Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 3581–3584

# Radioiodinated aza-diphenylacetylenes as potential SPECT imaging agents for β-amyloid plaque detection

Wenchao Qu,[a] Mei-Ping Kung,[a] Catherine Hou,[a] Lee-Way Jin[c] and Hank F. Kung[a,b,*]

[a]*Department of Radiology, University of Pennsylvania, Philadelphia, PA 19104, USA*
[b]*Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, USA*
[c]*Department of Pathology, University of California Health System, Sacramento, CA 95817, USA*

Received 9 March 2007; revised 17 April 2007; accepted 18 April 2007
Available online 25 April 2007

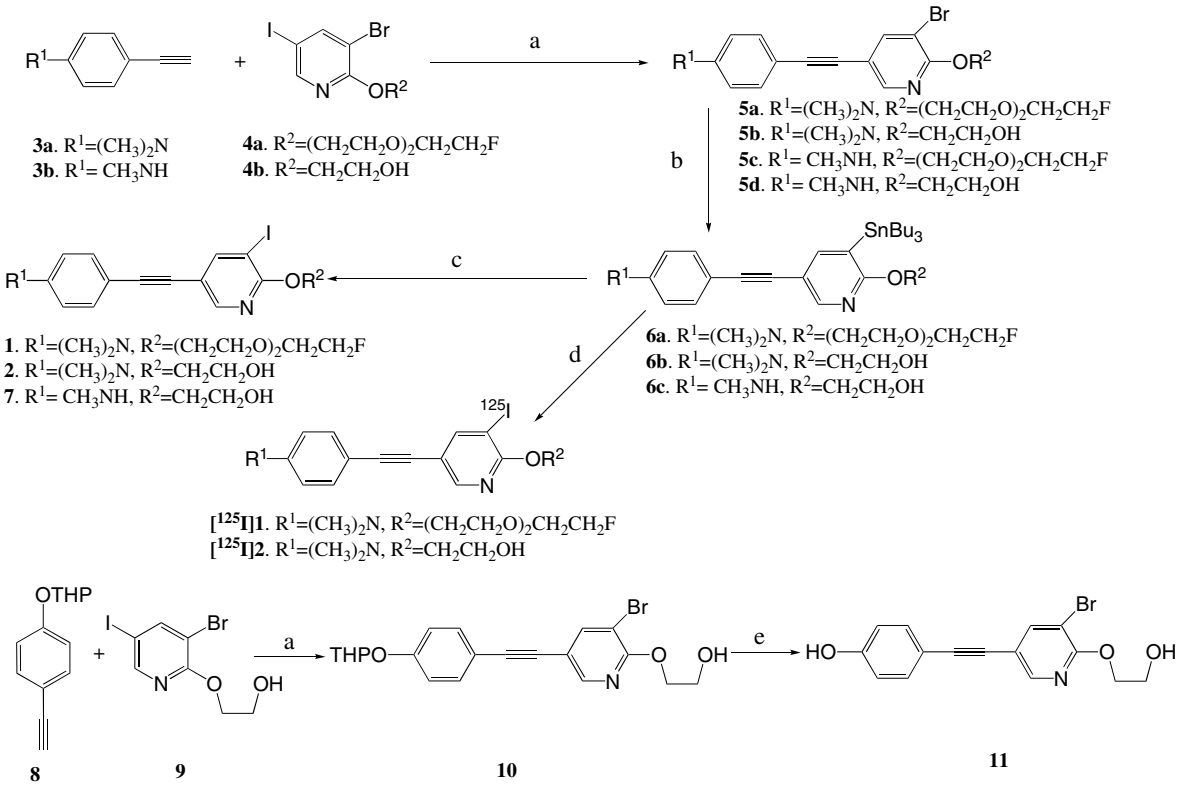
**Abstract**—Two new iodinated fluoro- and hydroxy-pegylated aza-diphenylacetylene derivatives, **1** and **2**, targeting β-amyloid (Aβ) plaques have been successfully prepared. In vitro binding carried out in tissue homogenates prepared from postmortem AD brains with [$^{125}$I]IMPY (6-iodo-2-(4′-dimethylamino)phenyl-imidazo[1,2-a]pyridine) as the radioligand indicated good binding affinities ($K_i$ = 9.2 and 16.8 nM for **1** and **2**, respectively). Brain penetrations of the corresponding radioiodinated ligands, evaluated in the normal mice, showed good initial brain penetrations (3.55% and 5.67% ID/g for [$^{125}$I]**1** and [$^{125}$I]**2** at 2 min post-injection). The washout from normal mice brain was relatively fast (0.33% and 0.91% ID/g at 2 h post-injection). The specific binding of these radioiodinated ligands to β-amyloid plaques was clearly demonstrated using film autoradiography of AD brain sections. Taken together, these preliminary results strongly suggest that these novel iodinated aza-diphenylacetylenes may be potentially useful for imaging Aβ plaques in the living human brain.


Formation and accumulation of aggregated protein deposits is a common feature of a number of neurodegenerative diseases.[1,2] Major neuropathology observations of postmortem Alzheimer's disease (AD) brains depict the presence of senile plaques (containing β-amyloid (Aβ) aggregates) and neurofibrillary tangles (highly phosphorylated tau proteins).[3,4] The exact mechanisms leading to the development of AD are not fully understood; however, the formation of Aβ plaques, consisting of β-sheets of Aβ protein aggregates, in the brain is a pivotal event in the pathogenesis of AD.[5–8] Thus, developing Aβ plaque-specific probes for in vivo imaging may be important for the diagnosis and treatment monitoring of AD.[4,9,10]

Positron emission tomography (PET) imaging with [$^{11}$C]PIB (2-(4′-(methylaminophenyl)-6-hydroxybenzothiazole)) has demonstrated the feasibility of visualizing Aβ plaques in patients suffering from AD.[11,12] However, the short half-life (20 min) of C-11 limits the usefulness of the agent for a widespread clinical application. To achieve a widespread availability, one major focus of our effort is in the development of I-123 ($T_{1/2}$ = 13 h) labeled Aβ plaque-specific imaging agents that can be used for single photon emission tomography (SPECT) imaging. The development of [$^{123}$I]IMPY (6-iodo-2-(4′-dimethylamino-)phenyl-imidazo[1,2-a]pyridine), a unique thioflavin derivative with an [1,2,a]imidazopyridine ring, showed the feasibility of developing SPECT imaging agents for targeting Aβ plaques.[13,14] However, certain undesirable characteristics of [$^{123}$I]IMPY, which include high lipophilicity, less in vivo stability, and insufficient signal to noise ratio, prompted us to pursue the development of a second generation of I-123 labeled SPECT imaging agents. In addition to the benzothiazole series, we have also explored radioiodinated derivatives of other backbone structures, including stilbenes[15] and fluorenes.[16] So far these attempts have met with a limited success. Recently, we reported a novel series of iodinated styrylpyridine derivatives showing promising results.[17] Based upon the successful results obtained from the fluorinated diphenylacetylene ligands for PET,[18] we decided to further extend our search of SPECT ligands using a similar aza-diphenylacetylene structure. Several iodinated aza-diphenylacetylene derivatives, in which one of the phenyl rings was replaced

*Keywords*: Alzheimer's disease; Radioiodination; Biodistribution; Autoradiography and Sonogashira coupling reaction.
* Corresponding author. Tel.: +1 215 662 3096; fax: +1 215 349 5035; e-mail: kunghf@sunmac.spect.upenn.edu

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.04.062




**Scheme 1.** Reagents and conditions: (a) $Pd(PPh_3)_4$, CuI, $Et_3N$, $CH_3CN$, 0 °C–rt; (b) $(Bu_3Sn)_2$, $Pd(PPh_3)_4$, toluene, 110 °C; (c) $I_2$, THF, 0 °C–rt; (d) $H_2O_2$, $Na^{125}I$, HCl, EtOH; (e) PPTS, EtOH, 55 °C, 3 h.

with a pyridine ring, were prepared and tested for their plaque binding affinities. Reported herein are the synthesis and the initial biological evaluations of these iodinated aza-diphenylacetylene ligands for targeting amyloid plaques.

Synthesis of the aza-diphenylacetylene derivatives is illustrated in Scheme 1. The key step in the synthesis is using a palladium catalyzed Sonogashira coupling reaction. All bromo-substituted compounds (**5a–d**, **10**) were readily assembled at room temperature by reacting alkynes (**3a–b**, **8**) with iodo-substituted pyridine compounds (**4a–b**). The phenol compound **11** was obtained from **10** through pyridinium *p*-toluenesulfonate catalyzed deprotection of tetrahydropyran (THP) protecting group. Next, the desired organotin compounds (**6a–c**) were successfully prepared by using the palladium catalyzed

**Table 1.** Potencies ($K_i$) of compounds on competition of [$^{125}$I]IMPY binding to amyloid plaques in AD brain homogenates

| Compound | $K_i$ (nM ± SEM) |
|---|---|
| **1** | 16.8 ± 1.8 |
| **2** | 9.2 ± 1.7 |
| **5a** | 11.2 ± 0.8 |
| **5b** | 6.7 ± 1.3 |
| **5c** | 13.1 ± 1.9 |
| **5d** | 1.6 ± 0.5 |
| **7** | 12.5 ± 2.5 |
| **11** | 6.2 ± 1.2 |
| IMPY | 5.0 ± 0.4[19] |

Each value was determined three times with duplicate for each measurement.

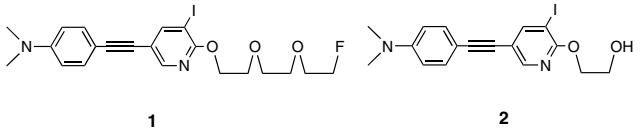


**Figure 1.** Structures of two iodinated aza-diphenylacetylene derivatives.

**Table 2.** Biodistribution in normal mice after an iv injection of [$^{125}$I]ligand (%ID/g, mean ± SD, $n$ = 3 mice per group)

| Organ | 2 min | 30 min | 1 h | 2 h |
|---|---|---|---|---|
| *[$^{125}$I]1* (*log P* = 2.60) | | | | |
| Blood | 4.23 ± 0.67 | 2.84 ± 0.32 | 3.19 ± 0.41 | 2.70 ± 0.09 |
| Heart | 15.8 ± 3.83 | 2.59 ± 0.68 | 1.53 ± 0.24 | 1.07 ± 0.03 |
| Muscle | 0.95 ± 0.27 | 1.43 ± 0.31 | 0.99 ± 0.20 | 0.67 ± 0.08 |
| Lung | 12.9 ± 3.20 | 4.00 ± 1.39 | 2.84 ± 0.43 | 2.07 ± 0.13 |
| Kidney | 16.3 ± 2.96 | 3.56 ± 0.82 | 2.78 ± 0.68 | 1.94 ± 0.10 |
| Spleen | 4.99 ± 0.21 | 1.76 ± 0.36 | 1.73 ± 0.34 | 1.46 ± 0.15 |
| Liver | 24.1 ± 4.06 | 11.2 ± 0.31 | 3.07 ± 0.62 | 1.76 ± 0.27 |
| Skin | 0.89 ± 0.07 | 2.12 ± 0.40 | 2.85 ± 0.23 | 2.75 ± 0.24 |
| Brain | 3.55 ± 0.91 | 3.10 ± 0.38 | 1.36 ± 0.10 | 0.33 ± 0.01 |
| *[$^{125}$I]2* (*log P* = 2.80) | | | | |
| Blood | 3.45 ± 0.29 | 3.38 ± 0.51 | 2.71 ± 0.43 | 3.29 ± 0.61 |
| Heart | 13.0 ± 2.47 | 2.31 ± 0.37 | 1.41 ± 0.33 | 1.33 ± 0.26 |
| Muscle | 0.98 ± 0.30 | 1.23 ± 0.20 | 0.71 ± 0.09 | 0.78 ± 0.11 |
| Lung | 13.0 ± 2.32 | 4.03 ± 0.41 | 2.60 ± 0.56 | 2.77 ± 0.86 |
| Kidney | 16.6 ± 2.48 | 4.10 ± 0.54 | 2.85 ± 0.50 | 2.40 ± 0.47 |
| Spleen | 6.33 ± 1.03 | 1.98 ± 0.34 | 1.42 ± 0.40 | 1.73 ± 0.45 |
| Liver | 17.2 ± 2.86 | 11.2 ± 1.59 | 5.21 ± 2.05 | 2.24 ± 0.45 |
| Skin | 0.87 ± 0.14 | 2.22 ± 0.64 | 2.46 ± 0.27 | 3.18 ± 0.17 |
| Brain | 5.67 ± 1.49 | 4.51 ± 0.56 | 2.14 ± 0.21 | 0.91 ± 0.17 |

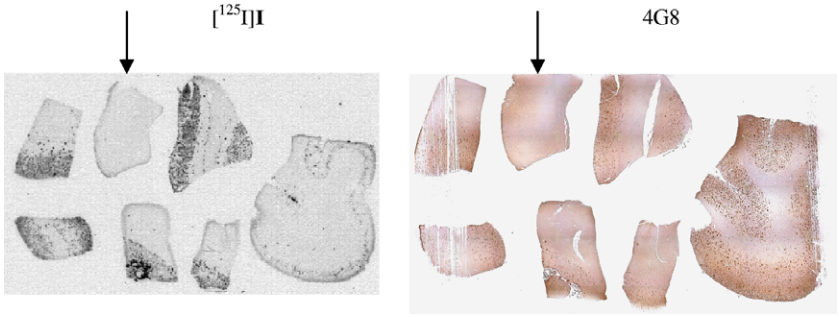



**Figure 2.** In vitro autoradiography to detect amyloid plaques with [$^{125}$I]**1**. The human macroarray brain sections were constructed from six postmortem AD cases plus one control (marked by arrowhead). The plaques were confirmed with 4G8 antibody immunohistochemistry staining.

*trans*-stannylation from their bromide precursors (**5a**–**b**, **5d**). The subsequent iododestannylation reaction afforded iodinated targets (**1**, **2**, **7**).[17,20]

The binding affinities of the non-radioactive ligands (**1**–**2**, **5a**–**d**, **7**, **11**) were screened via the binding competition with [$^{125}$I]IMPY using postmortem AD brain homogenates.[19] All the brominated and iodinated aza-diphenylacetylene derivatives examined displayed excellent to good binding affinities in comparison to [$^{125}$I]IMPY binding. It was evident that all of the brominated derivatives and the corresponding iodinated ligands displayed similar excellent binding affinities ($K_i$ values shown in Table 1 between series **5b** and **5d** vs **2** and **7**).

The hydroxyl pegylated derivatives, i.e., **5b**, **5d**, **11**, **2**, and **7**, competed effectively with [$^{125}$I]IMPY binding with $K_i$ values of 6.7 ± 1.3, 1.6 ± 0.5, 6.2 ± 1.2, 9.2 ± 1.7, and 12.5 ± 2.5 nM. The addition of a fluoropegylated chain to the 2′-position of the pyridine group displayed similar binding affinities to β-amyloid plaques. Compounds **5a**, **5c**, and **1** showed $K_i$ values of 11.2 ± 0.8, 13.1 ± 1.9, and 16.8 ± 1.8 nM (Table 1). Similarly, there is no significant difference in the binding affinities between *N*,*N*-dimethylamino derivatives, **5b**, **2**, and *N*-monomethylamino derivatives, **5d**, **7**. In addition, after replacing the substituted amino group with a hydroxy group attached to the 4-position of one end of the phenyl ring, the binding affinity remained the same ($K_i$ = 6.2 ± 1.2 and 6.7 ± 1.3 nM for **11** and **5b**, respectively) Figure. 1.

On the basis of the encouraging binding data observed for these series of ligands, we chose two representatives, **1** and **2**, to carry out further biological evaluations with the I-125 labeled probes. Radioiodination was successfully carried out with the corresponding tributyltin precursors, following the standard iododestannylation reaction, using hydrogen peroxide as the oxidant (Scheme 1).[13] The final HPLC-purified ligands, [$^{125}$I]**1** and [$^{125}$I]**2**, showed greater than 98% radiochemical purities with high overall yields (>60%) and high specific activities (~2000 Ci/mmol). The two radioiodinated probes measured under the experimental conditions showed moderate lipophilicity[17] (log *P* = 2.6 and 2.8), a desirable property for Aβ-targeting imaging agents. When evaluated for whole animal biodistribution after an iv injection in normal mice, [$^{125}$I]**1** and **2** displayed good initial penetrations of the intact blood–brain barrier with excellent initial brain uptakes (3.55% and 5.67% ID/g at 2 min after tracer injection). The high brain uptakes of these iodinated ligands were subsequently followed by relatively fast washouts with 0.33% and 0.91% ID/g remaining in the brain at 2 h after the tracer injection (Table 2). The kinetics of fast brain uptake and washout from normal brain (containing no Aβ plaques) is highly desirable for an Aβ plaque-targeting imaging agent.[10]

To confirm the specific labeling of radioiodinated azadiphenylacetylenes for Aβ plaques, we performed the in vitro film autoradiography. A human brain macroarray containing both AD and control cases allowed us to efficiently compare the plaque labeling with various radiolabeled probes. As shown in Figure 2, [$^{125}$I]**1** distinctively labeled plaques on AD brain sections with low background labeling, but not for the control section (indicated by an arrow), which is consistent with the immunohistochemistry staining with Aβ antibody 4G8.

In conclusion, we have demonstrated that iodinated azadiphenylacetylenes can be successfully prepared. They showed high binding affinities to β-amyloid plaques by in vitro binding assay. The radioiodinated derivatives, [$^{125}$I]**1** and [$^{125}$I]**2**, displayed desirable in vivo properties, with excellent brain penetrations and relatively fast rates of washout in normal mice (resulting in low background signal). Specific Aβ plaque labeling was clearly demonstrated by in vitro autoradiography of postmortem AD brain section. This series of SPECT probes warrants further investigation to confirm the usefulness for imaging amyloid plaques in AD.

## Acknowledgments

This work was supported by grants from the National Institutes of Health (AG-021868 to M.P.K.). The author thanks Pathology Core Laboratories at The Childrens Hospital of Philadelphia for assembling the human macro-array brain sections. The authors also thank Drs. Daniel Skovronsky and Rajesh Manchanda for their helpful discussion.

## References and notes


1. Kelly, J. W. *Curr. Opin. Struct. Biol.* **1996**, *6*, 11.
2. Shastry, B. S. *Neurochem. Int.* **2003**, *43*, 1.
3. Ginsberg, S. D.; Schmidt, M. L.; Crino, P. B.; Eberwine, J. H.; Lee, V. M.-Y.; Trojanowski, J. Q. Molecular pathology of Alzheimer's disease and related disorders. In *Cerebral Cortex: Neurodegenerative and Age-related Changes in Structure and Function of Cerebral Cortex*; Peters, A., Morrison, J. H., Eds.; Kluwer Academic/Plenum: New York, 1999; pp 603–654.
4. Selkoe, D. J. *JAMA* **2000**, *283*, 1615.
5. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
6. Hardy, J. *Curr. Alzheimer Res.* **2006**, *3*, 71.
7. Golde, T. E. *Brain Pathol.* **2005**, *15*, 84.
8. Marchesi, V. T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 9093.
9. Blennow, K.; Zetterberg, H. *Nat. Med.* **2006**, *12*, 753.
10. Mathis, C. A.; Wang, Y.; Klunk, W. E. *Curr. Pharm. Des.* **2004**, *10*, 1469.
11. 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. *Ann. Neurol.* **2004**, *55*, 306.
12. Mintun, M. A.; Larossa, G. N.; Sheline, Y. I.; Dence, C. S.; Lee, S. Y.; Mach, R. H.; Klunk, W. E.; Mathis, C. A.; DeKosky, S. T.; Morris, J. C. *Neurology* **2006**, *67*, 446.
13. Zhuang, Z.-P.; Kung, M.-P.; Hou, C.; Skovronsky, D.; Gur, T. L.; Trojanowski, J. Q.; Lee, V. M.-Y.; Kung, H. F. *J. Med. Chem.* **2001**, *44*, 1905.
14. Kung, M. P.; Hou, C.; Zhuang, Z.-P.; Zhang, B.; Skovronsky, D. M.; Gur, T.; Lee, V. M.-Y.; Trojanowski, J. Q.; Kung, H. F. *Brain Res.* **2002**, *956*, 202.
15. Kung, H. F.; Lee, C.-W.; Zhuang, Z. P.; Kung, M. P.; Hou, C.; Plossl, K. *J. Am. Chem. Soc.* **2001**, *123*, 12740.
16. Lee, C.-W.; Kung, M.-P.; Hou, C.; Kung, H. F. *Nucl. Med. Biol.* **2003**, *30*, 573.
17. Qu, W.; Kung, M.-P.; Hou, C.; Benedum, T. E.; Kung, H. F. *J. Med. Chem.* **2007**, *50*, 2157.
18. Chandra, R.; Oya, S.; Kung, M. P.; Hou, C.; Jin, L. W.; Kung, H. F. *J. Med. Chem.*, in press, **2007**.
19. Kung, M.-P.; Hou, C.; Zhuang, Z.-P.; Skovronsky, D.; Kung, H. F. *Brain Res.* **2004**, *1025*, 89.
20. Typical procedure for Sonogashira coupling reaction: **4-((5-bromo-6-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)pyridin-3-yl)ethynyl)-*N*,*N*-dimethylbenzeneamine** (**5a**). A mixture of 4-ethynyl-*N*,*N*-dimethylaniline (0.080 g, 0.55 mmol), **4a** (0.217 g, 0.5 mmol), and $Pd(Ph_3)_4$ (0.029 g, 0.025 mmol) in 3 mL $Et_3N$ and 3 mL $CH_3CN$ was deoxygenated by purging with nitrogen for 15 min. The reaction mixture was cooled with an ice bath and CuI (0.009 g, 0.05 mmol) was added into reaction flask. After 5 min at 0 °C, the reaction mixture was stirred at rt for 1 h. It was then concentrated and purified by flash chromatography (EtOAc/hexanes, 25:75) to yield **5a** as a light yellow solid (0.180 g, 79%). $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 8.20 (d, 1H, $J$ = 2.0 Hz), 7.92 (d, 1H, $J$ = 2.0 Hz), 7.39 (dt, 2H, $J_1$ = 8.9 Hz, $J_1$ = 2.3 Hz), 6.66 (dt, 2H, $J_1$ = 8.9 Hz, $J_1$ = 2.3 Hz), 4.69 (t, 1H, $J$ = 4.2 Hz), 4.55 (t, 2H, $J$ = 4.9 Hz), 4.45 (t, 1H, $J$ = 4.2 Hz), 3.94–3.68 (m, 8H), 3.01 (s, 6H). $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 158.3, 150.4, 143.5, 138.0, 129.6, 129.5, 127.7, 125.2, 118.8, 112.5, 107.5, 85.0, 81.6, 71.2, 71.0, 70.8, 70.4, 69.6, 66.7, 40.5. HRMS calcd for $C_{21}H_{24}BrFN_2O_3$ ($M^+$), 450.0954; found, 450.0944.