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N-Methyl-3-(1-Hydroxy-5-[^{123}I]Iodopent-4-enyl)-4-Acetoxypiperidine, A Novel Candidate of Acetylcholinesterase Activity Imaging Agent

Takao Ueda[1], Toshiaki Irie[2], Kiyoshi Fukushi[2], Nobuo Ikota[2], Keizo Takatoku[3], Isamu Yomoda[3] and Shin-Ichiro Nagatsuka[1,2]*

[1]ADME/TOX Research Institute, Daiichi Pure Chemicals, Co., Ltd., Ibaraki,
[2]Advanced Technology for Medical Imaging, National Institute of Radiological Sciences, Chiba,
[3]Research Laboratories, Daiichi Radioisotope Laboratories, Ltd., Chiba,
Japan

SUMMARY

A novel acetylcholine radioanalog, *N*-methyl-3-(1-hydroxy-5-[^{123}I]iodopent-4-enyl)-4-acetoxypiperidine, was prepared by radioiodination of the corresponding tributylstannyl precursor that was synthesized in eight steps from 4-piperidone. The tracer has three asymmetric carbons giving eight optical isomers. Two optical isomers were isolated in the precursor synthesis by diastereomeric and enantiomeric separation. In the incubation experiments using rat cerebral cortical homogenate, one optical isomer was hydrolyzed by acetylcholinesterase with high reactivity and selectivity. The tracer is a candidate for mapping cerebral regional acetylcholinesterase activity by single photon emission computed tomography.

Key Words: acetylcholine analog, acetylcholinesterase, synthesis, chiral separation, radioiodination, single photon emission computed tomography

INTRODUCTION

Extensive studies at necropsy have pointed out degeneration of cortical and hippocampal cholinergic projections accompanied with reduced activity of choline acetyltransferase and acetylcholinesterase (AChE) in the brain of patients with Alzheimer's disease (1-3). We have designed several acetylcholine analogs such as *N*-methylpiperidin-3-yl-acetate (MP3A), -3-yl-propionate (MP3P), -4-yl-acetate (MP4A) and -4-yl-propionate (MP4P or PMP) for mapping cerebral AChE activity in order to detect cholinergic degeneration *in vivo* (4). These tracers are lipophilic enough to pass through the brain-blood barrier. The tracers incorporated into the

*Correspondence to: Shin-Ichiro Nagatsuka, ADME/TOX Research Institute, Daiichi Pure Chemicals, Co., Ltd., 2,117 Muramatsu, Tokai-mura, Naka-gun, Ibaraki 319-1182, Japan; TEL: +81-29-282-0232; FAX: +81-29-282-0182; e-mail: nagatsuka@hkg.odn.ne.jp

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brain were partly washed out, and the rest were hydrolyzed selectively by AChE to form hydrophilic alcohol that was consequently trapped in the brain at the site of metabolic reaction (Fig.1) (4-7). Recently, carbon-11 labeled MP4A and PMP have been applied to positron emission tomography (PET), resulting in successfully quantitative measurement of AChE activity in living human brain (8-14). However, widespread clinical application of this method is quite restricted by not only extremely short half-life of carbon-11 but also limited availability of PET scanners. In the present study, we have synthesized a novel acetylcholine analog labeled with iodine-123, *N*-methyl-3-(1-hydroxy-5-[^{123}I]iodopent-4-enyl)-4-acetoxypiperidine (MHIP4A) **11**(I-123), with a view to developing an AChE activity imaging agent for single photon emission computed tomography (SPECT). The tracer possesses common structure to MP4A and additionally includes the side chain with double bond for iodine labeling together with hydroxyl group reducing the extremely high lipophilicity of the side chain to ensure the cerebral retention of metabolite. [^{123}I]MHIP4A was prepared by radioiodination from the tributylstannyl precursor. Two of the eight optical isomers of MHIP4A were separated during the precursor synthesis. Reactivity and selectivity in their hydrolysis by AChE were evaluated in the incubation experiments using rat cerebral cortical homogenate.

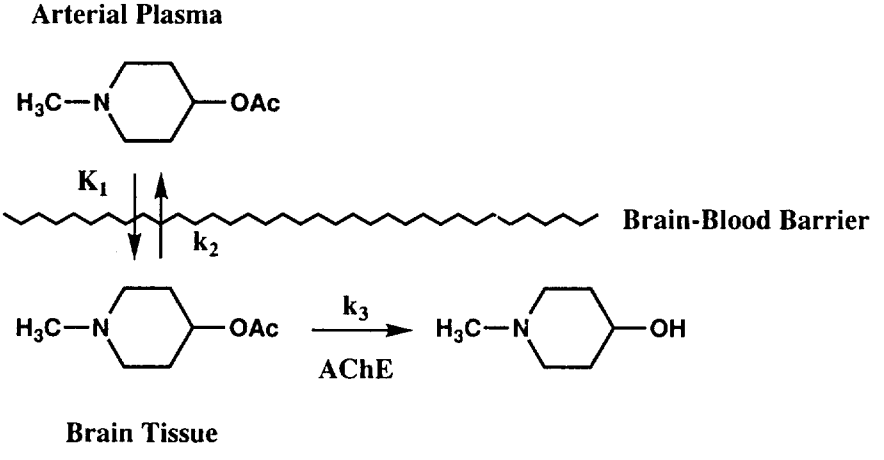
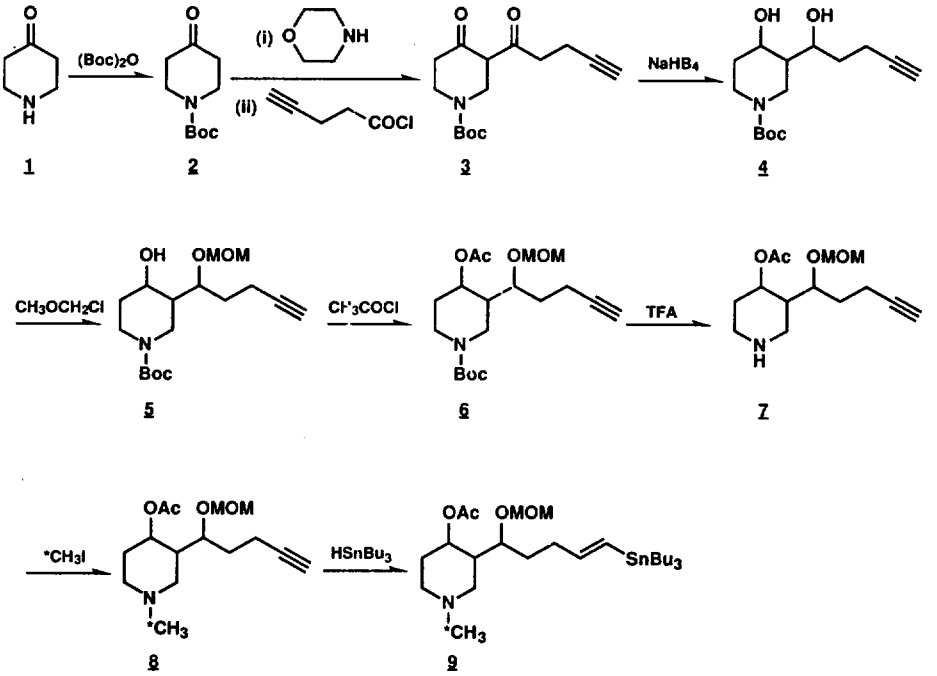


Fig.1: Metabolic trapping of an *N*-methylpiperidinyl derivative, MP4A, in the brain. MP4A is incorporated into the brain depending upon regional cerebral blood flow. A portion of MP4A is washed out, but the rest is hydrolyzed by AChE to *N*-methyl-4-piperidinol that is locally trapped in the brain. K_1, k_2 and k_3 are rate constants of tracer uptake, washout and metabolic reaction, respectively.

RESULTS AND DISCUSSION

The amine residue of the starting material, 4-piperidone **1**, was protected with *N*-t-butyloxycarbonyl (Boc) group. The Boc derivative **2** was acylated with 4-pentynoyl chloride *via* enamine to provide 3-acyl-4-piperidone **3**. The diketone **3** was reduced with sodium borohydride to give corresponding diol **4** with three asymmetric carbons. Diastereomeric separation of the diol **4** using silica gel chromatography gave three fractions of isomers. The diastereomer fraction eluted first (1st fraction) was thought to be a mixture of two enantiomers. Actually, methoxymethyl (MOM) derivative **5**



Scheme.1: Synthetic route of the tributylstannyl precursor of MHIP4A, *N*-methyl-3-(1-methoxymethyloxy-5-tributylstannylpent-4-enyl)-4-acetoxypiperidine **9**. *C shows ^{12}C or ^{14}C.

derived from the 1st fraction could be separated by chiral-HPLC into two isomer fractions 1:1 (Fig. 2), giving $[\alpha]_D$ values of +91.6° and -90.0°. The enantiomer with shorter retention time (Rt) **5a** gave the final material **11** that could not be hydrolyzed

by AChE at all. However, the final material **11** derived from the other enantiomer with longer Rt **5b** showed preferable reactivity and selectivity against AChE.

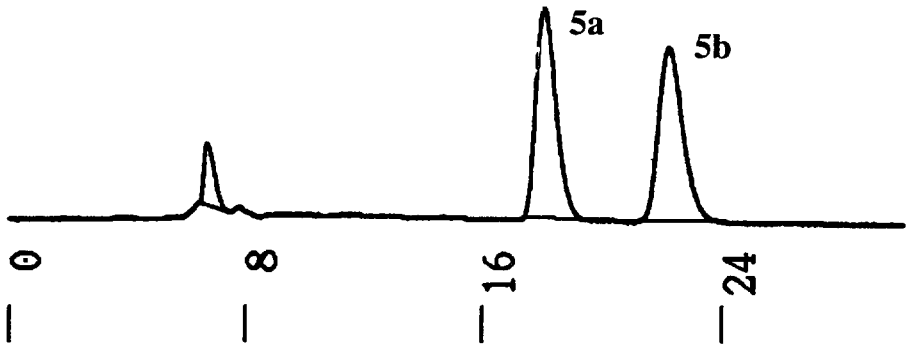
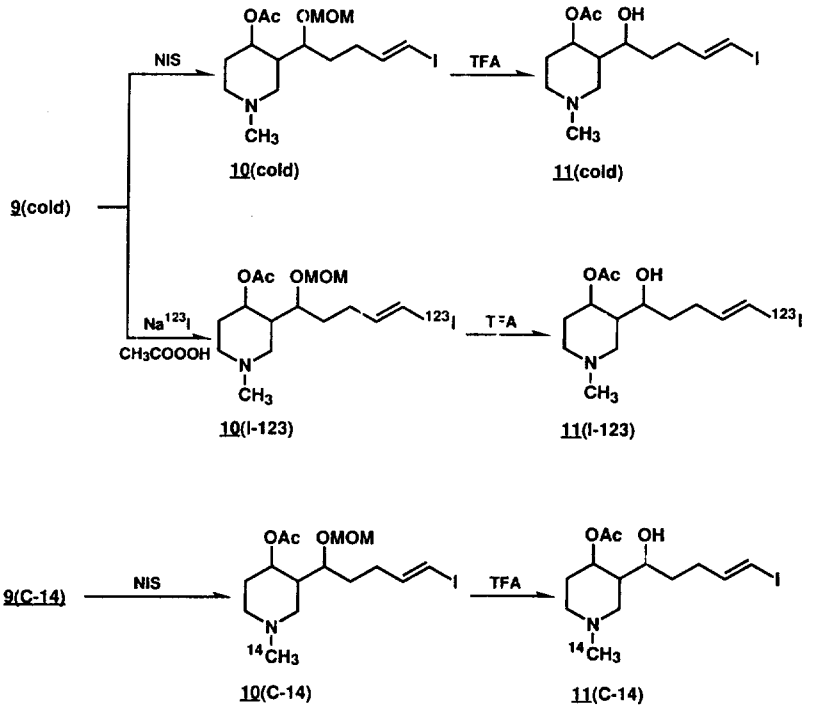


Fig.2: Chiral separation of two enantiomers **5** derived from the 1st fraction of **4**.
The enantiomer with shorter Rt (18.1min) **5a** showed $[\alpha]_D$ value of +91.6°, while the other with longer Rt (22.2min) **5b** showed $[\alpha]_D$ value of -90.0° (0.25% in ethyl acetate). Ratio of two isomers was 1:1.

The MOM derivative **5b** was acetylated to **6** with acetyl chloride. Removal of Boc group with trifluoroacetic acid (TFA) gave **7**, which was then *N*-methylated with cold or carbon-14 labeled methyl iodide to yield **8**(cold or C-14). The *N*-methylated material **8**(cold or C-14) was reacted with tributyltin hydride to provide the precursor, *N*-methyl-3-(1-methoxymethyloxy-5-tributylstannylpent-4-enyl)-4-acetoxypiperidine **9**(cold or C-14).

The precursor **9**(cold) was treated with *N*-iodosuccinimide (NIS) to yield **10**(cold) consisting of two geometrical isomers (*trans* and *cis* iodine at double bond in the pentenyl side chain). The *trans* and *cis* isomers separated by ODS-HPLC could be identified from the signal of olefinic proton in ^{1}H NMR. Removal of MOM group of **10**(cold) with TFA gave the authentic MHIP4A **11**(cold). Carbon-14 labeled MHIP4A **11**(C-14) was prepared from **9**(C-14) by the same procedure as the preparation of MHIP4A **11**(cold). Both geometrical isomers of **11** showed high selectivity against AChE with almost identical metabolic reactivity. In the present study, we have evaluated the *trans* isomer that was formed predominantly (*trans*:*cis* = 3:1).

The radioiodination was performed by adding sodium [^{123}I]iodide to 9(cold) in the presence of peracetic acid as an oxidizing agent. The reaction was terminated by addition of sodium metabisulfite, and the mixture was extracted with ethyl acetate and purified by ODS-HPLC to obtain *trans* 10(I-123). Removal of MOM group of the *trans* 10(I-123) with TFA gave the final material, MHIP4A 11(I-123). The overall radiolabeling process was completed within 5hrs with more than 50% of radiochemical yield (from sodium [^{123}I]iodide). Radiochemical purity was more than 98%.



Scheme.2: Preparation of cold, iodine-123 labeled and carbon-14 labeled MHIP4A 11 from their tributylstannyl precursor.

Reactivity and selectivity of MHIP4A in the hydrolysis by AChE were examined by incubating carbon-14 or iodine-123 labeled MHIP4A 11(C-14 or I-123) with rat cerebral cortical homogenate in the presence and absence of specific AChE inhibitor, BW284c51 (6). The reactivity of MHIP4A 11(C-14 or I-123) in the absence of

BW284c51 was 0.73mL/min/g tissue. The reactivity was greatly reduced to 0.058 mL/min/g tissue in the presence of the specific inhibitor. The selectivity of MHIP4A **11**(C-14 or I-123) against cerebral AChE was, therefore, calculated to be 92%. The final concentration of BW284c51 employed (0.016mM) inhibited rat cerebral AChE activity by 100% without inhibiting butyrylcholinesterase activity (15).

The selectivity of clinically used N-methylpiperidinyl esters against rat cerebral cortical AChE was more than 90%, and their reactivity ranged from about 0.4mL/min/g for MP4P and about 1.5mL/min/g for MP4A. The reactivity of MHIP4A **11**(C-14 or I-123) was within this range with high specificity against cerebral AChE. Metabolic profile of MHIP4A **11**(C-14 or I-123) was, therefore, quite preferable. *In vivo* distribution study, which will be published elsewhere, showed blood-flow dependent early distribution of MHIP4A **11**(I-123) followed by accumulation of metabolite depending upon cerebral regional AChE activity in rat brain. This material is, therefore, a candidate of SPECT tracer for mapping cerebral AChE activity.

EXPERIMENTAL

Materials. All chemicals used in the present study were of reagent grade. Solvents were dried over molecular sieves, and freshly distilled when required. Compositions of solvent system were all shown in volume by volume. Yields of chemical reaction were calculated on the substrate and product weight basis.

Thin-layer chromatography (TLC) to monitor the progress of chemical and metabolic reactions was run on silica gel TLC plates (Merck $60F_{254}$). Silica gel chromatography was run using Merck silica gel 60 (70-230 mesh) in an appropriate glass column. Uncorrected melting points were determined on an FP62 apparatus (Mettler, Greifensee, Switzerland). High resolution mass spectrometry (HRMS) was done as a commercial service in Hitachi Instruments Engineering Co., Ltd. (Ibaraki, Japan). Mass spectra were taken under electron impact (EI) or secondly ion mass spectrometry (SIMS) mode. ^{1}H NMR spectra were obtained by using a GX-400 spectrometer (JEOL, Tokyo, Japan) in deuterated chloroform. Chemical shifts are shown in δ (ppm) downfield compared to tetramethylsilane as an internal standard.

High performance liquid chromatography (HPLC) was run on an LC-6A HPLC system (Shimadzu, Kyoto, Japan) with UV detection at 215nm. Chiral-cel OJ column (20x250mm) was obtained from Daicel Chemical Industries Ltd. (Tokyo, Japan). Novapak ODS-C18 column (4.6x300mm) was obtained from Waters Co. (Milford, MA, USA).

Male Wistar rats (8 weeks old) were purchased from Imamichi Institute for Animal Reproduction (Ibaraki, Japan). Phosphor imaging plates (IP, Type-BASIII) and a bioimaging analyzer (BAS-2000) were obtained from Fuji Photo Film Co. (Tokyo, Japan).

***N*-t-butyloxycarbonyl-4-piperidone 2**: 4-Piperidone monohydrate hydrochloride **1** (75g, 488.2mmol) was dissolved in 250mL of distilled water. To this solution was added 1000mL of 1M sodium hydroxide and further added di-t-butyldicarbonate (120g) dropwise under vigorous stirring at room temperature for 6hr. The reaction mixture was then extracted with ethyl acetate. The extract was evaporated *in vacuo*, and the residual solid was recrystallized from hexane to yield 38.9g of **2** (195.3mmol, 40%). m.p.: 74.4-75.2℃.

***N*-t-butyloxycarbonyl-3-(1-oxo-4-pentynyl)-4-piperidone 3**: **2** (30g, 150.6mmol) was dissolved in 150mL of toluene followed by addition of 19mL of morpholine and heated under reflux for 20hr with removing water using a Dean-Stark apparatus. After cooling to room temperature, the mixture was evaporated *in vacuo* and redissolved in 150mL of anhydrous dioxane. To this solution was added 6.0g of 4-pentynoyl chloride dropwise, followed by heating under reflux for 16hr. After cooling to room temperature, the mixture was filtered and evaporated *in vacuo*. The residue was purified by silica gel chromatography (hexane:ethyl acetate = 4:1) and recrystallization from hexane to yield 6.03g of **3** (21.6mmol , 14%). m.p.: 76.8-77.6℃; HRMS (EI) calcd for $[M-C_4H_9]^+$: 222.0765, found 222.0744.

***N*-t-butyloxycarbonyl-3-(1-hydroxy-4-pentynyl)-4-piperidinol 4**: **3** (5.58g, 20.0 mmol) was dissolved in 80mL of ethyl acetate:ethanol (1:1) followed by addition of 1g of sodium borohydride portionwise and stirred at room temperature for 1hr. The mixture was diluted with water and extracted with ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated *in vacuo* to

yield 4.82g of **4** (17.0mmol, 85%). HRMS (SIMS) calcd for $[M+H]^+$: 284.1860, found 284.1855.

Silica gel chromatography (hexane:ethyl acetate = 1:1, column size 35x250mm) of **4** (2.6g) gave three diastereomer fractions. The elution volume of each fraction was 400-600mL for the 1st fraction (1.44g), 600-740mL for the 2nd fraction (0.7g) and 740-940mL for the 3rd fraction (0.4g), respectively. The 1st diastereomer fraction of **4** was subjected to further syntheses as described in the Results and Discussion section.

***N*-t-butyloxycarbonyl-3-(1-methoxymethyloxy-4-pentynyl)-4-piperidinol 5**: To the 1st fraction of **4** (1.44g, 5.08mmol) dissolved in 30mL of ethyl acetate and 8.9mL of diisopropylamine was added 2mL of MOM chloride dropwise and stirred at room temperature for 5hr. The reaction mixture was diluted with water under cooling in an ice-water bath, added 30mL of 1M hydrochloric acid dropwise and extracted with ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate and evaporated *in vacuo* to obtain crude **5**, which was then purified by silica gel chromatography (hexane:ethyl acetate = 1:1). The material **4** was then recovered from the chromatography and methoxymethylated again in the same manner. The methoxymethylation was performed twice under mild conditions to suppress the methoxymethylation of 4-hydroxy group of the piperidine ring. The overall yield was 0.60g (1.83mmol, 36%).

The MOM derivative **5** was subjected to chiral-HPLC using chiral-cel OJ (hexane:2-propanol = 100:5, flow rate 6mL/min) to obtain two isomer fractions with shorter and longer retention time. Enantiomer **5a** with shorter retention time: HRMS (SIMS) calcd for $[M+H]^+$: 328.2122, found 328.2105. $[\alpha]_D$ +91.6° (0.25% in ethyl acetate). Enantiomer **5b** with longer retention time. HRMS (SIMS) calcd for $[M+H]^+$: 328.2122, found 328.2114. $[\alpha]_D$ -90.0° (0.25% in ethyl acetate).

Both enantiomers were converted to the final material, however, MHIP4A derived from **5a** could not be hydrolyzed by AChE as described in the Results and Discussion section. Experimental details with respect to enantiomer **5b** are presented hereafter.

***N*-t-butyloxycarbonyl-3-(1-methoxymethyloxy-4-pentynyl)-4-acetoxypiperidine 6**: The enantiomer **5b** obtained from the chiral HPLC separation (216mg, 0.66mmol)

was dissolved in 40mL of hexane:dichloromethane (1:1) and 806mg of 4-*N*,*N*-dimethylaminopyridine was added. To this solution was added 0.235mL of acetyl chloride dropwise and stirred at room temperature for 5hr. The reaction mixture was diluted with water under cooling in an ice-water bath and 30mL 1M hydrochloric acid was added dropwise. The mixture was then extracted with ethyl acetate. The extract was washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate and evaporated *in vacuo* to obtain crude **6**, which was then purified by silica gel chromatography (hexane:ethyl acetate = 3:1) to yield 250mg of **6** (0.66 mmol, 100%). HRMS (EI) calcd for M^+: 369.2150, found 369.2160.

3-(1-methoxymethyloxy-4-pentynyl)-4-acetoxypiperidine 7: **6** (125mg, 0.34 mmol) was dissolved in 20mL of hexane:dichloromethane (1:1) followed by addition of 1.0mL of TFA dropwise and then stirred at room temperature for 16hr. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in 20mL of dichloromethane. To this solution was added several drops of conc. ammonia solution under stirring in an ice-water bath. The organic layer was dried over anhydrous sodium sulfate and evaporated *in vacuo* to yield 90mg of **7** (0.33mmol, 99%).

***N*-methyl-3-(1-methoxymethyloxy-4-pentynyl)-4-acetoxypiperidine 8**(cold): **7** (90mg, 0.33mmol) was dissolved in 20mL of acetone followed by addition of 25mg of cold methyl iodide dropwise at -80°C and subsequently stirred at room temperature for 2hr. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in 15mL of chloroform. To this solution was added one drop of conc. ammonia solution under stirring in an ice-water bath. The solution was dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residue was purified by silica gel chromatography (chloroform:methanol = 15:1) to yield 17mg of **8**(cold) (60μmol, 18%). HRMS (EI) calcd for M+: 283.1782, found 283.1818.

8(C-14) was prepared from **7** by the same manner except for the addition of [^{14}C]methyl iodide instead of cold methyl iodide.

***N*-methyl-3-(1-methoxymethyloxy-5-tributylstannylpent-4-enyl)-4-acetoxy-piperidine 9**(cold): **8**(cold) (17mg, 60μmol) and 2,2'-azo-bis-isobutyronitrile (5mg) were dissolved in 10mL of anhydrous toluene, 0.1mL of tributyltin hydride was added dropwise at 85°C under nitrogen atmosphere and the solution was stirred at

85°C for 1hr. The reaction mixture was then evaporated *in vacuo* and the residue was purified by silica gel chromatography (chloroform:methanol = 15:1) to yield 30mg of **9**(cold) (52μmol, 87%). ^{1}H NMR ($CDCl_3$) δ 5.90 (2H, s, olefinic), 5.08-5.04 (1H, m), 4.69 (1H, d, J=7.3Hz, OC**H**HOCH$_3$), 4.61 (1H, d, J=7.3Hz, OCH**H**OCH$_3$), 3.97 (1H, s), 3.39 (3H, s, OCH_2OCH_3), 3.30 (1H, d, J=12.5Hz), 3.15 (1H, d, J=9.5Hz), 2.99-2.91 (2H, m), 2.75 (3H, d, J=4.4Hz, N**CH**$_3$), 2.68-2.58 (1H, m), 2.46-2.39 (1H, m), 2.13-2.11 (2H, m), 2.09 (3H, s, **CH**$_3$COO), 1.83 (1H, br), 1.71-1.60 (2H, m), 1.52-1.44 (6H, m, $SnCH_2CH_2CH_2CH_3$ or $SnCH_2CH_2CH_2CH_3$), 1.41-1.26 (6H, m, $SnCH_2CH_2CH_2CH_3$ or $SnCH_2CH_2CH_2CH_3$), 0.94-0.84 (15H, m, Sn**CH**$_2$CH$_2$CH$_2$**CH**$_3$).

9(C-14) was prepared from **8**(C-14) by the same manner.

***N*-methyl-3-(5-iodopent-1-methoxymethyloxy-4-enyl)-4-acetoxypiperidine 10** (cold): **9**(cold) (30mg, 52μmol) was dissolved in 2.5mL of dichloromethane. NIS in dichloromethane (ca. 20mg/mL) was then added dropwise under stirring in an ice-water bath until completion of the reaction. The reaction mixture was evaporated *in vacuo* and the residue was purified by silica gel chromatography (chloroform: methanol = 15:1) to yield 16mg of **10**(cold) (39μmol, 74%). ODS-HPLC using Novapak ODS C-18 (methanol:water:triethylamine = 70:30:0.2, flow rate 0.6 mL/min) gave two geometric isomers of **10**(cold) (*trans*:*cis* = 3:1, retention time: *trans* 16.0min, *cis* 14.0min). ^{1}H NMR ($CDCl_3$) δ 6.50-6.43 (1H, m, olefinic), 6.06 (1H, d, olefinic, *trans* J=14.7Hz, *cis* J=8.1Hz), 5.08-5.03 (1H, m), 4.69 (1H, d, J=7.3Hz, OC**H**HOCH$_3$), 4.60 (1H, d, J=7.3Hz, OCH**H**OCH$_3$), 3.93 (1H, s), 3.39 (3H, s, OCH_2OCH_3), 3.31 (2H, d, J=9.5Hz), 3.06-2.87 (1H, m), 2.76 (3H, d, J=5.1Hz, N**CH**$_3$), 2.65-2.59 (1H, m), 2.45-2.38 (1H, m), 2.13-2.06 (4H, m), 2.10 (3H, s, **CH**$_3$COO), 1.88-1.85 (1H, m), 1.73-1.68 (1H, m); HRMS (EI) calcd for M^+: 411.0905, found 411.0908.

10(C-14) was prepared from **9**(C-14) by the same manner.

N-methyl-3-(1-hydroxy-5-iodopent-4-enyl)-4-acetoxypiperidine 11(cold): **10** (cold) (16mg, 39μmol) was dissolved in 1mL of dichloromethane followed by addition of 3mL of TFA and allowed to stand for 24hr at room temperature. The reaction mixture was evaporated *in vacuo* and the residue was purified by silica gel

chromatography (chloroform: methanol = 8:1) to yield 13mg of **11**(cold) (35μmol, 91%). The mixture of two geometric isomers of **11**(cold) could be separated by ODS-HPLC using Novapak ODS C-18 (methanol:water:triethylamine = 70:30:0.2, flow rate 0.6mL/min, retention time: *trans* 9.4min, *cis* 8.0min). HRMS (EI) calcd for M^+: 367.0642, found 367.0600.

11(C-14) was prepared from **10**(C-14) by the same manner.

Radioiodination of tributylstannyl precursor: **9**(cold) (0.1mg) was dissolved in 0.05mL of ethanol followed by addition of 50-200MBq of sodium [^{123}I]iodide, 0.05mL of 0.1M hydrochloric acid and 0.05mL of 0.32% peracetic acid. The reaction mixture was allowed to stand for 30min at room temperature with occasional stirring. To this mixture was added 0.05mL of sodium metabisulfite (100mg/mL) followed by addition of 1mL of saturated sodium carbonate solution. The mixture was extracted with ethyl acetate. The extract was dried over anhydrous sodium sulfate, evaporated *in vacuo* and the residue was purified by ODS-HPLC using Novapak ODS C-18 (methanol:water:triethylamine = 70:30:0.2, flow rate 0.6mL/min) to obtain two geometric isomers of **10**(I-123) (*trans*:*cis* = 3:1, radiochemical yield from sodium [^{123}I]iodide > 50%). The retention time of each geometric isomer was identical to that of corresponding cold sample.

Predominant *trans* isomer of **10** (I-123) was taken and treated with 0.5mL of TFA for 2hr at room temperature. The mixture was evaporated *in vacuo* and the residue was purified by ODS-HPLC using Novapak ODS C-18 (methanol:water:triethylamine = 70:30:0.2, flow rate 0.6mL/min) to obtain the final material **11**(I-123). The retention time of the tracer was identical to that of cold sample. Radiochemical purity was more than 98%. Theoretical specific radioactivity was 8.715PBq/mmol.

Incubation experiment using rat cerebral cortical homogenate: The buffer solution used was 0.1M potassium phosphate (pH 7.4). Rat cerebral cortex was homogenized using a glass homogenizer equipped with a Teflon-pestle and adjusted to 200mg tissue/mL in the buffer. The homogenate (0.2mL) was taken in a plastic tube, 0.01mL of the buffer with or without BW284c51 (final concentration 0.016mM) was added and preincubated at 37°C for 10min. Carbon-14 or iodine-123 labeled MHIP4A (ca. 37kBq) in 0.02mL was added to start metabolic reaction at 37°C.

After incubating designated time period (3-30min), the reaction was terminated by adding 0.5mL of ethanol, followed by centrifugation at 1800 x g for 15min. A portion of supernatant solution was directly spotted onto a TLC plate and developed using ethyl acetate:2-propanol: conc. ammonia solution = 15:5:1 as a solvent system. The plate was dried, covered with thin polyethylene foil (4μm thick) and exposed to an IP for 16hr. The IP was read by BAS-2000 to obtain the ratio of unchanged MHIP4A and its hydrolyzed metabolite in the incubation mixture. Carbon-14 labeled MP4A and MP4P, synthesized as reported previously (4), were also subjected to the incubation experiment by the same manner. Metabolic reactivity and selectivity was calculated as reported previously (6).

CONCLUSION

One of the eight optical isomers of *N*-methyl-3-(1-hydroxy-5-[^{123}I]iodopent-4-enyl)-4-acetoxypiperidine was isolated and confirmed to have preferable metabolic reactivity and selectivity against cerebral acetylcholinesterase. This acetylcholine radioanalog is a candidate of tracer for mapping regional cerebral acetylcholinesterase activity using single photon emission computed tomography.

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