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Diphenylpropynone derivatives as probes for imaging β-amyloid plaques in Alzheimer's brains

Masahiro Ono [a,b,*], Hiroyuki Watanabe [a,b], Rumi Watanabe [b], Mamoru Haratake [b], Morio Nakayama [b,*], Hideo Saji [a]

[a] Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan
[b] Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan

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ABSTRACT

A new series of diphenylpropynone (DPP) derivatives for use in vivo to image β-amyloid (Aβ) plaques in the brain of patients with Alzheimer's disease (AD) were synthesized and characterized. Binding experiments in vitro revealed high affinity for Aβ (1–42) aggregates at a K_i value ranging from 6 to 326 nM. Furthermore, specific labeling of plaques was observed in sections of brain tissue from Tg2576 transgenic mice stained using one of the compounds, **1**. In biodistribution experiments with normal mice, [^{125}I]**1** displayed moderate uptake (1.55% ID/g at 2 min) and clearance from the brain with time (0.76 ID/g at 60 min). Taken together, DPP can serve as a new molecular scaffold for developing novel Aβ imaging agents by introducing appropriate substituted groups.

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the elderly and is characterized clinically by dementia, cognitive impairment, and memory loss. The neuropathological hallmarks of AD include abundant deposits of β-amyloid (Aβ) plaques and neurofibrillary tangles. The deposition of Aβ plaques has been regarded as an initial event in the pathogenesis of AD.[1,2] Therefore, the quantitative evaluation of Aβ plaques in the brain with non-invasive techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) could lead to the presymptomatic detection of AD and new anti-amyloid therapies.[3–5]

Developing Aβ imaging probes is currently an emerging field of research. The basic requirements for suitable probes include: (i) good penetration of the blood–brain barrier, (ii) selectively binding or labeling of Aβ plaques, and (iii) clear and contrasting signals between plaques and non-plaques. Based on these requirements, several promising agents with the backbone structure of DDNP, thioflavin-T and Congo Red have been synthesized and evaluated for use in vivo as probes to image Aβ plaques in AD brain. Clinical trials in AD patients have been conducted with several agents including [^{18}F]FDDNP,[6,7] [^{11}C]6-OH-BTA-1,[8,9] [^{11}C]SB-13,[10,11] [^{18}F]BAY94-9172,[12,13] [^{123}I]IMPY,[14,15] [^{18}F]AV-45,[16–18] [^{11}C]AZD2184,[19] and [^{18}F]AZD4694[20] indicating the imaging of Aβ plaques in the living human brain to be useful for the diagnosis of AD.

Recently, to develop more useful PET/SPECT probes, a number of groups have reported new Aβ-binding probes without the basic structure of DDNP, thioflavin-T and Congo Red. Kung et al. reported several diphenylacetylenes as PET/SPECT probes for Aβ plaques, which are a simplified version of stilbene derivatives; the double bond in the stilbene derivative is replaced by a triple bond (Fig. 1).[21–23] Two reasons prompted them to investigate this class of compound. The first reason was that the compounds can be synthesized by a Sonogashira reaction, which is tolerant of a wide variety of functional groups. The second and more important reason

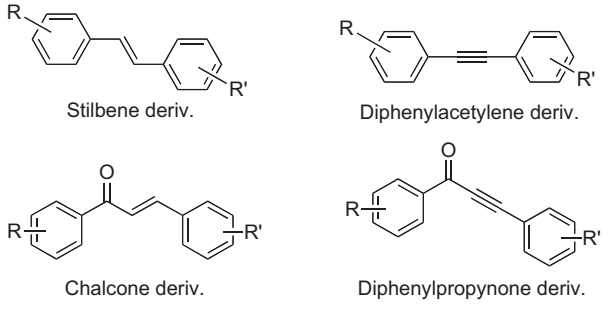

Figure 1. Chemical structure of the stilbene, diphenylacetylene, chalcone, and diphenylpropynone derivatives.

* Corresponding authors. Tel.: +81 75 753 4608; fax: +81 75 753 4568 (M.O.); tel./fax: +81 95 819 2441 (M.N.).
E-mail addresses: ono@pharm.kyoto-u.ac.jp (M. Ono), morio@nagasaki-u.ac.jp (M. Nakayama).

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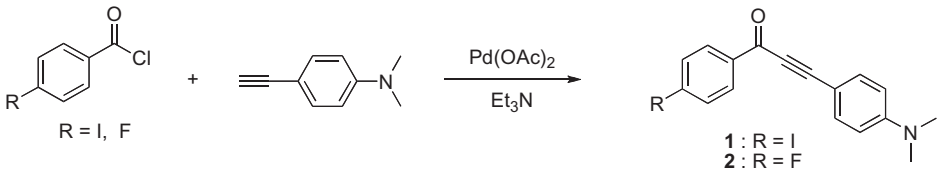
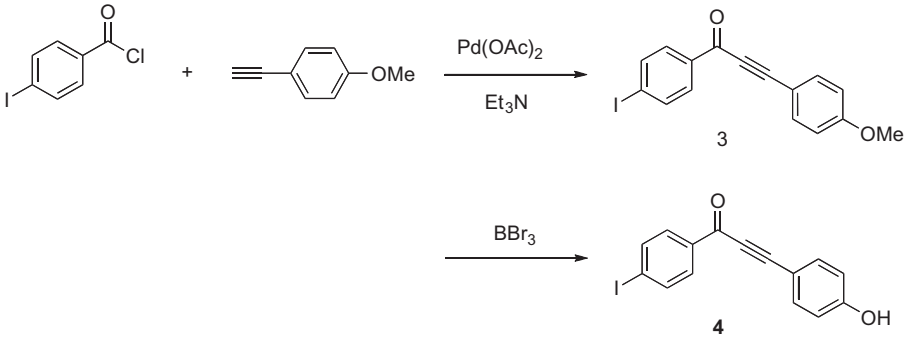
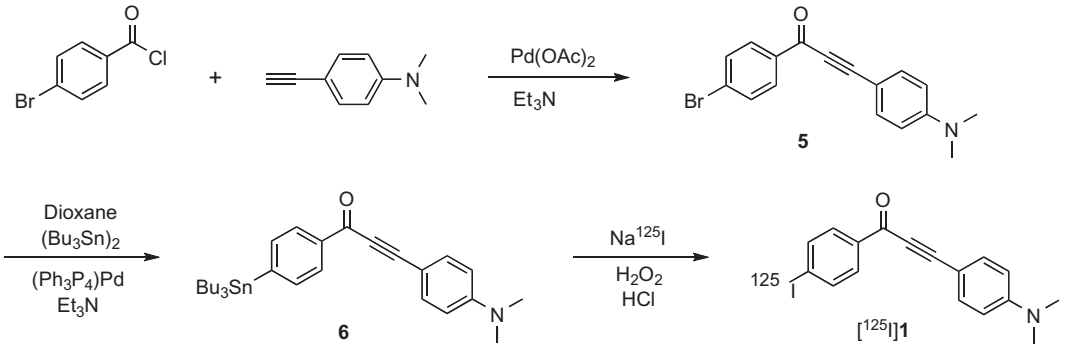


Scheme 1.



Scheme 2.



Scheme 3.

is the absence of geometrical isomers, a problem often encountered with their double-bonded counterparts.[24] Diphenylacetylenes are relatively rigid; therefore, the degree of freedom around the triple bond is limited, leading to a tight fit with the binding pocket at the β-sheet. Previous papers have reported that diphenylacetylenes displayed excellent binding to Aβ plaques and could be potentially useful for in vivo imaging of Aβ plaques in living human brain.[21–23]

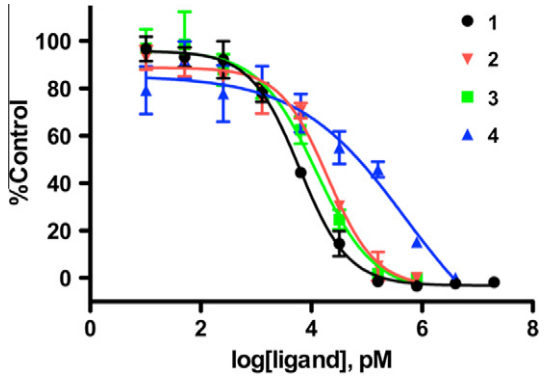


Figure 2. Competitive curves of [^{125}I]DMIC against the DPP derivatives Aβ (1–42) aggregates.

Based on the positive results reported previously, we applied the same molecular design to chalcone derivatives which we have recently reported to be useful as probes for the diagnosis of AD.[25–27] In this study, we designed and synthesized a new series of diphenylpropynone (DPP) derivatives by replacing the double bond in the chalcone scaffold with a triple bond, and evaluated their usefulness as probes for imaging Aβ (Fig. 1). To our knowledge, this is the first time the use of DPP derivatives in vivo as probes to image Aβ plaques in the AD brain has been proposed.

The synthesis of the DPP derivatives is outlined in Schemes 1–3. Various strategies have been developed for the synthesis of a DPP

Table 1
Inhibition by DPP derivatives of ligand binding to Aβ (1–42) aggregates

Compound	K_i[a] (nM)
1	6.0 ± 0.15
2	20.4 ± 1.3
3	13.7 ± 5.0
4	325.8 ± 13.8
IMPY	45.6 ± 11.5

[a] Values are the mean and standard error of the mean for three independent experiments.

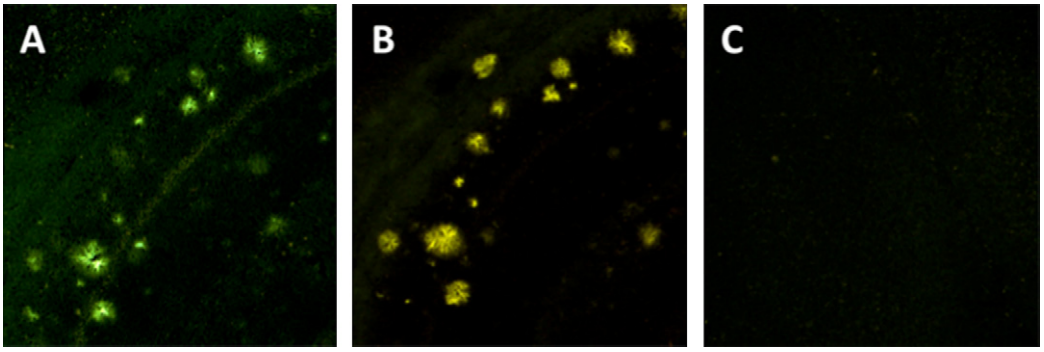


Figure 3. Fluorescent staining of **1** in 10-μm sections of the Tg2576 mouse brain (A). Labeled plaques were confirmed by staining of the adjacent sections with thioflavin S (B). No apparent staining of **1** was observed in the age-matched control mouse brain (C).

scaffold.[28–30] We selected a simple and efficient ligand-less, copper- and solvent-free palladium-catalyzed synthesis of ynones by coupling acyl chlorides with terminal alkynes which makes use of only 1 equiv of acid chloride and 1 equiv of Et_3N, and a relatively small amount of catalyst, reported previously.[31] With this process, the DPP derivatives (**1**, **2**, **3**, and **5**) were prepared by the coupling of benzoyl chloride (*p*-iodobenzoyl chloride, *p*-fluorobenzoyl chloride, or *p*-bromobenzoyl chloride) with phenyl acetylene (4-ethynyl-*N*,*N*-dimethylaniline or *p*-ethynyl anisole) in the presence of $Pd(OAc)_2$ as the catalyst and Et_3N as the base. **3** was converted to **4** by demethylation with BBr_3 in CH_2Cl_2 (32% yield) (Scheme 2). The tributyltin derivative **6** was prepared from the bromo compound **5** using a bromo to tributyltin exchange reaction catalyzed by Pd(0) in a yield of 54% (Scheme 3). The tributyltin derivative was used as the starting material for radioiodination in the preparation of [^{125}I]**1**. The novel radioiodinated DPP derivative [^{125}I]**1** was obtained by iododestannylation using hydrogen peroxide as the oxidant which produced the desired radioiodinated ligand. It was anticipated that the non-carrier-added preparation would result in a final product bearing a theoretical specific activity similar to that of ^{125}I (2200 Ci/mmol). The radiochemical identity of the radioiodinated ligand was verified by co-injection with non-radioactive **1** from its HPLC profile. The final radioiodinated compound, [^{125}I]**1**, showed a single peak of radioactivity at a retention time of 14.4 min. [^{125}I]**1** was obtained in ca. 50% radiochemical yield with a radiochemical purity of >95% after purification by HPLC.

(*E*)-4-Dimethylamino-4′-[^{125}I]iodo-chalcone ([^{125}I]DMIC) was synthesized and used as the radioligand for competition experiments (K_d value of [^{125}I]DMIC is 4.2 nM).[25] In vitro competitive binding experiments to evaluate the affinity of the DPP derivatives for Aβ aggregates were carried out in solutions with [^{125}I]DMIC as a competitive ligand. The DPP derivatives inhibited the binding of [^{125}I]DMIC in a dose-dependent manner (Fig. 2), and the affinity of DPP derivatives for Aβ (1–42) aggregates varied from 6 to 326 nM (Table 1). The DPP derivatives had affinity for Aβ (1–42) aggregates in the following order: **1** > **3** > **2** > **4**. The K_i values indicated that the affinity for Aβ (1–42) aggregates was affected by the substituted group in the DPP scaffold, and compounds **1** and **3** showed higher affinity than IMPY, a well known Aβ imaging probe (K_i = 45 nM). We have previously reported that the K_i value of a chalcone with the dimethylamino group was 2.9 nM,[25] indicating that the DPP derivative **1** has the same binding affinity as this chalcone derivative. We selected **1** with the highest affinity for Aβ (1–42) aggregates for radiolabeling and additional experiments.

Next, **1** was investigated for its affinity for Aβ plaques by in vitro neuropathological fluorescent staining in Tg2576 transgenic mouse brain sections as shown in Figure 3. Tg2576 mice show marked Aβ deposition in the cingulated cortex, entorhinal cortex, dentate gyrus, and hippocampus by 11–13 months of age[32] and have been frequently used to evaluate the specific binding of Aβ plaques in experiments in vitro and in vivo.[19,25,33–35] Many Aβ plaques were clearly stained with **1** (Fig. 3A), as reflected by the high affinity for Aβ aggregates in in vitro competition assays, while no labeling was observed in the wild-type mouse brain (Fig. 3C). The labeling pattern was consistent with that observed with thioflavin S, a pathological dye commonly used for staining Aβ plaques in the brain (Fig. 3B). These results suggest that **1** can bind to Aβ plaques in the mouse brain in addition to having affinity for synthetic Aβ (1–42) aggregates.

The biodistribution of the radioiodinated compound [^{125}I]**1** in vivo was tested in normal mice (Table 2). A biodistribution study provides important information on brain uptake. The ideal Aβ imaging probe should have good blood–brain penetration to deliver a sufficient dose into the brain while achieving rapid clearance from normal regions to result in a higher signal to noise ratio in the AD brain. The initial brain uptake of [^{125}I]**1** was 1.55% of injected dose/gram at 2 min postinjection, whereas the radioactivity accumulated in the brain was rapidly eliminated (0.76% of injected dose/gram at 60 min postinjection), indicating highly desirable properties for Aβ imaging probes. The radioactivity profile of [^{125}I]**1** in the brain was similar to that of chalcones and related derivatives reported previously.[25,26] The conversion of the double bond in the chalcone scaffold to the triple bond in the DPP scaffold affected neither the affinity for Aβ aggregates nor the pharmacokinetics of the radioactivity in the brain, indicating that the introduction of appropriate substituted groups into the DPP scaffold can lead to the development of new useful PET/SPECT probes for Aβ plaques like chalcone derivatives.

In conclusion, we successfully designed and synthesized a new series of DPP derivatives as probes for the imaging of Aβ plaques in the brain in vivo. Some of the derivatives displayed excellent affin-

Table 2
Biodistribution of radioactivity after injection of [^{125}I]**1** in normal mice[a]

Tissue	Time after injection (min)			
	2	10	30	60
Blood	6.19 (1.05)	5.44 (0.36)	3.94 (0.46)	3.07 (0.20)
Liver	19.96 (2.47)	15.29 (1.81)	12.02 (1.21)	10.06 (1.07)
Kidney	10.45 (1.82)	9.81 (1.31)	8.34 (0.77)	7.75 (0.71)
Intestine	2.73 (0.79)	8.44 (1.67)	12.04 (1.44)	14.02 (1.77)
Spleen	4.41 (0.95)	5.19 (0.93)	5.47 (0.27)	4.43 (1.64)
Stomach[b]	0.75 (0.16)	2.97 (2.55)	2.35 (1.38)	1.46 (0.38)
Pancreas	3.90 (0.44)	3.39 (0.31)	2.57 (0.30)	2.09 (0.13)
Heart	10.00 (1.19)	7.53 (1.14)	6.03 (0.46)	4.94 (0.54)
Brain	1.55 (0.26)	1.23 (0.09)	0.93 (0.06)	0.76 (0.07)

[a] Expressed as percent injected dose per gram. Each value represents the mean (SD) for 4-6 animals.
[b] Expressed as percent injected dose per organ.

ity for Aβ aggregates in binding experiments in vitro. The DPP derivatives clearly stained Aβ plaques in sections of brain from an AD patient. In biodistribution experiments using normal mice, the degree to which the DPP derivative **1** penetrated the brain was also very encouraging. Taken together, the present results suggest that DPP can function as a molecular scaffold with which to develop new Aβ imaging probes.

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Supplementary data

Supplementary data (procedures for the preparation of DPP derivatives, in vitro binding assay, in vitro fluorescent staining using brain sections from Tg2576 mice, and biodistribution experiments) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.058.

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