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Synthesis and bioactivity of fused- and spiro-β-lactone-lactam systems

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An investigation of the formation of fused- and spiro-β-lactone annulate to γ-lactams has shown that the fused systems are formed preferentially, under standard conditions, but that spiro systems are accessible only when the formation of the fused system is blocked and require careful optimisation of reaction conditions. These systems display both weak antibacterial activity and proteasome inhibition.

Introduction

The complex bicyclic framework of β-lactone-containing natural products, such as oxazolomycin,[1, 2] salinosporamide[3] and omuralide[4] (Figure 1), has attracted significant attention amongst synthetic organic chemists. Moreover, the reported ability of these compounds to inhibit the 20S proteasome,[5-7] leading to potential roles in diverse disease therapies, makes them of biological importance. Most synthetic routes rely on a late-stage formation of the β-lactone, accessed from a β-hydroxyacid precursor, which can be formed using HATU/DIPEA,[8] BOPCl/pyridine[9] or BOPCl/Et_3N.[4]

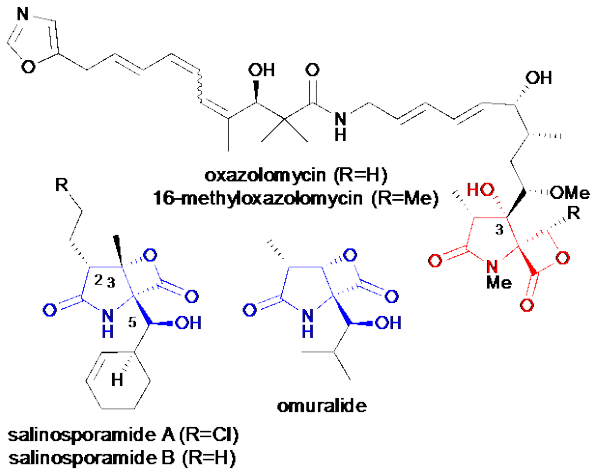
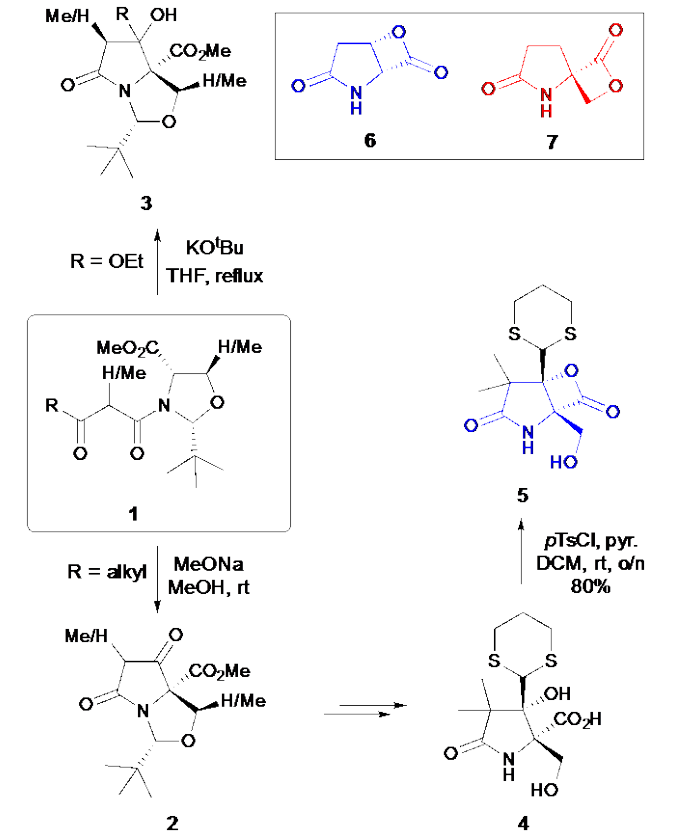


Figure 1



Scheme 1

Our research has led to the development of effective methodology to use oxazolidines of type **1** to construct bicyclic tetramate **2** via a chemo- and stereoselective Dieckmann cyclisation, and hydroxypyrrolidinone **3** via an aldol cyclisation (Scheme 1). Further functionalisation of tetramate **2** allowed the synthesis of β-hydroxyacid **4**, which was cyclised regioselectively to give fused β-lactone **5**, but without

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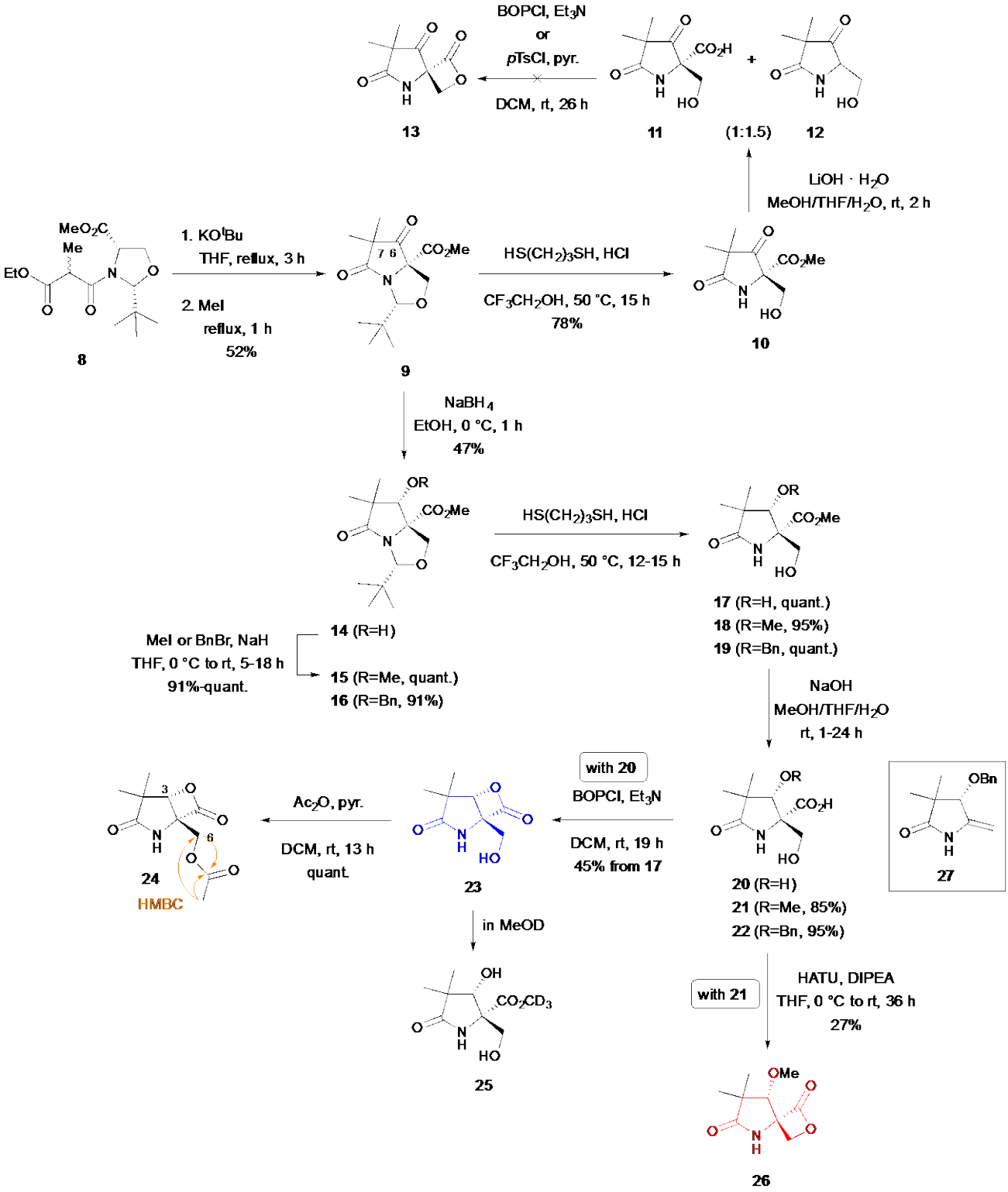
formation of the alternative spiro β-lactone.[10] Given the ease of preparation of the highly functionalised bicyclic systems **2** and **3**, it was of interest to examine whether selective and controlled introduction of fused- and spiro-β-lactones **6** and **7** could be achieved, which would be important for the general synthesis of mimics and analogues of the parent natural products.

Results and Discussion

Preparation of fused- and spiro-β-lactones with a tetramate core

Tetramate **9** (Scheme 2) was chosen as a simple system for the initial studies towards the formation of spiro-β-lactones, where the lack of alcohol group at *C*(6) would avoid the formation of the fused-β-lactone, and the double substitution at *C*(7) would



Scheme 2

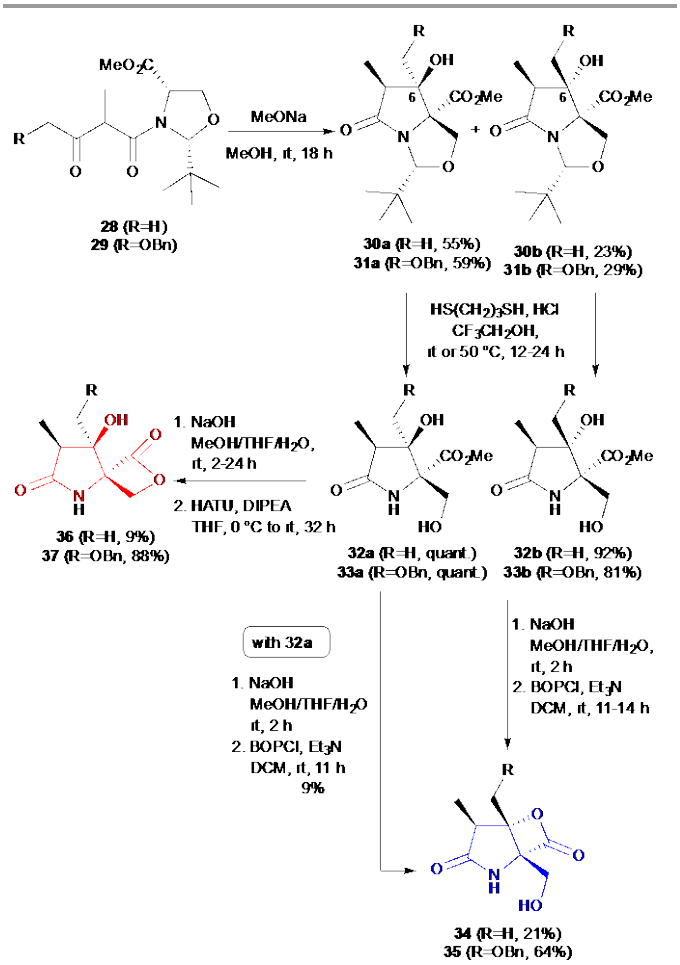
prevent enol-keto tautomerism. Dieckmann cyclisation of malonamide **8** followed by *in situ* methylation gave gem-dimethyl product **9**.[11] *N*,*O*-Acetal deprotection with 1,3-propanedithiol[12] furnished alcohol **10**, which upon saponification with LiOH gave carboxylic acid **11** as a 1:1.5 mixture with the decarboxylated product **12**. Attempted lactonisation of the crude mixture with *p*TsCl/pyridine or BOPCl/Et_3N gave a complex crude mixture from which no product **13** was obtained after *flash* column chromatography. Therefore, it was evident that a different substrate was required to study the preparation of spiro-β-lactones, which in particular lacked a carbonyl group at *C*(6) to avoid decarboxylation of the β-hydroxyacid.

Reduction of tetramate **9** with $NaBH_4$ in EtOH[11] gave alcohol **14** as a single *exo*-diastereomer, as indicated by NOE correlations. This diastereomeric outcome was consistent with our previous report[13] which showed that hydride attack to similar tetramates preferentially occurred from the *endo*-face of the bicyclic system, avoiding the nitrogen lone pair, the methyl ester and the bulky *tert*-butyl group. Subsequent *N*,*O*-acetal deprotection and ester hydrolysis gave acid **20**, which upon treatment with BOPCl and Et_3N gave fused-β-lactone **23** as a single product. The formation of the β-lactone was evidenced by observation in the HRMS spectrum of a fragment with the correct molecular weight and the characteristic IR absorption at 1833 cm^{-1} of the lactone carbonyl. The selective formation of the fused-, and not the spiro-β-lactone, was expected by analogy to Angelov *et al.*'s earlier results.[10] The assignment was further confirmed by HMBC spectroscopic analysis of the product **24** formed *via* acetylation of the free alcohol, which showed correlations of the acetyl carbonyl and methyl groups with both *H*(6)s but not with *H*(3). Of interest is that, while **23** was stable in $CDCl_3$, it slowly degraded to ester **25** when kept in CD_3OD, resulting from attack of the deuterated methanol to the labile β-lactone.

This observed regioselectivity for β-lactone ring closure indicated that formation of the alternative spiro-β-lactone would require initial alcohol protection, to leave the β-hydroxyacid with only one possible mode of cyclisation. For this purpose, methyl and benzyl ether analogues **15** and **16** were prepared and subjected to similar *N,O*-acetal deprotection and ester hydrolysis. While treatment with BOPCl and Et_3N only led to degradation products, the use of the alternative HATU and DIPEA conditions with methyl ether **21** successfully furnished spiro-β-lactone **26**. However, for the benzyl ether **22**, no product was observed, and the crude NMR spectrum of the product showed doublet signals in the olefinic region, which could correspond to enamine **27**. The lack of formation of the desired β-lactone could be due to the bulky benzyloxy group and illustrates that the delicate steric constraints in these ring and highly functionalised systems dominate the chemical outcome.

Preparation of fused- and spiro-β-lactones with a pyrrolidinone core

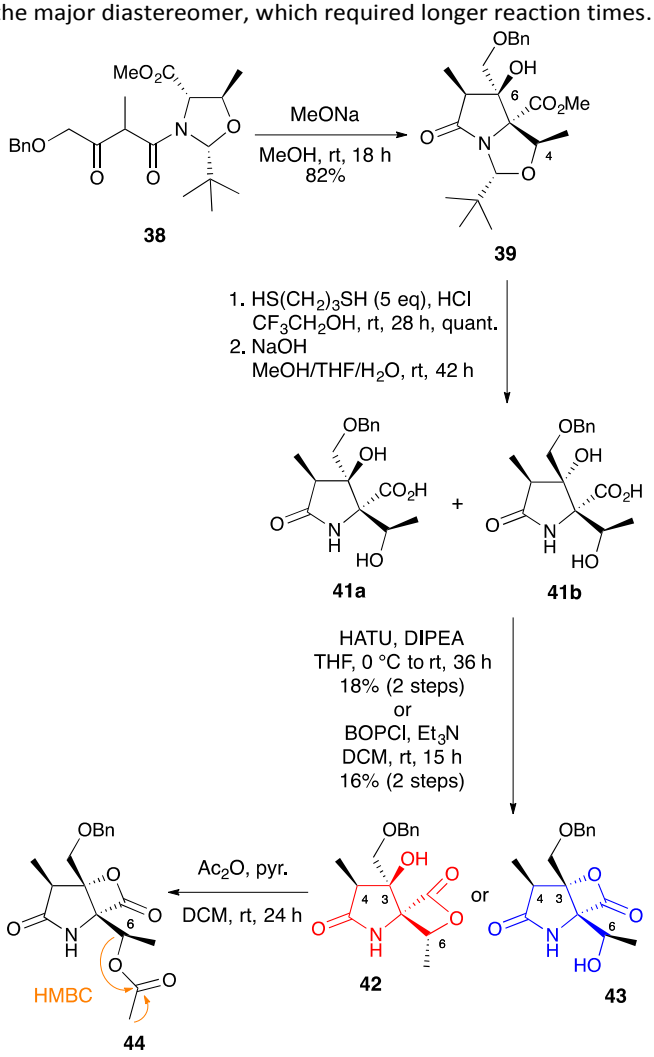
The synthesis of analogues bearing a hydroxypyrrolidinone core, closer to that of the γ-lactam-β-lactone natural products, requires an aldol ring closure of malonamide **28** (Scheme 3). Out of the four possible diastereomers from the cyclisation, a 2:1 mixture of only two isomers, separable by column chromatography, was obtained. NOE correlations revealed that **30a**, which possessed the same relative stereochemistry as oxazolomycin, was formed as the major product.[14] Alcohol **30b** was formed as the minor diastereomer, and no significant amounts of other isomers were observed. Interestingly, *N*,*O*-acetal deprotection, ester hydrolysis and β-lactonisation of either **30a** or **30b** with BOPCl/Et_3N gave fused-β-lactone **34** in both cases. This was due to epimerisation of **32a** during basic hydrolysis, probably via a retro-aldol reaction, a phenomenon in these systems which we had reported earlier.[11] Instead, when hydrolysing **32a** and treating the crude acid with HATU/DIPEA, spiro-β-lactone **36** was successfully isolated in 21% yield.



Scheme 3

Although a similar outcome was achieved with benzyloxy analogues **31a** and **31b**, which gave fused-β-lactone **35** and spiro-β-lactone **37** in good yields, interesting differences in the reactivity of both diastereomers were observed. The bulky benzyloxy group in the *endo*-face of the bicycle lowered the reactivity of the minor diastereomer towards *N*,*O*-acetal deprotection. While full conversion of **31a** was achieved with 1.5 equivalents of 1,3-propanedithiol, only 80% of **30b** was converted even with 5 equivalents. Conversely, for ester

hydrolysis, the bulky group hindered the methyl ester instead in the major diastereomer, which required longer reaction times.



Scheme 4

The additional methyl group found in 16-methyloxazolomycin (Figure 1) could be incorporated starting the synthesis from L-threonine instead of L-serine, as shown in Scheme 4.[14] In this case, aldol ring closure of malonamide **38** gave one single diastereomer **39**. This higher diastereoselectivity could most likely originate from steric hindrance between the *C*(4) methyl group and the *C*(6) substituent. However, after acetal deprotection and ester hydrolysis, a 2:1 mixture of isomers **41a** and **41b** was obtained, again as a result of epimerisation during a retro-aldol reaction. When the crude acid mixture was subjected to β-lactonisation and subsequent column chromatography purification, only one product was isolated, which displayed the correct MH^+ by HRMS analysis and C=O stretch at 1821 cm^{-1} corresponding to β-lactone formation. However, structural assignment as the spiro- or fused- structure in this case proved to be challenging. Cyclisation of the acids proceeded in similar yields for each diastereomer with either BOPCl and Et_3N, or HATU and DIPEA, which would suggest the formation of the fused-β-lactone, as found in earlier work (*vide supra*). NOE analysis, however, gave some contradictory data: strong correlations were observed between *H*(4) and C(3)CH_2, which would be consistent with spiro-**42**, but also between C(4)CH_3 and C(3)CH_2, indicative of fused-**43**. *H*(6) and C(3)CH_2 also showed some NOE interactions, which would agree with the structure of **43**. In order to gain further evidence for the assignment of the β-lactone, acetylation of the free alcohol was attempted. Even after stirring with an excess of acetic anhydride and pyridine overnight, the conversion was only 25%. This lack of reactivity is consistent with the hindered tertiary alcohol of the spiro- structure, however when the crude mixture was analysed by HMBC spectroscopy, clear correlations were found between the acetyl carbonyl and *H*(6), which would indicate fused-**43**. Thus, with more evidence to support the formation of the fused-β-lactone over the spiro- one, assignment as **44** was made, but this outcome shows the difficulty of assigning structure in such complex systems.

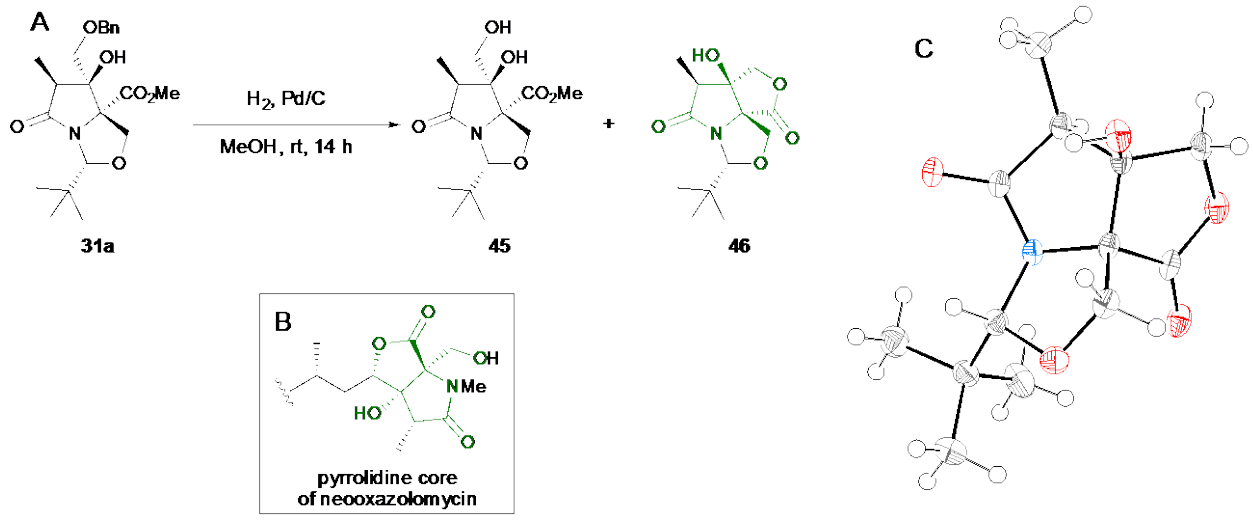
Of interest is that when benzyl deprotection of **31a** was attempted, γ-lactone **46** was also formed (Scheme 5). The formation of this unexpected side-product, confirmed by single crystal X-ray diffraction studies,[15] was noteworthy, as it possessed the same core as neooxazolomycin, another member of the oxazolomycin family.[16]

NMR characteristics of β-lactones

^1H and ^{13}C NMR analysis of the synthesised β-lactones revealed some consistent trends, in terms of differences between fused- and spiro-analogues and also with their methyl ester precursors. The most distinctive values were for *C*(3), *C*(6) and *H*(6), and are indicated in Table 1.

Spiro-β-lactones had *C*(6) chemical shifts 3.6-4.7 ppm higher than the parent methyl esters, while for the fused- ones these were 6.7-8.1 ppm lower. For *C*(3), the opposite trend was observed and the differences were less pronounced, but for **26** this pattern differed. It could be that the differences between the *C*(3) of fused- and spiro- structures are also a result of their different stereochemistry (3*S* and 3*R* respectively), rather than the nature of the β-lactone.

The chemical shifts of the ^1H NMR signals of *H*(6) were larger for spiro-β-lactones when compared to both the corresponding fused-β-lactone and their methyl ester precursor, while the *H*(6) values of fused-β-lactones were similar to the methyl ester. Another distinct feature is the coupling constant of *H*(6)s: this value was 11-13 Hz for fused-β-lactones and for the methyl esters, while there was a notable decrease for spiro-β-lactones, being halved to 6 Hz.



Scheme 5

Table 1 Comparison of relevant ^{1}H and ^{13}C NMR chemical shifts and *J* couplings for fused- and spiro-β-lactones (β-L) and their methyl ester (M. E.) precursors.

Comp	Chemical shift [ppm]						J coupling [Hz]	
	C(3)		*C*(6)		*H*(6)[a]		*H*(6)	
	β-L	M. E.	β-L	M. E.	β-L	M. E.	β-L	M. E.
23 fused	81.8	78.5	58.7	66.0	3.87	3.86	12.2	11.2
26 spiro	88.8	88.5	70.6	66.2	4.47	3.90	6.0	11.4
34 fused	87.5	79.4	57.0	65.1	3.88	3.84	12.6	10.8
36 spiro	77.5	79.4	67.7	64.1	5.03	4.01	6.3	11.6
35 fused	87.6	80.6	57.2	63.9	3.94	3.92	12.5	10.8
37 spiro	79.6	81.6	69.4	64.7	4.57	4.02	6.0	11.2
42/43	87.5	79.4	77.5	70.8	4.25	4.18	6.5[b]	6.5[b]

[a]Average chemical shift between $H(6_A)$ and $H(6_B)$
[b]Coupling between *H*(6) and C(6)CH_3

Therefore, while both NOE correlations and acetylation of the free alcohol can be used to assign these compounds, ^{1}H and ^{13}C NMR shifts showed clear differences between fused and spiro forms, and could be used to confirm the structure of newly synthesised β-lactones.

Biological activity

The calculated physicochemical properties of the synthesised analogues, which are summarised in Table 2, show that they comply with Lipinski's 'rule of five',[17] indicating that they possess 'drug likeness'. Their antibacterial properties against Gram-negative *E. coli* and Gram-positive *S. aureus* were evaluated using the hole-plate method.[18] Some of the compounds, and in particular bicyclic pyrrolidinones **31a** and **31b**, *gem*-dimethyl **10** and β-lactones **34** and **42/43** showed some *E. coli* activity, as well as weaker inhibition of *S. aureus*. The lack of activity of similar analogues **30** and **39**, and β-lactones **35**, **36** and **37** indicates that small modification of the structure and physicochemical properties of the compounds can have a pronounced effect on biological potency. Therefore, the preparation of a library of analogues which cover a larger chemical space and allow for SAR studies could provide more active compounds and might assist in the study of their mechanism of action.

The β-lactones were also assayed for proteasome inhibition. The 20S proteasome can degrade proteins at three different active sites: chymotrypsin-like (CT-L), trypsin-like (T-L) or caspase-like (CA-L), which target hydrophobic residues, basic aminoacids and acidic aminoacids respectively.[19, 20] Salinosporamide and analogues have been shown to possess the highest activity at the CT-L site. Moreover, it has been reported that inhibition of CT-L activity is often sufficient for a significant reduction of the proteasome activity, while the effect of inhibiting the T-L and CA-L sites is lower. Therefore, a chymotrypsin-like cell-based assay was chosen to test the β-lactones, with H460 and KMS cancer cells. Interestingly, again only **34** and **42/43** displayed some weak activity in KMS cells, with a 38% and 45% reduction of proteasome activity respectively at 50 μM. This outcome suggests that the β-lactone unit is not the primary moeity responsible for biological activity of the parent natural products.

Conclusions

With this work, we have shown that highly functionalised bicyclic tetramates and pyrrolidinones can be accessed in high levels of diastereo- and chemoselectivity. These systems can be derivatised to selectively generate spiro- and fused-β-lactones and γ-lactones, which contain core scaffolds of biologically active natural products such as oxazolomycin and salinosporamide. It is clear that small differences in the structure and stereochemistry of these compounds can have a profound effect on their reactivity and biological activity, and that where fused or spiro ring formation can occur, the former appears to be preferred, at least in these systems. The

synthesised analogues displayed weak antibacterial and proteasome inhibitory properties. However, further functionalisation of these systems, for instance via modification of the substituent at *C*(6), might lead to more active compounds.

Acknowledgements

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Table 2 Physicochemical properties and bioactivity of synthesised analogues

Compound	MW	clogP[a]	PSA[a]	MSA[a]	%PSA[b]	H-bond donor count	H-bond acceptor count	Zone size [mm][c,d,e]	
								E. coli	*S. aureus*
9	283.32	2.39	72.91	457.79	15.93	0	4	n.a.	n.a.
10	215.20	-0.26	92.70	321.29	28.85	2	4	16	14.5 H
14	285.34	1.47	76.07	468.48	16.24	1	4	n.a.	n.a.
15	299.37	2.11	65.07	503.81	12.92	0	4	13.5	14.5
17	217.22	-1.18	95.86	330.85	28.97	3	4	n.a.	n.a.
18	231.25	-0.53	84.86	367.98	23.06	2	4	n.a.	n.a.
21	217.22	-0.68	95.86	332.56	28.82	3	5	n.a.	n.a.
23	185.18	-0.66	75.63	267.30	28.29	2	3	14.5	n.a.
30a	285.34	1.19	76.07	467.09	16.29	1	4	n.a.	n.a.
30b	285.34	1.19	76.07	467.90	16.26	1	4	n.a.	n.a.
31a	391.46	2.51	85.30	619.71	13.76	1	5	20	14
31b	391.46	2.51	85.30	619.61	13.77	1	5	21	14
32a	217.22	-1.45	95.86	330.84	28.97	3	4	n.a.	n.a.
32b	217.22	-1.45	95.86	331.38	28.93	3	4	n.a.	n.a.
33a	323.34	-0.13	105.09	483.40	21.74	3	5	n.a.	n.a.
33b	323.34	-0.13	105.09	483.24	21.75	3	5	n.a.	n.a.
34	185.18	-0.93	75.63	268.04	28.22	2	3	18.5	13
35	291.30	0.39	84.86	420.48	20.18	2	4	n.a.	n.a.
37	291.30	0.39	84.86	418.55	20.27	2	4	14 H	13 H
39	405.49	2.93	85.30	648.37	13.16	1	5	n.a.	n.a.
40	337.37	0.28	105.09	510.91	20.57	3	5	n.a.	n.a.
42/43	305.33	0.80	84.86	447.21/448.56	18.98/18.92	2	4	15.5	n.a.
45	301.34	0.14	96.30	476.29	20.22	2	5	n.a.	n.a.
46	269.30	0.66	76.07	409.21	18.59	1	4	n.a.	14 H

[a]logP, polar surface area (PSA) and molecular surface area (MSA) were calculated using chemicalize.org; [b]%PSA = (PSA/MSA)*100%; [c]Compounds tested at 4 mg/mL for 20 h. Microbiological assays were performed by the hole-plate method with the test organism *Staphylococcus aureus* N.C.T.C. 6571 or Escherichia coli X580. Solutions (100 µL) of the compounds to be tested (4 mg/mL) were loaded into wells in bioassay plates and incubated overnight at 37 °C. The diameters of the resultant inhibition zones were measured; [d]n.a. = not active; [e]H = halo; denotes a ring of reduced bacterial density, but not a distinctly clear zone

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