Articles

Structure Elucidation of the Novel Glycopeptide Antibiotic UK-68,597

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The structure of a novel glycopeptide antibiotic has been determined using a combination of fast atom bombardment mass spectrometry and two-dimensional proton NMR techniques. The antibiotic belongs to the vancomycin group, and its structure is related to those of ristocetin and teicoplanin. The antibiotic is found to contain several unusual features including a high degree of aromatic chlorination, an aromatic sulfate ester, and an α-keto group in place of the more usual N-terminal amine.

Introduction

The vancomycin group of antibiotics has received considerable attention in recent years due to the lack of resistance to their antimicrobial action.[1] Vancomycin, the first member of the group to be isolated[2] and have its structure determined,[3a,b] is now of considerable clinical importance. However, it has to be administered in a relatively high daily dosage and also has undesirable side effects.[4] Thus, there is a continuing search for novel members of the group, which may provide a more effective antibiotic with lessened detrimental effects. In addition, various members of the group, such as avoparcin,[4] show performance-enhancing effects in food-producing animals.

The antibiotics are known to complex with mucopeptides terminating in D-alanyl-D-alanine,[5] and it seems likely that they inhibit bacterial cell wall biosynthesis by binding to such peptides in the developing peptidoglycan surrounding Gram-positive bacteria.[6] Thus the study of these antibiotics has also proved fruitful in the area of molecular recognition,[7-9] as they are of low molecular weight (commonly below 2000 Da), yet show a high degree of specificity in binding to peptides terminating with D-alanyl-D-alanine.

This paper describes the structure elucidation of UK-68,597, a novel member of this group of antibiotics. The antibiotic was obtained by fermentation of a strain of *Actinoplanes* sp. and initially purified by adsorption onto Amberlite XAD-2 resin directly from the broth filtrate. The antibiotic could be adsorbed from the resin eluates onto agarose-bound D-alanyl-D-alanine.[10,11] The compound was then eluted from the affinity gel, and the purification was completed by reverse-phase HPLC. The structure of the antibiotic was then determined using fast atom bombardment mass spectrometry (FAB-MS) and a variety of one- and two-dimensional NMR techniques. The strategy is similar to that used in this laboratory to determine the structure of teicoplanin[12] and A-40926.[13] In particular, a phase-sensitive double quantum filtered COSY experiment (DQF-COSY) is used to outline the *J* coupling networks within the sugars and peptide residues. The short range inter-proton distances obtained from 2D nuclear Overhauser enhanced spectra (NOESY) allow the coupling networks to be placed together to produce a completed structure.

The nomenclature used for the antibiotic protons is that used previously in this laboratory.[12,13] Peptide protons are designated by the residue number (counting from the N-terminus) and a small case letter corresponding to the position within the residue. Sugar protons are denoted by a large case letter for the sugar type, and a number which refers to its position within that sugar (counting from the anomeric position).

Discussion

Fast Atom Bombardment Mass Spectrometry. FAB-MS of glycopeptide antibiotics is well documented,[15,16] and positive molecular ions can routinely be observed using 10–20 nmol of material in glycerol/thio-

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Table I. Ions Observed from UK-68,597 in Positive and Negative Ion Fast Atom Bombardment Mass Spectra[a]

matrix	m/z	rel int	inference
TG/TCA	1758	0.2	$[M + H + TG]^+$
positive ion	1672	0.4	$[M + Na]^+$
	1650	0.6	$[M + H]^+$
	1570	1.0	$[M + H - SO_3]^+$
	1265	0.08	$[M + H - SO_3 - \text{sugars}]^+$
TG/GLY/	1648	1	$[M - H]^-$
5% 15c5	1614	0.16	$[M - H - Cl + H]^-$
negative ion	1343	0.14	$[M - H - \text{sugars}]^-$

[a] Abbreviations: TG = α-thioglycerol. [b] TCA = trichloroacetic acid. [c] 15c5 = 15/5 crown ether. [d] M = intact antibiotic.

glycerol matrices. However, UK-68,597 gave observable molecular ions only under very acidic matrix conditions (thioglycerol with added trichloroacetic acid), and these were still of low abundance relative to the chemical noise. Ions of approximately equal abundance 80 mass units apart were found at m/z 1650 and 1570. Analysis in the negative ion mode revealed a very abundant ion at m/z 1648, with no ion 80 mass units lower. This anomalous behavior, coupled with the presence of 1.9% sulfur in the compound (determined by microanalysis and corresponding to 1 atom of sulfur in a molecule of molecular weight 1649) suggested that UK-68,597 contained a sulfate ester. This was verified by the known fragmentations of peptide sulfate esters in positive and negative ion FAB-MS,[17] and also by spectrometric titrations revealing an ionizable group of $pK_A = -1$. One other member of the vancomycin group containing an aromatic sulfate ester has been previously reported.[18]

The greater signal to chemical noise ratio in the negative ion FAB spectrum allowed the observation of less abundant fragment ions containing further information. These ions are listed in Table I. The loss of 34 mass units was indicative of aromatic chlorine being replaced by hydrogen from the matrix. The broad spread of isotope peaks in the molecular ion suggested that there were at least three chlorines present in the antibiotic. The masses of the remaining fragment ions were rationalized in terms of cleavage of glycosidic bonds and revealed the nature of the attached sugars, as shown in Table I. No further fragment ions were seen below m/z 1343. Hence 1344 represents the molecular weight of the aglycon of UK-68,597. Further fragments were not expected due to the highly cross-linked nature of the aglycone.

Proton NMR Spectroscopy. The proton NMR spectrum of UK-68,597 in DMSO-d_6 was broad at room temperature. However, on warming to 335 K the line width decreased and a well-resolved spectrum resulted. A phase-sensitive double quantum filtered COSY (DQF-COSY) was acquired with a 5 mM sample at this temperature. The spectrum showed six NH-αCH couplings, as expected for the heptapeptide backbone commonly found in these antibiotics. One of the α-protons was further coupled to a βCH, while a second was coupled to a βCH_2. The lack of extended upfield coupling networks indicated that the side chains are all aromatic (and none aliphatic, as is the case for vancomycin[3]). This conclusion was verified by the seven aromatic spin systems observed in the DQF-COSY. They consisted of two 1,3,4-trisubstituted rings, one 1,3,5-trisubstituted ring, and four pairs of meta-coupled doublets.

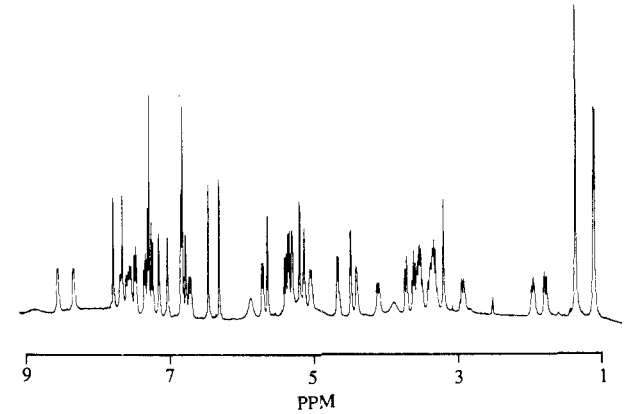

Figure 1. Proton NMR spectrum of UK-68,597 in DMSO-d_6 at 400 MHz and 320 K.

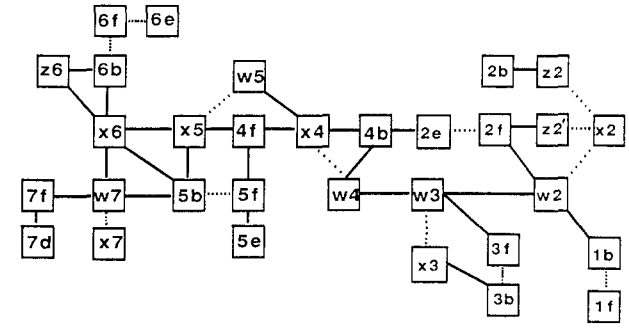

Figure 2. Schematic diagram of the NOEs and coupling connectivities used in the assignment of the aglycone of UK-68,597. Solid lines represent dipolar (NOE) couplings, broken lines represent scalar (J) couplings.

The coupling networks could next be assigned to particular portions of the aglycon by the use of the short-range spatial connectivities obtained from a phase-sensitive NOESY experiment. The assignment relies to some extent on the similarity of the C-terminal region of all known antibiotics of the vancomycin group.[19] However, it has been shown for other antibiotics in the group that the observed set of NOEs can only be satisfied by one particular set of stereochemistries and sites of cross linking of residues 2, 4, 5, 6, and 7.[20,21] The NOE data were obtained at a higher concentration of antibiotic (24 mM) to improve signal to noise, and at a lower temperature (320 K) to increase the magnitude of the observed negative NOEs (the decrease in temperature decreases its tumbling rate and hence pushes it further into the negative NOE regime). The slight increase in peak overlap and linewidth under these conditions did not present any serious problems of interpretation. The one-dimensional proton NMR spectrum of this sample is shown in Figure 1.

The assignment can be initiated from the relatively high field pair of meta-coupled aromatic doublets at 5.63 and 5.18 ppm. These are characteristic of the ring 4 protons, 4b and 4f, which experience large upfield ring current shifts from rings 2 and 6.[22] Both 4b and 4f have NOEs to the αCH of residue 4, whereas only one of them (the more

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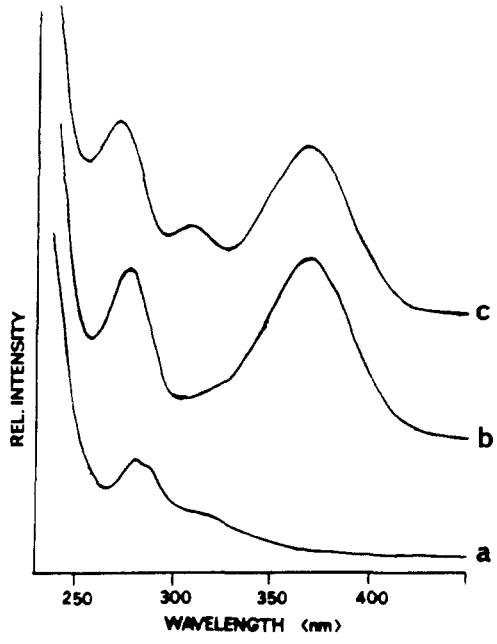

Figure 3. UV spectra of UK-68,597. 10^{-5} molar antibiotic at increasing pH. (a) pH 3.0, (b) pH 7.0, (c) pH 8.5.

downfield) has an NOE to the amide NH of residue 4, and must therefore correspond to 4b. Having established the resonance positions of residue 4, further NOEs and couplings can be used to assign the rest of the aglycon resonances. These are depicted schematically in Figure 2. Residue 3 is found to be the 1,3,5-trisubstituted ring, and the large NOE from 3f to a meta-coupled doublet (1b) locates ring 1, and is indicative of a biphenyl ether coupling between ring 1 and 3. However, the NOEs usually present between 1b or 1f and the singlet resonance x1, the αCH of the N-terminal residue, are absent in the NOESY spectrum. This lack of any resonance assignable to x1 is discussed in more detail below. A small NOE from 4b to 2e permits ring 2 to be identified as a 1,3,4-trisubstituted ring, with a chlorine at the rear of the ring in position 2c. The NH-αCH-βCH_2 is also found to be part of residue two by the intraresidue NOEs from 2f to z2′ and 2b to z2.

Assignment of the left-hand side of the aglycon is made possible by the NOEs from 4f to 6e, w5, and 5b. The pattern of interresidue NOEs involving the protons at the front of the molecule on the left-hand side are characteristic of these antibiotics and arise from the biphenyl linkage of rings 5 and 7, and the cis amide bond between residues 5 and 6.

When conducting the spectrometric titrations to detect the ionizable group of $pK_A = -1$, it was also noted that a further ionization was taking place at $pK_A = 4$–5 (see Figure 3). This second ionizable group could not be explained by the ionization of the C-terminal carboxylic acid or of phenolic hydroxyls, as the former is not usually UV active, whereas the latter occur at a higher pK_A and at 280 nm;[5] the novel ionization took place in association with the appearance of a band at the longer wavelength of 370 nm. This piece of information, together with the lack of any signal assignable to the αCH of residue 1, may be rationalized by proposing an α-keto group in place of the more usual N-terminal amine. Organisms producing such antibiotics have been noted in the patent literature,[23] and semisynthetic derivatives containing an α-keto amide have been prepared.[24] Thus, the pH-dependent chromophore

Table II. Chemical Shift of Hydroxyphenylglycine Residues of UK-68,597, with and without the Aryl Sulfate Ester

resonance	with OSO_3H[a]	without OSO_3H[a]	teicoplanin[b]
1b	7.02	6.85	6.67
1f	7.64	7.58	6.92
3b	6.83	6.34	6.33
3d	6.82	6.34	6.39
3f	6.77	6.44	6.29
5b	7.14	7.12	7.05
5d	6.82	6.77	6.70
7d	6.45	6.47	6.36
7f	6.30	6.23	6.29

[a] Data obtained at 320 K and 400 MHz. [b] Data from ref 12.

arises from conjugation of the α-keto group with the π-system of ring 1, producing a vinylogous acid with the para hydroxyl of ring 1.

This proposition is confirmed by two further pieces of evidence. First, the ^{13}C NMR spectrum in DMSO-d_6 at 320 K (spectrum not shown) revealed a peak at 185.4 ppm (relative to the internal DMSO carbon peak at 39.9 ppm) corresponding to an α-keto amide-like carbonyl. For comparison, in the aglycon of teicoplanin, the furthest downfield carbon resonance is at 169.9 ppm.[14]

Second, treatment of UK-68,597 with an excess of methanolic sodium borohydride for 8 h at room temperature resulted in the FAB negative molecular ion shifting to m/z 1650, an increase of two mass units relative to the starting material. HPLC showed the reaction mixture to be a mixture of two products, both having the increased molecular weight. The peaks are of approximately equal intensity, indicating that there is little facial selectivity of the borohydride's attack upon the α-keto amide.

The only remaining ambiguity in the structure of the aglycon is in the location of the aryl sulfate ester. In previous studies, this location has been deduced by the comparison of chemical shifts of antibiotics with and without the sulfate, both of which were fortuitously produced by the same microorganism.[18] It was found that the protons ortho to the sulfate ester were shifted downfield by more than 0.6 ppm relative to the equivalent proton adjacent to a phenol hydroxyl. During the isolation and purification of UK-68,597, the species without the sulfate ester was not isolated, and pulse hydrolysis proved unsuccessful in selectively removing the sulfate ester.

As an alternative, UK-68,597 was incubated with an aryl sulfatase enzyme. The antibiotic was found to act as a substrate for the enzyme, and after 2 days, HPLC showed that the incubation mixture contained two major species: unreacted starting material and a new compound of longer retention time. Milligram quantities of this new antibiotic were purified and analyzed in a similar manner to the parent antibiotic. The new compound produced a positive molecular ion at m/z 1570, and a negative molecular ion at m/z 1568 (corresponding to $(M + H)^+$ and $(M - H)^-$, respectively). There was no observable ion at m/z 1648 in the negative ion spectrum. A comparison of the chemical shifts of the hydroxyphenylglycine protons, with and without the sulfate ester, is made in Table II. The majority of other resonances changed by less than 0.1 ppm.

From the chemical shifts in Table II, it is apparent that the sulfate is attached to the phenolic hydroxyl of ring 3. Further proof of this assignment is shown in Figure 4. By cooling DMSO solutions of the antibiotics to room temperature and carefully adjusting the pH with hydrochloric

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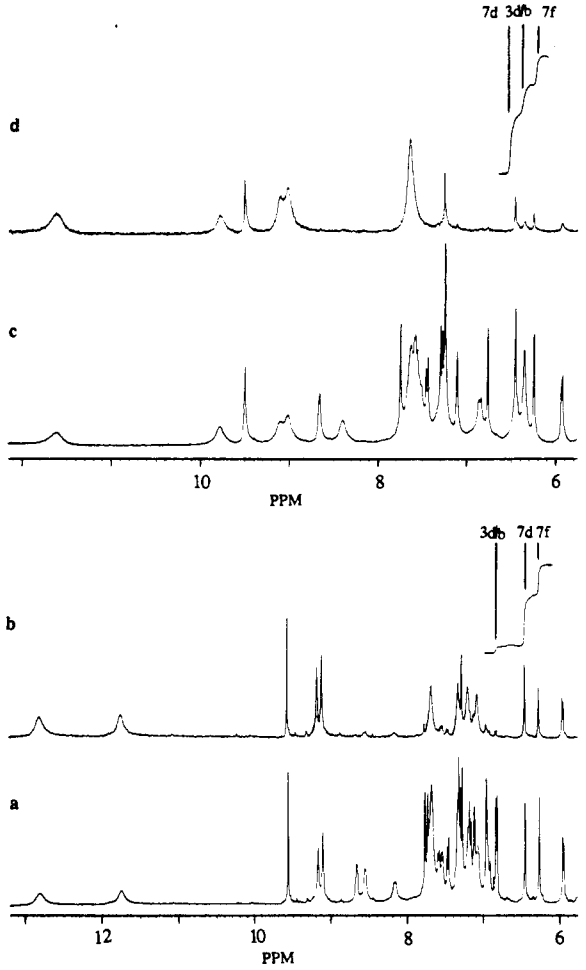

Figure 4. Saturation transfer experiments from the residual water peak in DMSO-d_6: (a) downfield region of UK-68,597; (b) saturation transfer difference spectrum of (a); (c) downfield region of UK-68,597 without sulfate; (d) saturation transfer difference spectrum of (c). All spectra obtained at 400 MHz and 293 K.

acid in DMSO and sodium methoxide, it was possible to place the phenolic hydroxyl protons into slow exchange with the residual water in the DMSO. Preirradiation of this residual water resulted in a transfer of magnetization to the phenolic hydroxyl protons. Partial saturation of thse protons then led to the build up of an NOE to the protons ortho to the hydroxyl group.[12] Hence, all protons adjacent to phenolic hydroxyls can be located. For the sample containing the sulfate ester, only 7d and 7f had any appreciable NOE. For the sample without the sulfate ester, 7d and 7f again had an NOE, but 3b and 3d also had an NOE comparable in size to that of 7f (3d and 3f have equivalent chemical shift at this particular temperature and pH, hence only their combined NOE can be observed). The NOE to 7d is much larger by virtue of it being adjacent to two hydroxyl groups. These saturation transfer experiments again point to the sulfate ester being attached to ring 3, and also indicate that the substituents at positions 1e and 5e are chlorines since no NOE is observed between the phenolic hydroxyls and protons of ring 1 or ring 5.

Having completely assigned the resonances of the aglycon, the remaining signals in the proton NMR must originate from the carbohydrate portion of the molecule. Two anomeric protons are immediately apparent in the DQF COSY and resonate at 5.33 and 5.29 ppm. Connectivity from G1, the lower field of these, to G2 is indicated, followed by further coupling from G2 to G3, G3 to G4, and G5 to G6/6′. Coupling between G4 and G5 cannot be

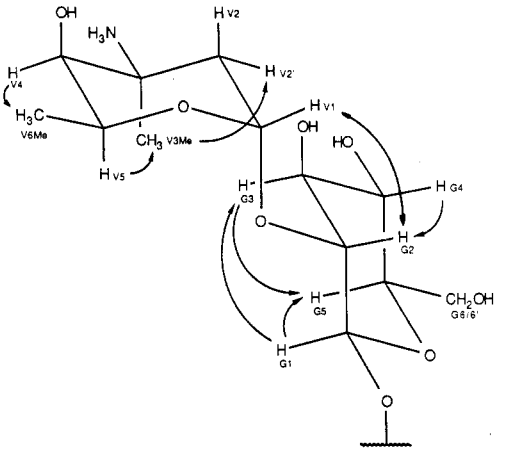

Figure 5. Structure of the disaccharide. The NOEs defining the stereochemistry are shown by arrows.

directly observed in the DQF COSY because of their almost identical chemical shifts. The resonances of G2 to G6/G6′ are all indicative of protons adjacent to oxygen, and thus the "G" resonances represent the hexose noted in the FAB fragments. The 7.6-Hz coupling between G1 and G2 can only arise from the trans diaxial coupling of a β anomer sugar. The NOEs listed in Table II can only be satisfied by protons G3, G4, and G5 also being axially disposed about a six-membered ring leading to the assignment of the hexose as β-glucopyranose, assumed to be of D absolute configuration.

The fragments noted in the FAB mass spectrum suggested that the second sugar contained in UK-68,597 was isomeric with the dideoxyamino sugar vancosamine. The couplings and NOEs of the unassigned resonances are also consistent with this proposition and also indicate that the relative stereochemistries are identical with those of vancosamine, as shown in Figure 5. In particular, the small coupling between V1 and V2 can only arise if the α anomer is present, i.e. V1 is equatorial. The small coupling constant between V4 and V5 is also consistent with the relative stereochemistry shown in Figure 5 (the coupling constant must be of the order of 0–2 Hz as the 1–3–3–1 quartet of V5, arising from the coupling to the V6 methyl, is not noticeably perturbed by any further coupling).

A connectivity between the vancosamine and the glucose is high-lighted by a large NOE between V1 and G2, indicating a vancosaminyl(2–1)-glucosyl disaccharide. No NOEs between G1 and the aglycon protons are observed to locate the site of attachment of this dissaccharide. However, the lack of observable NOE may arise as a result of the correlation time, τ_c, of the motion between G1 and the aglycon protons being such that $\tau_c\omega = 1$ (where ω is the Larmor frequency of the protons), in which case the size of the NOE would be very small despite G1 being spatially close to protons of the aglycon. This problem may be overcome by either changing conditions of temperature or solvent in order to alter the correlation time or by observing the NOEs in the presence of a spin locking field.[25] The first of these options would create the problem of reassignment of the spectrum under the new conditions. Hence, the second option was taken, and a 2D-CAMELSPIN spectrum acquired. Under the conditions of this experiment, all spatially close protons exhibit positive NOEs. Correspondingly, crosspeaks are observed between G1 and 2e, and between G1 and 6e. Thus, the disaccharide

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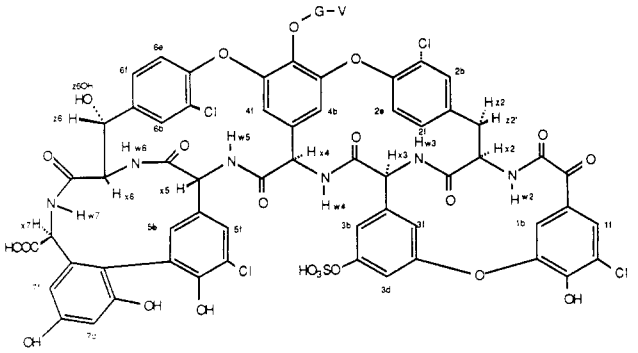

Figure 6. Structure of UK-68,597. -G-V = α-L-Vancosaminyl(1-2)-β-D-glucosyl.

is attached to ring 4, and at least two rotamers are populated, one with G1 adjacent to 2e, and the other with G1 adjacent to 6e. Similar evidence for the existence of these two rotamers in the identical disaccharide in vancomycin have been previously reported.[26] The latter of these two rotamers would also place the vancosamine V6 methyl group in a position close to 2e, explaining their observed NOE. This conformation of the vancosamine/glucose saccharide has been ascribed importance in the binding of peptides terminating in D-alanyl-D-alanine by the antibiotic vancomycin.[26]

The completed structure of the antibiotic, derived by the above methods of assignment, is shown in Figure 6. A tabulation of chemical shift, coupling constant and NOE data is presented in Table III, together with the corresponding chemical shifts of vancomycin.

Conclusion. UK-68,597 is found to be a glycopeptide antibiotic structurally related to vancomycin. The aglycon is similar to that of A 47934,[18] having the same positions of chlorination of rings 2, 5, and 6 and also containing an aryl sulfate ester. However, they differ in the location of the sulfate ester, in the type of sugars attached to the aglycon, and in the functionality at the α-carbon of residue one. UK-68,597 contains the unusual feature of having an α-keto group, instead of an amine. Despite the lack of an N-terminal ammonium group, the antibiotic retains antimicrobial action and is capable of binding to agarose-bound D-alanyl-D-alanine, thus confirming[24] that a charged N-terminus is not essential to the mode of action of these antibiotics. The reason for the presence of a sulfate ester in the antibiotic remains uncertain but may be involved in increasing the selectivity of the binding process rather than improving the magnitude of binding to peptides terminating in D-alanyl-D-alanine.

Experimental Section

The antibiotic was initially recovered by adsorption onto Amberlite XAD-2 resin directly from the broth filtrate. The glycopeptide could be selectively adsorbed from the resin eluates onto agarose-bound D-alanyl-D-alanine.[10,11] The compound was then desorbed from the affinity support by eluting with aqueous ammonia/acetonitrile (7:3). The purification was completed by preparative reverse-phase HPLC, using a Waters 10μ C-18μ-Bondapak column (19 × 150 mm), eluting with 0.1 M ammonium formate–acetonitrile (85:15) at a flow rate of 8 mL/min. Fractions containing the antibiotic were pooled, desalted, and lyophilized to give a pale yellow solid.

FAB-MS spectra were recorded on a Kratos MS-50 double focusing instrument fitted with an Iontech fast atom gun, a standard FAB source, a high-field magnet, and a PAD accelerator.

Table III. Chemical Shift Data for UK-68,597

resonance	chemical shift[a] ppm	couplin,[b] Hz	vancomycin[c] chemical shift, ppm	NOEs observed in UK-68,597[d]
1b	7.02		n/a	w2 (s), w3 (s), 3f (l)
1f	7.64	d 2.1	n/a	
w2	7.67	d 10.0+	8.00	z2 (s), 2f (m), 3f (s), w3 (s)
x2	5.03	bm	4.86	z2 (m), z2′ (l), 2b (s)
z2	2.91	bd 12.4	n/a	z2′ (l), x2 (l), w2 (s), 2f (l), 2b (m)
z2′	3.42	0	n/a	z2 (l), x2 (l), 2b (l), 2f (m)
2b	7.27	0	7.42	z2′ (l), z2 (m), x2 (s)
2e	7.23	d 8.1	7.20	2f (l), 4b (s)
2f	7.34	bd 8.1	7.57	2e (l), w2 (m), z2 (m), z2′ (s)
w3	7.58	d 10.4	n/a	w2 (m), 1b (m), 3f (l), 4b (m)
x3	5.38	d 10.3	n/a	3b (l)
3b	6.83	bs	n/a	x3 (l)
3d	6.77	bs	n/a	
3f	6.82	bs	n/a	1b (l), w2 (s), w3 (m), 4b (s), w4 (s)
w4	7.54	d 8.1	8.43	4d (l), 3f (m)
x4	5.70	d 7.9	5.71	w5 (l), 4b (m), 4f (m)
4b	5.63	d 12.7+	5.63	w4 (m), 2e (s), x4 (m)
4f	5.18	bs	5.21	x4 (m), w5 (m), 5b (s), x5 (m)
w5	8.55	d 5.8+	8.14	5f (m), 4x (l), 4f (m)
x5	4.39	d 5.6	4.50	4f (m), 5b (l), x6 (l), z6 (s), 6b (m), w7 (m)
5b	7.14	d 2.1+	7.19	4f (m), x5 (l), x6 (m), 6b (m), w7 (l)
5f	6.82	d 2.1+	6.78	x4 (s), w5 (m)
w6	6.71	d 11.6	6.50	6f (m)
x6	4.09	d 11.6	4.22	4f (s), 5b (s), x5 (l), 6b (m), z6 (l), w7 (l)
z6	5.12	bs	5.13	x5 (s), 5b (s), x6 (l), w7 (l), 6b (l)
z6OH	5.86	d 6.4+	n/a	z6 (s), w6 (s), 6f (s)
6b	7.77	d 1.68+	7.87	4f (s)
6e	7.29	0	7.28	4f (s), 6f (l)
6f	7.47	d 8.3	7.48	w6 (m), 6e (l), z6OH (m)
w7	8.32	d 6.1	8.39	x5 (m), 5b (l), x6 (m), z6 (m), 6b (m), 7f (s)
x7	4.47	d 6.1	4.50	
7d	6.45	d 2.1	6.44	
7f	6.30	d 2.1	6.30	w7 (s)
G1	5.33	d 7.6	5.35	G3 (l), G5 (l)
G2	3.60	0	3.62	G4 (l), V1 (l), V2/2′ (s)
G3	3.54	0		G1 (l), G5 (l)
G4	3.31	0		G2 (l)
G5	3.35	0		G1 (l), G3 (l), G6′ (m)
G6	3.55	0		G5 (l), G6′
G6′	3.70	0		G5 (l), G6 (l)
V1	5.29	d 3.8	5.30	V2/V2′ (m), G2 (l)
V2	1.73	d 12.8	1.98	V1 (m), V2′ (l), V3Me (m)
V2′	1.93	bd	–	
V3Me	1.34	s	1.41	V2 (m), V2- (s), V4 (l), V5 (l)
V4	3.21	d xx		V3Me (l), V5 (m), V6Me (m)
V4OH	3.42	d xx		
V5	4.65	q 6.6	4.70	V3Me (l), V4 (l), V6Me (l)
V6me	1.09	d 6.6	1.10	V4 (m) V5 (l), 2e (s)

[a] Data obtained at 335 K and 250 MHz in DMSO-d_6. Shifts measured relative to internal DMSO signal at 2.50 ppm. [b] Data obtained at 250 MHz and 335 K. + Denotes values obtained at 400 MHz and 320 K. s = singlet, d = doublet, m = multiplet, b = broad resonance, O = overlapping resonances preclude measurement. [c] Data from ref 26. [d] Data obtained from 2D-NOESY spectrum at 400 MHz and 320 K in DMSO-d_6. Magnitude of negative NOE judged by the intensity of the corresponding crosspeak (s = small, m = medium, l = large).

A sample of 10–20 nmol of analyte was dispersed in 2 μL of matrix. For positive ion spectra the matrix commonly employed was thioglycerol with 1 μL of 1 N trichloroacetic acid added to the matrix. In the negative ion mode, glycerol with 5% (v/v) 15c5 crown ether, was used as matrix. The sample was bombarded with xenon atoms accelerated to 6–9 keV, and the secondary ions accelerated to 8 keV for analysis by the electrostatic and magnetic

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sectors. Spectra were calibrated by reference to the oligomeric matrix ions.

UV spectra were recorded on a Uvikon 810P double beam spectrophotometer using 1 cm path length cells. Buffered solutions commonly contained 10^{-5} M antibiotic.

Determination of the sulfur content of the antibiotic was performed at Pfizer Central Research.

Proton NMR spectra were recorded with Brucker WM-250 or AM-400 spectrometers equipped with Aspect 2000 computers. Before analysis, the samples were dissolved in DMSO-d_6, which was then removed in vacuo, and the sample was dried under reduced pressure over phosphorus pentoxide. Spectra were then recorded with these samples dissolved in fresh DMSO-d_6 (99.96 atom %, Aldrich Chemical Co.). Spectral widths of 2500 or 4000 Hz were used (at 250 and 400 MHz, respectively) with quadrature detection employed throughout.

Two-dimensional NMR spectra were acquired in the phase-sensitive mode using quadrature detection in f_2. The spectra consisted of 400-512 increments of t_1. Each t_2 data set was composed of 2K data points, and was the result of 32 transients for the COSY spectra, and 64 transients for the NOESY and CAMELSPIN spectra. The sum of acquisition time and recycle delay was typically 2 s, approximately twice the T_1 value for molecules of this size. NOESY spectra were recorded with a mixing time of 400 ms, and a spin lock field strength of 4 kHz applied for 200 ms was used for the CAMELSPIN experiment. 2-D data sets were multiplied by Lorentzian-Gaussian functions and zero filled to 1K data points in f_1 prior to transformation.

One-dimensional saturation transfer experiments were performed using a recycle delay of 2 s and a preirradiation time of 1.5 s. Sets of 32 transients were acquired with the decoupler alternatively off resonance, and then on resonance with the residual water peak in the DMSO. The resulting FIDs were subtracted and then Fourier transformed.

^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer operating at 100.62 MHz. Waltz-16 was employed for broad-band proton decoupling, with a recycle delay of 2.5 s during which time the decoupler was left on for NOE enhancement. The data was collected over a spectral width of 21 000 Hz and 32K data points. A 2-Hz line broadening was applied prior to transformation.

Type V limpet arylsulfatase enzyme was obtained from Sigma Chemical Co. Typically, 100 mg of antibiotic in 10 mL of 0.2 M sodium acetate buffer, pH 5.0, was incubated with 2 mg (25 units) of enzyme at 36 °C for 48 h. After lyophilization, the mixture was purified by reverse-phase HPLC using a Waters C-18 μ-Bondapak column (9 × 30 mm) and eluted with acetonitrile-0.25 M aqueous ammonium acetate pH 7.8 (1:4) at a flow rate of 3 mL/min.

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Asymmetric Synthesis of Arylglycines

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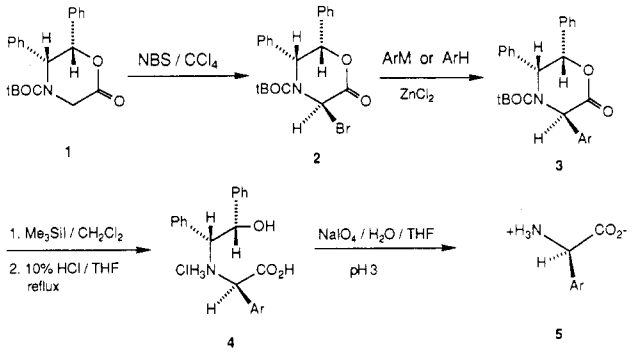
The asymmetric synthesis of several arylglycines are reported. The methodology deployed involves either cuprate or Friedel-Crafts couplings to chiral bromoglycinates. The % ee's range from 82 to 94%. Both an oxidative and reductive protocol are employed to unmask the oxazinone chiral auxilliary providing the free α-amino acids.

The arylglycines constitute an important class of non-proteinogenic α-amino acids.[1] For example, *p*-hydroxyphenylglycine is a side-chain constituent of the β-lactam antibiotic amoxicillin.[2] Numerous other, highly functionalized arylglycines are found in numerous peptide and glycopeptide antibiotics such as the vancomycins.[3] The apparent simplicity of the arylglycine structure is complicated by the ease of base-catalyzed racemization of the α-methine proton, rendering these substances challenging synthetic targets to obtain in optically pure form.

Numerous approaches to the synthesis of arylglycines have recently appeared, including: enzymatic resolution of racemic Strecker-derived amides and esters;[4] Friedel-Crafts additions to chiral cationic glycine equivalents;[5] asymmetric Strecker reactions;[6] electrophilic amination of chiral enolates;[7] and nucleophilic ring opening of aryl epoxy

Scheme I



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