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Modified 5′-Trityl Nucleosides as Inhibitors of *Plasmodium falciparum* dUTPase

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2′-Deoxyuridine triphosphate nucleotidohydrolase (dUTPase) is a potential drug target for the treatment of malaria. We previously reported the discovery of 5′-tritylated analogues of deoxyuridine as selective inhibitors of this *Plasmodium falciparum* enzyme. Herein we report further structure–activity studies; in particular, variations of the 5′-trityl group, the introduction of various substituents at the 3′-position of deoxyuridine, and modifications of the base. Compounds were tested against both the enzyme and the parasite. Variations of the 5′-trityl group and of the 3′-substituent were well tolerated and yielded active compounds. However, there is a clear requirement for the uracil base for activity, because modifications of the uracil ring result in loss of enzyme inhibition and significant decreases in antiplasmodial action.

Introduction

Malaria is a widespread, life-threatening tropical disease, with over 500 million clinical cases per year. It causes over a million deaths each year, mainly amongst children in sub-Saharan Africa.[1] Due to drug resistance developed by *Plasmodium falciparum* (the causative agent of the most severe form of malaria) against current treatments, there is an urgent need for new drugs that act on new molecular targets in order to circumvent the problem of increasing resistance.

A potential target of interest is the enzyme 2′-deoxyuridine triphosphate nucleotidohydrolase (dUTPase), which is an essential enzyme in nucleotide metabolism. The role of dUTPase is to hydrolyse dUTP to dUMP. This has two major effects on nucleotide metabolism: first, it decreases the level of dUTP; second, the enzyme provides a source of dUMP for biosynthesis of dTMP, a precursor of dTTP. The combination of these effects decreases the dUTP/dTTP ratio, and hence reduces the erroneous incorporation of uracil into DNA by DNA polymerases, as these enzymes can substitute dUTP for dTTP.

There is good evidence that dUTPase is an essential enzyme. Gene inactivation has been reported to be lethal in several organisms such as *S. cerevisiae*[2] and *E. coli*,[3] and therefore inhibition of the enzyme could represent a new avenue for chemotherapeutic development.

We previously reported a series of 5′-tritylated nucleosides as inhibitors of dUTPase that are leads for the development of potential medicines for the treatment of malaria.[4,5] These compounds are highly selective for the *Plasmodium* enzyme over the human enzyme. Further SAR studies were performed that show it is possible to prepare "acyclic" analogues in which the deoxyribose ring is replaced by an aliphatic linker, whilst retaining activity.[6] Herein we report additional studies to extend our SAR analysis to 5′-substituted trityl-2′-deoxyuridine analogues, 5′-heterotrityl-2′-deoxyuridine analogues (Figure 1 A), 3′-modified 5′-trityl nucleosides, and some base-modified trityl nucleosides (Figure 1 B,C).

Such structures would further probe the *P. falciparum* dUTPase active site, giving new insight into the development of more potent and selective inhibitors. Herein we present the synthesis of the listed structures and their biological evaluation against the *P. falciparum* dUTPase and the parasite cultured in vitro.

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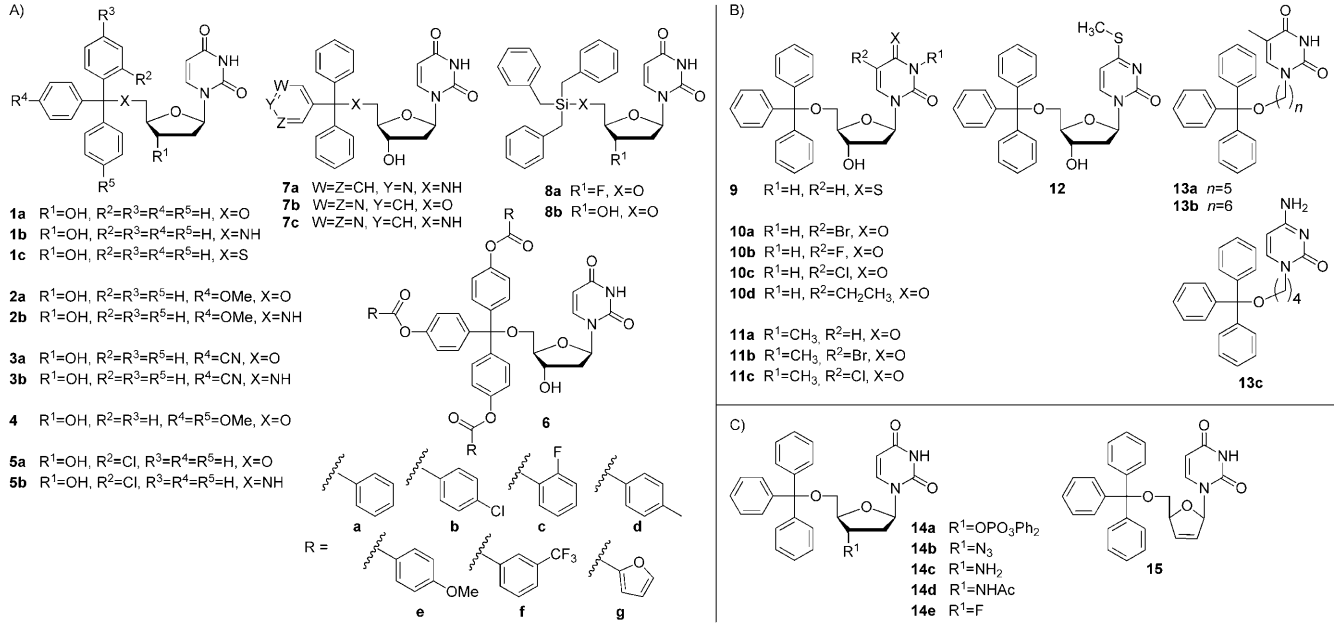
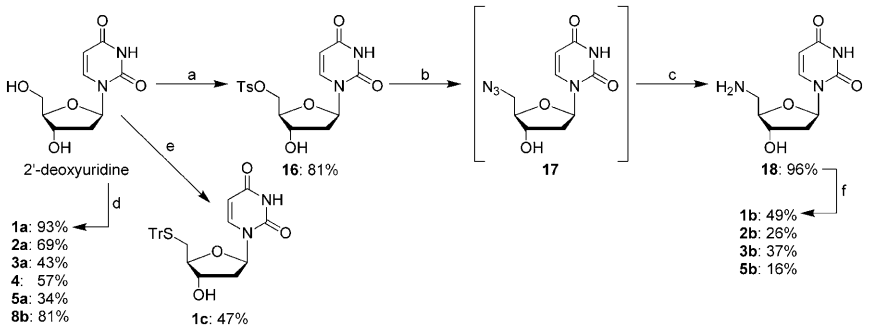


Figure 1. The modified nucleosides synthesised in this work (compounds **1a**, **1b**, **14a**, **14e**, and **15** were reported previously;[4,5] data are reported here for comparative purposes).

Chemistry

Substituted trityl analogues

Several modified 5′-trityl nucleosides were prepared from commercially available trityl chlorides (e.g., 4-methoxytrityl, 4,4′-dimethoxytrityl) by starting from either 2′-deoxyuridine or 5′-amino-2′-deoxyuridine. The latter was synthesised in a three-step procedure[7] (Scheme 1).



Scheme 1. a) TsCl, Py, 0 °C, 5 h; b) NaN_3, DMF, 90 °C, 16 h; c) H_2, 5% Pd/C, EtOH/H_2O; d) Ar_3C-Cl, Py, DMAP, 40–50 °C, 12–92 h, or Bn_3SiCl, Py, 0 °C, 2.5–3 h; e) PPh_3, Tr-SH, DEAD, 1,4-dioxane; f) Ar_3C-Cl, Py, 40–50 °C, 12–48 h.

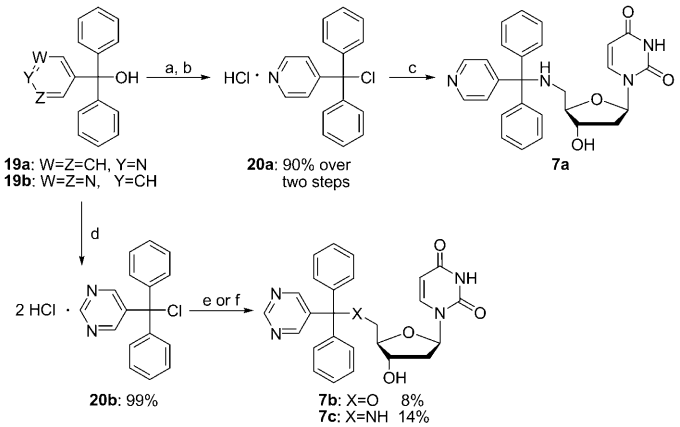
The modified trityl groups were introduced by using the general conditions reported for the synthesis of 5′-tritylated nucleosides[8] (heating in pyridine with, if necessary, catalytic 4-dimethylaminopyridine (DMAP)),[9] Scheme 1. The tribenzylsilyl derivatives **8a**,**b** were synthesised by the same procedure from the commercially available chloride (Scheme 1).

A 5′-*S*-trityl group is usually introduced into the nucleoside by direct tritylation of 5′-deoxy-5′-thionucleoside in the presence of base[10] or by reaction of trityl thiol with 5′-*O*-mesyl-,[10] 5′-*O*-tosyl-,[10] or 5′-deoxy-5′-halonucleoside.[11] To our knowledge there is no reported application of the Mitsunobu protocol for the introduction of *S*-trityl groups into nucleosides. We successfully applied this method for the synthesis of 5′-*S*-tritylthio-5′,2′-dideoxyuridine **1c** (Scheme 1).

Heterocyclic trityl derivatives

We investigated the replacement of one of the phenyl rings with an aromatic heterocycle, in particular, the 4-pyridyl and 5-pyrimidyl derivatives. These heterocycles are similar in size to the phenyl group; they may favour additional interactions with the enzyme active site through their nitrogen heteroatoms, should improve water solubility, and should also provide increased stability in acidic medium.

Diphenyl(4-pyridyl)methyl chloride itself was prepared from the corresponding carbinol (Scheme 2) by following the method of Tzerpos et al.[12] First diphenyl(4-pyridyl)methanol was converted into its hydrochloride, which was then treated with a mixture of thionyl chloride and acetyl chloride at room temperature to produce the desired chloride **20a** in quantitative yield. However, diphenyl(4-pyridyl)methyl chloride decomposed rapidly in contact with moisture, which limited storage even under vacuum. 5′-Amino-2′-deoxyuridine was successfully reacted with **20a** in the presence of triethylamine to form the diphenyl(4-pyridyl)methyl-



Scheme 2. a) HCl/H_2O, reflux, 2 h; b) $SOCl_2$, AcCl, RT, 72 h; c) 5′-amino-2′,5′-dideoxyuridine, TEA, Py, 40–70 °C; d) $SOCl_2$/AcCl, RT, 72 h; e) 5′-amino-2′,5′-dideoxyuridine, Py, TEA, microwave (1 min at 80 °C, 1 min at 100 °C); f) 2′-deoxyuridine, Py, DMAP, microwave (2×1 min at 100 °C).

amino target **7a**. The diphenyl(5-pyrimidyl)methoxy analogue **7b** and the diphenyl(5-pyrimidyl)methylamino derivative **7c** were similarly prepared from diphenyl(5-pyrimidyl)methyl chloride hydrochloride **20b** (prepared by reacting the corresponding carbinol[13] **19b** with thionyl chloride and acetyl chloride at room temperature).

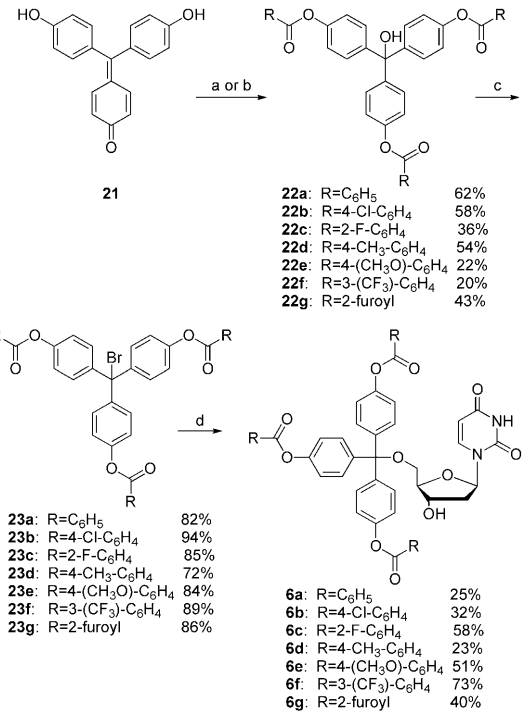
Polysubstituted trityl groups

We decided to extend the range of trityl groups examined, with some polysubstituted trityl groups. To extend application of trityl analogues, Sekine et al. introduced *tris*[4-(acyloxy)trityl] groups, which are base labile.[14–16] Only reports of acetoxy,[17] benzoyloxy,[15] and 4-methoxybenzoyl[16] derivatives have been published so far. We extended the reported methodology for the synthesis of a new series of aroyloxy analogues by starting from the commercially available aurine **21** (rosolic acid), as outlined in Scheme 3.

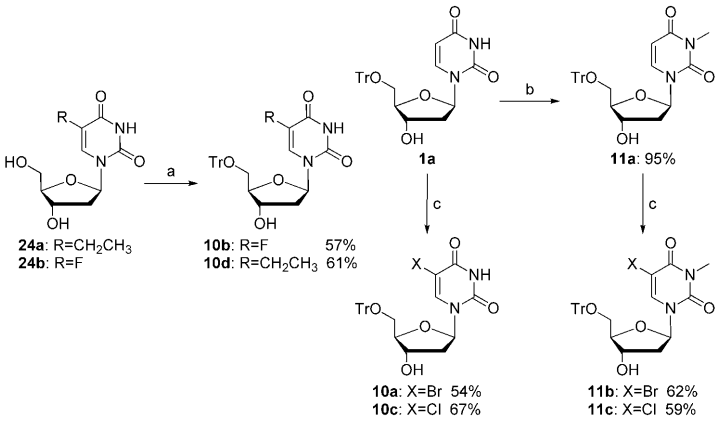
Starting from the corresponding aroyl chlorides, a series of *tris*[4-(substituted)]trityl methanols were prepared (**22a**–**g**). Treatment of compounds **22a**–**g** with acetyl bromide in toluene converted them into to the corresponding bromides **23a**[14] and **23b**–**g**. These were then coupled with the 2′-deoxyuridine in pyridine by conventional heating for compound **6a**,**b**, or by microwave irradiation with two cycles of 30 s at 100 °C, with the addition of extra tritylating agent after the first cycle, to give compounds **6c**–**g** (Scheme 3).

Modified base analogues

Bases modified at the 5-position, 5-fluoro-5′-*O*-trityl-2′-deoxyuridine[18] **10b** and 5-ethyl-5′-*O*-trityl-2′-deoxyuridine[19] **10d**, were synthesised in good yield by starting from their respective parent nucleosides: 5-ethyl-2′deoxyuridine[20] **24a** and 5-fluoro-2′-deoxyuridine **24b** (Scheme 4). Direct bromination of



Scheme 3. a) Benzoyl chloride (R: C_6H_5), tolyl chloride (R: 4-CH_3-C_6H_4), *para*-chlorobenzoyl chloride (4-Cl-C_6H_4), *para*-methoxybenzoyl chloride (R: 4-(OCH_3)-C_6H_4), *meta*-trifluoromethylbenzoyl chloride (R: 3-(CF_3)-C_6H_4), *ortho*-fluorobenzoyl chloride (R: 2-F-C_6H_4), 2-furoyl chloride (R: 2-furoyl), Py, 80–90 °C, 1.5 h; b) same as in a), *N*,*N*-dimethylaniline instead of Py; c) AcBr, PhMe, reflux, 1.5 h; d) 2′-deoxyuridine, Py, 80 °C, 3 h, or 2′-deoxyuridine, Py, microwave (2×30 s at 100 °C).

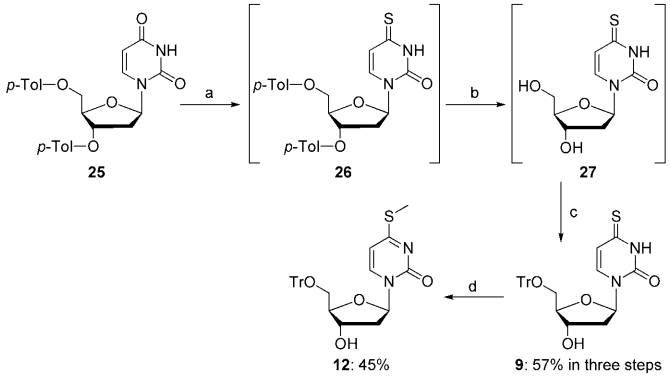


Scheme 4. a) TrCl, DMAP, Py, 80 °C, 3 h; b) CH_2N_2/Et_2O, CH_3OH; c) Py, NCS (X = Cl), or NBS (X = Br).

1a to 5-bromo-5′-*O*-trityl-2′-deoxyuridine **10a** with elemental bromine was unsuccessful and led only to decomposition. *N*-Halo-substituted succinimides in pyridine have been reported for the synthesis of 5-halogenated *N*-1-propargyluracils.[21] Chlorination of **1a** with *N*-chlorosuccinimide (NCS) by this method led to 5-chloro-5′-*O*-trityl-2′-deoxyuridine **10c** in good yield (Scheme 4). The 5-bromo analogue[22] **10a** was prepared in the same way with *N*-bromosuccinimide (NBS) instead of NCS. Direct treatment of **1a** with diazomethane resulted in

quantitative synthesis of 3-methyl-5′-*O*-trityl-2′-deoxyuridine **11a**, which was then used as the starting material for the synthesis of 3-methyl-5-bromo-5′-*O*-trityl-2′-deoxyuridine **11b** and 3-methyl-5-chloro-5′-*O*-trityl-2′-deoxyuridine **11c**.

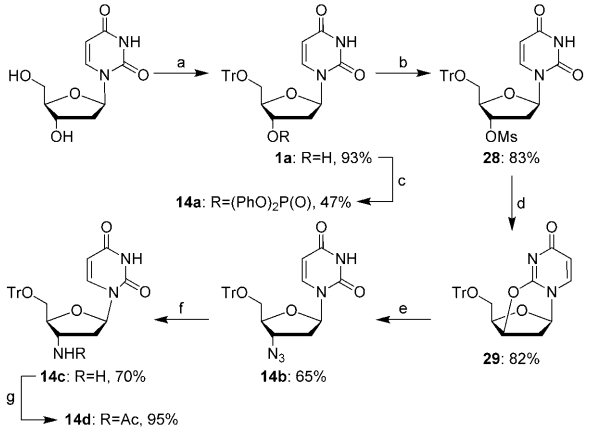
Our efforts in the direct thiation of **1a** with Lawesson's reagent[23] were unsuccessful. Therefore, we decided to tritylate 4-thio-2′-deoxyuridine **27** to 4-thio-5′-*O*-trityl-2′-deoxyuridine[24] **9**. Lawesson's-reagent-promoted thiation of 5′,3′-di-*O*-*p*-tolyl-2′-deoxyuridine[25] **25** resulted in the formation of 4-thio-5′,3′-di-*O*-*p*-tolyl-2′-deoxyuridine **26** in high yield. This intermediate was deprotected with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in methanol into **27**, which was used directly for tritylation. Methylation of **9** into its 4-methylthio derivative **12** was carried out in very good yield with methyl iodide (Scheme 5).



Scheme 5. a) Lawesson's reagent, dioxane; b) DBU, CH_3OH; c) TrCl, DMAP, Py; d) MeI, NaOH, TDA-1, dioxane.

Modified 3′-nucleosides

Modifications of the 3′-position of the sugar ring were based on the synthetic approaches developed by Horwitz and coworkers[26] for the synthesis of AZT and by Kumar et al.[27] for the synthesis of threothymidine, as illustrated in Scheme 6.



Scheme 6. a) TrCl, Py, 50 °C, 12 h; b) CH_3SO_2Cl, Py, 0 °C then RT, 4 h; c) $(PhO)_2P(O)Cl$, Py, RT, 12 h; d) DBU, CH_2Cl_2, RT, 30 h; e) LiF, TMEDA, Me_3SiN_3, DMF, 110 °C, 20 h; f) H_2, Lindlar's catalyst, EtOH, 5 h; g) Ac_2O, TEA, CH_2Cl_2, RT, 3 h.

The 5′-trityl-2′-deoxyuridine **1a** was converted into the 3′-diphenyl phosphate ester **14a** by reaction with diphenylchlorophosphate.[5] The azido derivative **14b** was prepared by mesylation, and then formation of the 5′-trityl anhydro nucleoside **29**. This was opened with lithium azide (generated in situ from lithium fluoride and azidotrimethylsilane in the presence of tetramethylethylenediamine (TMEDA), as suggested by Kirschenheuter and colleagues[28]) to form **14b** with retention of 2′-deoxyribose stereochemistry at the 3′-position. In the next stage, azide **14b** was converted into the amine **14c** by mild hydrogenation using Lindlar's catalyst. Previous attempts with 10% and 5% Pd/C were unsuccessful due to the lability of the trityl ether under the hydrogenation conditions. The 3′-amino nucleoside **14c** was then reacted with acetic anhydride to produce the acetamide derivative **14d**.

Results and Discussion

Biological results

All the compounds were evaluated against recombinant *P. falciparum* and human dUTPases in order to estimate inhibition constants and selectivity. They were also screened against cultures of the parasite to evaluate in vitro IC_{50} values.

Enzyme inhibition

The lead compounds for the inhibition of *P. falciparum* dUTPase are 5′-O-trityl-2′-deoxyuridine **1a** and 5′-tritylamino-2′-deoxyuridine **1b**, the latter of which is the most potent inhibitor reported so far ($K_i = 0.2$ μM).[4–6] The thio-linked analogue **1c** exhibited activity against the enzyme similar to that of the oxygen-linked analogue **1a**, but with slightly better selectivity (Table 1).

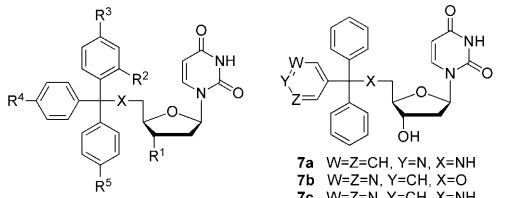
Methoxy, nitrile, and chloro substituents on the trityl group (compounds **1**–**5**) were generally well tolerated, with the 4-cyanotrityl compounds **3a** and **3b** and the 4-methoxytrityl **2b** presenting the best K_i values in the sub-micromolar range. Although the inhibition constants are similar, but not better than that of the lead **1b**, all these substituted trityl derivatives were selective for the *Plasmodium* dUTPase over the human enzyme.

None of the polysubstituted trityl derivatives **6a**–**g** showed any activity, with K_i values all greater than 1000 μM. This may be due to steric hindrance or hydrolysis with concomitant loss of the trityl group.

The "heterotrityl" derivatives **7a**–**c** had similar activity to the lead compounds. The pyridyldiphenyl derivative **7a** was the most active, with a K_i value of 0.23 μM comparable with the lead **1b**. Remarkably, all heterotrityl nucleosides **7a**–**c** were more selective for the parasitic enzyme, with inhibition constants >1000 μM for the human dUTPase, and consequently high selectivity indexes ($K_{i\,human}/K_{i\,P.fal.}$).

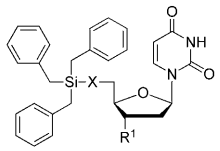
Replacement of the oxygen atom in the 5′-position with an amino group improved the activity in some cases, as shown by the pairs **1a**/**1b** and **2a**/**2b**, whilst in other cases the effect

Table 1. Biological results for the modified 5′-trityl nucleosides.



1a R_1=OH, R^2=R^3=R^4=R^5=H, X=O
1b R^1=OH, R^2=R^3=R^4=R^5=H, X=NH
1c R^1=OH, R^2=R^3=R^4=R^5=H, X=S
2a R^1=OH, R^2=R^3=R^5=H, R^4=OMe, X=O
2b R^1=OH, R^2=R^3=R^5=H, R^4=OMe, X=NH
3a R^1=OH, R^2=R^3=R^5=H, R^4=CN, X=O
3b R^1=OH, R^2=R^3=R^5=H, R^4=CN, X=NH
4 R^1=OH, R^2=R^3=H, R^4=R^5=OMe, X=O
5a R^1=OH, R^2=Cl, R^3=R^4=R^5=H, X=O
5b R^1=OH, R^2=Cl, R^3=R^4=R^5=H, X=NH

7a W=Z=CH, Y=N, X=NH
7b W=Z=N, Y=CH, X=O
7c W=Z=N, Y=CH, X=NH



8a R^1=F, X=O
8b R^1=OH, X=O

Compd	K_i [μM] *P. fal.* dUTPase	K_i [μM] Human dUTPase	IC_{50} [μM] *P. fal.*[a]	IC_{50} [μM] L6 cells[b]
1a	1.8[4]	17.7[4]	6[4]	192[4]
1b	0.2[4]	46.3[4]	4.5[4]	nd
1c	4.2	>1000	1.1	21
2a	4.7	>1000	1.9	nd
2b	0.68	351	6.2	nd
3a	0.94	696	1.3	44
3b	0.37	213	3.0	28
4	1.9	>1000	2.8	24
5a	2.3	>1000	2.0	35
5b	3.4	238	2.0	30
6a	>1000	>1000	3.7	108
6b	>1000	>1000	2.7	>96
6c	>1000	>1000	1.3	>102
6d	>1000	>1000	1.6	>103
6e	>1000	>1000	1.0	>98
6f	>1000	>1000	4.3	>87
6g	>1000	>1000	1.2	10
7a	0.23	>1000	>10	nd
7b	1.8	>1000	7.7	>190
7c	0.98	>1000	7.2	>190
8a	>1000	>1000	2.1	14
8b	103	>1000	1.2	29

[a] *P. falciparum K1*. [b] Cytotoxicity toward rat L6 myoblasts; nd: not determined. Controls: for *P. falciparum*, chloroquine, IC_{50} = 0.1 μM; for cytotoxicity, podophyllotoxin, IC_{50} = 0.012 μM. IC_{50} values are the means of four values of two independent assays performed in duplicate.

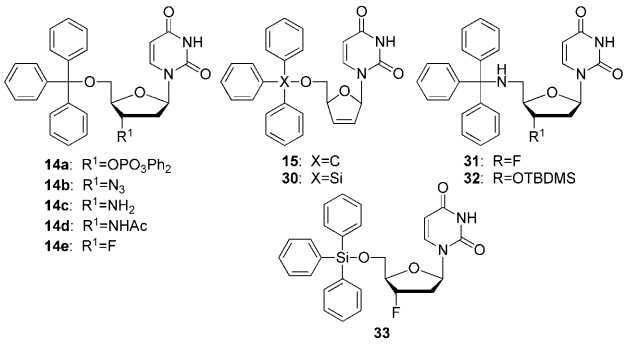
was marginal (**3a**/**3b** and **7b**/**7c**). With the sulfur, instead, a loss of activity was observed (K_i = 4.5 μM for **1c**).

3′ Variants

Substitution of the OH group at the 3′-position of the ribose with NH_2 rendered K_i values (K_i = 1.3 μM for **14c**) similar to that of the lead compound **1a**, and the N-acetylated derivative **14d** was equally active (K_i = 1.3 μM). Replacement of the 3′-OH group with fluorine led to a slight loss of inhibition as shown by compounds **14e** and **31**. These compounds had similar activity to the tritylated d4U **15**, with no substituent at the 3′-position (Table 2).[5]

Compounds with bulky lipophilic groups such as **14a** and **32** showed high K_i values, indicating that this kind of substituent does not favour enzyme inhibition. Interestingly, the 5′-triphenylsilyl-3′-fluoro-2′,3′-dideoxyuridine **33** was the least active of this series of compounds, with a K_i value greater than the highest concentration tested in the biological assay. This compares with **14e** and **30**, which have activity at ~1–5 μM.

Table 2. Biological results (enzymatic and cellular) for the 3′-modified nucleosides.



14a: R^1=OPO_3Ph_2
14b: R^1=N_3
14c: R^1=NH_2
14d: R^1=NHAc
14e: R^1=F

15: X=C
30: X=Si

31: R=F
32: R=OTBDMS

33

Compd	K_i [μM] *P. fal.* dUTPase	K_i [μM] Human dUTPase	IC_{50} [μM] *P. fal.*[a]	IC_{50} [μM] L6 cells[b]
14a	112	nd	0.8	18
14b	47	>1000	1.6	25
14c	1.3	>1000	0.99	3.1
14d	1.3	>1000	1.92	40
14e[4]	5.0	457.0	2.0	nd
15[4]	0.70	>1000	>11	nd
30[4]	1.3	>1000	1.0	85
31[4]	12.4	>1000	5.3	30
32[4]	313	>1000	1.8	8
33[4]	>1000	>1000	1.0	85

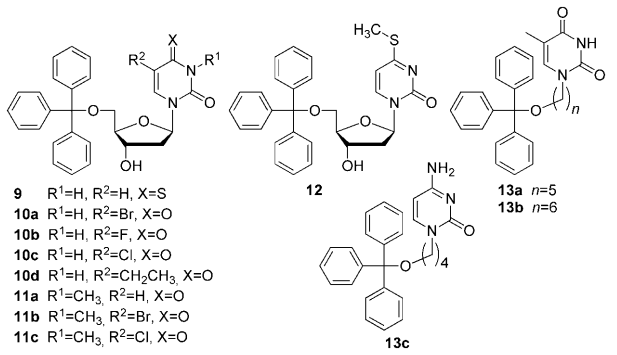
[a] *P. falciparum K1*. [b] Cytotoxicity toward rat L6 myoblasts; nd: not determined. Controls: for *P. falciparum*, chloroquine, IC_{50} = 0.1 μM; for cytotoxicity, podophyllotoxin, IC_{50} = 0.012 μM. IC_{50} values are the means of four values of two independent assays performed in duplicate. Compounds **14a**, **14e**, **15**, and **30**–**33** were published previously, and are listed here for comparative purposes only.

Base analogues

Modification of the base of the nucleosides was clearly not well tolerated, as all the resulting compounds were essentially inactive against the enzyme. This probably reflects the tight hydrogen-bonding network that binds the uracil in the dUTPase active site. The enzyme is very specific in its binding, only binding dUTP and not dTTP, dCTP, dATP, or dGTP. The only exception to this lack of inhibitory potency among the base-modified compounds is the 5-fluorouracil derivative **10b**, which has a K_i value of 22 μM. This is perhaps not surprising, as the fluorine has relatively small steric requirements (Table 3).

The acyclic analogues of thymidine **13a**,**b** and cytidine **13c** are completely devoid of activity, confirming the high selectivity of the enzyme for the uracil base. In fact, the latter three compounds are analogues of acyclic uracil derivatives, which were previously reported as very potent inhibitors of *P. falciparum* dUTPase.[6]

Table 3. Biological results for the base-modified nucleosides.



Compd	K_i [μM]		IC_{50} [μM]	
	P. fal. dUTPase	Human dUTPase	*P. fal.*[a]	L6 cells[b]
9	201	>1000	4.0	35
10a	>1000	480	9.1	35
10b	22	>1000	10.2	23
10c	>1000	800	9.9	29
10d	>1000	>1000	5.6	6
11a	>1000	>1000	2.6	14
11b	>1000	>1000	3.3	14
11c	>1000	>1000	4.8	12
12	>1000	>1000	0.4	18
13a	>1000	>1000	6.6	nd
13b	>1000	>1000	6.2	nd
13c	>1000	>1000	nd	nd

[a] *P. falciparum K1*. [b] Cytotoxicity toward rat L6 myoblasts; nd: not determined. Controls: for *P. falciparum*, chloroquine, $IC_{50}=0.1$ μM; for cytotoxicity, podophyllotoxin, $IC_{50}=0.012$ μM. IC_{50} values are the means of four values of two independent assays performed in duplicate.

In vitro assay

All compounds were assayed against intact *P. falciparum* parasite cultured in human erythrocytes and against mammalian L6 cells as a counterscreen for toxicity. A number of compounds showed activity against *P. falciparum* in the low or sub-micromolar levels, with good selectivity over the mammalian cells. However, others showed poor selectivity (<10-fold) and are probably generally toxic to the cells (notably compounds **3b**, **4**, **6g**, **8a**, **9**, **10a**–**d**, **11a**–**c**, **14c**, **31**, and **32**).

Discussion

5′-Trityl analogues

SAR analysis of the modified 5′-trityl nucleosides shows that substituents in the triphenyl moiety can be introduced without compromising enzyme inhibition, with the exception of the bulky aryl substituents in the series of compounds **6a**–**g**. The cyano derivative **3**, the chloro **5**, the methoxy **2**, and dimethoxy **4** all exhibit similar K_i values. The lack of activity of the large aryl groups (compounds **6a**–**g**) is probably due either to the group being too large to fit into the active site, or to a lack of chemical stability.

An interesting result was observed with the heteroaryldiphenyl derivatives **7a**–**c**. In these cases one of the phenyl rings could be substituted with a heteroaryl group without compromising enzyme inhibition, although there was a loss of in vitro activity for compound **7a** ($IC_{50}>10$ μM), whilst **7b** and **7c** retained activity. The introduction of a heteroaryl group such as pyridine or pyrimidine should increase water solubility and decrease the log *P* value.

The general lack of variation of enzyme inhibition as the substituent on the trityl group is modified, is consistent with the hydrophobic character of the trityl-binding pocket of *P. falciparum* dUTPase. Moreover, one of the phenyl rings is exposed to the solvent, and this is likely to be where the substituted phenyl or heteroaromatic ring of the inhibitors would sit upon binding the enzyme.

Increased enzyme inhibition is observed if the oxygen atom at the 5′-position is replaced by an amino group in compound pairs **1a**/**1b** and **2a**/**2b**. However, a similar trend was not obtained for growth inhibition in vitro, although all pairs gave IC_{50} values within the 1–6 μM range. This is presumably due to other factors such as cell permeability, which undoubtedly plays a major role in activity against the parasite. With the other pairs **3a**/**3b**, **5a**/**5b**, and **7b**/**7c**, there was no significant change observed in enzyme inhibition on replacing the 5′-oxygen with a 5′-nitrogen. The 5′-thiotrityl derivative **1c** showed moderate enzyme inhibition ($K_i=4.2$ μM), yet an IC_{50} value of 1.1 μM against the parasite. However, this result could be influenced by the slightly increased toxicity of **1c**, as shown by the L6 cell assay: $IC_{50}=21$ μM (Table 1).

3′ Variants

In analysis of the 3′-position of the sugar we observed that the replacement of the OH group with NH_2 retained enzyme inhibition, as seen for the 3′-amino derivative **14c**, but this also increased toxicity. Acylation of the amino moiety (compound **14d**) did not affect enzyme inhibition but decreased the toxicity against mammalian cells and retained antiplasmodial activity ($IC_{50}=2.1$ μM). The 3′-diphenyl phosphate ester **14a**, the 3′-azide **14b**, and the 3′-*tert*-butyldimethylsilyl ether **32** all have K_i values >40 μM; however, for two of these compounds the presence of a bulky lipophilic group could limit solubility and affect activity against the enzyme in vitro. Nonetheless, it is possible to replace the 3′-hydroxy group with a fluorine, as observed for **14e** (Table 2).

No significant conclusions on the requirements for antiparasitic activity can be obtained for the 3′-substituted compounds, as **14a**, **14b**, **14d**, **30**, and **33** are all active, with IC_{50} values ~1–2 μM. However, comparing these results with the previously reported 3′-fluoro **14e** and didehydro derivatives **15**,[5] we can deduce that the substitution of the 3′-hydroxy group with small polar groups does not affect inhibition of either the enzyme or parasite growth in vitro. This is in agreement with the structural data, which suggest that the 3′-substitutent can point toward the solvent and away from the active site, thus allowing substitutions at this position.[4]

Base analogues

The high selectivity of dUTPase for uridine analogues strongly limits the modifications that can be introduced on the nucleobase. As shown in Table 3, the 5-bromo **10a**, 5-chloro **10c**, and 5-ethyl **10d** nucleosides were all poor inhibitors, and none of the N3-methylated derivatives **11a**, **11b**, or **11c** gave K_i values less than 1000 μM. The best-tolerated substitution was the introduction of a fluorine atom at position 5 for compound **10b**. This compound showed a moderate inhibition of *P. falciparum* dUTPase ($K_i = 22$ μM). The base analogues (compounds **9**–**12**), in addition to showing poor inhibition of dUTPase, appear to be toxic in cell culture and lack selectivity.

We had previously reported some acyclic uracil derivatives as potent inhibitors of the parasitic enzyme, and it was therefore of interest to test whether the corresponding thymidine derivatives **13a**, **13b**, and cytosine derivative **13c** are active. As reported in Table 3, none of the three acyclic analogues showed K_i values lower than 1 mM, again indicating the strong requirement of the uracil ring.

Enzyme and cell activity

Compounds that showed inhibition of *P. falciparum* dUTPase also inhibited the growth of the intact parasite, with the exception of compounds **7a** and **15**. The reason for the lack of in vitro activity for these compounds is still unclear. This could possibly be due to instability in the cell culture medium or issues related to uptake/efflux or protein binding. The majority of compounds that are active and selective in the enzyme inhibition assays are also selective in vitro for *P. falciparum* over mammalian L6 cells, with the exception of compounds **3b**, **4**, **14c**, and **31**. This result is consistent with the selective inhibition of the *P. falciparum* dUTPase. Most of the compounds that are active against *P. falciparum* dUTPase show inhibition in the fairly narrow K_i value range of 0.2–5 μM. These compounds inhibit growth of the parasite in the range of 1–7 μM. Conversely, compounds that are not selective for *P. falciparum* dUTPase over the human enzyme are nonselective (toxic) at the cellular level.

The exceptions to this are compounds **6a**–**g**. These are inactive against *P. falciparum* dUTPase, yet are active and selective against the parasite. These compounds almost certainly act through a mechanism of action other than inhibition of dUTPase. A supposed mechanism of action could involve intracellular esterase-mediated hydrolysis of the ester groups at position 4 of the phenyl rings. This would lead to a 4,4′,4″-*tris*-(hydroxy)trityl group that could undergo cleavage under basic conditions, as reported by Sekine and co-workers,[14] releasing the unprotected nucleoside and rosolic acid. The latter is known to have toxic effects in mammals.[29] This cleavage presumably occurs more rapidly in the parasite than in L6 cells, accounting for the selectivity.

As we previously reported,[5] the trityl moiety itself has certain activity against *P. falciparum*, although not against the enzyme. Thus the trityl alcohol has an IC_{50} value of 12.3 μM against *P. falciparum*, whereas the triphenylsilyl alcohol has an IC_{50} value of 0.3 μM. However, there is clearly a base requirement for selective activity, because analogues (compounds **9**–**13**) in which the uracil is modified or replaced by another base are generally less active and toxic to both *P. falciparum* and L6 cells in culture.

Conclusions

Some new 5′-trityl-2′,3′-dideoxyuridine analogues were synthesised. The modifications were introduced on the oxygen atom at the 5′-position of the sugar moiety, on the phenyl rings of the trityl group, at the 3′-position of the sugar, and on the nucleobase. There is almost no tolerance for modifications on the base. However, there is room for variation of the trityl group and the 3′-position. Some of the compounds prepared failed to inhibit dUTPase, yet intracellular metabolism and additional modes of action could explain this. New compounds with improved activity and selectivity against *P. falciparum* dUTPase may give rise to nontoxic entities that show increased activity against the parasite.

Experimental Section

General methods

^{1}H NMR, ^{13}C NMR, ^{19}F NMR, and 2D NMR spectra were recorded either on a Bruker Avance DPX 300 spectrometer, on a Bruker Avance DPX 500 spectrometer, or on a Varian 400 MHz instrument. Chemical shifts (δ) are expressed in ppm. Signal splitting patterns are described as singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), multiplet (m), or a combination thereof; 2D NMR spectra were performed to aid in assignments.

Low-resolution electrospray (ES) mass spectra were recorded either on a Fisons VG Platform mass spectrometer, a Bruker MicroTof mass spectrometer, or on a Waters Micromass ESI Q-ToF spectrometer, run in either positive or negative ion modes, using either CH_3OH, CH_3OH/H_2O (95:5) or H_2O/CH_3CN (1:1) + 0.2% HCOOH as the mobile phase. For each spectrum, the most abundant and relevant peaks are listed with their relative intensities (%) given in brackets. High-resolution electrospray MS measurements were performed by the EPSRC Mass Spectrometry Centre, Swansea (UK).

Infrared (IR) spectra were recorded on a PerkinElmer 1600 FTIR spectrometer using NaCl plates for liquids (neat) and with a diffuse reflectance accessory using a KBr matrix for solids. IR data are given as wavenumbers expressed in cm^{-1}.

Melting points (mp) were measured on a Griffin analogue melting-point apparatus and are uncorrected. Thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} plates using UV light and/or PMA or ninhydrin for visualisation. TLC data are given as the R_f value with the corresponding eluent system specified in brackets. Column chromatography was performed either with prepacked silica columns (ISOLUTE SI columns) on a Jones Chromatography Flashmaster apparatus or with Fluka silica gel 60.

All reactions were carried out under dry and inert conditions (N_2 atmosphere) unless otherwise stated. Reactions using microwave irradiation were carried in a Biotage Initiator™ microwave.

Enzyme purification and inhibition assays

Both recombinant *P. falciparum* and human dUTPases were expressed in *E. coli* BL21 (DE3) cells that had been transformed with the pET11Pfdut and pET3Hudut (kindly provided by P. O. Nyman, Lund University, Sweden) expression vectors, respectively. For dUTPase purification, the same procedure was used for both the human and *Plasmodium* enzymes. Cell pellets from a 2-L IPTG-induced culture were resuspended in 40 mL buffer A (20 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) pH 5.5, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT) and 20 mM PMSF. The cells were lysed by sonication, and the cell extract was cleared by centrifugation at 16000 *g* for 45 min. The supernatant was loaded onto a 50 mL phosphocellulose (Whatman P-11) column at 4 °C and was eluted with a gradient of NaCl (50 mM→2 M) in buffer A. The enzyme was then dialyzed against buffer A prior to gel filtration chromatography on a Superdex 200 HA 10/30 column at 4 °C. Purified fractions contained dUTPase of ~96% purity.

Nucleotide hydrolysis was monitored by mixing enzyme and substrate with a rapid kinetic accessory (Hi-Tech Scientific) attached to a spectrophotometer (Cary 50) and connected to a computer for data acquisition and storage as described previously.[30] Protons, released through the hydrolysis of nucleotides, were neutralised by a pH indicator in weakly buffered medium with similar pK_a and monitored spectrophotometrically at the absorbance peak of the basic form of the indicator. The ratio between the indicator and the buffer concentration was 50:2000 (μM), and the absorbance changes were kept within 0.1 units. The indicator/buffer pair used was red cresol/bicine (pH 8, $\lambda = 573$ nm). Assay mixes contained 30 nM PfdUTPase, 50 μM dUTP, 5 mM $MgCl_2$, 1.25 mg mL^{-1} BSA, and 100 mM KCl. V_{max} and K_{Mapp} were calculated by fitting the resulting data to the integrated Michaelis–Menten equation. The apparent K_M values were plotted against inhibitor concentration, and K_i values (Table 2) were obtained according to Equation (1).

$$K_{Mapp} = K_M/K_i\,[I] + K_M \quad (1)$$

General procedure A for the synthesis of 5′-tritylated and modified 5′-trityl nucleosides 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, 5b, 10b, 10d, 13a, 13b, and 13c: To a stirred solution of nucleoside (1 equiv) in pyridine was added the trityl precursor (1.1–1.3 equiv) and DMAP (0.1 equiv) when stated. The reaction was stirred at 40–80 °C for 3–92 h (as reported for each compound). The progress of the reaction was monitored by TLC. The mixture was cooled to room temperature, quenched with H_2O or CH_3OH, and extracted with CH_2Cl_2 or EtOAc. The organic solution was washed with H_2O, saturated $NaHCO_3$, and diluted HCl or AcOH, or otherwise stated. The solution was dried over $MgSO_4$, concentrated, and purified by chromatography.

General procedure B for the synthesis of modified trityl nucleosides 6b–g: A microwave vial was charged with 2′-deoxyuridine (1 equiv), *tris*(substituted)trityl bromide (0.5 equiv) and pyridine. The reaction was heated at 100 °C under microwave irradiation for 30 s. After the first cycle more trityl precursor (0.5 equiv) was added to the solution, and the reaction was again irradiated at 100 °C for 30 s. The solvent was co-evaporated with toluene, and the crude residue was purified by chromatography. ISOLUTE SI column eluted with 0→5% CH_3OH in $CHCl_3$.

General procedure C for the synthesis of 5′-tribenzylsilyl nucleosides 8a and 8b: Tribenzylsilyl chloride (1.3 equiv) was added to a solution of 2′-deoxyuridine (1 equiv) in pyridine. The reaction was stirred at 0 °C for 2.5–3 h. The solvent was removed under reduced pressure. The residue was co-evaporated with toluene several times and purified by chromatography. ISOLUTE SI column eluted with 0→3% CH_3OH in $CHCl_3$.

General procedure F for the synthesis of 5-chloro- and 5-bromo-2′-deoxynucleosides: To a stirred solution of nucleoside (0.2 mmol) in 3 mL dry pyridine, protected from light, was added the appropriate *N*-halosuccinimide (NCS or NBS; 0.4 mmol), and the resulting mixture was kept at 100 °C for 30 min. The mixture was cooled, excess volatiles were removed by evaporation, and the crude residue was purified by PTLC in 10% CH_3OH in $CHCl_3$ and precipitated with hexane from *i*PrOH.

General procedure G for the synthesis of *tris*[4-(aroyloxy)phenyl]methanols in the presence of pyridine: To a solution of rosolic acid (10 g, 34.5 mmol) in dry pyridine (25 mL) was added the appropriate aroyl chloride (183.2 mmol). The mixture was heated at 90 °C for 1.5 h, and progress of the reaction was monitored by TLC in 3% $(CH_3)_2CO$ in $CHCl_3$. The mixture was cooled to room temperature, quenched with ice-H_2O and finally extracted with CH_2Cl_2. The organic phase was washed several times with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated under reduced pressure to a gum. The gummy residue was co-evaporated several times with toluene to remove the last traces of pyridine to give the crude product, which was recrystallised from toluene/cyclohexane or CH_3OH.

General procedure H for the synthesis of *tris*[4-(aroyloxy)phenyl]methanols in the presence of 4-(*N*,*N*-dimethyl)aniline: This procedure is similar to that mentioned above with the use of dry 4-(*N*,*N*-dimethyl)aniline (25 mL) instead of dry pyridine. The crude gummy residue was purified by column chromatography on silica gel in a stepwise gradient of toluene/$CHCl_3$ 1:1, $CHCl_3$, $CHCl_3$/acetone 98:2 (*v*/*v*), and finally recrystallised from toluene/cyclohexane or CH_3OH.

General procedure I for the synthesis of trityl bromides: To a suspension of appropriate trityl analogue (5.66 mmol) in dry toluene (10 mL) was added acetyl bromide (1 mL, 13.5 mmol). The mixture was heated at 85 °C for 1.5 h, and the hot solution was filtered to remove a small amount of insoluble material. Dry hexane was added portionwise to the hot filtrate. After cooling, the resulting precipitate was collected by filtration and washed with dry hexane/toluene (2:1 *v*/*v*) and then with dry hexane.

5′-*S*-Tritylthio-5′,2′-dideoxyuridine (1c): To a stirred solution of 2′-deoxyuridine (50 mg, 0.22 mmol) in 3 mL dry 1,4-dioxane were subsequently added in four portions PPh_3 (281.6 mg, 1.05 mmol; 70.4 mmol, 0.263 mmol in each portion) and TrSH (362.0 mg, 1.28 mmol; 90.5 mg, 0.32 mmol in each portion) followed by DEAD also in four portions (0.18 mL, 1.09 mmol; 45 μL, 0.272 mmol in each portion). The resulting mixture was stirred at room temperature. The mixture was concentrated to dryness. The residue was purified by PTLC eluting with 10% CH_3OH in $CHCl_3$ and crystallised from toluene to give **1c** (50.3 mg, 47%); mp: 91–93 °C; $R_f = 0.43$ (5% $CH_3OH/CHCl_3$); ^{1}HNMR ($CDCl_3$): $\delta = 1.98$, 2.31 (1H, ddd, $J =$ 6.8 Hz, $J = 13.8$ Hz, $J = 6.5$ Hz, and 1H, ddd, $J = 4.3$ Hz, $J = 6.4$ Hz, $J =$ 14.0 Hz, 2′-H an 2′′-H), 2.48, 2.60 (1H, dd, $J = 5.4$ Hz, $J = 12.9$ Hz and 1H, dd, $J = 6.5$ Hz, $J = 12.9$ Hz, 5′-H and 5′′-H), 3.68 (1H, m, 4′-H), 4.00 (1H, m, 3′-H), 5.70 (1H, d, $J = 7.6$ Hz, 5-H), 6.10 (dd, 1H, $J =$ 6.5 Hz, $J = 6.5$ Hz, 1′-H), 7.22–7.45 (16H, m, Ar*H*, 6-H), 8.29 (1H, bs, N*H*); MS (EI) *m*/*z* 509 $[M+Na]^+$; Anal. (%) calcd for ($C_{28}H_{26}N_2O_5S$, 1 CH_3OH): C 67.16, H 5.83, N 5.40, found: C 66.97, H 5.88, N 5.71.

5′-*O*-(4-Methoxytrityl)-2′-deoxyuridine (2a): This compound was prepared by following general procedure A, starting from 4-me-

thoxytrityl chloride (0.61 g, 1.98 mmol), 2′-deoxyuridine (0.41 g, 1.80 mmol), and pyridine (10 mL), stirred at 50 °C for 40 h. Purified by flash column chromatography, eluting the column (ISOLUTE SI) with a gradient of 0→5% CH_3OH in $CHCl_3$. The title compound was obtained as a white crystalline solid (0.625 g, 69%). R_f = 0.28 (10% $CH_3OH/CHCl_3$); mp: 96–97 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.27 (1H, m, 2′-H), 2.42 (1H, m, 2′-H), 2.57 (1H, bs, 3′-OH), 3.42 (2H, m, 5′-H), 3.77 (3H, s, OCH_3), 4.00 (1H, m, 4′-H), 4.54 (1H, m, 3′-H), 5.37 (1H, d, J = 8.1 Hz, 5-H), 6.29 (1H, t, J = 6.3 Hz, 1′-H), 6.82 (2H, d, J = 8.9 Hz, Ar-*H*), 7.18–7.38 (12H, m, Ph-*H* and Ar-*H*), 7.74 (1H, d, J = 8.1 Hz, 6-H), 9.20 (1H, bs, 3-NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 41.6 (2′-CH_2), 55.7 (5′-CH_2), 63.5 (OCH_3), 71.9 (3′-CH_2), 85.5 (4′-CH), 86.5 (1′-CH), 87.8 ($ArPh_2$-*C*), 102.7 (5-CH), 113.8 (Ar-CH), 127.8 (Ph-CH), 128.5 (Ph-CH), 128.8 (Ph-CH), 130.8 (Ar-CH), 135.1 (Ar-C), 140.6 (6-CH), 144.1 (Ph-C), 144.3 (Ar-CH), 150.8 (2-C), 159.3 (Ar-C), 163.8 (4-C); LRMS (ES$^+$) *m/z* 523 ([*M*+Na]$^+$, 100%); HRMS (ES$^+$) calcd for $C_{29}H_{28}N_2O_6Na^+$ [*M*+Na]$^+$: 523.1840, found: 523.1837; IR (KBr): $\tilde{\nu}$ = 3208, 3054, 1714, 1694, 1682, 1507, 147, 1250, 1092, 1034, 759, 702 cm^{-1}; Anal. (%) calcd for ($C_{29}H_{28}N_2O_6$, 1.42 HCl, 0.40 H_2O): C 62.25, H 5.44, N 5.01, Cl 9.00, found: C 62.17, H 5.05, N 4.85, Cl 8.86.

5′-(4-Methoxytrityl)amino-2′,5′-dideoxyuridine (2b): The compound was prepared following the general procedure A, starting from 5′-amino-2′,5′-dideoxyuridine **23** (0.20 g, 0.90 mmol), 4-methoxytrityl chloride (0.29 g, 0.99 mmol) and pyridine (5 mL), stirred at 50 °C for 40 h. Purified by flash column chromatography eluting the column (ISOLUTE SI) with a gradient of 0→5% CH_3OH in $CHCl_3$. Compound **2b** was obtained as a white solid (0.115 g, 26%). R_f = 0.29 (10% $CH_3OH/CHCl_3$); mp: 140–143 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.04 (2H, m, 2′-H), 2.29–2.48 (2H, m, 5′-H), 2.69 (1H, dd, J = 3.7, 12.1 Hz, 5′-NH), 3.83 (3H, s, OCH_3), 4.15 (1H, m, 4′-H), 4.30 (1H, m, 3′-H), 5.69 (1H, d, J = 8.1 Hz, 5-H), 6.32 (1H, t, J = 6.4 Hz, 1′-H), 6.88 (2H, m, Ar-*H*), 7.17 (1H, d, J = 8.1 Hz, 6-H), 7.21–7.61 (14H, m, Ph-*H* and Ar-*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 40.8 (2′-CH_2), 46.6 (5′-CH_2), 55.7 (OCH_3), 70.7 ($ArPh_2$-*C*), 73.0 (3′-CH), 85.4 (1′-CH), 86.8 (4′-CH), 103.2 (5-CH), 113.7 (Ar-CH), 126.9 (Ph-CH), 128.4 (Ph-CH), 128.9 (Ph-CH), 130.2 (Ar-CH), 138.0 (Ar-C), 139.9 (6-CH), 146.2 (Ph-C), 150.8 (2-C), 158.4 (Ar-C), 163.8 (4-C); LRMS (ES$^+$) *m/z* 522 ([*M*+Na]$^+$, 27%), 87 (100); HRMS (ES$^+$) calcd for $C_{29}H_{30}N_3O_5^+$ [*M*+H]$^+$: 500.2180, found: 500.2174; IR (KBr): $\tilde{\nu}$ = (3408), 3052, 1713, 1694, 1682, 1666, 1650, 1501, 1250, 1034, 760 cm^{-1}; Anal. (%) calcd for ($C_{29}H_{29}N_3O_5$, 0.88 HCl): C 65.52, H 5.66, N 7.90, Cl 5.87, found: C 65.69, H 5.52, N 7.86, Cl 6.05.

5′-*O*-(4-Cyanotrityl)-2′-deoxyuridine (3a): The compound was prepared following the general procedure A, starting from 4-cyanotrityl chloride (0.40 g, 1.31 mmol), 2′-deoxyuridine (0.23 g, 1.00 mmol), pyridine (5 mL) and DMAP (11 mg, 0.09 mmol) stirred at 50 °C for 92 h. Purified by flash column chromatography with a gradient of 0→6% CH_3OH in $CHCl_3$. The title compound was a white solid (0.215 g, 43%). R_f = 0.29 (10% $CH_3OH/CHCl_3$); mp: 92–95 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.19 (1H, m, 2′-C*H*H), 2.45 (1H, m, 2′- CH*H*), 2.94 (1H, bs, 3′-OH), 3.38 (2H, m, 5′-H), 4.06 (1H, m, 4′-H), 4.50 (1H, m, 3′-H), 5.45 (1H, d, J = 8.1 Hz, 5-H), 6.27 (1H, t, J = 6.2 Hz, 1′-H), 7.24–7.34 (10H, m, Ph-*H*), 7.53–7.60 (5H, m, 6-H and Ar-*H*), 9.50 (1H, bs, 3-NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 41.4 (2′-CH_2), 63.9 (5′-CH_2), 71.7 (3′-CH), 85.6 (1′-CH), 86.1 (4′-CH), 87.6 ($ArPh_2$-*C*), 102.8 (5-CH), 111.4 (Ar-C), 119.0 (C≡N), 128.6 (Ph-CH), 128.8 (Ph-CH), 129.0 (Ar-CH), 132.4 (Ar-CH), 140.3 (6-CH), 142.0 (Ph-C), 142.1 (Ph-C),150.1 (Ar-C), 150.8 (2-C), 163.8 (4-C); LRMS (ES$^+$) *m/z* 518 ([*M*+Na]$^+$, 23%), 268 (Ph_3C^+, 100%); LRMS (ES$^-$) *m/z* 494 ([*M*−H]$^+$, 100%); HRMS (ES$^+$) calcd for $C_{29}H_{29}N_4O_5^+$ [*M*+NH_4]$^+$ 513.2132, found: 513.2132; IR (KBr): $\tilde{\nu}$ = 3401, 3180, 3060, 2230 (CN), 1685, 1463, 1273, 1088 cm^{-1}; Anal. (%) calcd for ($C_{29}H_{25}N_3O_5$, 2.35 HCl) C 59.93, H 4.74, N 7.23, found: C 59.89, H 4.45, N 7.02.

5′-(4-Cyanotrityl)amino-2′,5′-dideoxyuridine (3b): The compound was prepared by following general procedure A, starting from 4-cyanotrityl chloride (0.41 g, 1.34 mmol), 5′-amino-2′,5′-dideoxyuridine (**23**) (0.24 g, 1.05 mmol) and pyridine (5 mL), stirred at 40 °C for 48 h. Purified by flash column chromatography eluting the column (ISOLUTE SI) with a gradient of 0→5% CH_3OH in $CHCl_3$. White crystalline solid (0.386 g, 37%). R_f = 0.31 (10% $CH_3OH/CHCl_3$); mp: 160–163 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.03 (2H, m, 2′-C*H*H and 5′-N*H*), 2.21 (1H, m, 5′-C*H*H), 2.37 (1H, m, 2′-C*H*H), 2.58 (1H, m, 5′-C*H*H), 3.31 (1H, bs, 3′-OH), 4.10 (1H, m, 4′-H), 4.24 (1H, m, 3′-H), 5.63 (1H, d, J = 8.1 Hz, 5-H), 6.24 (1H, t, J = 6.3 Hz, 1′-H), 7.00 (1H, dd, J = 2.1, 8.1 Hz, 6-H), 7.18–7.48 (10H, m, Ph-*H*), 7.54 (2H, d, J = 8.1 Hz, Ar-*H*), 7.66 (2H, d, J = 8.1 Hz, Ar-*H*), 9.74 (1H, bs, 3-NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 40.7 (2′-CH_2), 46.5 (5′-CH_2), 71.3 ($ArPh_2$-*C*), 72.8 (3′-CH), 85.6 (1′-CH), 86.5 (4′-CH), 103.3 (5-CH), 110.7 (Ar-CH), 119.2 (*C*N), 127.6 (Ph-CH), 128.7 (Ph-CH), 128.9 (Ph-CH), 129.1 (Ph-CH), 129.5 (Ar-CH), 132.4 (Ar-CH), 139.9 (6-CH), 144.3 (Ph-C), 145.0 (Ar-C), 150.8 (2-C), 151.6 (Ar-C), 163.8 (4-C); LRMS (ES$^+$) *m/z* 517 ([*M*+Na]$^+$, 19%), 495 ([*M*+H]$^+$, 9%), 517 ([$CNPh_3C^+$], 100%); HRMS (ES$^+$) calcd for $C_{29}H_{27}N_4O_4^+$ [*M*+H]$^+$ 495.2027, found: 495.2023; IR (KBr): $\tilde{\nu}$ = 3387, 3177, 3027, 2230 (CN), 1699, 1661, 1466, 1267, 1097, 1039 cm^{-1}; Anal. (%) calcd for ($C_{29}H_{26}N_4O_4$, 1.0 HCl, 0.20 H_2O) C 64.80, H 5.21, N 10.42, Cl 6.46, found: C 64.69, H 4.90, N 10.28, Cl 6.84.

5′-*O*-(4,4′-Dimethoxytrityl)-2′,5′-dideoxyuridine (4): The compound was prepared by following general procedure A, starting from 2′-deoxyuridine (0.51 g, 2.23 mmol), 4,4′-dimethoxytrityl chloride (0.98 g, 2.89 mmol), DMAP (32 mg, 0.26 mmol) and pyridine (10 mL), stirred at 40 °C for 48 h. Purified by flash column chromatography eluting the column (ISOLUTE SI) with a gradient of 0→5% CH_3OH in $CHCl_3$. Compound **4** was obtained as a pale-yellow solid (0.678 g, 57%). R_f = 0.33 (10% $CH_3OH/CHCl_3$); mp: 85–86 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.31 (1H, m, 2′-H), 2.51 (1H, m, 2′-H), 2.80–2.90 (1H, b signal, 3′-OH), 3.84 (6H, s, 2×OCH_3), 3.50 (2H, m, 5′-H), 4.10 (1H, m, 4′-H), 4.62 (1H, m, 3′-H), 5.46 (1H, d, J = 8.1 Hz, 5-H), 6.38 (1H, t, J = 6.3 Hz, 1′-H), 6.89 (4H, d, J = 8.7 Hz, Ar-*H*), 7.25–7.45 (9H, m, Ph-*H* and Ar-*H*), 7.85 (1H, m, 6-H), 9.50 (1H, bs, 3-NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 41.6 (2′-CH_2), 55.7 (OCH_3), 64.4 (5′-CH_2), 72.0 (3′-CH), 85.5 (4′-CH or 1′-CH), 86.5 (4′-CH or 1′-CH), 87.5 (Ar_2Ph-*C*), 102.7 (5-CH), 113.7 (Ar-CH), 127.6 (Ph-CH), 128.4 (Ph-CH), 128.5 (Ph-CH), 130.5 (Ar-CH), 135.6 (Ar-C), 135.8 (Ar-C), 140.7 (6-CH), 144.7 (Ph-C), 150.7 (2-C), 159.1 (Ar-C), 163.7 (4-C). (ES$^+$) *m/z* 553 ([*M*+Na]$^+$, 49%); HRMS (ES$^+$) calcd for $C_{30}H_{30}N_2O_7Na^+$ [*M*+Na]$^+$ 553.1945, found: 553.1948; Anal. (%) calcd for ($C_{30}H_{30}N_2O_7$, 2.20 HCl) C 58.99, H 5.31, N 4.59, Cl 12.77, found: C 58.72, H 5.13, N 4.33, Cl 12.68.

5′-*O*-(2-Chlorotrityl)-2′-deoxyuridine (5a): The compound was prepared by following general procedure A, starting from 2′-deoxyuridine (0.24 g, 1.04 mmol), 2-chlorotrityl chloride (0.64 g, 2.04 mmol), DMAP (18 mg, 0.15 mmol) and pyridine (5 mL), stirred at 40 °C for 50 h. Purified by flash column chromatography (ISOLUTE SI column) eluting with 0→5% CH_3OH in $CHCl_3$. The title compound was as a white solid (0.176 g, 34%). R_f = 0.14 (10% $CH_3OH/CHCl_3$); mp: 95–98 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.31 (1H, m, 2′-H), 2.49 (1H, m, 2′-H), 2.66 (1H, bs, 3′-OH), 3.48 (2H, m, 5′-H), 4.09 (1H, m, 4′-H), 4.71 (1H, m, 3′-H), 5.53 (1H, d, J = 8.1 Hz, 5-H), 6.37 (1H, t, J = 6.2 Hz, 1′-H), 7.25–7.50 (13H, m, Ph-*H* and Ar-*H*), 7.70–7.85 (2H, m, 6-H and Ar-*H*), 9.18 (1H, bs, 3-NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 41.4 (2′-CH_2), 64.0 (5′-CH_2), 71.6 (3′-CH), 85.3 (4′-CH or 1′-CH), 86.1 (4′-CH or 1′-CH), 87.6 ($ArPh_2$-*C*), 102.7 (5-CH),

127.0 (Ar-CH), 127.8 (Ph-CH), 127.9 (Ph-CH), 128.4 (Ph-CH), 128.5 (Ph-CH), 128.7 (Ph-CH), 128.8 (Ph-CH), 129.9 (Ar-CH), 131.1 (Ar-CH), 132.6 (Ar-CH), 134.7 (Ar-C), 140.2 (Ar-C), 140.7 (6-CH), 141.7 (Ar-C), 142.3 (Ph-C), 150.7 (2-C), 163.7 (4-C); LRMS (ES^+) *m/z* 529 ($[M+Na]^+$, 12%), 527 ($[M+Na]^+$, 29%), 87 (100%); HRMS (ES^+) calcd for $C_{28}H_{25}N_2O_5NaCl^+$ $[M+Na]^+$ 527.1344, found: 527.1350; Anal. (%) calcd for ($C_{28}H_{25}N_2O_5Cl$, 1.65 HCl, 0.30 H_2O) C 58.95, H 4.81, N 4.91, Cl 16.47, found: C 58.93, H 4.39, N 4.76, Cl 16.15.

5′-(2-Chlorotrityl)amino-2′,5′-dideoxyuridine (5b): The compound was prepared by following general procedure A, starting from 5′-amino-2′,5′-dideoxyuridine (**23**) (0.24 g, 1.04 mmol), 2-chlorotrityl chloride (0.41 g, 1.33 mmol) and pyridine (5 mL), stirred at 40 °C for 24 h. Purified by flash column chromatography eluting the column (ISOLUTE SI) with a gradient of 0→5% CH_3OH in $CHCl_3$. Compound **5b** was obtained as a white solid (85 mg, 16%). R_f=0.17 (10% $CH_3OH/CHCl_3$); mp: 129–131 °C; ^1H NMR (300 MHz, $CDCl_3$): δ=2.02 (1H, m, 2′-H), 2.20 (1H, m, 5′-H), 2.37 (1H, m, 2′-H), 2.53 (1H, m, 5′-H), 4.16 (1H, m, 3′-H), 4.24 (1H, m, 4′-H), 5.62 (1H, d, *J*=8.1 Hz, 5-H), 6.26 (1H, t, *J*=6.4 Hz, 1′-H), 7.05–7.45 (15H, m, 6-H and Ph-*H* and Ar-*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ=41.1 (2′-CH_2), 47.3 (5′-CH_2), 71.5 ($ArPh_2$-*C*), 72.9 (3′-CH), 85.5 (1′-CH), 86.8 (4′-CH), 103.0 (5-CH), 126.8 (Ar-CH), 126.9 (Ar-CH), 127.6 (Ar-CH), 128.4 (Ar-CH), 128.5 (Ar-CH), 128.6 (Ar-CH), 129.2 (Ar-CH), 132.3 (Ar-CH), 132.9 (Ar-CH), 134.7 (A-C), 140.0 (6-CH), 140.9 (Ar-C), 144.8 (Ar-C), 146.0 (Ar-C), 150.7 (2-C), 163.7 (4-C); LRMS (ES^+) *m/z* 526 ($[M+Na]^+$, 11%), 504 ($[M+H]^+$, 14%), 277 ($[Ph^2ArC]^+$, 100%); HRMS (ES^+) calcd for $C_{28}H_{27}N_3O_4Cl^+$ $[M+H]^+$ 504.1685, found: 504.1689.

4-(Chlorodiphenylmethyl)pyridine hydrochloride (20a): Diphenyl(4-pyridyl)methanol (2.12 g, 8.11 mmol) was taken in H_2O (15 mL). Concentrated HCl (1 mL) was added and the mixture was held at reflux for 2 h. The crude solution obtained was concentrated in vacuo to yield diphenyl(4-pyridyl)methanol hydrochloride as a white/cream solid (2.173 g, 90%); mp: 204–205 °C; ^1H NMR (300 MHz, $CDCl_3$): δ=7.37 (10H, m, Ph-*H*), 8.14 (2H, d, *J*=6.7 Hz, Ar-*H*), 8.81 (2H, d, *J*=6.7 Hz, Ar-*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ=82.6 ($ArPh_2C$), 127.7 (Ar-CH), 129.6 (Ph-H), 129.7 (Ph-H), 129.9 (Ph-H), 142.7 (Ar-H), 146.2 (Ph-C), 170.3 (Ar-C); LRMS (ES^+) *m/z* 262 ($[PyPh_2COH+H]^+$, 100%).

Diphenyl(4-pyridyl)methanol hydrochloride (1.01 g, 3.41 mmol), $SOCl_2$ (9 mL), and CH_3COCl (6 mL) were stirred at room temperature for 72 h. Excess $SOCl_2$ and CH_3COCl was removed under reduced pressure, and the crude product was co-evaporated several times with toluene to yield 4-(chlorodiphenylmethyl)pyridine hydrochloride (1.073 g, 100%); mp: 154–155 °C; ^1H NMR (300 MHz, $CDCl_3$): δ=7.19–7.44 (10H, m, Ph-*H*), 7.86 (2H, d, *J*=5.2 Hz, Ar-*H*), 8.95 (2H, d, *J*=5.2 Hz, Ar-*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ=78.5 ($ArPh_2C$), 127.8 (Ar-CH), 129.2 (Ph-H), 129.5 (Ph -H), 129.7 (Ph -H), 141.5 (Ar-H), 141.9 (Ph -C), 165.0 (Ar-C); LRMS (ES^+) *m/z* 303 ($[M+Na]^+$, 11%), 262 ($[PyPh_2COH+H]^+$, 100%).

5-(Chlorodiphenylmethyl)pyrimidine hydrochloride (20b): Diphenyl(5-pyrimidyl)methanol (1.06 g, 4.05 mmol), $SOCl_2$ (9 mL) and CH_3COCl (6 mL) were stirred at room temperature for 72 h. The excess of $SOCl_2$ and CH_3COCl was removed under reduced pressure and the residue was co-evaporated several times with toluene to yield 5-(chlorodiphenylmethyl)pyrimidine hydrochloride (**25b**) as a thick yellow oil (1.28 g, 99%); ^1H NMR (500 MHz, $CDCl_3$): δ=7.20–7.27 (4H, m, Ph-*H*), 7.37–7.45 (6H, m, Ph-*H*), 7.84 (2H, s, Ar-*H*), 9.20 (1H, s, Ar-*H*); ^{13}C NMR (125 MHz, $CDCl_3$): δ=75.7 (Ph^2Ar-*C*), 128.8 (Ph-CH), 129.1 (Ph-CH), 129.2 (Ph-CH), 141.3 (Ar-C), 141.8 (Ph-C), 1 (Ar-CH), 156.8 (Ar-CH); LRMS (ES^+) *m/z* 263 ($[M-Cl+OH+H]^+$, 100%).

5′-[Diphenyl(4-pyridyl)methyl]amino-2′,5′-dideoxyuridine (7a): 5′-Amino-2′,5′-dideoxyuridine (**18**) (0.11 g, 0.47 mmol) was added to a solution of diphenyl(4-pyridyl)methyl chloride (**20a**) (0.15 g, 0.47 mmol), pyridine (3 mL) and Et_3N (0.12 mL, 0.86 mmol). The reaction mixture was heated at 40 °C for 4 h then the temperature was increased to 70 °C for 10 h. The crude solution was partitioned between H_2O (5 mL) and EtOAc (3×7 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated in vacuo. The brown solid obtained was taken in CH_3OH and the remaining insoluble material was filtered off. The filtrate was concentrated under reduced pressure and further purified using flash column chromatography eluting the column (ISOLUTE SI) with a gradient of 0→8% CH_3OH in $CHCl_3$. The title compound was a pale-yellow solid (61 mg, 27%). R_f=0.24 (10% $CH_3OH/CHCl_3$); mp: 131–133 °C; ^1H NMR (300 MHz, CD_3OD): δ=2.15–2.60 (4H, m, 2-H and 5′-H), 4.03 (1H, m, 3′-H or 4′-H), 4.18 (1H, m, 3′-H or 4′-H), 5.63 (1H, d, *J*=8.0 Hz, 5-H), 6.21 (1H, t, *J*=6.4 Hz, 1′-H), 7.20–7.48 (11H, m, 6-H and Ph-*H*), 7.59 (2H, d, *J*=5.9 Hz, Ar-*H*), 8.42 (2H, d, *J*=5.9 Hz, Ar-*H*); ^{13}C NMR (75 MHz, CD_3OD): δ=40.9 (2′-CH_2), 47.6 (5′-CH_2), 72.2 ($ArPh_2$-*C*), 73.3 (3′-CH), 87.1 (1′-CH or 4′-CH), 87.9 (1′-CH or 4′-CH), 103.4 (5-CH), 125.7 (Ar-CH), 128.6 (Ph-CH), 129.7 (Ph-CH), 130.2 (Ph-CH), 130.3 (Ph-CH), 142.7 (6-CH), 145.8 (Ph-C), 146.1 (Ph-C), 150.2 (Ar-CH), 152.4 (Ar-C), 158.1 (2-C), 166.4 (4-C); LRMS (ES^+) *m/z* 963 ($[2M+Na]^+$, 13%), 493 ($[M+Na]^+$, 84%), 471 ($[M+H]^+$, 13%); HRMS (ES^+) calcd for $C_{27}H_{27}N_4O_4^+$ $[M+H]^+$, 471.2027, found: 471.2033.

5′-[Diphenyl(5-pyrimidyl)methyl]-2′-deoxyuridine (7b): 2′-Deoxyuridine (0.15 g, 0.47 mmol) was added to a solution of 5-(chlorodiphenylmethyl)pyrimidine hydrochloride (**20b**) (0.26 g, 0.83 mmol) and DMAP (10 mg, 0.08 mmol) in dry pyridine (1 mL). The reaction mixture was heated at 100 °C in a microwave twice for 1 min. The solvent was removed in vacuo and the residue co-evaporated with toluene. The crude product was then purified by flash column chromatography (ISOLUTE SI column) eluting with 0→8% CH_3OH in $CHCl_3$. The title compound was obtained a pale-yellow solid (26 mg, 8%). R_f=0.40 (10% $CH_3OH/CHCl_3$); mp: 103–108 °C; ^1H NMR (500 MHz, $CDCl_3$): δ=2.23 (1H, m, 2′-C*H*H), 2.48 (1H, m, 2′-CH*H*), 3.43 (2H, m, 5′-CH_2), 3.78 (1H, bs, 3′-OH), 4.10 (1H, m, 4′-H), 4.58 (1H, m, 3′-H), 5.51 (1H, d, *J*=8.1 Hz, 5-H), 6.26 (1H, t, *J*=6.2 Hz, 1′-H), 7.37 (10H, m, Ph-*H*), 7.57 (1H, d, *J*=8.1 Hz, 6-H), 8.85 (2H, s, Ar-*H*), 9.12 (1H, s, Ar-*H*), 9.94 (1H, bs, 3-NH); ^{13}C NMR (500 MHz, $CDCl_3$): δ=40.6 (2′-CH_2), 63.0 (5′-CH_2), 70.4 (3′-CH), 76.9 ($ArPh_2$-*C*), 84.9 (1′-CH), 85.1 (4′-CH), 102.1 (5-CH), 128.09 (Ph-CH), 128.14 (Ph-CH), 128.3 (Ph-CH), 128.4 (Ph-CH), 137.7 (Ar-C), 139.4 (6-CH), 140.2 (Ph-C), 140.9 (Ph-C), 150.1 (2-C), 156.0 (Ar-CH), 156.7 (Ar-CH), 163.2 (4-C); LRMS (ES^+) *m/z* 967 ($[2M+Na]^+$, 7%), 945 ($[2M+H]^+$, 8%), 495 ($[M+Na]^+$, 58%), 473 ($[M+H]^+$, 100%); HRMS (ES^+) calcd for $C_{26}H_{25}N_4O_5^+$ $[M+H]^+$ 473.1819, found: 473.1821.

5′-[Diphenyl-(5-pyrimidyl)methyl]amino-2′,5′-dideoxyuridine (7c): 5-(Chlorodiphenylmethyl)pyrimidine hydrochloride **20b** (0.25 g, 0.79 mmol) was taken up in dry pyridine (1 mL) in the presence of TEA (0.15 mL) under N_2. 5′-Amino-2′,5′-dideoxyuridine **18** (0.15 g, 0.66 mmol) was added and the reaction mixture was heated using microwave irradiation, first at 80 °C for 1 min and then at 100 °C for 1 min. The solvent was removed in vacuo and the residue co-evaporated with toluene. The crude product was then purified by flash column chromatography (ISOLUTE SI column) eluting with 0→12% CH_3OH in $CHCl_3$ yielding the title compound as a pale-yellow solid (44 mg, 14%). R_f=0.30 (10% $CH_3OH/CHCl_3$); mp: 121–126 °C; ^1H NMR (500 MHz, $CDCl_3$): δ=2.10–2.25 (2H, m, 2′-C*H*H and $ArPH_2$C-N*H*), 2.30–2.45 (2H, m, 2′-CH*H* and 5′-C*H*H), 2.64 (1H, m, 5′-CH*H*), 3.34 (1H, bs, 3′-OH), 4.10 (1H, m, 3′-H), 4.40 (1H, m, 4′-H), 5.68 (1H, d, *J*=8.1 Hz, 5-H), 6.20

(1H, t, $J=6.5$ Hz, 1′-H), 7.06 (1H, d, $J=8.1$ Hz, 6-H), 7.25–7.50 (10H, m, Ph-*H*), 8.94 (2H, s, Ar-*H*), 9.09 (1H, s, Ar-*H*), 9.51 (1H, bs, 3-NH); ^{13}C NMR (500 MHz, $CDCl_3$): $\delta=40.1$ (2′-CH_2), 45.6 (5′-CH_2), 68.4 (ArPH_2-*C*), 72.0 (3′-CH), 85.6 (1′-CH), 85.8 (4′-CH), 102.9 (5-CH), 127.5 (Ph-CH), 128.1 (Ph-CH), 128.3 (Ph-CH), 128.62 (Ph-CH), 128.64 (Ph-CH), 138.8 (Ar-C), 139.7 (6-CH), 143.5 (Ph-C), 143.9 (Ph-C), 150.2 (2-C), 156.5 (Ar-CH), 157.0 (Ar-CH), 163.1 (4-C); LRMS (ES^+) *m/z* 943 ([2*M*+H]$^+$, 8%), 494 ([*M*+Na]$^+$, 53%), 472 ([*M*+H]$^+$, 100%); HRMS (ES^+) calcd for $C_{26}H_{26}N_5O_4^+$ [*M*+H]$^+$ 472.1979, found: 472.1983.

3′-Azido-5′-*O*-trityl-2′,3′-dideoxyuridine (14b): LiF (0.14 g, 5.61 mmol) was suspended in DMF (3 mL) and heated at 105 °C with stirring. To the stirred suspension was added TMEDA (5 mL) followed by azidotrimethylsilane (0.64 g, 5.61 mmol). After stirring for 1 h, **29** (1.41 g, 3.11 mmol) dissolved in DMF (2 mL) was added, and the reaction was allowed to proceed for 20 h at 110 °C. The mixture was cooled, poured into $CHCl_3$ (110 mL) and filtered through Celite. The solvent was removed under reduced pressure, and the residue (brown oil) was taken in EtOAc (100 mL). The organic phase was washed with H_2O (4×180 mL), dried ($MgSO_4$), filtered and concentrated. The concentrated mixture was purified by column chromatography eluting with 3% CH_3OH in $CHCl_3$. The title compound was isolated as an orange solid (0.996 g, 65%). $R_f=0.78$ (5% $CH_3OH/CHCl_3$); mp: 67–70 °C; ^{1}H NMR (300 MHz; $CDCl_3$): $\delta=$ 2.41–2.61 (2H, m, 2′-H), 3.49 (1H, dd, $J=2.8$, 11.1 Hz, 5′-CH*H*), 3.61 (1H, dd, $J=1.9$, 11.1 Hz, 5′-C*H*H), 3.98–4.02 (1H, m, 3′-H-), 4.40 (1H, q, $J=6.3$ Hz, 4′-H), 5.44 (1H, dd, $J=1.9$, 8.1 Hz, 5-H), 6.25 (1H, t, $J=$ 5.3 Hz, 1′-H), 7.32–7.46 (15H, m, Ph-*H*), 7.90 (1H, d, $J=8.2$ Hz, 6-H), 8.41 (1H, s, N-H); ^{13}C NMR (75 MHz; $CDCl_3$)δ 36.8 (2′-CH_2), 59.8 (3′-CH), 64.0 (5′-CH_2), 84.1 (1′-CH), 85.2 (4′-CH), 88.2 (Ph_3-*C*), 102.8 (5-CH), 128.0 (Ph-*C*H), 128.6 (Ph-*C*H), 129.0 (Ph-*C*H), 140.2 (6-CH), 143.4 (Ph-*C*), 151.2 (2-C), 161.4 (2-C); LRMS (ES^+) *m/z* 518.0 ([*M*+Na]$^+$, 60%); HRMS (ES^+) calcd for $C_{28}H_{25}N_5O_4Na$ [*M*+Na]$^+$ 518.1804, found: 518.1824; IR (KBr): $\tilde{\nu}=3038$, 2108, 1691 cm^{-1}; Anal. (%) calcd for ($C_{28}H_{25}N_{4.5}O_4$, 0.6 H_2O) C 66.87, H 5.33, N 12.53, found: C 66.59, H 4.68, N 12.70.

3′-Amino-5′-*O*-trityl-2′,3′-dideoxyuridine (14c): Compound **14b** (0.10 g, 0.20 mmol) was hydrogenated in presence of Lindlar's catalyst (20 mg) in EtOH (5 mL) for 5 h. The suspension was filtered through Celite, and fresh Lindlar's catalyst (20 mg) was added to the filtrate. The black suspension was then hydrogenated for further 3 h. The solvent was evaporated and the concentrated solution was purified by column chromatography eluting with a gradient of 2→10% CH_3OH in CH_2Cl_2. The title compound was obtained as white solid (0.065 g, 70%). $R_f=0.3$ (10% CH_3OH/CH_2Cl_2); mp: 110–114 °C; ^{1}H NMR (300 MHz; $CDCl_3$): $\delta=2.18$–2.44 (2H, m, 2′-H), 3.38–3.73 (5H, m, 3′-H, 4′-H-, 5′-H), 5.41 (1H, d, $J=8.1$ Hz, 5-H), 6.20 (1H, q, $J=3.3$ Hz, 1′-H), 7.27–7.47 (15H, m, Ph-*H*), 7.97 (1H, d, $J=$ 8.2 Hz, 6-H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=42.7$ (2′-CH_2), 50.7 (3′-CH), 62.2 (5′-CH_2), 85.2 (1′-CH), 87.2 (4′-CH), 87.9 (Ph_3-*C*), 102.2 (5-CH), 127.8 (Ph-*C*H), 128.5 (Ph-*C*H), 129.1 (Ph-*C*H), 140.7 (6-CH), 143.7 (Ph-*C*), 150.5 (2-C), 163.5 (4-C); LRMS (ES^+) *m/z* 491.7 ([*M*+Na]$^+$, 45%); 243.2 ([Ph_3C]$^+$, 100%); HRMS (ES^+) calcd for $C_{28}H_{27}N_3O_4Na$ [*M*+Na]$^+$ 492.1899, found: 492.1902; Anal. (%) calcd for ($C_{28}H_{27}N_3O_4$, 0.5 H_2O) C 70.28, H 5.90, N 8.78, found: C 70.64, H 5.92, N 8.41.

3′-Aacetylamino-5′-*O*-trityl-2′,3′-dideoxyuridine (14d): Ac_2O (0.05 g, 0.044 mL, 0.469 mmol) and TEA (0.065 mL, 0.469 mmol) were added to suspension of **14c** (0.10 g, 0.213 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred at room temperature for 3 h. The solvent was evaporated to give a white solid, which was purified by column chromatography eluting with a gradient of 2→6% CH_3OH in CH_2Cl_2. Compound **14d** was obtained as white solid (0.103 g, 95%). $R_f=0.45$ (10% CH_3OH/CH_2Cl_2); mp: 135–140 °C; ^{1}H NMR (300 MHz; $CDCl_3$): $\delta=2.04$ (3H, s, COCH_3), 2.32–2.50 (2H, m, 2′-H-), 3.47–3.59 (2H, m, 5′-H), 4.07 (1H, s, 4′-H), 4.72–4.79 (1H, m, 3′-H), 5.39 (1H, d, $J=8.1$ Hz, 5-H), 6.34 (1H, t, $J=6.3$ Hz, 1′-H), 6.94 (1H, s, CON*H*), 7.28–7.46 (15H, m, Ph-*H*), 7.83 (1H, d, $J=$ 8.2 Hz, 6-H), 9.82 (1H, s, N-H); ^{13}C NMR (75 MHz; $CDCl_3$): $\delta=38.8$ (2′-CH_2), 50.9 (3′-CH), 62.1 (5′-CH_2), 85.2 (4′-CH), 87.3 (1′-CH), 88.1 (Ph_3-*C*), 103.1 (5-CH), 127.9 (Ph-CH), 128.5 (Ph-CH), 129.1 (Ph-CH), 140.5 (6-CH), 143.6 (Ph-C), 154.9 (2-C), 163.6 (4-C); LRMS (ES^+) *m/z* 533.8 ([*M*+Na]$^+$, 20%); HRMS (ES^+) calcd for $C_{30}H_{29}N_3O_5Na$ [*M*+Na]$^+$ 534.2005, found: 534.2009; Anal. (%) calcd for ($C_{30}H_{29}N_3O_5$, 1.0 HCl, 1.0 H_2O) C 63.66, H 5.70, N 7.42, found: C 63.20, H 5.15, N 7.11.

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