JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 647–664.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.585

Research Article

Synthesis and ^{11}C-labelling of (*E*,*E*)-1-(3′,4′-dihydroxystyryl)-4-(3′-methoxy-4′-hydroxystyryl) benzene for PET imaging of amyloid deposits†

Yanming Wang[1,*], Chester A. Mathis[1], Guo-feng Huang[1], Daniel P. Holt[1], Manik L. Debnath[2] and William E. Klunk[2]
[1] *P.E.T. Facility, Department of Radiology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA*
[2] *Laboratory of Molecular Neuropharmacology, Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA*

Summary

Carboxylic acid derivatives of the amyloid-binding dye Congo red do not enter the brain well and are thus unable to serve as *in vivo* amyloid-imaging agents. A neutral amyloid probe, (*E*,*E*)-1-(3′,4′-dihydroxystyryl)-4-(3′-methoxy-4′-hydroxystyryl)benzene (**3**), devoid of any carboxylate groups has been designed and synthesized via a 12-step reaction sequence with a total yield of 30%. The unsymmetric compound **3** has also been labelled with C-11 via [^{11}C]methyl iodide ([^{11}C]CH_3I) methylation of a symmetric 4,4′-dimesyl protected precursor followed by deprotection. Preliminary evaluation indicated that compound **3** selectively stained plaques and neurofibrillary tangles in post-mortem AD brain, and exhibited good binding affinity ($K_i = 38 \pm 8$ nM) for Aβ(1–40) fibrils *in vitro*. *In vivo* pharmacokinetic studies indicated that [^{11}C]**3** exhibited higher brain uptake than its carboxylic acid analogs and good clearance from normal control mouse brain. [^{11}C]**3** also exhibited specific *in vivo* binding to pancreatic amyloid deposits in the NOR-beta transgenic

*Correspondence to: Y. Wang, P.E.T. Facility, Department of Radiology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA. E-mail: wangy2@msx.upmc.edu

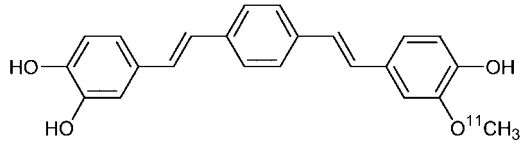
†Dedicated to Professor Dr Dieter Seebach on the occasion of his 65th birthday and in recognition of his many contributions to chemical science.

Received 18 December 2002
Revised 23 January 2002
Accepted 25 February 2002

mouse model. These results justify further investigation of **3** and similar derivatives as surrogate markers for *in vivo* quantitation of amyloid deposits. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: Alzheimer's disease; β-amyloid; ^{11}C-labelling; brain uptake; NOR-beta transgenic mouse model

Graphical abstract



Introduction

Progress has been made towards the development of new therapies based upon the amyloid cascade hypothesis of Alzheimer's disease (AD).[1] Optimal development of these anti-amyloid therapies,[2,3] such as β- and γ-secretase inhibition and β-amyloid (Aβ) immunization, awaits methods to assess amyloid deposition in the living brain. A useful biomarker of amyloid deposition in the brain could make possible identification of individuals at risk for AD and facilitate the evaluation of anti-amyloid therapies through molecular imaging techniques[4] such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT).

Towards this goal, efforts have been made to develop lipophilic Congo red (CR) analogs that have high affinity for amyloid and are capable of penetrating the blood–brain barrier (BBB).[5] CR analogs, such as Chrysamine G (CG) and X-34 (1,4-bis(3′-carboxy-4′-hydroxy-styryl)benzene, **1**) (Figure 1), are salicylic acid derivatives and exhibit only marginal brain entry.[6–11] This is likely due to the known detrimental effects of aromatic carboxylic acids on brain entry.[12] As a strategy to increase the permeability across the BBB, this study focused on the effects of replacing the acidic COOH groups of **1** with OH groups on both binding to amyloid fibrils and brain permeability. Unlike carboxylic acids, aromatic OH groups (pKa 8–11) are largely neutral at physiologic pH, but could potentially participate in polar interactions

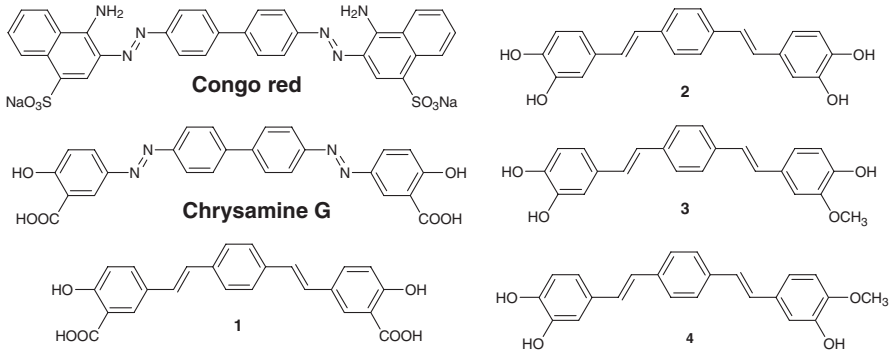


Figure 1. Structures of amyloid-binding agents: Congo red (CR), Chrysamine G (CG), (*E,E*)-1,4-bis(3′-carboxy-4′-hydroxystyryl)benzene 1, (*E,E*)-1,4-bis(3′,4′-dihydroxystyryl)benzene 2, (*E,E*)-1-(3′,4′-dihydroxystyryl)-4-(3′-methoxy-4′-hydroxystyryl)benzene 3, and (*E,E*)-1-(3′,4′-dihydroxystyryl)-4-(3′-hydroxy-4′-methoxystyryl)benzene 4

with Aβ in a manner similar to the COOH groups. In addition, the hydroxyl group can also be readily methylated with methyl iodide. With our ultimate goal being development of an *in vivo* PET amyloid imaging agent, we report here the design and synthesis of (*E,E*)-1-(3′,4′-dihydroxystyryl)-4-(3′-methoxy-4′-hydroxystyryl)benzene (**3**), which can be produced in a ^{11}C-labelled form via methylation of an aromatic OH group of the precursor (**19**). Preliminary evaluation indicated that **3** exhibited good permeability across the BBB and retained good affinity for Aβ fibrils *in vitro*.

Results and discussion

In order to examine the effect of replacing COOH groups of **1** with OH, we initially studied 1,4-bis(3′,4′-dihydroxystyryl)benzene **2**. Compared to the dicarboxylic acid **1**, compound **2** showed no significant loss of affinity for Aβ fibrils (Figure 2). Since our ultimate goal is the development of a ^{11}C-labelled compound for use with PET, we also studied the two mono-methoxy analogs **3** and **4** (Figure 1). Mono-methoxy analogs were studied because methylation of **2** with a tracer amount of no-carrier-added [^{11}C]CH_3I resulted only in mono-methoxy analogs for stoichiometric reasons. Preliminary studies indicated that rodent brain uptake of the 3′-O$^{11}CH_3$ analog (**3**) was *ca*. five-fold higher

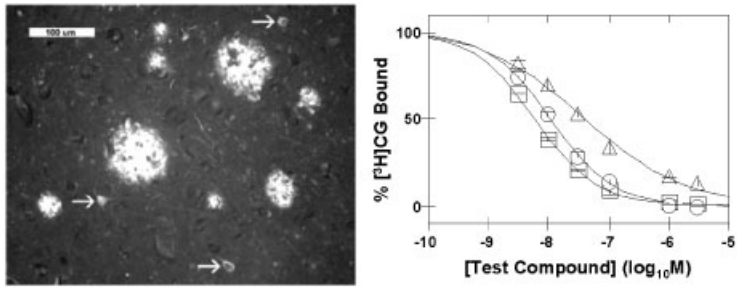
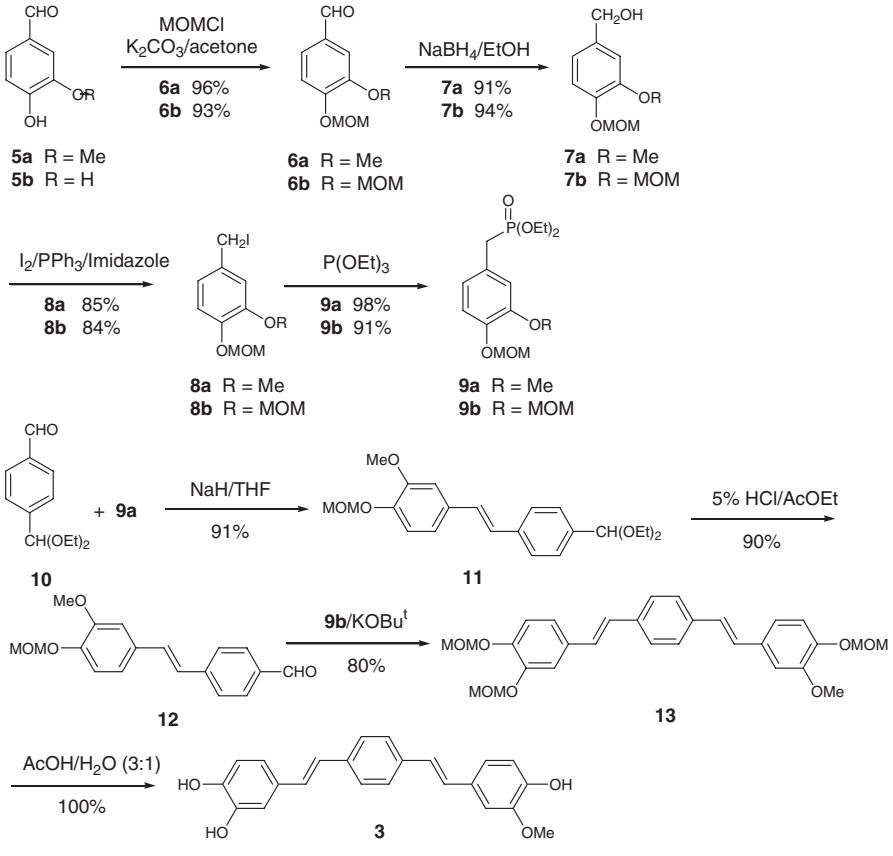


Figure 2. (Left) Fluorescent microscopic image of post-mortem AD brain stained with 3. Arrows indicate neurofibrillary tangles. The large, round, bright structures are amyloid plaques. Bar = 100 μm. (Right) Binding curves for the inhibition of [^{3}H]CG binding to Aβ(1–40) fibrils by 1 (squares), 2 (circles) and 3 (triangles)

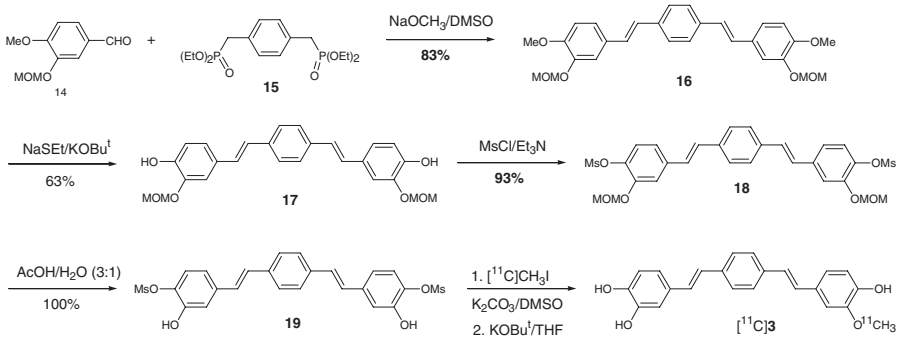
than the 4′-OMe analog (**4**) at 2 min post-injection. Therefore, we focused on the synthesis and ^{11}C-labelling of **3**, free from the 4′-O$^{11}CH_3$ analog.

Although the synthesis of symmetric distyrylbenzene compounds has been reported,[13] synthesis of the unsymmetric variants has been rarely documented.[14] We thus developed a convergent synthetic approach to **3** based on Wittig–Horner coupling. The total synthesis involved 12 steps with a total yield of 30% (Scheme 1). Beginning with methoxymethyl (MOM) protection of 3,4-dihydroxybenzaldehyde **5b** and 3-methoxy-4-hydroxybenzaldehyde **5a**, both MOM-protected aldehydes **6a** and **6b** were reduced with sodium borohydride ($NaBH_4$) to benzyl alcohols **7a** and **7b**. Iodination of the resulting benzyl alcohols with iodine in the presence of triphenyl phosphine (PPh_3)/imidazole afforded the corresponding benzyl iodides **8a** and **8b**. Subsequent Arbuzov reactions with triethyl phosphate ($P(OEt)_3$) yielded the benzylphosphonates **9a** and **9b**. Compound **9a** was first coupled with terephthaldehyde monoacetal **10** in the presence of sodium hydride (NaH) in good yield. After converting the acetal **11** to the aldehyde, the monostyrylbenzaldehyde **12** was then coupled with 3,4-diMOMO-benzylphosphonate **9b** to give the MOM-protected unsymmetric distyrylbenzene compound **13**. This second coupling proceeded well if potassium tert-butoxide was used as the base. Cleavage of the MOM groups led to **3** in quantitative yield. NMR analysis indicated that only the (*trans*, *trans*)-isomer of the product was obtained (Scheme 1).

In addition, we developed a method for radiolabelling **3** selectively at the 3′-position via [^{11}C]CH_3I methylation of symmetric **19** (Scheme 2).



Scheme 1. Synthesis of the unsymmetric distyrylbenzene derivative 3



Scheme 2. Synthesis of the symmetric precursor 19 and C-11-labelling of 3

Because the precursor was in great excess relative to the tracer amount of CH_3I ($\sim$100:1), only monomethylation in the 3′-position could be detected. Similar to the synthesis of the cold standard, the precursor **19** was synthesized through Wittig–Horner reaction of the xylylene diphosphonate **15** with 3-MOMO-4-methoxybenzaldehyde **14** in good yield. The 4-methyl groups of **16** were then replaced with mesyl groups to give **18**. Cleavage of the 3-MOM groups gave the corresponding **19**, which was then methylated with [^{11}C]CH_3I. The C-11-labelled intermediate was then treated with potassium tert-butoxide to cleave the mesyl groups. After purification by high-pressure liquid chromatography (HPLC), approximately 370–925 MBq (10–25 mCi) of [^{11}C]**3** was routinely produced with a radiochemical purity of $>95\%$, a chemical purity of $>80\%$, and specific activity >56 MBq/nmol at end of a 40 min synthesis (EOS). The radiochemical yield was $2.3\% \pm 0.9$ at EOS based on [^{11}C]CH_3I ($n=10$), which was not optimized.

Like **1**, compound **3** is fluorescent and stains amyloid plaques in AD brain tissue sections (Figure 2). Compound **3** also stains the other typical pathological feature found in post-mortem AD brain, the neurofibrillary tangle.[15] The predominant β-pleated sheet structure common to amyloid plaques and neurofibrillary tangles has been posited to mediate the binding of the Congo red class of compounds, including **1**. Compared to the absence of staining in age-matched normal control brain (not shown), this indicates a selective binding of **3** to AD pathology. In addition, compound **3** competed with [^{3}H]CG binding to synthetic Aβ(1–40) fibrils with an affinity ($K_i = 38$ nM) similar to that of CG ($K_i = 3 \pm 0.3$ nM), **1** ($K_i = 6 \pm 0.3$ nM), **2** ($K_i = 12 \pm 0.4$ nM), and CR ($K_i = 48 \pm 6$ nM) (Figure 2).

[^{11}C]**3** entered mouse brain in significant amounts, $2.25 \pm 0.98\%$ ID/g in whole brain ($7.16 \pm 3.07\%$ ID/g in whole blood) at 2 min after intravenous (i.v.) injection. This value is consistent with levels achieved by typical PET neuroimaging agents. At 30 min post-injection, the concentration of radioactivity in mouse brain dropped to $0.71 \pm 0.33\%$ ID/g (2:30 min ratio = 3.2:1), indicating good efflux of free and non-specifically bound [^{11}C]**3** out of normal mouse brain.

The *in vivo* pharmacokinetic profile of [^{11}C]**3** was further evaluated in the NOR-beta transgenic mouse pancreatic model of Aβ amyloid deposition.[16] As shown in Figure 3, by 60 min post-[^{11}C]**3** injection, the concentration of radioactivity in the amyloid-containing pancreas of the transgene positive mice (Tg^+, open circles) was found to be two-fold higher than that in the pancreas of transgene negative littermates

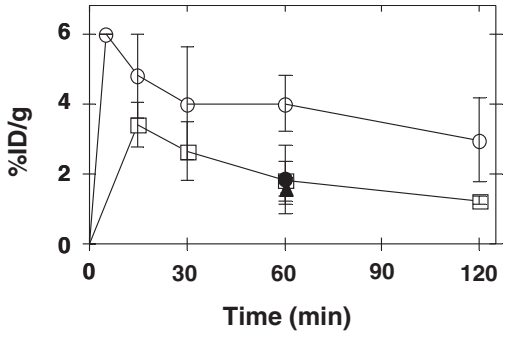


Figure 3. Time–radioactivity course of [^{11}C]3 in the pancreas of transgene positive amyloid-containing NOR-beta mice (open circles) and transgene negative littermates (open square). Filled symbols at 60 min represent tissue levels of [^{11}C]3 after pre-treatment with 40 mg/kg MeO-X-04 in transgene positive (filled circle) or transgene negative (filled triangle) mice

(Tg^-, open squares). As a further test of whether the difference in radioactivity concentrations between Tg^+ and Tg^- pancreas was due to saturable and specific binding of [^{11}C]**3** to Aβ deposits, a competitive *in vivo* binding experiment was carried out with (*E*,*E*)-2-methoxy-1,4-bis(4′-hydroxystyryl)benzene (MeO-X-04), another amyloid-binding agent with high affinity ($K_i = 27$ nM for Aβ(1–40)).[17] Mice were first treated with MeO-X-04 (40 mg/kg) through intraperitoneal (i.p.) injection 60 min prior to injection of [^{11}C]**3**. At 60 min post-[^{11}C]**3** injection, the concentration of radioactivity in Tg^+ mice pre-treated with MeO-X-04 (filled circle) was the same as that in the untreated Tg^- control mice (open square) (Figure 3). In addition, radioactivity levels in Tg^- control pancreas were not affected by pre-treatment with MeO-X-04 (filled triangle) (Figure 3). This suggests that the increased retention of radioactivity in Tg^+ pancreas was due to saturable and specific binding of [^{11}C]**3** to Aβ deposits.

In summary, we have synthesized and ^{11}C-labelled compound **3**, which showed good permeability across the BBB in normal control mice. Although the initial brain entry is less than that of recently reported thioflavin-T derivatives,[18,19] this compound represents a distinct chemical class of amyloid-binding agents, which have different binding properties compared to the thioflavin-T analogs. The *in vivo* pharmacokinetic studies in the NOR-beta transgenic model indicated that compound **3** may be useful in the study of peripheral amyloid deposits.

Experimental section

General remarks

4-Hydroxy-3-methoxybenzaldehyde (**5a**), 3,4-dihydroxybenzaldehyde (**5b**), terephthaldehyde mono-(diethyl acetal) (**10**), chloromethyl methyl ether (MOMCl), triethyl phosphite, triphenyl phosphine, imidazole, sodium hydride (powder, 95%), potassium tert-butoxide, sodium borohydride, sodium thioethoxide, iodine were purchased from Aldrich Chemical Company and used without further purification. Tetraethyl 1,4-xylylene diphosphonate (**15**) was obtained from TCI. Acetone (solvent grade) was dried over potassium carbonate (K_2CO_3) at room temperature (RT) for more than 24 h. K_2CO_3 was finely powdered before use. Tetrahydrofuran (THF) was freshly distilled over sodium metal and benzophenone.

The high-resolution mass spectra were taken under EI condition on a double-focusing high-resolution mass spectrometer (AUTOSPEC of Micromass Inc.). Samples were introduced by direct insertion probe. The ^{1}H-NMR spectra of all compounds were measured on a Bruker 300 and 500 MHz using TMS as internal reference and were in agreement with the assigned structures. The thin layer chromatography (TLC) was performed using Silica Gel 60 F_{254} from EM Sciences and detected under UV lamp. Flash chromatography was performed on silica gel 60 (230–400 mesh; purchased from Mallinckrodt Company; column sizes: 54.0 cm × 2.5 cm, 36.5 cm × 2.5 cm or 39.2 cm × 2.2 cm) using hexanes and ethyl acetate (EtOAc) as eluents.

*3-Methoxy-4-methoxymethoxybenzaldehyde (***6a***).*

4-Hydroxy-3-methoxybenzaldehyde (**5a**) (3.0 g, 20 mmol) was dissolved in acetone (100 ml). To this solution, K_2CO_3 (15 g, 109 mmol) and MOMCl (8.0 ml, 105 mmol) were added at RT. The reaction mixture was allowed to stir at RT overnight (~16 h). The suspension was filtered and the residue was washed with ethyl acetate (20 ml). After evaporation of the solvent, the residue was purified by flash chromatography on silica gel (70 g) eluted with ethyl acetate/hexanes (1:4) to give 4.0 g (96%) of **6a** as colorless oil with R_f 0.38 (hexanes/ethyl acetate 2:1).

^{1}H-NMR δ(300 MHz, $CDCl_3$): 3.48(3 H, s, OCH_3), 3.90(3 H, s, CH_3), 5.28(2 H, s, CH_2), 7.72–7.40(3 H, arom), 9.82(1 H, s, CHO). HRMS: m/z calcd for $C_{10}H_{12}O_4$ (M^+) 196.0736, found 196.0741.

*3,4-Dimethoxymethoxybenzaldehyde (***6b***)*

This compound was synthesized in a manner analogous to **6a** starting with **5b** to give 3.1 g (93%) of **6b** as white solid with R_f 0.71 (hexanes/ethyl acetate 1:1) and m.p. 40.5–45.5°C.

^{1}H-NMR δ(300 MHz, $CDCl_3$): 3.52(3 H, s, CH_3), 3.52(3 H, s, CH_3), 5.33(2 H, s, CH_2), 5.30(2 H, s, CH_2), 7.68–7.26(3 H, arom), 9.86(1 H, s, CHO). HRMS: *m/z* calcd for $C_{11}H_{14}O_5$ (M^+) 226.0841, found 226.0842.

*3-Methoxy-4-methoxymethoxybenzyl alcohol (***7a***)*

3-Methoxy-4-methoxymethoxybenzaldehyde (**6a**) (4.0 g, 20 mmol) was dissolved in anhydrous ethanol (EtOH) (100 ml). $NaBH_4$ (0.4 g, 11 mmol) was added. The reaction mixture was allowed to stir at RT for 10 min followed by the evaporation of solvent. The residue was dissolved in water (100 ml) and extracted with EtOAc (50 ml × 3). The organic layers were combined, dried over magnesium sulfate ($MgSO_4$) (10 g) and filtered. Evaporation of solvent gave 3.7 g (91%) of **7a** as colorless oil.

^{1}H-NMR δ(300 MHz, $CDCl_3$): 3.48(3 H, s, OCH_3), 3.84(3 H, s, OCH_3), 4.56(2 H, s, OCH_2), 5.18(2 H, s, CH_2), 6.80–7.10(3 H, arom), 9.86(1 H, s, CHO). HRMS: *m/z* calcd for $C_{10}H_{14}O_4$(M^+) 198.0892, found 198.0888.

*3,4-Dimethoxymethoxybenzyl alcohol (***7b***)*

This compound was synthesized in a manner analogous to **7a** starting with **6b** to give 2.8 g (94%) of **7b** as colorless oil with R_f 0.41 (hexanes/ethyl acetate 1:1).

^{1}H-NMR δ(300 MHz, $CDCl_3$): 3.54(3 H, s, OCH_3), 3.54(3 H, s, OCH_3), 4.63(2 H, s, CH_2O), 5.25(2 H, s, CH_2), 5.26(2 H, s, CH_2), 6.99–7.21(3 H, arom). HRMS: *m/z* calcd for $C_{11}H_{16}O_5$ (M^+) 228.0998, found 228.0996.

*3-Methoxy-4-methoxymethoxybenzyl iodide (***8a***)*

3-Methoxy-4-methoxymethoxybenzyl alcohol (**7a**) (3.7 g, 19 mmol) was dissolved in THF (100 ml) followed by addition of PPh_3 (5.4 g, 20 mmol) and imidazole (1.5 g, 22 mmol). The reaction mixture was cooled with ice-water bath. Iodine (5.2 g, 20 mmol) in THF (50 ml) was added over

20 min. After being stirred for an additional 30 min, the reaction was quenched by addition of water (0.5 ml) and the solvent was evaporated. The residue was purified by flash chromatography on silica gel (90 g), eluted with hexanes/ethyl acetate (85:15) to give 4.9 g (85%) of **8a** as white solid with R_f 0.23 (hexanes/ethyl acetate 4:1).

^{1}H-NMR δ(300 MHz, $CDCl_3$): 3.50(3 H, s, CH_3O), 3.89(3 H, s, CH_3O), 4.46(2 H, s, CH_2I), 5.22(2 H, s, CH_2O), 7.00–7.19(3 H, m, arom.).

*3,4-Dimethoxymethoxybenzyl iodide (***8b***)*

This compound was synthesized in a manner analogous to **8a** starting with **7b** to give 3.2 g (84%) of **8b** as colorless oil with R_f 0.46 (hexanes/ethyl acetate 3:1).

^{1}H-NMR δ(300 MHz, $CDCl_3$): 3.51(3 H, s, CH_3O), 3.53(3 H, s, CH_3O), 4.44(2 H, s, CH_2I), 5.22(2 H, s, CH_2O), 5.23(2 H, s, CH_2O). 7.00–7.19(3 H, m, arom.).

*Diethyl 3-methoxy-4-methoxymethoxybenzylphosphonate (***9a***)*

3-Methoxy-4-methoxymethoxybenzyl iodide (**8a**) (4.8 g, 15 mmol) was dissolved in triethyl phosphite (10 ml) in a vial (20 ml). The vial was sealed and heated at 140°C overnight ($\sim$16 h). The volatiles and excess triethyl phosphite were removed in a kugelrohr oven (100°C) under high vacuum ($\sim$1 mmHg) to give 4.8 g (98%) of **9a** as colorless oil.

^{1}H-NMR δ(300 MHz, $CDCl_3$): 1.23(6 H, t, J = 7.1 Hz, $2CH_3$), 3.06(2 H, d, J = 21.3 Hz, PCH_2), 3.47(3 H, s, CH_3O), 3.84(3 H, s, CH_3), 3.99(4 H, m, J = 7.17 Hz, $2CH_2OP$), 5.17(2 H, s, CH_2O), 6.74–7.06(3 H, m, arom.). HRMS: m/z calcd for $C_{14}H_{123}O_6P$ (M^+) 318.1232, found 318.1246.

*Diethyl 3,4-dimethoxymethoxybenzylphosphonate (***9b***)*

This compound was synthesized in a manner analogous to **9a** starting with **8b** to give 3.0 g (91%) of **9b** as colorless oil.

^{1}H-NMR δ(300 MHz, $CDCl_3$): 1.22(6 H, t, J = 7.1 Hz, $2CH_3$), 3.04(2 H, d, J = 21.3 Hz, PCH_2), 3.47(6 H, s, $2CH_3O$), 3.99(4 H, m, J = 7.26 Hz, $2CH_2OP$), 5.17(2 H, s, CH_2O), 5.18(2 H, s, CH_2O). 6.80–7.10(3 H, m, arom.). HRMS: m/z calcd for $C_{15}H_{25}O_7P$ (M^+) 348.1337, found 348.1344.

*(E)-4-diethoxymethyl-3′-methoxy-4′-methoxymethoxystilbene (***11***)*

Diethyl 3-methoxy-4-methoxymethoxybenzylphosphonate (**9a**) (2.0 g, 6.3 mmol) and terephthaldehyde mono-(diethyl acetal) (**10**) (1.3 g, 6.3 mmol) were dissolved in THF (150 ml) followed by addition of sodium hydride (0.5 g, 19 mmol). The reaction was refluxed for 3 h and then cooled to RT. The excess sodium hydride was hydrolyzed with water (0.5 ml) and the solvent was evaporated. The residue was dissolved in water (100 ml) and extracted with ethyl acetate (3×30 ml). The combined extracts were dried over $MgSO_4$ (5 g), filtered, and evaporated to give an oil which was purified by flash chromatography on silica gel (60 g). Elution with hexanes/ethyl acetate (85:15) gave 2.1 g (91%) of **7** as a light yellow oil with R_f 0.40 (hexanes/ethyl acetate 3:1).

^{1}H-NMR δ(300 MHz, $CDCl_3$): 1.25(6 H, t, $J = 6.72$ Hz, CH_3), 3.52(3 H, s, CH_3O), 3.54–3.66(4 H, m, $2CH_2O$), 5.23(2 H, s, CH_2O), 5.52(1 H, s, CHOEt), 6.90–7.55(9 H, m, 9CH).

*(E)-4-(3′-methoxy-4′-methoxymethoxystyryl)benzaldehye (***12***)*

(*E*)-4-diethoxymethyl-3′-methoxy-4′-methoxymethoxystilbene (**11**) (3.0 g, 8.0 mmol) was dissolved in ethyl acetate (50 ml), and 5% aqueous HCl solution (25 ml) was added. The reaction mixture was allowed to stir at RT for 30 min. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (30 ml $\times$ 3). The combined organic layers were dried over $MgSO_4$ (15 g) and evaporated to give 2.1 g (90%) of **12** as yellow solid with R_f 0.39 (hexanes/ethyl acetate 2:1).

^{1}H-NMR δ(300 MHz, $CDCl_3$): 3.53(3 H, s, CH_3O), 3.95(3 H, s, CH_3O), 5.26(2 H, s, CH_2O), 6.94–7.86(9 H, m, 9CH), 9.97(1 H, s, CHO). HRMS: *m*/*z* calcd for $C_{18}H_{118}O_4$ (M^+) 298.1205, found 298.1200.

*(E,E)-1-(3′,4′-dimethoxymethoxystyryl)-4-(3′-methoxy-4′-methoxymethoxystyryl)benzene (***13***)*

4-(3′-Methoxy-4′-methoxymethoxystyryl)-benzaldehye (**12**) (1.8 g, 6.2 mmol) and diethyl 3,4-dimethoxymethoxybenzyl phosphonate (**9b**) (2.2 g, 6.2 mmol) were dissolved in THF (100 ml). After addition of potassium tert-butoxide (2.1 g, 19 mmol), the reaction mixture was refluxed for 30 min and then cooled to RT. The solvent was evaporated and the residue was dissolved in water (150 ml) and extracted with ethyl acetate (6×50 ml). The combined extracts were dried over $MgSO_4$ and

filtered. The solvent was evaporated to give a yellow solid which was purified by flash chromatography on silica gel (80 g), eluted with hexanes/ethyl acetate (2:1) to give 2.4 g (80%) of **13** as a yellow solid with R_f 0.41 (hexanes/ethyl acetate 1:1) and m.p. 103.0–106.0°C.

^{1}H-NMR δ(300 MHz, $CDCl_3$): 3.55(6 H, s, $2CH_3O$), 3.58(3 H, s, CH_3O), 3.96(3 H, s, CH_3O), 5.26(4 H, s, $2CH_2O$), 5.31(2 H, s, CH_2O), 6.90–7.50(14 H, m, 14 CH). HRMS: m/z calcd for $C_{29}H_{32}O_7$ (M^+) 492.2148, found 492.2130.

*(E,E)-1-(3′,4′-dihydroxystyryl)-4-(3′-methoxy-4′-hydroxystyryl) benzene (**3**)*

(*E*,*E*)-1-(3′,4′-dimethoxymethoxystyryl)-4-(3′-methoxy-4′-methoxymethoxystyryl)benzene (**13**) (0.46 g, 0.85 mmol) was suspended in acetic acid (AcOH)/H_2O (3:1, 80 ml) and heated at 100°C for 1 h. After cooling to RT, the reaction mixture was poured into 800 ml of water. The aqueous solution was extracted with ethyl acetate (100 ml × 5). The combined extracts were dried over $MgSO_4$ (30 g), filtered, and evaporated. The residue was dried under high vacuum (~1 mm Hg) overnight (~16 h) to give 0.34 g of **3** as yellow solid with quantitative yield with R_f 0.15 (hexanes/ethyl acetate 1:1) and m.p. 170°C (dec.).

^{1}H-NMR δ(300 MHz, acetone-d_6): 3.91(3 H, s, CH_3O), 6.81–7.57(14 H, m, 14CH). HRMS: m/z calcd for $C_{23}H_{20}O_4$ (M^+) 360.1362, found 360.1357.

*3-Methoxymethoxy-4-methoxybenzaldehyde (**14**)*

To a solution of 3-hydroxy-4-methoxy-benzaldehyde (9.5 g, 63 mmol) in 175 ml of methylene chloride (CH_2Cl_2) was added dimethoxymethane (50 ml, 0.56 mol) followed by the addition of *p*-toluenesulfonic acid (73 mg). The reaction mixture was refluxed overnight while water generated as the reaction proceeded was removed using a soxhlet extractor containing 27 g of 3 Å molecular sieves. After the completion of the reaction, 0.5 ml of triethylamine was added to the dark, black–purple solution, which was then washed three times with 1 N NaOH and two times with 100 ml of water. The organic layer was dried over $MgSO_4$ and evaporated to give 2.0 g (16% yield) of 3-methoxymethoxy-4-methoxybenzaldehyde.

^{1}H-NMR (500 MHz, DMSO-d_6) 7.63(dd, $J_1 = 8.14$ Hz, $J_2 = 2.03$, 1 H, 5-H); 7.54(d, $J = 2.03$ Hz, 1 H, 2-H); 7.23(d, $J = 8.14$, 1 H, 6-H); 5.24(s, 2 H, CH_2); 3.89(s, 3 H, CH_3O). 3.40(s, 3 H, CH_3O).

*(E, E)-1,4-bis(3′-methoxymethoxy-4′-methoxystyryl)benzene (***16***)*

To a solution of tetraethyl 1,4-xylylene diphosphonate (**15**) (0.76 g 2.0 mmol) in 3 ml of dried dimethyl sulfate (DMSO) was added sodium methoxide solution in methanol (1.1 ml, 5 mmol) followed by addition of a solution of 3-methoxymethoxy-4-methoxybenzaldehyde (**14**) (0.78 g, 4.0 mmol) in 3 ml of dried DMSO. The reaction mixture was then stirred overnight at RT. It was then poured into 200 ml of water. After adjusting the pH to 5.1, the water layer was extracted with 200 ml of ethyl acetate. The organic layer was then washed with water (3×100 ml) and dried over $MgSO_4$. After evaporation of the solvent, the residue was recrystallized by dissolving in a minimal amount of THF followed by adding hexanes until the pure product precipitated giving 0.76 g (83% yield) of **16**.

^{1}H-NMR(500 MHz, DMSO-d_6): 7.57(s, 4 H, C_6H_4); 7.34(d, $J = 2.03$ Hz, 2 H, 2′-H), 7.22(dd, $J_1 = 8.65$ Hz, $J_2 = 2.03$ Hz, 2 H, 5′-H); 7.20(d, $J = 16.28$ Hz, 2 H, ethylene); 7.08(d, $J = 16.28$ Hz, 2 H, ethylene); 7.02(d, $J = 8.65$ Hz, 2 H, 6′-H); 5.21(s, 4 H, CH_2); 3.80(s, 6 H, 4′-OCH_3); 3.43(s, 6 H, OCH_3).

*(E, E)-1,4-bis(3′-methoxymethoxy-4′-hydroxystyryl)benzene (***17***)*

To a solution of the (*E, E*)-1,4-bis(3′-methoxymethoxy-4′-methoxystyryl)benzene (**16**) (0.40 g, 0.87 mmol) in 20 ml of dried *N*,*N*-dimethyl formamide (DMF) was added potassium tert-butoxide (0.98 g, 8.7 mmol) and sodium thioethoxide (NaSEt) (0.74 g, 8.7 mmol). The dark solution was then refluxed for 140 min. The reaction was complete according to HPLC analysis. The reaction mixture was poured into 200 ml of water (pH 3.8) and extracted with 200 ml of ethyl acetate. The organic layer was then washed with water (3×100 ml) and dried over $MgSO_4$. The residue was then purified by dissolving in minimal amount of ethyl acetate followed by adding hexanes until the pure product precipitated giving 0.21 g (63% yield) of **17**.

^{1}H-NMR(500 MHz, DMSO-d_6): 7.53(s, 4 H, C_6H_4); 7.28(d, $J = 2.03$ Hz, 2 H, 2′-H), 7.14(d, $J = 16.28$ Hz, 2 H, ethylene); 7.10(dd,

$J_1 = 8.65$ Hz, $J_2 = 2.03$, 2 H, 5′-H); 7.00(d, $J = 16.28$ Hz, 2 H, ethylene); 6.83(d, $J = 8.65$ Hz, 2 H, 6′-H); 5.20 (s, 4 H, CH_2); 3.44(s, 6 H, OCH_3).

*(E, E)-1,4-bis(3′-methoxymethoxy-4′-methanesulfonyloxystyryl)-benzene (***18***)*

To a solution of (*E*, *E*)-1,4-bis(3′-methoxymethoxy-4′-hydroxystyryl)-benzene (**17**) (0.18 g, 0.40 mmol) in 30 ml of dried methylene chloride was added triethylamine (1.7 ml, 12 mmol) and mesyl chloride (0.62 ml, 8.0 mmol). The reaction mixture was stirred at RT for 22 h. After complete conversion as monitored by HPLC, the reaction mixture was poured into 100 ml of 0.1 N HCl and extracted with methylene chloride. The organic layer was washed with water (3×20 ml) and dried over $MgSO_4$. The residue was then purified by dissolving in minimal amount of THF followed by adding hexanes until the pure product precipitated giving 0.19 g (83% yield) of **18**.

^{1}H-NMR(500 MHz, $CDCl_3$): 7.52(s, 4 H, C_6H_4); 7.39(d, $J = 2.03$ Hz, 2 H, 2′-H), 7.31(d, $J = 8.65$ Hz, 2 H, 6′-H); 7.19(dd, $J_1 = 8.65$ Hz, $J_2 = 2.03$, 2 H, 5′-H); 7.07(s, 4 H, ethylene); 5.30(s, 4 H, CH_2); 3.55 (s, 6 H, OCH_3); 3.22(s, 6 H, SCH_3).

*(E, E)-1,4-bis(3′-OH-4′-methanesulfonyloxystyryl)benzene (***19***)*

To cleave the MOM groups, the mesylated intermediate **18** (50 mg, 90 μmol) was added to 3 ml of glacial acetic acid in a 5-ml vial. After adding 1 ml of water, the reaction mixture was heated at 110°C for 85 min. It was then poured into 100 ml of water (pH 6.4). The product was then extracted with 100 ml of ethyl acetate. The organic layer was washed with water (3×100 ml) and dried over $MgSO_4$. The crude product was then purified dissolving in minimal amount of THF followed by adding hexanes until the pure product precipitated giving 39 mg (83% yield) of **19**.

^{1}H-NMR(500 MHz, DMSO): 7.63(s, 4 H, C_6H_4); 7.24(d, $J = 16.28$ Hz, 2 H, ethylene), 7.23(d, $J = 8.65$ Hz, 2 H, 6′-H); 7.19(d, $J = 2.03$ Hz, 2 H, 2′-H); 7.16(d, $J = 16.28$ Hz, 2 H, ethylene); 7.13(dd, $J_1 = 8.65$ Hz, $J_2 = 2.03$, 2 H, 5′-H); 3.36(s, 6 H, SCH_3).

*(E,E)-1,4-bis(3′,4,-dihydroxystyryl)benzene (***2***)*

To a solution of tetraethyl 1,4-xylylene diphosphonate (0.19 g, 0.5 mmol) in 5.0 ml of DMF was added potassium tert-butoxide

(0.56 g, 5.0 mmol) followed by the addition of 3,4-di(methoxymethoxy) benzaldehyde (0.16 g, 1.0 mmol) in 2.0 ml of DMF. After stirring overnight at RT, NaSEt (2.0 g, 24 mmol) and 20 ml of DMF were added. The reaction mixture was refluxed overnight and poured into 200 ml of water. After adjusting the pH to 6.7 with diluted HCl solution, the water layer was extracted with 200 ml of ethyl acetate. The organic layers were washed with water (3×100 ml) and dried over $MgSO_4$. The residue was recrystallized in THF and hexanes to give 0.13 g (77%) of **2**.

Binding studies with synthetic Aβ (1-40) fibrils.[18] The appropriate concentrations of cold inhibitors to be tested were combined with [^{3}H]CG (specific activity 35 Ci/mmol) in a volume of 0.95 ml of binding buffer (150 mM Tris–HCl, pH 7.0 containing 20% ethanol). The assay was begun by addition of 50 μl of 100 nM Aβ stock to achieve a final concentration of 0.30 nM [^{3}H]CG, 5.0 nM Aβ fibrils, and the appropriate concentration of test compound. After incubation for 60 min at RT, the binding mixture was filtered through a Whatman GF/B glass filter via a Brandel M-24R cell harvester (Gaithersburg, MD) and rapidly washed twice with 3.0 ml binding buffer. The filters were placed in Cytoscint-ES (ICN Biomedical, Irvine, CA) and immediately counted. Complete (100%) inhibition of binding was defined as the number of counts displaced by 10 μM unlabelled CG. All assays were done at least in triplicate.

Tissue staining. Tissue autofluorescence was quenched and staining was accomplished by the procedure used for **1** staining described in detail by Styren *et al*.[20] Briefly, deparaffinized, quenched tissue sections were taken from PBS into a 100 μM solution of **3** in 40% ethanol (adjusted to pH 10 with 0.1 N NaOH) for 10 min. The sections were then dipped briefly five times into tap water before differentiation in 0.2% NaOH in 80% ethanol for 2 min. The sections were then placed in tap water for 10 min prior to coverslipping with Fluoromount-G (Electron Microscopy Sciences, Fort Washington, PA). Fluorescent sections were examined using an Olympus Vanox AH-RFL-LB fluorescence microscope and were optimally viewed with a V-filter set (excites 400–410 nm, dichroic mirror DM455, 455 nm longpass filter).

Radiolabelling. The [^{11}C]methylation of **19** was performed via the reaction of no-carrier-added [^{11}C]CH_3I with 1.0 mg **19** in the presence of 1–2 mg K_2CO_3 in 0.4 ml of DMSO at 65°C for 5 min. After the reaction,

0.3 ml of potassium tert-butoxide (1.0 M in THF) was added and the mixture was heated for an additional 3 min at 65°C. The reaction mixture was diluted with 18 ml of 0.1 M ammonium formate and eluted through a Waters C8 Sep Pak Plus. The Sep Pak was subsequently eluted with 0.8 ml of acetonitrile followed by 0.5 ml of 0.1 M ammonium formate into a V-vial. The solution was purified by semi-preparative HPLC (Phenomenex Prodigy ODS Prep 10 μm 250 × 10 mm eluted with 50/50 acetonitrile/TEA buffer [TEA = 50 mM triethylammonium phosphate, pH 7.2]) at 5 ml/min. The fraction containing [^{11}C]**3** (k' 3.5) was diluted with 30 ml of 18.2 MΩ-cm deionized water and eluted through a C8 Sep Pak Plus. The Sep Pak was washed with 10 ml of deionized water and the product was eluted with 1.0 ml of absolute EtOH into a sterile vial containing 9 ml saline. Quality control was performed on the final formulation by analytical HPLC using a Waters 996 photodiode array detector set at 369 nm with Bioscan Radio-HPLC detector. [^{11}C]**3** was found to co-elute with an authentic sample of **3** ($k' = 3.6$) on a Phenomenex Prodigy ODS (3) 5 µm 250 × 4.6 mm column eluted with 50/50 acetonitrile/TEA buffer [TEA = 50 mM triethylammonium phosphate, pH 7.2] at 2 ml/min.

Brain uptake studies in mice. Female Swiss–Webster mice ($n = 3$–5 at each time point) weighing 20–30 g were injected with 5–30 µCi of [^{11}C]**3** (specific activity ~37 MBq/nmol) contained in 0.1 ml of isotonic saline solution (containing ~5% ethanol from the Sep Pak elution) into a lateral tail vein. The mice were anesthetized at 2 or 30 min post-injection and killed by cardiac excision following cardiac puncture to obtain a terminal arterial blood sample. The brains were rapidly removed and dissected into cerebellum and remaining whole brain (including brain stem) fractions. Radioactivity content of brain and blood samples was assayed using a gamma well-counter (Packard Instruments Model 5003, Meridan CT), and the counts were decay corrected to the time of injection relative to C-11 standards prepared from the injection solution to determine the percent injected dose (% ID) in the samples. The samples were weighed to determine the percent injected dose per gram tissue (% ID/g).

NOR-beta mouse studies. NOR-beta mice (Tg positive and Tg negative: $n = 3$–5 at each time point) weighing 20–30 g were injected with 5–30 µCi of [^{11}C]**3** (specific activity ~37 MBq/nmol) contained in 0.1 ml of isotonic saline solution (containing ~5% ethanol from the Sep Pak

elution) into a lateral tail vein. The mice were anesthetized at 2, 30, 60, and 120 min post-injection and killed by cardiac excision following cardiac puncture to obtain a terminal arterial blood sample. The mice were dissected and the pancreas was rapidly removed. Radioactivity content of the pancreas and blood samples was assayed using a gamma well-counter and the % ID/g was determined by the method described in the above procedure.

NOR-beta mouse blocking studies. NOR-beta mice (Tg positive and Tg negative $n = 3$) weighing 20–30 g were treated with an i.p. injection of 40 mg/kg unlabelled MeO-X-04[17] in sterile water (buffered to pH 8.0 using sodium bicarbonate for solubility). At 60 min post-treatment, the mice were injected with 50–100 μCi of [^{11}C]**3** (specific activity ~37 MBq/nmol) contained in 0.1 ml of isotonic saline solution (containing ~5% ethanol from the Sep Pak elution) into a lateral tail vein. The mice were anesthetized at 60 min post-injection and killed by cardiac excision following cardiac puncture to obtain a terminal arterial blood sample. Radioactivity content of the pancreas and blood samples was assayed using a gamma well-counter and the % ID/g was determined by the method described in the above procedure.

Acknowledgements

The NOR-beta transgenic mice were kindly made available to us by Aventis Pharmaceuticals, Inc. This work was supported in part by grants from the Alzheimer's Association (IIRG-95-076) and Aventis Pharmaceuticals, Inc (WEK).

References

1. Hardy JA, Higgins GA. *Science* 1992; **256**: 184–185.
2. Schenk DB, Seubert P, Lieberburg I, Wallace J. *Arch Neurol* 2000; **57**: 934–936.
3. Nunan J, Small DH. *FEBS Lett* 2000; **483**: 6–10.
4. Selkoe DJ. *Nat Biotechnol* 2000; **18**: 823–824.
5. Klunk WE. *Neurobiol Aging* 1998; **19**: 145–147.
6. Klunk WE, Debnath ML, Pettegrew JW. *Neurobiol Aging* 1995; **16**: 541–548.

7. Mathis CA, Mahmood K, Debnath ML, Klunk WE. *J Label Compd Radiopharm* 1997; **40**: 94–95.
8. Zhen W, Han H, Anguiano M, Lemere CA, Cho CG, Lansbury Jr PT. *J Med Chem* 1999; **42**: 2805–2815.
9. Dezutter NA, Dom RJ, de Groot TJ, Bormans GM, Verbruggen AM. *Eur J Nucl Med* 1999; **26**: 1392–1399.
10. Skovronsky DM, Zhang B, Kung MP, Kung HF, Trojanowski JQ, Lee VM. *Proc Natl Acad Sci USA* 2000; **97**: 7609–7614.
11. Zhuang ZP, Kung MP, Hou C, Skovronsky DM, Gur TL, Plossl K, Trojanowski JQ, Lee VM, Kung HF. *J Med Chem* 2001; **44**: 1905–1914.
12. Salminen T, Pulli A, Taskinen J. *J Pharm Biomed Anal* 1997; **15**: 469–477.
13. Meier H. *Angew Chem Int Ed Engl* 1992; **31**: 1399–1540.
14. Zerban G, Meier H. *Z Naturforsch* 1993; **48b**: 171–184.
15. Mann DM. *Ann Med* 1989; **21**: 133–136.
16. Shoji T, Sato M, Sasaki A, Matsubara E, Igeta Y, Kanai M, *et al. Neurobiol Aging* 1998; **19**: S59–63.
17. Klunk WE, Bacskai BJ, Mathis CA, Kajdasz ST, McLellan ME, Frosch MP, Debnath ML, Holt DP, Wang Y, Hyman BT. *J Neurophath Exp Neurol* 2002; in press.
18. Klunk WE, Wang Y, Huang GF, Debnath ML, Holt DP, Mathis CA. *Life Sci* 2001; **69**: 1471–1484.
19. Mathis CA, Bacskai BJ, Kajdasz ST, McLellan ME, Frosch MP, Hyman BT, Holt DP, Wang Y, Huang GF, Debnath ML, Klunk WE. *Bioorg Med Chem Lett* 2002; **12**: 295–298.
20. Styren SD, Hamilton RL, Styren GC, Klunk WE. *J Histochem Cytochem* 2000; **48**: 1223–1232.

