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The natural alkaloid isoanabasine: synthesis from 2,3′-bipyridine, efficient resolution with BINOL, and assignment of absolute configuration by Mosher's method

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Abstract—A highly efficient and practical resolution of racemic 1-benzylisoanabasine, which was synthesized by reduction of the benzyl salt of 2,3′-bipyridine, has been achieved through molecular complexation with (*R*)-BINOL or (*S*)-BINOL to afford pure enantiomers (100% ee). The two enantiomers of the natural alkaloid isoanabasine have been obtained by debenzylation of the corresponding enantiomeric 1-benzylisoanabasine. Using Mosher's method by NMR techniques, the absolute configuration of (−)-isoanabasine has been assigned as the (*R*)-configuration for the first time. Moreover, an unexpected rotamer ratio of Mosher's amide was observed. The *syn*-form of two rotamers of (*R*)-MTPA-(*R*)-isoanabasine was predominant over the *anti*-form.

1. Introduction

The rarely investigated minor natural alkaloid isoanabasine **1** (Fig. 1) has been discovered only in low quantities in the desert plant *Anabasis aphylla* L., which can be found in the northwest of China and Central Asia.[1] Recently, much attention has been paid to the synthesis and biological activities of its skeleton analogues including natural and synthetic alkaloids, such as anabasine, anatabine, cytisine, and some other pyridyl piperidines.[2,3] These analogues displayed potential as therapeutic agents for peripheral and central nervous system diseases and other disorders because of their affinity activities to neuronal nicotinic acetylcholine receptors (nAChRs) but with lower toxicities than the well-known alkaloid nicotine.[3] Experience has shown that important biological activities of chiral natural products are related with their absolute configuration, so that methods providing single enantiomers were of utmost importance. However, to the best of our knowledge, the enantiomers of **1** had never gained as much attention, no report has ever clarified the absolute configuration or focused on the preparation of its enantiomer. Limited preliminary studies related to **1** were conducted only on reactions of the pyridine or the piperidine ring during the 1970s in the former Soviet Union.[4] Herein, we report our approach to the two enantiomers of **1** via synthesis and resolution, plus assignment of absolute configuration of **1** by Mosher's method.

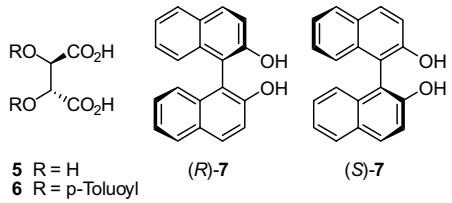
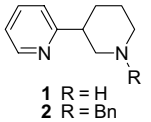


Figure 1. Chiral reagents bearing double acidic groups for resolution.



2. Results and discussion

2.1. Synthesis of racemic alkaloids

Syntheses of substituted piperidines have been reported using a variety of methods including stereoselective

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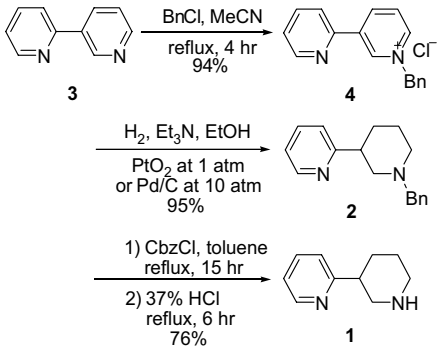
approaches in the development of new drugs.[5] However, those methods involving enantioselective reaction usually lead to a complex mixture of products, low yield, or quantitative consumption of the chiral auxiliary meaning that they are not practical. All reported preparations of **1** were based on the reduction of 2,3′-bipyridine **3** in two approaches. One involved the direct use of a large amount of reductive metals in low to moderate yields.[6a,b] The other method was the oxidation of 2,3′-bipyridine prior to catalytic hydrogenation with Pd/C.[6c,d] In view of the advantages of a pre-constructed heterocyclic skeleton upon reduction of bipyridines to pyridyl piperidines, we have utilized an improved reductive approach to **1** from **3**.

Formation of the piperidine ring of **1** was accomplished on a large scale by selective reduction of 3′-pyridyl through benzylation on less hindered nitrogen atom of **3** (Scheme 1). Benzyl salt **4**, obtained by heating a solution of **3** and benzyl chloride in acetonitrile, was elaborated to **2** via hydrogenation in the presence of triethylamine catalyzed with PtO_2 or Pd/C. Debenzylation of **2** was performed by substituent exchange reaction on nitrogen with benzyl chloroformate and then hydrolysis with 37% HCl to afford racemic **1** in total 67% yield.



Scheme 1.

2.2. Enantiomerically pure alkaloids from resolution

Molecular complexation, based on molecular recognition between host and guest molecules directed by steric complementary action and specific intermolecular forces (such as hydrogen bonding and second-order interaction), has proven to be an effective method for the resolution of organic molecules.[7] Optically active 1,1′-bi-2-naphthol **7** (BINOL) has shown excellent performances in this field. A few successful examples on resolution of alkaloids by forming host–guest complex with resolving reagent bearing double carboxylic or phenolic groups have been published.[8] The selectively formed diastereomeric salt in well-matched chirality, if possible, would display marked differences in physical or chemical properties such as solubility and stability compared with the alkali guest and acidic host themselves or the chirality-mismatched diastereomeric salt.

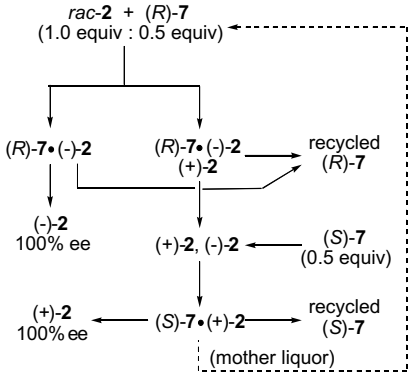
Three readily available chiral reagents bearing double acidic groups, *l*-tartaric acid **5**, *l*-O,O′-ditoluoyl tartaric acid **6** and (*R*)-**7** (Fig. 1), were selected as candidates to screen out better resolving process. As shown in Table 1, fortunately, (*R*)-**7** could form crystals with one enantiomer of **2** in ethanol in a molar ratio of 1:2 [(*R*)-**7**:*rac*-**2**]. The solvent was then changed with methanol to give a similar result. Acetonitrile and toluene were not suitable for the resolution. Varying the ratio of the resolving reagent and racemic alkaloid to 1:1 did not afford better results. Hence, we selected (*R*)-**7** and (*S*)-**7** as resolving reagents to optimize the resolution process.

Table 1. Screening of resolving reagents by formation of precipitates[a]

Alkaloids	Resolving reagents		
	L-**5**	L-**6**	(*R*)-**7**
1	–	–	–
2	–	–	+

[a] Those formed precipitates after mixing were marked with '+', otherwise with '–'.

The typical resolution process employed is as follows. A solution of (*R*)-**7** (0.5 equiv) and *rac*-**2** (1.0 equiv) in ethanol was stirred under reflux for 0.5 h. The crystals formed during cooling to room temperature were collected by filtration and purified by recrystallization. The resulting crystals were characterized as a 1:1 molecular complex of **2** and (*R*)-**7** by ^{1}H NMR and elemental analysis.[9] Decomposition of the resolving complex with 15% NaOH afforded the (−)-enantiomer of **2** (100% ee,[10] 79% yield based on half of the starting *rac*-**2**), which displayed $[\alpha]_D^{20} = -65.4$ (*c* 2.0, ethanol). All mother liquids from resolution and recrystallization, enriched in the (+)-enantiomer of **2**, were combined and washed with 15% NaOH to remove (*R*)-**7**. The resulting residues were applied to form molecular complex with (*S*)-**7** (0.5 equiv) to afford (+)-**2** (100% ee,[10] 83% yield based on half of the starting *rac*-**2**), which displayed $[\alpha]_D^{20} = +65.2$ (*c* 2.0, ethanol). Both (*R*)- and (*S*)-**7** were recovered in >80% yield by acidification of the corresponding alkali aqueous solution, respectively (Scheme 2).



Scheme 2. Schematic procedure of the resolution.

Unfortunately, no crystals of the molecular complex of (*R*)-**7** and (−)-**2** suitable for X-ray crystallography structure analysis have been obtained until now. However, by ^{1}H NMR (Fig. 2), we have observed that the hydroxy

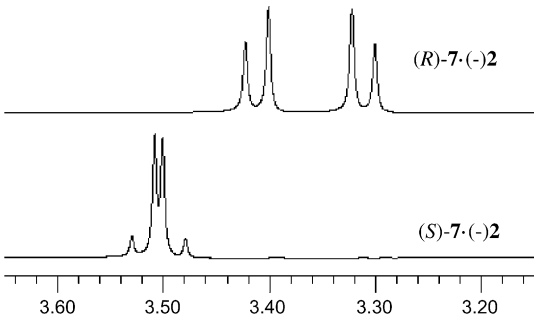


Figure 2. Partial ^{1}H NMR spectra of diastereomeric 1:1 mixtures of (−)-**2** with (*R*)-**7** and (*S*)-**7**.

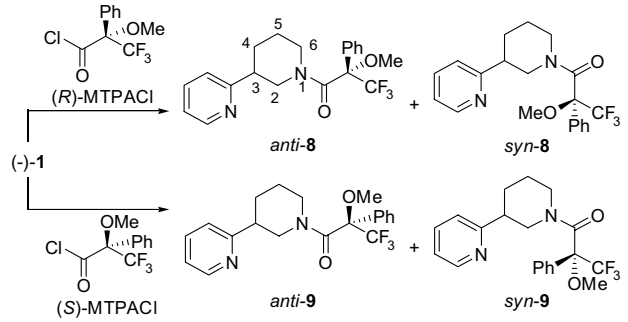
protons in the molecular complex (*R*)-**7**·(−)-**2** have shown a downfield shift from δ 5.02 [in (*R*)-**7**] to δ 6.80 ppm, while the protons on benzyl methylene showed a double doublet peak with a large coupling constant (J = 60.0 and 13.2 Hz). The 1:1 mixture of (*S*)-**7** and (−)-**2** in the same concentration[11] to the molecular complex (*R*)-**7**·(−)-**2** exhibited great differences and that the hydroxy protons also showed a downfield shift from δ 5.02 to δ 6.50 ppm and the protons on benzyl methylene showed double doublet with small split (J = 18.0 and 13.2 Hz).[12] The larger split in ^{1}H NMR spectra of the molecular complex (*R*)-**7**·(−)-**2** demonstrated the stronger hindered rotation of benzyl around its C–N bond, which should come from the stronger binding between (*R*)-**7** and (−)-**2**. These phenomena suggested that the hydrogen bonding present in both diastereomeric mixtures, and the stronger interactions in (*R*)-**7**·(−)-**2** originated from the matching of chirality of the host and guest in terms of chiral recognition, which was a fundamental driver to the stereoselectivity of (*R*)-**7** to (−)-**2** or (*S*)-**7** to (+)-**2** during resolution.

Based on the debenzylation procedure described in the last section, both enantiomers of **2** were converted to corresponding pure enantiomers of **1**, which displayed a specific rotation of 15.2 (*c* 1.0, ethanol) with the same sign to their benzyl precursors.

2.3. Assignment of absolute configuration of (−)-1

After pioneering work in the 1970s, Mosher's method has been one of the most useful techniques amongst those employed to determine the absolute configuration of organic compounds.[13] Hoye and Renner extended and modified Mosher's method to cyclic secondary amines, which have a stereogenic center on the ring.[14] It has been proven that Hoye's method was the most efficient technique for the assignment of the absolute configuration of substituted cycloamines[15a–f] even though the inconveniences presented in MTPA amide synthesis and NMR spectra analysis might limit its application.[15g,h]

In principle, the most stable conformation of the Mosher amides derived from the secondary amines (such as those derived from piperidine) has the trifluoromethyl group coplanar with the carbonyl group *syn*-periplanar and with the nitrogen *anti*-periplanar, the phenyl group on MTPA moiety of the amides shielded one of four possible quadrants of the molecule depending on the specific rotamer and absolute stereochemistry. Furthermore, it was reasonable to assume that the larger pyridyl group at the 3-position in the equatorial position was favorable over that at the axial position. For Mosher's amide isomers **8** and **9** (Scheme 3), one rotamer of **8** in which the axial protons shielded most at the 2- and 6-positions, should take the same rotation conformation around the amide bond to that of **9** in which most-shielded axial protons at 3- and 5-positions and vice versa.[16]



Scheme 3. Conversion of (−)-**1** to corresponding Mosher amides.[16]

Based on the above principles and in light of the ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, and ^{1}H–^{13}C HMQC, the chemical shifts of the rotamers were assigned and we easily knew that the minor of **8** and the major of **9** were a pair of rotamers, which had taken the *syn*-form while another pair were *anti*-rotamers (Table 2). Hence, the molar ratio[17] of *syn*-**8** to *anti*-**8** was 1:3.1 similar to what has generally been reported[14,15a–e] and that of *syn*-**9** to *anti*-**9** was unexpectedly 2.8:1, which had been presented only once in the literature.[15f] From the differences of chemical shifts ($\Delta\delta_{S-R}$), the axial 2′-proton in the *syn*-**8** and the axial 3′-proton in *syn*-**9** should take *syn*-form with phenyl on corresponding MTPA moiety based on the carbonyl plane. Thus, the absolute stereochemistry on stereogenic center of (−)-**1** was deduced to be an (*R*)-configuration. The rendered 3D representations have clearly exhibited the absolute configuration

Table 2. Assignment of chemical shifts (ppm) of protons on piperidinyl ring of Mosher amides

H[a]	*syn*-**8** (minor)	*anti*-**8** (major)	*syn*-**9** (major)	*anti*-**9** (minor)	$\Delta\delta_{S-R}$	
					syn	*anti*
2a	2.41	2.96	3.17	2.99	**−0.76**	−0.03
2e	4.10	4.83	4.08	4.80	0.02	0.05
3	2.86	2.78	1.67	3.05	**1.19**	−0.24
4a	1.70	1.75	1.87	1.76	−0.18	−0.01
4e	2.05	1.84	1.76	2.02	0.29	−0.18
5a	1.63	0.48	1.50	1.55	0.13	**−1.07**
5e	1.92	1.18	1.79	1.55	0.13	−0.37
6a	2.57	2.91	2.62	2.30	−0.05	**0.61**
6e	4.85	4.01	4.78	3.84	0.07	0.17

[a] Protons at axial position were marked with 'a' and those at equatorial position marked with 'e'.

and the shielding circumstance in Mosher amides (Fig. 3).

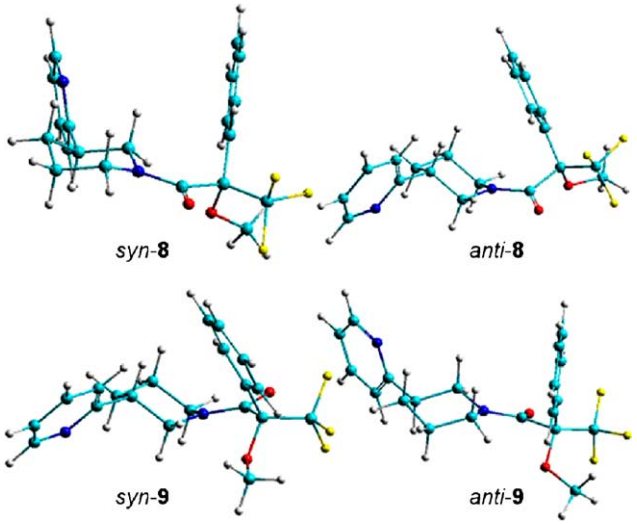


Figure 3. 3D representations of rotamers of Mosher amides **8** and **9** from minimizing energy.

3. Conclusions

In conclusion, we have synthesized the natural alkaloid isoanabasine **1** via a facile and practical route in which the intermediate 1-benzylisoanabasine **2** was resolved to both enantiomers in 100% ee efficiently by forming a molecular complex with (*R*)- and (*S*)-1,1′-bi-2-naphthol **7**. The preliminary investigation to the chiral recognition involving in resolution, studied by ^{1}H NMR, revealed that the matching of chirality between (*R*)-**7** and (*R*)-(−)-**2** [or (*S*)-**7** and (*S*)-(+)-**2**] strengthened the hydrogen bonding of the host–guest complex. Enantiomerically pure **1** was obtained by debenzylation of corresponding enantiomeric **2**. The absolute configuration of (−)-**1** was assigned as an (*R*)-configuration using Mosher's method by NMR techniques. During the deduction, it was observed from unexpected rotamer ratio of Mosher amide derived from (*R*)-(−)-**1** and (*S*)-MTPACl that the *syn*-rotamer predominated over the *anti*-one.

4. Experiments

Generally, chiral HPLC measurements were carried out on a Shimadzu Class-VP workstation through CHIRALCEL® OD-H column (Daicel Chemical Industries, Ltd) with a UV detector at 254 nm. Optical rotations were measured on a Perkin Elmer 341LC polarimeter. ESI-MS measurements were conducted with a LCQ instrument. Unless otherwise noted, ^{1}H spectra were recorded on a Bruker 600 MHz spectrometer and the proton chemical shifts referenced to TMS as internal standard at 0.00 ppm. ^{13}C NMR chemical shifts were reported in parts per million relative to the solvent $CDCl_3$ at 77.0 ppm or DMSO-d_6 at 39.5 ppm. Melting points were measured with a hot-stage microscope XT-4. Elemental analysis was carried out with a VarioEL instrument. TLC was performed on aluminum TLC-layers Silica gel GF-254. Detection was done by UV light (254 and 365 nm). All materials were available commercially without further purification. All products were dried in vacuum prior to further reaction and analysis.

4.1. Preparation of 1′-benzyl-2,3′-bipyridinium chloride 4

A stirring solution of 2,3′-bipyridine **3** (15.60 g, 0.10 mol) and benzyl chloride (13.8 mL, 0.12 mol) in acetonitrile (100 mL) was heated to reflux for 4 h. After being cooled to room temperature, the precipitates were collected by filtration, washed with acetonitrile (30 mL), and dried in vacuum to give 26.56 g of 1′-benzyl-2,3′-bipyridinium chloride **4** as buff granule (94% yield). Mp 78–80 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.15 (s, 1H), 9.38 (d, 1H, J = 6.0 Hz), 9.27 (d, 1H, J = 8.4 Hz), 9.79 (d, 1H, J = 4.2 Hz), 8.39 (d, 1H, J = 7.8 Hz), 8.30 (dd, 1H, J = 7.8, 6.0 Hz), 8.08 (dt, 1H, J = 7.8, 1.8 Hz), 7.68 (d, 2H, J = 6.6 Hz), 7.59 (dd, 1H, J = 7.2, 4.8 Hz), 7.44 (m, 3H), 6.10 (s, 2H). ^{13}C NMR (150 MHz): δ 150.1, 149.6, 144.4, 143.0, 142.5, 138.4, 138.0, 134.3, 129.2, 129.1, 128.9, 128.5, 125.1, 122.0, 63.2. ESI-MS (*m*/*z*): $[M-I]^+$ 247.0.

4.2. Preparation of racemic 1-benzylisoanabasine [i.e., 1-benzyl-3-(pyridin-2-yl)piperidine] *rac*-2

A suspension of **4** (11.28 g, 0.040 mol), triethylamine (6.2 mL, 0.044 mol), and 10% Pd/C (0.6 g) in ethanol (100 mL) was stirred in an autoclave under hydrogen atmosphere at 10 atm for 6–8 h at room temperature. Upon deflation of the hydrogen, the insoluble species were removed by filtration through Celite. After evaporation of the ethanol, the residues were dissolved in dichloromethane (100 mL) and washed with 10% NaOH (30 mL × 2) and water. The organic layer was dried over magnesium sulfate. The solvent was removed by evaporation to give 9.57 g of 1-benzylisoanabasine **2** as colorless oil (95% yield). ^{1}H NMR (600 MHz, $CDCl_3$): 8.52 (d, 1H, J = 4.2 Hz), 7.57 (dt, 1H, J = 7.8, 1.8 Hz), 7.35 (d, 2H, J = 7.2 Hz), 7.30 (t, 2H, J = 7.2 Hz), 7.24 (t, 1H, J = 7.2 Hz), 7.16 (d, 1H, J = 7.8 Hz), 7.09 (dd, 1H, J = 7.8, 4.8 Hz), 3.59 (s, 2H), 3.07 (m, 2H), 2.94 (d, 1H, J = 11.4 Hz), 2.28 (t, 1H, J = 10.8 Hz), 2.07 (m, 1H), 1.97 (dt, 1H, J = 12.6, 1.8 Hz), 1.78 (m, 2H), 1.61 (m, 1H). ^{13}C NMR (150 MHz): δ 163.6, 149.1, 138.1, 136.3, 129.2, 128.2, 127.0, 122.0, 121.3, 63.4, 59.0, 53.6, 44.6, 30.5, 25.3. ESI-MS (*m*/*z*): $[M+H]^+$ 253.1.

An alternative hydrogenation using PtO_2 as catalyst was carried out at ambient pressure in a similar procedure.

4.3. Preparation of racemic isoanabasine [i.e., 3-(pyridin-2-yl)piperidine] *rac*-1

A stirred solution of **2** (2.52 g, 0.010 mol) and benzyl chloroformate (1.7 mL, 0.012 mol) in toluene (30 mL) was heated to reflux for 10 h under a nitrogen atmosphere. After being cooled to room temperature, the

reaction mixture was filtered through silica gel (200 mesh) and eluted with ethyl acetate (100 mL) to remove tar and decolorizing. The filtrate was then washed with saturated $NaHCO_3$ (40 mL × 2) and water. The solvent was removed by evaporation and the residues dissolved in a mixture of acetic acid (20 mL) and 37% HCl (10 mL). The acidic solution was stirred and heated to reflux for 6 h and the acids then distilled off. The residues were suspended in 10% NaOH (30 mL) and extracted with dichloromethane (50 mL × 2). The organic layer was dried over magnesium sulfate and evaporated to give 1.23 g of *rac*-**1** as viscous oil (76% yield). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.49 (d, 1H, J = 4.8 Hz), 7.56 (dt, 1H, J = 7.8, 1.8 Hz), 7.12 (d, 1H, J = 7.8 Hz), 7.07 (dd, 1H, J = 7.2, 4.8 Hz), 3.23 (m, 1H), 3.15 (s, 1H), 3.08 (d, 1H, J = 12.8 Hz), 2.86 (m, 2H), 2.66 (dt, 1H, J = 12.2, 2.4 Hz), 2.00 (dd, 1H, J = 12.6, 3.0 Hz), 1.76 (m, 2H), 1.60 (m, 1H). ^{13}C NMR (150 MHz): δ 163.4, 149.0, 136.3, 121.6, 121.3, 51.7, 46.1, 45.2, 30.6, 26.1. ESI-MS (*m*/*z*): $[M+H]^+$ 163.1.

4.4. Resolution of racemic 1-benzylisoanabasine to enantiomers (*R*)-(−)-2 and (*S*)-(+)-2

A solution of *rac*-**2** (5.56 g, 0.022 mol) and (*R*)-1,1′-bi-2-naphthol (*R*)-**7** (3.15 g, 0.011 mol) in ethanol (25 mL) was stirred at 70 °C for 0.5 h and cooled naturally to room temperature. After 1 h, the precipitates were collected by filtration and purified by recrystallization in ethanol (15 mL). The resultant molecular complex was suspended in 15% NaOH (12 mL) and extracted with dichloromethane (30 mL × 3). The extract was washed with saturated $NaHCO_3$, dried over magnesium sulfate, and evaporated to dryness to give 2.20 g of (*R*)-(−)-**2** (79% yield). All filtrates from the separation of the crystals and recrystallization were combined and evaporated to remove ethanol. The residues were dissolved in dichloromethane (60 mL) and washed with 15% NaOH (15 mL × 2) to remove (*R*)-**7**. All aqueous solutions containing sodium salt of (*R*)-**7** were combined and acidified with 10% HCl to form precipitates, which were collected by filtration, washed with water, and recrystallized in toluene to give 2.57 g of (*R*)-**7** (82% yield). The organic phase enriched with (*S*)-(+)-**2** was then dried with magnesium sulfate and evaporated to give an oil, which was complexed with (*S*)-**7** (3.15 g, 0.011 mol) in ethanol to give 2.31 g of (*S*)-(+)-**2** (83% yield) in similar procedure as described above. (*S*)-**7** was recovered in 80% yield and the terminal residual oil, usually >0% ee, could be used for resolution again. (*R*)-(+)-**2**: $[\alpha]_D^{20} = -65.4$ (*c* 2.0, ethanol), 100% ee [t_R(*R*) = 5.5 min]. (*S*)-(−)-**2**: $[\alpha]_D^{20} = +65.2$ (*c* 2.0, ethanol), 100% ee [t_R(*S*) = 6.6 min].[9,10]

4.5. (*R*)-(−)-Isoanabasine (*R*)-(−)-1

Prepared from (*R*)-(−)-**2** in a similar procedure to *rac*-**1** from *rac*-**2**. $[\alpha]_D^{20} = -15.2$ (*c* 1.0, ethanol).

4.6. (*S*)-(+)-Isoanabasine (*S*)-(+)-1

Prepared from (*S*)-(+)-**2** in a similar procedure to *rac*-**1** from *rac*-**2**. $[\alpha]_D^{20} = +15.2$ (*c* 1.0, ethanol).

4.7. Preparation of Mosher's amide (3*R*,2′*S*)-1-(2-methoxy-2-trifluoromethyl-phenylaceto)-3-(pyridin-2-yl)piperidine *anti*- and *syn*-8

To a solution of (*R*)-(−)-**1** (21.0 mg, 0.12 mmol) and triethylamine (18.4 μL, 0.13 mmol) in dichloromethane (2.0 mL) was added dropwise a solution of (*R*)-(−)-2-methoxy-2-trifluoromethyl-phenylacetyl chloride [(*R*)-MTPACl, 24.5 μL, 0.13 mmol] in dichloromethane (1 mL) at 0 °C. The reaction mixture was stirred for another 4 h at room temperature and diluted with dichloromethane (5 mL) followed by quenching with water (3 mL). The organic layer was separated and washed with water (3 mL) again, dried over magnesium sulfate, and evaporated to dryness. The residues were purified with silica-gel chromatography column to give 40.0 mg of rotameric mixture of **8** as oil (86% yield), which showed a single R_f value on TLC. The molar ratio of *syn*-**8** to *anti*-**8** was 1:3.1 characterized by ^{1}H NMR. *anti*-**8**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.53 (d, 1H, J = 4.5 Hz), 7.62 (d, 2H, J = 7.4 Hz), 7.61 (m, 1H), 7.41 (m, 2H), 7.39 (d, 1H, J = 7.3 Hz), 7.18 (d, 1H, J = 7.8 Hz), 7.13 (dd, 1H, J = 7.3, 5.0 Hz), 4.83 (d, 1H, J = 12.9 Hz), 4.01 (d, 1H, J = 13.5 Hz), 3.68 (s, 3H), 2.96 (t, 1H, J = 12.4 Hz), 2.91 (dt, 1H, J = 13.6, 2.8 Hz), 2.78 (tt, 1H, J = 11.4, 3.7 Hz), 1.84 (d, 1H, J = 12.3 Hz), 1.75 (dt, 1H, J = 12.5, 4.0 Hz), 1.18 (dt, 1H, J = 13.6, 3.1 Hz), 0.48 (m, 1H). ^{13}C NMR (150 MHz): δ 163.7, 161.6, 149.1, 136.5, 134.1, 129.1, 128.1, 126.5, 123.6 (q, J = 288 Hz), 122.0, 121.7, 84.7 (q, J = 24.9 Hz), 55.3, 47.2, 45.1, 43.6, 30.3, 23.5. *syn*-**8**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.43 (d, 1H, J = 4.1 Hz), 7.55 (dt, 1H, J = 7.7, 1.5 Hz), 7.44 (dd, 2H, J = 8.0, 3.2 Hz), 7.30 (m, 3H), 7.08 (dd, 1H, J = 7.3, 5.0 Hz), 6.97 (d, 1H, J = 7.8 Hz), 4.85 (overlap with a peak of *anti*-**8**, 1H), 4.10 (dd, 1H, J = 13.5, 1.7 Hz), 3.85 (s, 3H), 2.86 (tt, 1H, J = 11.8, 3.7 Hz), 2.57 (dt, 1H, J = 13.0, 2.7 Hz), 2.41 (t, 1H, J = 12.5 Hz), 2.05 (d, 1H, J = 13.4 Hz), 1.92 (dt, 1H, J = 13.3, 2.5 Hz), 1.70 (m, 1H), 1.63 (m, 1H). ^{13}C NMR (150 MHz): δ 164.0, 161.3, 149.2, 136.5, 133.8, 129.0, 128.2, 126.3, 123.5 (q, J = 288 Hz), 121.8, 121.4, 84.9 (q, J = 24.9 Hz), 56.2, 50.5, 44.8, 42.9, 30.6, 25.2. ESI-MS (*m*/*z*): $[M+H]^+$ 378.3.

4.8. Preparation of Mosher's amide (3*R*,2′*R*)-1-(2-methoxy-2-trifluoromethyl-phenylaceto)-3-(pyridin-2-yl)piperidine *anti*- and *syn*-9

The synthesis of **9** was performed by amidation of (*R*)-(−)-**1** with (*S*)-MTPACl to give 41.8 mg of rotameric mixture (90% yield) in similar procedure to that of **8**. The molar ratio of *syn*-**9** to *anti*-**9** was 2.8:1. *syn*-**9**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.54 (d, 1H, J = 4.5 Hz), 7.58 (d, 2H, J = 7.6 Hz), 7.53 (dt, 1H, J = 7.7, 1.6 Hz), 7.43 (m, 3H), 7.16 (m, 1H), 6.21 (d, 1H, J = 7.8 Hz), 4.78 (dt, 1H, J = 13.0, 1.9 Hz), 4.08 (dt, 1H, J = 13.0, 1.9 Hz), 3.62 (s, 3H), 3.17 (dd, 1H, J = 11.7, 13.5 Hz), 2.62 (dt, 1H, J = 13.0, 2.4 Hz), 1.87 (m, 1H), 1.78 (m, 2H), 1.67 (tt, 1H, J = 11.9, 3.7 Hz), 1.50 (m, 1H). ^{13}C NMR (150 MHz): δ 163.5, 160.3, 147.8, 137.8, 134.5, 129.1, 128.3, 126.8, 123.6 (q, J = 288 Hz), 123.3, 122.3, 84.5 (q, J = 24.9 Hz), 55.4, 49.3, 43.0, 42.8, 29.7, 24.7.

anti-**9**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.64 (d, 1H, J = 4.5 Hz), 7.76 (dt, 1H, J = 7.7, 1.6 Hz), 7.47 (m, 2H), 7.36 (m, 3H), 7.29 (m, 2H), 4.80 (overlap with a peak of *syn*-**9**, 1H), 3.84 (d, 1H, J = 13.4 Hz), 3.78 (s, 3H), 3.05 (m, 1H), 2.99 (m, 1H), 2.30 (dt, 1H, J = 12.0, 4.5 Hz), 2.02 (d, 1H, J = 13.4 Hz), 1.76 (overlap with a peak of *syn*-**9**, 1H), 1.55 (m, 2H). ^{13}C NMR (150 MHz): δ 164.2, 160.8, 147.9, 138.3, 133.9, 129.1, 128.2, 126.4, 123.7 (q, J = 288 Hz), 122.6, 122.5, 84.9 (q, J = 24.9 Hz), 56.1, 46.7, 45.9, 43.5, 30.2, 25.0. ESI-MS (*m*/*z*): $[M+H]^+$ 378.3.

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9. The integrals of all proton peaks in ^{1}H NMR spectra and element contents were matched with the formula of molecular complex (*R*)-**7**·(−)-**2**. ^{1}H NMR (53.8 mg in 0.5 mL of $CDCl_3$): δ 8.37 (d, 1H, J = 4.2 Hz), 7.84 (d, 2H, J = 9.0 Hz), 7.81 (d, 2H, J = 8.4 Hz), 7.52 (dt, 1H, J = Hz), 7.29 (d, 4H, J = 9.0 Hz), 7.22 (m, 9H), 7.06 (m, 2H), 3.36 (dd, 2H, J = 60.0, 13.2 Hz), 3.02 (m, 1H), 2.97 (d, 1H, J = 11.4 Hz), 2.81 (d, 1H, J = 10.8 Hz), 2.13 (t, 1H, 10.8 Hz), 1.95 (dt, 1H, J = 11.4, 3.0 Hz), 1.86 (d, 1H, J = 11.4 Hz), 1.68 (m, 2H), 1.47 (qd, 1H, J = 12.0, 7.5 Hz). Elemental analysis result: calculated for $C_{37}H_{34}N_2O_2$: C, 82.50; H, 6.36; N, 5.20. Found: C, 82.41; H, 6.45; N, 5.19.
10. Enantiomeric excess analysis was performed with *n*-hexane/isopropanol/diethylamine (in volume ratio of 70/30/0.1) as mobile phases. The retention time of (−)-**2** was 5.5 min, while that of (+)-**2** was 6.6 min under a flow velocity of 1.0 mL/min.
11. The ^{1}H NMR spectra of the molecular complex of (*R*)-**7** and (−)-**2** were measured in concentration of 53.8 mg of the molecular complex in 0.6 mL of $CDCl_3$, and that of (*S*)-**7** and (−)-**2** in concentration of 25.2 mg of (−)-**2** and 28.6 mg of (*S*)-**7** in 0.6 mL of $CDCl_3$.
12. Besides protons on benzyl methylene, few of other protons also showed a few differences in chemical shifts between two diastereomeric complexes.
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16. Both amides were composed of two inseparable rotamers from hindered rotation around amido C–N bond. The rotamers showed two different groups of peaks and one predominated over another.
17. The rotamer ratios of Mosher amides measured by NMR after purification were consistent with those before purification.