HPLC-Based Method for Determination of Absolute Configuration of α-Chiral Amines

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We introduce a novel, HPLC-based method for facile determination of the absolute configuration of α-chiral amines. Our method is easily applied to a variety of compounds, including amino acid derivatives. The method involves initial derivatization of the chiral amine analyte with the chiral derivatizing reagent, *N*-succinimidyl α-methoxyphenylacetate (SMPA), to produce the corresponding diastereomeric adducts. Inspection of a particular rotomer of the SMPA adduct and application of simple rules correlates absolute configuration and HPLC elution order. A key aspect of our method is that it can be used to determine absolute configuration without using enantiomeric standards of the amine analytes. Furthermore, it is of utmost significance that our method can also be used to determine absolute configuration even when only one analyte enantiomer of unknown absolute configuration is present, as is often the case for enzymatic products, naturally derived compounds, or enantiomerically enriched compounds prepared via chiral syntheses. We have observed strict adherence between predicted and observed absolute configuration for a wide variety of α-chiral amines. The chromatographic method we present in this paper is very practical and has several important advantages over NMR-based approaches which have been previously developed. For example, microgram quantities of an analyte in a complex enzymatic mixture can be directly analyzed by our HPLC-based method while the impurities often preclude definitive proton assignments in the NMR approach.

Despite a long-standing awareness of the stereoselective nature of drug interactions in animals and man, synthetic drugs that contain one or more chiral centers are still developed and marketed as racemates.[1] It has been estimated that between 1983 and 1985, 95% of chiral drugs were introduced as racemates.[1] Uncertainties over the possibility of new regulatory requirements pertaining to drug stereochemistry and over the continued acceptability of racemic drugs by worldwide regulatory agencies are causing a reassessment of established developed strategies and planning for drugs currently under consideration. Thus, since the guidelines recently issued by the Food and Drug Administration require rigorous justification for FDA approval of racemates, many pharmaceutical companies have decided to limit future chiral drug development to single enantiomers.[2] Limitations in separation technologies have been among the most influential factors affecting stereoisomeric drug development. Thus, the critical need for analytical methods applicable to the development and control of processes leading to enantiomerically pure chiral drugs is becoming a pivotal element.

Raban and Mislow[3] first reported the general utility of *O*-methyl mandelates for determining enantiomeric purities and as resolving agents. Dale and Mosher[4] examined the use of *O*-methyl mandelyl chloride in further detail and developed an NMR model to predict absolute configuration. Their approach was based on a conformational model that rationalized diastereomeric NMR nonequivalence. We had previously introduced the chiral derivatizing reagent (*S*)-*N*-succinimidyl α-methoxyphenylacetate [(*S*)-SMPA] and demonstrated its utility in HPLC-based resolutions.[5] We demonstrated the utility of SMPA for the preparative separation of enantiomers from racemates, the determination of optical purity, the isolation and resolution of chiral amine-containing compounds from complex enzymatic assay mixtures, and the subsequent recovery of the resolved enantiomers in high optical purity.

With the determination of absolute configuration being traditionally limited to the relatively laborious method of X-ray crystallography, the development of alternative analytical and preparative methods for the determination of absolute configuration would be a significant contribution. HPLC has been used extensively for the determination of enantiomeric composition through the use of chiral mobile phases,[7-14] chiral stationary phases,[15-19] or derivatization by enantiomerically pure reagents resulting in resolvable diastereomeric adducts.[20-24] Additionally, chiral stationary phases have been used to identify the absolute configurations

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of analytes through an elution order comparison with enantiomeric standards of closely related analogs; the elution order of the analyte is assumed to be correlated with that of a known enantiomer of the analog. Obviously, this approach is limited to analytes where enantiomeric standards of structurally similar analogs are available. In contrast, HPLC has not been used for determining the absolute configurations of chiral compounds via diastereomeric adducts, when enantiomeric analyte standards are not available. In this paper we introduce a new method for facile determination of absolute configuration of a variety of α-chiral amines. The method involves SMPA derivatization followed by application of a simple model. A key aspect of our method is that it can be used to determine absolute configuration without the need for enantiomeric standards of the analyte or closely related analogs. We demonstrate the applicability of this method to a number of compounds—including amino acids— and we anticipate that it will be generally applicable to a wide variety of α-chiral amines.

EXPERIMENTAL SECTION

Methods. NMR spectra were obtained on a Gemini 300-MHz NMR spectrometer. Mass spectra were obtained on a Finnigan MAT 112S instrument with a SS200 data system. A Laboratory Data Control (Riviera Beach, FL) high-performance liquid chromatograph was used for separations of diastereomers, with a Constametric III pump and a Spectro Monitor III (Model 1204D) variable-wavelength detector. Separations were achieved using either a 25-cm (21.4-mm i.d.) Dynamax Macro semipreparative silica column or a 25-cm silica column (5 m, 4.6-mm i.d., analytical) from Rainin (Woburn, MA). Microanalyses were performed by Atlantic Microlabs, Atlanta, GA.

Syntheses. (*S*)-*N*-Succinimidyl α-methoxyphenylacetate [(*S*)-SMPA] was synthesized by the dropwise addition of 5.46 g (26.5 mmol, 1.1 equiv) of 1,3-dicyclohexylcarbodiimide in 30 mL of dry THF to a stirred solution of 4.0 g (24.1 mmol) of 1-phenyl-1-(*S*)-methoxyacetic acid and 2.77 g (24.1 mmol) of *N*-hydroxysuccinimide in 20 mL of dry THF under an argon atmosphere at room temperature. The solution was stirred for 3 h, over the course of which a precipitate (dicyclohexylurea) appeared. After filtration, the THF solution was evaporated to dryness and the residue redissolved in 50 mL of ethyl acetate (EtOAc). The extract was washed five times with H_2O (10 mL), five times with brine (10 mL), and twice, quickly, with ice-cold saturated $NaHCO_3$ (10 mL). In order to avoid hydrolysis of the ester, it was essential that the base washes be accomplished quickly and at reduced temperatures. The washed EtOAc extract was then evaporated to dryness and the SMPA recrystallized from hot ethanol to give 5.5 g of the product (22.1 mmol, 92% yield): mp 95.7–96.3 °C; $[\alpha]^{25} = +125°$ ($c = 3$, ethanol); ^{1}H NMR (acetone-d_6) δ 7.6 (m, 5 H), 5.33 (s, 1 H), 3.55 (s, 3 H), 2.9 (s, 4 H). Anal. Calcd for $C_{13}H_{13}NO_5$: C, 59.30; H, 4.99; N, 5.32. Found: C, 59.23; H, 5.02; N, 5.29.

(*R*)-*N*-Succinimidyl α-methoxyphenylacetate [(*R*)-SMPA] was synthesized from 1-phenyl-1-(*R*)-methoxyphenylacetic acid as described above for the *S* isomer: mp 98–99 °C; $[\alpha]^{25} = -130°$ ($c = 2$, ethanol); ^{1}H NMR (acetone-d_6) δ 7.6 (m, 5 H), 5.3 (s, 1 H), 3.6 (s, 3 H), 2.8 (s, 4 H).

(*R,S*)-2,4-Dimethyl-2-oxazoline. This compound was synthesized according to the general method of Meyers et al.[25] (*R,S*)-2-Amino-1-propanol (70 mmol) was added to a stirred mixture of ethyl acetimidate hydrochloride (87 mmol) and dry methylene chloride (60 mL) at 0 °C under argon. The resulting solution was stirred at 0 °C for 6 h and was then poured into ice/water (100 mL). The aqueous phase was extracted 3× with 20 mL of CH_2Cl_2, and the combined extracts were dried over Na_2SO_4. The solution was distilled through a 6-in. column to yield the product (55%): bp 109–112 °C; ^{1}H NMR ($CDCl_3$) δ 4.39–3.48 (m, 3 H), 1.89 (s, 3 H), 1.17 (d, 3 H).

(*R*)- and (*S*)-2,4-Dimethyl-2-oxazoline. These compounds were synthesized by the above procedure except that either (*R*)- or (*S*)-2-amino-1-propanol was employed. ^{1}H-NMR data were identical to those above.

(*R,S*)-1-(Phenylthio)-2-aminopropane Hydrochloride. This compound was prepared by a modification of the method of Wehrmeister.[26,27] (*R,S*)-2,4-Dimethyl-2-oxazoline (36 mmol) was added to thiophenol (40 mmol) under argon. After an initial exothermic reaction, the solution was warmed to 80–100 °C for 6 h. HCl (300 mL of 6 N) was added to the cooled reaction mixture, which was then refluxed for 6 h. The resulting solution was washed with $CHCl_3$ (3 × 50 mL) and the aqueous layer evaporated to dryness, yielding a white solid. The crude product was purified by recrystallization from ethanol/ether to yield the product (85%): mp 154–155.5 °C; ^{1}H NMR (D_2O) δ 7.45 (m, 5 H), 3.72–3.12 (m, 3 H), 1.38 (d, 3 H); mass spectrum (EI) *m/e* 167 (M^+); (CI) *m/e* 168 (M + 1). Anal. Calcd for $C_9H_{13}NS$·HCl: C, 53.06; H, 6.93; N, 6.87. Found: C, 52.99; H, 6.97; N, 6.86.

(*R*)-1-(Phenylthio)-2-aminopropane was synthesized from the appropriate chiral oxazoline and thiophenol using the synthetic procedure outlined above for (*R,S*)-1-(phenylthio)-2-aminopropane: $[\alpha]^{25}_D = -16.4°$ ($c = 1.0$, H_2O); mp 111–112 °C; ^{1}H-NMR data were identical to those of (*R,S*)-1-(phenylthio)-2-aminopropane. Anal. Calcd for $C_9H_{13}NS$·HCl: C, 53.06; H, 6.93; N, 6.87. Found: C, 53.12; H, 6.96; N, 6.85.

(*S*)-1-(Phenylthio)-2-aminopropane was synthesized from the appropriate chiral oxazoline and thiophenol using the synthetic procedure outlined above for (*R,S*)-1-(phenylthio)-2-aminopropane: $[\alpha]^{25}_D = +18.8°$ ($c = 1.1$, H_2O); mp 110–111 °C; ^{1}H-NMR data were identical to those of (*R,S*)-1-(phenylthio)-2-aminopropane. Anal. Calcd for $C_9H_{13}NS$·HCl: C, 53.06; H, 6.93; N, 6.87. Found: C, 52.97; H, 6.94; N, 6.87.

(*R,S*)-1-(Phenylseleno)-2-aminopropane was synthesized from the appropriate chiral oxazoline and benzeneselenol using the synthetic procedure outlined above for (*R,S*)-1-(phenylthio)-2-aminopropane: mp 145–146 °C; ^{1}H-NMR data were identical to those of (*R,S*)-1-(phenylthio)-2-aminopropane. Anal. Calcd $C_9H_{13}NSe$·HCl: C, 43.13; H, 5.63; N, 5.59. Found: C, 43.22; H, 5.64; N, 5.56.

(*R*)-1-(Phenylseleno)-2-aminopropane was synthesized from the appropriate chiral oxazoline and benzeneselenol using the synthetic procedure outlined above for (*R,S*)-1-(phenylthio)-2-aminopropane: mp 92–93 °C; ^{1}H-NMR data were identical to those of (*R,S*)-1-(phenylthio)-2-aminopropane. Anal. Calcd for $C_9H_{13}NSe$·HCl: C, 43.13; H, 5.63; N, 5.59. Found: C, 43.00; H, 5.68; N, 5.58.

(*S*)-1-(Phenylseleno)-2-aminopropane was synthesized from the appropriate chiral oxazoline and benzeneselenol using the synthetic procedure outlined above for (*R,S*)-1-(phenylthio)-2-aminopropane: mp 90–92 °C; ^{1}H-NMR data were identical to those of (*R,S*)-1-(phenylthio)-2-aminopropane. Anal. Calcd for $C_9H_{13}NSe$·HCl: C, 43.13; H, 5.63; N, 5.59. Found: C, 42.94; H, 5.70; N, 5.53.

Derivatization of α-Chiral Amines. Our typical procedure for derivatization of the various α-chiral amines was as follows. To a 1-mL reaction vial containing 0.8 mL of THF and 0.2 mL of H_2O were added 30 mmol of the amine hydrochloride and 15.6 mg (60 mmol) of (*S*)-SMPA. After addition of 4.1 mL (29.5 mmol) of triethylamine, the vial was sealed, heated on a steam bath for 15 min, quenched by addition of 0.5 mL of saturated sodium bicarbonate, and subsequently stored at −20 °C until analyzed.

In all cases, the identity of each diastereomer produced from the reaction of the various amine-containing compounds with (*S*)-SMPA was established as follows. First, the SMPA adduct of the racemic amine was injected onto an analytical silica HPLC column. In all cases, two peaks were observed which were found to comigrate with the SMPA derivative of either the authentic *R* or *S* enantiomer of the chiral amine. The various mobile phases employed and the capacity factors obtained are listed in Table I. Direct confirmation of the identity of the SMPA adduct was obtained for each enantiomer of all of the amines by isolation of

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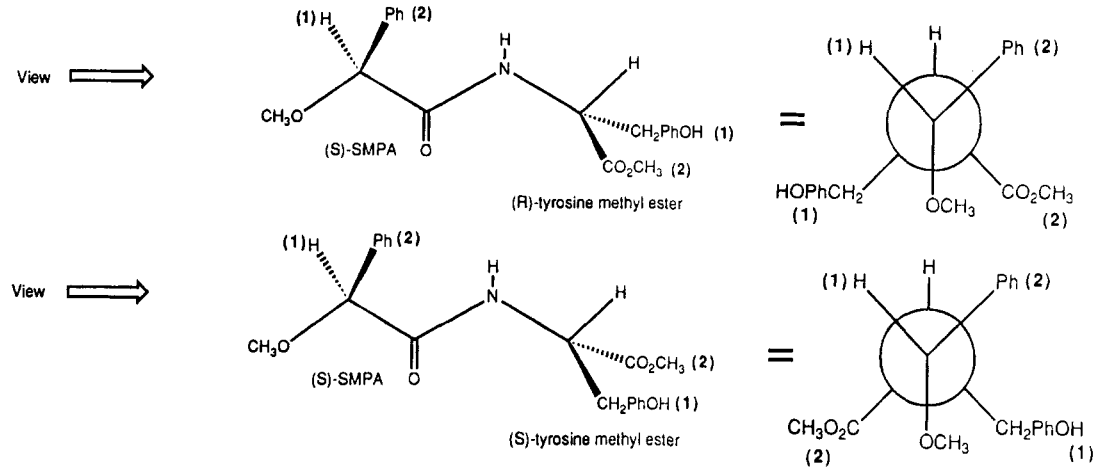


Figure 1. Extended conformations and Neuman projections for (top) (*R*)- and (bottom) (*S*)-tyrosine methyl ester derivatized with (*S*)-SMPA. The Neuman projections are visualized by viewing the extended conformation along the indicated axis. The substituents at each chiral center are labeled 1 or 2 using the criteria presented in the text.

the adducts using preparative-scale HPLC followed by mass spectral analysis. The mass spectral data obtained for each of the compounds listed in Table I is as follows. (Entry numbers correspond to the structures listed in Table I.) MS (CI) *m/z*: 1, 364 (MH^+, base); 2, 316 (MH^+, base); 3, 222 (MH^+, base); 4, 270 (MH^+, base); 5, 224 (MH^+, base); 6, 238 (MH^+, base); 7, 286 (MH^+, base); 8, 344 (MH^+, base); 9, 328 (MH^+, base); 10, 252 (MH^+, base).

RESULTS AND DISCUSSION

The use of *O*-methyl mandelate derivatives for determining enantiomeric purities and as resolving agents was first described by Rabon and Mislow.[3] Subsequently, Dale and Mosher[4] demonstrated the use of several mandelyl derivatizing agents, including *O*-methyl mandelyl chloride, for predicting absolute configuration of α-chiral amines and alcohols. Their approach makes use of NMR spectroscopy, and it is based on a conformational model whereby diastereomeric NMR nonequivalence is correlated to absolute configuration. In order to utilize the method of Dale and Mosher, one particular conformation of the diastereomeric adduct formed between *O*-methyl mandelyl chloride and the chiral amine or alcohol is examined and correlated with NMR data. The conformer on which the assignment of absolute configuration is made is used solely for the purpose of correlating proton nonequivalence to absolute configuration, and it does not necessarily represent the ground-state configuration of the molecule in question.

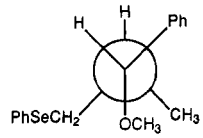
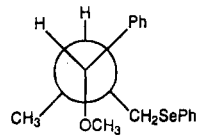
Herein, we introduce a new HPLC-based method for facile determination of absolute configuration of a variety of α-chiral amines. The compounds listed in Table I were used to develop this method, which entails correlating absolute configuration and HPLC elution order. First, each enantiomer of each amine was derivatized with (*S*)-SMPA to produce the corresponding diastereomeric adducts. Inspection of the various conformers of these adducts in light of previous conclusions from NMR studies led us to postulate a simple empirical model for predicting the HPLC elution order of the adducts from each pair of amine enantiomers. HPLC experiments were then carried out, and the predicted and experimentally determined elution orders were compared. In every case, the observed elution order was in complete agreement with that predicted from the model. We wish to emphasize that, as is the case for the NMR-based method of Dale and Mosher, our method utilizes a particular conformer of the diastereomeric adducts solely for the purpose of predicting absolute configuration from elution order, and not necessarily as a representation of the true ground-state conformation of the adducts.

The procedure for employing our method is as follows. The SMPA adduct of the amine of interest is examined, and the substituents α to the amide nitrogen and to the carbonyl are designated as either 1 or 2, based on their relative size and hydrogen bond donor capability. A substituent containing a functionality capable of being a hydrogen bond donor is always designated as 1; otherwise, the smaller group is designated as 1 and the larger group 2. The SMPA adducts from each chiral amine enantiomer are then rotated into the conformation shown in Figure 1 and compared in order to predict elution order. The diastereomer which contains both of the groups designated 1 on the same side of the amide bond is predicted to elute last.

As an example of the application of this method, consider the (*S*)-SMPA derivative of tyrosine methyl ester, shown in Figure 1. Focusing first on the (*S*)-SMPA-derived half of the adduct, the phenyl substituent is larger than the hydrogen substituent, and neither group has hydrogen bond donor capability; therefore, the hydrogen is assigned a priority number of 1. (This holds for all of the compounds listed in Table I, since they are all adducts formed from derivatization with SMPA.) Turning to the portion of the adduct derived from tyrosine methyl ester, the substituents to be compared are the *p*-hydroxybenzyl and carbomethoxy functionalities. Although the carbomethoxy group is the smaller of the two, the phenolic hydroxy group has hydrogen bond donor capability and consequently is assigned the priority number 1. Figure 1 shows that both substituents with priority number 1 are on the same side when the tyrosine has the *R* configuration; therefore, we predict that this diastereomer will elute last. This prediction was tested by derivatizing both (*R*)- and (*S*)-tyrosine esters with (*S*)-SMPA and chromatographing the resulting diastereomers. As shown in Figure 2, derivatized (*R*)-tyrosine methyl ester indeed elutes after the adduct from (*S*)-tyrosine methyl ester.

It is important to emphasize that determination of absolute configuration using our method does *not* require the availability of enantiomeric standards of the amine analyte. Moreover, a key aspect of our method is that it can be used both in cases where the sample to be analyzed is a racemate and in cases where the analyte is a single stereoisomer of unknown absolute configuration (see below). The procedure for determining absolute configuration when the racemate is available is outlined in Figure 3 for the case of 2-amino-1-butanol. First, the racemate is derivatized with (*S*)-SMPA, and then the resulting diastereomers are chromatographed. The SMPA-derivatized diastereomer of each chirality is drawn in the proper conformation, as depicted in Figure 3, and the elution order is then deduced using the previously defined

Table I. Configurational Correlation of (*S*)-SMPA Derivatives

entry	conformational views of diastereomeric pair: faster eluting isomer[a]	conformational views of diastereomeric pair: slower eluting isomer[a]	mobile phase[b]	capacity factors[a,c]: k'[d]	capacity factors[a,c]: k'[e]	config of faster eluting isomer[f]
						
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	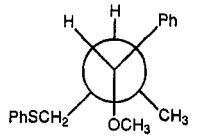	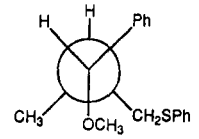				
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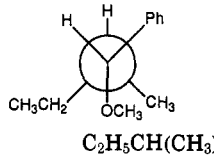
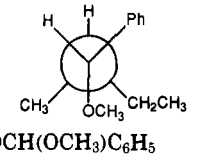
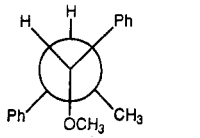
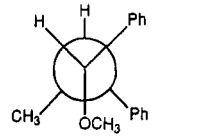
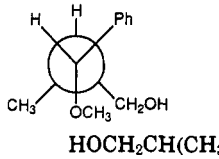
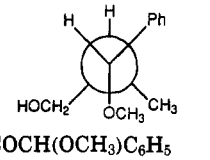
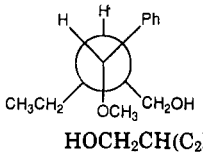
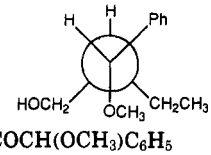
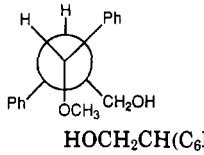
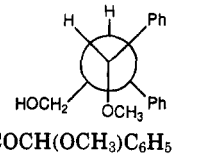
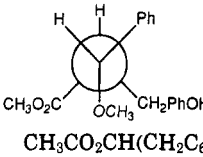
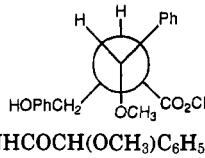
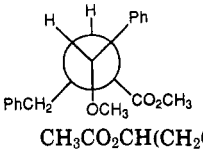
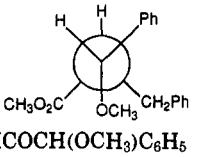
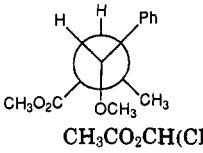
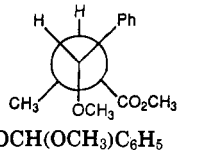
						
3	$C_2H_5CH(CH_3)NHCOCH(OCH_3)C_6H_5$		3:1	3.0	2.3	*S*
						
4	$C_6H_5CH(CH_3)NHCOCH(OCH_3)C_6H_5$		3:1	2.9	1.6	*S*
						
5	$HOCH_2CH(CH_3)NHCOCH(OCH_3)C_6H_5$		1:1	9.8	5.7	*R*
						
6	$HOCH_2CH(C_2H_5)NHCOCH(OCH_3)C_6H_5$		1:1	9.5	2.6	*R*
						
7	$HOCH_2CH(C_6H_5)NHCOCH(OCH_3)C_6H_5$		1:1	8.3	2.2	*R*
						
8	$CH_3CO_2CH(CH_2C_6H_4OH(NHCOCH(OCH_3)C_6H_5$		2:1	2.8	1.7	*S*
						
9	$CH_3CO_2CH(CH_2C_6H_5)NHCOCH(OCH_3)C_6H_5$		3:1	3.3	2.0	*R*
						
10	$CH_3CO_2CH(CH_3)NHCOCH(OCH_3)C_6H_5$		3:1	3.8	3.2	*S*

[a] Determined using authentic samples of each amine enantiomer. [b] The mobile phase is expressed as a ratio of hexane to ethyl acetate. Flow rate was 1.0 mL/min, and the column utilized was an analytical silica gel column using UV at 254 nm as the method of detection. [c] The reported parameters were calculated from chromatograms where measurements of peak retention time and void time were used to calculate the appropriate parameter. [d] The capacity factor of the slower eluting isomer. [e] The capacity factor of the faster eluting isomer. [f] In all cases, the predicted and experimentally determined elution orders were in complete agreement.

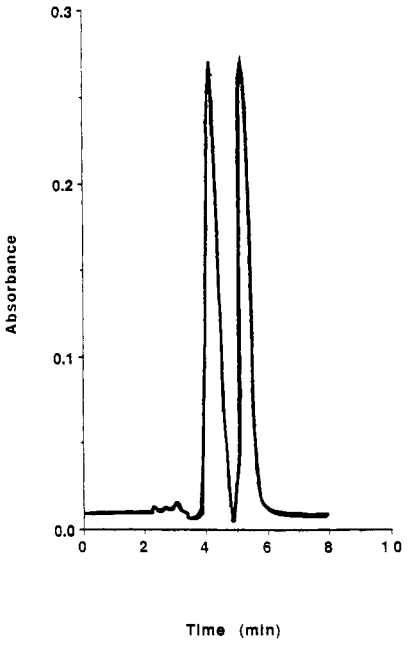


Figure 2. Resolution of (*R,S*)-tyrosine methyl ester on HPLC after (*S*)-SMPA derivatization. A 20-μL aliquot from the reaction of (*R,S*)-tyrosine methyl ester with excess (*S*)-SMPA was chromatographed on an analytical silica gel column. The peaks, with retention times of 4.5 and 6 min, were assigned as derivatized (*S*)- and (*R*)-tyrosine methyl esters, respectively, by comparison with the (*S*)-SMPA derivatives of authentic samples of (*S*)- and (*R*)-tyrosine esters. These experimental results correlate with the predictions of the model presented in this paper.

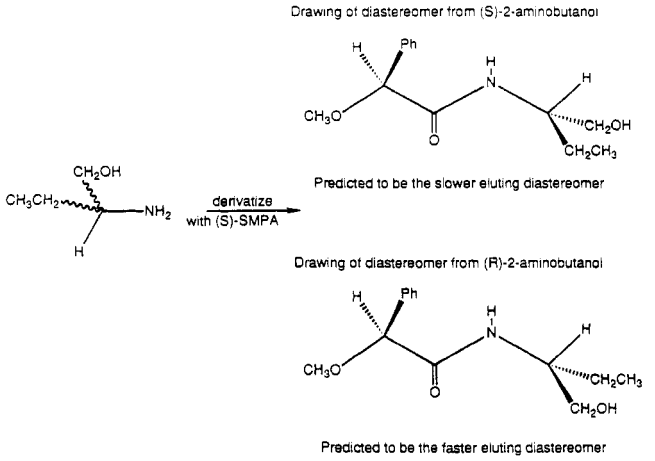


Figure 3. Determination of absolute configuration for an α-chiral amine in cases where the racemate is available. The derivatized enantiomers are rotated to the conformation shown above and assigned as the faster or slower eluting isomer, using the model discussed in the text. The derivatized analyte enantiomer coelutes with one of the two peaks from the derivatized racemate.

rules. In this example, the hydrogen and hydroxymethyl substituents—the groups assigned priority number 1 at the two chiral centers, respectively—are on the same side for the adduct derived from (*S*)-2-amino-1-butanol; the slower eluting and faster eluting peaks are therefore assigned to correspond to the SMPA derivatives of (*S*)- and (*R*)-2-amino-1-butanol, respectively. Thus, the absolute configuration of a 2-amino-1-butanol sample of unknown chirality is easily determined by simply spiking the derivatized racemate with the derivatized analyte. We emphasize that all that is needed for this type of determination is the racemate and the analyte of unknown chirality: No enantiomeric standards whatsoever are required.

The above example obviously holds for cases where the racemate of the amine of interest is in hand. However, very

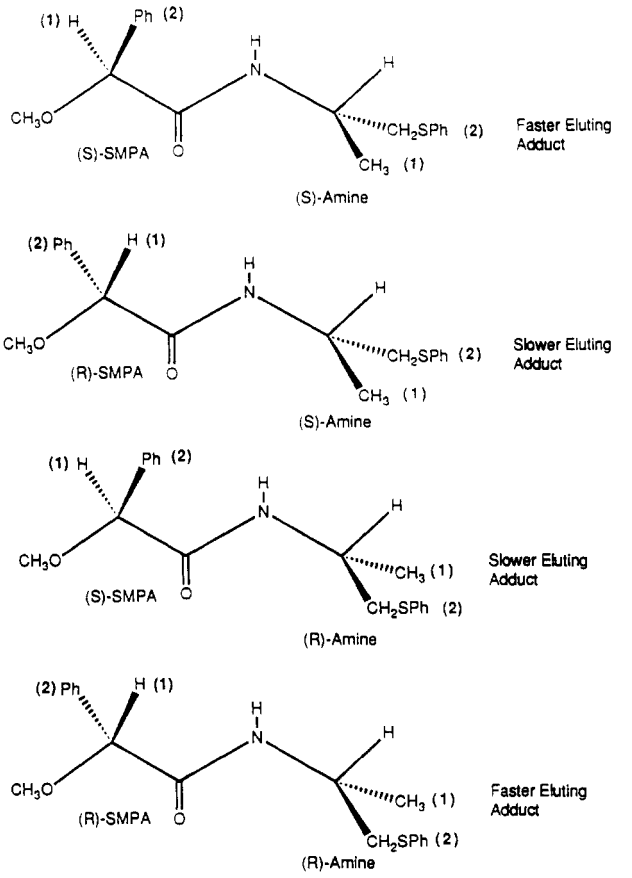


Figure 4. Determination of absolute configuration for an α-chiral amine in cases where only a single sample of unknown configuration is available. In this case (top) (*S*)- and (bottom) (*R*)-1-(phenylthio)-2-aminopropanes are derivatized with both (*R*)- and (*S*)-SMPA. The model predicts that, for the (*S*)-amine, the adduct formed from (*R*)-SMPA will be the slower eluting isomer, whereas for the (*R*)-amine, the adduct formed from (*S*)-SMPA will be the slower eluting isomer.

often only a *single* sample of unknown configuration is available, as is usually the case for enzymatic products or for compounds prepared via chiral syntheses. *It is therefore of utmost significance that our method can be used to determine absolute configuration even when only one enantiomer of the α-chiral amine is available. In such a case, absolute configuration is determined by comparing the adducts formed upon derivatization with both (R)- and (S)-SMPA.* In practice, rotomers of all four possible adducts—arising from derivatization of both amine enantiomers with (*R*)- and (*S*)-SMPA—are inspected and the elution orders predicted as described above. For one of the amine enantiomers, the (*R*)-SMPA adduct will be predicted to elute before the (*S*)-SMPA adduct; the opposite elution order will be predicted for the other amine enantiomer. The experiment is then performed; i.e., the amine sample of unknown configuration is derivatized with both (*R*)- and (*S*)-SMPA and chromatographed. The experimentally observed elution pattern will fit only one of the two predictions. Thus, the absolute configuration of the chiral amine of interest will have been deduced.

An example of such an absolute configuration determination from a single analyte enantiomer is illustrated in Figure 4 for the case of 1-(phenylthio)-2-aminopropane (entry 2 of Table I). The figure depicts the requisite conformations of the diastereomers formed from derivatization of (*R*)- and (*S*)-1-(phenylthio)-2-aminopropane with (*S*)- and (*R*)-SMPA, respectively. Using our rules, and recognizing that none of the substituents are hydrogen bond donors, priority number 1 is assigned to the hydrogen substituent on the SMPA-derived half of the adduct, and to the methyl substituent on

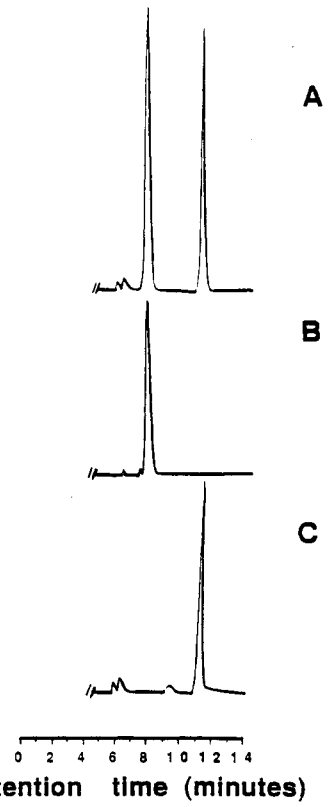


Figure 5. Elution profiles for (*S*)-1-(phenylthio)-2-aminopropane derivatized with (*R*)- and (*S*)-SMPA. (A) HPLC chromatogram of (*S*)-1-(phenylthio)-2-aminopropane after derivatizing with racemic SMPA. (B) HPLC chromatogram of the (*S*)-SMPA derivative of (*S*)-1-(phenylthio)-2-aminopropane, t_r = 8.5 min. (C) HPLC chromatograph of the (*R*)-SMPA derivative of (*S*)-1-(phenylthio)-2-aminopropane, t_r = 11.5 min. After the derivatizations were performed as described in the Experimental Section, a 10-μL aliquot of the solution was injected onto a silica column (4.6 × 250 mm) with 3:1 hexane/ethyl acetate as the eluent at a flow rate of 1 mL/min. Detection was at 254 nm.

the amine-derived portion of the adduct. It is evident that, for the (*S*)-amine, the adduct formed from (*R*)-SMPA is predicted to elute last, whereas the opposite elution order [i.e., the adduct from (*S*)-SMPA eluting last] is predicted for the (*R*)-amine. The predictions of Figure 4 are validated by the experimental results in Figure 5, which shows the elution profiles actually obtained for the derivatives of (*S*)-1-(phenylthio)-2-aminopropane with either (*S*)- or (*R*)-SMPA. It is evident that the observed elution order of the adducts is indeed in complete accord with the predictions of our model. Thus, this example illustrates how derivatization with both (*R*)- and (*S*)-SMPA allows determination of absolute configuration even in a case where only one enantiomer of the α-chiral amine of interest is available.

We wish to emphasize that our model depicts a particular conformation of the amine–SMPA adduct solely for the purpose of predicting elution order, and it should be clearly understood that this conformer does not necessarily represent the ground state of the molecule. However, the conformer employed in the model is consistent with accepted literature. The eclipsing of the methoxy and carbonyl groups has been observed[4,6] and may be a reflection of favorable interactions between these functionalities.[4] The geometry of the amide bond is well-known to be trans,[28] as depicted in Figures 1, 3, and 4, in all but the rarest cases; the resonance energy of 20 kcal/mol[28] precludes facile cis/trans isomerization. Furthermore, as depicted, the dihedral angle between the amide N–H and the α-chiral C–H is at an energy minimum.[28] Therefore, the rotomer employed in the model would be expected to represent an energetically favorable conformation.

The method described here was tested by examining both the racemates and the individual *R* and *S* enantiomers of 10 different compounds, and the results are summarized in Table I. As shown in the table, in all cases the experimental results were in complete agreement with the prediction of our model. Entries 1–4 illustrate examples where the relative size of the substituents is the primary factor affecting elution order. The powerful effects of hydrogen bond donor substituents are illustrated in entries 5–8. Finally, entries 9 and 10 illustrate that the presence of a hydrogen bond *acceptor* substituent has no effect on the correlation between elution order and absolute configuration.

It is clear from the results presented in Table I that elution order changes with respect to absolute configuration even though the compounds are stereotopically equivalent. For example, if the *R* configurations of the amines derived from entries 2 and 5 are superimposed so that the methyl and hydrogen groups of each correspond, the hydroxymethyl and the thiophenol groups are stereotopically equivalent. Since both these substituents are assigned priority number 1 in the *R,S* nomenclature system, no change in specification of absolute configuration has occurred. However, opposite elution orders are predicted for the *R* and *S* enantiomers of these two amines, because of the hydrogen bond donor capability of the hydroxymethyl group, despite the fact that it is larger than the methyl substituent in entry 5. Similarly, comparison of entries 8 and 9 underscores the remarkable effect of the hydroxyl moiety—even when it is present as a substituent on a large phenyl ring—on HPLC retention. The large phenyl group of entry 9 probably inhibits interactions with silica, but the phenolic hydroxyl group of entry 8 overwhelms any steric considerations, resulting in a reversal in the elution order of the amine enantiomers.

In conclusion, the chromatographic method we present in this paper is very practical and has several important advantages over the NMR-based approach previously developed by Dale and Mosher.[4] NMR techniques typically require milligram quantities of the analyte, whereas our HPLC-based method requires only microgram to nanogram quantities of analyte. Furthermore, NMR requires that the sample be very pure, which often proves to be a difficult task. In contrast, resolution of the individual components of complex mixtures is routinely achieved using HPLC.[29,30] Indeed, we have successfully used our chromatographic approach directly on enzymatic reaction mixtures. Finally, complicated NMR splitting patterns often preclude definitive assignments of pertinent protons and therefore render an absolute configuration assignment ambiguous; in practice, unless the proton of interest is a singlet, assignments may become prohibitively difficult. Therefore, we anticipate that our HPLC-based method will be generally applicable to a wide variety of α-chiral amines.

ACKNOWLEDGMENT

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