Chiral High-Pressure Liquid Chromatographic Stationary Phases. 3. General Resolution of Arylalkylcarbinols

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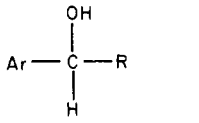
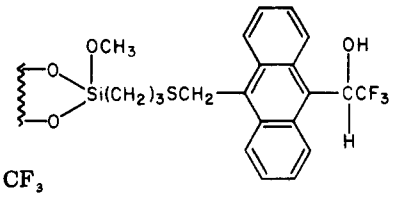
Enantiomers of arylalkylcarbinols (1) may be separated by chromatography upon a stationary phase comprised of chiral *N*-(3,5-dinitrobenzoyl)phenylglycine ionically bonded to γ-aminopropyl silanized silica. The order of elution of the enantiomers is related to the absolute configuration by a chiral recognition model. Hence, absolute configurations as well as enantiomeric purity can be conveniently determined on as little as nanogram quantities of carbinol. Alternatively, preparative separations can be performed upon the chiral phase, the scale being dictated by the column size. A convenient in situ method for preparation of efficient high-pressure liquid chromatography (HPLC) columns of this type is described.

The chromatographic separation of enantiomers upon chiral stationary phases (CSP's) is a challenge that has been taken up repeatedly by workers in the various areas of chemistry.[1] In general, those CSP's reported capable of efficiently separating enantiomers do so for but a modest range of closely related solutes, the intimate details of the chiral recognition mechanism rarely being understood. Unusual in this regard is a fluoro alcoholic CSP that separates the enantiomers of a broad range of solutes of assorted functionality.[2,3] This CSP, consisting of chiral alcohol **1a** bonded to silica as shown in **2**, was designed to



1a, Ar = 9-anthryl; R = CF_3



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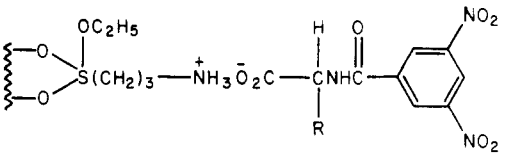
conform to a chiral recognition rationale that enumerates several features essential to a CSP if it is to have nonidentical affinities for solute enantiomers. For example, there must be at least three simultaneous interactions between the CSP and at least one of the solute enantiomers. Moreover, at least one of these interactions must be stereochemically dependent.[4] Understandably, any CSP will have a domain limited to those solute enantiomers capable of undergoing the simultaneous multiple interactions employed by that particular CSP. To the extent that one understands the nature of the chiral recognition interactions, one can rationally improve the design of a CSP so as to extend its domain and enhance its general utility.

It should be evident that there is a "reciprocal" aspect to chiral recognition. If an enantiomer of A can, in terms of affinity, distinguish between the enantiomers of B, then an enantiomer of B can similarly distinguish between the enantiomers of A. Thus, any enantiomer from a racemate resolvable upon fluoro alcohol derived CSP **2** is a potential candidate for incorporation into a reciprocal CSP intended to resolve racemic fluoro alcohol **1a**. An important consequence of this reciprocality is that one CSP can be used to evaluate the chiral recognition potential of other compounds (prospective CSP's), and an iterative procedure can be utilized for rapid optimization of CSP design.

We recently described[3] two reciprocal CSP's, both covalently bonded to silica. We now describe an ionically bonded reciprocal CSP that is more conveniently prepared than its predecessors and which offers superior performance in that it resolves not just anthrylalkylcarbinols such as **1a**, *but most other type 1 alcohols as well.*

Results and Discussion

Treatment of γ-aminopropyl-derivatized silica with (*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine (**3a**) affords ionically bonded CSP **4a**. This treatment may be conducted



4a, R = C_6H_5
b, R = isobutyl
c, R = isopropyl

either prior to column packing *or upon a highly efficient prepacked column.* The ionically bonded chiral acid does not leach from the column at significant rates so long as relatively nonpolar mobile phases are used. Figure 1 and Table I document the resolution of a variety of type 1 alcohols upon a 4.6 mm × 250 mm commercial aminopropyl column[5] made chiral by in situ modification. The resultant column allows one to assess enantiomeric purity and absolute configuration of minute quantities of type 1 alcohols, detection sensitivity being the limiting factor. Enantiomeric purities are determined from the relative peak areas of the enantiomers; absolute configuration is assessed from observed elution order, the known absolute configuration of CSP **4a**, and the chiral recognition mechanism (vide infra). Small-scale preparative resolutions (milligrams) of type 1 alcohols can be accomplished upon the aforementioned 4.6-mm column; gram quantities have been resolved upon a larger preparative column.[6]

Ionically bonded CSP **4** is a logical extension of our observation that the 3,5-DNB derivatives of a number of

(1) For recent reviews, see: (a) R. Audebert, *J. Liq. Chromatogr.*, 2, 1063 (1979); (b) G. Blaschke, *Angew. Chem., Int. Ed. Engl.*, 19, 13 (1980).
(2) W. H. Pirkle and D. W. House, *J. Org. Chem.*, 44, 1957 (1979).
(3) W. H. Pirkle, D. W. House, and J. M. Finn, *J. Chromatogr.*, 192, 143 (1980).
(4) The "three-point" conception did not originate with us. This requirement, nicely described in the review by Lochmüller and Souter [*J. Chromatogr.*, 113, 283 (1975)], is originally attributable to Dalgliesh [*J. Chem. Soc.*, 363 (1974)].
(5) Regis Chemical Co. furnished a Hi-Chrom Reversible column packed with 5-μm γ-aminopropyl-silanized spherical silica particles. These columns, chirally modified as described herein, are now available from Regis.
(6) A preliminary account of some preparative applications of CSP **4a** was presented at the 179th National Meeting of the American Chemical Society, Houston, TX, 1980.

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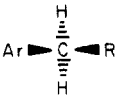
Table I. Resolutions of Type 1 Alcohols upon CSP 4a



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1	Ar	R	α^a	$k_1{}^b$	1	Ar	R	α^a	$k_1{}^b$
a	9-anth	CF_3	1.33*	5.5	x	1-naphthyl	CH_2CH_3	1.09*	9.8
b	9-anth	C_2F_5	1.40*	2.3	y	1-naphthyl	n-C_4H_9	1.11	6.7
c	9-anth	C_3F_7	1.46*	2.2	z	1-naphthyl	CCl_3	1.20	6.9
d	9-anth	CH_3	1.30*	6.8	aa	1-naphthyl	C_6H_5	1.06	17.7
e	9-anth	n-C_4H_9	1.48	3.9	bb	1-(2-ethoxy)naphthyl	n-C_4H_9	1.07	9.7
f	9-anth	CH_2-$COOC_2H_5$	1.27	9.0	cc	1-(2,7-dimethyl)naphthyl	CH_3	1.10	21.0
g	9-anth	$CH_2C_6H_5$	1.48	7.1	dd	2-naphthyl	CF_3	1.08*	14.8
h	9-anth	C_6H_5	1.57	6.4	ee	2-naphthyl	CH_3	1.13*	17.7
i	9-(10-CH_3)anth	CF_3	1.43*	7.3	ff	2-naphthyl	n-C_4H_9	1.15	8.2
j	9-(10-n-C_4H_9)anth	CF_3	1.44*	4.7	gg	2-naphthyl	CCl_3	1.08	12.0
k	9-(2-CH_3)anth	CF_3	1.39	5.3	hh	C_6H_5	CF_3	1.06*	5.3
l	9-(3-CH_3)anth	CF_3	1.33	4.9	ii	C_6H_5	CH_3	1.05*	5.5
m	9-(10-CH_3O)anth	CF_3	1.28*	5.3	jj	C_6H_5	C_2H_5	1.05*	5.5
n	9-(10-Br)anth	CF_3	1.39*	4.2	kk	C_6H_5	$CH(CH_3)_2$	1.04	2.6
o	9-(10-C_6H_5)	CF_3	1.16	2.4	ll	C_6H_5	$C(CH_3)_3$	1.08*	2.1
p	9-(4-C_6H_5)anth	CF_3	1.28*	4.6	mm	C_6H_5	n-C_4H_9	1.08*	3.1
q	9-(1-C_6H_5)anth	CF_3	1.31*	3.9	nn	C_6H_5	cyclopropyl	1.04	5.2
r	9-(10-benzyl)anth	CF_3	1.44	3.9	oo	C_6H_5	cyclohexyl	1.08	4.3
s	3-pyrenyl	CF_3	1.08*	17.0	pp	mesityl	CH_3	1.10	3.9
t	3-pyrenyl	CH_3	1.06*	29.6	qq	p-tolyl	CF_3	1.08*	5.1
u	3-pyrenyl	n-C_4H_9	1.16	14.0	rr	p-biphenyl	CH_3	1.03	9.9
v	1-naphthyl	CF_3	1.08*	12.9	ss	3,4-dimethoxyphenyl	i-C_3H_7	1.07	59.6
w	1-naphthyl	CH_3	1.14*	16.6					

[a] The enantiomer having the absolute configuration indicated above is known to elute first from CSP 4a for those entries marked with an asterisk. Elution orders of the remaining entries have not been established. [b] Entries a-u were chromatographed by using 5% isopropyl alcohol in hexane, 1% isopropyl alcohol in hexane being used for the remainder.

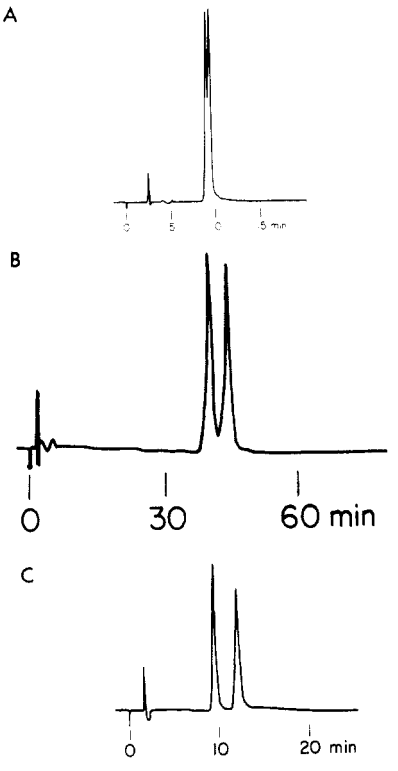
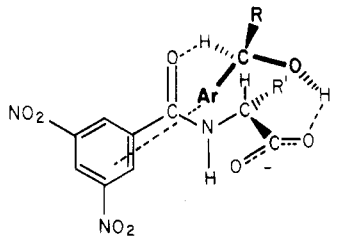


Figure 1. Chromatographic separation of the enantiomers of (A) racemic phenylisopropylcarbinol, (B) racemic (trifluoromethyl)-α-naphthylcarbinol, and (C) racemic (trifluoromethyl)-9-anthrylcarbinol, on the in situ modified commercial amino column.

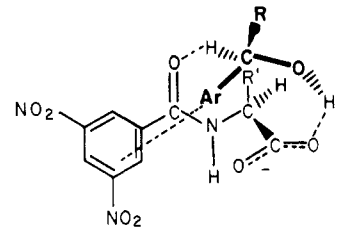
racemic amino acids can, as the carboxylate salts, be resolved upon fluoro alcohol CSP 2. The chiral recognition mechanism originally suggested to rationalize these resolutions is equally appropriate in the reciprocal sense. This mechanism, in addition to accounting for elution orders, also allows qualitative rationalization of the degrees of chiral recognition (α value magnitudes) and extent of retention (k' value).

Chiral Recognition Mechanism. Type 1 alcohols hydrogen bond to basic sites in other molecules. Should the second molecule contain an appropriately located auxiliary site of lesser basicity, the alcohol's carbinyl hydrogen may bond weakly thereto, affording transient chelate-like solvates.[6] In the case of *N*-3,5-DNB derivatives of α-amino acid carboxylates, the primary (hydroxyl) hydrogen bond is to the carboxylate group, the secondary carbinyl hydrogen interaction occurring at the less basic 3,5-DNB carbonyl oxygen. A tertiary interaction between the π-basic aryl substituent of the alcohol and the π-acidic 3,5-DNB group can occur if the aryl group can assume an appropriate orientation. The chiral recognition process can be described from either of two viewpoints. One might consider that the three aforementioned interactions occur simultaneously for both diastereomeric solvates, as shown in **5a,b**. In order for this to occur, however, the carbonyl oxygen of the DNB group is (essentially) eclipsed by either the aminyl hydrogen or the R′ group, as shown in solvates **5a** and **5b**, respectively. Eclipsing of the R′ group causes solvate **5b** to be of higher energy than **5a**. Hence, a CSP derived from the amino acid derivative having the absolute configuration depicted in **5a** should most strongly retain the type 1 alcohol enantiomer of the absolute configuration depicted in **5a**. This view thus invokes a fourth interaction (eclipsing) as the origin of the stereochemical dependence of the stability of the solvates.

From the second viewpoint, one takes cognizance of the preference for amides of amines having a single aminyl hydrogen to populate conformations placing the aminyl hydrogen near and essentially in the plane of the amide



5a



5b

carbonyl oxygen. Treating the *N*-3,5-DNB derivative as "locked" in the conformation depicted in **5a**, one can easily see for **5a** that exchanging the positions of R and the carbinyl hydrogen would disrupt the secondary interaction. Hence, the relative stereochemistry depicted in **5a** is again expected to afford the most stable solvate. Interchange of any pair of carbinyl substituents leads to this same conclusion. Hence, the stereochemical origin of the stability difference is evident, its magnitude being determined by the energetics of the various interactions and the rigidity of the amino acid derivative. To the extent that the structure of R′ influences conformational behavior of the derivative, the two viewpoints converge.

(A) Effect of Structural Variation within the Amino Acid. In solution, the stable conformation of *N*-acyl α-amino acids having an aminyl hydrogen is that which (essentially) eclipses this hydrogen with the acyl carbonyl oxygen. By influencing the energy difference between the two eclipsed conformations (H vs. R′), the structure of R′ influences α. It is observed that CSP **4a** affords larger α values for type 1 alcohols than does CSP **4b**, which, in turn, gives larger α's than does CSP **4c**.[7] In each instance where partially resolved, configurationally known alcohol samples were available, the elution orders of the enantiomers from CSP's **4a–c** were those expected from the chiral recognition model. Entries in Table I representing such samples bear asterisks. The presumption is that all alcohols in Table I elute in accordance with the chiral recognition model.

(B) Effect of Structural Variation within the Alcohol. The structure of the aryl substituent of a type 1 alcohol not only influences the π basicity of this group but, through the presence or absence of ortho substituents and peri hydrogens, also will influence its rotational behavior with respect to the chiral carbinyl group, CH(OH)R. This rotational behavior, also influenced by the nature of R, bears upon the degree of simultaneous occurrence of the multiple interactions (and the strength thereof) essential for chiral recognition. It may be seen from the table that the α and k_1' values for the 9-anthryl carbinols are greater than those of the naphthyl or phenyl analogues. This might be expected purely from considerations of π basicity. However, the k_1' values of the analogous 3-pyrenylcarbinols are greater than those of the anthrylcarbinols whereas the α values are smaller. We infer that the 3-pyrenyl group is a better π base than is the 9-anthryl group, but, owing to the presence of but one peri hydrogen, the 3-pyrenyl group is not as favorably oriented for the essential multiple simultaneous interactions. A "favorable" orientation is one in which the hydroxyl group and the carbinyl hydrogen are presented to the same face of the anthryl system. The hydroxyl and the R group of the carbinol preferentially "straddle" one of the two anthryl peri hydrogens. The more nearly the C–R–anthryl dihedral angle approaches 90°, the more favorable the arrangement for simultaneous multiple interaction. One does note an increase in α as the size of R increases, a trend explainable in terms of increasing dihedral angle. Interestingly, electronegative R groups afford somewhat smaller α's than simple alkyl groups of comparable size. This may stem from inductive diminishment of the π basicity of the anthryl system by the electronegative R group. This trend can be noted when the anthryl system is directly substituted with electronegative substituents. An additional function of the R group is to prevent π interactions with the wrong face of the anthryl system, interactions leading to retention but not chiral recognition. Extraneous retention "dilutes" the magnitude of α. Hence, bulky R groups play two roles in chiral recognition.

(C) Effects of Additional Functionality within the R Group of a Type 1 Alcohol. Although we have not had opportunity to study many type 1 alcohols having additional functionality within the R group, our experience to date suggests that additional functionality poses no serious problem in terms of enantiomer separability *unless* such functionality either diverts essential interactions with the CSP or adversely alters the conformational behavior of the alcohol. Should a "remote" functional group lead to additional but nonchirally recognizing interactions with the CSP, one expects k_1' values to increase and α values to decrease.

Separation of the Enantiomers of Non-Type-1 Alcohols. Although it is premature to discuss the underlying causes, we have noted that CSP **4a** is capable of separating the enantiomers of some non-type-1 alcohols and, indeed, other classes of solutes. Investigations of these solute classes are in progress and will be reported later.

Effects of Silica upon Chiral Recognition. Several types of silica have been used to prepare CSP **4a**, leading to columns displaying somewhat different α values for any given type 1 alcohol. Variations in surface area, pore size, and extent of loading are expected to influence k' values but not α values *so long as the surfaces of the adsorbents are identical.* The variation in α values presumably reflects the presence, in varying degrees, of nonchirally recognizing retention mechanisms. The presence of residual silanol groups on the silica surface is a possible source of this additional retention, the capping of these groups being a subject under investigation.

Conclusion

A simply prepared, high-efficiency, chiral HPLC column capable of separating the enantiomers of a large number of type 1 alcohols is described. A mechanism accounting for the origin and sense of the chiral recognition is presented. By use of the column described, enantiomeric purities and absolute configurations of a great many type 1 alcohols can be rapidly determined on submilligram quantities of samples. Conversely, larger preparative columns of this type can resolve gram quantities of racemic type 1 alcohols.[6]

(7) Ref 9–20, as cited in ref 3, are pertinent to this chelation.

(8) This result was anticipated since the enantiomeric 3,5-DNB derivatives of phenylglycine afford larger α values upon CSP **2** than do the corresponding derivatives of leucine, or in turn, valine. For example, the α and k_1' values upon CSP's **4a–c**, respectively, are as follows: 1.66, 8.8; 1.45, 3.4; 1.33, 2.5 for carbinol **1a**; 1.72, 3.0; 1.60, 2.5; 1.55, 1.8 for carbinol **1c**; 1.56, 7.5; 1.36, 4.0; 1.21, 3.0 for carbinol **1n**. These CSP's were prepared from Ventron silica which affords somewhat higher α and k_1' values than does 5-μm Spherisorb.

Experimental Section

Chromatography was performed isocratically by using an Altex 100 pump, a Valco 7000-psi injector with a 10-μL loop, and an Altex Model 152 dual wavelength (254 and 280 nm) detector. Hexane–isopropyl alcohol mixtures were used as mobile phases, the alcohol content controlling k' values but having little effect upon α values. The various type 1 alcohols used in this study were available either commercially, by borohydride reduction of the corresponding ketones, by Grignard addition to aldehydes, or from prior studies. Those type 1 alcohols indicated as being enantiomerically enriched were available from prior studies, absolute configurations being taken from the literature or being assigned by NMR methods.[9]

Preparation of *N*-(3,5-Dinitrobenzoyl) Amino Acids. A slurry of 2 mol of amino acid and 2 mol of 3,5-dinitrobenzoyl chloride in 2 L of dry THF was stirred at room temperature for 7–10 days. Unreacted amino acid was removed by filtration and washed with THF. The filtrate was concentrated under vacuum, and the residue was dissolved in 8–10 L of saturated sodium bicarbonate solution and extracted continuously with ether to remove neutral impurities. The pH of the solution was adjusted to ca. 5.3 with citric acid, and the liberated *N*-(3,5-dinitrobenzoyl) amino acid was isolated by continuous ether extraction. The pH of the aqueous solution was maintained at 5.3. The ethereal extract was dried over anhydrous $MgSO_4$ and evaporated to dryness. The yield of crystalline derivative is 50–80%.

Use of higher reaction temperatures may lead to partial racemization. Recrystallization of the derivatives removes the minor quantity of the second enantiomer that may be present. Enantiomeric purities were monitored by HPLC upon CSP **2a**.

(*R*)-(−)-(3,5-Dinitrobenzoyl)phenylglycine: mp 217–218 °C; NMR (acetone-d_6) δ 5.81 (d, 1 H), 7.30–7.65 (m, 5 H), 8.90–9.20 (m, 4 H); IR (KBr) 3400–3085, 1733 (vs), 1652 (vs), 1580 (vs), 1345, 1218, 1190, 1080, 920, 722, 722 cm^{-1}; $[\alpha]^{20}_D$ −90.0 (*c* 0.92, THF). Anal. Calcd for $C_{15}H_{11}N_3O_7$: C, 52.18; H, 3.21; N, 12.17. Found: C, 52.12; H, 3.24; N, 12.20.

(*S*)-(3,5-Dinitrobenzoyl)valine: mp 184–185 °C; NMR (acetone-d_6) δ 1.02 (d, 6 H), 2.28 (m, 1 H), 4.59 (m, 1 H), 8.35 (d, 1 H), 8.98 (s, 3 H); IR (KBr) 3345 (br), 3110, 1720 (vs), 1645 (vs), 1550 (vs), 1350, 1303, 1258, 1245, 1215, 1128, 1090, 926, 850, 733, 722 cm^{-1}. Anal. Calcd for $C_{12}H_{13}N_3O_7$: C, 46.30; H, 4.18; N, 13.50. Found: C, 46.63; H, 4.14; N, 13.49.

(*S*)-(3,5-Dinitrobenzoyl)leucine: mp 140 °C; NMR (acetone-d_6) δ 0.97 (d, 6 H), 1.67–2.00 (m, 3 H), 4.63–4.87 (m, 1 H), 8.48–8.67 (d, 1 H), 9.00–9.15 (s, 3 H); IR (KBr) 3382 (br), 3125, 1732 (vs), 1660 (vs), 1560 (vs), 1470, 1355 (vs), 1310, 1246, 1203, 1082, 923, 832, 732 cm^{-1}. Anal. Calcd for $C_{13}H_{15}N_3O_7$: C, 48.00; H, 4.65; N, 12.92. Found: C, 47.94; H, 4.63; N, 12.69.

Preparation of Bulk Chiral Packing. A slurry of 10 g of the chosen γ-aminopropyl-silanized HPLC silica was treated overnight at room temperature with 15 mmol of the amino acid DNB in 50 mL of THF. The silica was collected by filtration and washed with THF and ether. Samples of packing to be used for elemental analysis were dried under vacuum. The extent of loading of the bonded phase depends upon the silica used. "Large pore" (600 m^2/g) silica obtained from Ventron Chemical Co. and ball milled to a powder was used for exploratory work after treatment with (γ-aminopropyl)triethoxysilane as previously described.[3] Commercial 5-μm aminopropyl Spherisorb silica was similarly modified with phenylglycine DNB for comparison of loading levels. From elemental analysis, the ground Ventron silica was loaded with amino acid derivatives to the following extents: **3a**, 0.54 mmol/g of support (based on C); **3b**, 0.40 mmol/g of support (based on C and N); **3c**, 0.41 mmol/g of support (based on C and N). The Spherisorb-based packing contained 0.19 mmol of **3a**/g of support (based on N), 0.21 mmol/g (based on C).

Procedure for in Situ Modification of Packed Amino Columns. A solution of 2 mL of anhydrous triethylamine in 40 mL of dry THF was pumped through the column to ensure that the aminopropyl groups were present as the free base. Following a 20-mL wash with dry THF, a solution of 2.0 g of **3a** in 40 mL of dry THF was pumped through the column. Afterward, the column was washed with 30 mL of dry THF followed by 30 mL of anhydrous methanol. Finally, the column was washed with 10% isopropyl alcohol in hexane (ca. 100 mL) until the base line was relatively constant.

Acknowledgment. This work has been partially supported by the National Science Foundation.

Registry No. (±)-**1a**, 60686-64-8; (±)-**1b**, 74928-68-0; (±)-**1c**, 74928-69-1; (±)-**1d**, 74928-67-9; (±)-**1e**, 77495-10-4; (±)-**1f**, 77495-11-5; (±)-**1g**, 77495-12-6; (±)-**1h**, 77549-69-0; (±)-**1i**, 74958-72-8; (±)-**1j**, 74928-64-6; (±)-**1k**, 77495-13-7; (±)-**1l**, 74928-61-3; (±)-**1m**, 74928-65-7; (±)-**1n**, 74958-73-9; (±)-**1o**, 74958-75-1; (±)-**1p**, 77549-70-3; (±)-**1q**, 77495-14-8; (±)-**1r**, 74958-74-0; (±)-**1s**, 77549-71-4; (±)-**1t**, 77495-15-9; (±)-**1u**, 77495-16-0; (±)-**1v**, 17556-44-4; (±)-**1w**, 57605-95-5; (±)-**1x**, 77495-17-1; (±)-**1y**, 77495-18-2; (±)-**1z**, 77495-19-3; (±)-**1aa**, 38379-46-3; (±)-**1bb**, 77495-20-6; (±)-**1cc**, 77495-21-7; (±)-**1dd**, 17556-45-5; (±)-**1ee**, 40295-80-5; (±)-**1ff**, 77495-22-8; (±)-**1gg**, 77495-23-9; (±)-**1hh**, 340-05-6; (±)-**1ii**, 13323-81-4; (±)-**1jj**, 613-86-5; (±)-**1kk**, 63180-93-8; (±)-**1ll**, 57377-60-3; (±)-**1mm**, 21632-19-9; (±)-**1nn**, 63226-80-2; (±)-**1oo**, 21632-21-3; (±)-**1pp**, 63180-92-7; (±)-**1qq**, 77549-72-5; (±)-**1rr**, 25675-30-3; (±)-**1ss**, 77495-24-0; (*R*)-**3a**, 74927-72-3; (*L*)-**3b**, 77495-25-1; (*L*)-**3c**, 7495-01-4; (*R*)-phenylglycine, 875-74-1; (*L*)-valine, 72-18-4; (*L*)-leucine, 61-90-5.

(9) For examples, see: (a) W. H. Pirkle and J. R. Hauske, *J. Org. Chem.*, **42**, 1839 (1977); (b) W. H. Pirkle and T. G. Burlingame, *Tetrahedron Lett.*, 4039 (1967); (c) M. S. Pavlin, Ph.D. Thesis, University of Illinois, 1977.