01 Jan 1992

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Recommended Citation

A. Berthod et al., "Empirical Procedure That Uses Molecular Structure To Predict Enantioselectivity Of Chiral Stationary Phases," *Analytical Chemistry*, vol. 64, no. 4, pp. 395 - 404, American Chemical Society, Jan 1992.
The definitive version is available at https://doi.org/10.1021/ac00028a014

Empirical Procedure That Uses Molecular Structure To Predict Enantioselectivity of Chiral Stationary Phases

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A total of 121 racemic compounds were separated in the normal-phase mode on a (*S*)-(1-naphthylethyl)carbamoylated β-cyclodextrin (*S*-NEC–β-CD) bonded phase and 74 on the *R* equivalent (*R*-NEC) chiral stationary phase (CSP). All compounds are of the type that have four substituents on a stereogenic center, rather than an "axis of chirality". It is shown that the binary solvent pair used as the mobile phase has a significant influence on chiral recognition. However, the proportions of the components of a specific pair have little effect. From the results, the individual contributions to chiral recognition by these CSPs were estimated for 81 different substituents of the stereogenic center. Varying the arrangement of these 81 substituents could produce over 1.6 million compounds. Hydrogen was chosen as the reference substituent and was assigned a 0 cal/mol free energy. The chiral recognition increased when sp^2-hybridized carbons were connected to the stereogenic center. Conversely, sp^3-hybridized carbons decreased the enantioselectivity. Amido groups increased the chiral recognition, especially when associated with π-acid (3,5-dinitrobenzoyl) or π-basic (naphthyl) groups. This approach does not allow one to know which enantiomer elutes first. However, the "substituent energy" list for chiral compounds can be used to obtain an estimated value for the enantioselectivity of a compound by adding the energy contributions of the four substituents connected to the stereogenic center. In this way one can predict a priori whether or not a compound will separate on a CSP and estimate its separation factor (α). Theoretically, this approach can be used for most CSPs, provided a sufficient data base is generated on them.

INTRODUCTION

Each optical isomer of a racemic drug may have different therapeutic as well as toxicologic effects, in vivo.[1] The U.S. Food and Drug administration may soon require biotoxicity and bioefficacy studies on not only the racemate but also on each enantiomer before granting approval for marketing a new active chiral molecule.[2] High-performance liquid chromatography (HPLC) using commercially available chiral stationary phases (CSP) is now a recognized technique for stereochemical analysis.[3,4]

Chiral recognition, in chromatographic terms, means preferential interaction of one enantiomer with the CSP. To date, the trend is to design new CSPs capable of resolving different classes of compounds.[5] The problem is that the number and types of available CSPs has grown tremendously in the past few years. The user may be confused by a choice of more than 50 CSPs.[6] The enantioselectivity of many CSPs overlap, making some redundant. On the other hand there is a significant number of racemates that cannot be resolved by LC despite the large number of available CSPs.

We recently designed a naphthylethyl isocyanate derivatized β-cyclodextrin (β-CD) stationary phase.[7] The naphthylethyl isocyanate derivative is chiral and can be used in normal-phase or reversed-phase conditions to resolve different types of enantiomers.[8,9] Two CSPs were synthesized: the (*R*)-(−)-1-(1-naphthylethyl)carbamoylated β-CD (*R*-NEC–β-CD) and the corresponding *S*(+) phase (*S*-NEC–β-CD). The goal of this work is to try to answer the question a priori: Does a solute have a chance to be resolved by a NEC–β-CD CSP? By separating a significant number of enantiomers, we have obtained a data base that was used to extract the enantioselectivity contribution of each substituent from the stereogenic center. The two NEC–β-CD stationary phases (*R* and *S*) were studied in the normal-phase mode with apolar hexane–isopropyl alcohol mobile phases and more polar acetonitrile–ethanol mobile phases.

EXPERIMENTAL SECTION

Chemicals. The structures of the solutes are given in the tables. The derivatization procedure was extensively described elsewhere.[7,8] The derivatizing agents were obtained from various sources, Sigma (St. Louis, MO), Aldrich (Milwaukee, WI), Alfa (Ward Hill, MA), Fisher (St. Louis, MO), and Kodak (Rochester, NY). They consisted of carboxylic acid chlorides and thionyl chloride. Various amines and alcohols were also used.[8] The dansyl amino acids and dinitropyridinyl amino acid derivatives were obtained from Sigma.

Columns. The preparation of the β-CD bonded stationary phase and the (naphthylethyl)carbamoylation have been described.[7,8] The cyclodextrin silica coverage was approximately 0.2 μmol/m^2. The *R*-NEC derivatization added an average 6.7 (*R*)-(naphthylethyl)carbamoyl units/CD ring leading to a *R*-NEC coverage of 1.34 μmol/m^2. The *S*-NEC derivatization added only about 3.5 *S*-NEC units/CD ring which was a 0.7 μmol/m^2 coverage.[8] The *R*-NEC and *S*-NEC stationary phases (5 μm) were slurry packed in 25 cm, 4.6 mm i.d., columns. These columns can be obtained from Advanced Separation Technologies Inc. (Whippany, NJ).

Chromatographic Apparatus. A Shimadzu pump, Model LC-6A, a Shimadzu SPD-6A UV detector, and a Waters R401 differential refractometer detector were used with a Shimadzu CR2AX integrator. All experiments were done in the normal-phase mode with hexane–isopropyl alcohol or ethanol–acetonitrile mobile phases.

THEORY

Enantiomers are nonsuperimposible mirror images of one another. Although they differ in configuration in an isotropic environment, their properties that govern classical chromatographic retention, i.e., vapor pressure, solubility, density or ionization constants, are identical. In the presence of a chiral stationary phase (CSP) some interactions must occur differently between the two enantiomers and the stationary phase in order to obtain stereoselective resolution.[10] The three-point interaction model (Dalgliesh[11]) requires three points of attachment between the solute and some part of the stationary phase. Only one of the two enantiomers can better fulfill the three-point attachment, and it becomes more retained than the other.[12] This model is oversimplified since the interactions are not all necessarily attractive: steric hindrance, a repulsive

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0003-2700/92/0364-0395$03.00/0

Table I. Substituent Free Energy Contributions (cal/mol) for Enantiomeric Recognition on (*R*)- and (*S*)-(Naphthylethyl)carbamate-Substituted Cyclodextrin Chiral Stationary Phases[a]

	substituents		*S*-NEC-CSP		*R*-NEC-CSP	
no.	empirical formula	structure of R	Hex-IPA	ACN-EtOH	Hex-IPA	ACN-EtOH
1	Br	-Br	-27		-27	
2	Cl	-Cl	-40			
3	**H**	**reference substituent**	**0**	**0**	**0**	**0**
4	CHO_2	-COOH		7		0
5	CH_2NO	-NH-CHO		0		-7
6	CH_3	methyl	-74	-34	-81	-54
7	CH_3N_2O	$-NH-CO-NH_2$		27*,[b]		20*
8	CH_3O	$-CH_2-OH$	-122*		-128*	
9	CH_3O	$-O-CH_3$	-94*		-94*	
10	$CH_2H_3O_2$	$-COO-CH_3$	14		27	
11	C_2H_4NO	$-NH-CO-CH_3$	14	7	14	-7
12	C_2H_5	ethyl	-81		-81	
13	C_2H_5O	$-CH_2-O-CH_3$			40*	
14	C_2H_5O	$-CHOH-CH_3$	-115*		-142*	
15	C_2H_5S	$-CH_2-S-CH_3$	-27	-27	-14	-27
16	C_3H_5	cyclopropyl	-108*			
17	$C_3H_5O_2$	$-COO-CH_2-CH_3$	54		94	
18	C_3H_7	*n*-propyl	-81	-34	-88	-27
19	C_3H_7	isopropyl	-20*	-27	14*	-20
20	C_4H_9	*n*-butyl	-40	-34	-47	-27
21	C_4H_9	$-CH_2-CH-(CH_3)_2$	-47	-47	-68	-27
22	C_4H_9	$-CH(CH_3)-CH_2-CH_3$		-14*		0*
23	C_4H_9	*tert*-butyl	-81*		0*	
24	$C_5H_3N_4O_2S$	-NH-DNBPy		47		47
25	$C_5H_9O_2$	$-COO-(CH_2)_3-CH_3$	142*		135*	
26	$C_5H_9O_2$	solketal	-20@*,[c]			
27	$C_5H_{10}NO$	-CO-NH-*t*-Bu	68			
28	$C_5H_{10}NO_2$	--NH-CO-O-*t*-Bu	0*	-27		
29	C_5H_{11}	*n*-pentyl	-81		-54	
30	C_6H_5	phenyl (-Φ)	81	81*	74	61*
31	C_6H_5O	-Φ-OH	68*		68*	
32	C_6H_5O	-O-Φ	54*			
34	C_6H_{11}	-cyclohexyl	81		94	
35	C_6H_{13}	-*n*-hexyl	-81		-68	
36	$C_7H_3N_2O_6$	-O-CO-DNB	142		142	
37	$C_7H_4NO_4$	$-COO-Φ-NO_2$	14			
38	$C_7H_4N_3O_5$	-NH-CO-DNB	230	68	196	54
39	$C_7H_4N_3O_5$	-CO-NH-DNB	263			
40	$C_7H_4N_3O_6$	-O-CO-NH-DNB	182		162	
41	C_7H_6NO	-NH-CO-Φ	27	14	27	34
42	C_7H_6NO	-CO-NH-Φ	148			
43	$C_7H_6NO_2$	-NH-CO-O-Φ	27*	-27		
44	C_7H_6Cl	$-CH_2-Φ-Cl$	14		7	
45	C_7H_7	$-CH_2-Φ$	0	0	-81	0
46	C_7H_7O	$-CH_2-Φ-OH$	94*	27*	115*	-34*
47	C_7H_7O	$-CH_2-Φ-OH$(meta)	-27*		47*	
48	$C_7H_{12}NO$	-CH-NH-cyclohexyl	155			
49	C_7H_{15}	$-(CH_2)_6-CH_3$	-54*		-54*	
50	C_8H_4NO	$-CH-N(CH_3)$-cyclohexyl	148			
51	$C_8H_5N_2O_6$	$-CH_2-O-CO-DNB$	94		68	
52	$C_8H_6N_3O_5$	$-CH_2-NH-CO-DNB$	20*		20*	
53	$C_8H_6N_3O_5$	$-N(CH_3)-CO-DNB$	243		243	
54	$C_8H_6N_3O_6$	$-CH_2-O-CO-NH-DNB$	68*		47*	
55	C_8H_7	-1-benzocyclobutene	-122@*			
56	C_8H_8NO	$-CH-NH-CH_2-Φ$	122			
57	C_8H_8NO	$-CH-N(CH_3)-Φ$	94			
58	C_8H_9	$-CH_2-CH_2-Φ$		-7*		120*
59	C_8H_9O	$-CH_2-Φ-O-CH_3$		0*		-54*
60	C_9H_7N	$-CH_2-$(3-indole)	27	14*	-202	-20
61	C_9H_7NO	$-CH_2-O-$(7-indole)	14*			
62	C_9H_9	-(1-indanyl)			0@*	
63	$C_9H_{10}NO$	$-CH-NH-CH_2-CH_2-Φ$	142			
64	$C_9H_{10}NO$	$-CO-N(CH_3)-CH_2-Φ$	94			
65	$C_9H_{17}O_2$	$-CO-O-(CH_2)_7-CH_3$	230*		189*	
66	$C_{10}H_7$	-(1-naphthyl)	108		108	
67	$C_{10}H_{11}$	-(1-tetrahydronaphthyl)	-20@*		54	
68	$C_{10}H_{11}$	-(2-tetrahydronaphthyl)	-108@*			
69	$C_{10}H_{11}O$	$-CH_2-O-Φ-CH_2-CH=CH_2$	20*			
70	$C_{10}H_{11}O_2$	$-CH_2-O-Φ-O-CH_2-CH=CH_2$	14*			
71	$C_{10}H_{13}O_2$	$-CH_2-O-Φ-CH_2-CH_2OCH_3$	34*			
72	$C_{11}H_7O_2$	-CH-O-(1-naphthyl)	0			
73	$C_{11}H_8NO$	-NH-CO-(1-naphthyl)	54	34	34	20
74	$C_{11}H_8NO$	-CH-NH-(1-naphthyl)	162			
75	$C_{11}H_9$	$-CH_2-$(1-naphthyl)		7*		-34*

Table I (Continued)

no.	substituents: empirical formula	substituents: structure of R	S-NEC-CSP: Hex-IPA	S-NEC-CSP: ACN-EtOH	R-NEC-CSP: Hex-IPA	R-NEC-CSP: ACN-EtOH
76	$C_{11}H_9$	-CH_2-(2-naphthyl)		-7*		14*
77	$C_{11}H_9O$	-CH_2-O-(1-napthyl)	20*		20*	
78	$C_{12}H_9$	-(1-acenaphthenyl)	-34@*			
79	$C_{12}H_{13}N_2O_2S$	-NH-DNS	61		61	
80	$C_{12}H_{14}N_3O_5$	-CH_2-N(*t*-Bu)-CO-DNB	101		74	
81	$C_{13}H_9O$	-CH(Φ)-O-DNB	88		88	
		Synergistic and Antagonistic Substituent Interactions				
two identical substituents		no asymmetry		cancel all value		
38 and **30**		NHCO-DNB and phenyl, add	175		148	
39 and **30**		CONH-DNB and phenyl, add	175		27	
38 and **66**		NHCO-DNB and naphthyl, add	486*	432*		
38 and **34**		NHCO-DNB and cyclohexyl	-142*		0*	
two different DNB derivatives decrease the energy by			-230		-209	

[a] Relative free energy values were determined for two different mobile-phase solvent systems, hexane and isopropyl alcohol (Hex-IPA) and acetonitrile and ethanol (AcN-EtOH). In each case hydrogen is taken as the reference substituent and is arbitrarily assigned a free energy value of zero. The energy values listed in the table depend on the reference energy chosen for the hydrogen atom, no. 3. Abbreviations: DNB = $C_6H_3N_2O_4$ = 1-(3,5-dinitrobenzene); DNBPy = $C_5H_2N_3O_4$ = 2-(3,5-dinitropyridine); DNS = $C_{12}H_{12}NO_2S$ = dansyl derivative. All benzene rings (Φ) are para substituted, unless otherwise indicated. [b] Values marked with an asterisk refer to a nonredundant substituent, i.e. a substituent appearing in less than two compounds. [c] @ means the asymmetric carbon is part of the ring structure.

interaction, is often involved in chiral recognition mechanisms.

The capacity factor, k', of a solute is the chromatographic parameter directly related to all solute–stationary phase interactions. Expressing each solute–stationary phase interaction with its corresponding molecular free energy of adsorption, ΔG_i, yields

$$\sum \Delta G_i = -RT \log k'/\phi \quad (1)$$

in which ϕ is the volume ratio of stationary phase over mobile phase. The enantioselectivity, α, is expressed by the ratio of the capacity factors of the two enantiomers:

$$\alpha = k'_2/k'_1 \quad \text{with} \quad k'_2 > k'_1 \quad (2)$$

Using eq 1

$$\alpha = \exp[\textstyle\sum_{i1}(\Delta G_i/RT) - \sum_{i2}(\Delta G_i/RT)] \quad (3)$$

In the case of enantiomers, all solute–stationary phase interactions except the chiral interactions, $\sum \Delta G_c$, are identical and cancel.

$$\alpha = \exp\textstyle\sum[(\Delta G_{c1} - \Delta G_{c2})/RT] = \exp[(1/RT)\Delta(\Delta G_c)] \quad (4)$$

In eq 4, $\Delta(\Delta G_c)$ represents the difference in molecular free energy of the chiral interaction for the two enantiomers.[5] The $\Delta(\Delta G_c)$ energy difference is solely responsible for enantioselectivity (eq 4). $\Delta(\Delta G_c)$ is a measure of the degree of chiral recognition between two enantiomers and a given CSP. Without additional experiments to determine which enantiomer is selectively retained, only the absolute value of $\Delta(\Delta G_c)$ can be determined. The $|\Delta(\Delta G_c)|$ energy difference does not give any indication as to which enantiomer is most retained, i.e., the elution order.

Computational studies of the interactions of chiral molecules which chiral stationary phases were done by several authors.[13-17] For example, the $\Delta(\Delta G_c)$ energy difference of weakly bound diastereoisomeric complexes as transient species in chiral chromatography was fully computed for the retention of trifluoro-1-(9-anthryl)ethanol on a ((3,5-dinitrobenzoyl)-phenyl)glycine CSP.[16] These studies were done with CSPs and solutes able to form π–π interactions and hydrogen bonds. Although computational studies use molecular mechanics and quantum chemistry, the models used remain semiempirical and they sometimes lead to differing conclusions.[13,18]

In this work, we propose a different approach based only on experimental results. To estimate the $\Delta(\Delta G_c)$ of a given compound, we assumed that the free energy difference, $\Delta(\Delta G_c)$, is the sum of four terms, each term being related to one of the four different substituents attached to the stereogenic center:

$$\Delta(\Delta G_c) = (\Delta Gc_{11} - \Delta G_{c12}) + (\Delta G_{c21} - \Delta G_{c22}) + (\Delta G_{c31} - \Delta G_{c32}) + (\Delta G_{c41} - \Delta G_{c42}) \quad (5)$$

Although this is a simple model, it is not based on unreasonable assumptions. Somewhat analogous statistical mechanical approaches have been used in which a multitude of different additive interactions occur between a solute and the chiral stationary phase.[19]

The second assumption is that the chiral interaction contribution of a given substituent is independent of the others. Obviously, this assumption should be qualified. If two substituents are identical, the asymmetry disappears, which implies $\Delta(\Delta G_c) = 0$. For some compounds we found a synergistic effect on the chiral recognition energy due to two particular substituents. Such effects are noted in the tables and are discussed in the following section.

RESULTS AND DISCUSSION

Assessment of the Substituent Free Energies. Table I lists the substituents considered in this study. The empirical formulas are in alphabetical order according to the letters of the atomic symbols. Also, moieties with fewer atoms (within a group) are listed before those with more atoms. Table II lists the chromatographic results that were used to obtain the free energy contributions listed in Table I. A total of 126 chiral compounds were analyzed on the two CSPs. This was 126 × 4 = 504 contributions on the 126 stereogenic centers. There were only 81 different substituents because of redundancy. For example, hydrogen was one of the four substituents in 124 of the 126 compounds. For this reason, the hydrogen atom substituent was chosen as the reference substituent with the arbitrary value of 0 cal/mol for chiral free energy contribution. If a substituent has a positive energy value in Table I, it means that it increases somewhat the chiral recognition by the NEC-β-CD phase compared to hydrogen. A negative energy

Table II. Separation Data for a Variety of Derivatized Enantiomeric Solutes on *R*-NEC and *S*-NEC-β-CD Chiral Stationary Phases

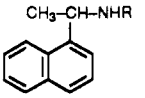
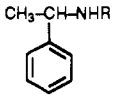
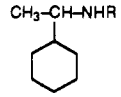
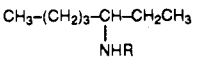
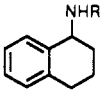
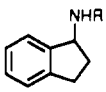
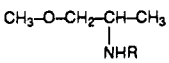
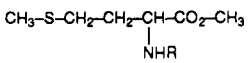
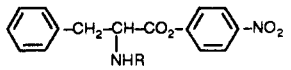
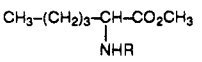
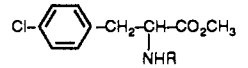
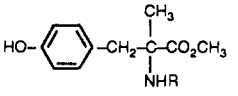
compd	R substituent	% v/v modifer	CSP	k'^a	R_s	α_{exp}	α_{calc}	% error
Mobile Phase, Hexane–Isopropyl Alcohol (IPA)								
Amines								
(1-(1-naphthyl)ethyl)amine	38*,[b]	30% IPA	*S*-NEC	5.82*R*	14	3.59	3.59	0
	38*	30% IPA	*R*-NEC	3.33*S*	13.4	3.05	3.05	0
	73	30% IPA	*S*-NEC	1.75*R*	1.4	1.14	1.14	0
	73	30% IPA	*R*-NEC	1.67*S*	1.4	1.14	1.14	0
	41	30% IPA	*S*-NEC	1.28*R*	1.3	1.11	1.11	0
	41	30% IPA	*R*-NEC	1.17*S*	1.1	1.09	1.10	0.9
	11	30% IPA	*S*-NEC	1.00*R*	0.5	1.04	1.08	3.8
	11	10% IPA	*S*-NEC	7.82*R*	0.7	1.04	1.08	3.8
	11	10% IPA	*R*-NEC	6.35*S*	0.8	1.06	1.07	0.9
(methylbenzyl)amine	38	30% IPA	*S*-NEC	4.56*R*	6.4	2.03	2.02	0.5
	38	30% IPA	*R*-NEC	2.85*R*	5.8	1.77	1.77	0
	73	10% IPA	*S*-NEC	6.90*R*	1.9	1.13	1.11	1.8
	73	10% IPA	*R*-NEC	7.39*S*	0.6	1.02	1.05	2.9
	41	10% IPA	*S*-NEC	435*R*	1.0	1.06	1.06	0
	41	10% IPA	*R*-NEC	5.08*S*	0	1.00	1.03	3.0
	11	10% IPA	*S*-NEC	3.83*R*	0.6	1.03	1.03	0
	11	10% IPA	*R*-NEC	4.50*S*	0.6	1.03	1.01	1.9
(1-(cyclohexyl)ethyl)amine	38	10% IPA	*S*-NEC	8.07*R*	2.7	1.18	1.17	0.9
	38	10% IPA	*R*-NEC	5.72*R*	4.5	1.43	1.43	0
	73	10% IPA	*S*-NEC	3.11*R*	1.2	1.09	1.11	1.8
	73	10% IPA	*R*-NEC	3.59*R*	1.0	1.08	1.08	0
	41	10% IPA	*S*-NEC	2.20*R*	1.0	1.08	1.06	1.8
	41	10% IPA	*R*-NEC	2.55*R*	1.0	1.08	1.07	0.9
	11	10% IPA	*S*-NEC	2.58*R*	0.7	1.06	1.04	1.9
	11	10% IPA	*R*-NEC	2.68*R*	0.7	1.06	1.05	0.9
3-aminoheptane	38	10% IPA	*S*-NEC	8.32	2.6	1.18	1.2	1.7
	38	10% IPA	*R*-NEC	6.14	2.0	1.12	1.12	0
	73	10% IPA	*S*-NEC	1.56	0	1.00	0.89∼[c]	0
	73	2% IPA	*S*-NEC	10.21	0	1.00	0.89∼	0
	41	2% IPA	*S*-NEC	6.47	0	1.00	0.85∼	0
(1,2,3,4-tetrahydro-1-naphthyl)amine*	38	30% IPA	*R*-NEC	1.20	4.2	1.83	1.80	1.6
								
1-aminoindan*	38	30% IPA	*R*-NEC	1.53	4.7	1.85	1.84	0.6
								
2-amino-1-methoxypropane*	38	20% IPA	*R*-NEC	4.76	3.5	1.31	1.3	0.8
								
Amino Esters								
DL-methionine methyl ester	38	40% IPA	*S*-NEC	5.03D	6.3	1.94	1.45	25
	38	40% IPA	*R*-NEC	3.39L	4.3	1.55	1.43	7.7
	73	30% IPA	*S*-NEC	1.75D	0.6	1.06	1.07	0.9
	73	10% IPA	*S*-NEC	6.33D	1.0	1.06	1.07	0.9
	73	30% IPA	*R*-NEC	6.67D	0.6	1.03	1.08	4.8
	41	10% IPA	*S*-NEC	5.36D	0.7	1.04	1.02	1.9
	41	10% IPA	*R*-NEC	5.49L	0.6	1.03	1.07	3.8
	11	10% IPA	*S*-NEC	5.75	0	1.00	1.00	0
	11	10% IPA	*R*-NEC	6.30	0	1.00	1.05	5
DL-phenylalanine *p*-nitrophenyl ester	43	10% IPA	*S*-NEC	4.42D	0.7	1.05	1.07	1.8
	28	10% IPA	*S*-NEC	1.68	0	1.00	1.02	2
DL-norleucine methyl ester	38	30% IPA	*R*-NEC	2.40	4.6	1.42	1.33	6.3
								
p-chlorophenylalanine methyl ester	38	40% IPA	*R*-NEC	4.00	1.6	1.15	1.47	27.8
								
α-methyl-*p*-tyrosine methyl ester	38	30% IPA	*R*-NEC	3.72	2.6	1.70	1.55	8.8
								

Table II (Continued)

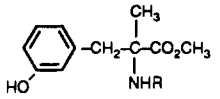
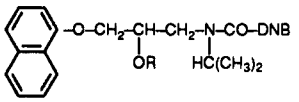
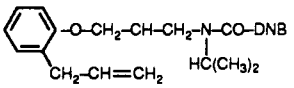
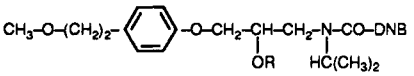
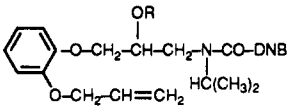
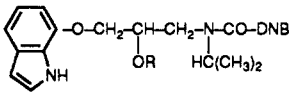
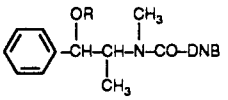
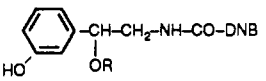
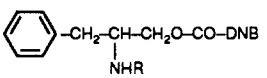
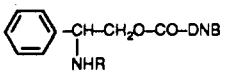
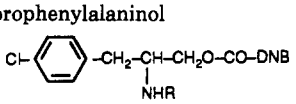
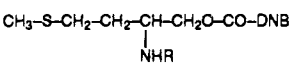
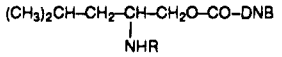
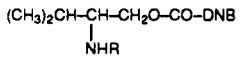
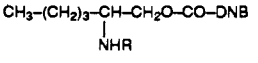
compd		R substituent	% v/v modifer	CSP	k'^a	R_s	α_{exp}	α_{calc}	% error
α-methyl-*m*-tyrosine methyl ester 		**38**	30% IPA	*R*-NEC	5.44	2.0	1.30	1.38	6.1
		Amino Alcohols							
propranolol* 		**36**	10% IPA	*S*-NEC	11.36*R*	0.8	1.06	1.05	0
		36	10% IPA	*R*-NEC	12.49*S*	0.7	1.05	1.05	0
alprenolol* 		**36**	10% IPA	*S*-NEC	5.29*R*	0.8	1.06	1.06	0
metoprolol* 		**36**	10% IPA	*S*-NEC	13.98	1.0	1.08	1.08	0
oxprenolol* 		**36**	10% IPA	*S*-NEC	7.98	0.7	1.05	1.05	0
pindolol* 		**36**	20% IPA	*S*-NEC	11.32	0.6	1.04	1.04	0
ephedrine 	(*S*,*R* and *R*,*S*)	**53**	10% IPA	*S*-NEC	15.38	0.6	1.03	1.05	1.8
		53	10% IPA	*R*-NEC	16.28	1.1	1.07	1.07	0
	(*R*,*R* and *S*,*S*)	**53**	10% IPA	*S*-NEC	11.65	0	1.00	1.05	5
		53	10% IPA	*R*-NEC	11.67	1.1	1.07	1.07	0
norphenylephrine* 		**36**	30% IPA	*S*-NEC	11.22	0	1.00	1.00	0
		36	30% IPA	*R*-NEC	12.78	0.6	1.04	1.04	0
phenylalaninol 		**38**	10% IPA	*S*-NEC	7.86*S*	2.0	1.16	1.17	0.8
		38	10% IPA	*R*-NEC	9.96	0	1.00	0.96~	0
2-phenylglycinol 		**38**	20% IPA	*S*-NEC	10.53*S*	2.5	1.27	1.34	5.5
		38	20% IPA	*S*-NEC	12.42*R*	1.7	1.21	1.24	2.5
p-chlorophenylalaninol 		**38**	20% IPA	*S*-NEC	8.93	1.5	1.15	1.20	4.3
		38	20% IPA	*R*-NEC	9.32	1.8	1.18	1.11	3.8
methionol 		**38**	20% IPA	*S*-NEC	9.23	0.7	1.05	1.12	6.7
		38	20% IPA	*R*-NEC	9.78	0.8	1.06	1.07	0.9
leucinol 		**38**	10% IPA	*S*-NEC	8.16*S*	1.2	1.08	1.08	0
		38	10% IPA	*R*-NEC	9.05*R*	0.8	1.06	0.98~	6.0
2-amino-3-methyl-1-butanol* 		**38**	10% IPA	*S*-NEC	8.66*R*	1.9	1.14	1.14	0
		38	10% IPA	*R*-NEC	9.26*S*	1.7	1.12	1.12	0
2-amino-1-hexanol 		**38**	10% IPA	*S*-NEC	7.81	1.5	1.09	1.10	0.9
		38	10% IPA	*R*-NEC	8.58	1.1	1.07	1.01	5.6

Table II (Continued)

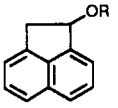
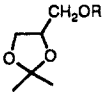
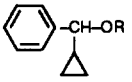
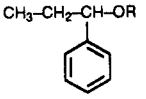
compd	R substituent	% v/v modifer	CSP	k'^a	R_s	α_{exp}	α_{calc}	% error
2-amino-1-pentanol	38	10% IPA	*S*-NEC	9.20	0	1.00	1.02	2
$CH_3-(CH_2)_2-CH(NHR)-CH_2O-CO-DNB$	38	10% IPA	*R*-NEC	11.11	0.7	1.05	0.95	5.0
2-amino-1-butanol	38	10% IPA	*S*-NEC	10.56*R*	0.7	1.05	1.02	2.8
$CH_3-CH_2-CH(NHR)-CH_2O-CO-DNB$	38	10% IPA	*R*-NEC	14.0	0	1.00	0.96~	0
2-amino-1-propanol	38	10% IPA	*S*-NEC	15.0	0.6	1.02	1.03	1.0
$CH_3-CH(NHR)-CH_2O-CO-DNB$	38	10% IPA	*R*-NEC	15.43	0.6	1.03	0.96~	3.0
1-amino-2-propanol	36	10% IPA	*S*-NEC	15.46	0	1.00	0.79~	0
$CH_3-CH(OR)-CH_2-NH-CO-DNB$	36	10% IPA	*R*-NEC	17.30	0.6	1.02	0.81~	2.0
		Alcohols						
1-acenaphthenol* 	36	10% IPA	*S*-NEC	10.21	1.9	1.12	1.12	0
1-indanol* 	36	10% IPA	*S*-NEC	8.24	3.2	1.28	1.28	0
solketal* 	54	10% IPA	*S*-NEC	11.54	1.4	1.09	1.09	0
cyclopropylbenzyl alcohol* 	40	10% IPA	*S*-NEC	6.92	2.1	1.22	1.22	0
1-phenyl-1-propanol 	40	10% IPA	*S*-NEC	6.55	4.0	1.32	1.27	3.8
		Carboxylic Acids						
2-chloropropionic acid	39	20% IPA	*S*-NEC	3.78	2.7	1.28	1.30	1.6
$CH_3-CH(Cl)-C(=O)-R$	74	2% IPA	*S*-NEC	12.39	1.6	1.08	1.08	0
	42	2% IPA	*S*-NEC	6.86	1.2	1.07	1.06	0.9
	57	2% IPA	*S*-NEC	2.61	0	1.00	0.97~	0
	56	2% IPA	*S*-NEC	7.67	0	1.00	1.01	1.0
	64	2% IPA	*S*-NEC	4.01	0	1.00	0.97~	0
	63	2% IPA	*S*-NEC	6.11	0.8	1.04	1.05	0.9
	48	2% IPA	*S*-NEC	2.58	1.0	1.07	1.07	0
	50	2% IPA	*S*-NEC	2.44	1.1	1.08	1.06	1.8
	72	2% IPA	*S*-NEC	10.16	0	1.00	0.82~	0
2-bromopropionic acid	74	2% IPA	*S*-NEC	21.64	1.9	1.11	1.11	0
$CH_3-CH(Br)-C(=O)-R$	42	2% IPA	*S*-NEC	11.6	1.7	1.10	1.08	1.8
	57	2% IPA	*S*-NEC	12.36	0	1.00	0.99~	0
	56	2% IPA	*S*-NEC	11.96	0.6	1.02	1.03	0.9
	64	2% IPA	*S*-NEC	4.60	0	1.00	0.99~	0
	63	2% IPA	*S*-NEC	8.6	1.2	1.06	1.07	0.9
	48	2% IPA	*S*-NEC	4.76	1.5	1.10	1.10	0
	50	2% IPA	*S*-NEC	2.53	1.2	1.08	1.08	0
	72	2% IPA	*S*-NEC	10.44	0	1.00	0.84~	0
	27	2% IPA	*S*-NEC	1.56	0	1.00	0.94~	0
2-bromobutyric acid	74	2% IPA	*S*-NEC	16.00	1.6	1.09	1.09	0
$CH_3-CH_2-CH(Br)-C(=O)-R$	42	2% IPA	*S*-NEC	9.00	1.8	1.08	1.07	0.9
	57	2% IPA	*S*-NEC	2.04	0	1.00	0.98~	0
	56	2% IPA	*S*-NEC	9.03	0.6	1.02	1.02	0
	64	2% IPA	*S*-NEC	2.50	0	1.00	0.98~	0
	63	2% IPA	*S*-NEC	6.50	1.0	1.06	1.06	0
	48	2% IPA	*S*-NEC	3.42	1.1	1.07	1.08	0.9
	50	2% IPA	*S*-NEC	1.96	1.0	1.06	1.07	0.9
	72	2% IPA	*S*-NEC	9.92	0	1.00	0.83~	0
	27	2% IPA	*S*-NEC	1.45	0	1.00	0.93~	0

Table II (Continued)

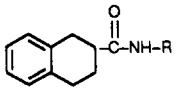
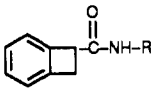
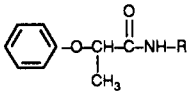
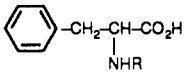
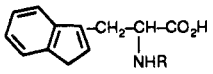
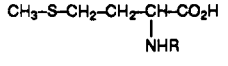
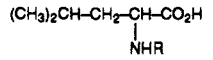
compd	R substituent	% v/v modifer	CSP	k'^a	R_s	α_{exp}	α_{calc}	% error
1,2,3,4-tetrahydro-2-naphthamide 	**39**	20% IPA	*S*-NEC	4.17	1.1	1.10	1.10	0
1-benzocyclobutenecarboxamide* 	**39**	20% IPA	*S*-NEC	6.60	0.7	1.04	1.04	0
2-phenoxypropionamide* 	**39**	20% IPA	*S*-NEC	6.18	5.9	1.52	1.53	0.7
Mobile Phase, Acetonitrile (ACN)–Ethanol (1% Acetic Acid v/v)								
	Amino Acids							
DL-phenylalanine 	**38**	50% ACN	*S*-NEC	5.12L	2.6	1.14	1.14	0
	38	50% ACN	*R*-NEC	7.98L	2.0	1.10	1.10	0
	73	50% ACN	*S*-NEC	3.83L	1.8	1.09	1.07	1.8
	73	50% ACN	*R*-NEC	4.89L	1.2	1.06	1.04	1.8
	41	50% ACN	*S*-NEC	3.00L	1.3	1.07	1.04	2.8
	41	50% ACN	*R*-NEC	4.05L	1.2	1.06	1.06	0
	11	50% ACN	*S*-NEC	2.39L	0.5	1.03	1.02	0.9
	11	50% ACN	*R*-NEC	2.78L	0	1.00	0.99~	0
	43	50% ACN	*S*-NEC	2.30	0	1.00	0.97~	0
	28	50% ACN	*S*-NEC	0.99	0	1.00	0.97~	0
	5	50% ACN	*S*-NEC	4.05L	0.6	1.07	1.01	5.6
	5	50% ACN	*R*-NEC	4.33L	0	1.00	0.99~	0
	7*	50% ACN	*S*-NEC	2.79	1.0	1.06	1.06	0
	7	50% ACN	*R*-NEC	2.85	0.6	1.03	1.03	0
	24	50% ACN	*S*-NEC	10.28	1.8	1.09	1.09	0
	24	75% ACN	*R*-NEC	14.42	0.6	1.02	1.08	5.9
	79	50% ACN	*S*-NEC	4.60L	2.5	1.13	1.12	0.9
	79	50% ACN	*R*-NEC	6.01L	2.7	1.15	1.11	3.5
DL-tryptophan 	**38**	50% ACN	*S*-NEC	4.16L	3.0	1.18	1.16	1.7
	38	50% ACN	*R*-NEC	6.22D	0.6	1.03	1.06	2.9
	73	50% ACN	*S*-NEC	2.91L	2.0	1.10	1.10	0
	73	50% ACN	*R*-NEC	4.07	0	1.00	1.00	0
	41	50% ACN	*S*-NEC	2.32L	1.2	1.06	1.06	0
	41	50% ACN	*R*-NEC	3.25L	1.7	1.09	1.02	6.4
	11	0% ACN	*S*-NEC	8.58L	0.6	1.04	1.05	0.9
	11	0% ACN	*R*-NEC	10.74	0	1.00	0.95~	0
	43	50% ACN	*S*-NEC	0.98	0	1.00	0.99~	0
	43	0% ACN	*S*-NEC	9.18	0	1.00	0.99~	0
	28	50% ACN	*S*-NEC	0.76	0	1.00	0.99~	0
	28	0% ACN	*S*-NEC	4.62	0	1.00	0.99~	0
	5	0% ACN	*S*-NEC	8.47	0.6	1.04	1.04	0
	5	0% ACN	*R*-NEC	10.65	0	1.00	0.95~	0
	24	50% ACN	*S*-NEC	6.74	0.6	1.02	1.12	9.8
	24	75% ACN	*R*-NEC	14.01	1.2	1.06	1.05	0.9
	79	50% ACN	*S*-NEC	3.16L	1.2	1.06	1.15	8.5
	79	50% ACN	*R*-NEC	4.30L	1.4	1.07	1.07	0
DL-methionine 	**38**	50% ACN	*S*-NEC	3.94L	2.0	1.10	1.08	1.8
	38	50% ACN	*R*-NEC	5.63D	2.0	1.10	1.05	4.5
	73	50% ACN	*S*-NEC	3.00L	0.6	1.02	1.02	0
	73	50% ACN	*R*-NEC	3.84	0	1.00	0.99~	0
	41	0% ACN	*S*-NEC	9.71	0	1.00	0.99~	0
	11	0% ACN	*S*-NEC	5.96	0	1.00	0.98~	0
	24	50% ACN	*S*-NEC	10.95	1.0	1.05	1.05	0
	24	75% ACN	*R*-NEC	19.40	0.6	1.03	1.03	0
	79	50% ACN	*S*-NEC	4.37L	1.0	1.05	1.07	1.8
	79	50% ACN	*R*-NEC	5.86L	0.7	1.04	1.06	1.8
DL-leucine 	**38**	50% ACN	*S*-NEC	2.12L	0.7	1.05	1.05	0
	38	50% ACN	*R*-NEC	2.55D	1.2	1.10	1.05	4.5
	73	50% ACN	*S*-NEC	1.32	0	1.00	0.99~	0
	73	50% ACN	*R*-NEC	1.61	0	1.00	0.99~	0
	41	50% ACN	*S*-NEC	1.03	0	1.00	0.96~	0
	24	50% ACN	*S*-NEC	4.58	0.6	1.03	1.01	1.9
	24	50% ACN	*R*-NEC	5.20	0.7	1.04	1.04	0
	79	50% ACN	*S*-NEC	1.68L	0.6	1.03	1.03	0
	79	50% ACN	*R*-NEC	2.12L	0.6	1.03	1.06	2.9

Table II (Continued)

compd	R substituent	% v/v modifer	CSP	k'^a	R_s	α_{exp}	α_{calc}	% error
DL-norleucine	24	50% ACN	*S*-NEC	5.63	0.7	1.04	1.03	0.9
$CH_3-(CH_2)_3-CH(NHR)-CO_2H$	24	50% ACN	*R*-NEC	7.60	0.7	1.04	1.04	0
	79	50% ACN	*S*-NEC	2.38	1.0	1.05	1.06	0.9
	79	50% ACN	*R*-NEC	3.12	0.7	1.04	1.06	1.8
DL-norvaline	24	50% ACN	*S*-NEC	5.65	0.7	1.04	1.04	0
$CH_3-(CH_2)_2-CH(NHR)-CO_2H$	24	50% ACN	*R*-NEC	8.20	0.7	1.04	1.04	0
	79	50% ACN	*S*-NEC	2.40	0.7	1.04	1.06	1.8
	79	50% ACN	*R*-NEC	2.99	1.0	1.05	1.06	0.9
DL-serine	24	50% ACN	*S*-NEC	10.05	0	1.00	1.01	1.0
$HO-CH_2-CH(NHR)-CO_2H$	24	50% ACN	*R*-NEC	17.00	0.7	1.04	1.04	0
	79	50% ACN	*S*-NEC	8.13	1.0	1.05	1.04	0.9
	79	50% ACN	*R*-NEC	10.89	1.0	1.05	1.06	0.9
DL-alanine	41	10% ACN	*S*-NEC	3.32	0.6	1.03	0.98~	3.0
$CH_3-CH(NHR)-CO_2H$	24	50% ACN	*S*-NEC	8.00	0.6	1.03	1.03	0
	24	50% ACN	*R*-NEC	16.00	0.7	1.04	0.99~	4.0
DL-valine	11	0% ACN	*S*-NEC	2.02	0	1.00	0.98~	0
$(CH_3)_2CH-CH(NHR)-CO_2H$	79	50% ACN	*S*-NEC	2.00L	1.7	1.09	1.07	1.8
	79	50% ACN	*R*-NEC	2.57L	1.5	1.08	1.07	0.9

[a] Capacity factor of the first eluted enantiomer; configuration indicated as *R*, *S*, L, or D, when known. [b] Entries marked with an asterisk refer to a compound containing a nonredundant substituent (see text). Values marked with a ~ mean the calculated α was lower than 1; for the error estimation α_{calc} was set to 1.00. [c] The 48 compounds, whose chromatographic data have been listed in ref 8, were also used in this study.

value in Table I means that the substituent somewhat decreases the chiral recognition relative to hydrogen. We stress that the hydrogen reference choice is completely arbitrary. Only the sum for the four substituents is known (eqs 4 and 5) and related to α. For example, choosing 60 cal/mol for the hydrogen atom substituent energy would have decreased by 20 cal/mol the free energy of all other substituents listed in Table I. Clearly, changing the arbitrary reference energy for hydrogen could also change some of the substituent values in Table I from positive to negative values. As the chiral data base increases to include more compounds without hydrogen substituents on the stereogenic center, the free energy value assigned to hydrogen can be refined along with all other energy values.

The results listed in Table II were put into a computer spreadsheet producing one equation (eq 4) per compound studied, with three unknown values corresponding to the free energy of the three substituents. As stated previously, the fourth substituent, H, has the reference value $\Delta G_H = 0$ cal/mol. The equations can be combined assuming that a substituent provides the same energy contribution regardless of the nature of the other three substituents (assumption 2). The equation system was solved while the following criterion was minimized:

$$E = \sum|\alpha_{calc} - \alpha_{exp}| \quad (6)$$

The error, E, is the sum of the absolute value of the difference between the calculated and experimental enantioselectivity factor for the set of compounds. When the calculated α value was lower than unity, α_{calc} was set to unity (no resolution). However, the actually computed α_{calc} values, lower than 1, are listed in Table II with a "~" symbol in order to provide more information on the precision of the method.

Table III lists the number of compounds that were used on each CSP and with each mobile phase. Some recently published results were included to enlarge the data base.[8] The average error between the experimental and calculated α values was lower than 3%. It should be noted that this error is lowered by the number of nonredundant substituents. A nonredundant substituent is one that exists only on one compound in the study. The computer fitting affects a free energy to that particular substituent which makes the calculated value exactly equal to the experimental α with a zero error. Free energy values obtained with nonredundant substituents are pointed out by an asterisk in Table I. Table III shows that there were approximately 20% nonredundant substituents. Taking into account the error decrease due to nonredundant substituents, the average error on calculated α values is in the 4% range.

Table III. Number of Compounds, Nonredundant Substituents, Error, and Maximum Error on the Calculated Enantioselectivity

stationary phase	no. of compds	no. of nonredund subst	% av α error	% max α error
Hexane–IPA Mobile Phases				
S-NEC–β-CD CSP	112	27	2.9	30
R-NEC–β-CD CSP	74	16	3.1	25
ACN–Ethanol Mobile Phases				
S-NEC–β-CD CSP	54	9	1.5	12
R-NEC–β-CD CSP	45	8	1.8	9

How Tables I and II Are Related. Consider, for example, the first compound of Table II which is the dinitrobenzoyl derivative of (1-(1-naphthyl)ethyl)amine. Its stereogenic center bears a hydrogen atom, a methyl group, a naphthyl group, and a (3,5-dinitrobenzoyl)amide group. These four substituents are listed in Table I as **3**, **6**, **66**, and **38**, respectively. Only substituent **38** is indicated in Table II because the three others are shown with the molecule. With the *S*-NEC–β-CD chiral stationary phase, the energy listed in Table I for the four substituents are 0, −74, 108, and 230 cal/mol, respectively. The sum of the four energies is 264 cal/mol. A 486 cal/mol "bonus" energy should be added because of the synergistic effect of substituents **66** and **38** when they are present in the same molecule (see end of Table I). An explanation of these additional effects is given in the subsequent section on $\pi-\pi$ interactions. Therefore, the total energy is 750 cal/mol for the compound. This energy should be divided by the product RT (586 cal/mol at 295 K), and eq 4 gives the calculated α value as $\alpha_{calc} = \exp(750/586) = 3.59$. This value is listed in Table II. With the *R*-NEC CSP, the energies of the four substituents are 0, −81, 108, and 196, respectively. The bonus is only 432 cal/mol, giving a total energy of 655 cal/mol. α_{calc} is $\exp(655/586) = 3.05$. Note that in these cases the calculated α_{calc} values correspond exactly

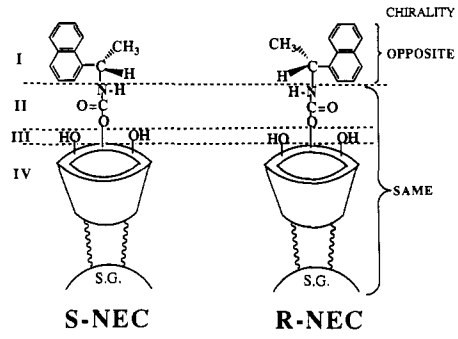


Figure 1. Simplified representation of the two stationary phases used: (left) (*S*)-(naphthylethyl)carbamoylated β-cyclodextrin stationary phase; (right) (*R*)-(naphthylethyl)carbamoylated β-cyclodextrin stationary phase. The chiralities of the naphthylethyl moieties are opposite (I). The chiralities of the secondary hydroxyl (III) and the β-CD cavity (IV) are identical. The carbamoyl groups (II) are not chiral. SG is silica gel.

to the experimental α values because substituents **66** and **38** appeared together only in this particular compound (see the nonredundant substituent discussion, vide supra).

Mobile-Phase Effects. We observed that moderate changes in the proportions of the components of a specific binary mobile phase could have very significant effects on solute retention times and capacity factors and little or no effect on enantioselectivity. Comparable observations were found in the literature.[20] The results in Table I were obtained for two binary mixtures: isopropyl alcohol (IPA)–hexane varying from 2% v/v to 50% v/v of IPA in hexane and acetonitrile (ACN)–ethanol. The ethanol percentage varied from 50% v/v to 100% v/v. Acetic acid (1%) was added to the acetonitrile–ethanol mobile phases. Table I shows that the absolute chiral free energy for a given substituent tends to be lower with the more polar acetonitrile–ethanol phases than with less polar hexane–IPA phases independent of the substituent polarity.

Stationary-Phase Effects. It has been shown that the chiral recognition process by NEC-derivatized β-CD involves two different processes: the so-called Pirkle-type recognition process (π–π interaction, hydrogen bonding, dipole stacking, and steric hindrance) and the CD inclusion capability.[8,9] Both processes could act synergistically or antagonistically or independently.[8] As shown by Figure 1, the NEC moieties are bonded to the chiral cyclodextrin molecule. The *S*-NEC CSP was less substituted (3.5 *S*-NEC/CD ring) than the *R*-NEC CSP (6.7 *S*-NEC/CD). However, the cyclodextrin seems to have a greater synergistic effect with the *S*-NEC moiety. Therefore more compounds were analyzed on this CSP (Table III). Alkyl groups (**6** (methyl), **18** (propyl), **20** (butyl), **21** (isobutyl)) seem to decrease the chiral recognition of the *R*-NEC CSP more than that of the *S*-NEC CSP. When a carbonyl group is attached to the stereogenic center, the chiral recognition seems to be enhanced on the *R*-NEC CSP (substituents **10** (–COO–CH_3), **17** (–COO–CH_2–CH_3), **65** (–CO–O–Oct)). Most substituents produced comparable energy values on both CSPs. The largest difference between the two CSPs is observed for substituent **60** (–CH_2–(3-indole)) which is the substituent in tryptophan. The positive 27 cal/mol on the *S*-NEC phase became a negative –202 cal/mol on the *R*-NEC phase.

Substituent Effects. The important point of this study is the relative free energy value of each substituent compared to the others since the absolute values depend on the reference chosen.

sp^3 versus sp^2. The highest free energy values (**39**, 263 cal/mol; **48**, 155 cal/mol; **63**, 142 cal/mol) were obtained when an sp^2-hybridized carbon was attached to the stereogenic center. The lowest free energy values (**6**, –74 cal/mol; **12**, –81 cal/mol; **18**, –81 cal/mol; **23**, –81 cal/mol) were obtained with alkyl substituents or when an sp^3-hybridized carbon was attached to the stereogenic carbon. It was noted previously that chiral recognition on various CSPs was enhanced when sp^2 carbons were connected to the stereogenic carbon.[3,4,21,22] The insertion of a methylene group between an sp^2-hybridized carbon and the stereogenic center decreased the free energy by about 80 cal/mol (compare substituents **30** and **45**, or **66** and **75**). An oxygen insertion seems also to decrease the free energy by more than 30 cal/mol (**30** and **32**; **39** and **40**).

π–π Interactions. The effect of π–π interactions in chiral recognition by Pirkle-type CSPs was extensively studied.[4,5,10,13,18] The derivatized NEC–β-CD phases bear a π-basic naphthyl group (Figure 1). π–π interactions with π-acid groups (nitro or dinitro groups) are possible. All substituents of Table I with a π-acid 3,5-dinitrobenzoyl group (DNB) have a highly positive free energy. It seems that acid–base π–π interactions increase the chiral recognition. However, when two different π-acid DNB derivatives were present in a compound, the enantioselectivity decreased significantly. Apparently, a compound having two different π-acidic groups can form diastereomeric complexes in which the enantioselectivity of one complex is opposite to that of the other. Therefore they tend to partially or totally cancel one another (antagonistic effect), resulting in a poorer enantiomeric separation. In a few cases, two substituents were found to have synergistic effects (e.g., –NHCO–DNB and naphthyl or phenyl). Only in theses cases (five in all) were the contributions of the substituents not additive. Hence a fifth contribution was included at the end of Table I under the heading Synergistic and Antagonistic Substituent Interactions for these special cases. For example, when two different competing π-acidic substituents are present in the same compound, the $\Delta(\Delta G_c)$ sum must be decreased by 230 cal/mol (Table I). Conversely, when certain π-acidic and π-basic groups are present in the same compound, vide supra, the $\Delta(\Delta G_c)$ sum is increased (Table I).

Hydrogen Bonding and Dipole Stacking. The amido group –CO–NH– is most often responsible for hydrogen bonding and/or dipole stacking. NEC amido groups and secondary hydroxyls at the mouth of the β-CD cavity can be involved in hydrogen bonding (Figure 1). Wainer showed that the solute–amido stationary phase–amido hydrogen bonds were most important.[23] Reversing the linkage between a solute and its π-acidic or π-basic moiety from "NH–CO–" to "–CO–NH–" reversed the elution order.[24] All substituents containing the amido sequence have a positive free energy. As pointed out earlier, the energy is higher if the carbonyl group, with its sp^2-hybridized carbon, is connected to the stereogenic center. The energy is lower if the NH group is first. For example, the –NH–CO–Φ substituent **41** has 27 cal/mol, its isomer –CO–NH–Φ **42** has 148 cal/mol. The phenyl group alone (**30**) has an intermediate value of 81 cal/mol. Similar results were obtained with the naphthyl substituents **73** (–NH–CO–naphthyl), **66** (naphthyl), and **74** (–CO–NH–naphthyl), with energies of 54, 108, and 162 cal/mol, respectively. However, when hydrogen bonds and π–π interactions were associated, the stereogenic center was less sensitive to the hybridization. Substituents **38** (–NH–CO–DNB) and **40** (–O–CO–NH–DNB) have 230 and 182 cal/mol energy values, respectively; **39** (–CO–NH–DNB) has 263 cal/mol. The decreasing effect of a methylene insertion is obvious with substituents **52** (–CH_2–NH–CO–DNB), only 20 cal/mol, and **54** (–CH_2–O–CO–NH–DNB), 68 cal/mol.

The replacement of the amido hydrogen by a methyl group does not significantly change the chiral recognition (substituents **48** and **50**, **38** and **53**, and also **56** and **64**). Steric effects may compensate for the loss of one hydrogen bond. Steric effects are likely responsible for the large energy increase

between substituent **52**, 20 cal/mol, and the *tert*-butyl–nitrogen-substituted equivalent **80**, with 101 cal/mol.

Ring Effects. The cyclohexyl energy (**34**, 81 cal/mol) is much higher than the hexyl homologue energy (**35**, −81 cal/mol). Although an sp^3-hybridized carbon is attached to the stereogenic center, steric effects and compactness of the cyclohexyl ring allow it to be recognized by the CSPs. When the asymmetric carbon is part of a ring (substituent indicated by @ in Table I), the method presented here did not allow distinction of one side of the ring from the other. The same energy value was assigned to both sides, although one side was often an sp^2-hybridized carbon while the other side was sp^3-hybridized. All ring substituent energies were negative (@, Table I).

Several Chiral Centers. The last substituent, **81** (–CH(Φ)–O–DNB), contains a stereogenic center. It is derived from ephedrine which has two stereogenic centers and can be resolved into four optical isomers. In this case, two substituents should be studied, the *R*-configuration and the *S*-configuration, with different free energies.

CONCLUSION

The purely empirical approach for chiral recognition presented in this work is not intended to replace fundamental studies of mechanism. However, it is useful for the end-user of enantiomeric separations in that the list of substituent energies can be used to predict the feasibility of other separation on the NEC CSPs. The 81 substituents listed in this study could make more than 1.6 million different chiral compounds. We studied only 121 of these. The DNB derivative of octyl ester (1-naphthyl)glycine has an asymmetric carbon with substituents **65** (+230 cal/mol), **66** (+108 cal/mol), and **39** (+230 cal/mol), with an additional 486 cal/mol bonus for associating **39** and **66**. We did not analyze a racemic mixture of this compound that would have the maximum theoretical free energy for enantiomeric recognition (1.05 kcal/mol) by the *S*-NEC–β-CD CSP. It should produce an enantioselectivity value α, as high as 6. This example shows how this work could be used. It is likely that this compound would be fully resolved on this CSP. If the addition of the energy contribution for the four substituents of a given racemate produces a positive energy value, the NEC–β-CD phase is likely appropriate and will resolve the racemate. If the calculation produces a close to zero or negative energy value, another CSP should be tried. This same type of empirical approach could be used for most other CSPs provided a sufficient data base is obtained.

ACKNOWLEDGMENT

Support of this work by the National Institute of Health (Grant NIH BMT 2R01 GM3692-05A2) is gratefully acknowledged. Also, we would like to acknowledge Gary Bertrand, University of Missouri—Rolla, for helpful discussions and manuscript proofreading.

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RECEIVED for review August 16, 1991. Accepted November 13, 1991.