Synthesis and Biological Activities of Optical Isomers of 2-(4-Chlorophenyl)-5,6-dihydro-(1)benzothiepino[5,4-*c*]pyridazin-3(2*H*)-one 7-Oxide[1)]

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Two enantiomers of 2-(4-chlorophenyl)-5,6-dihydro-(1)benzothiepino[5,4-*c*]pyridazin-3(2*H*)-one 7-oxide ((±)-1: Y-23684) were synthesized in high yields by asymmetric oxidation of the synthetic precursor (2) using modified Sharpless reagent. Among the oxidants tested, cumene hydroperoxide (CHP) gave the highest optical and chemical yields, while *tert*-butyl, *tert*-amyl, and 1,1,3,3-tetramethylbutyl hydroperoxides did not show such high enantio-selectivities. The absolute configuration of (+)-1 enantiomer synthesized from 2, Ti(O-iso-Pr)$_4$, (−)-diethyl tartarate, and CHP was determined to be *S* by X-ray crystallographic analysis. Both enantiomers, *S*-(+)-1 and *R*-(−)-1, and (±)-1 had approximately equivalent *in vivo* activities to antibicuculline test in mice and anticonflict test in rats, although *S*-(+)-1 showed about three times higher affinity to benzodiazepine receptor than *R*-(−)-1 in [^{3}H]diazepam binding assay.

Keywords asymmetric oxidation; sulfoxide; enantiomer; benzodiazepine receptor; anticonflict test; anxiolytic activity; X-ray crystallography; Y-23684

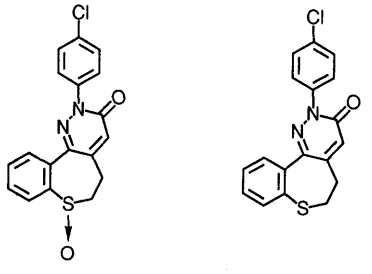
In our previous paper,[2)] we reported the synthesis and anxiolytic activity of 2-aryl-5,6-dihydro-(1)benzothiepino-[5,4-*c*]pyridazin-3(2*H*)-ones and related compounds. Among them, 2-(4-chlorophenyl)-5,6-dihydro-(1)benzothiepino[5,4-*c*]pyridazin-3(2*H*)-one 7-oxide ((±)-**1**, Y-23684) had a promising anxioselective anxiolytic activity, namely partial agonistic activity to benzodiazepine receptor. This compound has an asymmetric sulfur atom arising from sulfoxide at the 7-position of the (1)benzothiepino[5,4-*c*]pyridazine skeleton and therefore two enantiomeric forms can exist.

The biological activities of 1,4-benzodiazepines were found to be restricted to the enantiomer possessing the *S*-configuration in the compounds having a chirality center at the 3-position.[3)]

In this paper, we describe the synthesis of each enantiomer of (±)-**1** by asymmetric oxidation, determination of the absolute configuration of each enantiomer by X-ray crystallographic analysis, and their biological activity.

Chemistry We initially tried to prepare the enantiomers by the condensation of optically active 5-oxo-2,3,4,5-tetrahydro-1-benzothiepin-4-acetic acid 1-oxide and 4-chlorophenylhydrazine followed by oxidation with PbO in acetic acid. With this procedure it was difficult to obtain a requisite quantity of the optically pure enantiomers owing to racemization.

Kagan and his colleagues[4)] have reported that a useful reagent for the selective asymmetric sulfide-sulfoxide oxidation was obtained by addition of an adequate amount of water in the Sharpless reagent[5)] for asymmetric epoxidation of allylic alcohols. Thus, we attempted to prepare each enantiomer of (±)-**1** by the asymmetric oxidation of 2-(4-chlorophenyl)-5,6-dihydro-(1)benzothiepino[5,4-*c*]-pyridazin-3(2*H*)-one (**2**) with the use of various hydroperoxides, *i.e.*, cumene hydroperoxide (CHP), *tert*-butyl hydroperoxide (TBHP), *tert*-amyl hydroperoxide (TAHP), and 1,1,3,3-tetramethylbutyl hydroperoxide (TMBHP).



(±)-**1** (Y-23684) **2**

Chart 1

As shown in Table I, CHP gave the highest value with respect to both the optical (81.3% ee) and chemical (82.5%) yields compared with those of other oxidants tested (entry 1). Even when the water content was increased from 0.5 to 0.9 eq (entry 2), the optical and chemical yields were comparable to those of entry 1. These data suggest that the oxidation of CHP can tolerate to the change of reaction conditions which often takes place in large-scale production. When the reaction temperature was elevated to 0 °C (entry 4), high ee was retained while the sulfone formation was increased after 2 h.

In spite of the conditions (entry 5) which showed high ee for the asymmetric oxidation of methyl *p*-tolyl sulfide into the corresponding chiral sulfoxide when TBHP was used as an oxidant,[4)] the reaction resulted in low chemical

Table I. Asymmetric Oxidation of **2**

Entry	Oxidant	Reaction conditions[a)]	Composition[b)] (%)				ee(%)[c)]
			S	(−)SO	(+)SO	SO_2	
1	CHP	−20°C, 19 h	16.0	74.8	7.7	1.5	81.3
2	CHP	−20°C, 20 h[d)]	10.6	77.8	9.6	2.0	78.0
3	CHP	−20°C, 45 h	3.1	84.4	7.4	5.1	83.9
4	CHP	0°C, 2 h	1.1	80.5	7.8	10.6	82.3
5	TBHP	−20°C, 20 h[e)]	48.4	33.4	16.4	1.8	34.1
6	TBHP	−20°C, 20 h	17.9	64.5	14.9	2.7	62.5
7	TBHP	−24°C, 20 h	42.5	48.8	8.8	0	69.4
8	TBHP	0°C, 12 h	0	63.5	10.8	25.9	70.9
9	TBHP	23°C, 12 h	0	39.5	21.4	39.1	29.7
10	TAHP	−20°C, 17 h	36.1	44.7	14.3	4.9	51.5
11	TMBHP	−20°C, 17 h	36.0	51.0	11.4	1.6	63.5

a) **2** (2.9 mM) in CH_2Cl_2 (dried over molecular sieves), under N_2 atmosphere. Reagent: Ti (O-iso-Pr)$_4$–(+)-DET–H_2O–oxidant (1 : 2 : 0.5 : 2).[6)] *b*) After working up according to the method described in the Experimental section, the compositions of crude product were determined by HPLC using chiral cel OD column; S, sulfide; SO, sulfoxide; SO_2, sulfone. *c*) ee, enantiomeric excess. *d*) H_2O, 0.9 eq. *e*) H_2O, 1.1 eq.

yield of sulfoxide (49.8%) and low optical yield (34.1% ee). When the water content was reduced to 0.5 eq (entry 6), the chemical and optical yields were increased to 79.4% and 62.5% ee, respectively, though lower than those of CHP. Furthermore, the formation of sulfone which was difficult to separate from the sulfoxide was increased when the reaction temperature was raised to 0 °C (entry 8). In addition, a large scale of the reaction with TBHP gave lower optical yield because of difficulty in the control of the reaction conditions, especially the water content.

Neither TAHP (entry 10) nor TMBHP (entry 11) surpassed CHP and TBHP in terms of optical yield after the asymmetric oxidation of **2**, and the chemical yields were unimproved. From the results it was suggested that compound **2** could be oxidized asymmetrically using CHP as an oxidant with high chemical and optical yields. In this study, it was difficult to obtain a requisite quantity of the optically pure enantiomer by recrystallization when the optical purity was below 60% ee.

Thus, asymmetric oxidation of **2** was carried out to obtain large quantities of optically active products using CHP as shown in Chart 2. Compound (+)-**1** was predominantly obtained from **2** when (−)-diethyl tartarate (DET) was used as a chiral ligand. (+)-DET, on the contrary, preferentially gave (−)-**1**. Compounds (+)-**1** and (−)-**1** were obtained from **2** in 51.5% and 37.4% chemical yields, respectively, in over 99% ee from recrystallization of the corresponding crude products. The asymmetric oxidation of complicated cyclic sulfide **2** using CHP as an oxidant was found to proceed with high enantioselectivity in excellent chemical yield and was thus recognized as useful for the large-scale production of each enantiomer of (±)-**1**, similar to the oxidation of some aryl methyl sulfides to the corresponding sulfoxides.[4c]

The optical purity of these compounds was determined by high performance liquid chromatography (HPLC) analysis using chiral stationary phase column (Chiracel OD®). The absolute configuration of (+)-**1** enantiomer was determined to be *S* by X-ray crystallographic analysis. The crystal of (+)-**1** has two crystallographically independent molecules A and B as shown in Fig. 1. The determination also provided that the configuration of (−)-**1** was *R* because its optical rotation was opposite to that of (+)-**1**.

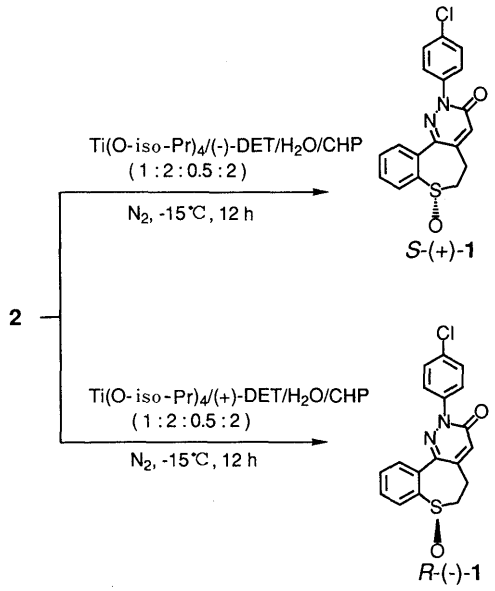


Chart 2

Biological Activities Table II gives comparative biological data of the compounds *R*-(−)-**1** and *S*-(+)-**1** with those of (±)-**1**.

In *in vitro* test, compound *S*-(+)-**1** showed three times higher affinity to the benzodiazepine receptor than *R*-(−)-**1**, and the affinity of (±)-**1** was intermediate between those of the *S*-(+)-**1** and *R*-(−)-**1** enantiomers. Thus the affinity decreased in the following order: *S*-(+)-**1** > (±)-**1** > *R*-(−)-**1**.

In *in vivo* test, compounds (±)-**1**, *S*-(+)-**1**, and *R*-(−)-**1** were approximately equipotent in the antibicuculline and anticonflict tests. Metabolic conversion of sulfoxides to sulfones is a well-known reaction.[7] From the metabolic

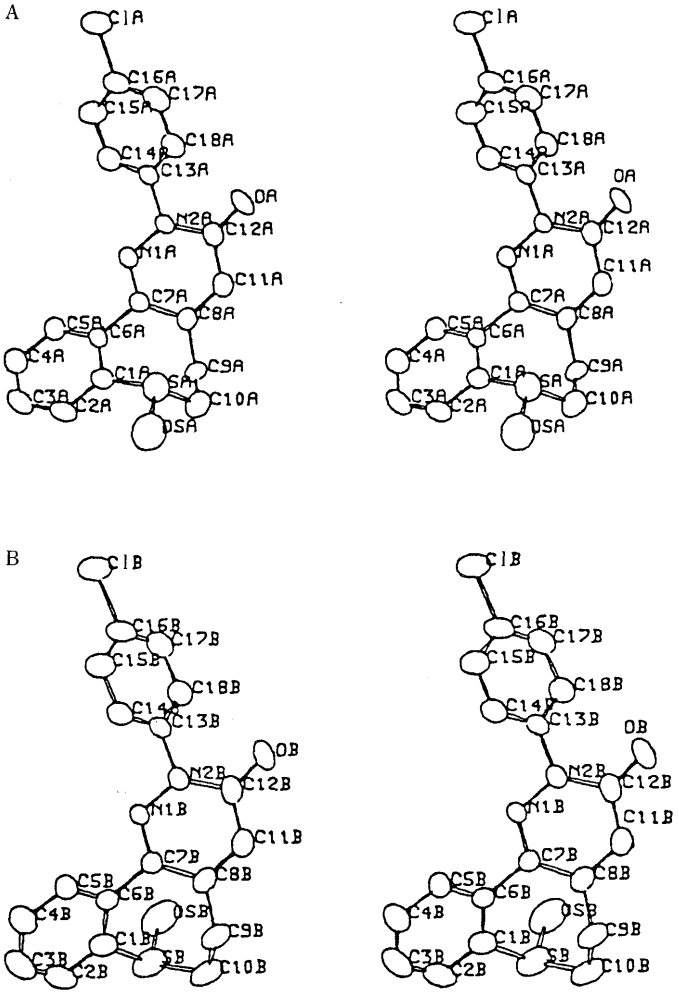


Fig. 1. Stereoscopic Drawing of *S*-(+)-**1**
A, molecule A; B, molecule B.

Table II. Biological Data of (+)-**1**, (−)-**1**, and (±)-**1**

Compd.	^{3}H–DZ binding K_i (nM)	Antibicuculline ED_{50} (mg/kg, *p.o.*)	Anticonflict (Water-lick test) MED (mg/kg, *p.o.*)
(+)-**1**	27	1.2	5
(−)-**1**	86	1.5	5
(±)-**1**	41	1.2	5

investigation of (±)-**1**,[8] the achiral sulfone compound, 2-(4-chlorophenyl)-5,6-dihydro(1)benzothiepino[5,4-*c*]-pyridazin-3(2*H*)-one 7,7-dioxide, has been proved to be an active main metabolite (K_i=9.6 nM, antibicuculline test; ED_{50}=0.3 mg/kg i.v.). These findings are support our observation that the pharmacological effects of each enantiomer are nearly equal to those of (±)-**1** (Y-23684).

Conclusion

We successfully synthesized the compounds *S*-(+)-**1** and *R*-(−)-**1** with high optical purities by asymmetric oxidation using a modified Sharpless reagent according to the method reported by Kagan and his colleagues. Among the oxidants tested, cumene hydroperoxide gave the highest chemical and optical yields, although the precise mechanisms of the reaction remain to be investigated. In the biological evaluation, *S*-(+)-**1** showed the highest affinity to the benzodiazepine receptor *in vitro*, while (±)-**1** and both enantiomers were equipotent in *in vivo* pharmacological tests.

Experimental

Melting points were determined on a Buchi 530 melting point apparatus and are uncorrected. Infrared (IR) spectra were taken on a JASCO IR-810 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a JNM-GSX400 spectrometer and chemical shifts were given in ppm with tetramethylsilane as the internal standard. Mass spectra (MS) were measured with a JEOL JMS-01SG-2 spectrometer. Optical rotations were measured on a JASCO DIP-181 digital polarimeter. The water content of the reaction mixture were determined by the Karl Fisher method using a Kyoto Electronic MKA-3. Merck Silica gel 60 (70—230 mesh) was used for column chromatography.

***S*-(+)-(4-Chlorophenyl)5,6-dihydro-(1)benzothiepino[5,4-*c*]pyridazin-3(2*H*)-one 7-Oxide (*S*-(+)-1)** Titanium(IV) isopropoxide ($Ti(O\text{-}iso\text{-}Pr)_4$, 97% (17.2 ml, 0.061 mol) and (−)-DET (24 g, 0.116 mol) were dissolved in CH_2Cl_2 (500 ml) at room temperature under nitrogen and stirred for 15 min. H_2O (0.6 ml, 0.033 mol) was introduced through a rubber spectrum *via* microsyringe. After the mixture was stirred for 30 min, a solution of 2-(4-chlorophenyl)-5,6-dihydro-(1)benzothiepino[5,4-*c*]pyridazin-3(2*H*)-one (**2**) (20 g, 0.058 mol) in CH_2Cl_2 (100 ml) was added. The solution was cooled to −20 °C and 80% CHP (25 ml, 0.13 mol) was added and stirred for 5 h. The mixture was allowed to stand overnight at −15 °C and then water was added dropwise to the solution at −15 °C. Vigorous stirring was maintained for 1 h at −15 °C and for an additional 1 h at room temperature. The resulting white gel was filtered through a filter agent (Celite®) and thoroughly washed with CH_2Cl_2. The filtrate was washed with aq. $NaHSO_3$ and water, and dried over $MgSO_4$. After evaporation of the solvent, the residue was chromatographed on a silica gel column with $CHCl_3$ as an eluent to give a crude product (16 g, 78.1% ee), which was recrystallized from acetonitrile. After concentration of the mother liquors, the residue was recrystallized from EtOH to give *S*-(+)-**1** (10.4 g, 51.5%), mp 216—217 °C, $[\alpha]_D^{25}$ +40.4 (c=1, $CHCl_3$). *Anal.* Calcd for $C_{18}H_{13}ClN_2O_2S$: C, 60.59; H, 3.67; N, 7.85. Found: C, 60.59; H, 3.80; N, 7.81. IR (KBr): 1675 cm^{-1} (C=O). MS *m/z*: 356 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 2.75 (1H, ddd, J=13.6, 9.5, 8.0 Hz), 2.88 (1H, ddd, J=13.6, 7.3, 2.9 Hz), 3.14 (1H, ddd, J=12.2, 8.0, 2.9 Hz), 3.97 (1H, ddd, J=12.2, 9.5, 7.3 Hz), 6.98 (1H, s), 7.45 (2H, d, J=8.9 Hz), 7.65 (1H, m), 7.66 (1H, m), 7.67 (2H, d, J=8.9 Hz), 7.74 (1H, m), 7.94 (1H, d, J=7.8 Hz).

***R*-(−)-2-(4-Chlorophenyl)-5,6-dihydro-(1)benzothiepino[5,4-*c*]-pyridazine-3(2*H*)-one 7-Oxide (*R*-(−)-1)** Compound *R*-(−)-**1** was prepared by the oxidation of **2** (18 g, 0.053 mol) with $Ti(O\text{-}iso\text{-}Pr)_4$, 97% (15.7 ml, 0.053 mol), (+)-DET (21.9 g, 0.106 mol), H_2O (0.5 ml, 0.03 mol), and 80% CHP (24 ml, 0.12 mol) in the same manner as described above. Yield of *R*-(−)-**1** was 7 g (47.4%), mp 216—217 °C, $[\alpha]_D^{25}$ −40.5 (c=1, $CHCl_3$). *Anal.* Calcd for $C_{18}H_{13}ClN_2O_2S$: C, 60.59; H, 3.67; N, 7.85. Found: C, 60.56; H, 3.78; N, 7.75.

Determination of Optical Purities of *S*-(+)-1 and *R*-(−)-1 The optical purities were measured by HPLC analyses using chiral stationary phase column, Chiracel OD (4.6 mm i.d. × 250 mm) [column temperature, 25 °C; mobile phase, ethanol; flow rate, 0.8 ml/min; detection, ultraviolet at 254 nm]. The optical purities were determined to be as follows: *R*-(−)-**1**,

Table III. Crystal Data of *S*-(+)-**1**

Chemical formula	:	$C_{18}H_{13}ClN_2O_2S$
Formula weight	:	356.83
Crystal system	:	Monoclinic
Space group	:	$P2_1$
Z	=	4
a	=	22.762 (8) Å
b	=	8.566 (4) Å
c	=	8.337 (2) Å
β	=	90.98 (2)°
V	=	1625.2 Å^3
μ (Cu$K\alpha$)	=	33.94 cm^{-1}
λ	=	1.5418 Å
D_{calcd}	=	1.458 Mgcm^{-3}
F (000)	=	736

Table IV. Final Atomic Potential Parameters of *S*-(+)-**1**

	x	y	z	B_{eq}
ClA	−2508 (1)	10826 (3)	10919 (2)	6.01 (5)
SA	424 (1)	9022 (2)	1981 (2)	4.27 (3)
OSA	903 (2)	9716 (6)	1013 (6)	6.82 (15)
OA	−2005 (1)	7122 (6)	4240 (6)	5.74 (13)
N1A	−594 (2)	8108 (6)	5799 (5)	3.14 (10)
N2A	−1190 (1)	7992 (6)	5584 (5)	3.06 (10)
C1A	744 (2)	8317 (7)	3808 (6)	3.16 (12)
C2A	1352 (2)	8516 (8)	4082 (7)	4.29 (17)
C3A	1589 (2)	8116 (9)	5548 (7)	4.49 (16)
C4A	1234 (2)	7514 (8)	6746 (7)	4.37 (16)
C5A	635 (2)	7325 (8)	6471 (6)	3.75 (15)
C6A	386 (2)	7713 (7)	5005 (6)	2.89 (12)
C7A	−264 (2)	7534 (6)	4708 (6)	2.98 (13)
C8A	−483 (2)	6747 (7)	3292 (6)	3.11 (13)
C9A	−63 (2)	6030 (7)	2126 (6)	3.33 (14)
C10A	248 (3)	7179 (8)	1032 (7)	4.21 (16)
C11A	−1079 (2)	6618 (7)	3125 (7)	3.46 (14)
C12A	−1472 (2)	7238 (7)	4291 (7)	3.67 (14)
C13A	−1517 (2)	8693 (6)	6875 (6)	2.94 (13)
C14A	−1377 (2)	8324 (8)	8416 (7)	4.16 (15)
C15A	−1677 (2)	8955 (9)	9681 (7)	4.76 (16)
C16A	−2120 (2)	10042 (8)	9346 (7)	4.27 (16)
C17A	−2263 (2)	10447 (8)	7786 (8)	4.40 (17)
C18A	−1960 (2)	9768 (7)	6522 (7)	3.76 (14)
ClB	7455 (1)	5676 (3)	11042 (2)	6.37 (5)
SB	4351 (1)	3996 (2)	1692 (2)	4.91 (4)
OSB	4859 (2)	5040 (6)	1852 (5)	6.74 (16)
OB	6953 (2)	2221 (6)	4104 (6)	5.89 (14)
N1B	5551 (1)	3312 (6)	5583 (5)	2.89 (10)
N2B	6147 (1)	3142 (6)	5415 (5)	3.26 (10)
C1B	4156 (2)	3395 (7)	3650 (6)	3.65 (14)
C2B	3565 (2)	3522 (8)	4031 (8)	4.97 (18)
C3B	3374 (2)	3171 (10)	5547 (8)	5.61 (19)
C4B	3774 (2)	2671 (8)	6699 (8)	4.69 (17)
C5B	4368 (2)	2536 (7)	6347 (6)	3.52 (14)
C6B	4565 (2)	2888 (6)	4813 (6)	2.72 (11)
C7B	5208 (2)	2734 (6)	4480 (6)	2.75 (12)
C8B	5416 (2)	1911 (7)	3111 (6)	3.19 (13)
C9B	4984 (3)	1120 (7)	1981 (6)	3.92 (15)
C10B	4637 (3)	2208 (8)	871 (7)	4.80 (17)
C11B	6010 (2)	1747 (8)	2988 (7)	3.94 (15)
C12B	6414 (2)	2371 (7)	4147 (7)	3.91 (15)
C13B	6484 (2)	3747 (7)	6768 (6)	3.02 (13)
C14B	6293 (2)	3442 (7)	8291 (6)	3.53 (14)
C15B	6592 (2)	4004 (9)	9598 (7)	4.35 (15)
C16B	7082 (2)	4933 (8)	9355 (7)	4.32 (16)
C17B	7272 (2)	5290 (8)	7849 (8)	4.29 (17)
C18B	6972 (2)	4678 (7)	6518 (7)	3.97 (15)

Final atomic positional parameters (×10^4) and equivalent isotropic thermal parameters (Å^2) with estimated standard deviations in parentheses.

TABLE V. Bond Distances (Å) of *S*-(+)-**1**

ClA–C16A	1.729 (6)	ClB–C16B	1.750 (6)
SA–OSA	1.492 (5)	SB–OSB	1.466 (6)
SA–C1A	1.783 (5)	SB–C1B	1.776 (6)
SA–C10A	1.807 (7)	SB–C10B	1.804 (7)
OA–C12A	1.217 (6)	OB–C12B	1.236 (6)
N1A–N2A	1.369 (5)	N1B–N2B	1.374 (5)
N1A–C7A	1.287 (6)	N1B–C7B	1.295 (6)
N2A–C12A	1.403 (7)	N2B–C12B	1.395 (7)
N2A–C13A	1.450 (6)	N2B–C13B	1.449 (6)
C1A–C2A	1.409 (7)	C1B–C2B	1.392 (7)
C1A–C6A	1.398 (7)	C1B–C6B	1.401 (7)
C2A–C3A	1.370 (8)	C2B–C3B	1.377 (9)
C3A–C4A	1.396 (8)	C3B–C4B	1.380 (9)
C4A–C5A	1.388 (7)	C4B–C5B	1.392 (7)
C5A–C6A	1.378 (7)	C5B–C6B	1.394 (7)
C6A–C7A	1.504 (6)	C6B–C7B	1.500 (6)
C7A–C8A	1.441 (7)	C7B–C8B	1.429 (7)
C8A–C9A	1.507 (7)	C8B–C9B	1.510 (8)
C8A–C11A	1.367 (7)	C8B–C11B	1.366 (8)
C9A–C10A	1.524 (8)	C9B–C10B	1.524 (9)
C11A–C12A	1.434 (8)	C11B–C12B	1.425 (8)
C13A–C14A	1.355 (7)	C13B–C14B	1.374 (7)
C13A–C18A	1.393 (7)	C13B–C18B	1.386 (7)
C14A–C15A	1.378 (8)	C14B–C15B	1.364 (8)
C15A–C16A	1.396 (9)	C15B–C16B	1.387 (8)
C16A–C17A	1.380 (9)	C16B–C17B	1.370 (9)
C17A–C18A	1.397 (9)	C17B–C18B	1.395 (8)

Bond distances (Å) with standard deviations in parentheses.

TABLE VI. Bond Angles (°) of *S*-(+)-**1**

OSA–SA–C1A	107.7 (3)	OSB–SB–C1B	107.6 (3)
OSA–SA–C10A	105.7 (3)	OSB–SB–C10B	105.3 (3)
C1A–SA–C10A	99.4 (3)	C1B–SB–C10B	101.5 (3)
N2A–N1A–C7A	118.0 (4)	N2B–N1B–C7B	118.2 (4)
N1A–N2A–C12A	125.0 (4)	N1B–N2B–C12B	124.8 (4)
N1A–N2A–C13A	113.2 (4)	N1B–N2B–C13B	113.2 (4)
C12A–N2A–C13A	121.7 (4)	C12B–N2B–C13B	121.9 (4)
SA–C1A–C2A	118.9 (4)	SB–C1B–C2B	116.4 (4)
SA–C1A–C6A	120.0 (3)	SB–C1B–C6B	123.6 (4)
C2A–C1A–C6A	120.8 (5)	C2B–C1B–C6B	119.9 (5)
C1A–C2A–C3A	119.0 (5)	C1B–C2B–C3B	120.9 (5)
C2A–C3A–C4A	120.4 (5)	C2B–C3B–C4B	119.5 (5)
C3A–C4A–C5A	120.3 (5)	C3B–C4B–C5B	120.7 (6)
C4A–C5A–C6A	120.3 (5)	C4B–C5B–C6B	120.2 (5)
C1A–C6A–C5A	119.2 (4)	C1B–C6B–C5B	118.8 (4)
C1A–C6A–C7A	120.3 (4)	C1B–C6B–C7B	122.5 (4)
C5A–C6A–C7A	120.6 (4)	C5B–C6B–C7B	118.7 (4)
N1A–C7A–C6A	115.4 (4)	N1B–C7B–C6B	114.4 (4)
N1A–C7A–C8A	124.0 (4)	N1B–C7B–C8B	123.5 (4)
C6A–C7A–C8A	120.6 (4)	C6B–C7B–C8B	122.0 (4)
C7A–C8A–C9A	120.3 (4)	C7B–C8B–C9B	119.9 (5)
C7A–C8A–C11A	116.8 (5)	C7B–C8B–C11B	117.0 (5)
C9A–C8A–C11A	122.8 (5)	C9B–C8B–C11B	122.8 (5)
C8A–C9A–C10A	115.4 (5)	C8B–C9B–C10B	115.4 (5)
SA–C10A–C9A	113.8 (4)	SB–C10B–C9B	118.4 (4)
C8A–C11A–C12A	122.0 (5)	C8B–C11B–C12B	122.5 (5)
OA–C12A–N2A	120.5 (5)	OB–C12B–N2B	121.1 (5)
OA–C12A–C11A	125.4 (5)	OB–C12B–C11B	124.8 (6)
N2A–C12A–C11A	114.1 (4)	N2B–C12B–C11B	114.1 (4)
N2A–C13A–C14A	119.4 (5)	N2B–C13B–C14B	118.6 (4)
N2A–C13A–C18A	119.7 (5)	N2B–C13B–C18B	120.2 (5)
C14A–C13A–C18A	120.8 (5)	C14B–C13B–C18B	121.1 (5)
C13A–C14A–C15A	121.5 (5)	C13B–C14B–C15B	120.6 (5)
C14A–C15A–C16A	118.3 (6)	C14B–C15B–C16B	118.5 (5)
ClA–C16A–C15A	118.9 (5)	ClB–C16B–C15B	118.1 (5)
ClA–C16A–C17A	120.1 (5)	ClB–C16B–C17B	119.9 (5)
C15A–C16A–C17A	121.0 (6)	C15B–C16B–C17B	122.0 (6)
C16A–C17A–C18A	119.6 (5)	C16B–C17B–C18B	119.1 (5)
C13A–C18A–C17A	118.8 (5)	C13B–C18B–C17B	118.7 (5)

Bond angles (°) with standard deviations in parentheses.

99.80% ee (t_R: 13.78 min): *S*-(+)-**1**, 99.26% ee (t_R: 17.79 min).

X-Ray Analysis After compound (+)-**1** was crystallized from the acetonitrile to give a colorless prism, its absolute configuration was determined to be *S* at the 7-position (*S*) as shown in Fig. 1. The crystal has two unique molecules A and B and belongs to a monoclinic system with space group $P2_1$. Crystal data are shown in Table III. Intensities were collected on an Enaraf-Nonius CAD_4 diffractometer with graphite-monochromate Cu$K\alpha$ radiation ($\lambda = 1.5418$ Å), and 2400 unique reflections with $1 > 2.36$ (I) were used for the refinement. The structure was solved by the direct method. Atomic parameters were refined by a block-diagonal method and the final R value was 0.039.

The final parameters, bond distances and angles are listed in Tables IV, V, and VI, respectively, along with their standard derivation.

Benzodiazepine Receptor Binding Assay Preparation of synaptosomal fraction and [^{3}H]diazepam ([^{3}H]DZ) binding studies were carried out according to the method of Möhler and Okada.[9] Crude synaptosomal membranes of rat brain were suspended in 50 mM Tris–HCl buffer (pH 7.4) containing 120 mM NaCl and 50 mM KCl. The reaction was started by the addition of a 900 μl aliquot of crude synaptosomal membranes to a 100 μl solution containing [^{3}H]DZ (final concentration was 2 nM) and a known concentration of test compounds. After the mixture was incubated for 20 min at 0 °C, the binding was stopped by adding 3 ml of ice-cold 50 mM Tris–HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl. The samples were then filtered under reduced pressure through Whatman GF/B filters and immediately washed 4 times with 3 ml of ice-cold buffer. The radioactivity on the filters was measured by a liquid scintillation counter. Binding in the presence of 1 mM unlabelled DZ was defined as nonspecific binding. Specific binding was defined as the difference between the total binding and the non-specific binding. The experiments were carried out in triplicate. K_i values were determined by the relationship $K_i = IC_{50}/(1 + c/K_d)$, where IC_{50} was the concentration of the test compounds which caused 50% reduction of the specific binding *vs.* control, c was the concentration of [^{3}H]DZ (2 nM) and K_d was the dissociation constant determined by Scatchard's plot.

Anticonvulsant Test (Antibicuculline Test) The experimental procedure was practiced with the modified method of Lippa and Regan.[10] Groups of 7—14 ddY male mice were challenged with bicuculline (0.6 mg/kg, i.v.) 1 h after the oral administration of the test compounds. The ED_{50} values were calculated by the probit method as the dose which prevented the tonic extension in half of the animals.

Anticonflict Test (Water-Lick Test) The experimental procedure was carried out with a modified method of Vogel *et al.*[11] Groups of 10—14 Wistar rats were deprived of water for 72 h before the tests began. The rats were placed in a Plexiglas conflict test box (light compartment: 38 × 38 × 20 cm, dark compartment: 10 × 10 × 20 cm). A water bottle with a stainless steel spout extended 3 cm into the box at 10 cm above the grid floor. A drinkometer circuit (Ohara Inc., Nihon Koden) was connected to the spout and the number of licks was counted. The rats were placed in the apparatus where an electric shock (0.2—0.3 mA, 0.3 s) was given once every 20th lick. After the rats received the first electric shock, the number of shocks was recorded during the subsequent 3 min text period. The test compounds were administered orally 1 h before the trial. The minimum effective dose (MED) was defined as the lowest dose producing a statistically significant difference in the punished responses from 0.5% methylcellulose-treated (one-way Anova test; $p < 0.05$).

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References and Notes

1) *a*) The 110th Annual Meeting of Pharmaceutical Society of Japan, Sapporo, Aug. 1990; *b*) Meeting of Kyushu Branch, Pharmaceutical Society of Japan, Fukuoka, Dec. 1990.
2) T. Nakao, M. Obata, M. Kawakami, K. Morita, H. Tanaka, Y. Morimoto, S. Takehara, T. Yakushiji and T. Tahara, *Chem. Pharm. Bull.*, **39**, 2556 (1991).
3) *a*) J. F. Blout, R. I. Fryer, N. W. Gilman and L. J. Todaro, *Mol. Pharmacol.*, **24**, 425 (1983); *b*) W. Haefely, E. Kyburz and M. Möhler, "Advances in Drug Research," Vol. 14, ed. by B. Testa, Academic

Press, New York, 1985, pp. 168—322.
4) *a*) P. Pitchen and H. B. Kagan, *Tetrahedron Lett.*, **25**, 1049 (1984); *b*) P. Pitchen, E. Dunach, M. N. Deshmukh and H. B. Kagan., *J. Am. Chem. Soc.*, **106**, 8188 (1984); *c*) S. H. Zhao, O. Samuel and H. B. Kagan, *Tetrahedron*, **43**, 5135 (1987).
5) *a*) K. B. Sharpless and T. Katsuki, *J. Am. Chem. Soc.*, **102**, 5974 (1980); *b*) B. E. Rossiter, T. Katsuki and K. B. Sharpless, *ibid.*, **103**, 464 (1981).
6) The water content was determined by Karl–Fisher method, though interaction between Karl–Fisher reagent and $Ti(O\text{-}iso\text{-}Pr)_4$ might be possible.
7) H. B. Hucker, S. C. Stauffer, S. D. White, R. E. Rhodes, B. H. Arison, E. R. Umbenhauer, R. J. Bower and F. G. McMahon, *Drug Metab. Dispos.*, **1**, 721 (1973).
8) The 110th Annual Meeting of Pharmaceutical Society of Japan, Sapporo, Aug. 1990.
9) M. Möhler and T. Okada, *Life Sci.*, **20**, 2101 (1970).
10) A. S. Lippa and B. Regan, *Life Sci.*, **21**, 1779 (1970).
11) J. R. Vogel, B. Beer and D. E. Clody, *Psychopharmacology*, **21**, 1 (1971).