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Synthesis of fluorescent probes based on stilbenes and diphenylacetylenes targeting β-amyloid plaques

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Abstract

Three fluorescent probes were synthesized aiming for optical imaging to detect amyloid plaques present in the patients with Alzheimer's disease (AD). These compounds were prepared via Sonogashira coupling of a well-defined fluorophore (4-bora-3a,4a-diaza-*s*-indacene, BODIPY) with the pharmacophore possessing either a stilbene or a diphenylacetylene moiety. Different polyethylene glycol chain lengths were used as linkers between the fluorophore and the pharmacophore to adjust the lipophilicity of these probes. These compounds exhibit strong fluorescence emission between 665 and 680 nm and have very high extinction coefficients comparable to the parent fluorophore, BODIPY dye.

Keywords: Fluorescent probes; Alzheimer's disease; BODIPY; Near-IR imaging

1. Introduction

The formation of β-amyloid (Aβ) plaques, consisting of β-sheets of Aβ protein aggregates, in the brain is a pivotal event in the pathogenesis of Alzheimer's disease (AD).[1,2] Thus, the development of specific agents targeting Aβ aggregates is important for the diagnosis, and it may be possible for the evaluation of the therapeutic course of the disease progression.[3,4] Recently, significant advancement for the detection of Aβ plaques in AD patients has been achieved in using positron emission tomography (PET) and single photon emission tomography (SPECT) imaging ligands.[5–8]

For our effort to develop complementary techniques, we envisioned to make new water-soluble neutral fluorescent compounds that would emit in the near-infrared region and be able to bind to Aβ plaques as tools for in vitro studies. To meet the requirements of such fluoroscent probes, we have employed a tether approach for designing the probes. All these fluorescent probes have three common structural units, that is, a fluorophore, a linker, and a pharmacophore, where the fluorophore is tethered to the pharmacophore through the polypegylated linker (Fig. 1). The polypegylated linker is added to improve the water-solubility. This series of fluorescent probes is not designed to penetrate intact blood-brain barrier; instead, due to their higher molecular weight, rather these will only bind to Aβ-plaques accumulated on the cerebral blood vessels, at the extravascular space, commonly observed in the patients with cerebral angiopathy.[9–11]

For all our probes prepared for this Letter, we opted for the same neutral fluorophore, a 4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dye, **1**, previously synthesized and characterized by Kim et al.[12] The reason for choosing **1** as the fluorophore owes to its strong red shifted, sharp fluorescence emission at 654 nm and its neutral nature. The choice of pharmacophores is based on our previous findings that a series of 4-amino-4′-hydroxy substituted stilbene[13] and diphenylacetylene derivatives[14] showed high binding affinities ($K_i = 1$–10 nM range) to Aβ aggregates. Similarly,

Abbreviations: *BODIPY, 4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-*indacene; PEG, poly ethylene glycol

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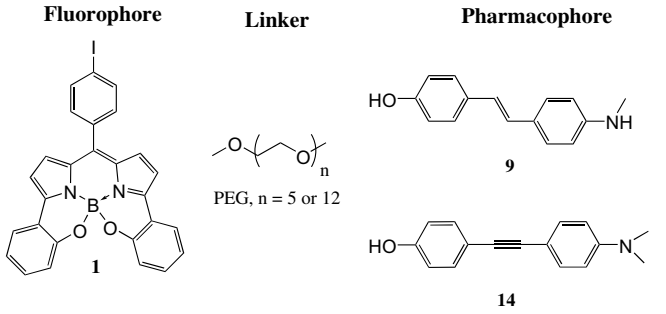


Fig. 1. Examples of fluorophore, linkers, and pharmacophores.

various units of polyethylene glycol (PEG, $n = 5$ or 12) as linkers on the 4′-hydroxyl group of stilbene and diphenylacetylene through which the fluorophore is attached at the end of PEG chain provides a flexible tool to adjust the lipophilicity of the dye.[15,13] Reported herein are the synthesis and spectroscopic properties of the three compounds as potential fluorescent probes targeting Aβ plaques.

The synthesis began with the preparation of the BODIPY dye **1** by slightly modifying the procedure reported by Kim et al.[16] Two common methods exist to build a boradiazoindacene core: (1) starting either from an aldehyde[17] or (ii) from an acyl chloride.[18,19] In the previously reported synthesis, dipyrromethene **4** was prepared by the condensation of 4-iodobenzoyl chloride with 2-aryl pyrrole **2**[20,21] in a moderate yield. Although this approach is attractive owing to the acid sensitive nature of the 2-aryl pyrrole **2**, the method is hampered by its very long reaction time (usually required 2–4 days of refluxing). Therefore we decided to explore the first approach as illustrated in Scheme 1.

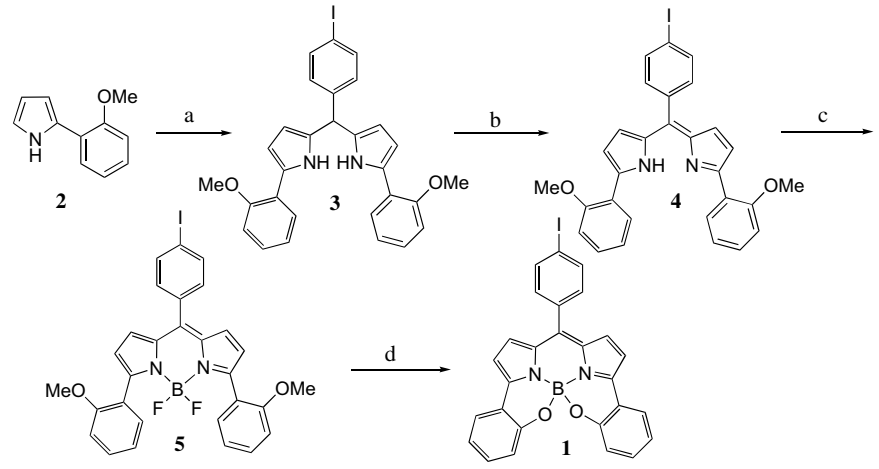
The condensation of *p*-iodobenzaldehyde with 2-aryl pyrrole **2** at 0 °C was found to be extremely fast in the presence of catalytic amounts of TFA. The corresponding dipyrromethane **3** could be isolated in 52% yield when the reaction was quenched within 5 min after the addition of TFA.[22] The subsequent oxidation to dipyrromethene **4** by *p*-chloroanil also proceeded rapidly.[23] These two steps can be accomplished within a day in good overall yield (more than 50%). Previously, the target compound, **1**, was prepared in a one-pot two-step reaction beginning with dipyrromethene **4**. Boron complexation of dipyrromethene **4** followed by the demethylation using BBr_3 furnished compound **1** in 93% yield.[16] In a slightly modified way, we isolated the borane complex **5** and upon treatment with 1.0 M BBr_3 in DCM afforded the target dye **1** as a green solid, which was identical with the reported compound in all aspects. This slight modification enabled us to use 1.0 M BBr_3 in DCM rather than neat BBr_3.

With the BODIPY dye **1** in hand, the next issue was to connect the fluorophore to the rest of molecule. Alkynyl connectors are thought to be the most appropriate because it is known that these connectors are particularly useful by favoring electronic communication and extending the conjugation length.[24] This is especially true when the connection is through the para position of the central benzene ring of the BODIPY dye. Moreover, the iodo-substituted BODIPY dye can be suitably utilized in the cross-coupling reaction with the terminal alkynes.[25] The terminal alkynes **10a** and **10b** were prepared by the reactions shown in Scheme 2.

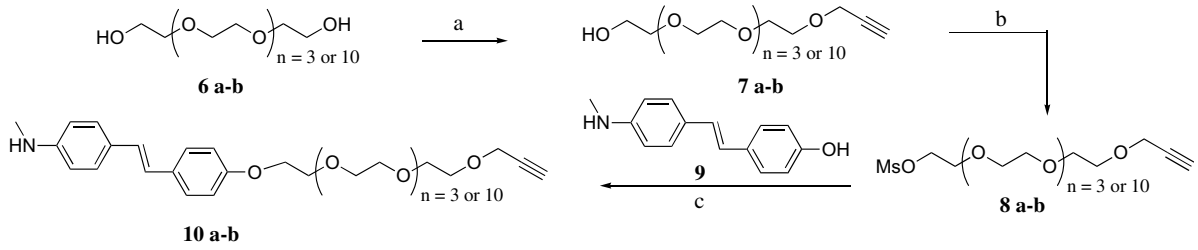
Firstly the PEGs ($n = 3$ or 10) were monoalkylated with propargyl bromide to afford the terminal alkynes **7a**–**b** in 45% and 39% yield, respectively. The free OH groups of **7a**–**b** were converted into mesylates **8a**–**b** by reacting with MsCl in the presence of triethyl amine. To prepare compounds with five or 12 ethoxy group as the PEG linkage, mesylates **8a**–**b** were coupled with the OH-group of 4-methyl amino-4′-hydroxy stilbene **9**[26] to obtain **10a**–**b** in 82% and 78% yield, respectively.

In order to introduce the PEG functionalized stilbene pharmacophore into the BODIPY core, compound **1** was coupled with the propargyl-PEG-stilbene **10a**–**b** (Scheme 3).

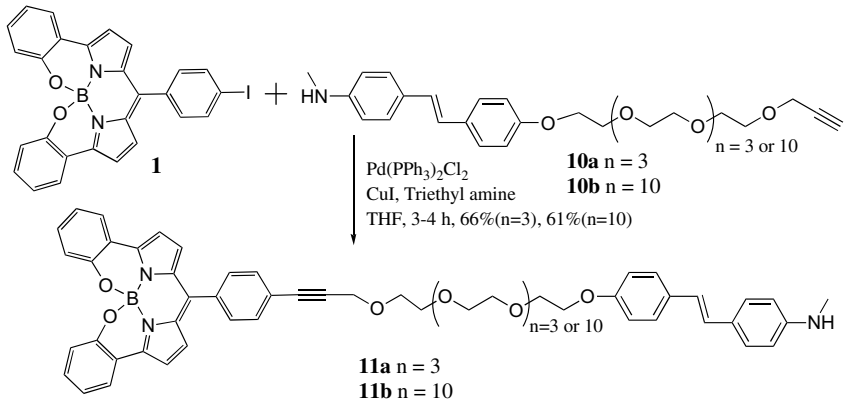
Thus 4-iodo substituted dye **1** was treated with **10a**–**b** under catalyzed condition using $Pd(PPh_3)_2Cl_2$ and CuI,



Scheme 1. Synthesis of 4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dye **1**. Reagents and conditions: (a) *p*-iodo benzaldehyde, TFA, DCM, 0 °C, 52%; (b) *p*-chloroanil, DCM, 1 h, 85%; (c) Et_3N, BF_3–Et_2O, MePH, 80 °C, 88%; (d) 1.0 M BBr_3 in DCM, 0 °C to rt, 4 h, 82%.



Scheme 2. Preparation of terminal alkynes **10a** and **10b**. Reagents and conditions: (a) NaH, propargyl bromide, THF, 45% ($n = 3$), 39% ($n = 10$); (b) MsCl, Et_3N, DCM, 91% ($n = 2$), 88% ($n = 10$); (c) K_2CO_3, DMF, 100 °C, 12 h, 82% ($n = 3$), 78% ($n = 10$).



Scheme 3. Sonogashira coupling to synthesize compounds **11a**–**b**.

and yielded the target compounds **11a**–**b** in 66% and 61% yield, respectively, as green solids. These compounds were isolated via chromatography on deactivated alumina column. Extensive decomposition occurred when silica gel was used for chromatography. Similarly, the synthesis of the fluorescent compound **16** via Sonogashira coupling is shown in Scheme 4.

Palladium-catalyzed coupling of alkyne **12** with 4-iodobenzaldehyde **13** produced the internal alkyne **15** in 51% yield. The alkylation of the OH group of 4,4-dimethyl amino 4′-hydroxy diphenylacetylene **15** with mesylate **8a** furnished the terminal alkyne **15** in 62% yield. Sonogashira coupling of **15** with **1** gave the target compound **16** in 67% yield, again as a green solid.

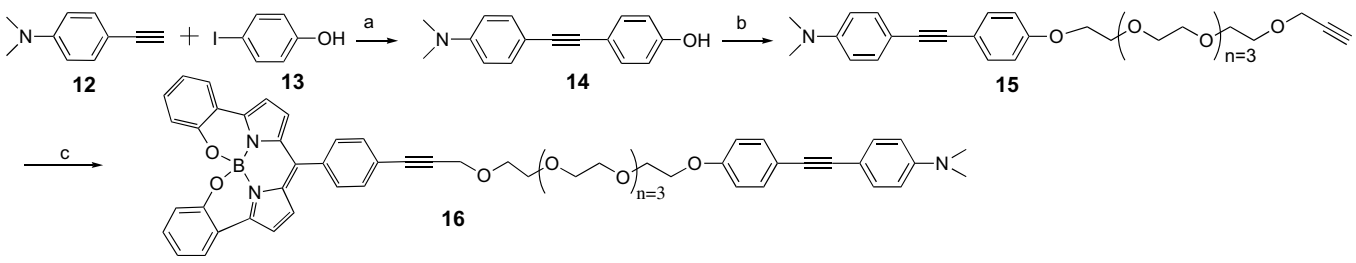
Spectroscopic data relevant to the present discussion are shown in Table 1. A typical fluorescence emission spectrum as shown by compound **16** is given in Figure 2.

Table 1
Spectroscopic data for **11a**, **11b**, and **16**

Compound	Solvent	λ_{max}/nm (abs)	ε/M^{-1} cm^{-1}	λ_{max}/nm (em)
11a	Water	338	38,000	680
11b	Water	364	34,000	665
16	Water	345	35,000	678

The fluorescence properties were examined under ambient conditions either in water or in ethanol. It is important to point out that all dyes are soluble in water and exhibit the expected absorption and emission patterns typical of BODIPY dye **1**.

While compound **1** exhibits fluorescence emission at 654 nm in chloroform, all newly synthesized compounds **11a**, **11b**, and **16** are red-shifted by 10–20 nm relative to the parent compound **1**. This is probably due to the



Scheme 4. Synthesis of Fluorescent probe **16**. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 3 h, 51%; (b) **8a**, K_2CO_3, DMF, 62%; (c) **1**, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 4 h, 67%.

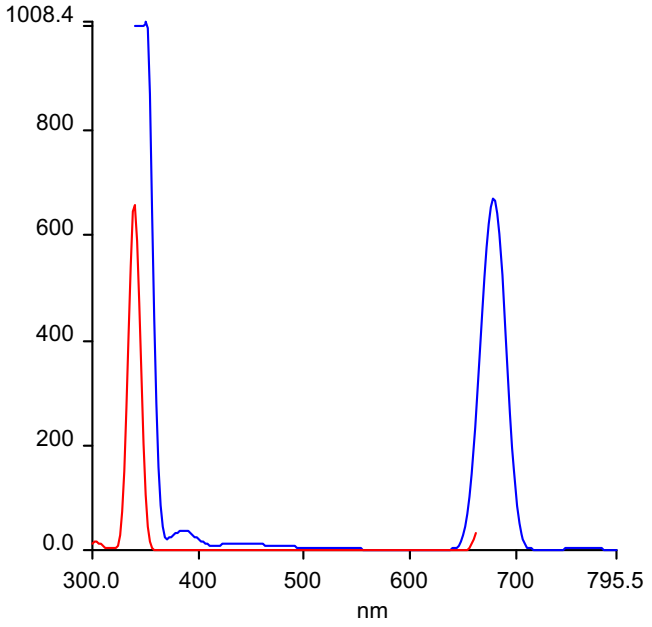


Fig. 2. Excitation and emission (250 nM in ethanol) spectra of **16**.

extended conjugation with the alkyne in these compounds. Compounds **11a**, **11b**, and **16** have similar molar extinction coefficients in water ($\varepsilon_{max} = 38{,}000\ M^{-1}\,cm^{-1}$, $34{,}000\ M^{-1}\,cm^{-1}$, and $35{,}000\ M^{-1}\,cm^{-1}$, respectively) compared to compound **1** ($\varepsilon_{max} = 46{,}000\ M^{-1}\,cm^{-1}$) in chloroform.

In conclusion, we present here examples of water-soluble fluorescent agents with emission in the near-IR, for targeting β-amyloid plaques. These compounds were successfully prepared and characterized. They have high extension coefficients showing emission around 665–680 nm. The in vitro binding affinity and other biological properties of these compounds will be reported later.

2. General procedure for Sonogashira coupling

To a solution of bodipy dye **1** in THF and triethyl amine were added the catalyst $Pd(PPh_3)_2Cl_2$ (10 mol %) and CuI (5 mol %). The alkyne (1.2 equiv) in THF was added dropwise and the reaction mixture was stirred at room temperature for 3–4 h. The solvents were evaporated and the crude product was purified by flash column chromatography on basic alumina using EtOAc/hexane mixture (1:1) as eluant giving the pure products as green solids.

Compound (**11a**): ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.79–7.61 (m, 6H), 7.39–7.26 (m, 6H), 7.08–6.85 (m, 12H), 6.56 (d, 2H, $J = 8.0$ Hz), 4.47 (s, 2H), 4.13 (t, 2H, $J = 4.0$ Hz), 3.87–3.67 (m, 18H), 2.83 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 158.1, 154.5, 150.4, 149.0, 137.9, 134.3, 134.1, 132.5, 132.4, 132.2, 131.4, 130.7, 130.0, 128.8, 128.6, 127.6, 127.3, 127.2, 126.0, 125.4, 124.2, 120.7, 120.0, 119.9, 116.8, 115.1, 112.7, 88.1, 85.8, 71.1, 70.9, 70.7, 70.0, 69.6, 67.8, 59.4, 30.9. HRMS (ESI) calcd for $C_{55}H_{52}BN_3O_6$ [M^+] 894.3926 obsd 894.3965.

Compound (**11b**): ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.76–7.62 (m, 6H), 7.40–7.30 (m, 6H), 7.07–6.97 (m, 4H), 6.94–6.87 (m, 8H), 6.58 (d, 2H, $J = 8.0$ Hz), 4.48 (s, 2H), 4.13 (t, 2H, $J = 4.0$ Hz), 3.87–3.53 (m, 46H), 2.85 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 158.2, 154.5, 150.4, 148.0, 137.8, 134.3, 134.1, 132.5, 132.2, 131.3, 130.7, 130.0, 128.1, 127.7, 127.4, 127.0, 126.0, 125.4, 124.7, 120.7, 120.0, 119.9, 116.8, 115.1, 113.4, 88.1, 85.8, 71.1, 70.8, 70.7, 70.0, 69.6, 67.7, 59.4, 31.4. HRMS (ESI) calcd for $C_{69}H_{80}BN_3O_{15}$ [M^+] 1202.576 obsd 1202.577.

Compound (**16**): ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.75–7.62 (m, 6H), 7.43–7.35 (m, 6H), 7.07–7.04 (m, 5H), 6.93–6.84 (m, 5H), 6.64 (d, 2H, $J = 9.0$ Hz), 4.48 (s, 2H), 4.13 (t, 2H), 3.87–3.67 (m, 18H), 2.97 (s, 6H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 158.5, 154.5, 150.4, 150.2, 137.9, 134.3, 134.1, 132.9, 132.7, 132.5, 132.4, 132.2, 132.1, 132.0, 130.7, 130.0, 128.8, 128.6, 126.0, 125.4, 120.7, 120.0, 116.8, 114.8, 112.1, 110.7, 89.3, 88.1, 87.3, 85.8, 71.1, 70.9, 70.8, 70.7, 69.9, 69.6, 67.7, 59.4, 40.4. HRMS (ESI) calcd for $C_{56}H_{52}BN_3O_8$ [M^+] 906.3926 obsd 906.396.

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Supplementary data

Supplementary data (experimental procedure and characterization of some intermediate compounds and copies of ^{1}H, ^{13}C, and UV spectra of some intermediate compounds and all fluorescent compounds) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.03.130.

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