

## Synthetic Access to Hydrophilic Tetramate Derivatives of Cysteine

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### Abstract

The synthesis, structural and antibacterial evaluation of bicyclic tetramate derivatives of cysteine rendered hydrophilic with pendant heterocyclic substituents is reported; effective synthetic protocols and antibacterial activity for a small library of polar derivatives was found, and direct evidence for strong metal chelation in these systems was obtained. A computational study has developed a detailed understanding of the controlling factors of the key Dieckmann cyclisation step.

### Introduction

The emergence of antimicrobial resistance (AMR) leading to the loss of efficacy of clinically relevant antibiotics, particularly in the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species),<sup>1</sup> has clearly demonstrated that new antibacterial small molecule entities are urgently needed.<sup>2-4</sup> Antibacterial natural products have traditionally provided a valuable and

accessible start point for drug discovery,<sup>5-9</sup> and our interest in this area has focussed on tetramate-containing systems, since such natural products may exhibit wide ranging antibacterial activity coupled with low levels of toxicity.<sup>10-13</sup> New synthetic routes to these compounds continue to be developed.<sup>10, 14-17</sup> We have previously reported detailed investigations of bicyclic tetramates which have both demonstrated their ease of synthesis and potent antibacterial activity, at least for Gram positive systems,<sup>18</sup> and more recently in particular that cysteine-derived tetramate analogues with functionalisation at C-2 and C-7 are highly effective (Figure 1);<sup>19, 20</sup> however, one key limitation of this and much of our earlier work has been the reliance on a *t*-butyl ring substituent which has been required for good chemical stability of intermediates, since only limited alternatives appear to be tolerated, and especially for *O,N*-systems.<sup>18</sup> However, the better stability of *S,N*-systems offered the possibility to move away from such highly hydrophobic substituents, but even in these cases, *in vitro* activities diminished when tested in presence of blood, suggesting that plasma protein binding might be impacting upon free blood concentration and hence potency.<sup>19</sup> Hansch noted in 1987 that “Without convincing evidence to the contrary, drugs should be made as hydrophilic as possible without loss of efficacy.”<sup>21</sup> With this in mind, we sought to increase compound polarity by incorporation of heterocyclic rings at C-2 and C-7 amide pendant positions of the tetramate core **1** (Figure 1), which was expected also to correlate with a decrease in lipophilicity and an increase in aqueous solubility. In particular, C-2 *t*-butyl or phenyl groups (*R*<sup>1</sup>) present in previously synthesised tetramate analogues were replaced with pyridine rings (*R*<sup>1</sup>), and C-7 amide pendants (*R*<sup>2</sup>) were 4-chloro-2-methyl-benzyl, cyclohexyl, adamantyl and polar tetrahydropyranyl groups.

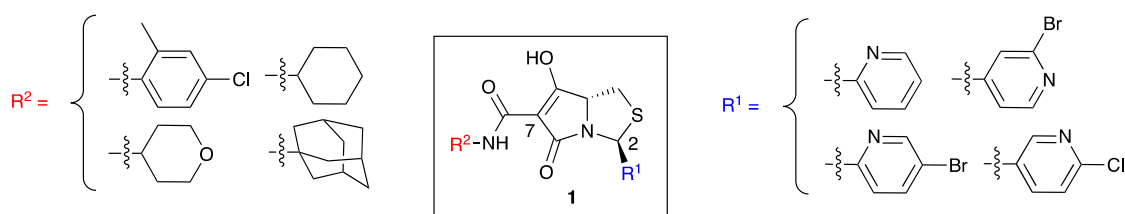


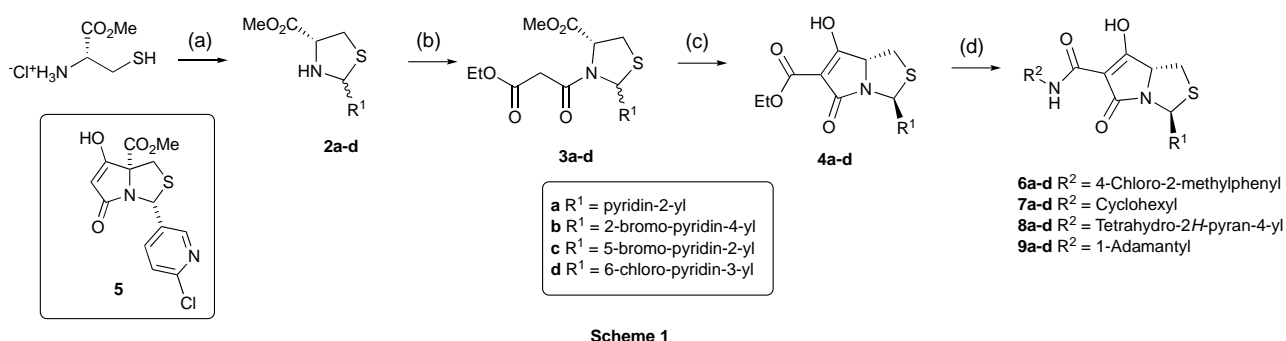
Figure 1

**Figure 1.** Tetramate analogues

## Results and Discussion

We have earlier reported a synthetic pathway leading to highly functionalised bicyclic tetramic acid derivatives with high enantiopurity, starting from L-serine, L-threonine and L-cysteine,<sup>18</sup> beginning with a method developed by Seebach and co-workers,<sup>22, 23</sup> in which methyl esters of respective amino acids were condensed with pivaldehyde (*t*-BuCHO) to yield oxazolidines or thiazolidines as a mixture of *cis/trans*-2,5 diastereomers. This was followed by *N*-acylation and Dieckmann cyclisation to give bicyclic tetramates (Scheme 1,  $R^1 = t\text{-Bu}$ ); the chemo-, diastereo- and enantioselectivity of this process is controlled by the bulky C-2 *t*-butyl group.<sup>18</sup> Although there was some reported precedent of the synthesis of highly functionalised bicyclic lactam systems with *endo*-substituents, they were found to be much less stable than the corresponding *exo*-substituents.<sup>24, 25</sup> This route of cyclisation was favoured even though the reaction proceeded *via* a relatively unstable enolate formation. It was later shown that this sequence could be run with isobutyraldehyde or substituted benzaldehydes in both L-serine-derived oxazolidine systems and L-cysteine-derived thiazolidine systems, and while the oxazolidine systems were found not to be as stable, thiazolidine systems derived from L-cysteine and substituted benzaldehydes allowed greater variations at C-2 without compromising the heterocyclic stability (Scheme 1,  $R^1 = \text{Ar}$ ).<sup>20</sup> Noteworthy was that this substitution modified the chemoselectivity of the Dieckmann cyclisation to favour the formation of tetramate C-5 esters, allowing for convenient late-stage functionalisation to tetramate carboxamides by transamidation reactions.<sup>19, 20</sup> With this in mind, the immediate question was whether this approach could be extended to heterocyclic aldehydes

(Scheme 1, R<sup>1</sup>= Het), allowing incorporation of heterocycles at both C-2 and C-7, generating the target cysteine-derived tetramate library to increase molecular polarity.



**Scheme 1.** Synthesis of tetramate analogues from L-cysteine methyl ester HCl. *Reagents and conditions:* (a) R<sup>1</sup>CHO, Et<sub>3</sub>N, petrol 40/60, reflux under Dean-Stark conditions, 18 h; (b) mono-ethyl malonate, DCC, DMAP, DCM, 0 °C to r.t., 18 h; (c) KO<sup>t</sup>Bu, THF, reflux, 4 h; (d) R<sup>2</sup>NH<sub>2</sub>, THF/toluene (1 : 4), reflux, 18 h.

Condensation of L-cysteine methyl ester hydrochloride with four different pyridine-carboxaldehydes gave the corresponding stable thiazolidines **2a-d** in good yield (75 - 86%, Scheme 1 and Table 1, SI) as a mixture of *cis*- and *trans*-diastereomers in approximately equal ratio. They were readily distinguished by a difference in the chemical shifts of H-2 and H-5, with that of the *trans*-isomer being more downfield than the *cis*-isomer (Table 1, SI), consistent with that previously found in thiazolidines with C-2 substituted aromatic rings.<sup>20</sup> The assignment of stereochemistry was further confirmed by NOE analysis, where the presence of an enhancement between H-5 proton and an aromatic proton suggested *trans*-stereochemistry and an enhancement between H-5 and H-2 protons suggested *cis*-stereochemistry (Figure 1, SI).

Thiazolidines **2a-d**, as a diastereomeric mixture, were converted by DCC coupling with mono-ethyl malonate to the corresponding *N*-acylthiazolidines **3a-d** in high yield (77 - 92%,

Scheme 1 and Table 2, SI) with a *cis/trans* ratio favouring the former, as has been found previously.<sup>20</sup>

Similar to thiazolidines **2a-d**, characteristic H-2 chemical shifts of the acylated products **3a-d** were observed, with each diastereomer appearing as a rotameric pair. For **3a**, the *cis*- and *trans*-diastereomers could be separated by *flash* column chromatography, and the assignment of relative stereochemistry was supported by 1D NOE analysis (Figure 1, SI). Although 2D NOESY experiments were not sensitive enough to detect weak nOe effects in this case, they nonetheless also confirmed the presence of rotamers around the amide bond in the *N*-acylthiazolidine series (Figure 2); thus, between the pair of rotamers from the same diastereomer, there were reproducible and dynamic proton exchanges, which could be distinguished from NOE signals in the 2D NOESY spectrum (Figure 2, SI). In 2D NOESY spectra of small molecules ( $MW \leq 500$ ) in low viscosity solvent (e.g.  $CDCl_3$ ), peaks arising from chemical exchange processes have the same sign as the diagonal (red) while NOE cross peaks (blue) are of opposite sign.<sup>26</sup> Cross peaks (6.48, 6.20) or (6.20, 6.48) of H-2 chemical shifts, which were of the same sign as the diagonal peaks, indicated that they arose from dynamic chemical exchange and hence supported that these chemical shifts belonged to a pair of rotamers arising from the same diastereomer.

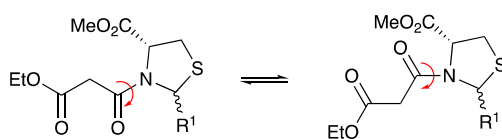


Figure 3

**Figure 2.** Rotameric behaviour of *N*-acylthiazolidines from amide bond rotation.

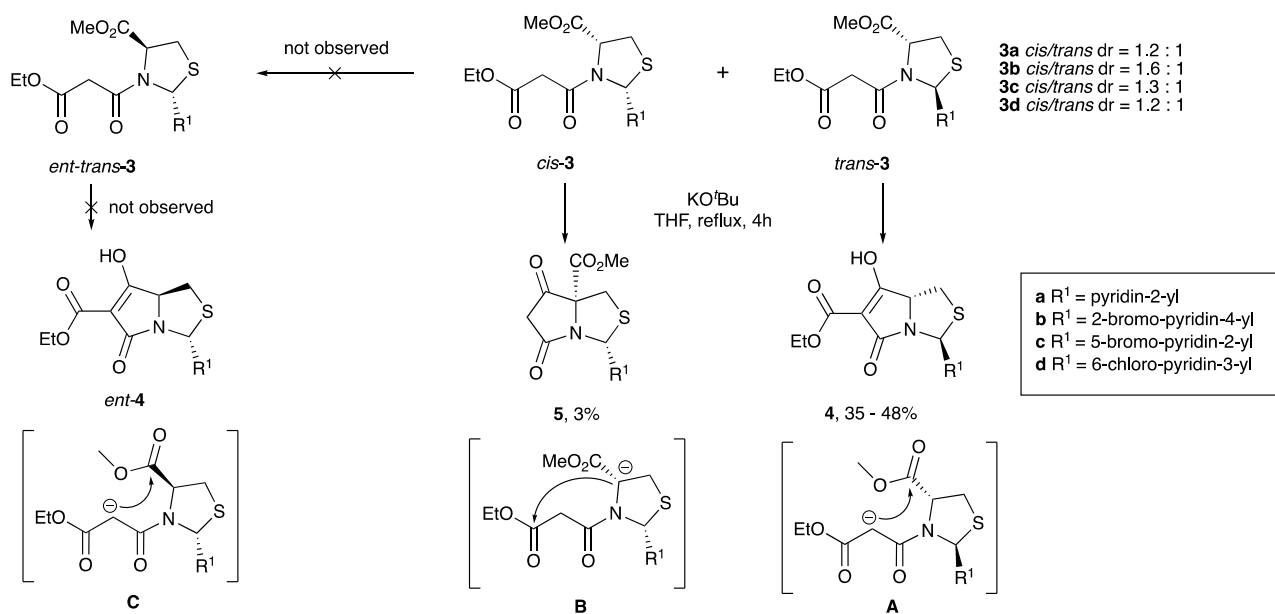
Moreover, it was observed that for the major and minor rotamers of *N*-acylthiazolidines **3a-d**, the H-2 chemical shift of the *trans*-isomer was invariably more upfield than that of the *cis*-isomer (Table 2, SI), which was opposite to that observed in the thiazolidine series (Table 1, SI). The change in the *cis/trans* ratio from the starting thiazolidines **2a-d** to *N*-acylthiazolidines **3a-d**

suggested an interconversion between the two diastereomers during the course of acylation, with the equilibrium leading to a preferred formation of the *cis*-malonylthiazolidines, and allowing an all-equatorial arrangement around the heterocyclic ring; this type of behaviour has been observed previously in C-2 *t*-butyl and isopropyl series.<sup>18</sup>

Dieckmann cyclisation of *N*-acylthiazolidines **3a-d** with KO<sup>t</sup>Bu successfully gave stable bicyclic tetramate ester products **4a-d** in 35-48% yield, which existed as the enol tautomer as observed by <sup>1</sup>H NMR spectroscopy, and with characteristic H-2 and H-5 chemical shift values for the bicyclic ring system consistent to those previously reported in thiazolidine-derived tetramate esters with C-2 substituted aromatic rings (Table 3, SI).<sup>20</sup> These were formed as single diastereomers with a *trans* relationship between H-2 and H-5 across the bicyclic ring systems, which was confirmed by NOE analysis for **4a** (Figure 1, SI). On the basis of the consistent and characteristic H-2 chemical shifts, the same stereochemistry was assigned for other tetramate esters **4b-d** in the series.

The predominant pathway of cyclisation starting from the *trans*-malonylthiazolidines, *trans*-**3a-d** to tetramates **4a-d** suggested that the reaction proceeded preferentially by closure of the side chain malonamide enolate onto the C-5 ester (Scheme 2, type **A**), which also placed the C-2 heterocycles on the less hindered *exo*-face of **4**. This preferred route of ring closure was consistent with that previously observed in thiazolidine derived systems with C-2 aromatic rings.<sup>20</sup> The alternatively cyclised product **5** (Scheme 1) was detected by mass spectrometry from the reaction of **3d** in 3% yield, the NOE analysis of which suggested a relative *cis*-stereochemistry between C-2 substituent and C-5 methyl ester (Figure 1, SI); in this case, cyclisation starting from *cis*-**3d** proceeded by closure of the less stable C-5 enolate onto the ethyl ester (type **B**, Scheme 2), which preferentially placed the C-2 group on the *exo*-face. This minor route of cyclisation was also reported previously in thiazolidine derived systems with C-2 aromatic rings to occur in < 1% yield.<sup>20</sup> Furthermore, a further Dieckmann cyclisation could occur after epimerisation at C-5 of *cis*-

**3** under the basic conditions of the reaction giving *ent-trans*-**3**. Since the *cis*- and *trans*-diastereomers of malonamides **3b-d** could not be easily separated and the mixture was used for the subsequent Dieckmann cyclisation, this simultaneous epimerisation and cyclisation gave concerns about erosion of enantiopurity for the products. In the case of **3a**, the two diastereomers were able to be separated by *flash* column chromatography and each was used for separate cyclisation. Reaction of malonamide *trans*-**3a** with KO<sup>t</sup>Bu successfully gave the cyclised product **4a** as expected. However, attempted cyclisation of *cis*-**3a** only gave degraded product, indicating that cyclisation after epimerisation of *cis*-**3a** was not observed (cyclisation via type **C** enolate, Scheme 2).



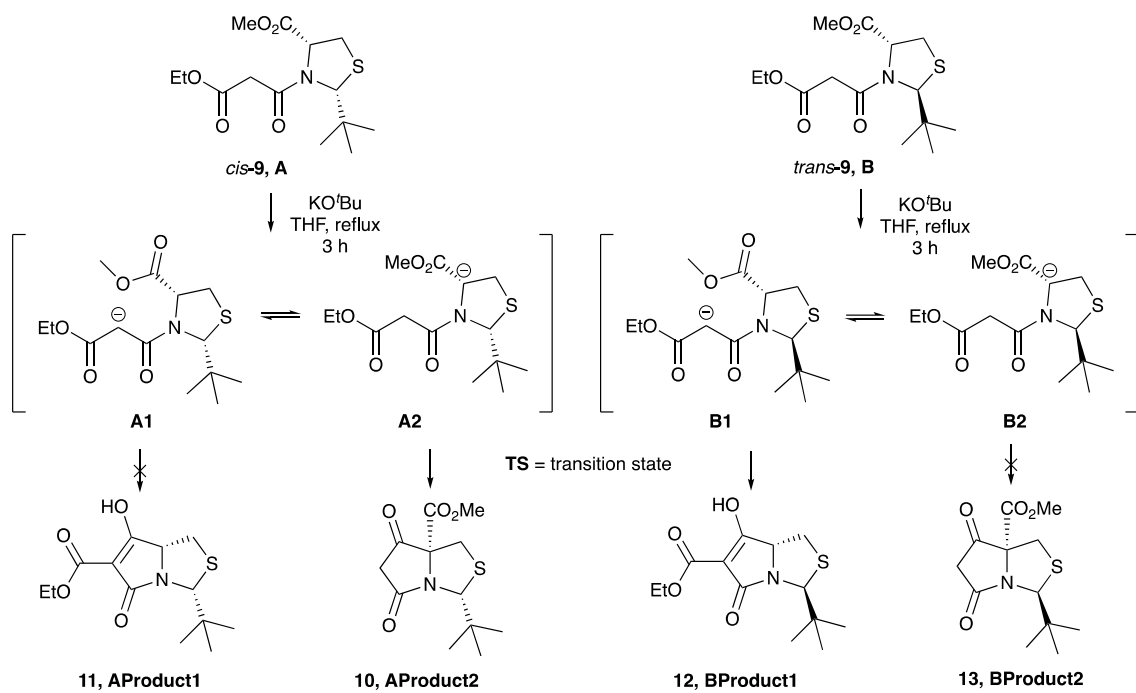
Scheme 2

**Scheme 2.** Synthesis of thiazolidine-derived tetramates with C-2 pyridine rings.

In order to understand the switch of chemoselectivity of Dieckmann cyclisation after substituent change at C-2 from a bulky *t*-butyl or isopropyl group to a planar aromatic system (substituted phenyl rings or heterocycles), density-functional theory (DFT) calculations were carried out to model the energy profile of the cyclisation process in thiazolidine systems with C-2 *t*-butyl group and phenyl ring. To simplify the model, the calculations assumed that the various

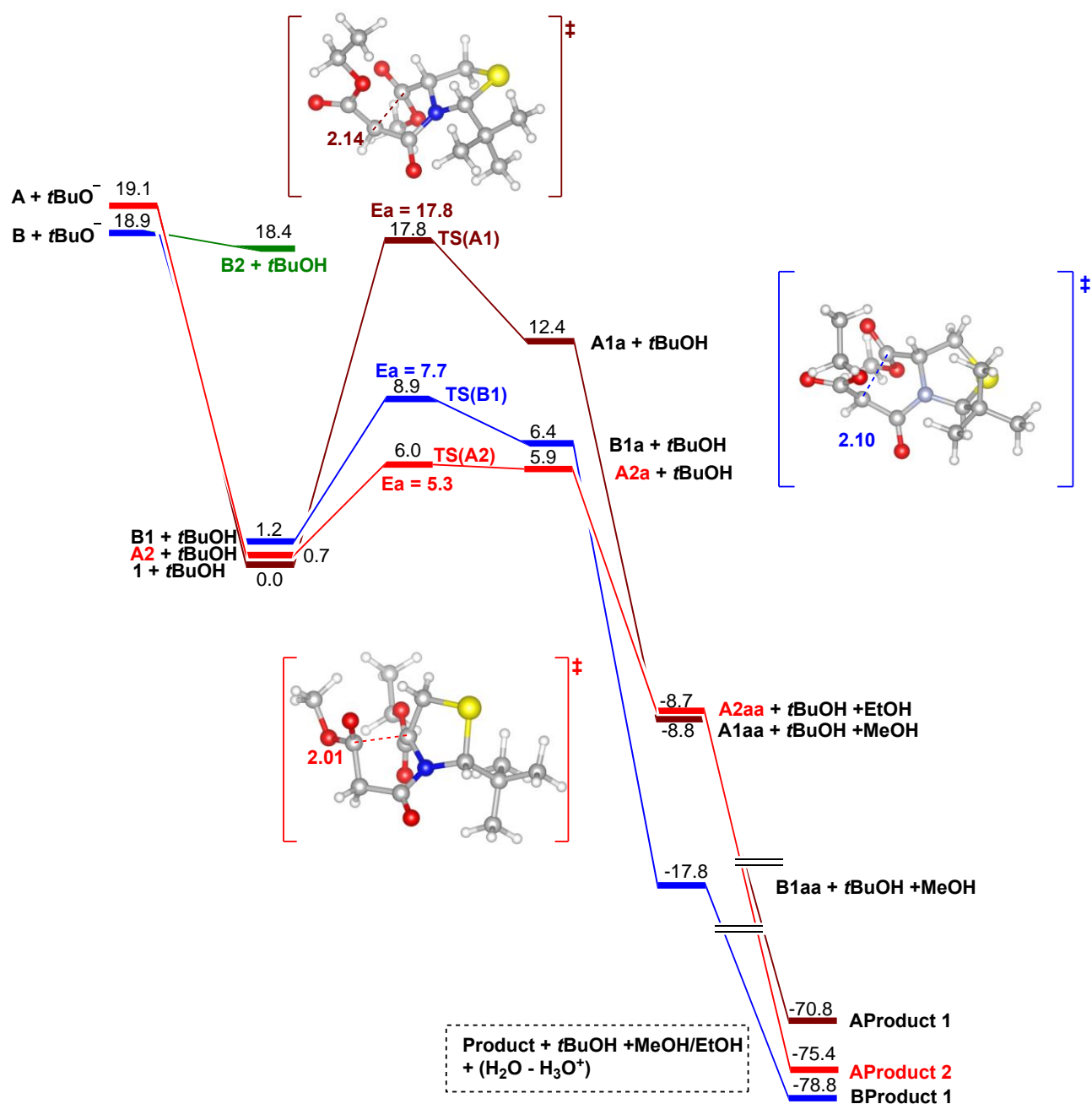
enolates were in free equilibrium (Scheme 3 and Figure 3). In the cyclisation of *N*-acylthiazolidines **9** with a C-2 *t*-butyl group, the major C-5 methyl ester tetramate product **10 (AProduct2)**, 57% yield) formed from *cis*-**9 (A)** following the energy pathway with the lowest activation energy ( $E_a = 5.3$  kcal/mol). The alternative pathway from the same starting material **9** to **11 (AProduct1)**, which placed the C-2 *t*-butyl group onto the *endo*-face of the bicyclic ring, required a much higher activation energy ( $E_a = 17.8$  kcal/mol). The significant difference in these activation energies ( $\Delta E_a = 12.5$  kcal/mol) strongly kinetically favoured the pathway leading to **10 (AProduct2)**, which was also the more stable product (minimum energy =  $-75.4$  kcal/mol) as compared to **11 (AProduct1)** (minimum energy =  $-70.8$  kcal/mol). The other tetramate product **12 (BProduct1)** from *trans*-**9** was not observed because the previous step of *N*-acylation resulted in an exclusive formation of *cis*-**9**. Given that **12 (BProduct1)** possessed the lowest energy (minimum energy =  $-78.8$  kcal/mol) and that the activation energy ( $E_a = 7.7$  kcal/mol) was not significantly higher ( $E_a = 7.7$  kcal/mol), **12 (BProduct1)** could potentially form if the *trans*-**9** had been present; the formation of an analogous product was indeed observed in related work, but only with 4% yield.<sup>27</sup> This model suggested that for malonamides with C-2 *t*-butyl group, the Dieckmann cyclisation operated under strong kinetic control.





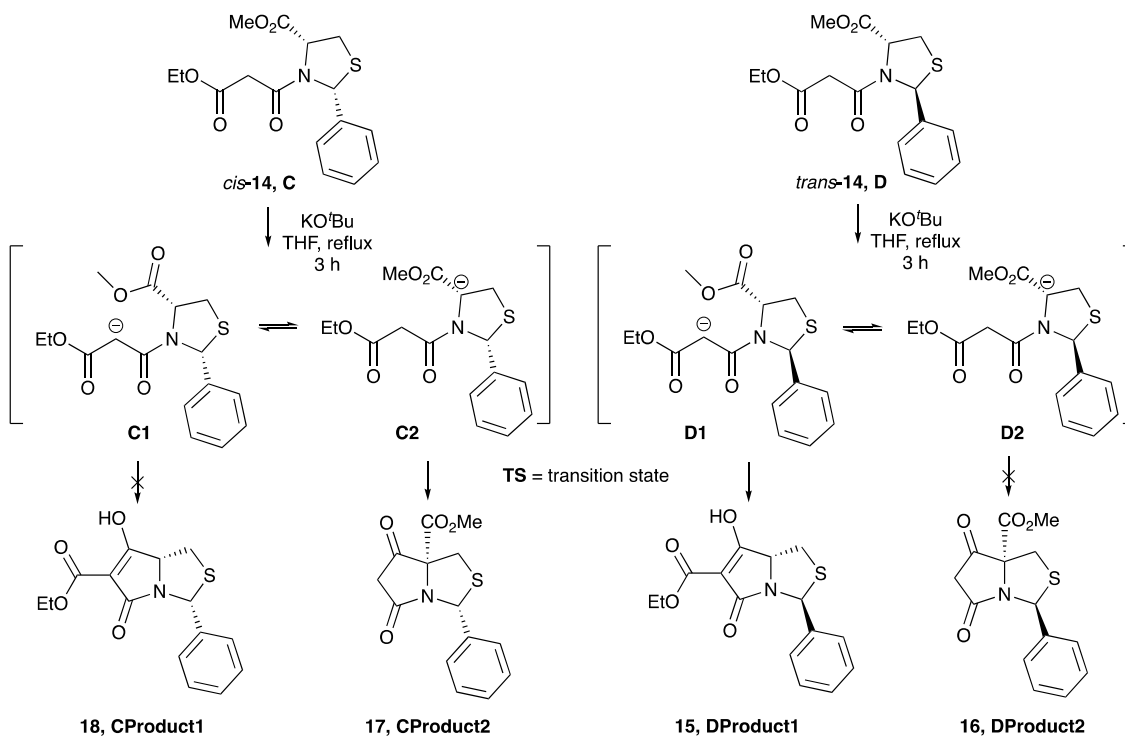
Scheme 3

**Scheme 3.** Cyclisation pathway of malonamides **9**.



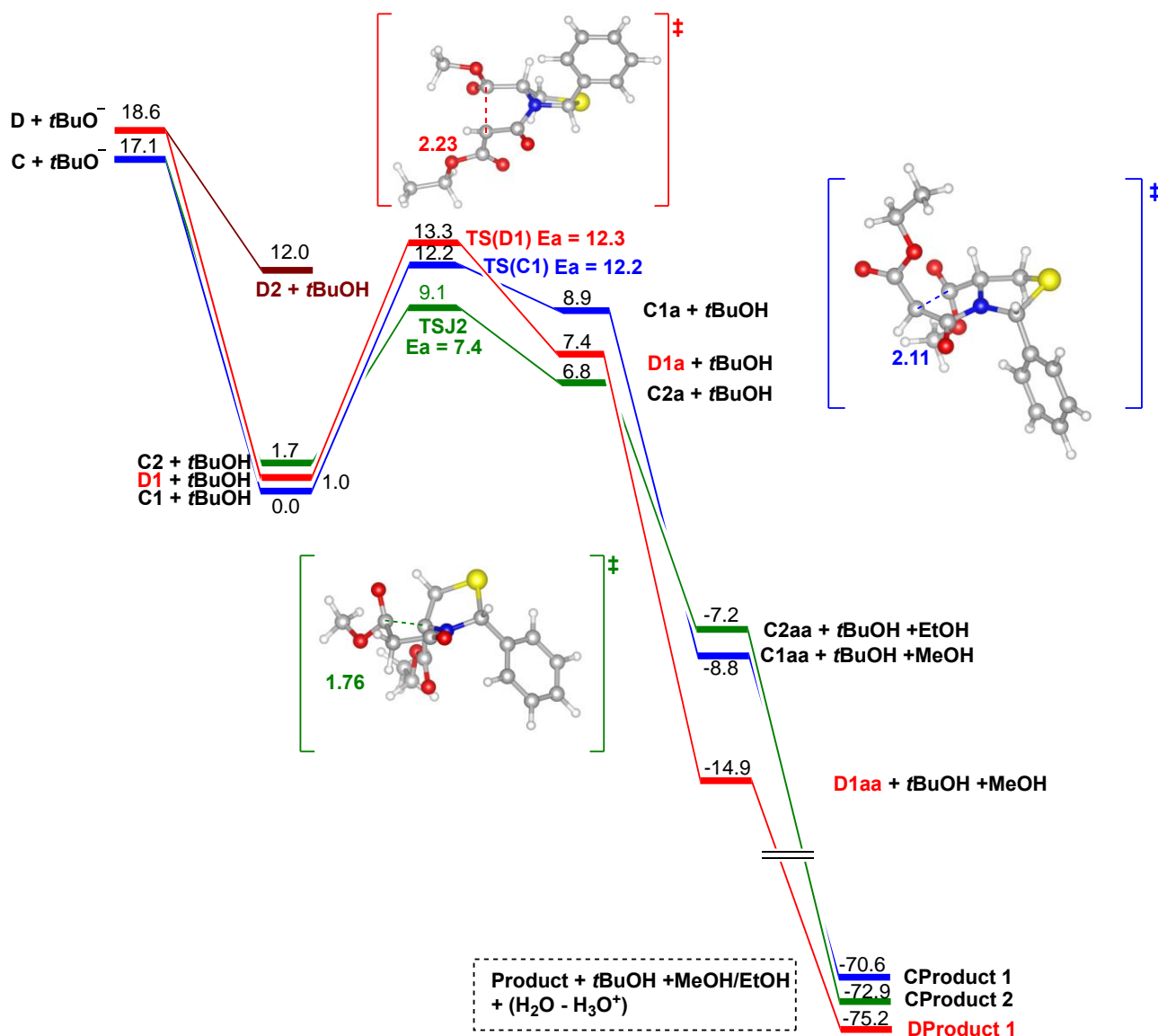
**Figure 3.** Reaction energy profile of Dieckmann cyclisation for malonamides of C-2 *t*-butyl group. Calculated potential energy surfaces at the B3LYP/6-311++G (d,p) level of theory with SMD solvation (THF). All energies are reported in kcal/mol and referenced to the lowest energy carbanion generated after initial deprotonation. Labels: A=*cis*-9, B=*trans*-9, AProduct1=11, AProduct2=10, BProduct1=12, BProduct2=13; TS = Transition State (see Scheme 3). Energy values in kcal/mol.

By comparison, tetramate ester **15 (DProduct1)** was formed as the major product (43% yield) in the cyclisation of *cis*- and *trans*-malonylthiazolidines **14** with C-2 phenyl group (Scheme 4 and Figure 4), the pathway starting from *trans*-**14 (D)**, with the more stable side chain enolate intermediate (**D1**) closing onto the C-5 ester. Product **15 (DProduct1)** appeared to be the thermodynamic product of this process with minimum energy at -75.2 kcal/mol, although the difference in energy between the alternatives **18 (CProduct1)** and **17 (CProduct2)** was small. The pathway from *cis*-**14 (C)** favoured the formation of **17 (CProduct2)** with the least activation energy ( $E_a = 7.4$  kcal/mol) of all represented pathways and a smaller minimum energy as compared to **18 (CProduct1)**. The reason why **17 (CProduct1)** was only observed in < 1% yield from a starting mixture enriched in *cis*-**14 (J)** (*cis/trans* = 1.7 : 1)<sup>20</sup> could not be explained by this model. In contrast to the C-2 *t*-butyl series, Dieckmann cyclisation for malonamides with C-2 aromatic rings appeared to be no longer under a strong kinetic control; thermodynamic control appeared to account for the observed chemoselectivity in the reaction in this case.



Scheme 4

**Scheme 4.** Cyclisation pathway of malonamides **14**.

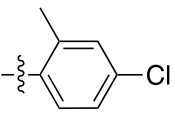
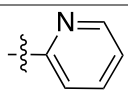
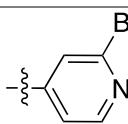
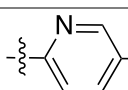
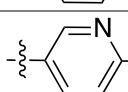
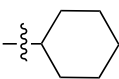
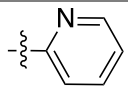
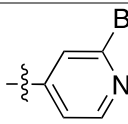
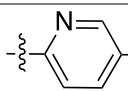
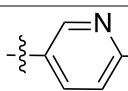
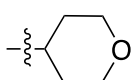
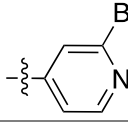
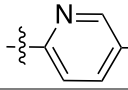
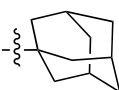
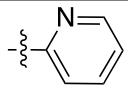
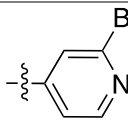
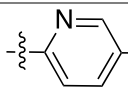
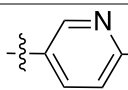


**Figure 4.** Reaction energy profile of Dieckmann cyclisation for malonamides with C-2 phenyl group. Calculated potential energy surfaces at the B3LYP/6-311++G (d,p) level of theory with SMD solvation (THF). All energies are reported in kcal/mol and referenced to the lowest energy carbanion generated after initial deprotonation. Labels: C=*cis*-**14**, D=*trans*-**14**, DProduct1=**15**, DProduct2=**16**, CProduct1=**18**, CProduct2=**17** (see Scheme 4). Energy values in kcal/mol.

The tetramate esters **4a-d** were further functionalised by direct ester to amide conversion with primary amines to give carboxamides **6a-d**, **7a-d**, **8a-d**, and **9a-d**, a process which had been found to be effective in other systems.<sup>19</sup> Some reactions did not go to completion when run in

THF/toluene at reflux for 18 h and yields varied (10-70%, Table 3). The *trans*-ring stereochemistry was expected to be conserved during the course of reaction and products all showed characteristic H-2 and H-5 chemical shift data (Table 3). The *trans*- stereochemistry was further supported by NOE analysis of **9d**, indicating an enhancement between H-5 and aromatic H-2' protons (Figure 1, SI).

**Table 1.** Yield, H2 and H5 chemical shifts (CDCl<sub>3</sub>, 400 MHz) for tetramate carboxamides.

	R <sup>2</sup> =	R <sup>1</sup> =	$\delta_{\text{H}}$ (ppm)		% Yield
			H-2	H-5	
6a			6.35	5.11 (br. s)	70
6b			6.18	4.81 (app t)	52
6c			6.28	5.08 (app t)	62
6d			6.24	4.82 (app t)	67
7a			6.33	4.84 (br. s)	41
7b			6.16 (major) 6.24 (minor)	4.61 (major, br. s) 4.73 (minor, br. s)	15
7c			6.25	4.81 (br.s)	31
7d			6.24	4.59 (br. s)	24
8b			6.14 (major) 6.20 (minor)	4.67 (major, br. s) 4.39 (minor, dd)	12
8c			6.30 (major) 6.25 (minor)	4.90 (major, app t) -	10
9a			6.31	4.84 (br. s)	44
9b			6.17	4.48 (br. s)	33
9c			6.25 (major) 6.33 (minor)	4.81 (app t) 4.57 (app t)	38
9d			6.23 (major) 6.31 (minor)	4.59 (app t) -	35

Tautomeric behaviour was known to exist for 3-acyltetramic acids in solution.<sup>13, 28</sup> <sup>1</sup>H NMR spectra for these tetramate carboxamides indicated complete enolisation, with no chemical shift

for H-7 being detected. Thus, tautomeric pairs **AB** and **CD** are internal tautomers which rapidly exchange with each other through proton transfer, and averaged signals were observed for **AB** and **CD** on the NMR time scale (Figure 5). Only the external tautomers, between the *endo*-enolic pair **A/C** and between the *exo*-enolic pair **B/D**, could be detected by NMR spectroscopy, with distinct  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts. The assignment of the chemical shifts for carbonyl functional groups C-6, C-8 and C-9 was supported by HMBC analysis, which also confirmed the assignment of the tautomeric forms. The chemical shifts of H-2 and carbonyl carbons (C-6, C-8, C-9) showed a similar pattern for the major/minor tautomers across the series (Table 2), which was also consistent with that observed in previously reported tetramate carboxamides with C-2 aromatic rings.<sup>19</sup>

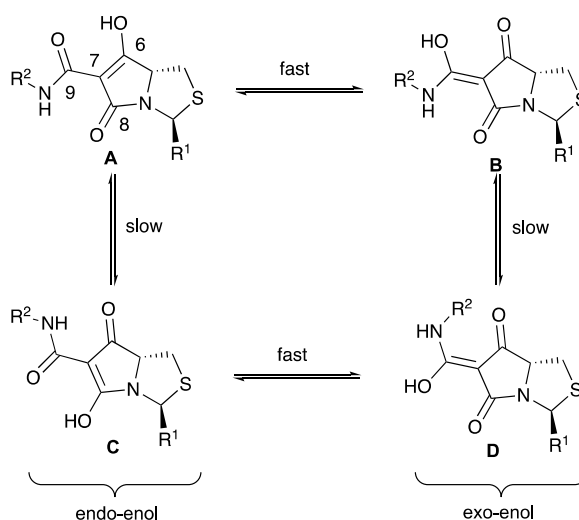


Figure 7

Figure 5. Tautomeric behaviour of tetramate carboxamides.

Table 2. H-2 and C-6, C-8, C-9 chemical shifts ( $\text{CDCl}_3$ ) of tetramate carboxamides.

	$\text{R}^2 =$	$\text{R}^1 =$	$\delta_{\text{H}}$ (ppm)				AB/CD <sup>[a]</sup>
			H-2	C-6	C-8	C-9	
6a			6.35	188.0	-	163.8	<b>AB</b> only
6b			6.18	184.9	172.3	163.6	<b>AB</b> only

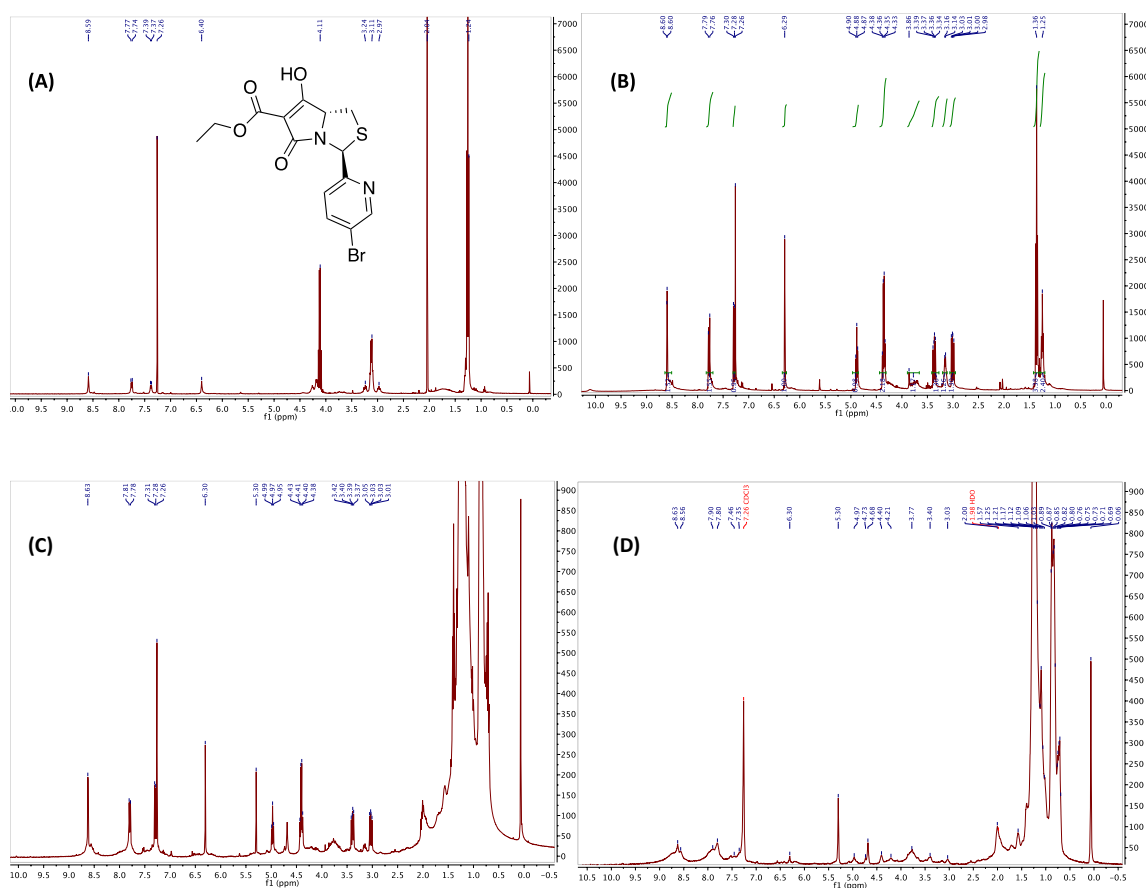
<b>6c</b>				6.28	185.0	171.9	163.7	<b>AB</b> only
<b>6d</b>				6.24	184.7	172.3	163.7	<b>AB</b> only
<b>7a</b>				6.33	-	-	165.5	<b>AB</b> only
<b>7b</b>			<b>AB</b>	6.16	189.1	172.8	165.4	3.3 : 1
			<b>CD</b>	6.24	191.2	178.4	-	
<b>7c</b>				6.25	188.5	172.3	165.4	<b>AB</b> only
<b>7d</b>				6.24	188.7	173.3	165.4	<b>AB</b> only
<b>8b</b>			<b>AB</b>	6.14	187.2	172.5	165.3	3.3 : 1
			<b>CD</b>	6.20	193.0	176.9	165.9	
<b>8c</b>			<b>AB</b>	6.30	177.9	173.0	167.9	3.4 : 1
			<b>CD</b>	6.25	-	-	-	
<b>9a</b>				6.31	188.8	-	166.3	<b>AB</b> only
<b>9b</b>				6.17	190.0	-	166.2	<b>AB</b> only
<b>9c</b>			<b>AB</b>	6.25	189.2	172.5	166.2	2.3 : 1
			<b>CD</b>	6.33	-	-	-	
<b>9d</b>			<b>AB</b>	6.23	189.5	173.0	166.2	3.2 : 1
			<b>CD</b>	6.31	191.1	-	167.9	

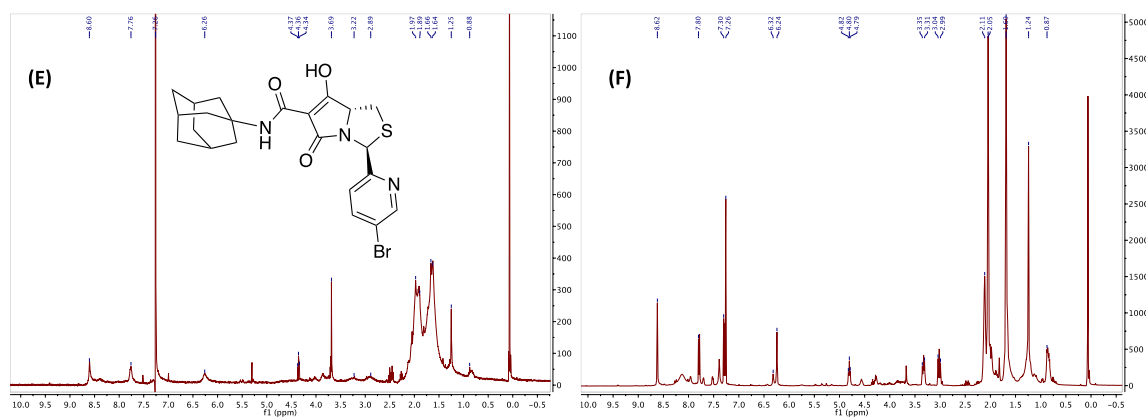
<sup>[a]</sup> The tautomeric ratio **AB/CD** was calculated as the ratio of integration of H-2. – in most cases where sample mass was low, routine <sup>13</sup>C NMR was not sensitive enough to detect all <sup>13</sup>C signals for minor tautomers.

The purification of tetramate esters and carboxamides by *flash* silica column chromatography has been reported to result in broadening of signals in <sup>1</sup>H NMR spectra; this has been attributed to the formation of metal chelates during the chromatographic process from trace amounts of metal impurities.<sup>19, 20, 28</sup> This problem could be solved by running chromatography with 1% Et<sub>3</sub>N in the eluant, and subsequently washing with 5% citric acid to give metal free-tetramates with improved signal sharpness and resolution.<sup>19, 20</sup> However, the presence of a basic



pyridine ring ( $pK_a = 5.23$ ) in the tetramate systems **4-9** limited the acid strength for post-column washing and also inevitably resulted in loss of yield to high solubility in the acidic aqueous layer. In order to probe metal chelating properties further, after characterisation of the post-column and acid-washed compound by  $^1H$  NMR spectrometry, a metal-free sample of tetramate ester **4c** was re-dissolved in DCM and washed with 1M aqueous solutions of metal ions. Comparison of the  $^1H$  NMR spectra clearly showed line broadening of metal chelated forms, relative to the clean material **4c** (Figure 6); thus, tetramate carboxamides appeared to bind more strongly with metals than tetramate esters (Figure 6, A versus E), and stronger binding to  $Fe^{2+}$  as compared to  $Ca^{2+}$  (Figure 6, C versus D).





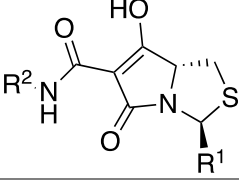
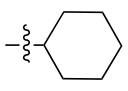
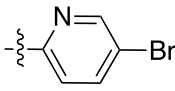
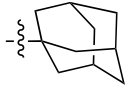
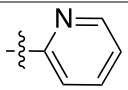
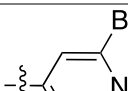
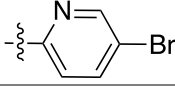
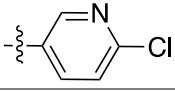
**Figure 6.**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz) spectra indicating metal-chelation abilities of tetramate ester **4c** and tetramate carboxamide **9c**. (A) Tetramic ester **4c** post-column, before acidic wash; (B) tetramic ester **4c** post-column, after 5% citric acid wash; (C) metal-free **4c** washed with aq. 1M  $\text{Ca}(\text{NO}_3)_2$  solution; (D) metal-free **4c** washed with aq. 1M  $\text{FeSO}_4$  solution; (E) Tetramic carboxamide **9c** post-column, before acidic wash; (F) tetramic carboxamide **9c** post-column, after 5% citric acid wash.

## Biological evaluation

The tetramate esters **4a-d** and carboxamides **6a-d**, **7a-d**, **8a-d** and **9a-d** were tested for their biological activities against Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative *Escherichia coli* (*E. coli*) by minimum inhibitory concentration (MIC) assay; data for the only active compounds is given (Table 3). While good activity was seen against the former, no activity was seen against Gram-negative *E. coli*. In keeping with earlier observations,<sup>29</sup> none of the tetramate esters **4a-d** showed any activity. Primary evaluation indicated that a hydrophobic pendant at C-7 was essential for bioactivity while substitutions with heterocycles at C-2 successfully increased compound polarity as measured by PSA without compromising bioactivity. Unfortunately, these tetramate analogues were rendered inactive when tested in the presence of blood. We had earlier found that antibacterially active 3-carboxamides exhibited characteristic physicochemical properties ( $530 < \text{MSA} < 600$ ,  $65 < \text{PSA} < 80$ ,  $11 < \text{rel-PSA} < 14$ ,  $1.2 < \text{ClogP} < 2.5$ ,  $-1.1 <$

$\text{ClogD}_{7.4} < 0$ )<sup>29</sup> and the corresponding data for compounds **9a-d** fit comfortably within these bounds; the limited structural scope of this library, however, does not permit a meaningful SAR analysis to be made, although the inactivity of the tetrahydropyranyl and chloromethylphenyl series is consistent with our earlier observation that polar groups at this position generally compromise phenotypic antibacterial activity.<sup>29</sup> That distinct antibacterial classes exhibit characteristic mean values for their physicochemical properties has been reported by O'Shea and Moser.<sup>30</sup>

**Table 3.** Biologically active cysteine-derived tetramate analogues.

	$R^2 =$	$R^1 =$	MIC against MRSA ug/ml	MW	clogP <sup>[a]</sup>	PSA <sup>[a]</sup>
<b>7c</b>			7.80	438	2.41	82.5
<b>9a</b>			1.95	412	1.72	82.5
<b>9b</b>			1.95	490	2.85	82.5
<b>9c</b>			3.91	490	2.72	82.5
<b>9d</b>			0.24	446	2.55	82.5

<sup>[a]</sup> Relevant chemical properties of the active compounds were calculated using MarvinSketch 16.10.

## Conclusion

The synthesis of cysteine-derived bicyclic tetramate analogues with C-2 and C-7 substitutions, which is highly chemoselective and stereoselective, leading to bicyclic tetramate esters and tetramate carboxamides with a *trans* relationship between H-2 and H-5, has been achieved. The Dieckmann cyclisation proceeded in the same direction to that previously observed

in C-2 aromatic series,<sup>20</sup> but in a different manner to that in the C-2 *t*-butyl and isopropyl series,<sup>18</sup> highlighting the steric control exerted by C-2 substituents on the chemoselectivity of the cyclisation process. Although this allowed increasing polarity of tetramate carboxamides to be achieved by introducing heterocycles at C-2 and C-7 without diminishing *in vitro* bioactivity, tetramate analogues with polar C-7 pendants were inactive, suggesting that the C-7 amide pendant was not a suitable position for polarity modulation. The tetramate analogues exhibited structure dependent metal chelation properties, influencing compound purification and biological activities. Despite their increase in polarity, these cysteine-derived tetramate analogues lost their potent *in vitro* bioactivity when tested in the presence of blood.

## Experimental

All reagents were obtained either from Sigma Aldrich, Alfa Aesar or Fluorochem and used without further purification. All reactions were carried out in oven-dried reaction flasks under inert (N<sub>2</sub>) atmosphere unless not using dry solvents. Reaction times were recorded in minutes (min), hours (h) or days (d). Reactions left overnight (o.n.) lasted for 16 - 20 h. 'Petroleum ether' refers to that fraction of light petroleum ether boiling at 40-60 °C and was used as received. Temperatures below room temperature were obtained using cold baths: 0 °C (ice/water), -15 °C (ice/NaCl salt) and -78 °C (dry ice/acetone). Temperatures above room temperature were obtained with heating with oil bath. Solvents were evaporated at 40 °C unless otherwise stated under reduced pressure on a Buchi RE 111 Rotavapour attached to a Vacuubrand CVC2 pump and pressure control system. Concentrations (c) in the general procedures referred to the limiting reagent and were given in mmol/mL. Thin layer chromatography (TLC) was performed using Merck aluminium foil backed sheets precoated with 0.2 mm Kieselgel 60 F<sub>254</sub>. Product spots were visualized by UV fluorescence (max 254 nm, for conjugated systems), staining with a KMnO<sub>4</sub> solution and heating (for unsaturated systems) or staining with ninhydrin solution and heating (for primary and secondary

amines). Retention factors ( $R_f$ ) were quoted to the nearest 0.01. Column chromatography was carried out using Sigma Aldrich silica gel 60, 0.040-0.063 (230-400 mesh particle size). The eluents used were determined based on the  $R_f$  values. Melting points were recorded using a Stuart Scientific SMP1 melting point instrument in open capillaries and were uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter at the stated temperature (25 °C) using the D line of sodium (wavelength at 589 nm) and a path length of 1 dm. Specific rotations  $[\alpha]_D^{25}$  were calculated and reported in  $10^{-1} \text{ } ^\circ\text{C cm}^2 \text{ g}^{-1}$  with concentration  $c$  given in g/100 ml. Infrared (IR) spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer with thin film (oil) or powder (solid). Absorption maxima ( $\nu_{\text{max}}$ ) were reported in wavenumbers ( $\text{cm}^{-1}$ ) and only selected peaks were reported.  $^1\text{H}$  NMR spectra were recorded on AVF (400 MHz) or AVC (500 MHz) spectrometers.  $^{13}\text{C}$  NMR spectra were recorded on Bruker AVF spectrometer at 101 MHz or on AVC spectrometer at 126 MHz with proton decoupling and cryogenic detection probe. Chemical shifts ( $\delta$ ) were reported in parts per million (ppm) and were referenced to the residual solvent peak. The abbreviations used to describe multiplicity were as follows: s (singlet), br. s (broad singlet), d (doublet), dd (double doublet), t (triplet), td (triple doublet), dt (doublet triplet), q (quartet), sept (septet), m (multiplet). Coupling constants ( $J$ ) were given in Hertz (Hz). 2D-NOESY and 1D-NOE experiments were performed using Bruker AVB400 or AVC (500 MHz) spectrometer. HMBC experiments were recorded on Bruker AVC spectrometer. Low resolution mass spectra ( $m/z$ ) were recorded on a Fisons Platform spectrometer using electrospray ionisation (ESI). High resolution mass spectra (HRMS) were recorded on a Bruker  $\mu\text{TOF}$  (ESI or APCI) spectrometer by the internal service at the Department of Chemistry, University of Oxford. The  $m/z$  values of major peaks were reported in Daltons and their intensities given as percentages of the base peaks.

#### **General procedure for synthesis of thiazolidines 2a-d**

Et<sub>3</sub>N (1.2 eq.) and respective aldehyde (1.0 eq.) were added to L-cysteine methyl ester hydrochloride (1.2 eq.) in petroleum ether (*c* = 0.3). The mixture was heated under reflux at 110 °C with continuous removal of water using a Dean-Stark apparatus for 18 h. The reaction mixture was then filtered and washed with diethyl ether and the combined filtrates were concentrated *in vacuo* and residue was purified by *flash* column chromatography to give thiazolidines **2a-d**.

#### General procedure for synthesis of *N*-acylthiazolidines **3a-d**

A solution of mono-ethyl malonate (1.2 eq.) in dichloromethane (DCM) was added dropwise to a stirred solution of thiazolidine (1.0 eq.), *N,N'*-dicyclohexylcarbodiimide (DCC, 1.2 eq.) and 4-dimethylaminopyridine (DMAP, 0.1 eq.) in DCM (*c* = 0.2) at 0 °C. The mixture was stirred at 0 °C for 15 min and then at room temperature for 18 h. The reaction mixture was then filtered and the solid washed with DCM and the combined filtrates were concentrated *in vacuo* and residue was purified by *flash* column chromatography to give *N*-acylated thiazolidines **3a-d**.

#### General procedure for synthesis of tetramate esters **4a-d**

Potassium *tert*-butoxide (KO<sup>t</sup>Bu, 1.2 eq.) was added to a stirred solution of *N*-acylated thiazolidine (1.0 eq.) in dry THF (*c* = 0.1) and the mixture was heated under reflux for 4 h. It was then cooled to room temperature, concentrated *in vacuo* and partitioned between diethyl ether and water. The aqueous layer was carefully acidified with 2M hydrochloric acid to pH = 3 and extracted with ethyl acetate. The combined organic layers were washed with brine, dried with MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography with 1% Et<sub>3</sub>N in the eluent to give the desired product. The product was dissolved in DCM and washed with 5% citric acid. The combined organic fractions were dried with MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield the desired tetramate esters **4a-d**.

### General procedure for synthesis of tetramate carboxamides 5a-d, 6a-d, 7a-d and 8a-d

Amine (1.2 eq.) was added to a stirred solution of tetramate ester (1.0 eq.) in THF/toluene (1 : 4,  $c = 0.05$ ) and the mixture was heated at reflux for 18 h. It was then cooled to room temperature, concentrated *in vacuo* and residue was purified by flash column chromatography with 1% Et<sub>3</sub>N in the eluent to give the desired product. The product was dissolved in DCM and washed with 5% citric acid. The combined organic fractions were dried with MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to yield the desired tetramate carboxamides **5a-d**, **6a-d**, **7a-d** and **8a-d**.

### General Procedure for the Evaluation of Antibacterial Activity

The samples were prepared as 4 mg/mL solutions of 70 % DMSO in MeOH, with serial dilution to the desired concentrations where necessary. A 100  $\mu$ L aliquot of each sample solution to be tested was loaded into 10 mm wells in agar plates and incubated for 24 h at 35 °C. The assays were repeated in triplicates and the diameters of the resultant inhibition zones were measured ( $\pm 1$  mm) along two perpendicular axes and then averaged to obtain the zone of inhibition. A 'blank' was run with solvent alone, to ensure the solvent made no contribution to the zone of inhibition. The relative potency was estimated by reference to standards prepared with cephalosporin C.

### (5*R*)-5-Methoxycarbonyl-2-(pyridin-2-yl)-1,3-thiazolidine, 2a

Yield (1.95 g, 75%); orange oil; inseparable 1.2 : 1 *cis* and *trans* diastereomers;  $R_f = 0.44$  (petrol : EtOAc; 1 : 4);  $\nu_{\max}/\text{cm}^{-1}$  1740 (s, C=O), 3291 (br, N-H); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): major isomer (*cis*):  $\delta$  8.62 (d, 1H,  $J = 4.8$  Hz, H6'), 7.67 (dd, 1H,  $J = 7.7, 1.8$  Hz, H4'), 7.29 (d, 1H,  $J = 7.8$  Hz, H3'), 7.20 – 7.27 (m, 1H, H5'), 5.65 (s, 1H, H2), 4.03 (dd, 1H,  $J = 9.7, 6.7$  Hz, H5), 3.82 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.44 (dd, 1H,  $J = 10.1, 6.6$  Hz, H4<sub>B</sub>), 3.09 (app t, 1H,  $J = 9.9$  Hz, H4<sub>A</sub>); minor isomer (*trans*):  $\delta$  8.57 (d, 1H,  $J = 4.8$  Hz, H6'), 7.63 – 7.66 (m, 1H, H4'), 7.33 (d, 1H,  $J = 7.8$  Hz, H3'), 7.16 – 7.23 (m, 1H, H5'), 5.84 (s,

1H, H2), 4.55 (dd, 1H,  $J = 6.6, 4.4$  Hz, H5), 3.80 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.32 – 3.40 (m, 2H, H<sub>4A</sub> and H<sub>4B</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): major isomer (*cis*):  $\delta$  170.8 (CO<sub>2</sub>CH<sub>3</sub>), 156.4 (C2'), 149.4 (C6'), 136.5 (C4'), 123.0 (C3'), 121.7 (C5'), 71.2 (C5), 65.8 (C2), 52.2 (CO<sub>2</sub>CH<sub>3</sub>), 39.0 (C4); minor isomer (*trans*):  $\delta$  171.8 (CO<sub>2</sub>CH<sub>3</sub>), 158.3 (C2'), 149.2 (C6'), 136.5 (C4'), 122.6 (C5'), 121.2 (C3'), 70.7 (C5), 65.3 (C2), 52.2 (CO<sub>2</sub>CH<sub>3</sub>), 38.4 (C4);  $m/z$  (ESI<sup>+</sup>) 225 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$ : [M+H]<sup>+</sup> Calcd for C<sub>10</sub>H<sub>13</sub>O<sub>2</sub>N<sub>2</sub>S 225.0692; Found 225.0690.

**(5*R*)-2-(2-Bromopyridin-4-yl)-5-methoxycarbonyl-1,3-thiazolidine, 2b**

Yield (3.04 g, 86%); yellow oil; inseparable 0.6 : 1 *cis* and *trans* diastereomers;  $R_f = 0.15$  (petrol : EtOAc; 4 : 1);  $\nu_{\max}/\text{cm}^{-1}$  (neat) 1736 (s, C=O), 3315 (br, N-H); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): major isomer (*trans*):  $\delta$  8.29 (d, 1H,  $J = 5.2$  Hz, H6'), 7.61 (s, 1H, H3'), 7.32 (d, 1H,  $J = 5.2$  Hz, H5'), 5.75 (s, 1H, H2), 3.99 (q, 1H,  $J = 8.1, 7.1$  Hz, H5), 3.81 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.35 (dd, 1H,  $J = 10.6, 6.8$  Hz, H<sub>4B</sub>), 3.04 – 3.16 (m, 1H, H<sub>4A</sub>); minor isomer (*cis*):  $\delta$  8.37 (d, 1H,  $J = 5.2$  Hz, H6'), 7.65 (s, 1H, H3'), 7.38 (d, 1H,  $J = 5.2$  Hz, H5'), 5.47 (s, 1H, H2), 3.99 (q, 1H,  $J = 8.1, 7.1$  Hz, H5), 3.81 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.46 (dd, 1H,  $J = 10.2, 6.8$  Hz, H<sub>4B</sub>), 3.04 – 3.16 (m, 1H, H<sub>4A</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): major isomer (*trans*):  $\delta$  171.6 (CO<sub>2</sub>CH<sub>3</sub>), 154.7 (C6'), 150.0 (C4'), 142.3 (C2'), 125.7 (C3'), 121.0 (C5'), 68.0 (C5), 64.0 (C2), 52.7 (CO<sub>2</sub>CH<sub>3</sub>), 38.1 (C4); minor isomer (*cis*):  $\delta$  171.0 (CO<sub>2</sub>CH<sub>3</sub>), 150.6 (C6'), 150.3 (C4'), 142.4 (C2'), 126.5 (C3'), 121.5 (C5'), 69.6 (C5), 65.4 (C2), 52.7 (CO<sub>2</sub>CH<sub>3</sub>), 38.7 (C4);  $m/z$  (ESI<sup>+</sup>) 303 and 305 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$ : [M+H]<sup>+</sup> Calcd for C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>N<sub>2</sub>BrS 302.9797 and 304.9777; Found 302.9795 and 304.9772.

**(5*R*)-2-(5-Bromopyridin-2-yl)-5-methoxycarbonyl-1,3-thiazolidine, 2c**

Yield (2.83 g, 80%); yellow oil; inseparable 1 : 1 *cis* and *trans* diastereomers;  $R_f = 0.31$  (petrol : EtOAc; 3 : 1);  $\nu_{\max}/\text{cm}^{-1}$  1736 (s, C=O), 3301 (br, N-H); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): *trans* isomer:  $\delta$



8.60 (d, 1H,  $J = 2.2$  Hz, H6'), 7.77 (td, 1H,  $J = 8.2, 2.3$  Hz, H4'), 7.20 (d, 1H,  $J = 8.0$  Hz, H3'), 5.80 (s, 1H, H2), 4.47 (dd, 1H,  $J = 6.7, 4.7$  Hz, H5), 3.79 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.42 (dd, 1H,  $J = 10.1, 6.6$  Hz, H4<sub>B</sub>), 3.28 (dd, 1H,  $J = 10.6, 4.7$  Hz, H4<sub>A</sub>); *cis* isomer:  $\delta$  8.66 (d, 1H,  $J = 2.2$  Hz, H6'), 7.77 (td, 1H,  $J = 8.2, 2.3$  Hz, H4'), 7.25 (d, 1H,  $J = 8.3$  Hz, H3'), 5.60 (s, 1H, H2), 4.01 (dd, 1H,  $J = 9.6, 6.6$  Hz, H5), 3.81 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.35 (dd, 1H,  $J = 10.6, 6.8$  Hz, H4<sub>B</sub>), 3.02 – 3.09 (m, 1H, H4<sub>A</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): *cis* and *trans* isomers:  $\delta$  172.0, 171.1 (CO<sub>2</sub>CH<sub>3</sub>), 157.8, 155.5 (C2'), 151.0, 150.7 (C6'), 139.4 (C4'), 123.3, 122.6 (C3'), 120.2, 119.7 (C5'), 70.9, 70.4 (C5), 66.3, 65.6 (C2), 52.7 (CO<sub>2</sub>CH<sub>3</sub>), 39.5, 38.8 (C4);  $m/z$  (ESI<sup>+</sup>) 303 and 305 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$ : [M+H]<sup>+</sup> Calcd for C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>N<sub>2</sub>BrS 302.9797 and 304.9777; Found 302.9800 and 304.9777.

#### **(5*R*)-2-(6-Chloropyridin-3-yl)-5-methoxycarbonyl-1,3-thiazolidine, 2d**

Yield (2.49 g, 83%); yellow oil; inseparable 0.8 : 1 *cis* and *trans* diastereomers;  $R_f = 0.48$  (petrol : EtOAc; 3 : 2);  $\nu_{\max}/\text{cm}^{-1}$  1736 (s, C=O), 3304 (br, N-H); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): major isomer (*trans*):  $\delta$  8.43 (s, 1H, H2'), 7.73 (d, 1H,  $J = 8.3$  Hz, H4'), 7.23 (d, 1H,  $J = 8.2$  Hz, H5'), 5.77 (s, 1H, H2), 4.02 (app t, 1H,  $J = 6.7$  Hz, H5), 3.75 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.32 (dd, 1H,  $J = 11.4, 7.0$  Hz, H4<sub>B</sub>), 3.03 – 3.15 (m, 1H, H4<sub>A</sub>); minor isomer (*cis*):  $\delta$  8.45 (s, 1H, H2'), 7.82 (d, 1H,  $J = 8.3$  Hz, H4'), 7.30 (d, 1H,  $J = 8.3$  Hz, H5'), 5.48 (s, 1H, H2), 3.96 (app t, 1H,  $J = 8.1$  Hz, H5), 3.75 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.43 (dd, 1H,  $J = 10.1, 7.2$  Hz, H4<sub>B</sub>), 3.03 – 3.15 (m, 1H, H4<sub>A</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): major isomer (*trans*):  $\delta$  171.8 (CO<sub>2</sub>CH<sub>3</sub>), 150.6 (C2'), 148.5 (C6'), 137.8 (C4'), 136.6 (C3'), 123.8 (C5'), 67.3 (C5), 64.0 (C2), 52.8 (CO<sub>2</sub>CH<sub>3</sub>), 38.0 (C4); minor isomer (*cis*):  $\delta$  171.3 (CO<sub>2</sub>CH<sub>3</sub>), 151.6 (C2'), 149.0 (C6'), 138.1 (C4'), 133.2 (C3'), 124.3 (C5'), 68.9 (C5), 65.5 (C2), 52.8 (CO<sub>2</sub>CH<sub>3</sub>), 39.1 (C4);  $m/z$  (ESI<sup>+</sup>) 259 ([M+H]<sup>+</sup>, 100%) and 261 ([M+H]<sup>+</sup>, 33%); HRMS (ESI<sup>+</sup>)  $m/z$ : [M+H]<sup>+</sup> Calcd for C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>N<sub>2</sub>ClS 259.0303 and 261.0273; Found 259.0303 and 261.0271.

**(5R)-1-(3-Ethoxy-3-oxopropanoyl)-5-methoxycarbonyl-2-(pyridin-2-yl)-1,3-thiazolidine, 3a**

Yield (2.41 g, 84%); yellow oil; separable 1.2 : 1 *cis* and *trans* diastereomers; major isomer (*cis*, a 1 : 0.2 mixture of rotamers in CDCl<sub>3</sub>):  $R_f$  = 0.36 (petrol : EtOAc; 1 : 1);  $[\alpha]_D^{25}$  = + 67.7 ( $c$  = 0.94, CHCl<sub>3</sub>);  $\nu_{\max}/\text{cm}^{-1}$  1664 (s, C=O), 1740 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  8.55 (d, 1H,  $J$  = 4.8 Hz, H6' major rotamer), 8.47 (d, 1H,  $J$  = 4.4 Hz, H6' minor rotamer), 8.08 (app d, 1H,  $J$  = 7.9 Hz, H4' major rotamer), 7.71 – 7.79 (m, 1H, H3' major rotamer), 7.59 – 7.63 (m, 1H, H3' minor rotamer), 7.23 (dd, 1H,  $J$  = 8.1, 5.3 Hz, H5' major rotamer), 7.10 – 7.14 (m, 1H, H5' minor rotamer), 6.45 (s, 1H, H2 minor rotamer), 6.18 (s, 1H, H2 major rotamer), 5.07 (dd, 1H,  $J$  = 6.7, 3.7 Hz, H5 minor rotamer), 5.01 (dd, 1H,  $J$  = 8.3, 6.6 Hz, H5 major rotamer), 4.18 (q, 2H,  $J$  = 7.2 Hz, OCH<sub>2</sub>CH<sub>3</sub> minor rotamer), 4.11 (q, 2H,  $J$  = 7.1 Hz, OCH<sub>2</sub>CH<sub>3</sub> major rotamer), 3.80 (s, 3H, CO<sub>2</sub>CH<sub>3</sub> major rotamer), 3.78 (s, 3H, CO<sub>2</sub>CH<sub>3</sub> minor rotamer), 3.37 (d, 1H,  $J$  = 15.6 Hz, H2''<sub>B</sub> major rotamer), 3.30 (dd, 1H,  $J$  = 12.0, 6.6 Hz, H4<sub>B</sub> major rotamer), 3.22 (dd, 1H,  $J$  = 12.0, 8.3 Hz, H4<sub>A</sub> major rotamer), 3.13 (d, 1H,  $J$  = 15.6 Hz, H2''<sub>A</sub> major rotamer), 1.26 (t, 3H,  $J$  = 7.1 Hz, OCH<sub>2</sub>CH<sub>3</sub> minor rotamer), 1.21 (t, 3H,  $J$  = 7.1 Hz, OCH<sub>2</sub>CH<sub>3</sub> major rotamer); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  170.5 and 170.1 (CO<sub>2</sub>CH<sub>3</sub>), 167.0 and 166.4 (C3''), 165.6 and 165.1 (C1''), 159.3 and 158.5 (C2'), 149.7 and 149.1 (C6'), 137.7 and 136.8 (C4'), 123.3 and 122.7 (C3'), 120.7 and 120.4 (C5'), 67.7 and 67.6 (C5), 64.8 and 63.9 (C2), 61.8 and 61.7 (OCH<sub>2</sub>CH<sub>3</sub>), 53.2 and 52.8 (CO<sub>2</sub>CH<sub>3</sub>), 42.8 and 42.1 (C2''), 31.8 (C4), 14.1 and 14.1 (OCH<sub>2</sub>CH<sub>3</sub>);  $m/z$  (ESI<sup>+</sup>) 339 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$ : [M+H]<sup>+</sup> Calcd for C<sub>15</sub>H<sub>19</sub>O<sub>5</sub>N<sub>2</sub>S 339.1009; Found 339.1008.

Minor isomer (*trans*, a 1 : 0.7 mixture of rotamers in CDCl<sub>3</sub>):  $R_f$  = 0.27 (petrol : EtOAc; 1 : 1);  $[\alpha]_D^{25}$  = -117.3 ( $c$  = 0.99, CHCl<sub>3</sub>);  $\nu_{\max}/\text{cm}^{-1}$  1661 (s, C=O), 1739 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  8.57 (d, 1H,  $J$  = 4.1 Hz, H6' major rotamer), 8.51 (d, 1H,  $J$  = 4.6 Hz, H6' minor rotamer), 7.72 (td, 1H,  $J$  = 7.7, 1.8 Hz, H4' major rotamer), 7.64 (app td, 1H,  $J$  = 7.6, 1.4 Hz, H4' minor rotamer), 7.29 (d, 1H,  $J$  = 7.8 Hz, H3' major rotamer), 7.23 (d, 1H,  $J$  = 7.6 Hz, H3' minor rotamer), 7.22 – 7.26 (m, 1H, obscured, H5' major rotamer), 7.16 (dd, 1H,  $J$  = 7.4, 5.0 Hz, H5' minor rotamer), 6.25 (s, 1H, H2

minor rotamer), 6.16 (s, 1H, H2 major rotamer), 5.32 (app d, 1H,  $J = 7.1$  Hz, H5 major rotamer), 5.18 (app d, 1H,  $J = 6.1$  Hz, H5 minor rotamer), 4.15 – 4.22 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub> minor rotamer), 4.09 (q, 2H,  $J = 7.1$  Hz, OCH<sub>2</sub>CH<sub>3</sub> major rotamer), 3.86 (s, 3H, CO<sub>2</sub>CH<sub>3</sub> minor rotamer), 3.80 (s, 3H, CO<sub>2</sub>CH<sub>3</sub> major rotamer), 3.83 – 3.89 (dd, 1H, obscured, H4<sub>B</sub> minor rotamer), 3.60 (dd, 1H,  $J = 12.3$ , 7.2 Hz, H4<sub>B</sub> major rotamer), 3.39 – 3.45 (m, 3H, H4<sub>A</sub>, H2''<sub>A</sub>, H2''<sub>B</sub> minor rotamer), 3.32 (d, 1H,  $J = 15.2$  Hz, H2''<sub>B</sub> major rotamer), 3.20 (app d, 1H,  $J = 12.4$  Hz, H4<sub>A</sub> major rotamer), 3.07 (d, 1H,  $J = 15.2$  Hz, H2''<sub>A</sub> major rotamer), 1.26 (t, 3H,  $J = 7.2$  Hz, OCH<sub>2</sub>CH<sub>3</sub> minor rotamer), 1.22 (t, 3H,  $J = 7.2$  Hz, OCH<sub>2</sub>CH<sub>3</sub> major rotamer); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  170.4 and 169.8 (CO<sub>2</sub>CH<sub>3</sub>), 167.1 and 166.4 (C3''), 165.1 and 164.7 (C1''), 160.4 and 160.2 (C2'), 150.1 and 149.6 (C6'), 137.5 and 136.8 (C4'), 123.3 and 122.7 (C3'), 119.8 and 119.5 (C5'), 66.7 and 66.2 (C5), 64.2 and 64.0 (C2), 61.8 and 61.6 (OCH<sub>2</sub>CH<sub>3</sub>), 53.5 and 52.9 (CO<sub>2</sub>CH<sub>3</sub>), 43.3 and 42.5 (C2''), 31.5 (C4), 14.1 and 14.1 (OCH<sub>2</sub>CH<sub>3</sub>);  $m/z$  (ESI<sup>+</sup>) 339 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$ : [M+Na]<sup>+</sup> Calcd for C<sub>15</sub>H<sub>18</sub>O<sub>5</sub>N<sub>2</sub>NaS 361.0829; Found 361.0826.

**(5R)-2-(2-Bromopyridin-4-yl)-1-(3-ethoxy-3-oxopropanoyl)-5-methoxycarbonyl-1,3-thiazolidine,**

**3b**

Yield (3.80 g, 92%); yellow oil; inseparable 1.6 : 1 *cis* and *trans* diastereomers;  $R_f = 0.33$  (petrol : EtOAc; 1 : 1);  $\nu_{\max}/\text{cm}^{-1}$  1661 (s, C=O), 1738 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): *cis* isomer as a 1 : 0.3 mixture of rotamers, *trans* isomer 1 : 0.8 as a mixture of rotamers in CDCl<sub>3</sub>:  $\delta$  8.39 (app t, 1H,  $J = 4.8$  Hz, H6' *trans* isomer), 8.30 (d, 1H,  $J = 5.2$  Hz, H6' *cis* isomer), 7.39 (s, 1H, H3' *cis* isomer), 7.34 (s, 1H, H3' *trans* isomer), 7.18 (d, 1H,  $J = 6.7$  Hz, H5' *cis* isomer), 7.12 (d, 1H,  $J = 6.5$  Hz, H5' *trans* isomer), 6.22 (s, 1H, H2 *cis* isomer minor rotamer), 6.14 (s, 1H, H2 *cis* isomer major rotamer), 6.12 (s, 1H, H2 *trans* isomer major rotamer), 6.12 (s, 1H, H2 *trans* isomer minor rotamer), 5.20 (app t, 1H,  $J = 3.4$  Hz, H5 *cis* isomer major rotamer), 4.19 – 4.28 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub> *cis* isomer), 4.08 – 4.19 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub> *trans* isomer), 3.86 (s, 3H, CO<sub>2</sub>CH<sub>3</sub> *cis* isomer, minor rotamer), 3.76 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>

*trans* isomer, minor rotamer), 3.74 (s, 3H, CO<sub>2</sub>CH<sub>3</sub> *cis* isomer, major rotamer), 3.72 (s, 3H, CO<sub>2</sub>CH<sub>3</sub> *trans* isomer, major rotamer), 3.72 – 3.79 (m, 2H, obscured, H<sub>4B</sub>), 3.44 – 3.50 (m, 2H, H<sub>4A</sub>), 3.12 – 3.39 (m, 4H, H<sub>2''A</sub>, H<sub>2''B</sub>), 1.31 (t, 3H, *J* = 7.0 Hz, OCH<sub>2</sub>CH<sub>3</sub>, *trans* isomer major rotamer), 1.22 – 1.28 (m, 3H, OCH<sub>2</sub>CH<sub>3</sub>, *cis* isomer major rotamer); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 169.9, 169.8, 169.7, 168.9 (CO<sub>2</sub>CH<sub>3</sub> major, minor and rotamers), 167.2, 167.1, 166.7, 166.3 (C3'' major, minor and rotamers), 165.9, 165.4, 165.1, 165.0 (C1'' major, minor and rotamers), 153.9, 153.6, 152.4, 151.2 (C6' major, minor and rotamers), 150.9, 150.6, 150.2, 150.0 (C4' major, minor and rotamers), 143.0, 142.7, 142.3, 142.1 (C2' major, minor and rotamers), 125.9, 125.4, 123.9 (C3' major, minor and rotamers), 120.9, 120.3, 119.3, 119.1 (C5' major, minor and rotamers), 67.8, 67.1, 65.2, 64.7 (C5 major, minor and rotamers), 64.1, 63.9, 63.8, 63.6 (C2 major, minor and rotamers), 62.0, 61.9, 61.7, 61.4 (OCH<sub>2</sub>CH<sub>3</sub> major, minor and rotamers), 53.6, 53.4, 52.9, 49.0 (CO<sub>2</sub>CH<sub>3</sub> major, minor and rotamers), 43.0, 42.7, 42.3, 42.0 (C2'' major, minor and rotamers), 34.3, 33.7, 33.2, 33.1 (C4 major, minor and rotamers), 14.1, 14.0, 14.0, 13.9 (OCH<sub>2</sub>CH<sub>3</sub> major, minor and rotamers); *m/z* (ESI<sup>+</sup>) 415 and 417 ([M-H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) *m/z*: [M+Na]<sup>+</sup> Calcd for C<sub>15</sub>H<sub>17</sub>O<sub>5</sub>N<sub>2</sub>BrNaS 438.9934 and 440.9913; Found 438.9929 and 440.9908.

**(5*R*)-2-(5-Bromopyridin-2-yl)-1-(3-ethoxy-3-oxopropanoyl)-5-methoxycarbonyl-1,3-thiazolidine, 3c**

Yield (3.28 g, 85%); yellow oil; inseparable 1.3 : 1 *cis* and *trans* diastereomers; *R<sub>f</sub>* = 0.30 (petrol : EtOAc; 1 : 1); *ν*<sub>max</sub>/cm<sup>-1</sup> 1662 (s, C=O), 1739 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): *cis* isomer as a 1 : 0.2 mixture of rotamers, *trans* isomer 1 : 0.9 as a mixture of rotamers in CDCl<sub>3</sub>: δ 8.51 (d, 1H, *J* = 2.5 Hz, H<sub>6'</sub> *trans* isomer major rotamer), 8.43 (d, 1H, *J* = 2.5 Hz, H<sub>6'</sub> *trans* isomer minor rotamer), 8.28 (d, 1H, *J* = 2.6 Hz, H<sub>6'</sub> *cis* isomer major rotamer), 8.24 (d, 1H, *J* = 2.3 Hz, H<sub>6'</sub> *cis* isomer minor rotamer), 8.03 (dd, 1H, *J* = 8.4, 2.7 Hz, H<sub>4'</sub> *trans* isomer major rotamer), 7.74 (dd, 1H, *J* = 8.3, 2.5 Hz, H<sub>4'</sub> *trans* isomer minor rotamer), 7.43 – 7.49 (m, 4H, H<sub>3'</sub> *trans* isomer minor and major

rotamers, H4' *cis* isomer major and minor rotamers), 7.37 (d, 1H, *J* = 8.2 Hz, H3' *cis* isomer major rotamer), 7.22 (d, 1H, *J* = 8.2 Hz, H3' *cis* isomer minor rotamer), 6.21 (s, 1H, H2 *cis* isomer minor rotamer), 6.19 (s, 1H, H2 *cis* isomer major rotamer), 6.18 (s, 1H, H2 *trans* isomer minor rotamer), 6.15 (s, 1H, H2 *trans* isomer major rotamer), 5.24 (app d, 1H, *J* = 6.3 Hz, H5 *cis* isomer minor rotamer), 5.15 (app t, 1H, *J* = 3.3 Hz, H5 *cis* isomer major rotamer), 5.04 (dd, 1H, *J* = 6.2, 2.6 Hz, H5 *trans* isomer minor rotamer), 5.00 (app t, 1H, *J* = 7.1 Hz, H5 *trans* isomer major rotamer), 3.97 – 4.20 (m, 8H, OCH<sub>2</sub>CH<sub>3</sub> *cis* and *trans* isomers), 3.80 (s, 3H, CO<sub>2</sub>CH<sub>3</sub> *cis* isomer major rotamer), 3.78 (s, 3H, CO<sub>2</sub>CH<sub>3</sub> *trans* isomer major rotamer), 3.77 (s, 3H, CO<sub>2</sub>CH<sub>3</sub> *trans* isomer minor rotamer), 3.74 (s, 3H, CO<sub>2</sub>CH<sub>3</sub> *cis* isomer minor rotamer), 3.09 – 3.49 (m, 16H, H4<sub>A</sub>, H4<sub>B</sub>, H2''<sub>A</sub>, H2''<sub>B</sub> *cis* and *trans* isomers), 1.14 – 1.26 (m, 12H, OCH<sub>2</sub>CH<sub>3</sub> *cis* and *trans* isomers with rotamers); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  170.0, 170.0, 169.8, 169.0 (CO<sub>2</sub>CH<sub>3</sub> major, minor and rotamers), 167.1, 166.8, 166.3, 165.9 (C3'' major, minor and rotamers), 165.2, 165.1, 165.0, 165.0 (C1'' major, minor and rotamers), 149.4, 148.4, 147.2, 146.8 (C2' major, minor and rotamers), 142.2, 142.0, 141.4, 140.7 (C6' major, minor and rotamers), 137.8, 137.3, 137.1, 137.1 (C4' major, minor and rotamers), 135.7, 135.5, 135.4, 134.1 (C3' major, minor and rotamers), 128.5, 128.3, 127.7, 127.6 (C5' major, minor and rotamers), 64.6, 64.2, 64.1, 63.9 (C5 major, minor and rotamers), 63.7, 63.3, 62.9, 62.3 (C2 major, minor and rotamers), 61.9, 61.8, 61.8, 61.7 (OCH<sub>2</sub>CH<sub>3</sub> major, minor and rotamers), 53.5, 53.3, 52.9, 49.0 (CO<sub>2</sub>CH<sub>3</sub> major, minor and rotamers), 43.0, 42.8, 42.3, 42.0 (C2'' major, minor and rotamers), 34.2, 33.7, 33.2, 32.1 (C4 major, minor and rotamers), 14.1, 14.0, 14.0, 14.0 (OCH<sub>2</sub>CH<sub>3</sub> major, minor and rotamers); *m/z* (ESI<sup>+</sup>) 417 and 419 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) *m/z*: [M+H]<sup>+</sup> Calcd for C<sub>15</sub>H<sub>18</sub>O<sub>5</sub>N<sub>2</sub>BrS 417.0114 and 419.0094; Found 417.0109 and 419.0087.

**(5*R*)-2-(6-Chloropyridin-3-yl)-1-(3-ethoxy-3-oxopropanoyl)-5-methoxycarbonyl-1,3-thiazolidine,**

**3d**

Yield (2.66 g, 77%); yellow oil; inseparable 1.2 : 1 *cis* and *trans* diastereomers;  $R_f = 0.39$  (petrol : EtOAc; 1 : 1);  $\nu_{\max}/\text{cm}^{-1}$  1660 (s, C=O), 1737 (s, C=O);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz): *cis* isomer as a 1 : 0.5 mixture of rotamers, *trans* isomer 1 : 0.9 as a mixture of rotamers in  $\text{CDCl}_3$ :  $\delta$  8.53 (s, 1H, H2' *trans* isomer major rotamer), 8.46 (s, 1H, H2' *trans* isomer minor rotamer), 8.31 (s, 1H, H2' *cis* isomer major rotamer), 8.27 (s, 1H, H2' *cis* isomer minor rotamer), 8.13 – 8.19 (m, 1H, H4' *trans* isomer major rotamer), 7.85 (d, 1H,  $J = 10.2$  Hz, H4' *trans* isomer minor rotamer), 7.58 (d, 1H,  $J = 10.5$  Hz, H4' *cis* isomer major rotamer), 7.54 (d, 1H,  $J = 10.5$  Hz, H4' *cis* isomer minor rotamer), 7.31 – 7.37 (m, 2H, H5' *trans* isomers with rotamers), 7.20 – 7.26 (m, 2H, H5' *cis* isomers with rotamers), 6.23 (s, 1H, H2 *cis* isomer with rotamers), 6.21 (s, 1H, H2, *trans* isomer major rotamer), 6.17 (s, 1H, H2 *trans* isomer minor rotamer), 5.26 (app d, 1H,  $J = 6.7$  Hz, H5 *cis* isomer minor rotamer), 5.16 (app t, 1H,  $J = 2.9$  Hz, H5 *cis* isomer major rotamer), 5.03 – 5.08 (m, 1H, H5 *trans* isomer major rotamer), 5.01 (app d, 1H,  $J = 7.0$  Hz, H5 *trans* isomer minor rotamer), 4.00 – 4.22 (m, 8H,  $\text{OCH}_2\text{CH}_3$  *cis* and *trans* isomers with rotamers), 3.82 (s, 3H,  $\text{CO}_2\text{CH}_3$  *cis* isomer major rotamer), 3.80 (s, 3H,  $\text{CO}_2\text{CH}_3$  *trans* isomer minor rotamer), 3.79 (s, 3H,  $\text{CO}_2\text{CH}_3$  *trans* isomer major rotamer), 3.75 (s, 3H,  $\text{CO}_2\text{CH}_3$  *cis* isomer minor rotamer), 3.09 – 3.53 (m, 16H, H4<sub>A</sub>, H4<sub>B</sub>, H2''<sub>A</sub>, H2''<sub>B</sub> *cis* and *trans* isomers with rotamers), 1.15 – 1.28 (m, 12H,  $\text{OCH}_2\text{CH}_3$  *cis* and *trans* isomers with rotamers);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  170.1, 170.1, 169.9, 169.1 ( $\text{CO}_2\text{CH}_3$  major, minor and rotamers), 167.2, 166.9, 166.4, 166.0 (C3'' major, minor and rotamers), 166.0, 165.2, 165.0 (C1'' major, minor and rotamers), 151.8, 151.6, 150.9, 150.4 (C2' major, minor and rotamers), 149.1, 148.1, 146.8, 146.5 (C6' major, minor and rotamers), 138.1, 137.4, 136.9, 136.8 (C4' major, minor and rotamers), 135.7, 135.7, 135.1, 133.7 (C3' major, minor and rotamers), 124.8, 124.7, 124.1, 123.9 (C5' major, minor and rotamers), 64.7, 64.3, 64.2, 64.0 (C5 major, minor and rotamers), 63.8, 63.4, 63.0, 62.3 (C2 major, minor and rotamers), 62.0, 62.0, 61.9, 61.8 ( $\text{OCH}_2\text{CH}_3$  major, minor and rotamers), 53.6, 53.4, 53.0, 49.0 ( $\text{CO}_2\text{CH}_3$  major, minor and rotamers), 43.2, 43.0, 42.4, 42.1 (C2'' major, minor and rotamers), 34.3, 34.0, 33.3, 32.2 (C4 major, minor and rotamers), 14.1, 14.1, 14.0, 14.0 ( $\text{OCH}_2\text{CH}_3$

major, minor and rotamers);  $m/z$  (ESI<sup>+</sup>) 373 ([M+H]<sup>+</sup>, 100%) and 375 ([M+H]<sup>+</sup>, 33%); HRMS (ESI<sup>+</sup>)  $m/z$ : [M+H]<sup>+</sup> Calcd for C<sub>15</sub>H<sub>18</sub>O<sub>5</sub>N<sub>2</sub>ClS 373.0620 and 375.0590; Found 373.0613 and 375.0582.

**(2S,5R)-1-Aza-7-ethoxycarbonyl-6-hydroxy-8-oxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]oct-6-ene, 4a**

Yield (870 mg, 48%); brown oil;  $R_f$  = 0.23 (MeOH : EtOAc; 1 : 6);  $[\alpha]_D^{25}$  = -196.7 ( $c$  = 0.55, MeOH);  $\nu_{\max}/\text{cm}^{-1}$  1589 (s, C=C), 1636 (s, C=O); <sup>1</sup>H NMR (methanol-*d*<sub>4</sub>, 500 MHz):  $\delta$  (C6-OH not seen), 8.44 (d, 1H,  $J$  = 5.6 Hz, H6'), 7.78 (app td, 1H,  $J$  = 7.7, 1.8 Hz, H4'), 7.47 (d, 1H,  $J$  = 7.8 Hz, H3'), 7.27 (dd, 1H,  $J$  = 7.4, 5.0 Hz, H5'), 6.36 (s, 1H, H2), 4.40 (app t, 1H,  $J$  = 7.7 Hz, H5), 4.11 – 4.27 (m, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 3.24 (dd, 1H,  $J$  = 10.8, 7.3 Hz, H4<sub>B</sub>), 2.91 (dd, 1H,  $J$  = 10.9, 8.1 Hz, H4<sub>A</sub>), 1.28 (t, 3H,  $J$  = 7.3 Hz, OCH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C NMR (methanol-*d*<sub>4</sub>, 101 MHz):  $\delta$  194.9 (C6), 179.5 (C8), 168.1 (C9), 162.3 (C2'), 149.8 (C6'), 139.0 (C4'), 123.9 (C3'), 121.2 (C5'), 91.3 (C7), 71.3 (C5), 65.2 (C2), 60.2 (OCH<sub>2</sub>CH<sub>3</sub>), 34.6 (C4), 15.0 (OCH<sub>2</sub>CH<sub>3</sub>);  $m/z$  (ESI<sup>-</sup>) 305 ([M-H]<sup>-</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$ : [M+H]<sup>+</sup> Calcd for C<sub>14</sub>H<sub>15</sub>O<sub>4</sub>N<sub>2</sub>S 307.0747; Found 307.0747.

**(2S,5R)-1-Aza-2-(2-bromopyridin-4-yl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 4b**

Yield (1.20 g, 35%); orange foamy solid, mp 165 – 171 °C;  $R_f$  = 0.36 (MeOH : EtOAc; 1 : 6);  $[\alpha]_D^{25}$  = -68.8 ( $c$  = 0.35, CHCl<sub>3</sub>);  $\nu_{\max}/\text{cm}^{-1}$  (neat) 1586 (s, C=C), 1635 (s, C=O); <sup>1</sup>H NMR (methanol-*d*<sub>4</sub>, 400 MHz):  $\delta$  (C6-OH not seen), 8.27 (d, 1H,  $J$  = 5.2 Hz, H6'), 7.63 (s, 1H, H3'), 7.44 (d, 1H,  $J$  = 5.2 Hz, H5'), 6.30 (s, 1H, H2), 4.17 – 4.24 (m, 3H, OCH<sub>2</sub>CH<sub>3</sub> and H5), 3.21 – 3.25 (dd, 1H, H4<sub>B</sub> obscured by solvent peak), 2.97 (dd, 1H,  $J$  = 11.1, 7.2 Hz, H4<sub>A</sub>), 1.26 (t, 3H,  $J$  = 7.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C NMR (methanol-*d*<sub>4</sub>, 101 MHz):  $\delta$  194.6 (C6), 179.7 (C8), 167.7 (C9), 156.4 (C6'), 151.2 (C4'), 142.8 (C2'), 126.7 (C3'), 122.2 (C5'), 91.5 (C7), 70.2 (C5), 63.3 (C2), 60.2 (OCH<sub>2</sub>CH<sub>3</sub>), 34.6 (C4), 15.1 (OCH<sub>2</sub>CH<sub>3</sub>);

$m/z$  (ESI<sup>-</sup>) 383 and 385 ([M-H]<sup>-</sup>, 100%); HRMS (ESI<sup>-</sup>)  $m/z$ : [M-H]<sup>-</sup> Calcd for C<sub>14</sub>H<sub>12</sub>O<sub>4</sub>N<sub>2</sub>BrS 382.9707 and 384.9686; Found 382.9712 and 384.9690.

**(2S,5R)-1-Aza-2-(5-bromopyridin-2-yl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 4c**

Yield (1.18 g, 40%); brown foamy solid, mp 157 – 163 °C;  $R_f$  = 0.26 (MeOH : EtOAc; 1 : 6);  $[\alpha]_D^{25}$  = -129.8 ( $c$  = 1.01, CHCl<sub>3</sub>);  $\nu_{\max}/\text{cm}^{-1}$  (neat) 1581 (s, C=C), 1638 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  (C6-OH not seen), 8.60 (d, 1H,  $J$  = 2.2 Hz, H6'), 7.78 (dd, 1H,  $J$  = 8.3, 2.3 Hz, H4'), 7.29 (d, 1H,  $J$  = 8.3 Hz, H3'), 6.29 (s, 1H, H2), 4.88 (app t, 1H,  $J$  = 7.7 Hz, H5), 4.36 (q, 2H,  $J$  = 7.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.37 (dd, 1H,  $J$  = 10.9, 7.3 Hz, H4<sub>B</sub>), 3.01 (dd, 1H,  $J$  = 10.9, 8.1 Hz, H4<sub>A</sub>), 1.36 (t, 3H,  $J$  = 7.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  187.2 (C6), 170.0 (C8), 166.9 (C9), 158.4 (C2'), 150.7 (C6'), 139.5 (C4'), 121.5 (C3'), 119.8 (C5'), 97.8 (C7), 66.4 (C5), 62.6 (C2), 61.5 (OCH<sub>2</sub>CH<sub>3</sub>), 32.9 (C4), 14.3 (OCH<sub>2</sub>CH<sub>3</sub>);  $m/z$  (ESI<sup>-</sup>) 383 and 385 ([M-H]<sup>-</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$ : [M+Na]<sup>+</sup> Calcd for C<sub>14</sub>H<sub>13</sub>O<sub>4</sub>N<sub>2</sub>BrNaS 406.9672 and 408.9651; Found 406.9673 and 408.9652.

**(2S,5R)-1-Aza-2-(6-chloropyridin-3-yl)-7-ethoxycarbonyl-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 4d**

Yield (974 mg, 41%); orange oil;  $R_f$  = 0.36 (MeOH : EtOAc; 1 : 6);  $[\alpha]_D^{25}$  = -168.7 ( $c$  = 0.13, CHCl<sub>3</sub>);  $\nu_{\max}/\text{cm}^{-1}$  1585 (s, C=C), 1653 (s, C=O), 1704 (s, C=O); <sup>1</sup>H NMR (methanol-*d*<sub>4</sub>, 400 MHz):  $\delta$  (C6-OH not seen), 8.47 (s, 1H, H2'), 7.94 (d, 1H,  $J$  = 8.2 Hz, H4'), 7.45 (d, 1H,  $J$  = 8.3 Hz, H5'), 6.28 (s, 1H, H2), 4.83 (app t, 1H,  $J$  = 7.5 Hz, H5), 4.28 (q, 2H,  $J$  = 7.5 Hz, OCH<sub>2</sub>CH<sub>3</sub>), 3.32 – 3.38 (m, 1H, H4<sub>B</sub>), 3.07 (dd, 1H,  $J$  = 10.9, 8.3 Hz, H4<sub>A</sub>), 1.31 (t, 3H,  $J$  = 7.1 Hz, OCH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C NMR (methanol-*d*<sub>4</sub>, 101 MHz):  $\delta$  185.5 (C6), 173.5 (C8), 164.7 (C9), 151.7 (C2'), 149.0 (C6'), 139.4 (C4'), 137.8 (C3'), 125.4 (C5'), 98.8 (C7), 67.6 (C5), 61.5 (C2), 60.9 (OCH<sub>2</sub>CH<sub>3</sub>), 33.7 (C4), 14.7 (OCH<sub>2</sub>CH<sub>3</sub>);  $m/z$  (ESI<sup>-</sup>) 339 ([M-H]<sup>-</sup>, 100%)



and 341 ( $[M-H]^-$ , 33%); HRMS (ESI $^-$ )  $m/z$ :  $[M-H]^-$  Calcd for  $C_{14}H_{12}O_4N_2ClS$  339.0212 and 341.0182; Found 339.0208 and 341.0177.

**(2*R*,5*R*)-1-Aza-2-(6-chloropyridin-3-yl)-5-methoxycarbonyl-6,8-dioxo-3-thiabicyclo[3.3.0]-octane, 5**

Yield (70 mg, 3%); orange oil;  $R_f$  = 0.21 (MeOH : EtOAc; 1 : 6);  $[\alpha]_D^{25}$  = -113.9 ( $c$  = 1.00, MeOH);  $\nu_{max}/cm^{-1}$  1583 (s, C=C), 1695 (s, C=O);  $^1H$  NMR (methanol- $d_4$ , 400 MHz):  $\delta$  8.48 (s, 1H, H2'), 7.95 (d, 1H,  $J$  = 8.3 Hz, H4'), 7.45 (d, 1H,  $J$  = 8.3 Hz, H5'), 6.40 (s, 1H, H2), 4.17 – 4.30 (m, 2H, H7), 3.70 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 3.21 – 3.28 (obscured, H4<sub>B</sub>), 3.03 (dd, 1H,  $J$  = 11.2, 7.2 Hz, H4<sub>A</sub>);  $^{13}C$  NMR (methanol- $d_4$ , 101 MHz):  $\delta$  194.6 (C6), 180.0 (C8), 167.1 (CO<sub>2</sub>CH<sub>3</sub>), 151.2 (C2'), 148.9 (C6'), 139.4 (C4'), 139.1 (C3'), 125.2 (C5'), 70.4 (C5), 62.5 (C2), 59.5 (CO<sub>2</sub>CH<sub>3</sub>), 50.3 (C7), 34.8 (C4);  $m/z$  (ESI $^-$ ) 325 ( $[M-H]^-$ , 100%) and 327 ( $[M-H]^-$ , 33%); HRMS (ESI $^+$ )  $m/z$ :  $[M+H]^+$  Calcd for  $C_{13}H_{12}O_4N_2ClS$  327.0201 and 329.0171; Found 327.0203 and 329.0172.

**(2*S*,5*R*)-1-Aza-7-(4-chloro-2-methylphenylaminocarbonyl)-6-hydroxy-8-oxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]oct-6-ene, 6a**

Yield (183 mg, 70%); brown oil;  $R_f$  = 0.34 (MeOH : EtOAc; 1 : 6);  $[\alpha]_D^{25}$  = -114.6 ( $c$  = 0.67, CHCl<sub>3</sub>);  $\nu_{max}/cm^{-1}$  1644 (s, C=O), 1678 (s, C=O);  $^1H$  NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  9.39 (br. s, 1H, NH), 8.61 (br. d, 1H, H6'), 8.02 (br. d, 1H, H6''), 7.72 (t, 1H,  $J$  = 7.6 Hz, H4'), 7.41 (d, 1H,  $J$  = 7.7 Hz, H3'), 7.19 (br. s, 3H, H3'', H5'' and H5'), 6.35 (s, 1H, H2), 5.11 (br. s, 1H, H5), 3.45 (dd, 1H,  $J$  = 18.7, 8.1 Hz, H4<sub>B</sub>), 3.06 – 3.16 (m, 1H, H4<sub>A</sub>), 2.33 (s, 3H, CH<sub>3</sub>);  $^{13}C$  NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  188.0 (C6), C8 not shown, 163.8 (C9), 159.1 (C2'), 149.7 (C6'), 138.2 (C4'), 133.7 (C2''), 130.5 (C4''), 130.1 (C1'', C3''), 126.8 (C5''), 123.2 (C3'), 122.8 (C6''), 120.2 (C5'), C7 not shown, 67.1 (C5), 62.2 (C2), 32.4 (C4), 17.8 (CH<sub>3</sub>);  $m/z$  (ESI $^-$ ) 400 ( $[M-H]^-$ , 100%) and 402 ( $[M-H]^-$ , 33%); HRMS (ESI $^+$ )  $m/z$ :  $[M+H]^+$  Calcd for  $C_{19}H_{17}O_3N_3ClS$  402.0674 and 404.0644; Found 402.0674 and 404.0643.

**(2*S*,5*R*)-1-Aza-2-(2-bromopyridin-4-yl)-7-(4-chloro-2-methylphenylaminocarbonyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 6b**

Yield (130 mg, 52%); orange oil;  $R_f$  = 0.30 (100% EtOAc);  $[\alpha]_D^{25}$  = -151.6 ( $c$  = 1.01,  $\text{CHCl}_3$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  1587 (s, C=C), 1634 (s, C=O), 1695 (s, C=O);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  9.28 (br. s, 1H, NH), 8.37 (d, 1H,  $J$  = 5.1 Hz, H6'), 8.00 (d, 1H,  $J$  = 8.6 Hz, H6''), 7.59 (s, 1H, H3'), 7.33 (d, 1H,  $J$  = 4.9 Hz, H5'), 7.19 (app d, 2H,  $J$  = 7.0 Hz, H3'', H5''), 6.18 (s, 1H, H2), 4.81 (app t, 1H,  $J$  = 6.9 Hz, H5), 3.29 – 3.34 (m, 1H, H4<sub>B</sub>), 3.10 (dd, 1H,  $J$  = 11.2, 8.3 Hz, H4<sub>A</sub>), 2.33 (s, 3H, CH<sub>3</sub>);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  184.9 (C6), 172.3 (C8), 163.6 (C9), 151.9 (C6'), 150.6 (C4'), 142.9 (C2'), 133.3 (C2''), 131.0 (C4''), 130.6 (C3''), 130.2 (C1''), 126.9 (C5''), 125.5 (C3'), 122.9 (C6''), 120.6 (C5'), 98.9 (C7), 66.5 (C5), 60.2 (C2), 32.6 (C4), 17.7 (CH<sub>3</sub>);  $m/z$  (ESI<sup>-</sup>) 478, 480 ([M-H]<sup>-</sup>, 100%) and 482 ([M-H]<sup>-</sup>, 33%); HRMS (ESI<sup>-</sup>)  $m/z$ : [M-H]<sup>-</sup> Calcd for C<sub>19</sub>H<sub>14</sub>O<sub>3</sub>N<sub>3</sub>BrClS 477.9633, 479.9613 and 481.9583; Found 477.9636, 479.9612 and 481.9580.

**(2*S*,5*R*)-1-Aza-2-(5-bromopyridin-2-yl)-7-(4-chloro-2-methylphenylaminocarbonyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 6c**

Yield (154 mg, 62%); orange oil;  $R_f$  = 0.39 (100% EtOAc);  $[\alpha]_D^{25}$  = -142.1 ( $c$  = 0.63,  $\text{CHCl}_3$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  1483 (s, C=C), 1587 (s, C=C), 1635 (s, C=O), 1693 (s, C=O);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  9.34 (br. s, 1H, NH), 8.65 (s, 1H, H6'), 8.03 (d, 1H,  $J$  = 9.2 Hz, H6''), 7.83 (d, 1H,  $J$  = 8.3 Hz, H4'), 7.56 (br. s, 1H, OH), 7.31 (d, 1H,  $J$  = 8.3 Hz, H3'), 7.19 (br. s, 2H, H3'', H5''), 6.28 (s, 1H, H2), 5.08 (app t, 1H,  $J$  = 7.6 Hz, H5), 3.44 (dd, 1H,  $J$  = 10.8, 7.5 Hz, H4<sub>B</sub>), 3.10 (dd, 1H,  $J$  = 10.6, 8.5 Hz, H4<sub>A</sub>), 2.33 (s, 3H, CH<sub>3</sub>);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  185.0 (C6), 171.9 (C8), 163.7 (C9), 157.8 (C2'), 151.1 (C6'), 139.7 (C4'), 133.5 (C2''), 130.5 (C4''), 130.3 (C3''), 130.1 (C1''), 126.9 (C5''), 122.8 (C6''), 121.5 (C3'), 120.1 (C5'), 99.0 (C7), 67.0 (C5), 61.8 (C2), 32.3 (C4), 17.7 (CH<sub>3</sub>);  $m/z$  (ESI<sup>-</sup>) 478 and 480 ([M-H]<sup>-</sup>, 100%) and 482

( $[M-H]^-$ , 33%); HRMS ( $ESI^-$ )  $m/z$ :  $[M-H]^-$  Calcd for  $C_{19}H_{14}O_3N_3BrClS$  477.9633, 479.9613 and 481.9583; Found 477.9632, 479.9608 and 481.9575.

**(2S,5R)-1-Aza-7-(4-chloro-2-methylphenylaminocarbonyl)-2-(6-chloropyridin-3-yl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 6d**

Yield (171 mg, 67%); orange oil;  $R_f$  = 0.31 (100% EtOAc);  $[\alpha]_D^{25}$  = -145.4 ( $c$  = 1.01,  $CHCl_3$ );  $\nu_{max}/cm^{-1}$  1585 (s, C=C), 1633 (s, C=O), 1693 (s, C=O);  $^1H$  NMR ( $CDCl_3$ , 400 MHz):  $\delta$  9.29 (br. s, 1H, NH), 8.90 (br. s, 1H, OH), 8.52 (s, 1H, H2'), 8.00 (d, 1H,  $J$  = 8.9 Hz, H6''), 7.78 (d, 1H,  $J$  = 8.3, H4'), 7.34 (d, 1H,  $J$  = 8.3 Hz, H5'), 7.19 (app d, 2H,  $J$  = 7.0 Hz, H3'', H5''), 6.24 (s, 1H, H2), 4.82 (app t, 1H,  $J$  = 7.4 Hz, H5), 3.33 (dd, 1H,  $J$  = 11.3, 7.1 Hz, H4<sub>B</sub>), 3.10 (dd, 1H,  $J$  = 11.3, 8.4 Hz, H4<sub>A</sub>), 2.33 (s, 3H, CH<sub>3</sub>);  $^{13}C$  NMR ( $CDCl_3$ , 101 MHz):  $\delta$  184.7 (C6), 172.3 (C8), 163.7 (C9), 151.7 (C2'), 148.3 (C6'), 137.6 (C4'), 134.8 (C3'), 133.5 (C2''), 130.7 (C4''), 130.5 (C3''), 130.3 (C1''), 127.0 (C5''), 124.5 (C5'), 123.0 (C6''), 99.2 (C7), 66.6 (C5), 59.5 (C2), 32.7 (C4), 17.8 (CH<sub>3</sub>);  $m/z$  ( $ESI^-$ ) 434 ( $[M-H]^-$ , 100%) and 436 ( $[M-H]^-$ , 65%); HRMS ( $ESI^-$ )  $m/z$ :  $[M-H]^-$  Calcd for  $C_{19}H_{14}O_3N_3Cl_2S$  434.0138 and 436.0109; Found 434.0134 and 436.0104.

**(2S,5R)-1-Aza-7-(cyclohexylaminocarbonyl)-6-hydroxy-8-oxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]oct-6-ene, 7a**

Yield (96 mg, 41%); orange oil;  $R_f$  = 0.47 (MeOH : EtOAc; 1 : 6);  $[\alpha]_D^{25}$  = +116.9 ( $c$  = 1.00,  $CHCl_3$ );  $\nu_{max}/cm^{-1}$  1557 (s, C=C), 1620 (s, C=O), 1688 (s, C=O);  $^1H$  NMR ( $CDCl_3$ , 400 MHz):  $\delta$  (NH and OH obscured), 8.58 (d, 1H,  $J$  = 4.2 Hz, H6'), 7.67 (app td, 1H,  $J$  = 7.7, 1.7 Hz, H4'), 7.38 (d, 1H,  $J$  = 7.8 Hz, H3'), 7.20 (dd, 1H,  $J$  = 7.0, 5.2 Hz, H5'), 6.33 (s, 1H, H2), 4.84 (br. s, 1H, H5), 3.79 – 3.89 (m, 1H, H10), 3.35 (dd, 1H,  $J$  = 10.9, 7.3 Hz, H4<sub>B</sub>), 3.03 (dd, 1H,  $J$  = 10.9, 8.4 Hz, H4<sub>A</sub>), 1.87 – 1.98 (m, 2H, 2 x H11), 1.69 – 1.80 (m, 2H, 2 x H12), 1.56 – 1.65 (m, 2H, 2 x H13), 1.17 – 1.44 (m, 2 x H11, 2 x H12);  $^{13}C$  NMR ( $CDCl_3$ , 101 MHz):  $\delta$  (C8 and C6 not shown), 165.5 (C9), 159.8 (C2'), 149.9 (C6'), 137.1

(C4'), 122.9 (C3'), 119.9 (C5'), 100.1 (C7), 68.3 (C5), 62.8 (C2), 46.0 (C10), 38.8 (C11), 32.8 (C4), 25.4 (C13), 24.6 (C12);  $m/z$  (ESI<sup>-</sup>) 358 ([M-H]<sup>-</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$ : [M+H]<sup>+</sup> Calcd for C<sub>18</sub>H<sub>22</sub>O<sub>3</sub>N<sub>3</sub>S 360.1376; Found 360.1378.

**(2S,5R)-1-Aza-2-(2-bromopyridin-4-yl)-7-(cyclohexylaminocarbonyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 7b**

Yield (34 mg, 15%); orange oil;  $R_f$  = 0.65 (MeOH : EtOAc; 1 : 9);  $[\alpha]_D^{25}$  = -128.0 ( $c$  = 0.60, CHCl<sub>3</sub>);  $\nu_{\max}/\text{cm}^{-1}$  1586 (s, C=C), 1624 (s, C=O), 1691 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): a 3.3 : 1 mixture of AB/CD tautomers:  $\delta$  8.34 (d, 1H,  $J$  = 5.0 Hz, H6'), 7.86 (br. s, 1H, OH), 7.57 (s, 1H, H3'), 7.42 (br. s, 1H, NH), 7.31 (d, 1H,  $J$  = 4.8 Hz, H5'), 6.24 (s, 1H, H2 minor tautomer), 6.16 (s, 1H, H2 major tautomer), 4.73 (br. s, 1H, H5 minor tautomer), 4.61 (br. s, 1H, H5 major tautomer), 3.79 – 3.92 (m, 1H, H10), 3.21 – 3.32 (m, 1H, H4<sub>B</sub>), 3.01 – 3.11 (m, 1H, H4<sub>A</sub>), 1.88 – 1.97 (m, 2H, 2 x H11), 1.71 – 1.80 (m, 2H, 2 x H12), 1.55 – 1.68 (m, 2H, 2 x H13), 1.21 – 1.48 (m, 4H, 2 x H11, 2 x H12); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz):  $\delta$  191.2 (C6 minor), 189.1 (C6 major), 178.4 (C8 minor), 172.8 (C8 major), 165.4 (C9), 152.6 (C6'), 150.5 (C4'), 142.8 (C2'), 125.6 (C3'), 120.7 (C5'), 95.8 (C7 minor), 93.8 (C7 major), 70.5 (C5 minor), 67.6 (C5 major), 61.2 (C2 minor), 60.6 (C2 major), 49.9 (C10 minor), 48.6 (C10 major), 32.8 (C4), 32.1 (C11), 25.3 (C13), 24.5 (C12);  $m/z$  (ESI<sup>-</sup>) 436 and 438 ([M-H]<sup>-</sup>, 100%); HRMS (ESI<sup>-</sup>)  $m/z$ : [M-H]<sup>-</sup> Calcd for C<sub>18</sub>H<sub>19</sub>O<sub>3</sub>N<sub>3</sub>BrS 436.0336 and 438.0316; Found 436.0336 and 438.0315.

**(2S,5R)-1-Aza-2-(5-bromopyridin-2-yl)-7-(cyclohexylaminocarbonyl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 7c**

Yield (71 mg, 31%); yellow oil;  $R_f$  = 0.81 (MeOH : EtOAc; 1 : 6);  $[\alpha]_D^{25}$  = -87.3 ( $c$  = 1.01, CHCl<sub>3</sub>);  $\nu_{\max}/\text{cm}^{-1}$  1558 (s, C=C), 1648 (s, C=O), 1690 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  9.96 (br. s, 1H, NH), 8.60 (s, 1H, H6'), 7.77 (d, 1H,  $J$  = 8.3 Hz, H4'), 7.44 (br. s, 1H, OH), 7.28 (d, 1H,  $J$  = 8.3 Hz, H3'),

6.25 (s, 1H, H<sub>2</sub>), 4.81 (br. s, 1H, H<sub>5</sub>), 3.72 – 3.91 (m, 1H, H<sub>10</sub>), 3.32 (dd, 1H, *J* = 10.9, 7.4 Hz, H<sub>4B</sub>), 3.01 (dd, 1H, *J* = 10.8, 8.4 Hz, H<sub>4A</sub>), 1.78 – 2.09 (m, 2H, 2 x H<sub>11</sub>), 1.63 – 1.78 (m, 2H, 2 x H<sub>12</sub>), 1.48 – 1.65 (m, 2H, 2 x H<sub>13</sub>), 1.21 – 1.48 (m, 4H, 2 x H<sub>11</sub>, 2 x H<sub>12</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 188.5 (C<sub>6</sub>), 172.3 (C<sub>8</sub>), 165.4 (C<sub>9</sub>), 158.4 (C<sub>2'</sub>), 150.8 (C<sub>6'</sub>), 139.5 (C<sub>4'</sub>), 121.3 (C<sub>3'</sub>), 119.7 (C<sub>5'</sub>), 107.3 (C<sub>7</sub>), 67.9 (C<sub>5</sub>), 62.2 (C<sub>2</sub>), 48.3 (C<sub>10</sub>), 32.7 (C<sub>4</sub>), 32.0 (C<sub>11</sub>), 25.3 (C<sub>13</sub>), 24.5 (C<sub>12</sub>); *m/z* (APCI<sup>+</sup>) 438 and 440 ([M+H]<sup>+</sup>, 100%); HRMS (APCI<sup>+</sup>) *m/z*: [M+H]<sup>+</sup> Calcd for C<sub>18</sub>H<sub>21</sub>O<sub>3</sub>N<sub>3</sub>BrS 438.0482 and 440.0461; Found 438.0477 and 440.0456.

**(2*S*,5*R*)-1-Aza-7-(cyclohexylaminocarbonyl)-2-(6-chloropyridin-3-yl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 7d**

Yield (55 mg, 24%); yellow oil; *R<sub>f</sub>* = 0.62 (MeOH : EtOAc; 1 : 6); [α]<sub>D</sub><sup>25</sup> = -141.1 (*c* = 1.00, CHCl<sub>3</sub>); *v*<sub>max</sub>/cm<sup>-1</sup> 1560 (s, C=C), 1624 (s, C=O), 1687 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ (NH obscured), 8.48 (s, 1H, H<sub>2'</sub>), 7.75 (d, 1H, *J* = 8.3 Hz, H<sub>4'</sub>), 7.30 (d, 1H, *J* = 8.3 Hz, H<sub>5'</sub>), 6.89 (br. s, 1H, OH), 6.24 (s, 1H, H<sub>2</sub>), 4.59 (br. s, 1H, H<sub>5</sub>), 3.76 – 3.90 (m, 1H, H<sub>10</sub>), 3.26 (dd, 1H, *J* = 11.2, 7.4 Hz, H<sub>4B</sub>), 3.05 (dd, 1H, *J* = 11.2, 8.2 Hz, H<sub>4A</sub>), 1.84 – 2.00 (m, 2H, 2 x H<sub>11</sub>), 1.69 – 1.80 (m, 2H, 2 x H<sub>12</sub>), 1.52 – 1.71 (m, 2H, 2 x H<sub>13</sub>), 1.20 – 1.43 (m, 4H, 2 x H<sub>11</sub>, 2 x H<sub>12</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz): δ 188.7 (C<sub>6</sub>), 173.3 (C<sub>8</sub>), 165.4 (C<sub>9</sub>), 151.3 (C<sub>2'</sub>), 148.1 (C<sub>6'</sub>), 137.5 (C<sub>4'</sub>), 135.3 (C<sub>3'</sub>), 124.2 (C<sub>5'</sub>), 100.1 (C<sub>7</sub>), 67.4 (C<sub>5</sub>), 59.9 (C<sub>2</sub>), 48.5 (C<sub>10</sub>), 32.7 (C<sub>4</sub>), 32.0 (C<sub>11</sub>), 25.3 (C<sub>13</sub>), 24.5 (C<sub>12</sub>); *m/z* (ESI<sup>-</sup>) 392 ([M-H]<sup>-</sup>, 100%) and 394 ([M-H]<sup>-</sup>, 33%); HRMS (ESI<sup>-</sup>) *m/z*: [M-H]<sup>-</sup> Calcd for C<sub>18</sub>H<sub>19</sub>O<sub>3</sub>N<sub>3</sub>ClS 392.0841 and 394.0812; Found 392.0843 and 394.0811.

**(2*S*,5*R*)-1-Aza-2-(2-bromopyridin-4-yl)-6-hydroxy-8-oxo-7-(tetrahydro-2*H*-pyran-4-aminocarbonyl)-3-thiabicyclo[3.3.0]oct-6-ene, 8b**

Yield (28 mg, 12%); orange oil; *R<sub>f</sub>* = 0.44 (MeOH : EtOAc; 1 : 6); [α]<sub>D</sub><sup>25</sup> = -127.7 (*c* = 1.30, CHCl<sub>3</sub>); *v*<sub>max</sub>/cm<sup>-1</sup> 1587 (s, C=C), 1623 (s, C=O), 1648 (s, C=O), 1691 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): a

3.3 : 1 mixture of AB/CD tautomers:  $\delta$  9.29 (br. s, 1H, NH), 8.53 (d,  $J$  = 5.0 Hz, H6' minor), 8.35 (d, 1H,  $J$  = 5.1 Hz, H6' major), 8.07 (s, H3' minor), 7.83 (d,  $J$  = 5.0 Hz, H5' minor), 7.57 (s, 1H, H3'), 7.41 (br. s, 1H, OH), 7.32 (d, 1H,  $J$  = 5.0 Hz, H5' major), 6.20 (s, H2 minor), 6.14 (s, 1H, H2 major), 4.67 (br. s, 1H, H5 major), 4.39 (dd,  $J$  = 7.1, 2.2 Hz, H5 minor), 4.03 – 4.11 (m, 1H, H10), 3.98 (d, 2H,  $J$  = 11.3 Hz, 2 x H12), 3.46 – 3.52 (m, 2H, 2 x H12), 3.27 (dd, 1H,  $J$  = 11.2, 7.2 Hz, H4<sub>B</sub>), 3.05 (dd, 1H,  $J$  = 11.2, 8.1 Hz, H4<sub>A</sub>), 1.92 (d, 2H,  $J$  = 11.2 Hz, 2x H11), 1.54 – 1.67 (m, 2H, 2x H11);  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 126 MHz):  $\delta$  193.0 (C6 minor), 187.2 (C6 major), 176.9 (C8 minor), 172.5 (C8 major), 165.9 (C9 minor), 165.3 (C9 major), 152.4 (C6' minor), 151.0 (C6' major), 150.5 (C4' major), 150.1 (C4' minor), 142.8 (C2' major), 140.5 (C2' minor), 128.3 (C3' minor), 125.6 (C3' major), 122.4 (C5' minor), 120.7 (C5' major), 95.7 (C7 major), 83.9 (C7 minor), 67.0 (C5), 66.4 (C12), 60.4 (C2), 45.7 (C10), 32.7 (C4), 32.0 (C11);  $m/z$  (ESI<sup>+</sup>) 440 and 442 ( $[\text{M}+\text{H}]^+$ , 100%); HRMS (ESI<sup>+</sup>)  $m/z$ :  $[\text{M}+\text{H}]^+$  Calcd for  $\text{C}_{17}\text{H}_{17}\text{O}_4\text{N}_3\text{BrS}$  438.0129 and 440.0108; Found 438.0134 and 440.0111.

**(2S,5R)-1-Aza-2-(5-bromopyridin-2-yl)-6-hydroxy-8-oxo-7-(tetrahydro-2H-pyran-4-aminocarbonyl)-3-thiabicyclo[3.3.0]oct-6-ene, 8c**

Yield (23 mg, 10%); orange oil;  $R_f$  = 0.50 (MeOH : EtOAc; 1 : 6);  $[\alpha]_{\text{D}}^{25}$  = -119.8 ( $c$  = 0.74,  $\text{CHCl}_3$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  1578 (s, C=C), 1644 (s, C=O), 1676 (s, C=O);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz): a 3.4 : 1 mixture of AB/CD tautomers:  $\delta$  (NH and OH obscured), 8.63 (s, 1H, H6' minor), 8.61 (s, 1H, H6' major), 7.78 (d, 1H,  $J$  = 8.3 Hz, H4' major), 7.29 (d, 1H,  $J$  = 8.3 Hz, H3'), 6.30 (s, 1H, H2 major), 6.25 (s, 1H, H2 minor), 4.90 (app t, 1H,  $J$  = 7.6 Hz, H5), 4.37 (app q, 1H,  $J$  = 7.0 Hz, H10), 3.90 – 4.02 (m, 2H, 2 x H12), 3.42 – 3.54 (m, 2H, 2 x H12), 3.37 (dd, 1H,  $J$  = 10.9, 7.3 Hz, H4<sub>B</sub>), 2.97 – 3.07 (m, 1H, H4<sub>A</sub>), 1.82 – 1.98 (m, 2H, 2x H11), 1.52 – 1.68 (m, 2H, 2x H11);  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 126 MHz):  $\delta$  177.9 (C6), 173.0 (C8), 167.9 (C9), 159.1 (C2' minor), 158.2 (C2' major), 150.9 (C6' major), 150.8 (C6' minor), 139.7 (C4'), 121.6 (C3' minor), 121.4 (C3' major), 120.7 (C5' minor), 119.9 (C5' major), 96.2 (C7), 66.9, 66.7, 66.5, 66.1 (C5 and C12, major and minor tautomers), 62.4 (C2), 45.9 (C10), 32.8 (C4), 32.1 (C11);

$m/z$  (ESI<sup>+</sup>) 440 and 442 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$ : [M-H]<sup>-</sup> Calcd for C<sub>17</sub>H<sub>17</sub>O<sub>4</sub>N<sub>3</sub>BrS 438.0129 and 440.0108; Found 438.0132 and 440.0110.

**(2S,5R)-7-(Adamantylaminocarbonyl)-1-aza-6-hydroxy-8-oxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]oct-6-ene, 9a**

Yield (118 mg, 44%); brown oil;  $R_f$  = 0.70 (MeOH : EtOAc; 1 : 6);  $[\alpha]_D^{25}$  = -21.6 ( $c$  = 1.00, CHCl<sub>3</sub>);  $\nu_{\max}/\text{cm}^{-1}$  1560 (s, C=C), 1633 (s, C=O), 1684 (C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  (OH obscured), 10.15 (br. s, 1H, NH), 8.58 (d, 1H,  $J$  = 4.3 Hz, H6'), 7.67 (app td, 1H,  $J$  = 7.7, 1.7 Hz, H4'), 7.38 (d, 1H,  $J$  = 7.8 Hz, H3'), 7.19 (dd, 1H,  $J$  = 7.7, 5.2 Hz, H5'), 6.31 (s, 1H, H2), 4.84 (br. s, 1H, H5), 3.33 (dd, 1H,  $J$  = 10.9, 7.3 Hz, H4<sub>B</sub>), 3.01 (dd, 1H,  $J$  = 10.9, 8.5 Hz, H4<sub>A</sub>), 2.11 (br. s, 3H, Adamantyl-CH), 2.05 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.69 (br. s, 6H, Adamantyl-CH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  188.8 (C6), C8 not shown, 166.3 (C9), 159.8 (C2'), 149.8 (C6'), 137.1 (C4'), 122.9 (C3'), 119.8 (C5'), C7 not shown, 68.0 (C5), 62.7 (C2), 53.3 (Adamantyl-C), 41.7 (Adamantyl-CH<sub>2</sub>), 36.1 (Adamantyl-CH<sub>2</sub>), 32.7 (C4), 29.4 (Adamantyl-CH);  $m/z$  (ESI<sup>-</sup>) 410 ([M-H]<sup>-</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$ : [M+H]<sup>+</sup> Calcd for C<sub>22</sub>H<sub>26</sub>O<sub>3</sub>N<sub>3</sub>S 412.1689; Found 412.1691.

**(2S,5R)-7-(Adamantylaminocarbonyl)-1-aza-2-(2-bromopyridin-4-yl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 9b**

Yield (84 mg, 33%); orange oil;  $R_f$  = 0.62 (MeOH : EtOAc; 1 : 9);  $[\alpha]_D^{25}$  = -135.6 ( $c$  = 1.01, CHCl<sub>3</sub>);  $\nu_{\max}/\text{cm}^{-1}$  1584 (s, C=C), 1648 (s, C=O), 1688 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  (OH obscured), 8.99 (br. s, 1H, NH), 8.32 (d, 1H,  $J$  = 5.1 Hz, H6'), 7.55 (s, 1H, H3'), 7.30 (d, 1H,  $J$  = 5.2 Hz, H5'), 6.17 (s, 1H, H2), 4.48 (br. s, 1H, H5), 3.24 (dd, 1H,  $J$  = 11.2, 7.4 Hz, H4<sub>B</sub>), 3.04 (dd, 1H,  $J$  = 11.2, 8.1 Hz, H4<sub>A</sub>), 2.12 (br. s, 3H, Adamantyl-CH), 2.05 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.69 (br. s, 6H, Adamantyl-CH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  190.0 (C6), C8 not shown, 166.2 (C9), 152.8 (C6'), 150.4 (C4'), 142.8 (C2'), 125.5 (C3'), 120.7 (C5'), 100.1 (C7), 68.4 (C5), 60.8 (C2), 53.9 (Adamantyl-C), 41.7

(Adamantyl-CH<sub>2</sub>), 36.1 (Adamantyl-CH<sub>2</sub>), 32.9 (C<sub>4</sub>), 29.4 (Adamantyl-CH); *m/z* (ESI<sup>-</sup>) 488 and 490 ([M-H], 100%); HRMS (ESI<sup>-</sup>) *m/z*: [M-H]<sup>-</sup> Calcd for C<sub>22</sub>H<sub>23</sub>O<sub>3</sub>N<sub>3</sub>BrS 488.0649 and 490.0629; Found 488.0652 and 490.0629.

**(2S,5R)-7-(Adamantylaminocarbonyl)-1-aza-2-(5-bromopyridin-2-yl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 9c**

Yield (97 mg, 38%); brown oil; *R<sub>f</sub>* = 0.56 (MeOH : EtOAc; 1 : 9); [ $\alpha$ ]<sub>D</sub><sup>25</sup> = -97.0 (*c* = 0.53, CHCl<sub>3</sub>);  $\nu_{\text{max}}$ /cm<sup>-1</sup> 1559 (s, C=C), 1648 (s, C=O), 1687 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): a 2.3 : 1 mixture of AB/CD tautomers:  $\delta$  8.63 (s, 1H, H6'), 7.80 (dd, 1H, *J* = 8.3, 2.2 Hz, H4'), 7.30 (d, 1H, *J* = 8.3 Hz, H3'), 6.53 (br. s, 2H, OH and NH), 6.33 (s, H2 minor), 6.25 (s, 1H, H2 major), 4.81 (t, 1H, *J* = 7.8 Hz, H5 major), 4.57 (t, *J* = 7.9 Hz, H5 minor), 3.33 (app dt, 1H, *J* = 11.0, 6.8 Hz, H4<sub>B</sub>), 2.99 – 3.07 (m, 1H, H4<sub>A</sub>), 2.12 (br. s, 3H, Adamantyl-CH), 2.05 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.70 (br. s, 6H, Adamantyl-CH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  189.2 (C6 major), 172.5 (C8 major), 166.2 (C9 major), 158.4 (C2'), 150.9 (C6'), 140.9 (C4' minor), 139.6 (C4' major), 121.3 (C3'), 119.8 (C5'), 94.3 (C7 major), 68.1 (C5 major), 66.3 (C5 minor), 62.9 (C2 minor), 62.2 (C2 major), 53.4 (Adamantyl-C), 44.3 (Adamantyl-CH<sub>2</sub> minor), 41.7 (Adamantyl-CH<sub>2</sub> major), 36.2 (Adamantyl-CH<sub>2</sub> major), 36.0 (Adamantyl-CH<sub>2</sub> minor), 33.0 (C4 minor), 32.6 (C4 major), 29.8 (Adamantyl-CH minor), 29.4 (Adamantyl-CH major); *m/z* (ESI<sup>+</sup>) 490 and 492 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) *m/z*: [M+H]<sup>+</sup> Calcd for C<sub>22</sub>H<sub>25</sub>O<sub>3</sub>N<sub>3</sub>BrS 490.0795 and 492.0774; Found 490.0795 and 492.0773.

**(2S,5R)-7-(Adamantylaminocarbonyl)-1-aza-2-(6-chloropyridin-3-yl)-6-hydroxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 9d**

Yield (92 mg, 35%); orange oil; *R<sub>f</sub>* = 0.70 (MeOH : EtOAc; 1 : 9); [ $\alpha$ ]<sub>D</sub><sup>25</sup> = -90.5 (*c* = 1.10, CHCl<sub>3</sub>);  $\nu_{\text{max}}$ /cm<sup>-1</sup> 1562 (s, C=C), 1627 (s, C=O), 1686 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): a 3.2 : 1 mixture of AB/CD tautomers:  $\delta$  (NH obscured), 8.49 (s, 1H, H2'), 7.75 (d, 1H, *J* = 8.3, H5' major), 7.39 (br. s, 1H,



OH), 7.32 (d, 1H,  $J$  = 8.3 Hz, H4' major tautomer), 6.31 (s, H2 minor), 6.23 (s, 1H, H2 major), 4.59 (app t, 1H,  $J$  = 7.7 Hz, H5), 3.26 (dd, 1H,  $J$  = 11.2, 7.2 Hz, H4<sub>B</sub>), 3.05 (dd, 1H,  $J$  = 11.1, 8.2 Hz, H4<sub>A</sub>), 2.13 (br. s, 3H, Adamantyl-CH), 2.06 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.70 (br. s, 6H, Adamantyl-CH<sub>2</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz):  $\delta$  191.1 (C6 minor), 189.5 (C6 major), 173.0 (C8 major), 167.9 (C9 minor), 166.2 (C9 major), 151.3 (C2'), 148.2 (C6'), 137.5 (C4'), 135.3 (C3'), 124.2 (C5'), 93.9 (C7 major), 67.7 (C5), 59.9 (C2), 53.7 (Adamantyl-C), 41.7 (Adamantyl-CH<sub>2</sub>), 36.2 (Adamantyl-CH<sub>2</sub> major), 36.0 (Adamantyl-CH<sub>2</sub> minor), 32.8, 32.1 (C4 major and minor tautomers), 29.8 (Adamantyl-CH minor), 29.4 (Adamantyl-CH major);  $m/z$  (ESI<sup>-</sup>) 444 ([M-H]<sup>-</sup>, 100%) and 446 ([M-H]<sup>-</sup>, 33%); HRMS (ESI<sup>-</sup>)  $m/z$ : [M-H]<sup>-</sup> Calcd for C<sub>22</sub>H<sub>23</sub>O<sub>3</sub>N<sub>3</sub>ClS 444.1154 and 446.1125; Found 444.1157 and 446.1125.

## Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.xxxx. Supporting Tables; <sup>1</sup>H and <sup>13</sup>C NMR spectra; calculated energies (PDF).

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### Graphical abstract

