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Role of the Carbohydrate Moieties in Chiral Recognition on Teicoplanin-Based LC Stationary Phases

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For this study, we used the macrocyclic antibiotic teicoplanin, a molecule consisting of an aglycone peptide "basket" with three attached carbohydrate (sugar) moieties. The sugar units were removed and the aglycone was purified. Two chiral stationary phases (CSPs) were prepared in a similar way, one with the native teicoplanin molecule and the other with the aglycone. Twenty-six compounds were evaluated on the two CSPs with seven RPLC mobile phases and two polar organic mobile phases. The compounds were 13 amino acids or structurally related compounds (including DOPA, folinic acid, etc.) and 13 other compounds (such as carnitine, bromacil, etc.). The chromatographic results are given as the retention, selectivity, and resolution factors along with the peak efficiency and the enantioselective free energy difference corresponding to the separation of the two enantiomers. The polarities of the two CSPs are similar. It is clearly established that the aglycone is responsible for the enantioseparation of amino acids. The difference in enantioselective free energy between the aglycone CSP and the teicoplanin CSP was between 0.3 and 1 kcal/mol for amino acid enantioseparations. This produced resolution factors 2−5 times higher with the aglycone CSP. Four non amino acid compounds were separated only on the teicoplanin CSP. Six and five compounds were better separated on the teicoplanin and aglycone CSPs, respectively. Although the sugar units decrease the resolution of α-amino acid enantiomers, they can contribute significantly to the resolution of a number of non amino acid enantiomeric pairs.

After the 1928 discovery of penicillin, it was thought that life-threatening infections could be completely controlled. Soon, the appearance of penicillin-resistant bacteria prompted the search for different molecules such as methicillin, the first manmade antibiotic. Strains of methicillin-resistant *Staphylococcus aureus* (MRSA) also appeared, and a neverending struggle against infectious agents began. Today, strains of MSRA and *Enterococcus faecalis* are resistant to almost all antibiotics and have become killers throughout the world.[1] Currently, the antibiotics of last resort are glycopeptides of the vancomycin family. It was demonstrated that the mode of action of these antibiotics was different from that of earlier compounds.[2] The vancomycin-related antibiotics bind to the bacterial cell wall D-alanyl-D-alanine terminal group, blocking the process of wall building. Several members of the group, vancomycin, avoparcin, ristocetin, and teicoplanin, are used more wisely today to contain the "superbug" MSRA proliferation.

Since the target of these antibiotics is the D-alanyl-D-alanine group, we thought early on that they could be used in the separation of amino acid enantiomers.[3,4] It turned out that chiral stationary phases (CSPs) based on these macrocyclic antibiotics were very successful, not only in amino acid enantioresolution[5,6] but also in the resolution of a wide variety of different enantiomers,[7,8] using both HPLC and capillary electrophoresis (CE) techniques.[3−11]

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In some antibiotic studies, it was found that vancomycin derivatives with altered carbohydrate moieties killed bacteria by a mechanism differing from that of native vancomycin.[12] In some cases, carbohydrate units alone had antibiotic activity.[12] Transposing these findings to the chiral recognition process is a logical step. The question arises as to the exact role of the sugar moieties in the highly successful teicoplanin CSP. To answer this question and investigate the role of the teicoplanin sugar units, the three carbohydrate units of the teicoplanin molecule were removed and the teicoplanin aglycone "basket" was isolated and purified. Two CSPs were prepared in a similar way: one with the complete teicoplanin molecule and the other with only the aglycone "basket." The enantiorecognition capability of the two CSPs was evaluated using an identical set of racemic compounds containing a variety of functionalities.

EXPERIMENTAL SECTION

Chemicals. The stationary-phase base was LiChrospher Si-(100) silica gel (5 μm particle size, 10 nm pore diameter, 400 m^2/g specific surface area (Merck, Darmstadt, Germany)). The reagents (3-aminopropyl)triethoxysilane and 1,6-diisocyanatohexane were obtained from Fluka (Buchs, Switzerland). Table 1 lists the racemic compounds that were tested on the CSPs, each with its structure and a reference number used in the other tables. They were all obtained from Sigma-Aldrich (St. Louis, MO). The chemical solvents dry toluene and pyridine were from J. T. Baker (Phillipsburg, NJ). The chromatographic solvents methanol and acetonitrile and the buffer additives (acetic acid, ammonium acetate, and triethylamine) were from Fisher Scientific (Fair Lawn, NJ). Teicoplanin was a gift of the LePetit Research Center (Gerenzano, Italy).

Preparation of the Teicoplanin Aglycone. A 5 g sample of the teicoplanin complex (2.7 mmol) was dissolved with magnetic stirring at room temperature in 30 mL of DMSO and 1 mL of 80% H_2SO_4. The mixture was heated to 65 °C for 1.5 h. The phenoxy linkage of the nonylglucosamine unit was then hydrolyzed (Figure 1); disappearance of the main teicoplanin peak from the reaction liquid phase was checked by HPLC (column 250 × 4 mm i.d. ODS Hypersil; mobile phases (A) 0.1 M ammonium acetate and (B) 0.1 M ammonium acetate/acetonitrile, 20/80; linear gradient from 10% B to 50% B in 20 min, to 75% B in 15 min, to 100% B in 5 min; flow rate 1.00 mL/min; T = 20 °C; UV detection at 254 nm). A 1 mL aliquot of 80% H_2SO_4 was added, and the reaction mixture was kept at 65 °C for 3 h. The phenoxy linkage of the mannose unit was then hydrolyzed. To remove the *N*-acetyl-β-D-glucosamine unit, it was necessary to raise the temperature to 80 °C for 24 h. Appearance of the aglycone peak from the reaction liquid phase was checked by HPLC, as described above (same conditions except isocratic elution; A/B = 75/25, by volume). After cooling to 30 °C, the DMSO solution was diluted with 63 mL of demineralized water, and the resulting pH was 1.5. To this cloudy solution was added 750 mg of activated carbon (Darco G 60, Aldrich Catalog No. 24227-6), and the stirring was continued for 1 h. The mixture was filtered on Celite, and the filtrate was neutralized with 10% NaOH aqueous solution. The suspension obtained was cooled to 5 °C and left overnight. Then the precipitated solid was recovered by filtration, washed with water (5 mL), and dried under vacuum (0.1 mbar) at 60 °C for 15 h, giving 2.60 g of crude light brown aglycone (molar yield 80%). The molecular weight of each product was checked by high-resolution MS after each step as indicated in Figure 1. The crude aglycone (2.60 g) was further purified as follows. It was dissolved, with stirring, in 15 mL of DMSO, plus 15 mL of demineralized water and 0.6 mL of 20% HCl(aq). When the dissolution was complete, another 9 mL of water was added and the pH of the solution was brought to 7 by adding 20% NaOH(aq) solution. The suspension was then cooled to 5 °C for 12 h, and the solid aglycone was filtered, washed with water (15 mL) and acetone, and dried under vacuum (0.1 mbar) at 50 °C, giving 1.82 g of pure aglycone (molar yield 70%).

Preparation of the Chiral Stationary Phases. The full procedure was recently reported.[9] A 5 g sample of LiChrospher Si(100) was dried at 150 °C for 1 h under vacuum (0.1 mbar) in a round-bottom flask. A 120 mL portion of dry toluene was added, and the mixture was heated to reflux to azeotropically remove any residual water. Then, 2.5 mL of (3-aminopropyl)triethoxysilane (11 mmol) was added dropwise and the mixture was heated to reflux for 4 h. After cooling, the modified silica was filtered off, and washed with toluene, methanol, and dichloromethane, and dried at 90 °C (0.1 mbar, 1 h). The elemental analysis gave 4.18% C and 1.14% N, corresponding to 1045 μmol/g of aminopropyl groups or 2.61 μmol/m^2 based on the N percentage.

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 dry toluene. Then, 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 teicoplanin (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. Disappearance of the main teicoplanin peak from the reaction liquid phase was checked by HPLC, as previously described.[9] After cooling, the teicoplanin-bonded silica was washed with 50 mL sequential portions of pyridine, water, methanol, acetonitrile, and dichloromethane. It was then dried under vacuum (70 °C, 0.1 mbar, 2 h). The elemental analysis gave 15.6% C and 3.61% N, corresponding to 142 μmol/g of teicoplanin or 0.36 μmol/m^2 based on the C percentage.

The very same procedure was followed to prepare the aglycone CSP. The 1,6-diisocyanatohexane-activated phase was prepared and reacted in situ with a suspension of 0.70 g of the aglycone (0.58 mmol) in 70 mL of dry pyridine. The final elemental analysis gave 13.64% C and 3.37% N, corresponding to 151 μmol/g of aglycone or 0.38 μmol/m^2 based on the C percentage.

Column Preparation. A classical packing procedure was used: A 3.3 g sample of the bonded LiChrospher was suspended in 60 mL of a 50/50 acetone/chloroform mixture with 15% acetic acid. After 5 min of ultrasonication, the slurry was packed in a 250 × 4.6 mm stainless steel column at 700 bar with a Haskel DSTV-122 pump using methanol as the pressurizing agent. The column efficiencies were in the 40 000 plate/m of column range, checked with a 90/10 hexane/

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Table 1. Solutes Used in the Study

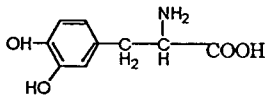
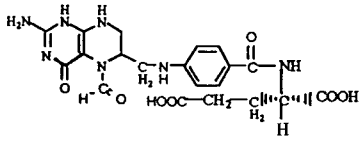
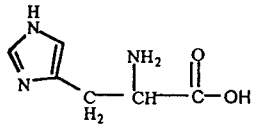
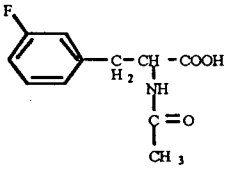
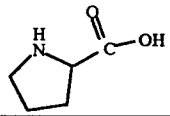
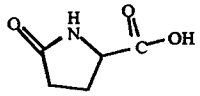
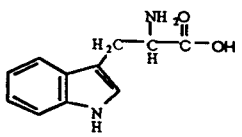
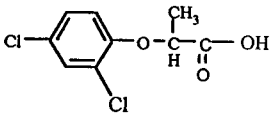
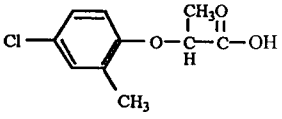
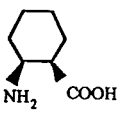
Code	solute	structure	m.w.	use
Class A - α-Amino Acids				
A1	Aspartic acid	COOH-CH_2-CH(NH_2)-COOH	133	
A2	DOPA		197	antiparkisonian
A3	Folinic acid		473	antianemic
A4	Histidine		155	essential AA
A5	Leucine	(CH_3)$_2$CH-CH_2-CH(NH_2)-COOH	131	essential AA
A6	Phenylalanine	ϕ-CH_2-CH(NH_2)-COOH	165	essential AA
A7	N-acetyl-m-fluorophenylalanine			
A8	Proline		115	
A9	2-pyrrolidone-5-carboxylic acid		129	
A10	Threonine	CH_3-CHOH- CH(NH_2)-COOH	119	essential AA
A11	Tryptophan		204	essential AA
A12	Dichlorprop		235	herbicide
A13	Mecoprop		214.5	herbicide
Class B - Stereogenic Center(s) in the Ring				
B1	Cis-2-amino cyclohexane carboxylic acid		345	

Table 1. (Continued)

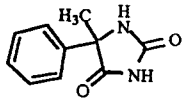
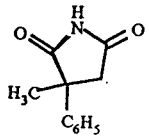
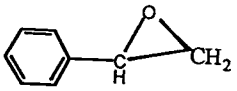
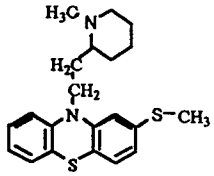
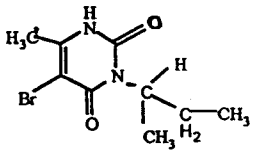
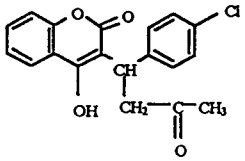
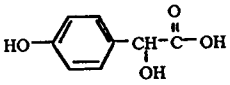
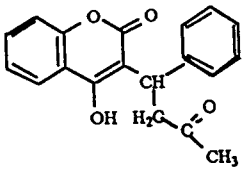
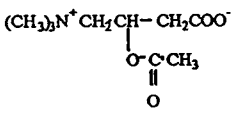
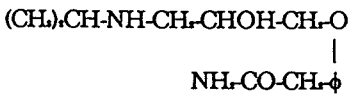
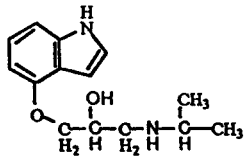
Code	solute	structure	m.w.	use
B2	5-methyl-5-phenyl hydantoin		190	
B3	α-methyl α-phenyl succinimide		189	antiurolithic
B4	Styrene oxide		120	
B5	Thioridazine		370.5	antipsychotic
Class C - Aromatic Ring Connected to the Stereogenic Center				
C1	Bromacil		261	herbicide
C2	Coumachlor		343	rodenticide
C3	4-Hydroxy mandelicacid		168	urinary antiseptic
C4	Warfarin		308	rodenticide
Class D - R-CH2-C*HOH-CH2-R'				
D1	Acetyl carnitine		203	
D2	Atenolol		266	antiarhytmic

Table 1. (Continued)

Code	solute	structure	m.w.	use
D3	Carnitine	$(CH_3)_3N^+$-CH_2-CHOH-CH_2-COO^-	161	fat fighter
D4	Pindolol		248	antihypertensive

chloroform mobile phase and acetophenone ($k' > 11$).

Chromatographic System. The 26 compounds were eluted on two 25 cm columns with nine different mobile phases at 22 °C as listed in Table 2. The results are listed in Tables 2 and 3. The chromatographic system consisted of a Shimadzu SCL-10A System Controller and an SIL-10A Auto Injector, a Shimadzu LC-10AT pump, and a Shimadzu SPD-10A UV detector with a Chromatopac CR 501 integrator (Shimadzu, Kyoto, Japan; obtained from Delta Instruments, Mountain View, MO).

RESULTS AND DISCUSSION

Analyte Selection. Table 1 shows that the selected solutes can be arranged in four classes. Class A contains α-amino acids and structurally related compounds. All have the general structure R−C*H(COOH)−NH−R′, with R and R′ differing by atoms or groups. Two non amino acid herbicide molecules (i.e., **A12** and **A13**) with the general structure R−C*H(COOH)−O−R′ were added as a subsection of class A because of their similar stereogenicity. Class B consists of non amino acid enantiomeric compounds where the stereogenic center is part of a ring structure that introduces some rigidity into the molecule. Class C compounds are non amino acid molecules with one or more aromatic moieties directly connected to the stereogenic center. An aromatic structure is not necessarily a benzene ring. It can be any other π-electron-rich ring such as uracil or a pyrimidinedione ring (bromacil) or a pyranone ring (coumachlor and warfarin). Class D compounds are non amino acid solutes that contain an alcohol moiety connected to the stereogenic center (i.e., a general structure R−CH_2−C*HOH−CH_2−R′). Acetylcarnitine also was placed in class D even though its hydroxyl group was acetylated. These compounds were selected as a result of previous studies with the teicoplanin CSP.[3−5,8−10] Table 2 lists the results for the class A compounds (amino acids). Table 3 lists the results obtained for the three other compound classes. The retention factor, selectivity, resolution factor, peak efficiency, and enantioselective energy differences are given for each compound, in addition to the mobile phase used.

Mobile Phases and Results Presentation. All compounds in Table 1 were evaluated with seven different RPLC mobile phases plus a 100% methanol mobile phase. Also, a polar organic mobile phase (acetonitrile/methanol, 95%/5% v/v, with 0.2% triethylamine (TEA) and 0.3% acetic acid) was evaluated due to its low hydrogen-bonding capability. A total of 234 chromatograms were obtained on each of the two CSPs (26 compounds × 9 mobile phases). To simplify the presentation, Tables 2 and 3 list only the chromatographic results obtained when an enantiomeric separation was achieved on at least one CSP.

Stationary-Phase Polarity and Solute Retention. Considering the nature of the bonded chiral selectors on the silica surface, it is apparent that different functional groups are present in the two CSPs. The linkage between the silica surface and the antibiotic has nine apolar methylene units and either a ureido or carbamate linkage.[9] The polar groups of the free teicoplanin antibiotic are 14 hydroxyl groups, of which 4 are phenolic groups, 1 free amino group, and 1 free carboxylic acid group. Its apolar groups are the nine methylene units of its sugar alkyl chain and the row of six amide linkages in the macrocyclic portion of the molecule (see Figure 1) and the seven benzene rings attached to it. For the aglycone molecule, the basket itself has only seven polar hydroxyl groups, of which six are phenols, and it lacks the apolar alkyl chain connected to a sugar. It has the free amino and carboxylic acid groups, the apolar row of six amide linkages, and the seven benzene rings (Figure 1). However, it is difficult to evaluate the polarities of these two stationary phases by considering just their molecular structure. The retention factors of the different test molecules should give a better idea of the relative polarity of these stationary phases.

The retention factors of the first eluting enantiomers of the intermediate-polarity compounds of the four classes are somewhat lower with the same RPLC mobile phase on the aglycone phase than on the teicoplanin phase (Tables 2 and 3). However, the retention factors of the whole set of compounds are relatively similar on the two CSPs with the same mobile phase. For example, the k' values of the first enantiomer of pindolol (**D4**) are 10.0 and 10.7 with the 85% methanol mobile phase on the teicoplanin and aglycone CSPs, respectively. These k' values both drop to 4.03 and 3.45, respectively, with the 100% methanol mobile phase (Table 3). For the more polar 5-methyl-5-phenylhydantoin solute (**B2**), the k' values are 4.00 and 3.96 with the 20% methanol mobile phase at pH 4.1. They drop to 0.30 and 0.26 when the 85% methanol mobile phase is used. These retention factors are almost identical between the two CSPs (Table 3). Since the stationary-phase coverages of the two CSPs are fairly similar, it can be stated that the overall polarity of the aglycone stationary phase used in this study is somewhat similar to the polarity of the corresponding teicoplanin stationary phase. The retention factors of the second eluting isomers differ much more widely. This will be discussed in the enantioselectivity section of this article.

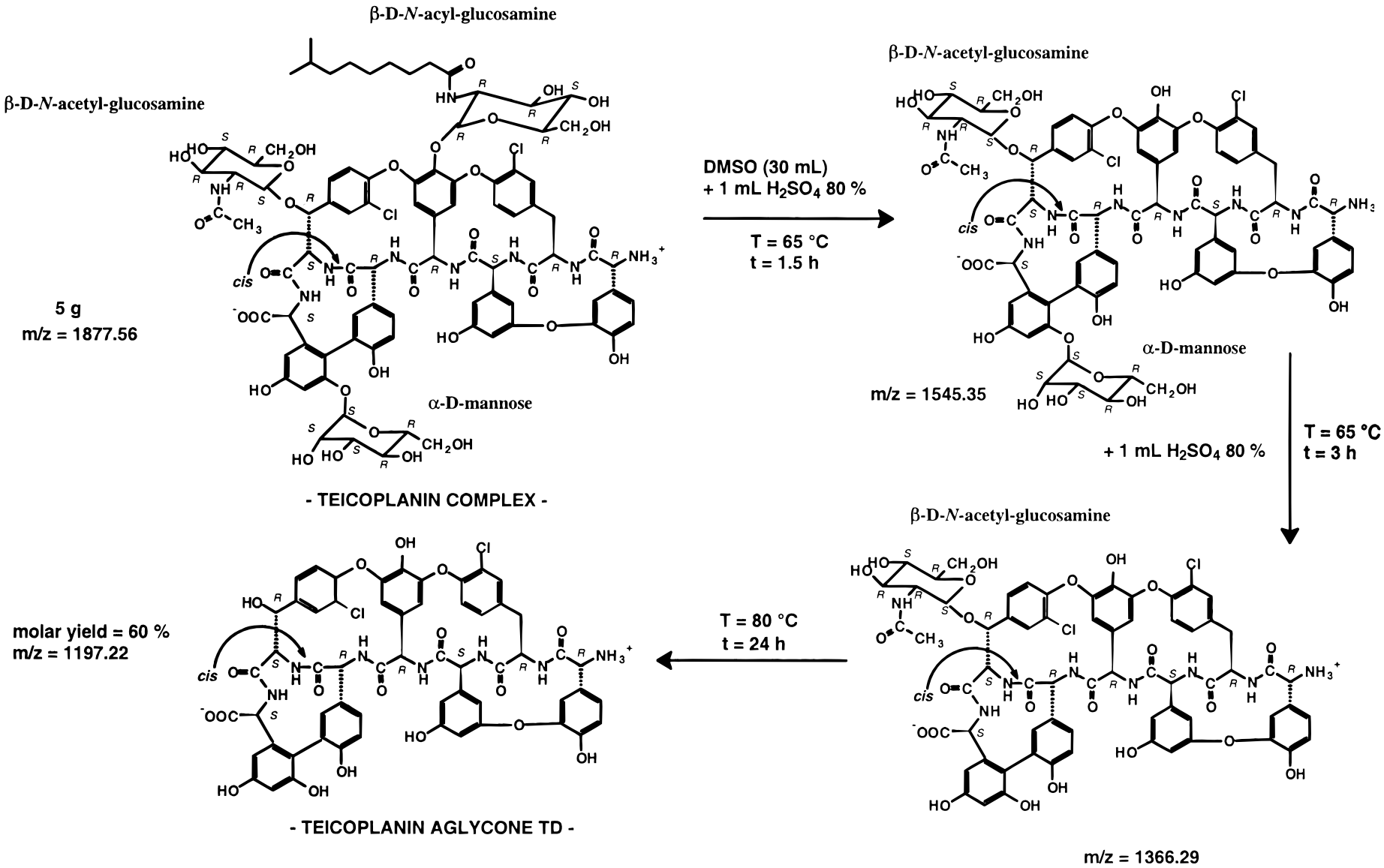


Figure 1. Preparation of the aglycone and its molecular structure.

Table 2. Chromatographic Results Obtained at 22 °C on the Two CSPs with Nine Different Mobile Phases for the Class A Compounds (Amino Acids and Related Compounds)

compd	CSP[a]	mobile phase[b]	k'_1	k'_2	α	R_s	N_1 (plates)	N_2 (plates)	ΔG^c (kcal/mol)
				Amino Acid Compounds					
A1	T	60-3.8	0.56	0.82	1.46	0.7	350	300	0.22
	A		nd[d]	nd					
	T	100	2.20	4.44	2.02	1.25	100	90	0.41
	A		nd	nd					
A2	T	20	0.54	0.96	1.78	1.5	700	600	0.33
	A		0.51	4.52	8.86	5.3	1500	200	1.29
	T	20-4.1	0.40	0.66	1.65	1.8	2900	1300	0.29
	A		0.40	3.9	9.75	6.0	2200	350	1.33
	T	40	0.66	1.08	1.64	2.0	1600	1100	0.29
	A		0.48	4.55	9.48	7.0	1400	600	1.32
	T	60	0.53	1.09	2.06	2.4	1300	900	0.42
	A		0.57	5.76	10.1	8.2	1500	600	1.35
	T	60-3.8	0.45	0.9	2.00	2.3	1300	1100	0.41
	A		0.38	4.29	11.3	8.2	2000	600	1.42
	T	60-4.1	0.50	1.16	2.32	3.2	2100	900	0.49
	A		0.45	5.31	11.8	8.3	1700	600	1.44
	T	85	1.10	3.04	2.76	4.2	1800	500	0.59
	A		1.26	14.4	11.4	7.7	700	400	1.43
A3	T	20-4.1	9.43	15.1	1.60	1.5	210	180	0.27
	A		2.39	2.39	1.00	0			0
	T	40	6.31	12.1	1.92	3.4	750	500	0.38
	A		3.84	3.84	1.00	0			0
	T	60	1.93	3.53	1.83	2.6	650	600	0.35
	A		2.29	2.29	1.00	0			0
	T	60-3.8	2.72	5.17	1.90	3.6	1200	700	0.38
	A		2.42	2.42	1.00	0			0
	T	60-4.1	1.79	3.79	2.12	3.5	800	500	0.44
	A		2.45	2.45	1.00	0			0
	T	85	0.53	1.25	2.36	1.2	200	120	0.50
	A		1.13	1.13	1.00	0			0
	T	100	2.25	4.07	1.81	2.8	650	650	0.35
	A		2.68	2.68	1.00	0			0
A4	T	60-3.8	0.67	0.67	1.00	0			0
	A		0.41	0.66	1.61	1.2	900	800	0.28
	T	60-4.1	2.70	2.70	1.00	0			0
	A		1.83	2.76	1.51	2.1	1200	700	0.24
	T	85	2.32	4.13	1.78	2.2	500	400	0.34
	A		8.45	16.4	1.94	2.3	300	250	0.39
A5	T	20	0.32	0.51	1.59	1.2	2400	900	0.27
	A		0.92	2.88	3.13	4.8	2000	800	0.67
	T	40	1.56	2.14	1.37	2.2	2900	1400	0.18
	A		0.28	1.32	4.71	4.2	2000	600	0.91
	T	60	0.36	0.80	2.22	2.8	1900	1500	0.47
	A		0.30	1.33	4.43	4.6	1400	900	0.87
	T	60-3.8	0.26	0.63	2.42	2.6	2000	1400	0.28
	A		0.16	1.30	8.12	5.3	1100	1000	0.52
	T	85	0.51	1.34	2.63	3.6	1600	900	0.57
	A		0.60	4.02	6.70	6.5	1700	500	1.11
A6	T	20	0.72	0.72	1.00	0			0
	A		0.46	1.03	2.24	3.0	2200	1000	0.47
	T	20-4.1	0.63	0.74	1.17	0.5	2200	1300	0.09
	A		0.48	1.02	2.13	2.6	2000	800	0.44
	T	40	0.80	0.80	1.00	0			0
	A		0.57	1.43	2.51	4.8	2000	1500	0.54
	T	60	0.98	1.30	1.33	1.25	1500	800	0.17
	A		0.64	1.75	2.73	5.1	2600	1300	0.59
	T	60-3.8	0.64	0.75	1.17	0.6	1900	1100	0.09
	A		0.46	1.36	2.96	5.0	2600	1500	0.63
	T	60-4.1	0.51	0.66	1.29	0.9	1600	1200	0.15
	A		0.49	1.42	2.90	4.9	2100	1500	0.62
	T	85	1.02	1.55	1.52	1.6	800	700	0.25
	A		0.98	3.11	3.17	5.2	1000	800	0.68
	T	100	1.78	2.75	1.54	1.5	700	280	0.26
	A		1.68	5.10	3.04	4.2	1000	360	0.65
A7	T	200.1	2.00	11.2	5.60	8.0	1000	650	1.01
	A		2.82	14.7	5.21	3.6	400	120	0.97
	T	40	1.55	4.34	2.80	1.6	80	70	0.60
	A		0.47	6.07	12.9	11	500	350	1.50
	T	60	1.21	4.11	3.40	1.7	90	50	0.72
	A		0.98	4.49	4.58	2.0	80	70	0.90
	T	60-3.8	2.25	9.68	4.30	6.5	800	550	0.85
	A		2.16	10.28	4.76	6.0	600	450	0.91
	T	60-4.1	0.81	6.11	7.54	9	1000	700	1.18
	A		0.70	5.85	8.36	5.5	550	350	1.25
	T	85	0.05	2.00	40	5.7	1300	450	2.16
	A		0.01	1.65	165	5.8	1300	550	2.75

Table 2. Continued

compd	CSP[a]	mobile phase[b]	k'_1	k'_2	α	R_s	N_1 (plates)	N_2 (plates)	ΔG^c (kcal/mol)
				Amino Acid Compounds					
A8	T	20	0.27	0.79	2.93	3.1	1300	1200	0.63
	A		0.43	1.88	4.37	6.2	2300	1100	0.86
	T	40	0.40	0.40	1.00	0			0
	A		0.77	3.35	4.35	6.2	2200	600	0.86
	T	60-3.8	0.58	1.68	2.90	5.0	2400	1200	0.62
	A		0.98	4.34	4.43	6.0	1200	800	0.87
A9	T	60-3.8	1.56	1.89	1.21	0.7	600	500	0.11
	A		2.02	2.61	1.29	1.0	500	500	0.15
	T	60-4.1	0.15	0.34	2.27	1.5	1800	1100	0.49
	A		0.17	0.41	2.41	1.8	1500	1300	0.52
	T	100	1.13	1.44	1.27	0.7	500	400	0.14
	A		1.00	1.62	1.62	2.0	1000	700	0.28
A10	T	40	0.06	0.14	2.33	0.7	1700	1500	0.48
	A		0.07	0.23	3.28	1.5	1900	1700	0.70
	T	60	0.12	0.23	1.92	1.2	4400	1900	0.38
	A		0.03	0.15	5.00	1.4	2700	2300	0.95
	T	60-3.8	0.39	0.39	1.00	0			0
	A		0.07	0.30	4.28	2.0	1700	1600	0.85
	T	85	0.71	1.14	1.60	1.7	1000	900	0.28
	A		0.46	1.51	3.28	4.0	1100	800	0.70
	T	100	2.20	2.20	1.00	0			0
	A		0.93	2.76	2.97	3.7	1100	400	0.64
A11	T	20	1.74	1.93	1.11	0.5	1800	600	0.06
	A		1.50	3.33	2.22	2.6	1000	250	0.47
	T	20-4.1	1.38	1.53	1.11	0.7	3800	1100	0.06
	A		1.04	2.89	2.78	4.2	1700	500	0.60
	T	40	1.59	1.99	1.25	1.4	2600	1200	0.13
	A		1.40	3.21	2.29	4	2000	600	0.49
	T	60	1.28	1.66	1.30	1.3	1300	1000	0.15
	A		1.43	3.26	2.28	3.8	1700	500	0.48
	T	60-3.8	0.90	1.19	1.32	1.4	2000	1300	0.16
	A		0.77	2.04	2.65	4.4	1700	900	0.57
	T	60-4.1	0.76	1.13	1.49	1.5	1100	900	0.23
	A		0.85	2.29	2.69	4.8	2100	900	0.58
	T	85	1.21	1.95	1.61	1.7	1000	400	0.28
	A		1.37	4.08	2.98	4.7	800	600	0.64
	T	100	2.23	3.64	1.63	1.5	400	230	0.29
	A		2.05	14.2	6.93	6.5	650	300	1.13
				Non Amino Acid Compounds					
A12	T	20-4.1	12.4	14.1	1.14	0.7	1300	400	0.07
	A		2.6	2.6	1.00	0			0
	T	60-4.1	1.42	1.69	1.19	0.7	900	600	0.10
	A		0.97	0.97	1.00	0			0
	T	100	0.27	0.38	1.41	0.8	1400	1300	0.20
	A		0.4	0.4	1.00	0	1800	1500	0.49
	T	95ACN	1.61	2.09	1.30	1.3	1900	600	0.15
	A		0.06	0.06	1.00	0			0
A13	T	20-4.1	10.2	11.3	1.11	0.6	800	500	0.06
	A		2.12	2.12	1.00	0			0
	T	100	2.25	4.07	1.81	2.4	500	500	0.35
	A		0.3	0.3	1.00	0	1800	1500	0.43
	T	95ACN	1.65	2.03	1.23	1.0	1600	600	0.12
	A		0.03	0.03	1.00	0			0

[a] T = teicoplanin chiral stationary phase (CSP); A = aglycone CSP. [b] Mobile phase code: 20 = 20% methanol/80% water v/v; 20-4.1 = 20% methanol/80% water buffered at pH 4.1 by 1% TEAA; 40 = 40% methanol/60% water v/v; 60 = 60% methanol/40% water v/v; 60-3.8 = 60% methanol/40% water buffered at pH 3.8 by acetic acid; 60-4.1 = 60% methanol/40% water buffered at pH 4.1 by 1% TEAA; 85 = 85% methanol/15% water with 2.5×10^{-2} M ammonium acetate; 100 = 100% methanol with 0.1% TEA and 0.1% v/v acetic acid; 95ACN = 95% acetonitrile/5% methanol with 0.2% TEA and 0.3% acetic acid. [c] Enantioselective energy ($-RT \ln \alpha$). [d] nd = not detected, weak absorbance, and/or $k' > 40$.

Figure 2 shows the retention factors of three compounds versus the mobile-phase composition. For the amino acid phenylalanine (**A6**), an increase in the retention factor is observed when the mobile-phase methanol content increases. This behavior was observed on other stationary phases.[7] It is due to the reduced solubility of amino acids in methanol-rich mobile phases. For most compounds, a linear decrease of the log k' value is observed with an increase in the methanol content of the mobile phase (bromacil, **C1**, Figure 2). No retention factors higher than 26 (retention time 80 min) were listed in the tables because this retention time was considered as a maximum acceptable value. When the injected solute did not elute after 90 min, the column was rinsed with pure methanol and reequilibrated with the mobile phase for another solute.

Enantioselectivity. Tables 2 and 3 list the enantioselectivity factors ($\alpha = k'_2/k'_1$), the peak efficiencies (N), and the resolution factors (R_s). The classical equation for the resolution factor calculation was not used because the peak efficiency observed

Table 3. Chromatographic Results Obtained at 22 °C on the Two CSPs with Nine Different Mobile Phases for the Class B−D Compounds

compd	CSP[a]	mobile phase[b]	k'_1	k'_2	α	R_s	N_1 (plates)	N_2 (plates)	ΔG^c (kcal/mol)
Class B Compounds: Structures Where the Stereogenic Center is Part of a Ring System									
B1	T	85	1.03	1.11	1.08	0.4	2300	1200	0.05
	A		1.42	1.42	1.00	0			0
B2	T	20	4.37	8.39	1.92	3.7	1300	600	0.38
	A		3.55	6.92	1.95	3.5	1300	500	0.39
	T	20-4.1	4.00	7.92	1.98	5.4	2300	1200	0.40
	A		3.96	7.56	1.91	3.7	1300	600	0.38
	T	40	1.90	3.70	1.95	4.2	1400	1100	0.39
	A		1.64	3.85	2.35	5.2	2000	1000	0.50
	T	60	0.75	1.82	2.43	5.0	1500	2200	0.52
	A		0.63	1.68	2.67	5.9	2900	2100	0.57
	T	60-3.8	0.86	1.96	2.28	4.6	2500	1300	0.48
	A		0.61	1.60	2.62	5.5	2800	1900	0.56
	T	60-4.1	0.55	1.41	2.56	5.0	1700	2500	0.55
	A		0.56	1.48	2.64	5.5	3000	2000	0.57
	T	85	0.30	0.75	2.50	2.5	2400	2400	0.54
	A		0.26	0.91	3.50	3.5	2400	2400	0.73
	T	100	0.15	0.53	3.53	3.6	2700	2500	0.74
	A		0.29	0.93	3.21	4.8	2000	2600	0.68
	T	95ACN	1.31	1.70	1.30	1.3	1200	800	0.15
	A		1.16	1.68	1.45	1.4	1300	1200	0.22
B3	T	20	4.63	5.05	1.09	0.8	2300	1800	0.05
	A		3.32	3.65	1.10	0.8	1600	2200	0.06
	T	20-4.1	4.20	4.51	1.07	0.7	800	800	0.04
	A		3.80	4.14	1.09	0.8	2500	1500	0.05
	T	40	1.77	1.88	1.06	0.5	3300	1700	0.03
	A		1.47	1.68	1.14	1.1	3200	2900	0.08
	T	60	0.65	0.71	1.09	0.3	1300	1100	0.05
	A		0.50	0.61	1.22	0.9	2100	2100	0.12
	T	60-3.8	0.77	0.86	1.12	0.4	1100	1200	0.06
	A		0.49	0.69	1.41	1.1	1400	1200	0.20
	T	60-4.1	0.57	0.57	1.00	0			0
	A		0.46	0.55	1.20	0.6	1800	1800	0.11
B4	T	20	4.55	4.91	1.08	0.8	2600	2500	0.05
	A		4.25	4.25	1.00	0			0
	T	20-4.1	4.07	4.27	1.05	0.4	1800	1500	0.03
	A		3.55	3.55	1.00	0			0
	T	40	1.86	2.01	1.08	0.5	1600	1500	0.05
	A		1.41	1.41	1.00	0			0
B5	T	85	14.7	15.3	1.04	1.4	2600	1000	0.02
	A		23.1	25.2	1.09	1.4	4200	4300	0.05
	T	100	6.80	6.80	1.00	0			0
	A		8.60	9.38	1.09	1.3	4500	4500	0.05
Class C Compounds: Non-AA Compounds with at Least One Aromatic Ring									
C1	T	20	8.71	11.7	1.34	3.1	2200	2000	0.17
	A		6.51	6.90	1.06	0.6	3100	1700	0.03
	T	20-4.1	8.00	10.5	1.31	2.9	2800	2000	0.16
	A		7.01	7.49	1.07	0.7	2200	1700	0.04
	T	40	2.62	3.20	1.22	1.6	1800	2000	0.12
	A		2.30	2.30	1.00	0			0
	T	60	1.06	1.20	1.13	0.6	1500	1300	0.07
	A		0.68	0.68	1.00	0			0
	T	60-3.8	1.13	1.32	1.17	0.8	1800	1100	0.09
	A		0.71	0.71	1.00	0			0
	T	60-4.1	0.89	1.01	1.13	0.6	1600	1400	0.07
	A		0.61	0.61	1.00	0			0
C2	T	60	3.07	3.62	1.18	0.8	700	600	0.10
	A		3.19	3.19	1.00	0			0
	T	60-3.8	6.53	8.10	1.24	1.8	1400	1500	0.13
	A		4.20	4.62	1.10	0.7	1300	1300	0.06
C3	T	20-4.1	0.83	2.96	3.57	3.8	700	350	0.74
	A		0.93	2.60	2.80	3.6	700	500	0.60
	T	40	1.76	3.13	1.78	1.2	300	100	0.34
	A		0.53	0.53	1.00	0			0
	T	60	1.21	2.20	1.82	0.8	60	70	0.35
	A		0.16	0.16	1.00	0			0
	T	60-3.8	2.08	5.03	2.42	2.6	500	200	0.52
	A		2.32	4.36	1.88	2.0	200	360	0.37
	T	60-4.1	0.38	2.77	7.29	5.4	340	650	1.16
	A		0.31	1.33	4.29	3.9	700	800	0.85
	T	85	0.25	2.19	8.76	5.6	400	250	1.28
	A		0.42	1.71	4.07	3.1	700	700	0.83
	T	100	0.78	4.21	5.40	4.5	350	330	0.99
	A		0.98	2.60	2.65	2.2	150	300	0.57
	T	95ACN	4.16	19.1	4.59	5.0	300	140	0.89
	A		8.00	14.16	1.77	2.0	260	240	0.33

Table 3. Continued

compd	CSP[a]	mobile phase[b]	k'_1	k'_2	α	R_s	N_1 (plates)	N_2 (plates)	ΔG^c (kcal/mol)
			Class C Compounds: Non-AA Compounds with at Least One Aromatic Ring						
C4	T	40	12.4	16.1	1.30	1.8	700	1000	0.15
	A		3.84	3.84	1.00	0			0
	T	60	2.00	2.66	1.33	1.5	800	900	0.17
	A		1.84	1.84	1.00	0			0
	T	60-3.8	3.50	4.48	1.28	1.9	1400	1600	0.14
	A		2.06	2.32	1.13	1.0	2000	2100	0.07
	T	60-4.1	2.55	3.06	1.20	1.3	1600	1400	0.11
	A		1.56	1.68	1.08	0.7	1400	1200	0.04
			Class D Compounds: R−CH_2−CHOH−CH_2−R′						
D1	T	40	0.37	0.63	1.69	1.7	1100	2300	0.31
	A		1.47	2.07	1.41	2.5	2000	2200	0.20
	T	60	4.50	4.50	1.00	0			0
	A		1.63	2.45	1.50	4.0	3600	3500	0.24
	T	85	1.08	2.19	2.03	4.6	1800	2000	0.41
	A		1.50	4.50	3.00	7.0	1200	1500	0.64
D2	T	85	10.3	11.2	1.09	1.1	4900	2200	0.05
	A		5.00	5.00	1.00	0			0
	T	100	5.93	7.06	1.19	1.5	2100	1300	0.10
	A		5.30	5.72	1.08	0.9	3400	2800	0.05
D3	T	85	0.80	0.95	1.19	0.9	2100	2000	0.10
	A		1.21	1.73	1.43	1.8	1000	1300	0.21
	T	100	3.06	3.37	1.10	0.5	1500	500	0.06
	A		2.68	3.56	1.33	1.6	450	500	0.17
D4	T	85	10.0	10.6	1.06	1.3	9500	8500	0.03
	A		10.7	11.3	1.06	0.7	5500	5000	0.02
	T	100	4.03	4.78	1.19	1.7	2600	2500	0.10
	A		3.45	3.45	1.00	0			0

[a] T = teicoplanin chiral stationary phase (CSP); A = aglycone CSP. [b] The mobile phase code is the same as that given in Table 2. [c] Enantioselective energy ($-RT \ln \alpha$).

with the first eluting enantiomer often differed widely from the efficiency observed with the second enantiomer. The resolution factor was calculated using

$$R_s = {}^1/_2(t_{R_2} - t_{R_1})/(t_{R_2}/(\sqrt{N_2}) + t_{R_1}/(\sqrt{N_1}))$$

where t_R is the retention time of an enantiomer and N, the corresponding peak efficiency. The difference in enantioselective free energy was estimated using[13]

$$-\Delta\Delta G = RT \ln \alpha$$

The highest enantioselectivity factors obtained on the aglycone CSP were α = 110 and 11.8 for *N*-acetylfluorophenylalanine (**A7**) and DOPA (**A2**), respectively. The highest enantioselectivity factors obtained on the teicoplanin CSP were α = 40 and 8.8 for *N*-acetylfluorophenylalanine (**A7**) and 4-hydroxymandelic acid (**C3**), respectively. The very high values for compound **A7** may have high errors because the first enantiomer of this phenylalanine derivative is very poorly retained on both CSPs. The near-zero k'_1 values for this first enantiomer were difficult to estimate accurately. However, several α values higher than 10 were obtained for both CSPs. This corresponds to a difference in enantioselective free energy in the 1.4 kcal/mol range, which is indicative of the very high enantiorecognition capability of these two stationary-phase chiral selectors. Tables 2 and 3 show that the resolution factors associated with these high enantioselectivity factors can be as high as 8. These stationary phases are sometimes so selective in resolving enantiomers that it may take several tens of minutes after the first enantiomer appears to see the peak representing the second enantiomer. Figure 3 shows the chromatograms of DOPA (**A2**) on the two CSPs with the 60%/40% MeOH/H_2O unbuffered mobile phase. A 2 min span separates the first eluting L-DOPA enantiomer from the D-enantiomer on the teicoplanin CSP. This time difference increases to 16 min for these enantiomers on the aglycone CSP. As already described in our previous work, the L-forms of all the common amino acids elute first and the D-forms elute second.[3−5,7]

For the amino acid compounds, the change in mobile-phase composition induced relatively small variations in enantioselectivity factors and enantioselective free energies (Table 2). The difference in enantioresolution free energy of nonionizable compounds is almost independent of the methanol content in the mobile phase on both CSPs (Table 3).

Role of the Carbohydrate Moieties. The aglycone of teicoplanin is not water soluble. The teicoplanin solubility in water is in the gram per liter range.[14] Thus, at least one of nature's roles for the three sugar units is to render the teicoplanin molecule water soluble. The nonyl chain of the glucosamine unit is responsible for its surfactant properties. This would tend to enhance the concentration of teicoplanin at various interfaces, including the bacterial cell wall. These features, water solubility and surfactant properties, are not essential for a chiral stationary phase. However, the three carbohydrate units are themselves

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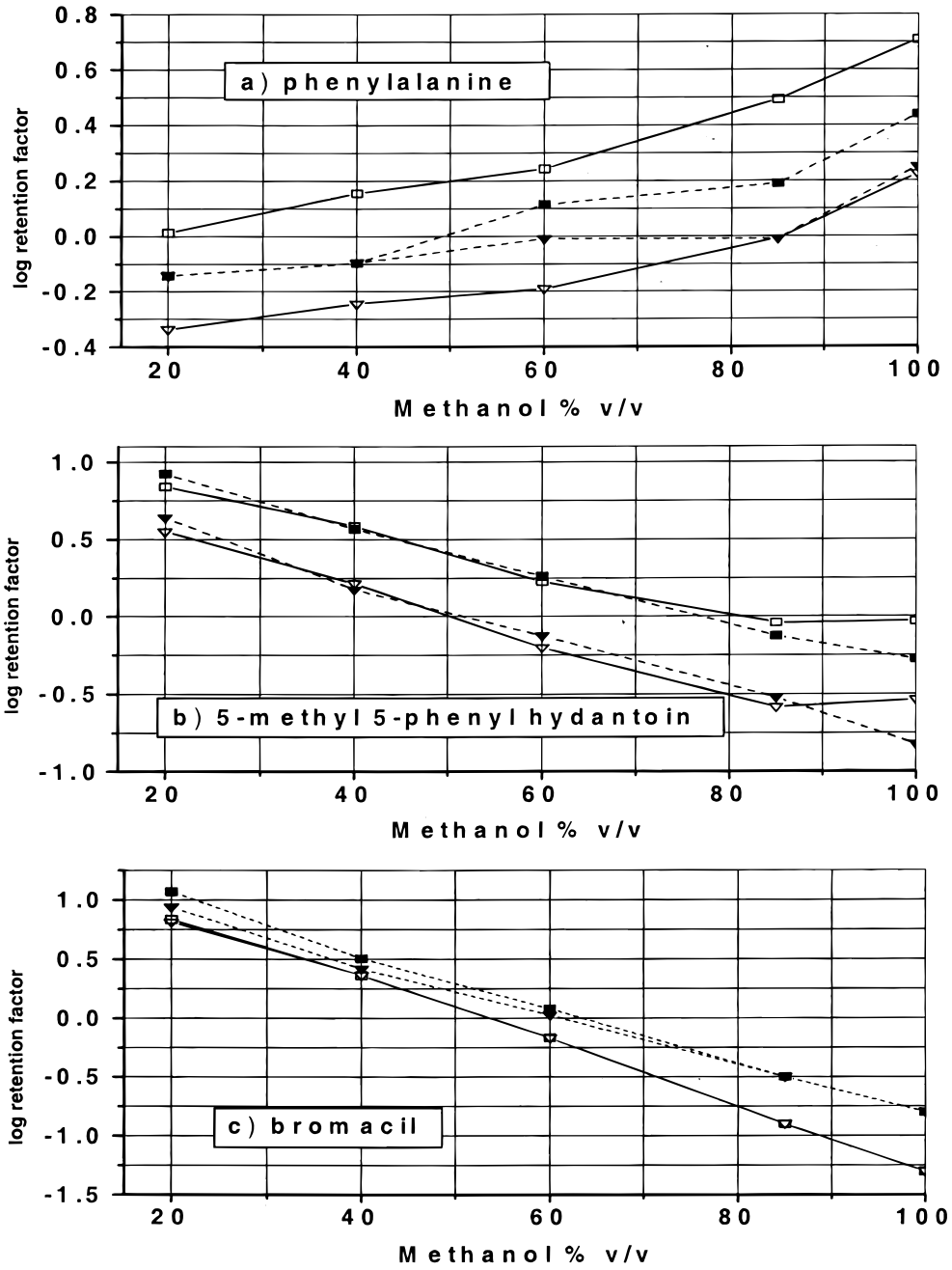

Figure 2. Retention factors versus methanol mobile-phase content: (a) phenylalanine (**A6**); (b) 5-methyl-5-phenylhydantion (**B2**); (c) bromacil (**C1**); (▼) k'_1 for teicoplanin; (■) k'_2 for teicoplanin; (▽) k'_1 for aglycone; (□) k'_2 for aglycone.

chiral. Comparing the results obtained on the two CSPs may help to determine the role of the pendant sugar moieties in enantiorecognition.

To quantify the effect of the sugar units, the differences in enantioselective free energies, ΔG, listed in Tables 2 and 3, between the two CSPs were used. The values obtained for a given compound were summed for every mobile phase, and the rounded average values for the compounds were plotted as shown in Figure 4. For example, for D,L-proline (compound A8), three mobile phases were shown to produce successful enantioseparation. The ΔG energies were 0.63, 0.00, and 0.62 kcal/mol on the teicoplanin CSP, that is, a total of 1.25 kcal/mol. The corresponding values were 0.86, 0.86, and 0.87 kcal/mol on the aglycon CSP, a total of 2.59 kcal/mol. The energy difference between the two CSPs is $1.25 - 2.59 = -1.34$ kcal/mol for three different mobile phases or an average value of -0.447 kcal/mol that is rounded to -0.4 kcal/mol for Figure 4. A negative number means that the compound is better separated on the aglycone CSP. A positive number means that the compound is better separated on the native teicoplanin CSP, which contains the carbohydrate moieties. Note that most of the negative numbers are for amino acids (the class A compounds) while most of the positive numbers are for compounds that do not contain carboxylate functionalities.

Cases Where the Carbohydrate Units Decrease Enantiorecognition. Clearly, the α-amino acids are much better resolved by the aglycone CSP than by the native teicoplanin CSP. Figure 3 shows the DOPA (**A2**) enantioseparations on the two CSPs. The 1 kcal/mol energy difference corresponds to a 4 times higher α value

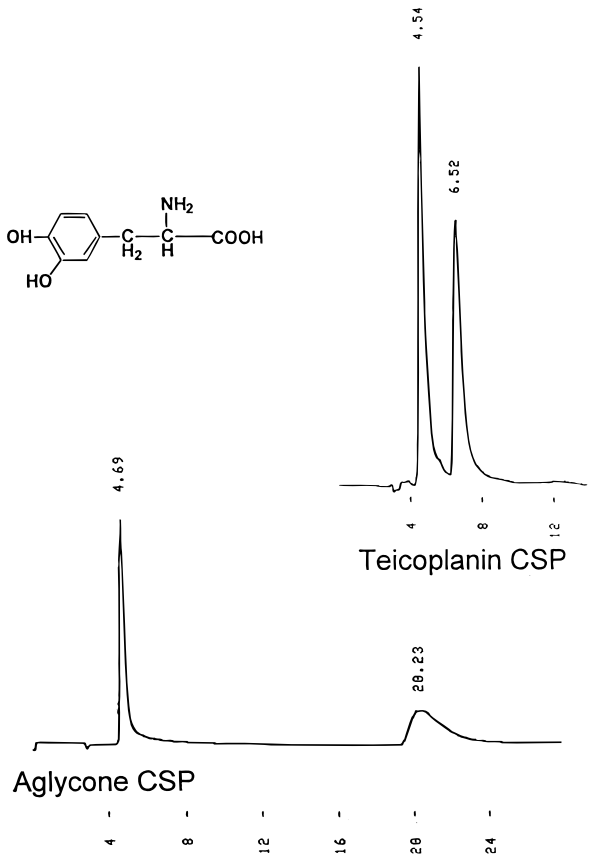

Figure 3. Chromatograms of D,L-DOPA on the native teicoplanin CSP (top) and the aglycone CSP (bottom). Conditions: mobile phase 60% methanol/40% water, pH 4.1 with TEA + acetic acid; 1 mL/min; UVdetection at 254 nm; $T = 22$ °C.

on the aglycone CSP compared to the teicoplanin CSP. For the α-amino acids, the difference in enantioselective free energy is about 0.5 kcal/mol higher on the aglycone CSP than on the teicoplanin CSP. The α values of amino acids are 2−3 times higher on the aglycone CSP than on the native teicoplanin CSP. This significant energy difference means that the sugar units decrease the amino acid enantiorecognition. This also indicates that it is the aglycone basket of the teicoplanin molecule that is solely responsible for enantiorecognition of the common α-amino acids. The sugar units may be present to decrease the affinity for amino acids other than D-ala, since the teicoplanin target is the D-ala-D-ala peptide termination in the Gram(+) bacterial cell wall.[2] It was shown for vancomycin, ristocetin, and eremomycin that the sugar units promoted the dimerization of the antibiotic molecules, enhancing the binding to the target L-lysine-D-alanine-D-alanine.[15]

From a chiral separations point of view, the sugar units of the native teicoplanin molecule may intervene in the chiral recognition process in at least three ways: (i) steric hindrance, where the sugar units occupy room inside the "basket", which limits the access of other molecules to binding sites; (ii) blocking possible interaction sites on the aglycone, where two sugars are linked through phenol hydroxyl groups and the third sugar is linked through an alcohol moiety (Figure 1); (iii) offering competing interaction sites, where the three sugars are themselves chiral and have hydroxyl, ether, and amido functional groups.

The high free energy difference between the two related CSPs is mainly observed with amino acids (Table 2). This may be due to the effect of steric hindrance, but the two other possibilities should be considered as well. The 1 kcal/mol enantiorecognition energy difference in the case of DOPA (**A2**) compared to the 0.5 kcal/mol value for phenylalanine (**A6**) clearly indicates that the hydroxyl groups of the benzene ring of DOPA interact with a hydroxyl group of the aglycone that was formerly occupied by a sugar unit in the teicoplanin molecule. This interaction enhances the enantiorecognition of DOPA.

In general, it seems that the steric hindrance effect of the sugar moieties is predominant for α-amino acids, which are thought to "dock" and bind inside the cleft of the aglycone near its amine (or ureido—if attached to a linkage chain) functional group.[5,9,16] This constitutes a highly specific binding site for α-D-amino acids. It appears that most D-amino acids can associate more strongly and easily with this active binding site of the aglycone than they can on the native teicoplanin molecule. This closer approach produces stronger enantiointeraction and better enantioselectivity. This can be seen in Figure 2, which shows that the L-enantiomer of phenylalanine (**A6**) is slightly less retained on the aglycone CSP than on the teicoplanin CSP. However, the opposite trend is seen for the D-enantiomer. The addition of these two effects (slightly reduced L-retention and increased D-retention on the aglycone CSP) produces the large difference in the observed enantioselectivities of the two CSPs. Besides steric hindrance, the two phenols and the hydroxyl group created on the aglycone seem to further enhance the interaction with the amino acids.

The cases of the carnitine and acetylcarnitine compounds (**D1** and **D3**) are different: they are partially resolved on the teicoplanin CSP, and the enantioresolution is enhanced on the aglycone CSP (Table 3). Once again, steric hindrance may be invoked to explain the better enantioresolution of carnitine by the aglycone CSP. However, carnitine is a γ-amino acid whose stereogenic center bears a hydroxyl group (Table 1). Since the acetylation of this hydroxyl group (acetylcarnitine, **A1**) produces better enantiorecognition on both CSPs (Table 3), it is likely that the primary stereoselective interactions take place at or near the active binding site for α-amino acids[15] and the carboxylate moiety of the carnitine molecules. Reducing the capability of the alcohol group to hydrogen-bond to the carboxylic group helps the enantiorecognition in this case.

Cases Where the Carbohydrate Units Are Necessary for Enantiorecognition. Of the class A compounds, only folinic acid (**A3**) is better resolved by the native teicoplanin CSP. However, compound **A3** (which is a metabolite of folic acid) is quite different from the common protein amino acids in Table 1. Its nitrogen atom bears a large amido substitutent (Table 1). Compounds **A12** and **A13** are structurally related to amino acids, but the amino group is replaced by oxygen. This substitution significantly decreases the binding energy of the amino acid binding site. Thus, their enantioresolution factors are lower than those of the amino acids. Consequently, the mobile phase must be optimized to obtain baseline resolution (i.e., >99% methanol, Table 2). There are no clear differences between the two CSPs, suggesting that the stereoselective binding site, in this case, may not be the amino

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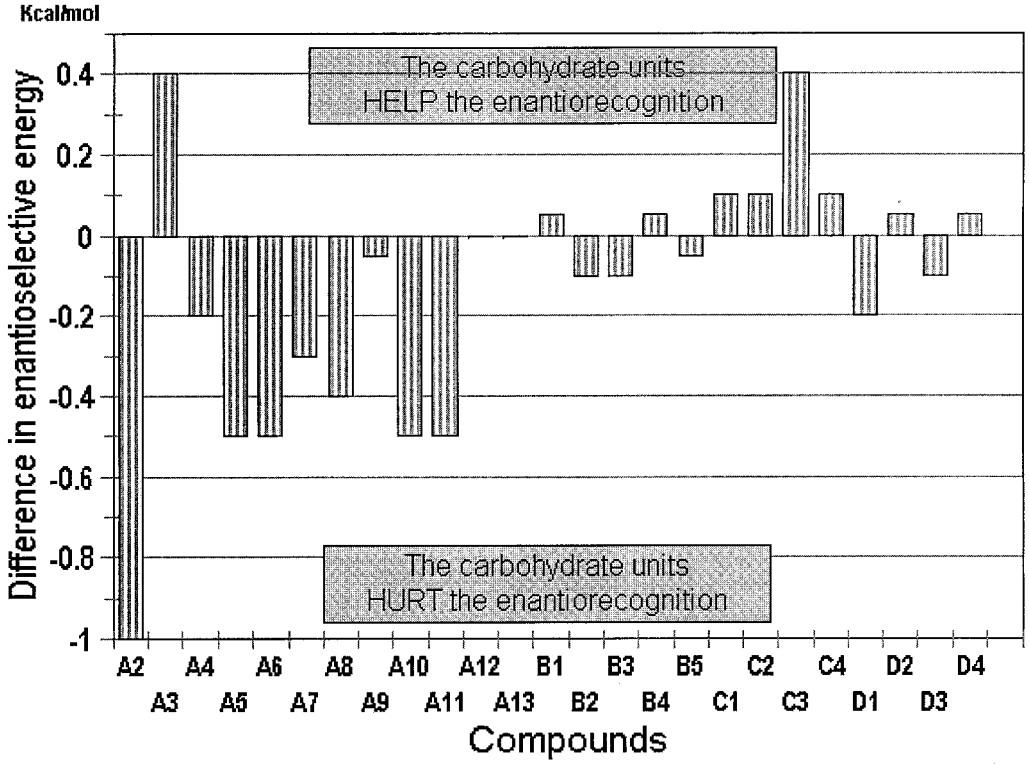

Figure 4. Average enantioselectivity energy differences between the aglycone and the native teicoplanin CSPs. A positive value corresponds to a better enantioresolution by the teicoplanin column. A negative value corresponds to a better enantioresolution by the aglycone column. For compound codes, see Table 1.

acid binding site. Excluding the two carnitines, of the 13 non amino acid compounds, 8 are better enantioseparated by the teicoplanin CSP, that is, ~70%. Four compounds are better resolved by the aglycone CSP. Of the eight racemates better resolved by the teicoplanin CSP, two are not separated by the aglycone CSP. This means that 2-aminocyclohexane carboxylic acid (**B1**) and styrene oxide (**B4**) need the carbohydrate moieties to be enantioresolved. The six other compounds are separated by both CSPs. For example, 4-hydroxymandelic acid (**C3**) shows values of 3.56, 7.30, and even 18 on the teicoplanin CSP. The corresponding α values are 2−3 times lower on the aglycone stationary phase. This demonstrates that the sugar units often help the enantioresolution process in the case of non amino acid enantiomers.

Enantioselective Association. It is well-known that a small change in a molecule can cause a large change in its enantiorecognition by a CSP.[7] This effect is observed among phenylalanine (**A6**), DOPA (**A2**), and *N*-acetylfluorophenylalanine (**A7**). The addition of two hydroxy groups to the benzene ring of phenylalanine produces DOPA (Table 1). The enantiorecognition free energy increased by about 0.3 kcal/mol on the teicoplanin CSP and by more than 0.8 kcal/mol on the aglycone phase. A fluorine atom on the benzene ring and acetylation of the free amino group produce compound **A7**, whose enantiorecognition free energy increased by almost 1 kcal/mol on the two CSPs (Table 2), proving that additional interactions take place with the aglycone basket. These could be $\pi-\pi$ interactions between the chlorine-substituted benzene ring of the basket and the fluorine-substituted benzene ring of **A7** or hydrogen-bond interactions between the acetylated amine and an amido group in the basket. Similar observations can be made with non amino acid compounds such as methylphenylhydantoin (**B2**) and methylphenylsuccinimide (**B3**), which differ by a methylene group in **B3** and an NH group in **B2**. This simple molecular change destroys the enantiorecognition of hydantoin. The corresponding energy is reduced by 0.4−0.5 kcal/mol for the succinimide derivative. But the resolution factors that were in the 3−5 range on the two CSPs for the hydantoin derivative drop to 0.8 down to 0, indicating no separation for the succinimide compound. These energy changes are independent of the mobile-phase composition. The positive point about teicoplanin-based CSPs is that if these stationary phases have a clear receptor accepting amino acid substrates, they also have several other enantioselective binding sites that accommodate, more loosely, a variety of other enantiomers (Table 3).

Kinetic Considerations. Tables 2 and 3 list the chromatographic efficiencies, *N*, roughly estimated using the classical equation

$$N = 5.54(t_r/W_{0.5h})^2$$

in which t_r is the peak retention time and $W_{0.5h}$ is the peak width at half-height. This equation is exact only if the peak is perfectly symmetric. The obtained efficiencies are in the thousand plate range, which is low for a modern 25 cm HPLC column. These values are 10−40 times lower than the efficiencies of these columns when new and tested with a *n*-hexane/chloroform normal phase (~40 000 plates/m). Since the chromatographic system was not optimized for band-broadening minimization, our goal is not to give the exact values of the peak efficiencies but to show that

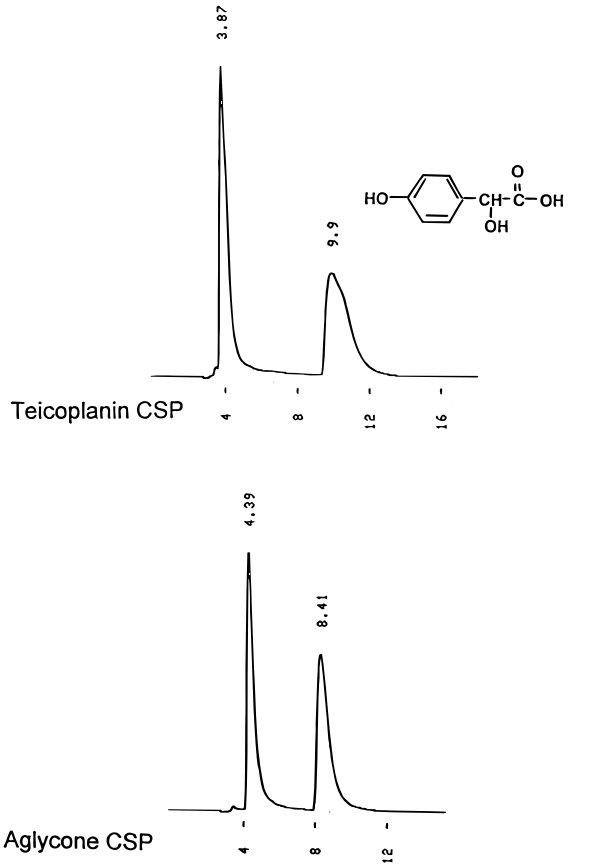

Figure 5. Chromatograms of 4-hydroxymandelic acid on the native teicoplanin CSP (top) and the aglycone CSP (bottom). Conditions: mobile phase 85% methanol/15% water, pH 4.1 with TEA + acetic acid; 1 mL/min; UV detection at 254 nm; $T = 22$ °C.

there is often a significant difference in efficiency between the first eluting enantiomer and the second (Figures 3 and 5). For D,L-DOPA and the 60% methanol mobile phase (**A2** and Figure 3), the efficiencies on the teicoplanin CSP were 1300 plates for the first eluting enantiomer and 800 plates for the second eluting enantiomer, a 38% drop. This trend was even more pronounced for the aglycone CSP, with 1500 and 600 plates for the first and second enantiomers, respectively, a 60% drop. It was even lower with the more polar 20% methanol mobile phase, where the efficiencies were 2200 plates for the first eluting peak and dropped to a mere 350 plates for the second, a 7-fold lower value. The mass transfer of the more retained enantiomer is much slower than that of the first eluting enantiomer. The interaction between the D-amino acids is thermodynamically strong, producing high retention times and slow adsorption−desorption kinetics, which result in poor mass transfer and kinetics. This trend is observed to a much lesser extent for the non amino acid solutes (Table 3 and Figure 5). This phenomenon was described previously for the teicoplanin CSP.[5] Indeed it can even be used to distinguish between D- and L-amino acids when no standards are available.

CONCLUSION

It is clear that the carbohydrate units on teicoplanin are not needed for the enantioresolution of the common α-amino acids. The cleft near the amine end (or ureido group—if attached to a linkage chain) of the aglycone basket is an important part of the receptor site for amino acid recognition. Amino acids appear to have an easier access to this site on the aglycone CSP, which produces much higher enantioselectivities and resolution factors for these compounds compared to those obtained on the native teicoplanin CSP. Non amino acid compounds that have carboxylate groups also can associate with the aglycone amino acid binding site. Sometimes, these analytes are better resolved by the aglycone CSP. However, many non amino acid compounds were enantioresolved by a combination of interactions involving both the aglycone basket and its attached sugar units. These compounds are better resolved, or resolved only, on the native teicoplanin CSP. They are often neutral compounds or amine-containing compounds lacking an anionic group that could enhance their ability to bind at the amino acid site of the teicoplanin "basket".

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