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Functionalised Bicyclic Tetramates Derived from Cysteine as Antibacterial Agents

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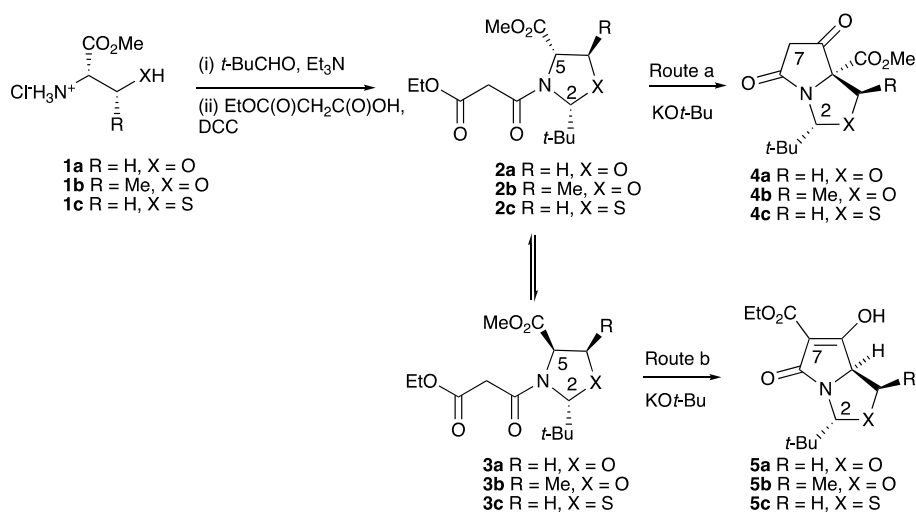
Routes to bicyclic tetramates derived from cysteine permitting ready incorporation of functionality at two different points around the periphery of heterocyclic skeleton are reported. This has enabled the identification of systems active against Gram-positive bacteria, some of which show gyrase and RNA polymerase inhibitory activity. In particular, tetramates substituted with glycosyl side chains, chosen to impart polarity and aqueous solubility, show high antibacterial activity coupled with modest gyrase/polymerase activity in two cases. An analysis of physicochemical properties indicates that the antibacterially active tetramates generally occupy physicochemical space with MW of 300-600, clogD_{7.4} of -2.5 to 4 and rel. PSA of 11-22%. This work demonstrates that biologically active 3D libraries are readily available by manipulation of a tetramate skeleton.

Introduction

Tetramates are of interest¹⁻⁴ principally for their antibacterial activity,^{5, 6} and we have reported recently that systems derived from the amino acids, serine **1a**,⁷ threonine **1b**,⁸ and cysteine **1c**⁹ provide useful templates for application in synthetic and medicinal chemistry, which in some cases exhibit potent antibacterial activity. Amongst various methodologies to these derivatives,¹⁰ our route makes use of the preferential formation of the malonamides of *t*-butyl oxazolidine/thiazolidine templates, formed as the *cis*-2,5-isomer **2a-c** rather than the alternative *trans*-2,5- **3a-c**, which mimises the steric

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impact of the bulky *t*-butyl group, to direct a very efficient chemoselective Dieckmann ring closure (Route a, Scheme 1) in preference to the alternative (Route b, Scheme 1).¹¹ Of interest, though, is that the analogous thiazolidine system derived from cysteine and an aromatic aldehyde behaves differently (Scheme 2), not least because there is a shift in preference away from the *cis*-thiazolidine **6** to the *trans*- isomer **7**, the latter of which now preferentially cyclizes to give tetramate **8**, equivalent to Route b in Scheme 1; this shift is, however, not complete and some of the *cis*-isomer **6**, after epimerization at C5, closes under these conditions leading to the enantiomer of **8**, and therefore giving some erosion of enantioselectivity.¹² The overall outcome is that acyl tetramates **8** (Scheme 2),¹³ known for their metal chelating¹⁴ and antibacterial activity,¹⁴ are accessed directly, rather than by acylation of an unfunctionalised tetramate nucleus.¹⁵ Since we had earlier explored in detail the structure activity relationship of tetramates substituted at C-7 but limited to *t*-butyl substitution at C-2, these thiazolidine compounds provided access for the first time to compounds with an aryl substituent at C-2 and this gave the opportunity to explore the scope of antibacterial bioactivity at this position. The results of that work are reported here.



Scheme 1

Results and Discussion

Synthesis of the required tetramate proceeded from cysteine **1c** to a mixture of the *cis*- and *trans*- diastereomeric malonamides **6a-g** and **7a-g**, with a preference for the latter *trans*- isomers in three cases, followed by Dieckmann cyclisation to 7-ethoxycarbonyltetramates **8a-g** (Scheme 2); there is up to 20% attrition of e.e. during this process, arising from cyclisation of the minor but unseparated *cis*-isomer **6a-g**.¹² These esters were expected to be suitable for conversion to the amide by direct aminolysis, a process which had been used successfully previously,¹⁶ and this was immediately successful for synthesis of systems **9a-v** (see Table 1) despite the high level of functional group density around the ring system which might not have given a chemoselective process, although elevated temperature was needed for successful reaction.

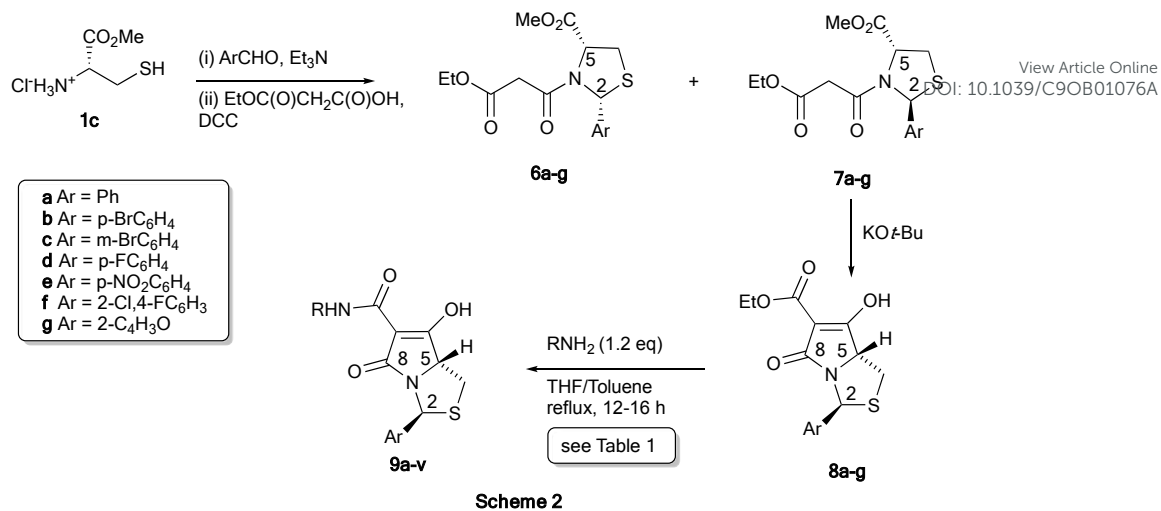
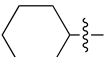


Table 1: Synthesis of tetramates **9a-v** according to Scheme 2.

Compound	Ar	R	Yield (%)	Tautomeric ratio ^a
9a	Ph	1-Adamantyl	45	2.7:1
9b	p-BrC ₆ H ₄		70	2.6:1
9c	m-BrC ₆ H ₄		38	2:1
9d	p-FC ₆ H ₄		56	2.7:1
9e	p-NO ₂ C ₆ H ₄		40	2.4:1
9f	2Cl-4-FC ₆ H ₃		60	2.5:1
9g	2-C ₄ H ₃ O		92	3:1
9h	Ph	4-Cyclohexylphenyl	54	15:1
9i	p-FC ₆ H ₄		45	13:1
9j	p-NO ₂ C ₆ H ₄		59	12.5:1
9k	2Cl-4-FC ₆ H ₃		73	15:1
9l	Ph	4-Chloro-2-methylphenyl	53	only 1 form
9m	p-FC ₆ H ₄		40	only 1 form
9n	p-NO ₂ C ₆ H ₄		37	only 1 form
9o	p-BrC ₆ H ₄		40	only 1 form
9p	m-BrC ₆ H ₄		38	only 1 form
9q	2Cl-4-FC ₆ H ₃		35	only 1 form
9r	p-BrC ₆ H ₄	4-Morpholinophenyl	69	only 1 form
9s	2-C ₄ H ₃ O		75	only 1 form
9t	p-BrC ₆ H ₄	4-Aminotetrahydropyranyl	70	5.2:1

9u	Ph	Cyclohexyl	49	3.1:1
9v	2Cl-4-FC ₆ H ₃		40	3.1:1

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^a Major form is tautomer pair AB -see Figure 4 and Table 2.

Chemoselective formation of the amide product, and not the alternative enamine arising by attack at the ketone, was evident by loss of the ester function, and also confirmed by HMBC correlation (Figure 1); thus, C-6 correlates with H-4 and H-5, while C-8 correlates with H-2 and H-5 as expected. For compounds **9s** and **9t**, C-9 correlates with the β -CH in the amine with respect to C-9, confirming the assignment of the carbonyl functional groups.

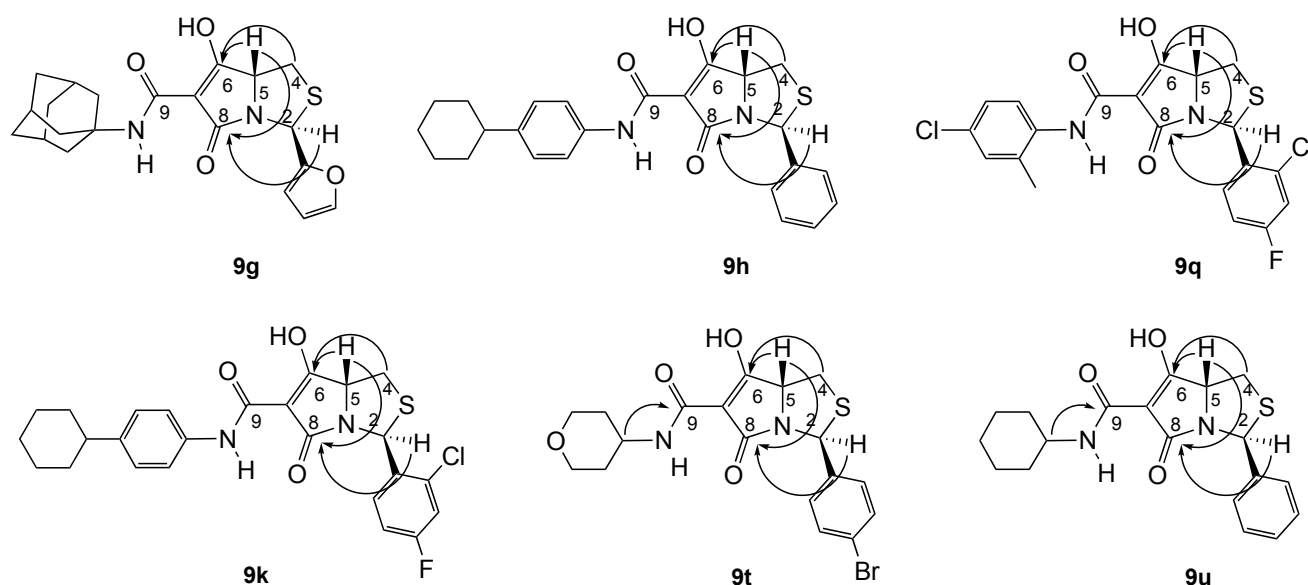


Figure 1. HMBC correlation data of selected carboxamide tetramates.

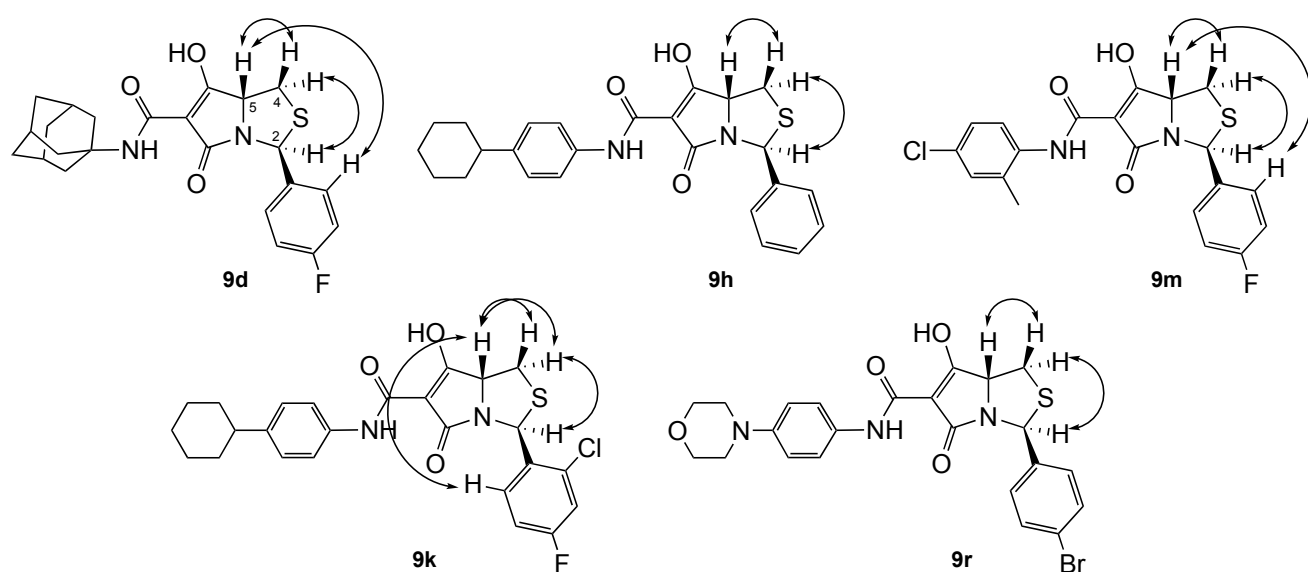


Figure 2. NOE analysis of selected carboxamide tetramates.

As anticipated, the stereochemical relationship of the starting bicyclic tetramate esters **8** was conserved in the amide systems, and a *trans*- relationship across the bicyclic ring between H-2 and H-5 was determined from NOE analysis (Figure 2); this outcome was further confirmed by single crystal X-ray diffraction studies of adamantyl derivative **9f** (Figure 3).¹⁷

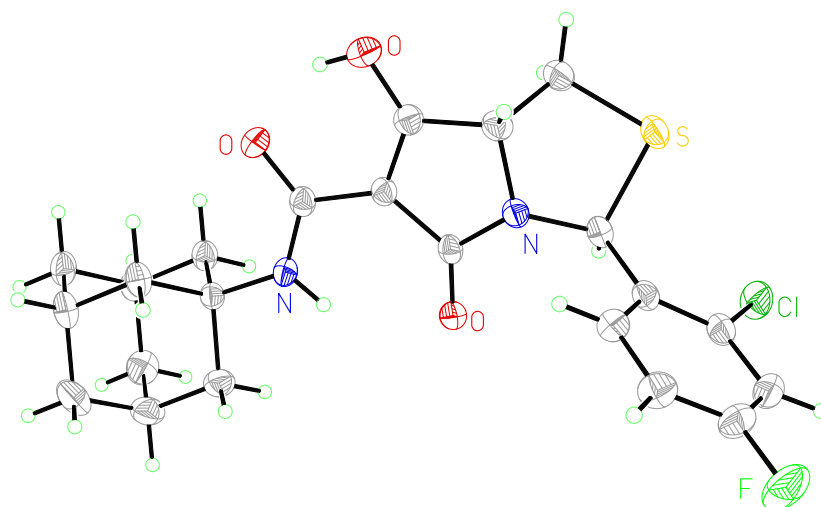


Figure 3: The structure of **9f** from single crystal X-ray diffraction studies. Displacement ellipsoids drawn at 50% probability.

The products were generally found to exist as tautomeric forms, a phenomenon which has been observed previously, and which could be assigned from careful NMR examination.^{15, 18} In their NMR spectra, the internal tautomeric pairs (A and B, C and D) could not be distinguished and were observed as an averaged hybrid, while the external tautomeric pairs (AB and CD) had distinct ¹³C chemical shift differences of C-6, C-8 and C-9 (Figure 4), so that the tautomeric forms could be readily distinguished (Table 2).

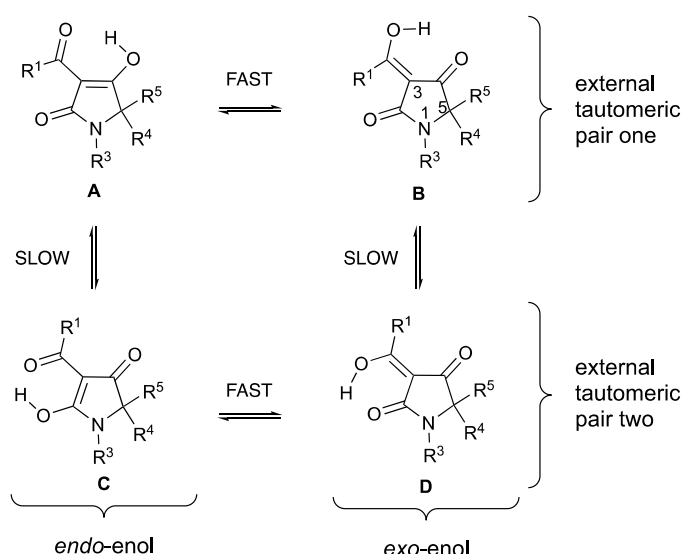


Figure 4

Table 2. ¹³C chemical shifts of tautomers of selected examples of carboxamide tetramates.

Compound	Solvent	¹³ C chemical shifts (ppm)	Tautomeric form
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Number		C-6	C-8	C-9	
9a	CDCl ₃	187.8	172.2	165.8	major (AB)
		191.1	178.0	166.4	minor (CD)
9b	CDCl ₃	188.4	172.4	165.9	major (AB)
		191.1	178.2	166.4	minor (CD)
9f	CDCl ₃	188.7	172.0	166.0	major (AB)
		191.0	177.8	166.5	minor (CD)
	MeOD	189.5	175.2	166.9	only 1 form (AB)
9k	CD ₂ Cl ₂	185.2	171.7	164.3	major (AB)
		191.5	178.2	165.4	minor (CD)
9q	CD ₂ Cl ₂	184.7	171.7	164.3	only 1 form (AB)
9t	CD ₂ Cl ₂	186.6	172.5	165.9	major (AB)
		191.6	178.8	166.5	minor (CD)

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The ¹³C chemical shifts of C-6, C-8 and C-9 of the minor tautomers were invariably more downfield than their major tautomers, with the smallest $\Delta\delta^{13}\text{C}$ observed for the exocyclic amide C-9. The tautomeric behaviour is also solvent dependent, as seen from the different chemical shift values of **9f** in CDCl₃ and CD₃OD. In polar solvents (e.g. methanol-d₄, acetone-d₆, DMSO-d₆ and CD₃CN), only one set of resonances was observed for each carbon, and appears to be that of an averaged hybrid structure arising from facile equilibrium of the tautomeric forms. NOESY data obtained for **9k** in dry CD₂Cl₂ revealed that the major tautomer for **9k** was A (Figure 4), based on the correlation from H-5 to proximal enol O-H, which would not have been possible in other tautomeric forms B, C and D (Figure 4). This conclusion is supported by single crystal X-ray diffraction studies of tetramate **9f** (Figure 3, *vide supra*), which was found to exist in the endoenolic form A. Since the chemical shift pattern observed for the major/minor tautomers is similar for all compounds, they are all assumed to adopt the major tautomeric form A, and this finding is similar to that found in related systems.¹⁵ Minimum energy conformations were generated for the four tautomeric forms A-D for compounds **9k** using MM2 methodology and confirmed tautomer A to be the thermodynamically more stable (Table 3 and Table 1, SI).

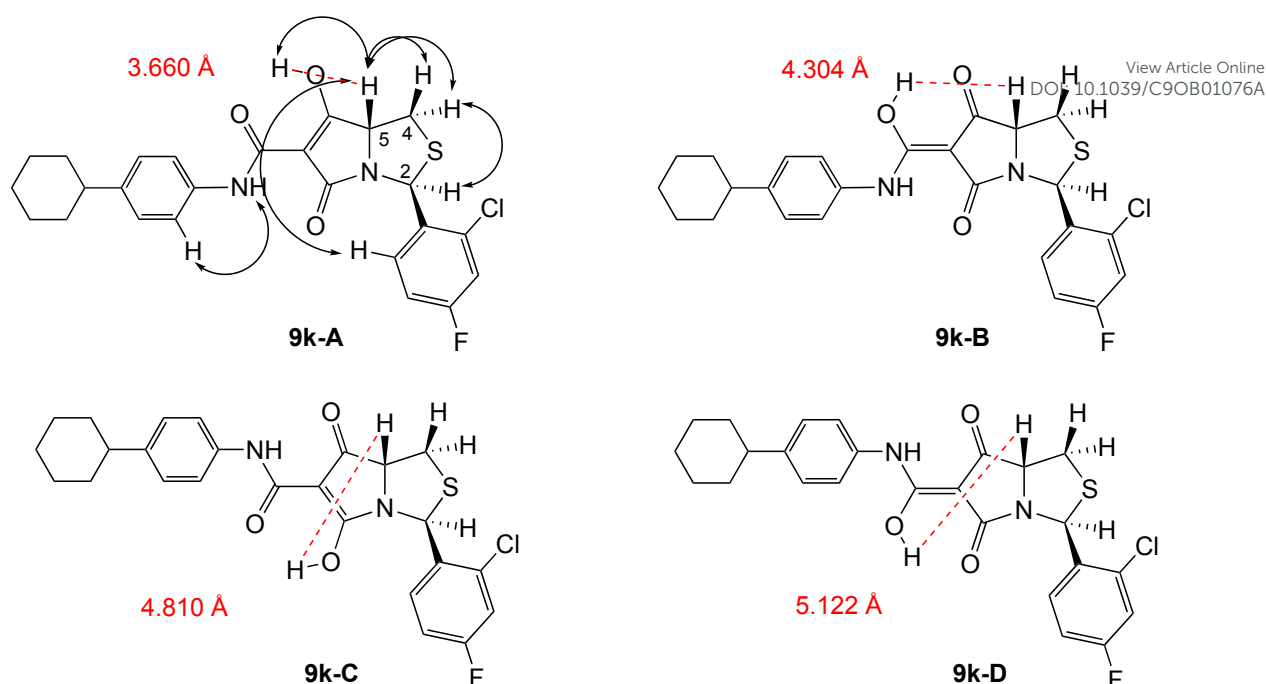


Figure 4. NOESY correlation data for **9k** suggesting tautomer A as the major tautomer. The distance between H-5 and enol O-H for each tautomer was calculated for the MM2-energy minimized molecular model using Chem3D v.15.

Table 3. MM2-minimized energy of tautomeric forms A-D for selected examples **9f**, **9k** and **9q**.

Compound Number	Tautomeric ratio	Minimum Energy of tautomeric forms (kcal/mol)			
		A	B	C	D
9f	2.5:1	18.93	39.86	26.83	39.32
9k	15:1	6.93	31.28	13.04	30.75
9q	only 1 form	0.80	25.54	7.05	26.23

Having demonstrated the generality and selectivity of this approach, of interest was its application to more elaborate side chain systems; to this end, the products **9w-g'** were accessed by direct aminolysis of esters **8a**, **b** and **f** using the required amines (Scheme 2 and Table 4), mostly available from commercial sources. In order to incorporate a higher level of polarity, mono-Fmoc protected **10b**, prepared from dapsone **10a**, an inhibitor of dihydropteroate synthetase,¹⁹ was used for the aminolysis of tetramate ester **8a** to give carboxamide tetramate **9a'**, which on subsequent deprotection gave **9b'** in 40 % yield (Scheme 3). The design of carboxamide **9d'** was inspired by naturally occurring tetramates kibelomycin²⁰ and amycolamicin,²¹ and was synthesised by aminolysis of tetramate **8a** with glycosylated amine derivative **12b** (Scheme 4). Although it has been previously reported that the desired glycosylated amine **12b** could be directly synthesised from 4-aminophenol **11a** and bromogalactose,^{22, 23} Fmoc-protected amine **11b** was found to be more suitable for this purpose by Lewis acid-mediated (BF₃·OEt₂) glycosylation; the equatorial substitution of the aryl group was confirmed by the *trans*-diaxial ³J_{H1-H2} coupling constant (8.0 Hz). Base-mediated

deprotection of the amine gave the desired product **12b** which was reacted with tetramate **8a** to yield carboxamide **9d'** in 60 % yield, and methanolysis under basic conditions afforded **9e'**. DOI: 10.1039/C9OB01076A

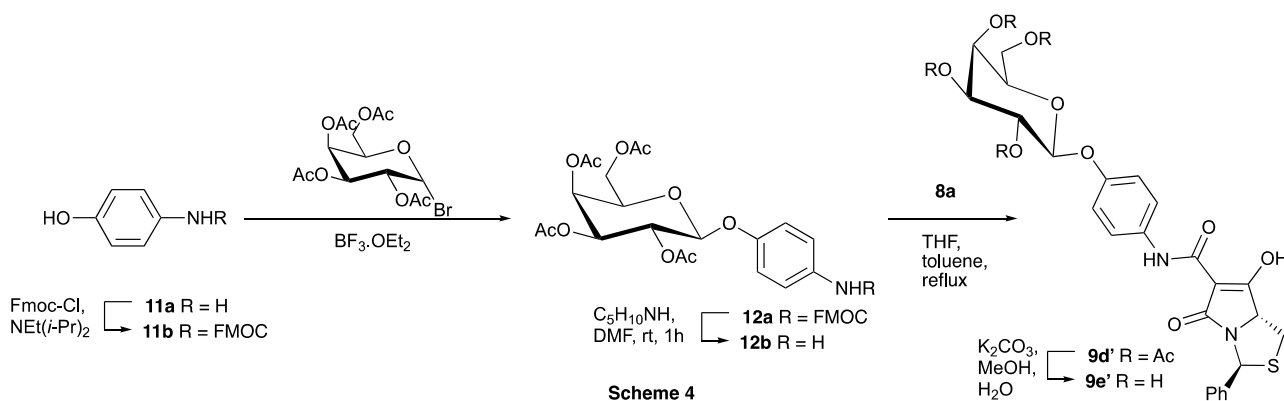
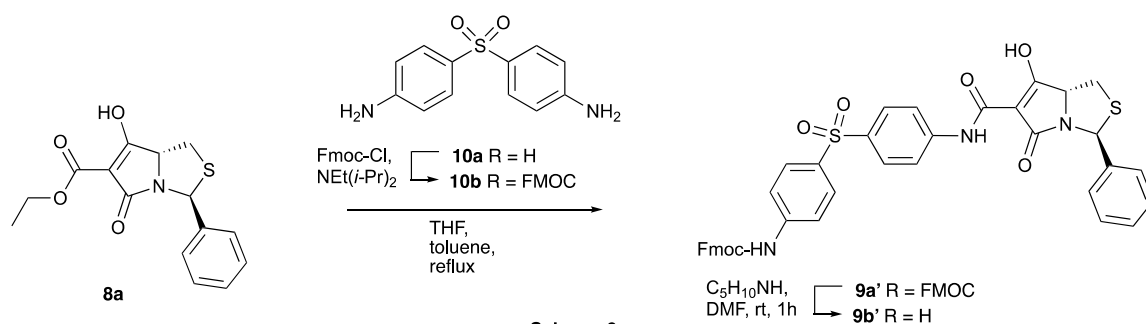
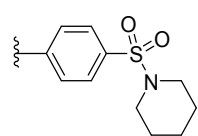
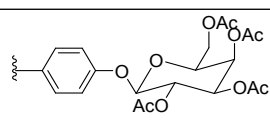
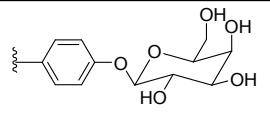
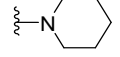
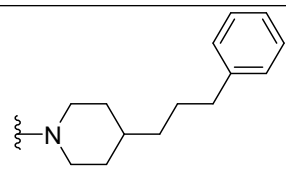


Table 4: Synthesis of tetramates **9w-g'**

Compound	Ar	R	Yield (%)
9w	Ph		30
9x			74
9y			75
9z			72
9a'			30
9b'			40

9c'	p-BrC ₆ H ₄		40
9d'	Ph		60
9e'	Ph		54
9f'	2Cl-4-FC ₆ H ₃		30
9g'	2Cl-4-FC ₆ H ₃		36

The carboxamidotetramates **9a-g'**, when purified by silica gel column chromatography, gave broad signal resonances in their ¹H NMR spectra, consistent with metal chelation, as has been observed previously.⁷ As a result, carboxamides were routinely purified on silica column with 1 % Et₃N in the eluent. Residual Et₃N was removed by washing with 5 % citric acid, giving the purified product with sharp and well-resolved ¹H spectra; an example is given for compound **9d** (Figure 5). Alternatively, it was also possible to purify these carboxamides on silica column without any Et₃N in the eluent, provided that they were subsequently washed with 2 M HCl to give the metal-free form.

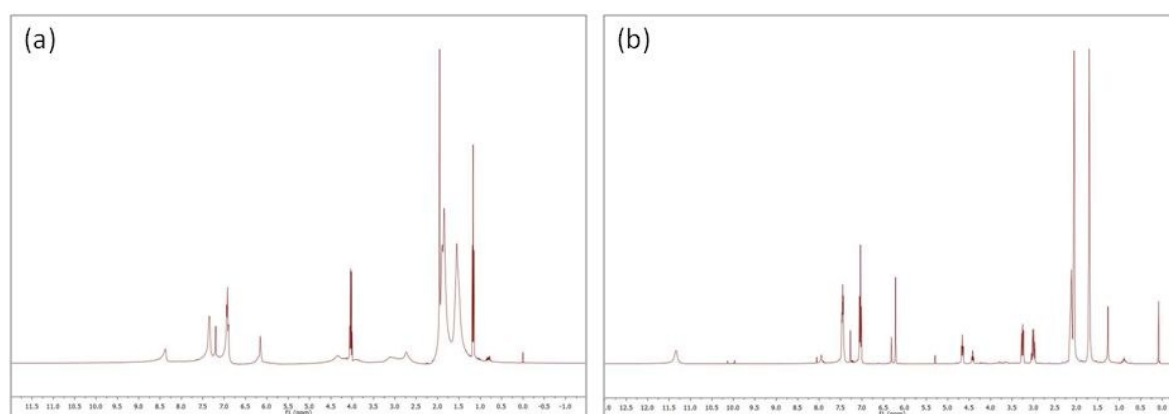


Figure 5. ¹H NMR spectra of tetramate **9d** (a) post-column purification, before acid-wash and (b) post-column purification, after acid-wash.

Since peak broadening was a recurrent observation after column purification, the metal-chelated tetramate and acid-washed tetramate for compound **9a** were analysed by Inductively Coupled Plasma Mass Spectrometry. The metals Na, Mg, Fe and Zn but especially Ca were found in high abundance in

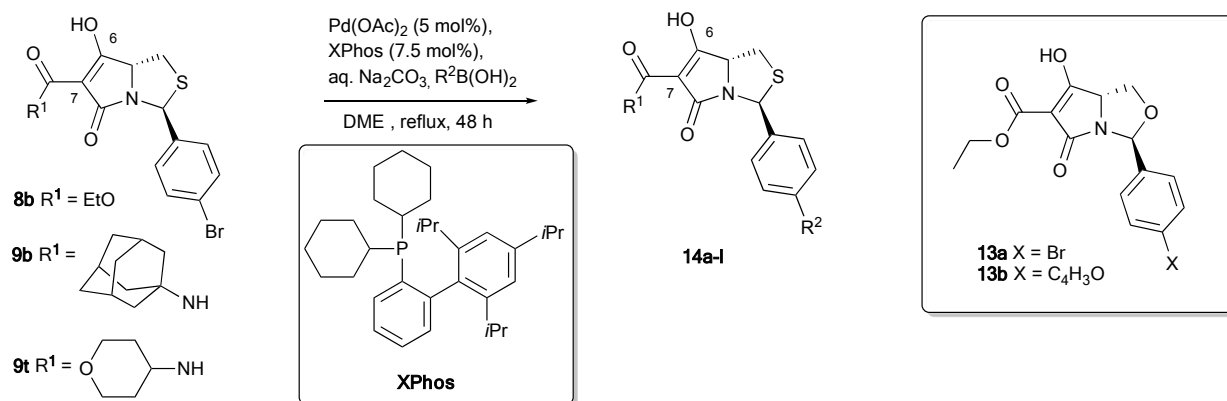
the metal-chelated tetramate, which were substantially removed by washing with 2M HCl (Table 5 and Table 2, SI). Clearly these tetramates are easily capable of forming metal salts with diverse cations; the metal chelating behaviour of 3-acyltetramates has been reviewed recently,^{2, 6, 13, 14, 24} and chelation both of natural products²⁵⁻²⁹ especially with antibacterial activity³⁰ and of simpler systems³¹⁻³⁶ has also been reported while in our own work, calcium salt formation (but not other metals) during the isolation of tetramates had been observed earlier.³⁷

Table 5. Metal content observed in metal-chelated and acid-washed forms of carboxamide **9a**.

	Metal content (ppm)				
	Mg	Ca	Fe	Zn	Na
Metal-chelated	7026.1	20483.7	38.2	71.2	3385.6
Acid-washed	15.9	24.8	34.8	9.9	18.8

In an attempt to make use of the C-2 bromoaryl substituent for Suzuki-Miyaura (SM) cross-coupling reactions for further derivatisation, reaction of both thiazolidines **8b** and **9b** with 2-furanylboronic acid was examined, but only unreacted starting material was recovered; this was in contrast to oxazolidine **13a** (Scheme 5) which gave the expected coupled product **13b** under the same conditions. This failure of SM cross-coupling on thiazolidine-derived tetramic acids was attributed to catalyst deactivation by the cyclic thioether function, and alternative reaction conditions were clearly necessary. While successful SM cross-coupling on thiophene systems had been documented using PPh₃ and AsPh₃,³⁸ no reports on systems containing non-aromatic thioethers such as those of the thiazolidine-derived tetramates could be found in the literature. However, a more recent report on Suzuki cross-coupling on biotin-derived systems³⁹ together with a detailed review by Buchwald et al.⁴⁰ suggested XPhos as an alternative, and this ligand proved to be successful, with the desired product **14a** being formed along with the return of some unreacted starting material (Scheme 5 and Table 6). Although it has been reported that the active form of the catalyst for SM cross-coupling involving dialkylbiaryl phosphine ligands is the monoligated L₁Pd(0) species rather than the more highly coordinated L₂Pd(0),^{40, 41} a ratio of L:Pd=1.5:1 was not sufficient to drive the reaction to completion even after a reaction time of 48 h. Increasing the L:Pd ratio to 3:1 led to completion of the reaction and this approach was successfully applied to the synthesis of a range of carboxamide tetramates **14a-l** with elaborated pendant groups at C-2 derived from **9b** and **9s** (Table 6). The boron reagents used for the above transformations were commercially available acids or their pinacol esters, except for **14c** where the boron reagent was the glycol ester derived from the corresponding boronic acid, prepared according to literature procedure.⁴² Carboxamide tetramate **14k** was synthesised as a mixture of diastereomers from the corresponding commercially available racemic 1-(tetrahydropyran-

2-yl)-1H-pyrazole-5-boronic acid pinacol ester. It would appear the additional nucleophilicity of XPhos gives a higher electron density at Pd(0) in the monoligated $L_1Pd(0)$ species, and increases the rate of oxidative addition leading to overall successful coupling.⁴⁰



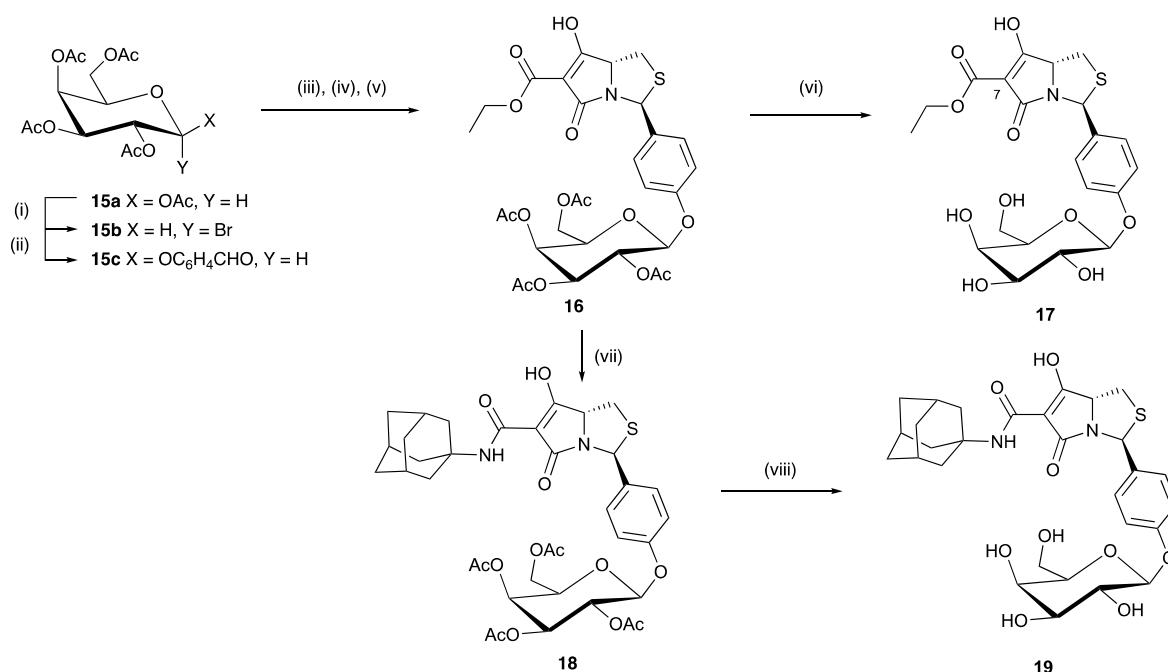
Scheme 5

Table 6: Suzuki-Miyaura cross-coupling of carboxamide tetramates **9b** and **9t**.

Carboxamide tetramate (R=)	Compound Number	R ₁	% Yield	Compound Number	R ₁	% Yield
	14a		72	14g		70
	14b		74	14h		78
	14c		80	14i		53
	14d		45	14j		70
	14e		78	14k		60
	14f		74			
	14l		30			

Several naturally occurring tetramic acids bearing glycosyl residues are known, including virgineone,⁴³ kibdelomycin,⁴⁴ amycolamicin,⁴⁵ aurantoside^{46, 47} and streptolydigin.⁴⁸ While the exact role of the sugar moiety of these tetramates is not known, it may give improved bioavailability with increased solubility and transportation, or help modulate toxicity and aid in bacterial target interaction.⁴⁹ Thus, investigating the effect of glycosylated tetramates on antibacterial activity was of

interest, and the systems developed thus far offered the feasibility of C-2 substitution of the tetramate ring. In order to demonstrate an alternative process using late-stage ring formation, bicyclic tetramates with C-2 pendant glycones were derived from β -D-galactose pentaacetate (Scheme 6). Acetobromo- α -D-galactose **15b** was prepared from β -D-galactose pentaacetate **15a** according to the literature procedure;⁵⁰ axial bromination at the anomeric carbon was confirmed from the $^3J_{(H1-H2)}$ -coupling constant of H-1 (d, $J=4.1$ Hz). Solid-liquid phase transfer-catalysed aryl glycosylation of 4-hydroxybenzaldehyde with acetobromo- α -D-galactose **15b** gave the desired aldehyde **15c**, according to a modified literature procedure; BnNBu_3Cl proved to be a better phase transfer catalyst than Bu_4NBr while the presence of H_2O led to a remarkable reduction in yield.⁵¹ The β -anomer was obtained exclusively, where the equatorial substitution of the phenoxide at the anomeric carbon was confirmed by 3J -coupling constant of the anomeric C-H (d, $J=8.0$ Hz). The glycosylated aldehyde **15c** was then condensed with L-cysteine methyl ester hydrochloride to yield the expected thiazolidine in 85 % yield with a *cis:trans* diastereomeric ratio of 1.6:1, and this material was *N*-acylated to give the corresponding malonamide in 52 % yield, which was in turn cyclised to afford the glycosylated tetramate **16** but only if the crude product was isolated without acidic work-up. Base-catalysed methanolysis gave tetraol **17** in quantitative yield (Scheme 6); neither hydrolysis nor transesterification of the ethyl ester at C-7 was observed under these reaction conditions. Aminolysis of tetramate ester **16** afforded carboxamide tetramates **18** with the acyl groups of the sugar moiety intact; methanolysis of the former resulted in the very polar carboxamide tetramate **19**.

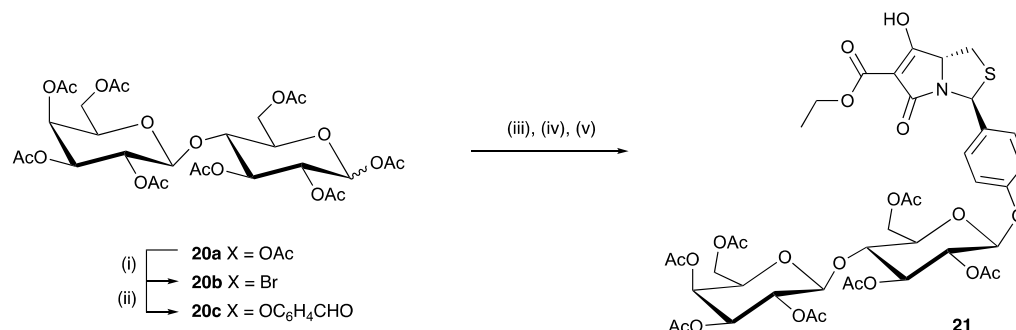


(i) BiBr_3 (0.05 eq), TMSBr (4 eq), CH_2Cl_2 , 1 h; (ii) $p\text{-H}(\text{O})\text{CC}_6\text{H}_4\text{OH}$, Bu_3NBnCl (0.1 eq), K_2CO_3 (5 eq), CHCl_3 , rt, 24 h; (iii) L-Cysteine methyl ester hydrochloride (1.2 eq), Et_3N (1.2 eq), Petrol, reflux, 19 h; (iv) Ethylhydrogen malonate (1.2 eq), DCC (1.2 eq), DMAP (0.1 eq), CH_2Cl_2 , rt, 15 h; (v) KOtBu (1.2 eq), THF, reflux, 3h; (vi) K_2CO_3 (1.2 eq), $\text{MeOH:H}_2\text{O}$ (4:1), rt, 5 min; (vii) 2-adamantylamine, THF/Toluene, reflux, 16 h; (viii) K_2CO_3 (1.2 eq), $\text{MeOH:H}_2\text{O}$ (4:1), rt, 10-15 min

Scheme 6

A tetramate with a disaccharide pendant group was derived from commercially available lactose octaacetate (Scheme 7); this followed a similar approach to the monosaccharide derivative

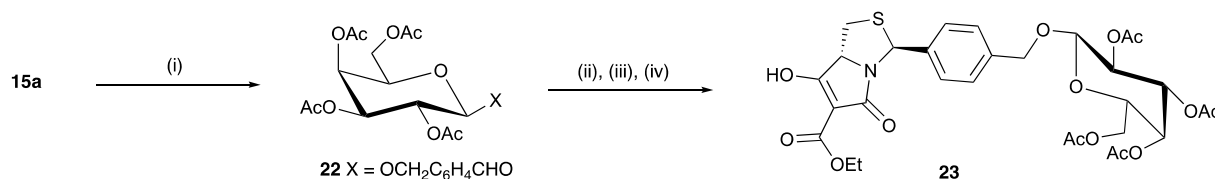
above. Commercially available lactose octaacetate **20a** (as a 2.9:1 mixture of α - and β - anomers at the glucopyranosyl end) was subjected to BiBr_3 -catalysed bromination and afforded **20b** as the α -anomer in 92 % yield, confirmed by 3J -coupling constant of the anomeric C-H. Aryl glycosylation gave aldehyde **20c**, which was condensed with L-cysteine methyl ester hydrochloride, acylated and cyclised to afford tetramate **21**.

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(i) BiBr_3 (0.05 eq), TMSBr (4 eq), CH_2Cl_2 , 1 h; (ii) $p\text{-H}(\text{O})\text{CC}_6\text{H}_4\text{OH}$, Bu_3BnNCl (0.1 eq), K_2CO_3 (5 eq), CHCl_3 , rt, 24 h; (iii) L-Cysteine methyl ester hydrochloride (1.2 eq), Et_3N (1.2 eq), Petrol, reflux, 19 h; (iv) Ethyl hydrogen malonate (1.2 eq), DCC (1.2 eq), DMAP (0.1 eq), CH_2Cl_2 , rt, 15 h; (v) KOtBu (1.2 eq), THF, reflux, 3 h

Scheme 7

Due to the acid-labile nature of phenyl glycosides prepared above and the resulting difficulty associated with the acidic work-up procedure required to remove chelating metal ions, an alternative route to the incorporation of glycones via a benzyl alcohol linking group was explored (Scheme 8). The controlled reduction of terephthalaldehyde with NaBH_4 between $0\text{--}2^\circ\text{C}$ afforded 4-(hydroxymethyl)benzaldehyde in 70 % yield according to a modified literature procedure.⁵² Lewis acid-catalysed glycosylation with β -D-galactosepentaacetate **15a** gave aldehyde **22** as the β -anomer in 71% yield along with the byproduct 4-(acetoxymethyl)benzaldehyde in 28 % yield. Following the usual synthetic route to access the bicyclic tetramate core, condensation of aldehyde **22** with cysteine ethyl ester yielded the expected thiazolidine, which was acylated and cyclized under basic conditions to afford benzyl glycosylated tetramate **23**, which proved to be stable to acidic work-up.

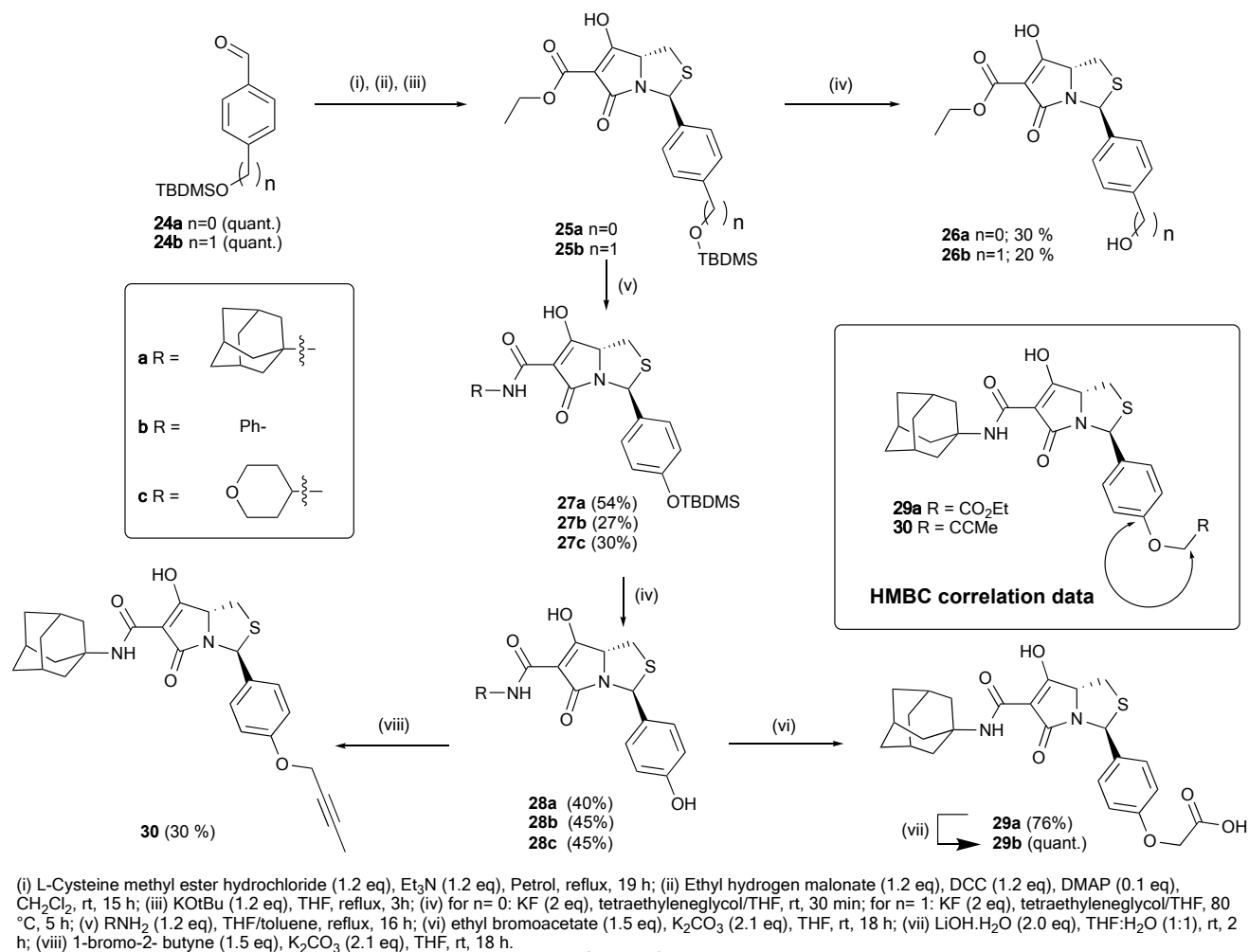


(i) $p\text{-H}(\text{O})\text{CC}_6\text{H}_4\text{CH}_2\text{OH}$, $\text{BF}_3\cdot\text{OEt}_2$ (1.2 eq), CH_2Cl_2 , rt, 24 h; (ii) L-Cysteine methyl ester hydrochloride (1.2 eq), Et_3N (1.2 eq), Petrol, reflux, 19 h; (iii) ethyl hydrogen malonate (1.2 eq), DCC (1.2 eq), DMAP (0.1 eq), CH_2Cl_2 , rt, 15 h; (iv) KOtBu (1.2 eq), THF, reflux, 3 h.

Scheme 8

In order to permit late stage C-2 substituent manipulation, tetramates **26a** and **26b** were derived from tetramates **25a,b** by silyl deprotection, which were obtained from the silyl ether of 4-hydroxybenzaldehyde **24a** or 4-(hydroxymethyl)benzaldehyde **24b** respectively (Scheme 9). Conversion of **25a** to the corresponding carboxamides **27a-c** by aminolysis with the required amine proceeded using the approach described above, in modest to good yield. The deprotection of silyl ether **27a** to obtain tetramate **28a**, however, required some optimisation. While TBAF was a successful desilylating agent, chromatographic purification was difficult, possibly due to the basicity

of fluoride ion in an aprotic solvent⁵³ leading to deprotonation of the product tetramic acid. However, alternative conditions using KF as a fluoride source were more successful. Two other carboxamide tetramates **28b** and **28c** were also synthesised using this approach.

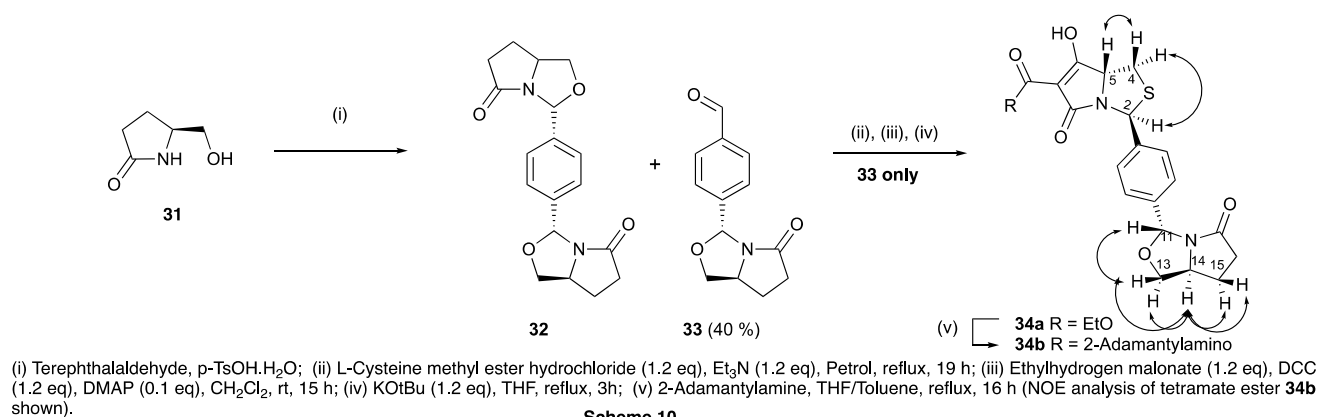


Scheme 9

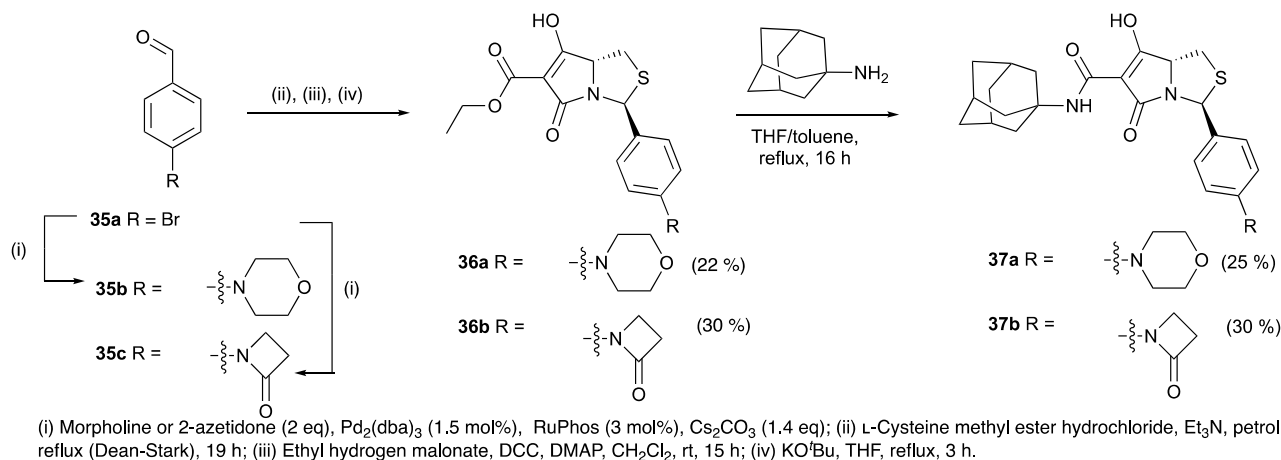
The presence of a phenolic group at the C-2 position provided a platform for further functionalization of carboxamide tetramates via Williamson ether synthesis. In the presence of K_2CO_3 , nucleophilic substitution of ethyl bromoacetate and 1-bromo-2-butyne by the phenoxide derived from **28a** gave carboxamide tetramates **29a** and **30** respectively (Scheme 9). HMBC correlation data confirmed the substitution at the phenolic but not at the enolic alcohol group. Ester hydrolysis of **29a** afforded the carboxylic acid bearing tetramate **29b** with improved hydrophilicity.

Since the pyroglutamate skeleton is found in antibacterial natural products (e.g. oxazolomycin^{54, 55} and pramanicin⁵⁶), the synthesis of a pyroglutamate-tetramate hybrid system was also examined. This was achieved by condensing pyroglutaminol **31**,⁵⁷ prepared by the literature procedure,^{58, 59} with terephthalaldehyde by reflux in toluene using a Dean-Stark apparatus to give a mixture of products **32** and **33** in 1:2.4 ratio (Scheme 10); the lactam-functionalized aldehyde **33** was isolated by column chromatography as a single diastereomer, whose stereochemical assignment was in agreement with previous reports on *N,O*-acetal formation of **31** with benzaldehyde.^{60, 61} Aldehyde **33** was in turn converted to the desired bicyclic tetramate ester **34a** in the usual way, which was

converted to carboxamide **34b** with 1-adamantylamine. The stereochemical assignment of the tetramate system was confirmed by NOE experiments (Scheme 10). The relative stereochemistry of both heterocyclic systems was found to be *trans*-, in keeping with structures reported in the literature.⁶²

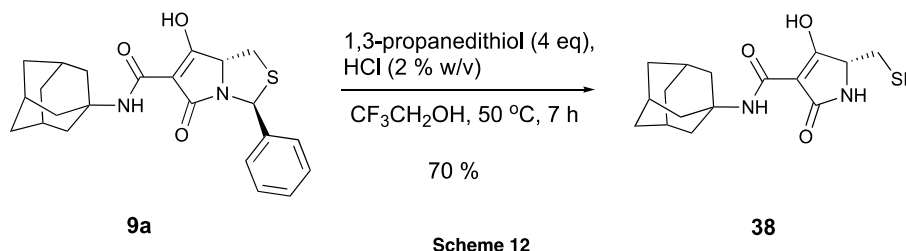


Of interest is that direct Buchwald-Hartwig amination of bromo-substituted tetramates **8b** and **9b** using any of XPhos, RuPhos and XantPhos was not successful. Instead, *p*-bromobenzaldehyde **35a** was functionalized via Buchwald amination/amidation to give aldehydes **35b,c** and these were then used to synthesise the tetramate core *de novo* using a similar route to that described above, giving esters **36a,b** which were converted to carboxamides **37a,b** as before (Scheme 11).



In one case, deprotection of the *N,S*-acetal was examined; in comparison to oxazolidine-based *N,O*-acetal systems, thiazolidine-based *N,S*-acetals have been shown to have higher stability to acids,⁶³ and their deprotection often requires harsh conditions such as heating under reflux with 5M HCl for 72 h.⁶⁴ However, Onoda *et al.* have shown that Boc-protected thiazolidines can be deprotected at the *N,S*-acetal in good yield with 15 eq of TFA in water-saturated CH₂Cl₂ under ambient conditions within 2 h.⁶⁵ Deprotection of carboxamide tetramate **9a** was investigated with TFA in CH₂Cl₂, but even after a reaction time of 24 h, only starting material remained, and while the addition of water accelerated the reaction, complete deprotection was not observed in 24 h. However, deprotection of thiazolidine via the Corey-Reichard protocol⁶⁶ was successful giving

carboxamidotetramate **38** in 70 % yield (Scheme 12). This observation highlights the need for stronger conditions for the deprotection of the more stable thiazolidine-derived carboxamide tetramates.



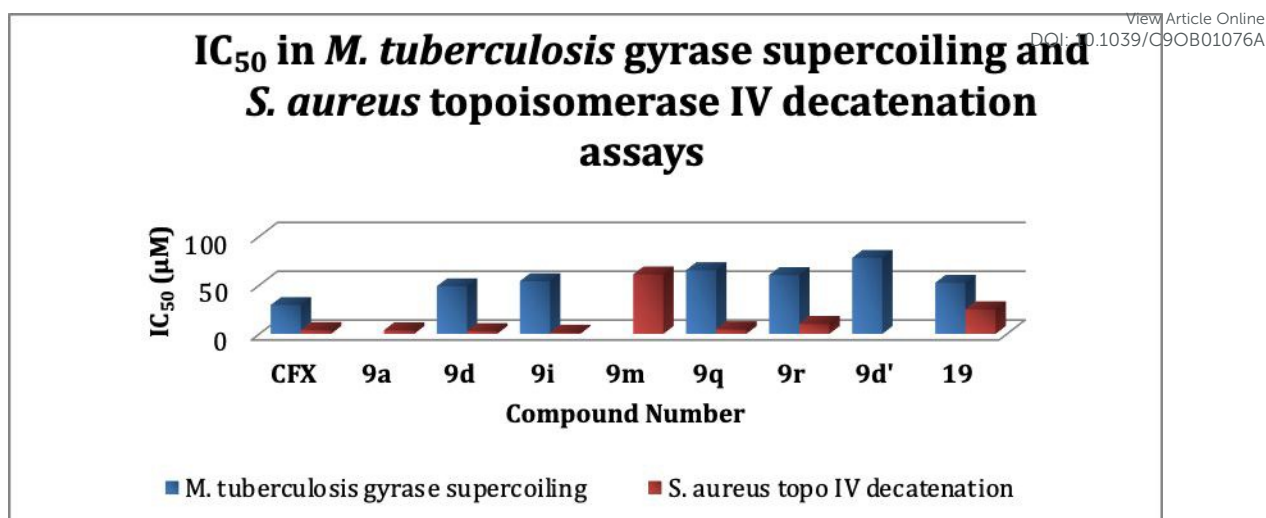
Antibacterial properties

Mode of action of tetramates

Naturally-occurring tetramates, such as kibelomycin and amycolamycin, are inhibitors of bacterial topoisomerase IV (topo IV) and DNA gyrase,^{20, 44, 67} whereas the tetramates streptolydigin and tirandamycin are inhibitors of RNA polymerase (RNAP).^{68, 69} Some tetramates have been shown to provide dual inhibition of both RNAP and undecaprenyl pyrophosphate synthase (UPPS).⁷ All four of these enzymes are essential for viability. The primary function of topo IV is the decatenation of daughter chromosomes that are multiply-linked, during the final stages of DNA replication,⁷⁰ and the role of DNA gyrase is catalysis of negative supercoiling of DNA.⁷¹ Bacterial RNAP is an attractive target in antibacterial chemotherapy as the bacterial RNAP subunit sequences are highly conserved while being different from eukaryotic RNAP subunit sequences. This permits broad-spectrum activity and therapeutic selectivity.⁷² An examination of the mode of action of the tetramates prepared in the current work was therefore of interest.

Inhibition of possible bacterial target enzymes by some bicyclic tetramates was studied for *Staphylococcus aureus* topo IV and RNAP, *Escherichia coli* RNAP and *Mycobacterium tuberculosis* gyrase. A selection of compounds (full data is given in Tables 3-5, SI) initially was tested at a fixed concentration of 100 μ M for the inhibition of topo IV and gyrase, and the percentage of DNA decatenated or supercoiled in the presence of each test compound was calculated. A lower percentage of decatenation or supercoiling indicated a higher level of inhibition of the target enzymes. The data were compared to those for the known topo IV and gyrase inhibitor, ciproflaxacin. Concentrations resulting in half-maximal inhibition (IC_{50} s) for some of the more potent inhibitors (*vide infra*) were determined and are presented in Figure 6(a). Tetramates **9a**, **9h** and **9m** were tested for their inhibition of *S. aureus* and *E. coli* RNAP,⁷³⁻⁷⁵ and the data obtained are presented in Figure 6(b).

(a)



(b)

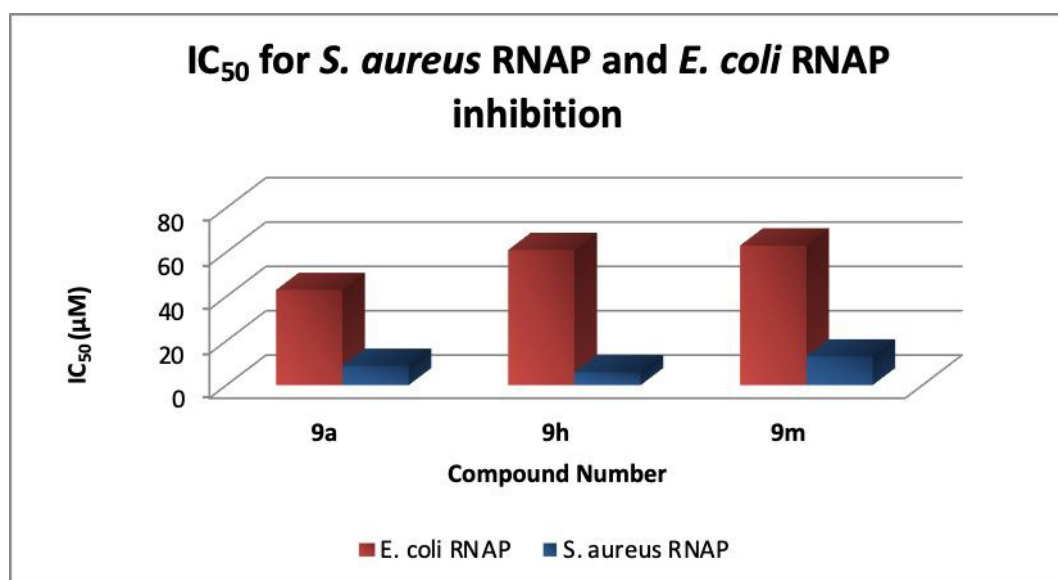


Figure 6.(a) IC₅₀ values of tetramates against *M. tuberculosis* gyrase and *S. aureus* topoisomerase IV. Compounds **9a** and **9m** were weak inhibitors of *M. tuberculosis* gyrase and **9d'** was a weak inhibitor of *S. aureus* topoisomerase IV. (b) IC₅₀ values of tetramates against *S. aureus* RNAP and *E. coli* RNAP.

Comparison of minimal inhibitory concentrations (MICs) for antibacterial activity against methicillin-resistant *S. aureus* (Table 10, SI; see also Figure 7; *vide infra*) with IC₅₀s for inhibition of *S. aureus* RNAP and topo IV (Figure 6) indicates that compounds exhibiting high antibacterial activities also exhibit high *S. aureus* RNAP and topo IV inhibitory activities, consistent with the possibility that antibacterial activity may be attributable to RNAP and topo IV inhibitory activity. Compounds **9a**, **9h** and **9m** inhibited both RNAP and *S. aureus* topo IV, consistent with a possible dual mode of action for these compounds. Some compounds that showed high efficacy in enzyme inhibition assays did not show high efficacy in whole-cell assays (e.g **9d'**, **19**). This potentially may

be due to low cell permeability or elimination via efflux mechanisms, leading to reduced bioavailability in the bacterial cell. In general, *E. coli* RNAP was less sensitive to tetramates than *S. aureus* RNAP, and the *M. tuberculosis* gyrase was less sensitive to tetramates than *S. aureus* topo IV.

Antibacterial activity: hole-plate method

Whole-cell antibacterial assays were performed using the hole-plate method with Gram-positive *S. aureus* DS267 or Gram-negative *E. coli* X580, using Cephalosporin C as a positive control. The samples were prepared as 4 mg/mL solutions of 70% DMSO in MeOH, with serial dilution to the desired concentrations where necessary. The relative potency was estimated by reference to positive controls prepared with Cephalosporin C.⁷⁶ Many of the carboxamide analogues **9a-v** showed antibacterial activity against *S. aureus*, but none showed activity against *E. coli* (Table 6-9, ESI). The presence of an adamantyl carboxamide side chain on the tetramate core resulted in a significant enhancement of antibacterial activity and such tetramates **9a-f** were active at a concentration of 1 µg/mL. Tetramates **9h-9k** with a 4-cyclohexylphenyl group or **9l-9q** with a 4-chloro-2-methylphenyl group demonstrated good antibacterial activity, although much less potent compared to their adamantyl analogues, with the 4-cyclohexylphenyl group being the more active. The decrease in antibacterial activity observed for **9r**, **9s** and **9w** further confirms the requirement for a bulky pendant group at C-7 of the bicyclic tetramate core.

Since some tetramates have been shown to lose antibacterial activity in the presence of human serum albumin (HSA),⁷ further assays were run in the presence of HSA. These data were compared with the bioactivity of each sample in the absence of HSA and selected examples are given in Figure 7 along with full data in Table 6-9 (ESI). While **9a-9f** showed good antibacterial activity at 1 µg/mL in the absence of HSA, there was a complete loss of activity in the presence of 4 mg/mL of HSA (where the tetramate:HSA ratio is approximately 1:30, data not shown). These data suggest that carboxamides are possible ligands for HSA. Nevertheless, of interest was the return of bioactivity for these tetramates when the concentration of the test compounds was increased to 5 µg/mL (in which tetramate:HSA ratio is approximately 1:6). Similarly, tetramates **9h-9q** exhibited no antibacterial activity at their original concentrations when tested in the presence of HSA, but their activity was regained by increasing the compound concentrations. Interestingly, tetramates **9g**, **9r**, **9s** and **9w** which were less potent compared to the other tetramates discussed above retained their potency in the presence of HSA.

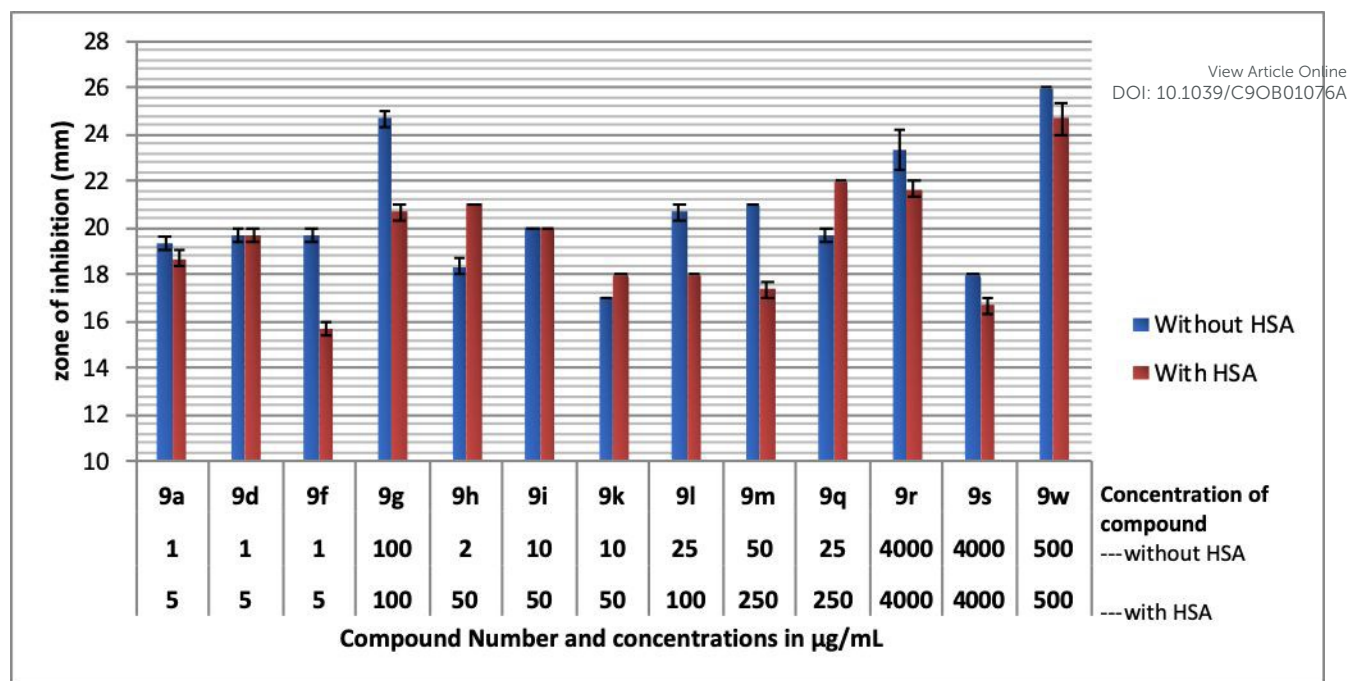


Figure 7. Bioactivity of carboxamide tetramates with/without HSA.

The assay samples were prepared in 70% DMSO in MeOH for reasons of solubility, followed by mixing with albumin solution, and resulted in a final solvent composition of 35:15:50 of DMSO:MeOH:H₂O in the well (35% DMSO). It is known that higher concentrations of DMSO can lead to protein denaturation,^{77, 78} and this might have interfered with the above analysis. Carboxamides **9a-9f** showed good antibacterial activity at 1 µg/mL, and these samples could be dissolved in a minimum amount of DMSO at such low concentrations. Thus, the antibacterial activity of carboxamides **9a-9f** in the presence of HSA was tested where the final solvent composition was 2% DMSO in H₂O. The zones of inhibition measured were in agreement with the data obtained in the presence of 35% DMSO, confirming that the antibacterial activity observed for the tetramates in the presence of HSA was not a result of the compound unbound to denatured HSA.

Broth dilution method

Broth assays were performed to determine MICs of compounds (Table 10, ESI). The carboxamido tetramates **9a-g'** showed either weak (MIC= 250 µg/mL) or no activity against Gram-(-) strains, in agreement with the data obtained from the hole-plate method, while some exhibited promising antibacterial activity against Gram-(+) strains. Among the C-7 functionalized tetramates, the most potent carboxamide tetramates **9b-f, h, l-m, q, u-v, z** showed an MIC of 0.49 µg/mL against at least one organism, significantly better than the tetramate ester analogues **8a-g**,¹² highlighting the importance of a carboxamide side chain group for whole-cell antibacterial activity. However, the presence of more polar carboxamides at C-7 led to a loss of activity (see **9d'** and **9e'**). Since an MIC

value of 16-32 $\mu\text{g/mL}$ against a test organism is considered a suitable minimum for optimisation,⁷⁹ several tetramates therefore offer suitable starting points for core scaffold elaboration. The selectivity of these tetramates for prokaryotic cells over mammalian cells was analysed via a primary cytotoxicity screening against HaCaT cell line (see ESI). Compounds that are worth progressing from this stage should possess ≥ 10 -fold higher antibacterial activity over cytotoxicity,⁷⁹ and on this basis, carboxamido tetramates **9e**, **9f**, **9g**, **9h**, **9l**, **9m** and **9p** are of interest.

The tetramates with C-2 functionalization obtained from SM cross-coupling were assayed for antibacterial activity against Gram(-) bacterial strains *E. coli*, *K. pneumoniae* and *P. aeruginosa* and Gram(+) strains MRSA, *Enterococcus* and *Streptococcus pneumoniae* but no activity was observed. There was, however, potent antibacterial activity against Gram(+) strains MRSA, *Enterococcus* and *Streptococcus pneumoniae*. In particular, compounds **14d**, **14h** and **14k** displayed improved antibacterial activity against MRSA while **14a**, **14d** and **14h** showed improved activity against *Enterococcus sp.* compared to **9a**. The glycosylated tetramates were either inactive or only weakly active (MIC= 250 $\mu\text{g/mL}$) against Gram(-) strains tested, but displayed potent antibacterial activity against Gram(+) strains MRSA, *Enterococcus* and *Streptococcus pneumoniae*. Contrary to the trend in antibacterial activity observed for the tetramate esters **8a-g**,¹² tetramate ester **16** proved to be more active than its adamantyl analogue **18**. Increasing polarity at C-2 as in **17** conferred no bioactivity. Introduction of a disaccharide analogue also led to the complete loss of antibacterial activity of tetramate ester **21**. Further, it was interesting to note that the aglycone analogue **26a** was slightly more potent than the glycosylated tetramate **16**. In the case of glycosyl tetramate **23**, in which an acylated galactose is linked to the tetramate via a benzyl ether, improved antibacterial activity was observed compared to **16**. Both **23** and its aglycone analogue **26b** displayed similar level of antibacterial activity. Thus, while the presence of a glycone does not lead to a significant compromise in bioactivity, it seems that a significant increase in polarity at C-2 leads to a detrimental reduction in the antibacterial activity (e.g. **17**). The exact role of the glycosidic residue is not known, but it may assist in improving polarity, bioavailability, modulating toxicity and bacterial target interaction.⁴⁹ Conversion of the tetramate ester **16** to its carboxamide analogues **18** led to a loss in activity. However, the absence of the glycone moiety in these carboxamide tetramates restored antibacterial activity as observed for **28a**, indicating that the presence of a glycone is not favourable for antibacterial activity of carboxamide tetramates. This outcome for amides is in contrast to that observed for tetramate esters.

Etherification of **28a** to **29a** resulted in a 64-fold reduction in antibacterial activity. Ethyl ester hydrolysis of **29a** to give carboxylic acid **29b** resulted in a further 8-fold reduction in potency (MIC of **29b** against MRSA was 250 $\mu\text{g/mL}$). However, etherification of **28a** with 1-bromo-2-butyne afforded **30** without much compromise in antibacterial activity. It was also interesting to note that replacement

of the adamantyl side chain with either phenyl (**28b**) or tetrahydropyranyl (**28c**) groups led to a reduction in potency, with no antibacterial activity being observed for the latter. An evaluation of adamantyl carboxamide tetramates with aliphatic heterocycles **34b**, **37a** and **37b** appended at C-2 showed good antibacterial activity when compared to the corresponding tetramate esters. This observation is in agreement with the general trend observed for tetramate esters and their carboxamide analogues, highlighting the need for a bulky, hydrophobic C-7 pendant group. In a separate comparison of C-7 adamantyl carboxamides bearing morpholine, pyroglutamate and β -lactam moieties at C-2, morpholine substituted tetramate **37a** was the most active while pyroglutamate substituted tetramate **34b** was the least potent.

In the absence of a C-2 substituent, antibacterial activity of tetramate **38** against Gram-positive *S. aureus* is significantly reduced, and to obtain a similar zone of inhibition to that of **9a**, a 1000-fold increase in concentration of **38** was necessary. However, upon deprotection, activity against Gram-negative *E. coli* could be observed; thus, at a concentration of 4 mg/mL, **38** showed activity, while **9a** was completely inactive. It is noteworthy that deprotected tetramate **38** does not exhibit significant binding to albumin as its antibacterial activity is retained when treated with HSA. This improvement in activity observed for *E. coli* could be due at least in part to its increased polarity, which in turn leads to increased cell membrane permeability.

O'Shea and Moser have analysed the major antibacterial classes of compounds and have shown that mean values for physicochemical properties define distinct classes;⁸⁰ antibacterial efficacy does not, however, necessarily correspond to oral bioavailability codified in the Lipinski Rule of 5,⁸¹ primarily as a result of bacterial cell wall penetration.^{82, 83} A correlation of calculated physicochemical properties with the antibacterial activity of some known tetramates was undertaken (using Marvin (16.4.18.0), 2016, ChemAxon (<http://www.chemaxon.com>)) (Table 11, SI), and their physicochemical properties show general resemblance to fluoroquinolones, with relative PSA values of 13-25% and a MW range of 300-600Da. A similar comparison of physicochemical properties (MW, clogD_{7.4} and rel. PSA) and antibacterial activity (where 'activity' was defined as MIC of ≤ 8 $\mu\text{g/mL}$ ⁸⁰ against at least one bacterial strain) of the tetramate compound library (Figure 8 and Table 11, SI) showed compounds that are active largely occupy physicochemical space with MW of 300-600, clogD_{7.4} of -2.5 to 4 and rel. PSA of 11-22; in this, they also generally resemble fluoroquinolones.⁸⁰ The 'active' tetramate esters **16** and **23** with glycone pendants appear as outliers due to their higher MW in the region of 650-670. Compounds that are more hydrophilic (that is, with lower clogD_{7.4} and higher relative PSA) did not exhibit antibacterial activity against Gram-positive strains, even though the mean MW of known Gram-positive antibacterial agents is around 800.⁸⁰ However, not all polar tetramates that fit in to the optimal chemical space of antibacterials with Gram-negative activity showed the expected activity, although the antibacterial activity of *N,S*-acetal deprotected tetramate **38**

against *E. coli* suggests suitable polarity improves Gram-negative activity of tetramates, since uptake/penetration through the cell membrane might be better.⁷⁰ Tetramates with antibacterial activity possessed desired physicochemical properties required by Lipinsky's Ro5, and therefore have potential to be developed as oral drug candidates, but these highly hydrophobic compounds are likely to be penetrative of the blood brain barrier on the basis of calculation of log BB values using a published algorithm.⁸⁴ Furthermore, the Fsp³ value (Fsp³ = sp³ hybridized carbon count/ total carbon count⁸⁵) of the tetramate scaffold **8** is 0.43; Lovering and co-workers have demonstrated that complexity as measured by Fsp³ increased for molecules in clinical progression and that drugs have an average Fsp³ of 0.47.⁸⁵ This places the tetramate scaffold in an ideal region of chemical space for development. Moreover, increased complexity appears to reduce toxicity of drug candidates,⁸⁶ and this was consistent with our observations: for the antibacterially active tetramates for which cytotoxicity against HaCaT mammalian cell line was determined, the mean Fsp³ calculated for tetramates with low toxicity (Fsp³ = 0.41), defined as those that possess ≥ 10 -fold antibacterial activity (against at least one bacterial strain) over cytotoxicity, was higher than those with higher toxicity levels (Fsp³ = 0.38).⁸⁶ No reaction of tetramate **9f** with benzylamine and cysteine under ambient conditions was observed, consistent with the lack of toxicity observed in these systems.

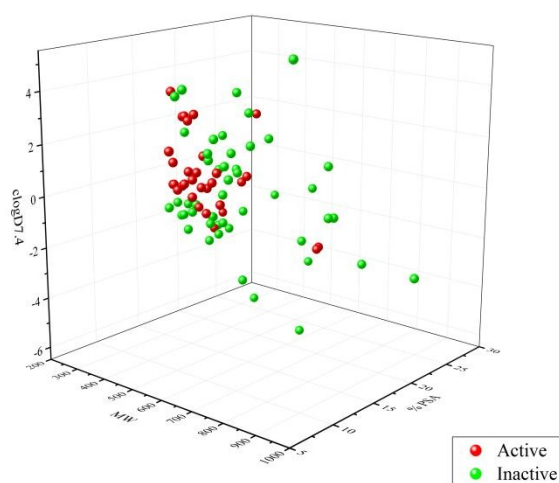


Figure 8. Correlation of physicochemical properties (MW, clogD_{7.4}, and rel. PSA) with antibacterial activity.

Experimental

General procedure: Esterification of L-serine and DL-cysteine.¹²

To a suspension of the amino acid (1.0 eq) in MeOH (2 mL/mmol) at 0 °C, SOCl₂ (1.2 eq) was added drop-wise under continuous stirring and warmed to rt, then heated at reflux for 1-3 h. The reaction mixture was concentrated *in vacuo* to obtain the respective amino ester.

General procedure: Synthesis of *N*-acylated thiazolidines **6,7**.¹²

Step 1: To L-cysteine methyl ester hydrochloride (1.0 eq) in petrol (25 mL/1 g), Et₃N (1.2 eq) and aldehyde (1.2 eq) were added. The mixture was heated at reflux, with continuous removal of water using a Dean-Stark apparatus, for 19 h. It was then filtered and washed with Et₂O. The combined filtrates were concentrated *in vacuo* and residue was purified by silica gel flash column chromatography (eluent: EtOAc/petrol) to give the required thiazolidine.

Step 2: A solution of ethyl hydrogen malonate (1.2 eq) in CH₂Cl₂ (2.5 mL/mmol) was added to a stirred solution of thiazolidine (1.0 eq), DCC (1.2 eq) and DMAP (0.1 eq) in CH₂Cl₂ (5 mL/mmol) at 0 °C. The mixture was stirred at 0 °C for 15 min and then at rt for 15 h. The reaction mixture was filtered to remove dicyclohexylurea and the residue was washed with CH₂Cl₂. The combined filtrates were concentrated *in vacuo* and purified by silica gel flash column chromatography (eluent: EtOAc/petrol) to give *N*-acylated thiazolidines **6,7**.

General procedure: Synthesis of bicyclic tetramates **8**.¹²

KO^tBu (1.2 eq) was added to a solution of the *N*-acylated thiazolidine in THF and heated at reflux for 3 h. It was then cooled to rt, concentrated *in vacuo* and partitioned between Et₂O and water. The aqueous layer was extracted and acidified with 2M HCl (to pH 1) and extracted with EtOAc. The combined EtOAc extracts were washed with brine, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (with 1% Et₃N) to give the desired product. The product was then dissolved in CH₂Cl₂ and washed with 5% citric acid. The organic fractions were dried over Na₂SO₄, filtered and concentrated *in vacuo* to yield the desired bicyclic tetramates **8a-g**.

General procedure: Synthesis of carboxamide tetramates **9a-g**'

To tetramic acid (1.0 eq) dissolved in THF/toluene or DMSO/toluene (1:9, 10 mL/mmol), amine (1.5 eq) was added. The solution was heated at reflux for 16 h, cooled to rt and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (eluent: EtOAc/MeOH/1 % Et₃N). The product was dissolved in CH₂Cl₂ and washed with 5% citric acid. The organic fractions were dried over Na₂SO₄, filtered and concentrated *in vacuo* to yield the bicyclic carboxamide tetramate.

Conclusion

Thiazolidine derived tetramate esters were readily elaborated to carboxamido tetramates by direct uncatalyzed ester to amide exchange, and conditions were identified which permit skeletal elaboration by Suzuki-Miyaura coupling despite the presence of a cyclic thioether function. While tetramate esters were inactive against both Gram-positive and Gram-negative bacterial strains, the

presence of a carboxamide pendant group at C-7 of the tetramate core conferred antibacterial activity against Gram-positive strains; however, the introduction of more polar substituents led to reduced or no activity as observed for **17** and **29b**. The antibacterial activity spectrum observed for various C-2 functionalized tetramates demonstrated the ability to accommodate a range of substituents while retaining antibacterial activity, providing scope for physicochemical property optimization, and an analysis of physicochemical properties indicates that bioactive tetramates generally occupy physicochemical space with MW of 300-600, clogD_{7.4} of -2.5 to 4 and rel. PSA of 11-22%. With regards to cytotoxicity, compounds with low toxicity had a higher Fsp3 value compared to those with high toxicity. Evaluation of antibacterial activity of tetramates in the presence of HSA suggests possible binding of tetramates to albumin may occur. Although the major tautomeric pair of these species has been identified in solution, it should not be assumed that this is also the binding tautomer at its enzyme target site, since a number of tautomeric forms may be in free equilibrium.

More generally, the identification of novel, non-planar, synthetically accessible heterocyclic systems has become of interest in drug discovery.^{85, 87, 88} The work described here shows that highly functionalised heterocyclic skeletons with several points of diversity, modelled on bioactive natural products, are available in a short synthetic sequence, and that further functional group elaboration may give structures which exhibit similar bioactivity profiles to that of the parent natural product, in this case antibacterial activity. Importantly, these compounds provide well-defined 3D templates which minimise aromatic ring count,⁸⁸ but maintain acceptable starting values of MW, PSA, numbers of rotatable bonds, H-bond acceptors and H-bond donors, while leaving ample scope for lead optimisation in the drug discovery process. They therefore offer suitable skeletons for application in fragment-based drug design^{89, 90} which allow escape “from flatland”.⁸⁵

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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