PAPER

Tetramate derivatives by chemoselective Dieckmann ring closure of *allo*-phenylserines, and their antibacterial activity†

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A general route which provides direct access to substituted bicyclic tetramates, making use of Dieckmann cyclisation of oxazolidine derivatives derived from *allo*-phenylserines, is reported. Of interest is the high level of diastereoselectivity observed for the *N*-acylation reaction of oxazolidines and the complete chemoselectivity of their ring closure in the Dieckmann cyclisation. Significantly, the sense of the chemoselectivity is different to earlier reported *threo*-phenylserine systems, showing the importance of steric bulk around the bicyclic ring system. The derived C7-carboxamidotetramates, but not C7-acyl systems, exhibited potent antibacterial activity against MRSA, with the most active compounds exhibiting well-defined physicochemical and structure–activity properties. This work clearly demonstrates that densely functionalised tetramates are both readily available and may exhibit high levels of antibacterial activity.

Introduction

The tetramate system occurs as a core skeleton in natural products which exhibit a wide range of bioactivity,[1–3] and we have shown that bicyclic tetramates, which are accessible by chemoselective and stereoselective Dieckmann ring closures of oxazolidine or thiazolidine templates **1** (Fig. 1),[4,5] may also exhibit potent antibacterial activity, mostly against Gram-positive bacteria. The chemoselectivity of this cyclisation depends upon the identity of R^1, R^2, X, along with the 2,5-relative stereochemistry of the *N*-acyloxazolidine or thiazolidine starting material **1**. Normally, path A mode of cyclisation is preferred and predominates for 2,5-*cis*-malonyloxazolidines (X = O, R^1 = ^{t}Bu, R^2 = H/Me) derived from serine[5,6] and threonines[7,8] leading to tetramates **2**, although 2,5-*trans*-malonyloxazolidines (X = O) if formed, usually close by path B leading to tetramates **3**. This intrinsic chemoselectivity may be controlled in some cases by adjusting the conditions for the Dieckmann cyclisation, thus tetramates (X = O, R^2 = H) arising by path A were most readily accessed in good yield using the reaction conditions (KO^{t}Bu (1.1 eq.), dry ^{t}BuOH, reflux), while those from path B were available by the use of alternate conditions (KO^{t}Bu (2.2 eq.), wet ^{t}BuOH, reflux).[9] On the other hand, *N*-acylthiazolidines derived from cysteine that had been condensed with aromatic aldehydes (X = S, R^1 = Ar, R^2 = H) gave tetramate products by path B, that is, in a different direction to the *t*-butyl series (X = S, R^1 = ^{t}Bu, R^2 = H) which close by path A, and this arises due to differing stabilities of the *cis*- and *trans*-oxa(or thia)zolidine starting materials.[4,10,11] However, by contrast to threonines (X = O, R^1 = ^{t}Bu, R^2 = Me) which close by path A, *threo*-β-arylserines ((2S*,3R*)-2-amino-3-hydroxy-3-arylpropanoic acids, X = O, R^1 = ^{t}Bu, R^2 = Ar) close by path B as a result of the steric influence of the C-4 aryl group.[12] Of interest was the chemoselectivity which would be observed for *allo*-β-arylserines ((2S*,3S*)-2-amino-3-hydroxy-3-arylpropanoic acid), which are epimers at C-4 of *threo*-β-arylserines; we report here the results of that investigation.

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Results and discussion

The initial requirement was access to *allo*-β-hydroxy-β-aryl-α-amino acids, and such systems have previously been reported by Chang *et al.*[13] along with Inoue *et al.*[14] who exploited fully diastereoselective catalytic reductions of oximes using palladium on carbon, controlled by formation of a substrate–catalyst complex where the palladium coordinates with both the oxygen and nitrogen atoms of the substrate.[13,14] The

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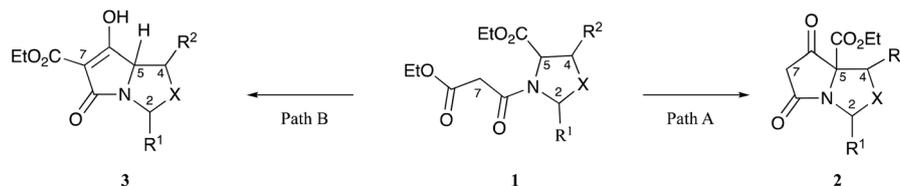*Org. Biomol. Chem.*, 2023, **21**, 4061–4071 | **4061**

Fig. 1 Chemoselectivity of Dieckmann cyclisation leading to tetramates.

required ethyl aroylacetate starting materials were either commercially available, or could be readily prepared using the methodology of Clay *et al.*[15] Compound **5a** is commercially available, but for the others, ethyl potassium malonate **4** was dissolved in acetonitrile and was reacted with acid chlorides

using a MgCl$_2$–Et$_3$N base system to obtain β-oxoesters **5b–g** (Scheme 1), typically in quantitative yields and with a very high level of purity, not requiring chromatography; more than 3 eq. of Et$_3$N was required to suppress the formation of side products.[15] NMR analysis indicated that these compounds existed
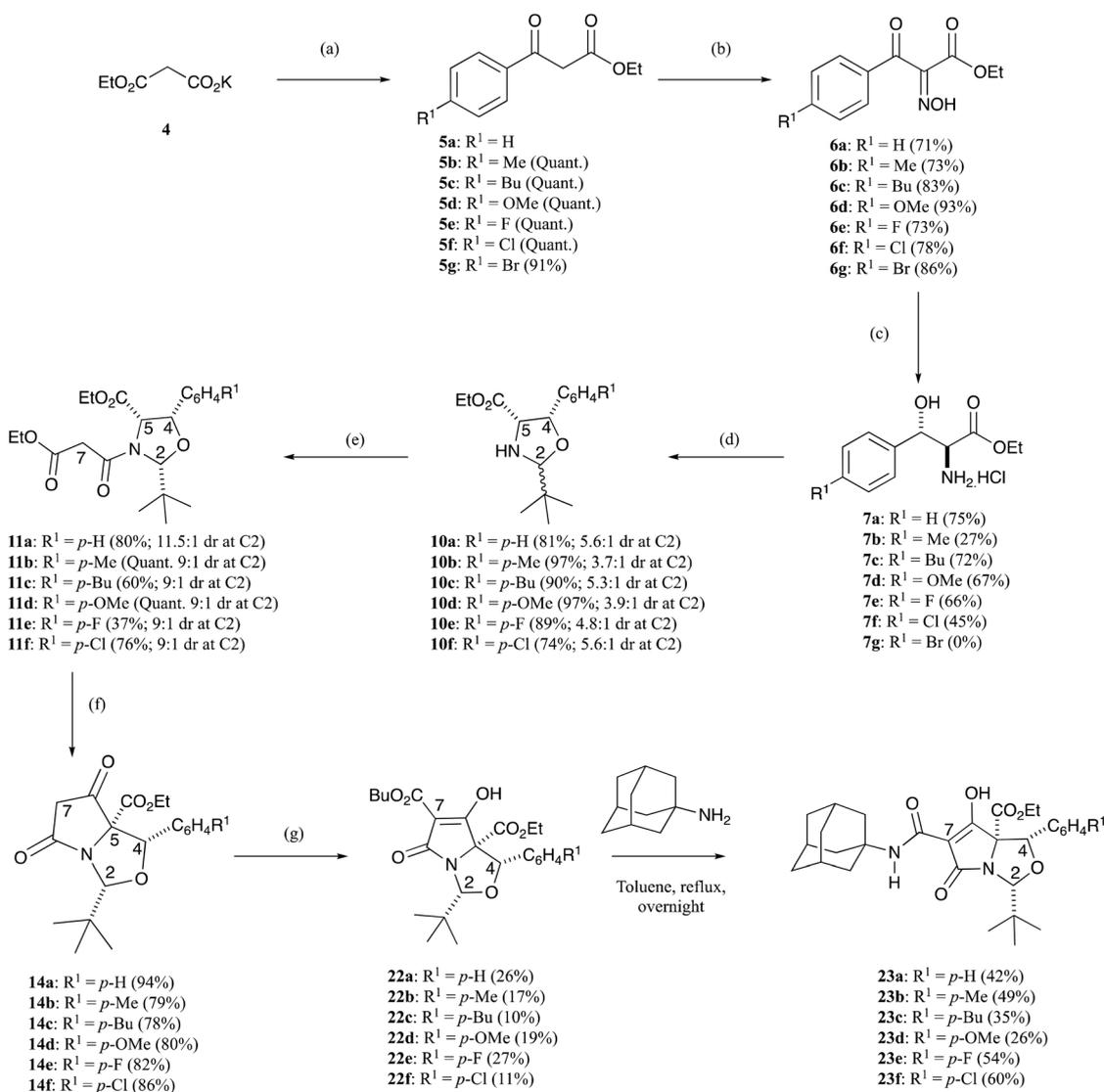
Scheme 1 Synthetic route to tetramates. Synthesis of tetramates **14**, **22** and **23** from *allo*-phenylserine derivatives **7a–f**; (a) MeCN, MgCl$_2$, Et$_3$N, 10–25 °C, 2.5 h then R^{1}C$_6$H$_4$C(O)Cl, Et$_3$N, rt, overnight; (b) NaNO$_2$ in H$_2$O, AcOH, rt, 30 min-overnight; (c) EtOH, conc. HCl, H$_2$, Pd–C, rt, 1–3 d; (d) pivaldehyde, petroleum ether 40 : 60, Et$_3$N, Dean–Stark, >100 °C, overnight; (e) ethyl malonyl chloride, pyridine, DCM, reflux, overnight; (f) DBU, THF, rt, overnight; (g) butyl chloroformate, DCM, DMAP, reflux, overnight.

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as a mixture of keto–enol tautomers (Table S1, ESI†). These were readily converted to their oxime derivatives **6a–g** by reaction with sodium nitrite in glacial acetic acid using the conditions of Fell *et al.* (Scheme 1 and Table S2, ESI†), which required the temperature to be kept below 10 °C, giving the products in yields of between 71–93% in excellent purity, again not requiring chromatography.[16] The major isomer for **6a** was assumed to be *E*, based upon the work of Fell *et al.*,[16] although this assignment was not material for subsequent work. Oximes **6a–g** were dissolved in ethanol and conc. HCl and hydrogenated at ambient temperature and pressure, using palladium on carbon as a catalyst, for 1–3 days, and this gave *allo*-arylserines **7a–f** fully diastereoselectively, in variable yields but again of high purity, not requiring chromatography (Scheme 1 and Table S3, ESI†).[13,14] A sizeable amount of catalyst was required to produce the desired product in acceptable yields, and as a result, oxime **6g** gave compound **7a** instead of the desired bromo **7g** by concomitant hydrogenolysis. Confirmation that the phenylserine derivatives were indeed the *allo*-isomer was achieved by conversion of phenylserine **7a** to *cis*-oxazolidinone **8** and comparison to literature data (Scheme 2 and Table S4, ESI†).[17] Furthermore, *allo*-phenylserine **7a** was converted to crystalline Boc-protected *allo*-phenylserine **9a** (Scheme 2), and a single-crystal X-ray structure confirmed the *allo*-assignment (Fig. S1, ESI†).[18]
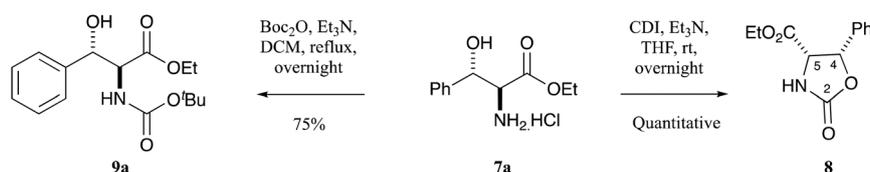
With amino esters **7a–f** in hand, reaction with pivaldehyde in the presence of Et₃N and petroleum ether 40 : 60 with heating to over 100 °C under a Dean–Stark trap for 16–24 h formed oxazolidines **10a–f** in good yields – typically between 74–97% – as roughly a 5 : 1 mixture of diastereomers at the C2-centre, as determined from NMR analysis (Scheme 1 and Table S5, ESI†).[5,7] Confirmation of their relative stereochemistry was achieved by nOe analysis. Since we had earlier found that oxazolidines derived from *threo*-phenylserines were not very stable to chromatographic purification,[12] these products were taken directly into the next reaction sequence. Oxazolidines **10a–f** were readily *N*-acylated by reaction with ethyl malonyl chloride under basic conditions to form malonamides **11a–f** in 37% up to quantitative yields (Scheme 1). Once again, the crude products were pure by NMR analysis but could easily be chromatographically purified if required to obtain malonamides **11a–f** with high levels of purity (Table S6, ESI†), being typically a 9 : 1 mixture of diastereomers at the C2-position. nOe studies clearly demonstrated the *cis*-relationship between the H2, H4 and H5-protons for malonamide **11a** and this was also confirmed by a single-crystal X-ray structure (Fig. S1, ESI†).[18] This stereochemical outcome was also

assumed for the compound series as a whole on the basis of the similarity of their NMR spectra.

Interestingly, the *N*-acylation of oxazolidines **10a–f** was highly diastereoselective in favour of the *all cis* product (Scheme 1 and Fig. 2), and much more so than the related *threo*-phenylserine derivatives.[12] Therefore, it was apparent that the *cis*-C4–C5 stereochemistry had a significant effect on the diastereoselectivity of both the oxazolidine cyclisation and *N*-acylation reactions. While the *all cis* oxazolidine diastereomer **10a–f** is the major one, favoured *ca.* 5 : 1 over the minor one, it is also the most reactive in the *N*-acylation reaction, since there is an exposed face for reactions which is away from all three ring substituents, and this gives the major *N*-acyl derivative **11a–f**. The minor oxazolidine is not so reactive to *N*-acylation, since both sides of attack on the oxazolidine experience some steric hindrance from ring substituents, allowing for equilibration to the major oxazolidine and onward reaction to the major *N*-acyl product, leading to an overall 9 : 1 mixture of malonamide diastereomers **11a–f**, starting from roughly a 5 : 1 mixture of oxazolidine diastereomers **10a–f**.

When oxazolidine **10a** was reacted with ethyl methylmalonyl chloride **12c** (Scheme 3),[19] malonamide **13a** was obtained as a 1.8 : 1 mixture of diastereomers at the C7-position, which could be partially separated by careful chromatographic purification, and whose ring relative stereochemistry was confirmed by nOe and NOESY analysis with full assignment achievable by a single-crystal X-ray analysis (Fig. S1, ESI†).[18] Similarly, oxazolidine **10a** was reacted with ethyl phenylmalonyl chloride **12d** (Scheme 3) and malonamide **13b** was obtained as a 1.7 : 1 mixture of diastereomers at the C7-position in good yield, but unlike methyl derivatives **13a**, were inseparable by chromatographic purification.[19] The relative stereochemistry of the ring protons of malonamides **13b** was confirmed as being *all cis* by a single-crystal X-ray structure of one of the diastereomers (Fig. S1, ESI†).[18] Equilibration of malonamide diastereomers **13a** and **13b** in CDCl₃ solution over several weeks was observed.

Malonamides **11a–f** were subjected to the Dieckmann cyclisation to form the desired bicyclic tetramates **14a–f** (Scheme 1). Initially, malonamide **11a** was reacted with KOʰBu in THF at reflux overnight, but this led to a complex mixture of unidentifiable products. However, changing the base to DBU, following the procedure of Josa-Culleré *et al.*,[20] effectively gave tetramates **14a–f** (Table S7, ESI†) in excellent yields as single diastereomers (Scheme 1) and with full chemoselectivity, following path A for ring closure (Fig. 1). This corresponds to a

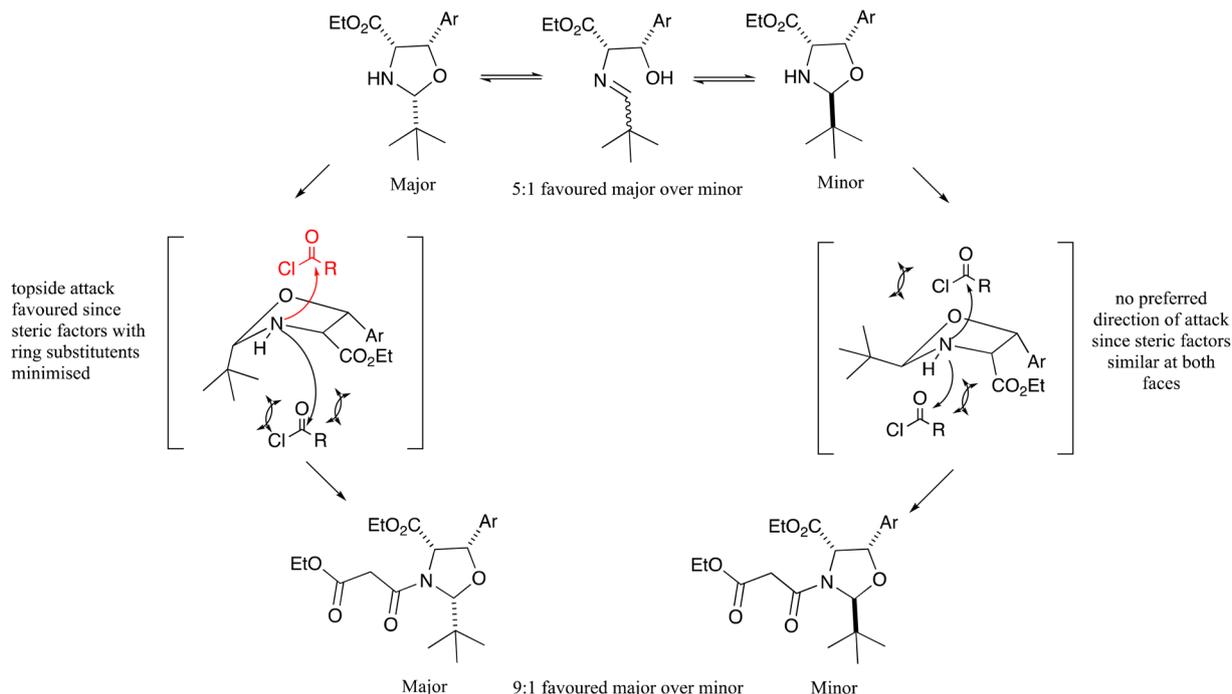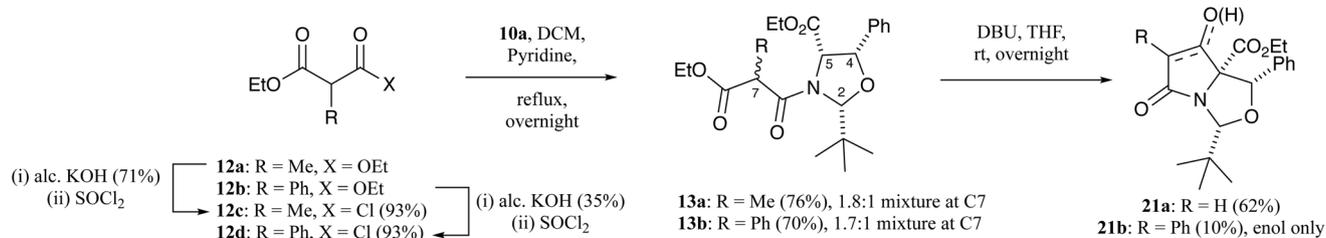**Scheme 2** Confirmation of relative stereochemistry of phenylserine.

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Fig. 2 Diastereoselectivity in oxazolidine ring closure and *N*-acylation.

Scheme 3 Synthetic route to C-7 alkyl and aryl tetramates.

switch in the chemoselectivity observed for *threo*-phenylserines[12] and this is discussed in more detail below. These tetramates did not require chromatographic purification, being pure by NMR analysis, since aqueous extraction of the highly acidic tetramate salts proved to be effective. Overall, pure tetramates **14a–f** could be synthesised from β-oxoesters **5a–g** in 5 synthetic steps without the need for purification by chromatography at any stage of the reaction sequence. Additionally, tetramates **14a–f** were stable in CDCl₃ solution and as an oil for many months. Of interest is that long-range coupling between the H2 and H7-protons (coupling constant of 0.8 Hz) was observed for tetramates **14** (Fig. S2, ESI†). The C5-ethyl ester stereochemistry was later confirmed from a single-crystal X-ray structure of C7-carboxamides **23a** and **23d** (Fig. S1, ESI†).[18] Substituted tetramates **21a** and **21b** were formed *via* the Dieckmann cyclisation of malonamides **13a** and **13b** respectively (Scheme 3). Tetramate **21a** was obtained in 62% yield as a mixture of keto/enol tautomers, but in the case of tetramate

21b, a yield of only 10% was obtained, and the product was observed exclusively in the enolic form in CDCl₃ solvent.

Of interest was both the high level of, and the switch in, chemoselectivity in the Dieckmann cyclisation of malonamides **11a–f** derived from *allo*-phenylserines (Fig. 3) when compared with those derived from *threo*-phenylserine derivatives.[12] In the formation of enolates **15a–f** and then **16a–f** from malonamides **11a–f** which ultimately led to the formation of the observed bicyclic tetramates **14a–f**, both the ᵗBu and aryl groups are on the *exo*-face in *pseudo*-diequatorial locations, thus minimising steric interactions. Tetramates **17a–f** which might have formed *via* the ring closure of enolates **18a–f** would require C-5 epimerisation in the original substrate, in which both the ᵗBu and aryl groups would be found on the *exo*-face of the bicyclic lactam, but this material was not observed experimentally. Of interest is that tetramates **19a–f** and **20a–f** were also not observed experimentally and this is most likely due to the fact that the ᵗBu and aryl groups are on
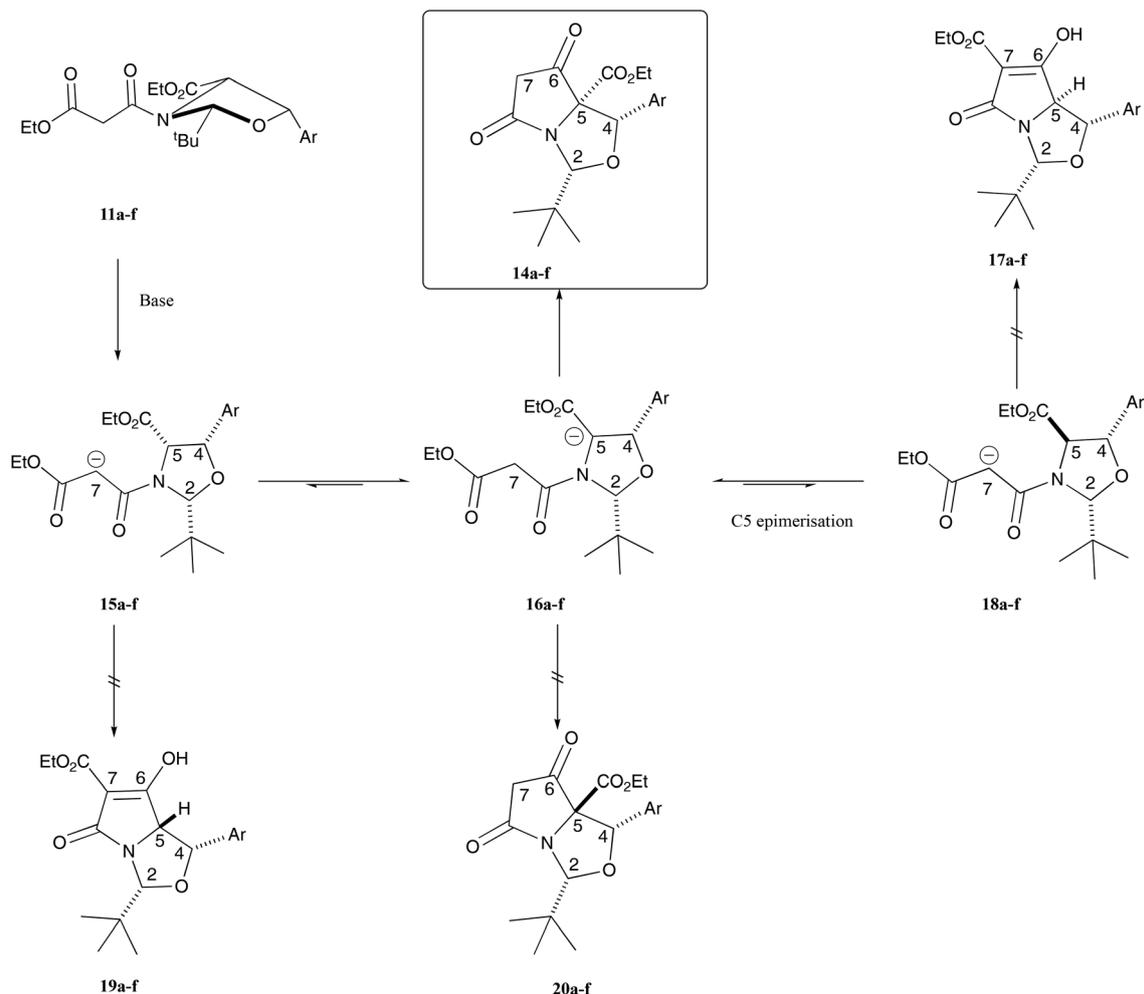
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Fig. 3 Dieckmann ring closure leading to tetramates.

the *endo*-face of the bicyclic lactam in both cases, causing unfavourable steric congestion – consistent with that observed for the *threo*-phenylserine-derived tetramic acids, for which detailed theoretical calculations were made.[12] Overall, this work demonstrates that *allo*-phenylserines behave in a similar manner to the reactions of L-serine, L-threonine, L-*allo*-threonine and L-cysteine-derived products that were condensed with pivaldehyde,[5,7] reacting by path A (Fig. 1) but very differently to *threo*-phenylserine derivatives in Dieckmann ring closures,[12] reacting by path B (Fig. 1).

The chemoselective formation of tetramates **14a–f** from malonamides **11a–f** led to unfunctionalised C7-tetramates. In order to incorporate functionality at this position, tetramates **14a–f** were reacted with butyl chloroformate under basic conditions, following the reaction conditions of Jeong *et al.*,[21] to form C7-butyl ester tetramates, to allow differentiation of C5-ethyl esters **22a–f** (Scheme 1 and Table S8, ESI†). The yields for these conversions were poor – typically between 10–27%, with a considerable amount of starting material being present in the reaction mixture after 24 h, probably reflecting the low reactivity of the intermediate tetramate enolate. Moreover, the

C7-butyl ester tetramates **22a–f** were extremely unstable in CDCl$_3$ solution – analogous to C7-ethyl ester tetramates of type **3** (Fig. 1) reported earlier,[12] needing to be both chromatographically purified and reacted on very quickly; this instability could also explain the presence of starting material in the initial reaction mixture. These compounds also picked up trace metal ions during chromatographic purification, a phenomenon we had observed earlier, but which could be readily removed with a 10% citric acid (aq.) workup. The importance of metal chelation in these systems has recently become increasingly apparent and has been reviewed.[22,23]

These compounds could be converted to C7-carboxamidotetramic acids **23a–f** by reaction with adamantylamine in yields that ranged from 26–60% (Scheme 1 and Table S9, ESI†) and which were suitable for chromatographic purification as well as being stable in both the pure form and in CDCl$_3$ solution for many months. The aminolysis reaction was fully chemoselective for the C7-butyl ester, with no exchange at the more hindered C5-ethyl ester. Single-crystal X-ray structures of compounds **23a** and **23d** confirmed their relative stereochemistry, and that no epimerisation had occurred at any of the ring

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stereogenic centres during the aminolysis reactions (Fig. S1, ESI†).[18] Compounds **23a–f**, after chromatographic purification on silica gel, picked up trace metal ions to form metal-chelates across the tri-carbonyl unit; this was immediately evident in the ^{1}H NMR spectrum, where broad peaks were observed after chromatographic purification, a phenomenon which has been seen previously.[4,21] In the case of compound **23a**, the H2 and H4-signals were relatively sharp – indicating these protons were relatively far away from the metal-chelation site – whereas the adamantyl and aromatic peaks were relatively broad – indicating these groups were relatively close to the metal-chelation site (Fig. S3a, ESI†). The acid-washed tetramic acids **23a–f** existed as approximately a 50 : 50 mixture of AB : CD tautomers in CDCl₃ solution (Fig. 4), as has been observed in related systems,[21] but this differed from C7-carboxamides **24** that lacked a C5-ethyl ester (*i.e.* those derived from *threo*-phenylserine), whose ratio was typically an 80 : 20 AB : CD tautomeric mixture in CDCl₃ solution (Fig. 4).[12] It appears that the *allo*-derived **23a** binds metal ions with less affinity than *threo*-derived carboxamidotetramate **24a**,[12] since the NMR peaks were far sharper for tetramate **23a** (Fig. S3, ESI†) than for tetramate **24a**. Since the only difference between these two compounds was a missing C5-ethyl ester in the case of tetramate **24a**, it seems that the C5-ethyl esters might play an important (steric) role in the reduction of metal-chelation across the proximal tri-carbonyl core. The NMR peaks of tetramates **23a–f** could be further sharpened by washing metal-chelated tetramic acids with 10% citric acid solution (aq.) (Fig. S3b, ESI†). Interestingly, the peaks for both the adamantyl and aromatic groups were sharpened by a simple acid wash, and changes in the chemical shift values for the ^{t}Bu, adamantyl and H4-groups before and after acid wash (Fig. S3b, ESI†) were observed.

Inspired by an earlier report of the direct acylation of tetramates using carboxylic acids,[24] tetramate **14a** was coupled with aromatic and aliphatic carboxylic acids using DCC and stoichiometric amounts of DMAP (1.3 eq.) to form C7-acyltetramates **25a–s** in acceptable yields (Scheme 4 and Table S10, ESI†). In this process, substituents were tolerated at the *para*-position of the aromatic ring system, but unsurprisingly less so at the *ortho*-position. Moreover, more hindered acids such as 1-naphthoic acid, 2,6-dichlorobenzoic acid, 2-chlorobenzoic acid and 9-anthracenecarboxylic acid gave no products. While the 2-thienyl substituent was obtained in poor yield, aliphatic acids were obtained in moderate to good yields. NOESY studies suggested no epimerisation had occurred at any of the stereogenic centres after DCC/DMAP coupling. Once again, purification of these tetramic acids on silica gel led to the introduction of trace metal ions, evidenced by very broad resonances in their ^{1}H NMR spectra (Fig. S4, ESI†), and which were much broader than metal-chelated C7-carboxamides **23a** described above (Fig. S3, ESI†). For example, in the case of carboxamidotetramate **23a**, while discernible peaks are visible, for acyltetramate **25d**, no assignable peaks were initially visible but sharp peaks arose after a simple acid wash with 10% citric acid solution (aq.).

Additionally, the products in all cases were obtained as the CD tautomers, with the exception of **25o–q**, which were observed as a mixture of tautomers but in which the CD tautomers were the major ones. Tan *et al.* had earlier demonstrated that the major tautomers for C7-acyltetramates **26** was the CD pair, in which the C6-carbonyl peaks from the AB tautomers were more downfield due to deshielding from hydrogen bond effects (Fig. 5),[21,24–26] and the spectra for **25a–s** were assigned by analogy.

Although the above described synthesis of *allo*-phenylserines **7a–f** from β-oxoesters **5a–f** *via* Pd–C catalysed hydrogenation was very diastereoselective, it suffered from four major drawbacks. Firstly, all the β-oxoesters **5b–f** and oximes **6a–f** –

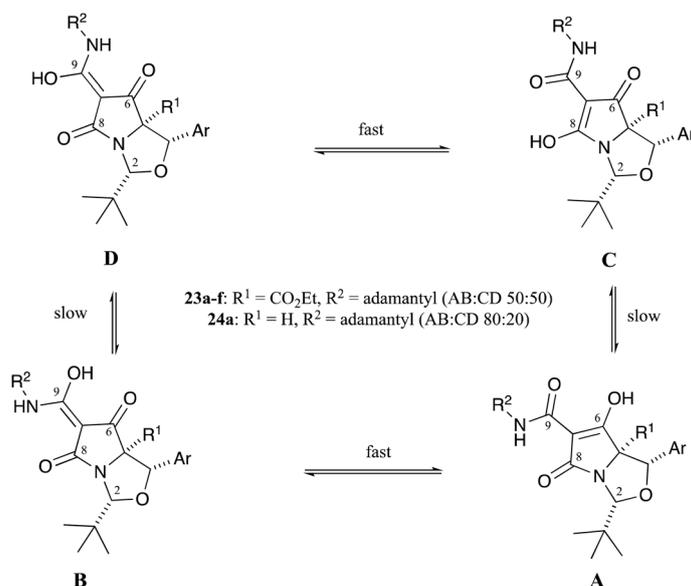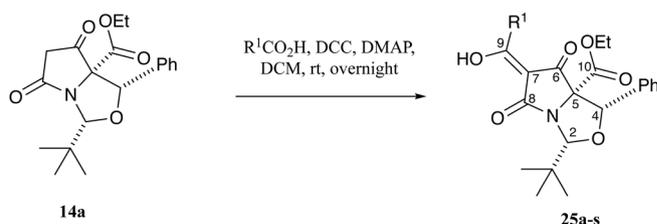**Fig. 4** Tautomeric equilibria in carboxamidotetramates.

23a–f: R¹ = CO₂Et, R² = adamantyl (AB:CD 50:50)
24a: R¹ = H, R² = adamantyl (AB:CD 80:20)

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25a: R^1 = Ph (32%)
25b: R^1 = p-Me-C$_6$H$_4$ (26%)
25c: R^1 = p-OMe-C$_6$H$_4$ (18%)
25d: R^1 = p-Cl-C$_6$H$_4$ (39%)
25e: R^1 = p-Br-C$_6$H$_4$ (35%)
25f: R^1 = p-I-C$_6$H$_4$ (37%)
25g: R^1 = p-NO$_2$-C$_6$H$_4$ (30%)
25h: R^1 = p-CF$_3$-C$_6$H$_4$ (41%)
25i: R^1 = m-OMe-C$_6$H$_4$ (44%)
25j: R^1 = o-Br-C$_6$H$_4$ (12%)

25k: R^1 = m,m-OMe-C$_6$H$_3$ (17%)
25l: R^1 = 2-thienyl (13%)
25m: R^1 = CH$_2$Br (37%)
25n: R^1 = c-C$_6$H$_{11}$ (57%)
25o: R^1 = CH$_2$-c-C$_6$H$_{11}$ (44%)
25p: R^1 = CH$_2$-adamantyl (57%)
25q: R^1 = Me (51%)
25r: R^1 = C$_5$H$_{11}$ (47%)
25s: R^1 = C$_3$H$_6$CH=CH$_2$ (41%)

Scheme 4 Synthetic route to C-7 acyl tetramates.

25q: R^1 = Ph, R^2 = Et   C6: 188.3 ppm; C8: 179.9 ppm
                                   C9: 188.5 ppm; C10: 165.3 ppm
26: R^1 = H, R^2 = Me   C6: 188.0 ppm; C8: 179.8 ppm
                                   C9: 188.4 ppm; C10: 167.4 ppm

25q: R^1 = Ph, R^2 = Et   C6: 195.8 ppm; C8: 172.6 ppm
                                   C9: 190.7 ppm; C10: 165.1 ppm
26: R^1 = H, R^2 = Me   C6: 195.5 ppm; C8: 172.3 ppm
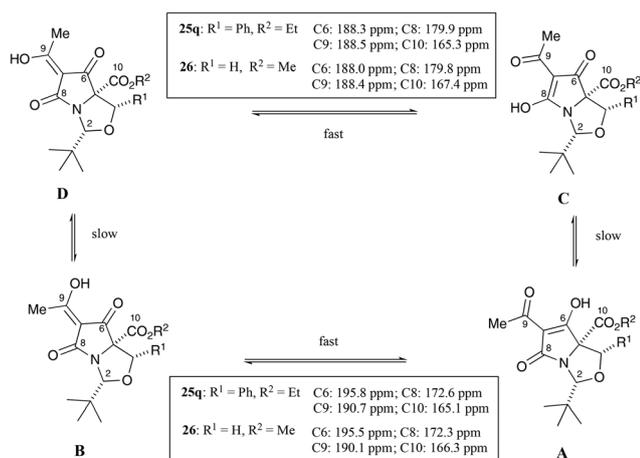   C9: 190.1 ppm; C10: 166.3 ppm

Fig. 5 Tautomeric equilibria in acyltetramates.

with the exception of **5a** – had to be synthesised. Secondly, a sizeable amount of Pd–C was required to convert the oximes **6a–f** to *allo*-phenylserines **7a–f**, and thirdly, the yields were modest. Finally, chloro- and bromo-substituted *allo*-phenylserine derivatives further reacted by hydrogenolysis under the reduction conditions to give dehalogenated side-products. An alternative, based upon a report by Chaumont *et al.*[27] of the *erythro*-diasteroselective synthesis of Boc-protected *allo*-phenylserine derivatives *via* a decarboxylative aldol reaction between the hemi-malonate derivative of diethyl (Boc-amino)malonate **27c** and various aldehydes (Scheme 5),[27] was therefore examined; starting **27c** could be readily synthesised by reacting inexpensive diethyl aminomalonate hydrochloride **27a** with Boc$_2$O. Boc-protected *allo*-phenylserine **9a** synthesised this way was fully identical to that both prepared by Chaumont *et al.*[27] and the material prepared earlier (Scheme 1). This approach proved to be applicable to a wide variety of substituted *allo*-phenylserine derivatives **9a–m** (Scheme 5 and Table S11, ESI†).

Boc-protected-*allo*-phenylserine derivatives **9a–l** were deprotected using TFA and were stirred at room temperature for 2 h to furnish *allo*-phenylserines **28a–k** as the trifluoroacetate ammonium salts in quantitative yields (Scheme 5 and Table S12 ESI†). The exception was **9l**, which gave a complex mixture of side-products, and was thus not used further, and **9m** was discontinued due to its low yield. The *allo*-phenylserine derivatives **28a–k** were then reacted with pivaldehyde using the established protocol outlined above, to obtain oxazolidines
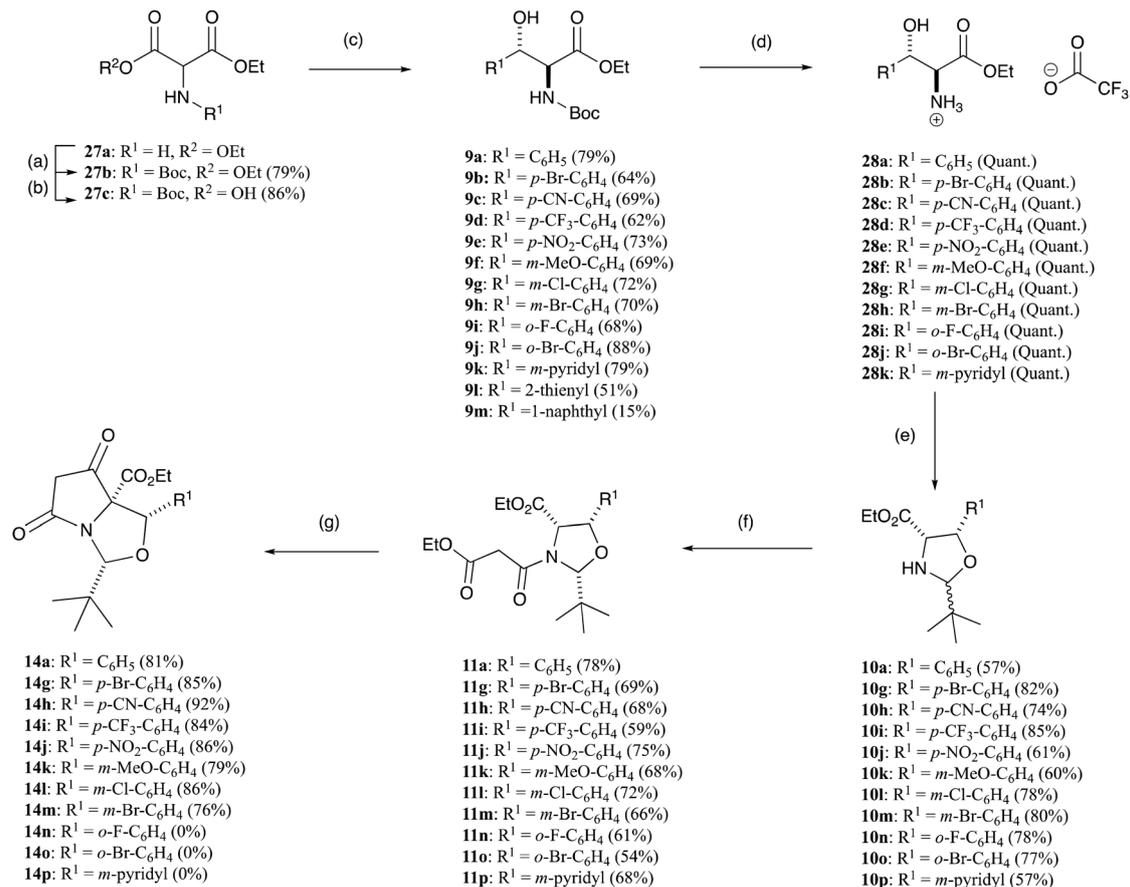
10a,g–p in good yields as mixtures of diastereomers (Scheme 5 and Table S13, ESI†). The spectroscopic consistencies of oxazolidines **10a,g–p** with those of **10a–f** derived from β-oxoesters **5a–f** prepared above confirmed their *cis*-relationship (see Tables S5 and S13, ESI†). The major and minor diastereomers of oxazolidines **10g–p** were readily assigned by comparison to the independently prepared and characterised materials described above. These compounds were then *N*-acylated with ethyl malonyl chloride using the established protocol to obtain malonamides **11a,g–p** in good yields as predominantly one diastereomer (Scheme 5 and Table S14, ESI†), again consistent with that of malonamides **11a–f**. Comparison to earlier compounds and NOESY studies confirmed their relative stereochemistry. These malonamides **11a,g–p** were then reacted with DBU in THF overnight (Scheme 5) to form tetramates **14a,g–m** in good yields (Table S15, ESI†). However, surprisingly, tetramate **14n** was formed as a very minor product, and was not used further, and tetramate **14o** did not form at all, presumably due to hindrance at the *ortho*-position. Additionally, the Dieckmann cyclisation of the 3-pyridyl substituent **11p** was not successful.

Evaluation of antibacterial activity

While C7-unfunctionalised tetramates have no intrinsic antibacterial activity against Gram (+) and Gram (−) bacteria,[28] compounds **14a–f** showed appreciable activity against MRSA, with MIC values ranging between 7.8–15.6 µg mL^{-1}, whereas **14g** and **14k** was more weakly acting with MIC of 31 µg mL^{-1} (Table 1 and Table S16, ESI†). However, tetramates **14h–j** were much less active and had activity ranging between 125–250 µg mL^{-1}, clearly indicating that substitution around the C4-aromatic ring system imparts antibacterial activity. However, no activity was observed against *E. coli*, a feature commonly observed with such tetramates, and which is likely to arise due to poor bacterial cell membrane permeability and/or efficient efflux. Surprisingly, C7-acyltetramates **25** – which were more analogous to natural product antibacterial tetramates – were relatively inactive against both MRSA and *E. coli* (Table S16, ESI†).

However, C7-carboxamides **23a–f** displayed potent antibacterial activities against MRSA, similar to previously reported C7-carboxamides,[12,21] and of interest was that a C5-ethyl ester group was well tolerated, particularly when compared with *threo*-derived C7-carboxamide **24a**,[12] although none exhibited antibacterial activity against *E. coli*. The antibacterial activity

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Scheme 5 Expanded functionalisation of C4-aromatic tetramates **14a,g–m** via the decarboxylative aldol reactions of hemimalonate **27c**; (a) Boc$_2$O, 1 M NaOH (aq.), 1,4-dioxane, rt, overnight; (b) KOH, EtOH, rt, overnight; (c) R^{1}CHO, Et$_3$N, 50 °C, 2 d; (d) TFA, DCM, rt, 2 h; (e) petroleum ether 40 : 60, pivaldehyde, Et$_3$N, >100 °C, Dean–Stark, overnight; (f) ethyl malonyl chloride, pyridine, DCM, reflux, overnight; (g) DBU, THF, rt, overnight.

Table 1 Biological activity (antibacterial activity and cytotoxicity) for selected tetramates

Compound	MIC$_{(S. aureus)}$ (µg mL^{-1})	Cell lines EC$_{50}$ (µg mL^{-1})				Therapeutic ratio[a]
		HeLa	heK-293	CaCo	MDCK	
14a	15.6	15.6	31.3	62.5	31.3	1
14b	7.8	15.6	15.6	31.3	31.3	2
14c	15.6	7.8	3.9	31.3	15.6	0.5
14d	15.6	15.6	31.3	62.5	31.3	1
14e	15.6	15.6	31.3	62.5	31.3	1
14f	15.6	15.6	15.6	31.3	31.3	1
23a	0.25	15.6	15.6	31.3	31.3	62
23b	0.25	15.6	15.6	31.3	31.3	62
23c	3.9	15.6	31.3	31.3	62.5	4
23d	0.25	7.8	15.6	7.8	31.3	31
23e	0.25	7.8	15.6	15.6	31.3	31
23f	0.25	7.8	7.8	15.6	15.6	31

[a] Calculated from MIC against *S. aureus* using the HeLa cell line (EC$_{50}$ HeLa cell line/MIC$_{(S. aureus)}$).

against MRSA for C7-carboxamides **23a,b,d–f** was extremely potent – MIC values of 0.25 µg mL^{-1} – with the exception of tetramate **23c**, which had a MIC value of 3.9 µg mL^{-1} (Table 1), suggesting that hydrophobic groups at the *para*-position worsen bioactivity. The physicochemical property space of the most potent compounds (MIC < 4 µg mL^{-1}) was confined to a small area of chemical structure space (523 < M_w < 579 g mol^{-1}; 3.9 < clog$P$ < 5.9; 105 < PSA < 114 Å^2; 777 < MSA < 898 Å^2; HBD = 2; HBA = 5, see Fig. 6 and Table S16, ESI†), with many compliant with the Ro5 – even though the initial design
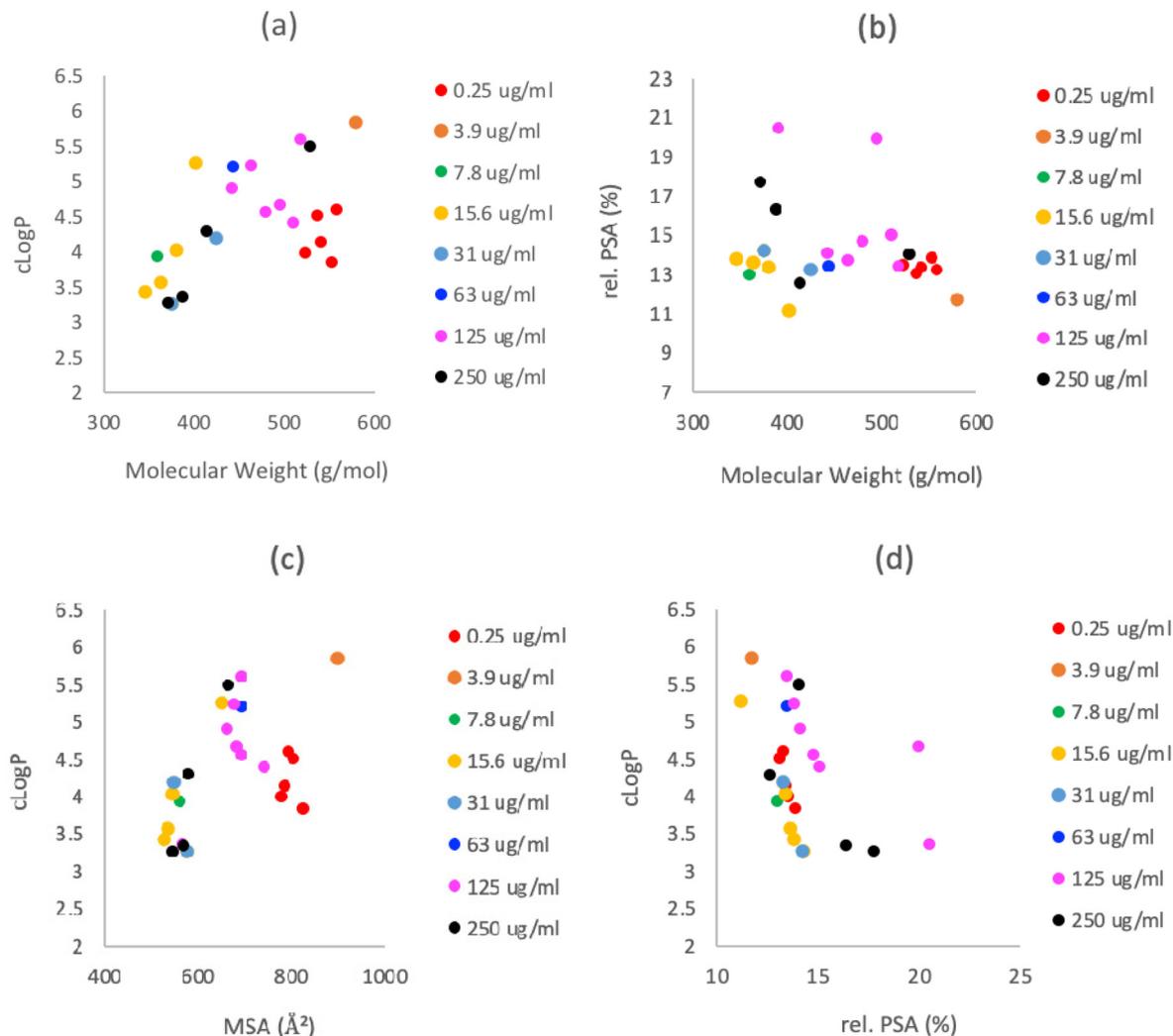
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Fig. 6 Physicochemical property space of tetramates; (a) clog P plotted against M_w; (b) rel. PSA plotted against M_w; (c) clog P plotted against MSA and (d) clog P plotted against rel. PSA. clog P, MSA and PSA were calculated using Marvin (19.9.0), 2019, ChemAxon.

of these compounds did not explicitly seek to obey Lipinski's parameters. The high clog P values of many of these may also explain, at least in part, the lack of activity observed against Gram (−) *E. coli* bacteria. These bicyclic tetramates were significantly more lipophilic and less polar than other antibiotic classes (Fig. S5, ESI†) and the structure–activity profile indicates distinct regions of the bicyclic nucleus responsible for antibacterial activity and/or metal chelation (Fig. 7).

This compound series showed some cytotoxicity against HeLa, heK-293, CaCo and MDCK human cell lines, and the therapeutic ratio (calculated from MIC against *S. aureus* using the HeLa cell line (EC$_{50}$ $_{HeLa \; cell \; line}$/MIC$_{(S. \; aureus)}$) (Table 1) shows that C5-ethyl ester tetramates **14a–f** possessed low selectivity, although this was mainly due to the lack of potency observed against MRSA. However, carboxamides **23a,b,d–f** showed high selectivity for bacteria, with a therapeutic ratio of either 31 or 62. Panduwawala *et al.*[4] and Jeong *et al.*[21] had earlier demonstrated that carboxamidotetramic acids typically
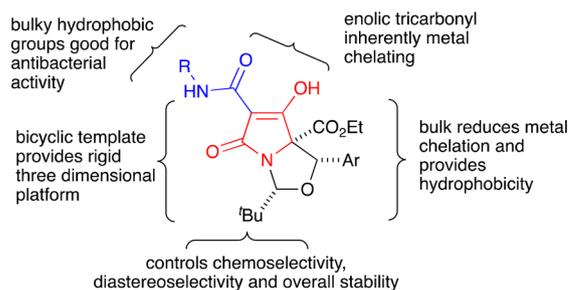

Fig. 7 Overview of the chemical components necessary for potent antibacterial activity against MRSA, derived from an in-depth SAR analysis of the *N,O*-bicyclic tetramates.

lost their antibacterial activity in the presence of human serum albumin (HSA). When bicyclic tetramates **23a–f** were tested against MRSA in the presence of 1/3 the serum albumin concentration as that found in human blood, for carboxamides

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23a,b, **23d–f** the activity dropped 16-fold, and in the case of **23e**, 33-fold. However, compound **23c** only showed a 4-fold decrease in antibacterial activity and noteworthy is that it is the most lipophilic in the carboxamide series **23a–f**. It was clear from these results that MIC shifted to higher values in the presence of HSA, consistent with the known behaviour of plasma protein binding small molecules; it has earlier been shown that PPB strongly correlates with increased hydrophobicity.[29] However, good bioactivity (MIC < 4 µg mL^{-1}) is still observed in the presence of 1/3 the HSA concentration as that found in blood, and this result is important, indicating that the aromatic substitution placed at the C4-position of the bicyclic tetramate could not only be tolerated, but could also be crucial to adjusting the physicochemical properties of these bicyclic tetramates to lend itself towards potential *in vivo* studies and therapeutic applications.

Conclusion

It has been demonstrated that oxazolidines derived from *allo*-phenylserines efficiently close by Dieckmann ring closure giving tetramates with full diastereocontrol; the sense of this closure follows analogous reactions of serine, threonine and cysteine but is different to *threo*-phenylserines. Importantly, the oxazolidine ring substituents have been shown to reduce metal cation chelation by steric interaction. C7-unfunctionalised tetramates **14a–f** had appreciable activity against MRSA, and conversion to C7-carboxamides **23** led to much improved antibacterial activity. A large, bulky, hydrophobic carboxamide pendant group at the C7-position led to more potent antibacterial activity. All the compounds screened showed no antibacterial activity against *E. coli*. C7-adamantylcarboxamidotetramic acids **23a–f** were very selective for bacteria over mammalian cells. Many of the compounds synthesised were within the constraints of Ro5. These compounds occupied a distinct chemical property space different from that occupied by other known antibiotics, with the most potent compounds occupying 523 < M_w < 579 g mol^{-1}; 3.9 < clog P < 5.9; 11.7 < rel-PSA < 13.9%. Unfortunately, these compounds suffered from PPB, leading to the MIC values being shifted to higher concentrations when tested in the presence of HSA. From a consideration of physicochemical properties, potency and toxicity of these compounds, the most promising candidates for further optimisation were **23a,b,d–f**.

Conflicts of interest

There are no conflicts to declare.

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