N-[^{18}F]Fluoroethylpiperidin-4ylmethyl Acetate, a Novel Lipophilic Acetylcholine Analogue for PET Measurement of Brain Acetylcholinesterase Activity

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The reduction of acetylcholinesterase (AChE) activity in the brain has been measured in dementia disorders such as Alzheimer's disease and dementia with Lewy bodies using ^{11}C-labeled acetylcholine analogues, *N*-[^{11}C]methylpiperidin-4-yl acetate and propionate, and positron emission tomography (PET). Our aim was to develop an ^{18}F-labeled acetylcholine analogue useful for brain AChE mapping with PET, since ^{18}F, with a longer half-life, has advantages over ^{11}C. In a preliminary study, a series of *N*-[^{14}C]ethylpiperidin-3-yl or -4-ylmethanol esters (acetyl and propionyl esters) were newly designed and evaluated in vitro regarding the reactivity with and specificity to AChE using purified human enzymes, leading to a novel ^{18}F-labeled acetylcholine analogue, *N*-[^{18}F]fluoroethylpiperidin-4-ylmethyl acetate. In rat experiments, the ^{18}F-labeled candidate showed desirable properties for PET AChE measurement: high brain uptake of the authentic ester, high AChE specificity, a moderate hydrolysis rate, and low membrane permeability (metabolic trapping) of the metabolite.

Introduction

Biochemical analyses of postmortem brain tissues have revealed that the activity of acetylcholinesterase (AChE) was lower in Alzheimer's disease compared with age-matched controls.[1] For quantitative measurement of AChE activity in the brain using positron emission tomography (PET), ^{11}C-labeled lipophilic acetylcholine analogues, *N*-[^{11}C]methylpiperidin-4-yl acetate ([^{11}C]-MP4A) and propionate ([^{11}C]MP4P), were developed[2] and applied to PET studies of dementia disorders such as Alzheimer's disease,[3,4] Parkinson's disease with dementia,[5] and dementia with Lewy bodies.[6,7]

Since ^{18}F has a longer half-life (110 min vs 20 min) than ^{11}C, ^{18}F-labeled derivatives of [^{11}C]MP4A and [^{11}C]-MP4P have advantages over [^{11}C]MP4A and [^{11}C]MP4P such as better image quality and delivery of tracer to other PET centers. Though displacement of *N*-[^{11}C]-methyl group of [^{11}C]MP4A and [^{11}C]MP4P with [^{18}F]-fluoromethyl group would provide only a minimal biochemical change, due to the molecular similarity and biosteric property of the *N*-CH_2F and *N*-CH_3 groups, it is not applicable to the piperidine ring due to the instability of the fluoromethyl group attached to the secondary amine.[8] Recently, using [^{18}F]fluoroethyl bromide as an ^{18}F-labeling reagent, ^{18}F-labeled derivatives of [^{11}C]MP4A and [^{11}C]MP4P such as *N*-[^{18}F]fluoroethylpiperidin-4-yl acetate ([^{18}F]FEtP4A),[9] *N*-[^{18}F]fluoroethylpiperidin-3-yl acetate, and *N*-[^{18}F]fluoroethylpiperidin-4-yl propionate[10] were synthesized. Though the *N*-[^{18}F]fluoroethyl moiety in the derivatives was resistant to in vivo defluorination, all of the compounds showed a much lower hydrolysis rate and lower specificity to AChE than [^{11}C]MP4A and [^{11}C]MP4P.[10,11]

It has been reported that primary alcohol esters are more rapidly hydrolyzed by cholinesterases than secondary alcohol esters.[12] Modification of the ester group on *N*-[^{18}F]fluoroethylpiperidines from the secondary to the primary ester form is expected to produce an ^{18}F-labeled acetylcholine analogue with appropriate AChE reactivity. In this study, we synthesized a series of *N*-[^{14}C]ethylpiperidin-3-yl and -4-ylmethanol esters (primary alcohol esters) and evaluated their reactivity and specificity for AChE in vitro using purified human enzymes to determine the compound most suitable for ^{18}F-labeling. After the screening, the selected piperidinemethanol ester was labeled with [^{18}F]fluoroethyl bromide and its reactivity and AChE specificity were evaluated in vitro using rat tissue and an AChE specific inhibitor. Furthermore, an in vivo study was also performed in rat for evaluating the retention of the metabolite in the brain.

Rationale. Figure 1 shows a compartmental model for the lipophilic acetylcholine analogue illustrating the principle of PET measurement of AChE activity in the brain based on the metabolic trapping of the acetylcholine analogue. In this model, the k_3 represents the first-order hydrolysis rate of the acetylcholine analogue with AChE. In PET, the k_3 value is not measured directly but is estimated through mathematical analysis from the time−activity curve of tracer in the brain measured by PET. Therefore, the accuracy of AChE measurement with PET depends on the tracer used for k_3 estimation. The ideal tracer must satisfy the following conditions: First, the unchanged tracer must be sufficiently lipophilic to cross the blood−brain barrier. Second, the tracer must have a high specificity for the target enzyme. Third, the hydrolysis rate of the tracer must be moderate. It is known that precise estimation of k_3

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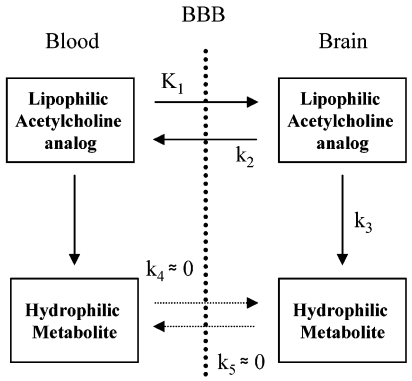


Figure 1. Schematic diagram representing the rationale of tracer design for measuring AChE activity in the brain using $^{11}C/^{18}F$-labeled lipophilic acetylcholine analogue and PET. In this model, K_1 and k_2 represent the rate constants of transfer of the lipophilic analogue across the blood−brain barrier (BBB), k_3 represents the hydrolysis rate of the ester by AChE (an index of AChE activity) and k_4 and k_5 represent the rate constants of transfer of the hydrophilic metabolite across the BBB. Both k_4 and k_5 must be small.

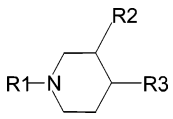
is possible under the condition that the ratio of k_3 to k_2 is about 0.5.[13] Finally, the radiolabeled metabolite must be sufficiently hydrophilic not to cross the blood−brain barrier; thus both k_4 and k_5 must be as small as possible.

Synthesis. The esters evaluated in this study were prepared from corresponding *N*-Boc piperidinol or piperidinemethanols as starting materials. The optical separation of the *R* and *S* isomers of *N*-Boc-3-piperidinemethanol was performed by the literature method with lipase PS.[14] The optical purity was 90% for *R* isomer and more than 99% for *S* isomer. *N*-[^{14}C]-ethyl and *N*-[^{18}F]fluoroethyl labeling was performed with [^{14}C]ethyl iodide (2.0 MBq/μmol) and [^{18}F]fluoroethyl bromide (740 GBq/μmol), respectively. The radiochemical purities of the labeled compounds were more than 98%. Though some primary esters at the second position of the piperidine or pyrrolidine ring are known to migrate to the N atom,[15,16] all the primary esters synthesized in this study were stable (decomposition rate; <0.0001 min^{-1}) in a pH 7.4 phosphate buffer.

In Vitro Study. The hydrolysis rate with and specificity to AChE of the ^{14}C and ^{18}F-labeled esters were evaluated in vitro using two assay systems, either a pure enzyme system or a crude brain tissue homogenate system. In the former system, since it is known that BChE hydrolyzes acetylcholine at about one-third the rate of AChE, BChE (human serum) and AChE (human erythrocytes) were used to examine the quantitative relationships between the structure and hydrolysis rate of the esters. In the latter system, to evaluate the AChE specificity of esters more rigorously, the hydrolysis rate of the esters was measured in rat (Wistar, male, 8 weeks) brain tissue homogenates with or without a specific inhibitor of AChE (BW284c51, 30 nM). The octanol−water partition coefficients of the esters and the metabolites were measured using ^{14}C- and ^{18}F-labeled compounds.

In Vivo Study. To evaluate bidirectional transport rates of the hydrophilic metabolite of [^{18}F]**6** across the blood−brain barrier (k_4 and k_5 in Figure 1), rat in vivo studies were performed. Time courses of radioactivity concentration in three brain regions (cerebral cortex, cerebellum, and striatum) were measured at 1, 5, 15, 30, and 60 min after intravenous administration of [^{18}F]-**6** and the metabolite of [^{18}F]**6** in rats. The k_5, the elimination rate constant of radioactivity from the brain, was estimated from the curve during 15 and 60 min after intravenous injection of [^{18}F]**6** in rats. The brain uptake was expressed as % dose per g tissue after correction for cerebral blood pool (4%) radioactivity.

Table 1. Structure of Compounds



compound	R1	R2	R3
		Secondary Esters	
MP4A	Me	H	$OCOCH_3$
MP4P	Me	H	$OCOCH_2CH_3$
FEtP4A	CH_2CH_2F	H	$OCOCH_3$
1	Et	H	$OCOCH_3$
		Primary Esters	
2	Et	H	CH_2OCOCH_3
3R	Et	(*R*)CH_2OCOCH_3	H
3S	Et	(*S*)CH_2OCOCH_3	H
4	Et	H	$CH_2OCOCH_2CH_3$
5R	Et	(*R*)$CH_2OCOCH_2CH_3$	H
5S	Et	(*S*)$CH_2OCOCH_2CH_3$	H
6	CH_2CH_2F	H	CH_2OCOCH_3

Results and Discussion

In preliminary studies, to determine the primary ester structure suitable for ^{18}F-labeling, a series of *N*-[^{14}C]-ethylpiperidin-3-yl or -4-ylmethanol esters were synthesized and evaluated in vitro with respect to reactivity with and specificity to AChE. The ^{14}C-labeled esters were derived from the previously reported ^{18}F-labeled acetylcholine analogue, [^{18}F]FEtP4A, by modifying the structure in three different ways (Table 1): (1) conversion from the secondary acetyl ester to the primary ester to increase the hydrolysis rate by AChE; (2) conversion of the acyl group from acetyl (**2**, **3R**, **3S**) to propionyl (**4**, **5R**, **5S**) to slightly decrease the rate; (3) positional change of an acyl group on the piperidine ring (3-yl, 4-yl) including optical isomers (*R*, *S*) to further modify the hydrolysis rate as well as the specificity to AChE.

Using purified human AChE and BChE, the relative hydrolysis rates of *N*-[^{14}C]ethylpiperidinemethanol esters were measured (Figure 2). With AChE (Panel A), all the primary esters synthesized showed a rather narrow range of hydrolysis rates (AChE-rates), ranging from 0.30 to 0.85 min^{-1} in unit enzyme concentration per mL. Among the six esters, the AChE rates were different from each other ($P < 0.01$, Games−Howel test) in descending order of **3R** > **2** > **5R** > **5S** > **3S** > **4**. Under the same condition, the hydrolysis rate of [^{11}C]-MP4A was 0.59 min^{-1} (data not shown). The acetyl esters (**2**, **3R**, **3S**) showed AChE-rate of about 2-fold that of the corresponding propionyl esters (**4**, **5R**, **5S**). As for the positional effect between 4yl- and 3yl-esters, no consistent tendency was observed. Regarding the enantiomer effect, *R* isomers of acetyl and propionyl ester (**3R**, **5R**) showed 1.8-fold and 1.4-fold higher AChE-rates than the corresponding *S* isomers (**3S**, **5S**). It is also known that in the case of the secondary esters, *N*-[^{14}C]-methylpiperidin-3-yl acetate ([^{14}C]MP3A) and propionate ([^{14}C]MP3P), *R* isomers are more rapidly hy-

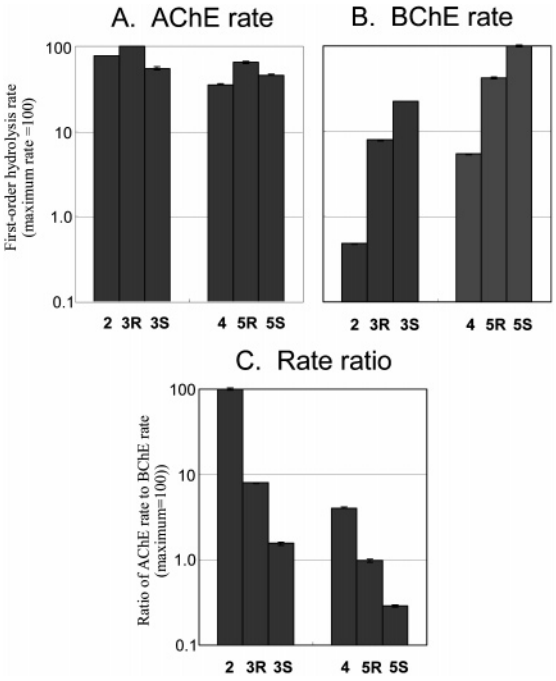


Figure 2. Relative hydrolysis rates of *N*-[^{14}C]ethylpiperidinemethanol esters, acetates (**2**, **3R** and **3S**) and propionates (**4**, **5R** and **5S**), with pure human AChE (Panel A; AChE-rate) and BChE (Panel B; BChE-rate), and the ratio of the AChE-rate to BChE-rate (Panel C) (mean±SD, triplicate). In each panel, the highest value is taken as 100. The AChE-rates and BChE-rates of the six esters were significantly different from each other ($P < 0.01$, Games-Howel test).

drolyzed by AChE than *S* isomers.[17] The reported *R*/*S* ratios of [^{14}C]MP3A and [^{14}C]MP3P are 3 and 8, respectively, which are much larger than the observed R/S ratios (1.4−1.8 times) of primary esters examined in this study. This difference may be associated with the more flexible nature of the primary ester bond compared with the secondary ester bond.

Regarding the BChE-rate (Figure 2; Panel B), completely inverse structure−activity relationships were observed: (1) a wide range (about 220-fold) of BChE-rates among the esters, (2) higher rates of the propionyl esters than those of acetyl esters, and (3) inverse stereospecificity of BChE, that is, *S* isomer > *R* isomer with BChE versus *R* isomer > *S* isomer with AChE. Because of such differences in the AChE- and BChE-rates among the *N*-[^{14}C]ethyl esters, the dynamic range of the ratio of AChE-rate/BChE-rate reached almost 400-fold among the six derivatives (Figure 2; Panel C). Of the six ^{14}C-labeled esters examined, **2** showed the highest value of the AChE-rate/BChE-rate ratio. In addition, the hydrolysis rate of **2** was almost the same as that of [^{11}C]MP4A (one of the established tracers in human PET studies), which is known to have the hydrolysis rate appropriate for AChE in the human cerebral cortices. Based on these results, the structure of **2** was considered to be most suitable for ^{18}F-labeling, and thus *N*-[^{18}F]fluoroethylpiperidin-4-ylmethyl acetate, [^{18}F]**6**, was synthesized.

Table 2 shows the biochemical properties of [^{18}F]**6** as compared with two established tracers, [^{11}C]MP4A and [^{11}C]MP4P, and the previously developed [^{18}F]FEtP4A performed using rat brain tissue homogenates. For the total hydrolysis rate, [^{18}F]**6** showed almost the same

Table 2. Comparison of [^{18}F]**6** with Previously Synthesized [^{18}F]- or [^{11}C]-Labeled *N*-Alkylpiperidinol Esters

compd	total rate[a]	AChE specificity,[b] %	log P ester[b]	log P metabolite[c]
[^{18}F]**6**	1.67	86	0.04	−1.3
[^{11}C]MP4A	1.73	97	−0.30	−2.2
[^{11}C]MP4P	0.41	82	−0.22	−2.2
[^{18}F]FEtP4A	0.10	65	−0.11	−1.9

[a] The first-order hydrolysis rate constant (min^{-1}) in rat cerebral cortical homogenate at a unit tissue concentration (g/mL) without inhibitor. [b] The AChE-mediated hydrolysis rate was measured using AChE-specific inhibitor, BW284c51. [c] Partition coefficients of authentic esters and their metabolites measured in a mixture of 1-octanol/phosphate buffer (0.1 M, pH 7.4). The values for [^{18}F]FEtP4A are literature values.

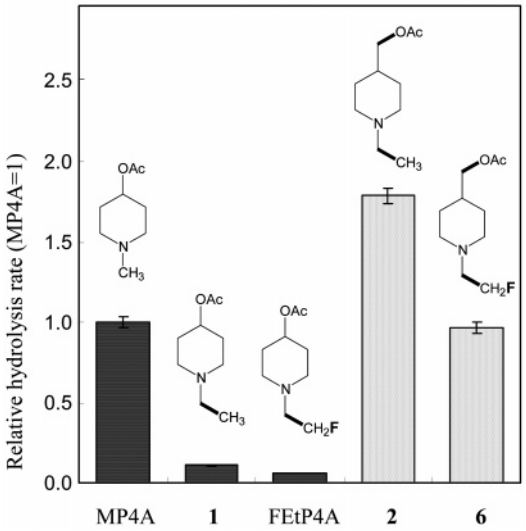


Figure 3. Comparison of enzymatic hydrolysis rates of secondary (gray bar) and primary (dotted bar) alcohol acetyl esters with different *N*-alkyl groups (MP4A; *N*-CH_3, **1** and **2**; *N*-C_2H_5, FEtP4A and **6;** *N*-C_2H_4F) measured in rat cerebral cortical homogenates. The values (mean±SD, n=3) are presented by taking the rate of [^{11}C]MP4A as 1. The rates were significantly different from each other ($P < 0.01$, Games-Howel test).

value as that of [^{11}C]MP4A. In the present method, since the accuracy of AChE measurement is highly dependent on the ratio of the hydrolysis rate (k_3) to the washout rate (k_2) of tracer in the target brain region, we must select a tracer with an appropriate k_3/k_2 ratio, which is proportional to k_3 when k_2 is constant. In human cerebral cortices which is the major target region in the diagnosis of Alzheimer's disease, [^{11}C]MP4A is known to have a suitable k_3/k_2 ratio. The result indicates that the total hydrolysis rate of [^{18}F]**6** may be suitable for the human cerebral cortex. With AChE specificity, though the value (86%) of [^{18}F]**6** was lower than that (97%, data not shown) of corresponding *N*-C_2H_5 compound 2, the value was intermediate between [^{11}C]-MP4A (97%) and [^{11}C]MP4P (82%), and much higher than that (65%) of [^{18}F]FEtP4A. These results suggest that [^{18}F]**6** may be a promising candidate as a tracer for quantification of human brain AChE activity in vivo using PET, so far as biochemical properties are concerned.

Figure 3 shows a summary of the relationships between the ester structure and the hydrolysis rate of ester in rat brain homogenates. In the case of secondary esters, the conversion of *N*-alkyl group from *N*-CH_3 in

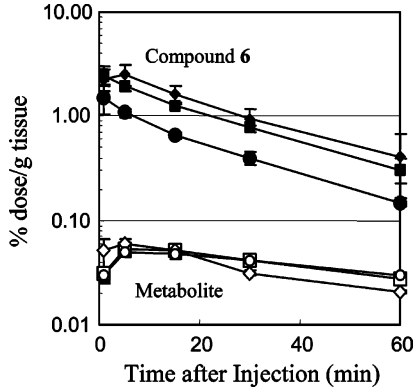


Figure 4. Time-activity curves of ^{18}F uptake in the three brain regions following intravenous administration of [^{18}F]**6** and the ^{18}F-labeled metabolite of **6** in rats. Square; cerebral cortex, diamond; striatum, circle; cerebellum.

[^{14}C]MP4A to *N*-C_2H_5 in **1** and *N*-C_2H_4F in FEtP4A resulted in 8-fold and 16-fold reductions in the hydrolysis rate, respectively, indicating that the hydrolysis rate of FEtP4A is too low. Apparently, introduction of the fluorine atom to the *N*-ethyl group reduced the hydrolysis rate by about 2-fold. On the other hand, conversion of the ester structure from the secondary ester to the primary ester resulted in a 16-fold increase in the hydrolysis rate, in both cases of *N*-ethyl and *N*-fluoroethyl compounds. The same fluorine effect, i.e., 50% reduction in the rate, was also observed with the primary esters examined. Because of such compensating effects of dual modifications, i.e., ester conversion from the secondary to primary form (16-fold increase) and conversion of *N*-alkyl group from *N*-CH_3 to *N*-C_2H_4F (16-fold decrease), **6** had consequently almost the same hydrolysis rate as [^{11}C]MP4A.

To evaluate the lipophilicity of [^{18}F]**6** and its metabolite in vitro, partition coefficients (Log *P*) of [^{18}F]**6** and its metabolite were measured in a mixture of 1-octanol and a phosphate buffer (0.1 M, pH 7.4) and compared with those of the reference compounds (Table 2). With the ester form, the Log *P* values were in the decreasing order of [^{18}F]**6** > [^{18}F]FEtP4A > [^{11}C]MP4P > [^{11}C]-MP4A and with the metabolite, [^{18}F]**6** > [^{18}F]FEtP4A > [^{11}C]MP4P = [^{11}C]MP4A (the metabolites of [^{11}C]-MP4A and [^{11}C]MP4P are identical), which correlated well with the molecular volume. Since [^{11}C]MP4A and [^{11}C]MP4P are known to have relatively high extraction fractions by the brain tissues, it is expected that [^{18}F]**6** is sufficiently lipophilic to cross the blood−brain barrier.

To examine whether the metabolite from [^{18}F]**6** is hydrophilic enough not to cross the blood−brain barrier, we have performed rat in vivo studies to measure transport rates (k_4 and k_5 in Figure 1) of the ^{18}F-labeled metabolite across the blood−brain barrier. When the ^{18}F-labeled metabolite was intravenously injected in rats (Figure 4; lower curves), the uptake of ^{18}F was almost the same among the three brain regions and remained low until 60 min after injection, indicating that k_4 (transport rate of the metabolite from blood to brain; Figure 1) is slow. When [^{18}F]**6** was injected in rats (Figure 4; upper curves), the uptake of ^{18}F was much higher than that of the metabolite and it was different among the three regions. The estimated k_5 (elimination rate of the metabolite from brain to blood; Figure 1) was 0.035 min^{-1} (half time; 20 min), which was about 3-fold larger than those of [^{11}C]MP4A and [^{11}C]MP4P in rats (half time; 60 min).[18] As for [^{11}C]MP4A and [^{11}C]MP4P, large species difference in the k_5 value is known between rats and humans; k_5 in humans is almost 10-fold smaller than in rats.[19] Based on such a species difference, in humans, half time of metabolite clearance might be longer than the 20 min observed in rats. Considering the duration time of PET scan (about 60 min), the metabolite of **6** formed within the brain might have a sufficiently longer residence time for k_3 estimation in a human PET study. Furthermore, the rank order of ^{18}F uptake from **6** during the stationary phase, striatum > cortex > cerebellum, was consistent with the known distribution of the AChE activity in the brain and was different from that of the blood flow, cortex > striatum > cerebellum.

Conclusion

We have developed a novel ^{18}F-labeled radiotracer, [^{18}F]fluoroethylpiperidin-4-ylmethyl acetate (**6**), as a tracer for the quantification of AChE activity in the brain using PET, through a simple screening test of the *N*-[^{14}C]ethyl esters synthesized using purified human enzymes in vitro. In experiments using rats, it was confirmed that **6** possessed desirable properties for accurate and quantitative measurement of AChE activity using PET based on the metabolic trapping principle.

Experimental Section

Synthesis. [^{14}C]Ethyl iodide (2.04 MBq/μmol) was obtained from Amersham International Ltd. All starting materials for chemical synthesis were purchased from Aldrich Chemical Co. Other chemicals were of the highest grade available commercially. Authentic samples were analyzed by ^{1}H and ^{13}C nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectroscopy using a JNM AL-300 (300 MHz) spectrometer and also by elemental analysis. Optical rotations were measured on a JASCO DIP-360 polarimeter.

Preparation of (*R*)- and (*S*)-*N*-*tert*-butoxycarbonyl 3-piperidinemethanol. (*R*)- and (*S*)-*N*-*tert*-Butoxycarbonyl 3-piperidinemethanol were prepared with the reported method.[1] The racemic *N*-*tert*-butoxycarbonyl 3-piperidinemethanol (5.0 g, 23 mmol) was stirred in 100 mL $CHCl_3$ for 2 h at room temperature with 15 mL vinyl acetate and 2 g lipase PS (pseudomonas sp.; Amano Pharmaceutical Co., Ltd., Japan). The residue, that was obtained by filtering to remove the insoluble solid and evaporated under diminished pressure to remove the solvent, was then purified by column chromatography (silica gel C-200, hexane/ethyl acetate = 1/3) to yield (S)-*N*-*tert*-butoxycarbonylpiperidin-3-ylmethyl acetate (2.5 g, 42%, $[\alpha]_D^{20}$ +17.8° (c = 1, $CHCl_3$)).

Additionally, about 2.8 g of recovered hydroxyl compounds enriched with nonreacted *R* isomer was treated twice with lipase PS in the same manner as described above and purified by column chromatography to obtain optically pure (*R*)-*N*-*tert*-butoxycarbonyl 3-piperidinemethanol (2.0 g, 40%, $[\alpha]_D^{20}$ −11.2° (c = 1, $CHCl_3$)).

(*S*)-*N*-*tert*-Butoxycarbonylpiperidin-3-ylmethyl acetate (2.0 g, 7.8 mmol) was dissolved in 5 mL EtOH, and 2.5 mL 2 M NaOH was added and reacted for 2 h at room temperature. Then 50 mL ethyl acetate was added and washed with water. The organic layer was dried over anhydrous $MgSO_4$, and the residue that was obtained by evaporating the solvent was purified by column chromatography (silica gel C-200, hexane/ethyl acetate = 1/1), and yielding (*S*)-*N*-*tert*-butoxycarbonyl 3-piperidinemethanol (1.6 g, 97%, $[\alpha]_D^{20}$ +12.5° (c = 1, $CHCl_3$)).

Preparation of *N*-Ethylpiperidin-4-yl Acetate (1). General Method. *N*-*tert*-Butoxycarbonyl-4-hydroxypiperidine (1.0

g, 5.0 mmol) was dissolved in 10 mL methylene chloride, and pyridine (790 mg, 10 mmol) was added. To this solution, acetyl chloride (630 mg, 8.0 mmol) was added and the reaction mixture was kept at room temperature for 3 h. After adding 100 mL ethyl acetate, the organic layer of the mixture was washed with water, 5% HCl, water, saturated $NaHCO_3$, and finally water. The organic solution was then dried over anhydrous $MgSO_4$. The residue that was obtained by evaporating the solvent was purified by column chromatography (silica gel C-200, hexane/ethyl acetate = 4/1), yielding *N-tert*-butoxycarbonylpiperidin-4-yl acetate (1.01 g, 83%). The compounds were assigned according to the previously reported NMR spectra.[1]

N-tert-Butoxycarbonylpiperidin-4-yl acetate (500 mg, 2.06 mmol) was dissolved in 5 mL ethyl acetate, and 5 mL 4 M HCl/ethyl acetate was added under ice-cold condition. After the solution was allowed to stand for 1 h at room temperature, ethyl acetate was evaporated under vacuum at less than room temperature, yielding piperidin-4-yl acetate hydrochloride (380 mg). This compound was dissolved in 5 mL chloroform, and 5 mL saturated NH_3−chloroform solution was added under ice-cold conditions. After the mixture was reacted for 5 min at room temperature, the solution was filtered to remove the insoluble solid and the solvent was then evaporated under diminished pressure, yielding piperidin-4-yl acetate. The compounds were assigned according to the reported NMR spectra.[1]

Piperidin-4-yl acetate (200 mg, 1.4 mmol) was dissolved in 40 mL methylene chloride and 10 mL 30% acetaldehyde and triacetoxy borohydride (850 mg, 4.0 mmol) were added. The solution was allowed to stand for 2 h at room temperature. After adding 100 mL ethyl acetate, the organic layer of the mixture was washed with water. The organic solution was then dried over anhydrous $MgSO_4$. The residue that was obtained by evaporating the solvent was purified by column chromatography (Chromatorex NH silica gel, hexane/ethyl acetate = 4/1) to yield *N*-ethylpiperidin-4-yl acetate (1) (165 mg, 69%).

N-Ethylpiperidin-4-yl acetate (1): ^{1}H NMR (δ, $CDCl_3$): 1.03 (3H, *t*, CH_3), 1.59−1.70 (2H, *m*, 2×CH), 1.82−1.89 (2H, *m*, 2×CH,), 1.99 (3H, *s*, CH_3), 2.11−2.19 (2H, *m*, 2×CH), 2.35 (2H, *q*, CH_2), 2.63−2.69 (2H, *m*, 2×CH), 4.68−4.74 (1H, *m*, CH). ^{13}C NMR ($CDCl_3$): 12.19, 21.29, 30.81, 50.47, 52.15, 70.43, 170.52. Anal. ($C_9H_{17}NO_2$): C, H, N. IR (neat) cm^{-1} 1705.

Other *N*-ethyl compounds (**2**−**5S**) were synthesized by the same manner as described above from the corresponding *N-tert*-butoxycarbonylpiperidinemethanols as a starting material. The reference compounds (MP4A, MP4P, and FEtP4MA) were prepared using the method in the literature including radiolabeling.[2,3]

N-tert-Butoxycarbonylpiperidin-4-ylmethyl acetate: ^{1}H NMR (δ, $CDCl_3$): 1.08−1.22 (2H, *m*, 2×CH), 1.43 (9H, *s*, t-Bu), 1.64−1.68 (2H, *m*, 2×CH), 1.72−1.81 (1H, *m*, CH), 2.03 (3H, *s*, CH_3), 2.63−2.71 (2H, *m*, 2×CH), 3.90 (2H, *d*, CH_2), 4.07−4.11 (2H, *m*, 2×CH). ^{13}C NMR ($CDCl_3$): 20.82, 28.37, 28.61, 35.47, 43.28, 68.40, 79.35, 154.72, 171.02. Anal. ($C_{13}H_{23}NO_4$): C, H, N. IR (neat) cm^{-1} 1689, 1738.

N-tert-Butoxycarbonylpiperidin-4-ylmethyl propionate: ^{1}H NMR (δ, $CDCl_3$): 1.09−1.23 (5H, *m*, CH_3, 2×CH), 1.42 (9H, *s*, t-Bu), 1.63−1.67 (2H, *m*, 2×CH), 1.72−1.81 (1H, *m*, CH), 2.30 (2H, *q*, CH_2), 2.62−2.71 (2H, *m*, 2×CH), 3.90 (2H, *d*, CH_2), 4.07−4.10 (2H, *m*, 2×CH). ^{13}C NMR ($CDCl_3$): 9.11, 27.49, 28.40, 28.65, 35.56, 43.34, 68.26, 79.37, 154.76, 174.42. Anal. ($C_{14}H_{25}NO_4$): C, H, N. IR (neat) cm^{-1} 1697, 1722.

(*R*)- and (*S*)- *N-tert*-Butoxycarbonylpiperidin-3-ylmethyl acetate; *R* isomer: $[\alpha]_D^{20}$ −16.2° (*c* = 1, $CHCl_3$), *S* isomer $[\alpha]_D^{20}$ +17.8° (*c* = 1, $CHCl_3$). The compounds were assigned according to the reported NMR spectra[1].

(*R*)- and (*S*)- *N-tert*-Butoxycarbonylpiperidin-3-ylmethyl propionate; *R* isomer: $[\alpha]_D^{20}$ −14.7° (*c* = 1, $CHCl_3$), *S* isomer: $[\alpha]_D^{20}$ +16.3° (*c* = 1, $CHCl_3$). ^{1}H NMR (δ, $CDCl_3$): 1.12 (3H, *t*, CH_3), 1.19−1.25 (1H, *m*, CH), 1.43 (10H, *s*, t-Bu, CH), 1.60−1.67 (1H, *m*, CH), 1.73−1.79 (2H, *m*, 2×CH), 2.29 (2H, *q*, CH_2), 2.59 (1H, *broad s*, CH), 2.74−2.81 (1H, *m*, CH), 3.83−3.99 (4H, *m*, CH_2, 2×CH). ^{13}C NMR ($CDCl_3$): 9.13, 24.43, 27.23, 27.50, 28.40, 35.34, 44.05, 46.93, 66.15, 79.42, 154.79, 174.38. Anal. ($C_{14}H_{25}NO_4$): C, H, N. IR (neat) cm^{-1} 1692, 1742.

Piperidin-4-ylmethyl acetate: ^{1}H NMR (δ, $CDCl_3$): 1.13−1.22 (2H, *m*, 2×CH), 1.63−1.74 (3H, *m*, 2×CH, CH), 2.00 (3H, *s*, CH_3), 2.05 (1H, *s*, NH), 2.51−2.61 (2H, *m*, 2×CH), 3.03−3.09 (2H, *m*, 2×CH), 3.85−3.88 (2H, *m*, CH_2). ^{13}C NMR ($CDCl_3$): 20.83, 29.83, 46.03, 53.64, 69.02, 171.1. Anal. ($C_8H_{15}NO_2$): C, H, N. IR (neat) cm^{-1} 3374, 1736.

Piperidin-4-ylmethyl propionate: ^{1}H NMR (δ, $CDCl_3$): 1.09 (3H, *t*, CH_3), 1.07−1.12 (1H, *m*, CH), 1.39−1.48 (1H, *m*, CH), 1.59−1.65 (1H, *m*, CH), 1.66 (1H, *s*, NH), 1.71−1.79 (2H, *m*, 2×CH), 2.24−2.35 (3H, *m*, CH, CH_2), 2.46−2.55 (1H, *m*, CH), 2.92−2.97 (1H, *m*, CH), 3.02−3.06 (1H, *m*, CH), 3.80−3.93 (2H, *m*, CH_2). ^{13}C NMR ($CDCl_3$): 9.10, 25.90, 27.49, 27.76, 36.63, 46.84, 49.89, 67.05, 174.42. Anal. ($C_9H_{17}NO_2$): C, H, N. IR (neat) cm^{-1} 1734, 3372.

(*R*)- and (*S*)-Piperidin-3-ylmethyl acetate; *R* isomer: $[\alpha]_D^{20}$ +13.9° (*c* = 1, $CHCl_3$), *S* isomer: $[\alpha]_D^{20}$ −15.4° (*c* = 1, $CHCl_3$). ^{1}H NMR (δ, $CDCl_3$): 1.06−1.11 (1H, *m*, CH), 1.30−1.48 (1H, *m*, CH), 1.59−1.65 (1H, *m*, CH), 1.72−1.76 (2H, *m*, 2×CH), 1.99 (3H, *s*, CH_3), 2.00 (1H, *s*, NH), 2.27−2.34 (1H, *m*, CH), 2.46−2.54 (1H, *m*, CH), 2.93−3.06 (2H, *m*, 2×CH), 3.78−3.91 (2H, *m*, 2×CH). ^{13}C NMR ($CDCl_3$): 20.78, 25.74, 27.67, 36.44, 46.72, 49.73, 67.16, 171.01. Anal. ($C_8H_{15}NO_2$): C, H, N. IR (neat) cm^{-1} 1735.

(*R*)- and (*S*)-Piperidin-3-ylmethyl propionate; *R* isomer: $[\alpha]_D^{20}$ +10.5° (*c* = 1, $CHCl_3$), *S* isomer: $[\alpha]_D^{20}$ −11.9° (*c* = 1, $CHCl_3$). ^{1}H NMR (δ, $CDCl_3$): 0.86 (3H, *t*, CH_3), 1.06−1.12 (1H, *m*, CH), 1.36−1.49 (1H, *m*, CH), 1.61−1.71 (1H, *m*, CH), 1.76−1.80 (2H, *m*, 2×CH), 1.86 (3H, *s*, CH_3), 2.05 (1H, *s*, NH), 2.31−2.36 (1H, *m*, CH), 2.41−2.53 (1H, *m*, CH), 2.99−3.12 (2H, *m*, 2×CH), 3.81−3.96 (2H, *m*, 2×CH). ^{13}C NMR ($CDCl_3$): 16.52, 21.28, 24.90, 28.10, 37.22, 48.23, 49.30, 66.79, 172.25. Anal. ($C_9H_{17}NO_2$): C, H, N. IR (neat) cm^{-1} 1735.

N-Ethylpiperidin-4-ylmethyl acetate (**2**): ^{1}H NMR (δ, $CDCl_3$): 1.02 (3H, *t*, CH_3), 1.20−1.34 (2H, *m*, CH_2), 1.59−1.68 (3H, *m*, 2×CH, CH), 1.79−1.87 (2H, *m*, 2×CH), 1.99 (3H, *s*, CH_3), 2.33 (2H, *q*, CH_2), 2.88−2.92 (2H, *m*, 2×CH), 3.87 (2H, *d*, CH_2). ^{13}C NMR ($CDCl_3$): 12.06, 20.82, 28.83, 35.28, 52.51, 52.84, 68.78, 171.06. Anal. ($C_{10}H_{19}NO_2$): C, H, N. IR (neat) cm^{-1} 1736.

N-Ethylpiperidin-4-ylmethyl propionate (**4**): ^{1}H NMR (δ, $CDCl_3$): 1.04 (3H, *t*, CH_3), 1.10 (3H, *t*, CH_3),1.28−1.32 (2H, *m*, CH_2), 1.59−1.67 (3H, *m*, 2×CH, CH), 1.80−1.88 (2H, *m*, 2×CH), 2.28 (2H, *q*, CH_2), 2.35 (2H, *q*, CH_2), 2.89−2.93 (2H, *m*, 2×CH), 3.89 (2H, *d*, CH_2). ^{13}C NMR ($CDCl_3$): 9.11, 12.10, 27.50, 28.88, 35.38, 52.55, 52.89, 68.66, 174.49. Anal. ($C_{11}H_{21}NO_2$): C, H, N. IR (neat) cm^{-1} 1723.

(*R*)- *and* (*S*)-*N*-Ethylpiperidin-4-ylmethyl acetate (**3R**, **3S**); *R* isomer: $[\alpha]_D^{20}$ +7.8° (*c* = 0.4, $CHCl_3$), *S* isomer: $[\alpha]_D^{20}$ −8.7° (*c* = 1, $CHCl_3$). ^{1}H NMR (δ, $CDCl_3$): 1.01 (3H, *t*, CH_3), 1.24−1.32 (3H, *m*, CH, CH_2), 1.50−1.69 (2H, *m*, 2×CH), 1.75−1.88 (2H, *m*, 2×CH) 1.98 (3H, *s*, CH_3), 2.33 (2H, *q*, CH_2), 2.78−2.87 (2H, *m*, 2×CH), 3.78−3.84 (1H, *m*, CH), 3.89−3.94 (1H, *m*, CH). ^{13}C NMR ($CDCl_3$): 11.97, 20.88, 24.81, 27.40, 38.83, 52.75, 53.62, 56.77, 67.37, 171.11. Anal. ($C_{10}H_{19}NO_2$): C, H, N. IR (neat) cm^{-1} 1718.

(*R*)- *and* (*S*)-*N*-Ethylpiperidin-4-ylmethyl propionate (**5R**, **5S**); *R* isomer: $[\alpha]_D^{20}$ +8.0° (*c* = 1.1, $CHCl_3$), *S* isomer: $[\alpha]_D^{20}$ −8.9° (*c* = 1.1, $CHCl_3$).^{1}H NMR (δ, $CDCl_3$): 0.98−1.10 (6H, *m*, 2×CH_3) 1.19−1.33 (3H, *m*, CH, CH_2), 1.54−1.69 (3H, *m*, 3×CH), 1.74−1.97 (2H, *m*, 2×CH), 2.22−2.36 (3H, *m*, CH, CH_2), 2.77−2.87 (2H, *m*, 2×CH), 3.78−3.95 (2H, *m*, 2×CH). ^{13}C NMR ($CDCl_3$): 9.11, 11.95, 24.81, 27.40, 27.51, 35.87, 52.71, 53.60, 56.77, 67.14, 174.41. Anal. ($C_{11}H_{21}NO_2$): C, H, N. IR (neat) cm^{-1} 1731.

Preparation of *N*-Fluoroethylpiperidin-4-ylmethyl Acetate (6). Piperidin-4-ylmethyl acetate (500 mg, 3.2 mmol), 1-fluoro-2-tosylethane (1.38 g, 6.35 mmol), and 150 mg K_2CO_3 were dissolved in 4 mL dimethylformamide for 2.5 h at 80 °C. After adding 100 mL ethyl acetate, the organic layer of the mixture was washed with water, 27% NH_3, and finally saturated NaCl. The organic solution was then dried over anhydrous $MgSO_4$. The residue that was obtained by evapo-

rating the solvent was purified by column chromatography (silica gel C-200, chloroform/ethyl acetate = 1/1) to yield *N*-fluoroethylpiperidin-4-ylmethyl acetate (472 mg, 73%).

N-Fluoroethylpiperidin-4-ylmethyl acetate (**6**): ^{1}H NMR (δ, $CDCl_3$): 1.21−1.37 (2H, *m*, 2×CH), 1.62−1.70 (3H, *m*, CH, CH_2), 2.02 (3H, *s*, CH_3), 2.01−2.08 (2H, *m*, 2×CH), 2.61 (1H, *m*, CH), 2.71 (1H, *m*, CH), 2.93 (2H, *d*, CH_2), 3.88−3.91 (2H, *m*, 2×CH), 4.46 (1H, *m*, CH), 4.62 (1H, *m*, CH). ^{13}C NMR ($CDCl_3$): 20.90, 28.73, 34.93, 53.56, 58.30, 58.52, 68.70, 80.78, 83.04, 171.10. Anal. ($C_{10}H_{18}FNO_2$): C, H, N. IR (neat) cm^{-1} 1735.

N-[^{14}C]Ethylation (^{14}C-labeled **1**−**5S**). A mixture of precursor ester (demethyl piperidinemethanol ester, 100 μmol), 5 mg K_2CO_3, and [^{14}C]ethyl iodide (10 MBq) was heated in 2 mL acetone at 50 °C with a water bath for 2 h. An ethyl acetate solution containing anhydrous HCl (in excess of ester) was added to the reaction solution, and the mixture was dried under nitrogen gas. The residue was dissolved in 200 μL MeOH and was subjected to thin-layer chromatography (TLC) with a silica gel plate and a mixture of ethyl acetate:2-propanol:ammonia (100:10:1; vols) as a developing solvent. The radioactive zone with R_f value corresponding to the authentic compound was collected and extracted with a 1:2 mixture of MeOH and chloroform. Radiochemical yields based on [^{14}C]-methyl iodide were more than 70% and purities were more than 99%.

N-[^{18}F]Fluoroethylation (^{18}F-labeled **6**). *N*-[^{18}F]Fluoroethylpiperidin-4-ylmethyl acetate was prepared with reported method using piperidin-4-ylmethyl acetate and 1-bromo-2-[^{18}F]-fluoroethane. [^{18}F]F$^-$ was produced by the ^{18}O (p, n) ^{18}F reaction on 10−20 atom % $H_2{}^{18}O$ using 18 MeV protons (14.2 MeV on target) from the cyclotron and separated from [^{18}O]-H_2O using Dowex 1-X8 anion-exchange resin in an irradiating room. The [^{18}F]F$^-$ was eluted from the resin with aqueous potassium carbonate (3.3 mg/0.3 mL) into a glass vial containing CH_3CN (1.5 mL)/Kryptofix 2.2.2. (25 mg) and transferred into a reaction vessel in a hot cell. After the [^{18}F]F$^-$ was dried to remove H_2O and CH_3CN from the reaction vessel, 2-bromoethyl triflate ($BrCH_2CH_2OTf$) (8 μL) in *o*-dichlorobenzene (500 μL) was added to the radioactive solution. Then, the resulting 1-bromo-2-[^{18}F]fluoroethane in this vessel was distilled under a helium flow (90−100 mL/min) at 130 °C for 2 min and bubbled into another vessel containing a solution of anhydrous DMF (300 μL) and piperidin-4-ylmethyl acetate (1 mg) at −20 °C. After maximum radioactivity was reached into the solution, the reaction mixture was warmed to 130 °C and maintained for 30 min. The content was diluted with a high performance liquid chromatography (HPLC) mobile phase (500 μL) and applied onto a semipreparative column (10 mm ID × 250 mm, Megapack SIL C18) attached to the JASCO HPLC system. Elution with 5 mM CH_3COONH_4 (pH = 4.9)/CH_3CN (9/1) at flow rate of 4 mL/min gave a radioactive fraction corresponding to pure ^{18}F-labeled 6 (t_R = 13 min). The fraction was collected in a rotary evaporator and evaporated to dryness at 90 °C under reduced pressure. The residue was redissolved in 10 mL of saline and passed through a Millipore filter (GS, 0.22 μm) to provide an injectable solution of ^{18}F-labeled **6**. All of the above procedures were carried out automatically by the use of a previously reported system. Total synthesis time was 92−96 min from the end of bombardment. At the end of synthesis, 347−420 MBq of ^{18}F-labeled **6** was obtained. The radiochemical purity of ^{18}F-labeled **6** was assayed by analytical HPLC (column: Finepak Sil C18−10, JASCO, 4.6 mm ID × 250 mm, Megapack SIL C18). The mobile phase was 50 mM CH_3COONH_4 (pH = 3.1)/CH_3CN (3/7). The t_R was 7.3 min for ^{18}F-labeled **6**. The radiochemical purity of the compound was consistently more than 98%.

Hydrolysis Rate with Pure Human AChE and BChE. Purified enzymes were obtained from Sigma Chemical Co. The purified human AChE was added to 0.5% Triton X-100 phosphate buffer (0.1 M, pH 7.4), and then the solution (100 μL) was placed in tubes and preincubated at 37 °C for 30 min. Each ^{14}C-labeled ester solution (15 kBq in 20 μL buffer) was added to each tube to initiate the reaction. At a designated interval, 200 μL EtOH was added to each tube to stop the reaction. 5 μL of the solution was then applied to a silica gel TLC plate and developed with a mixture of ethyl acetate:2-propanol:ammonia (100:10:1; vols). The air-dried TLC plate was covered with a 5 μm thick foil and placed in a cassette in contact with the imaging phosphor plate for 1 h. The radioactivities corresponding to the R_f value of the unchanged esters and metabolites were quantitated using the BAS 1800 system. The enzymatic hydrolysis rate by AChE (AChE rate) of each compound was calculated as: AChE rate = $(-\ln(A_2/A_1))/(T_2 - T_1)/C$, where A_1 and A_2 represent the ^{14}C radioactivity of the unchanged ester remaining at times T_1 and T_2, respectively; and C represents the enzyme concentration (Unit/mL) in the reaction solution. The hydrolysis rates by BChE (BChE rate) were measured in the same manner. Nonenzymatic hydrolysis rates of each ester were negligibly slow (<0.0001 min^{-1}). The assay was performed in triplicate, and the statistical comparison, Games−Howel test ($p < 0.01$) was made.

Hydrolysis Rate and Specificity with Rat Cerebral Cortical Homogenate. Experimental animals used in the present study were treated and handled according to the "Recommendations for Handling of Laboratory Animals for Biomedical Research", compiled by the Committee on Safety and Ethical Handling Regulations for Laboratory Animal Experiments, NIRS.

Tissue homogenates were prepared as follows. Rats (Wistar, male, 8 weeks, 210−230 g, n = 3) were anesthetized with diethyl ether and killed by decapitation. The brain was removed and dissected, and the cerebral cortex was obtained and weighed. The tissues were homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4). The homogenates were diluted to the appropriate concentration, and 100 μL homogenate of cerebral cortex was placed in the reaction tubes. Each compound solution (15 kBq in 20 μL buffer) was added to each tube to initiate the reaction. The following procedure was the same as that described above. Diethyl ether, used as anesthesia, did not affect the measurement of enzyme activity. The specificity of compounds for AChE was measured by addition of BW284c51 (30 nM). The AChE specificities of the compounds in the cerebral cortex homogenate were measured from the ratio of the noninhibited hydrolysis rate to inhibited hydrolysis rate. Statistical comparison was made using the Games−Howel test ($p < 0.01$).

Partition Coefficient. ^{18}F-labeled 6, MP4A, MP4P, and their metabolites were added to a 1:1 mixture of 1-octanol and 0.1 M phosphate buffer (pH 7.4), shaken vigorously, and allowed to equilibrate for 1 h at room temperature. The concentrations and purity of the radioactivity in the organic and aqueous phases were measured with a gamma or liquid scintillation counter and TLC, respectively. The partition coefficient was calculated as the ratio of the concentration in the organic phase to that in the aqueous phase.

Brain Regional Kinetics of Compound 6 and Its Metabolite. ^{18}F-Labeled **6** or metabolite (370 kBq/200 μL) were injected intravenously to rats (Wistar, male, 8 weeks, 210−230 g, n = 3 per time point), and the rats were anesthetized with diethyl ether and killed by decapitation at 1, 5, 15, 30, and 60 min after injection. The striatum, cerebral cortex, and cerebellum were dissected out and weighed, and the radioactivity was measured with a gamma counter. The brain uptake of radioactivity was calculated as % dose per g tissue.

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Supporting Information Available: Elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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