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

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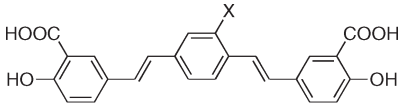
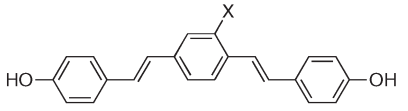
Starting from bisphenolic bis-styrylbenzene DF-9 (**4**), β-amyloid (Aβ) binding affinity and specificity for phenolic bis-styrylbenzenes, monostyrylbenzenes, and alkyne controls were determined by fluorescence titration with β-amyloid peptide $A\beta_{1-40}$ and a fluorescence assay using APP/PS1 transgenic mouse brain sections. Bis-styrylbenzene SAR is derived largely from work on symmetrical compounds. This study is the first to describe Aβ 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, changing the orientation about the central phenyl ring, replacing the alkene with alkyne bonds, or eliminating the central phenyl ring. The only compound with an Aβ binding affinity and specificity comparable to **4** was its 3-hydroxy regioisomer **8**. Like **4**, **8** crossed the blood−brain barrier and bound to Aβ plaques in vivo. By use of 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[a])[1] is a common neurodegenerative disorder in the elderly[2] characterized by an accumulation of β-amyloid (Aβ) plaques in the brain parenchyma and neurofibrillary tangles (NFTs) in the neuron.[3,4] Although Aβ and NFTs are commonly observed in nondemented elderly, 80 years and older, the molecular pathogenesis of AD is believed to be affected by Aβ production and its clearance.[5,6] The amyloid cascade hypothesis[7] for AD pathogenesis proposes that accumulation and aggregation of Aβ trigger 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 Aβ accumulation and aggregation are elevated by mutations of the APP, PS1, and PS2 genes.[8]

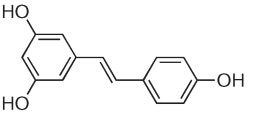
The discovery of the diazo dye Congo red as an Aβ binding ligand[9] provided a starting point in the search for potential in vivo imaging agents using positron emission tomography (PET), single photon emission computed tomography (SPECT), and magnetic resonance imaging (MRI). Investigation of Congo red led to the discovery of bis-styrylbenzenes as a class of compounds with strong Aβ plaque binding properties. These compounds are exemplified by the salicylate bis-styrylbenzenes X-34 (**1**)[10] and FSB (**2**),[11] which have been shown to penetrate the blood−brain barrier (BBB) and bind to Aβ 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 Aβ 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 Aβ-binding SAR has been elucidated based largely on symmetrical bis-styrylbenzenes.



1, (X-34) X = H
2, (FSB) X = F



3, (K114) X = Br
4, (DF-9) X = H



5, Resveratrol

Design

Previously, we showed that the bis-styrylbenzene, (*E*,*E*)-1,4-bis(4-hydroxystyryl)benzene (**4**), binds to Aβ 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

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[a] Abbreviations: APP/PS1, amyloid precursor protein/presenlin 1; PS2, presenilin 2; PDAPP, plate-derived growth factor-β chain promoter driving amyloid presursor protein transgenic mice; AD, Alzheimer's disease; Aβ, amyloid β; 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, fluorescence intensity.

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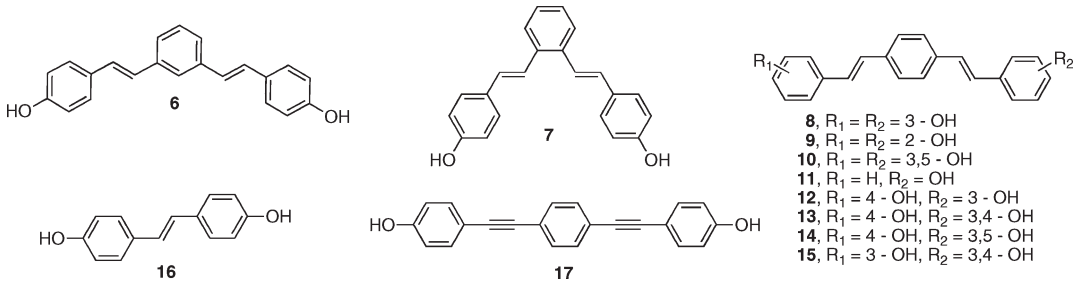
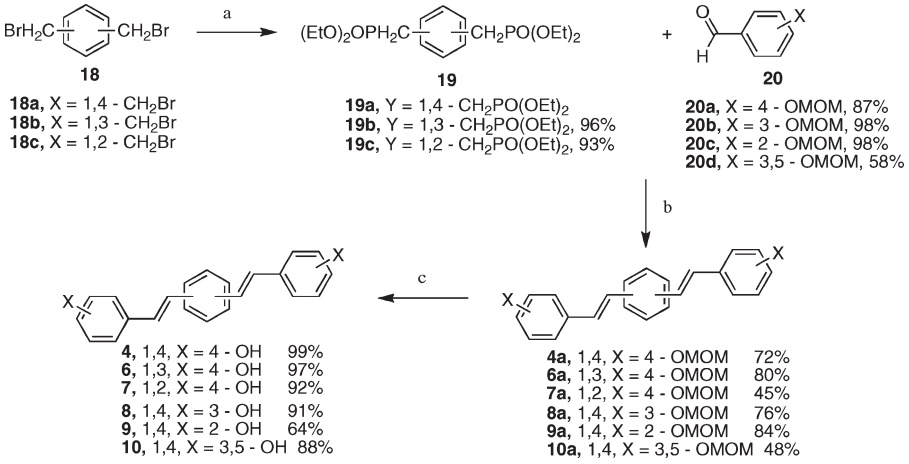


Figure 1. Target compounds **6**−**17**.

Scheme 1. Synthesis of Bis-styrylbenzenes **4** and **6**−**10**[a]



[a] Reagents and conditions: (a) triethyl phosphite, 140 °C, 4 h; (b) $NaOCH_3$, DMF, 80 °C, 1 h; (c) HCl, $CHCl_3$/MeOH (3:1), room temp, 24 h.

(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 Aβ-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 monostyryl (**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 Aβ-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

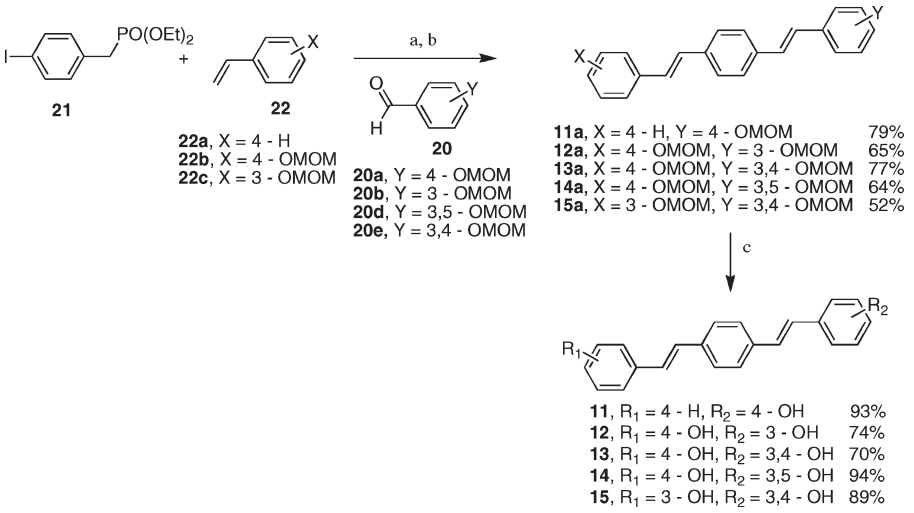
Compounds **4**[13] and **6**−**10** (Scheme 1) were obtained using a previously reported procedure.[13] The preparation of **6** was an improvement on the previously published procedure[19] in which a low-yielding (20%) double-Heck coupling was used. The overall yields of **8** (69%) and **10** (42%) 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**−**c** were coupled with methoxymethoxy (MOM) substituted benzaldehydes **20a**−**d** 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**−**c** 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 benzaldehyde **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 (Aβ) Binding Affinity and Specificity

β-Amyloid (Aβ) binding affinity and specificity for bis-styrylbenzenes **6**−**17**, with **4** and **5** as controls, were determined by means of fluorescent titrations[27] 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

Scheme 2. Synthesis of Bis-styrylbenzenes **11**−**15**[a]



[a] Reagents and conditions: (a) $Pd(OAc)_2$, phenylurea, K_2CO_3, DMF, 110 °C, 22 h; (b) $NaOCH_3$, DMF, 80 °C, 1 h; (c) HCl, $CHCl_3$/MeOH (3:1), room temp, 24 h.

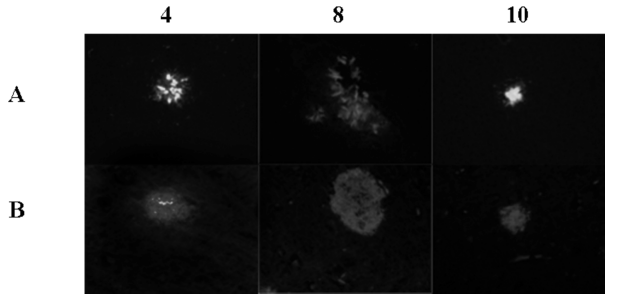


Figure 2. Fluorescence imaging of in vitro mouse and human brain sections: (A) Aβ plaque in (Aβ) aged APP/PS1 mouse and (B) human AD brain sections. Frozen brain sections (10 μm thickness) were stained with 50 μM of **4**, **8**, and **10** for 30 min in 9:1 PBS/EtOH, washed successively with 75% aqueous EtOH, 95% aqueous EtOH, and xylene. Fluorescence images were taken using a Nikon TE-2000, 60× Pan Fluor objective, and Roper HQ CCD camera (600× original magnification). A 360/460 (DAPI filter set) was used for imaging.

Tg2576 strain,[28] which expresses the K670N/M671L mutant of APP695 found as the Swedish familial AD gene, and the M146L 6.1 strain,[29] which expresses the M146L mutant of PS1 found as the early onset familial AD gene. The specificity is determined by measuring the fluorescent intensity of Aβ plaques (specific signal) compared to that of background normal brain tissue (noise) (Figure 2).

The only compound with an Aβ 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 Aβ 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 Aβ 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 Aβ binding affinity (370- and 660-fold) than **4** and **8**.

Table 1. Amyloid Peptide $A\beta_{1-40}$ Binding Affinity (K_d) and Aβ Plaque Binding Specificity (S/N Ratio) to APP/PS1 Transgenic Mouse Brain Slices for **4**−**17**

compd	K_d [a] (μM)	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

[a] Values represent the average ± SD of 3 determinations. [b] Values represent the average ± SD of 10 determinations. [c] Reported K_d = 11 ± 2 nM and S/N = 17 ± 1.3.[13]

The 2 orders of magnitude decrease in Aβ 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 antiaggregation properties of 1,3-bis-styrylpyridines and 1,3-bis-styrylbenzenes[30] and Aβ -binding properties of 1,3-bis-styrylquinaldines[31] have been investigated, this is the first time that Aβ-binding affinity and specificity hasve been assessed as a function of the central ring substitution pattern of bis-styrylbenzenes.

As evidenced by the 17- to 27-fold loss in Aβ 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**, monophenol **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 analogue **17** had a 6-fold loss in Aβ binding affinity and a 2.4-fold loss in specificity, possibly due to an inability to attain a conformer orientation complementary to the Aβ fibril surface. Of all of the target compounds, the symmetrical monostyrylbenzene **16** had the weakest Aβ binding

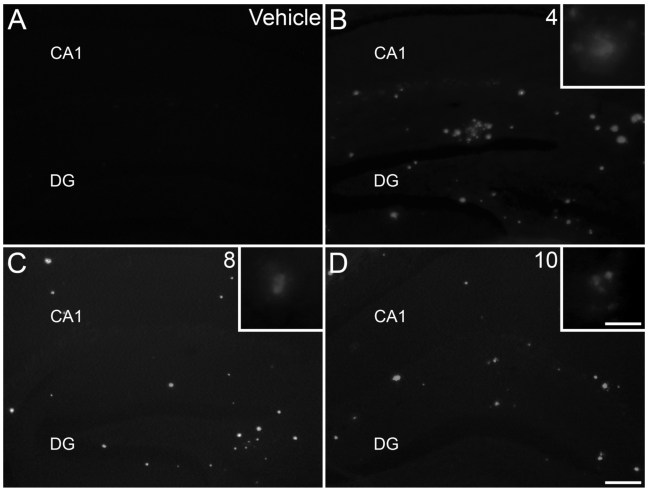


Figure 3. In vivo labeling of Aβ plaques via intravenous injection of **4**, **8**, or **10** in PDAPP mice. Ten-month-old PDAPP mice were injected intraperitoneally with vehicle only (Aβ) (A), or single 50 mg/kg doses of **4** (B), **8** (C), or **10** (D) and sacrificed at 48 h after injection. Fluorescence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

affinity, 3 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 Aβ fibril surface or a combination of the two. Surprisingly, monostyrylbenzene **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 Aβ 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 Aβ 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 Aβ plaques. Bis-styrylbenzenes **4**, **8**, and **10** (50 mg/kg) were injected into 10-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 Aβ plaques in vivo. Each section showed intense Aβ plaque signals with high specificities. Control nontransgenic 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 Aβ 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). Compounds **4**, **6**, **12**, and **16** were no less effective than quercetin ($IC_{50} = 1.3 \pm 1.1$ μM) 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 ($IC_{50} > 100$ μM) in this assay, monostyrylbenzene **5** and bis-alkyne **17** were the least effective radical scavengers with IC_{50} values 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 seems 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.

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

compd	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

[a] Values represent the average ± SD of three determinations. [b] Reported $IC_{50} = 9.1$ μM.[35] [c] Reported $IC_{50} = 74 \pm 5.3$ μM.[43]

Summary

These data extend the SAR of bis-styrylbenzene Aβ binding and free-radical scavenging activity and provide further direction for the development of noninvasive Aβ 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 seems 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. ^{1}H (500 MHz) and ^{13}C (125.7 MHz) NMR spectra were measured on a Varian spectrometer using $CDCl_3$ and DMSO-d_6 as solvents. All chemical shifts are reported in parts per million (ppm) and are relative to internal $(CH_3)_4Si$ for ^{1}H and $CDCl_3$ (77.0 ppm) and DMSO-d_6 (39.7 ppm) for ^{13}C NMR. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. All target compounds had purities of ≥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(diethylphosphonylmethyl)benzene (**19a**) (1.12 g, 2.96 mmol) in DMF (15 mL) at room temperature. This mixture was heated to 80 °C for 2 h. The reaction was then quenched with H_2O (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%). ^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCl_3$) 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 $CHCl_3$/MeOH (25 mL) were stirred at room temperature until **4a** was completely dissolved before dropwise addition of 12.0 M HCl (0.812 mL, 9.74 mmol) at room temperature. The reaction was quenched with H_2O (20 mL) after 24 h. The $CHCl_3$/MeOH layer was removed in vacuo to afford **4** as a white solid (0.379 g, 99%): mp dec 312 °C. ^{1}H 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). ^{13}C 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. ^{1}H 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). ^{13}C 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). ^{1}H 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). ^{13}C 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. ($C_{22}H_{18}O_2 \cdot {}^1/_3H_2O$) 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. ^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCl_3$) 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. ^{1}H 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). ^{13}C NMR (DMSO-d_6) δ 115.6, 122.7, 126.0, 127.4, 128.4, 128.5, 130.8, 135.7, 157.6. Anal. ($C_{22}H_{18}O_2$) 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. ^{1}H 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). ^{13}C 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). ^{1}H 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). ^{13}C 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. ($C_{22}H_{18}O_2$) 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%): ^{1}H NMR ($CDCl_3$) 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, 2 H). ^{13}C NMR ($CDCl_3$) 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). ^{1}H 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). ^{13}C 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. ($C_{22}H_{18}O_2$) 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. ^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCl_3$) 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). ^{1}H 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). ^{13}C 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. ^{1}H 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). ^{13}C 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-iodobenzylphosphonate (**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 mixture 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 mixture was stirred for 1 h at 80 °C and then cooled to room temperature and quenched with H_2O (15 mL). The solid was then filtered and rinsed with H_2O and ether to afford **11a** (0.317 g, 79%): mp 240−243 °C. ^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCl_3$) 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. ^{1}H 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). ^{13}C 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) and **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 the mixture was cooled to 80 °C, compound **20b** (0.146 g, 0.880 mmol) and sodium

methoxide solution (0.330 mL, 1.76 mmol) were added. The mixture 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. ^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCl_3$) 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. ^{1}H 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). ^{13}C 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) and **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 the mixture was cooled to 80 °C, compound **20e** (0.221 g, 0.977 mmol) and sodium methoxide solution (0.366 mL, 1.95 mmol) were added. The mixture 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. ^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCl_3$) 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. ^{1}H 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). ^{13}C 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) and **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 the mixture was cooled to 80 °C, compound **20d** (0.218 g, 0.965 mmol) and sodium methoxide solution (0.362 mL, 1.93 mmol) were added. The mixture 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. ^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCl_3$) 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. ^{1}H 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). ^{13}C 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) and **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 the mixture was cooled to 80 °C, compound **20e** (0.229 g, 1.01 mmol) and sodium methoxide solution (0.381 mL, 2.03 mmol) were added. The mixture 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. ^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCl_3$) 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 Aβ_{1-40} Binding Affinity Determination by Fluorescence Titration. Amyloid (Aβ) binding affinities (K_d) for **5**−**17** and **4** as a control were determined by means of fluorescence titrations,[13,27] with amyloid peptide Aβ_{1-40} at 23 °C. Intrinsic fluorescence intensity (FLINT) changes associated with ligand binding to aggregated Aβ_{1-40} were recorded on a Cary Eclipse fluorescence spectrophotometer (Varian) using excitation wavelengths determined for each compound. Fixed concentrations of Aβ_{1-40} (500 nM for **5**−**7**, **9**, **10**−**16**; 40 nM for **4** and **17**; 20 nM for **8**) were diluted to 500 μL in 9:1 PBS/EtOH in a 10 mm quartz fluorescence cuvette. Concentrations of Aβ_{1-40} were selected to be no more than 10-fold higher than compound binding affinities to obtain accurate K_d values. To the Aβ_{1-40} PBS/EtOH solution in the cuvette, aliquots of test compounds in PBS/EtOH were titrated using a 2.0 μL of 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. Aβ_{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 μM 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 brain samples were cryoprotected in 20% sucrose in PBS and subjected to cryostat sectioning (Leica). Frozen brain sections (10 μm 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% aqueous EtOH, 95% aqueous 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: 400×) 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 μm 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% aqueous 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 Aβ 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 mm thickness, postfixed with 4% PFA for 5 min, washed in PBS, and mounted on microscope slides with coverslips using VECTASHIELD 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 solublized 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 to 5 mM), 100 μM DPPH, and MeOH (300 μL total volume). Each concentration was performed in triplicate, and IC_{50} values were determined using the sigmoidal dose-response fit in Prism 4.0c software (GraphPad, Inc.)

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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] **19c**,[36] **20a**−**e**,[22,32,37−39] **22b**,[22] **22c**,[40] **24**,[41] **25**,[41] and **28**.[42] This material is available free of charge via the Internet at http://pubs.acs.org.

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