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The Ugi reaction in the generation of new nucleosides as potential antiviral and antileishmanial agents

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Abstract

5-Formyl-2′-deoxyuridine-3′,5′-diacetate was converted to a small library of 5-substituted pyrimidine nucleoside *N*-acylamino acid amides by means of a Ugi multicomponent reaction. The reaction allowed introduction of various substituents at the acyl moiety, at the amino acid α-amide group, and at the amino acid carboxyl function. Evaluation of these novel 5-substituted nucleosides against vaccinia virus and cowpox virus provided one compound with discernable activity against cowpox virus but five- to eightfold less active than the Cidofovir standard. More promising activity was seen for the inhibition of *Leishmania donovani* promastigotes. Several synthetic products showed antileishmanial activity in the 10^{-5} M range. When compared to earlier studies demonstrating anti-orthopoxviral and antileishmanial activity of 5-substituted pyrimidine nucleosides, these results imply that the 5-(*N*-acylamino acid amide)-derivatized pyrimidine nucleosides may possess more steric bulk, greater hydrophobicity, and more flexibility than is compatible with these particular biological activities.

Keywords: Pyrimidines; Aldehyde derivatives; Inhibitors; Vaccinia virus; Cowpox virus; Smallpox; Neglected diseases; Parasitic diseases

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1. Introduction

The last natural case of smallpox occurred in 1977 and the World Health Organization declared smallpox an eradicated disease in 1980 [1,2]; nonetheless, this devastating disease remains a bioterrorist and biowarfare threat to humanity [3–6]. There is no drug treatment for this disease although Cidofovir has US Food and Drug Administration Investigational New Drug status for the treatment of smallpox vaccination complications and would likely be used on a compassionate basis in the event a smallpox outbreak [7–9]. Although several other potential therapeutic agents are in various stages of development, the search for effective smallpox antivirals remains an urgent priority [7–9].

The causative agent of smallpox is the variola virus; however, because of the inherent dangers of experiments with this virus, the nearly genetically identical surrogates of vaccinia virus (VV) and cowpox virus (CV) are employed to screen for activity against this

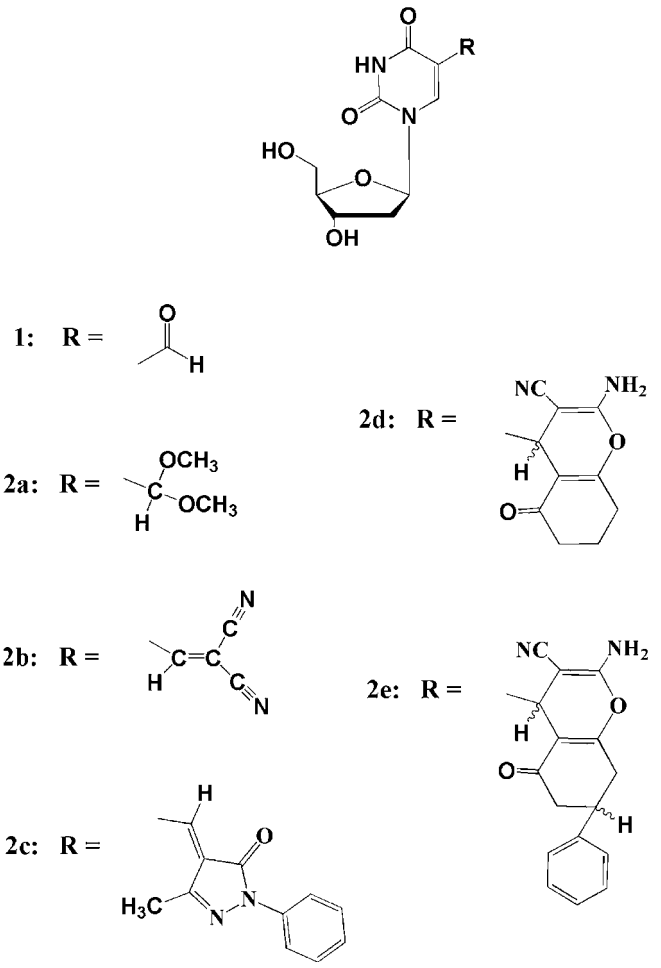


Fig. 1. Structure of 5-formyl-2′-deoxyuridine (**1**) and selected derived nucleosides (**2a**–**2e**) with significant activity against orthopoxviruses and the *Leishmania donovani* parasite.

orthopoxvirus family [7–9]. To generate novel candidate molecules for potential anti-orthopoxvirus activity, we have exploited the versatile aldehyde synthon of 5-formyl-2′-deoxyuridine (Fig. 1, **1**) [10–13]. As part of this exploration, we have examined synthetic products derived from the reaction of **1** (or closely related derivatives) in gem-diether formation (e.g., compound **2a**) [10], the Knoevenagel reaction (compound **2b**) [11], pyrazolone conjugation (e.g., compound **2c**) [12], and multicomponent Knoevenagel reactions (e.g., compounds **2d** and **2e**) [13]. Recently, we have described the anti-orthopoxvirus activity of a number of these structurally diverse novel nucleosides (Fig. 1) [10–13]. At least a subset of these agents owe their antiviral activity to phosphorylation by the orthopoxvirus thymidine kinase [13], which appears to be even more promiscuous than the herpes virus thymidine kinase upon which anti-herpes virus chemotherapy is substantially based.

Additional interest in this group of compounds has been generated by the discovery of their significant antileishmanial activity (Torrence, Fan, Zhang, and Loiseau, in press) prompted by the observations of Peyron et al. [14]. Leishmaniasis affects 12 million people worldwide, and there is a great need for more effective less toxic drug options [15,16].

Because elaboration of the aldehyde synthon has provided a rich source of new modified nucleosides with significant anti-orthopoxvirus and antileishmanial activities, we extended this exploratory chemistry to another reaction strategy. In addition, since the novel antiviral nucleosides described in Fig. 1 possess highly bulky and relatively hydrophobic substituents at the 5 position of the nucleoside pyrimidine ring, we have sought to explore additional diversity at this substituent position. Herein, we describe the application of the multicomponent Ugi reaction to generate a series of previously undescribed hypermodified nucleosides that in turn have been evaluated for their activity against vaccinia virus, cowpox virus, and the parasite *Leishmania donovani*.

2. Results and discussion

The structural array presented in Fig. 1 did not appear to present an upper limit of steric bulk and hydrophobicity relative to either anti-orthopoxvirus activity or to antileishmanial activity. Insofar as all of the structures of Fig. 1 were synthesized from the intermediate 5-formyl-2′-deoxyuridine, we employed the aldehyde group in another of its diverse chemistries; namely, the Ugi multicomponent reaction [17–19]. This metathesis generates peptide-like, flexible molecules which could be considered probes of the biological receptor molecules responsible for the antiviral and antiparasitic activities under investigation.

The construction of the peptide-like molecules was based on the well-established Ugi four components condensation (U-4CC) of an oxo compound (5-formyl-2′-deoxyuridine), an amine, a carboxylic acid, and an isocyanide. This results in a pyrimidine nucleoside *N*-acylamino acid amide product containing a pyrimidine 5 position side-chain bearing three potentially different substituents, one each arising from the amine, carboxylic acid, and isocyanide reactants. To the best of our knowledge, this is the first example in which the 5-formyl-2′-deoxyuridine has been involved as the source of oxo compound in the Ugi multicomponent reaction chemistry.

Initially, the condensation of stoichiometric amounts of 3′,5′-diacetyl-5-formyl-2′-deoxyuridine, aniline, benzoic acid, and benzyl isocyanide was investigated in anhydrous methanol at room temperature. The nucleoside moiety could be smoothly incorporated into the

desired *N*-acylamino acid amide through this four component condensation in good yield. Because a new chiral carbon was created in the acylamino amide products, this condensation process gave rise to two diastereomers (**3a** and **3b**: $R_1 = R_3 =$ phenyl; $R_2 =$ benzyl). Fortunately, the polarity difference between the two diastereomers was large enough in most cases to obtain a separation through column chromatography which gave two isomers with a ratio of 1:1. While no absolute configuration could be assigned, both isomers were fully characterized with ^{1}H NMR, ^{13}C NMR and high resolution mass spectrometry (HRMS).

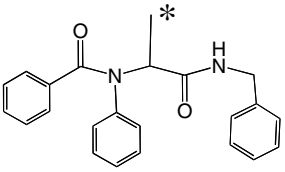
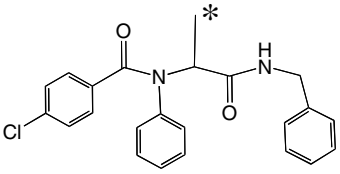
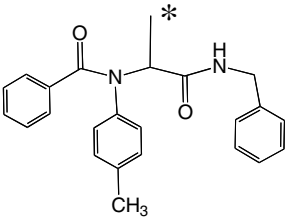
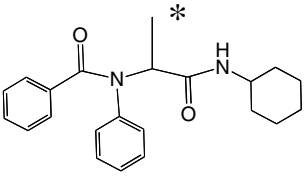
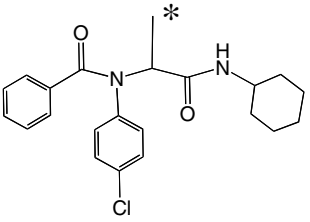
Based on the foregoing success, readily available amine, acid and isocyanide components were screened as reaction substrates to study the scope and generality of the present method and to build a small library of *N*-acylamino acid amides. Various amine, carboxylic acid and isocyanides reacted smoothly with the nucleoside aldehyde to give the corresponding products in high yields.

As a variation on this theme, by employing trimethylsilylazide instead of carboxylic acid in this condensation process, novel tetrazole derivatives could be produced (Table 1, compounds **15**, **16a**, **16b**). These are of interest since they would be, by virtue of the tetrazole moiety, less conformationally flexible compared to the *N*-acylamino acid amides.

Table 1 presents the results of antiviral and anti-leishmanial evaluation of the 25 synthetic products **3a**–**16b**. Several conclusions can be drawn from that data.

1. None of the compounds evaluated possessed significant antiviral activity against either vaccinia virus or cowpox virus. Several compounds did demonstrate some detectable antiviral activity according to the cytopathogenic effect assay. Among these was compound **10b** with an EC_{50} against cowpox virus of 45 μM. That this may represent a specific activity was suggested by the complete lack of activity of the other diastereomer **10a**; however, it was five- to eightfold less active than Cidofovir against cowpox (see Table 1 and Table 1 legend). Marginal antiviral activity against one or both orthopoxviruses was demonstrated by compounds **9a**, **9b**, **11a**, and **16b**; however, these EC_{50}s were in the range of 100 μM or greater. Greater antiviral activity would have warranted confirmation of these observations by plaque reduction assays.
2. Several of these nucleoside-peptide hybrids were, according the Neutral red uptake, significantly toxic to the human foreskin fibroblasts used for virus assays. Thus, compounds **12a**, **12b**, **13a**, and **13b** displayed a CC_{50} in the range of 10 μM. It is noteworthy that the greatest toxicity associated with the investigated compounds arose in those analogues that possessed an alkyne modification in the acyl moiety.
3. A subset of the examined compounds possessed moderate anti-leishmanial activity. Compounds **3b**, **7a**, **7b**, **9a**, **9b**, **10a**, and **10b** all displayed moderate activity in the range of 12–44 μM. Minimal activity was also detectable with compounds **4a**, **4b**, **8a**, **12a**, **12b**, **15**, **16a**, and **16b**. In the instances available for comparison, there did not appear to be a specific association of the anti-leishmanial activity with only one member of a diastereomeric pair. Notably, the alkyne-containing congeners **12a**, **12b**, **13a**, and **13b** were significantly less toxic to *Leishmania donovani* than to the cultured human foreskin fibroblast used in the antiviral studies.
4. In regard to structure–activity relationships, it can be pointed out that the most active anti-leishmanial *N*-acylamino acid amide (compound **3b**) bore three unsubstituted phenyl rings. Introduction of a *p*-chloro substituent to the phenyl ring of the *N*-acyl group of compound **3b** brought about a large reduction in activity (compound **4a**

Table 1
Anti-orthopoxviral and anti-leishmanial activities of (2′-deoxyuridin-5-yl)-*N*-acylamino acid amides

Compound	R Group[a]	Vaccinia virus[b] EC_{50} (μM)	Cowpox virus[b] EC_{50} (μM)	Host cell toxicity[b] CC_{50} (μM)	*Leishmania donovani*[c] IC_{50} (μM)[d]
3a		>60	>60	245	ND
3b	Diastereoisomer of **3a**	>60	>60	243	12.1 ± 1.4
4a		>300	>300	245	98.8 ± 9.3
4b	Diastereoisomer of **4a**	>300	>300	290	70.4 ± 6.8
5		>300	>300	250	ND[d]
6a		>300	>300	206	ND[d]
6b	Diastereoisomer of **6a**	>300	>300	246	ND[d]
7a		>300	>300	294	33.1 ± 4.2
7b	Diastereoisomer of **7a**	>300	>300	255	33.9 ± 3.7

(*continued on next page*)

Table 1 (*continued*)

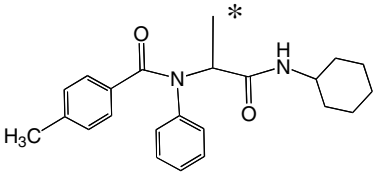
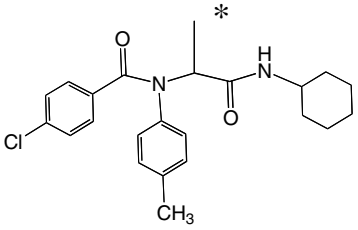
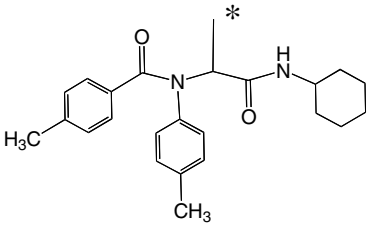
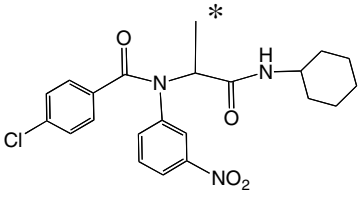
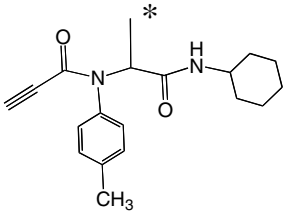
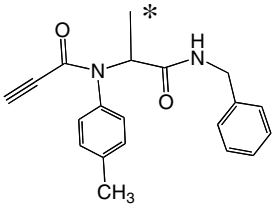
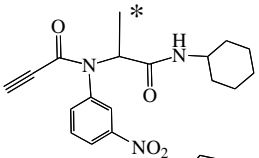
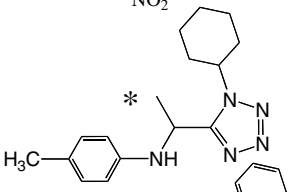
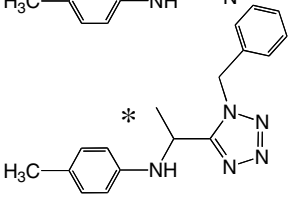
Compound	R Group[a]	Vaccinia virus[b] EC_{50} (μM)	Cowpox virus[b] EC_{50} (μM)	Host cell toxicity[b] CC_{50} (μM)	*Leishmania donovani*[c] IC_{50} (μM)
8a		>300	>300	272	70.1 ± 7.5
8b	Diastereoisomer of **8a**	>300	>300	273	ND[d]
9a		150	263	>300	23.2 ± 1.7
9b	Diastereoisomer of **9a**	178	>300	>300	23.7 ± 2.2
10a		>300	>300	>300	38.0 ± 4.1
10b	Diastereoisomer of **10a**	>300	45.3	>300	43.8 ± 4.9
11a		246	163	>300	>100
11b	Diastereoisomer of **11a**	>300	>300	>300	ND[d]
12a		>12	>12	26.2	94.3 ± 8.9
12b	Diastereoisomer of **12a**	>2.4	>2.4	5.1	75.1 ± 7.3

Table 1 (*continued*)

Compound	R Group[a]	Vaccinia virus[b] EC_{50} (μM)	Cowpox virus[b] EC_{50} (μM)	Host cell toxicity[b] CC_{50} (μM)	*Leishmania donovani*[c] IC_{50} (μM)
13a		>12	>12	19.4	>100
13b	Diastereoisomer of **13a**	>12	>12	24.6	>100
14		>60	>60	139	>100
15		>300	>300	>300	90.5 ± 8.9
16a		>300	>300	>300	97.1 ± 9.8
16b	Diastereoisomer of **16a**	>300	58	>300	70.2 ± 6.9

[a] * indicates the point of attachment of the acylamino acid amide chain to the pyrimidine nucleoside.

[b] Antiviral assays were performed through the laboratory of Dr. Earl Kern at the University of Alabama according to the procedures described previously for activity against vaccinia virus (Copenhagen) and cowpox virus (Brighton) and for cytotoxicity (Neutral Red uptake assay) in human foreskin fibroblast (HFF) cells. e. EC_{50}, concentration that brings about a 50% reduction in virus cytopathogenic effect; CC_{50}, concentration which causes a cytotoxic effect (as ascertained by Neutral Red uptake) on 50% of uninfected cells. Under these conditions, Cidofovir showed an EC_{50} of 5.6–8.9 μM against cowpox virus and an EC_{50} of 4.9–7.0 against vaccinia virus.

[c] Procedures for in vitro evaluation on promastigote forms. Promastigote forms of a *Leishmania donovani* LV9 clone and the standard drugs pentamidine and amphotericin B were used for the assay. M-199 medium supplemented with 40 mM Hepes, 100 μM adenosine, 0.5 mg/ml hemin, 10% heat-inactivated fetal calf serum (hi-FCS) and 50 μg/ml gentamycin was added to each well of a 96-well microtiter plate. Serial drug dilutions were added to the wells. Then 2×10^5 promastigote forms of *L. donovani* in 100 μl were added to each well and the plate incubated at 27 °C (under a 5% CO_2 atmosphere) for 72 h. Each concentration was screened in triplicate. The viability of promastigotes was checked using the tetrazolium-dye (MTT) colorimetric method. The MTT cell proliferation assay is a colorimetric assay system, which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. After incubation of the cells with the MTT reagent, a detergent solution was added to lyse the cells and solubilize the coloured crystals. The samples were read using an ELISA plate reader at a wavelength of 570 nm. The amount of color produced was directly proportional to the number of viable cells. The results are expressed as the concentrations inhibiting parasite growth by 50% (IC_{50}) after a 3-day incubation period. Under these conditions, the IC_{50} of pentamidine was 6.6 ± 0.7 μM and the IC_{50} of Amphotericin B was 0.10 ± 0. 02 μM.

[d] ND, not determined.

and **4b**). When compound **3b** was modified by replacing the benzyl group with a cyclohexyl group together with the simultaneous introduction of a *p*-chloro to the R_1 phenyl or a *p*-methyl to the R_3 phenyl, a three- to sixfold decrease in activity was observed (compounds **7a**, **7b**, **8a**). Somewhat surprisingly, double modifications provided compounds of significant activity. Thus, analogues (**9a**, **9b**, **10a**, **10b**) bearing a cyclohexyl moiety at R_2, a *p*-toluyl group at R_1 and either a *p*-chlorophenyl group or a *p*-toluyl group at R_3 resulted in antileishmanial activities approximately one-quarter to one-half that of compound **3b**. Substitution of the *p*-toluyl group of compound **9a** or **9b** with a *p*-nitrophenyl group effected a complete loss of detectable activity. Analogues (compound **12a**–**16b**) containing the *N*-propynoyl moiety or the tetrazole modification were either much less active or devoid of detectable antileishmanial activity.

5. With reference to the anti-orthopoxvirus evaluation, at this stage it appears that the considerable steric bulk and hydrophobicity that characterizes compounds **3a**–**16b** are not compatible with activity against this group of viruses. While, certain of the pyrimidine 5-substituents of the anti-orthopoxvirus agents of Fig. 1 (**2c**, **2d**, **2e**) possess considerable steric bulk and hydrophobicity, they also are relatively conformationally constrained because of their cyclic structure and they may be considered to present both a hydrophobic face (phenyl and/or methylenes) as well as a more polar one (e.g., nitrile and amino groups). This double-faced nature of sidedness may be critical to their activity, especially as viewed in the context of the results of the present study. These structural factors may be related to the established requisite phosphorylation of compounds **2b**–**2e** by the orthopoxvirus thymidine kinase.

Based upon the foregoing results and discussion, at least two paths should be followed in further exploration of this group of nucleosidic peptides. First, the most active of these compounds such as compound **10b** (active against cowpox virus) and compound **3b** (active against *Leishmania donovani*) should be synthesized as their unprotected 3′,5′-ribose hydroxyl analogues since previous studies have shown that maximum antiviral and maximum anti-leishmanial activity is obtained when the ribose hydroxyls are free. Secondly, the synthesis of additional analogues of structures **3a**–**16b** should be pursued, especially with reference to antileishmanial activity. Variations should be pursued that examine a wider range of R_1, R_2, and R_3 substituents than reported in this study.

3. Conclusions

This study has demonstrated that the Ugi multicomponent reaction can be used to generate structural diversity at the 5 position of the pyrimidine ring of 2′-deoxyuridine and that such products are formed in high yield. At least a subset of these Ugi products may be considered as leads for further explorations as orthopoxvirus antivirals and anti-leishmanial agents as well as other biological activities.

4. Experimental

Melting points were recorded with a Barnstead 1201D electrothermal melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer. $CDCl_3$ is used as solvent for different compounds. The chemical shifts are reported in parts per million (∂) and signals are quoted as s (singlet),

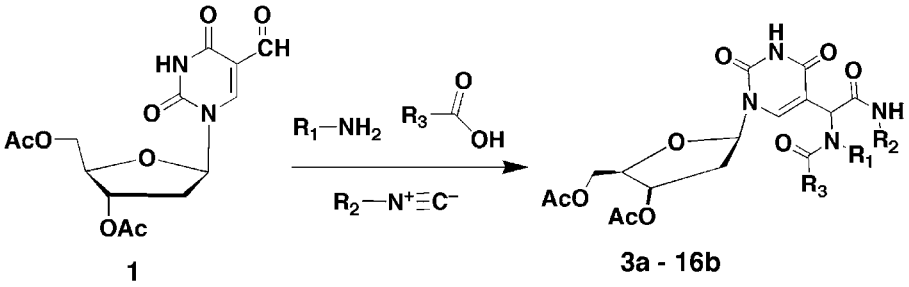


Fig. 2. Ugi multicomponent reaction as applied to 5-formyl-2′-deoxyuridine.

d (doublet), t (triplet), m (multiplet), dd (doublet of doublets), and ddd (doublet of doublets of doublets). The mass spectra were performed on a HP 1100 MSD spectrometer at the HT Laboratories, San Diego. The HRMS (High Resolution Mass Spectra) were performed on a JEOL HX 110 A spectrometer at the Department of Chemistry, University of Arizona. Silica gel column chromatography was conducted with Sigma–Aldrich silica gel (70–230 mesh). 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine (**1**, Fig. 2) was accessed through the known oxidation of 3′,5′-di-*O*-acetylthymidine with potassium peroxysulfate ($K_2S_2O_8$) in the presence of $CuSO_4$ and 2,6-lutidine in aqueous acetonitrile. The amines, isocyanides, and the trimethylsilyl azide used herein were from commercial sources.

4.1. General procedure for the preparation of the Ugi adducts

In a round bottom flask, 1 mmol 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine was dissolved in 8 mL methanol. To this solution was added 1 mmol amine. The solution gradually turned into a light yellow color. About 20 min later, 1 mmol of isocyanide was added and followed by the addition of 1 mmol of carboxylic acid. The formed solution was stirred at room temperature for 2–3 days till the full conversion of the starting materials (monitored by TLC). At completion, the solvent was removed under reduced pressure, and the residue was separated through column chromatography (ethyl acetate–hexane, 2:1, v/v) to give the corresponding product. Tetrazole compounds (**15**, **16a** and **16b**, Table 1) were prepared in a similar manner by using trimethylsilylazide instead of carboxylic acid.

N-[(benzylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-phenylbenzamide (**3a**): mp 96–98 °C; ^{1}H NMR (CD_3Cl) δ: 1.64–1.71 (m, 1H, H2′-1), 2.05 (s, 3H, –OCO*CH*$_3$), 2.08–2.16 (m, 4H, H2′-2, –OCO*CH*$_3$), 4.09–4.11 (m, 3H, H5′, H4′), 4.37 (dd, 1H, $J1 = 15.2$ Hz, $J2 = 6.0$ Hz, –CH_2-1), 4.45 (dd, 1H, $J1 = 15.2$ Hz, $J2 = 6.0$ Hz, –CH_2-2), 5.04 (d, 1H, $J = 6.4$ Hz, H3′), 6.12 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.6$ Hz, H1′), 6.22 (s, 1H, –CH), 7.02–7.23 (m, 16H, –Ar*H*, –N*H*), 7.92 (s, 1H, H6), 8.52 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 21.03, 21.11, 37.35, 43.70, 56.65, 63.82, 74.34, 82.27, 84.78, 109.21, 127.38, 127.64, 128.70, 128.79, 128.99, 129.81, 130.59, 135.96, 138.46, 141.30, 142.56, 149.99, 163.04, 168.96, 170.50, 171.26, 171.45. ESI LRMS *m*/*e*: 655 (MH^+), 677 (MNa^+).

N-[(benzylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-phenylbenzamide (**3b**): mp 95–97 °C; ^{1}H NMR (CD_3Cl) δ: 2.03–2.10 (m, 7H, H2′-1,2 –OCO*CH*$_3$), 2.28 (ddd, 1H, $J1 = 14.0$ Hz, $J2 = 5.2$ Hz, $J3 = 1.2$ Hz, H2′-2), 4.05 (dd, $J1 = 12.0$ Hz, $J2 = 3.2$ Hz,

H5′-1), 4.09–4.11 (m, 1H, H4′), 4.20 (dd, $J1 = 13.6$ Hz, $J2 = 3.6$ Hz, H5′-2), 4.44 (dd, 1H, $J1 = 14.8$ Hz, $J2 = 6.0$ Hz, –CH_2-1), 4.51 (dd, 1H, $J1 = 15.2$ Hz, $J2 = 6.0$ Hz, –CH_2-2), 5.15–5.17 (m, 1H, H3′), 6.16 (dd, 1H, $J1 = 9.2$ Hz, $J2 = 5.2$ Hz, H1′), 6.43 (s, 1H, –CH), 6.91–7.33 (m, 15H, –ArH), 7.32 (t, 1H, $J = 6.0$ Hz, –NH), 7.90 (s, 1H, H6), 8.61 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 21.08, 21.15, 37.36, 43.72, 56.70, 63.81, 74.36, 82.31, 84.81, 109.20, 127.44, 128.10, 128.88, 128.91, 128.99, 129.90, 130.66, 136.06, 138.44, 141.55, 142.60, 149.86, 162.96, 169.11, 170.52, 171.26, 171.66. ESI LRMS *m*/*e*: 655 (MH^+), 677 (MNa^+); HRMS (FAB) Calcd for $C_{35}H_{35}N_4O_9$: 655.2405 $(MH)^+$, found: 655.2426.

N-[(benzylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-phenyl-4-chlorobenzamide (**4a**): mp 99–101 °C; ^{1}H NMR (CD_3Cl) δ: 1.67–1.74 (m, 1H, H2′-1), 2.08 (s, 3H, –OCOCH_3), 2.16–2.21 (m, 4H, H2′-2, –OCOCH_3), 4.12–4.15 (m, 3H, H5′, H4′), 4.45 (dd, 2H, $J1 = 5.6$ Hz, $J2 = 2.0$ Hz, –CH_2), 5.10 (d, $J = 6.4$ Hz, H3′), 6.16 (dd, 1H, $J1 = 8.4$ Hz, $J2 = 5.2$ Hz, H1′), 6.25 (s, 1H), 7.04–7.28 (m, 15H, –ArH), 7.87 (s, 1H, H6). ^{13}C NMR (CD_3Cl) δ: 21.02, 21.10, 31.14, 37.39, 43.80, 56.69, 63.81, 72.26, 82.31, 84.83, 108.84, 127.58, 127.69, 128.17, 128.87, 129.24, 130.21, 130.40, 134.23, 136.03, 138.21, 141.01, 142.68, 149.70, 162.68, 168.70, 170.34, 170.46, 171.17. HRMS (FAB) Calcd for $C_{35}H_{34}ClN_4O_9$: 689.2015 $(MH)^+$, found: 689.1999.

N-[(benzylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-phenyl-4-chlorobenzamide (**4b**): mp 103–105 °C; ^{1}H NMR (CD_3Cl) δ: 1.87–1.95 (m, 1H, H2′-1), 2.01 (s, 3H, –OCOCH_3), 2.02 (s, 3H, –OCOCH_3), 2.09–2.16 (m, 1H, H2′-2), 3.96–4.12 (m, 3H, H5′, H4′), 4.34–4.43 (m, 2H, –CH_2), 5.06 (d, 1H, $J = 6.4$ Hz, H3′), 6.05 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.2$ Hz, H1′), 6.29 (s, 1H, –CH), 6.89–7.22 (m, 14H, –ArH), 7.46 (t, 1H, $J = 6.4$ Hz, –NH), 7.73 (s, 1H, H6), 9.48 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 21.00, 21.12, 31.14, 37.35, 43.83, 56.11, 64.09, 74.62, 82.29, 84.91, 108.42, 127.68, 127.76, 128.19, 128.24, 128.88, 129.37, 130.08, 130.27, 134.02, 136.17, 138.45, 140.30, 143.43, 149.79, 162.96, 168.88, 170.53, 170.65, 171.18. HRMS (FAB) Calcd for $C_{35}H_{34}ClN_4O_9$: 689.2015 $(MH)^+$, found: 689.2042.

N-[(benzylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-4-methylphenylbenzamide (**5**): mp 93–95 °C; ^{1}H NMR (CD_3Cl) δ: 1.97–2.28 (m, 11H, H2′, CH_3, 2 –OCOCH_3), 4.00 (dd, 1H, $J1 = 12.0$ Hz, $J2 = 3.2$ Hz, H5′-1), 4.07–4.09 (m, 1H, H4′), 4.19 (dd, 1H, $J1 = 12.0$ Hz, $J2 = 3.6$ Hz, H5′-2), 4.42–4.45 (m, 2H, –CH_2), 5.13 (d, 1H, $J = 6.0$ Hz, H3′), 6.16 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.6$ Hz, H1′), 6.40 (s, 1H, –CH), 6.79 (d, 2H, $J = 7.6$ Hz, –ArH), 6.87 (d, 2H, $J = 7.6$ Hz, –ArH), 7.07–7.30 (m, 10H, –ArH), 7.56 (t, 1H, $J = 6.0$ Hz, –NH), 7.82 (s, 1H), 9.23 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 20.97, 21.14, 21.22, 37.36, 43.80, 55.88, 64.09, 74.77, 82.30, 84.90, 108.49, 127.64, 127.80, 127.86, 128.77, 128.88, 129.76, 129.85, 129.96, 135.73, 137.76, 137.90, 138.53, 143.51, 149.84, 162.75, 169.07, 170.55, 171.20, 171.92. HRMS (FAB) Calcd for $C_{36}H_{37}N_4O_9$: 669.2561 $(MH)^+$, found: 669.2589.

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-phenylbenzamide (**6a**): mp 111–113 °C; ^{1}H NMR (CD_3Cl) δ: 0.88–1.82 (m, 11H, H2′-1, –$(CH_2)_5$–), 2.05 (s, 3H, –OCOCH_3), 2.13–2.22 (m, 4H, H2′-2, –OCOCH_3), 3.64–3.72 (m, 1H, –NHC*H*–), 4.12–4.25 (m, 3H, H5′, H4′), 5.12–5.13 (m, 1H, H3′), 6.11 (s, 1H, –CH), 6.24 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.2$ Hz, H1′), 6.90 (d, 1H, $J = 8.0$ Hz, –N*H*CH–), 7.04–7.21 (m, 10H, ArH), 7.85 (s, 1H, H6), 9.89 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 21.02, 21.09, 24.98, 25.52, 32.72, 37.38, 48.94, 57.18, 63.99, 74.39, 82.23, 84.51, 109.55,

127.78, 128.60, 128.96, 129.63, 130.59, 136.27, 141.74, 142.77, 150.00, 163.47, 167.74, 170.50, 171.27, 171.32. ESI LRMS *m/e*: 647 (MH^+), 669 (MNa^+).

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-phenylbenzamide (**6b**): mp 116–118 °C; ^{1}H NMR (CD_3Cl) δ: 1.09–1.88 (m, 10H, –$(CH_2)_5$–), 2.06 (s, 3H, –OCOCH_3), 2.07–2.13 (m, 4H, H2′-1, –OCOCH_3), 2.25–2.30 (m, 1H, H2′-2)P, 3.69–3.76 (m, 1H, –CH), 4.07–4.11 (m, 2H, H5-1, H4′), 4.20 (dd, 1H, $J1 = 12.4$ Hz, $J2 = 4.8$ Hz, H5′-2), 5.15–5.17 (m, 1H, H3′), 6.16 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.6$ Hz, H1′), 6.32 (s, 1H, –CH), 7.02–7.24 (m, 11H, ArH, –NH), 7.87 (s, 1H, H6), 9.74 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 21.00, 21.12, 24.90, 25.60, 32.88, 32.98, 37.38, 48.72, 55.96, 64.18, 74.68, 82.27, 84.98, 108.65, 127.86, 128.96, 128.64, 129.18, 129.89, 130.17, 135.91, 140.71, 143.67, 149.94, 163.17, 168.06, 170.53, 171.15, 171.72. HRMS (FAB) Calcd for $C_{34}H_{39}N_4O_9$: 647.2718 $(MH)^+$, found: 647.2747.

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-4-chlorophenylbenzamide (**7a**): mp 112–114 °C; ^{1}H NMR (CD_3Cl) δ: 0.93–1.84 (m, 11H, H2′-1, –$(CH_2)_5$–), 2.08 (s, 3H, –OCOCH_3), 2.15–2.23 (m, 4H, H2′-2, –OCOCH_3), 3.65–3.72 (m, 1H, –CH), 4.15–4.17 (m, 2H, H5-1, H4′), 4.23 (dd, 1H, $J1 = 12.4$ Hz, $J2 = 4.8$ Hz, H5′-2), 5.12–5.14 (m, 1H, H3′), 6.10 (s, 1H, H1′), 6.23 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.6$ Hz, H1′), 6.75 (d, 1H, $J = 8.0$ Hz, –NH), 7.05–7.20 (m, 9H, –ArH), 7.85 (s, 1H, H6), 9.60 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 21.02, 21.10, 24.92, 25.58, 32.84, 37.45, 48.99, 56.98, 63.96, 74.27, 82.27, 84.66, 109.12, 128.17, 129.01, 129.23, 130.42, 131.75, 134.49, 135.93, 141.26, 142.87, 149.74, 163.08, 167.60, 170.27, 170.47, 171.19. HRMS (FAB) Calcd for $C_{34}H_{38}ClN_4O_9$: 681.2328 $(MH)^+$, found: 681.2319.

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-4-chlorophenylbenzamide (**7b**): mp 110–112 °C; ^{1}H NMR (CD_3Cl) δ: 1.13–1.89 (m, 10H, –$(CH_2)_5$–), 2.07 (s, 3H, –OCOCH_3), 2.11–2.15 (m, 4H, H2′-1, –OCOCH_3), 2.29 (dd, 1H, $J1 = 14.0$ Hz, $J2 = 5.6$ Hz, H2′-2), 3.69–3.76 (m, 1H, –CH), 4.08–4.12 (m, 2H, H5-1, H4′), 4.21 (dd, 1H, $J1 = 12.8$ Hz, $J2 = 4.8$ Hz, H5′-2), 5.17 (d, 1H, $J = 6.8$ Hz), 6.16 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.6$ Hz, H1′), 6.28 (s, 1H), 6.89 (d, 1H, $J = 8.0$ Hz, –NH), 7.04–7.18 (m, 9H, –ArH), 7.88 (s, 1H, H6), 9.65 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 20.98, 21.11, 24.87, 25.60, 32.91, 33.00, 37.41, 48.75, 56.07, 64.17, 74.61, 82.29, 85.02, 108.43, 128.21, 129.41, 130.04, 130.19, 134.23, 136.11, 140.44, 143.70, 149.82, 163.00, 167.88, 170.53, 170.59, 171.13. ESI LRMS *m/e*: 681 (MH^+), 703 (MNa^+).

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-phenyl-4-methylbenzamide (**8a**): mp 112–114 °C; ^{1}H NMR (CD_3Cl) δ: 1.05–1.86 (m, 11H, H2′-1, –$(CH_2)_5$–), 2.08 (s, 3H, –CH_3), 2.20–2.23 (m, 7H, H2′-2, 2 –OCOCH_3), 3.67–3.74 (m, 1H, –CH), 4.17–4.26 (m, 3H, H4′, H5′), 5.13 (d, 1H, $J = 6.4$ Hz, H3′), 6.15 (s, 1H, -CH), 6.23 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.6$ Hz, H1′), 6.75 (d, 1H, $J = 8.0$ Hz, –NH), 6.89 (d, 2H, $J = 8.0$ Hz, –ArH), 7.11–7.19 (m, 7H, –ArH), 7.94 (s, 1H, H6), 9.27 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 21.03, 21.12, 21.51, 24.94, 25.58, 32.83, 37.37, 48.87, 56.90, 63.95, 74.37, 82.24, 84.68, 109.28, 127.72, 128.51, 128.84, 129.04, 130.39, 133.14, 140.04, 141.85, 142.92, 149.81, 162.97, 167.82, 170.48, 171.24, 171.34. HRMS (FAB) Calcd for $C_{35}H_{41}N_4O_9$: 661.2874 $(MH)^+$, found: 661.2894.

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-phenyl-4-methylbenzamide (**8b**): mp 109–111 °C; ^{1}H NMR (CD_3Cl) δ: 1.07–1.89 (m, 10H, –$(CH_2)_5$–), 2.02–2.31 (m, 11H, –CH_3, 2 –OCOCH_3, H2′), 3.68–3.76 (m, 1H, –CH), 4.07–4.10 (m, 2H, H5-1, H4′), 4.20 (dd, 1H, $J1 = 12.8$ Hz, $J2 = 4.8$ Hz, H5′-2), 5.17 (d, 1H, $J = 6.0$ Hz), 6.17 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.6$ Hz, H1′),6.30 (s, 1H, –CH), 6.88

(d, 2H, $J = 8.0$ Hz, –ArH), 6.99–7.15 (m, 8H, –ArH), 7.89 (s, 1H, H6), 9.66 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 20.98, 21.12, 21.50, 24.88, 25.61, 32.88, 32.98, 37.36, 48.66, 56.09, 64.19, 74.71, 82.27, 84.96, 108.69, 127.84, 128.52, 128.89, 129.20, 130.06, 132.88, 140.21, 141.03, 143.69, 149.96, 163.10, 168.12, 170.53, 171.16, 171.70. ESI LRMS m/e: 661 (MH^+), 683 (MNa^+).

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridine-5-yl)methyl]-*N*-4-methylphenyl-4-chlorobenzamide (**9a**): mp 121–123 °C; ^{1}H NMR (CD_3Cl) δ: 0.90–1.80 (m, 11H, H2′-1, –$(CH_2)_5$–), 2.07 (s, 3H, –CH_3), 2.13–2.22 (m, 7H, H2′-2, 2 –OCOCH_3), 3.62–3.70 (m, 1H, –CH), 4.14–4.18 (m, 2H, H4′, H5′-1), 4.24 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.2$ Hz, H5′-2), 5.12 (d, 1H, $J = 6.0$ Hz, H3′), 6.07 (s, 1H, –CH), 6.23 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.6$ Hz, H1′), 6.81 (d, 1H, $J = 8.0$ Hz, –NH), 6.94 (d, 2H, $J = 7.6$ Hz, –ArH), 7.05–7.16 (m, 6H, –ArH), 7.80 (s, 1H, H6), 9.76 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 21.01, 21.09, 21.20, 24.94, 24.97, 25.53, 32.77, 37.27, 48.94, 57.15, 63.97, 74.34, 82.23, 84.47, 109.47, 128.09, 129.79, 130.11, 134.76, 135.66, 138.02, 138.71, 142.73, 149.93, 163.36, 167.67, 170.26, 170.47, 171.21. HRMS (FAB) Calcd for $C_{35}H_{40}ClN_4O_9$: 695.2485 $(MH)^+$, found: 695.2501.

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-4-methylphenyl-4-chlorobenzamide (**9b**): mp 113–115 °C; ^{1}H NMR (CD_3Cl) δ: 1.12–1.87 (m, 10H, –$(CH_2)_5$–), 2.06–2.15 (m, 7H, H2′-1, –CH_3, –OCOCH_3), 2.13–2.31 (m, 4H, H2′-2, –OCOCH_3), 3.68–3.73 (m, 1H, -CH), 4.04–4.10 (m, 2H, H5′-1, H4), 4.21 (dd, 1H, $J1 = 11.6$ Hz, $J2 = 3.2$ Hz, H5′-2), 5.17 (d, 1H, $J = 6.0$ Hz, H3′), 6.17 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.6$ Hz, H1′), 6.26 (s, 1H, –CH), 6.88–6.94 (m, 5H, –ArH, –NH), 7.07 (d, 2H, $J = 8.8$ Hz, –ArH), 7.17 (d, 2H, $J = 8.8$ Hz, –ArH), 7.86 (s, 1H, H6). ^{13}C NMR (CD_3Cl) δ: 20.95, 21.12, 21.25, 24.87, 25.60, 32.91, 33.01, 37.39, 48.70, 56.06, 64.12, 74.69, 82.28, 85.02, 108.51, 128.18, 129.75, 130.03, 130.18, 134.38, 135.96, 137.72, 138.19, 143.71, 149.94, 163.08, 167.93, 170.53, 170.69, 171.13. HRMS (FAB) Calcd for $C_{35}H_{40}ClN_4O_9$: 695.2485 $(MH)^+$, found: 695.2479.

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-4-methylphenyl-4-methylbenzamide (**10a**): mp 113–116 °C; ^{1}H NMR (CD_3Cl) δ: 0.91–1.80 (m, 11H, H2′-1, –$(CH_2)_5$–), 2.05 (s, 3H, –CH_3), 2.11–2.21 (m, 10H, –CH_3, 2 –OCOCH_3, H2′-2), 3.62–3.69 (m, 1H, –CH), 4.13–4.17 (m, 2H, H5′-1, H4), 4.22 (dd, 1H, $J1 = 12.8$ Hz, $J2 = 5.2$ Hz, H5′-2), 5.11 (d, 1H, $J = 6.0$ Hz, H3′), 6.08 (s, 1H, –CH), 6.24 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.6$ Hz, H1′), 6.85–7.11 (m, 9H, –ArH, –NH), 7.81 (s, 1H, H6). ^{13}C NMR (CD_3Cl) δ: 21.01, 21.09, 21.18, 21.49, 25.00, 25.52, 32.71, 37.19, 48.84, 57.06, 63.96, 74.45, 82.18, 84.49, 109.63, 128.41, 128.80, 129.58, 130.26, 133.41, 137.48, 139.26, 139.71, 142.70, 150.07, 163.40, 167.94, 170.49, 170.27, 171.31. HRMS (FAB) Calcd for $C_{36}H_{43}N_4O_9$: 675.3031 $(MH)^+$, found: 675.3005.

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-4-methylphenyl-4-methylbenzamide (**10b**): mp 108–110 °C; ^{1}H NMR (CD_3Cl) δ: 1.12–1.86 (m, 10H, –$(CH_2)_5$–), 2.06 (s, 3H, –CH_3), 2.13–2.21 (m, 10H, –CH_3, 2 –OCOCH_3, H2′-1), 2.29 (dd, 1H, $J1 = 14.4$ Hz, $J2 = 4.8$ Hz, H2′-2), 3.66–3.72 (m, 1H, –CH), 4.04 (dd, 1H, $J1 = 12.0$ Hz, $J2 = 3.6$ Hz, H5′-1), 4.07–4.10 (m, 1H, H4′), 4.22 (dd, 1H, $J1 = 12.0$ Hz, $J2 = 3.6$ Hz, H5′-2), 5.17 (d, 1H, $J = 6.0$ Hz, H3′), 6.20 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.6$ Hz, H1′), 6.30 (s, 1H, –CH), 6.88–6.93 (m, 6H, –ArH), 7.03 (d, 1H, $J = 8.4$ Hz, –NH), 7.12 (d, 2H, $J = 8.0$ Hz, –ArH), 7.88 (s, 1H, H6). ^{13}C NMR (CD_3Cl) δ: 20.95, 21.13, 21.25, 21.53, 24.87, 25.62, 32.89, 33.02, 37.37, 48.60, 56.02, 64.15, 74.80, 82.27, 84.96, 108.67, 128.52, 128.90, 129.70, 129.86, 132.96, 137.72, 138.26, 140.14,

143.75, 150.02, 163.03, 168.18, 170.55, 171.16, 171.81. ESI LRMS *m/e*: 675 (MH^+), 697 (MNa^+).

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-3-nitrophenyl-4-chlorobenzamide (**11a**): mp 125–128 °C; ^{1}H NMR (CD_3Cl) δ: 0.87–1.72 (m, 10H, –$(CH_2)_5$–), 1.98–2.29 (m, 8H, H2′, 2 –OCOCH_3), 3.62–3.68 (m, 1H, –CH), 4.12 (dd, 1H, $J1 = 12.0$ Hz, $J2 = 2.8$ Hz, -H5′-1), 4.16–4.19 (m, 1H, H4′), 4.35 (dd, 1H, $J1 = 12.0$ Hz, $J2 = 3.2$ Hz, –H5′-2), 5.19 (d, 1H, $J = 4.8$ Hz, H3′), 5.98 (s, 1H, –CH), 6.20–6.23 (m, 1H, H1′), 6.78 (d, 1H, $J = 6.4$ Hz, –NH), 7.12 (d, 2H, $J = 8.4$ Hz, –ArH), 7.18 (d, 2H, $J = 8.4$ Hz, –ArH), 7.29 (t, 1H, $J = 7.6$ Hz, –ArH), 7.53 (s, 1H, H6), 7.94 (dd, 2H, $J1 = 8.0$ Hz, $J2 = 1.6$ Hz, –ArH), 8.33 (s, 1H, –ArH), 10.18 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 20.99, 21.08, 24.92, 25.51, 32.57, 32.72, 37.71, 49.32, 58.38, 64.15, 74.62, 82.68, 84.76, 109.11, 122.51, 124.59, 128.57, 129.72, 130.31, 133.76, 136.55, 136.69, 142.10, 143.44, 148.47, 149.64, 163.99, 166.61, 169.98, 170.52, 171.33. HRMS (FAB) Calcd for $C_{34}H_{37}ClN_5O_{11}$: 726.2179 $(MH)^+$, found: 726.2197.

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-3-nitrophenyl-4-chlorobenzamide (**11b**): mp 132–134 °C; ^{1}H NMR (CD_3Cl) δ: 1.14–1.88 (m, 10H, –$(CH_2)_5$–), 2.07–2.16 (m, 7H, H2′-1, 2 –OCOCH_3), 2.29–2.33 (m, 1H, H2′-2), 3.72–3.76 (m, 1H, –CH), 4.11–4.25 (m, 3H, H4′, H5′), 5.17 (d, 1H, $J = 5.6$ Hz, H3′), 6.12 (t, 1H, $J = 6.4$ Hz, H1′), 6.20 (s, 1H, –CH), 6.73 (d, 1H, $J = 7.6$ Hz, –NH), 7.13 (d, 2H, $J = 8.0$ Hz, –ArH), 7.20 (d, 2H, $J = 8.0$ Hz, –ArH), 7.32 (t, 1H, $J = 8.0$ Hz, –ArH), 7.42 (s, 1H, H6), 7.91–8.09 (m, 3H, –ArH), 9.47 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 20.95, 21.09, 24.91, 25.55, 32.93, 33.00, 37.61, 49.02, 56.82, 63.93, 74.36, 82.35, 85.12, 108.44, 122.86, 125.10, 128.68, 130.01, 130.26, 133.50, 136.40, 136.78, 142.08, 143.38, 148.39, 149.50, 162.86, 167.31, 170.36, 170.51, 171.06; ESI LRMS *m/e*: 727 (MH^+), 749 (MNa^+).

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-4-methyl-phe- nylpropiolamide (**12a**): mp 115–117 °C; ^{1}H NMR (CD_3Cl) δ: 1.08–1.89 (m, 11H, H2′-1, –$(CH_2)_5$–), 2.06 (s, 3H, –CH_3), 2.10–2.16 (m, 1H, H2-2), 2.21 (s, 3H, –OCOCH_3), 2.32 (s, 3H, –OCOCH_3), 2.78 (s, 1H, CH), 3.65–3.68 (m, 1H, –CH), 4.09–4.16 (m, 3H, H4′, H5′), 5.06 (d, 1H, $J = 6.4$ Hz, H3′), 5.95 (s, 1H, –CH), 6.16 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.6$ Hz, H1′), 6.77 (d, 1H, $J = 8.0$ Hz, –NH), 7.12 (d, 2H, $J = 8.4$ Hz, –ArH), 7.20 (d, 2H, $J = 8.4$ Hz, –ArH), 7.57 (s, 1H, H6), 9.70 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 21.01, 21.07, 21.34, 24.92, 25.67, 32.65, 37.15, 49.01, 55.17, 63.85, 74.23, 76.36, 80.66, 82.13, 84.37, 108.74, 129.85, 130.51, 136.58, 139.29, 142.43, 149.82, 153.77, 162.83, 167.16, 170.46, 171.22. ESI LRMS *m/e*: 609 (MH^+), 631 (MNa^+). HRMS (FAB) Calcd for $C_{31}H_{37}N_4O_9$: 609.2561 $(MH)^+$, found: 609.2575.

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-4-methylphenyl-propiolamide (**12b**): mp 111–113 °C; ^{1}H NMR (CD_3Cl) δ: 1.17–1.91 (m, 10H, –$(CH_2)_5$–), 2.02–2.09 (m, 4H, H2′-1, –CH_3), 2.17 (s, 3H, –OCOCH_3), 2.27–2.34 (m, 4H, H2′-2, –OCOCH_3), 2.83 (s, 1H, CH), 3.66–3.73 (m, 1H, –CH), 3.98 (dd, 1H, $J1 = 12.0$ Hz, $J2 = 4.0$ Hz, H5′-1), 4.06–4.08 (m, 1H, H4′), 4.21 (dd, 1H, $J1 = 11.6$ Hz, $J2 = 3.6$ Hz, H5′-2), 5.15 (d, 1H, $J = 6.4$ Hz, H3′), 6.12 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.2$ Hz, H1′), 6.21 (s, 1H, –CH), 6.76 (d, 1H, $J = 8.4$ Hz, –NH), 6.99 (d, 2H, $J = 8.0$ Hz, –ArH), 7.11 (d, 2H, $J = 8.0$ Hz, –ArH), 7.65 (s, 1H, H6), 9.26 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 20.95, 21.11, 21.44, 24.87, 25.63, 32.77, 32.84, 37.51, 48.94, 53.72, 64.04, 74.65, 76.06, 81.42, 82.21, 84.83, 107.40, 130.03, 130.09, 135.31, 139.50, 143.68, 149.63, 154.31, 162.62, 167.32, 170.55, 171.16. ESI LRMS *m/e*: 609 (MH^+), 631 (MNa^+).

N-[(benzylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-4-methylphenylpropiolamide (**13a**): mp 98–100 °C; ^{1}H NMR (CD_3Cl) δ: 1.48–1.55 (m, 1H, H2′-1), 2.05–2.16 (m, 7H, H2′-2, –CH_3, –OCOCH_3), 2.31 (s, 3H, –OCOCH_3), 2.74 (s, 1H, CH), 4.04–4.10 (m, 3H, H5′, H4′), 4.38 (dd, 1H, $J1 = 14.8$ Hz, $J2 = 6.0$ Hz, –CH_2-1), 4.47 (dd, 1H, $J1 = 14.8$ Hz, $J2 = 6.0$ Hz, –CH_2-2), 5.04 (d, 1H, $J = 6.0$ Hz, H3′), 6.07–6.11 (m, 2H, H1′, –CH), 7.09–7.25 (m, 9H, –ArH), 7.45 (t, 1H, $J = 6.0$ Hz, –NH), 7.56 (s, 1H, H6), 9.77 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 21.00, 21.08, 21.34, 37.18, 43.75, 55.03, 63.69, 74.17, 76.25, 80.89, 82.16, 84.54, 108.51, 127.37, 127.66, 128.79, 129.87, 130.54, 136.35, 138.29, 139.35, 142.31, 149.83, 153.92, 162.68, 168.26, 170.46, 171.22. ESI LRMS *m*/*e*: 617 (MH^+), 639 (MNa^+).

N-[(benzylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-4-methylphenylpropiolamide (**13b**): mp 103–105 °C; ^{1}H NMR (CD_3Cl) δ: 1.84–1.92 (m, 1H, H2′-1), 2.05 (s, 3H, –CH_3), 2.09 (s, 3H, –OCOCH_3), 2.19–2.25 (m, 1H, H2′-2), 2.30 (s, 3H, –OCOCH_3), 2.79 (s, 1H, CH), 3.94 (dd, 1H, $J1 = 12.0$ Hz, $J2 = 3.6$ Hz, -H5′-1), 4.02–4.05 (m, 1H, H4′), 4.16 (dd, 1H, $J1 = 12.0$ Hz, $J2 = 3.6$ Hz, –H5′-2), 4.36 (dd, 1H, $J1 = 14.8$ Hz, $J2 = 6.0$ Hz, –CH_2-1), 4.47 (dd, 1H, $J1 = 14.8$ Hz, $J2 = 6.0$ Hz, –CH_2-2), 5.08 (d, 1H, $J = 6.0$ Hz, H3′), 6.07 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 4.8$ Hz, H1′), 6.24 (s, 1H, –CH), 6.97 (d, 2H, $J = 8.4$ Hz, –ArH), 7.04 (d, 2H, $J = 8.0$ Hz, –ArH), 7.22–7.31 (m, 5H, –ArH), 7.48 (s, 1H, H6), 7.52 (t, 1H, $J = 6.0$ Hz, –NH), 9.64 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 20.94, 21.12, 21.42, 37.40, 43.86, 54.12, 63.98, 74.65, 76.12, 81.30, 82.19, 84.76, 107.76, 127.57, 127.82, 128.84, 129.99, 130.16, 135.41, 138.44, 139.35, 143.11, 149.77, 154.27, 162.72, 168.34, 170.56, 171.24. HRMS (FAB) Calcd for $C_{32}H_{33}N_4O_9$: 617.2248 $(MH)^+$, found: 617.2278.

N-[(cyclohexylcarbamoyl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-*N*-3-nitrophenylpropiolamide (**14**): mp 122–125 °C; ^{1}H NMR (CD_3Cl) δ: 1.13–1.96 (m, 10H, –$(CH_2)_5$–), 2.06–2.37 (m, 8H, H2′, 2 –OCOCH_3), 2.85 (s, 0.5, CH, one isomer), 2.88 (s, 0.5H, CH, another isomer), 3.69–3.73 (m, 1H, –CH), 4.08–4.29 (m, 3H, H4′, H5′), 5.12–5.14 (m, 1H, H3′), 5.82 (s, 0.5 H, –CH, one iomer), 6.03 (dd, 0.5 H, $J1 = 8.8$ Hz, $J2 = 5.2$ Hz, H1, one isomer), 6.08 (s, 0.5H, CH, another isomer), 6.17 (dd, 0.5 H, $J1 = 8.8$ Hz, $J2 = 5.2$ Hz, H1, another isomer), 6.66 (d, 0.5H, $J = 7..2$ Hz, -NH, one isomer), 6.81 (d, 0.5H, $J = 7.6$ Hz, -NH, another isomer), 7.51–7.85 (m, 3H, –ArH, H6), 8.16–8.41 (m, 2H, –ArH), 9.66 (br s, 0.5H, –NH, one isomer), 9.85 (br s, 0.5H, –NH, another isomer). ^{13}C NMR (CD_3Cl) δ: 20.92, 21.00, 21.06, 24.92, 25.63, 32.67, 32.77, 32.85, 37.57, 37.68, 49.19, 49.38, 54.93, 57.14, 63.81, 64.04, 74.25, 74.35, 75.81, 75.94, 81.51, 82.04, 82.28, 82.54, 84.59, 85.01, 107.76, 108.40, 123.95, 124.14, 125.73, 126.00, 129.93, 130.10, 137.01, 137.10, 137.09, 139.68, 140.81, 142.43, 143.00, 148.51, 149.55, 149.65, 153.08, 153.29, 162.63, 162.83, 166.41, 166.86, 170.54, 171.11, 171.39. HRMS (FAB) Calcd for $C_{30}H_{34}N_5O_{11}$: 640.2256 $(MH)^+$, found: 640.2245.

N-[(1-cyclohexyl-1*H*-tetrazol-5-yl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-4-methylbenzeneamine (**15**): mp 99–101 °C; ^{1}H NMR (CD_3Cl) δ: 1.25–1.93 (m, 10H, –$(CH_2)_5$–), 1.97–2.10 (m, 7H, H2′-1, –CH_3, –OCOCH_3), 2.26 (s, 3H, –OCOCH_3), 2.42–2.47 (m, 1H, H2′-2), 4.08 (dd, 1H, $J1 = 12.0$ Hz, $J2 = 3.6$ Hz, H5′-1), 4.23–4.26 (m, 1H, H4′), 4.40–4.49 (m, 2H, H5′-2, –CH), 5.04 (d, 1H, $J = 6.0$ Hz, H3′), 5.82 (s, 1H, –CH), 6.22 (dd, 1H, $J1 = 8.8$ Hz, $J2 = 5.2$ Hz, H1′), 6.63 (d, 2H, $J = 8.0$ Hz, –ArH), 7.01 (d, 2H, $J = 8.0$ Hz, –ArH), 7.83 (s, 1H, H6), 8.92 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 20.65, 21.04, 21.10, 25.07, 25.47, 25.52, 32.95, 33.41, 37.80, 46.27, 58.58, 63.49, 74.32, 82.84, 86.30, 112.11, 115.04, 129.65, 130.36, 138.39, 142.88, 149.76, 154.17, 162.45, 170.54,

171.40. ESI LRMS *m/e*: 582 (MH^+), 604 (MNa^+). HRMS (FAB) Calcd for $C_{28}H_{35}N_7O_7$: 581.2598 $(M)^+$, found: 581.2604.

N-[(1-benzyl-1*H*-tetrazol-5-yl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-4-methylbenzeneamine (**16a**): mp 90–93 °C; ^{1}H NMR (CD_3Cl) δ: 1.86–1.94 (m, 1H, H2′-1), 1.99 (s, 3H, –CH_3), 2.07 (s, 3H, -OCOCH_3), 2.19 (s, 3H, –OCOCH_3), 2.41 (dd, 1H, $J1 = 14.4$ Hz, $J2 = 5.2$ Hz, H2′-2), 4.06 (dd, 1H, $J1 = 12.0$ Hz, $J2 = 3.2$ Hz, H5′-1), 4.18–4.22 (m, 1H, H4′), 4.34 (dd, 1H, $J1 = 12.0$ Hz, $J2 = 6.4$ Hz, H5′-2), 4.93 (d, 1H, $J = 9.6$ Hz, –NH), 5.03 (d, 1H, $J = 6.0$ Hz, H3′), 5.67–5.77 (m, 3H, –CH, –CH_2), 6.15 (dd, 1H, $J1 = 8.0$ Hz, $J2 = 5.2$ Hz, H1′), 6.42 (d, 2H, $J = 8.0$ Hz, –ArH), 6.89 (d, 2H, $J = 8.0$ Hz, –ArH), 7.13–7.26 (m, 5H, –ArH), 7.71 (s, 1H, H6), 10.11 (br s, 1H, -NH). ^{13}C NMR (CD_3Cl) δ: 20.63, 20.96, 21.10, 37.79, 46.35, 51.66, 63.62, 74.40, 82.86, 86.29, 111.87, 114.66, 127.98, 128.85, 129.19, 129.25, 130.19, 133.61, 138.59, 142.83, 150.08, 155.14, 163.17, 170.57, 171.19. HRMS (FAB) Calcd for $C_{29}H_{31}N_7O_7$: 589.2285 $(M)^+$, found: 589.2289.

N-[(1-benzyl-1*H*-tetrazol-5-yl)(3′,5′-di-*O*-acetyl-2′-deoxyuridin-5-yl)methyl]-4-methylbenzeneamine (**16b**): mp 91–93 °C; ^{1}H NMR (CD_3Cl) δ: 2.05–2.12 (m, 7H, H2′-1, –CH_3, –OCOCH_3), 2.21 (s, 3H, –OCOCH_3), 2.49 (dd, 1H, $J1 = 14.0$ Hz, $J2 = 5.6$ Hz, H2′-2), 4.19–4.31 (m, 3H, H4′, H5′), 5.16 (d, 1H, $J = 6.0$ Hz), 5.68–5.78 (m, 3 H, –CH_2, –CH), 6.16 (dd, 1H, $J1 = 8.4$ Hz, $J2 = 5.6$ Hz, H1′), 6.42 (d, 2H, $J = 8.0$ Hz, –ArH), 6.91 (d, 2H, $J = 8.0$ Hz, –ArH), 7.18–7.26 (m, 5H, –ArH), 7.82 (s, 1H, H6), 9.70 (br s, 1H, –NH). ^{13}C NMR (CD_3Cl) δ: 20.64, 21.10, 21.20, 38.05, 45.85, 51.71, 63.91, 74.57, 83.21, 86.19, 112.06, 114.58, 127.98, 128.07, 128.99, 129.30, 130.18, 133.54, 138.68, 142.60, 149.96, 155.19, 163.00, 170.58, 171.17. ESI LRMS *m/e*: 590 (MH^+), 612 (MNa^+).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bioorg.2006.08.004.

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