Expedited Articles

Isomerization of (*Z,Z*) to (*E,E*)1-Bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)-styrylbenzene in Strong Base: Probes for Amyloid Plaques in the Brain

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In developing probes for detecting β-amyloid (Aβ) plaques in the brain of Alzheimer's disease (AD), we have synthesized 1-bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (**5**, BSB). Due to the presence of two double bonds, formation of four different isomers is possible. Four isomers, ***E,E*-5**, ***E,Z*-5**, ***Z,E*-5**, and ***Z,Z*-5**, were prepared. Surprisingly, all showed strong fluorescent labeling of Aβ plaques in the brain of postmortem brain sections of patients with confirmed AD. In vitro binding assay also showed that all four isomers of BSB (***E,E*-5**, ***E,Z*-5**, ***Z,E*-5**, and ***Z,Z*-5**) displayed a similar high binding affinity inhibiting the binding of [^{125}I]***E,E*-6**, 1-iodo-2,5-bis-(3-hydroxycarbonyl-4-methoxy)styrylbenzene (IMSB) to Aβ_{1-40} aggregates. The inhibition constants (K_i) of ***E,E*-5**, ***E,Z*-5**, ***Z,E*-5**, and ***Z,Z*-5** were 0.11 ± 0.01, 0.19 ± 0.03, 0.27 ± 0.06, and 0.13 ± 0.02 nM, respectively. Due to the fact that geometric stability of these styrylbenzenes is unknown, and the conversion of ***Z,Z*-5** to ***E,E*-5** may occur automatically in the binding or labeling assaying conditions, we have investigated the kinetics of conversion of ***Z,Z*-5** to ***E,E*-5** by NMR in D_2O/NaOD at elevated temperatures (70, 95, and 115 °C). The activation energy was determined to be 14.15 kcal/mol. The results strongly suggest that the isomeric conversion at room temperature in aqueous buffer solution is unlikely. All of the styrylbenzene isomers clearly showed potential as useful tools for studying Aβ aggregates in the brain. The data suggest that, despite the rigidity of this series of styrylbenzenes, the binding sites on Aβ aggregates may have certain flexibility and the binding pockets could be adaptable for binding to other smaller ligands. Such information could be exploited to develop new ligands for detecting amyloid plaques in AD.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease of the brain characterized by dementia, cognitive impairment, and memory loss. β-Amyloid (Aβ) peptides are involved in the development and progression of AD. The fibrillar aggregates of amyloid peptides, Aβ_{1-40} and Aβ_{1-42}, found in senile plaques and cerebrovascular amyloid deposits in AD patients[1] are major metabolic products of amyloid precursor protein. Prevention and reversal of Aβ plaque formation are being targeted as treatments for this disease.[2−8] Ligands exhibiting specific high binding affinity to Aβ aggregates may be useful for diagnosis and treatment of AD.[9] Since AD is now becoming one of the most devastating illnesses affecting a large number of older patients and their families, there are urgent needs for better diagnosis and treatment, and we have synthesized and evaluated potentially useful compounds based on small neutral molecules.

Previously, ligands for staining amyloid aggregates were based on highly conjugated dyes, such as Congo Red (CR), Thioflavin T and S, Chrysamine G (CG), and X-34[10,11] (Chart 1). Thioflavins and CR have been used in fluorescent staining of plaques and tangles in postmortem AD brain sections.[12] A more abbreviated form of CG, X-34 (Chart 1), has been reported as another fluorescent dye for staining amyloid aggregates.[13,14] Previously, a highly conjugated bromo derivative, ***E,E*-5** (BSB), had been evaluated as a fluorescent probe for AD.[15] Several lines of evidence suggest that BSB is an appropriate starting point for future efforts to generate an imaging agent for monitoring AD. It labels Aβ plaques in human AD brain sections with high sensitivity and specificity, and it crosses the blood-brain barrier and labels numerous AD-like Aβ plaques throughout the brain of transgenic mice following iv injection. Recently, the synthesis of ***E,E*-5** (BSB) and the corresponding iodinated BSB were reported.[21] This series of compounds with strong fluorescent signals may have great

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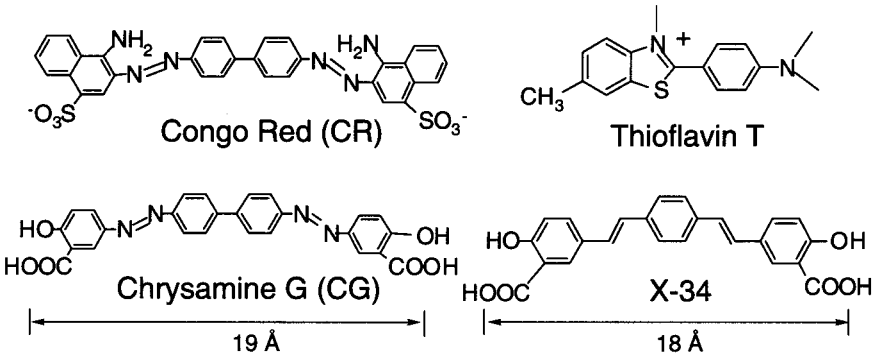
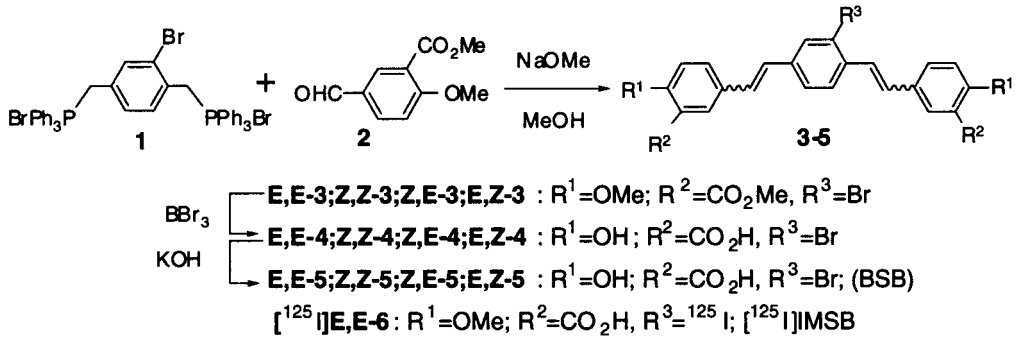
Chart 1. Chemical Structures of Congo Red (CR), Thioflavin T, Chrysamine G (CG) and X-34.



Scheme 1. Synthesis of Styrylbenzenes



potential as tools for staining Aβ aggregates by in vitro labeling methods. On the basis of these exciting findings, efforts were made in the synthesis of all four possible isomers of ***E,E*-5** (BSB) produced in the Wittig reaction to investigate the influence of geometry on the binding affinity toward Aβ plaques. We report herein the synthesis and characterization of four isomers of **5**: ***E,E***, ***Z,Z***, ***Z,E***, and ***E,Z***. Isomeric conversion from ***Z,Z*** to ***E,E*** and their binding affinities toward preformed Aβ aggregates as well as fluorescent staining of Aβ plaques in AD brain sections by these isomers were also evaluated.

Results and Discussion

For this particular series of styrylbenzenes, the chemical synthesis of the isomers has never been reported before. The key step in the synthesis of the styrylbenzene derivatives was the Wittig reaction (Scheme 1), by which aldehyde **2** and triphenylphosphonium salt **1** were condensed. During this condensation reaction, the ***E*,*E*-3** isomer conveniently precipitated from the reaction mixture in 11% yield. The other three isomers, ***Z*,*E*-3**, ***Z*,*E*-3**, and ***E*,*Z*-3** were isolated by flash chromatography from the remaining mother liquor in 24, 13, and 11% yield, respectively (Scheme 1).

All four isomers of **3** were converted to the corresponding acid, BSB, **5** in two steps: demethylation with BBr_3 in CH_2Cl_2 followed by hydrolysis of ester groups in EtOH in the presence of KOH. All four of these compounds are strongly fluorescent, a property making them useful for staining. The excitation and emission wavelengths were between 394−431 nm and 489−532 nm, respectively (see Table 1).

Surprisingly, all four isomers of BSB (***E,E*-5**, ***E,Z*-5**, ***Z,E*-5**, and ***Z,Z*-5**) displayed strong inhibition on [^{125}I]-***E,E*-6** 1-iodo-2,5-bis-(3-hydroxycarbonyl-4-methoxy)-styrylbenzene (IMSB)[21] binding to Aβ_{1-40} aggregates (peptide concentrations 10−20 nM). Inhibition constants (K_i) of ***E,E*-5** (BSB), ***E,Z*-5**, ***Z,E*-5**, and ***Z,Z*-5** were 0.11 ± 0.01, 0.19 ± 0.03, 0.27 ± 0.06, and 0.13 ± 0.02 nM, respectively, while CG and salicylic acid displayed K_i values of 0.14 ± 0.04 and >1800 nM, respectively.

Table 1. Excitation and Emission Wavelengths of Isomers of BSB

compound	excitation [nm] maximum	emission [nm] maximum
***E,E*-5** (BSB)	432[a]	532[b]
***E,Z*-5** (BSB)	418[a]	502[c]
***Z,E*-5** (BSB)	416[a]	498[c]
***Z,Z*-5** (BSB)	394[a]	498[d]

[a] Emission set to 510 nm. [b] Excitation set to 430 nm. [c] Excitation set to 420 nm. [d] Excitation set to 410 nm.

In brain sections of confirmed AD cases, Thioflavin S, as expected, showed strong fluorescent staining of the Aβ aggregates. Using the same concentration (0.05 mM), all four isomers of **5** displayed excellent fluorescent staining comparable or better than that observed with Thioflavin S (Figure 1). The images of these isomers substantiate the excellent binding affinity observed by the in vitro binding assay using Aβ_{1-40} aggregates.

One remaining question on the observed high binding affinity for all isomers is the possibility that isomerization of ***E,Z*-5**, ***Z,E*-5**, and ***Z,Z*-5** to ***E,E*-5** during the assaying condition may occur automatically. As a consequence, the high binding affinity and excellent staining seen in AD brain section may be from only one active isomer, ***E,E*-5** (BSB). By close examination of the chemical structure, one may hypothesize that the salicylic moiety on both ends of the molecule could promote the conversion of the cis form to the trans form, especially, when the phenol groups are ionized (Scheme

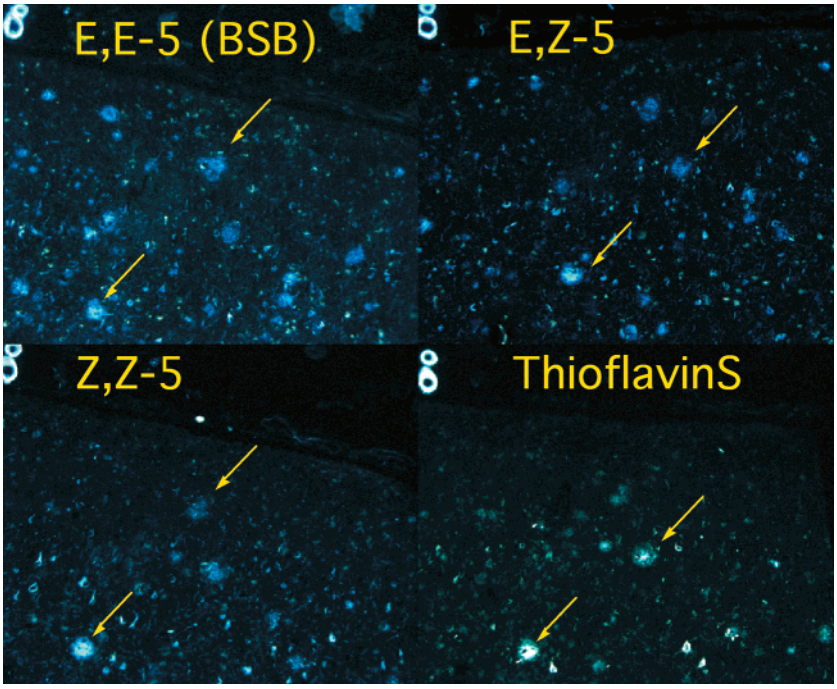
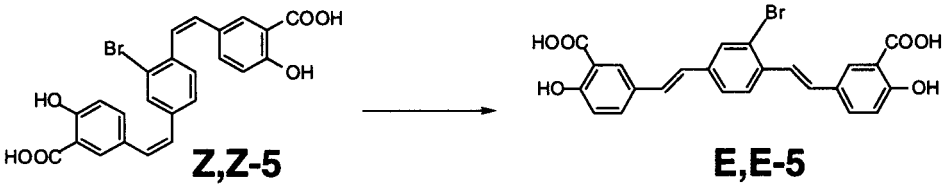


Figure 1. Fluorescent images of ***E,E*-5**, ***E,Z*-5**, ***Z,Z*-5**, and Thioflavin S using adjacent sections of postmortem AD brain. All of the images were obtained under similar incubating conditions using a concentration of 0.05 mM. The vascular amyloid plaques at the upper left-hand corner of each picture were used as markers to align the images.

Scheme 2. Isomerization of ***Z,Z*-5** to ***E,E*-5**



2). In general, it is assumed that the trans isomer, ***E,E*-5**, is more stable than the cis isomer, ***Z,Z*-5**. Isomerization will only happen from ***Z,Z*-5** to ***E,E*-5**. It was reported previously that a similar cis−trans isomerization of diethylstilbestrol (DES) in basic condition was a first-order reaction. The isomerization from ***Z,Z*-5** to ***E,E*-5** most likely will go through a mechanism similar to that of DES.[16,17]

To investigate this possibility we first dissolved ***Z,Z*-5** and ***E,E*-5** in D_2O in the presence of a small drop of NaOD to enhance the solubility. At room temperature the NMR spectra of ***Z,Z*-5** and ***E,E*-5** in D_2O showed very little change for 6 h (data not shown). However, after overnight at room temperature the sample for ***Z,Z*-5** did show some indication that indeed the conversion to the ***E,E*-5** isomer did happen.

To further confirm the isomerization reaction, a more detailed kinetic study was performed. An NMR tube, containing 11 mg of ***Z,Z*-5**, was dissolved in 0.1 mL of 1.4 M NaOD in D_2O and followed by the addition of D_2O to bring the final volume to 0.6 mL (0.23 M). The NMR tube was then placed in a heating block filled with sand to control the temperature at either 70, 95, or 115 °C. The changes can be readily quantified by measuring the same NMR sample and observing the shifting of peaks in the range of 5.9∼6.4 ppm (Figure 2). The aromatic proton signals of the ^1H NMR for ***Z,Z*-5** exhibited definite changes with time, although in nearly all cases the NMR signals are difficult to interpret because of overlapping and broadening. Fortunately, signals in the olefinic-proton region (5.9∼6.4 ppm, box in Figure 2) are unique for ***Z,Z*-5** without any interference from other signals. The reduction of peaks in this region was proportional to the changes of ***Z,Z*-5** to ***E,E*-5** at various time points. Eventually, ***Z,Z*-5** was completely converted to ***E,E*-5**, and the NMR spectrum of ***Z,Z*-5** appeared identical to that of authentic ***E,E*-5** in the same solvent.

A representative sample of data is presented in Figure 2. The kinetics of this isomerization of BSB was evaluated by calculating the fraction of isomerization (F), which was calculated based on the following equation:

$$F_t = (I_0 - I_t)/(I_0 - I_\infty)$$

I: integral ratio of olefinic peaks (6.06∼5.91 ppm) to whole aromatic peaks (7.35∼5.91 ppm)

I_t: integral ratio at time t

I_0: integral ratio at time 0

I_∞: integral ratio at time ∞

At time ∞, the integral of the regions representing olefinic peaks of ***Z,Z*-5** was very close to zero.

Therefore:

$$F_t = (I_0 - I_t)/(I_0)$$

A logarithmic plot of $(1 - F)$ at different temperatures

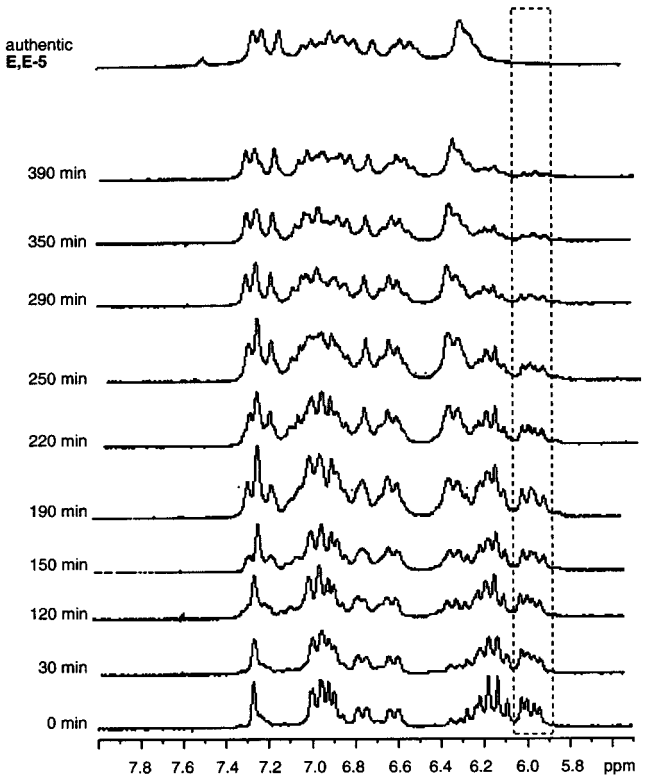


Figure 2. ^{1}H NMR spectra of a selected region of ***Z,Z*-5** (0.02 mmol) in D_2O/NaOD (0.23 M). The solution in the NMR test tube was heated at 95 °C, and the spectra were obtained at various time points after heating. The NMR spectrum of a solution of authentic ***E,E*-5** (BSB) (0.02 mmol) in D_2O/NaOD (0.23 M) is shown on the top.

vs the time *t* gave linear curves, suggesting a first-order reaction under basic conditions:

$$\textbf{\textit{Z,Z}-5} \rightarrow \textbf{\textit{E,E}-5}$$

and

$$\ln(1-F)_t = -kt + \ln(1-F)_0 \quad \text{(see Figure 3a)}$$

The rate constants *k* at various temperatures can be determined from measuring the slopes of the curves.

The activation energy E_a can then be calculated by fitting the Arrhenius equation where *R* is the gas constant and *T* is the temperature in kelvin.

$$\ln(k) = -\frac{E_a}{R}\left(\frac{1}{T}\right) + \ln(A) \quad \text{(see Figure 3b)}$$

A plot of ln(*k*) vs 1/*T* showed a straight line (R^2 = 0.9912), and E_a was determined to be 14.15 kcal/mol.

On the basis of this result it is reasonable to assume that at room temperature the kinetics of isomerization from ***Z,Z*-5** to ***E,E*-5** will be very slow. Therefore, it is likely that the in vitro binding data and staining of AD brain sections in Figure 2 are due to the isomers as indicated. It was reported previously that highly conjugated fluorescent probes, such as CG and its related derivatives, such as X-34, exhibited binding predominantly to the β-sheet structure. Due to the relatively rigid structure of these probes, the distance between the two carboxylic groups is about 18 Å.[10,11] It was suggested that the binding affinity was largely determined by

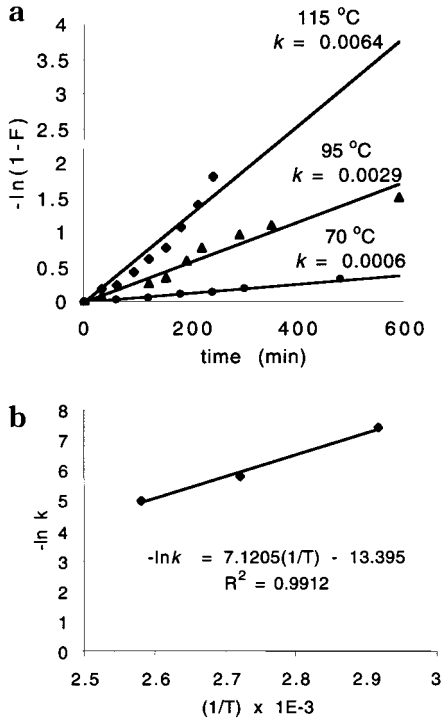


Figure 3. (a) Effects of temperature (70, 95, and 115 °C) on isomerization of ***Z,Z*-5** to ***E,E*-5**. (b) Plot of reaction rates vs 1/*T*. By measuring the slope for the plot of ln *k* versus 1/*T* and fitting the Arrhenius equation: $-\ln k = E_a/(RT) + \ln A$ (where *R* is the gas constant and *T* is the temperature in kelvin). The activation energy (E_a) for the isomerization of ***Z,Z*-5** to ***E,E*-5** was calculated to be 14.15 kcal/mol.

these two fixed negative charges. Since all four possible isomers of BSB (***E,E*-5**, ***E,Z*-5**, ***Z,E*-5**, and ***Z,Z*-5**) showed a similar binding affinity toward the aggregates, and the relative distance between the two negative charges is unlikely to be the same for all four of the isomers, conceptual alternatives for the high binding affinity of these compounds to the β-sheet structure may be necessary. The nature of the interactions between these small ligands with the macromolecular Aβ aggregates remains to be explored. Such information will be extremely useful in designing new ligands as probes for Aβ aggregates.

In conclusion, synthesis of novel styrylbenzenes as ligands for binding Aβ_{1-40} aggregates is reported. Four possible isomers of 1-bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (BSB), **5**, were prepared and elucidated. The kinetics of conversion of ***Z,Z*-5** to ***E,E*-5** was evaluated by NMR under strong basic conditions (D_2O/NaOD) at elevated temperatures. The activation energy was determined to be 14.15 kcal/mol. It is likely that no conversion of ***Z,Z*-5** to ***E,E*-5** would happen during binding assays at room temperature. On the basis of their exquisite binding affinity and selectivity to Aβ aggregates, they are extremely useful tools for studying the aggregates in vitro by binding assay and fluorescent staining of the brain sections of AD patients.

Experimental Section

All reagents used in the synthesis were commercial products and were used without further purification unless otherwise indicated. Anhydrous Na_2SO_4 was used as a drying agent. Flash column chromatography was performed on 230−400 mesh silica gel. ^{1}H NMR spectra were obtained on Bruker

spectrometers (Bruker DPX 200 and AMX 500). Chemical shifts are reported as δ values with respect to residual protons in $CDCl_3$ unless otherwise mentioned. Coupling constants are reported in hertz. Kinetic experiments were maintained at constant temperature by a heating block (Thermolyne, Type 17600) equipped with multiple wells (the stability of the heating block is ±0.7 °C).

Synthesis of 1-Bromo-2,5-bis-(3-methoxylcarbonyl-4-methoxy)styrylbenzene (3). To a mixture of 5-formyl-2-methoxy-benzoic acid methyl ester **2** (1.16 g, 6.0 mmol, prepared from 5-formylsalicylic acid[18]) and phosphonium salt **1** (2.6 g, 3.0 mmol, prepared from 2-bromo-1,4-*p*-xylene[19]) in MeOH (12 mL) was added a solution of sodium methoxide (1.3 mL, 25 wt % in MeOH) dropwise at 0 °C in an ice bath. The mixture was stirred at room temperature overnight. The solid formed was filtered and washed with methanol to give ***E,E*-3** (175 mg 10.9%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.907 (s, 3H, CH_3O-), 3.909 (s, 3H, CH_3O-), 3.915 (s, 3H, CH_3O-), 3.919 (s, 3H, CH_3O-), 6.91 (d, J = 16.3 Hz, 1H, olefinic), 6.97(d, J = 8.5 Hz, 1H, Ar*H*), 6.98 (d, J = 8.5 Hz, 1H, Ar*H*), 6.99 (d, J = 16.4 Hz, 1H, olefinic), 7.03 (d, J = 16.3 Hz, 1H, olefinic), 7.34 (d, J = 16.2 Hz, 1H, olefinic), 7.40 (d, J = 8.2 Hz, 1H, Ar*H*), 7.59 (d,d, J = 8.5, 2.1 Hz, 1H, Ar*H*), 7.60 (d, J = 8.3 Hz, 1H, Ar*H*), 7.65 (d,d, J = 8.7, 2.2 Hz, 1H, Ar*H*), 7.69 (s,1H, Ar*H*), 7.94 (s, 1H, Ar*H*), 7.95 (s, 1H, Ar*H*); MS m/z 559 (MNa^+). HRMS m/z Calcd for $C_{28}H_{26}BrO_6(MH^+)$: 537.0913. Found: 537.0963.

The filtrate was neutralized with HCl (10%). MeOH was removed, and water was added to the residue. The mixture was extracted with CH_2Cl_2. The organic phase was concentrated to give crude product. Flash chromatography (Hex: EtOAc, 8:1) gave (*Z*,*Z*)-1-bromo-2,5-bis-(3-methoxylcarbonyl-4-methoxy)styrylbenzene ***Z,Z*-3**, ***Z,E*-3**, and ***E,Z*-3**.

***Z,Z*-3** (385 mg, 24.0%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.80 (s, 3H, CH_3O-), 3.81 (s, 3H, CH_3O-), 3.83 (s, 3H, CH_3O-), 3.85 (s, 3H, CH_3O-), 6.42 (d, J = 12.2 Hz, 1H, olefinic), 6.51 (d, J = 12.2 Hz, 1H, olefinic), 6.52 (d, J = 12.1 Hz, 1H, olefinic), 6.57 (d, J = 12.0 Hz, 1H, olefinic), 6.75 (d, J = 8.7 Hz, 1H, Ar*H*), 6.80 (d, J = 8.7 Hz, 1H, Ar*H*), 6.94 (d,d, J = 8.1, 1.2 Hz, 1H, Ar*H*), 7.01 (d, J = 8.0 Hz, 1H, Ar*H*), 7.21 (d,d, J = 8.7, 2.3 Hz, 1H, Ar*H*), 7.31 (d,d, J = 8.6, 2.2 Hz, 1H, Ar*H*), 7.50 (d, J = 1.3 Hz, 1H, Ar*H*), 7.60 (d, J = 2.3 Hz, 1H, Ar*H*), 7.68 (d, J = 2.2 Hz, 1H, Ar*H*); MS m/z 554 ($M^+ + NH_4$).

***Z,E*-3** (204 mg, 12.7%)**:** ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.85 (s, 3H, CH_3O-), 3.89 (s, 3H, CH_3O-), 3.92 (s, 3H, CH_3O-), 3.93 (s, 3H, CH_3O-), 6.45 (d, J = 12.2 Hz, 1H, olefinic), 6.56 (d, J = 12.1 Hz, 1H, olefinic), 6.83 (d, J = 8.7 Hz, 1H, Ar*H*), 6.96 (d, J = 16.5 Hz, 1H, olefinic), 6.98 (d, J = 8.1 Hz, 1H, Ar*H*), 7.15 (d, J = 8.1 Hz, 1H, Ar*H*), 7.33 (d, J = 16.1 Hz, 1H, olefinic), 7.35 (d,d, J = 8.6, 2.3 Hz, 1H, Ar*H*), 7.48 (d, J = 8.1 Hz, 1H, Ar*H*), 7.49 (d, J = 1.1 Hz, 1H, Ar*H*), 7.64 (d,d, J = 8.9, 2.3 Hz, 1H, Ar*H*), 7.72 (d, J = 2.2 Hz, 1H, Ar*H*), 7.95 (d, J = 2.3 Hz, 1H, Ar*H*); MS m/z 554 ($M^+ + NH_4$).

***E,Z*-3** (176 mg, 11.0%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.83 (s, 3H, CH_3O-), 3.85 (s, 3H, CH_3O-), 3.92 (s, 3H, CH_3O-), 3.93 (s, 3H, CH_3O-), 6.56 (d, J = 11.9 Hz, 1H, olefinic), 6.62(d, J = 12.2 Hz, 1H, olefinic), 6.77 (d, J = 8.7 Hz, 1H, Ar*H*), 6.89 (d, J = 16.2 Hz, 1H, olefinic), 6.98 (d, J = 8.4 Hz, 1H, Ar*H*), 7.04 (d, J = 16.0 Hz, 1H, olefinic),7.15 (d, J = 8.1 Hz, 1H, Ar*H*), 7.22 (d,d, J = 8.5, 1.5 Hz, 1H, Ar*H*), 7.24 (d,d, J = 8.5, 2.4 Hz, 1H, Ar*H*), 7.58 (d,d, J = 8.7, 2.3 Hz, 1H, Ar*H*), 7.66 (d, J = 2.3 Hz, 1H, Ar*H*), 7.73 (s, 1H, Ar*H*), 7.95 (d, J = 2.3 Hz, 1H, Ar*H*); MS m/z 554 ($M^+ + NH_4$).

General Procedure for 1-Bromo-2,5-bis-(3-methoxycarbonyl-4-hydroxy)styrylbenzene (4). To a solution of ***E,E*-3** (730 mg, 1.36 mmol) in CH_2Cl_2 (250 mL) was added BBr_3 (10.5 mL, 1 M solution in hexane) dropwise at −78 °C in a dry ice−acetone bath. The mixture was allowed to warm to room temperature. Water was added while the reaction mixture was cooled at 0 °C in an ice bath. The mixture was extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated to afford 636 mg of ***E,E*-4** (92%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.99 (s, 6H, CH_3O-, −COOCH_3), 6.99 (d, J = 16.3 Hz, 1H, olefinic), 6.98 (d, J = 16.5 Hz, 1H, olefinic), 7.01 (d, d, J = 8.3, 2.6 Hz, 2H, Ar*H*), 7.04 (d, J = 16.3 Hz, 1H, olefinic), 7.33 (d, J = 16.5 Hz, 1H, olefinic), 7.40 (d, J = 8.7 Hz, 1H, Ar*H*), 7.60−7.73 (m, 4H, Ar*H*), 7.97 (s, 2H, Ar*H*), 10.81 (s, 1H, O*H*), 10.83 (s, 1H, O*H*); MS m/z 509 ($M^+ + H$).

***Z,Z*-4**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.82 (s, 3H, −COOCH_3), 3.83 (s, 3H, −COOCH_3), 6.36 (d, J = 12.1 Hz, 1H, olefinic), 6.45 (d, J = 12.1 Hz, 1H, olefinic), 6.46 (d, J = 12.1 Hz, 1H, olefinic), 6.51 (d, J = 12.1 Hz, 1H, olefinic), 6.68 (d, J = 8.7 Hz, 1H, Ar*H*), 6.74 (d, J = 8.7 Hz, 1H, Ar*H*), 6.90 (d, J = 8.1 Hz, 1H, Ar*H*), 6.97 (d, J = 8.1 Hz, 1H, Ar*H*), 7.14 (d, d, J = 8.7, 2.3 Hz, 1H, Ar*H*), 7.25 (d, d, J = 8.7, 2.3 Hz, 1H, Ar*H*), 7.46 (s, 1H, Ar*H*), 7.60 (d, J = 2.2 Hz, 1H, Ar*H*), 7.69 (d, J = 2.2 Hz, 1H, Ar*H*), 10.72 (s, 1H, O*H*), 10.74 (s, 1H, O*H*).

***Z,E*-4**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.98 (s, 3H, −COOCH_3), 3.99(s, 3H, −COOCH_3), 6.44 (d, J = 12.5 Hz, 1H, olefinic), 6.54-(d, J = 12.5 Hz, 1H, olefinic), 6.82−7.03 (m, 4H, Ar*H*), 7.16−7.50 (m, 3H, Ar*H*), 7.60−7.77 (m, 3H, Ar*H*), 7.95 (d, J = 2.2 Hz, 1H, Ar*H*), 10.77 (s, 1H, O*H*), 10.83 (s, 1H, O*H*).

***E, Z*-4**: ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.98 (s, 3H, −COOCH_3), 3.99(s, 3H, −COOCH_3), 6.57 (d, J = 12.5 Hz, 1H, olefinic), 6.59 (d, J = 12.5 Hz, 1H, olefinic), 6.80−7.03 (m, 5H, Ar*H*), 7.16−7.62 (m, 2H, Ar*H*), 7.60−7.74 (m, 3H, Ar*H*), 7.96 (d, J = 2.2 Hz, 1H, Ar*H*), 10.73 (s, 1H, O*H*), 10.80 (s, 1H, O*H*).

General Procedure for 1-Bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (5). To a solution of ***E,E*-4** (534 mg, 1.0 mmol) in EtOH (60 mL) was added KOH (1.2 g) in solid form. The mixture was stirred under reflux for 5 h. The cold mixture was acidified with HCl (10% solution) and extracted with mixed solvent (CH_2Cl_2:MeOH, 9:1). The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated to give 480 mg of ***E,E*-5** (95%): ^{1}H NMR (200 MHz, MeOD) δ 6.96 (d,d, J = 3.4, 8.7 Hz, 2H, Ar*H*), 6.99 (d, J = 16.3 Hz, 1H, olefinic), 7.12 (d, J = 16.3 Hz, 1H, olefinic), 7.17 (d, J = 16.3 Hz, 1H, olefinic), 7.34 (d, J = 16.3 Hz, 1H, olefinic), 7.56 (d, J = 8.4 Hz, 1H, Ar*H*), 7.73−7.80 (, m, 4H Ar*H*), 8.02 (t, J = 2.6 Hz, 2H, Ar*H*); MS m/z 480 (M^+). Anal. Calcd for $C_{24}H_{17}BrO_6 \cdot 0.5\ H_2O$: C, 58.79; H, 3.70. Found: C, 58.89, H, 3.34.

***Z,Z*-5**: ^{1}H NMR (200 MHz, MeOD) δ 6.48 (d, J = 12.1 Hz, 1H, olefinic), 6.49 (d, J = 12.1 Hz, 1H, olefinic), 6.58 (d, J = 12.1 Hz, 1H, olefinic), 6.61 (d, J = 12.1 Hz, 1H, olefinic), 6.73 (d, J = 8.6 Hz, 1H, Ar*H*), 6.77 (d, J = 8.6 Hz, 1H, Ar*H*), 7.01 (d, J = 8.1 Hz, 1H, Ar*H*), 7.06 (d, J = 8.1 Hz, 1H, Ar*H*), 7.23 (d, d, J = 8.6, 2.3 Hz, 1H, Ar*H*), 7.33 (d, d, J = 8.6, 2.3 Hz, 1H, Ar*H*), 7.51 (s, 1H, Ar*H*), 7.68 (d, J = 2.3 Hz, 1H, Ar*H*), 7.78 (d, J = 2.3 Hz, 1H, Ar*H*). HRMS m/z Calcd for $C_{24}H_{17}BrO_6$ (MH^+): 480.0208. Found: 480.0201.

***Z,E*-5**: ^{1}H NMR (200 MHz, MeOD) δ 6.48 (d, J = 12.1 Hz, 1H, olefinic), 6.54 (d, J = 12.1 Hz, 1H, olefinic), 6.93−7.38 (m, 4H, Ar*H*), 7.47−7.79 (m, 5H, Ar*H*), 8.01 (s, 2H, Ar*H*).

***E,Z*-5**: ^{1}H NMR (200 MHz, MeOD): δ 6.52 (d, J = 12.1 Hz, 1H, olefinic), 6.63 (d, J = 12.1 Hz, 1H, olefinic), 6.91−7.03 (m, 3H, Ar*H*), 7.08−7.37 (m, 3H, Ar*H*), 7.58−7.18 (m, 3H, Ar*H*), 8.0 (s, 2H, Ar*H*).

Binding Assays Using Aggregated Peptide Fibrils in Solution. A solid form of peptide Aβ_{1-40} was purchased from Bachem (King of Prussia, PA). Aggregation of peptides was carried out by gently dissolving the peptide (0.5 mg/mL) in a buffer solution (pH 7.4) containing 10 mM sodium phosphate and 1 mM EDTA. The solutions were incubated at 37 °C for 36−42 h with gentle and constant shaking. Binding studies were carried out in 12 × 75 mm borosilicate glass tubes according to the procedure described[10] with some modifications. For inhibition studies, 1 mL of the reaction mixture contained 40 μL of inhibitors (10^{-5}−10^{-10} M in 10% EtOH) and 0.05 nM radiotracer in 40% EtOH. Nonspecific binding was defined in the presence of 800 nM of CG. The mixture was incubated at room temperature for 3 h, and the bound and the free radioactivity were separated by vacuum filtration through Whatman GF/B filters using a Brandel M-24R cell harvester followed by 2 × 3 mL washes of 10% ethanol at room temperature. Filters containing the bound I-125 ligand were counted in a γ counter (Packard 5000) with 70% counting efficiency. The results of inhibition experiments were subjected

to nonlinear regression analysis using software EBDA [20] by which K_i values were calculated.

Fluorescent Staining. Brains of Alzheimer's disease (AD) patients ($n = 3$) were obtained at autopsy, and neuropathological diagnosis was confirmed by current criteria. We performed a variety of studies using 6 μm thick serial sections of paraffin-embedded blocks of hippocampus and cortex from brains fixed in 70% ethanol (EtOH)/150 mM NaCl. For BSB staining, sections were immersed in 0.05 mM of ***E,E*-5** (BSB), ***E,Z*-5**, or ***Z,Z*-5**, in 50% EtOH for 30 min. Sections were quickly differentiated in saturated Li_2CO_3 and rinsed in 50% EtOH before examination by fluorescent microscopy. For staining with Thioflavin-S (TF-S), serial sections of brain tissue were immersed in 10% neutral buffered formalin (NBF) for 1 h, followed by bleaching of lipofuscin in 0.05% $KMnO_4$ and destaining in 0.2% $K_2S_2O_5$/0.2% oxalic acid prior to staining with TF-S for 3 min (0.0125% 3 TF-S in 40% EtOH). Sections were quickly differentiated in 50% EtOH prior to examination by fluorescent microscopy with a UV filter set (wide excitation filter at 360 nm and long pass emission filter at 420 nm). Data for ***Z,E*-5** are not shown in Figure 2; it stains the brain section as well as the other three isomers at 0.05 mM.

Isomerization of *Z,Z*-5 to *E,E*-5 at Different Temperatures. Variable time NMR spectra of ***Z,Z*-5** at 70, 95, and 115 °C were measured in order to determine the activation energy for the isomerization (Figures 2 and 3). An NMR tube, containing 11 mg of ***Z,Z*-5**, which was dissolved in 0.1 mL of 1.4 M NaOD in D_2O, followed by adding D_2O to bring final volume to 0.6 mL (0.23 M), was placed in a heating block filled with sand. The temperature was maintained constant throughout the experiment. Separate NMR samples were prepared for each temperature. At various time points, the NMR spectra were acquired, the integral of each isomer was calculated, and the data were plotted (Figure 3a,b).

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