Enantiomer-Differentiating Ability of Cyclo(Phe–Pro)$_4$ for Noradrenaline Hydrochloride and Preparation of Complexes with Various Amine Hydrochlorides

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Cyclo(Phe–Pro)$_4$ (1) formed a 1:1 complex with noradrenaline hydrochloride (NAd·HCl) through intermolecular hydrogen bonds and a hydrophobic effect. The formation constant of the 1:1 complex of 1 with *l*-NAd·HCl was approximately 12.3 times that of the complex with *d*-NAd·HCl. It was presumed that the secondary hydroxy group of *l*-NAd·HCl formed an intermolecular hydrogen bond with Pro^2 CO of 1, whereas that of *d*-NAd·HCl did not. The 1:1 complexes of 1 with various amine hydrochlorides were prepared.

Key words noradrenaline; cyclopeptide; enantiomer differentiation; intermolecular hydrogen bond; NMR

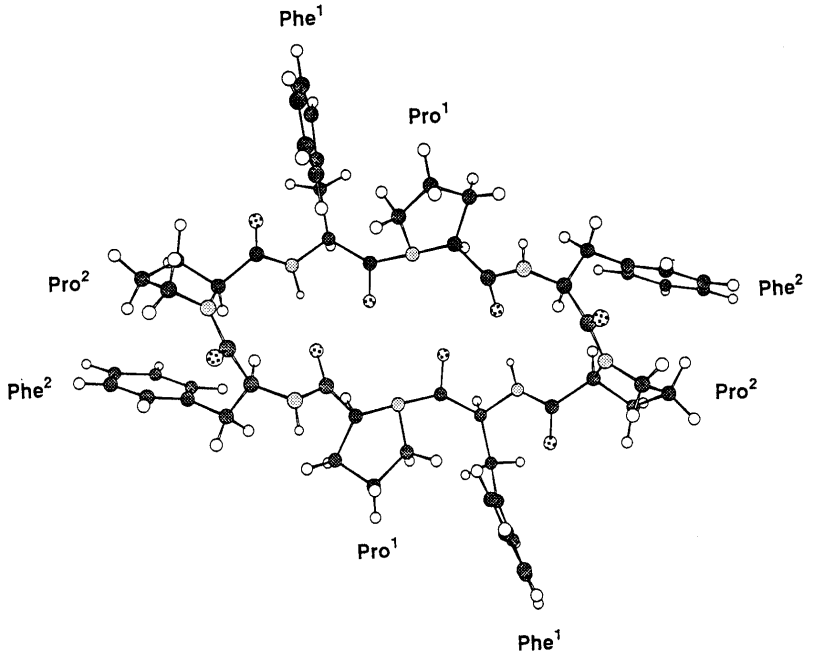
One of the superior functions of a living organism is molecular recognition, in particular, enantiomer differentiation. Noradrenaline (NAd) is a postganglionic neurotransmitter in the sympathetic nervous system, and a strong sympathomimetic drug. Its *l*-form exhibits much higher physiological activity than its *d*-form.

An approach to elucidate the mechanism of enantiomer differentiation is to synthesize various kinds of artificial receptor probes and investigate the difference in interaction between the *d*- and *l*-form of NAd in complex formation. Thus, we chose a cyclopeptide as an artificial receptor probe.[1] Cyclooctapeptide cyclo(Phe–Pro)$_4$ (**1**), which was synthesized previously as a model for an ionophore, has a rigid skeleton[2] and a chiral cavity which is sufficiently large to incorporate a molecule of NAd. In this study, the difference in interaction between **1** and the *d*- and *l*-forms was investigated.

Results and Discussion

Cyclo(Phe–Pro)$_4$ (**1**) takes a C_2-symmetric conformation in $CDCl_3$ and dimethyl sulfoxide (DMSO)-d_6. Residues are termed Phe^1, Pro^1, Phe^2, and Pro^2, and the peptide bonds between Phe^2 and Pro^2 are *cis* form. **1** has two intramolecular hydrogen bonds between Phe^1 NH and Pro^1 CO, and forms β turns.[2] The benzene rings of Phe^2 face the pyrrolidines of Pro^2.[1d] The structure of **1** as shown in Chart 1 was constructed based on the above information using molecular modeling software.[3]

Solutions of **1** (9.76 mg, 0.01 mmol) in $CDCl_3$ added to solutions of *dl*-NAd·HCl (2.06 mg, 0.01 mmol) in DMSO-d_6 were prepared. In three solutions, solution (A) [$CDCl_3$ (500 μl) and DMSO-d_6 (100 μl)], solution (B) [$CDCl_3$ (300 μl) and DMSO-d_6 (300 μl)], and solution (C) [$CDCl_3$ (100 μl) and DMSO-d_6 (500 μl)], the complex formation of **1** with *dl*-NAd·HCl was investigated by



Cyclo(Phe-Pro)$_4$ (1)

Chart 1

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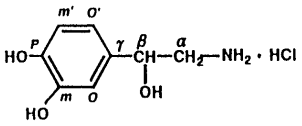


noradrenaline hydrochloride (NAd · HCl)

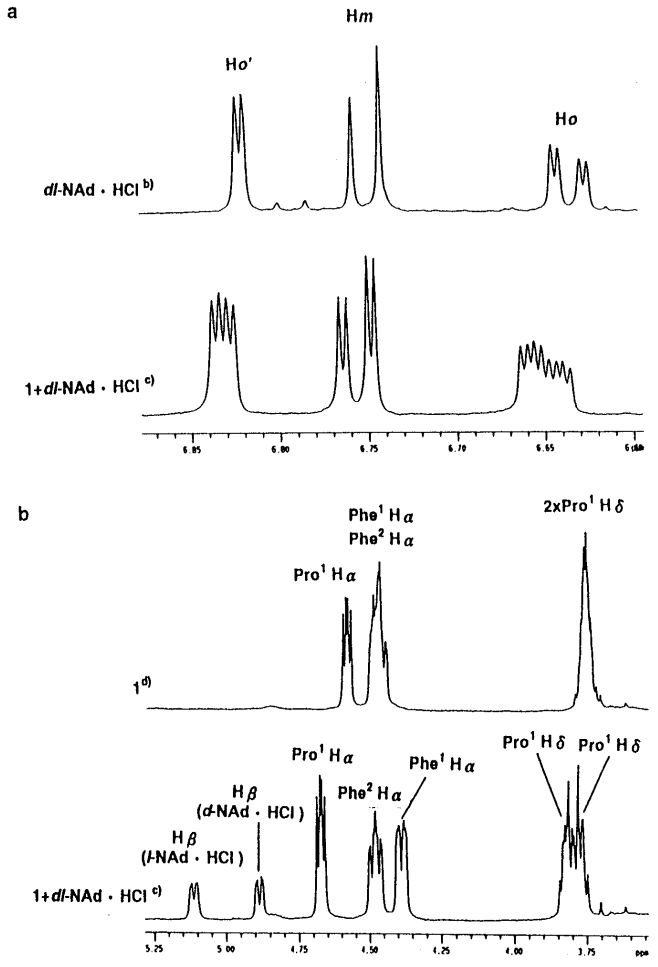


Fig. 1. Splitting of the Signals of NAd·HCl (a) and Separation of the Signals of **1** (b) in ^{1}H-NMR Spectra[a)]

a) NMR solvent: solution (B) [$CDCl_3$ (300 μl)+DMSO-d_6 (300 μl)], b) *dl*-NAd·HCl (2.06 mg, 0.01 mmol), c) **1** (9.76 mg, 0.01 mmol) and *dl*-NAd·HCl (2.06 mg, 0.01 mmol), d) **1** (9.76 mg, 0.01 mmol).

monitoring with ^{1}H-NMR. In solutions (A) and (B), the splitting of ^{1}H-NMR signals of *dl*-NAd·HCl was observed, indicating that complexes had formed (Fig. 1a). The splitting resulted from the formation of diastereomeric pairs of complexes. Previously, similar splitting of the ^{13}C-NMR signals of DL-amino acid ester salts was reported by Deber.[4)] The signals of Phe^1 and Phe^2 H_α, and the two Pro^1 H_δ were separated due to complex formation (Fig. 1b). However, in solution (C) such splitting and the separation of the signals of *dl*-NAd·HCl were not observed, indicating that the complexes did not form.

Information regarding the structural flexibility of **1** can be experimentally obtained from the relaxation times of the carbon resonances (T_1). The NT_1 values (N=number of attached protons, T_1=longitudinal relaxation time) are correlated directly with molecular mobility.[5)] Each NT_1 value of the carbons of *d*- and *l*-NAd·HCl was decreased by the formation of a complex with **1**. This finding

Table 1. NT_1 Values (ms) of NAd·HCl

Sample	C_α	C_β	C_o	$C_{m'}$	$C_{o'}$
l-NAd·HCl	1792	1685	924	1209	926
l-NAd·HCl in complex with **1**	622	540	268	493	411
d-NAd·HCl in complex with **1**	512	605	342	342	390

Sample: a solution of NAd·HCl (2.06 mg 0.01 mmol) in the absence and presence of **1** (9.76 mg, 0.01 mmol) in $CDCl_3$ (500 μl) and DMSO-d_6 (100 μl) [solution (A)].

Table 2. ^{13}C-NMR Spectra of **1**

Sample	Phe^1 CO	Phe^2 CO	Pro^2 CO	Pro^1 CO
In solution (A)				
1	167.588	170.130	170.780	172.375
1+*d*-NAd·HCl	167.893	169.990	170.895	172.269
	(0.305)	(−0.140)	(0.115)	(−0.106)
1+*l*-NAd·HCl	168.205	169.694	171.413	172.104
	(0.617)	(−0.436)	(0.633)	(−0.271)
1+*dl*-NAd·HCl	168.090	169.768	171.035	172.137
	(0.502)	(−0.362)	(0.255)	(−0.238)
1+*d*-Pro·HCl	167.958	170.031	170.903	172.310
	(0.370)	(−0.099)	(0.123)	(−0.065)
1+*l*-Pro·HCl	167.908	170.080	170.878	172.325
	(0.320)	(−0.050)	(0.098)	(−0.050)
In solution (B)				
1	167.284	169.908	170.360	172.310
1+*d*-NAd·HCl	167.383	169.900	170.377	172.285
	(0.099)	(−0.008)	(0.017)	(−0.025)
1+*l*-NAd·HCl	167.399	169.834	170.401	172.269
	(0.115)	(−0.074)	(0.051)	(−0.041)

Solution (A): $CDCl_3$ (500 μl)+DMSO-d_2 (100 μl). Solution (B): $CDCl_3$ (300 μl)+DMSO-d_6 (300 μl). Sample: **1** (9.76 mg, 0.01 mmol) without and with NAd·HCl (2.06 mg, 0.01 mmol) or Prop·HCl (2.96 mg, 0.01 mmol). ( ) is the shift value (ppm) following complex formation with NAd·HCl or Prop·HCl.

indicated that *d*- and *l*-NAd·HCl entered the cavity of **1** (Table 1).

Upon complex formation with NAd·HCl, no change in the chemical shift of each ^{13}C-NMR signal of **1** was observed with the exception of the signals of the four carbonyl carbons, indicating that a conformational change did not occur due to the rigidity of the skeleton. Thus, **1** maintained its C_2-symmetric conformation.[1b,c)] In particular, no shift or disappearance of the ^{1}H-NMR signal of Phe^1 NH (9.060 ppm) was observed, implying that the intramolecular hydrogen bonds of **1** were still present. The signal of Phe^2 NH (8.199 ppm) disappeared after about 2 h upon dissolution of **1** in solution (A) due to exposure to the solvent.

Table 2 lists the chemical shifts of the four carbonyl carbons of **1** in the ^{13}C-NMR spectra. The samples analyzed were **1** in the absence and presence of *d*-, *l*-, and *dl*-NAd·HCl in solutions (A) and (B). The NMR signal is shifted downfield upon hydrogen bond formation, indicating that Phe^1 CO and Pro^2 CO formed intermolecular hydrogen bonds with NAd·HCl. Considering the existence of intramolecular hydrogen bonds between Phe^1 NH and Pro^1 CO, it was expected that the intermolecular hydrogen bond between Pro^2 CO and NAd·HCl would be very weak. These carbonyl groups were bonded with the protons of the amino group of NAd·HCl which were stronger donors of hydrogen bond than those

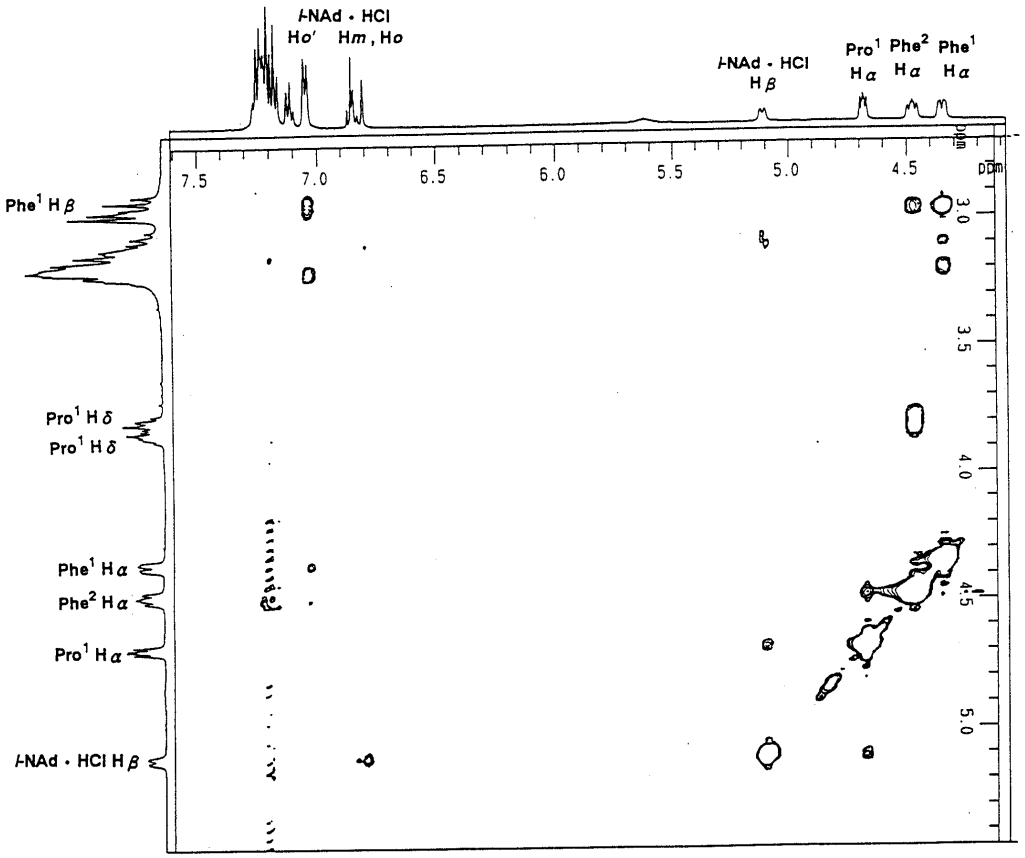


Fig. 2. ROESY Spectrum of 1 : 1 Complex of **1** with *l*-NAd·HCl

The sample solution contains **1** (9.76 mg, 0.01 mmol) and *l*-NAd·HCl (2.06 mg, 0.01 mmol) in $CDCl_3$ (500 μl) and DMSO-d_6 (100 μl) [solution (A)].

of the two catechol hydroxy groups.

Each shift value in the chemical shift of solution (B) is smaller than that of solution (A), implying that the intermolecular hydrogen bonds in solution (B) are weaker than those in solution (A). That is, an increase in the amount of DMSO-d_6 results in the weakening of intermolecular hydrogen bonds, and in solution (C) these bonds were not formed.

Upon plotting the shift values of the chemical shift of Phe^1 CO in ^{13}C-NMR measurements *vs.* the molar ratio of NAd·HCl, the type of complex formed was determined using the molar method to be 1 : 1 in both *d*- and *l*-NAd·HCl. The formation constants[1b,6] of the 1 : 1 complex of **1** with *d*- and *l*-NAd·HCl were calculated by a non-linear least-squares method as 231.52 and 2838.4 M^{-1}, respectively. The formation constant of **1** with *l*-NAd·HCl was approximately 12.3 times that with *d*-NAd·HCl.

The rotating frame nuclear overhauser effect spectroscopy (ROESY)[7] spectrum of the complex of **1** with *l*-NAd·HCl in solution (A) is shown in Fig. 2. Two important correlations are the rotating frame nuclear overhauser effects (ROEs) between Phe^1 H_α, H_β and $H_{o'}$. That with *d*-NAd·HCl afforded the same two ROEs. These ROEs indicated that the catechol moiety of NAd·HCl inserted into a hydrophobic space consisting of the pyrrolidine of Pro^2 and the benzyl group of Phe^1 (Chart 2).[3] We reported previously that the benzene ring of PheOMe·HCl inserted into this hydrophobic space, and was fixed upon the face of the pyrrolidine of Pro^2 in the 1 : 1 complex.[1c]

Upon 1 : 1 complex formation in solution (A), the ^{1}H-NMR signal (5.640 ppm) of the proton of the secondary hydroxy group of *l*-NAd·HCl was shifted downfield by 0.102 ppm; on the other hand, that of *d*-NAd·HCl was shifted upfield by 0.027 ppm. Furthermore, upon plotting the shift values of the chemical shift of the carbons of *l*-NAd·HCl in ^{13}C-NMR measurements *vs.* the molar ratio of **1** (Fig. 3), the downfield shift of C_β (69.489 ppm) is larger than those of other carbons. On the other hand, a similar downfield shift of C_β of *d*-NAd·HCl and dopamine hydrochloride was not observed. These findings support the conclusion that the secondary hydroxy group of *l*-NAd·HCl formed an intermolecular hydrogen bond with Pro^2 CO, whereas that of *d*-NAd·HCl did not.

The nitrogen atom of NAd·HCl was positioned almost in the center of the cavity of **1** due to the formation of the intermolecular hydrogen bonds and the catechol moiety was fixed in the hydrophobic space consisting of the pyrrolidine of Pro^2 and the benzyl group of Phe^1. As a result, it was presumed that the proton of the secondary hydroxy group of the *l*-form was sufficiently close to form an intermolecular hydrogen bond with Pro^2 CO (Chart 2).

For the purpose of comparison with the interaction between **1** and NAd·HCl, we also investigated the interaction between **1** and propranolol hydrochloride (Prop·HCl) (Chart 3). In mixtures of **1** with *dl*-Prop·HCl in solutions (A) and (B), splitting of the ^{1}H-NMR signals of *dl*-Pro·HCl was observed. **1** formed a 1 : 1 complex with Prop·HCl. The formation constants with *d*- and *l*-Prop·

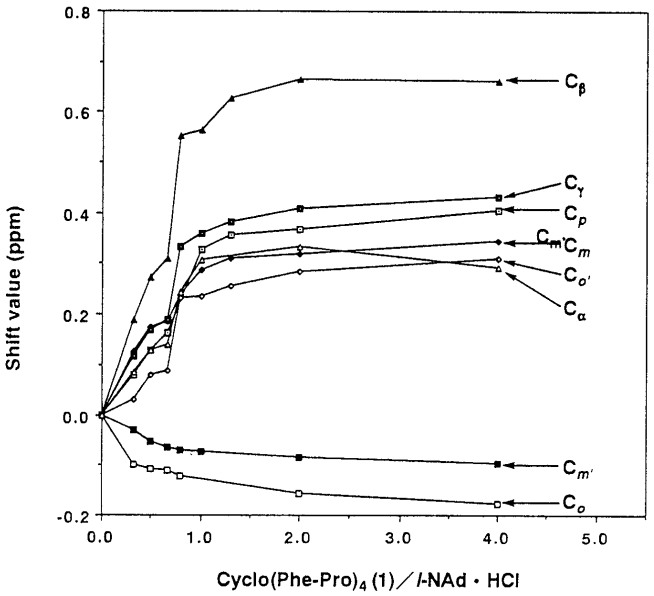
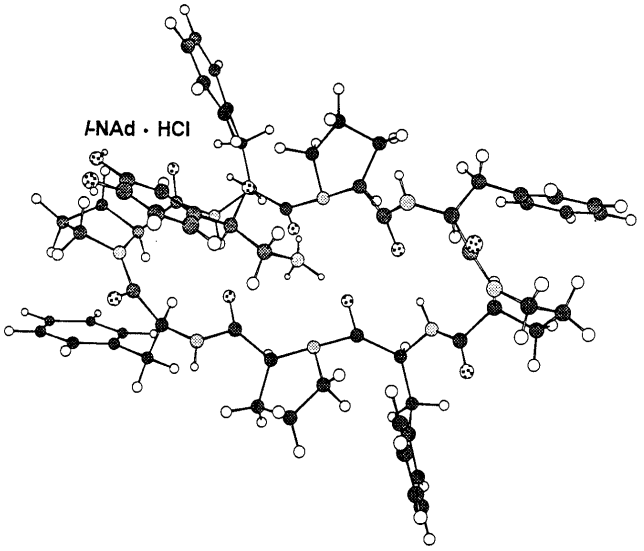


Fig. 3. The Shifts of the ^{13}C-NMR Signals of *l*-NAd·HCl upon Addition of **1**

Initial condition: *l*-NAd·HCl (2.06 mg, 0.01 mmol) in solution (A) [$CDCl_3$ (500 μl) and DMSO-d_6 (100 μl) [solution (A)].

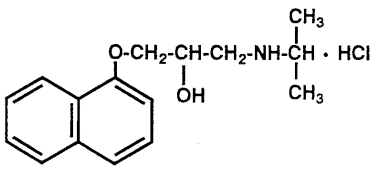


1:1 Complex of Cyclo(Phe-Pro)$_4$ (1) with *l*-NAd · HCl

Chart 2

HCl in solution (A) were 298.51 and 341.60 M^{-1}, respectively. Therefore, **1** in complex with Prop·HCl did not show a pronounced enantiomer differentiation.

Upon complex formation of **1** with Prop·HCl, the chemical shifts of the four carbonyl carbons in the ^{13}C-NMR spectra exhibited similar changes to those observed upon complex formation with NAd·HCl (Table 1). However, the intermolecular ROEs between Phe^1 H_α, H_β of **1** and the naphthalene moiety of Prop·HCl, and the downfield shift in the signal of the proton of the secondary hydroxy group were not observed. These findings indicated that the interaction between **1** and Prop·HCl in the 1:1 complex was only that of the intermolecular hydrogen bonds between Phe^1 CO, Pro^2 CO and the protons of the amino group of Prop·HCl.



propranolol hydrochloride (Prop · HCl)

Chart 3

Table 3. Melting Points and Yields of the 1:1 Complexes of **1** with Amine Hydrochlorides

Amine hydrochloride	1:1 Complex	
	mp (°C)	Yield (%)
Phenethylamine·HCl	252—253	83.9
Dopamine·HCl	209—211	92.2
d-Noradrenaline·HCl	203 dec.	66.9
l-Noradrenaline·HCl	234—236	62.0
d-Propranolol·HCl	169—171	77.8
l-Propranolol·HCl	173—175	75.9

Isolation of the 1:1 complexes of **1** with NAd·HCl, Prop·HCl, and other amine hydrochlorides was attempted. Solutions of **1** and the amine hydrochlorides in $CHCl_3$ and MeOH afforded 1:1 complexes. The mp (°C) and yield (%) of these complexes are listed in Table 3. Only the 1:1 complex of **1** with *l*-NAd·HCl was obtained from the solution of **1** with *dl*-NAd·HCl in $CHCl_3$ and DMSO.

Experimental

Melting points were determined using a Yanaco MP apparatus and are uncorrected. NMR spectra were obtained on a JEOL JMN-LA500 spectrometer operating at 500.00 MHz for ^{1}H and 125.65 MHz for ^{13}C at 25 °C. Chemical shift values are expressed in ppm downfield using tetra- methylsilane (TMS) as an internal standard. Samples were dissolved in $CDCl_3$ and DMSO-d_6 (99.8% D, Aldrich Chemical Company, Inc.) in a 5 mm i.d. sample tube. The possibility of peptide aggregation for **1** was investigated by recording the ^{1}H-NMR spectra of solutions containing 1.82—54.55 mM (**1**) in solutions (A)—(C). No significant changes in chemical shifts or line widths were observed over this concentration range. T_1 values were estimated using the standard inversion-recovery sequence to determine the null in signal intensity. ROESY experiments[7] were carried out using a mixing time of 250 ms in the phase-sensitive mode.

Materials *l*-NAd·HCl and *d*- and *l*-Prop·HCl were purchased from Sigma Chemical Company (St Louis, MO), and *d*-NAd·HCl from Kankyoukagaku Center Co., Ltd. (Yokohama). **1** was prepared according to the reported method.[2]

Preparation of 1:1 Complexes of 1 with Amine Hydrochlorides A solution of **1** (9.76 mg, 0.01 mmol) in $CHCl_3$ (500 μl) was added to solutions of amine hydrochlorides (0.01 mmol) in MeOH (150 μl). The mixtures were filtered and the filtrates were left for 0.5 h at room temperature and concentrated under reduced presure to give colorless powders of 1:1 complexes of **1** with amine hydrochlorides (Table 3).

Isolation of 1:1 Complex of 1 with *l*-NAd·HCl A solution of **1** (29.28 mg, 0.03 mmol) in $CHCl_3$ (1.5 ml) was added to a solution of *dl*-NAd·HCl (6.18 mg, 0.03 mmol) in DMSO (300 μl) and the mixture was left for 2 h at room temperature. A colorless powder precipitated and was filtered through a membrane filter (PTFE, 0.2 μm, Toyo Roshi Kaisha, Ltd.) and washed with ethyl ether to give a 1:1 complex of **1** with *l*-NAd·HCl (5.35 mg, 15.1%).

References and Notes

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