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Comparison and Modeling Study of Vancomycin, Ristocetin A, and Teicoplanin for CE Enantioseparations

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The structurally related glycopeptide antibiotics vancomycin, ristocetin A, and teicoplanin can all be used as chiral selectors in capillary electrophoresis (CE). Both experimental and modeling studies were done to elucidate their similarities and differences. There are identifiable morphological differences in the aglycon macrocyclic portions of these three compounds. In addition, there are other structural distinctions that can affect their CE enantioselectivity, migration times, and efficiency. Teicoplanin is the most distinct of the three and is the only one that is surface active. Its aggregational properties appear to affect its enantioselectivity among other things. The similar but not identical structures of the three glycopeptides produce similar but not identical enantioselectivities. This leads to the empirically useful "principle of complementary separations", in which a partial resolution with one chiral selector can be brought to baseline with one of the others. Overall, ristocetin A appears to have the greatest applicability for CE enantioseparations.

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Many new chiral run buffer additives for capillary electrophoresis (CE) have been introduced in the past few years. Polysulfated carbohydrates such as heparin and dextran sulfate were found to baseline resolve a variety of antihistamines as well as antimalarial compounds.[1,2] The regiospecific monosubstituted 2-(1-naphthyl)ethylcarbamoylated β-cyclodextrin, one of the most widely employed HPLC-derivatized cyclodextrin stationary phases,[3] was successfully evaluated as a chiral CE electrolyte additive.[4] Anionic cyclodextrin derivatives such as sulfobutyl ether β-cyclodextrin (SBE-β-CD) and sulfonated cyclodextrins have made an impact on enantioselective CE separations.[5−8] Two recent comprehensive reviews on CE separations have appeared.[9,10] Until 1994, ~90% of all chiral CE separations were done with cyclodextrins or their derivatives.

Few new chiral selectors have had as immediate and dramatic an impact as have the macrocyclic antibiotics.[11−25] There are literally hundreds of macrocyclic antibiotics, and their versatility in separating enantiomers, isomers, and routine compounds is becoming well known. The fact that a new family or class of chiral selectors can be used effectively in so many different ways (i.e., in HPLC, TLC, CE) to separate a wide variety of anionic, neutral, and cationic compounds can be intimidating as well as confusing. Nevertheless, these chiral antibiotics are not merely analogues or replacements for cyclodextrins or other chiral selectors. They have unique structural features and functionalities that allow the resolution of many classes of compounds and often produce separations with far greater selectivity.[11−25]

Among the most useful macrocyclic antibiotics added to the background electrolyte (BGE) in CE have been the ansamycins and the oligophenolic glycopeptides. Rifamycin B and rifamycin SV are *ansa* compounds (i.e., compounds that contain a chromophore spanned by an aliphatic "bridge") that differ only in the type and location of the substituents on their naphthohydroquinine ring.[17,23] Rifamycin B is negatively charged at the pH values commonly studied and is known to separate a number of cationic, pharmacologically active compounds, including β-adreneric blocking agents, vasoconstrictors, bronchodilators, and several barbiturates.[17,23] Ward et al. have shown rifamycin SV and rifamycin B to be complementary to each other in that the latter separates positively charged, single-ring compounds, while the former

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resolves positively and negatively charged compounds containing two rings.[23]

Glycopeptide macrocyclic antibiotics appear to be among the more useful chiral selectors available for use in capillary electrophoresis.[17,24] Typical glycopeptide antibiotics contain a peptide core of complex amino acids and linked phenolic moieties, one or more neutral sugar moieties, and one or more amino saccharides.[30] Currently, over 350 acidic or anionic racemic solutes have been electrophoretically resolved with glycopeptide chiral selectors including numerous nonsteroidal antiinflammatory compounds,[18−21] antineoplastics,[18−21] lactic acids,[18−21] herbicides,[18−22] rodenticides,[22] and most N-blocked amino acids.[18−21] The highest enantioresolution ever reported in CE (e.g., Rs > 20) was first obtained using vancomycin.[18] Subsequently, a resolution > 22 was obtained for the herbicide 2-(3-chlorophenoxy)propionic acid on a relatively short capillary (effective length of 25 cm).[19]

Glycopeptide antibiotics have been widely studied as chiral selectors in HPLC, TLC, and CE for several reasons: (1) they are amphoteric (i.e., contain ionizable groups with acidic and basic p*K*a values, (2) each has the functionalities that make stereoselective association feasible, (3) most have relatively low background absorbance at wavelengths > 250 nm, permitting the use of direct UV detection, (4) each contains hydrophobic and hydrophilic moieties, making them water soluble, soluble in aqueous buffers, and slightly soluble in many hydroorganic solvents, and (5) they are indefinitely stable as a solid under anhydrous conditions at 0 °C, sufficiently stable in aqueous solution and CE buffers, and stable with specific HPLC covalent bonding chemistries and packing procedures.[11−25] Although the glycopeptide chiral selectors most often separate neutral and anionic compounds, the separation mechanism is not well understood. Very little has been reported on teicoplanin-based CE separations, and only a single paper involving ristocetin A has appeared.[19,20] The primary interaction of glycopeptide-based CE chiral separations is thought to involve charge−charge or electrostatic interactions.[18−22] Proposed secondary interactions include hydrogen bonding, steric repulsion, hydrophobic, dipole−dipole, and $\pi-\pi$. Unfortunately, little is known about the geometry, secondary structure, and conformation of the glycopeptide antibiotics.

In this work, we examine the more common and useful macrocyclic antibiotics: vancomycin, ristocetin A, and teicoplanin. Molecular modeling of these compounds is done. This allows a direct description and comparison of the structural features that affect chiral recognition as well as the other physicochemical properties of these compounds. Also, other relevant operational CE separation parameters are compared.

EXPERIMENTAL SECTION

Materials. Vancomycin hydrochloride salt, ristocetin A sulfate salt, nonsteroidal antiinflammatory compounds, methotrexate, amino acids, amino acid derivatives, 9-fluorenylmethyl chloroformate (FMOC-Cl), sodium dihydrogen phosphate, and potassium hydroxide were purchased from Sigma Chemical Co. (St. Louis, MO). Teicoplanin was a generous gift donated by Marian Merrill Dow Research Institute (Cincinnati, OH). All other chiral compounds containing carboxylic acid functional groups were obtained from Aldrich (Milwaukee, WI). HPLC grade acetonitrile and 2-propanol, sodium hydroxide, and reagent grade hydrochloric acid were purchased from Fisher (St. Louis, MO). (6-Aminoquinolyl)-*N*-hydroxysuccinimidyl carbamate (AQC) derivatizingreagent was supplied by Waters (Milford, MA). Sodium dodecyl sulfate (SDS) used in the studies was obtained from Bio-Rad (Richmond, CA).

Methods. Waters provided a Quanta 4000 capillary electrophoresis apparatus equipped with a fixed-wavelength UV lamp. All chiral separations were performed using a 32.5 cm × 50 μm i.d. (25 cm to the detector) fused-silica capillary obtained from Quadrex (New Haven, CT). All analytes were monitered using direct UV detection at 254 or 280 nm. This CE unit is equipped with a forced air convection cooling system. The capillaries were prepared by burning off the polyimide coating from a 1 cm section of the fused-silica capillary to form a window and then inserting the capillary into the capillary holder. The capillary was conditioned with 0.1 M potassium hydroxide solution for 10 min. The capillary was further purged with doubly distilled water for 5 min, followed by a 5 min equilibration with the desired composition and pH of the running buffer. The sodium phosphate buffer stock solution was prepared by volume and adjusted to the appropriate pH with either sodium hydroxide or hydrochloric acid. The chiral selector solutions were prepared by dissolving them in the desired concentration of sodium phosphate buffer. All solutions were degassed by sonication. The aqueous buffer−organic modifiers (i.e., acetonitrile or 2-propanol) were prepared by volume. Therefore, all percent concentrations are actually volume percents prior to mixing.

All samples were dissolved in doubly distilled water or the running buffer and filtered with 0.45 μm nylon syringe filters purchased from Alltech (Deerfield, IL) prior to analysis. Samples were hydrostatically injected for 3 s. Chiral electrophoretic separations were achieved with solutions of 0.1 mg/mL and at ambient temperature (~22 °C). The run voltage for all separations was +5 kV. All chiral run buffer solutions were stored in a refrigerator at 4 °C when not in use. The derivatization procedures for AQC and FMOC amino acid compounds have been described previously.[27] The absorbance spectra were measured using a Hitachi Model U-2000 double-beam UV−visible spectrophotometer. Methanol and acetone were used as the electroosmotic flow (EOF) markers.

Micellar studies were performed on a Beckman P/ACE 2000 CE unit (Beckman, Palo Alto, CA), equipped with an 50 μm × 30 cm fused-silica capillary (23 cm to the detection window). Data acquisition was done using System Gold software. Direct UV detection was accomplished at 254 nm. Separations were done in 0.1 M phosphate buffer pH 6 or 7, at 20 °C. Capillary pretreatment and conditioning were the same as in the previous system. Methanol was used as neutral marker for SDS studies. It has been previously noted that macrocyclic antibiotics tend to interact electrostatically with the capillary wall.[18−20] For this reason, the capillary was rinsed with 0.5 M potassium hydroxide for 3 min between sample runs, followed by doubly distilled water

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and run buffer for an additional 2 min. This capillary conditioning ensures the reproducibility of the EOF velocities and migration times. Samples were introduced into the capillary by the pressure injection mode at 0.5 psi for 1−3 s. Binding constants for vancomycin, ristocetin A, and teicoplanin to SDS micelles were determined using the equation and methods outlined in ref 21.

Critical micelle concentration studies of teicoplanin were measured using a surface tensiometer (Fisher, St. Louis, MO). The software used for molecular modeling studies was Alchemy III, obtained from Tripos Associates (St. Louis, MO). The program allows computation of the structural conformation of the macrocyclic antibiotics corresponding to the minimal energy while taking into consideration the atom size, electrostatic and dipole interactions, bond lengths, and bond angles in three dimensions.

RESULTS AND DISCUSSION

Vancomycin, ristocetin A, and teicoplanin macrocyclic antibiotics are produced as fermentation products of *Streptomyces orientalis*, *Nocardia lurida*, and *Actinoplanes teichomyceticus*, respectively.[26,28−35] These antibiotics are primarily active against aerobic and anaerobic Gram-positive microorganisms both in vitro and in vivo. They are known to inhibit cell wall synthesis.[31−33,36−43] Teicoplanin is a mixture of five closely related analogues, designated T-A_{1-1} through T-A_{1-5}.[35,43] They differ by approximately 20 molecular mass units because of the variation of the length (i.e., C_{10}−C_{11}) and substituent groups of the acyl side chain that is attached to the amino saccharide. Teicoplanin A_{2-2} is the most common or abundant product produced. It has been reported to be between 2 and 4 times more active against Gram-positive bacteria than vancomycin in vitro.[40,41,44] However, teicoplanin A_{1-1} has shown some activity against several Gram-negative pathogenic bacteria.[39]

Macrocyclic glycopeptides are soluble in water, buffers, and acidic aqueous solutions and less soluble at neutral pH.[26,30,44] They are moderately soluble in polar aprotic solvents (i.e., DMSO, DMF) but insoluble in most nonpolar organic solvents.[26] The heptaphenolic glycopeptides used in this study are white solids. The absorbance spectra of all the antibiotics are somewhat similar and have been published previously.[18−20] In the pH range from 4 to ∼8, the spectra for these compounds nearly overlap. At wavelengths <250, the UV absorbance increases sharply. Between 250 and 310 nm, the absorbance is weak, with a small maximum near 280 nm. Above 310 nm, the absorbance is negligible. The absorbance of the glycopeptides at alkaline pH values is somewhat greater at all wavelengths, and there appears to be a red shift of 20 nm for the teicoplanin and ristocetin A spectra, but less for vancomycin.[18−20] It is believed that this is due to ionization of the phenolic groups and/or partial decomposition of the macrocyclic antibiotics.[36] We previously determined that direct UV absorbance can be used in CE with all three of the glycopeptide antibiotics studied because of their relatively low extinction coefficients at wavelengths >250 nm and the fact that very dilute chiral selector concentrations (i.e., typically between 1 and 5 mM) are needed in order to achieve enantioselectivity.[18−20]

Structure and Morphology. Table 1 gives a comparison of the structural features and physicochemical properties of the vancomycin, ristocetin A, and teicoplanin glycopeptide antibiotics. All three of these related compounds consist of an aglycon "basket" made up of fused macrocyclic rings (Figure 1) and pendant carbohydrate moieties. The macrocycles contain both ether and peptide linkages (Figure 1B). The aglycons of vancomycin and teicoplanin contain two chloro-substituted aromatic rings, while the analogous portion of ristocetin A contains no chloro substituents (Figure 1B). Vancomycin is the smallest of the three compounds (MW 1449, 18 stereogenic centers). It consists of three macrocyclic rings and an attached disaccharide consisting of D-glucose and vancosamine. The other two glycopeptides (teicoplanin, MW 1877, and ristocetin A, MW 2006) are somewhat larger. They have four, rather than three, fused macrocyclic rings and a greater number and different types of pendant sugar moieties. For example, teicoplanin has three attached monosaccarides, two of which are D-glucosamine and one of which is D-mannose. Ristocetin A has a pendant tetrasaccharide and two monosaccharide moieties. These saccharides include D-arabinose, D-mannose, D-glucose, and D-rhamnose. Teicoplanin has one unique characteristic: it has a hydrophobic acyl side chain ("hydrophobic tail") attached to a 2-amino-2-deoxy-β-D-glucopyranosyl moiety (Figure 1, Table 1). Consequently, teicoplanin is surface active and aggregates to form micelles. Neither of the other two glycopeptides has shown this type of behavior under the experimental conditions used in this study (Table 1). Teicoplanin's critical micelle concentration in unbuffered aqueous solutions is ∼0.18 mM.

All three glycopeptides have analogous ionizable groups which control their charge and are thought to play a role in their association with and chiral recognition of chiral analytes. For example, there is an amine on the aglycon portion of each compound (Figure 1B). Vancomycin has a secondary amine, while the other two compounds have primary amine groups. All three compounds also have amino saccharide moieties. However, teicoplanin is unique in that it has two amino saccharides, both of which are N-acylated (Figure 1). There is a carboxylic acid moiety on the aglycon of both vancomycin and teicoplanin, while the equivalent group on ristocetin A is esterified (Figure 1). The only other ionizable groups on these compounds are the phenolic moieties. At operational pH values (∼3.5−7.5), these are generally protonated and probably serve mainly as hydrogen bonding sites.

Figure 1 shows both the molecular "space-filling" model (Figure 1A) and the corresponding "stick" model (Figure 1B) for vancomycin, teicoplanin, and ristocetin A (see the Experimental

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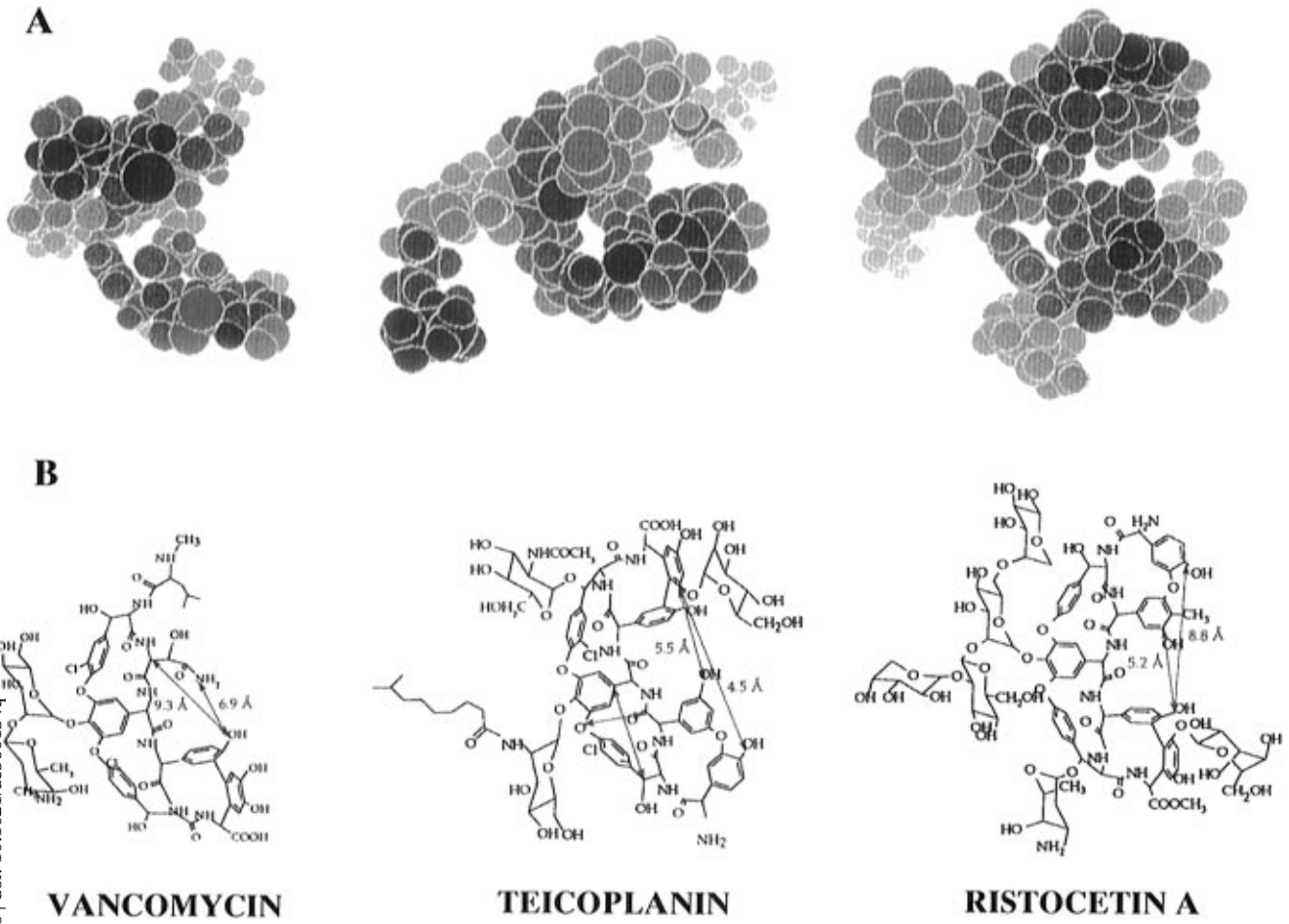

Figure 1. Structures of the macrocyclic antibiotics vancomycin, teicoplanin, and ristocetin A showing a profile view of the aglycon "basket" using (A) space-filling molecular models produced through energy minimization and (B) stick figures. The colored atoms in part A denote the hydrophilic moieties, while the black portion designates the more hydrophobic regions. Red represents carboxylate groups, green are ammonium groups, and blue are hydroxyls. Black regions include the aromatic rings, connecting carbons, and amido linkages.

Table 1. Comparison of the Physicochemical Properties of Vancomycin, Ristocetin A, and Teicoplanin Macrocyclic Antibiotics

characteristic	vancomycin	ristocetin A	teicoplanin
molecular weight	1449	2066[a]	1877[b]
no. of stereogenic centers	18	38	23
produced from fermentation product of	*Streptomyces orientalis*	*Nocardia lurida*	*Actinoplanes teicomyceticus*
no. of macrocycles	3	4	4
no. of monomer sugar moieties	2	6	3
hydrophobic tail	0	0	1
no. of OH groups[c]	9 (3)	21 (4)	15 (4)
no. of amine groups	2	2	1
no. of carboxylic acids	1	0	1
no. of amido groups	7	6	7
no. of aromatic groups[d]	5 (2)	7	7 (2)
methyl esters	0	1	0
p*I*[e]	7.2	7.5	4.2, 6.5
relative stability	1−2 weeks	3−4 weeks	2−3 weeks
current cost	$100.00/g	$1600.00/g	not available in U.S.
aggregational behavior[f]	no	no	yes

[a] Ristocetin A is a mixture of two structurally similar compounds differing by the number of carbons in one of its sugar moieties. The mixture used in this study is >90% ristocetin A. [b] Teicoplanin is a mixture of five closely related compounds differing by the number of carbons (i.e., (C_{10}−C_{11}) and substituted groups attached to the fatty acid side chain terminating off the amino sugar (see Figure 1). [c] The number in parentheses correspond to phenolic moieties. [d] The number in parentheses correspond to the number of chlorinated substituents attached to the aglycon baskets. [e] The p*I* values were determined using dilute solutions of the respective macrocyclic antibiotics in 0.1 M phosphate buffer. Methanol was used as the EOF marker. These values were taken from refs 17−19 and 33. [f] This is the aggregational behavior under the buffered conditions reported in this paper.

Section for modeling details). The antibiotics are arranged by size and appear from the smallest (vancomycin) to the largest (ristocetin A). These three structurally related compounds are rotated to show a profile view of the "C-shaped" aglycon "basket". The colored regions in Figure 1A are hydrophilic groups. The hydroxyl groups are depicted in blue, the ammonium groups in

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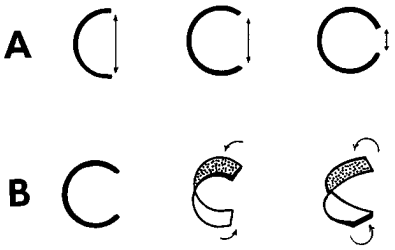

Figure 2. Simplified schematic showing two important morphological characteristics of the aglycon part of glycopeptide antibiotics. (A) End-to-end distance (represented by the length of the arrow) decreases from left to right. (B) The "C-shaped" aglycon also can be twisted to different degrees. The helical twist increases from left to right in this series of three figures.

green, and the carboxylate groups in red. The black area denotes the more hydrophobic regions, including amido linkages, aromatic rings, and apolar connecting carbons. These figures provide a more accurate three-dimensional representation of these compounds, as well as a color coding of many of their important functional groups. As can be seen in Figure 1, the glycopeptides are chiral molecules differing in their size and shape as well as the number and type of substituent groups attached to the macrocyclic peptide core (i.e., the aglycon basket). Also, there is a helical twist to the aglycon portion of each of the three molecules.

As mentioned previously, the aglycon portion of vancomycin consists of three fused macrocyclic rings and an attached amino acid, while teicoplanin and ristocetin A have four fused macrocyclic rings (Table 1). A chiral selector's size and shape and the geometric arrangement of its functional groups help to determine its enantioselective properties. While all three of these macrocyclic glycopeptides have semirigid aglycon baskets, each has distinct morphological characteristics. Two of the more important of these characteristics are (1) the openness of the C-shaped aglycon basket and (2) its degree of helical twist. Both of these features are illustrated in Figure 2. When the glycopeptides are observed in profile (as seen in Figures 1 and 2), "openness" is related to the distance between opposite ends of the aglycon. This is shown schematically in Figure 2A, where the figure on the left is the most open (i.e., greatest distance between ends), and the figure on the right is the most closed (indeed, it is nearly circular). The analogous end-to-end distances have been measured for vancomycin, teicoplanin, and ristocetin A. They are shown next to the red arrows in Figure 1B. Clearly, vancomycin has the most open aglycon, with its shortest end-to-end distance being ~9.3 Å. Teicoplanin appears to have the most closed aglycon (almost cyclic) with the end-to-end distances varying from ~4.5 to 5.5 Å. Ristocetin A is intermediate, with end-to-end distances varying from ~5.2 to 8.8 Å. These variations in the end-to-end distances result from the helical twist of the aglycon, which makes one portion of the macrocycle's terminal edge closer than another.

It is more difficult to measure the degree of helical twist of the aglycon, as shown schematically in Figure 2B. However, it appears that the degree of twist is one of the factors that affects the shape of the individual macrocyclic rings that form the aglycon "basket". For example, an unstrained macrocyclic ring can be nearly circular. Strained or deformed rings may be more elongated or oval-shaped. The various diameters of the individual macrocyclic rings were measured and compared, as shown for the teicoplanin structure in Figure 1B (i.e., the crossed red

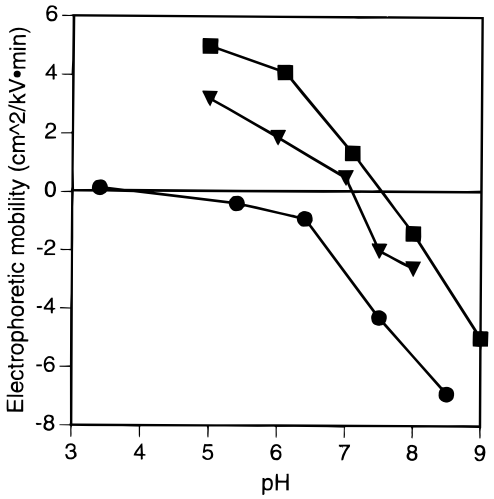

Figure 3. Plot showing the effect of solution pH on the electrophoretic mobility of ristocetin A (■), vancomycin (▲), and teicoplanin (●) macrocyclic antibiotics using 0.1 M phosphate buffer. The capillary for ristocetin A and vancomycin studies was 32.5 cm × 50 μm i.d. (25 cm to the detector window). The voltage was +5 kV. The electrophoretic mobility of teicoplanin was obtained using a 44 cm × 50 μm i.d. capillary (36.5 cm to the detector) and a run voltage of +10 kV. Either acetone or methanol was used as the electroosmotic flow marker.[18−20]

arrows). We can define an "axial ratio" (AR) for these rings as AR = length of the major axis/length of the minor axis. Axial ratios close to 1 indicate that the macrocyclic ring is close to circular. The larger the ratio, the more elongated and strained are the individual rings. The range of axial ratios for vancomycin is ~1.3−1.7, that for teicoplanin is ~1.38−1.42, and that for ristocetin A is ~1.36−2.13. Clearly, none of the macrocyclic ring axial ratios approach 1.0; however, some of the macrocyclic rings in ristocetin A and vancomycin are significantly more deformed (elongated) than others in the same molecule. This could indicate a greater degree of helical twist in these molecules. Oddly enough, vancomycin and ristocetin A are the smallest and largest glycopeptides, respectively. This shows that the degree of helical twist may not be strictly a function of the molecule's size or the number of fused macrocyclic rings. The axial ratios of all four of teicoplanin's rings were similar. The other two glycopeptides had greater variation in the axial ratios of their individual macrocyclic rings.

Comparison of CE Properties of Vancomycin, Ristocetin A, and Teicoplanin. The electrophoretic mobilities of vancomycin, ristocetin A, and teicoplanin as a function of pH (using phosphate buffer) are shown in Figure 3. As can be seen, the curves for ristocetin A and vancomycin are somewhat similar, and their p*I* values are close under these experimental conditions (7.5 and 7.2, respectively). It has been reported that, under different experimental conditions (e.g., different buffers, buffer strengths, etc.), the measured or apparent p*I* values can shift slightly.[25] The electrophoretic mobility versus pH curve and the p*I* for teicoplanin are clearly different from those of the other two glycopeptides (Figure 3). Between pH ~6.5 and 3.0, the teicoplanin curve is fairly flat and very close to the line of zero electrophoretic mobility. Though it is difficult to tell with any great accuracy, the p*I* appears to be approximately 3.8 under these experimental conditions. This is significantly different from an earlier reported p*I* of 6.5.[34] At least two factors that can account for the shape of teicoplanin's electrophoretic mobility versus pH curve, as well as its relatively

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Table 2. Comparison of Migration Times, Effective Mobilities, and Resolution of Enantiomers Using Different Structurally Related Glycopeptide Macrocyclic Antibiotics as Chiral Run Buffer Additives

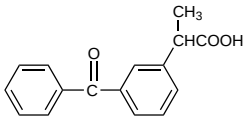
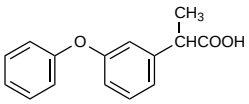
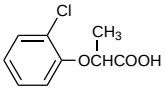
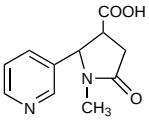
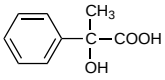
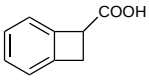
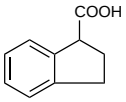
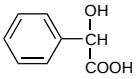
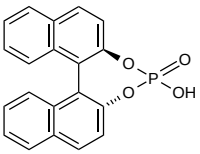
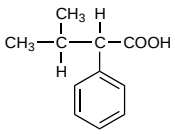
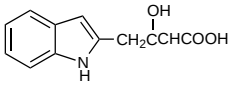
compound		resolution	time(1)[b]	time(2)[c]	$\mu_e(1)$[d]	$\mu_e(2)$[d]
1. ketoprofen 	(V)	6.2	22.1	26.3	−6.2	−7.4
	(R)	5.7	12.3	14.3	−8.5	−10.3
	(T)	1.1	12.5	13.0	−5.1	−5.6
2. fenoprofen 	(V)	3.0	27.5	29.6	−7.6	−8.1
	(R)	0.9	16.1	16.5	−11.6	−11.8
	(T)	1.1	16.3	16.7	−8.1	−8.3
3. 2-(2-chlorophenoxy)propionic acid 	(V)	1.3	47.5	50.3	−10.1	−10.3
	(R)	2.1	19.5	21.5	−13.3	−14.1
	(T)	0.5	26.0	27.0	−11.8	12.0
4. ±-*trans*-4-cotininecarboxylic acid 	(V)	6.0	40.2	47.6	−9.5	−10.1
	(R)	3.2	16.3	19.8	−11.7	−13.5
	(T)	1.1	16.6	18.0	−8.3	9.0
5. atrolactic acid 	(V)	1.0	34.0	35.2	−8.8	−8.9
	(R)	0.4	28.6	29.1	−16.0	16.1
	(T)	0.9	19.3	20.0	−9.6	−9.9
6. 1-benzocyclobutenecarboxylic acid 	(V)	2.2	49.4	54.9	−10.3	−10.6
	(R)	1.1	21.0	22.5	−13.9	−14.4
	(T)	0.6	23.8	24.4	−11.3	−11.4
7. 3-oxo-2-indanacarboxylic acid 	(V)	6.3	56.5	75.5	−10.7	−11.4
	(R)	2.6	27.3	32.5	−15.7	−16.7
	(T)	11	24.5	25.9	−11.4	−11.8
8. mandelic acid 	(V)	0.0	51.5		−10.4	
	(R)	2.0	23.0	28.4	−14.6	−15.9
	(T)	3.4	31.5	37.1	−12.9	−13.7
9. 1,1′-binaphthyl-2,2′-diyl hydrogen phosphate 	(V)	0.6	21.6	22.0	−6.0	−6.2
	(R)	4.1	17.4	19.5	−12.3	−13.3
	(T)	0.0	18.2		−9.1	
10. 3-methyl-2-phenylbutyric acid 	(V)	0.8	42.0	43.5	−9.6	−9.8
	(R)	6.4	25.0	30.6	−15.2	−16.4
	(T)	0.0	16.0		−7.9	
11. indoleacetic acid 	(V)	1.7	36.1	38.6	−9.0	−9.0
	(R)	3.4	12.5	17.1	−8.7	−12.2
	(T)	0.4	21.3	21.6	−10.4	−10.5

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Table 2 (Continued)

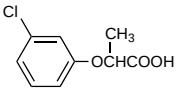
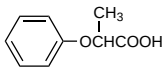
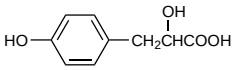
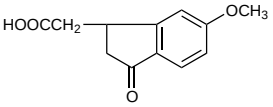
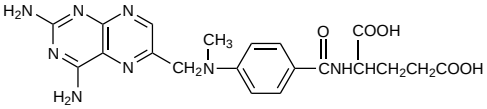
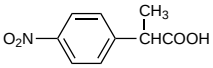
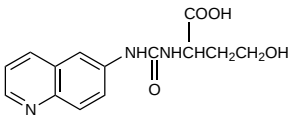
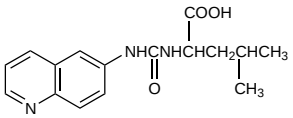
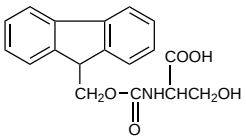
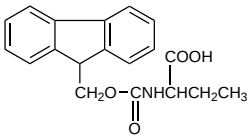
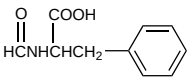
compound		resolution	time(1)[b]	time(2)[c]	$\mu_e(1)$[d]	$\mu_e(2)$[d]
12. 2-(3-chlorophenoxy)propionic acid 	(V)	1.0	41.4	43.2	−9.6	−9.7
	(R)	8.9	11.2	21.4	−7.2	−14.1
	(T)	2.0	16.1	17.7	−8.0	−8.8
13. 2-phenoxypropionic acid 	(V)	1.2	47.0	49.8	−10.1	10.3
	(R)	0.8	21.0	21.7	−13.9	−14.1
	(T)	0.0	24.2		−11.3	
14. 3-(4-hydroxyphenyl)lactic acid 	(V)	3.4	34.5	38.0	−8.8	−9.3
	(R)	12.2	11.9	19.0	−8.0	−13.1
	(T)	0.2	14.7		−7.0	
15. 5-methoxy-1-indanacetic acid 	(V)	2.9	28.0	30.4	−7.7	−8.2
	(R)	4.1	10.3	12.0	−5.9	−8.1
	(T)	0.8	13.4	13.8	−5.9	−6.3
16. methotrexate 	(V)	2.3	22.0	25.0	−6.2	−7.0
	(R)	11.4	12.5	16.5	−8.7	−11.8
	(T)	11.0	14.0	19.5	−6.4	−9.7
17. 2-(4-nitrophenyl)propionic acid 	(V)	3.8	69.5	84.4	−11.2	−11.6
	(R)	1.6	16.0	16.7	−11.5	−11.9
	(T)	0.0	25.2		−11.6	
18. AQC-DL-homoserine 	(V)	7.9	27.2	33.1	−7.6	−8.6
	(R)	11.1	12.3	16.7	−8.5	−11.9
	(T)	6.8	13.0	15.6	−5.6	−7.6
19. AQC-DL-leucine 	(V)	2.5	17.3	18.7	−4.1	4.9
	(R)	6.4	10.0	12.9	−5.4	−9.1
	(T)	3.7	11.4	14.1	−3.8	−6.5
20. FMOC-DL-serine[e] 	(V)	1.4	18.3	20.0	−4.7	−5.4
	(R)	2.4	10.9	12.1	−6.8	8.2
	(T)	1.0	12.4	13.0	−5.0	−5.6
21. FMOC-DL-α-amino-*n*-butyric acid 	(V)	2.9	15.8	17.1	−3.3	−4.0
	(R)	5.1	8.9	10.7	−3.4	−6.5
	(T)	0.5	10.6	11.3	−2.7	−3.7
22. *N*-formyl-DL-phenylalanine 	(V)	2.3	49.5	62.0	−10.3	−10.9
	(R)	0.7	14.0	14.6	−10.1	−10.5
	(T)	1.1	16.7	17.5	−8.3	−8.8

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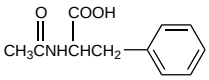
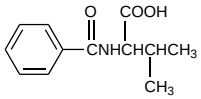
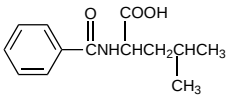
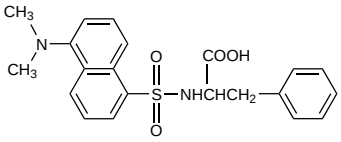
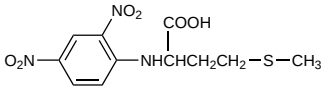
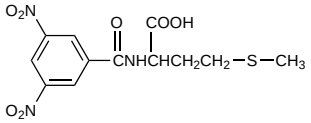
Table 2 (Continued)

compound		resolution	time(1)[b]	time(2)[c]	$\mu_e(1)$[d]	$\mu_e(2)$[d]
23. *N*-acetyl-DL-phenylalanine 	(V)	4.8	27.0	49.0	−7.5	−10.2
	(R)	0.6	14.2	15.0	−10.2	−10.8
	(T)	1.5	14.8	16.6	−7.1	−8.2
24. *N*-benzoyl-DL-valine 	(V)	1.7	38.1	40.6	−9.3	−9.5
	(R)	5.5	10.9	20.6	−6.8	−13.8
	(T)	1.4	14.7	15.6	−7.0	−7.6
25. *N*-benzoyl-DL-leucine 	(V)	1.9	31.3	33.6	−8.3	8.7
	(R)	3.7	11.0	13.6	−6.9	−9.7
	(T)	2.8	12.7	14.8	−5.3	−7.1
26. dansyl-DL-phenylalanine 	(V)	2.6	15.9	17.2	−3.3	−4.1
	(R)	9.9	10.1	13.3	−5.6	−6.9
	(T)	0.0	13.4		−5.9	
27. 2,4-DNP-DL-methionine 	(V)	2.3	40.7	44.4	−9.5	9.9
	(R)	7.1	8.2	11.0	−1.8	−6.9
	(T)	0.0	11.1		−3.4	
28. PHTH-DL-methionine 	(V)	2.5	34.7	37.0	−8.9	−9.1
	(R)	2.7	11.7	13.0	−7.8	−6.9
	(T)	0.0	12.9		−5.5	

[a] The run buffer was 0.1 M phosphate buffer, pH 6.0 (unless otherwise indicated), containing 2 mM vancomycin (V), 2 mM ristocetin (R), and 2 mM teicoplanin (T), as indicated. The run voltage for all separations was +5 kV, and the capillary was 32.5 cm × 50 μm (25 cm to the detector window). [b] Migration time (in minutes) of the first-eluting enantiomer. [c] Migration time (in minutes) of the second-eluting enantiomer. [d] $\mu_e(1)$ and $\mu_e(2)$ are the effective electrophoretic mobilities of the first- and second-eluting enantiomers, respectively, in $cm^2 \cdot kV^{-1} \cdot min^{-1}$. [e] 2 mM ristocetin added to run buffer at pH 7.0.

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low (compared to the other glycopeptides) p*I*. First, teicoplanin lacks the additional amine moiety on its pendant sugar groups (Figure 1). Both of teicoplanin's aminosaccharides are N-acylated. Hence, its main ionizable groups are the single carboxylate and the primary amine on the aglycon. The phenolic moieties may have some less pronounced effects under certain conditions. Overall, it appears that teicoplanin is nearly zwitterionic or slightly negative in the pH range of ~3.5−6.0. Therefore, at pH values above ~3.8, teicoplanin is slightly negatively charged and migrates in the opposite direction as vancomycin, ristocetin A, and the EOF. Second, micelle formation or self-association is known to affect the apparent pK_a values, p*I* values, reaction rates, spectral properties, etc. of a wide variety of compounds.[45] This is undoubtedly true for teicoplanin as well. Aggregation also affects several of teicoplanin's solution properties. As will be described in a subsequent section of this paper (on the effect of organic modifiers), aggregation also appears to affect teicoplanin's enantioselectivity.

A comparison of CE separation data under analogous experimental conditions is given in Table 2 for vancomycin, ristocetin A, and teicoplanin. All of the glycopeptides seem to prefer anionic, acidic, or neutral compounds under these experimental conditions.[19−22] Under comparable conditions the use of vancomycin almost always produced separations with the longest migration times. For any given analyte, migration times with vancomycin were usually twice those of ristocetin A, while those with teicoplanin usually were intermediate (Table 2). For example, the enantiomers of 2-(2-chlorophenoxy)propionic acid (entry 3, Table 2) eluted in approximately 50 min with vancomycin, 22 min with ristocetin A, and 27 min with teicoplanin. The reason separations utilizing vancomycin resulted in longer separation times than those that use comparable concentrations of the other glycopeptides is directly related to the EOF velocity in these systems. Vancomycin-based separation systems have lower EOFs than either teicoplanin- or ristocetin A-based systems. Obviously, the EOF velocity is inversely related to the experimentally measured EOF times. The EOF times used to calculate the data in Table 2 for vancomycin, ristocetin A, and teicoplanin were approximately 12.0, 7.5, and 9.0 min, respectively.

In comparable CE experiments at pH values between ~4 and 7, the vancomycin-based separations not only had the longest EOF times (i.e., the slowest EOF velocities) but also produced the

(45) Nieto, M.; Perkins, H. R. *Biochem. J.* **1971**, *123*, 773.
(46) Corti, A.; Soffientini, A.; Cassani, G. *J. Appl. Biochem.* **1985**, *7*, 133.

greatest band broadening.[18] Also, it has been demonstrated that increasing the run buffer concentration of any of these three glycopeptides tends to further decrease the EOF velocities.[18−20] Viscosity differences in these experiments were minimal (because of the low concentrations used) and could not be used to account for the EOF changes.[18−20] The reason for the decreased EOF velocities is that all of these glycopeptides have amine functional groups and can associate with the capillary wall (see Figure 1). Vancomycin appears to associate most strongly with the capillary wall. It is the smallest glycopeptide, it has two amine groups, and it is positively charged under the conditions of this study (Figure 1). Neither teicoplanin nor ristocetin A appears to bind to the capillary as extensively as vancomycin (as indicated by the EOF times, the elution times, and the band broadening of the analyte concentration profiles). The reasons for this involve their individual structures. For example, at least three structural features help to limit the interaction of teicoplanin (Figure 1) with the capillary wall. First, teicoplanin has only a single amine group on the aglycon "basket". Consequently, it tends to be zwitterionic or slightly anionic at the pH values used for CE separations. Teicoplanin also has more steric bulk (in the form of an additional sugar moiety, a hydrophobic tail, and a fourth macrocyclic ring), which helps to limit a close approach between its lone amine group and the capillary silanol groups. Finally, teicoplanin aggregates to form micelles.[20,46] It was recently shown that incorporation of glycopeptides into micelles greatly decreases wall binding effects by lowering the monomer concentration in the run buffer.[21] Although ristocetin A does not aggregate, it is significantly larger than the other two glycopeptides. It contains more and larger hydrophilic saccharide moieties. This steric bulk must help to limit its wall interactions. Recently, Ward et al. found that coated capillaries effectively suppressed the Columbic interactions between vancomycin and the capillary wall.[24]

Another way to minimize interactions between the glycopeptides and the capillary wall, as well as to decrease separation times, is to increase the concentration (i.e., ionic strength) of the background electrolyte and to increase the applied voltage, respectively.[18−20] Unfortunately, both of these actions result in increased Joule heating. It is well known that Joule heating decreases the efficiency of CE separations. However, there is an additional concern when using glycopeptide antibiotics. The stability of these compounds decreases substantially at temperatures above 35 °C.[18−20] Consequently, previous publications involving the glycopeptide antibiotics tended to use somewhat higher than normal (for CE) background electrolyte concentrations but lower than normal run voltages.[18−20] Typical separation conditions are shown in Table 3. Note that there is a compromise between using higher ionic strength to decrease the wall interaction of vancomycin and using lower voltages (+5 kV) to decrease Joule heating. Also, shorter capillaries are typically used in these separations, since the enantioselectivities of these macrocyclic antibiotics are greater than those of most other chiral selectors.[18−20] Clearly, the optimization of any CE separation involving glycopeptides must take these additional factors into account. This is particularly true for vancomycin, which is the least stable of the three glycopeptides and binds most extensively with the capillary wall.

Effect of Organic Modifiers and pH. Both the presence of organic modifiers and variations in pH are known to affect CE enantioseparations when using macrocyclic antibiotics as chiral selectors.[17−22] Since these effects have been discussed previously for the individual glycopeptides, they will only be summarized here. However, the effect of the self-association of teicoplanin on enantioselectivity has not been considered previously. There is a major difference in the effect of small amounts of added organic cosolvents on glycopeptide-based enantioseparations. Over 75% of all teicoplanin-based separations require small amounts of acetonitrile in order to achieve the best enantiosparations (or often any separation), as seen in Figure 4. Alcohol organic modifiers tend to precipitate teicoplanin from solution.[20] Conversely, vancomycin-based enantioseparations are rarely enhanced and frequently are degraded when small amounts of miscible, organic cosolvents are added to the run buffer.[18] Figure 5 shows the effect of organic solvents on ristocetin A-based enantioseparations. In this case, the increase in enantioresolution of 2-(3-chlorophenoxy)-propionic acid is thought to result from the decrease in electroosmotic flow that occurs upon addition of organic cosolvents. As can be seen, there is the expected increase in migration times (Figure 5C). The reason organic modifiers produce different effects for different glycopeptides is thought, in part, to be that teicoplanin aggregates in aqueous solution while vancomycin and ristocetin A do not.[18,20] Preliminary evidence shows that as little as 5% added acetonitrile can inhibit the aggregation of teicoplanin.[15] Apparently self association can sometimes hinder or alter chiral recognition. This notion is supported by other recent reports involving a completely different class of compounds.[17,23] Rifamycin B and rifamycin SV are surface-active *ansa* compounds that were used in CE to resolve several chiral amine-containing analytes.[17,23] In virtually every experiment, miscible organic cosolvents were needed to obtain or enhance the enantioseparation.

pH can have a significant effect on enantioseparations when ionizable chiral selectors, such as glycopeptide antibiotics, are used. Unlike for neutral cyclodextrins, the pH of the running electrolyte governs not only the charge and migration behavior of the chiral analytes but also that of the chiral selector. Therefore, the role of pH is much more complex with glycopeptide macrocyclic antibiotics than with other nonionizable chiral selectors used in CE. In general, decreasing the pH of the run buffer increases the enantioseparations of the test solutes. This is shown in Figure 6 for the compounds FMOC- (A) and AQC-α-amino-DL-pimellic acid (B), utilizing vancomycin and ristocetin A, respectively, as the chiral selector. Interestingly, the change in the EOF velocity with pH was relative small with all three of the glycopeptide antibiotics. It is important to note that there is a limit to this pH effect, since lowering the pH below 5 can result in protonation of the chiral analyte, making it a neutral species. Also vancomycin, teicoplanin, and ristocetin A are not stable in solution below pH 4 or above pH 9. Enhanced enantioseparations for vancomycin and ristocetin A were found at pH values slightly below their p*I* values (pH 7.2 and 7.5, respectively). However, optimum enantioseparations using teicoplanin as an electrolyte additive were achieved at pH values above its isoelectric point (pH ∼3.8). It is well known that maximum enantioselectivities are most often achieved when the electromigrations of the chiral selector and chiral solutes are opposite to one another.[18,47]

Micellar Effects. Recently, sodium dodecyl sulfate (SDS) micelles were shown to have a profound effect on the vancomycin-based enantioseparations.[21] The addition of SDS micelles to the

(47) Wren, S. A.; Rowe, R. C. *J. Chromatogr.* **1992**, *609*, 363.

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Table 3. Typical CE Separation Conditions (Using Vancomycin as a Chiral Selector) for Several Racemates Not Previously Reported[a]

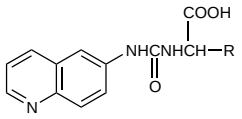
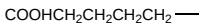
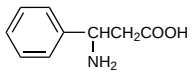
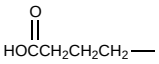
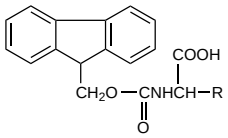
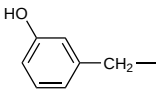
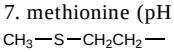
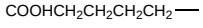
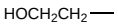
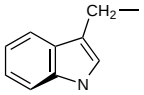
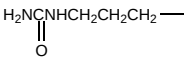
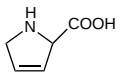
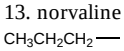
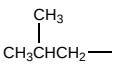
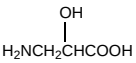
compound (R)	resolution	time(1)[b]	time(2)[c]	$\mu_e(1)$[d]	$\mu_e(2)$[d]
AQC Amino Acids					
					
1. α-aminopimellic acid (pH 7.0) 	10.1	28.6	40.0	−7.9	−9.5
2. 3-aminophenylpropionic acid (pH 7.0) 	2.7	16.5	19.5	−3.7	−5.2
3. α-aminoadipic acid (pH 7.0) 	5.5	44.0	53.0	−9.8	−10.5
FMOC Amino Acids					
					
4. valine pH 7.0 	1.6	14.6	15.3	−2.4	−2.9
5. *m*-tyrosine (pH 7.0) 	4.9	11.6	14.1	−0.8	−3.2
6. alanine (pH 7.0) 	3.0	13.5	14.9	−1.5	−2.6
7. methionine (pH 7.0) 	2.5	12.9	13.9	−0.9	−1.9
8. α-aminopimellic acid (pH 7.0) 	7.7	20.0	30.4	−5.4	−8.2
9. homoserine (pH 7.0) 	1.2	13.4	14.0	−1.4	−1.9
10. tryptophan (pH 7.0) 	10.6	12.7	14.5	−0.4	−34
11. citrulline (pH 7.0) 	2.9	14.1	15.1	−2.0	−2.8
12. 3,4-dehydroproline (pH 7.0) 	0.8	16.0	16.4	−3.4	−3.6
13. norvaline (pH 7.0) 	2.3	14.3	15.3	−2.2	−2.9
14. leucine (pH 7.0) 	1.8	14.7	15.5	−2.5	−3.1
15. isoserine (pH 7.0) 	1.8	14.7	15.5	−2.3	−3.2

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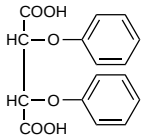
Table 3 (Continued)

compound (R)	resolution	time(1)[b]	time(2)[c]	$\mu_e(1)$[d]	$\mu_e(2)$[d]
16. 2,3-dibenzoyl-DL-tartaric acid (pH 7.0)	2.3	32.3	34.7	−8.5	−8.9



[a] The run buffer was 0.1 M sodium phosphate buffer at pH 7.0, and the capillary was 50 μm × 32.5 cm (effective length of 25 cm). A 2 mM solution of vancomycin was used, and the run voltage was +5 kV. The migration times, effective mobilities, and enantioresolutions are shown. [b] Migration time (in minutes) of the first-eluting enantiomer. [c] Migration time (in minutes) of the second-eluting enantiomer. [d] $\mu_e(1)$ and $\mu_e(2)$ are the effective electrophoretic mobilities of the first- and second-eluting enantiomers, respectively, in $cm^2 \cdot kV^{-1} \cdot min^{-1}$.

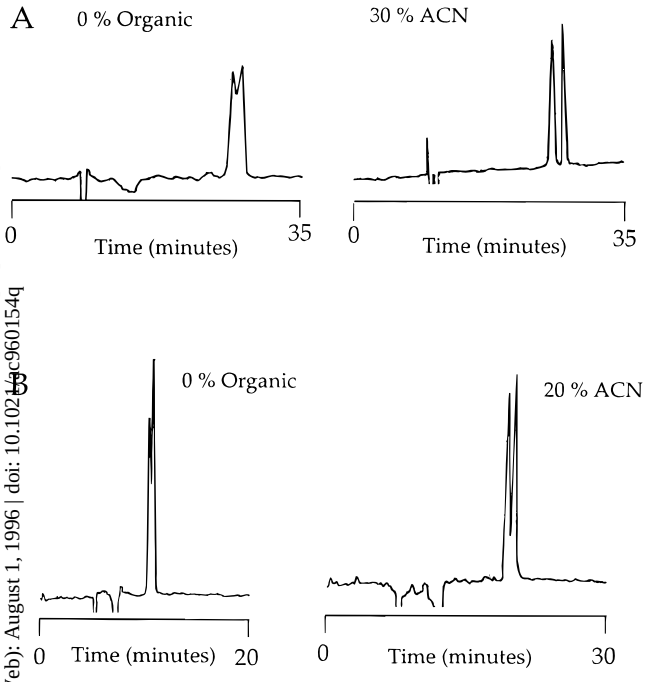


Figure 4. Capillary electropherograms comparing the effects of organic modifiers on the enantiomeric resolution of the racemates of (A) 2-(2-chlorophenoxy)propionic acid and (B) *N*-benzoyl-DL-valine, using a 2 mM solution of teicoplanin at pH 6.0. The designated concentrations of acetonitrile are shown above. Other experimental conditions were as follows: run voltage, +5 kV; temperature, 22 °C; UV detection, 254 nm; and capillary length, 32.5 cm × 50 μm i.d., or 25 cm to the detection window.

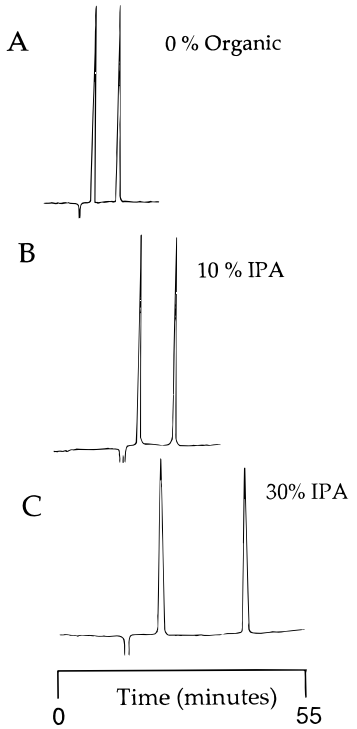


Figure 5. Capillary electropherograms showing the effect of organic cosolvents on the enantioseparation of (A) 2-(3-chlorophenoxy)-propionic acid and (B) *trans*-4-cotininecarboxylic acid. 2 mM ristocetin A in 0.1 M phosphate buffer was used at pH 7.0. Other experimental conditions were the same as in Figure 7.

CE run buffer was shown to enhance efficiency by an order of magnitude, decrease analysis time, reverse the enantiomeric elution order, and decrease the overall enantioselectivity.[21] Typical effects of SDS micelle concentration on migration times and electrophoretic mobilites are shown in Figure 7. The theory and mechanism of these phenomena were described in detail.[21] Until now, SDS effects on teicoplanin and ristocetin A CE separations have not been considered.

To understand the effect of SDS micelles on glycopeptide-based enantioseparations, the interactions between these two species must be considered. As can be seen in Table 4, all three glycopeptides bind to SDS micelles, thereby forming comicelles of sorts. However, the magnitudes of their binding constants (K_bs) are quite different, with teicoplanin's K_b approximately 4 times that of vancomycin. Under the conditions of these experiments, vancomycin is approximately 90% micelle bound, leaving 10% in free solution.[21] Usually, the binding of an enantiomer to the free glycopeptide is significantly different than that to the SDS−glycopeptide co-micelle. This is to be expected, since the SDS micelle has an overall negative fraction of charge.[48] Also, the close association between the micellar surfactants and the glycopeptide could affect an analyte's interaction with the chiral selector. For example, dansyl-L-valine has a K_b of 143 to vancomycin and a K_b of 21 to the vancomycin−SDS comicelle.[21] Consequently, there is a reversal in the enantiomeric elution order when SDS is added to the run buffer. In general, acidic or anionic analytes tend to bind more strongly to the positively charged free vancomycin than to the negatively charged SDS−vancomycin complex or comicelle. Also, the presence of micelles tends to decrease the amount of glycopeptide binding to the wall of the capillary.[21] This has two beneficial effects: (1) it increases the electroosmotic flow, which decreases migration times, and (2) it increases separation efficiency.

As shown in Figure 8A, the addition of SDS to a teicoplanin-based separation produces effects very similar to those of the

(48) Rosen, M. J. *Surfactants and Interfacial Phenomena*; John Wiley & Sons: New York, 1978.

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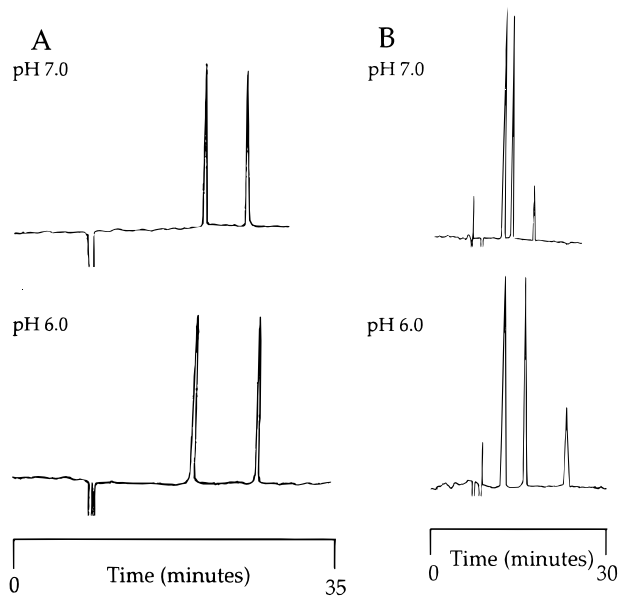

Figure 6. (A) Electropherograms showing the effect of pH on the resolution of FMOC-α-amino-DL-pimellic acid using vancomycin as a chiral selector. In part B the pH effects on enantioresolution of AQC-α-amino-DL-pimellic acid are shown using ristocetin A as a chiral selector added to the running buffer. The run voltage for all separations was +5 kV, and the analytes were detected at 254 nm. The capillary was 50 μm × 32.5 cm, 25 cm to the detector.

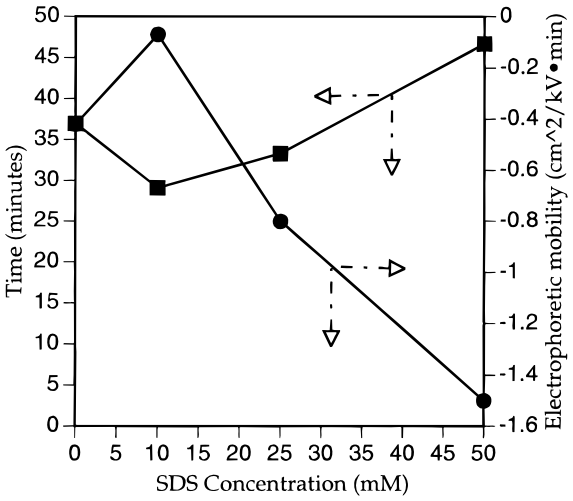

Figure 7. Plots of the electrophoretic mobility (●) and migration time (■) versus SDS concentration for ketoprofen using vancomycin as a chiral selector. Note that, at surfactant concentrations near but exceeding the critical micelle concentration (cmc) the electrophoretic mobility increases, and the migration time decreases. However, at higher [SDS], the trend reverses.

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vancomycin experiments. Chiral analytes with larger binding constants to teicoplanin often show a reversal in the enantiomeric elution order (Figure 8A). However, SDS-mediated reversal is not a universal phenomenon, as shown in Figure 8B. Some analytes (particularly those with smaller K_b values, Figure 8B) can have the same retention order.

Ristocetin A appears to behave somewhat differently than the other two glycopeptides. Although it binds to SDS micelles (Table 4), it does not bind strongly to the capillary wall. Consequently, one does not see the extensive increase in efficiency and decrease in migration times found for vancomycin. There is a significant concentration-dependent effect on selectivity, however. By controlling the ratio of SDS to ristocetin A, an enantioseparation can be enhanced or degraded. This is shown in Figure 9B and C. The best separation is achieved at 25 mM SDS for both racemic ketoprofen and indoprofen. Higher or lower concentrations of SDS produce poorer separations. Also, reversal of enantiomeric retention does not appear to be as frequent in the SDS−ristocetin A system. Clearly, this system is interesting and unusual. Ristocetin A has a greater number of hydrophilic moieties than the other glycopeptides. Also, it has two amine groups but no carboxylate group (Figure 1). Consequently, it may associate mainly with the surface of the SDS micelle, like a large counterion. More extensive work must be done before CE separations in the SDS−ristocetin A system can be completely explained.

Table 4. Equilibrium Association Data for Three Structurally Related Glycopeptide Macrocyclic Antibiotics with Sodium Dodecyl Sulfate

chiral selector	equilibrium constant (K)	partition coefficient (P)
vancomycin	290	1200
ristocetin A	480	1900
teicoplanin	1300	5300

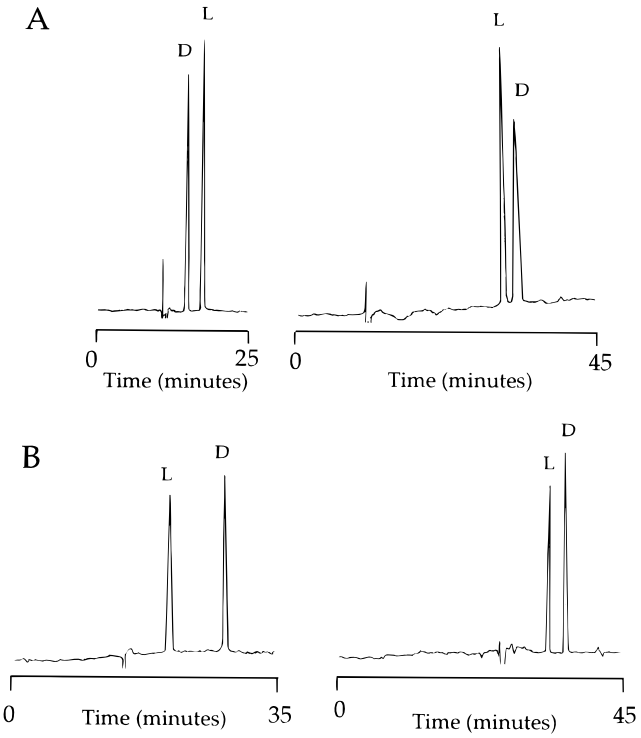

Figure 8. Electropherograms showing the effect of SDS micelles on the enantiomeric elution order. The run buffer contained 25 mM SDS and 2 mM chiral selector. The other experimental conditions were as follows: run voltage, +5 kV; temperature, 20 °C; UV detection, 254 nm; and capillary length, 27 cm × 50 μm i.d., 20 cm to the detection window. (A) The enantiomeric elution order reversed for the compound AQC-DL-methionine using the glycopeptide, teicoplanin. (B) The enantiomeric elution order remained the same with the addition of SDS micelles to the system using ristocetin A as the chiral selector.

Enantioselectivity and the Principle of Complementary Separations. The glycopeptide antibiotics vancomycin, ristocetin A, and teicoplanin are related to one another by structure and action. These similarities extend to their enantioselectivities as well. In most cases, the glycopeptides have the highest enantio-

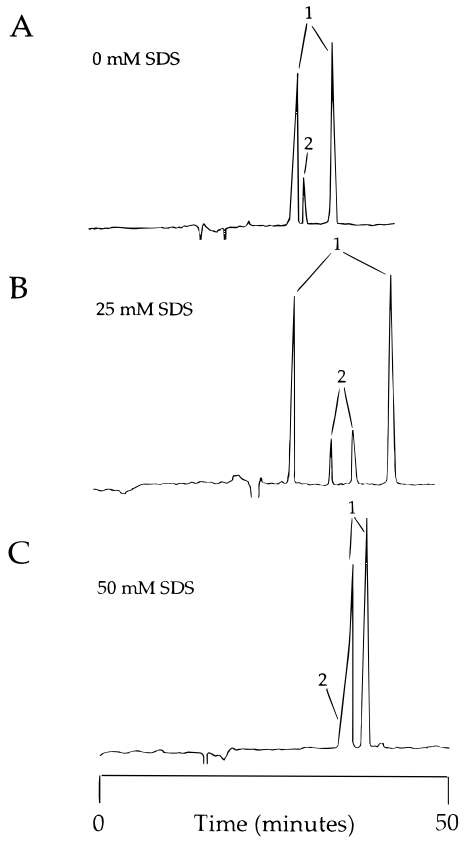

Figure 9. Electropherograms showing the effect of SDS on the enantiomeric separation of a racemic mixture of ketoprofen and indoprofen using ristocetin A as a chiral selector. All separations were performed with 2 mM solutions in 0.1 M, pH 6 phosphate buffer at 20 °C, with the indicated concentration of SDS. The run voltage was +15 kV, and solutes were detected directly at 254 nm. The capillary was 50 μm × 27 cm, effective length of 20 cm. Peak designations are as follows: (1) ketoprofen and (2) indoprofen.

selectivities for compounds containing an acidic or anionic moiety (e.g., carboxylate, phosphate, sulfonate groups, etc.). It appears to be beneficial when these groups are α or β to the stereogenic center (Tables 2 and 3). Enantioselective recognition seems to be enhanced when a chiral compound contains a carbonyl group, an aromatic ring, or an amide nitrogen in close proximity to the stereogenic center (i.e., α, β, or γ position). In the case of 1,1′-binaphthyl-2,2′-diyl dihyrogen phosphate (entry 9, Table 2), the acidic group is an integral part of the stereogenic axis. It appears that among the most important primary sites of interaction are the glycopeptide amine groups. The initial contact or "docking" of an analyte to these glycopeptide antibiotics may well be via these groups. Conversely, the carboxylic acid moieties on the aglycon (Figure 1) do not seem to play a significant role in chiral recognition as far as the compounds and conditions of this study are concerned. In the case of ristocetin A, the carboxylate is esterified, and yet it seems to have the broadest enantioselectivity and produce the shortest migration times. Furthermore, it was noted in a recent LC paper involving teicoplanin that the carboxylate group seemed to have no effect or an adverse effect on the enantioseparation of some amino acids.[14] However, the carboxylate group appeared to play a significant role in the nonenantioselective retention of amine-containing chiral compounds.[14]

Additional simultaneous interactions must occur between the glycopeptides and the chiral analytes if an enantioselective separation is to occur. The most likely of these are a hydrophobic association with the aglycon pocket and hydrogen bonding or dipolar interactions with the pendant sugar moieties, the phenolic hydroxyls, and/or the peptide linkages. Clearly these interactions will not be identical among the glycopeptides, since their aglycons differ somewhat in size and shape. The number, type, and location of their saccharide moieties differ as well. The repulsion of these bulky groups may provide additional steric interactions in some cases. The exact nature and location of these "secondary" interactions are not known at the present time. It is complicated by the fact that the saccharide moieties are free to rotate and can assume a variety of configurations. It is believed that on-going high-field NMR studies will provide additional information in the near future.

While the glycopeptide antibiotics belong to the same structural family and have somewhat analogous enantioselectivities, they have some distinct differences as well. These structural differences have been thoroughly described in the preceding portion of this work. As can be seen from the results in Table 2, the variations in glycopeptide structure can sometimes produce distinct selectivities. Among the 28 compounds evaluated in Table 2, only the antineoplastic compound, methotrexate, and a few derivatized amino acids were baseline resolved with all three glycopeptides under analogous conditions. Ristocetin A seemed to have the broadest enantioselectivities (separates the most compounds), while teicoplanin resolves the fewest. However, there are specific cases where only one or another macrocyclic antibiotic can achieve a separation. For example, the greatest enantioresolution of 1-benzocyclobutenecarboxylic acid (entry 6, Table 2) was obtained using vancomycin as a chiral selector, while both ristocetin A and teicoplanin produced only partial separations. Mandelic acid (entry 8) is an interesting example, where teicoplanin prevailed as the most enantioselective chiral additive. A resolution of 3.4 was obtained with teicoplanin, and 2.0 was obtained with ristocetin A, while vancomycin was unable to resolve the enantiomers of mandelic acid or any other substituted mandelic acids. Test solutes 3, 9−12, and 14−16 are examples where ristocetin A provided the highest enantioselectivities and, in most cases, had the shortest elution times. In general, ristocetin A resolves about as many racemates in CE as teicoplanin and vancomycin combined.[18−22]

The last 10 compounds in Table 2 are a few examples of the large number of N-functionalized amino acids that were enantioresolved. For the majority of compounds studied, the greatest enantioresolutions for the aromatic derivatized amino acids (i.e., AQC, FMOC, *N*-benzoyl, dansyl, PHTH, and 2,4-DNP) were obtained with ristocetin A as a chiral selector, whereas the aliphatic derivatized amino acids (i.e., *N*-formyl and *N*-acetyl) were best resolved using vancomycin. In general, vancomycin enantioresolved more derivatized amino acids, while teicoplanin separated the fewest of the three glycopeptides studied.

The data in Table 2 indicate that the glycopeptide antibiotic chiral selectors are complementary to one another. They have somewhat similar but not identical enantioselectivities. Consequently, if only a partial enantioresolution can be obtained with one glycopeptide, there is a high probability that a baseline or better separation can be obtained with one of the others. This "principle of complementary separations" is shown in Figure 10. Note that the "principal of complementary separations" (in this context) means that one or another of the glycopeptide selectors will produce a superior enantioseparation for any compound

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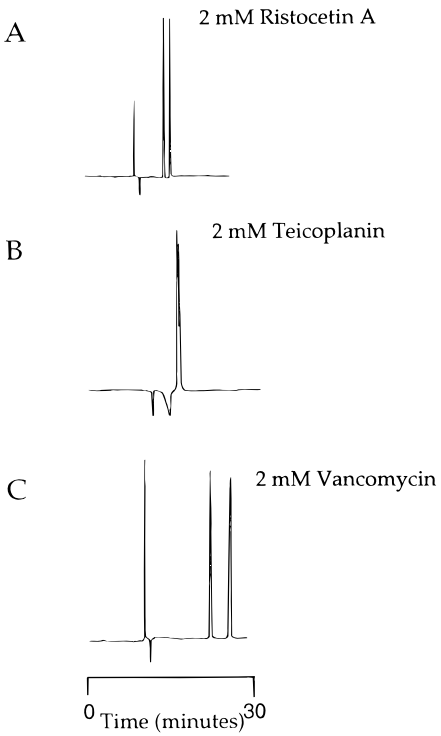

Figure 10. Electropherograms comparing the enantioresolution of racemic ketoprofen using (A) 2 mM ristocetin A, (B) 2 mM teicoplanin, and (C) and 2mM vancomycin at pH 6.0 in 0.1 M phosphate buffer. Detection was at 254 nm and the capillary was 32.5 cm × 50 μm i.d. capillary (25 cm to the detector window). The run voltage was +5 kV and separation were carried out at ambient temperature.

belonging to one structural class (i.e., acids or anionic solutes in this case). This means that their basic enantioselective retention mechanisms are related. While we cannot yet predict a priori which glycopeptide antibiotic will give the best resolution for a given analyte, it appears that a partial resolution with one selector usually can be improved upon by using one of the structurally related macrocyclic glycopeptide chiral selectors. Previously, this has shown to be a very useful optimization approach in HPLC using related chiral stationary phases.[13] This is the first time that it has been demonstrated that a similar approach can be used in CE.

Stability. The stability of macrocyclic antibiotics in aqueous and in hydroorganic solvents is a major concern when choosing a chiral selector for CE. All of the glycopeptide macrocyclic selectors decomposed with time, under the conditions of this study. Upon degradation, the migration times of the test solutes usually increased.[18−20] Vancomycin was determined to be the least stable macrocyclic antibiotic. Solutions between pH 5 and 7 deteriorated within 6−7 days when refrigerated at 4 °C. Teicoplanin solutions in the same pH range were somewhat more stable since the EOF velocities and enantioresolutions were reproducible for approximately 2−3 weeks. The most hydrolytically stable macrocyclic antibiotic was found to be ristocetin A. Solutions were successfully utilized for more than 4 weeks when certain precautions were taken. For example, glycopeptide solutions should be refrigerated at 4 °C overnight or when not in use. Excessive temperature (≥ 35 °C) and pH extremes (pH greater than 7 or less than 4) should be avoided. The stabilities of vancomycin, teicoplanin, and ristocetin A solutions at room temperature (22 °C) were reduced to 2−3 days, 3−4 days, and 6−7 days, respectively. Complete antibiotic deterioration occurs upon heating in neutral or basic conditions (pH 7−12) and at temperatures slightly above 22 °C. Deactivation is believed to occur due to the cleaving of the sugar moieties and/or by the opening of at least one macrocyclic ring through hydrolysis of amide bond(s).[18−20] Deterioration of the antibiotic/electrolyte solution is evidenced by the appearance of a slightly yellow color. Eventually, a murky solution forms, and the glycopeptide products may precipitate or fall out of solution. CE baseline noise increases significantly when solutions of the glycopeptide begin to degrade. Also, the precipitate formed can cause plugging or other irregularities in separation performance. Degradation of the solid antibiotic substance has been observed after storing for 2 months under hydrated conditions at 4 °C. The best way to store the solid material is under anhydrous condition and at <0 °C.

It is important to note that the semidegraded solutions sometimes can still produce enantiomeric separations. However, it is not uncommon to observe an increase in migration times and baseline noise and a decrease in the enantiomeric resolutions. For example, when a resolution of 10 is obtained with a "fresh" antibiotic solution, a semidegraded mixture may still provide a more than adequate baseline resolution between 2 and 5. Interestingly, the solution stability of the glycopeptides increases with increasing number of attached saccharide moieties. However, it is not known whether or not this is coincidental.

CONCLUSIONS

The three related macrocyclic glycopeptides antibiotics (vancomycin, ristocetin A, and teicoplanin) differ from one another in terms of the morphology of their aglycon "baskets" as well as the number, type, and location of their substituents. Consequently, they tend to have similar but not identical enantioselectivities. Presumably, any of their unique selectivities can be related to the differences in their morphology and/or subtituent groups. In addition, teicoplanin is the only one of these three glycopeptides that has an appended hydrocarbon chain, which rendered it surface active. Self-aggregation appears to inhibit or decrease enantioselective CE separations. Limiting or inhibiting self-aggregation tends to enhance enantioselectivity. Addition of a secondary achiral surfactant (SDS) just over its critical micelle concentration tends to decrease migration times, increase efficiency, and reverse the retention order for analytes that have large binding constants to vancomycin and teicoplanin. However, addition of SDS to a ristocetin A-based CE separation can either increase or decrease enantioselectivity. These effect are highly variable and dependent on the SDS concentration. Overall, ristocetin A appears to be the most useful CE chiral selector, since it can resolve the greatest variety and number of racemates in the shortest time.

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