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On the antibiotic activity of oxazolomycin

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ABSTRACT

Structural analysis of oxazolomycin and simpler fragments containing a common 3-hydroxy-2,2-dimethylpropanamide moiety has indicated that a U-shaped conformation is preferred, in some cases stabilised by hydrogen bonding between the N–H and O–H residues, as shown by a combination of molecular modelling, NMR spectroscopic and single crystal X-ray analysis. A direct synthesis of this unit has been established via the opening of β-lactones by a range of amines, and their antibacterial activity been shown to vary with the hydrophobic character of the substituents.

The oxazolomycins[1] **1**, the first originally isolated in 1985,[2] and the most recent, lajollamycin **2**, isolated much later in 2005,[3] are structurally novel antibiotic and antiviral agents, whose bioactivity has been proposed to arise from their protonophoric properties and whose biosynthesis has been the recent focus of attention.[4,5] Only one member, neooxazolomycin, has been successfully synthesised,[6,7] although a number of groups have established strategies for the synthesis of core components, including the lactam,[8–13] the middle component,[14] and the left-hand portion.[15–18] Related compounds, with various structural elements common to the oxazolomycins, such as inthomycin[19–21] and clathrynamide,[22] have also been the focus of attention. It has been reported that the observed antimicrobial properties of the oxazolomycins are a result of their protonophoric activity,[23,24] similar to the lipopeptaibols, a family of membrane active antimicrobial peptides.[25]

Amongst a number of unusual structural features of this compound, the central amide function is remarkable for the proximal geminal dimethyl and β-hydroxy functions. Although it is clearly not so hindered that amide bond formation is not possible, as the two extant syntheses of neooxazolomycin testify,[6,7] it seemed to us that this *gem*-dimethyl amide motif was likely to enforce a U-shaped structure on the molecule on the basis of the Thorpe–Ingold effect,[26] and that this might in turn be an important element to the observed bioactivity of the oxazolomycins; such an arrangement would be expected to give a polar lactam-lactone head group, and a non-polar tail. We report here results which suggest that oxazolomycin is in fact likely to adopt such a conformation, as do suitably substituted but simpler *gem*-dimethyl amides, and that the latter can exhibit antibacterial activity in their own right.

Molecular modeling[27] of the truncated structure **3** of oxazolomycin generated an energy minimised structure in which the geminal dimethylamide portion induced a turn, likely to be stabilised by a hydrogen bond as a result of the predicted close proximity of the N–H and C-3 hydroxyl groups (2.07 Å) (Fig. 1). Of interest was that in the modelling of oxazolomycin B **1b** itself, the minimum energy conformation (see Fig. 2) generated by AM1 semi-empirical (SPARTAN) calculation gave a U-shaped preferred conformation with an NH–O distance of 4.07 Å, too long for effective hydrogen bonding, but that this was indeed the preferred conformation was suggested by modelling a more linear structure, which was nearly 6 kcal/mol higher in energy and for which the NH. . .O distance was 4.65 Å. Similarly, the modelling of inthomycin B **4**, a primary amide lacking the dienyl substituent of oxazolomycin, indicated a structure which (Fig. 3) has an NH-O distance of 2.07 Å but which is clearly linear. A similar trimethyl substitution pattern to that found in the oxazolomycins has been reported to effectively control conformation in cyclic prodrugs,[28] and the potential influence of acyclic stereocontrol for the maintenance of antibacterial bioactivity is illustrated by the importance of intramolecular hydrogen bonds in the non-peptidic antibacterial antimycin A[29] and of conformational effects in pediocin.[30] On this basis, a more detailed examination of the synthesis and structure of the *gem*-dimethyl amide motif was warranted; desirable in any synthesis was the capacity to readily modify the amide flanking groups in order to explore their effect on antibacterial activity.

We found that the required *gem*-dimethyl amides were most readily generated by reaction of the enolate of phenyl isobutyrate **5** with aldehydes according to the literature method[31]; when

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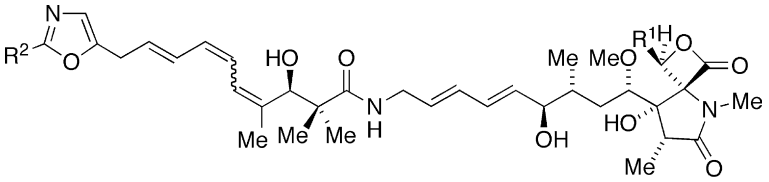
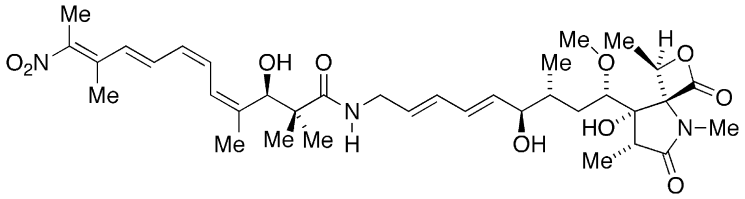


1a Oxazolomycin A: R^1 = H, R^2 = H; 4′*Z*,6′*Z*,8′*E*
1b Oxazolomycin B: R^1 = H, R^2 = H; 4′*E*,6′*E*,8′*E*
1c Oxazolomycin C: R^1 = H, R^2 = H; 4′*Z*,6′*E*,8′*E*
1d 16-Methyloxazolomycin: R^1 = Me, R^2 = H; 4′*Z*,6′*Z*,8′*E*
1e Curromycin A: R^1 = CH_3OCH_2, R^2 = Me; 4′*Z*,6′*Z*,8′*E*
1f Curromycin B: R^1 = Me, R^2 = Me; 4′*Z*,6′*Z*,8′*E*



2 Lajollamycin

applied to benzaldehyde, the corresponding β-lactone **6** was generated in 75% yield (Scheme 1). This could be opened by treatment with primary saturated and unsaturated amines, to give the amides 7a–**e** in good to excellent yield,[32] although this reaction could be sluggish, as might be expected of such a hindered lactone, requiring extended reaction time at 40 °C to force the reaction to completion, whilst avoiding the expected competing decarboxylation side reaction which would occur at high temperature. That these structures exist in a U-shaped conformation in solution was suggested by nOe analysis of **7a**, which showed enhancements of N–H, Me and C(H)OH protons (Scheme 1), and in the case of **12b**, single crystal X-ray analysis (Fig. 4a and Tables 1 and 3)[33] clearly indicated a hairpin structure, stabilised by an intramolecular hydrogen bond as a result of the predicted close proximity of the N–H and C-8 hydroxyl groups (2.29 Å), consistent with the in silico result. The sequence was also applicable to ketones, as exemplified by the reaction of cyclohexanone with phenyl isobutyrate **5** followed by ring opening with allylamine, to give amide **9** in excellent overall yield. Variation in the acyl side chain substitution pattern was also possible, as shown by the reaction of aldehydes **10a–e** giving amides **12a–e** in excellent overall yield (see Scheme 2). Although the aldehyde **10d** was easily prepared as shown in Scheme 3, attempted coupling of bromide **11e** with the same styrylboronate gave not the expected β-lactone, but the alkene (*E*)-1-(2-methylprop-1-enyl)-2-styrylbenzene, from Suzuki coupling followed by direct decarboxylation, in 34% yield as a result of the high temperature which was used. In the case of α,β-unsaturated aldehydes (see Scheme 4), the aldol adducts **13** and **14** rather than the expected β-lactones were formed,

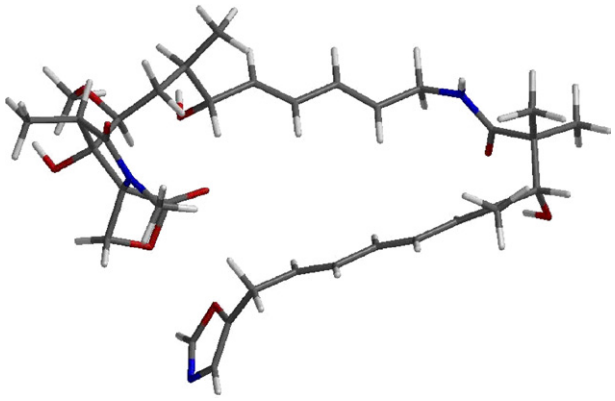


Figure 2. Minimum energy conformation for oxazolomycin B **1b** (energy = −252.4 kcal/mol, NH. . .O distance 4.07 Å).

but only in the presence of zinc chloride (2 equivalents); in the absence of zinc chloride, only the elimination products **15** and **16** were obtained in low yield, resulting from spontaneous decarboxylation of the β-lactone which was initially formed, along with unreacted starting material. It would appear that the zinc chloride acts as a Lewis acid, promoting the initial aldol addition, but also preventing the subsequent cyclisation by chelation across the β-hydroxycarbonyl group. These esters could be easily converted to amides **17** and **18** by aminolysis. Single crystal X-ray analysis of

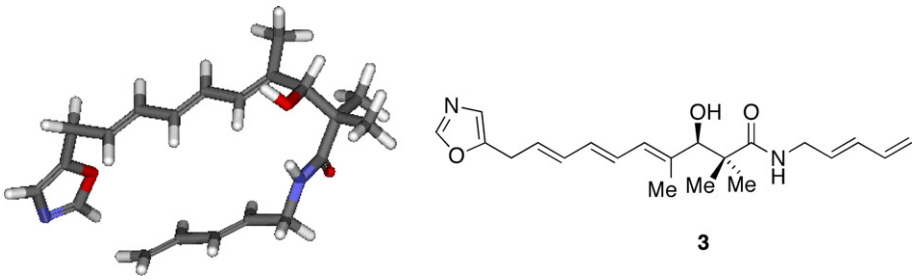


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Figure 1. Minimum energy conformation for amide **3**.

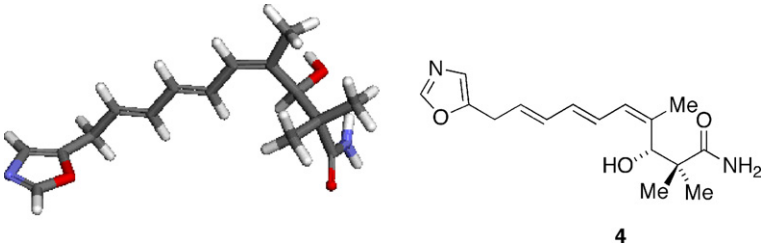
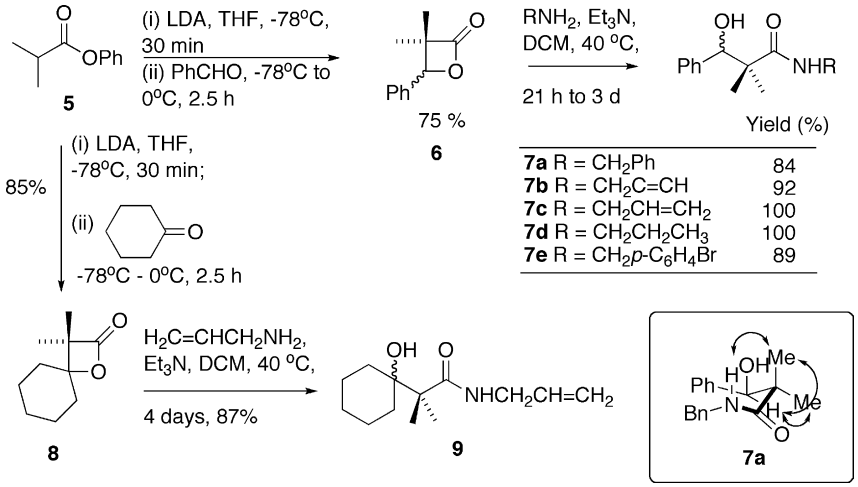
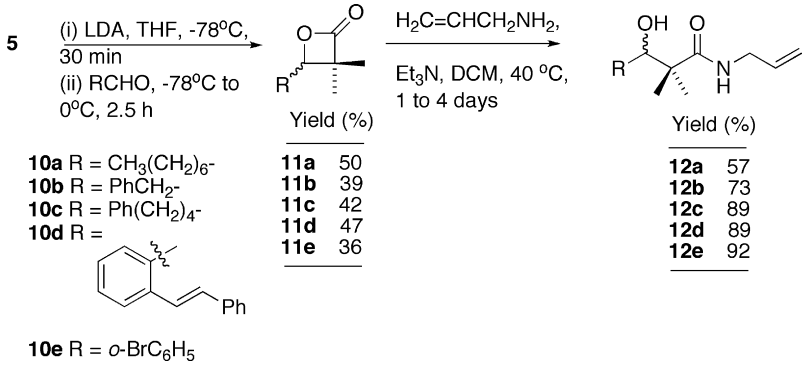


Figure 3. Minimum energy conformation for inthomycin **4**.



Scheme 1.



Scheme 2.

17 clearly indicated a linear structure, stabilised by an intermolecular hydrogen bond (Fig. 4b and Table 1).[33]

Bioassay against *Staphylococcus aureus* and *Escherichia coli* gave the results indicated in Table 2.[34] Derivative **12c** showed the best activity against *S. aureus*, although both **12d** and **18** exhibited weaker activity, with potency values relative to cephalosporin C of some 5–8%; the minimum inhibitory concentration of the most active compound **12c** was found to be 4 mg/ml, making this compound approximately 80-fold less active than KSM-2690, a member of the oxazolomycin family reported to be active against *S. aureus* at 50 μg/ml.[35] Much higher levels of activity were found against *E. coli*, and **12a**, **12c**, **12d** and **18** in particular exhibited potent activity at 4 mg/ml; their minimum inhibitory concentration was found to be in the range 0.5–1 mg/ml. Since the reported MIC values for oxazolomycins A–C **1a–c** are in excess of 100 μg/ml,[36] the most active fragments **7a**, **12c** and **12d** are approximately only 5 times lower in activity. The bioactivity of these compounds broadly correlates with their hydrophobicity, as shown from their Log*P* values; the exceptional compounds are bromophenyl derivative **7e** and amide **18**.

It is noteworthy that the common 3-hydroxy-2,2-dimethylpropanamide motif present in these compounds, and the parent oxazolomycins, complies with the Lipinski 'Rule of Five'[37] and is a versatile lead-like template suitable for library generation with at least two points of chemical diversity. The value of such Lipinski-compliant natural products with the required physicochemical properties for lead structures for drug discovery has recently been elaborated.[38]

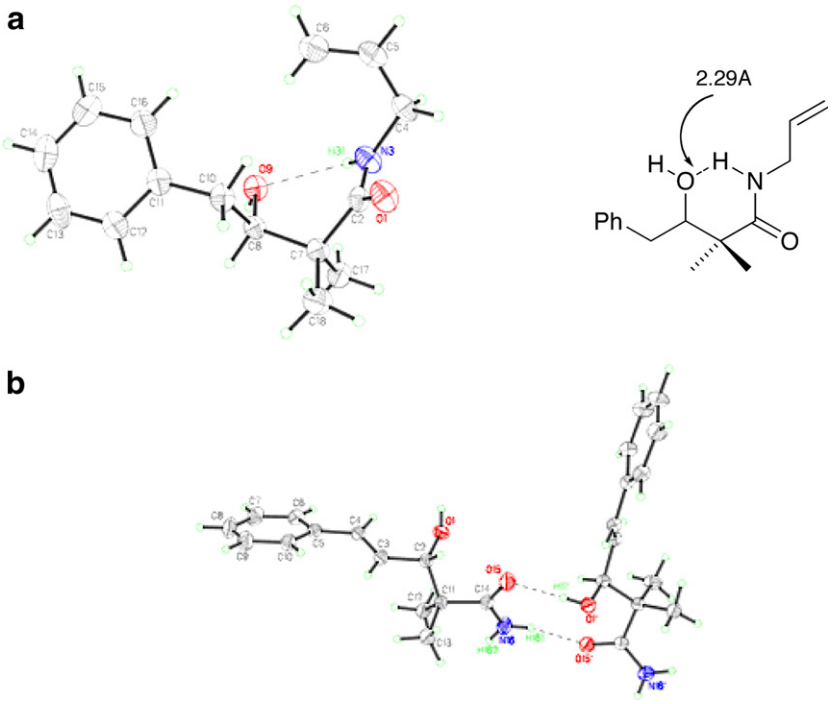
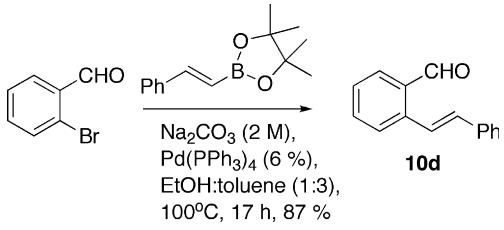


Figure 4. Crystal structures of (a) compound **12b** and (b) compound **17** showing the hydrogen bonding interactions detailed in Table 1. Thermal ellipsoids shown at 15% probability level.

Table 1
Hydrogen bonds for compounds **12b** and **17** (in Å and °; symmetry transformations used to generate equivalent atoms: #1 $-x+1, y+1/2, -z+2$ #2 $-x+1, y-1/2, -z+2$)

Compound	D–H...A	D(D–H)	D(H...A)	D(D...A)	<(DHA)
12b	N(3)–H(31)...O(9)	0.89	2.29	2.851(2)	121
17	N(16)–H(161)...O(15)#1	0.90	2.27	3.104(3)	154
	O(1)–H(11)...O(15)#2	0.86	1.90	2.739(3)	168



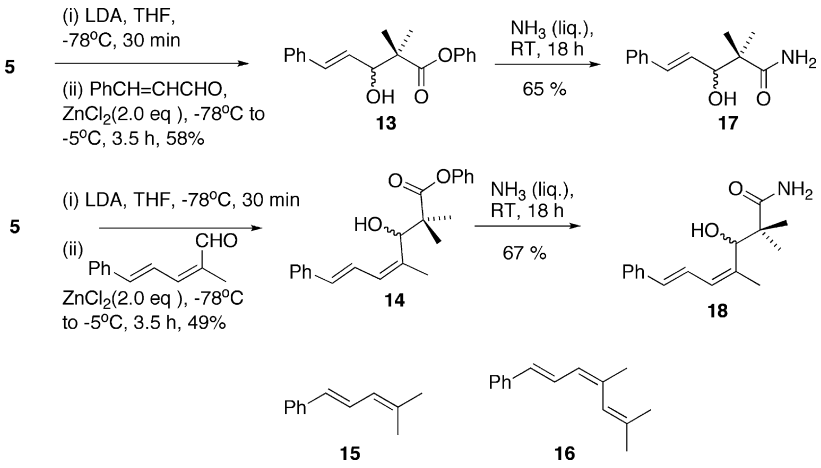
Scheme 3.

In conclusion, we have shown that the 3-hydroxy-2,2-dimethylpropanamide motif present in the oxazolomycins is likely to induce a turn in the preferred molecular conformation, and that, appropriately substituted, it can exhibit antibacterial activity. This natural product inspired motif is therefore of interest, since it may provide a template for library generation capable of ready optimisation against a number of bacterial targets.

Table 2
Bioassay of compounds **4–9** against *S. aureus* and *E. coli* using the hole plate method

Compound	Log*P*[a]	Bioactivity[b]					
		S. aureus			*E. coli*		
		Zone size/mm	Relative potency[c]	MIC[d]	Zone size/mm	Relative potency[c]	MIC[d] (mg/ml)
7a	3.59	Inactive	–	–	17	0.005	0.5
7b	2.07	Inactive	–	–	15	0.003	nd
7c	2.55	Inactive	–	–	Inactive	–	–
7d	2.68	Inactive	–	–	Inactive	–	–
7e	4.42	Inactive	–	–	Inactive	–	–
9	2.26	Inactive	–	–	Inactive	–	–
12a	3.73	Inactive	–	–	22	0.01	1.0
12b	2.83	Inactive	–	–	Inactive	–	–
12c	4.08	17	0.08	4	23	0.01	0.5
12d	4.74	14	0.06	nd	20	0.01	0.5
17	2.14	Inactive	–	–	Inactive	–	–
18	2.83	14	0.05	–	28	0.03	1.0

[a] Log*P* calculated using ChemBioDraw Ultra 11.0.
[b] Hole plate bioassay at 4 mg/ml (7:3 DMSO/H_2O).[34]
[c] Expressed as zone size per mg/ml, relative to cephalosporin C standard.
[d] Minimum inhibitory concentration estimated by serial dilution until no inhibition zone is observed; nd, not determined.



Scheme 4.

Table 3
Crystals structure data for compounds **12b** and **17**

Compound	**12b**	**17**
Chemical formula	$C_{15}H_{21}NO_2$	$C_{13}H_{17}NO_2$
Formula weight	247.34	219.28
Temperature (K)	150	150
Wavelength (Å)	0.71073	0.71073
Crystal system	Monoclinic	Orthorhombic
Space group	$P\,2_1/c$	$P\,2_1$
a (Å)	13.9977(3)	6.0960(2)
b (Å)	7.9134(2)	8.2767(3)
c (Å)	12.5315(5)	11.9649(5)
α (°)	90	90
β (°)	91.3997(12)	102.1584(12)
γ (°)	90	90
Cell volume (Å^3)	1387.69(7)	590.14(4)
Z	4	2
Calculated density (mg/m^3)	1.184	1.234
Absorption coefficient (mm^{-1})	0.078	0.083
F_{000}	536	236
Crystal size (mm)	0.20 × 0.15 × 0.07	0.18 × 0.18 × 0.18
Reflections measured	17901	10880
Unique reflections	3152	1435
R_{int}	0.058	0.045
Observed reflections ($I > 2\sigma(I)$)	2368	1356
Parameters refined	163	145
Goodness of fit	0.9845	0.9995
$R(I > 2\sigma(I))$	0.0488	0.0348
$wR(I > 2\sigma(I))$	0.1268	0.0859
Residual electron density (min,max) (eÅ^{-3})	−0.29, 0.25	−0.17, 0.16

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.105.

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32. Representative procedure for the ring opening of β-lactones with amine nucleophiles: (±)-***N*-Allyl-3-hydroxy-2,2-dimethyl-3-phenylpropanamide** **7c**. A solution of 6 (40 mg, 0.23 mmol), dry Et_3N (23 mg, 0.25 mmol) and allylamine (66 mg, 1.16 mmol) in dry DCM (1 ml) in a flask fitted with a reflux condenser was stirred at 40 °C for 24 h. The solvent was removed in vacuo and the remaining residue loaded onto a flash chromatography column of silica gel. Elution with 7:3 petrol/EtOAc furnished the desired product **7c** (54 mg, 100%) as a thick, colourless oil: R_f 0.18 (7:3 petrol/EtOAc); υ_{max}(film)/cm^{-1} 3345, 2978, 1642, 1538, 1177 and 1048; δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.06 (3H, s, 2-CH_3), 1.20 (3H, s, 2 − CH'_3), 3.83 (2H, dt, J 5.6 and 1.4, 1′-CH_2), 4.60 (1H, s, H-3), 4.66 (1 H, br s, OH), 5.07–5.15 (2H, m, 3′-CH_2), 5.68–5.85 (1 H, m, H-2′), 6.28 (1H, br s, NH) and 7.22–7.34 (5 H, m, 5× ArCH); δ_C (100.6 MHz; $CDCl_3$; Me_4Si) 20.6 (2-CH_3), 24.4 (2 − CH'_3), 41.8 (C-1′), 46.2 (C-2), 79.9 (C-3), 116.3 (C-3′), 127.6 (*ortho*- or *meta*-CH), 127.7 (*para*-CH), 127.8 (*ortho*- or *meta*-CH), 133.9 (C-2′), 140.8 (*ipso*-C) and 177.6 (C-1); m/z (ESI+) 291 ([M+CH_3CN+NH_4]$^+$, 36%) and 256 (16, [M+Na]$^+$); HRMS (ESI+): found 256.1296, $C_{14}H_{19}NNaO_2$ requires 256.1308. (±)-***N*-Allyl-3-hydroxy-2,2-dimethyl-7-phenylheptanamide** **12c**. Thick,

colourless oil, 89%: R_f 0.14 (7:3 petrol/EtOAc); υ_{max}(film)/cm^{-1} 3338, 3085, 3026, 2934, 2859, 1640, 1537, 1454, 1259, 1181, 1079 and 990; δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.14 (3H, s, 2-CH_3), 1.25 (3H, s, 2 − CH_3'), 1.26–1.40, 1.51–1.54, 1.61–1.69 (6H, series of m, 4-, 5- and 6-CH_2), 2.62 (2H, m, 7-CH_2), 3.46 (1H, dd, *J* 9.5 and 6.3, *H*-3), 3.66 (1H, br s, O*H*), 3.87 (2H, m, 1′-CH_2), 5.13 (1H, d, *J* 10.2, *H*-3′), 5.19 (1H, d, *J* 17.1, *H*′-3′), 5.84 (1H, ddt, *J* 17.1, 10.2 and 5.5, *H*-2′), 6.47 (1H, br s, N*H*) and 7.16–7.30 (5H, m, 5× ArC*H*); δ_C (100.6 MHz; $CDCl_3$; Me_4Si) 21.1 (2-CH_3), 24.5 (2 − CH_3'), 26.4, 31.5, 31.7 (*C*-4, -5 and -6), 36.0 (*C*-7), 41.6 (*C*-1′), 45.9 (*C*-2), 77.7 (*C*-3), 116.1 (*C*-3′), 125.6 (*para*-CH), 128.3, 128.4 (*ortho*- and *meta*-CH), 134.3 (*C*-2′), 142.6 (*ipso*-*C*) and 177.8 (*C*-1); *m/z* (ESI+) 312 ([M+Na]$^+$, 23%), 290 (45,[M+H]$^+$) and 103 (55); HRMS (ESI+): found 312.1934, $C_{18}H_{27}NNaO_2$ requires 312.1934.

33. Crystals of **12b** and **17** were grown by slow diffusion. Single crystal X-ray diffraction data were collected using graphite monochromated Mo Kα radiation (λ = 0.71073 Å) on an Enraf-Nonius KappaCCD diffractometer. The diffractometer was equipped with a Cryostream N2 open-flow cooling device,[39] and the data were collected at 150(2) K. Series of ω-scans were performed in such a way as to cover a sphere of data to a maximum resolution of 0.77 Å. Cell parameters and intensity data were processed using the DENZO-SMN package[40] and the structures were solved by direct methods and refined by full-matrix least squares on F^2 using the CRYSTALS suite.[41] Intensities were corrected for absorption effects by the multi-scan method, based on multiple scans of identical and Laue equivalent reflections. All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were initially positioned geometrically and optimised using restraints, then refined using a riding model. Crystal data and structure refinement parameters are included in Table 3. Crystallographic data (excluding structure factors) for the structures in this letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 687802 and 687803. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk.
34. *Bioassay of penicillin products.*[42–44] Microbiological assays were performed by the hole-plate method with the test organism *Staphylococcus aureus* N.C.T.C. 6571 or *E. 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, and relative potency estimated by reference to standards prepared with Cephalosporin C. Minimum inhibitory concentrations were estimated by serial dilution until no inhibition zones were obtained after bioassay.
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