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ORIGINAL PAPER

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Separation of the enantiomers of substituted dihydrofurocoumarins by HPLC using macrocyclic glycopeptide chiral stationary phases

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Abstract Enantiomer separations by HPLC using the macrocyclic glycopeptides teicoplanin (Chirobiotic T), teicoplanin aglycon (Chirobiotic TAG), and ristocetin A (Chirobiotic R) chiral stationary phases (CSP) have been achieved on a unique series of potentially biologically active racemic analogues of dihydrofurocoumarin. The macrocyclic glycopeptides have proven to be very selective for this class of compound. All of the 28 chiral analogues examined afforded baseline separation on at least one of the macrocyclic glycopeptide CSP. The teicoplanin CSP showed the broadest enantioselectivity with 24 of the compounds baseline separated. The TAG and the R CSP produced 23 and 14 baseline separations respectively. All three mobile phase modes, i.e. normal phase (NP), reversed phase (RP), and new polar organic modes (PO), have been evaluated. The NP mode proved to be most effective for the separation of chiral dihydrofurocoumarins on all CSP tested. In the reversed phase (RP) mode, all three CSP separated a similar number of compounds. It was observed that the structural characteristics of the analytes and steric effects are very important factors leading to chiral recognition. Hydrogen bonding was found to play a secondary role in chiral discrimination in the normal phase and polar organic modes. Hydrophobic interactions are important for chiral separation in the reversed-phase mode. Chromatographic retention data does not provide information on the absolute configuration of these chiral dihydrofurocoumarin derivatives. However, when coupled with circular dichroism using the exciton coupling chirality method, the enantiomer elution order and the absolute configuration of some chiral dihydrofurocoumarins were successfully determined.

Keywords HPLC · Macrocyclic glycopeptide · Chiral stationary phases · Enantiomer separation · Dihydrofurocoumarin

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Introduction

The first documented use of dihydrofurocoumarins (Fig. 1) was in ancient Egypt, where dihydrofurocoumarin-rich extracts were used for the treatment of skin disorders, such as psoriasis and vitiligo [1, 2, 3]. Extensive investigation of this class of compounds started in the late 1970s and a variety of useful medical effects were found [4, 5, 6]. Recently, different chiral dihydrofurocoumarins have been isolated as natural products, and shown to be active against a number of diseases [7]. For example, marmesin and columbionetin derivatives have been shown to exhibit cytotoxicity against KB cells, to inhibit c-AMP (which affects coronary vasodilation) and to mediate the action of acetylcholinesterase (which plays a role in Alzheimer's disease) [4, 5, 6, 7, 8, 9]. Dihydrofurocoumarins appear to

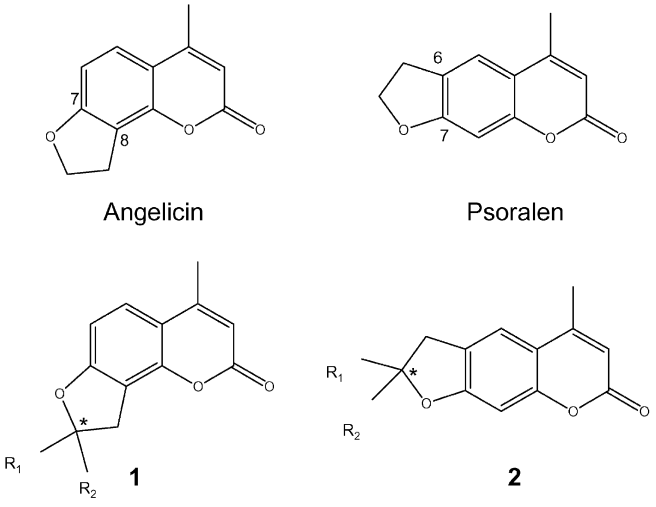

Fig. 1 The top two structures are angelicin and psoralen, which are often found in nature. Structure *1* is a substituted dihydroangelicin and structure *2* is a substituted dihydropsoralen. R_1 and R_2 can be various types of aliphatic or aromatic substituents. Note, when $R_1 \neq R_2$, the carbon marked with an asterisk is the stereogenic center

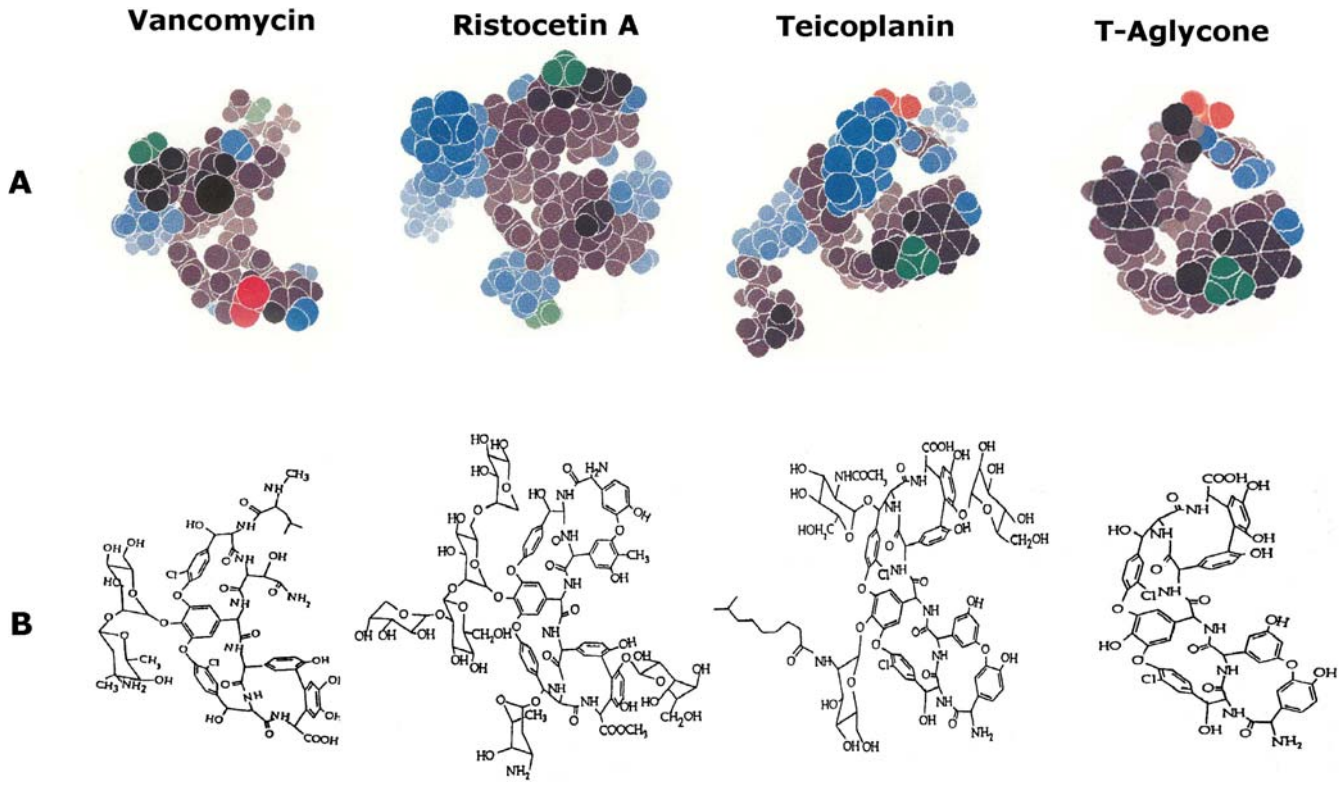

Fig. 2 Structures of the four macrocyclic antibiotics (vancomycin, teicoplanin, ristocetin A, and teicoplanin aglycon) tested in this study showing a profile view of the aglycon "basket" using (*A*) space-filling molecular models produced through energy minimization, and (*B*) stick figures. The *colored* atoms in part *A* denote the hydrophilic moieties, while the *black* portion designates the more hydrophobic regions. *Red* represents carboxylate groups, *green* designates ammonium groups, and *blue* indicates hydroxyls. *Black* regions include the aromatic rings, connecting carbons, and amido linkages (revised from Ref. [19])

be nontoxic [4, 10, 11, 12]. Clearly, the potential pharmaceutical applications of these compounds are promising. However, the pharmacological activity of both enantiomers must be assessed. This means that asymmetric synthesis or enantioselective separations must be used to prepare the pure enantiomers. Methods for the asymmetric synthesis of these compounds are under development, but are proving to be very difficult and afford only low yield [13].

The palladium-catalyzed annulation of 1,3-dienes by *o*-iodoacetoxycoumarins has produced racemic substituted dihydrofurocoumarins (Fig. 1) in high yields [14]. Effective methods for separating and identifying these synthetic products, as well as the stereoisomers of related natural products are desperately needed.

Macrocyclic glycopeptides are one of the fastest growing classes of chiral selectors. Since their first introduction as CSP for HPLC, TLC, and as buffer additives for CE in 1994 by Armstrong [15, 16, 17, 18], enantiomer separations of over a thousand different compounds have been reported [19]. The structure of the macrocyclic glycopeptides includes many functional groups, including aromatic, hydroxyl, amine, carboxylic acid moieties, amide linkages, hydrophobic pockets, etc. (Fig. 2). A complete description of this family of compounds has been given previously [15, 18, 19, 20, 21, 22, 23]. All possible molecular interactions, including ionic interactions, hydrogen bonding, steric, dipole–dipole and π–π interactions, as well as hydrophobic interactions responsible for chiral recognition, are available within their relatively compact structures. This class of CSP is multimodal, which means they can be utilized in any of the known mobile phase modes including normal-phase, reverse-phase and polar organic phase modes [15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56].

Two of the most common coumarin-based pharmacologically active compounds are warfarin and coumachlor. Their racemates have been separated by a wide variety of CSP. However, to our knowledge, no enantioseparations of chiral dihydrofurocoumarins (Fig. 1) have been reported on any CSP. In this study, 28 chiral dihydrofurocoumarins have been evaluated. The first part of this work discusses the overall separation performance using different macrocyclic glycopeptide CSP and different mobile phase conditions. Subsequently, the effect of analyte structure on the enantioselective separation is discussed. The information gleaned from the separation of structurally related compounds using different mobile phases and closely related CSP provides some insight into the chiral recognition mechanism for the substituted dihydrofurocoumarins.

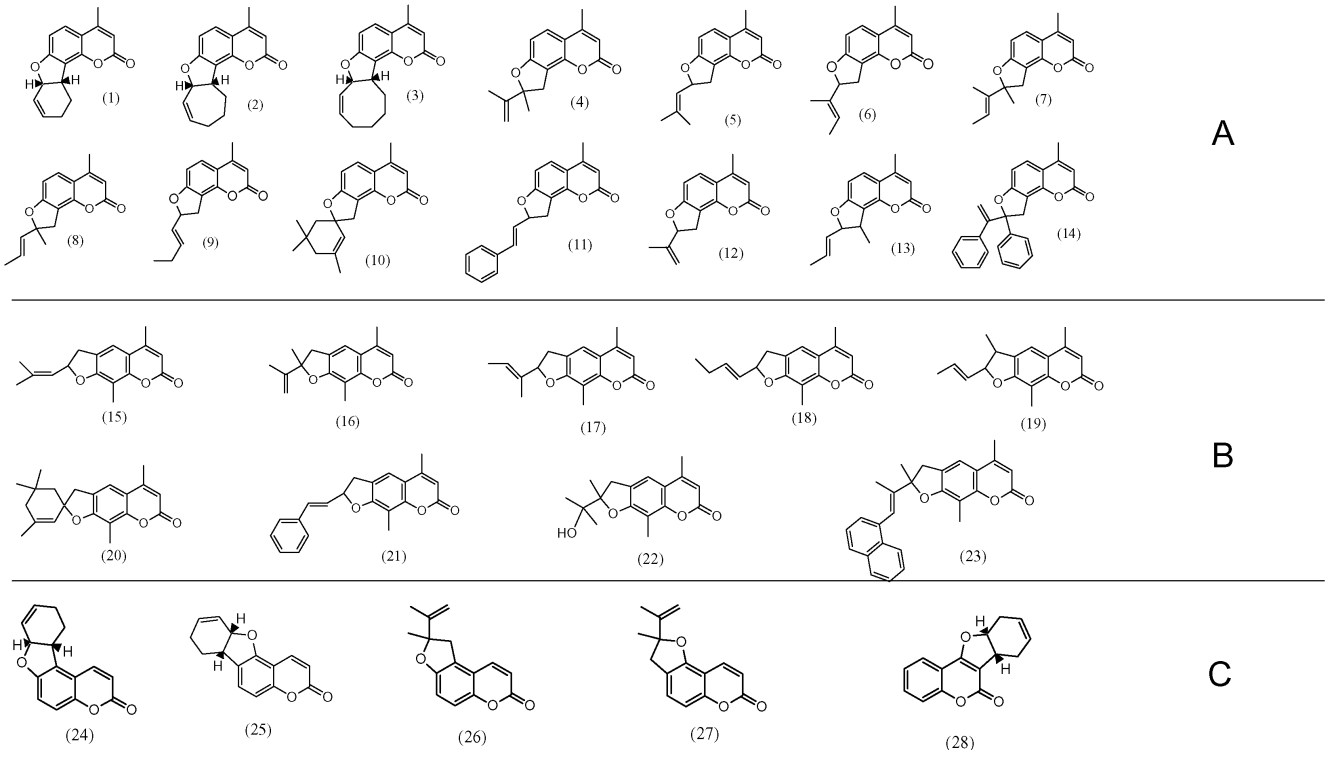

Fig. 3 Structures of the chiral dihydrofurocoumarins evaluated in this study. Compounds listed in group *A* consist of fourteen dihydroangelicins, group *B* contains nine dihydropsoralens, and group *C* consists of five additional dihydrofurocoumarin derivatives

Experimental

Dihydrofurocoumarin derivatives

All of the racemic dihydrofurocoumarin derivatives were synthesized and purified as previously reported [14]. The structures of the 28 chiral coumarin derivatives used in this study are given in Fig. 3. The dihydrofurocoumarins shown in Fig. 3 can be divided into three structural categories. The compounds in the first group A are dihydroangelicin derivatives. The furan ring is fused to the 7 and 8 positions of the coumarin giving these molecules a "bent" appearance. The second group B (Figs. 1 and 3) is referred to as the dihydropsoralens. The furan ring in these compounds is fused to the 6 and 7 positions of the coumarin giving these molecules a linear orientation. The last group (C) of compounds has the furan ring fused to the 5 and 6 positions or 3 and 4 positions (Fig. 3). All 28 dihydrofurocoumarins have stereogenic centers located in the furan ring. Note that both compounds 13 and 19 have two stereogenic centers (Fig. 3). Separations of two pairs of enantiomers were achieved in both of these cases.

Other chemicals

HPLC-grade acetonitrile (ACN), methanol (MeOH), 2-propanol (IPA), *n*-heptane (Hep), as well as certified-grade triethylamine (TEA) and acetic acid (AA) were purchased from Fisher (St Louis, MO, USA) and/or EM (Gibbstown, NJ, USA). Ethanol (EtOH), 200 proof, was purchased from AAPER Alcohol and Chemical (Shelbyville, KY, USA). Water was deionized and filtered through active charcoal and a 5 µm filter.

HPLC system and the chiral stationary phases

Separations were achieved using a HP 1050 HPLC system with UV detector, auto injector, using computer controlled Chem-station data processing software. All three CSP, trade named Chirobiotic T, Chirobiotic TAG, and Chirobiotic R columns (250 mm× 4.6 mm i.d.) were obtained from Advanced Separation Technologies (Astec, Whippany, NJ, USA). The detailed structures of the chiral selectors used in these CSP are shown in Fig. 2. The chiral stationary phases were prepared by bonding the chiral selectors to a 5 µm spherical porous silica gel through a linkage chain [15, 53]. All separations were repeated at least three times with very good reproducibility. Detection wavelengths were varied between 220 nm and 327 nm, which correspond to the two molecular absorption maxima of the dihydrofurocoumarins. The injection volume was 2 µL. Sample concentration is about 1 mg mL^{-1}. Separations were carried out under isocratic conditions at a flow rate of 1 mL min^{-1} or 0.5 mL min^{-1} at 21 °C. The mobile phase was premixed and degassed under vacuum conditions.

Mobile phase compositions

Three different mobile phase modes (i.e., normal phase, reversed phase and new polar organic modes) were used and compared. In the normal-phase mode, *n*-heptane was used as the non-polar solvent and the polar organic modifier was ethanol, which proved to give better resolution than using isopropanol as the modifier. In the reversed-phase mode, a mixture of pure deionized water and methanol was used throughout the study. Aqueous buffer solutions of 1% triethylamine (TEA, 0.07 mol L^{-1}), with a pH of 4.1 adjusted by acetic acid, were tried, but did not produce any significant difference in the separations. Reversed phase separations using acetonitrile as the modifier were compared to the separations achieved using methanol as the modifier. It was found that using methanol as the modifier gave much better selectivity and resolution. In the polar organic mode, 100% pure methanol and acetonitrile were used and compared. Addition with some acid (acetic acid) and base (TEA) at various ratios to the mobile phase was tried, but did not produce any improvements in the separations. All the reported mobile phase compositions used are not necessarily the optimum con-

Table 1 Chromatographic results obtained with chiral dihydrofurocoumarin derivatives on three macrocyclic antibiotic CSP

Compound number[a] Mobile phase[b]	Ristocetin A			Teicoplanin			Teicoplanin aglycone		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
1									
NP: Heptane/EtOH	6.69	1.03	0.5	5.13	1.15	1.93	3.48	1.35	4.23
RP: H_2O/MeOH	5.57	1.03	0.45	4.35	1.08	0.95	7.41	1.13	1.5
PO: IPA	0.68	1.14	0.56	0.67	1.34	1.4	1.36	1.62	1.93
PO: EtOH	0.28	1.11	0.55	0.21	1.27	1.32	0.62	1.41	2.84
PO: MeOH	0.16	1	0	0.1	1.14	0.2	0.46	1.34	2.91
2									
NP: Heptane/EtOH	5.68	1.08	1.12	4.35	1.25	3.53	3.09	1.49	5.27
RP: H_2O/MeOH	7.89	1	0	10.64	1.09	1.48	14.01	1.15	1.51
PO: IPA	0.55	1.3	1.05	0.56	1.62	2.01	1.16	1.89	2.37
PO: EtOH	0.23	1.26	1.06	0.18	1.49	1.65	0.56	1.61	3.68
PO: MeOH	0.15	1	0	0.1	1.27	0.63	0.47	1.4	3.27
3									
NP: Heptane/EtOH	5.12	1.08	1.2	4.05	1.16	2.45	2.84	1.33	3.71
RP: H_2O/MeOH	11.9	1	0	17.20	1.02	0.61	20.43	1.06	0.55
PO: IPA	0.51	1.29	0.87	0.51	1.45	1.5	1.13	1.53	1.51
PO: EtOH	0.22	1.23	0.8	0.17	1.24	0.88	0.58	1.34	2.07
PO: MeOH	0.15	1	0	0.1	1	0	0.52	1.11	1.0
4									
NP: Heptane/EtOH	5.83	1	0	5.13	1.1	1.61	3.43	1.13	1.79
RP: H_2O/MeOH	6.04	1	0	5.43	1.03	0.46	7.96	1	0
PO: IPA	0.87	1	0	1.11	1.23	1.24	1.84	1.17	0.6
PO: EtOH	0.31	1	0	0.29	1.15	0.69	0.7	1.1	0.76
PO: MeOH	0.11	1	0	0.1	1	0	0.42	1	0
5									
NP: Heptane/EtOH	7.76	1.07	0.8	7.27	1.18	2.32	4.43	1.12	1.58
RP: H_2O/MeOH	7.11	1	0	6.96	1	0	10.57	1	0
PO: IPA	0.89	1.12	0.46	1.23	1.31	1.42	2.07	1.15	0.55
PO: EtOH	0.31	1.17	0.67	0.32	1.21	1.35	0.79	1.1	0.76
PO: MeOH	0.14	1	0	0.12	1	0	0.51	1.05	0.5
6									
NP: Heptane/EtOH	7.36	1	0	7.47	1	0	4.38	1.04	0.56
RP: H_2O/MeOH	7.13	1.15	1.5	6.25	1.27	2.74	9.39	1.2	1.95
PO: IPA	0.94	1	0	1.29	1	0	2.15	1	0
PO: EtOH	0.32	1.1	0.3	0.35	1	0	0.76	1.14	1.01
PO: MeOH	0.14	1	0	0.11	1.22	0.65	0.49	1.17	1.5
7									
NP: Heptane/EtOH	4.88	1.03	0.2	4.48	1.18	2.68	2.76	1.18	2.15
RP: H_2O/MeOH	7.31	1.03	0.3	6.2	1.04	0.46	9.43	1	0
PO: IPA	0.72	1	0	0.88	1.39	1.62	1.5	1.2	0.63
PO: EtOH	0.24	1.13	0.45	0.23	1.33	1.45	0.58	1.17	1.5
PO: MeOH	0.1	1	0	0.1	1	0	0.39	1.08	0.6
8									
NP: Heptane/EtOH	6.07	1	0	5.96	1.05	1.42	3.73	1.06	0.84
RP: H_2O/MeOH	5.81	1.1	1.25	5.21	1.11	1.42	7.89	1.08	0.64
PO: IPA	0.78	1	0	1.24	1.13	0.61	2.04	1	0
PO: EtOH	0.3	1	0	0.32	1.06	0.4	0.73	1	0
PO: MeOH	0.11	1	0	0.1	1	0	0.46	1	0
9									
NP: Heptane/EtOH	7.14	1.02	0.2	6.78	1.12	0.73	4.26	1.05	1.33
RP: H_2O/MeOH	7.47	1.14	1.55	7.1	1.07	0.73	10.57	1.06	0.6
PO: IPA	0.82	1.11	0.43	1.1	1.23	1.12	1.89	1.1	0.2
PO: EtOH	0.29	1.09	0.5	0.29	1.1	0.56	0.74	1	0
PO: MeOH	0.13	1	0	0.1	1	0	0.48	1	0

Table 1 (continued)

Compound number[a] Mobile phase[b]	Ristocetin A			Teicoplanin			Teicoplanin aglycone		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
10									
NP: Heptane/EtOH	4.41	1.06	0.7	4.48	1.09	1.52	2.24	1.12	1.65
RP: H_2O/MeOH	11.1	1.07	0.59	11.13	1.07	0.67	16.57	1.1	0.85
PO: IPA	0.59	1	0	0.81	1.2	0.88	1.32	1.27	0.76
PO: EtOH	0.2	1.1	0.41	0.2	1.24	1.2	0.53	1.23	1.4
PO: MeOH	0.1	1	0	0.1	1	0	0.38	1.13	0.76
11									
NP: Heptane/EtOH	14.14	1.04	0.2	13.9	1.07	1.02	8.58	1.03	0.53
RP: H_2O/MeOH	19.00	1.22	2.16	17.75	1.06	0.95	27.75	1.15	1.5
PO: IPA	1.28	1.12	0.5	1.86	1.1	0.47	3.58	1	0
PO: EtOH	0.43	1	0	0.47	1	0	1.22	1	0
PO: MeOH	0.19	1	0	0.19	1	0	0.81	1	0
12									
NP: Heptane/EtOH	7.36	1	0	7.65	1	0	4.89	1	0
RP: H_2O/MeOH	5.86	1.07	0.95	5.04	1.28	2.69	7.61	1.18	1.77
PO: IPA	1.03	1	0	1.35	1	0	2.18	1	0
PO: EtOH	0.38	1	0	0.37	1	0	0.76	1.1	0
PO: MeOH	0.15	1	0	0.11	1	0	0.48	1.15	0
13									
NP: Heptane/EtOH	4.13	1.07	0.8	3.23	1.17	2.61	2.07	1.38	4.88
	5.08	1.07	1.0	4.03	1.24	3.37	2.52	1.49	6.35
RP: H_2O/MeOH	4.95	1	0	3.94	1	0	6.39	1	0
				4.16	1	0	7.29	1	0
PO: IPA	0.46	1	0	0.36	1.56	1.4	0.8	1.62	2.4
	0.56	1	0	0.42	1.75	2	0.86	2.08	2.5
PO: EtOH	0.17	1	0	0.1	1.3	0.5	0.33	1.58	2.35
	0.21	1	0	0.11	1.64	1.5	0.37	1.67	2.92
PO: MeOH	0.1	1	0	0.05	1	0	0.23	1.51	2.6
				0.07	1	0	0.26	1.53	2.6
14									
NP: Heptane/EtOH	8.35	1.07	0.8	7.07	1.21	3.07	4.27	1.09	1.24
RP: H_2O/MeOH	24.62	1.12	1.2	42.94	1.19	1.88	49.0	1.21	1.55
PO: IPA	0.95	1	0	1.04	1.29	1.3	2.08	1	0
PO: EtOH	0.31	1.1	0.52	0.28	1.32	1.54	0.75	1.12	0.67
PO: MeOH	0.14	1	0	0.11	1.36	1.12	0.58	1.17	1.5
15									
NP: Heptane/EtOH	11.0	1.0	0.3	8.38	1.11	1.71	4.53	1.09	1.5
RP: H_2O/MeOH	9.27	1.0	0.41	7.59	1	0	10.93	1	0
PO: IPA	0.77	1	0	0.77	1.21	0.6	1.42	1.1	0.37
PO: EtOH	0.32	1	0	0.2	1.12	0.4	0.59	1.09	0.6
PO: MeOH	0.15	1	0	0.1	1	0	0.44	1	0
16									
NP: Heptane/EtOH	8.45	1.0	0.3	6.88	1.02	0.7	3.87	1	0
RP: H_2O/MeOH	10.1	1.0	1.21	6.09	1	0	8.82	1.05	0.45
PO: IPA	0.58	1	0	0.7	1	0	1.21	1	0
PO: EtOH	0.26	1	0	0.19	1	0	0.53	1	0
PO: MeOH	0.12	1	0	0.1	1	0	0.37	1	0
17									
NP: Heptane/EtOH	10.8	1.0	0.5	8.69	1.11	1.61	4.57	1.09	1.45
RP: H_2O/MeOH	10.9	1.0	0.46	7.5	1	0	10.75	1	0
PO: IPA	0.79	1	0	0.77	1.21	0.67	1.33	1.12	0.2
PO: EtOH	0.32	1	0	0.21	1.11	0.5	0.58	1.09	0.6
PO: MeOH	0.15	1	0	0.1	1	0	0.44	1	0

Table 1 (continued)

Compound number[a] Mobile phase[b]	Ristocetin A			Teicoplanin			Teicoplanin aglycone		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
18									
NP: Heptane/EtOH	10.1	1	0	7.90	1.17	2.24	4.33	1.08	1.51
RP: H_2O/MeOH	10.1	1	0	7.34	1.07	0.62	11.07	1	0
PO: IPA	0.72	1	0	0.67	1.33	1.26	1.27	1.1	0.44
PO: EtOH	0.29	1	0	0.18	1.23	0.79	0.55	1.08	0.54
PO: MeOH	0.14	1	0	0.1	1.21	0.2	0.41	1	0
19									
NP: Heptane/EtOH	8.66	1.0	0.5	5.47	1.06	1.2	3.5	1.1	1.5
	9.55	1.0	0.5	5.59	1.12	2.6	3.86	1.21	3.14
RP: H_2O/MeOH	7.79	1	0	6.41	1	0	8.64	1	0
PO: IPA	0.68	1	0	0.68	1	0	1.15	1	0
				0.91	1	0	1.4	1	0
PO: EtOH	0.26	1	0	0.17	1	0	0.48	1	0
	0.29	1	0	0.22	1	0	0.60	1	0
PO: MeOH	0.11	1	0	0.05	1	0	0.34	1	0
							0.39	1	0
20									
NP: Heptane/EtOH	6.66	1	0	5.52	1.05	1.22	2.57	1	0
RP: H_2O/MeOH	14.3	1	0	11.17	1	0	18.04	1	0
PO: IPA	0.45	1	0	0.51	1	0	0.98	1	0
PO: EtOH	0.19	1	0	0.11	1	0	0.43	1	0
PO: MeOH	0.1	1	0	0.05	1	0	0.33	1	0
21									
NP: Heptane/EtOH	19.5	1.0	0.5	11.46	1.17	2.6	8.38	1.09	1.46
RP: H_2O/MeOH	25.9	1	0	13.06	1.18	1.1	29.07	1	0
PO: IPA	1.07	1	0	1.01	1.4	1.2	2.38	1.05	0.2
PO: EtOH	0.4	1	0	0.27	1.27	1.1	0.88	1.09	0.6
PO: MeOH	0.2	1	0	0.12	1.25	0.68	0.69	1	0
22									
NP: Heptane/EtOH	3.68	3.42	4.62	47.31	1.75	5.8	16.37	1.27	2.69
RP: H_2O/MeOH	2.85	1.8	4.97	4.7	1.41	0.7	4.9	1.1	0.65
PO: IPA	1.3	3.6	1.71	0.12	1.56	1.5	2.33	1.95	2.51
PO: EtOH	0.4	3.66	3.76	0.39	1.71	3.01	1.3	1.3	1.95
PO: MeOH	0.15	2.03	2.72	0.39	1.71	2.99	0.55	1.15	1.21
23									
NP: Heptane/EtOH	12.4	1.05	0.7	7.66	1.07	1.45	14.8	1	0
RP: H_2O/MeOH	28.5	1.35	1.51	16.8	1	0	53.3	1.16	1.52
PO: IPA	0.65	1	0	0.84	1.13	0.54	1.75	1.1	0.2
PO: EtOH	0.25	1	0	0.26	1.07	0.38	0.67	1.1	0.3
PO: MeOH	0.12	1	0	0.15	1	0	0.57	1.12	1.1
24									
NP: Heptane/EtOH	20.88	1.21	2.5	14.16[c]	1.03	0.65	17.14	1	0
RP: H_2O/MeOH	7.47	1.09	0.6	14.23	1	0	8.93	1.15	1.61
PO: IPA	1.44	1.34	0.82	3.33	1.13	0.8	4.5	1.08	0.2
PO: EtOH	0.43	1.21	1.35	0.64	1.12	0.4	1.56	1	0
PO: MeOH	0.19	1.11	0.5	0.64	1.12	0.3	0.83	1.05	0.62
25									
NP: Heptane/EtOH	12.42	1.46	3.34	11.5	1.11	1.45	7.49	1.15	2.03
RP: H_2O/MeOH	1.54	1.31	2.61	16.81	1.09	1.1	12.71	1.10	1.45
PO: IPA	1.93	1.51	1.2	2.13	1.14	0.7	2.62	1.16	0.58
PO: EtOH	0.66	1.54	2.3	0.57	1.13	0.58	1.08	1.17	1.5
PO: MeOH	0.24	1.36	1.6	0.56	1.12	0.58	0.70	1.25	2.53

Table 1 (continued)

Compound number[a] Mobile phase[b]	Ristocetin A			Teicoplanin			Teicoplanin aglycone		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
26									
NP: Heptane/EtOH	11.62	1.08	1.5	10.44	1.02	0.2	7.95	1.03	0.51
RP: H_2O/MeOH	4.71	1	0	3.88	1	0	5.08	1	0
PO: IPA	0.79	1.19	0.95	2.44	1	0	2.43	1	0
PO: EtOH	0.26	1.25	0.65	0.45	1	0	0.84	1	0
PO: MeOH	0.21	1	0	0.45	1	0	0.47	1	0
27									
NP: Heptane/EtOH	7.63	1.11	1.5	6.63	1	0	5.18	1	0
RP: H_2O/MeOH	1.08	1.1	0.76	6.45	1.04	0.52	11.32	1	0
PO: IPA	0.99	1.35	0.76	1.76	1	0	2.22	1	0
PO: EtOH	0.31	1.22	0.79	0.43	1	0	0.87	1	0
PO: MeOH	0.12	1	0	0.43	1	0	0.56	1	0
28									
NP: Heptane/EtOH	5.84	1.32	3.0	3.83	1.19	2.69	2.92	1.32	3.66
RP: H_2O/MeOH	1.1	2.6	9.05	5.99	1.26	3.6	6.94	1.16	1.82
PO: IPA	0.65	1.75	1.5	0.53	1.68	1.96	0.85	1.7	1.8
PO: EtOH	0.24	1.86	2.10	0.17	1.66	1.81	0.46	1.47	2.75
PO: MeOH	0.16	2.12	3.44	0.16	1.68	1.81	0.39	1.4	3.07

[a]The number of the compound corresponds to the structure shown in Fig. 3

[b]NP=normal phase, heptane/EtOH for R is 98.5/1.5, for T is 97.5/2.5, and for TAG is 90/10 (0.5 mL min^{-1}) at 1 mL min^{-1} unless otherwise indicated. RP=reversed phase, H_2O/MeOH for R is 70/30, for T is 65/35, and for TAG is 50/50 at 1 mL min^{-1}. PO=polar organic mode, all 100% alcohol, flow rate 0.5 mL min^{-1}. k_1=retention factor of the first eluting enantiomer, α=enantioselectivity factor, R_S=enantioresolution factor. All the data are average values from triplicate analyses, standard deviation <0.06.

[c]Hep/EtOH=95/5 because of the long retention time. Data were usually obtained using Hep/EtOH=75/25 because of strong retention. When Hep/EtOH=95/5 was applied, the first peak eluted at 67.05 min, corresponding to k_1=20.8 and the second peak was too broad.

ditions for the enantiomer separations. The mobile phase compositions were adjusted in order to achieve a reasonable elution time and selectivity. In this way, direct comparison in the separation of this family of compounds can be made. Optimized separations for individual compounds can be done easily if the need arises.

Calculations of the chromatographic parameters

The dead volume (t_0×flow rate) was estimated using the change in refractive index caused by different compositions of the injection solvent. All other related parameters were calculated using the following formulas. The retention factor (k_1) was calculated using the equation $k_1=(t_{r1}-t_0)/t_0$, where t_{r1} is the corresponding retention time for the first eluted enantiomer. The enantioselectivity factor (α) was calculated using $\alpha=k_2/k_1$, where k_2 is the retention factor for the second eluted enantiomer. The resolution factor (R_S) was calculated using the equation $R_S=2\times(t_{r2}-t_{r1})/(w_1+w_2)$, where t_{r2} and t_{r1} are the retention times of the second and first eluted enantiomers and w_1 and w_2 are the corresponding base peak widths. The efficiency or number of theoretical plates (n) was calculated using the equation $n=16(t_r/w)^2$.

Results and discussion

Comparison of the performance of the three CSP

Table 1 and Fig. 4 summarize the overall number of observable ($\alpha\geq1.02$) and baseline ($R_S\geq1.5$) enantiomer separations obtained on each CSP with the five mobile phases tested. In Fig. 4, the lighter bar represents the number of observable enantioseparations and the black bar indicates the number of baseline enantioseparations. As can be seen from Fig. 4, the Chirobiotic T and TAG CSP appear to be the most broadly useful for separating enantiomers of dihydrofurocoumarins. The teicoplanin CSP resolved enantiomers of the entire set of 28 compounds with 24 of them being baseline separated. The teicoplanin aglycon CSP resolved the enantiomers of 26 compounds with 23 of them baseline separations. These two closely related CSP show similar selectivities for most of the compounds. However, there are some slight differences. Dihydropsoralens, which have a "straight shape structure" (Fig. 3) tend to be better resolved on the teicoplanin based CSP (Table 1). This indicates the importance of the sugar units on teicoplanin in the chiral discrimination of these compounds. The teicoplanin aglycon (TAG) CSP showed better selectivities for dihydroangelicins and other "bent-shaped" molecules (Table 1) using the same mobile phase conditions. This in turn implies that the steric effect of the bulky sugar units on the teicoplanin CSP decreases chiral recognition for some of these compounds. This effect has been discussed in a previous paper [53]. Neither the Chirobiotic T, nor the TAG CSP, could baseline resolve compounds 16, 26 and 27 (Table 1, Fig. 3). However, all of these compounds were baseline separated on the ristocetin A CSP (i.e., Chirobiotic R), which baseline separated 14 out of the 28

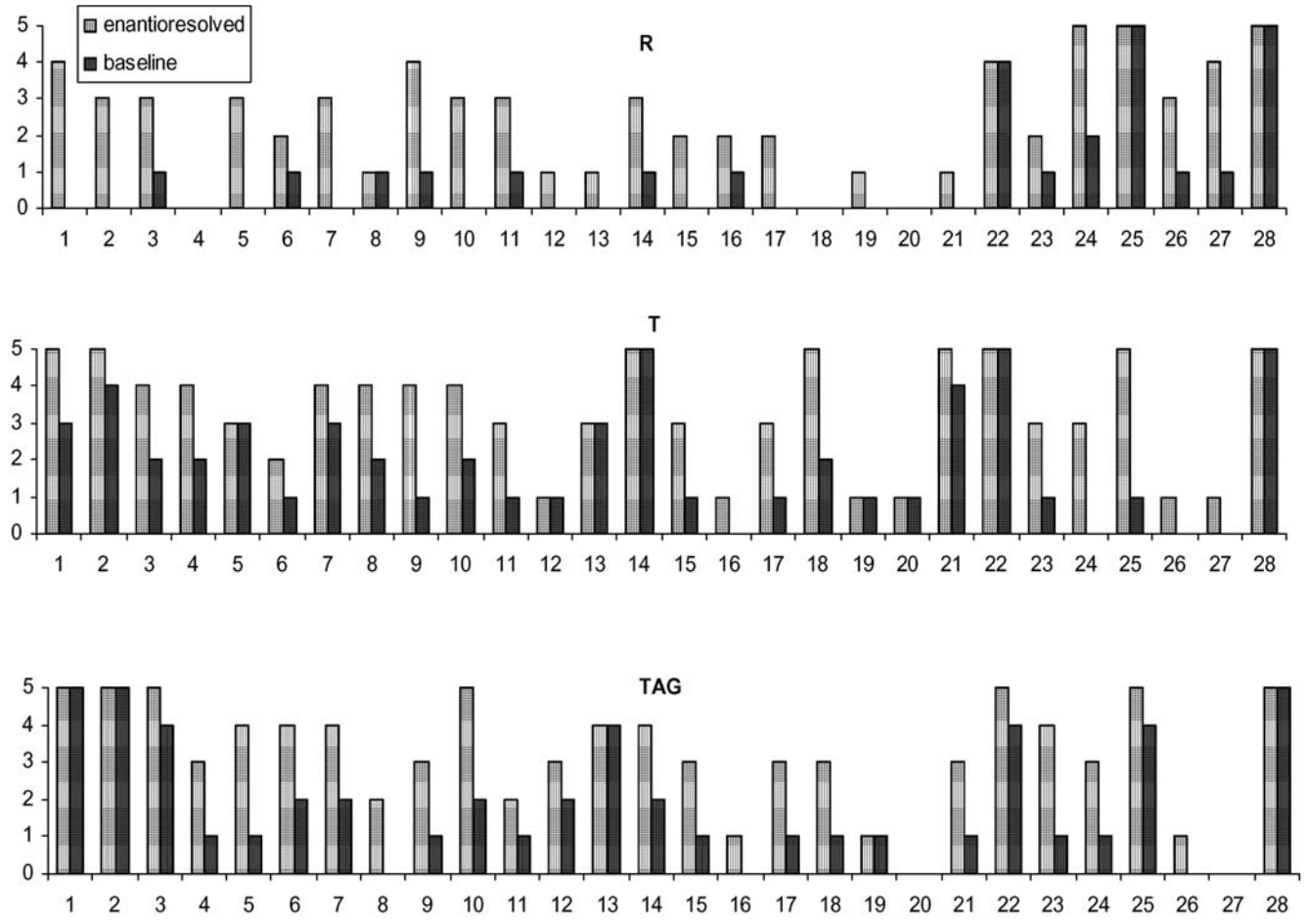

Fig. 4 Summary of the number of baseline and partial separations obtained on different CSP. The Y axis represents the number of separations achieved using the five different mobile phase modes listed in Table 1. The X axis indicates the compound numbers assigned corresponding to Table 1 and Fig. 3. The *light bar* stands for the number of mobile phase modes that resolved (α>1.01) the corresponding compound. The *black bar* indicates the number of baseline separations (R_S≥1.5) achieved

compounds. The ristocetin A CSP shows better selectivity toward most of the third group of compounds (Fig. 3), some of which (compounds 26 and 27) did not separate very well on either the teicoplanin or teicoplanin aglycon CSP. Compounds that could not be separated on the Chirobiotic R column were all baseline resolved on either or both the T and TAG columns. This demonstrates the complementary nature of these CSP, which has also been demonstrated in previous studies [29]. The principle of complementary separation says that if a partial enantiomer separation is obtained with one glycopeptide-based CSP, there is a strong probability that a baseline or better separation can be obtained with a related macrocyclic glycopeptide CSP using the same or similar mobile phase conditions [29]. This allows for improved resolution by simply switching to a related Chirobiotic column.

Figure 5 illustrates the complementary nature of the Chirobiotic TAG and R columns for the separation of compounds 1 and 22. As can be seen, compound 22 was not baseline separated on the Chirobiotic TAG column in the RP mode, but was well separated on the related Chirobiotic R column in the same mobile phase condition. Conversely, compound 1 was well separated with the Chirobiotic TAG column in the NP mode, but not with the Chirobiotic R column.

Effects of mobile phase modes

It is well known that CSP based on macrocyclic glycopeptides are multimodal, which means they can be used in any mobile phase mode and can achieve different separations in each mode [15, 56]. This can be advantageous, since the chiral recognition mechanisms in different separation modes are different and this allows the CSP to separate a greater variety of chiral analytes. Compounds that do not separate in one chromatographic mode are often easily separated in another mode on the same CSP.

Figure 6 summarizes the number of baseline separations achieved on the three CSP in different mobile phase modes, i.e. the normal-phase mode (NP), reversed-phase mode (RP) and polar organic mode (PO). Clearly the normal-phase mode with hexane/ethanol is the most effective approach for separating substituted dihydrofurocoumarins when using teicoplanin and teicoplanin aglycon CSP. The teicoplanin (T) and teicoplanin aglycon (TAG) CSP base-

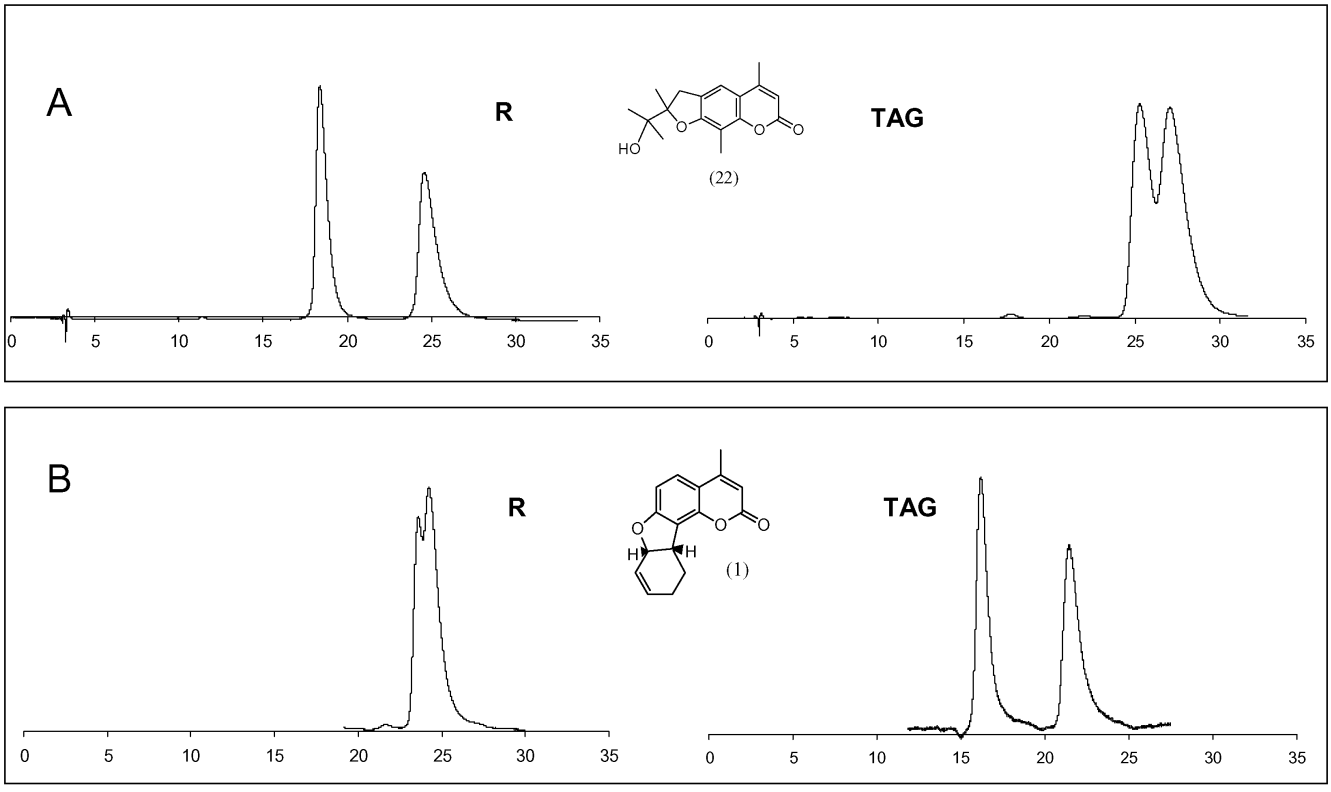

Fig. 5 Chromatograms that demonstrate the complementary nature of Chirobiotic R versus Chirobiotic TAG columns. **A**. The separation of compound 22 using the Chirobiotic R versus Chirobiotic TAG in the reversed-phase mode (H_2O/MeOH 60/40, 1 mL min^{-1}). **B**. Comparison of the separation for compound 1 using the same two columns in the normal-phase mode (hexane/EtOH 95/5, 1 mL min^{-1}). It is obvious that the two columns are complementary to each other for the separation of these two compounds

line separated 23 and 21 compounds respectively using this single mobile phase. Teicoplanin (T) is the most effective chiral selector with all mobile phases except 100% methanol. The ristocetin A CSP was less effective for the chiral dihydrofurocoumarins regardless of the mobile phase conditions. In the reversed-phase mode (RP), all three CSP were less effective, but they separated similar numbers of compounds. In the polar organic mode (PO), however, both the teicoplanin and teicoplanin aglycon again showed much better selectivity compared to the ristocetin A CSP (Fig. 6).

Normal-phase mode separations

More and better enantioseparations of the dihydrofurocoumarins were achieved in the normal-phase mode than

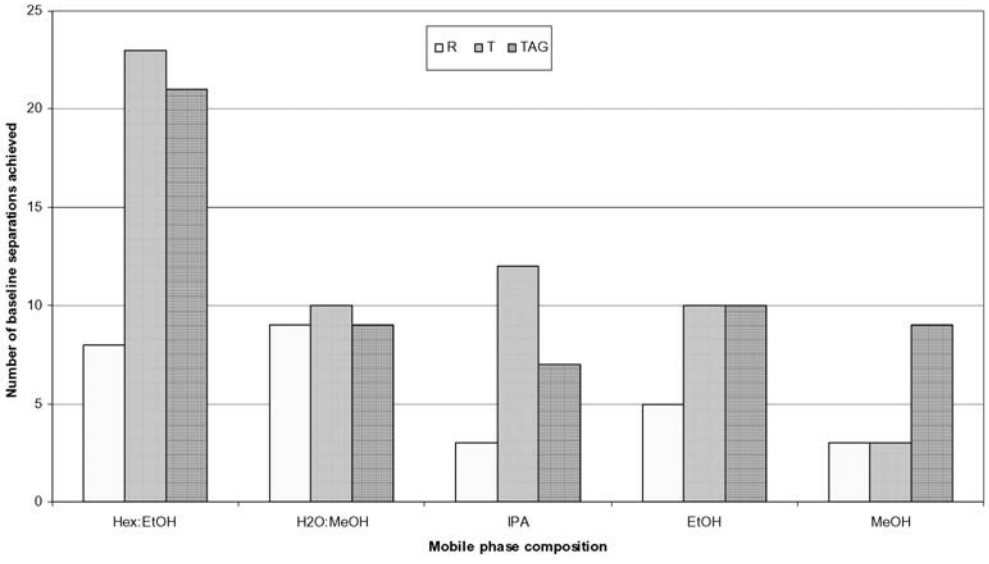

Fig. 6 Summary of the number of baseline separations (after optimization) achieved using different mobile phase modes (i.e. NP, RP, and PO modes) on Chirobiotic R (*R*), Chirobiotic T (*T*) and Chirobiotic TAG (*TAG*) columns

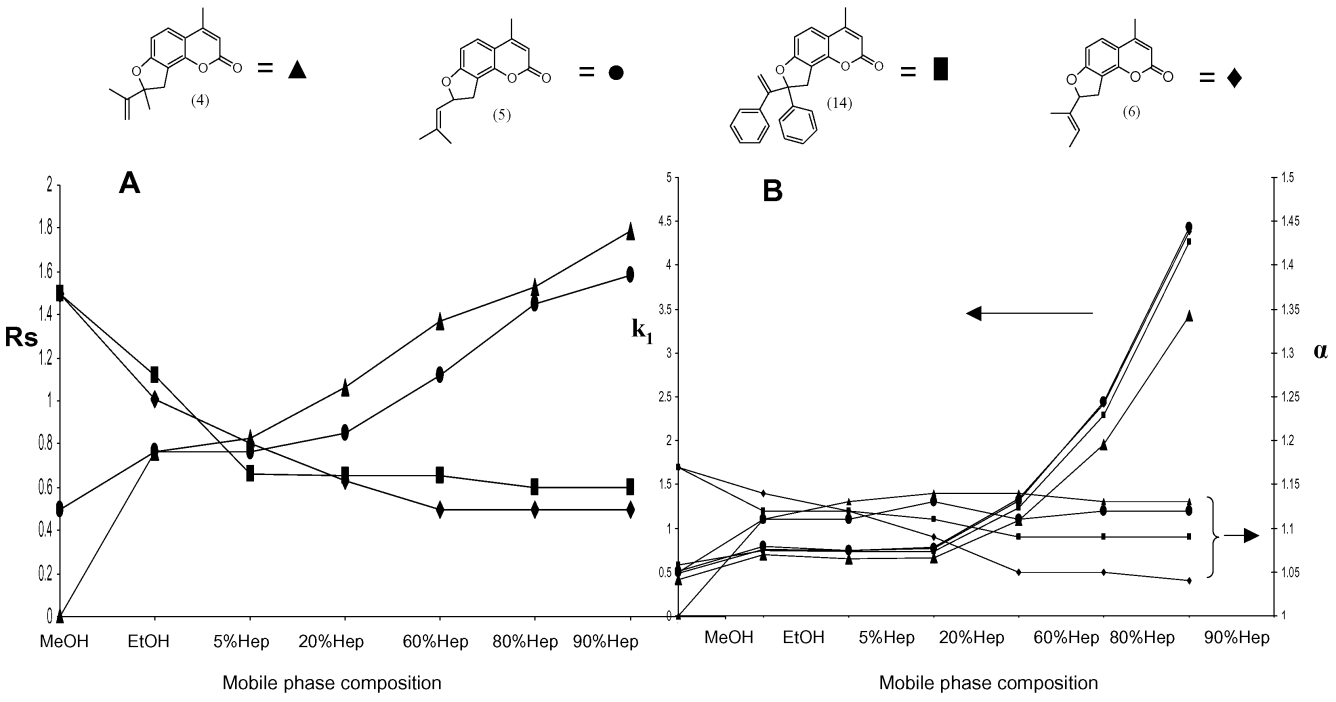

Fig. 7 Plots showing the effect of the type of mobile phase modifier on retention, selectivity, and resolution factors. **A**. Changes in resolution factor as the mobile phase becomes less polar. The Y axis indicates the unit of resolution factors and the X axis is the mobile phase composition. Note that ethanol is the modifier in all heptane-containing mobile phases. It can be seen that compounds 4 and 5 show different trends compared to compounds 6 and 14 when the mobile phase becomes less polar. **B**. Changes in the selectivity factor and resolution factor as the mobile phase composition changes

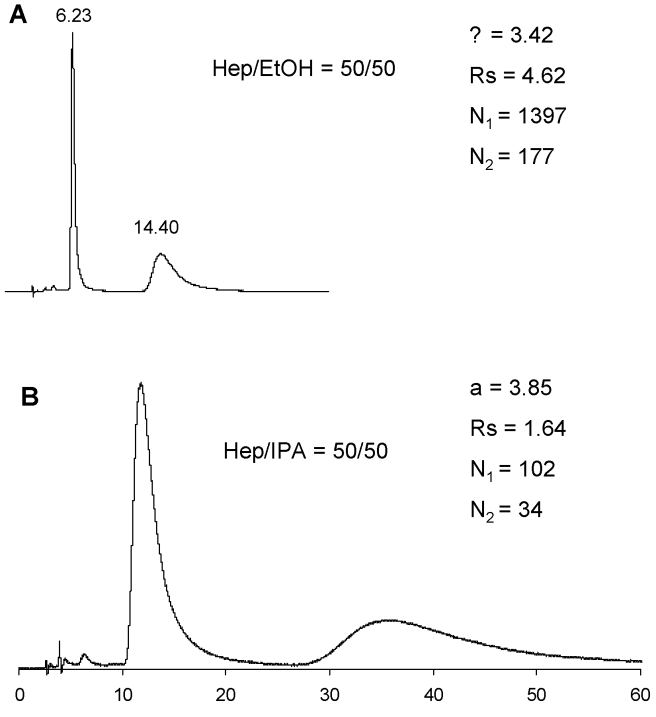

Fig. 8 Comparison of separations of compound 22 using either ethanol or isopropanol as a modifier in the NP mode using the Chirobiotic R column. **A**. Chromatogram obtained using heptane/ethanol 50/50 (*v*/*v*). **B**. Chromatogram obtained using heptane/isopropanol 50/50 (*v*/*v*). Both use a flow rate of 1 mL min^{-1}

in other chromatographic modes. In the normal-phase mode, the CSP behaves as a polar stationary phase. The strongly polar functional groups and aromatic rings of the CSP provide the interactions needed for both retention and chiral recognition. In normal phase HPLC, retention is controlled by adjusting the percentage of a polar organic modifier, such as ethanol or propanol.

Figure 7 shows the effect of added organic modifier on the retention and selectivity, as well as the resolution factors for selected compounds in the normal-phase mode on the TAG CSP. In Fig. 7A and 7B, the X-axis represents the mobile phase compositions starting with the most polar solvent (100% methanol) and extending to the most nonpolar solvent mixture (i.e. 90% hexane+10% ethanol by volume). The Y axis in Fig. 7A represents resolution factor R_S, and that in Fig. 7B represents retention factors, k_1, and enantioselectivity factors, α, for compounds 4, 5, 6, and 14. The TAG CSP showed a very typical normal phase behavior. When the mobile phase becomes less polar, the retention increases. However, the selectivity factor stays almost constant (Fig. 7B). Clearly, increasing the retention time does not necessarily increase the enantiomer resolution. As can be seen from Fig. 7A, improved resolutions were achieved for compounds 4 and 5 by simply decreasing the polarity of the mobile phase. Most of the compounds in Table 1 behaved in this way. In contrast, some of the dihydrofurocoumarins (such as compounds 6 and 14) behaved in just the opposite way (Fig. 7A).

Ethanol was selected as the organic modifier of choice, since it produced improved enantioresolution for the en-

Table 2 The relevant properties of the solvents used in this study [57–60]

Solvent	Formula weight (Dalton)	Density ($g\,cm^{-3}$)	Boiling point	Dipole moment	Dielectric constant	Viscosity (cP)	Polarity index P' (Snyder)	Hydrogen bond acidity	Hydrogen bond basicity
Water	18.02	0.998	100	1.87	80.1	1.00	10.2 (9)	1.17	0.18
Methanol	32.04	0.791	64.7	1.7	32.70	0.55	5.1 (6.6)	0.93	0.62
Ethanol	46.07	0.858	78.0	1.69	25.30	1.2	(5.2)	0.83	0.77
Isopropanol	60.09	0.654	82.26	1.68	18.62	2.4	3.9 (4.3)	0.76	0.95
Acetonitrile	41.05	0.782	81.6	3.44	37.5	0.37	5.8 (6.2)	0.19	0.31
n-Hexane	86.18	0.659	68.7	0.00	1.89	0.31	0.1 (0)	0.00	0.00
n-Heptane	100.21	0.684	98.4	0.00	1.92	0.41	0.1	0.00	0.00

tire set of compounds in the normal-phase mode. Figure 8 is a comparison of the separations for compound 22 using ethanol and isopropanol (50% in heptane by volume) as modifiers on the ristocetin A CSP. As can be seen from these two chromatograms, the selectivity factors are very similar (3.42 and 3.85 using ethanol and isopropanol respectively), but the resolution factors are much different (4.62 and 1.64 respectively). The efficiencies (N, number of theoretical plates) for peak 1 are 1397 and 102 using ethanol versus isopropanol, respectively. Using ethanol as the organic modifier improved the separation efficiency of all compounds separated. For convenience, Table 2 lists the relevant properties of the solvents used in this study [57, 58, 59, 60].

As can be seen from Table 2, the biggest difference in property between ethanol and isopropanol is viscosity. Isopropanol is about twice as viscous as ethanol. Viscosity contributes to the band broadening by two mass transfer terms found in the van Deemter equation. One is the mobile phase mass transfer term, and the other is the stagnant mobile phase mass transfer term. However, poor stationary phase mass transfer may be the most important factor affecting band broadening when using isopropanol versus ethanol as modifiers in the normal-phase mode. Ethanol more effectively competes with the analyte for strong adsorption sites on the CSP. The polarity index for ethanol is greater than that of isopropanol. One may note that the two 50/50 volume ratio mixtures do not contain the same mole fraction of alcohol and therefore of hydroxyl groups, because the molar mass and density of the two alcohols are different. It was calculated that heptane/ethanol 50/50 (*v*/*v*) is heptane 0.278 molar fraction (0.722 for ethanol) and heptane/2-propanol is 0.335 molar fraction (~20% higher) with only 0.665 for propanol. The lower content in OH groups associated with the higher viscosity (restricted access) explains the difference in elutropic strength between the two 50/50 (*v*/*v*) alcohol/heptane mobile phases. In addition, this accounts for the shorter retention times obtained for all of these analytes when ethanol is used as a modifier. The Ristocetin A CSP is extremely selective for the compounds of the third group in Fig. 3 (miscellaneous dihydrofurocoumarins) in the normal-phase mode. Additionally, compound 22, a dihydropsoralen, is well separated by the Chirobiotic R CSP. Few other baseline separations were observed on this CSP in the NP mode.

Reversed-phase mode separations

The reversed-phase mode (RP) is not as effective as the normal-phase mode (NP) for the separation of the dihydrofurocoumarins with teicoplanin based CSP. Interestingly, the ristocetin A CSP separated a great number of compounds in this mode. In fact, the best separation, with a resolution factor of 9.05 for compound 28, was achieved in the reversed-phase mode using the Chirobiotic R column. Hydrophobic interactions are believed to be one of the more important intermolecular interactions between this class of CSP and these analytes in the reversed-phase mode [15]. Compounds 11, 14, 21 and 23 (with one or more aromatic groups attached directly or indirectly to the stereogenic center) are the most hydrophobic compounds in this study. Note the large k_1 of 53 for compound 23 (Table 1) when a mobile phase of 50/50 methanol/water was used. The strong hydrophobic interactions for compounds 11, 14 and 23 did improve their enantioselectivity. But for compound 21, which is a dihydropsoralen, the strong hydrophobic interaction, indicated a large k_1 of 29 on the TAG CSP, but no enantioselectivity was observed for this compound in the reversed-phase mode. This means that the hydrophobic interactions between this analyte and the CSP in the reversed-phase mode do not contribute to chiral recognition as they do for compounds 11, 14 and 23. Clearly, some hydrophobic interactions contribute to nonselective retention, as for compound 21. If retention is dominated by nonselective hydrophobic interactions between the analytes and the CSP, these dominant interactions reduce the access of the analyte to favorable chiral recognition sites.

While baseline enantiomer separations occurred more frequently in the NP mode, there are a few cases where a baseline separation of enantiomers was only achieved in the RP mode (compounds 6, 9, 11 and 12) when using macrocyclic glycopeptide CSP. This is probably due to steric effects as will be discussed in the section "Steric effects".

New polar organic mode separations

The new polar-organic mode is a modification of the polar organic mode that was originally developed for chiral separations with cyclodextrin bonded phases [61, 62]. Generally, the main solvent component in the new polar-

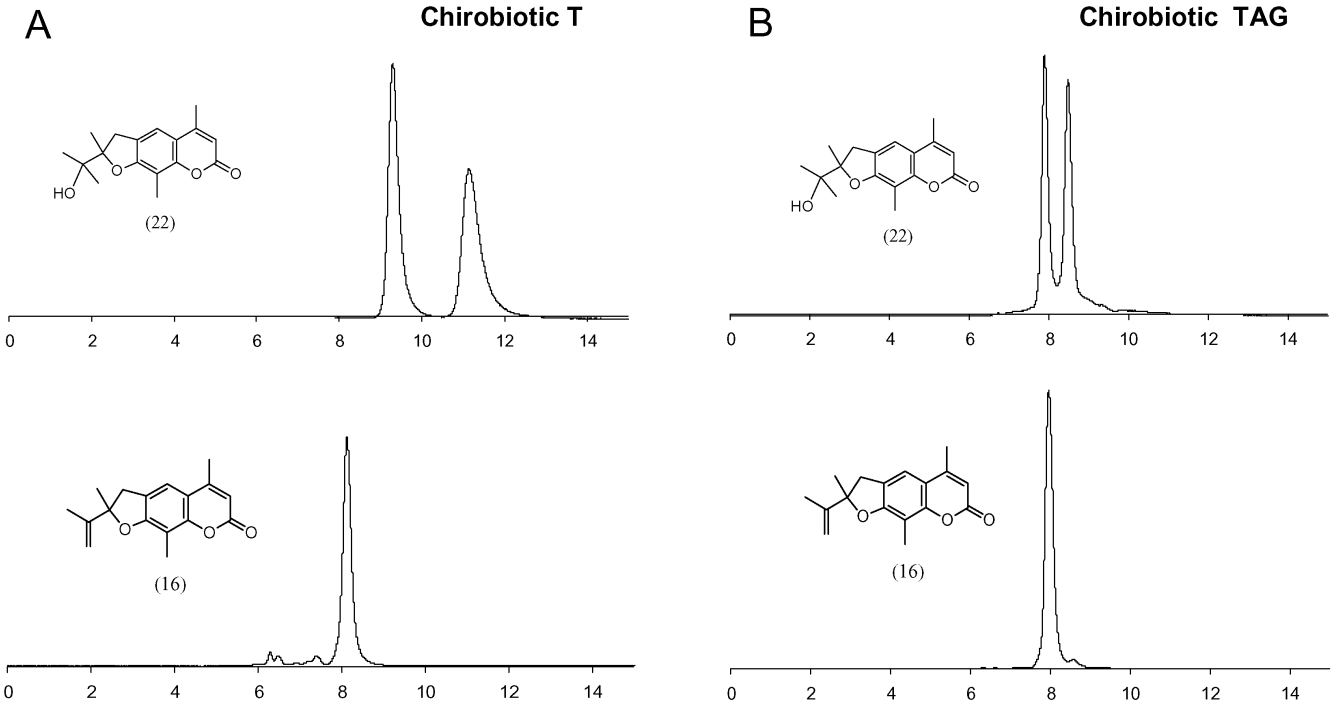

Fig. 9 Effect of hydrogen bonding ability of the analytes on chiral recognition in the polar organic mode on (**A**) Chirobiotic T column and (**B**) Chirobiotic TAG column. The mobile phase was 100% ethanol with a flow rate of 1 mL min^{-1}

organic mobile phase is an alcohol (e.g. methanol, ethanol or isopropanol) with a very small amount of acid/base added to affect retention and selectivity. The retention time can also be adjusted by adding acetonitrile. Fig. 6 shows that the teicoplanin and teicoplanin aglycon CSP separated the largest number of dihydrofurocoumarins using this simple mobile phase mode (100% alcohol). The ristocetin A CSP separated fewer of these compounds in the polar organic mode. It was noted that optimization of the mobile phase by adjusting the acid to base ratio did not improve enantioselectivity in the PO mode. This indicates that ionic interactions do not play a role in the chiral recognition of these neutral molecules in this mode. Instead, dipole–dipole, H-bonding, π–π interactions and steric effects (or some combination thereof) are the main driving forces for chiral recognition.

Note that the selectivity factors for the separations of all dihydrofurocoumarins using MeOH, EtOH and IPA are quite similar (Table 1), while the resolution and retention factors are very different. Narrower and sharper peaks were always obtained for the separations using EtOH or MeOH as the mobile phases as compared to IPA. As can be seen from Table 2, this may be caused by the higher viscosity of isopropanol.

When one compares the separations achieved using pure methanol and pure ethanol, an interesting observation was made (Fig. 7 and Table 1). When a better separation of enantiomers [enantioresolution factor (R_S) and enantioselectivity factor (α)] was achieved using pure methanol (compared to pure ethanol), it was found that the reversed-phase mode was better than the normal-phase mode for separating these enantiomers. This is true for compounds 6, 14, and 24. When a better separation of enantiomers was achieved using pure ethanol (compared to methanol), then the normal-phase mode was more effective than the reversed-phase mode in separating these enantiomers. This is true for most of the remaining compounds. When pure methanol produced results that were approximately equal to those obtained while using pure ethanol, both the NP and the RP modes were found to work well. These observations hold true for the Chirobiotic T and TAG CSP when analyzing the dihydrofurocoumarin derivatives shown in Fig. 3. This observation may be useful in choosing a mode of separation (the RP or NP mode) for the teicoplanin and teicoplanin aglycon CSP. Unfortunately, the results from the Chirobiotic R CSP do not follow a discernable trend.

Role of hydrogen bonding in chiral discrimination

In the polar organic mode, when the pure alcohol mobile phase was mixed with acetonitrile, the number of separations was significantly decreased for the dihydrofurocoumarins. In fact, when using 100% acetonitrile as the mobile phase, no separations were achieved on any CSP for the entire set of analytes, even though analyte retention was similar to that found with methanol. One can note from Table 2 that the polarity of acetonitrile is very similar to that of methanol. However, acetonitrile is a dipolar aprotic solvent, while all the alcohols are polar protic solvents. Therefore, the hydrogen bond acidity (i.e. the ability to donate an H-bond) of acetonitrile is small, while the alcohols are both hydrogen-bond donors and acceptors

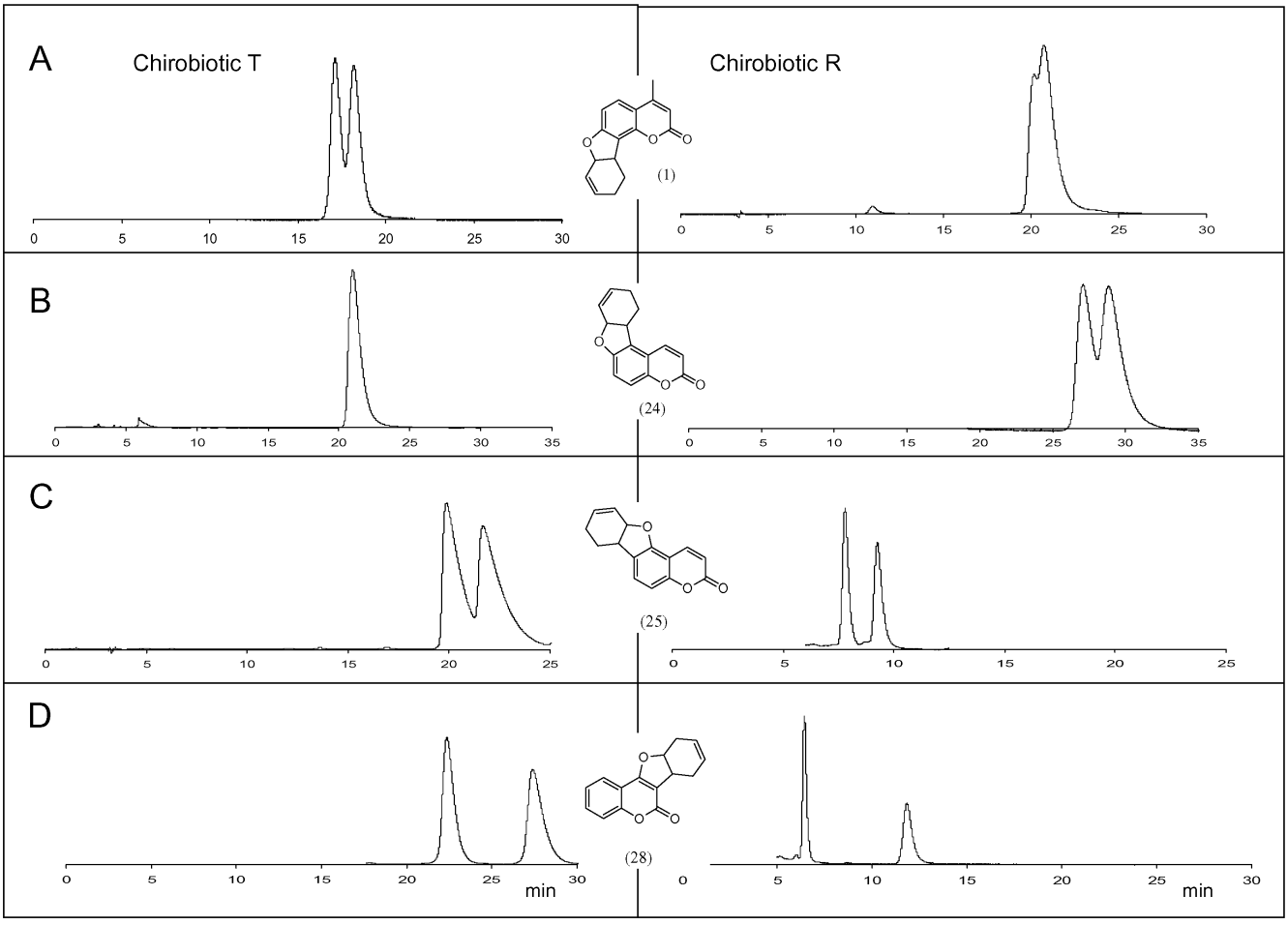

Fig. 10 Effects of an analyte's molecular spatial orientation on its enantioselectivity. **A**, **B**, **C**, and **D** are a comparison of the separations achieved on the Chirobiotic T and R columns in the RP mode (H_2O/MeOH 65/35) for compounds 1, 23, 24, and 27, respectively; flow rate 1 mL min^{-1}

(Table 2). This fact taken together with the fact that no separations were observed when using pure acetonitrile as the mobile phase, supports the contention that the hydrogen bonding between the mobile phase molecules and the analyte or the CSP plays a role in chiral recognition in the polar organic mode.

When the separations of compounds 16 and 22 are compared using the teicoplanin and teicoplanin aglycon CSP in 100% ethanol, the effect of hydrogen bonding is obvious (Fig. 9). With an hydroxyl group attached to the carbon next to the stereogenic center, compound 22 was separated on all CSP with high selectivity and resolution in any of the pure alcohol mobile phases (Fig. 9, Table 1 and Fig. 4). However, removing this hydroxyl group produces compound 16, which cannot be separated on any CSP in the PO mode (Fig. 9, Table 1 and Fig. 4). In the normal-phase mode, the separation of compound 22 was much improved compared to compound 16 on all CSP examined (Table 1, Fig. 8). This illustrates the additional benefits of hydrogen bonding between the analyte and the chiral selector for chiral recognition in the normal-phase mode. In the reversed-phase mode, however, the hydrogen bonding ability of the aqueous mobile phase is too strong. The hydrogen bonding interaction between the analytes and the CSP is much less pronounced in the reversed-phase mode (RP) where the hydrophobic interactions predominate. Separations of compound 22 were greatly diminished on the teicoplanin and teicoplanin aglycon CSP in the RP mode. This is likely due to the H-bonding sites on the chiral selector being preferentially associated with the polar mobile phase molecules. Therefore, one can conclude that hydrogen bonding between the hydroxyl group on the analyte and the hydrogen bonding groups on the CSP in the PO mode, as well as in the NP mode, is one of the key interactions, which leads to chiral recognition.

Effects due to the nature of the individual compounds

Geometry of the dihydrofurocoumarins

As discussed in the section "Effects of mobile phase modes", dihydroangelicins, dihydropsoralens, and the third group of analogues (Fig. 3) have different enantioselectivities on different CSP. It was also observed that the orientation of the furan oxygen in relation to the coumarin effects the chiral separation. Figure 10 shows the chro-

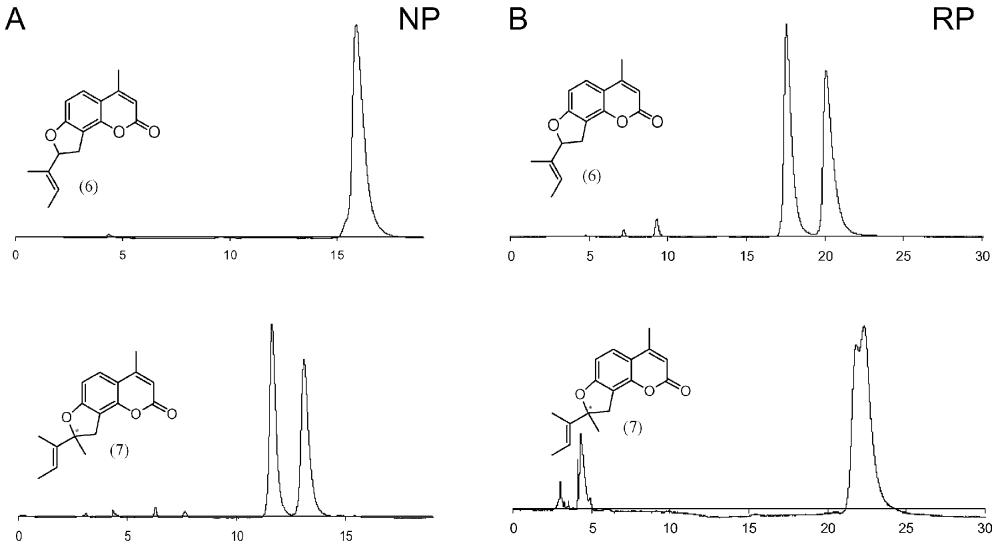

Fig. 11 Comparison of the role of steric effects for enantioseparations on the Chirobiotic T column using the NP versus the RP mode. **A**. A pair of chromatograms obtained in NP mode (hexane/EtOH 95/5, flow rate 1 mL min^{-1}). **B**. A pair of chromatograms obtained in RP mode (H_2O/MeOH 60/40, flow rate 1 mL min^{-1}). Clearly, steric effects generated by the extra methyl group of compound 7 (in the position alpha to the stereogenic center) have the opposite effect in the NP mode versus in the RP mode

matograms of compounds 1, 24, 25 and 28 obtained on the Chirobiotic T and Ristocetin A columns in the RP mode. The only difference for these analytes is the positional substitution of the furan ring on the coumarin body. Note that the only difference between compounds 24 and 25 is the location of the ether linkage. It can be seen from the chromatograms that the best separations for the 7,8-substituted dihydrofurocoumarins (Fig. 1) were achieved on the Chirobiotic T and TAG columns (i.e. compound 1), while the other kinds of substituted dihydrofurocoumarins (i.e. 5,6-substituted and 6,7-substituted, Fig. 1) were better separated on the Chirobiotic R column (i.e. compounds 24, 25, and 28). Another series of examples, which showed the exact same effects, are compounds 4, 16, 26, and 27 (Table 1 and Fig. 3).

Steric effects

Steric repulsive effects are important interactions responsible for chiral recognition in any mobile phase mode for the dihydrofurocoumarins. Steric effects in the normal-phase mode were found to produce results which were contrary to the trends observed in the reversed-phase mode. Steric bulk in positions α or β to the stereogenic center can either enhance or diminish the enantioselectivity.

In the normal-phase mode, there are four dihydroangelicins (compounds 6, 9, 11, and 12) that were not well separated on the teicoplanin and teicoplanin aglycon (TAG) CSP. They have in common smaller substituents attached to the stereogenic center compared to many of the other dihydroangelicins (compounds 4, 7, and 8 for example). Many of the well resolved dihydroangelicins have methyl groups as one of the substituents. However, compounds 6, 9, 11, and 12 have hydrogen atoms instead. Conversely, many of the compounds in the second group of Fig. 3 (dihydropsoralens) are better separated into enantiomers when a hydrogen atom is present as one of the substituents on the stereogenic center (compounds 15, 17, and 18 versus compound 16). An exception to this is compound 22, which may interact with the CSP quite differently from the rest of compounds due to the hydroxyl group attached alpha to the stereogenic center.

In the reversed-phase mode, compounds 6, 9, 11, and 12 (which have less bulk around the stereogenic center) were all separated better than compounds 4, 7 and 8, which is opposite from the trend found in the normal-phase mode. This indicates that the steric repulsive interactions between the analyte and the CSP in the reversed-phase mode play a negative role in the chiral discrimination of dihydroangelicins, while the steric effects play a positive role for the same compounds in the NP mode. Similarly, for dihydropsoralens (in Fig. 3), steric bulk (compound 16) did improve the enantioselectivity with ristocetin A and teicoplanin aglycon CSP. Therefore, steric effects in the RP mode behave in just the opposite way from those seen in the NP mode for the enantiomer separation of dihydrofurocoumarins.

Figure 11 shows a comparison of the enantioseparations of compounds 6 and 7. The opposite role of steric effects for enantioseparations in the NP mode and the RP mode observed on the Chirobiotic T column are shown in this figure. The only difference between these two compounds is the fact that compound 7 has an extra methyl group connected directly to the stereogenic center, which greatly enhances the enantioselectivity in the NP mode. However, in the RP mode, the extra methyl group on compound 7 significantly diminishes the enantioselectivity. Identical behavior can also be found for another pair of compounds 4 and 12 (Table 1).

Absolute configuration determination and enantiomer elution order

The absolute configuration of the separated enantiomers for selected dihydrofurocoumarin derivatives has been determined using the exciton coupling chirality method [8]. It has been shown that the first eluted peak on the Chirobi-

otic T column in the NP mode for compounds 4 and 11 had the *R* configuration. The first eluted peak of compound 8 on the Chirobiotic R column in the RP mode has the *S* configuration [8]. Part of this results has been published [8] and more work is on-going.

Conclusions

The macrocyclic glycopeptide CSP have been shown to be very selective for resolution of the enantiomers of a series of newly synthesized, potentially biologically active chiral dihydrofurocoumarin derivatives. The teicoplanin and teicoplanin aglycon CSP are most effective for the enantioseparation of this class of compounds. The ristocetin A CSP separated fewer overall compounds, but produced the best separations for those dihydrofurocoumarins that were not easily separated on the teicoplanin-based CSP. The normal-phase mode is the most broadly effective and useful separation mode for all of these CSP. Hydrogen bonding is believed to play a key role in the normal phase and the polar organic mode chiral separations. Hydroxyl groups on the rim of the aglycon portion of all CSP are responsible for hydrogen-bonding interactions with the analyte. An hydroxyl group on the analyte near the stereogenic center greatly enhanced the enantioselectivity of all CSP in all mobile phase modes. Hydrophobic interactions are important in the RP mode. Dihydroangelicins, dihydropsoralens, and a related third group of dihydrofurocoumarins (Fig. 3) behave very differently with regard to enantioselectivity on the different CSP. Steric repulsive effects are very important for achieving chiral recognition on all three CSP, and in both the NP and RP modes. However, the steric bulk near the chiral center of the dihydroangelicin tends to enhance the NP enantiomer separations and inhibit the corresponding RP separations. The exact opposite trend is seen for dihydropsoralens. The absolute configuration of selected, collected enantiomers has been determined and therefore the enantiomer elution orders for these particular compounds on a particular CSP under specific mobile phase conditions have been determined.

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