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라세미 화합물의 거울상 이성질체 구분을 위한 간단하고 효과적인 방법

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Simple and Efficient Method for the Enantiomeric discrimination of Racemates

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요 약: 라세미 혼합물 (헤미에스테르)의 키랄 순도를 효율적으로 측정하기 위한 방법으로 이작용기 (티오우레아와 3차 아민)를 갖는 키랄 이동제 (**3**)에 대한 연구이다. 다양한 헤미에스테르와 키랄 이동제 (**3**)의 결합으로 형성된 부분 입체 이성질체에 의해서 헤미에스테르의 메톡시 양성자의 신호가 분명하게 분리되었다. ^{1}H NMR에서 헤미에스테르의 거울상 이성질체에 대한 분명한 신호 분리는 헤미에스테르의 카르보닐기와 키랄 이동제 (**3**)의 이작용기(티오우레아와 3차 아민) 사이의 수소 결합에 기인한다. 본 연구는 키랄 이동제 (**3**)를 사용하여 헤미에스테르의 키랄 순도를 빠르고 간단하게 결정할 수 있는 방법을 제공한다.

Abstract: The efficient use of a chiral shift agent (**3**) containing bifunctional group (thiourea and tertiary amine) for the determination of the enantiomeric purity of racemic mixture (Hemiesters) has been studied. The diastereomeric complexes derived from a chiral shift agent (**3**) with various hemiesters gave rise to well separate signals of the methoxy protons of hemiesters. Good splitting signals for enantiomers of hemiesters in ^{1}H NMR are originated form the hydrogen bonds between carbonyl groups of hemiester and bifunctional groups of a chiral shift agent (**3**) such as thiourea moiety and tertiary amine. This study provides a quick and simple way to determine the chiral purity of hemiester using chiral transfer agent (**3**).

Keywords: *chiral shift agent, thiourea, tertiary amine, hemiesters*

1. Introduction

Because of the importance of chirality in pharmaceutical and biological industry, a simple and fast method for the measurement of enantiomeric purity is an important research area. Natural living things are composed of chiral biological materials and they interact with each stereoisomer of a racemic mixture. Although chiral chromatographic methods are widely used. ^{1}H NMR spectroscopy is still a useful alternative for determining enantiomeric excess of organic molecules[1,2]. Because it has the advantages of simple performance and accessibility. Chiral hemiesters, and derivatives thereof, have proven to be versatile building blocks in asymmetric synthesis[3-5]. They contain one or more stereogenic centres and two chemically differentiated carbonyl functionalities. Because of the importance of chiral hemiesers, methods to determine the enantiomeric purities are also important. Several methods have been reported for the

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determination of enantiomeric excess (ee) of hemiesters[6-8]. The most common method is *via* the reaction of the chiral amine reagent such as (*R*)-(+)-1-(1-naphthyl)-ethylamine, with carboxylic acid of hemiesters (Scheme 1).

The enantiomeric excess was determined by HPLC or ^{1}H NMR analysis of a diasteroisomeric mixture of the corresponding amide. However, this method utilizes highly expensive chiral amine reagents and tedious time-wasting processes such as amide coupling reaction, work-up and chromatographic isolation were necessary. Thus, there is still a strong need for more convenient method for the determination of chiral hemiesters.

2. Experimental Procedure

2.1. General

^{1}H-NMR analyses were performed on Varian Mercury 500 (USA, 500 MHz for ^{1}H) instrument.

2.2. Prepartion of Chiral Shift Agent (3)

Under an nitrogen atmosphere, to a solution of 3,5-bis (trifluoromethyl)phenyl) isothiocyanate (1.0 eq, 605 mg) in dry THF (2.0 mL) was added (*R*,*R*)-N,N-dimethyl- *trans*-diaminocyclohexane (317 mg, 2.23 mmol). After the reaction mixture was stirred for 3 h, the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on silica gel (Ethylacetate/n-Hexane = 1/2 as eluant) gave the desired thiourea **3** (597 mg, 65%) as white amorphous solid. ^{1}H NMR (500 MHz, DMSO-d_6) d 10.0 (s, 1H), 8.21 (s, 1H), 8.17 (s, 2H), 7.66 (s, 1H), 4.09 (br s, 1H), 2.54 (br s, 1H), 2.21 (S, 7H), 1.82 (br s, 1H), 1.74 (br s, 1H), 1.63 (br d, J = 11.0 Hz, 1H), 1.31- 1.01 (m, 4H).

2.3. Procedure for the Determination of ee

The chiral shift agent **3** (5 mg) was mixed **4a** and **ent-4a** (5 mg) in 0.5 mL of $CDCl_3$. The resulting solution was stirred for 10 min and transferred to an NMR tube for measurement.

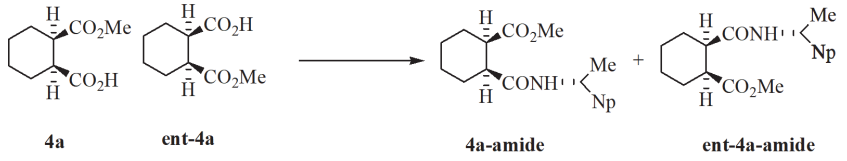
3. Results and Discussion

3.1. Synthesis of Chiral Shift Agent (3)

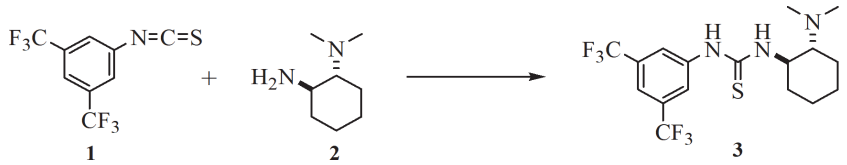
(*R*,*R*)-N,N-dimethyl-*trans*-diaminocyclohexane (**1**) was reacted with 3,5-bis(trifluoromethyl)phenyl) isothiocyanate (**2**) in THF to give chiral shift agent **3** [9]. The synthetic pathways are shown in Scheme 2.

3.2. Enantiomeric Discrimination

Molecular recognition of carboxylic acid or carboxylate has been gained considerable research interest for the enantio-discrimination of amino acids[10,11]. Several receptors possessing urea[12-14] and thiourea[15,16] moieties as a binding site for anion recognition have been reported, in which the binding takes place *via* hydrogen bonding interaction. Chiral amines are also used for the enantio-



Scheme 1. Reagents and condition; *R*-(+)-1-(1-naphthyl)-ethylamine, EDCI, CH_2Cl_2, room temperature.



Scheme 2. Reagents and condition; 3,5-bis(trifluoromethyl)phenyl) isothiocyanate, (*R*,*R*)-N,N-dimethyl-*trans*-diaminocyclohexane, THF, room temperature.

discrimination of racemic mixture. Mono- and bis-alkaloid chiral auxiliaries with anthraquinone or phenanthryl cores were proven as chiral shift agents fort the enantio-discrimination of chiral hemiesters[17]. In this study, we describe that compound **3** containing bifunctional moiety (thiourea and tertiary amine) can be used as a chiral shift agent for the enantiomeric discrimination of hemiesters (Figure 1).

Several urea and thiourea containing a base moiety have been recently proven to be useful bifunctional organocatalyst [18]. Previously, a bifunctional organocatalyst containing thiourea moiety was used in methanolysis of *meso*-cyclic anhydride in order to get chiral hemiesers[19]. Cinchona alkaloid-derived thiourea catalyst can bind and activate the anhydride electrophile by hydrogen bonding to the thiourea moiety and subsequently encourage attack at a single anhydride carbonyl moiety through general-base catalysis by the suitably positioned chiral quinuclidine base. However, to the best of our knowledge, there has been no report that bifunctional organocatalyst containing thiourea moiety have been used as a chiral shift agent for the enantiomeric discrimination of hemiesters. The binding properties of **3** in solution (chloroform-d_1) with hemiesters were studied by the ^{1}H NMR (500 MHz). Hemiester has both a strong hydrogen bond acceptor (ester) and a strong hydrogen bond donor (carboxylic acid). The catalyst **3** also has hydrogen bond donating (thiourea) and hydrogen bond acceptor (tertiary amine). There are three possible interactions between bifunctional chiral shift agent and hemiesters such as thiourea binding, chiral amine binding, bifunctional binding of thiourea and tertiary amine. Thus enantiomeric discrimination may be anticipated by three possible interactions. A typical spectrum that illustrates the use of this methodology is shown in Figure 2A. To verify chiral shifting efficiency of **3**, nonequivalent chemical shifts is compared with those of diasteroisomeric

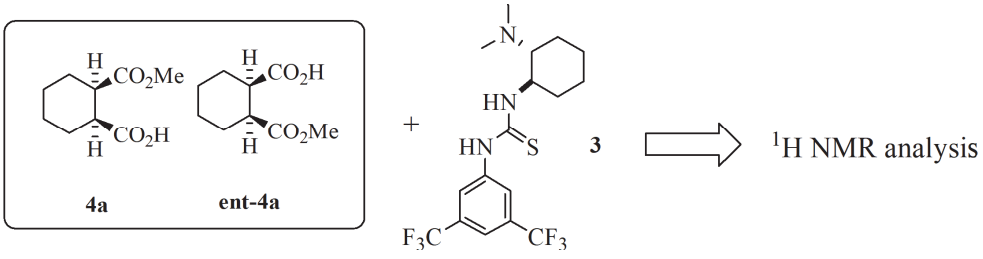


Figure 1. Structure of the hemiesters (**4a** and **ent-4a**) and chiral shift reagent (**3**).

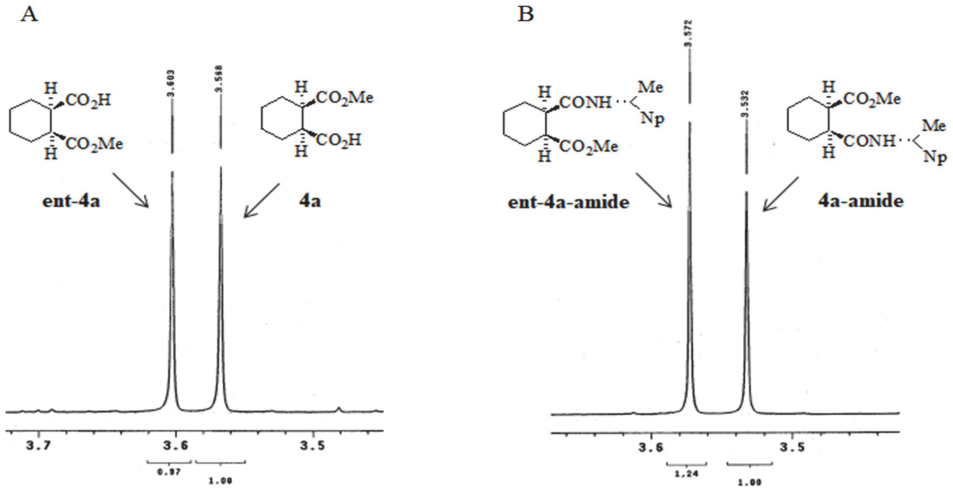
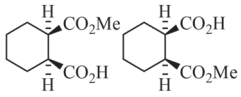
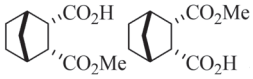
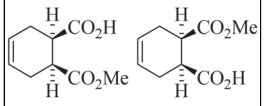
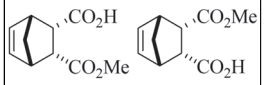
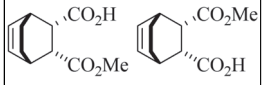
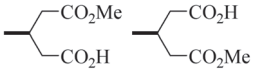


Figure 2. Partial ^{1}H NMR spectra (500 MHz) highlighting the methoxy proton signal in (A) racemic hemiester (**4a** and **ent-4a**) with 1.1 equiv of **3**. (B) amide derivative of racemic hemiester (**4a-amide** and **ent-4a-amide**).

Table 1. ^{1}H NMR Chemical Shift

Entry	Hemiesters	Chemical shifts (d, ppm)		
		4a – 4f	**ent-4a – 4f**	**Difference**
1	 **4a** **ent-4a**	3.568	3.603	0.035
2	 **4b** **ent-4b**	3.548	3.618	0.070
3	 **4c** **ent-4c**	3.612	3.620	0.008
4	 **4d** **ent-4d**	3.577	3.587	0.010
5	 **4e** **ent-4e**	3.535	3.585	0.050
6	 **4f** **ent-4f**	3.626	3.646	0.020

mixture of amide (Figure 2B).

The methoxy proton of hemiesters appear as sharp singlet and do not overlap with other proton signals. The addition of **3** in hemiesters (**4a** and **ent-4a**) solution, a splitting signal of methoxy proton of hemiesters and two nonequivalent chemical shift (d_1 = 3.603 ppm, d_2 = 3.568 ppm) were observed in the ^{1}H NMR spectra as a consequence of the formation of diastereomeric complexes. It can be noticed that the **ent-4a** exhibits the chemical shift at higher frequency (lower field) than that of **4a**. Its efficiency was similar to that of the amidation method using (*R*)-(+)-1-(1-naphthyl)-ethylamine. In the view of this observation, we decided to investigate the properties of **3** as the chiral shift reagent in the determination of the optical purity of other hemiesters. The results are shown in Table 1. More pronounced enantiomeric discrimination was achieved in bicyclic hemiesters (**4b** and **ent-4b**). The splitting signal of **4b** and **ent-4b** in ^{1}H NMR spectrum is shown in Figure 3A. Hemiesters containing an olefin moiety showed reduced separation (entry 3 and 4). However, increased separation was detected in case of the high steric bicyclic hemiester possessing the olefin moiety (**4e** and **ent-4e**). The chiral shift agent **3** was also enough for the enantiomeric discrimination of monocyclic hemiester (entry 6). The splitting

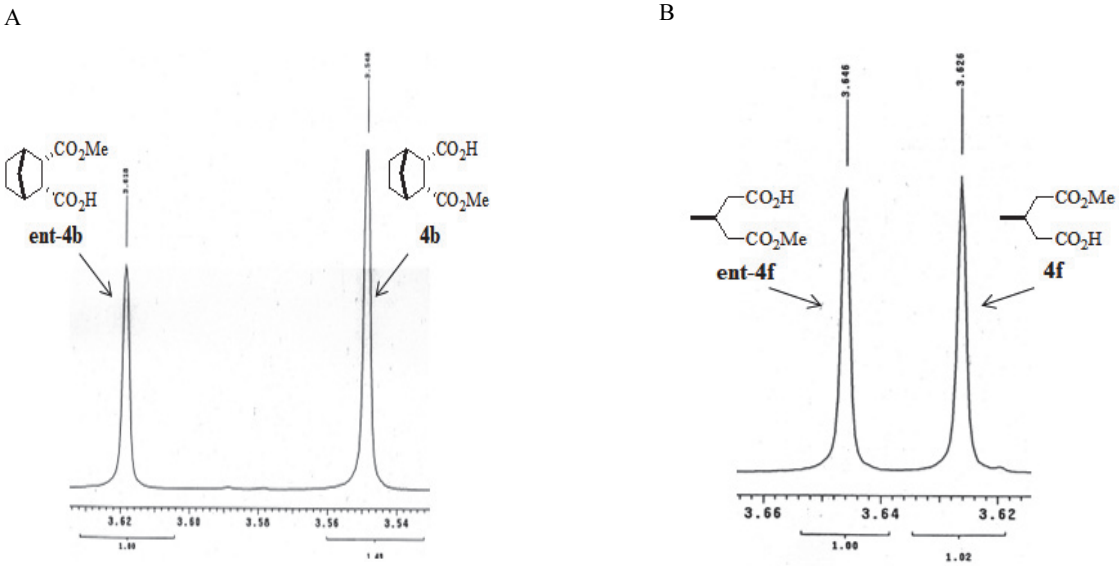


Figure 3. Partial ^{1}H NMR spectra (500 MHz) highlighting the methoxy proton signal in (A) racemic hemiester (**4b** and **ent-4b**) with 1.1 equiv of **3**. (B) racemic hemiester (**4f** and **ent-4f**) with 1.1 equiv of **3**.

signal of **4f** and **ent-4f** in ^{1}H NMR spectrum is shown in Figure 3B.

4. Conclusion

In the view of this observation, we decided to investigate the properties of **3** as the chiral shift agent in the determination of the optical purity of other hemiesters. The results are shown in Table 1. More pronounced enantiomeric discrimination was achieved in bicyclic hemiesters (**4b** and **ent-4b**). Hemiesters containing an olefin moiety showed reduced separation (entry 3 and 4). However, increased separation was detected in case of the high steric bicyclic hemiester possessing the olefin moiety (**4e** and **ent-4e**). The chiral shift agent **3** was also enough for the enantiomeric discrimination of monocyclic hemiester (entry 6). Further studies on the enantiomeric discrimination of a-hydroxy acids and amino acids are underway.

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