Synthetic Studies directed towards the Vancomycin Carboxylate Binding Pocket

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The synthesis of the diastereoisomeric lactones 12a and 12b as models for the carboxylate binding pocket of vancomycin is described; the structure of 12a, which corresponds to the diphenyl ether rotamer found in vancomycin, is established by X-ray crystallographic analysis.

The glycopeptide antibiotic, vancomycin, is active against most gram-positive bacteria and, indeed, remains the drug of choice for the treatment of infections due to methicillin-resistant staphylococci.[1] The investigation of the structure and mode of action of the vancomycin class of antibiotics, particularly by Williams and coworkers, has made a significant contribution to our appreciation of the variety of interactions which are important for effective molecular recognition.[2,3] The interaction of vancomycin with the N-acyl-D-ala-D-ala terminal sequence of cell-wall peptides is a process characterised by a complex series of hydrogen-bonding and electrostatic interactions. Central to this process is the structural feature of vancomycin known as the carboxylate-binding pocket 1, as shown in Fig. 1. This feature has attracted interest from a number of groups in terms of total synthesis and as a vehicle for probing the precise nature of the interaction that occurs between the antibiotic and its peptide guest.[4–6]

In this communication we describe the results of a preliminary synthetic investigation directed towards this carboxylate-binding structural feature. In this study we have replaced a lactam linkage (a hydrogen-bond donor site) by a lactone, but we have successfully established the 16-membered macrocycle by a lactonisation procedure. This is of interest given recent reports of difficulties that have been encountered in similar processes, but the chemistry described below has yet to be extended to the generation of a macrocyclic lactam.[7†]

The synthesis of the target lactones 12 was carried out in several phases, the first of which is shown in Scheme 1.‡ Ullmann coupling, under standard conditions, served to generate the diphenyl ether 2, which then underwent selective chlorination in the presence of tin(iv) chloride to give the monochloro adduct 3 in 50% overall yield.§ Standard func-

tional group manipulations and protection then led smoothly to aldehyde 4 in 61% yield from 3.

Using the elegant methods developed by Evans et al.,[9] reaction of 4 with the enantiomerically pure boron-based enolate 5 gave the syn bromo aldol adduct 6 in 83% isolated
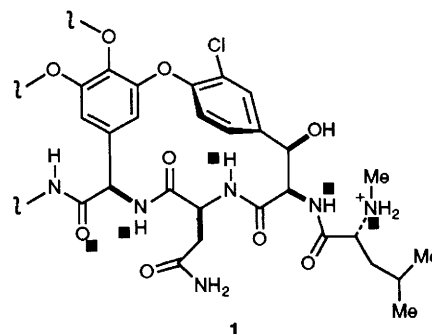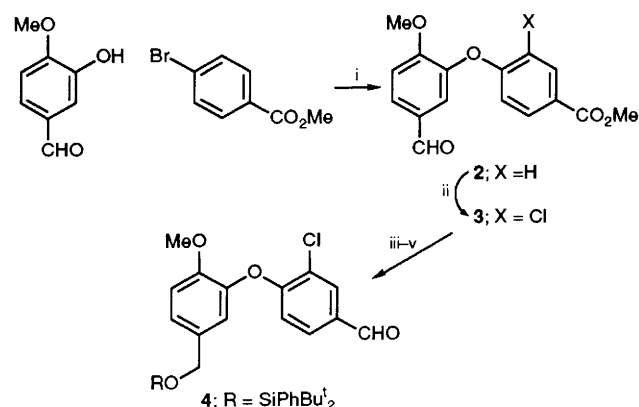

Fig. 1 Partial structure of vancomycin showing central features of the carboxylate binding site. (■ indicates key hydrogen-bonding/electrostatic sites)
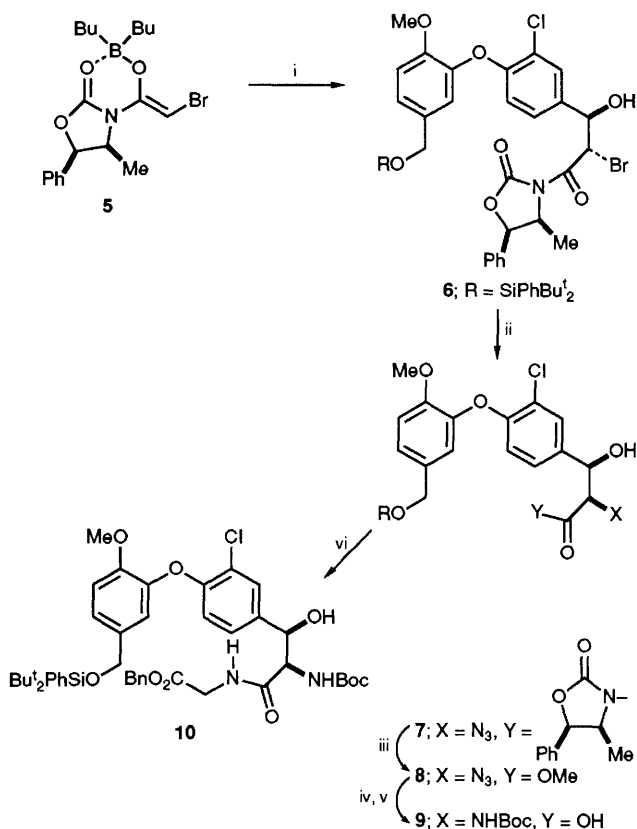

Scheme 1 Reagents and conditions: i, CuO, K_2CO_3, pyridine, reflux, 12 h (63%); ii, Cl_2, $SnCl_4$, CH_2Cl_2, room temp. (80%); iii, NaBH$_4$, EtOH, 0 °C then ClSiPhBu$^t_2$, imidazole, dimethylformamide (DMF), room temp.; iv, LiAlH$_4$, tetrahydrofuran (THF), 0 °C; v, MnO$_2$, C_6H_6, reflux (61% from 3)

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† The synthesis of a 14-membered biaryl ether lactone by lactonisation has been reported.[8]

‡ All new compounds gave satisfactory spectral data (IR, ^{1}H and ^{13}C NMR) and were further characterised by elemental analysis or in the case of lactones 12a, b, by high resolution mass measurement.

§ This reaction was accompanied by the formation of a small amount of dichlorinated product corresponding to reaction ortho to the methoxy substituent of 3.
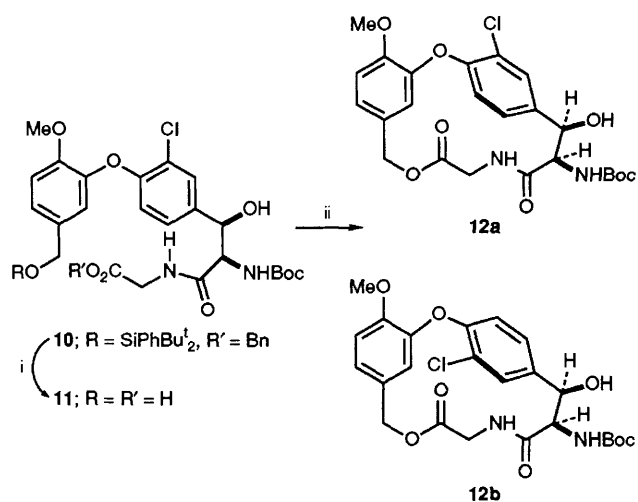
yield (Scheme 2). Although azide displacement of the bromo function of 6 to give 7 proceeded smoothly, we encountered difficulties with the removal of the oxazolidin-2-one chiral auxiliary. Using standard cleavage procedures,[10] we observed

significant levels of retroaldol reaction but removal of the auxiliary was eventually achieved (over a 10 day period) using a buffered (pH 4) methanolysis process to give 8 in high yield. Manipulation of 8 to give the corresponding N-Boc protected *anti* β-hydroxy-α-amino acid 9 was straightforward and this intermediate was coupled with glycine benzyl ester to give ester 10 in excellent overall yield.

The third phase of this study was concerned with establishing the macrocycle (Scheme 3). Hydrogenolysis of the benzyl ester component of 10 followed by fluoride-mediated desilylation gave the key hydroxy acid 11. Macrolactonisation was achieved under the conditions previously described by Boden and Keck[11] using the water-soluble carbodiimide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, in the presence of N-hydroxybenzotriazole and DMAP to give a 1.2:1 mixture of the diastereoisomeric lactones 12a and 12b in 41% combined yield after separation by HPLC (Lichrosorb Diol;

Scheme 2 *Reagents and conditions*: i, 4, Et$_2$O, −78 to 0 °C (83%); ii, NaN$_3$, DMF, room temp.; iii, MeOH, pH 4, 10 days, room temp. (76% from 6); iv, H$_2$, 10% Pd on C, (Bu^{t}OCO)$_2$O (Boc$_2$O), EtOAc (82%); v, LiOH, H$_2$O, THF (100%); BnO$_2$CCH$_2$NH$_2$, CH$_2$Cl$_2$, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, N-hydroxybenzotriazole, room temp. (96%)

Scheme 3 *Reagents and conditions*: i, H$_2$, 10% Pd on C, EtOAc, room temp. then Bu$_4$NF, THF, 0 °C (98%); ii, N-hydroxybenzotriazole, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, 4-(dimethylamino)-pyridine (DMAP), DMAP·HCl, CHCl$_3$, reflux (41%)
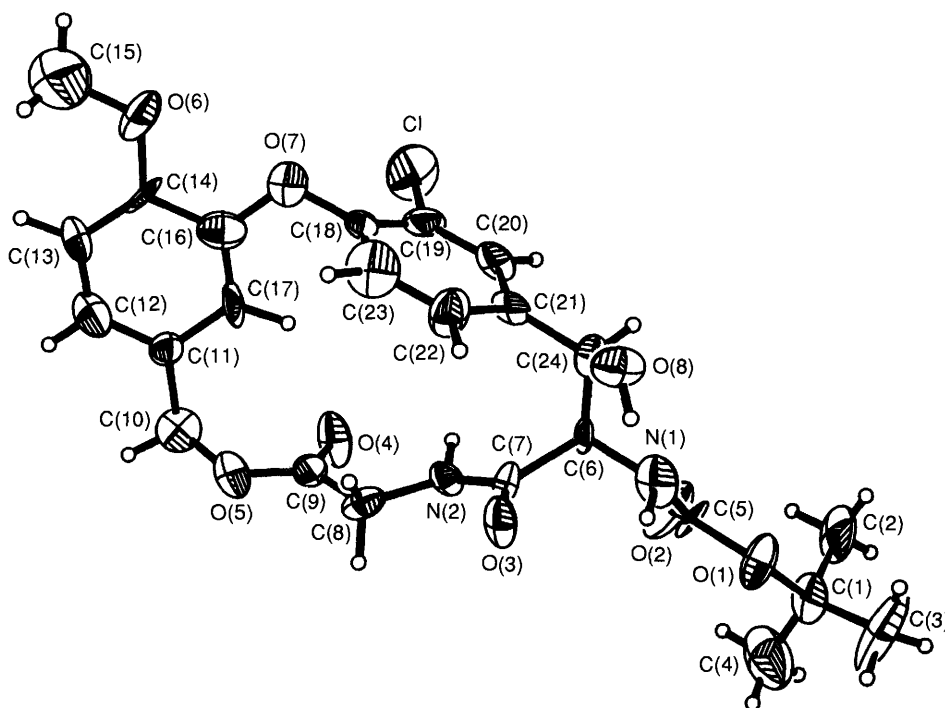
Fig. 2 ORTEP diagram of lactone 12a

heptane–ethanol).¶ The structure of **12a**, which corresponds to the 'outside' chlorine isomer (as in vancomycin[12]), was determined by X-ray crystallographic analysis (Fig. 1).‖ This served both to confirm the relative and absolute stereochemistry of the β-hydroxy-α-amino acid component, as well as to establish beyond doubt the spatial relationship between the chlorine substituent and the macrocyclic ring.

N-Methyl-L-leucine, though not incorporated within the macrocyclic framework of **1**, is nevertheless thought to be important for effective carboxylate binding. We have examined the incorporation of this unit into lactones **12a, b**, however, attempts to remove the *N*-Boc residue under acidic conditions led to extensive decomposition, which is presumably due to the lability of the benzylic nature of the lactone linkage. Introduction of this essential *N*-methyl amino acid component prior to closure of the macrocyclic ring should be feasible and we shall also examine the possibilities of achieving a macrolactamisation using the methodology outlined above.

¶ The cyclisation reaction shown in Scheme 3 was accompanied by a 50% yield of a complex mixture of diastereoisomeric dimeric lactone products, evidence for which was obtained by fast atom bombardment (FAB) mass spectral analysis.

‖ *Crystal data:* $C_{24}H_{27}ClN_2O_8$, $M = 506.94$, hexagonal, $P6_522$, $a = b = 9.672(4)$, $c = 101.37(6)$ Å, $V = 8212(11)$ Å^3, $\lambda = 1.54178$Å, $Z = 12$, $D_c = 1.23$ g cm^{-3}, $F(000) = 3192$, μ(Cu-Kα) $= 16.3$ cm^{-1}.

Crystals were obtained from acetone–water as colourless hexagonal prisms. Siemens R3m/V diffractometer, 3745 independent reflections measured ($3 < 2\theta < 115°$) of which 1135 reflections had $I > 3.0 \sigma(I)$. Full-matrix least-squares refinement with anisotropic thermal parameters was used for all non-hydrogen atoms. Hydrogen atoms, with the exception of that attached to N(2), were refined in riding mode but with individual isotropic temperature factors. Small residual peaks, probably due to the presence of solvent in the crystal lattice, appeared in the difference Fourier maps but these could not be satisfactorily refined. Individual weights were applied according to the scheme $w = [\sigma^2(F_o) + 0.001|F_o|^2]^{-1}$, refinement converged at R 0.061, R_w 0.058, goodness-of-fit $= 1.27$. The space group and hence the absolute structure were confirmed using the eta test of Rogers[13] [$\eta = 1.0(2)$]. All computations were carried out using the SHELXTL PLUS[14] (µ-VAX II) system of programs. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

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