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Synthesis and binding affinities of methylvesamicol analogs for the acetylcholine transporter and sigma receptor

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Abstract—We synthesized methylvesamicol analogs **13**–**16** and investigated the binding characteristics of 2-[4-phenylpiperidino]cyclohexanol (vesamicol) and methylvesamicol analogs **13**–**16**, with a methyl group introduced into the 4-phenylpiperidine moiety, to sigma receptors (σ-1, σ-2) and to vesicular acetylcholine transporters (VAChT) in membranes of the rat brain and liver. In competitive inhibition studies, (−)-*o*-methylvesamicol [(−)-OMV] (**13**) (K_i = 6.7 nM), as well as (−)-vesamicol (K_i = 4.4 nM), had a high affinity for VAChT. (+)-*p*-Methylvesamicol [(+)-PMV] (**16**) (K_i = 3.0 nM), as well as SA4503 (K_i = 4.4 nM), reported as a σ-1 mapping agent for positron emission tomography (PET), had a high affinity for the σ-1 receptor. The binding affinity of (+)-PMV (**16**) for the σ-1 receptor (K_i = 3.0 nM) was about 13 times higher than that for the sigma-2 (σ-2) receptor (K_i = 40.7 nM). (+)-PMV (**16**) (K_i = 199 nM) had a much lower affinity for VAChT than SA4503 (K_i = 50.2 nM) and haloperidol (K_i = 41.4 nM). These results showed that the binding characteristics of (−)-OMV (**13**) to VAChT were similar to those of (−)-vesamicol and that (+)-PMV (**16**) bound to the σ-1 receptor with high affinity. In conclusion, (−)-OMV (**13**) and (+)-PMV (**16**), which had a suitable structure, with a methyl group for labeling with ^{11}C, may become not only a new VAChT ligand and a new type of σ receptor ligand, respectively, but may also become a new target compound of VAChT and the σ-1 receptor radioligand for PET, respectively.
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1. Introduction

Several radiolabeled analogs of vesamicol[1–5] have been proposed as VAChT mapping agents for diagnosing dementia characterized by the degeneration of the cholinergic neurotransmitter system, such as Alzheimer's disease. We also reported that radioiodinated (−)-*o*-iodovesamicol [(−)-*o*-IV], radioiodinated at the *ortho*-position of the 4-phenylpiperidine moiety of (−)-enantiomer of vesamicol, was suitable as a VAChT mapping agent for SPECT.[6,7] On the other hand, Efange et al.[2] and Custer et al.[8] reported that there were several vesamicol analogs showing great affinity not only for VAChT, but also for sigma receptors. Then, we investigated the relationships between the position of the substituent into the 4-phenylpiperidine moiety of vesamicol and a kind of optical isomer, and the affinity for VAChT or sigma receptors.[6] As a result, we found that radioiodinated (+)-*p*-iodovesamicol [(+)-*p*-IV] radioiodinated at the *para*-position into the 4-phenylpiperidine moiety of the (+)-enantiomer of vesamicol bound to sigma receptor (σ-1) with high affinity and high selectivity.[9] In this study, with the aim of extending human VAChT or sigma receptor imaging studies to a higher level of precision with positron emission tomography (PET), we synthesized methylvesamicol analogs that had a suitable structure for labeling with ^{11}C and investigated the in vitro binding characteristics of these analogs to VAChT and sigma receptors (σ-1, σ-2). Furthermore, we synthesized trialkylstannyl vesamicol analogs, which were used as precursors for the synthesis of $^{11}CH_3$-labeled vesamicol analogs.

2. Results

2.1. Chemistry

The key intermediates for the synthesis of (−)-*o*-methylvesamicol ((−)-OMV) (**13**) and (+)-*p*-methylvesamicol

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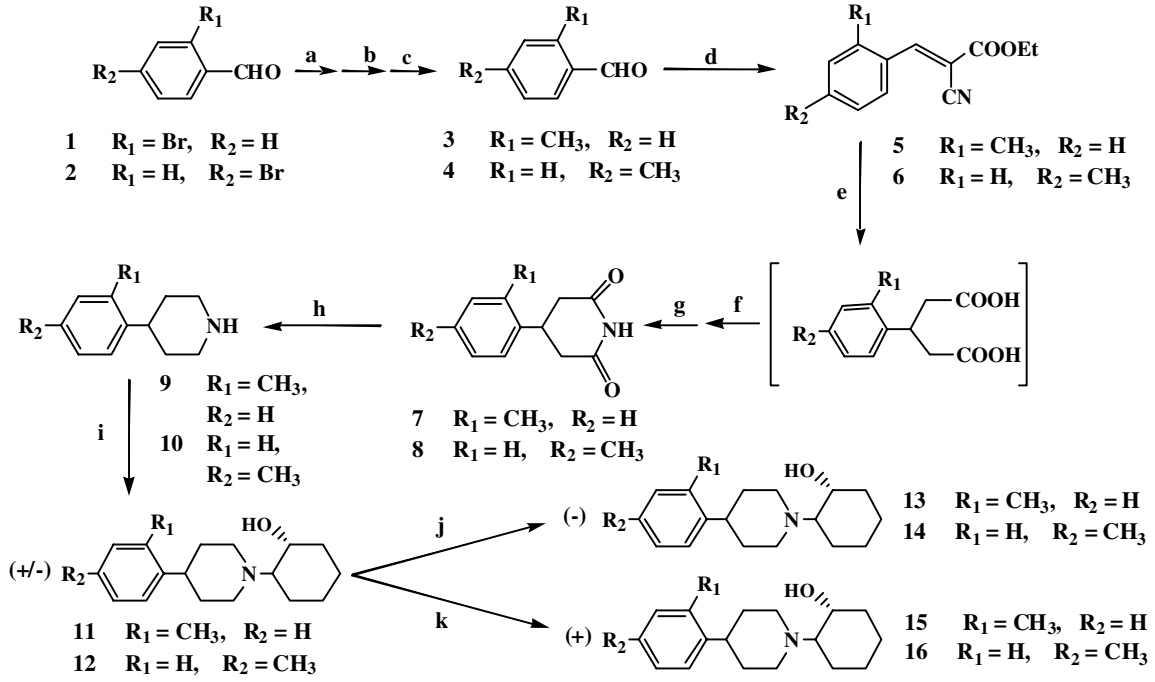
((+)-PMV) (**16**) were 4-(2-methylphenyl)piperidine (**9**) and 4-(4-methylphenyl)piperidine (**10**), respectively. These methylvesamicol analogs were prepared as outlined in Scheme 1. 2-Methylbenzaldehyde (**3**) was prepared from 2-bromobenzaldehyde (**1**) by a three-step reaction, the protection of the aldehyde with ethylene glycol, formation of the Grignard reagent, and methylation with methyl iodide. The reaction of 2-methylbenzaldehyde (**3**) with ethyl cyanoacetate under Claizen–Schmidt conditions gave **5** in 55.3% yield by purification using column chromatography on silica gel. The conjugate addition of the diethylmalonate carbanion onto **5**, followed by acid-catalyzed hydrolysis, provided the crude diacid. The diacid was converted first to the corresponding diester and then to imide **7** with an overall yield of 61.2%. Reduction of the imide **7** with borane-tetrahydrofuran (BH_3–THF) afforded the desired key intermediate, **9**, in 66.5% yield. The racemic *o*-methylvesamicol (**11**) was obtained in the usual manner by refluxing **9** with cyclohexene oxide in ethanol. Each enantiomer of *o*-methylvesamicol (**13** and **14**) was obtained by recrystallizing the corresponding diastereoisomeric salts with (−)-di-*p*-toluoyl-L-tartaric acid and (+)-di-*p*-toluoyl-D-tartaric acid, respectively. Each enantiomer of *p*-methylvesamicol, **15** and **16**, was prepared by the same method as for **13** and **14** with 4-bromobenzaldehyde (**2**). Each enantiomer of the methylvesamicol analogs **13**–**16** from the corresponding starting materials (**1** and **2**) via 10-step reactions produced respective overall yields of 1.3–4.1%.

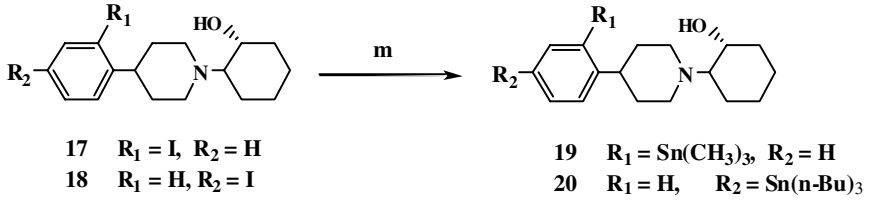
Trialkylstannyl compounds, **19** and **20**, used as precursors for $^{11}CH_3$-labeled tracers, were prepared by a palladium-catalyzed reaction of the corresponding iodovesamicols, **17** and **18** (Scheme 2). Modest yields of trialkylstannyl compounds (33–71%) were obtained after purification through thin-layer chromatography on silica gel.

2.2. Binding studies

The binding affinities of methylvesamicol analogs and reference compounds to the VAChT binding sites and sigma receptors (σ-1, σ-2) are shown in Table 1. *o*-Methylvesamicol, as well as vesamicol, bound enantioselectively to the VAChT binding sites. The binding



Scheme 1. Synthesis of methylvesamicol analogs. Reagents and conditions: (a) ethylene glycol, *p*-TsOH, benzene, reflux; (b) Mg, THF, CH_3I, reflux; (c) 5% HCl, rt; (d) ethylcyanoacetate, AcOH, pyrrolidine, reflux; (e) i—NaH, diethylmalonate, THF, 4 °C, ii—6 N HCl, reflux; (f) $SOCl_2$, MeOH, 4 °C; (g) urea, NaOEt, EtOH, reflux; (h) BH_3–THF, reflux; (i) cyclohexene oxide, EtOH, reflux; (j) (−)-di-*p*-toluoyl-L-tartaric acid, acetone, rt; (k) (+)-di-*p*-toluoyl-D-tartaric acid, acetone, rt.



Scheme 2. Synthesis of trialkylstannyl-vesamicol analogs. Reagents: (m) $[(alkyl)_3Sn]_2$, $(Ph_3P)_4Pd(0)$, toluene, reflux.

Table 1. Affinities of vesamicol derivatives and sigma receptor ligands for vesicular acetylcholine transporter (VAChT) and sigma receptors (σ-1, σ-2)

	VAChT (K_i)	Sigma receptor	
		σ-1 (K_i)	σ-2 (K_i)
(−)-Vesamicol	4.4 ± 0.6	73.8 ± 11.2	346 ± 37
(+)-Vesamicol	135 ± 14	31.5 ± 11.3	330 ± 36
(−)-OMV	6.7 ± 0.4	33.7 ± 5.9	266 ± 28
(+)-OMV	22.5 ± 2.0	10.7 ± 0.2	218 ± 20
(−)-PMV	22.9 ± 0.4	8.1 ± 2.0	42.4 ± 6.3
(+)-PMV	199 ± 32	3.0 ± 0.2	40.7 ± 2.9
SA4503	50.2 ± 7.2	4.4 ± 1.0	242 ± 17
Haloperidol	41.4 ± 17.6	2.6 ± 0.8	167 ± 19
(+)-Pentazocine	315 ± 121	5.5 ± 2.0	2470 ± 150
DTG	165 ± 26	—	20.7 ± 4.8

K_i values derived from IC_{50} values according to the equation: $K_i = IC_{50}/(1 + C/K_d)$, where C is the concentration of the radioligand and each K_d is the dissociation constant of the corresponding radioligand (K_d of (−)-[^{3}H]vesamicol to VAChT (K_d = 7.40 nM), K_d of [^{3}H]pentazocine to σ-1 (K_d = 19.9 nM), K_d of [^{3}H]DTG to σ-2 (K_d = 22.3 nM)).

affinity of (−)-OMV (**13**) (K_i = 6.7 nM) to the VAChT binding sites was greater than that of (+)-OMV (**14**) (K_i = 22.5 nM). Also, the binding affinity of (−)-OMV (**13**) (K_i = 33.7 nM) to the σ-1 receptor was less than that of (+)-OMV (**14**) (K_i = 10.7 nM). (+)-PMV (**16**) (K_i = 3.0 nM) displayed a slightly higher affinity for the σ-1 receptor than SA4503 (K_i = 4.4 nM) and (+)-pentazocine (K_i = 5.5 nM), which are known as sigma ligands. The binding affinity of (+)-PMV (**16**) to the σ-1 receptor (K_i = 3.0 nM), was about 14 times higher than the sigma-2 (σ-2) receptor (K_i = 40.7 nM). Also, (+)-PMV (**16**) displayed a lower affinity for the VAChT binding sites (K_i = 199 nM) than SA4503 (K_i = 50.2 nM) and haloperidol (K_i = 41.4 nM).

3. Discussion

Radiolabeled vesamicol analogs have been proposed not only to be VAChT, but also sigma receptor, mapping agents.[10–13] In this study, we synthesized methylvesamicol analogs (**13**–**16**) and investigated the binding affinity of these analogs to VAChT binding sites or sigma receptors (σ-1, σ-2). First, we attempted to synthesize 4-(2-methylphenyl)piperidine (**9**) and 4-(2-methylphenyl)piperidine (**10**) as the key intermediates for the synthesis of methylvesamicol analogs (**13**–**16**). To introduce a methyl group into the ortho- or *para*-position of the 4-phenylpiperidine, two methylbenzaldehydes (**3** and **4**) were prepared from bromobenzaldehydes (**1** and **2**) with a Grignard reaction, and **9** and **10** were prepared from the corresponding compounds (**3** and **4**) via a five-step reaction. Methylvesamicol analogs (**13**–**16**) were synthesized from these key intermediates (**9** and **10**) in the same manner as reported by Roger et al.[14]

The results of binding studies showed that the introduction of a methyl group, as well as iodine, into the *ortho*-position of the 4-phenylpiperidine moiety had no influence on the affinity of methylvesamicol analog binding for VAChT. The affinity for VAChT was in the order of (−)-vesamicol ≧ (−)-OMV (**13**) > (+)-OMV (**14**) > (−)-PMV (**15**) > haloperidol > SA4503 > (+)-vesamicol > DTG > (+)-PMV (**16**) > (+)-pentazocine. The VAChT binding affinity of (−)-OMV (**13**) (K_i = 6.7 nM) was slightly less than that of (−)-vesamicol (K_i = 4.4 nM). However, the affinity of (−)-OMV (**13**) to VAChT was thought to be sufficiently high for the VAChT imaging.

Then, the binding affinity of (−)-OMV (**13**) to the VAChT binding sites (K_i = 6.7 nM) was much higher than those of the σ-1 receptor (K_i = 33.7 nM) and the σ-2 receptor (K_i = 266 nM), which showed that (−)-OMV bound to VAChT binding sites with high specificity. Thus, (−)-OMV (**13**), which had a suitable structure with a methyl group for labeling with ^{11}C, had similar characteristics for VAChT as (−)-vesamicol. Because the labeling of vesamicol with ^{11}C is structurally difficult, (−)-OMV (**13**) may become a new target compound of the VAChT radioligand for PET.

Regarding the introduction of the methyl group into the *para*-position of the 4-phenylpiperidine moiety, *p*-methylvesamicol (**15** and **16**) exhibited a higher affinity for sigma receptors (σ-1, σ-2) and a lower affinity for VAChT binding sites than vesamicol and *o*-methylvesamicol (**3** and **14**). Namely, the introduction of the methyl group into the *para*-position of vesamicol caused its binding affinity for sigma receptors (σ-1, σ-2) to increase, and its binding affinity for VAChT binding sites to decrease. The *p*-methylvesamicol bound enantioselectively to the σ-1 receptor. The σ-1 receptor binding affinity of (+)-PMV (**16**) (K_i = 3.0 nM) was greater than that of (−)-PMV (**15**) (K_i = 8.1 nM). Furthermore, the σ-1 receptor binding of (+)-PMV (**16**) was comparable to that of haloperidol (K_i = 2.6 nM), and thought to be sufficiently high to be used as a σ-1 receptor imaging agent, because the selectivity of (+)-PMV (**16**) (σ-2/σ-1 = about 14) for the σ-2 and σ-1 receptors was comparable to that of (+)-*p*-IV (σ-2/σ-1 = about 16), which was reported previously to be a sigma-1 receptor ligand.[9] (+)-PMV (**16**) (K_i = 199 nM) showed a lower affinity for VAChT binding sites than SA4503 (K_i = 50.2 nM) and haloperidol (K_i = 41.4 nM). (+)-Pentazocine and haloperidol are thought to bind to other neuroreceptors.[15,16] In addition, (+)-[^{3}H]pentazocine showed low accumulation in the rat brain.[17] (+)-PMV (16) has a structure similar to that of (+)-*p*-IV. Judging from the above, (+)-PMV (**16**), as well as SA4503, was thought to be a superior σ-1 receptor ligand. [^{11}C]SA4503 has already been reported to be a potential radioligand for mapping of the σ-1 receptor.[18,19] In the future, (+)-[^{11}C]PMV should be evaluated as to its potential as a PET radioligand for mapping the σ-1 receptor, as compared with [^{11}C]SA4503.

Trialkylstannyl compounds (**19** and **20**), precursors of (−)-[^{11}C]OMV and (+)-[^{11}C]PMV, were prepared from the corresponding iodovesamicol reported previously.[6,9] (−)-[^{11}C]OMV and (+)-[^{11}C]PMV, labeled with ^{11}C, will be synthesized by methylation of the corresponding trialkylstannyl compounds (**19** and **20**) with [^{11}C]methyl

iodide in a palladium-promoted cross-coupling reaction.[20]

In conclusion, the introduction of the methyl group, as well as iodine, into the ortho- or *para*-position of vesamicol varied the binding characteristics of methylvesamicol analogs for VAChT binding sites and sigma receptors (σ-1, σ-2). (−)-OMV (**13**) and (+)-PMV (**16**) may become not only a new VAChT ligand and a new type of σ receptor ligand, respectively, but may also become a new target compound of VAChT and a σ-1 receptor positron imaging agent, respectively. At present, (−)-[^{11}C]OMV and (+)-[^{11}C]PMV are prepared by the substitution of the trialkylstannyl group with [^{11}C]methyl iodide in a palladium-promoted cross-coupling reaction[20] and we evaluated these in vivo characteristics in the brain of rats and monkeys.

4. Experimental

Melting points were measured with a Yanagimoto micromelting point apparatus. NMR spectra were recorded on a JEOL JNM-GSX500 (taken in deuterated chloroform with tetramethylsilane as the internal standard). Mass spectra(MS) recorded on a JEOL JMS-SX102 spectrometer coincided well with the proposed structures. Elemental analyses were performed on a Yanaco CHN Corder MT-5; all results were within ±0.3% of theoretical values. Specific rotation was obtained on a Nippon Bunko DIP-181 digital polarimeter. (−)-[^{3}H]Vesamicol (1.30 TBq/mmol), [^{3}H]1,3-di-tolylguanidine ([^{3}H]DTG) (1.1 TBq/mmol), [^{3}H]pentazocine (1.0 TBq/mmol), and [^{125}I]sodium iodide (644 GBq/mg) were purchased. Other drugs and reagents, except for SA4503, were purchased from Sigma-Aldrich Japan (Tokyo, Japan). SA4503 was prepared by M's Science (Kobe, Japan).

4.1. 2-Methylbenzaldehyde (3)

A mixture of 2-bromobenzaldehyde (**1**) (25 g, 135 mmol), ethylene glycol (41.9 g, 675 mmol), and *p*-toluenesulfonic monohydrate (2.57 g, 13.5 mmol) in benzene (300 mL) was refluxed for 3 h with a Dean–Stark phase separating head and the water was removed as formed. The cooled reaction mixture was diluted with an equal volume of ether, and this mixture was washed with 10% sodium carbonate solution and then with water. The washed organic layer was dried by filtration through anhydrous Mg_2SO_4 and evaporated to near dryness under reduced pressure to provide a quantitative yield of the crude corresponding ketal.

Next, in a three-necked flask fitted with a reflux condenser and a separatory funnel containing Mg turnings (3.64 g, 150 mmol) and dry THF (50 mL), the crude dry ketal (22.9 g, 100 mmol) in dry THF (50 mL) was added dropwise under Argon gas. After the addition was complete, the whole mixture was refluxed for 1 h. After the flask was cooled in ice-cold water, methyl iodide (71 g, 500 mmol) in dry THF (60 mL) was added dropwise and the mixture was refluxed for 2 h. The reaction mixture was cooled in an ice-bath and treated with 25% aq NH_4Cl (150 mL) and extracted with ether. The organic extract was dried over anhydrous Mg_2SO_4 and concentrated under reduced pressure to provide the corresponding methyl-ketal as a yellow syrup. This syrup was dissolved in acetone (50 mL) and treated with 5% HCl. The mixture was stirred for 30 min at room temperature. After removing the acetone under reduced pressure, the reaction mixture was diluted with water, and then made alkaline with K_2CO_3. The mixture was extracted with ethyl acetate. The organic solvent was dried over $MgSO_4$ and evaporated to leave a syrup, which was purified by column chromatography on silica gel using ethyl acetate/hexane (1:9, v/v) as the eluent to yield **3** (11.5 g, 70.8% from 2-bromobenzaldehyde, **1**).

Compound **3**: ^{1}H NMR ($CDCl_3$): δ 2.66 (s, 3H), 7.84–7.31 (m, 4H), 10.26 (br s, 1H). Mass spectrum (*m*/*e*): 120 $[M]^+$.

4.2. Ethyl 2-cyano-3-(2-methylphenyl)acrylate (5)

Glacial acetic acid (5.6 mL) and pyrrolidine (3.07 mL) were added to a mixture of **3** (17.8 g, 148 mmol) and ethylcyanoacetate (20.1 g, 177.7 mmol) in benzene (150 mL). The resulting mixture was refluxed with the azeotropic removal of water for 120 min, cooled to room temperature, diluted with benzene (50 mL), and washed consecutively with water (100 mL) and saturated $NaHCO_3$ (100 mL). The organic extract was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with ethyl acetate/hexane (1:4, v/v). The solvent was removed under reduced pressure to provide 17.62 g (55.3%) of **5**.

Compound **5**: ^{1}H NMR ($CDCl_3$): δ 1.52–1.29 (t, J = 7.02 Hz, 3H), 2.45 (s, 3H), 4.58–4.22 (dd, J = 7.02 Hz, J = 14.3 Hz, 2H), 7.38–7.34 (m, 3H), 8.56 (s, 1H), 8.18–8.09 (m, 1H). Mass spectrum (*m*/*e*): 215 (M^+).

4.3. 3-(2-Methylphenyl)piperidine-2,6-dione (7)

Sodium hydride (6.36 g, of a 60% oil immersion, 160 mmol) was washed with dry THF (20 mL) under argon and resuspended in dry THF (50 mL). A solution of diethyl malonate (25.92 g, 162 mmol) in dry THF (60 mL) was added dropwise with cooling (ice bath), over 20 min. The ice bath was removed and the resulting solution was stirred for an additional 15 min. At this time, a solution of **5** (17.12 g, 79.6 mmol) in dry THF (100 mL) was added dropwise over 30 min. After stirring for 13 h, the reaction mixture was treated with 20% aq NH_4Cl (200 mL) and the layers were separated. The aq layer was reextracted with ethyl acetate and set aside. The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated to a syrup. The latter was treated with 6 N HCl (200 mL) and the mixture was refluxed for 60 h. Upon cooling, the diacid crystallized from the solution. Thionyl chloride (21.62 g, 181.7 mmol) was added dropwise over 30 min to a cooled (ice bath) solution of the crude diacid product in dry MeOH (90 mL). Cooling was continued for an

additional 60 min and the ice bath was then removed. Stirring was then continued for 16 h, at which time the solvent was removed under reduced pressure and the resulting syrup was redissolved in CH_2Cl_2 (20 mL). The solution was washed with saturated aq $NaHCO_3$ (2× 75 mL), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to provide the crude diester as a syrup. The latter was used without further purification.

A mixture of urea (5.28 g, 88.0 mmol) and the diester in dry EtOH (50 mL) was quickly added to a solution of sodium ethylate (80 mL) prepared from 2.03 g (0.088 g-atm) of sodium. The resulting mixture was refluxed for 17 h, cooled to room temperature, and filtered to remove the insoluble material, which was later shown to be **7**. The latter was washed consecutively with 10% aq NH_4Cl and ethyl acetate to yield 9.90 g (61.2%) of the imide.

Compound **7**: ^{1}H NMR (CD_3OD): δ 2.36 (s, 3H), 2.83–2.70 (m, 4H), 3.76–3.52 (m, 1H), 7.19 (s, 4H). Mass spectrum (*m*/*e*): 203 (M^+).

4.4. 4-(2-Methylphenyl)piperidine (9)

150 mL (150 mmol) of 1.0 M BH_3–THF was introduced into a freshly dried three-necked flask fitted with a reflux condenser, magnetic stirrer, and dropping funnel; this flask was placed in an ice-water bath. Then, the compound **7** (9.46 g, 46.6 mmol) in dry THF (25 mL) was added to the borane solution under argon. After the addition was complete, the reaction mixture was stirred for 20 min at below 20 °C and then refluxed for 19 h. At this time, the mixture was allowed to cool to room temperature, and the reaction was quenched by the careful addition of 6 N HCl (20 mL). After the evolution of hydrogen had subsided, the mixture was concentrated under reduced pressure and the residue was treated with more 6 N HCl (100 mL). The resulting solution was refluxed for 22 h, cooled to room temperature, and concentrated to a white solid that was redissolved in water (100 mL). The solution was adjusted to pH 11 with NaOH pellets and extracted successively with CH_2Cl_2. The organic extracts were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to provide 6.60 g (81.0%) of **9** as a syrup, which solidified upon standing.

Compound **9**: ^{1}H NMR (CD_3OD): δ 2.05 (m, 4H), 2.35 (s, 3H), 7.19 (s, 4H), 4.15–3.54 (m, 5H). Mass spectrum (*m*/*e*): 175 (M^+).

4.5. (±)-2-(4-(2-Methylphenyl)piperidino)cyclohexanol ((±)-*o*-methylvesamicol) ((±)-OMV) (11)

Compound **9** (4.65 g, 26.6 mmol) and cyclohexene oxide (10.1 mL, 100 mmol) were dissolved in 30 mL of dry EtOH and refluxed for 20 h. Upon cooling of the solution, white crystals formed, which were collected by filtering and washed with cold EtOH. The crude product was recrystallized from EtOH. Yield of product **11** was 2.32 g (31.9%).

Compound **11**: mp: 116–118 °C. ^{1}H NMR ($CDCl_3$): δ 1.38–1.19 (m, 4H), 1.84–1.71 (m, 8H), 2.33–2.07 (m, 3H), 2.33 (s, 3H), 2.86–2.69 (m, 3H), 3.70–3.42 (m, 1H), 7.25–7.09 (m, 4H). Mass spectrum (*m*/*e*): 273 (M^+). Anal. Calcd for $C_{18}H_{27}NO$: C, 79.07; H, 9.95; N, 5.12. Found: C, 78.88; H, 10.15; N, 5.06.

4.6. (−)-2-(4-(2-Methylphenyl)piperidino)cyclohexanol ((−)-OMV) (13)

Racemic *o*-methylvesamicol (**11**) (1.63 g, 5.96 mmol) in 25 mL of acetone was added dropwise with stirring to (−)-di-*p*-toluoyl-L-tartaric acid monohydrate (2.54 g, 6.56 mmol) in 25 mL of acetone and set aside to crystallize at 23 °C. Crude crystals were collected by filtering and recrystallized from EtOH. The yield of product **13** was 0.541 g (66.5%). The filtrate was concentrated under reduced pressure and used to obtain the (+)-isomer of **11**.

Compound **13**: mp: 116–118. Anal. Calcd for $C_{18}H_{27}NO$: C, 79.07; H, 9.95; N, 5.12. Found: C, 78.94; H, 10.20; N, 5.06. Specific rotation: $[\alpha]_D^{30}$ −28.4 (c = 0.502, $CHCl_3$).

4.7. (+)-2-(4-(2-Methylphenyl)piperidino)cyclohexanol ((+)-OMV) (14)

The filtrate containing the (+)-isomer of **11** was added dropwise with stirring to (+)-di-*p*-toluoyl-D-tartaric acid monohydrate (5.54 g, 6.56 mmol) in 25 mL of acetone and set aside to crystallize at 23 °C. Crystals were collected by filtering and recrystallized from EtOH. The yield of product **14** was 0.168 g (20.6%).

Compound **14**: mp: 116–118 °C. Anal. Calcd for $C_{18}H_{27}NO$: C, 79.07; H, 9.95; N, 5.12. Found: C, 79.01; H, 10.04; N, 4.97. Specific rotation: $[\alpha]_D^{30}$ +28.3 (c = 0.504, $CHCl_3$).

4.8. (±)-2-(4-(4-Methylphenyl)piperidino)cyclohexanol ((±)-*p*-methylvesamicol) ((±)-PMV) (12)

Synthesis was carried out as for compound **12**, with 4-bromobenzaldehyde (**2**). Compound **12** from **2** via an eight-step reaction produced respective overall yields of 5.0%.

Compound **12**: mp: 128–130 °C. NMR ($CDCl_3$): δ 1.23 (4H, m), 1.57–1.84 (8H, m), 2.13 (1H, m), 2.22–2.2 (2H, m), 2.32 (3H, s), 2.43–2.45 (1H, m), 2.72–2.74 (2H, m), 2.92 (1H, m), 3.39 (1H, m), 7.12 (4H, s). Anal. Calcd for $C_{18}H_{27}NO$: C, 79.07; H, 9.95; N, 5.12. Found: C, 78.84; H, 9.96; N, 4.91. Mass spectrum (*m*/*e*): 273 (M^+).

4.9. (−)-2-(4-(4-Methylphenyl)piperidino)cyclohexanol ((−)-*p*-methylvesamicol) ((−)-PMV) (15)

This compound, **15**, was prepared using the same method as for **13**. **15** was obtained in 65.5% yield.

Compound **15**: mp: 128–130 °C. Mass spectrum (*m*/*e*): 273 (M^+). Anal. Calcd for $C_{18}H_{27}NO$: C, 79.07; H,

9.95; N, 5.12. Found: C, 78.79; H, 10.11; N, 4.97. Specific rotation: $[\alpha]_D^{30}$ −26.4 (c = 0.568, $CHCl_3$).

4.10. (+)-2-(4-(4-Methylphenyl)piperidino)cyclohexanol ((+)-*p*-methylvesamicol) ((+)-PMV) (16)

This compound, **16**, was prepared using the same method as for **14**.

Compound **16** was obtained in 30.0% yield.

Compound **16**: mp: 128–130 °C. Mass spectrum (*m*/*e*): 273 (M^+). Anal. Calcd for $C_{18}H_{27}NO$: C, 79.07; H, 9.95; N, 5.12. Found: C, 78.77; H, 10.02; N, 4.94. Specific rotation: $[\alpha]_D^{30}$ +26.7 (c = 0.500, $CHCl_3$).

4.11. (−)-2-(4-(2-Trimethylstannylphenyl)piperidino)cyclohexanol ((−)-*o*-trimethylstannyl-vesamicol) (19)

To a solution of (−)-*o*-iodovesamicol **13** (116 mg (0.3 mmol)) and hexamethylditin (246 mg (0.75 mmol)) in 5 mL dry toluene was added tetrakis(triphenylphosphine)palladium(0) (20 mg (17.5 μmol)). The mixture was degassed by bubbling argon through the reaction mixture for 5 min and then refluxed for 15 h under an argon atmosphere. As the reaction proceeded, the reaction mixture changed in color from yellow to black. The black precipitate was concentrated under reduced pressure and then purified by preparative thin-layer chromatography on silica gel using ethylacetate/hexane (1:5, v/v) as the eluent to yield **19** (42 mg, 33.0%).

Compound **19**, colorless oil. NMR ($CDCl_3$): δ 0.30 (9H, s), 1.42–1.60 (4H, m), 1.60–1.95 (8H, m), 2.02–2.40 (3H, m), 2.62–3.00 (3H, m), 4.22–4.68 (1H, m), 6.95–7.50 (4H, m). Mass spectrum (*m*/*e*): 421,423 $[M]^+$.

4.12. (+)-2-(4-(4-Tributylstannylphenyl)piperidino)cyclohexanol ((+)-*p*-tributylstannyl-vesamicol) (20)

This compound, **20**, was prepared using the same method as for compound **19** with (+)-*p*-iodovesamicol **18** and hexabutylditin. Compound **20** was obtained in 71.2% yield.

Compound **20**, colorless oil. NMR ($CDCl_3$): δ 0.86–0.91 (m. 9), 1.00–1.06 (m, 6), 1.12–1.26 (m, 4), 1.29–1.37 (m, 6), 1.48–1.60 (m, 6), 1.62–1.71 (m, 2), 2.13–2.27 (m, 3), 2.42–2.50 (m, 1), 2.73–2.76 (m, 2), 2.91–2.95 (m, 1), 3.39 (m, 1). Mass spectrum (*m*/*e*): 547,549 $[M]^+$.

4.13. Tissue preparation

Animal experiments were carried out in compliance with the Guidelines for the Care and Use of Laboratory Animals at the Takara-machi Campus of Kanazawa University. Rat brain membranes and rat liver membranes were prepared from rat brains without a cerebellum and livers from male Sprague–Dawley rats (250–300 g), each using a method described previously.[6]

5. In vitro competitive binding study

5.1. VAChT binding

The assays were performed using a method reported previously,[6] except that 504–710 μg protein of rat cerebral membranes was used. Briefly, 10 nM (−)-[^{3}H]vesamicol was used as a radioligand. Various concentrations of enantiomers of methylvesamicol analogs or sigma ligands (from 10^{-10} to 10^{-5} M) were used as subject compounds. The mixture was incubated for 60 min at 37 °C in the presence of 100 nM haloperidol to mask sigma receptors.

5.1.1. σ-1 Receptor binding. Rat cerebral membranes (504–710 μg protein) were incubated in triplicate with 3 nM (+)-[^{3}H]pentazocine and various concentrations of enantiomers of methylvesamicol analogs or sigma ligands (from 10^{-10} to 10^{-5} M) in 0.5 mL of 50 mM Tris–HCl (pH 7.8) for 90 min at 37 °C. Nonspecific binding was determined in the presence of 10 μM (+)-pentazocine. The incubated samples were treated in the same manner as described for the VAChT binding assays.

5.1.2. σ-2 Receptor binding. Rat liver membranes (155–296 μg protein) were incubated in triplicate with 3 nM [^{3}H]DTG and various concentrations of enantiomers of methylvesamicol analogous or sigma ligands (from 10^{-10} to 10^{-5} M) in 0.5 mL of 50 mM Tris–HCl (pH 7.8) for 90 min at 37 °C in the presence of 1 μM (+)-pentazocine to mask σ-1 sites. Nonspecific binding was determined in the presence of 10 μM DTG and 1 μM (+)-pentazocine. The incubated samples were treated in the same manner as described for VAChT binding assays.

6. Data analysis

K_i values in inhibition studies and K_d and B_{max} values in saturation binding studies were calculated with the 'Graphpad Prism' computer program (GraphPad Software, Inc. San Diego, USA).

References and notes

1. Wieden, L.; Eriksson, L.; Ingvar, M.; Parsons, S. M.; Rogers, G. A.; Stone-Elander, S. *Neurosci. Let.* **1992**, *13*, 1–4.
2. Efange, S. M. N.; Michelson, R. H.; Khare, A. B.; Thomas, J. R. *J. Med. Chem.* **1993**, *36*, 1754–1760.
3. Kuhl, D. E.; Koeppe, R. A.; Fessler, J. A.; Minoshima, S.; Ackermann, R. J., et al. *J. Nucl. Med.* **1994**, *35*, 405–410.
4. Khare, A. B.; Langason, R. B.; Parsons, S. M.; Mach, R. H.; Efange, S. M. N. *Nucl. Med. Biol.* **1999**, *26*, 609–617.
5. Bando, K.; Taguchi, K.; Cinoza, Y.; Naganuma, T.; Tanaka, Y.; Koike, K.; Takatoku, K. *Nucl. Med. Biol.* **2001**, *28*, 251–260.
6. Shiba, K.; Yano, T.; Sato, W.; Mori, H.; Tonami, N. *Life Sci.* **2002**, *71*/*13*, 1591–1598.
7. Shiba, K.; Mori, H.; Tonami, N. *Ann. Nucl. Med.* **2003**, *17*, 451–456.
8. Custer, F. G. J.; Leysen, J. E.; Stoof, J. C.; Herscheid, J. D. M. *Eur. J. Pharmacol.* **1997**, *338*, 177–183.

9. Shiba, K.; Ogawa, K.; Mori, H. *Bioorg. Med. Chem.* **2005**, *13*, 1095–1099.
10. Jung, Y. W.; Van Dort, M. E.; Gildersleeve, D. L.; Wieland, D. M. *J. Med. Chem.* **1990**, *33*, 2065–2068.
11. Efange, S. M. N.; Dutta, A. K.; Michelson, R. H.; Kung, H. F.; Thomas, J. R.; Billing, J.; Boudreau, R. *J. Nucl. Med. Biol.* **1992**, *19*/*3*, 337–348.
12. Efange, S. M. N.; Mach, R. H.; Smith, C. R.; Khare, A. B.; Foulon, C.; Akella, S. K.; Childers, S. R.; Parsons, S. M. *Biochem. Pharmacol.* **1995**, *49*, 791–797.
13. Jung, Y.-W.; Frey, K. A.; Mulholland, G. K.; Rosario, R. D.; Sherman, P. S.; Raffel, D. M.; Van Dort, M. E.; Kuhl, D. E.; Gildersleeve, D. L.; Wieland, D. M. *J. Med. Chem.* **1996**, *39*, 3331–3342.
14. Rogers, G. A.; Parsons, S. M.; Anderson, D. C.; Nilsson, L. M.; Bahr, B. A.; Kornreich, W. D.; Kaufman, R.; Jacobs, R. S.; Kirtman, B. *J. Med. Chem.* **1990**, *32*, 1217–1230.
15. De Costa, B. R.; Xiao-shu, H. Peter Jenner., Eds.; Academic Press, Horcourt Brace: New York, 1994; pp 45–111.
16. Weissman, A. D.; Broussolle, E. P.; London, E. D. *J. Chem. Neuroanat.* **1990**, *3*, 347–354.
17. Kawamura, K.; Kobayashi, T.; Matsuno, K.; Ishiwata, K. *Synapse* **2003**, *48*, 80–86.
18. Ishii, K.; Ishiwata, K.; Kimura, Y.; Kawamura, K.; Oda, K.; Senda, M. *NeuroImage* **2001**, *13*, S984 (Number 6 Part 2 of 2 Parts) (abstract).
19. Mishina, M.; Ishiwata, K.; Ishii, K.; Kitamura, S.; Kimura, Y.; Kawamura, K.; Oda, K.; Sasaki, S.; Sakayori, O.; Hamamoto, M.; Kobayashi, S.; Katayama, Y. *Acta Neurol.* **2005**, *112*, 103–107.
20. Suzuki, M.; Doi, H.; Bjokman, M.; Andersson, Y.; Lagstrom, B.; Watanabe, Y.; Noyori, R. *Chem. Eur. J.* **1997**, *3*, 2039–2042.