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Novel chiral pyrrolidinone scaffolds derived from threonine with antibacterial activity

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Dedicated to Professor Henri Kagan on the occasion of his 80th birthday

ABSTRACT

The synthesis of chiral pyrrolidinones derived from threonine, making use of a Dieckmann or aldol ring closure, is described. Compounds were found to exhibit antibacterial activity, for which the correlation with various physiochemical parameters was examined. This chiral tetramate scaffold may provide a useful template for fragment-based drug design providing rapid access to novel antibacterial compound libraries.

1. Introduction

The discovery of new antibiotics has become imperative by reason of the emergence of bacterial strains resistant to current clinically effective drugs.[1,2] Amongst a number of strategies applied to drug development, high throughput[3] and diversity-oriented synthesis[4] has yielded some leads.[5] However, re-examination of the function and availability of natural products have proved to be a key impetus in recent innovation,[6] and natural product inspired synthesis is gaining renewed acceptance[7–13] and its application for drug development has been demonstrated.[14–17] It has recently been reported that a natural product library can allow the identification of privileged structures which are suitable leads for optimisation in drug discovery,[18,19] since they are Lipinski-compliant,[20] and this analysis can be used to inform the design and synthesis of novel scaffolds.[21] In fact, given the paucity of New Chemical Entities (NCE) entering the antibacterial pipeline, a sense of urgency to ensure that the benefits of such an approach become available in suitable timeframes is emerging.[22] It has recently been realised that the physicochemical characteristics of antibacterial compounds differ sufficiently from the property space of commonly available compound libraries, that re-investigation of natural products to identify novel antibacterials is fully justified,[23] and that the structural information thus obtained will be useful for the design of NCE suitable for fragment-based drug design.[24,25] Libraries based upon such leads have several key benefits which do not apply to combinatorially-derived systems: they will have benefited from the optimisation of bioactivity for a given receptor as a result of natural selection; they will be expected to provide an enhanced rate of positive hits for a given library size; they will likely provide novel structural chemotypes not currently in use in existing therapeutic regimes; they would not be immediately susceptible to resistance-conferring genes in the bacterial and DNA pools and they are likely to provide novel new target proteins and receptors.[26,27] Some time ago, we identified pyrrolidin(on)es as a common structural motif in a variety of antibiotics, and set about the development of direct synthetic approaches for this structural core and the assessment of their intrinsic bioactivity.[28–30] The tetramic acid nucleus, as distinct from pyrrolidin(on)es, is a common functional entity found in natural products that exhibit a range of bioactivity.[31] A potentially general solution to the problem of introduction of functional diversity in a tetramate has recently appeared[32] and the chemistry related to the synthesis of cyclic quaternary amino acids has been reviewed.[33] We have developed effective synthetic methodology for the preparation of optically active pyrrolidinone analogues derived from serine.[34–37] This exploits Seebach's concept of Self-Regeneration of Stereocentre reactions[38] and the propensity of oxazolidines to control both the chemoselectivity and stereoselectivity of intramolecular Dieckmann and aldol reactions, giving a cyclisation leading to tetramate and pyroglutamate products, which are heterocyclic fragments of the natural product, oxazolomycin A–C **1a–c** (Fig. 1). Since related classes of antibacterial compounds exist which contain an additional methyl substituent at C-16,[39,40] namely 16-methyloxazolomycin **1d** and KSM-B and C **1e,f**, we considered it important to demonstrate that this strategy could be extended to threonine derivatives, and report herein the results of that investigation; part of this work has appeared earlier.[41] Furthermore, we evaluated the antibacterial activity of the products against two organisms, in order to gain activity data which might be of use in the design of novel libraries, using the chiral tetramate and pyrrolidinone structure as a core skeleton. This is of particular relevance since we believed that the libraries derived from this skeleton would obey the 'rule of three' (M <300; No H bond donors $\leqslant$3 and acceptors $\leqslant$3; *c*Log P = 3; No of rotatable bonds $\leqslant$3; Polar surface area = 60 Å^2), which has been suggested to be optimal for the construction of fragment libraries.[25,42]

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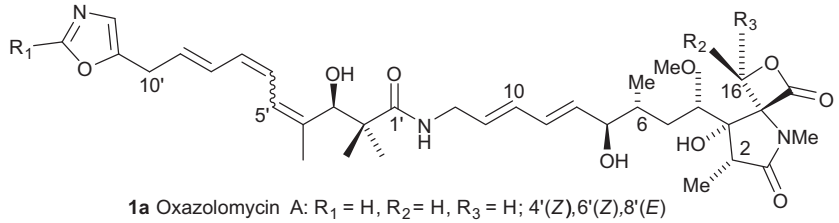


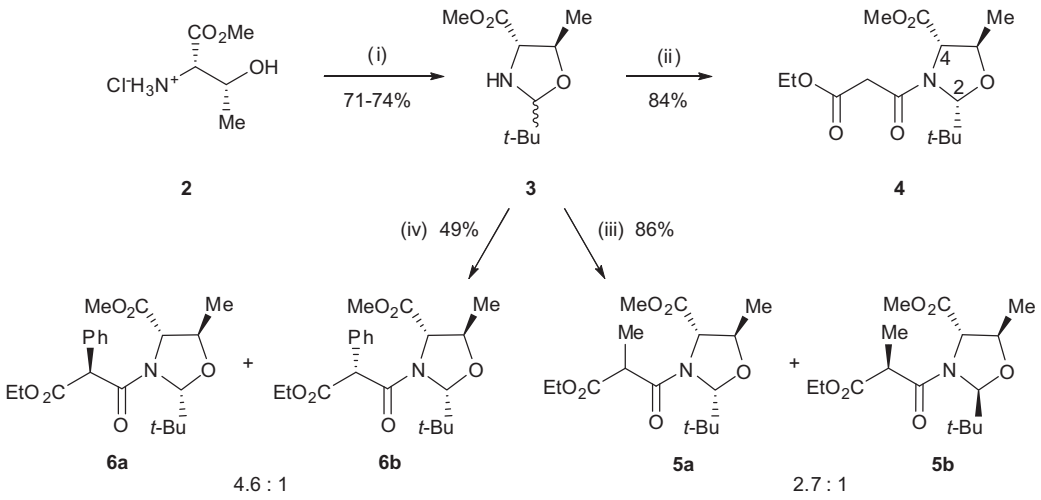
1a Oxazolomycin A: R_1 = H, R_2 = H, R_3 = H; 4'(*Z*),6'(*Z*),8'(*E*)
1b Oxazolomycin B: R_1 = H, R_2 = H, R_3 = H; 4'(*E*),6'(*E*),8'(*E*)
1c Oxazolomycin C: R_1 = H, R_2 = H, R_3 = H; 4'(*Z*),6'(*E*),8'(*E*)
1d 16-Methyloxazolomycin: R_1 = H, R_2 = Me, R_3 = H; 4'(*Z*),6'(*Z*),8'(*E*)
1e KSM-2690 B: R_1 = H, R_2 = H, R_3 = Me; 4'(*Z*),6'(*Z*),8'(*E*)
1f KSM-2690 C: R_1 = H, R_2 = H, R_3 = Me; 4'(*Z*),6'(*E*),8'(*E*)

Figure 1.

2. Results and discussion

According to the method developed by Seebach et al.,[43] L-threonine methyl ester hydrogen chloride **2** or DL-threonine methyl ester hydrogen chloride was condensed with pivaldehyde in the presence of triethylamine with continuous removal of water to give oxazolidine **3** as 1:1 mixture of *cis*-2,4 and *trans*-2,4 isomers in 71–74% yield (Scheme 1). This was readily acylated with the required malonate or β-ketoacid in the presence of DCCI, or EDAC and DMAP in DCM, or with the acyl chloride in pyridine and DCM, to give N-acylated oxazolidines **4, 5** and **6** in high yield as either principally or exclusively the *cis*-2,4-oxazolidine diastereomer; this stereochemistry was readily shown by NOE analysis (Fig. 2), in which enhancements were typically observed between H(2), H(4) and C(5)Me, all located on the same face, and between H(5) and the methyl of the ester, indicating their proximity on the other side of the oxazolidine ring. In some cases, enhancement between the methyl ester and the *t*-butyl groups was observed, confirming their facial co-location. In the case of acyl derivatives **5a,b**, unusually the *cis*- and *trans*-oxazolidines in 3:1 diastereomeric ratio were obtained, as a colourless oil and a white crystalline solid, respectively, and recrystallisation of the minor *trans*-oxazolidine **5b** from ethyl acetate gave crystals which were suitable for single crystal X-ray diffraction studies (Fig. 3), allowing confirmation of the unusual *trans*-stereochemistry of C-2 and C-4 and establishment of the side chain (*S*)-stereochemistry. In the case of *cis*-**5a**, the side chain stereochemistry could not be established. On the other hand, **6a,b** was obtained as a single *cis*-oxazolidine diastereomer, but epimeric at the *N*-acyl side chain; recrystallisation of the major diastereomer **6a** from ethyl acetate gave crystals, which were subjected to X-ray analysis (Fig. 4), and this both confirmed the *cis*-oxazolidine ring assignment and established the *N*-acyl side chain stereochemistry to be (*S*). The substituents on the nitrogen atom are not planar but partially pyramidalised, as has been previously observed in other *N*-acyl oxazolidines.[37,44] This pyramidalisation presumably occurs to move the *N*-acyl side chain away from C-2 and C-4 substituents, effectively giving an all *trans*-arrangement of the oxazolidine ring substitutents. Direct acylation of oxazolidine **3** with phenylacetyl chloride and cyanoacetic acid gave derivatives **7** and **8,** respectively (Scheme 2).

In the case of ketoamides **9a,b**, readily prepared from ester **10** via acid **11**, the product existed as a keto–enol mixture in a ratio of 5:3, but the α-methylacetoacetyl , also prepared from ester **10** via acid **12**, gave a mixture of *cis*-oxazolidine **13** and *trans*-oxazolidine **14** in a 2.4:1 ratio (Scheme 3). The *cis*-oxazolidine **13** was obtained as a 1:1 mixture of side chain epimers and *trans*-oxazolidine **14** was formed as a 1:2 mixture of side chain epimers, but did not exhibit any keto–enol tautomerism as observed by ^{1}H NMR spectroscopy. The stereochemistry of these oxazolidines could not be established by single crystal X-ray diffraction techniques so was instead determined by NOE analysis (Fig. 2). The *cis*-oxazolidines prepared as outlined above were characterised by typical chemical shifts (δ) for H-2 at 5.4, H-4 at 4.1–4.5 and H-5 at 4.8, but the one example of the *trans*-ring (compound **5b)** showed H-2 at 5.11, H-4 at 3.8 and H-5 at 4.1.



Scheme 1. Reagents and conditions: (i) Me_3CCHO, petrol (40/60), reflux, 16–20 h; (ii) DCCI, DMAP, $EtO_2CCH_2CO_2H$, DCM, 4 h; (iii) ethyl-methylmalonyl chloride, pyridine, DCM, rt, 5 h; (iv) $EtO_2CCH(Ph)CO_2H$, DCCI, DMAP, DCM, rt, 4 h.

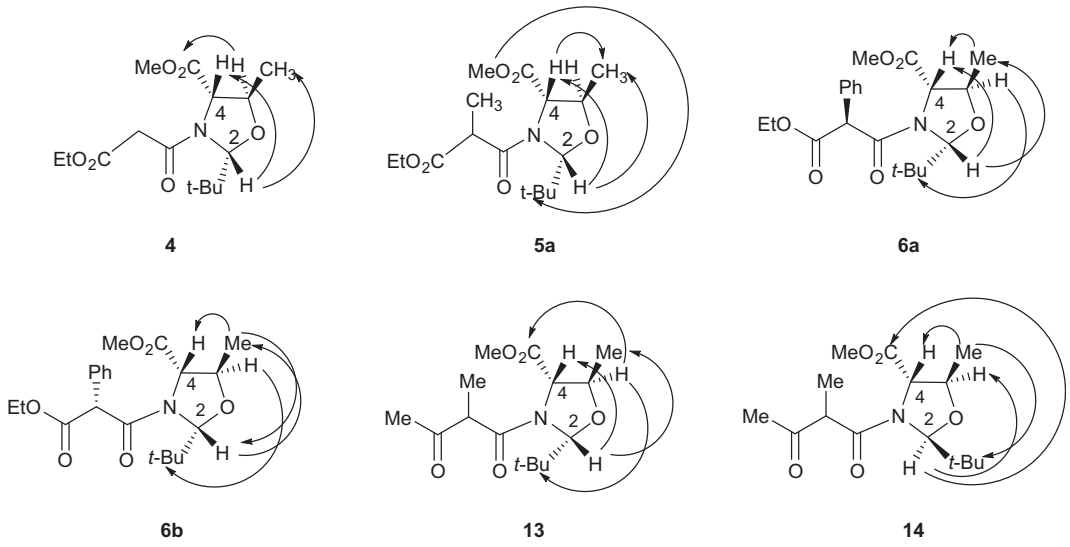


Figure 2.

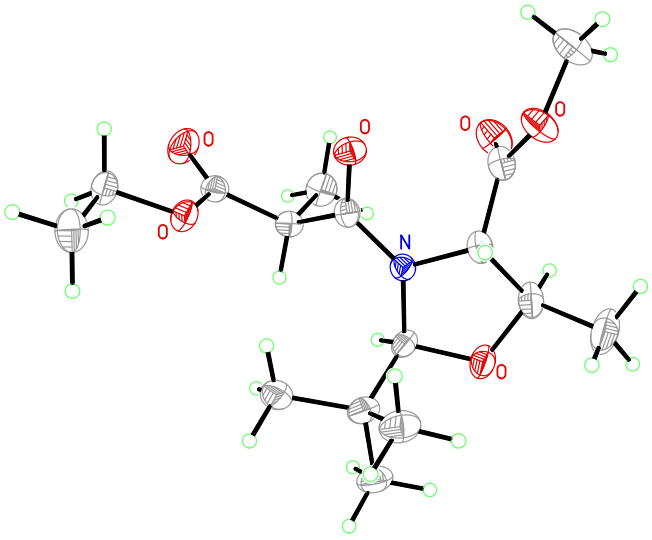


Figure 3. X-ray structure for **5b**.

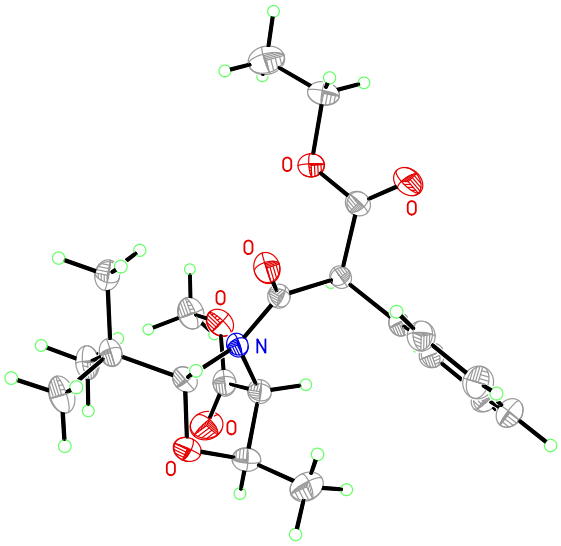


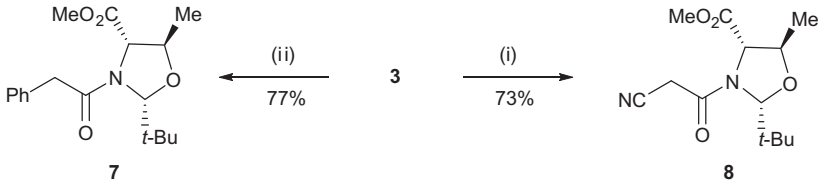
Figure 4. X-ray structure for **6a**.

2.1. Dieckmann-type cyclisations

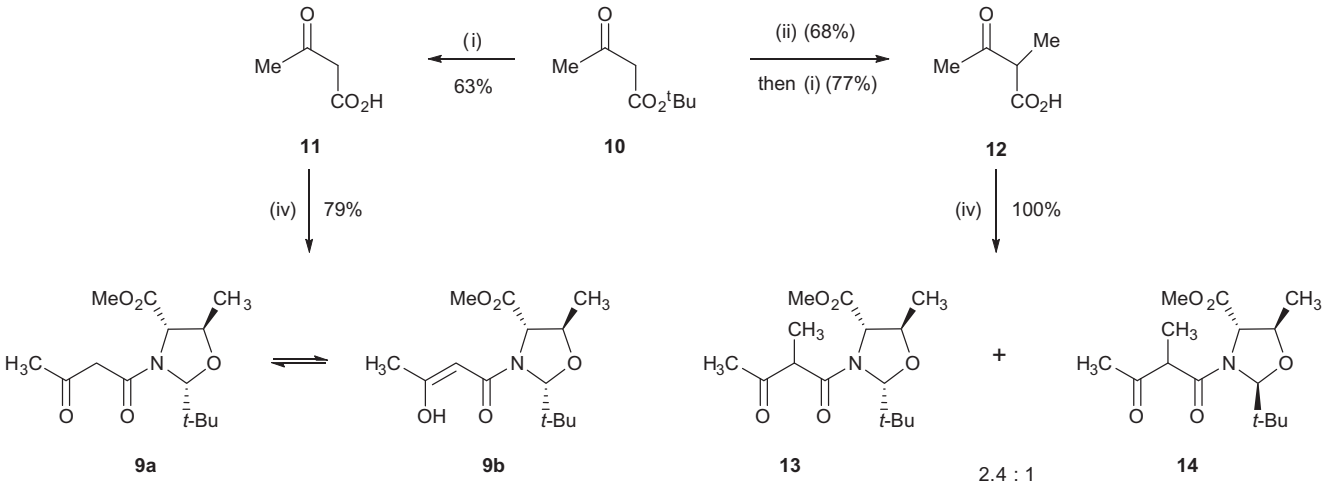
When the reported conditions for Dieckmann closure of related serine-derived oxazolidines (KO^{t}Bu in ^{t}BuOH for 3 h) were applied,[34] oxazolidine **4** did not undergo cyclisation, but when reacted with potassium *tert*-butoxide for 8 h at reflux, tetramic acid **15** was obtained in 42% yield along with 7% of decarboxylated tetramic acid **16** (Scheme 4). Although compound **16** did not form when the reaction time was decreased, the yield of tetramic acid **15** also reduced to 23%. The stereochemistry of tetramic acids **15** and **16** was established by NOE experiment (Fig. 5).

The treatment of oxazolidine **5a** with potassium *tert*-butoxide in *tert*-butanol at reflux gave tetramic acid **17** in 43% yield, which existed in equilibrium with enol form **18** (Scheme 4). The *N*-acyl oxazolidine mixture **6a,b** was cyclised to tetramic acid **19** in 67% yield, obtained in the enol form, as indicated by the absence of the C(7)H proton in the ^{1}H NMR spectrum. The tetramic acid **19** was hydrolysed with 1 M NaOH by heating at reflux and decarboxylated by heating in vacuo to give tetramic acid **20** in 51% yield with complete retention of stereochemistry at C-5. In the case of oxazolidine **7** (Scheme 5), the only mode of cyclisation was by attack of the enolate from the *N*-acyl side chain to the methyl ester, and reaction with sodium methoxide in refluxing methanol gave only tetramic acid **20** in 53% yield which was identical to that obtained from **19** (Scheme 4).

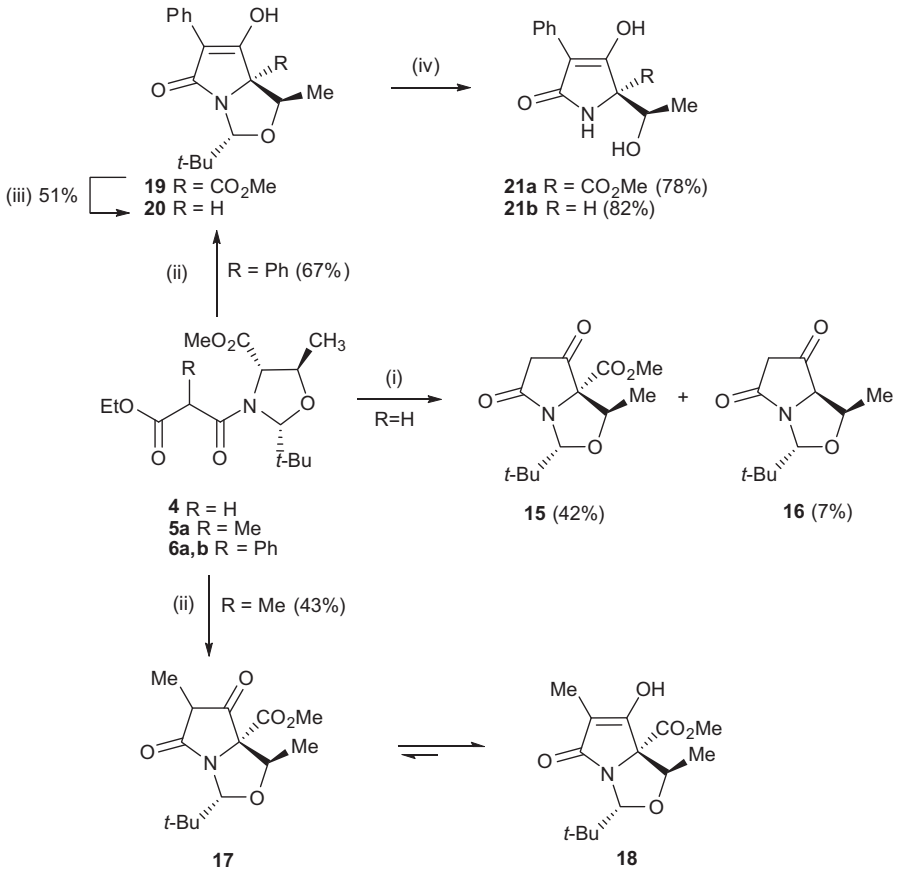
When nitrile ester **8** was treated with sodium methoxide in methanol, a clean Thorpe–Ziegler reaction giving enamine **22** in 71% yield occurred (Scheme 5). When this reaction was carried out with potassium *tert*-butoxide in *tert*-butanol, enamine **23** was obtained as the major product, along with **22** as a minor product. The major product **23** formed in higher yield when wet *tert*-butanol was used. This could presumably be due to the formation of potassium hydroxide, which under the reaction conditions both hydrolysed the ester and decarboxylated the intermediate acid to give the observed enamine **23**. The enamine **23** on basic hydrolysis gave tetramic acid **16** in 43% yield, identical to material previously obtained (Scheme 4). The stereochemistry of enamine **22** and tetramic acid **16** was confirmed by NOE analysis (Fig. 5); irradiation of the H-2 proton gave an enhancement to the methyl at C-4 and enhancement of the methyl of CO_2Me gave an enhancement to



Scheme 2. Reagents and conditions: (i) $NCCH_2CO_2H$, DCCI, DMAP, DCM, CH_3CN; (ii) $PhCH_2COCl$, pyridine, DCM, rt, 4 h.



Scheme 3. Reagents: (i) TFA; (ii) KO*t*-Bu, MeI, THF; (iii) (i) **3**, DCCI, DMAP, DCM; (iv) (i) **3**, EDAC, DMAP, DCM.



Scheme 4. Reagents and conditions: (i) KO*t*-Bu, *t*-BuOH, reflux, 8 h; (ii) KO*t*-Bu, *t*-BuOH, reflux, 3 h; (iii) 1 N NaOH, reflux, 5 h; (iv) $HS(CH_2)_3SH$, CF_3CH_2OH, 2% w/v HCl.

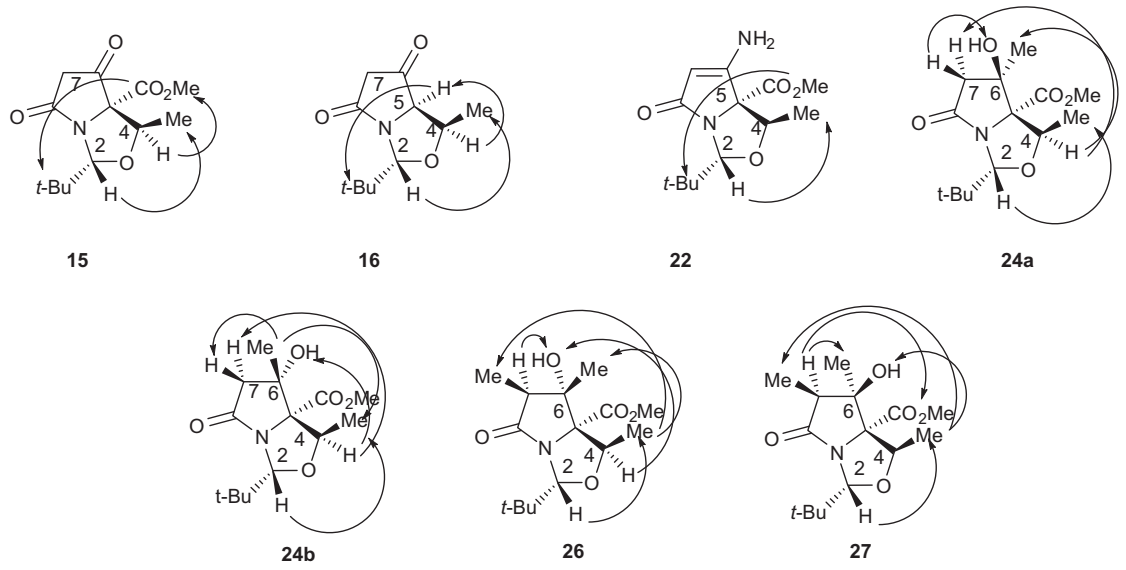
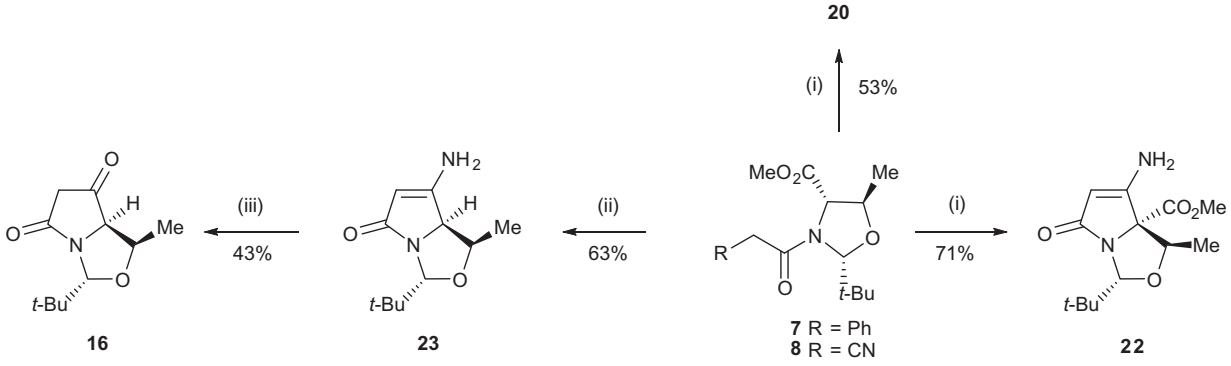


Figure 5.



Scheme 5. Reagents: (i) NaOMe, MeOH; (ii) KO*t*Bu, *t*-BuOH (wet) then H_2O; (iii) NaOH/H_2O, heat.

the *tert*-butyl group, thus indicating their co-location on the *exo* face of the bicyclic system.

2.2. Intramolecular aldol cyclisation

cis-Oxazolidine **9** on treatment with sodium methoxide in methanol underwent aldol condensation to give two products **24a** and **24b** in 73% overall yield in 5:1 ratio (Scheme 6), readily separable by flash column chromatography eluting with EtOAc and petrol (1:3). The stereochemistry of these products was established by NOE experiment (Fig. 5). Recrystallisation of the major diastereomer **24a** from ethyl acetate gave crystals which were subjected to single crystal X-ray diffraction studies (Fig. 6), which confirmed the stereochemistry previously established by NOE analysis. When the above-mentioned reaction was carried out in refluxing methanol in the presence of NaOMe, the elimination product **25** was also isolated in 7% yield from the reaction in addition to the aldol products **24a,b** which were obtained in 71% yield.

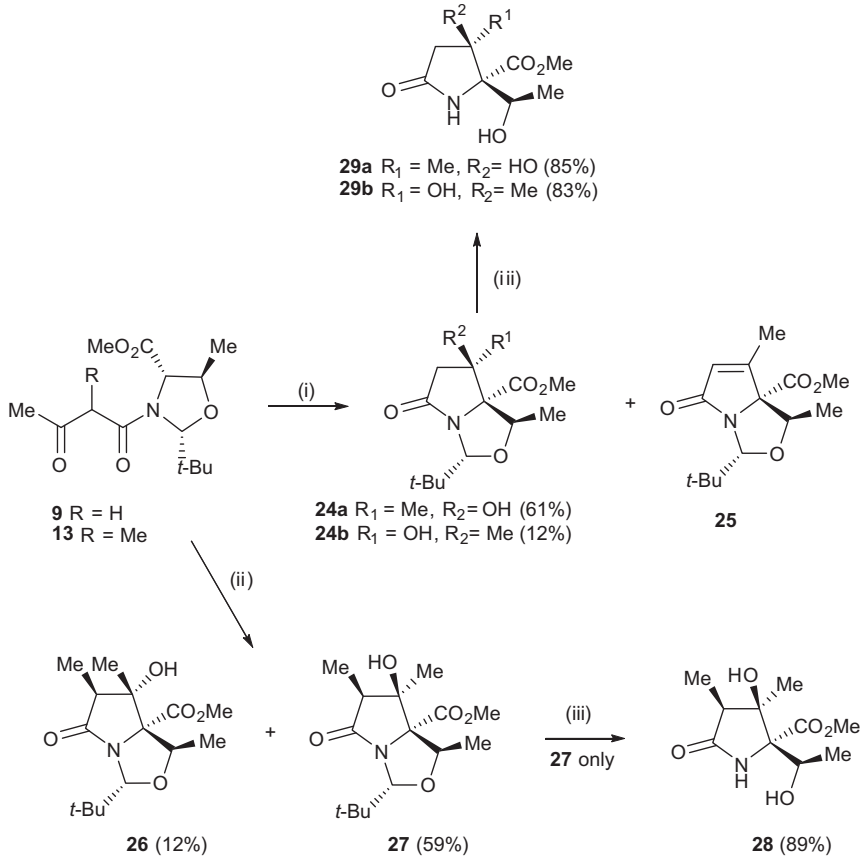
Cyclisation of *cis*-oxazolidine **13** with sodium methoxide in methanol gave only two diastereomers (Scheme 6). Purification by column chromatography gave major diastereomer **27** in 59% yield and minor diastereomer **26** in 12% yield, the relative stereochemistry of which were again determined by NOE experiment (Fig. 5). The major diastereomer **27** was recrystallised from ethyl acetate and analysed by single X-ray crystallography (Fig. 7), and this confirmed the NOE assignment.

2.3. Deprotection

The Corey-Reichard et al.[45] protocol (propane-1,3-dithiol in a solution of 2,2,2-trifluoroethanol acidified with HCl) was applied for the deprotection of bicyclic compounds **19**, **20**, **24a,b** and **27** to give the amido alcohols **21a,b**, **28** and **29a,b** in excellent yield (78–89%) (Schemes 4 and 6). These were purified by partitioning the reaction mixture between ethyl acetate and water (for **21a,b**) or purified by flash column chromatography eluting with ethyl acetate and methanol (4:1) (for **28** and **29a,b**). The structure of alcohol **29a** was determined by X-ray analysis (Fig. 8).

2.4. Bioassay

The tetramic and pyroglutamic acids obtained as described above were tested for their bioactivity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* using the disc diffusion method, and the results are given in Table 1. The value of whole-cell assays for finding bioactive lead compounds with antibacterial activity has been recently highlighted, prior to screening in biochemical and genetic assays to establish method of action;[46–48] although not giving quantitative MIC values, these assays were nonetheless suitable for our purpose, giving active/not active outcomes enabling rapid assessment of the structural variations of the fragment modification. Activity was seen for most compounds against the Gram-positive *Staphylococcus* and *Bacillus* strains. No



Scheme 6. Reagents and conditions: (i) NaOMe, MeOH, 18 h, rt; (ii) NaOMe, MeOH; (iii) $HS(CH_2)_3SH$, CF_3CH_2OH, 2% w/v HCl.

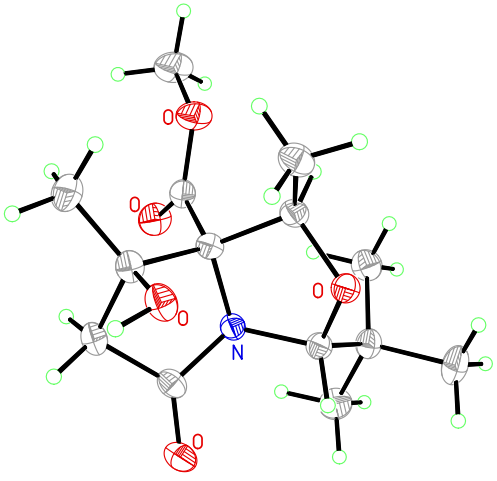


Figure 6. X-ray structure for **24a**.

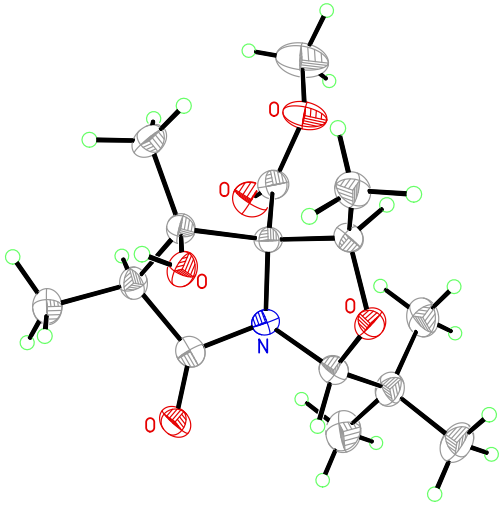


Figure 7. X-ray structure for **27**.

activity was seen against the Gram-negative *Escherichia coli*, with the exception of compound **29a**, which exhibits strong activity. The significantly lower cell wall permeability of Gram-negative bacteria, as a result of the presence of the lipopolysaccharide outer membrane, is well known, and this makes the development of novel Gram-negative antibacterials particularly challenging.[23]

Chemical informatics analysis[49] is instructive; log *P* and log $D_{7.4}$ values confirm the lipophilic character of protected oxazolidines **16**, **24a** and **27** but the significantly more hydrophilic character of alcohols **21b** and **29a**, and in the case of the latter, its ready ionisation at physiological pH, leading to a significant increase in polarity. All compounds have similar calculated van der Waals and solvent accessible (SASA) molecular surface area,[51] as would be expected from their common structural skeleton, with the protected oxazolidines **16**, **24a** and **27** possessing larger areas than the deprotected alcohols **21b** and **29a** (Table 1). The polar surface area (PSA) parameter, which correlates the presence of polar atoms with membrane permeability and therefore gives an indication of drug transport properties,[52] has been reported to have an optimal value of 70 < PSA < 120 Å^2 for a non-CNS orally absorbable drug,[53] and of interest is that the least active compound **16** possessed a

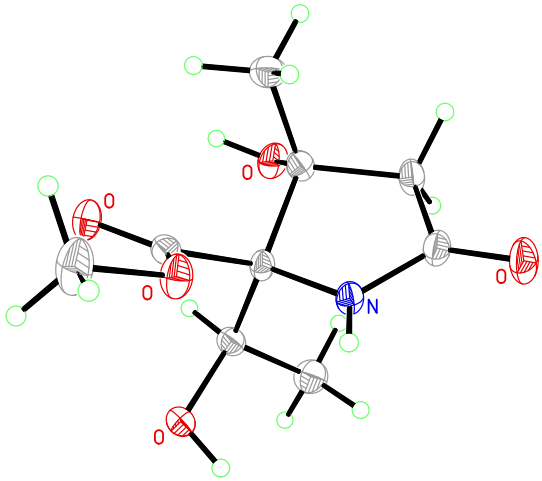


Figure 8. X-ray structure for **29a**.

PSA value outside this range. The higher activity of **21b, 24a, 27** and **29a** is likely to result from improved membrane permeability due to their higher polarity; these compounds possess relative PSA values of 18.1–29.5%, whereas for **16**, this value is as low as 13.4%. On the other hand, the *Bacillus* strains appeared to be particularly sensitive to the more hydrophobic compounds **24a** and **27**. These results suggest that the observed antibacterial activity of the oxazolomycins may be derived from both the right hand lactam and the central amide fragment, which we have recently shown to be independently active,[54] and although at this stage we have no target information for these compounds, we note the report of broad spectrum antibacterial activity of pyrrolidine-2,5-diones, which have a novel mode of action as inhibitors of acetyl-CoA carboxylase.[55]

Of interest is that this skeleton exhibits a similar physicochemical profile to other small molecule antibacterials,[23] and may provide a useful fragment for further optimisation of antibacterial activity by chemical manipulation at accessible points of the heterocyclic nucleus, using the 'rule of three';[25,42] noteworthy by comparison is the low levels of antibacterial activity we have recently reported for other simple spirocyclic bislactams derived from pyroglutamic acid,[30] and of simple tetramates[28] and pyrrolidines.[29]

Recently there has been interest not only in the identification of novel but synthetically tractable heterocyclic systems[56] but also in the identification of increasing molecular complexity by moving away from planar systems in the process of drug discovery.[57,58] The neglect of chirality has recently been recognised as a key deficiency of contemporary drug discovery methodology.[59] Our work demonstrates that enantiopure skeletons, which might be considered to be simpler mimics of natural products, with several points of diversity, are available in less than 3 synthetic steps, and that further simple modification gives structures which exhibit bioactivity properties akin to that of the parent natural product. Moreover, these systems, despite their three dimensional complexity, offer a small MW, PSA and numbers of rotatable bonds, H-bond acceptors and H-bond donors, and this leaves ample scope for optimisation in the drug discovery process using the Lipinski parameters. There is no doubt therefore that 'escaping from flatland'[57] may be more readily synthetically accessible than has hitherto been assumed, enabling the proposed benefits of such liberation to be realised.

3. Experimental

The following were prepared by literature methods: L-threonine methyl ester hydrochloride,[60,61] DL-threonine methyl ester hydrochloride **2**,[61,60] (4*S*,5*R*)-2-(*t*-butyl)-4-methoxycarbonyl-5-methyl-oxazolidine **3**[43] and (±)-2-(*t*-butyl)-4-methoxycarbonyl-5-methyl-oxazolidine **3**.[43] Ethyl-α-methylmalonic acid and ethyl-α-methylmalonyl chloride were prepared according to the method of Kissenger.[62] Acetoacetic acid **11** was prepared by the method of Ohta.[63] Ethyl α-phenylmalonate acid was prepared using the literature procedure.[64]

3.1. Acylation general method A1

To a solution of 2-*tert*-butyl-4-methoxycarbonyl-5-methyl oxazolidine **3** and pyridine in DCM (7/10 of volume) at 0 °C was added a solution of the appropriate acid chloride in DCM (3/10 of volume). The solution was stirred at 0 °C for 15 min and then at room temperature for 3–5 h. The solution was diluted with DCM (50 ml) and then washed with NH_4Cl (aq) (40 ml), 10% $NaHCO_3$ (aq) (40 ml) and brine (40 ml), dried over $MgSO_4$ and evaporated in vacuo.

3.2. Acylation general method A2

To a solution of 2-*tert*-butyl-4-methoxycarbonyl-5-methyl oxazolidine **3**, DCCI or EDAC and DMAP in DCM (7/10 ml volume) at 0 °C was added a solution of the appropriate acid in DCM or CH_3CN (3/10 ml of volume). The mixture was stirred at 0 °C for 15 min and then at room temperature for 3–5 h. The reaction mixture was filtered to remove dicyclohexyl urea, the residue being washed with DCM (3 × 15 ml) and the combined filtrates were evaporated in vacuo.

3.2.1. (2*R*,4*S*,5*R*)-2-(*tert*-Butyl)-3-(3-ethoxy-3-oxopropanoyl)-4-methoxycarbonyl-5-methyl-oxazolidine 4

According to Section 3.2, oxazolidine **3** (1.02 g, 5.06 mmol), DCCI (1.08 g, 5.26 mmol) and DMAP (43 mg, 0.35 mmol) in DCM (15 ml) were reacted with ethyl hydrogen malonate (695 mg, 5.26 mmol) in DCM (10 ml). The reaction mixture was filtered, washed with 1 M KH_2PO_4 (2 × 20 ml), dried over $MgSO_4$ and evaporated in vacuo. The crude mixture was purified by flash column

Table 1
Cheminformatic and bioassay data of selected compounds[a]

Compound	log *P*	log $D_{7.4}$	PSA (Å^2)	MSA (Å^2)	SASA (Å^2)	Zone size (cm)[b]		
						E. coli	*S. aureus*	*B. subtilis*
16	1.7	1.7	46.6	343	351	Inactive	2.89	Inactive
24a	1.1	1.1	76.1	461	420	Inactive	2.56	6.3
27	1.6	1.6	76.1	491	441	Inactive	3.03	5.87
21b	0.36	−0.69	72.4	293	363	—	3.24	Inactive
29a	−1.6	−1.6	95.9	325	341	4.86	Inactive	Inactive

[a] log *P*, log $D_{7.4}$, PSA, MSA and SASA values calculated using MARVIN.[49]
[b] Diameter of inhibition zone (±1 mm) from disc diffusion assay.[50]

chromatography (EtOAc/petrol, 1:3) to give the *cis*-oxazolidine **4** (1.3 g, 84% yield) as a light brown oil.

R_f = 0.21 (EtOAc/petrol, 1:3); $[\alpha]_D^{22} = -71.1$ (*c* 1.6, $CHCl_3$); ν_{max}/cm^{-1} (KBr) 2980 (m), 2960 (m), 2875 (w), 1745 (s), 1675 (s), 1390, 1370 and 1240; δ_H (400 MHz, $CDCl_3$) (1.5:1 mixture of conformers) Major: 0.88 (9H, s, C(CH_3)$_3$), 1.23–1.27 (3H, m, CH_2CH_3), 1.32 (3H, d, *J* 3.8 Hz, $CHCH_3$), 3.41 (1H, d, *J* 15.4 Hz, COCHHCO), 3.55 (1H, d, *J* 15.4 Hz, COCHHCO), 3.78 (3H, s, CO_2CH_3), 4.12–4.20 (2H, m, CH_2CH_3), 4.29 (1H, d, *J* 3.8 Hz, C(4)*H*), 4.72–4.76 (1H, m, C(5)*H*), 5.40 (1H, s, C(2)*H*); δ_C (100.6 MHz) 14.0 (CH_2CH_3), 20.1 ($CHCH_3$), 25.8 (C(CH_3)$_3$), 37.8 (*C*(CH_3)$_3$), 42.8 (*C*(7)), 52.8 (CO_2CH_3), 61.6 ($COCH_2CO$), 65.4 (*C*(4)), 76.1 (*C*(5)), 96.1 (*C*(2)), 166.6, 167.2, 167.3 and 170.0 (amide and ester carbonyls); *m/z* (ES^+) 338 ($M+Na^+$, 100%), 316 ($M+H^+$, 70%); ($M+H^+$) Found 316.1764, $C_{15}H_{26}NO_6$ requires 316.1760.

The racemic form (±)-4 was similarly prepared from **(±)-3** in 77% yield and exhibited similar spectroscopic data.

3.2.2. (2*R*,4*S*,5*R*)-2-(*tert*-Butyl)-3-(3-ethoxy-2-methyl-3-oxo propanoyl)-4-methoxycarbonyl-5-methyloxazolidine 5a and (2*S*,4*S*,5*R*,2′*S*)-2-(*tert*-butyl)-3-(3-ethoxy-2-methyl-3-oxopropanoyl)-4-methoxycarbonyl-5-methyl-oxazolidine 5b

According to Section 3.1, oxazolidine **3** (0.30 g, 1.5 mmol) was reacted with pyridine (0.25 ml, 3.15 mmol) and ethyl-α-methylmalonyl chloride (0.49 g, 3.0 mmol) in DCM (15 ml). Purification by flash column chromatography (EtOAc/petrol, 1:4) gave oxazolidine **5a** (0.31 g, 63% yield) as a viscous oil and oxazolidine **5b** (0.11 g, 23%) as a colourless crystalline solid.

Compound **5a**: R_f = 0.33 (EtOAc/petrol, 1:4); $[\alpha]_D^{22} = +95.2$ (*c* 2.2, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 2980 (m), 2960 (m), 2875 (w), 1744 (s), 1675 (s), 1395, 1370 and 1240; δ_H (400 MHz, $CDCl_3$) 0.88 (9H, s, C(CH_3)$_3$), 1.24 (3H, t, *J* 7.1 Hz, CH_2CH_3), 1.34 (3H, d, *J* 6.2 Hz, $CHCH_3$), 1.44 (3H, d, *J* 6.8 Hz, $COCHCH_3$), 3.63 (1H, q, *J* 6.8 Hz, $COCHCH_3$), 3.86 (3H, s, CO_2CH_3), 4.12 (2H, q, *J* 7.1 Hz, CH_2CH_3), 4.52 (1H, d, *J* 3.5 Hz, C(4)*H*), 4.78–4.83 (1H, m, C(5)*H*), 5.47 (1H, s, C(2)*H*); δ_C (100.6 MHz, $CDCl_3$) 14.1 (CH_2CH_3), 14.5 ($COCHCH_3$), 20.2 ($CHCH_3$), 25.7 (C(CH_3)$_3$), 37.7 (*C*(CH_3)$_3$), 45.5 ($COCHCH_3$), 52.8 (CO_2CH_3), 61.6 (CH_2CH_3), 65.2 (*C*(4)), 75.8 (*C*(5)), 95.7 (*C*(2)), 169.9, 170.3 and 171.7 (amide and ester carbonyls); *m/z* (ES^+) 388 ($M+H_2O-CH_3CN^+$, 100%), 330 ($M+H^+$, 10%), ($M+H^+$) Found 330.1845, $C_{16}H_{28}NO_6$ requires 330.1838.

Compound **5b**: R_f = 0.21 (EtOAc/petrol, 1:4); mp 92–93 °C; $[\alpha]_D^{22} = +87.2$ (*c* 2.2, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 2980 (m), 2960 (m), 2875 (w), 1744 (s), 1675 (s), 1395, 1370 and 1240; δ_H (400 MHz, $CDCl_3$) 1.03 (9H, s, C(CH_3)$_3$), 1.27 (3H, t, *J* 7.2 Hz, CH_2CH_3), 1.34 (3H, d, *J* 6.1 Hz, $CHCH_3$), 1.42 (3H, d, *J* 7.2 Hz, $COCHCH_3$), 3.47 (1H, q, *J* 6.7 Hz, $COCHCH_3$), 3.76 (3H, s, CO_2CH_3), 3.85 (1H, d, *J* 8.8 Hz, C(4)*H*), 4.06–4.22 (3H, m, C(5)*H* and CH_2CH_3), 5.10 (1H, s, C(2)*H*); δ_C (100.6 MHz, $CDCl_3$) 14.1 (CH_2CH_3), 15.2 ($COCHCH_3$), 20.2 ($CHCH_3$), 25.8 (C(CH_3)$_3$), 38.8 (*C*(CH_3)$_3$), 45.8 ($COCHCH_3$), 52.8 (CO_2CH_3), 61.6 (CH_2CH_3), 65.8 (*C*(4)), 75.8 (*C*(5)), 96.6 (*C*(2)), 169.7, 170.0 and 171.4 (amide and ester carbonyls); *m/z* (ES^+) 330 ($M+H^+$, 100%), ($M+H^+$) Found 330.1834, $C_{16}H_{28}NO_6$ requires 330.1838. The crystal structure was determined from X-ray diffraction data.

The racemic forms **(±)-5a,b** were similarly prepared from **(±)-3** in 77% yield and exhibited similar spectroscopic data.

3.2.3. (2*R*,4*S*,5*R*,2′*R*)-2-(*tert*-Butyl)-3-(3-ethoxy-2-phenyl-3-oxo-propanoyl)-4-methoxycarbonyl-5-methyl-oxazolidine 6a and (2*R*,4*S*,5*R*,2′*S*)-2-(*tert*-butyl)-3-(3-ethoxy-2-phenyl-3-oxopropanoyl-4-methoxycarbonyl-5-methyl-oxazolidine 6b

According to Section 3.2, oxazolidine **3** (350 mg, 1.74 mmol), DCCI (392 mg, 1.91 mmol) and DMAP (16 mg, 0.13 mmol) in DCM (10 ml) were reacted with ethyl α-phenylmalonic acid (252 mg, 1.91 mmol) in DCM (4 ml). The reaction mixture was filtered, dried over $MgSO_4$ and evaporated in vacuo. Recrystallisation from ethyl acetate gave oxazolidine **6a** (334 mg, 49%) as colourless crystals.

Compound **6a**: mp 130–132 °C; R_f = 0.34 (EtOAc/petrol, 1:3); $[\alpha]_D^{22} = -43.4$ (*c* 2.0, $CHCl_3$); ν_{max}/cm^{-1} (KBr) 2990 (m), 2960 (m), 2922 (w), 2875 (w), 1745 (s), 1675 (s), 1390, 1370 and 1170; δ_H (400 MHz, $CDCl_3$) 0.79 (3H, d, *J* 6.3 Hz, $CHCH_3$), 0.93 (9H, s, C(CH_3)$_3$), 1.27 (3H, t, *J* 7.1 Hz, $CO_2CH_2CH_3$), 3.89 (3H, s, CO_2CH_3), 4.05 (1H, d, *J* 3.0 Hz, C(4)*H*), 4.16–4.22 (2H, q, *J* 7.1 Hz, CH_2CH_3), 4.65–4.70 (1H, m, C(5)*H*), 4.90 (1H, s, *CH*Ph), 5.40 (1H, s, C(2)*H*), 7.30–7.37 (5H, m, Ar*H*); δ_C (100.6 MHz, $CDCl_3$) 14.0 ($CO_2CH_2CH_3$), 19.2 ($CHCH_3$), 25.7 (C(CH_3)$_3$), 37.7 (*C*Me$_3$), 52.9 (CO_2CH_3), 58.2 (*C*HPh), 61.7 ($COCH_2CH_3$), 64.4 (*C*(4)), 75.3 (*C*(5)), 95.9 (*C*(2)), 127.9, 128.2, 128.5, 129.2 and 132.1 (Ar*C*), 168.3, 169.0, 170.0 (amide and ester carbonyls); *m/z* (ES^+) 392 ($M+H^+$, 100%), ($M+H^+$) Found 392.2069, $C_{21}H_{30}NO_6$ requires 392.2073. The crystal structure was determined from X-ray diffraction data.

Purification of the residue from the above-mentioned crystallisation with flash column chromatography (EtOAc/petrol) gave oxazolidine **6a** (29 mg, 4.2%), mixture of oxazolidines **6a** and **6b** (1:1) (40 mg, 5.8%) and oxazolidine **6b** (75 mg, 11%) as a colourless oil.

Compound **6b**: R_f = 0.31 (EtOAc/petrol, 1:3); $[\alpha]_D^{22} = +87$ (*c* 2.0, $CHCl_3$); ν_{max}/cm^{-1} (KBr) 2990 (m), 2958 (m), 2920 (w), 2870 (w), 1745 (s), 1677 (s), 1390, 1375 and 1170; δ_H (400 MHz, $CDCl_3$) 0.82 (9H, s, C(CH_3)$_3$), 1.27 (3H, t, *J* 7.1 Hz, $CO_2CH_2CH_3$), 1.39 (3H, d, *J* 6.2 Hz, $CHCH_3$), 3.75 (3H, s, CO_2CH_3), 4.23 (2H, q, *J* 7.1 Hz, $CO_2CH_2CH_3$), 4.49 (1H, d, *J* 3.2 Hz, C(4)*H*), 4.83–4.85 (1H, m, C(5)*H*), 4.92 (1H, s, *CH*Ph), 5.51 (1H, s, C(2)*H*), 7.33–7.39 (3H, m, Ar*H*), 7.43–7.48 (2H, m, Ar*H*); δ_C (100.6 MHz, $CDCl_3$) 14.1 ($CO_2CH_2CH_3$), 19.9 ($CHCH_3$), 25.7 (C(CH_3)$_3$), 37.7 (*C*Me$_3$), 52.7 (CO_2CH_3), 57.3 (*C*HPh), 62.0 ($COCH_2CH_3$), 65.6 (*C*(4)), 76.0 (*C*(5)), 96.0 (*C*(2)), 128.0, 128.3, 128.6, 129.3, 129.7 and 133.6 (Ar*C*), 168.4, 169.5, 170.0 (amide and ester carbonyls); *m/z* (ES^+) 392 ($M+H^+$, 100%), HRMS ($M+H^+$) Found 392.2065, $C_{21}H_{30}NO_6$ requires 392.2073.

3.2.4. (2*R*,4*S*,5*R*)-2-(*tert*-Butyl)-3-(2-phenylacetyl)-5-methyl-4-methoxycarbonyl-oxazolidine 7

According to Section 3.1, oxazolidine **3** (1.0 g, 4.98 mmol) and pyridine (0.59 g, 7.46 mmol) in DCM (15 ml) were reacted with phenylacetyl chloride (0.93 g, 5.97 mmol) in DCM (8 ml). Purification by flash column chromatography on silica gel (DCM/petrol, 1:1 gradually increasing polarity to DCM) gave the title compound **7** (1.22 g, 77%) as a white crystalline solid.

R_f = 0.34 (DCM); $[\alpha]_D^{22} = -4.9$ (*c* 1.56, $CHCl_3$) ν_{max}/cm^{-1} (KBr) 2956 (s), 2870 (m), 1746 (s), 1436 (s), 1208 (s), 1173 (s); δ_H (400 MHz, $CDCl_3$) 0.93 (9H, s, C(CH_3)$_3$), 1.12 (3H, d, *J* 5.9 MHz, $CHCH_3$), 3.68 (1H, d, *J* 15.0 Hz, *CH*HPh), 3.78 (1H, d, *J* 15.0 Hz, CH*H*Ph), 3.81 (3H, s, CO_2CH_3), 4.20 (1H, d, *J* 3.5 Hz, C(4)*H*), 4.67–4.70 (1H, m, C(5)*H*), 5.43 (1H, s, C(2)*H*), 7.21–7.37 (5H, m, Ar*H*); δ_C (100.6 MHz, $CDCl_3$) 20.0 ($CHCH_3$), 25.9 (C(CH_3)$_3$), 37.9 (*C*Me$_3$), 42.3 (CH_2Ph), 52.7 (CO_2CH_3), 65.2 (*C*(4)), 76.0 (*C*(5)), 96.4 (*C*(2)), 127.1, 128.8, 129.0, 129.2 and 134.1 (Ar*C*), 170.3 and 172.0 (amide and ester carbonyls); *m/z* (ES^+) 320 ($M+H^+$, 100%), 264 ($M+H^+-{}^tBu$), ($M+H^+$) Found 320.1871, $C_{18}H_{26}NO_4$ requires 320.1862.

The racemic form **(±)-7** was similarly prepared from **(±)-3** in 77% yield and exhibited similar spectroscopic data.

3.2.5. (2*R*,4*S*,5*R*)-2-(*tert*-Butyl)-3-(2-cyanoacetyl)-4-methoxycarbonyl-5-methyl-oxazolidine 8

According to Section 3.2, a solution of oxazolidine **3** (0.6 g, 3.0 mmol), DMAP (0.04 mg, 0.3 mmol) and DCCI (0.62 g, 3.0 mmol) in DCM (10 ml) at 0 °C were reacted with cyanoacetic acid (0.26 mg, 3.0 mmol) in CH_3CN (2 ml). The reaction mixture was stirred at room temperature for 4 h and then filtered, the residue was washed with DCM (3 × 15 ml) and combined filtrates were

concentrated in vacuo. Purification by flash column chromatography (EtOAc/DCM, 1:9) gave oxazolidine **8** (0.59 g, 73%) as a white crystalline solid.

R_f = 0.31 (EtOAc/DCM, 1:9); mp 82–83 °C; $[\alpha]_D^{22} = -41.7$ (*c* 1.2, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 2960 (m), 1750 (s), 1730 (s) and 1665 (s); δ_H (mixture of rotamers) (400 MHz, $CDCl_3$) 0.92 (9H, *s*, $C(CH_3)_3$), 1.38 and 1.40 (3H, s, $CHCH_3$), 3.57 (1H, d, *J* 19.4 Hz, CHHCN), 3.66 (1H, d, *J* 18.1 Hz, CHHCN), 3.80 and 3.83 (3H, s, CO_2CH_3), 4.09–4.14 (1H, br s, C(4)*H*), 4.75–4.79 (1H, m, C(5)*H*), 5.42 (1H, s, C(2)*H*); δ_C (100.6 MHz, $CDCl_3$) 20.5 ($CHCH_3$), 25.9 ($C(CH_3)_3$), 26.5 (CH_2CN), 33.9 ($C(CH_3)_3$), 53.2 (CO_2CH_3), 65.5 (*C*(4)), 76.5 (*C*(5)), 97.1 (*C*(2)), 113.5 (CN), 162.7 and 169.3 (amide and ester carbonyls); *m/z* (ES^+) 269 ($M+H^+$, 100%), 184 ($M+H^+ - {}^t$BuCHO, 45%); ($M+H^+$) Found 269.1513, $C_{13}H_{21}N_2O_5$ requires 269.1501.

The racemic form **(±)-8** was similarly prepared from **(±)-3** in 73% yield and exhibited similar spectroscopic data.

3.2.6. *tert*-Butyl-α-methylacetoacetate

To a solution of *tert*-butyl acetoacetate **10** (4.00 g, 25.3 mmol) in THF (25 ml) at 0 °C was added KO^{t}Bu (2.97 g, 26.5 mmol). The mixture was cooled to room temperature and stirred for 40 min, and then a solution of MeI (1.74 ml, 27.9 mmol) in THF (6 ml) was added dropwise over 10 min. The mixture was stirred for further 5 h and partitioned between ether (30 ml) and water (30 ml). The aqueous layer was extracted with ether (30 ml) and the combined organic extracts were washed with brine (40 ml), dried over $MgSO_4$ and evaporated in vacuo. Purification by Kugelrohr distillation gave *tert*-butyl-α-methyl acetoacetate (2.1 g, 68%) as a colourless liquid. ν_{max}/cm^{-1} (film) 2980 (m), 2940 (m), 1740 (s), 1715 (s), 1370 (s) and 1150 (s); δ_H (200 MHz, $CDCl_3$) 1.29 (3H, d, *J* 7.0 Hz, $CHCH_3$), 1.46 (9H, s, $C(CH_3)_3$), 2.23 (3H, s, CH_3CO), 3.41 (1H, q, *J* 7.0 Hz, $CHCH_3$); *m/z* (GCMS) 190 ($M + NH_4^+$, 5%), 173 ($M+H^+$, 100%).

3.2.7. α-Methylacetoacetic acid 12

A solution of *tert*-butyl-α-methylacetoacetate (2.0 g, 11.6 mmol) in TFA (5 ml) was stirred at room temperature for 24 h. TFA was removed by co-evaporation with toluene in vacuo to give α-methylacetoacetic acid **12** (1.05 g, 77%) as a dark brown oil. ν_{max}/cm^{-1} (film) 2980 (m), 2940 (m), 1740 (s), 1715 (s), 1370 (s) and 1150 (s); δ_H (200 MHz, $CDCl_3$) 1.38 (3H, d, *J* 7.0 Hz, $CHCH_3$), 2.32 (3H, s, CH_3CO), 3.59 (1H, q, *J* 7.0 Hz, $CHCH_3$), 11.82 (1H, br s, CO_2H).

3.2.8. (2*R*,4*S*,5*R*)-3-Acetoacetyl-2-(*tert*-butyl)-4-methoxycarbonyl-5-methyl-oxazolidine 9a and 9b

According to Section 3.2, oxazolidine **3** (0.9 g, 4.49 mmol), DMAP (44.0 mg, 0.36 mmol) and DCCI (1.09 g, 5.29 mmol) in DCM (20 ml) were reacted with acetoacetic acid (0.54 g, 5.29 mmol) in DCM (8 ml). Purification by flash column chromatography (EtOAc/petrol, 1:3) gave *cis*-oxazolidine **9a,b** (0.96 g, 79%), as mixture of keto enol tautomers (10:7), as a light brown oil. R_f = 0.27 (EtOAc/petrol, 1:3); ν_{max}/cm^{-1} (thin film) 2960 (s), 2860 (s), 2118 (s), 1746 (s), 1436 (s), 1208 (s), 1173 (s); $[\alpha]_D^{22} = -34.7$ (*c* 1, $CHCl_3$); Keto tautomer: δ_H (400 MHz, $CDCl_3$) 0.89 (9H, s, $C(CH_3)_3$), 1.23 (3H, d, *J* 7.1 Hz, $CHCH_3$), 2.29 (3H, s, $COCH_3$), 3.56 (1H, d, *J* 15.2 Hz, COCHHCO), 3.61 (1H, d, *J* 14.9 Hz, COCHHCO), 3.77 (3H, s, CO_2CH_3), 4.22 (1H, d, *J* 4.1 Hz, C(4)*H*), 4.72–4.77 (1H, m, C(5)*H*), 5.41 (1H, s, C(2)*H*); δ_C (100.6 MHz, $CDCl_3$) 20.1 ($CHCH_3$), 23.4 ($COCH_3$), 25.8 ($C(CH_3)_3$), 37.9 ($C(CH_3)_3$), 51.5 ($COCH_2CO$), 52.8 (CO_2CH_3), 65.4 (*C*(4)), 76.1 (*C*(5)), 96.1 (*C*(2)), 168.0, 170.0, 170.3, 176.4 (amide and ester carbonyls), 202.3 (CH_2COCH_3).

Enol tautomer δ_H (100.6 MHz, $CDCl_3$) 0.95 (9H, s, $C(CH_3)_3$), 1.25 (3H, d, *J* 7.1 Hz, $CHCH_3$), 1.96 (3H, s, $COCH_3$), 3.79 (3H, s, CO_2CH_3), 4.22 (1H, d, *J* 4.1, C(4)*H*), 4.60–4.64 (1H, m, C(5)*H*), 4.97–5.02 (1H, br s, CHOH), 5.41 (1H, s, C(2)*H*); δ_C 20.5 ($CHCH_3$), 26.1 ($C(CH_3)_3$), 30.5 ($COCH_3$), 37.9 ($C(CH_3)_3$), 52.6 (CO_2CH_3), 65.4 (*C*(4)), 77.2 (*C*(5)), 89.1 (CHOH), 96.1 (*C*(2)); *m/z* (ES^+) 286 ($M+H^+$, 100%); ($M+H^+$) Found 286.1651, $C_{14}H_{24}NO_5$ requires 286.1654.

The racemic form **(±)-9a,b** was similarly prepared from **(±)-3** in 71% yield as a mixture of keto/enol tautomers (4:3), and exhibited similar spectroscopic data.

3.2.9. (2*R*,4*S*,5*R*)-3-(Acetoacetyl-2-(*tert*-butyl)-4-methoxycarbonyl-5-methyl-oxazolidine 13 and (2*S*,4*S*,5*R*)-3-(Acetoacetyl-2-(*tert*-butyl)-4-methoxycarbonyl-5-methyl-oxazolidine 14

According to Section 3.2, oxazolidine **3** (0.30 g, 1.49 mmol), DMAP (21.0 mg, 0.18 mmol) and EDAC (0.34 g, 1.79 mmol) in DCM (5 ml) were reacted with α-methyl acetoacetic acid (0.21 g, 1.79 mmol) in DCM (2 ml). The mixture was then partitioned between DCM (20 ml) and saturated NH_4Cl (aq) (20 ml), organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification by flash column chromatography (EtOAc/petrol, 1:3) gave oxazolidine **13** (0.21 g, 71%) and oxazolidine **14** (0.09 g, 29%) as a light brown oil.

Compound **13**: R_f = 0.32 (EtOAc/petrol, 1:3); ν_{max}/cm^{-1} ($CHCl_3$) 2960 (m), 1750 (s), 1730 (s) and 1665 (s); δ_H (400 MHz, $CDCl_3$) (mixture of epimers (1:1)); 0.95, 1.01 (9H, 2 s, $C(CH_3)_3$,), 1.30 and 1.32 (3H, 2 d, *J* 6.3 Hz and *J* 6.9 Hz, C(5)HCH_3 and COCHCH_3CO), 1.36 (3H, d, *J* 6.3 Hz, C(5)HCH_3, epimer a), 1.50 (3H, d, *J* 6.8 Hz, COCHCH_3CO), 2.22 (3H, s, $COCH_3$, epimer a), 2.26 (3H, s, $COCH_3$, epimer b), 3.46 (1H, q, *J* 7.0 Hz, COC*H*CH_3CO, epimer a), 3.64 (1H, q, *J* 7.0 Hz, COC*H*CH_3CO, epimer b), 3.82 and 3.85 (3H, 2 s, CO_2CH_3, epimers a and b), 4.12 (1H, d, *J* 4.3 Hz, C(4)*H*, epimer a), 4.43 (1H, d, *J* 4.3 Hz, C(4)*H*, epimer b), 4.73–4.98 (2H, m, C(5)*H*, epimers a and b), 5.43 and 5.44 (2H, 2 s, C(2)*H*, epimers a and b); δ_C (100.6 MHz, $CDCl_3$) 13.0, (COCHCH_3), 20.1, 20.4 (C(5)HCH_3), 25.8, 25.9 ($C(CH_3)_3$), 27.1, 27.5 ($COCH_3$), 37.7, 38.1 ($C(CH_3)_3$), 52.8, 52.9, 53.2, 54.5 (COCHCO and CO_2CH_3), 64.9, 65.8 (*C*(4), 75.9, 76.0 (*C*(5)), 95.9, 96.3 (*C*(2)), 170.0, 170.4, 172.8 (amide and ester carbonyls), 203.0 ($COCH_3$); *m/z* (ES^+) 300 ($M+H^+$, 55%), 215 ($M+H^+ - {}^t$BuCHO, 100%); ($M+H^+$) Found 300.1793, $C_{15}H_{26}NO_5$ requires 300.1811.

Compound **14**: R_f = 0.27 (EtOAc/petrol, 1:3); $[\alpha]_D^{20} = +11.6$ (*c* 3, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 2960 (m), 1750 (s), 1730 (s) and 1665 (s); δ_H (200 MHz, $CDCl_3$) (1:1 mixture of epimers) 0.87, 0.94 (9H, 2 s, $C(CH_3)_3$), 1.28, 1.29, 1.34, 1.36 (3H, 4 d, *J* 6.3, 6.8 Hz, $COCHCH_3$-CO, C(5)HCH_3), 2.22, 2.54 (3H, 2 s, $COCH_3$), 3.48 (1H, q, *J* 6.9 Hz, COC*H*CH_3, epimer a), 3.63 (1H, q, COC*H*CH_3, epimer b), 3.78, 3.80 (3H, 2 s, $COCH_3$), 4.10 (1H, d, *J* 4.3 Hz, C(4)*H*, epimer a), 4.42 (1H, d, *J* 4.4 Hz, C(4)*H*), 4.73–4.81 (1H, m, C(5)*H*), 5.42, 5.43 (1H, s, C(2)*H*); *m/z* (ES^+) 300 ($M+H^+$, 100%); ($M+H^+$) Found 300.1816, $C_{15}H_{26}NO_5$ requires 300.1811.

The racemic form **(±)-13,14** was similarly prepared from **(±)-3** in 69% yield as a mixture of keto/enol tautomers (8:3), and exhibited similar spectroscopic data.

3.2.10. (2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-5-methoxycarbonyl-4-methyl-6,8-dioxo-3-oxabicyclo[3.3.0]-octane 15 and (2*R*,4*R*,5*R*)-1-aza-2-(*tert*-butyl)-4-methyl-6,8-dioxo-3-oxabicyclo[3.3.0] octane 16

To a solution of oxazolidine **4** (0.2 g, 0.64 mmol) in ^{t}BuOH (15 ml) was added KO^{t}Bu (78 mg, 0.70 mmol) and the solution was heated at reflux for 7 h, cooled to room temperature and partitioned between ether (15 ml) and water (2 × 15 ml). The aqueous layer was acidified with 2 M HCl and extracted with ether (2 × 20 ml). The organic extracts were washed with brine, dried over $MgSO_4$ and evaporated in vacuo to give a brown oil which was purified by flash column chromatography (EtOAc/petrol, 1:1 increasing polarity to neat EtOAc) to give pure tetramic acid **15** as a light yellow solid (72 mg, 42% yield) and dicarbonyl **16** (11 mg, 7%) as a white solid.

Compound **15**: mp 145–147 °C; R_f = 0.22 (EtOAc/petrol, 1:1); $[\alpha]_D^{22} = +47.2$ (*c* 1.5, MeOH); ν_{max}/cm^{-1} ($CHCl_3$) 2960 (m), 1750 (s), 1730 (s) and 1665 (s); δ_H (400 MHz, $CDCl_3$) 0.91 (9H, s, C(C*H*$_3$)$_3$), 1.06 (3H, d, *J* 6.7 Hz, C(4)HC*H*$_3$), 3.15 (1H, d, *J* 21.7 Hz, C(7)*H*H), 3.65 (1H, d, *J* 21.7 Hz, C(7)H*H*), 3.81 (3H, s, CO_2CH_3), 5.10 (1H, q, *J* 6.7 Hz, C*H*CH$_3$), 5.13 (1H, s, C(2)*H*); δ_C (100.6 MHz, $CDCl_3$) 15.1 (CHC*H*$_3$), 24.7 (C(C*H*$_3$)$_3$), 35.4 (*C*(CH$_3$)$_3$), 45.2 (*C*(7)), 53.6 (CO_2CH_3), 73.9 (*C*(5)), 75.0 (*C*(4)), 95.8 (*C*(2)), 162.7, 173.4 and 199.5 (carbonyls); *m/z* (ES^-) 268 (M−H$^-$, 35%), 201 (M−H$^-$−^{t}BuH, 100%); (M−H$^-$) Found 268.1179, $C_{13}H_{18}NO_5$ requires 268.1185.

Compound **16**: R_f = 0.17 (EtOAc); $[\alpha]_D^{22} = +95.4$ (*c* 1.5, MeOH); ν_{max} 2970 (m), 1775 (s), 1720 (s), 1370 (s), 1360 (s), 1245 (s); δ_H (400 MHz, $CDCl_3$) 0.96 (9H, s, C(C*H*$_3$)$_3$), 1.02 (3H, d, *J* 6.6 Hz, CHC*H*$_3$), 3.10 (1H, d, *J* 22.1 Hz, COC*H*H), 3.39 (1H, d, *J* 22.1 Hz, COCH*H*), 4.37 (1H, d, *J* 6.2 Hz, C*H*CO), 4.53 (1H, q, *J* 6.6 Hz, C*H*CH$_3$), 5.20 (1H, s, C(2)*H*); δ_C (100.6 MHz) 15.7 (CHC*H*$_3$), 24.7 (C(C*H*$_3$)$_3$), 36.2 (*C*(CH$_3$)$_3$), 45.4 (*C*(7)), 71.8 (*C*(5)), 73.6 (*C*(4)), 94.2 (*C*(2)), 172.9 (N*C*O) and 203.6 (*C*(6)); *m/z* (ES^-) 211 (M−H$^-$, 100%); (M−H$^-$) Found 211.1198, $C_{11}H_{17}NO_3$ requires 211.1208.

The racemic form **(±)-15,16** was similarly prepared from **(±)-3** in 46 and 6% yield, respectively, and exhibited similar spectroscopic data.

3.2.11. (2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6,8-dioxo-4,7-dimethyl-5-methoxycarbonyl-3-oxabicyclo-[3.3.0]octane 17

To a solution of oxazolidine **5a** (0.15 g, 0.46 mmol) in ^{t}BuOH (10 ml) was added KO^{t}Bu (57 mg, 0.51 mmol) and the resultant solution was heated at reflux for 3 h, cooled to room temperature and partitioned between ether (15 ml) and water (15 ml). The aqueous layer was acidified with 2 M HCl and extracted with ether (2 × 15 ml). The combined organic extracts were washed with brine, dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by flash column chromatography (EtOAc/petrol, 1:3 increasing polarity to neat EtOAc) to give the product **17** (as mixture of tautomers) in 43% yield (55 mg).

Mp 131–133 °C; R_f = 0.16 (EtOAc); ν_{max}/cm^{-1} 3675 (w), 3550 (br w), 3410 (br w), 2960 (m), 1780 (m), 1725 (s); δ_H (400 MHz, $CDCl_3$) (enol tautomer) 0.93 (9H, s, C(C*H*$_3$)$_3$), 1.23 (3H, d, *J* 6.7 Hz, C(4)HC*H*$_3$), 1.69 (3H, s, C(7)C*H*$_3$), 3.80 (3H, s, CO_2CH_3), 4.97 (1H, s, C(2)*H*), 5.09 (1H, q, *J* 6.7 Hz, C(4)*H*CH$_3$); δ_C (100.6 MHz, $CDCl_3$) 6.1 (C(7)*C*H$_3$), 15.3 (C(4)H*C*H$_3$), 24.8 (C(*C*H$_3$)$_3$), 36.7 (*C*(CH$_3$)$_3$), 52.8 (CO_2CH_3), 75.2 (C(5)), 79.9 (C(4), 96.0 (C(2)), 103.7 (C(7)), 169.1 and 169.3 (amide and ester CO) and 181.2 (C(6)); (keto tautomer) 0.92 (9H, s, C(C*H*$_3$)$_3$), 1.21 (3H, d, *J* 6.9 Hz, C(4)HC*H*$_3$), 1.27 (3H, d, *J* 7.6, C(7)HC*H*$_3$), 3.69 (1H, q, *J* 7.6, C(7)*H*CH$_3$), 3.82 (3H, s, CO_2CH_3), 4.69 (1H, q, *J* 6.9, C(4)*H*CH$_3$) and 5.07 (1H, s, C(2)H); δ_C (100.6 MHz, $CDCl_3$) 15.7 (C(7)*C*H$_3$), 16.3 (C(4)*C*H$_3$), 24.6 (C(*C*H$_3$)$_3$), 35.5 (*C*(CH$_3$)$_3$), 49.4 (C(7)), 53.4 (CO_2CH_3), 80.1, 82.2 (C4) and C5), 97.8 (C(2)), 167.7 and 175.0 (amide and ester CO) and 202.1 (C(6)); *m/z* (ES^+) 284 (M+H$^+$); Found 284.1491, $C_{14}H_{22}NO_5$ requires 284.1492.

3.2.12. (2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-4-methyl-8-oxo-7-phenyl-3-oxabicyclo[3.3.0]oct-6-ene 19

To a solution of oxazolidine **6a** (130 mg, 0.33 mmol) in dry *tert*-butanol was added KO^{t}Bu (141 mg, 0.36 mmol) and the mixture was heated to reflux for 3 h. The mixture was then cooled to room temperature and partitioned between ether (20 ml) and water (20 ml). The aqueous layer, after re-extraction with ether (15 ml), was acidified with 2 M HCl and extracted with EtOAc (2 × 20 ml). The organic extracts were washed with brine (25 ml), dried over $MgSO_4$ and evaporated in vacuo to give the crude brown oil which was purified by flash column chromatography eluting with EtOAc/MeOH (4:1) to give the title compound **19** (76 mg, 67%) as a brown semi-solid. R_f = 0.37 (EtOAc/MeOH, 6:1); $[\alpha]_D^{20} = +87.2$ (*c* 1.2, MeOH); ν_{max}/cm^{-1} ($CHCl_3$) 3520 (w), 2960 (m), 2875 (w), 1750 (s), 1730 (s), 1665 (s), 1285 and 1176; δ_H (400 MHz, CD_3OD) 0.92 (9H, s, C(C*H*$_3$)$_3$), 1.09 (3H, d, *J* 6.7 Hz, CHC*H*$_3$), 3.78 (3H, s, CO_2CH_3), 4.84 (1H, s, C(2)*H*), 5.10 (1H, q, *J* 6.7 Hz, C*H*CH$_3$), 7.14 (1H, t, *J* 7.2 Hz, Ar*H*), 7.30 (2H, t, *J* 7.7 Hz, Ar*H*), 7.83 (2H, d, *J* 7.5 Hz, Ar*H*); δ_C (100.6 MHz, CD_3OD) 14.1 (CHC*H*$_3$), 24.5 (C(C*H*$_3$)$_3$), 35.3 (*C*(CH$_3$)$_3$), 52.3 (CO_2CH_3), 74.6 (*C*(4)), 94.4 (*C*(2)), 104.2 (*C*(7)), 125.5, 127.2, 127.8 and 132.8 (C_6H_5), 171.3, 175.6 and 1821.0 (*C*(6) and carbonyls); *m/z* (ES^-) 344 (M−H$^-$, 100%), (M−H$^-$) Found 344.1487, $C_{19}H_{22}NO_5$ requires 344.1498.

3.2.13. (2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-4-methyl-8-oxo-7-phenyl-3-oxabicyclo [3.3.0] oct-6-ene 20

To a solution of oxazolidine **7** (0.50 g, 1.56 mmol) in methanol (5 ml) was added NaOMe (0.09 g, 1.72 mmol) and the mixture was heated at reflux for 16 h. After cooling to room temperature the mixture was partitioned between DCM (20 ml) and water (20 ml). The DCM layer was dried over $MgSO_4$ and evaporated in vacuo. Purification by flash column chromatography eluting with DCM gave starting material (0.13 g, 25%). The aqueous layer was acidified with 2 M HCl and extracted with ether (2 × 25 ml). The ether extracts were washed with brine (15 ml), dried over $MgSO_4$ and evaporated in vacuo. Purification by flash column chromatography (EtOAc/DCM, 1:6) gave the title tetramic acid **20** (0.24 g, 53%) as a white solid.

Mp 179–180 °C; R_f = 0.29 (EtOAc/DCM, 1:1); $[\alpha]_D^{22} = +63.2$ (*c* 1.12, MeOH); ν_{max}/cm^{-1} (KBr) 3310–2800 (m), 2960 (m), 2875 (w), 1625 (s), 1595, 1400, 1370 and 1340; δ_H (400 MHz, $CDCl_3$) 0.98 (9H, s, C(C*H*$_3$)$_3$), 1.42 (3H, d, *J* 5.8 Hz, CHC*H*$_3$), 3.56–3.62 (1H, m, C*H*CH$_3$), 3.78 (1H, d, *J* 8.4 Hz, C(5)*H*), 4.77 (1H, s, C(2)*H*), 7.22–7.51 (5H, m, ArH); δ_C (100.6 MHz, $CDCl_3$) 17.4 (CHCH$_3$), 24.8 (C(*C*H$_3$)$_3$), 35.6 (*C*(CH$_3$)$_3$), 67.6 (*C*(5)), 76.9 (*C*(4)), 94.3 (*C*(2)), 108.1 (*C*(7)), 127.6, 128.2, 128.4, 128.7 and 129.3 (Ar*C*), 168.3 (*C*(6)), 177.5 (*C*(8)); *m/z* (ES^-) 286 (M−H$^-$, 100%), (M−H$^-$) found 286.1518, $C_{17}H_{21}NO_3$ requires 286.1521.

The racemic form **(±)-20** was similarly prepared from **(±)-7** in 58% yield, and exhibited similar spectroscopic data.

3.2.14. (2*R*,4*R*,5*S*)-1-Aza-6-amino-2-(*tert*-butyl)-5-methoxycarbonyl-4-methyl-8-oxo-3-oxabicyclo-[3.3.0]-oct-6-ene 22

To a solution of nitrile **8** (200 mg, 0.75 mmol) in MeOH (8 ml) was added NaOMe (54 mg, 0.80 mmol). The mixture was stirred at room temperature for 15 h then partitioned between ether (20 ml) and water (20 ml). The aqueous layer was acidified with 2 M HCl and extracted with ether (3 × 10 ml). The combined organic extracts were washed with brine (15 ml), dried over $MgSO_4$ and concentrated in vacuo to give the title compound **22** (142 mg, 71%) as a pale yellow crystalline solid. Mp 139–141 °C; R_f = 0.36 (EtOAc); $[\alpha]_D^{22} = +23.2$ (*c* 1.3, $CHCl_3$); ν_{max}/cm^{-1} 3520 (w), 3405 (w), 2870 (m), 1695 (m), 1640 (s); δ_H (400 MHz, $CDCl_3$) 0.92 (9H, s, C(C*H*$_3$)$_3$), 1.11 (3H, d, *J* 6.7 Hz, CHC*H*$_3$), 3.82 (3H, s, CO_2Me), 4.56 (2H, br s, N*H*$_2$), 4.79, 4.89 (2H, 2 s, C(2)*H* and C(7)*H*), 4.96 (1H, q, *J* 6.7 Hz, C(4)*H*); δ_C (100.6 MHz, $CDCl_3$) 14.1 (CHCH$_3$), 24.9 (C(CH$_3$)$_3$), 35.1 (*C*(CH$_3$)$_3$), 53.2 (*CO*$_2$Me), 74.7 (*C*(4)), 78.6 (*C*(5)), 92.7 and 94.3 [*C*(2) and C(7)], 159.4, 171.6 and 179.5 (*C*NH$_2$ and carbonyls); *m/z* (ES^-) 269 (M+H$^+$, 100%); HRMS Found 269.1497, $C_{13}H_{21}N_2O_4$ requires 269.1501.

3.2.15. (2*R*,4*R*,5*S*)-1-Aza-6-amino-2-(*tert*-butyl)-4-methyl-8-oxo-3-oxabicyclo[3.3.0]-oct-6-ene 23

To a solution of oxazolidine **8** (55 mg, 0.20 mmol) in 1% water in ^{t}BuOH (5 ml) was added KO^{t}Bu (25 mg, 0.22 mmol). The mixture was heated at reflux for 2 h, cooled to room temperature and poured into water. The aqueous layer was extracted with ether (2 × 10 ml). The combined ether extracts were washed with brine

(10 ml), dried over $MgSO_4$ and evaporated in vacuo. Purification by flash column chromatography (EtOAc/petrol, 1:1) gave enamine (5%). The aqueous layer was acidified with 2 M HCl and extracted with ether (2 × 10 ml). The ether extracts were washed with brine (10 ml), dried over $MgSO_4$ and evaporated in vacuo to give crude enamine **23** (26 mg, 63%) as a white solid, which was recrystallised from $CHCl_3$. Mp 142–144 °C; R_f = 0.17 (EtOAc); $[\alpha]_D^{20} = +104.6$ (*c* 1.5, MeOH); ν_{max}/cm^{-1} 3520 (w), 3415 (m), 2970 (m), 1685 (s), 1640 (s), 1600; δ_H (400 MHz, $CDCl_3$) 0.94 (9H, s, C(C*H*$_3$)$_3$), 1.01 (3H, d, *J* 6.8 Hz, CHC*H*$_3$), 4.41–4.45 (2H, m, C(4)*H* and C(5)*H*), 4.58 (2H, br s, N*H*$_2$), 4.84 and 4.86 (2H, 2 s, C(2)*H* and C(7)*H*); δ_C (100.6 MHz, $CDCl_3$) 14.3 (CHCH$_3$), 24.7 (C(*C*H$_3$)$_3$), 35.9 (*C*(CH$_3$)$_3$), 66.7 and 72.2 [*C*(4) and *C*(5)], 92.2 and 94.0 [*C*(2) and C(7)], 168.8 and 178.2 (*C*NH$_2$ and *C*O); *m/z* (ES$^-$) 209 (M−H$^-$, 100%); Found 209.1297, $C_{11}H_{17}N_2O_2$ requires 209.129.

3.2.16. (2*R*,4*R*,5*R*)-1-Aza-2-(*tert*-butyl)-6,8-dioxo-4-methyl-3-oxabicyclo[3.3.0]octane 16

A solution of enamine **23** (45 mg, 0.21 mmol) in *i*-PrOH (1 ml) and 2 M NaOH (1 ml) was heated at reflux for 18 h, cooled to room temperature and partitioned between 2 M HCl (5 ml) and EtOAc (5 ml). The aqueous layer was extracted with EtOAc (5 ml) and the combined organic extracts were washed with brine (10 ml), dried over $MgSO_4$ and evaporated in vacuo. Purification by flash column chromatography on silica gel (EtOAc/petrol, 1:1, increasing polaity to EtOAc) gave the tetramic acid **16** (20 mg, 43%) as a white solid, with identical spectroscopic properties to those reported above.

3.2.17. (2*R*,4*R*,5*R*,6*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxy carbonyl-4,6-dimethyl-8-oxo-3-oxabicyclo[3.3.0]octane 24a and (2*R*,4*R*,5*R*,6*R*)-1-aza-2-(*tert*-butyl)-6-hydroxy-5-methoxy-carbonyl-4,6-dimethyl-8-oxo-3-oxabicyclo[3.3.0]octane 24b

To a solution of oxazolidine **9a,b** (0.45 g, 1.58 mmol) in dry methanol was added NaOMe (86 mg, 1.59 mmol) and the solution was stirred for 24 h at room temperature and then partitioned between ether (15 ml) and NH_4Cl (aq) (15 ml). The ether layer was washed with brine (15 ml), dried over $MgSO_4$ and evaporated in vacuo to give crude product which was purified by flash column chromatography (EtOAc/petrol, 1:2) to give alcohol **24a** (0.27 g, 61%) as a crystalline white solid and alcohol **24b** (0.05 g, 12%) as a white solid.

Compound **24a**: mp 79–80 °C; R_f = 0.32 (EtOAc/petrol, 1:4); $[\alpha]_D^{20} = +38.2$ (*c* 1.5, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3400 (w), 2960 (m), 1750 (s), 1730 (s) and 1665 (s); δ_H (400 MHz, $CDCl_3$) 0.87 (9H, s, C(C*H*$_3$)$_3$), 1.33 (3H, s, CC*H*$_3$), 1.68 (3H, d, *J* 6.6 Hz, CHC*H*$_3$), 2.30 (1H, d, *J* 15.9 Hz, C(7)*H*H), 3.05 (1H, d, *J* 15.8 Hz, C(7)H*H*), 3.77 (3H, s, CO$_2$C*H*$_3$), 4.74 (1H, q, *J* 6.5 Hz, C(4)*H*), 5.03 (1H, s, C(2)*H*); δ_C (100.6 MHz, $CDCl_3$) 15.1 (CHCH$_3$), 22.9 (C(OH)*C*H$_3$), 25.7 (C(*C*H$_3$)$_3$), 37.4 (*C*(CH$_3$)$_3$), 49.5 (*C*(7)), 52.7 (CO$_2$*C*H$_3$), 78.4 (*C*(4)), 80.5 and 82.2 [*C*(5) and *C*(6)], 96.0 (*C*(2)), 172.2, 179.0 (carbonyls); *m/z* (ES$^-$) 284 (M−H$^-$, 100%), 198 (M−H$^-$−^{t}BuCHO, 95%); Found 284.1490, $C_{14}H_{22}NO_5$ requires 284.1498.

Compound **24b**: R_f = 0.27 (EtOAc/petrol, 1:4); $[\alpha]_D^{20} = +29.0$ (*c* 1.35, $CHCl_3$); ν_{max}/cm^{-1} 3420 (w), 3370 (br w), 2960 (m), 1740 (s), 1710 (s), 1115 (s); δ_H (400 MHz, $CDCl_3$) 0.93 (9H, s, C(C*H*$_3$)$_3$), 1.38 (3H, s, C(OH)C*H*$_3$), 1.71 (3H, d, *J* 6.6 Hz, CHC*H*$_3$), 2.29 (1H, d, *J* 15.8 Hz, C(7)*H*H), 2.69 (1H, br s, O*H*), 3.10 (1H, d, *J* 15.8 Hz, C(7)H*H*), 3.81 (3H, s, CO$_2$C*H*$_3$), 4.77 (1H, q, *J* 6.6 Hz, *CH*CH$_3$), 5.31 (1H, s, C(2)*H*); δ_C (100.6 MHz, $CDCl_3$) 15.1 (CHCH$_3$), 22.8 (C(OH)CH$_3$), 25.8 (C(*C*H$_3$)$_3$), 37.4 (*C*(CH$_3$)$_3$), 49.6 (*C*(7)), 52.7 (CO$_2$*C*H$_3$), 78.3 (*C*(4)), 80.2 and 82.3 [*C*(5) and *C*(6)], 96.5 (*C*(2)), 171.9 and 178.8 (ester and amide carbonyls); *m/z* (ES$^-$) 284 (M−H$^-$, 100%), 198 (M−H$^-$−^{t}BuCHO, 95%); Found 284.1494, $C_{14}H_{22}NO_5$ requires 284.1498. The crystal structure was determined from X-ray diffraction data.

3.2.18. (2*R*,4*R*,5*R*,6*R*,7*S*)-1-Aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-4,6,7-trimethyl-8-oxo-3-oxabicyclo [3.3.0]octane 27 and (2*R*,4*R*,5*R*,6*S*,7*S*)-1-aza-2-(*tert*-butyl)-6-hydroxy-5-methoxycarbonyl-4,6,7-trimethyl-8-oxo-3-oxabicyclo[3.3.0]octane 26

To a solution of oxazolidine **13** (172 mg, 0.64 mmol) in methanol (4 ml) was added NaOMe (36 mg, 0.66 mmol) and the mixture was stirred at room temperature for 20 h and then partitioned between ether (15 ml) and NH_4Cl (aq) (15 ml). The organic layer was washed with brine (15 ml), dried over $MgSO_4$ and evaporated in vacuo to give crude alcohol which was purified by flash column chromatography (EtOAc/petrol, 1:3) to give pure alcohol **27** (101 mg, 59%) as an off white crystalline solid and alcohol **26** (21 mg, 12%) as a viscous oil.

Compound **26**: R_f = 0.21 (EtOAc/petrol, 1:4); $[\alpha]_D^{20} = +33.4$ (*c* 1.1, $CHCl_3$); ν_{max}/cm^{-1} 3520 (w), 3410 (br), 1720 (s), 1710 (s), 1165 (m); δ_H (400 MHz, $CDCl_3$) 0.91 (9H, s, C(C*H*$_3$)$_3$), 1.03 (3H, d, *J* 7.1 Hz C(7)C*H*$_3$), 1.30 (3H, s, C(OH)C*H*$_3$), 1.73 (3H, d, *J* 6.5 Hz, C(4)C*H*$_3$), 2.98 (1H, q, *J* 7.1, C(7)*H*), 3.81 (3H, s, CO$_2$C*H*$_3$), 4.71 (1H, q, *J* 6.5 Hz, C(4)*H*), 5.03 (1H, s, C(2)*H*); δ_C (100.6 MHz, $CDCl_3$) 6.3 (C(7)CH$_3$), 15.5 (C(4)CH$_3$), 21.4 (C(6)CH$_3$), 26.3 (C(CH$_3$)$_3$), 38.0 (*C*Me$_3$), 49.1 (*C*(7)), 53.1 (CO$_2$CH$_3$), 79.3 (*C*(4)), 79.5 and 80.3 [*C*(5) and *C*(6)), 97.5 (*C*(2)), 172.4 and 182.0 (ester and amide carbonyls); *m/z* (ES$^-$) 298 (M−H$^-$, 100%); (M−H$^-$) Found 298.1648, $C_{15}H_{24}NO_5$ requires 284.1654.

Compound **27**: mp 109–110 °C; R_f = 0.26 (EtOAc/petrol, 1:4); $[\alpha]_D^{20} = +63.7$ (*c* 1.2, $CHCl_3$); ν_{max}/cm^{-1} 3580 (w), 3360 (br w), 1740 (s), 1710 (s), 1115 (m); δ_H (400 MHz, $CDCl_3$) 0.93 (9H, s, C(C*H*$_3$)$_3$), 1.05 (3H, d, *J* 7.1 Hz C(7)C*H*$_3$), 1.32 (3H, s, C(OH)C*H*$_3$), 1.72 (3H, d, *J* 6.5 Hz, C(4)C*H*$_3$), 3.01 (1H, q, *J* 7.1, C(7)*H*), 3.80 (3H, s, CO$_2$C*H*$_3$), 4.74 (1H, q, *J* 6.5 Hz, C(4)*H*), 5.03 (1H, s, C(2)*H*); δ_C (100.6 MHz, $CDCl_3$) 6.3 (C(7)CH$_3$), 15.5 (C(4)CH$_3$), 21.5 (C(6)CH$_3$), 26.2 (C(CH$_3$)$_3$), 37.9 (*C*Me$_3$), 49.0 (*C*(7)), 53.1 (CO$_2$CH$_3$), 79.1 (*C*(4)), 79.4 and 80.2 [*C*(5) and *C*(6)), 97.2 (*C*(2)), 172.3 and 181.2 (ester and amide carbonyls); *m/z* (ES$^-$) 298 (M−H$^-$, 100%), 212 (M−H$^-$−^{t}BuCHO, 90%); (M−H$^-$) Found 298.1650, $C_{15}H_{24}NO_5$ requires 298.1654. The crystal structure was determined from X-ray diffraction data.

3.2.19. (2*R*,1′*R*)-Methyl 3-hydroxy-2-(1′-hydroxyethyl)-5-oxo-4-phenyl-2,5-dihydro-1*H*-pyrrole-2-carboxylate 21a

To a solution of β-keto amide **19** (85 mg, 0.25 mmol) in 1.5% w/v HCl in trifluoroethanol (5 ml) was added propane-1,3-dithiol (25 μl, 0.25 mmol). The mixture was stirred at room temperature for 7 h and then partitioned between water (5 ml) and EtOAc (5 ml). The aqueous layer was concentrated in vacuo to give alcohol **21a** (53 mg) in 78% yield. R_f = 0.27 (EtOAc/MeOH, 4:1); $[\alpha]_D^{20} = +43.2$ (*c* 1.0, MeOH); ν_{max}/cm^{-1} (KBr) 3420 (w), 2597 (w), 1737 (s), 1658 (s), 1582 (s), 1237 (s); δ_H (400 MHz, CD_3OD) 1.31 (3H, d, *J* 8.1 Hz, CHC*H*$_3$), 3.73 (3H, s, CO$_2$CH$_3$), 4.14 (1H, q, *J* 8.0 Hz, C*H*OH), 7.21–7.25 (1H, m, Ar*H*), 7.32 (2H, t, *J* 7.7 Hz, Ar*H*), 7.75 (2H, d, *J* 7.8 Hz, Ar*H*) ; δ_C (100.6 MHz, CD_3OD) 18.3 (CHCH$_3$), 53.2 (CO$_2$CH$_3$), 68.3, 71.3 (C(2) and C2′), 98.3, (*C*(4)), 126.1, 127.9, 128.2, 129.4, 130.1(Ar*C*), 169, 171.4, 179.5 (CO$_2$CH$_3$, *C*(3) and *C*(5)); *m/z* (ES$^+$) 278 (M+H$^+$, 100%): Found 278.1026, $C_{14}H_{16}NO_5$ requires 278.1023.

3.2.20. (2*R*,1′*R*)-3-Hydroxy-2-(1′-hydroxyethyl)-4-phenyl-1*H*-pyrrol-2(5*H*)-one 21b

To a solution of β-keto amide **20** (130 mg, 0.45 mmol) in 1.5% w/v HCl in trifluoroethanol (7 ml) was added propane-1,3-dithiol (51 μl, 0.51 mmol). The mixture was stirred at room temperature for 7 h and then partitioned between water (10 ml) and EtOAc (10 ml). The aqueous layer was concentrated in vacuo to give crude alcohol **21b** (83 mg, 82%) as an off white solid. R_f = 0.34 (EtOAc/MeOH, 3:1); $[\alpha]_D^{20} = +51.1$ (*c* 0.9, MeOH); ν_{max}/cm^{-1} (KBr) 3428

(w), 2597 (w), 2039 (s), 1704 (s), 1563 (s), 1257 (s), 720 (s); δ_H (400 MHz, CD_3OD) 1.34 (3H, d, *J* 8.3 Hz, CHC*H*$_3$), 4.12 (1H, s, C(2)*H*), 4.17–4.23 (1H, m, C*H*OH), 7.22–7.26 (1H, m, Ar*H*), 7.35 (2H, t, *J* 7.5 Hz, Ar*H*), 7.74 (2H, d, *J* 7.9 Hz, Ar*H*) ; δ_C (100.6 MHz, CD_3OD) 19.0 (CHCH_3), 62.3 (*C*(2)), 66.4 (*C*HCH$_3$), 97.1 (*C*(4)), 126.7, 128.0, 128.4 and 131.1 (ArC), 167.3 and 184.6 (*C*(3) and *C*(5)); *m/z* (ES$^-$) 218 (M−H$^-$, 100%),: Found 218.0837, $C_{12}H_{12}NO_3$ requires 218.0817.

3.2.21. (2*R*,3*R*,4*S*,1′*R*)-2,3-Dimethyl-3-hydroxy-2-(*R*-1-hydroxyethyl)-2-methoxycarbonyl-5-pyrrolidinone 28

To a solution of alcohol **27** (180 mg, 0.63 mmol) in 2% HCl in trifluoroethanol (5 ml) was added 1,3-propanedithiol (0.06 ml, 0.60 mmol) and the mixture was stirred at room temperature for 7 h. The solvent was evaporated in vacuo and the residue was purified by flash column chromatography (EtOAc/MeOH, 4:1) giving diol **28** (138 mg, 89%) as a white solid.

Mp 141–142 °C; R_f = 029 (MeOH/EtOAc, 1:4); $[\alpha]_D^{20} = +60.1$ (*c* 0.6, MeOH); ν_{max}/cm^{-1} (KBr) 3428 (w), 2597 (w), 2039 (s), 1704 (s), 1563 (s), 1257 (s), 720 (s); δ_H (200 MHz, CD_3OD) 1.11 (3H, d, *J* 7.3 Hz, CH(OH)C*H*$_3$), 1.33 (3H, d, *J* 6.6 Hz, CHC*H*$_3$), 1.42 (3H, s, CC*H*$_3$), 2.41 (1H, q, *J* 7.1, C(4)*H*), 3.81 (3H, s, CO_2CH_3), 4.22 (1H, q, *J* 6.4 Hz, C*H*OH); δ_C (50.3 MHz, CD_3OD) 9.1 (C(4)*C*H3), 17.3 (CH*C*H$_3$), 23.4 (*C*CH$_3$), 48.1 (*C*(4)), 51.3 (CO_2CH_3), 71.1 (*C*HCH$_3$), 75.8 and 79.0 (*C*(2) and *C*(3)), 172.8 and 176.4 (ester and amide carbonyls); *m/z* (ES$^-$) 230 (M−H$^-$, 100%); (M−H$^-$) Found 230.1023, $C_{10}H_{16}NO_5$ requires 230.1028.

3.2.22. (2*R*,3*R*,1′*R*)-3-Hydroxy-2-(hydroxyethyl)-2-methoxycarbonyl-3-methyl-5-pyrrolidinone 29a

To a solution of tetramic acid **24a** (0.20 g, 0.70 mmol) in 2% HCl in trifluoroethanol (5 ml) was added 1,3-propanedithiol (0.07 ml, 0.70 mmol) and the mixture was stirred at room temperature for 5 h. The solvent was evaporated in vacuo and the residue was purified by flash column chromatography (EtOAc/MeOH, 4:1) to give diol **29a** (0.13 g, 85%) as a white solid. Mp 156–160 °C; R_f = 0.31 (EtOAc/MeOH, 1:4); ν_{max}/cm^{-1} (KBr) 3428 (w), 2597 (w), 2039 (s), 1704 (s), 1563 (s), 1257 (s), 720 (s); δ_H (200 MHz, CD_3OD) 1.29 (3H, d, *J* 8.3 Hz, CHC*H*$_3$), 1.32 (3H, s, CC*H*$_3$), 2.30 (1H, d, *J* 17.0 Hz, C(4)*H*H), 2.66 (1H, d, *J* 17.0 Hz, C(4)H*H*), 3.80 (3H, s, CO_2CH_3), 4.48 (1H, q, *J* 6.3 Hz, *C*HCH$_3$); δ_C (50.3 MHz, CD_3OD) 17.6 (CH*C*H$_3$), 26.5 (*C*CH$_3$), 46.3 (*C*(4)), 51.5 (CO_2CH_3), 70.4 (*C*HCH$_3$), 75.5 and 77.6 (*C*(2) and *C*(3)), 172.2 and 176.1 (ester and amide carbonyls); *m/z* (ES$^-$) 216 (M−H$^-$, 100%), HRMS: Found 216.0863, $C_9H_{14}NO_5$ requires 216.0872. The crystal structure was determined from X-ray diffraction data.

3.2.23. (2*R*,3*S*,2′*R*)-3-Hydroxy-2-(1-hydroxyethyl)-2-methoxycarbonyl-3-methyl-5-pyrrolidinone 29b

To a solution of alcohol **24b** (0.37 g, 1.3 mmol) in 2% HCl in trifluoroethanol (5 ml) was added 1,3-propanedithiol (0.13 ml, 1.3 mmol) and the mixture was stirred for 5 h at room temperature. The solvent was then evaporated in vacuo and the residue was purified by flash column chromatography (EtOAc/MeOH, 4:1) to give the title compound **29b** which after crystallisation gave a white solid in 83% yield (0.24 g). Mp 158–160 °C; R_f = 0.34 (MeOH/EtOAc, 1:4); $[\alpha]_D^{20} = +79.6$ (*c* 1.7, MeOH); ν_{max}/cm^{-1} 3428 (w), 2597 (w), 2039 (s), 1704 (s), 1563 (s), 1257 (s), 720 (s); δ_H (400 MHz, CD_3OD) 1.30 (3H, d, *J* 6.3 Hz, CHC*H*$_3$), 1.33 (3H, s, C(OH)C*H*$_3$), 2.30 (1H, d, *J* 17.0 Hz, C(4)*H*H), 2.66 (1H, d, *J* 17.0 Hz, C(4)H*H*), 3.80 (3H, s, CO_2CH_3), 4.48 (1H, q, *J* 6.2 Hz, *C*HCH$_3$); δ_C (100.6 MHz, $CDCl_3$) 17.7 (CH*C*H$_3$), 26.6 (C(OH)CH$_3$), 46.3 (*C*(4)), 51.6 (CO_2CH_3), 70.4 (*C*HCH$_3$), 75.5 and 77.7 [*C*(2) and *C*(3)], 172.3 and 176.1 (amide and ester carbonyls); *m/z* (ES$^+$) 216 (M−H$^-$, 100%); HRMS Found 216.0871, $C_9H_{14}NO_5$ requires 216.0872.

3.3. Structure determination

Single crystal diffraction data were collected using an Enraf-Nonius KappaCCD diffractometer (Mo Kα radiation (λ = 0.71073 Å) at 150(2) K with an Oxford Cryosystems Cryostream N2 open-flow cooling device[65] and processed using the DENZO-SMN package,[66] including inter-frame scaling (which was carried out using SCALEPACK within DENZO-SMN). The structures were solved using SIR92.[67] Refinement was carried out using full-matrix least-squares within the CRYSTALS suite.[68] All non-hydrogen atoms were refined with anisotropic displacement parameters and hydrogen atoms were visible in the difference map. The OH hydrogen atoms were located in a difference Fourier map and coordinates and isotropic thermal parameter subsequently refined. All other hydrogen atoms were positioned geometrically after each cycle of refinement. In each case, a 3-term Chebychev polynomial weighting scheme was applied prior to the final refinement.[69–71] Full crystallographic data for all structures have been deposited with the Cambridge Crystallographic Data Centre, CCDC 758790–758794. Copies of these data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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References

1. Kotra, L. P.; Golemi, D.; Vakulenko, S.; Mobashery, S. *Chem. Ind.* **2000**, 341–344.
2. Niccolai, D.; Tarsi, L.; Thomas, R. J. *Chem. Commun.* **1997**, 2333–2342.
3. Cheng, C. C.; Shipps, G. W., Jr.; Yang, Z.; Sun, B.; Kawahata, N.; Soucy, K. A.; Soriano, A.; Orth, P.; Xiao, L.; Mann, P.; Black, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6507–6514.
4. Galloway, W. R. J. D.; Bender, A.; Welch, M.; Spring, D. R. *Chem. Commun.* **2009**, 2446–2462.
5. Cordier, C.; Morton, D.; Murrison, S.; Nelson, A.; O'Leary-Steele, C. *Nat. Prod. Rep.* **2008**, *25*, 719–737.
6. Gullo, V. P.; McAlpine, J.; Lam, K. S.; Baker, D.; Petersen, F. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 523–531.
7. Rouhi, A. M. *Chem. Eng. News* **2003**, *81*, 77–91.
8. Breinbauer, R. B.; Vetter, I. R.; Waldmann, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 2878.
9. Ganesan, A. *Curr. Opin. Biotechnol.* **2004**, *15*, 584–590.
10. Koch, M. A.; Waldmann, H. *Drug Discovery Today* **2005**, *10*, 471.
11. Shang, S.; Tan, D. S. *Curr. Opin. Chem. Biol.* **2005**, *9*, 248–258.
12. Nören-Müller, A.; Reis-Correa, I., Jr.; Prinz, H.; Rosenbaum, C.; Saxena, K.; Schwalbe, H. J.; Vestweber, D.; Cagna, G.; Schunk, S.; Schwarz, O.; Schiewe, H.; Waldmann, H. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 10606.
13. Baltz, R. H. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 507–513.
14. Njardarson, J. T.; Gaul, C.; Shan, D.; Huang, X.-Y.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 1038–1040.
15. Nussbaum, F. v.; Brands, M.; Hinzen, B.; Weigand, S.; Habich, D. *Angew. Chem., Int. Ed.* **2006**, *45*, 5072–5129.
16. Koehn, F. E.; Carter, G. T. *Nat. Rev. Drug Disc.* **2005**, *4*, 206–220.
17. Hemkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. *Tetrahedron* **1997**, *53*, 5643.
18. Quinn, R. J.; Carroll, A. R.; Pham, N. B.; Baron, P.; Palframan, M. E.; Suraweera, L.; Pierens, G. K.; Muresan, S. *J. Nat. Prod.* **2008**, *71*. 464-46.
19. Costantino, L.; Barlocco, D. *Curr. Med. Chem.* **2006**, *13*, 65–85.
20. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **2001**, *46*, 3–26.
21. Jenkins, I. D.; Lacrampe, F.; Ripper, J.; Alcaraz, L.; Van Le, P.; Nikolakopoulos, G.; Leone, P. D.; White, R. H.; Quinn, R. J. *J. Org. Chem.* **2009**, *74*, 1304–1313.
22. Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2007**, *70*, 461–477.
23. O'Shea, R.; Moser, H. E. *J. Med. Chem.* **2008**, *51*, 2871–2878.
24. Hajduk, P. J. *J. Med. Chem.* **2006**, *49*, 6972–6976.
25. Rees, D. C.; Congreve, M.; Murray, C. W.; Carr, R. *Nat. Rev. Drug Disc.* **2004**, *3*, 660–672.
26. Balamurugan, R.; Dekkerab, F. J.; Waldmann, H. *Mol. BioSyst.* **2005**, *1*, 36–45.
27. Koch, M. A.; Wittenberg, L. O.; Basu, S.; Jeyaraj, D. A.; Gourzoulidou, E.; Reinecke, K.; Odermatt, A.; Waldmann, H. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 16721.
28. Jeong, Y.-C.; Moloney, M. G. *Synlett* **2009**, 2487–2491.

29. Chandan, N.; Moloney, M. G. *Org. Biomol. Chem.* **2008**, *6*, 3664–3666.
30. Hill, T.; Kocis, P.; Moloney, M. G. *Tetrahedron Lett.* **2006**, *47*, 1461–1463.
31. Royles, B. J. L. *Chem. Rev.* **1996**, *95*, 1961–2001.
32. Paintner, F. F.; Metz, M.; Bauschke, G. *Synlett* **2003**, 627–630.
33. Cativiela, C.; Ordóñez, M. *Tetrahedron: Asymmetry* **2009**, *20*, 1–63.
34. Andrews, M. D.; Brewster, A. G.; Crapnell, K. M.; Ibbett, A. J.; Jones, T.; Moloney, M. G.; Prout, K.; Watkin, D. *J. Chem. Soc., Perkin Trans. 1* **1998**, 223–235.
35. Andrews, M. D.; Brewster, A. G.; Chuhan, J.; Ibbett, A. J.; Moloney, M. G.; Prout, K.; Watkin, D. *Synthesis* **1997**, 305–308.
36. Andrews, M. D.; Brewster, A. G.; Moloney, M. G. *Synlett* **1996**, 612–614.
37. Andrews, M. D.; Brewster, A.; Moloney, M. G. *Tetrahedron: Asymmetry* **1994**, *5*, 1477–1478.
38. Seebach, D.; Sting, A. R.; Hoffmann, M. *Angew. Chem., Int. Ed.* **1996**, *35*, 2708–2748.
39. Ryu, G.; Hwang, S.; Kim, S.-K. *J. Antibiot.* **1997**, *50*, 1064–1066.
40. Ryu, G.; Kim, S.-K. *J. Antibiot.* **1999**, *52*, 193–197.
41. Anwar, M.; Moloney, M. G. *Tetrahedron Lett.* **2007**, *48*, 7259–7262.
42. Congreve, M.; Carr, R.; Murray, C.; Jhoti, H. *Drug Discovery Today* **2003**, *8*, 876–877.
43. Seebach, D.; Stucky, G. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 1351–1353.
44. Seebach, D.; Lamatsch, B.; Amstutz, R.; Beck, A. K.; Dobler, M.; Egli, M.; Fitzi, R.; Gautschi, M.; Herradon, B.; Hidber, P. C.; Irwin, J. J.; Locher, R.; Maestro, M.; Maetzke, T.; Mourino, A.; Pfammatter, E.; Plattner, D. A.; Schlickli, C.; Schweizer, W. B.; Seiler, P.; Stucky, G.; Petter, W.; Escalante, J.; Juaristi, E.; Quintana, D.; Miravitlles, C.; Molins, E. *Helv. Chim. Acta* **1992**, *75*, 913.
45. Corey, E. J.; Reichard, G. A. *J. Am. Chem. Soc.* **1992**, *114*, 10677–10678.
46. Payne, D. J.; Gwynn, M. N.; Holmes, D. J.; Pompliano, D. L. *Nat. Rev. Drug Disc.* **2007**, *6*, 29–40.
47. Puri, A. A.; Bogyo, M. *ACS Chem. Biol.* **2009**, *4*, 603–616.
48. Frearson, J. A.; Collie, I. T. *Drug Discovery Today* **2009**, *14*, 1150–1158.
49. Marvin was used for drawing, displaying and characterising chemical structures, substructures and reactions, Marvin 5.2.1, 2009, ChemAxon (http://www.chemaxon.com). Calculator Plugins were used for structure property prediction and calculation, Marvin 5.2.5.1, 2009, ChemAxon (http://www.chemaxon.com).
50. Disc diffusion assay on nutrient agar plates inoculated with bacterial culture, incubated at 37 °C for 12 h, using Kirby-Bauer antibiotic testing method[73] (disc diffusion method). The 6 mm disc was loaded with 100 mL of a 4 mg/mL solution of the analyte. Solvent (DMSO) controls gave zone sizes of 1.21, 0.63 and 1.02 cm for *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*, respectively.
51. Ferrara, P.; Apostolakis, J.; Caflisch, A. *Proteins Struct. Funct. Bioinform.* **2002**, *46*, 24–33.
52. Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714–3717.
53. Ertl, P. In *Molecular Drug Properties*; Mannhold, R., Ed.; Wiley-VCH: Weinheim, 2007; pp 111–126.
54. Bagwell, C. L.; Moloney, M. G.; Thompson, A. L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4081–4086.
55. Freiberg, C.; Brunner, N. A.; Schiffer, G.; Lampe, T.; Pohlmann, J.; Brands, M.; Raabe, M.; Habich, D.; Ziegelbauer, K. *J. Biol. Chem.* **2004**, *279*, 26066–26073.
56. Pitt, W. R.; Parry, D. M.; Perry, B. G.; Groom, C. R. *J. Med. Chem.* **2009**, *52*, 2952–2963.
57. Lovering, F.; Bikker, J.; Humblet, C. *J. Med. Chem.* **2009**, *52*, 6752–6756.
58. Ritchie, T. J.; MacDonald, S. J. F. *Drug Discovery Today* **2009**, *14*, 1011–1020.
59. Feher, M.; Schmidt, J. M. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 218–227.
60. Seebach, D.; Aebi, J. D.; Coquaz, M. G.; Naef, R. *Helv. Chim. Acta* **1987**, *70*, 1194–1216.
61. Seebach, D.; Aebi, J. D. *Tetrahedron Lett.* **1984**, *25*, 2545–2548.
62. Kissenger, L. W.; Ungnade, H. E. *J. Org. Chem.* **1958**, *23*, 1340.
63. Ohta, S.; Tsujimara, A.; Okamoto, M. *Chem. Pharm. Bull.* **1981**, *29*, 2762–2768.
64. Bentrude, W. G.; Day, R. O.; Holmes, J. M.; Quin, G. S.; Setzer, W. N.; Sopchik, A. E.; Holmes, R. R. *J. Am. Chem. Soc.* **1984**, *106*, 106.
65. Cosier, J.; Glazer, A. M. *J. Appl. Crystallogr.* **1986**, *19*, 105–107.
66. Otwinowski, Z.; Minor, W. *Methods Enzymol.* **1997**, *276*, 307–326.
67. Altomare, A.; Cascarano, G.; Giacovazzo, G.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *J. Appl. Crystallogr.* **1994**, *27*, 435.
68. Betteridge, P. W.; Carruthers, J. R.; Cooper, R. I.; Prout, K.; Watkin, D. J. *J. Appl. Crystallogr.* **2003**, *36*, 1487.
69. Prince, E. *Mathematical Techniques in Crystallography and Materials Science*; Springer: New York, 1982.
70. Carruthers, J. R.; Watkin, D. J. *Acta Crystallogr., Sect. A* **1979**, *35*, 698–699.
71. Watkin, D. J. *Acta Crystallogr., Sect. A* **1994**, *50*. 411-43.
72. Fletcher, D. A.; McMeeking, R. F.; Parkin, D. *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 746–749.
73. Bauer, A. W.; Kirby, W. M.; Sherris, J. C.; Turck, M. *Am. J. Clin. Pathol.* **1966**, *45*, 493–496.