Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 39 (2004) 573–578
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Fluoro-substituted and ^{13}C-labeled styrylbenzene derivatives for detecting brain amyloid plaques

Kumi Sato [a], Makoto Higuchi [b], Nobuhisa Iwata [b], Takaomi C. Saido [b], Kazumi Sasamoto [a,*]

[a] *Dojindo Laboratories, Tabaru 2025-5, Mashiki-machi, Kumamoto 861-2202, Japan*
[b] *Laboratory for Proteolytic Neuroscience, RIKEN Brain Science Institute, Wako-shi, Saitama 351-0198, Japan*

Received 13 October 2003; received in revised form 19 February 2004; accepted 26 February 2004

Available online 28 May 2004

Abstract

Two styrylbenzene derivatives, (*E,E*)-1-fluoro-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (FSB) and (*E,E*)-1-bromo-2,5-bis(3-hydroxycarbonyl-4-hydroxy)styrylbenzene-α,α′-$^{13}C_2$ ([^{13}C]BSB), were synthesized for use as a histochemical stain to detect amyloid plaques of Alzheimer's disease (AD) brain sections. An analysis of fluorescence spectra demonstrated that FSB shows approximately twofold fluorescence intensity relative to the conventional styrylbenzene derivative, (*E,E*)-1-bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy) styrylbenzene (BSB). Moreover, FSB was found to stain amyloid plaques and neurofibrillary tangles of AD brains with greater fluorescence intensity and a lower level of background signals compared to BSB. These finding indicate that FSB can be an excellent fluorescent compound to label human amyloid lesions with high sensitivity and specificity. Because of the possession of a nuclide with a quantized angular momentum, both FSB and [^{13}C]BSB are also potential contrast agents for magnetic resonance imaging to locate AD pathologies in vivo.
© 2004 Elsevier SAS. All rights reserved.

Keywords: Alzheimer's disease; Histochemical staining; Strylbenzenes; Magnetic resonance imaging

1. Introduction

Alzheimer's disease (AD) has now become one of the most common diseases among the elderly, affecting more than 4 million people in United States alone. The disease is neuropathologically characterized by the formations of senile plaques (SPs), which consist of mainly amyloid β (Aβ) peptide, and of filamentous tau lesions including neurofibrillary tangles (NFTs), neuropil threads (NPs) and plaque neurites, and finally loss of neurons. The definitive diagnosis of AD is based on histochemical and/or immunohistochemical staining of these neuropathological hallmarks in postmortem brain sections. Congo red and thioflavin-S have been most widely used as standard stains that detect brain amyloids by fluorescence [1]. Recently, however, much effort have been directed toward derivatization of Congo red to develop a small ligand molecule that binds brain amyloid in vivo as a possible antemortem diagnostic tool for AD [2,3]. Lee and her co-workers [4,5] reported one of such ligands, (*E,E*)-1-bromo-2,5-bis(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (BSB), with which they were able to detect amyloid plaques in vivo in a mouse model of AD. BSB was also found to be useful as a stain with bright fluorescence for detecting amyloid deposits in systemic amyloidosis both in vitro and in vivo [6].

We were interested in exploiting an analogous compound to BSB that contains a fluoro group, instead of bromo, because fluoro-substitution has several advantages that the fluorescence intensity is normally greater than that of the corresponding bromo-substituted compounds (known as 'heavy atom effect') and that the smaller molecular weight is favorable for enhanced permeability of the blood-brain barrier (BBB) as well as a rapid clearance from the body when used as an in vivo probe molecule. In addition, fluoro-substituted probes have a potential to be used as a contrast agent in magnetic resonance imaging (MRI), by which detection of AD amyloid plaques has never been achieved to date. Toward this goal, we synthesized fluoro-substituted and ^{13}C-labeled BSB analogs, (*E,E*)-1-fluoro-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (FSB) and (*E,E*)-1-bromo-2,5-bis(3-hydroxycarbonyl-4-hydroxy)styrylbenzene-

Abbreviations: Aβ, amyloid β; AD, Alzheimer's disease; NFTs, neurofibrillary tangles; NPs, neuropil threads; SPs, senile plaques.

* Corresponding author.
E-mail address: sasamoto@dojindo.co.jp (K. Sasamoto).

© 2004 Elsevier SAS. All rights reserved.
doi:10.1016/j.ejmech.2004.02.013

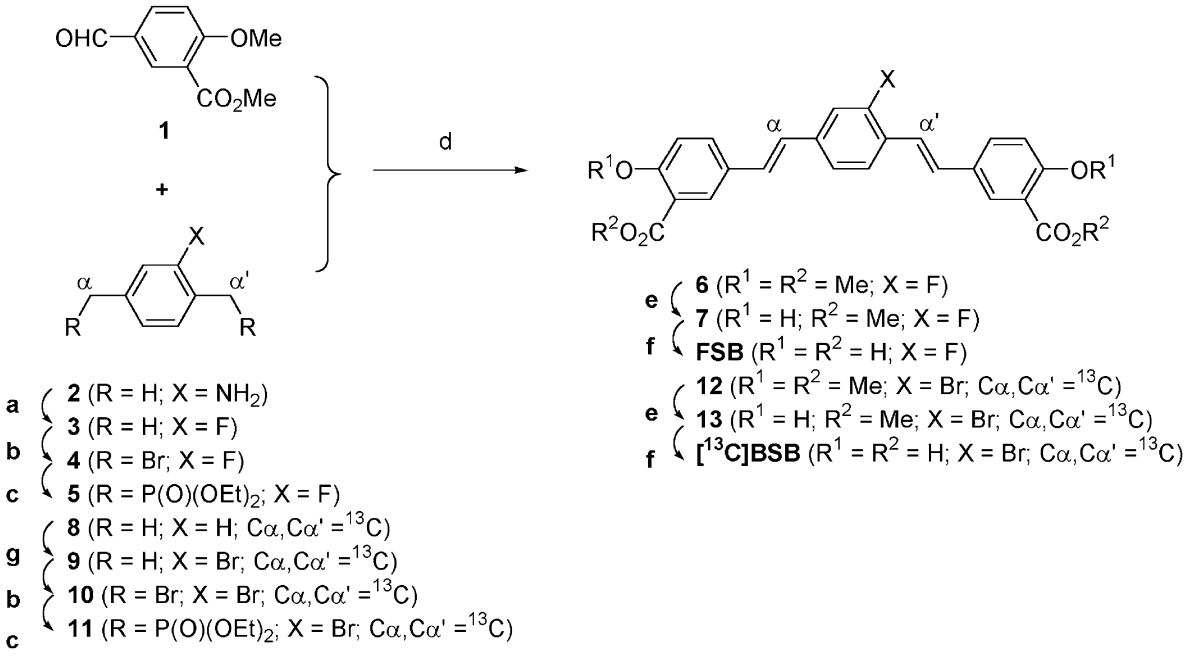
α,α′-$^{13}C_2$ ([^{13}C]BSB), and report herein our preliminary results obtained with these analogs in staining AD brain amyloid lesions.

2. Results and discussion

The syntheses of FSB and [^{13}C]BSB were carried out in a similar manner to BSB [5], starting from 2,5-dimethylaniline or *p*-xylene-α,α′-$^{13}C_2$, respectively, as outlined in Scheme 1. Since initial attempts to fluorinate *p*-xylene using various electrophilic fluorinating reagents failed due primarily to difficulties in purification, we finally converted 2,5-dimethylaniline to fluoride **3** via the corresponding diazonium tetrafluoroborate, which decomposed spontaneously to give the desired product. The key step was the Wittig–Horner coupling reaction between phosphate ester **5** and aldehyde **1** that produced (*E,E*)-isomer **6** as a sole product as confirmed by ^{1}H-NMR. Deprotection of **6** in two steps gave FSB in a moderate yield, whose NMR spectra were very similar to those of BSB except a strong peak at –118 ppm in ^{19}F-NMR spectrum. In the synthesis of [^{13}C]BSB, *p*-xylene-α,α′-$^{13}C_2$, in which the two methyl groups of *p*-xylene are ^{13}C-labeled, was used as a starting material because it is commercial available and ^{13}C-labeling of **1** at its aldehyde group turned out to be of very low yield. To install a bromo group into this compound, we employed a method reported by Kitagawa et al. [7] that utilized a ferrocenium complex, affording bromide **9** in 59% yield. From **9** to the final product [^{13}C]BSB, the synthesis was done in an identical manner to BSB. [^{13}C]BSB showed very strong peaks in ^{13}C-NMR spectrum at 124.8 and 125.5 ppm, those corresponding to the vinylic carbons; it also showed ^{13}C–H couplings in its ^{1}H-NMR spectrum.

The emission and excitation spectra of FSB and [^{13}C]BSB, in comparison with BSB, are shown in Fig. 1. FSB fluoresces with maximum emission and excitation wavelengths identical to those of BSB (λ_{ex} = 390 nm; λ_{em} = 520 nm) but with intensities being roughly twofold. The increased fluorescence intensities can be attributed to the heavy atom effect, which is well known particularly with halide-substituted fluorophores whose fluorescence is quenched in the order Cl < Br < I. These fluorescence properties seem to indicate that FSB is more sensitive as a stain for histochemical localization of AD pathology than BSB, although it is not clear whether and to what extent the fluorescence is enhanced upon binding to amyloid fibrils.

Fig. 2compares FSB and [^{13}C]BSB with BSB in staining temporal cortex and hippocampus tissue sections from AD brains, both of which were ethanol-fixed. The same protocol was used for the FSB and BSB stains, and thus was not optimized for FSB. FSB clearly stained SPs, NFTs, NPs and plaque neurites with a greater intensity than BSB. The difference in the contrast of staining between FSB and BSB was particularly pronounced in some regions of the temporal cortex, where relatively immature, non-neuritic plaques were abundant (Fig. 2A, C). As BSB was reported to label a variety of amyloid forms such as SPs and NFTs, it is likely that FSB



Reagents and conditions: (a) i) HCl, $NaNO_2$, 5 °C. ii) rt; (b) NBS, ABIN, $CHCl_3$, reflux; (c) $(EtO)_3P$, Δ; (d) **1**, NaOMe, MeOH, 0 °C to reflux; (e) BBr_3, -78 °C, CH_2Cl_2; (f) aq.KOH, reflux; (g) $Cp_2FeB[3,5\text{-}C_6H_3(CF_3)_2]_4$, ZnO, CH_2Cl_2, Br_2, -50 °C to 0 °C.

Scheme 1. Synthesis of FSB and [^{13}C]BSB.

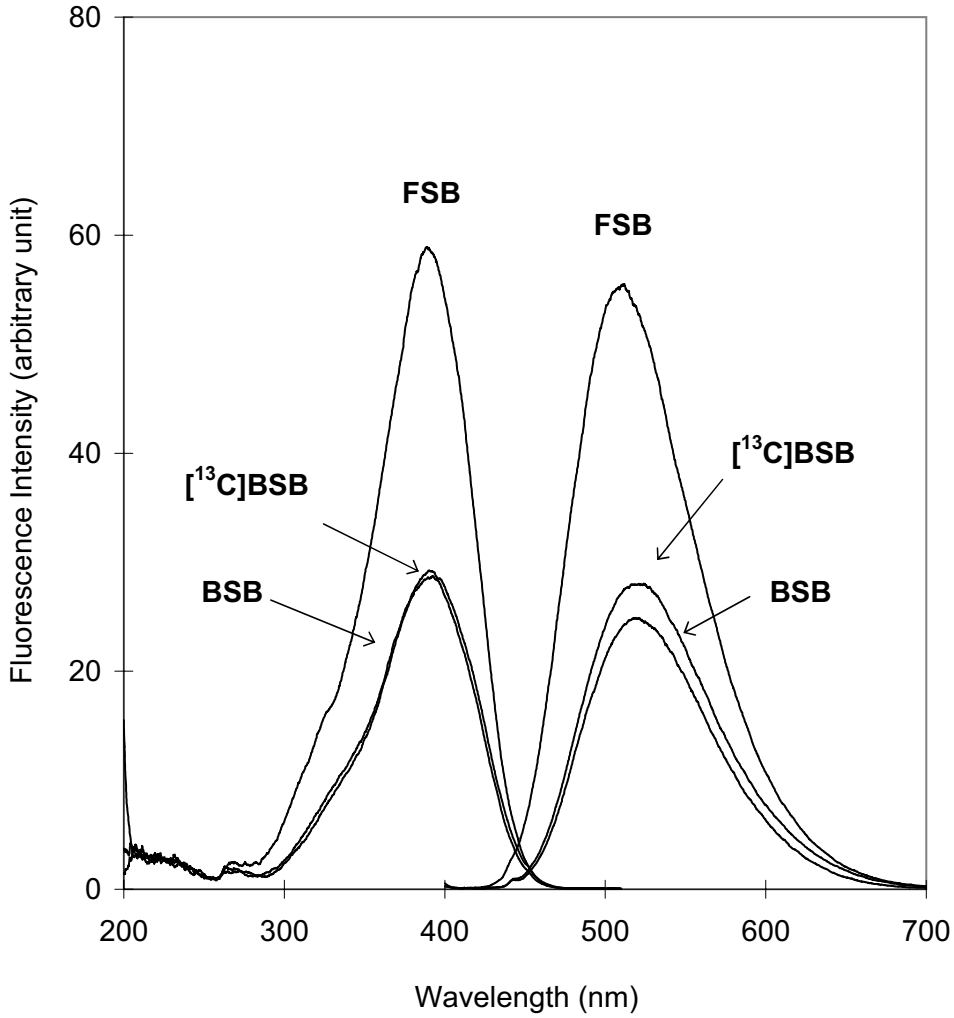


Fig. 1. Fluorescence spectra of FSB and BSB taken at 1.0 μM in DMSO.

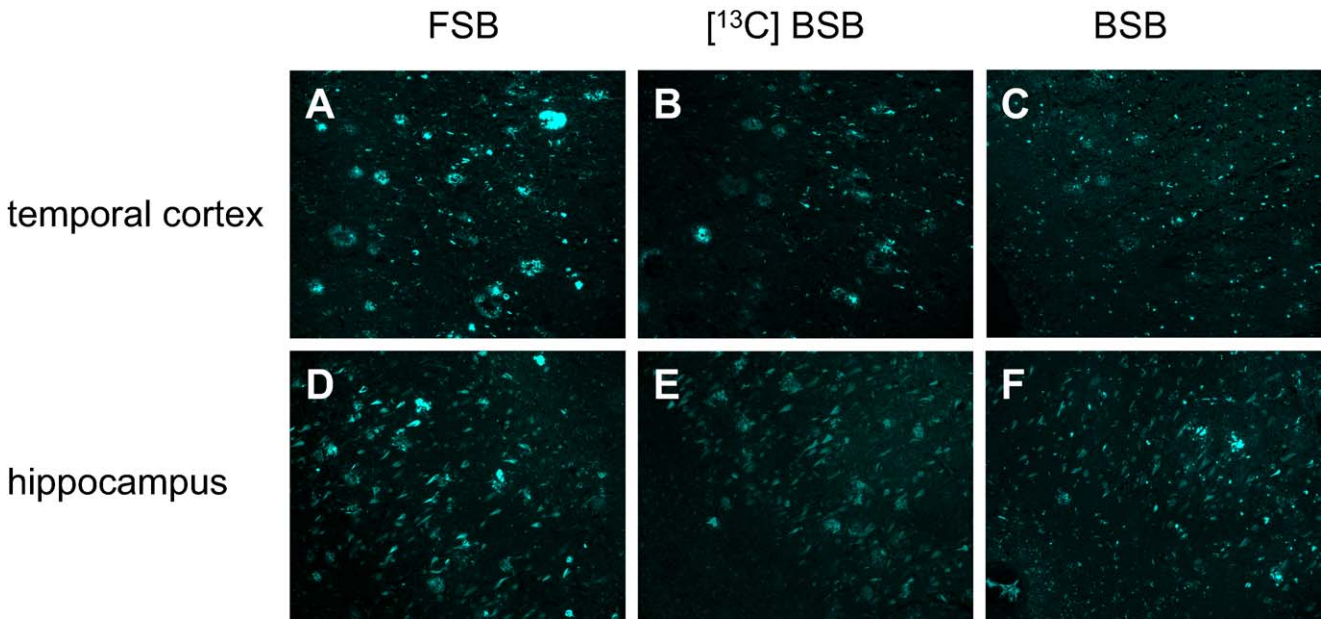


Fig. 2. Temporal cortex (A–C) or hippocampal (D–F) sections from AD brains (ethanol-fixed) were stained with FSB (A and D), [^{13}C]BSB (B and E) or BSB (C and F).

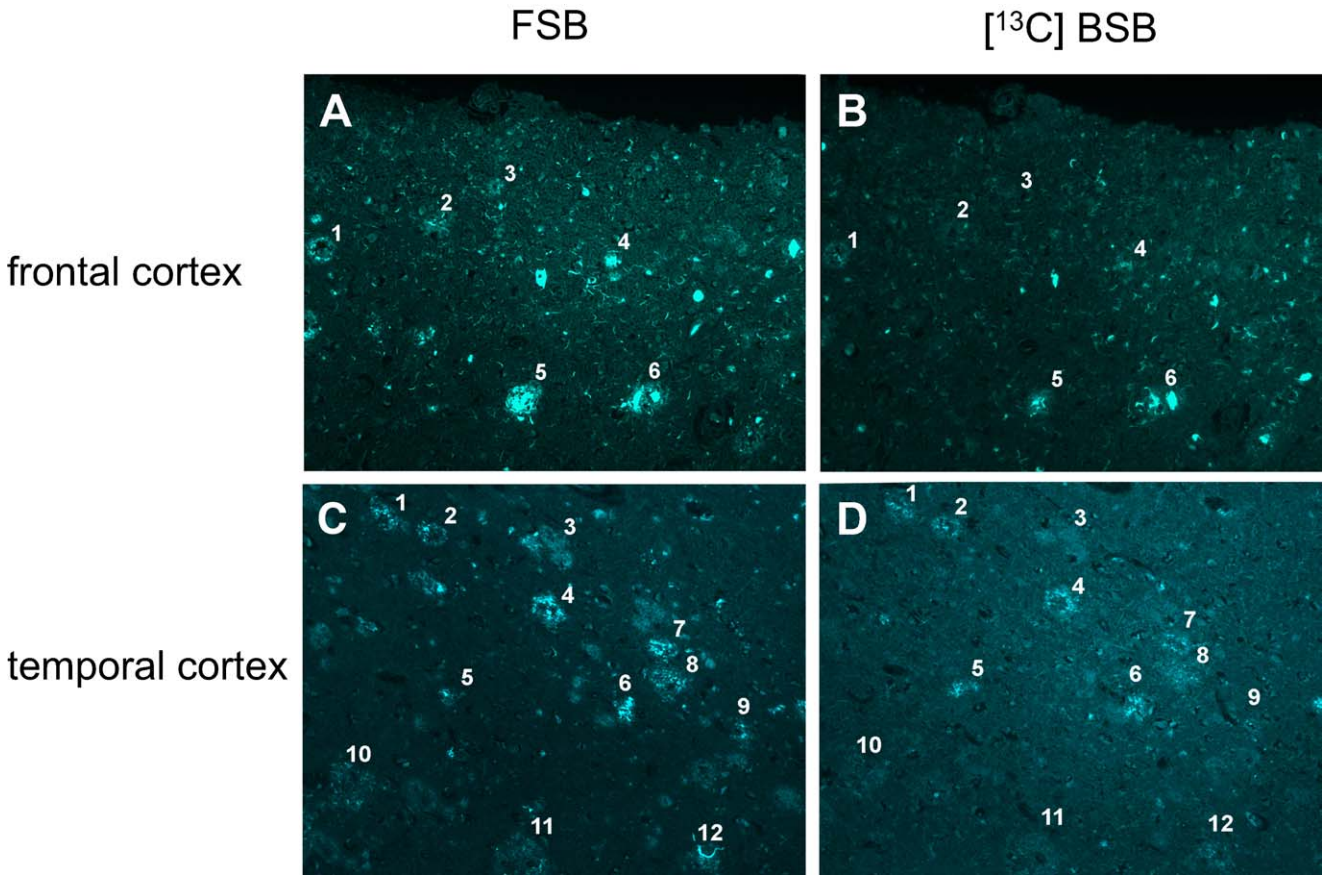


Fig. 3. Subadjacent sections of frontal (A and B) or temporal (C and D) cortex from AD brains, fixed, respectively, in isotonic ethanol or NBF, were stained with FSB (A and C) or [^{13}C]BSB (B and D). The number indicates the same plaque in FSB- and [^{13}C]BSB-stained sections.

also has similar affinities for these amyloid forms containing the β-pleated sheet structure. [^{13}C]BSB showed fluorescence staining that was comparable to the staining with BSB in both hippocampus and temporal cortex.

Using subadjacent ethanol-fixed frontal and NBF-fixed temporal cortex sections, staining of the same amyloid plaque with FSB and [^{13}C]BSB was compared (Fig. 3). Again, FSB gave a better contrast than [^{13}C]BSB with a greater fluorescence intensity and a lower background level.

The observations of the present study clearly demonstrate that both FSB and [^{13}C]BSB effectively stain hallmark lesions of AD including SPs and NFTs. Those BSB analogs were designed in the hope that they can be used as a sensitive MRI probe for an antemortem diagnosis of AD. Since ^{19}F and ^{13}C are NMR active, amyloid lesions can be located by detecting MR signals from these nuclides. Notably, FSB was found to label these AD pathologies with higher sensitivity and specificity than BSB, suggesting its superiority in the use for neuropathological examinations. Our observation showed that [^{13}C]BSB binds to amyloid plaques and NFTs with an affinity similar to BSB. The biodistribution and pharmacokinetics of [^{13}C]BSB are supposed to be identical to those of BSB, and thus it may be useful for imaging amyloid plaques in living AD patients and animal models as BSB has been demonstrated to visualize these lesions in vivo [4,5]. Although it will be required to clarify the kinetics FSB in animals and humans, and both FSB and BSB exist possibly as a monocarboxylate anion due to their similar pK_a values of the carboxylic groups, it is likely that FSB penetrates intact BBB with a higher permeability than BSB because of its less bulky structure. In addition, our results of histochemical staining imply high specificity of FSB in labeling amyloid plaques of living AD patients compared to BSB. It gives a bluish green fluorescence under UV light, which allows the plaques to be readily distinguished. FSB is also in contrast to Congo red that fluoresces pink to orange-red, with which small plaques are unlikely to be detected because of this fluorescence. FSB might also be useful as a histochemical stain for systemic amyloidosis [6]. In vitro binding studies as well as in vivo studies for FSB and [^{13}C]BSB are currently underway and will be reported elsewhere.

3. Experimental section

p-Xylene-α,α′-$^{13}C_2$ was purchased from Isotec (Miamisburg, Ohio, USA) and used as purchased. ^{1}H-Nuclear magnetic resonance (NMR) spectra were taken on a JEOL JNM ECP-300 spectrometer operating at 300 MHz. Mass spectra were recorded on a JEOL JMS-AX505W spectrometer. IR

spectra were measured on a Hitachi 270-30 spectrometer. Fluorescence spectra were taken on a Hitachi F-4500 spectrometer. Fluorescent tissue sections were viewed using a Zeiss Axiophot 2 fluorescence microscope (Göttingen, Germany).

*3.1. 3-Methoxycarbonyl-4-methoxybenzaldehyde (**1**)*

5-Formylsalicylic acid (22.0 g, 132.4 mmol) was dissolved in acetone (1100 ml) with heating, to which was added K_2CO_3 (36.5 g, 264.1 mmol) and methyl iodide. The mixture was heated at 50 °C for 12 h, during which time a total of 253.4 g of methyl iodide (1.78 mol) was added every 2 h. After the solvent was stripped off, water (200 ml) was added to leave crude methylated product, which was crystallized from ethanol to give 16.59 g (65%) of **1** as a white crystalline solid. ^{1}H-NMR ($CDCl_3$): δ 3.93 (s, 3H), 4.06 (s, 3H), 7.12 (d, J = 8.7 Hz, 1H), 8.03 (dd, J = 8.5, 2.2 Hz, 1H), 8.33 (d, J = 2.1 Hz, 1H), 9.92 (s, 1H).

*3.2. 2-Fluoro-p-xylene (**3**)*

Concentrated HCl (17 ml) was slowly added to a stirred suspension of 2,5-dimethylaniline (10 g, 82.5 mmol) in water (30 ml). An aqueous solution (10 ml) of $NaNO_2$ (7.0 g, 101.4 mmol) was then added dropwise with stirring to this suspension at 5 °C. After being stirred for 1 h at 5 °C, the solution was filtered. Addition of $NaBF_4$ (11.0 g, 100.2 mmol) to this filtrate caused precipitation of the corresponding diazonium salt, which decomposed spontaneously at room temperature to leave the crude fluorinated product. Column chromatography on SiO_2 (hexane) afforded 1.51 g (15%) of **3** as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 2.22 (s, 3H), 2.30 (s, 3H), 6.79–6.83 (m, 2H), 7.03 (t, J = 8.0 Hz, 1H); ^{19}F-NMR ($CDCl_3$): –118.5 ppm.

*3.3. 1-Fluoro-2,5-bis(bromomethyl)benzene (**4**)*

A chloroform solution (100 ml) containing **3** (1.02 g, 8.2 mmol), *N*-bromosuccinimide (3.1 g, 17.4 mmol) and 2,2′-azobis(isobutyronitrile) (10 mg, 0.8 mol%) was refluxed for 100 min. After being cooled, the chloroform solution was washed with water, dried ($MgSO_4$), and concentrated to leave colored solids, which crystallized by addition of hexane to give 0.99 g (43%) of **4** as off-white crystalline powder. ^{1}H-NMR ($CDCl_3$): δ 4.43 (s, 2H), 4.49 (s, 2H), 7.08–7.41 (m, 3H); ^{19}F-NMR ($CDCl_3$): –115.9 ppm; MS: *m/z* 281 (M + 1). 3

*3.4. 2-Fluoro-1,4-bis(diethylphosphonato)xylene (**5**)*

A mixture of **4** (0.99 g, 3.5 mmol) and triethyl phosphite (1.16 g, 7.0 mmol) was heated at 160 °C for 4 h. The reaction mixture was chromatographed on SiO_2 (5% MeOH in CH_2Cl_2) to afford 1.18 g (85%) of **5** as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 1.23–1.29 (m, 12H), 3.11 (d, J = 20.3 Hz, 2H), 3.17 (d, J = 20.6 Hz, 2H), 3.98–4.10 (m, 8H), 7.04 (d, J = 9.9 Hz, 1H), 7.27–7.34 (m, 2H); ^{19}F-NMR ($CDCl_3$): –117.1 ppm; MS: *m/z* 397 (M + 1).

*3.5. (E,E)-1-Fluoro-2,5-bis(3-methoxycarbonyl-4-methoxy) styrylbenzene (**6**)*

Sodium methoxide in methanol (28 wt.%, 2 ml) was added to an anhydrous methanol solution (5 ml) containing **1** (1.16 g, 6.0 mmol) and **5** (1.18 g, 3.0 mmol) at 0 °C, and the solution was refluxed overnight. Precipitates formed were collected and suspended in water (100 ml). The pH of this suspension was adjusted to 3 with 1 M HCl, and the solution was extracted with chloroform. The chloroform layer was washed with water, dried ($MgSO_4$) and concentrated to give a crude coupling product, which was chromatographed on SiO_2 (10% EtOAc in $CHCl_3$) affording 0.42 g (30%) of **6** as yellow powder. ^{1}H-NMR ($CDCl_3$): δ 3.93 (s, 6H), 3.94 (s, 6H), 6.94–7.26 (m, 8H), 7.55 (t, J = 7.9 Hz, 1H), 7.61 (dd, J = 9.1, 2.2 Hz, 1H), 7.64 (dd, J = 9.3, 2.2 Hz, 1H), 7.97 (d, J = 2.2 Hz, 1H), 7.98 (d, J = 2.2 Hz, 1H); ^{19}F-NMR ($CDCl_3$): –118.1 ppm; MS: *m/z* 476 (M^+).

*3.6. (*E,E*)-1-Fluoro-2,5-bis(3-methoxycarbonyl-4-hydroxy) styrylbenzene (**7**)*

To **6** (0.42 g, 0.87 mmol) in dichloromethane (50 ml) was added 1.0 M BBr_3 in dichloromethane (9.0 ml, 9.0 mmol) at –78 °C, and the mixture was stirred overnight at room temperature. Water (30 ml) and methanol (~5 ml) were added to the reaction mixture, and the organic layer was separated. The aqueous layer was extracted with dichloromethane; the combined organic layer was washed with water, dried ($MgSO_4$) and concentrated to give **7** in almost quantitative yield as yellow powder. ^{1}H-NMR (DMSO-d_6): δ 3.93 (s, 6H), 7.03 (d, J = 8.7 Hz, 1H), 7.04 (d, J = 8.5 Hz, 1H), 7.15 (d, J = 16.5 Hz, 1H), 7.16 (d, J = 16.5 Hz, 1H), 7.36 (d, J = 16.5 Hz, 1H), 7.37 (d, J = 16.5 Hz, 1H), 7.43–7.52 (m, 2H), 7.75–7.89 (m, 3H), 7.98 (s, 2H), 10.59 (s, 2H); ^{19}F-NMR (DMSO-d_6): –118.6 ppm.

*3.7. (*E,E*)-1-Fluoro-2,5-bis(3-hydroxycarbonyl-4-hydroxy) styrylbenzene (FSB)*

A suspension of **7** (0.42 g, 0.94 mmol) in 0.06 M aqueous KOH (150 ml) was refluxed for 4 h, and the pH of the solution was adjusted to 2 by slow addition of 6 M HCl. After being cooled, precipitates were collected to give 0.23 g (64% from **6**) of FSB as yellow powder. mp 294 °C (decomp.); ^{1}H-NMR (DMSO-d_6): δ 6.95 (d, J = 8.5 Hz, 1H), 6.96 (d, J = 8.5 Hz, 1H), 7.12 (d, J = 16.2 Hz, 1H), 7.13 (d, J = 16.5 Hz, 1H), 7.33 (d, J = 16.5 Hz, 1H), 7.34 (d, J = 16.2 Hz, 1H), 7.42–7.49 (m, 2H), 7.74–7.76 (m, 1H), 7.79 (d, J = 8.8 Hz, 1H), 7.80 (d, J = 8.8 Hz, 1H), 7.98 (d, J = 2.2 Hz, 1H), 8.00 (d, J = 2.2 Hz, 1H); ^{19}F-NMR (DMSO-d_6): –118.7 ppm (s); MS: *m/z* 419 (M – 1); IR (KBr): 3430 (OH), 3025 (CO_2H), 1670 (C=O), 1210, 1190, 955 cm^{-1}.

3.8. 2-Bromo-p-xylene-α,α′-$^{13}C_2$ (**9**)

Bromination of *p*-xylene-α,α′-$^{13}C_2$ was carried out according to a method reported by Kitagawa et al. [7]. Briefly, bromine (1.65 g, 10.3 mmol) was added to a dichloromethane solution (5 ml) containing ferrocenium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate [7] (0.49 g, 0.46 mmol) and ZnO (0.84 g, 10.3 mmol). The solution was stirred at room temperature for 1 h, to which was then added *p*-xylene-α,α′-$^{13}C_2$ (1.0 g, 9.2 mmol) in dichloromethane (10 ml) at –50 °C. After being stirred for 2 h at 0 °C, the reaction mixture was partitioned between ethyl acetate and saturated $Na_2S_2O_3$. Ethyl acetate layer was washed with brine, dried ($MgSO_4$) and concentrated in vacuo. Chromatography on SiO_2 (hexane) gave 1.03 g (59%) of **9** as a colorless oil. ^{1}H-NMR ($CDCl_3$): δ 2.28 (d, J = 127.0 Hz, 3H), 2.33 (d, J = 127.0 Hz, 3H), 6.99 (dd, J = 7.7, 4.1 Hz, 1H), 7.09 (dd, J = 7.7, 4.7 Hz, 1H), 7.34 (d, J = 4.4 Hz, 1H).

3.9. 1-Bromo-2,5-bis(bromomethyl)benzene-α,α′-$^{13}C_2$ (**10**)

The compound was synthesized in the same manner as for **4** in 43% yield as a white crystalline solid. ^{1}H-NMR ($CDCl_3$): δ 4.41 (d, J = 154.0 Hz, 2H), 4.58 (d, J = 154.0 Hz, 2H), 7.32 (ddd, J = 7.7, 4.7, 1.7 Hz, 1H), 7.43 (dd, J = 7.7, 4.7 Hz, 1H), 7.61 (dd, J = 5.5, 1.7 Hz, 1H).

3.10. 2-Bromo-1,4-bis(diethylphosphonato)xylene-α,α′-$^{13}C_2$ (**11**)

The compound was synthesized in the same manner as for **5** in 94% yield as yellow oil. ^{1}H-NMR ($CDCl_3$): δ 1.25 (t, J = 7.1 Hz, 12H), 3.08 (dd, J = 128.0, 21.0 Hz, 2H), 3.37 (dd, J = 128.0, 21.0 Hz, 2H), 3.98–4.09 (m, 8H), 7.20–7.26 (m, 1H), 7.39–7.43 (m, 1H), 7.50–7.52 (m, 1H).

3.11. (E,E)-1-Bromo-2,5-bis(3-methoxycarbonyl-4-methoxy) styrylbenzene-α,α′-$^{13}C_2$ (**12**)

The compound was synthesized in the same manner as for **6** in 50% yield as yellow powder. ^{1}H-NMR ($CDCl_3$): δ 3.93 (s, 6H), 3.94 (s, 6H), 6.94 (dd, J = 154.0, 16.0 Hz, 1H), 6.98–7.14 (m, 4H), 7.43–7.45 (m, 1H), 7.60–7.72 (m, 5H), 7.97 (s, 1H), 7.98 (s, 1H).

3.12. (E,E)-1-Bromo-2,5-bis(3-methoxycarbonyl-4-hydroxy) styrylbenzene-α,α′-$^{13}C_2$ (**13**)

The compound was synthesized in the same manner as for **7** in 97% yield as yellow powder. ^{1}H-NMR (DMSO-d_6): δ 3.93 (s, 6H), 6.85–7.54 (m, 6H), 7.62–7.68 (m, 1H), 7.82–7.88 (m, 4H), 7.97 (d, J = 2.0 Hz, 1H), 7.99 (d, J = 2.1 Hz, 1H), 10.59 (s, 1H), 10.61 (s, 1H).

3.13. (E,E)-1-Bromo-2,5-bis(3-hydroxycarbonyl-4-hydroxy) styrylbenzene-α,α′-$^{13}C_2$ ([^{13}C]BSB)

The compound was synthesized in the same manner as for FSB in 50% yield as yellow-brown powder. mp 303 °C (decomp.); ^{1}H-NMR (DMSO-d_6): δ 6.86–7.55 (m, 6H), 7.61–7.68 (m, 1H), 7.81–7.89 (m, 4H), 8.00 (d, J = 2.2 Hz, 1H), 8.02 (d, J = 2.2 Hz, 1H); ^{13}C-NMR (DMSO-d_6): 124.8, 125.5 ppm; MS: *m/z* 483(M – 2); IR (KBr): 3450 (OH), 3025 (CO_2H), 1670 (C=O), 1210, 1180, 950 cm^{-1}.

3.14. Staining

Brain tissues from four AD patients obtained at autopsy were used in this study. Tissues were fixed either in 70% ethanol containing 150 mM NaCl or in 10% neutral buffered formalin (NBF) overnight. Four-micrometre-thick sections of parafin-embedded blocks were stained with FSB or [^{13}C]BSB according to a protocol for BSB [4]. Briefly, tissues sections were deparaffinized and immersed in a 0.01% FSB or [^{13}C]BSB in 50% ethanol for 30 min. Sections were then differentiated in saturated Li_2CO_3 and rinsed in 50% ethanol to be examined with the fluorescence microscopy. Upon the staining, autofluorescence from endogenous lipofuscin was not quenched by using an oxidizing step as reported by Barden [8].

References

[1] H. Puchtler, F. Sweat, M. Levine, On the binding of Congo red by amyloid, J. Histochem. Cytochem. 10 (1962) 355–364.
[2] S.D. Styren, R.H. Hamilton, G.C. Styren, W.E. Klunk, X-34, A fluorescent derivative of Congo red: a novel histochemical stain for Alzheimer's disease pathology, J. Histochem. Cytochem. 48 (2000) 1223–1232.
[3] W.E. Klunk, M.L. Debnath, J.W. Pettegrew, Development of small molecule probes for the beta-amyloid protein of Alzheimer's disease, Neurobiol. Aging 15 (1994) 691–698.
[4] D.M. Skovronsky, B. Zhang, M.-P. Kung, H.F. Kung, J.Q. Trojanowski, V.M.-Y. Lee, In vivo detection of amyloid plaques in a mouse model of Alzheimer's disease, Proc. Natl. Acad. Sci. 97 (2000) 7609–7614.
[5] Z.-P. Zhuang, M.-P. Kung, C. Hou, D.M. Skovronsky, T.L. Gur, K. Plossl, J.Q. Trojanowski, V.M.-Y. Lee, H.F. Kung, Radioiodinated styrylbenzenes and thioflavins as probes for amyloid aggregates, J. Med. Chem. 44 (2001) 1905–1914.
[6] Y. Ando, Y. Tanoue, K. Haraoka, K. Ishikawa, S. Katsuragi, M. Nakamura, X. Sun, K. Nakagawa, K. Sasamoto, K. Takesako, T. Ishizaki, K. Doh-ura, A novel tool for detecting amyloid deposits in systemic amyloidosis in vitro and in vivo, Lab. Invest. 83 (2003) 1751–1759.
[7] H. Kitagawa, T. Shibata, J. Matsuo, T. Mukaiyama, Efficient method for the preparation of aromatic bromides and iodides by ferrocenium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate—catalyzed halogenation with bromine and iodine monochloride, Bull. Chem. Soc. Jpn. 75 (2002) 339–345.
[8] H. Barden, The presence of ethylenic bonds and vic-glycol groups in neuromelanin and lipofuscin in the human brain, J. Histochem. Cytochem. 31 (1983) 849–858.