

23 Mar 1994

## Macrocyclic Antibiotics as a New Class of Chiral Selectors for Liquid Chromatography

Christine Bagwill

Follow this and additional works at: <https://scholarsmine.mst.edu/oure>

 Part of the [Chemistry Commons](#)

---

### Recommended Citation

Bagwill, Christine, "Macrocyclic Antibiotics as a New Class of Chiral Selectors for Liquid Chromatography" (1994). *Opportunities for Undergraduate Research Experience Program*. 2.  
<https://scholarsmine.mst.edu/oure/2>

This Presentation is brought to you for free and open access by Scholars' Mine. It has been accepted for inclusion in Opportunities for Undergraduate Research Experience Program by an authorized administrator of Scholars' Mine. This work is protected by U. S. Copyright Law. Unauthorized use including reproduction for redistribution requires the permission of the copyright holder. For more information, please contact [scholarsmine@mst.edu](mailto:scholarsmine@mst.edu).

**MACROCYCLIC ANTIBIOTICS AS A NEW CLASS OF  
CHIRAL SELECTORS FOR LIQUID CHROMATOGRAPHY**

**Christina S. Bagwill, Chemistry Department**

## ABSTRACT

In the field of chromatographic separations, the need for new chiral stationary phases is always in demand. Many stationary phases already exist, some of the more popular being cyclodextrin or protein based phases. During this research, a new class of chiral selectors were discovered and evaluated for chiral recognition. Vancomycin, thiostrepton, and rifamycin B all belong to the macrocyclic antibiotic family. Not only can this new class of chiral selectors be used to resolve compounds that cannot be separated on any other column, but it is also more stable and has much greater capacity than protein based columns.

## INTRODUCTION

In order to understand the significance of this research, some relevant information is needed regarding the importance of chiral separation technology. All of the stationary phases mentioned previously are selective for chiral compounds. A chiral compound is one that is not superposable on its mirror reflection (e.g., your left and right hands are mirror images but are not superposable on one another). Some examples of compounds which have chiral centers are listed in Table 1 and Table 5. Mirror image compounds that are not superposable are called enantiomers. They have identical physical and chemical properties in an isotropic environment. Since they have identical properties it is difficult to distinguish one from another through 'normal' chemical methods. However, using a chromatography column containing a chiral selector sometimes allows certain enantiomers to be successfully separated. Choosing a chiral selector is dependent upon the functionality of the enantiomer and the interactions taking place between the enantiomers and the stationary phase.

A typical enantiomeric separation is given in Figure 1. The notation system for distinguishing between two enantiomers use the letters: (R), (S) or (D), (L). The (D) and (L) labeling is the older system but is still used today. This notation is usually used with amino acids, sugars and related compounds. The (R) and (S) system is newer and more widely used. The letter (R) is for the Latin "rectus" (i.e., right or clockwise) and (S) "sinister" is for left or counter clockwise. These terms are used to describe the absolute conformation of a chiral compound according to their sequence rules.

Chiral separations are very important to the pharmaceutical, food and beverage, and environmental industries. Many drugs that the pharmaceutical industries produce are chiral. Over the past two years chiral drug

technology have been given the cover story in several issues of *Chemical and Engineering News*. The September 27, 1993 issue talks about the effects of enantiomers on the pharmaceutical industry. When antibiotic drugs are produced by fermentation they will only consist of one enantiomer. However when chemists synthesize chiral compounds without including a chiral enantiomeric reagent or chiral catalysts, the result is a 50-50 mixture of enantiomers which is called a racemate or a racemic mixture. Usually one enantiomer is responsible for the pharmacological activity, and the other may be inactive or toxic. Until recently, because of cheaper production costs, drug researchers were satisfied with manufacturing racemic forms of the chiral drugs. Ibuprofen is an example of a drug which has been produced as a racemic mixture. Independently, several companies are preparing to market the S (+) enantiomer of ibuprofen because it is the "active" enantiomer. Conversely, both enantiomers of ketoprofen play different medicinal roles. The S-(+)-ketoprofen is used as an analgesic/anti-inflammatory whereas the (R) enantiomer of ketoprofen is active against bone loss in periodontal disease.<sup>2</sup>

Carvone is an example of a chiral compound that is both a natural or synthetic chiral flavor and fragrance component found in most foodstuffs. The (R) enantiomer of carvone smells like mint, and the (S) enantiomer smells like caraway. Enantiomeric analyses allow the only accurate evaluation of flavors and fragrances.<sup>6</sup>

## EXPERIMENTAL SECTION

**Materials.** All racemic analytes resolved in this study were obtained from Aldrich (Milwaukee, WI) or Sigma (St. Louis, MO). All HPLC grade solvents and N,N-dimethylformamide (DMF) were obtained from Fisher (Pittsburgh, PA). All organosilane compounds were obtained from Petrarch (Bristol, PA). Cyclic antibiotics containing amine, hydroxyl or carboxylic acid functionalities can be linked to silica gel in a variety of different ways. The three cyclic antibiotics discussed in this work (vancomycin, thiostrepton and rifamycin B from Sigma) all contain one or more of these functional groups. Structures for these compounds are shown in Figure 2. Carboxylic acid terminated organosilanes (e.g., 10-carbomethoxy)-ethylmethyldichlorosilane, 2-(carbomethoxy)-ethyltrichlorosilane, etc.) can be used to immobilize vancomycin, and thiostrepton while amine terminated organosilanes (e.g., 3-aminopropyldimethylethoxysilane, 3-aminopropyltriethoxysilane, etc) can be used for rifamycin B. In a typical reaction, four grams of dry silica gel is slurried on 50 mL of dry toluene in a 250 mL 3 neck flask. Two grams of the desired organosilane is dissolved in approximately 15 mL of dry toluene contained in a dropping flask. The organosilane

solution is added drop-wise over approximately 30 minutes to the refluxing toluene-silica gel slurry. The mixture is allowed to reflux (~ 110°C) for two hours, then cooled, filtered and washed with methanol, 50% aqueous methanol, methanol again, and then dried. The silanized silica gel can be slurried in anhydrous DMF. One gram of the appropriate cyclic antibiotic is added along with an appropriate carbodiimide dehydrating agent. After six hours the chiral stationary phase material is filtered and washed with methanol and then aqueous methanol.

The cyclic antibiotics also can be attached to silica gel via epoxy terminated organosilanes as has been described previously for cyclodextrin.<sup>3</sup> Another approach involves reacting the macrocycle with a 2 to 3 molar excess of an isocyanate terminated organosilane (e.g., 3-isocyanatopropyltriethoxysilane) in anhydrous DMF. This product is then added to a dry DMF slurry of silica gel (approximately 2 grams of modified cyclic antibiotic to 4 grams of silica gel). The solution is stirred and allowed to react for 20 hours at 107°C. Subsequently the chiral stationary phase was filtered and washed as indicated previously. Although other attachment chemistries are possible, these are the ones used in the initial studies.

Methods. The cyclic antibiotic chiral stationary phases ( 5  $\mu$  particles) were slurry packed into 5 cm x 0.44 cm i.d. stainless steel columns. Separations were achieved using a Shimadzu LC 6A liquid chromatograph with UV detection (254 nm) and a C-R3A chromatopac data station or with a Waters model 590 HPLC with a 745B data module. Separations were carried out at a flow rate of 1.0 mL/min. and at room temperature (~ 22°C) unless noted otherwise. Mobile phase compositions are listed in the appropriate tables and figures.

## RESULTS AND DISCUSSION

Vancomycin is produced by *Streptomyces orientalis* (Figure 2).<sup>7</sup> It has a molecular weight of 1,449. There are three macrocyclic portions to the molecule which also contains five aromatic rings. Also, there are two side chains, one of which is a carbohydrate dimer and the other a N-methyl-amino acid. Upon heating in neutral or basic conditions, aspartic acid is lost thereby opening at least one of the macrocyclic rings. This is thought to occur during the silica gel immobilization reaction step. Native vancomycin contains 18 stereogenic centers, 9 hydroxyl groups, 2 amine groups, 7 amido groups and 2 chlorine moieties. These groups are known to be useful for stereoselective molecular interactions with chiral analytes.

Rifamycin B is produced by *Nocardia mediterranei* and has a molecular weight of 755.8 (Figure 2).<sup>8,9</sup> It

has 9 stereogenic centers, 4 hydroxyl groups one carboxylic acid moiety and one amide bond. This particular macrocycle may be particularly useful as a chiral mobile phase additive for the separation of chiral amino-alcohols.

Thiostrepton is produced by *Streptomyces azureus* and consists of two joined macrocyclic rings.<sup>10,11</sup> It has a molecular weight of 1,665. It has 17 stereogenic centers, 5 hydroxyl groups, 10 amide linkages and one secondary amine.

All of the cyclic antibiotic stationary phases were tested for stability by switching back and forth between normal and reversed phase modes. All appeared to be stable in both chromatographic modes. Also the enantioselectivity of these stationary phases appears to be different in the two chromatographic modes. Hence, chiral recognition mechanism in the reversed phase mode is not the same as that in the normal phase mode. Table 1 lists a number of reversed phase data for the macrocyclic antibiotic columns.

Optimization of reversed phase separations are done in much the same way as for cyclodextrin-based columns.<sup>3,4,5</sup> Retention is adjusted by controlling the amount of organic modifiers added. Selectivity is effected by both the type of organic modifier and the pH of the mobile phase. Efficiency and selectivity can be effected by ionic strength, buffer type, other additives, and flow rate (Table 4). Lower temperatures usually enhance chiral separations (i.e., increase  $\alpha$ , Table 3).

The effect of pH on enantioselective retention can be seen in Table 2. Ionizable solutes such as coumachlor are significantly affected by pH while nonionizable molecules, such as devrinol are not as affected. However, it should be noted that since these chiral stationary phases are ionizable as well, pH effects for neutral molecules are possible if they result from changes in the chiral selector. Coumachlor, as seen in Table 2, is best resolved at acidic pHs where it exists as the protonated neutral molecule. Clearly the enhanced resolution of coumachlor from pH 7.6 to 4.5 is mainly due to greater efficiency and somewhat longer retention times. The selectivity changes ( $\alpha$ s) are not appreciable in this region. Below pH 4.5 there is a discontinuity for both the retention ( $k'$ ) and  $\alpha$ . Since this occurs for the neutral molecule devrinol it is believed that is due to the protonation of the vancomycin stationary phase. Unlike proteins, macrocyclic antibiotics can be used in the normal phase mode without denaturation or any irreversible change in enantioselectivity. Table 5 lists a number of normal phase data for the macrocyclic antibiotic columns. The racemates resolved in the normal phase mode generally cannot be resolved in the reversed phase mode

and vice versa. Again some of these separations have not been achieved on any other chiral stationary phase (Compound 8, PROXYL, Table 5). Also because of the lower molecular weight and greater loadability of the macrocyclic chiral stationary phases, as compared to proteins, semipreparative and preparative scale-ups are much more feasible.

### CONCLUSIONS

Macrocyclic antibiotics are viable chiral selectors for HPLC. They can be bonded to silica gel via linkage chains using a variety of chemistries. They can be used in either the reversed phase mode or the normal phase mode and have different enantioselectivities in each. Also, cyclic antibiotics can be derivatized in order to change interactions taking place between the analyte and the stationary phase. Macrocyclic antibiotic bonded phases have many of the characteristics of protein-based stationary phases, but with greater stability and much higher capacities. As a results of their relatively small size and the fact that their structures are known, basic studies on chiral recognition should be feasible.

### ACKNOWLEDGEMENTS

I would like to acknowledge the other co-participants: D.W. Armstrong, Y. Tang, S. Chen, Y. Zhou, and J.-R. Chen. Also a special appreciation to Mary Gasper for her help in preparing this paper.

## REFERENCES

- (1) Armstrong, D. W.; Tang, Y.; Chen, S.; Zhou, Y.; Bagwill, C.; and Chen, J.-R. *Anal. Chem.* Accepted for publication May 1, 1994.
- (2) Stinson, S. *Chemical and Engineering News* September 27, 1993.
- (3) Armstrong, D. W.; DeMond, W. *J. Chromatogr. Sci.* **1984**, 22, 411
- (4) Armstrong, D. W.; Stalcup, A.M.; Hilton, M. L.; Duncan, J.D.; Faulkner Jr., J. R.; Chang, S.-C. *Anal. Chem.* **1990**, 62, 1612.
- (5) Armstrong, D. W.; Hilton, M.; Coffin, L. *LC-GC* **1992**, 9, 646.
- (6) Armstrong, D.W.; Chang, C.-D.; Li, W. Y. *Journal of Agricultural and Food Chemistry* **1990**, 38, 1674
- (7) Higgins, H. M.; Harrison, W. H.; Wild, G. M.; Bungay, H.R.; McCormick, M. H. *Antibiotics Ann.* **1958-59**, 906.
- (8) Sensi, P. et al., *Antibiotics Ann.* **1959-60**, 262.
- (9) Rinehart, Jr., K. L.; Shield, L S. *Fortschritte Chem. Naturstoffe* **1976**, 33, 231.
- (10) Pagano, J.F.; Weinstein, M.J.; Stout, H.A.; Donovick, R, *Antibiotics Ann.* **1955-56**, 554.
- (11) Vandeputte, J.; Dutcher, J. D. *Antibiotics Ann.* **1955-56**, 560.



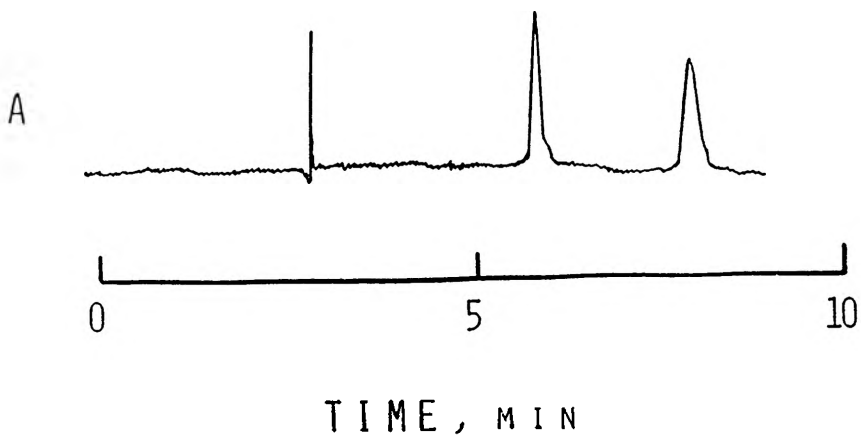
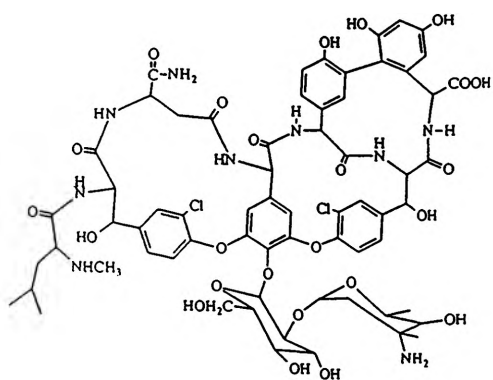
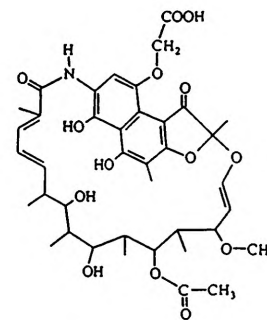


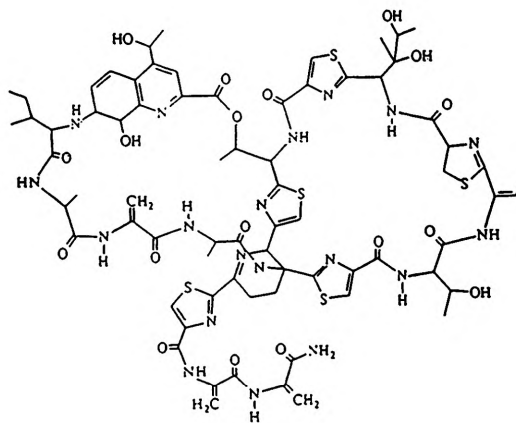
Figure 1



VANCOMYCIN



RIFAMYCIN B



THIOSTREPTON

Figure 2

**Table 1. Chromatographic Data for the Reversed-Phase Resolution of Racemic Compounds on Macrocyclic Antibiotic Bonded Stationary Phases**

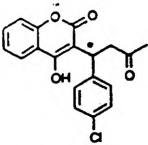
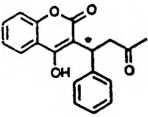
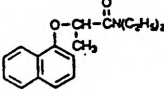

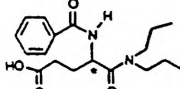
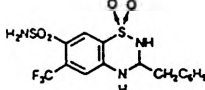
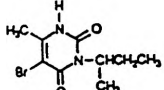

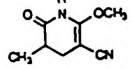
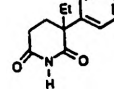
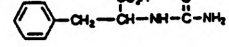
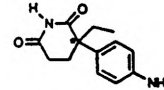
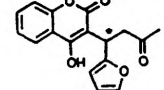
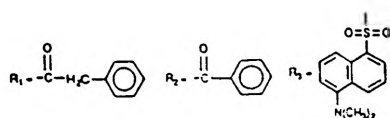
	compounds <sup>a,c</sup>	$k_1'$ <sup>b</sup>	$\alpha$	mobile phase <sup>c</sup>	pH	column <sup>d</sup>
(1)	coumachlor 	3.27	2.0	10:90	4.1	Van
(2)	warfarin 	1.98 2.27	1.70 1.44	10:90 10:90	7.0 4.1	Van Van
(3)	devrinol 	1.40	1.62	10:90	4.1	Van
(4)	5-methyl-5-phenylhydantoin 	0.38 0.24	1.41 1.36	10:90 10:90	7.0 4.1	Van Van
(5)	proglumide 	1.17 1.18	1.40 1.75	10:90 10:90	7.0 4.1	Van Van
(6)	$\alpha$ -(1-aminoethyl)-4-hydroxybenzyl alcohol $\text{HOC}_6\text{H}_4\text{CH}(\text{CH}(\text{NH}_2)\text{CH}_3)\text{OH}$	0.39	1.30	10:90	7.0	Van
(7)	bendroliumethiazide 	1.58	1.25	10:90	7.0	Van
(8)	bromacil 	0.67	1.21	10:90	7.0	Van
(9)	idazoxan 	0.38	1.21	10:90	7.0	Rif
(10)	3-methyl-5-cyano-6-methoxy-3,4-dihydro-2-pyridone 	0.32	1.21	10:90	7.0	Van
(11)	pyridoglutethimide 	0.84	1.20	10:90	7.0	Van
(12)	<i>N</i> -carbamyl-D,L-phenylalanine 	0.31	1.20	10:90	4.1	Van
(13)	aminoglutethimide 	0.79	1.15	10:90	7.0	Van
(14)	<i>N</i> -benzoylalanine methyl ester $\text{CH}_3\text{-CH}(\text{NH}_2)\text{-CO}_2\text{CH}_3$	0.47	1.15	10:90	4.1	Van
(15)	coumafuryl 	0.68	1.15	10:90	7.0	Van

Table 1 (Continued)

	compounds <sup>a,c</sup>	$k_1'$ <sup>b</sup>	$\alpha$	mobile phase <sup>c</sup>	pH	column <sup>d</sup>
(16)	dansyl- $\alpha$ -amino-n-butyric acid 	3.29	1.15	10:90	4.1	Van
(17)	dansylaspartic acid 	3.00	1.15	10:90	4.1	Van
(18)	<i>N</i> -(3,5-dinitrobenzoyl)phenylglycine 	1.55	1.15	10:90	4.1	Van
(19)	thioridazine 	22.0	1.15	10:90	7.0	Thio
(20)	dansylnorleucine 	6.17	1.14	10:90	4.1	Van
(21)	5-(4-hydroxyphenyl)-5-phenylhydantoin 	0.78	1.14	10:90	7.0	Van
(22)	dansylserine 	2.09	1.12	10:90	4.1	Van
(23)	indapamide 	1.35	1.12	10:90	7.0	Van
(24)	benzoin methyl ester $C_6H_5CH(OCH_3)COC_6H_5$	0.80	1.11	10:90	7.0	Van
(25)	<i>N</i> -benzoylleucine 	1.90	1.10	10:90	4.1	Van
(26)	<i>N</i> -(3,5-dinitrobenzoyl)leucine 	1.44	1.10	10:90	4.1	Van
(27)	methsuximide 	0.39	1.10	10:90	7.0	Van
(28)	dansylvaline 	3.97	1.09	10:90	4.1	Van
(29)	indoprofen 	1.55 2.85	1.09 1.06	10:90 10:90	4.1 7.0	Van Van
(30)	<i>N</i> -benzoylphenylalanine 	2.10	1.08	10:90	4.1	Van
(31)	<i>N</i> -benzoylvaline 	0.68	1.08	10:90	4.1	Van
(32)	1,1-binaphthyl-2,2'-diyl hydrogen phosphate 	3.75 1.63	1.07 1.05	10:90 10:90	4.1 7.0	Van Van
(33)	<i>N</i> -t-Boc- <i>p</i> -chlorophenylalanine 	2.06	1.07	10:90	4.1	Van

<sup>a</sup> The compounds in this table are listed in order of their  $\alpha$ -values from highest to lowest. <sup>b</sup> This is the  $k'$  of the first eluted enantiomer. <sup>c</sup> The mobile-phase compositions indicated are the volume ratios of acetonitrile to 1% triethylammonium acetate buffer. <sup>d</sup> The abbreviations for the columns are as follows: Van, vancomycin bonded stationary phase; Thio, thiostrepton bonded stationary phase; Rif, rifamycin B bonded stationary phase. <sup>e</sup>



**Table 2. Effect of pH on the Chromatographic Retention and Separation of Coumachlor and Devrinol on a 25 cm × 0.44 cm (i.d.) Vancomycin CSP<sup>a</sup>**

pH	coumachlor			devrinol		
	<i>k'</i>	$\alpha$	Rs	<i>k'</i>	$\alpha$	Rs
7.6	1.00	1.69	1.6	1.15	1.80	3.2
6.2	1.21	1.64	2.0	1.16	1.77	3.3
5.5	2.00	1.60	2.7	1.36	1.76	3.5
4.5	3.00	1.64	4.1	1.46	1.70	3.6
3.6	1.65	2.42	3.9	1.19	1.41	3.7

<sup>a</sup> The mobile phase was 10:90, acetonitrile–1% triethylammonium acetate buffer (by volume). The temperature was 22 °C.

**Table 3. Effect of Temperature on the Reversed-Phase Retention and Resolution of Enantiomers of Proglumide, 5-Methyl-5-phenylhydantoin, and *N*-Carbamyl-D,L-phenylalanine<sup>a</sup>**

temp (°C)	<i>k'</i>	$\alpha$	Rs
Proglumide			
0	1.33	2.27	3.6
5	1.33	2.11	3.3
15	1.31	1.87	2.4
22	1.18	1.75	2.1
35	0.93	1.57	1.8
45	0.76	1.44	1.6
5-Methyl-5-phenylhydantoin			
0	0.35	1.38	1.5
5	0.27	1.36	1.0
22	0.24	1.34	1.0
35	0.24	1.30	0.9
45	0.19	1.32	0.7
<i>N</i> -Carbamyl-D,L-phenylalanine			
0	0.51	1.39	1.5
5	0.39	1.34	1.3
15	0.38	1.23	1.0
22	0.31	1.20	0.8
35	0.27	1.11	0.7
45	0.22	1.00	0

<sup>a</sup> The column was a 25 cm × 0.44 cm (i.d.) vancomycin CSP. The mobile phase was 10:90 acetonitrile–1% triethylammonium acetate buffer (pH 4.1). The flow rate was 1.0 mL/min.

**Table 4. Effect of Flow Rate on the Normal-Phase Enantiomeric Separation of 3a,4,5,6-Tetrahydrosuccinimido[3,4-*b*]acenaphthen-10-one<sup>a</sup>**

flow rate (mL/min)	$\alpha$	Rs
0.50	1.31	1.28
0.75	1.31	1.19
1.00	1.27	1.14
1.50	1.30	1.13
2.00	1.29	1.11

<sup>a</sup> The column was a 25 cm × 0.44 cm (i.d.) vancomycin CSP. The mobile phase was 50:50 2-propranol–hexane (by volume).

**Table 5. Chromatographic Data for the Normal-Phase Resolution of Racemic Compounds on Macrocyclic Antibiotic Bonded Stationary Phases**

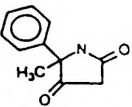
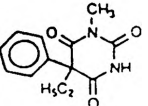
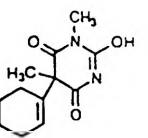
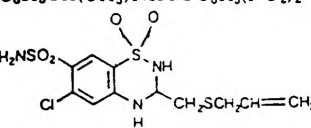
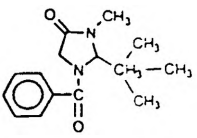
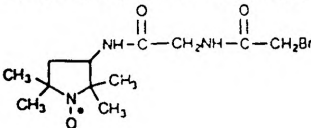
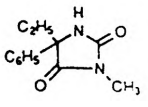
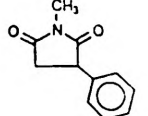
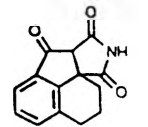
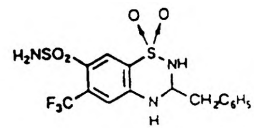
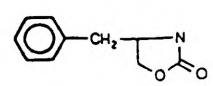
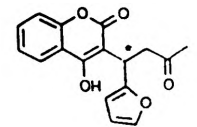
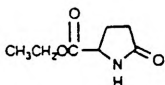
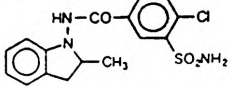
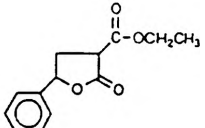
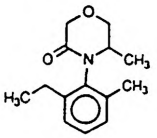
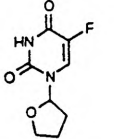
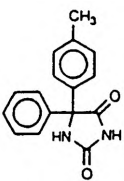
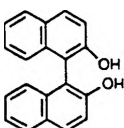
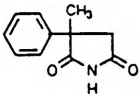
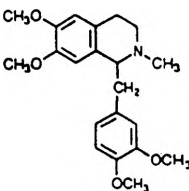
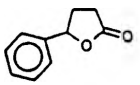
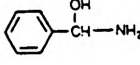
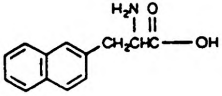
compounds <sup>a</sup>		$k_1^{r,b}$	$\alpha$	mobile phase <sup>c</sup>	column <sup>d</sup>	
(1)	5-methyl-5-phenylhydantoin		2.50	1.67	50:50	Van
(2)	mephobarbital		0.58	1.62	50:50	Van
(3)	hexobarbital		0.75	1.61	50:50	Van
(4)	<i>N</i> -(3,5-dinitrobenzoyl)- $\alpha$ -methylbenzylamine	$C_6H_5CH(CH_3)NHCOC_6H_3(NO_2)_2$	0.82	1.36	50:50	Van
(5)	althiazide		5.30	1.35	65:35	Thio
(6)	1-benzoyl-2- <i>tert</i> -butyl-3-methyl-4-imideazolidinone		2.67	1.35	50:50	Van
(7)	<i>N</i> -(3,5-dinitrobenzoyl)- $\alpha$ -methylbenzylamine	$C_6H_5CH(CH_3)NHCOC_6H_3(NO_2)_2$	2.00	1.33	10:90	Van
(8)	3-[2-(2-bromoacetamido)acetamido]-PROXYL		2.63	1.30	50:50	Van
(9)	mephentoin		1.64	1.30	10:90	Van
(10)	phensuximide		3.84	1.26	10:90	Van
(11)	3a,4,5,6-tetrahydrosuccinimido[3,4- <i>b</i> ]acenaphthen-10-one		2.00	1.25	50:50	Van
(12)	bendroflumethiazide		2.95	1.24	50:50	Van
(13)	4-benzyl-2-oxazolidinone		4.2 1.30	1.10 1.21	65:35 50:50	Thio Van
(14)	coumafuryl		0.30	1.20	50:50:10 <sup>e</sup>	Rif

Table 5 (Continued)

compounds <sup>a</sup>		$k_1'$ <sup>b</sup>	$\alpha$	mobile phase <sup>c</sup>	column <sup>d</sup>	
(15)	ethyl-2-pyrrolidone-5-carboxylate		3.05	1.15	50:50	Van
(16)	<i>N,N'</i> -bis( $\alpha$ -methylbenzyl)sulfamide	$[\text{C}_6\text{H}_5\text{CH}(\text{CH}_3)\text{NH}]_2\text{SO}_2$	0.66	1.13	10:90	Van
(17)	indapamide		2.5	1.13	50:50	Van
(18)	$\alpha$ -carbethoxy- $\gamma$ -phenyl- $\gamma$ -butyrolactone		0.58	1.12	50:50	Van
(19)	CGA-40919		0.72	1.11	10:90	Van
(20)	ftorafur		4.68	1.11	50:50	Van
(21)	5-(4-Methylphenyl)-5-phenylhydantoin		6.00	1.10	90:10	Van
(22)	1,1'-bi-2-naphthol		0.80	1.09	10:90	Van
(23)	$\alpha$ -methyl- $\alpha$ -phenylsuccinimide		3.02	1.09	10:90	Van
(24)	laudanosine		2.37	1.13	10:90	Rif
(25)	$\gamma$ -phenyl- $\gamma$ -butyrolactone		4.43 4.05	1.08 1.08	40:60 10:90	Van Van
(26)	mandelamide		2.50	1.04	50:50	Van
(27)	3-(2-naphthyl)alanine		1.28	1.04	50:50	Van

<sup>a</sup> The compounds in this table are listed in order of their  $\alpha$ -values from highest to lowest (except for the last four compounds which use a different mobile phase). <sup>b</sup> This is the  $k'$  of the first eluted enantiomer. <sup>c</sup> The mobile-phase composition represents the volume ratio of 2-propanol to hexane. <sup>d</sup> The abbreviations for the columns are as follows: Van, vancomycin bonded stationary phase; Thio, thiostrongon bonded stationary phase; and Rif, rifamycin B bonded stationary phase. <sup>e</sup> This mobile phase composition represents the volume ratios of 2-propanol-hexane-acetonitrile.