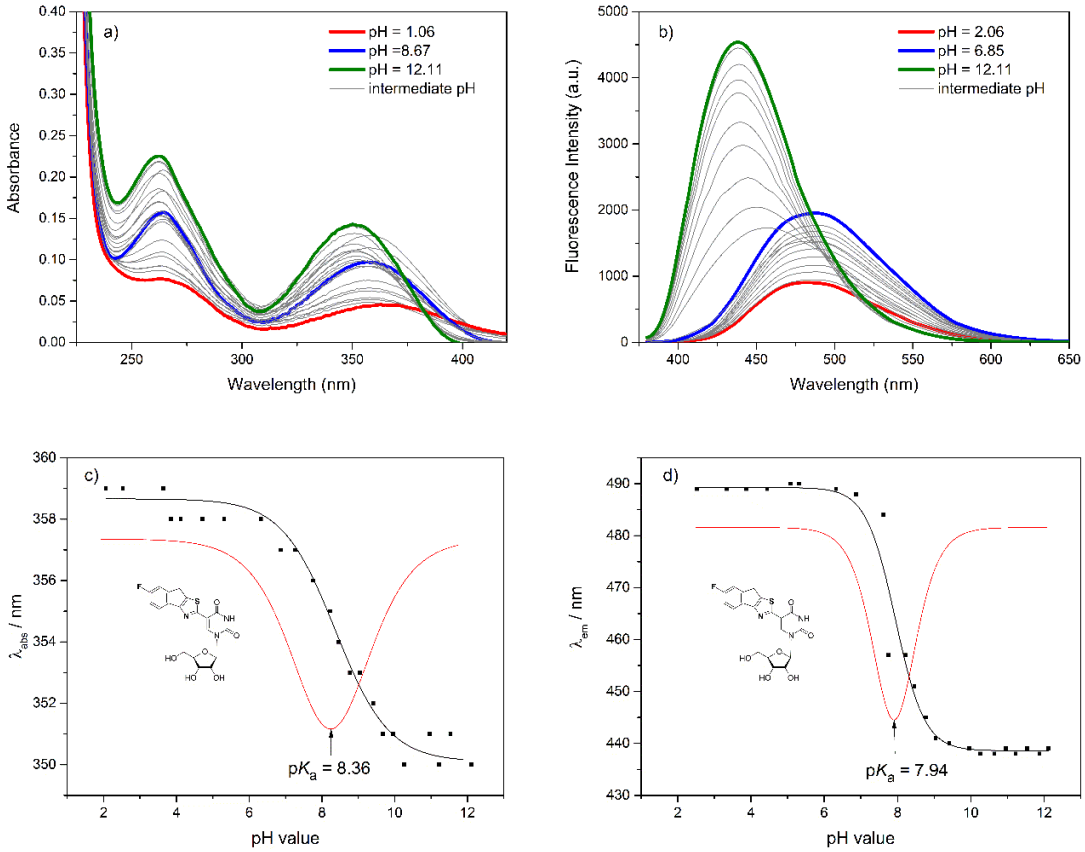


Figure 4. pH-Dependent UV spectra and fluorescence spectra of **7c** measured in 0.1 M sodium phosphate buffer with (a) UV-spectra at pH values from 1.99 to 12.03 (b) Emission spectra at pH values from 1.99 to 12.03. (c) Graph of the absorption (abs) maxima against pH value (black line) and its first derivative (red line) using data from (a). (d) Graph of the emission (em) maxima against pH value (black line) and its first derivative (red lines) using data from (b).

Table 2. pK_a values of uridine and its derivatives

Compound	U[31, 32]	7a	7b	7c	7d	7e	7g	7h	7j	7k	7l	7m
pK_a (abs)	9.20-9.25	8.66	8.40	8.36	8.30	8.32	8.91	7.74	8.41	8.04	8.32	8.86
pK_a (em)		8.62	8.51	7.94	8.21	8.08	8.46	8.15	8.73	8.31	8.19	8.43

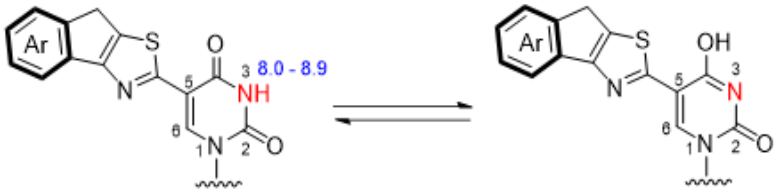


Figure 5. Schematic representation of possible pyrimidine nucleoside tautomers with labelled nitrogen atoms (red) and suggested p*K*a values (blue) corresponding to possible deprotonation sites.

Conclusion

In conclusion, the new fluorescent uridine analogues whose emission maximum is in the visible region are obtained. They exhibit remarkable solvent and pH dependent fluorescence, indicating their promising ability to be fluorescent probes. We have also described a novel protocol for the preparation of 5-thioamide-uridine, a valuable intermediate for polyaromatic heterocycle. Further studies on the application of new uridine analogues are currently underway in our laboratory.

Experimental Section

General Experimental Methods. All materials and solvents were of laboratory grade as obtained from commercial suppliers (Energy Chemical, Leyan, Tianjin ZhiYuan Reagent Co. Ltd) and were used without further purification. Thin-layer chromatography (TLC) was covered with silica gel 60 F254 (0.2 mm). Flash column chromatography (FC): silica gel (40−60 μM) at 0.4 bar. ^{1}H NMR spectra were recorded on 400 MHz spectrometers (Bruker). The *J* values are given in Hz, and δ values are in ppm relative to Me_4Si as internal standard residual solvent peaks were used as internal standards: DMSO (quint, δ^H = 2.50 ppm). ^{13}C-NMR spectra were recorded on 100 MHz spectrometers (Bruker); δ relative to DMSO (δ 40.5 ppm).The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, m = multiplet, dd = doublet-

doublet, ddd = doublet- doublet -doublet, dt = doublet-triplet, dq =doubletquartet, br = broad. Mass spectra were obtained on a quadrupole ion trap instrument equipped with an atmospheric pressure ion source (Agilent 6230B).

Photophysics. For all photophysical experiments, fluorescent nucleosides **7a-m** were prepared as 20 mM DMSO stock solutions and stored at -20 °C. Measurements were performed with spectrophotometric grade 1, 4-dioxane, DMSO, ethanol, methanol and deionized H_2O. For all values reported, final DMSO concentrations after dilution were always less than 0.1%. Absorption spectra were measured on a UV spectrophotometer (Speco5d50 Plus, Analytik Jena AG, Germany) with 1 nm resolution and corrected for the blank. Fluorescence spectra were recorded on a fluorescence spectrophotometer (F-4500, Hitachi, Japan) with a 1 nm resolution. Molar extinction coefficient measurements were conducted in deionized H_2O using Beer's Law plots of concentration versus absorbance. Quantum yield measurements were performed with optical densities adjusted to 0.1 ± 0.05 at the indicated λ_{abs} of each nucleoside and corresponding standard quinine sulfate in 0.5 M H_2SO_4 (Φ_{fl} = 0.55) was used as the standard for all compounds **7a-m**.[33] The relative quantum yields of each compound were determined using the formula: $\Phi_{ref} = \Phi_s \times (A_s/A_{ref}) \times (I_{ref}/I_s) \times (n_{ref}^2/n_s^2)$, Here Φ, A, I. and *n* stand for quantum yield, integrated emission intensity, optical density at λ_{ex} and refractive index (n_{water} = 1.333, $n_{dioxane}$ = 1.417, $n_{methanol}$ = 1.326, $n_{ethanol}$ = 1.361, n_{DMSO} = 1.480), respectively. Sample and reference are denoted by s and ref, respectively. [34]

5-Cyano-1-[(2′,3′,5′-O-tri-acetyl)-*β*-D-ribofuranosyl)]-uracil (4).

5-Iodouridine[35] (15.0 g, 40.5 mmol) was dissolved in pyridine (80 mL) and Ac_2O (17.0 g, 162.0 mmol, 5 eq.) was added. The mixture was stirred at at room temperature overnight. When the reaction was completed, the solvent was removed in vacuo. The resultant mixture was extracted with CH_2Cl_2 (80 mL × 3). The combined organic layers were washed with brine (100 mL × 3) and dried over $MgSO_4$. The solvent was removed in vacuum and the residue was suspended in HMDS (100 mL) and heated at 130 °C for 10 h on an oil bath. The excess of HMDS was removed under reduced pressure and the resulting clear colourless oil was dissolved in dry pyridine (200 mL). Cuprous cyanide (25.4 g, 283.5 mmol, 7 eq.) was added and the solution heated under argon for 8 h at 120 °C on an oil bath. Pyridine was removed under reduced pressure and 200 mL of ethyl acetate was added .The solution was filtered through a pad of

celite and extensively washed with ethyl acetate. The filtrate was washed by water (100 mL × 3) and the organic layers were combined and dried with Na_2SO_4 and concentrated on a rotary evaporator. The residue was purified by column chromatography on silica gel (Petroleum ether/EtOAc, 2:1 to 1:1) to give the compound **4** as light yellow foam (13.0 g, 83%). TLC (silica gel, CH_2Cl_2/MeOH, 20:1) R_f 0.50; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (br s, 1H, NH), 8.67 (s, 1H), 5.88 (d, *J* = 4.0 Hz, 1H), 5.50 (dd, *J* = 6.3Hz, 4.0 Hz, 1H), 5.36 (dd, *J* = 6.2 Hz, 1H), 4.42 – 4.21 (m, 3H), 2.14 – 1.99 (m, 9H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 170.0, 169.2, 159.9, 150.5, 148.9, 114.0, 89.2, 88.9, 79.2, 72.5, 68.8, 62.5, 20.5, 20.2, 20.2; HR-ESI MS (*m*/*z*): [M - H]$^-$ calcd for $C_{16}H_{16}N_3O_9$ 394.0892, found 394.0915.

5-Carbothioamide-1-[(2′,3′-O-di-acetyl-*β*-D-ribofuranosyl)]-uracil (5).

A solution of P_2S_5 (4.9 g, 11.1 mmol, 2 eq.) in EtOH (80 mL) was stirred at rt. for 1 h. 5-Cyano-(2′, 3′, 5′-triaceyl)-Uridine (2.2 g, 5.6 mmol) was added and the resulting solution was stirred at 85 °C for 7 h on an oil bath. The mixture was concentrated in vacuo and purified by a silica gel column (methanol/ dichloromethane 1:80) to give the compound **5** as a yellow solid (1.4 g, 65%). TLC (silica gel, CH_2Cl_2/MeOH, 20:1) R_f 0.45; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05 (s, 1H), 10.14 (d, *J* = 4.4 Hz, 1H), 9.90 (d, *J* = 4.4 Hz, 1H), 9.30 (s, 1H), 6.06 (d, *J* = 5.5 Hz, 1H), 5.46 (t, *J* = 5.5 Hz, 1H), 5.35 (dd, *J* = 5.6 Hz, 3.7 Hz, 1H), 4.25 – 4.21 (m, 1H), 4.02 – 3.91(m, 1H), 3.67 (t, *J* = 3.6 Hz, 2H), 2.10 (s, 3H), 2.04 (s, 3H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 191.3, 169.4, 169.3, 162.4, 150.2, 149.2, 110.3, 87.5, 83.6, 73.3, 71.3, 71.1, 60.8, 20.4, 20.3; HR-ESI MS (m/z): [M + H]$^+$ calcd for $C_{14}H_{18}N_3O_8S$ 388.0809, found 388.0778.

The synthesis of 2-bromo 1-indanones (**6c**, **6e – m**):

To a solution of indanones (2 mmol) in ethyl acetate (15 mL) was added with copper (II) bromide (4 mmol, 2eq.) and refluxed on an oil bath until the reaction completed (monitored by TLC), The solution was filtered and concentrated. The resultant mixture was extracted with ethyl acetate (10 mL × 3). The combined organic layers were washed with brine (30 mL × 3) and dried over $MgSO_4$ and removed in *vacuo*. The residue was purified by FC (silica gel; petroleum ether / ethyl acetate, 25:1). The compound **6f** was purified by recrystallization from ethanol. The compound **6j** was used in the next step without further purification because it was easy to decompose.

2-bromo-5-fluoro-2,3-dihydro-1*H*-inden-1-one (6c)

Compound **6c** (360 mg, 78%) was obtained as a white solid. TLC (silica gel, n-Hexane / ethyl acetate, 10:1) R_f 0.52; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (dd, J = 8.5 Hz, 5.4 Hz, 1H), 7.47 – 7.42 (m, 1H), 7.37 – 7.30 (m, 1H), 5.03 (dd, J = 7.4 Hz, 2.9 Hz, 1H), 3.89 (dd, J = 18.5, 7.4 Hz, 1H), 3.32 (dd, J = 18.6 Hz, 2.8 Hz, 1H). The NMR data are identical to earlier reported literature values.[36]

2-bromo-6-methoxy-2,3-dihydro-1*H*-inden-1-one (6e)

Compound **6e** (400 mg, 83%) was obtained as a white solid. TLC (silica gel, n-Hexane / ethyl acetate, 5:1) R_f 0.50; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49 (d, J = 8.4 Hz, 1H), 7.35 (dd, J = 8.4 Hz, 2.6 Hz, 1H), 7.19 (d, J = 2.6 Hz, 1H), 5.03 (dd, J = 7.2 Hz, 2.9 Hz, 1H), 3.86 – 3.79 (m, 7H), 3.80 – 3.76 (m, 1H), 3.23 (dd, J = 17.9 Hz, 2.7 Hz, 1H). The NMR data are identical to earlier reported literature values.[37]

2-bromo-5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one (6f)

Compound **6f** (440 mg, 81%) was obtained as a yellow solid. TLC (silica gel, n-Hexane / ethyl acetate, 1:1) R_f 0.53; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.14 (d, J = 9.7 Hz, 2H), 4.96 (dd, J = 7.2 Hz, 2.7 Hz, 1H), 3.89 (s, 3H), 3.82 (s, 3H), 3.80 – 3.73 (m, 1H), 3.21 (dd, J = 18.1 Hz, 2.5 Hz, 1H). The NMR data are identical to earlier reported literature values.[38]

6-bromo-2,3,5,6-tetrahydro-7*H*-indeno[5,6-*b*]furan-7-one (6g)

Compound **6g** (430 mg, 85%) was obtained as a white solid. TLC (silica gel, n-Hexane / ethyl acetate, 5:1) R_f 0.54; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41 (s, 1H), 6.95 (s, 1H), 5.00 (dd, J = 7.2 Hz, 2.8 Hz, 1H), 4.61 (t, J = 8.7 Hz), 3.77 (dd, J = 17.8 Hz, 7.2 Hz, 1H), 3.28 (t, J = 8.6 Hz, 2H), 3.20 (dd, J = 17.6 Hz, 2.6 Hz, 1H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 198.6, 160.08, 145.1, 138.9, 133.1, 123.1, 102.0, 71.6, 46.2, 37.0, 29.3; HR-ESI MS (m/z): $[M + H]^+$ calcd for $C_{12}H_7BrO$ 252.9859 found 252.9861.

2-bromo-2,3-dihydro-1*H*-cyclopenta[*a*]naphthalen-1-one (6h)

Compound **6h** (460 mg, 88%) was obtained as a white solid. TLC (silica gel, n-Hexane / ethyl acetate, 10:1) R_f 0.54; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (d, J = 8.3 Hz, 1H), 8.32 (d, J = 8.4 Hz, 1H), 8.09 (d, J = 8.1 Hz, 1H), 7.74 – 7.79 (m, 1H), 7.66 (t, J = 7.6 Hz, 2H), 5.11 (dd, J = 7.1 Hz, 2.6 Hz, 1H), 3.99 (dd, J = 18.6 Hz, 7.1 Hz, 1H), 3.45 (dd, J = 18.7 Hz, 2.6 Hz, 1H); ^{13}C{^{1}H} NMR (100 MHz,

DMSO-d_6) δ 199.9, 155.4, 137.2, 132.4, 129.5, 128.8, 128.6, 127.1, 126.8, 124.1, 122.7, 45.9, 37.8; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for $C_{13}H_9BrO$ 260.9910 found 260.9911.

2-bromoacenaphthylen-1(2*H*)-one (6i)

Compound **6i** (440 mg, 91%) was obtained as a yellow solid. TLC (silica gel, n-Hexane / ethyl acetate, 15:1) R_f 0.45; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (d, *J* = 8.1 Hz, 1H), 8.08 (d, *J* = 7.3 Hz, 2H), 7.89 (dd, *J* = 8.1 Hz, 7.0 Hz, 1H), 7.83 – 7.74 (m, 2H), 6.20 (s, 1H). The NMR data are identical to earlier reported literature values.[39]

5-bromo-5,6-dihydro-4*H*-cyclopenta[*b*]thiophen-4-one (6k)

Compound **6k** (380 mg, 87%) was obtained as a white solid. TLC (silica gel, n-Hexane / ethyl acetate, 10:1) R_f 0.52; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (d, *J* = 5.1 Hz, 1H), 7.24 (d, *J* = 5.1 Hz, 1H), 5.22 (dd, *J* = 6.7 Hz, 2.4 Hz, 1H), 3.99 (dd, *J* = 18.5 Hz, 6.7 Hz, 1H), 3.42 (dd, *J* = 18.5 Hz, 2.1 Hz, 1H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 191.1, 168.0, 141.4, 133.5, 119.4, 119.4, 49.4, 36.3; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for C_7H_5BrOS 216.9317 found 216.9320.

5-bromo-4,5-dihydro-6*H*-cyclopenta[*b*]thiophen-6-one (6l)

Compound **6l** (375 mg, 85%) was obtained as a white solid. TLC (silica gel, n-Hexane / ethyl acetate, 10:1) R_f 0.52; ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.46 (d, J = 4.7 Hz, 1H), 7.26 (d, J = 4.8 Hz, 1H), 5.23 (dd, J = 6.7 Hz, 2.4 Hz, 1H), 3.82 (dd, J = 18.1 Hz, 6.7 Hz, 1H), 3.24 (dd, J = 18.1 Hz, 2.4 Hz, 1H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-*d6*) δ 190.15, 166.10, 143.99, 136.50, 124.74, 49.35, 36.10; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for C_7H_5BrOS 215.9244 found 216.9320.

5-bromo-5,6-dihydro-4*H*-cyclopenta[*b*]thiophen-4-one (6m)

Compound **6m** (375 mg, 85%) was obtained as a white solid. TLC (silica gel, n-Hexane / ethyl acetate, 10:1) R_f 0.52; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (d, *J* = 2.0 Hz, 1H), 6.80 (d, *J* = 2.0 Hz, 1H), 5.19 (dd, *J* = 6.5 Hz, 2.1 Hz, 1H), 3.85 (dd, *J* = 18.4 Hz, 6.5 Hz, 1H), 3.29 (dd, *J* = 18.5, 1.9 Hz, 1H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 188.6, 179.7, 151.8, 124.2, 105.4, 50.1, 34.3. HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for $C_7H_5BrO_2$ 200.9546, found 200.9561.

The synthesie of compounds (**7a - m**):

Compounds **6a - l** (1.2 mmol, 1.1 eq.) were added to a solution of mixture of compound **5** (300 mg, 0.77 mmol) in 20 mL of ethanol. The reaction mixture was stirred for 3 h at 85 °C on an oil bath. After the mixture was cooled to room temperature and concentrated in vacuo. The residue was treated with 0.05 M K_2CO_3 (in methanol, 20 mL) and the mixture was refluxed for 10 mins to remove the protecting groups. The organic solvent was neutralized with a solution of HCl (1N) and evaporated to dryness. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 10:1) or flash chromatography using a C18 reverse phase column (C18 Redi*SepRf* 43 g column, 10–25% acetonitrile in water for 30 min).

5-(4,5-Diphenylthiazole-2-yl)-1-(*β*-D-*ribofuranosyl*)–uracil (7a).

Compound **7a** (300 mg, 81%) was obtained as a white solid. TLC (silica gel, CH_2Cl_2/MeOH, 5:1) R_f 0.64; UV (MeOH): λ_{max} 343 nm (22000); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (br s, 1H), 9.07 (s, 1H), 7.69 – 7.48 (m, 2H), 7.46 – 7.24 (m, 8H,), 5.94 (d, *J* = 4.7 Hz, 1H), 5.52 (d, *J* = 5.4 Hz, 1H,), 5.23 – 5.12 (m, 2H), 4.21 (dd, *J* = 10.0 Hz, 4.8 Hz, 1H), 4.08 (dd, *J* = 10.0 Hz, 5.0 Hz, 1H), 4.00 – 3.93 (m, 1H), 3.78 – 3.63 (m, 2H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 159.9, 161.4, 155.9, 149.6, 147.8, 139.0, 134.5, 132.3, 129.2, 129.0, 128.5, 128.3, 127.7, 107.6, 88.9, 85.1,74.2, 69.8, 60.6; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for $C_{24}H_{22}N_3O_6S$ 480.1224, found 480.1248.

5-(8*H*-indeno[1,2-*d*]thiazol-2-yl)-1-(*β*-D-*ribofuranosyl*)–uracil (7b).

Compound **7b** (297 mg, 80%) was obtained as a light yellow solid. TLC (silica gel, CH_2Cl_2/MeOH, 5:1) R_f 0.59; UV (MeOH): λ_{max} 355 nm (22900); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (br s, 1H), 9.12 (s, 1H), 7.77 (d, *J* = 7.3 Hz, 1H), 7.57 (d, *J* = 7.4 Hz, 1H), 7.39 (t, *J* = 7.4 Hz, 1H), 7.31 – 7.22 (m, 1H), 5.91 (d, *J* = 4.6 Hz,1H), 5.50 (d, *J* = 5.3 Hz, 1H), 5.25 (t, *J* = 4.5 Hz,1H), 5.16 (d, *J* = 5.3 Hz, 1H), 4.20 (dd, *J* = 9.9 Hz, 4.9 Hz, 1H), 4.07 (dd, *J* = 9.9 Hz, 4.9 Hz, 1H), 3.99 – 3.97 (m, 1H), 3.92 (s, 2H), 3.82 – 3.74, 3.72 – 3.64 (2m, 2H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 166.0, 159.3,155.5, 146.4, 137.6, 137.2, 136.2, 126.7, 125.1, 124.7, 118.2, 108.0, 89.1, 84.59,7.78, 70.1, 61.3, 31.9; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for $C_{19}H_{18}N_3O_6S$ 416.0911, found 416.0911.

5-(6-Fluoro-8*H*-indeno[1,2-*d*]thiazol-2-yl)-1-*(β-D-ribofuranosyl)*–uracil (7c).

Compound **7c** (275 mg, 82%) was obtained as a white solid. TLC (silica gel, CH2Cl2/MeOH, 5:1) R_f 0.54; UV (MeOH): λ_{max} 358 nm (13400); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.98 (br s, 1H), 9.14 (s, 1H), 7.75 (dd, *J* = 8.3 Hz, 5.3 Hz, 1H), 7.45 (dd, *J* = 9.2 Hz, 2.4 Hz, 1H), 7.29 – 7.19 (m, 1H), 5.92 (d, *J* = 4.5 Hz, 1H), 5.52 (br s, 1H), 5.27 (br s, 1H), 5.16 (br s, 1H), 4.20 (t, *J* =

4.7 Hz, 1H), 4.08 (t, *J* = 4.8 Hz, 1H), 3.98 (d, *J* = 7.5 Hz, 3H), 3.79 (d, *J* = 11.7 Hz), 3.68 (d, *J* = 12.3 Hz, 1H); $^{13}C\{^{1}H\}$ NMR (100 MHz, DMSO-d_6) δ 162.4, 162.0, 161.3, 159.6, 159.0, 149.6, 148.9, 148.8, 139.1, 136.6, 136.6, 133.1, 133.0, 119.5, 119.4, 113.6, 113.41, 113.1, 112.9, 108.1, 88.9, 84.9, 74.3, 69.7, 60.5, 32.4; HR-ESI MS (m/z): [M + H]+ calcd for $C_{19}H_{17}FN_3O_6S$ 434.0817, found 434.0840.

5-(6-Methoxy-8*H*-indeno[1,2-*d*]thiazol-2-yl)-1-(*β*-D-*ribofuranosyl*)–uracil (7d).

Compound **7d** (258 mg, 75%) was obtained as a light yellow solid. TLC (silica gel, CH_2Cl_2/MeOH, 5:1) R_f 0.54; UV (MeOH): λ_{max} 351 nm (15100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.98 (br s, 1H), 9.08 (s, 1H), 7.65 (d, *J* = 8.3 Hz, 1H), 7.20 (d, *J* = 2.3 Hz, 1H), 6.96 (dd, *J* = 8.3 Hz, 2.4 Hz, 1H), 5.92 (d, *J* = 4.7 Hz, 1H), 5.51 (br s, 1H), 5.26 (t, *J* = 4.5 Hz, 1H), 5.17 (br s, 1H), 4.20 (d, *J* = 5.1 Hz, 1H), 4.08 (t, *J* = 4.8 Hz, 1H), 3.98 (dd, *J* = 4.8 Hz, 2.8 Hz, 1H), 3.90 (s, 2H), 3.83 – 3.74 (m, 4H, CH_3), 3.72 – 3.64 (m, 1H); $^{13}C\{^{1}H\}$ NMR (100 MHz, DMSO-d_6) δ 161.8, 161.19, 159.9, 157.9, 149.5, 148.3, 138.7, 134.8, 129.6, 119.1, 112.3, 111. 7, 108.3, 88.8, 84.9, 74.2, 69.8, 60.6, 55.3, 32.2; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for $C_{20}H_{20}N_3O_7S$ 446.1016, found 446.1029.

5-(5-Methoxy-8*H*-indeno[1,2-*d*]thiazol-2-yl)-1-(*β*-D-*ribofuranosyl*)–uracil (7e).

Compound **7e** (265 mg, 77%) was obtained as a white solid. TLC (silica gel, CH_2Cl_2/MeOH, 5:1) R_f 0.59; UV (MeOH): λ_{max} 358 nm (12000); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.00 (br s, 1H), 9.13 (s, 1H), 7.45 (d, *J* = 8.3 Hz, 1H), 7.33 (d, *J* = 2.5 Hz, 1H), 6.83 (dd, *J* = 8.3 Hz, 2.5 Hz, 1H), 5.93 (d, *J* = 4.7 Hz, 1H), 5.52 (br s, 1H), 5.31 (t, *J* = 4.3 Hz, 1H), 5.17 (br s, 1H), 4.22 (t, *J* = 4.8 Hz, 1H), 4.09 (t, *J* = 4.8 Hz, 1H), 4.01 – 3.95 (m, 1H), 3.90 – 3.75 (m, 6H, CH_3, CH_2), 3.72 – 3.65 (m, 1H); $^{13}C\{^{1}H\}$ NMR (100 MHz, DMSO-d_6) δ 162.1, 161.3, 159.8, 158.8, 149.6, 139.0, 138.3, 138.2, 137.78, 125.7, 110.837, 108.2, 104.7, 88.8, 84.9, 74.2, 69.7, 60.5, 55.3, 31.4; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for $C_{20}H_{20}N_3O_7S$ 446.1016, found 446.1032.

5-(5,6-Dimethoxy-8*H*-indeno[1,2-*d*]thiazol-2-yl)-1-(*β*-D-*ribofuranosyl*)-uracil (7f).

Compound **7f** (240 mg, 65%) was obtained as a bright yellow solid. TLC (silica gel, CH_2Cl_2/MeOH, 5:1) R_f 0.54; UV (MeOH): λ_{max} 371 nm (21200); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (br s, 1H), 9.08 (s, 1H), 7.34 (s, 1H), 7.25 (s, 1H), 5.92 (d, *J* = 4.9 Hz, 1H), 5.51 (d, *J* = 5.4 Hz, 1H), 5.29 (t, *J* = 4.4 Hz, 1H), 5.16 (d, *J* = 5.3 Hz, 1H), 4.22 (dd, *J* = 10.1 Hz, 5.0 Hz, 1H), 4.09 (dd, *J* = 9.8 Hz, 4.9 Hz, 1H), 4.00 – 4.95 (m, 1H), 3.86 (s, 3H), 3.84 (s, 2H), 3.81 (s,

3H), 3.80 – 3.75, 3.72 – 3.64 (2m, 2H); $^{13}C\{^{1}H\}$ NMR (100 MHz, DMSO-d_6) δ 161.6, 161.9, 160.3, 149.5, 148.3, 147.5, 138.7, 138.7, 135.4, 129.3, 110.0, 108.4, 103.2, 88.8, 84.9, 74.0, 69.8, 60.5, 55.9, 55.8, 31.9; HR-ESI MS (m/z): $[M + H]^+$ calcd for $C_{21}H_{22}N_3O_8S$ 476.1122, found 476.1123.

5-(7,9-Dihydro-6*H*-furo[3',2':5,6]indeno[1,2-d]thiazol-2-yl)-1-(*β*-D-*ribofuranosyl*)-uracil (7g).

Compound **7g** (265 mg, 75%) was obtained as a white solid. TLC (silica gel, CH_2Cl_2/MeOH, 5:1) R_f 0.65; UV (MeOH): λ_{max} 364 nm (11200); ^1H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H, H-6), 7.27 (s, 1H), 7.05 (s, 1H), 5.99 (d, *J* = 5.4 Hz, 1H), 5.23 (br s, 1H), 4.66 – 4.50 (m, 2H), 4.20 (t, *J* = 5.4 Hz, 1H), 4.06 (t, *J* = 4.7 Hz, 1H), 3.93 (dd, *J* = 7.5 Hz, 3.7 Hz 1H), 3.75 – 3.63 (m, 2H), 3.52 – 3.41 (m, 1H), 3.25 – 3.16 (t, *J* = 8.9 Hz, 2H); $^{13}C\{^{1}H\}$ NMR (100 MHz, DMSO-d_6) δ 168.8, 165.4, 159.1, 159.0, 155.7, 138.4, 137.5, 137.1, 136.4, 123.7, 121.6, 108.1, 99.6, 88.8, 84.6, 73.9, 71.1, 70.1, 61.2, 31.2, 29.1; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for $C_{21}H_{20}N_3O_7S$ 458.1016, found. 458.0948.

5-(7*H*-benzo[6,7]indeno[1,2-*d*]thiazol-9-yl)-1-(*β*-D-*ribofuranosyl*)-uracil (7h).

Compound **7h** (288 mg, 80%) was obtained as a light green solid. TLC (silica gel, CH_2Cl_2/MeOH, 5:1) R_f 0.65; UV (MeOH): λ_{max} 370 nm (12900); ^1H NMR (400 MHz, DMSO-d_6) δ 9.19 (d, *J* = 8.2 Hz, 1H), 8.75 (s, 1H), 8.00 (d, *J* = 8.2 Hz, 1H), 7.76 (d, *J* = 8.3 Hz, 1H), 7.72 – 7.64 (m, 1H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.49 (d, *J* = 8.3 Hz, 1H), 6.11 (d, *J* = 5.6 Hz, 1H), 5.31 (br s, 2H), 4.37 (t, *J* = 5.4 Hz, 1H), 4.17 (t, *J* = 4.3 Hz, 1H), 4.05 – 3.95 (m, 1H), 3.81 (s, 2H), 3.69 (d, *J* = 22.4 Hz, 1H), 3.30 – 3.15 (m, 1H); $^{13}C\{^{1}H\}$ NMR (100 MHz, DMSO-d_6) δ 169.8, 166.0, 159.9, 156.6, 143.7, 137.4, 136.2, 132.6, 132.3, 127.8, 126.4, 125.9, 125.4, 125.1, 124.3, 123.5, 108.2, 88.7, 85.2, 74.0, 70.7, 61.9, 32.3; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for Chemical Formula: $C_{23}H_{20}N_3O_6S$ 466.1067 found. 466.1083.

5-(Acenaphtho[1,2-*d*]thiazol-8-yl)–1-(*β*-D-*ribofuranosyl*)-uracil (7i).

The residue was purified by flash chromatography using a C18 reverse phase column (C18 Redi*SepRf* 43 g column, 10-20% acetonitrile in water for 30 min) to afford the compound **7i** (185 mg, 53%) as a bright yellow solid. TLC (silica gel, CH_2Cl_2/MeOH, 5:1) R_f 0.13; UV (MeOH): λ_{max} 348 nm (29300); ^1H NMR (400 MHz, DMSO-d_6) δ 12.02 (br s, 1H), 9.21 (s, 1H), 8.09 (d, *J* = 6.8 Hz, 1H), 8.03 – 7.90 (m, 3H), 7.68 (ddd, *J* = 19.9, 8.3, 6.9 Hz, 2H), 5.94 (d, *J* = 4.5 Hz, 1H), 5.55 (br s,1H), 5.31(br s, 1H), 5.20 (br s, 1H), 4.24 (t, *J* = 4.7 Hz, 1H), 4.12 (t, *J* = 4.9 Hz, 1H), 4.04 – 3.9 8 (m, 1H), 3.88 – 3.78, 3.77 – 3.67 (2m, 2H); $^{13}C\{^{1}H\}$ NMR (100 MHz, DMSO-

d_6) δ 161.8, 161.5, 159.6, 149.6, 139.3, 134.1, 131.6, 130.4, 128.8, 128.2, 127.9, 127.6, 127.3, 122.7, 121.6, 108.1, 89.0, 84.9, 74.3, 69.7, 60.5; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for $C_{22}H_{18}N_3O_6S$ 452.0911, found. 452.0909.

5-(8*H*-thiazolo[5',4':4,5]cyclopenta[1,2-*b*]pyridin-2-yl)-1-(*β*-D-*ribofuranosyl*)-uracil (7j).

The residue was purified by flash chromatography using a C18 reverse phase column (C18 column, 10-30% acetonitrile in water for 30 min) to afford compound **7j** (132 mg, 41%) as a gray solid .TLC (silica gel, CH_2Cl_2/MeOH, 5:1) R_f 0.65; UV (MeOH): λ_{max} 346 nm (13100); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 – 8.40 (m, 2H), 7.85 (d, *J* = 7.1 Hz, 1H), 7.18 (dd, *J* = 7.5 Hz, 5.0 Hz, 1H), 5.92 (d, *J* = 5.7 Hz, 1H), 5.12 (br s, 2H), 4.14 (t, *J* = 5.6 Hz, 1H), 3.99 (t, *J* = 4.8 Hz, 1H), 3.92 – 3.80 (m, 3H), 3,66 – 3.56 (m, 2H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 169.4, 166.8, 158.5, 156.2, 155.6, 147.2, 141.7, 140.3, 137.6, 132.0, 119.3, 108.0, 88.5, 84.9, 73.5, 70.3, 61.7, 29.8; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for $C_{18}H_{17}N_4O_6S$ 417.0863, found. 417.0867.

5-(7*H*-thieno[3',2':4,5]cyclopenta[1,2-*d*]thiazol-2-yl)-1-(*β*-D-*ribofuranosyl*)-uracil (7k).

Compound **7k** (143 mg, 44%) was obtained as a bright yellow solid.TLC (silica gel, CH_2Cl_2/MeOH, 5:1) R_f 0.65; UV (MeOH): λ_{max} 371 nm (11400); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (br s, 1H), 8.91 (s, 1H), 7.53 (d, *J* = 4.8 Hz, 1H), 7.23 (d, *J* = 4.8 Hz, 1H), 5.91 (d, *J* = 5.2 Hz, 1H), 5.49 (d, *J* = 5.5 Hz, 1H), 5.23 – 5.11 (m, 2H), 4.21 (dd, *J* = 10.3 Hz, 5.2 Hz, 1H), 4.05 – 4.20 (m, 1H), 3.96 – 3.90 (m, 1H), 3.84 (s, 2H), 3.73 – 3.60 (m, 2H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 161.3, 161.2, 156.0, 150.7, 149.5, 138.6, 137.5, 136.1, 126.1, 123.5, 108.3, 88.6, 85.1, 73.8, 69.8, 60.8, 30.6; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for $C_{17}H_{16}N_3O_6S_2$ 422.0475, found 422.0475.

5-(4*H*-thieno[2',3':4,5]cyclopenta[1,2-*d*]thiazol-2-yl)-1-(*β*-D-*ribofuranosyl*)-uracil (7l).

Compound **7l** (170 mg, 52%) was obtained as a bright yellow solid. TLC (silica gel, CH_2Cl_2/MeOH, 5:1) R_f 0.59; UV (MeOH): λ_{max} 363 nm (10000); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (br s, 1H), 9.02 (s, 1H), 7.63 (d, *J* = 5.0 Hz, 1H), 7.35 (d, *J* = 5.0 Hz, 1H), 5.91 (d, *J* = 4.9 Hz, 1H), 5.51 (d, *J* = 5.4 Hz, 1H), 5.21 (t, *J* = 4.4 Hz, 1H), 5.17 (d, *J* = 5.2 Hz, 1H), 4.25 – 4.16 (m, 1H), 4.10 – 4.03 (m, 1H), 4.05 – 3.90 (m, 3H), 3.80 – 3.71, 3.69 – 3.61 (2m, 2H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 161.2, 160.7, 157.1, 149.5, 145.5, 141.7, 138.5, 137.0, 129.0, 117.8, 108.4, 88.7, 84.9, 74.0, 69.8, 60.6, 31.1; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for $C_{17}H_{16}N_3O_6S_2$ 422.0475, found 422.0478.

5-(4*H*-furo[2',3':4, 5]cyclopenta[1,2-*d*]thiazol-2-yl)-1-(*β*-D-*ribofuranosyl*)-uracil (7m).

Compound (**6m**) (1.2 mmol, 1.1 eq.) was added to a solution of mixture of compound **5** (300 mg, 0.77 mmol) in 10 mL of DMF. The reaction mixture was stirred for 6 h at 105 °C on an oil bath. After the mixture was cooled to room temperature and concentrated in vacuo. The residue was treated with 0.05 M K_2CO_3 (in methanol, 20 mL) and the mixture was refluxed for 10 mins to remove the protecting groups. The organic solvent was neutralized with a solution of HCl (1N) and evaporated to dryness. The residue purified flash chromatography using a C18 reverse phase column (C18 Redi*SepRf* 43 g column, 10–25% acetonitrile in water for 30 min) to afford compound **7m** (160 mg, 51%) as a light yellow solid. TLC (silica gel, CH_2Cl_2/MeOH, 5:1) R_f 0.54; UV (MeOH): λ_{max} 365 nm (11300); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.74 (s, 1H), 7.78 (d, J = 1.9 Hz, 1H), 6.79 (d, J = 2.0 Hz, 1H), 5.92 (d, J = 5.4 Hz, 1H), 4.18 (t, J = 5.3 Hz, 1H), 4.05 (t, J = 4.7 Hz, 1H), 3.94 – 3.89 (m, 1H), 3.85 (s, 2H), 3.72 – 3.60 (m, 2H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6) δ 164.1, 163.0, 160.8, 153.8, 151.9, 146.8, 137.7, 130.7, 123.3, 108.4, 104.8, 88.7, 84.8, 73.7, 69.9, 61.0, 28.5; HR-ESI MS (*m*/*z*): $[M + H]^+$ calcd for $C_{17}H_{16}N_3O_7S$ 406.0703 found 406.0681.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Description of new compound's sensitivity to polarity and pH, LC chromatograms of compound **7b**, **7c**, **7e** and **7l** at pH 1, 7 and 13 and NMR spectra of new and known compounds.

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