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Original article

Design, synthesis and evaluation of novel uracil acetamide derivatives as potential inhibitors of *Plasmodium falciparum* dUTP nucleotidohydrolase

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Abstract

The ubiquitous enzyme dUTP nucleotidohydrolase (dUTPase) catalyses the hydrolysis of dUTP to dUMP and can be considered as the first line of defence against incorporation of uracil into DNA. Inhibition of this enzyme results in over-incorporation of uracil into DNA, leading to DNA fragmentation and cell death and is therefore lethal. By taking advantage of structural differences between the human and *Plasmodium* dUTPase, selective inhibitors of the enzyme can be designed and synthesised with the aim of being developed into novel anti-parasitic drugs. Analogue based design was used to target the *Plasmodium falciparum* dUTPase (*Pf*dUTPase). The structures of previously discovered selective inhibitors of the *Pf*dUTPase were modified by insertion of an amide bond. A series of tritylated uracil acetamide derivatives were synthesised and assessed for inhibition of the enzyme and parasite growth *in vitro*. These compounds were weak inhibitors of the *Pf*dUTPase.

Keywords: dUTP nucleotidohydrolase; Uracil acetamide; Anti-plasmodial; Anti-malarial

1. Introduction

Malaria is a health problem in more than 90 countries, placing about 40% of the world's population at risk of contracting the disease. It is endemic to tropical and subtropical regions. Annually, there are over 300 million cases of malaria and more than 1 million deaths, mainly amongst children [1]. The parasite *Plasmodium falciparum*, transmitted to the human host through the female *Anopheles* mosquito, is the main cause of severe clinical malaria. Resistance of the pathogenic parasite to commonly used chemotherapeutic agents is a major issue and the constant need for new drugs to treat this disease is urgent.

dUTP nucleotidohydrolase, also called dUTP pyrophosphatase or dUTPase is an enzyme which catalyses the hydrolysis of dUTP to dUMP in the presence of magnesium ions [2]. The enzyme has two major roles in maintaining the correct free nucleotide balance in the cell. It provides dUMP, a major cellular source for dTMP which is in turn needed for dTTP formation. It also maintains the concentration of dUTP 10^{-5} times lower than that of dTTP thereby minimizing mistaken incorporation of dUTP into DNA

Abbreviations: *Pf*dUTPase, *Plasmodium falciparum* dUTPase; dUTPase, dUTP nucleotidohydrolase; TBDPS, *tert*-butyl diphenyl silyl; EDC, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide; CbZ, carbobenzoxy; DCC, dicyclohexylcarbodiimide; DhbtOH, 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine.

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[3]. dUTPase is an ubiquitous enzyme and it has been shown to be essential for cell viability in many organisms [4–6].

Initially, we have shown tritylated deoxyuridine analogues and then subsequently tritylated acyclic uridine analogues were able to inhibit the *P. falciparum* dUTPase (*Pf*dUTPase) enzyme selectively in the low micromolar range (Fig. 1). These compounds also inhibited parasite growth in the low micromolar range [7–9]. This enzyme is a good target for development of novel anti-malarials.

In this paper we report an analogue based design, in which an amide bond was inserted into the scaffold of these previously discovered selective acyclic inhibitors. It was thought that through amide coupling methodology, a wide range of functional variability could be introduced to these molecules and therefore this modification would be advantageous for diversification studies and ultimately to increase the potency of our inhibitors. Furthermore, the presence of an amide bond would introduce rigidity into the conformationally flexible acyclic inhibitors.

2. Chemistry

The synthesis of the *tert*-butyl diphenyl silyl (TBDPS) uracil acetamides (**3a**, **3b**) was carried out by coupling the TBDPS protected amino alcohol (**2a**, **2b**) to 1-carboxymethyl uracil (**1**) [10] using EDC as a coupling reagent (Scheme 1).

The tritylamino analogues were synthesised by mono-tritylation of the relevant diamines (**4a–c**) followed by coupling to 1-methylcarboxyuracil (**1**) again using EDC as a coupling reagent (Scheme 2).

Subsequent to the synthesis of monoalkyl chain acetamide uracil derivatives, dialkyl chain derivatives were synthesised. Diamino uracil acetamides **11a** and **11b** were synthesised as shown in Scheme 3. The first step was selective protection of the terminal amino functions of the starting linear triamines which was carried out using carbobenzyloxy imidazole (CbZ-imidazole) [11]. CbZ-imidazole was synthesised from the reaction of CbZ–Cl with imidazole at 0 °C. In the case of compound **9a**, the uracil acetamide was synthesised from the reaction of compound **1** with the protected triamine **7a** using dicyclohexylcarbodiimide (DCC) as a coupling reagent and 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine (DhbtOH) as an additive [12]. Compound **9b**, however, was synthesised from the reaction of the activated succinimidyl ester, **8** of compound **1** with the protected triamine **7b** [12]. Subsequent deprotection of the terminal amino groups was carried out by hydrogenation over 5% Pd/C catalyst. NMR analyses of compounds **10a** and **10b**, however, revealed that acyl migration to the terminal amino groups had occurred to some extent (see Scheme 4). Tritylation was then carried out. However, the final product was not the expected product. During the tritylation process migration of the uracil acetamide to the terminal amino group was observed. Migration from the secondary amine to the terminal amine is presumably an equilibrium process, and addition of the bulky trityl group to one of the terminal amines pushes the equilibrium in favour of migration of the uracil acetamide from the secondary amine to the other terminal amine (Scheme 4). The migration was detected by studying the NMR spectra. If no migration had occurred in **11a** or **11b**, then there would be 4 protons in the spectra corresponding to C(O)NCH_2– and 2 protons corresponding to –CH_2NH-trityl and 2 protons corresponding to –CH_2NH_2. The C(O)NCH_2– ($\delta \sim 3.4$ ppm) protons are distinctive (downfield) compared to the –CH_2NH-trityl ($\delta \sim 2.2$–2.4 ppm) and –CH_2NH_2 ($\delta \sim 2.8$–2.9 ppm). However, in the spectra

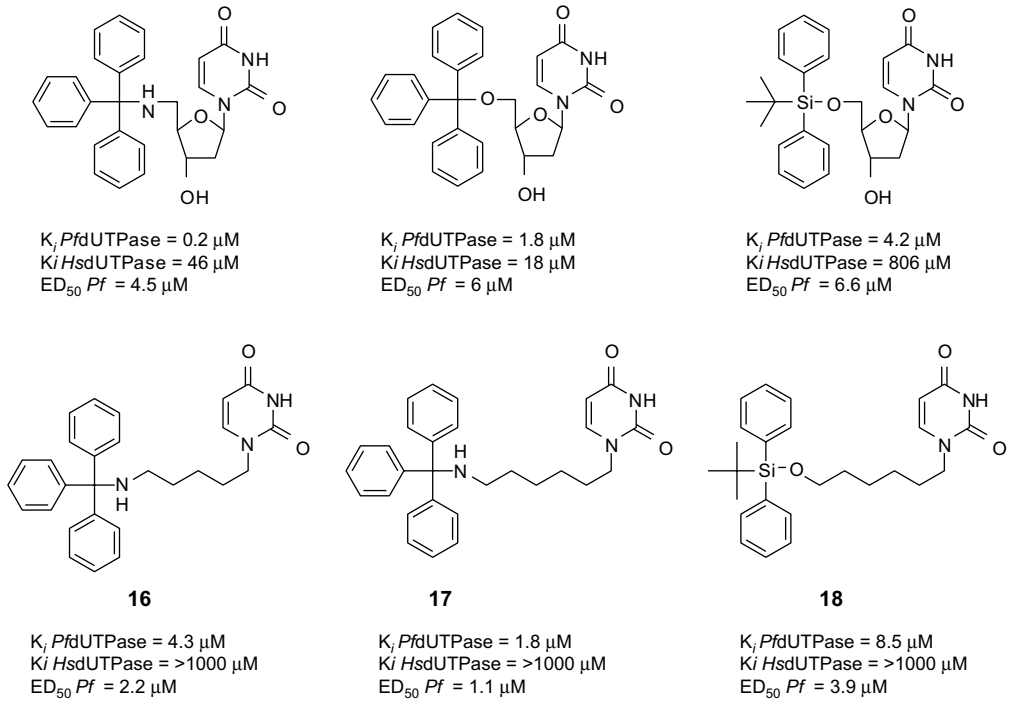
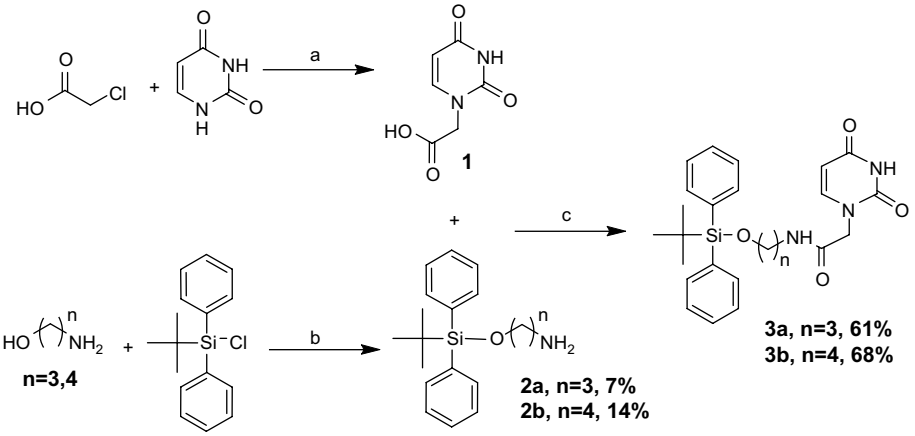


Fig. 1. Previously reported PfdUTPase inhibitors [8,9].



Scheme 1. Synthesis of TBDPS uracil acetamide derivatives (a) H_2O, KOH, reflux 1 h, HCl, 4 °C, 16 h; (b) DMF, imidazole, RT, 18 h; (c) EDC, DMF, 18 h.

for **11a** or **11b**, there are 2 protons in the spectra corresponding to C(O)NCH_2– and 2 protons corresponding to –CH_2NH-trityl and 4 protons corresponding to –C$H_2$$NH_2$.

Various attempts were made to prevent this migration. Tritylation of **10b** was attempted at 0 °C in pyridine to prevent further acyl migration from occurring, but there was no reaction at this temperature; heating was required (70 °C for 8 h) and migration was again observed. It was found that carrying out the reaction at RT in DCM using Et_3N as base reduced reaction time to 2 h and this procedure was used for the synthesis of compound **11a**. However, it did not prove possible to isolate the compound where migration of the uracil acetamide did not occur.

Hydroxy functionalised branched chain derivatives were synthesised as shown in Scheme 5. The starting diamino alcohol was protected at the hydroxy function using the TBDPS group. Selective tritylation of the terminal amine was then carried out by slow addition of a dilute solution of trityl chloride to diamines **13** at 0 °C followed by the addition of Et_3N. Coupling to 1-carboxymethyl uracil to yield the uracil acetamide **14** was again carried out using the DCC, DhbtOH methodology. Finally, removal of the TBDPS group was carried out using TBAF to yield the desired uracil acetamide **15**.

3. Biological activity

The compounds were assayed against the recombinant form of the *Pf*dUTPase and the human dUTPase as reported previously [8]. A continuous spectrophotometric method was used for the enzyme assays using indicator, based on the fact that the hydrolysis of dUTP to dUMP is coupled to release of protons.

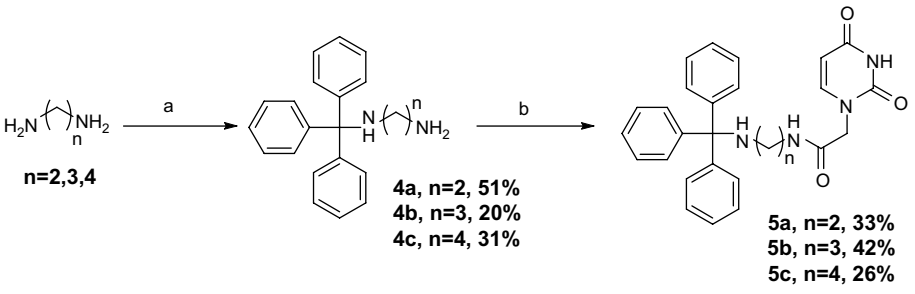
The compounds were also tested for *in vitro* activity against the erythocytic stages of the chloroquine resistant K1 strain of *P. falciparum* and against rat L6 cells for toxicity.

4. Results and discussion

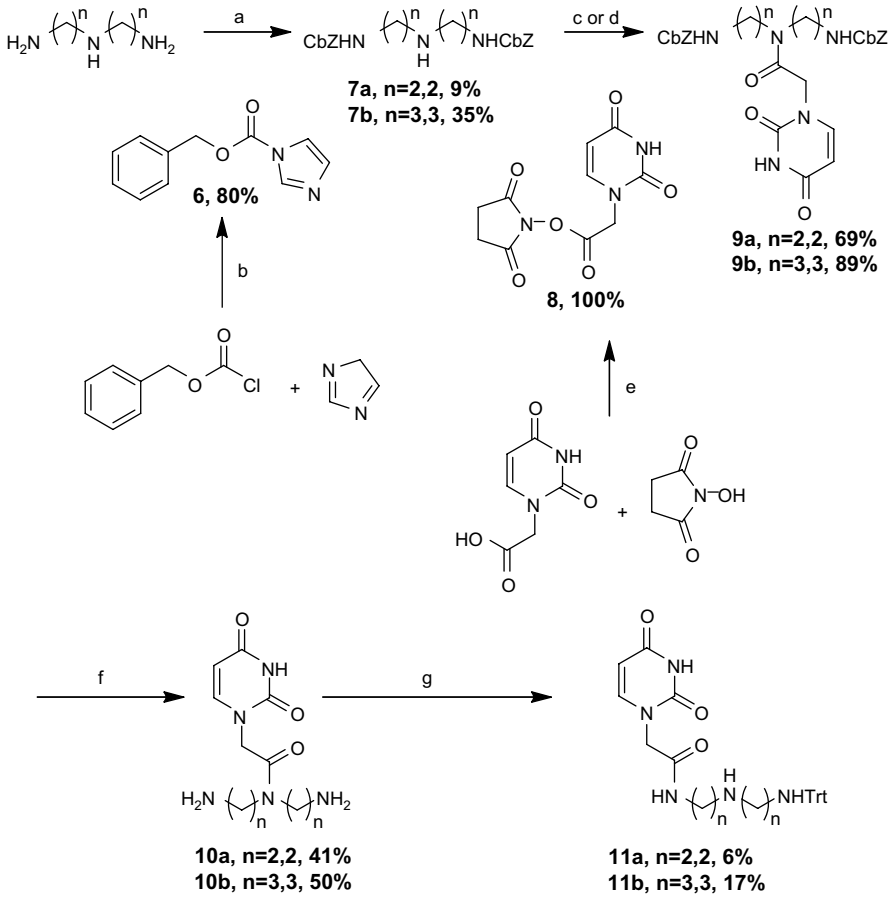
The results of the biological assays for the uracil acetamide derivatives synthesised are shown in Table 1. Compounds **16**–**18** are some of the lead inhibitors previously synthesised and reported [9] in which the amide bond is not present, the structures of which are shown in Fig. 1.

Two of the uracil acetamide compounds **5a** and **5b** exhibited weak but selective inhibition of the *Pf*dUTPase enzyme ($K_i = 37.3$, 23.1 μM, respectively). However, the activity of these compounds against the enzyme was about 10-fold lower than their acyclic analogues **17** and **18** ($K_i = 4.3$, 1.84 μM, respectively). It would therefore seem that introduction of an amide bond is unfavourable for the inhibitory action of these nucleoside derivatives against the *Pf*dUTPase.

Despite their lack of activity as inhibitors of the dUTPase enzyme, some of these compounds show quite potent activity against *P. falciparum* cultured in red blood cells. These compounds may therefore be exerting their effect on a different target which has not yet been identified.



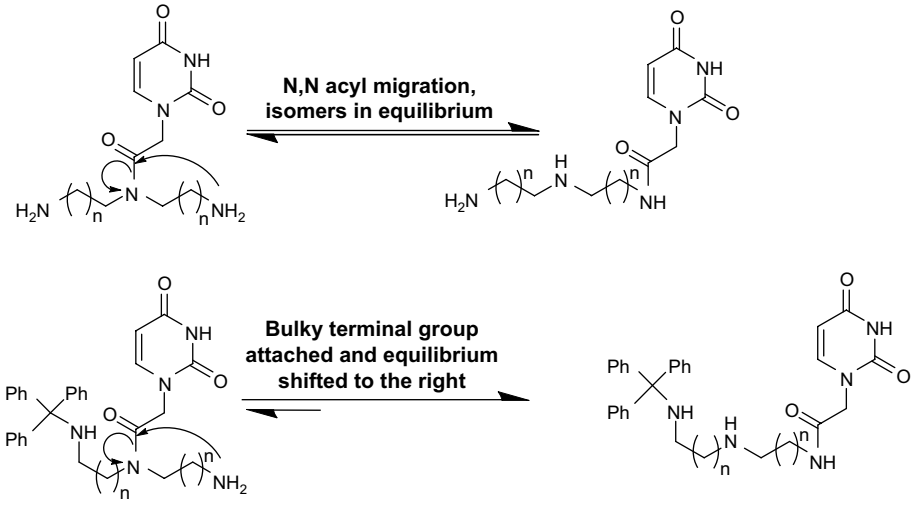
Scheme 2. Synthesis of tritylamino uracil acetamide derivatives (a) TrtCl, DCM, RT, 18 h; (b) EDC, DMF, RT, 18 h.



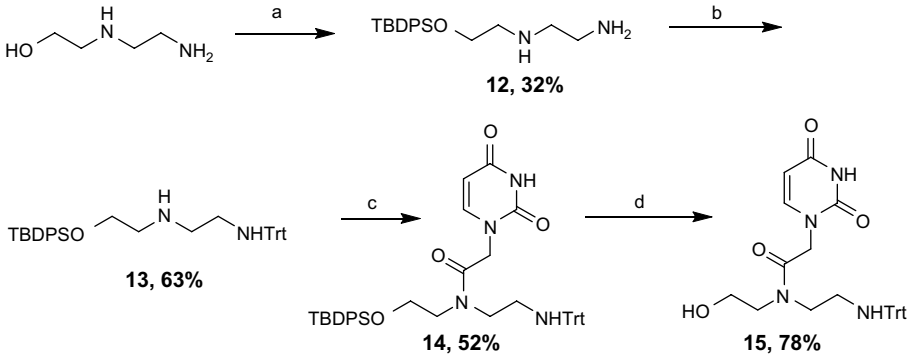
Scheme 3. Synthesis of branched chain trityldiamino uracil acetamide derivatives (a) DCM, 2 d, RT; (b) DCM, 0 °C, RT, 10 min; (c) DMF, DMAP, 3 d, 60 °C; (d) 1-carboxymethyl uracil, DMF, DCC, DhbtOH, 0 °C, 1 h, RT 2d; (e) DMF, DCC, 0 °C, 3 h, RT, 24 h; (f) MeOH/EtOH, H_2, 5% Pd/C; (g) TrtCl, Et_3N, DCM, RT, 2 h.

4.1. Molecular modelling

In order to try and understand the lack of activity, some molecular modelling studies were undertaken. Attempts to dock compound **5b** in the active site failed to reproduce the orientations found with a tritylated deoxyuridine analogue [7]. This was in comparison to other acyclic derivatives, which were docked in conformations consistent with the crystallographic data [9]. Therefore **5b** was manually placed into the active site in a conformation consistent with the crystallographic



Scheme 4. Equilibrium of *N*,*N* acyl migration.



Scheme 5. Synthesis of branched chain hydroxyl tritylamino uracil acetamide derivatives (a) TBDPSCl, pyridine, 24 h, RT; (b) TrtCl, DCM, Et_3N, 0 °C, RT, 24 h; (c) 1-carboxymethyl uracil, DCC, DhbtOH, 0 °C, 1 h, RT, 24 h; (d) THF, TBAF, RT, 30 min.

structure of the tritylated deoxyuridine analogue. Compound **5b** was then minimised in the active site using the MAB force field in Moloc [13] in order to identify any large conformational change due to interaction between the protein and ligand. These studies suggest the reason for the loss of activity of the uracil acetamides may be due to an unfavourable conformation the molecules must adopt to bind in the active site of the enzyme. The required conformation forces the O-3 of the uracil ring to be in close proximity to the carbonyl oxygen of the amide bond causing unfavourable electrostatic repulsion. There also may be unfavourable interactions between the carbonyl oxygen of the amide bond and the carbonyl oxygen of Glu115 and phenyl OH of Tyr112 (Fig. 2).

In fact, as calculated using the Tripos force field, the energy difference between the lowest energy conformation of compound **5b** *in vacuo* and the conformation it would have to adopt in the active site of the PfdUTPase is 7.5 kcal/mol.

5. Conclusion

The introduction of an amide bond into the scaffold of previously discovered selective inhibitors of the *P. falciparum* dUTPase led to the synthesis of a range of uracil acetamide derivatives. Introduction of an amide bond at the β-C to the N-1 of the uracil ring resulted in a loss of activity against the *Pf*dUTPase enzyme. Molecular modelling and conformational studies showed that this may have been due to the fact that the low energy conformation of the uracil acetamide compounds must overcome an energy barrier to adopt the required binding conformation. Additionally, there may be an unfavourable interaction between the O-2 of the uracil ring and the carbonyl oxygen of the amide bond in the proposed bound conformation leading to a decrease in potency of these compounds.

The uracil acetamide compounds do retain good activity *in vitro*, however. Their mode of action has not yet been ascertained.

Table 1
Biological results of uracil acetamides against the trimeric dUTPase and *in vitro* results against malaria parasites

Compound	Enzyme assay K_i (μM)			*In vitro* assay IC_{50} (μM)	
	P. falciparum	Human	SI[a]	*P. falciparum*	Toxicity[b]
3a	>1 mM	>1 mM	>1	4.6	88.9
3b	>1 mM	>1 mM	>1	3.7	33.4
5a	37.3	>1 mM	>26	4.2	53.0
5b	23.1	>1 mM	>43	2.5	102.2
5c	>1 mM	>1 mM	>1	2.9	42.7
11a	>1 mM	>1 mM	>1	4.5	52.7
11b	>1 mM	>1 mM	>1	3.9	76.7
15	>1 mM	>1 mM	>1	>83.0	>373
16	4.3	>1 mM	>232	2.2	39
17	1.8	>1 mM	>555	1.1	40
18	8.5	>1 mM	>117	3.9	18.0

Data for **16-18** has been previously reported [9].
[a] SI = selectivity index [K_i(human dUTPase)/K_i(*Pf*dUTPase)].
[b] Toxicity tests were carried out on L6 cells.

6. Experimental

Low resolution mass spectra were recorded at a Platform II mass spectrometer (Micromass) from Fisons where ionisation was achieved in the positive and negative electrospray modes using a 1:1 mixture of acetonitrile and water plus 0.2% formic acid or a 95:5 mixture of methanol and water plus 0.2% formic acid as a mobile phase. High resolution mass spectra were recorded by the National Mass Spectrometry Service Centre in Swansea at a MAT 900 XLT high resolution double focussing mass spectrometer from Finnigan or by University of Birmingham at a Micromass LCT spectrometer. High resolution mass spectra were also recorded at a MicrOTOF mass spectrometer from Bruker Daltonics. Accurate mass measurement was performed by peak matching. NMR spectra were recorded at a Bruker 300 MHz NMR spectrometer or at a Bruker 500 MHz NMR spectrometer. Purification by column chromatography was performed on Sorbosil C60A silica gel 40–60 μm from Merck. Flash chromatography was performed using the Flashmaster II from Jones Chromatography and Isolute® propylene columns which were prepacked with silica. Qualitative thin layer chromatography (TLC) was done on precoated aluminium sheets Silica gel 60 F254 from Merck. Compounds were detected either with ninhydrin or 254 nm UV light. Elemental analysis was carried out by the analytical and chemical consultancy services MEDAC Ltd. Found percentage values are reported together with calculated values

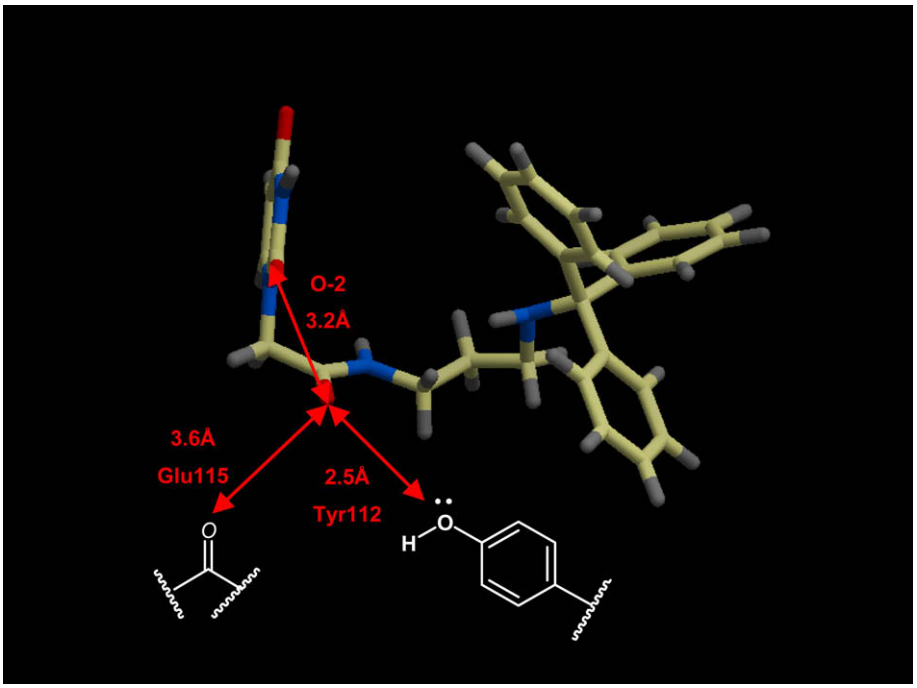


Fig. 2. Possible repulsive interactions to the carbonyl oxygen of the amide bond in uracil acetamides compounds in the PfdUTPase active site.

for different salt compositions as indicated by the preceding formula. Melting points were determined with a Gallenkamp melting point apparatus and are not corrected. Solvents and reagents were purchased from Sigma–Aldrich, Fluka or Lancaster and were used without further purification. Dry solvents were purchased in sure sealed bottles stored over molecular sieves. Deuterated solvents were purchased from Goss. All molecular modelling was carried out on a silicon graphics work station or a Mac OS using SYBYL 6.9 or 7.2.

6.1. Chemistry

6.1.1. Synthesis of 1-carboxymethyl uracil (**1**) [10]

Uracil (2.98 g, 26.64 mmol), chloroacetic acid (4.41 g, 47.67 mmol) and KOH (6.57 g, 117.12 mmol) were dissolved in 100 mL water and refluxed for 1 h. The reaction mixture was cooled to room temperature and acidified to pH 2 with HCl (32% w/w in water). The mixture was left at 4 °C overnight. The white precipitate formed was collected by filtration and washed with water (50 mL), ethanol (50 mL) and EtOAc (50 mL) to afford 3.16 g of the product as a white powder.

Yield: 71%. Melting point: 287–290 °C (Lit. 287 °C [10]). R_f: 0.1 in $CHCl_3$/MeOH, 9:1. ^{1}H NMR (300 MHz, DMSO) δ 4.43 (s, 2H, CH_2), 5.60 (d, $J = 7.8$, 1H, =C*H*), 7.62 (d, $J = 7.8$, 1H, =C*H*). ^{13}C NMR (75 MHz, DMSO) δ 49.4 (CH_2), 101.7 (CO_2H), 146.9 (*C*=C), 151.9 (C=*C*), 164.7(NH*C*O), 170.4 (HN*C*O). LRMS (ES^-): $m/z = 168.8$ $[M - H^+]$.

6.1.2. General procedure for tert-butyl diphenyl silylation of hydroxyl group using DMF and imidazole

TBDPSCl (1.1 eq.) dissolved in DMF (0.2 mL/mmol) was added to a mixture of imidazole (2.2 eq.) and the alcohol (1 eq.) dissolved in DMF (0.1 mL/mmol) under N_2 or Ar atmosphere. The mixture was left stirring at RT until the disappearance of the starting material was observed by TLC.

6.1.2.1. Synthesis of 3-(tert-butyl diphenyl silanyloxy)-propylamine (**2a**).

Yield: 7%. R_f: 0.3 in CH_2Cl_2/MeOH/NH_3, 90:8:2. ^{1}H NMR (300 MHz, MeOD) δ 0.95 (s, 9H, $CH_3 \times 3$), 1.71 (qn, $J = 6.50$, 2H, $CH_2CH_2CH_2$), 2.78 (t, $J = 7.1$, 2H, CH_2NH_2), 3.73 (t, $J = 6.01$, 2H, CH_2O), 7.36 (m, 6H, *meta*,*para*-PhH), 7.71 (m, 4H, *ortho*-PhH). ^{13}C NMR (75 MHz, MeOD) δ 20.5 (tC–Si), 27.9 ($CH_3 \times 3$), 36.5 ($CH_2CH_2CH_2$), 40.3 (CNH_2), 63.6 (*C*O), 129.3 (*meta*-Ph*C*), 131.4 (*para*-Ph*C*), 135.2 (Ph*C*–Si), 136.7–137.4 (*ortho*-Ph*C*). LRMS (ES^+): $m/z = 314.2$ $[M + H^+]$, 636.9 2 $[M + H^+]$. HRMS (ES^+): found: 314.1935 $[M + H^+]$ $C_{19}H_{28}ONSi$ requires 314.1935.

6.1.2.2. Synthesis of 4-(tert-butyl diphenyl silanyloxy)-butylamine (**2b**).

Yield: 14%. R_f: 0.2 in CH_2Cl_2/MeOH/NH_3, 90:8:2. ^{1}H NMR (300 MHz, MeOD) δ 1.05 (s, 9H, $CH_3 \times 3$), 1.01 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.63 (t, $J = 6.65$, 2H, CH_2NH_2), 3.69 (m, 2H, CH_2O), 7.36 (m, 6H, *meta*,-*para*-PhH), 7.60 (m, 4H, *ortho*-PhH). ^{13}C NMR (75 MHz, MeOD) δ 20.5 (*C*–Si), 27.9 ($CH_3 \times 3$), 30.5 ($CH_2CH_2CH_2$), 31.5 ($CH_2CH_2CH_2$), 42.8 (CNH_2), 65.0 (CO), 129.3 (*meta*-Ph*C*), 131.3 (*para*-Ph*C*), 135.3 (Ph*C*–Si), 137.1 (*ortho*-Ph*C*). LRMS (ES^+): $m/z = 328.2$ $[M + H^+]$. HRMS (ES^+): found: 328.2098 $[M + H]^+$ $C_{20}H_{30}ONSi$ requires 328.2091.

6.1.3. General procedure for the mono-tritylation of diamines

TrtCl (0.1 eq.) dissolved in DCM (2.5 mL/mmol) was added to the diamines in DCM (0.1 mL/mmol) and left stirring

under N_2 at RT overnight. The mixture was poured into 50 mL ice water and extracted with ether (1 × 100 mL, 2 × 50 mL). The organic layer was washed with water (2 × 50 mL), brine (1 × 50 mL) and dried with $MgSO_4$. The solvent was evaporated off. The product was purified by flash chromatography.

*6.1.3.1. Synthesis of 2-(triphenylmethylamino) ethylamine (**4a**).* Yield: 51%. R_f: 0.6 in CH_2Cl_2/MeOH/NH_3, 90:7:3. ^{1}H NMR (300 MHz, MeOD) δ 2.14 (t, $J = 6.19$, 2H, CH_2NHTrt), 2.64 (t, $J = 6.26$, 2H, CH_2NH$_2$), 7.19 (m, 3H, *para*-Ph*H*), 7.29 (m, 6H, *meta*-Ph*H*), 7.5 (m, 6H, *ortho*-Ph*H*). ^{13}C NMR (75 MHz, MeOD) δ 43.4 (*C*H$_2$NH$_2$), 47.6 (*C*H$_2$NHTrt), 83.3 (Trt quat. C), 127.7 (*para*-Ph*C*), 129.1 (*ortho*-Ph*C*), 129.7 (*meta*-Ph*C*), 147.9 (Ph quat. *C*). LRMS (ES$^+$): $m/z = 303$ [M + H$^+$]. HRMS (ES$^+$): found: 303.1853 [MH$^+$] $C_{21}H_{23}N_2^+$ requires 303.1856.

*6.1.3.2. Synthesis of 3-tritylamino propylamine (**4b**).* Yield: 20%. R_f: 0.4 in CH_2Cl_2/MeOH/NH_3, 92:6.4:1.6. ^{1}H NMR (300 MHz, MeOD) δ 1.63 (qn, $J = 7.05$, 2H, CH$_2$C*H*$_2$CH$_2$), 2.15 (t, $J = 6.96$, 2H, CH_2NHTrt), 2.67 (t, $J = 7.27$, 2H, CH$_2$NH$_2$), 7.15 (m, 3H, *para*-PhH), 7.24 (m, 6H, *meta*-PhH), 7.42 (m, 66*ortho*6H, *ortho*-PhH). ^{13}C NMR (75 MHz, MeOD) δ 35.1 (CH$_2$*C*H$_2$CH$_2$), 41.4 (*C*H$_2$NH$_2$), 43.3 (*C*H$_2$NHTrt), 72.6 (Trt quat. C), 127.7 (*para*-Ph*C*), 129.1 (*ortho*-Ph*C*), 130.3 (*meta*-Ph*C*), 147.9 (Ph quat. C). LRMS (ES$^+$): $m/z = 317$ [M + H$^+$]. HRMS (ES$^+$): found: 317.2015 [MH$^+$] $C_{22}H_{25}N_2^+$ requires 317.2012.

*6.1.3.3. Synthesis of 4-(triphenylamino) butylamine (**4c**).* Yield: 31%. R_f: 0.3 in CH_2Cl_2/MeOH/NH_3, 90:7:3. ^{1}H NMR (300 MHz, MeOD) δ 1.51 (m, 4H, CH$_2$C*H*$_2$C*H*$_2$CH$_2$), 2.15 (d, $J = 6.76$, 2H, CH_2NHTrt), 2.60 (d, $J = 6.79$, 2H, CH_2NH$_2$), 7.17 (m, 3H, *para*-PhH), 7.26 (m, 6H, *meta*-PhH), 7.47 (m, 6H, *ortho*-PhH). ^{13}C NMR (75 MHz, MeOD) δ 29.3 (*C*H$_2$), 31.7 (*C*H$_2$), 42.8 (*C*H$_2$NH$_2$), 45.2 (*C*H$_2$NHTrt), 72.6 (Trt quat. C), 127.7 (*para*-Ph*C*), 129.1 (*ortho*-Ph*C*), 130.3 (*meta*-Ph*C*), 147.9 (Ph quat. C). LRMS (ES$^+$): $m/z = 331$ [M + H$^+$]. HRMS (ES$^+$): found: 331.2005 [M + H]$^+$ $C_{23}H_{27}N_2^+$ requires 331.2169.

6.1.4. General procedure for EDC mediated coupling to form amide bonds

1-Carboxyuracil **1** (1.3 eq.) and EDC (1.4 eq.) dissolved in DMF (2 mL/mmol) was added to the amine (1 eq.) under N_2 or Ar atmosphere. The reaction was left at RT, usually overnight. The solvent was evaporated off and the crude purified by column chromatography.

*6.1.4.1. Synthesis of N-[3-(tert-butyl diphenyl silanyloxy)-propyl]-2-uracil acetamide (**3a**).* Yield: 61%. Melting point: 186 °C. R_f: 0.5 in CH_2Cl_2/MeOH, 90:10. ^{1}H NMR (300 MHz, MeOD) δ 1.05 (s, 9H, CH_3 × 3), 1.79 (qn, $J = 6.63$, 2H, CH$_2$C*H*$_2$CH$_2$), 3.37 (m, 2H, CH_2NH$_2$), 3.75 (t, $J = 6.11$, 2H, CH_2O), 4.35 (s, 2H, COCH_2uracil), 5.67 (d, $J = 7.9$, 1H, =C*H*), 7.40 (m, 6H, *meta*,*para*-Ph*H*), 7.42 (d, $J = 7.44$, 1H, =C*H*), 7.68 (m, 4H, *ortho*-Ph*H*). ^{13}C NMR (75 MHz, DMSO) δ 18.8 (3 × *C*H$_3$), 26.6 (*C*–Si), 32.0 (CH$_2$*C*H$_2$CH$_2$), 45.1 (*C*H$_2$NH), 49.4 (CO*C*H$_2$uracil), 61.2 (*C*H$_2$O), 100.4 (*C*=C), 127.9 (*para*-Ph*C*), 129.8 (*meta*-Ph*C*), 133.2 (*ortho*-Ph*C*), 135.0 (Si–Ph*C*), 146.6 (C=*C*), 151.0, (N*C*ONH), 163.9 (C*C*ONH), 166.5 (NH*C*OCH$_2$). LRMS (ES$^-$): $m/z = 464.0$ [M − H$^+$]. HRMS (ES$^+$): found: 466.2159 [M + H$^+$] $C_{25}H_{32}O_4N_3Si_1^+$ requires 466.2157. Analysis: found: C, 64.35; H, 6.75; N, 8.86%; calc. for $C_{25}H_{31}N_3O_4Si \cdot 0.1$ mol H_2O: C, 64.24; H, 6.73, N, 8.99%.

6.1.4.2. Synthesis of N-[4-(tert-butyl diphenyl silanyloxy)-butyl]-2-uracil acetamide (3b). Yield: 68%. Melting point: 174 °C. R_f: 0.5 in CH_2Cl_2/MeOH, 9:1. ^{1}H NMR (300 MHz, MeOD) δ 1.05 (s, 9H, CH_3 × 3), 1.63 (m, 4H, CH$_2$C*H*$_2$C*H*$_2$CH$_2$), 3.23 (t, $J = 6.53$, 2H, CH_2NH$_2$), 3.71 (t, $J = 5.77$, 2H, CH_2O), 4.41 (s, 2H, COCH_2uracil), 5.65 (d, $J = 7.65$, 1H, =C*H*), 7.40 (m, 6H, *meta*,*para*-PhH), 7.51 (d, $J = 7.87$, 1H, =C*H*), 7.66 (m, 4H, *ortho*-PhH). ^{13}C NMR (75 MHz, MeOD) δ 20.4 (Me$_3$*C*–Si), 27.8 (*C*H$_3$ × 3), 30.3 (CH$_2$*C*H$_2$CH$_2$), 31.3 (CH$_2$*C*H$_2$CH$_2$), 40.8 (*C*H$_2$NH$_2$), 51.6 (*C*H$_2$uracil), 65.0 (*C*H$_2$O), 102.6 (*C*=C), 129.2 (*meta*-Ph*C*), 131.2 (*para*-Ph*C*), 135.4 (Ph*C*–Si), 137.0 (*ortho*-Ph*C*), 148.3 (C=C), 153.1 (N*C*ONH), 156.7 (C*C*ONH), 169.5 (NH*C*OCH$_2$). LRMS (ES$^-$): $m/z = 478$ [M − H$^+$]. HRMS (ES$^+$): found: 480.2306 [M + H$^+$] $C_{26}H_{34}O_4N_3Si_1^+$ requires: 480.2313 [MH]. Analysis: found: C, 64.43; H, 6.92; N, 8.69%; calc. for $C_{26}H_{33}O_4N_3Si \cdot 0.2$ mol H_2O: C, 64.62; H, 6.97; N, 8.70%.

*6.1.4.3. Synthesis of 1-[N-(2-triphenylmethylaminoethyl)-acetamide] uracil (**5a**).* Yield: 33%. Melting point: 219 °C. R_f: 0.4 in CH_2Cl_2/MeOH, 95:5. ^{1}H NMR (300 MHz, MeOD) δ 2.28 (t, $J = 6.30$, 2H, CH_2NHTrt), 3.37 (t, $J = 6.33$, 2H, CH_2NHCO), 4.43 (s, 2H, CH_2CO), 5.66 (d, $J = 7.86$, 1H, =C*H*), 7.18 (m, 1H, =C*H*), 7.18 (m, 3H, *para*-PhH), 7.29 (m, 6H, *meta*-PhH), 7.45, (m, 6H, *ortho*-PhH). ^{13}C NMR (75 MHz, MeOD) δ 41.4 (*C*H$_2$NHTrt), 45.5 (*C*H$_2$NHCO), 50.3 (*C*H$_2$uracil), 70.5 (Trt quat. C), 100.8 (*C*=C), 126.4 (*ortho*-Ph*C*), 128.1 (*para*-Ph*C*), 128.7 (*meta*-Ph*C*), 145.4 (C=*C*), 146.4 (Ph quat. C), 164.3 (C*C*ONH), 167.2 (NH*C*OCH$_2$). LRMS (ES$^-$): $m/z = 453$ [M − H$^+$]. HRMS (ES$^-$): found: 453.1940 [M + H$^+$] $C_{27}H_{27}N_4O_3$ requires 453.1927.

*6.1.4.4. Synthesis of 1-[N-(3-triphenylmethylaminopropyl)-acetamide] uracil (**5b**).* Yield: 42%. Melting point: 189 °C. R_f: 0.6 in CH_2Cl_2/MeOH, 90:10. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62 (m, 2H, CH$_2$C*H*$_2$CH$_2$), 2.13 (m, 2H, CH_2NHTrt), 3.31 (m, 2H, CH_2NHCO), 4.18 (s, 2H, CH$_2$CO), 5.61 (d, $J = 7.76$, 1H, =C*H*), 7.19 (m, 1H, =C*H*), 7.14 (m, 3H, *para*-PhH), 7.22 (m, 6H, *meta*-PhH), 7.40, (m, 6H, *ortho*-PhH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.6 (CH$_2$*C*H$_2$CH$_2$), 38.8 (*C*H$_2$NHTrt), 41.6 (*C*H$_2$NHCO), 51.1 (*C*H$_2$uracil), 71.4 (Trt quat. C), 102.9 (*C*=C), 126.8 (*para*-Ph*C*), 128.3 (*ortho*-Ph*C*), 129.1 (*meta*-Ph*C*), 145.7 (C=*C*), 146.4 (Ph quat. C), 151.7 (HN*C*ONH), 164.5 (C*C*ONH), 166.6 (HN*C*OCH$_2$). LRMS (ES$^+$): $m/z = 469$ [M + H$^+$]. HRMS (ES$^+$): found: 469.2239 [M + H$^+$]

$C_{28}H_{29}N_4O_3^+$ requires 469.2240. Analysis: found: C, 69.16; H, 5.91; N, 11.43%; calc. for $C_{28}H_{28}N_4O_3 \cdot 1.0H_2O$: C, 69.12; H, 6.21; N, 11.51%.

*6.1.4.5. Synthesis of 1-[N-(3-triphenylmethylaminobutyl)-acetamide] uracil (**5c**).* Yield: 26%. Melting point: 180 °C. R_f: 0.3 in CH_2Cl_2/MeOH, 90:10. ^{1}H NMR: (300 MHz, $CDCl_3$) δ 1.57 (m, 4H, $CH_2CH_2CH_2CH_2$), 2.19 (m, 2H, CH_2NHTrt), 3.27 (m, 2H, CH_2NHCO), 4.29 (s, 2H, CH_2CO), 5.75 (d, $J = 7.90$, 1H, =C*H*), 7.25 (m, 1H, =C*H*), 7.23 (m, 3H, *para*-Ph*H*), 7.32 (m, 6H, *meta*-Ph*H*), 7.50 (m, 6H, *ortho*-Ph*H*). ^{13}C NMR: (75 MHz, $CDCl_3$) δ 27.6, 28.6 ($CH_2CH_2CH_2CH_2$), 40.4 (CH_2NHTrt), 43.6 (CH_2NHCO), 51.3 (CH_2CO), 71.3 (Trt quat. C), 102.9 (*C*=C), 126.7 (*para*-Ph*C*), 128.2 (*ortho*-Ph*C*), 129.0 (*meta*-Ph*C*), 145.6 (C=*C*), 146.5 (quat. Ph−*C*), 151.5 (HN*C*ONH), 164.1 (C*C*ONH), 166.4 (HN*C*OCH_2). LRMS (ES^+): $m/z = 483$ [M + H$^+$]. HRMS (ES^+): found: 483.2396 [M + H$^+$] $C_{29}H_{31}N_4O_3^+$ requires 483.2391. Analysis: found: C, 71.35; H, 6.23; N, 11.37%; calc. for $C_{29}H_{30}N_4O_3 \cdot 0.3H_2O$: C, 71.38; H, 6.32; N, 11.48%.

*6.1.5. Synthesis of benzyl 1H-imidazole-1-carboxylate (**6**) [11]*

Benzyl chloroformate (19.92 g, 117.23 mmol) was cooled to 0 °C in an ice bath. Imidazole (15.96 g, 234.47 mmol) in DCM (70 mL) was added slowly. The reaction was left at RT for 15 min. The mixture was washed with citric acid (3 × 150 mL) and the organic layer was dried to give the pure product as a colourless oil (20.60 g). The product could not be detected by ES MS as it is more than likely too unstable to the ionisation conditions.

Yield: 87%. Melting point: 41 °C. R_f: 0.7 in CH_2Cl_2/MeOH 90:10. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39 (s, 2H, CH_2), 7.02 (m, 1H, =C*H*), 7.34−7.43 (m, 5H, Ph*C*H), 7.49 (m, 1H, =C*H*), 8.20 (m, 1H, C*H*). ^{13}C NMR (125 MHz, $CDCl_3$) δ 70.3 (CH_2), 117.6 (N*C*H=), 128.8−129.6 (Ph*C*), 131.1 (N*C*H=), 134.4 (quat. Ph−C), 137.6 (N*C*H=), 149.1 (O*C*ON).

6.1.6. General procedure for carbobenzoxy protection of terminal NH groups of triamines

The relevant triamine (1 eq.) and DMAP (0.2 eq.) were dissolved in DCM (0.7 mL/mmol). Compound **6** (2 eq.) in DCM (1 mL/mol) was added under N_2 atmosphere. The reaction was left stirring overnight at 50 °C. The solvent was evaporated off under reduced pressure and the product purified by flash chromatography.

*6.1.6.1. Synthesis of N^1,$N^{1'}$-(dicarbobenzoxy) diethylene triamine (**7a**).* Yield: 35%. Melting point: 53−55 °C. R_f: 0.9 in CH_2Cl_2/MeOH/NH_3, 80:18:2. ^{1}H NMR (500 MHz, MeOD) δ 2.64 (t, $J = 6.2$, 4H, 2 × CH_2NH), 3.19 (t, $J = 6.2$, 4H, 2 × CH_2NHCbZ), 5.01 (s, 4H, 2 × CH_2CbZ), 7.28 (m, 10H, PhC*H*). ^{13}C NMR (125 MHz, MeOD) δ 41.1, (CH_2NH), 49.5 (CH_2NHCbZ), 67.5 (CH_2CbZ), 128.5−129.5 (Ph*C*H), 138.3 (quat. Ph−C), 158.9 (NH*C*OO). LRMS (ES^+): $m/z = 372.2$ [M + H]$^+$.

*6.1.6.2. Synthesis of $N^1N^{1'}$-(dicarbobenzoxy) di (n-propyl) triamine (**7b**).* Yield: 38%. Melting point: 208 °C. R_f: 0.4in CH_2Cl_2/MeOH, 75:25. ^{1}H NMR (500 MHz, MeOD) δ 1.85 (qn, $J = 6.9$, 4H, 2 × $CH_2CH_2CH_2$), 2.94 (t, $J = 7.5$, 4H, 2 × CH_2NH), 3.25 (t, $J = 6.5$, 4H, 2 × CH_2NHCbZ), 5.10 (s, 4H, 2 × CH_2CbZ), 7.36 (m, 10H, PhCH). ^{13}C NMR (125 MHz, MeOD) δ 26.4 ($CH_2CH_2CH_2$), 38.8 (CH_2NH), 46.2 (CH_2NHCbZ), 65.9 (CH_2O), 126.6−128.8 (Ph*C*H), 137.0 (quat. Ph−C), 157.5 (NH*C*OO). LRMS (ES^+): $m/z = 399.4$ [M + H]$^+$.

*6.1.7. Synthesis of 1-carboxymethyluracil succinimidyl ester (**8**) [12]*

1-Carboxymethyl uracil **1** (2.95 g, 17.35 mmol) and *N*-hydroxysuccinimide (2.21 g, 19.09 mmol) were dissolved in DMF (20 mL) and cooled to 0 °C in an ice bath. A solution of DCC (3.94 g, 19.09 mmol) in DMF (5 mL) was added to the mixture under N_2 atmosphere. The solution was left stirring at 0 °C for 3 h and then at RT for a further 24 h. The pure product was precipitated from MeOH to yield a white solid (4.70 g). The product could not be detected by ES MS as it was too unstable to ionisation.

Yield: quantitative. Melting point: 218−222 °C. R_f: 0.2 in CH_2Cl_2/MeOH/NH_3, 80:18:2. ^{1}H NMR (500 MHz, DMSO) δ 2.83 (s, 4H, CH_2succ), 5.02 (s, 2H, CH_2uracil), 5.85 (d, $J = 7.9$, 1H, =C*H*), 7.74 (d, $J = 7.9$, 1H, =C*H*). ^{13}C NMR (125 MHz, DMSO) δ 25.8 (2 × CH_2succ), 46.9 (CH_2uracil), 102.1 (=*C*), 145.6 (=*C*), 151.0 (N*C*ONH), 164.0 (C*C*ONH), 165.3 (CH_2*C*ON), 170.1 (2 × *C*Osucc).

*6.1.8. Synthesis of 1-[$N^{11}N^{11'}$-(dicarbobenzoxy) diamino diethylacetamide] uracil (**9a**)*

Compound **7a** (1.53 g, 4.12 mmol) was dissolved in DMF (5 mL). 1-Carboxymethyluracil (700 mg, 4.12 mmol) in DMF (5 mL) was added under N_2 atmosphere, followed by DhbtOH (770 mg, 4.74 mmol) in DMF (5 mL). The solution was cooled to 0 °C and DCC (1.02 g, 4.95 mmol) in DMF (5 mL) was added under N_2 atmosphere. The mixture was left stirring at 0 °C for 1 h and then at RT for 48 h after which time a precipitate had formed. The solvent was evaporated off under reduced pressure and DCU was precipitated out using EtOAc. On addition of diethyl ether to the EtOAc solution, a further gel precipitate formed. This was collected by decantation. On evaporation of the remaining solvent, a foamy white solid formed and identified as the pure product (783 mg).

Yield: 36%. R_f: 0.4 in CH_2Cl_2/MeOH, 90:10. ^{1}H NMR (500 MHz, MeOD) δ 3.25−3.30 (m, 4H, 2 × CH_2NHCbZ), 3.40−3.45 (m, 4H, 2 × CH_2NHuracil), 4.57 (s, 2H, CH_2uracil), 5.10 (m, 4H, 2 × CH_2CbZ), 5.65 (d, $J = 7.9$, 1H, =C*H*), 7.25−7.39 (m, 11H, PhCH + =CH). ^{13}C NMR (125 MHz, MeOD) δ 35.0, 39.4, 40.0 (CH_2NH), 49.9 (CH_2uracil), 67.6 (CH_2CbZ), 102.2 (=C), 129.0−130.0 (Ph*C*H), 147.9 (=C), 154.7, 159.0 (quat. CO). LRMS

(ES$^+$): $m/z = 524$ $[M+H]^+$. HRMS (ES$^+$): found: 546.1953 $[M+Na]^+$ $C_{26}H_{29}N_5O_7Na^+$ requires 546.1965.

6.1.9. Synthesis of 1-[$N^{12}N^{12'}$-(dicarbobenzoxy) diaminodi (n-propyl) acetamide] uracil (**9b**)

Compound **7b** (1.67 g, 6.26 mmol) and DMAP (10% w/w) were dissolved in DMF (10 mL). Compound **8** (2.50 g, 6.26 mmol) was added under N_2 atmosphere. The reaction was heated to 60 °C and left stirring for 4 d. The solvent was removed *in vacuo*. White crystals were recrystallised from EtOAc and were washed with H_2O (10 mL) to yield the pure product (2.37 g).

Yield: 69%. Melting point: 141–144 °C. R_f: 0.4 in CH_2Cl_2/MeOH, 90:10. ^{1}H NMR (500 MHz, MeOD) δ 1.73, 1.88 (qn, $J = 6.7$, $J = 6.9$, 4H, $2 \times CH_2CH_2CH_2$), 3.11, 3.20 ($2 \times$ t, $J = 6.49$, 4H, CH_2NH), 3.38 (m, 4H, $2 \times CH_2NHCbZ$), 4.58 (s, 2H, CH_2uracil), 5.08 (m, 4H, $2 \times CH_2CbZ$), 5.67 (d, $J = 7.8$, 1H, =C*H*), 7.28–7.35 (m, 10H, Ph*CH*), 7.43 (d, $J = 7.8$, =C*H*). ^{13}C NMR (125 MHz, MeOD) δ 27.2, 28.2 ($2 \times CH_2CH_2CH_2$), 37.6 (CH_2NHCbZ), 43.3, 44.3 (CH_2NHuracil), 48.5 (CH_2uracil), 65.9 (CH_2CbZ), 100.6 (=C), 127.3–128.0 (Ph*CH*), 137.0 (quat. Ph–C), 146.6 (=C), 151.4 (NH*C*ONH), 157.4 (NH*C*OO), 165.4 (HC*C*ONH), 167.2 (H_2CCON). LRMS (ES$^+$): $m/z = 552.4$ $[M+H]^+$.

6.1.10. General procedure for the removal of the CbZ protecting group by hydrogenation

5% Pd/C (0.1 eq. w/w) was pre-activated with hydrogen in a round-bottomed flask using a hydrogen balloon. The relevant protected amine dissolved in either MeOH or a 1:1 mixture of MeOH:EtOH was added. The mixture was flushed with hydrogen three times using a hydrogen balloon and finally left stirring under hydrogen until the disappearance of the starting material was observed by TLC. The catalyst was removed by filtration after flushing the system with N_2.

*6.1.10.1. Synthesis of 1-[diaminodiethylacetamide] uracil (**10a**).* Yield: 41%. R_f: 0.2 in CH_2Cl_2/MeOH/NH_3, 80:16:4. ^{1}H NMR (500 MHz, MeOD) δ 2.70–2.97 (m, 4H, CH_2NH_2), 3.30–3.59 (m, 4H, CH_2Nuracil), 4.46, 4.75 ($2 \times$ s, 2H, CH_2uracil), 5.70 (m, 1H, =CH), 7.53 (m, 1H, =CH). ^{13}C NMR (175 MHz, MeOD) δ 39.8–41.2 (CH_2NH_2), 49.1–51.3 (CH_2Nuracil + CH_2uracil), 102.3 (=C), 147.9 (=C). LRMS (ES$^+$): $m/z = 256$ $[M+H]^+$. HRMS (ES$^+$): found: 256.1400 $[M+H]^+$ $C_{10}H_{18}N_5O_3^+$ requires 256.1404.

*6.1.10.2. Synthesis of 1-[diaminodi (n-propyl) acetamide] uracil (**10b**).* Yield: 35%. R_f: 0.2 in CH_2Cl_2/MeOH/NH_3, 80:16:4. ^{1}H NMR (500 MHz, MeOD) δ 1.78–1.94 (m, 4H, $CH_2CH_2CH_2$), 2.70–2.83 (m, 4H, CH_2NH_2), 3.40–3.51 (m, 4H, CH_2Nuracil), 4.34, 4.72 ($2 \times$ s, 2H, CH_2uracil), 5.69 (m, 1H, =CH), 7.51 (m, 1H, =CH). ^{13}C NMR (175 MHz, MeOD) δ 25.1–26.0 ($CH_2CH_2CH_2$), 38.1–38.8 (CH_2NH_2), 45.1–45.7 (CH_2Nuracil), 48.5 (CH_2uracil), 101.2 (=C), 147.6 (=C), 150.4 (N*C*ONH), 165.4 (NH*C*OCH), 167.2 (N*C*OCH_2). LRMS (ES$^+$): $m/z = 284$ $[M+H]^+$. HRMS (ES$^+$): found: 284.1715 $[M+H]^+$ $C_{12}H_{22}N_5O_3^+$ requires 284.1717.

6.1.11. Synthesis of 1-[N-(triphenylmethylaminoethyl)-N-(aminoethyl) acetamide] uracil (**11a**)

A suspension of **10a** (100 mg, 0.39 mmol) in DCM (30 mL) was placed under N_2 atmosphere and Et_3N (80 mg, 0.78 mmol) in DCM (2 mL) was added followed by TrtCl (110 mg, 0.39 mmol) in DCM (10 mL). The solution which became cloudy was left stirring at RT for 2 h. The solvent was removed *in vacuo* and both mono- and ditritylated products were observed by mass spectrometry. The crude was washed with diethyl ether and water and purified by flash chromatography (CH_2Cl_2/MeOH/NH_3, 100:0:0 → 80:18.6:0.4). The product was collected as a white solid (11 mg).

Yield: 6%. R_f: 0.5 in CH_2Cl_2/MeOH/NH_3, 85:14:1. ^{1}H NMR (500 MHz, MeOD) δ 2.39 (m, 2H, CH_2NHTrt), 2.88 (m, 4H, $2 \times CH_2NHCH_2$), 3.42 (m, 2H, CH_2NHuracil), 4.45 (s, 2H, CH_2uracil), 5.67 (d, $J = 7.87$, 1H, =C*H*), 7.18–7.50 (m, 16H, Ph*CH* + =C*H*). ^{13}C NMR (175 MHz, MeOD) δ 30.7 (CH_2NHTrt), 39.1, 43.2 (CH_2NHCH_2), 51.4 (CH_2NHuracil), 54.8 (CH_2uracil), 72.1 ($2 \times$ quat. Trt C), 102.4 (=C), 127.5–129.9 (Ph–CH), 147.8 (=C^6), 147.2 (quat. Ph C), 147.2 (N*C*ONH), 170.2 (NH*C*OCH_2). LRMS (ES$^+$): $m/z = 498$ $[M+H]^+$. HRMS (ES$^+$): found: 498.2498 $[M+H]^+$ $C_{29}H_{32}N_5O_3^+$ requires 498.2500.

6.1.12. Synthesis of 1-[N-(triphenylmethyl) diaminodi (n-propyl) acetamide] uracil (**11b**)

Compound **10b** (140 mg, 0.49 mmol) and Et_3N (0.03 mg, 0.25 mmol) were dissolved in pyridine (50 mL). TrtCl (138 mg, 0.49 mmol) in pyridine (10 mL) was added over 3 h. After 1 h the solution had begun to turn cloudy. After addition had ceased the mixture was heated to 70 °C, left stirring for 2 d and monitored periodically by TLC. The solvent was evaporated off under reduced pressure. The remaining starting material was removed by precipitation firstly from EtOH (10 mL) and subsequently EtOAc (10 mL). The remaining filtrate was purified by flash chromatography (CH_2Cl_2/MeOH/NH_3, 100:0:0 → 97:2.4:0.6) to yield **11b** (45 mg) as a yellow crystalline solid.

Yield: 17%. Melting point: 103–105 °C. R_f: 0.1 in CH_2Cl_2/MeOH/NH_3, 85:14:1. ^{1}H NMR (500 MHz, MeOD) δ 1.75–1.80 (m, 4H, $2 \times CH_2CH_2CH_2$), 2.23–2.27 (m, 2H, CH_2NHTrt), 2.75–2.81 (m, 4H, $2 \times CH_2NH$), 3.37–3.51 (m, 2H, CH_2NHuracil), 4.41 (s, 2H, CH_2uracil), 5.67 (d, $J = 7.9$, 1H, =CH), 7.19–7.52 (m, 16H, Ph–CH + =CH). ^{13}C NMR (175 MHz, MeOD) δ 29.2, 30.0 ($2 \times CH_2CH_2CH_2$), 38.0 (CH_2NHTrt), 43.1 (CH_2NHCH_2), 58.6 (CH_2NHuracil), 51.5 (CH_2uracil), 72.3 (quat. Trt-C), 102.3 (=C), 127.3–129.9 (Ph–CH), 147.9 (=C), 147.4 (quat. Ph–C), 169.8 (NH*C*OCH_2). LRMS (ES$^+$): $m/z = 526$ $[M+H]^+$. HRMS (ES$^+$): found: 548.2639 $[M+Na]^+$ $C_{31}H_{35}N_5O_3Na^+$ requires 548.2632.

*6.1.13. Synthesis of N-(tert-butyl diphenyl silyloxy) ethyl ethylenediamine (**12**)*

The amino alcohol, *N*-(2-hydroxyethyl) ethylenediamine (2.13 mL, 21.07 mmol) was dissolved in pyridine (20 mL), and *tert*-butyl diphenyl silyl chloride (5.50 mL, 21.15 mmol) in pyridine (30 mL) was added. The reaction was left stirring at RT overnight. The solvent was removed *in vacuo* and the crude product was purified by column chromatography (CH_2Cl_2/MeOH/NH_3, 100:0:0 → 90:8:2) to yield the pure product as a yellow oil (2.27 g). Yield: 32%. R_f: 0.7 in CH_2Cl_2/MeOH/NH_3, 80:18:2. ^{1}H NMR (250 MHz, MeOD) δ 1.08 (s, 9H, CH_3*t*-Bu), 2.76, 2.80, 2.84 (3 × t, $J = 6.2$, $J = 5.7$, $J = 6.00$, 3 × CH_2NH), 3.81 (t, $J = 5.6$, CH_2O), 7.42, 7.71 (m, 10H, PhCH). ^{13}C NMR (125 MHz, MeOD) δ 20.1 (*t*-Bu CH_3), 27.4 (*t*-Bu quat. C), 41.1 (*C*H_2NH_2), 50.5, 52.0 (2 × *C*H_2NH), 64.2 (*C*H_2O), 128.9, 131.1, 136.7 (Ph−*C*H), 134.6 (quat. Ph−C). LRMS (ES^+): $m/z = 343.4$ $[M + H]^+$.

*6.1.14. Synthesis of N-(tert-butyl diphenyl silyloxy) ethyl-N-(triphenylmethylamino) ethyl amine (**13**)*

A solution of **12** (500 mg, 1.46 mmol) in DCM (50 mL) was cooled to 0 °C under N_2 atmosphere. TrtCl (407 mg, 1.46 mmol) in DCM (80 mL) was added slowly. The reaction was brought to RT and Et_3N was added (0.20 mL, 1.46 mmol). The reaction was left stirring at RT overnight over which time the solution turned cloudy. The solvent was removed *in vacuo*. The crude was redissolved in DCM (30 mL), washed with H_2O and dried with $MgSO_4$ to yield a sticky yellow oil (613 mg). This crude product was used for the subsequent synthetic step without further purification. Yield: 72%. R_f: 0.6 in CH_2Cl_2/MeOH/NH_3, 95:4.5:0.5. ^{1}H NMR (500 MHz, MeOD) δ 0.97 (s, 9H, CH_3*t*-Bu), 2.20 (t, $J = 5.65$, 2H, CH_2NHTrt), 2.57, 2.61 (2 × t, $J = 5.6$, $J = 6.0$, 2H, 2 × CH_2NH), 3.70 (t, $J = 5.6$, 2H, CH_2O), 7.05−7.37 (m, 25H, PhCH). ^{13}C NMR (125 MHz, MeOD) δ 20.1 (*t*-Bu quat. C), 27.4 (*t*-Bu CH_3), 44.2 (*C*H_2NHTrt), 50.7, 52.1 (2 × *C*H_2NH), 63.9 (*C*H_2O), 72.1 (Trt quat. C), 127.4, 130.5 (TrtPh*C*H), 128.9, 130.5, 136.7 (TBDPS Ph*C*H), 134.6 (TBDPS quat. Ph−C), 147.5 (TrtPh quat. C). LRMS (ES^+): $m/z = 585$ $[M + H]^+$.

*6.1.15. Synthesis of 1-[N-(tert-butyl diphenyl silyloxy) ethyl-N-(triphenylmethylamino) ethyl-acetamide] uracil (**14**)*

Compound **13** (614 mg, 1.05 mmol) was dissolved in DMF (3 mL) and cooled to 0 °C under N_2 atmosphere. A solution of **1** (180 mg, 1.05 mmol) and DhbtOH (200 mg, 1.21 mmol) in DMF (3 mL) was added while stirring under N_2 atmosphere followed by a solution of DCC (260 mg, 1.25 mmol) in DMF (6 mL). The mixture was left stirring at 0 °C for 1 h and then at RT overnight. The solvent was evaporated off under reduced pressure. The crude was redissolved in DCM (30 mL) and washed with $NaHCO_3$ (3 × 30 mL), NaCl (2 × 30 mL) and dried with $MgSO_4$. The solvent was removed *in vacuo* and the pure product was precipitated from MeOH as a white solid (365 mg). NMR showed splitting of peaks due to restricted rotation about the amide bond. Yield: 52%. R_f: 0.8 in CH_2Cl_2/MeOH, 95:5. ^{1}H NMR (500 MHz, MeOD) δ 1.02, 1.08 (2 × s, 9H, CH_3*t*-Bu), 2.15, 2.22 (2 × m, 2H, CH_2NHTrt), 3.41, 3.47 (2 × m, 4H, CH_2NHuracil), 3.71, 3.85 (2 × m, 2H, CH_2O), 4.58, 4.75 (2 × s, 2H, CH_2uracil), 5.56, 5.66 (2 × d, $J = 7.8$, =CH), 7.20−7.69 (m, 26H, PhCH + =CH). ^{13}C NMR (75 MHz, MeOD) δ 19.8, 20.3 (*t*-Bu quat. C), 26.5, 27.0 (*t*-Bu CH_3), 43.2, 43.6, (*C*H_2NHTrt), 47.3, 48.2 (2 × *C*H_2Nuracil), 50.5, 51.6 (CH_2uracil), 63.7, 64.3 (*C*H_2O), 72.3 (Trt quat. C), 127.4−135.0 (Ph*C*H), 147.5 (TrtPh quat. C), 154.2 (NCONH), 166.5 (NHCOC=), 168.8 (NCOCH_2). LRMS (ES^+): $m/z = 738$ $[M + H]^+$.

*6.1.16. Synthesis of 1-[N-hydroxyethyl-N-(triphenylmethyl amino) ethyl-acetamide] uracil (**15**)*

Compound **14** (365 mg, 0.495 mmol) was dissolved in THF (5 mL) and TBAF in THF (1.0 mL, 1.0 mmol). After 30 min, no starting material could be seen by TLC. The solvent was removed *in vacuo* and purified by flash chromatography (CH_2Cl_2/MeOH, 100:0 → 95:5) and the pure product was isolated as a white solid (200 mg). NMR again showed peak splitting. It was proven, however, by variable temperature NMR that this was due to restricted rotation around the nitrogen bond as *J* C(7)H_2 reduced from 0.4 to 0.1 ppm on heating to 40 °C. Yield: 79%. R_f: 0.25 in CH_2Cl_2/MeOH, 95:5. ^{1}H NMR (500 MHz, MeOD) δ 2.38, 2.41 (2 × t, $J = 6.0$, 2H, CH_2NHTrt), 3.45, 3.55 (2 × m, 4H, CH_2Nuracil), 3.63, 3.73 (2 × t, $J = 6.0$, 2H, CH_2O), 4.78, 4.79 (2 × s, 2H, CH_2uracil), 5.57 (d, $J = 7.9$, 1H, =CH), 7.17−7.48 (m, 16H, PhCH + =CH). ^{13}C NMR (125 MHz, MeOD) δ 43.1, 43.7 (*C*H_2NHTrt), 48.7 (*C*H_2uracil), 50.4, 51.2, (*C*H_2NHuracil), 60.4 (*C*H_2O), 72.6 (Trt quat. C), 102.1 (=C), 127.4, 128.9, 129.9 (Ph*C*H), 147.4 (Ph quat. C), 147.9 (=C), 152.9 (N*C*ONH), 166.9 (HN*C*OC=), 169.7 (N*C*OCH_2). LRMS (ES^+): $m/z = 499$ $[M + H]^+$. HRMS (ES^+): found: 499.2361 $[M + H]^+$ $C_{29}H_{31}N_4O_4^+$ requires 499.2340.

6.2. Biological assays

Methods used for the enzyme assays and parasite assays have been described previously [7–9].

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