Published in final edited form as:

J Med Chem. 2010 November 25; 53(22): 7992–7999. doi:10.1021/jm1006929.

Phenolic Bis-Styrylbenzenes as β -Amyloid Binding Ligands and Free Radical Scavengers

Daniel P. Flaherty[†], Tomomi Kiyota[‡], Yuxiang Dong[†], Tsuneya Ikezu[‡], and Jonathan L. Vennerstrom^{†,*}

[†] University of Nebraska Medical Center, College of Pharmacy, 986025 Nebraska Medical Center, Omaha, NE, 68198

[‡] University of Nebraska Medical Center, College of Medicine, 985880 Nebraska Medical Center, Omaha, NE, 68198

Abstract

Starting from bisphenolic bis-styrylbenzene DF-9 (**4**), β -amyloid ($A\beta$) binding affinity and specificity for phenolic bis-styrylbenzenes, mono-styrylbenzenes and alkyne controls were determined by fluorescence titration with β -amyloid peptide $A\beta_{1-40}$ and a fluorescence assay using APP/PS1 transgenic mouse brain sections. Bis-styrylbenzene SAR is derived largely from work on symmetrical compounds; this study is the first to describe $A\beta$ binding data for bis-styrylbenzenes unsymmetrical in the outer rings. With one exception, binding affinity and specificity were decreased by adding and/or changing the substitution pattern of phenol functional groups, changes to the orientation about the central phenyl ring, replacing the alkene with alkyne bonds, or elimination of the central phenyl ring. The only compound with an $A\beta$ binding affinity and specificity comparable to **4** was its 3-hydroxy regioisomer **8**. Like **4**, **8** crossed the blood-brain-barrier and bound to $A\beta$ plaques in vivo. Using a DPPH assay, phenol functional groups with *para* orientations seem to be a necessary, but insufficient, criterion for good free radical scavenging properties in these compounds.

Alzheimer's disease (AD)¹ is a common neurodegenerative disorder in the elderly² characterized by an accumulation of β -amyloid ($A\beta$) plaques in the brain parenchyma and neurofibrillary tangles (NFTs) in the neuron.^{3,4} Although $A\beta$ and NFTs are commonly observed in nondemented elderly, 80 years and older, the molecular pathogenesis of AD is believed to be effected by $A\beta$ production and its clearance.^{5,6} The amyloid cascade hypothesis⁷ for AD pathogenesis proposes that accumulation and aggregation of $A\beta$ triggers a cascade that leads to the characteristic pathologies of AD. Evidence in support of this hypothesis is obtained from studies of familial AD, which reveal that $A\beta$ accumulation and aggregation are elevated by mutations of the APP, PS1 and PS2 genes.⁸

The discovery of the diazo dye Congo red as an $A\beta$ binding ligand⁹ provided a starting point in the search for potential in vivo imaging agents using positron emission tomography (PET), single photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI). Investigation of Congo red led to the discovery of bis-styrylbenzenes as a class of compounds with strong $A\beta$ plaque binding properties. These compounds are exemplified by the salicylate bis-styrylbenzenes X-34 (**1**)¹⁰ and FSB (**2**),¹¹ which have been

tel: 402.559.5362, fax: 402.559.9543, jvenners@unmc.edu.

Supporting Information Available. Elemental analysis data for **4a**, **6a–10a**, **12a–15a**, **6–16** and **29**. Synthetic procedures for **16**,²⁵ **17**,²⁶ **19b**,³⁷ and **19c**,³⁷ **20a–20e**,^{22,32,38–40} **22b**,²² and **22c**,⁴¹ **24**,⁴² **25**,⁴² **28**.⁴³

shown to penetrate the blood – brain barrier (BBB) and bind to A β plaques deposited in APP or APP/PS1 mice.¹¹ As shown by K114 (**3**) and DF-9 (**4**), acidic functional groups are not necessary for good A β binding.^{12,13} Several stilbene-based compounds, such as SB-13 and BAY94-9172, have been studied for early detection in AD.^{14,15} While the structure-activity relationship (SAR) of bis-styrylbenzene compounds has been extensively investigated, the common thread in these studies is that A β -binding SAR has been elucidated based largely on symmetrical bis-styrylbenzenes.

Design

Previously, we showed that the bis-styrylbenzene, (*E,E*)-1,4-*bis*(4-hydroxystyryl) benzene (**4**), binds to A β with high affinity and specificity.¹³ In recent years, many^{16,17} have recognized the antioxidant and potential anti-AD properties of the polyphenolic styrylbenzene (stilbene) resveratrol (**5**), a well-known component of red wine. Therefore, we also included structural elements of **5** in the design of target compounds (Figure 1), especially since oxidative stress is considered to play an important role in AD pathology.¹⁸

Little has been studied regarding substitution patterns in the central benzene ring of A β -binding bis-styrylbenzenes. For this purpose we designed target compounds **6** and **7** with a 1,3 and 1,2 orientation about the central benzene ring as opposed to the more extended 1,4 orientation in **4** and other reported bis-styrylbenzenes.^{11,12} Target compounds **8** and **9** are the *meta* and *ortho* bis-phenolic regioisomers of **4**. Tetraphenol **10** is a symmetrical bis-styrylbenzene that combines the resorcinol substructures of **5**. Unsymmetrical target compounds **11–15** contain from one to three phenol functional groups, but each maintains at least one of the *para* phenols of prototype **4**. The final target compounds are the mono-styryl (**16**) and bis-alkyne (**17**) derivatives of **4**. A secondary objective in designing these unsymmetrical bis-styrylbenzenes was an attempt to increase the solubility of this class of compounds as we have observed that symmetrical bis-styrylbenzenes have poor aqueous solubilities. For each target compound, we determined A β -binding affinity and specificity and antioxidant properties in addition to BBB penetration for selected compounds. We suggest that these data will be useful in the design and discovery of new early diagnosis probes for AD using PET, SPECT or MRI and potentially for AD therapeutics.

Chemistry

Compounds **4**¹³ and **6–10** (Scheme 1) were obtained using a previously reported procedure.¹³ The preparation for **6** was an improvement on the previously published procedure¹⁹ for this compound in which a low-yielding (20%) double-Heck coupling was used. The overall yields of **8** (69%) and **10** (42%) were better than those (49 and 28%) previously reported^{20,21} for these compounds. 1,3- and 1,2-*Bis*(diethylphosphonylmethyl)benzenes **19b** (96%) and **19c** (93%) were synthesized using the Arbuzov reaction with *o*- and *m*-xylylene dibromide. *Bis*-diethylphosphonates **19a–19c** were coupled with methoxymethoxy (MOM)-substituted benzaldehydes **20a–20d** to afford **4a** and **6a–10a** in 48–84% yields, which were then deprotected to afford **4** and **6–10** in 64–99% yields.

Compounds **11–15** (Scheme 2) were synthesized via successive Heck and Horner–Wadsworth–Emmons reactions using a new one-pot procedure.²² Heck couplings between 4-iodobenzyl phosphonate (**21**) and styrenes **22a–22c** were accomplished with palladium(II) acetate, phenylurea and potassium carbonate in DMF at 110 °C for 22 h.²² The reaction mixtures were cooled to 80 °C followed by addition of benzaldehydes **20a**, **20b**, **20d** or **20e**

and 30% w/v sodium methoxide in MeOH and then stirred for an additional 1 h to afford **11a–15a** in 52–79% yield. HCl deprotection of **11a–15a** afforded **11–15** in 70 – 94% yield after filtration and washing. Although **11** was previously synthesized in 31% overall yield in a five-step procedure,²⁴ we were able to synthesize this same compound in 73% overall yield using this new one-pot method. Compounds **16** and **17** were synthesized by modified literature^{25,26} procedures (see Supporting Information).

β -Amyloid ($A\beta$) Binding Affinity and Specificity

β -Amyloid ($A\beta$) binding affinity and specificity for bis-styrylbenzenes **6–17**, with **4** and **5** as controls, were determined by means of fluorescent titrations²⁷ with aggregated amyloid peptide $A\beta_{1-40}$ and an *in vitro* fluorescence-based assay using APP/PS1 transgenic mouse brain sections.¹³ The APP/PS1 transgenic mice were developed by crossing the Tg2576 strain,²⁸ which express the K670N/M671L mutant of APP695 found as the Swedish familial AD gene, and the M146L 6.1 strain,²⁹ which express the M146L mutant of PS1 found as the early onset familial AD gene. The specificity is determined by measuring the fluorescent intensity of $A\beta$ plaques (specific signal) compared to that of background normal brain tissue (noise) (Figure 2).

The only compound with an $A\beta$ binding affinity and specificity comparable to that of **4** was its 3-hydroxy regioisomer **8**, which had a measured K_d of 5.3 ± 0.3 nM and a specificity of 9.6 ± 3.0 (Table 1). In contrast, **9**, the 2-hydroxy regioisomer of **4**, had an 84-fold weaker $A\beta$ binding affinity, although it had good binding specificity. Apparently, the 1,4 orientation about the central benzene ring in compounds **4**, **8**, and **9** endows them with the extended geometry required to maintain strong binding interactions in the hydrophobic binding site(s) of the $A\beta$ fibril, but only **4** and **8** have the required phenol functional group orientations to potentially form strong H-bonding interactions. However, **12**, the unsymmetrical regioisomeric hybrid of **4** and **8**, had an unexpectedly much weaker $A\beta$ binding affinity (370 and 660-fold) compared to that of **4** and **8**.

The two order of magnitude decrease in $A\beta$ binding affinity for **6** and **7** show that the optimal geometry about the central benzene ring is a 1,4 orientation as opposed to 1,3 (**6**) or 1,2 (**7**). Even though the anti-aggregation properties of 1,3-bis-styrylpyridines and 1,3-bis-styrylbenzenes³⁰ and $A\beta$ -binding properties of 1,3-bis-styrylquinaldines³¹ have been investigated, this is the first time that $A\beta$ -binding affinity and specificity has been assessed as a function of the central ring substitution pattern of bis-styrylbenzenes.

As evidenced by the 17 to 27-fold loss in $A\beta$ binding affinity for the more polar tetraphenol **10** and triphenols **13** and **14**, additional phenol functional groups decreased affinity and tended to decrease specificity. Compared to bis-phenol **4**, mono-phenol **11** showed a 16-fold reduction in binding affinity, due most likely to the loss of a H-bond formed by the second phenol functional group in **4**. Compared to **4**, the more rigid linear alkyne analog **17** had a 6-fold loss in $A\beta$ binding affinity and a 2.4-fold loss in specificity, due possibly to an inability to attain a conformer orientation complementary to the $A\beta$ fibril surface. Of all of the target compounds, the symmetrical mono-styrylbenzene **16** had the weakest $A\beta$ binding affinity – three orders of magnitude weaker than that of **4**. This could be attributed either to the loss of the central benzene ring eliminating a critical binding interaction, or to the shorter distance between phenol functional groups, reducing the effectiveness of **16** to form strong H-bonds with the $A\beta$ fibril surface, or a combination of the two. Surprisingly, mono-styrylbenzene **5** displayed only a 52-fold loss in binding affinity compared to **4**. Conceivably, the 3,5-dihydroxy substituted **5**, but not the 4-hydroxy substituted **16**, provides conformers with the geometry required to form strong H-bonds to the aggregated $A\beta$ fibril.

Human AD Brain Section Staining

Bis-styrylbenzenes **8** and **10**, along with the control bis-styrylbenzene **4**, were used to stain human AD brain sections (Figure 2). Each compound was shown to bind specifically to A β plaques in human brain sections. Age matched controls were also stained and showed no staining of normal brain tissue (data not shown).

In Vivo BBB Delivery in Plaque-Bearing Mice

We next determined whether interperitoneally administered **8** and **10** (with **4** as a control) could cross the BBB and bind to A β plaques. Bisstyrylbenzenes **4**, **8** and **10** (50 mg/kg) were injected into ten-month-old plate-derived growth factor- β chain promoter driving APP (PDAPP) transgenic mice. As shown in Figure 3, all compounds crossed the BBB and bound to A β plaques in vivo. Each section showed intense A β plaque signals with high specificities. Control non-transgenic animals showed no specific signals in the brain for any of the tested compounds after intraperitoneal injection (data not shown). These data confirm that these bis-styrylbenzene derivatives retain the BBB penetration capabilities of **4**, and that **8** and **10** have the potential as lead compounds for early A β detection research.

Free Radical Scavenging Activity

The relative free radical scavenging properties of the target bis-styrylbenzenes, along with quercetin as a control, were measured using the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) following the procedure of Yang et al.³² with some modifications (Table 2). Compound **4**, **6**, **12**, and **16** were no less effective than quercetin ($IC_{50} = 1.3 \pm 1.1 \mu M$) in DPPH free radical scavenging activity, whereas compounds **7**, **9**, **10**, **14** and **15** were 3 to 7-fold less potent ($p < 0.05$) than quercetin. Aside from **8** and **13**, which had no antioxidant activity ($IC_{50s} > 100 \mu M$) in this assay, mono-styrylbenzene **5** and bis-alkyne **17** were the least effective radical scavengers with IC_{50s} of 62 and 45 μM , respectively. No particular SAR is apparent from these data aside from the general observation that a phenol functional group with a *para* orientation seem to be a necessary, but insufficient, criterion for good free radical scavenging properties of bis-styrylbenzenes. With polyphenolic antioxidants such as **5**¹⁷ of increasing interest in AD research, this set of compounds may prove beneficial in future research.

Summary

These data extend the SAR of bis-styrylbenzene A β binding and free-radical scavenging activity and provide further direction for the development of non-invasive A β binding ligands for early detection of AD. One new bis-styrylbenzene prototype (**8**) was identified that could serve as a building block for the design and discovery of new early diagnosis probes for AD using PET, SPECT or MRI and potentially for AD therapeutics. Finally, we observed that a phenol functional group with a *para* orientation seem to be a necessary, but insufficient, criterion for good free radical scavenging properties in these bis-styrylbenzenes.

Experimental Section

General

Starting materials were purchased from Aldrich, TCI or Acros. All reactions were run under a positive pressure of Ar. Melting points were determined on a Stanford Research Systems E-Z Melt apparatus and are uncorrected. ¹H (500 MHz) and ¹³C (125.7 MHz) NMR spectra were measured on a Varian spectrometer using CDCl₃ and DMSO-*d*₆ as solvents. All chemical shifts are reported in parts per million (ppm) and are relative to internal (CH₃)₄Si for ¹H, CDCl₃ (77.0 ppm) and DMSO-*d*₆ (39.7 ppm) for ¹³C NMR. Elemental analyses

were performed by M-H-W Laboratories, Phoenix, AZ. All target compounds had purities $\geq$ 95%.

(*E,E*)-1,4-bis(4-hydroxystyryl)benzene (**4**)¹³

Step 1. A 30 wt % solution of sodium methoxide in methanol (1.39 mL, 7.40 mmol) was added to a stirred mixture of **20a** (0.983 g, 5.92 mmol) and 1,4-bis(diethylphosphorylmethyl)benzene (**19a**) (1.12 g, 2.96 mmol) in DMF (15 mL) at rt. This mixture was heated to 80 °C for 2 h. The reaction was then quenched with H₂O (10 mL) and the solid precipitate was filtered and rinsed with ether to afford (*E,E*)-1,4-bis-(4-methoxymethoxy)styrylbenzene **4a** (0.858 g, 72%). ¹H NMR (CDCl₃) δ 3.50 (s, 6H), 5.20 (s, 4H), 6.98 (d, J = 16.1 Hz, 2H), 7.04 (d, J = 8.8 Hz, 4H), 7.08 (d, J = 16.1, 2H), 7.45 (d, J = 8.3 Hz, 4H), 7.48 (s, 4H). ¹³C NMR (CDCl₃) δ 56.0, 94.4, 116.4, 126.6, 126.7, 127.7, 127.8, 131.3, 136.6, 156.8. **Step 2.** **4a** (0.490 g, 1.22 mmol) and 4:1 CHCl₃:MeOH (25 mL) were stirred at rt until **4a** was completely dissolved before dropwise addition of 12.0 M HCl (0.812 mL, 9.74 mmol) at rt. The reaction was quenched H₂O (20 mL) after 24 hr. The CHCl₃:MeOH layer was removed in vacuo to afford **4** as a white solid (0.379 g, 99%): mp dec. 312 °C. ¹H NMR (DMSO-*d*₆) δ 6.77 (d, J = 8.3 Hz, 4H), 7.00 (d, J = 16.6 Hz, 2H), 7.15 (d, J = 16.6 Hz, 2H), 7.43 (d, J = 8.8 Hz, 4H), 7.52 (s, 4H), 9.57 (s, 2H). ¹³C NMR (DMSO-*d*₆) δ 115.8, 125.0, 126.5, 128.0, 128.2, 128.3, 136.5, 157.6.

(*E,E*)-1,3-bis(4-hydroxystyryl)benzene (**6**)¹⁸

Step 1. As described above for **4a**, **20a** (0.677 g, 4.07 mmol) and **19b** (0.773 g, 2.04 mmol) yielded **6a** (0.660 g, 80%): mp 123 – 126 °C. ¹H NMR (DMSO-*d*₆) δ 3.39 (s, 6H), 5.22 (s, 4H), 7.05 (d, J = 8.8 Hz, 4H), 7.14 (d, J = 16.1 Hz, 2H), 7.28 (d, J = 16.6, 2H), 7.36 (t, J = 7.3 Hz, 1H), 7.45 (d, J = 8.3 Hz, 2H), 7.56 (d, J = 8.8 Hz, 4H), 7.80 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ 55.8, 94.0, 116.6, 124.0, 125.6, 126.7, 127.9, 128.4, 129.2, 130.9, 137.8, 156.7. **Step 2.** As described above for **4**, **6a** (0.406 g, 1.01 mmol) was deprotected using 12.0 M HCl (0.672 mL, 8.07 mmol) to yield **6** (0.309 g, 97%): mp dec. 218 – 220 °C (lit.¹⁸ mp 232 – 234 °C). ¹H NMR (DMSO-*d*₆) δ 6.78 (d, J = 8.8 Hz, 4H), 7.03 (d, J = 16.1 Hz, 2H), 7.21 (d, J = 16.1 Hz, 2H), 7.32 (t, J = 6.8 Hz, 1H), 7.40 (d, J = 7.3 Hz, 2H), 7.44 (d, J = 8.3 Hz, 4H), 7.72 (s, 1H), 9.59 (s, 2H). ¹³C NMR (DMSO-*d*₆) δ 116.0, 124.0, 125.2, 125.4, 128.23, 128.5, 129.0, 129.3, 138.3, 157.8. Anal. (C₂₂H₁₈O₂·1/3 H₂O) C, H.

(*E,E*)-1,2-bis(4-hydroxystyryl)benzene (**7**)

Step 1. As described above for **4a**, **20a** (0.310 g, 1.87 mmol) and **19c** (0.353 g, 0.993 mmol) yielded **7a** (0.169 g, 45%): mp 67 – 70 °C. ¹H NMR (CDCl₃) δ 3.50 (s, 6H), 5.20 (s, 4H), 6.95 (d, J = 16.1 Hz, 2H), 7.04 (d, J = 8.8 Hz, 4H), 7.26 (dd, J_1 = 5.8 Hz, J_2 = 3.4 Hz, 2H), 7.33 (d, J = 16.1 Hz, 2H), 7.46 (d, J = 8.8 Hz, 4H), 7.55 (dd, J_1 = 5.9 Hz, J_2 = 3.4 Hz, 2H). ¹³C NMR (CDCl₃) δ 55.0, 94.3, 116.4, 125.0, 126.5, 127.4, 127.8, 130.6, 131.5, 136.0, 156.9. **Step 2.** As described above for **4**, **7a** (0.151 g, 0.375 mmol) was deprotected using 12.0 M HCl (0.250 mL, 3.00 mmol) to yield **7** (0.108 g, 92%): mp 262 – 265 °C. ¹H NMR (DMSO-*d*₆) δ 6.78 (d, J = 7.8 Hz, 4H), 7.00 (d, J = 16.1 Hz, 2H), 7.25 (brs, 2H), 7.42 (d, J = 16.1 Hz, 2H), 7.50 (d, J = 7.8 Hz, 4H), 7.62 (brs, 2H), 9.58 (s, 2H). ¹³C NMR (DMSO-*d*₆) δ 115.6, 122.7, 126.0, 127.4, 128.4, 128.5, 130.8, 135.7, 157.6. Anal. (C₂₂H₁₈O₂) C, H.

(*E,E*)-1,4-bis(3-hydroxystyryl)benzene (**8**)¹⁹

Step 1. As described above for **4a**, **20b** (0.388 g, 2.33 mmol) and **19a** (0.442 g, 1.17 mmol) yielded **8a** (0.359 g, 76%): mp 136 – 140 °C. ¹H NMR (DMSO-*d*₆) δ 3.41 (s, 6H), 5.24 (s, 4H), 6.92 – 6.95 (m, 2H), 7.24 – 7.28 (m, 8H), 7.31 (t, J = 7.8 Hz, 2H), 7.62 (s, 4H). ¹³C NMR (DMSO-*d*₆) δ 56.0, 94.4, 114.0, 115.6, 120.3, 126.9, 128.3, 128.6, 129.7, 136.6, 138.8, 157.6. **Step 2.** As described above for **4**, **8a** (0.136 g, 0.338 mmol) was deprotected

using 12.0 M HCl (0.230 mL, 2.70 mmol) to yield **8** (0.097 g, 91%): mp 281 – 283 °C (lit.¹⁹ mp 240 °C). ¹H NMR (DMSO-*d*₆) δ 6.69 (dd, *J* = 7.8 Hz, 2.0 Hz, 2H), 6.98 (brs, 2H), 7.04 (d, *J* = 7.8 Hz, 2H), 7.12 – 7.22 (m, 6H), 7.60 (s, 4H). ¹³C NMR (DMSO-*d*₆) δ 113.2, 115.1, 117.8, 127.1, 128.0, 128.7, 129.8, 136.5, 138.6, 157.8. Anal. (C₂₂H₁₈O₂) C, H.

(*E,E*)-1,4-bis(2-hydroxystyryl)benzene (9**).³³**

Step 1. As described above for **4a**, **20c** (1.05 g, 6.31 mmol) and **19a** (1.19 g, 3.17 mmol) yielded **9a** (1.07 g, 84%): ¹H NMR (CDCl₃) δ 3.53 (s, 6H), 5.26 (s, 4H), 7.03 (t, *J* = 7.3 Hz, 2H), 7.11 (d, *J* = 16.6 Hz, 2H), 7.13 (d, *J* = 7.8 Hz, 2H), 7.22 (td, *J* = 8.3 Hz, 1.5 Hz, 2H), 7.52 (d, *J* = 15.1 Hz, 2H), 7.53 (s, 4H), 7.63 (dd, *J* = 7.8 Hz, 1.5 Hz, 2H). ¹³C NMR (CDCl₃) δ 56.2, 94.8, 114.9, 122.1, 123.1, 126.2, 126.8, 127.3, 128.6, 128.9, 137.1, 154.6.

Step 2. As described above for **4**, **9a** (0.634 g, 1.58 mmol) was deprotected using 12.0 M HCl (0.230 mL, 2.70 mmol) to yield **9** (0.316 g, 64%): mp 262 – 265 °C (lit.³³ mp 230 – 231 °C). ¹H NMR (DMSO-*d*₆) δ 6.82 (t, *J* = 7.8 Hz, 2H), 6.88 (d, *J* = 7.8 Hz, 2H), 7.10 (t, *J* = 7.8 Hz, 2H), 7.21 (d, *J* = 16.1 Hz, 2H), 7.43 (d, *J* = 16.6 Hz, 2H), 7.55 (s, 4H), 7.59 (d, *J* = 7.8 Hz, 2H), 9.75 (s, 2H). ¹³C NMR (DMSO-*d*₆) δ 116.0, 119.5, 123.5, 124.0, 126.6, 126.8, 127.6, 128.8, 136.9, 155.2. Anal. (C₂₂H₁₈O₂) C, H.

(*E,E*)-1,4-bis(3,5-dihydroxystyryl)benzene (10**).²¹**

Step 1. As described above for **4a**, **20d** (0.173 g, 0.765 mmol) and **19a** (0.145 g, 0.382 mmol) yielded **10a** (0.096 g, 48%): mp 101 – 106 °C. ¹H NMR (CDCl₃) δ 3.51 (s, 12H), 5.20 (s, 8H), 6.66 (t, *J* = 2.4 Hz, 2H), 6.88 (d, *J* = 2.4 Hz, 4H), 7.04 (d, *J* = 16.1 Hz, 2H), 7.09 (d, *J* = 16.1 Hz, 2H), 7.50 (s, 4H). ¹³C NMR (CDCl₃) δ 56.1, 94.5, 104.3, 107.8, 126.9, 128.3, 128.9, 136.5, 139.5, 158.5. **Step 2.** As described above for **4**, **10a** (0.084 g, 0.16 mmol) was deprotected using 12.0 M HCl (0.214 mL, 2.57 mmol) to yield **10** (0.049 g, 88%) mp 290 – 305 °C (lit.²¹ mp >300 °C). ¹H NMR (DMSO-*d*₆) δ 6.16 (s, 2H), 6.45 (s, 4H), 7.04 (d, *J* = 16.1 Hz, 2H), 7.09 (d, *J* = 16.1 Hz, 2H), 7.57 (s, 4H). ¹³C NMR (DMSO-*d*₆) δ 104.9, 127.0, 127.7, 129.0, 136.5, 139.0, 158.7.

(*E,E*)-1-styryl-4-(4-hydroxystyryl)benzene (11**).²²**

As described above for **4**, **11a** (0.654 g, 1.91 mmol) was deprotected using 12.0 M HCl (1.05 mL, 12.6 mmol) to afford **11** (0.53 g, 93%): mp 281 – 285 °C. ¹H NMR (DMSO-*d*₆) δ 6.78 (d, *J* = 8.3 Hz, 2H), 7.03 (d, *J* = 16.1 Hz, 2H), 7.18 (d, *J* = 16.6 Hz, 2H), 7.26 (s, 2H), 7.27 (t, *J* = 7.3 Hz, 1H), 7.38 (t, *J* = 7.8 Hz, 2H), 7.44 (d, *J* = 8.3 Hz, 2H), 7.55 (d, *J* = 8.3 Hz, 2H), 7.59 (d, *J* = 8.3 Hz, 2H), 7.61 (d, *J* = 7.9 Hz, 2H), 9.59 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ 115.8, 124.9, 126.5, 126.6, 127.0, 128.1, 128.3, 128.7, 128.9, 131.8, 131.9, 135.9, 137.2, 137.3, 157.6, 167.2.

(*E,E*)-1-styryl-4-[4-(methoxymethoxy)styryl]benzene (11a**).²²**

Diethyl 4-iodobenzyl phosphonate (**21**) (0.414 g, 1.17 mmol) and **22a** (0.260 g, 2.34 mmol) were mixed with palladium (II) acetate (0.008 g, 0.035 mmol), phenylurea (0.010 g, 0.070 mmol) and potassium carbonate (0.323 g, 2.34 mmol) in DMF (5 mL) and stirred at 110 °C for 22 h. The reaction temperature was then cooled to 80 °C and to this mixture were added **20a** (0.195 g, 1.17 mmol) and 30% w/v sodium methoxide in methanol (0.440 mL, 2.34 mmol). The reaction was stirred for 1 h at 80 °C and then cooled to rt and quenched with H₂O (15 mL). The solid was then filtered and rinsed with H₂O and ether to afford **11a** (0.317 g, 79%): m.p. 240 – 243 °C. ¹H NMR (CDCl₃) δ 3.50 (s, 3H), 5.20 (s, 2H), 6.99 (d, *J* = 16.6 Hz, 1H), 7.04 (d, *J* = 8.3 Hz, 2H), 7.09 (d, *J* = 16.6 Hz, 1H), 7.12 (s, 2H), 7.26 (t, *J* = 7.3 Hz, 1H), 7.36 (t, *J* = 7.3 Hz, 2H), 7.46 (d, *J* = 8.3 Hz, 2H), 7.48 (d, *J* = 7.8 Hz, 2H), 7.49 (s, 4H), 7.52 (d, *J* = 7.8 Hz, 2H). ¹³C NMR (CDCl₃) δ 56.0, 94.4, 116.4, 126.5, 126.6, 126.7, 126.8, 127.6, 127.7, 128.0, 128.3, 128.4, 128.7, 131.3, 136.3, 136.9, 137.3, 156.9.

(*E,E*)-1-(4-hydroxystyryl)-4-(3-hydroxystyryl)benzene (12)

As described above for **4**, **12a** (0.280 g, 0.696 mmol) was deprotected using 12.0 M HCl (0.696 mL, 8.35 mmol) to afford **12** (0.161 g, 74%): mp 297 – 301 °C. ¹H NMR (DMSO-*d*₆) δ 6.68 (d, *J* = 8.3 Hz, 1H), 6.78 (d, *J* = 8.8 Hz, 2H), 6.98 (s, 1H), 7.02 (d, *J* = 16.6 Hz, 1H), 7.04 (d, *J* = 7.3 Hz, 1H), 7.12 – 7.22 (m, 4H), 7.44 (d, *J* = 8.8 Hz, 2H), 7.54 (d, *J* = 8.3 Hz, 2H), 7.58 (d, *J* = 8.8 Hz, 2H). ¹³C NMR (DMSO-*d*₆) δ 113.2, 115.0, 115.8, 117.7, 125.0, 126.6, 127.1, 128.1, 128.3, 128.6, 129.8, 135.9, 137.1, 138.7, 157.6, 157.8.

(*E,E*)-1-[4-(methoxymethoxy)styryl]-4-[3-(methoxymethoxy)styryl]benzene (12a)

As described above for **11a**, **21** (0.312 g, 0.880 mmol), **22b** (0.289 g, 1.76 mmol) were mixed with palladium (II) acetate (0.006 g, 0.03 mmol), phenylurea (0.007 g, 0.05 mmol) and potassium carbonate (0.243 g, 1.76 mmol) and stirred for 22 h at 110 °C in DMF. After cooling to 80 °C, compound **20b** (0.146 g, 0.880 mmol) and sodium methoxide solution (0.330 mL, 1.76 mmol) were added and the reaction was stirred for 1 h at 80 °C and worked up as described above to yield **12a** (0.23 g, 65%): mp 142 – 152 °C. ¹H NMR (CDCl₃) δ 3.50 (s, 3H), 3.51 (s, 3H), 5.20 (s, 2H), 5.22 (s, 2H), 6.95 (dd, *J*₁ = 7.8 Hz, *J*₂ = 2.0 Hz, 1H), 6.99 (d, *J* = 16.6 Hz, 1H), 7.04 (d, *J* = 8.3 Hz, 2H), 7.08 (d, *J* = 16.1 Hz, 1H), 7.09 (s, 2H), 7.18 (d, *J* = 7.3 Hz, 1H), 7.21 (s, 1H), 7.28 (t, *J* = 8.3 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 2H), 7.50 (s, 4H). ¹³C NMR (CDCl₃) δ 55.9, 94.2, 94.3, 113.9, 115.4, 116.3, 120.3, 126.6, 126.8, 127.6, 127.9, 128.0, 128.6, 129.6, 131.2, 136.1, 136.9, 138.8, 156.8, 157.5.

(*E,E*)-1-(4-hydroxystyryl)-4-(3,4-dihydroxystyryl)benzene (13)

As described above for **4**, **13a** (0.100 g, 0.216 mmol) was deprotected using 12.0 M HCl (0.252 mL, 3.03 mmol) to afford **13** (0.050 g, 70%): mp 341 – 350 °C. ¹H NMR (DMSO-*d*₆) δ 6.73 (d, *J* = 7.8 Hz, 1H), 6.77 (d, *J* = 8.3 Hz, 2H), 6.87 (d, *J* = 8.3 Hz, 1H), 6.91 (d, *J* = 16.6 Hz, 1H), 7.01 (d, *J* = 16.6 Hz, 1H), 7.08 (d, *J* = 16.6 Hz, 1H), 7.15 (d, *J* = 16.6 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.51 (s, 4H). ¹³C NMR (DMSO-*d*₆) δ 113.5, 115.8, 115.9, 118.8, 124.9, 125.1, 126.6, 126.6, 128.1, 128.2, 128.4, 128.6, 129.0, 136.4, 136.5, 145.6, 145.8, 157.5.

(*E,E*)-1-[4-(methoxymethoxy)styryl]-4-[3,4-di(methoxymethoxy)styryl]benzene (13a)

As described above for **11a**, **21** (0.346 g, 0.977 mmol), **22b** (0.321 g, 1.95 mmol) were mixed with palladium (II) acetate (0.007 g, 0.03 mmol), phenylurea (0.008 g, 0.06 mmol) and potassium carbonate (0.270 g, 1.95 mmol) and stirred for 22 h at 110 °C in DMF. After cooling to 80 °C, compound **20e** (0.221 g, 0.977 mmol) and sodium methoxide solution (0.366 mL, 1.95 mmol) were added and the reaction was stirred for 1 h at 80 °C and worked up as described above to yield **13a** (0.35 g, 77%): mp 128 – 138 °C. ¹H NMR (CDCl₃) δ 3.50 (s, 3H), 3.53 (s, 3H), 3.56 (s, 3H), 5.20 (s, 2H), 5.26 (s, 2H), 5.30 (s, 2H), 6.98 (d, *J* = 16.1, 1H), 6.98 (d, *J* = 16.1 Hz, 1H), 7.04 (d, *J* = 8.3 Hz, 2H), 7.05 (d, *J* = 16.1 Hz, 1H), 7.08 (d, *J* = 16.1 Hz, 1H), 7.12 (dd, *J*₁ = 8.3 Hz, *J*₂ = 2.0 Hz, 1H), 7.15 (d, *J* = 8.3 Hz, 1H), 7.35 (d, *J* = 1.5 Hz, 1H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.48 (s, 4H). ¹³C NMR (CDCl₃) δ 56.0, 56.2, 94.4, 95.3, 95.4, 114.2, 116.4, 116.6, 121.0, 126.6, 126.7, 127.2, 127.6, 127.8, 127.8, 131.3, 132.2, 136.4, 136.7, 146.9, 147.4, 156.8.

(*E,E*)-1-(4-hydroxystyryl)-4-(3,5-dihydroxystyryl)benzene (14)

As described above for **4**, **14a** (0.113 g, 0.244 mmol) was deprotected using 12.0 M HCl (0.285 mL, 3.42 mmol) to afford **14** (0.076 g, 94%): mp 295 – 305 °C. ¹H NMR (DMSO-*d*₆) δ 6.16 (s, 1H), 6.45 (d, *J* = 2.0 Hz, 2H), 6.78 (d, *J* = 8.8 Hz, 2H), 7.02 (d, *J* = 16.6 Hz, 1H), 7.04 (s, 1H), 7.08 (d, *J* = 16.6 Hz, 1H), 7.17 (d, *J* = 16.1 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 2H), 7.53 (d, *J* = 8.3 Hz, 2H), 7.56 (d, *J* = 8.8 Hz, 2H), 9.27 (s, 2H), 9.61 (s, 1H). ¹³C NMR

(DMSO-*d*₆) δ 102.5, 104.9, 115.8, 125.0, 126.6, 127.0, 127.8, 128.1, 128.4, 128.6, 128.7, 135.9, 137.1, 139.1, 157.6, 158.8.

(*E,E*)-1-[4-(methoxymethoxy)styryl]-4-[3,5-di(methoxymethoxy)styryl]benzene (14a)

As described above for **11a**, **21** (0.342 g, 0.965 mmol), **22b** (0.317 g, 1.93 mmol) were mixed with palladium (II) acetate (0.007 g, 0.03 mmol), phenylurea (0.008 g, 0.06 mmol) and potassium carbonate (0.267 g, 1.93 mmol) and stirred for 22 h at 110 °C in DMF. After cooling to 80 °C, compound **20d** (0.218 g, 0.965 mmol) and sodium methoxide solution (0.362 mL, 1.93 mmol) were added and the reaction was stirred for 1 h at 80 °C and worked up as described above to yield **14a** (0.285 g, 64%): mp 114 – 119 °C. ¹H NMR (CDCl₃) δ 3.49 (s, 3H), 3.51 (s, 6H), 5.20 (s, 6H), 6.66 (s, 1H), 6.88 (d, *J* = 2.0 Hz, 2H), 6.98 (d, *J* = 16.1 Hz, 1H), 7.03 (d, *J* = 16.6 Hz, 1H), 7.04 (d, *J* = 8.8 Hz, 2H), 7.08 (d, *J* = 16.6 Hz, 2H), 7.45 (d, *J* = 8.8 Hz, 2H), 7.48 (s, 4H). ¹³C NMR (CDCl₃) δ 56.1, 94.3, 94.4, 104.3, 107.8, 116.4, 126.6, 126.9, 127.7, 128.0, 128.1, 129.0, 131.2, 136.1, 137.0, 139.5, 156.9, 158.5.

(*E,E*)-1-(3-hydroxystyryl)-4-(3,4-dihydroxystyryl)benzene (15)

As described above for **4**, **15a** (0.114 g, 0.246 mmol) was deprotected using 12.0 M HCl (0.246 mL, 2.96 mmol) to afford **15** (0.072 g, 89%): mp 285 – 289 °C. ¹H NMR (DMSO-*d*₆) δ 6.69 (d, *J* = 7.8 Hz, 1H), 6.74 (d, *J* = 8.3 Hz, 1H), 6.89 (d, *J* = 8.3 Hz, 1H), 6.93 (d, *J* = 16.1 Hz, 1H), 6.98 (s, 1H), 7.01 (s, 1H), 7.04 (d, *J* = 7.8 Hz, 1H), 7.11 (d, *J* = 16.6 Hz, 1H), 7.16 (s, 2H), 7.17 (t, *J* = 8.3 Hz, 1H), 7.54 (d, *J* = 7.8 Hz, 2H), 7.57 (d, *J* = 8.3 Hz, 2H). ¹³C NMR (DMSO-*d*₆) 113.2, 113.5, 115.0, 115.9, 117.7, 118.9, 124.8, 126.6, 127.0, 128.1, 128.3, 128.9, 129.0, 129.8, 135.9, 137.1, 138.7, 145.6, 145.9, 157.8.

(*E,E*)-1-[3-(methoxymethoxy)styryl]-4-[3,4-di(methoxymethoxy)styryl]benzene (15a)

As described above for **11a**, **21** (0.359 g, 1.01 mmol), **22c** (0.333 g, 2.03 mmol) were mixed with palladium (II) acetate (0.007 g, 0.03 mmol), phenylurea (0.008 g, 0.06 mmol) and potassium carbonate (0.281 g, 2.03 mmol) and stirred for 22 h at 110 °C in DMF. After cooling to 80 °C, compound **20e** (0.229 g, 1.01 mmol) and sodium methoxide solution (0.381 mL, 2.03 mmol) were added and the reaction was stirred for 1 h at 80 °C and worked up as described above to yield **15a** (0.244 g, 52%): mp 94 – 101 °C. ¹H NMR (CDCl₃) δ 3.51 (s, 3H), 3.53 (s, 3H), 3.56 (s, 3H), 5.22 (s, 2H), 5.26 (s, 2H), 5.30 (s, 2H), 6.95 (dd, *J*₁ = 7.8 Hz, *J*₂ = 2.4 Hz, 1H), 6.98 (d, *J* = 16.6 Hz, 1H), 7.06 (d, *J* = 16.1 Hz, 1H), 7.09 (s, 2H), 7.12 (dd, *J*₁ = 8.8 Hz, *J*₂ = 2.0 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 1H), 7.17 (d, *J* = 8.8 Hz, 1H), 7.21 (s, 1H), 7.28 (t, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 1.5 Hz, 1H), 7.49 (s, 4H). ¹³C NMR (CDCl₃) δ 56.0, 56.2, 94.4, 95.3, 95.4, 114.0, 114.2, 115.5, 116.6, 120.3, 121.1, 126.7, 126.8, 127.1, 128.0, 128.1, 128.7, 129.7, 132.1, 136.3, 136.8, 138.9, 146.9, 147.4, 157.6.

In Vitro A β ₁₋₄₀ Binding Affinity Determination by Fluorescence Titration

Amyloid (A β) binding affinities (*K*_d) for **5–17** and **4** as a control, were determined by means of fluorescence titrations,^{13,27} with amyloid peptide A β ₁₋₄₀ at 23 °C. Intrinsic fluorescence intensity (FLINT) changes associated with ligand binding to aggregated A β ₁₋₄₀ were recorded on a Cary Eclipse fluorescence spectrophotometer (Varian) using excitation wavelengths determined for each compound. Fixed concentrations of A β ₁₋₄₀ (500 nM for **5–7**, **9**, **10–16**; 40 nM for **4** and **17**; 20 nM for **8**) were diluted to 500 μ L in 9:1 PBS:EtOH in a 10 mm quartz fluorescence cuvette. Concentrations of A β ₁₋₄₀ were selected to be no more than 10-fold higher than compound binding affinities to obtain accurate *K*_d values. To the A β ₁₋₄₀ PBS:EtOH solution in the cuvette, aliquots of test compounds in PBS:EtOH were titrated using a 2.0 μ L Hamilton syringe with a reproducibility adapter along a concentration gradient of test compound (0.8 nM-saturation). Fluorescence spectra were recorded until the fluorescence no longer increased with increasing compound

concentration (saturation). The FLINT at these wavelengths was plotted versus compound concentrations to yield binding isotherms. A β_{1-40} K_d values were determined (Prism 4.0c software, Graphpad, Inc.).

In Vitro A Plaque Binding Specificity Using APP/PS1 Transgenic Mouse Brain Slices

DMSO stock solutions of **4–17** were diluted to 0.50 mM with EtOH and then diluted with 9:1 PBS:EtOH to prepare 50 μ M solutions. Eleven-month old transgenic APP/PS1 mice derived from crossing the Tg2576 line expressing APP Swedish mutant and the M146L 6.1 line expressing presenilin-1 mutant^{28,29} were anesthetized and transcardially perfused with 4% paraformaldehyde in PBS under the guidance of Institutional Animal Care and Use Committee at the University of Nebraska Medical Center. Fixed brains samples were cryoprotected in 20% sucrose in PBS and subjected to cryostat sectioning (Leica). Frozen brain sections (10 μ m thickness) of aged APP/PS1 transgenic mice (three sections per dilution point) were stained with compound solutions for 30 min, and then washed successively with 75% aq. EtOH, 95% aq EtOH and xylene. Fluorescence imaging of the stained and washed brain sections were carried out using a DAPI filter (Chroma) and a Roper HQ CCD camera (original magnification: 400x) following a standard FSB staining protocol.³⁴ Fluorescence images of three plaques per section were systematically captured using the same image acquisition setting (laser power, capturing time, photomultiplier setting) to obtain comparable fluorescent intensities of five plaque regions (specific signal) and five background regions (noise signal) to calculate signal-to-noise (S/N) ratios.

Fluorescence Staining of AD and Control Brain Sections by **4**, **8** and **10**

Human adult AD brain sections and age-matched controls were stained with compounds **4**, **8** and **10**. The frozen brain sections (10 μ m thickness) were stained with compound solutions (same concentrations and PBS:EtOH content as mouse brain section staining solutions) for 30 min and then washed successively with 75% aq. EtOH, 95% ethanol and xylene. Fluorescence imaging was performed as described in the previous section.

In Vivo BBB Delivery of **4**, **8** and **10** to A β Plaque-Bearing PDAPP Mouse Brain

Ten-month- old plate-derived growth factor- β chain promoter driving (PDAPP) mice were injected intraperitoneally (IP) with vehicle only (10% DMSO/PBS) or with single 50 mg/kg doses IP of **4**, **8**, or **10**, and sacrificed at 48 h after injection by transcardial perfusion with 4% paraformaldehyde (PFA) in PBS. After cryoprotection, fixed frozen brains were sectioned with 30 μ m thickness, postfixed with 4% PFA for 5 min., washed in PBS, and mounted on microscope slides with coverslips using Vectorshield mounting medium (Vector Laboratories, Burlingame, CA). Fluorescence images were captured with a digital Olympus DP71 camera (Olympus, Center Valley, PA) connected to a TE-300 microscope (Nikon, Garden City, NJ).

DPPH Free Radical Scavenging Assay

Relative free radical quenching for **4–17**, along with quercetin as a control, was assayed spectrophotometrically using the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) following the procedure of Yang et al.^{32,34} with some modifications. Abstraction of a proton from a free radical scavenger (target compound) decreases the DPPH absorption at 517 nm. Reactions were run in a 96-well plate and the absorbance at 517 nm was recorded on a plate reader. All compounds were first solubilized in DMSO to form concentrated stock solutions (~15 mM) that were then diluted into working solutions in MeOH. Mixtures in the wells contained increasing concentrations of target compounds (0 nM–5 mM), 100 μ M DPPH and MeOH (300 μ L total volume). Each concentration was performed in triplicate and IC₅₀

values were determined using the sigmoidal doseresponse fit in Prism 4.0c software (Graphpad, Inc.)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This investigation received financial support from the Vada Oldfield Alzheimer Research Foundation (T.I, T.K.), National Institute of Health (P01 NS043985 (T.I.), R01 MH083523 (T.I.), R21 AG032600 (T.I.)), UNMC Brain Bank Core Fund (T.I.), an American Foundation of Pharmaceutical Education (AFPE) pre-doctoral fellowship (D. P. F.), a Josiah Kirby Lilly, Sr. Memorial AFPE pre-doctoral fellowship (D. P. F) and a Nancy and Ronald Reagan Alzheimer's Scholarship Award (D. P. F.) and the Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center (D. P. F.). We thank Dr. L. Mucke for providing PDAPP mice (line J20). We also thank Sarah A. Flaherty for preparing figures of brain staining and in vivo data.

Non-standard Abbreviations

APP/PS1	Amyloid Precursor Protein/Presenilin 1
PS2	Presenilin 2
PDAPP	Plate-Derived Growth Factor- β Chain Promoter Driving Amyloid Presursor Protein Transgenic Mice
AD	Alzheimer's Disease
Aβ	Amyloid Beta
NFT	Neurofibrillary Tangles
PET	Positron Emission Tomography
SPECT	Single Photon Emission Computed Tomography
BBB	Blood-Brain Barrier
SAR	Structure Activity Relationship
MRI	Magnetic Resonance Imaging
MOM	Methoxymethyl
FLINT	Flourescence Intensity

References

- Goedert M, Spillantini G. A Century of Alzheimer's Disease. *Science* 2006;314:777–781. [PubMed: 17082447]
- Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer's Disease in the United States in the U. S. Population: Prevalence Estimates Using the 2000 Census. *Arch Neurol* 2003;60:1119–1122. [PubMed: 12925369]
- Alzheimer A. Ueber eine eigenartige Erkankung der Hirnrinde. *Allg Z Psychiatr* 1907;64:146–148.
- Selkoe DJ. Alzheimer's Disease: Genotypes, Phenotypes and Treatments. *Science* 1997;275:630–631. [PubMed: 9019820]
- Berg L, McKeel DW Jr, Miller JP, Baty J, Morris JC. Neuropathological Indexes of Alzheimer's Disease in Demented and Nondemented Persons Aged 80 Years or Older. *Arch Neurol* 1993;50:349–358. [PubMed: 8460956]

6. Schmitt FA, Davis DG, Wekstein DR, Smith CD, Ashford JW, Markesbery WR. "Preclinical" AD Revisited: Neuropathology of Cognitively Normal Older Adults. *Neurology* 2000;55:370–376. [PubMed: 10932270]
7. Hardy J, Selkoe DJ. The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics. *Science* 2002;297:353–356. [PubMed: 12130773]
8. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD. Apolipoprotein E. High-Avidity Binding to Amyloid and Increased Frequency of Type 4 Allele in Late-Onset Familial Alzheimer Disease. *Proc Natl Acad Sci US A* 1993;90:1977–1981.
9. Puchtler H, Sweat F, Levine M. On the Binding of Congo Red by Amyloid. *J Histochem Cytochem* 1962;10:355–364.
10. Styren SD, Hamilton RL, Styren GC, Klunk WE. X-34, a Fluorescent Derivative of Congo Red: A Novel Histochemical Stain for Alzheimer's Disease Pathology. *J Histochem Cytochem* 2000;48:1223–1232. [PubMed: 10950879]
11. Sato K, Higuchi M, Iwata N, Saido TC, Sasamoto K. Fluoro-Substituted and ¹³C-Labeled Styrylbenzene Derivatives for Detecting Brain Amyloid Plaques. *Eur J Med Chem* 2004;39:573–578. [PubMed: 15236837]
12. Crystal AS, Giasson BI, Crowe A, Kung MP, Zhuang ZP, Trojanowski JQ, Lee VMY. A Comparison of Amyloid Fibrillogenesis using the Novel Fluorescent Compound K114. *J Neurochem* 2003;86:1359–1368. [PubMed: 12950445]
13. Flaherty DP, Walsh SM, Kiyota T, Dong Y, Ikezu T, Vennerstrom JL. Polyfluorinated Bis-styrylbenzene β -Amyloid Plaque Binding Ligands. *J Med Chem* 2007;50:4986–4992. Compound **4** was prepared previously in our lab using a Horner-Wadsworth-Emmons coupling with 1,4-bis(diethyl phosphonylmethyl)benzene and *p*-anisaldehyde to form (*E,E*)-1,4-bis(4-methoxy)styrylbenzene which was deprotected with 1.0 M boron tribromide in DCM to yield **4**. [PubMed: 17845017]
14. Kung HM, Choi SR, Qu W, Zhang W, Skovransky D. ¹⁸F Stilbenes and Styrylpyridines for PET Imaging of A β Plaques in Alzheimer's Disease: A Miniperspective. *J Med Chem* 2010;53:933–941. [PubMed: 19845387]
15. Huynh THV, Mantel MLH, Mikkelsen K, Lindhardt AT, Nielson NC, Otzen D, Skrydstrup T. A Versatile Approach to β -Amyloid Fibril-Binding Compounds Exploiting the Shirakawa/Hayashi Protocol for *trans*-Alkene Synthesis. *Org Lett* 2009;11:999–1002. [PubMed: 19161320]
16. Villano D, Fernandez-Pachon MS, Troncoso AM, Garcia-Parrilla MC. Comparison of Antioxidant Activity of Wine Phenolic Compounds and Metabolites In Vitro. *Analyt Chim Acta* 2005;538:391–398.
17. Feng Y, Wang XP, Yang SG, Wang YJ, Zhang X, Du XT, Sun XX, Zhao M, Huang L, Liu RT. Resveratrol Inhibits β -Amyloid Oligomeric Cytotoxicity but does not Prevent Oligomer Formation. *Neurotoxicology* 2009;30:986–995. [PubMed: 19744518]
18. Sayre LM, Perry G, Smith MA. Oxidative Stress and Neurotoxicity. *Chem Res Toxicol* 2008;21:172–188. [PubMed: 18052107]
19. Bruza, KJ. Bis((aryl(vinyl)benzenes. US Patent. 4,529,556 1985.
20. Solntsev KM, McGrier PL, Fahrni CJ, Tolbert LM, Bunz UHF. Anomalous Photophysics of Bis(hydroxystyryl)benzenes: A Twist on the Para/Meta Dichotomy. *Org Lett* 2008;10:2429–2432. [PubMed: 18476714]
21. Gerold J, Holzenkamp U, Meier H. Bis-, Tris- and Tetrakis(squaraines) Linked by Stilbenoid Scaffolds. *Eur J Org Chem* 2001:2757–2763.
22. (a) Flaherty DP, Dong Y, Vennerstrom JL. A One-Pot Synthesis of Unsymmetrical Bis-styrylbenzenes. *Tetrahedron Lett* 2009;50:6228–6230. (b) Mantel MLH, Søjberg LS, Huynh THV, Ebran J-P, Lindhardt (né Hansen) AT, Nielsen NC, Skrydstrup T. An Expedient Synthesis of the Fibril Binding Compounds FSB via Sequential Pd-Catalyzed Couplings Reactions. *J Org Chem* 2008;73:3570–3573. [PubMed: 18363363]
23. Cui X, Zhou Y, Wang N, Liu L, Guo Q-X. SOMETHING IS MISSING HERE???
24. Tew GN, Pralle MU, Stupp SI. Supramolecular Materials from Triblock Rodcoil Molecules Containing Phenylene Vinylene. *J Am Chem Soc* 1999;121:9852–9866.

25. Wood JH, Bacon JA, Meibohm AW, Throckmorton WH, Turner GP. The Synthesis of Symmetrical Diaryl Ethylenes. *J Am Chem Soc* 1941;63:1334–1335.
26. Yam CM, Kakkar AK. Molecular Self-Assembly of Dihydroxy-Terminated Molecules via Acid-Base Hydrolytic Chemistry of Silica Surfaces: Step-by-step Multilayered Thin Film Construction. *Langmuir* 1999;15:3807–3815. Compound **17** was prepared by this group via a Heck reaction between 1,4-diethynylbenzene and 4-iodophenol in 50% yield. Our attempts at synthesizing **17** using this method failed and we isolated only the mono-Heck product and an insoluble impurity.
27. Lockhart A, Ye L, Judd DB, Merritt AT, Lowe PN, Morgenstern JL, Hong G, Gee AD, Brown J. Evidence for the Presence of Three Binding Sites for the Thioflavin T Class of Alzheimer's Disease PET Imaging Agents on Amyloid Peptide Fibrils. *J Biol Chem* 2005;280:7677–7684. [PubMed: 15615711]
28. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. Correlative Memory Deficits, A β Elevation, and Amyloid Plaques in Transgenic Mice. *Science* 1996;274:99–102. [PubMed: 8810256]
29. Duff K, Eckman C, Zehr C, Yu X, Prada CM, Perez-tur J, Hutton M, Buee L, Harigaya Y, Yager D, Morgan D, Gordon MN, Holcomb L, Refolo L, Zenk B, Hardy J, Younkin S. Increased Amyloid-42(43) in Brains of Mice Expressing Mutant Presenilin 1. *Nature* 1996;383:710–713. [PubMed: 8878479]
30. Byeon SR, Lee JH, Sohn JH, Kim DC, Shin KJ, Yoo KH, Mook-Jung I, Lee WK, Kim DJ. Bis-Styrylpyridine and Bis-Styrylbenzene Derivatives as Inhibitors for A β Fibril Formation. *Bioorg Chem Lett* 2007;17:1466–1470.
31. Li Q, Min J, Ahn YH, Namm J, Kim EM, Lui R, Kim HY, Ji Y, Wu H, Wisniewski T, Chang YT. Styryl-Based Compounds as Potential In Vivo Imaging Agents for β -Amyloid Plaques. *ChemBioChem* 2007;8:1679–1687. [PubMed: 17705341]
32. Yang LX, Huang KX, Li HB, Gong JX, Wang F, Feng YB, Tao QF, Wu YH, Li XK, Wu XM, Zeng S, Spencer S, Zhao Y, Qu J. Design, Synthesis, and Examination of Neuron Protective Properties of Alkenylated and Amidated Dehydro-Silybin Derivatives. *J Med Chem* 2009;52:7732–7752. [PubMed: 19673490]
33. Tewari R, Kumari N, Kendurkar P. Studies on Ylides: Stereospecific Synthesis of *trans*, *trans*-Distyrylbenzenes and *trans*, *trans*-Divinylbenzenes. *Indian J Chem, Sect B: Org Chem Incl Med Chem* 1977;15:753–755.
34. Blois MS. Antioxidant Determinations by the use of a Stable Free Radical. *Nature* 1958;181:1199–1200.
35. Higuchi M, Iwata N, Matsuba Y, Sato K, Sasamoto K, Saido TC. ^{19}F and ^1H MRI Detection of Amyloid Plaques In Vivo. *Nat Neurosci* 2005;8:527–533. [PubMed: 15768036]
36. Maksimovic Z. In Vitro Antioxidant Activity of Ragweed (*Ambrosia artemisiifolia* L., Asteraceae) Herb. *Ind Crops and Prod* 2008;28:356–360.
37. Winter A, Friebe C, Hager MD, Schubert US. Synthesis of Rigid π -Conjugated Mono-, Bis-, Tris-, and Tetrakis(terpyridine)s: Influence of the Degree and Pattern of Substitution on the Photophysical Properties. *Eur J Org Chem* 2009;6:801–809.
38. Barbasiewicz M, Makosza M. Intermolecular Reactions of Chlorohydrine Anions: Acetalization of Carbonyl Compounds Under Basic Conditions. *Org Lett* 2006;8:3745–3748. [PubMed: 16898807]
39. Hintermann L, Masuo R, Suzuki K. Solvent-Controlled Leaving-Group Selectivity in Aromatic Nucleophilic Substitution. *Org Lett* 2008;10:4859–4862. [PubMed: 18841994]
40. Townsend CA, Christensen SB, Davis SG. Synthesis of Averufin and its Role in Aflatoxin B₁ Biosynthesis. *J Chem Soc Perkin Trans I* 1988;4:839–861.
41. Frazier, KA.; Renga, JM. Preparation of Ethenylidenes. US Patent. 4,713,458. 1987.
42. Thakkar K, Geahlen RL, Cushman M. Synthesis and Protein-Tyrosine Kinase Inhibitory Activity of Polyhydroxylated Stilbene Analogues of Piceatannol. *J Med Chem* 1993;36:2950–2955. [PubMed: 8411012]
43. Bull SR, Palmer LC, Fry NJ, Greenfield MA, Messmore BW, Meade TJ, Stupp SI. A Templating Approach for Monodisperse Self-Assembled Organic Nanostructures. *J Am Chem Soc* 2008;130:2742–2743. [PubMed: 18266371]

44. Fauonneau B, Waffo-Teguo P, Huguet F, Barrier L, Decendit A, Merillon JM. Comparative Study of Radical Scavengers and Antioxidant Properties of Phenolic Compounds from *Vitis Vinifera* Cell Cultures Using In Vitro Tests. Life Sci 1997;61:2103–2110. [PubMed: 9395251]

Figure 1.
Target compounds **6–17**.

Figure 2. Fluorescence imaging of in vitro mouse and human brain sections

Fluorescence imaging of A β plaque in (A β) aged APP/PS1 mouse and (B) human AD brain sections. Frozen brain sections (10 μ m thickness) were stained with 50 μ M of **4**, **8** and **10** for 30 min in 9:1 PBS:EtOH, washed successively with 75% aq. EtOH, 95% aq. EtOH, and xylene. Fluorescence images were taken using a Nikon TE-2000, 60x Pan Fluor objective, and Roper HQ CCD camera (600x original magnification). A 360/460 (DAPI filter set) was used for imaging.

Figure 3. In vivo labeling of A β plaques via intravenous injection of 4, 8 or 10 in PDAPP mice
Ten-month-old PDAPP mice were injected intraperitoneally with vehicle only (A β), or single 50 mg/kg doses of **4** (B), **8** (C) or **10** (D) and sacrificed at 48 h after injection. Fluorescence images of the CA1 and dentate gyrus (DG) fields of the hippocampi were captured using 10x or 40x (insets) objectives on a Nikon TE-300 with a digital camera. Insets show representative fluorescent staining of A β plaques by **4**, **8** or **10**. Bar = 200 μ m or 50 μ m (insets).

Scheme 1. Synthesis of Bis-styrylbenzenes **4** and **6–10**.^a^aReagents and Conditions: (a) triethylphosphite, 140 °C, 4 h, (b) NaOCH₃, DMF, 80 °C, 1 h, (c) HCl, CHCl₃/MeOH (3:1), rt, 24 h.

Scheme 2.

Synthesis of Bis-styrylbenzenes **11–15**.^a

^aReagents and conditions (a) Pd(OAc)₂, phenylurea, K₂CO₃, DMF, 110 °C, 22 h (b) see b from Scheme 1, (c) see c from Scheme 1.

Table 1

Amyloid Peptide A β_{1-40} Binding Affinity (K_d) and A β Plaque Binding Specificity (S/N Ratio) to APP/PS1 Transgenic Mouse Brain Slices for **4–17**.

Compound	K_d^a (μ M)	S/N ratio ^b
4	0.0095 ± 0.0003^c	14 ± 1.1
5	0.50 ± 0.04	3.5 ± 0.7
6	2.5 ± 0.1	3.1 ± 0.7
7	6.3 ± 0.1	5.1 ± 1.4
8	0.0053 ± 0.0003	9.6 ± 3.0
9	0.80 ± 0.02	11 ± 2.8
10	0.16 ± 0.01	9.6 ± 1.8
11	0.16 ± 0.01	8.1 ± 2.3
12	3.5 ± 0.2	9.0 ± 1.5
13	0.26 ± 0.02	9.1 ± 0.6
14	0.19 ± 0.01	3.8 ± 0.9
15	0.20 ± 0.02	5.6 ± 1.3
16	9.2 ± 0.2	5.2 ± 1.2
17	0.054 ± 0.005	5.9 ± 0.4

^aValues represent the average $\pm$ SD of three determinations.

^bValues represent the average $\pm$ SD of ten determinations.

^cReported $K_d = 11 \pm 2$ nM and S/N = 17 ± 1.3 ¹³

Table 2Free Radical Scavenging Activity measured by DPPH Assay for Quercetin, and **4–17**.

Compound	DPPH IC ₅₀ (μM) ^a
Quercetin	1.3 ± 1.1 ^b
4	3.1 ± 1.2
5	62 ± 1.4 ^c
6	3.4 ± 1.1
7	4.0 ± 1.1
8	> 100
9	4.0 ± 1.1
10	5.1 ± 1.0
11	5.6 ± 1.1
12	3.5 ± 1.2
13	>100
14	4.5 ± 1.1
15	9.1 ± 1.1
16	1.8 ± 1.2
17	45 ± 1.0

^aValues represent the average ± SD of three determinations.^bReported IC₅₀ = 9.1 μM³⁶^cReported IC₅₀ = 74 ± 5.3 μM⁴⁴