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Title: Molecular neuroimaging of Alzheimer's disease
Issue Date: 2014-05-28
Chapter 10

Polyfluorinated bis-styrylbenzenes as amyloid-β plaque binding ligands

adapted from Bioorg Med Chem 2014, doi: 10.1016/j.bmc.2014.02.054
Abstract

Detection of cerebral β-amyloid (Aβ) by targeted contrast agents remains of great interest to aid the *in vivo* diagnosis of Alzheimer’s disease (AD). Bis-styrylbenzenes have been previously reported as potential Aβ imaging agents. To further explore their potency as $^{19}$F MRI contrast agents we synthetized several novel fluorinated bis-styrylbenzenes and studied their fluorescent properties and amyloid-β binding characteristics. The compounds showed a high affinity for Aβ plaques on murine and human brain sections. Interestingly, competitive binding experiments demonstrated that they bound to a different binding site than chrysamine G. Despite their high logP values, many bis-styrylbenzenes were able to enter the brain and label murine amyloid *in vivo*. Unfortunately initial post-mortem $^{19}$F NMR studies showed that these compounds as yet do not warrant further MRI studies due to the reduction of the $^{19}$F signal in the environment of the brain.
Introduction

Alzheimer’s disease (AD) is the predominant form of dementia in the aging population. The disease is marked by neuronal degeneration associated with deposits of tau proteins in intraneuronal neurofibrillary tangles (NFTs) and of amyloid-β (Aβ) peptides in extracellular amyloid plaques. Although the precise role of amyloid in AD pathology is still not completely understood, accumulation of amyloid plaques is thought to precede the onset of the first clinical symptoms by up to two decades. A clinical imaging technique capable of visualizing and quantifying these early changes thus may enable early diagnosis and better understanding of the pathophysiology.

Over the past years progress has been made in the development of Aβ-targeting imaging ligands suitable for visualization by positron emission tomography (PET), single positron emission tomography (SPECT), fluorescence microscopy or magnetic resonance imaging (MRI). For clinical use, the [11C]-benzothiazole derivative Pittsburgh compound B (PiB, Figure 10.1) is the best characterized in vivo PET radiotracer. However, as the short half-life of 11C limits its use to medical centers with an on-site cyclotron, alternatives are desired. Therefore, several longer-lived 18F radiofluorinated derivatives have been designed, like flutemetamol (2), florbetapir (3) and florbetaben (4), of which the first two recently have been the first to be admitted for commercial use with the last one expected to follow soon thereafter. Despite the inherent high sensitivity of PET, the development of Aβ-targeted imaging probes suitable for clinical MRI remains attractive as this would lower the threshold for performing amyloid scans given the wider availability of MRI systems as compared to PET systems, the lack of ionizing irradiation and the lower costs involved in performing clinical MRI as compared to PET. Furthermore, such agents could be used in one scan session comprising a comprehensive structural and functional scan protocol as well as a scan to detect the molecular imaging tracers, whereas one PET examination only provides one biomarker.

Based on congo red (5), several bis-styrylbenzenes have been reported to show strong Aβ binding affinities and serve as potential backbones for in vivo PET or SPECT imaging probes, like 6 (chrysamine G), 7 (X-34), 8 (ISB) and 10 (Methoxy-X04) (Figure 10.1 and Scheme 10.1). The styrylbenzene backbone has also been explored for the development as an MRI contrast agent. The initial breakthrough came with the design of compound 9 (FSB), which was specifically designed for in vivo detection of Aβ using 19F MRI. Since normal biological tissue completely lacks fluorine, 19F MRI would allow direct imaging of the amyloid binding compound, without any endogenous background signal (‘hot spot imaging’). Initial in vivo animal studies with compound 9 showed promising results. Unfortunately, 19F MRI in vivo experiments suffer from the inherently low sensitivity of MRI with a detection limit in the micromolar to millimolar range depending merely on the voxel size and magnetic field strength. As initial 19F compound only carried a single fluorine atom this makes 19F MRI a technical challenge requiring long acquisition times. The incorporation of multiple magnetically equivalent 19F atoms could aid with respect to this aspect, as the spin of each individual 19F nucleus directly adds to the MR signal. The study therefore aimed to further exploit the bis-styrylbenzene backbone as a specific amyloid-β targeting MR contrast agent by increasing the number of fluorine atoms positioned to maintain favorable NMR characteristics as well as solubility.
Synthesized compounds were evaluated with respect to their Aβ binding and specificity, and their fluorescent properties. Partition coefficients (logP) were determined to assess hydrophobicity. Compounds were injected systemically in transgenic AD mice to determine BBB passage and affinity for amyloid plaques in vivo. For the most promising compound, in vitro and post-mortem 19F NMR studies were performed.

Design

Previously Flaherty et al. have designed a similar series of polyfluorinated compounds, based on a bis-styrylbenzene structure with either a non-substituted core with fluorine substitutions on the outer rings, or four 19F atoms on the inner ring with a polar substituent on the outer ring. These compounds were found to target Aβ in vivo. As was already proven for Methoxy-X04
POLYFLUORINATED BIS-STYRYLBENZENES

Scheme 10.1 General synthesis route of envisioned fluorinated bis-styrylbenzenes

Reagents and conditions: a) N-bromosuccinimide, benzoyl peroxide, CCl₄, reflux, 16 h. b) Triethyl phosphite, 150 °C, 16 h. c) 1. KOtBu or NaH, THF, -10 or 0 °C, 20 min. then D, rt, 16 h. d) tetra-n-butylammonium fluoride, THF, 0 °C, rt, 16 h.

Table 10.1 Summary of envisioned fluorinated bis-styrylbenzenes

<table>
<thead>
<tr>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>R⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (Methoxy-X04)</td>
<td>OMe</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>11</td>
<td>OMe</td>
<td>OMe</td>
<td>OH</td>
</tr>
<tr>
<td>12</td>
<td>OMe</td>
<td>H</td>
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</tr>
<tr>
<td>13</td>
<td>OMe</td>
<td>H</td>
<td>CF₃</td>
</tr>
<tr>
<td>14</td>
<td>OMe</td>
<td>H</td>
<td>OCF₃</td>
</tr>
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<td>15</td>
<td>OMe</td>
<td>H</td>
<td>CF₃CF₂H</td>
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<tr>
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<td>OH</td>
<td>H</td>
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<td>H</td>
<td>CF₃CF₂H</td>
</tr>
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<td>OH</td>
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<td>O(CH₂)₃CF₃</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>22</td>
<td>O(CHF₂)₂CF₃</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>23</td>
<td>OH</td>
<td>H</td>
<td>OMe</td>
</tr>
<tr>
<td>24</td>
<td>OMe</td>
<td>H</td>
<td>OMe</td>
</tr>
</tbody>
</table>

(10), acidic functional groups are not required for high affinity Aβ binding, with the apparent Kᵦ’s of these fluorinated compounds being ~300 fold lower compared to 9. Despite their ability to label amyloid in vivo, however, the Flaherty compounds are very hydrophobic, and therefore their solubility and blood-brain barrier (BBB) passage are likely to limit their full potential as a ¹⁹F MRI agent. Furthermore, positioning of the ¹⁹F group directly on the planar backbone may have a detrimental effect on its relaxometry following binding, while it has been suggested that in addition to the inherent sensitivity of MRI also ¹⁹F relaxivity plays an important role in the
detection, due to a reduction of the transverse relaxation time ($T_2$) caused by binding to amyloid, or by a hydrophobic environment like brain tissue. Therefore, we set out to extend the existing bis-styrylbenzene library by adding one or more polar moieties to improve solubility and increase the number of (magnetically equivalent) fluorine atoms positioned such to maintain favorable NMR characteristics.

As a starting point, Methoxy-X04 (10) was chosen. With an affinity for Aβ in the nanomolar range ($K_i = 26.8$ nM), this fluorescent small molecule is frequently used for intravital microscopy studies as it has high affinity for Aβ plaques in vivo following intravenous or intraperitoneal injection. We designed a series of bis-styrylbenzenes with incorporating multiple preferably magnetically equivalent fluorine atoms (11 – 24) or additional minor modifications. (Scheme 10.1)

Compounds 11 – 24 are accessible from the general synthesis route given in Scheme 10.1, with the key building blocks being a diphosphonate (C) and an aromatic aldehyde (D). Substituted p-xylene (A) is subjected to radical bromination to yield dibromide (B). This dibromide is then treated with triethyl phosphite in an Arbuzov reaction to give diphosphonate (C). A Horner-Wadsworth-Emmons (HWE) reaction between diphosphonate (C) and an aldehyde (D) yields the (E, E)-bis-styrylbenzene (E). If necessary, an additional deprotection step with TBAF is carried out to remove the silyl protecting groups (only in the cases where the final compounds have hydroxyl substituents). Independently, a similar synthesis route for the design of NFT and amyloid probes based on styrylbenzenes was recently published by Boländer et al.

The building blocks needed for the synthesis of compounds 10 – 24 are depicted in Figure 10.2, and were synthetized or commercially available. (For synthesis of all building blocks, see Appendix I).

Results and Discussion

Fluorescent properties

All synthesized compounds are expected to have fluorescent properties based on their conjugated ring structures. We therefore determined excitation and emission wavelengths of 300 nM solutions and the corrected emission intensities were compared to that of Methoxy-X04. (Table 10.1)

It has been reported that binding to amyloid may have a significant effect on fluorescence properties, and therefore the fluorescence was also measured in the presence of synthetic Aβ fibrils. The parent compound 10 showed the highest intrinsic fluorescence with a 10-fold increase in the presence of amyloid fibrils. A similar increase was typically only observed for those compounds that only had minor modifications on the outer rings. Several previously reported Aβ-targeting fluorophores have shown a clear red-shifted emission spectrum following binding. Some of our compounds showed a similar red-shift; though resulting in multiple emission peaks in the spectrum. (Figure 10.3) This observation suggests the presence of multiple binding sites, similar to what has previously been reported, for example for Thioflavin.
Qualitative assessment of human and murine amyloid plaques binding

Fluorescence microscopy was used for a qualitative assessment of the amyloid-binding properties. A concentration series of each compound (1 – 10 – 100 μM) was applied to brain slices of APP/PS1 mice and human AD patients. At 100 μM all compounds, except 11 and 21, showed characteristic staining of amyloid plaques on both human and murine sections. At the lower concentrations, however, clear differences in affinity were found. (Table 10.1 and Figure 10.4) Amyloid plaques in humans and mice differ in composition and compactness. This is likely the reason that all compounds showed higher affinity for murine plaques as they were visible even after staining with 1 μM concentration, whereas compounds 19 – 21 and 23 – 24 showed no detectable amyloid plaques in the human sections at this concentration. To illustrate these differences, Table 10.1 and Figure 10.4 highlight the results of the 10 μM staining on human sections and the 1 μM concentration used to stain the murine sections. In general, planarity is considered one of the criteria for binding to Aβ plaques. The introduction of an additional substitution on the middle ring, which disturbs the planar conformation, has a detrimental effect on the binding affinity, as virtually no binding was seen using 11.

Although previous studies have reported possible interaction of styrylbenzenes with NFTs, for none of our compounds NFTs staining was observed in the human AD cortex at these concentrations. Furthermore, with no background staining in either human or murine control brain sections this suggested their specificity to amyloid. As stated in the previous paragraph, the fluorescence intensity with and without amyloid fibrils differed significantly for the various compounds. Therefore, the fluorescence intensity in the presence of fibrils was calculated and expressed relative to the intensity of Methoxy-X04 (10). (Table 10.1)
Table 10.1 Fluorescent and binding characteristics

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>Ex vivo Aβ bindinga</th>
<th>λex(^b)</th>
<th>λem</th>
<th>Fluorescence intensity(^c)</th>
<th>Fluorescence intensity on binding to fibrillar Aβ(^d)</th>
<th>Increase on binding to fibrillar Aβ</th>
<th>Ki (nM)</th>
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<tr>
<td>10</td>
<td>344.403</td>
<td>+</td>
<td>372</td>
<td>451</td>
<td>100</td>
<td>100</td>
<td>9.8</td>
<td>24.2</td>
</tr>
<tr>
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<td>436</td>
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<tr>
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<td>471</td>
<td>9</td>
<td>1</td>
<td>~1</td>
<td>n.d.</td>
</tr>
<tr>
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<td>456</td>
<td>10</td>
<td>1</td>
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<tr>
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<td>456</td>
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<td>1</td>
<td>~1</td>
<td>n.d.</td>
</tr>
<tr>
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<td>465</td>
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<td>472</td>
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<td>472</td>
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<td>-1</td>
<td>n.d.</td>
</tr>
<tr>
<td>24</td>
<td>508.452</td>
<td>-</td>
<td>355</td>
<td>494</td>
<td>37</td>
<td>4</td>
<td>-1</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

\(^a\) Staining of amyloid plaques in human (10 µM) and APP-PS1 murine (1μM) was scored whether the compound stained less (-), similar (+) or more (++) for amyloid plaques in comparison to 10. \(^b\) Ex/Em wavelength maxima were determined of 300 nM solutions. \(^c\) Fluorescence intensity was calculated relative to 10. \(^d\) Fluorescence intensity was calculated relative to intensity of 10 after binding to fibrillar Aβ. n.d. = not detected; n.p. = not performed.
Assuming that the fluorescence yield in the presence of synthetic Aβ is representative for that in the presence of amyloid plaques, the data in Figure 10.4 can be interpreted as follows. Despite having a lower fluorescent intensity for the bound compound, compounds 16 – 18 and 22 labeled more human amyloid, thus suggesting an improved binding compared to Methoxy-X04. Similar analysis of the stained APP/PS1 sections revealed the affinity of all compounds, except 11 and 21, to be significantly higher than that of our lead compound Methoxy-X04 (K<sub>d</sub> 26.8 nM). Compounds 13 and 15 were found to have the most efficient binding properties for murine amyloid plaques. With the lowest fluorescent intensity after binding to amyloid, being 100 x less than Methoxy-X04, the murine amyloid plaques nonetheless appeared very bright.

Affinity for synthetic amyloid-β fibrils
A competition assay with [³H]chrysamine G (6) was used to determine the binding inhibition coefficient (K<sub>i</sub>) for the compounds. Based on this assay reproducible results could only be obtained for those compounds with a hydroxyl substituent on the outer rings. (Figure 10.5) The ex vivo stainings clearly showed that many of the other compounds show affinity for amyloid, implying that these compounds probably use different binding sites than chrysamine G. Even the provided Ki values for compounds 19 – 23 only reflect competition against the [³H] chrysamine G binding sites, and therefore most likely underestimate the overall affinity of these compounds for synthetic Aβ fibrils.

LogP values
The blood-brain barrier (BBB) is a tight layer of endothelial cells in the wall of cerebral blood vessels that limits the passage of blood compounds into the brain. It is traditionally stated that for optimal passive BBB passage, compounds should preferably have moderately hydrophobicity (logD or logP 2.0 - 3.5) however a number of successful radiopharmaceuticals do not meet this requirement. Therefore LogP or LogD should be considered carefully as selection criterion,
Figure 10.4 Staining for murine and human amyloid plaques
Shown paraffin embedded 8 µm thick human AD sections are stained using 10 µM, whereas APP/PS1 murine sections are stained using 1 µM to best depict the differences in amyloid staining between the different compounds. Thirty micrometer thick murine APP/PS1 sections following in vivo administration are shown with two times less magnification to allow visualization of a larger cortical region. All images were digitized using the same settings.
but it is a valid parameter for selection nonetheless when applied within one series of compounds. For each of our compounds 10 – 24, logP values were determined with an HPLC-based method according to Benhaim et al.\textsuperscript{23-25} The found logP values are shown in Table 10.2. The limitation of this method is that logP values > 7 cannot be measured reliably. Not surprisingly, many of the compounds actually do show logP values > 7, which is a logical consequence of the fact that Methoxy-X04 (10) itself already has a logP value of 5.05 and that the introduction of fluorine makes the molecules more hydrophobic.

\textbf{In vivo amyloid plaque labeling in transgenic AD mice}

To assess the ability of the compounds to pass the BBB \textit{in vivo} and subsequently bind to amyloid, solutions of each compound were injected intravenously in living transgenic APP/PS1 mice that had extensive cerebral amyloid plaques. The mice were sacrificed, their brains were removed and post-mortem sections were studied using the same fluorescence microscopy set-up as for the stained brain sections. \textit{In vivo} labeling of amyloid plaque was observed for almost all compounds, except 11, 23 and 24, showing their ability to pass the BBB. (Figure 10.4 and Table 10.2) Apparently, a high logP value does not necessarily prohibit BBB passage. Some compounds, particularly 13, showed a comparable signal intensity after intravenous injection compared to Methoxy-X04, despite having a 100-fold lower fluorescence yield. This suggests that these compounds have a high affinity and/or cross the BBB more efficiently than Methoxy-X04.

<table>
<thead>
<tr>
<th>Compound</th>
<th>logP (calc)*</th>
<th>logP (determined)</th>
<th>\textit{In vivo} amyloid labeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>5.55</td>
<td>4.84</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>5.46</td>
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<td>12</td>
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<td>13</td>
<td>9.06</td>
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<td>8.44</td>
<td>&gt;7</td>
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<td>15</td>
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<td>16</td>
<td>8.74</td>
<td>6.18</td>
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<tr>
<td>17</td>
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<td>24</td>
<td>8.89</td>
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<td>-</td>
</tr>
</tbody>
</table>

*LogP values were calculated used web-based methods: www.molinspiration.com and http://intro.bio.umb.edu/111-112/OLLM/111F98/logp/test.html. The ability to label amyloid plaques in the brains of APP-PS1 mice following intravenous injection were scored absent (-) or present (+).
In vitro and post-mortem fluorine NMR

Based on the above experiments, compounds 13 and 22 were identified as the most promising leads, based on favorable binding, and positive staining after intravenous injection in vivo indicating good BBB passage. Initial $^{19}$F NMR studies were only conducted with compound 22, which had the highest number of magnetically equivalent $^{19}$F atoms. NMR spectra were acquired for 1.92 mM of 22 with or without an excessive amount of fibrillar Aβ. Binding to fibrillar amyloid did not result in significant line broadening of the NMR spectrum. (Figure 10.6A) The NMR spectrum corresponding to compound 22 mixed with homogenized APP/PS1 brain, however, revealed a small chemical shift of 0.03 ppm and a severe reduction in $T_2$ as observed by the line broadening of the peak. As suggested previously, most likely the lipophilic environment of the brain tissue itself results in a reduced relaxation time and thereby signal loss. Finally, 30 µmol/kg of 22 was injected intravenously in APP/PS1 mice. The brains were removed after 24 hours and a $^{19}$F NMR spectrum was obtained of the brain homogenate. No $^{19}$F signal was observed, although antemortem intravital microscopy showed clear labeling of cerebral amyloid. (Figure 10.6B-C)

The severe attenuation of the $^{19}$F NMR signal due the hydrophobic nature of both the fluorinated compounds as well as the brain itself seemed to hampered the detection of the $^{19}$F signal in the mouse brain. This is contrary to the initial publication of a $^{19}$F amyloid ligand by Higuchi et al., but in agreement with the findings of Amatsubo et al. Our design balanced between the planarity needed for amyloid binding and free rotation for the fluorine groups to maintain favourable NMR characteristics, however, this data still suggests that the hydrophobic interaction between the brain and the $^{19}$F groups are responsible for line broadening. As recently pointed out in vivo by Yanagisawa et al. this problem might be overcome by the use of a polyethylene glycol (PEG) linker to attach the fluorine groups further away from the amyloid binding core.
Summary and conclusion

In this work, a series of 15 analogs of Methoxy-X04 (10) with various number of fluorine atoms has been synthesized and evaluated for their Aβ binding properties and ability to pass the BBB. The incorporation of suitably placed fluoro substitutions could improve the current (MRI) contrast agents for the diagnosis of Alzheimer’s disease. It was concluded that the introduction of a second substitution on the inner ring was not well tolerated, whereas single bulky modifications on both the outer and inner rings were well tolerated. Despite the observed high logP values, brain entry did not seem to be inhibited for most compounds. Based on all findings, compounds 13 and 22 were considered most promising for the development of Aβ imaging agents. However, the post-mortem NMR results leave us to conclude that there seems to be no role for these compounds as MR imaging agents for the diagnosis of AD. To our opinion, it remains doubtful whether the incorporation of other fluorine moieties or higher field magnetic field strength will help to overcome these hurdles next to those set by the inherent relatively low sensitivity of 19F MRI in combination with known cerebral Aβ concentrations.

Nevertheless, this study expands the existing knowledge on bis-styrylbenzenes as amyloid targeting agents in general and creates opportunities for their application as fluorescent amyloid ligands for preclinical optical imaging. Recent advances in fluorine chemistry create further opportunities to radiolabel compounds of our series with 18F. These compounds might provide additional information regarding accumulation of cerebral amyloid, especially since we have found that our series of compounds bind to a distinct binding site.
Materials and Methods

**Preparation of Aβ<sub>1-40</sub> fibrils**

Aβ fibrils were prepared by stirring a 0.5 mg/ml solution of Aβ peptide (1-40) (RPeptide, Bogart, GA) at 37 ºC for 3 days, which resulted in a cloudy solution. The presence of fibrils was confirmed by the appearance of an emission peak at 482 nm (excitation 440 nm) upon addition of a 5 μM solution in PBS of Thioflavin T (Sigma, Germany) to a small amount of fibrils. Aliquots of 10 μl were transferred to Eppendorf vials and stored at -80 ºC until the assay was to be performed.

**Fluorescence spectra**

All compounds were dissolved in DMSO at 0.3 mM and diluted to 300nM with 9:1 PBS:ethanol. Fluorescence spectra were measured on a Varian Cary Eclipse fluorescence spectrophotometer to obtain peak excitation and emission wavelengths, which were used to select the correct fluorescence filter settings for further microscopic evaluation. All measurements were carried out at 20 ºC and in triplicate. 3D fluorescence spectra were obtained by adding 500 μL of the dissolved compounds to previously prepared Aβ aliquots. After manual shaking for 30 sec, 300 μL samples were measured (Infinity M1000, Tecan, Switzerland).

**Staining of human and transgenic AD brain sections**

Stock solutions of 3 mM in DMSO were diluted to 1 – 10 – 100 µM in 2:3 PBS:ethanol and sonicated for 15 minutes. Paraffin sections (8 µm) of the medial temporal lobe cortex of human AD subject that was assigned as Braak IV, 14 months old transgenic murine APP/PS1 brain and age-matched control cortices were deparaffinized prior to staining for 10 minutes in absolute darkness. After gently rinsing with tap water, sections were placed in 0.1 % NaOH in 80% ethanol for 2 minutes, air dried and coverslipped using Aqua / Polymount. Fluorescence of the stained sections was analyzed using a whole microscopic slide scanner (Pannoramic MIDI, 3DHistech, Hungary) with a DAPI filter cube (Ex 365 nm; Em 445/50 nm) using the same intensity setting throughout all experiments.

**In vivo Aβ plaque labeling in transgenic AD mice**

Twelve-to-fourteen-month-old APP/PS1 mice or age-matched wildtype animals (n=2 per compound) were injected intravenously with 0.05 M dissolved in 1:1 DMSO:Cremophor diluted with PBS to a total volume of 200 μl, resulting in a total dose of 30μmol/kg. One day after injection, animals were sacrificed using 200 μl Euthanasol (AST Pharma) prior to transcardial perfusion with 4% paraformaldehyde in PBS. Brains were removed and cryoprotected in 4% PFA with 10% sucrose for 4 hours followed by immersion in 4% PFA with 30% sucrose overnight. Snap-frozen brains were cryosectioned (30 µm) and fluorescence images were analyzed as described above.

**Competition binding assay**

Competition binding experiments were conducted at room temperature, in a final volume of 1 ml assay buffer (150 mM Tris-HCl, 20% ethanol, pH 7.0). Compounds were dissolved as 3 mM stock solutions in DMSO, sonicated for 15 min, and used in a final concentration range of 30
μM to 30 pM. 10 μl of unlabeled test compounds was combined with 890 μl of assay buffer and 50 μl of 100 nM [3H]-chrysamine G stock (specific activity 33.8 Ci/m mole). The mixture was sonicated for 10 min, and the assay was subsequently started by the addition of 50 μl synthetic Aβ1-40 fibrils, to achieve final concentrations of 5 nM [3H]chrysamine G and 50 nM fibrils. Nonspecific binding was determined in the presence of 1 μM Methoxy-X04. Incubations were terminated after 1 h via filtration through Whatman GF/B filters (pre-soaked in binding buffer), using a 48-well Brandel harvester. The filters were washed two times with 3 ml of ice-cold binding buffer (pH 7.0), and radioactivity was determined by liquid scintillation spectrometry in 5 ml of Optiphase-HiSafe 3, at an efficiency of 40 %.

\[ K_i \] values were determined by nonlinear regression analysis using the equation: 
\[
\log EC_{50} = \log [10^{\log K_i} \times (1 + \text{RadioligandNM}/\text{HotKdNM})]
\]

where \( K_i = 200 \text{ nM} \), and radioligand = 5nM. (GraphPad Software Inc., San Diego, CA)

**Procedure for logP determinations**

LogP determinations were performed using literature procedures. The measurements were performed on a Jasco HPLC-system (detection simultaneously at 214 and 254 nm) coupled to a Perkin Elmer Sciex API 165 mass instrument with a custom-made Electrospray Interface (ESI). An analytical Gemini C18 column (Phenomenex, 50 x 4.60 mm, 3 micron) was used in combination with buffers A) phosphate buffer of pH = 7.0 (0.02 M Na₂HPO₄ adjusted to pH = 7.0 with phosphoric acid) and B) 0.25% octanol in methanol.

Of all compounds to be evaluated, stock solutions of 0.5 mg/ml were prepared in methanol. These stock solutions were then diluted with water, making sure that the volume percentage of water was such that the compounds did not precipitate (max 40% water). A 0.25 mg/ml solution of NaNO₃ in water was used as a non-retaining compound to determine the dead time of the system. For calibration purposes known compounds were taken from the literature. Of these compounds, 0.25 mg/ml solutions in either 75% H₂O/MeOH or 50% H₂O/MeOH were made.

For each sample, a series of four isocratic runs was performed, (for instance 55, 60, 65, 70% B), and the retention times (from UV-detection at 214 nm) thus obtained were converted to the retention factor \( k' \) according to the formula 
\[
k' = (t_R - t_0)/t_0
\]
with \( t_R \) being the retention time of the compound and \( t_0 \) being the retention time of NaNO₃. The retention factors were extrapolated to 0% B, yielding \( k'_w \). As there is a linear relationship between logP and log \( k'_w \), plotting logP values of known compounds (Table 10.3) against obtained log \( k'_w \) values in this system yields a calibration curve. From this curve, logP values of unknown compounds can be calculated from their \( k'_w \) values.

**In vitro ¹⁹F NMR analyses**

To investigate the possible effect on its NMR properties caused by either binding to amyloid or the lipophilic environment of the brain several samples were prepared similar to the protocol described previously. The in vitro ¹⁹F NMR analyses were performed using a Bruker DMX400 NMR spectrometer (Bruker, Germany). All samples were diluted with 10% D₂O in 0.1 M PBS with one EDTA-free protease inhibitor tablet (Complete Mini, Roche Diagnostics) added to every 10 ml. Aliquots (500 μl) of only the solvent, the solvent containing 22 μM of aggregated Aβ or the
Brain homogenate were added to 20 µl of 0.05 M of compound 22 in 1:1 DMSO:Cremophor to achieve a final concentration of 1.92 mM of compound 22. The mixtures were transferred to a standard 5 mm NMR tube. NMR spectra were obtained using a single pulse sequence with a 22,573 Hz spectral width (SW) and 100 scans. The chemical shifts of the 19F NMR signals were identified by setting the reference trifluoroacetic acid (TFA) at 0 ppm.

Post-mortem 19F NMR in APP/PS1 mice

Twelve-to-fourteen-month-old APP/PS1 mice (n=2) received an intravenous injection with 0.05 M of compound 22 dissolved in 1:1 DMSO:Cremophor diluted with PBS to a total volume of 200 µl to achieve a total dose of 30 µmol/kg. Prior injection the animals underwent cranial window surgery.21 Twenty four hours post injection the animals underwent in vivo multiphoton microscopy (LSM 710 MP, Carl Zeiss, Germany) operating at 750 nm to validate the presence and labeling of cerebral amyloid. After perfusion the resected brain was snap frozen and prepared for NMR similar to the above brain homogenates. A similar fluorine NMR spectrum was obtained using a single pulse sequence with a 94340 Hz SW and 1200 scans.

### Table 10.3 Reference logP values

<table>
<thead>
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<th>compound</th>
<th>logP</th>
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<tr>
<td>resorcinol</td>
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<td>p-nitroaniline</td>
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<td>phenol</td>
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</tr>
<tr>
<td>naphtalene</td>
<td>3.37</td>
</tr>
</tbody>
</table>

Based on known literature values, the logP of several reference compounds yield a required calibration curve to determine the logP values of our compounds.21-23

### Synthetic Procedures

**General**

Reagents and solvents were used as provided, unless stated otherwise. 4-trifluoromethylbenzaldehyde (35), 4-trifluoromethoxybenzaldehyde (36), 4-(1,1,2,2-tetrafluoroethyl) benzaldehyde (37), 3-trifluoromethyl-4-methoxybenzaldehyde (38) (Figure 10.2) were purchased at standard suppliers. The other building blocks were prepared following literature procedures (see Appendix I). THF was distilled over LiAlH₄ prior to use. Reactions were carried out under inert conditions and ambient temperature, unless stated otherwise. Prior to performing a reaction, traces of water were removed from the starting materials by repeated coevaporation with anhydrous 1,4-dioxane or anhydrous toluene. These solvents were stored over 4 Å molsieves. Reactions were monitored by thin layer chromatography on aluminum coated silica sheets (Merck, silica 60 F254), using visualization either with iodine, or spraying with a solution of 25 g (NH₄)₆MoO₄, 10 g (NH₄)₂Ce(SO₄)₃ in 100 ml H₂SO₄ and 900 ml H₂O, or a
solution of 20% H$_2$SO$_4$ in ethanol, followed by charring at ~150 °C. Column chromatography was carried out with silica gel (Screening Devices bv, 40-63 μm particle size, 60 Å), using technical grade solvents. NMR spectra were recorded at 298K on a Bruker AV400 using deuterated solvents. All carbon spectra are proton-decoupled. Chemical shifts (δ) are given in ppm, in $^{13}$C spectra relative to the solvent peaks of CDCl$_3$ (77.0 ppm), CD$_2$OD (49.0 ppm), DMF-d$_7$ (29.76 ppm), acetone-d$_6$ (29.9 ppm) or DMSO-d$_6$ (39.51 ppm), in $^1$H spectra relative to the solvent peak of tetramethyldisilane (0.0 ppm), CD$_2$OD (3.31 ppm), DMF-d$_7$ (2.75 ppm), acetone-d$_6$ (2.05 ppm) or DMSO-d$_6$ (2.50 ppm), in $^{19}$F spectra relative to the solvent peak of TFA (0 ppm). Coupling constants are given in Hz. IR spectra were recorded on a Perkin Elmer Paragon 1000 FT-IR Spectrometer. High resolution mass spectra were recorded by direct injection (2 µL of a 2 µM solution in H$_2$O/MeCN; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylphthalate (m/z = 391.28428) as a “lock mass”. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). It should be noted that, with the exception of compound 9 (data not shown) and only certain intermediates ionized correctly, none of the target compounds provided useful (HR)MS data.

LC-MS analysis was performed on a Finnigan Surveyor HPLC system with a Gemini C$_{18}$ 50 × 4.6 mm column (3 micron, Phenomenex, Torrance, CA, USA) (detection at 200-600 nm), coupled to a Thermo Finnigan LCQ Advantage Max mass spectrometer (Breda, The Netherlands) with electrospray ionization (ESI; system 1), with as eluents (A): H$_2$O; (B): MeCN and (C): 1% aq. TFA.

**General procedure A: Horner-Wadsworth-Emmons reaction with NaH**
The diphosphonate (0.83 mmol) was dissolved in THF (2.6 ml) and cooled to 0 °C. NaH (60% wt. dispersion in mineral oil, 0.17 g, 4.17 mmol) was added and the mixture stirred for 30 min at 0°C. The aldehyde (2.08 mmol) was dissolved in THF and added to the reaction mixture, which was subsequently stirred for 16 h at rt. After cooling and quenching with water, the mixture was extracted three times with EtOAc and the combined organic layers were washed with sat. aq. NaHCO$_3$, dried (Na$_2$SO$_4$), filtered and concentrated. The crude product was subjected to column chromatography to yield the pure product.

**General procedure B: Horner-Wadsworth-Emmons reaction with KOTBu**
The diphosphonate (0.95 mmol) was dissolved in THF (20 ml) and cooled to -10 °C. KOTBu (0.30 g, 2.45 mmol) was added and the black mixture stirred for 20 min. A solution of the aldehyde (2.37 mmol) in THF (6 ml) was added and the reaction stirred at room temperature for 16 h. The reaction was cooled to 0 °C, quenched with water and extracted five times with EtOAc. The combined organic layers were dried (Na$_2$SO$_4$), filtered and concentrated. The crude product was subjected to column chromatography to yield the pure product.

**General procedure C: silyl deprotection**
A solution of protected bis-styrylbenzene (0.63 mmol) in THF (2 ml) was cooled to 0 °C and TBAF (1 M in THF, 3.14 ml, 3.14 mmol) was added. The blood-red solution was stirred for 16 h after
which water was added and the reaction mixture was extracted with EtOAc. To the aqueous layer 1 M HCl was added, followed by two times extracting with EtOAc. The combined organic layers were dried (Na$_2$SO$_4$), filtered and concentrated. The pure product was obtained by column chromatography (0 → 25% EtOAc/light petroleum).

(E, E)-1-methoxy-2,5-bis(4-hydroxy)styrylbenzene; Methoxy-X04 (10)

Methoxy-X04 was prepared according to general procedures A and C, using diphosphonate 25 and aldehyde 33. Physical data corresponded to those reported by Klunk et al.$^{10}$

(E, E)-1,4-dimethoxy-2,5-bis(4-hydroxy)styrylbenzene (11)

Following general procedure A, diphosphonate 26 (0.34 g, 0.78 mmol) was reacted with aldehyde 33 (0.46 g, 1.9 mmol). A bright yellow solid was isolated (221 mg) by column chromatography (10 → 30% EtOAc/light petroleum) of which 100 mg was purified by preparative HPLC (40:60 → 80:20 of 20 mM NH$_4$OAc/MeOH), to yield 22 mg (0.059 mmol, 8%) of compound 11.

$^1$H NMR (CD$_3$OD, 400 MHz): δ 7.43 (d, $J = 8.6$ Hz, 4H); 7.33 (d, $J = 16.5$ Hz, 2H); 7.27 (s, 2H); 7.19 (d, $J = 16.5$ Hz, 2H); 6.82 (d, $J = 8.6$ Hz, 4H); 3.93 (s, 6H). $^{13}$C NMR (CD$_3$OD, 100 MHz): δ 158.5; 152.6; 130.9; 129.7; 128.9; 127.6; 121.1; 116.6; 109.9; 56.8. IR (neat): 3359.6; 1605.3; 1515.7; 1495.6; 1463.6; 1435.4; 1408.7; 1260.0; 1196.0; 1171.8; 1022.4; 958.3; 849.6; 819.7; 790.1; 685.9; 551.0; 521.6. LC-MS retention time: 8.99 min (10 → 90% MeCN, 15 min run).

(E, E)-1-methoxy-2,5-bis(4-trifluoromethyl)styrylbenzene (13)

Following general procedure A, diphosphonate 26 (0.20 g, 0.5 mmol) was reacted with aldehyde 34 (0.31 g, 1.25 mmol). The crude product was subjected to column chromatography (0 → 2% EtOAc/light petroleum) to yield the intermediary bis-TBDMS protected styrylbenzene (0.28 g, 0.48 mmol, 96%) as a bright yellow solid. $^1$H NMR (CDCl$_3$, 400 MHz): δ 7.58 (d, $J = 8.1$ Hz, 1H); 7.51 (d, $J = 6.1$ Hz, 2H); 7.49 (d, $J = 6.3$ Hz, 2H); 7.44 (s, 1H); 7.34-7.28 (m, 4H); 7.16-7.13 (m, 2H); 7.13-7.09 (m, 2H); 7.03 (d, $J = 1.4$ Hz, 1H); 4.75 (d, $J = 2.4$ Hz, 4H); 3.95 (s, 3H); 0.95 (s, 9H); 0.95 (s, 9H); 0.11 (s, 6H); 0.11 (s, 6H). $^{13}$C NMR (CDCl$_3$, 100 MHz): δ 157.0; 141.0; 140.7; 137.9; 136.7; 136.0; 128.7; 128.4; 128.1; 126.4; 126.4; 126.0; 122.7; 119.3; 108.6; 64.9; 64.8; 55.6; 26.0; 18.4; -5.2. IR (neat): 2953.7; 2927.5; 2855.3; 1593.8; 1553.7; 1515.3; 1458.3; 1421.9; 1377.6; 1248.7; 1206.0; 1084.2; 1037.1; 1005.7; 967.3; 837.4; 773.9; 668.1; 504.2. The bis-TBDMS protected (E, E)-styrylbenzene (0.27 g, 0.46 mmol) was treated with TBAF according to general procedure C. Compound 12 was obtained by column chromatography (50% EtOAc/light petroleum → 10% MeOH/EtOAc) as a bright yellow solid (0.14 g, 0.37 mmol, 79%).

$^1$H NMR (DMF-d$_7$, 400 MHz): δ 7.68 (d, $J = 8.0$ Hz, 1H); 7.59 (d, $J = 8.1$ Hz, 2H); 7.55 (d, $J = 8.1$ Hz, 2H); 7.49 (d, $J = 16.6$ Hz, 1H); 7.39-7.34 (m, 4H); 7.31 (d, $J = 5.0$ Hz, 2H); 7.28 (d, $J = 4.9$ Hz, 1H); 7.25-7.21 (m, 2H). $^{13}$C NMR (DMF-d$_7$, 100 MHz): δ 157.7; 141.0; 140.7; 137.9; 136.7; 136.0; 128.7; 128.4; 128.1; 126.4; 126.4; 126.0; 122.7; 119.3; 108.6; 64.9; 64.8; 55.6; 26.0; 18.4; -5.2. IR (neat): 2953.7; 2927.5; 2855.3; 1593.8; 1553.7; 1515.3; 1458.3; 1421.9; 1377.6; 1248.7; 1206.0; 1084.2; 1037.1; 1005.7; 967.3; 837.4; 773.9; 668.1; 504.2. The bis-TBDMS protected (E, E)-styrylbenzene (0.27 g, 0.46 mmol) was treated with TBAF according to general procedure C. Compound 12 was obtained by column chromatography (50% EtOAc/light petroleum → 10% MeOH/EtOAc) as a bright yellow solid (0.14 g, 0.37 mmol, 79%).

(E, E)-1-methoxy-2,5-bis(4-trifluoromethyl)styrylbenzene (13)

Following general procedure A, diphosphonate 26 (0.20 g, 0.5 mmol) was reacted with aldehyde
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35 (0.22 g, 1.25 mmol). After work-up, the crude product was purified by column chromatography (0 → 1% EtOAc/light petroleum) and the compound 13 was obtained as a bright yellow solid (80 mg, 0.18 mmol, 36%).

1H NMR (acetone-d₆, 400 MHz): δ 7.82 (d, J = 8.2 Hz, 2H); 7.79 (d, J = 8.5 Hz, 2H); 7.76-7.69 (m, 5H); 7.66 (d, J = 16.7 Hz, 2H); 7.43 (s, 2H); 7.38 (d, J = 16.4 Hz, 2H); 7.29 (dd, J = 8.1 Hz, J = 1.3 Hz, 1H); 4.00 (s, 3H).

13C NMR (acetone-d₆, 100 MHz): δ 158.5; 143.0; 142.4; 139.3; 132.1; 128.3; 128.3; 127.9; 127.9; 127.8; 126.7; 126.5; 126.5; 126.5; 120.7; 110.3; 56.1.

19F NMR (acetone-d₆, 375 MHz): δ 12.70 (s, 3F); 12.65 (s, 3F). IR (neat): 1608.4; 1464.0; 1420.1; 1323.6; 1246.5; 1158.5; 1116.6; 1059.5; 1065.5; 1036.4; 1014.1; 967.0; 954.2; 866.5; 848.8; 829.9; 759.2; 744.8; 622.2; 593.3; 508.4.

(E, E)-1-methoxy-2,5-bis(4-trifluoromethoxy)styrylbenzene (14)
Following general procedure A, diphosphonate 25 (0.20 g, 0.5 mmol) was reacted with aldehyde 36 (0.24 g, 1.25 mmol). After work-up, the product 14 was obtained by column chromatography (0 → 1.5% EtOAc/light petroleum) as a bright yellow solid (83 mg, 0.17 mmol, 35%).

1H NMR (CDCl₃, 400 MHz): δ 7.56 (d, J = 8.0 Hz, 1H); 7.54-7.50 (m, 4H); 7.44 (d, J = 16.5 Hz, 1H); 7.19 (t, J = 7.8 Hz, 4H); 7.14-7.10 (m, 2H); 7.09-7.05 (m, 2H); 7.01 (d, J = 1.3 Hz, 1H); 3.94 (s, 3H).

13C NMR (CDCl₃, 100 MHz): δ 157.2; 137.7; 136.7; 135.9; 127.7; 127.5; 126.6; 125.9; 124.1; 121.2; 119.4; 110.3; 55.5.

19F NMR (CDCl₃, 375 MHz): δ 19.80 (s, 3F); 19.79 (s, 3F). IR (neat): 1593.1; 1557.6; 1510.2; 1421.9; 1392.9; 1302.4; 1274.0; 1195.2; 1115.9; 1104.9; 1034.0; 1015.7; 962.2; 921.6; 838.6; 673.8; 620.1; 530.0; 505.9.

(E, E)-1-methoxy-2,5-bis(4-{1,1,2,2-tetrafluoroethyl})styrylbenzene (15)
Following general procedure A, diphosphonate 25 (0.20 g, 0.5 mmol) was reacted with aldehyde 37 (0.28 g, 1.25 mmol). After work-up, compound 15 was isolated by column chromatography (0 → 7.5% EtOAc/light petroleum) as a yellow solid (0.14 g, 0.25 mmol, 50%).

1H NMR (CDCl₃, 400 MHz): δ 7.56 (d, J = 7.3 Hz, 1H); 7.55-7.50 (m, 4H); 7.33 (d, J = 16.5 Hz, 1H); 7.24-7.16 (m, 4H); 7.13-7.04 (m, 4H); 7.01 (d, J = 2.3 Hz, 1H); 6.07-5.76 (m, 2H); 3.94 (s, 3H).

13C NMR (CDCl₃, 100 MHz): δ 157.1; 148.5; 148.2; 137.7; 136.4; 135.6; 129.2; 128.7; 127.9; 127.8; 127.6; 127.5; 126.6; 125.8; 122.4; 121.9; 121.8; 121.8; 121.7; 119.4; 119.1; 116.7; 116.4; 116.2; 115.0; 110.2; 108.7; 108.1; 107.7; 107.3; 55.5. 19F NMR (CDCl₃, 375 MHz): δ -10.5 (s, 4F); -59.06 (t, J = 5.5 Hz, 2F); -59.20 (t, J = 5.5 Hz, 2F). IR (neat): 1510.5; 1463.9; 1421.9; 1392.9; 1302.4; 1274.0; 1195.2; 1115.4; 1035.7; 1015.7; 961.8; 837.0; 784.1; 766.2; 709.4; 623.3; 600.0; 544.1.

(E, E)-1-hydroxy-2,5-bis(4-trifluoromethyl)styrylbenzene (16)
Following general procedure B, diphosphonate 27 (0.85 g, 1.34 mmol) was reacted with aldehyde 35 (0.59 g, 3.36 mmol). After work-up, the crude product was purified by column chromatography (0 → 6% EtOAc/light petroleum) and product 16 was obtained as a bright yellow solid (0.2 g, 0.48 mmol, 36%).

1H NMR (CD₃OD, 400 MHz): δ 7.67-7.52 (m, 10H); 7.21 (d, J = 16.5 Hz, 1H); 7.14 (d, J = 2.2Hz, 2H); 7.07 (dd, J = 8.1 Hz, J = 1.5 Hz, 1H); 7.02 (d, J = 1.6 Hz, 1H). 13C NMR (CD₃OD, 100 MHz): δ 156.2; 142.7; 141.9; 138.6; 131.7; 129.2; 127.8; 127.8; 127.5; 127.4; 127.2; 126.9; 126.2; 126.2; 126.1; 126.1; 125.0; 119.5; 114.3. 19F NMR (CD₃OD, 375 MHz): δ 14.98 (s, 3F); 14.91 (s, 3F). IR (neat):
(E, E)-1-hydroxy-2,5-bis(4-trifluoromethoxy)styrylbenzene (17)
Following general procedure B, diphosphonate 27 (0.99 g, 1.56 mmol) was reacted with aldehyde 36 (0.74 g, 3.91 mmol). The crude product was purified by column chromatography (0 → 8% EtOAc/light petroleum) and product 17 was obtained as a bright yellow solid (0.47 g, 1.01 mmol, 65%).

1H NMR (CD3OD, 400 MHz): δ 7.63 (d, J = 5.8 Hz, 2H); 7.61 (d, J = 5.6 Hz, 2H); 7.48 (d, J = 16.5 Hz, 1H); 7.40-7.34 (m, 1H); 7.27-7.18 (m, 5H); 7.12 (d, J = 3.2 Hz, 2H); 7.07 (dd, J = 8.1 Hz, J = 1.5 Hz, 1H); 7.02 (d, J = 1.6 Hz, 1H). 13C NMR (CD3OD, 100 MHz): δ 156.7; 149.7; 149.4; 149.4; 139.2; 139.0; 138.1; 133.0; 130.8; 129.0; 128.8; 128.1; 127.9; 126.0; 125.4; 122.3; 121.6; 119.7; 114.4. 19F NMR (CD3OD, 375 MHz): δ 18.85 (s, 6F). IR (neat): 1604.3; 1557.6; 1515.8; 1505.7; 1269.9; 1158.4; 1099.9; 1015.6; 967.6; 928.0; 839.2; 676.6; 624.0; 525.1.

(E, E)-1-hydroxy-2,5-bis(4-{1,1,2,2-tetrafluoroethyl})styrylbenzene (18)
Following general procedure B, diphosphonate 27 (0.74 g, 1.17 mmol) was reacted with aldehyde 37 (0.65 g, 2.92 mmol). After work-up, the crude product was subjected to column chromatography (0 → 10% EtOAc/light petroleum) to yield product 18 as a bright yellow solid (0.27 g, 0.5 mmol, 43%).

1H NMR (CD3OD, 400 MHz): δ 7.61 (d, J = 5.3 Hz, 2H); 7.59 (d, J = 5.2 Hz, 2H); 7.56 (d, J = 8.1 Hz, 1H); 7.25-7.19 (m, 5H); 7.12 (d, J = 5.5 Hz, 2H); 7.08 (dd, J = 7.9 Hz, J = 1.7 Hz, 1H); 7.02 (d, J = 1.5 Hz, 1H); 6.46-6.14 (m, 2H). 13C NMR (CD3OD, 100 MHz): δ 156.6; 149.2; 139.2; 138.4; 137.6; 130.4; 128.8; 128.6; 128.1; 128.0; 127.9; 126.0; 125.4; 123.2; 121.6; 119.7; 114.4.

(E, E)-1-trifluoromethyl-2,5-bis(4-hydroxy)styrylbenzene (19)
Following general procedure A, diphosphonate 28 (0.37 g, 0.83 mmol) was reacted with aldehyde 33 (0.49 g, 2.1 mmol). After work-up, the crude product was purified by column chromatography (0 → 4% EtOAc/light petroleum) and the intermediary bis-TBDMS protected styrylbenzene was obtained as a bright yellow solid (0.36 g, 0.54 mmol, 71%). 1H NMR (CDCl3, 400 MHz): δ 7.77-7.72 (m, 2H); 7.61 (d, J = 5.3 Hz, 2H); 7.59 (d, J = 5.2 Hz, 2H); 7.56 (d, J = 8.1 Hz, 1H); 7.48 (d, J = 16.6 Hz, 1H); 7.25-7.19 (m, 5H); 7.12 (d, J = 5.5 Hz, 2H); 7.08 (dd, J = 7.9 Hz, J = 1.7 Hz, 1H); 7.02 (d, J = 1.5 Hz, 1H); 6.46-6.14 (m, 2H). 13C NMR (CDCl3, 100 MHz): δ 156.0, 155.9, 136.5, 135.0; 131.7, 130.3, 130.2, 129.6, 129.0, 128.1, 127.9, 126.9, 125.0, 123.8, 122.0, 120.4, 29.7, 25.7, -4.4. 19F NMR (CDCl3, 375 MHz): δ 18.17 (s, 3F). IR (neat): 1598.9; 1508.0; 1471.8; 1327.9; 1314.0; 1251.5; 1170.0; 1154.7; 1131.4; 1115.4; 1102.1; 1051.1; 962.1; 938.5; 906.8; 834.5; 778.2; 700.2; 667.7; 660.3; 638.4; 554.5; 534.7. The bis-TBDMS protected styrylbenzene (0.34 g, 0.54 mmol) was treated with TBAF according to general procedure C, and following column chromatography (0 → 30% EtOAc/light petroleum), the impure product was purified by HPLC (CN column, 35:65 → 10:90 of 0.2% aq. TFA/MeOH) and product 19 was obtained as a yellow solid (83 mg, 0.22 mmol, 40%).

1H NMR (CD3OD, 400 MHz): δ 7.81 (d, J = 8.2 Hz, 1H); 7.73 (s, 1H); 7.70 (d, J = 8.5 Hz, 1H); 7.42 (d, J = 8.0 Hz, 2H); 7.38 (d, J = 8.0 Hz, 2H); 7.23 (d, J = 16.0 Hz, 1H); 7.14 (t, J = 16.9, 2H); 6.99 (d, J =
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16.3 Hz, 1H); 6.80 (dd, J = 7.8 Hz, J = 3.5 Hz, 4H).

13C NMR (CD3OD, 100 MHz): δ 159.1; 158.9; 138.3; 136.2; 133.3; 131.1; 130.2; 130.0; 129.3;
129.2; 128.4; 128.0; 127.5; 125.0; 124.9; 124.7; 124.7; 124.6; 124.6; 121.7; 116.7; 116.6. 19F NMR
(CD3OD, 375 MHz): δ 17.64 (s, 3F). IR (neat): 1605.0; 1512.1; 1441.0; 1313.9; 1257.1; 1239.9;
1199.4; 1171.9; 1153.6; 1108.8; 1079.5; 1049.9; 959.8; 869.9; 837.2; 812.7; 671.7; 552.7; 522.1.

(E, E)-1-(2,2,2-trifluoroethoxy)-2,5-bis(4-hydroxy)styrylbenzene (20)

Following general procedure B, diposphonate 29 (0.45 g, 0.95 mmol) was reacted with aldehyde 33 (0.56 g, 2.37 mmol). After work-up, the crude product was subjected to column chromatography (0 → 5% EtOAc/light petroleum) and the bis-TBDMS protected styrylbenzene was obtained as a bright yellow solid (0.4 g, 0.63 mmol, 66%). 1H NMR (CDCl3, 400 MHz): δ 7.59-
7.51 (m, 4H); 7.43 (d, J = 16.5 Hz, 1H); 7.31 (d, J = 7.7 Hz, 1H); 7.69 (d, J = 8.0 Hz, 1H); 7.24 (d, J =
16.4 Hz, 1H); 7.18 (d, J = 16.3 Hz, 1H); 7.00 (dd, J = 8.6 Hz, J = 2.0 Hz, 4H); 4.56 (q, J = 8.2 Hz,
J = 8.1 Hz, 2H); 1.16 (s, 18H); 0.38 (s, 6H); 0.38 (s, 6H).

13C NMR (CDCl3, 100 MHz): δ 155.6; 155.5; 154.7; 137.9; 131.8; 131.0; 130.3; 129.3; 128.6; 127.7;
126.7; 126.7; 125.8; 121.0; 120.4; 120.3; 120.1; 110.7; 67.2; 66.8; 66.5; 66.1; 29.7; 25.6; -4.4. 19F NMR (CDCl3, 375 MHz): δ
3.80 (t, J = 8.1 Hz, 3F).

The bis-TBDMS protected styrylbenzene (0.4 g, 0.63 mmol) was treated with TBAF according to
general procedure C. After work-up, the crude product was purified by column chromatography
(0 → 25% EtOAc/light petroleum) and product 20 was obtained as a bright yellow solid (0.127
0.31 mmol, 49%). 1H NMR (CD3OD, 400 MHz): δ 7.51 (d, J = 8.1 Hz, 1H); 7.40-7.29 (m, 4H); 7.20 (d,
J = 16.5 Hz, 1H); 7.13 (dd, J = 1.1 Hz, 1H); 6.87 (d, J = 16.3 Hz, 1H); 6.81-6.75 (m, 4H); 4.48 (q, J = 8.3 Hz, J = 8.3 Hz, 2H).

13C NMR (CD3OD, 100 MHz): δ 157.8; 157.6; 155.5; 138.9; 130.4; 130.0; 129.7; 126.7; 126.7;
125.8; 121.0; 120.4; 120.3; 120.1; 110.7; 67.2; 66.8; 66.5; 66.1; 29.7; 25.6; -4.4. 19F NMR (CDCl3, 375 MHz): δ
3.80 (t, J = 8.1 Hz, 3F).

(E, E)-1-(4,4,4-trifluorobutoxy)-2,5-bis(4-hydroxy)styrylbenzene (21)

Following general procedure B, diposphonate 30 (0.50 g, 1 mmol) was reacted with aldehyde 33 (0.59 g, 2.5 mmol). After work-up, the crude product was purified by column chromatography
(0 → 2% EtOAc/light petroleum) and the bis-TBDMS protected styrylbenzene was obtained as
a bright yellow solid (0.3 g, 0.46 mmol, 46%). 1H NMR (CDCl3, 400 MHz): δ 7.53 (d, J = 8.1 Hz, 1H);
7.38 (d, J = 8.4 Hz, 4H); 7.28 (d, J = 16.5 Hz, 1H); 7.12-7.05 (m, 2H); 7.03 (d, J = 16.2 Hz, 1H); 6.95
(d, J = 1.1 Hz, 1H); 6.92 (d, J = 16.3 Hz, 1H); 6.86-6.80 (m, 4H); 4.13 (t, J = 6.0 Hz, 2H); 2.46-2.31 (m,
2H); 2.20-2.10 (m, 2H); 0.99 (s, 18H); 0.21 (s, 12H). 13C NMR (CDCl3, 100 MHz): δ 155.9; 155.6;
155.4; 137.9; 131.3; 130.6; 128.7; 128.3; 127.7; 127.6; 126.5; 126.5; 126.1; 120.9; 120.4; 120.3;
119.6; 109.6; 66.6; 31.1; 30.8; 29.4; 25.7; 22.4; 22.3; 18.2; -4.4. 19F NMR (CDCl3, 375 MHz): δ
11.39 (t, J = 10.9 Hz, 3F). IR (neat): 1599.9; 1508.7; 1471.7; 1250.8; 1166.6; 1153.9; 1027.7; 908.7;
833.2; 798.6; 779.1; 700.5; 661.8; 623.5; 531.4; 504.4.
The silylated bis-styrylbenzene (0.3 g, 0.46 mmol) was deprotected according to general procedure C. After work-up, the crude product was purified using column chromatography (10 → 22.5% EtOAc/light petroleum) and product 21 was obtained as a bright yellow solid (0.12 g, 0.27 mmol, 58%).

\[ ^{1}H\text{ NMR (CD}_{3}\text{OD, 400 MHz): } \delta 7.50 (d, J = 8.1 Hz, ^{1}H); 7.38 (d, J = 8.6 Hz, 2H); 7.34 (d, J = 8.6 Hz, 2H); 7.24 (d, J = 16.5 Hz, ^{1}H); 7.11-7.02 (m, 4H); 6.92 (d, J = 16.3 Hz, ^{1}H); 6.80-6.75 (m, 4H); 4.13 (t, J = 6.0 Hz, 2H); 2.50-2.35 (m, 2H); 2.16-2.06 (m, 2H). \]

\[ ^{13}C\text{ NMR (CD}_{3}\text{OD, 100 MHz): } \delta 158.4; 158.2; 157.3; 139.4; 131.1; 130.4; 129.7; 129.5; 128.9; 128.6; 127.3; 127.1; 126.6; 121.1; 120.4; 116.5; 110.8; 67.8; 32.1; 31.8; 31.5; 31.2; 23.5; 23.4; 23.4. \]

\[ ^{19}F\text{ NMR (CD}_{3}\text{OD, 375 MHz): } \delta 3.48 (t, J = 11.3 Hz, 3F). \]

IR (neat): 3325.9; 1605.69; 1593.7; 1515.5; 1505.9; 1447.9; 1385.8; 1338.1; 1241.0; 1171.3; 1026.0; 961.8; 826.7; 621.1; 519.8.

\[(E, E)-1-(nonafluoro-tert-butoxy)-2,5-bis(4-hydroxy)styrylbenzene (22)\]

Following general procedure B, diphosphonate 32 (0.55 g, 0.89 mmol) was reacted with aldehyde 33 (0.53 g, 2.2 mmol). After work-up, the crude product was purified by column chromatography (0 → 2% EtOAc/light petroleum) and the bis-TBDMS protected styrylbenzene was obtained as a bright yellow solid (0.22 g, 0.28 mmol, 32%).

\[ ^{1}H\text{ NMR (CDCl}_{3}\text{, 400 MHz): } \delta 7.27-7.20 (m, 4H); 7.20-7.12 (m, 2H); 6.90-6.81 (m, 2H); 6.79-6.73 (m, 2H); 6.72-6.64 (m, 4H); 7.48 (d, J = 8.2 Hz, ^{1}H); 0.84 (s, ^{1}H); 0.06 (s, 6H); 0.06 (s, 6H). \]

\[ ^{13}C\text{ NMR (CDCl}_{3}\text{, 100 MHz): } \delta 155.8; 155.8; 150.5; 137.9; 130.5; 130.3; 130.2; 130.0; 129.3; 127.9; 127.8; 126.1; 125.3; 124.2; 120.5; 119.0; 31.9; 29.4; 25.7; -4.4. \]

\[ ^{19}F\text{ NMR (CDCl}_{3}\text{, 375 MHz): } \delta 8.81 (s, 9F). \]

IR (neat): 1600.0; 1508.4; 1472.0; 1249.8; 1169.5; 1155.1; 1123.8; 998.7; 965.7; 904.5; 833.8; 779.5; 726.0; 700.6; 537.4; 504.5.

The crude product was treated with TBAF according to general procedure C. The crude product was subjected to column chromatography (10 → 25% EtOAc/light petroleum) and product 22 was obtained as a bright yellow solid (72 mg, 0.13 mmol, 47%).

\[ ^{1}H\text{ NMR (CD}_{3}\text{OD, 400 MHz): } \delta 7.72 (d, J = 8.3 Hz, ^{1}H); 7.46-7.34 (m, 5H); 7.31 (s, ^{1}H); 7.22 (d, J = 16.5 Hz, ^{1}H); 7.05 (d, J = 16.4 Hz, ^{1}H); 7.10 (d, J = 12.4 Hz, ^{1}H); 6.96-6.90 (m, 1H); 6.82-6.77 (m, 4H). \]

\[ ^{13}C\text{ NMR (CD}_{3}\text{OD, 100 MHz): } \delta 159.0; 158.9; 151.6; 139.6; 131.8; 131.7; 130.9; 130.1; 129.9; 129.3; 129.1; 127.4; 125.5; 125.1; 123.0; 120.1; 119.8; 118.7; 116.7; 116.6. \]

\[ ^{19}F\text{ NMR (CD}_{3}\text{OD, 375 MHz): } \delta 10.93 (s, 9F). \]

IR (neat): 3332.1; 1606.3; 1515.6; 1250.3; 1171.8; 1123.0; 965.1; 829.3; 727.4; 668.0; 529.1.

\[(E, E)-1-hydroxy-2,5-bis(3-trifluoromethyl-4-methoxy)styrylbenzene (23)\]

Following general procedure B, diphosphonate 27 (0.52 g, 1 mmol) was reacted with aldehyde 38 (0.51 g, 2.5 mmol). After work-up, the crude product was purified by column chromatography (0 → 2% EtOAc/light petroleum) and the bis-TBDMS protected styrylbenzene was obtained as a bright yellow solid (0.22 g, 0.28 mmol, 32%).

\[ ^{1}H\text{ NMR (acetone-d}_{6}\text{, 400 MHz): } \delta 7.80-7.71 (m, 4H); 7.54 (d, J = 8.0 Hz, ^{1}H); 7.39 (d, J = 16.6 Hz, ^{1}H); 7.26-7.16 (m, 3H); 7.13 (d, J = 16.4 Hz, ^{1}H); 6.90-6.88 (m, 1H); 6.82-6.77 (m, 4H). \]

\[ ^{13}C\text{ NMR (acetone-d}_{6}\text{, 100 MHz): } \delta 156.2; 139.0; 132.5; 132.2; 131.9; 131.1; 129.0; 127.8; 127.4; 125.8; 125.7; 125.6; 125.5; 125.0; 124.1; 119.3; 114.3; 113.9; 56.6; 56.6. \]

\[ ^{19}F\text{ NMR (acetone-d}_{6}\text{, 375 MHz): } \delta 7.62 (s, 3F); 7.60 (s, 3F). \]

IR (neat): 3523.0; 1615.5; 1512.0; 1501.5; 1428.0; 1327.6; 1274.3; 1261.6; 1119.8; 1058.0; 1017.1; 963.1; 823.7; 667.6; 645.9; 569.8; 544.1.
(E, E)-1-methoxy-2,5-bis(3-trifluoromethyl-4-methoxy)styrylbenzene (24)

Following general procedure B, diphosphonate 25 (0.41 g, 1 mmol) was reacted with aldehyde 38 (0.51 g, 2.5 mmol). After work-up, the crude product was purified by column chromatography (0 → 10% EtOAc/light petroleum) and product 24 was obtained as a bright yellow solid (0.14 g, 0.27 mmol, 27%).

$^1$H NMR (DMSO-d$_6$, 400 MHz): δ 7.97-7.83 (m, 3H); 7.81 (dd, $J = 7.8$ Hz, $J = 1.7$ Hz, $^1$H); 7.76 (d, $J = 1.7$ Hz, $^1$H); 7.65 (d, $J = 8.1$ Hz, $^1$H); 7.46 (d, $J = 16.8$ Hz, $^1$H); 7.40-7.20 (m, 6H); 3.93 (s, 3H); 3.92 (s, 3H).

$^{13}$C NMR (DMSO-d$_6$, 100 MHz): δ 156.1; 137.9; 131.8; 131.5; 130.0; 129.7; 129.2; 128.8; 127.8; 127.3; 127.0; 126.6; 125.6; 125.1; 122.4; 122.2; 120.7; 119.3; 117.5; 117.2; 114.6; 113.4; 109.1; 56.3; 56.2.

$^{19}$F NMR (DMSO-d$_6$, 375 MHz): δ 17.26 (s, 3F); 17.2 (s, 3F). IR (neat): 1615.6; 1510.4; 1505.8; 1463.9; 1328.5; 1260.0; 1118.3; 1054.9; 1021.0; 959.3; 818.0; 667.8; 645.5; 541.1.
References


PART TWO | Development of Molecular Imaging strategies

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