Synthesis of Biphenyltrienes as Probes for β-Amyloid Plaques

Zhi-Ping Zhuang,† Mei-Ping Kung,† and Hank F. Kung*,†,‡

Departments of Radiology, and Pharmacology, University of Pennsylvania, Room 305, 3700 Market Street, Philadelphia, Pennsylvania 19104

Received October 11, 2005

We report a series of *p*-hydroxy-, *p*-amino-, *p*-monomethylamino-, and *p*-monofluoroethylamino-substituted biphenyltrienes that displayed high binding affinities to β-amyloid plaques. In an in vitro binding assay using postmortem brain homogenates of Alzheimer's patients and [^{125}I]9, the triene compounds showed excellent binding affinities. When labeled with suitable radionuclides, they are useful as in vivo imaging agents for detecting Aβ plaques in the brains of Alzheimer's patients.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease affecting millions of elderly people. Common clinical symptoms of AD include cognitive decline, irreversible memory loss, disorientation, language impairment, etc. Major neuropathology observations of postmortem AD brains include the presence of senile plaques, neurofibrillary tangles, and neurophil threads containing β-amyloid (Aβ) aggregates and highly phosphorylated tau proteins.[1] The exact mechanisms leading to the development of AD are not fully understood; however, the formation of Aβ plaques in the brain is a pivotal event in the pathology of Alzheimer's disease. Significant circumstantial evidence suggests that fibrillary Aβ plaques predominately consisting of the aggregates of Aβ40 and Aβ42 peptides play a major role in AD pathogenesis.[2] The formation of Aβ aggregates in the brain is now considered a significant event that produces various toxic effects in neuronal cells leading to the formation of neuritic plaques.[2] In view of the critical roles Aβ plaques play in AD, Aβ-plaque-specific imaging agents may be useful for early detection or for monitoring the progression and effectiveness of treatment of AD.[3−5]

Several research groups have reported Aβ-plaque-specific imaging agents based on highly conjugated dyes, such as Congo Red and Chrysamine G.[6,7] Thioflavin T, **5** as well as Congo Red have been used in the fluorescent staining of plaques and tangles in postmortem AD brain sections.[8] More abbreviated forms of Chrysamine G, such as styrylbeneze, including **1**−**4** (Figure 1), have been reported as fluorescent dyes for staining amyloid aggregates.[9,10] Although these molecules displayed desirable properties, such as highly conjugated, high binding affinity and moderate lipophilicity, their partial charge prevents them from getting across the intact blood−brain barrier (BBB). A recent report has suggested that it may be possible to prepare near-infrared fluorescent imaging agents for imaging the plaques inside the brain.[11]

To overcome the observed brain penetration deficit associated with the styrylbenzene series (**1**−**4**), we focused on benzothiazole and other backbone structures. Recently, successful uses of a C-11-labeled benzothiazole derivative, [^{11}C]PIB (**8**) and a highly lipophilic F-18-labeled probe, [^{18}F]FDDNP (**10**) for plaque and tangle visualization in living AD patients have demonstrated the potential usefulness of in vivo plaque imaging.[12,13] Parallel to these efforts, we have similarly prepared [^{11}C]-SB-13 (**7**), a stilbene derivative, for plaque imaging.[14] As expected, **7** displayed high accumulation in the affected cortical areas of the brain in mild to moderate AD patients but not in the age-matched control subjects.[15]

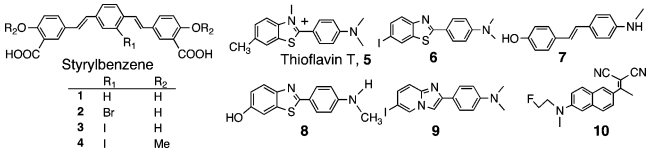


Figure 1. Chemical structures of various probes for Aβ aggregates.

To investigate the significance of the distance between the two phenyl groups of stilbenes, we have added additional double bonds (trienes) instead of one vinyl bond. None of the previously reported ligands contains a highly conjugated polyene backbone. To our surprise, the addition of a triene bond between the biphenyl groups produces highly selective compounds showing excellent binding affinity for homogenates of brain tissues from AD patients. Reported herein are the syntheses and structure−activity relationships of a series of derivatives as selective probes potentially useful for detecting amyloid aggregates in the brain.

Results and Discussion

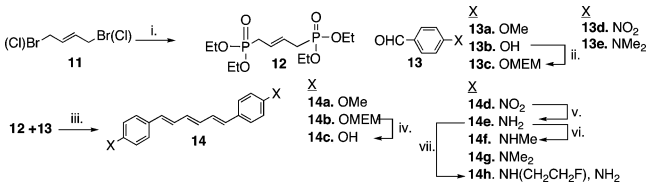
Chemistry. The key step for the synthesis of biphenyltrienes is the Wittig reaction between various aldehydes, **13**, and bisdiethyl phosphonate **12**, which was readily prepared from 1,4-dibromo (or chloro)-2-butene (Scheme 1). The Wittig reagents, **12**, and various aldehydes **13** were readily reacted in the presence of NaH and THF under a refluxing conditions.[16,17] The hydroxyl phenyl triene **14c** was obtained from hydrolysis of the MEM protected precursor **14b**. The bisamino compound **14e** and bismonomethylamino compound **14f** were synthesized from the correspondent nitro compound **14d** through reduction and monomethylation. The bis-*p*-(*N*,*N*-dimethylamino) derivative **14g** was prepared directly through the Wittig reaction between the *p*-(*N*,*N*-dimethylamino)benzaldehyde (**13e**) and the bisdiethyl phosphonate **12**. One unsymmetrically substituted derivative, *N*-monofluoroethyl compound **14h**, was prepared by reacting the di-amino derivative **14e** with 1-bromo-2-fluoroethane in the presence of potassium carbonate in DMF.

In Vitro Binding of Aβ plaques in AD Brain Tissue Homogenates. Using the in vitro binding of preformed Aβ peptide aggregates and two different ligands, [^{125}I](*E*,*E*)-1-iodo-2,5-bis-(3-hydroxycarbonyl-4-methoxy)styrylbenzene ([^{125}I]**4**), and [^{125}I] 2-[4′-dimethyl-aminophenyl]-6-iodobenzothiazole ([^{125}I]-**6**), the presence of at least two mutually exclusive binding sites

* Corresponding author. Tel: (215) 662-3096. Fax: (215) 349-5035. E-mail:kunghf@sunmac.spect.upenn.edu.
† Department of Radiology.
‡ Department of Pharmacology.

10.1021/jm051020k CCC: $33.50
Published on Web 03/29/2006

Scheme 1[a]



[a] i. $P(OEt)_3$; ii. MEMCl/DIEA, CH_2Cl_2; iii. NaH, THF, reflux; iv. HCl, THF/MeOH(2:1); v. $SnCl_2$/EtOH; vi. NaOMe/$(CH_2O)_n$; $NaBH_4$/MeOH; vii. $BrCH_2CH_2F$, K_2CO_3/DMF; Except for **14h**, all are symmetrical.

Table 1. Inhibition Constants (K_i, nM)[a] of Compounds on Ligand Binding to Homogenates of Brain Tissue

compd	vs[^{125}I]**4**	vs[^{125}I]**9**
14a −OMe	>8000	>4000
14c −OH	150 ± 30	9.0 ± 2.1
14d $-NO_2$	>4000	500 ± 30
14e $-NH_2$	375 ± 150	9.0 ± 3.2
14f −NHMe	122 ± 40	7.5 ± 2.5
14g $-NMe_2$	>7000	837 ± 80
14h-$NH(CH_2CH_2F)_2$	217 ± 20	12 ± 3
4	5.1 ± 1.2	>1000
9	>5000	5.0 ± 0.4
7	>3000	1.2 ± 0.2
8	>10000	2.8 ± 0.5
10	>8000	152 ± 20

*Values are the mean ± SEM of three independent experiments, each in duplicate.

on these aggregates was demonstrated.[18] Recently our laboratory has reported that [^{125}I] 2-(4′-dimethylaminophenyl)-6-iodo-imidazo[1,2-a]pyridine ([^{125}I]**9**) binds to the same binding sites as those for **6** in transgenic mice as well as in postmortem homogenates of AD brain tissues, and **9** has a better in vivo biodistribution profile as an imaging agent.[18−20] Specific in vitro binding of [^{125}I]**9** can be clearly measured in cortical gray matter but not in the white matter of AD cases. The location and density of the specific signal detected by [^{125}I]**9** correlated with the distribution of amyloid plaques in these brain specimens, as confirmed by thioflavin-S staining.

In this study, we have extended the in vitro binding assay to use the homogenates of AD brain tissue and the two ligands for two different and mutually exclusive binding sites using two different radiolabeled ligands, [^{125}I]**4** and [^{125}I]**9** (Table 1). We demonstrated that the new biphenyltrienes showed variable binding affinities depending on the substitution groups at the p position of the phenyl groups. Generally, the biphenyltrienes preferentially bind to the binding sites for **9**. Specifically, compounds **14c**, **14e**, **14f**, and **14h** containing substitution groups, −OH, $-NH_2$, −NHMe, and $-NH(CH_2CH_2F)$ displayed moderate binding affinities toward [^{125}I]**4**, giving K_i values of 150, 375, 122, and 217 nM, respectively. However, these four compounds exhibited highly potent binding toward [^{125}I]**9**, showing K_i values of 9.0, 9.0, 7.5, and 12 nM, respectively. The binding data suggests that the biphenyltrienes are highly competitive for the binding sites of **9** on the Aβ aggregates, whereas the affinity for the binding sites for **4** is 15−50-fold lower. It is expected that the compound with a [^{18}F]fluoroethyl group on **14h** can be prepared and that the labeled [^{18}F]**14h** may be useful as a PET imaging agent specifically targeting Aβ plaques in the brain.

There are several unexpected findings for this series of compounds. There is a dramatic difference in binding affinities between −OMe and **14a**, and −OH and **14c** (>4000 vs 9.0 nM using [^{125}I]**9** and >8000 vs 150 nM using [^{125}I]**4**). The addition of the methyl group significantly reduces the binding affinities to both binding sites. The same dramatic difference in binding affinities is observed between −NHMe and **14f**, and $-NMe_2$ and **14g** (7.5 vs 837 nM using [^{125}I]**9** and 122 vs >7000 nM using [^{125}I]**4**). This is a striking difference in binding affinity between two seemingly related compounds; **14f** has a mono-*N*-methylamino, and **14g** has a *N*,*N*-dimethylamino substitution at the p position of the phenyl rings. In the same assay system, as expected, nonradioactive **9** showed a high binding affinity toward [^{125}I]**9** binding (K_i = 5.0 nM), whereas it displayed a very low binding affinity to the [^{125}I]**4** sites suggesting that there are two distinct biding sites. Both **9** and **7** showed strong competitive binding to [^{125}I]**9** binding sites (K_i = 5.0 and 1.2 nM, respectively). The results are consistent with previously reported values.[18] Recently, **8** has been tested in AD patients as a Aβ-plaque-specific imaging agent.[13] It has been reported previously that [^{3}H]**8** binds to the homogenates of postmortem AD brain tissue with a high binding affinity (K_d = 1.4 nM).[3] In our laboratory, the competition of the binding sites of **8** to [^{125}I]**9** showed a measured K_i value of 2.8 nM, which is comparable to that reported previously. It was also observed that in our study another PET imaging agent **10** that binds to plaques as well as tangles showed a moderate binding affinity toward the [^{125}I]**9** binding site (K_i = 152 nM) [18,19] indicating **10** might not compete with [^{125}I]**9** for identical binding pockets on amyloid plaques. There may be a third binding site not related to **4** or **9**. This observation has not been reported previously. Significantly, the multiple binding sites on the Aβ plaques in human AD brains may be important and should be carefully considered in the future when developing in vivo imaging agents.

In summary, the results of the binding study suggest that this series of novel ligands based on biphenyltrienes showed potent binding toward Aβ plaques in human AD brain tissue homogenates. The competitive binding study showed excellent binding affinities with biphenyltrienes containing −OH, $-NH_2$, −NHMe, and $-NH(CH_2CH_2F)$ groups. When labeled with suitable short-lived radionuclides, they may be useful as imaging agents for detecting amyloid aggregates in the living human brain.

Experimental Section

The reagents used in the synthesis were purchased from Aldrich or Fluka and used without further purification unless otherwise indicated. Anhydrous Na_2SO_4 was used as a drying agent. ^{1}H NMR spectra were obtained on a Bruker spectrometer (Bruker AC 200). The chemical shifts are reported as δ values with chloroform as the internal reference unless otherwise mentioned. The coupling constants are reported in Hz. Multiplicity is defined by s (singlet), d (doublet), t (triplet), br (broad), and m (multiplet). PTLC: preparative thin-layer chromatography, silica gel, GF, 2000 microns from Analtech, Inc. Elemental Analysis was performed by Atlantic Microlab, Inc.

General Procedure for the Wittig Reaction. 1,6-Bis(4′-methoxyphenyl)-hexa-1,3,5-triene (14a). To a suspension of NaH (60 mg, 60% in mineral oil, 1.5 mmol) in THF (5 mL) was added dropwise a solution of tetraethyl-2-butene-1,4-diphosphonate (**12**) (164 mg, 0.5 mmol) in THF (5 mL) followed by a solution of 4-methoxybenzaldehyde (136 mg, 1 mmol) in THF (10 mL). The mixture was stirred under reflux overnight. Isopropyl alcohol was added to destroy the excess NaH after cooling. Ice water was added, and the precipitate was collected by suction,washed with water, and dried. The crude product was recrystallized in benzene to afford 34 mg of product (mp 244−245 °C, 23%). ^{1}HNMR (200 MHz, $CDCl_3$): δ 3.81 (s, 6H), 6.46 (s, 2H), 6.52 (d, J = 15.6 Hz, 2H), 6.74 (m, 2H), 6.85 (d, J = 8.5 Hz, 4H), 7.34 (d, J = 8.7 Hz, 4H). Anal($C_{20}H_{20}O_2$) H, C: calcd, 82.16; found, 81.24.

1,6-Bis[4′-(2″-methox-ethoxy)-methoxyphenyl]-hexa-1,3,5-triene (14b). Prepared as described above from 4-(2′-methoxy-

ethoxy-)methoxy-benzaldehyde (**13c**, 2.1 g, 10 mmol) and **12** (1.64 g, 5 mmol) to afford 1.18 g of product (29%). ^{1}HNMR (200 MHz, $CDCl_3$): δ 3.37 (s, 6H), 3.33 (m, 4H), 3.81 (m, 4H), 5.28 (s, 4H), 6.45 (d,d, J = 6.9, 2.8 Hz, 2H), 6.51 (d, J = 15.5 Hz, 2H), 6.76 (d,d,d, J = 15.5, 7.0, 3.2 Hz, 2H), 7.00 (d, J = 8.7 Hz, 4H), 7.33 (d, J = 8.7 Hz, 4H). Anal($C_{26}H_{32}O_6$) C, H.

1,6-Bis(4′-nitrophenyl)-hexa-1,3,5-triene (14d). Prepared as described above from 4-nitro-benzaldehyde (302 mg, 2 mmol) and **12** (328 mg, 1 mmol) to afford 200 mg of product (62%). ^{1}HNMR (200 MHz, $CDCl_3$): δ 6.64 (d,d, J = 7.0, 3.0 Hz, 2H), 6.70 (d, J = 15.6 Hz, 2H), 7.04 (d,d,d, J = 15.4, 7.0, 2.9 Hz, 2H), 7.54 (d, J = 8.8 Hz, 4H), 8.19 (d, J = 8.8 Hz, 4H). Anal($C_{18}H_{14}N_2O_4$) C, H, N.

1,6-Bis(4′-dimethylaminophenyl)-hexa-1,3,5-triene (14g). Prepared as described above from 4-dimethyaminobenzaldehyde (149 mg, 1 mmol) and **12** (164 mg, 0.5 mmol) to afford 60 mg of product (38%). ^{1}HNMR (200 MHz, $CDCl_3$): δ 2.97 (s, 12 H), 6.42 (d,d, J = 6.9, 2.9 Hz, 2H), 6.47 (d, J = 13.8 Hz, 2H), 6.68 (d, J = 8.8 Hz, 4H), 6.70 (m, 2H), 7.30 (d, J = 8.9 Hz, 4H). Anal($C_{22}H_{26}N_2$) C: calcd, 82.97; found, 76.55. H: calcd, 8.23; found, 8.65. N: calcd, 8.8; found, 7.89.

4-(2′-Methoxyethoxy-)methoxybenzaldehyde (13c). To a solution of 4-hydroxybenzaldehyde (3.7 g, 30 mmol) and diisopropylethylamine (7.6 mL) in CH_2Cl_2 (60 mL) was added dropwise MEMCl (5.0 mL) in CH_2Cl_2 (17 mL) at 0 °C. The mixture was stirred at RT for 3 h and quenched with HCl (60 mL, 0.5 N). The mixture was extracted with CH_2Cl_2. The organic phase was washed with NaOH solution (1 N) and water. The separated organic phase was dried under Na_2SO_4, filtered, and concentrated to give 6.2 g of product (97%), which was pure enough to be used in the next reaction. ^{1}HNMR (200 MHz, $CDCl_3$): δ 3.36 (s, 3H), 3.54 (m, 2H), 3.83 (m, 2H), 5.41 (s, 2H), 7.16 (d, J = 8.7 Hz, 2H), 7.83 (d, J = 8.7 Hz, 2H), 9.90 (s, 1H).

1,6-Bis(4′-hydroxyphenyl)-hexa-1,3,5-triene (14c). To a solution of **14b** (237 mg, 0.54 mmol) in a mixed solvent (15 mL, THF/MeOH = 2:1) was added HCl (2 mL, concentrated). The mixture was stirred overnight at RT. Water was added, and the mixture was extracted with ethyl acetate. In general, the work up gave the crude product, which was purified by PTLC (Hex/EA = 2:1 as the developing solvent) to give 44 mg of product (31%). ^{1}HNMR (200 MHz, $CDCl_3$): δ 6.44 (d,d, J = 6.7, 2.7 Hz, 2H), 6.49 (d, J = 14.5 Hz, 2H), 6.70 (m, 2H), 6.75 (d, J = 8.6 Hz, 4H), 7.27 (d, J = 8.6 Hz, 4H), 7.55 (s, 2H). Anal($C_{18}H_{16}O_2 \cdot 1/2\ H_2O$) C, H.

1,6-Bis(4′-aminophenyl)-hexa-1,3,5-triene (14e). A mixture of **14d** (180 mg, 0.56 mmol) and $SnCl_2$ (1.05 g) in EtOH (20 mL) was refluxed overnight. Water was added, and the mixture was made basic with an NaOH solution (40%). The mixture was extracted with EA. The separated organic phase was dried under Na_2SO_4, filtered, and concentrated to give the crude product, which was purified by PTLC (Hexane/Ethyl acetate = 1:1) to give 50 mg of product (34%). ^{1}HNMR (200 MHz, $CDCl_3$): δ 6.39 (d,d, J = 7.0, 3.0 Hz, 2H), 6.44 (d, J = 15.2 Hz, 2H), 6.59 (d, J = 8.5 Hz, 4H), 6.70 (d,d,d, J = 15.5, 7.0, 3.0 Hz, 2H), 7.18 (d, J = 8.5 Hz, 4H). Anal($C_{18}H_{18}N_2$) H, N. C: calcd, 82.41; found, 80.09.

1,6-Bis(4′-methylaminophenyl)-hexa-1,3,5-triene (14f). To a suspension of **14e** (15 mg, 0.06 mmol) in MeOH (3 mL) was added NaOMe (30 mg) in solid form followed by $(CH_2O)_n$ (18 mg, 0.6 mmol) in solid form. The mixture was stirred under reflux for 2 h. $NaBH_4$ (44 mg, 1.2 mmol) was added in portions after the reaction mixture was cooled. The resulting mixture was stirred under reflux for 1 h. Ice water was added, and the mixture was extracted with CH_2Cl_2. The organic phase was dried under Na_2SO_4, filtered, concentrated, and purified by PTLC (Hexane/Ethyl acetate = 2:1) to give 15 mg of product (90%). ^{1}HNMR (200 MHz, $CDCl_3$): δ 2.85 (s, 6H), 6.40 (d,d, J = 6.9, 2.9 Hz, 2H), 6.46 (s, J = 15.6 Hz, 2H), 6.55 (d, J = 8.6 Hz, 4H), 6.68 (m, 2H), 7.26 (d, J = 8.9 Hz, 4H). Anal($C_{20}H_{22}N_2 \cdot H_2O$) C, H, N.

1-(4′-Aminophenyl)-6-[4′-(2′-fluoroethylamino)phenyl]-hexa-1,3,5-triene (14h). To a solution of **14e** (30 mg, 0.11 mmol) and 1-bromo-2-fluoroethane (100 mg, 0.78 mmol) in DMF (3 mL) was added K_2CO_3 (160 mg, 5 eq) and KI (5 mg). The mixture was stirred overnight at 90 °C. Water was added, and the mixture was extracted with CH_2Cl_2. The solvent was removed, and the residue was purified by PTLC (Hexane/Ethyl acetate = 2:1) to give 7 mg of product (20%). ^{1}HNMR (200 MHz, $CDCl_3$): d 3.45 (t,d, J=26.6, 4.5 Hz 2H), 4.62 (t,d, J = 47.3, 4.5 Hz, 2H), 6.27−6.67 (m, 10 H), 7.20−7.34 (m, 4H). Anal($C_{20}H_{21}FN_2$) H. C: calcd, 77.89; found, 74.04. N: calcd, 9.08; found, 8.41.

Preparation of the Radioiodinated Ligands. The desired ^{125}I-labeled ligands **9** and **4** were prepared using iododestannylation reactions. Hydrogen peroxide (50 μL, 3% w/v) was added to a mixture of 50 μL of the correspondent tributyltin precursor (1 μg/μL of EtOH), 50 μL of 1 N HCl, and [^{125}I]NaI (1−5 mCi) in a closed vial. The reaction was allowed to proceed for 10 min at room temperature and then terminated by the addition of 100 μL of sat. $NaHSO_3$. The reaction mixture was either directly extracted (styrylbenzenes) with ethyl acetate (3 × 1 mL) or extracted after neutralization with a saturated sodium bicarbonate solution (thioflavins). The combined extracts were evaporated to dryness. For styrylbenzenes, the residues were dissolved in 100 μL of EtOH and purified by HPLC using a reversed phase column (Waters μbondpad, 3.9 × 300 mm) with an isocratic solvent of 65% acetonitrile−35% trifluoroacetic acid (0.1%) in a flow rate of 0.8 mL/min. [^{125}I]**9** was purified on a PRP-1 column (Hamilton, 4.1 × 250 mm) eluted with an isocratic solvent of 90% acetonitrile−10% 3,3-dimethyl-glutaric acid (5 mM, pH 7.0) and a flow rate of 1.0 mL/min. The desired fractions containing the product were collected, condensed, and re-extracted with ethyl acetate. The no-carrier-added products were evaporated to dryness and redissolved in 100% EtOH (1 μCi/μL). The final ^{125}I probes, with a specific activity of 2200 Ci/mmol and a greater than 95% radiochemical purity, were stored at −20 °C up to six weeks for in vitro binding studies.

Binding Assays Using Homogenates of AD Brain Tissue. Postmortem brain tissues were obtained from AD patients during autopsies, and the neuropathological diagnosis was confirmed by current criteria (NIA-Reagan Institute Consensus Group, 1997). The homogenates were then prepared from dissected gray matters in phosphate buffered saline (PBS at pH 7.4) at a concentration of approximately 100 mg wet tissue/mL (motor-driven glass homogenizer with a setting of 6 for 30 s). The homogenates were aliquoted into 1 mL-portions and stored at −70 °C for three to six months without the loss of the binding signal.

Binding assays were carried out in 12 × 75 mm borosilicate glass tubes. For competition studies, the reaction mixture contained 50 μL of AD brain tissue homogenates (containing 20−50 μg protein), 50 μL of [^{125}I]**9** (diluted in PBS, 0.02−0.04 nM for [^{125}I]-**9**), and 50 μL of inhibitors (10^{-7}−10^{-10} M diluted serially in PBS containing 0.1% bovine serum albumin) in a final volume of 1 mL. Similarly, [^{125}I]**4** (diluted in PBS, 0.02−0.04 nM) was used for the binding assay. Nonspecific binding was defined in the presence of 600 nM **9** or **4** in the same assay tubes. The mixture was incubated at 37 °C for 2 h, and the bound and free radioactivities were separated by vacuum filtration through Whatman GF/B filters using a Brandel M-24R cell harvester followed by 2 × 3 mL washes of PBS at room temperature. The filters containing the bound I-125 ligand were counted in a gamma counter (Packard 5000) with 70% counting efficiency. The results of the inhibition experiments were based on the assumption that the compounds under evaluation competed for the same binding site with the hot ligand, and the data was subjected to nonlinear regression analysis with the EBDA and Ligand programs [21] by which the K_i values were calculated.

Acknowledgment. This work was supported by grants from the National Institutes of Health (AG022559 to H.F.K and AG-21868 to M-P. K.) and the Institute for the Study of Aging (M-P. K.). We thank Dr. George Barrio for kindly providing cold FDDNP for the competition study.

Supporting Information Available: Elemental analysis data for the compounds used in this study. This material is avaliable free of charge via the Internet at http://pubs.acs.org.

References

(1) Selkoe, D. J. Alzheimer disease: mechanistic understanding predicts novel therapies. *Ann. Intern. Med.* **2004**, *140*, 627−638.

(2) Hardy, J.; Selkoe, D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **2002**, *297*, 353−356.

(3) Mathis, C. A.; Wang, Y.; Klunk, W. E. Imaging b-amyloid plaques and neurofibrillary tangles in the aging human brain. *Curr. Pharm. Des.* **2004**, *10*, 1469−1492.

(4) Selkoe, D. J. Imaging Alzheimer's amyloid. *Nat. Biotechnol.* **2000**, *18*, 823−824.

(5) Mathis, C. A.; Klunk, W. E.; Price, J. C.; DeKosky, S. T. Imaging technology for neurodegenerative diseases: progress toward detection of specific pathologies. *Arch. Neurol. (Chicago)* **2005**, *62*, 196−200.

(6) Klunk, W. E.; Debnath, M. L.; Pettegrew, J. W. Small-molecule beta-amyloid probes which distinguish homogenates of Alzheimer's and control brains. *Biol. Psychiatry* **1994**, *35*, 627.

(7) Klunk, W. E.; Debnath, M. L.; Koros, A. M.; Pettegrew, J. W. Chrysamine-G, a lipophilic analogue of Congo red, inhibits Aβ-induced toxicity in PC12 cells. *Life Sci.* **1998**, *63*, 1807−1814.

(8) Elhaddaoui, A.; Pigorsch, E.; Delacourte, A.; Turrell, S. Competition of congo red and thioflavin S binding to amyloid sites in Alzheimer's diseased tissue. *Biospectroscopy* **1995**, *1*, 351−356.

(9) Styren, S. D.; Hamilton, R. L.; Styren, G. C.; Klunk, W. E. X-34, a fluorescent derivative of Congo Red: a novel histochemical stain for Alzheimer's disease pathology. *J. Histochem. Cytochem.* **2000**, *48*, 1223−1232.

(10) Link, C. D.; Johnson, C. J.; Fonte, V.; Paupard, M.-C.; Hall, D. H.; Styren, S.; Mathis, C. A.; Klunk, W. E. Visualization of fibrillar amyloid deposits in living, transgenic *Caenorhabditis elegans* animals using the sensitive amyloid dye, X-34. *Neurobiol. Aging* **2001**, *22*, 217−226.

(11) Hintersteiner, M.; Enz, A.; Frey, P.; Jaton, A. L.; Kinzy, W.; Kneuer, R.; Neumann, U.; Rudin, M.; Staufenbiel, M.; Stoeckli, M.; Wiederhold, K. H.; Gremlich, H. U. In vivo detection of amyloid-beta deposits by near-infrared imaging using an oxazine-derivative probe. *Nat. Biotechnol.* **2005**, *23*, 577−83.

(12) Agdeppa, E. D.; Kepe, V.; Liu, J.; Flores-Torres, S.; Satyamurthy, N.; Petric, A.; Cole, G. M.; Small, G. W.; Huang, S. C.; Barrio, J. R. Binding characteristics of radiofluorinated 6-dialkylamino-2-naphthylethylidene derivatives as positron emission tomography imaging probes for β-amyloid plaques in Alzheimer's disease. *J. Neurosci.* **2001**, *21*, RC189.

(13) Klunk, W. E.; Engler, H.; Nordberg, A.; Wang, Y.; Blomqvist, G.; Holt, D. P.; Bergstrom, M.; Savitcheva, I.; Huang, G.-f.; Estrada, S.; Ausen, B.; Debnath, M. L.; Barletta, J.; Price, J. C.; Sandell, J.; Lopresti, B. J.; Wall, A.; Koivisto, P.; Antoni, G.; Mathis, C. A.; Langstrom, B. Imaging Brain Amyloid in Alzheimer's Disease with Pittsburgh Compound-B. *Ann. Neurol.* **2004**, *55*, 306−319.

(14) Ono, M.; Wilson, A.; Nobrega, J.; Westaway, D.; Verhoeff, P.; Zhuang, Z.-P.; Kung, M.-P.; Kung, H. F. ^{11}C-Labeled Stilbene Derivatives as Aβ-aggregate-specific PET Imaging Agents for Alzheimer's Disease. *Nucl. Med. Biol.* **2003**, *30*, 565−571.

(15) Verhoeff, N. P.; Wilson, A. A.; Takeshita, S.; Trop, L.; Hussey, D.; Singh, K.; Kung, H. F.; Kung, M.-P.; Houle, S. In vivo imaging of Alzheimer disease beta-amyloid with [11C]SB-13 PET. *Am. J. Geriatr. Psychiatry* **2004**, *12*, 584−595.

(16) Kauffman, J. M.; Moyna, G. Diarylamino groups as photostable auxofluors in 2-benzoxazolylfluorene, 2,5-diphenyloxazoles, 1,3,5-hexatrienes, 1,4-distyrylbenzenes, and 2,7-distyrylfluorenes. *J. Org. Chem.* **2003**, *68*, 839−853.

(17) Chen, C. H.; Doney, J. J.; Reynolds, G. A.; Saeva, F. D. New donors with two-electron oxidation. Synthesis and electrochemical properties of highly conjugated bis(4H-thiopyrans) and bis(flavenes). *J. Org. Chem.* **1983**, *48*, 2757−2761.

(18) Kung, M.-P.; Hou, C.; Zhuang, Z.-P.; Skovronsky, D.; Kung, H. F. Binding of two potential imaging agents targeting amyloid plaques in postmortem brain tissues of patients with Alzheimer's disease. *Brain Res.* **2004**, *1025*, 89−105.

(19) Kung, M.-P.; Hou, C.; Zhuang, Z.-P.; Cross, A. J.; Maier, D. L.; Kung, H. F. Characterization of IMPY as a potential imaging agent for β-amyloid plaques in double transgenic PSAPP mice. *Eur. J. Nucl. Med. Mol. Imaging* **2004**, *31*, 1136−1145.

(20) Zhuang, Z. P.; Kung, M. P.; Wilson, A.; Lee, C. W.; Plossl, K.; Hou, C.; Holtzman, D. M.; Kung, H. F. Structure−activity relationship of imidazo[1,2-a]pyridines as ligands for detecting beta-amyloid plaques in the brain. *J. Med. Chem.* **2003**, *46*, 237−243.

(21) Munson, P. J.; Rodbard, D. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **1980**, *107*, 220−239.

JM051020K