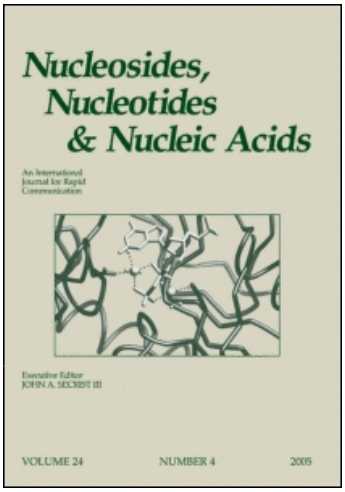

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Synthesis, Protonation Behavior, Conformational Analysis, and Regioselective Enzymatic Acylation of the Novel Diamino Analogue of (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVDU)

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ABSTRACT

(*E*)-3′,5′-Diamino-5-(2-bromovinyl)-2′,3′,5′-trideoxyuridine (**5**), the diamino analogue of BVDU (**1**), was synthesized from BVDU. The protonation behavior of **5** has been studied by means of pH-metric measurements and NMR spectroscopy. This study allows the determination of the basicity constants and the stepwise protonation sites. Thus, the main species at physiological pH is the monoprotonated form. The conformational analysis of this nucleoside analogue was also carried out through ^{1}H NMR spectroscopy. In addition, a convenient synthesis of N-3′ and N-5′ acylated derivatives was developed by regioselective enzymatic acylation. Thus, *Candida antarctica* lipase B (CAL-B) selectively acylated the 5′-amino group, thus furnishing nucleosides **8**. On the other hand, immobilized *Pseudomonas cepacia* lipase (PSL-C) exhibited the opposite selectivity, conferring acylation at the 3′-amino group, thus affording derivatives **9**.

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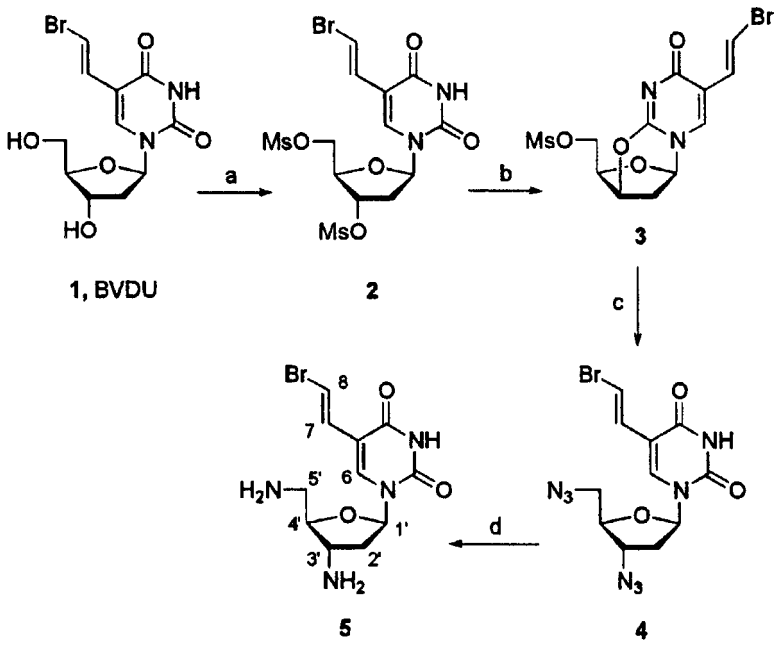
Key Words: Aminodeoxynucleosides; Enzymatic acylation; Lipases; Protonation of diamino-BVDU.

INTRODUCTION

Nucleoside analogues have figured prominently in the search for effective antiviral agents despite concerns over the toxicity generally associated with this class of compounds. This has resulted in an explosion of synthetic activity in the field of nucleosides and in the discovery of a number of derivatives with potent antitumor and antiviral activities.[1] Nucleoside derivatives are present in most of the treatment protocols for human viral infections. Thus, 3′-azido-3′-deoxythymidine (AZT, Zidovudine)[2] was the first anti-HIV nucleoside analogue approved by the FDA to treat AIDS patients.

Various 5-substituted-2′-deoxyuridine derivatives have shown interesting biological properties.[3] Among them (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU, Brivudin®) (**1**, Sch. 1) has emerged as a potent and selective inhibitor of herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV).[4] Its mechanism of action[5] is based on the intracellular phosphorylation to its 5′-diphosphate derivative by HSV-1 or VZV-encoded thymidine kinase (TK), further conversion to the triphosphate derivative by cellular enzymes, and incorporation into viral DNA. Owing to its



(a) MsCl, Py, 0 °C, 37 h (99%); (b) Et_3N, EtOH, 80 °C, 18 h (84%); (c) NaN_3, *p*-$O_2NC_6H_4OH$, DMF, 110 °C, 4h (67%); (d) Ph_3P, THF-H_2O, 30 h, rt (71%).

Scheme 1.

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lower affinity for other cellular and viral TKs, BVDU exhibits low cytotoxicity and poor inhibitory activity against viral infections caused by HSV-2 or TK-lacking HSV-1 strains.

Recently, we have accomplished a short and convenient synthesis of pyrimidine 3′,5′-diaminonucleosides from the parent natural nucleosides.[6] As an extension of this work, here we describe the preparation, for the first time, of (*E*)-3′,5′-diamino-5-(2-bromovinyl)-2′,3′,5′-trideoxyuridine (**5**) from BVDU. Examples of amino sugar nucleosides are known to possess anticancer, antibacterial, and antimetabolic activities.[7] In addition, protonation, conformational analysis, and regioselective enzymatic acylation of this novel derivative were also carried out.

RESULTS AND DISCUSSION

For the synthesis of (*E*)-3′,5′-diamino-5-(2-bromovinyl)-2′,3′,5′-trideoxyuridine (**5**), BVDU (**1**) was first converted into its 3′,5′-di-*O*-mesyl derivative **2** by treatment with methanesulfonyl chloride in pyridine at 0°C (Sch. 1). When this dimesyl derivative was heated under reflux with an excess of Et_3N in EtOH solution, anhydronucleoside **3** was isolated in 84% yield. Conversion of **3** into the diazide **4** was attempted by treatment with sodium azide in DMF solution at 120°C. However, under these conditions **3** was transformed into a new derivative, which was identified by NMR and MS spectroscopy as **7** (Sch. 2). This compound appeared to have been formed by the nucleophilic displacement of bromide by a negatively charged oxygen at the C-4 of the pyrimidine ring (intermediate **III**, Sch. 2). This nucleophilic displacement has been previously observed by Jones and his coworkers[8] in the treatment of 5-(2-bromovinyl) uracil with potassium *tert*-butoxide.

To avoid the formation of **7**, stabilization of the negative charge at N-3 was necessary (intermediate **II**, Sch. 2).

To protonate the negative charge, a compound with a p*K*a lower than the pyrimidine bases (p*K*a between 9–10), but higher than for HN_3 (4.68), which is explosive, should be added to the reaction mixture. Thus, a notable improvement in the reaction of **3** with sodium azide and *p*-nitrophenyl alcohol (p*K*a = 7.15) at 110°C was achieved, affording **4** in 67% yield after flash chromatography. Temperatures below 110°C led to substantial formation of **6**. Subsequent reduction of diazide **4** by Ph_3P gave the diamino nucleoside **5** in 71% yield, since reduction by catalytic hydrogenation afforded side reactions at the double bond in the bromovinyl moiety.

To study the main species present at physiological pH, the behavior of **5** towards protonation was studied using pH-metric measurements and NMR spectroscopy. To determine the stepwise protonation sites we recorded its ^1H and ^{13}C NMR spectra at different pD values (pD = pH + 0.4) (Fig. 1). As a general rule, when pD is decreased, proton atoms in the α-position to protonated nitrogen move downfield while β-C signals move upfield.[9]

In the range pD 12–10, no appreciable variations in the chemical shifts of ^1H and ^{13}C NMR spectra occurred, but between pD 10 and 8 a significant downfield of H-5′ and an upfield of C-4′ were observed. The shift of C-4′ can be ascribed to protonation at N-5′ or N-3′, but the fact that C-2′ did not experience a clear shift suggests that the first proton binding to the molecule occurs mainly at the amino

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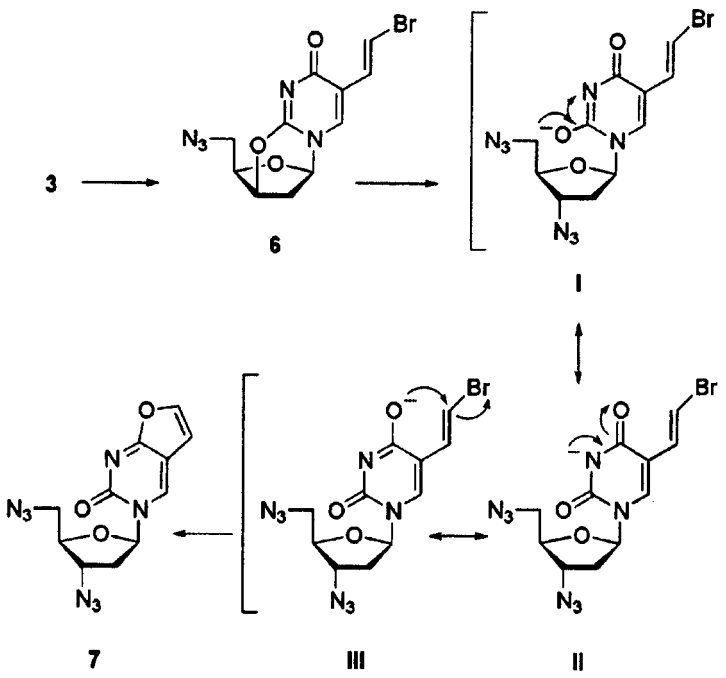

Scheme 2.

group of the 5′-position. In the pD range 8–5, the most significant shifts are shown by H-3′, C-4′, and C-2′, which confirms that the second protonation to be predominantly at the N-3′ position. As seen in Fig. 1, **5** occurs mainly in its diprotonated form at pD = 5.

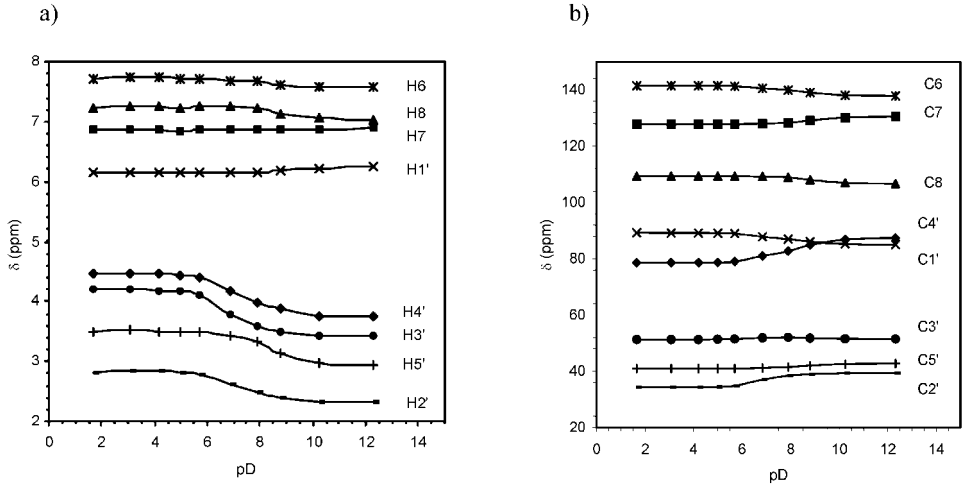

Figure 1. ^{1}H and ^{13}C NMR chemical shifts of **5** as a function of pD.

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The potentiometric titrations were carried out in 0.1 M Me_4NCl aqueous solution at 298 K. From the basicity constants ($pKa = 8.95 \pm 0.03$ corresponding to the first protonation, and $pKa = 6.41 \pm 0.03$ for the second one), the distributions of the protonated species of **5** formed as a function of pH were calculated using the computer program SUPERQUAD (Fig. 2). Between pH 2 and 5, almost exclusively the diprotonated form exists. At a pH of *ca.*7–8.5 the main species in solution is the monoprotonated form, whereas the free amine predominates above pH = 9. Thus, the main species at physiological pH is the monoprotonated form.

We also performed the conformational analysis of 3′,5′-diamino-BVDU to clarify its sugar conformation depending on the degree of protonation of its amino groups. Generally, ^{1}H NMR measurements of nucleoside analogues give useful conformational information. It is well known that the nucleosides and their analogues exist in equilibrium between S- and N-type conformations based on their furanose-ring puckering. ^{1}H-^{1}H coupling constants bear a relationship with furanose ring conformation, and several types of equations to predict the probability of an S-type conformation of the furanose ring were also proposed by Altona and co-workers.[10] In the case of deoxyribose units, the coupling constants can be interpreted in the same manner as for the much commoner ribofuranoses, except that the absence of the 2′-hydroxyl group necessitates the application of a small electronegativity correction to the calculated couplings, in which the protons H-2′ participate. Also, the splitting patter of the H-1′ signal in deoxyribosides yields the sum (J_s) of the coupling $J_{1'2'}(\text{cis}) + J_{1'2'}(\text{trans})$, which already allows one to draw conclusions concerning the equilibrium composition, especially where a series of analogous compounds are compared. Therefore, the equation $S(\%) = 100(J_s - 7.1)/9$ can be obtained.[10c]

Observed coupling constants $J_{1'2'}$(cis) and $J_{1'2'}$(trans) values and calculated *S*(%) values are summarized in Table 1. In these ^{1}H NMR measurements, diprotonated-, monoprotonated- and diamino-BVDU showed *S*(%) values of 67, 57, and 56%, respectively. These results indicate that the deoxyfuranose-ring conformation was predominantly in S-type conformation, the diprotonated species being the highest,

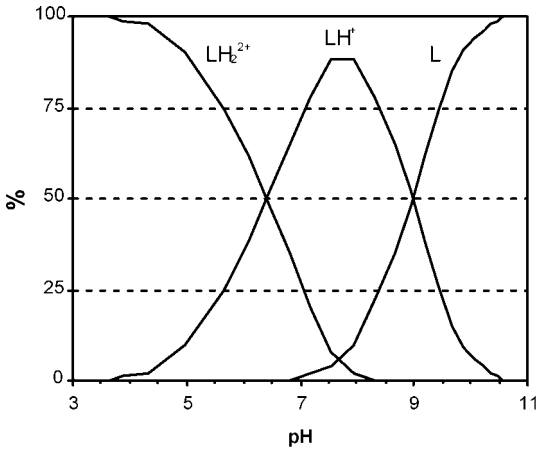


Figure 2. Distribution diagram of protonated species of **5** (=L) as a function of pH.

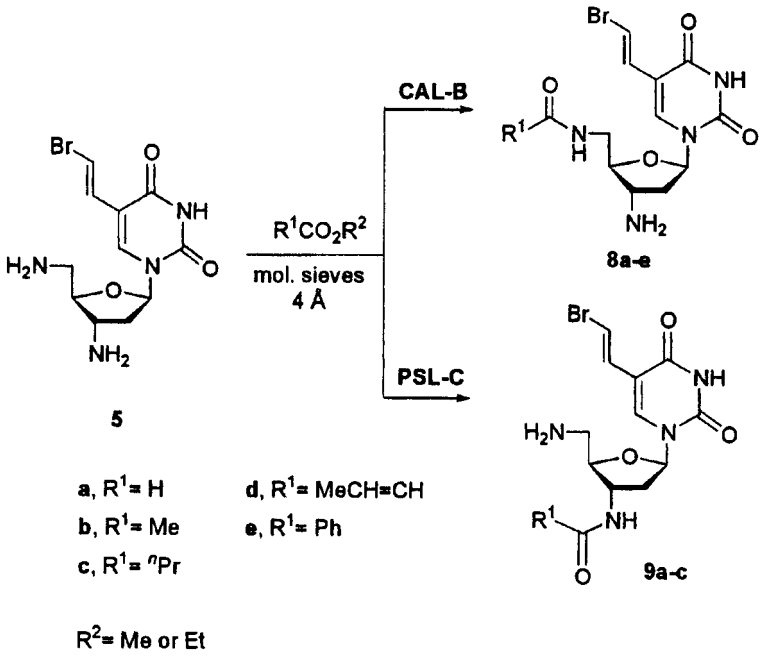
Table 1. Coupling constants and *S*(%) values of species depending on pD.

Species	pD[a]	$J_{1'2'}$(cis) (Hz)	$J_{1'2'}$(trans) (Hz)	J_s (Hz)[b]	*S*(%)[c]
5-H_2^{2+}	1.2	7.7	5.4	13.1	67
5-H^+	7.2	7.4	4.8	12.2	57
5	12.0	7.1	5.0	12.1	56

[a]pD = pH + 0.4.
[b]$J_s = J_{1'2'}(\text{cis}) + J_{1'2'}(\text{trans})$.
[c]$S(\%) = 100(J_s - 7.1)/9$, from Ref.[10c].

probably due to charge repulsions, and, in fact, molecular modelling studies[11] have confirmed this conformation.

Antiviral activity measurements of nucleoside **5** revealed that this compound did not exhibit any appreciable activity against any of the viruses tested.[12] It is known that in some cases a simple acylation in a nucleoside can result in an increase of their biological activity compared with the unmodified derivative.[13] For this reason we describe here the regioselective acylation of the amino groups in **5** by means of biocatalysts (Sch. 3), traditional chemical methods failing to distinguish between them. Similarly to previous reports for other nucleoside derivatives,[6,14] *Candida antarctica* lipase B (CAL-B, Novozym 435) showed excellent regioselectivity toward the 5′-amino group in the acetylation reaction with ethyl acetate, **8b** being isolated with 92% yield after flash chromatography (entry 2, Table 2). It is noteworthy that no



Scheme 3.

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Table 2. Enzymatic acylation of **5** in THF.

		Acylating agent					Isolated yields (%)		
Entry	Enzyme	R^1	R^2	equiv.	T (°C)	t (h)	N-3′	N-5′	N-3′,5′
1	CAL-B	H	Et	2	40	9		49	15
2	CAL-B	Me	Et	8	40	12		92	
3	CAL-B	^{n}Pr	Et	10	40	41		69	
4	CAL-B	MeCH=CH	Me	20	60	33		49	
5	CAL-B	Ph	Me	40	60	144		63	
6	PSL-C	H	Et	5	60	21	38[a]		30
7	PSL-C	Me	Et	50	60	34	79		traces
8	PSL-C	^{n}Pr	Et	50	60	94	55	40	

[a]Isolated as a mixture of monoacylated compounds 5′:3′ (2:1) by ^{1}H NMR.

N-3′ or N-3′,5′ acetylated derivatives were formed. This lipase retains the high selectivity at the 5′-position with butyrate, crotonate, and benzoate esters, giving rise to the corresponding N-5′ acylated nucleosides **8c**–**e** in moderate/high yields (entries 3–5, Table 2). In these cases, due to the lower reactivity of the esters, larger ratios of acylating agents and higher temperatures were used to bring the conversions close to 100%. In spite of this, acylation reactions with methyl benzoate and methyl crotonate revealed that part of the starting material remain unchanged. In contrast, due to the higher reactivity of ethyl formate, CAL-B exhibited lower selectivity with this ester, and **8a** was obtained in 49% yield, together with a considerable amount of N-3′,5′ acylated compound (entry 1, Table 2).

Gratifyingly, selective 3′-amino acetylation was accomplished by reaction of **5** with ethyl acetate and immobilized *Pseudomonas cepacia* lipase (PSL-C) at 60°C, furnishing **9b** in 79% yield (entry 7, Table 2). Traces of diacetylated derivative were formed. On the other hand, when ethyl formate or butyrate were used, the process took place with lower selectivity (entries 6 and 8, Table 2). In the cases of methyl crotonate or benzoate, which have already shown low reactivity with CAL-B, the reaction did not occur.

In summary, we have synthesized (*E*)-3′,5′-diamino-5-(2-bromovinyl)-2′,3′,5′-trideoxyuridine (**5**) from BVDU. The potentiometric study and the analysis of ^{1}H and ^{13}C NMR at different pD values allows the determination of the ionization constants and the sequence of the protonation sites. The first protonation takes place mainly at the 5′-amino group, giving the monoprotonated form as the predominant species in the pH range 7–8.5. At pH = 5, the nucleoside derivative is in its diprotonated form. Conformational analysis revealed that is the diprotonated species in which the deoxyfuranose ring shows the highest percentage of S-type conformation. In order to increase the biological activity of this BVDU derivative, regioselective enzymatic acylation of the amino groups at 3′ and 5′ position was carried out. In particular, CAL-B was found to be selective in catalyzing the acylation of 5′-amino group in diamino derivative **5**. In contrast, regioselective acetylation of the 3′-amino group was accomplished with PSL-C. Biological assays with these acylated compounds are in progress.

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EXPERIMENTAL

Candida antarctica lipase B (CAL-B, Novozym 435, 7300 PLU/g) and immobilized *Pseudomonas cepacia* lipase (PSL-C, 783 U/g) were purchased from suppliers. Melting points were taken on samples in open capillary tubes and are uncorrected. IR spectra were recorded on an Infrared Fourier Transform spectrophotometer using KBr pellets. Flash chromatography was performed using silica gel 60 (230–400 mesh). ^{1}H-, ^{13}C-NMR, and DEPT were obtained using AC-200 (^{1}H, 200.13 MHz and ^{13}C, 50.3 MHz), and AC-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz), or DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) spectrometers for routine experiments. An AMX-400 spectrometer operating at 400.13 and 100.61 MHz for ^{1}H and ^{13}C, respectively, was used for the acquisition of ^{1}H-^{1}H and ^{1}H-^{13}C correlation experiments. The chemical shifts are given in delta (δ) values and the coupling constants (J) in Hertz (Hz). ESI^{+} was used to record mass spectra (MS). Microanalyses were performed on a Perkin-Elmer model 2400 instrument. For the NMR titrations, the samples were prepared with known amounts of the diaminonucleoside, the pD was adjusted by addition of DCl or NaOD solutions in D_2O and the correction $pD = pH^{*} + 0.4$ was used, where pH^{*} is the direct measurement of a pH-meter calibrated with non-deuterated buffer solutions. For the pH-metric titrations, a Metrohm TITROPROCESSOR-636 titrimeter was used, the reference electrode was an Ag/AgCl electrode in sat. aq. KCl, the cell was thermostated at 298 ± 0.1 K, and the measurements were performed under nitrogen atmosphere. The protonation constants were determined by titration with 0.1N NaOH of a solution containing 10^{-3}M of the HCl salt of the diamine in the presence of Me_4NCl (0.1M). The measurements were carried out twice, and the data analysis was performed with the computer program SUPERQUAD.[15]

(*E*)-5-(2-Bromovinyl)-3′,5′-di-*O*-(methanesulfonyl)-2′-deoxyuridine 2. Methanesulfonyl chloride (0.42 mL, 5.4 mmol) was added dropwise to a solution of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (**1**, BVDU, 300 mg, 0.9 mmol) in dry pyridine (5 mL) under nitrogen at 0°C. The mixture was stirred at this temperature for 37 h. The reaction crude was poured into a vigorously stirred ice-water mixture (30–40 mL). The precipitated solid was collected by filtration, washed with cold water (3×5 mL) and dried in vacuo, affording **2** (437 mg, 99%) as a brown solid. R_f (EtOAc) 0.77; mp 142–144°C (decomposed); found: C, 31.7; H, 3.7; N, 5.6. $C_{13}H_{17}N_2O_9S_2Br$ requires C, 31.9; H, 3.5; N, 5.7%; υ_{max}(KBr)/cm^{-1} 3420, 3026, 2937, 1716, 1678, 1617, and 1353; δ_H (300 MHz; DMSO-d_6) 2.70 (2H, m, 2′-H), 3.39 (3H, s, *Me*S), 3.46 (3H, s, *Me*S), 4.53 (1H, m, 4′-H), 4.60 (2H, m, 5′-H), 5.44 (1H, br s, 3′-H), 6.33 (1H, apparent t, 1′-H, J 6.9), 6.98 (1H, d, 7-H, J 13.6), 7.44 (1H, d, 8-H, J 13.6), 7.94 (1H, s, 6-H), and 11.69 (1H, s, NH); δ_C(50.3 MHz, DMSO-d_6) 36.5 (C-2′), 36.9 (*Me*S), 37.7 (*Me*S), 68.4 (C-5′), 79.0 (C-3′), 80.9 (C-4′), 84.7 (C-1′), 107.4 (C-8), 110.4 (C-5), 129.6 (C-7), 139.3 (C-6), 149.3 (C-2), and 161.6 (C-4); m/z (ESI^{+}) 529 [$(M^{81}Br + K)^{+}$, 100%], 527 [$(M^{79}Br + K)^{+}$, 97], 513 [$(M^{81}Br + Na)^{+}$, 52], 511 [$(M^{79}Br + Na)^{+}$, 48], 491 [$(M^{81}Br + H)^{+}$, 12], and 489 [$(M^{79}Br + H)^{+}$, 9].

(*E*)-2,3′-Anhydro-5-(2-bromovinyl)-5′-*O*-(methanesulfonyl)-2′-deoxyuridine 3. Dry triethylamine (0.8 mL, 5.72 mmol) was added to a solution of **2** (400 mg, 0.82 mmol)

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in ethanol (12 mL) under nitrogen, and then was heated, under reflux, for 18 h. The reaction crude was cooled, and the precipitate was collected by filtration and washed with cold ethanol (3×5 mL) affording, after vacuum drying **3** (234 mg, 84%) as a white solid. R_f (20% MeOH/EtOAc) 0.43; mp 161–162°C (decomposed); found: C, 33.9; H, 3.3; N, 6.5. $C_{12}H_{13}N_2O_6SBr$ requires C, 33.9; H, 3.1; N, 6.6%; υ_{max}(KBr)/cm^{-1} 1636, 1617, 1588, 1473, and 1171; δ_H (300 MHz, DMSO-d_6) 2.50–2.70 (2H, m, 2′-H), 3.21 (3H, s, *Me*S), 4.27 (1H, m, 4′-H), 4.50 (2H, m, 5′-H), 5.41 (1H, br s, 3′-H), 5.94 (1H, d, 1′-H, *J* 3.4), 6.83 (1H, d, 7-H, *J* 13.4), 7.55 (1H, d, 8-H, *J* 13.4), and 7.95 (1H, s, 6-H); δ_C (75.5 MHz, DMSO-d_6) 32.7 (C-2′), 36.7 (*Me*S), 68.0 (C-5′), 77.5 (CH), 82.1 (CH), 87.7 (C-1′), 109.4 (C-8), 115.1 (C-5), 130.2 (C-7), 139.7 (C-6), 152.2 (C-2), and 168.3 (C-4); *m/z* (ESI$^+$) 433 [$(M^{81}Br + K)^+$, 100%], 431 [$(M^{79}Br + K)^+$, 95], 417 [$(M^{81}Br + Na)^+$, 53], 415 [$(M^{79}Br + Na)^+$, 50], 395 [$(M^{81}Br + H)^+$, 20], and 393 [$(M^{79}Br + H)^+$, 18].

(*E*)-3′,5′-Diazido-5-(2-bromovinyl)-2′,3′,5′-trideoxyuridine 4. *p*-Nitrophenyl alcohol (435 mg, 3.13 mmol) first, and then sodium azide (1.02 g, 15.64 mmol), were added to a suspension of anhydronucleoside **3** (615 mg, 1.56 mmol) in dry dimethylformamide (20 mL) under nitrogen. The solution was stirred for 4 h at 110°C, then 2 mL of water were added, and the solvent was evaporated in vacuo. The crude residue was disolved in $CHCl_3$, and extracted with water (6×5 mL). The organic layer was dried with Na_2SO_4, filtered off and the solvent was evaporated under reduced pressure. The crude was purified by flash cromatography column (33% EtOAc/Hexane) affording after vacuum drying **4** (400 mg, 67%) as a white solid. R_f (EtOAc) 0.92; mp 118–121°C (decomposed); found: C, 34.2; H, 2.6; N, 29.3. $C_{11}H_{11}N_8O_3Br$ requires C, 34.5; H, 2.9; N, 29.2%; υ_{max}(KBr)/cm^{-1} 3412, 2926, 2105, 1721, 1681, 1617, 1465, and 1276; δ_H (300 MHz, $CDCl_3$) 2.35–2.57 (2H, m, 2′-H), 3.59–3.87 (2H, m, 5′-H), 3.98 (1H, m, 4′-H), 4.25 (1H, m, 3′-H), 6.14 (1H, apparent t, 1′-H, *J* 6.2), 6.67 (1H, d, 7-H, *J* 13.7), 7.41 (1H, d, 8-H, *J* 13.7), 7.55 (1H, s, 6-H), and 9.26 (1H, s, NH); δ_C (75.5 MHz, $CDCl_3$) 38.0 (C-2′), 51.6 (C-5′), 60.0 (C-3′), 82.2 (CH), 85.4 (CH), 110.5 (C-8), 111.8 (C-5), 127.9 (C-7), 137.0 (C-6), 148.8 (C-2), and 161.0 (C-4); *m/z* (ESI$^+$) 423 [$(M^{81}Br + K)^+$, 31%], 421 [$(M^{79}Br + K)^+$, 29], 407 [$(M^{81}Br + Na)^+$, 100], 405 [$(M^{79}Br + Na)^+$, 100], 385 [$(M^{81}Br + H)^+$, 14], and 383 [$(M^{79}Br + H)^+$, 14].

(*E*)-3′,5′-Diamino-5-(2-bromovinyl)-2′,3′,5′-trideoxyuridine 5. Water (43 μL, 2.37 mmol) and triphenylphosphine (342 mg, 1.30 mmol) were added to a solution of **4** (227 mg, 0.59 mmol) in tetrahydrofurane (7 mL). The solution was stirred for 30 h at room temperature. Then the solvent was evaporated and the crude residue was purified by flash chromatography column [1% NH_3(aq.)/MeOH] affording after vacuum drying **5** (140 mg, 71%) as a white solid. R_f [5% NH_3(aq.)/MeOH] 0.30; mp 189–191°C (decomposed); $[\alpha]_D^{20} + 19$ (c 0.5 in MeOH); υ_{max}(KBr)/cm^{-1} 3542, 3466, 3414, 3366, 3286, 2935, 1712, 1674, 1450, and 1283; found: C, 40.1; H, 4.3; N, 17.1. $C_{11}H_{15}N_4O_3Br$ requires C, 39.9; H, 4.6; N, 16.9%; δ_H (300 MHz, MeOH-d_4) 2.35–2.60 (2H, m, 2′-H), 3.07–3.26 (2H, m, 5′-H), 3.55 (1H, m, 3′-H), 3.86 (1H, m, 4′-H), 6.32 (1H, dd, 1′-H, *J* 7.4, *J* 4.9), 7.05 (1H, d, 7-H, *J* 13.4), 7.56 (1H, d, 8-H, *J* 13.4), and 7.97 (1H, s, 6-H); δ_C (75.5 MHz, MeOH-d_4) 41.3 (C-2′), 44.1 (C-5′), 53.2 (C-3′), 86.6 (C-4′), 88.5 (C-1′), 109.0 (C-8), 112.2 (C-5), 130.5 (C-7), 140.5

(C-6), 151.2 (C-2), and 163.9 (C-4); m/z (ESI^+) 355 [$(M^{81}Br + Na)^+$, 30%], 353 [$(M^{79}Br + Na)^+$, 30], 333 [$(M^{81}Br + H)^+$, 97], and 331 [$(M^{79}Br + H)^+$, 100].

(*E*)-2,3′-Anhydro-5′-azido-5-(2-bromovinyl)-2′,5′-dideoxyuridine 6. This compound was obtained as a byproduct in the synthesis of **4** when temperatures beyond 110°C were used. Flash chromatography column (10% MeOH/EtOAc) afforded **6** as a white solid. R_f (20% MeOH/EtOAc) 0.63; mp 202–204°C (decomposed); υ_{max}(KBr)/cm^{-1} 3024, 2108, 1636, 1617, 1592, and 1477; found: C, 38.7; H, 2.9; N, 20.8. $C_{11}H_{10}N_5O_3Br$ requires C, 38.8; H, 3.0; N, 20.6%; δ_H (300 MHz, DMSO-d_6) 2.50–2.70 (2H, m, 2′-H), 3.50–3.65 (2H, m, 5′-H), 4.41 (1H, br s, 4′-H), 5.33 (1H, br s, 3′-H), 5.94 (1H, br s, 1′-H), 6.84 (1H, d, 7-H, J 13.4), 7.54 (1H, d, 8-H, J 13.4), and 7.97 (1H, s, 6-H); δ_C (75.5 MHz, DMSO-d_6) 32.8 (C-2′), 49.9 (C-5′), 77.7 (C-3′), 83.4 (C-4′), 87.6 (C-1′), 109.4 (C-8), 115.1 (C-5), 130.2 (C-7), 139.7 (C-6), 152.3 (C-2), and 168.3 (C-4); m/z (ESI^+) 380 [$(M^{81}Br + K)^+$, 9%], 378 [$(M^{79}Br + K)^+$, 16], 364 [$(M^{81}Br + Na)^+$, 91], and 362 [$(M^{79}Br + Na)^+$, 100].

3-(3′,5′-Diazido-2′,3′,5′-trideoxy-β-D-ribofuranosyl)-2,3-dihydrofuro[2,3-d] pyrimidin-2-one 7. Sodium azide (33 mg, 0.51 mmol) was added to a suspensión of anhydronucleoside **3** (50 mg, 0.13 mmol) in dry DMF (1 mL) under nitrogen. The solution was stirred for 3 h at 120°C, then 100 μL of water were added, and the solvent was evaporated in vacuo. The crude residue was purified by flash chromatography column (10% MeOH/EtOAc) affording after vacuum drying **7** (16 mg, 42%) as a brown solid. R_f (EtOAc) 0.60; mp 103–104°C; υ_{max}(KBr)/cm^{-1} 3100, 2106, 1638, 1617, 1573, and 1393; found: C, 43.7; H, 3.4; N, 37.0. $C_{11}H_{10}N_8O_3$ requires C, 43.7; H, 3.3; N, 37.1%; δ_H (300 MHz, $CDCl_3$) 2.44 (1H, m, 2′-H), 2.80 (1H, m, 2′-H), 3.68–3.95 (2H, m, 5′-H), 4.07 (1H, m, 4′-H), 4.17 (1H, m, 3′-H), 6.21 (1H, dd, 1′-H, J 6.5, J 4.8), 6.58 (1H, d, 7-H, J 2.9), 7.37 (1H, d, 8-H, J 2.9), and 8.49 (1H, s, 6-H); δ_C (75.5 MHz, $CDCl_3$) 38.7 (C-2′), 51.3 (C-5′), 59.2 (C-3′), 82.5 (C-4′), 87.7 (C-1′), 104.5 (C-8), 105.9 (C-5), 136.7 (C-7), 144.8 (C-6), 154.2 (C-2), and 171.8 (C-4); m/z (ESI^+) 341 [$(M + K)^+$, 100%], 325 [$(M + Na)^+$, 22], and 303 [$(M + H)^+$, 7].

General Procedure for the Enzymatic Acylation of 5. Synthesis of 8 and 9. Corresponding ester (ethyl formate, ethyl acetate, ethyl butyrate, methyl crotonate, or methyl benzoate) was added to a suspension of **5** (20 mg, 0.060 mmol), lipase (10 mg of CAL-B or 130 mg of PSL-C), and molecular sieves 4 Å (20 mg) in dry THF (4.5 mL) under nitrogen, and the mixture was stirred at 250 rpm (temperature and reaction time are indicated in Table 2). Then, the enzyme and molecular sieves were filtered off and washed with MeOH (3 × 2 mL). The filtrate was evaporated to dryness, and the crude residue was purified by flash chromatography column [gradient eluent 10% MeOH/EtOAc-MeOH for compounds **8a–e** and gradient eluent 10% MeOH/EtOAc-MeOH-10% NH_3(aq.)/MeOH for compounds **9a–c**]. To crystalize the final products, Et_2O was used.

(*E*)-3′-Amino-5-(2-bromovinyl)-5′-formylamino-2′,3′,5′-trideoxyuridine 8a. R_f [5% NH_3(aq.)/MeOH] 0.65; mp 66–68°C (decomposed); υ_{max}(KBr)/cm^{-1} 3429, 1600, and 1565; found: C, 40.3; H, 4.0; N, 15.8. $C_{12}H_{15}N_4O_4Br$ requires C, 40.2; H,

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4.2; N, 15.6%; δ_H (200 MHz, MeOH-d_4) 2.33–2.60 (2H, m, 2′-H), 3.53 (1H, m, 3′-H), 3.79 (2H, m, 5′-H), 3.92 (1H, m, 4′-H), 6.30 (1H, dd, 1′-H, *J* 7.1, *J* 5.1), 7.08 (1H, d, 7-H, *J* 13.7), 7.57 (1H, d, 8-H, *J* 13.7), 7.98 (1H, s, 6-H), and 8.34 (1H, s, *H*CO); δ_C (50.3 MHz, MeOH-d_4) 40.4 (CH_2), 41.0 (CH_2), 53.3 (C-3′), 86.7 (2C, 2CH), 108.8 (C-8), 112.2 (C-5), 130.7 (C-7), 140.2 (C-6), 151.7 (C-2), 164.3 (C=O), and 164.6 (C-4); *m/z* (ESI^+) 383 [$(M^{81}Br + Na)^+$, 56%], 381 [$(M^{79}Br + Na)^+$, 57], 361 [$(M^{81}Br + H)^+$, 99], and 359 [$(M^{79}Br + H)^+$, 100].

(*E*)-5′-Acetylamino-3′-amino-5-(2-bromovinyl)-2′,3′,5′-trideoxyuridine 8b. R_f [5% NH_3(aq.)/MeOH] 0.69; mp 100–102°C (decomposed); υ_{max}(KBr)/cm^{-1} 3627, 1600, and 1560; found: C, 41.7; H, 4.7; N, 15.2. $C_{13}H_{17}N_4O_4Br$ requires C, 41.9; H, 4.6; N, 15.1%; δ_H (300 MHz, MeOH-d_4) 2.18 (3H, s, *Me*CO), 2.35–2.57 (2H, m, 2′-H), 3.50 (1H, m, 3′-H), 3.75 (2H, m, 5′-H), 3.91 (1H, m, 4′-H), 6.28 (1H, dd, 1′-H, *J* 6.8, *J* 4.2), 7.07 (1H, d, 7-H, *J* 13.7), 7.56 (1H, d, 8-H, *J* 13.7), and 7.92 (1H, s, 6-H); δ_C (75.5 MHz, MeOH-d_4) 22.6 (*Me*CO), 41.0 (CH_2), 41.7 (CH_2), 53.0 (C-3′), 86.8 (CH), 87.0 (CH), 108.7 (C-8), 112.1 (C-5), 130.8 (C-7), 140.2 (C-6), 152.2 (C-2), 165.3 (C-4), and 173.8 (C=O); *m/z* (ESI^+) 397 [$(M^{81}Br + Na)^+$, 100%], 395 [$(M^{79}Br + Na)^+$, 100], 375 [$(M^{81}Br + H)^+$, 18], and 373 [$(M^{79}Br + H)^+$, 19].

(*E*)-3′-Amino-5-(2-bromovinyl)-5′-butyrylamino-2′,3′,5′-trideoxyuridine 8c. R_f [5% NH_3(aq.)/MeOH] 0.51; mp 72–74°C (decomposed); υ_{max}(KBr)/cm^{-1} 3510, 1698, 1607, 1568, and 1472; found: C, 45.1; H, 5.3; N, 13.7. $C_{15}H_{21}N_4O_4Br$ requires C, 45.0; H, 5.3; N, 14.0%; δ_H (300 MHz, MeOH-d_4) 1.15 (3H, t, 4″-H, *J* 7.1), 1.84 (2H, m, 3″-H), 2.30–2.56 (4H, m, 2′-H + 2″-H), 3.47 (1H, m, 3′-H), 3.65–3.83 (2H, m, 5′-H), 3.92 (1H, m, 4′-H), 6.27 (1H, dd, 1′-H, *J* 7.1, *J* 4.6), 7.07 (1H, d, 7-H, *J* 13.7), 7.58 (1H, d, 8-H, *J* 13.7), and 7.94 (1H, s, 6-H); δ_C (75.5 MHz, MeOH-d_4) 14.1 (C-4″), 20.4 (C-3″), 39.0 (CH_2), 40.8 (CH_2), 41.4 (CH_2), 52.9 (C-3′), 86.8 (CH), 87.1 (CH), 109.0 (C-8), 112.0 (C-5), 130.6 (C-7), 140.3 (C-6), 151.3 (C-2), 164.0 (C-4), and 176.7 (C=O); *m/z* (ESI^+) 441 [$(M^{81}Br + K)^+$, 6%], 439 [$(M^{79}Br + K)^+$, 5], 425 [$(M^{81}Br + Na)^+$, 98], 423 [$(M^{79}Br + Na)^+$, 100], 403 [$(M^{81}Br + H)^+$, 97], and 401 [$(M^{79}Br + H)^+$, 93].

(*E*)-3′-Amino-5-(2-bromovinyl)-5′-crotonylamino-2′,3′,5′-trideoxyuridine 8d. R_f [5% NH_3(aq.)/MeOH] 0.60; mp 129–131°C (decomposed); υ_{max}(KBr)/cm^{-1} 3527, 1702, 1676, 1601, 1466, and 1279; found: C, 45.1; H, 4.9; N, 13.8. $C_{15}H_{19}N_4O_4Br$ requires C, 45.2; H, 4.8; N, 14.1%; δ_H (300 MHz, MeOH-d_4) 2.09 (3H, dd, 4″-H *J* 6.8, |*J*| 1.7), 2.40–2.54 (2H, m, 2′-H), 3.50 (1H, m, 3′-H), 3.80 (2H, m, 5′-H), 3.94 (1H, m, 4′-H), 6.17 (1H, dq, 2″-H, *J* 15.4, |*J*| 1.7), 6.29 (1H, dd, 1′-H, *J* 6.8, *J* 4.3), 6.99–7.11 (2H, m, 3″-H + 7-H), 7.55 (1H, d, 8-H, *J* 13.7), and 7.88 (1H, s, 6-H); δ_C (75.5 MHz, MeOH-d_4) 18.1 (C-4″), 41.0 (2C, 2CH_2), 52.7 (C-3′), 86.6 (CH), 87.2 (CH), 108.8 (C-8), 112.0 (C-5), 125.8 (C-2″), 130.8 (C-7), 140.0 (C-6), 141.8 (C-3″), 151.7 (C-2), 164.6 (C-4), and 169.1 (C=O); *m/z* (ESI^+) 423 [$(M^{81}Br + Na)^+$, 33%], 421 [$(M^{79}Br + Na)^+$, 38], 401 [$(M^{81}Br + H)^+$, 100], and 399 [$(M^{79}Br + H)^+$, 97].

(*E*)-3′-Amino-5′-benzoylamino-5-(2-bromovinyl)-2′,3′,5′-trideoxyuridine 8e. R_f [5% NH_3(aq.)/MeOH] 0.66; mp 148–149°C (decomposed); υ_{max}(KBr)/cm^{-1} 3454, 1708, 1600, 1566, and 1264; found: C, 49.6; H, 4.5; N, 13.1. $C_{18}H_{19}N_4O_4Br$ requires C, 49.8; H, 4.4; N, 12.9%; δ_H (300 MHz, MeOH-d_4) 2.39–2.58 (2H, m, 2′-H), 3.56 (1H, m, 3′-H), 3.89 (1H, m, 5′-H), 4.04 (2H, m, 5′-H + 4′-H), 6.29 (1H, dd, 1′-H, *J* 6.8, *J* 4.3), 6.88 (1H, d, 7-H, *J* 13.7), 7.52 (1H, d, 8-H, *J* 13.7), 7.65–7.78 (3H, m, *m*-H + *p*-H), 7.92 (1H, s, 6-H), and 8.06 (2H, m, *o*-H); δ_C (75.5 MHz, MeOH-d_4) 40.9 (CH_2), 41.7 (CH_2), 52.8 (C-3′), 86.7 (CH), 87.2 (CH), 109.0 (C-8), 112.0 (C-5), 128.3 (CH), 129.7 (CH), 130.4 (C-7), 133.0 (C-*p*), 135.2 (C-*i*), 140.1 (C-6), 151.2 (C-2), 163.8 (C-4), and 170.7 (C=O); *m*/*z* (ESI^+) 459 [$(M^{81}Br + Na)^+$, 64%], 457 [$(M^{79}Br + Na)^+$, 62], 437 [$(M^{81}Br + H)^+$, 97], and 435 [$(M^{79}Br + H)^+$, 100].

(*E*)-3′-Acetylamino-5′-amino-5-(2-bromovinyl)-2′,3′,5′-trideoxyuridine 9b. R_f [5% NH_3(aq.)/MeOH] 0.63; mp: 186–187°C (decomposed); υ_{max}(KBr)/cm^{-1} 3478, 1716, 1601, 1566, and 1474; found: C, 42.0; H, 4.9; N, 15.2. $C_{13}H_{17}N_4O_4Br$ requires C, 41.9; H, 4.6; N, 15.1%; δ_H (300 MHz, MeOH-d_4) 2.15 (3H, s, *Me*CO), 2.46–2.65 (2H, m, 2′-H), 3.05–3.19 (2H, m, 5′-H), 3.94 (1H, m, 4′-H), 4.57 (1H, m, 3′-H), 6.34 (1H, dd, 1′-H, *J* 6.8, *J* 5.4), 7.06 (1H, d, 7-H, *J* 13.7), 7.55 (1H, d, 8-H, *J* 13.7), and 8.06 (1H, s, 6-H); δ_c (75.5 MHz, MeOH-d_4) 22.6 (*Me*CO), 38.1 (C-2′), 44.2 (C-5′), 51.0 (C-3′), 86.3 (CH), 86.5 (CH), 109.0 (C-8), 112.4 (C-5), 130.6 (C-7), 140.4 (C-6), 151.7 (C-2), 164.4 (C-4), and 173.4 (C=O); *m*/*z* (ESI^+) 413 [$(M^{81}Br + K)^+$, 4%], 411 [$(M^{79}Br + K)^+$, 3], 397 [$(M^{81}Br + Na)^+$, 27], 395 [$(M^{79}Br + Na)^+$, 25], 375 [$(M^{81}Br + H)^+$, 97], and 373 [$(M^{79}Br + H)^+$, 100].

(*E*)-5′-Amino-5-(2-bromovinyl)-3′-butyrylamino-2′,3′,5′-trideoxyuridine 9c. R_f [5% NH_3(aq.)/MeOH] 0.45; mp 149–151°C (decomposed); υ_{max}(KBr)/cm^{-1} 3467, 1699, 1604, 1568, and 1475; found: C, 44.8; H, 5.5; N, 13.7. $C_{15}H_{21}N_4O_4Br$ requires C, 45.0; H, 5.3; N, 14.0%; δ_H (200 MHz, MeOH-d_4) 1.13 (3H, t, 4″-H, *J* 7.3), 1.82 (2H, m, 3″-H), 2.38 (2H, t, 2″-H, *J* 7.1), 2.54 (2H, m, 2′-H), 3.13 (2H, m, 5′-H), 3.94 (1H, m, 4′-H), 4.59 (1H, m, 3′-H), 6.35 (1H, dd, 1′-H, *J* 6.7, *J* 5.4), 7.07 (1H, d, 7-H, *J* 13.7), 7.56 (1H, d, 8-H, *J* 13.7), and 8.07 (1H, s, 6-H); δ_C (75.5 MHz, MeOH-d_4) 14.0 (C-4″), 20.3 (C-3″), 38.1 (CH_2), 38.9 (CH_2), 44.2 (CH_2), 50.8 (C-3′), 86.3 (CH), 86.5 (CH), 109.0 (C-8), 112.4 (C-5), 130.5 (C-7), 140.4 (C-6), 151.4 (C-2), 164.1 (C-4), and 176.3 (C=O); *m*/*z* (ESI^+) 441 [$(M^{81}Br + K)^+$, 5%], 439 [$(M^{79}Br + K)^+$, 3], 425 [$(M^{81}Br + Na)^+$, 42], 423 [$(M^{79}Br + Na)^+$, 44], 403 [$(M^{81}Br + H)^+$, 97], and 401 [$(M^{79}Br + H)^+$, 100].

(*E*)-5-(2-Bromovinyl)-3′,5′-diformylamino-2′,3′,5′-trideoxyuridine 10a. R_f [5% NH_3(aq.)/MeOH]: 0.90; found: C, 40.4; H, 3.9; N, 14.2. $C_{13}H_{15}N_4O_5Br$ requires C, 40.3; H, 3.9; N, 14.5%; δ_H (200 MHz, MeOH-d_4) 2.51–2.69 (2H, m, 2′-H), 3.66–3.90 (2H, m, 5′-H), 4.12 (1H, m, 4′-H), 4.60 (1H, m, 3′-H), 6.33 (1H, dd, 1′-H, *J* 6.8), 7.09 (1H, d, 7-H, *J* 13.7), 7.60 (1H, d, 8-H, *J* 13.7), 7.99 (1H, s, 6-H), 8.28 (1H, s, *H*CO), and 8.31 (1H, s, *H*CO); *m*/*z* (ESI^+) 427 [$(M^{81}Br + K)^+$, 3%], 425 [$(M^{79}Br + K)^+$, 4], 411 [$(M^{81}Br + Na)^+$, 100], 409 [$(M^{79}Br + Na)^+$, 98], 389 [$(M^{81}Br + H)^+$, 4], and 387 [$(M^{79}Br + H)^+$, 5].

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