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Tritium-labeled (*E,E*)-2,5-Bis(4'-hydroxy-3'-carboxystyryl)benzene as a Probe for β-Amyloid Fibrils

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Abstract

Accumulation of Aβ in the brains of Alzheimer disease (AD) patients reflects an imbalance between Aβ production and clearance from their brains. Alternative cleavage of amyloid precursor protein (APP) by processing proteases generates soluble APP fragments including the neurotoxic amyloid Aβ40 and Aβ42 peptides that assemble into fibrils and form plaques. Plaque-buildup occurs over an extended time-frame, and the early detection and modulation of plaque formation are areas of active research. Radiolabeled probes for the detection of amyloid plaques and fibrils in living subjects are important for noninvasive evaluation of AD diagnosis, progression, and differentiation of AD from other neurodegenerative diseases and age-related cognitive decline. Tritium-labeled (*E,E*)-1-[^{3}H]-2,5-bis(4'-hydroxy-3'-carbomethoxystyryl)benzene possesses an improved level of chemical stability relative to a previously reported radioiodinated analog for radiometric quantification of Aβ plaque and tau pathology in brain tissue and *in vitro* studies with synthetic Aβ and tau fibrils.

Accumulation of extracellular Aβ senile plaques in brain tissue and tangles of hyperphosphorylated tau protein inside brain neurons are the classical histopathological signs of Alzheimer's disease (AD).[1,2] According to the AD β-amyloid hypothesis,[3,4] the imbalance between the production and clearance of Aβ from brains of AD patients results from the alternative processing of the amyloid precursor protein[5,6] (APP) by β- and γ-

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secretases resulting in the generation of soluble APP fragments:[7] amyloid $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$, which assemble into plaques.[8] Tau protein hyperphosphorylation is induced by Aβ leading to neurofibrillary tangle formation.[9,10] The soluble oligomers of $A\beta_{42}$ that result from the assembly of monomer produced by the sequential proteolytic cleavage of APP by β- and γ-secretase are neurotoxic,[11] cause neuroinflammation and neuronal death, and ultimately result in cognitive impairment, irreversible memory loss, and disorientation in AD.

Because Aβ plaque formation occurs over a lengthy time period, methods for the early and conclusive detection[12], monitoring, and prevention[13–16] of Aβ deposition are of considerable importance. The development of probes for amyloid plaques and tangles[17,18] provides one avenue for the noninvasive diagnosis and evaluation of AD progression in living subjects and its differentiation from age-related cognitive decline in AD patients.[19,20] We report the synthesis of a radiolabeled Congo Red analog, (*E,E*)-1-[^{3}H]-2,5-bis(4'-hydroxy-3'-carboxystyryl)benzene (**1a**), which has not been reported previously, and its non-radiolabeled counterpart[21] **1b**, which is commonly known as X-34 (Fig. 1). Both ligands bind to fibrillar forms of both Aβ and tau, and we demonstrate the utility of **1a** in studies of synthetic $A\beta_{40}$ and $A\beta_{42}$ fibrils. We also report a cautionary note regarding the oxidative sensitivity of an intermediate in this route, namely 2,5-bis(4'-hydroxy-3'-carbomethoxystyryl)-1-iodobenzene (**2**) (Fig. 1) and by analogy, a radioiodinated version of **2**, which has also been reported[22] as a probe for Aβ deposition.

Plans for the synthesis of **1a** or **1b** focused on the regioselective, catalytic hydrogenolysis of a suitable iodinated 4-styrylstilbene, namely (*E,E*)-2,5-bis(4'-hydroxy-3'-carbomethoxystyryl)-1-iodobenzene (**2**) (Fig. 2), using tritium gas and a palladium catalyst or sodium borohydride and a palladium catalyst, respectively. Initial efforts, as a consequence, focused on the synthesis of **2**. Following the procedure of Zhuang,[22] the benzylic bromination of 2-bromo-*para*-xylene (**3**) and Arbusov reaction with triethyl phosphite provided tetraethyl (2-bromo-1,4-phenylene)bis(methylene)-diphosphonate. Wadsworth-Emmons condensation of this phosphonate with 3-carbomethoxy-4-methoxybenzaldehyde and demethylation of the intermediate bisanisole **4** secured the bisphenol **5** (Fig. 2). Efforts, however, to effect the tri-nbutylstannylation of **5** were unsuccessful, contrary to a report[22] claiming a 25% yield. Suspecting that this failure was a consequence of the phenolic hydroxyl groups in **5**, we converted **5** to the bisacetate **6** and were successful in converting **6** to the arylstannane **7**, albeit only in 18–23% yields. Iodination of **7** using sodium iodide and hydrogen peroxide, and saponification of **8** furnished the iodinated 4-styrylstilbene **2** in very poor yield. The low yield in the iodination reaction was unexpected until subsequent work, as discussed below, brought to our attention the oxidative sensitivity of **2**.

The poor yields in the stannylation and iodination reactions led us to explore an alternate route to the iodinated 4-styrylstilbene **2**. Elaboration of 2-iodo-*p*-xylene (**9**) to the iodinated bisphenol **11** followed a similar sequence to that described earlier (Fig. 2). The acetylation of **11** afforded the bisacetate **8** that was identical to material prepared from the route originating with 2-bromo-*p*-xylene (**3**). The saponification of **8** and subsequent acidification

gave a precipitate that was unambiguously identified as the iodinated 4-styrylstilbene **2** according to NMR and mass spectral data. Efforts, however, to purify **2** by recrystallization or chromatography were complicated by the apparent instability of **2** on exposure to air. For example, the ^{1}H NMR of a chromatographed sample of the iodinated 4-styrylstilbene **2** did not display the sharp signals seen in either the non-chromatographed sample of **2** or in the brominated analog, 2,5-bis(4'-hydroxy-3'-carbomethoxystyryl)-1-bromobenzene (**12**). Oxidative cyclization[23–25] of other 1,4-bis(styryl)benzenes to 3-styrylphenanthrenes suggested at least one avenue for the oxidation of **2**, and indeed, the treatment of non-chromatographed, well characterized **2** with hydrogen peroxide alone led to a plethora of products. In summary, we developed an unambiguous route to 2,5-bis(4'-hydroxy-3'-carbomethoxystyryl)-2-iodobenzene (**2**) that did not involve an oxidative process, and we utilized **2**, without further purification, in the synthesis of the desired **1a** and **1b**, as discussed below. In our opinion, investigators opting to use the radioiodinated [^{125}I]-version[22] of **2** as a probe for Aβ deposition should be aware of this chemical instability, avoid chromatographic purification on silica gel, and consider the tritiated ligand **1a** (Fig. 1) as a preferable alternative. It should also be noted that the "cold" ligand **1b** is sufficiently fluorescent to find application in its own right as a probe for Aβ deposition.[26]

Catalytic hydrogenolysis[27] of iodinated 4-styrylstilbene **2** using sodium borohydride and a catalytic amount of tetrakis(triphenylphosphine)palladium(0) furnished 2,5-bis(4'-hydroxy-3'-carbomethoxystyryl)benzene (**1b**) or X-34,[26,28] as it is commonly known in the literature (Fig. 3). The "cold" 4-styrylstilbene (**1b**) was also synthesized independently using the Horner-Emmons condensation of 5-formylsalicylic acid with *p*-xylenediphosphonic acid tetraethyl ester.[28] In order to achieve high specific activity, hydrogenolysis of **2** was performed using tritium gas and a palladium catalyst to provide radiolabeled (*E*,*E*)-1-[^{3}H]-2,5-bis(4'-hydroxy-3'-carbomethoxystyryl)benzene (**1a**) at 23 Ci/mmole. Tritiated sodium borohydride and the palladium catalyst could be used to secure **1a**, albeit at lower specific activities than those reported here.

The binding of tritiated **1a** was selective for the Congo Red-binding site on $A\beta_{40}$ and $A\beta_{42}$ fibrils and was displaced only by those unlabeled ligands, including the "cold" **1b** that mimicked Congo Red (Table 1). Non-specific retention of **1a** on the GF/B filter material was very low, and as expected, ligands with structures similar to Congo Red (*i.e.*, possess extended π systems) vied for binding against **1a** with efficacy values (EC_{50}, Table 1) for either $A\beta_{40}$ or $A\beta_{42}$ fibrils (*i.e.*, displayed varied EC_{50} values) that varied with substitution patterns. In contrast, benzothiazole ligands, such as Thioflavine T, Pittsburgh Compound B (PIB), or 2-(4′-methylaminophenyl)benzothiazole (BTA-1), were ineffective in displacing **1a** as were several other molecules reported to bind to Aβ fibrils but with structures unlike the Congo Red structure. From the Scatchard analysis, the binding stoichiometry of tritiated **1a** approached one ligand per two peptides. The similarity of the Scatchard K_d to the EC_{50} for "cold" **1b** competition against tritiated **1a** suggested that the EC_{50}'s were equivalent to K_d values, namely for $A\beta_{40}$, K_d = 0.60 μM and the ratio of molecules of **1b**/molecules of $A\beta_{40}$ (as monomers) = 0.43 and for $A\beta_{42}$, K_d = 0.25 μM and the ratio of molecules of **1b**/molecules of $A\beta_{42}$ (as monomers) = 0.63.

The structure and conformation of amyloid fibrils reflects the conditions under which they are assembled. This polymorphism is most evident in comparing the Aβ deposits from animal models of AD and human AD brain. A large amount of high affinity PIB binding is found in AD brain, but binding of this ligand is virtually undetectable in brain tissue of transgenic mouse models[29] or non-human primate brains[30] with similar amounts of Aβ deposition, as judged by immunohistochemistry or Congo Red binding. Since (*E*,*E*)-1-[^{3}H]-2,5-bis(4'-hydroxy-3'-carbomethoxystyryl)benzene possesses an extended π system like the panfibrillar amyloid, Congo Red ligand, tritiated **1a** will be useful for standardizing the quantitative assessment of relationships between chemical structures of existing and future amyloid probes with the ligand binding site types present on amyloid deposits.[31,32] Ligands, like the one reported here, will be important for understanding the molecular mechanisms of deposition linked to AD and other neurodegenerative diseases with misfolded protein pathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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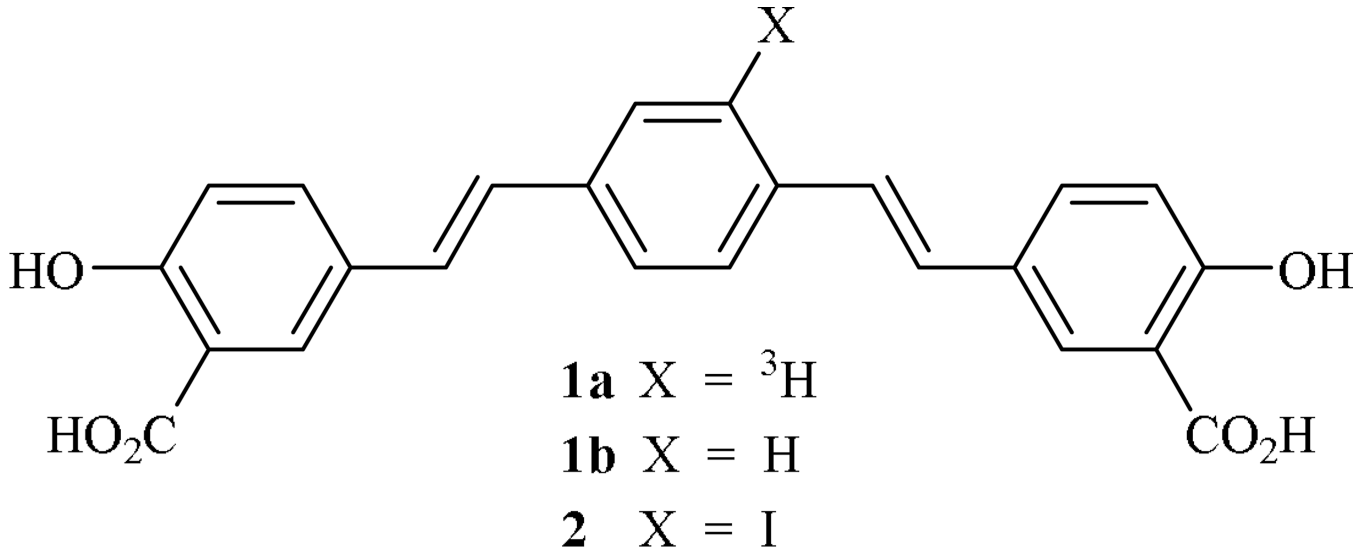

Figure 1. Iodinated and tritiated (*E*,*E*)-bis-1,4-styrylbenzenes required as imaging agents

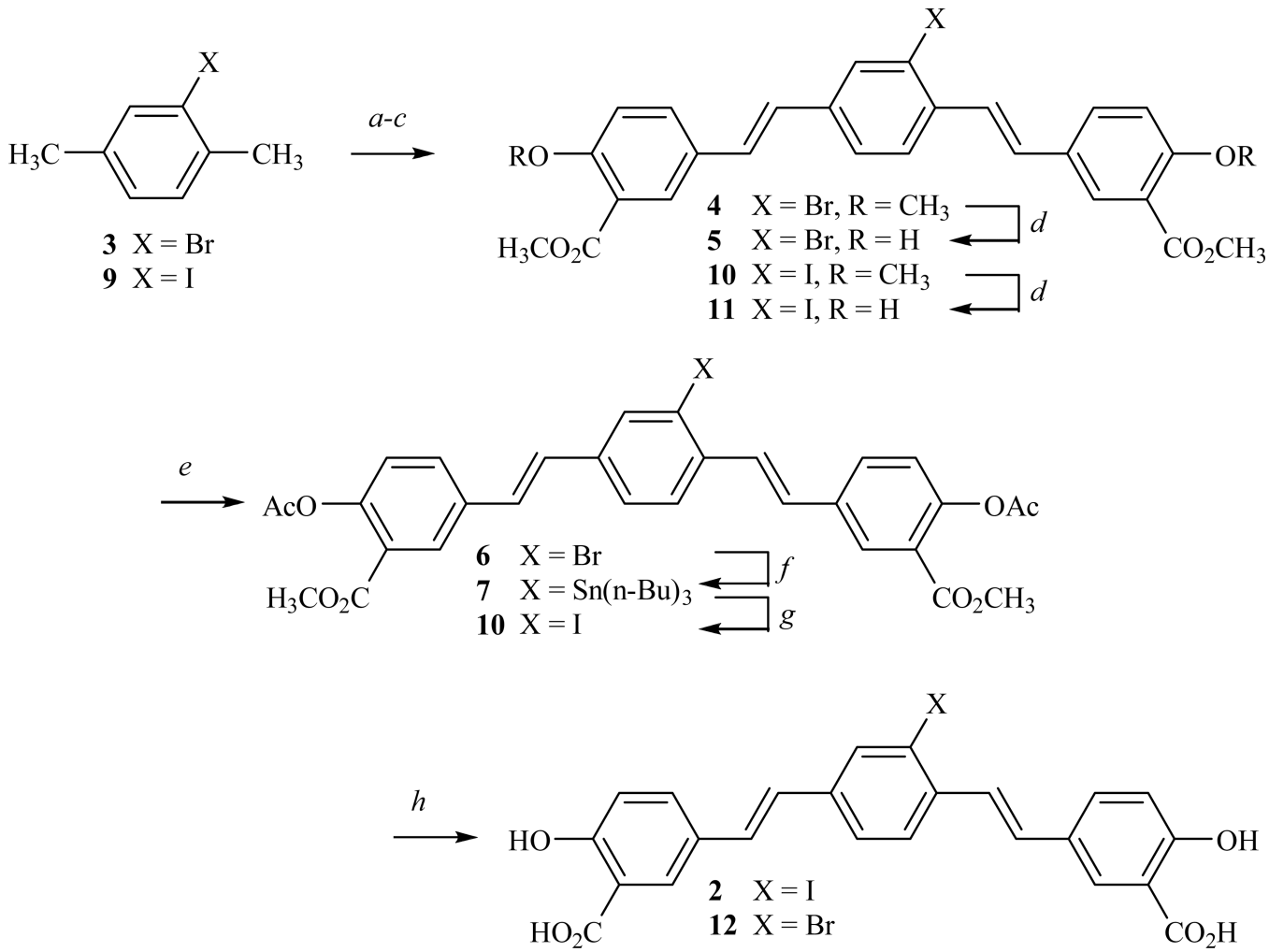

Figure 2. Synthesis of (*E,E*)-2,5-bis(4'-hydroxy-3'-carboxystyryl)-1-iodobenzene (2)

a, NBS (recrystallized), AIBN (cat), CCl_4; *b*, $P(OCH_2CH_3)_3$; *c*, $NaOCH_3$, 3-carbomethoxy-4-methoxybenzaldehyde, CH_3OH; d, BBr_3, CH_2Cl_2, −78°C; e, Ac_2O, Py; f, $Pd(PPh_3)_4$, $(n\text{-}Bu)_3SnSn(n\text{-}Bu)_3$; *g*, NaI, H_2O_2; *h*, NaOH, aq CH_3OH.

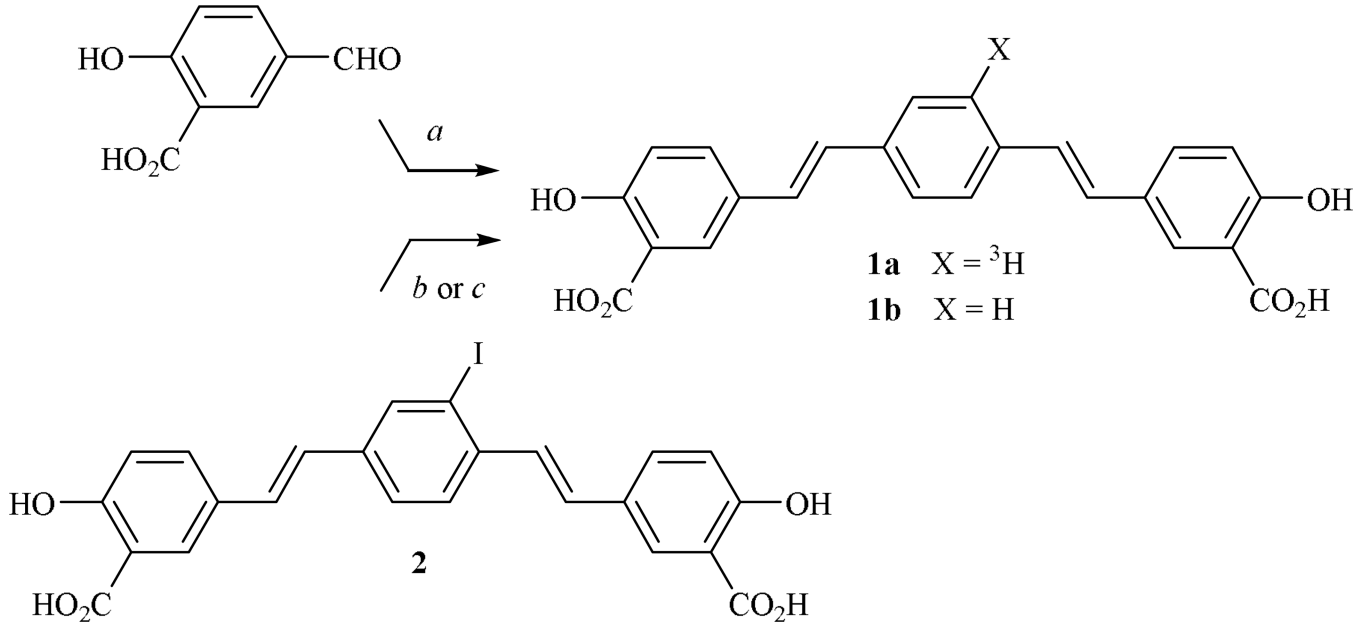

Figure 3. Synthesis of (*E*,*E*)-1-[^{3}H]-2,5-bis(4'-methoxy-3'-carboxystyryl)benzene (1a) and its "cold" analog (1b or X-34)

a, $NaOCH_3$, *p*-xylenediphosphonic acid tetraethyl ester; *b*, tritium gas, proprietary palladium catalyst (ViTrax, Placentia, CA) or [^{3}H]-$NaBH_4$, $Pd(PPh_3)_4$ to give **1a**; *c*, $NaBH_4$, $Pd(PPh_3)_4$ to give **1b**.

Table 1

Displacement of 5 nM of (*E*,*E*)-1-[^{3}H]-2,5-bis(4'-hydroxy-3'-carbomethoxystyryl)benzene (1a) from synthetic Aβ fibrils by unlabeled compounds

[^{3}H]-X-34 is another name for (*E*,*E*)-1-[^{3}H]-2,5-bis(4'-hydroxy-3'-carbomethoxystyryl)benzene (**1a**); X-34 is another name for (*E*,*E*)-2,5-bis(4'-hydroxy-3'-carbomethoxystyryl)benzene (**1b**); BSB, (*E*,*E*)-1-bromo-2,5-bis(4'-hydroxy-3'-carbomethoxystyryl)benzene; ISB, (*E*,*E*)-1-iodo-2,5-bis(4'-hydroxy-3'-carbomethoxystyryl)benzene; K114, (*E*,*E*)-1-bromo-2,5-bis(4'-hydroxystyryl)benzene; BMB, (*E*,*E*)-1-methoxy-2,5-bis(4'-aminostyryl)benzene or as it also known 1,4-bis(4-aminophenylethenyl)-2-methoxybenzene; PIB, Pittsburgh Compound B; BTA-1, 2-(4´-methylaminophenyl)benzothiazole; IMPY, 2-(4'-dimethylaminophenyl)-6-iodoimidazo[1,2-a]pyridine; ThT, Thioflavine T; ThS, Thioflavin S.

Compound	**Aβ(1–40) EC_{50}, μM**	**Aβ(1–42) EC_{50}, μM**
Chrysamine G	9	0.9
Congo Red	2.3	0.7
1b or X-34	0.53	0.2
BSB	0.23	0.12
ISB	0.2	0.15
K114	>10	3
BMB	>10	3
PIB	>10	>10
BTA-1	>10	>10
IMPY	>10	>10
ThT	>10	>10
ThS	>10	>10
primulin	>10	>10
curcumin	>10	4
resveratrol	>10	>10
resorufin	>10	>10
naproxen	>10	>10
ibuprofen	>10	>10