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Nuclear magnetic resonance studies for the chiral recognition of (+)-(*R*)-18-crown-6-tetracarboxylic acid to amino compounds

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Dedicated to Professor Terumichi Nakagawa on the occasion of his retirement and 63rd birthday.

Abstract

Chiral recognition capability of (+)-(*R*)-18-crown-6-tetracarboxylic acid ($18C6H_4$) to various amino compounds containing 16 amino acids, five alkyl amines, seven aminoalcohols and other amino compounds in nuclear magnetic resonance (^{1}H-NMR) analysis was investigated. In general, amino compounds having an aromatic ring were well chiral recognized with $18C6H_4$ compared with those having no aromatic ring. Effects of $18C6H_4$ concentration and the kind of deuterated solvents (D_2O, CD_3OD and CD_3CN) for measurement on the chiral recognition was investigated in detail. Concentration of 5 equivalent $18C6H_4$ to the amino compounds was found to be sufficient for the chiral recognition. On the other hand, an effective deuterated solvent (D_2O, CD_3OD or CD_3CN) for measurement was different in each compound. Distinguishment of 1.0% of the minor enantiomer (D-form) in L-alanine-β-naphthylamide was found to be possible by ^{1}H-NMR employing $18C6H_4$ as a chiral shift reagent.

Keywords: Chiral discrimination; Enantiomer separation; Chiral crown ether; Nuclear magnetic resonance spectrometry; Chiral NMR shift reagents

1. Introduction

The separation of enantiomers is a subject of great interest because the antipode of a chiral drug is regarded as one of the impurities from the viewpoint of quality control [1,2]. For these purposes, powerful, widely, and selectively applicable analytical tools are needed to determine the enantiomeric purity of the chiral drugs. During the past few decades, several analytical methods, such as gas chromatography (GC) [3,4], high-performance liquid chromatography (HPLC) [5–8], capillary electrophoresis (CE) [9–11], nuclear magnetic resonance (NMR) [12,13] etc. have been developed. Among them, NMR is a still

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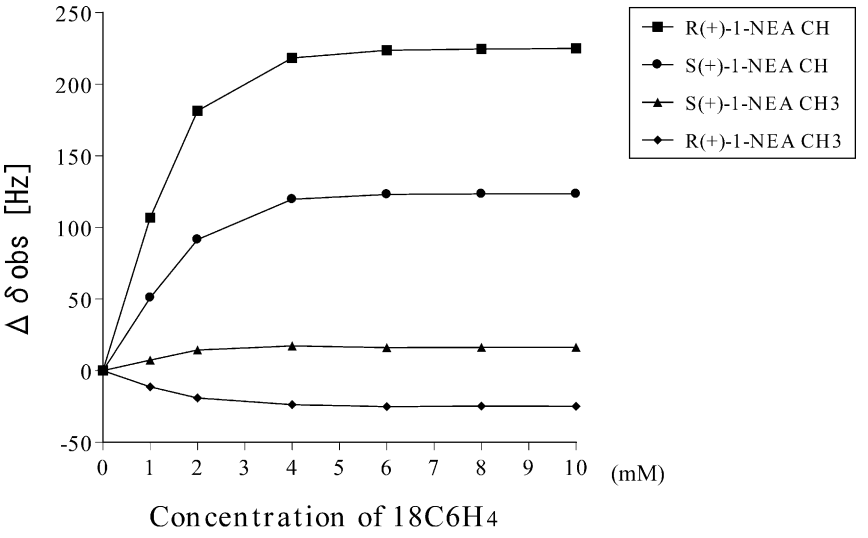

Fig. 1. The change of chemical shift (2 mM 1-NEA) by the addition of $18C6H_4$.

useful method although most of the enantiomer separation has been performed by HPLC or CE.

In NMR method, chiral derivatizing reagents or chiral shift reagents are employed. (*R*) or (*S*)-2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA), which was introduced by Mosher in 1969 as a chiral derivatizing reagent in ^{1}H-NMR, has been widely used [14–16]. There is no hydrogen for racemization, so racemization of MTPA under the derivatization is impossible. MTPA reacts readily with primary and secondary alcohols or amines to form diastereoisomeric amides or esters that may be recognized by ^{1}H-NMR. Camphor-based chiral lanthanide shift reagents, $[Eu(pvc)_2]$ etc. are also useful for the determination of enantiomers [17]. Enantiomeric purity of acetyl-L-carnitine, which is one of the biological substances localized in various tissues of mammals,

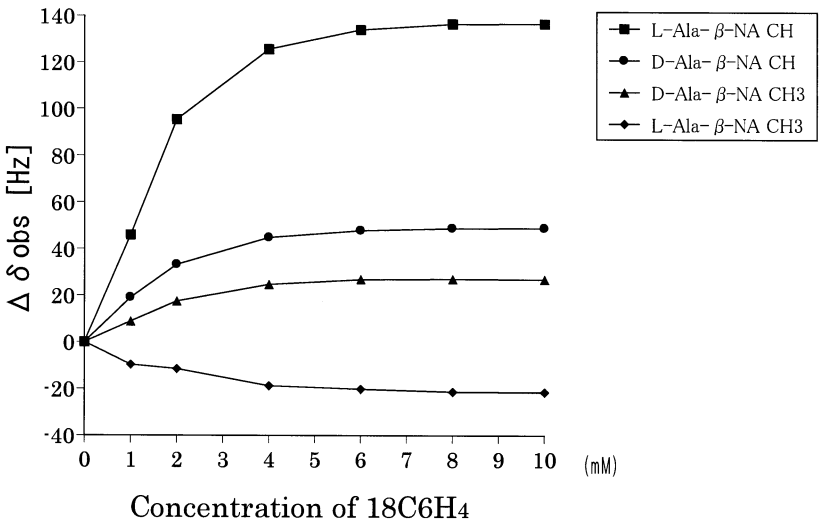

Fig. 2. The change of chemical shift (2 mM Ala-β-NA) by the addition $18C6H_4$.

Table 1
Effect of deuteration solvents on chemical shift change (R^1R^2–CH–NH_2)

Entry	Compounds R^1, R^2	Solvent	CH proton without 18C6H$_4$	$\Delta\delta$ (CH proton with 18C6H$_4$)	CH_3 proton without 18C6H$_4$	$\Delta\delta$ (CH_3 proton with 18C6H$_4$)
1	Phenylglycine (PheG) C_6H_5, COOH	D_2O	5.21	+0.07(L), +0.17(D)	–	–
2		CD_3OD	5.08	+0.38(L), +0.50(D)	–	–
3		CD_3CN	5.11	+0.20(L), +0.42(D)	–	–
4	1-Phenyethyl amine, (1-PEA) C_6H_5, CH_3	D_2O	4.54	+0.05(*S*), +0.03(*R*)	1.64	±0.00
5		CD_3OD	4.45	+0.29(*R*), +0.33(*S*)	1.63	−0.02(*S*), +0.06(*R*)
6		CD_3CN	4.50	+0.09(*R*), +0.16(*S*)	1.64	−0.03(*S*), ±0.00(*R*)
7	Alanine-β-Naphthylamide (Ala-β-NA) $C_{10}H_7NHCO$, CH_3	D_2O	4.30	+0.04	1.68	+0.00(L), +0.01(D)
8		CD_3OD	4.17	+0.15(D), +0.32(L)	1.65	−0.03(L), +0.06(D)
9		CD_3CN	4.34	Overlapped	1.66	−0.02(L), ±0.00 (D)
10	1-(1-Naphthyl) ethylamine (1-NEA) $C_{10}H_7$, CH_3	D_2O	5.46	+0.01(*S*), +0.09(*R*)	1.78	−0.02(*R*), +0.01(*S*)
11		CD_3OD	5.39	+0.21(*S*), +0.47(*R*)	1.76	−0.06(*R*), ±0.00(*S*)
12		CD_3CN	5.39	+0.14(*S*), +0.30(*R*)	1.77	−0.08(*R*), ±0.00(*S*)

All chemical shifts reported in ppm relative to TPS at 27 °C.

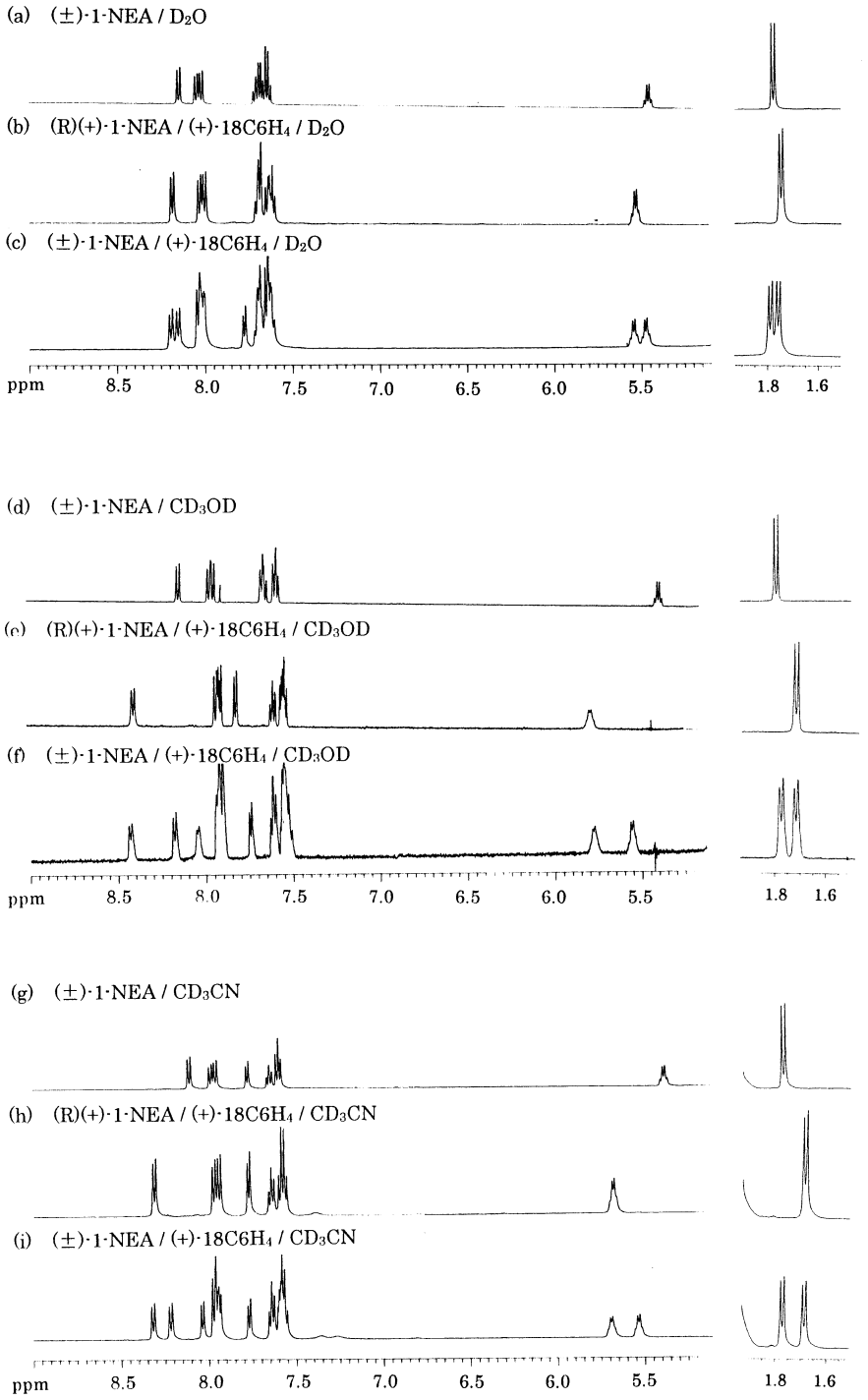

Fig. 3. ^{1}H-NMR spectra of (±)-1-NEA and (+)-18C6H$_4$ mixtures in D_2O, CD_3OD and CD_3CN.

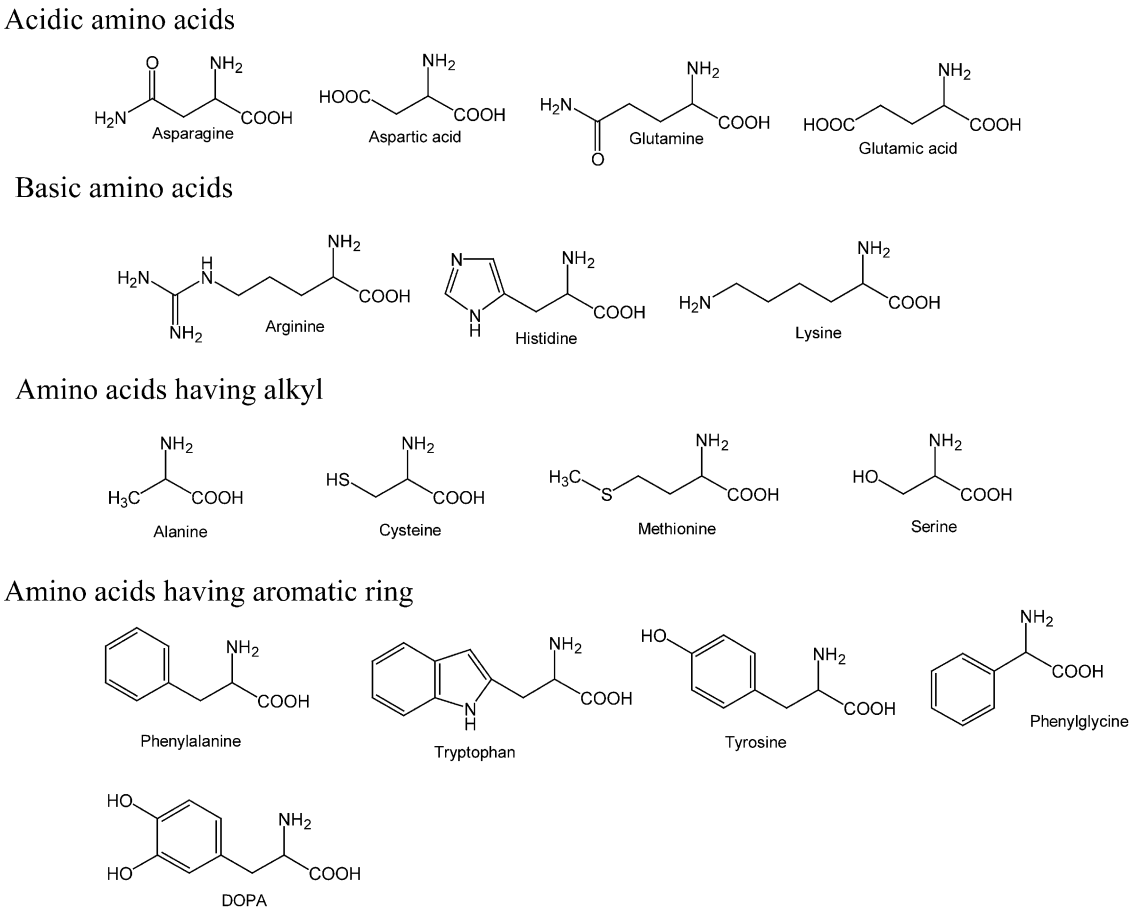

Fig. 4. Structures of amino acids investigated.

was successfully determined by using [Eu(hfc)$_3$] [18]. (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol has been widely used as a chiral shift reagent [19–22]. The chiral shift reagent method has advantages such as simplicity, rapid analysis, etc.

Crown ethers, first introduced by Pedersen in 1967 [23,24], are synthetic macrocyclic polyethers that can form selective complexes with suitable cations. Chiral (+)-(*R*)-18-crown-6-tetracarboxylic acid (18C6H$_4$), first synthesized by Lehn and his co-workers [25,26], was successfully employed for the CE enantiomer separation of amino acids and amino compounds as chiral selectors [27–29], because of its high solubility in water. Recently, 18C6H$_4$ was also employed for the chiral stationary phases (CSPs) in HPLC [30–35].

In our previous works [30,31,33], we reported the synthesis and the evaluation of the novel CSPs chemically immobilized 18C6H$_4$. NMR spectroscopy was also employed for the investigation of the interaction between 1-(1-naphthyl)ethylamine (1-NEA) and 18C6H$_4$ [31].

In this work, we further investigated the capability of 18C6H$_4$ as a chiral shift reagent in NMR. Effects of the concentration of 18C6H$_4$ and deuterated solvents (D_2O, CD_3OD and CD_3CN) for measurement on the chiral recognition were investigated. Sixteen amino acids, five alkyl amines, seven aminoalcohols and eight other amino compounds were applied in this study. Finally, a possibility of this method as a quality testing (optical purity testing) method of chiral compounds was investigated.

Table 2
$\Delta\delta$ of amino acids [R–CH(NH_2)COOH] in D_2O

Entry	Compounds	*R*	CH proton without 18C6H_4	$\Delta\delta$ (CH proton with 18C6H_4)
Acidic amino acids				
13	Asparagine	H_2NCOCH_2–	4.37	+0.03(L), +0.05(D)
14	Aspartic acid	$HOOCCH_2$–	4.41	+0.03(L), +0.05(D)
15	Glutamine	$H_2NCOCH_2CH_2$–	4.15	+0.02(L), +0.06(D)
16	Glutamic acid	$HOOCCH_2CH_2$–	4.12	+0.02(L), +0.06(D)
Basic amino acids				
17	Arginine	$^+NH_2$=C(NH_2)$NHCH_2CH_2CH_2$–	4.12	+0.02(L), +0.11(D)
18	Histidine	$C_3N_2H_3CH_2$–	4.41	+0.02(L), +0.08(D)
19	Lysine	$^+H_3NCH_2CH_2CH_2CH_2$–	4.11	+0.02(L), +0.06(D)
Amino acids having alkyl				
20	Alanine	CH_3–	4.14	+0.03(L), +0.05(D)
21	Cysteine	$HSCH_2$–	4.37	+0.03(L), +0.09(D)
22	Methionine	$CH_3SCH_2CH_2$–	4.24	+0.01(L), +0.05(D)
23	Serine	$HOCH_2$–	4.22	+0.03(L), +0.06(D)
Amino acids having aromatic ring				
24	Phenylalanine	$C_6H_5CH_2$–	4.38	±0.00(L), +0.03(D)
25	Tryptophan	$C_8H_6NCH_2$–	4.42	±0.00(L), +0.03(D)
26	Tyrosine	4-$OHC_6H_4CH_2$–	4.32	±0.00(L), +0.03(D)
27	DOPA	3,4-di$OHC_6H_3CH_2$–	4.31	±0.00(L), +0.03(D)
28	Phenylglycine	C_6H_5–	5.21	+0.07(L), +0.17(D)

All chemical shifts reported in ppm relative to TPS at 27 °C.

2. Materials and methods

2.1. NMR equipment

^{1}H-NMR spectra were taken in D_2O, CD_3OD and CD_3CN on Brucker DRX-500 FT-NMR spectrometer (Rheinstetten, Germany) operating at 500 MHz in the ^{2}H lock mode. Chemical shifts were reported in parts per million (ppm) relative to sodium 3-(trimethysilyl)propane sulfonate (TSP), and coupling constants were reported in Hz. Typical acquisition parameters included sweep width (SWH) of 6000 Hz, temperature of 27 °C digital resolution of 0.1834 Hz per point. Typical processing parameters included window type (WDW) of exponential multiplication (EM), sine bell shift (SSB) of 0, line broadening (LB) of 0.30 Hz, and gaussian broadening (GB) of 0. Spinning tubes of 5 mm i.d. containing 600 μl of a solution were employed.

2.2. Materials

18C6H_4 was purchased from Aldrich Chemicals (Milwaukee, WI, USA). D_2O, CD_3OD, CD_3CN, DCl and TSP were purchased from ISOTEC Inc. (OH, USA). DL-alanine-β-naphthylamide (DL-Ala-β-NA) was purchased from Aldrich Chemicals. L-alanine-β-naphthylamide (L-Ala-β-NA) was purchased from USB (Cleveland, OH, USA), and the other amino compounds used were purchased from Aldrich Chemicals, Katayama Kagaku Kogyo (Osaka, Japan), Tokyo Kasei Kogyo (Tokyo, Japan) and Wako Pure Chemicals (Tokyo, Japan).

2.3. Optical purity testing of L-Ala-β-NA (general procedure)

Spinning tubes of 5 mm i.d. containing 600 μl of a solution were employed. Two milimolar (for 600 μl) of the testing compounds was taken in tube, and 18C6H_4 solution (10 mM, 600 μl) was added.

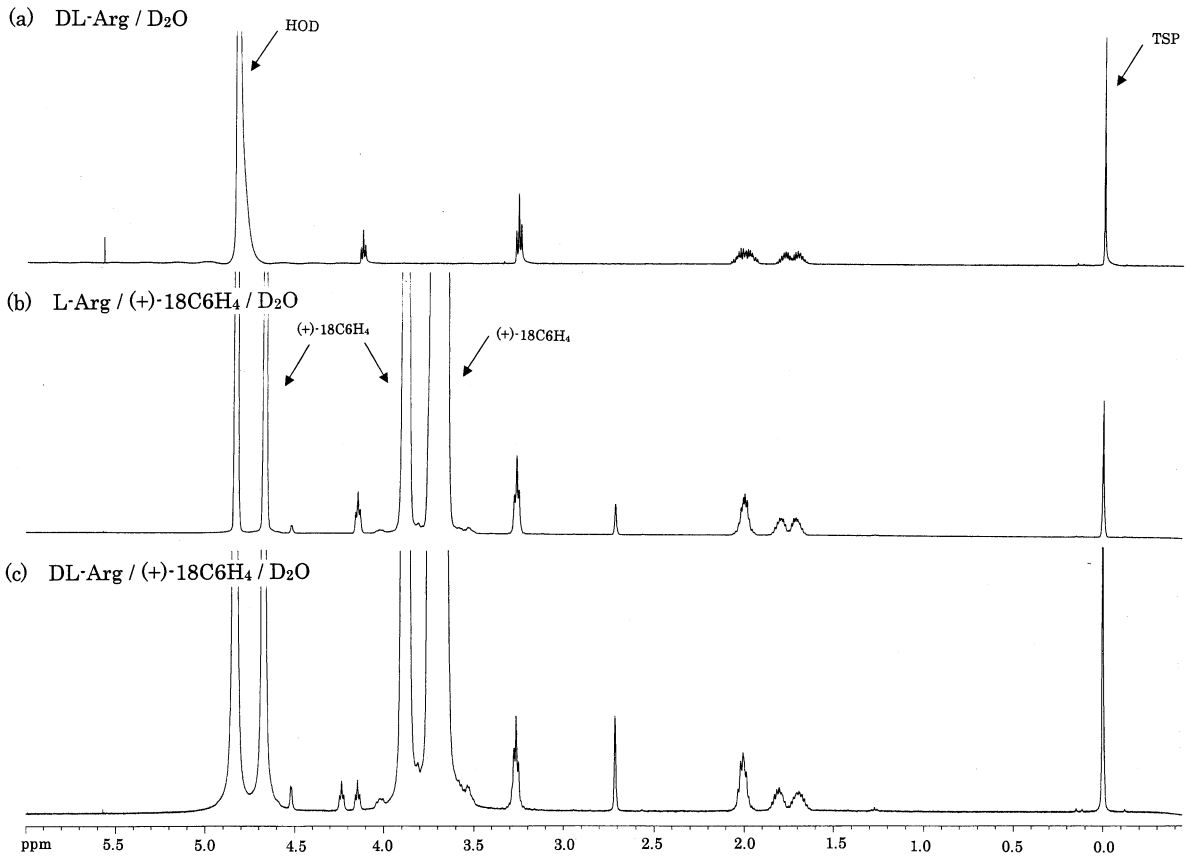

Fig. 5. ^{1}H-NMR spectra of DL-Arginine and (+)-18C6H$_4$ mixtures in D_2O.

Then, 6 μl of DCl and 6 μl of 1% TSP were added. DCl was employed for the protonation of tetracarboxylic acids and a primary amine. The pH of sample solutions in case of D_2O had been adjusted to around 1.

3. Results and discussion

3.1. Characterization of 18C6H$_4$ as a chiral shift reagent

3.1.1. Concentration of 18C6H$_4$

Effect of the concentration of 18C6H$_4$ (from 1 to 10 mM) on the change of chemical shift ($\Delta\delta_{obs}$) of 1-NEA (2 mM) and Ala-β-NA (2 mM) was investigated. The well resolved doublet at $\delta = 1.77$ ppm (CH_3, methyl protons, 1-NEA) or $\delta = 1.66$ ppm (Ala-β-NA), and qualtet at $\delta = 5.39$ ppm (CH, methine proton, 1-NEA) or $\delta = 4.17$ ppm ($J = 7.0$ Hz, Ala-β-NA) were evaluated. The $\Delta\delta_{obs}$ versus the concentration of 18C6H$_4$ curves for 1-NEA and Ala-β-NA are shown in Figs. 1 and 2, respectively. The saturation of $\Delta\delta_{obs}$ to 2 mM of the compounds were obtained above 8 mM of the chiral shift reagent. The concentration of 5 equivalent of the chiral shift reagent to the compounds was found to be sufficient for the chiral recognition of 18C6H$_4$.

3.1.2. Effect of deuterated solvents (D_2O, CD_3OD and CD_3CN) for measurement

Effect of deuterated solvents such as water (D_2O), methanol (CD_3OD) and acetonitrile

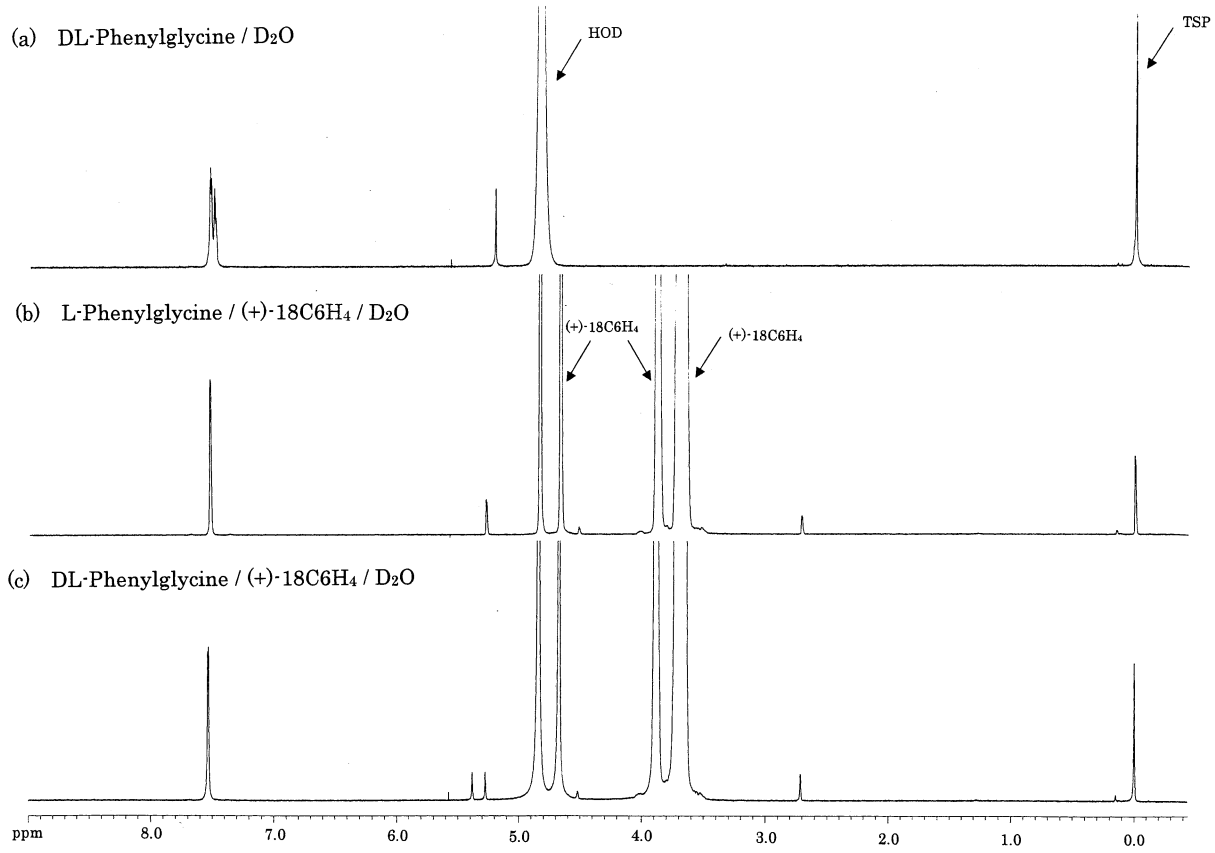

Fig. 6. ^{1}H-NMR spectra of DL-phenylglycine and (+)-18C6H$_4$ mixtures in D_2O.

(CD_3CN), on the chiral recognition of 18C6H$_4$ was investigated by employing phenylglycine (PheG), 1-phenylethylamine (1-PEA), Ala-β-NA and 1-NEA as amino compounds. Chiral recognition of PheG, 1-PEA, Ala-β-NA and 1-NEA was observed in all solvents (D_2O, CD_3OD and CD_3CN). The results are summarized in Table 1. The comparison of the ^{1}H-NMR spectra of 1-NEA in the presence (Fig. 3c, f and i) of 18C6H$_4$ and in the absence (Fig. 3a, d and g) of 18C6H$_4$ revealed two sets of each proton or group of RS-1-NEA. These peaks observed in Fig. 3(c, f and i), were simply superposition of each spectrum from the individual diastereomeric inclusion complexes that have formed. The methyl (δ 1.7–1.8 ppm) and the methine (δ 5.5–5.9 ppm) protons of the enantiomers of 1-NEA were significantly separated into the two sets of resonance in the presence of (+)-18C6H$_4$. Aromatic protons (δ 7.5–8.5 ppm) were also splited with relatively small chemical shift differences (Fig. 3). Since the methyl and the methine protons are adjacent to the ammonium cation, these protons must be significantly influenced by the chiral moiety (carboxylic acids) and shifted. It seems that the methyl and methine protons can utilize to investigate the trend of shift change in each enantiomer, and to measure the enantiomeric purity of the chiral compounds.

The comparison of the chemical shift change ($\Delta\delta$ value) of the methine and the methyl protons revealed that the methine proton shifted larger than the methyl protons (entries 4–12). The methine proton in PheG (entries 1–3) downshifted in D_2O, CD_3OD and CD_3CN, and the methine

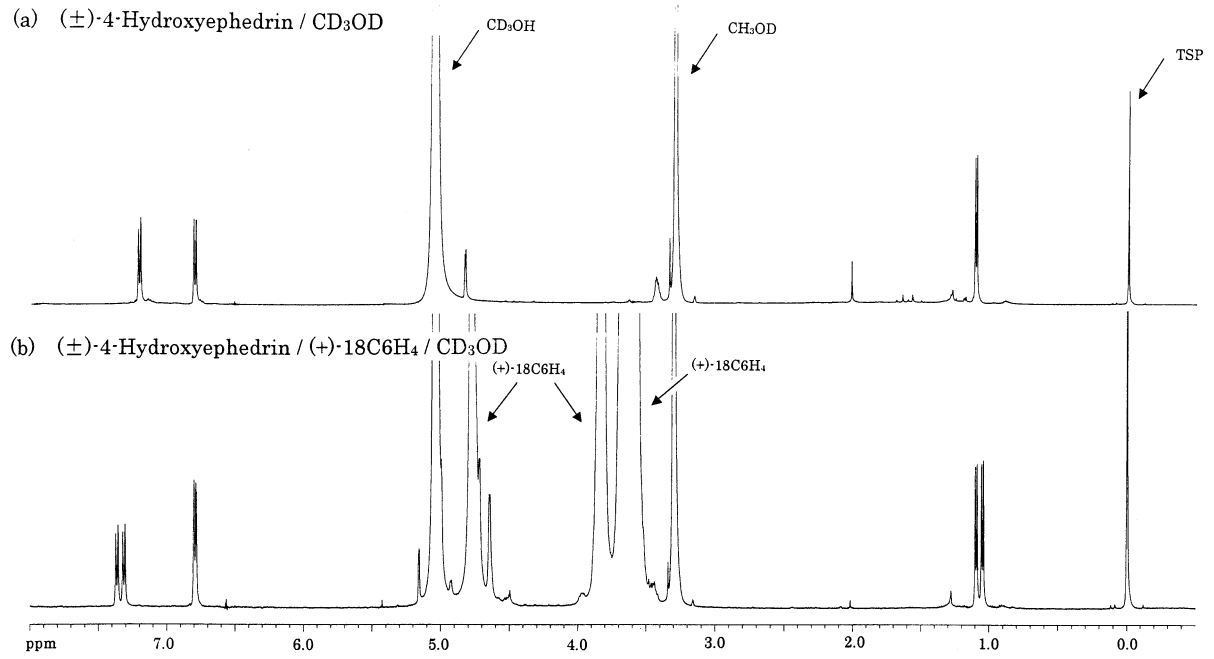

Fig. 7. ^{1}H-NMR spectra of (±)-4-hydroxyephedrin and (+)-18C6H$_4$ mixtures in CD_3OD.

proton of D-form in PheG significantly shifted than that of L-form. $\Delta\delta$ (D−L) values of the methine proton in D_2O, CD_3OD and CD_3CN were 0.10 ppm (=0.17−0.07), 0.12 ppm (=0.50−0.38) and 0.22 ppm (=0.42−0.20), respectively. High chiral recognition of 18C6H$_4$ to PheG was obtained by using CD_3CN as a deuterated solvent. The methine proton of 1-PEA (entries 4–6) much downshifted in CD_3OD. However, $\Delta\delta$ ($S-R$) value of the methine proton in CD_3OD was 0.04 ppm and $\Delta\delta$ ($S-R$) value of the methine proton in CD_3CN was 0.07 ppm (=0.16−0.09). High chiral recognition of 18C6H$_4$ to 1-PEA was also shown by using CD_3CN. The methine proton of *S*-1-PEA in D_2O downshifted larger than that of *R*-1-PEA. The trend in D_2O (entry 4) was

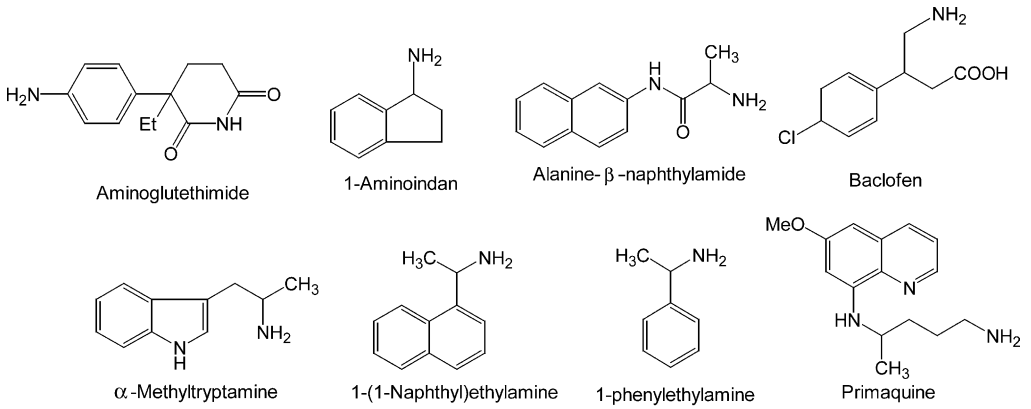

Fig. 8. Structures of other amino compounds investigated.

Table 3
$\Delta\delta$ of Alkylamines (R^1R^2–CH–NH_2) in CD_3OD

Entry	Compounds	R^1, R^2	CH_3 (R^1) proton without 18C6H4	$\Delta\delta$ (CH_3 (R^1) proton with 18C6H4)	CH_3 (R^2) proton without 18C6H4	$\Delta\delta$ (CH_3 (R^2) proton with 18C6H4)
29	sec-Butylamine	CH_3, CH_3CH_2	1.28	−0.02, +0.01	1.00	−0.03, −0.02
30	2-Aminopentane	CH_3, $CH_3(CH_2)_2$	1.28	−0.01, +0.01	0.92	±0.00
31	2-Heptylamine	CH_3, $CH_3(CH_2)_4$	1.28	−0.02, ±0.00	0.97	±0.00, +0.01
32	1,3-Dimethyl-*n*-butylamine	CH_3, $(CH_3)_2CHCH_2$	1.26	±0.00, +0.02	0.94	±0.00, +0.02
					0.97	+0.01
33	3-Amino-2,2-dimethylbutane	CH_3, $(CH_3)_3C$	1.28	±0.00, +0.02	1.01	+0.02

All chemical shifts reported in ppm relative to TPS at 27 °C.

different from that in CD_3OD and CD_3CN (entries 5, 6). $\Delta\delta$ (D–L) value of the methine proton of DL-Ala-β-NA in D_2O (entry 7) was the same, and chemical shift of DL-Ala-β-NA in CD_3CN (entry 9) overlapped with proton peaks of 18C6H4. Chiral recognition of 18C6H4 to the methine proton in Ala-β-NA could confirm by using CD_3OD (entry 8). The choice of solvent for the measurement was very important. $\Delta\delta$ ($R-S$) value of the methine proton in CD_3OD was 0.26 ppm, which was larger than that in D_2O (0.08 ppm) and CD_3CN (0.16 ppm).

3.2. Application for the chiral recognition of amino compounds

3.2.1. Amino acids

Chiral recognition capability of 18C6H4 (5 equivalent) to 16 kinds of amino acids (Fig. 4) was investigated by using D_2O as a deuterated solvent. D_2O was selected as a solvent, because the methine proton of amino acids often overlapped with 18C6H4 proton in CD_3OD. The results are summarized in Table 2. Typical spectra are shown in Figs. 5 and 6. Arg (Fig. 5) and PheG (Fig. 6) gave large $\Delta\delta$ values. In general, the methine proton in D-form amino acids was observed in the low field compared with that in L-form. This trend can be usable as the decision of absolute configuration having an amino acid moiety. Moreover, $\Delta\delta$ (D−L) values in Phe, Try, Tyr and DOPA (entries 24–27) were all 0.03 ppm. These compounds have the same structural part in the molecule. $\Delta\delta$ (D−L) value of the methine proton must depend on the structure of neighboring to the amine function.

3.2.2. Alkyl amines and aminoalcohols

Alkyl amines (entries 29–33) in Table 3 are important reagents as a chiral building block. However, it is not easy to measure the optical purity of alkyl amines. These compounds have no UV absorbance to detect in the usual HPLC–UV method. Therefore, the optical purity of these compounds have been measured by the chiral GC method after derivatization of analytes. The maximum $\Delta\delta$ ($R-S$ or $S-R$) value observed in NMR with 18C6H4 as a chiral shift reagent was

Table 4
$\Delta\delta$ of Aminoalcohols (R^1–CH(OH)–CH(R^2)–NH_2)

Entry	Compounds	R^1	R^2	Solvent	CH_3 proton without 18C6H4	$\Delta\delta$ (CH_3 proton with 18C6H4)	CH(OH) proton without 18C6H4	$\Delta\delta$ (CH(OH) proton with 18C6H4)
34	2-Amino-1-propanol	CH_3	H	CD_3OD	1.26	+0.05, +0.07	Not asigned	Overlapped
35	2-Amino-1-butanol	CH_3CH_2	H	CD_3OD	0.99	+0.03	Not asigned	Overlapped
36	2-Amino-1-phenylethanol	C_6H_5	H	CD_3OD	–	–	4.90	Overlapped
				D_2O	–	–	5.01	+0.01, +0.03
37	Norphenylepherine	3-OHC_6H_4–	H	CD_3OD	–	–	4.83	Overlapped
38	2-Amino-1-phenylpropanol	C_6H_5–	CH_3	CD_3OD	1.08	−0.04, ±0.00	4.96	+0.13, +0.29
				D_2O	1.19	−0.01, ±0.00	4.99	Overlapped
39	4-Hydroxynorephedrine	4-OH·C_6H_4–	CH_3	CD_3OD	1.11	−0.06, −0.01	4.84	Overlapped
40	2-Amino-1,2-dipheny-lethanol	C_6H_5	C_6H_5	CD_3OD	–	–	5.23	−0.03, +0.27

All chemical shifts reported in ppm relative to TPS at 27 °C.

0.02 ppm (entry 31, 32, 33). Chiral recognition capability of 18C6H4 to alkyl amines was low.

The results for aminoalcohols are summarized in Table 4. Aminoalcohols having no aromatic ring (entries 34–36) also were not recognized with 18C6H4 well. On the other hand, $\Delta\delta$ (1*R*,2*S*−1*S*,2*R*) values of methine proton in aminoalcohols having an aromatic ring were 0.16 ppm (= 0.29 − 0.13, entry 38), 0.30 ppm (= 0.27 + 0.03, entry 40), larger than that having no aromatic ring as might have been expected. Typical spectra obtained from 4-hydroxyephedrin are shown in Fig. 7.

3.2.3. Other amino compounds

Chiral recognition capability of 18C6H4 to amino compounds having an aromatic ring, drug substances and/or chiral derivatizing reagents, etc. was investigated through the comparison of the spectrum of each compound in the presence and in the absence of 18C6H4. The results are summarized in Table 5. Structures of amino compounds investigated are shown in Fig. 8. The large $\Delta\delta$ (*R*−*S* or D−L) values were obtained for 1-NEA (0.26 ppm), baclofen (0.19 ppm) and Ala-β-NA (0.17 ppm). The protons showing large shift change were adjacent to (one or two bonds distance between amine function and shifted protons) the amine function as we expected. Shift change of methyl protons in primaquine was also observed, although there was five bonds distance between the amine function and the methyl function (methyl protons). There must be a contribution of hydrogen-bonding association between the secondary amine function of the analyte and tetracarboxylic acids of 18C6H4, because the distance between the secondary amine function and the methyl function (methyl protons) shifted is two bonds.

3.2.4. Validation and application to the enantiomeric purity determination of L-Ala-β-NA

The optical purity testing of L-Ala-β-NA was investigated with this method. Some validations (Fig. 9) such as linearity and limit of detection (LOD) were also demonstrated for the new testing method. The linearity was investigated through the intensities of the D-form in L-form standards, which were mixed with D-form over the range

Table 5
$\Delta\delta$ of Other amino compounds in CD_3OD

Entry	Compounds	Position	Shifted proton without $18C6H_4$	$\Delta\delta$ (Shifted proton with $18C6H_4$)	Position	Shifted proton without $18C6H_4$	$\Delta\delta$ (Shifted proton with $18C6H_4$)
41	Aminoglutethimide, (aromatase inhibitors)	CH (aromatic)	7.52	+0.14, +0.16	–	–	–
42	1-Aminoindan, (anti-Parkinson drug metabolite)	CH	4.86	+0.01, +0.06	–	–	–
43	Alanine-β-naphthylamide (chiral derivatizing reagent)	CH	4.17	+0.15(D), +0.32(L)	CH_3	1.65	−0.03(L), +0.06(D)
44	Baclofen (skeketal muscle relaxant)	$CH_2(NH_2)$	2.81	−0.04, +0.23	$CH_2(NH_2)$	2.66	−0.03, +0.02
45	α-Methyltryptamine (monoamine oxidase inhibitor)	CH	3.59	Overlapped	CH_3	1.31	−0.11, −0.09
46	1-(1-Naphthy)lethylamine (chiral derivatizing reagent)	CH	5.39	+0.21(*S*), +0.47(*R*)	CH_3	1.76	−0.06(*R*), ±0.00(*S*)
47	1-Phenylethylamine (chiral derivatizing reagent)	CH	4.45	+0.29(*R*), +0.33(*S*)	CH_3	1.63	−0.02(*S*), +0.06(*R*)
48	Primaquine (antimalarial drug)	–	–	–	CH_3	1.37	+0.07, +0.08

All chemical shifts reported in ppm relative to TPS at 27 °C.

from 1.0 to 10.0%. The relationship between the found values (y) and the theoretical values (x) was found to be a straight line ($y = 1.087x - 0.254$) (correlation coefficient = 0.9928); the good linearity was confirmed in this method. The detection limit of D-form was investigated by visual evaluation, and 1.0% D-form in L-Ala-β-NA could be determined. Probably, thorough an increase of the concentration of an analyte, around 0.2% of LOD will be obtained. Finally, optical purity testing of L-Ala-β-NA (USB), which was purchased from the commercial source, was performed by the method. D-Ala-β-NA was not detected (i.e. not more than 1.0%), indicating that this reagent can be usable as the chiral derivatizing reagent as it is.

4. Conclusion

Chiral recognition capability of $18C6H_4$ to several amino acids was investigated. The chiral methine proton in amino acids shifted selectively by adding $18C6H_4$ as a chiral shift reagent. The methine proton signal of D-form of amino acids was observed in the low field compared with that of L-form. In the other words, chiral recognition capability of $18C6H_4$ to D-amino acids was strong than that to L-amino acids as definite laws. This trend can be usable for the decision of absolute configuration having an amino acid moiety. The results obtained in this study will prove to be useful for the absolute configuration determination of amino compounds. Moreover, in the complexes formed by macrocyclic polyethers of 18-crown-6 type, the cationic substrates interact with oxygen of polyether ring and are held in the center of the circular cavity, chirality of amino acids is recognized by tetracarboxylic acids of $18C6H_4$. Alkyl amines and aminoalcohols having no aromatic ring were not well recognized with $18C6H_4$. In primaquine, the shift change was observed in spite of five bonds distance between the amine function and the methyl function (methyl protons). The NMR method with $18C6H_4$ was successfully applied for the determining the enantiomeric purity of Ala-β-NA.

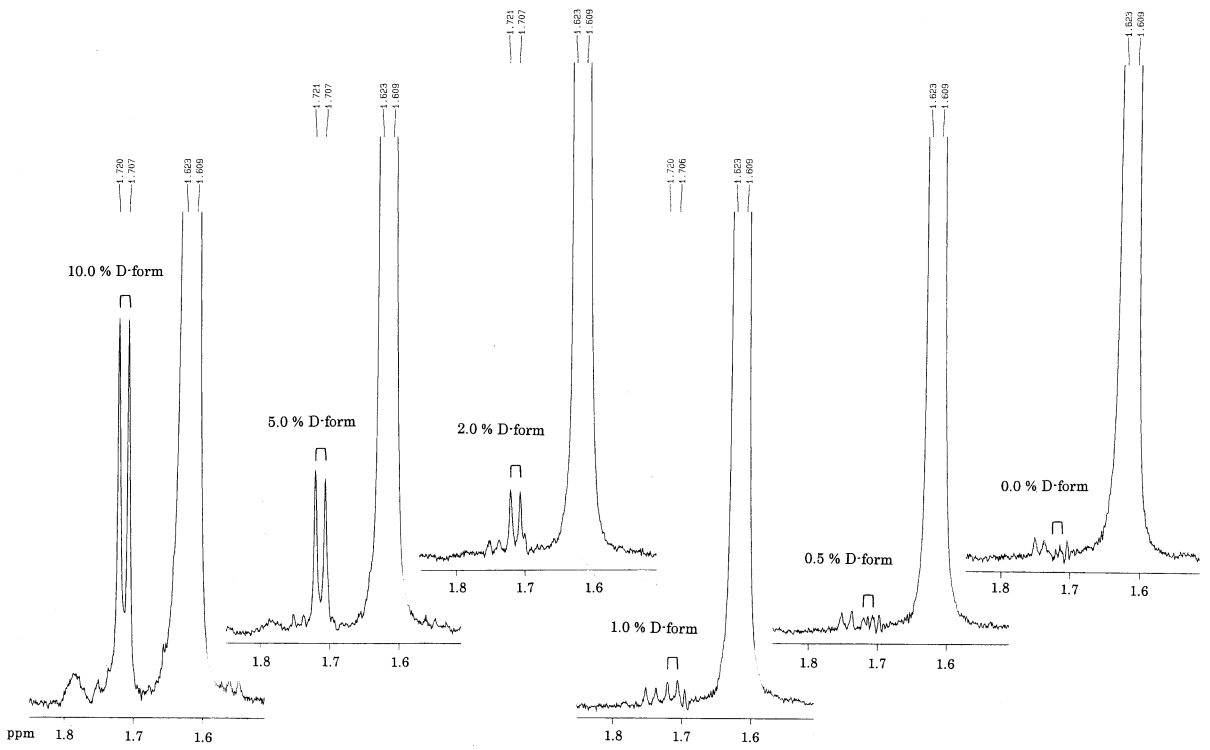

Fig. 9. Linearity and detection limit of D-form in L-Ala-β-NA.

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