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Evaluation of Dalbavancin as Chiral Selector for HPLC and Comparison with Teicoplanin-Based Chiral Stationary Phases

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ABSTRACT Dalbavancin is a new compound of the macrocyclic glycopeptide family. It was covalently linked to 5 μm silica particles using two different binding chemistries. Approximately 250 racemates including (a) heterocyclic compounds, (b) chiral acids, (c) chiral amines, (d) chiral alcohols, (e) chiral sulfoxides and sulfilimines, (f) amino acids and amino acid derivatives, and (g) other chiral compounds were tested on the two new chiral stationary phases (CSPs) using three different mobile phases. As dalbavancin is structurally related to teicoplanin, the same set of chiral compounds was screened on two commercially available teicoplanin CSPs for comparison. The dalbavancin CSPs were able to separate some enantiomers that were not separated by the teicoplanin CSPs and also showed improved separations for many racemates. However, there were other compounds only separated or better separated on teicoplanin CSPs. Therefore, the dalbavancin CSPs are complementary to the teicoplanin CSPs. *Chirality 22:495–513, 2010.*

KEY WORDS: dalbavancin; teicoplanin; chiral stationary phases; HPLC; macrocyclic glycopeptides; racemates; enantiomeric separation

INTRODUCTION

Macrocyclic antibiotics were first introduced as a new class of chiral selectors for enantioseparations by HPLC and capillary electrophoresis in 1994.[1] The ANSA family (rifamycin B and SV)[2,3] and glycopeptide group (vancomycin, ristocetin, and teicoplanin[4–8]) were demonstrated to have the most advantageous structures for enantiomeric separations. There are many structurally related oligophenolic glycopeptides belonging to the later group that have proven to be useful. Thus far, vancomycin, ristocetin, teicoplanin, A82846B,[9] LY307599,[10] avoparcin,[11] and A40926[12] of the macrocyclic glycopeptide family have been evaluated as chiral selectors. These chiral selectors can be further divided into two groups, according to the number of fused rings in the aglycone part of their structure. In comparison, vancomycin types have a three ring aglycone, whereas the teicoplanin-type glycopeptides have one more ring in the aglycone which makes it "semirigid." They all show great selectivity over a wide rage of chiral molecules including amino acids, carboxylic acids, and neutral compounds. Their excellent enantioselective separation capabilities has been attributed to the richness of different functional groups in their structures, such as aromatic rings with and without chloro-substituents, ionizible phenolic moieties, amino groups, amide groups, carboxylates, and carbohydrate moieties. Therefore, many kinds of intermolecular interactions, such as π–π and dipole–dipole interactions, hydrophobic interactions, and hydrogen bonding, can be involved in the chiral recognition via association with these functional groups.[13]

Although all the macrocyclic glycopeptides are within the same family of compounds, small changes in their structure can result in significant differences in their enantiorecognition abilities. For example, α-amino acids are better separated on the teicoplanin aglycone-based chiral stationary phases (CSPs), which is produced by cleaving all the carbohydrate moities from teicoplanin.[14] In the case of A40926, there are only a few small structural variations compared with teicoplanin. However, it is found that some compounds can only be separated or better separated on the HPLC CSP based on A40926, whereas the teicoplanin column separates a larger number of racemates.[12]

Dalbavancin is a new semisynthetic lipoglycopeptide derived from A40926, a naturally occurring glycopeptide produced by actinomycete *Nonomuraea* species.[15] It has enhanced activity against gram-positive bacteria and unique pharmacokinetics compared with existing drugs in its class.[16] In this work, two CSPs were prepared by binding dalbavancin to two different 5 μm spherical silica gels, respectively, as to mirror the synthesis and make up of the Chirobiotic T and T2 columns. They are designated as D1 and D2. Their enantioseparation capabilities were evaluated with 250 pairs of enantiomers containing different functional groups. These analytes were also screened on

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the commercial teicoplanin CSPs (i.e., Chirobiotic T and Chirobiotic T2) for comparison, and these two CSPs are designated as T1 and T2.

MATERIALS AND METHODS

Materials

All the racemic analytes tested in this study were purchased from Sigma-Aldrich, and all HPLC-grade solvents were obtained from VWR (Bridgeport, NJ). HPLC-grade Kromasil silica gel (particle size 5 μm, pore size 100 Å, and surface area 310 m^2/g) was obtained from Akzo Nobel (EKA Chemicals, Bohus, Sweden), and LiChrospher Si(100) silica gel (particle size 5 μm, pore size 100 Å, and surface area 400 m^2/g) was purchased from Merck (Darmstadt, Germany). All organosilane compounds were obtained from Silar Laboratories (Wilmington, NC). These include: (3-aminopropyl) dimethylethoxysilane, (3-aminopropyl) triethoxysilane, [2-(carbomethoxy) ethyl] trichlorosilane, [1-(carbomethoxy)ethyl] methyldichlorosilane, (3-isocyanatopropyl) triethoxysilane, and (3-glycidoxypropyl) triethoxysilane. Dalbavancin was the generous gift of Pfizer (Washington, MO).

Methods

Preparation of the D1 CSP. One gram of dried dalbavancin (0.53 mmol) was dissolved in 55 ml of anhydrous DMF in a 250-ml three-neck round flask with mechanical stirring. Then, triethylamine (0.72 ml, 5.16 mmol) and 3-(triethoxysilyl)propyl isocyanate (0.865 ml, 3.50 mmol) were added into the solution at room temperature under argon protection. The solution was heated to 95°C for 5 h and cooled to 60°C. The dried Kromasil silica (3.50 g, 5 μm, 100 A°) was added into the solution. The mixture was heated to 105°C over night and then cooled to room temperature and filtered. The CSP was washed by methanol, methanol/water (50/50, v/v), pure water, and methanol (50 ml for each solvent), and dried in oven at 100°C overnight. Elemental analysis showed 8.0% carbon loading.

Preparation of the D2 CSP. The D2 stationary phase was prepared as previously described for the teicoplanin CSP. Five grams of Lichrospher silica gel was first dried at 150°C under vacuum, and then it is heated in toluene to reflux to remove azaeotropically all residual water. It is followed by adding 2.5 ml of 3-aminopropyl triethoxysilane, and the reaction mixture was heated to reflux for 4 h. The modified silica gel was filtered, washed with toluene, methanol, and dichloromethane, and dried at 90°C overnight. Elemental analysis showed that the derivatized silica gel has 4.0% carbon loading. A 2.5 ml portion of 1,6-diisocyanatohexane (15 mmol) was added to an ice-bath-cooled slurry of 2.5 g of 3-aminopropyl-Lichrospher in 50 ml of anhydrous toluene. Next, the mixture was heated at 70°C for 2 h. After cooling, the supernatant toluene phase was removed under an argon atmosphere. The excess reactant was removed by dry toluene washing. A suspension of 1 g of dalbavancin (0.53 mmol) in 100 ml of dry pyridine was added dropwise to the wet activated silica. Next, the mixture was heated at 70°C for 12 h with stirring under an argon atmosphere. After cooling, the dalbavancin bonded silica was washed with 50 ml portions in the sequence pyridine, water, methanol, acetonitrile, and dichloromethane. It was dried under vacuum. Elemental analysis showed 11.0% carbon loading (increased by 7.0%).

Chromatographic condition. CSPs were slurry packed into 250 mm × 4.6 mm (i.d.) stainless steel columns at 600 bar. Evaluation of the columns was conducted on HP 1090 HPLC system with a DAD UV detector and autosampler. Detection wavelengths were selected at 220, 230, and 254 nm. The injection volumes were 5 μl. All sample concentrations were 1 mg/ml. Separations were carried out under isocratic conditions at a flow rate of 1 ml/min at 21°C. The mobile phases were premixed and degassed under vacuum for 10 min. The column dead times were tested by injection of solution of 1,3,5-tri-*tert*-butylbenzene in 100% methanol.

RESULTS AND DISCUSSION

The Structure of Dalbavancin

Dalbavancin is a second generation glycopeptide antibiotic molecule (see Fig. 2). The major difference between dalbavancin and teicoplanin are as follows: (a) different phenyl rings are chloro-substituted (see rings 2 and 3, Figs. 1 and 2); (b) the β-D-*N*-acety-glucosamine unit of teicoplanin (see ring 5, Figs. 1 and 2) is replaced by a simple hydroxyl group; (c) the primary hydroxyl group of *N*-acyl-glucosamine unit of teicoplanin has been oxidized to a carboxylic acid, which can generate an anion; (d) the primary amine group on the aglycone portion of teicoplanin is a secondary amine substituted by methyl group; (e) the carboxylic group close to phenyl ring 7 is converted to an amide group connected with three methylene groups and it has a dimethylamino group at the end (in dalbavancin); and (f) dalbavancin has 10 carbons in the carbon chain of β-D-*N*-acyl-glucosamine, whereas teicoplanin has only nine carbons. The last difference noted earlier is the least likely to affect enantioseparation because one more methylene group does not provide any additional interactions that are beneficial to chiral recognition. Previous studies by our group have shown that the teicoplanin carbohydrate units play an important role in chiral recognition. It helps in the separation of nonamino acid compounds. However, they also decrease the separation of many α-amino acid enantiomers.[14] Thus, the elimination of the β-D-*N*-acety-glucosamine unit in dalbavancin can substantially affect its enantioselectivity. The other changes made to carboxylic groups, hydroxyl group, and amino groups can also contribute to differences in the enantioselectivity of dalbavancin relative to teicoplanin. Dalbavancin has one tertiary amine and secondary amine, respectively, and one carboxylic group on the *N*-acyl-glucosamine (Figs. 1 and 2). Whereas, teicoplanin has only one carboxylic group connected to the aglycone and one primary amino group. As amine and carboxylic acids group are ionizable in aqueous solution and can interact via electrostatic interactions with charged analytes, these changes could lead to different

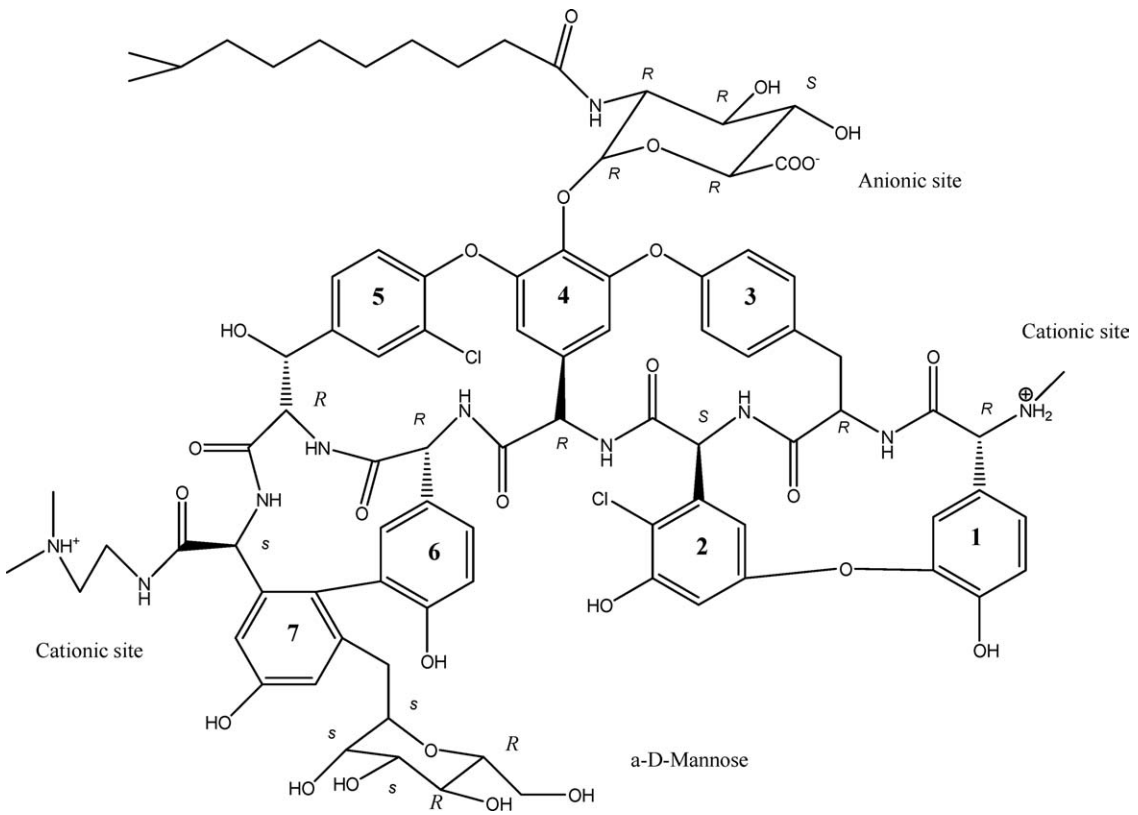


Fig. 1. The structure of the macrocyclic glycopeptide dalbavancin.

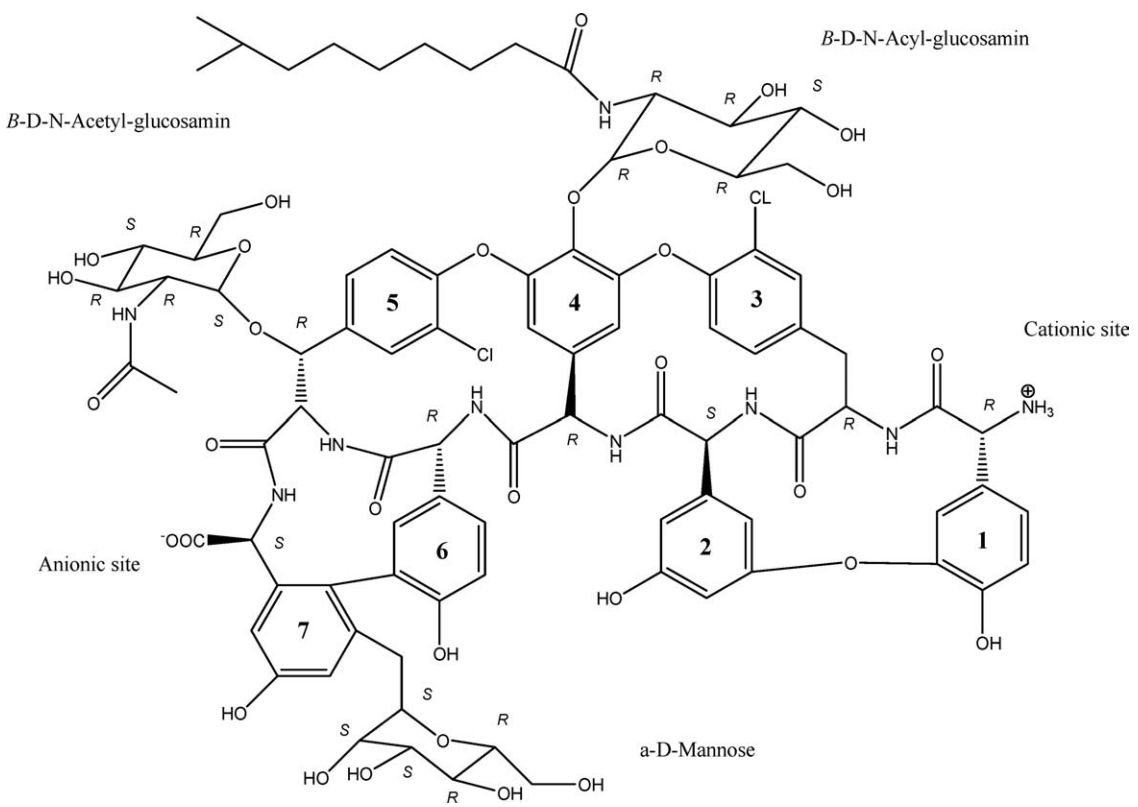


Fig. 2. The structure of the macrocyclic glycopeptide teicoplanin.

TABLE 1. Chromatographic data for the normal phase resolution of racemic compounds on D1, D2, T1, and T2 columns

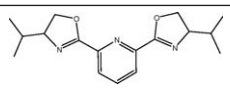
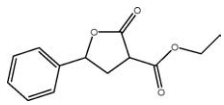
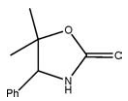
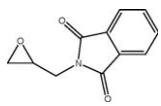
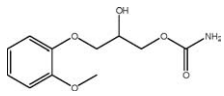
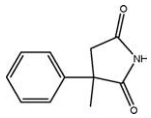
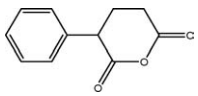
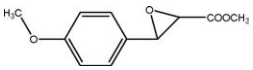
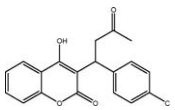
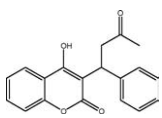
			80% Heptane/20% ethanol		
Compound name	Structure	CSPs	k_1	a	R_s
2,6-Bis(4-isopropyl-2-oxazolin-2-yl)pyridine		D1	1.04	1.18	1.0
2-Carbethoxy-gamma-phenyl-gamma-butyrolactone		D1 D2 T1 T2	1.34 2.90 1.47 1.15	1.13 1.35 1.03 1.07	0.9 1.5 0.5 0.5
5,5-Dimethyl-4-phenyl-2-oxazolidinone		D1 D2 T2	3.93 10.72 4.19	1.38 1.57 1.44	1.4 1.8 1.4
N-(2,3-Epoxypropyl)-phthalimide		D2 T1	2.28 1.99	1.12 1.07	0.9 1.0
Guaiacol glyceryl ether carbamate		T1	7.72	1.05	0.5
alpha-Methyl-alpha-phenyl-succinimide		D1 D2 T1 T2	2.02 4.52 2.37 2.40	1.11 1.44 1.23 1.21	0.9 2.2 1.5 1.4
2-Phenylglutaric anhydride		T1	3.27	1.06	0.5
Methyl trans-3-(4-methoxyphenyl)glycidate		D2	1.07	1.58	1.8
3-(alpha-Acetonyl-4-chlorobenzyl)-4-hydroxycoumarin		D1 D2 T2	0.88 0.85 0.89	1.39 1.21 1.21	1.4 0.7 1.2
Warfarin		D1 D2 T2	0.83 1.91 0.89	1.44 1.17 1.17	1.5 0.8 1.0

TABLE 1. Continued

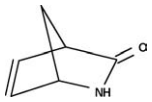
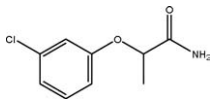
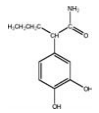
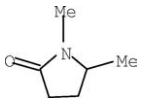
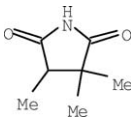
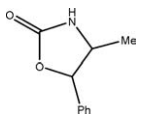
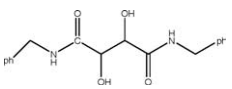
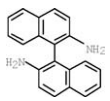
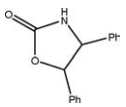
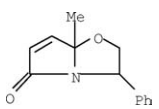
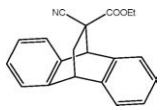
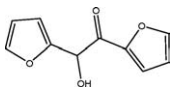
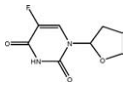
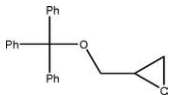
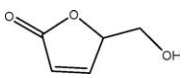
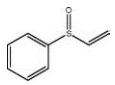
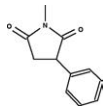
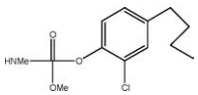
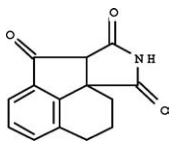
Compound name	Structure	CSPs	80% Heptane/20% ethanol k_1	a	R_s
2-Azabicyclo[2.2.1]-hept-5-en-3-one		D1	4.26	1.29	1.5
		D2	14.52	1.16	0.8
		T1	7.59	1.06	0.7
2-(3-Chlorophenoxy)propionamide		D1	1.49	1.13	1.0
		D2	2.97	1.36	2.2
		T1	2.21	1.09	1.2
		T2	2.18	1.12	1.3
DL-3,4-Dihydroxyphenyl-alfa-propylacetamide		D1	7.43	1.07	0.6
1,5-Dimethyl-2-pyrrolidinone		D2	2.62	1.26	1.3
		T1	3.31	1.03	1.4
alpha,alpha-Dimethyl-beta-methylsuccinimide		D1	1.05	1.33	1.5
		D2	2.10	1.51	2.5
		T1	1.69	1.10	1.4
		T2	1.55	1.16	1.3
1,5-Dimethyl-4-phenyl-2-imidazolidinone		D1	1.57	1.26	1.4
		D2	4.86	1.65	3.6
		T1	3.24	1.70	2.3
		T2	2.78	1.11	1.0
N,N′-Dibenzyl-tartramide		T1	6.41	1.04	0.5
2,2′-Diamino-1,1′-binaphthalene		D2	5.03	1.05	0.5
		T2	3.40	1.16	1.3
cis-4,5-Diphenyl-2-oxazolidinone		D1	4.52	1.66	2.6
2,3-Dihydro-7a-methyl-3-phenylpyrrolo[2,1-b]-oxazol-5(7aH)-one		T1	2.29	1.04	0.8
		T2	1.90	1.05	0.5

TABLE 1. Continued

Compound name	Structure	CSPs	80% Heptane/20% ethanol k_1	a	R_s
Ethyl 11-cyano-9,10-dihydro-endo-9, 10-ethanoanthracene-11-carboxylate		D2	1.03	1.17	0.8
		T1	0.72	1.05	0.5
Furoin		D1	2.61	1.04	1.0
		D2	6.45	1.28	1.3
		T1	3.28	1.22	2.3
		T2	3.88	1.20	1.8
Ftorafur		D1	7.58	1.33	1.4
		T1	15.90	1.17	0.9
Glycidyl trityl ether		T2	0.24	1.15	0.5
5-Hydroxymethyl-2(5H)-furanone		D2	7.77	1.27	1.5
Phenyl vinyl sulfoxide		D1	1.41	1.09	0.8
Phensuximide		D2	2.55	1.12	0.9
		T1	2.28	1.11	1.4
		T2	2.13	1.22	1.6
Ruelene		D2	0.79	1.09	0.5
		T2	0.66	1.71	1.2
3a,4,5,6-Tetrahydro-succininido[3,4-b]acenaphthen-10-one		D1	5.93	1.44	1.8
		T1	6.79	1.25	1.4
		T2	0.59	1.94	0.9

chiral recognition abilities especially in the reversed phase mode.

Chromatographic Evaluation

The four columns, D1, D2, T1, and T2, were evaluated in three mobile phase modes: the normal-phase, polar organic, and reversed-phase modes. In the normal-phase mode, a mixture of 20% ethanol and 80% heptane was used as mobile phase. In the polar organic mode, 100% methanol was evaluated. In the reversed-phase mode, methanol and water were mixed at the ratio of 1:1, and 0.1% NH4OAc was used as buffer to adjust to pH 4.2. To compare the behavior of the different CSPs, results presented were obtained with the same mobile phase compositions

TABLE 2. Chromatographic data for the polar organic phase resolution of racemic compounds on D1, D2, T1, and T2 columns

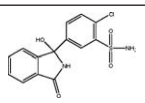
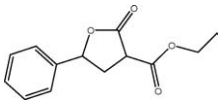
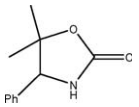
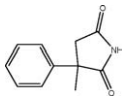
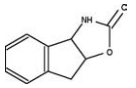
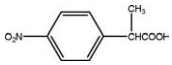
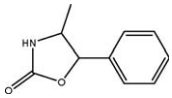
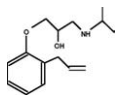
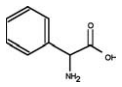
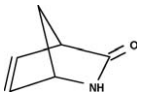
			100% MeOH		
Compound name	Structure	CSPs	k_1	a	R_s
Chlorthalidone		D2	0.44	1.28	0.7
2-Carbethoxy-gamma-phenyl-gamma-butyrolactone		D2	0.13	1.51	0.7
5,5-Dimethyl-4-phenyl-2-oxazolidinone		D1 T2	0.22 0.13	1.38 2.03	1.0 1.4
alpha-Methyl-alpha-phenyl-succinimide		D2	0.23	1.30	0.7
(cis)-(±)−3,3a,8,8a-Tetrahydro-2H-indeno[1,2-d]oxazol-2-one		D1 D2 T1 T2	0.84 2.74 1.02 0.70	1.98 1.96 1.23 1.15	3.0 2.7 1.0 0.9
2-(4-Nitrophenyl)propionic acid		T2	0.17	1.35	0.7
4-Methyl-5-phenyl-2-oxazolidinone		D1 D2 T1	0.26 0.53 0.28	2.36 4.24 2.79	2.5 5.5 4.4
Alprenolol		T2	3.46	1.25	2.7
DL-alpha-Aminophenyl-acetic acid		T2	0.30	3.77	1.3
2-Azabicyclo[2.2.1]-hept-5-en-3-one		D1 D2	0.24 0.61	1.20 1.19	0.8 0.9

TABLE 2. Continued

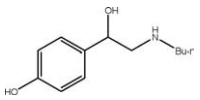
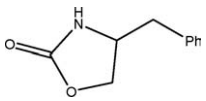
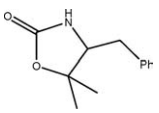
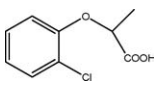
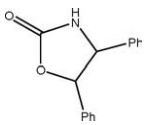
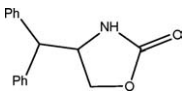
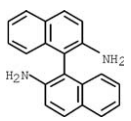
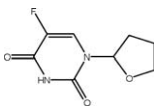
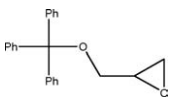
Compound name	Structure	CSPs	100% MeOH k_1	100% MeOH a	100% MeOH R_s
Bamethane		T2	3.88	1.19	1.5
1.1′-Binaphthyl-2,2′-diyl hydrogenphosphate		T1	0.21	1.87	3.2
4-Benzyl-2-oxazolidinone		D1 D2 T1 T2	0.61 2.07 0.72 0.39	1.16 1.08 1.29 1.23	0.9 0.5 1.5 0.9
4-Benzyl-5, 5-dimethyl-2-oxazolidinone		D1 D2 T1 T2	0.29 0.65 0.36 0.15	1.20 1.78 2.73 1.84	0.8 2.3 4.8 1.4
dl-2-(2-Chlorophenoxy)-propionic acid		T2	0.04	3.85	1.3
cis-4,5-Diphenyl-2-oxazolidinone		D1 T1 T2	0.22 0.25 0.21	1.62 1.75 1.71	1.4 3.2 1.5
4-(Diphenylmethyl)-2-oxazolidinone		D1 D2 T1	0.38 1.08 0.41	1.45 1.47 1.55	1.2 1.5 1.6
2,2′-Diamino-1,1′-binaphthalene		T2	0.24	1.10	0.5
Ftorafur		D1 T1	0.29 0.50	1.20 1.08	0.8 0.6
Glycidyl trityl ether		T1	0.09	1.23	0.5

TABLE 2. Continued

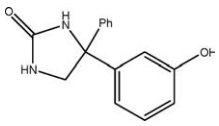
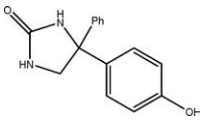
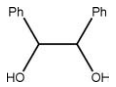
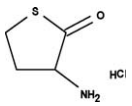
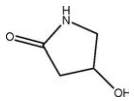
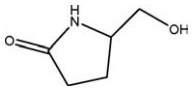
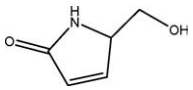
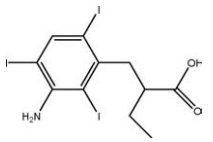
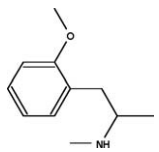
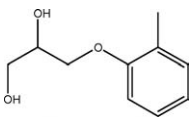
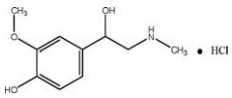
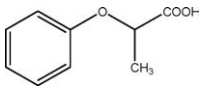
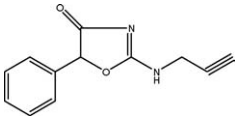
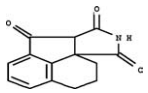
Compound name	Structure	CSPs	100% MeOH k_1	100% MeOH a	100% MeOH R_s
5-(4-Hydroxyphenyl)-5-phenylhydantoin		D1 D2 T1 T2	0.51 2.59 0.36 0.68	1.95 4.07 1.08 1.44	2.5 7.0 0.5 1.4
5-(3-Hydroxyphenyl)-5-phenylhydantoin		D2 T1 T2	1.51 0.32 0.49	1.32 1.11 1.23	1.3 0.6 0.9
Hydrobenzoin		T1	0.09	1.22	0.5
DL-Homocysteine thiolactone hydrochloride		T2	3.06	1.19	1.8
4-Hydroxy-2-pyrrolidinone		D2	0.60	1.37	1.3
5-(Hydroxymethyl)-2-pyrrolidinone		D2 T1	0.68 0.50	2.06 1.19	2.9 1.4
5-Hydroxymethyl-2(5H)-furanone		D2 T1	0.42 0.29	1.54 1.08	1.4 0.5
Iopanoic acid or (3-[3-amino-2,4,6-triiodophenyl]-2-ethyl-propanoic acid		D1	0.26	1.23	0.9
Methoxyphenamine		D2 T1 T2	0.50 0.31 0.34	1.78 1.27 1.23	2.2 1.2 0.8
Mephenesin		T1	0.09	1.32	0.6

TABLE 2. Continued

Compound name	Structure	CSPs	100% MeOH k_1	100% MeOH a	100% MeOH R_s
Metanephrine hydrochloride		T2	1.83	1.29	1.0
2-Phenoxypropionic acid		T2	0.02	6.80	1.2
5-Phenyl-2-(2-propynyl-amino)-2-oxazolin-4-one		D1 D2 T1	0.27 0.46 0.31	1.88 3.81 1.46	1.3 3.0 0.9
3a,4,5,6-Tetrahydro-succininido[3,4-b]-acenaphthen-10-one		D1 D2 T1 T2	0.30 0.81 0.31 0.33	1.14 1.24 1.16 1.19	0.7 0.9 1.0 0.7

for all of the CSPs. However, these conditions are not necessarily optimal for all the enantiomeric separations. Better separations can be obtained in specific cases if the mobile phase compositions and organic modifiers are optimized. The elution order of amino acids is "L" before "D." Other compounds for which standards are not available have not yet be determined.

Approximately 250 compounds were injected on these columns. These analytes include (a) heterocyclic compounds, (b) chiral acids, (c) chiral amines, (d) chiral alcohols, (e) chiral sulfoxides and sulfilimines, (f) amino acid and amino acid derivatives, and (g) other chiral compounds. To simplify the presentation, Tables 1–3 list only the chromatographic results obtained when an enantiomeric separation was achieved.

Comparison of CSPs in the Normal-Phase Mode

Table 1 lists the separations achieved on the four columns when used in the normal-phase mode. The number of successful enantioseparations achieved on D1, D2, T1, and T2 is 16, 17, 17, and 15, respectively. Interestingly, D2 always gives much greater retention for most of the analytes than the other three columns. Conversely, D1 has the least retention for most compounds. In the case of 2-azabicyclo[2.2.1]-hept-5-en-3-one, the retention factor *(k)* on D2 is three times as great as it is on D1. According to elemental analysis results of the CSPs, the carbon loading of D2 is higher than D1 by 3%. This can be caused either by more chiral selector loading or more unreacted linkages. And both of these factors can contribute to longer retention of analytes. Also, the additional ureic group of the D2 linkage can interact with analytes and increase the retention time. However, longer retention does not necessarily result in better resolution of racemates. Among the racemates that both D1 and D2 can separate, six are better separated on D2 and four are better separated on D1, according to the separation factors (α). The enantiomeric separations of 2,6-bis(4-isopropyl-2-oxazolin-2-yl)pyridine, DL-3,4-dihydroxyphenyl-alpha-propylacetamide, cis-4,5-diphenyl-2-oxazol-idinone, and phenyl vinyl sulfoxide were only achieved on the D1 CSP in the normal-phase mode. While methyl trans-3-(4-methoxyphenyl) glycidate and 5-hydroxymethyl-2(5H)-furanone enantiomers were separated on the D2 CSP only. Thus, it is obvious that the binding chemistry not only affects the retention factors but also changes the enantioselectivity in same cases. The influences of the nonchiral spacers on chiral separation were first studied for β-cyclodextrin CSPs.[17] Other studies have been done by several research groups.[4,18–21] It was found that different types of chiral selectors favor linkages with different nature and length. However, for macrocyclic glycopeptide chiral selectors, each binding method has its own advantages, and sometimes unique selectivity.

Teicoplanin-based columns can separate five compounds that the dalbavancin-based CSPs did not. 2-Carbethoxy-gamma-phenyl-gamma-butyrolactone was separated barely by T1 and T2. However, its separation was greatly improved to baseline on the D2 CSP. Among the total 29 compounds separated by these four columns in the normal-phase mode, 19 compounds are better or only separated by the dalbavancin-based CSPs. There are no obvious structural differences between the solutes sepa-

TABLE 3. Chromatographic data for the reversed phase resolution of racemic compounds on D1, D2, T1, and T2 columns

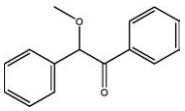
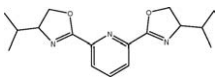
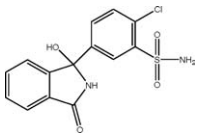
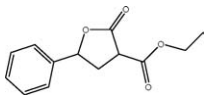
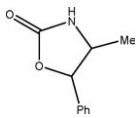
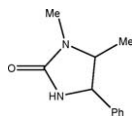
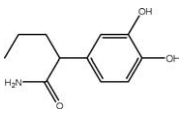
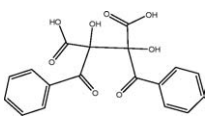
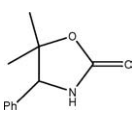
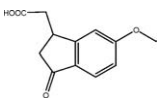
			NH_4OAc Buffer 50%/ MeOH 50%		
Compound name	Structure	CSPs	K_1	A	R_s
Benzoin methyl ether		2	6.25	1.29	1.4
2,6-Bis(4-isopropyl-2-oxazolin-2-yl)pyridine		D2 T2	1.74 0.79	1.58 1.94	2.3 3.0
Chlorthalidone		D2 T1 T2	1.86 0.45 0.93	1.41 1.12 1.06	1.3 0.8 0.5
2-Carbethoxy-gamma-phenyl-gamma-butyrolactone		D2	5.21	1.11	0.8
1,5-Dimethyl-4-phenyl-2-imidazolidinone		D2 T1 T2	2.80 0.57 2.06	1.21 1.05 1.10	1.4 0.5 0.9
1,5-Dimethyl-4-phenyl-2-imidazolidinone		D2 T1 T2	2.80 0.57 2.06	1.21 1.05 1.10	1.4 0.5 0.9
3,4-Dihydroxyphenyl-2-propylacetamide		D2	2.19	0.90	0.6
2,3-Dibenzoyl-dl-tartaric acid		D2	3.01	1.10	0.5
5,5-Dimethyl-4-phenyl-2-oxazolidinone		D1	0.75	1.84	1.2
5-Methoxy-1-indanone-3-acetic acid		D2	1.41	1.10	0.6

TABLE 3. Continued

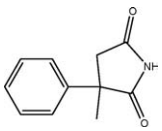
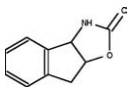
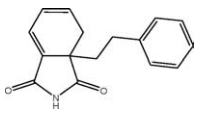
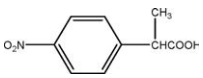
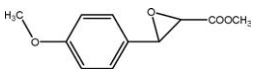
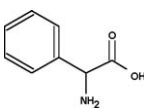
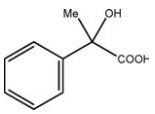
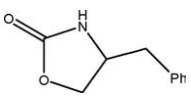
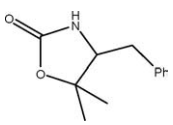
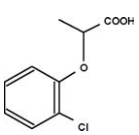
Compound name	Structure	CSPs	NH_4OAc Buffer 50%/ MeOH 50% K_1	A	R_s
alpha-Methyl-alpha-phenyl-succinimide		D2 T1 T2	1.20 0.49 0.69	1.23 1.13 1.12	1.2 1.0 0.8
3,3a,8,8a-Tetrahydro-2H-indeno[1,2-d]-oxazol-2-one		D1	1.52	1.61	1.5
(1-Phenethyl)phthalimide		D2 T1 T2	4.81 0.89 2.38	1.10 1.07 1.06	0.8 0.8 0.5
2-(4-Nitrophenyl)propionic acid		D1 D2	1.29 3.33	1.30 1.17	1.4 1.0
Methyl trans-3-(4-methoxyphenyl)glycidate		D2	1.49	1.10	0.6
dl-alpha-Aminophenyl-acetic acid		T2	0.50	5.05	4.6
Atrolactic acid hemihydrate		D1 D2	0.80 0.59	1.82 5.05	2.4 4.9
4-Benzyl-2-oxazolidinone		T1 T2	8.50 1.29	1.03 1.30	0.9 1.5
(−/+)-4-Benzyl-5, 5-dimethyl-2-oxazolidinone		D1 T1	1.46 1.00	1.31 3.57	1.5 5.9
2-(2-Chlorophenoxy)-propionic acid		D1 D2 T2	1.00 1.10 0.04	1.42 2.43 0.62	1.2 2.4 0.4

TABLE 3. Continued

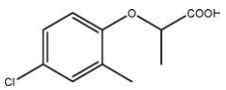
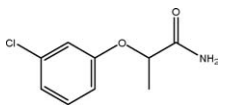
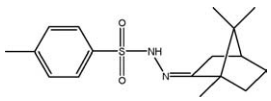
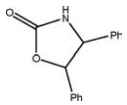
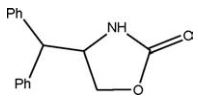
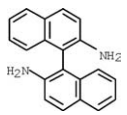
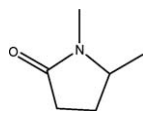
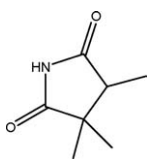
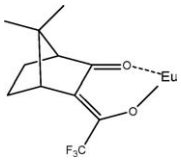
Compound name	Structure	CSPs	NH_4OAc Buffer 50%/ MeOH 50% K_1	A	R_s
2-(4-Chloro-2-methyl-phenoxy)propionic acid		D1 D2	1.21 1.63	1.33 1.64	1.2 2.0
2-(3-Chlorophenoxy)propionamide		D2 T2	1.42 0.77	1.18 1.04	1.0 0.4
(±)Camphor *p*-tosyl hydrazon		T1	0.97	1.14	1.0
cis-4,5-Diphenyl-2-oxazolidinone		D1 T1 T2	1.63 0.87 1.94	1.23 1.42 1.44	1.3 2.9 2.7
4-(Diphenylmethyl)-2-oxazolidinone		D1 T1 T2	2.28 1.31 2.06	1.20 1.59 1.22	1.2 1.8 1.3
2,2′-Diamino-1,1′-binaphthalene		T1	1.01	1.11	1.0
1,5-Dimethyl-2-pyrrolidinone		D2	0.35	1.16	0.6
alpha,alpha-Dimethyl-beta-methylsuccinimide		D2 T1 T2	0.37 0.30 0.29	1.52 1.07 1.14	1.4 0.5 0.6
Europium tris[3-(trifluoromethylhydroxy-methylene)]-(−) camphorate		D2	2.09	1.33	0.8

TABLE 3. Continued

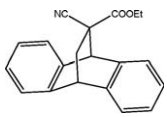
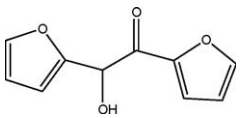
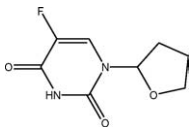
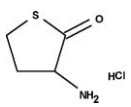
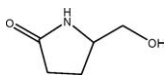
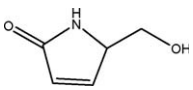
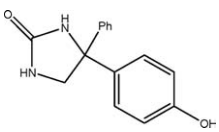
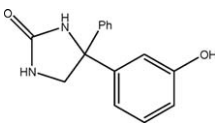
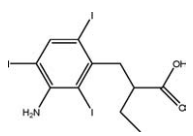
Compound name	Structure	CSPs	NH_4OAc Buffer 50%/ MeOH 50% K_1	A	R_s
Ethyl 11-cyano-9,10-dihydro-endo-9,10-ethanoanthracene-11-carboxylate		D2 T2	4.66 2.07	1.04 1.07	0.5 0.6
Furoin		D2 T2	0.73 0.42	1.09 1.07	0.5 0.4
Ftorafur		T1	0.94	1.08	0.9
DL-Homocysteine thiolactone hydrochloride		D2 T2	0.67 1.40	1.15 1.04	0.8 0.5
5-(Hydroxymethyl)-2-pyrrolidinone		D2	0.24	1.99	1.4
5-Hydroxymethyl-2(5H)-furanone		D2 T2	0.31 0.15	1.79 1.20	1.4 0.5
5-(4-Hydroxyphenyl)-5-phenylhydantoin		D1 T1	3.00 1.27	1.48 1.17	1.8 1.2
5-(3-Hydroxyphenyl)-5-phenylhydantoin		D1 T1 T2	2.36 1.15 4.11	1.18 1.15 1.36	1.2 1.2 1.5
Iopanoic acid or (3-[3-amino-2,4,6-triiodophenyl]-2-ethyl-propanoic acid		D1	1.94	1.16	0.9

TABLE 3. Continued

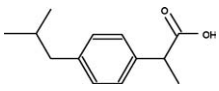
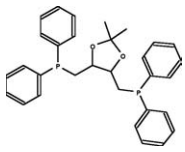
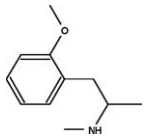
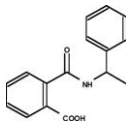
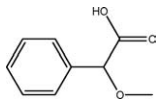
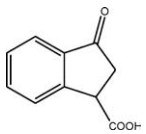
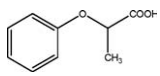
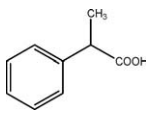
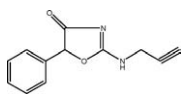
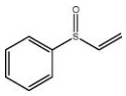
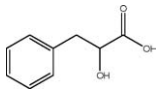
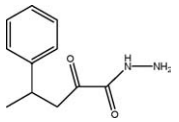
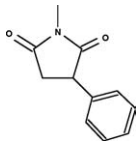
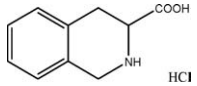
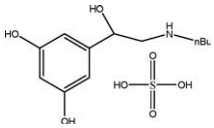
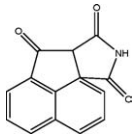
Compound name	Structure	CSPs	NH_4OAc Buffer 50%/ MeOH 50% K_1	A	R_s
4-Isobutyl-alpha-methylphenylacetic acid		D1	1.91	1.08	0.7
(±)2,3-O-Isopropylidene 2,3-dihydroxy-1,4-bis-(disphenylphosphino)butane		D2	4.67	1.21	1.0
Methoxyphenamine		D2 T2	1.68 9.30	2.00 1.03	1.2 0.4
N-(alpha-Methylbenzyl)phthalic acid monoamide		D1	0.71	1.66	2.0
alpha-Methoxyphenylacetic acid		D1	0.66	1.07	0.5
3-Oxo-1-indancarboxylic acid		D2	1.64	1.50	1.5
2-Phenoxypropionic acid		D2	0.57	2.73	3.3
2-Phenylpropionic acid		D2	1.10	1.10	0.5
5-Phenyl-2-(2-propynyl-amino)-2-oxazolin-4-one		D1	0.86	1.69	1.5

TABLE 3. Continued

Compound name	Structure	CSPs	NH_4OAc Buffer 50%/ MeOH 50% K_1	A	R_s
Phenyl vinyl sulfoxide		D2	1.08	1.25	1.3
		T1	0.52	1.13	1.0
		T2	0.56	1.27	1.4
DL-beta-Phenyllactic acid		D1	0.41	1.14	0.6
(±)-5-(alpha-Phenethyl)semioxamazide		D2	0.70	1.07	0.5
		T2	0.50	1.13	0.7
Phensuximide		D2	1.01	1.31	1.4
		T2	1.00	1.31	1.4
1,2,3,4-Tetrahydro-3-isoquinolinecarboxylic acid hydrochloride		D1	0.73	1.65	1.5
Terbutaline hemisulfate salt		T2	5.63	1.22	3.1
3a,4,5,6-Tetrahydro-succininido[3,4-b]acenaphthen-10-one		D1	1.06	1.30	1.2
		T1	0.93	1.42	5.0

rated by one CSP versus another CSP. Representative chromatograms of analytes separated on the macrocyclic glycopeptide CSPs are shown in Figure 3.

Comparison of CSPs in the Polar Organic Mode

Methanol is used as mobile phase for the polar organic mode because it can elute analytes faster than acetonitrile for teicoplanin-type CSPs.[4] A total of 13, 17, 18, and 18 racemates have been separated on D1, D2, T1, and T2, respectively. These analytes include carboxylic acids, amine, alcohol, and neutral compounds. The results are listed in Table 2 and representative chromatograms are shown in Figure 4. According to the enantioselectivity factors, three enantiomers are best separated on the D1 CSP, including one that was separated only on this CSP, 12 solutes were best separated by the D2 CSP, including four that were separated only on this stationary phase, 10 racemates were best separated by the T1 column, including four that were separated only by this CSP, 10 analytes were best separated by the T2 CSP, including nine that were separated only by this CSP. There are five compounds that can be separated by all of the four CSPs. All of them are neutral molecules containing a hetero-five-member ring in the structure. For the compound 5-(4-hydroxyphenyl)-5-phenylhydantoin, both the D1 and D2 CSPs gave much higher enantioselectivities and reso-

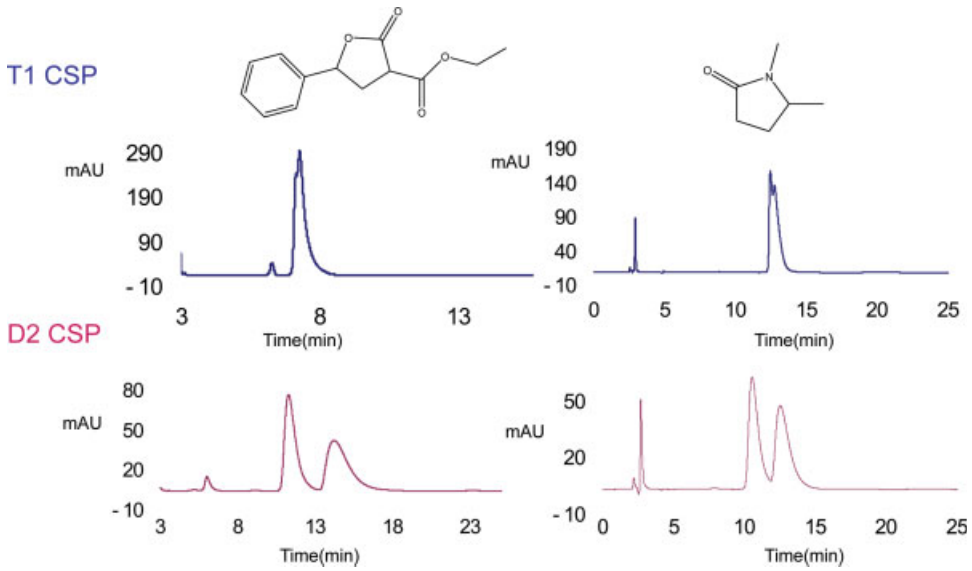


Fig. 3. Representative chromatograms of two analytes on the T1 and D2 CSPs in the normal-phase mode: heptane/ethanol 80/20 v/v; flow rate 1 ml/min. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

lutions than those of T1 and T2 CSPs. The enantioselectivity factors for D1 and D2 are 1.95 and 4.07, respectively, and their resolutions correspond to 2.5 and 7.0, respectively, which indicates the excellent chiral resolving capabilities of dalbavancin. Interestingly, the separation results changed significantly if a small alteration is made to the analyte's structure. For example, the only structural difference between 5-(3-hydroxyphenyl)-5-phenylhydantoin and 5-(4-hydroxyphenyl)-5-phenylhydantoin is position of the phenolic group. However, the previous baseline separation (R_s 7.0) achieved on D2 for 5-(4-hydroxyphenyl)-5-phenylhydantoin was downgraded to a partial separation (R_s 1.3) on D2 for 5-(3-hydroxyphenyl)-5-phenylhydantoin. The substantial decline in the enantiomeric selectivity and resolution indicates that the position of phenol group is very important for chiral recognition. Some of the compounds separated in the polar organic mode can also be separated in the normal-phase mode. For example, enantiomers of 2-carbethoxy-gamma-phenyl-gamma-butyrolactone can be separated on all of the four CSPs and was baseline separated by D2. However, it was only partially separated on D2 in the polar organic mode (R_s 0.7). This is because the analyte does not retain long enough to interact with the chiral selectors in the polar organic mode.

Comparison of CSPs in the Reversed-Phase Mode

Previous studies have revealed that reversed phase separations are among the most successful for the glycopeptide CSPs. Clearly, dalbavancin and teicoplanin CSPs follow this trend (see Fig. 5). Fifty-four racemates have been

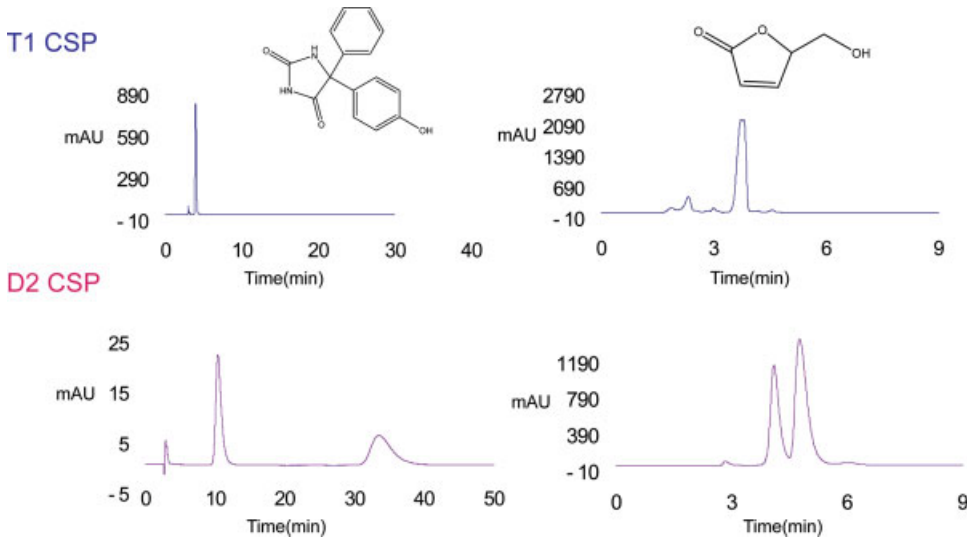


Fig. 4. Representative chromatograms of two analytes on the T1 and D2 CSPs in the polar organic phase mode: 100% methanol; flow rate 1 ml/min. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

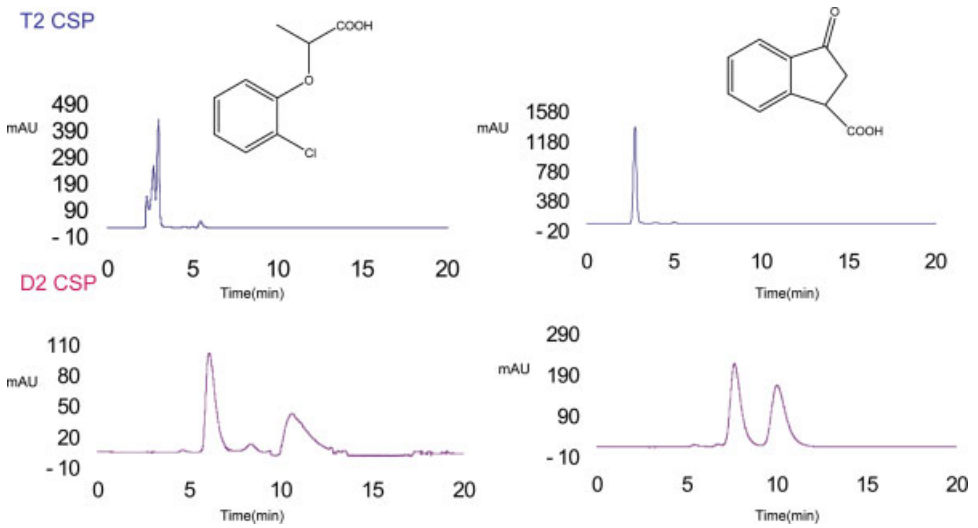


Fig. 5. Representative chromatograms of two analytes on the T2 and D2 CSPs in the reversed-phase mode: 20 mM NH_4NO_3 buffer/methanol 1:1 v/v; flow rate 1 ml/min. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

separated by these four columns together, and the results are listed in Table 3. Twenty-three racemic solutes can be separated on both dalbavancin and teicoplanin CSPs. This suggests that these two chiral selectors have somewhat analogous chiral recognition capabilities because of their similar structures. Fourteen racemates were only separated on the dalbavancin columns. This also demonstrates that these two classes of CSPs are complimentary to each other. Atrolactic acid hemihydrate was baseline separated by D1 ($\alpha = 1.82$, $R_s = 2.4$) and D2 ($\alpha = 5.05$, $R_s = 4.9$) CSPs, but it was not separated on either of the teicoplanin-based columns. These differences in enantioselective Gibbs energy correspond to 0.3 kcal/mol for D1, 0.9 kcal/mol for D2, and 0 kcal/mol for T1 and T2. In this particular example, the dalbavancin columns are much more effective. Interestingly, many of the analytes that are only separated on dalbavancin-based CSPs have a free carboxylic group in their structure, such as *N*-(alpha-methylbenzyl)phthalic acid monoamide, alpha-methoxyphenylacetic acid, 3-oxo-1-indancarboxylic acid, 2-phenoxypropionic acid, 2-phenylpropionic acid, beta-phenyllactic acid, and 1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid hydrochloride. This improved enantioselectivity toward carboxylic acids may be partly attributed to the tertiary amino group coupled to dalbavancin via an amide linkage (Fig. 1). In aqueous solution at pH 4.2, this group is protonated and carries a positive charge. This cationic site can interact with deprotonated carboxylic anions through charge–charge interactions, which is an important process in chiral recognition. In contrast, the teicoplanin-based CSPs (i.e., T2) only separated one of the tested amino acids, DL-alpha-aminophenyl-acetic acid and one of the tested carboxylic acids, 2-(2-chlorophenoxy)-propionic acid ($R_s = 0.4$). Although there is one cationic site on native teicoplanin, it can be converted to a carbamate group when bonded to silica gel. Thus, the teicoplanin chiral selector only has one anionic site after linked to silica gel. The poor enantioselectivity of teicoplanin to some of the carboxylic acids in this study should be partially due to the lack of cationic sites on the teicoplanin molecule.

CONCLUSIONS

Two dalbavancin-based CSPs were made using two different linkages to silica gel. Their enantiomeric separation capabilities have been investigated by comparison of the separations achieved on Chirobiotic T and T2 commercial columns. The structural differences in the chiral selectors and linkages between the four CSPs presented in this work do not make one superior to another. All of them can separate some racemic solutes that cannot be separated by the other CSPs tested. It is as expected that they show similar enantiomeric separation abilities to many analytes because their structures are very closely related. However, dalbavancin-based CSPs exhibit enhanced enantioselectivities to carboxylic acids, where the additional cationic site of the chiral selector may play an important role during the chiral recognition process. Thus, it is obvious that these four CSPs are complementary to one another. If a racemate is poorly separated on one CSP, it is possible the other related CSPs will produce an enhanced separation. This is the principal of complementary separation that was model for the class of chiral selectors. Future work will involve the detailed study of elution order of enantiomers and binding linkage effects on dalbavancin-based CSPs.

ACKNOWLEDGMENTS

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