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Synthesis and Activity of 5′-Uridinyl Dipeptide Analogues Mimicking the Amino Terminal Peptide Chain of Nucleoside Antibiotic Mureidomycin A

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Abstract—A series of 5′-uridinyl dipeptides were synthesised which mimic the amino terminal chain of nucleoside antibiotic mureido omycin A. Aminoacyl-β-alanyl- and aminoacyl-*N*-methyl-β-alanyl- dipeptides were attached either via an ester linkage to the 5′-hydroxyl of uridine, or via an amide linkage to 5′-amino-5′-deoxyuridine. The most active inhibitor of *Escherichia coli* phospho-MurNAc-pentapeptide translocase (MraY) was 5′-*O*-(L-Ala-*N*-methyl-β-alanyl)-uridine (**13l**), which also showed 97% enzyme inhibition at 2.35 mM concentration, and showed antibacterial activity at 100 μg/mL concentration against *Pseudomonas putida*. Both the central *N*-methyl amide linkage and a 5′ uridine ester linkage were required for highest biological activity. Enzyme inhibition was shown to be competitive with Mg^{2+}. It is proposed that the primary amino terminus of the inhibitor binds in place of the Mg^{2+} cofactor at the MraY active site, positioned via a *cis*-*N*-methyl amide linkage.

Introduction

Mureidomycins A–D (**1**–**4**) are peptidyl nucleoside antibiotics isolated from *Streptomyces flavidovirens* SANK 60486 (see Fig. 1A).[1–3] Their structure consists of a 3′-deoxyuridine nucleoside linked via a 4′,5′-enamide linkage to a peptide chain containing two *meta*-tyrosine residues, one methionine residue, and one *N*-methyl-diaminobutyric acid residue.[2] They show antibacterial activity against strains of *Pseudomonas* (MIC 0.1–3 μg/mL), and have been shown to protect mice against infection by *Pseudomonas aeruginosa* (ED_{50} 50–100 mg/kg).[3] Two closely related families of natural products, the pacidamycins 1–7,[4–6] and the napsamycins A–D,[7] also with selective anti-pseudomonal activity, have been isolated from *Streptomyces coeruleorubidus* AB1183F-64 and *Streptomyces* sp. HIL Y-82, respectively.

The mureidomycins selectively inhibit phospho-MurNAc-pentapeptide translocase (MraY), an enzyme which catalyses the first step of the lipid cycle of bacterial peptidoglycan biosynthesis, namely the reaction of UDPMurNAc-L-Ala-γ-D-Glu-*m*-DAP-D-Ala-D-Ala with lipid carrier undecaprenyl phosphate, to give lipid intermediate I (see Fig. 1B).[8] They show no inhibition of bacterial teichoic acid or mammalian *N*-linked glycoprotein biosynthesis,[9] unlike MraY inhibitor tunicamycin, which is a potent inhibitor of the mammalian phospho-GlcNAc transferase enzyme.[10] Using a continuous fluorescent assay, Brandish et al. have reported previously that mureidomycin A is a slow-binding inhibitor (K_i 35 nM, K_i* 2 nM) of solubilised *Escherichia coli* MraY.[11,12] The molecular basis for the EI to EI* transition during slow-binding inhibition is unknown.

We have previously studied the role of the unusual enamide functional group in the biological activity of mureidomycin A. Although an enamide-containing model compound based on a tetrahydrofuran ring showed high chemical reactivity, a uridine-based enamide analogue showed low chemical reactivity, and showed no inhibition of *E. coli* MraY.[13] In the pacidamycin family, chemical reduction of the enamide has been reported, to give a dihydropacidamycin-D which showed only slightly reduced activity towards MraY.[14]

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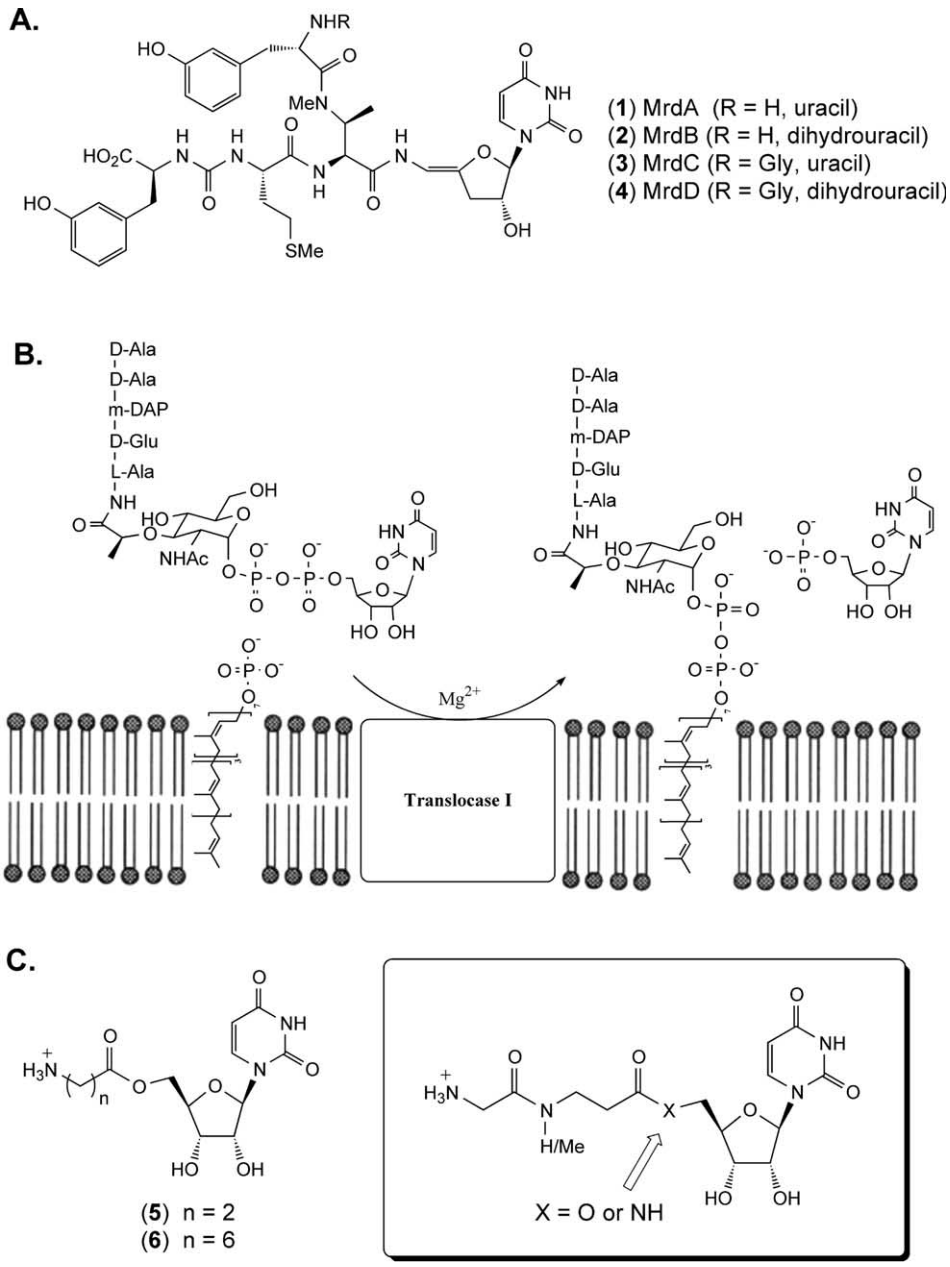


Figure 1. (A) Structures of mureidomycins A–D. Absolute stereochemistry is based on stereochemical elucidation of pacidamycins.[14] (B) Reaction catalysed by phosphoMurNAc-pentapeptide translocase (MraY, translocase I). (C) Structures of target 5′-uridinyl dipeptides.

Thus, the enamide functional group does not appear to be primarily responsible for the biological activity of these compounds.

We have also reported previously that 3-aminopropionyl (**5**) and 7-aminoheptanoyl (**6**) 5′-uridine esters showed some inhibitory activity (IC_{50} 0.26 and 1.5 mM, respectively) towards *E. coli* MraY, suggesting that the amino terminus of the peptide chain may be important for enzyme inhibition.[15] A number of synthetic dihydropacidamycins containing various amino acid sidechains have been reported to show comparable anti-pseudomonal activity and MraY inhibition,[14,16] indicating that the nature of the amino acid sidechains is not critical for activity in this series. However, dihydropacidamycin[17] and dihydromureidomycin[18] analogues lacking the *N*-methyl and *C*-methyl groups of the *N*-methyl-diaminobutryic acid moiety show no biological activity at 100 μg/mL concentration, suggesting an important role for these methyl groups.

In order to investigate the role of the amino-terminal peptide chain of mureidomycin A for MraY inhibition and antibacterial activity, we decided to synthesise a series of 5′-uridinyl dipeptide analogues in which the diaminobutyric acid unit is simplified to β-alanine or *N*-methyl β-alanine (see Fig. 1C). We report the synthesis and biological evaluation of a series of 5′-*O*-(aminoacyl-β-alanyl)-uridine derivatives in which the dipeptide is attached via either an ester or an amide linkage to the 5′-position of uridine.

Results

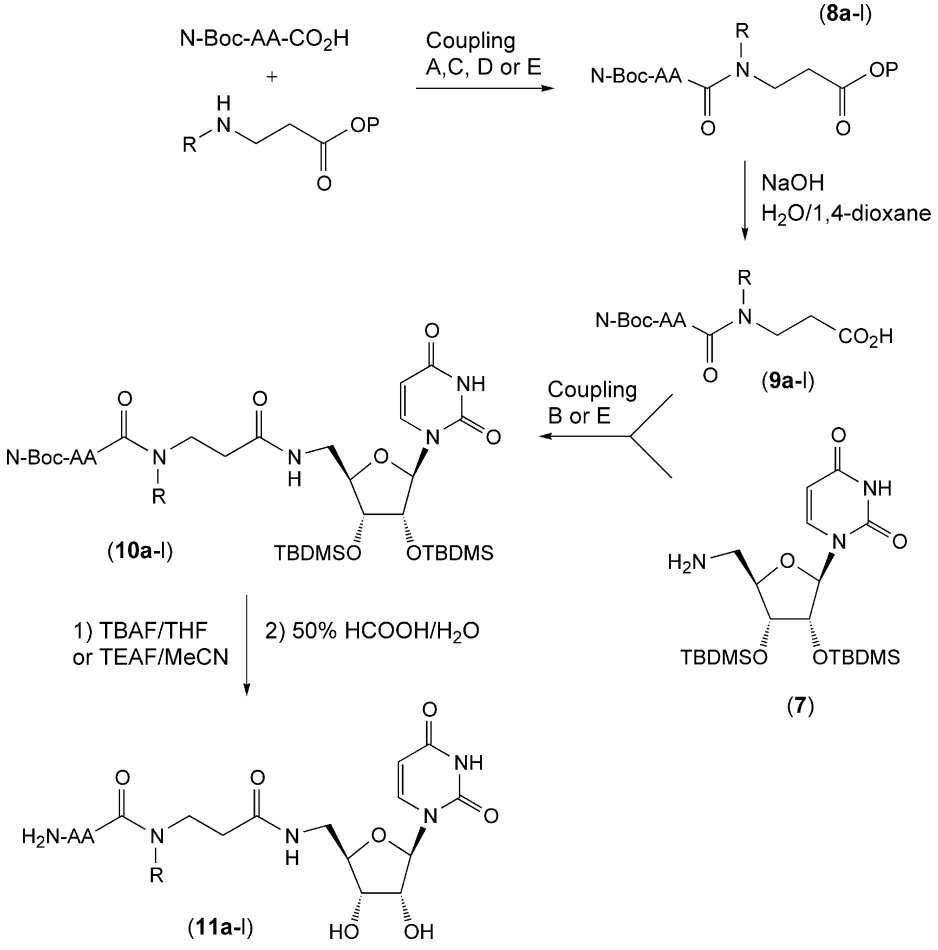
Synthesis of 5′-uridinyl dipeptides

Attachment of the dipeptide via an amide linkage required the preparation of 5′-amino-5′-deoxyuridine. Attempts to couple the known 2′-*O*,3′-*O*-isopropylidene derivative[18] to a carboxylic acid using DCC or mixed anhydride couplings gave in our hands only 10–20% yields of coupled product. The major side product was found to be a secondary amine arising from intramolecular Michael addition of the free amine to C-6 of the uracil base, observed previously in the literature.[19] Therefore, 5′-azido-5′-deoxyuridine was prepared by treatment of uridine with di-isopropyl-azodicarboxylate, triphenylphosphine and HN_3, in 64% yield.[20] Protection of the 2′- and 3′-hydroxyl groups with *t*-butyldimethylsilyl chloride,[21] followed by reduction of the azide using H_2S via the method of Dempcy et al.,[22] gave the known TBDMS-protected amine[21] (**7**) in 80% yield. The TBDMS-protected amine (**7**) was found not to suffer from intramolecular reaction during amide bond couplings.

N-Methyl β-alanine *t*-butyl ester was prepared by addition of *N*-benzylmethylamine to *t*-butyl acrylate,[23] followed by debenzylation via catalytic hydrogenation, in 76% overall yield. The *t*-butyl ester was found to couple satisfactorily to carboxylic acids, whereas the corresponding methyl ester suffered from intramolecular reaction to form β-lactam by-products.

As illustrated in Scheme 1, six *N*-Boc-amino acids (L-Phe, D-Phe, L-Tyr-O′Bu, Gly, L-Ala, β-Ala) were coupled with β-alanine methyl ester and *N*-methyl β-alanine *t*-butyl ester to give 12 protected dipeptides (**8a–l**). Three coupling methods were used: mixed anhydride coupling using isobutyl chloroformate[24] (method A) or isopropenyl chloroformate[25] (method B); formation of the *N*-hydroxysuccinimide active ester by isobutyl chloroformate mixed anhydride coupling (method C) or using isopropenyl succinimido carbonate[26] (method D), followed by coupling; or PyBOP coupling[27] (method E). Coupling of the *N*-methyl amine was problematic using method A, but gave yields of 70–95% using methods C or E. Each of the protected dipeptides was treated with 1 M sodium hydroxide in 1:1 H_2O/dioxane to give the free acids (**9a–l**) in 70–99% yield.

Amide coupling of the 12 free acids to protected 5′-amino-5′-deoxyuridine (**8**) proceeded slowly, presumably due to steric hindrance. Mixed anhydride coupling with isopropenyl chloroformate (method B)

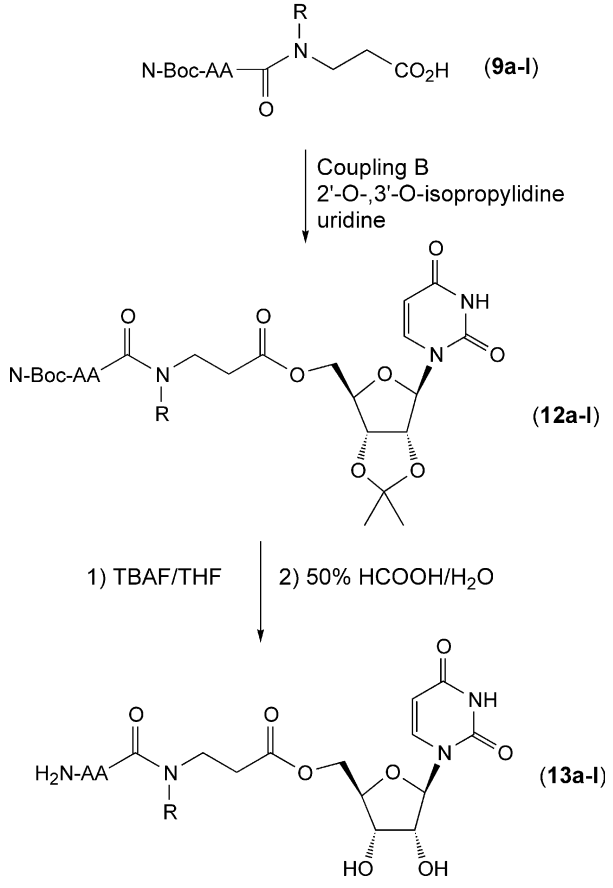


Scheme 1. Synthesis of amide-linked 5′-uridinyl dipeptides. Method A: isobutyl chloroformate, Et_3N; method B: isopentenyl chloroformate, Et_3N; method C: coupling of *N*-hydroxysuccinimide ester to amine; method D: isopropenyl succinimidocarbonate; method E: PyBOP coupling. AA, amino acid (**a**,**b**, L-Phe; **c**,**d**, D-Phe; **e**,**f**, L-Tyr; **g**,**h**, Gly; **i**,**j**, β-Ala; **k**,**l**, L-Ala; see Table 1); R, H (**a**,**c**,**e**,**g**,**i**,**k**) or CH_3 (**b**,**d**,**f**,**h**,**j**,**l**); P, CH_3 or ^{t}Bu.

gave 33–66% yields of **10a–l**; also PyBOP coupling (method E) gave the desired coupled products **10f** and **10k** in 60 and 48%, respectively. Deprotection was achieved by treatment with tetrabutylammonium fluoride (31–71% yield), followed by acidic deprotection in 50% aqueous formic acid (92–99% yield), followed by purification via cation exchange chromatography on Dowex 50W resin, to give the desired products **11a–l** (see Scheme 1).

Ester coupling of the 12 free acids to 2′-*O*,3′-*O*-isopropylidene uridine also proceeded slowly. Best yields were obtained via a mixed anhydride coupling method using isopropenyl chloroformate (method B), which gave 20–74% yields of the 12 protected products **12a–l**, as shown in Scheme 2. Coupling to the 5′-*O* position, rather than the uracil imide N, was confirmed in each case by a shift of the H-5′ ^{1}H NMR signal from 3.6 to 4.2 ppm, and corresponding shifts in the ^{13}C NMR spectra of the coupled products. Deprotection was achieved using 50% aqueous formic acid, in 83–99% yield, to give the desired ester linked products **13a–l**.

From inspection of the ^{13}C NMR spectra for the uridine 5′-dipeptides (see Tables 3–6 in the Experimental), it is apparent that the compounds containing *N*-methyl-β-alanine show duplication of signals, due to *cis* and *trans* rotamers of the *N*-methyl amide linkage. This is especially apparent in the 5′-esters, where there is duplication of signals for ribose carbons (by 0.4–1.0 ppm for **12a–l**, and by 0.2–0.6 ppm for **13a–l**) as well as for β-alanine and amino acid carbon signals. The two observed signals for the *N*-methyl carbon are typically separated by 2.0–3.0 ppm, for example 36.3 and 34.1 ppm in **12l**, and 35.3 and 33.3 ppm in **13l**. No apparent changes were observed when NMR spectra were recorded at 37 °C.



Scheme 2. Synthesis of ester-linked 5′-uridinyl dipeptides. Method B: isopentenyl chloroformate, Et_3N. AA, amino acid (**a**,**b**, L-Phe; **c**,**d**, D-Phe; **e**,**f**, L-Tyr; **g**,**h**, Gly; **i**,**j**, β-Ala; **k**,**l**, L-Ala; see Table 1); R, H (**a**,**c**,**e**,**g**,**i**,**k**) or CH_3 (**b**,**d**,**f**,**h**,**j**,**l**).

Biological evaluation

The compounds were assayed as inhibitors of solubilised *E. coli* phospho-MurNAc-pentapeptide translocase, using a radiochemical assay, monitoring transfer of ^{14}C from ^{14}C-UDPMurNAc-pentapeptide to lipid-linked product.[11,15] Inhibitors were added at 2.35 mM and 235 μM concentrations. The majority of the amide-linked analogues (see Table 1) showed only 15–30% inhibition at 2.35 mM concentration, with **13j** (β-Ala-*N*-Me-β-Ala-) and **13e** (L-Tyr-β-Ala-) showing 43 and 48% inhibition, respectively. The majority of the ester-linked analogues (see Table 1) also showed 15–30% inhibition at 2.35 mM concentration, however **15l** (L-Ala-*N*-Me-β-Ala-) showed 97% inhibition at 2.35 mM.

The latter compound **15l**, which was consistently the most potent enzyme inhibitor, was tested at variable concentrations of $MgCl_2$. As shown in Table 2, at 235 μM inhibitor and 25 mM Mg^{2+}, 8% inhibition was observed, but 33% inhibition was observed at 5 mM Mg^{2+}, consistent with the hypothesis that the amino terminus of MrdA binds in place of the Mg^{2+} cofactor

Table 1. Inhibition (%) of solubilised *E. coli* MraY by 5′-uridinyl dipeptide analogues **11a–l** and **13a–l**[a]

	AA	R	MraY inhibition of amide **11** (%) @ 2.35 mM	MraY inhibition of ester **13** (%) @ 2.35 mM
a	L-Phe	H	27	13
b	L-Phe	Me	NT	15
c	D-Phe	H	22	27
d	D-Phe	Me	NT	8
e	L-Tyr	H	48	24
f	L-Tyr	Me	NT	18
g	Gly	H	NT	40
h	Gly	Me	NT	23
i	β-Ala	H	16	34
j	β-Ala	Me	43	33
k	L-Ala	H	21	24
l	L-Ala	Me	14	97

[a]Assays as described in the Experimental. AA, amino-terminal amino acid; R, beta-alanine N-substituent; NT, not tested.

Table 2. Inhibition (%) of solubilised *E. coli* MraY by analogues **13l** at 5–25 mM concentrations of $MgCl_2$[a]

Concentration of $MgCl_2$ (mM)	Inhibition @ 2.35 mM **13l** (%)	Inhibition @ 235 μM **13l** (%)
5	97	33
12.5	96	28
25	97	8

[a]Assays as described in the Experimental. Data corrected for variation in enzyme activity at 5–25 mM $MgCl_2$.

at the MraY active site.[15] Under the same assay conditions, 235 μM mureidomycin A showed 97% inhibition of MraY at 25 mM $MgCl_2$.

Compounds **11a–l** and **13a–l** were tested for antibacterial activity at 100 μg/mL against *E. coli* K12, *Pseudomonas putida*, *Bacillus subtilis*, and *Streptococcus pneumoniae*, using a filter disk assay on Luria Broth agar plates. The only compound to show antibacterial activity was the L-Ala-*N*-Me-β-Ala-containing analogue **13l**, which showed strong activity (27 mm zone of inhibition) against *P. putida* at 100 μg/mL. The anti-pseudomonal selectivity of this compound matches that of the parent compound mureidomycin A.[3]

Synthesis of conformationally restrained analogues

As described above, highest biological activity was shown by a compound containing the *N*-methyl-amide functional group, also found in mureidomycin A. A plausible hypothesis is that the *N*-methyl amide forms a *cis*–amide bond which positions the amino terminus correctly for enzyme inhibition. As mentioned above, NMR spectra of *N*-methyl-β-alanine-containing analogues show duplication of peaks, corresponding to *cis* and *trans* amide rotamers which interconvert slowly.

In order to examine this hypothesis, conformationally restrained analogues **14–16** were designed, as shown in Scheme 3, in which the amino functional group is held in the desired conformation. Analogue **14**, containing a piperidine-3-carboxylic acid unit attached to the 5′-position of uridine, was synthesised by coupling of *N*-Boc-nipecotic acid to 2′-*O*,3′-*O*-isopropylidene-uridine, followed by acidic deprotection. Analogues **15** and **16** contain a δ-lactam ring formed from L- or D-ornithine, attached via a two-carbon linkage to the 5′-position of uridine. The δ-lactam ring was formed by cyclisation of L- or D-ornithine, using the method of Pellegata et al.[28] and alkylated using ethyl bromoacetate, after Semple et al.[29] After alkaline deprotection, the acids were coupled to 2′-*O*,3′-*O*-isopropylidene-uridine using the isopropenyl chloroformate mixed anhydride method, followed by acidic deprotection.

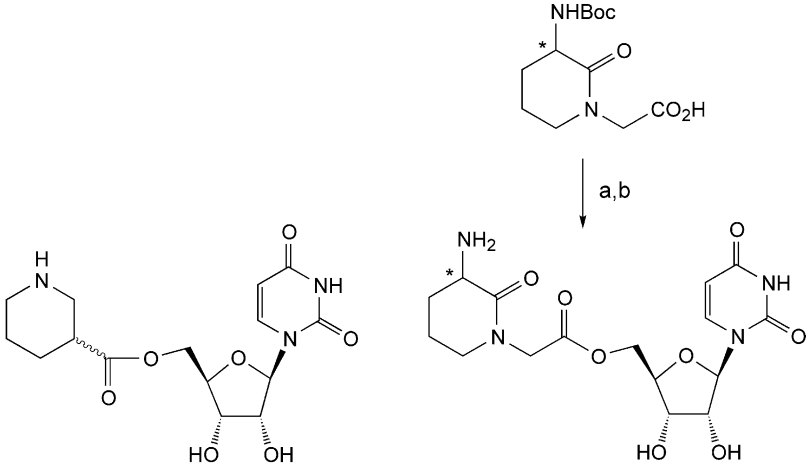
Biological evaluation

Compounds **14–16** were assayed as inhibitors of solubilised *E. coli* phospho-MurNAc-pentapeptide translocase, using the radiochemical assay. They showed 14, 27 and 21% enzyme inhibition respectively at 2.35 mM concentration, thus the conformational restraint has not apparently assisted the biological activity of these compounds. No antibacterial activity was observed for **14–16** at 100 μg/mL against *E. coli* K12, *P. putida*, *B. subtilis*, and *S. pneumoniae*, using a filter disk assay.

Discussion

In order to examine the role of the amino terminal peptide chain of mureidomycin A, we have synthesised a series of 5′-uridinyl dipeptide analogues. Most potent inhibition of MraY is observed with analogue **11l** containing *N*-methyl-β-alanine and an ester linkage to the 5′-position of uridine. Significantly lower activity is observed if either the *N*-methyl amide or the ester linkage is not present, implying that both functional groups are important for biological activity. The same analogue shows anti-pseudomonal activity, with a similar selectivity to the natural product. Enzyme assays at variable Mg^{2+} concentrations indicate that increasing concentrations of Mg^{2+} reduce the potency of enzyme inhibition, consistent with the hypothesis that the amino terminus binds in place of the Mg^{2+} cofactor.[15]

On the basis of these and other literature data, a hypothesis can be made for the molecular mechanism of action of mureidomycin A, illustrated in Figure 2. It has been demonstrated previously that the unusual enamide functional group is not primarily responsible for biological activity.[13,14] It appears that the α-amino terminus



Scheme 3. Synthesis of conformationally restrained analogues **14–16**. Conditions: (a) 2′,3′-isopropylidene uridine, coupling method B; (b) 50% HCO_2H/H_2O.

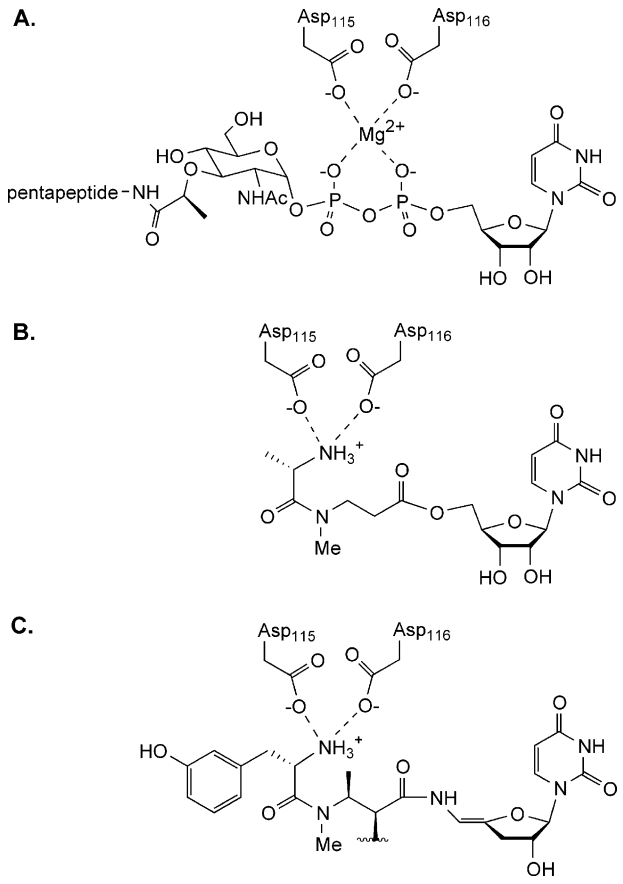


Figure 2. (A) Proposed Mg^{2+} binding site at the active site of MraY. (B) Proposed binding of analogue **13l** to Mg^{2+} binding site of MraY, via *cis*-amide linkage. (C) Binding of mureidomycin A to Mg^{2+} binding site of MraY, via *cis*-amide linkage.

is important for biological activity; thus we propose that the protonated ammonium ion binds in place of the Mg^{2+} cofactor at the MraY active site. The MraY amino acid sequence contains a DDxxD motif at residues 115–119 which, as proposed previously,[15] may form a Mg^{2+} binding site at Asp-115 and Asp-116, as illustrated in Figure 2A. The *N*-methyl amide functional group is also important for biological activity, consistent with observations by other researchers that analogues lacking this group are biologically inactive.[17,18] Since two rotamers are observed in solution by ^{13}C NMR spectroscopy for compounds containing the *N*-methyl amide, we propose that this compound exists partly in a *cis*-amide conformer, whose structure positions the amino terminus in the Mg^{2+} binding site (see Fig. 2B). Our initial attempts to synthesise conformationally restrained analogues have not resulted in increased biological activity; however, in principle this strategy could lead to the design of more potent inhibitors for MraY.

Mureidomycin A could also adopt a *cis*-amide conformer, as shown in Figure 2C. The terminal amino acid in MrdA is *meta*-tyrosine, whereas in this study analogues containing L-Phe (**13b**) or L-Tyr (**13f**) at this position were less active than **13l** containing L-Ala, found in pacidamycin D.[14] Analogues **13i** and **13j** containing β-alanine, matching the length of the acyl chain of 5′-*O*-7-aminoheptanoyl-uridine (**6**) shown previously to inhibit MraY,[15] were also less active than **13l**.

We have found that the chemical nature of the linkage of the dipeptide to the 5′-position of uridine is important for biological activity. Thus, replacement of the 5′-ester linkage of **13l** with a 5′-amide linkage (**13l**) leads to a loss of biological activity. Since the amide linkage is held in a *trans* conformation, but the ester linkage is conformationally flexible, it appears that the ester linkage permits an active conformation which is not possible for the amide. This in turn implies that a (secondary) role of the 5′-enamide linkage in the natural product is to permit the adoption of an active conformation, rather than to promote reactivity.

The molecular basis for the slow-binding inhibition kinetics shown by mureidomycin A has yet to be established, a plausible explanation being a conformational change of the enzyme–inhibitor complex to form the EI* complex. Although these synthetic analogues of MrdA are not potent enzyme inhibitors, they are the first simplified synthetic analogues to show antibacterial activity, thus inhibition of MraY is a realistic target for antibacterial chemotherapy.

Experimental

General

Chemicals were purchased from Acros, Aldrich, Avocado, BDH, Lancaster, Novobiochem, or Sigma Chemical Companies, unless otherwise stated. Flash chromatography was performed using Merck Kieselgel 60 (230–400 mesh). 300 MHz NMR spectra were recorded on either a Bruker AC300 or Bruker DPX 300 Fourier Transform Spectrometer. 2′,3′-Di-*tert*butyldimethylsilyl-5′-amino-5′-deoxyuridine (**7**) was prepared by the route of Peterson et al.,[21] but using hydrogen sulfide gas to reduce 5′-azide substituent as described by Dempcy et al.[22] β-Alanine methyl ester was prepared by treatment of β-alanine with thionyl chloride and methanol, in 89% yield. Isopropenyl succinimido carbonate was prepared by the method of Takeda et al.[26]

Preparation of *N*-methyl β-alanine *tert*-butyl ester. A modification of the method of Leonard and Durand was used.[23] A solution of *tert*-butyl acrylate (14.65 mL, 0.1 mol) in anhydrous methanol (20 mL) was added to a solution of *N*-benzylmethylamine (14.2 mL, 0.11 mol) in anhydrous methanol (20 mL) at 0 °C. After 15 min, the reaction was allowed to warm to room temperature and the solution stirred for 20 h. The solvent was carefully removed under reduced pressure and the resulting oil purified by distillation to give *N*-methyl, *N*-benzyl-β-alanine *tert*-butyl ester as a colourless oil, 22.66 g, 91%. δ_H (300 MHz, $CDCl_3$) 7.25–7.33 (5H, m), 3.51 (2H, s, Ph$\underline{CH_2}$), 2.72 (2H, t, $J = 6.9$ Hz, $C\underline{H_2}N$), 2.46 (2H, t, $J = 6.9$ Hz, $C\underline{H_2}CO$), 2.42 (3H, s, NCH_3), 1.46 (9H, s, ^{t}Bu) ppm δ_C (75 MHz, $CDCl_3$) 172.1, 139.2, 129.1, 128.5, 128.4, 127.1, 80.4, 62.2, 53.2, 42.0, 34.2, 28.3 ppm; *m*/*z* (ES^+) 250.1 (MH^+). 10% Palladium on car-

bon (1.50 g) was suspended in a solution of *N*-phenyl-*N*-methyl-β-alanine-*tert*-butyl ester (15.00 g, 60.16 mmol) and anhydrous methanol (100 mL). The solution was degassed, then saturated with hydrogen gas, and stirred for 2 days. The reaction mixture was filtered through a Celite pad, and solvents carefully removed under reduced pressure. The resulting oil was purified by Kugelrohr distillation to give *N*-methyl-β-alanine-*tert*-butyl ester as a colourless oil, 9.01 g, 84%. δ_H (300 MHz, $CDCl_3$) 2.75 (2H, t, $J=6.9$ Hz, $C\underline{H}_2N$), 2.37 (5H, $C\underline{H}_2CO$ and $NC\underline{H}_3$), 1.91 (1H, br, NH), 1.40 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 172.3, 80.6, 47.3, 36.3, 35.6, 28.2 (^{t}Bu) ppm; *m*/*z* (ES^+) 160.0 (MH^+).

Preparation of *N*-Boc-aminoacyl-β-alanyl esters (8a–l). *N*-Boc-amino acids (L-phenylalanine, D-phenylalanine, L-tyrosine(*O*-^{t}Bu), glycine, β-alanine, or L-alanine) were coupled with either β-alanine methyl ester or *N*-methyl β-alanine *t*-butyl ester, using coupling method A, C, D or E, as described below.

Method A (isobutyl chloroformate mixed anhydride coupling).[24] General method. *N*-^{t}Boc-amino acid (2 mmol) and *N*-methyl morpholine (219 L, 2 mmol) were dissolved in anhydrous THF (10 mL), and the solution cooled to 0 °C. Isobutylchloroformate (286 μL, 2.2 mmol) was added, the resulting suspension was stirred for 20 min, and a solution of β-alanine methyl ester hydrochloride (279 mg, 2 mmol) and *N*-methyl morpholine (219 μL, 2 mmol) in anhydrous DMF (5 mL) was added, and the solution stirred for 30 min at 0 °C, then allowed to warm to room temperature, and stirred for 20–24 h. The solvents were removed in vacuo, and the residue taken up in ethyl acetate (50 mL) and washed with water (25 mL), 5% potassium hydrogen sulphate solution (25 mL), 5% sodium bicarbonate solution (25 mL), water (25 mL), brine (25 mL). The organic phase was dried (Na_2SO_4), concentrated in vacuo, and residue purified by flash column chromatography (3% MeOH/DCM) to give the requisite protected dipeptides.

N-Boc-L-Phe-β-Ala-OMe (**8a**) was prepared by method A, in 91% yield. δ_H (300 MHz, CD_3OD) 7.11–7.22 (5H, m, Ar), 4.21 (1H, t, $J=4.1$ Hz, Phe α-CH), 3.61 (3H, s, OMe), 3.28–3.49 (2H, m, NCH_2), 3.05 and 2.79 (2 × 1H, d, $J=4.1$, 10.2 Hz, Phe β-CH_2), 2.34–2.48 (2H, m, CH_2CO), 1.32 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 174.3, 173.5, 157.5, 138.6, 130.4, 129.4, 127.7, 80.6, 57.4, 52.2, 39.4, 36.2, 34.5, 28.6 ppm; *m*/*z* (ES^+) 351 (MH^+).

N-Boc-D-Phe-β-Ala-OMe (**8c**) was prepared by method A, in 71% yield. δ_H (300 MHz, $CDCl_3$) 7.08–7.22 (5H, m, Ar), 6.68 (1H, br, NH), 5.41 (1H, d, $J=6.23$ Hz, NH), 4.26 (1H, m, Phe α-CH), 3.51 (3H, s, OMe) 3.18–3.48 (2H, m, NCH_2), 2.90 (2H, d, $J=4.2$ Hz, Phe β-CH_2), 2.22–2.45 (2H, m, CH_2CO), 1.31 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 172.8, 171.8, 155.7, 137.2, 129.6, 128.8, 127.1, 80.1, 56.2, 52.0, 39.2, 35.1, 34.0, 28.6 ppm; *m*/*z* (ES^+) 351 (MH^+).

N-Boc-L-Tyr(*O*-^{t}Bu)-β-Ala-OMe (**8e**) was prepared by method A, in 81% yield. δ_H (300 MHz, $CDCl_3$) 7.00 (2H, d, $J=8.3$ Hz, Ar), 6.81 (2H, d, $J=8.2$ Hz, Ar), 6.37 (1H, t, $J=3.6$ Hz, NH), 5.12 (1H, d, $J=4.0$ Hz, NH), 4.17 (1H, br, Tyr α-CH), 3.56 (3H, s, OMe), 3.22–3.47 (2H, m, NCH_2), 2.91 (2H, d, $J=6.2$ Hz, Tyr β-CH_2), 2.28–2.42 (2H, m, CH_2CO), 1.32 (9H, s, ^{t}Bu), 1.24 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 172.9, 171.7, 154.6, 131.9, 130.0, 124.6, 80.3, 78.7, 56.3, 52.1, 38.5, 35.1, 34.0, 29.2, 28.6 ppm; *m*/*z* (FAB) 423 (MH^+).

N-Boc-β-Ala-β-Ala-OMe (**8i**) was prepared by method A, in 60% yield. δ_H (300 MHz, $CDCl_3$) 6.81 (1H, br, NH), 5.40 (1H, br, NH), 3.54 (3H, s, OCH_3), 3.37 (2H, q, $J=6.9$, NCH_2), 3.23 (2H, q, $J=6.9$ Hz, NCH_2), 2.42 (2H, t, $J=6.9$ Hz, CH_2CO), 2.24 (2H, t, $J=6.9$ Hz, CH_2CO), 1.30 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 173.0, 171.9, 156.4, 79.3, 52.0, 36.4, 35.9, 34.6, 33.6, 28.6 ppm; *m*/*z* (CI^+, NH_3) 275 (MH^+).

Method C (*N*-hydroxysuccinimide ester coupling). Preparation of *N*-Boc-L-Phe-*N*-Me-β-Ala-*O*^{t}Bu (8b). ^{t}Boc-L-phenylalanine (265 mg, 1.0 mmol), isopropenyl succinimido carbonate (199 mg, 1.0 mmol) and 4-dimethylaminopyridine (12 mg, 0.1 mmol) were dissolved in 1,4-dioxane (6.0 mL) and stirred at room temperature for 19 h. The solvent was removed in vacuo, and the residue taken up in ethyl acetate (100 mL), and washed with 1 M hydrochloric acid (50 mL), water (50 mL), 5% sodium bicarbonate solution (50 mL), brine (50 mL). The organic phase was dried (Na_2SO_4), and evaporated in vacuo to give Boc-L-Phe *N*-hydroxysuccinimide ester as a white solid (351 mg, 97%). δ_H (300 MHz, $CDCl_3$) 7.15–7.32 (5H, m, Ar), 4.98 (1H, d, $J=4.0$ Hz, NH), 4.83 (1H, q, $J=4.0$ Hz, Phe α-CH), 3.22 and 3.09 (2 × 1H, dd, $J=4.1$, 11.8 Hz, Phe β-CH_2), 2.76 (4H, s, succ-CH_2), 1.31 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 169.3, 168.2, 155.1, 135.2, 130.1, 129.0, 127.7, 80.8, 53.1, 38.4, 28.6, 25.9 ppm. *N*-^{t}Boc-L-phenylalanine-*N*-hydroxysuccinimide ester (362 mg, 1.0 mmol) and *N*-methyl-β-alanine-*tert*-butyl ester (159 mg, 1.0 mmol) were dissolved in acetonitrile (6 mL) and stirred at room temperature for 24 h. The solvent was removed in vacuo, and the residue taken up in ethyl acetate (100 mL) and washed with 1 M hydrochloric acid (50 mL), water (50 mL), 5% sodium bicarbonate solution (50 mL), brine (50 mL). The organic phase was dried (Na_2SO_4), evaporated in vacuo, and the residue taken up in ethyl acetate and purified by flash column chromatography (1:1 ethyl acetate/hexane) to give a white solid, 306 mg, 75%. δ_H (300 MHz, $CDCl_3$) 7.08–7.26 (5H, m, Ar), 5.56 (1H, d, $J=6.2$ Hz, NH), 4.70 (1H, m, Phe α-CH), 3.30–3.60 (2H, m, NCH_2), 2.89 (2H, m, Phe β-CH2), 2.79, 2.66 (2 × s, rotamers, N–CH_3), 2.15–2.40 (2H, m, CH_2CO), 1.26 (9H, s, ^{t}Bu), 1.21 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 171.8, 171.2, 155.3, 136.8, 129.7, 128.6, 127.1, 80.9, 79.6, 51.7, 44.8, 40.1, 35.9, 33.7, 33.6, 28.5, 28.3 ppm; *m*/*z* (CI^+, NH_3) 407 (MH^+).

Method D (isopropenyl succinimido carbonate coupling). Preparation of *N*-Boc-L-Ala-*N*-Me-β-Ala-*O*^{t}Bu (8i). ^{t}Boc-L-alanine (189 mg, 1.0 mmol), isopropenyl succinimido carbonate[26] (199 mg, 1.0 mmol) and 4-dimethylaminopyridine (12 mg, 0.1 mmol) were dissolved in dry acetonitrile (6 mL) and stirred at room temperature for 5 h. A solution of *N*-methyl-β-alanine-*tert*-butyl ester

(159 mg, 1.0 mmol) in acetonitrile/1,4-dioxane (3 mL) (2:1 v/v) was added and the solution stirred for 20 h. The solvent was evaporated in vacuo, and the residue taken up in ethyl acetate (100 mL), and washed with 1 M hydrochloric acid (50 mL), water (50 mL), 5% sodium bicarbonate solution (50 mL), brine (50 mL). The organic phase was dried (Na_2SO_4), evaporated to give a yellow oil, which was taken up in ethyl acetate and purified by flash column chromatography (hexane/ethyl acetate 1:1) to give a colourless oil (126 mg, 38%). δ_H (300 MHz, $CDCl_3$) 5.60 (1H, br, NH), 4.50 (1H, m, Ala α-CH), 3.41–3.75 (2H, m, NCH_2), 3.02 and 2.86 (2 × s, rotamers, N–CH_3), 2.35–2.52 (2H, m, CH_2CO), 1.36 and 1.39 (2 × 9H, s, ^{t}Bu), 1.21 (3H, d, $J = 6.8$ Hz, Ala CH_3) ppm; δ_C (75 MHz, $CDCl_3$) 173.0, 171.4, 155.5, 81.1, 79.8, 46.7, 45.0, 36.1, 34.9, 33.8, 28.7, 28.4, 19.1 ppm; m/z (CI^+, NH_3) 331 (MH^+).

Method E (PyBOP coupling).[27] General method. *N*-^{t}Boc-amino acid (2 mmol), β-alanine methyl ester hydrochloride (278 mg, 2 mmol) or N-methyl-β-alanine-*tert*-butyl ester (318.5 mg, 2 mmol), PyBOP (1.040 g, 2 mmol) were dissolved in anhydrous dichloromethane (10 mL). Diisopropylethylamine (1.045 mL, 6 mmol) in anhydrous dichloromethane (2 mL) was added dropwise. The solution was stirred for 2 days. The solution was diluted with ethyl acetate (30 mL), and washed with water (20 mL), 5% potassium hydrogen sulphate solution (20 mL), 5% sodium bicarbonate solution (20 mL), brine (20 mL). The organic phase was dried (Na_2SO_4), and concentrated in vacuo. The residue was taken up in ethyl acetate and purified by flash column chromatography (ethyl acetate) to give the requisite protected dipeptide.

N-Boc-L-Ala-β-Ala-OMe (**8c**) was prepared by method E, in 75% yield. δ_H (300 MHz, $CDCl_3$) 7.01 (1H, br, NH), 5.51 (1H, br, NH), 4.04 (1H, m, Ala α-CH), 3.59 (3H, s, OMe), 3.42 (2H, m, NCH_2), 2.47 (2H, t, $J = 6.9$ Hz, CH_2CO), 1.33 (9H, s, ^{t}Bu), 1.24 (3H, d, $J = 6.91$ Hz, Ala CH_3) ppm; δ_C (75 MHz, $CDCl_3$) 173.8, 173.2, 155.9, 80.2, 52.1, 50.6, 35.2, 34.1, 28.6, 18.7 ppm; m/z (FAB) 275 (MH^+).

N-Boc-D-Phe-*N*-Me-β-Ala-*O*^{t}Bu (**8d**) was prepared by method E, in 98% yield. δ_H (300 MHz, $CDCl_3$) 7.08–7.26 (5H, m, Ar), 6.04 (1H, d, $J = 6.2$ Hz, NH), 4.71 (1H, m, Tyr α-CH), 3.32–3.74 (2H, m, NCH_2), 2.80–3.08 (5H, m, Tyr β-CH2 and NCH_3), 2.25–2.50 (2H, m, CH_2CO), 1.41 (9H, s, ^{t}Bu), 1.32 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 172.0, 171.8, 171.3, 155.3, 137.0, 136.8, 129.8, 128.6, 127.1, 80.9, 79.6, 51.8, 45.3, 44.9, 40.5, 40.1, 36.0, 33.7, 34.6, 33.6, 28.6, 28.3 ppm; m/z (FAB) 407 (MH^+).

N-Boc-L-Tyr(*O*-^{t}Bu)-*N*-Me-β-Ala-*O*^{t}Bu (**8f**) was prepared by method E, in 91% yield. δ_H (300 MHz, $CDCl_3$) 6.98 (2H, d, $J = 8.4$ Hz), 6.71 (2H, d, $J = 8.4$ Hz), 5.52 (1H, d, $J = 6.9$ Hz, NH), 4.59 (1H, m, Tyr α-CH), 3.18–3.49 (2H, m, NCH_2), 2.79 (2H, m, Tyr β-CH_2), 2.70, 2.53 (2 × s, rotamers, NCH_3), 2.10–2.35 (2H, m, CH_2CO), 1.25 (9H, s, ^{t}Bu), 1.23 (9H, s, ^{t}Bu), 1.13 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 171.9, 171.1, 170.3, 155.3, 154.4, 131.9, 131.7, 130.2, 125.2, 81.2, 80.8, 79.5, 51.8, 45.3, 44.8, 39.9, 39.6, 35.8, 33.6, 34.6, 33.7, 29.1, 28.3, 28.2 ppm; m/z (FAB) 479 (MH^+).

N-Boc-Gly-β-Ala-OMe (**8g**) was prepared by method E, in 49% yield. δ_H (300 MHz, $CDCl_3$) 7.03 (1H, br, NH), 5.70 (1H, br, NH), 3.71 (2H, d, $J = 6.9$ Hz, Gly α-CH_2), 3.59 (3H, s, OCH_3), 3.43 (2H, q, $J = 6.9$ Hz, NCH_2), 2.49 (2H, t, $J = 6.9$ Hz, CH_2CO), 1.33 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 173.0, 170.2, 156.5, 80.2, 52.0, 44.4, 35.2, 34.1, 28.6 ppm; m/z (CI^+, NH_3) 261 (MH^+).

N-Boc-Gly-*N*-Me-β-Ala-*O*^{t}Bu (**8h**) was prepared by method E, in 71% yield. δ_H (300 MHz, $CDCl_3$) 5.48 (1H, br, NH), 3.79, 3.68 (2 × 1H, d, $J = 6.9$ Hz, Gly α-CH_2), 3.40, 3.29 (2H, t, $J = 6.9$ Hz, NCH_2), 2.79, 2.69 (2 × s, rotamers, NCH_3), 2.27 (2H, m, CH_2CO), 1.22 (18H, s, 2 × ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 171.1, 170.2, 168.5, 156.0, 81.4, 80.8, 79.4, 44.7, 44.5, 42.6, 42.2, 34.9, 33.2, 34.2, 33.8, 28.4, 28.1 ppm; m/z (FAB) 317 (MH^+).

N-Boc-β-Ala-*N*-Me-β-Ala-*O*^{t}Bu (**8j**) was prepared by method E, in 97% yield. δ_H (300 MHz, $CDCl_3$) 5.46 (1H, br, NH), 3.52 (2H, m, NCH_2), 3.30 (2H, m, NCH_2), 2.96 and 2.88 (2 × s, rotamers, NCH_3), 2.52 and 2.27 (4H, m, 2 × CH_2CO), 1.34 (9H, s, ^{t}Bu), 1.32 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 171.8, 171.3, 170.5, 156.2, 81.5, 80.8, 79.0, 45.6, 44.4, 36.5, 36.0, 36.0, 33.3, 34.6, 34.0, 33.2, 28.6, 28.2 ppm; m/z (FAB^+) 331 (MH^+).

Preparation of *N*-Boc-aminoacyl-β-alanines (9a–l). General procedure. The fully protected dipeptide (**8a–l**) was dissolved in 1 M sodium hydroxide/1,4-dioxane (1:1 v/v) (8 mL/mmol) and stirred at room temperature for 15–16 h. The solution was acidified to pH 2 with 1 M hydrochloric acid and extracted with ethyl acetate (20 mL/mmol). The organic phase was dried (Na_2SO_4) and concentrated in vacuo to give the free acid.

N-^{t}Boc-L-Phe-β-Ala (**9a**). 90% yield. δ_H (300 MHz, $CDCl_3$) 7.73 (1H, br, NH), 7.09 (5H, br, Ar), 5.94 (1H, br, NH), 4.38 (1H, br, Phe α-CH), 3.29 (2H, br, NCH_2), 2.96 and 2.77 (2 × 1H, dd, $J = 4.1$, 10.2 Hz, Phe β-CH_2), 2.20 (2H, br, CH_2CO), 1.20 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 178.5, 172.9, 156.4, 137.4, 129.8, 128.7, 127.0, 80.2, 56.2, 39.5, 37.0, 28.7 ppm; m/z (ES^+) 337.2 (MH^+).

N-^{t}Boc-L-Phe-*N*-Me-β-Ala (**9b**) 98% yield. δ_H (300 MHz, acetone-d_6) 7.11–7.32 (5H, m, Ar), 6.34 (1H, br, NH), 6.25 (1H, d, $J = 6.9$ Hz, NH), 4.73–4.89 (1H, m, Phe α-CH), 3.39–3.72 (2H, m, NCH_2), 2.83–3.03 (5H, m, Phe β-CH_2 and N–CH_3), 2.42–2.69 (2H, m, CH_2CO), 1.32 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, acetone-d_6) 174.2, 173.2, 156.6, 138.7, 138.5, 130.8, 129.5, 127.8, 79.8, 52.9, 46.2, 45.7, 39.8, 39.6, 36.5, 34.2, 33.7, 32.7, 28.9 ppm; m/z (FAB) 351 (MH^+).

N-^{t}Boc-D-Phe-β-Ala (**9c**) 76% yield. δ_H (300 MHz, acetone-d_6) 7.45 (1H, br, NH), 7.11–7.28 (5H, m, Ar), 6.11 (1H, br, NH), 4.29 (1H, br, Phe α-CH), 3.41 (2H, m, NCH_2), 3.12 and 2.89 (2 × 1H, dd, $J = 4.1$, 10.2 Hz, Phe

β-CH_2), 2.45 (2H, t, $J=6.9$ Hz, CH_2CO), 1.30 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, acetone-d_6) 173.9, 173.0, 156.6, 139.1, 130.6, 129.4, 127.6, 79.7, 57.1, 39.4, 36.2, 34.6, 28.9 ppm; *m/z* (FAB) 337 (MH^+).

N-^{t}Boc-D-Phe-*N*-methyl-β-Ala (**9d**). 99% yield. δ_H (300 MHz, $CDCl_3$) 7.12–7.29 (5H, m, Ar), 5.96 (1H, d, $J=6.9$ Hz, NH), 4.75–4.96 (1H, m, Phe α-CH), 3.39–3.73 (2H, m, NCH_2), 2.82–3.02 (2H, m, Phe β-CH_2), 2.83, 2.74 (2 × s, rotamers, N–CH_3), 2.36–2.59 (2H, m, CH_2CO), 1.35 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 174.7, 173.6, 172.3, 156.6, 136.1, 136.0, 129.2, 128.2, 126.7, 79.8, 51.4, 44.9, 44.5, 39.5, 39.3, 35.5, 33.6, 32.4, 31.6, 28.1 ppm; *m/z* (FAB) 351 (MH^+).

N-^{t}Boc-L-Tyr(*O*-^{t}Bu)-β-Ala (**9e**) 99% yield. δ_H (300 MHz, $CDCl_3$) 6.37 (1H, br, NH), 7.01 (2H, d, $J=8.3$ Hz), 6.83 (2H, d, $J=8.3$ Hz), 5.94 (1H, br, NH), 4.39 (1H, br, Tyr α-CH), 3.30 (2H, br, NCH_2), 2.96 and 2.73 (2 × 1H, m, Tyr β-CH_2), 2.22 (2H, m, CH_2CO), 1.24 (9H, s, ^{t}Bu), 1.21 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 178.9, 171.7, 156.3, 154.6, 132.5, 130.2, 124.6, 80.0, 78.7, 56.1, 38.9, 36.6, 29.1, 28.7 ppm; *m/z* (FAB) 409 (MH^+).

N-^{t}Boc-L-Tyr(*O*-^{t}Bu)-*N*-methyl-β-Ala (**9f**) 99% yield. δ_H (300 MHz, $CDCl_3$) 7.03 (2H, d, $J=8.3$ Hz), 6.79 (2H, d, $J=8.3$ Hz), 5.84 (1H, d, $J=6.9$ Hz, NH), 4.70 (1H, m, Tyr α-CH), 3.25–3.60 (2H, m, NCH_2), 2.76–2.92 (2H, m, Tyr β-CH_2), 2.65 (3H, s, NCH_3), 2.35–2.51 (2H, m, CH_2CO), 1.32 (9H, s, ^{t}Bu), 1.21 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 174.8, 173.9, 172.8, 155.7, 154.5, 131.6, 130.2, 124.6, 80.1, 78.8, 52.0, 50.4, 45.6, 45.1, 39.6, 39.4, 36.3, 33.5, 33.2, 32.2, 29.1, 28.6 ppm; *m/z* (FAB) 423 (MH^+).

N-^{t}Boc-Gly-β-Ala (**9g**) 72% yield. δ_H (300 MHz, CD_3OD) 3.71 (2H, s, Gly α-CH_2), 3.45 (2H, t, $J=6.9$ Hz, NCH_2), 2.43 (2H, t, $J=6.9$ Hz, CH_2CO), 1.47 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, CD_3OD) 178.5, 172.8, 158.8, 81.2, 45.1, 37.5, 29.1 ppm; *m/z* (FAB) 247 (MH^+).

N-^{t}Boc-Gly-*N*-methyl-β-Ala (**9h**) 87% yield. δ_H (300 MHz, $CDCl_3$) 5.69 (1H, br, NH), 3.89 and 3.76 (2 × d, rotamers, $J=6.8$ Hz, Gly α-CH_2), 3.35–3.51 (2H, m, NCH_2), 2.87, 2.75 (2 × s, rotamers, NCH_3), 2.40 (2H, m, CH_2CO), 1.24 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 174.5, 173.4, 169.5, 156.6, 79.9, 46.4, 45.0, 42.6, 42.2, 35.3, 33.5, 32.7, 32.3, 28.5 ppm; *m/z* (FAB) 261 (MH^+).

N-^{t}Boc-β-Ala-β-Ala (**9i**) 97% yield. δ_H (300 MHz, $CDCl_3$) 7.05 (1H, br, NH), 5.48 (1H, br, NH), 3.43 (2H, q, $J=6.9$ Hz, NCH_2), 3.29 (2H, q, $J=6.9$ Hz, NCH_2), 2.52 (2H, t, $J=6.9$ Hz, CH_2CO), 2.32 (2H, t, $J=6.9$ Hz, CH_2CO), 1.34 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 175.6, 172.7, 156.9, 80.0, 38.8, 37.3, 35.4, 34.1, 28.7 ppm; *m/z* (ES^+) 261 (MH^+).

N-^{t}Boc-β-Ala-*N*-methyl-β-Ala (**9j**) 99% yield. δ_H (300 MHz, $CDCl_3$) 5.57 (1H, br, NH), 3.56 (2H, m, NCH_2), 3.32 (2H, m, NCH_2), 2.99, 2.88 (2 × s, rotamers, NCH_3), 2.45–2.62 (4H, m, CH_2CO), 1.36 (9H, s, ^{t}Bu) ppm; δ_C (75 MHz, $CDCl_3$) 175.1, 173.9, 172.6, 156.6, 79.6, 45.7, 44.7, 36.7, 36.6, 36.5, 33.5, 34.0, 33.3, 33.1, 32.6, 28.4 ppm; *m/z* (ES^+) 275 (MH^+).

N-^{t}Boc-L-Ala-β-Ala (**9k**) 75% yield. δ_H (300 MHz, CD_3OD) 3.98 (1H, m, Ala α-CH), 3.36 (2H, m, NCH_2), 2.33 (2H, t, $J=6.9$ Hz, CH_2CO), 1.43 (9H, s, ^{t}Bu), 1.26 (3H, d, $J=6.8$ Hz, Ala CH_3) ppm; δ_C (75 MHz, CD_3OD) 179.1, 176.2, 158.0, 81.1, 50.4, 37.7, 37.8, 29.2, 19.1 ppm; *m/z* (FAB) 261 (MH^+).

N-^{t}Boc-L-Ala-*N*-methyl-β-Ala (**9l**) 85% yield. δ_H (300 MHz, $CDCl_3$) 5.81 (1H, m, NH), 4.47–4.68 (1H, m, Ala α-CH), 3.46–3.74 (2H, m, NCH_2), 3.08 and 2.89 (2 × s, rotamers, N–CH_3), 2.49–2.70 (2H, m, CH_2CO), 1.35 (9H, 2 × s, ^{t}Bu), 1.25 and 1.21 (2 × d, $J=6.8$ Hz, Ala CH_3) ppm; δ_C (75 MHz, $CDCl_3$) 175.0, 173.8, 155.8, 79.9, 47.8, 46.7, 45.6, 45.2, 36.3, 34.1, 33.3, 32.3, 28.6, 18.6, 18.1 ppm; *m/z* (FAB) 275 (MH^+).

Preparation of 5′-*N*-(*N*-Boc-aminoacyl-β-alanyl)-5′-amino-2′-*O*,3′-*O*-di(*tert*-butyldimethylsilyl) 5′-deoxyuridines (10a–l). *N*-Boc-aminoacyl-β-alanine (**9a–l**) was coupled with 5′-amino-2′-*O*-,3′-*O*-di(*tert*-butyldimethylsilyl) 5′-deoxyuridine (**7**), using coupling method B or E. See Table 3 for ^{13}C NMR data.

Method B (isopropenyl chloroformate mixed anhydride coupling). Preparation of 5′-*N*-(*N*-Boc-L-Phe-β-Ala-)-5′-amino-2′-*O*-,3′-*O*-di(*tert*-butyldimethylsilyl) 5′-deoxyuridine (10a). *N*-^{t}Boc-L-Phe-β-Ala (**9a**) (183 mg, 561 μmol) was dissolved in anhydrous THF (2.0 mL) and neutralised with *N*-methyl morpholine (62 μL, 561 μmol). The solution was cooled to 0 °C and isopropenyl-chloroformate (62 μL, 561 μmol) was added, and the resulting suspension stirred for 4 min. A solution of 5′-amino-2′,3′-*O*-bis(*tert*-butyldimethyl silyl)-5′-deoxyuridine (**8**) (265 mg, 561 μmol) and triethylamine (78 μL, 561 μmol) in anhydrous THF (2.0 mL) was added, and the suspension stirred at 0 °C for 15 min, then room temperature for 2 days. The solvent was removed in vacuo, and the residue taken up in ethyl acetate (50 mL), and washed with water (20 mL), 5% potassium hydrogen sulphate solution (20 mL), 5% sodium bicarbonate solution (20 mL), water (20 mL), and brine (20 mL). The organic phase was dried (Na_2SO_4), and concentrated in vacuo. The residue was taken up in 3% MeOH/DCM and purified by flash column chromatography (3% MeOH/DCM) to give **10a** as a white foam, 214 mg, 48%. δ_H (300 MHz, $CDCl_3$) 7.29 (1H, d, $J=8.2$ Hz, uracil H-6), 7.08–7.22 (5H, m, Ar), 6.89 (1H, br, NH), 5.69 (1H, d, $J=8.2$ Hz, uracil H-5), 5.42 (1H, br, NH), 5.37 (1H, br, H-1′), 4.42 (1H, br, H-2′), 4.20 (1H, br, Phe α-CH), 4.05 (1H, br, H-4′), 3.89 (1H, br, H-3′), 3.58 (1H, m, H-5′), 3.31 (3H, br, H-5′ and β-Ala NCH_2), 2.96 (2H, br, Phe β-CH_2), 2.26 (2H, br, β-Ala CH_2CO), 1.29 (9H, s), 0.83 (9H, s), 0.78 (9H, s), 0.04 (3H, s), −0.01 (3H, s), −0.02 (3H, s), −0.04 (3H, s) ppm; *m/z* (FAB) 790 (MH^+); HRMS 790.4243, calcd 790.4243.

5′-*N*-(*N*-Boc-D-Phe-β-Ala-)-5′-amino-2′-*O*-,3′-*O*-di(*tert*-butyldimethylsilyl) 5′-deoxyuridine (**10c**) was prepared

by method B from acid **9c** in 56% yield. δ_H (300 MHz, $CDCl_3$) 7.08–7.26 (6H, m, Ar and uracil H-6), 6.69 (1H, br, NH), 5.67 (1H, d, $J=8.2$ Hz, uracil H-5), 5.24 (1H, br, H-1′), 5.17 (1H, br, NH), 4.50 (1H, br, H-2′), 4.19 (1H, br, Phe α-CH), 3.99 (1H, m, H-4′), 3.83 (1H, m, H-3′), 3.55 (1H, m, H-5′), 3.20–3.48 (3H, br, H-5′ and β-Ala NCH_2), 2.93 (2H, br, Phe β-CH_2), 2.28 (2H, br, β-Ala CH_2CO), 1.30 (9H, s), 0.82 (9H, s), 0.77 (9H, s), 0.02 (3H, s), −0.02 (3H, s), −0.03 (3H, s), −0.06 (3H, s) ppm; *m/z* (FAB) 790 (MH^+); HRMS 790.4242, calcd 790.4243.

5′-*N*-(N-Boc-L-Tyr(*O*-^{t}Bu)-β-Ala-)-5′-amino-2′-*O*-,3′-*O*-di(*tert*-butyldimethylsilyl) 5′-deoxyuridine (**10e**) was prepared by method B from acid **9e** in 66% yield. δ_H (300 MHz, $CDCl_3$) 7.31 (1H, d, $J=8.2$ Hz, uracil H-6), 7.11 (1H, br, NH), 6.99 (2H, d, $J=8.3$ Hz, Ar), 6.85 (1H, br, NH), 6.80 (2H, d, $J=8.3$ Hz, Ar), 5.69 (1H, d, $J=8.2$ Hz, uracil H-5), 5.43 (1H, br, NH), 5.38 (1H, br, H-1′), 4.42 (1H, br, H-2′), 4.18 (1H, br, Tyr α-CH), 4.07 (1H, br, H-4′), 3.87 (1H, br, H-3′), 3.59 (1H, m, H-5′), 3.33 (3H, br, H-5′ and β-Ala NCH_2), 2.86–2.99 (2H, m, Tyr β-CH_2), 2.26 (2H, br, β-Ala CH_2CO), 1.30 (9H, s), 1.22 (9H, s), 0.81 (9H, s), 0.76 (9H, s), 0.01 (3H, s), −0.02 (3H, s), −0.04 (3H, s), −0.06 (3H, s) ppm; *m/z* (FAB) 862 (MH^+).

5′-*N*-(*N*-Boc-L-Tyr(*O*-^{t}Bu)-*N*-methyl-β-Ala-)-5′-amino-2′-*O*-,3′-*O*-di(*tert*-butyldimethylsilyl) 5′-deoxyuridine (**10f**) was prepared by method E from acid **9f** in 60% yield. δ_H (300 MHz, $CDCl_3$) 10.42 (NH), 7.33 (2H, m, uracil H-6, NH), 7.01 (2H, d, $J=8.2$ Hz, Ar), 6.79 (2H, d, $J=8.2$ Hz, Ar), 5.79 (1H, d, $J=6.1$ Hz, NH), 5.69 (1H, d, $J=8.1$ Hz, uracil H-5), 5.48 (1H, d, $J=2.7$ Hz, H-1′), 4.62 (1H, m, H-2′), 4.22 (1H, t, $J=4.1$ Hz, Tyr α-CH), 4.02 (1H, br, H-4′), 3.89 (1H, br, H-3′), 3.29–3.47 (4H, br, H-5′, β-Ala NCH_2), 2.82 (2H, m, Tyr β-CH_2), 2.79 and 2.62 (2 × s, rotamers, N–CH_3), 2.38 (2H, br, β-Ala CH_2CO), 1.32 (9H, s), 1.22 (9H, s), 0.84 (9H, s), 0.79 (9H, s), −0.01 (3H, s), −0.03 (3H, s), −0.04 (3H, s), −0.07 (3H, s) ppm; *m/z* (FAB) 786 (MH^+).

5′-*N*-(*N*-Boc-β-Ala-β-Ala-)-5′-amino-2′-*O*-,3′-*O*-di(*tert*-butyldimethylsilyl) 5′-deoxyuridine (**10i**) was prepared by method B from acid **9i** in 35% yield. δ_H (300 MHz, $CDCl_3$) 9.39 (1H, br, NH), 7.20 (1H, d, $J=8.2$ Hz, uracil H-6), 7.09 (1H, br, NH), 6.55 (1H, br, NH), 5.69 (1H, d, $J=8.2$ Hz, uracil H-5), 5.21 (1H, br, NH), 5.18 (1H, d, $J=4.8$ Hz, H-1′), 4.53 (1H, t, $J=4.7$ Hz, H-2′), 4.02 (1H, m, H-4′), 3.89 (1H, m, H-3′), 3.61 (1H, m, H-5′), 3.41 (2H, q, $J=6.7$ Hz, NCH_2), 3.31 (3H, m, H-5′ and NCH_2), 2.39 (2H, m, CH_2CO), 2.30 (2H, t, $J=6.6$ Hz, CH_2CO), 1.32 (9H, s), 0.83 (9H, s), 0.78 (9H, s), −0.01 (3H, s), −0.03 (3H, s), −0.03 (3H, s), −0.06 (3H, s) ppm; *m/z* (FAB) 714 (MH^+); HRMS 714.3929, calcd 714.3930.

5′-*N*-(*N*-Boc-β-Ala-*N*-methyl-β-Ala-)-5′-amino-2′-*O*-,3′-*O*-di(*tert*-butyldimethylsilyl) 5′-deoxyuridine (**10j**) was prepared by method B from acid **9j** in 33% yield. δ_H (300 MHz, $CDCl_3$) 9.62 (1H, br, NH), 7.20 (1H, br, NH), 7.29 and 7.09 (2 × d, $J=8.2$ Hz, rotamers, uracil H-6), 5.69 (1H, d, $J=8.2$ Hz, uracil H-5), 5.39 (1H, br, NH), 5.30 and 5.05 (2 × d, $J=2.9$ Hz, H-1′), 4.72 and 4.40 (2 × dd, $J=2.9$, 3.5 Hz, H-2′), 3.98–4.10 (1H, m, H-4′), 3.89 (1H, m, H-3′), 3.79 (1H, m, H-5′), 3.45–3.65 (2H, m, NCH_2), 3.15–3.43 (3H, m, H-5′ and NCH_2), 2.91 and 2.88 (2 × s, N–CH_3), 2.42–2.72 (4H, m, CH_2CO), 1.33 and 1.32 (9H, 2 × s), 0.82 (9H, s), 0.78 and 0.76 (9H, 2 × s), −0.01 (3H, s), −0.03 (3H, s), −0.03 (3H, s), −0.06 (3H, s) ppm; *m/z* (FAB) 728 (MH^+); HRMS 728.4086, calcd 728.4086.

5′-*N*-(*N*-Boc-L-Ala-β-Ala-)-5′-amino-2′-*O*-,3′-*O*-di(*tert*-butyldimethylsilyl) 5′-deoxyuridine (**10k**) was prepared by method E from acid **9k** in 48% yield. δ_H (300 MHz, $CDCl_3$) 9.92 (1H, br, NH), 7.37 (1H, d, $J=8.2$ Hz, uracil H-6), 7.14 (1H, br, NH), 6.96 (1H, br, NH), 5.72 (1H, d, $J=8.2$ Hz, uracil H-5), 5.39 (1H, d, $J=2.9$ Hz, H-1′), 5.30 (1H, br, NH), 4.42 (1H, br, H-2′), 3.96–4.09 (2H, m, H-4′ and Ala α-CH), 3.88 (1H, m, H-3′), 3.43–3.72 (2H, m, H-5′), 3.20–3.39 (2H, m, β-Ala NCH_2), 2.36 (2H, t, $J=6.9$ Hz, CH_2CO), 1.33 (9H, s), 1.26 (3H, d, $J=6.9$ Hz, Ala CH_3), 0.83 (9H, s), 0.79 (9H, s), −0.01 (3H, s), −0.03 (3H, s), −0.03 (3H, s), −0.06 (3H, s) ppm; *m/z* (FAB) 714 (MH^+); HRMS 714.3929, calcd 714.3930.

5′-*N*-(*N*-Boc-L-Ala-*N*-methyl-β-Ala-)-5′-amino-2′-*O*-,3′-*O*-di(*tert*-butyldimethylsilyl) 5′-deoxyuridine (**10l**) was prepared by method B from acid **9l** in 46% yield. δ_H (300 MHz, $CDCl_3$) 9.69 (1H, br, NH), 7.29 and 7.11 (2 × d, $J=8.2$ Hz, rotamers, uracil H-6), 7.17 (1H, br, NH), 5.69 (2H, m, uracil H-5, and NH), 5.38 and 5.07 (2 × d, $J=4.9$ Hz, H-1′), 4.78, and 4.47 (2 × t, $J=6.1$ Hz, Ala α-CH), 4.67 and 4.34 (2 × t, $J=5.0$ Hz, H-2′), 3.80–4.13 (3H, m, H-3′, H-4′, H-5′), 3.30–3.72 (3H, m, H-5′ and NCH_2), 3.03 and 2.90 (2 × s, N–CH_3), 2.34–2.58 (2H, m, CH_2CO), 1.42 and 1.37 (9H, 2 × s), 1.25 and 1.19 (3H, 2 × d, $J=6.8$ Hz, Ala CH_3), 0.83 (9H, s), 0.78 (9H, s), −0.01 (3H, s), −0.03 (3H, s), −0.06 (3H, s) ppm; *m/z* (FAB) 728 (MH^+); HRMS 728.4086, calcd 728.4086.

Preparation of 5′-*N*-(aminoacyl-β-alanyl-)-5′-amino-5′-deoxyuridines (11a–l). General procedure. The fully protected 5′-uridinyl-dipeptide (**10a–l**) was dissolved in either (a) THF (3 mL/100 μmol), and tetrabutylammonium fluoride (1 M in THF, 1.5 equivalents) added, or (b) acetonitrile (3 mL/100 μmol), and tetraethylammonium fluoride (0.5 M in MeCN, 1.5 equivalents) added, and the solution stirred for 16–20 h. The solvents were removed in vacuo, and the residue taken up in 10% MeOH/DCM, and purified by flash column chromatography (10% MeOH/DCM) to give the desilylated product. The *N*-^{t}Boc protected 5′-uridinyl-dipeptides were then dissolved in 50% aqueous formic acid (∼10 mL/mmol) and stirred at room temperature for 16 h. The resulting solutions were applied to an ion-exchange chromatography column (Dowex® 50WX8-100, 3 × 1 cm) and eluted with 50% aqueous formic acid (3 × 5 mL), 3% ammonia solution (3 × 5 mL), and 5% ammonia solution (4 × 5 mL). The fractions that contained compounds that were both UV active and ninhydrin positive were combined and freeze-dried to give the free amine product. See Table 4 for ^{13}C NMR data.

Table 3. ^{13}C NMR data for **10a–l** (75 MHz, CDCl3)

		10a	10c	10e	10f	10i	10j	10k	10l
Uracil	C-2	151.1	151.0	151.0	151.0	150.9	150.9	151.0	151.9
	C-4	164.0	163.5	164.0	164.0	163.4	163.7	163.8	163.4
	C-5	103.0	103.1	103.0	102.9	103.1	103.0	103.1	103.0
	C-6	143.3	143.9	143.2	142.5	144.3	143.2	143.3	142.9
Ribose	C-1′	94.4	95.8	94.4	93.1	96.3	94.9	94.7	94.1
	C-2′	73.4	73.4	73.4	74.0	73.4	73.3	73.6	73.4
	C-3′	73.4	72.9	73.4	73.4	72.7	71.4	73.3	72.0
	C-4′	84.4	84.9	84.4	84.2	85.0	84.5	84.4	84.4
	C-5′	41.5	41.4	41.5	41.6	41.3	41.5	41.4	41.3
	Si-*t*Bu	18.4	18.4	18.4	18.3	18.4	18.4	18.4	18.4
		18.3	18.3	18.3	18.2	18.3	18.3	18.3	18.4
		26.1	26.1	26.1	26.1	26.1	26.1	26.1	26.1
	Si-Me	−4.0	−4.0	−4.0	−4.0	−4.1	−4.2	−4.0	−4.0
		−4.2	−4.2	−4.3	−4.1	−4.2	−4.4	−4.3	−4.1
		−4.4	−4.3	−4.4	−4.4	−4.3	−4.6	−4.4	−4.4
		ND	−4.4	ND	ND	−4.5			
β-Ala	C=O	172.4	172.1	172.4	172.3	172.5	171.9	172.7	173.2
	α-C	39.1	39.0	38.4	34.7	37.2	36.7	36.4	37.0/34.9
	β-C	36.1	36.1	36.1	46.7/46.3	36.0	47.4/45.3	36.4	47.1/46.1
	N-Me	—	—	—	36.4/34.8	—	36.4/33.8	—	36.6/34.4
Boc	C=O	155.8	155.8	155.8	155.8	156.5	156.6	155.9	155.8
	t-Bu	80.3	80.3	80.2	80.4	80.3	79.9	80.3	79.9
		28.7	28.7	28.7	28.7	28.8	28.8	28.8	28.7
AA	C=O	172.2	171.8	172.2	171.8	172.0	171.7	173.6	170.5
	α-C	56.5	56.3	56.6	52.2/51.9	36.7	36.1/35.1	50.8	46.9/46.6
	β-C	36.0	36.1	36.1	39.6/39.3	35.9	34.2/33.2	18.9	20.9/18.7
		137.1	137.2	132.0	131.7				
		129.6	129.7	130.1	130.2				
		124.6	124.6	124.6	124.6				
		128.9	127.2	154.4	154.6				
				78.7	78.8				
				29.2	29.1				

5′-*N*-(L-Phe-β-Ala-)-5′-amino-5′-deoxyuridine (**11a**) was obtained as a white foam (48 mg, 46%). δ_H (300 MHz, D_2O) 7.42 (1H, d, $J=8.2$ Hz, uracil H-6), 6.96–7.23 (5H, m, Ar), 5.63 (1H, d, $J=8.2$ Hz, uracil H-5), 5.54 (1H, d, $J=3.9$ Hz, H-1′), 4.12 (1H, t, $J=4.1$ Hz, H-2′), 3.99 (1H, m, H-4′), 3.94 (1H, t, $J=4.1$ Hz, H-3′), 3.68 (1H, t, $J=6.6$ Hz, Phe α-CH), 3.30–3.48 (3H, m, H-5′, NCH_2), 3.17 (1H, td, $J=6.5$, 13.1 Hz, H-5′), 2.89 and 2.77 (2 × 1H, dd, $J=6.5$, 13.1 Hz, Phe β-CH_2), 2.22 (2H, t, $J=6.6$ Hz, CH_2CO) ppm; *m*/*z* (FAB) 462 (MH^+); HRMS 462.1988, calcd 462.1989.

5′-*N*-(D-Phe-β-Ala-)-5′-amino-5′-deoxyuridine (**11c**) was obtained as a white foam (18 mg, 67%). δ_H (300 MHz, D_2O) 7.47 (1H, d, $J=8.2$ Hz, uracil H-6), 7.02–7.28 (5H, m, Ar), 5.68 (1H, d, $J=8.1$ Hz, uracil H-5), 5.58 (1H, d, $J=4.0$ Hz, H-1′), 4.13 (1H, t, $J=4.1$ Hz, H-2′), 3.96 (2H, m, H-3′ and H-4′), 3.88 (1H, t, $J=6.5$ Hz, Phe α-CH), 3.39 (3H, m, H-5′ and NCH_2), 3.19 (1H, dt, $J=6.5$, 11.8 Hz, H-5′), 2.92 (2H, m, Phe β-CH_2), 2.31 (2H, m, CH_2CO) ppm; *m*/*z* (FAB) 462 (MH^+).

5′-*N*-(L-Tyr-β-Ala-)-5′-amino-5′-deoxyuridine (**11e**). For this compound, the desilylated *N*-′Boc-*O*-*tert*-butyl ether protected compound was dissolved in 95% trifluoroacetic acid (10 mL/mmol) and stirred at 0 °C for 30 min. The solution was diluted with water (30 mL/mmol) and freeze-dried. The residue was dissolved in 50% formic acid and purified by ion-exchange chromatography as above, to give **11e** as a white foam (7 mg, 51%). δ_H (300 MHz, D_2O) 7.42 (1H, d, $J=8.2$ Hz, uracil H-6), 6.91 (2H, d, $J=8.2$ Hz, Ar), 6.69 (2H, d, $J=8.2$ Hz, Ar), 5.62 (1H, d, $J=8.1$ Hz, uracil H-5), 5.53 (1H, d, $J=1.1$ Hz, H-1′), 4.10 (1H, dd, $J=1.1$, 4.2 Hz, H-2′), 4.00 (2H, m, H-4′ and Tyr α-CH), 3.89 (1H, t, $J=4.1$ Hz, H-3′), 3.30–3.48 (3H, m, H-5′ and NCH_2), 3.22 (1H, dt, $J=6.5$, 13.1 Hz, H-5′), 2.96 and 2.82 (2 × 1H, dd, $J=4.0$, 10.3 Hz, Tyr β-CH_2), 2.33 (2H, t, $J=6.6$ Hz, CH_2CO) ppm; *m*/*z* (FAB) 478 (MH^+); HRMS 478.1938, calcd 478.1938.

5′-*N*-(β-Ala-β-Ala-)-5′-amino-5′-deoxyuridine (**11i**) was obtained as a white foam (6 mg, 43%). δ_H (300 MHz, D_2O) 7.54 (1H, d, $J=8.1$ Hz, uracil H-6), 5.74 (1H, d, $J=8.2$ Hz, uracil H-5), 5.63 (1H, d, $J=2.7$ Hz, H-1′), 4.23 (1H, t, $J=2.9$ Hz, H-2′), 3.97 (2H, m, H-3′ and H-4′), 3.42 (2H, m, H-5′), 3.33 (2H, t, $J=6.8$ Hz, NCH_2), 3.11 (2H, m, NCH_2), 2.54 (2H, t, $J=6.8$ Hz, CH_2CO), 2.38 (2H, t, $J=6.7$ Hz, CH_2CO) ppm; *m*/*z* (FAB) 386 (MH^+).

5′-*N*-(β-Ala-*N*-methyl-β-Ala-)-5′-amino-5′-deoxyuridine (**11j**) was obtained as a white solid (12 mg, 39%). δ_H (300 MHz, D_2O) 7.54 (1H, d, $J=8.1$ Hz, uracil H-6), 5.74 (1H, d, $J=8.1$ Hz, uracil H-5), 5.64 (1H, d, $J=1.9$ Hz, H-1′), 4.26 (1H, dd, $J=2.0$, 5.9 Hz, H-2′), 3.98 (2H, m, H-3′ and H-4′), 3.35–3.60 (4H, m, H-5′ and NCH_2), 3.10 (2H, m, NCH_2), 2.89 and 2.78 (2 × s, N–CH_3), 2.68 and 2.49 (2 × t, $J=6.8$ Hz, CH_2CO), 2.39 (3H, t, $J=6.6$ Hz, CH_2CO) ppm; *m*/*z* (FAB) 400 (MH^+).

5′-*N*-(L-Ala-β-Ala-)-5′-amino-5′-deoxyuridine (**11k**) was obtained as a white foam (18 mg, 30%). δ_H (300 MHz, D_2O) 7.58 (1H, d, $J=8.2$ Hz, uracil H-6), 5.79 (1H, d, $J=8.1$ Hz, uracil H-5), 5.68 (1H, d, $J=2.9$ Hz, H-1′), 4.23 (1H, t, $J=3.0$ Hz, H-2′), 4.01 (2H, m, H3′ and H4′), 3.87 (1H, q, $J=6.8$ Hz, Ala α-CH), 3.24–3.54 (4H, m, H-5′ and NCH_2), 2.39 (2H, t, $J=6.6$ Hz, CH_2CO), 1.32 (3H, d, $J=6.8$ Hz, Ala CH_3) ppm; *m*/*z* (FAB) 386 (MH^+); HRMS 386.1676, calcd 386.1676.

5′-*N*-(L-Ala-*N*-methyl-β-Ala-)-5′-deoxy-uridine (**11l**) was obtained as a white foam (43 mg, 68%). δ_H (300 MHz, D_2O) 7.53 (1H, d, $J=8.1$ Hz, uracil H-6), 5.78 (1H, d, $J=8.2$ Hz, uracil H-5), 5.65 (1H, d, $J=1.7$ Hz, H-1′), 4.21 (1H, m, H-2′), 4.18 (1H, m, Ala α-CH), 3.92 (2H, m, H-3′ and H-4′), 3.64 (1H, q, $J=6.9$ Hz, H-5′), 3.24–3.50 (3H, m, H-5′ and NCH_2), 2.91 and 2.79 (2 × s, rotamers, N–CH_3), 2.52 and 2.39 (2 × t, $J=6.8$ Hz, CH_2CO), 1.23 and 1.22 (2 × d, $J=6.8$ Hz, Ala CH_3) ppm; *m*/*z* (FAB) 400 (MH^+); HRMS 400.1832, calcd 400.1852.

Preparation of 5′-*O*-(*N*-Boc-aminoacyl-β-alanyl)-2′-*O*-, 3′-*O*-isopropylidine uridines (12a–l). General procedure. The *N*-^{t}Boc-protected dipeptide (**9a–l**) was dissolved in anhydrous DCM (20 mL/g), neutralised with *N*-methyl morpholine (1 equiv), and the resulting solution cooled to 0 °C. Isopropenylchloroformate (1 equiv) was added, and the solution stirred for 4 min. A solution of 2′, 3′-*O*-isopropylideneuridine (1 equiv) and 4-dimethyl-aminopyridine (5 mg/mmol dipeptide) in anhydrous THF (10 mL/mmol) was added, and the resulting suspension stirred for 20 min at 0 °C, then 2 days at room temperature. The solvents were removed in vacuo, and the residue taken up in ethyl acetate (25 mL/mmol) and washed with water (20 mL/mmol), 5% potassium hydrogen sulphate solution (20 mL/mmol), 5% sodium bicarbonate solution (20 mL/mmol), water (20 mL/mmol), and brine (20 mL/mmol). The organic phase was dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash column chromatography (3% MeOH/DCM) to give the coupled product. See Table 4 for ^{13}C NMR data.

Table 4. ^{13}C NMR data for **11a–l** (75 MHz, D_2O)

		11a	11c	11e	11j	11k	11l
Uracil	C-2	151.5	ND	ND	151.9	ND	152.4
	C-4	166.5	ND	ND	166.4	164.5	167.3
	C-5	102.5	102.4	102.4	102.6	102.6	102.6
	C-6	142.0	142.0	141.8	142.6	142.6	142.5
Ribose	C-1′	90.7	91.0	90.6	91.0	91.1	91.0
	C-2′	73.7	73.6	73.8	73.7	73.3	73.3
	C-3′	71.0	71.0	71.1	70.8	70.9	70.9
	C-4′	81.9	81.6	82.1	82.1	82.1	82.0
	C-5′	41.6	41.6	41.9	41.1	41.1	41.0
β-Ala	C=O	174.2	ND	ND	174.7	ND	174.5
	α-C	36.0	37.5	36.7	30.2	35.3	34.4/33.9
	β-C	39.4	38.0	39.4	46.3/45.1	36.2	45.9/45.7
	N-Me	—	—	—	35.9/33.2	—	35.6/33.6
AA	C=O	173.4	ND	ND	171.3	ND	171.3
	α-C	55.5	54.7	54.7	35.9	49.3	47.1/46.9
	β-C	35.3	36.0	36.1	34.4	16.9	17.5/16.5
		135.6	135.6	131.0			
		129.5	129.4	116.1			
		129.2	129.0				
		127.8	127.4				

5′-*O*-(*N*-Boc-L-Phe-β-Ala)-2′-*O*-,3′-*O*-isopropylidine uridine (**12a**) was obtained as a colourless oil (495 mg, 72%). δ_H (300 MHz, $CDCl_3$) 10.32 (1H, br, NH), 7.09–7.26 (6H, m, uracil H-6, Ar), 6.77 (1H, br, NH), 5.68 (1H, d, $J=8.2$ Hz, uracil H-5), 5.50 (1H, s, H-1′), 5.44 (1H, d, $J=8.0$ Hz, NH), 5.04 (1H, d, $J=4.9$ Hz, H-2′), 4.78 (1H, t, $J=4.9$ Hz, H-3′), 4.11–4.40 (4H, m), 3.40 (2H, m, NCH_2), 2.94 (2H, d, $J=6.2$ Hz, Phe β-CH_2), 2.39 (2H, m, CH_2CO), 1.49 (3H, s), 1.32 (9H, s), 1.29 (3H, s) ppm; *m*/*z* (FAB) 603 (MH^+); HRMS 603.2665, calcd 603.2666.

5′-*O*-(*N*-Boc-L-Phe-*N*-methyl-β-Ala)-2′-*O*-,3′-*O*-isopropylidine uridine (**12b**) was obtained as a colourless oil (458 mg, 74%). δ_H (300 MHz, $CDCl_3$) 10.42 (1H, br, NH), 7.28 (1H, d, $J=8.2$ Hz, uracil H-6), 7.05–7.25 (5H, m, Ar), 5.58–5.79 (3H, m), 5.09 and 4.99 (1H, d, $J=5.3$ Hz, rotamers, H-2′), 4.82 (1H, H-3′), 4.72 (1H, q, $J=6.1$ Hz, Phe α-CH), 4.01–4.32 (3H, m, H-4′ and H-5′), 3.51 and 3.40 (2H, m, NCH_2), 2.89 (2H, m, Phe β-CH_2), 2.79 and 2.66 (2 × s, N–CH_3), 2.45 (2H, m, CH_2CO), 1.49 (3H, s), 1.31 and 1.28 (9H, 2 × s), 1.21 (3H, s) ppm; *m*/*z* (FAB) 617 (MH^+); HRMS 617.2822, calcd 617.2823.

5′-*O*-(*N*-Boc-D-Phe-β-Ala)-2′-*O*-,3′-*O*-isopropylidene uridine (**12c**) was obtained as a white foam (19 mg, 49%). δ_H (300 MHz, $CDCl_3$) 9.64 (1H, br, NH), 7.04–7.24 (6H, m, uracil H-6, Ar), 6.49 (1H, br, NH), 5.68 (1H, d, $J=8.2$ Hz, uriacil H-5), 5.42 (1H, d, $J=0.8$ Hz, H-1′), 5.20 (1H, d, $J=7.1$ Hz, NH), 5.07 (1H, dd, $J=0.8$, 5.8 Hz, H-2′), 4.79 (1H, t, $J=5.9$ Hz, H-3′), 4.11–4.31 (4H, m), 3.49 (1H, br), 3.29 (1H, br), 2.92 (2H, m, Phe β-CH_2), 2.33 (2H, m, CH_2CO), 1.50 (3H, s), 1.33 (9H, s), 1.27 (3H, s) ppm; *m*/*z* (FAB) 603 (MH^+); HRMS 603.2665, calcd 603.2666.

5′-*O*-(*N*-Boc-D-Phe-*N*-methyl-β-Ala)-2′-*O*-,3′-*O*-isopropylidene uridine (**12d**) was obtained as a white foam (49 mg, 72%). δ_H (300 MHz, $CDCl_3$) 10.42 (1H, br, NH), 7.05–7.30 (6H, m, uracil H-6, Ar), 5.70 (2H, m, uracil H-5, NH), 5.54 (1H, s, H-1′), 5.02 (1H, d, $J=4.9$ Hz, H-2′), 4.80 (1H, m, H-3′), 4.73 (1H, q, $J=5.9$ Hz, Phe α-CH), 4.12–4.36 (3H, m), 3.59–3.34 (2H, m, NCH_2), 2.88 (2H, d, $J=6.0$ Hz, Phe β-CH_2), 2.80 and 2.69 (3H, 2 × s, rotamers, N–CH_3), 2.48 (2H, m, CH_2CO), 1.51 (3H, s), 1.34 (9H, s), 1.31 (3H, s) ppm; *m*/*z* (FAB) 617 (MH^+); HRMS 617.2824, calcd 617.2822.

5′-*O*-(*N*-Boc-L-Tyr(*O*-^{t}Bu)-β-Ala)-2′-*O*-,3′-*O*-isopropylidene uridine (**12e**) was obtained as a colourless oil (70 mg, 21%). δ_H (300 MHz, $CDCl_3$) 7.21 (1H, d, $J=8.2$ Hz, uracil H-6), 7.03 (2H, d, $J=8.2$ Hz, Ar), 6.83 (2H, d, $J=8.2$ Hz, Ar), 6.66 (1H, br, NH), 5.67 (1H, d, $J=8.2$ Hz, uracil H-5), 5.45 (1H, br, NH), 5.27 (1H, s, H-1′), 5.05 (1H, dd, $J=0.8$, 6.1 Hz, H-2′), 4.79 (1H, t, $J=6.0$ Hz, H-3′), 4.14–4.34 (4H, m, H4′), 3.37 (2H, m, NCH_2), 2.87 (2H, d, $J=6.2$ Hz, Tyr β-CH_2), 2.20–2.47

(2H, br, CH_2CO), 1.50 (3H, s), 1.36 (9H, s), 1.30 (3H, s), 1.23 (9H, s) ppm; *m/z* (FAB) 675 (MH^+).

5′-*O*-(*N*-Boc-L-Tyr(*O*-^{t}Bu)-*N*-methyl-β-Ala)-2′-*O*-,3′-*O*-isopropylidene uridine (**12f**) was obtained as a colourless oil (336 mg, 55%). δ_H (300 MHz, $CDCl_3$) 10.32 (1H, br, NH), 7.24, 7.20 (1H, d, $J=8.2$ Hz, uracil H-6), 7.06 (2H, d, $J=8.2$ Hz, Ar), 6.81 (2H, d, $J=8.2$ Hz, Ar), 5.59–5.75 (3H, m), 5.02 (1H, d, $J=6.2$ Hz, H-2′), 4.82 (1H, m, H-3′), 4.68 (1H, q, $J=6.1$ Hz, Tyr α-CH), 4.01–4.35 (3H, m), 3.45 (2H, m, NCH_2), 2.84 (2H, m, Tyr β-CH_2), 2.79, 2.60 (3H, 2 × s, rotamers, N–CH_3), 2.48 (2H, m, CH_2CO), 1.52 (3H, s), 1.36 (9H, s), 1.29 (3H, s), 1.25 (9H, s) ppm; *m/z* (FAB) 689 (MH^+); HRMS 689.3398, calcd 689.3398.

5′-*O*-(*N*-Boc-Gly-β-Ala)-2′-*O*-,3′-*O*-isopropylidene uridine (**12g**) was obtained as a colourless oil (56 mg, 34%). δ_H (300 MHz, $CDCl_3$) 9.89 (1H, br, NH), 7.22 (1H, d, $J=8.1$ Hz, uracil H-6), 6.82 (1H, br, NH), 5.67 (1H, d, $J=8.2$ Hz, uracil H-5), 5.49 (1H, d, $J=0.8$ Hz, H-1′), 5.37 (1H, br, NH), 5.08 (1H, dd, $J=0.8$, 5.0 Hz, H-2′), 4.83 (1H, t, $J=4.9$ Hz, H-3′), 4.18–4.35 (3H, m), 3.70 (2H, d, $J=6.1$ Hz, Gly α-CH_2), 3.47 (2H, q, $J=6.1$ Hz, NCH_2), 2.53 (2H, t, $J=6.1$ Hz, CH_2CO), 1.49 (3H, s), 1.39 (9H, s), 1.31 (3H, s) ppm; *m/z* (FAB) 513 (MH^+).

5′-*O*-(*N*-Boc-Gly-*N*-methyl-β-Ala)-2′-*O*-,3′-*O*-isopropylidene uridine (**12h**) was obtained as a white foam (190 mg, 56%). δ_H (300 MHz, $CDCl_3$) 10.05 (1H, br, NH), 7.20 (1H, d, $J=8.1$ Hz, uracil H-6), 5.72 (1H, d, $J=8.2$ Hz, uracil H-5), 5.64 (1H, br, NH), 5.75 (1H, s, H-1′), 5.13 and 5.04 (2 × dd, rotamers, $J=0.7$, 6.1 Hz, H-2′), 4.84 (1H, m, H-3′), 4.15–4.53 (3H, m), 4.04 and 3.91 (2 × d, $J=6.1$ Hz, Gly α-CH_2), 3.68 and 3.57 (2 × m, NCH_2), 2.96 and 2.93 (2 × s, N–CH_3), 2.62 (2H, t, $J=6.0$ Hz, CH_2CO), 1.55 (3H, s), 1.42 (9H, s), 1.33 (3H, s) ppm; *m/z* (FAB) 527 (MH^+); HRMS 527.2354, calcd 527.2353.

5′-*O*-(*N*-Boc-β-Ala-β-Ala)-2′-*O*-,3′-*O*-isopropylidene uridine (**12i**) was obtained as a white foam (74 mg, 49%). δ_H (300 MHz, $CDCl_3$) 9.72 (1H, br, NH), 7.21 (1H, d, $J=8.1$ Hz, uracil H-6), 6.49 (1H, br, NH), 5.69 (1H, d, $J=8.1$ Hz, uracil H-5), 5.49 (1H, d, $J=0.78$ Hz, H-1′), 5.22 (1H, br, NH), 5.05 (1H, dd, $J=0.8$, 5.9 Hz, H-2′), 4.83 (1H, dd, $J=4.2$, 5.9 Hz, H-3′), 4.18–4.34 (3H, m), 3.47 (2H, q, $J=6.0$ Hz, NCH_2), 3.32 (2H, q, $J=6.0$ Hz, NCH_2), 2.54 (2H, t, $J=6.0$ Hz, CH_2CO), 2.30 (2H, t, $J=6.1$ Hz, CH_2CO), 1.50 (3H, s), 1.37 (9H, s), 1.31 (3H, s) ppm; *m/z* (FAB) 527 (MH^+); HRMS 527.2352, calcd 527.2353.

5′-*O*-(*N*-Boc-β-Ala-*N*-methyl-β-Ala)-2′-*O*-,3′-*O*-isopropylidene uridine (**12j**) was obtained as a colourless oil (73 mg, 20%). δ_H (300 MHz, $CDCl_3$) 10.05 (1H, br, NH), 7.23 (1H, d, $J=8.1$ Hz, uracil H-6), 5.73 (1H, d, $J=8.1$ Hz, uracil H-5), 5.60 (1H, d, $J=0.8$ Hz, H-1′), 5.49 (1H, br, NH), 5.12 and 5.09 (1H, dd, $J=0.9$, 6.2 Hz, H-2′), 4.90 (1H, m, H-3′), 4.21–4.41 (3H, m), 3.60 (2H, q, $J=6.1$ Hz, NCH_2), 3.39 (2H, q, $J=5.9$ Hz, NCH_2), 2.95 and 2.89 (2 × s, rotamers, N–CH_3), 2.59 (3H, m), 2.49 (1H, t, $J=5.9$ Hz, CH_2CO), 1.56 (3H, s), 1.43 (9H, s), 1.36 (3H, s) ppm; *m/z* (FAB) 541 (MH^+); HRMS 541.2510, calcd 541.2509.

5′-*O*-(*N*-Boc-L-Ala-β-Ala)-2′-*O*-,3′-*O*-isopropylidene uridine (**12k**) was obtained as a colourless oil (85 mg,

Table 5. ^{13}C NMR data for **12a–l** (75 MHz, $CDCl_3$)

		12a	12b	12c	12d	12e	12f	12g	12h	12i	12j	12k	12l
Uracil	C-2	150.8	150.6	150.5	150.6	150.7	150.6	150.6	149.9	150.5	150.5	150.7	150.4
	C-4	164.1	164.2	163.5	164.2	163.9	164.1	164.0	163.5	163.9	164.0	164.1	163.9
	C-5	103.1	102.9	103.2	102.9	103.1	102.9	103.1	102.3	103.1	103.0	103.1	103.0
	C-6	143.6	143.7	143.6	143.5	143.4	143.7	143.5	143.3	143.5	143.7	143.5	143.7
Ribose	C-1′	95.9	96.0/95.4	96.4	95.8/95.5	95.9	96.1/95.5	96.2	95.9/95.1	96.3	96.7/95.7	96.0	96.4/95.7
	C-2′	84.6	84.8/84.4	84.6	84.8/84.6	84.6	84.8/84.3	84.8	84.2/83.7	84.8	84.9/84.6	84.8	84.8/84.3
	C-3′	80.9	81.5/80.9	80.8	81.5/81.3	80.9	81.4/80.6	81.4	80.7/80.1	81.5	81.4	81.3	81.3/80.5
	C-4′	85.5	85.9/85.4	85.5	85.8/85.6	85.5	85.8/85.3	85.9	85.2/84.7	85.9	86.0/85.9	85.8	85.9/85.4
	C-5′	64.0	64.6/63.8	64.0	64.6/64.5	64.0	64.5/63.5	64.3	63.8/63.2	64.3	64.6/64.3	64.3	64.3/63.6
	Isoprop	114.9	114.8	115.1	114.8	114.9	114.7	115.0	114.2	115.0	114.8	114.9	115.0
		27.5	27.5	27.6	27.5	27.6	27.5	27.5	26.9	27.5	27.5	27.5	27.6
		25.8	25.6	25.7	25.6	25.7	25.6	25.7	25.0	25.7	25.7	25.7	25.7
β-Ala	C=O	171.9	172.4	171.7	172.3	172.0	172.5	172.3	171.1	172.3	172.3	173.5	173.4
	α-C	34.9	33.2/32.2	35.0	33.1/32.2	35.0	33.3/32.2	35.2	32.4/32.0	35.3	34.1/33.3	34.4	33.6/32.4
	β-C	39.3	45.2/45.0	39.5	45.2/44.9	38.7	45.2/45.0	34.4	42.2/41.9	37.1	45.4/44.6	35.2	45.4/45.1
	N-Me	—	36.2/32.2	—	36.3/34.0	—	36.2/34.0	—	34.7/33.1	—	36.3/33.5	—	36.3/34.1
Boc	C=O	155.8	155.5	155.8	155.5	155.8	155.5	156.5	155.8	156.5	156.5	155.8	155.8
	t-Bu	80.6	80.2	80.8	80.1	80.9	80.3	80.6	79.7	80.6	79.5	80.4	80.4
		28.7	28.7	28.7	28.7	28.7	28.7	28.7	28.1	28.8	28.8	28.7	28.8
AA	C=O	ND	171.6	171.8	171.6	171.9	171.6	170.2	170.0	172.3	171.8	172.2	171.7
	α-C	56.1	51.9/51.7	56.2	51.9/51.8	56.1	52.0/51.6	44.5	44.4/43.9	34.6	32.7	50.3	46.8/46.5
	β-C	34.4	40.5/40.1	34.4	40.4/40.1	34.5	40.1/39.8			36.6	36.7/36.5	19.1	20.1/19.2
		137.1	136.9	137.1	136.9	132.0	131.7						
		129.7	129.8	129.6	129.8	130.1	130.3						
		128.9	128.7	129.0	128.8	124.6	124.6						
		127.2	127.2	127.3	127.2	154.6	154.5						
						78.8	78.8						
						29.1	29.1						

25%). δ_H (300 MHz, $CDCl_3$) 10.17 (1H, br, NH), 7.29 (1H, d, $J=8.1$ Hz, uracil H-6), 6.95 (1H, br, NH), 5.68 (1H, d, $J=8.1$ Hz, uracil H-5), 5.54 (1H, d, $J=0.8$ Hz, H-1′), 5.34 (1H, br, NH), 5.04 (1H, dd, $J=0.9$, 5.9 Hz, H-2′), 4.81 (1H, dd, $J=6.0$ Hz, H-3′), 4.19–4.30 (3H, m), 4.09 (1H, br, Ala α-CH), 3.46 (2H, q, $J=5.9$ Hz, NCH_2), 2.53 (2H, t, $J=6.0$ Hz, CH_2CO), 1.51 (3H, s), 1.37 (9H, s), 1.32 (3H, s), 1.28 (3H, d, $J=5.9$ Hz, Ala CH_3) ppm; *m/z* (FAB) 527 (MH^+); HRMS 527.2353, calcd 527.2354.

5′-*O*-(*N*-Boc-L-Ala-*N*-methyl-β-Ala)-2′-*O*-,3′-*O*-isopropylidene uridine (**12l**) was obtained as a colourless oil (66 mg, 38%). δ_H (300 MHz, $CDCl_3$) 9.72 (1H, br, NH), 7.25 and 7.13 (2 × d, $J=8.1$ Hz, rotamers, uracil H-6), 5.69 and 5.66 (2 × d, $J=8.2$ Hz, uracil H-5), 5.54 and 5.51 (2 × s, H-1′), 5.08 and 4.99 (2 × d, $J=6.1$ Hz, H-2′), 4.85 (1H, m, H-3′), 4.12–4.72 (4H, m), 3.74 and 3.55 (2H, q, $J=5.0$ Hz, NCH_2), 3.02 and 2.90 (2 × s, N–CH_3), 2.64 and 2.58 (2 × t, $J=6.1$ Hz, CH_2CO), 1.50 (3H, s), 1.39 and 1.37 (9H, 2 × s), 1.29 (3H, s), 1.26 and 1.20 (2 × d, $J=6.1$ Hz, Ala CH_3) ppm; *m/z* (FAB) 541 (MH^+); HRMS 541.2510, calcd 541.2509.

Preparation of 5′-*O*-(aminoacyl-β-alanyl)-uridines (13a–l). General procedure. The protected 5′-*O*-uridinyl-dipeptide (**12a–l**) was dissolved in 50% aqueous formic acid (20 mL/mmol) and stirred at room temperature for 20–24 h. The resulting solutions were diluted with water (100 mL/mmol), and freeze-dried. The residues were dissolved in water, filtered through a cotton wool plug, and the filtrate freeze-dried to give the requisite product. See Table 6 for ^{13}C NMR data.

5′-*O*-(L-Phe-β-Ala) uridine (**13a**) was obtained as a white foam (88 mg, 97%). δ_H (300 MHz, D_2O) 7.44 (1H, d, $J=8.1$ Hz, uracil H-6), 6.95–7.22 (5H, m, Ar), 5.67 (2H, m, H-1′, uracil H-5), 3.92–4.27 (6H, m), 3.40–3.09 (1H, m, NCH_2), 2.94 (2H, m, Phe β-CH_2), 2.32 (2H, m, CH_2CO) ppm; *m/z* (FAB) 463 (MH^+); HRMS 463.1828, calcd 483.1829.

5′-*O*-(L-Phe-*N*-methyl-β-Ala) uridine (**13b**) was obtained as a white foam (322 mg, 84%). δ_H (300 MHz, D_2O) 7.50 (1H, d, $J=8.1$ Hz, uracil H-6), 7.02–7.22 (5H, m, Ar), 5.64 (2H, m, H-1′, uracil H-5), 4.49 (1H, t, $J=6.1$ Hz, Ala α-CH_2), 4.11–4.23 (4H, m), 4.06 (1H, t, $J=6.1$ Hz, H-4′), 3.73 and 3.22 (2 × 1H, td, $J=5.9$, 11.9 Hz, NCH_2), 2.99 and 2.94 (2H, d, $J=6.15$ Hz, Phe β-CH_2), 2.74 and 2.69 (2 × s, rotamers, N–CH_3), 2.50 (2H, t, $J=6.0$ Hz, CH_2CO) ppm; *m/z* (FAB) 477 (MH^+); HRMS 477.1985, calcd 477.1985.

5′-*O*-(D-Phe-β-Ala) uridine (**13c**) was obtained as a white foam (13 mg, 94%). δ_H (300 MHz, D_2O) 7.52 (1H, d, $J=8.2$ Hz, uracil H-6), 7.03–7.28 (5H, m, Ar), 5.68 (2H, m, H-1′ and uracil H-5), 3.96–4.29 (6H, m), 3.45 and 3.18 (2 × 1H, td, $J=6.1$, 12.3 Hz, NCH_2), 2.97 (2H, d, $J=6.1$ Hz, Phe β-CH_2), 2.43 (2H, t, $J=6.0$ Hz, CH_2CO) ppm; *m/z* (FAB) 463 (MH^+); HRMS 463.1830, calcd 463.1829.

5′-*O*-(D-Phe-*N*-methyl-β-Ala) uridine (**13d**) was obtained as a white foam (372 mg, 91%). δ_H (300 MHz, D_2O) 7.52 (1H, d, $J=8.2$ Hz, uracil H-6), 7.04–7.26 (5H, m, Ar), 5.68 (2H, m, H-1′ and uracil H-5), 4.55 (1H, t, $J=6.1$ Hz, Phe α-CH_2), 4.21–4.32 (3H, m), 4.02–4.20 (2H, m), 3.79 and 3.23 (2 × 1H, td, $J=5.9$, 11.9 Hz, NCH_2), 3.02 and 2.96 (2H, d, $J=6.20$ Hz, Phe β-CH_2), 2.79 and 2.73 (3H, 2 × s, rotamers, N–CH_3), 2.52 (2H, t, $J=5.9$ Hz, CH_2CO) ppm; *m/z* (FAB) 477 (MH^+); HRMS 477.1986, calcd 477.1985.

5′-*O*-(Gly-β-Ala) uridine (**13f**) was obtained as a pale yellow solid (29 mg, 99%). δ_H (300 MHz, D_2O) 7.60 (1H, d, $J=8.1$ Hz, uracil H-6), 5.76 (1H, d, $J=8.1$ Hz, uracil H-5), 5.71 (1H, d, $J=3.8$ Hz, H-1′), 4.23–4.32 (3H, m, H-2′ and H-5′), 4.12–4.19 (2H, m, H-3′ and H-4′), 3.63 (2H, s, Gly α-CH_2), 3.40 (2H, t, $J=6.5$ Hz, NCH_2), 2.56 (2H, t, $J=6.5$ Hz, CH_2CO) ppm; *m/z* (FAB) 373 (MH^+); HRMS 373.1360, calcd 373.1359.

5′-*O*-(Gly-*N*-methyl-β-Ala) uridine (**13g**) was obtained as a white foam (140 mg, 94%). δ_H (300 MHz, D_2O) 7.59 and 7.53 (2 × d, $J=8.1$ Hz, rotamers, uracil H-6), 5.69 (2H, m, H-1′ and uracil H-5), 4.23 (3H, m, H-2′ and H-5′), 4.10 (2H, m, H-3′ and H-4′), 3.98 and 3.82 (2 × s, Gly α-CH_2), 3.45–3.58 (2H, m, NCH_2), 2.87 and 2.79 (2 × s, rotamers, N–CH_3), 2.67 and 2.58 (2 × t, $J=6.0$ Hz, CH_2CO) ppm; *m/z* (FAB) 387 (MH^+); HRMS 387.1515, calcd 387.1516.

5′-*O*-(β-Ala-β-Ala) uridine (**13h**) was obtained as a white foam (20 mg, 94%). δ_H (300 MHz, D_2O) 7.57 (1H, d, $J=8.1$ Hz, uracil H-6), 5.75 (2H, m, H-1′ and uracil H-5), 4.27 (3H, m, H-2′ and H-5′), 4.11 (2H, br, H-3′ and H-4′), 3.33 (2H, t, $J=5.9$ Hz, NCH_2), 3.10 (2H, t, $J=5.9$ Hz, NCH_2), 2.52 (4H, m, CH_2CO) ppm; *m/z* (FAB) 387 (MH^+); HRMS 387.1516, calcd 387.1516.

5′-*O*-(β-Ala-*N*-methyl-β-Ala) uridine (**13i**) was obtained as a white foam (52 mg, 89%). δ_H (300 MHz, D_2O) 7.59 and 7.56 (2 × d, $J=8.1$ Hz, rotamers, uracil H-6), 5.82 (2H, m, H-1′ and uracil H-5), 4.23 (3H, m, H-2′ and H-5′), 4.11 (2H, m, H-3′ and H-4′), 3.52 (2H, m, NCH_2), 3.11 (2H, t, $J=5.9$ Hz, NCH_2), 2.94 and 2.74 (2 × s, rotamers, N–CH_3), 2.77 and 2.66 (2H, t, $J=6.0$ Hz, CH_2CO), 2.53 (2H, t, $J=6.0$ Hz, CH_2CO) ppm; *m/z* (FAB) 401 (MH^+); HRMS 401.1673, calcd 401.1672.

5′-*O*-(L-Ala-β-Ala) uridine (**13k**) was obtained as a white foam (59 mg, 93%). δ_H (300 MHz, D_2O) 7.55 (1H, d, $J=8.1$ Hz, uracil H-6), 5.76 (1H, d, $J=8.1$ Hz, uracil H-5), 5.70 (1H, d, $J=1.9$ Hz, H-1′), 4.25 (3H, m, H-2′ and H-5′), 4.12 (2H, m, H-3′ and H-4′), 3.87 (1H, q, $J=6.1$ Hz, Ala α-CH), 3.39 (2H, m, NCH_2), 2.52 (2H, t, $J=6.0$ Hz, CH_2CO), 1.31 (3H, d, $J=6.1$ Hz, Ala CH_3) ppm; *m/z* (FAB) 387 (MH^+); HRMS 387.1516, calcd 387.1516.

5′-*O*-(L-Ala-*N*-methyl-β-Ala) uridine (**13l**) was obtained as a white foam (79 mg, 84%). δ_H (300 MHz, D_2O) 7.61 and 7.57 (2 × d, $J=8.1$ Hz, uracil H-6), 5.79 (1H, d, $J=8.1$ Hz, uracil H-5), 5.73 (1H, d, $J=1.5$ Hz, H-1′), 4.30 (4H, m), 4.12 (2H, m, H-3′ and H-4′), 3.65 and 3.47 (2 × 1H, td, $J=6.0$, 12.0 Hz, NCH_2), 2.97 and 2.82 (2 ×

s, rotamers, N–CH_3), 2.70 and 2.58 (2H, t, $J=5.9$ Hz, CH_2CO), 1.38 and 1.36 (2 × d, $J=6.2$ Hz, Ala CH_3) ppm; *m/z* (FAB) 401 (MH^+); HRMS 401.1674, calcd 401.1672.

In the cases of 5′-*O*-(L-Tyr-β-Ala) uridine (**13e**) and 5′-*O*-(L-Tyr-β-Ala) uridine (**13f**), the *N*-^{t}Boc/*O*-*tert*-butyl ether/isopropylidene protected compound **12e/f** was dissolved in 95% trifluoroacetic acid (10 mL/mmol) and stirred at 0 °C for 30 min. The solutions were diluted with water (50 mL/mmol) and freeze-dried. The residues were dissolved in water, filtered through a cotton wool plug, and the filtrate freeze-dried to give the requisite product.

5′-*O*-(L-Tyr-β-Ala) uridine (**13e**) was isolated as a white foam (30 mg, 92%). δ_H (300 MHz, D_2O) 7.46 (1H, d, $J=8.1$ Hz, uracil H-6), 6.88 (2H, d, $J=8.2$ Hz, Ar), 6.63 (2H, d, $J=8.2$ Hz, Ar), 5.64 (2H, m, H-1′ and uracil H-5), 4.23 (2H, m, H-5′), 4.15 (2H, m, H-2′ and H-4′), 4.07 (1H, t, $J=4.9$ Hz, H-3′), 3.94 (1H, t, $J=6.0$ Hz, Tyr α-CH), 3.48 and 3.10 (2 × 1H, m, NCH_2), 2.89 (2H, m, Tyr β-CH_2), 2.27–2.53 (2H, m, CH_2CO) ppm; *m/z* (ES+) 479 (MH^+); HRMS (FAB) 479.1777, calcd 479.1778.

5′-*O*-(L-Tyr-*N*-methyl-β-Ala) uridine (**15f**) was isolated as a white foam (98 mg, 83%). δ_H (300 MHz, D_2O) 7.40 (1H, d, $J=8.1$ Hz, uracil H-6), 6.84 (2H, d, $J=8.3$ Hz, Ar), 6.56 (2H, d, $J=8.3$ Hz, Ar), 5.58 (2H, m, H-1′ and uracil H-5), 4.37 (1H, t, $J=6.1$ Hz, Tyr α-CH), 4.18 (2H, m, H-5′), 4.11 (2H, m, H-2′ and H-4′), 3.96 (1H, m, H-3′), 3.65 and 3.18 (2 × 1H, td, $J=6.0$, 12.0 Hz, NCH_2), 2.89 and 2.83 (2H, d, $J=6.1$ Hz, Tyr β-CH_2), 2.65 and 2.60 (3H, 2 × s, rotamers, N–CH_3), 2.43 (2H, t, $J=5.9$ Hz, CH_2CO) ppm; *m/z* (FAB) 493 (MH^+); HRMS 493.1935, calcd 493.1934.

Preparation of 5′-*O*-(3-piperidinecarboxyl) uridine (14). 1-(^{t}Boc)-3-piperidinecarboxylic acid (807 mg, 3.52 mmol) in anhydrous THF (15 mL) was neutralised with *N*-methyl morpholine (774 μL, 7.04 mmol), and isopropenyl chloroformate (385 μL, 3.52 mmol) added. The resulting suspension was stirred for 2 min, and a solution of 2′, 3′-*O*-isopropylideneuridine (1.00 g, 3.52 mmol) in anhydrous THF (10 mL) was added. The suspension was stirred for 2 days. Solvents were removed in vacuo, and the residue taken up in ethyl acetate (50 mL) and washed with water (25 mL), 5% potassium hydrogen sulphate solution (25 mL), 5% sodium bicarbonate solution (35 mL), and brine (25 mL). The organic phase was dried (Na_2SO_4) and concentrated in vacuo. The residue was taken up in 3% MeOH/DCM and purified by flash column chromatography (3% MeOH/DCM) to give 5′-*O*-(*N*-^{t}Boc-3-piperidinecarboxyl)- 2′,3′-*O*-isopropylideneuridine as a white solid, 625 mg, 36%. δ_H (300 MHz, $CDCl_3$) 10.29 (1H, br, NH), 7.27 (1H, d, $J=8.1$ Hz, uracil H-6), 5.68 (1H, d, $J=8.1$ Hz, uracil H-5), 5.60 (1H, s, H-1′), 4.99 (1H, d, $J=6.8$ Hz, H-2′), 4.78 (1H, br, H-3′), 4.23 (3H, m, H-4′ and H-5′), 4.02 (1H, m, NCH_2), 3.84 (1H, d, $J=10.6$ Hz, NCH_{equiv}), 2.91 (1H, dd, $J=8.7$, 10.6 Hz, NCH_{ax}), 2.72 (1H, td, $J=9.1$, 0.8 Hz, NCH_{ax}), 2.38 (1H, m, CHCO), 1.93 (1H, m), 1.61 (2H, m), 1.48 (3H, s), 1.34 (10H, s), 1.24 (3H, s) ppm; *m/z* (FAB) 496 (MH^+); HRMS 496.2296, calcd 496.2295.

The protected 5′-ester (100 mg, 202 μmol), was dissolved in aqueous formic acid (6 mL, 50% v/v) and stirred at room temperature for 16 h. The solution was diluted with water (5 mL) and freeze-dried to give 5′-*O*-(3-piperidinecarboxyl) uridine (**14**) as a white foam, 72 mg, 99%. δ_H (300 MHz, D_2O) 7.54 (1H, d, $J=8.2$ Hz, uracil H-6), 5.75 (1H, d, $J=8.2$ Hz, uracil H-5), 5.68 (1H, d, $J=0.7$ Hz, H-1′), 4.29 (3H, m, H-2′ and H-5′), 4.12 (2H, br, H-3′ and H-4′), 3.38 (1H, dd, $J=1.3$, 13.4 Hz, NCH_{equiv}), 3.17 (2H, m), 2.89 (2H, m), 1.99 (1H, br), 1.79 (1H, m), 1.63 (2H, m) ppm; δ_C (75 MHz, D_2O) 173.6, 166.4, 151.6, 142.4, 102.5, 91.1, 81.1, 73.5, 69.6, 64.5, 44.3, 44.1, 38.3, 24.7, 20.9 ppm; *m/z* (FAB) 356 (MH^+); HRMS 356.1457, calcd 356.1458.

Table 6. ^{13}C NMR data for **13a–l** (75 MHz, D_2O)

		13a	13b	13c	13d	13e	13f	13g	13h	13i	13j	13k	13l
Uracil	C-2	151.4	151.4	151.4	151.0	151.5	151.4	151.8	151.7	150.5	151.7	151.7	151.7
	C-4	166.2	166.3	166.3	166.3	166.2	166.1	166.5	166.4	164.6	166.6	166.4	166.5
	C-5	103.1	102.5	102.5	102.5	102.4	102.4	102.5	102.5	102.5	102.5	102.5	102.5
	C-6	141.8	141.9	142.0	142.0	141.8	141.7	142.2	142.1	142.1	142.2	142.1	142.2
Ribose	C-1′	90.6	91.1/90.8	90.8	91.1/90.7	90.7	91.0/90.6	90.8	90.7	90.7	90.9/90.7	90.7	91.1/90.8
	C-2′	73.7	73.7/73.5	73.6	73.6	73.7	73.7/73.5	73.6	73.6	73.6	73.7/73.5	73.6	73.6/73.5
	C-3′	69.7	69.7/69.5	69.7	69.6	69.7	69.6/69.4	69.6	69.7/69.6	69.6	69.6	69.6	69.6
	C-4′	81.3	81.3/81.1	81.2	81.2	81.3	81.1/80.8	81.4	81.3/81.1	81.4	81.4/81.3	81.3	81.3
	C-5′	64.3	64.6/64.0	64.4	64.3/64.0	64.5	64.4	64.0	64.0	64.0	64.2/64.0	64.0	64.1
β-Ala	C=O	173.3	173.5	173.5	173.8	173.4	173.4	173.7	173.6	173.8	173.9	173.6	173.6
	α-C	35.1	31.9/31.7	35.3	31.9	35.2	31.8	33.6	31.8	33.7	32.2/30.2	33.6	32.0/31.8
	β-C	37.2	45.0/44.7	37.3	45.1/44.7	36.4	44.9	35.4	40.3	36.0	45.3/44.2	35.4	44.8/44.6
	N-Me	—	36.1/33.2	—	36.0/33.3	—	36.0/33.2	—	34.6/32.9	—	35.7/33.0	—	35.3/33.3
AA	C=O	169.2	169.3	169.2	169.2	169.3	169.3	167.2	167.0	166.5	172.2	171.0	170.7
	α-C	54.6	51.9	54.6	51.9/51.8	54.5	51.8	40.6	44.4	32.2	29.7	49.3	47.4
	β-C	33.3	36.6	33.5	36.6	33.4	35.7			35.2	36.0/35.8	16.8	16.3/15.5
		134.0	133.6	133.9	133.6	125.5	125.1						
		129.6	129.7	129.6	129.8	116.0	115.9						
		129.3	129.4	129.4	129.4	130.0	131.1						
		128.2	128.4	128.2	128.4	155.4	155.6						

Preparation of 5′-*O*-((*S*)-3-amino-2-piperidin-1-yl)acetyl) uridine (15). 2′-[*N*-7-′Boc-(*S*)-3-amino-2-oxo-piperidin-1-yl] acetic acid (87 mg, 320 μmol), prepared from L-ornithine by the literature methods,[28,29] was dissolved in anhydrous dichloromethane (3 mL), neutralised with *N*-methyl morpholine (35 μL, 320 μmol) and cooled to 0 °C. Isopropenyl chloroformate (35 μL, 320 μmol) was added and the solution stirred for 4 min. A solution of 2′,3′-*O*-isopropylideneuridine (91 mg, 320 μmol) and 4-dimethylaminopyridine (3 mg) in anhydrous tetrahydrofuran (4 mL) was added. The solution was stirred for 20 min at 0 °C, than allowed to warm to room temperature and stirred for 2 days. Solvents were removed in vacuo, and the residue taken up in ethyl acetate (20 mL), and washed with water (15 mL), 5% potassium hydrogen sulphate (15 mL), 5% sodium bicarbonate (15 mL), water (15 mL), and brine (15 mL). The organic phase was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by flash column chromatography (3% MeOH/DCM) to give the protected 5′-ester as a colourless oil, 26 mg, 15%.

δ_H (300 MHz, $CDCl_3$) 9.40 (1H, br, NH), 7.21 (1H, d, $J = 8.4$ Hz, uracil H-6), 5.71 (1H, d, $J = 8.3$ Hz, uracil H-5), 5.63 (1H, d, $J = 6.8$ Hz, H-1′), 5.44 (1H, d, $J = 5.0$ Hz, NH), 5.00 (1H, m, H-2′), 4.79 (1H, m, H-3′), 4.33 (1H, m, H-5′), 4.14–4.30 (3H, m), 4.04 (1H, br, pip α-CH), 3.87 and 3.81 (2 × d, $J = 13.3$ Hz, NCH_2CO), 3.30 (2H, m, NCH_2), 2.38 (1H, m), 1.90 (2H, m), 1.71 (1H, m), 1.47 (3H, s), 1.36 (9H, s), 1.28 (3H, s) ppm; *m*/*z* (FAB) 539 (MH^+); HRMS 539.2352, calcd 539.2353.

The protected 5′ ester (20 mg, 37 μmol) was dissolved in aqueous formic acid (4 mL, 50% v/v) and stirred at room temperature for 36 h. The solution was diluted with water (6 mL) and freeze-dried to give 5′-*O*-((*S*)-3-amino-2-piperidin-1-yl)acetyl) uridine (**15**) as a white foam (16 mg, 98%). δ_H (300 MHz, D_2O) 7.54 (1H, d, $J = 8.3$ Hz, uracil H-6), 5.79 (1H, d, $J = 8.3$ Hz, uracil H-5), 5.71 (1H, s, H-1′), 3.88–4.45 (8H, m), 3.32 (2H, m, pip NCH_2), 2.21 (1H, m), 1.91 (3H, m) ppm; δ_C (75 MHz, D_2O) 170.4, 168.2, 166.5, 152.0, 142.3, 102.6, 90.8, 81.3, 73.5, 69.6, 64.6, 50.2, 49.6, 49.4, 25.0, 20.2 ppm; *m*/*z* (FAB) 399 (MH^+); HRMS 399.1516, calcd 399.1516.

5′-*O*-((*R*)-3-Amino-2-piperidin-1-yl)acetyl) uridine (**16**) was prepared as above from *N*-7-′Boc-(*R*)-3-amino-2-piperidinone, and was obtained as a white foam (1.6 mg, 3%). δ_H (300 MHz, D_2O) 7.54 (1H, d, $J = 8.3$ Hz, uracil H-6), 5.79 (1H, d, $J = 8.3$ Hz, uracil H-5), 5.71 (1H, s, H-1′), 3.88–4.45 (8H, m), 3.32 (2H, m, pip NCH_2), 2.21 (1H, m), 1.91 (3H, m) ppm; *m*/*z* (FAB) 399 (MH^+); HRMS 399.1515, calcd 399.1516.

Enzyme assays

E. coli phospho-MurNAc-pentapeptide translocase was expressed and solubilised as described by Brandish et al.[11] Radiochemical assays (volume 50 μL) were performed, as previously described,[11] in the presence of 40 μg solubilised *E. coli* translocase I, 18 μM (3.5 nCi) ^{14}C-UDPMurNAc-pentapeptide, 10 μM undecaprenyl phosphate, 150 μg phosphatidylglycerol and 25 mM $MgCl_2$ in 100 mM Tris buffer pH 7.5, and were incubated at 37 °C for 30 min. Assays were stopped by addition of 50 μL 6 M pyridinium acetate pH 4.5. Lipid-linked product was extracted into 100 μL *n*-butanol, and ^{14}C determined by scintillation counting. All assays were performed in duplicate.% Inhibition was calculated as {(cpm in the absence of inhibitor − cpm in the presence of inhibitor)/cpm in the absence of inhibitor} × 100.

Antibacterial testing

Stock 100 μg/mL aqueous solutions of the inhibitor samples were prepared. 50 μL of sample was placed on a 15 mm round filter paper and the filter paper placed on a 'lawn' of bacterial culture (100 μL of culture at A_{600} 0.1) on a Luria Broth agar plate and incubated at 37 °C for 16 h. Ampicillin (100 μg/mL) and mureidomycin A (100, 10, 1 μg/mL) controls were used. Antibacterial activity was measured by the size of the zone of clearance around the filter paper. The strains used for testing were *E. coli* K12, *P. putida* ATCC 4359 (Gram-negative); *B. subtilis* W23 and *S. pneumoniae* (Gram-positive).

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