Structure of the Pseudoaglycon of A35512B

Ann H. Hunt

Contribution from the Lilly Research Laboratories, Indianapolis, Indiana 46285.
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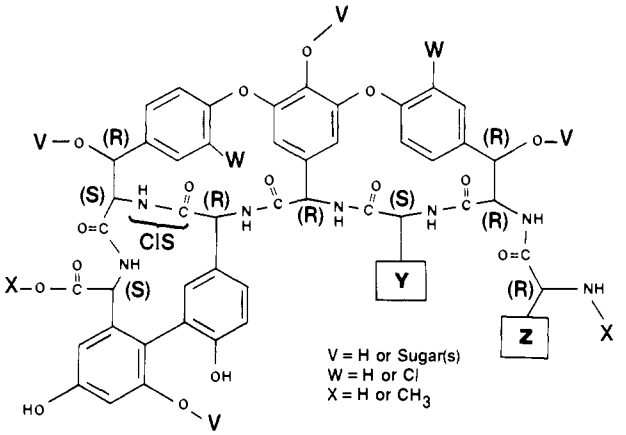
Abstract: The structure of the pseudoaglycon of the glycopeptide antibiotic A35512B is described. The structure elucidation is based on ^{1}H NMR studies of A35512B and A35512B ψ-aglycon in dimethyl sulfoxide solution; negative nuclear Overhauser effects and analogies between A35512B and other glycopeptides have led to the structural proposals. The A35512B aglycon differs from the aglycon of ristocetin A only in the structure of the diphenyl ether amino acid. The amino sugar of the ψ-aglycon is linked to the peptide core through an aliphatic hydroxyl group, and intact A35512B contains neutral sugars attached to the ψ-aglycon at three phenolic sites.

A35512B is a glycopeptide antibiotic produced by *Streptomyces candidus*.[1,2] The antibiotics in this class act by binding to cell-wall mucopeptides containing the terminal dipeptide D-alanyl-D-alanine; this interaction inhibits cross-linking of the bacterial cell wall and leads to eventual lysis of the organism.[3] Vancomycin,[4] avoparcin,[5] ristocetin,[6] and A35512B[7] have all been shown to form complexes with the mucopeptide analogue acetyl-D-Ala-D-Ala; such binding to a common substrate suggests that the glycopeptides have binding sites with similar or identical structural features, including their stereochemical details. Indeed, general structure **1** is shared by



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all the glycopeptide antibiotics for which structures have been reported.[8-13] The glycopeptides may be divided into three subclasses, based on the structure of the groups Y and Z in **1**.

(1) Y,Z = Aliphatic. The only reported example of this glycopeptide type is vancomycin, where Y and Z are the side chains of asparagine and *N*-methylleucine, respectively.[8-10] A crystalline degradation product from vancomycin, CDP-I, is the only glycopeptide-like molecule for which a structure has been determined by X-ray crystallography;[9] CDP-I differs from the general structure **1** in that it contains an isoaspartyl residue rather than aspartate, but Harris and Harris have demonstrated that an aspartyl → isoaspartyl rearrangement occurs during hydrolysis of vancomycin to form CDP-I.[8] No asymmetric centers are involved in this rearrangement, however, and the absolute configurations deduced for CDP-I (from the crystallographic results and the known configurations of L-aspartic acid and *N*-methyl-D-leucine[14]) should be unchanged for vancomycin; these configurations are indicated in **1**. An unusual structural feature in the CDP-I peptide backbone is the existence of a cis amide linkage,[9] as shown in **1**; Williamson and Williams have demonstrated the presence of the same cis bond in vancomycin on the basis of NMR evidence alone, without reference to the known structure of CDP-I.[10]

(2) Y, Z = Aromatic. Two glycopeptide families of this type have been reported: actinoidin A and B [where Y and Z are the side chains of L-phenylalanine and a (4-hydroxyphenyl)-D-glycine][3,11] and α- and β-avoparcin [where Y and Z are the side chains of a (4-hydroxyphenyl)glycine and *N*-methyl(4-hydroxyphenyl)glycine rhamnoside].[12] Ellestad and co-workers have used NMR evidence to argue that avoparcin also contains the cis amide linkage shown in **1** and that avoparcin and vancomycin have the same relative stereochemistry at all positions (except possibly for the N-terminal moiety, which remains uncertain in avoparcin).[12c]

(3) Y,Z = a Diphenyl Ether. The glycopeptide antibiotic which has been studied the most extensively (after vancomycin) is ristocetin A (ristomycin A).[13,15] The ristocetins A and B differ only in the nature of the sugars attached to **1**. In glycopeptides of the ristocetin type, the groups Y and Z in structure **1** are linked together by an ether bond; in ristocetin, the substituent has the structure **2**.[15,16] Kalman and Williams have argued from NMR

(1) (a) Michel, K. H.; Shah, R. M.; Hamill, R. L. *J. Antibiot.* **1980**, *33*, 1397-1406. (b) Debono, M.; Molloy, R. M.; Barnhart, M.; Dorman, D. E. *J. Antibiot.* **1980**, *33*, 1407-1416.

(2) Later cell-wall studies have shown the producing organism to be a non-streptomycete; M. Hoehn, personal communication.

(3) For a recent review, see: Williams, D. H.; Rajananda, V.; Williamson, M. P.; Bojesen, G. *Top. Antibiot. Chem.* **1980**, *5*, 119-139.

(4) (a) Perkins, H. R. *Biochem. J.* **1969**, *111*, 195-205. (b) Brown, J. P.; Feeney, J.; Burgen, A. S. V. *Mol. Pharmacol.* **1975**, *11*, 119-125. (c) Convert, O.; Bongini, A.; Feeney, J. *J. Chem. Soc., Perkin Trans. 2* **1980**, 1262-1270. (d) Williams, D. H.; Butcher, D. W. *J. Am. Chem. Soc.* **1981**, *103*, 5697-5700.

(5) Fesik, S. W.; Armitage, I. M.; Ellestad, G. A.; McGahren, W. J. "Book of Abstracts", 184th National Meeting of the American Chemical Society, Kansas City, MO, Sept 1982; American Chemical Society: Washington, DC, 1982; MEDI 66.

(6) (a) Nieto, M.; Perkins, H. R. *Biochem. J.* **1971**, *124*, 845-852. (b) Kalman, J. R.; Williams, D. H. *J. Am. Chem. Soc.* **1980**, *102*, 906-912.

(7) Hunt, A. H.; Vernon, P. D. *J. Antibiot.* **1981**, *34*, 469-471.

(8) Harris, C. M.; Harris, T. M. *J. Am. Chem. Soc.* **1982**, *104*, 4293-4295.

(9) Sheldrick, G. M.; Jones, P. G.; Kennard, O.; Williams, D. H.; Smith, G. A. *Nature (London)* **1978**, *271*, 223-225.

(10) Williamson, M. P.; Williams, D. H. *J. Am. Chem. Soc.* **1981**, *103*, 6580-6585.

(11) Sztaricskai, F.; Harris, C. M.; Harris, T. M. *Tetrahedron Lett.* **1979**, 2861-2864.

(12) (a) McGahren, W. J.; Martin, J. H.; Morton, G. O.; Hargreaves, R. T.; Leese, R. A.; Lovell, F. M.; Ellestad, G. A. *J. Am. Chem. Soc.* **1979**, *101*, 2237-2239. (b) McGahren, W. J.; Martin, J. H.; Morton, G. O.; Hargreaves, R. T.; Leese, R. A.; Lovell, F. M.; Ellestad, G. A.; O'Brien, E.; Holker, J. S. E. *J. Am. Chem. Soc.* **1980**, *102*, 1671-1684. (c) Ellestad, G. A.; Leese, R. A.; Morton, G. O.; Barbatschi, F.; Gore, W. E.; McGahren, W. J.; Armitage, I. M. *J. Am. Chem. Soc.* **1981**, *103*, 6522-6524.

(13) (a) Kalman, J. R.; Williams, D. H. *J. Am. Chem. Soc.* **1980**, *102*, 897-905. (b) Williams, D. H.; Rajananda, V.; Bojesen, G.; Williamson, M. P. *J. Chem. Soc., Chem. Commun.* **1979**, 906-908. (c) Harris, C. M.; Harris, T. M. *J. Am. Chem. Soc.* **1982**, *104*, 363-365.

(14) Marshall, F. J. *J. Med. Chem.* **1965**, *8*, 18-22.

(15) (a) Williams, D. H.; Rjananda, V.; Kalman, J. R. *J. Chem. Soc., Perkin Trans. 1* **1979**, 787-792. (b) Harris, C. M.; Kibby, J. J.; Fehlner, J. R.; Raabe, A. B.; Barber, T. A.; Harris, T. M. *J. Am. Chem. Soc.* **1979**, *101*, 437-445. (c) Sztaricskai, F.; Harris, C. M.; Harris, T. M. *J. Antibiot.* **1979**, *32*, 446-452.

(16) Harris, C. M.; Harris, T. M. *Tetrahedron Lett.* **1979**, 3905-3908.

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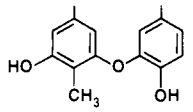

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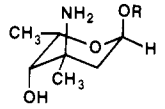
comparisons that ristocetin A has the peptide backbone shown in structure **1**, including the cis peptide bond, and that ristocetin A and vancomycin have the same absolute stereochemistry at eight of the nine asymmetric centers (excluding the N-terminal site).[13a] Harris and Harris have shown that the N-terminal asymmetric site in ristocetin has an *R* configuration, in agreement with structure **1**, based on circular dichroism studies of a ristocetin A degradation product.[13c]

Antibiotic A35512B is a glycopeptide of the ristocetin type; it contains four neutral sugars (rhamnose, glucose, fucose, and mannose),[1b] the amino sugar 3-epi-L-vancosamine (**3**),[17] and three



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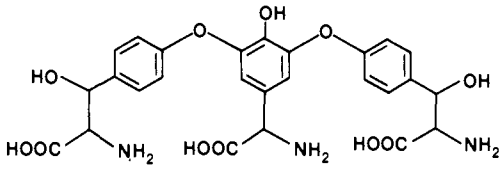
complex amino acids. Debono et al. have characterized the products of chemical degradation of A35512B; they have proposed that the amino acid constituents of the glycopeptide are dechlorovancomycinic acid (**4**), actinoidinic acid (**5**), and a novel



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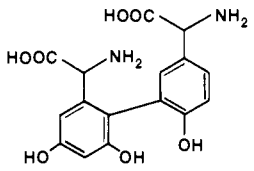

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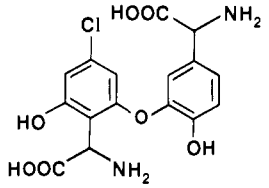
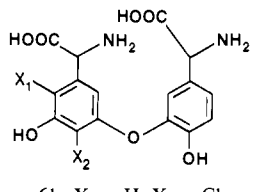
chlorinated bis(amino acid) having a diphenyl ether unit similar to **2**.[1b] Two possible structures for the diphenyl ether amino acid were proposed (**6a,b**), while structures such as **6c** were thought



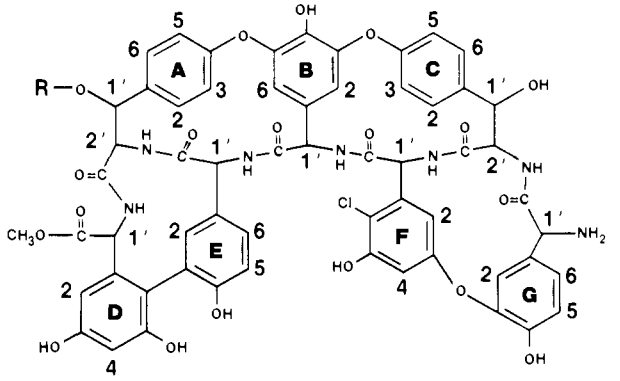
6a



6b, X_1 = H; X_2 = Cl
6c, X_1 = Cl; X_2 = H

to be inconsistent with some of the earlier NMR data. The NMR studies described in the present paper show that **6a** is not present in A35512B, but the results described here are consistent with both **6b** and **6c**. Harris and Harris, however, have recently offered evidence for **6c** as the correct structure, based on synthesis of a **6c** degradation product by Ullmann condensation.[18]

Under appropriate hydrolysis conditions, the neutral sugars may be removed from a typical glycopeptide, leaving only an amino sugar attched to the peptide core, thus producing the pseudoaglycon ("ψ-aglycon"). The ψ-aglycon of A35512B has the structure **7**; it differs from the ψ-aglycon of ristocetin A only in the identity



R = 3-*epi*-L-vancosamine

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of the amino sugar attached at A-1′ and in the structure of the diphenyl ether substituent corresponding to **2**. Three sugar moieties are attached to **7** to constitute the complete antibiotic, A35512B; the sugars are attached through phenolic linkages at B4, D-5, and F-5. Proton NMR evidence which supports these structural conclusions is described in this paper.

Experimental Section

The ψ-aglycon was prepared by acid hydrolysis of A35512B (5% HCl in refluxing methanol),[13c] followed by precipitation from aqueous acetonitrile as the dihydrochloride salt. Solutions were prepared in either Me_2SO-d_6 or Me_2SO-d_6 containing D_2O; for the latter solvent the ψ-aglycon was lyophilized 3 times from D_2O before use. Spectra were recorded at temperatures ranging from ambient (~23 °C) up to ~50 °C, with a Bruker WH 360 spectrometer in the Fourier transform mode. Difference NOE (nuclear Overhauser effect) spectra were obtained by subtracting free induction decays accumulated with the decoupler off resonance from similar accumulations with particular resonances irradiated, followed by Fourier transformation of the difference signals. The procedure was not optimized for maximum NOE measurement; the usual irradiation period was 2.0 s, followed by a preaccumulation delay of 0.03 s. Typical decoupler power settings were 16-dB attentuation (0.2-W level) for normal decoupling and 22–26 dB for difference NOE measurements; when overlap of the irradiating frequency to neighboring resonances was suspected, the decoupling was repeated at a lower power level. NOE observations which disappear at lower power levels were not considered in the structure elucidation reported here. The problem of large NOEs arising by spin migration due to the exchange of –OH and –NH protons[10] has been addressed by doing full decoupling studies in both Me_2SO and Me_2SO/D_2O; NOEs between nonexchangeable protons which are significantly reduced by the addition of D_2O have been discarded. The NOE percentages reported in the text were computed from decreases in peak height (negative NOEs); these values have only qualitative significance, since they were measured under nonoptimized conditions.

Plasma desorption mass spectroscopy results have been obtained for A35512B and for the ψ-aglycon by Professor R. D. Macfarlane (Texas A&M University). A35512B: calculated for $C_{90}H_{101}N_8O_{39}Cl$ = 1954.3; found = 1954 ± 2. A35512B ψ-aglycon: calculated for $C_{66}H_{61}N_8O_{21}Cl$ = 1337.7; found = 1338.5 ± 0.2.

Results

The 360-MHz ^1H NMR spectrum of the A35512B ψ-aglycon dihydrochloride in Me_2SO-d_6 is shown in Figure 1. Several pieces of structural information may be obtained from this spectrum: (1) The three-proton singlet at 3.70 ppm (which occurs in both the ψ-aglycon and the intact glycopeptide) indicates that A35512B, like ristocetin,[13a] has a methyl ester at its carboxy terminal. (2) The resonances of the amino sugar **3** (marked AS in Figure 1) are listed in Table I. The coupling of the anomeric proton (4.85 ppm) to either proton at position 2 is small, indicating that the α anomer **3** is present in the ψ-aglycon. (3) The amino acid structures **4**, **5**, and **6** contain six phenols, and the spectrum in Figure 1 shows six phenolic resonances (10.59, 10.17, 9.56, 9.43, 9.39, and 8.83 ppm) for the ψ-aglycon. The site of attachment

(17) Debono, M.; Molloy, R. M. *J. Org. Chem.* **1980**, *45*, 4685–4687.
(18) (a) Harris, C. M.; Harris, T. M. *Tetrahedron* **1983**, *39*, 1661–1666. (b) X-ray crystallographic evidence indicates that structure **6c** is correct; N. Jones, unpublished results.

Table I. NMR Parameters for 3 in the A35512B ψ-Aglycon Dihydrochloride

proton	chemical shift, δ	remarks
1	4.85	slightly broadened singlet
2a, 2e	1.99, 1.89	broadened AB quartet
3-CH_3	1.25	singlet
3-NH_3^+	7.77	broadened singlet
4	3.19	doublet, coupled to OH
4-OH	5.55	overlapped, coupled to 4
5	3.64	broadened quartet
5-CH_3	1.17	doublet

for amino sugar **3**, therefore, must be one of the aliphatic hydroxyl groups of amino acid **4**. The resonance of the remaining –OH overlaps a CH doublet at 5.94 ppm in Figure 1. All six of the phenolic resonances have been assigned by observation of negative NOEs produced at adjacent ring protons when the phenolic peak is irradiated; these assignments are included in Table II. The resonance at 9.43 pppm is the only one of the six for which no NOE is observed, indicating that this peak arises from the phenol of amino acid **4**, which has no adjacent protons. Assignment of the phenols on amino acids **5** and **6** will be discussed below. (4) The ψ-aglycon dihydrochloride spectrum contains two $-NH_3^+$ resonances (8.61 and 7.77 ppm), one of which arises from the amino sugar. The 8.61-ppm peak is from the amino terminal of the peptide core (irradiation of this resonance causes a broad singlet at 5.50 ppm to sharpen, while no coupling would be expected from the $-NH_3^+$ group of **3**).

Tetrasubstituted Aromatic Rings. The amino acids **4**, **5**, and **6** each contain an aromatic ring having two meta protons and four other substituents. Decoupling experiments were used to locate three pairs of meta-coupled proton resonances in the A35512B ψ-aglycon spectrum, and difference NOE experiments involving the phenolic peaks were used to distinguish between the rings, which are labeled B, D, and F to correspond to their positions in structure **7** (NOEs are represented by arrows from the irradiated

Table II. Proton NMR Assignments[a] for the A35512B Aglycon, Compared with Corresponding Assignments for Ristocetin A,[13a] Vancomycin,[19] and β-Avoparcin[12c]

	chemical shifts, δ (*J*, Hz)				
	A35512B ψ-aglycon·2HCl				
proton	Me_2SO	Me_2SO/D_2O	ristocetin A	vancomycin	β-avoparcin
A-NH	7.10 (11)		7.20 (12)	6.50 (12)	
A-2′	4.31 (11)	4.34	4.38 (12)	4.22 (12)	4.33 (12)
A-1′	5.10 (~0)	5.16	5.17 (~0)	5.13 (~0)	5.11
A-2	7.50 (8)	7.52	7.55 (8.2)	7.87	7.73
A-3	6.89 (8)	6.94	6.88 (8, 2)	Cl	Cl
A-5	7.15	7.16	7.21 (8, 2)	7.28 (8)	
A-6	7.41 (8)	7.46	7.41 (8)	7.48 (8)	
B-NH	7.07 (8)		7.66 (8.2)	8.14 (8)	
B-1′	5.56 (8)	5.59	5.65 (8.2)	5.71 (8)	5.65 (7.5)
B-2	5.84	5.90	5.85	5.63	5.75
B-4 (OH)	9.43				
B-6	5.18	5.22	5.38	5.21	5.25
C-NH	7.74 (8)		7.21 (9)	8.00 (9)	
C-2′	5.10	5.19	5.09 (9, 5)	4.86 (4)	5.05 (4.4)
C-1′	5.12	5.14	5.19 (5)	5.15 (4)	5.40 (4.4)
C-1′ (OH)	5.94			5.85	
C-2	7.96 (8)[b]	7.92	7.86 (8)	7.57 (8)	7.61 (9, 1.5)
C-3	7.07	7.13	7.25 (8)	7.20 (8)	
C-5	~7.14	7.24	7.29 (8)	Cl	
C-6	~7.10	7.11	7.12 (8, 2)	7.42	
D-NH	9.06		9.26 (5)	8.39 (7)	
D-1′	4.39 (5.5)	4.43	4.55 (5)	4.50 (7)	4.39 (5)
D-2	6.06	6.10	6.32 (2)	6.30 (2)	6.29 (2.2)
D-3 (OH)	9.56				
D-4	6.44	6.47	6.85 (2)	6.44 (2)	6.42 (2.2)
D-5 (OH)	8.83				
$COOCH_3$	3.70	3.72			
E-NH	8.77		8.58 (6.5)	8.43 (6)	
E-1′	4.59 (5)	4.62	4.73 (6.5)	4.50 (6)	4.63 (4.5)
E-2	7.26	7.29	7.26 (2)	7.19	
E-4 (OH)	9.35				
E-5	6.72 (8)	6.76	6.77 (8)	6.73 (8)	6.71 (8)
E-6	6.74 (8)	6.80	6.84 (8, 2)	6.78 (8, 1)	6.88 (8, 2)
F-NH	7.60 (10.5)		7.41 (10)	6.59 (7)	
F-1′	5.94 (10.5)	6.00	5.25 (10)	4.38 (7)	5.32 (10)
F-2	6.54	6.57	6.42		
F-4	6.66	6.69	CH_3		
F-5 (OH)	10.59				
F-6	Cl	Cl	6.45		
G-NH_3^+	8.61				
G-1′	5.50	5.45	4.83		4.08
G-2	6.63	6.69	6.59		
G-4 (OH)	10.17				
G-5	7.14	7.15	7.03 (8)		
G-6	7.26	7.24	7.18 (8.2)		

[a] 360-MHz spectra recorded at ~23 °C. Chemical shifts are listed vs. internal Me_4Si. The slight downfield shift of many of the resonances in the Me_2SO/D_2O column may reflect an interaction of D_2O with the reference peak, since the chemical shift of Me_2SO in these spectra is 2.57 ppm (for the spectra in the Me_2SO column, Me_2SO = 2.50 ppm). [b] Resonances on ring C are numbered so that the most downfield peak is C-2.

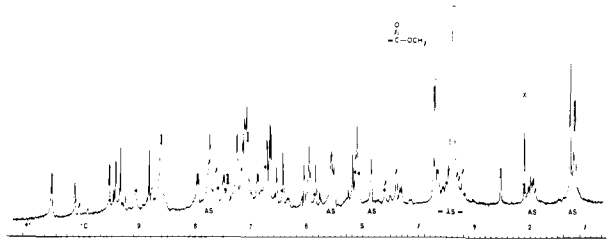
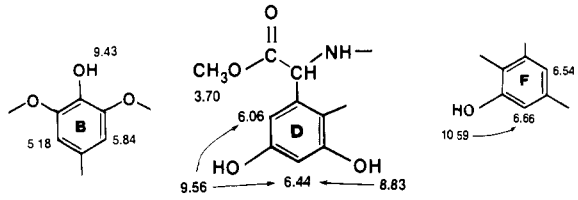
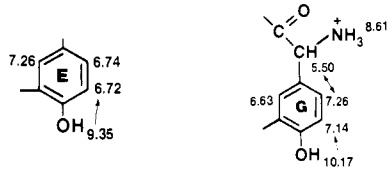


Figure 1. 360-MHz ^{1}H NMR spectrum of A35512B ψ-aglycon dihydrochloride (in Me_2SO-d_6, ambient temperature). Peaks marked AS arise from the amino sugar. The peak marked X is from residual acetonitrile; it disappears after lyophilization.

site to the NOE site). Ring D is attached to the carboxy terminal of the peptide chain; the results shown for ring F could be consistent with any of the three structures for **6**.



Trisubstituted Aromatic Rings. Amino acids **5** and **6** each contain an aromatic ring with protons at positions 2, 5, and 6, a phenol at position 4, and other substituents at positions 1 and 3. Decoupling and NOE experiments were used to locate two such sets of resonances, those arising from rings E and G in structure



7. The two rings can be distinguished on the basis of chemical shiift comparisons with the corresponding portions of ristocetin (see Table II), as well as by NOE patterns involving ring E (which will be discussed later). Irradiation of the amino-terminal CH (5.50 ppm) causes a negative NOE at the 7.26-ppm doublet on ring G (the doublet structure is seen most clearly in a difference NOE experiment); this NOE is observed in both directions after removal of exchangeable protons by the addition of D_2O.

Disubstituted Aromatic Rings. The amino acid **4** contains two identical para-disubstituted rings; the eight proton resonances arising from these rings have been identified by decoupling experiments carried out at a variety of temperatures and in both Me_2SO and Me_2SO/D_2O. Amino acid **4** is present in both A35512B and ristocetin,[13,15] and the rings A and C can be dis-

tinguished on the basis of chemical shift comparisons presented in Table II. Even more useful distinctions between rings A and C can be made on the basis of NOE observations; these will be discussed below.

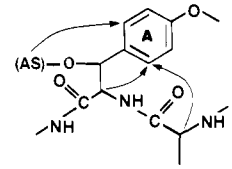
Construction of the General Structure 1. Williams and coworkers have shown that the regions of vancomycin[4d] and ristocetin A[6b] which take part in complex formation with Ac-D-Ala-D-Ala are contained in the common structure **1**. Many of the protons in the binding site region are held in close proximity, and extensive "nests" of NOEs involving hydrogens or substituents on rings A, D, and E have been observed for both vancomycin[10] and ristocetin A.[13a] An analogous NOE pattern occurs for A35512B, involving

Table III. NOEs[a] for A35512B ψ-Aglycon Dihydrochloride, Ristocetin A,[13a] and Vancomycin[10]

proton(s) irradiated	resonances reduced, %		
	A35512B ψ	ristocetin A	vancomycin
A-2′	A-1′ (21)[b]	A-1′ (10)	A-1′ (32)
A-2′	A-2 (25)	A-2 (20)	A-2 (40)
A-2′	D-NH (46)		D-NH (43)
A-2′	E-1 (57)	E-1′ (60)	E-1′ (35)
A-2′	E-2 (27)	E-2 (25)	E-2 (48)
D-NH	A-2′ (30)		A-2′ (11)
D-NH	A-2 (13)		A-2 (8)
D-NH	E-1′ (28)		E-1′ (2)
D-NH	E-2 (27)		E-2 (6)
E-1′	A-2′ (50)	A-2′ (40)	A-2′ (56)
E-1′	A-2 (19)		A-2 (33)
E-1′	B-6 (26)		B-6 (30)
E-1′	D-NH (31)		D-NH (38)
E-1′	E-2 (27)	E-2 (30)	E-2 (56)
E-2	A-2′ (40)		A-2′ (47)[b]
E-2	D-NH (38)		
E-2	E-1′ (36)	E-1′ (25)	E-1′ (24)[b]
E-NH	B-1′ (31)	B-1′ (25)	B-1′ (53)
E-NH	E-6 (19)		
B-1′	E-NH (33)		E-NH (49)
B-1′	B-2 (26)		B-2 (39)
B-1′	B-6 (18)		B-6 (17)
AS no. 1	A-6 (34)[b]		
C-NH/F-NH	C-2 (39)	C-2 (30)[c]	
C-NH/F-NH	F-2 (55)	F-2 (20)[d]	
C-NH/F-NH	G-2 (34)	G-2 (12)[d]	
G-1′	G-6 (15)[b]	G-6 (15)	

[a] Spectra recorded in Me_2SO at 23 °C for A35512B ψ-aglycon, variable temperatures for ristocetin A, and 35 °C for vancomycin. [b] Measured in Me_2SO/D_2O. [c] C-NH. [d] F-NH.

the following resonances in particular: A-2′, -1′, and -2; D-NH; E-1′ and -2. These and other NOE observations which are important in assigning the A35512B resonances are collected in Table III, where they are compared with similar results from vancomycin and ristocetin. (NOEs from A-1′ have not been listed, since this resonance is highly overlapped; a number of difference NOEs to A-1′ have been observed, however.) The amino acid **4** is symmetrical, but rings A and C may be distinguished not only by chemical shift comparisons but also by the NOEs to proton A-2 from several nearby hydrogens, including A-2′ and E-1′ in particular. The A-6 doublet (7.41 ppm) is reduced in intensity when either the anomeric proton (4.85 ppm) or the 2 axial proton (1.99 ppm) of amino sugar **3** is irradiated, indicating A-1′ as the site of attachment for **3**.



A35512B is analogous to ristocetin and avoparcin in this respect; both of these glycopeptides have ristosamine attached to their peptide cores through the A-1′ hydroxyl group.[12c,13a]

The coupling patterns in Table II and the NOE patterns in Table III allow direct assignment of the 1′-NH pairs attached to rings B, D, E, and G, thus leaving the 1′-NH pair at F and the 2′–1′-NH group at C to be assigned by elimination; the validity of using such chemical shift and NOE comparisons to assign structures in closely related molecules has been examined carefully and demonstrated by Williamson and Williams.[10] The cis peptide bond in **1** has been shown to be required by the intense NOE between A-2′ and E-1′ in vancomycin (NOE ~ 56%, E-1′ → A-2′; half-time for maximum NOE buildup = 0.14 s),[10] granted that the general connectivity is as shown in **1** and that the dihedral angle between the A-2′ and A-NH protons is ~180° ($J_{A\text{-}2'\text{-}NH}$

= 12 Hz for vancomycin[19]). The presence of the same cis bond in the other glycopeptides is required by their NMR data as well: ristocetin A[13a] (NOE E-1′ → A-2′ = 40%; A-2′ → E-1′ = 60%; J = ~12 Hz); β-avoparcin[12c] (NOE ~50%; J = 12 Hz); A35512B ψ-aglycon (E-1′ → A-2′ = ~50%; A-2′ → E-1′ = ~57%; J = 11 Hz).

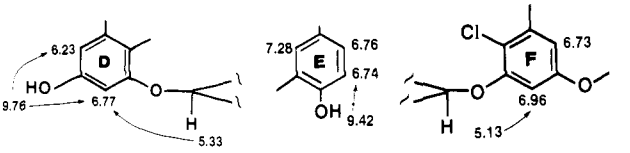
Williamson and Williams have shown that the correct relative stereochemistry can be deduced for vancomycin, on the basis of NMR results, for five of the seven "unknown" asymmetric centers (Y and Z in **1** are attached to sites having known configurations for vancomycin): the carbons bearing the protons A-1′, A-2′, B-1′, D-1′, and E-1′.[10] The similar nests of NOEs for ristocetin and vancomycin (as suggested in Table III) and the similarities in chemical shiits and coupling constants for avoparcin, ristocetin, and vancomycin (as illustrated in Table II) have been taken as indications that these glycopeptides share the same relative[12c] configurations at their chiral centers, or the same absolute[13a] stereochemistry when their common ability to form complexes with Ac-D-Ala-D-Ala is considered. The fact that A35512B binds to Ac-D-Ala-D-Ala with an affinity equal to or even greater than that of vancomycin for the peptide[7] strongly suggests that the two antibiotics have the same absolute structure (shown in **1**); comparison of the A35512B ψ-aglycon data in Tables II and III with data from the other glycopeptides supports this contention for the centers which carry the protons A-1′, A-2′, B-1′, D-1′, and E-1′ (*R*, *S*, *R*, *S*, and *R*).[9] Furthermore, the absolute configurations of the sites in A35512B where Y and Z are attached in **1** have been determined independently by Harris and Harris (by hydrolysis and reduction of the amino acids to cyclohexylglycines): *S* for the site attached to Y (and F-1′) and *R* for the center linked to Z (and G-1′).[18a]

The two remaining stereochemical centers in the A35512B aglycon (the carbons at C-1′ and C-2′) would be expected to have the same configurations (*R*, *R*) as in vancomycin and the other glycopeptides; these centers are near the proposed carboxylate binding site in the Ac-D-Ala-D-Ala complexes with both vancomycin[4d] and ristocetin A.[6b] It is difficult to examine this suggestion with the data in Tables II and III because the resonances of interest are highly overlapped in the ψ-aglycon; in the intact antibiotic, however, the resonances are more spread out. When A35512B is examined at 50 °C in Me_2SO plus a trace addition of TFA-d_1, the C-1′/-2′/-NH protons have the following parameters: C-1′ (5.28 ppm, d, J = 4.5 Hz); C-2′ (5.06 ppm, dd, J = 4.5, 9 Hz); C-NH (7.32 ppm, d, J = 9 Hz). These coupling constants suggest that A35512B and the glycopeptides listed in Table II have similar structures in the C region; in addition, the C-2 and C-NH protons experience similar NOEs in ristocetin and A35512B, as listed in Table III. These comparison support the conclusion, based on the similarities in their complexation behavior, that A35512B and the other glycopeptides have identical absolute configurations at the C-1′ and C-2′ sites and thus that A355512B shares structure **1** with all the glycopeptides for which structures have been published.

Linkage of Y and Z to 1. The groups Y and Z for A35512B are the aromatic ether portion of one of the structures **6a**, **6b**, or **6c**, and the Z portion is the same for each of these—ring G—as has already been shown above. The large NOE experienced by proton F-2 when C-NH/F-NH is irradiated (~55%) requires that structure **6a** be ruled out, but it is consistent with either **6b** or **6c**. In structure **6b** (X_1 = H), NOEs might be expected between the protons at the F-1′ and F-6 positions, and such NOEs have been observed in both directions in ristocetin A (see **2**) by Kalman and Williams.[13a] In A35512B, however, no such NOE has been observed, which is consistent with structure **6c** as proposed by Harris and Harris.[18] The A35512B ψ-aglycon, therefore, has the complete structure **7**, with the stereochemical details as shown in **1**.

Sites of Attachment for Neutral Sugars. Although A35512B and ristocetin A are quite similar at the aglycon level, the intact antibiotics differ in both their neutral sugar content and in the sites where the sugars are joined to the peptide core. In ristocetin A there are two attachment sites: mannose is linked to the ψ-aglycon through the D-5 phenol, and a branched tetrasaccharide is attached through the phenol on ring B.[13a,b] In A35512B, the four neutral sugars (rhamnose, glucose, fucose, and mannose)[1b] are attached at three sites;[20] these sites are the aglycon phenols at B-4, D-5, and F-5. The assignment of these phenols as the locations for carbohydrate attachment to the ψ-aglycon is based on two kinds of evidence from the NMR spectra: (1) observations of nuclear Overhauser effects involving either unsubstituted phenolic OHs or the anomeric protons of sugars in the intact antibiotic and (2) comparisons of chemical shifts for aromatic protons in the ψ-aglycon and in intact A35512B.

The resonances of the aglycon in the spectra of the intact factor A35512B have been assigned by the same procedures described for the ψ-aglycon; results for three of the rings are shown.



For ring E (phenol unsubstituted), the chemical shifts for all three protons are virtually unchanged in A35512B in comparison with the ψ-aglycon, while for rings D and F, large and similar shifts are observed for the protons which are ortho (0.33 and 0.30 ppm downfield) and para (0.17 and 0.19 ppm downfield) to the sites where sugars are attached.

Two sugar sites and two free phenols have been accounted for; there should be one more of each. The third OH, however, is apparently susceptibile to exchange processes, since the third phenolic peak is missing from the spectra of A35512B. When the D-3 phenol (9.76 ppm) is irradiated, the G-5 resonance (7.06 ppm) appears to be diminished slightly, suggesting that perhaps the remaining OH is at G-4. Chemical shift comparisons for the ring G protons support this suggestion: G-5 (ortho to the potential carbohydrate attachment site) is shifted upfield by 0.08 ppm in A35512B, while G-2 and G-6 are both 0.12 ppm upfield in comparison to their positions in the ψ-aglycon. These shifts are smaller than those observed for rings D and F, and they are in the opposite direction from the shifts produced by sugar attachment to D and F, leading to the conclusion that the G-4 phenol is not the site for the third sugar connection. Therefore, the remaining site must be the B-4 OH.[21] The B-4 phenol is a sugar attachment site for all the glycopeptides for which sites have been reported: vancomycin,[9] ristocetin,[13a] avoparcin,[12b] and actinoidin.[11a] The carbohydrate attached at B-4 in these antibiotics is a disaccharide or larger in every case. If the A35512B structure is analogous to those of other members of this class in terms of the distribution of sugars, it is likely that B-4 is the disaccharide site in this antibiotic, as well.

Conclusion

Antibiotic A35512B is a glycopeptide with structural similarities to ristocetin A; the A355128B ψ-aglycon, **7**, differs from that of ristocetin A only in the identity of the amino sugar attached at A-1′ and in the identity and arrangement of substitutents on ring F. Three sugar moieties are attached to **7** to constitute the complete antibiotic; the sugars are attached through phenolic linkages at B-4, D-5, and F-5. A35512B differs from all previously

(19) Williams, D. H.; Kalman, J. R. *J. Am. Chem. Soc.* **1977**, *99*, 2768–2774.

(20) The disaccharide rhamnosylglucose has been isolated following partial acid hydrolysis of A35512B, indicating that the maximum number of sugar sites is three; unpublished results from D. Dorman, G. Maciak, M. A. Bogan, and T. K. Elzey.

(21) The presence of sugar residues on rings B and F of A35512B is supported by analysis of the products of the following sequence of reactions: methylation with CH_3I, hydrolysis, methylation with CD_3I, and oxidative cleavage; M. Debono, personal communication.

reported glycopeptide antibiotics by having a site of carbohydrate attachment on ring F.

Acknowledgment. The A35512B ψ-aglycon was prepared by Dr. E. J. Fornefeld, and all NMR spectra were recorded by T. K. Elzey. Dr. D. E. Dorman initiated the NMR studies on A35512B and has been a participant in many useful discussions concerning the work reported here. I thank Dr. C. M. Harris and Dr. T. M. Harris for access to ref 18a prior to publication.

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Communications to the Editor

Strategic Design of Organic Conductors. Structure of a Prototypical Molecule

James Y. Becker, Joel Bernstein,* Shmuel Bittner, Nurit Levi, and Sason S. Shaik*

The Organic Metals Group, Department of Chemistry Ben-Gurion University of the Negev Beer-Sheva 84120, Israel

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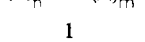
To design efficient organic conductors composed of donor (D) and acceptor (A) moieties one must achieve the following necessary conditions:[1,2] (a) enforce a segregated mode of stacking (··· DD···| |···AA···) in the solid state; (b) control the D:A stoichiometry; (c) encode ab initio the desired degree of electron transfer (ρ) into the molecular unit; (d) stabilize the delocalized state, ··· $D^{\rho+}D^{\rho+}$···| |···$A^{\rho-}A^{\rho-}$···, below the localized ones (e.g., ···$D^{+}D$··· ||···$A^{-}A$···); (e) permit and control the degree of interchain coupling. The systematic control of these conditions will allow the preparation of organic conductors with *predesigned properties*.

The archetypal molecular unit (**1**), which contains both donor



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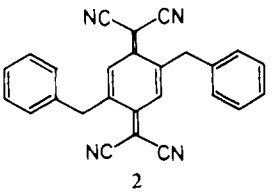
and acceptor moieties in a *prefixed* stoichiometric ratio (n:m), is potentially endowed with the necessary properties that can be manipulated to meet requirements b–d. Thus, for example, a gradual increase of the donor–acceptor abilities of the moieties in **1** will eventually result in an electron transfer whose degree (per D and A) is *predetermined* by the stoichiometric ratio.

The packing of this molecular unit in the solid state may be manipulated by changing the intramolecular connectivity (of D's and A's) in a manner that will eventually increase the propensity of **1** to aggregate in the desired segregated mode of stacking (see a above).

We report here the structure of the primitive member of such a class (D_2A) of molecular units, 2,5-dibenzyl-7,7,8,8-tetracyano-*p*-quinodimethane (DBTCNQ, **2**).[3] As a prototype



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molecule containing a weak donor (D = phenyl), this material was expected not to be a ground-state conductor ("organic metal") but rather to provide an archetypal D_2A framework for modifications of charge-transfer properties and crystal packing motifs. In both these regards, the crystal structure[4-6] does show promise

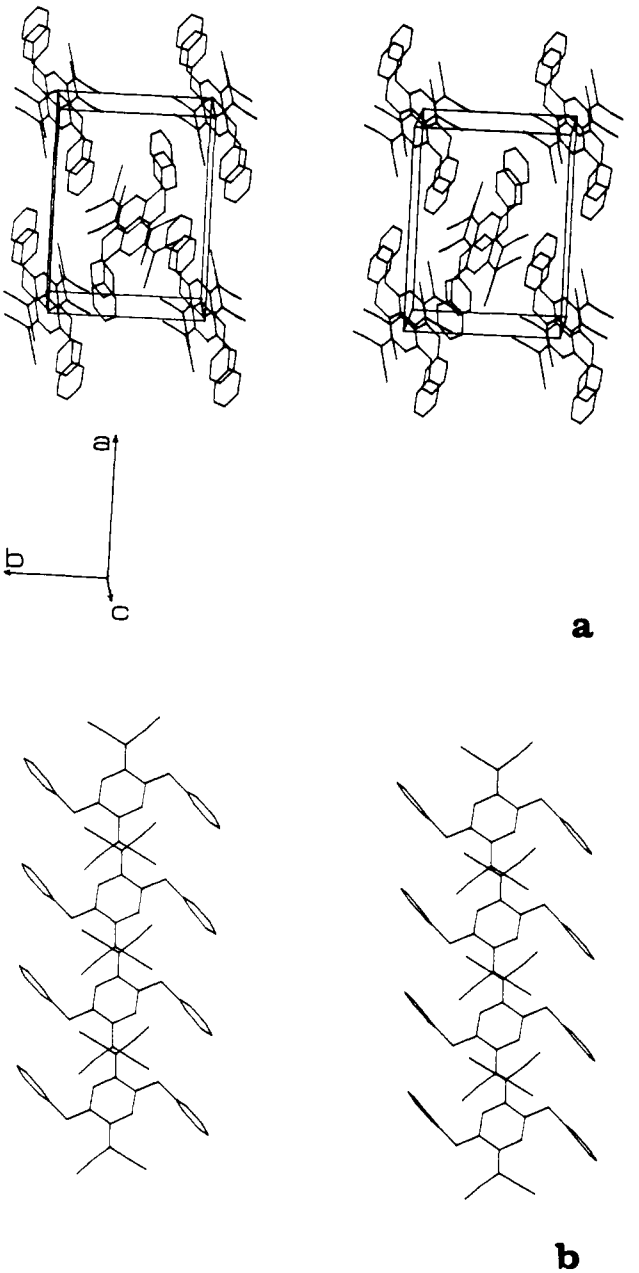

Figure 1. Stereoviews of (a) the unit cell viewed on the *ab* plane (focus on the back face of the cell) and (b) the ···AA··· stacking along the *c* axis which is the line of centers of TCNQ moieties.

(1) Shaik, S. S. *J. Am. Chem. Soc.* **1982**, *104*, 5328.

(2) (a) Perlstein, J. H. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 519. (b) Torrance, J. B. *Acc. Chem. Res.*, **1979**, *12*, 79.

(3) Synthesis will be reported separately in full. It involves dibenzylation of 2,5-bis(ethoxycarbonyl)cyclohexane-1,4-dione with $PhCH_2Br$, hydrolysis, and decarboxylation to yield the 2,5-dibenzyl-*p*-benzoquinone. Treatment of the quinone with $CH_2(CN)_2$ followed by dehydrobromination yields **2** as deep orange crystals (recrystallized from CH_3CN), mp 226–227 °C (Mettler hot stage). Redox potentionals from a voltammogram recorded vs. Ag wire at 100 mV/s in CH_3CN–0.1 M $LiClO_4$ gives two reversible couples: $E_{P_1}^{ox} = -0.09$ V, $E_{P_1}^{red} = -0.01$ V; $E_{P_2}^{ox} = -0.39$ V, $E_{P_2}^{red} = -0.32$ V. IR shows ν_{CN} 2213 cm^{-1}.

0002-7863/83/1505-4468$01.50/0