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Development of dual functional SPECT/fluorescent probes for imaging cerebral β-amyloid plaques

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ABSTRACT

The imaging of β-amyloid (Aβ) aggregates in the brain may lead to the early detection of Alzheimer's disease (AD) and monitoring of the progression and effectiveness of treatment. The purpose of this study was to develop dual modality SPECT and fluorescent probes based on boron dipyrromethane (BODIPY) as a core structure. We designed and synthesized an ^{125}I-labeled derivative of BODIPY (BODIPY7). BODIPY7 had a K_i value of 108 nM for Aβ(1–42) aggregates and exhibited peaks of absorption/emission at 606/613 nm. It detected Aβ plaques in sections of brain tissue from an animal model of AD and displayed low uptake in the brain and high uptake in the liver in normal mice. Although additional modifications of the BODIPY scaffold are necessary to improve brain uptake, these results should aid the development of dual functional SPECT/fluorescent probes for the imaging of Aβ plaques in the brain.

The formation of β-amyloid (Aβ) plaques is a key neurodegenerative event in Alzheimer's disease (AD).[1,2] Since the imaging of these plaques in vivo may lead to the presymptomatic diagnosis of AD, many molecular probes, including positron emission tomography (PET)[3–6] and single photon emission computed tomography (SPECT)[7,8] tracers labeled with a radioisotope (RI), have been developed. The PET ligand [^{11}C]-2-(4-(methylamino)phenyl)-6-hydroxybenzothiazole (PIB) with a benzothiazole backbone has shown particular promise in early clinical trials and is currently being used in a number of human studies.[9,10] Nuclear imaging with PET/SPECT probes is an established clinical modality that offers good sensitivity deep in tissue, which permits whole body quantitative imaging not only in small animals but also in humans. However, it is limited by factors such as a time-consuming data acquisition process, expensive equipment, exposure to radioactivity, the need for highly skilled personnel, and a relatively poor spatial resolution.[11]

In addition to PET/SPECT probes, much attention has focused on the development of near-infrared fluorescent (NIRF) probes targeting Aβ plaques.[12–14] Optical imaging with NIRF probes is a relatively new modality that offers real-time, nonradioactive, and, depending on the technique, high-resolution imaging. Among NIRF probes reported to date, NIAD-4[13] and CRANAD-2[15] cross the blood–brain barrier, selectively bind Aβ with high affinity, clear quickly from the brain, and absorb and emit within the near-infrared region (650–900 nm), often called the 'optical window'.[16] Optical imaging techniques are not quantitative, especially when the object is located deep to the skin because of significant signal attenuation in tissue, but NIRF imaging has the potential to provide a rapid, inexpensive, and nonradioactive drug screening system for AD.

Currently, in vivo imaging of Aβ plaques in AD brains is primarily performed using nuclear imaging techniques such as SPECT and PET. We hypothesize that the development of dual functional nuclear/fluorescent imaging probes not only can provide complementary information that may lead to improve diagnosis and management of AD patients, but also can facilitate the validation of optical imaging by standard nuclear imaging techniques. Although several recent papers demonstrate the usefulness of dual nuclear and fluorescent imaging probes targeting tumor,[17,18] such dual functional probes for Aβ plaques have not been reported. Here, we propose a design strategy for the development of a dual SPECT/fluorescent probe for Aβ plaques in the brain. In this study, we selected boron dipyrromethane (BODIPY), one of the most useful fluorophores,[19–23] and introduced the radiolabeled moiety

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NIAD-4, 2-(4-[^{125}I]iodophenyl)-5-thiophene, at the 3-position. Described herein is the synthesis and characterization of this radioiodinated derivative of BODIPY. To our knowledge, this is the first time dual SPECT/fluorescent probes have been proposed as probes for imaging Aβ plaques in the brain.

The target BODIPY derivative was prepared as shown in Scheme 1. The compound **1** was synthesized in a yield of 21.4% by the Suzuki coupling reaction. After reduction of the aldehyde to an alcohol by $NaBH_4$, the desired Wittig reagent **3** was readily prepared from **2** and triphenylphosphine. The compound **3** was produced by a Wittig reaction between **3** and pyrroaldehyde. The key step in the formation of the BODIPY backbone was accomplished by the condensation of pyrrole-2-carboxylaldehyde and **4** at low temperature, followed by the addition of $BF_3{\cdot}OEt_2$. The bromo compound (**5**) was reacted with bis(tributyltin) using Pd(0) as a catalyst, and the corresponding tributyltin derivative (**6**) was obtained in a yield of 17.0%. The tributyltin derivative (**6**) was readily reacted with iodine in chloroform at room temperature to give the iodo derivative (**7**) in a yield of 20.0%. The radioiodination was achieved by the same iododestannylation reaction using hydrogen peroxide as the oxidant, which produced the desired radioiodinated ligand, [^{125}I]BODIPY7, in a yield of 25% and with greater than 95% radiochemical purity. It is anticipated that the no-carrier-added preparation resulted in the final product bearing similar theoretical specific activity to ^{125}I.

To quantify the affinity of BODIPY7 for Aβ plaques, we carried out inhibition assays on the binding to Aβ(1–42) aggregates with

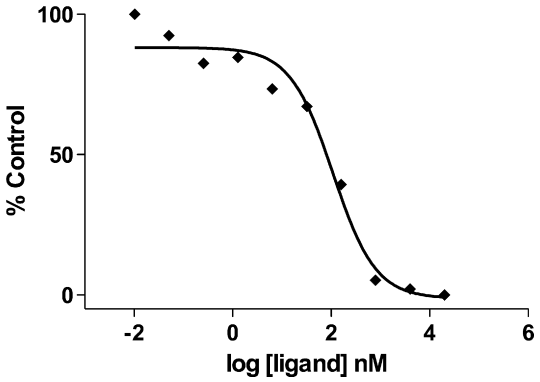
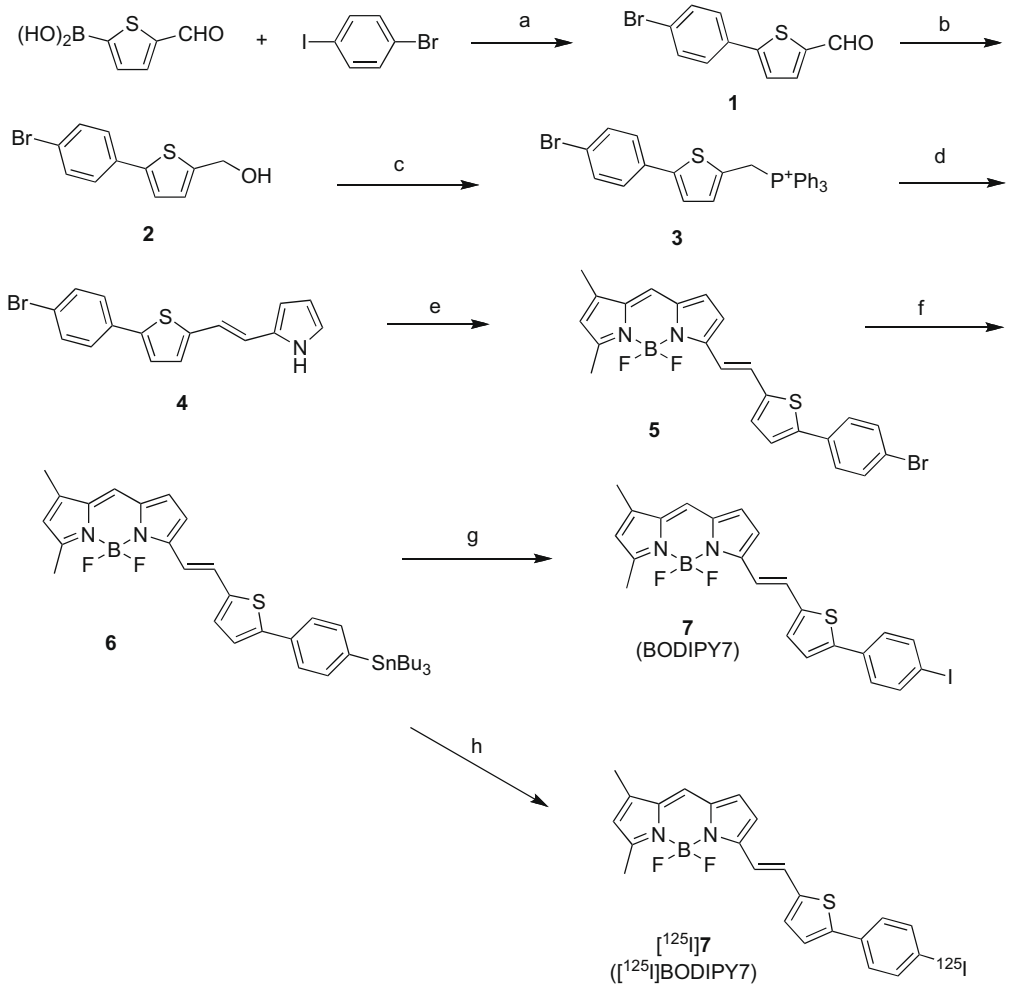


Figure 1. Curve of BODIPY7's inhibition of the binding of [^{125}I]IMPY to Aβ(1–42) aggregates.

[^{125}I]IMPY as a competing radioligand. BODIPY7 inhibited the binding of [^{125}I]IMPY in a dose-dependent manner, indicating that it has affinity for Aβ(1–42) aggregates (Fig. 1). This result suggests that the binding sites of BODIPY7 and IMPY partly overlap. BODIPY7 had a K_i value of 108 nM, indicating sufficiently high affinity for Aβ aggregates. A recent paper reported that the introduction of the triazole moiety into the BODIPY scaffold afforded dyes that allowed for the unambiguous differentiation of an unordered conformation, which is mostly a benign form, from an ordered



Scheme 1. Reagents: (a) dioxane, $(Ph_3P)_4Pd$, Na_2CO_3; (b) MeOH, $NaBH_4$; (c) $CHCl_3$, $Ph_3P{\cdot}HBr$; (d) MeOH, NaOMe, 2-formylpyrrole; (e) CH_2Cl_2, 3,5-dimethylpyrrole-2-carboxaldehyde, $POCl_3$, Et_3N, $EtOBF_3$; (f) dioxane, $(Bu_3Sn)_2$, $(Ph_3P)_4Pd$, Et_3N; (g) $CHCl_3$, I_2; (h) EtOH, HCl, H_2O_2, [^{125}I]NaI.

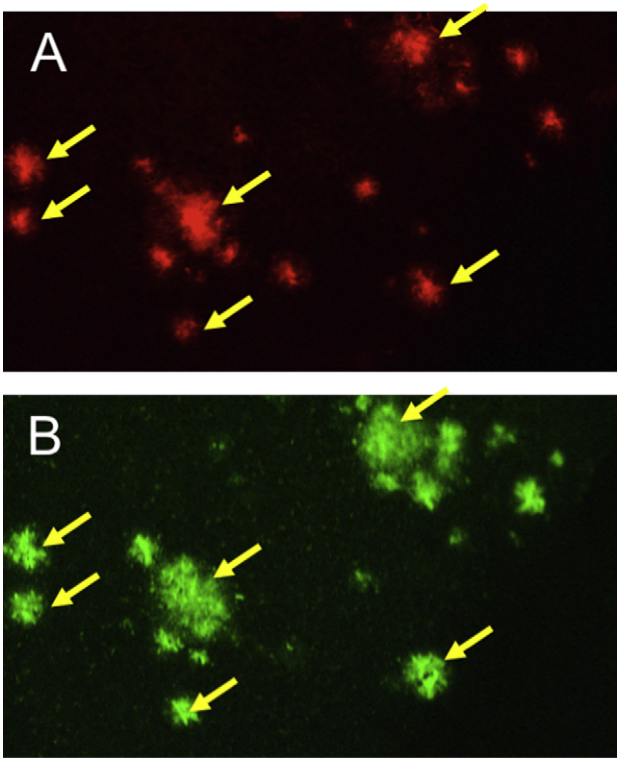


Figure 2. Neuropathological staining of BODIPY7 (A) in a 10-μm section from a Tg2576 mouse brain. Labeled plaques were confirmed by staining of the adjacent section with thioflavin S (B).

conformation, largely a neurotoxic form, of Aβ(1–42) soluble oligomers.[24] Although this Letter did not report the K_i value of the triazole-containing BODIPY, we consider BODIPY7 to potentially be suitable for monitoring conformational transitions of amyloid species in vitro. Also, these results support the validity of using BODIPY as a scaffold for probes to image Aβ plaques in vivo.

Next, the usefulness of BODIPY7 for neuropathological staining of Aβ plaques was investigated in an animal model of AD, the Tg2576 mouse, specifically engineered to overproduce Aβ plaques in the brain.[25] BODIPY7 clearly stained the plaques as reflected by its high affinity for Aβ aggregates in in vitro inhibition assays (Fig. 2A). The labeling pattern was consistent with that observed with thioflavin S (Fig. 2B). In contrast, wild-type mice displayed no remarkable accumulation of BODIPY7 in brain sections (data not shown). These results suggest that BODIPY7 can function as a probe for detecting Aβ plaques in the brain and deserves further investigation as a potential dual Aβ imaging probe.

Radioiodinated BODIPY7 was tested in normal mice to assess its ability to cross the blood–brain barrier (BBB) (Table 1). The uptake of [^{125}I]BODIPY7 in the brain was 0.4%ID/g at 2 min postinjection, and most radioactivity had been washed out from the brain by 30 min postinjection (0.14%ID/g). Biodistribution experiments also demonstrated high and persistent levels of radioactivity in the liver and spleen, and a decrease in uptake into the lung with time. Despite its suitable lipophilicity (log P = 2.2) and reasonable molecular size (mol wt 363),[10] the compound's uptake in the brain was relatively low, which may be explained by its rapid trapping in the liver. An initial brain uptake higher than 0.5%ID/organ at 2 min postinjection is preferred for Aβ imaging probes, and this initial uptake should remain at less than 30% at 30 min in normal mouse brain because of the absence of Aβ plaques. Although its initial uptakes fell short of these criteria, [^{125}I]BODIPY7 showed a rapid washout (30 min) with a value equal to 35% of the initial brain uptake. Despite good affinity for synthetic Aβ(1–42) aggregates

Table 1
Biodistribution of radioactivity after injection of [^{125}I]BODIPY7 in normal mice[a]

Tissue	Time after injection (min)			
	2	10	30	60
Blood	11.67 (1.70)	4.09 (0.25)	3.09 (0.17)	2.44 (1.25)
Liver	35.51 (3.62)	46.02 (1.80)	45.87 (2.60)	45.78 (3.58)
Kidney	5.39 (0.34)	4.79 (0.50)	4.46 (0.41)	4.43 (0.41)
Intestine	0.48 (0.07)	0.93 (0.16)	1.91 (0.17)	3.04 (0.18)
Spleen	16.12 (3.89)	26.90 (3.98)	28.21 (3.79)	21.52 (8.99)
Pancreas	1.19 (0.35)	0.74 (0.17)	0.72 (0.15)	0.92 (0.28)
Heart	5.59 (1.74)	2.95 (0.50)	2.31 (0.53)	2.30 (0.35)
Stomach[b]	1.40 (0.10)	4.73 (1.86)	4.79 (1.12)	5.50 (0.51)
Brain	0.40 (0.05)	0.19 (0.02)	0.14 (0.01)	0.14 (0.01)

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

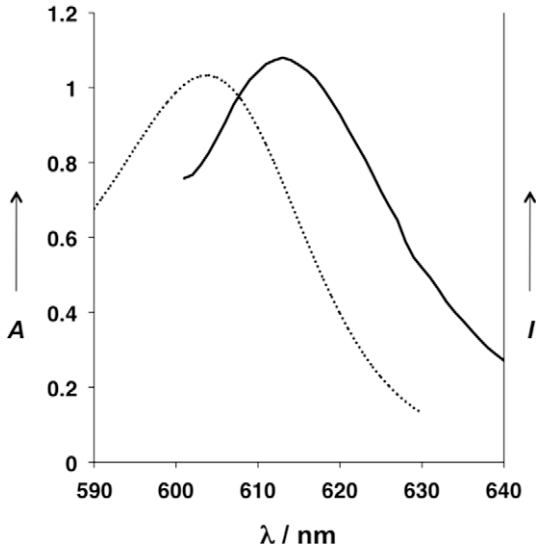


Figure 3. Absorption (dotted line) and emission (line) spectra of BODIPY7.

and the clear labeling of Aβ plaques in mouse brain sections, the radioiodinated BODIPY did not have characteristics for the imaging of Aβ plaques. Recently, it was reported that CRANAD-2 can penetrate the BBB and bind to Aβ plaques in the brain in vivo, though it possesses a difluoroboron core like BODIPY and a larger molecular weight than BODIPY7.[15] Therefore, additional structural changes may modify the properties of BODIPY derivatives to improve their suitability for imaging.

Although BODIPY7 had a high fluorescent quantum yield (φ = 0.36), it exhibited shorter wavelengths of absorption/emission at 606/613 nm than are appropriate for optical imaging in vivo (Fig. 3). Recent papers have reported the development of BODIPY derivatives with absorption/emission bands in the near-infrared region.[26] By introducing additional structural changes to extend the near-infrared wavelength simultaneously with modifications to improve penetration for the BBB, more useful SPECT/fluorescent probes may be developed in the future.

In conclusion, we designed and synthesized a radioiodinated BODIPY derivative as a dual SPECT/fluorescent probe for imaging Aβ plaques in the brain. In binding experiments in vitro, the BODIPY derivative showed high affinity for Aβ(1–42) aggregates. BODIPY7 clearly stained Aβ plaques in mouse brain, reflecting its affinity for Aβ aggregates in vitro. These findings suggest that additional structural changes to the BODIPY backbone may be applied to potential dual SPECT/fluorescent probes for the imaging of Aβ plaques.

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Supplementary data

Supplementary data (procedure for the preparation of new BODIPY derivatives, in vitro binding assay, in vitro fluorescent staining using Tg2576 mouse brain sections, and biodistribution studies) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.027.

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