

## Metal Binding and its Amelioration in Tetramates

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### Abstract

Metal chelation in tetramates may be ameliorated by change of ligating group and by steric blocking, and leads to a change in antibacterial properties; the former was achieved by replacement of an amide with a C-9 C=N bond and the latter by the synthesis of cysteine-derived tetramates with functionalisation at the C-6 or C-9 enolic groups. In both cases, metal-chelating ability was weak and loss of antibacterial activity was observed. Tetramate alkylations with an extended tricarbonyl conjugated system could be achieved under Mitsunobu conditions which led to regioisomers, distinguishable by careful HMBC correlation and carbonyl carbon chemical shift analysis.. C-9 and C-6 *O*-alkylation were observed but not C-8 *O*-alkylation for tetramate carboxamides; interestingly, C-7 alkylation with allyl and prenyl derivatives was also observed, and this arose by rearrangement of initially formed *O*-alkyl products. Only the C-7 alkylated tetramate derivatives **13a** and **13d** with no metal chelating ability demonstrated promising antibacterial activity against MRSA, with the most active analogue exhibiting an MIC against MRSA  $\leq 1.95$   $\mu\text{g/ml}$ , suggesting a mechanism of action independent from metal chelation. Otherwise, modifications at C-6/C-9 of tetramates led to a complete loss of metal chelating ability, which

correlated with a loss of antibacterial activity. This work further confirms that metal chelating capability is of fundamental importance in the biological activity of tetramates.

## Introduction

Antibacterial natural products provide an important start point for drug discovery,<sup>1-4</sup> and tetramate-containing systems<sup>5</sup> exhibit wide ranging antibacterial activity coupled with low levels of toxicity.<sup>6-9</sup> New compounds continue to be found<sup>10, 11</sup> and synthesised,<sup>12</sup> but one defining characteristic is their metal chelating ability.<sup>13</sup> We have earlier reported that cysteine-derived tetramates demonstrated excellent antibacterial activities against MRSA, but which diminished when tested *in vitro* in the presence of blood, and this seemed to be likely to be associated either with plasma protein binding or metal chelation.<sup>14</sup> The former was considered in an earlier publication,<sup>14</sup> but in order to address the latter, approaches to modulate metal chelation in cysteine-derived tetramates, and an investigation of its effect on antibacterial activity, was warranted.

Naturally occurring 3-acyltetramates, with distinct antibacterial activities, were reported to exhibit metal-binding abilities<sup>13, 15-18</sup> and different possible modes of metal complexation of 3-acyltetramates are known (Figure 1).<sup>15</sup> The X-ray crystal structure of a Cu(II) chelate of a 3-acyltetramic acid<sup>19</sup> revealed that it existed in a Z-enol square planar complex,<sup>20, 21</sup> and Group II metal ions such as Mg(II) and Ca(II) were reported to form complexes of the same Z-configuration.<sup>22</sup> IR analysis showed that an Fe(III) complex of a tetramic acid existed mainly in the Z-form while its Ni(II) complex mainly in its E-form.<sup>21</sup> Various other metal chelates of 3-acyltetramates (Zn(II), Ga(III), La(III), Ru(II),<sup>23</sup> Al(III),<sup>24</sup> Pd(II),<sup>25</sup> Co(II),<sup>26</sup> Rh(I),<sup>27</sup> Na(I), K(I),<sup>28</sup> Cs(I),<sup>29</sup> Cd(II), Pb(II),<sup>30</sup> Ba(II),<sup>31</sup> Hg(II),<sup>32</sup> and Mn(II)) have all been reported.<sup>33</sup> It was also noted that in amorphous solid state or in solution, metal chelates of tetramic acids might exist in a mixture of the two possible configurations.<sup>21</sup>

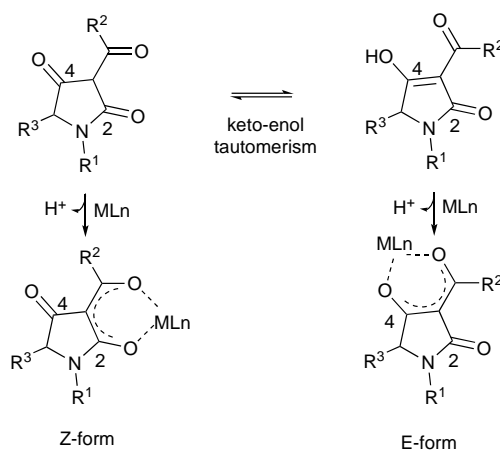
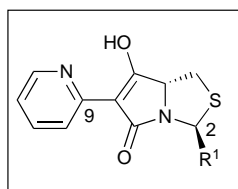



Figure 1: Tautomerism and metal chelation in acetyltetramates


The reality of metal chelating properties of tetramates in our work was indicated by broadening of  $^1\text{H}$  NMR spectra of chromatographically purified material which could only be removed by acid wash.<sup>14, 34-36</sup> Although metal salts of the tetramates were not routinely observable by MS, X-ray photoelectron spectroscopic (XPS) analysis of some derivatives isolated without acid wash clearly identified the presence of  $\text{Ca(II)}$ ,<sup>37</sup> and the high abundance of  $\text{Ca(II)}$ , together with  $\text{Na(I)}$ ,  $\text{Mg(II)}$ ,  $\text{Fe(III)}$  and  $\text{Zn(II)}$ , was also found in metal-chelated tetramates using inductively coupled plasma mass spectrometry (ICP-MS).<sup>14</sup> By comparison of  $^1\text{H}$  NMR spectra of post-column, acid-washed and metal-chelated tetramates, metal complexation behaviour of tetramates derivatives was also found to be structure dependent and metal-ion dependent.<sup>38</sup>

The metal chelation of tetramates has an immediate consequence for their biological activities.<sup>15, 17</sup> Metal sequestration was found to be one of the modes of antibacterial action of tetramates, suggesting metal binding ability could be essential for their biological activities.<sup>15, 39</sup> Metal chelates of a 3-acyltetramic acid, melophlin C, with  $\text{Ga(III)}$ ,  $\text{La(III)}$  and  $\text{Ru(II)}$ , all of which were considered to be  $\text{Fe(III)}$  mimics, were found to be active against *Micrococcus luteus* (*M. luteus*).<sup>23</sup> Interestingly, the antibacterial activity was not observed with the metal-free melophlin C.<sup>23</sup> Streptolydigin, a tetramate-containing natural product, was reported to inhibit bacterial RNA polymerase (RNAP) but not eukaryotic RNA polymerases; non-catalytic  $\text{Mg(II)}$  ion present in bacterial RNAP was found to be essential for its binding,<sup>40</sup> indicating that metal chelation is not

only important for the potency and efficacy of tetramate derivatives, but also for their selectivity and safety as antibacterial agents.<sup>41</sup> Some tetramic acids demonstrated increased toxicity as a metal complex such as magnesidin,<sup>22</sup> while in others, toxicity was attenuated by metal chelation.<sup>42</sup> On the other hand, aqueous solubility and stability of metal complexes of tetramate derivatives were also reported to influence their biological activity.<sup>15, 17</sup> A tetramate has been found which bound weakly with Cu(II) with the resultant complex being unstable in water,<sup>21</sup> but which, by contrast, formed a strong and stable chelate with Fe(III), which was poorly soluble.<sup>21</sup> Similarly, tetramate carboxamides were found to precipitate out of aqueous solution upon titration of Fe(III) solution.<sup>14</sup> Metal chelates of tetramic acids generally exhibit greater lipophilicity than the free acid forms, therefore impacting aqueous solubility and permeability through the cell membrane.<sup>13</sup> The synthetic goal of the work described here-in was to alter metal chelation ability of tetramates by modification of the functional groups expected to be directly participating in metal. To this end, the C-9 carbonyl group was first replaced with a C=N bond, the idea being to reduce metal binding ability and acidity of the system (Figure 2A). Additionally, change in the tetramate to introduce enol functionalisation and therefore directly block chelating behaviour was examined (Figure 2B). The effect of these changes was examined using a phenotypic assay for assessing antibacterial activities of the tetramate library.





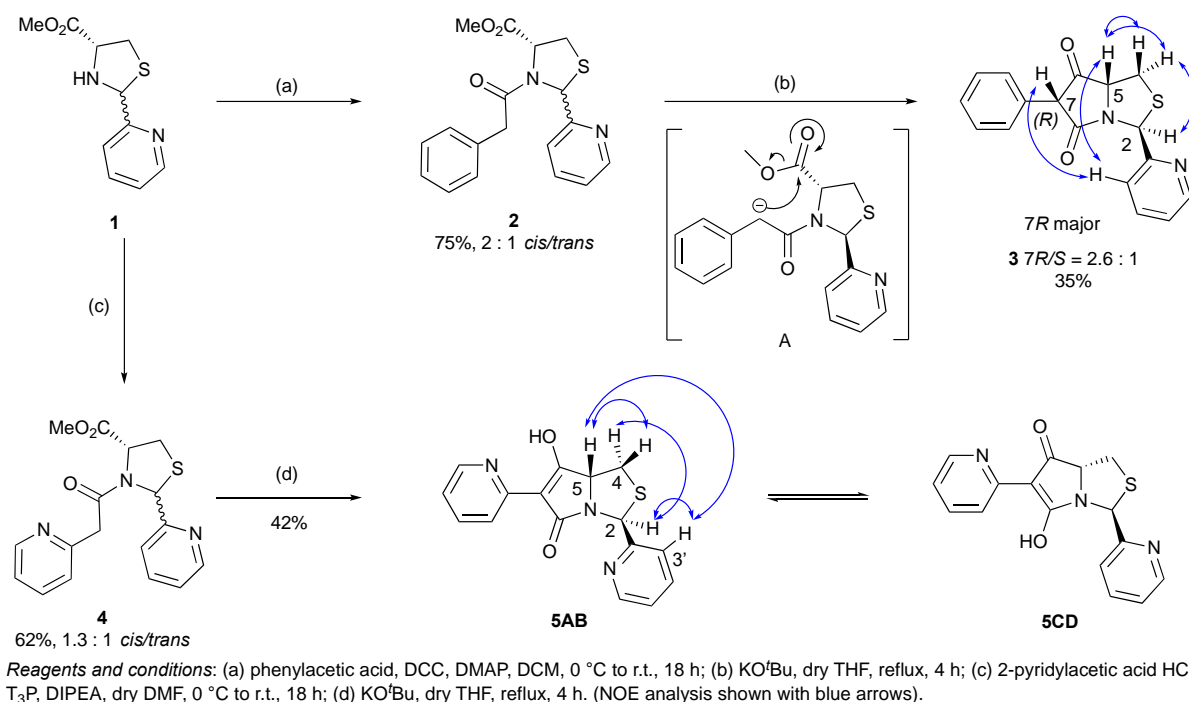
$R^3 =$ 


## Results and Discussion

Access to C-9 substituted aryl and heteroaryl systems was achieved using thiazolidine **1**, where *N*-acylation with phenylacetic acid resulted in the formation of *N*-acylthiazolidine **2** as a 2 : 1 mixture of *cis/trans* diastereomers, each as a pair of rotamers, in 75% yield, and Dieckmann

cyclisation led to the formation of the C-7 phenyl substituted bicyclic tetramate **3** in 35% yield. In this case, the Dieckmann cyclisation is forced from the side chain enolate to the C-5 methyl ester (enolate type A, Scheme 1) to form the stable 5-membered ring of **3**. The tetramate **3** existed as an inseparable mixture of keto/enol tautomers in organic solvent (CDCl<sub>3</sub>) with a keto/enol tautomeric ratio of 7 : 3. The keto form was an inseparable mixture of diastereomers (2.6 : 1 *7R/S*) by observation of the C-7 protons in <sup>1</sup>H NMR spectrum, with a *trans*-relationship between H-2 and H-5, where the relative stereochemistry was confirmed by NOE analysis (Scheme 1).

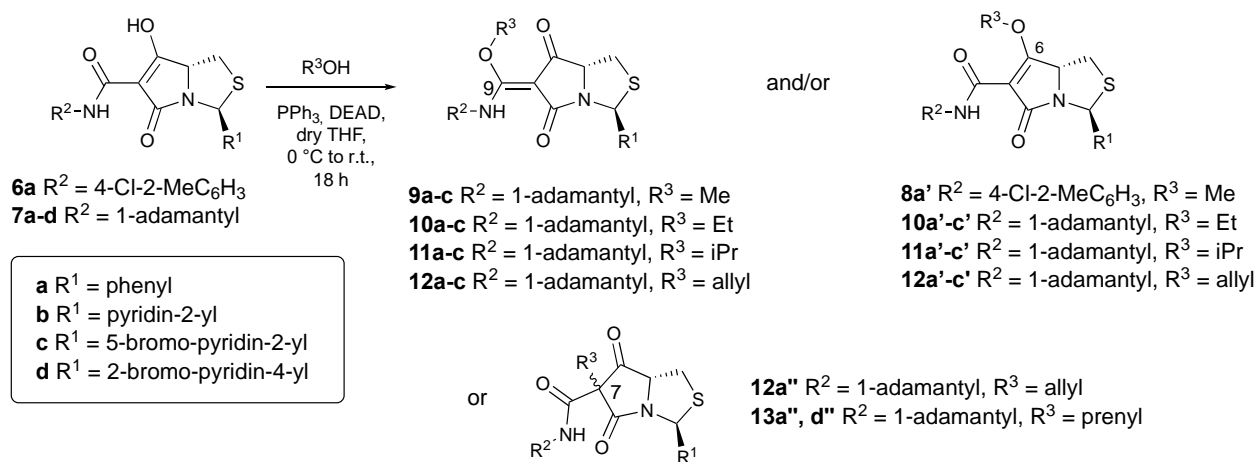
This route was repeated with 2-pyridylacetic acid·HCl, but its insolubility in the original reaction using DCM solvent hindered the *N*-acylation reaction, and no product was isolated, even with addition of Et<sub>3</sub>N to the reaction mixture. The coupling condition was thus changed to T<sub>3</sub>P/DIPEA in the more polar DMF solvent, which successfully produced *N*-acylthiazolidine **4** as a inseparable 1.3 : 1 mixture of *cis/trans* diastereomers, each as a pair of rotamers, in 62% yield. Subsequent Dieckmann cyclisation resulted in the desired tetramate **5** in 42% yield, with cyclisation only observed for the *trans* diastereomer which placed the C-2 bulky pyridyl group at the less hindered *exo* face of the bicyclic system (the *cis* isomer did not cyclise and was recovered). In this case, the tetramate derivative **5** showed complete enolisation, since no C-7 proton was observed and it existed as a 1.5 : 1 inseparable mixture of **AB** : **CD** tautomers with a *trans*-relationship between H-2 and H-5, where the relative stereochemistry was confirmed by NOE analysis (Scheme 1). These model compounds were later studied for their metal chelation ability and tested for antibacterial activity.



**Scheme 1: Synthesis of tetramates**

We wished in particular to access C-6 enolic derivatives, but had earlier found that direct alkylation of a tetramate ester and tetramate carboxamide by reactions with alkyl halides under basic conditions had been completely unsuccessful, probably due to the very low nucleophilicity of the extensively conjugated C-6 hydroxyl group in these systems.<sup>37</sup> However, we found that the C-6 enol group, with its acidity conferred by the extended tricarbonyl conjugated system, could be functionalised with suitable alcohols under Mitsunobu conditions as a mixture of C-6 *O*-alkylated and C-9 *O*-alkylated products,<sup>36</sup> although their yield decreased for increasingly bulky alcohols.<sup>43</sup> Use of these conditions for **6a** and **7a-d** gave tetramate carboxamides **8-13** (Scheme 2). A general observation from this and earlier work<sup>43</sup> was that tetramate carboxamides were less reactive under these conditions than the corresponding tetramate esters, probably due to a changed electronic nature of C-6 enol as well as the steric hindrance posed by C-9 bulky amide pendants. H-2 and H-5 chemical shifts of the alkylated tetramate carboxamides **8-13**, characteristic of a bicyclic tetramate ring, are tabulated together with the reaction yield (Table 1). The varied yield of the reactions and increased difficulty encountered with bulky alcohols again pointed to the unreactive nature of the C-6 enol group. The relative stereochemistry at C-2 and C-5 of the

alkylated carboxamides was confirmed by NOE analysis of selected examples, where the *trans*-relationship between H-2 and H-5 was conserved from the starting carboxamides. Given the highly conserved H-2 and H-5 chemical shifts across the series **8-12** (Table 1), the *trans*-2,5 stereochemistry was assigned to all tetramates **8-12**.



Scheme 2: Synthesis of O-alkyl tetramates

**Table 1.** Yield & H-2, H-5 chemical shifts (CDCl<sub>3</sub>, 400 MHz) for alkylated tetramates **8-13** (see Scheme 2).



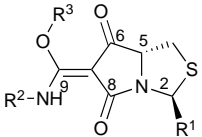
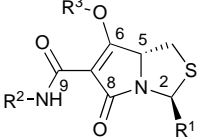
	$R^2 = / R^3 =$	$R^1 =$	$\delta_H$ (ppm)		% Yield <sup>a</sup>	Ratio <sup>b</sup>
			H-2	H-5		
<b>8a'</b>	$R^2 =$ $R^3 =$		6.33	4.88 (dd)	30	-
<b>9a</b>	$R^2 =$ $R^3 =$		6.43	4.42 (t)	31	-
<b>9b</b>			6.49	4.57 (t)	43	-
<b>9c</b>			6.43	4.54 (t)	11	-
<b>10a</b>	$R^2 =$ $R^3 =$		6.43	4.41 (t)	32	10 : 1
<b>10a'</b>			6.14	Obscured <sup>c</sup>		
<b>10b</b>			6.49	4.56 (t)	29	5 : 1
<b>10b'</b>			6.17	Obscured <sup>c</sup>		
<b>10c</b>			6.43	4.53 (t)	17	5 : 1
<b>10c'</b>			6.10	Obscured <sup>c</sup>		
<b>11a</b>	$R^2 =$ $R^3 =$		6.43	4.41 (t)	27	1.1 : 1
<b>11a'</b>			6.23	4.66 (dd)	24	
<b>11b</b>			6.49	4.57 (t)	17	1.7 : 1
<b>11b'</b>			6.29	4.85 (dd)		
<b>11c</b>			6.43	4.53 (t)	15	1 : 1
<b>11c'</b>			6.23	4.80 (dd)		
<b>12a</b>	$R^2 =$ $R^3 =$		6.43	4.41 (t)	21	1 : 2
<b>12a'</b>			6.21	4.74 (dd)	38	
<b>12b</b>			6.49	4.57 (t)	5	1 : 2.8
<b>12b'</b>			6.29	4.93 (dd)	14	
<b>12c</b>			6.43	4.53 (t)	11	5 : 1
<b>12c'</b>			6.24	4.71 (m)		
<b>7R-13a''</b>	$R^2 =$ $R^3 =$		6.58	4.62 (dd)	3	1 : 5
<b>7S-13a''</b>			6.55	4.33 (dd)	15	
<b>13d''</b>			6.47 6.45	4.17 – 4.27 (m)	10	1 : 1

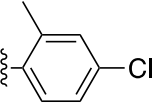

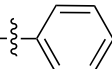
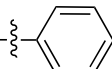


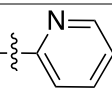
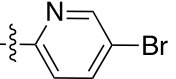
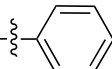


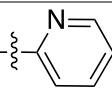
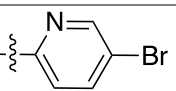
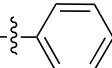


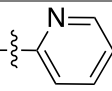
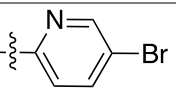
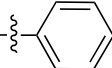
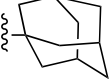

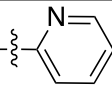
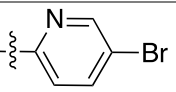

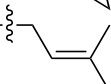
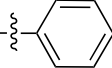
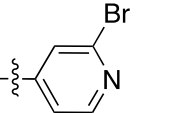
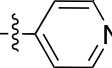
<sup>[a]</sup> Isolated yield after column chromatography; <sup>[b]</sup> The ratio of the two isomers **10-12:10-12'** and diastereomers **13''** was calculated from the isolated yield or integration ratio of H-2 chemical shifts in the isolated mixture. <sup>[c]</sup> Obscured by the chemical shift of OCH<sub>2</sub>CH<sub>3</sub> at 4.70 - 4.80 ppm.



Products **11a** and **11a'** were obtained as two distinct species and NOE analysis showed enhancement between H-5 and protons on the isopropyl side chain (H-10 and H-11) in **11a'** but not in **11a**, suggesting that they were regioisomers with different positions of alkylation (Figure S1, ESI). The presence of regioisomers was further supported by tabulation of the relevant carbon chemical shifts, which gave two distinct sets of data across alkylated carboxamides (Table 2). In some cases, the two isomers were separable by flash column chromatography, and since the distinct sets of chemical shifts did not equilibrate, that they were not rotamers. The positions of alkylation were elucidated by HMBC correlation analysis. For example, from the reaction of **6a** and methanol under the Mitsunobu conditions, an exclusive C-6 *O*-alkylated enol ether **8a'** was observed in which C-6 correlated with H-4, H-5 and methoxy protons, C-8 correlated with H-2 and H-5 protons and C-9 weakly correlated with the N-H proton (Figure S2, ESI). By contrast, **9a** was a C-9 *O*-alkylated product exclusively with C-9 correlating strongly with the methoxy protons (Figure S3, ESI). In some cases of the alkylated tetramate carboxamides, a large number of scans were required to observe the C-6, C-7, C-8 and C-9 chemical shifts in  $^{13}\text{C}$  NMR spectra; the detection and assignment of such chemical shifts were assisted by enhanced sensitivity of a cryogenic  $^{13}\text{C}$  detection probe as well as HMBC correlation analysis.

**Table 2.** C-6, C-7, C-8, C-9 chemical shifts (CDCl<sub>3</sub>) for the alkylated tetramates **8-13** (see Scheme 2).


and/or


	$R^2 = / R^3 =$	$R^1 =$	$\delta$ (ppm)				Site of alkylation
			C-6	C-7	C-8	C-9	
<b>8a'</b>	$R^2 =$  $R^3 =$ 		178.8	102.4	173.3	158.7	C-6 O
<b>9a</b>			192.2	87.1	174.9	169.0	C-9 O
<b>9b</b>	$R^2 =$  $R^3 =$ 		193.6	87.0	176.6	169.1	C-9 O
<b>9c</b>			192.4	86.9	174.8	169.1	C-9 O
<b>10a</b>			196.1	87.5	181.1	167.9	C-9 O
<b>10a'</b>			-	97.3	175.1	160.5	C-6 O
<b>10b</b>	$R^2 =$  $R^3 =$ 		196.2	87.4	180.7	167.9	C-9 O
<b>10b'</b>			179.2	96.9	177.2	162.1	C-6 O
<b>10c</b>			196.1	87.3	180.6	167.9	C-9 O
<b>10c'</b>			-	97.0	175.0	161.0	C-6 O
<b>11a</b>			192.4	87.7	175.6	166.7	C-9 O
<b>11a'</b>			175.6	103.1	174.3	160.2	C-6 O
<b>11b</b>	$R^2 =$  $R^3 =$ 		192.8	87.6	176.2	166.8	C-9 O
<b>11b'</b>			175.5	102.8	174.4	160.5	C-6 O
<b>11c</b>			192.7	87.6	176.2	166.8	C-9 O
<b>11c'</b>			176.6	102.8	174.5	160.0	C-6 O
<b>12a</b>			192.4	87.6	175.1	167.6	C-9 O
<b>12a'</b>			176.0	103.8	173.7	159.9	C-6 O
<b>12b</b>	$R^2 =$  $R^3 =$ 		191.2	87.4	173.1	167.6	C-9 O
<b>12b'</b>			176.7	103.6	173.7	159.9	C-6 O
<b>12c</b>			192.5	87.4	173.2	167.6	C-9 O
<b>12c'</b>			175.0	100.2	169.5	159.6	C-6 O
<b>7R-13a''</b>	$R^2 =$  $R^3 =$ 		201.8	68.0	170.9	162.9	C-7 C
<b>7S-13a''</b>			200.1	69.1	171.7	160.3	C-7 C
<b>13d''</b>			201.4	69.9	172.1	163.1	C-7 C
			199.6	70.0	172.2	160.1	C-7 C

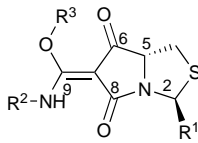
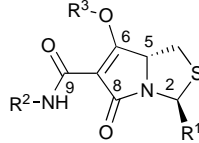
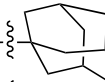
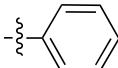
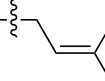
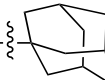
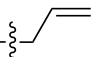
[–] In <sup>13</sup>C NMR spectra of **10a,c**, C-6 chemical shift was not observed for the minor isomer, due to the intrinsic difficulty to detect C-6 as well as its relatively low proportion in the isolated mixture (9% and 17% respectively).

The relative proportion of two regioisomers was also strongly influenced by the steric bulkiness of the reacting alcohol, with the ratio conserved within a series of the same C-9 amide pendant and the same alcohol (i.e. same R<sup>2</sup> and R<sup>3</sup> groups, Scheme 2). By comparing the set of carbon chemical shifts to that observed in the model compounds **8a'** and **9a**, structures of the tetramates were assigned (Table 2). C-6, C-8 and C-9 chemical shifts were consistently more downfield in the C-9 *O*-alkylated isomers than in the C-6 *O*-alkylated ones, with one exception where C-8 chemical shift in the C-9 *O*-alkylated isomer **12b** was more upfield than that of the C-6 *O*-alkylated **12b'** but only with a difference of 0.6 ppm. The assignment of C-8 chemical shifts in **12b** and **12b'** was unambiguous because the two isomers were separated by column chromatography and could be analysed individually. This trend was reversed in C-7 chemical shifts, with those of the C-9 *O*-alkylated series being invariably more upfield. The set of chemical shifts was also distinct from that observed in the C-8 *O*-alkylated tetramate ketone analogues.<sup>36</sup> Tetramates **8-12** demonstrated consistent trends in the H-2 and H-5 chemical shifts, with H-2 being invariably more downfield in C-9 *O*-alkylated isomers than in C-6 *O*-alkylated isomers. This trend was reversed for H-5 chemical shifts (Table 1).

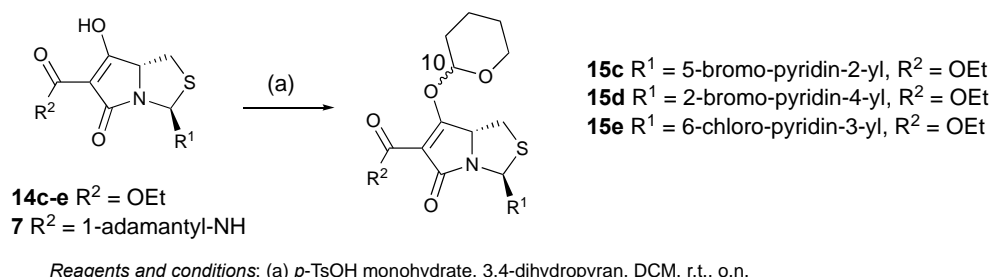
However, in the case of **13a''**, **d''**, the sets of proton and carbon chemical shifts did not conform to either of the two sets previously observed in C-9 or C-6 *O*-alkylated series (Tables 1 and 2), suggesting that they were alkylated at a different position other than C-9 or C-6. The more downfield C-6 chemical shifts at around 200 ppm also indicated the presence of a ketone functional group instead of a conjugated enol group. HMBC data for **13a''** indicated that the C-6 carbon was correlated with H-4 and H-5 protons, C-8 correlated with H-2, H-5 and weakly with H-4 protons and C-9 correlated with N-H proton. The HMBC correlations between the protons on the alkylated side chain and C-7, but not with any carbonyl carbons, supported a tetramate with C-7 alkylation (Scheme 2). The chemical shifts corresponding to protons on the alkylated side chain,

consistent with vinyl protons instead of allylic protons, further confirmed the structure as drawn (Figure S4, ESI). The HMBC correlations to C-7 were also stronger with the methyl protons (2-bond correlation) than the two vinyl protons (3-bond correlation). The C-7 alkylated tetramate carboxamide **13a''** was synthesised as a 1 : 5 mixture of 7*R*/*S* diastereomers with a *trans*-relationship between H-2 and H-5. The major 7*S* diastereomer was isolated in 15% yield and its relative stereochemistry confirmed by NOE analysis (Scheme 2). Compound **13d''** was however a 1 : 1 7*R*/*S* mixture of diastereomers. That this C-7 alkylation proceeded by initial *O*-alkylation followed by migration was suggested when it was observed that when **12a'** was kept in CDCl<sub>3</sub> solution over an extended period, the rearranged C-alkylated **12a''** was obtained as a single diastereomer (of unassigned stereochemistry) at C-7 (Table 3 and Fig S5, ESI).

**Table 3.** Comparison of characteristic chemical shifts (CDCl<sub>3</sub>) between **13a''** and **12a''** (see Scheme 2).

		and/or						
$R^2 = / R^3 =$		$R^1 =$	$\delta$ (ppm)					
			H-2	H-5	C-6	C-7	C-8	C-9
<b>7R-13a''</b>	$R^2 =$ 		6.58	4.54 (m)	200.5	68.6	170.4	160.8
<b>7S-13a''</b>	$R^3 =$ 		6.55	4.33 (dd)	200.1	69.1	171.6	160.3
<b>12a''</b>	$R^2 =$ 		6.60	4.30 (dd)	200.9	69.7	171.6	162.6
	$R^3 =$ 							

were reactive to further *O*-alkylation in moderate yields (53 - 59%), although carboxamides failed to react, with recovery of the starting materials (Scheme 3). The THP-protected tetramate esters were synthesised as a mixture of 1 : 1 diastereomers at C-10, and the characteristic and consistent chemical shifts of the tetramates **15** are summarised in Table 4.<sup>44</sup>



Scheme 3: Synthesis of *O*-alkyl tetramates

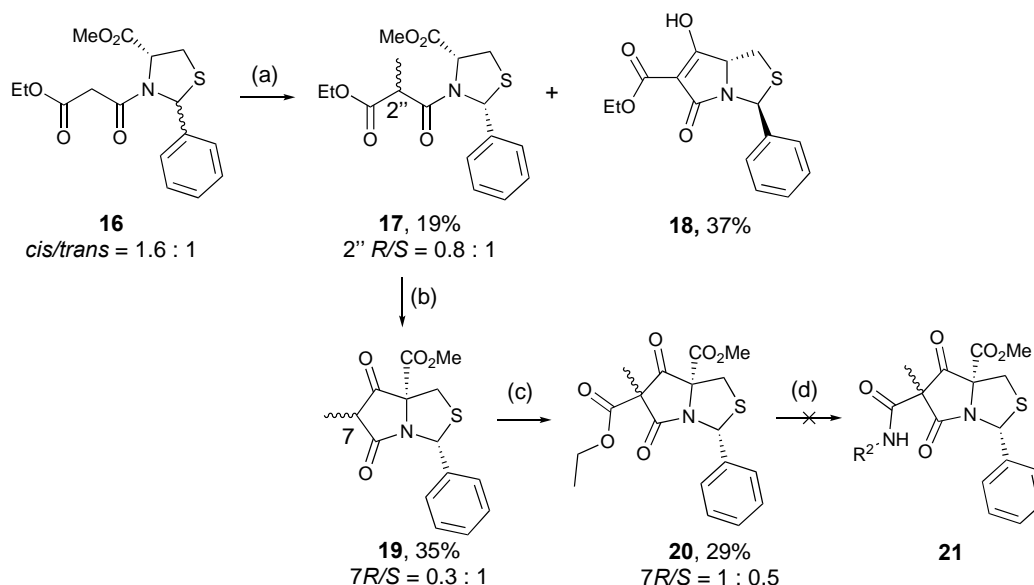
**Table 4.** Yield, H-2, C-6/7/8/9 chemical shifts ( $\text{CDCl}_3$ ) for THP-protected tetramate esters (see Scheme 3).

	$R^1 =$	$\delta$ (ppm)					Ratio <sup>a</sup>	% Yield <sup>b</sup>
		H-2	C-6	C-7	C-8	C-9		
<b>15c</b>		6.58	201.7	80.4	167.8	163.5	1 : 1	59
		6.52	201.2	79.3	167.2	163.4		
<b>15d</b>		6.50	200.8	80.5	167.6	163.3	1 : 1	57
		6.44	200.3	79.3	166.7	163.2		
<b>15e</b>		6.58	201.2	80.3	167.8	163.4	1 : 1	53
		6.52	200.7	79.2	167.3	163.3		

<sup>[a]</sup> The ratio of two diastereomers (C10*R*/*S*) was calculated from the integration ratio of H-2 chemical shifts in the isolated mixture. <sup>[b]</sup> Isolated yield from flash column chromatography.

*C*-Alkylation of the side chain of the *N*-acylthiazolidine **16** was, however, possible, which with simultaneous Dieckmann cyclisation under basic conditions produced tetramate ester **18** as a major product in 37% yield (Scheme 4) along with *C*-alkylated *N*-acylthiazolidine **17** as a minor product. This was isolated in 19% yield and used for a separate Dieckmann cyclisation with  $\text{KO}^t\text{Bu}$  in THF under reflux. Of interest is that the presence of the additional methyl group altered the direction of cyclisation, leading to C-5 methyl ester tetramate **19** in 35% yield as a 0.3 : 1 mixture

of diastereomers (7*R/S*) with a *cis*-relationship between H-2 and H-5. This tetramate **19** was reacted with ethyl chloroformate under the basic conditions of DMAP to create a C-7 quaternary centre in 29% yield. However, both ester groups in the resultant tetramate **20** were unreactive and did not give **21** under transamidation conditions, with recovery of the starting material.

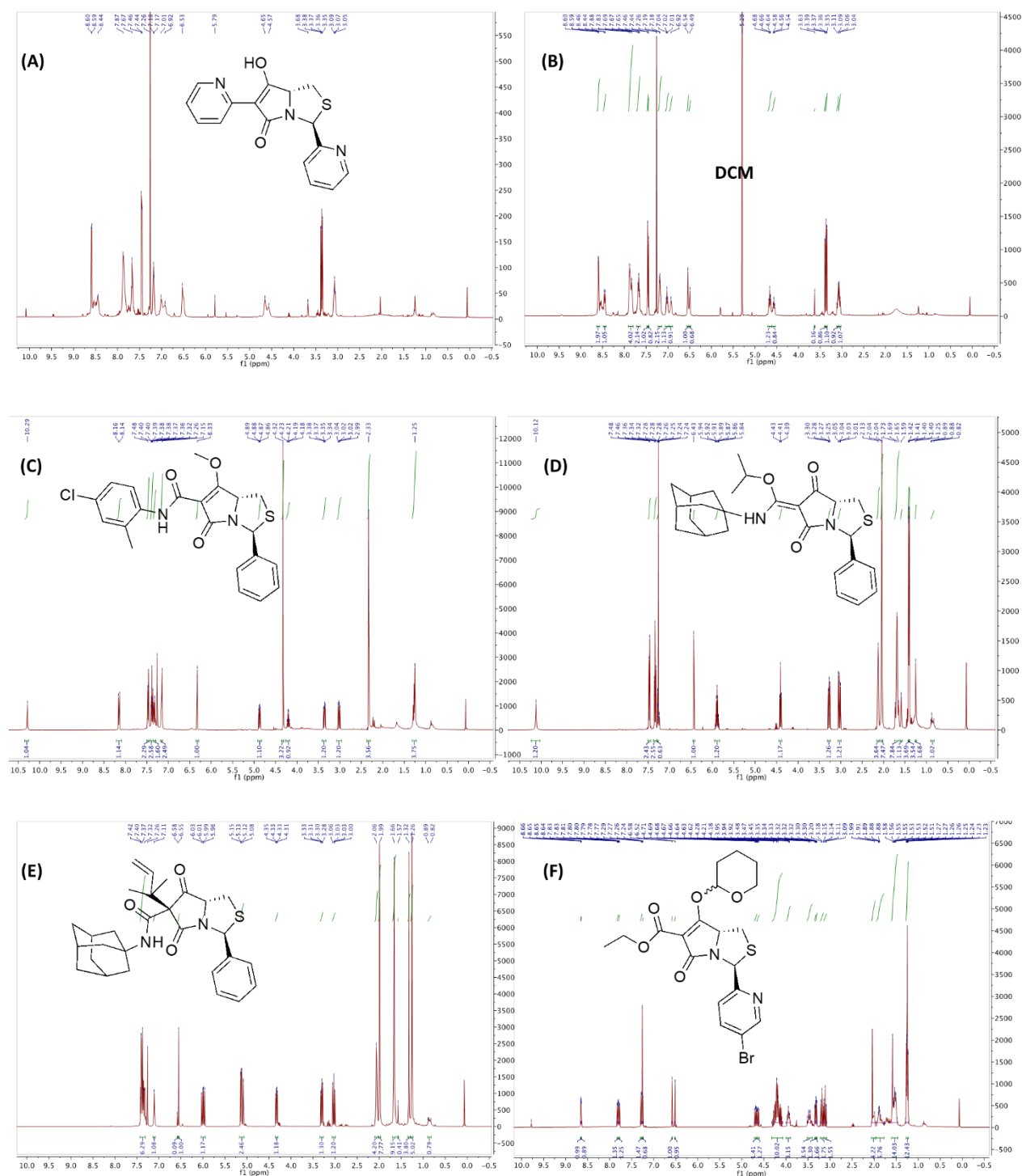


Reagents and conditions: (a) NaH, MeI, dry THF, 0 °C to r.t., o.n.; (b) KO<sup>t</sup>Bu, dry THF, reflux, 4 h; (c) ethyl chloroformate, DMAP, DCM, 0 °C to r.t., o.n.; (d) R<sup>2</sup>NH<sub>2</sub> (R<sub>2</sub> = 1-adamantyl), THF/toluene, reflux, o.n.

Scheme 4: Synthesis of C-alkyl tetramates

A primary goal of this project was to modulate metal binding properties of the tetramates. Tetramate-containing natural products and their derivatives were well-known for their metal-binding properties, which are often apparently correlated with their biological activities.<sup>15, 16</sup> The synthesised tetramates also exhibited structure-dependent metal chelation, consistently demonstrated by peak broadening of <sup>1</sup>H NMR spectra after column chromatography. In contrast to the previously reported tetramate carboxamides,<sup>38</sup> which exhibited strong metal chelation abilities, tetramate **5** with a C-9 C=N bond replacing a carbonyl group was found to be only weakly metal-binding, as indicated by slight broadening of peaks in its <sup>1</sup>H NMR spectrum after purification with flash silica column chromatography (Figure 3A). It could be washed with 5% aqueous citric acid solution to easily render metal-free material (Figure 3B). Tetramate esters **15** and carboxamides **8-13** were all non-metal chelating as suggested by the sharp and resolved <sup>1</sup>H NMR

spectra directly after column chromatography (Figure 3C-F). The reduction in metal chelation properties of tetramates with modifications at C-9 carbonyl and C-6 enol functionalities indicated the importance of these groups for metal complexation.



**Figure 3.**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz) spectra indicating metal-chelation abilities of tetramates. (A) **5**, with a C-9 C=N bond, post-column, before acidic wash; (B) **5** post-column, after 5% citric acid

wash; (C) C-6 *O*-alkylated **8a'** post-column; (D) C-9 *O*-alkylated **11a** post-column; (E) C-7 Alkylated **7S-13a''** post-column; (F) Tetramate ester **15c** post-column.

In order to quantify the metal chelation property, width at half height ( $W_{1/2}$ ) values for the H-2 signals in post-column  $^1\text{H}$  NMR spectra as estimates of peak broadening were used (Table 5); H-2 was chosen for its universal presence in all structures and its singlet structure. Although H-2 signals of metal-chelated tetramates did not have perfect Lorentzian shape, their widths gave a good estimate for the extent of peak broadening. In cases where two sets of H-2 signals were present in the NMR spectrum of a single compound due to tautomeric behaviour, the major peak was taken for calculation.

**Table 5.** Post-column widths at half height/Hz for H-2 signals of tetramates.<sup>a</sup>

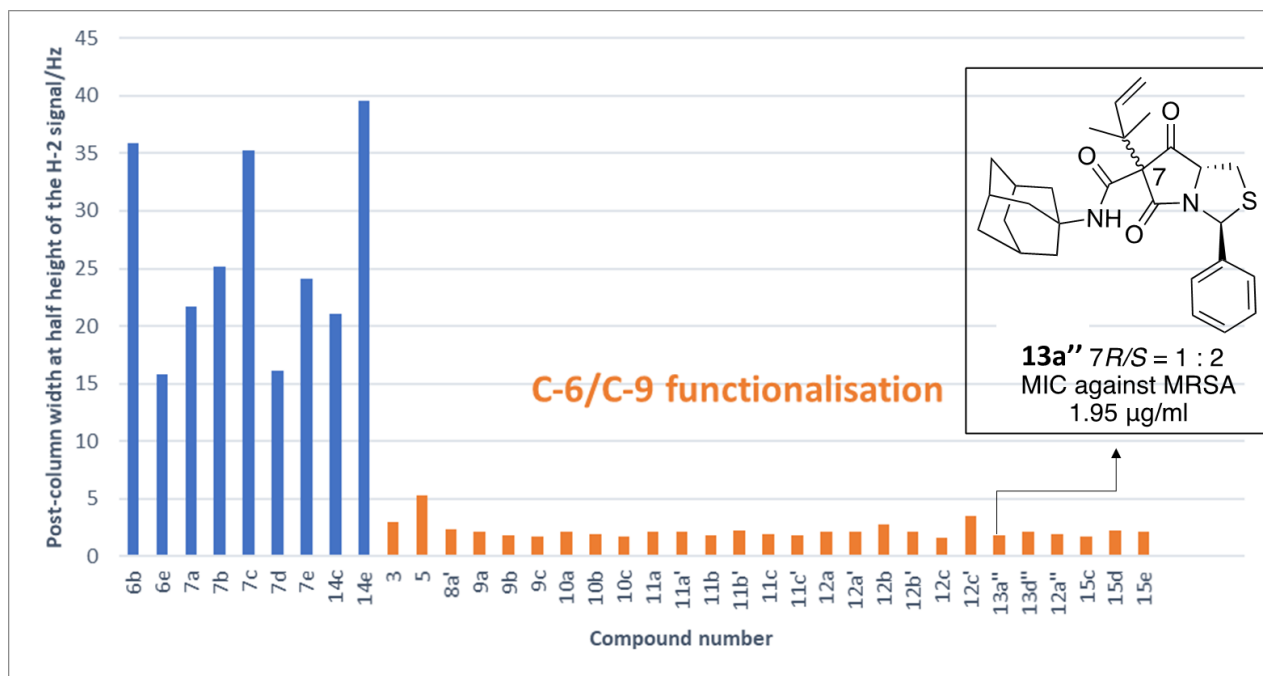
Cpd No.	$W_{1/2}/\text{Hz}$	Cpd No.	$W_{1/2}/\text{Hz}$	Cpd No.	$W_{1/2}/\text{Hz}$
<b>3</b>	3.0	<b>9c</b>	1.7	<b>12b</b>	2.8
<b>5</b>	5.3	<b>10a</b>	2.2	<b>12b'</b>	2.2
<b>6b</b>	35.9	<b>10b</b>	1.9	<b>12c</b>	1.7
<b>6e</b>	15.8	<b>10c</b>	1.8	<b>12c'</b>	3.5
<b>7a</b>	21.7	<b>11a</b>	2.1	<b>7S-13a''</b>	1.8
<b>7b</b>	25.2	<b>11a'</b>	2.2	<b>13d''</b>	2.1
<b>7c</b>	35.2	<b>11b</b>	1.9	<b>12a''</b>	2.0
<b>7d</b>	16.1	<b>11b'</b>	2.3	<b>14c</b>	21.1
<b>7e</b>	24.1	<b>11c</b>	2.0	<b>14e</b>	39.5
<b>8a'</b>	2.4	<b>11c'</b>	1.9	<b>15c</b>	1.8
<b>9a</b>	2.2	<b>12a</b>	2.2	<b>15d</b>	2.3
<b>9b</b>	1.8	<b>12a'</b>	2.1	<b>15e</b>	2.1

<sup>[a]</sup> Data for tetramates were consistently obtained from 400 MHz NMR spectroscopy with samples in  $\text{CDCl}_3$ .

Direct functionalisation at C-6 and C-9 clearly reduced metal binding, and tetramate derivatives **3**, **5**, **8-13** and **15** had an average post-column H-2 width of only 2.3 Hz (Table 5 and Figure 4). Significant improvement in post-column peak resolution was visually discernable. This supported the direct involvement of C-6 and C-9 oxygen in metal complexation. Interestingly,



tetramate derivative **13a''** was non-metal binding but demonstrated good antibacterial activity (see below), with the lowest MIC value against MRSA at 1.95 µg/ml, indicating a mode of action independent from metal chelation (Figure 4).

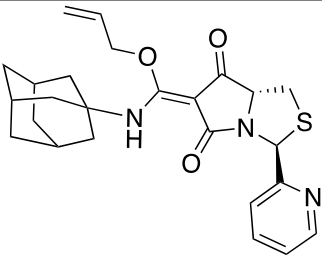
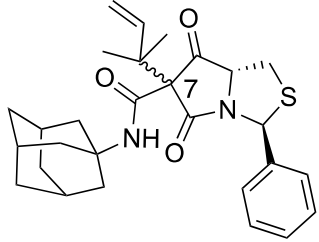
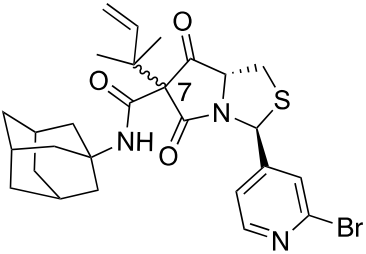


**Figure 4.** Plot of post-column H-2 widths at half height ( $W_{1/2}$ , Hz) for tetramates **6 - 7, 14** and derivatives **8 - 13, 15** with C-6/C-9 functionalisation.

The tetramate derivatives **3** and **5** and carboxamides **8-13** were tested for their biological activities against Gram-positive MRSA (some were also tested against *Streptococcus*) and Gram-negative *E. coli* by MIC assays. No activity was seen against *E. coli*. Neither **3** nor **5** showed any biological activity, and noteworthy is their low metal chelating ability as discussed earlier. Alkylated tetramate carboxamides **12b** was found to be only weakly active against Gram-positive MRSA (and *Streptococcus*) (Table 6). All THP-protected tetramate carboxamides were inactive, but interestingly C-7 alkylated tetramate carboxamides **13a'',d''** demonstrated promising antibacterial activities against Gram-positive MRSA (and *Streptococcus*). These tetramate derivatives were non-metal chelating as indicated by the sharp and resolved post column  $^1\text{H}$  NMR spectra (Figure 3), suggesting that they might act via a mechanism independent from metal binding, and differently

from the previously synthesised tetramates.<sup>41</sup> The activity of **13a''** was further mitigated in the presence of blood due to its high lipophilicity and hence extensive binding to plasma protein HSA; the extent of plasma protein binding could be reduced with the more polar analogue **13d''**, although with a less potent biological activity, thus warranting further optimisation. It was also important to note the effect of C-7 configuration on antibacterial activity, with 7*S* diastereomer appeared to be more active than 7*R* diastereomer. As the relative proportion of 7*S* diastereomer increased by 7%, there was a two-fold decrease in the MIC value, suggesting that the 1 : 2 7*R/S*-**13a''** mixture was twice as active as the 1 : 1.5 mixture. The sample of **13d''** was however a 1:1 mixture of 7*R/S* diastereomers, thus rendering the MIC values not directly comparable between **13a'',d''**.

**Table 6.** Biologically active cysteine-derived tetramates.

	Structure	MIC <sup>c</sup> (µg/ml) against		MW	clogP <sup>a</sup>	PSA <sup>a</sup> /Å <sup>2</sup>
		MRSA	<i>Streptococcus</i>			
<b>12b</b>		31.3	31.3	452	3.29	71.5
<b>13a''</b>				479	4.98	66.5
	1 : 2 7 <i>R/S</i>	1.95	Not tested			
	1 : 1.5 7 <i>R/S</i>	3.91	Not tested			
	7 <i>S</i> only <sup>b</sup>	7.81	3.95			
<b>13d''</b>		15.6	Not tested	559	4.85	79.4
	1 : 1 7 <i>R/S</i>					

[a] Relevant chemical properties of the active compounds were calculated using MarvinSketch 20.3.0, 2020, ChemAxon; [b] The third sample of **13a''** with exclusively 7S diastereomer was tested in a different batch than the other two samples, thus making the MIC values not directly comparable; [c] MIC assays run using Linezolid as a positive control.

Safety in human use is another important selection criterion for good antibacterial drugs.<sup>45</sup>

The selectivity of the synthesised tetramates to prokaryotic bacteria cells over eukaryotic mammalian cells was analysed using cytotoxicity screens against Hela, HEK-239, CaCo and MDCK cell lines (Table 7), chosen since Hela is a highly durable and proliferative human cervical cancer cell line;<sup>46</sup> HEK-239 is a human embryonic kidney cell line to model renal toxicity; CaCo is a human colorectal adenocarcinoma cell line as a model of intestinal epithelium<sup>47</sup> to test for gastrointestinal toxicity; and MDCK is a dog kidney cell line as a non-human mammalian model. For interpretation of MIC values, an MIC against MRSA of < 8 µg/ml was considered to be active.<sup>48</sup> An IC<sub>50</sub> of 62.5 - 125 µg/ml against selected cell lines in the cytotoxicity screen was considered to be non-toxic, with the weak toxicity more likely to be contributed by DMSO introduced during the testing procedure;<sup>49, 50</sup> an IC<sub>50</sub> of 15.6 - 31.3 µg/ml was considered less toxic while a IC<sub>50</sub> < 8 µg/ml was toxic. The selectivity index for MRSA was calculated as the ratio of IC<sub>50</sub> in respective cell lines against the MIC value (IC<sub>50</sub>/MIC).

**Table 7.** Cytotoxicity screen of selected cysteine-derived tetramates.

	MIC (µg/ml) against MRSA	IC <sub>50</sub> (µg/ml) against				MRSA Selectivity
		Hela	HEK-239	CaCo	MDCK	
<b>13a'', 7R/S = 1:2</b>	1.95	3.91	3.91	7.81	7.81	2.0 - 4.0
<b>13a'', 7R/S = 1:1.5</b>	3.91	7.81	7.81	15.6	15.6	2.0 - 4.0
<b>13d''</b>	15.6	7.81	7.81	31.3	31.3	0.5 - 2.0

Drug-likeness of active tetramates was further assessed by calculation of their physiochemical properties, for which high oral availability, aqueous solubility and low protein plasma binding are considered to be important.<sup>45</sup> The parameters molecular weight MW (Da),

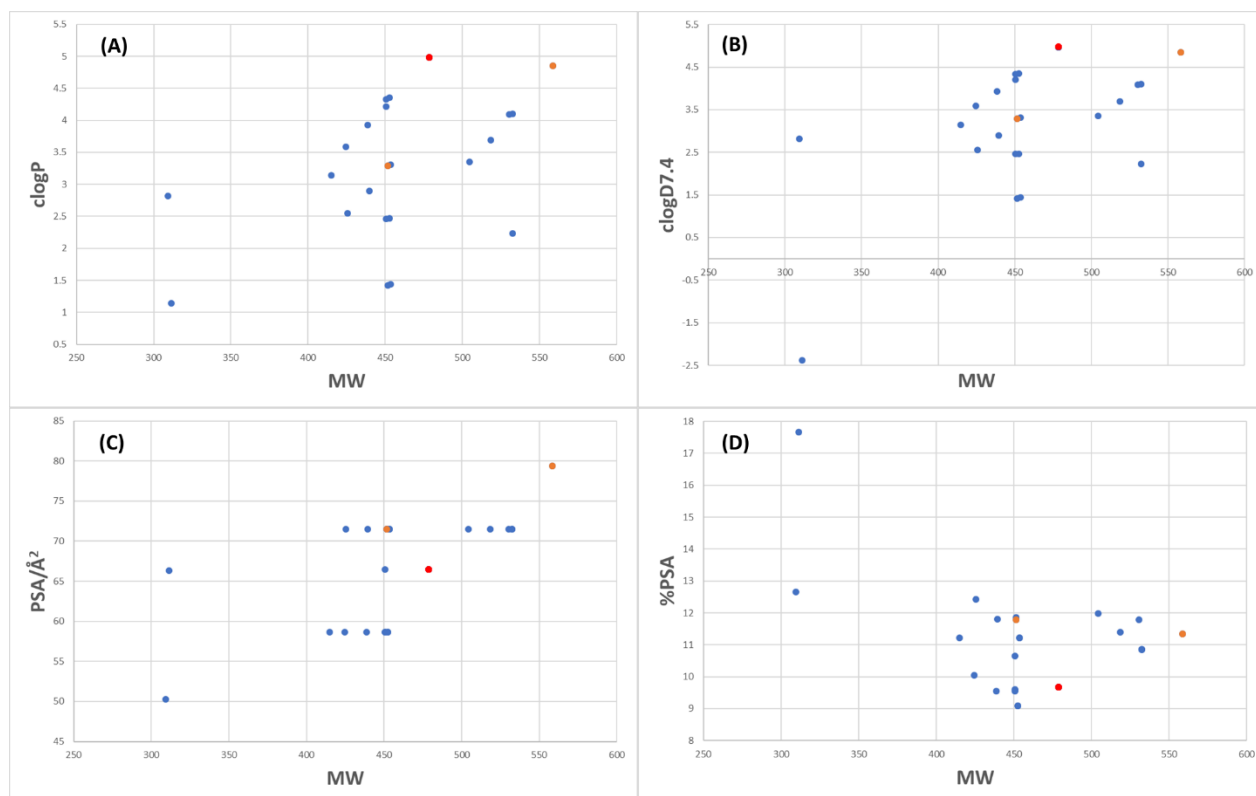
clogP, clogD<sub>7.4</sub>, polar surface area (PSA/Å<sup>2</sup>) and relative PSA (PSA as a % of molecular surface area, MSA/Å<sup>2</sup>) of the tetramates were calculated (Table 8).<sup>51</sup> While an MIC of 16 - 32 µg/ml against tested pathogenic organism is considered a suitable minimum at a preliminary evaluation stage,<sup>52</sup> effective antibacterial activity of cysteine-derived tetramates synthesised in this project was defined by an MIC against MRSA of < 8 µg/ml.<sup>48</sup>

**Table 8. Physiochemical properties and activity of tetramates.<sup>a</sup>**

Cpd No.	MW /Da	clogP	clogD <sub>7.4</sub>	PSA /Å <sup>2</sup>	MSA /Å <sup>2</sup>	% PSA	HBD	HBA	MIC against MRSA	
									µg/ml	µM
<b>3</b>	309.38	2.82	2.82	50.27	397.45	12.6	0	3	-	-
<b>5</b>	311.36	1.14	-2.38	66.32	375.60	17.7	1	4	-	-
<b>8a'</b>	414.90	3.14	3.14	58.64	522.40	11.2	1	3	-	-
<b>9a</b>	424.56	3.59	3.59	58.64	583.38	10.1	1	4	-	-
<b>9b</b>	425.55	2.55	2.55	71.53	576.01	12.4	1	5	-	-
<b>9c</b>	504.44	3.35	3.35	71.53	596.60	12.0	1	5	-	-
<b>10a</b>	438.59	3.93	3.93	58.64	614.18	9.54	1	4	-	-
<b>10b</b>	439.57	2.90	2.90	71.53	606.18	11.8	1	5	-	-
<b>10c</b>	518.47	3.69	3.69	71.53	627.25	11.4	1	5	-	-
<b>11a</b>	452.61	4.35	4.35	58.64	645.93	9.08	1	4	-	-
<b>11a'</b>	452.61	2.47	2.47	58.64	645.69	9.08	1	3	-	-
<b>11b</b>	453.60	3.31	3.31	71.53	637.88	11.2	1	5	-	-
<b>11b'</b>	453.60	1.44	1.44	71.53	638.11	11.2	1	4	-	-
<b>11c</b>	532.50	4.10	4.10	71.53	658.51	10.9	1	5	-	-
<b>11c'</b>	532.50	2.23	2.23	71.53	659.15	10.9	1	4	-	-
<b>12a</b>	450.60	4.33	4.33	58.64	613.95	9.55	1	4	-	-
<b>12a'</b>	450.60	2.46	2.46	58.64	611.04	9.60	1	3	-	-
<b>12b</b>	451.59	3.29	3.29	71.53	606.47	11.8	1	5	31.3	69.2
<b>12b'</b>	451.59	1.42	1.42	71.53	603.19	11.9	1	4	-	-
<b>12c</b>	530.48	4.09	4.09	71.53	606.47	11.8	1	5	-	-
<b>13a'', 7R/S 1:2</b>	478.65	4.98	4.98	66.48	687.00	9.68	1	3	1.95	4.07
<b>13a'', 7R/S 1:1.5</b>	478.65	4.98	4.98	66.48	687.00	9.68	1	3	3.91	8.17
<b>7S-13a''</b>	478.65	4.98	4.98	66.48	687.00	9.68	1	3	7.81	16.3
<b>13d''</b>	558.54	4.85	4.85	79.37	699.89	11.3	1	4	15.6	27.9
<b>12a''</b>	450.60	4.21	4.21	66.48	624.09	10.7	1	3	-	-

<sup>[a]</sup> MW was calculated by ChemDraw 19.1.1.32; clogP, clogD<sub>7.4</sub>, PSA, MSA, hydrogen bond donors (HBD) and acceptors (HBA) were calculated with Marvin (20.3.0), 2020, ChemAxon; % PSA was calculated by % PSA/MSA. (-) means tested but not active. MW = Molecular Weight, PSA = polar surface area, MSA = molecular surface area, rel PSA (PSA/MSA %).

The ranges of physiochemical properties for tetramates exhibiting antibacterial activity (with MIC against MRSA < 32 µg/ml) were MSA of 606 - 670 Å<sup>2</sup>, MW of 451 - 559 Da, clogP of 3.2 – 5.0, clogD<sub>7.4</sub> of 3.2 – 5.0, PSA of 66 - 80 Å<sup>2</sup> and relative PSA of 9 - 12%. These physiochemical boundaries differ significantly in clogD values than previously reported antibacterial active tetramate carboxamides, as might be expected from the functionalisation of the acidic C-6 enol group.<sup>41</sup> The chemical space occupied by these tetramates was further delineated by plots of clogP, clogD<sub>7.4</sub>, PSA and relative PSA against MW (Figure 5). Of interest was that for tetramates with antibacterial activity, their clogP and clogD<sub>7.4</sub> values positively correlate with MW in an approximately linear relationship (Figure 5A and B). Such trends in structure-activity relationships were previously noted for antibacterial active tetramate carboxamides.<sup>41</sup> The preservation of PSA boundaries and narrow ranges of relative PSA indicated the small variation in polarity which is required to maintain antibacterial activity against MRSA.



**Figure 5.** Plots of (A) clog P, (B) clogD<sub>7.4</sub>, (C) PSA/Å<sup>2</sup> and (D) %PSA against MW/Da. Data representing the active tetramate is highlighted in red (MIC against MRSA < 8 µg/ml); tetramates

exhibiting mild activity ( $8 \mu\text{g/ml} < \text{MIC against MRSA} \leq 32 \mu\text{g/ml}$ ) are represented in yellow. Colour code: Blue – inactive,  $\text{MIC} > 32 \mu\text{g/ml}$ ; Yellow – mild,  $8 < \text{MIC} < 32$ ; Red – active,  $\text{MIC} < 8 \mu\text{g/ml}$

While a small subset of the synthesised tetramates demonstrated good antibacterial activity against Gram-positive strains of bacteria including MRSA, no activity in Gram-negative *E. coli* was observed. This is in accordance with previous observations that Gram-positive and Gram-negative activities have very different requirements of physiochemical properties.<sup>48</sup> Among tetramates that showed activity against MRSA, while none of their MW exceeds 620 Da, they do have  $\text{clogP} > 3$ ,  $\text{clogD}_{7.4} > 1$  and relative PSA were  $< 12\%$ , which increased their likelihood of being transported out by efflux pumps in Gram-negative bacteria. Most tetramate-containing natural products exhibit selective activity against Gram-positive bacteria while only a few such as epicoccarine A, zopfiellamide A and kibdelomycin have been reported with antibacterial activities against Gram-negative bacteria and mycobacteria which have more complex cell wall and membrane structures.<sup>53-60</sup> This suggests that tetramate-based derivatives generally occupy a chemical space more intrinsically in accordance with the requirement for Gram-positive activity. The physiochemical properties of **13a''** which has an MIC against MRSA  $\leq 1.95 \mu\text{g/ml}$  are MW = 478 Da,  $\text{clogP} = 4.98$  and relative PSA = 10%, which fit better into the chemical space as described by O'Shea and Moser for selective Gram-positive activities.<sup>48</sup>

## Conclusion

We report here that change of ligating group and steric blocking impacts upon metal chelation in tetramates. Replacement of a side chain amide with a C-9 C=N bond both leads to loss of metal-chelating ability and antibacterial activity. The alkylations of tetramates with an extended tricarbonyl conjugated system required in this study could be achieved under Mitsunobu

conditions, the products of which could be assigned by careful HMBC and carbonyl carbon chemical shift analysis. C-6 and C-9 *O*-alkylation were observed but not C-8 *O*-alkylation for tetramate carboxamides; C-7 alkylation with allyl and prenyl derivatives was also observed, and this arose by rearrangement of the initially formed *O*-alkyl products. Alkylation at C-6/C-9 of tetramates led to a complete loss of metal chelating ability, and this was also found to result in a loss of antibacterial activity. This result suggests that the antibacterial activity of tetramates may indeed crucially depend on their metal chelating ability. By contrast, some C-7 alkylated tetramate derivatives with no metal chelating ability demonstrated promising antibacterial activity against MRSA, with a lowest MIC against MRSA  $\leq 1.95 \mu\text{g/ml}$ , suggesting a mechanism of action independent from metal chelation.

## Experimental

### General techniques

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 ( $\text{N}_2$ ) 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 Rotavapor 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  $\text{KMnO}_4$  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 ATR FT-IR spectrometer with thin film (oil sample prepared in 10 mg/1ml  $\text{CHCl}_3$ ) 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 in  $^1\text{H}$  NMR or deuterated solvent peak in  $^{13}\text{C}$  NMR. 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. Structural assignments were made with additional information from gCOSY, gHSQC, and gHMBC experiments. 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. When halogens (X = Cl or Br) are present, MS peaks for isotopomers were included, with the relative abundance corresponded to the isotopic ratios of  $^{35}\text{Cl} : ^{37}\text{Cl} = 3 : 1$  and  $^{79}\text{Br} : ^{81}\text{Br} = 1 : 1$ .

Screening of compounds were performed by Oxford Antibiotic Group, Austria. For MIC determination by broth dilution assay, the samples were tested in a primary 96 well plates screening assay. The compounds were diluted in Mueller Hinton Broth (MHB) for bacterial screening to a stock solution of 1000  $\mu\text{g/mL}$ , serial diluted and overlaid with a microbe solution in a concentration of  $10^4$  CFU/ml. The plates were incubated for 24 h at 35 °C, after which MIC values were read from the plates. For cytotoxicity testing, the synthesized compounds were tested against four different cell lines: HeLa, HEK 293, MDCK and CaCo. The cells were seeded in a 96 well plate and incubated until a confluence of 80% was achieved (under physiological conditions - 37°C, 5%  $\text{CO}_2$  and 95% humidity). The samples were tested by serial dilution in triplicates with starting concentration of 250  $\mu\text{g/mL}$ . After 24 and 48 hours the survival of cells was evaluated by microscope and measured with Alamar Blue.  $\text{IC}_{50}$  values were obtained from the calibration curves. Compounds **1**, **6**, **7**, **14**, **16** and **18** have been published in the literature.<sup>14, 35, 38</sup> These compounds were synthesised according to the literature published procedures and used as starting materials for the relevant reactions.

### Synthesis of *N*-acylthiazolidine **2**

Phenyl acetic acid (1.2 eq.) was added dropwise to a stirred solution of thiazolidine **1** (1.0 eq., 0.9 mmol), *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 filtered and washed with DCM and the combined

filtrates were concentrated *in vacuo* and residue was purified by flash column chromatography to give the *N*-acylated thiazolidine **2**.

#### General procedure of Dieckmann cyclisation for the synthesis of **3**, **5** and **19**

Potassium *tert*-butoxide (KO<sup>t</sup>Bu, 1.2 eqv.) was added to a stirred solution of *N*-acylated thiazolidine (1.0 eqv., 8 mmol) in dry THF (*c* = 0.1) and the mixture was heated under reflux using an oil bath 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 (HCl) to pH = 3 and extracted with ethyl acetate (EtOAc). 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 product.

#### Synthesis of *N*-acylthiazolidine **4**

T<sub>3</sub>P (1.2 eqv., 50% w/v in DMF) was added dropwise to a stirred solution of pyridylacetic acid HCl (1.2 eqv.), DIPEA (5 eqv.) and thiazolidine **1** (1.0 eqv., 1 mmol) in dry DMF (*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 diluted with EtOAc and washed with distilled water and brine. The organic layers were separated, combined, dried with MgSO<sub>4</sub> and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography to give *N*-acylthiazolidine **4**.

#### Synthesis of tetramate derivatives **8-13**

Diethyl azodicarboxylate (DEAD, 1.4 eqv.) was added dropwise to a cooled solution of alcohol (1.2 eqv.), triphenylphosphine (PPh<sub>3</sub>, 1.2 eqv.) and the respective tetramate carboxamides **6a** or **7a-c** (1.0 eqv., 0.2 mmol) in dry THF (*c* = 0.1) at 0 °C. The mixture was stirred at 0 °C for 15 min and then at room temperature for 20 h. It was then concentrated *in vacuo* and residue was purified by flash column chromatography to produce tetramates **8-13**.

### Synthesis of THP-protected tetramate esters **15**

A mixture of the tetramate (1 eqv., 100 mg), 3,4-dihydro-2H-pyran (1.2 eqv.) and catalytic *p*-TsOH monohydrate (0.03 eqv.) in DCM (*c* = 0.1) was stirred at room temperature for 16 h. The mixture was diluted with DCM and washed sequentially with sat. aq. NaHCO<sub>3</sub> and brine. The organic layer was separated, dried with MgSO<sub>4</sub> and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography to yield THP-protected tetramate esters **15c-e**.

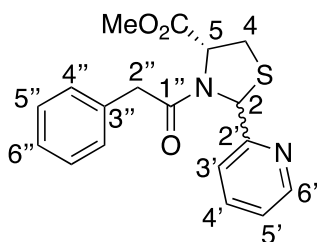
### Synthesis of side chain C-methylated *N*-acylthiazolidine **17**

MeI (1.3 eqv.) was added dropwise to a stirred solution of NaH (1.3 eqv., 60% w/w oil suspension) and *N*-acylthiazolidine **16** (1 eqv., 1 g, 3 mmol) in dry THF (*c* = 0.03) at 0 °C. The mixture was warmed to room temperature and stirred for 16 h. It was then neutralised with sat aq. NH<sub>4</sub>Cl and extracted with EtOAc. The organic layer was separated, dried with MgSO<sub>4</sub> and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography to give **17**.

### General procedure of acylation for synthesis of **20**

Ethyl chloroformate (1.2 eqv.) was added dropwise to a stirred solution of dry Et<sub>3</sub>N or DMAP (1.2 eqv.) and the respective tetramate **19** (1 eqv.) in dry DCM (*c* = 0.1) at 0 °C. The mixture was warmed to room temperature and stirred for 18 h. The reaction mixture was then diluted with DCM and washed with 2M HCl solution. The combined organic layers were dried with MgSO<sub>4</sub> and concentrated *in vacuo* and resulting residue was purified by flash column chromatography to give the desired product.

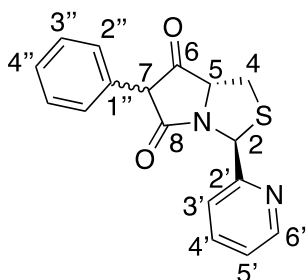
### (5*R*)-5-methoxycarbonyl-1-(2-phenylacetyl)-2-(pyridin-2-yl)-1,3-thiazolidine, **2**



Synthesised from **1** (200 mg, 0.9 mmol); yield (221 mg, 75%); yellow oil; inseparable 2 : 1 *cis* and *trans* diastereomers; *R*<sub>f</sub> = 0.23 (petrol : EtOAc; 3 : 2); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): *cis* as a 1 : 0.1 mixture of rotamers in

CDCl<sub>3</sub>, *trans* as a 1 : 0.7 mixture of rotamers in CDCl<sub>3</sub>:  $\delta$  8.53 (d,  $J$  = 4.8 Hz, 1H, H6' *cis* major rotamer), 8.05 (d,  $J$  = 7.9 Hz, 1H, H3' *cis* major rotamer), 7.66 (td,  $J$  = 7.7, 1.7 Hz, 1H, H4' *cis* major rotamer), 7.56 – 7.66 (m, H4' *trans* major and minor rotamers), 7.21 – 7.30 (m, 2 x H5'', H6'' and H5', *trans* major and minor rotamers), 7.14 – 7.23 (m, 4H, 2 x H5'', H6'' and H5', *cis* major rotamers), 7.03 (d,  $J$  = 6.5 Hz, 2H, H4'', *cis* major rotamer), 6.44 (s, H2, *cis* minor rotamer), 6.21 (s, 1H, H2, *cis* major rotamer), 5.79 (s, H2, *trans* minor rotamer), 5.60 (s, H2, *trans* major rotamer), 4.92 – 4.96 (m, H5, *cis* minor rotamer), 4.89 (dd,  $J$  = 9.4, 6.5 Hz, 1H, H5, *cis* major rotamer), 4.49 (dd,  $J$  = 6.4, 4.6 Hz, H5, *trans* major rotamer), 3.99 (dd,  $J$  = 9.7, 6.7 Hz, H5, *trans* minor rotamer), 3.78 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>, *cis* major rotamer), 3.77 (s, CO<sub>2</sub>CH<sub>3</sub>, *trans* major rotamer), 3.75 (s, CO<sub>2</sub>CH<sub>3</sub>, *trans* minor rotamer), 3.73 (s, CO<sub>2</sub>CH<sub>3</sub>, *cis* minor rotamer), 3.59 (s, H2'', *trans* and minor major rotamer), 3.48 (d,  $J$  = 9.6 Hz, 2H, H2'', *cis* major and minor rotamer), 3.38 – 3.44 (m, H4<sub>B</sub>, *trans* major and minor rotamers), 3.30 (dd,  $J$  = 5.5, 3.8 Hz, H4<sub>A</sub>, *trans* major rotamer), 3.21 (dd,  $J$  = 12.0, 6.5 Hz, 1H, H4<sub>B</sub>, *cis* major and minor rotamer), 3.12 (dd,  $J$  = 12.0, 9.4 Hz, 1H, H4<sub>A</sub>, *cis* major and minor rotamer), 3.02 – 3.09 (m, H4<sub>A</sub>, *trans* minor rotamers); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  175.1, 171.0, 170.5 (CO<sub>2</sub>CH<sub>3</sub> major, minor and rotamers), 160.0 (C1''), 157.4, 156.7 (C2' major and minor isomers), 149.9, 149.6, 149.5 (C6' major, minor and rotamers), 137.8, 137.0, 137.0 (C4' major, minor and rotamers), 134.4, 133.3 (C3'' major and minor), 129.5, 129.1 (C5'' major, minor and rotamers), 128.7, 128.6 (C6'' major, minor and rotamers), 127.3, 127.1, 127.1 (C4'' major, minor and rotamers), 123.5, 123.2, 123.2 (C3' major, minor and rotamers), 122.2, 121.7, 120.6 (C5' major, minor and rotamers), 71.5, 71.0, 67.2 (C5 major, minor and rotamers), 66.3, 65.7, 65.2 (C2 major, minor and rotamers), 53.2, 52.9, 52.7 (CO<sub>2</sub>CH<sub>3</sub> major, minor and rotamers), 41.7, 41.5, 39.4, 38.8 (C2'' major, minor and rotamers), 33.9, 31.4 (C4 *cis* and *trans*);  $m/z$  (ESI<sup>+</sup>) 343 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>)  $m/z$  calcd for C<sub>18</sub>H<sub>19</sub>O<sub>3</sub>N<sub>2</sub>S [M+H]<sup>+</sup> 343.1111; found 343.1106.

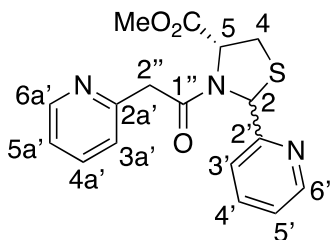
**(2*S*,5*R*)-1-aza-6,8-dioxo-7-phenyl-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]octane, 3**



Synthesised from **2** (220 mg, 0.64 mmol); yield (70 mg, 35%); brown oil;

$R_f$  = 0.25 (100% EtOAc);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz): an inseparable mixture of 5 : 3 : 2 *7R* keto/*7S* keto/enol forms:  $\delta$  8.33 (d,  $J$  = 4.7 Hz, 1H, H6'), 7.74 (app d,  $J$  = 7.6 Hz, 2H, H3' and H4'), 7.66 (app t,  $J$  = 7.7 Hz, 1H, H5'), 7.46 (d,  $J$  = 7.8 Hz, 1H, H4''), 7.28 (d,  $J$  = 7.3 Hz, 2H, H3''), 7.16 (d,  $J$  = 7.2 Hz, 2H, H2''), 6.25 (s, 1H, H2), 4.80 (dd,  $J$  = 8.7, 6.6 Hz, 1H, H5), 3.62 (s, H7 minor, *7S* keto), 3.56 (s, 1H, H7 major, *7R* keto), 3.20 (dd,  $J$  = 10.8, 6.3 Hz, 1H, H4<sub>B</sub>), 2.88 (q,  $J$  = 10.8 Hz, 1H, H4<sub>A</sub>);  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  (C6 not shown), 176.1 (C8), 159.4 (C2'), 148.3 (C6'), 138.4 (C4'), 130.7 (C1''), 129.5, 129.3 (C3''), 128.3, 127.9 (C4''), 127.8, 126.9 (C2''), 123.3 (C3'), 121.1 (C5'), 104.8 (C7, enol), 67.3 (C5), 62.1 (C2), 43.2 (C7, keto), 33.7 (C4);  $m/z$  (ESI<sup>+</sup>) 311 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{17}\text{H}_{15}\text{O}_2\text{N}_2\text{S}$  [M+H]<sup>+</sup> 311.0896; found 311.0852.

#### (5*R*)-5-methoxycarbonyl-1-(2-pyridylacetyl)-2-(pyridin-2-yl)-1,3-thiazolidine, **4**



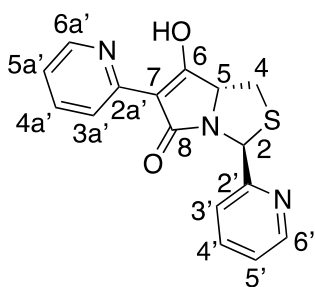
Synthesised from **1** (220 mg, 1 mmol); yield (212 mg, 62%); greenish oil;

inseparable 1.3 : 1 *cis* and *trans* diastereomers;  $R_f$  = 0.51 (petrol : EtOAc; 6 : 1);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz): *cis* as a 1 : 0.2 mixture of rotamers in  $\text{CDCl}_3$ , *trans* as a 1 : 0.7 mixture of rotamers in  $\text{CDCl}_3$ :  $\delta$

8.39 – 8.61 (m, 6H, H6' and H6a'), 8.10 (d,  $J$  = 7.9 Hz, 6H, H3' and H3a'), 7.46 – 7.76 (m, 6H, H4' and H4a'), 7.04 – 7.35 (m, 6H, H5' and H5a'), 6.58 (s, 1H, H2, *cis* major rotamer), 6.40 (s, H2, *cis* minor rotamer), 6.33 (s, 1H, H2, *trans* major rotamer), 6.23 (s, 1H, H2, *trans* minor rotamer), 5.64 (dd,  $J$  = 6.6, 3.1 Hz, H5, *cis* minor rotamer), 5.53 (app d,  $J$  = 5.9 Hz, 1H, H5, *trans* minor rotamer), 5.29 (app d,  $J$  = 7.9 Hz, 1H, H5, *trans* major rotamer), 4.94 (dd,  $J$  = 9.3, 6.6 Hz, 1H, H5, *cis* major rotamer), 3.80 (s, 3H,  $\text{CO}_2\text{CH}_3$ , *trans* major rotamer), 3.80 (s, 3H,  $\text{CO}_2\text{CH}_3$ , *cis* major rotamer), 3.77 (s, 3H,  $\text{CO}_2\text{CH}_3$ , *trans* minor rotamer), 3.76 (s,  $\text{CO}_2\text{CH}_3$ , *cis* minor rotamer), 3.72 (s, 2H, H2'', *cis* major rotamer), 3.60 (s, 2H, H2'', *trans* major rotamer), 3.56 (d,  $J$  = 2.1 Hz, 2H, H2'', *trans* minor rotamer),

3.52 (s, H2'', *cis* minor rotamer), 3.34 – 3.42 (m, 2H, H4<sub>B</sub> *trans*, major and minor rotamers), 3.26 (dd, *J* = 11.9, 6.6 Hz, 1H, H4<sub>B</sub> *cis*, major and minor rotamers), 3.11 – 3.21 (m, 3H, H4<sub>A</sub>, major, minor and rotamers); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 101 MHz): δ 170.9, 170.8, 170.1 (CO<sub>2</sub>CH<sub>3</sub> major, minor and rotamers), 169.7, 169.0, 169.0 (C1'' major, minor and rotamers), 160.7, 160.5, 160.0 (C2' major and minor isomers), 155.0, 154.5, 154.4 (C2a' major and minor isomers), 150.0, 149.8, 149.5, 149.4, 149.2, 149.1 (C6' and C6a' major, minor and rotamers), 137.5, 137.3, 137.0, 136.8, 136.8, 136.7 (C4' and C4a' major, minor and rotamers), 124.3, 124.0, 123.8 (C3' major, minor and rotamers), 123.1, 122.5, 122.2 (C3a' major, minor and rotamers), 120.5, 119.8, 119.6 (C5' and C5a' major, minor and rotamers), 67.8, 67.4, 66.4, 65.9 (C5 major, minor and rotamers), 65.0, 64.2, 64.0, 63.8 (C2 major, minor and rotamers), 53.3, 53.1, 52.8 (CO<sub>2</sub>CH<sub>3</sub> major, minor and rotamers), 45.5, 44.8, 44.3 (C2'' major, minor and rotamers), 31.6, 31.5, 31.4 (C4 major, minor and rotamers); *m/z* (ESI<sup>+</sup>) 344 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>) *m/z* calcd for C<sub>17</sub>H<sub>18</sub>O<sub>3</sub>N<sub>3</sub>S [M+H]<sup>+</sup> 344.1063; found 344.1064.

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



Synthesised from **4** (210 mg, 0.6 mmol); yield (80 mg, 42%); yellow oil;

*R*<sub>f</sub> = 0.43 (EtOAc : MeOH; 6 : 1); *v*<sub>max</sub>/cm<sup>-1</sup> 1583 (s, C=C), 1631 (s, C=O),

1670 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): inseparable 1.5 : 1 AB/CD

tautomers: δ (C6-OH not observed), 8.60 (d, *J* = 4.4 Hz, 2H, H6' and H6a',

major tautomer), 8.45 (d, *J* = 8.9 Hz, 2H, H6' and H6a', minor tautomer), 7.80 – 7.91 (m, 4H, H4'

and H4a' minor and major tautomers), 7.64 – 7.72 (m, 2H, H3' and H3a' major tautomer), 7.45 (d, *J*

= 7.8 Hz, 2H, H3' and H3a' minor tautomer), 7.19 (d, *J* = 5.2 Hz, 2H, H5' and H5a' major tautomer),

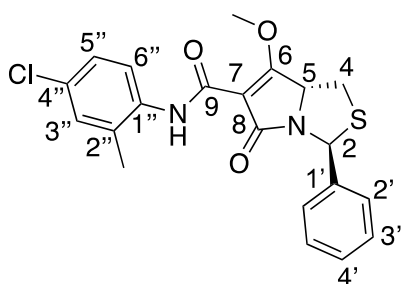
7.02 (t, *J* = 6.5 Hz, 1H, H5' minor tautomer), 6.92 (br. s, 1H, H5a' minor tautomer), 6.54 (s, 1H, H2

major tautomer), 6.49 (s, 1H, H2 minor tautomer), 4.66 (app t 1H, *J* = 7.8 Hz, H5, major tautomer),

4.56 (app t, 1H, *J* = 7.7 Hz, H5, minor tautomer), 3.37 (dd, *J* = 10.8, 7.3 Hz, 2H, H4<sub>B</sub>, major and

minor tautomer), 3.08 (q,  $J = 10.4$  Hz, 2H, H<sub>4A</sub>, major and minor tautomer);  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 126 MHz):  $\delta$  194.3 (C6 major tautomer), 191.4 (C6 minor tautomer), 178.3 (C8 minor tautomer), 174.0 (C8 major tautomer), 160.8 (C2'), 149.7 (C6' and C6a'), 141.8 (C2a'), 137.0 (C4'), 134.7 (C4a'), 122.6 (C3' and C3a'), 119.8 (C5' and C5a'), 91.0 (C7 minor tautomer), 86.5 (C7 major tautomer), 71.0 (C5 minor tautomer), 69.9 (C5 major tautomer), 64.2 (C2), 33.9 (C4);  $m/z$  (ESI<sup>+</sup>) 312 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{16}\text{H}_{14}\text{O}_2\text{N}_3\text{S}$  [M+H]<sup>+</sup> 312.0801; found 312.0802.

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

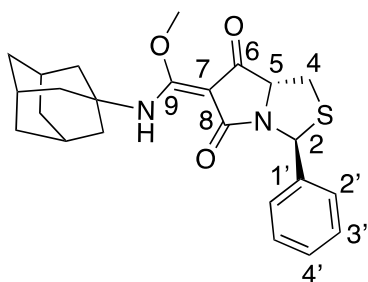


Yield (25 mg, 30%); yellow oil;  $R_f = 0.43$  (petrol : EtOAc; 1 : 4);

$[\alpha]_D^{25} = -114.0^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  1585 (s, C=C), 1618 (s, C=O), 1694 (s, C=O), 3258 (br, N-H);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  10.29 (s, 1H, NH), 8.15 (d,  $J = 9.4$  Hz, 1H, H6''), 7.47 (d,  $J = 7.4$  Hz,

2H, H2'), 7.38 (t,  $J = 7.3$  Hz, 2H, H3'), 7.32 (t,  $J = 7.2$  Hz, 1H, H4'), 7.12 – 7.19 (m, 2H, H3'', H5''), 6.33 (s, 1H, H2), 4.88 (dd,  $J = 9.0, 6.5$  Hz, 1H, H5), 4.32 (s, 3H, OCH<sub>3</sub>), 3.36 (dd,  $J = 10.9, 6.5$  Hz, 1H, H4<sub>B</sub>), 3.02 (dd,  $J = 10.8, 9.1$  Hz, 1H, H4<sub>A</sub>), 2.33 (s, 3H, CH<sub>3</sub>);  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  178.8 (C6), 173.3 (C8), 158.7 (C9), 139.7 (C1'), 135.1 (C2''), 130.2 (C4''), 130.0 (C3''), 129.0 (C1''), 128.9 (C3'), 128.5 (C4'), 126.6 (C5''), 126.4 (C2'), 122.7 (C6''), 102.4 (C7), 65.5 (C5), 62.0 (OCH<sub>3</sub>), 61.5 (C2), 33.5 (C4), 18.1 (CH<sub>3</sub>);  $m/z$  (ESI<sup>+</sup>) 415 ([M+H]<sup>+</sup>, 100%) and 417 ([M+H]<sup>+</sup>, 33%); HRMS (TOF, ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{21}\text{H}_{20}\text{O}_3\text{N}_2\text{ClS}$  [M+H]<sup>+</sup> 415.0878 and 417.0848; found 415.0879 and 417.0847.

**(2S,5R)-7-(Adamantylaminomethylene)-1-aza-9-methoxy-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 9a**

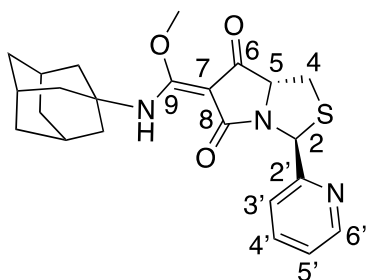


Yield (22 mg, 31%); pale yellow oil;  $R_f = 0.27$  (petrol : EtOAc; 4 : 1);

$[\alpha]_D^{25} = -110.3^\circ$  ( $c = 1.00$ ,  $\text{CHCl}_3$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  1636 (s, C=O), 1682 (s, C=O), 3298 (br. N-H);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  9.80 (br. s, 1H, NH), 7.47 (d,  $J = 7.0$  Hz, 2H, H2'), 7.34 (t,  $J = 7.4$  Hz, 2H, H3'), 7.26 –

7.28 (m, 1H, H4'), 6.43 (s, 1H, H2), 4.42 (app t,  $J = 7.6$  Hz, 1H, H5), 4.31 (s, 3H,  $\text{OCH}_3$ ), 3.28 (dd,  $J = 11.1$ , 7.4 Hz, 1H, H4<sub>B</sub>), 3.04 (dd,  $J = 11.1$ , 8.0 Hz, 1H, H4<sub>A</sub>), 2.13 (br. s, 3H, Adamantyl-CH), 2.01 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.69 (br. s, 6H, Adamantyl-CH<sub>2</sub>);  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 126 MHz):  $\delta$  192.2 (C6), 174.9 (C8), 169.0 (C9), 141.4 (C1'), 128.7 (C3'), 127.9 (C4'), 126.5 (C2'), 87.1 (C7), 69.6 (C5), 63.9 (C2), 62.4 ( $\text{OCH}_3$ ), 55.1 (Adamantyl-C), 42.2 (Adamantyl-CH<sub>2</sub>), 36.1 (Adamantyl-CH<sub>2</sub>), 34.1 (C4), 29.5 (Adamantyl-CH);  $m/z$  (ESI<sup>+</sup>) 425 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{24}\text{H}_{29}\text{O}_3\text{N}_2\text{S}$  [M+H]<sup>+</sup> 425.1893; found 425.1892.

**(2S,5R)-7-(Adamantylaminomethylene)-1-aza-9-methoxy-6,8-dioxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]octane, 9b**

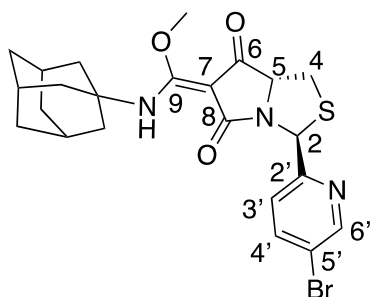


Yield (37 mg, 43%); yellow oil;  $R_f = 0.57$  (petrol : EtOAc; 1 : 3);  $[\alpha]_D^{25} = -110.0^\circ$  ( $c = 1.00$ ,  $\text{CHCl}_3$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  1635 (s, C=O), 1684 (s, C=O);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  9.80 (br. s, 1H, NH), 8.58 (d,  $J = 4.7$  Hz, 1H, H6'), 7.66 (app td,  $J = 7.7$ , 1.8 Hz, 1H, H4'), 7.40 (d,  $J = 7.9$  Hz, 1H, H3'), 7.18 (dd,  $J = 7.5$ , 4.9 Hz, 1H, H5'), 6.49 (s, 1H, H2), 4.57 (app t,  $J = 7.7$  Hz, 1H, H5), 4.31 (s, 3H,  $\text{OCH}_3$ ), 3.35 (dd,  $J = 10.9$ , 7.4 Hz, 1H, H4<sub>B</sub>), 3.05 (dd,  $J = 10.9$ , 8.0 Hz, 1H, H4<sub>A</sub>), 2.13 (br. s, 3H, Adamantyl-CH), 2.01 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.69 (br. s, 6H, Adamantyl-CH<sub>2</sub>);  $^{13}\text{C}\{^1\text{H}\}$  NMR

( $\text{CDCl}_3$ , 101 MHz):  $\delta$  193.6 (C6), 176.6 (C8), 169.1 (C9), 160.5 (C2'), 149.8 (C6'), 137.0 (C4'), 122.7 (C3'), 119.9 (C5'), 87.0 (C7), 70.1 (C5), 64.6 (C2), 62.4 ( $\text{OCH}_3$ ), 55.1 (Adamantyl-C), 42.2 (Adamantyl-CH<sub>2</sub>), 36.1 (Adamantyl-CH<sub>2</sub>), 34.0 (C4), 29.5 (Adamantyl-CH);  $m/z$  (TOF, ESI<sup>+</sup>) 426 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{23}\text{H}_{28}\text{O}_3\text{N}_3\text{S}$  [M+H]<sup>+</sup> 426.1846; found 426.1847.



**(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-2-(5-bromopyridin-2-yl)-9-methoxy-6,8-dioxo-3-thiabicyclo[3.3.0]octane, 9c**



Yield (13 mg, 11%); pale yellow oil;  $R_f$  = 0.32 (petrol : EtOAc; 7 : 3);

$[\alpha]_D^{25}$  = -132.2° ( $c$  = 1.0,  $\text{CHCl}_3$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  1600 (s, C=C), 1636 (s,

C=O), 1687 (s, C=O), 3273 (br, N-H);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$

9.78 (br. s, 1H, NH), 8.63 (d,  $J$  = 1.9 Hz, 1H, H6'), 7.78 (dd,  $J$  = 8.3, 2.3

Hz, 1H, H4'), 7.32 (d,  $J$  = 8.3 Hz, 1H, H3'), 6.43 (s, 1H, H2), 4.54 (app t,  $J$  = 7.7 Hz, 1H, H5), 4.30 (s,

3H,  $\text{OCH}_3$ ), 3.34 (dd,  $J$  = 11.0, 7.4 Hz, 1H, H4<sub>B</sub>), 3.05 (dd,  $J$  = 11.0, 8.0 Hz, 1H, H4<sub>A</sub>), 2.13 (br. s, 3H,

Adamantyl-CH), 2.01 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.69 (br. s, 6H, Adamantyl-CH<sub>2</sub>);  $^{13}\text{C}\{^1\text{H}\}$  NMR

( $\text{CDCl}_3$ , 126 MHz):  $\delta$  192.4 (C6), 174.8 (C8), 169.1 (C9), 159.1 (C2'), 150.8 (C6'), 139.5 (C4'), 121.4

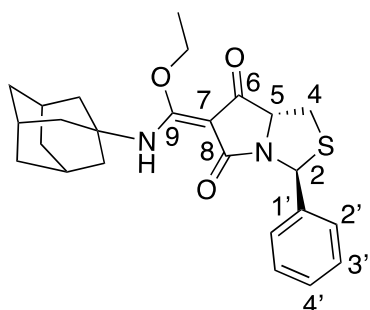
(C3'), 119.6 (C5'), 86.9 (C7), 70.1 (C5), 64.1 (C2), 62.4 ( $\text{OCH}_3$ ), 55.1 (Adamantyl-C), 42.2

(Adamantyl-CH<sub>2</sub>), 36.1 (Adamantyl-CH<sub>2</sub>), 34.1 (C4), 29.5 (Adamantyl-CH);  $m/z$  (ESI<sup>+</sup>) 504 and 506

( $[\text{M}+\text{H}]^+$ , 100%); HRMS (TOF, ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{23}\text{H}_{27}\text{O}_3\text{N}_3\text{BrS}$   $[\text{M}+\text{H}]^+$  504.0951 and 506.0931;

found 504.0952 and 506.0930.

**(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-9-ethoxy-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 10a**



Yield (24 mg, 32%, an inseparable 10 : 1 mixture of C-9/C-6 O-

alkylated isomers); pale yellow oil;  $R_f$  = 0.33 (petrol : EtOAc; 4 : 1);

$\nu_{\text{max}}/\text{cm}^{-1}$  1635 (s, C=O), 1685 (s, C=O);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):

major C-9 O-alkylated isomer:  $\delta$  9.89 (br. s, 1H, NH), 7.46 (d,  $J$  = 7.1

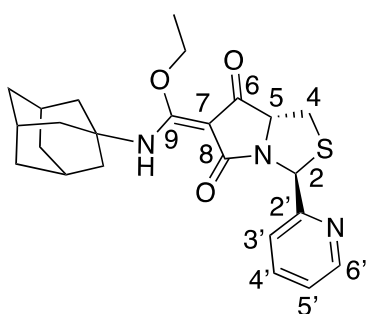
Hz, 2H, H2'), 7.34 (t,  $J$  = 7.4 Hz, 2H, H3'), 7.24 – 7.28 (m, 1H, H4'),

6.43 (s, 1H, H2), (s, 6.14, H2 minor, C-6 O-alkylated isomer), 4.69 – 4.79 (m, 2H,  $\text{OCH}_2\text{CH}_3$ ), 4.41

(app t,  $J$  = 7.6 Hz, 1H, H5), 3.27 (dd,  $J$  = 11.1, 7.4 Hz, 1H, H4<sub>B</sub>), 3.03 (dd,  $J$  = 11.1, 7.9 Hz, 1H, H4<sub>A</sub>),

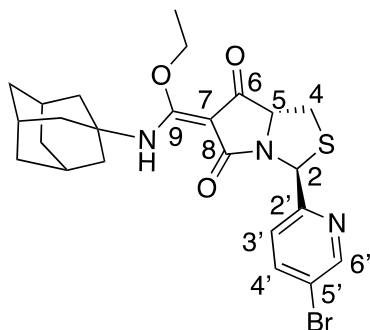
2.13 (br. s, 3H, Adamantyl-CH), 2.04 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.69 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.46 (t, *J* = 7.2 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): major C-9 *O*-alkylated isomer: δ 196.1 (C6), 181.1 (C8), 167.9 (C9), 141.4 (C1'), 128.7 (C3'), 127.9 (C4'), 126.5 (C2'), 87.5 (C7), 72.0 (OCH<sub>2</sub>CH<sub>3</sub>), 69.6 (C5), 63.9 (C2), 54.8 (Adamantyl-C), 42.2 (Adamantyl-CH<sub>2</sub>), 36.1 (Adamantyl-CH<sub>2</sub>), 34.1 (C4), 29.5 (Adamantyl-CH), 15.5 (OCH<sub>2</sub>CH<sub>3</sub>); minor C6 *O*-alkylated isomer (not all peaks shown): δ 175.1 (C8), 160.5 (C9), 97.3 (C7); *m/z* (TOF, ESI<sup>+</sup>) 439 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) *m/z* calcd for C<sub>25</sub>H<sub>31</sub>O<sub>3</sub>N<sub>2</sub>S [M+H]<sup>+</sup> 439.2050; found 439.2048.

**(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-9-ethoxy-6,8-dioxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]octane, 10b**



Yield (26 mg, 29%, an inseparable 5 : 1 mixture of C-9/C-6 *O*-alkylated isomers); yellow oil; *R*<sub>f</sub> = 0.51 (petrol : EtOAc; 2 : 3); *v*<sub>max</sub>/cm<sup>-1</sup> 1636 (s, C=O), 1682 (s, C=O), 3298 (br. s, N-H); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): major C-9 *O*-alkylated isomer: δ 9.89 (br. s, 1H, NH), 8.59 (d, *J* = 4.7 Hz, 1H, H6'), 7.66 (app td, *J* = 7.7, 1.8 Hz, 1H, H4'), 7.40 (d, *J* = 7.9 Hz, 1H, H3'), 7.18 (dd, *J* = 8.0, 5.3 Hz, 1H, H5'), 6.49 (s, 1H, H2), (s, 6.17, H2 minor, C-6 *O*-alkylated isomer), 4.75 (br. s, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.56 (app t, *J* = 7.8 Hz, 1H, H5), 3.37 – 3.31 (m, 1H, H4<sub>B</sub>), 3.04 (dd, *J* = 10.9, 8.1 Hz, 1H, H4<sub>A</sub>), 2.13 (br. s, 3H, Adamantyl-CH), 2.04 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.68 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.47 (t, *J* = 7.1 Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): major C-9 *O*-alkylated isomer: δ 196.2 (C6), 180.7 (C8), 167.9 (C9), 160.5 (C2'), 149.8 (C6'), 137.0 (C4'), 122.7 (C3'), 119.9 (C5'), 87.4 (C7), 72.1 (OCH<sub>2</sub>CH<sub>3</sub>), 70.1 (C5), 64.6 (C2), 54.8 (Adamantyl-C), 42.2 (Adamantyl-CH<sub>2</sub>), 36.1 (Adamantyl-CH<sub>2</sub>), 34.1 (C4), 29.5 (Adamantyl-CH), 15.5 (OCH<sub>2</sub>CH<sub>3</sub>); minor C6 *O*-alkylated isomer (not all peaks shown): δ 196.2 (C6), 177.2 (C8), 162.1 (C9), 96.9 (C7); *m/z* (ESI<sup>+</sup>) 440 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>) *m/z* calcd for C<sub>24</sub>H<sub>30</sub>O<sub>3</sub>N<sub>3</sub>S [M+H]<sup>+</sup> 440.2008; found 440.2002.

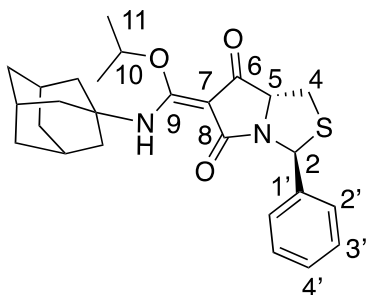
**(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-2-(5-bromopyridin-2-yl)-9-ethoxy-6,8-dioxo-3-thiabicyclo[3.3.0]octane, 10c**



Yield (18 mg, 17%, an inseparable 5 : 1 mixture of C-9/C-6 *O*-alkylated isomers); pale yellow oil;  $R_f$  = 0.33 (petrol : EtOAc; 7 : 3);  $\nu_{\max}/\text{cm}^{-1}$  1635 (s, C=O), 1685 (s, C=O);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz): major C-9 *O*-alkylated isomer:  $\delta$  9.87 (br. s, 1H, NH), 8.62 (d,  $J$  = 2.3 Hz, 1H, H6'), 7.78 (dd,  $J$  = 8.3, 2.3 Hz, 1H, H4'), 7.32 (d,  $J$  = 8.3 Hz, 1H,

H3'), 6.43 (s, 1H, H2), (s, 6.10, H2 minor, C-6 *O*-alkylated isomer), 4.62 – 4.85 (m, 2H,  $\text{OCH}_2\text{CH}_3$ ), 4.53 (app t,  $J$  = 7.7 Hz, 1H, H5), 3.34 (dd,  $J$  = 11.0, 7.4 Hz, 1H, H4<sub>B</sub>), 3.04 (dd,  $J$  = 11.0, 8.0 Hz, 1H, H4<sub>A</sub>), 2.13 (br. s, 3H, Adamantyl-CH), 2.04 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.69 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.47 (t,  $J$  = 7.1 Hz, 3H,  $\text{OCH}_2\text{CH}_3$ );  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 126 MHz): major C-9 *O*-alkylated isomer:  $\delta$  196.1 (C6), 180.6 (C8), 167.9 (C9), 159.2 (C2'), 150.8 (C6'), 139.5 (C4'), 121.4 (C3'), 119.6 (C5'), 87.3 (C7), 72.1 ( $\text{OCH}_2\text{CH}_3$ ), 70.0 (C5), 64.2 (C2), 54.9 (Adamantyl-C), 42.2 (Adamantyl-CH<sub>2</sub>), 36.1 (Adamantyl-CH<sub>2</sub>), 34.1 (C4), 29.5 (Adamantyl-CH), 15.5 ( $\text{OCH}_2\text{CH}_3$ ); minor C6 *O*-alkylated isomer (not all peaks shown):  $\delta$  175.0 (C8), 161.0 (C9), 97.0 (C7);  $m/z$  (ESI<sup>+</sup>) 518 and 520 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{24}\text{H}_{29}\text{O}_3\text{N}_3\text{BrS}$  [M+H]<sup>+</sup> 518.11075 and 520.10870; found 518.1107 and 520.1086.

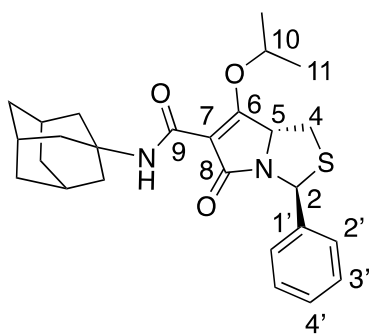
**(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-9-isopropoxy-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 11a**



Yield (24 mg, 27%); pale yellow oil;  $R_f$  = 0.33 (petrol : EtOAc; 4 : 1);  $[\alpha]_{\text{D}}^{25}$  = -118.5° ( $c$  = 1.00,  $\text{CHCl}_3$ );  $\nu_{\max}/\text{cm}^{-1}$  1633 (s, C=O), 1684 (s, C=O);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  10.12 (br. s, 1H, NH), 7.47 (d,  $J$  =

7.1 Hz, 2H, H2'), 7.34 (t,  $J = 7.4$  Hz, 2H, H3'), 7.24 – 7.28 (m, 1H, H4'), 6.43 (s, 1H, H2), 5.89 (sept,  $J = 6.1$  Hz, 1H, H10), 4.41 (app t,  $J = 7.6$  Hz, 1H, H5), 3.27 (dd,  $J = 11.1, 7.4$  Hz, 1H, H4<sub>B</sub>), 3.03 (dd,  $J = 11.1, 7.9$  Hz, 1H, H4<sub>A</sub>), 2.13 (br. s, 3H, Adamantyl-CH), 2.04 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.69 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.41 (d,  $J = 6.1$  Hz, 3H, H11<sub>B</sub>), 1.40 (d,  $J = 6.1$  Hz, 3H, H11<sub>A</sub>);  $^{13}\text{C}\{^1\text{H}\}$  NMR (CDCl<sub>3</sub>, 126 MHz):  $\delta$  192.4 (C6), 175.6 (C8), 166.7 (C9), 141.4 (C1'), 128.7 (C3'), 127.9 (C4'), 126.5 (C2'), 87.7 (C7), 80.1 (C10), 69.5 (C5), 64.1 (C2), 54.6 (Adamantyl-C), 42.2 (Adamantyl-CH<sub>2</sub>), 36.2 (Adamantyl-CH<sub>2</sub>), 34.2 (C4), 29.5 (Adamantyl-CH), 23.0, 22.9 (C11);  $m/z$  (ESI<sup>+</sup>) 453 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>)  $m/z$  calcd for C<sub>26</sub>H<sub>33</sub>O<sub>3</sub>N<sub>2</sub>S [M+H]<sup>+</sup> 453.2206; found 453.2208.

**(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-6-isopropoxy-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 11a'**



Yield (22 mg, 24%); pale yellow oil;  $R_f = 0.30$  (petrol : EtOAc; 4 : 1);

$[\alpha]_D^{25} = -58.3^\circ$  ( $c = 1.00$ , CHCl<sub>3</sub>);  $\nu_{\max}/\text{cm}^{-1}$  1654 (s, C=O), 1686 (s,

C=O);  $^1\text{H}$  NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  8.17 (br. s, 1H, NH), 7.45 (d,  $J =$

7.6 Hz, 2H, H2'), 7.35 (t,  $J = 7.4$  Hz, 2H, H3'), 7.27 – 7.32 (m, 1H, H4'),

6.23 (s, 1H, H2), 5.67 (sept,  $J = 6.1$  Hz, 1H, H10), 4.66 (dd,  $J = 8.9, 6.5$

Hz, 1H, H5), 3.25 (dd,  $J = 10.9, 6.5$  Hz, 1H, H4<sub>B</sub>), 2.90 (dd,  $J = 10.9, 8.9$  Hz, 1H, H4<sub>A</sub>), 2.06 (br. s, 9H,

Adamantyl-CH<sub>2</sub> and Adamantyl-CH), 1.69 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.39 (d,  $J = 6.1$  Hz, 3H, H11<sub>B</sub>),

1.35 (d,  $J = 6.1$  Hz, 3H, H11<sub>A</sub>);  $^{13}\text{C}\{^1\text{H}\}$  NMR (CDCl<sub>3</sub>, 126 MHz):  $\delta$  175.6 (C6), 174.3 (C8), 160.2 (C9),

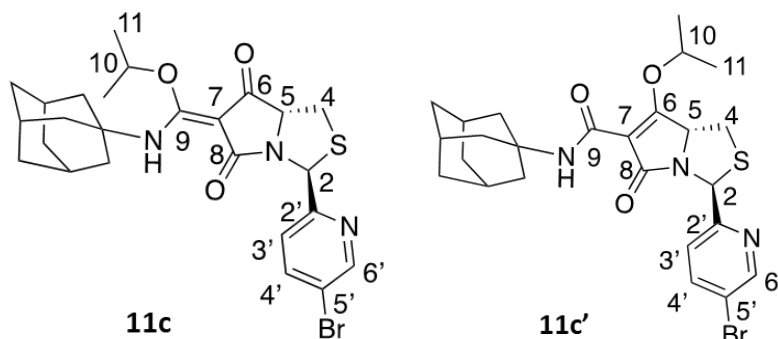
140.2 (C1'), 128.9 (C3'), 128.3 (C4'), 126.5 (C2'), 103.1 (C7), 78.9 (C10), 66.6 (C5), 62.0 (C2), 51.8

(Adamantyl-C), 41.7 (Adamantyl-CH<sub>2</sub>), 36.6 (Adamantyl-CH<sub>2</sub>), 34.0 (C4), 29.6 (Adamantyl-CH), 22.7,

22.6 (C11);  $m/z$  (TOF, ESI<sup>+</sup>) 453 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>26</sub>H<sub>33</sub>O<sub>3</sub>N<sub>2</sub>S [M+H]<sup>+</sup>

453.2206; found 453.2208.

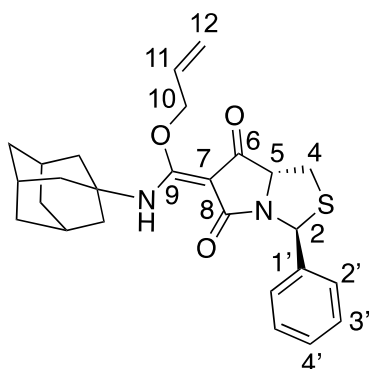
**(2*S*,5*R*)-7-(Adamantylaminomethylene)-1-aza-2-(5-bromopyridin-2-yl)-9-isopropoxy-6,8-dioxo-3-thiabicyclo[3.3.0]octane, 11c** and **(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-1-aza-2-(5-bromopyridin-2-yl)-6-isopropoxy-8-oxo-3-thiabicyclo[3.3.0]oct-6-ene, 11c'**



Yield (12 mg, 15%, an inseparable 1 : 1 mixture of C9/C6 O-alkylated isomers); pale yellow oil;  $R_f$  = 0.30 (petrol : EtOAc; 9 : 1);  $\nu_{\max}/\text{cm}^{-1}$  1635 (s, C=O), 1687 (s, C=O);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz): C-9 O-alkylated isomer **11c**:  $\delta$  10.09 (br. s, 1H, NH), 8.63 (s, 1H, H6'), 7.79 (app td,  $J$  = 8.1, 2.3 Hz, 1H, H4'), 7.33 (d,  $J$  = 8.3 Hz, 1H, H3'), 6.43 (s, 1H, H2), 5.87 (sept,  $J$  = 6.1 Hz, 1H, H10), 4.53 (app t,  $J$  = 7.6 Hz, 1H, H5), 3.33 – 3.37 (m, 1H, H4<sub>B</sub>), 3.05 (dd,  $J$  = 11.0, 7.8 Hz, 1H, H4<sub>A</sub>), 2.13 (br. s, 3H, Adamantyl-CH), 2.06 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.68 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.41 (d,  $J$  = 6.1 Hz, 3H, H11<sub>B</sub>), 1.40 (d,  $J$  = 6.1 Hz, 3H, H11<sub>A</sub>); C6 O-alkylated isomer **11c'**:  $\delta$  8.63 (s, 1H, H6'), 8.10 (br. s, 1H, NH), 7.79 (app td,  $J$  = 8.1, 2.3 Hz, 1H, H4'), 7.29 (d,  $J$  = 8.3 Hz, 1H, H3'), 6.23 (s, 1H, H2), 5.63 (sept,  $J$  = 6.1 Hz, 1H, H10), 4.80 (dd,  $J$  = 8.7, 6.8 Hz, 1H, H5), 3.29 – 3.33 (m, 1H, H4<sub>B</sub>), 2.94 (dd,  $J$  = 10.8, 8.7 Hz, 1H, H4<sub>A</sub>), 2.04 – 2.06 (br. s, 3H, Adamantyl-CH), 2.04 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.68 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.36 (d,  $J$  = 6.1 Hz, 3H, H11<sub>B</sub>), 1.32 (d,  $J$  = 6.1 Hz, 3H, H11<sub>A</sub>);  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 126 MHz): C-9 O-alkylated isomer **11c**:  $\delta$  192.7 (C6, only seen by HMBC), 176.2 (C8), 166.8 (C9), 159.2 (C2'), 150.8 (C6'), 139.5 (C4'), 121.5 (C3'), 119.6 (C5'), 87.6 (C7), 80.3 (C10), 69.9 (C5), 64.4 (C2), 54.7 (Adamantyl-C), 42.2 (Adamantyl-CH<sub>2</sub>), 36.6 (Adamantyl-CH<sub>2</sub>), 34.2 (C4), 29.6 (Adamantyl-CH), 23.0, 22.8, (C11); C6 O-alkylated isomer **11c'**:  $\delta$  176.6 (C6, only seen by HMBC), 174.5 (C8), 160.0 (C9), 158.1 (C2'), 151.0 (C6'), 139.7 (C4'), 121.6 (C3'), 120.0 (C5'), 102.8 (C7), 79.0 (C10), 66.8 (C5), 62.1 (C2), 51.8 (Adamantyl-C), 41.7 (Adamantyl-CH<sub>2</sub>), 36.2 (Adamantyl-CH<sub>2</sub>), 33.9

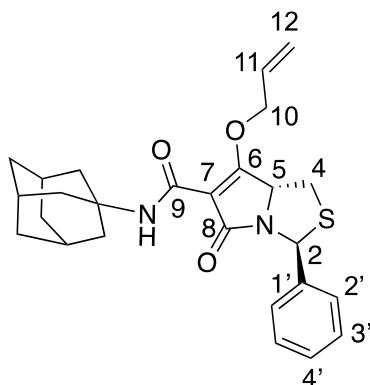
(C4), 29.5 (Adamantyl-CH), 22.9, 22.7 (C11);  $m/z$  (TOF, ESI<sup>+</sup>) 532 and 534 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$  calcd for C<sub>25</sub>H<sub>31</sub>O<sub>3</sub>N<sub>3</sub>BrS [M+H]<sup>+</sup> 532.1264 and 534.1244; found 532.1266 and 534.1246.

**(2S,5R)-7-(Adamantylaminomethylene)-9-alloxy-1-aza-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 12a**



Yield (25 mg, 21%); yellow oil;  $R_f$  = 0.32 (petrol : EtOAc; 4 : 1);  $[\alpha]_D^{25}$  = -110.5° ( $c$  = 1.00, CHCl<sub>3</sub>);  $\nu_{\max}/\text{cm}^{-1}$  1635 (s, C=O), 1685 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  9.95 (br. s, 1H, NH), 7.47 (d,  $J$  = 7.5 Hz, 2H, H2'), 7.34 (t,  $J$  = 7.6 Hz, 2H, H3'), 7.25 – 7.28 (m, 1H, H4', obscured by solvent), 6.43 (s, 1H, H2), 6.01 – 6.12 (m, 1H, H11), 5.43 (d,  $J$  = 17.1 Hz, 1H, H12<sub>B</sub>), 5.34 (d,  $J$  = 10.3 Hz, 1H, H12<sub>A</sub>), 5.20 (br. s, 2H, H10), 4.41 (app t,  $J$  = 7.6 Hz, 1H, H5), 3.27 (dd,  $J$  = 11.1, 7.4 Hz, 1H, H4<sub>B</sub>), 3.03 (dd,  $J$  = 11.1, 8.0 Hz, 1H, H4<sub>A</sub>), 2.12 (br. s, 3H, Adamantyl-CH), 2.02 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.68 (br. s, 6H, Adamantyl-CH<sub>2</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz):  $\delta$  192.4 (C6), 175.1 (C8), 167.6 (C9), 141.3 (C1'), 131.8 (C11), 128.7 (C3'), 127.9 (C4'), 126.5 (C2'), 120.3 (C12), 87.6 (C7), 76.2 (C10), 69.5 (C5), 63.9 (C2), 54.9 (Adamantyl-C), 42.2 (Adamantyl-CH<sub>2</sub>), 36.1 (Adamantyl-CH<sub>2</sub>), 34.1 (C4), 29.5 (Adamantyl-CH);  $m/z$  (ESI<sup>+</sup>) 451 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>)  $m/z$  calcd for C<sub>26</sub>H<sub>31</sub>O<sub>3</sub>N<sub>2</sub>S [M+H]<sup>+</sup> 451.2050; found 451.2049.

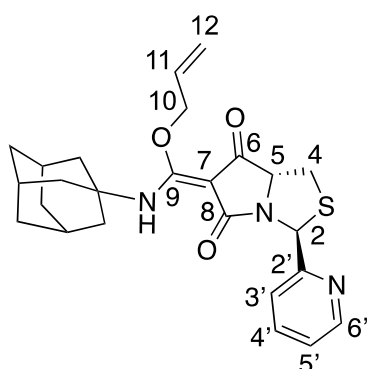
**(2S,5R)-7-(Adamantylaminocarbonyl)-6-alloxy-1-aza-8-oxo-2-phenyl-3-thiabicyclo[3.3.0]oct-6-ene, 12a'**



Yield (46 mg, 38%); pale yellow oil;  $R_f$  = 0.16 (petrol : EtOAc; 4 : 1);  $[\alpha]_D^{25}$  = -50.2° ( $c$  = 1.00, CHCl<sub>3</sub>);  $\nu_{\max}/\text{cm}^{-1}$  1656 (s, C=O), 1686 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  8.10 (br. s, 1H, NH), 7.44 (d,  $J$  = 7.2 Hz, 2H, H2'), 7.29 – 7.37 (m, 3H, H4' and H3'), 6.21 (s, 1H, H2),

5.92 – 6.06 (m, 1H, H11), 5.43 (d,  $J = 17.2$  Hz, 1H, H12<sub>B</sub>), 5.33 (d,  $J = 10.4$  Hz, 1H, H12<sub>A</sub>), 5.13 (d,  $J = 5.7$  Hz, 2H, H10), 4.74 (dd,  $J = 9.1, 6.4$  Hz, 1H, H5), 3.26 (dd,  $J = 10.9, 6.4$  Hz, 1H, H4<sub>B</sub>), 2.92 (dd,  $J = 10.9, 9.1$  Hz, 1H, H4<sub>A</sub>), 2.05 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.96 (br. s, 3H, Adamantyl-CH), 1.67 (br. s, 6H, Adamantyl-CH<sub>2</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  176.0 (C6), 173.7 (C8), 159.9 (C9), 140.0 (C1'), 131.8 (C11), 128.8 (C3'), 128.4 (C4'), 126.5 (C2'), 119.9 (C12), 103.8 (C7), 75.4 (C10), 66.1 (C5), 61.7 (C2), 51.8 (Adamantyl-C), 41.6 (Adamantyl-CH<sub>2</sub>), 36.5 (Adamantyl-CH<sub>2</sub>), 33.8 (C4), 29.5 (Adamantyl-CH);  $m/z$  (ESI<sup>+</sup>) 451 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>)  $m/z$  calcd for C<sub>26</sub>H<sub>31</sub>O<sub>3</sub>N<sub>2</sub>S [M+H]<sup>+</sup> 451.2050; found 451.2049.

**(2*S*,5*R*)-7-(Adamantylaminomethylene)-9-alloxy-1-aza-6,8-dioxo-2-(pyridin-2-yl)-3-thiabicyclo[3.3.0]octane, 12b**

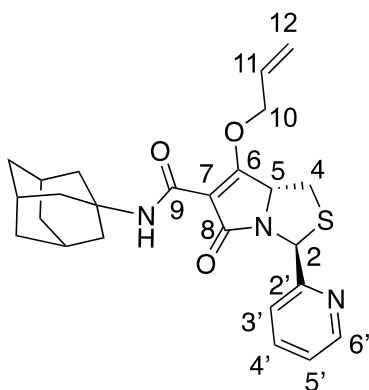


Yield (7 mg, 5%, calculated from crude <sup>1</sup>H NMR as the isolated product was contaminated with reduced DEAD); yellow oil;  $R_f = 0.65$  (petrol : EtOAc; 1 : 1);  $\nu_{\max}/\text{cm}^{-1}$  1635 (s, C=O), 1719 (s, C=O), 3299 (br. s, N-H); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  9.94 (br. s, 1H, NH), 8.59 (d,  $J = 4.9$  Hz, 1H, H6'), 7.67 (app td,  $J = 7.7, 1.7$  Hz, 1H, H4'), 7.40 (d,  $J =$

7.8 Hz, 1H, H3'), 7.18 (dd,  $J = 7.5, 4.9$  Hz, 1H, H5'), 6.49 (s, 1H, H2), 5.99 – 6.13 (m, 1H, H11), 5.43 (d,  $J = 17.1$  Hz, 1H, H12<sub>B</sub>), 5.34 (d,  $J = 10.3$  Hz, 1H, H12<sub>A</sub>), 5.21 (br. s, 2H, H10), 4.57 (app t,  $J = 7.7$  Hz, 1H, H5), 3.35 (dd,  $J = 10.9, 7.4$  Hz, 1H, H4<sub>B</sub>), 3.04 (dd,  $J = 10.9, 8.1$  Hz, 1H, H4<sub>A</sub>), 2.12 (br. s, 3H, Adamantyl-CH), 2.03 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.67 (br. s, 6H, Adamantyl-CH<sub>2</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz):  $\delta$  191.2 (C6), 173.1 (C8), 167.6 (C9), 160.4 (C2'), 149.8 (C6'), 137.0 (C4'), 131.8 (C11), 122.7 (C3'), 120.3 (C12), 119.9 (C5'), 87.4 (C7), 76.3 (C10), 70.1 (C5), 64.6 (C2), 55.0 (Adamantyl-C), 42.2 (Adamantyl-CH<sub>2</sub>), 36.1 (Adamantyl-CH<sub>2</sub>), 34.1 (C4), 29.5 (Adamantyl-CH);  $m/z$  (ESI<sup>+</sup>) 452 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>)  $m/z$  calcd for C<sub>25</sub>H<sub>30</sub>O<sub>3</sub>N<sub>2</sub>S [M+H]<sup>+</sup> 452.2202; found 452.2204.

**(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-6-allyloxy-1-aza-8-oxo-2-(pyridin-2-yl)-3-**

**thiabicyclo[3.3.0]oct-6-ene, 12b'**

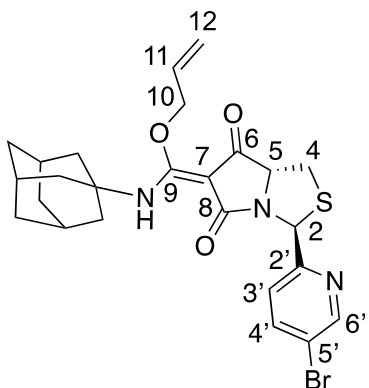


Yield (12 mg, 14%); yellow oil;  $R_f$  = 0.51 (petrol : EtOAc; 1 : 1);  $[\alpha]_D^{25}$  = -42.4° ( $c$  = 0.60,  $\text{CHCl}_3$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  1655 (s, C=O), 1687 (s, C=O), 3300 (br. s, N-H);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz):  $\delta$  8.59 (d,  $J$  = 4.9 Hz, 1H, H6'), 8.08 (br. s, 1H, NH), 7.68 (app td,  $J$  = 7.7, 1.8 Hz, 1H, H4'), 7.37 (d,  $J$  = 7.8 Hz, 1H, H3'), 7.21 (dd,  $J$  = 7.5, 4.9 Hz, 1H, H5'), 6.29 (s, 1H, H2), 5.96 – 6.05 (m, 1H, H11), 5.44 (d,  $J$  = 17.2 Hz, 1H, H12<sub>B</sub>), 5.34 (d,

$J$  = 10.5 Hz, 1H, H12<sub>A</sub>), 5.08 – 5.19 (m, 2H, H10), 4.93 (dd,  $J$  = 8.9, 6.8 Hz, 1H, H5), 3.34 (dd,  $J$  = 10.7, 6.8 Hz, 1H, H4<sub>B</sub>), 2.96 (dd,  $J$  = 10.7, 8.9 Hz, 1H, H4<sub>A</sub>), 2.06 (br. s, 9H, Adamantyl-CH<sub>2</sub> and Adamantyl-CH), 1.67 (br. s, 6H, Adamantyl-CH<sub>2</sub>);  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 126 MHz):  $\delta$  176.7 (C6), 173.7 (C8), 159.9 (C9), 159.3 (C2'), 150.0 (C6'), 137.2 (C4'), 131.8 (C11), 123.1 (C3'), 120.1 (C12), 120.0 (C5'), 103.6 (C7), 75.4 (C10), 66.3 (C5), 62.3 (C2), 51.8 (Adamantyl-C), 41.7 (Adamantyl-CH<sub>2</sub>), 36.6 (Adamantyl-CH<sub>2</sub>), 33.6 (C4), 29.6 (Adamantyl-CH);  $m/z$  (TOF, ESI<sup>+</sup>) 452 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>)  $m/z$  calcd for  $\text{C}_{25}\text{H}_{30}\text{O}_3\text{N}_2\text{S}$  [M+H]<sup>+</sup> 452.2202; found 452.2204.

**(2*S*,5*R*)-7-(Adamantylaminomethylene)-9-allyloxy-1-aza-2-(5-bromopyridin-2-yl)-6,8-dioxo-3-**

**thiabicyclo[3.3.0]octane, 12c**

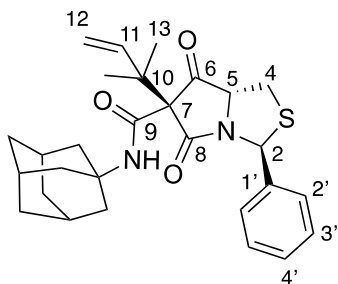


Yield (10 mg, 11%, isolated as 5 : 1 mixture of C-9/C-6 O-alkylated isomers); pale yellow oil;  $R_f$  = 0.54 (petrol : EtOAc; 7 : 3);  $\nu_{\text{max}}/\text{cm}^{-1}$  1636 (s, C=O), 1693 (s, C=O), 3295 (br. s, N-H);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz): major C-9 O-alkylated isomer:  $\delta$  9.92 (br. s, 1H, NH), 8.63 (d,  $J$  = 2.3 Hz, 1H, H6'), 7.78 (dd,  $J$  = 8.3, 2.3 Hz, 1H, H4'), 7.32 (d,  $J$  = 8.3



Hz, 1H, H3'), 6.43 (s, 1H, H2), 6.24 (s, H2 minor, C-6 *O*-alkylated isomer), 5.99 – 6.15 (m, 1H, H11), 5.43 (d,  $J = 17.1$  Hz, 1H, H12<sub>B</sub>), 5.35 (d,  $J = 10.4$  Hz, 1H, H12<sub>A</sub>), 5.19 (br. s, 2H, H10), 4.71 (m, H5 minor), 4.53 (app t,  $J = 7.5$  Hz, 1H, H5), 3.34 (dd,  $J = 11.0, 7.4$  Hz, 1H, H4<sub>B</sub>), 3.04 (dd,  $J = 11.0, 8.0$  Hz, 1H, H4<sub>A</sub>), 2.12 (br. s, 3H, Adamantyl-CH), 2.03 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.68 (br. s, 6H, Adamantyl-CH<sub>2</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): major C-9 *O*-alkylated isomer:  $\delta$  192.5 (C6), 173.2 (C8), 167.6 (C9), 159.1 (C2'), 150.8 (C6'), 139.5 (C4'), 131.7 (C11), 121.4 (C3'), 120.4 (C5'), 119.6 (C12), 87.4 (C7), 76.3 (C10), 70.0 (C5), 64.2 (C2), 55.0 (Adamantyl-C), 42.2 (Adamantyl-CH<sub>2</sub>), 36.1 (Adamantyl-CH<sub>2</sub>), 34.1 (C4), 29.5 (Adamantyl-CH); minor C-6 *O*-alkylated isomer (not all peaks shown):  $\delta$  175.0 (C6), 169.5 (C8), 159.6 (C9), 100.2 (C7);  $m/z$  (ESI<sup>+</sup>) 530 and 532 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>)  $m/z$  calcd for C<sub>25</sub>H<sub>29</sub>O<sub>3</sub>N<sub>3</sub>BrS [M+H]<sup>+</sup> 530.1108 and 532.1087; found 530.1109 and 532.1087.

**(2*S*,5*R*,7*S*)-7-(Adamantylaminocarbonyl)-1-aza-7-(2-methylbut-3-en-2-yl)-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 7*S*-13a''**

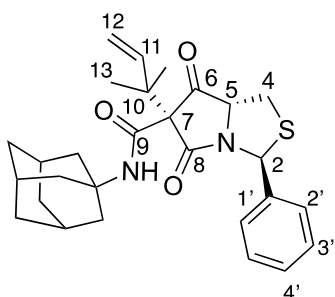


Synthesised from **7a** (330 mg, 0.7 mmol); yield (49 mg, 15%); pale yellow oil;  $R_f = 0.34$  (petrol : EtOAc; 9 : 1);  $[\alpha]_D^{25} = -115.8^\circ$  ( $c = 1.00$ , CHCl<sub>3</sub>);  $\nu_{\max}/\text{cm}^{-1}$  1661 (s, C=O), 1697 (s, C=O), 1770 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  7.38 – 7.42 (m, 2H, H2'), 7.34 – 7.38 (m, 2H, H3'),

7.31 – 7.35 (m, 1H, H4'), 7.11 (br. s, 1H, NH), 6.55 (s, 1H, H2), 6.00 (dd,  $J = 17.3, 10.8$  Hz, 1H, H11), 5.07 – 5.15 (m, 2H, H12), 4.33 (dd,  $J = 9.9, 6.7$  Hz, 1H, H5), 3.30 (dd,  $J = 10.8, 6.7$  Hz, 1H, H4<sub>B</sub>), 2.99 – 3.07 (m, 1H, H4<sub>A</sub>), 2.06 (br. s, 3H, Adamantyl-CH), 1.99 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.66 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.26 (s, 3H, H13<sub>A</sub>), 1.32 (s, 3H, H13<sub>B</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 101 MHz):  $\delta$  200.1 (C6), 171.7 (C8), 160.3 (C9), 142.1 (C11), 139.5 (C1'), 129.0 (C3'), 128.7 (C4'), 126.4 (C2'), 115.5 (C12), 70.1 (C5), 69.1 (C7), 62.9 (C2), 52.9 (Adamantyl-C), 44.6 (C10), 41.3 (Adamantyl-CH<sub>2</sub>), 36.4

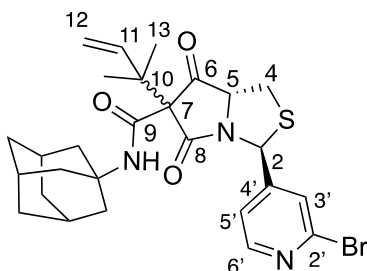
(Adamantyl-CH<sub>2</sub>), 34.0 (C<sub>4</sub>), 29.5 (Adamantyl-CH), 23.4 (C<sub>13<sub>B</sub></sub>), 23.0 (C<sub>13<sub>A</sub></sub>); *m/z* (ESI<sup>+</sup>) 479 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>) *m/z* calcd for C<sub>28</sub>H<sub>35</sub>O<sub>3</sub>N<sub>2</sub>S [M+H]<sup>+</sup> 479.2363; found 479.2365.

**(2*S*,5*R*,7*R*)-7-(Adamantylaminocarbonyl)-1-aza-7-(2-methylbut-3-en-2-yl)-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 7*R*-13a''**



Synthesised from **7a** (330 mg, 0.7 mmol); yield (3%, calculated from crude <sup>1</sup>H NMR, characterisation based a 1.3 : 1 mixture of 7*R*/*S* diastereomers of 8 mg isolated from column chromatography); pale yellow oil; *R<sub>f</sub>* = 0.59 (petrol : EtOAc; 9 : 1); *ν*<sub>max</sub>/cm<sup>-1</sup> 1661 (s, C=O), 1698 (s, C=O), 1772 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ (NH not observed), 7.27 – 7.43 (m, 5H, H<sub>4'</sub>, H<sub>3'</sub> and H<sub>2'</sub>), 6.58 (s, 1H, H<sub>2</sub>), 5.99 (dd, *J* = 17.3, 10.7 Hz, 1H, H<sub>11</sub>), 5.04 – 5.17 (m, 2H, H<sub>12</sub>), 4.62 (dd, *J* = 8.9, 7.6 Hz, 1H, H<sub>5</sub>), 3.27 (m, 1H, H<sub>4<sub>B</sub></sub>), 2.90 (dd, *J* = 10.6, 8.9 Hz, 1H, H<sub>4<sub>A</sub></sub>), 2.05 (br. s, 3H, Adamantyl-CH), 1.93 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.65 (br. s, 6H, Adamantyl-CH<sub>2</sub>), 1.25 (s, 3H, H<sub>13<sub>B</sub></sub>), 1.24 (s, 3H, H<sub>13<sub>A</sub></sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): δ 201.8 (C<sub>6</sub>), 170.9 (C<sub>8</sub>), 162.9 (C<sub>9</sub>), 142.2 (C<sub>11</sub>), 139.3 (C<sub>1'</sub>), 129.0 (C<sub>3'</sub>), 128.6 (C<sub>4'</sub>), 126.2 (C<sub>2'</sub>), 116.9 (C<sub>12</sub>), 69.5 (C<sub>5</sub>), 68.0 (C<sub>7</sub>), 62.3 (C<sub>2</sub>), 53.0 (Adamantyl-C), 44.6 (C<sub>10</sub>), 41.3 (Adamantyl-CH<sub>2</sub>), 36.3 (Adamantyl-CH<sub>2</sub>), 32.9 (C<sub>4</sub>), 29.5 (Adamantyl-CH), 26.0 (C<sub>13</sub>); *m/z* (ESI<sup>+</sup>) 479 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, ESI<sup>+</sup>) *m/z* calcd for C<sub>28</sub>H<sub>35</sub>O<sub>3</sub>N<sub>2</sub>S [M+H]<sup>+</sup> 479.2363; found 479.2365.

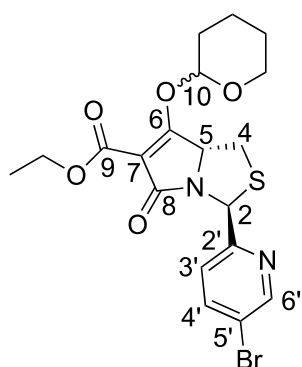
**(2*S*,5*R*)-7-(Adamantylaminocarbonyl)-2-(2-bromopyridin-4-yl)-1-aza-7-(2-methylbut-3-en-2-yl)-6,8-dioxo--3-thiabicyclo[3.3.0]octane, 13d''**



Synthesised from **7d** (200 mg, 0.4 mmol); yield (23 mg, 10%, an inseparable 1 : 1 mixture of 7*R*/*S* diastereomers); yellow oil; *R<sub>f</sub>* = 0.21 (petrol : EtOAc; 9 : 1); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 8.38 (d, *J* =

5.1 Hz, 1H, H6'), 8.36 (d,  $J = 5.5$  Hz, 1H, H6'), 7.51 (s, 1H, H3'), 7.43 (s, 1H, H3'), 7.22 (d,  $J = 5.1$  Hz, 1H, H5', another set of H5' of the other diastereomer obscured by solvent peak), 6.96 (br. s, 2H, NH), 6.47 (s, 1H, H2, 7R), 6.45 (s, 1H, H2, 7S), 6.19 (br. s, 1H, H11), 6.00 (dd,  $J = 17.4, 10.7$  Hz, 1H, H11), 5.09 – 5.28 (m, 4H, H12), 4.17 – 4.27 (m, 2H, H5), 3.32 (dd,  $J = 10.8, 6.8$  Hz, 1H, H4<sub>B</sub>, 7S), 3.23 – 3.29 (m, 1H, H4<sub>B</sub>, 7R), 3.03 – 3.11 (m, 1H, H4<sub>A</sub>, 7S), 2.87 (dd,  $J = 13.6, 7.7$  Hz, 1H, H4<sub>A</sub>, 7R), 2.08 (br. s, 6H, Adamantyl-CH), 1.99 (br. s, 12H, Adamantyl-CH<sub>2</sub>), 1.67 (br. s, 12H, Adamantyl-CH<sub>2</sub>), 1.36 (s, 3H, H13<sub>B</sub>, 7S), 1.31 (s, 3H, H13<sub>A</sub>, 7S), 1.29 (s, 3H, H13<sub>B</sub>, 7R), 1.25 (s, 3H, H13<sub>A</sub>, 7R); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 126 MHz): **7R-13d''**:  $\delta$  201.4 (C6), 172.1 (C8), 163.1 (C9), 151.6 (C6'), 150.7 (C4'), 142.1 (C2'), 139.4 (C11), 125.0 (C3'), 120.4 (C5'), 116.1 (C12), 69.9 (C7), 64.4 (C5), 60.5 (C2), 53.1 (Adamantyl-C), 44.7 (C10), 41.3 (Adamantyl-CH<sub>2</sub>), 36.3 (Adamantyl-CH<sub>2</sub>), 33.8 (C4), 29.5 (Adamantyl-CH), 26.3 (C13<sub>B</sub>), 25.9 (C13<sub>A</sub>); **7S-13d''**:  $\delta$  199.6 (C6), 172.2 (C8), 160.1 (C9), 151.3 (C6'), 150.8 (C4'), 143.0 (C2'), 139.4 (C11), 125.4 (C3'), 120.5 (C5'), 115.4 (C12), 70.0 (C7), 66.2 (C5), 61.1 (C2), 53.1 (Adamantyl-C), 44.7 (C10), 41.4 (Adamantyl-CH<sub>2</sub>), 36.4 (Adamantyl-CH<sub>2</sub>), 34.2 (C4), 29.5 (Adamantyl-CH), 23.5 (C13<sub>B</sub>), 23.0 (C13<sub>A</sub>);  $m/z$  (TOF, ESI<sup>+</sup>) 558 and 560 ([M+H]<sup>+</sup>, 100%), C<sub>27</sub>H<sub>33</sub>O<sub>3</sub>N<sub>3</sub>BrS ([M+H]<sup>+</sup>).

**(2S,5R)-1-Aza-2-(5-bromopyridin-2-yl)-7-ethoxycarbonyl-8-oxo-6-((tetrahydro-2H-pyran-2-yl)oxy)-3-thiabicyclo[3.3.0]oct-6-ene, 15c**

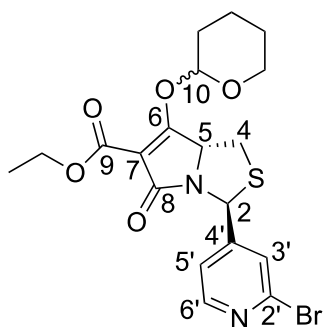


Synthesised from **14c** (100 mg, 0.3 mmol) by general procedure I; yield (72 mg, 59%); yellow oil; inseparable 1 : 1 10R/S diastereomers;  $R_f = 0.42$  (petrol : EtOAc; 4 : 1);  $\nu_{\max}/\text{cm}^{-1}$  1707 (s, C=O), 1748 (s, C=O), 1779 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  8.65 (d,  $J = 2.3$  Hz, 1H, H6'), 8.64 (d,  $J = 2.2$  Hz, 1H, H6'), 7.82 (dd,  $J = 8.3, 2.3$  Hz, 1H, H4'), 7.79 (dd,  $J = 8.3, 2.3$

Hz, 1H, H4'), 7.28 (d,  $J = 8.3$  Hz, 1H, H3'), 7.24 (d, 1H, H3', obscured by solvent peak), 6.58 (s, 1H, H2), 6.52 (s, 1H, H2), 4.69 (dd,  $J = 9.9, 6.8$  Hz, 1H, H10), 4.64 (dd,  $J = 9.9, 6.8$  Hz, 1H, H10), 4.07 –

4.32 (m, 6H,  $\text{OCH}_2\text{CH}_3$  and H5), 3.90 – 3.99 (m, 2H, Tetrahydropyranyl  $\text{OCH}_2$ ), 3.41 – 3.54 (m, 2H, Tetrahydropyranyl  $\text{OCH}_2$ ), 3.34 (dd,  $J = 6.8, 2.1$  Hz, 1H,  $\text{H}_{4\text{B}}$ ), 3.31 (dd,  $J = 6.8, 2.1$  Hz, 1H,  $\text{H}_{4\text{A}}$ ), 3.18 (t,  $J = 10.1$  Hz, 1H,  $\text{H}_{4\text{B}}$ ), 3.11 (t,  $J = 10.1$  Hz, 1H,  $\text{H}_{4\text{A}}$ ), 1.99 (br. s, 4H, Tetrahydropyranyl  $\text{CH}_2$ ), 1.77 – 1.96 (m, 4H, Tetrahydropyranyl  $\text{CH}_2$ ), 1.46 – 1.60 (m, 4H, Tetrahydropyranyl  $\text{CH}_2$ ), 1.22 – 1.27 (m, 6H,  $\text{OCH}_2\text{CH}_3$ );  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  201.7, 201.2 (C6), 167.8, 167.2 (C8), 163.5, 163.4 (C9), 157.6, 157.5 (C2'), 151.3, 151.3 (C6'), 139.8, 139.8 (C4'), 120.3, 120.2 (C3'), 120.0 (C5'), 80.4, 79.3 (C7), 71.4, 71.2 (C10), 70.1, 69.9 (Tetrahydropyranyl  $\text{OCH}_2$ ), 69.7, 69.6 (C5), 62.7, 62.7 (C2), 62.2, 62.1 ( $\text{OCH}_2\text{CH}_3$ ), 33.8, 33.7 (C4), 26.2, 25.9 (Tetrahydropyranyl  $\text{CH}_2$ ), 25.7, 25.2 (Tetrahydropyranyl  $\text{CH}_2$ ), 23.1, 22.9 (Tetrahydropyranyl  $\text{CH}_2$ ), 14.0, 14.0 ( $\text{OCH}_2\text{CH}_3$ );  $m/z$  (APCI $^+$ ) 469 and 471 ( $[\text{M}+\text{H}]^+$ , 100%); HRMS (TOF, APCI $^+$ )  $m/z$  calcd for  $\text{C}_{19}\text{H}_{22}\text{O}_5\text{N}_2\text{BrS}$   $[\text{M}+\text{H}]^+$  469.0427 and 471.0407; found 469.0420 and 471.0399.

**(2*S*,5*R*)-1-Aza-2-(2-bromopyridin-4-yl)-7-ethoxycarbonyl-8-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-3-thiabicyclo[3.3.0]oct-6-ene, 15d**

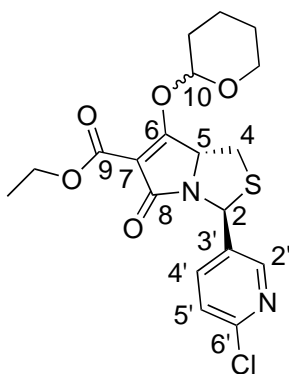


Synthesised from **14d** (100 mg, 0.3 mmol); yield (70 mg, 57%); pale yellow oil; inseparable 1 : 1 10*R*/*S* diastereomers;  $R_f = 0.34$  (petrol : EtOAc; 4 : 1);  $\nu_{\text{max}}/\text{cm}^{-1}$  1709 (s, C=O), 1747 (s, C=O), 1780 (s, C=O);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  8.34 (d,  $J = 5.2$  Hz, 1H,  $\text{H}_{6'}$ ), 8.33 (d,  $J = 5.2$  Hz, 1H,  $\text{H}_{6'}$ ), 7.56 (s, 1H,  $\text{H}_{3'}$ ), 7.50 (s, 1H,  $\text{H}_{3'}$ ), 7.15 – 7.21 (m, 2H,  $\text{H}_{5'}$ ),

6.50 (s, 1H, H2), 6.44 (s, 1H, H2), 4.50 (dd,  $J = 10.2, 6.7$  Hz, 1H, H10), 4.45 (dd,  $J = 10.0, 6.8$  Hz, 1H, H10), 4.00 – 4.31 (m, 6H,  $\text{OCH}_2\text{CH}_3$  and H5), 3.65 – 3.89 (m, 2H, Tetrahydropyranyl  $\text{OCH}_2$ ), 3.33 – 3.57 (m, 2H, Tetrahydropyranyl  $\text{OCH}_2$ ), 3.27 – 3.33 (m, 2H,  $\text{H}_{4\text{B}}$ ), 3.06 – 3.19 (m, 2H,  $\text{H}_{4\text{A}}$ ), 1.88 – 1.99 (m, 4H, Tetrahydropyranyl  $\text{CH}_2$ ), 1.58 – 1.82 (m, 4H, Tetrahydropyranyl  $\text{CH}_2$ ), 1.52 (br. s, 4H, Tetrahydropyranyl  $\text{CH}_2$ ), 1.21 – 1.26 (m, 6H,  $\text{OCH}_2\text{CH}_3$ );  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  200.8, 200.3 (C6), 167.6, 166.7 (C8), 163.3, 163.2 (C9), 151.8, 151.7 (C6'), 150.7, 150.6 (C4'), 143.4, 143.1

(C2'), 124.5, 124.4 (C3'), 120.3 (C5'), 80.5, 79.3 (C7), 70.6, 70.3 (C10), 69.8, 69.7 (Tetrahydropyranyl OCH<sub>2</sub>), 67.2, 67.1 (C5), 62.7, 62.7 (C2), 59.3, 59.1 (OCH<sub>2</sub>CH<sub>3</sub>), 34.0, 33.9 (C4), 26.2, 25.8, 25.7, 25.6, 25.2 (Tetrahydropyranyl CH<sub>2</sub>), 22.9, 22.9 (Tetrahydropyranyl CH<sub>2</sub>), 14.0, 13.9 (OCH<sub>2</sub>CH<sub>3</sub>); *m/z* (APCI<sup>+</sup>) 469 and 471 ([M+H]<sup>+</sup>, 100%); HRMS (TOF, APCI<sup>+</sup>) *m/z* calcd for C<sub>19</sub>H<sub>22</sub>O<sub>5</sub>N<sub>2</sub>BrS [M+H]<sup>+</sup> 469.0427 and 471.0407; found 469.0428 and 471.0405.

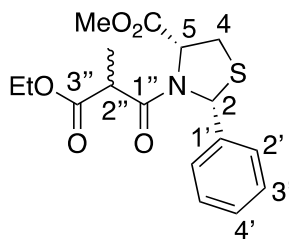
**(2*S*,5*R*)-1-Aza-2-(6-chloropyridin-3-yl)-7-ethoxycarbonyl-8-oxo-6-((tetrahydro-2*H*-pyran-2-yl)oxy)-3-thiabicyclo[3.3.0]oct-6-ene, 15e**



Synthesised from **14e** (80 mg, 0.2 mmol) by general procedure I; yield (53 mg, 53%); greenish yellow oil; inseparable 1 : 1 10*R*/*S* diastereomers; *R<sub>f</sub>* = 0.31 (petrol : EtOAc; 4 : 1); *v*<sub>max</sub>/cm<sup>-1</sup> 1711 (s, C=O), 1749 (s, C=O), 1779 (s, C=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 8.45 (s, 1H, H2'), 8.42 (s, 1H, H2'), 7.62 – 7.70 (m, 2H, H4'), 7.29 – 7.35 (m, 2H, H5'), 6.58 (s, 1H, H2), 6.52 (s, 1H,

H2), 4.50 – 4.58 (m, 1H, H10), 4.47 (dd, *J* = 9.7, 6.9 Hz, 1H, H10), 4.11 – 4.28 (m, 6H, OCH<sub>2</sub>CH<sub>3</sub> and H5), 3.80 – 3.97 (m, 2H, Tetrahydropyranyl OCH<sub>2</sub>), 3.43 – 3.52 (m, 2H, Tetrahydropyranyl OCH<sub>2</sub>), 3.36 – 3.43 (m, 1H, H4<sub>B</sub>), 3.28 – 3.36 (m, 1H, H4<sub>B</sub>), 3.16 – 3.23 (m, 1H, H4<sub>A</sub>), 3.08 – 3.16 (m, 1H, H4<sub>A</sub>), 1.75 – 2.03 (m, 4H, Tetrahydropyranyl CH<sub>2</sub>), 1.58 – 1.75 (m, 4H, Tetrahydropyranyl CH<sub>2</sub>), 1.45 – 1.58 (m, 4H, Tetrahydropyranyl CH<sub>2</sub>), 1.24 (t, *J* = 7.1 Hz, 6H, OCH<sub>2</sub>CH<sub>3</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (CDCl<sub>3</sub>, 101 MHz): δ 201.2, 200.7 (C6), 167.8, 167.3 (C8), 163.4, 163.3 (C9), 151.5 (C2'), 147.6, 147.5 (C6'), 137.1, 137.1 (C4'), 134.7, 134.5 (C3'), 124.4 (C5'), 80.3, 79.2 (C7), 70.9, 70.8 (C10), 70.3, 70.1 (Tetrahydropyranyl OCH<sub>2</sub>), 67.1 (C5), 62.8, 62.7 (C2), 59.2, 59.1 (OCH<sub>2</sub>CH<sub>3</sub>), 33.9, 33.8 (C4), 26.2 (Tetrahydropyranyl CH<sub>2</sub>), 25.2 (Tetrahydropyranyl CH<sub>2</sub>), 23.0, 22.9 (Tetrahydropyranyl CH<sub>2</sub>), 13.9 (OCH<sub>2</sub>CH<sub>3</sub>); *m/z* (ESI<sup>+</sup>) 425 ([M+H]<sup>+</sup>, 100%); and 427 ([M+H]<sup>+</sup>, 33%); HRMS (TOF, APCI<sup>+</sup>) *m/z* calcd for C<sub>19</sub>H<sub>22</sub>O<sub>5</sub>N<sub>2</sub>ClS [M+H]<sup>+</sup> 425.0933 and 427.0903; found 425.0937 and 427.0907.

**(2*R*,5*R*)-1-(3-Ethoxy-2-methyl-3-oxopropanoyl)-5-methoxycarbonyl-2-phenyl-1,3-thiazolidine, 17**



Synthesised from **16** (1 g, 3 mmol); yield (195 mg, 19%); yellow oil;

inseparable 0.8: 1 C2'' *R/S* diastereomers;  $R_f$  = 0.18 (petrol : EtOAc; 4 : 1);

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  7.62 (d,  $J$  = 7.0 Hz, 2H, H2' major), 7.24 – 7.40

(m, 8H, H4' and H3' major and minor and H2' minor, obscured by solvent

peak), 6.30 (s, 1H, H2 major), 6.08 (s, 1H, H2 minor), 5.16 – 5.33 (m, 1H, H5 minor), 4.85 – 5.04 (m,

1H, H5 major), 4.01 – 4.32 (m, 4H,  $\text{OCH}_2\text{CH}_3$ ), 3.77 (s, 3H,  $\text{CO}_2\text{CH}_3$  major), 3.72 (s, 3H,  $\text{CO}_2\text{CH}_3$

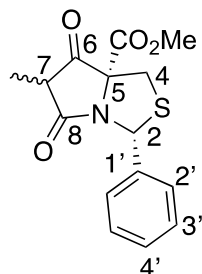
minor), 3.26 – 3.40 (m, 3H, H2'' and H4), 3.10 – 3.26 (m, 3H, H2'' and H4), 1.31 – 1.42 (m, 3H,

$\text{OCH}_2\text{CH}_3$ ), 1.16 – 1.28 (m, 3H,  $\text{OCH}_2\text{CH}_3$ ), 1.04 – 1.19 (d,  $J$  = 6.9 Hz, 3H,  $\text{CH}_3$  major), 0.99 – 1.06 (m,

3H,  $\text{CH}_3$  minor);  $m/z$  ( $\text{ESI}^+$ ) 374 ( $[\text{M}+\text{Na}]^+$ , 100%); HRMS (TOF,  $\text{ESI}^+$ )  $m/z$  calcd for  $\text{C}_{17}\text{H}_{21}\text{O}_5\text{N}_2\text{NaS}$

$[\text{M}+\text{Na}]^+$  374.1033; found 374.1033.

**(2*R*,5*R*)-1-Aza-6,8-dioxo-7-methyl-5-methoxycarbonyl-2-phenyl-3-thiabicyclo[3.3.0]octane, 19**



Synthesised from **17** (890 mg, 2.5 mmol) by Dieckmann cyclisation; yield (272

mg, 35%, an inseparable 0.3 : 1 mixture of diastereomers, 7*R/S*); yellow oil;  $R_f$  =

0.25 (EtOAc: MeOH; 8 : 1);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  7.50 (d,  $J$  = 7.2 Hz, 2H,

H2'), 7.26 – 7.37 (m, 3H, H4' and H3'), 6.61 (s, H2 minor), 6.57 (s, 1H, H2 major),

3.99 (d,  $J$  = 11.1 Hz, H4<sub>B</sub> minor), 3.92 (d,  $J$  = 11.5 Hz, 1H, H4<sub>B</sub> major), 3.65 (s, 3H,  $\text{CO}_2\text{CH}_3$  major),

3.55 (d,  $J$  = 7.4 Hz, 1H, H7), 3.52 (s,  $\text{CO}_2\text{CH}_3$  minor), 3.16 (d,  $J$  = 11.5 Hz, 1H, H4<sub>A</sub> major), 3.09 – 3.13

(m, H4<sub>A</sub> minor), 1.51 (d,  $J$  = 7.8 Hz,  $\text{CH}_3$  minor), 1.33 (d,  $J$  = 7.4 Hz, 3H,  $\text{CH}_3$  major);  $^{13}\text{C}\{^1\text{H}\}$  NMR

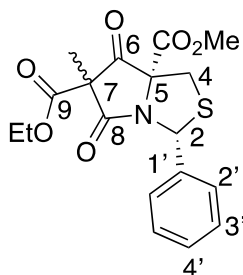
( $\text{CDCl}_3$ , 101 MHz):  $\delta$  201.4 (C6), 171.6 (C8), 166.8 ( $\text{CO}_2\text{CH}_3$ ), 137.9 (C1'), 128.7, 128.7, 127.3 (C2', C3'

and C4'), 82.7 (C5), 64.2 (C2 major), 63.7 (C2, minor), 53.9 ( $\text{CO}_2\text{CH}_3$ ), 48.3 (C7), 35.7 (C4), 8.5 ( $\text{CH}_3$ );

$m/z$  ( $\text{ESI}^+$ ) 306 ( $[\text{M}+\text{H}]^+$ , 100%); HRMS ( $\text{ESI}^+$ )  $m/z$  calcd for  $\text{C}_{15}\text{H}_{16}\text{O}_4\text{NS}$   $[\text{M}+\text{H}]^+$  306.0795; found

306.0797.

**(2*R*,5*R*)-1-Aza-7-ethoxycarbonyl-7-methyl-5-methoxycarbonyl-6,8-dioxo-2-phenyl-3-thiabicyclo[3.3.0]octane, 20**



Synthesised from **19** (180 mg, 0.6 mmol) by acylation with ethyl chloroformate; yield (66 mg, 29%, an inseparable 1 : 0.5 mixture of diastereomers, *C7*R*/S*); pale yellow oil;  $R_f$  = 0.25 (petrol : EtOAc; 85 : 15);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  7.57 (d,  $J$  = 7.2 Hz, 2H, H2' major), 7.52 (d,  $J$  = 7.2 Hz,

2H, H2' minor), 7.26 – 7.40 (m, 6H, H4' and H3' major and minor), 6.60 (s, 1H, H2 major), 6.19 (s, 1H, H2 minor), 4.32 (q,  $J$  = 7.1 Hz, 2H,  $\text{OCH}_2\text{CH}_3$  minor), 4.20 (q,  $J$  = 7.1 Hz, 2H,  $\text{OCH}_2\text{CH}_3$  major), 3.85 (d,  $J$  = 11.5 Hz, 1H, H4<sub>B</sub> major), 3.76 (d,  $J$  = 11.5 Hz, 1H, H4<sub>B</sub> minor), 3.69 (s, 3H,  $\text{CO}_2\text{CH}_3$  minor), 3.59 (s, 3H,  $\text{CO}_2\text{CH}_3$  major), 3.16 (d,  $J$  = 11.5 Hz, 1H, H4<sub>A</sub> major), 3.15 (d,  $J$  = 11.5 Hz, 1H, H4<sub>A</sub> minor), 1.79 (s, 3H,  $\text{CH}_3$  minor), 1.59 (s, 3H,  $\text{CH}_3$  major), 1.38 (t,  $J$  = 7.1 Hz, 3H,  $\text{OCH}_2\text{CH}_3$  minor), 1.24 (t,  $J$  = 7.1 Hz, 3H,  $\text{OCH}_2\text{CH}_3$  major);  $^{13}\text{C}\{^1\text{H}\}$  NMR ( $\text{CDCl}_3$ , 101 MHz):  $\delta$  196.8 (C6), 172.6 (C8), 169.3, 168.2, 165.8, 164.6 ( $\text{CO}_2\text{CH}_3$  and C9 major and minor), 138.6, 137.5 (C1' major and minor), 128.6, 128.5, 128.5, 128.3, 127.3, 127.0 (C2', C3' and C4' major and minor), 82.6 (C5 major), 78.9 (C5 minor), 66.4 (C7), 64.7, 63.3 (C2 major and minor), 62.5, 62.1 ( $\text{OCH}_2\text{CH}_3$  major and minor), 53.7, 53.5 ( $\text{CO}_2\text{CH}_3$  major and minor), 37.8, 37.0 (C4 major and minor), 16.6, 14.1 ( $\text{OCH}_2\text{CH}_3$  major and minor), 7.9 ( $\text{CH}_3$ );  $m/z$  ( $\text{ESI}^+$ ) 378 ( $[\text{M}+\text{H}]^+$ , 100%); HRMS (TOF,  $\text{ESI}^+$ )  $m/z$  calcd for  $\text{C}_{18}\text{H}_{20}\text{O}_6\text{NS}$   $[\text{M}+\text{H}]^+$  378.1006; found 378.1013.

## Supporting Information

The supporting Information is available free of charge on the ACS Publications website at DOI: xxxx  
Copies of  $^1\text{H}$  and  $^{13}\text{C}$  spectra of new compounds (PDF).

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## Abstract

