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A Chiral Anisotropic Reagent for Determination of the Absolute Configuration of a Primary Amino Compound

Ken-ichi Harada*, Youhei Shimizu and Kiyonaga Fujii

Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468-8503, Japan

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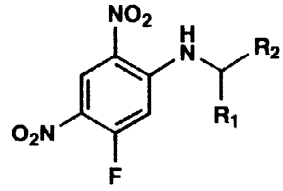
Abstract: A chiral anisotropic reagent, 1-fluoro-2,4-dinitrophenyl-5-(*R*,*S*)-phenylethylamine ((*R*,*S*)-FDPEA, **2**), was developed for determination of the absolute configuration of the α-carbon of primary amino compounds. This reagent has advantages over conventional reagents in terms of reactivity, reliability and effectiveness, and was successfully applied to a peptide, microginin, produced by cyanobacteria.

Only a few methods are known for the determination of the absolute configuration of the α-carbon of primary amino compounds; however, the modified Mosher's method has been widely used for this purpose.[1] In a previous paper, we also proposed another method using LC/MS based on the advanced Marfey's method, which makes it possible to non-empirically determine the absolute configuration of primary amino compounds including amino acids.[2,3] In this method, D- and L-FDLA (1-fluoro-2,4-dinitrophenyl-5-D-leucinamide and -L-leucinamide, **1**) are used as the derivatization reagent to easily give the diastereomeric derivatives which can be separated. Their absolute configuration can then be determined based on their elution order under reversed phase conditions. We have proposed a separation mechanism for the resolution of both resulting diastereomers, which includes a stable and fixed conformation assisted by the intramolecular hydrogen bondings between the nitro groups and α-amino groups of the target compound and the leucinamide moiety. This conformation can be readily confirmed by UV spectral analysis and an NOE experiment.[4] If this fixed conformation can also be formed in the case of 1-fluoro-2,4-dinitrophenyl-5-(*R*,*S*)-phenylethylamine ((*R*,*S*)-FDPEA, **2**), it is expected to be available as a chiral anisotropic reagent for the determination of the absolute configuration of primary amino compounds.



1: $R_1 = CH_2CH(CH_3)_2$, $R_2 = CONH_2$

2: $R_1 = CH_3$, $R_2 = C_6H_5$

*Corresponding author. Fax: 81-52-834-8780. E-mail: kiharada@meijo-u.ac.jp.

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The reagents **2** were prepared according to the method of Marfey.[5,6] The FDPEA derivatives were usually prepared by treatment of the primary amino compound with FDPEA under slightly basic conditions followed by TLC preparative separation.[7] The conformation of the resulting derivatives was investigated using UV and NMR spectral methods. The obtained UV spectra are characterized by two absorption maxima around 340 and 415 nm, which are derived from the bridge chromophore between the nitro groups of the dinitrobenzene (DNB) and the amino groups of phenylethyamine (PEA) and the tested primary amino compound .[4] After the assignment of each signal in the NMR spectra of the FDPEA derivative, an NOE experiment was carried out, showing that NOEs ranging from 10 to 20% are observed between the α-proton of PEA and H-6 of DNB and between the α-proton of the tested primary amino compound and H-6 of DNB. These experimental results indicated that both α-protons are spatially situated near H-6 of DNB in the FDPEA derivatives shown in Fig. 1 (A) and the resulting conformation is quite similar to those of the FDLA derivatives of amino acids and primary amino compounds.[4]

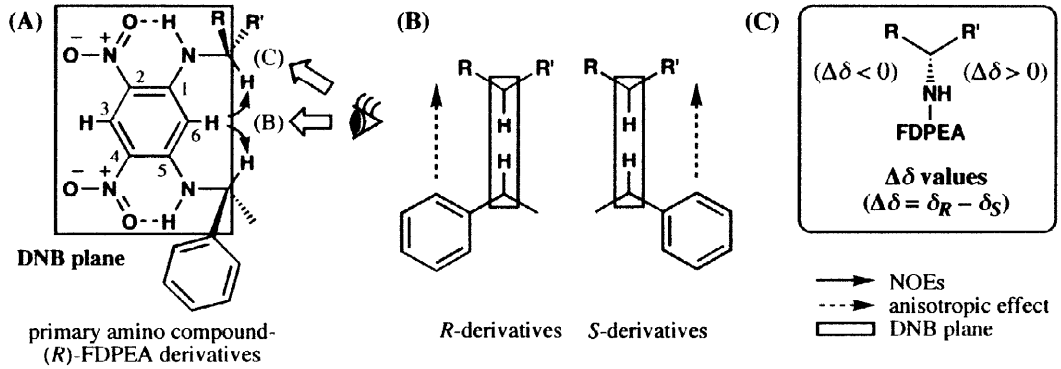

Figure 1. **(A)** Conformation of primary amino compound-(*R*)-FDPEA derivatives. **(B)** and **(C)** Views of the (*R* and *S*)-FDPEA derivatives drawn from the direction shown by the outlined arrow in **(A)**.

Figure 1 (B) shows another view of the conformation of the (*R*,*S*)-FDPEA derivatives of a primary amino compound. When (*R*)-FDPEA is used, substituent R is more strongly affected by the phenyl group, whereas the opposite phenomenon occurs in the case of (*S*)-FDPEA. Here, the Δδ value is defined as ($\Delta\delta = \delta_R - \delta_S$), where δ_R is the chemical shift of each proton in the (*R*)-FDPEA derivative and δ_S is the chemical shift of each proton in the (*S*)-FDPEA derivative. As is obvious from Figs. 1 (B) and (C), the Δδ values for the protons oriented on the left side of the DNB plane are all negative, while those located on the right side of the DNB plane are positive.

Several primary amino compounds were analyzed by this method after the derivatization using (*R*,*S*)-FDPEA. The obtained data for L-isoleucinol and the D- and L-phenylalaninols are summarized as typical examples together with those of the MTPA (2-methoxy-2-phenyl-2-trifluoromethylacetyl) derivatives by the modified Mosher's method[1] in Table 1. From these experiments, the following results were obtained. As expected, the sign of the Δδ value is clearly separated on the boundary of a carbon atom bearing a primary amino group, indicating that the desired conformation is favorably formed. The Δδ values obtained by the present method are larger than those determined by the modified Mosher's method, indicating that an anisotropic effect in the present method is more effective. Although the Δδ values for the (*R*,*S*)-FDPEA

derivatives of the D- and L-phenylalaninols should be ideally the same with opposite signs, those of each proton are slightly different, suggesting that the resulting conformation under the operating conditions is slightly different from that depicted in Fig. 1. Through these experiments, we have ascertained that the proposed method is applicable to non-empirically determine the absolute configuration of primary amino compounds including amino acids.

Table 1. Comparison of $\Delta\delta$ values between (*R,S*)-FDPEA and (*R,S*)-MTPA derivatives (ref. 1) of L-isoleucinol and D- and L-phenylalaninols in ^{1}H-NMR spectra.

	L-isoleucinol		L-phenylalaninol		D-phenylalaninol
Position	FDPEA	MTPA	FDPEA	MTPA	FDPEA
α CH	0.0045	–0.0200	–0.0725	0.0400	0.0240
β CH	0.3120	0.0000			
β CH$_2$			0.2410	–0.0270	–0.2880
			0.2855	–0.0010	–0.3410
β CH$_3$	0.2260	–0.0420			
β'CH$_2$	–0.4375	0.0500	–0.4825	0.0570	0.4355
	–0.5085	0.0700	–0.5360	0.0540	0.4880
γ CH$_2$	0.3910	–0.0840			
	0.3095	–0.0900			
γ CH$_3$	0.2830	–0.0560			
α NH	0.0020	–0.1420	–0.0230	0.1210	–0.0695

In order to demonstrate the applicability of the present method, we tried to confirm and determine the absolute configuration of a constituent primary amino compound in microginin produced by cyanobacteria. Microginin is a linear peptide containing a 3-amino-2-hydroxydecanoic acid (Ahda) unit and the absolute configuration at the C-3 of Ahda had not been elucidated in the first report (Fig. 2).[8] We have now also determined the absolute configuration at the C-3 of Ahda as *R* by our proposed LC/MS method combined with the advanced Marfey's method.[3] Microginin was separately treated with (*R,S*)-FDPEA in 1 M $NaHCO_3$ (aq) at 57 °C for 1 hr to give the corresponding tris-FDPEA derivatives. These resulting derivatives were then purified by silica gel chromatography.[9] After the desired compounds, whose molecular weights were confirmed by FABMS,[10] were subjected to UV and NOESY analyses to confirm the fixed conformation, the $\Delta\delta$ values of each proton (DMSO-d_6) were estimated as shown in Fig. 2.[11] This result indicates that the absolute configuration at the C-3 of Ahda is *R* and this conclusion is completely consistent with that obtained by synthesis[12] and our previous method.[3] Thus, this proposed method is assured to be very practical and reliable.

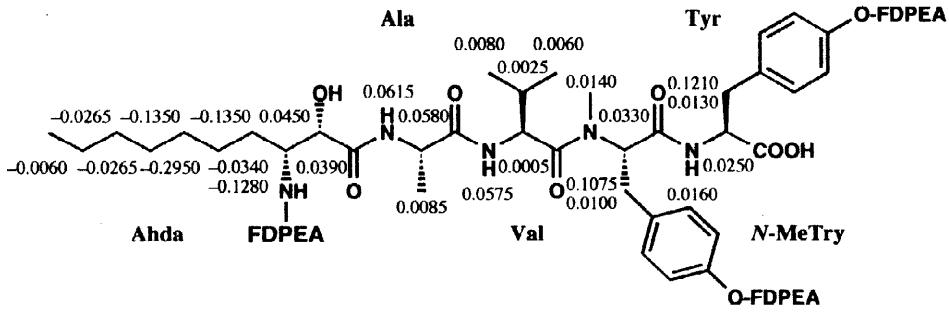

Figure 2. $\Delta\delta$ values in ^{1}H-NMR spectra obtained for microginin-((*R,S*)-FDPEA)$_3$ (400MHz, DMSO-d_6).

In the present study, we established another non-empirical method for the determination of the absolute configuration of a primary amino compound using NMR spectroscopy, which has the following advantages over conventional methods: (1) derivatization with (*R*, *S*)-FDPEA is much easier than with a conventional reagent, e.g., MTPA, (2) conformation of the resulting FDPEA derivative is definitely fixed by the characteristic hydrogen bonding and arrangement of related functional groups, (3) such a conformation can be easily confirmed using UV spectral analysis and NOE measurement, and (4) the $\Delta\delta$ values determined by this method are larger than those obtained by the modified Mosher's method. In addition, the obtained results can also support the fact that a primary amino compound derivatized with FDLA or FDPEA has the definite conformation shown in Fig. 1.[4]

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REFERENCES AND NOTES

1. Kusumi, T.; Fukushima, T.; Ohtani, I.; Kakisawa, H. *Tetrahedron Lett.* **1991**, *32*, 2939-2942.
2. Fujii, K.; Ikai, Y.; Oka, H.; Suzuki, M.; Harada, K.-I. *Anal. Chem.* **1997**, *69*, 5146-5151.
3. Fujii, K.; Shimoya, T.; Ikai, Y.; Oka, H.; Harada, K.-I. *Tetrahedron Lett.* **1998**, *39*, 2579-2582.
4. Fujii, K.; Ikai, Y.; Mayumi, T.; Oka, H.; Suzuki, M.; Harada, K.-I. *Anal. Chem.* **1997**, *69*, 3346-3352.
5. Marfey, P. *Carlsberg Res. Commun.*, **1984**, *49*, 591-596.
6. To a solution of 1 M NaOH (3.9 mL), acetone (60 mL) and anhydrous magnesium sulfate was added (*R,S*)-1-phenylethylamine (3.81 mmol, 461 mg), and the solution was allowed to stand at room temperature for 3 hr. A solution of 1,5-difluoro-2,4-dinitrobenzene (FFDNB) in acetone (3.27 mmol, 667.1 mg/15 mL) was added, then after 30 min of standing, water (88.9 mL) was added to produce 1-fluoro-2,4-dinitrophenyl-5-(*R*)-phenylethylamine ((*R*)-FDPEA, 713.9 mg, 61.4%) and 1-fluoro-2,4-dinitrophenyl-5-(*S*)-phenylethylamine ((*S*)-FDPEA, 816.3 mg, 70.2%). (*R*)-FDPEA; yellow crystals, mp 82-84 °C, $[\alpha]_D$ –209.76° (*c* 0.5, acetone), FABMS *m/z* 306 $[M+H]^+$, ^{1}H-NMR (CD_3OD) : δ 9.04 (1H, d, *J* = 7.6 Hz), δ 7.24-7.27 (5H, m), δ 6.67 (1H, d, *J* = 14 Hz), δ 4.89 (1H, q, *J* = 6.8 Hz), δ 1.67 (3H, d, *J* = 6.8 Hz). (*S*)-FDPEA ; yellow crystals, mp 82-84 °C, $[\alpha]_D$ 209.46° (*c* 0.5, acetone), FABMS *m/z* 306 $[M+H]^+$, ^{1}H-NMR (CD_3OD) : δ 9.03 (1H, d, *J* = 8 Hz), δ 7.24-7.27 (5H, m), δ 6.67 (1H, d, *J* = 14.4 Hz), δ 4.90 (1H, q, *J* = 6.4 Hz), δ 1.67 (3H, d, *J* = 6.4 Hz).
7. The following is a typical preparation of a primary amino compound-(*R,S*)-FDPEA derivative: L-isoleucinol-(*R*)-FDPEA; L-isoleucinol (0.05 mmol, 5.85 mg) in 400 μL of 1 M $NaHCO_3$ was treated with 1% (*R*)-FDPEA acetone solution (2 mL (20 mg, 0.0655 mmol)) and the mixture was allowed to stand at 37 °C for 1 hr. After cooling, 1 M HCl (400 μL) was added and the residue obtained after evaporation of the reaction mixture was subjected to preparative TLC (benzene:acetone = 8:2) to give the (*R*)-FDPEA derivative (9.3 mg, 46%).
8 Okino, T.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *Tetrahedron Lett.* **1993**, *34*, 501-504.
9. Microginin-((*R,S*)-FDPEA)$_3$: microginin (9.8 μmol, 7 mg x 2) in 400 μL of 1 M $NaHCO_3$ was treated with 1% (*R,S*)-FDPEA acetone solution (each 2 mL (20 mg, 0.0655 mmol)) and the mixture was allowed to stand at 57 °C for 1 hr, then after cooling, 1 M HCl (400 μL) was added. The residue obtained after evaporation of the reaction mixture was applied to silica gel chromatography twice using ($CHCl_3$:CH_3OH:H_2O = 65:10:5 (lower layer), AcOEt:*i*-PrOH:H_2O = 8:1:2 (upper layer) as the mobile phase to give the pure (*R*)-FDPEA derivative (3.2 mg, 20.9%) and (*S*)-FDPEA derivative (7.4 mg, 48.4%).
10. Microginin-((*R*)-FDPEA)$_3$; FABMS *m/z* 1569 $[M+H]^+$, *m/z* 1591 $[M+Na]^+$. Microginin-((*S*)-FDPEA)$_3$; FABMS *m/z* 1569 $[M+H]^+$, *m/z* 1591 $[M+Na]^+$.
11 Almost the same result was obtained in CD_3OD.
12. Matsuura, F.; Hamada, Y.; Shioiri, T. *Tetrahedron* **1994**, *50*, 11303-11314.