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Pleuromutilins. Part 1: The Identification of Novel Mutilin 14-Carbamates

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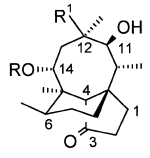
Abstract—A novel series of mutilin 14-carbamates has been discovered as a result of structure–activity studies on the naturally occurring antibiotic pleuromutilin (**1**). In particular, the 4-methoxybenzoylcarbamate, SB-222734 (**15o**) displays potent antibacterial activity against a number of bacterial pathogens which are resistant to currently used agents and shows enhanced metabolic stability when compared to earlier pleuromutilin derivatives. Such derivatives therefore have the potential to provide a new class of antibacterial agents for human therapy which address the threat of bacterial resistance.

Introduction

The emergence and spread of resistance to existing antibiotics (e.g. β-lactams, macrolides, quinolones) is of major concern world-wide. Although initially viewed as a problem for the hospital setting, bacterial resistance is now also an accepted issue for community acquired infections.[1] There is, therefore, an urgent need to identify and exploit new classes of antibacterial agents, with modes of action which are distinct from those of established classes, in order to circumvent the issue of bacterial resistance.

One approach to this goal is to re-investigate those known antibiotics, and their targets, which have so far found little or no utility in human medicine. One such under-exploited antibiotic is pleuromutilin (**1**) which was first isolated in 1951 from the basidiomycete *Pleurotus mutilus*.[2] The unusual tricyclic diterpenoid structure of the natural product was elucidated in the 1960s by Arigoni[3] and Birch[4] and subsequently confirmed by X-ray crystallography.[5] Pleuromutilin exerts its antibacterial activity by inhibiting bacterial protein synthesis through an interaction with the prokaryotic ribosome.[6] Although the precise molecular interactions are not yet known its mode of action appears to be unique since, in laboratory studies, no clinically relevant cross-resistance is seen with other classes of antibacterial agents. However, to date, no pleuromutilin derivative has been successfully developed for human therapy (Chart 1).



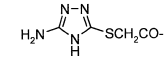
Pleuromutilin	(**1**)	R = $HOCH_2CO$-	$R^1 = {}^{19}CH={}^{20}CH_2$
Tiamulin	(**2**)	R = $Et_2NCH_2CH_2SCH_2CO$-	$R^1 = CH=CH_2$
TDM 85,530	(**3**)	R = 	$R^1 = CH_2CH_3$
Mutilin	(**4**)	R = H	$R^1 = CH=CH_2$

Chart 1.

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Semi-synthetic pleuromutilins were investigated during the 1970s and early 1980s by the then Sandoz group. This work led to a number of publications detailing some of the intriguing chemistry of the mutilin nucleus, although details of SAR remained sparse. Those SAR studies which have been reported consist mainly of manipulations of the pleuromutilin C14 glycolic acid side chain.[7] As a result of these efforts, two thioacetate derivatives, tiamulin (**2**) and TDM-85,530 (**3**), were identified, both of which have improved antibacterial activity compared to pleuromutilin itself. Tiamulin was successfully developed as a veterinary antibiotic, whereas TDM-85,530 entered phase I studies in volunteers but was not progressed.[8]

Semi-synthetic pleuromutilins display many of the properties appropriate for an oral antibiotic for human therapy (e.g. appropriate spectrum of activity; efficacy following oral administration in in vivo infection models) but suffer from having only moderate potency against *Haemophilus influenzae* (an important respiratory tract pathogen) and from being rapidly and extensively metabolised in vivo, thus limiting their oral bioavailability. Here, we describe some of our own investigations in the pleuromutilin area. These studies culminated in the discovery of a novel series of mutilin 14-carbamates with potent antibacterial activity, including activity against strains resistant to current therapies, and with enhanced metabolic stability over previous analogues.

Results and Discussion

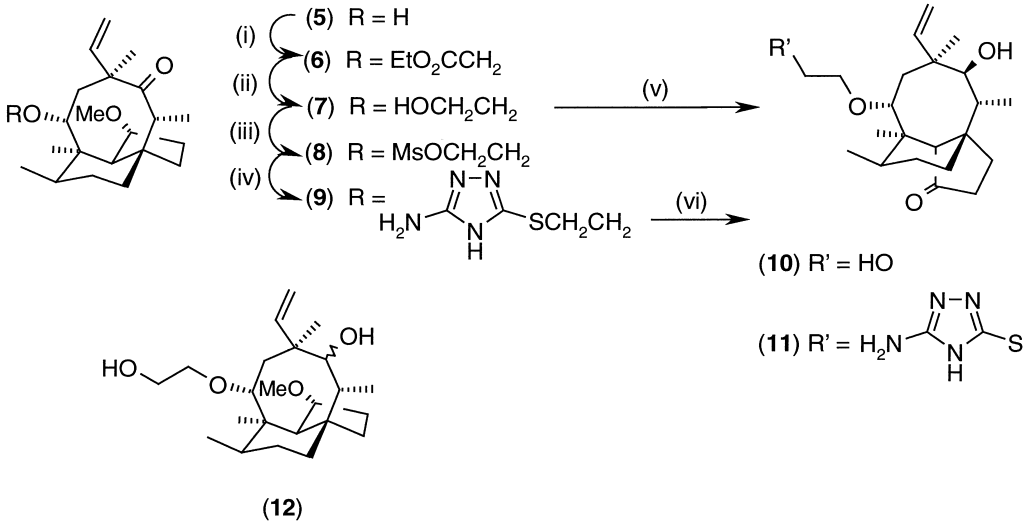
The early studies by the Sandoz group had demonstrated that a C14 side chain was essential for antibacterial activity; the tricyclic diol, mutilin (**4**), did not inhibit protein synthesis on isolated *Escherichia coli* ribosomes and was devoid of useful antibacterial activity.[7b] We wished to further explore the SAR associated with this side chain so as to assess the importance of the C14 acyloxy group per se, rather than substituted variants thereof. To this end, we targeted a number of novel C14 ether, carbamate, amide and urea linked derivatives.

Chemistry

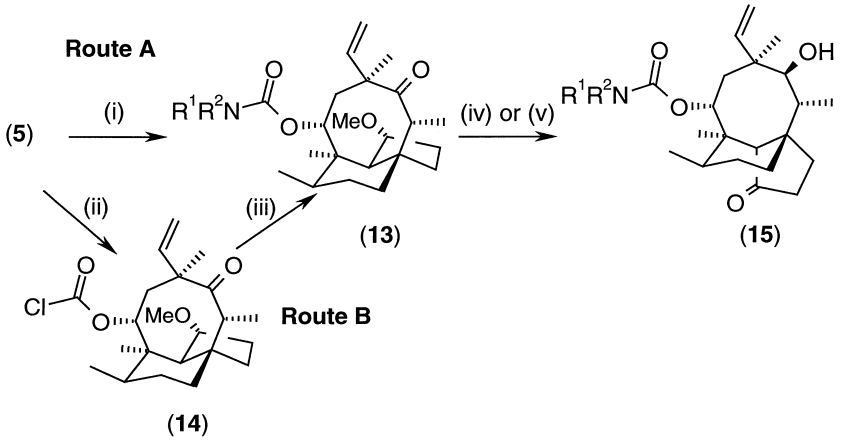
Central to the synthesis of the targeted *O*-linked analogues (ethers and carbamates) is the skeletally rearranged intermediate, colloquially known as 4-*epi*-mutilin (**5**). 4-*epi*-Mutilin is readily available in two steps from pleuromutilin via an acid-catalysed 1,5-hydride shift[9] and serves as a mutilin surrogate in which the C11 hydroxyl group has been protected, thus allowing selective functionalisation at the less reactive C14 hydroxyl group.

C14 ether analogues of pleuromutilin and TDM-85,530 were prepared as shown in Scheme 1. Reaction of 4-*epi*-mutilin (**5**) with ethyl diazoacetate in the presence of a rhodium II catalyst generated the mutilin-oxyacetate derivative (**6**). Subsequent reduction of this keto-ester with lithium aluminium hydride resulted in a 3:2 mixture of the desired keto-alcohol (**7**) and the diol (**12**), as a result of the steric protection afforded to the C11 ketone. Treatment of **7** with a saturated solution of zinc chloride in concentrated hydrochloric acid then effected a reverse 1,5-hydride shift[9] regenerating the mutilin configuration and, in this particular case, the pleuromutilin ether analogue (**10**). The 19,20 didehydro-ether analogue of TDM-85,530 (**11**) was also prepared from the keto-alcohol (**7**) via the 4-*epi*-mutilin derivatives (**8**) and (**9**).

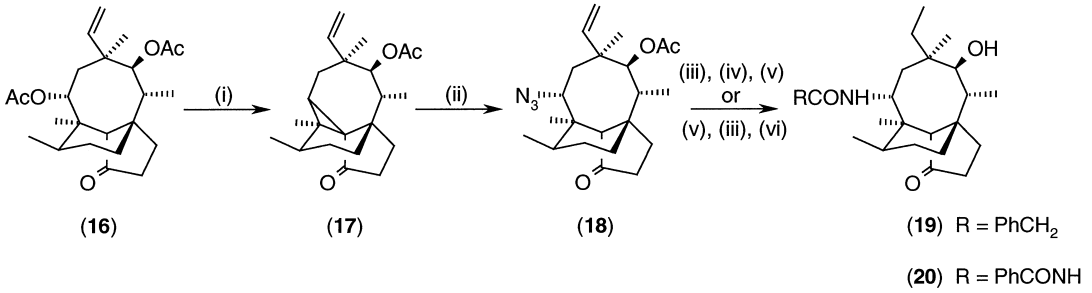
The C14-carbamate derivatives were prepared by one of two routes shown in Scheme 2. In route A, 4-*epi*-mutilin (**5**) was reacted with an isocyanate or a carbamoyl chloride to form a 4-*epi*-mutilin 14-carbamate derivative (**13**). Unactivated isocyanates and carbamoyl chlorides reacted very slowly with 4-*epi*-mutilin, requiring 5 to 7 days at room temperature for complete reaction. The



Scheme 1. Synthesis of mutilin 14-ether derivatives. Reagents and conditions: (i) ethyl diazoacetate, $Rh_2(OAc)_4$, dichloromethane; (ii) lithium aluminium hydride, diethyl ether; (iii) methanesulphonyl chloride, pyridine, dichloromethane; (iv) 3-Amino-5-mercapto-1,2,4-triazole, sodium methoxide, acetone/methanol; (v) zinc chloride, conc. hydrochloric acid, dioxane; (vi) conc. hydrochloric acid, dioxane.



Scheme 2. Synthesis of mutilin 14-carbamates. Reagents and conditions: (i) R^1NCO or R^1R^2NCOCl, THF; (ii) trichloromethyl chloroformate, triethylamine, THF; (iii) R^1R^2NH, dichloromethane; (iv) zinc chloride, conc. hydrochloric acid, dioxane; (v) conc. hydrochloric acid, dioxane.



Scheme 3. Synthesis of 14-acylamino mutilin derivatives. Reagents and conditions: (i) potassium *tert* butoxide, DMF; (ii) sodium azide, DMF, 150 °C; (iii) hydrogen gas, 10% palladium on charcoal, THF; (iv) phenylacetyl chloride, triethylamine, chloroform; (v) sodium hydroxide, ethanol; (vi) benzoylisocyanate, dichloromethane.

reaction with activated sulphonyl- or acylisocyanates,[10] on the other hand, was complete within minutes. The alternative, three-step process, route B, proceeds via the stable, crystalline 4-*epi*-mutilin 14-chloroformate (**14**) which is formed by treatment of 4-*epi*-mutilin with trichloromethyl chloroformate. Reaction of the chloroformate (**14**) with primary or secondary amines then leads to a variety of 4-*epi*-mutilin 14-carbamate derivatives, in high yield. The later route has the advantage over route A, in that it allows access to a more diverse range of carbamates due to the ready availability of primary and secondary amines. Once more, strong acid treatment effects rearrangement of the 4-*epi*-mutilin 14-carbamates (**13**) to the corresponding mutilin derivatives (**15**).

In contrast to the ether and carbamate derivatives previously discussed, the synthesis of the *N*-linked analogues proved far more taxing (Scheme 3). Substitution, as opposed to functionalisation, of the mutilin C14 hydroxyl group is extremely difficult; the appropriate trajectory for an incoming nucleophile during S_N2 displacement of the C14 hydroxyl group (or other leaving group) is blocked by C10 and attempts to introduce substituents by an S_N1 type mechanism are thwarted by another transannular 1,5-hydrogen shift from C10 to C14 (Fig. 1) which results in expulsion of the C14 leaving group.[11] In an attempt to circumvent these problems, we proposed to make use of yet another of the vagaries of mutilin chemistry, namely the 4,14-cyclopropane (**17**),[12]

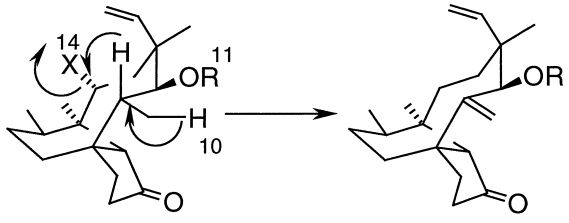


Figure 1.

Table 1. Antibacterial activity of novel mutilin derivatives

Compound	IVT[b]	MIC (μg/mL)[a]					
		S.a. 1	*S.a.* 2	*S.p*	*H.i.*	*M.c.*	*E.c.*
1	71	0.5	0.25	2	1	0.25	2
2	77	0.25	0.125	0.125	8	≤0.06	8
3	65	0.25	0.125	0.25	4	0.125	8
10	nd	>64	>64	32	>64	64	>64
11	18	64	32	32	>64	32	>64
19	29	>4	>4	>4	>64	>4	>64
20	0	>4	>4	>4	>64	>4	>64

[a]*S.a.* 1, *Staphylococcus aureus* Oxford (methicillin sensitive). *S.a.* 2, *Staphylococcus aureus* V573 (methicillin resistant); *S.p.*, *Streptococcus pneumoniae* ERY2 (macrolide resistant); *H.i., Haemophilus influenzae* WM493 (penicillin resistant); *M.c., Moraxella catarrhalis* Ravasio (penicillin resistant); *E.c,. Escherichia coli* DC2, permeability mutant.
[b]IVT, percent inhibition of in vitro protein synthesis (*poly* Phe) in presence of 1 μM drug. nd, not determined.

since such conjugated cyclopropanes are known to be susceptible to nucleophilic ring opening.[13] The cyclopropane (**17**) was conveniently prepared in 32% yield by treatment of mutilin 11,14-diacetate (**16**) with potassium *tert*-butoxide in DMF. However, this cyclopropane proved stubbornly resistant to a variety of ring opening conditions. Only treatment with sodium azide in DMF at 150 °C proved successful, generating the C14-azide (**18**) in low yield. This enabled us to complete the synthesis of the desired C14 amino derivatives by reduction of the azide, along with concomitant reduction of the 19,20 double bond, followed by acylation of the resultant amine and saponification of the 11-acetate.

Biological results

The antibacterial activity of the ethers (**10** and **11**), amide (**19**) and acylurea (**20**) mutilin derivatives are shown in Table 1, with pleuromutilin, tiamulin and TDM-85,530 included for comparative purposes. These novel derivatives all display considerably reduced antibacterial activity, which is also reflected in the lack of, or significantly reduced, activity in an in vitro translation assay.

The carbamate derivatives (**15**), on the other hand, display potent antibacterial activity against Gram-positive and the more permeable Gram-negative bacteria, in some cases surpassing that of the standard agents. The activity of a representative selection of such derivatives is shown in Table 2, thus demonstrating the SAR observed for this series. The *N*-aryl and *N*-arylalkyl carbamates (**15a–d**) display good to excellent anti-Gram-positive activity, including activity against strains of methicillin-resistant *Staphylococcus aureus* and macrolide resistant *Streptococcus pneumoniae*. Within the series of *N*-aryl derivatives, electron donating substituents are advantageous whereas electron withdrawing substituents have a deleterious effect on antibacterial activity. The *N*-alkyl carbamates (**15e,f**) display broad-spectrum activity but are in general less potent than the *N*-aryl derivatives against the Gram-positive pathogens. *N,N*-disubstituted carbamates (**15g,h**), including cyclic derivatives (**15i**), are in all cases less potent than the corresponding mono-substituted

Table 2. Antibacterial activity of mutilin-14-carbamates

Compound	R^1	R^2	Route	MIC (μg/mL)[a]					
				S.a. 1	*S.a.* 2	*S.p*	*H.i.*	*M.c.*	*E.c.*
15a	Ph	H	A	≤0.06	≤0.06	2	64	1	64
15b	4-MeO.Ph	H	A	≤0.06	≤0.06	0.5	>64	4	>64
15c	4-NO_2.Ph	H	A	2	2	64	>64	64	>64
15d	$PhCH_2$	H	A	≤0.06	≤0.06	0.25	8	0.125	4
15e	Me	H	A	0.5	0.5	2	8	0.5	16
15f	$(CH_2)_2OH$	H	B	1	1	2	16	1	8
15g	Ph	Me	B	1	1	8	>64	16	>64
15h	Me	Me	B	4	2	4	>64	4	>64
15i	$CH_2CH_2OCH_2CH_2$		B	4	4	4	>64	8	>64
15j	H	H	A[b]	0.5	0.125	0.5	4	0.125	2
15k	HO	H	B	0.5	0.25	1	2	0.25	0.5
15l	Me_2N	H	B	0.5	0.25	1	8	0.25	1
15m	MsNH	H	B	1	0.5	4	16	0.5	nd
15n	$PhSO_2$	H	A	≤0.06	≤0.06	0.5	8	1	32
15o	4-MeO.PhCO	H	A	≤0.06	≤0.06	≤0.06	1	≤0.06	1

[a]See footnote to Table 1 for abbreviations.
[b]**15j** was prepared by the reaction of 4-*epi*-mutilin with trichloroacetyl isocyanate followed by hydrolysis of the trichloroacetylcarbamate.

Table 3. Antibacterial activity of mutilin 14-(4-methoxybenzoyl)carbamate (**15o**, SB-222734)[a]

		MIC (μg/mL)			
		15o	*Amoxycillin*	*Clarithromycin*	*Chloramphenicol*
S. aureus ($n=40$)	MIC_{90}	0.06	>128	>64	16
	MIC_{50}	≤0.015	64	>64	8
	MIC range	≤0.015–4	≤0.125–>128	≤0.06–>64	0.06–>32
S. pneumoniae ($n=40$)	MIC_{90}	0.5	No data	>512	16
	MIC_{50}	0.25	No data	0.06	4
	MIC range	0.03–0.5	No data	≤0.03–>512	2–16
H. influenzae ($n=40$)	MIC_{90}	2	64	32	8
	MIC_{50}	1	4	16	0.5
	MIC range	0.125–4	0.125–>64	4–64	0.25–16

[a]The extended panel of organisms have the following resistance profiles. *S. aureus*: macrolide, fusidic acid and ciprofloxacin resistant strains, many with multi-resistant characteristics, including 22 methicillin-resistant organisms. *S. pneumoniae*: 18 penicillin, 16 clarithromycin, 17 chloramphenicol and six ofloxacin resistant strains including many with multi-resistant profiles. *H. influenzae*: 12 β-lactamase producers, 11 clarithromycin and six tetracycline resistant strains.

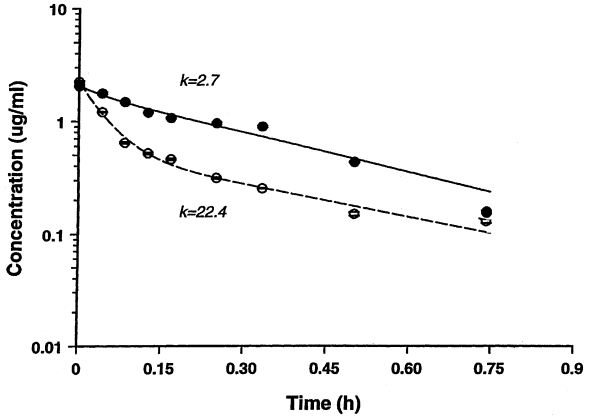


Figure 2. Comparative disappearance curves for SB-222734 (**15o**, closed circles) and tiamulin (open circles) in a mouse liver microsome preparation.

derivatives. The primary carbamate (**15j**) and those derivatives bearing small polar substituents (**15k**–**m**) on the other hand, show improved potency against *Haemophilus influenzae* but at the expense of some of the anti-Gram-positive activity displayed by the aryl derivatives. However, the *N*-arylsulphonyl (**15n**) and, in particular, the *N*-aroyl carbamates (**15o**, SB-222734) combine the best features of all of the derivatives previously discussed, displaying excellent broad-spectrum activity. The activity of this latter derivative was further challenged through evaluation against an expanded selection of bacteria with differing susceptibilities to existing agents (Table 3). The novel aroyl carbamate (**15o**) was shown to possess potent in vitro antibacterial activity against *S. aureus, S. pneumoniae* and *H. influenzae* and was clearly superior to the comparator agents used in the study.

As previously mentioned, pleuromutilins such as tiamulin are rapidly eliminated in vivo by cytochrome P450-mediated hydroxylation of the tricyclic nucleus. The rate of metabolism of the novel *N*-aroylcarbamate (**15o**) was therefore compared to that of tiamulin, in an in vitro mouse live microsome preparation (Fig. 2). This study demonstrated that, in addition to its superior antibacterial activity, **15o** was significantly more stable, being eliminated at one tenth of the rate of tiamulin.

Conclusion

Our studies have thus extended knowledge of the SAR associated with the pleuromutilins as a class and, as a result, identified a novel series of potent antibacterials. In doing so, we have also demonstrated that the nature of the mutilin C14 substituent is crucial to the activity of such derivatives. Clearly the carbonyl group is an essential feature of this C14 substituent, since both the pleuromutilin and TDM-85,530 ether analogues (**10** and **11**) are devoid of activity. However, the linker atom also appears to be of significance since both the amide and acylurea derivatives are inactive, whereas the ester and carbamates are active.

The novel mutilin 14-carbamates described have potent antibacterial activity, displaying a spectrum of activity which encompasses the major respiratory tract pathogens. The 4-methoxybenzoyl carbamate (**15o**) is particularly impressive, demonstrating potent activity against a panel of organisms which are refractory to treatment with established antibacterial agents. Furthermore, **15o** also displays improved stability to mouse liver microsomes, when compared to previous semi-synthetic derivatives.

Experimental

General conditions

NMR spectra were recorded on a Bruker AC250 or AM250 spectrometer in $CDCl_3$ (unless otherwise stated) with tetramethylsilane as an internal standard. Infra-red spectra were recorded in dichloromethane solution on a Perkin-Elmer 1600 series FTIR. Mass spectra were recorded on either a Finnigan TSQ700, VG ZAB1F or VG Trio-2 spectrometer in electron impact (EI), electrospray (ESI), chemical ionisation (NH_3DCI) or fast atom bombardment (FAB) modes. Chromatography was performed on Merck silica 60, (230–400 mesh) eluting with ethyl acetate/hexane mixtures, unless otherwise specified.

(3*R*)-3-Deoxo-11-deoxy-14-*O*-(ethoxycarbonylmethyl)-3-methoxy-11-oxo-4-*epi*-mutilin (6). Ethyl diazoacetate (2.5 mL, 22.8 mmol) in dichloromethane (20 mL) was slowly added, over 6 h, to a mixture of (**5**, 2.0 g, 6 mmol) and rhodium acetate dimer (8 mg) in dichloromethane (5 mL) under argon. The reaction mixture was then concentrated and the residue purified by silica gel chromatography, eluting with dichloromethane, to give (**6**, 1.1 g, 42%); ν_{max} 1753, 1697 cm^{-1}; ^{1}H NMR 0.97 (3H, d, $J=6.7$ Hz), 0.98 (3H, d, $J=6.4$ Hz), 1.23 (3H, s), 1.24 (3H, s), 1.29 (3H, t, $J=7.2$ Hz), 1.03–1.34 (4H, m), 1.47 (2H, m), 1.67 (1H, d, $J=15.8$ Hz), 1.70 (1H, d, $J=11.2$ Hz), 1.97 (2H, m), 2.18 (1H, m), 2.42 (1H, dd, $J=15.8$, 9.5 Hz), 2.86 (1H, q, $J=6.4$ Hz), 3.21 (3H, s), 3.48 (1H, ddd, $J=11.2$, 8.0, 5.2 Hz), 3.92 (1H, d, $J=15.2$ Hz), 4.08 (1H, d, $J=9.5$ Hz), 4.15 (1H, d, $J=15.2$ Hz), 4.21 (2H, dq, $J=5.8$, 7.1 Hz), 5.10 (1H, d, $J=17.4$ Hz), 5.27 (1H, d, $J=10.7$ Hz), 6.14 (1H, dd, $J=17.4$, 10.7 Hz); MS (NH3DCI) m/z 421 (MH^+).

(3*R*)-3-Deoxo-11-deoxy-14-*O*-(2-hydroxyethyl)-3-methoxy-11-oxo-4-*epi*-mutilin (7). A solution of (**6**, 520 mg, 1.24 mmol) in THF (7 mL) was added to an ice-cooled suspension of lithium aluminium hydride (70 mg, 1.84 mmol) in THF (3 mL) under an argon atmosphere. After 4 h, the reaction was carefully quenched by the addition of water (0.1 mL), 15% sodium hydroxide (0.1 mL) and water (0.3 mL) and the mixture rapidly stirred until a fine suspension formed. The mixture was filtered and the residue washed with brine, dried (magnesium sulphate) concentrated and purified by silica gel chromatography, eluting with 1/19 ethyl acetate/dichloromethane, to give (**7**, 170 mg, 36%); ν_{max} 2930, 1697 cm^{-1}; ^{1}H NMR 0.97 (6H, d, $J=6.5$ Hz), 1.17 (3H,

s), 1.23 (3H, s), 1.02–1.37 (5H, m), 1.47 (1H, m), 1.66 (1H, d, $J=15.6$ Hz), 1.69 (1H, d, $J=11.3$ Hz), 1.98 (3H, m), 2.18 (1H, m), 2.37 (1H, dd, $J=15.6$, 9.5 Hz), 2.87 (1H, q, $J=6.5$ Hz), 3.32 (3H, s), 3.34 (1H, m), 3.52 (1H, ddd, $J=11.3$, 5.1, 2.6 Hz), 3.67 (3H, m), 4.02 (1H, d, $J=9.5$ Hz), 5.09 (1H, d, $J=17.5$ Hz), 5.27 (1H, d, $J=10.8$ Hz), 6.19 (1H, dd, $J=17.5$, 10.8 Hz); MS (NH_3DCI) m/z 379 (MH^+).

Also formed was (3*R*)-3-deoxo-14-*O*-(2-hydroxyethyl)-3-methoxy-4-*epi*-mutilin (**12**, 21%); ν_{max} 2930 cm^{-1}; ^1H NMR 0.86 (3H, d, $J=6.3$ Hz), 1.07 (3H, d, $J=6.8$ Hz), 1.09 (3H, s), 1.13 (3H, s), 1.05–1.52 (6H, m), 1.71 (1H, d, $J=11.2$ Hz), 1.92 (1H, m), 2.05 (3H, m), 2.26 (1H, m), 2.64 (1H, dd, $J=15.9$, 7.5 Hz), 3.16 (1H, m), 3.31 (3H, s), 3.61 (5H, m), 4.63 (1H, ddd, $J=11.2$, 7.3, 5.3 Hz), 5.05 (1H, d, $J=17.7$ Hz), 5.12 (1H, d, $J=11.1$ Hz), 6.07 (1H, dd, $J=17.7$, 11.3 Hz); MS (NH_3DCI) m/z 398 (MNH_4^+).

(3*R*)-3-Deoxo-11-deoxy-14-*O*-(2-methanesulphonyloxyethyl)-3-methoxy-11-oxo-4-*epi*-mutilin (8). Methanesulphonyl chloride (0.15 mL, 1.94 mmol) was added to an ice-cooled solution of (**7**, 133 mg, 0.35 mmol) and pyridine (0.3 mL, 3.7 mmol) in dichloromethane (15 mL) and the resultant solution stirred at room temperature for 18 h. The reaction mixture was then washed with 1 M hydrochloric acid and brine. The organic phase was then dried (magnesium sulphate), concentrated and the residue purified by silica gel chromatography, eluting with 1/19 ethyl acetate/dichloromethane, to give (**8**, 136 mg, 85%); ν_{max} 1698 cm^{-1}; ^1H NMR 0.95 (3H, d, $J=6.7$ Hz), 0.96 (3H, d, $J=6.4$ Hz), 1.14 (3H, s), 1.23 (3H, s), 1.02–1.36 (4H, m), 1.48 (2H, m), 1.60 (1H, d, $J=15.6$ Hz), 1.68 (1H, d, $J=11.3$ Hz), 1.98 (2H, m), 2.17 (1H, m), 2.37 (1H, dd, $J=15.6$, 9.5 Hz), 2.84 (1H, q, $J=6.5$ Hz), 3.04 (3H, s), 3.21 (3H, s), 3.48 (2H, m), 3.81 (1H, ddd, $J=10.5$, 6.8, 3.3 Hz), 3.99 (1H, d, $J=9.4$ Hz), 4.28 (1H, ddd, $J=11.2$, 5.1, 3.1 Hz), 4.36 (1H, ddd, $J=11.2$, 6.7, 3.1 Hz), 5.10 (1H, d, $J=17.5$ Hz), 5.27 (1H, d, $J=10.8$ Hz), 6.15 (1H, dd, $J=17.5$, 10.8 Hz); MS (NH_3DCI) m/z 457 (MH^+).

(3*R*)-14-*O*-[2-(3-Amino-1,2,4-triazol-5-yl-thio)-ethyl]-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin (9). A solution of 3-amino-5-mercapto-1,2,4-triazole (50 mg, 0.43 mmol) in 1 M sodium methoxide in methanol (0.6 mL, 0.6 mmol) was added to an ice-cooled solution of (**8**, 133 mg, 0.29 mmol) in acetone (4 mL). The cooling bath was then removed and the reaction mixture stirred at room temperature for 24 h before being concentrated to dryness. The residue was then partitioned between ethyl acetate and water, dried (magnesium sulphate), concentrated and purified by silica gel chromatography, eluting with ethyl acetate, to give (**9**, 41 mg, 30%); ν_{max} 1697 cm^{-1}; ^1H NMR 0.96 (6H, d, $J=6.5$ Hz), 1.12 (1H, m), 1.20 (3H, s), 1.21 (3H, s), 1.31 (3H, m), 1.47 (2H, m), 1.65 (1H, d, $J=15.6$ Hz), 1.68 (1H, d, $J=11.2$ Hz), 1.94 (2H, m), 2.17 (1H, m), 2.38 (1H, dd, $J=15.5$, 9.7 Hz), 2.83 (1H, q, $J=6.3$ Hz), 3.20 (3H, s), 3.21 (2H, m), 3.46 (1H, m), 3.62 (1H, m), 3.82 (1H, m), 4.00 (1H, d, $J=9.4$ Hz), 4.64 (2H, br), 5.07 (1H, d, $J=17.5$ Hz), 5.25 (1H, d, $J=10.7$ Hz), 6.15 (1H, dd, $J=17.5$, 10.7 Hz); MS (NH_3DCI) m/z 477 (MH^+).

14-*O*-(2-Hydroxyethyl)-mutilin (10). A solution of (**7**,34 mg, 0.09 mmol) in dioxane (2 mL) was treated with Lukas reagent (concentrated hydrochloric acid saturated with zinc chloride, 0.5 mL) and stirred at room temperature for 3 h. The reaction mixture was then partitioned between ethyl acetate and brine and the aqueous phase re-extracted with ethyl acetate. The combined organic extracts were washed with water, dried (magnesium sulphate) concentrated and the residue purified by silica gel chromatography to give (**10**, 21 mg, 64%); ν_{max} 1733 cm^{-1}; ^1H NMR 0.89 (6H, d, $J=7.0$ Hz), 1.19 (1H, m), 1.24 (3H, s), 1.37 (3H, s), 1.35–1.49 (4H, m), 1.62–1.85 (5H, m), 1.97 (1H, dd, $J=16.1$, 8.3 Hz), 2.10 (1H, brs), 2.25 (3H, m), 3.23 (1H, ddd, $J=9.1$, 5.3, 3.6 Hz), 3.39 (1H, dd, $J=9.3$, 6.8 Hz), 3.53 (1H, ddd, $J=9.2$, 6.1, 3.3 Hz), 3.65 (2H, br), 3.46 (1H, m), 3.84 (1H, d, $J=8.3$ Hz), 5.33 (1H, d, $J=17.3$, 1.3 Hz), 5.39 (1H, dd, $J=11.5$, 1.3 Hz), 6.20 (1H, dd, $J=17.4$, 11.1 Hz); MS (NH_3DCI) m/z 382 (MNH_4^+), $C_{22}H_{36}O_4$.

14-*O*-[2-(3-Amino-1,2,4-triazol-5-yl-thio)-ethyl]-mutilin (11). Concentrated hydrochloric acid (0.5 mL) was added to a solution of (**9**, 38.6 mg, 0.08 mmol) in dioxane (2 mL) and the resultant solution stirred at room temperature for 20 h. The reaction mixture was then partitioned between ethyl acetate and water and the mixture basified with saturated sodium hydrogen carbonate solution. The aqueous phase was re-extracted with ethyl acetate and the combined organic fractions washed with water, dried (magnesium sulphate) and concentrated. The resulting residue was purified by silica gel chromatography, eluting with ethyl acetate, to give (**11**, 18 mg, 48%); ν_{max} 1733 cm^{-1}; ^1H NMR 0.87 (3H, d, $J=6.8$ Hz), 0.88 (3H, d, $J=7.0$ Hz), 1.20 (3H, s), 1.36 (3H, s), 1.33–1.48 (6H, m), 1.66 (3H, m), 1.96 (1H, dd, $J=16.0$, 8.3 Hz), 2.07 (1H, brs), 2.20 (3H, m), 3.15 (2H, m), 3.38 (1H, d, $J=6.4$ Hz), 3.48 (1H, m), 3.66 (1H, m), 3.80 (1H, d, $J=8.2$ Hz), 4.60 (2H, br), 5.28 (1H, d, $J=17.6$ Hz), 5.34 (1H, d, $J=11.3$ Hz), 6.15 (1H, dd, $J=17.3$, 11.3 Hz); MS (NH_3DCI) m/z 463 (MH^+), $C_{24}H_{38}N_4O_3S$.

4-*epi*-mutilin 14-carbamates (13): route A

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(*N*-phenylcarbamate) (13a). A solution of (**5**, 1 g, 3 mmol) in dry dichloromethane (3 mL) was treated with phenyl isocyanate (0.65 mL, 6 mmol) and *N,N*-diisopropylethylamine (1 drop) and the solution was stirred at room temperature, with exclusion of moisture, for 7 days. The solution was then diluted with ethyl acetate and was washed with 1 M hydrochloric acid, water and saturated sodium hydrogen carbonate solution. The solution was then dried (sodium sulphate) and concentrated to a colourless oil which was purified by silica gel chromatography yielding (**13a**, 1.25 g, 92%); ν_{max} 1724, 1695 cm^{-1}; ^1H NMR 0.89 (3H, d, $J=6.9$ Hz), 1.00 (3H, d, $J=6.4$ Hz), 1.20 (3H, s), 1.25 (3H, s), 1.07–1.37 (5H, m), 1.49 (1H, m), 1.70 (1H, d, $J=15.3$ Hz), 1.73 (1H, d, $J=11.3$ Hz), 2.01 (2H, m), 2.21 (1H, m), 2.48 (1H, dd, $J=15.2$, 10.0 Hz), 2.97 (1H, q, $J=6.4$ Hz), 3.24 (3H, s), 3.48 (1H, ddd, $J=11.3$, 8.1,

5.2 Hz), 5.02 (1H, d, $J=17.5$ Hz), 5.32 (1H, d, $J=10.2$ Hz), 5.79 (1H, d, $J=9.9$ Hz), 6.56 (1H, brs), 6.75 (1H, dd, $J=17.5$, 10.2 Hz), 7.08 (1H, t, $J=7.1$ Hz), 7.32 (2H, m), 7.42 (2H, m); MS (ESI−ve ion) m/z 452 ($(M-H)^-$).

The following compounds were prepared using the above method (**5**) and the appropriate isocyanate.

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(*N*-4-methoxyphenyl carbamate) (13b). In 95% yield from (**5**, 3 mmol) and 4-methoxyphenyl isocyanate (6 mmol); ν_{max} 1722, 1697 cm^{-1}; ^1H NMR 0.89 (3H, d, $J=6.1$ Hz), 0.99 (3H, d, $J=6.4$ Hz), 1.07–1.29 (5H, m), 1.20 (6H, s), 1.34–1.37 (1H, m), 1.70 (1H, d, $J=15.2$ Hz), 1.73 (1H, d, $J=11.3$ Hz), 1.94–2.05 (2H, m), 2.15–2.24 (1H, m), 2.46 (1H, dd, $J=15.2$, 10.0 Hz), 2.96 (1H, q, $J=6.4$ Hz), 3.23 (3H, s), 3.47 (1H, m), 3.80 (3H, s), 5.01 (1H, d, $J=17.5$ Hz), 5.31 (1H, d, $J=10.6$ Hz), 5.77 (1H, d, $J=10.0$ Hz), 6.43 (1H, broad s), 6.75 (1H, dd, $J=17.5$, 10.6 Hz), 6.86 (2H, d, $J=8.9$ Hz), 7.31 (2H, broad d); MS (ESI–ve ion) m/z 482 ($(M-H)^-$).

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(*N*-4-nitrophenylcarbamate) (13c). In 47% yield from (**5**, 3 mmol) and 4-nitrophenyl isocyanate (6 mmol); ν_{max} 1733, 1698 cm^{-1}; ^1H NMR 0.87 (3H, d, $J=6.9$ Hz), 1.01 (3H, d, $J=6.4$ Hz), 1.10–1.90 (6H, m), 1.21 (3H, s), 1.26 (3H, s), 1.68 (1H, d, $J=15.2$ Hz), 1.75 (1H, d, $J=11.5$ Hz), 1.94–2.06 (2H, m), 2.16–2.25 (1H, m), 2.51 (1H, dd, $J=15.2$, 10.1 Hz), 2.94 (1H, q, $J=6.3$ Hz), 3.23 (3H, s), 3.47–3.49 (1H, m), 5.04 (1H, d, $J=17.5$ Hz), 5.32 (1H, d, $J=10.6$ Hz), 5.82 (1H, d, $J=10.1$ Hz), 6.70 (1H, dd, $J=17.5$, 10.6 Hz), 6.93 (1H, broad s), 7.61 (2H, d, $J=9.1$ Hz), 8.22 (2H, d, $J=9.1$ Hz); MS (NH_3DCI) m/z 499 (MH^+), m/z 516 (MNH_4^+).

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(*N*-benzylcarbamate) (13d). In 95% yield from (**5**, 3 mmol) and benzyl isocyanate (6 mmol); ν_{max} 1711, 1698 cm^{-1}; ^1H NMR 0.87 (3H, d, $J=6.8$ Hz), 0.98 (3H, d, $J=6.4$ Hz), 1.18 (3H, s), 1.19 (3H, s), 1.02–1.54 (6H, m), 1.67 (1H, d, $J=15.2$ Hz), 1.70 (1H, d, $J=11.3$ Hz), 1.93–2.04 (2H, m), 2.15–2.23 (1H, m), 2.42 (1H, dd, $J=15.1$, 10.0 Hz), 2.95 (1H, q, $J=6.4$ Hz), 3.22 (3H, s), 3.42–3.51 (1H, m), 4.32 (1H, dd, $J=14.9$, 5.5 Hz), 4.52 (1H, dd, $J=14.9$, 6.4 Hz), 4.95 (1H, broad s), 5.01 (1H, d, $J=17.6$ Hz), 5.32 (1H, d, $J=10.7$ Hz), 5.69 (1H, d, $J=9.8$ Hz), 6.79 (1H, dd, $J=17.6$, 10.7 Hz), 7.26–7.37 (5H, m); MS(EI) m/z 467 (M^+).

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(*N*-methylcarbamate) (13e). In 37% yield from (**5**, 1 mmol) and methyl isocyanate (2 mmol); ν_{max} 1711 cm^{-1}; ^1H NMR 0.85 (3H, br d, $J=6.9$ Hz), 0.99 (3H, d, $J=6.4$ Hz), 1.20 (6H, s), 1.00–1.30 (4H, m), 1.47 (1H, m), 1.65 (3H, m), 2.02 (2H, m), 2.20 (1H, m), 2.40 (1H, dd, $J=15.3$, 9.8 Hz), 2.83 (3H, br d, $J=4.8$ Hz), 2.95 (1H, q, $J=6.4$ Hz), 3.23 (3H, s), 3.46 (1H, m), 4.55 (1H, br), 5.01 (1H, d, 17.5 Hz), 5.31 (1H, d, $J=10.5$ Hz), 5.65 (1H, d, $J=9.9$ Hz), 6.79 (1H, dd, $J=17.5$, 10.5 Hz); MS(EI) m/z 391 (M^+).

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(*N*-phenylsulphonyl carbamate) (13n). In 71% yield from (**5**, 1 mmol) and benzenesulphonyl isocyanate (2 mmol); ν_{max} 1745, 1698 cm^{-1}; ^1H NMR 0.62 (3H, d, $J=6.9$ Hz), 0.95 (3H, d, $J=6.4$ Hz), 1.10 (3H, s), 1.15 (3H, s), 1.48–1.02 (7H, m), 1.67 (1H, d, $J=11.3$ Hz), 1.99 (2H, m), 2.16 (1H, m), 2.32 (1H, dd, $J=15.3$, 10.0 Hz), 2.77 (1H, q, $J=6.4$ Hz), 3.21 (3H, s) 3.37 (1H, ddd, $J=11.1$, 8.3, 5.1 Hz), 4.96 (1H, d, $J=17.5$ Hz), 5.25 (1H, d, $J=10.7$ Hz), 5.67 (1H, d, $J=10.0$ Hz), 6.42 (1H, dd, $J=17.5$, 10.7 Hz), 7.57 (2H, m), 7.68 (1H, t, $J=7.3$ Hz), 8.05 (2H, d, $J=7.1$ Hz); MS(EI) m/z 517 (M^+).

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-[*N*-(4-methoxybenzoyl) carbamate] (13o). A suspension of (**5**, 335 mg, 1.0 mmol), 4-methoxybenzoyl-chloride (682 mg, 4.0 mmol), and silver cyanate (689 mg, 4.6 mmol) in dry dichloromethane (50 mL) was stirred for 1 h at room temperature under an atmosphere of argon. The reaction mixture was then diluted with dichloromethane, filtered and the filtrate washed with 1M hydrochloric acid, water and saturated sodium chloride solution. After drying (magnesium sulphate) the crude material was purified by silica gel chromatography to give (**13o**, 488 mg, 95%); ν_{max} 1774, 1697 cm^{-1}; ^1H NMR 0.90 (3H, d, $J=6.9$ Hz), 1.00 (3H, d, $J=6.4$ Hz), 1.07–1.56 (12H, m), 1.20 (3H, s), 1.32 (3H, s), 1.72 (1H, d, $J=15.3$ Hz), 1.74 (1H, d, $J=11.2$ Hz), 1.94–2.04 (2H, m), 2.16–2.24 (1H, m), 2.53 (1H, dd, $J=15.2$, 10.1 Hz), 2.91 (1H, q, $J=6.2$ Hz), 3.23 (3H, s), 3.42–3.50 (1H, m), 3.87 (3H, s), 5.00 (1H, d, $J=17.5$ Hz), 5.29 (1H, d, $J=10.7$ Hz), 5.84 (1H, d, $J=9.9$ Hz), 6.73 (1H, dd, $J=17.5$, 10.7 Hz), 6.97 (2H, d, $J=8.9$ Hz), 7.81 (2H, d, $J=8.9$ Hz); MS (EI) m/z 511 (MH^+); (NH_3DCI) m/z 512 (MH^+).

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(*N*-trichloroacetyl carbamate) precursor to (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-carbamate, (13j). In quantitative yield from (**5**, 1 mmol) and trichloroacetyl isocyanate (3.3 mmol); ν_{max} 1737, 1698 cm^{-1}; ^1H NMR (acetone-d_6) 0.85–0.91 (3H, m), 1.02 (3H, d, $J=6.4$ Hz), 1.11–1.79 (14H, m), 1.90–2.23 (3H, m),2.42–2.63 (1H, m), 3.01 (1H, q, $J=6.4$ Hz), 3.18–3.27 (5H, m), 3.50–3.59 (1H, m), 4.04–4.18 (2H, m), 4.99 (1H, d, $J=17.6$ Hz), 5.30 (1H, d, $J=10.8$ Hz), 5.83–5.87 (1H, m), 6.82–7.91 (4H, m); MS (NH_3DCI) m/z 521 (MH^+), m/z 539 (MNH_4^+).

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(*N*,*N*-dimethylcarbamate) (13h). A solution of (**5**, 336 mg, 1.0 mmol) and *N*,*N*-dimethylcarbamoyl chloride (0.12 mL, 1.3 mmol) in pyridine (10 mL) was heated to reflux under an atmosphere of argon. Further portions of *N*,*N*-dimethylcarbamoyl chloride (0.12 mL, 1.3 mmol) were added to the reaction at daily intervals during its duration. After 14 days at reflux, the reaction was allowed to cool and then partitioned between ethyl acetate and 1 M hydrochloric acid. The organic phase was separated and washed with water followed by saturated sodium chloride solution. After drying (magnesium sulphate) the crude material was purified by

silica gel chromatography to give (**13h**, 158 mg, 40%); ν_{max} 1693 cm^{-1}; ^1H NMR 0.87 (3H, d, $J=6.7$ Hz), 0.98 (3H, d, $J=6.4$ Hz), 1.07–1.74 (6H, m), 1.20 (3H, s), 1.26 (3H, s), 1.99–2.04 (2H, m), 2.16–2.24 (1H, m), 2.82 (3H, s), 2.92 (3H, s), 2.92 (1H, m), 3.21 (3H, s), 3.23 (3H, s), 3.46–3.56 (1H, m), 4.28 and 4.76 (ABq, $J=15.2$ Hz) with 4.32 and 4.76 (ABq, $J=15.7$ Hz) (total 2H), 5.01 (1H, d, $J=17.6$ Hz), 5.32 (1H, d, $J=10.2$ Hz), 5.72 (1H, d, $J=9.9$ Hz), 6.79–6.90 (1H, m), 7.22–7.31 (5H, m); MS(EI) m/z 405 (M^+).

4-*epi*-Mutilin 14-carbamates: route B

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-chloroformate (14). Trichloromethylchloroformate (0.215 mL, 1.48 mmol) followed by triethylamine (0.495 mL, 3.56 mmol) were added to an ice-cooled solution of (**5**, 1.0 g, 2.97 mmol) in THF (10 mL) under an atmosphere of argon. The heterogeneous mixture was stirred at room temperature for 2 h and then treated with further quantities of trichloromethylchloroformate (0.215 mL, 1.48 mmol) and triethylamine (0.495 mL, 3.56 mmol). After a further 2 h, the reaction was diluted with ethyl acetate and washed with saturated sodium chloride solution. The organic phase was then dried (magnesium sulphate) and concentrated to afford (**14**, 1.42 g, quant) as a yellow oil which crystallised on standing. The chloroformate (**14**) was generally used without further purification, although this could be readily achieved by silica gel chromatography; ν_{max} 1765, 1732, 1699 cm^{-1}; ^1H NMR 0.92 (3H, d, $J=6.8$ Hz), 1.00 (3H, d, $J=6.4$ Hz), 1.12 (1H, m), 1.22 (3H, s) 1.26 (3H, s), 1.22–1.41 (4H, m), 1.73 (2H, m), 1.98 (1H, m), 2.01 (2H, m), 2.19 (1H, m), 2.58 (1H, dd, $J=15.4$, 10.3 Hz), 2.77 (1H, q, $J=6.4$ Hz), 3.22 (3H, s), 3.44 (1H, ddd, $J=11.3$, 8.0, 5.3 Hz), 5.04 (1H, d, $J=17.5$ Hz), 5.31 (1H, d, $J=10.7$ Hz), 5.76 (1H, d, $J=10.2$ Hz), 6.45 (1H, dd, $J=17.5$, 10.7 Hz).

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-[*N*-(2-hydroxyethyl)carbamate] (13f). Ethanolamine (0.14 mL, 2.25 mmol) was added to a solution of (**14**, 300 mg, 0.75 mmol) in dichloromethane (5 mL) under an atmosphere of argon. After stirring for 1 h, the solution was diluted with dichloromethane and washed with 1M hydrochloric acid, water and saturated sodium chloride solution. The solution was then dried (magnesium sulphate), concentrated and the crude residue purified by silica gel chromatography to afford (**13f**, 323 mg, 100%); ν_{max} 1699 cm^{-1}; ^1H NMR ($CDCl_3$) 0.85 (3H, d, $J=6.9$ Hz), 0.95–1.72 (7H, m), 0.98 (3H, d, $J=6.4$ Hz), 1.23 (6H, s), 1.61 (1H, brs) 1.93–2.04 (2H, m), 2.14–2.36 (1H, m), 2.41 (1H, dd, $J=15.2$, 10.1 Hz), 2.93 (1H, q, $J=6.4$ Hz), 3.22 (3H, s), 3.37–3.48 (3H, m), 3.72 (2H, m), 5.00 (1H, d, $J=17.6$ Hz), 5.04 (1H, broad s) 5.29 (1H, d, $J=10.6$ Hz), 5.69 (1H, d, $J=9.9$ Hz), 6.73 (1H, dd, $J=17.6$, 10.6 Hz); MS (NH_3DCI) m/z 422 (MH^+), m/z 439 (MNH_4^+).

The following compounds were prepared using the above method from (**14**) and the appropriate amine, or amine hydrochloride.

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(*N*-methyl-*N*-phenylcarbamate) (13g). In 81% yield from (**14,** 0.75 mmol) and *N*-methylaniline (2.32 mmol); ν_{max} 1693 cm^{-1}; ^1H NMR 0.82 (3H, m), 0.97 (3H, d, $J=6.4$ Hz), 1.02–1.38 (11H, m), 1.58–1.74 (3H, m), 1.99 (2H, m), 2.18 (1H, m), 2.41 (1H, m), 2.92 (1H, m), 3.19 (3H, s), 3.32 (3H, s), 3.45 (1H, m), 5.00 (1H, d, $J=17.5$ Hz), 5.30 (1H, d, $J=10.7$ Hz), 5.69 (1H, m), 6.83 (1H, m), 7.24 (3H, m), 7.37 (2H, m); MS(EI) m/z 467 (M^+).

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-14-*O*-(morpholinocarbonyl)-4-*epi*-mutilin (13i). In 57% yield from (**14,** 0.75 mmol) and morpholine (2.3 mmol); ν_{max} 1691 cm^{-1}, ^1H NMR 0.89 (3H, d, $J=6.9$ Hz), 0.98 (3H, d, $J=6.4$ Hz), 1.09 (1H, m), 1.20 (3H, s), 1.23 (3H, s), 1.20–1.52 (5H, m), 1.63 (1H, d, $J=15.2$ Hz), 1.72 (1H, d, $J=11.3$ Hz), 1.99 (2H, m), 2.20 (1H, m), 2.43 (1H, dd, $J=15.2$, 10.0 Hz), 2.93 (1H, q, $J=6.4$ Hz), 3.22 (3H, s), 3.49 (5H, m), 3.66 (4H, m), 5.01 (1H, d, $J=17.6$ Hz), 5.31 (1H, d, $J=10.7$ Hz), 5.86 (1H, d, $J=9.9$ Hz), 6.79 (1H, dd, $J=17.6$, 10.7 Hz); MS(EI), m/z 447 (M^+).

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(*N*-hydroxycarbamate) (13k). In 54% yield from (**14,** 0.38 mmol) and hydroxylamine hydrochloride (0.72 mmol); ν_{max} 1720, 1698 cm^{-1}; ^1H NMR 0.84 (3H, d, $J=6.9$ Hz), 0.99 (3H, d, $J=6.4$ Hz), 1.19 (6H, s), 1.03–1.35 (4H, m), 1.49 (2H, m), 1.62 (1H, d, $J=15.2$ Hz), 1.72 (1H, d, $J=11.3$ Hz), 1.99 (2H, m), 2.19 (1H, m), 2.45 (1H, dd, $J=15.2$, 10.1 Hz), 2.89 (1H, q, $J=6.4$ Hz), 3.21 (3H, s), 3.44 (1H, ddd, $J=11.2$, 8.0, 5.4 Hz), 5.02 (1H, d, 17.5 Hz), 5.29 (1H, d, $J=10.7$ Hz), 5.73 (1H, d, $J=9.9$ Hz), 6.67 (2H includes 1H, dd, $J=17.5$, 10.6 Hz), 7.18 (1H, s); MS(FAB) m/z 416 (MNa^+).

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(*N*-dimethylamino carbamate) (13l). In 73% yield from (**14,** 0.42 mmol) and 1,1-dimethylhydrazine (0.52 mmol); ν_{max} 1729, 1696 cm^{-1}, ^1H NMR 0.84 (3H, d, $J=6.9$ Hz), 0.98 (3H, d, $J=6.4$ Hz), 1.18 (6H, s), 1.05–1.53 (5H, m), 1.64 (3H, m), 1.98 (2H, m), 2.18 (1H, m), 2.40 (1H, dd, $J=14.9$, 10.2 Hz), 2.58 (6H,s), 2.92 (1H, q, $J=6.4$ Hz), 3.21 (3H, s), 3.46 (1H, ddd, $J=11.2$, 4.7, 2.9 Hz), 4.98 (1H, d, 17.5 Hz), 5.26 (1H, d, $J=10.7$ Hz), 5.54 (1H, br s), 5.66 (1H, d, $J=9.9$ Hz), 6.78 (1H, dd, $J=17.5$, 10.7 Hz); MS(EI) m/z 420 (M^+).

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-[*N*-(methanesulphonylamino) carbamate] (13m). In 89% yield from (**14,** 0.43 mmol) and methanesulphonyl hydrazide (0.85 mmol); ν_{max} 1716, 1698 cm^{-1}, ^1H NMR 0.87 (3H, d, $J=6.9$ Hz) 1.00 (3H, d, $J=6.4$ Hz), 1.05–1.54 (6H, m), 1.21 (3H, s), 1.33 (3H, s), 1.67 (1H, d, $J=15.3$ Hz), 1.76 (1H, d, $J=11.3$ Hz), 2.00 (2H, m), 2.21 (1H, m), 2.57 (1H, dd, $J=15.3$, 10.1 Hz), 2.87 (1H, q, $J=6.4$ Hz), 3.22 (3H, s), 3.33 (3H, s), 3.47 (1H, ddd, $J=11.3$, 8.1, 5.3 Hz), 4.32 (2H, s), 5.03 (1H, d, 17.5 Hz), 5.31 (1H, d, $J=10.7$ Hz), 5.85 (1H, d, $J=10.1$ Hz), 6.63 (1H, dd, $J=17.5$, 10.7 Hz); MS(EI) m/z 470 (M^+).

Mutilin 14-carbamates

Mutilin 14-(*N*-phenylcarbamate) (15a). A solution of (**13a**, 160 mg) in dioxane (3 mL) was treated with a saturated solution of zinc chloride in concd hydrochloric acid (1.2 mL) and the solution was stirred at room temperature for 3.5 h. The mixture was partitioned between ethyl acetate and saturated sodium chloride solution then the organic phase washed with sodium hydrogen carbonate solution. The solution was dried (magnesium sulphate), concentrated and the crude residue purified by silica gel chromatography to give (**15a**, 145 mg, 93%); ν_{max} 1726 cm^{-1}; ^1H NMR 0.78 (3H, d $J=6.2$ Hz), 0.89 (3H, d, $J=7.0$ Hz), 1.14 (1H, m), 1.19 (3H, s), 1.34–1.52 (4H, m), 1.46 (3H, s), 1.72 (4H, m) 2.12 (2H, m), 2.24 (2H, m), 2.41 (1H, quint, $J=6.9$ Hz), 3.37 (1H, dd, $J=10.9$, 6.6 Hz), 5.22 (1H, dd, $J=17.5$, 1.5 Hz), 5.37 (1H, dd, $J=11.0$, 1.5 Hz), 5.79 (1H, d, $J=8.3$ Hz), 6.50 (1H, s), 6.60 (1H, dd, $J=17.4$, 11.0 Hz), 7.06 (1H, t, $J=7.1$ Hz), 7.33 (4H, m); MS(EI) m/z 439 (M^+), $C_{27}H_{37}NO_4$.

The following compounds were prepared using the general procedure described above.

Mutilin 14-[*N*-(4-methoxyphenyl)]-carbamate (15b). In 86% yield (**13b**); ν_{max} 1725 cm^{-1}; ^1H NMR 0.79 (3H, broad d), 0.87 (3H, d, $J=7.0$ Hz), 1.18 (3H, s), 1.14–1.82 (13H, m), 2.04–2.26 (3H, m), 2.37 (1H, quint, $J=6.9$ Hz), 3.36 (1H, dd, $J=10.9$, 6.7 Hz), 3.78 (3H, s), 4.81 (1H, dd, $J=17.4$, 1.6 Hz), 5.36 (1H, dd, $J=10.9$, 1.4 Hz), 5.73 (1H, d, $J=8.3$ Hz), 6.39 (1H, broad s), 6.59 (1H, dd, $J=17.4$, 10.9 Hz), 6.85 (2H, d, $J=8.9$ Hz), 7.26 (2H, broad d); MS (EI) m/z 469 (M^+), $C_{28}H_{39}NO_5$.

Mutilin 14-[*N*-(4-nitrophenyl)]-carbamate (15c). In 82% yield from (**13c**); ν_{max} 1733 cm^{-1}; ^1H NMR 0.78 (3H, d, $J=6.5$ Hz), 0.92 (3H, d, $J=7.0$ Hz), 1.20–1.84 (10H, m), 1.20 (3H, s), 1.46 (3H, s), 2.09–2.28 (3H, m), 2.39 (1H, quintet, $J=7.0$ Hz), 3.38 (1H, dd, $J=10.7$, 6.6 Hz), 5.23 (1H, dd, $J=17.5$, 1.4 Hz), 5.39 (1H, dd, $J=10.9$, 1.4 Hz), 5.80 (1H, d, $J=9.3$ Hz), 6.56 (1H, dd, $J=17.4$, 10.9 Hz), 6.88 (1H, broad s), 7.56 (2H, d, $J=9.2$ Hz), 8.20 (2H, d, $J=9.2$ Hz); MS (EI) m/z 484 (M^+), $C_{27}H_{36}N_2O_6$.

Mutilin 14-(*N*-benzyl)-carbamate (15d). In 82% yield from (**13d**); ν_{max} 1718 cm^{-1}; ^1H NMR 0.77 (3H, d, $J=5.9$ Hz), 0.86 (3H, d, $J=7.0$ Hz), 1.08–1.80 (8H, m), 1.17 (3H, s), 1.39 (1H, s) 1.99–2.07 (3H, m), 2.17–2.24 (2H, m), 2.39 (1H, quintet., $J=6.9$ Hz), 3.35 (1H, dd, $J=10.8$, 6.7 Hz), 4.31 (1H, dd, $J=16.0$, 5.9 Hz), 4.41 (1H, dd, $J=16.0$, 6.2 Hz), 4.90 (1H, broad t), 5.20 (1H, d, $J=17.3$ Hz), 5.36 (1H, d, $J=10.9$ Hz), 5.69 (1H, d, $J=8.4$ Hz), 6.61 (1H, dd, $J=17.3$, 10.9 Hz), 7.24–7.43 (5H, m); MS (EI) m/z 391 (M^+); MS (NH_3DCI) m/z 454 (MH^+), $C_{28}H_{39}NO_4$.

Mutilin 14-(*N*-methyl)-carbamate (15e). In 69% yield from (**13e**); ν_{max} 1732, 1714 cm^{-1}; ^1H NMR 0.76 (3H, d, $J=6$ Hz), 0.86 (3H, d, $J=7.0$ Hz), 1.23 (3H, s), 1.42 (6H, m), 1.70 (4H, m), 2.02 (2H, m), 2.21 (4H, m), 2.37 (1H, quintet, $J=6.8$ Hz), 2.78 (3H, br d, $J=4.8$ Hz), 3.34 (1H, dd, $J=11.0$, 6.7 Hz), 4.47 (1H, br), 5.21 (1H, dd, $J=17.4$, 1.6 Hz), 5.37 (1H, br d, $J=11.0$ Hz), 5.64 (1H, d, $J=8.4$ Hz), 6.61 (1H, dd, $J=17.4$, 11.0 Hz); MS(EI) m/z 377 (M^+), $C_{22}H_{35}NO_4$.

Mutilin 14-[*N*-(2-hydroxyethyl)carbamate] (15f). In 47% yield from (**13f**); ν_{max} 1733, 1712 cm^{-1}; ^1H NMR 0.76 (3H, d, $J=6.4$ Hz), 0.86 (3H, d, $J=7.0$ Hz), 1.08–1.81 (10H, m) 1.16 (3H, s), 1.40 (3H, s), 2.0 (1H, m), 2.08 (1H, broad s), 2.18–2.24 (2H, m), 2.39 (1H, quintet, $J=6.9$ Hz), 3.31–3.38 (3H, m), 3.68 (2H, m), 4.98 (1H, m), 5.20 (1H, dd, $J=17.5$, 1.5 Hz), 5.35 (1H, dd, $J=11.3$, 1.5 Hz), 5.64 (1H, d, $J=8.3$ Hz), 6.56 (1H, dd, $J=17.5$, 11.3 Hz); MS(EI) m/z 407 (M^+), $C_{23}H_{37}NO_5$.

Mutilin 14-(*N*-methyl-*N*-phenyl)-carbamate (15g). In 66% yield from (**13g**); ν_{max} 1734, 1691 cm^{-1}; ^1H NMR 0.74 (3H, m), 0.85 (3H, d, $J=7.0$ Hz), 1.07–1.78 (9H, m), 1.18 (3H, s), 1.58 (3H, s), 2.05–2.38 (5H, m), 3.28 (3H, s), 3.33 (1H, dd, $J=11.2$, 6.7 Hz), 5.23 (1H, d, $J=17.6$ Hz), 5.38 (1H, d, $J=10.7$ Hz), 5.71 (1H, m), 6.64 (1H, dd, $J=17.6$, 10.7 Hz), 7.20 (3H, m), 7.34 (2H, m); MS(EI) m/z 453 (M^+), $C_{28}H_{39}NO_4$.

Mutilin 14-(*N*,*N*-dimethyl)-carbamate (15h). In 49% yield from (**13h**); ν_{max} 1734, 1692 cm^{-1}; ^1H NMR 0.73 (3H, d, $J=6.4$ Hz), 0.84 (3H, d, $J=7.1$ Hz), 1.08–1.80 (5H, m), 1.16 (3H, s), 1.36 (1H, d, $J=16.0$ Hz), 1.45 (3H, s) 2.00–2.10 (2H, m), 2.18–2.26 (2H, m), 2.37 (1H, quintet, $J=6.9$ Hz), 2.86 (3H, s), 2.90 (3H, s), 3.34 (1H, dd, $j=11.3$, 6.6 Hz), 5.20 (1H, dd, $J=17.4$, 1.7 Hz), 5.36 (1H, dd, $J=11.0$, 1.6 Hz), 5.67 (1H, d, $J=8.4$ Hz), 6.65 (1H, dd, $J=17.4$, 11.0 Hz); MS(EI) m/z 391 (M^+); MS (NH_3DCI) m/z 392 (MH^+), $C_{23}H_{37}NO_4$.

14-*O*-(Morpholinocarbonyl)-mutilin (15i). In 55% yield from (**13i**); ν_{max} 1733, 1689 cm^{-1}, ^1H NMR 0.74 (3H, d, $J=6.5$ Hz), 0.86 (3H, d, $J=7.0$ Hz), 1.17 (3H, s), 1.19 (1H, m), 1.43 (3H, s), 1.34–1.54 (4H, m), 1.57–1.81 (4H, m), 2.04 (1H, m), 2.10 (1H, br), 2.22 (2H, m), 2.36 (1H, quintet, $J=7.0$ Hz), 3.35 (1H, dd, $J=11.2$, 6.6 Hz), 3.43 (4H, m), 3.62 (4H, m), 5.21 (1H, dd, $J=17.4$, 1.6 Hz), 5.37 (1H, dd, $J=11.0$, 1.6 Hz), 5.70 (1H, d, $J=8.4$ Hz), 6.62 (1H, dd, $J=17.4$, 11.0 Hz); MS(EI) m/z 433 (M^+), $C_{25}H_{39}NO_5$.

Mutilin 14-(*N*-hydroxy)-carbamate (15k). In 68% yield from (**13k**); ν_{max} (KBr) 1728 cm^{-1}, ^1H NMR 0.67 (3H, br d, $J=5.7$ Hz), 0.81 (3H, d, $J=6.8$ Hz), 1.06 (4H, includes 3H, s), 1.26 (3H, m), 1.33 (3H, s), 1.49 (2H, m), 1.65 (2H, m), 2.09 (4H, m), 2.36 (1H,br s), 3.40 (1H, m,), 4.46 (1H, d, $J=6.1$ Hz), 5.04 (1H, dd, $J=11.1$, 1.8 Hz), 5.11 (1H, dd, $J=17.7$, 1.8 Hz), 5.46 (1H, d, $J=8.0$ Hz), 6.24 (1H, dd, $J=17.7$, 11.1 Hz), 8.59 (1H, s), 9.38 (1H, s); MS(NH_3DCI) m/z 397 (MNH_4^+), $C_{21}H_{33}NO_5$.

Mutilin 14-(*N*-dimethylamino)-carbamate (15l). In 89% yield from (**13l**); ν_{max} 1732 cm^{-1}, ^1H NMR 0.76 (3H, d, $J=6.2$ Hz), 0.87 (3H, d, $J=7.0$ Hz), 1.12 (1H, m), 1.16 (3H, s), 1.42 (7H, m), 1.59–1.81 (4H, m), 2.03 (2H, m), 2.22 (2H, m), 2.36 (1H, quintet, $J=6.9$ Hz), 2.55 (6H, s),

3.34 (1H, dd, $J=10.9$, 6.6 Hz), 5.19 (1H, dd, $J=17.4$, 1.5 Hz), 5.34 (1H, dd, $J=11.0$, 1.5 Hz), 5.41 (1H, br s), 5.65 (1H, d, $J=8.4$ Hz), 6.60 (1H, dd, $J=17.4$, 11.0 Hz); MS(EI) m/z 406 (M^+), $C_{23}H_{38}N_2O_4$.

Mutilin 14-(*N*-methanesulphonylamino)-carbamate (15m). In 85% yield from (**13m**); ν_{max} 1733 cm^{-1}, ^1H NMR 0.77 (3H, d, $J=6.8$ Hz), 0.89 (3H, d, $J=7.0$ Hz), 1.17 (1H, m), 1.19 (3H, s), 1.41–1.81 (8H, m), 1.59 (3H, s), 2.12 (1H, br s), 2.24 (4H, m), 3.29 (3H, s), 3.37 (1H, dd, $J=10.6$, 6.7 Hz), 4.28 (2H, s), 5.23 (1H, dd, $J=17.4$, 1.4 Hz), 5.37 (1H, dd, $J=11.0$, 1.4 Hz), 5.81 (1H, d, $J=8.6$ Hz), 6.48 (1H, dd, $J=17.4$, 11.0 Hz); MS(NH_3DCI) m/z 474 (MNH_4^+), $C_{22}H_{36}N_2O_6S$.

Mutilin 14-(*N*-benzenesulphonyl)-carbamate (15n). In 88% yield from (**13n**); ν_{max} 1736 cm^{-1}; ^1H NMR 0.51 (3H, d, $J=6.7$ Hz), 0.85 (3H, d, $J=7.0$ Hz), 1.07 (3H, s), 1.08 (1H, m), 1.23–1.75 (8H, m), 1.33 (3H, s), 1.95 (2H, m), 2.19 (3H, m), 3.18 (1H, dd, $J=10.1$, 6.7 Hz), 5.07 (1H, dd, $J=17.5$, 1.3 Hz), 5.23 (1H, dd, $J=11.0$, 1.3 Hz), 5.61 (1H, d, $J=8.4$ Hz), 6.26 (1H, dd, $J=17.5$, 11.0 Hz), 7.54 (2H, d, $J=7.5$ Hz), 7.65 (1H, t, $J=7.4$ Hz), 8.00 (2H, d, $J=7.4$ Hz); MS(EI) m/z 503 (M^+), $C_{27}H_{37}NO_6S$.

Mutilin 14-[*N*-(4-methoxybenzoyl)]-carbamate (15o). In 33% yield from (**13o**); ν_{max} 1776, 1733, 1710 cm^{-1}; ^1H NMR 0.81 (3H, d, $J=6.6$ Hz), 0.88 (3H, d, $J=7.0$ Hz), 1.10–1.81 (9H, m), 1.15 (3H, s), 1.51 (3H, s), 2.09–2.26 (2H, m), 2.12 (1H, bs), 2.35 (1H, quintet $J=6.9$ Hz), 3.36 (1H, dd, $J=11.0$, 6.6 Hz), 3.86 (3H, s), 5.22 (1H, dd, $J=17.3$, 1.5 Hz), 5.37 (1H, dd, $J=11.0$, 1.5 Hz), 5.83 (1H, d, $J=8.5$ Hz), 6.56 (1H, dd, $J=17.3$, 11.0 Hz), 6.95 (2H, d, $J=8.8$ Hz), 7.77 (2H, d, $J=8.8$ Hz), 7.88 (1H, bs); MS(NH_3DCI) m/z 498 (MH^+), $C_{29}H_{39}NO_6$.

Mutilin 14-(*N*-trichloroacetylcarbamate). In 60% yield from (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(*N*-trichloroacetylcarbamate); ν_{max} 1803, 1736 cm^{-1}; ^1H NMR (acetone-d_6) 0.89 (3H, d, $J=6.8$ Hz), 1.01 (3H, d, $J=6.4$ Hz), 1.11–2.22 (17H, m), 2.55 (1H, dd, $J=15.4$, 10.1 Hz), 2.91–2.96 (1H, m), 3.19 (3H, s), 3.45–3.55 (1H, m), 5.00 (1H, d, $J=17.6$ Hz), 5.31 (1H, d, $J=10.7$ Hz), 5.88 (1H, d, $J=10.0$ Hz), 6.74 (1H, dd, $J=17.6$, 10.7 Hz), 10.59 (1H broad s); MS (ESI−ve ion) m/z 506 ($(M-H)^-$).

Mutilin 14-carbamate (15j). Mutilin 14-(*N*-trichloroacetylcarbamate) (300 mg, 0.59 mmol) was dissolved in dichloromethane (2 mL) and methanol (2 mL) then treated with potassium carbonate (122 mg, 0.9 mmol). The reaction was stirred at rt for 4 h before diluting with dichloromethane. The organic phase was washed with water followed by saturated sodium chloride solution, dried (magnesium sulphate), concentrated and triturated with diethyl ether to give (**15j**, 179 mg, 85%); ν_{max} 1725 cm^{-1}; ^1H NMR 0.79 (3H, d, $J=6.4$ Hz), 0.86 (3H, d, $J=7.0$ Hz), 1.17 (3H, s), 1.39 (3H, s), 1.38–1.79 (10H, m), 2.14 (1H, d, $J=8.6$ Hz), 2.09 (1H, broad s), 2.17–2.31 (2H, m), 2.36 (1H, quint, $J=6.9$ Hz), 3.35 (1H, broad t), 4.52 (2H, broad s), 5.21 (1H, dd, $J=17.4$, 1.5 Hz), 5.36 (1H, dd, $J=11.0$, 1.5 Hz), 5.62 (1H, d, $J=8.5$ Hz) 6.57 (1H, dd, $J=17.4$, 11.0 Hz); MS(NH_3DCI) m/z 364 (MH^+), $C_{21}H_{33}NO_4$.

4,14-Cyclomutilin-11-acetate (17). An ice-cooled solution of (**16**, 12.1 g, 30 mmol) in DMF (120 mL) was purged with argon before treatment with potassium *tert*-butoxide (5 g, 50 mmol). The mixture was then stirred for 2 h, whilst warming to room temperature, diluted with ethyl acetate and washed with water. The organic fraction was dried (magnesium sulphate), concentrated and purified by silica gel chromatography to give (**17**, 3.35 g, 32%); ν_{max} 1725, 1697 cm^{-1}; ^1H NMR 0.78 (3H, d, $J=7.1$ Hz), 0.90 (3H, s), 0.94 (3H, d, $J=6.4$ Hz), 0.95–1.2 (3H, m), 1.25–1.55 (5H, m), 1.68–1.8 (2H, m), 1.90 (1H, dd, $J=13.4$, 1.4 Hz), 2.0–2.2 (5H, m), 2.2–2.4 (2H, m), 2.6–2.8 (1H, m), 4.53 (1H, d, $J=8.9$ Hz), 5.1–5.25 (2H, m), 6.04 (1H, dd, $J=17.5$, 11.3 Hz); MS(NH_3DCI) m/z 345 (MH^+).

14-Azido-14-deoxy-mutilin 11-acetate (18). A suspension of (**17**, 2.54 g, 7.4 mmol) and sodium azide (2 g, 30.7 mmol) in DMF (30 mL) was heated under argon at 150 °C for 20 h. After cooling the mixture was diluted with ethyl acetate and washed with water. The organic fraction was dried (magnesium sulphate), concentrated and purified by silica gel chromatography to give (**18**, 125 mg, 4.5%); ν_{max} 2098, 1728 cm^{-1}; ^1H NMR 0.83 (3H, d, $J=7.1$ Hz), 0.94 (3H, d, $J=7.3$ Hz), 1.08 (3H, s), 1.15 (1H, m), 1.35 (3H, s), 1.36–1.45 (3H, m), 1.65–1.75 (2H, m), 1.85–1.95 (2H, m), 2.0–2.1 (1H, m), 2.11 (3H, s), 2.13–2.23 (2H, m), 2.25–2.35 (1H, m), 3.93 (1H, d, $J=6.9$ Hz), 4.89 (1H, d, $J=6.9$ Hz), 5.3–5.4 (2H, m), 6.13 (1H, dd, $J=18.1$, 11.4 Hz); MS(NH_3DCI) m/z 405 (MNH_4^+).

***N*-(14-Amino-14-deoxy-19,20-dihydromutilin) 11-acetate-14-phenylacetamide.** A solution of (**18**, 30 mg, 0.08 mmol) in THF (2 mL) was treated with 10% palladium on charcoal (50 mg) and shaken under an atmosphere of hydrogen for 2 h. The mixture was then filtered through kieselguhr and concentrated. The residue was then dissolved in chloroform, cooled in an ice-bath and treated with triethylamine (0.02 mL, 0.16 mmol) and phenylacetyl chloride (0.02 mL, 0.16 mmol). After 2 h, the solution was washed with saturated sodium hydrogen carbonate, dried (magnesium sulphate) and concentrated. The residue was then purified by silica gel chromatography to give *N*-(14-amino-14-deoxy-19,20-dihydromutilin) 11-acetate-14-phenylacetamide (25 mg, 68%); ν_{max} 1725, 1664 cm^{-1}; ^1H NMR 0.6–0.9 (12H, m), 0.9–1.2 (5H, m), 1.2–1.4 (2H, m), 1.5–2.0 (7H, m), 2.07 (3H, s), 2.1–2.3 (3H, m), 2.7 (1H, m), 3.49 (2H, d, $J=16$ Hz), 4.7–4.9 (2H, m), 5.49 (1H, d, $J=10.2$ Hz), 7.1–7.4 (5H, m); MS(EI) m/z 481 (MH^+).

***N*-(14-Amino-14-deoxy-19,20-dihydromutilin) 14-phenylacetamide (19).** A solution of *N*-(14-amino-14-deoxy-19,20-dihydromutilin) 11-acetate-14-phenylacetamide (23 mg, 0.048 mmol) in ethanol (1 mL) was treated with 10 M sodium hydroxide (0.1 mL, 1 mmol) and heated to reflux for 5 h. After cooling, the mixture was diluted

with ethyl acetate and washed with water. The organic phase was then dried (magnesium sulphate), concentrated and purified by silica gel chromatography to give (**19**, 7.5 mg, 35%); ν_{max} 1732, 1664 cm^{-1}; ^1H NMR 0.7–0.8 (6H, m), 0.8–1.9 (21H, m), 2.04 (1H, s), 2.1–2.25 (2H, m), 2.61 (1H, quintet, $J = 6.9$ Hz), 3.32 (1H, d, $J = 6.2$ Hz), 3.48 (2H, d, $J = 16$ Hz), 4.73 (1H, dd, $J = 9.5$, 6.7 Hz), 5.44 (1H, d, $J = 10.2$ Hz), 7.15–7.4 (5H, m); MS(EI) m/z 439 (MH^+), $C_{28}H_{41}NO_3$.

14-Azido-14-deoxymutilin

A solution of 14-azido-14-deoxy-mutilin 11-acetate (100 mg, 0.26 mmol) in ethanol (1 mL) was treated with an ethanolic solution of potassium hydroxide. After stirring at rt for 18 h, the mixture was partitioned between ethyl acetate and water. The organic phase was then dried (magnesium sulphate), concentrated and the residue purified by silica gel chromatography to give 14-azido-14-deoxy-mutilin (87 mg, 98%); ν_{max} 2098, 1735 cm^{-1}; ^1H NMR 0.94 (3H, d, $J = 7.1$ Hz), 0.97 (3H, d, $J = 7.3$ Hz), 1.05–1.25 (4H, m), 1.32 (3H, s), 1.35–1.85 (9H, m), 1.95–2.1 (2H, m), 2.15–2.3 (2H, m), 3.44 (1H, t, $J = 6.5$ Hz), 4.03 (1H, d, $J = 7.2$ Hz), 5.30–5.45 (2H, m), 6.16 (1H, dd, $J = 17.7$, 11.4 Hz); MS(NH_3DCI) m/z 346 (MNH_4^+).

***N*-(14-Amino-14-deoxy-19,20-dihydromutilin)-*N*-(4-methoxybenzoyl)-urea (20).** A solution of 14-azido-14-deoxy-mutilin (40 mg, 0.116 mmol) in THF (3 mL) was treated with 10% palladium on charcoal (50 mg) and shaken under an atmosphere of hydrogen for 2 h. The mixture was then filtered through kieselguhr and concentrated before being redissolved in dichloromethane (1.5 mL). The solution was then added to an ice-cooled solution of 4-methoxybenzoyl isocyanate (generated from 4-methoxybenzoyl chloride (341 mg, 2 mmol) and silver cyanate (345 mg, mmol)) then stirred at rt for 15 min. After washing with saturated sodium hydrogen carbonate solution, the reaction mixture was dried (magnesium sulphate), concentrated and the residue purified by silica gel chromatography to give (**20**, 45 mg, 77%); ν_{max} 1732, 1705, 1686 cm^{-1}; ^1H NMR 0.65 (3H, d, $J = 7.4$ Hz), 0.80 (3H, d, $J = 6.4$ Hz), 0.85–0.95 (6H, m), 1.0–1.19 (15H, m), 2.0–2.3 (3H, m), 2.45 (1H, quintet, $J = 6.7$ Hz), 3.38 (1H, m), 3.86 (3H, s), 4.69 (1H, dd, $J = 9.3$, 6.5 Hz), 6.94 (2H, d, $J = 8.9$ Hz), 8.05 (2H, d, $J = 8.9$ Hz), 9.27 (1H, d, $J = 9.3$ Hz), 10.01 (1H, s); MS (NH_3DCI) m/z 499 (MNH_4^+), $C_{29}H_{42}N_2O_5$.

Antibacterial activity

The susceptibility of the strains used in this study were measured by a broth microdilution assay in accordance with the procedures recommended by the National Committee for Clinical Laboratory Standards.[14] Data are presented as the minimum inhibitory concentration (MIC in μg/mL) required to inhibit the growth of the stated organism.

In vitro translation assay (IVT)

Poly(U)-dependent ^3H-*poly*(Phe) synthesis directed by *E. coli* ribosomes was used to determine the effect of novel pleuromutilin analogues on in vitro translation. Results are expressed as percent inhibition relative to untreated control calculated from the trichloroacetic acid precipitated ^3H-poly(Phe) produced.

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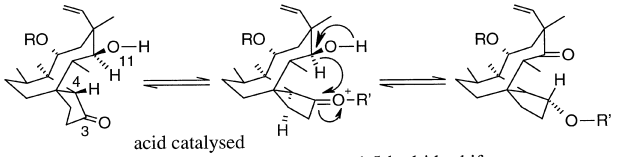

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