# Synthesis and Structure–Activity Studies of a Series of 1-Oxa-8-azaspiro[4.5]decanes as $M_1$ Muscarinic Agonists

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**2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one (17), designed by incorporating the tetrahydrofuran ring moiety of muscarone into an 8-azaspiro[4.5]decane skeleton, and related 1-oxa-8-azaspiro[4.5]decanes were synthesized and assessed as $M_1$ muscarinic agonists for the symptomatic treatment of dementia of Alzheimer's type. The compounds were tested for central muscarinic $M_1$ and $M_2$ receptor affinity and *in vivo* muscarinic activities: namely, amelioration of scopolamine-induced impairment in rat passive avoidance tasks, and induction of hypothermia, tremor, and salivary secretion. Compound 17 exhibited potent muscarinic activities *in vitro* and *in vivo* with no selectivity. Systematic modifications of 17 were conducted, and a number of compounds, including the 2-ethyl analogue (18), 3-methylene analogue (29), 3-dithioketal analogues (26, 28), and 3-oxime analogue (37) were found to display preferential affinity for $M_1$ receptors over $M_2$ receptors and, in addition, to exhibit potent antiamnesic activity sufficiently separated from hypothermia-inducing activity, taken as an index of cholinergic side effects, compared with the reference compound RS86 (1). Structure–activity relationships are discussed in comparison with those for muscarone analogues. Of these compounds only two, 2-ethyl-8-methyl-1-oxa-8-azaspiro[4.5]decan-3-one (18) and 2,8-dimethyl-3-methylene-1-oxa-8-azaspiro[4.5]decane (29), stimulated phosphoinositide hydrolysis in rat hippocampal slices, indicating partial agonistic activity for $M_1$ muscarinic receptors.**

**The optical resolution of 18 and 29 was performed. Eudismic ratios of both compounds in binding affinity were low, but $M_1$ agonist activity resided preferentially in the (−)-isomers. The absolute configuration of (−)-29 was determined by X-ray crystal structure analysis to be *S*, being the same as that of muscarone. Based on the *in vivo* selectivity, (−)-29 was selected for clinical studies.**

**Key words** muscarinic $M_1$ agonist; 1-oxa-8-azaspiro[4.5]decane; YM796; subtype selectivity; scopolamine-induced amnesia; structure–activity relationship

Learning and memory disabilities in dementia of Alzheimer's type (DAT) have been shown to be associated with marked cholinergic deficits in the cortical and hippocampal areas.[1] The activity of choline acetyltransferase, a marker of presynaptic cholinergic function, is consistently reduced.[2] However, postsynaptic muscarinic receptors, especially the $M_1$ subtype [with high affinity for pirenzepine (PZ)], appear largely intact.[3] $M_1$ muscarinic receptors are abundant in the cerebral cortex and hippocampus, both of which are associated with memory and learning. On the other hand, $M_2$ (with low affinity for PZ) muscarinic receptors are distributed in the cerebellum and peripheral tissues.[4] These findings suggest that a centrally active $M_1$-selective agonist might have potential as a safe antidementia drug.

In the last decade, the use of muscarinic cholinergic agonists such as arecoline and RS86 (**1**) has been investigated in clinical studies of DAT.[5] Some improvement in cognitive function was observed but the cholinergic side effects of these drugs precluded their further clinical development. The separation of the antiamnesic effect from side effects is therefore important in the development of muscarinic agonists as antidementia drugs.

We previously reported that a 4-oxa-analogue of **1**, 3-ethyl-8-methyl-1-oxa-3,8-diazaspiro[4.5]decane-2,4-dione (**2**), exhibited potent antiamnesic activity with higher selectivity for this activity over cholinergic side effects, namely hypothermia and salivation, as compared with **1**.[6] Structure–activity studies of spirooxazolidine-2,4-diones suggested that the 8-azaspiro[4.5]decane skeleton of **1** or **2** is a useful template for designing new muscarinic agonists. Selectivity for antiamnesic activity might be improved by modification of the five-membered ring of **1** or **2**, hence possibly leading to the creation of a selective $M_1$ agonist without adverse cholinergic effects.

From comparison of the structures of **1** and **2** with those of the well-known muscarinic agonists muscarine (**3**) and muscarone (**4**), we designed and synthesized 3-hydroxy-2,8-dimethyl-1-oxa-8-azaspiro[4.5]decane (**23**) and 2,8-dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one (**17**), both of which have the tetrahydrofuran ring moiety of muscarine or muscarone incorporated into the 8-azaspiro[4.5]decane skeleton. Interestingly, compound **17**, but not **23**, displayed potent muscarinic activity, whereas selectivity for antiamnesic activity over cholinergic side effects was low. In order to improve selectivity and to obtain information about structure–activity relationships, various modifications were carried out. We report herein the synthesis and pharmacological evaluation of a series of 1-oxa-8-azaspiro[4.5]decanes and their thia analogues. From these investigations, 2-ethyl-8-methyl-1-oxa-8-azaspiro[4.5]-decan-3-one (**18**) and 2,8-dimethyl-3-methylene-1-oxa-8-azaspiro[4.5]decane (**29**) were found to be $M_1$ partial agonists and to have substantially higher selectivity for antiamnesic effect over cholinergic side effects, namely hypothermia, tremor, and salivation, in comparison with **1**, although their $M_1$ selectivity in binding affinity was modest. This paper also describes resolution and pharmacological evaluation of the enantiomers of **18** and **29**.

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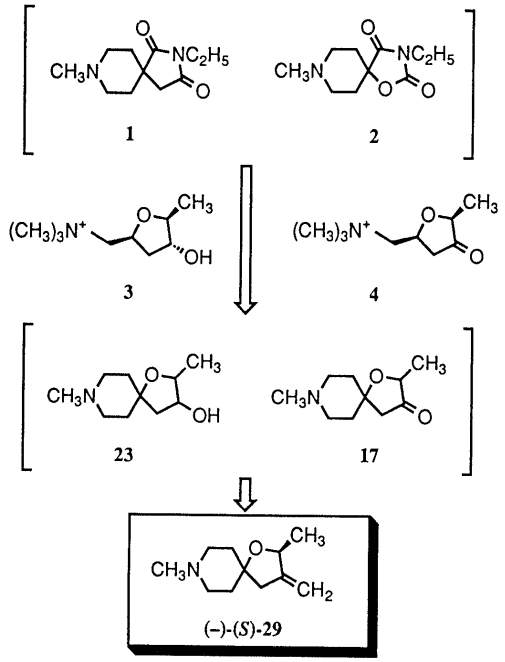
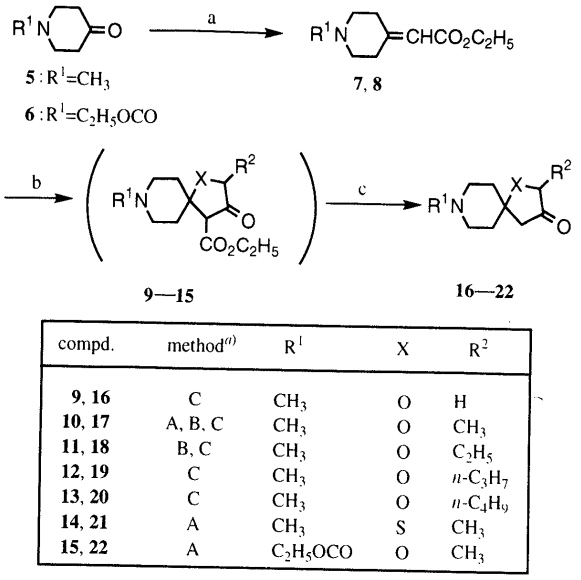



Chart 1

## Chemistry

The 1-oxa-8-azaspiro[4.5]decan-3-ones **16**—**22** were synthesized *via* the Michael addition–Dieckmann sequence, reported to be a convenient procedure for the preparation of simple tetrahydrofuranones (Chart 2).[7] Treatment of ethyl lactate sodium salt in dimethylsulfoxide (DMSO) with ethyl (1-methyl-4-piperidinylidene)acetate (**7**), readily available by Horner–Emmons olefination of 1-methyl-4-piperidone (**5**),[8] afforded the spiro *β*-keto ester **10** in 11% yield. The decarboxylative hydrolysis of **10** in aqueous HCl gave 2,8-dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one (**17**) in 82% yield (method A, Chart 2). In order to improve the total yield, the reaction conditions were modified. When the reaction mixture of the first step in method A was subjected to decarboxylative hydrolysis without the isolation of **10**, the total yield was improved from 9% in method A to 18.5% (method B). In addition, the reaction solvent was changed to tetrahydrofuran (THF) to avoid troublesome manipulations owing to the use of DMSO. The addition–cyclization reaction took more than 2 d in THF, whereas it took only about 20 h in DMSO. Subsequent decarboxylative hydrolysis of the crude *β*-keto ester, however, afforded **17** in 30% yield (method C). Using method C, **17** was readily prepared in kilogram quantities in 30—40% yield from **7**. Though ethyl (−)-(*S*)-lactate was used as a starting material, the compound **17** thus obtained did not rotate the polarization plane, indicating that complete racemization had occurred during the addition–cyclization and/or decarboxylative hydrolysis reactions. These methods were applicable to several derivatives. 2-Demethylated (**16**), 2-ethyl (**18**), 2-propyl (**19**), and 2-butyl (**20**) analogues of **17** were prepared by method C from the corresponding *α*-hydroxycarboxylate in 22.5, 28, 20, and 14% yields, respectively. The attempted reaction of ethyl 2-methyl-2-hydroxypropionate with **7** failed to give the 2,2-dimethyl analogue, probably due to steric hindrance. The 1-thia




| compd. | method[a] | $R^1$ | X | $R^2$ |
|---|---|---|---|---|
| **9, 16** | C | $CH_3$ | O | H |
| **10, 17** | A, B, C | $CH_3$ | O | $CH_3$ |
| **11, 18** | B, C | $CH_3$ | O | $C_2H_5$ |
| **12, 19** | C | $CH_3$ | O | $n$-$C_3H_7$ |
| **13, 20** | C | $CH_3$ | O | $n$-$C_4H_9$ |
| **14, 21** | A | $CH_3$ | S | $CH_3$ |
| **15, 22** | A | $C_2H_5OCO$ | O | $CH_3$ |

a) $(C_2H_5O)_2P(O)CH_2CO_2C_2H_5$, NaH, dimethoxyethane; b , c) see method A–C.[a]

*a*) method A: b) $R^2CH(XH)CO_2R^3$ (X = O or S, $R^3$ = $CH_3$ or $C_2H_5$), NaH, DMSO; c) aqueous HCl or NaCl, DMF.
method B: same reagents as method A, but the spiro β-ketoester is not isolated.
method C: b) $R^2CH(OH)CO_2C_2H_5$, NaH, THF; c) aqueous HCl, the spiro β-ketoester is not isolated.

Chart 2

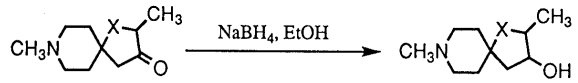
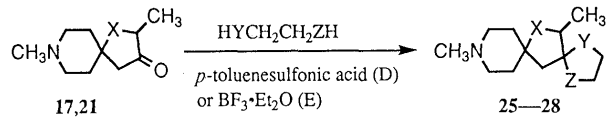



Chart 3

analogue **21** was also obtained by method A in 22% yield. In the case of the *N*-ethoxycarbonyl derivative **22**, decarboxylation of the *β*-keto ester **15** was carried out in the presence of sodium chloride under neutral conditions, since removal of the ethoxycarbonyl group occurred during acid-catalyzed hydrolysis. After our patent for these 1-oxa-8-azaspiro[4.5]decan-3-ones and their thia analogues was disclosed,[9] an alternative synthesis of **21** comprising 8 steps (36% yield from ethyl lactate) was reported by Shapiro and Lavi.[10] In spite of its low yields, our method represents a convenient synthesis of 2-monosubstituted 1-oxa-8-azaspiro[4.5]decan-3-ones and their thia analogues. This is because it requires only 2 steps and the starting materials are readily available.

Muscarine-based derivatives **23** and **24** were prepared by reduction of 1-oxa-8-azaspiro[4.5]decan-3-ones **17** and **21** with sodium borohydride (Chart 3).

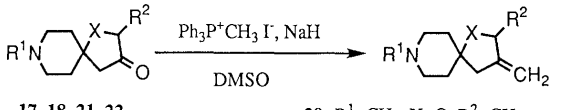
Modifications at the 3-position of muscarone-based compounds were carried out by the usual methods, as shown in Charts 4, 5, and 6, to yield ketals (**25**, **27**), thioketals (**26**, **28**), olefines (**29**—**33**), oximes (**34**—**39**), and a hydrazone (**40**). The demethylated analogue **33** was prepared by deprotection of the *N*-ethoxycarbonyl derivative **32**. Oximes were isolated as *syn* and *anti* isomers by column chromatography and recrystallization. Configuration of the isomers was assigned on the basis of $^{13}$C-NMR analyses and thin-layer chromatography as




17,21 25—28

| compd. | method | X | Y | Z |
|---|---|---|---|---|
| 25 | D | O | O | O |
| 26 | E | O | S | S |
| 27 | D | S | O | O |
| 28 | E | S | S | S |

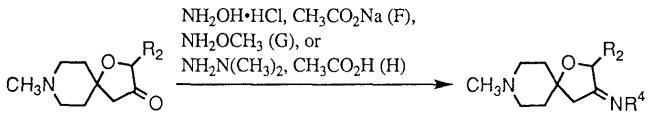
Chart 4




17, 18, 21, 22

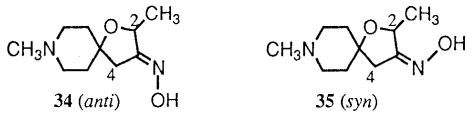
29 : $R^1$=$CH_3$, X=O, $R^2$=$CH_3$
30 : $R^1$=$CH_3$, X=O, $R^2$=$C_2H_5$
31 : $R^1$=$CH_3$, X=S, $R^2$=$CH_3$
32 : $R^1$=$C_2H_5OCO$, X=O, $R^2$=$CH_3$
33 : $R^1$=H, X=O, $R^2$=$CH_3$

Chart 5




17, 18 34—40

| compd. | method | $R^2$ | $R^4$ |
|---|---|---|---|
| 34 | F | $CH_3$ | OH(*anti*) |
| 35 | F | $CH_3$ | OH(*syn*) |
| 36 | F | $C_2H_5$ | OH(*anti*) |
| 37 | F | $C_2H_5$ | OH(*syn*) |
| 38 | G | $CH_3$ | $OCH_3$(*anti*) |
| 39 | G | $CH_3$ | $OCH_3$(*syn*) |
| 40 | H | $CH_3$ | $N(CH_3)_2$ |



34 (*anti*) 35 (*syn*)

Chart 6

follows. In the $^{13}$C-NMR spectrum of compound **34**, signals of carbons at the 2- and 4-positions were observed at 72.518 and 38.044 ppm, respectively. On the other hand, compound **35** exhibited C-2 and C-4 peaks at 71.139 and 40.513 ppm, respectively. These differences in chemical shifts were regarded as high-field shifts owing to the steric constraint induced by the hydroxy group on the imino bond ($\gamma$ effect). Thus, the geometries of **34** and **35** were assigned as *anti* and *syn*, respectively. On silica gel thin-layer chromatography using $CHCl_3$–MeOH–27% aqueous $NH_3$ (10 : 1 : 0.1, v/v) as an eluent, **34** exhibited a higher *Rf* value than **35**. In other pairs (**36** and **37**, **38** and **39**), the isomers with higher *Rf* values were assigned as *anti* isomers.

The optical resolution of the racemic **18** and **29** was effected by means of a diastereomer salt method using a chiral acid. The di-*p*-toluoyl-L-tartrate of racemic **18** was recrystallized four times from mixed solvent ($CH_2Cl_2$–ether, 1 : 1, v/v) to afford the di-*p*-toluoyl-L-tartrate of

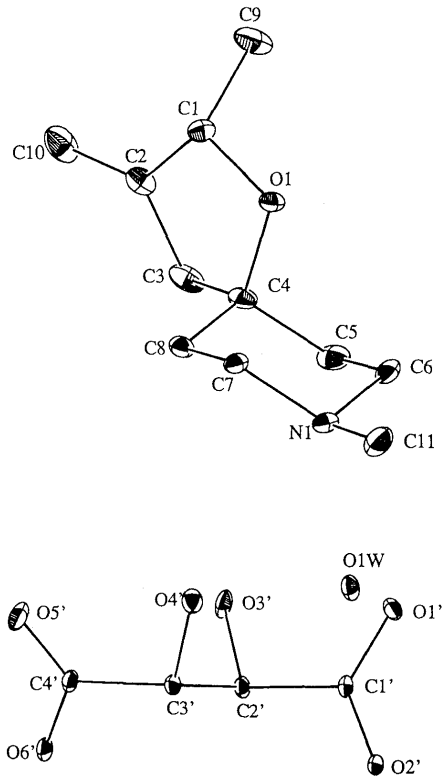



Fig. 1

(−)-**18**. The free base, (−)-**18**, was obtained through a silica gel column ($CHCl_3$–MeOH–27% aqueous $NH_3$, 10 : 1 : 0.1, v/v) and converted to the maleate. The antipode, (+)-**18**, was obtained by using di-*p*-toluoyl-D-tartaric acid. The di-*p*-toluoyl-D-tartrate of racemic **29** was recrystallized nine times from a mixed solvent (MeOH–water, 2 : 1, v/v) to afford the di-*p*-toluoyl-D-tartrate of (−)-**29**. The free base, (−)-**29**, was obtained by treatment of the salt with aqueous NaOH and converted to the sesquifumarate and tartrate monohydrate. The antipode, (+)-**29**, was obtained using di-*p*-toluoyl-L-tartaric acid. The optical purities (>97% ee) of the four optical isomers were determined by 500 MHz $^1$H-NMR analysis of the corresponding di-*p*-toluoyl-L or D-tartrate. The absolute configuration of (−)-**29** was determined to be *S* by single-crystal X-ray diffraction studies of the L-tartrate monohydrate of (−)-**29**, the structure of which is illustrated in Fig. 1.

## Pharmacology

The desired activity profile of compounds was as follows: (1) high affinity for $M_1$ receptors with high selectivity over $M_2$ receptors; (2) antiamnesic effect in the scopolamine-induced amnesia model with high selectivity over muscarinic side effects; and (3) efficacy as an $M_1$ agonist on the second messenger system, as demonstrated by stimulation of phosphoinositide (PI) hydrolysis. The affinities of compounds for $M_1$ and $M_2$ receptors were evaluated in terms of the abilities of the compounds to displace [$^3$H]PZ, an $M_1$ selective ligand, from rat cerebral cortex membrane[11] and [$^3$H]quinuclidinyl benzilate (QNB) from rat cerebellum membrane,[12] respectively. Muscarinic receptors on cerebellum membrane are re-

ported to be mostly of the $M_2$ subtype.[13] The affinities are given as $K_i$ values in comparison with those of carbachol and **1** in Table 1. The ratio of affinities, [$K_i$ (QNB)/$K_i$ (PZ)], gave a measure of $M_1$ selectivity. Antiamnesic effects were evaluated in terms of the ability to ameliorate scopolamine-induced impairments in one-trial passive avoidance tasks of rats.

As muscarinic agonists are known to induce hypothermia and tremor, effects suggested to be associated with central $M_2$ receptors,[14] and to increase salivary secretion *via* peripheral muscarinic receptors, the effects of test compounds on rectal temperature, behavior, and salivary secretion of mice were also observed, as described in Experimental. These three effects were regarded not only as indices of muscarinic activity, but also as indices of cholinergic side effects in anticipation of future clinical use. In Table 1, only the hypothermia-inducing activity of test compounds is shown for simplicity. The dose ratio, minimum effective dose (MED) (antiamnesic effect)/ $ED_{\Delta 2^{\circ}C}$ (hypothermia), can be regarded as a therapeutic index.

$M_1$ receptors in the hippocampus have been suggested to mediate PI hydrolysis as a second messenger system.[15] The ability of several compounds to stimulate PI metabolism was examined in rat hippocampal slices in comparison with carbacol as a full agonist.

## Results and Discussion

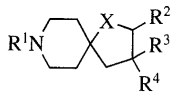
**Muscarine- and Muscarone-Based Compounds** As shown in Table 1, muscarone-based compound **17** exhibited high affinity for both $M_1$ and $M_2$ receptors with $K_i$ values of 0.37 and 0.12 $\mu$M, respectively, and showed potent antiamnesic and hypothermia-inducing effects. In contrast to **17**, muscarine-based compound **23** showed 50-fold less potent $M_1$ receptor binding affinity and had no effect in *in vivo* tests at the doses tested. These results suggest that **17**, but not **23**, is a potent centrally active muscarinic agonist (even though **23** is a mixture of diastereomers). As shown in Table 1, this relation of **17** and **23** seems to be retained in their 1-thia analogues (**21** *vs.* **24**). Since tertiary amine congeners of muscarine and muscarone are regarded as having only weak muscarinic activity,[16] compound **17** is notable as the first tertiary amine-type muscarinic agonist with a muscarone skeleton. The lack of activity in muscarine-based compounds is reminiscent of the low activity of muscarine in comparison with muscarone.[17] Replacement of an oxygen atom at the 2-position with a sulfur atom (**21** *vs.* **17**) had less effect on the affinity but slightly reduced the activity *in vivo*. The effects of the replacement of an oxygen atom with a sulfur atom at the 2-position seem to be consistent with those observed with muscarone and thiomuscarone.[18]

**Alteration of 2-Substituents** In order to find more $M_1$-selective compounds, we investigated a series of 1-oxa-8-azaspiro[4.5]decanes with various substituents at the 2- and 3-positions. First, the substituent at the 2-position of compound **17** was varied from hydrogen to *n*-butyl (**16**, **18**—**20**). The demethylated derivative **16** exhibited very low affinity for both $M_1$ and $M_2$ receptors. This finding is again consistent with the observation that the 2-methyl group on the tetrahydrofuran ring of muscarone is important for its muscarinic activity.[18] In comparison with the results for **17**, affinity for $M_1$ receptors increased when the 2-alkyl substituent was elongated to *n*-butyl. Moreover, affinity for $M_2$ receptors decreased in the case of the ethyl and *n*-propyl congeners, **18** and **19**, and was the same as that of **17** in the case of the *n*-butyl congener **20**. As a result, these alterations gave compounds with preferential affinity for $M_1$ receptors over $M_2$ receptors. Among compounds **18**—**20**, however, only the ethyl congener **18** induced hypothermia at the doses tested. The intrinsic activity of compounds **19** and **20** may be inferior to that of **18**. Interestingly, compound **18** exhibited a potent antiamnesic effect, and showed a therapeutic index 100- and 10-fold higher than those of compounds **17** and **1**, respectively.

**Alteration of 3-Substituents** The results for muscarone- and muscarine-based compounds suggest that a carbonyl group at the 3-position plays an important role in activation of the muscarinic receptor. To obtain more information about structural requirements for this locus, we performed systematic modifications at the 3-position, as shown in Table 1. Ketal and thioketal derivatives **25**—**28** exhibited interesting structure–activity relationships. The ketal derivative **25** displayed preferential affinity for $M_1$ receptors over $M_2$ receptors in contrast to **17**, despite having lower binding affinity than **17**. Corresponding with the affinity, **25** exhibited weak antiamnesic and hypothermia-inducing activities. Sequential replacement of oxygen atoms with sulfur atoms increased the affinity for both $M_1$ and $M_2$ receptors (**27**, **26**, **28**); **28** showed more than 100-fold higher affinity for both $M_1$ and $M_2$ receptors than **25**. These compounds, **26**—**28**, had the same degree of $M_1$ selectivity as **25**, and exhibited both antiamnesic and hypothermia-inducing activities, with high selectivity for the former as compared with **25**. Among them, compound **28** had the highest affinity, as well as the highest selectivity for $M_1$ receptors and 100-fold higher therapeutic indices than **1**. These results suggest that bulky substituents such as a five-membered ketal are tolerated in the interaction with muscarinic receptors.

In the next step, the carbonyl group of muscarone-based compounds was altered to a methylene or an imino group, both of which are similar to a carbonyl group in that they have $\pi$-electrons. Compound **29**, having a methylene group, unexpectedly exhibited high affinity and a degree of selectivity for $M_1$ receptors. In addition, **29** had potent antiamnesic and modest hypothermia-inducing activity, resulting in a therapeutic index 27-fold higher than that of **1**. Replacement of an oxygen atom with a sulfur atom (**31**) and that of a methyl group at the 2-position with an ethyl group (**30**) had little effect on $M_1$ binding affinity but diminished the antiamnesic activity. *N*-Demethylation decreased both activities *in vitro* and *in vivo*. The carbonyl group of muscarone has been regarded as being important in the interaction of this compound with muscarinic receptors[17a,19] and a hydrogen bond[20] or dipole–dipole interaction[19] was suggested to be involved in this interaction. Though the olefin group seems to be a poor hydrogen bond acceptor and to be less polar than a carbonyl group, these results suggest that an olefin group is tolerated as a substituent of a carbonyl group in the

Table 1. Muscarinic Activities of 1-Oxa-8-azaspiro[4.5]decane Derivatives and Their Thia Analogues



| Compound | Structure | | | | Binding data: $K_i$,[a] $\mu$M | | | Antiamnesic effect,[e] MED (mg/kg, s.c.) | Hypothermia $ED_{A2\,°C}$ (mg/kg, s.c.) | Ratio[f] |
|---|---|---|---|---|---|---|---|---|---|---|
| | $R^1$ | X | $R^2$ | $R^3$, $R^4$ | [$^3$H]PZ[b] | [$^3$H]QZB[c] | $M_2/M_1$ index[d] | | | |
| **16**·HCl | $CH_3$ | O | H | =O | 10 (9.9—11) | 18 (16—20) | 1.8 | NT | 30 | |
| **17**·HCl | $CH_3$ | O | $CH_3$ | =O | 0.37 (0.36—0.38) | 0.12 (0.11—0.12) | 0.3 | 0.3 | 0.3 | 1 |
| **18**·maleate | $CH_3$ | O | $C_2H_5$ | =O | 0.49 (0.48—0.50) | 0.89 (0.84—0.95) | 1.8 | 0.03 | 3 | 100 |
| **19**·maleate | $CH_3$ | O | $n$-$C_3H_7$ | =O | 0.18 (0.17—0.19) | 0.51 (0.50—0.52) | 2.8 | NT | >30 | |
| **20**·maleate | $CH_3$ | O | $n$-$C_4H_9$ | =O | 0.067 (0.062—0.072) | 0.17 (0.17—0.18) | 2.5 | NT | >30 | |
| **21**·HCl | $CH_3$ | S | $CH_3$ | =O | 0.32 (0.25—0.39) | 0.27 (0.23—0.31) | 0.8 | 1 | 3 | 3 |
| **23**·HCl | $CH_3$ | O | $CH_3$ | H, OH | 19 (19—19) | NT | | >10 | >30 | |
| **24**·HCl | $CH_3$ | S | $CH_3$ | H, OH | 15 (11—20) | NT | | >30[g] | >30 | |
| **25**·maleate | $CH_3$ | O | $CH_3$ | $-OCH_2CH_2O-$ | 5.3 (4.9—5.7) | 17 (15—19) | 3.2 | 1 | 3 | 3 |
| **26**·maleate | $CH_3$ | O | $CH_3$ | $-SCH_2CH_2S-$ | 0.19 (0.19—0.20) | 0.56 (0.55—0.57) | 2.9 | 0.1 | 30 | 300 |
| **27**·maleate | $CH_3$ | S | $CH_3$ | $-OCH_2CH_2O-$ | 2.6 (2.6—2.6) | 5.6 (5.1—6.3) | 2.2 | 0.1 | 3 | 30 |
| **28**·maleate | $CH_3$ | S | $CH_3$ | $-SCH_2CH_2S-$ | 0.030 (0.029—0.030) | 0.13 (0.13—0.14) | 4.3 | 0.03 | 30 | 1000 |
| **29**·fumarate | $CH_3$ | O | $CH_3$ | $=CH_2$ | 1.6 (1.5—1.6) | 3.1 (2.7—3.6) | 1.9 | 0.03 | 8 | 270 |
| **30**·HCl | $CH_3$ | O | $C_2H_5$ | $=CH_2$ | 0.94 (0.93—0.96) | 1.9 (1.8—2.0) | 2.0 | 0.3 | 30 | 100 |
| **33**·HCl | H | O | $CH_3$ | $=CH_2$ | 5.3 (4.9—5.6) | 4.2 (3.4—5.3) | 0.8 | NT | >30 | |
| **31**·HCl | $CH_3$ | S | $CH_3$ | $=CH_2$ | 1.8 (1.8—1.8) | 1.4 (1.3—1.5) | 0.8 | 1 | 30 | 30 |
| **34** | $CH_3$ | O | $CH_3$ | =NOH (*anti*) | 0.62 (0.60—0.64) | 0.46 (0.44—0.49) | 0.7 | 0.03 | 3 | 100 |
| **35** | $CH_3$ | O | $CH_3$ | =NOH (*syn*) | 0.18 (0.17—0.18) | 0.095 (0.076—0.118) | 0.5 | >1 | 3 | |
| **36** | $CH_3$ | O | $C_2H_5$ | =NOH (*anti*) | 0.34 (0.31—0.37) | 0.83 (0.81—0.85) | 2.4 | 3 | >30 | >10 |
| **37** | $CH_3$ | O | $C_2H_5$ | =NOH (*syn*) | 0.034 (0.033—0.036) | 0.054 (0.052—0.055) | 1.6 | 0.3 | >30 | >100 |
| **38**·maleate | $CH_3$ | O | $CH_3$ | $=NOCH_3$ (*anti*) | 1.6 (1.6—1.7) | 3.6 (3.4—3.7) | 2.3 | 10 | 30 | 3 |
| **39**·maleate | $CH_3$ | O | $CH_3$ | $=NOCH_3$ (*syn*) | 0.57 (0.50—0.65) | 0.88 (0.75—1.03) | 1.5 | 3 | 1 | 0.3 |
| **40**·fumarate | $CH_3$ | O | $CH_3$ | $=NN(CH_3)_2$ | 0.65 (0.63—0.66) | 0.97 (0.94—1.00) | 1.5 | NT | 0.3 | |
| **1**·HBr | | | | | 0.46 (0.45—0.46) | 0.97 (0.95—1.00) | 2.1 | 0.1 | 1 | 10 |
| Carbachol | | | | | 1.5 (1.4—1.6) | 0.11 (0.11—0.12) | 0.073 | | | |

*a*) $K_i$ values were determined by experiments performed in triplicate. Values in parentheses are 95% confidence intervals. *b*) Displacement of [$^3$H]pirenzepine from rat cortex membrane preparations. *c*) Displacement of [$^3$H]quinuclidinyl benzilate from rat cerebellum membrane preparations. *d*) The ratio of QNB/PZ, $K_i$'s. *e*) Ameliorating effects on scopolamine-induced impairment of rat passive avoidance tasks. *f*) The ratio of $ED_{A2\,°C}$ (hypothermia)/MED (antiamnesic effect). *g*) Mice were used as described previously.[6] NT: Not tested.

interaction with muscarinic receptors, especially $M_1$ receptors. These findings are consistent with the observation by De Amici *et al.* that a methylene analogue of muscarone possessed a potency similar to that of muscarine.[17a]

As stable imino derivatives, oxime (**34—39**) and hydrazone (**40**) derivatives were examined. The oximes were prepared as pure geometrical isomers, *syn* and *anti* isomers. Most of these compounds exhibited affinity for $M_1$ receptors as high as those of the corresponding

Table 2. Effects of Selected Compounds on Phosphoinositide Turnover in Rat Hippocampal Slices[a]

| Compound | Control | Test compound | | | Carbachol ($10^{-4}$ M)[b] |
|---|---|---|---|---|---|
| | | $10^{-5}$ M | $10^{-4}$ M | $10^{-3}$ M | |
| **17** | 0.172±0.003 | 0.361±0.016*** (62%)[c] | 0.452±0.006*** (92%) | 0.460±0.012*** (94%) | 0.478±0.019*** |
| **18** | 0.181±0.004 | 0.203±0.0035** (6%) | 0.232±0.011*** (14%) | 0.234±0.007*** (14%) | 0.542±0.006*** |
| **29** | 0.180±0.005 | 0.185±0.006 | 0.216±0.008** (11%) | 0.208±0.004* (8%) | 0.512±0.015*** |
| **1** | 0.180±0.005 | 0.229±0.006*** (15%) | 0.251±0.011*** (21%) | 0.268±0.007*** (27%) | 0.512±0.015*** |

*a*) Results are expressed as [$^3$H]IPs/([$^3$H]lipids + [$^3$H]IPs) ± S.E. of triplicate experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ *vs.* control. *b*) Effects of carbachol ($10^{-4}$ M) determined in the same experiment with the test compound are shown as an index of a full agonist. *c*) The increase of [$^3$H]IPs/([$^3$H]lipids + [$^3$H]IPs) is expressed as a percentage of that elicited by carbachol ($10^{-4}$ M).

carbonyl congeners. The *syn* isomers seemed to have slightly higher affinity for both $M_1$ and $M_2$ receptors than the corresponding *anti* isomers, but these binding data did not parallel the results of *in vivo* tests. Among these compounds, only two, **34** (*anti* isomer) and **37** (*syn* isomer), had potent antiamnesic activity. The *syn* isomer (**35**) of **34** did not have antiamnesic activity, although it had higher affinity than **34**. The hydrazone derivative **40** exhibited potent hypothermia-inducing activity, suggesting a potent $M_2$ agonistic activity. Overall, although substitution of a carbonyl group with various imino groups increased $M_1$ binding affinity or $M_1$ selectivity, the binding data for these compounds were reflected in *in vivo* activity in a complicated manner. To obtain a better understanding of these phenomena, the efficacy of these compounds as $M_1$ and $M_2$ agonists and bioavailability should be assessed.

**Stimulation of PI Hydrolysis** Modification of the muscarone-based compound **17** afforded several compounds with a degree of selectivity in both *in vitro* and *in vivo* tests. To assess their efficacy as $M_1$ agonists, some of these compounds were evaluated in a functional model of $M_1$ receptor activation, namely stimulation of PI hydrolysis. Data for compounds that exhibited significant potentiation are shown in Table 2 (carbachol and compound **1** were used as reference compounds). Muscarone-based compound **17** enhanced the hydrolysis of PI with an efficacy similar to that of carbachol and higher than that of **1**. Compound **18** had an efficacy similar to that of **1**. Compound **29** consistently exhibited significant, though marginal, stimulation of PI hydrolysis. The ethyl analogue (**30**) of **29**, the ketal **25**, the thioketal **26**, and the oxime **37** did not enhance PI hydrolysis. It should be noted that stimulation of PI hydrolysis is one of several methods used for assessment of $M_1$ agonistic activity.[21] Although more investigations would be necessary to understand the potent antiamnesic effects of the compounds that are negative in the stimulation of PI hydrolysis, one probable explanation is that they have weak partial agonistic activity undetectable in this system and such weak partial agonists also elicit antiamnesic effects in an amnesia model induced by the muscarinic antagonist scopolamine.[22]

Apparently, compounds with high selectivity for antiamnesic effects over hypothermia-inducing effects tend to have low efficacy as $M_1$ agonists. The nonselective compound **17** was the most efficacious. Compounds **18** and **29**, with high selectivity, exhibited rather low efficacy. These results seem to be in line with previously reported observations that low-efficacy muscarinic agonists display functional subtype selectivity.[22] The degree of muscarinic efficacy appropriate for the treatment of DAT remains controversial.[22,23] We thought that candidate compounds for further evaluation should have sufficient efficacy to stimulate PI hydrolysis. Thus, compounds **18** and **29** were selected for further studies.

Table 3. $M_1$ and $M_2$ Receptor Binding Affinities of Enantiomers of **18** and **29**

| Compound | Binding data: $K_i$,[a] μM | |
|---|---|---|
| | [$^3$H]PZ[b] | [$^3$H]QNB[c] |
| (−)-**18** | 0.32 (0.31—0.32) | 0.47 (0.39—0.57) |
| (+)-**18** | 0.57 (0.56—0.57) | 1.7 (1.7—1.8) |
| (−)-**29** | 1.2 (1.1—1.2) | 2.1 (1.9—2.3) |
| (+)-**29** | 3.8 (3.5—4.1) | 12 (12—13) |

*a*—*c*) See footnotes for Table 1.

**Enantiomers of 18 and 29** Both compounds **18** and **29** have a stereogenic center at the 2-position. Thus, it is important to investigate the activity of their optical isomers. As shown in Table 3, the eudismic ratios of **18** and **29** in binding affinity were small and both (−)-isomers exhibited a slightly higher affinity than the (+)-isomers. It should be noted that (−)-**29**, but not (−)-**18**, exhibited selectivity for $M_1$ receptors similar to that observed with the racemate. Interestingly, only the (−)-isomers stimulated PI-hydrolysis, as shown in Table 4, indicating that the efficacy of both racemates resides in the (−)-isomers. The absolute configuration of (−)-**29** is *S*, which is the same as that of muscarone.[17b] These results, together with the similarity in structure–activity relationships, suggest that **29** and the related derivatives interact with $M_1$ receptors in a manner similar to muscarone.

As shown in Table 5, oral administrations of (−)-**18** and (−)-**29** elicited muscarinic effects with higher selectivity for antiamnesic effect over the other muscarinic effects than that of **1**, being similar to the results observed with the racemates. Although the $M_1$ selectivity of (−)-**29** in binding affinity may not be enough to explain the high selectivity *in vivo*, functional studies in transfected cells

Table 4. Effects of Enantiomers of **18** and **29** on Phosphoinositide Turnover in Rat Hippocampal Slices[a]

| Compound | Control | Test compound | | | Carbachol ($10^{-4}$ M)[b] |
|---|---|---|---|---|---|
| | | $10^{-5}$ M | $10^{-4}$ M | $10^{-3}$ M | |
| (−)-**18** | 0.152±0.006 | 0.209±0.002*** (20%)[c] | 0.209±0.010** (20%) | 0.216±0.006*** (23%) | 0.435±0.012*** |
| (+)-**18** | 0.152±0.006 | 0.161±0.005 | 0.163±0.004 | 0.152±0.007 | 0.435±0.012*** |
| (−)-**29** | 0.228±0.004 | 0.247±0.008* (4.4%) | 0.279±0.012*** (12%) | 0.258±0.009** (7%) | 0.660±0.012***[d] |
| (+)-**29** | 0.228±0.004 | 0.232±0.007 | 0.238±0.005 | 0.228±0.007 | 0.660±0.012***[d] |

*a*—*c*) See footnotes for Table 2. *d*) Carbachol $10^{-3}$ M.

Table 5. Pharmacological Profiles of (−)-**18** and (−)-**29** in Comparison with Reference Compound **1**

| Compd. | Antiamnesic effect[a] MED (mg/kg, *p.o.*) | Hypothermia[b] $ED_{\Delta 2\,°C}$ (mg/kg, *p.o.*) | Tremor[b] MED (mg/kg, *p.o.*) | Salivation[b] $ED_{30\,mg}$ (mg/kg, *p.o.*) |
|---|---|---|---|---|
| (−)-**18** | 0.13 | 1 | 5 | 0.5 |
| (−)-**29**[c] | 0.03 | 23 | >64 | 11 |
| **1** | 0.5 | 0.45 | 5 | 0.2 |

*a*) In rats. *b*) In mice. *c*) Tartrate monohydrate was used.

Table 6. Postitional Parameters and *B*(eq) for (−)-**29** L-Tartrate Monohydrate

| Atom | *x* | *y* | *z* | *B*(eq) |
|---|---|---|---|---|
| O(1) | 0.1710 (02) | 0.80294 (12) | −0.3125 (04) | 3.73 (5) |
| N(1) | 0.0500 (02) | 0.6392 (02) | −0.1312 (05) | 4.51 (8) |
| C(1) | 0.1875 (03) | 0.8570 (02) | −0.4650 (06) | 3.94 (8) |
| C(2) | 0.2306 (02) | 0.8071 (02) | −0.6131 (06) | 4.7 (1) |
| C(3) | 0.2482 (03) | 0.7258 (03) | −0.5290 (08) | 5.7 (1) |
| C(4) | 0.1798 (02) | 0.7207 (02) | −0.3751 (06) | 4.25 (8) |
| C(5) | 0.2081 (03) | 0.6715 (03) | −0.2095 (09) | 6.0 (1) |
| C(6) | 0.1378 (04) | 0.6712 (03) | −0.0594 (07) | 5.8 (1) |
| C(7) | 0.0185 (03) | 0.6881 (02) | −0.2924 (06) | 3.81 (8) |
| C(8) | 0.0881 (03) | 0.6900 (02) | −0.4443 (06) | 3.86 (8) |
| C(9) | 0.2446 (05) | 0.9255 (03) | −0.3961 (10) | 7.4 (2) |
| C(10) | 0.2456 (05) | 0.8321 (05) | −0.7835 (09) | 7.8 (2) |
| C(11) | −0.0194 (05) | 0.6351 (03) | 0.0159 (07) | 6.4 (1) |
| C(1′) | 0.0772 (02) | 0.4191 (02) | −0.1029 (04) | 2.38 (6) |
| C(2′) | 0.0831 (02) | 0.4135 (02) | −0.3130 (04) | 2.43 (6) |
| C(3′) | −0.0105 (02) | 0.4253 (02) | −0.3980 (04) | 2.37 (6) |
| C(4′) | −0.0083 (02) | 0.4244 (02) | −0.6075 (04) | 2.57 (6) |
| O(1′) | 0.1067 (02) | 0.48087 (13) | −0.0290 (03) | 3.63 (5) |
| O(2′) | 0.0426 (02) | 0.35913 (12) | −0.0222 (03) | 2.73 (4) |
| O(3′) | 0.1407 (02) | 0.4737 (02) | −0.3858 (03) | 3.77 (5) |
| O(4′) | −0.0469 (02) | 0.50005 (14) | −0.3388 (03) | 3.59 (5) |
| O(5′) | −0.0409 (02) | 0.4794 (01) | −0.6930 (03) | 4.23 (5) |
| O(6′) | 0.0289 (02) | 0.36068 (13) | −0.6797 (03) | 3.19 (5) |
| O(1W) | 0.3049 (02) | 0.4707 (02) | −0.2296 (04) | 3.31 (5) |

expressing the gene for $M_1$ and $M_2$ muscarinic receptors also indicated high functional selectivity of (−)-**29** for $M_1$ over $M_2$ subtype.[24] In addition, (−)-**29** exhibited little or no affinity for various other receptors related to learning behavior, namely adrenoceptors ($\alpha_1$, $\alpha_2$, $\beta$), nicotinic, dopamine ($D_1$, $D_2$), 5-hydroxytryptamine (5-HT) (5-$HT_1$, 5-$HT_2$), $\gamma$-aminobutyric acid (GABA) ($GABA_A$), $\mu$-opioid, and benzodiazepine receptors (unpublished data). Thus the high selectivity *in vivo* of (−)-**29** is presumably ascribed to its functional selectivity and efficacy for a specific muscarinic receptor subtype.

## Conclusion

Compound **17**, designed to combine features from the structure of muscarone and an 8-azaspiro[4.5]decane skeleton, is a potent but nonselective muscarinic agonist. Systematic modifications of **17** gave a number of muscarinic ligands with a degree of $M_1$ receptor selectivity and potent ameliorating activity on scopolamine-induced impairment in passive avoidance tasks, sufficiently separated from cholinergic side effects. These muscarinic activities of 1-oxa-8-azaspiro[4.5]decanes depend on the structure of the five-membered ring moiety in a similar way to that reported for muscarone and its derivatives. These results suggest that compound **17** and related compounds interact with the muscarinic receptor in a similar manner to muscarone and muscarine, and support the usefulness of the 8-azaspiro[4.5]decane skeleton as a basic template for designing novel muscarinic agonists.

A functional test for assessing $M_1$ agonistic activity revealed that the structural requirements for $M_1$ receptor activation are strict when compared to the apparently considerable tolerance in *in vivo* muscarinic activity, and only **18** and **29** among the selected compounds are $M_1$ partial agonists.[25] Investigations of the enantiomers of **18** and **29** revealed the $M_1$ agonistic activity resides on the both levorotatory isomers. These results, together with the high therapeutic indices in comparison with that of **1**, suggest that (−)-**29** could be a candidate drug for the symptomatic treatment of DAT. The tartrate monohydrate of (−)-**29** is under clinical trial as YM796.[25]

### Experimental

All melting points were determined with a Yanaco MP-3 melting point apparatus and are uncorrected. $^1$H-NMR spectra were measured with a JEOL FX90Q or a FX100 spectrometer; chemical shifts are recorded in $\delta$ units from tetramethylsilane as an internal standard and the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad, and dd=double doublet. $^{13}$C-NMR spectra were recorded with a JEOL FX90Q at 22.5 MHz and were referenced to internal tetramethylsilane. Mass spectra were recorded with a Hitachi M-80 or JEOL JMS-DX300 spectrometer. Infrared (IR) spectra were recorded on a Hitachi 270-30 IR spectrophotometer. All solutions were dried over anhydrous magnesium sulfate.

**2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one (17) [Method A]** (i) Ethyl 2,8-Dimethyl-3-oxo-1-oxa-8-azaspiro[4.5]decane-4-carboxylate (**10**): Sodium hydride (60% mineral oil dispersion, 4 g, 100 mmol) was washed with *n*-hexane and suspended in anhydrous ether (150 ml). A solution of ethyl lactate (11.8 g, 100 mmol) in anhydrous ether (50 ml) was added to the suspension at 5—10 °C. The suspension was stirred at room temperature for 3 h, by which time the evolution of hydrogen gas ceased. The ether was distilled off under reduced pressure and the residue

was dissolved in DMSO. This solution was cooled to 15 °C, and ethyl (1-methyl-4-piperidinylidene)acetate (**7**, 18.3 g, 100 mmol)[8] was added to it. After being stirred at room temperature for about 20 h, the mixture was poured into ice-water (200 ml) and acidified with aqueous 36% HCl to pH 4. Then the mixture was basified to about pH 8 with $NaHCO_3$, saturated with NaCl, and extracted with $CHCl_3$ (3 × 300 ml). The combined extract was washed, dried, and concentrated *in vacuo*. The residue was purified through a silica gel column ($CHCl_3$–MeOH–27% aqueous $NH_3$, 10 : 1 : 0.1, v/v) to give 2.9 g of **10** in 11% yield as a solid; IR (KBr): 1672, 1552 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 1.16—1.48 (6H, m), 1.70—2.00 (4H, m), 2.32 (3H, s), 2.30—2.80 (4H, m), 4.00—4.20 (3H, m). MS *m/z*: 255 ($M^+$), 181, 136. *Anal.* Calcd for $C_{13}H_{21}NO_4$: C, 61.16; H, 8.29; N, 5.49. Found: C, 61.03; H, 8.24; N, 5.42.

(ii) 2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one (**17**): Compound **10** (3.08 g, 12 mmol) was dissolved in 1 N HCl (50 ml) and the solution was heated under reflux for 8 h. The mixture was cooled in an ice-water bath, basified with 20% aqueous NaOH and extracted with $CHCl_3$. The combined extract was washed, dried, and concentrated in vacuo. The residue was purified through a silica gel column ($CHCl_3$–MeOH, 20 : 1, v/v) to give 1.8 g (82% yield) of **17** as a pale yellow oil. Hydrochloride; mp 179—181 °C (dec.). IR (KBr): 1754 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 1.30 (3H, d, $J$=7 Hz), 1.80—2.50 (4H, m), 2.48 (2H, s), 2.80 (3H, d), 3.00—3.50 (4H, m), 3.98 (1H, q, $J$=7 Hz). MS *m/z*: 183 ($M^+$), 110. *Anal.* Calcd for $C_{10}H_{17}NO_2 \cdot HCl$: C, 54.67; H, 8.26; N, 6.38. Found: C, 54.40; H, 8.27; N, 6.31.

**[Method B]** The reaction mixture containing the ketoester **10** obtained from 50 mmol of ethyl lactate by method A was poured into ice-water (100 ml) and acidified with 36% aqueous HCl to pH 2. Additional 36% aqueous HCl (4 ml) was added, and the mixture was heated for 6 h at 130 °C (oil-bath temperature), then treated as described for method A (ii) to give **17** in 18.5% yield.

**[Method C]** Ethyl lactate (67.7 g, 0.57 mol) was added to a suspension of sodium hydride (60% mineral oil dispersion, 23 g, 0.57 mol) in anhydrous THF (230 ml) at 5—8 °C and the mixture was stirred for 1.5 h at room temperature. It was then cooled to 5 °C and **7** (100 g, 0.55 mol) was added at once. After being stirred for 2 d at 25 °C, the mixture was poured into 860 ml of ice-water and washed with ethyl acetate twice. The aqueous solution was acidified with 315 ml of 36% aqueous HCl and heated for 4 h at 100 °C (oil-bath temperature). The same treatment of the mixture as in method A (ii), followed by distillation, afforded 31.5 g of **17** (30% yield) as a pale yellow oil; bp 80 °C (1.8 mmHg).

**8-Methyl-1-oxa-8-azaspiro[4.5]decan-3-one (16)** Compound **16** was prepared by method C in 22.5% yield from ethyl hydroxyacetate and **7**. Hydrochloride; $^1$H-NMR ($CDCl_3$) $\delta$: 1.80—2.20 (2H, m), 2.47 (2H, s), 2.30—3.80 (6H, m), 2.84 (3H, d, $J$=5 Hz), 4.05 (2H, s). MS *m/z*: 170 ($[M+1]^+$). *Anal.* Calcd for $C_9H_{15}NO_2 \cdot HCl \cdot 0.7H_2O$: C, 49.52; H, 8.03; N, 6.42. Found: C, 49.56; H, 8.54; N, 6.50.

**2-Ethyl-8-methyl-1-oxa-8-azaspiro[4.5]decan-3-one (18)** Compound **18** was prepared by method B in 14% yield and by method C in 28% yield from ethyl 2-hydroxybutyrate and **7**. Maleate; mp 126—128 °C (EtOH). $^1$H-NMR ($CDCl_3$) $\delta$: 1.00 (3H, t, $J$=7 Hz), 1.50—2.60 (6H, m), 2.46 (2H, m), 2.88 (3H, s), 3.10—3.60 (4H, m), 3.95 (1H, dd, $J$=5, 6 Hz) 6.32 (2H, s). MS *m/z*: 197 ($M^+$), 168, 110. *Anal.* Calcd for $C_{11}H_{19}NO_2 \cdot C_4H_4O_4$: C, 57.50; H, 7.40; N, 4.47. Found: C, 57.47; H, 7.27; N, 4.46.

**Optical Resolution of Racemic 2-Ethyl-8-methyl-1-oxa-8-azaspiro-[4.5]decan-3-one (18)** The racemate **18** (14 g, 71 mmol) and di-*p*-toluoyl-L-tartaric acid monohydrate (29.5 g, 71 mmol) were dissolved in $CH_2Cl_2$ and the solution was concentrated. The residue was treated with ether to yield the di-*p*-toluoyl-L-tartaric acid salt of **18** as crystals. The crystals were recrystallized **4** times from mixed solvents ($CH_2Cl_2$–ether, 1 : 1) to yield the di-*p*-toluoyl-L-tartaric acid salt of (−)-**18** (3.59 g, 9% yield). This product was purified through a silica gel column ($CHCl_3$–MeOH–27% aqueous $NH_3$, 10 : 1 : 0.1, v/v) to afford the free base of (−)-**18** (1.18 g) as an oil. This was treated with an equimolar amount of maleic acid in MeOH to afford colorless crystals (0.67 g) after recrystallization from MeOH. mp 128—130 °C. $[\alpha]_D^{20}$ −59.5 ($c$=0.62, MeOH). *Anal.* Calcd for $C_{11}H_{19}NO_2 \cdot C_4H_4O_4$: C, 57.50; H, 7.40; N, 4.47. Found: C, 57.21; H, 7.31; N, 4.45. Optical purity was determined to be >97% ee by 500 MHz $^1$H-NMR of the di-*p*-toluoyl-L-tartaric acid salt of (−)-**18**. The peaks due to a proton at the 2-position were used.

The antipode, (+)-**18** maleate, was also obtained using di-*p*-toluoyl-D-tartaric acid in a similar manner. mp 128—130 °C. $[\alpha]_D^{20}$ +59.7 ($c$=0.60, MeOH). *Anal.* Calcd for $C_{11}H_{19}NO_2 \cdot C_4H_4O_4$: C, 57.50; H, 7.40; N, 4.47. Found: C, 57.37; H, 7.35; N, 4.43. Optical purity >97% (500 MHz $^1$H-NMR of the di-*p*-toluoyl-D-tartaric acid salt of (+)-**18**).

**8-Methyl-2-propyl-1-oxa-8-azaspiro[4.5]decan-3-one (19)** Compound **19** was prepared by method C in 20% yield from ethyl 2-hydroxypentanoate and **7**. Maleate; mp 129—131 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 0.94 (3H, m), 1.30—1.80 (4H, m), 1.80—2.60 (4H, m), 2.80 (3H, s), 3.00—3.60 (4H, m), 3.93 (1H, dd, $J$=5, 6 Hz), 2.50 (2H, s), 6.28 (2H, s). MS *m/z*: 211 ($M^+$), 110. *Anal.* Calcd for $C_{12}H_{21}NO_2 \cdot C_4H_4O_4$: C, 58.70; H, 7.70; N, 4.28. Found: C, 58.61; H, 7.79; N, 4.28.

**2-Butyl-8-methyl-1-oxa-8-azaspiro[4.5]decan-3-one (20)** Compound **20** was prepared by method C in 14% yield from ethyl 2-hydroxyhexanoate. Maleate; mp 142.0—142.5 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 0.92 (3H, m), 1.20—1.80 (6H, m), 1.80—2.50 (4H, m), 2.44 (2H, s), 2.84 (3H, s), 3.00—3.64 (4H, m), 3.92 (1H, m), 6.28 (2H, s). MS *m/z*: 225 ($M^+$), 196, 138, 110. *Anal.* Calcd for $C_{13}H_{23}NO_2 \cdot C_4H_4O_4$: C, 59.81; H, 7.97; N, 4.10. Found: C, 59.76; H, 7.88; N, 4.02.

**2,8-Dimethyl-1-thia-8-azaspiro[4.5]decan-3-one (21)** Compound **21** was prepared according to method A except for the use of MeOH–ether mixed solvent in the formation of the sodium salt of ethyl thiolactate.

(i) Ethyl 2,8-Dimethyl-3-oxo-1-thia-8-azaspiro[4.5]decane-4-carboxylate (**14**): **14** was obtained in 31% yield. Hydrochloride; mp 161—164 °C. IR (KBr): 1660, 1620 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 1.50 (3H, t, $J$=7 Hz), 1.54 (3H, d, $J$=7 Hz), 1.70—2.00 (4H, m), 2.78 (3H, s), 2.90—3.60 (4H, m), 4.19 (1H, q, $J$=7 Hz), 4.44 (2H, q, $J$=7 Hz). MS *m/z*: 271, 238, 225. *Anal.* Calcd for $C_{13}H_{21}NO_3S \cdot HCl \cdot 0.8H_2O$: C, 48.45; H, 7.38; N, 4.35. Found: C, 48.50; H, 7.01; N, 4.32.

(ii) 2,8-Dimethyl-1-thia-8-azaspiro[4.5]decan-3-one (**21**): Compound **21** was obtained in 71% yield as an oil. Hydrochloride; mp 210—213 °C. IR (KBr): 1753 $cm^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 1.44 (3H, d, $J$=7 Hz), 1.90—2.80 (4H, m), 2.74 (2H, s), 2.80 (3H, s), 2.90—3.50 (4H, m), 3.66 (1H, q, $J$=7 Hz). MS *m/z*: 199 ($M^+$), 166, 110. *Anal.* Calcd for $C_{10}H_{17}NOS \cdot HCl \cdot 0.5H_2O$: C, 49.07; H, 7.82; N, 5.72. Found: C, 49.15; H, 7.63; N, 5.77.

**3-Hydroxy-2,8-dimethyl-1-oxa-8-azaspiro[4.5]decane (23)** Sodium borohydride (25 mg) was added to a solution of **17** (200 mg, 1.1 mmol) in EtOH (7 ml), and the mixture was stirred for 2 h at room temperature. The reaction mixture was cooled in an ice-water bath, acidified with 6 N HCl, stirred for 20 min, and concentrated *in vacuo*. The residue was purified through a silica gel column ($CHCl_3$–MeOH–27% aqueous $NH_3$, 5 : 1 : 0.1, v/v) to give 29 mg of **23** as a colorless oil in 14% yield. The NMR data shown below indicated that this product consisted of diastereomers. Hydrochloride; mp 174—178 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 1.25 (3H, m), 1.60—2.60 (6H, m), 3.74 (3H, d, $J$=5 Hz), 3.00—3.40 (4H, m), 3.80—4.30 (2H, m). MS *m/z*: 185 ($M^+$), 168, 110. *Anal.* Calcd for $C_{10}H_{19}NO_2 \cdot HCl$: C, 54.17; H, 9.09; N, 6.32. Found: C, 53.90; H, 9.22; N, 6.27.

**3-Hydroxy-2,8-dimethyl-1-thia-8-azaspiro[4.5]decane (24)** Compound **24** was prepared by the method described for **23** in 98% yield. The NMR data shown below indicated that this product consisted of diastereomers. Hydrochloride; mp 225—229 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 1.32 (3H, d, $J$=7 Hz), 1.75—2.70(6H, m), 2.77 (3H, d, $J$=5 Hz), 2.90—3.90 (5H, m), 4.04 (0.3H, m), 4.38 (0.7H, m). MS *m/z*: 201 ($M^+$), 168, 110. *Anal.* Calcd for $C_{10}H_{19}NOS \cdot HCl$: C, 50.51; H, 8.48; N, 5.89. Found: C, 50.37; H, 8.38; N, 5.81.

**10,14-Dimethyl-1,4,13-trioxa-10-azadispiro[4.1.5.2]tetradecane (25). [Method D]** A mixture of **17** (730 mg, 4 mmol), ethylene glycol (2.25 ml), *p*-toluenesulfonic acid monohydrate (836 mg, 4.4 mmol), and toluene (30 ml) was heated under reflux for 3 h with a Dean–Stark azeotropic dehydration apparatus. The reaction mixture was poured into an aqueous solution (30 ml) of $NaHCO_3$ (1.26 g) and extracted with $CHCl_3$. The extract was dried and concentrated *in vacuo*, and the residue was purified through a silica gel column ($CHCl_3$–MeOH–27% aqueous $NH_3$, 10 : 1 : 0.1, v/v) to give 640 mg of **25** as an oil in 72% yield. Maleate; mp 106—108 °C ($CH_2Cl_2$–ether). $^1$H-NMR ($CDCl_3$) $\delta$: 1.15 (3H, d, $J$=7 Hz), 1.90—2.10 (6H, m), 2.78 (3H, s), 3.00—3.50 (4H, m), 3.80—4.10 (5H, m), 6.28 (2H, s). MS *m/z*: 227 ($M^+$), 182, 110. *Anal.* Calcd for $C_{12}H_{21}NO_3 \cdot C_4H_4O_4$: C, 55.97; H, 7.34; N, 4.08. Found: C, 55.81; H, 7.14; N, 4.04.

**10,14-Dimethyl-1,4-dioxa-13-thia-10-azadispiro[4.1.5.2]tetradecane (27)** Compound **27** was prepared according to method D as an oil in 82% yield. Maleate; mp 143—145 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 1.24 (3H, d, $J$=7 Hz), 2.08—2.30 (6H, m), 2.76 (3H, s), 2.70—3.10 (2H, m), 3.30—3.60 (3H, m), 4.00 (4H, s), 6.18 (2H, s). MS *m/z*: 243($M^+$), 210. *Anal.* Calcd for $C_{12}H_{21}NO_2S \cdot C_4H_4O_4$: C, 53.46; H, 7.01; N, 3.90.

Found: C, 53.21; H, 6.86; N, 3.74.

**10,14-Dimethyl-13-oxa-1,4-dithia-10-azadispiro[4.1.5.2]tetradecane (26). [Method E]** Boron trifluoride etherate (2.0 ml, 16 mmol) was added to a solution of **17** (500 mg, 2.73 mmol) and 1,2-dimercaptoethane (0.45 ml, 5.4 mmol) in $CH_2Cl_2$ (10 ml) at 0—5 °C. The mixture was stirred for 1 h at room temperature and poured into aqueous 20% NaOH (30 ml). The mixture was extracted with AcOEt, and the extract was dried and concentrated *in vacuo*. The residue was purified through a silica gel column ($CHCl_3$–MeOH–27% aqueous $NH_3$, 20 : 1 : 0.1, v/v) to give 445 mg of **26** as an oil in 63% yield. Maleate; mp 114—115 °C (MeOH–ether). $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.23 (3H, d, $J$=7 Hz), 1.60—2.04 (4H, m), 2.49 (2H, s), 2.76 (3H, s), 3.00—3.42 (8H, m), 4.06 (1H, q, $J$=7 Hz), 6.03 (2H, s). MS *m/z*: 259 ($M^+$), 231, 187. *Anal.* Calcd for $C_{12}H_{21}NOS_2 \cdot C_4H_4O_4$: C, 51.18; H, 6.71; N, 3.73. Found: C, 50.87; H, 6.57; N, 3.66.

**10,14-Dimethyl-1,4,13-trithia-10-azadispiro[4.1.5.2]tetradecane (28)** Compound **28** was prepared according to method E as an oil in 79% yield. Maleate; mp 110—115 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 1.40 (3H, d, $J$=7 Hz), 2.40—2.80 (2H, m), 2.80 (3H, s), 3.30 (4H, s), 3.69 (1H, q, $J$=7 Hz), 6.28 (2H, s). MS *m/z*: 275 ($M^+$), 242, 110. *Anal.* Calcd for $C_{12}H_{21}NS_3 \cdot C_4H_4O_4 \cdot 0.3H_2O$: C, 48.41; H, 6.50; N, 3.53. Found: C, 48.33; H, 6.40; N, 3.66.

**2,8-Dimethyl-3-methylene-1-oxa-8-azaspiro[4.5]decane (29)** Sodium hydride (60% mineral oil dispersion, 272 mg, 6.8 mmol) was washed with hexane, suspended in DMSO (8 ml) and heated at 60 °C for 1 h. The faint-green solution thus obtained was cooled to *ca.* 20 °C. Methyltriphenylphosphonium bromide (2.43 g, 6.8 mmol) was added, and the mixture stirred for 30 min. Compound **17** (590 mg, 3.2 mmol) was added, and the mixture was stirred for 2 h at room temperature, poured into ice-water (50 ml) and extracted with $CHCl_3$. The extract was washed, dried, and concentrated *in vacuo*. The residue was purified through a silica gel column ($CHCl_3$–MeOH–27% aqueous $NH_3$, 10 : 1 : 0.1, v/v) to afford 320 mg of **29** as a pale yellow oil in 55% yield. Fumarate; mp 112—117 °C (acetonitrile). $^1$H-NMR ($CDCl_3$) $\delta$: 1.28 (3H, d, $J$=6 Hz), 1.60—2.38 (4H, m), 2.48 (2H, m), 2.77 (3H, s), 2.82—3.20 (2H, m), 3.30—3.60 (2H, m), 4.42 (1H, m), 4.86 (1H, m), 4.98 (1H, m), 6.82 (2H, s), 11.31 (2H, br). MS *m/z*: 181 ($M^+$), 166, 96. *Anal.* Calcd for $C_{11}H_{19}NO \cdot C_4H_4O_4$: C, 60.59; H, 7.80; N, 4.71. Found: C, 60.30; H, 7.72; N, 4.59.

**Optical Resolution of Racemic 2,8-Dimethyl-3-methylene-1-oxa-8-azaspiro[4.5]decane (29)** The racemate **29** (190 g, 1.05 mol) and di-*p*-toluoyl-D-tartaric acid monohydrate (425 g, 1.05 mol) were dissolved in MeOH (2.09 l) and the solution was cooled to below 5 °C. To this solution, water (1.02 l) was added with stirring. The whole was allowed to stand for 3 d, then the precipitated crystals were collected and recrystallized **8** times from a mixed solvent (MeOH–water, 2 : 1, v/v) as described above to afford the di-*p*-toluoyl-D-tartaric acid salt of (−)-**29** (61 g, 10% yield). A suspension of the di-*p*-toluoyl-D-tartaric acid salt of (−)-**29** in water (200 ml) was basified with 25% NaOH and extracted with ether. The organic layer was dried and evaporated to give a pale yellow oil (18.8 g). The oil was dissolved in acetonitrile (250 ml) and fumaric acid (16.3 g, 0.14 mol) was added. The mixture was warmed to yield a clear solution, which was allowed to stand at room temperature for 3 h and at below 5 °C for 15 h to yield (−)-**29** sesquifumarate (27.6 g) as colorless crystals. mp 128—129 °C. $[\alpha]_D^{20}$ −28.3 ($c$=1.10, MeOH). $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.20 (3H, d, $J$=6 Hz), 1.60—2.00 (4H, m), 2.48 (2H, m), 2.58 (3H, s), 2.90—3.10 (4H, m), 4.40 (1H, m), 4.87 (1H, m), 4.95 (1H, m), 6.50 (3H, s), 10.96 (3H, br s). MS *m/z*: 181 ($M^+$), 152. *Anal.* Calcd for $C_{11}H_{19}NO \cdot C_6H_6O_6$: C, 57.45; H, 7.09; N,3.94. Found: C, 57.26; H, 7.04; N, 3.88. Optical purity was determined to be >97% ee by 500 MHz $^1$H-NMR spectroscopy of the di-*p*-toluoyl-D-tartaric acid salt of (−)-**29**. The C-2 methyl signals were used.

The antipode, (+)-**29** sesquifumarate, was also obtained using di-*p*-toluoyl-L-tartaric acid in a similar manner. mp 128—129 °C. $[\alpha]_D^{20}$ +28.0 ($c$=1.23, MeOH). *Anal.* Calcd for $C_{11}H_{19}NO \cdot C_6H_6O_6$: C, 57.45; H, 7.09; N,3.94. Found: C, 57.31; H, 7.08; N, 3.77. Optical purity >97% ee (500 MHz $^1$H-NMR spectroscopy of the di-*p*-toluoyl-L-tartaric acid salt of (+)-**29**).

The free base, (−)-**29**, was treated with an equimolar amount of L-tartaric acid in 90% aqueous EtOH and recrystallized from 90% aqueous EtOH to give the tartrate monohydrate of (−)-**29**; mp 97 °C. $[\alpha]_D^{20}$ −16.4 ($c$=0.988, MeOH). *Anal.* Calcd for $C_{11}H_{19}NO \cdot C_4H_6O_6 \cdot H_2O$: C, 51.57; H, 7.79; N,4.01. Found: C, 51.44; H, 7.75; N, 3.91.

(+)-**29** Tartrate monohydrate was obtained in a similar manner. mp 97 °C. $[\alpha]_D^{20}$ +16.3 ($c$=1.054, MeOH). *Anal.* Calcd for $C_{11}H_{19}NO \cdot C_4H_6O_6 \cdot H_2O$: C, 51.57; H, 7.79; N, 4.01. Found: C, 51.65; H, 7.81; N, 3.98.

**2,8-Dimethyl-3-methylene-1-thia-8-azaspiro[4.5]decane (31)** Compound **31** was prepared by the method described for **29** as an oil in 87% yield. Hydrochloride; mp 197—200 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 1.42 (3H, d, $J$=6 Hz), 1.80—2.00 (2H, m), 2.40—3.20 (6H, m), 2.76 (3H, d, $J$=5 Hz), 3.40—3.60 (2H, m), 4.00 (1H, m), 4.94 (1H, m), 5.00 (1H, m). MS *m/z*: 197 ($M^+$), 164, 96. *Anal.* Calcd for $C_{11}H_{19}NS \cdot HCl$: C, 56.51; H, 8.62; N, 5.99. Found: C, 55.82; H, 8.32; N, 5.85.

**2-Methyl-3-methylene-1-oxa-8-azaspiro[4.5]decane (33)** (i) Ethyl 8-Ethoxycarbonyl-2-methyl-3-oxo-1-oxa-8-azaspiro[4.5]decane-4-carboxylate (**15**): Compound **15** was prepared by method A described for **17** in 25% yield, IR (neat): 2990, 1776, 1738, 1704 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 1.18—1.50 (9H, m), 1.50—2.10 (4H, m), 4.00—4.40 (5H, m). MS *m/z*: 313 ($M^+$), 284, 268, 239.

(ii) 8-Ethoxycarbonyl-2-methyl-1-oxa-8-azaspiro[4.5]decan-3-one (**22**): Sodium chloride (512 mg, 8.75 mmol) and water (315 $\mu$l) were added to a solution of **15** (2.74 g, 8.75 mmol) in dimethylformamide (DMF) (10 ml). The mixture was heated for 2 h at 140—150 °C and poured into ice-water (30 ml). The resulting mixture was extracted with $CHCl_3$, and the extract was washed, dried, and concentrated *in vacuo*. The residue was purified through a silica gel column (hexane–ethyl acetate, 1 : 1, v/v) to give 1.54 g of **22** as an oil in 73% yield, IR (neat): 1764, 1700 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 1.28 (3H, t, $J$=7 Hz), 1.32 (3H, d, $J$=7 Hz), 1.50—1.90 (4H, m), 2.38 (2H, s), 3.28—3.90 (4H, m), 4.03 (1H, q, $J$=7 Hz), 4.15 (2H, q, $J$=7 Hz). MS *m/z*: 241 ($M^+$), 212, 196.

(iii) 2-Methyl-3-methylene-1-oxa-8-azaspiro[4.5]decane (**33**): 8-Ethoxycarbonyl-2-methyl-3-methylene-1-oxa-8-azaspiro[4.5]decane (**32**) was prepared by the method described for **29** in 63% yield. A solution of **32** (0.7 g) in MeOH (7.6 ml) was added to an aqueous solution (1.5 ml) of KOH (1.64 g) and the mixture was heated under reflux for 24 h, then concentrated *in vacuo*. The residue was purified through a silica gel column ($CHCl_3$–MeOH–27% aqueous $NH_3$, 8 : 1 : 0.1, v/v) to give 0.29 g of **33** as an oil. The oil was treated with 4 N ethanolic HCl to give a hydrochloride, mp 133 °C (dec.). $^1$H-NMR ($CDCl_3$) $\delta$: 1.27 (3H, d, $J$=7 Hz), 1.60—3.20 (4H, m), 2.48 (2H, m), 3.30 (4H, m), 4.43 (1H, m), 4.92 (2H, m), 9.48 (2H, m). MS *m/z*: 197 ($M^+$), 164, 96. *Anal.* Calcd for $C_{10}H_{17}NO \cdot HCl \cdot 0.2H_2O$: C, 57.94; H, 8.95; N, 6.76. Found: C, 57.76; H, 8.78; N, 6.65.

***anti*- and *syn*-3-Hydroxyimino-2,8-dimethyl-1-oxa-8-azaspiro[4.5]decane (34, 35). [Method F]** A solution of **17** (1.3 g, 7.1 mmol) in MeOH (20 ml) was added dropwise to a solution of hydroxylamine hydrochloride (567 mg, 8.1 mmol) and sodium acetate (670 mg, 8.1 mmol) in water (3 ml), and the mixture was heated at 80 °C for 1 h, then concentrated *in vacuo*. The residue was purified through a silica gel column ($CHCl_3$–MeOH–27% aqueous $NH_3$, 10 : 1 : 0.1, v/v) to give *anti* isomer-rich fractions (690 mg, eluted first), *syn* isomer-rich fractions (220 mg), and a mixture of both isomers (460 mg) as a solid (total yield: 98%).

Recrystallization of *anti* isomer-rich fractions from ether gave the pure *anti* isomer **34** (560 mg, 40% yield); mp 155—157 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 1.34 (3H, d, $J$=7 Hz), 1.60—1.90 (4H, m), 2.30 (3H, s), 2.30—2.80 (6H, m), 4.45 (1H, q, $J$=7 Hz). MS *m/z*: 198 ($M^+$), 137, 96. $^{13}$C-NMR ($CDCl_3$) $\delta$: 19.8 (C2-$CH_3$), 34.9, 37.8 (C6, C10), 38.0 (C4), 45.9 (N-$CH_3$), 52.5, 52.7 (C7, C9), 72.5 (C2), 78.7 (C5), 164.1 (C3). *Anal.* Calcd for $C_{10}H_{18}N_2O_2 \cdot 0.1H_2O$: C, 60.04; H, 9.17; N, 14.00. Found: C, 59.94; H, 8.95; N, 13.99.

Recrystallization of *syn* isomer-rich fractions from ether–hexane gave the pure *syn* isomer **35** (100 mg, 7% yield); mp 118—120 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 1.44 (3H, d, $J$=7 Hz), 1.53—1.91 (4H, m), 2.31 (3H, s), 2.38—2.63 (6H, m), 4.75 (1H, q, $J$=7 Hz). MS *m/z*: 198 ($M^+$), 137, 96. $^{13}$C-NMR ($CDCl_3$) $\delta$: 18.6 (C2-$CH_3$), 34.0, 37.0 (C6, C10), 40.5 (C4), 45.8 (N-$CH_3$), 52.0, 52.4 (C7, C9), 71.1 (C2), 78.2 (C5), 164.4 (C3). *Anal.* Calcd for $C_{10}H_{18}N_2O_2$: C, 60.58; H, 9.15; N, 14.13. Found: C, 60.15; H, 9.08; N, 13.97.

***anti*- and *syn*-2-Ethyl-3-hydroxyimino-8-methyl-1-oxa-8-azaspiro[4.5]decane (36, 37)** Compound **36** and **37** were prepared according to method F in 86% total yield and separated by silica gel column chromatography into the *anti* isomer (42% yield, eluted first), the *syn* isomer (20% yield), and their mixture.

Compound **36**: mp 129—131 °C (ether–hexane). $^1$H-NMR ($CDCl_3$) $\delta$: 0.96 (3H, t, $J$=7 Hz), 1.50—1.90 (6H, m), 2.28 (3H, s), 2.24—2.90 (6H, m), 4.35 (1H, m). MS *m/z*: 212 ($M^+$), 137, 96. *Anal.* Calcd for

$C_{11}H_{20}N_2O_2 \cdot 0.3H_2O$: C, 60.69; H, 9.54; N, 12.87. Found: C, 60.28; H, 9.24; N, 12.66.

Compound **37**: mp 92—94 °C (hexane). $^1$H-NMR ($CDCl_3$) $\delta$: 0.94 (3H, t, $J$=7 Hz), 1.55—2.00 (6H, m), 2.32 (3H, s), 2.30—2.70 (6H, m), 4.72 (1H, m). MS $m/z$: 212 ($M^+$), 137, 96. *Anal.* Calcd for $C_{11}H_{20}N_2O_2$: C, 62.24; H, 9.50; N, 13.20. Found: C, 61.87; H, 9.49; N, 13.08.

***anti-* and *syn*-3-Methoxyimino-2,8-dimethyl-1-oxa-8-azaspiro[4.5]decane (38, 39). [Method G]** Sodium (57 mg, 2.5 mmol) was dissolved in MeOH (2 ml), followed by the addition of *O*-methylhydroxylamine hydrochloride (209 mg, 2.5 mmol). This solution was added to a solution of **17** (425 mg, 2.3 mmol) in MeOH and the mixture was stirred at room temperature for 2 d and at 50 °C for 1 d. To this mixture was added *O*-methylhydroxylamine solution prepared by the procedure described above, using 310 mg of *O*-methylhydroxylamine hydrochloride. The resulting mixture was stirred for 12 h at 50 °C. The reaction mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified through a silica gel column ($CHCl_3$–MeOH–27% aqueous $NH_3$, 20 : 1 : 0.1, v/v) to give 140 mg of the *anti* isomer **38** (29% yield, eluted first) and 60 mg of the *syn* isomer **39** (12% yield).

Compound **38** Maleate: mp 146 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.28 (3H, d, $J$=7 Hz), 1.72—1.98 (4H, m), 2.56—2.72 (2H, m), 2.79 (3H, s), 3.09—3.36 (4H, m), 3.81 (3H, s), 4.53 (1H, q, $J$=7 Hz), 6.03 (2H, s). MS $m/z$: 212 ($M^+$), 137, 96. *Anal.* Calcd for $C_{11}H_{20}N_2O_2 \cdot C_4H_4O_4$: C, 54.87; H, 7.37; N, 8.53. Found: C, 54.71; H, 7.41; N, 8.41.

Compound **39** Maleate: mp 145 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.33 (3H, d, $J$=7 Hz), 1.64—2.06 (4H, m), 2.61—2.68 (2H, m), 2.79 (3H, s), 3.08—3.28 (4H, m), 3.79 (3H, s), 4.68 (1H, q, $J$=7 Hz), 6.04 (2H, s). *Anal.* Calcd for $C_{11}H_{20}N_2O_2 \cdot 1.3C_4H_4O_4$: C, 53.58; H, 6.99; N, 7.71. Found: C, 53.76; H, 6.73; N, 7.87.

**2,8-Dimethyl-3-(*N*,*N*-dimethyl)hydrazono-1-oxa-8-azaspiro[4.5]decane (40). [Method H]** *N*,*N*-Dimethylhydrazine (220 mg, 3.7 mmol) and acetic acid (0.2 ml) were added to a solution of **17** (550 mg, 3 mmol) in EtOH (4 ml). The mixture was stirred for 30 min and concentrated *in vacuo*. The residue was purified through a silica gel column ($CHCl_3$–MeOH–27% aqueous $NH_3$, 190 : 9 : 1, v/v) to give 600 mg of **40** as an oil in 89% yield. Fumarate; mp 118—121 °C (dec., acetonitrile). $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.35 (3H, d, $J$=7 Hz), 1.70—2.40 (4H, m), 2.47 (6H, s), 2.68 (3H, s), 2.80—3.50 (4H, m), 4.45 (1H, q, $J$=7 Hz), 6.87 (2H, s), 9.35 (2H, m). MS $m/z$: 225 ($M^+$), 210. *Anal.* Calcd for $C_{12}H_{23}N_3O \cdot C_4H_4O_4 \cdot 0.6H_2O$: C, 54.56; H, 8.07; N, 11.93. Found: C, 54.51; H, 7.72; N, 11.78.

**X-Ray Analysis of (−)-29 L-Tartrate Monohydrate** The colorless prismatic crystal used for the X-ray study was obtained from an EtOH and water solution and had dimensions of approximately 0.5 × 0.1 × 0.1 mm. All measurements were made on a Rigaku AFC5R diffractometer with graphite-monochromated Cu$K_\alpha$ ($\lambda$=1.54184 Å). Crystal data: $C_{15}H_{27}NO_8$, $M_r$=349.38, orthorhombic, space group $P2_12_12_1$, $a$=14.827(2), $b$=16.604(2), $c$=7.237(1) Å, $V$=1781.6(3) Å$^3$, $Z$=4, $D_x$=1.30 g/cm$^3$, $F$(000)=752 and $\mu$(Cu$K_\alpha$)=8.94 cm$^{-1}$. The data were collected at room temperature using the $\omega$–2$\theta$ technique to a maximum 2$\theta$ of 120.1°. A total of 1574 reflections was collected. The intensities of three representative reflections were measured after every 150 reflections. No decay correction was applied. The data was corrected for Lorentz and polarization effects, but no absorption correction was made.

The structure was solved by direct methods[26] and expanded using Fourier techniques.[27] The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined isotropically. The final cycle of full-matrix least-squares refinement was based on 1405 observed reflections ($I$>3$\sigma$($I$)) and 109 variable parameters and converged with $R$=0.034 and $R_w$=0.041. The standard deviation of an observation of unit weight was 1.49. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.10 and −0.15 $e$/Å$^3$, respectively. Atomic scattering factors were taken from "International Tables for X-ray Crystallography."[28] All calculations were performed using the teXsan[29] crystallographic software package. The final positional parameters of non-H atoms are given in Table 6.

**Biological Methods** Doses are expressed in terms of the free base. The following were obtained commercially: scopolamine hydrochloride (Tokyo Kasei Co., Ltd.), and [$^3$H]PZ and [$^3$H]QNB (Du Pont-New England Nuclear).

**Receptor Binding Assay** Membrane preparation and tritium-labeled ligand receptor binding assay were described previously.[25a] The cerebral cortex and the cerebellum from male Wistar rats were homogenized separately in 0.32 M ice-cold sucrose (1 : 10, w/v) using a motor-driven Teflon/glass homogenizer. The homogenates were centrifuged at 900 *g* for 10 min at 4 °C. The supernatants were then recentrifuged at 11500 *g* for 20 min at 4 °C. The pellets thus obtained were washed twice in 5 mM Tris–HCl buffer (pH 7.4) by resuspension and recentrifugation. Membranes were stored at −80 °C until required.

[$^3$H]PZ binding for $M_1$ receptors was performed according to the method of Watson *et al.*[11] Frozen rat cerebral cortex membrane was resuspended in an assay buffer (8.3 mM Tris–HCl, 1.25 mM $MnCl_2$, pH 7.4). The membrane suspension, corresponding to 250 $\mu$g of protein determined by the method of Lowry with bovine serum albumin as the standard, was incubated with approximately 1.0 nM [$^3$H]PZ at 25 °C for 60 min. Test compounds were added in a volume of 50 $\mu$l to give a final assay volume of 1.0 ml. Nonspecific binding was determined using 10 $\mu$M atropine. Assays were terminated by rapid filtration under vacuum through a Whatman GF/B filter. The filters were washed immediately four times with 4 ml each of 50 mM Tris–HCl and 120 mM NaCl buffer (pH 7.4). The filter was placed in a scintillation vial, to which 10 ml of NEN Aquasol-2 cocktail was added. Radioactivity retained on the filter was determined by liquid scintillation spectrometry. All receptor binding assays were performed in triplicate. Competition binding data were analyzed by logit-log analysis using an RS1 package (BBN Software Products Corp.) to provide the $IC_{50}$. The $IC_{50}$ values were corrected for receptor occupancy by [$^3$H]PZ as described by Cheng and Prusoff[30] to give $K_i$ values (concentration of nonlabeled ligand that causes half-maximal receptor occupancy in the absence of [$^3$H]PZ).

[$^3$H]QNB binding for $M_2$ receptors was carried out according to the method of Yamamura and Snyder.[12] The incubation mixture consisted of 50 mM $Na^+/K^+$ phosphate buffer (pH 7.4), 0.06 nM [$^3$H]QNB, 150 $\mu$g protein of resuspended rat cerebellum membrane, and a test drug in a total volume of 4.0 ml. Nonspecific binding was defined with 10 $\mu$M atropine. The mixture was incubated for 60 min at 25 °C, and the incubation was terminated by filtration through Whatman GF/B glass filters. The filters were washed three times with 3 ml of 50 mM $Na^+/K^+$ phosphate buffer (pH 7.4). The estimations of filter-bound radioactivity and the data analyses were similar to those in the case of [$^3$H]PZ binding.

**PI Hydrolysis** Rat hippocampal slices were prepared and prelabeled with [$^3$H]*myo*-inositol as described by Brown *et al.*[31] The slices were incubated for 60 min at 37 °C in the presence of a test compound and 10 mM LiCl in Krebs–Henseleit buffer containing 18.7 mM $K^+$. The [$^3$H]inositol phosphates (IPs) and [$^3$H]lipids (lipids) were separated and measured by the same method as described by Brown *et al.*[31] Results were expressed as [$^3$H]IPs/([$^3$H]lipids + [$^3$H]IPs).

**Passive Avoidance Tasks with Scopolamine-Treated Rats** Six male Wistar rats (Japan Slc. Inc.) were used for each dose of test compounds. A test compound or vehicle was administered subcutaneously or orally 30 min before the training session simultaneously with scopolamine (1 mg/kg i.p.). In the training session, each rat was placed in the light box of a two-compartment passive avoidance apparatus (O'Hara Co., Ltd.). When the rat entered the dark compartment, a foot-shock (70 V, AC) was applied for 1 s through the metal grid bars of the floor. Retention was assessed 24 h after the training session. Latency to enter the dark compartment was measured up to a maximum cut-off of 300 s. When the latencies of at least 4 rats were more than 60 s, the compound was evaluated as active. About 90% of vehicle-treated rats exhibited a latency of less than 60 s.

Each compound was initially tested at 1, 3, or 10 mg/kg. When a compound was found to be active at one of these doses, it was retested at a three-fold lower dose, and the minimal dose required to improve the impairment of the passive avoidance tasks (MED) was thereby determined.

**Hypothermia and Tremor** Male ICR mice (Japan Slc. Inc.) were used for these experiments. Before and 30 min after the subcutaneous or oral administration of a test compound, rectal temperature was measured with a thermoprobe (MC-111, Omron), and the dose required to reduce the rectal temperature by 2 °C ($ED_{\Delta 2°C}$) was determined. Each test compound was initially tested at 30 mg/kg; the dose was then consecutively decreased to one-third of the preceding dose until hypothermia over 2 °C was not observed. Each dose group contained 3—6 mice.

The occurrence of tremor was observed for 30 min after the administration of a test compound. The minimal dose required to induce tremor in at least one mouse (MED) was determined. Each dose group contained 4—6 mice.

**Salivation** Male ICR mice (Japan Slc. Inc.) were used. Saliva was

collected 15 and 45 min after the subcutaneous or oral administration of a test compound. The dose producing more than 30 mg of saliva as a total amount ($ED_{30\,mg}$) was determined in the same manner as in the case of hypothermia. Each dose group contained 3—6 mice.

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