

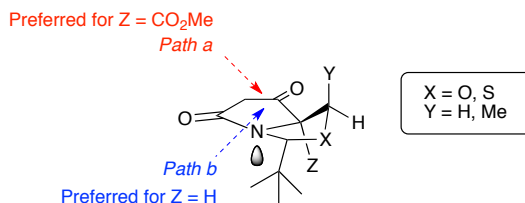
Stereoselectivity in the Reduction of Bicyclic Tetramates

Laia Josa-Culleré^a
Mark G. Moloney^{*,a}
Amber L. Thompson^a

^a Department of Chemistry, Chemistry Research Laboratory,
The University of Oxford, 12 Mansfield Road, Oxford. OX1 3TA.

* indicates the main/corresponding author.

e-mail: mark.moloney@chem.ox.ac.uk



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Abstract. The reduction of bicyclic tetramates can be achieved with high levels of diastereocontrol, but small changes in the substitution of the bicyclic lactam system can lead to changes in the steric bias of the concave/convex system. The tetramates and pyroglutamates prepared in this work exhibited limited antibacterial activity.

Key words tetramate, reduction, lactam, stereoselectivity, pyroglutamate

Hydroxylated pyrrolidinones are a key structural feature in many biologically active natural products, such as lactacystin,¹ oxazolomycin² and pramanicin.³ We have earlier developed effective methodology for the construction of tetramates, which can be used as scaffolds to generate libraries of potent antibacterial analogues,⁴ and since the reduction of this tetramate directly leads to mimics of hydroxylated pyrrolidinones, a study of the stereoselectivity of this transformation was of interest.

Firstly, L-serine, L-cysteine and L-threonine methyl esters were converted to the corresponding *O,N*- or *S,N*-heterocycles **1a-c** and thence to tetramates **2a-c** using the literature protocols.⁵ The Dieckmann cyclisation of *N*-oxazolidine **1a**, derived from L-serine, to give tetramic acid **2a** has been found to proceed with high levels of chemo- and enantioselectivity,⁵ but the optical purity of the corresponding cysteine and threonine systems **2b** and **2c** has not yet been evaluated. For this purpose, racemic samples of tetramates **2a-c** were also prepared starting from DL-serine, cysteine, and threonine respectively, and compared with the enantiopure ones by chiral HPLC. The *ee* value of **2a** was confirmed to be high (96%) and both **2b** and **2c** similarly showed no detectable presence of the other enantiomer. These results confirm the effectiveness of Seebach's protocol for the control of such enolate-mediated cyclisations,⁶ and further

emphasise the value of *t*-butyl oxazolidines/thiazolidines in chemoselective Dieckmann ring closures; it would appear that the *t*-butyl group imparts both unusual stability to the heminaminal ether ring and chemoselective and steric bias in the cyclisation.

Reduction of the ketone group in the tetramate **2a** with sodium acetoxyborohydride generated *in situ*⁷ has been reported to give alcohol **3a** as the major diastereomer, with high levels of diastereoselectivity.⁸ Of interest was the outcome in the reduction of the alternative tetramate cores **2b** and **2c**, which have a sulphur atom and a methyl group respectively that might sterically bias the attack of the hydride even further, and lead to an alternative diastereoisomeric preference. We initially reproduced the reported results for **2a** and found that the (6*S*)-alcohol of **3a** is indeed formed in 96% diastereomeric excess over the (6*R*)-alcohol **3a'**, as determined by LC-MS, and in quantitative yield (Table 1 and Scheme 1).²⁰ The presence of the bulkier sulphur atom in **2b** gave the product (6*S*)-**3b** in quantitative yield, with similar stereoselectivity (Figure 1) but the alternative epimer (6*R*)-**3b'** was not observed at all (stereochemistry established by NOE analysis (Figure 2). With **2c**, however, while diastereomer (6*S*)-**3c** was still the major one formed, some (6*R*)-**3b'** was also obtained, with a *de* of 68%. However, while the reduction of tetramates **2a** and **2b** was completed in good yield in only 2 h (Table 1), tetramate **2c** required much longer, and even after 2 days, there was still starting material left, indicative of greater steric hindrance at the C(6) position from the C(4) substituent, and the best yield which could be achieved was 51%. The absolute configuration of alcohol **3b** was confirmed by single crystal X-ray diffraction (Figure 3).⁹

The high stereoselectivity observed for the reduction of **2a-c** is probably due to a preference for *endo*- (that is, concave face) hydride addition (Scheme 1, Inset Path a) arising from either

repulsion from the nitrogen lone pair or steric blocking from the methyl ester in the bicyclic system, or a combination of both, leading to the *exo*-hydroxyl product being favoured. To confirm this, and to determine whether this diastereoselectivity could be changed, decarboxylation of the tetramic acids **2a-c** was examined; treatment with sodium hydroxide under reflux and then decarboxylation gave tetramates **4a-c** in yields of 32–66% (Scheme 1).¹⁹ While the decarboxylated products **4a** and **4b** were formed as single diastereoisomers, with relative stereochemistry demonstrated by NOE analysis (Figure 2), decarboxylation of **2c** furnished a 2.8:1 mixture of **4c** and its epimer **6**, respectively. MM2 calculations suggested an energy difference between **4c** and its **6** of only 1 kcal/mol, consistent with a facile elimination-re-addition sequence of the intermediate enolate generated from **2c** after decarboxylation. With compounds **4a-c** in hand, reduction with sodium acetoxyborohydride was examined, and found to give compounds (6*S*)-**5a-c** and (6*R*)-**5a'-c'**, whose stereochemistry was assigned by NOE analysis (Figure 1). However, a significant decrease in stereoselectivity was observed when compared to the methoxycarbonyl substituted systems **2a-c** (Table 1 and Figure 1): for serine, while (6*S*)-**5a** was still the major product, the diastereomeric excess was reduced to 53% over the other possibility, (6*R*)-**5a'**. For cysteine and threonine, the selectivity was fully inverted and (6*R*)-isomers **5b'** and **5c'** were obtained in preference to (6*S*)-**5b** and **5c**, with *de* values of 31% and 92% (Figure 1).

In the addition of hydride to these systems, it would appear then that for tetramates **2a-c**, which possess an immediately adjacent bridgehead carboxymethyl group, the dominant route of entry is by *Path a* (Scheme 1, Inset), that is to the *endo*-face of the concave system, which also happens to avoid the *N*-lone pair and the *exo-t*-butyl group. However, for tetramates **4a-c**, the absence of the bridgehead carboxymethyl group at C-5 but also the presence of the bulky C-4 methyl of threonine or the large ring sulphur atom is enough to force hydride entry to the *exo*-face of the convex bicycle, giving the (6*S*)-isomer, so much so that for **4c**, *Path b* becomes the dominant mode of entry (Scheme 1, Inset); similar steric control in alkylations of related bicyclic lactams has been observed.¹⁰ This outcome confirms the significantly greater steric influence of the ring heteroatom and *endo*-methyl groups in **4b** and **4c** respectively, relative to the oxazolidine system **4a**. Attempted inversion of configuration of alcohol **3a** was unsuccessful. The use of Mitsunobu conditions¹¹ with DEAD, triphenylphosphine and either benzoic, 4-nitrobenzoic or the less bulky acetic acid instead gave the elimination product **7** exclusively.

Of interest was that the ¹H and ¹³C NMR spectra of the alcohol products showed some consistent differences: the (6*R*) alcohols **3a'-c'** and **5a'-c'** showed *H*(6) at lower field and the *C*(6) at higher field than the alternative (6*S*) **3a-c** and **5a-c** diastereomers (Table 1). Also, as observed by Andrews *et al.* for similar compounds,⁸ the difference in chemical shift between *H*(4_{endo}) and *H*(4_{exo}) was significantly bigger for the (6*S*) alcohols **3a-c** and **5a-c** than for (6*R*) alcohols **3a'-c'** and **5a'-c'**, but the opposite is true for *H*(7).

The prepared analogues were assayed against *S. aureus* and *E. coli* using the hole-plate method,¹² and the results are shown in Table 2. This phenotypic assay, which does not provide MIC values, nonetheless allows a rapid qualitative assessment of the antibacterial activity. Most of the synthesised compounds were inactive, consistent with earlier findings showing that the pyroglutamate or tetramate cores alone are insufficient for activity.¹³ Of interest, however, is that the decarboxylated tetramate systems **4a-c** exhibited significant Gram-positive activity, and analysis of their chemoinformatic parameters¹⁴ (Table 2) indicated that the most active possessed somewhat lower clogP values (1.28–1.73) but very similar %PSA values (11.6–14.7) to other active tetramate which have been previously reported,⁴ and this might be due to their favourable membrane permeability.

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Supporting Information

Yes

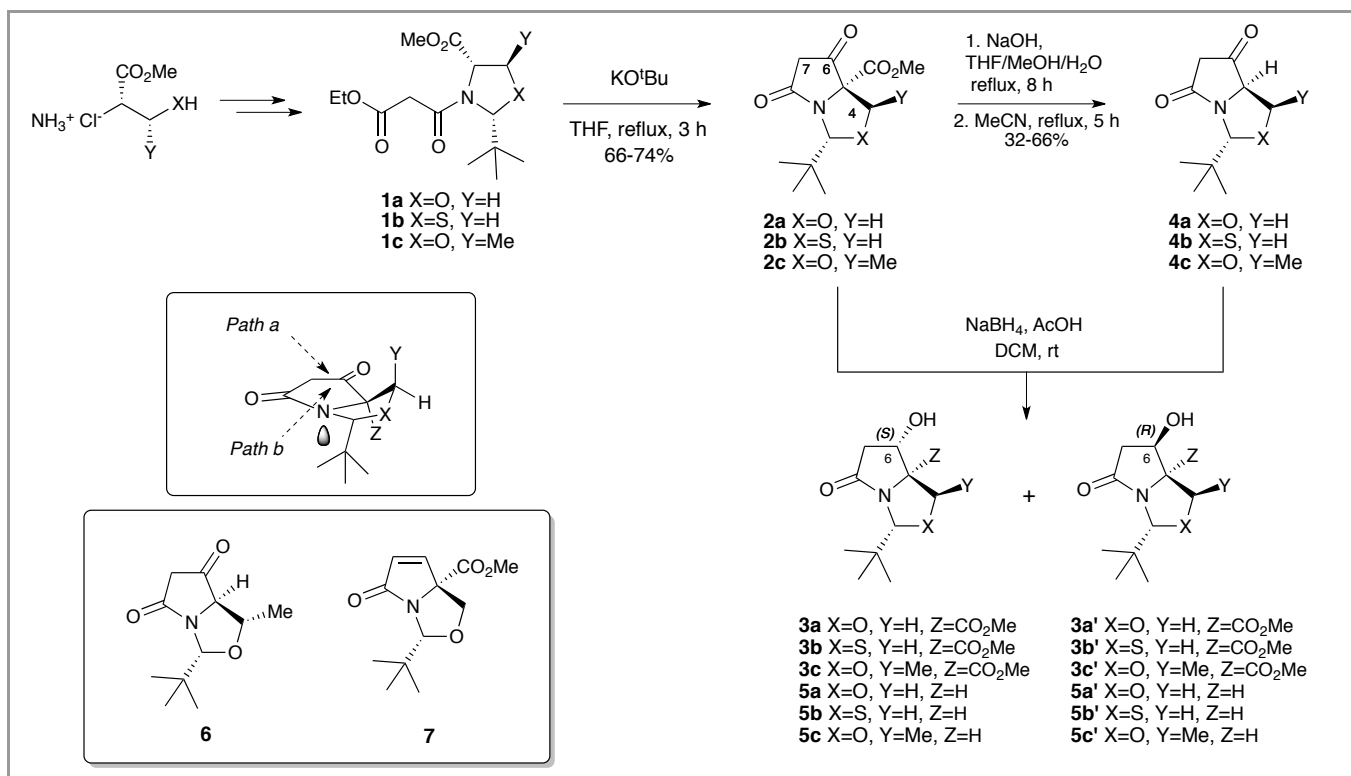
Primary Data

No

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- Single crystal X-ray diffraction data were collected at 150 K with an Oxford Diffraction (Rigaku) SuperNova diffractometer (using $\lambda=1.54180$ Å) and processed with CrysAlisPro as per the SI (CIF). The structure was solved with SIR92¹⁵ and refined with CRYSTALS¹⁶ including the Flack *x* parameter¹⁷ which refined to -0.008(8) (unrestrained). Bayesian analysis¹⁸ of the Bijvoet pairs gave the Hooft *y* parameter as -0.002(4) and the probability that the structure was the correct hand of >99.99% given that the crystal is either enantiopure or a racemic twin. The asymmetric unit contains three molecules, all with the same stereochemistry.

- Full crystallographic data (in CIF format) is available as ESI and has been deposited with the Cambridge Crystallographic Data Centre (reference code CCDC 1452987).
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- (19) **General procedure for the decarboxylation of tetramic acids.** Tetramic acid **2a-c** (1.0 eq) and NaOH (2.0 eq) in a 4:1:1 mixture of THF/MeOH/H₂O was heated at reflux for 8 h and then cooled to room temperature. The mixture was acidified with 2 M aqueous HCl, extracted with EtOAc, dried over Na₂SO₄ and concentrated *in vacuo*. The reaction crude was dissolved in CH₃CN and heated at reflux for 5 h then cooled to room temperature. The mixture was acidified with 2 M aqueous HCl, extracted with EtOAc, dried over Na₂SO₄ and concentrated under reduced pressure. The reaction crude was purified by *flash* column chromatography to give decarboxylated tetramic acids **4a-c**. Data for (**4b**). Yield 32% (33 mg); off-white solid; m.p. 142-144 °C. R_f (50% EtOAc in DCM) 0.50; [α]_D²⁰ +71.2 (c 0.8, DCM); ν_{max}/cm⁻¹ 2960 (C-H), 1769 (C=O), 1699 (C=O); δ_H (400 MHz, CDCl₃) 1.00 (9H, s, C(CH₃)₃), 2.84 (1H, t, J 10.4, C(4)H_AH_B), 3.13 (1H, app dd, J 22.0, 1.5, C(7)H_AH_B), 3.19 (1H, dd, J 10.4, 7.0, C(4)H_AH_B), 3.31 (1H, d, J 22.0, C(7)H_AH_B), 4.44 (1H, dd, J 10.0, 7.0, C(5)H), 5.30 (1H, s, C(2)H); δ_C (100 MHz, CDCl₃) 26.4 (C(CH₃)₃), 33.3 (C(4)), 37.8 (C(CH₃)₃), 43.0 (C(7)), 71.5 (C(2)), 72.4 (C(5)), 170.1 (C(8)), 203.5 (C(6)); m/z (ESI⁺) 214.1 (MH⁺, 91%), 236.1 (MNa⁺, 100%); HRMS (ESI⁺) found 214.08959, C₁₀H₁₆NO₂S requires (MH⁺) 214.08963.
- (20) **General procedure for the reduction of tetramic acids.** NaBH₄ (2.2 eq) was added portionwise at 0 °C to a solution of tetramic acids **2a-c** or **4a-c** (1.0 eq) and acetic acid (9.0 eq) in anhydrous DCM. The reaction mixture was stirred at 0 °C for 15 min and then at room temperature until starting material disappeared by TLC (typically 2 h). The reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc, washed with brine, dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The crude product was purified by *flash* column chromatography to give the desired alcohol **3a-c** and **5a-c**. Data for (**3b**). Yield quant. (101 mg); white solid; m.p. 75-77 °C. R_f (50% EtOAc in DCM) 0.38; [α]_D²⁰ +18.6 (c 1.0, DCM); ν_{max}/cm⁻¹ 3380 (O-H), 2956 (C-H), 1744 (C=O), 1686 (C=O); δ_H (500 MHz, CDCl₃) 0.92 (9H, s, C(CH₃)₃), 2.63 (1H, d, J 4.5, OH), 2.74 (1H, dd, J 15.5, 8.0, C(7)H_AH_B), 2.98 (1H, dd, J 16.0, 10.5, C(7)H_AH_B), 3.03 (1H, d, J 11.5, C(4)H_AH_B), 3.84 (3H, s, CO₂CH₃), 3.90 (1H, d, J 11.5, C(4)H_AH_B), 4.43 (1H, ddd, J 10.5, 8.0, 4.0, C(6)H), 5.04 (1H, s, C(2)H); δ_C (125 MHz, CDCl₃) 26.6 (C(CH₃)₃), 38.0, 38.7 (C(4), C(CH₃)₃), 40.9 (C(7)), 53.1 (CO₂CH₃), 72.8, C(2)), 76.6 (C(6)), 82.0 (C(5)), 171.2 (CO₂CH₃), 173.8 (C(8)); m/z (ESI⁺) 274.1 (MH⁺, 52%), 296.1 (MNa⁺, 100%); HRMS (ESI⁺) found 296.0936, C₁₂H₁₉NNaO₄S requires (MNa⁺) 296.0927. Data for (**5b**). Yield 86% (32 mg); white solid; 1.9:1 mixture of diastereomers. R_f (50% EtOAc in DCM) 0.21; ν_{max}/cm⁻¹ 3369 (O-H), 2962 (C-H), 1674 (C=O); δ_H (500 MHz, CDCl₃) major isomer (**5b'**): 0.96 (9H, s, C(CH₃)₃), 2.46 (1H, dd, J 17.2, 3.0, C(7)H_AH_B), 2.93 (1H, dd, J 17.2, 6.5, C(7)H_AH_B), 2.97 (1H, dd, J 10.8, 7.2, C(4)H_AH_B), 3.21 (1H, dd, J 10.8, 7.8, C(4)H_AH_B), 4.33 (1H, td, J 7.5, 5.5, C(5)H), 4.58 (1H, ddd, J 6.5, 5.5, 3.0, C(6)H), 5.05 (1H, s, C(2)H); minor isomer (**5b**): 0.98 (9H, s, C(CH₃)₃), 2.51 (1H, t, J 10.2, C(4)H_AH_B), 2.61 (1H, dd, J 18.0, 3.5, C(7)H_AH_B), 2.83 (1H, dd, J 18.0, 7.5, C(7)H_AH_B), 3.11 (1H, dd, J 10.5, 6.0, C(4)H_AH_B), 4.08 (1H, ddd, J 10.0, 6.0, 2.0, C(5)H), 4.29 (1H, ddd, J 7.5, 3.5, 2.0, C(6)H), 5.01 (1H, s, C(2)H); δ_C (100 MHz, CDCl₃) major isomer (**5b'**): 26.5 (C(CH₃)₃), 30.6 (C(4)), 38.2 (C(CH₃)₃), 42.4 (C(7)), 66.7 (C(6)), 68.8 (C(5)), 70.8 (C(2)), 174.1 (C(8)); minor isomer (**5b**): 26.5 (C(CH₃)₃), 35.0 (C(4)), 37.6 (C(CH₃)₃), 41.8 (C(7)), 69.5 (C(6)), 71.0 (C(2)), 73.8 (C(5)), 175.0 (C(8)); m/z (ESI⁺) 216.1 (MH⁺, 97%), 238.1 (MNa⁺, 90%); HRMS (ESI⁺) found 216.10514, C₁₀H₁₈NO₂S (MH⁺) requires 216.10528.



Scheme 1 Synthesis of key intermediates

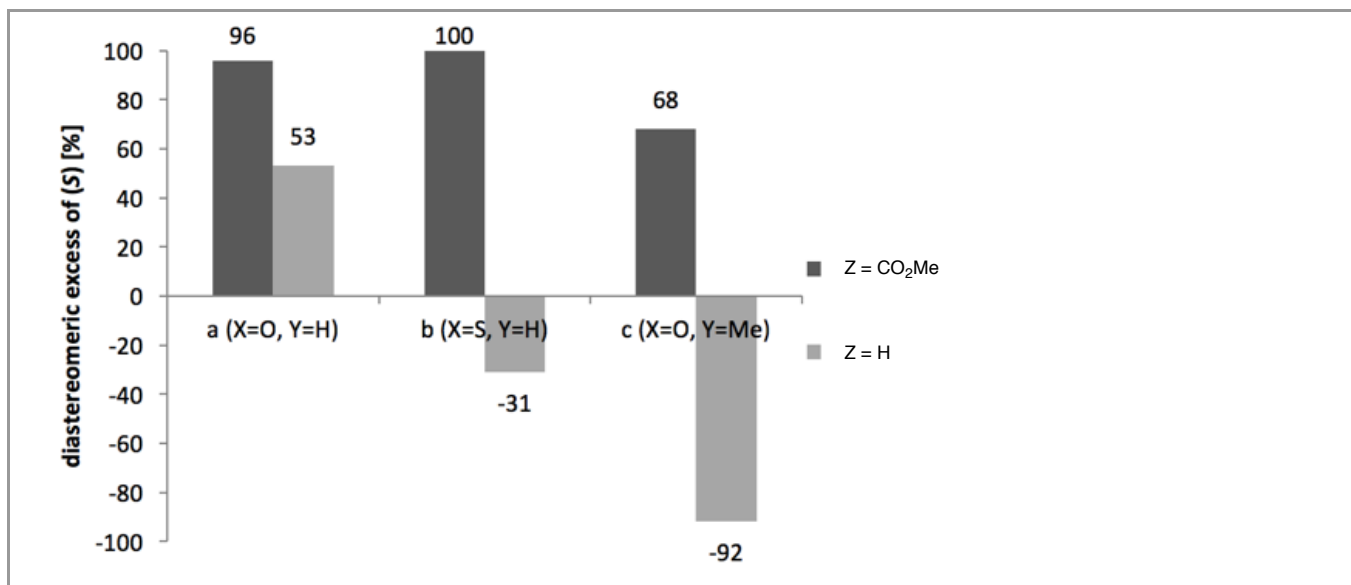


Figure 1 Diastereoselectivity of reduction reactions

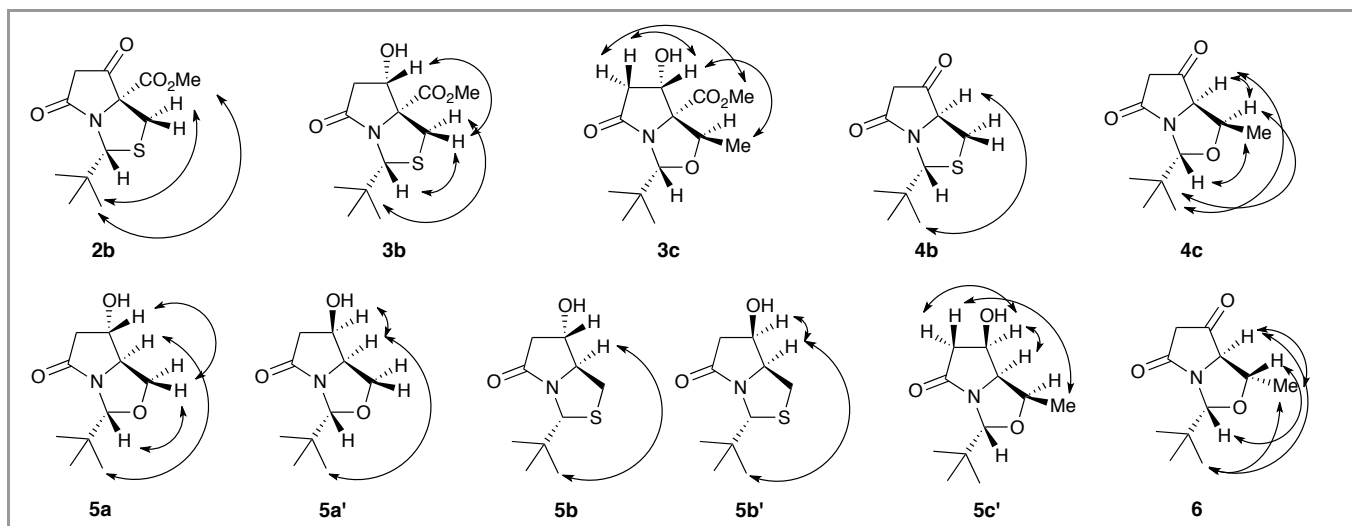


Figure 2 NOE Data for key intermediates

Figure 3 Structure of compound **3b** determined from single crystal X-ray diffraction data; displacement ellipsoids drawn at 50% probability and one equivalent is shown for clarity.Table 1. Yield and representative chemical shifts of (*S*) and (*R*) alcohols **3a-c** and **5a-c**.

Compound	Yield(%)	H(6) [ppm]		C(6) [ppm]		H(4 _{endo}) –H(4 _{exo}) [ppm]		H(7 _A) –H(7 _B) [ppm]	
		(6 <i>S</i>)	(6 <i>R</i>)	(6 <i>S</i>)	(6 <i>R</i>)	(6 <i>S</i>)	(6 <i>R</i>)	(6 <i>S</i>)	(6 <i>R</i>)
3a	quant.	4.56	-	73.8	-	1.32	-	0.34	-
3b	quant.	4.43	-	76.6	-	0.87	-	0.24	-
3c	51	4.61	-	69.7	-	-	-	0.31	-
5a	99	4.36	4.55	69.1	66.5	1.15	0.0	0.06	0.63
5b	86	4.29	4.58	68.8	66.7	0.60	0.24	0.22	0.47
5c	92	-	4.95	-	65.6	-	-	-	0.42

Table 2. Physicochemical properties and bioactivity of synthesised analogues

Compound	MW	clogP ^a	PSA ^a	MSA ^a	%PSA ^b	Zone size [mm] ^c	
						<i>S. aureus</i>	<i>E. coli</i>
2a	255.27	1.29	72.9	393.2	18.5	N.A.	N.A.

2b	271.33	1.74	63.7	399.0	16.0	N.A.	N.A.
2c	269.30	1.70	72.9	422.6	17.3	N.A.	N.A.
3a	257.29	0.37	76.1	402.2	18.9	N.A.	N.A.
3b	273.35	0.82	66.8	409.2	16.3	N.A.	N.A.
3c	271.31	0.78	76.1	431.2	17.6	N.A.	N.A.
4a	197.23	1.28	46.6	317.6	14.7	20	N.A.
4b	213.30	1.73	37.4	322.9	11.6	28	14
4c	211.26	1.70	46.6	347.1	13.4	15	N.A.
5a	199.25	0.51	49.8	327.2	15.2	N.A.	N.A.
5b	215.31	0.97	40.5	332.7	12.2	N.A.	N.A.
5c	213.28	0.93	49.8	356.2	14.0	N.A.	N.A.

^a clogP, polar surface area (PSA) and molecular surface area (MSA) were calculated using chemicalize.org

^b %PSA = (PSA/MSA)*100%; ^c N.A. = Not Active