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Practical and efficient synthesis of the (*R*)-atropisomer of a 4-phenyl 1,2,4-triazole derivative as a selective GlyT1 inhibitor

Takashi Sugane *, Noritaka Hamada, Takahiko Tobe, Wataru Hamaguchi, Itsuro Shimada, Kyoichi Maeno, Junji Miyata, Takeshi Suzuki, Tetsuya Kimizuka, Shuichi Sakamoto, Shin-ichi Tsukamoto

Drug Discovery Research, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan

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ABSTRACT

Herein we describe a novel and efficient method for synthesizing the (*R*)-atropisomer of 3-[3-ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl]-2-methylbenzonitrile **1**, a novel GlyT1 inhibitor. The diastereomeric salt formation of 3-[3-ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl]-2-methylbenzoic acid **7** with (1*R*,2*S*)-(−)-2-amino-1,2-diphenylethanol afforded the desired (*R*)-atropisomer. We also report the determination of the absolute configuration of (*R*)-**7** by powder X-ray diffraction.

1. Introduction

In the central nervous system, specific sodium/chloride-dependent transporters regulate glycine levels in the synapse. Glycine activity here is terminated by reuptake via two high-affinity glycine transporters: glycine transporters 1 (GlyT1) and 2 (GlyT2).[1,2] GlyT1 is expressed in forebrain areas such as the hippocampus and cerebral cortex, and co-localized with the *N*-methyl-D-aspartate (NMDA) receptors. Glycine acts as a co-agonist of the NMDA receptor, where it has been suggested that GlyT1 modulates NMDA receptor activity by regulating glycine concentration in the vicinity of the NMDA receptors. As a result, agents which inhibit the activity of GlyT1 and thus activate the NMDA receptors may be useful for treating schizophrenia, dementia, and related disorders.[3–5]

We have previously identified 3-[3-ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl]-2-methylbenzonitrile **1** as one such selective GlyT1 inhibitor.[6] Compound **1** has a restricted rotation between the triazole-4-yl and 3-cyano-2-methylphenyl groups, resulting in two enantiomeric rotational isomers. In order to evaluate the thermal stability for interconversion, solutions of (+)-**1** were maintained at various temperatures, and racemization was monitored by HPLC. The barrier was found to be 30.5 kcal/mol, which correlates with a half-life of 21 years under physiological conditions (i.e. 37 °C).[6] Subsequent biological studies revealed that only the (−)-atropisomer (−)-**1** showed potent GlyT1 inhibitory activity, with the corresponding (+)-atropisomer (+)-**1** exhibiting far less potent activity (Table 1).[6] Since nearly all of the inhibitory activity resided in one rotational isomer and racemization was not observed for several months at ambient temperatures, we selected (−)-**1** as a candidate for further preclinical studies. It was therefore important for us to establish a practical synthetic method to provide enough enantiomerically pure test samples for preclinical studies.

Herein we report a concise method for synthesizing (−)-**1**, including resolution via diastereomeric salt formation of intermediate **7** using (1*R*,2*S*)-(−)-2-amino-1,2-diphenylethanol (ADPE). In addition, we have also determined the absolute stereochemistry of these atropisomes via powder X-ray diffraction.

2. Results and discussion

2.1. Synthesis of benzoic acid 7

In order to obtain enantiomerically pure (*R*)-**1**, we first focused on the resolution *via* the diastereomeric salts of (*RS*)-**1** with 15 different optically active carboxylic acids, but we found this approach to be infeasible. We therefore considered utilizing carboxylic acid (*RS*)-**7** for the resolution (Scheme 1).

Before conducting the resolution, we investigated an efficient synthetic route to intermediate **7**. We have previously showed that the cyclization of the triazole ring involves the addition of anilines to 1,2,4-oxadiazoles or reactions of methyl thioimidates with acyl hydrazides (Scheme 2).[7,8] Although these methods were of great use for preparing a wide variety of triazoles for structure optimization, they required extreme thermal conditions (>160 °C) and chromatographic purification, neither of which are suitable for large-scale synthesis. We therefore attempted the cyclization of the triazole using the reaction of carboximidohydrazide **5** with Et-C(OEt)$_3$[9] under conditions that are milder than those shown in Scheme 2.

* Corresponding author. Tel.: +81 29 863 6678; fax: +81 29 852 5387.
E-mail address: takashi.sugane@astellas.com (T. Sugane).

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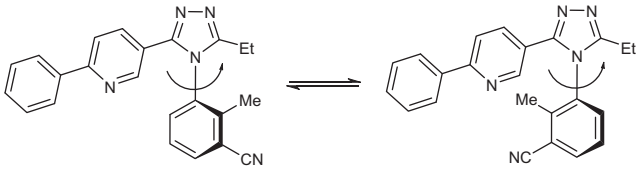
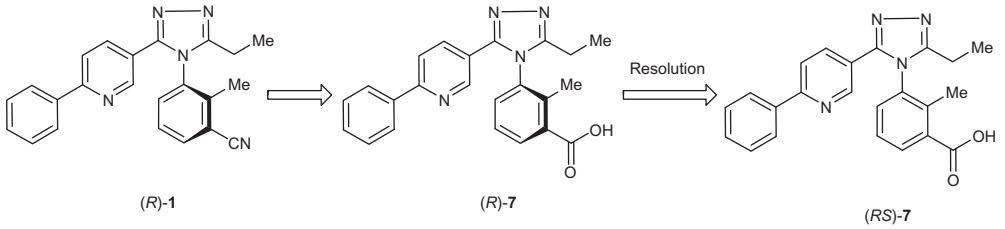
Table 1
In vitro GlyT1 inhibitory activity



(*R*)-(-)-**1** (*S*)-(+)-**1**

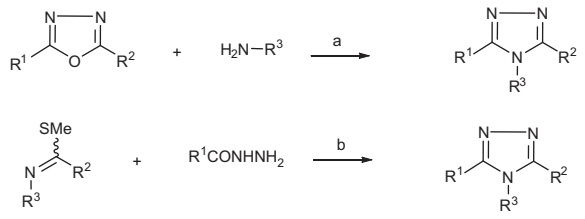
Compound	$[\alpha]_D^{25}$ (*c* 1.0, $CHCl_3$),	Rat GlyT1 IC_{50}[a] (μM)	Rat GlyT2 IC_{50}[b] (μM)	Selectivity[c]
(*RS*)-**1**	—	0.16 ± 0.030	>30	>187
(*S*)-(+)-**1**	+213	20 ± 4.8	>30	>1.5
(*R*)-(−)-**1**	−211	0.064 ± 0.023	>30	>469

[a] [^{3}H]glycine uptake assay using rat C6 cells. Values are mean ± standard error for three experiments.
[b] [^{3}H]glycine uptake assay using rat brainstem cells.
[c] Ratio of the IC_{50} (μM) values rat GlyT2/rat GlyT1.



(*R*)-**1** (*R*)-**7** (*RS*)-**7**

Scheme 1. Approach for the synthesis of (*R*)-**1**.



Scheme 2. Reagents and conditions: (a) TsOH·H_2O or (1*S*)-10-camphorsulfonic acid, neat, >160 °C; (b) TsOH·H_2O or (1*S*)-10-camphorsulfonic acid, DMF, >160 °C.

The preparation of **7** started with nicotinic acid **2** (Scheme 3). Compound **2** was converted into the acid chloride by using $SOCl_2$, followed by reaction with aniline **3** to afford amide **4** in 77% yield. Using $SOCl_2$, the amide moiety of **4** was then converted into the corresponding imidoyl chloride, which was further treated with hydrazine hydrate to give carboximidohydrazide **5** in 70% yield. Cyclization of the triazole ring proceeded smoothly using $EtC(OEt)_3$ with carboximidohydrazide **5** in EtOH to give the desired triazole **6** in 80% yield. Finally, hydrolysis of the methyl ester of **6** afforded benzoic acid **7** in 93% yield. It should be noted that no severe thermal conditions were required for any of these 4 steps, and the desired benzoic acid **7** was obtained without chromatographic purification.

2.2. Resolution of 7

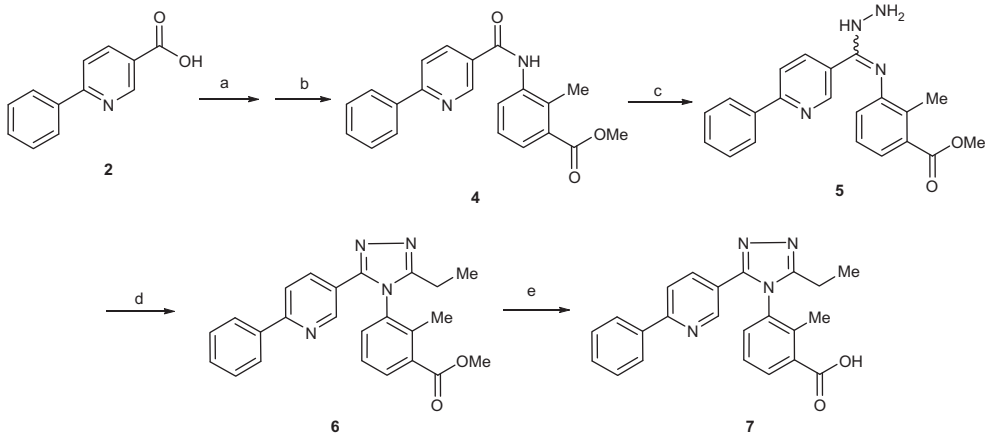
The benzoic acid **7** was subjected to resolution via diastereomeric salt formation. We screened a panel of 36 commercially available optically active amines and resolving conditions to maximize both the yield and enantiomeric purity (ee) and identified (1*R*,2*S*)-(−)-2-amino-1,2-diphenylethanol (1*R*,2*S*)-ADPE as a suitable and the most efficient resolving agent for (*R*)-**7** (Scheme 4).[10] The (1*R*,2*S*)-ADPE salt formation with (*RS*)-**7** was accomplished in EtOH/H_2O (1:3), and after 4 h of stirring, the precipitated salt (*R*)-**7**·(1*R*,2*S*)-ADPE was obtained in good diastereomeric purity (93% de). A single recrystallization of the salt obtained in EtOH/H_2O (1:3) increased the enantiomeric purity to 99.5% de with good yield (41% yield, E = 82%).[11] Treatment of the diastereomeric salt (*R*)-**7**·(1*R*,2*S*)-ADPE in ethanol with 1 equivalent of HCl, followed by filtration of the resultant precipitate gave the desired optically active benzoic acid (*R*)-**7** (95% yield, 99.5% ee).

Undesired (*S*)-**7** can be recycled using the axially chiral nature of **7**. The salt containing 86% ee of undesired (*S*)-**7** was dissolved in *N*,*N*-dimethylacetamide (DMA) and then heated to 155 °C. Under these conditions, racemization was completed in 9 h, yielding racemate **7**. The undesired (*S*)-**7** was almost quantitatively recycled to the racemate (*RS*)-**7** and could be used to prepare an additional batch of desired (*R*)-**7**. This resolution process involving recycling is valuable, as a large amount of the desired atropisomer can be efficiently obtained without resorting to large-scale chiral HPLC resolution.

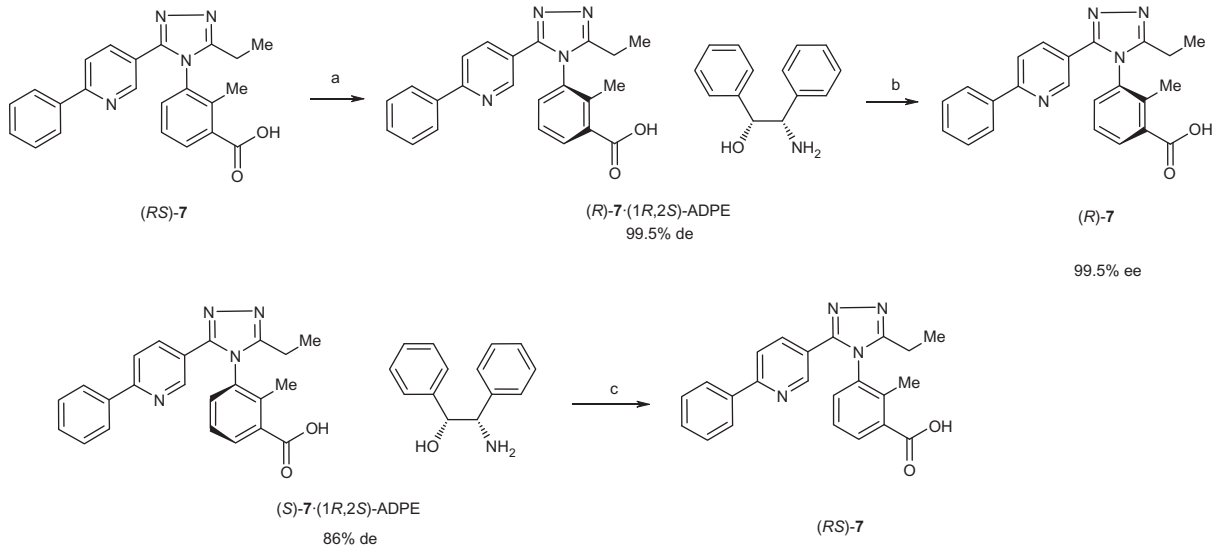
2.3. Determination of the absolute configuration of (*R*)-7

Since attempts to prepare single crystals of (*R*)-**1** or (*R*)-**7** suitable for X-ray diffraction were unsuccessful, we attempted to determine the absolute configuration by powder X-ray diffraction (PXRD)[12] using the powder sample of the salt (*R*)-**7**·(1*R*,2*S*)-ADPE. Generally PXRD can only determine relative stereochemistry. It can, however, determine the absolute configuration by comparison with the known absolute configuration of the compound in the same crystal.[12b,d]

The PXRD data analysis was carried out using Accelrys Materials Studio software packages (X-Cell, DMol3, Reflex Plus).[13] The X-ray powder diffraction data collected for (*R*)-**7**(1*R*,2*S*)-ADPE were indexed by X-Cell.[14] The intensities were integrated by Pawley method[15] implemented within Reflex Plus.



Scheme 3. Reagents and conditions: (a) $SOCl_2$, 60 °C; (b) methyl 3-amino-2-methylbenzoate **3**, pyridine, THF, rt, 77%; (c) (i) $SOCl_2$, 80 °C, (ii) hydrazine hydrate, EtOH, rt, 70%; (d) $EtC(OEt)_3$, EtOH, reflux, 80%; (e) 1 M NaOH, MeOH, rt, 93%.



Scheme 4. Resolution and recycling of atropisomer **7**. Reagents and conditions: (a) (i) (1*R*,2*S*)-(−)-2-amino-1,2-diphenylethanol, EtOH/H_2O (1:3); (ii) recrystallization from EtOH/H_2O (1:3), 41%; (b) 1 M HCl aq, 95%; (c) (i) DMA, 155 °C; (ii) 1 M HCl aq, 98%.

After initial geometrical optimization using DMol3, the structure solution was performed by a direct-space method using parallel tempering method[16] (Reflex Powder Solve). We used both (*R*)-**7** and (*S*)-**7** as initial structures for the structure refinement, where the bond at the 4-position of the triazole (chiral axis) was able to rotate freely in the structure solutions. As a result, both initial structures with (*R*)-**7** and (*S*)-**7** yielded the same solution with an (*R*)-configuration. The lattice constants after Rietveld refinement[17] (Reflex Powder Refinement) corresponded to a primitive orthorhombic cell ($P2_12_12_1$, For $Z = 4$) with dimensions: $a = 26.0848$ Å, $b = 25.0880$ Å, $c = 6.1007$ Å, $V = 3992.39$ Å^3. The calculated density was 1.00 g/cm^3. The final Rietveld refinement was $R_{wp} = 5.62\%$, $Rp = 3.82\%$. The molecular structure is represented in Figure 1, and the (*R*)-configuration was determined by comparison with the known absolute configuration of (1*R*,2*S*)-ADPE.

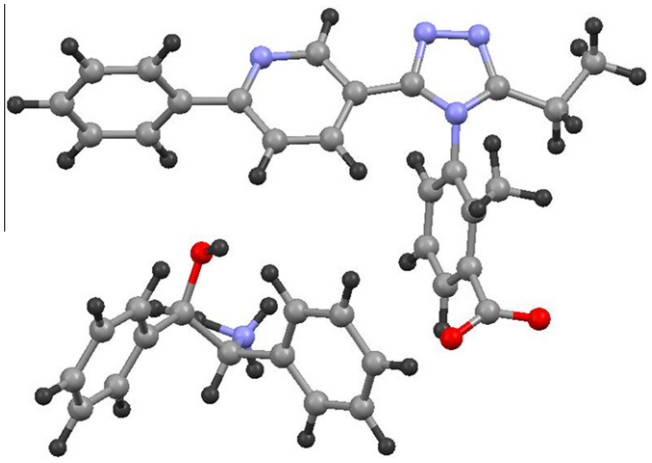
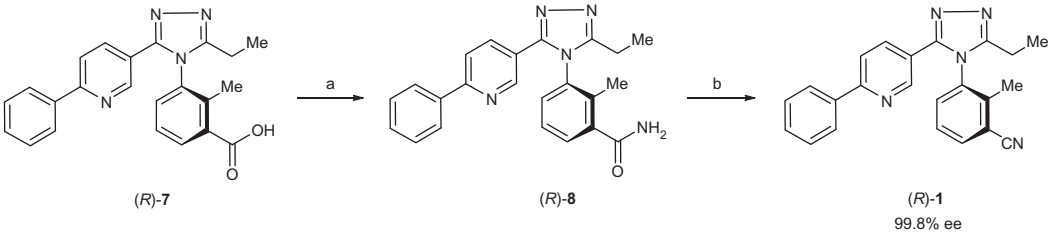


Figure 1. The molecular structure of the salt composed of (*R*)-**7** and (1*R*,2*S*)-ADPE.

2.4. Conversion of (*R*)-7 to (*R*)-1

The conversion of (*R*)-**7** to (*R*)-**1** was carried out as shown in Scheme 5. First, carboxylic acid (*R*)-**7** was converted into the corresponding carboxamide (*R*)-**8** in 86% yield using *N*-ethyl-*N*′-(3-



Scheme 5. Reagents and conditions: (a) EDCI·HCl, HOBt, NH_4Cl, Et_3N, DMF, rt, 86%; (b) $POCl_3$, DMF, 0 °C, 81%.

dimethylaminopropyl)carbodiimide hydrochloride (EDCI·HCl) and 1-hydroxybenzotriazole (HOBt) as the condensation agents. Carboxamide (*R*)-**8** was then reacted with $POCl_3$ in DMF at 0 °C to yield the desired (*R*)-**1** with 81% yield. No chromatographic purification was required in these two steps, nor was racemization observed with the (*R*)-**1** obtained.

3. Conclusion

In conclusion, we have developed a novel and efficient route for synthesizing the (*R*)-atropisomer of **1**, a potent and selective GlyT1 inhibitor. The resolution of the desired (*R*)-atropisomer was achieved via the formation of a diastereomeric salt of **7** with (1*R*,2*S*)-ADPE, and the opposite atropisomer could be recycled to the racemate. It is noteworthy that neither column chromatographic purification nor extreme thermal conditions were required at any point during the procedure, with (*R*)-**1** being obtained without racemization. In addition, we have determined the absolute stereochemistry of these analogues to be (*R*) via PXRD. By using this route, we were able to obtain a sufficient amount of the compound for further preclinical studies in pharmacology and drug safety. We believe that this route could be applied for manufacture-scale synthesis, and further optimization is currently under investigation.

4. Experimental

4.1. General

All commercial materials were used without further purification. Uncorrected melting points (mp) were determined using BÜCHI B-545 micro melting apparatuses. NMR spectra were recorded on a JEOL JMN-EX-400 at 400 MHz (^1H) and 100 MHz (^{13}C); the chemical shifts are expressed in δ (ppm) values with trimethylsilane as an internal reference (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad peak). Mass spectra (MS) were recorded on a JEOL GCmate II or JEOL JMS-LX2000 spectrometer. Elemental analyses were performed using a Yanaco MT-6 and were within ±0.4% of theoretical values. HPLC analyses were conducted on Hitachi D-7500 system. Enantiomeric excess (%ee) was determined using Chiralpak AD column (4.6 × 250 mm, eluent:hexane/EtOH [1:1], flow rate: 0.5 mL/min, UV detection: 254 nm) for compound **1**, or Chiralcel OJ-RH column (4.6 × 250 mm, eluent:CH_3CN/phosphate buffer [20 mM, pH 2.3] [3:7], flow rate: 0.5 mL/min, UV detection at 254 nm) for compound **7**.

4.2. Methyl 2-methyl-3-{[(6-phenylpyridin-3-yl)carbonyl]amino}benzoate 4

A mixture of 6-phenylnicotinic acid **2** (9.65 g, 48 mmol) and $SOCl_2$ (100 mL) was stirred at 60 °C for 1 h. After cooling at room temperature, the mixture was concentrated in vacuo to give an acid chloride as solid. The suspension of the acid chloride in THF (50 mL) was added to a mixture of methyl 3-amino-2-methylbenzoate **3** (8.0 g, 48 mmol), pyridine (3.9 mL, 48 mmol), and THF (150 mL) at 0 °C, and then stirred at room temperature for 1 h. Next, it was concentrated in vacuo, and the residue was washed with water to give crude **4** as a solid. The solid was recrystallized with EtOH/AcOEt/H_2O to give the title compound **4** (13 g, 77%) as a pale yellow crystal. Mp 167–168 °C. ^1H NMR (DMSO-*d6*, 400 MHz): δ 2.39 (3H, s), 3.86 (3H, s), 7.37 (1H, t, *J* = 8.0 Hz), 7.48–7.58 (4H, m), 7.68 (1H, d, *J* = 7.6 Hz), 8.16–8.22 (3H, m), 8.42 (1H, dd, *J* = 2.0, 8.4 Hz), 9.24 (1H, d, *J* = 2.0 Hz), 10.29 (1H, s). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 15.2, 52.1, 119.7, 125.8, 126.9, 127.6, 128.1, 128.8, 129.8, 130.5, 131.6, 134.6, 136.5, 137.1, 137.7, 148.8, 158.4, 163.9, 167.7. MS (FAB) *m*/*z* 347 [(M+H)$^+$]. Anal. Calcd for $C_{21}H_{18}N_2O_3$: C, 72.82; H, 5.24; N, 8.09. Found: C, 72.67; H, 5.29; N, 8.09.

4.3. Methyl 3-{[hydrazino(6-phenylpyridin-3-yl)methylene]amino}-2-methylbenzoate 5

A mixture of **4** (3.0 g, 8.7 mmol) and $SOCl_2$ (30 mL) was stirred at 80 °C for 1 h. After it was cooled to room temperature, the mixture was concentrated in vacuo to give a chloro imidate as a pale yellow solid (3.47 g, 100%). The solid obtained above (500 mg, 1.24 mmol) was added to a solution of hydrazine hydrate (0.30 mL, 6.2 mmol) in EtOH (10 mL), and then stirred at room temperature for 1 h. After adding water, the mixture was extracted with $CHCl_3$ and washed with water. The organic layer was extracted with 1 M HCl aqueous solution, and the aqueous layer was washed with $CHCl_3$. The aqueous layer was then treated with saturated $NaHCO_3$ solution, extracted with $CHCl_3$, dried over $MgSO_4$, and concentrated in vacuo to give the title compound **5** (314 mg, 70%) as a white solid. Mp 109–110 °C. ^1H NMR (DMSO-d_6, 400 MHz): δ 2.47 (3H, s), 3.83 (3H, s), 6.43 (1H, d, *J* = 8.0 Hz), 6.45–7.30 (3H, m), 7.01 (1H, t, *J* = 8.0 Hz), 7.13 (1H, d, *J* = 7.6 Hz), 7.38–7.49 (3H, m), 7.81 (1H, dd, *J* = 2.4, 8.8 Hz), 7.88 (1H, d, *J* = 8.4 Hz), 8.04–8.09 (2H, m), 8.71 (1H, d, *J* = 1.6 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.6, 51.9, 119.4, 120.7, 125.6, 125.7, 126.2, 126.9, 128.6, 128.9, 130.1, 131.8, 134.0, 135.4, 138.1, 141.8, 147.0, 154.4, 168.3. MS (FAB) *m*/*z* 361 [(M+H)$^+$]. Anal. Calcd for $C_{21}H_{20}N_4O_2$: C, 69.98; H, 5.59; N, 15.55. Found: C, 69.96; H, 5.65; N, 15.41.

4.4. Methyl 3-[3-ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl]-2-methylbenzoate 6

To a solution of **5** (335 mg, 0.93 mmol) in EtOH (4.0 mL) was added $EtC(OEt)_3$, (0.37mL, 1.86mmol) and the mixture was refluxed for 1 h. After cooling at room temperature, the mixture was concentrated in vacuo to give a crude solid. This solid was triturated with diethyl ether to give the title compound **6** (297 mg, 80%) as a white solid. Mp 184–185 °C. ^1H NMR (DMSO-d_6, 400 MHz): δ 1.17 (3H, t, *J* = 7.6 Hz), 2.01 (3H, s), 2.46–2.55 (2H, m), 3.84 (3H, s), 7.42–7.51 (3H, m), 7.60 (1H, t, *J* = 8.0 Hz), 7.78 (1H, dd, *J* = 2.4, 8.8 Hz), 7.87 (1H, d, *J* = 8.0 Hz), 7.99 (1H, d, *J* = 8.8 Hz), 8.02 (1H, d, *J* = 7.6 Hz), 8.05–8.08 (2H, m), 8.60 (1H, d,

J = 2.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 11.1, 14.4, 18.0, 52.3, 119.9, 121.8, 126.6, 127.7, 128.8, 129.6, 131.9, 132.3, 134.2, 135.5, 135.9, 137.3, 147.4, 150.6, 156.5, 156.7, 166.5. MS (FAB) m/z 399 [(M+H)$^+$]. Anal. Calcd for $C_{24}H_{22}N_4O_2{\cdot}0.1H_2O$: C, 72.02; H, 5.59; N, 14.00. Found: C, 71.95; H, 5.65; N, 14.01.

4.5. 3-[3-Ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl] -2-methylbenzoic acid 7

To a solution of **6** (274 mg, 0.69 mmol) in MeOH (5.0 mL) was added 1 M aqueous NaOH solution (1.38 mL, 1.38 mmol), and the mixture was stirred for 17 h at room temperature. To the reaction mixture was added 1 M aqueous HCl solution (1.38 mL, 1.38 mmol), followed by adding water (25 mL) to give a precipitate. The precipitate was collected by filtration, washed with water, and dried in vacuo to give the title compound **7** (246 mg, 93%) as a white solid. Mp 257–258 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.18 (3H, t, J = 7.3 Hz), 2.03 (3H, s), 2.45–2.52 (2H, m), 7.42–7.51 (3H, m), 7.57 (1H, t, J = 7.8 Hz), 7.78 (1H, dd, J = 1.9, 8.3 Hz), 7.83 (1H, d, J = 6.8 Hz), 7.99 (1H, d, J = 8.3 Hz), 8.03 (1H, dd, J = 0.9, 7.8 Hz), 8.05–8.10 (2H, m), 8.60 (1H, d, J = 1.9 Hz), 13.35 (1H, br s). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 11.2, 14.6, 18.1, 120.0, 121.9, 126.6, 127.6, 128.8, 129.7, 131.9, 132.0, 133.5, 134.2, 135.6, 135.9, 137.4, 147.5, 150.6, 156.6, 156.8, 167.9. MS (FAB) m/z 385 [(M+H)$^+$]. Anal. Calcd for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57. Found: C, 71.65; H, 5.30; N, 14.59.

4.6. Screening of optically active amines for the resolution of 7

General procedure: A mixture of **7** (30 mg, 0.08 mmol) and an optically active amine (0.08 mmol) was dissolved in CH_3CN/water (1:1, 4 mL). After the solution was kept at ambient temperature for 7–10 days, the precipitate was collected by filtration, washed with CH_3CN/water, and dried in vacuo to afford the corresponding diastereomeric salt. The diastereomeric salts (*R*)-**7**·(1*R*,2*S*)-ADPE (15%, 66% de) and (*S*)-**7**·(1*S*,2*R*)-ADPE (15%, 33% de) were obtained.

The following amines did not form solid diastereomeric salts with **7**: (*R*)-(+)-1-methylbenzylamine, (*S*)-(−)-1-methylbenzylamine, (*R*)-(−)-2-phenylglycinol, (*S*)-(+)-2-phenylglycinol, (1*S*,2*S*)-(+)-2-amino-1-phenyl-1,3-propanediol, (1*R*,2*R*)-(−)-2-amino-1-phenyl-1,3-propanediol, (*R*)-(+)-1-(1-naphthyl)ethylamine, (*S*)-(−)-1-(1-naphthyl)ethylamine, D-phenylalaninol, L-phenylalaninol, (1*S*,2*S*)-(+)-2-amino-1-(4-nitrophenyl)-1,3-propanediol, (1*R*,2*R*)-(−)-2-amino-1-(4-nitrophenyl)-1,3-propanediol, (1*R*,2*R*)-(+)-1,2-diphenyl-1,2-ethanediamine, (1*S*,2*S*)-(−)-1,2-diphenyl-1,2-ethanediamine, (1*R*,2*S*)-(+)-*cis*-1-amino-2-indanol, (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol, (1*R*,2*R*)-(−)-cyclohexanediamine, (1*S*,2*S*)-(+)-cyclohexanediamine, (*R*)-1-(3-methoxyphenyl)ethylamine, (*S*)-1-(3-methoxyphenyl)ethylamine, (*R*)-1-(4-methoxyphenyl)ethylamine, (*S*)-1-(4-methoxyphenyl)ethylamine, (*R*)-1,2,3,4-tetrahydro-1-naphthylamine, (*S*)-1,2,3,4-tetrahydro-1-naphthylamine, (1*R*,2*S*)-2-di-*n*-butylamino-1-phenyl-1-propanol, (1*S*,2*R*)-2-di-*n*-butylamino-1-phenyl-1-propanol, (*R*)-(−)-1-aminoindane, (*S*)-(+)-1-aminoindane, (*S*)-(−)-α,4-dimethylbenzylamine, (*R*)-1-(4-methylphenyl)ethylamine, (*S*)-1-phenyl-2-(*p*-tolyl)ethylamine, (*R*)-(−)-2-amino-1-phenylethanol, (*S*)-(−)-3-amino-1,2-propanediol, and D-threoninol.

4.7. (1*R*,2*S*)-2-Amino-1,2-diphenylethanol(−)-(*R*)-3-[3-ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl]-2-methylbenzoate(*R*)-7·(1*R*,2*S*)-ADPE

Racemic **7** (2.0 g, 5.2 mmol) and (1*R*,2*S*)-2-amino-1,2-diphenylethanol (1.1 g, 5.2 mmol) were dissolved in refluxing EtOH/water (1:1, 44 mL). To this refluxing solution was added water (44 mL), and the mixture was stirred at room temperature for 4 h. The resultant white solid was collected by filtration, washed with EtOH/water (1:4), and dried in vacuo to give a crude solid (1.47 g, 2.46 mmol, 93%, de). The crude solid was dissolved in refluxing EtOH/water (1:3, 40 mL), and the mixture was then stirred overnight at room temperature. The resultant solid was collected by filtration, washed with EtOH/water (1:4), and dried in vacuo to give the salt (*R*)-**7**·(1*R*,2*S*)-ADPE (1.26 g, 2.11 mmol, 41% yield, 99.5% de, *E* 82%) as a white solid. $[\alpha]_D^{25} = -163$ (c 1.0, MeOH). Mp 110–111 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.17 (3H, t, J = 7.2 Hz), 1.99 (3H, s), 2.43–2.51 (2H, m), 4.30 (1H, d, J = 4.0 Hz), 5.02 (1H, d, J = 4.4 Hz), 7.09–7.22 (11H, m), 7.41–7.49 (4H, m), 7.56 (1H, d, J = 7.6 Hz), 7.77–7.82 (2H, m), 7.98 (1H, d, J = 8.8 Hz), 8.05–8.08 (2H, m), 8.62 (1H, d, J = 1.6 Hz). MS (FAB) m/z 385 [(M+H)$^+$]. Anal. Calcd for $C_{23}H_{20}N_4O_2{\cdot}C_{14}H_{15}NO{\cdot}1.5H_2O$: C, 71.13; H, 6.13; N, 11.21. Found: C, 71.18; H, 6.37; N, 11.27. The filtrate obtained from the above salt formation was collected, and evaporated in vacuo to afford (*S*)-**7**·(*1R,2S*)-ADPE (1.66 g, 2.8 mmol, 86% de) as a colorless amorphous solid.

4.8. Thermal racemization of (*S*)-7

The salt (*S*)-**7**·(1*R*,2*S*)-ADPE (1.66 g, 2.8 mmol, 86% de) was dissolved in DMA (10 mL), and the mixture was stirred at 155 °C for 9 h. After cooling to room temperature, 1 M aqueous HCl (2.8 mL, 2.8 mmol) was added, and the mixture was diluted with water. The resulting precipitate was collected by filtration, washed with water, and dried in vacuo to give racemic **7** (1.05 g, 98%, 0% ee).

4.9. (−)-(*R*)-3-[3-Ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl]-2-methylbenzoic acid(*R*)-7

To a solution of diastereomeric salt (*R*)-**7**·(1*R*,2*S*)-ADPE (1.76 g, 2.8 mmol) in EtOH (15 mL) was added a 1 M aqueous HCl solution (2.8 mL, 2.8 mmol). Water was then added, and the resulting precipitate was collected by filtration, washed with water, and dried in vacuo to give the title compound (*R*)-**7** (1.03 g, 95%) as a white solid. $[\alpha]_D^{25} = -200$ (c 1.0, $CHCl_3$). Mp 235–236 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.18 (3H, t, J = 7.2 Hz), 2.03 (3H, s), 2.45–2.52 (2H, m), 7.42–7.51 (3H, m), 7.57 (1H, t, J = 8.0 Hz), 7.78 (1H, dd, J = 2.0, 8.4 Hz), 7.83 (1H, d, J = 7.6 Hz), 7.99 (1H, d, J = 8.8 Hz), 8.02 (1H, d, J = 7.6 Hz), 8.05–8.09 (2H, m), 8.61 (1H, d, J = 1.6 Hz), 13.33 (1H, br s). MS (FAB) m/z 385 [(M+H)$^+$]. Anal. Calcd for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57. Found: C, 71.62; H, 5.28; N, 14.57. HPLC retention time (min): 11.1 [(*R*)-**7**], 16.3 [(*S*)-**7**].

4.10. (−)-(*R*)-3-[3-Ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl]-2-methylbenzamide(*R*)-8

A mixture of (*R*)-**7** (800 mg, 2.1 mmol), NH_4Cl (333 mg, 6.2 mmol), Et_3N (0.43 mL, 3.1 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI·HCl) (498 mg, 2.6 mmol), and 1-hydroxy-1*H*-benzotriazole (HOBt) (351 mg, 2.6 mmol) in DMF (5.0 mL) was stirred at room temperature for 12 h. The reaction mixture was then diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, and dried in vacuo to give a crude solid. The solid was dissolved in refluxing EtOAc (10 mL), and the mixture was then stirred overnight at room temperature. The resultant solid was collected by filtration, washed with EtOAc, and dried in vacuo to give the title compound (*R*)-**8** (687 mg, 86%) as a white solid. $[\alpha]_D^{25} = -170$ (c 1.0, $CHCl_3$). Mp 207–208 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.19 (3H, t, J = 7.2 Hz), 1.90 (3H, s), 2.40–2.55 (2H, m), 7.42–7.53 (4H, m), 7.59–6.61 (2H, m), 7.67 (1H, d, J = 7.6 Hz), 7.82 (1H, dd, J = 2.0, 8.0 Hz), 7.96 (1H, br s), 7.99 (1H, d, J = 8.8 Hz), 8.05–8.09 (2H, m), 8.60 (1H, d, J = 2.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 11.2, 13.9, 18.0, 119.9, 121.9, 126.6, 127.3, 128.7, 129.0, 132.6, 133.6, 135.5, 137.4, 139.6, 147.3, 150.4, 156.5, 156.7, 169.6. MS (FAB) m/z 384 [(M+H)$^+$]. Anal.

Calcd for $C_{23}H_{21}N_5O$: C, 72.04; H, 5.52; N, 18.26. Found: C, 71.89; H, 5.56; N, 18.26.

4.11. (−)-(*R*)-3-[3-Ethyl-5-(6-phenylpyridin-3-yl)-4*H*-1,2,4-triazol-4-yl]-2-methylbenzonitrile(*R*)-1

To a solution of (*R*)-**8** (300 mg, 0.78 mmol) in DMF (3.0 mL) was added $POCl_3$ (0.087 mL, 0.94 mmol) at 0 °C, and the mixture was stirred for 20 min. The reaction was quenched by adding 1 M aqueous NaOH, and then extracted with EtOAc, washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo to give a crude solid. The solid was dissolved in refluxing EtOH/water (1:4, 5 mL), and the mixture was stirred overnight at room temperature. The resultant solid was collected by filtration, washed with EtOH/water (1:4), and dried in vacuo to give the title compound (*R*)-**1** (230 mg, 81%, 99.8% ee) as a white solid. $[\alpha]_D^{25} = -211$ (*c* 1.0, $CHCl_3$), mp 162–163 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.19 (3H, t, J = 7.4 Hz), 2.08 (3H, s), 2.41–2.59 (2H, m), 7.43–7.52 (3H, m), 7.66 (1H, t, J = 7.8 Hz), 7.76 (1H, dd, J = 1.5, 8.3 Hz), 7.97–8.02 (2H, m), 8.05–8.12 (3H, m), 8.64 (1H, d, J = 1.9 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 11.1, 15.6, 18.0, 114.4, 116.8, 119.9, 121.5, 126.6, 128.75, 128.78, 129.7, 133.7, 133.9, 134.9, 135.7, 137.4, 139.2, 147.7, 150.5, 156.7. MS (FAB) *m*/*z* 366. Anal. Calcd for $C_{23}H_{19}N_5$: C, 75.59; H, 5.24; N, 19.16. Found: C, 75.73; H, 5.23; N, 19.14. HPLC retention time (min): 22.7 [(*R*)-**1**], 33.1 [(*S*)-**1**].

4.12. Powder X-ray diffraction (PXRD)

PXRD data was collected using BL19B2 of SPring-8 with the approval of the Japan Synchrotron Radiation Research Institute (JASRI) (Proposal No. 2005A0922-S2-np). The powder sample was filled in a glass capillary and measured with the rotation. The structure was resolved by Materials Studio software.[13]

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