Role of the enamide linkage of nucleoside antibiotic mureidomycin A: synthesis and reactivity of enamide-containing analogues

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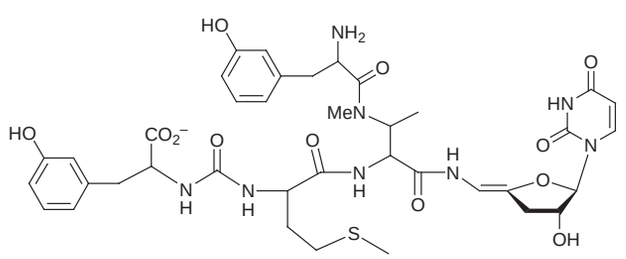
The reactivity of an unusual enamide functional group in the nucleoside antibiotic mureidomycin A (MRD A) has been investigated by synthesis of enamide-containing analogues. Enamides based on 2-methoxyethylamine and tetrahydrofurfurylamine were found to be unstable and reactive, whereas a uridine-based analogue showed high stability and low reactivity. Samples of mureidomycin A and the uridine-based analogue were found to be stable towards acid-catalysed tautomerisation and nucleophilic attack. The lack of reactivity and lack of enzyme inhibition shown by the uridine-based analogue are not consistent with the involvement of the enamide group in slow-binding inhibition of translocase I.

Introduction

The mureidomycin family of nucleoside antibiotics[1] (also reported as the pacidamycins[2] and napsamycins[3]) were isolated in 1989 by Isono and co-workers from *Streptomyces flavidoviridens*. They were found to possess potent anti-pseudomonal activity (MIC 0.125 μg ml^{-1}), and showed low toxicity in mice.[4] The mureidomycins have been shown to disrupt bacterial cell wall peptidoglycan biosynthesis[5] by inhibiting the translocation reaction of peptidoglycan precursor UDPMurNAc-L-Ala-γ-D-Glu-*m*-DAP-D-Ala-D-Ala † onto the lipid carrier undecaprenyl phosphate, catalysed by the enzyme phospho-MurNAc-pentapeptide translocase (translocase I).[6] We have previously shown that mureidomycin A (**1**) acts as a potent slow-binding inhibitor



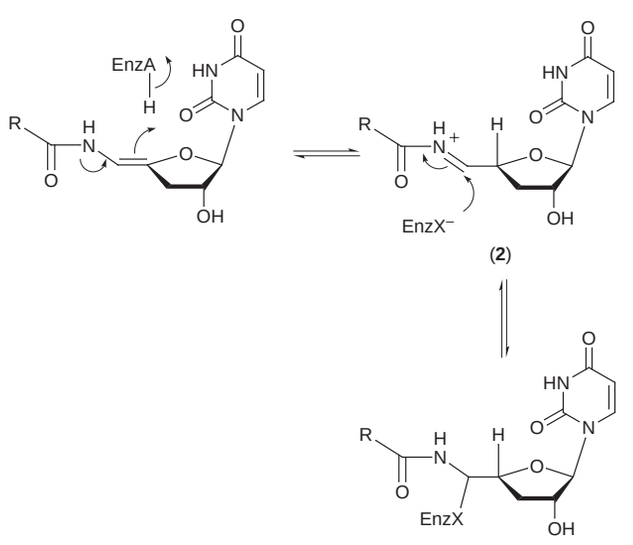
Mureidomycin A

(**1**)

of solubilised translocase I from *Escherichia coli*, *via* isomerisation of an initial EI complex (K_i 36 nM) to a more tightly bound EI* complex (K_i* 2 nM).[7] An understanding of the molecular basis of action for mureidomycin A could lead to the design of new antimicrobial agents, which are urgently needed to combat increasing bacterial resistance to clinical antibiotics.[8]

The structure of mureidomycin A (**1**) contains an unusual enamide functional group positioned at the 4′ and 5′ positions of a 3′-deoxyuridine nucleoside skeleton.[1] The presence of this unusual structural feature in a natural product of considerable biological activity prompted us to analyse the chemical reactivity of this system. Although enamides have been used for several purposes in organic synthesis,[9] a survey of the chemical literature showed that most reported examples were based on cyclic secondary amines, with very few examples arising from primary amines, as in mureidomycin A. A further unusual feature was the presence of a β-oxygen substituent (the endocyclic oxygen of ribose) which might be expected to increase the reactivity of the enamide.

Two observations suggested that this enamide functional group might be important for biological activity of mureidomycin A. Firstly, enamides are able to tautomerise under acidic conditions to generate reactive *N*-acyliminium ion intermediates, which are readily attacked by a range of nucleophiles.[10] Secondly, the reaction catalysed by translocase I is believed to occur *via* a two-step mechanism involving the attack of an active site nucleophile onto the β-phosphate of the substrate UDPMurNAc-pentapeptide.[11] Hence an attractive hypothesis for the slow-binding inhibition of translocase I by mureidomycin A is the protonation of the enamide moiety at the enzyme active site to give an *N*-acyliminium ion **2**, followed by attack of a nucleophilic amino acid side chain to give a covalent adduct **3**, as shown in Scheme 1. This paper describes



Scheme 1 Hypothesis for the mechanism of slow-binding inhibition by mureidomycin A involving tautomerisation to an *N*-acyliminium ion **2**, followed by nucleophilic attack by an enzyme active site nucleophile. Protonation at C-5′ to give an oxonium ion is also possible, however *N*-acylimines have been observed in our model studies.

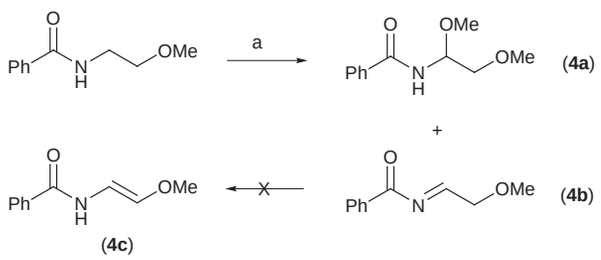
the synthesis of enamide analogues, based on primary amines containing a β-oxygen substituent, of increasing complexity in order to assess the reactivity of this functional group, and to investigate this mechanistic hypothesis.

† MurNAc = *N*-acetylmuramyl, *m*-DAP = *meso*-diaminopimelic acid.

Results

Model based on 2-methoxyethylamine

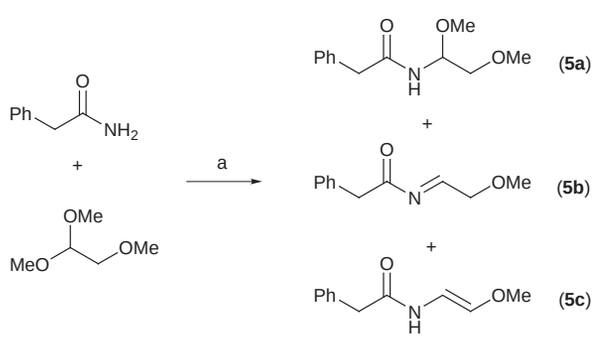
Enamides can be synthesised by anodic oxidation of amides to give the corresponding α-methoxy amide,[12,13] which can be converted by elimination into the corresponding enamide.[14] The simplest model enamide based on a primary amine which contains a β-oxygen substituent is enamide **4c**, derived from 2-methoxyethylamine. Accordingly, (2-methoxyethyl)-benzamide was subjected to anodic oxidation in methanol using tetrabutylammonium toluene-*p*-sulfonate as electrolyte,[13] giving in 37% yield a 9:2 mixture of α-methoxy amide **4a** and *N*-acylimine **4b**, whose structures were verified by 2D NMR spectroscopy (see Scheme 2). Attempts to tautomerise the



Scheme 2 Anodic oxidation of *N*-(2-methoxyethyl)benzamide: a, $Bu_4N^+TsO^-$, Na_2CO_3, MeOH, 19%.

N-acyl imine **4b** into enamide **4c** under basic conditions using DBU, KO^{t}Bu, or HMDS (1,1,1,3,3,3-hexamethyldisilazane) yielded only the starting material **4b**, indicating in this case a high stability for the *N*-acylimine.

There is also literature precedent for the preparation of enamides by reaction of amides with acetals.[15] Accordingly, phenylacetylamide was condensed with 1,1,2-trimethoxyethane in the presence of toluene-*p*-sulfonic acid (see Scheme 3).

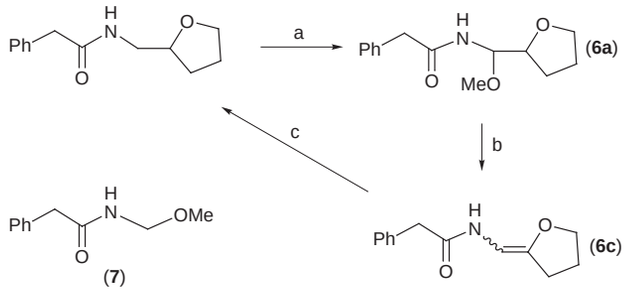


Scheme 3 Reaction of phenylacetamide with 1,1,2-trimethoxyethane: a, MeOH, Δ, cat. TsOH, 22%.

Despite isolation of a single component by column chromatography in 22% yield, analysis by NMR spectroscopy showed a mixture of α-methoxy amide **5a** (δ_H 4.65 dt for α proton), *N*-acylimine **5b** (δ_H 6.44 t for vinylic proton), and enamide **5c** (δ_H 5.73 and 5.46 for vinylic protons). Further attempts to purify the enamide component by column chromatography gave identical mixtures, suggesting that in this case there is a facile enamide–*N*-acylimine equilibrium.

Enamide model based on tetrahydrofurfuryl amine

In view of the observed instability of enamide **5c**, a model enamide **6c** based on tetrahydrofurfurylamine was conceived, in which the tetrahydrofuran ring would mimic the ribose ring of uridine, and the enamide alkene would be stabilised by additional substitution. Phenylacetyl(tetrahydrofurfuryl)amide was subjected to anodic oxidation, giving after column chromatography the α-methoxy amide **6a** in 13% yield, and a 21% yield of by-product **7** arising from free radical-mediated C–C



Scheme 4 Synthesis of tetrahydrofuran-2-ylenamide **6c**: a, $Bu_4N^+TsO^-$, Na_2CO_3, MeOH, 13%; b, silica gel, EtOAc, 25%; c, $NaBH_3CN$, MeOH, pH 4, 63%.

cleavage (see Scheme 4). Treatment of **6a** with silica gel gave a new component whose electrospray mass spectrum (MH^+ 218.0) was indicative of the desired enamide **6c**. Analysis of this new component by NMR spectroscopy identified a mixture of *E*- and *Z*-isomers, showing signals corresponding to the vinylic hydrogen at δ_H 5.82 and 5.69 ppm (*cf*. 5.77 ppm for **1**[1]). Notably however a LR-ESMS analysis performed two days after isolation of the product contained major new signals ($2M + H_2O + H^+$ 453.3, $2M + H_2O + Na^+$ 475.3) suggesting that some form of dimerisation process was occurring. Dimerisation of enamides is precedented in the chemical literature,[16] *via* tautomerisation to the *N*-acyliminium ion and reaction with a second equivalent of enamide.

Reduction of a crude sample of **6c** using sodium cyanoborohydride at pH 4 regenerated in 63% yield the saturated amide (see Scheme 4), which was identical by thin layer chromatography and NMR spectroscopy with an authentic sample. The behaviour of enamide **6c** therefore implied a high degree of instability and chemical reactivity.

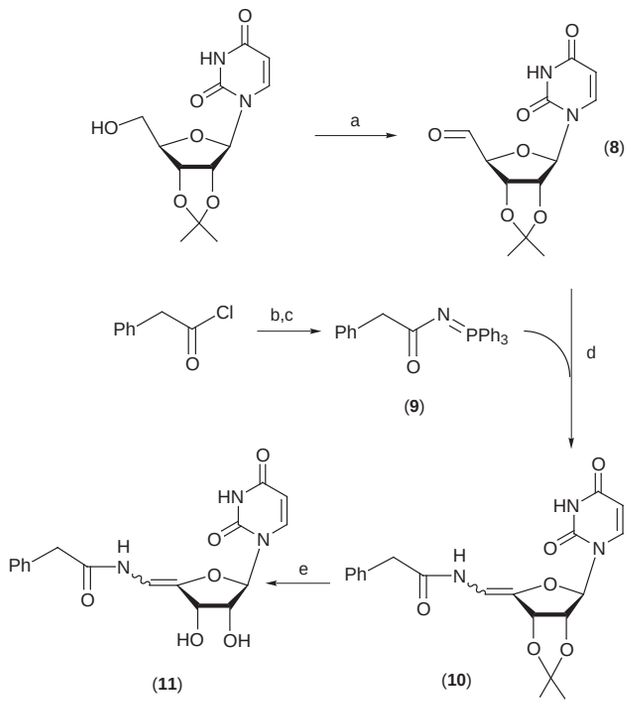
An enamide model based on uridine

A synthetic route was devised for a uridine-based enamide analogue which avoided acidic or basic reaction conditions which might tautomerise the resulting enamide. *N*-acylimines have been synthesised *via* aza-Wittig reactions between aldehydes and imino-phosphoranes under mild reaction conditions.[17] This methodology was applied to the synthesis of a uridine-based enamide, as shown in Scheme 5.

Oxidation of the readily prepared 2′,3′-*O*-(isopropylidene)-uridine by reaction with a 2:1 complex of chromium trioxide–pyridine in the presence of acetic anhydride afforded the 5′-aldehyde **8**.[18] The imino-phosphorane **9** was prepared by reaction of phenylacetyl chloride with trimethylsilyl azide at 4 °C (the low temperature needed to avoid Curtius rearrangement of the acyl azide), followed by *in situ* addition of triphenylphosphine, in 91% overall yield.[17]

In the aza-Wittig coupling a solution of iminophosphorane **9** in toluene was treated with a solution of 1 equiv. of aldehyde **8** in THF, and the resultant mixture heated to reflux. The reaction proceeded very slowly; a new component was observed by thin layer chromatography but conversion was incomplete even in reactions at reflux for six days. Separation of the new product from unreacted **9** proved very difficult but repeated column chromatography allowed the isolation of a white solid as a single component in 13% yield. LR-ESMS analysis of the product showed a molecular ion (MH^+ 400.3) corresponding to the required unsaturated adduct.

^{1}H NMR analysis showed a doubling of signals consistent with a 3:1 mixture of isomers. Notably a pair of vinylic signals, in a 3:1 ratio, were present at 6.50 and 6.65 ppm. This data is consistent with that expected for the enamide tautomer **10**, and the new signals may be assigned as the enamide protons of geometric isomers. These signals are slightly further downfield than the corresponding protons in mureidomycin A (5.77 ppm[1]) and pacidamycin 1 (5.93 ppm[2]), however the assignment is



Scheme 5 Synthesis of uridine-based enamides **10** and **11**: a, CrO_3, pyridine, Ac_2O, 53%; b, Me_3SiN_3, THF, 4 °C; c, PPh_3, THF, 4 °C, 91% overall; d, THF, reflux, 144 h, 13%; e, Dowex-H^+, MeOH–H_2O, 89%.

supported by the observed coupling patterns. Both signals exhibited a 10 Hz coupling to the adjacent NH. In the case of the minor isomer a further 1 Hz splitting was observed, consistent with an allylic coupling to the 3′-H, as observed in pacidamycin 1.[2] The downfield shift of these signals relative to the corresponding protons in the mureidomycins and pacidamycins could be attributed to the presence of the 3′-hydroxy which is absent in the antibiotics. The 3′-hydroxy may be expected to exert a slight electron withdrawing effect on the enamide moiety, and may also induce a change in the conformation of the ribose ring, thereby altering the orbital overlap of the enamide π-system and the lone pair of the ring oxygen.

The ^{13}C NMR spectrum also exhibited doubling of signals, and contained a pair of new quaternary carbon signals at 142.36 and 142.24 ppm (*cf*. MRD A enamide C at 142.39 ppm[1]) and new CH signals at 103.28 and 103.03 ppm (*cf*. MRD A enamide CH at 96.5 ppm[1]). This data confirms the presence of the enamide rather than the *N*-acylimine tautomer. Analysis by reversed phase HPLC gave two peaks at retention times 12.3 min and 12.6 min in a 2:1 ratio. Unlike the earlier enamide models, **10** was found to be stable towards storage, and was therefore suitable for analysis of its reactivity towards acid and nucleophiles.

Reactivity of mureidomycin a and model enamide 10

The methods utilised to synthesise enamides **5c** and **6c** highlighted a facile enamide–*N*-acylimine equilibrium, which is well precedented for enamides under acidic conditions.[10] It therefore seemed quite plausible that such a tautomerisation might be responsible for the slow-binding inhibition of translocase I by mureidomycin A, as illustrated in Scheme 1. It was therefore of great interest to investigate the reactivity of mureidomycin A and enamide model **10** towards acidic conditions and towards nucleophiles.

Solutions containing 1 mg mL^{-1} MRD A were incubated at room temperature with three different concentrations of acetic acid. Sodium acetate was also included in two incubations to increase the concentration of acetate ions and so promote trapping of any *N*-acyliminium ion as the α-acetoxy adduct (see Scheme 6). Aliquots were removed at regular time intervals and analysed by LR-ESMS for the presence of an acetic acid adduct (MH^+ 901) or any degradation products. The incubations were also analysed by RP-HPLC and compared to a standard of unreacted MRD A, all peaks were collected and characterised individually by LR-ESMS. This method of analysis would be expected to distinguish between isomers of MRD A formed as the result of an acid-catalysed equilibrium. The results of the RP-HPLC analyses are summarised in Table 1.

Table 1 HPLC-MS analysis of MRDA incubations under acidic conditions

Incubation conditions	R_t/min[a]	MH^+	Inference
Control	27.4	841	MRD A standard
10% AcOH	27.4	841	MRD A
50% AcOH	26.8	841	isomer of MRD A
	27.4	841	MRD A
Glacial AcOH	27.4	841	MRD A
50 mM NaOAc,	27.4	841	MRD A
50% AcOH	28.0	905, 903, 841	AcOH adduct
100 mM NaOAc,	27.4	841	MRD A
50% AcOH	28.2	905, 841	AcOH adduct

[a] Zorbax ODS C18 column, 10 mm × 250 mm, 0.1 M NH_4OAc–H_2O→0.1 M NH_4OAc–1:1 H_2O–MeCN, 30 min gradient, 2.5 mL min^{-1}, observing at 260 nm.

All analyses showed a major signal at MH^+ 841 consistent with the presence of MRD A. In the incubations containing 50 mM or 100 mM NaOAc a new peak was observed whose electrospray mass spectrum showed new peaks at MH^+ 903 and 905, close to the value of 901 expected for an acetate adduct, suggesting that certain amounts of an acetate adduct had formed in these cases, although in each case the major peak was mureidomycin A itself. In separate studies (see following paper) we were also unable to reduce the alkene component of **1** using a wide range of reducing agents, re-isolating **1** in each case by analytical HPLC/MS. The enamide core of mureidomycin A therefore exhibits low chemical reactivity towards acids, nucleophiles, and other chemical reagents.

Enamide **10** could be separated by reversed phase HPLC into two peaks at 12.4 and 12.8 min, both exhibiting molecular masses of MH^+ 400.4 by electrospray MS, corresponding to the *E*- and *Z*-isomers of the enamide. Collection of individual peaks followed by incubation for several hours in solvent containing 0.1% trifluoroacetic acid and re-injection revealed *no interconversion* of the two peaks, indicating that tautomerisation to the corresponding *N*-acyliminium ion is very slow (see Fig. 1).

The stability of the enamide **10** under acidic conditions was investigated. The enamide was incubated over a range of pH values in the presence of ammonium acetate buffer with the aim of trapping out any *N*-acyliminium ion formed as the α-acetoxy adduct (see Scheme 6). In addition the reactivity of the enamide with respect to nucleophiles, with and without acid catalysis, was investigated. Ethylamine and ethanethiol were employed as nucleophiles, as analogues of possible nucleophilic lysine and cysteine residues in the enzyme active site. The effect of a stoichiometric amount of Lewis acid on the reaction with ethanethiol was also investigated, in an attempt to mimic any polarisation of the enamide of MRD A by binding interactions with the enzyme. All experiments were performed with 1 mg mL^{-1} enamide **10** at room temperature for a minimum of 24 h. The incubations were analysed at regular time intervals by LR-ESMS and RP-HPLC. The results are summarised in Table 2.

No new products were observed in any of the experiments under mild acidic conditions or in the presence of added nucleophiles. In conjunction with the observation that the two isomers of the enamide do not interconvert in 0.1% trifluoroacetic acid, this suggests that the *N*-acyliminium ion is not readily formed in solution. Neither is there any evidence for reaction in the

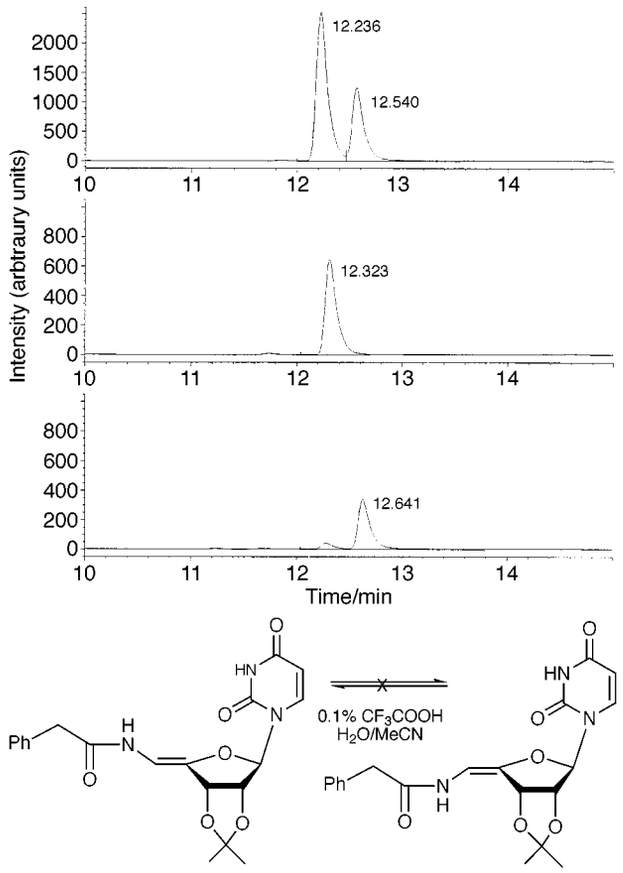
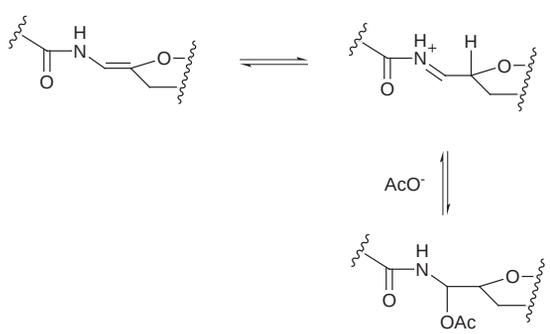


Fig. 1 Acid-stability of *E*- and *Z*-tautomers of uridine-based analogue **10**. A, HPLC traces of: mixture of *E*- and *Z*-isomers (top panel); isolated peaks re-injected onto column after incubation in presence of 0.1% CF_3COOH. B, Scheme illustrating *E*- and *Z*-isomers.



Scheme 6 Formation of α-acetoxy adduct *via* tautomerisation of enamide (of MRD A or **10**) to *N*-acyliminium ion, followed by attack of acetate. Protonation at C-5′ followed by attack at C-4′ is also possible.

presence of Lewis acids. Only under much more forcing conditions were any changes observed. In 10% trifluoroacetic acid a faster eluting component was seen to form over the course of the incubation, constituting 13% of the mixture after 24 h. The ratio of the two enamide isomers remained unchanged, indicating either that both were reacting at the same rate or that an equilibrium had been set up between the two forms. When the enamide was heated with glacial acetic acid a slower eluting product was observed. A component with the same R_t was seen to form when 0.1 M ammonium acetate was included in the incubation, but in an increased yield of 24% (*versus* 14%) after 24 h. It is tempting to assign this product as the acetic acid adduct formed as the result of acetate addition to the *N*-acyliminium ion. Unfortunately, in each of these reactions, no characteristic peaks were observed upon LR-ESMS analyses.

These experiments indicate that enamide (**10**) shows a similar chemical inertness to MRD A, and markedly less reactivity than the tetrahydrofuran-based enamide models. In turn this suggests that the uracil base exerts an important influence on the properties of the enamide functionality, resulting in an increase in chemical stability.

Table 2 HPLC-MS analysis of incubations of enamide **10** under acidic conditions and/or with nucleophiles

Reaction conditions	Observations
Control	R_t[a] 12.4 min (65%), 12.8 min (35%) MH^+ 400.3
1:1 MeOH–H_2O	no change by LR-ESMS or RP-HPLC analysis
0.1 M NH_4OAc, pH 6.8	
0.1 M NH_4OAc, pH 5.0	
0.1 M NH_4OAc, pH 3.8	
1:1 MeOH–g·AcOH	
1% $EtNH_2$ ± H^+	
1% EtSH ± H^+	
1 equiv. $AlCl_3$, EtSH, THF	
1 equiv. BF_3·OEt_2, EtSH, THF	
1 equiv. $ZnBr_2$, EtSH, THF	
10% TFA, 1:1 MeOH–H_2O	new peak R_t[a] 9.6 min (13%)
g·AcOH, 50 °C	new peak R_t[a] 13.4 min (14%)
0.1 M NH_4OAc, g·AcOH, 50 °C	new peak R_t[a] 13.4 min (24%)

[a] (Phenomenex Prodigy ODS3, 3 mm × 150 mm, 0.1% TFA–H_2O→0.04% TFA–MeCN, 20 min gradient, 0.5 mL min^{-1}, observing 254 nm).

Enzyme inhibition assays

A small sample of enamide **10** was carefully deprotected using Dowex-H^+ in methanol–water to give the corresponding diol **11**. NMR spectroscopic analysis verified that the isopropylidene group had been removed (no signal <3.0 ppm), and that the enamide linkage was intact (δ_H 6.07, 6.36 ppm for NC*H*=C). Diol **11** was assayed against solubilised *E. coli* translocase I using a radiochemical assay.[7] No enzyme inhibition was observed at a concentration of 1.1 mM. Under the same assay conditions mureidomycin A shows an IC_{50} of <0.1 μM.

Conclusions

These studies provide the first insight into the chemistry of the unusual 4′,5′-enamide linkage found in the mureidomycin family of nucleoside antibiotics. As mentioned above, there are few literature examples of enamides based on primary amines. The behaviour of the simple 2-methoxyethylamine analogues implies that such "primary" enamides are prone to tautomerisation to the corresponding *N*-acylimine, and that in this case the enamide–*N*-acylimine interconversion is facile. The additional β-substitution present in the tetrahydrofuranyl analogue **6c** appears to shift the position of the enamide–*N*-acylimine equilibrium in favour of the enamide, since no *N*-acylimine was detected in this case. However, the instability of this enamide suggests that the enamide–*N*-acylimine interconversion is still quite facile.

The behaviour of analogue **10** which is based on a uridine skeleton implies that there is a peculiar stability of the enamide functional group found in mureidomycin A. Unlike the tetrahydrofuranyl analogue **6c** which was reactive and unstable, both the natural product **1** and the uridine analogue **10** showed low reactivity towards acid and/or nucleophiles, and high stability. It appears that there is a stereoelectronic effect of nitrogen substitution at the anomeric centre of **1** and **10** which stabilises the enamide functional group. The dominant stereoelectronic effect in the ribose ring of **1** and **10** is likely to be the overlap of an oxygen lone pair with the σ^*_{C-N} orbital, which may force the ribose ring into a conformation in which neither oxygen lone pair overlaps with the π bond of the enamide. This type of marked dependence of chemical reactivity upon subtle changes

of conformation is reminiscent of the latent reactivity of the ene-diyne anticancer agents.[19]

The lack of reactivity of **1** and **10** under a range of acidic conditions, coupled with the lack of inhibition showed by analogue **10**, does not support the hypothesis for slow-binding inhibition of translocase I *via* an enamide–*N*-acyliminium ion tautomerisation, although it is possible that the enamide group in **1** is activated within the confines of the translocase I active site. It may be that the enamide skeleton simply provides a novel three-dimensional scaffold for this family of natural products, without contributing directly to the mechanism of slow-binding inhibition. An alternative mechanism for slow-binding inhibition would involve a conformational change of the EI complex to form the tightly bound EI* complex, which is precedented in other cases of slow-binding enzyme inhibition.[20] We note that a further nucleoside natural product liposidomycin B also acts as a slow-binding inhibitor of solubilised translocase I, yet does not contain an enamide functional group.[21] The following paper contains further structure–function studies on mureidomycin A which seek to define the structural features required for slow-binding inhibition of translocase I.

Experimental

General

Infra-red spectra were recorded on a 1600 series Perkin-Elmer FTIR spectrometer. NMR spectra were recorded on a Bruker AC300 Fourier Transform Spectrometer. Mass spectra were recorded on a VG platform quadrupole E.S.I. mass spectrometer. Analytical RP-HPLC was performed on a Hewlett Packard HP1100 ChemStation Chromatograph using a Phenomenex Prodigy ODS3 column, 3 mm × 150 mm, 0.1% TFA–H_2O→0.04% TFA–MeCN, 20 min gradient, 0.5 mL min^{-1}, observing at 254 nm, unless stated otherwise.

N-(2-Methoxyethyl)benzamide was prepared by Schotten–Baumann acylation of 2-methoxyethylamine using benzoyl chloride, in 96% yield.[22] *N*-Phenylacetyl-2-aminomethyltetrahydrofuran (mp 60–61 °C) was prepared by Schotten–Baumann acylation of tetrahydrofurfuryl amine using phenylacetyl chloride, in 52% yield.[22] 2′,3′-(*O*-isopropylidene)uridine 5′-aldehyde was prepared in 53% yield by oxidation of 2′,3′-*O*-(isopropylidene)uridine by the method of Corey and Samuelsson.[18] ^{14}C-UDPMurNAc-pentapeptide (specific activity 160 μCi $μmol^{-1}$) was a gift from SmithKline Beechams Pharmaceuticals.

Anodic oxidation of *N*-(2-methoxyethyl)benzamide

N-(2-Methoxyethyl)benzamide (500 mg, 2.79 mmol, 1 equiv.) and tetrabutylammonium toluene-*p*-sulfonate (345 mg, 0.04 M) were dissolved in methanol (20 mL). A graphite rod anode and a platinum mesh cathode were inserted into the solution and a steady current of 75 mA applied to give a current density of 10 mA cm^{-2}. The reaction mixture was stirred at room temperature for 7 h then the methanol removed *in vacuo*. The residue was taken up in dichloromethane and washed with saturated brine. The aqueous layer was re-extracted with dichloromethane (2 × 20 mL) and the combined organics dried over $MgSO_4$ and concentrated *in vacuo* to yield a brown oil. TLC (2:1 EtOAc–cyclohexane) showed three components, R_f 0.41, 0.31 and 0.00. Column chromatography allowed the isolation of a single component (R_f 0.31), shown to contain a 1:2 mixture of α-methoxy amide **4a** and *N*-acylimine **4b**, as an orange oil, 191 mg, 19%; LR-ESMS (+ve ion) MH^+ 178.0; IR (liquid film) 3333 (NH), 1649 (C=O) cm^{-1}; δ_H (300 MHz, $CDCl_3$, COSY analysis) **4b**, major product: 7.82 (2H, m), 7.58–7.40 (3H, m), 7.05 (1H, br s, *H*C=N, cross-peak to δ_H 4.92), 4.92 (2H, d, J = 7 Hz, N=CHC*H*$_2$, cross-peak to δ_H 7.05), 3.40 (3H, s, OC*H*$_3$); **4a**, minor product: 7.82 (2H, m), 7.58–7.40 (3H, m), 6.95 (1H, br d, J = 10 Hz, NH, cross-peak to δ_H 5.51), 5.51 (1H, dt, J = 10, 3 Hz, C*H*CH$_2$OMe, cross-peaks to δ_H 6.95, 3.6), 3.68 (1H, dd, J = 10, 3 Hz) and 3.52 (1H, dd, J = 10, 3 Hz, C*H*$_2$OMe, cross-peak to δ_H 5.51), 3.45 (3H, s, OMe), 3.40 (3H, s, OMe); δ_C (75.42 MHz, $CDCl_3$) (of mixture) 167.98, 133.83, 131.97, 128.67, 127.12, 127.09, 79.40, 73.84, 71.88, 59.64, 56.21, 56.17.

Anodic oxidation of *N*-phenylacetyl-2-aminomethyltetrahydrofuran

N-Phenylacetyl-2-aminomethyltetrahydrofuran (500 mg, 2.28 mmol, 1 equiv.), tetrabutylammonium toluene-*p*-sulfonate (432 mg), and sodium carbonate (242 mg, 2.28 mmol, 1 equiv.) were dissolved in methanol (20 mL). A graphite rod anode and a platinum mesh cathode were inserted into the solution and a steady current of 40 mA applied to give a current density of 10 mA cm^{-2}. The reaction mixture was stirred at room temperature for 7.5 h, then the solvent was removed *in vacuo*. The residue was taken up in dichloromethane and washed with brine. The aqueous layer was re-extracted with dichloromethane (2 × 20 mL) and the combined fractions dried over $MgSO_4$ and concentrated *in vacuo* to yield a brown oil. TLC showed three components (EtOAc; R_f 0.44, 0.28, 0.14). Column chromatography allowed the isolation of the product at R_f 0.28 as a light yellow oil (74 mg, 13%), which was identified by electrospray mass spectrometry as predominantly the α-methoxy amide (**6a**, MH^+ 250.2). The product at R_f 0.44 was isolated as a white solid (84 mg, 21%), and was identified by NMR spectroscopy as *N*-methoxymethyl(phenyl)acetamide **7**. The sample of **6a** was dissolved in ethyl acetate (5 mL) and stirred over silica gel (500 mg) for 5.5 h. The silica gel was removed by filtration and the solvent removed *in vacuo* to yield a colourless oil. Column chromatography (EtOAc) allowed the isolation of a component at R_f 0.28 as a colourless oil (16 mg, 25% from **6a**), found by spectroscopic analysis to be enamide **6c**, present as a mixture of *E*/*Z* isomers. Samples of **6c** were found to be unstable towards storage.

Found for **6c**: LR-ESMS (+ve ion) MH^+ 218.0; δ_H (300 MHz, $CDCl_3$) (isomer A) 7.45–7.20 (5H, m), 6.23 (1H, br s, NH), 5.82 (1H, br s, NC*H*=C), 4.13 (2H, d, J = 5 Hz, C*H*$_2$O), 3.63 (2H, s, PhC*H*$_2$), 2.55 (2H, t, J = 7 Hz, C*H*$_2$C=C), 1.96–1.80 (2H, m); δ_H (300 MHz, $CDCl_3$) (isomer B) 7.45–7.20 (5H, m, aromatic H), 6.23 (1H, br s, NH), 5.69 (1H, br s, NC*H*=C), 4.09 (2H, d, J = 4 Hz, C*H*$_2$O), 3.63 (2H, s, PhC*H*$_2$), 2.38 (2H, t, J = 7 Hz, C*H*$_2$C=C), 1.82–1.68 (2H, m).

Found for **7**: LR-ESMS (+ve ion) MH^+ 165.9, MNa^+ 188.0; IR (liquid film) 3241 (NH), 1639 (C=O) cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.82 (2H, dt, J = 2, 7 Hz), 7.54 (1H, tt, J = 2, 7 Hz), 7.45 (2H, tt, J = 2, 7 Hz), 7.02 (1H, br s, NH), 4.91 (2H, d, J = 7 Hz, NHC*H*$_2$OMe), 3.40 (3H, s, OC*H*$_3$); δ_C (75.42 MHz, $CDCl_3$) 168.22, 133.98, 132.11, 128.80, 127.27, 72.02, 56.27.

Reduction of 2-(phenylacetamidomethylene)tetrahydrofuran

Crude 2-(phenylacetamidomethylene)tetrahydrofuran **6c** (16 mg, 0.07 mmol, 1 equiv.) was dissolved in methanol (1 mL) and a trace of bromocresol green added. The yellow colour was maintained by the addition of 2 M HCl–methanol whilst sodium cyanoborohydride (8 mg, ~2 equiv.) was added. Over the next 2 h a further 2 equiv. of sodium cyanoborohydride were added whilst the reaction was stirred at room temperature. The reaction was then neutralised with 2 M NaOH and the solvent removed *in vacuo*. The residue was partitioned between water and ethyl acetate (3 × 25 mL), the combined fractions were washed with saturated brine, dried over $MgSO_4$ and concentrated *in vacuo* to yield a colourless oil. Column chromatography (2:1 EtOAc–cyclohexane) allowed the isolation of 2-(phenylacetamidomethyl)tetrahydrofuran as a white solid, R_f 0.43, 10 mg, 63%; LR-ESMS (+ve ion) MH^+ 220.1, MNa^+ 242.2; δ_H (300 MHz, $CDCl_3$) 7.32–7.18 (5H, m), 5.68 (1H, br s, NH), 3.85 (1H, qd, J = 7, 3 Hz, OC*H*CH$_2$N), 3.61 (2H, t, J = 6

Hz, NHCH_2), 3.52 (2H, s, PhCH_2), 3.43 (1H, ddd, J = 14, 6, 3 Hz, CH_2O), 3.14 (1H, dt, J = 14, 6 Hz, CH_2O), 1.94–1.65 (3H, m) and 1.49–1.38 (1H, m).

Reaction of phenylacetamide with 1,1,2-trimethoxyethane

Phenylacetamide (200 mg, 1.48 mmol, 1 equiv.), 1,1,2-trimethoxyethane (178 mg, 1.48 mmol, 1 equiv.) and toluene-*p*-sulfonic acid (10 mg) were dissolved in methanol (10 ml) and heated to reflux. After 30 min the reaction was cooled and the solvents removed *in vacuo*. Column chromatography (silica gel pre-washed with 1% diethylamine, EtOAc) allowed the isolation of a beige solid R_f 0.27, 62 mg, 22%. ^{1}H NMR spectroscopy showed a mixture of α-methoxy amide **5a**, imine **5b**, and enamide **5c**, which were assigned by comparison with spectra obtained for **4a** and **4b**. After storage at −20 °C TLC showed degradation to four components (EtOAc R_f 0.43, 0.22, 0.18, 0.0); δ_H (300 MHz, $CDCl_3$) (of mixture) 7.41–7.25 (6H, m), 6.44 (1H, d, J = 6 Hz, N=CH of imine **5b**), 6.20 (1H, br s, NH of α-methoxy amide **5a**), 5.45 (1H, br s) and 5.73 (1H, br s, HC=CH of enamide **5c**), 4.65 (1H, m, CHOCH$_3$ of **5a**), 4.19 (2H, d, J = 6 Hz, CH_2OCH$_3$ of **5b**), 3.76 (1H, dd, J = 4, 9 Hz) and 3.46 (1H, dd, J = 4, 9 Hz, CH_2OCH$_3$ of **5a**), 3.61, 3.60, 3.59 (5H, s, PhCH_2 + OCH$_3$), 3.23 (3H, s, CHOCH_3 of **5a**).

Triphenylphosphine *N*-Phenylacetylimide[23]

Phenylacetyl chloride (428 μL, 500 mg, 3.2 mmol, 1 equiv.) was dissolved in freshly distilled THF (1 mL) and the solution cooled in an ice bath. Trimethylsilyl azide (559 mg, 4.9 mmol, 1.5 equiv.) was added and the reaction was stirred at 0 °C under an atmosphere of nitrogen. A further 2 equiv. of trimethylsilyl azide was added over 6 h whilst the reaction was kept at 4 °C. IR analysis was used to monitor the disappearance of acid chloride ($\nu_{C=O}$ 1805.6 cm^{-1}) and the appearance of isocyanate ($\nu_{N=C=O}$ 2270.1 cm^{-1}) and acyl azide ($\nu_{C=O}$ 1716.5 cm^{-1}, $\nu_{N=N=N}$ 2137.6 cm^{-1}). The reaction was cooled to 0 °C and a solution of triphenylphosphine (1.87 g, 7.1 mmol, 2.2 equiv.) in dry THF (5 mL) was added dropwise over 15 min causing effervescence. The resultant yellow solution was stirred for a further 30 min, then solvent was removed *in vacuo*. Column chromatography (2:1 EtOAc–cyclohexane) allowed the isolation of triphenylphosphine *N*-phenylacetylimide **9** as a white solid, 1.17 g, 91%, R_f 0.40: mp 87–88 °C; LR-ESMS (+ve ion) MH$^+$ 396.5, MNa$^+$ 418.5; IR (nujol mull) 1598, 1461, 1347, 1255 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.75–7.66 (6H, m), 7.58–7.51 (2H, m), 7.49–7.40 (9H, m), 7.32 (2H, t, J = 7 Hz, 2-H, 6-H of PhCH$_2$), 7.22 (1H, t, J = 7 Hz, 4-H of PhCH$_2$), 3.82 (2H, d, J = 2 Hz, PhCH_2); δ_C (75.42 MHz, $CDCl_3$) 183.01 (d, J = 10 Hz, C-5′), 138.59, 133.20 (d, J = 11 Hz), 132.31 (d, J = 9 Hz), 129.72, 128.95, 128.71 (d, J = 13 Hz), 128.22, 127.65, 126.03, 48.04 (d, J = 20 Hz, PhCH$_2$).

5′-(*N*-Phenylacetyl)amino-5′-deoxy-4′,5′-didehydro-2′,3′-*O*-(isopropylidene)uridine

Triphenylphosphine *N*-phenylacetylimide **9** (250 mg, 0.6 mmol, 1 equiv.) in dry toluene (10 mL) was treated with a solution of 2′,3′-*O*-(isopropylidine)uridine 5′-aldehyde (178 mg, 0.6 mmol, 1 equiv.) in dry THF (1 mL). After 48 h at reflux the reaction was cooled and solvent was removed *in vacuo*. Repeated column chromatography (2:1 EtOAc–cyclohexane, R_f 0.31; 25:1 DCM–MeOH R_f 0.10) allowed the isolation of enamide (**10**) as a white solid, 30 mg, 12.5%: analytical RP-HPLC R_t 12.4 min (65%), R_t 12.8 min (35%); LR-ESMS (+ve ion) MH$^+$ 400.3, MNa$^+$ 422.3; HR-FABMS (+ve ion) MH$^+$ 400.1539 (calc. 400.1506); IR (nujol mull)/cm^{-1} 3445 (NH), 1652 (br, C=O); δ_H (300 MHz, $CDCl_3$) (major isomer) 9.50 (1H, br s, NH), 7.23–7.42 (5H, m), 7.12 (1H, br d, J = 10 Hz, NHC=C), 7.03 (1H, d, J = 8 Hz, uracil 6-H), 6.50 (1H, d, J = 10 Hz, NCH=C), 5.72 (1H, d, J = 8 Hz, uracil 5-H), 5.73 (1H, s, 1′-H), 5.32 (1H, d, J = 6 Hz, 3′-H), 5.02 (1H, dd, J = 1, 6 Hz, 2′-H), 3.62 (2H, s, PhCH_2), 1.50 and 1.38 (2 × 3H, s, CH$_3$); δ_H (300 MHz, $CDCl_3$) (minor isomer) 9.50 (1H, br s, NH), 7.23–7.42 (5H, m), 7.19 (1H, br d, J = 10 Hz, NHC=C), 7.14 (1H, d, J = 8 Hz, uracil 6-H), 6.65 (1H, dd, J = 1, 10 Hz, NCH=C), 5.72 (1H, d, J = 8 Hz, uracil 5-H), 5.62 (1H, s, 1′-H), 5.38 (1H, dd, J = 1, 6 Hz, 3′-H), 4.95 (1H, d, J = 7 Hz, 2′-H), 3.67 (2H, s, PhCH_2), 1.46 and 1.35 (2 × 3H, s, CH$_3$); δ_C (75.42 MHz, $CDCl_3$) (mixture of isomers) 167.75, 163.32, 150.03, 142.51 and 142.39 (C-4′), 141.79, 134.32, 132.32, 132.19, 129.67, 129.47, 129.12, 128.77, 128.61, 127.59, 114.16, 103.28 and 103.03 (C-5′), 101.91, 96.13, 83.27, 79.00, 43.53, 27.00, 26.03, 25.14 and 24.26.

5′-(*N*-Phenylacetyl)-amino-5′-deoxy-4′,5′-(didehydro)uridine

A solution of 5′-(*N*-phenylacetyl)amino-5′-deoxy-4′,5′-didehydro-2′,3′-*O*-(isopropylidene)uridine **10** (5 mg, 13 μmol, 1 equiv.) in 2:1 H_2O–MeOH (1.5 mL) was stirred over Dowex-H$^+$ ion exchange resin (50 mg) at 50 °C for 5 h. The cooled reaction mixture was filtered to remove resin, then the solvent was removed *in vacuo* and the residue lyophilised to yield the title compound as a white solid, 4 mg, 89%. LR-ESMS (+ve ion) MH$^+$ 360.4; ^{1}H NMR δ_H (300 MHz, D_2O) (major isomer) 7.4–7.5 (5H, m), 7.29 (1H, d, J = 8 Hz, uracil 6-H), 6.07 (1H, s, NCH=C), 5.96 (1H, d, J = 4 Hz, 1′-H), 5.69 (1H, d, J = 8 Hz, uracil 5-H), 5.1–5.3 (2H, m), 3.48 (2H, s, PhCH$_2$); δ_H (300 MHz, D_2O) (minor isomer) 7.4–7.5 (5H, m), 7.38 (1H, d, J = 8 Hz, uracil 6-H), 6.36 (1H, s, NCH=C), 5.84 (1H, d, J = 4 Hz, 1′-H), 5.57 (1H, d, J = 8 Hz, uracil 5-H), 5.1–5.3 (2H, m), 3.51 (2H, s, PhCH$_2$).

RP-HPLC investigation of enamide 10 isomers

A 25 μL aliquot of solution of 5′-(*N*-phenylacetyl)amino-5′-deoxy-4′,5′-didehydro-2′,3′-*O*-(isopropylidene)uridine **10** (1.0 mg, 2.5 μmol) in 1:1 MeOH–H_2O (1 mL) was diluted in 225 μL H_2O. 100 μL of this sample was analysed by analytical RP-HPLC. The two observed peaks were collected [R_t 12.4 min (65%), R_t 12.8 min (35%)] analysed by LR-ESMS (+ve ion) and then allowed to stand at room temperature for 2 h. The samples were then re-analysed by RP-HPLC as before. Peak (a): R_t 12.4 min, MH$^+$ 400.3; re-injection after 2 h, R_t 12.4 min (100%). Peak (b): R_t 12.8 min, MH$^+$ 400.3; re-injection after 2 h, R_t 12.8 min (100%).

Stability studies on 5′-(*N*-phenylacetyl)amino-5′-deoxy-4′,5′-didehydro-2′,3′-*O*-(isopropylidene)uridine 10

A series of 1 mL incubations of enamide **10** (1.0 mg, 2.5 μmol, 1 equiv.) were stirred at room temperature (unless otherwise stated), under the following conditions: (i) 1:1 MeOH–H_2O; (ii) 0.1 M NH_4OAc, pH 6.8, 1:1 MeOH–H_2O; (iii) 0.1 M NH_4OAc, pH 5.0, 1:1 MeOH–H_2O; (iv) 0.1 M NH_4OAc, pH 3.8, 1:1 MeOH–H_2O; (v) 1:1 MeOH–g·AcOH; (vi) 1% $EtNH_2$, 1:1 MeOH–H_2O, (10 μL g·AcOH added after 20 h); (vii) 1% EtSH, 1:1 MeOH–H_2O, (10 μL g·AcOH added after 20 h); (viii) 1 equiv. $AlCl_3$, 1.1 equiv. EtSH, dry THF; (ix) 1 equiv. $ZnBr_2$, 1.1 equiv. EtSH, dry THF; (x) BF_3.OEt_2, 1.1 equiv. EtSH, dry THF; (xi) 10% TFA, 1:1 MeOH–H_2O; (xii) g·AcOH, 50 °C; (xiii) 0.1 M NH_4OAc, g·AcOH, 50 °C. For LR-ESMS (+ve ion) analysis 10 μL samples were removed and diluted into 1 mL MeCN. After 24 h, 25 μL aliquots were removed and diluted with 225 μL H_2O for analysis by analytical RP-HPLC and LR-ESMS. A standard of 1 mg mL^{-1} **10** was analysed under identical conditions.

Stability studies on MRD A

Five solutions containing 1 mg mL^{-1} mureidomycin A **1** were prepared under the following conditions, and were stirred at room temperature: (i) 10% aqueous AcOH; (ii) 50% aqueous AcOH; (iii) glacial AcOH; (iv) 50% aqueous AcOH, 50 mM

NaOAc; (v) 50% aqueous AcOH, 100 mM NaOAc. At time points ($t = 0$, 5, 15, 30 min, 1, 2, 6, 48 h), 20 μL samples were removed and diluted into 1 mL water for LR-ESMS (+ve ion) analysis. After 48 h samples were stored at −20 °C before analysis by semi-prep RP-HPLC and LR-ESMS. A standard of 1 mg mL^{-1} aqueous MRD A was analysed under identical conditions.

Characterisation of compounds as inhibitors in the radiochemical assay

Aqueous solutions of enamide diol **11** were prepared at known concentrations, as determined by UV absorbance at 262 nm ($\varepsilon_{\text{uridine}} = 10^4$ M^{-1} cm^{-1}). *Escherichia coli* translocase 1 was prepared from overexpression construct JM109/pBROC525, as previously described,[7] and was solubilised in 0.5% Triton X-100. Radiochemical assays were performed, as previously described,[7] in the presence of 10 μL solubilised *E. coli* translocase I (4 mg mL^{-1} protein), 12.5 μM ^{14}C-UDP-MurNAc-pentapeptide (64 nCi $nmol^{-1}$) and 10 μM dodecaprenyl phosphate. All assays were performed in triplicate.

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References

1 F. Isono, M. Inukai, S. Takahashi, T. Haneishi, T. Kinoshita and H. Kuwano, *J. Antibiot.*, 1989, **42**, 667.
2 R. H. Chen, A. M. Buko, D. N. Whittern and J. B. McAlpine, *J. Antibiot.*, 1989, **42**, 512.
3 S. Chatterjee, S. R. Nadkarni, E. K. S. Vijayakumar, M. V. Patel, B. N. Ganguli, H. W. Fehlhaber and L. Vertesy, *J. Antibiot.*, 1994, **47**, 595.
4 F. Isono, T. Katayama, M. Inukai and T. Haneishi, *J. Antibiot.*, 1989, **42**, 674.
5 T. D. H. Bugg and C. T. Walsh, *Nat. Prod. Rep.*, 1992, **9**, 199.
6 F. Isono and M. Inukai, *Antimicrob. Agents Chemother.*, 1991, **35**, 234.
7 P. E. Brandish, M. K. Burnham, J. T. Lonsdale, R. Southgate, M. Inukai and T. D. H. Bugg, *J. Biol. Chem.*, 1996, **271**, 7609.
8 M. Michel and L. Gutmann, *Lancet*, 1997, **349**, 1901.
9 G. R. Lenz, *Synthesis*, 1978, 489.
10 W. N. Speckamp and H. Hiemstra, *Tetrahedron*, 1985, **41**, 4367.
11 M. G. Heydanek, W. G. Struve and F. C. Neuhaus, *Biochemistry*, 1969, **8**, 1214.
12 T. Shono, H. Hamaguchi and Y. Matsumara, *J. Am. Chem. Soc.*, 1975, **97**, 4264.
13 T. Shono, Y. Matsumara and K. Tsubata, *Org. Synth.*, 1985, **53**, 206.
14 T. Shono, Y. Matsumara, K. Tsubata, Y. Sugihara, S. Yamane, T. Kanazawa and T. Aoki, *J. Am. Chem. Soc.*, 1982, **104**, 6697.
15 H. Bohme and G. Berg, *Chem. Ber.*, 1966, **99**, 2127.
16 H. E. Schoemaker, T. Boer-Terpstra, J. Dijkink and W. N. Speckamp, *Tetrahedron*, 1980, **36**, 143.
17 T. Okano, T. Sakaida and S. Eguchi, *J. Org. Chem.*, 1996, **61**, 8826.
18 E. J. Corey and B. Samuelsson, *J. Org. Chem.*, 1984, **49**, 4735.
19 K. C. Nicolaou and W. M. Dai, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1387.
20 J. F. Morrison and C. T. Walsh, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 1987, **57**, 201.
21 P. E. Brandish, K. I. Kimura, M. Inukai, R. Southgate, J. T. Lonsdale and T. D. H. Bugg, *Antimicrob. Agents Chemother.*, 1996, **40**, 1640.
22 A. I. Vogel, *Vogel's Textbook of Practical Organic Chemistry*, 1989, Longman Scientific, London.
23 P. Froyen, *Phosphorus, Sulfur Silicon Relat. Elem.*, 1993, **78**, 161.

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