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Design, Synthesis, and Evaluation of 5′-Diphenyl Nucleoside Analogues as Inhibitors of the *Plasmodium falciparum* dUTPase

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Deoxyuridine 5′-triphosphate nucleotidohydrolase (dUTPase) is a potential drug target for malaria. We previously reported some 5′-tritylated deoxyuridine analogues (both cyclic and acyclic) as selective inhibitors of the *Plasmodium falciparum* dUTPase. Modelling studies indicated that it might be possible to replace the trityl group with a diphenyl moiety, as two of the phenyl groups are buried, whereas the third is exposed to solvent. Herein we report the synthesis and evaluation of some diphenyl analogues that have lower lipophilicity and molecular weight than the trityl lead compound. Co-crystal structures show that the diphenyl inhibitors bind in a similar manner to the corresponding trityl derivatives, with the two phenyl moieties occupying the predicted buried phenyl binding sites. The diphenyl compounds prepared show similar or slightly lower inhibition of *Pf*dUTPase, and similar or weaker inhibition of parasite growth than the trityl compounds.

Introduction

Approximately half of the world's population is at risk from malaria, which is a health problem in more than 100 countries. Approximately 90% of the one million deaths that occur each year from this disease occur in sub-Saharan Africa, mainly amongst children. The causative agent of malaria is the parasitic protozoan of the genus *Plasmodium*. This is transmitted to humans via the bite of the *Anopheles* mosquito, whereby *Plasmodium falciparum* is responsible for the most severe form of the disease.[1]

Deoxyuridine 5′-triphosphate nucleotidohydrolase (dUTPase) is a ubiquitous enzyme[2] that is found in prokaryotes and eukaryotes, in addition to a variety of viruses.[3,4] Its function is to catalyse the hydrolysis of dUTP to dUMP,[2,3] fulfilling two main roles within the cell. Firstly it provides dUMP, an essential precursor required for the production of dTMP, and secondly it maintains a low dUTP/dTTP concentration, thus minimising uracil incorporation into DNA.[3,4,5] The dUTPases have been shown to be essential for cell viability in all organisms studied to date, including *E. coli* and *S. cerevisiae*.[6] As a validated drug target,[6,7,8] and owing to its essentiality in cell viability, dUTPase is therefore a good choice with which to proceed in the development of novel and selective inhibitors.

We previously reported potent inhibitors of *P. falciparum* dUTPase (*Pf*dUTPase; Table 1), which show good selectivity over the corresponding human enzyme.[4,9] These consist of both cyclic and acyclic nucleoside analogues in which the potency and selectivity arises from those that carry the trityl functionality. Complete removal of the trityl group results in a significant decrease in both biological activity and selectivity of these compounds for the *Plasmodium* over the human enzyme (Figure 1).

The reason for this selectivity lies in the different dUTPase sequences between the two species. Val 42 and Gly 87 in the human enzyme are respectively replaced by Phe 46 and Ile 117 in the *Plasmodium* dUTPase. This has been shown crystallographically in a previous publication reporting the co-crystalli-

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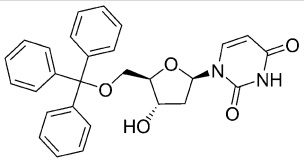
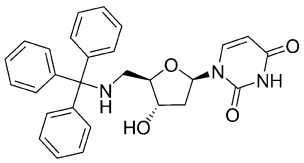
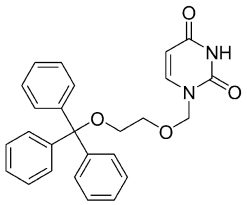
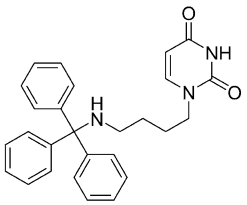
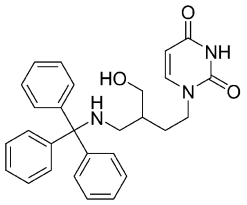
Table 1. Biological results for selected cyclic and acyclic *Pf*dUTPase inhibitors.

Compd	Structure	M_r [Da]	clog*P*	Enzyme assays: K_i [μM]			In vitro assays: EC_{50} [μM]		
				P. fal.[a]	Human	SI[b]	*P. fal.*	L6 cells[c]	SI[d]
A		470	3.0	1.8	17.7	10	6.0	192	32
B		469	2.8	0.2	46.3	232	4.5	–	–
C		428	3.1	0.7	17.0	24	0.9	70	78
D		425	3.8	0.9	>1 mM	>1111	3.8	33	9
E		455	3.2	0.2	5.7	29	3.2	55	17

[a] *P. falciparum* K1 chloroquine- and pyrimethamine-resistant strain. [b] Enzyme selectivity index (SI) calculated as [K_i *Hs*dUTPase/K_i *Pf*dUTPase]. [c] Cytotoxicity on rat L6 myoblasts; controls: for *P. falciparum*, chloroquine, EC_{50} = 0.1 μM; for cytotoxicity, podophyllotoxin, EC_{50} = 0.012 μM. The EC_{50} values are the means of two independent assays; the individual values vary by less than a factor of 2. [d] Cell selectivity index (SI) calculated as [EC_{50} L6/EC_{50} *P. falciparum*]. Calculated log*P* values were determined with StarDrop® 2009 ver. 4.2.1 (http://www.optibrium.com).

sation of *P. falciparum* dUTPase with a cyclic trityl inhibitor, compound **F** (Figure 2).[3] In the *Pf*dUTPase structure there is a hydrophobic pocket, which includes residues Phe46 and Ile117, in which the trityl group binds. There is no corresponding pocket in the human enzyme.

However, a major issue with the trityl analogues is their lipophilicity. To improve their solubility, water-solubilising groups can be included in the molecule, but extra groups result in an increase in molecular weight. For example, cyclic compounds **A** and **B** (Table 1) have molecular weights near 500 Da. The acyclic derivatives have lower molecular weights, but lipophilicity and water solubility remain an issue.

Our crystallographic and molecular modelling studies indicate that only two of the three phenyl rings of the trityl group have significant interactions with the enzyme; the third phenyl ring has only limited interaction with the protein and is directed outwards to the solvent (Figure 3). The two buried phenyl rings interact with Phe46. This suggests that compounds which lack the exposed ring may still be able to interact in the same manner and retain their activity. We have experimental evidence to support the postulate that at least two phenyl rings are a minimum requirement. We previously reported some triphenylsilyl and *tert*-butyldiphenylsilyl (TBDPS) derivatives (Figure 4).[4,9] By comparing appropriate pairs of compounds, it is evident that the two-phenyl-ring derivatives retain K_i values similar to that of the triphenyl analogues.

Herein we report a series of acyclic and cyclic nucleoside diphenyl derivatives to probe this further. These compounds should have decreased lipophilicity relative to the triphenyl derivatives, and so their water solubility should be increased. The compounds also have lower molecular weights. It is anticipated that these will contribute to the creation of derivatives that are more drug-like in accordance with the Lipinski parameters.[10]

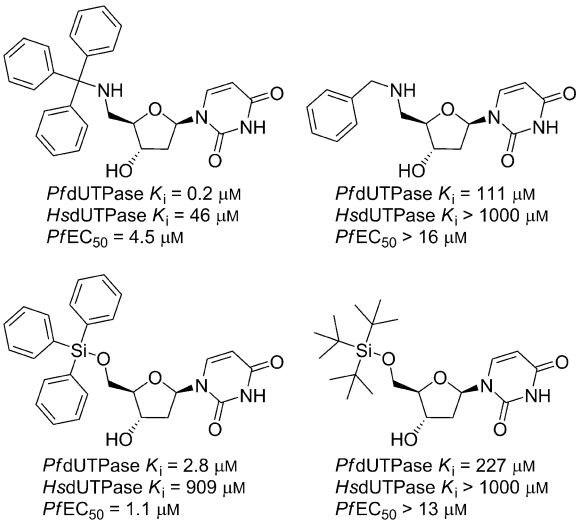


Figure 1. Comparison of biological results for selected *Pf*dUTPase inhibitors with and without the trityl group.

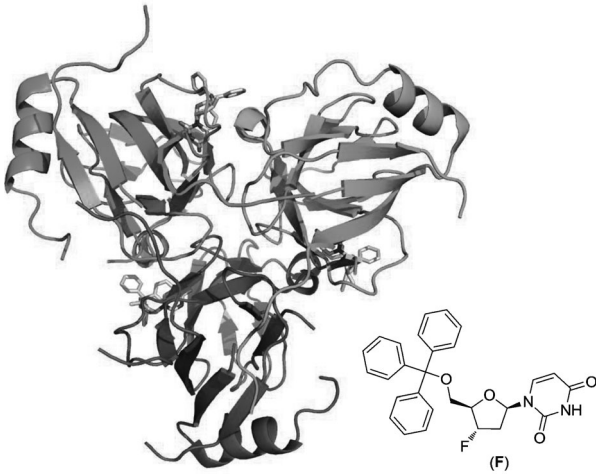


Figure 2. Published trityl *Pf*dUTPase inhibitor (**F**) and *Pf*dUTPase with compound **F** bound into the three active sites.

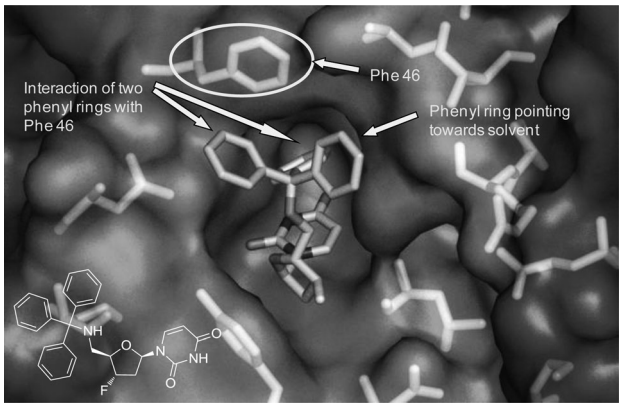


Figure 3. Trityl *Pf*dUTPase inhibitor interaction with Phe 46 in the active site.

From our previous work, the optimum length for the acyclic derivatives was shown to be a four-carbon chain, so we made the appropriate diphenyl derivatives. We also prepared three-carbon chain acyclic compounds for comparison (Figure 5). With the cyclic analogues, in addition to preparation of the diphenyl derivatives, we prepared derivatives in which one of the phenyl groups is replaced by a heteroaromatic ring, with the aim of increasing solubility. Furthermore, we investigated the effect of adding simple alkyl groups to the 5′-amino group.

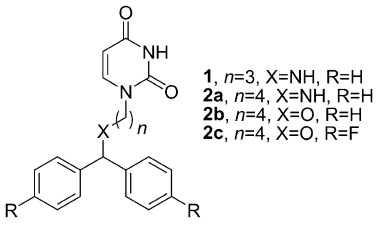


Figure 5. Structures of acyclic compounds prepared.

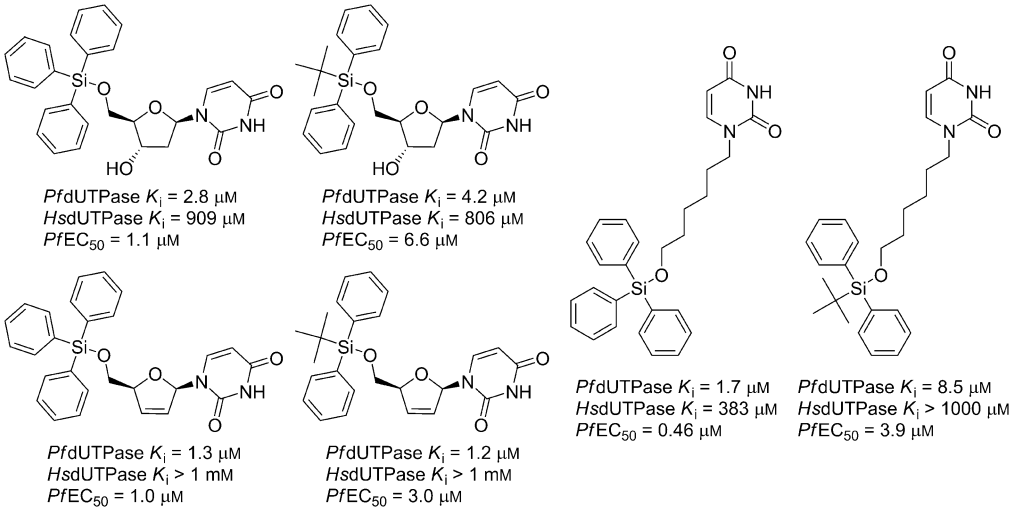


Figure 4. Comparison of biological activities for previously reported triphenylsilyl and TBDPS analogues.

Chemistry

Acyclic derivatives

Following some initial work, we decided to develop our methodology with the acyclic series and then apply this to the cyclic series.

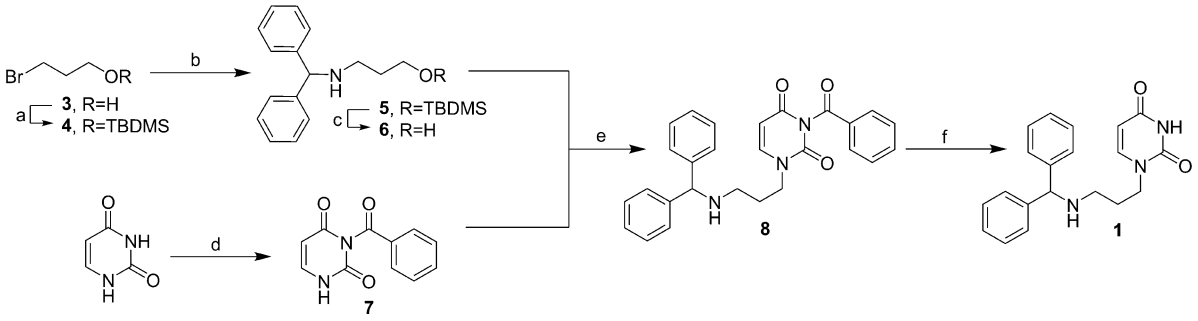
Three-C analogue

The three-carbon diphenylamino derivative **1** (Scheme 1) was synthesised by initially protecting the hydroxy functionality of 3-bromopropanol as a *tert*-butyldimethylsilyl ether. Attachment of the diphenyl moiety was then carried out with the use of benzhydrylamine and heating at 120 °C in acetonitrile. Having completed the diphenyl linker chain, attachment of the uracil was carried out with Mitsunobu methodology. The use of polymer-supported triphenylphosphine, a procedure previously used in our laboratories,[9] ensured a cleaner reaction without the triphenylphosphine oxide by-product, which is sometimes difficult to remove. To ensure monoalkylation at the N1 position of uracil, N3-protected uracil was used. Finally, benzoyl deprotection was carried out with sodium methoxide in methanol to yield the desired compound, **1**.

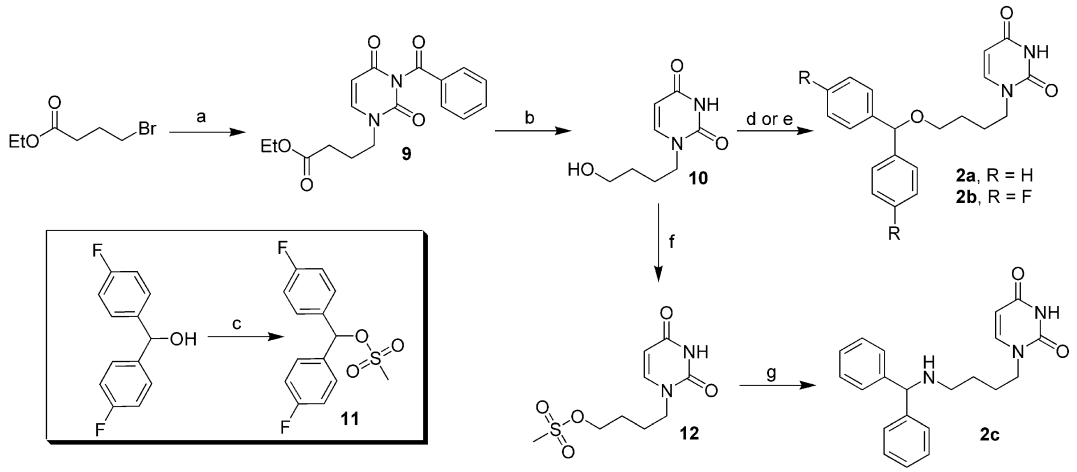
Four-C analogues

The preparation of the four-carbon derivatives was carried out through an alternate synthesis, summarised in Scheme 2, with the intention of producing compound **10** as a common intermediate. Initially monobenzoyl-protected 1,4-butanediol was used following a similar methodology to the three-carbon route. However, following Mitsunobu coupling and benzoyl deprotection, a low yield of alcohol intermediate **10** was obtained. It was found that coupling of ethyl 4-bromobutanoate to N3 benzoyl-protected uracil **7**[9] could be achieved cleanly in good yield after heating in *N*,*N*-dimethylformamide for 1 h. Reduction of the ester using a sodium borohydride/methanol system reported by Da Costa et al.[11] led to simultaneous reduction and benzoyl deprotection to give **10**.

This was then used to synthesise each acyclic derivative. Holding compound **10** at reflux with diphenyl carbinol in the presence of *para*-toluenesulfonic acid (PTSA) gave **2a**. Fluorinated compound **2b**, however, could not be prepared in the same manner. As an alternate procedure, bis(4-fluorophenyl)-

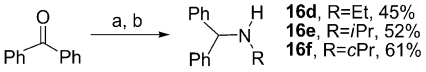


Scheme 1. Synthetic route for the three-carbon derivative. *Reagents and conditions:* a) TBDMSCl, imidazole, DMF, 0 °C→RT, 87%; b) $(C_6H_5)_2CHNH_2$, Et_3N, CH_3CN, 120 °C, 39%; c) TBAF, THF, RT, 84%; d) 1. PhCOCl, pyridine, CH_3CN, RT, 2. K_2CO_3, dioxane, 59%; e) PS–PPh_3, DIAD, THF, RT, 35%; f) $NaOCH_3$, CH_3OH, RT, 28%.



Scheme 2. Synthetic route for the four-carbon derivatives. *Reagents and conditions:* a) **7**, Cs_2CO_3, DMF, 60 °C, 1 h, 88%; b) $NaBH_4$, CH_3OH, THF, reflux, 1 h, 39%; c) $(p\text{-}FC_6H_5)_2CHOH$, MsCl, pyridine, RT; d) $(C_6H_5)_2CHOH$, PTSA, toluene, 4 Å MS, reflux, 20%; e) **11**, Et_3N, CH_3CN, 120 °C, 12% (two steps from 4,4′-difluorobenzhydrol); f) MsCl, pyridine, RT, 49%; g) $(C_6H_5)_2CHNH_2$, Et_3N, CH_3CN, 120 °C, 7%.

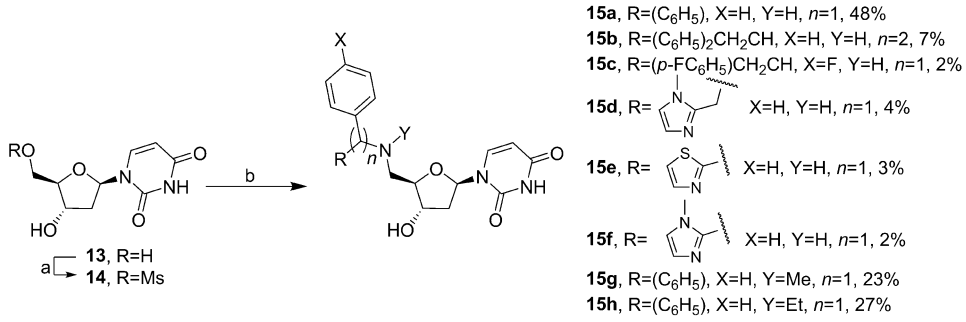
methanol was initially mesylated (compound **11**) and subsequently displaced by the acyclic alcohol, **10**. Finally the four-carbon amino diphenyl analogue **2c** was synthesised in a similar manner by mesylating alcohol **10** followed by reaction with benzhydrylamine.



Scheme 5. Synthetic route for **16d**–**16f**. *Reagents and conditions:* a) $TiCl_4$, RNH_2 0 °C→RT, 3 h; b) $NaBH_3CN$, RT.

Cyclic derivatives

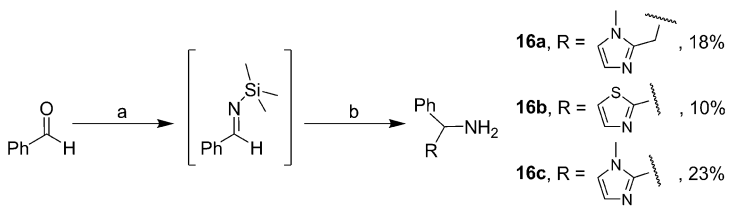
Following on from the methodology used for the acyclouridine diphenyl derivatives, mesylation of the 2′-deoxyuridine followed by displacement with the relevant diphenyl derivative was chosen as the route to synthesise the cyclic diphenyl analogues (Scheme 3). Displacement of the mesylate with the corresponding diphenyl methanamines was successful, although a similar displacement with the diphenyl carbinols was not.



Scheme 3. Synthesis of cyclic derivatives. *Reagents and conditions:* a) MsCl, pyridine, RT, 52%; b) amine, Et_3N, CH_3CN, 120 °C.

The diphenyl methanamines used to prepare compounds **15a**–**c** and **15g** were obtained commercially. The diaryl methanamines for compounds **15d**–**f** and **15h** were prepared in house. The diaryl methanamines **16a**–**c** were prepared by using the two-step procedure of Torregrosa et al.[12] (Scheme 4), which consists of formation of the N-silylated imine prepared from benzaldehyde and lithium hexamethyldisilazanide. The corresponding lithiated heterocycle was then added to this, which upon workup generated the primary amino compounds **16a**–**c**. These were isolated and used as a racemic mixture in the coupling reaction with the base.

Diphenyl methanamines **16d**–**f** were prepared as shown in Scheme 5 from benzophenone.[13] Reductive aminations were carried out with sodium cyanoborohydride in combination with titanium(IV) chloride to yield the desired products. For purposes of the initial in vitro screening, these were used as a racemic mixture as shown in Scheme 3. Upon reaction with 5′-mesyl-2′-deoxyuridine, the isopropyl (**16e**) and cyclopropyl (**16f**) derivatives failed to react; only the ethyl derivative **16d** reacted successfully to give **15h**.



Scheme 4. Synthetic route for phenylmethanamines. *Reagents and conditions:* a) $(Me_3Si)_2NLi$, THF, 0 °C; b) RLi, THF, 0 °C.

Results and Discussion

Compounds were evaluated for biological activity by testing against the recombinant *Pf*dUTPase and recombinant human dUTPase in order to determine inhibition and selectivity between the two enzymes. Compounds were also tested in vitro against the chloroquine-resistant K1 strain of *P. falciparum* and additionally against mammalian L6 cells as a measure of cytotoxicity (Table 1, Table 2, and Table 3).

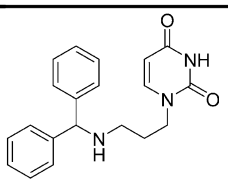
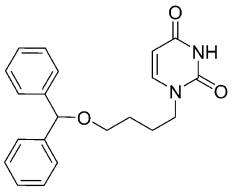
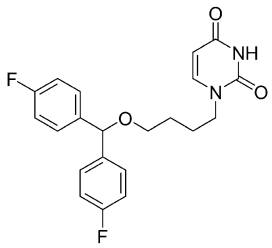
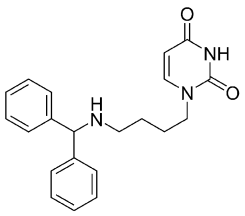
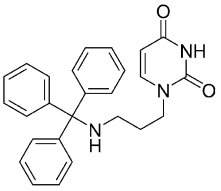
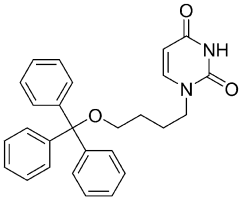
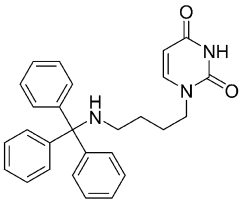
Acyclic analogues

For the three-carbon linked compound **1**, loss of one of the phenyl groups led to a significant (30-fold) loss in *Pf*dUTPase inhibition relative to the trityl derivative **21** ($K_i = 5.7$ versus 0.2 μM). However, for the four-carbon linked molecules, the effect on inhibition of *Pf*dUTPase was less marked. Thus the ether-linked compounds **2a** and **22** had reasonably similar activity ($K_i = 0.5$ versus 1.6 μM, respectively), although the amino-linked compounds **2c** and **23** ($K_i = 5.7$ versus 0.9 μM, respectively) showed a sixfold decrease in activity. The activity against parasites for the trityl and diphenyls are all similar, with EC_{50} values in the range 2.6–14 μM.

Cyclic analogues

Removal of one phenyl ring from the trityl derivative **24** gave compound **15a**, which retained the same inhibition of *Pf*dUTPase ($K_i = 0.2$ μM), whereas the presence of an extra methylene unit (in **15b**) led to a significant decrease ($K_i = 14$ μM). Replacing one of the phenyl rings with a heteroaromatic ring (compounds **15d**–**f**) resulted in decreased activity (~70-fold) relative to the trityl derivative **24**, with K_i values in the range 1.7–3.8 μM. Finally, addition of an alkyl group to the 5′-amino position (for **15g** and **15h**)

Table 2. Activity data for the acyclic derivatives.

Compd	Structure	Enzyme assays: K_i [μM] *P. fal.*[a]	Human	SI[b]	In vitro assays: EC_{50} [μM] *P. fal.*	L6 cells[c]	SI[d]
1		5.7	10	2	5.5	269	49
2a		0.5	49	98	14	104	7
2b		3.6	46	13	6.8	87	13
2c		5.7	63	11	2.6	99	38
21[e]		0.2	1.4	7	4.4	107	24
22[e]		1.6	>1000	>625	4.9	42	9
23[e]		0.9	>1000	>1111	3.8	33	9

[a] *P. falciparum* K1 chloroquine- and pyrimethamine-resistant strain. [b] Enzyme selectivity index (SI) calculated as [K_i *Hs*dUTPase/K_i *Pf*dUTPase]. [c] Cytotoxicity on rat L6 myoblasts; controls: for *P. falciparum*, chloroquine, EC_{50} = 0.1 μM; for cytotoxicity, podophyllotoxin, EC_{50} = 0.012 μM. The EC_{50} values are the means of two independent assays; the individual values vary by less than a factor of 2. [d] Cell selectivity index (SI) calculated as [EC_{50} L6/EC_{50} *P. falciparum*]. [e] Compounds **21**–**23** have been published,[9] but are included here for comparison.

gave compounds with only slightly lower activity (K_i = 0.5 and 0.9 μM) than compound **24**. Selectivity of *Pf*dUTPase over the human enzyme was retained in compound **15a** versus the trityl derivative **24** (> 500 versus 230). Introduction of the addi-

Table 3. Biological results for cyclic derivatives.

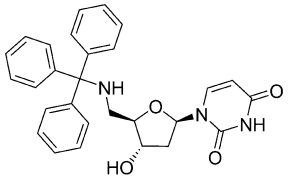
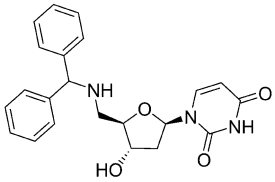
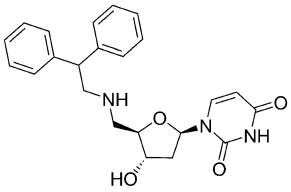
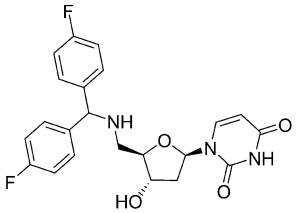
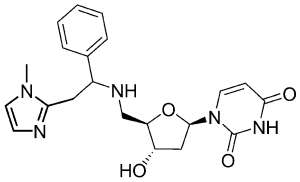
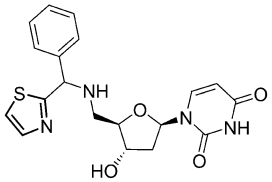
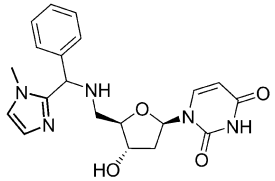
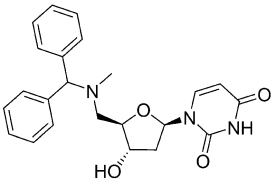
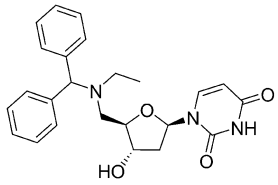
Compd	Structure	Enzyme assays: K_i [μM]			In vitro assays: EC_{50} [μM]		
		P. fal.[a]	Human	SI[b]	*P. fal.*	L6 cells[c]	SI[d]
24[e]		0.2	46	230	4.5	–	–
15a		0.2	>100	>500	12	>229	>20
15b		14	33	2	>12	219	>18
15c		3.8	>100	>26	10	145	15
15d		1.7	>100	>58	>12	>219	>18
15e		2.3	>100	>43	>13	215	17
15f		3.0	>100	>33	>12.6	>226	18
15g		0.5	26	52	5.8	>222	>38

Table 3. (Continued)

Compd	Structure	Enzyme assays: K_i [μM]			In vitro assays: EC_{50} [μM]		
		P. fal.[a]	Human	SI[b]	*P. fal.*	L6 cells[c]	SI[d]
15 h		0.9	33	37	9.1	117	13

[a] *P. falciparum* K1 chloroquine- and pyrimethamine-resistant strain. [b] Enzyme selectivity index (SI) calculated as [K_i *Hs*dUTPase/K_i *Pf*dUTPase]. [c] Cytotoxicity on rat L6 myoblasts; controls: for *P. falciparum*, chloroquine, EC_{50} = 0.1 μM; for cytotoxicity, podophyllotoxin, EC_{50} = 0.012 μM. The EC_{50} values are the means of two independent assays; the individual values vary by less than a factor of 2. [d] Cell selectivity index (SI) calculated as [EC_{50} L6/EC_{50} *P. falciparum*]. [e] Compound **24** has been published,[9] but is included here for comparison.

tional methylene group led to a significant decrease in selectivity, with only twofold preference for *Pf*dUTPase observed over the human enzyme. In the cellular assay, all the compounds showed similar or reduced activity compared to the starting trityl derivative **24**.

Water solubility

A comparison of water solubility between the original tritylated compound **24** and the methylated diphenyl compound **15 g** is shown in Figure 6. A decrease in the molecular weight

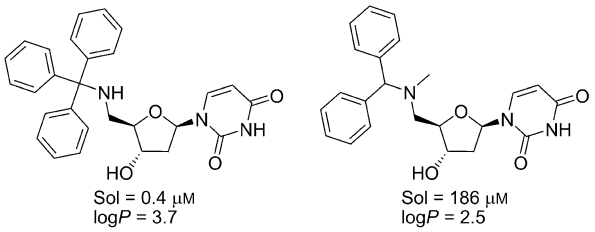


Figure 6. Measured water solubility [μM] comparison of trityl and diphenyl analogues **24** and **15 g**.

and lipophilicity of these compounds by removal of just one phenyl ring is reflected in the significant improvement in aqueous solubility. The solubility of the trityl derivative **24**, which is <0.5 μM, is increased to >180 μM for the diphenyl **15 g**. This is additionally reflected in the clog*P* value, which decreases from 3.7 to 2.5.

Crystal structures of the ligand complexes

*Pf*dUTPase was co-crystallised with each of the four ligands **24**, **15 a**, **15 f**, and **15 g**, and the structures of the complexes were refined. All are in the same space group, $P4_1$, with similar cell dimensions, each with a trimer in the asymmetric unit. The overall fold is closely similar to that of the complex with the trityl ligand, compound **F** (Figure 2).[3] Superposition of the trimers of the four ligand complexes on that of compound **F** gives an RMSD of 0.5–0.6 Å on ~400 equivalent Cα atoms. The RMSD drops to ~0.25 Å if the present four ligands in the same space group are superposed, showing only a minor effect of crystal packing. The N terminus is well ordered, from residue Met 1. There is a disordered loop with no visible density from residue 58 to 81 (the latter number varies slightly over the individual subunits of the four complexes), corresponding to the low complexity region in the sequence, well removed from the active site. As often observed in dUTPase structures, there is no electron density for the C terminus of each subunit: chains A and C are ordered up to residue 165, chain B only to 155. The ordering of residues 156–165 in chains A and C is a result of crystal packing against a symmetry-related trimer in a similar way, and these point away from the body of the trimer. There are no such contacts in subunit B, so this is likely to represent the conformation in solution, and our description of the structures focuses on this active site.

Compound 24 (Figure 7 A): Compound 24 binds in an essentially identical manner to that of the previously published trityl compound **F**, Figure 2. The ligand is well ordered and in the same orientation in all three active sites. The uracil and sugar rings occupy the same positions, making the same hydrogen bonds to the protein. Two trityl rings stack against Phe 46, with one ring (R1) pointing down into a hydrophobic pocket, a second (R2) stacking against the hydrophobic protein surface, while the third (R3) points out towards the solvent and is more exposed. The position of Phe 46 is rotated somewhat from its position in the original compound **F** complex. Tyr 112 stacks against the hydrophobic side of the sugar. The substitution of the linking O atom by an NH group has little impact on the position of the three aromatic rings of the trityl moiety. There is a water (W103) in the compound **24** complex, hydrogen bonded to the NH group of the ligand, which is not present in the complex of compound **F**.

Compound 15 a (Figure 7 B): This structure has the highest resolution (1.65 Å) of the *Pf*dUTPase complexes to date, with the ligand again well defined in all three active sites. The uracil and the sugar rings are bound in a very similar manner to those in the compound **F** and compound **24** complexes, Figure 7. The diphenyl moiety is oriented so that the two phenyl groups lie in the "buried" sites (R1 and R2). R1 superposes almost exactly on the compound **F**/compound **24** ring,

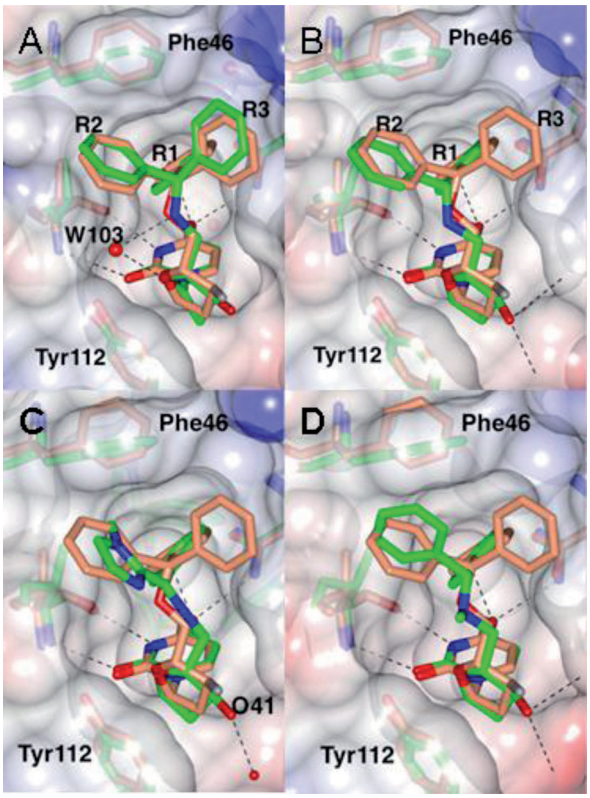


Figure 7. Views of the compounds bound to *Pf*DUTPase in the chain B active site. The compounds A) 24, B) **15a**, C) **15f**, and D) **15g** are coloured by atom type, each superimposed on compound **F** with its carbon atoms shown in coral. The electrostatic surface of the protein and the hydrogen bonds from the compound to the protein are shown, together with key protein residues in contact with the compound. The protein residues around the ligand site are shown, colour coded as for the compounds. Stereoviews of these structures are given in the Supporting Information.

while the orientation of the R2 ring is slightly rotated, probably reflecting the greater mobility of the diphenyl over the trityl unit. As expected, the R3 site is empty in this complex. The position of Tyr112 is the same as that observed in the compound **F** and compound **24** complexes. The plane of Phe46 has rotated even further in this complex relative to compound **F** and compound **24**, reflecting the small change in position and orientation of R2. This structure confirms that a diphenyl group is sufficient to bind as a substrate mimic, occupying the hydrophobic site present in the *Plasmodium* enzyme but absent in other species.

Compound 15f (Figure 7C): There is well-defined density for all the methylimidazole diaryl derivative ligands in all three active sites. The phenyl ring is buried in site R1, while the methylimidazole ring is accommodated in R2. The exposed R3 site is empty. There is a hydrogen bond from the ligand O41 to a water molecule. As in the complex with **15a**, Phe46 is rotated by ~90° from its position in the compound **F** complex, presumably reflecting the change in the nature of the ring at the R2 position, and lies in roughly the same position as in the complex with compound **15a**.

Compound 15g (Figure 7D): For the **15g** ligand—the diphenyl compound with the methylated 5′-N substituent—there is once more good density in all three active sites, with the two aromatic rings of the ligand occupying sites R1 and R2. The ligand binds in a very similar position to that observed for **15a**, with rotation of Phe46 from its position in the complex with compound **F**, and almost identical positions of the uracil and sugar rings. The R2 aromatic ring is rotated slightly from its position in **15a**.

In summary, all four ligands bind in a very similar manner, with the trityl or diphenyl and analogue moieties in the hydrophobic site identified in the original complex with compound **F**. The trityl amino compound **24** binds in a very similar manner to the original ligand **F**, showing that the substitution of oxygen by nitrogen in the linkage to the trityl group is easily accommodated. The three other complexes with **15a**, **15f**, and **15g** confirm that two aromatic rings are sufficient to provide binding in this site. As expected, if one ring is deleted from the trityl group, it is the exposed R3 position which is unfilled in the resulting complex. Most of the protein conformation is unchanged, but the side chain of Phe46 can rotate to accommodate the various ligands. Modified rings in the diphenyl complexes are bound at the half-exposed R2 position.

Conclusions

In an attempt to decrease lipophilicity and molecular weight of the trityl derivatives, we decided to remove one of the phenyl rings. A selection of both acyclic and cyclic derivatives were synthesised, both for comparison and in order to serve as a validation for the diphenyl-type analogues. The simple diphenyl derivatives **15a** and **2a** showed only slightly lower activity against both dUTPase and parasite than the corresponding trityl derivatives. The more complex heteroaromatic replacements generally showed lower activity against the enzyme and the parasite. Alkylation of the 5′-amino group appeared to be tolerated (**15g** and **15h**). Furthermore, compound **15g** showed improved solubility, a key goal of the project.

Crystallographic data show that the compounds bound in a very similar manner to the trityl derivatives. For the diphenyl derivatives, the two phenyl rings were bound in the "buried" sites occupied by phenyls from the trityl group. The position occupied by the solvent-exposed phenyl in the trityl group is not occupied in the diphenyl derivatives, as predicted. Therefore, it may be feasible in certain circumstances to replace the trityl group with a diphenyl group with relatively small loss in activity against the enzyme and parasite. Further optimisation is required in order to exploit these compounds as potential anti-malarial agents.

Experimental Section

Both recombinant *P. falciparum* and human dUTPases were expressed in *E. coli* BL21 (DE3) cells which had been transformed with the pET11Pfdut and pET3Hudut expression vectors, respectively (kindly provided by P. O. Nyman, Lund University, Sweden). For dUTPase purification, the same procedure was used for both the human and *Plasmodium* enzymes. Cell pellets from a 2.8 L

IPTG-induced culture were resuspended in 70 mL buffer A (20 mM MES, 50 mM NaCl, 1 mM DTT, pH 5.5) containing a protease inhibitor cocktail. The cells were lysed by sonication, and the cell extract was cleared by centrifugation at 14000 rpm (23700 *g*) for 30 min. The supernatant was loaded onto a 40 mL phosphocellulose (Whatman P-11) column at 4 °C and eluted with a gradient of NaCl (50 mM→1 M) in buffer A. Protein was further purified by gel filtration chromatography on a Superdex 200 HA 10/30 column at 4 °C. Pooled fractions were concentrated by centrifugation at 4 °C and desalted using a PD-10 column. The enzyme was stored in 10 mM bicine and 5 mM $MgCl_2$, pH 8 at −80 °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.[6] Protons, released through the hydrolysis of nucleotides, were neutralised by a pH indicator in weak buffered medium with similar pK_a and monitored spectrophotometrically at the absorbance peak of the basic form of the indicator. The molar ratio between indicator and buffer concentration was 5:200, and the absorbance changes were kept within 0.1 units. The indicator/buffer pair used was red cresol/bicine (pH 8, λ 573 nm). Assay mixes contained 30 nM *Pf*dUTPase, 50 μM dUTP, 5 mM $MgCl_2$, 1 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 1) were obtained according to Equation (1).

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

Activity against the *P. falciparum* K1 strain and cytotoxicity assessment against L6 (rat skeletal myoblast) cells was determined as previously reported.[9]

Chemistry

Solvents and reagents were purchased from commercial suppliers and used without further purification. Dry solvents were purchased in sure-sealed bottles stored over molecular sieves. Reactions were performed in a pre-dried apparatus under an atmosphere of argon unless otherwise stated. Normal-phase TLC was carried out on pre-coated silica plates (Kieselgel 60 F_{254}, BDH) with visualisation by either ninhydrin, PMA, or 254 nm UV light. Flash chromatography was performed using Combiflash Companion or Combiflash R_f and pre-packed columns (silica gel) purchased from Redisep (Presearch) or Sylicycle (Anachem). HPLC was performed using a Gilson instrument (321-Pump, 153-UV/Vis Detector) equipped with a Gilson liquid handler for injection and fraction collection, and an XBridge Prep C_{18} column (5 μm, ODB, 19×100 mm; Waters) with 0.1% NH_3 in H_2O (solvent A) and CH_3CN (solvent B) as mobile phase. Melting points (mp) were measured on a Gallenkamp melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DPX500 spectrometer or on a Bruker Avance DPX300 using the applied solvent simultaneously as internal standard. Deuterated solvents were purchased from Goss. Chemical shifts (δ) are given in ppm together with the multiplicity, relative frequency, coupling constants (*J*, in Hz), and assignment. Low-resolution electrospray (ES) mass spectra were recorded on a Bruker MicroTof mass spectrometer, run in positive or negative ion mode. High-resolution mass spectra were performed on a Bruker MicroTof mass spectrometer at University of Dundee. LC–MS analysis and chromatographic separation were conducted with a Bruker MicroTof mass spectrometer using an Agilent HPLC 1100 with a diode array detector in series, or using an Agilent 6130 quadrupole mass spectrometer in series with an Agilent 1200 binary pump, using one of the following methods:

Method 1. Phenomenex Gemini 5.0×50 mm column, acidic gradient running 5→95% CH_3CN/H_2O + 0.1% formic acid (FA), run time 6.1 min;

Method 2. Waters Xbridge C_{18}, 3.5 μm particle size, 2.1×50 mm column, acidic gradient running 5→95% CH_3CN/H_2O + 0.1% FA, run time 6.1 min;

Method 3. Waters Xbridge 5.0×50 mm column, basic gradient running 5→95% CH_3CN/H_2O + 0.1% NH_3;

Method 4. Waters Xbridge C_{18}, 3.5 μm particle size, 2.1×50 mm column, basic gradient running 20→95% CH_3CN/H_2O + 0.1% NH_3, run time 5.1 min;

Method 5. Waters Xbridge C_{18}, 3.5 μm particle size, 2.1×50 mm column, acidic gradient running 20→95% + 0.1% FA in CH_3CN, run time 6.5 min.

(3-Bromopropyloxy)*tert*-butyldimethylsilane (4): 3-Bromopropan-1-ol (1.31 mL, 15.0 mmol) was added to a cooled solution of TBDMSCl (2.49 g, 16.5 mmol) and imidazole (1.23 g, 18.0 mmol) in DMF (10 mL). The mixture was left to warn to room temperature overnight. The mixture was then diluted with Et_2O (20 mL) and extracted with $NaHCO_3$ (2×20 mL) and 3 M HCl (2×20 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to yield a colourless oil (3.3 g, 87%) that did not require further purification. R_f = 0.22 (hexane/EtOAc 90:10); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.00 (s, 6H, CH_3), 0.83 (s, 9H, CH_3), 1.96 (m, 2H, OCH_2CH_2), 3.45 (t, J = 6.4 Hz, 2H, CH_2Br), 3.67 (t, J = 5.8 Hz, 2H, OCH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ = −6.5 (CH_3), 17.2 (C), 24.8 (CH_3), 29.5 (CH_2), 34.4 (CH_2), 59.2 (CH_2).

***N*-Benzhydryl-3-(*tert*-butyldimethylsilyloxy)propan-1-amine (5):** Et_3N (3.51 mL, 25.3 mmol) and compound **4** (1.28 g, 5.06 mmol) in CH_3CN (8 mL) were added to a solution of benzhydrylamine (3.93 g, 4.55 mmol) in CH_3CN (10 mL). The mixture was heated at reflux until reaction completion. After cooling, the solvent was removed to leave the crude as an oil, which was purified by flash chromatography (EtOAc/hexane 0→4%) to give the product as a yellow oil (0.627 g, 39%); R_f = 0.33 (hexane/EtOAc 90:10); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.00 (s, 6H, CH_3), 0.83 (s, 9H, CH_3), 1.70 (m, 2H, OCH_2CH_2), 2.64 (m, 2H, CH_2NH), 3.66 (m, 2H, OCH_2), 4.75 (s, 1H, NHCH), 7.15 (m, 2H, H-Ar), 7.22 (m, 4H, H-Ar), 7.35 (m, 4H, H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$): δ = −5.2 (CH_3), 18.4 (C), 26.1 (CH_3), 33.1 (CH_2), 45.8 (CH_2), 32.1 (CH_2), 67.8 (CH), 127.0–128.5 (CH), 144.4 (C); LRMS (ES^+): *m/z* 167.0 $[Ph_2C]^+$, 356.2 $[M+H]^+$; HRMS (ES^+): found 356.2416 $[M+H]^+$ $C_{22}H_{34}NOSi^+$ requires 356.2404.

3-(Benzhydrylamino)propan-1-ol (6): TBAF (600 μL, 1.94 mmol) was added to a solution of **5** (0.625 g, 1.76 mmol) in THF (5 mL). This was left to stir at room temperature until reaction completion. Upon removal of the solvent, the crude was purified by flash chromatography (EtOAc/hexane 30→100%) to give the product as an off-white solid (0.358 g, 84%); R_f = 0.39 (hexane/EtOAc 40:60); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.63 (m, 2H, $NHCH_2CH_2$), 2.72 (m, 2H, $NHCH_2$), 3.69 (m, 2H, CH_2OH), 4.69 (d, J = 4.8 Hz, 1H, CHNH), 7.11–7.28 (m, 10H, H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 32.7 (CH_2), 48.3 (CH_2), 64.1 (CH_2), 68.0 (CH), 127.7–128.7 (CH), 143.3 (C); LRMS (ES^+): *m/z* 242.3 $[M+H]^+$.

3-*N*-Benzoyluracil (7): Benzoyl chloride (5.2 mL, 44.8 mmol) was added to a suspension of uracil (2.24 g, 20 mmol) in CH_3CN (20 mL)

and pyridine (8 mL) under argon. After 5 min the suspension turned clear green, which was left to stir overnight at room temperature. After 24 h the solution, which contained a precipitate, was concentrated under reduced pressure. The resultant residue was partitioned between H_2O (100 mL) and CH_2Cl_2 (100 mL), and the organic layer was concentrated. The residue was taken into a mixture of dioxane (40 mL) and K_2CO_3 (0.5 M, 20 mL) and stirred for 30 min. The solution was lowered to pH 5 with glacial acetic acid to leave a residue that was then dissolved in saturated $NaHCO_3$ (100 mL). This was stirred at room temperature for 1 h, and the precipitate was filtered off and washed with cold H_2O. This was recrystallised from aqueous acetone (70% *v/v*) to yield an off-white solid (2.54 g, 59%); $R_f = 0.11$ ($CHCl_3/CH_3OH$ 95:5); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): $\delta = 5.75$ (d, $J = 7.7$ Hz, 1H, C*H*CO), 7.61 (t, $J = 7.4$ Hz, 2H, H-Ar), 7.68 (d, $J = 7.8$ Hz, 1H, C*H*N), 7.79 (t, $J = 7.6$ Hz, 1H, H-Ar), 7.97 (d, $J = 7.2$ Hz, 2H, H-Ar), 11.63 (bs, 1H, NH); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): $\delta = 100.5$ (CH), 129.9 (CH), 130.7 (CH), 131.7 (C), 135.9 (CH), 143.8 (CH), 150.5 (C), 163.4 (C), 170.5 (C).

1-(3-(Benzhydrylamino)propyl)-3-benzoyluracil (8): Polymer-supported PPh_3 (2.5 equiv, 3 mmol g^{-1}) was swelled in THF for 15 min. Compound **6** (0.356 g, 1.48 mmol) and the 3-*N*-benzoyluracil **7** (0.640 g, 2.96 mmol) were added, and the mixture was shaken at room temperature for a further 15 min before addition of DIAD (2 equiv) in THF. The reaction was shaken until consumption of the alcohol was observed by TLC. The resin was then filtered off and washed with THF, and the solvent was removed under reduced pressure. The crude product was then purified by flash chromatography (hexane/EtOAc 40:60) to give the product as a white foam (0.230 g, 35%); $R_f = 0.11$ (EtOAc/hexane 60:40); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.89$ (m, 2H, CH_2CH$_2$N), 2.65 (t, $J = 6.3$ Hz, 2H, NHCH_2), 3.90 (t, $J = 6.7$ Hz, 2H, CH_2N), 4.80 (s, 1H, C*H*NH), 5.68 (d, $J = 7.9$ Hz, 1H, C*H*CO), 7.19 (d, $J = 8.0$ Hz, 1H, C*H*N), 7.25 (t, $J = 7.3$ Hz, 2H, H-Ar), 7.33 (t, $J = 7.6$ Hz, 4H, H-Ar), 7.41 (d, $J = 7.4$ Hz, 4H, H-Ar), 7.51 (t, $J = 7.8$ Hz, 2H, H-Ar), 7.67 (d, $J = 7.5$ Hz, 1H, H-Ar), 7.93 (d, $J = 7.2$ Hz, 2H, H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 29.2$ (CH_2), 44.2 (CH_2), 47.1 (CH_2), 67.6 (CH), 101.7 (CH), 127.3–143.7 (C-Ar), 144.9 (CH), 149.8 (C), 162.6 (C), 169.0 (C); LRMS (ES+): *m/z* 440.2 $[M+H]^+$; HRMS (ES+): found 440.1954 $[M+H]^+$ $C_{27}H_{26}N_3O_3^+$ requires 440.1969.

1-(3-(Benzhydrylamino)propyl)uracil (1): Compound **8** (0.200 g, 0.455 mmol) was dissolved in a solution of 0.2 M $NaOCH_3$ in CH_3OH, and the reaction was stirred at room temperature overnight until the disappearance of the starting esters was observed (TLC). The solution was neutralised with Dowex H^+ ion-exchange resin, filtered, and washed with CH_3OH. The solution was concentrated in vacuo, and the crude residue was purified by column chromatography (0→2% $CH_3OH/CHCl_3$). The title compound was obtained as an off-white crystalline foam (0.043 g, 28%). Purity by LCMS (UV chromatogram, λ 190–450 nm): 93%, $t_R = 0.7$ min (Method 4); $R_f = 0.36$ ($CHCl_3/CH_3OH$ 90:10); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): $\delta = 1.6$ (m, 2H, CH_2CH$_2$N), 2.40 (t, $J = 6.5$ Hz, 2H, NHCH_2), 3.73 (t, $J = 7.0$ Hz, 2H, CH_2N), 4.76 (s, 1H, C*H*NH), 5.50 (d, $J = 7.8$ Hz, 1H, C*H*CO), 7.18 (t, $J = 7.3$ Hz, 2H, H-Ar), 7.28 (t, $J = 7.6$ Hz, 4H, H-Ar), 7.41 (d, $J = 7.3$ Hz, 4H, H-Ar), 7.61 (d, $J = 7.8$ Hz, 1H, C*H*N), 11.22 (bs, 1H, N*H*CO); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): $\delta = 28.5$ (CH_2), 44.1 (CH_2), 45.6 (CH_2), 66.4 (CH), 100.6 (CH), 126.6 (CH), 127.0 (CH), 128.2 (CH), 144.7 (C), 145.8 (CH), 150.9 (C), 163.7 (C); LRMS (ES+): *m/z* 167.0 $[Ph_2C]^+$, 336.1 $[M+H]^+$; HRMS (ES+): found 336.1715 $[M+H]^+$ $C_{20}H_{22}N_3O_2^+$ requires 336.1707.

3-Benzoyl-1-(3-(ethoxycarbonyl)propyl)uracil (9): 3-*N*-Benzoyluracil **7** (1.00 g, 4.63 mmol) was combined with ethyl 4-bromobutanoate (1.35 g, 6.95 mmol) and Cs_2CO_3 (1.66 g, 5.09 mmol) in DMF (10 mL). This was left to stir at room temperature overnight, or alternatively was heated at 60 °C for 1 h. The mixture was then diluted with EtOAc (50 mL) and extracted with H_2O (5×30 mL). The aqueous layers were then combined and back extracted with EtOAc (50 mL). The organic layers were then combined, dried ($MgSO_4$), and the solvent removed. Purification was carried out by flash chromatography (EtOAc/hexane 0→100%) to give the title compound as a viscous yellow oil (1.336 g, 87%). Purity by LCMS (UV chromatogram, λ 190–450 nm): >99%, $t_R = 3.3$ min (Method 1); $R_f = 0.63$ ($CHCl_3/CH_3OH$ 90:10); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.18$ (t, $J = 7.7$ Hz, 3H, CH_3CH$_2$O), 1.95 (quint, $J = 7.1$ Hz, 2H, CH_2CH$_2$N), 2.31 (t, $J = 7.0$ Hz, 2H, COCH_2), 3.73 (t, $J = 7.1$ Hz, 2H, CH_2N), 4.07 (q, $J = 7.1$ Hz, 2H, CH_2O), 5.69 (d, $J = 8.0$ Hz, 1H, C*H*CO), 7.29 (d, $J = 8.0$ Hz, 1H, C*H*N), 7.45 (t, $J = 7.5$ Hz, 2H, H-Ar), 7.61 (t, $J = 7.5$ Hz, 1H, H-Ar), 7.90 (d, $J = 8.4$ Hz, 2H, H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 14.1$ (CH_3), 23.9 (CH_2), 30.7 (CH_2), 48.2 (CH_2), 60.6 (CH_2), 101.8 (CH), 129.2 (CH), 130.4 (CH), 131.4 (C), 135.2 (CH), 144.7 (CH), 149.8 (C), 162.5 (C), 169.1 (C), 172.4 (C); LRMS (ES+): *m/z* 331.1 $[M+H]^+$.

3-Benzoyl-1-(4-hydroxybutyl)uracil (10): $NaBH_4$ (3.65 g, 96.39 mmol) was added to a solution of **9** (3.53 g, 10.71 mmol) in THF (20 mL). This was heated at reflux for 15 min. CH_3OH (21.7 mL, 0.536 mmol) was then added very slowly over 25 min. After 1 h, the reaction was complete as evidenced by TLC. After cooling, H_2O (30 mL) was added to quench the reaction and stirred for a further 15 min. This was washed with CH_2Cl_2 (3×50 mL), and the aqueous layer was concentrated to an oil. This was further purified by flash chromatography (CH_3OH/CH_2Cl_2 0→10%) to give **10** (0.604 g, 31%) as a white wax. $R_f = 0.13$ ($CHCl_3/CH_3OH$ 90:10); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.55$ (m, 2H, HOCH$_2$CH_2), 1.75 (m, 2H, CH_2CH$_2$N), 3.65 (q, $J_1 = 11.1$ Hz, $J_2 = 6.1$ Hz, 2H, HOCH_2), 3.72 (m, 2H, CH_2N), 5.63 (dd, $J_1 = 7.9$ Hz, $J_2 = 1.8$ Hz, 1H, C*H*CO), 7.12 (d, $J = 7.9$ Hz, 1H, C*H*N), 8.19 (bs, 1H, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 25.8$ (CH_2), 29.0 (CH_2), 48.7 (CH_2), 62.2 (CH_2), 102.2 (CH), 144.4 (CH), 150.6 (C), 163.1 (C); LRMS (ES+): *m/z* 185.1 $[M+H]^+$, 369.2 $[2M+H]^+$.

Bis(4-fluorophenyl)methyl methanesulfonate (11): MsCl (0.527 mL, 6.81 mmol) was added to a solution of 4,4′-difluorobenzhydrol (1 g, 4.54 mmol) in pyridine (5 mL). This was left to stir at room temperature overnight. Upon reaction completion, the crude product was used in the following reaction as such. $R_f = 0.78$ ($CHCl_3/CH_3OH$ 90:10); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.78$ (s, 3H, CH_3), 4.68 (s, 1H, CHO), 7.09 (m, 4H, H-Ar), 7.35 (m, 4H, H-Ar).

1-(4-Mesyloxybutyl)uracil (12): MsCl (1.25 mL, 16.08 mmol) was added to a solution of **10** (0.305 g, 1.66 mmol) in pyridine (10 mL) cooled to 0 °C. This was left to warm to room temperature overnight. The solvent was concentrated in vacuo and purified by flash chromatography (EtOAc/hexane 0→100%) to give **12** (0.215 g, 49%). Purity by LCMS (UV chromatogram, λ 190–450 nm): 91%, $t_R = 0.6$ min (Method 2); $R_f = 0.57$ ($CHCl_3/CH_3OH$ 90:10); ^{1}H NMR (500 MHz, $(CD_3)_2SO$): $\delta = 1.65$ (m, 4H, CH_2CH_2N), 3.18 (s, 3H, CH_3), 3.69 (t, $J = 6.4$ Hz, 2H, CH_2N), 4.22 (t, $J = 5.8$ Hz, 2H, OCH_2CH$_2$), 5.56 (dd, $J = 7.8$, 2.3 Hz 1H, C*H*CO), 7.67 (d, $J = 7.8$ Hz, 1H, C*H*N), 11.27 (bs, 1H, N*H*); ^{13}C NMR (125 MHz, $(CD_3)_2SO$): $\delta = 24.6$ (CH_2), 25.4 (CH_2), 36.4 (CH_3), 46.8 (CH_2), 69.9 (CH_2), 100.9 (CH), 145.6 (CH), 150.9 (C), 163.7 (C); LRMS (ES+): *m/z* 263.0 $[M+H]^+$, 525.1 $[2M+H]^+$; HRMS (ES+): found 263.0686 $[M+H]^+$ $C_9H_{15}N_2O_5S^+$ requires 263.0696.

1-(4-(Benzhydryloxy)butyl)uracil (2a): 1-(4-Hydroxybutyl)uracil **10** (0.068 g, 0.372 mmol), benzhydrol (0.068 g, 0.370 mmol), and PTSA (0.070 g, 0.372 mmol) were combined in toluene (10 mL). Molecular

sieves (4 Å) were then added, and this was heated at reflux. After 30 min a further portion of benzhydrol (0.013 g, 0.074 mmol) was added, and heating at reflux continued for 1 h. The crude mixture was filtered, and the filtrate crude was purified by flash chromatography (EtOAc/hexane 60:40) to give **2a** as a colourless oil (0.026 g, 20%). Purity by LCMS (UV chromatogram, λ 190–450 nm): 92%, t_R = 3.5 min (Method 4); R_f = 0.18 (hexane/EtOAc 40:60); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.60 (m, 2H, OCH_2CH_2), 1.75 (m, 2H, CH_2CH_2N), 3.42 (t, J = 6.0 Hz, 2H, OCH_2), 3.68 (t, J = 7.3 Hz, 2H, CH_2N), 5.25 (s, 1H, *CH*O), 5.58 (dd, J = 7.9, 1.8 Hz, 1H, *CH*CO), 7.02 (d, J = 7.9 Hz, 1H, *CH*N), 7.15–7.26 (m, 10H, H-Ar), 9.09 (bs, 1H, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.2 (CH_2), 26.6 (CH_2), 48.7 (CH_2), 68.4 (CH_2), 83.9 (CH), 102.0 (CH), 126.4–128.4 (C-Ar), 142.1 (CH), 144.6 (CH), 150.8 (C), 163.7 (C); LRMS (ES+): m/z 167.0 $[Ph_2C]^+$, 351.1 $[M+H]^+$, 701.3 $[2M+H]^+$; HRMS (ES+): found 351.1716 $[M+H]^+$ $C_{21}H_{23}N_2O_3^+$ requires 351.1703.

1-(4-(4,4′-Difluorobenzhydryloxy)butyl)uracil (2b): Compounds **10** (0.194 g, 1.05 mmol), **11** (0.480 g, 2.18 mmol), and Et_3N (0.152 mL, 1.09 mmol) were combined in CH_3CN (5 mL). This was heated at reflux overnight. The solvent was then concentrated under reduced pressure, and the crude residue was purified by semi-preparative HPLC (t_R = 5.7 min, 1 min hold 95% A, 8 min ramp to 95% B, 2 min hold 95% B) to give **2b** (0.071 g, 17%). Purity by LCMS (UV chromatogram, λ 190–450 nm): 94%, t_R = 4.9 min (Method 2); R_f = 0.72 ($CHCl_3$/CH_3OH 90:10); ^{1}H NMR (500 MHz, CH_3OD): δ = 1.65–1.68 (m, 2H, CH_2CH_2N), 1.79–1.82 (m, 2H, OCH_2CH_2), 3.47–3.49 (t, J = 6.1 Hz, 2H, CH_2N), 3.77–3.79 (t, J = 7.3 Hz, 2H, OCH_2), 5.40 (s, 1H, *CH*O), 5.64 (d, J = 7.8 Hz, 1H, CHCO), 7.05–7.07 (m, 4H, H-Ar), 7.35–7.38 (m, 4H, H-Ar), 7.56 (d, J = 7.8 Hz, 1H, CHN); ^{13}C NMR (125 MHz, CH_3OD): δ = 27.0 (CH_2), 27.7 (CH_2), 49.5 (CH_2), 69.4 (CH_2), 83.5 (CH), 102.2 (CH), 116.1 (CH), 129.8 (CH), 140.0 (C), 147.38 (CH), 163.5 (d, J = 242.6 Hz, C), 166.8 (C); ^{19}F NMR (470 MHz, CH_3OD): δ = −117.2 (F); LRMS (ES^+): m/z 203.0 $[FPh_2C]^+$, 387.0 $[M+H]^+$, 773.3 $[2M+H]^+$; HRMS (ES^+): found 387.1519 $[M+H]^+$ $C_{21}H_{21}F_2N_2O_3^+$ requires 387.1515.

1-(4-(Benzhydrylamino)butyl)uracil (2c): 1-(4-Mesyloxybutyl)uracil **12** (0.050 g, 0.191 mmol), benzhydrylamine (0.039 mg, 0.229 mmol) and Et_3N (0.039 mL, 0.287 mmol) were combined in CH_3CN (2 mL). This was heated at reflux overnight. The mixture was diluted with CH_2Cl_2 (10 mL) and extracted with H_2O (3×10 mL). The organic layer was dried ($MgSO_4$) and concentrated. This was purified by flash chromatography (EtOAc/hexane 0→100%), followed by semi-preparative HPLC [t_R = 5.2 min, 1 min hold 95% A, 8 min ramp to 95% B, 2 min hold 95% B to give a white wax (0.009 g, 14%)]. Purity by LCMS (UV chromatogram, λ 190–450 nm): 93%, t_R = 4.3 min (Method 2); R_f = 0.68 ($CHCl_3$/CH_3OH 90:10); ^{1}H NMR (500 MHz, CH_3OD): δ = 1.46 (m, 2H, CH_2CH_2N), 1.58 (m, 2H, $NHCH_2CH_2$), 2.45 (m, 2H, CH_2N), 3.62 (t, J = 7.2 Hz, 2H, $NHCH_2$), 4.71 (s, 1H, *CH*NH), 5.52 (d, J = 7.8 Hz, 1H, *CH*CO), 7.10 (m, 2H, H-Ar), 7.19 (m, 4H, H-Ar), 7.28 (d, J = 7.2 Hz, 4H, H-Ar), 7.43 (d, J = 7.8 Hz, 1H, *CH*N); ^{13}C NMR (125 MHz, CH_3OD): δ = 27.3 (CH_2), 27.7 (CH_2), 48.5 (CH_2), 48.6 (CH_2), 68.5 (C), 102.1 (CH), 128.0 (C), 128.4 (CH), 128.7 (C), 129.4 (CH), 145.0 (CH), 147.3 (C), 166.8 (C); LRMS (ES^+): m/z 167.0 $[Ph_2C]^+$, 350.2 $[M+H]^+$; HRMS (ES^+): found 350.1864 $[M+H]^+$ $C_{21}H_{24}N_3O_2^+$ requires 350.1863.

5′-*O*-Mesyl-2′-deoxyuridine (14): MsCl (0.169 mL, 2.19 mmol) was added to a cooled solution of 2′-deoxyuridine (0.500 g, 2.19 mmol) in pyridine (10 mL), and this was left at room temperature overnight. The reaction mixture was concentrated and purified by flash chromatography (CH_3OH/$CHCl_3$ 0→5%) to give the title compound as a white crystalline solid (0.347 g, 52%). Purity by LCMS (UV chromatogram, λ 190–450 nm): 99%, t_R = 0.5 min (Method 2); R_f = 0.27 ($CHCl_3$/CH_3OH 90:10); mp: 172–174 °C; ^{1}H NMR (500 MHz, DMSO): δ = 2.18 (m, 2H, H-2′), 3.23 (s, 3H, CH_3), 3.98 (m, 1H, H-4′), 4.24 (m, 1H, H-3′), 4.36 (m, 2H, H-5′), 5.53 (bs, 1H, 3′-OH), 5.66 (dd, J = 8.1, 2.2 Hz, 1H, H-5), 6.20 (t, J = 6.9 Hz, 1H, H-1′), 7.65 (d, J = 8.1 Hz, 1H, H-6), 11.38 (bs, 1H, 3-NH); ^{13}C NMR (125 MHz, DMSO): δ = 36.6 (CH_3), 38.3 (CH_2), 69.4 (CH_2), 70.0 (CH), 83.4 (CH), 84.3 (CH), 102.0 (CH), 140.5 (CH), 150.3 (C), 162.9 (C); LRMS (ES^+): m/z 307.0 $[M+H]^+$, 613.1 $[2M+H]^+$; HRMS (ES^+): found 329.0413 $[M+H]^+$ $C_{10}H_{15}N_2O_7S^+$ requires 329.0414.

5′-(Benzhydrylamino)-2′,5′-dideoxyuridine (15a): Benzhydrylamine (0.178 mL, 1.03 mmol) and Et_3N (0.045 mL, 0.326 mmol) were added to a solution of 5′-mesyl-2′-deoxyuridine **14** (0.100 g, 0.326 mmol) in CH_3CN (2 mL), and the mixture was heated at 120 °C overnight. After cooling, this was diluted with CH_2Cl_2 (10 mL) and extracted with H_2O (3×10 mL). The organic layer was dried ($MgSO_4$), the solvent was removed, and purification by flash chromatography (CH_3OH/$CHCl_3$ 0→5%) gave **15a** as a pale-orange powder (0.061 g, 48%). Purity by LCMS (UV chromatogram, λ 190–450 nm): 91%, t_R = 4.0 min (Method 2); R_f = 0.40 ($CHCl_3$/CH_3OH 90:10); mp: 188–190 °C; ^{1}H NMR (500 MHz, DMSO): δ = 2.10 (m, 2H, CH-2′), 2.63 (m, 3H, CH-5′ + NH), 3.86 (m, 1H, CH-4′), 4.21 (m, 1H, CH-3′), 4.83 (d, J = 4.1 Hz, 1H, *CH*NHCH$_2$), 5.24 (d, J = 4.3 Hz, 1H, OH), 5.48 (d, J = 8.1 Hz, 1H, H-5), 6.12 (t, J = 6.7 Hz, 1H, H-1′), 7.20 (t, J = 7.3 Hz, 2H, H-Ar), 7.30 (t, J = 7.6 Hz, 4H, H-Ar), 7.42 (d, J = 7.2 Hz, 4H, H-Ar), 7.76 (d, J = 8.1 Hz, 1H, H-6), 11.28 (s, 1H, NH-3); ^{13}C NMR (125 MHz, DMSO): δ = 38.9 (CH_2), 49.5 (CH_2), 66.4 (CH), 71.2 (CH), 84.0 (CH), 85.7 (CH), 101.6 (CH), 126.6 (CH), 126.9 (CH), 128.3 (CH), 140.8 (CH), 144.5 (C), 150.3 (C), 163.0 (C); LRMS (ES^+): m/z 167.0 $[Ph_2C]^+$, 394.1 $[M+H]^+$; HRMS (ES^+): found 394.1747 $[M+H]^+$ $C_{22}H_{24}N_3O_4^+$ requires 394.1761.

5′-(2,2-Diphenylethylamino)-2′,5′-dideoxyuridine (15b): Following the procedure for **15a**, compound **14** (0.075 g, 0.244 mmol), 2,2′-diphenylethylamine (0.073 g, 0.366 mmol), and Et_3N (0.052 mL, 0.366 mmol) were suspended in CH_3CN (2 mL) and heated at 120 °C overnight. The solvent was removed, and purification by semi-preparative HPLC (t_R = 5.1 min, 1 min hold 95% A, 8 min ramp to 95% B, 2 min hold 95% B) gave **15b** (0.007 g, 7%). Purity by LCMS (UV chromatogram, λ 190–450 nm): 95%, t_R = 4.2 min (Method 2); R_f = 0.36 ($CHCl_3$/CH_3OH 90:10); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.92 (m, 1H, C*H*H-2′), 2.16 (m, 1H, CH*H*-2′), 2.70 (dd, J = 12.5, 6.1 Hz 1H, C*H*H-5′), 2.79 (dd, J = 12.5, 5.1 Hz 1H, CH*H*-5′), 3.11 (m, 2H, $CHCH_2NH$), 3.64 (m, 1H, H-4′), 3.97 (t, J = 7.7 Hz, 1H, *CH*CH$_2$NH), 4.03 (m, 1H, H-3′), 5.43 (d, J = 8.1 Hz, 1H, H-5), 5.97 (t, J = 6.3 Hz, 1H, H-1′), 7.02–7.13 (m, 11H, H-Ar + H-6); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 40.1 (CH_2), 51.1 (CH_2), 51.2 (CH_2), 54.7 (CH), 72.9 (CH), 84.5 (CH), 84.7 (CH), 102.6 (CH), 126.8–128.7 (C-Ar), 139.5 (CH), 142.3 (C), 142.5 (C); LRMS (ES^+): m/z 408.2 $[M+H]^+$, 815.3 $[2M+H]^+$; HRMS (ES^+): found 408.1901 $[M+H]^+$ $C_{23}H_{26}N_3O_4^+$ requires 408.1918.

5′-(Bis(4-fluorophenyl)methylamino)-2′,5′-dideoxyuridine (15c): 5′-Amino-2′-deoxyuridine (0.200 g, 0.881 mmol), **11** (0.194 g, 0.881 mmol), and Et_3N (0.124 mL, 0.891 mmol) were combined in CH_3CN (5 mL). This was heated at 120 °C overnight. The solvent was then concentrated under reduced pressure, and the crude residue was purified by semi-preparative HPLC (t_R = 4.9 min, 1 min hold 95% A, 8 min ramp to 95% B, 2 min hold 95% B) to give **15c** (0.006 g, 2%). Purity by LCMS (UV chromatogram, λ 190–450 nm): 91%, t_R = 4.4 min (Method 2); R_f = 0.52 ($CHCl_3$/CH_3OH 90:10); ^{1}H NMR (500 MHz, CH_3OD): δ = 2.28 (m, 2H, CH-2′), 2.79 (m, 2H, CH-5′), 3.99 (dt, J = 4.2, 6.9 Hz,1H, CH-4′), 4.29 (m, 1H, CH-3′), 4.90 (s, 1H, *CH*NHCH$_2$), 5.62 (d, J = 8.1 Hz, 1H, H-5), 6.22 (t, J = 6.5 Hz, 1H, H-1′), 7.05 (m, 4H, H-Ar), 7.43 (m, 4H, H-Ar), 7.73 (d, J = 8.1 Hz,

1H, H-6); ^{13}C NMR (125 MHz, CH_3OD): δ = 40.5 (CH_2), 50.6 (CH_2), 67.0 (CH), 73.1 (CH), 86.7 (CH), 87.2 (CH), 102.7 (CH), 116.1 (CH), 130.1 (CH), 142.6 (CH), 152.0 (C), 162.2 (C); ^{19}F NMR (470 MHz, CH_3OD): δ = −117.9 (F); LRMS (ES^+): *m/z* 203.0 $[(FPh)_2C]^+$, 430.1 $[M+H]^+$; HRMS (ES^+): found 430.1580 $[M+H]^+$ $C_{22}H_{22}F_2N_3O_4^+$ requires 430.1573.

5′-(2-(1-Methyl-1*H*-imidazole-2-yl)-2-phenylethylamino]-2′,5′-dideoxyuridine (15 d): Following the procedure for **15 a**, compounds **14** (0.100 g, 0.326 mmol), **16 a** (0.085 g, 0.424 mmol), and Et_3N (0.045 mL, 0.326 mmol) were suspended in CH_3CN (2 mL) and heated at 120 °C overnight. After cooling, the mixture was concentrated and purification by semi-preparative HPLC (t_R = 1.6 min, 1 min hold 95% A, 8 min ramp to 95% B, 2 min hold 95% B) gave **15 d** (0.005 g, 4%), isolated as a mixture of two diastereomers. Purity by LCMS (UV chromatogram, λ 190–450 nm): 100%, t_R = 0.6 min (Method 2); R_f = 0.26 ($CHCl_3/CH_3OH$ 90:10); ^{1}H NMR (500 MHz, CH_3OD): δ = 2.17–2.28 (m, 4H, H-2′), 2.59–2.80 (m, 4H, H-5′), 2.29–3.01 (m, 2H, CH_2CHNH), 3.12–3.18 (m, 2H, CH_2CHNH), 3.23 (s, 6H, CH_3), 3.92–3.96 (m, 2H, H-4′), 4.07–4.13 (m, 2H, CH_2*CH*NH), 4.18–4.23 (m, 2H, H-3′), 5.65 (d, *J* = 8.1 Hz, 1H, H-5), 5.68 (d, *J* = 8.1 Hz, 1H, H-5), 6.19 (d, *J* = 6.7 Hz, 1H, H-1′), 6.20 (d, *J* = 6.7 Hz, 1H, H-1′), 6.84–6.87 (m, 4H, (N)C*H*C*H*(N)CH_3), 7.23–7.34 (m, 10H, H-Ar), 7.62 (d, *J* = 8.1 Hz, 1H, H-6), 7.72 (d, *J* = 8.1 Hz, 1H, H-6); ^{13}C NMR (125 MHz, CH_3OD): δ = 31.3 (CH_3), 31.4 (CH_3), 34.3 (CH_2), 34.4 (CH_2), 39.1 (CH_2), 39.2 (CH_2), 49.0 (CH_2), 49.2 (CH_2), 62.4 (CH), 62.7 (CH), 71.9 (CH), 85.2 (CH), 85.3 (CH), 85.6 (CH), 86.3 (CH), 101.4 (CH), 120.7 (CH), 125.7 (CH), 125.8 (CH), 126.8 (CH), 127.2 (CH), 127.3 (CH), 128.3 (CH), 140.9 (C), 141.1 (CH), 142.3 (CH), 145.6 (C), 150.7 (C),164.8 (C), 164.9 (C); LRMS (ES^+): *m/z* 412.2 $[M+H]^+$; HRMS (ES^+): found 412.1985 $[M+H]^+$ $C_{21}H_{26}N_5O_4^+$ requires 412.1979.

5′-(Phenyl(thiazole-2-yl)methylamino)-2′-deoxyuridine (15 e): Following the procedure for **15 a**, compounds **14** (0.200 g, 0.652 mmol), **16 b** (0.124 g, 0.848 mmol), and Et_3N (0.165 mL, 0.652 mmol) were suspended in CH_3CN (2 mL) and heated at 120 °C overnight. After cooling, the mixture was diluted with CH_2Cl_2 (2 mL), and washed with H_2O (2 mL). The organic layer was concentrated and purification by semi-preparative HPLC (t_R = 4.1 min, 1 min hold 95% A, 8 min ramp to 95% B, 2 min hold 95% B) gave **15 e** (6.70 mg, 3%), isolated as a mixture of two diastereomers. Purity by LCMS (UV chromatogram, λ 190–450 nm): 94%, t_R = 0.7 min (Method 2); R_f = 0.47 ($CHCl_3/CH_3OH$ 90:10); ^{1}H NMR (500 MHz, CH_3OD): δ = 2.25–2.31 (m, 2H, H-2′), 2.84–2.89 (m, 1H, H-5′), 2.93–2.97 (m, 1H, H-5′), 3.99–4.02 (m, 1H, H-4′), 4.33–4.36 (m, 1H, H-3′), 5.26 (d, *J* = 6.2 Hz, 1H, C*H*NHCH$_2$), 5.64 (d, *J* = 8.1 Hz, 1H, H-5), 5.65 (d, *J* = 8.1 Hz, 1H, H-5), 6.22–6.25 (m, 1H, H-1′), 7.32 (m, 1H, H-Ar), 7.38 (m, 2H, H-Ar), 7.48 (d, *J* = 7.5 Hz, 2H, H-Ar), 7.54(d, *J*=3.2 Hz, 1H, (N)CHC*H*(S)), 7.72 (m, 1H, (N)C*H*CH(S)), 7.75 (d, *J* = 8.1 Hz, 1H, H-6), 7.81 (d, *J* = 8.1 Hz, 1H, H-6); ^{13}C NMR (125 MHz, CH_3OD): δ = 40.5 (CH_2), 50.5 (CH_2), 50.6 (CH_2), 66.2 (CH), 66.3 (CH), 73.0 (CH), 73.1 (CH), 86.7 (CH), 86.8 (CH), 87.2 (CH), 87.3 (CH), 102.8 (CH), 121.0 (CH), 128.7 (CH), 129.1 (CH), 129.8 (CH), 129.9 (CH), 142.5 (CH), 143.0 (CH), 152.1 (C), 166.2 (C), 177.2 (C), 177.3(C); LRMS (ES^+): *m/z* 401.1 $[M+H]^+$; HRMS (ES^+): found 401.1264 $[M+H]^+$ $C_{19}H_{21}N_4O_4S^+$ requires 401.1278.

5′-[(Phenyl)(1-methyl-1*H*-imidazol-2-yl)methylamino)]-2′,5′-dideoxyuridine (15 f): Following the procedure for **15 a**, compounds **14** (0.200 g, 0.652 mmol), **16 c** (0.124 g, 0.848 mmol), and Et_3N (0.165 mL, 0.652 mmol) were suspended in CH_3CN (2 mL) and heated at reflux overnight. After cooling the mixture was diluted with CH_2Cl_2 (2 mL) and washed with H_2O (2 mL). The organic layer was concentrated, and purification by semi-preparative HPLC (t_R = 4.0 min, 1 min hold 95% A, 8 min ramp to 95% B, 2 min hold 95% B) gave **15 f** as a white wax (0.008 g, 3%), isolated as a mixture of two diastereomers. Purity by LCMS (UV chromatogram, λ 190–450 nm): 100%, t_R = 0.6 min (Method 2); R_f = 0.15 ($CHCl_3/CH_3OH$ 90:10); ^{1}H NMR (500 MHz, CH_3OD): δ = 2.24–2.32 (m, 4H, H-2′), 2.77–2.93 (m, 4H, H-5′), 5.50 (s, 3H, CH_3), 3.53 (s, 3H, CH_3), 3.99–4.02 (m, 2H, H-4′), 4.28–4.31 (m, 2H, H-3′), 5.12 (s, 2H, C*H*NH), 5.58 (d, *J* = 8.0 Hz, 1H, H-5), 5.64 (d, *J* = 8.0 Hz, 1H, H-5), 6.23 (t, *J* = 6.4 Hz, 2H, H-1′), 7.00–7.01 (m, 4H, (N)C*H*C*H*(N)), 7.30–7.38 (m, 10H, H-Ar), 7.61 (d, *J* = 8.0 Hz, 1H, H-6), 7.84 (d, *J* = 8.0 Hz, 1H, H-6); ^{13}C NMR (125 MHz, CH_3OD): δ = 33.2 (CH_3), 33.3 (CH_3), 40.4 (CH_2), 40.5 (CH_2), 50.2 (CH_2), 50.6 (CH_2), 60.2 (CH), 60.5 (CH), 73.0 (CH), 73.2 (CH), 86.6 (CH), 86.7 (CH), 87.2 (CH), 87.4 (CH), 102.9 (CH), 123.0 (CH), 123.2 (CH), 127.3–129.9 (C-Ar), 141.0 (CH), 141.1 (CH), 142.4 (C), 142.7 (C), 149.5 (C), 149.6 (C), 152.7 (C), 167.0 (C); LRMS (ES^+): *m/z* 398.1 $[M+H]^+$, 795.3 $[2M+H]^+$; HRMS (ES^+): found 398.1807 $[M+H]^+$ $C_{20}H_{24}N_5O_4^+$ requires 398.1823.

5′-(*N*-Methylbenzhydrylamino)-2′,5′-dideoxyuridine (15 g): 5′-Mesyldeoxyuridine **14** (0.050 g, 0.163 mmol) was combined with *N*-methylbenzhydrylamine (0.094 g, 0.489 mmol) and Et_3N (0.050 g, 0.489 mmol) in CH_3CN (2 mL) and heated at 120 °C for five days until reaction completion. Upon cooling, the crude was diluted with CH_2Cl_2 (15 mL) and washed with H_2O (3×15 mL). The organic layer was dried ($MgSO_4$) and concentrated. Purification was carried out by flash chromatography (CH_3OH/CH_3Cl 0→5%) to give the product as a pale-brown crystalline powder (0.019 g, 29%). Purity by LCMS (UV chromatogram, λ 190–450 nm): 96%, t_R = 4.3 min (Method 5); R_f = 0.16 ($CH_3OH/CHCl_3$ 10:90); mp: 99–101 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.97 (ddd, *J* = 13.9, 7.1, 5.0 Hz, 1H, C*H*H-2′), 2.24 (s, 3H, H-12), 2.33 (m, 1H, CH*H*-2′), 2.57 (m, 2H, H-5′), 3.94 (q, *J* = 6.4 Hz, 1H, H-4′), 4.09 (dd, *J* = 12.6, 6.3 Hz, 1H, H-3′), 4.36 (s, 1H, C*H*NCH$_3$), 5.54 (d, *J* = 8.1 Hz, 1H, H-5), 6.11 (dd, *J* = 6.8, 5.1 Hz, 1H, H-1′), 7.08 (d, *J* = 8.1 Hz, 1H, H-6), 7.14–7.33 (m, 10H, H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 39.4 (CH_2), 41.8 (CH_3), 57.3 (CH_2), 73.0 (CH), 76.3 (CH), 83.0 (CH), 84.9 (CH), 102.4 (CH), 127.3–128.7 (CH), 139.4 (CH), 142.2 (C), 150.0 (C), 163.0 (C); LRMS (ES^+): *m/z* 408.2 $[M+H]^+$; HRMS (ES^+): found 408.1922 $[M+H]^+$ $C_{23}H_{26}N_3O_4^+$ requires 408.1918.

5′-(*N*-Ethylbenzhydrylamino)-2′,5′-dideoxyuridine (15 h): To a solution of 5′-mesyl-2′-deoxyuridine (83 mg, 0.270 mmol) in anhydrous DMF (4 mL), benzhydryl ethylamine (171 mg, 0.810 mmol) and Et_3N (0.141 mL, 0.810 mmol) were added. The reaction was stirred at 120 °C for four days in a sealed tube. After four days, further 5′-mesyl-2′-deoxyuridine (83 mg, 0.270 mmol) was added, and the mixture was stirred at 140 °C overnight. The reaction was then allowed to reach room temperature, and the solvents were removed under reduced pressure. The product was purified by semi-preparative HPLC (t_R = 6.7 min, 1 min hold 95% A, 9 min ramp to 95% B, 3 min hold 95% B) gave the title compound as a white solid (31 mg, 27%). Purity by LC–MS (UV chromatogram, λ 190–450 nm) 97% (Method 3); R_f = 0.27 (CH_3OH/CH_2Cl_2 10:90); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.07 (t, *J* = 7.1 Hz, 3H, CH_3), 2.06 (ddd, *J* = 13.9, 7.2, 5.0 Hz, 1H, H-2′), 2.39 (td, *J* = 13.7, 6.8 Hz, 1H, H-2′), 2.72–2.80 (m, 3H, H-5′ and CH_2CH$_3$), 2.91 (dd, *J* = 13.6, 5.7 Hz, 1H, H-5′), 3.52 (s, 1H, OH), 3.90 (dd, *J* = 12.8, 5.8 Hz, 1H, H-4′), 4.14 (dd, *J* = 12.9, 6.5 Hz, 1H, H-3′), 5.60 (d, *J* = 8.1 Hz, 1H, H-5), 6.17 (dd, *J* = 6.9, 5.0 Hz, 1H, H-1′), 7.09 (d, *J* = 8.2 Hz, 1H, H-6), 7.25–7.41 (m, 10H, H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 10.8 (CH_3), 39.7 (CH_2), 45.3 (CH_2), 51.9 (CH_2), 71.3 (CH), 72.8 (CH), 83.2 (CH), 84.6 (CH), 102.3 (CH), 127.4 (CH), 128.5 (CH), 128.6 (CH), 139.4 (CH), 141.6 (C), 149.8 (C), 162.7 (C); LRMS (ES^+) *m/z* 422.2 $[(M+H)^+$, 100%]; MS (ES^+) found 422.2066, $C_{24}H_{28}N_3O_4^+$ requires 422.2074.

2-(1-Methyl-1*H*-imidazol-2-yl)-1-phenylethanamine (16a): A solution of lithium hexamethyldisilazanide (LiHMDS; 1 M in THF, 20.3 mL, 20.3 mmol), was added dropwise to a solution of benzaldehyde (1.5 mL, 14.87 mmol) in THF at 0 °C under argon. The mixture was stirred for 30 min at the same temperature. In a separate round-bottom flask, 1,2-dimethylimidazole (1.3 g, 13.52 mmol) was dissolved in THF, and *n*-butyllithium (1.6 M in hexane, 9.3 mL, 14.87 mmol) was added dropwise at 0 °C under argon. The resulting mixture was stirred for 30 min at the same temperature. After this time, this solution was added dropwise to the benzylimine solution 0 °C. Upon completion of the addition, the reaction mixture was allowed to reach room temperature, hydrolysed with HCl (3 M, 90 mL), and washed with EtOAc (3×120 mL). The aqueous layer was then made alkaline with NaOH (2.5 M, 150 mL), extracted with EtOAc (3×150 mL), and the combined organic layers were dried over $MgSO_4$, and the solvents removed under reduced pressure. The product was purified by flash chromatography using the following gradient: 1 min hold at 100% CH_2Cl_2, 20 min ramp to 10% CH_3OH/NH_3 in CH_2Cl_2, 5 min hold to 10% CH_3OH/NH_3 in CH_2Cl_2. After removing the solvents, the title compound was obtained as pale-yellow oil (488 mg, 18%). Purity by LC–MS (UV chromatogram, λ 190–450 nm) 99% (Method 3); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 1.95$ (bs, 2H, NH_2), 2.94–2.96 (m, 2H, CH_2), 3.31 (s, 3H, CH_3), 4.59 (dd, $J = 7.7$, 5.4 Hz, 1H, CH), 6.77 (d, $J = 1.2$ Hz, 1H, CHN), 7.00 (d, $J = 1.2$ Hz, 1H, C*H*NCH$_3$), 7.26–7.37 (m, 5H, H-Ar); ^{13}C NMR (125 MHz, CDCl3) $\delta = 32.4$ (CH_3), 37.0 (CH_2), 53.8 (CH), 120.5 (CH), 126.3 (CH), 127.30 (CH), 127.35 (CH), 128.5 (CH), 145.3 (C), 146.0 (C); LRMS (ES$^+$) *m/z* 202.1 [$(M+H)^+$, 100%].

Phenyl(thiazol-2-yl)methanamine (16b): A solution of LiHMDS (1 M in THF, 17.6 mL, 17.6 mmol), was added dropwise to a solution of benzaldehyde (1.39 mL, 12.92 mmol) in THF at 0 °C under argon. The mixture was stirred for 30 min at the same temperature. In a separate round-bottom flask, thiazole (1 g, 11.75 mmol) was dissolved in THF, and *n*-butyllithium (1.6 M in hexane, 8.61 mL, 13.79 mmol) was added dropwise at 0 °C under argon. The resulting mixture was stirred for 30 min at the same temperature. After this time, this solution was added dropwise to the benzylimine solution 0 °C. Upon completion of the addition, the reaction mixture was allowed to reach room temperature, hydrolysed with HCl (3 M, 90 mL) and washed with EtOAc (3×120 mL). The aqueous layer was then made alkaline with NaOH (2.5 M, 150 mL), extracted with EtOAc (3×150 mL), and the combined organic layers were dried over $MgSO_4$, and the solvents were removed under reduced pressure. The product was purified by flash chromatography using the following gradient: 5 min hold at 100% CH_2Cl_2, 15 min ramp to 5% CH_3OH/NH_3 in CH_2Cl_2, 5 min hold to 5% CH_3OH/NH_3 in CH_2Cl_2. After removing solvents, the title compound was obtained as a yellow oil (217 mg, 10%). Purity by LC–MS (UV chromatogram, λ 190–450 nm) 84% (Method 3); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 2.20$ (bs, 2H, NH_2), 5.51 (s, 1H, CH), 7.25 (d, $J = 3.3$ Hz, 1H, CHS), 7.29–7.48 (m, 5H, H-Ar), 7.73(d, $J = 3.3$ Hz, 1H, CHN); ^{13}C NMR (125 MHz, CDCl3) $\delta = 58.3$ (CH), 119.0 (CH), 126.9 (CH), 127.9 (CH), 128.8 (CH), 142.7 (CH), 143.1 (C), 176.3 (C); LRMS (ES$^+$) *m/z* 174 [$(M-NH_2)^+$, 100%], 191.1 [$(M+H)^+$, 13%].

(1-Methyl-1*H*-imidazol-2-yl)(phenyl)methanamine (16c): A solution of LiHMDS (1 M in THF, 18.8 mL, 18.8 mmol) was added dropwise to a solution of benzaldehyde (1.39 mL, 12.92 mmol) in THF at 0 °C under argon. The mixture was stirred for 30 min at the same temperature. In a separate round-bottom flask, *N*-methylimidazole (1 g, 12.18 mmol) was dissolved in THF and *n*-butyllithium (1.6 M in hexane, 8.6 mL, 13.79 mmol) was added dropwise at −78 °C under argon. The resulting mixture was stirred for 1 h at the same temperature. After this time, this solution was added dropwise to the benzylimine solution 0 °C. Upon completion of the addition, the reaction mixture was allowed to reach room temperature, hydrolysed with HCl (3 M, 90 mL), and washed with EtOAc (3× 120 mL). The aqueous layer was then made alkaline with NaOH (2.5 M, 150 mL), extracted with EtOAc (3×150 mL), and the combined organic layers were dried over $MgSO_4$, and the solvents were removed under reduced pressure. The product was purified by flash chromatography using the following gradient: 5 min hold at 100% CH_2Cl_2, 15 min ramp to 20% CH_3OH/NH_3 in CH_2Cl_2, 5 min hold to 20% CH_3OH/NH_3 in CH_2Cl_2. After removing solvents, the title compound was obtained as a yellow oil (532 mg, 23%). Purity by LC–MS (UV chromatogram, λ 190–450 nm) 93% (Method 3); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 2.20$ (bs, 2H, NH_2), 3.41 (s, 3H, CH_3), 5.20 (s, 1H, CH), 6.84 (d, $J = 1.1$ Hz, 1H, CHN), 7.04 (d, $J = 1.2$ Hz, 1H, C*H*NCH$_3$), 7.27–7.37 (m, 5H, H-Ar); ^{13}C NMR (125 MHz, CDCl3) $\delta = 32.7$ (CH_3), 53.5 (CH), 121.5 (CH), 126.9 (CH), 127.0 (CH), 127.6 (CH), 128.9 (CH), 142.7 (C), 149.7 (C); LRMS (ES$^+$) *m/z* 171.1 [$(M-NH_2)^+$, 100%], 188.1 [$(M+H)^+$, 22%].

Benzhydryl ethylamine (16d): Benzophenone (1 g, 5.5 mmol) was dissolved in dry CH_2Cl_2 (30 mL), and a 1 M solution of $TiCl_4$ in CH_2Cl_2 (6 mL, 6 mmol) was added. The mixture was placed in an ice bath, and 2 M ethylamine solution in THF (6 mL, 12 mmol) was added. The resulting yellow suspension was stirred at room temperature for 3 h under argon. $NaCNBH_3$ (1 M solution in THF, 6.5 mL, 6.5 mmol) was then added to the reaction followed by 10 mL anhydrous CH_3OH, and the mixture was stirred for 1 h at room temperature. The mixture was made basic (pH ≈ 10) with a 1.5 M solution of NaOH and filtered. The filtrate was partitioned between EtOAc (100 mL) and H_2O (100 mL). The organic layer was separated, washed with H_2O (100 mL) and brine (100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in Et_2O (100 mL), acidified with concentrated HCl, and extracted with H_2O (100 mL). The aqueous extract was adjusted to pH ≈ 10 with aqueous NH_3 and extracted with Et_2O (2× 100 mL). The organic layer was dried over $MgSO_4$, and solvents were removed under reduced pressure to give the title compound as a yellow oil (518 mg, 45%). Purity by LC–MS (UV chromatogram, λ 190–450 nm) 93% (Method 3); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 1.16$ (t, $J = 7.1$ Hz, 3H, C*H*$_3$), 1.58 (bs, 1H, N*H*), 2.64 (q, $J = 7.1$ Hz, 2H, C*H*$_2$), 4.86 (s, 1H, C*H*), 7.21–7.43 (m, 10H, *H*-Ar); ^{13}C NMR (125 MHz, $CDCl_3$) $\delta = 15.4$ (CH_3), 42.6 (CH_2), 67.5 (CH), 126.9 (CH), 127.3 (CH), 128.5 (CH), 144.2 (C); LRMS (ES$^+$) *m/z* 167.1 [$(Ph_2C+H)^+$, 100%], 212.1 [$(M+H)^+$, 26%].

Benzhydryl isopropylamine (16e): Prepared as described above for **16d** from benzophenone (1 g, 5.5 mmol) and isopropylamine (1.0 mL, 12 mmol) to give the title compound as a yellow oil (636 mg, 52%); ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 1.11$ (d, 6H, $J = 6.3$ Hz, 2×C*H*$_3$), 2.77 (sept, 1H, $J = 6.3$ Hz, C*H*), 4.99 (s, 1H, C*H*), 7.21–7.42 (m, 10H, *H*-Ar); ^{13}C NMR (125 MHz, $CDCl_3$) $\delta = 23.2$ (2× CH_3), 46.1 (CH), 64.2 (CH), 126.8 (CH), 127.4 (CH), 128.4 (CH), 144.5 (C).

Benzhydryl cyclopropylamine (16f): Prepared as described above for **16d** from benzophenone (1 g, 5.5 mmol) and isopropylamine (0.8 mL, 12 mmol) to give the title compound as a yellow oil (755 mg, 61%). Purity by LC–MS (UV chromatogram, λ 190–450 nm) 99%; ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 0.18$–0.19 (m, 4H, 2× CH_2), 1.16 (t, 3H, $J = 7.1$ Hz, C*H*$_3$), 1.79 (bs, 1H, N*H*), 1.83–1.88 (m, 1H, CH_2CHCH_2), 4.70 (s, 1H, C*H*), 6.98–7.15 (m, 10H, *H*-Ar); ^{13}C NMR (125 MHz, $CDCl_3$) $\delta = 6.7$ (CH_2), 22.6 (CH), 67.2 (CH), 126.9 (CH), 127.4 (CH), 128.4 (CH), 144.2 (C); LRMS (ES$^+$) *m/z* 167.1 [$(Ph_2C+H)^+$, 100%], 224 [$(M+H)^+$, 14%].

Crystallography

Protein constructs: For co-crystallisation of the complex with **24**, a construct corresponding to the full-length enzyme was overexpressed, and the protein was purified as described for the first complex of a trityl derivative.[3] In brief, this involved a tag-free protein overexpressed in *E. coli* and then purified by using a phosphocellulose matrix followed by gel filtration. For the other three complexes, a second construct was used in which the six C-terminal residues, which were disordered in the first crystal structures, were replaced by a non-cleavable His_6 tag. This allowed a simplified purification protocol with no tag cleavage required. Crystals grew more rapidly and reproducibly with this construct, and were isomorphous to those for **24**. The construct is referred to as *Pf*dUTPase⊿C.

Crystallisation: To obtain the **24** complex, the ligand was first dissolved in ethanol. This was then added in threefold molar excess to a $\approx$1 mg mL^{-1} solution of the protein in 20 mM Tris-HCl pH 7.5, 10 mM NaCl, 5 mM $MgCl_2$. The other inhibitors were less soluble, and a method was devised for producing a crystallisation solution by first dissolving the ligand to a stock concentration of 10–20 mM in DMSO. An aliquot of stock ligand solution was slowly added to the protein with continuous mixing to give a crystallisation solution of 15 mg mL^{-1} protein, 1.5 mM ligand in 50 mM Tris-HCl pH 8.5, 50 mM NaCl, and 5 mM TCEP. The protein/ligand molar ratio was 1:2. A centrifugation step was performed after 20 min incubation to remove any precipitate (14000 *g*). Crystallisation of complexes was performed by sitting-drop vapour diffusion with 300 nL drops (1:1 or 3:2 protein/inhibitor solution: Ammonium Sulfate screen) using a Mosquito robot (TTP Labtech) in 96-well trays (SwissCi). Crystals of each complex grew to a maximum dimension of 100–150 μm. Crystals were obtained under the following conditions: **24**: 2.0 M ammonium sulfate, 0.1 M sodium acetate, pH 4.6; **15a** and **15f**: 2.2 M ammonium sulfate, 0.2 M disodium phosphate; **15g**: 2.2 M ammonium sulfate alone. Crystals of each complex were vitrified at 110 K. For **24** the crystal was cryoprotected using 40% glycerol/60% reservoir; for **15a**, 19% glycerol/78% well/3% ligand. The others were taken straight from the drop without cryoprotectant.

X-ray data collection and structure refinement: X-ray data were collected from single crystals at 110 K on station ID23eh1 at the ESRF, Grenoble (France) using an ADSC CCD detector for **24** and **15a**. For **15g** and **15f**, data were collected at the Diamond Light Source, on beam lines I02 and I24, respectively. The images were integrated using MOSFLM, and all crystals were isomorphous in space group is $P4_1$ with cell dimensions $a=b\approx77$ Å and $c\approx106$ Å, with a trimer of the complex in the asymmetric unit. Calculations were performed using the CCP4 suite.[14] The compound **24** structure was solved by molecular replacement using the known structure of the enzyme reported earlier[3] (PDB code: 1VYQ) as a search model. The other three started from the refined structure of **24**. The models were refined using REFMAC[15] alternating with rebuilding using COOT.[16] The coordinates and refinement dictionary for the ligands were generated using SKETCHER. The data and refinement statistics are listed in Table 4. X-ray crystallographic data and coordinates have been deposited with the RCSB Protein Data Bank (PDB codes: compounds **15a**, 3T64; **15f**, 3T6Y; **15g**, 3T70; **24**, 3T60).

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Table 4. Data processing and refinement statistics for the complexes **15a**, **15f**, **15g**, and **24**.

Data collection	15a	15f	15g	24
Beamline/wavelength [Å]	ID23eh1/1.072	I24/0.9778	IO2/0.976	ID23eh1/0.976
$P4_1$ cell dimensions: *a*, *c* [Å]	77.01, 106.28	76,83, 106.13	76.75, 105.83	76.97, 106.92
Resolution limits [Å]	36.20–1.65	37.96–2.60	43.56–1.80	40.00–2.40
No. unique reflections	74358	19028	56711	24538
Multiplicity	5.5	5.6	7.4	3.9
Completeness [%]	100.0	100.0	100.0	99.3
R-merge [%]	8.20	11.80	4.80	9.00
$I/\sigma(I)$	11.5	10.7	12.5	13.6
Refinement statistics[a]				
R-cryst/*R*-free [%]	19.14/21.56	15.90/22.65	20.09/23.23	19.91/25.36
No. protein atoms	3602	3459	3556	3226
No. water molecules	322	122	395	96
No. ligand atoms	87	87	90	105
No. sulfate atoms	20	25	30	n/a
No. glycerol atoms	n/a	n/a	n/a	18
RMS ⊿ bond lengths [Å]	0.011 (0.022)	0.025 (0.022)	0.008 (0.022)	0.024 (0.022)
RMS ⊿ bond angles [°]	1.335 (2.010)	2.563 (2.021)	1.133 (2.023)	2.508 (2.023)
RMS ⊿ chiral volume [Å^3]	0.093 (0.200)	0.166 (0.200)	0.072 (0.200)	0.430 (0.200)
B-average on protein atoms [Å^2]	23.12	37.48	26.60	36.36
B-average on water molecules [Å^2]	35.13	36.85	38.64	36.87
B-average on ligand atoms [Å^2]	23.73	40.32	25.93	36.57
B-average on sulfate atoms [Å^2]	45.54	69.72	66.44	n/a
B-average on glycerol atoms [Å^2]	n/a	n/a	n/a	51.82
Ramachandran outliers [%]	0.8	0.8	0.8	0.8

[a] For the refinement statistics, average ideal values are given in parentheses.

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