Axially Chiral 1,7-Naphthyridine-6-carboxamide Derivatives as Orally Active Tachykinin NK_1 Receptor Antagonists: Synthesis, Antagonistic Activity, and Effects on Bladder Functions

Hideaki Natsugari,*,† Yoshinori Ikeura,† Izumi Kamo,† Takenori Ishimaru,‡ Yuji Ishichi,† Akira Fujishima,† Toshimasa Tanaka,† Fumiko Kasahara,† Mitsuru Kawada,§ and Takayuki Doi†

Pharmaceutical Research Division and Technology Development Department, Takeda Chemical Industries, Ltd., 2-17-85, Jusohonmachi, Yodogawa-ku, Osaka 532-8686, Japan, and Discovery Research Division, Takeda Chemical Industries, Ltd., 10 Wadai, Tsukuba-shi, Ibaraki 300-4293, Japan

Received May 3, 1999

Cyclic analogues of *N*-[3,5-bis(trifluoromethyl)benzyl]-7,8-dihydro-*N*,7-dimethyl-5-(4-methylphenyl)-8-oxo-1,7-naphthyridine-6-carboxamide (**1**) having a 6−9-membered ring (**6**−**9**) were synthesized and evaluated for NK_1 antagonistic activities. The 8-membered ring compound with a β-methyl group at the $C_{(9)}$-position, (*aR*,9*R*)-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,-11-tetrahydro-9-methyl-5-(4-methylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]naphthyridine-6,13-dione [(*aR*,9*R*)-**8b**], was atropodiastereoselectively synthesized by cyclization of a chiral intermediate, **10g**. On the other hand, the 7-membered ring compound with a β-methyl group at the $C_{(9)}$-position [(9*S*)-**7b**] was obtained as an equilibrium mixture of atropisomers with a ratio of ca. 3:2 in solution at room temperature (measured by NMR in $CDCl_3$). Compounds (9*S*)-**7b** and (*aR*,9*R*)-**8b** exhibited excellent antagonistic activities both in vitro [IC_{50} (inhibition of [^{125}I]BH-SP binding in human IM-9 cells) = 0.28 and 0.45 nM, respectively] and in vivo (iv and po). Significantly, the in vitro activity of (*aR*,9*R*)-**8b** was ca. 750-fold higher than that of its enantiomer (*aS*,9*S*)-**8b**, ca. 40-fold higher than its atropisomer (*aS*,9*R*)-**8b**, and ca. 20-fold higher than its diastereomer (*aR*,9*S*)-**8b**. The structure−activity relationships in this series, along with the X-ray analysis of (*aR*,9*R*)-**8b**, indicated that the stereochemistry around the $-C_{(6)}(=O)-N_{(7)}-CH_2Ar$ moiety is important for NK_1 receptor recognition. The NK_1 antagonists showed effects on bladder functions in guinea pigs upon intravenous injection: i.e., the antagonists increased the shutdown time of distension-induced rhythmic bladder contractions and the bladder volume threshold, and the effects on the shutdown time were found to correlate well with the NK_1 antagonistic activities. Compound (*aR*,9*R*)-**8b** has been identified as a potential clinical candidate for the treatment of bladder function disorders.

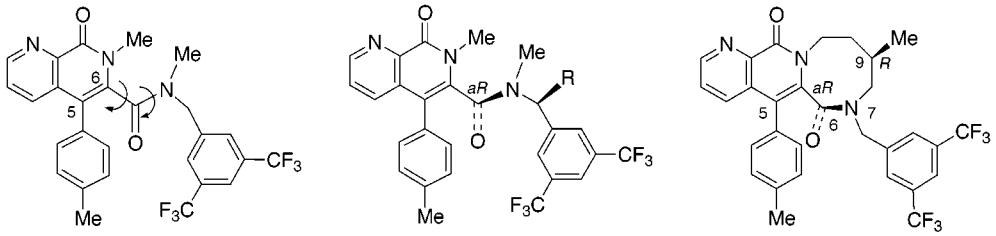
Introduction

Substance P (SP) is an endogenous undecapeptide belonging to the tachykinin peptide family.[1] By binding to the neurokinin-1 (NK_1) receptor, this neuropeptide elicits a wide variety of biological responses in both the central nervous system and peripheral tissues, including the transmission of pain and stress signals, the induction of neurogenic inflammation, and the contraction of smooth muscles.[1] Since the disclosure of the first nonpeptide NK_1 antagonist, CP-96,345,[2] interest in this area has dramatically increased, and recently publications describing orally active NK_1 antagonists, with diverse structures, have appeared (e.g., SR140333,[3] RPR100893,[4] CP-99,994,[5] CP-122,721,[6] CGP49823,[7] GR205171,[8] LY303870,[9] L-741,671,[10] L-742,694,[11] MK-869[12]), suggesting potential clinical utility in the treatment of pain, inflammation, rheumatoid arthritis, asthma, emesis, migraine, and depression.

In our preceding papers,[13] we described the discovery of a potent, orally active NK_1 antagonist, *trans*-*N*-[3,5-bis(trifluoromethyl)benzyl]-7,8-dihydro-*N*,7-dimethyl-5-(4-methylphenyl)-8-oxo-1,7-naphthyridine-6-carboxamide (*trans*-**1**[13a]; Figure 1). Since *trans*-**1** has a tertiary carboxamide group at the sterically hindered position (at $C_{(6)}$), it exhibits two notable structural features (Scheme 1). First, the *trans*- and *cis*-amide rotamers of **1** are separable at room temperature;[13a] the compound isolated by conventional workup is the *trans*-amide rotamer (*trans*-**1**), while the thermodynamically unstable *cis*-isomer (*cis*-**1**) was also isolated as a minor product by careful separation procedures. Both isomers are interconverted and reach an equilibrium state of a ca. 7:1 (*trans:cis*) ratio in solution. Second, *trans*-**1** and *cis*-**1** exist as a mixture of two separable and stable axially chiral isomers (atropisomers) [(*aR*)-*trans*-**1**, (*aS*)-*trans*-**1** and (*aR*)-*cis*-**1**, (*aS*)-*cis*-**1**, respectively][14,15] arising from the restricted rotation around the $-C_{(6)}-C(=O)-$ bond.[13c] The atropisomers (*aR*)-*trans*-**1** and (*aS*)-*trans*-**1**, which were separated by preparative high-performance liquid chromatography (HPLC) using a chiral column as oily substances, have significant stability in solution; e.g., they were not interconverted in dimethyl sulfoxide (DMSO) at 37 °C for 16 h and underwent racemization only after storage at 50 °C for 6 days. The potency of the *trans*-**1** is ca. 7−20-fold higher than that of *cis*-**1**, and the atropisomer (*aR*)-*trans*-**1** has ca. 6−13-fold higher potency than (*aS*)-*trans*-**1** (Table 1). These results indicate that the conformation of (*aR*)-*trans*-**1** is preferentially recognized by the receptor.

* Corresponding author. Phone: +81-6-6300-6541. Fax: +81-6-6300-6306. E-mail: Natsugari_Hideaki@takeda.co.jp.
† Pharmaceutical Research Division.
‡ Discovery Research Division.
§ Technology Development Department.

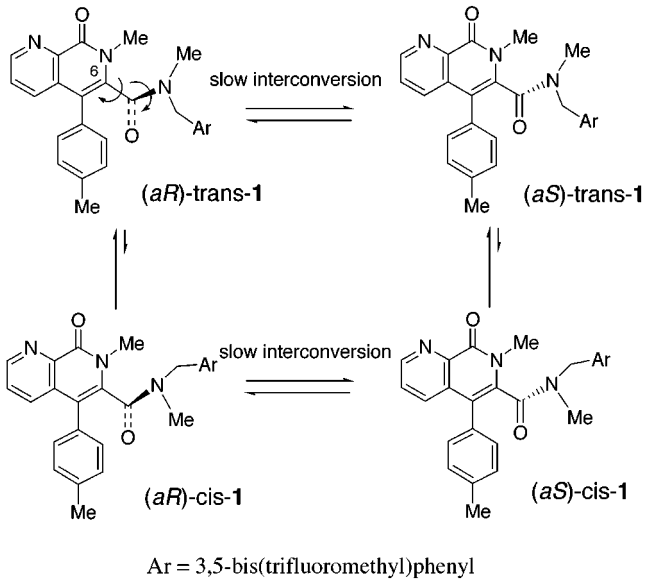
10.1021/jm990220r CCC: $18.00
Published on Web 09/04/1999



trans-**1** (*aR*)-trans-**1** R = H (*aR*,*S*)-trans-**1Me** R = Me (*aR*,9*R*)-**8b**

Figure 1. Potent NK_1 antagonists: *trans*-**1** and active atropisomers (*aR*)-*trans*-**1**, (*aR*,*S*)-*trans*-**1Me**, and (*aR*,9*R*)-**8b** (for the schematic drawings, see the footnote of Scheme 1).

Scheme 1[a]



[a] The four stereoisomers of **1**: *trans* and *cis* are used to indicate the relative configuration between the *N*-methyl group and the carbonyl oxygen in the amide moiety, and (*aR*) and (*aS*) denote the axial chirality originating from the restricted rotation around the $C_{(6)}$–C(O) bond. In the schematic drawings, the axial chirality is shown by indicating the steric position of the amide oxygen and nitrogen with respect to the plane of the 1,7-naphthyridine ring.

From the SAR data obtained for its methyl analogue [(*aR*)-*trans*-**1Me**; Figure 1] and its stereoisomers, the absolute axial stereochemistry reqired for the receptor binding was deduced to be (*aR*) with the amide oxygen below (and the nitrogen above) the plane of the 1,7-naphthyridine ring.[13c]

From a practical point of view, however, separation of the atropisomers (*aR*)-*trans*-**1** and (*aS*)-*trans*-**1** is difficult, and further study using *trans*-**1** as a racemate would meet with difficulty especially at the stage of pharmaceutical development. Thus, in the search for new compounds with an improved stereochemical profile, we designed cyclic analogues of **1** having 6–9-membered rings (the general formula **6**–**9**; Scheme 2). We also expected cyclization to give these derivatives the constrained structure desirable for receptor binding and hence high potency.

In this paper, we describe the discovery of a new, orally effective NK_1 antagonist, (*aR*,9*R*)-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]-naphthyridine-6,13-dione [(*aR*,9*R*)-**8b**] (Figure 1), which was atropodiastereoselectively synthesized by cyclization of a chiral intermediate, **10g** (Schemes 2, 3).[13d] Studies on the effects of the NK_1 antagonists on bladder functions in guinea pigs, which implied the clinical potential of the antagonists in the treatment of pollakiuria and urinary incontinence, are also described.

Synthetic Chemistry

The synthesis of the tricyclic analogues of **1** (**6**–**9**) is outlined in Scheme 2. First, 5-(4-methylphenyl)-8-oxo-8*H*-pyrano[3,4-*b*]pyridine-6-carboxylic acid (**3**) was prepared from 3-(4-methylbenzoyl)-2-pyridinecarboxylic acid (**2**) according to a procedure similar to that reported[13a] for the synthesis of isocoumarin-3-carboxylic acids from 2-benzoylbenzoic acids: i.e., **2** was condensed with diethyl hydroxymalonate via the acid chloride to obtain diethyl 5,6-dihydro-5-hydroxy-5-(4-methylphenyl)-8-oxo-8*H*-pyrano[3,4-*b*]pyridine-6,6-dicarboxylate, which was heated under reflux in concentrated HCl–AcOH to give **3**. The acid **3** was then amidated with 3,5-bis(trifluoromethyl)benzylamine to provide the amide **4**. Treatment of **4** with the appropriate hydroxy amines **5a**–**f** (P = H) followed by dehydration with 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) gave the *N*-[3,5-bis(trifluoromethyl)benzyl]-7-(hydroxyalkyl)-7,8-dihydro-5-(4-methylphenyl)-8-oxo-1,7-naphthyridine-6-carboxamides **10a**–**f**. Similarly, the hydroxy compounds **10g**–**h** were prepared by treatment of **4** with the protected hydroxyamines **5g**–**h** [P = tetrahydropyranyl (THP)] followed by deprotection with *p*-toluenesulfonic acid and dehydration with DBU. Cyclization of **10** was accomplished by mesylation of the hydroxy group followed by treatment with sodium hydride in tetrahydrofuran to give **6**–**9** in good yields. The stereochemical features of the tricyclic analogues **6**–**9** are described in parts (a) and (b), Results and Discussion.

Biology

The compounds prepared were evaluated in vitro for inhibition of [^{125}I]Bolton-Hunter (BH)-SP binding in human IM-9 cells[16] and in vivo for inhibition of capsaicin-induced plasma extravasation in the trachea of guinea pigs[17] upon iv and po administration. The NK_1 antagonists were also administered iv to urethane-anesthetized guinea pigs to examine the effects on bladder function using distension-induced rhythmic bladder contractions and the bladder volume threshold as the parameters.

Results and Discussion

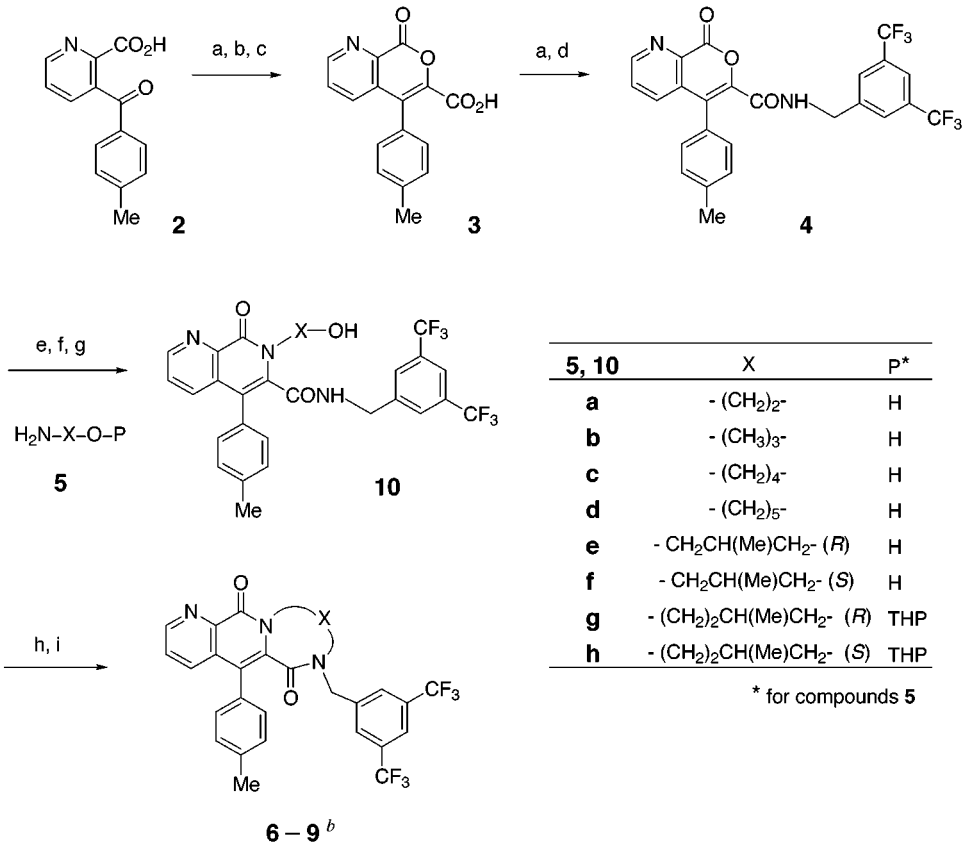
(a) Cyclic Analogues of 1 (6, 7a, 8a, and 9). The cyclic analogues of **1** with 6–9-membered rings (**6**, **7a**,

Table 1. Biological Properties of *N*-[3,5-Bis(trifluoromethyl)benzyl]-*N*-methyl-1,7-naphthyridine-6-carboxamide Isomers (**1**) and Their Cyclic Analogues (**6**−**9**)

	NK_1 antagonist activities				
		ID_{50} (μg/kg)[c]		effects on bladder functions (iv)	
compd no.[a]	IC_{50}[b] (nM)	iv	po	shutdown time[d] (min)	increase in bladder threshold[e] ED_{30} (mg/kg)
trans-**1**	0.34 ± 0.07	30 (18−54)	110 (76−178)	12.9 ± 3.9* (8)	0.10 (0.056−0.165)
(*aR*)-*trans*-**1**	0.24[f]	7.7 (4.1−12.5)	−[g]	16.2 ± 3.1** (10)	−
(*aS*)-*trans*-**1**	1.4[f]	100 (69−149)	−	5.8 ± 1.2 (10)	−
cis-**1**	4.2[f]	220 (130−2160)	−	11.7 ± 2.6**[h] (9)	17.3 ± 11.3%[i]
6	2.3 ± 0.3	45.4%[j]	−	−	−
7a	0.85 ± 0.08	7.2 (1.5−15.0)	73 (61−92)	10.1 ± 1.2 (10)	0.37 (0.091−0.573)
8a	0.68 ± 0.05	4.2 (1.0−8.4)	19 (9.9−29.3)	15.7 ± 3.4* (10)	0.066 (0.020−0.123)
(*aR*)-**8a**	0.38[k]	2.6 (2.1−3.1)	−	19.2 ± 2.9** (8)	−
(*aS*)-**8a**	7.1[f]	17 (10−31)	−	7.4 ± 1.5 (5)	−
9	1.8 ± 0.1	11 (5.2−17.3)	40 (7.2−74.3)	17.1 ± 2.9** (10)	0.086 (0.037−0.149)
(9*S*)-**7b**	0.28 ± 0.02	3.2 (1.9−5.5)	33 (18−71)	17.9 ± 3.3** (10)	0.16 (0.11−0.22)
(9*R*)-**7b**	9.5[k]	41 (20−145)	−	5.9 ± 1.2 (8)	19.9 ± 10.4%[l]
(*aR*,9*R*)-**8b**	0.45 ± 0.10	4.3 (2.4−11.4)	33 (13−98)	21.8 ± 3.0** (10)	0.051 (0.021−0.083)
(*aS*,9*R*)-**8b**	20 ± 16	26 (6.2−69.0)	−	−	−
(*aS*,9*S*)-**8b**	340 ± 64	>300	−	6.7 ± 3.0 (10)	3.8 ± 9.4%[i]
(*aR*,9*S*)-**8b**	8.6 ± 2.9	−	−	−	−

[a] For the structures, see Schemes 1, 3, and 4 and Figure 2. [b] Inhibition of [^{125}I]BH-SP binding in human IM-9 cells (lymphoblast cells). Values are the mean ± SD determined by at least three independent experiments (n = 3−6) run in duplicate unless otherwise noted. [c] Capsaicin-induced trachea extravasation in guinea pigs. To determine ID_{50} values, 5−8 animals were used at each dose. 95% Confidence limits are given in parentheses. [d] Mean ± SE value of shutdown time of distention-induced rhythmic bladder contractions in urethane-anesthetized guinea pigs at 0.3 mg/kg (iv) unless otherwise noted. Numbers of animals are given in parentheses. Dunnett's test: $^{**}p < 0.01$, $^{*}p < 0.05$. [e] Increasing effect on volume threshold in urethane-anesthetized guinea pigs. ED_{30} values are given unless otherwise noted. To determine the ED_{30} values, 6−12 animals were used at each dose. 95% confidence limits are given in parentheses. [f] Mean value of two independent experiments run in duplicate. [g] −, not tested. [h] Mean ± SE value at 1.0 mg/kg, iv. [i] Mean ± SE value of bladder volume increase (%) at 0.3 mg/kg (iv) [n = 6 for *cis*-**1** and n = 8 for (*aS*,9*S*)-**8b**]. [j] Inhibition (%) at 0.1 mg/kg (n = 6). [k] IC_{50} value determined by a single experiment run in duplicate. [l] Mean ± SE value of bladder volume increase (%) at 1.0 mg/kg (iv) (n = 10).

Scheme 2[a]



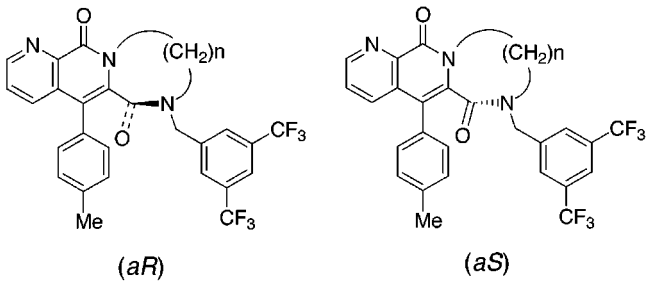
5, 10	X	P*
a	-$(CH_2)_2$-	H
b	-$(CH_3)_3$-	H
c	-$(CH_2)_4$-	H
d	-$(CH_2)_5$-	H
e	-$CH_2CH(Me)CH_2$- (*R*)	H
f	-$CH_2CH(Me)CH_2$- (*S*)	H
g	-$(CH_2)_2CH(Me)CH_2$- (*R*)	THP
h	-$(CH_2)_2CH(Me)CH_2$- (*S*)	THP

* for compounds **5**

[a] Reagents: (a) $SOCl_2$/THF; (b) diethyl hydroxymalonate, NaH/THF; (c) concd HCl−AcOH; (d) 3,5-bis(trifluoromethyl)benzylamine, Et_3N/THF; (e) **5**/THF−MeOH; (f) DBU/toluene−CH_3CN; (g) *p*-TosOH/MeOH (in the case of preparing **10g**−**h** via the THP ethers: P = THP); (h) MsCl, Et_3N/THF; (i) NaH/THF. [b]For specification of the residue X in **6**−**9** including stereochemistry originating from atropisomerism, see Figure 2 and Schemes 1, 3, and 4.

8a, and **9**) (Figure 2) have an apparent advantage in that they are expected to exist as single amide rotamers; the cyclic structure would serve to constrain the naphthyridine and *N*-benzyl groups bound to the amide moiety so as to be disposed in the *trans* conformation. In fact, the presence of the amide isomers was not observed in their NMR spectra.

As for the atropisomers in these compounds, we



6 n = 2 ; **7a** n = 3 ; **8a** n = 4 ; **9** n = 5

Figure 2. Atropisomers [(*aR*) and (*aS*)] of cyclic analogues of **1** having 6−9-membered rings (**6**, **7a**, **8a**, and **9**).

initially supposed that the flipping of these new rings would be too rapid to enable the separation of stable isomers at room temperature.[18] The pattern of the *N*-benzylic methylene protons in the NMR spectra was first examined for detection of the atropisomers. The methylene protons in compound **6** with a 6-membered ring appeared as a singlet, indicating that the conformers are rapidly interconverted even on the NMR time scale, whereas the other compounds with 7−9-membered rings (**7a**, **8a**, and **9**) showed distinct AB patterns for the methylene protons, indicating slow interconversion of the conformers on the NMR time scale. Compounds **6**, **7a**, **8a**, and **9** were next analyzed by chiral HPLC (CHIRALPAK AD).[19] Compound **6** showed, as expected, a single peak at room temperature, indicating again rapid interconversion of the conformers. Analysis of **7a** at room temperature also showed a single peak at room temperature. However, analysis of **7a** at or below −20 °C did show two equal peaks of the atropisomers [(*aR*)-**7a** and (*aS*)-**7a**], as had been anticipated from the NMR study. Although they were separated by preparative HPLC at that temperature, each separated fraction, obtained after rapid and careful workup at ca. 0 °C, again showed two equal peaks by HPLC analysis at −20 °C, indicating rapid interconversion of the conformers at or above ca. 0 °C. The HPLC analysis of the compounds having 8- and 9-membered rings (**8a** and **9**) showed two peaks at room temperature, indicating the presence of separable atropisomers. In fact, **8a** was separated by preparative HPLC using a chiral column to give the atropisomers (*aR*)-**8a** and (*aS*)-**8a**, which have opposite $[\alpha]_D$ values (+45.6° and −41.3°, respectively) and show considerable stability in solution; e.g., they are gradually interconverted in DMSO to a ca. 70% enantiomeric excess (i.e., ca. 15% conversion) at 37 °C over 40 h and undergo racemization after storage at 50 °C for ca. 2 days.

The NK_1 antagonistic activities of the tricyclic compounds [**6**, **7a**, **8a**, **9**, (*aR*)-**8a**, and (*aS*)-**8a**] are shown in Table 1. The in vitro potencies of **6**, **7a**, **8a**, and **9** correspond well to the in vivo (iv) potencies, and they vary with the ring size in the order of **8a** (ring size 8) ≥ **7a** (7) > **9** (9) > **6** (6), which presumably reflects the order of conformations desirable for receptor recognition. The low potency of **6** can be explained by the conformational studies on *trans*-**1**, **6**, **7a**, **8a**, and **9**, in which the 3,5-bis(trifluoromethyl)phenyl group in **6** was found not to overlap with that in *trans*-**1** (Figure 3). It is noteworthy that, although **7a**, **8a**, and **9** showed lower in vitro potencies than *trans*-**1**, their in vivo (iv) potencies are higher than that of *trans*-**1**, and also the high in vivo potency observed upon oral administration of **8a** is remarkable. The atropisomers of **8a** [(*aR*)-**8a** and (*aS*)-**8a**] exhibited, as expected, different affinities for the NK_1 receptor both in vitro and in vivo (iv). The potency of (*aR*)-**8a** was ca. 6−19-fold higher than that of (*aS*)-**8a**. The absolute stereochemistry of the active atropisomer of **8a** is presumed to have the same conformation (*aR*) as (*aR*)-*trans*-**1** with the amide oxygen below (and the nitrogen above) the plane of the naphthyridine ring [see part (b), Results and Discussion].

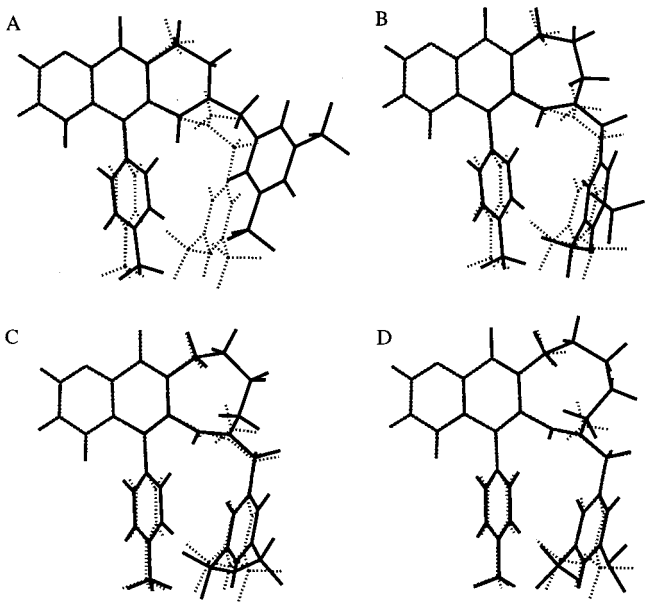


Figure 3. Overlapping between the most stable conformer of *trans*-**1** (dotted line) and those of the cyclic analogues (bold line) [A, **6**; B, **7a**; C, **8a**; and D, **9**] as determined by the conformational analysis using Discover CVFF force field.

(b) Cyclic Analogues of 1 with a Chiral Methyl Group [(9*S*)-7b, (9*R*)-7b, (9*R*)-8b, and (9*S*)-8b]. The results described in Results and Discussion (a) indicate that, although having favorable in vivo activity, **8a** is a racemate, making development as a clinical candidate difficult. Thus, the stereoselective synthesis of the active atropisomer of the 8-membered ring compound became our next goal. Since a practical route for the atropodiastereoselective synthesis of (*aR*)-**8a** itself could not be easily found, we selected the $C_{(9)}$-methyl analogues of **8a** [i.e., (9*R*)- and (9*S*)-**8b**] as the target compounds, expecting asymmetric induction from the $C_{(9)}$-chiral center to obtain the desirable axial chirality (*aR*). We thus achieved the stereoselective synthesis of the atropisomer [(*aR*,9*R*)-**8b**] by cyclization of an intermediate with a chiral methyl group, **10g** (Scheme 3). The ratio of the atropisomer (*aR*,9*R*)-**8b** to its isomer (*aS*,9*R*)-**8b** was ca. 98:2, and a single recrystallization step gave (*aR*,9*R*)-**8b** with >99% diastereomeric excess (de). The minor isomer (*aS*,9*R*)-**8b**, with 98.6% de, was isolated as a powdery substance by repeated preparative HPLC followed by a rapid and careful workup (e.g., evaporation of solvent at low temperature); at present, the purity of (*aS*,9*R*)-**8b** does not exceed 99% de due to the instability of (*aS*,9*R*)-**8b** in solution (see discussion below).

The stereochemistry of (*aR*,9*R*)-**8b** was determined by single-crystal X-ray structural analysis (Figure 4), which showed that the $-N_{(7)}-C_{(8)}-C_{(9)}-C_{(10)}-$ moiety

Scheme 3

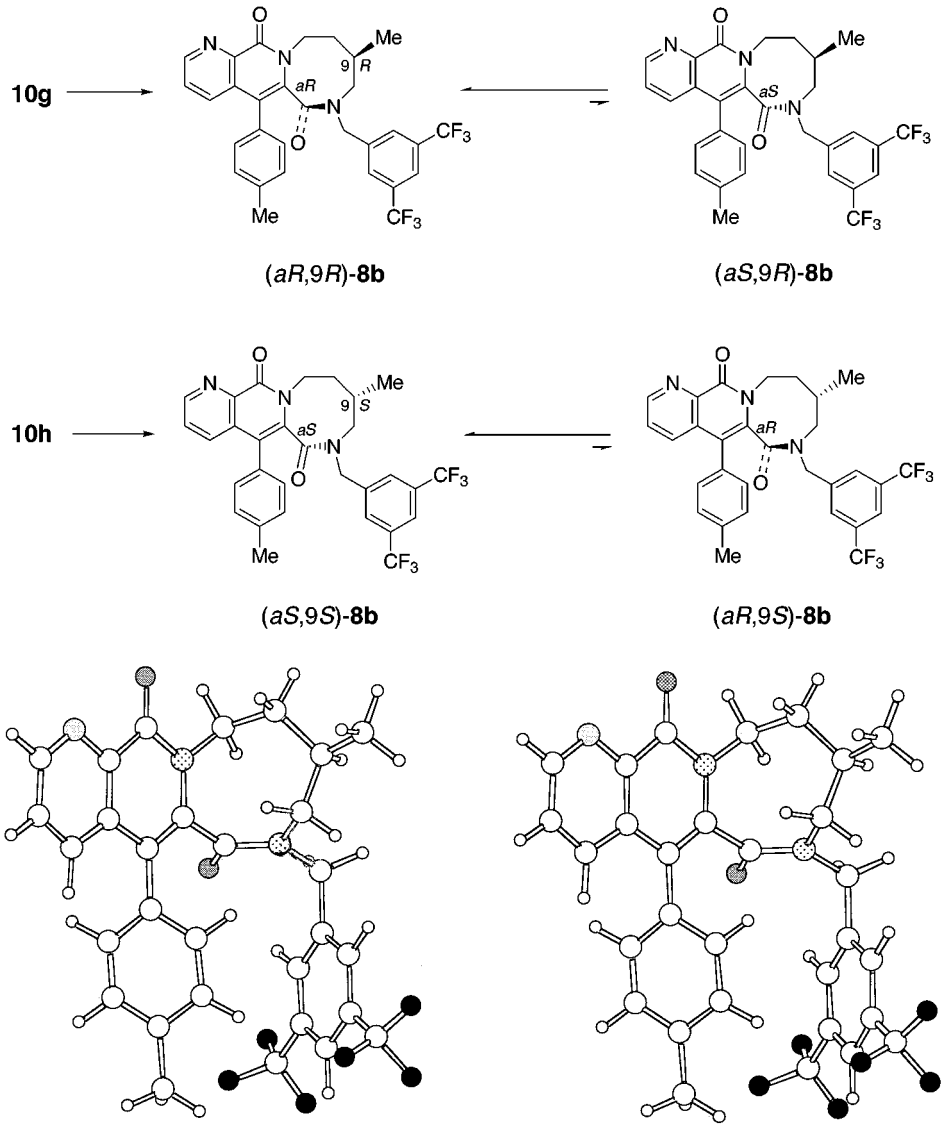


Figure 4. Stereoscopic molecular view of (*aR*,9*R*)-**8b** as determined by X-ray crystallographic analysis.

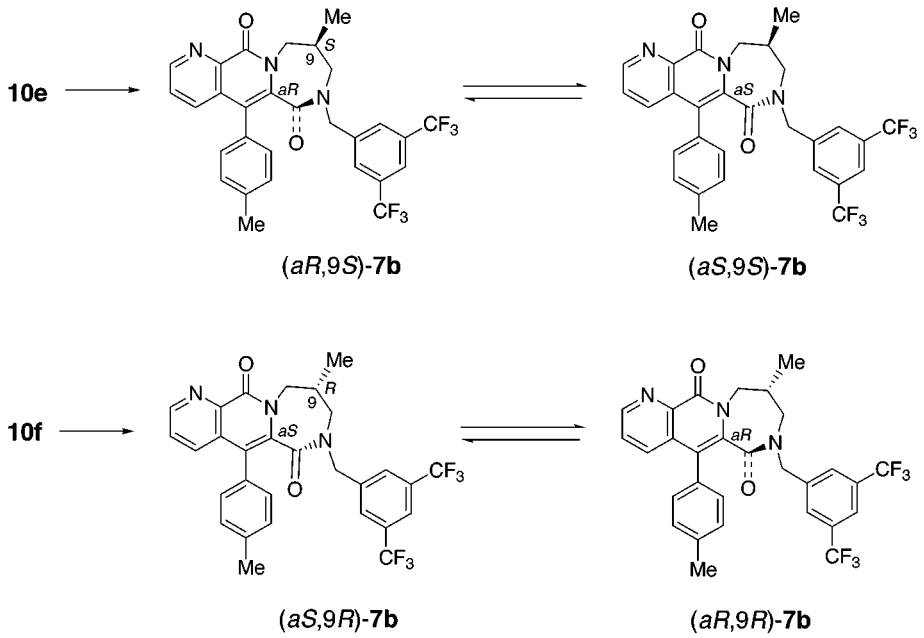
in the 8-membered ring is disposed above the plane of the adjacent 1,7-naphthyridine ring and the amide oxygen ($C_{(6)}$=O) is below the ring [i.e., (*aR*) stereochemistry]. The analysis also revealed the *trans*-conformation of the naphthyridine and *N*-benzyl groups at the amide bond and a stacking conformation between the $C_{(5)}$-phenyl and the $N_{(7)}$-benzylic phenyl groups as observed in the X-ray analysis of *trans*-**1**.[13a] The relative spatial orientation of the $C_{(9)}$-methyl group and the *N*-[3,5-bis-(trifluoromethyl)benzyl] group in (*aR*,9*R*)-**8b** is presumed to be important for high atropodiastereoselectivity. These two groups in (*aR*,9*R*)-**8b** are disposed in opposite directions, both in the crystalline form and in solution, as shown by the X-ray analysis and by NMR studies (i.e., the NOE between the $C_{(9)}$-proton and a benzylic methylene proton), respectively. The same two groups in (*aS*,9*R*)-**8b** are shown to be disposed in the same orientation in solution as observed by the NOE between the $C_{(9)}$-methyl protons and a benzylic methylene proton. The repulsion of these groups in (*aS*,9*R*)-**8b** may cause steric instability leading to the preferential formation of the thermodynamically stable atropisomer (*aR*,9*R*)-**8b** in the cyclization of **10g**.

Both atropisomers, (*aR*,9*R*)-**8b** and (*aS*,9*R*)-**8b**, were found to be interconverted in solution to reach the same equilibrium state [(*aR*,9*R*)-**8b**/(*aS*,9*R*)-**8b** = ca. 98:2] with the interconversion rate being temperature-dependent; e.g., in ethanol, both (*aR*,9*R*)-**8b** and (*aS*,9*R*)-**8b** reached equilibrium at 37 °C in ca. 60 h and at 50 °C in ca. 15 h.[20]

The enantiomer of (*aR*,9*R*)-**8b** [(*aS*,9*S*)-**8b**], with >99% de, was similarly obtained by the cyclization of the corresponding intermediate **10h** followed by a single recrystallization step. The minor diastereomer [(*aR*,9*S*)-**8b**] with 97.5% de was also isolated as a powdery substance by preparative HPLC (Scheme 3).

The 7-membered ring compounds with a chiral methyl group [(9*S*)-**7b** and its enantiomer (9*R*)-**7b**] were prepared by cyclization of the intermediates containing a chiral methyl group (**10e** and its enantiomer **10f**) (Scheme 4) to look for improved biological activity accompanied by improved stereochemical and pharmacokinetic profiles. The stereochemical nature of (9*S*)-**7b** and (9*R*)-**7b** is also of interest. Their NMR spectra showed signals due to two diastereomers in a ratio of ca. 3:2, indicating slow interconversion of the atropiso-

Scheme 4



mers [i.e., the diastereomers (*aR*) and (*aS*) of **7b**] on the NMR time scale. The chiral HPLC analysis of (9*S*)-**7b** gave results similar to those obtained with the unsubstituted 7-membered ring compound **7a**, i.e., a single peak at room temperature and two peaks at or below −20 °C (ca. 7:3 ratio at −40 °C), indicating rapid interconversion of the atropisomers [(*aR*,9*S*)-**7b** and (*aS*,9*S*)-**7b**] at room temperature as observed with **7a** [(*aR*)-**7a** and (*aS*)-**7a**].

The SAR in the series of 8-membered ring compounds with a chiral $C_{(9)}$-methyl group [(*aR*,9*R*)-**8b**, (*aS*,9*R*)-**8b**, (*aR*,9*S*)-**8b**, and (*aS*,9*S*)-**8b**] (Table 1) is especially useful for better understanding the stereochemistry required for receptor recognition. Compound (*aR*,9*R*)-**8b** exhibited excellent antagonistic activities both in vitro and in vivo. Significantly, the in vitro activity of (*aR*,9*R*)-**8b** is ca. 750-fold higher than that of the enantiomer (*aS*,9*S*)-**8b**, ca. 40-fold higher than that of the atropisomer (*aS*,9*R*)-**8b**, and ca. 20-fold higher than that of the diastereomer (*aR*,9*S*)-**8b**. These striking differences in potency between (*aR*,9*R*)-**8b** and its isomers, along with the SAR described above and in our preceding papers,[13] indicate that the stereochemistry around the $-C_{(6)}(=O)-N_{(7)}-CH_2-Ar$ moiety is the most important factor for receptor recognition; the amide moiety functions as a hydrogen bond acceptor center in the interaction with the receptor as reported for the tryptophan ester NK_1 antagonist L-732,138 [3,5-bis(trifluoromethyl)benzyl ester of *N*-acetyl-L-tryptophan].[21] The conformational studies on (*aR*,9*R*)-**8b** and L-732,138 indicated that their most stable conformers overlap well as shown in Figure 5, suggesting that the key determinants for the receptor recognition are similar each other. Furthermore, in compound (*aR*,9*R*)-**8b**, the β-methyl group serves to constrain the stereochemistry so as to dispose the amide oxygen below the plane of the 1,7-naphthyridine ring and to make the benzylic phenyl group adopt a stacking conformation with the $C_{(5)}$-phenyl group. We suppose that the weak activity of the diastereomer (*aR*,9*S*)-**8b**, which has (*aR*) configuration desirable for receptor recognition, may be ascribed to the *cis*-relationship between the benzyl moiety and the $C_{(9)}$-α-methyl group [cf. *trans* in (*aR*,9*R*)-**8b**] to produce a deleterious effect on receptor binding.

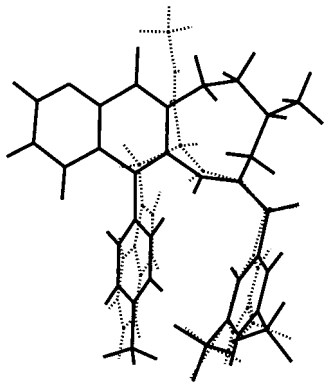


Figure 5. Overlapping between the most stable conformers of (*aR*,9*R*)-**8b** (bold line) and L-732,138 (dotted line) as determined by the conformational analysis using Discover CVFF force field.

Importantly, the 7-membered ring compound with a β-methyl group (9*S*)-**7b** showed excellent in vitro and in vivo (iv) activities. The potencies are similar to or slightly higher than those predicted from the activities of the unsubstituted compound **7a**. This highly improved activity of (9*S*)-**7b** may be explained by the conformational change at the benzyl moiety, which is affected by the $C_{(9)}$-methyl substituent so as to be better oriented for receptor recognition. The in vitro activity of (9*S*)-**7b** is ca. 30-fold higher than that of the α-methyl derivative (9*R*)-**7b**. The smaller difference in the potency between the 7-membered ring enantiomers [(9*S*)-**7b** and (9*R*)-**7b**], compared with the potency difference between the 8-membered enantiomers [i.e., ca. 500-fold between (*aR*,9*R*)-**8b** and (*aS*,9*R*)-**8b**], may reside in the greater conformational flexibility of the 7-membered ring.

Both the active atropisomer with an 8-membered ring (*aR*,9*R*)-**8b** and the active enantiomer with a 7-membered ring (9*S*)-**7b** have potent inhibitory effects upon oral administration, with ID_{50} values of 33 μg/kg.

Compounds (*aR*,9*R*)-**8b** and (9*S*)-**7b** exhibited more than ca. 500−1000-fold selectivity for the human NK_1 receptor (IM-9 cells) over the rat NK_1 receptor (rat forebrain: IC_{50} value 85 and 310 nM, respectively).[22] The selectivity of (*aR*,9*R*)-**8b** and (9*S*)-**7b** for the tachykinin NK_1, NK_2, and NK_3 receptors was assessed by functional assays using guinea pig ileum contraction induced by SP and NKA (the pA_2 values were determined to be 9.5 and 9.4 for NK_1 and 6.0 and 4.9 for NK_2, respectively) and [^{125}I]Me-Phe^7-NKB binding in guinea pig cerebral cortex membranes[23] (the IC_{50} values for NK_3 were determined to be $>1\ \mu$M for both compounds), indicating that (*aR*,9*R*)-**8b** and (9*S*)-**7b** are selective for NK_1 over NK_2 and NK_3.[24,25] In the SP-induced guinea pig ileum contraction assay of (*aR*,9*R*)-**8b** and (9*S*)-**7b**, increasing concentrations of these compounds produced parallel shifts of the log concentration−response curves for SP to the right, and the magnitude of the maximum responses to SP remained unchanged, indicating that the compounds behave as competitive antagonists.

(c) Effects of the NK_1 Antagonists on Bladder Functions in Guinea Pigs. So far, a number of potential clinical uses have been suggested for NK_1 antagonists; these are the treatment of pain, inflammation, rheumatoid arthritis, asthma, emesis, migraine, and depression. In our examination of the clinical potential of our new NK_1 antagonists, we have extensively studied the pharmacological profiles of these compounds. Several lines of evidence indicate that tachykinins play an important role in the activation of micturition-related reflexes in rats,[26] which prompted us to investigate the effects of our NK_1 antagonists on micturition for their possible use in the treatment of pollakiuria and urinary incontinence. Thus, we examined the effects of the NK_1 antagonists on bladder functions in urethane-anesthetized guinea pigs upon iv administration by measuring the shutdown time of the distension-induced rhythmic bladder contractions and the bladder volume threshold (Table 1).

(c-1) Effect on Distension-Induced Rhythmic Bladder Contractions. Distension of the urinary bladder of urethane-anesthetized guinea pigs by injection of saline (3−4.5 mL) induced high amplitude (>15 mmHg) rhythmic contractions with intervals of ca. 4−9 min. The contractions were completely eliminated by dropping tetrodotoxin onto the surface of the bladder dome, and the contractile pressure was reduced by intravenous injection of atropine. These results indicate that the distension-induced contractions are of neurogenic, not myogenic, origin. The effects of the compounds on the bladder contractions were evaluated using the shutdown time (i.e., the time interval between the contractions) and the contractile intravesical pressure. In this assay, iv injection of *trans*-**1** increased in a dose-dependent manner the shutdown time to 12.9 and 26.3 min at doses of 0.3 and 1.0 mg/kg, respectively [control value (vehicle: DMSO), 4.1 min], without affecting the contractile pressure. Table 1 shows the shutdown time when compounds were administered at 0.3 mg/kg, iv (in the case of *cis*-**1**, at 1.0 mg/kg, iv). Significant nonparametric correlations[27] between the shutdown time and the NK_1 antagonistic activities [IC_{50} (in vitro correlation coefficient (r) = − 0.650, $P < 0.05$) and ID_{50} (in vivo r = − 0.825, $P < 0.001$)] were observed. Especially noteworthy are the differences in the activity between pairs of optically active compounds [i.e., (*aR*)-*trans*-**1** vs (*aS*)-*trans*-**1**, (*aR*)-**8b** vs (*aS*)-**8a**, (9*S*)-**7b** vs (9*R*)-**7b**, and (*aR*,9*R*)-**8b** vs (*aS*,9*S*)-**8b**] and between the amide isomers (*trans*-**1** vs *cis*-**1**); in all cases, the compound with higher affinity for the NK_1 receptor caused a greater increase in shutdown time. None of the compounds had an effect on the contractile intravesical pressure, suggesting that the site of action of these NK_1 antagonists is in the central nervous system.

(c-2) Effect on Bladder Volume Threshold. To ascertain whether the suppressive effects on distension-induced rhythmic bladder contractions associated with the NK_1 antagonistic effects may be related to an increase in bladder storage function, the effect on the bladder volume threshold (i.e., volume to which the bladder can be filled before voiding) in urethane-anesthetized guinea pigs was evaluated for typical compounds. In this assay, *trans*-**1** showed potent activity upon iv injection with an ED_{30} value (the dose of the antagonist that increases the bladder volume threshold by 30%) of 0.10 mg/kg. Table 1 shows the ED_{30} values or percent increase in volume threshold compared with the pretreatment value. The activity was found to correlate relatively well with the effects on the shutdown time (r = − 0.805, $P < 0.05$); especially noteworthy are differences in the activities between pairs of enantiomers [i.e., (9*S*)-**7b** vs (9*R*)-**7b** and (*aR*,9*R*)-**8b** vs (*aS*,9*S*)-**8b**]. In the general, compounds with NK_1 antagonistic activity [in vitro and in vivo (iv)] equal to that of *trans*-**1** exhibited activity similar to [**9** and (9*S*)-**7b**] or more potent than [**8a** and (*aR*,9*R*)-**8b**] that of *trans*-**1**, whereas compounds *cis*-**1**, (9*R*)-**7b**, and (*aS*,9*S*)-**8b** showed weak activities, corresponding well to their low NK_1 antagonistic activities. The high potency of (*aR*,9*R*)-**8b** (ED_{30} = 0.051 mg/kg, iv) is remarkable and presumably reflects its good pharmacokinetic profile. The results reported in parts (c-1) and (c-2), Results and Discussion, suggest that the NK_1 receptor is involved in the micturition mechanisms in guinea pigs, where NK_1 antagonists act to increase the interval time of bladder contractions and the bladder volume threshold.

Conclusions

Studies on the atropisomers of the 6-(*N*-benzyl-*N*-methylcarboxamide) derivative of 1,7-naphthyridine (**1**), a potent and orally active NK_1 antagonist, led us to prepare a series of cyclic analogues of **1** (**6**−**9**). We have investigated the chemical properties and antagonistic activities of these derivatives and succeeded in preparing atropisomerically pure NK_1 antagonists. The following points should be emphasized: (1) the 8-membered ring compound with a β-methyl group [(*aR*,9*R*)-**8b**] and its enantiomer [(*aS*,9*S*)-**8b**] are atropodiastereoselectively synthesized by cyclization of the chiral intermediates **10g** and **10h**, respectively; (2) the 7-membered ring compound with a β-methyl group [(9*S*)-**7b**] and its enantiomer [(9*R*)-**7b**] exist as an equilibrium mixture of atropisomers in solution at room temperature; (3) (9*S*)-**7b** and (*aR*,9*R*)-**8b** have excellent antagonistic activities both in vitro and in vivo (iv and po), and (*aR*,9*R*)-**8b** has ca. 750-fold higher in vitro activity than its enantiomer (*aS*,9*S*)-**8b**; (4) the X-ray structural

analysis of (*aR*,9*R*)-**8b** exhibits the active conformation required for NK_1 receptor recognition, and this conformationally restricted molecule is useful as an aid in better understanding the interactions between the antagonists and the NK_1 receptor; and (5) the NK_1 antagonists increased the shutdown time of distention-induced bladder contractions and the bladder volume threshold in guinea pigs upon iv injection, and the effects on the shutdown time were found to correlate well with the NK_1 antagonistic activities, implying the clinical potential of NK_1 antagonists in the treatment of pollakiuria and urinary incontinence. Compound (*aR*,9*R*)-**8b**, designated as TAK-637, has been identified as a potential clinical candidate. Further pharmacological studies on (*aR*,9*R*)-**8b** will be reported in due course.

Experimental Section

Chemistry. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H NMR spectra were taken on a Varian Gemini 200 (200 MHz) spectrometer in $CDCl_3$ unless otherwise noted. Chemical shifts are given in ppm with tetramethylsilane as the internal standard, and coupling constants (*J*) are given in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, dd = double doublet, bs = broad singlet, bt = broad triplet. IR spectra were obtained on a Hitachi IR-215 spectrometer. Mass spectra were obtained on a JEOL JMS-AX505W spectrometer. Optical rotations were determined with a JASCO DIP-370 digital polarimeter. Elemental analyses were carried out by Takeda Analytical Laboratories Ltd. and are within ±0.4% of the theoretical values for the elements indicated unless otherwise noted. Extracted solutions were dried over anhydrous $MgSO_4$ or anhydrous Na_2SO_4. The yields reported are not optimized.

3-(4-Methylbenzoyl)-2-pyridinecarboxylic Acid (2). Compound **2** was prepared from 2,3-pyridinedicarboxylic acid anhydride and 4-bromotoluene by the method previously reported.[13a]

5-(4-Methylphenyl)-8-oxo-8*H*-pyrano[3,4-*b*]pyridine-6-carboxylic Acid (3). A mixture of **2** (3.0 g, 12.4 mmol), DMF (1 drop), thionyl chloride (4.5 mL, 61.7 mmol), and THF (30 mL) was heated under reflux for 2 h. The solvent was evaporated and the crystalline residue was dissolved in THF (50 mL). To the solution were added diethyl hydroxymalonate (4.1 g, 23.3 mmol) and then sodium hydride (60% dispersion in oil) (646 mg, 16.2 mmol) portionwise with stirring and cooling at −10 °C. After being stirred for 30 min at −10 °C, the reaction mixture was added to a solution of EtOAc (100 mL) and H_2O (100 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc. The organic layer and the extract were combined, washed with H_2O and brine, dried, and evaporated to give diethyl 5,6-dihydro-5-hydroxy-5-(4-methylphenyl)-8-oxo-8*H*-pyrano[3,4-*b*]pyridine-6,6-dicarboxylate as colorless crystals (4.0 g, 81%). Recrystallization from EtOAc−diisopropyl ether (IPE) gave colorless crystals: mp 148−149 °C; ^{1}H NMR 1.06 (3 H, t, *J* = 7.1), 1.21 (3 H, t, *J* = 7.1), 2.31 (3 H, s), 3.95−4.30 (4 H, m), 4.65 (1 H, s), 7.15 (2 H, d, *J* = 8.3), 7.55 (1 H, dd, *J* = 8.0, 4.8), 7.65 (2 H, d, *J* = 8.3), 8.47 (1 H, dd, *J* = 8.0, 1.4), 8.86 (1 H, dd, *J* = 4.8, 1.4). Anal. ($C_{21}H_{21}NO_7$) C, H, N.

A mixture of the crystals thus obtained (14.1 g, 35.3 mmol), AcOH (100 mL), and concentrated HCl (100 mL) was refluxed for 3 h. After evaporation of the solvent, the residue was diluted with H_2O. The colorless crystals precipitated were collected and washed with H_2O to give **3** (8.45 g, 85%). Recrystallization from THF−IPE gave colorless crystals: mp 274−277 °C (gradually turned to brown from ca. 240 °C); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) 2.43 (3 H, s), 6.10 (1 H, bs, COOH), 7.16 (2 H, d, *J* = 8.0), 7.29 (2 H, d, *J* = 8.0), 7.50−7.70 (2 H, m), 8.94 (1 H m). Anal. ($C_{16}H_{11}NO_4 \cdot 0.1H_2O$) C, H, N.

***N*-[3,5-Bis(trifluoromethyl)benzyl]-5-(4-methylphenyl)-8-oxo-8*H*-pyrano[3,4-*b*]pyridine-6-carboxamide (4).** A mixture of **3** (300 mg, 1.07 mmol), DMF (catalytic amount), thionyl chloride (1.0 mL, 13.7 mmol), dichloroethane (5 mL), and THF (5 mL) was heated under reflux for 1.5 h. The solvent was evaporated and the residue was dissolved in THF (15 mL). To the solution were added 3,5-bis(trifluoromethyl)benzylamine (278 mg, 1.14 mmol) and Et_3N (0.5 mL, 3.59 mmol), and the mixture was stirred at room temperature for 30 min. After dilution with EtOAc, the mixture was washed successively with H_2O, diluted HCl, aqueous $NaHCO_3$, and brine. The organic layer was dried and concentrated to give **4** as colorless crystals (383 mg, 71%). Recrystallization from EtOAc−ethyl ether gave colorless crystals: mp 182−183 °C; ^{1}H NMR 2.44 (3 H, s), 4.63 (2 H, d, *J* = 6.4), 7.17 (2 H, d, *J* = 8.1), 7.34 (2 H, d, *J* = 8.1), 7.50−7.65 (3 H, m), 7.73 (2 H, s), 7.80 (1 H, s), 8.96 (1 H, m). Anal. ($C_{25}H_{16}F_6N_2O_3$) C, H, N.

(*R*)-4-Amino-2-methyl-1-butanol THP Ether (5g). To a stirred suspension of $LiAlH_4$ (3.70 g, 97.5 mmol) in ethyl ether (200 mL) was added a solution of (*R*)-3-cyano-2-methyl-1-propanol THP ether[28b] (22.7 g, 124 mmol) in ethyl ether (100 mL) dropwise at 0 °C and the mixture stirred at room temperature for 1 h. To the mixture were added successively H_2O (3 mL), 15% aqueous NaOH (3 mL), and H_2O (10 mL) and the whole was stirred for 30 min. Insolubles were filtered off and washed with EtOAc. The filtrate and washings were combined and washed successively with aqueous K_2CO_3 and saturated aqueous NaCl. The organic layer was dried and evaporated to give **5g** as a colorless oil (21.7 g, 94%): ^{1}H NMR 0.94 (3 H × 1/2, d, *J* = 6.8), 0.95 (3 H × 1/2, d, *J* = 6.8), 1.2−1.9 (11 H, m), 3.13−3.90 (6 H, m), 4.57 (1 H, b).

(*S*)-4-Amino-2-methyl-1-butanol THP Ether (5h). Compound **5h** was prepared from (*S*)-3-cyano-2-methyl-1-propanol THP ether[28a] according to the same procedure described for the preparation of **5g**.

(*R*)-*N*-[3,5-Bis(trifluoromethyl)benzyl]-7,8-dihydro-7-(4-hydroxy-3-methylbutyl)-5-(4-methylphenyl)-8-oxo-1,7-naphthyridine-6-carboxamide (10g). To a solution of **4** (12.0 g, 23.7 mmol) in THF (75 mL)−MeOH (75 mL) was added **5g** (7.4 g, 39.5 mmol), and the mixture was stirred at room temperature for 16 h. After evaporation of the solvent, the residue was diluted with EtOAc. The mixture was washed successively with H_2O, 5% aqueous H_3PO_4, and brine, dried, and evaporated. To the residue were added acetonitrile (20 mL), toluene (125 mL), and DBU (5.0 mL, 33.4 mmol), and the mixture was refluxed for 1 h. After evaporation of the solvent, the residue was diluted with EtOAc and washed successively with H_2O, 5% aqueous H_3PO_4, and brine. The organic layer was dried and concentrated to give THP ether of **10g** as a pale yellow oil. To a solution of this oil in MeOH (50 mL) was added *p*-toluenesulfonic acid monohydrate (5.0 g, 26.3 mmol), and the mixture was stirred at room temperature for 30 min. After evaporation of the solvent, the residue was diluted with EtOAc and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried and concentrated to give **10g** as colorless crystals (7.74 g, 55%). Recrystallization from EtOAc−IPE gave colorless crystals: mp 123−125 °C (once melted and then solidified), 205−206 °C; ^{1}H NMR 0.79 (3 H, d, *J* = 6.6), 1.4−1.8 (3 H, m), 2.28 (3 H, s), 3.03 (1 H, t, *J* = 6.6, OH), 3.2−3.7 (4 H, m), 4.49 (2 H, d, *J* = 5.8), 7.0−7.3 (4 H, m), 7.30 (1 H, dd, *J* = 8.4, 4.4), 7.53 (1 H, dd, *J* = 8.4, 1.4), 7.68 (2 H, s), 7.78 (1 H, s), 8.48 (1 H, t, *J* = 6.0), 8.61 (1 H, dd, *J* = 4.4, 1.4); $[\alpha]_D^{20}$ +1.2° (*c* = 0.471, $CHCl_3$). Anal. ($C_{30}H_{27}F_6N_3O_3$) C, H, N.

***N*-[3,5-Bis(trifluoromethyl)benzyl]-7,8-dihydro-7-(2-hydroxyethyl)-5-(4-methylphenyl)-8-oxo-1,7-naphthyridine-6-carboxamide (10a).** Compound **4** (2.53 g, 5.0 mmol) was treated according to a procedure similar to that described for the preparation of **10g** using 2-aminoethanol (**5a**) in place of **5g** to afford **10a** as colorless crystals (1.92 g, 70%). Recrystallization from EtOAc gave colorless crystals: mp 123−125 °C; ^{1}H NMR 2.28 (3 H, s), 3.71 (2 H, m), 3.97 (2 H, m), 4.46 (2 H, d, *J* = 5.2), 7.00−7.20 (4 H, m), 7.37 (1 H, dd, *J* = 8.4, 4.2), 7.52 (1 H, dd, *J* = 8.4, 1.6), 7.66 (2 H, s), 7.76 (1 H, s), 8.51 (1 H, bs), 8.61 (1 H, dd, *J* = 4.2, 1.6). Anal. ($C_{27}H_{21}F_6N_3O_3$) C, H, N.

***N*-[3,5-Bis(trifluoromethyl)benzyl]-7,8-dihydro-7-(3-hydroxypropyl)-5-(4-methylphenyl)-8-oxo-1,7-naphthyridine-6-carboxamide (10b).** Compound **4** (8.29 g, 16.4 mmol) was treated according to a procedure similar to that described for the preparation of **10g** using 3-amino-1-propanol (**5b**) in place of **5g** to afford **10b** as colorless crystals (6.53 g, 71%). Recrystallization from THF-MeOH−ethyl ether gave colorless crystals: mp 127−129 °C; ^{1}H NMR 1.89 (2 H, m), 2.28 (3 H, s), 3.45 (2 H, m), 3.70 (2 H, m), 4.49 (2 H, d, J = 6.0), 7.07 (2 H, d, J = 8.0), 7.20 (2 H, d, J = 8.0), 7.41 (1 H, dd, J = 8.2, 4.4), 7.57 (1 H, dd, J = 8.2, 1.7), 7.67 (2 H, s), 7.78 (1 H, s), 8.30 (1 H, bt), 8.68 (1 H, dd, J = 4.4, 1.7). Anal. ($C_{28}H_{23}F_6N_3O_3$) C, H, N.

***N*-[3,5-Bis(trifluoromethyl)benzyl]-7,8-dihydro-7-(4-hydroxybutyl)-5-(4-methylphenyl)-8-oxo-1,7-naphthyridine-6-carboxamide (10c).** Compound **4** (200 mg, 0.39 mmol) was treated according to a procedure similar to that described for the preparation of **10g** using 4-amino-1-butanol (**5c**) in place of **5g** to afford **10c** as colorless crystals (144 mg, 63%). Recrystallization from EtOAc−IPE gave colorless crystals: mp 187−188 °C; ^{1}H NMR 1.3−1.5 (2 H, m), 1.6−1.9 (2 H, m), 2.29 (3 H, s), 2.82 (1 H, bs), 3.55 (2 H, t, J = 5.7), 3.69 (2 H, m), 4.48 (2 H, d, J = 5.8), 7.08 (2 H, d, J = 8.1), 7.21 (2 H, d, J = 8.1), 7.29 (1 H, dd, J = 8.4, 4.2), 7.52 (1 H, dd, J = 8.4, 1.4), 7.68 (2 H, s), 7.78 (1 H, s), 8.39 (1 H, bt), 8.61 (1 H, dd, J = 4.2, 1.4). Anal. ($C_{29}H_{25}F_6N_3O_3$) C, H, N.

***N*-[3,5-Bis(trifluoromethyl)benzyl]-7,8-dihydro-7-(5-hydroxypentyl)-5-(4-methylphenyl)-8-oxo-1,7-naphthyridine-6-carboxamide (10d).** Compound **4** (200 mg, 0.39 mmol) was treated according to a procedure similar to that described for the preparation of **10g** using 5-amino-1-pentanol (**5d**) in place of **5g** to afford **10d** as colorless crystals (190 mg, 81%). Recrystallization from EtOAc−ethyl ether gave colorless crystals: mp 136−137 °C; ^{1}H NMR 1.10−1.35 (2 H, m), 1.35−1.55 (2 H, m), 1.6−1.9 (2 H, m), 2.28 (3 H, s), 3.50−3.70 (4 H, m), 4.47 (2 H, d, J = 5.8), 7.06 (2 H, d, J = 8.0), 7.19 (2 H, d, J = 8.0), 7.35 (1 H, dd, J = 8.3, 4.4), 7.50 (1 H, d, J = 8.3, 1.4), 7.69 (2 H, s), 7.78 (1 H, s), 8.29 (1 H, bt), 8.64 (1 H, dd, J = 4.4, 1.4). Anal. ($C_{30}H_{27}F_6N_3O_3$) C, H, N.

(*R*)-*N*-[3,5-Bis(trifluoromethyl)benzyl]-7,8-dihydro-7-(3-hydroxy-2-methylpropyl)-5-(4-methylphenyl)-8-oxo-1,7-naphthyridine-6-carboxamide (10e). Compound **4** (1.0 g, 1.97 mmol) was treated according to a procedure similar to that described for the preparation of **10g** using (*R*)-3-amino-2-methyl-1-propanol (**5e**) in place of **5g** to afford **10e** as colorless crystals (932 mg, 82%). Recrystallization from EtOAc−IPE gave colorless crystals: mp 123−125 °C (once melted and then solidified), 215−216 °C; ^{1}H NMR 0.79 (3 H, d, J = 7.0), 2.13 (1 H, m), 2.28 (3 H, s), 3.10−3.70 (4 H, m), 4.48 (2 H, d, J = 6.2), 7.00−7.25 (4 H, m), 7.43 (1 H, dd, J = 8.4, 4.2), 7.59 (1 H, dd, J = 8.4, 1.6), 7.69 (2 H, s), 7.79 (1 H, s), 8.38 (1 H, bt), 8.70 (1 H, dd, J = 4.2, 1.6); $[\alpha]_D^{20}$ −9.0° (c = 0.346, $CHCl_3$). Anal. ($C_{29}H_{25}F_6N_3O_3 \cdot 0.5H_2O$) C, H, N.

(*S*)-*N*-[3,5-Bis(trifluoromethyl)benzyl]-7,8-dihydro-7-(3-hydroxy-2-methylpropyl)-5-(4-methylphenyl)-8-oxo-1,7-naphthyridine-6-carboxamide (10f). Compound **4** (1.0 g, 1.97 mmol) was treated according to a procedure similar to that described for the preparation of **10g** using (*S*)-3-amino-2-methyl-1-propanol (**5f**) in place of **5g** to afford **10f** as colorless crystals (788 mg, 69%). Recrystallization from EtOAc−IPE gave colorless crystals: mp 123−125 °C (once melted and then solidified), 215−216 °C; ^{1}H NMR 0.79 (3 H, d, J = 7.0), 2.13 (1 H, m), 2.28 (3 H, s), 3.10−3.70 (4 H, m), 4.48 (2 H, d, J = 6.2), 7.00−7.25 (4 H, m), 7.43 (1 H, dd, J = 8.4, 4.2), 7.59 (1 H, dd, J = 8.4, 1.6), 7.69 (2 H, s), 7.79 (1 H, s), 8.38 (1 H, bt), 8.70 (1 H, dd, J = 4.2, 1.6); $[\alpha]_D^{20}$ +11.1° (c = 0.350, $CHCl_3$). Anal. ($C_{29}H_{25}F_6N_3O_3 \cdot 0.5H_2O$) C, H, N.

(*S*)-*N*-[3,5-Bis(trifluoromethyl)benzyl]-7,8-dihydro-7-(4-hydroxy-3-methylbutyl)-5-(4-methylphenyl)-8-oxo-1,7-naphthyridine-6-carboxamide (10h). Compound **4** (1.0 g, 1.97 mmol) was treated according to a procedure similar to that described for the preparation of **10g** using **5h** in place of **5g** to afford **10h** as colorless crystals (900 mg, 77%). Recrystallization from EtOAc−IPE gave colorless crystals: mp 123−125 °C (once melted and then solidified), 207−208 °C; ^{1}H NMR 0.79 (3 H, d, J = 6.6), 1.4−1.8 (3 H, m), 2.28 (3 H, s), 3.03 (1 H, t, J = 6.6, OH), 3.2−3.7 (4 H, m), 4.49 (2 H, d, J = 5.8), 7.0−7.3 (4 H, m), 7.30 (1 H, dd, J = 8.4, 4.4), 7.53 (1 H, dd, J = 8.4, 1.4), 7.68 (2 H, s), 7.78 (1 H, s), 8.48 (1 H, t, J = 6.0), 8.61 (1 H, dd, J = 4.4, 1.4); $[\alpha]_D^{20}$ −2.7° (c = 0.391, $CHCl_3$). Anal. ($C_{30}H_{27}F_6N_3O_3 \cdot 0.5H_2O$) C, H, N.

(*aR*,9*R*)-7-[3,5-Bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]naphthyridine-6,13-dione [(*aR*,9*R*)-8b]. To a solution of **10g** (7.64 g, 12.9 mmol) and Et_3N (3.3 mL, 23.7 mmol) in THF (100 mL) was added methanesulfonyl chloride (1.8 mL, 23.3 mmol) at 0 °C, and the mixture was stirred at 0 °C for 30 min. The mixture was added to aqueous $NaHCO_3$ (50 mL), stirred at room temperature for 30 min, and extracted with EtOAc. The extract was washed successively with H_2O, diluted HCl, and brine, dried, and evaporated to give the mesylate of **10g** as a pale yellow foam. To a solution of this mesylate in THF (150 mL) was added NaH (60% dispersion in oil) (800 mg, 20.0 mmol) and the mixture was refluxed for 1 h. After dilution with EtOAc, the mixture was washed successively with H_2O, diluted HCl, H_2O, aqueous $NaHCO_3$, and brine, dried, and concentrated to give (*aR*,9*R*)-**8b** as colorless crystals (5.15 g, 69%). Recrystallization from EtOAc−IPE gave colorless crystals: mp 226−228 °C; ^{1}H NMR 0.91 (3 H, d, J = 6.8), 1.73 (1 H, m), 1.95−2.40 (2 H, m), 2.37 (3 H, s), 2.97 (1 H, d, J = 15), 3.35−3.62 (2 H, m), 3.99 (1 H, d, J = 15), 5.10 (1 H, dd, J = 14, 5.3), 5.46 (1 H, d, J = 15), 6.83 (1 H, dd, J = 7.8, 1.6), 7.05 (1 H, d, J = 7.8), 7.25 (1 H, d, J = 7.8), 7.34 (1 H, dd, J = 7.8, 1.6), 7.46 (1 H, dd, J = 8.4, 4.2), 7.47 (2 H, s), 7.55 (1 H, dd, J = 8.4, 1.8), 7.81 (1 H, dd, J = 4.2, 1.8), 8.91 (1 H, dd, J = 4.2, 1.8); an NOE, taken on a JEOL JNM-GX400 (400 MHz) spectrometer in $CDCl_3$, was observed between a benzylic methylene−H (δ 3.999) and $C_{(9)}$−H (δ 2.095); $[\alpha]_D^{20}$ +109.4° (c = 0.541, MeOH). Anal. ($C_{30}H_{25}F_6N_3O_2$) C, H, N.

7-[3,5-Bis(trifluoromethyl)benzyl]-8,9-dihydro-5-(4-methylphenyl)-7*H*-[1,4]pyrazino[2,1-*g*][1,7]naphthyridine-6,11-dione (6). Compound **10a** (200 mg, 0.36 mmol) was treated according to a procedure similar to that described for the preparation of (*aR*,9*R*)-**8b** to afford **6** as colorless crystals (109 mg, 56%). Recrystallization from EtOAc−ethyl ether gave colorless crystals: mp 270−271 °C; ^{1}H NMR 2.46 (3 H, s), 3.67 (2 H, t like, J = 5.4), 4.51 (2 H, t like, J = 5.4), 4.81 (2 H, s), 7.13 (2 H, d, J = 8.1), 7.33 (2 H, d, J = 8.1), 7.52 (1 H, dd, J = 8.4, 4.4), 7.64 (1 H, dd, J = 8.4, 1.6), 7.70 (2 H, s), 7.84 (1 H, s), 8.97 (1 H, dd, J = 4.4, 1.6). Anal. ($C_{27}H_{19}F_6N_3O_2$) C, H, N.

7-[3,5-Bis(trifluoromethyl)benzyl]-7,8,9,10-tetrahydro-5-(4-methylphenyl)-[1,4]diazepino[2,1-*g*][1,7]naphthyridine-6,12-dione (7a). Compound **10b** (11.1 g, 19.7 mmol) was treated according to a procedure similar to that described for the preparation of (*aR*,9*R*)-**8b** to afford **7a** as colorless crystals (6.95 g, 65%). Recrystallization from EtOAc−IPE gave colorless crystals: mp 194−195 °C; ^{1}H NMR 2.16 (2 H, m), 2.42 (3 H, s), 3.25−3.70 (3 H, m), 4.12 (1 H, d, J = 15), 5.34 (1 H, d, J = 15), 5.52 (1 H, m), 6.93 (1 H, d, J = 8.2), 7.20 (1 H, d, J = 8.2), 7.30−7.45 (2 H, m), 7.51 (1 H, dd, J = 8.4, 4.4), 7.62 (2 H, s), 7.70 (1 H, dd, J = 8.4, 1.6), 7.84 (1 H, s), 8.93 (1 H, dd, J = 4.4, 1.6). Anal. ($C_{28}H_{21}F_6N_3O_2$) C, H, N.

7-[3,5-Bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-5-(4-methylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]-naphthyridine-6,13-dione (8a). Compound **10c** (15.9 g, 27.5 mmol) was treated according to a procedure similar to that described for the preparation of (*aR*,9*R*)-**8b** to afford **8a** as colorless crystals (12.1 g, 79%). Recrystallization from EtOAc−IPE gave colorless crystals: mp 192−193 °C; ^{1}H NMR 1.7−2.5 (4 H, m), 2.37 (3 H, s), 3.25 (1 H, m), 3.40−3.72 (2 H, m), 4.01 (1 H, d, J = 15), 5.13 (1 H, dd, J = 14, 5.4), 5.46 (1 H, d, J = 15), 6.85 (1 H, d, J = 7.9), 7.05 (1 H, d, J = 7.9), 7.26 (1 H, d, J = 7.8), 7.34 (1 H, d, J = 7.8), 7.42−7.60 (2 H, m), 7.47 (2 H, s), 7.81 (1 H, s), 8.92 (1 H, m). Anal. ($C_{29}H_{23}F_6N_3O_2$) C, H, N.

7-[3,5-Bis(trifluoromethyl)benzyl]-7,8,9,10,11,12-hexahydro-5-(4-methylphenyl)-7*H*-[1,4]diazonino[2,1-*g*][1,7]-naphthyridine-6,14-dione (9). Compound **10d** (170 mg, 0.29

mmol) was treated according to a procedure similar to that described for the preparation of (*aR*,9*R*)-**8b** to afford **9** as colorless crystals (47 mg, 29%). Recrystallization from EtOAc−IPE gave colorless crystals: mp 177−179 °C; ^{1}H NMR 1.45−1.95 (4 H, m), 2.10 (2 H, m), 2.33 (3 H, s), 3.06−3.24 (1 H, m), 3.32−3.56 (2 H, m), 3.86 (1 H, d, J = 15), 4.95 (1 H, dt, J_d = 15, J_t = 4.8), 5.38 (1 H, d, J = 15), 6.86 (1 H, dd, J = 8.0, 1.5), 7.00 (1 H, d, J = 8.0), 7.17 (1 H, d, J = 8.2), 7.29 (1 H, dd, J = 8.2, 1.5), 7.40−7.54 (2 H, m), 7.44 (2 H, s), 7.79 (1 H, s), 8.89 (1 H, dd, J = 3.8, 2.0). Anal. ($C_{30}H_{25}F_6N_3O_2$) C, H, N.

(9*S*)-7-[3,5-Bis(trifluoromethyl)benzyl]-7,8,9,10-tetrahydro-9-methyl-5-(4-methylphenyl)-[1,4]diazepino[2,1-*g*]-[1,7]naphthyridine-6,12-dione [(9*S*)-7b]. Compound **10e** (840 mg, 1.45 mmol) was treated according to a procedure similar to that described for the preparation of (*aR*,9*R*)-**8b** to afford (9*S*)-**7b** as colorless crystals (406 mg, 50%). Recrystallization from EtOAc−IPE gave colorless crystals: mp 179−180 °C; ^{1}H NMR 1.05 (3 H × 3/5, d, J = 7.0), 1.22 (3 H × 2/5, d, J = 7.0), 2.39 (3 H × 2/5, s), 2.42 (3 H × 3/5, s), 2.52 (1 H, m), 3.0−3.3 (2 H, m), 3.48 (1 H × 3/5, dd, J = 14, 4.6), 3.71 (1 H × 2/5, dd, J = 16, 5.2), 4.06 (1 H × 2/5, d, J = 15), 4.12 (1 H × 3/5, d, J = 15), 5.28−5.65 (2 H, m), 6.83 (1 H × 2/5, d, J = 7.4), 6.96 (1 H × 3/5, d, J = 7.6), 7.09 (1 H × 2/5, d, J = 7.4), 7.20 (1 H × 3/5, d, J = 7.6), 7.35 (2 H, m), 7.42−7.75 (4 H, m), 7.83 (1 H, s), 8.92 (1 H, d, J = 3.6); in the major component, an NOE (taken on a JEOL JNM-GX400 (400 MHz) spectrometer in $CDCl_3$) was observed between a benzylic methylene−H (δ 4.119) and $C_{(9)}$−H (δ ca. 2.52), and in the minor component, an NOE was observed between a benzylic methylene−H (δ 4.064) and $C_{(9)}$−CH_3 (δ 1.217), whose absolute stereochemistry (*aR* or *aS*), however, has not been clarified yet; $[\alpha]_D^{20}$ +58.2° (*c* = 0.353, MeOH). Anal. ($C_{29}H_{23}F_6N_3O_2$) C, H, N. HPLC analysis [column, CHIRALPAK AD (4.6 mm i.d. × 250 mm);[19] eluant, hexane:2-propanol = 7:3; temperature, −40 °C; flow rate, 1.0 mL/min; detection, 254 nm] showed two peaks with the retention time of 11.3 and 22.2 min in a ratio of ca. 7:3; although these two peaks were separated by preparative HPLC at −40 °C, each fraction, obtained after rapid and careful workup at 0 °C, showed again the same two peaks as those of the original (9*S*)-**7b** by HPLC analysis at −40 °C.

(9*R*)-7-[3,5-Bis(trifluoromethyl)benzyl]-7,8,9,10-tetrahydro-9-methyl-5-(4-methylphenyl)-[1,4]diazepino[2,1-*g*]-[1,7]naphthyridine-6,12-dione [(9*R*)-7b]. Compound **10f** (700 mg, 1.21 mmol) was treated according to a procedure similar to that described for the preparation of (*aR*,9*R*)-**8b** to afford (9*R*)-**7b** as colorless crystals (408 mg, 60%). Recrystallization from EtOAc−IPE gave colorless crystals: mp 179−180 °C; ^{1}H NMR spectrum was identical with that of (9*S*)-**7b**; $[\alpha]_D^{20}$ −60.2° (*c* = 0.348, MeOH). Anal. ($C_{29}H_{23}F_6N_3O_2$) C, H, N.

(*aS*,9*S*)-7-[3,5-Bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]naphthyridine-6,13-dione [(*aS*,9*S*)-8b]. Compound **10h** (840 mg, 1.42 mmol) was treated according to a procedure similar to that described for the preparation of (*aR*,9*R*)-**8b** to afford (*aS*,9*S*)-**8b** as colorless crystals (540 mg, 66%). Recrystallization from EtOAc−IPE gave colorless crystals: mp 227−228 °C; ^{1}H NMR spectrum was identical with that of (*aR*,9*R*)-**8b**; $[\alpha]_D^{20}$ −107.1° (*c* = 0.521, MeOH). Anal. ($C_{30}H_{25}F_6N_3O_2$) C, H, N.

Atropisomers of 7-[3,5-Bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-5-(4-methylphenyl)-7*H*-[1,4]diazocino-[2,1-*g*][1,7]naphthyridine-6,13-dione [(*aR*)-8a and (*aS*)-8a]. Separation of **8a** into its atropisomers was carried out, similarly to the separation of *trans*-**1** into its atropisomers (*aR*)-*trans*-**1** and (*aS*)-*trans*-**1** described in our previous paper,[13c] by preparative HPLC using CHIRALPAK AD (10.0 mm i.d. × 250 mm)[19] under detection at 254 nm. Elution with a mixture of hexane:2-propanol (90:10) at a flow rate of 4.72 mL/min at 24 °C gave (*aR*)-**8a** and (*aS*)-**8a** as white powdery substances, respectively. (*aR*)-**8a**: retention time = 24.6 min; $[\alpha]_D^{20}$ +45.6° (*c* = 0.096, $CHCl_3$). (*aS*)-**8a**: retention time = 31.4 min; $[\alpha]_D^{20}$ −41.3° (*c* = 0.1, $CHCl_3$).

(*aS*,9*R*)-7-[3,5-Bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]naphthyridine-6,13-dione [(*aS*,9*R*)-8b, atropisomer of (*aR*,9*R*)-8b]. The mother liquor obtained after crystallization of (*aR*,9*R*)-**8b** was concentrated. The concentrate, which contained (*aR*,9*R*)-**8b** and (*aS*,9*R*)-**8b** in a ratio of ca. 5:1, was subjected to preparative HPLC [CHIRALCEL OD (10 mm i.d. × 250 mm);[19] eluant, hexane:ethanol (85:15) (4 mL/min); temperature, 0 °C; retention time, 15.7 min (cf. (*aR*,9*R*)-**8b**: 21.4 min)] to give (*aS*,9*R*)-**8b** as a white powdery substance (98.6% de): ^{1}H NMR 1.24 (3 H, d, J = 7.0), 1.8−2.4 (3 H, m), 2.27 (3 H, s), 3.37 (1 H, dd, J = 15, 4.4), 3.65 (1 H, m), 3.85 (1 H, d, J = 15), 4.07 (1 H, d, J = 15), 5.08 (1 H, m), 5.33 (1 H, d, J = 15), 6.72 (1 H, d, J = 8.2), 6.82 (1 H, d, J = 8.2), 7.09 (1 H, d, J = 8.2), 7.21 (1 H, d, J = 8.2), 7.45 (2 H, m), 7.51 (2 H, s), 7.77 (1 H, s), 8.89 (1 H, m); an NOE, taken on a JEOL JNM-GX400 (400 MHz) spectrometer in $CDCl_3$, was observed between a benzylic methylene−H (δ 4.080) and $C_{(9)}$−CH_3 (δ 1.252); MS (electron impact) *m*/*z* 573 (M^+), 554, 346, 310, 263, 235; $[\alpha]_D^{20}$ −156.8° (*c* = 0.305, MeOH).

(*aR*,9*S*)-7-[3,5-Bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]naphthyridine-6,13-dione [(*aR*,9*S*)-8b, atropisomer of (*aS*,9*S*)-8b]. The second crystals obtained after the first crystals of (*aS*,9*S*)-**8b**, which contained (*aS*,9*S*)-**8b** and (*aR*,9*S*)-**8b** in a ratio of ca. 5:1, were subjected to preparative HPLC [CHIRALCEL OD (20 mm i.d. × 250 mm);[19] eluant, hexane:ethanol (85:15) (8 mL/min); temperature, 24 °C; retention time, 25.8 min (cf. (*aS*,9*S*)-**8b**: 21.9 min)] to give (*aR*,9*S*)-**8b** as a white powdery substance (97.5% de): ^{1}H NMR spectrum was identical with that of (*aS*,9*R*)-**8b**; MS (electron impact) *m*/*z* 573 (M^+), 554, 346, 310, 263, 235; $[\alpha]_D^{20}$ +153.2° (*c* = 0.280, MeOH).

Single-Crystal X-ray Analysis of (*aR*,9*R*)-8b. Brief summary of the crystal data has been reported in the previous paper,[13d] and the details have been deposited with the Cambridge Structural Database (CCDC 182/988). Experimental procedures are as follows. Crystals of (*aR*,9*R*)-**8b** were grown from acetone−IPE. Data were collected on a diffractometer, Rigaku AFC5R, with Cu Kα radiation. Psi-scan empirical absorption correction was applied. The structure was determined by direct methods with the aid of TEXSAN[29] and refined by SHEXL93.[30]

Molecular Modeling Studies. A generation of low-energy conformers was carried out by the procedures described in the previous papers.[13a−c] Figures 3 and 5 show the overlapping between the most stable conformers of the molecules as determined by the analysis using Discover CVFF force field.

[^{125}I]BH-SP Binding in Human IM-9 Cells and Rat Forebrain. The binding activities were determined according to the protocol previously reported.[13a]

Guinea Pig Ileum Contraction Assay. The assay induced with SP or NKA was performed according to the protocol previously reported.[13a]

Inhibitory Effect on Capsaicin-Induced Plasma Extravasation in the Trachea of Guinea Pigs. The inhibitory effect was determined according to the protocol in the literature[17] with minor modification. Guinea pigs (Hartley, male) (n = 5−8) were anesthetized with 35 mg/kg pentobarbital injected intraperitoneally, and the test sample was then administered intravenously. Five minutes after administration, a solution of capsaicin (150 μg/kg) and Evans blue dye (20 mg/kg) in ethanol−saline (3:7) was administered (iv) to cause the reaction. In the case of the oral administration test, the sample was administered 60 min prior to reaction induction. Ten minutes after the reaction was induced, test animals were sacrificed by cutting the inferior vena cava, and the pulmonary artery was perfused with 50 mL of physiological saline. The trachea was excised, and its wet weight was measured. Evans blue dye was extracted by incubation in 1 mL of acetone−0.3% sodium sulfate (7:3) overnight (for more than 12 h). After centrifugation at 2800 rpm for 20 min, the concentration of Evans blue dye in the supernatant was quantified by measuring the absorbance at 620 nm. Plasma

extravasation was expressed in terms of the amount of extracted Evans blue dye (μg) relative to the weight of the trachea (g). The efficacy of the sample was evaluated by calculating the percent inhibition in accordance with the following formula: % inhibition $= [1 - (A - B)/(C - B)] \times 100$, in which A, B, and C represent the amount of Evans blue dye (μg/g) obtained in the test animal, in the group not treated with capsaicin (blank) (mean value), and in the control group (mean value), respectively.

Effects on Distention-Induced Rhythmic Bladder Contractions in Urethane Anesthetized Guinea Pigs. Male guinea pigs (Hartley, 250−350 g) were used. The animals were anesthetized with an intraperitoneal injection of urethane (1.2 g/kg). The urinary bladder was exposed through an incision in the abdomen, and the urethra was ligated. A 23-gauge needle connected to a polyethylene tube (PE90) was inserted into the bladder dome so the intravesical pressure could be recorded. In each animal, warmed physiological saline (39 °C) was injected into the bladder stepwise (0.2 mL) until regular isovolumetric bladder contractions appeared. Test compounds were administered intravenously after confirming stable rhythmic contractions. The effects of the test compounds were evaluated using both the shutdown time with a cutoff time of 30 min and the contractile intravesical pressure. The test compounds were dissolved in DMSO and injected in a volume of 0.05 mL/100 g (body weight). As the vehicle (DMSO) [0.05 mL/100 g (body weight), iv] affected the shutdown time in some preparations, statistical comparisons were made with the mean of the shutdown time in the vehicle-treated groups (Dunnett test). On the other hand, as intravesical pressure was not sensitive to the vehicle, statistical comparison was made between the predrug mean value and postdrug value (paired t-test). The significance of relationships between the effects on the bladder functions and the NK_1 antagonistic activities was determined by Spearman's rank correlation coefficient (r).[27]

Increasing Effect on Bladder Volume Threshold in Urethane Anesthetized Guinea Pigs. Male guinea pigs (Hartley, 250−350 g) were used. The animals were anesthetized with an intraperitoneal injection of urethane (1.2 g/kg). The urinary bladder was exposed through an incision in the abdomen. A 23-gauge needle connected to a polyethylene tube (PE90) was inserted into the bladder dome so physiological saline could be infused into the bladder. Before infusing saline into the bladder, the bladder was emptied via the polyethylene tube. Warm saline (39 °C) was continuously infused at a rate of 0.3 mL/min using an infusion pump. The volume threshold (volume to which the bladder can be filled before voiding) was measured at least two times before testing. After confirming stable responses, the mean value of the last two trials was taken as the control volume threshold, and the volume threshold was then measured 10 min after intravenous injection of the test compound. The effects of the test compounds were expressed as percent increase in volume threshold compared with the predrug value. The ED_{30} value was determined by measuring the dose of the test compound that increases the bladder volume threshold by 30%. In this assay, oxybutynin, a peripherally acting anti-pollakiuria agent, showed the ED_{30} value of 0.22 (0.0059−1.953) mg/kg, iv. The test compounds were dissolved in 80% DMSO and injected in a volume of 0.1 mL/100 g (body weight).

Acknowledgment. The authors wish to thank Mr. Kazuichi Umemoto for separation of the atropisomers, Ms. Keiko Higashikawa for X-ray analysis, Ms. Mika Murabayashi for NMR analysis, Mr. Osamu Saga and Mr. Yasukazu Tajima for in vitro screening, and Mr. Satoshi Okanishi and Mr. Shigemitsu Imai for in vivo screening.

References

(1) Review articles: (a) Otsuka, M.; Yoshioka, K. Neurotransmitter Functions of Mammalian Tachykinins. *Physiol. Rev.* **1993**, *73*, 229−308. (b) Maggi, C. A.; Patacchini, R.; Rovero, P.; Giachetti, A. Tachykinin Receptors and Tachykinin Receptor Antagonists. *J. Auton. Pharmacol.* **1993**, *13*, 23−93. (c) Regoli, D.; Boudon, A.; Fauchere, J.-L. Receptors and Antagonists for Substance P and Related Peptides. *Pharmacol. Rev.* **1994**, *46*, 551−599. (d) MacLean, S. Nonpeptide Antagonists of the NK_1 Tachykinin Receptor. *Med. Res. Rev.* **1996**, *16*, 297−317. (e) Lowe, J. A., III. Nonpeptide Tachykinin Antagonists: Medicinal Chemistry and Molecular Biology. *Med. Res. Rev.* **1996**, *16*, 527−545.

(2) (a) Snider, R. M.; Constantine, J. W.; Lowe, J. A., III; Longo, K. P.; Lebel, W. S.; Woody, H. A.; Drozda, S. E.; Desai, M. C.; Vinick, F. J.; Spencer, R. W.; Hess, H.-J. A Potent Nonpeptide Antagonist of the Substance P (NK_1) Receptor. *Science* **1991**, *251*, 435−437. (b) McLean, S.; Ganong, A. H.; Seeger, T. F.; Bryce, D. K.; Pratt, K. G.; Reynolds, L. S.; Siok, C. J.; Lowe, J. A., III; Heym, J. Activity and Distribution of Binding Sites in Brain of a Nonpeptide Substance P (NK1) Receptor Antagonist. *Science* **1991**, *251*, 437−439. (c) Lowe, J. A., III; Drozda, S. E.; Snider, R. M.; Longo, K. P.; Zorn, S. H.; Morrone, J.; Jackson, E. R.; McLean, S.; Bryce, D. K.; Bordner, J.; Nagahisa, A.; Kanai, Y.; Suga, O.; Tsuchiya, M. The Discovery of (2S, 3S)-*cis*-2-(Diphenylmethyl)-N-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine as a Novel, Nonpeptide Substance P Antagonist. *J. Med. Chem.* **1992**, *35*, 2591−2600.

(3) (a) Emonds-Alt, X.; Doutremepuichi, J.-D.; Heaulme, M.; Neliat, G.; Santucci, V.; Steinberg, R.; Vilain, P.; Bichon, D.; Ducoux, J.-P.; Proietto, V.; Broeck, D. V.; Soubri, P.; Fur, G. L.; Brelière, J.-C. In Vitro and In Vivo Biological Activities of SR140333, a Novel Potent Non-peptide Tachykinin NK_1 Receptor Antagonist. *Eur. J. Pharmacol.* **1993**, *250*, 403−413. (b) Jung, M.; Calassi, R.; Maruani, J.; Barnouin, M. C.; Souilhac, J.; Poncelet, M.; Gueudet, C.; Emonds-Alt, X.; Soubri, P.; Brelière, J. C.; Fur, G. L. Neuropharmacological Characterization of SR140333, a Non Peptide Antagonist of NK_1 Receptors. *Neuropharmacology* **1994**, *33*, 167−179.

(4) (a) Tabart, M.; Peyronel, J.-F. Synthesis of RPR100893, Prototype of a New Series of Potent and Selective Non Peptide NK_1 Antagonists: The Triarylperhydroisoindolols. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 673−676. (b) Fardin, V.; Carruette, A.; Menager, J.; Bock, M.; Flamand, O.; Faucoult, F.; Heuillet, E.; Moussaoui, S. M.; Tabart, M.; Peyronel, J. F.; Garret, C. In Vitro Pharmacological Profile of RPR100893, a Novel Non-peptide Antagonist of the Human NK_1 Receptor. *Neuropeptides* **1994**, *26* (Suppl. 1), 34. (c) Moussaoui, S. M.; Montier, F.; Carruette, A.; Fardin, V.; Floch, A.; Garret, C. In Vivo Pharmacological Profile of RPR100893, a Novel Non-peptide Antagonist of the Human NK_1 Receptor. *Neuropeptides* **1994**, *26* (Suppl. 1), 35.

(5) (a) Desai, M. C.; Lefkowitz, S. L.; Thadeio, P. F.; Longo, K. P.; Snider, R. M. Discovery of a Potent Substance P Antagonist: Recognition of the Key Molecular Determinant. *J. Med. Chem.* **1992**, *35*, 4911−4913. (b) McLean, S.; Ganong, A.; Seymour, P. A.; Snider, R. M.; Desai, M. C.; Rosen, T.; Bryce, D. K.; Longo, K. P.; Reynolds, L. S.; Robinson, G.; Schmidt, A. W.; Siok, C.; Heym, J. Pharmacology of CP-99,994; a Nonpeptide Antagonist of the Tachykinin Neurokinin-1 Receptor. *J. Pharmacol. Exp. Ther.* **1993**, *267*, 472−479. (c) Desai, M. C.; Vincent, L. A.; Rizzi, J. P. Importance of Parallel Vectors and "Hydrophobic Collapse" of Aligned Aromatic Rings: Discovery of a Potent Substance P Antagonist. *J. Med. Chem.* **1994**, *37*, 4263−4266.

(6) McLean, S.; Ganong, A.; Seymour, P. A.; Bryce, D. K.; Crawford, R. T.; Morrone, J.; Reynolds, L. S.; Schmidt, A. W.; Zorn, S.; Watson, J.; Fossa, A.; DePasquale, M.; Rosen, T.; Nagahisa, A.; Tsuchiya, M.; Heym, J. Characterization of CP-122,721; A Nonpeptide Antagonist of the Neurokinin NK_1 Receptor. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 900−908.

(7) (a) Vassout, A.; Schaub, M.; Gentsch, C.; Ofner, S.; Schilling, W.; Veenstra, S. CGP49823, a Novel NK_1 Receptor Antagonist: Behavioural Effects. *Neuropeptides* **1994**, *26* (Suppl. 1), 38; (b) Ofner, S.; Hauser, K.; Schilling, W.; Vassout, A.; Veenstra, S. J. SAR of 2-Benzyl-4-aminopiperidines: CGP49823, An Orally and Centrally Active Non-peptide NK_1 Antagonist. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1623−1628.

(8) (a) Gardner, C. J.; Armour, D. R.; Beattie, D. T.; Gale, J. D.; Hawcock, A. B.; Kilpatrick, G. J.; Twissell, D. J.; Ward, P. GR205171: A novel antagonist with high affinity for the tachykinin NK_1 receptor, and potent broad-spectrum anti-emetic activity. *Regul. Pept.* **1996**, *65*, 45−53. (b) Armour, D. R.; Chung, K. M. L.; Congreve, M.; Evans, B.; Guntrip, S.; Hubbard, T.; Kay, C.; Middlemiss, D.; Mordaunt, J. E.; Pegg, N. A.; Vinader, M. V.; Ward, P.; Watson, S. P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1015−1020.

(9) (a) Hipskind, P. A.; Howbert, J. J.; Bruns, R. F.; Cho, S. S. Y.; Crowell, T. A.; Foreman, M. M.; Gehlert, D. R.; Iyengar, S.; Johnson, K. W.; Krushinski, J. H.; Li, D. L.; Lobb, K. L.; Mason, N. R.; Muehl, B. S.; Nixon, J. A.; Phebus, L. A.; Regoli, D.; Simmons, R. M.; Threlkeld, P. G.; Waters, D. C.; Gitter, B. D. 3-Aryl-1,2-diacetamidopropane Derivatives as Novel and Potent NK-1 Receptor Antagonists. *J. Med. Chem.* **1996**, *39*, 736−748. (b) Gitter, B. D.; Bruns, R. F.; Howbert, J.; Waters, D. C.; Threlkeld, P. G.; Cox, L. M.; Nixon, J. A.; Lobb, K. L.; Mason,

N. R.; Stengel, P. W.; Cockerham, S. L.; Silbaugh, S. A.; Gehlert, D. R.; Schober, D. A.; Iyengar, S.; Calligaro, D. O.; Regoli, D.; Hipskind, P. A. Pharmacological Characterization of LY303870: A Novel, Potent and Selective Nonpeptide Substance P (Neurokinin-1) Receptor Antagonist. *J. Pharmacol. Exp. Ther.* **1996**, *275*, 737−744.

(10) Ladduwahetty, T.; Baker, R.; Cascieri, M. A.; Chabers, M. S.; Haworth, K.; Keown, L. E.; MacIntyre, D. E.; Metzger, J. M.; Owen, S.; Rycroft, W.; Sadowski, S.; Seward, E. M.; Shepheard, S. L.; Swain, C. J.; Tattersall, F. D.; Watt, A. P.; Williamson, D. W.; Hargreaves, R. J. *N*−Heteroaryl-2-phenyl-3-(benzyloxy)-piperidines: A Novel Class of Potent Orally Active Human NK1 Antagonist. *J. Med. Chem.* **1996**, *39*, 2907−2914.

(11) Hale, J. J.; Mills, S. G.; MacCoss, M. M.; Shah, S. K.; Qi, H.; Mathre, D. J.; Cascieri, M. A.; Sadowski, S.; Strader, C. D.; MacIntyre, D. E.; Metzger, J. M. 2-(*S*)-((3,5-Bis(trifluoromethyl)-benzyl)-oxy)-3-(*S*)-phenyl-4-((3-oxo-1,2,4-triazol-5-yl)methl)morpholine (1): A Potent, Orally Active, Morpholine-Based Human Neurokinin-1 Receptor Antagonist. *J. Med. Chem.* **1996**, *39*, 1760−1762.

(12) (a) Kramer, M. S.; Cutler, N.; Feighner, J.; Shrivastava, R.; Carman, J.; Sramek, J. J.; Reines, S. A.; Liu, G.; Snavely, D.; W.-Knowles, E.; Hale, J. J.; Mills, S. G.; MacCoss, M.; Swain, C. J.; Harrison, T.; Hill, R. G.; Hefti, F.; Scolnick, E. M.; Cascieri, M. A.; Chicchi, G. G.; Sadowski, S.; Williams, A. R.; Hewson, L.; Smith, D.; Carlson, E. J.; Hargreaves, R. J.; Rupniak, N. M. J. Distinct Mechanism for Antidepressant Activity by Blockade of Central Substance P Receptors. *Science* **1998**, *281*, 1640−1645. (b) Hale, J. J.; Mills, S. G.; MacCoss, M.; Finke, P. E.; Cascieri, M. A.; Sadowski, S.; Ber, E.; Chicchi, G. G.; Kurtz, M.; Metzger, J. M.; Eiermann, G.; Tsou, N. N.; Tattersall, F. D.; Rupniak, N. M. J.; Williams, A. R.; Rycroft, W.; Hargreaves, R.; MacIntyre, D. E. Structural Optimization Affording 2-(*R*)-(1-(*R*)-3,5-Bis(trifluoromethyl)phenylethoxy)-3-(*S*)-(4-fluoro)phenyl-4-(3-oxo-1,2,4-triazol-5-yl)methlmorpholine, a Potent, Orally Active, Long-Acting Morpholine Acetal Human NK-1 Receptor Antagonist. *J. Med. Chem.* **1998**, *41*, 4607−4614.

(13) (a) Natsugari, H.; Ikeura, Y.; Kiyota, Y.; Ishichi, Y.; Ishimaru, T.; Saga, O.; Shirafuji, H.; Tanaka, T.; Kamo, I.; Doi, T.; Otsuka, M. Novel, Potent, and Orally Active Substance P Anagonists: Synthesis and Antagonist Activity of *N*-Benzylcarboxamide Derivaives of Pyrido[3,4-*b*]pyridine. *J. Med. Chem.* **1995**, *38*, 3106−3120. (b) Ikeura, Y.; Tanaka, T.; Kiyota, Y.; Morimoto, S.; Ogino, M.; Ishimaru, T.; Kamo, I.; Doi, T.; Natsugari, H. Potent NK_1 Receptor Antagonists: Synthesis and Antagonistic Activity of Various Heterocycles with an *N*-[3,5-Bis(trifluoromethyl)-benzyl]-*N*-methylcarbamoyl Substituent. *Chem. Pharm. Bull.* **1997**, *45*, 1642−1652. (c) Ikeura, Y.; Ishichi, Y.; Tanaka, T.; Fujishima, A.; Murabayashi, M.; Kawda, M.; Ishimaru, T.; Kamo, I.; Doi, T.; Natsugari, H. Axially Chiral 1,7-Naphthyridine-6-carboxamide Derivatives as Tachykinin NK_1 Receptor Antagonists: Determination of the Absolute Stereochemical Requirements. *J. Med. Chem.* **1998**, *41*, 4232−4239. (d) A preliminary communication: Ikeura, Y.; Ishimaru, T.; Doi, T.; Kawda, M.; Fujishima, A.; Natsugari, H. Enantioselective synthesis of an axially chiral 1,7-naphthyridine-6-carboxamide derivative having potent antagonist activity at the NK_1 receptor. *Chem. Commun.* **1998**, 2141−2142.

(14) The italic letter *a* before *R* and *S* denotes an axial chirality as suggested by Cahn et al.: Cahn, R. S.; Ingold, C.; Prelog, V. Specification of Molecular Chirality. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 385−415.

(15) As for the chemistry and separation of atropisomers of some aromatic carboxamides, see: (a) Ohno, A.; Kashiwagi, M.; Ishihara, Y.; Ushida, S.; Oka, S. NAD(P)$^+$-NAD(P)H Models. 57. Stereochemistry in (Net) Hydride Transfer From and NAD(P)$^+$-NAD(P)H Models: Chirality Sink. *Tetrahedron* **1986**, *42*, 961−973. (b) Cuyegkeng, M. A.; Mannschreck, A. Liquid Chromatography on Triacetylcellulose, 14. Chromatographic Separation on Enantiomers and Barriers to Enantiomerization of Axially Chiral Aromatic Carboxamides. *Chem. Ber.* **1987**, *120*, 803−809. (c) Thayumanavan, S.; Beak, P.; Curran, D. P. Asymmetric Deprotonation of *N*,*N*-Dihexyl-1-Naphthamides to Provide Atropisomers of *N*,*N*-Dihexyl-2-Alkyl-1-Naphthamides. *Tetrahedron Lett.* **1996**, *37*, 2899−2902. (d) Clayden, J.; Westlund, N.; Wilson, F. X. Asymmetric Induction using Atropisomers: Diastereoselective Additions to 2-Acyl-1-Naphthamides. *Tetrahedron Lett.* **1996**, *37*, 5577−5580. (e) Clayden, J. Stereocontrol with Rotationally Restricted Amides. *Synlett* **1998**, 810−816.

(16) Cascieri, M. A.; Ber, E.; Fong, T. N.; Sadowski, S.; Basal, A.; Swain, C.; Seward, E.; Frances, B.; Burns, D.; Strader, C. D. Characterization of the Binding of a Potent, Selective Radioiodonated Antagonist to the Human Neurokinin-1 Receptor. *Mol. Pharmacol.* **1992**, *42*, 458−463.

(17) Eglezos, A.; Giuliani, S.; Viti, G.; Maggi, C. A. Direct Evidence That Capsaicin-induced Plasma Protein Extravasation is Mediated through Tachykinin NK_1 Receptors. *Eur. J. Pharmacol.* **1991**, *209*, 277−279.

(18) As for atropisomerism in 7-membered heterocycles, see: Gilman, N. W.; Rosen, P.; Earley, J. V.; Cook, C.; Todaro, L. J. Atropisomers of 1,4-Benzodiazepines. Synthesis and Resolution of a Diazepam-Related 1,4-Benzodiazepine. *J. Am. Chem. Soc.* **1990**, *112*, 3969−3978.

(19) DAICEL Chemical Industries, Ltd., Japan.

(20) The conversion of (*aS*,9*R*)-**8b** to (*aR*,9*R*)-**8b** is apparently faster than that from (*aR*,9*R*)-**8b** to (*aS*,9*R*)-**8b**. An accurate time course of conversion from (*aR*,9*R*)-**8b** to (*aS*,9*R*)-**8b** has not been estimated due to the small change (within a range of 2%).

(21) (a) Lewis, R. T.; Macleod, A. M.; Merchant, K. J.; Kelleher, F.; Sanderson, I.; Herbert, R. H.; Cascieri, M. A.; Sadowski, S.; Ball, R. G.; Hoogsteen, K. Tryptophan-Derived NK_1 Antagonists: Conformationally Constrained Heterocyclic Bioisosters of the Ester Linkage. *J. Med. Chem.* **1995**, *38*, 923−933. (b) Macleod, A. M.; Cascieri, M. A.; Merchant, K. J.; Sadowski, S.; Hardwicke, S.; Lewis, R. T.; MacIntyre, D. E.; Metzger, J. M.; Fong, T. M.; Shepheard, S.; Tattersall, F. D.; Hargreaves, R.; Baker, R. Synthesis and Biological Evaluation of NK_1 Antagonists Derived from L-Tryptophan. *J. Med. Chem.* **1995**, *38*, 934−941.

(22) Quirion, R.; Pilapil, C. Comparative Potencies of Substance P, Substance K, and Neuromedine K on Brain Substance P Receptors. *Neuropeptides* **1984**, *4*, 325−329.

(23) Renzetti, A. R.; Barsacchi, P.; Criscuoli, M.; Lucacchini, A. Characterization of NK-3 Binding Sites in Rat and Guinea Pig Cortical Membranes by the Selective Ligand [^{3}H]Senktide. *Neuropeptides* **1991**, *18*, 107−114.

(24) The selectivity for NK_1 over NK_2 and NK_3 in the compounds related to **1** has been confirmed: Hosoki, R.; Yanagisawa, M.; Onishi, Y.; Yoshioka, K.; Otsuka, M. Pharmacological Profiles of New Orally Active Nonpeptide Tachykinin NK_1 Receptor Antagonists. *Eur. J. Pharmacol.* **1998**, *341*, 235−241.

(25) Compounds *trans*-**1** and (*aR*,9*R*)-**8b** did not show effects on the binding of [^{3}H]nitrendipine to membranes from rat brain cortices at a concentration of 10 μM, indicating that this series of compounds does not interact with the L-type Ca^{2+} channels.

(26) (a) Maggi, C. A. The role of peptides in the regulation of the micturition reflex: an update. *Gen. Pharmacol.* **1991**, *22*, 1−24. (b) Lecci, A.; Giuliani, S.; Garret, C.; Maggi, C. A. Evidence for a role of tachykinins as sensory transmitters in the activation of micturition reflex. *Neuroscience* **1993**, *54*, 827−837.

(27) (a) Spearman, C. The proof and measurement of association between two things. *Am. J. Psychol.* **1904**, *15*, 72−101. (b) Snedecor, G. W.; Cochran, W. G. *Statistical Methods*; The Iowa State University Press: Ames, IA, 1967.

(28) (a) Mori, K. Revision of the Absolute Configuration of A-Factor. The Inducer of Stretomycin Biosynthesis, Basing on the Reconfirmed (*R*)-Configuration of (+)-Paraconic Acid. *Tetrahedron* **1983**, *39*, 3107−3109. (b) Mattes, H.; Hamada, K.; Benezra, C. Stereospecificity in Allergic Contact Dermititis to Simple Substituted Methylene Lactone Derivatives. *J. Med. Chem.* **1987**, *30*, 1948−1951.

(29) *TEXSAN: Single-Crystal Structure Analysis Software*, version 1.9; Molecular Structure Corp.: The Woodlands, TX, 1998.

(30) Sheldrick, G. M. *SHELXL93, Program for the Refinement of Crystal Structures*; University of Göttingen: Germany, 1993.

JM990220R