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New Chiral Derivatizing Agents: Convenient Determination of Absolute Configurations of Free Amino Acids by ^{1}H NMR

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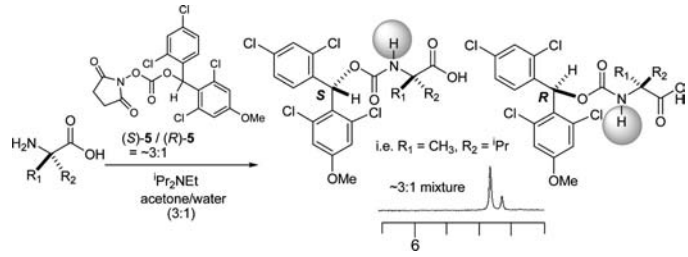
ABSTRACT



The chiral carbonate reagents 5 allow for the direct and unambiguous determination of the absolute configurations of a wide range of free amino acids using ^{1}H NMR. By using a ~3:1 mixture of (*S*)-5 and (*R*)-5, absolute configurations of the corresponding carbamates are determined by only analyzing the nitrogen protons.

Isosteric replacement of a scissile peptide bond represents a viable and popular approach in the rational design of peptidomimetics, which find applications as drugs.[1] To date, numerous synthetic methods for the preparation of optically active unnatural amino acid building blocks have been reported, and development of efficient synthetic methods for unnatural amino acids is still a subject of interest in modern organic synthesis.[2] In synthetic studies on such molecules, the unambiguous determination of absolute configurations of target molecules often requires comparison of physical properties of pure authentic samples or their derivatives. An indirect method via chiral derivatizing agents (i.e., Marfey's reagent) and subsequent HPLC analysis is a standard for determination of the absolute configurations or enantiomeric purities of amino acids.[3] On the other hand, the methods for determination of absolute configurations of free amino acids based on NMR analysis are limited, due to the lack of (1) proper chiral derivatizing reagents which react quantitatively with free amino acids in water containing solvents and (2) understanding of conformational preference of amino acid derivatives.[4] In addition, it is not possible to unambiguously determine the absolute configuration of chiral nonracemic amines or alcohols in a single chemical derivatization step with NMR spectroscopic analysis.[5] We now report a con-

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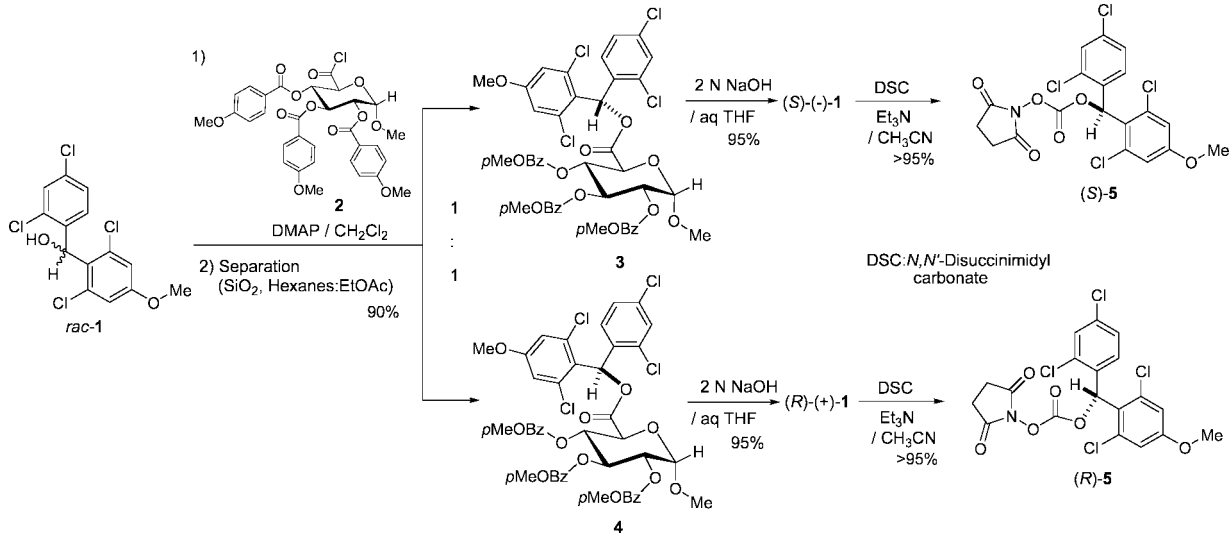
venient and reliable method for the determination of the absolute configurations of free amino acids via a single chemical operation followed by ^{1}H NMR analysis of the nitrogen protons (−OCON*H*−) of the resulting carbamate derivatives. To our knowledge, this is the first observation that the nitrogen proton can reliably be utilized for determination of the absolute configurations of nonracemic chiral primary amines by ^{1}H NMR.

We have developed an unsymmetrical (2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methanol (**1**, Scheme 1) which is stable against Brønsted and Lewis acids (i.e., 15% TFA, 30% HF, 2 N HCl, HBr/AcOH, $TiCl_4$, $ZnCl_2$, $AlCl_3$, $B(C_6F_5)_3$, BCl_3, BBr_3, BF_3•OEt_2, TMSOTf, and $La(OTf)_3$ at rt), Brønsted bases (i.e., NH_4OH (in aq THF), LiOH (in aq THF−MeOH), and DBU (in aq THF) at rt), and a wide variety of nucleophiles. Analysis of a lowest energy conformer of the acetate of **1** via a single-crystal X-ray analysis revealed that the −COO− linkage and the ether methine proton formed by two planar chloro-substituted benzenes showed a deviation of ∼20° out of a preferential common plane (Figure 1). Extensive 2D-NOESY experiments of the esters of **1** supported that the conformation in solution ($CDCl_3$) agreed with that observed in the solid state.[7] There must be a significant electronic repulsion between the *o*-chloro atoms in the two phenyl rings. The *o*-chloro atom in the dichlorobenzene ring (the phenyl ring A in Figure 1) is directed toward the carbonyl ester plane. Thus, the chloro atoms at the ortho positions in both phenyl groups hinder nucleophilic attack at the ester carbonyl from either the *re*- or *si*-face. In addition, the 3,5-dichloro atoms in the anisole moiety (the phenyl ring B) attenuate the electron-donating character of the methoxy group. Therefore, it was concluded that the esters derived from **1** would exhibit unusual stability against bases, nucleophiles, and acids.[8] Based on these conformational analyses of the esters of **1**, we speculated that the optically pure (*S*)- and (*R*)-(2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methanol, (*S*)-**1** and (*R*)-**1**, would be very useful chiral derivatizing agents for determination of absolute configurations of chiral nonracemic carboxylic acids and amino acids.

Scheme 1. Resolution of *rac*-**1** and Syntheses of the Chiral Carbonate (*S*)-**5** and (*R*)-**5**



All attempts to selectively reduce (2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methanone via chiral reducing agents[9] or to resolve diastereomers of *rac*-**1** using known chiral derivatizing agents did not provide satisfactory selectivity or separation.[10] Gratifyingly, (*S*)-**1** and (*R*)-**1** could readily be obtained through the resolution of *rac*-**1** using the glucose derivative **2**[11] as illustrated in Scheme 1.[12] Absolute

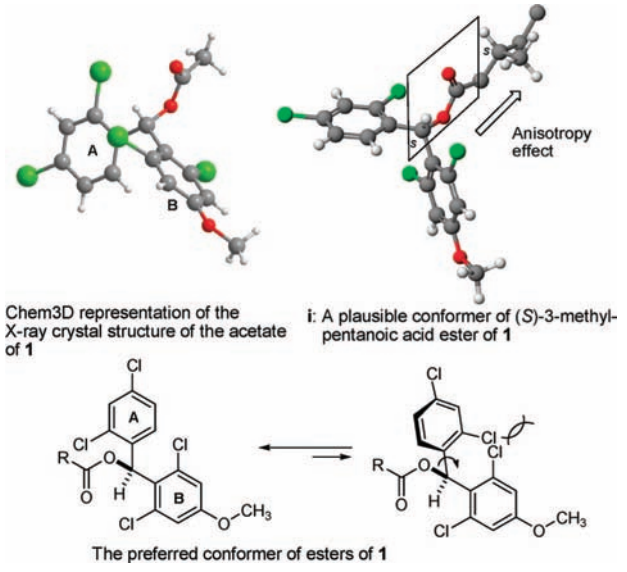


Figure 1. Preferred conformations of the esters of **1**.[6]

configurations of (*S*)-**1** and (*R*)-**1** were established through the advanced Mosher method and NOESY correlations of the L-alanyl carbamate derivative.[13]

^{1}H NMR analyses of the (*S*)-3-methylpentanoic acid esters of (*S*)-**1** and (*R*)-**1** revealed that the *syn*-substituent with respect to the dichloromethoxybenzene moiety (the phenyl ring B in Figure 1) in the ester derived from (*S*)-**1** was downfield-shifted due to the anisotropy effects of the phenyl ring; a large enough chemical shift nonequivalence of the β-methyl group between the esters of (*S*)- and (*R*)-**1** was observed (**i** in Figure 1).[14] Encouraged by these observations of the esters of **1**, we synthesized a series of carbamates by the treatment of free L- and D-amino acids with the carbonate (*S*)-**5**, which was synthesized with *N*,*N*′-disuccinimidyl carbonate (DSC) in the presence of ^{i}Pr$_2$NEt in acetone−water (3/1) (Scheme 1). Conformational analyses of the carbamates of amino acids via 2D-NOESY experiments revealed that the α-residues of L-amino acid derivatives exhibited a correlation with the diphenyl methine proton (examples of L- and D-Ala are shown in Figure 2). In addition, the

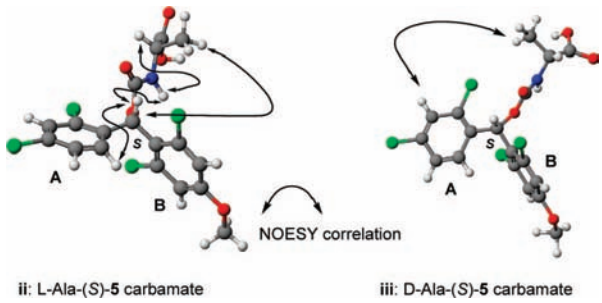


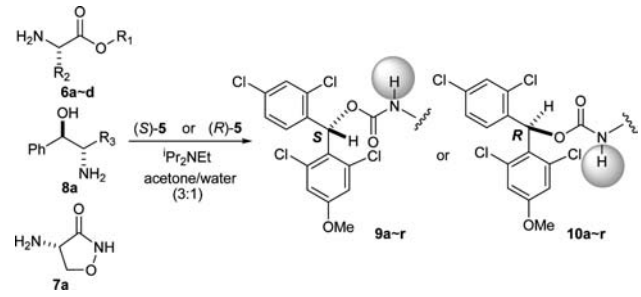
Figure 2. Plausible preferred conformers of the carbamates of (*S*)-**1**.

carbamate nitrogen proton exhibited strong NOESY correlations with the α-proton and the diphenyl methine proton. Therefore, the nitrogen proton of the L-amino acid derivatives should be located toward the dichloromethoxybenzene moiety (the phenyl ring B in **ii** in Figure 2); thus, the nitrogen proton is shifted downfield due to the anisotropy effect in the ^{1}H NMR spectra. On the contrary, the protons of the α-residue and nitrogen proton of the carbamates derived from D-amino acids did not show NOESY correlations with the diphenyl methine proton, but the proton(s) of the α-residue exhibited a NOESY correlation with the dichlorobenzene proton (the phenyl ring A in **iii**). These observations in the NOESY experiments led to the preferred conformation of the D-amino acid derivatives as illustrated in **iii** (Figure 2); thus, the nitrogen protons of D-Ala-(*S*)-**5** carbamates may not be suitably influenced by the anisotropic effect of the dichloromethoxybenzene ring (the phenyl ring B).

In order to generalize the use of (*S*)-**5** and (*R*)-**5** for the determination of absolute configurations of nonracemic chiral primary amines by ^{1}H NMR, over 50 carbamate derivatives were synthesized from natural and unnatural free amino acids and their esters and from amino alcohols. In all cases, reactions of the primary amines with (*S*)-**5** and (*R*)-**5** furnished the corresponding carbamates in quantitative yield (determined by ^{1}H NMR) regardless of the structure of the α-residue or the protection or nonprotection of carboxylic acid group. The indole, imidazole, alcohol, and phenol groups did not react with **5** under the conditions used for the carbamation reactions. Thus, the carbonates **5** serve as selective chiral carbamate reagents.[15]

Selected examples of the ^{1}H NMR analyses of the nitrogen protons of carbamate synthesized from L- (or (*S*)-)configuration substrates are summarized in Table 1. The nitrogen

Table 1. Chemical Shift Difference of the −O(CO)N*H*− proton[a]



entry	substrate	products	δ (*S*), δ (*R*)	Δδ (*S*-*R*)
1	**6a**: $R_1 = CH_3$, $R_2 = CH_3$	**9a**, **10a**	5.52, 5.41	+0.11
2	**6b**: $R_1 = {}^t$Bu, $R_2 = CH_2Ph$	**9b**, **10b**	5.45, 5.38	+0.07
3	**6c**: $R_1 = CH_3$, $R_2 = (CH_2)_2CO_2CH_3$	**9c**, **10c**	5.56, 5.54	+0.02
4	**6d**: L-Ala	**9d**, **10d**	5.63, 5.55	+0.08
5	**6e**: L-His[b]	**9e**, **10e**	6.10, 6.04	+0.06
6	**6f**: L-Phe	**9f**, **10f**	5.57, 5.47	+0.10
7	**6g**: L-Thr[b]	**9g**, **10g**	5.85, 5.83	+0.02
8	**6h**: L-Trp[b]	**9h**, **10h**	5.60, 5.50	+0.10
9	**6i**: L-Tyr[b]	**9i**, **10i**	5.71, 5.53	+0.18
10	**6j**: L-Lys[c]	**9j**, **10j**	5.51, 5.42	+0.09
11	**6k**: L-Val	**9k**, **10k**	5.48, 5.41	+0.07
12	**6l**: $R_1 = H$, $R_2 = {}^t$Bu	**9l**, **10l**	5.50, 5.45	+0.05
13	**6m**: $R_1 = H$, $R_2 = CH_2CN$	**9m**, **10m**	5.53, 5.49	+0.04
14	**6n**: $R_1 = H$, $R_2 = CH_2CH_3$	**9n**, **10n**	5.53, 5.42	+0.11
15	**6o**: $R_1 = H$, $R_2 = CH_2$-2-furyl	**9o**, **10o**	5.66, 5.61	+0.05
16	**6p**: $R_1 = H$, $R_2 = CH_2$-cyclohexyl	**9p**, **10p**	5.37, 5.28	+0.09
17	**7a**	9q, **10q**	5.99, 5.53	+0.46
18	**8a**: $R_3 = Ph$	**9r**, **10r**	5.79, 5.68	+0.11

[a] ^{1}H NMR spectra were recorded on a 300 or 400 MHz NMR spectrometer using $CDCl_3$ as solvent. [b] The hydroxy or amino group in the residue is intact. [c] The N^ϵ group of lysine formed the carbamate: its $\Delta\delta$ ($\delta_S - \delta_R$) value = ±0.

protons of carbamates derived with (*S*)-**5** were shifted downfield relative to those obtained with (*R*)-**5**. In all cases, good signal separations of the carbamate nitrogen protons were observed in the ^{1}H NMR spectra. It is worth mentioning that unlike other carbamate derivatives, concentration-dependent changes in chemical shift of the nitrogen proton of our carbamates have never been observed in $CDCl_3$. This could be attributed to the enhancement of the hydrophobic physicochemical property of free amino acids via incorporation of **1**. Due to the fact that (1) the absolute configuration of amino acids can conveniently be determined solely on the basis of ^{1}H NMR analysis of the carbamate nitrogen protons (Table 1), full assignments of all protons are not

necessary,[5,16] and (2) chemical shifts of the nitrogen protons of carbamates are largely separated from the other protons, we envisioned using a mixture of (*S*)-**5** and (*R*)-**5** (a ∼3:1 ratio) to determine the absolute configurations of nonracemic chiral primary amines via a single chemical operation and subsequent ^{1}H NMR analysis. In this method, the relative ratio of the signal area of L-amino acid derivatives should afford L-(*S*)/L-(*R*) ratio of ∼3:1,[17] whereas D-amino acid derivatives are expected to give a D-(*R*)/D-(*S*) ratio of ∼1:3. As summarized in Figure 3, the method for determination

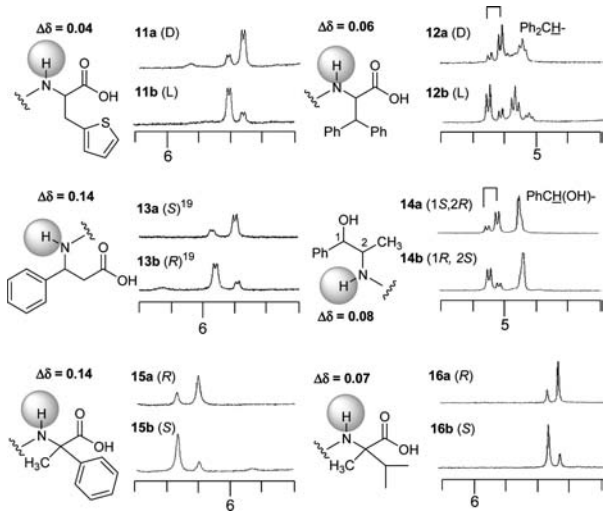


Figure 3. Chemical shifts of the nitrogen protons of carbamates derivatized using a ∼3:1 mixture of (*S*)-**5** and (*R*)-**5** (400 MHz spectra).

of the absolute configurations via a (*S*)-**5**/(*R*)-**5** ratio of ∼3:1 could apply to a wide range of chiral nonracemic primary amines; the unnatural α-amino acids, a *β*-amino acid, and an amino alcohol agreed with this empirical rule (**11**−**14** in Figure 3). Significantly, large enough chemical shift nonequivalences of the carbamate nitrogen protons of the α,α-disubstituted amino acids[18] were observed, and thus, the absolute configurations of nonracemic α,α-disubstituted amino acids can be conveniently determined by this method (**15** and **16** in Figure 3).[19]

In conclusion, we have developed unprecedented chiral derivatizing agents that allow for the determination of the absolute configurations of a wide range of amino acids by only analyzing the carbamate nitrogen protons in ^{1}H NMR spectra. The carbonate reagents (*S*)-**5** and (*R*)-**5** react selectively with alkylamines in aqueous conditions and form the corresponding carbamates in quantitative yield. Signal separations of the carbamate nitrogen protons are large enough, and thus, as summarized in Figure 3, the absolute configurations of nonracemic amino acids can be determined unambiguously by the ^{1}H NMR analysis of the relative ratio of the nitrogen protons of carbamates, which are synthesized quantitatively by the carbamation reactions using a ∼3:1 mixture of (*S*)-**5** and (*R*)-**5**. The method using these new derivatizing agents appears to have a significant advantage over currently available methods for determination of the absolute configuration of free amino acids via ^{1}H NMR spectroscopy.[4] The further scope and limitations of this method will be reported elsewhere.

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Supporting Information Available: Experimental procedures and copies of NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) Absolute configurations of esters have been determined by careful analyses of $\Delta\delta$ ($\delta_S - \delta_R$) values of esters derived from both (*S*)- and (*R*)-chiral derivatizing agents (CDA) via high-field ^{1}H NMR spectroscopy. Thus, two separate transformations of alcohol with CDA are necessary.

(6) A plausible conformer of the (*S*)-3-methylpentanoic acid ester of **1** (Figure 1) was obtained by using the semiempirical AM1 method.

(7) For the NOESY correlations of the ester of **1**, see the Supporting Information.

(8) Kurosu, M.; Biswas, K.; Narayanasamy, P.; Crick, D. C. *Synthesis* **2007**, *16*, 2513.

(9) For example, asymmetric reduction of (2,6-dichloro-4-methoxyphenyl)(2,4-dichlorophenyl)methanone via a chiral oxazaborolidine provided a 2.5:1 mixture of alcohols.

(10) Kasai, Y.; Taji, H.; Fujita, T.; Yamamoto, Y.; Akagi, M.; Sugio, A.; Kuwahara, S.; Watanabe, M.; Harada, N.; Ichikawa, A.; Schring, V. *Chirality* **2004**, *16*, 569.

(11) Kurosu, M.; Li, K. Unpublished data.

(12) The esters **3** and **4** could be separated via flash column chromatography (SiO_2, hexanes/EtOAc; see the Supporting Information).

(13) For determination of the absolute stereochemistries of (*S*)-**1**, see the Supporting Information.

(14) For assignment of the absolute configuration of α-chiral carboxylic acids by ^{1}H NMR, see: (a) Freire, F.; Quinoa, E.; Riguera, R. *Chem. Commun.* **2008**, *35*, 4147. (b) Dickins, R. S.; Badari, A. *Dalton Trans.* **2006**, *25*, 3088. (c) Yabuuchi, T.; Kusumi, T. *J. Org. Chem.* **2002**, *65*, 397. (d) Ferreiro, M. J.; Latypov, S. K.; Quiñoá, E.; Riguera, R. *J. Org. Chem.* **2000**, *65*, 2658. (e) Ferreiro, M. J.; Latypov, S. K.; Quinoa, E.; Riguera, R. *Tetrahedron: Asymmetry* **1997**, *8*, 1015. (f) Tyrrell, E.; Tsanga, M. W. H.; Skinnera, G. A.; Fawcettb, J. *Tetrahedron* **1996**, *52*, 9841.

(15) Neither kinetic resolution nor racemization has been observed during the carbamation reactions.

(16) For a general method for determination of the absolute configurations by ^{1}H NMR, see: (a) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.

(17) "L-(*R*)" denotes the L- or (*S*)-amino acid−(*R*)-**1** carbamate derivative.

(18) The α,α-disubstituted amino acids utilized in this study were synthesized according to the reported procedures; see: (a) Davis, F. A.; Lee, S.; Zhang, H.; Fanelli, D. L. *J. Org. Chem.* **2000**, *65*, 8704.

(19) In this empirical rule, the carboxylic acid attached group is considered as the second highest priority of the substituent groups: $-NH_2$ > the carboxylic acid attached group > the other residue. Thus, the relative ratio of the nitrogen protons of **13b** (*R*-configuration) agrees with that of the L-amino acid derivatives.