Structure of Antibiotic A41030A

Ann H. Hunt,* Douglas E. Dorman, Manuel Debono, and R. Michael Molloy

Lilly Research Laboratories, Indianapolis, Indiana 46285

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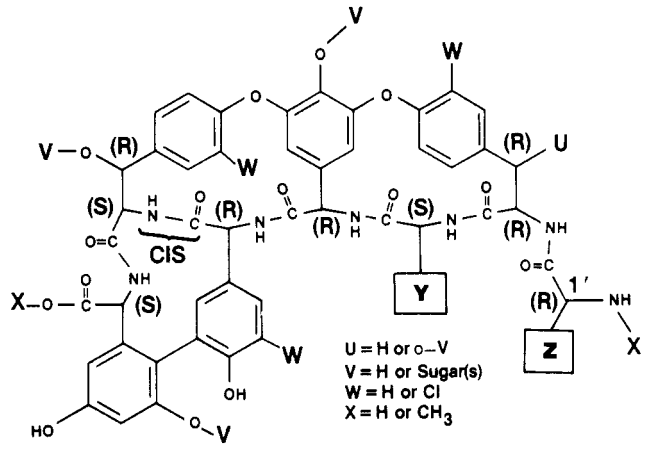
The structure of antibiotic A41030A is described. The structure elucidation is based on ^1H NMR studies of A41030A and of *t*-BOC-A41030A in dimethyl sulfoxide solution, analogies with the structures of vancomycin and related antibiotics, and degradation studies on A41030A. Antibiotic A41030A is a complex peptide structurally related to the aglycons of glycopeptide antibiotics; it most closely resembles the aglycons of teicoplanin and actaplanin. A41030A is the first such peptide that is chlorinated on the biphenyl unit.

The glycopeptide antibiotics are a class of complex molecules with distinct structural similarities; a typical glycopeptide contains a peptide core of aromatic amino acids (the aglycon), neutral sugars, and an amino sugar. The first glycopeptide to be discovered, vancomycin,[1] has been in clinical use for more than 20 years; one of the newest members of the class, teicoplanin,[2,3] is currently being evaluated for use in man. A goal of structural studies of these compounds is to define the minimum structural requirements for biological activity, and the structures of several of the glycopeptides have been described: vancomycin,[4,5] ristocetin (ristomycin),[6,7] avoparcin,[8] A35512B,[9] actaplanin,[10] and teicoplanin.[3] All of these antibiotics share the general structure **1**, including the absolute configurations where they are known.[4,11] (The groups Y and Z in **1** are variable sites.) Antibiotic A41030A is the first compound to be reported which shares structure **1** but which contains no sugars; i.e., V = H at every site in **1**. In addition, A41030A is the first compound of this class to contain a chlorinated biphenyl unit. For A41030A, U = V = X = H, W = Cl; Y and Z in **1** are a diphenyl ether in A41030A.



Experimental Section

Antibiotic A41030A is the major factor in a complex of antibiotics produced by *Streptomyces virginiae* NRRL 12525.[12] The material examined in the NMR studies described below contained 93.4% A41030A, based on HPLC analysis with UV detection at 235 nm; no other A41030 factors were detected among the minor UV-absorbing species.

Oxidative Degradation of A41030A. A41030A (200 mg) was dissolved in 0.5 N HCl and refluxed for 15 min. The solution was cooled and evaporated under reduced pressure; the residue was redissolved in a small amount of hot H_2O and reprecipitated with 4 N HCl. The filtrate gave a negative Molisch test, indicating that no sugars had been released. After drying, the solid was dissolved in methanol (10.8 mL), followed by addition of 216 mg of K_2CO_3 and 2.7 mL of CH_3I. The mixture was refluxed for 3 h and then concentrated to dryness under reduced pressure. The insoluble residue was washed twice with H_2O, filtered, and dried over P_2O_5 under vacuum for 24 h.

The methylated A41030A was suspended in 7.3 mL of H_2O, and 2.6 mL of 2 N NaOH and 730 mg of $KMnO_4$ in 16.7 mL of H_2O were added; the reaction mixture was heated for 4 h at 80 °C. After 4 h MnO_2 and excess $KMnO_4$ were destroyed by addition of $NaHSO_3$ and 1 N HCl. The mixture was extracted twice with ethyl acetate, and the extract was dried over Na_2SO_4.

After evaporation of the ethyl acetate, the residue was redissolved in methanol and methylated with CH_2N_2 in ether. Evaporation of the solvents gave a residue which was applied to a column of silica gel in toluene. The column was eluted with ethyl acetate–toluene mixtures; the major product, **2**, was eluted with 5% ethyl acetate in toluene: FD/MS, M^+ m/z 534 (isotope peaks showed two Cl); EI/MS, M^+ m/z 534; ^1H NMR (100 MHz) spectrum identical with that of methyl 3,5-bis[2-chloro-4-(methoxycarbonyl)phenoxy]-4-methoxybenzoate, obtained by a similar oxidative degradation of vancomycin.[13]

Acid Hydrolysis and Derivatization of A41030A. A41030A (983 mg) was dissolved in 30 mL of H_2O and 5 mL of 1 N KOH, and the mixture was cooled to 0 °C under N_2, followed by separate and simultaneous dropwise addition of 1.4 g of Me_2SO_4 and a solution of 0.62 g of KOH in 1 mL of H_2O. After 30 min the same additions of Me_2SO_4 and KOH were repeated at 25 °C. The reaction mixture was stirred for 1 h, followed by acidification with

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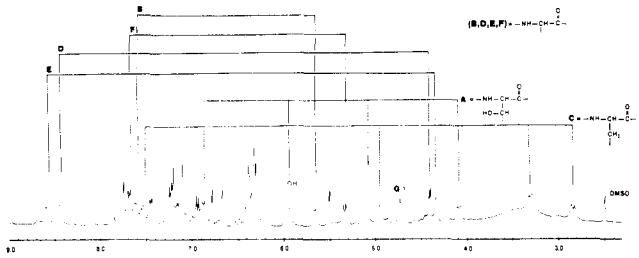

Figure 1. 360-MHz ^1H NMR spectrum of zwitterionic A41030A in Me_2SO-d_6, ambient temperature. The labeled substructures have been identified by spin–spin decoupling experiments.

HCl. A solid separated and was collected.

The methylated A41030A (862 mg) was dissolved in 30 mL of 6 N HCl plus 15 mL of acetic acid. The reaction mixture was refluxed for 48 h under N_2 and then evaporated to dryness under vacuum. The residue was dissolved in 1 N NaOH (10 mL) and cooled to 0 °C, followed by addition of 5 mL of acetic anhydride over a 1-h interval and under N_2. Stirring was continued for an additional 1 h at room temperature. A quantity of ice mixed with 20 mL of concentrated HCl was added, and the mixture was stirred for 15 min. After the mixture had warmed to room temperature the volatile components were removed under vacuum, yielding 580 mg of a foam.

The foam was dissolved in 100 mL of methanol and treated with excess ethereal CH_2N_2. After standing for 18 h, the reaction mixture yielded 500 mg of *N*-acetyl amino acid esters following workup. This mixture was chromatographed on 200 g of silica gel in toluene. Elution with $CHCl_3$ gave 210 mg of an amorphous material which was a mixture of two *N*-acetyl amino acid methyl esters, **3** and **4**: FD/MS, M^+ m/z 488, M^+ 536; the latter component showed isotope peaks indicative of monochlorination.

Preparation of *t*-BOC-A41030A. Zwitterionic A41030A (60 mg) and 15 mg of BOC-ON [2-(((*tert*-butoxycarbonyl)oxy)imino)-2-phenylacetonitrile][14] were suspended in 2 mL of acetone plus 2 mL of methanol, and 1 drop of triethylamine was added. The suspension was stirred until all components were in solution, plus one additional hour (total time ~5 h). The solution was evaporated under N_2, and the residue was triturated three times with ethyl acetate, yielding approximately 60 mg of the triethylammonium salt of *t*-BOC-A41030A. The product was dissolved in methanol and stirred for 15 min with 1–2 mL of IR-120 ion-exchange resin (prewashed with methanol). The mixture was filtered and evaporated to yield *t*-BOC-A41030A free acid.

NMR Spectroscopy. Proton NMR spectra were recorded at ambient temperature (~23 °C) or at elevated temperatures up to ~60 °C, using a Bruker WH360 spectrometer in the Fourier transform mode. Solutions were prepared in Me_2SO-d_6. Nuclear Overhauser effect (NOE) measurements were performed under nonoptimized conditions, as described previously;[9,10a] NOE values reported in the text have only qualitative significance.

Plasma Desorption Mass Spectrometry. PDMS results have been obtained for A41030A by Prof. R. D. Macfarlane (Texas A&M University): calcd for $C_{58}H_{44}O_{18}N_7Cl_3$ 1233.4; found 1233.6.

Elemental Analysis of A41030A. Calcd for $C_{58}H_{44}O_{18}N_7Cl_3$: Cl, 8.63. Found: Cl, 8.48 (tests indicated zero residue and zero ionic Cl).

Results and Discussion

Antibiotic A41030A exhibits antimicrobial activity against Gram-positive bacteria typical of a glycopeptide antibiotic;[12] however, inspection of the proton NMR spectrum of A41030A (Figure 1 and Table I) reveals that the material contains no sugar moieties (i.e., V = H at all sites in structure **1**).[15] The sugar proton region between 3 and 4 ppm is essentially blank; resonances near this region are at 2.85 and 3.33 ppm, the CH_2 portion of the substructure **5**. The resonances of **5** are labeled C in

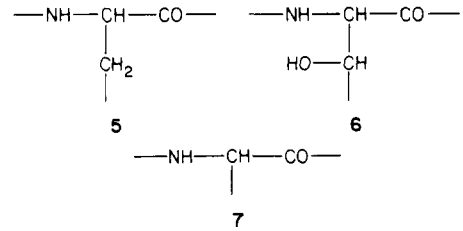

Figure 1 and Table I; such a substructure has also been observed in actaplanin[10] and teicoplanin.[3] The broadened doublet at 4.09 ppm is coupled to an amide doublet at 6.90 ppm (J = 12 Hz) and to a second doublet at 5.08 ppm (J ~ 0 Hz). The latter resonance is coupled to a hydroxyl proton at 5.94 ppm (J = 7 Hz); these groups make up

Table I. Proton NMR Assignments[a] for Zwitterionic A41030A Compared with Corresponding Assignments for Vancomycin,[2a] Teicoplanin ψ-Aglycon Methyl Ester,[3b] and Actaplanin ψ-Aglycon[10a]

	chemical shifts, δ (J, Hz)			
proton	A41030A	vancomycin	teicoplanin ψ	actaplanin ψ
A-NH	6.90 (12)	6.50 (12)	6.02 (12)	7.49 (11)
A-2′	4.09 (12)	4.22 (12)	4.11 (12)	4.26 (~10)
A-1′	5.08	5.13 (~0)	5.27 (~0)	5.07 (~0)
A-OH	5.94 (7)	5.88 (~6)		
A-2	7.75 (~1)	7.87	7.84	7.70
A-3	Cl	Cl	Cl	Cl
A-5	7.25 (8)	7.28 (8)	7.07 (8)	7.31 (8)
A-6	7.45 (8, 1.5)	7.48 (8)	7.25 (8)	7.33 (8, 1)
B-NH	7.61 (8.5)	8.14 (8)	7.47 (8)	7.60 (~8)
B-1′	5.67 (8.5)	5.71 (8)	5.57	5.60
B-2	5.50	5.63	5.56	5.63
B-4(OH)	9.60[b]		9.39	9.47
B-6	5.08	5.21	5.07	5.03
C-NH	7.45 (9)	8.00 (9)	8.12 (8)	7.98 (8)
C-2′	4.97	4.86 (4)	4.94	4.92
C-1′	3.33 (14, 4.5)	5.15 (4)	3.33	3.33
C-1″	2.83 (14, 2.5)	OH	2.88 (14)	2.85 (13)
C-2	7.67 (8)	7.57 (8)	7.82	7.85 (8)
C-3	7.25 (8)	7.20 (8)	7.13 (8)	6.90 (8, 2)
C-5	Cl	Cl	Cl	7.19 (8)
C-6	7.21 (1.5)	7.42	7.20	7.05 (8)
D-NH	8.47 (6)	8.39 (7)	8.47 (5)	9.05
D-1′	4.41 (6)	4.50 (7)	4.56 (5)	4.41 (5.5)
D-2	6.28 (2)	6.30	6.09	6.06
D-3(OH)	9.52[b]		9.28	9.51
D-4	6.38 (~1.5)	6.44 (2)	6.40	6.42 (2)
D-5(OH)	9.07[b]		8.73	8.97
E-NH	8.60 (5)	8.43 (6)	8.35 (5)	8.57
E-1′	4.34 (5)	4.50 (6)	4.28 (5)	4.49 (6)
E-2	7.11 (~2)	7.19	7.03	7.19 (1)
E-4(OH)	9.09[b]		9.06	9.31
E-5	Cl	6.73 (8)	6.67 (8)	6.69. (8)
E-6	6.78 (2)	6.78 (8, 1)	6.71 (8, 1)	6.74 (8, 1)
F-NH	7.70 (~10)		7.63 (10)	7.62 (10)
F-1′	5.32 (10)		5.35 (10)	5.27 (10)
F-2	6.36 (1.5)		6.36	6.40
F-4	6.31 (1.5)		6.36	CH_3
F-5(OH)	9.69[b]		9.55	9.57
F-6	6.34		6.33	6.38
G-1′	4.60		5.45	5.51
G-2	6.65 (~2)		6.80	6.72 (1)
G-4(OH)	9.43[b]		9.78	9.98
G-5	6.92 (8)		7.02	7.09 (8)
G-6	7.12 (8, ~1.5)		7.22	7.19 (8)

[a] 360-MHz spectra in Me_2SO-d_6; chemical shifts are listed vs. internal Me_4Si. Ambient temperature (~23 °C) for A41030A, teicoplanin ψ, and actaplanin ψ; 70 °C for vancomycin. [b] Phenolic resonances are from the spectrum of the *t*-BOC-A41030A derivatives; B-4 and E-4 may be reversed.

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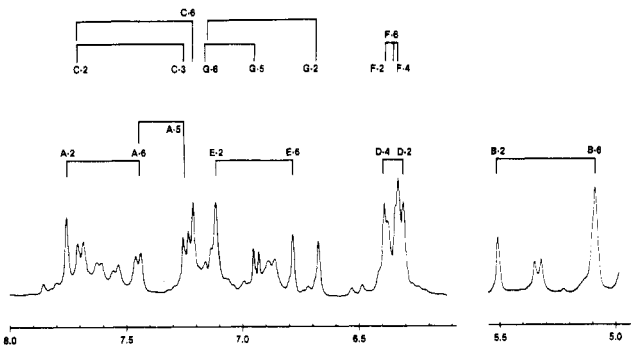
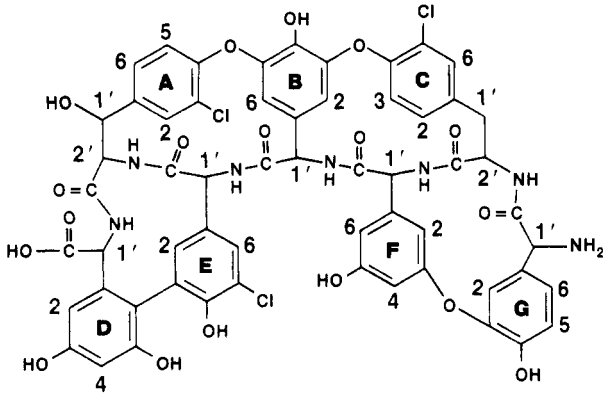


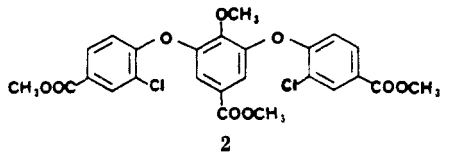
Figure 2. Aromatic protons from the spectrum in Figure 1; the labels on the substructural groups refer to the numbering scheme in structure 8.

substructure 6, labeled A in Table I and Figure 1. Four other substructure groups of type 7 were also located by decoupling experiments; these are labeled B, D, E, and F in Figure 1 and Table I. [The coupling constants and chemical shifts referred to in the discussion are those from Table I (concentration $\simeq$ 5 mg/mL or less); several couplings listed in Table I, especially involving exchangeable protons, are not apparent in the spectrum of Figure 1 (concentration $\simeq$ 20 mg/mL or higher) due to more rapid proton exchange and the general broadening due to the increased solute concentration.] The presence of group 5 implies that U = H in 1, and the absence of CH_3 resonances in Figure 1 indicates that X = H in 1 for A4103A, as well.

The A41030A aromatic proton resonances were also sorted into substructure groups by homonuclear decoupling observations. The aromatic region of Figure 1 is expanded in Figure 2, showing the seven such subgroups found for A41030A. Resonances are numbered in Figure 2 (and in Table I) according to the numbering scheme shown in 8; 8 is the structure of A41030A and is derived from the data being discussed in this report. Details of the structure elucidation will be presented below.



Degradation Product 2. Oxidative degradation of a glycopeptide antibiotic typically yields a triphenyl ether such as compound 2 as a characteristic indicator of the glycopeptide class; 2 has been obtained from vancomycin[13]



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and teicoplanin,[16] while the monochloro equivalent of 2

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Table II. NOEs[a] for A41030A, Vancomycin,[21] A35512B ψ-Aglycon,[9] and Actaplanin ψ-Aglycon[10a]

	resonances reduced (%)			
proton irradiated	A41030A	vancomycin	A35512B ψ	actaplanin ψ
A-2′	A-2 (27)	A-2 (40)	A-2 (25)	A-2 (37)
A-2′	D-NH (35)	D-NH (43)	D-NH (46)	D-NH (31)
A-2′	E-1′ (50)	E-1′ (35)	E-1′ (57)	E-1′ (41)
A-2′	E-2 (33)	C-2 (48)	E-2 (27)	E-2 (27)
B-1′	E-NH (36)	E-NH (49)	E-NH (33)	
B-1′	E-6 (20)			
C-1′	C-6 (40)			C-6 (33)
C-2′	C-6 (8)	C-6 (20)		
D-NH	A-2′ (33)	A-2′ (11)	A-2′ (30)	A-2′ (22)
D-NH	A-2 (12)	A-2 (8)	A-2 (13)	A-2 (20)
D-NH	E-1′ (18)	E-1′ (2)	E-1′ (28)	E-1′ (12)
D-NH	E-2 (19)	E-2 (6)	E-2 (27)	E-2 (6)
E-1′	A-2′ (50)	A-2′ (56)	A-2′ (50)	A-2′ (50)
E-1′	A-2 (12)	A-2 (33)	A-2 (19)	A-2 (34)
E-1′	D-NH (20)	D-NH (38)	D-NH (31)	D-NH (25)
E-1′	E-2 (30)	E-2 (56)	E-2 (27)	E-2 (31)
E-2	A-2′ (40)	A-2′ (47)	A-2′ (40)	
E-2	D-NH (34)		D-NH (38)	
E-2	E-1′ (30)	E-1′ (24)	E-1′ (36)	
E-NH	B-1′ (55)	B-1′ (53)	B-1′ (31)	B-1′ (43)
E-NH	E-6 (41)		E-6 (19)	
F-1′	F-6 (12)			F-6 (28)
F-NH	F-2 (28)		F-2 (55)	F-2 (36)
F-NH	G-2 (15)		G-2 (34)	

[a] Spectra recorded in Me_2SO-d_6 at ~23 °C for A41030A, A35512B ψ-aglycon, and actaplanin ψ-aglycon; 35 °C for vancomycin.

has been found for some glycopeptides (actaplanin,[10a] avoparcin,[17] and actinoidin[18]) and the nonchlorinated triphenyl ether derivative is isolated in other cases (A35512B[19] and ristocetin[20]). The three aromatic rings of 2 represent the rings A, B, and C in structure 8. Chemical shift comparisons for the various glycopeptides listed in Table I indicate that the aromatic protons on ring B occur at unusually high field, as shown in Figure 2. The two proton groups between 7.2 and 8.0 ppm in Figure 2 have likewise been assigned to rings A and C on the basis of their chemical shifts and coupling patterns. A number of negative NOEs (nuclear Overhauser effects) are usually observed during decoupling studies on glycopeptide antibiotics; NOEs to the proton labeled C-6 from the CH_2 and CH groups of 5 indicate that 5 is attached to the C ring. These and several other NOEs to be discussed below are listed in Table II, where they are compared to similar observations for other glycopeptides. Irradiation of either CH group of 6 leads to a reduction of the A-2 resonance intensity, suggesting that 6 is attached to ring A. The resonances A-2 and A-2′ are involved in an extensive "nest" of NOEs which will be discussed further below. The chemical shift comparisons in Table I indicate that the B-1′ proton occurs at low field for an α-CH; the group 7 having the most downfield CH resonance is labeled B in Figure 2 on this basis. This assignment is confirmed by NOE comparisons in Table II.

***t*-BOC-A41030A. Location of Phenols.** At the time of the structure elucidation being described in this report, fast-atom bombardment mass spectrometry was not yet

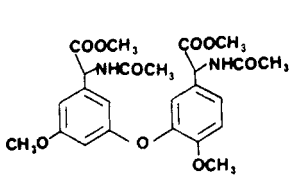
(17) McGahren, W. J.; Martin, J. H.; Morton, G. O.; Hargreaves, R. T.; Leese, R. A.; Lovell, F. M.; Ellestad, G. A.; O'Brien, E.; Holker, J. S. E. *J. Am. Chem. Soc.* **1980**, *102*, 1671–1684.

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

(19) Debono, M.; Molloy, R. M.; Barnhart, M.; Dorman, D. E. *J. Antibiot.* **1980**, *33*, 1406–1416.

(20) Williams, D. H.; Rajananda, V.; Kalman, J. R. *J. Chem. Soc., Perkin Trans. 1* **1979**, 787–792.

available in our laboratory, and the molecular weight of antibiotic A41030A was unknown. The lack of a firm molecular weight value led to the following two uncertainties, in particular: (1) uncertainty over the number of oxygens present as phenols and/or ether linkages, and (2) uncertainty over the number of chlorines in the molecule (elemental analysis results on early samples were ambiguous). Zwitterionic A41030A does not show well-resolved phenolic resonances in Me_2SO solution, having instead three broad peaks at 9.7, 9.5, and 9.3 ppm. The structure 1 contains at least four phenols (with V = H), and one or two additional phenols are present on the groups Y and Z for those glycopeptides that have aromatic groups at Y and Z (i.e., all reported glycopeptides except vancomycin). An ether linkage between Y and Z requires an additional oxygen in some cases (ristocetin,[6,7] A35512B,[9] actaplanin,[10] and teicoplanin[3]), while such a linkage is absent in others (avoparcin[8] and actinoidin[18]). The oxygen count for A41030A was aided by examination of the NMR spectrum of *t*-BOC-A41030A, which contains six sharp phenolic peaks in Me_2SO. Observation of NOEs from these peaks to resonances of adjacent aromatic protons was an important step in assigning the resonances of the substructure groups D–G in Figure 2. The six phenolic resonances of *t*-BOC-A41030A occur at 9.69, 9.60, 9.52, 9.43, 9.09, and 9.07 ppm. In zwitterionic A41030A (dilute solution), the aromatic resonances in the region ~6.3–6.4 ppm are five distinct peaks (6.28, 6.31, 6.34, 6.36, and 6.38 ppm), and the two outside resonances are meta-coupled to each other. In *t*-BOC-A41030A free acid the two outside peaks occur at 6.26 and 6.40 ppm, and the three inside resonances are overlapped in a broad three-proton group at ~6.33 ppm. This composite peak is reduced in amplitude when the 9.69-ppm phenolic resonance is irradiated, indicating that one or more of the three protons is adjacent to the phenol; the assignment of this group of peaks will be discussed below. Irradiation of the phenolic peak at 9.60 ppm produces no NOEs, but irradiation at 9.52 ppm produces two of them, at the meta-coupled peaks at 6.26 and 6.40 ppm. The 6.40-ppm resonance is also reduced when the two phenols near 9.08 ppm are irradiated. Such an NOE pattern, shown in **9**, is typical for the



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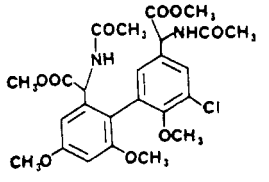


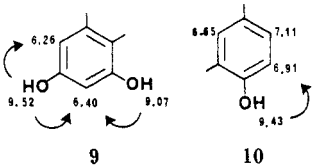
4

protons of ring D (see the ψ-aglycons of teicoplanin,[3b] actaplanin,[10a] and A35512B[9]), and the assignment of these peaks as D-2 and D-4 (see Figure 2) is made on this basis. (Only one NOE is observed when the two phenols near 9.08 ppm are irradiated; the 9.07-ppm phenol peak is assigned to ring D in Table I arbitrarily; the two peaks are too close to distinguish).

Irradiation of the 9.43-ppm phenolic resonance causes a small reduction in the ortho-coupled doublet at 6.91 ppm; the 6.91-ppm resonance is coupled to an overlapped resonance at 7.11 ppm which in turn is meta-coupled to a peak at 6.65 ppm, suggesting the substructure **10**. Group **10** matches the expected structure for ring E (if W = H in 1), but the pattern of chemical shifts is quite different from the values for ring E found for other glycopeptides (see Table I). Furthermore, the E-2 proton usually is involved in a number of NOE observations (see Table II), and these are not found for the A41030A resonance at 6.65 ppm, suggesting that group **10** is not ring E. However, glycopeptides that have a diphenyl ether for the groups Y and Z contain a second structure which matches group **10**, the ring G (Z in 1). The chemical shifts listed in **10** are similar to values for ring G in other glycopeptides (see Table I), and the group **10** is labeled as G in Figure 2, with the suggestion that it may be part of a diphenyl ether.

Two of the six phenols of *t*-BOC-A41030A caused no NOEs to adjacent protons when irradiated, those at 9.60 and 9.09 ppm. One of these must be the phenol at position B-4, which has no proton neighbors; the other must be on one of the two rings not yet identified, E and F (the substituent Y in 1). The remaining aromatic resonances are a meta-coupled pair of peaks at 7.11 and 6.78 ppm and the three resonances near 6.35 ppm; the latter group is associated with the phenol at 9.69 ppm.

Rings E and F. Degradation Products 3 and 4. The structure 1 is derived from the analysis by X-ray crystallography of a vancomycin degradation product, CDP-I,[4] followed by NMR comparisons of CDP-I, vancomycin, and other glycopeptides (differences between CDP-I and vancomycin have recently been described by Harris and Harris[5]). The CDP-I crystal structure indicates that a number of the protons of 1 are held in close proximity, and this is reflected in the NMR spectra of glycopeptides by the observation of extensive "nests" of NOEs; the largest group of protons involved in such a collection of NOEs usually includes A-1′, A-2′, A-2, D-NH, E-1′, and—in particular—E-2.[3b,6a,9,10a,21,22] Comparison of the observed NOEs for A41030A with similar results for other glycopeptides indicates that the group **7** α-CH/NH (4.41/8.47 ppm) is the pair D-1′/D-NH, the group α-CH/NH (4.34/8.60 ppm) is E-1′/E-NH, and the meta-coupled aromatic resonance at 7.11 ppm must be from proton E-2 (see Tables I and II). Identification of E-2 (7.11 ppm) and thus E-6 (6.78 ppm) is supported by a second, smaller set of NOEs; the E-NH and B-1′ protons show mutual NOEs in many glycopeptides, with smaller intensity changes sometimes produced at proton E-6. Irradiation of either E-NH or B-1′ in A41030A produces a negative NOE at the 6.78-ppm resonance (see Figures 1 and 2 and Table II). The 6.78-ppm resonance is not ortho-coupled, and therefore the substituent in position E-5 is not a proton. The 6.78-ppm resonance does not experience an NOE during irradiation of any of the A41030A phenols, indicating that the E-5 substituent is probably not OH. The possibility that substituent E-5 might be a third chlorine was confirmed by new elemental analysis data and by the mass spectral results for degradation products 3 and 4; these results will be discussed below.



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Three of the four groups **7** have been identified (B, D, E); the remaining α-CH/NH pair (5.32/7.70 ppm) must be F-1′/F-NH, and the remaining three aromatic protons (6.31, 6.34, and 6.36 ppm) must belong to ring F. The aromatic resonances show meta-coupling at the most, suggesting that they are symmetrically arranged around the ring. The 6.34-ppm peak is reduced when F-1′ (5.32

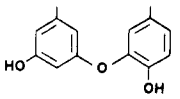
(21) Williams, M. P.; Williams, D. H. *J. Am. Chem. Soc.* **1981**, *103*, 6580–6585.
(22) Fesik, S. W.; Armitage, I. M.; Ellestad, G. A.; McGahren, W. J. *Mol. Pharmacol.* **1984**, *25*, 275–280.

ppm) is irradiated, and the 6.36-ppm peak is decreased when 7.70 ppm (F-NH) is decoupled; the assignments of the three ring protons have been made on the basis of these NOEs.

Confirmation that rings D and E are linked in a chlorinated biphenyl moiety and that rings F and G are part of a diphenyl ether was provided by the field desorption mass spectrum of the mixture of degradation products **3** and **4**. The methylated *N*-acetyl amino methyl esters (see Experimental Section) would be expected to have the following structures, with the masses as observed: **3**, $C_{24}H_{28}N_2O_9$, 488; **4**, $C_{25}H_{29}N_2O_9Cl$, 536.[23]

These results indicate that A41030A has the structure **8**; the molecular formula for **8** is $C_{58}N_{44}N_7O_{18}Cl_3$, M_r = 1233.4. Presence of the third Cl was also supported by elemental analysis (theoretical, 8.63%, found, 8.48%). Plasma desorption mass spectrometry results also agreed with $C_{58}H_{44}N_7O_{18}Cl_3$: found, 1233.6.[24]

Relationships between Structures 1 and 8. The structure **8** is the first example of a complex peptide having the general structure **1** that (a) contains no sugars (V = H at all sites) and (b) contains chlorine in the biphenyl bis(amino acid). The groups Y and Z are the diphenyl ether moiety **11**, which has previously been observed in teicoplanin.[3,16]



11

The absolute configurations shown in **1** are those found for vancomycin CDP-I by X-ray crystallography;[4] the unusual cis peptide bond is also observed in the CDP-I crystal structure. The cis bond is present in all glycopeptides for which structures have been reported; such a bond is required by the observations of (1) a dihedral angle between the A-2′ and A-NH protons of ~180° ($J_{A\text{-}2'\text{-}NH}$ = 10–12 Hz) and (2) close proximity of the A-2′ and E-1′ protons (large NOEs between them). The NMR parameters in Tables I and II indicate that A41030A shares this feature with the glycopeptides.

Williamson and Williams[21] have shown that the correct relative stereochemistry for most of the asymmetric centers in **1** can be deduced for vancomycin from NMR data alone, and the similarities of chemical shifts, coupling constants, and NOE observations shown in Tables I and II support the extension of the CDP-I results to the analogous centers on the other compounds, including A41030A. The glycopeptide antibiotics act by binding to cell-wall mucopeptides containing the terminal dipeptide D-alanyl-D-alanine, inhibiting cross-linking of the bacterial cell wall and leading to eventual lysis of the organism, and a number of the glycopeptides have been shown to form complexes with the mucopeptide analogue acetyl-D-Ala-D-Ala.[25] Such binding to a common substrate suggests that the antibiotics of this group have binding sites with similar or identical structural features, including their stereochemical details. The one asymmetric center for which NMR observations provide little or no stereochemical detail is the amino-terminal site, G-1′. However, epimerization of this site has been shown to lead to a substantial inactivation of the avoparcin glycopeptides[8,11c] and ristocetin.[26] Although no determinations of absolute stereochemistry have been attempted for A41030A, we believe that its high antibiotic activity[12,27] indicates identity with structure **1** at all of the asymmetric centers, including G-1′.

Conclusion

Antibiotic A41030A (**8**) is the smallest of the "glycopeptide-like" antibiotics yet described. It is a glycopeptide aglycon; sugar moieties are not included in the minimum structural requirements for glycopeptide biological activity. In addition, the presence of a third chlorine atom in A41030A helps to define regions of the peptide core that are probably unimportant for antibiotic activity, since addition of the bulky chlorine to ring E appears not to be detrimental to antibiotic activity.

Acknowledgment. We thank T. K. Elzey for recording several of the NMR spectra described in this report.

Registry No. **2**, 55033-62-0; **3**, 96228-22-7; **4**, 96228-23-8; **8**, 89139-41-3; **8** *t*-BOC deriv, 96245-27-1; [2-(((*tert*-butoxycarbonyl)oxy)imino)-2-phenylacetonitrile], 58632-95-4.

(23) Degradation products closely related to **3** have been isolated from teicoplanin.[16]

(24) The integral molecular weight of zwitterionic A41030A has been determined in our laboratories by fast-atom bombardment mass spectrometry: *m/z* 1232 (1231 + H); calculated for $C_{58}H_{44}N_7O_{18}Cl_3$, 1231; personal communication from John Occolowitz.

(25) (a) Hunt, A. H.; Vernon, P. D. *J. Antibiot.* **1981**, *34*, 469–471. (b) Fesik, S. W.; Armitage, I. M.; Ellestad, G. A.; McGahren, W. J. *Mol. Pharmacol.* **1984**, *25*, 281–286. (c) Williams, D. H.; Williamson, M. P.; Butcher, D. W.; Hammond, S. J. *J. Am. Chem. Soc.* **1983**, *105*, 1332–1339.

(26) Herrin, T. R.; Thomas, A. M.; Fesik, S. W. "Program and Abstracts", 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Las Vegas, NV 1983; American Society for Microbiology: Washington, DC, 1983; Abstr. 445.

(27) An account of the isolation and biological properties of A41030A is being prepared for publication by Michel et al.

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