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Separation of chiral sulfoxides by liquid chromatography using macrocyclic glycopeptide chiral stationary phases

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Abstract

A set of 42 chiral compounds containing stereogenic sulfur was prepared. There were 31 chiral sulfoxide compounds, three tosylated sulfilimines and eight sulfinate esters. The separations were done using five different macrocyclic glycopeptide chiral stationary phases (CSPs), namely ristocetin A, teicoplanin, teicoplanin aglycone (TAG), vancomycin and vancomycin aglycone (VAG) and seven eluents, three normal-phase mobile phases, two reversed phases and two polar organic mobile phases. Altogether the macrocyclic glycopeptide CSPs were able to separate the whole set of the 34 sulfoxide enantiomers and tosylated derivatives. Five of the eight sulfinate esters were also separated. The teicoplanin and TAG CSPs were the most effective CSPs able to resolve 35 and 33 of the 42 compounds. The three other CSPs each were able to resolve more than 27 compounds. The normal-phase mode was the most effective followed by the reversed-phase mode with methanol–water mobile phases. Few of these compounds could be separated in the polar organic mode with 100% methanol mobile phases. Acetonitrile was also not a good solvent for the resolution of enantiomers of sulfur-containing compounds, neither in the reversed-phase nor in the polar organic mode. The structure of the chiral molecules was compared to the enantioselectivity factors obtained with the teicoplanin and TAG CSP. It is shown that the polarity, volume and shape of the sulfoxide substituents influence the solute enantioselectivity factor. Changing the oxidation state of the sulfur atom from sulfoxides to sulfinate esters is detrimental to the compound's enantioselectivity. The enantiomeric retention order on the teicoplanin and TAG CSPs was very consistent: the (*S*)-(+)-sulfoxide enantiomer was always the less retained enantiomer. In contrast, the (*R*)-(−)-enantiomer was less retained by the ristocetin A, vancomycin and vancomycin aglycone columns, showing the complementarity of these CSPs. The macrocyclic glycopeptide CSPs provided broad selectivity and effective separations of chiral sulfoxides. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Glycopeptides; Sulfoxides

1. Introduction

Trivalent sulfur compounds such as sulfoxides and sulfinate esters have non-planar geometries and, when asymmetrically substituted, can be found as stable enantiomers at room temperature [1]. Traditionally, the sulfoxide group has been represented in illustrations as S=O, implying the existence of a

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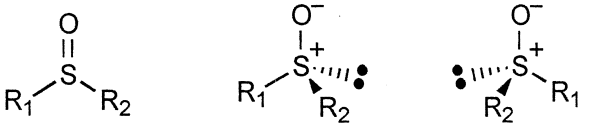
second bond between the two atoms. A more modern understanding is that the S–O bond is more ylide-like, i.e., the molecule bears no overall charge but has a negatively charged oxygen atom bonded to a positively charged sulfur atom [2]. The sulfur center is pyramidal, with a lone pair occupying the fourth position of the pseudotetrahedral center. The barrier to inversion depends on substituents, but for sulfoxides, it is in the neighborhood of 40 kcal/mol [3]. Thus, if the two substituents are different, stable stereoisomers exist:



traditional structure

sulfoxide enantiomers with currently accepted structure

Since the first report on the separation of chiral sulfoxides in 1926 [4], this family of compounds has received much attention given the usefulness of these compounds in organic synthesis [5–7]. Consequently, an effective separation of the enantiomers of racemic sulfoxides is of analytical and preparative interest. In 1959, using column liquid chromatography and an α-lactose home made stationary phase, an Italian research group was able to partially resolve a few racemic sulfoxides [8]. Some chiral sulfoxides were used to test early π–π association-type liquid chromatography (LC) chiral stationary phases (CSPs) [9]. Subsequently, this class of CSPs was used to separate a limited number of compounds containing stereogenic sulfur [10–14]. Protein bonded CSPs were also found to be able to separate some chiral sulfoxides [15,16]. Polysaccharide based CSPs were also used successfully to resolve a dozen sulfoxide enantiomeric pairs [17–21]. This appears to be the most useful class of CSPs for the enantioseparation of chiral sulfoxides thus far [22,23]. Cyclodextrin-based CSPs were also found to provide effective and efficient resolution of enantiomers of these compounds [24].

A recent review did not mention the use of the macrocyclic glycopeptide CSPs for the LC separation of sulfur containing compounds [25]. The goal of this work is to evaluate the capabilities of the macrocyclic glycopeptide-based CSPs for the separation of chiral sulfoxides. In order to obtain a thorough evaluation, the largest number of chiral sulfoxide molecules ever examined as well as several sulfinate esters and sulfilimines were especially synthesized and assayed for compound enantioselectivity. The first part of this work describes and discusses the separation results obtained on five different macrocyclic glycopeptide CSPs in three different mobile phase modes. These results are also compared with those found in previous reports. The second part of the study will focus on specific solute–stationary phase interactions. By relating the solute structures to the separation data, the factors involved in the chiral recognition process can sometimes be identified.

2. Experimental

2.1. S-containing compounds

The 42 chiral sulfur-containing compounds used in this study are listed in Table 1. The chiral sulfoxide compounds were sorted by increasing molecular mass from 1 to 31. 32 to 34 are the tosylated forms of 1, 16 and 22, respectively. 35 to 42 are sulfinate ester containing the R_1–SO–O–R_2 group. 1, 4 and 5 were obtained from Aldrich (St. Louis, MO, USA). All other 39 compounds were prepared and purified according to published methods by the group of Jenks at Iowa State University, Ames, IA, USA [26–30].

2.2. Other chemicals

HPLC-grade acetonitrile (ACN), methanol (MeOH), ethanol (EtOH), 2-propanol (IPA), *n*-hexane (hex) and methyl-*tert*.-butyl ether (MTBE) were purchased from Fisher (St. Louis, MO, USA) and/or EM (Gibbstown, NJ, USA). Water was deionized and filtered on active charcoal and a 5 μm filter. Triethylamine (TEA) and acetic acid (AA) were from Sigma (St. Louis, MO, USA).

2.3. Chiral stationary phases

Five different macrocyclic glycopeptide chiral selectors were evaluated. They were ristocetin A, $C_{95}H_{110}N_8O_{44}$, M_r 2066, teicoplanin,

Table 1
Structures of 42 stereogenic sulfur-containing compounds and the number of observable enantiomeric separations achieved for each

Compound number	R_1[a]	R_2[a]	SO[a]	M_r	Separations[b]
1	C6H5	CH3		140	17
2	CH3CH2CH2CH2CH=CH	CH3		146	13
3	CH3CH2CH2CH2CH2CH2	CH3		148	7
4	C6H5	CH2=CH		152	8
5	pCH3C6H4	CH3		154	10
6	mCH3C6H4	CH3		154	14
7	oCH3C6H4	CH3		154	15
8	FC6H4	CH3		159	16
9	pClC6H4	CH3		174.5	12
10	mClC6H4	CH3		174.5	13
11	oClC6H4	CH3		174.5	23
12	C6H5	(CH3)3C		182	20
13	aC10H7	CH3		190	21
14	C6H5CH2CH2CH=CH	CH3		194	15
15	CF3C6H4	CH3		208	10
16	C6H5	C6H5CH2		216	18
17	C6H5C6H4	CH3		216	17
18	pBrC6H4	CH3		219	12
19	mBrC6H4	CH3		219	14
20	oBrC6H4	CH3		219	22
21	CH3C6H4	C6H5CH2		230	10
22	C6H5	C6H5CH2CH2		230	18
23	C6H5	CH3C6H4CH2		230	14
24	CH3C6H4	C6H5CH2CH2		244	13
25	C6H4OCH3	C6H5CH2		246	12
26	ClC6H4	C6H5CH2		250.5	19
27	CH3C6H4	C6H5CH2CH2CH2		258	2
28	bC10H7	C6H5CH2		266	19
29	C6H5	C6H5CH2CH2C(CH3)2		272	28
30	CH3C6H4	C6H5CH2CH2C(CH3)2		286	14
31	C6H5	(C6H5)2CH		292	24
32	C6H5	CH3	Tosyl	279	12
33	C6H5	C6H5CH2	Tosyl	355	7
34	C6H5	C6H5CH2CH2	Tosyl	369	3
35	CH3O	C6H5CH2	s.e.	170	7
36	CH3CH2O	CH3C6H4	s.e.	184	0
37	C6H5CH2CH2CH2O	CH3	s.e.	198	1
38	CH3(CH3)CHO	CH3C6H4	s.e.	198	0
39	CH3CH2CH2O	CH3C6H4	s.e.	198	0
40	CH3CH2CH2CH2O	CH3C6H4	s.e.	212	3
41	CH3CH(CH3)CH2O	CH3C6H4	s.e.	226	1
42	CH3CH2CH(CH3)O	CH3C6H4	s.e.	226	3

C6H5 = phenyl ring; p, m, o = *para*, *meta*, *ortho* phenyl substitution, C10H7 = naphthalenyl group; a, b = α or β connected to the SO group, s.e. = sulfinate ester with the R1–O–SO–R2 asymmetric center.

[a] The general structure is $R_1–SO–R_2$. Compounds 1 to 31 are sulfoxides; compounds 32 to 35 are tosylated sulfoxides (tosyl = *p*-toluene sulfonate, SO was replaced by $SN–SO_2–C_6H_4–CH_3$); compounds 36 to 42 are sulfinate esters.

[b] Separation = cumulative number of observable enantioseparations on the five CSPs with the seven mobile phases (total = 35 essays per compound).

$C_{88}H_{97}Cl_2N_9O_{33}$, M_r 1878, vancomycin, $C_{66}H_{75}Cl_2N_9O_{24}$, M_r 1449, and the aglycone forms of the latter two: teicoplanin aglycone (TAG), $C_{58}H_{45}Cl_2N_7O_{18}$, M_r 1197 and vancomycin aglycone (VAG), $C_{53}H_{52}Cl_2N_8O_{17}$, M_r 1142. The complete structural description of these chiral selectors has been given in several articles [25,31–34]. The chiral stationary phases were prepared by bonding the chiral selectors to a 5 μm HPLC spherical porous silica gel through a linking chain [34,35]. The bonding chemistry was done by Astec (Whippany, NJ, USA). The chiral stationary phases were slurry-packed in 250×4.6 mm columns. These columns are marketed by Astec under the trade names: Chirobiotic R, T, V, TAG and VAG for the five glycopeptides, respectively.

2.4. Mobile phase compositions

The macrocyclic glycopeptide based CSPs were used in three different chromatographic modes: (1) the normal-phase mode with a low polar mobile phase, (2) the reversed-phase mode with hydro–organic mobile phases and (3) the polar organic mode that uses 100% polar organic solvent mobile phases. Three different low polarity normal mobile phases were used: *n*-hexane with 10% ethanol, *n*-hexane with 10% IPA and MTBE with 10% ACN (all % are given in v/v). Two compositions for reversed-phase separations were used: methanol and aqueous buffer of 1% TEA (0.07 *M*), adjusted to pH 4.1 with acetic acid, and the same pH 4.1 aqueous buffer but with ACN as the organic modifier. The methanol contents were 10% (v/v) with the vancomycin and VAG CSPs, 20% with the teicoplanin CSP, 30% with the ristocetin A CSP and 50% with the VAG CSP. The ACN content was 10% with the vancomycin, VAG and teicoplanin CSPs, 20% with the ristocetin A CSP and 30% with the TAG CSP. Two mobile phase compositions for polar organic mode were used: 100% methanol or 100% ACN, plus 0.025% (v/v) TEA (2 m*M*) and AA which were added to the organic solvents for use with the vancomycin, VAG and TAG CSPs. 0.05% TEA and AA (4 m*M*) and 0.1% TEA and AA (8 m*M*) were added when using the ristocetin A and teicoplanin CSPs, respectively. The mobile phase compositions for the reversed-phase and polar organic mode analyses were adapted to the CSPs to increase elution strength and reduce analyses duration. They are not necessarily the optimal mobile phase composition giving the best enantioselectivity.

3. Results and discussion

The 42 compounds listed in Table 1 were run in triplicate on five columns with seven different mobile phases. This produced 4410 chromatograms (i.e., 3 runs×1470 analyses). The experimental reproducibility was good. The 1470 average values for the 4410 analyses gave relative standard deviations lower than 0.08. Exactly 500 analyses showed some resolution of enantiomers of the racemic sulfur-containing compounds. Although the mobile phase compositions were not optimized for maximal resolution of enantiomers, the separation of the enantiomers was excellent with a baseline return between peaks ($R_s>1.5$) for 154 separations, that is almost one third (exactly 31%) of the chromatograms with an observable enantioseparation. It is notable that every partial enantioseparation could be improved by optimizing the corresponding mobile phase composition. In this study, only seven distinct mobile phase compositions, in the three chromatographic modes, were used in order to obtain a general view of the capability of the macrocyclic glycopeptide based CSPs for the resolution of enantiomers of chiral sulfur containing compounds. Table 2 lists the average value of the retention factor of the first enantiomer, k_1, the enantioselectivity factor, $\alpha = k_2/k_1$, and the resolution factor, R_s, for selected compounds on three CSPs: ristocetin A, teicoplanin and TAG. These three CSPs were the most widely applicable of the columns tested.

3.1. Stationary phase performance

Fig. 1 shows the number of observable enantioseparations ($\alpha>1.01$) obtained (for the 42 compounds listed in Table 1) on each CSPs with the seven mobile phases that were tested. The black bars indicate the number of baseline separation ($R_s>1.5$) obtained with the unoptimized mobile phases. Table 3 lists the cumulative number of enantioseparation ($\alpha>1$) obtained with each stationary phase along with the number of baseline separations ($R_s\geq1.5$) obtained.

Table 2
Chromatographic results obtained with selected chiral sulfur-containing compounds on three macrocyclic antibiotic CSPs

Mobile phase	Ristocetin A			Teicoplanin			Teicoplanin aglycone		
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
(1) Methyl phenyl sulfoxide									
RP MeOH	0.68	1	0	1.27	1.11	0.5	1.15	1.13	1.2
PO MeOH	0.21	1	0	0.15	1.40	0.8	0.46	1.38	1.3
PO ACN	1.23	1.94	3.8	1.79	1.14	0.6	2.17	1	0
NP hex–EtOH	3.15	1.15	1.7	8.90	1.11	2.1	3.36	1.34	2.7
NP hex–IPA	11.7	1.05	1.3	19.34	1.22	4.3	13.1	2.02	2.2
NP MTBE–ACN	8.13	1.11	1.5	9.80	1.03	0.2	15.8	1	0
(5) *p*-Toluyl methyl sulfoxide									
RP MeOH	1.23	1	0	2.50	1.12	1.3	2.15	1.19	1.7
PO MeOH	0.19	1	0	0.21	1.43	1.0	0.56	1.50	1.9
NP hex–EtOH	2.72	1	0	6.90	1.17	2.7	3.14	1.64	3.3
NP hex–IPA	3.52	1.16	0.8	7.17	1.57	2.1	6.93	2.08	2.3
NP MTBE–ACN	5.80	1.02	0.3	6.90	1	0	11.5	1	0
(6) *m*-Toluyl methyl sulfoxide									
RP MeOH	2.00	1	0	1.82	1.17	1.3	1.08	1.33	2.1
RP ACN	1.07	1	0	1.37	1.07	1.0	1.34	1	0
PO MeOH	0.13	1	0	0.24	1.41	1.2	0.39	1.59	2.9
NP hex–EtOH	5.44	1.06	1.25	6.87	1.32	3.4	13.2	1.53	3.2
NP hex–IPA	18.9	1	0	28.45	2.00	3.0	7.90	2.15	3
NP MTBE–ACN	7.47	1.05	0.9	8.40	1	0	57.0	1.16	1.8
(7) *o*-Toluyl methyl sulfoxide									
RP MeOH	1.47	1	0	4.93	1.07	1.3	1.59	1.16	1.4
PO MeOH	0.17	1	0	0.21	1.29	1.0	0.53	1.33	1.5
PO ACN	1.52	1.05	0.8	2.00	1	0	1.55	1	0
NP hex–EtOH	2.48	1.12	1.4	6.00	1.09	1.0	4.90	1.31	3
NP hex–IPA	8.60	1.10	0.5	8.07	1.29	1.0	13.6	1.70	2.3
NP MTBE–ACN	31.8	1.15	1.2	7.20	1.11	1.4	14.1	1	0
(8) *p*-Fluorophenyl methyl sulfoxide									
RP MeOH	0.68	1	0	1.23	1.14	1.3	1.07	1.28	1.7
PO MeOH	0.19	1	0	0.19	1.48	1.0	0.42	1.67	2.4
NP hex–EtOH	3.35	1	0	8.40	1.26	1.6	3.21	1.69	3.7
NP hex–IPA	19.6	1.11	1.1	6.13	1.71	1.9	13.1	2.02	2.6
NP MTBE–ACN	7.13	1.04	0.6	8.00	1	0	12.3	1.08	0.6
(9) *p*-Chlorophenyl methyl sulfoxide									
RP MeOH	1.03	1	0	2.00	1.13	1.6	1.74	1.21	1.8
PO MeOH	0.17	1	0	0.19	1.48	1.0	0.49	1.61	2
NP hex–EtOH	2.72	1	0	6.70	1.19	3.1	2.93	1.68	3.3
NP hex–IPA	3.45	1.18	0.8	6.93	1.63	2.0	14.5	2.34	2.9
NP MTBE–ACN	5.80	1.02	0.4	6.70	1	0	11.0	1	0
(10) *m*-Chlorophenyl methyl sulfoxide									
RP MeOH	2.32	1	0	2.18	1.18	1.6	1.68	1.28	1.5
RP ACN	1.28	1	0	1.67	1.05	1.2	1.62	1	0
PO MeOH	0.14	1	0	0.22	1.23	1.0	0.42	1.62	2.2
NP hex–EtOH	5.27	1.03	0.5	6.76	1.22	3.2	11.9	1.59	4.8
NP hex–IPA	14.6	1	0	20.9	1.57	2.9	7.00	2.69	3.1
NP MTBE–ACN	4.44	1.05	0.8	4.50	1	0	36.1	1.09	1.1

Table 2. Continued

Mobile phase	Ristocetin A			Teicoplanin			Teicoplanin aglycone		
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
(11) *o*-Chlorophenyl methyl sulfoxide									
RP MeOH	2.70	1	0	2.73	1.19	1.6	2.15	1.24	1.6
RP ACN	1.44	1	0	1.96	1.07	1.2	1.91	1	0
PO MeOH	0.15	1	0	0.28	1.34	1.2	0.60	1.67	3.4
PO ACN	0.50	1.16	0.7	0.88	1	0	1.17	1.11	0.6
NP hex–EtOH	3.10	1.04	0.6	4.37	1.16	2.2	9.80	1.40	3.5
NP hex–IPA	7.00	1	0	12.0	1.38	1.6	8.08	2.00	2.4
NP MTBE–ACN	2.95	1.11	1.4	3.00	1.11	1.5	30.5	1.06	0.6
(12) Phenyl *tert*.-butyl sulfoxide									
RP MeOH	1.64	1.08	0.8	2.13	1.06	0.8	1.30	1.20	1.5
PO MeOH	0.00	1	0	0.02	1	0	0.11	1.76	0.6
PO ACN	0.91	1.04	0.5	1.40	1	0	0.88	1	0
NP hex–EtOH	0.79	1	0	1.47	1.30	3.4	0.93	1.56	4.1
NP hex–IPA	1.46	1.20	1.3	0.80	2.50	1.9	1.50	1.81	2.5
NP MTBE–ACN	4.80	1.33	2.4	2.23	1.12	0.8	4.50	1.18	1.2
(13) α-Naphthalenyl methyl sulfoxide									
RP MeOH	3.50	1	0	11.61	1.18	3.0	3.56	1.43	4.8
RP ACN	0.94	1	0	3.83	1.03	0.3	3.05	1.07	0.7
PO MeOH	0.21	1	0	0.29	1.53	2.0	0.79	1.65	3.5
PO ACN	1.28	1.09	1.45	1.67	1	0	1.66	1	0
NP hex–EtOH	2.04	1.14	1.4	5.80	1.44	3.6	5.56	1.70	4.4
NP hex–IPA	7.80	1	0	7.13	2.18	2.5	16.5	1	0
NP MTBE–ACN	8.50	1.20	2.5	6.13	1.07	0.8	12.8	1.12	0.9
(18) *p*-Bromophenyl methyl sulfoxide									
RP MeOH	1.23	1	0	2.50	1.12	1.3	2.15	1.19	1.7
PO MeOH	0.19	1	0	0.21	1.43	1.0	0.56	1.50	1.9
NP hex–EtOH	2.72	1	0	6.90	1.17	2.7	3.14	1.64	3.3
NP hex–IPA	3.52	1.16	0.8	7.17	1.57	2.1	6.93	2.08	2.2
NP MTBE–ACN	5.80	1.02	0.3	6.90	1	0	11.5	1	0
(19) *m*-Bromophenyl methyl sulfoxide									
RP MeOH	2.70	1	0	2.71	1.17	1.3	1.75	1.32	3.0
RP ACN	1.51	1	0	1.81	1.09	1.2	0.66	1	0
PO MeOH	0.16	1	0	0.26	1.25	1.2	0.48	1.67	4.9
NP hex–EtOH	6.10	1.01	0.2	6.76	1.24	3.6	13.7	1.72	4.5
NP hex–IPA	19.1	1.04	0.5	24.5	1.80	2.9	7.50	2.73	3.1
NP MTBE–ACN	4.37	1.04	0.6	4.70	1	0	37.5	1.11	1.2
(20) *o*-Bromophenyl methyl sulfoxide									
RP MeOH	5.20	1	0	3.55	1.20	1.7	2.66	1.28	2.4
RP ACN	1.78	1	0	2.42	1.09	1.3	2.34	1	0
PO MeOH	0.17	1	0	0.31	1.32	1.3	0.68	1.56	3.9
PO ACN	0.82	1	0	0.88	1	0	1.38	1.09	0.6
NP hex–EtOH	3.30	1.50	1	4.78	1.17	2.1	12.4	1.49	3.2
NP hex–IPA	7.55	1	0	13.43	1.47	1.7	9.33	2.02	3.0
NP MTBE–ACN	3.13	1.11	1.45	3.20	1.11	1.2	34.2	1.07	0.7

Table 2. Continued

Mobile phase	Ristocetin A			Teicoplanin			Teicoplanin aglycone		
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
(22) Phenylethyl phenyl sulfoxide									
RP MeOH	5.20	1	0	7.20	1	0	3.90	1	0
RP ACN	1.44	1	0	4.20	1.06	0.7	3.95	1	0
PO MeOH	0.05	1	0	0.14	1	0	0.26	1.26	1.0
PO ACN	0.62	1.10	0.5	0.67	1	0	1.33	1	0
NP hex–EtOH	2.40	1.09	1.3	3.48	1	0	7.05	1.26	1.8
NP hex–IPA	4.66	1.12	1.4	9.54	1.17	0.7	3.92	1.47	1.3
NP MTBE–ACN	2.90	1.18	1.7	2.93	1.10	0.9	34.5	1	0
(26) *p*-Chlorophenyl benzyl sulfoxide									
RP MeOH	2.06	1.80	8	4.64	1.05	0.8	4.20	1	0
RP ACN	1.15	1.25	1.8	4.64	1.05	0.7	4.62	1	0
PO MeOH	0.05	3.10	1.5	0.06	1	0	0.37	1	0
NP hex–EtOH	1.17	1.16	1.2	2.57	1.09	1.0	1.36	1.16	1.2
NP hex–IPA	1.13	1	0	2.65	1.17	0.7	6.38	1.29	1.0
NP MTBE–ACN	2.33	1	0	2.10	1.06	0.3	4.90	1	0
(28) β-Naphthalenyl benzyl sulfoxide									
RP MeOH	7.40	1.45	4	11.5	1	0	7.00	1.11	1.25
RP ACN	1.69	1.29	2.2	8.92	1.05	0.5	7.60	1	0
PO MeOH	0.06	1.50	0.6	0.11	1	0	0.52	1.07	0.3
NP hex–EtOH	1.51	1	0	3.20	1.08	1.3	2.80	1.22	2.2
NP hex–IPA	3.44	1	0	3.47	1.38	0.8	6.00	1.33	1.1
NP MTBE–ACN	7.00	1	0	2.83	1.06	0.4	7.70	1.04	0.2
(29) 1,1-Dimethyl 3-phenylpropyl phenyl sulfoxide									
RP MeOH	5.00	1.15	1.5	6.67	1.42	1.4	3.90	1.19	1.6
RP ACN	1.42	1.13	1.4	6.25	1.20	1.7	5.64	1	0
PO MeOH	0.00	1	0	0.02	1	0	0.14	1.50	1.1
PO ACN	0.55	1.27	1.5	0.63	1	0	0.67	1	0
NP hex–EtOH	0.55	1.27	1.0	1.20	1.64	4.3	0.85	1.57	2.4
NP hex–IPA	0.39	1.33	1.2	1.40	3.96	4.6	3.55	1.95	2.1
NP MTBE–ACN	3.40	1.47	4.7	1.67	1.16	0.9	3.50	1.20	1
(31) Diphenylmethyl phenyl sulfoxide									
RP MeOH	5.00	1.27	2.7	8.53	1.15	1.1	5.26	1.10	0.8
RP ACN	1.36	1.24	1.5	8.00	1.16	1.3	6.90	1.05	0.6
PO ACN	0.32	1.28	1.5	0.29	1	0	0.67	1	0
NP hex–EtOH	0.79	1.25	1.6	1.93	1.05	0.7	1.26	1.06	0.6
NP hex–IPA	0.52	1.56	1.6	2.85	1	0	6.38	1	0
NP MTBE–ACN	1.27	2.00	6.4	1.30	1.41	3.2	3.70	1	0

RP = Reversed phase, PO = polar organic mode, NP = normal phase; k_1 = retention factor of the first eluting enantiomer, α = enantioselectivity factor, R_s = enantioresolution factor. Average values from triplicate analyzes, standard deviation below 0.08. A missing mobile phase line means that no separations were obtained on any CSPs.

The two teicoplanin based CSPs are clearly the most effective chiral stationary phases being able to resolve 35 and 33 chiral sulfur analytes for the teicoplanin and TAG CSPs, respectively. They produced almost twice as many observable separations as the three other CSPs. The teicoplanin CSP separated three compounds (25, 27 and 34) that the TAG CSP could not separate. In contrast, 40 was separated by the TAG and not by the teicoplanin CSP. 53% of the compounds enantioresolved by the TAG CSP were baseline separated. Even though unoptimized mobile phases were used, this figure is significantly

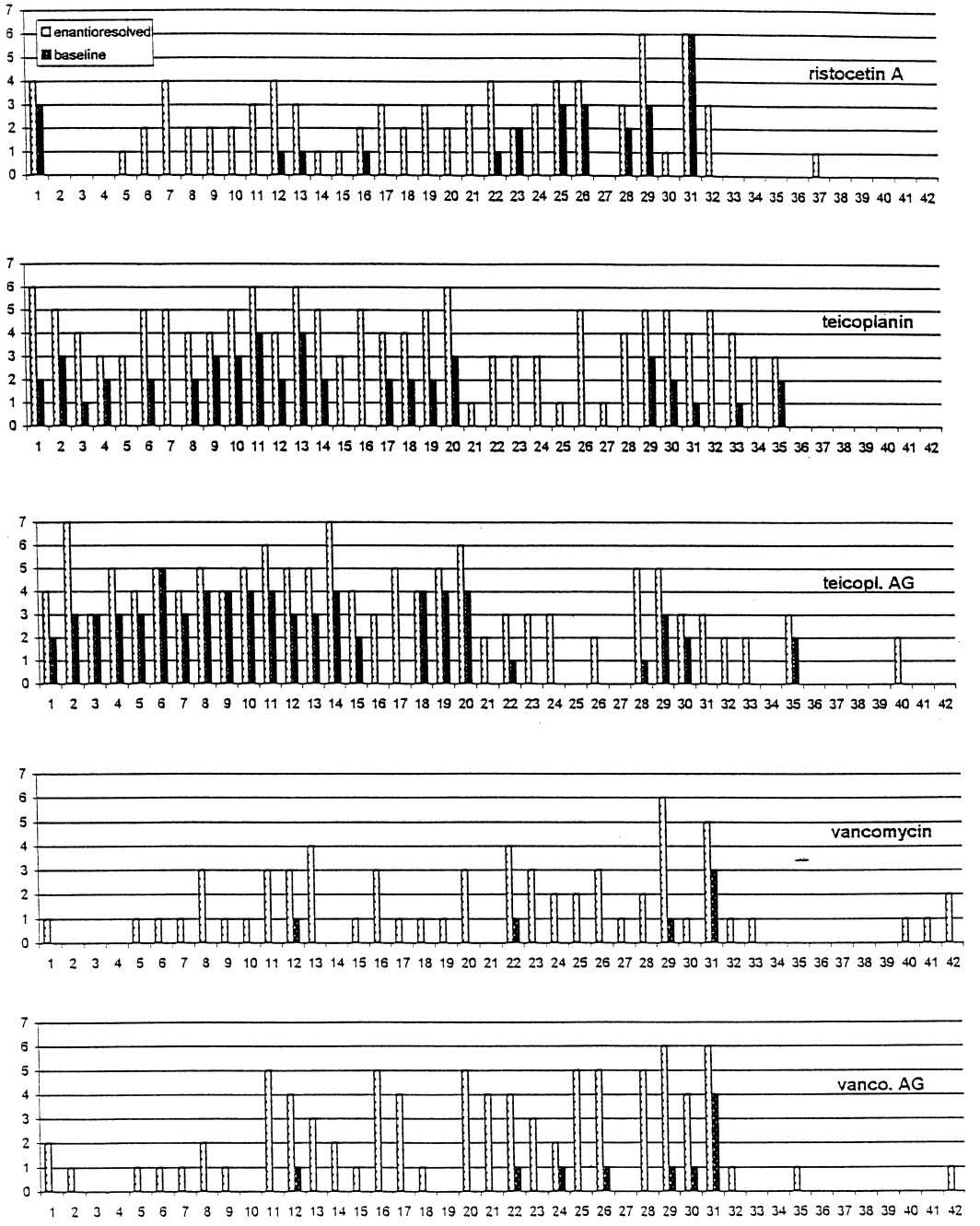


Fig. 1. Overview of the results arranged per solute and per CSP. The length of the bars indicates how many mobile phase were capable to produce observable separation of the enantiomers of the solute. White bars: number of observable enantioselective separations ($\alpha > 1.02$); dark bars: number of baseline separations ($R_s > 1.5$).

Table 3
Observable enantioseparations sorted by stationary phase type and mobile phase composition

	Compounds separated		Separation ($\alpha > 1.02$)		Baseline separation ($R_s \geq 1.5$)	
	Number	Percentage	Number	Percentage[a]	Number	Percentage[b]
Stationary phases						
Ristocetin A	29	69	81	28	26	32
Teicoplanin	35	83	142	48	48	34
TAG	33	79	134	46	71	53
Vancomycin	30	71	60	24	3	5
VAG	27	64	83	28	6	7
Mobile phases						
RP MeOH–buffer	35	83	92	43	30	33
RP ACN–buffer	26	62	56	27	6	11
PO MeOH	28	67	42	20	14	33
PO ACN	13	31	19	9	3	16
NP hex–EtOH	34	81	97	46	45	47
NP hex–IPA	34	81	87	41	39	45
NP MTBE–ACN	31	74	107	50	17	16
NP MTBE–MeOH	29[c]	69	50[c]	59[c]	11	22
Total	39	93	500	34	154	31

RP = Reversed phase, PO = polar organic mode, NP = normal phase.
[a] Percentage of observable separations from the total number of analyses done (42 compounds × 7 mobile phases = 294 analyses per stationary phase, and 42 compounds × 5 mobile phases = 210 analyses per mobile phase).
[b] Percentage of baseline separations obtained from the non optimized separations.
[c] Results obtained with the 97/3 optimized mobile phase composition but on vancomycin and VAG CSPs only (84 analyses instead of 210).

higher than the corresponding value for the teicoplanin CSP results (~34% baseline resolutions). This means that the enantioselectivity factors obtained with the TAG CSP often were higher that the corresponding values obtained with the native teicoplanin column (Table 3).

The vancomycin and VAG columns were able to separate 31 and 29 compounds, respectively. However, the number of chromatograms with observable enantioseparations is significantly lower with these two CSPs than with the teicoplanin and TAG CSPs (Table 3). Fig. 1 shows that most compounds could be resolved with one or two mobile phase compositions only. The number of compounds baseline resolved is dramatically lower; seven compounds on the VAG column and only four on the vancomycin column (12, 22, 29 and 31) (Fig. 1). The vancomycin CSP could partly resolve five compounds (10, 19, 27, 40 and 41) that the VAG CSP could not separate. Conversely, the VAG CSP could partly resolve 21 and 35, which vancomycin did not separate. It should be noted, however, that vancomycin is the only CSP that could partly separate the enantiomers of the sulfinate esters 41 and 42 with the apolar MTBE–MeOH (97:3, v/v) mobile phase. The results obtained with these two CSPs and the normal-phase MTBE–ACN (90:10) mobile phase were optimized. Methanol was substituted for ACN to decrease slightly the solute–CSP hydrogen bonding interactions. Shorter retention times and sharper peaks were obtained with a MTBE–MeOH (97:3, v/v) mobile phase compared to the MTBE–ACN (90:10, v/v) mobile phase (Table 3).

The glycopeptide antibiotics teicoplanin and vancomycin are naturally produced by the fermentation of *Actinoplanes teicomyceticus* and *Streptomyces orientalis*, respectively. Both molecules have an aglycone 'basket' core bearing three or two carbohydrate substituents, respectively. To answer whether the carbohydrate moieties are useful for compound enantioselectivity of stereogenic sulfur-containing compounds, the aglycone form of teicoplanin, TAG, and vancomycin, VAG, were prepared [33]. It was found that a few more sulfur containing compounds

could be resolved on the carbohydrate containing columns (teicoplanin or vancomycin) than on their aglycone counterpart columns (TAG or VAG). However, for the compounds that were separated on both CSPs with identical mobile phases, most had higher enantioselectivity and resolution factors on the aglycone columns (Table 2). Fig. 2 illustrates this observation for the vancomycin and teicoplanin based CSPs. 26 (Fig. 2, top left) is not resolved on the vancomycin CSP with the MTBE–ACN (90:10, v/v) mobile phase; its α and R_s values are 1.08 and 1.3, respectively, when the VAG column is used with the same mobile phase. For 31 (Fig. 2, top right), going from the vancomycin to the VAG CSP with the same mobile phase, the α and R_s values increase from 1.15 to 1.3 and from 1.2 to 1.4, respectively.

A similar improvement with the teicoplanin and TAG columns is also shown in Fig. 2 (bottom) for compounds 1 and 8 and the hex–EtOH (50:50, v/v) mobile phase. However, in some cases, the teicoplanin and vancomycin CSPs performed better than their aglycone counterparts. For example, compounds 25, 27 and 34 are separated by the teicoplanin CSP and not by the TAG column; compounds 10, 19, 27, 40, 41 and 42 are separated by the vancomycin CSP and not by the VAG column. The two forms of the macrocyclic glycopeptide selectors should be considered. The native form of glycopeptide seems capable of separating, at least partially, more enantiomeric pairs than the corresponding aglycone form. Similar results were reported previously for amino acids and the teicoplanin and TAG columns [33].

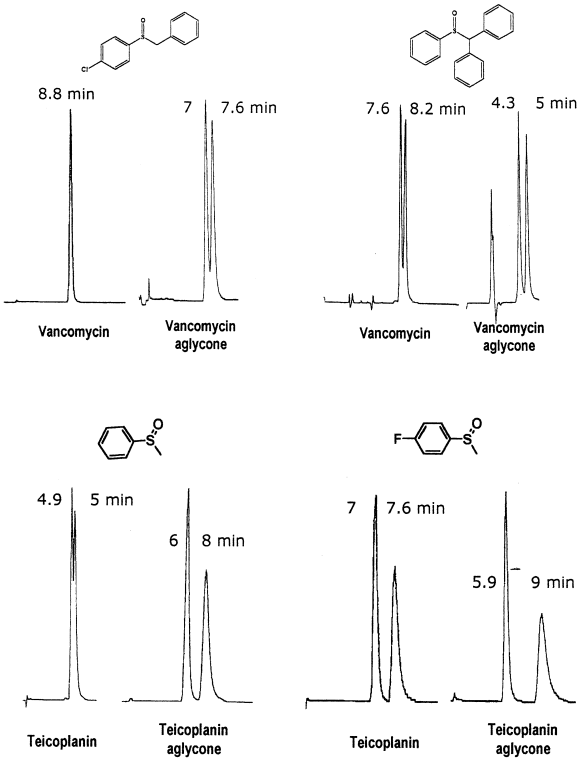


Fig. 2. Effect of the macrocyclic glycopeptide sugar units on compound enantioselectivity. Top: Vancomycin and compound 26 (left) and compound 31 (right). Mobile phase: MTBE–ACN (90:10, v/v), 1 ml/min. Bottom: Teicoplanin and compound 1 (left) and compound 8 (right). Mobile phase: hex–EtOH (50:50, v/v), 2 ml/min.

The ristocetin A column was able to resolve 29 of the 42 chiral sulfoxide compounds. This is only four compounds fewer than the TAG CSP and not significantly different from the number of compounds that the vancomycin and VAG columns could resolve. Of the 83 chromatograms obtained on the ristocetin A CSP that showed observable separation, 26 had baseline separation of the enantiomers (Table 3). This is significantly higher than the figures obtained with the vancomycin or VAG columns, especially in the number of baseline resolutions. It is, however, significantly lower than the figures obtained with the teicoplanin or TAG columns. The ristocetin column is useful in the separation of chiral sulfoxide and sulfinate esters. It is the only column able to separate the sulfinate methyl ester, compound 37. However, the mobile phase composition must be properly optimized.

The complementary nature of the macrocyclic glycopeptide CSPs is well known [34]. A partial separation obtained on one CSP is often converted in a baseline separation when changing to a related glycopeptide chiral selector. This effect was observed for the sulfoxides 25 and 31 that were poorly separated by the teicoplanin based CSPs and well separated by the ristocetin A CSP (Fig. 1) and, to a lesser extent, for several sulfinate esters (39, 40 and 42) that were partly separated by the vancomycin CSP only.

3.2. Mobile phase performance

Table 3 lists the cumulative number of separations

obtained with the different mobile phases. Clearly, the three normal-phase mobile phases and the methanol-containing reversed mobile phases are the most useful in separating these compounds with the macrocyclic glycopeptide CSPs. The *n*-hexane–alcohol mobile phases were both able to separate 34 compounds from the set of 42. Ethanol seems to be a better polar organic modifier than isopropanol since 97 observable chromatograms were obtained with *n*-hexane–ethanol normal mobile phases on the five CSPs. Forty-five of these separations were complete with a baseline return between peaks. The *n*-hexane–ethanol mobile phases were most useful with the TAG CSP. The *n*-hexane–IPA mobile phases could separate the same 34 compounds but on a lower number of stationary phases; only 87 chromatograms showed some separation with only 39 of them being baseline separations (Table 3). The MTBE–ACN mobile phases are low polarity mobile phases made of dipolar aprotic solvents. They were able to separate 31 compounds only, but with many different CSPs since they have the highest number of successful hits: 107. If these 107 separations are compared to the 31 compounds separated by this mobile phase, it means that an average of 3.5 CSPs were producing a chromatogram with observable enantioseparation for each 31 compounds. However 84% of these chromatograms were partial separations since only 17 were baseline separations. The MTBE–ACN mobile phases were most useful with the ristocetin A, vancomycin and VAG CSPs. It was shown that methanol, a polar hydrogen bond donor solvent, was a better organic modifier in the MTBE based mobile phase. The MTBE–MeOH (97:3, v/v) mobile phase showed a 10% improvement in the number of enantioseparated compounds and a doubling of the number of baseline separations compared to the MTBE–ACN (90:10, v/v) composition (study done on vancomycin and VAG CSPs only, Table 3).

The reversed-phase mode (with methanol–buffer mobile phases) was highly effective for the separation of chiral sulfoxides and related compounds as well. A total of 35 compounds, 32 of which belonged to the set of compounds separated by the normal mobile phases, were separated. 24 was not separated by the methanol–buffer mobile phase, but 34, a tosyl derivative, and 37, a sulfinate ester, were separated with the methanol reversed-phase and not with the normal-phase mobile phases. If 92 chromatograms showed enantioselectivity, only 30 were baseline separations ($R_s > 1.5$) (Table 3). Table 3 shows that the methanol–buffer mobile phase are much more effective than the acetonitrile–buffer mobile phases. The ACN–buffer mobile phases could resolve only 26 solutes of the set of sulfur-containing compounds. Fifty-six chromatograms showed observable enantioselectivity, that is an average value of two different CSPs per resolved compounds. Only six of the 56 chromatograms showed baseline separation. The ACN–buffer reversed-phase systems are not as effective in separating sulfoxide enantiomers with the TAG CSP than the methanol–buffer mobile phases. Table 2 shows that each time a partial resolution is obtained with an ACN–buffer mobile phase a higher α value is obtained with the corresponding methanol–buffer mobile phase. Note that when the RP ACN line is missing for a compound in Table 2, it means that no resolution was obtained on all CSPs ($\alpha = 1$). One exception should be mentioned: the sulfinate ester enantiomers of 41 could be partially resolved only by the vancomycin CSP in association with a ACN–buffer reversed mobile phase (Fig. 1).

The polar organic methanol mobile phases could separate 28 compounds with 48 chromatograms with observable separation (14 were baseline separations). All the 28 compounds could be better separated by another mobile phase either in the normal- or in the reversed-phase modes (Table 2). The teicoplanin based CSPs where the most compatible with the polar organic methanol mobile phases. The results were worse with the polar organic ACN mobile phases that could only separate 13 compounds and only three were completely resolved. The ristocetin CSP gave the best results with the polar organic ACN mobile phases (Fig. 4).

3.3. Effectiveness of the macrocyclic glycopeptide CSPs

Altogether the macrocyclic glycopeptide CSPs were able to separate the whole set of the 34 sulfoxide enantiomers and tosylated derivatives. Five of the eight sulfinate esters were also separated. The largest set of chiral sulfoxide compounds studied for enantioselective separation found in the literature contained 23 sulfoxide derivatives [36]. Only five of these compounds were also present in our set of 42

compounds. These compounds were separated on four different cellulose or amylose polysaccharide CSPs with a *n*-hexane–IPA (90:10, v/v) normal mobile phase only. The best CSP was able to resolve 16 compounds, of which only two were baseline separated. The compounds in this previous study that are common with ours, are 1, 4, 5, 16 and 21 (Table 1). With the same *n*-hexane–IPA normal mobile phase, 1 is baseline resolved by the teicoplanin and TAG columns and almost baseline resolved by the ristocetin A column ($R_s = 1.3$, Table 2). This compound is partially resolved by the best polysaccharide CSP of Ref. [36] with an R_s value of 0.8. It is not resolved by the three other CSPs. Similar observations can be made with the other four common compounds.

In another article, the capability of eight commercial cellulose-based sorbents were screened for the enantiomeric separation of a set of 10 chiral sulfoxides [18]. Our compounds 1, 4 and 18 were part of this set. The mobile phase used was *n*-hexane–IPA (90:10, v/v). The best enantioseparation of 1 was obtained with CSP Chiralcel OB with an α value of 1.72 giving a R_s value of 3.6. With the same mobile phase, teicoplanin resolves the enantiomers of 1 with an α value of 1.22 giving an R_s value of 4.27 (better efficiency) and TAG gave $\alpha = 2.02$ and $R_s = 2.2$ (better enantioselectivity) (Table 2). Similarly, 4 was also best separated by Chiralcel OB ($\alpha = 1.58$ and $R_s = 3.11$). The teicoplanin and TAG CSPs were also able to separate it with a baseline resolution ($\alpha =$ 1.44 and 1.47, $R_s = 1.8$ and 2.1, respectively). However, the values were slightly lower. Methyl dodecyl sulfoxide was part of the set of compounds in Ref. [14] and was not separated by Chiralcel OB and poorly separated by the Chiralcel OD column ($\alpha =$ 1.07, $R_s = 0.6$). Our compound 3, methyl hexyl sulfoxide is closely related. It was baseline resolved by both the teicoplanin and TAG CSPs in either the normal- or reversed-phase mode.

In a recent study, polar organic mobile phases (100% alcohol), reversed alcoholic mobile phases and *n*-hexane–IPA mobile phases were used with six polysaccharide phenylcarbamate CSPs to separate a set of five sulfoxide compounds [22]. Our compounds 1 and 5 were part of this set. Every time a comparison was possible (same mobile phase), there was a macrocyclic glycopeptide CSP that could match the polysaccharide CSPs used [22].

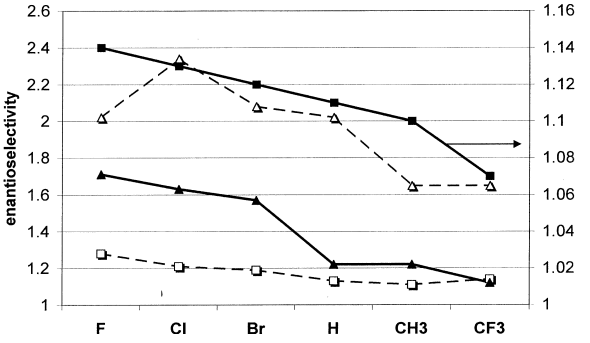


Fig. 3. Effect of the substituents of the *para*-substituted phenyl methyl sulfoxides on the compounds enantioselectivity. Thick lines: teicoplanin CSP, dotted lines: TAG CSP; squares: methanol–buffer (20:80, v/v) reversed mobile phase, triangles: *n*-hexane–IPA (90:10, v/v) normal mobile phase.

3.4. Structure–enantioselectivity relationships

3.4.1. Nature of the benzene ring substituent

Fig. 3 shows the enantioselectivity factors obtained for a variety of substituted aryl methyl sulfoxides with the teicoplanin and TAG columns with two mobile phases: *n*-hexane–IPA (90:10, v/v) and methanol–pH 4 buffer (50:50, v/v). The α factor is plotted versus the *para* substituent on the benzene ring. The enantioselectivity factors decrease in the order: $F > Cl > Br > H > CH_3 > CF_3$ for the two CSPs and for the two mobile phases (normal-phase mode and reversed-phase mode). Clearly, the order corresponds to the decreasing electronegativity order for the halogen atoms, the hydrogen atom and the methyl group. The trifluoromethyl group does not fit in this correlation. With three strongly electronegative atoms, it has a strong electron withdrawing capability. Also, the size of the substituents should be noted. The fluorine atom is the smallest halogen; the CF_3 group is slightly bigger than the CH_3 group and the H atom.

3.4.2. Position of the benzene ring substituents

Fig. 4 shows that the enantioselectivity factors obtained with the *meta*-substituted molecules were significantly higher than the values obtained with the corresponding *ortho* or *para* isomers. The exception is *ortho*-bromophenyl methyl sulfoxide separated by the teicoplanin CSP in the reversed-phase mode. It has an α value of 1.2, slightly higher than that of *meta*-bromophenyl methyl sulfoxide ($\alpha = 1.17$, Table

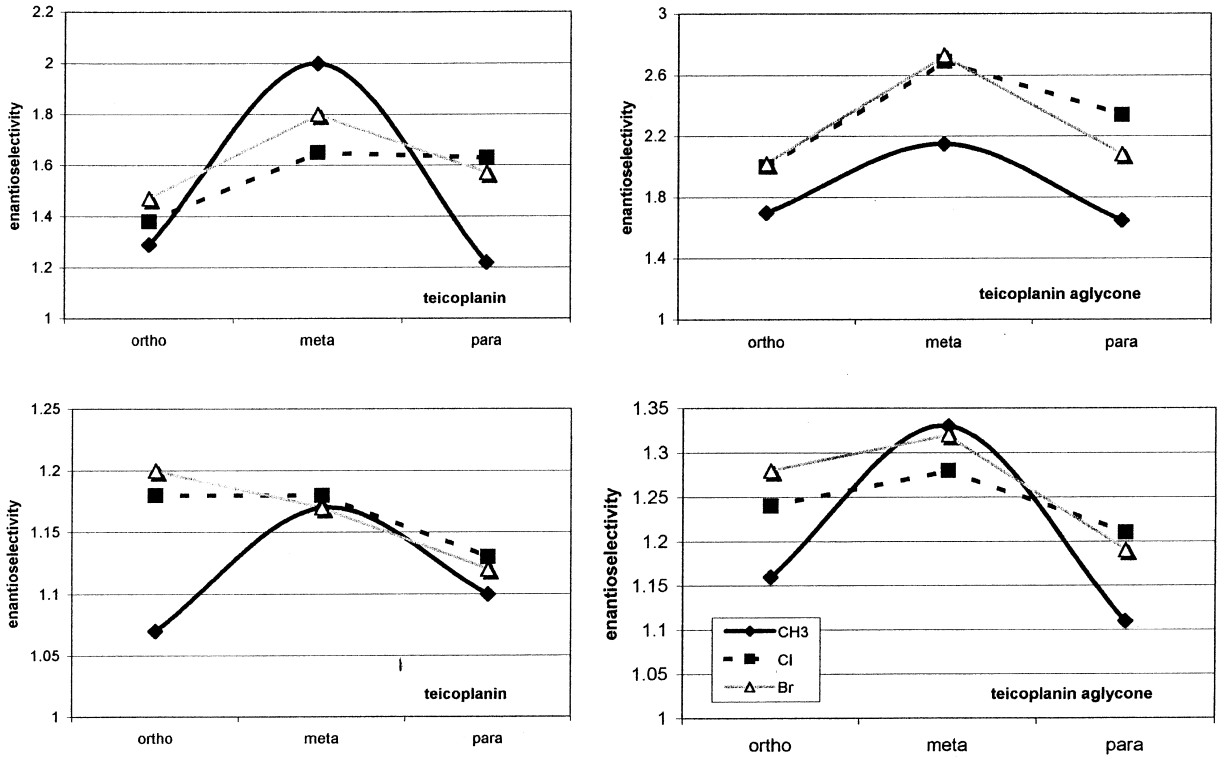


Fig. 4. Structural effect of the phenyl substituents of the *ortho*-, *meta*- or *para*-substituted phenyl methyl sulfoxides on the compounds enantioselectivity. Top figures: *n*-hexane–IPA (90:10, v/v) normal mobile phase; bottom figures: methanol–buffer (20:80, v/v) reversed mobile phase.

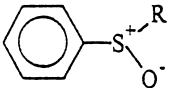
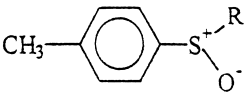
2). In organic synthesis it is known that the methyl group and halogen substituents are *ortho* and *para* directors for electrophilic aromatic substitutions. The electron deficient intermediate complex can be stabilized only by *ortho* and *para* substituents. It is possible that the *ortho* and *para* positions of the methyl, chloro or bromo substituents produce an electron density on the sulfur atom less favorable for compound enantioselectivity than that obtained when the substituents are in the *meta* position. However, the aromatic electron density and the electron environment of the sulfur atom are certainly not the only parameters acting on the compound enantioselectivity of sulfoxides by teicoplanin CSPs. Shape and steric repulsion are certainly involved in the mechanism [18].

3.4.3. Steric factors

Using a thermodynamic approach, Küsters et al. claimed that steric hindrance was the main reason for chiral discrimination of sulfoxides by polysaccharide based CSPs in the normal-phase mode [19]. Table 4 lists the enantioselectivity factors for a variety of phenyl sulfoxides and *para*-tolyl sulfoxides obtained with the teicoplanin and TAG CSPs and with normal- and reversed-phase mobile phases. Four substituents in the table are common to phenyl and *para*-toluyl sulfoxides. They are the methyl, benzyl, (2-phenyl) ethyl and (1,1-dimethyl 2-phenyl) ethyl substituents. Every time the enantiomers of these sulfoxides are separated ($\alpha > 1$), the α factor is better in the same experimental conditions for the phenyl sulfoxide than for the corresponding tolyl compound. It seems that the main part of this effect is due to steric hindrance. The slight change in molecular volume between the phenyl and the *para*-tolyl group makes a significant difference for the chiral selector.

Steric bulk seems to be the dominant factor explaining the increased α values obtained when the bulky *tert.*-butyl group replaces the methyl group (1

Table 4
Enantioselectivity factor for phenyl and toluyl sulfoxide chiral compounds

CSP compound number	R substituent	Teicoplanin		TAG	
		hex–IPA (90:10, v/v)	MeOH–buffer (20:80, v/v)	hex–IPA (90:10, v/v)	MeOH–buffer (50:50, v/v)
					
1	CH_3	1.22	1.11	2.02	1.13
4	Vinyl	1.44	1.06	1.46	1.19
12	t-Bu	2.50	1.06	1.81	1.20
16	Benzyl	1.09	1.00	1.12	1.07
22	$CH_2CH_2\phi$	1.17	1.00	1.47	1.00
23	$CH_2\phi CH_3$	1.00	1.00	1.09	1.07
29	$C(CH_3)_2CH_2CH_2\phi$	4.00	1.42	1.95	1.19
31	$CH(\phi)_2$	1.00	1.15	1.00	1.10
					
5	CH_3	1.22	1.10	1.65	1.11
21	Benzyl	1.00	1.00	1.00	1.08
24	$CH_2CH_2\phi$	1.00	1.02	1.30	1.00
27	$CH_2CH_2CH_2\phi$	1.00	1.00	1.00	1.00
30	$C(CH_3)_2CH_2CH_2\phi$	2.24	1.31	1.33	1.15
35	OCH_3	1.71	1.09	2.00	1.20
36	OCH_2CH_3	1.00	1.00	1.00	1.00
38	$OCH(CH_3)CH_3$	1.00	1.00	1.00	1.00
39	$OCH_2CH_2CH_3$	1.00	1.00	1.00	1.00
40	$OCH_2CH_2CH_2CH_3$	1.00	1.00	1.30	1.00
41	$OCH_2CH(CH_3)CH_3$	1.00	1.00	1.00	1.00
42	$OCH(CH_3)CH_2CH_3$	1.00	1.00	1.00	1.00

Vinyl = $CH{=}CH_2$; t-Bu = $C(CH_3)_3$; benzyl = $CH_2\phi$; ϕ = phenyl ring.

and 12, Table 4). Intramolecular stacking may also be part of steric repulsion. Comparing the results obtained with 22 and 29 (phenyl sulfoxides) and 24, 27 and 30 (tolyl sulfoxides), a dramatic increase of the enantioselectivity factor for the dimethylaryl-α-substituted sulfoxides can be noted (Table 4). Fig. 5 illustrates the intramolecular π–π stacking that could be favored with 29 and 30 having two methyl groups that promote bending of the alkyl chain, allowing the two aromatic rings to interact. 22, 24 and 27 have also two aromatic rings that could stack but possible free rotations around the CH_2 groups decrease the stacking. The stacked form of the compounds seems to interact strongly with some chiral selector sites.

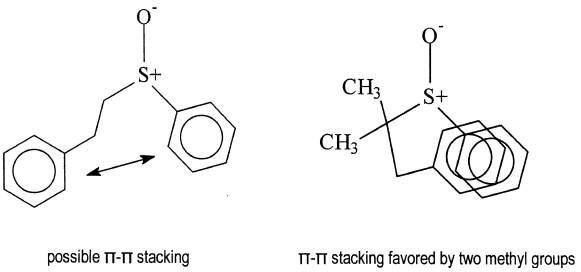


Fig. 5. Left: Possible intramolecular stacking by π–π interactions. Right: The stacking is favored by the two methyl groups in the α position of the sulfur atom.

Either reduced or no enantiorecognition is obtained when stacking is reduced (Table 4). The stacking effect is more pronounced with the apolar *n*-hexane–IPA mobile phase (where π–π interactions are favored) than with the polar methanol–buffer mobile phase.

3.4.4. Altering the sulfoxide group

Table 5 lists the results obtained with the teicoplanin CSP for the analyses of different compounds in which the sulfoxide group has been modified. For example, 32 is the N-tosyl analog of 1. The $S^+–O^-$ group of 1 is replaced by $S^+–N^-–SO_2–\phi\text{-}CH_3$. Changing the sulfoxide group to the N-tosylated form significantly decreased the chromatographic polarity of the molecule. Apparently, the N^- group is much more hindered than a lone O^- group which is very active for H-bonding. The N-tosyl analogs are less retained in the normal-phase mode and more retained in the reversed-phase mode (Table 5). The enantioselectivity factor, α, was less for all of the tosylated compounds except one in the normal-phase mode (Table 5). In the reversed-phase mode, the results were mixed. Clearly the tosyl group alters the interaction between the chiral analyte and the CSPs in both normal- and reversed-phase modes. Steric interactions will increase in both modes. While hydrophobic interactions are more significant for the N-tosylated compounds in the reversed-phase mode (Table 5), the corresponding π–π interactions do not seem to be enhanced in the normal-phase mode except for 34. Apparently, the increased retention and possible stacking with this molecule results in the elimination of enantioselective recognition.

35 to 42 are sulfinate esters. The oxidation number of sulfur in these compounds is different from that of the sulfoxide compounds. This change is detrimental for compound enantioselectivity. Only the enantiomers of methyl *p*-toluene sulfinate (35) could be resolved by the teicoplanin and TAG columns. Three of the seven sulfinate esters could not be resolved by any of the five CSPs studied. Ristocetin A was the only CSP able to partially resolve 37 and the vancomycin CSP could also partially resolve 40, 41 and 42 (Fig. 1). All partial separations were obtained in the reversed-phase mode. It should be pointed out that there are very few, if any, previously reported LC enantiomeric separations of sulfinate esters. However, these compounds are easily resolved by gas chromatography using cyclodextrin-based CSPs [37].

3.4.5. Enantiomeric retention order

The enantiomeric elution order was determined for all separations using a laser-based polarimetry detector and/or by injecting an enantiomer standard of known configuration. The first eluted enantiomer, for all compounds separated on the teicoplanin and TAG CSPs (but one) was the (*S*)-(+)-enantiomer. The one exception was methyl *p*-biphenyl sulfoxide (17) where the (*R*)-(−)-enantiomer eluted first with all mobile phases. Its long and rigid *para*-biphenyl substituent is very likely sterically altering its interaction with the teicoplanin or TAG chiral selector. Also, the *ortho* derivatives, 11 and 20, showed a reversed enantiomeric retention order, i.e., the (*R*)-(−)-enantiomer eluted first, but only in the normal-

Table 5
Chromatographic results obtained with sulfoxides and the corresponding N-tosylated compounds and sulfinate esters with the teicoplanin CSP

Mobile phase number	Formula	Hexane–IPA (90:10, v/v)				Methanol–buffer (20:80, v/v)			
		k_1	k_2	α	R_s	k_1	k_2	α	R_s
1	$\phi\text{-SO–CH}_3$	19.3	23.6	1.22	4.3	1.27	1.41	1.11	0.5
32	$\phi\text{-SNTs–CH}_3$	4.60	5.20	1.13	0.7	3.00	3.33	1.11	1.1
16	$\phi\text{-SO–CH}_2\phi$	6.30	6.86	1.09	0.6	3.50	3.50	1.00	0.0
33	$\phi\text{-SNTs–CH}_2\phi$	4.73	5.44	1.15	0.7	9.83	11.1	1.13	1.1
22	$\phi\text{-SO–CH}_2\text{CH}_2\phi$	9.54	11.2	1.17	0.7	7.21	7.21	1.00	0.0
34	$\phi\text{-SNTs–CH}_2\text{CH}_2\phi$	17.8[a]	17.8[a]	1.00	0.0	13.4	14.6	1.09	1.0
35	$\text{CH}_3\text{–}\phi\text{-SO–O–CH}_3$	0.47	0.80	1.71	1.5	0.20	0.22	1.09	1.1
36	$\text{CH}_3\text{–}\phi\text{-SO–O–CH}_2\text{CH}_3$	0.27	0.27	1.00	0.0	3.17	3.17	1.00	0.0

ϕ = Phenyl ring; NTs = $N–SO_2–\phi\text{-}CH_3$. Column Chirobiotic T, 25 cm × 4.6 mm I.D., 5 μm silica particle size.
[a] Experiments done with a hexane–IPA (80:20, v/v) normal mobile phase.

phase mobile phase of acetonitrile–methyl *tert*.-butyl ether only. With the six other mobile phases, the (*S*)-(+)-enantiomer of these two analytes eluted first.

Conversely, all chiral sulfoxides separated on the ristocetin A, the vancomycin and the VAG CSPs showed the (*R*)-(−)-enantiomer eluting first. This behavior was maintained with the optimized MTBE–MeOH mobile phase and the vancomycin and VAG CSPs. Fig. 6 shows the separation of the enantiomers of 29 on the TAG column (left) and the VAG column (right). The trace of the optical rotation detector shows that the enantiomeric retention order of the two enantiomers is reversed. The (*S*)-(+)-enantiomer elutes first on the TAG column and last on the VAG column. There were no exceptions for the vancomycin and VAG columns. It should be recalled, however, that these columns could only separate 30 and 27 compounds, respectively, from the set of 42. With the ristocetin A column, the only exceptions found were the benzyl derivatives, 16, 25 and 26, and the diphenylmethyl derivative, 31. The (*S*)-(+)-enantiomers of these four analytes eluted first but with the reversed-phase mode methanol–buffer system, only.

These results show again the complementarity of the macrocyclic glycopeptide CSPs. There are multiple chiral interaction sites on a given CSP and the individual chiral selectors of this class of compounds are not the same. For a given compound, if no enantioseparation is obtained in the different mobile phase modes with a particular CSP, chances are that another macrocyclic glycopeptide based CSP will separate the enantiomers [38]. Furthermore, for neutral sulfoxides, at least two different glycopeptide CSPs can have the opposite enantiomeric retention order.

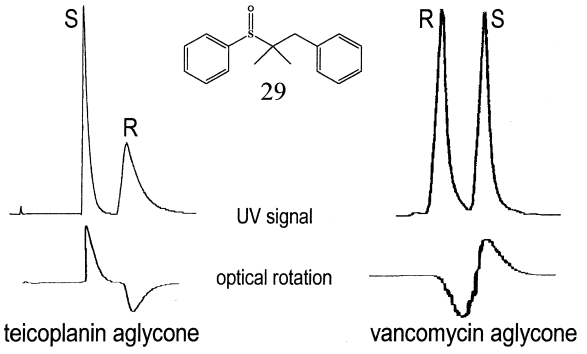


Fig. 6. Illustration of the enantiomeric retention order reversal. Left: Compound 29 separated with the TAG column, the (*S*)-(+)-enantiomer elutes first, normal mobile phase (*n*-hexane–EtOH, 50:50, v/v); right: on the VAG column, the (*R*)-(−) enantiomer elutes first, normal mobile phase (MTBE–ACN, 90:10, v/v).

4. Summary and conclusion

The macrocyclic glycopeptide CSPs are very useful for the separation of enantiomers of chiral sulfoxides. The teicoplanin and TAG CSPs with the *n*-hexane–IPA mobile phase (i.e., normal-phase mode) and the methanol–buffer mobile phase in the reversed-phase mode are the most effective CSP–mobile phase associations for the enantioseparation of these compounds. An important feature involving the chiral recognition mechanism of sulfoxide compounds seems to be steric repulsion. Also it appears that intramolecular stacking of some of the larger chiral sulfoxides can greatly affect its enantiorecognition. Compared to chiral sulfoxides, the sulfinate esters, with an increased oxidation state of the sulfur atom, are poorly separated by the macrocyclic antibiotic CSPs. The enantiomeric retention order of the enantiomer showed a great deal of consistency for any single CSP and mobile phase. However, reversing the enantiomeric retention order is possible by changing the CSP.

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References

[1] K. Mislow, A.J. Gordon, D.R. Rayner, J. Am. Chem. Soc. 90 (1968) 4854.
[2] J.A. Dobado, H. Martinez-Garcia, J.M. Molina, M.R. Sundberg, J. Am. Chem. Soc. 121 (1999) 3156.

[3] D.R. Rayner, A.J. Gordon, K. Mislow, J. Am. Chem. Soc. 90 (1968) 4854.
[4] P.W.B. Harrison, J. Keynon, H. Phillips, J. Chem. Soc. (1926) 2079.
[5] M.C. Carreño, Chem. Rev. 95 (1995) 1717.
[6] H. Matsuyama, Sulfur Rep. 22 (1999) 85.
[7] J.C. Carretero, R.G. Arrayas, N.D. Buezo, J.L. Garrido, I. Alonso, J. Adrio, Phosphorus Sulfur Silicon Relat. Elem. 153–4 (1999) 259.
[8] G. Farina, F. Montanari, A. Negrini, Gazz. Chim. Ital. 89 (1959) 1548.
[9] W.H. Pirkle, D.W. House, J. Org. Chem. 44 (1979) 1957.
[10] W.H. Pirkle, J.M. Finn, J.L. Schreiner, B.C. Hamper, J. Am. Chem. Soc. 103 (1981) 3964.
[11] S. Allenmark, L. Nielsen, W.H. Pirkle, Acta Chem. Scand. 37 (1983) 325.
[12] G. Gargaro, F. Gasparrini, D. Misiti, G. Palmieri, M. Pierini, D. Villani, Chromatographia 24 (1987) 505.
[13] M. Lienne, M. Caude, R. Rosset, A. Tambute, P. Delatour, Chirality 1 (1989) 142.
[14] C.J. Welch, T. Szczerba, S.R. Perrin, J. Chromatogr. A 758 (1997) 93.
[15] S. Allenmark, B. Bomgren, J. Chromatogr. 252 (1982) 297.
[16] K. Balmer, B.A. Persson, P.O. Lagerström, J. Chromatogr. A 660 (1994) 269.
[17] A. Ichida, T. Shibata, Y. Okamoto, Y. Yuki, H. Namikoshi, Y. Toga, Chromatographia 19 (1984) 280.
[18] I. Wainer, M.C. Alembik, C.R. Johnson, J. Chromatogr. 361 (1986) 374.
[19] E. Küsters, V. Loux, E. Schmid, P. Floersheim, J. Chromatogr. A 666 (1994) 421.
[20] M. Tanaka, H. Yamazaki, H. Hakusui, Chirality 7 (1995) 612.
[21] M.I. Donnoli, S. Superchi, C. Rosini, Enantiomers 5 (2000) 181.
[22] B. Chankvetadze, C. Yamamoto, Y. Okamoto, J. Chromatogr. A 922 (2001) 127.
[23] S.A. Matlin, M.E. Tiritan, Q.B. Cass, D.B. Boyd, Chirality 8 (1996) 147.
[24] E. Küsters, G. Gerber, Chromatographia 44 (1997) 91.
[25] T.J. Ward, A.B. Farris, J. Chromatogr. A 906 (2001) 73.
[26] S. Oae, N. Furukawa, in: Sulfilimines and Related Derivatives, ACS Symposium Series, Vol. 179, 1983, p. 2.
[27] J.M. Klunder, K.B. Sharpless, J. Org. Chem. 52 (1987) 2598.
[28] G. Capozzi, J. Drabowicz, D. Kiełbasinski, S. Menichetti, M. Mikołajczyk, C. Nativi, K. Schank, N. Schott, U. Zoller, Synthesis of Sulphones, Sulphoxides and Cyclic Sulphides, Wiley, Chichester, 1994.
[29] W.S. Jenks, W. Lee, D. Schutters, J. Phys. Chem. 98 (1994) 2282.
[30] W. Lee, W.S. Jenks, J. Org. Chem. 66 (2001) 474.
[31] M.P. Gasper, A. Berthod, U.B. Nair, D.W. Armstrong, Anal. Chem. 68 (1996) 2501.
[32] A. Berthod, Y. Liu, C. Bagwill, D.W. Armstrong, J. Chromatogr. A 731 (1996) 123.
[33] A. Berthod, X. Chen, J.P. Kullman, D.W. Armstrong, F. Gasparrini, I. D'Acquarica, C. Villani, A. Carotti, Anal. Chem. 72 (2000) 1727.
[34] U.B. Nair, S.C. Chang, D.W. Armstrong, Y.Y. Rawjee, D.S. Eggleston, J.V. McArdle, Chirality 8 (1996) 590.
[35] D.W. Armstrong, Y. Tang, S. Chen, Y. Zhou, C. Bagwill, J.R. Chen, Anal. Chem. 66 (1994) 1473.
[36] C.A. Montanari, Q.B. Cass, M.E. Tiritan, A.L.S. De Souz, Anal. Chim. Acta 419 (2000) 93.
[37] J.A. Anderson, J. Ding, R. McCulla, W.S. Jenks, D.W. Armstrong, J. Chromatogr. A 947 (2002) 197.
[38] T.E. Beesley, R.P.W. Scott, Chiral Chromatography, Separation Science Series, Wiley, Chichester, 1998.