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Short communication

(*S*)-(+)-1-Methyl-2-(6,7,-dimethoxy-2,3-naphthalimido)ethyl trifluoromethanesulfonate as a fluorescence chiral derivatizing reagent for carboxylic acid enantiomers in high-performance liquid chromatography

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Abstract

A new triflate-type fluorescence chiral derivatizing reagent, (*S*)-(+)-1-methyl-2-(6,7-dimethoxy-2,3-naphthalimido)ethyl trifluoromethanesulfonate, [*S*-(+)-MDNE-OTf], has been developed for the determination of the enantiomers of carboxylic acids. By introducing the two methoxy groups on the naphthalimido ring moiety, the red shift in the fluorescence spectrum and a high resolution in reversed-mode separation of the diastereomers of chiral carboxylic acids have been achieved. The detection limits ($S/N=3$) with ultraviolet and fluorescence detection are 8 fmol ($\lambda_{max}=283$ nm) and 4 fmol ($\lambda_{ex}=283$ nm, $\lambda_{em}=467$ nm), respectively.

Keywords: Enantiomer separation; Derivatization, LC; (*S*)-(+)-1-Methyl-2-(6,7,-dimethoxy-2,3-naphthalimido)ethyl trifluoromethanesulfonate; Carboxylic acids

1. Introduction

The two enantiomers of a natural chiral compound or a chiral synthetic drug can substantially differ in their pharmacological activity, depending on their absolute configuration. Often, only one of the enantiomers is pharmacologically active, while the other may be inactive or even harmful. In analytical chemistry, the analysis of enantiomers is therefore gaining ever more interest. High-performance liquid chromatography (HPLC) has been widely used for the separation of enantiomers. There are three general procedures for the enantiomeric separation of chiral compounds by HPLC: (a) direct separation on a chiral stationary phase (CSP), (b) direct separation on an achiral stationary phase with a chiral mobile phase additive, and (c) separation as the diastereomers derivatized with a chiral reagent on an achiral stationary phase. Although direct separation using a CSP column is a straightforward method, the CSP columns have disadvantages, such as low column efficiency, higher cost, over conventional ODS columns. On the other hand, chiral derivatization followed by HPLC is a versatile method for the separation of enantiomers having reactive functional groups. Many chiral derivatizing reagents have been developed for the chromatographic separation of various enantiomers [1–8].

We have previously developed (*S*)-(+)-1-methyl-2-(2,3-naphthalimido)ethyl trifluoromethanesulfonate

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[*S*-(+)-MNE-OTf] as a derivatizing reagent for chiral separation of carboxylic acid enantiomers in HPLC [9]. This reagent is highly reactive toward carboxylic acids even at room temperature. No racemization of the acids thus occurs during their derivatization, and the resulting diastereomers having intense fluorescence at 394 nm can be well separated in reversed-phase HPLC. In the case of a direct analysis of a biological sample, it is more favorable to obtain fluorescence in the longer wavelength ranges because naturally occurring metabolites such as purines show their fluorescence near 390 nm. In a previous paper, we have also reported that the introduction of two methoxy groups at the 6- and 7-positions of the 2,3-naphthalimido ring has resulted in a red shift in the fluorescence spectrum and reduced retention times of the derivatives in reversed-mode separation [10]. In this paper, we describe a new triflate-type chiral derivatizing reagent for carboxylic acid enantiomers, *S*-(+)-MDNE-OTf.

2. Experimental

2.1. Apparatus

The HPLC system was comprised of a Tosoh CCPS pump, a Rheodyne Model 7725 injector valve, a Tosoh UV-8020 spectrophotometer, a Hitachi F-1050 fluorescence spectrophotometer, and a Tosoh Chromatocorder 21 integrator. The analytical column used was a Wakosil-II-RS (5 μm, 150×4.6 mm I.D.). The melting points were measured with a Yanaco melting-point apparatus and were uncorrected. The fluorescence spectra were measured with a Shimazu RF-5300PC. The optical rotations were measured with a Perkin-Elmer 241 Polarimeter. An Erma ERC-3510 degasser was utilized for continuous degassing of the mobile phase.

2.2. Reagents and materials

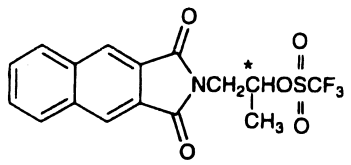
(*S*)-(+)-1-Amino-2-propanol (98%), (*S*)-(+)-ibuprofen (99%), potassium carbonate (99.99%) and potassium fluoride (99.99%) were purchased from Aldrich (Milwaukee, WI, USA). *tert*.-Butyloxycarbonyl-L-alanine (enantiomeric purity >99.8% ee) was obtained from Peptide Institute (Osaka, Japan). A Chiralcel OD column (5 μm, 250×4.6 mm I.D.) was obtained from Daicel (Osaka, Japan). HPLC grade acetonitrile was purchased from Wako (Osaka, Japan) and used in the preparation of the chromatographic mobile phases. All of the other chemicals were purchased from Aldrich and Tokyo Kasei (Tokyo, Japan). Water was purified using a Milli-Q water purification unit (Millipore, Beford, MA, USA). Eppendorf Safe-Lock micro-centrifuge tubes (2.0 ml) were used as reaction tubes.

2.3. Synthesis

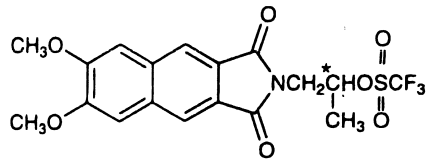
6,7-Dimethoxy-2,3-naphthalenedicarboxylic anhydride was prepared from 1,2-dimethoxybenzene according to a literature method [11,12]. (*S*)-(+)-1-Methyl-2-(6,7-dimethoxy-2,3-naphthalimido)ethanol [*S*-(+)-MDNE-OH] was synthesized as follows: in a 300-ml flask fitted with a water separator and a reflux condenser were placed 1.5 g (5.8 mmol) of 6,7-dimethoxy-2,3-naphthalenedicarboxylic anhydride, 0.52 g (7 mmol) of (*S*)-(+)-1-amino-2-propanol and 300 ml of dry toluene. The mixture was heated for 2 h with vigorous refluxing in an oil bath. After cooling, the solid product was filtered and then washed with three 50-ml portions of cold water. Two recrystallizations from an ethanol–water mixture gave transparent needles of *S*-(+)-MDNE-OH: yield 87.4%; m.p. 293°C; $[\alpha]_D^{20} = +18°$ (*C*=1, dichloromethane); anal. calculated for $C_{17}H_{17}NO_5$ 64.76% C, 5.40% H, 4.44% N; found: 63.92% C, 5.42% H, 4.34% N.

The resulting *S*-(+)-MDNE-OH was further enantiospecifically purified on a Chiralcel OD column with an eluent of hexane–2-propanol (8:2) and the combined effluents was evaporated under reduced pressure.

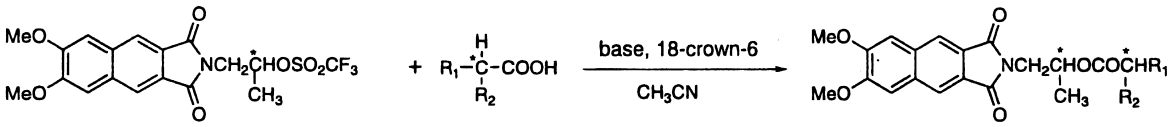
To a solution of trifluoromethanesulfonic anhydride (0.56 g, 2 mmol) in dichloromethane (50 ml) was carefully added dropwise a mixture of pyridine (0.16 g, 2 mmol) and *S*-(+)-MDNE-OH (0.5 g, 1.6 mmol) in dichloromethane (50 ml) at a rate such as to maintain the temperature of the reaction mixture below −5°C. The addition required about 1 h. After this, stirring was continued for 2 h. The resulting solution was washed three times with cold deionized water and then dried over anhydrous magnesium



S-(+)-MNE-OTf



S-(+)-MDNE-OTf



Scheme 1.

sulfate. After removing the dichloromethane under reduced pressure, the crude product was recrystallized twice from dichloromethane. *S*-(+)-MDNE-OTf was obtained as transparent flakes: yield 48%; m.p. 120°C (dec); anal. calculated for $C_{18}H_{16}NSO_7F_3$, 48.32% C, 3.58% H, 3.13% N; found: 49.05% C, 3.92% H, 3.35% N; IR: 1200 and 1400 cm^{-1} (–O–SO_2–), 1160 cm^{-1} (–C–O–); MS: m/z=448 (MH^+); $[\alpha]_D^{20}$=+16.9 (C=1, dichloromethane). [See Scheme 1 for the structures of *S*-(+)-MNE-OTf and *S*-(+)-MDNE-OTf.]

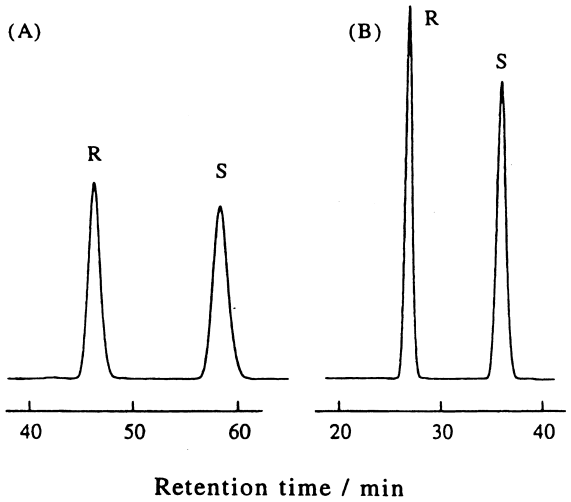


Fig. 1. Comparison of the chromatograms of the diastereomeric derivatives of ibuprofen with *S*-(+)-MNE-OTf (A) and *S*-(+)-MDNE-(+)-OTf (B). Chromatographic conditions: mobile phase, acetonitrile–water (60:40); flow-rate, 1.0 ml/min; fluorescence detection.

2.4. Derivatization procedure

The typical derivatization procedure was as follows. To 0.1 ml of a test solution of chiral carboxylic acids ($1\cdot10^{-3}$ M) in acetonitrile placed in a reaction tube were added 0.1 ml of 18-crown-6 ($1\cdot10^{-2}$ M) in acetonitrile and ca. 5 mg of potassium fluoride. After slightly vortexing the tube, 0.1 ml of *S*-(+)-DMNE-OTf ($1\cdot10^{-2}$ M) in acetonitrile was combined with it. The mixture was then vortexed for 20 min at room temperature. The resulting solution was diluted 100-fold with acetonitrile, and an aliquot (10 μl) of the supernatant was directly injected into the chromatograph. (See Scheme 1 for reaction.)

3. Results and discussion

One of the most important requirements in the derivatization of chiral compounds is no racemization of both analytes and derivatization reagents in the derivatization process. Because the α-hydrogen atom of carboxylic acids is susceptible to racemization under strong basic conditions, there is the risk of racemization in the derivatization of chiral carboxy-

Table 1
Chromatographic data for the diastereomers of carboxylic acids by derivatization with *S*-(+)-MNE-OTf and *S*-(+)-MDNE-OTf

Carboxylic acid	*S*-(+)-MNE-OTf			*S*-(+)-MDNE-OTf		
	k'	α	R_s	k'	α	R_s
Mandelic acid	2.75			2.09		
	3.57	1.30	2.7	2.77	1.32	2.8
α-Methoxyphenylacetic acid	5.73			3.97		
	7.52	1.31	4.1	5.23	1.32	4.1
Ketoprofen	14.07			9.60		
	17.84	1.27	4.7	12.35	1.29	4.7
Flurbiprofen	28.45			15.55		
	35.55	1.25	4.5	21.03	1.35	6.0
Ibuprofen	42.23			23.50		
	53.51	1.27	4.8	23.50	1.36	6.4
Lactic acid	25.76			20.22		
	27.35	1.06	1.3	21.49	1.06	1.4

Elution conditions: flow-rate, 0.8 ml/min for lactic acid and 1.0 ml/min for others; mobile phase, acetonitrile–water (30:70 for lactic acid and 60:40 for others).

lic acids using bases as a catalyst. This risk of racemization was examined by derivatizing *tert*.-butyloxycarbonyl-L-alanine using potassium fluoride and potassium carbonate. Higher enantiomeric purity (only 0.4% ee higher) of *tert*.-butyloxycarbonyl-L-alanine was observed for the use of potassium fluoride. Although the derivatizing reactions of carboxylic acids with triflate-type reagents proceed more rapidly in acetone than in acetonitrile, acetonitrile is purified more easily by a partial-freezing method at −80°C in a freezer. Therefore, acetonitrile and potassium fluoride were used as the reaction solvent and as the base catalyst, respectively, in this work.

In addition to the red shift in the fluorescence spectra, the introduction of two methoxy groups onto the 6- and 7-positions of the 2,3-naphthalimido ring has resulted in better resolution of the diastereomers formed, accompanying reduced retention times (Fig. 1 and summarized in Table 1).

Because the diastereomers are intrinsically different in their physicochemical properties, the intensities of UV or fluorescence spectra of the diastereomers derivatized from racemic compounds are

Table 2
Detection response ratios of the diastereomers derived from racemic carboxylic acids

Carboxylic acid	*S*-(+)-MNE-OTf		*S*-(+)-MDNE-OTf	
	UV	Fluorescence	UV	Fluorescence
Mandelic acid	1.10	1.10	1.14	1.17
α-Methoxyphenylacetic acid	1.12	1.13	1.07	1.08
Ketoprofen	1.10	3.68	1.11	1.36
Flurbiprofen	1.13	1.62	1.17	1.21
Ibuprofen	1.11	1.13	1.08	1.07
Lactic acid	0.97	0.97	0.98	0.98

Detection response ratio=peak area of the 1st eluted peak/peak area of the 2nd eluted peak.
Elution conditions as in Table 1.

therefore not more or less equal. Table 2 lists the peak area ratios obtained for the racemic acids by both UV and fluorescence detection; for example, in the extreme case of ketoprofen in fluorescence detection, the ratio of 3.68 in *S*-(+)-MNE-OTf has been improved to that of 1.36 by derivatizing with *S*-(+)-MDNE-OTf.

The enantiomeric purity of *S*-(+)-MDNE-OTf was estimated to be >99.0% ee by derivatizing *tert*.-butyloxycarbonyl-L-alanine. A calibration graph of peak-area ratios of the diastereomers derivatized with *S*-(+)-MDNE-OTf versus the *S*/*R* ratios of ibuprofen (*S*/*R* ratios=1 to 5) showed quite good linearity (correlation coefficient of $r=0.999$). The detection limits ($S/N=3$) for ibuprofen were 4 fmol for fluorescence detection ($\lambda_{ex}=283$ nm, $\lambda_{em}=467$ nm) and 8 fmol for UV detection ($\lambda_{max}=283$ nm, $\varepsilon_{max}=7.3\cdot10^4$), respectively.

In conclusion, 6,7-dimethoxylation of *S*-(+)-MNE-OTf brought about not only the expected red shift in the fluorescence spectra but also favorable chromatographic properties, such as better resolution and reduced retention times of the diastereomers formed. This reagent is considered to be useful for the determination of carboxylic acid enantiomers by reversed-phase HPLC.

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