Published in final edited form as:

Chemistry. 2017 December 01; 23(67): 17127–17135. doi:10.1002/chem.201703846.

Synthesis of thiophene based optical ligands that selectively detect tau pathology in Alzheimer's disease

Dr. Hamid Shirani^a, Dr. Hanna Appelqvist^a, Dr. Marcus Bäck^a, Dr. Therése Klingstedt^a, Prof. Nigel J. Cairns^b, and Prof. K. Peter R. Nilsson^{*,a}

^aDivision of Chemistry, Department of Physics, Chemistry and Biology, Linköping University, SE-581 83 Linköping, Sweden

^bDepartment of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri, U.S.A

Abstract

The accumulation of protein aggregates is associated with many devastating neurodegenerative diseases and the development of molecular ligands able to detect these pathological hallmarks is essential. Here, we report the synthesis of thiophene based optical ligands, denoted bi-thiophene-vinyl-benzothiazoles (bTVBTs) that can be utilized for selective assignment of tau aggregates in brain tissue with Alzheimer's disease (AD) pathology. The ability of the ligands to selectively distinguish tau deposits from the other AD associated pathological hallmark, senile plaques consisting of aggregated amyloid- β (A β) peptide, were reduced when the chemical composition of the ligands were altered, verifying that specific molecular interactions between the ligands and the aggregates are necessary for the selective detection of tau deposits. Our findings provide the structural and functional basis for the development of new fluorescent ligands that can distinguish between aggregated proteinaceous species consisting of different proteins. In addition, the bTVBT scaffold might be utilized to create powerful practical research tools for studying the underlying molecular events of tau aggregation and for creating novel agents for clinical imaging of tau pathology in AD.

A specific pathological partner

bi-thiophene-vinyl-benzothiazoles (bTVBTs) are superior thiophene-based optical ligands for selective detection of tau pathology in Alzheimer's disease (AD). Alternating distinct chemical motifs of the bTVBT scaffold greatly reduced the ability of the dye to distinguish A β and tau deposits, the two pathological hallmarks in AD.

* petni@ifm.liu.se.

Supporting information for this article is given via a link at the end of the document.

Keywords

fluorescent ligands; protein aggregates; tau; amyloid- β ; Alzheimer's disease

Introduction

Protein deposits are the pathological hallmarks of a wide range of neurodegenerative diseases,^[1] including Alzheimer's disease (AD), and small hydrophobic ligands that are selective for protein aggregates having an extensive cross β -pleated sheet conformation and sufficient structural regularity have been developed.^[2-7] The most common ligands are derivatives of Congo Red or Thioflavins and in addition to these conventional ligands, a variety of other molecular scaffolds has also been reported.^[8] However, most of these ligands detect disease-associated protein aggregates in general and the next scientific challenge is to develop small molecular ligands that target specific disease-associated protein aggregates consisting of a distinct protein.

In AD, the two major proteinaceous deposits are extracellular senile plaques consisting of aggregated amyloid- β (A β) peptide and intraneuronal neurofibrillary tangles (NFTs) composed of aggregated tau.^[1,9] Thus, the development of ligands specifically targeting A β or tau deposits are essential for clinical diagnosis of AD, as well as for evaluating the contribution of these respective aggregated species to the complex molecular pathology in AD brain. Molecular scaffolds enabling visualization of A β deposits in humans with AD by positron emission tomography (PET) imaging have been presented.^[10-12] More recently, some molecular scaffolds targeting the other pathological hallmark, tau deposits, in AD have also been recognized.^[13-18] However, different morphotypes of A β and tau aggregates have been reported^[19-23] and the existence of distinct aggregate morphotypes has been suggested to explain the heterogeneous phenotype reported for several neurodegenerative protein aggregation diseases. Hence, a variety of ligands might be necessary to achieve an accurate assessment of the diversity of pathological protein deposits present in AD.

Luminescent conjugated oligothiophenes (LCOs) have been utilized for fluorescence imaging of protein aggregates and compared to conventional ligands, LCOs have been shown to detect a wider range of disease-associated protein aggregates.^[24-26] In addition, LCOs having distinct chemical compositions can also be utilized for spectral assessment of different protein aggregates, such as A β or tau deposits in AD.^[27,28] Lately, a thiophene based tetrameric ligand, q-FTAA-CN (Figure 1A) with a striking higher affinity for A β deposits than aggregated species composed of tau was also identified.^[29]

Herein, we explored if a thiophene-based ligand with a high selectivity toward tau pathology in AD could be synthesized. As a template, a previously reported tau specific ligand, PBB3 (Figure 1B)^[17] was selected and two classes of thiophene-based ligands, bi-thiophene-vinyl-benzothiazoles/benzimidazoles (bTVBTs/bTVBIs) and tert-thiophene-benzothiazoles (tTBTs), were generated (Figure 1C). The ligands were applied on human brain tissue sections with AD pathology and bTVBTs were identified as a novel class of optical ligands that selectively identified tau pathology in AD. Furthermore, subtle changes in the chemical composition of the bTVBTs were shown to reduce or eliminate their capacity for selective identification of tau deposits. Thus, these findings might aid in the chemical design of ligands recognizing aggregated proteinaceous species consisting of a distinct protein, as well as in the development of novel agents for clinical imaging of tau pathology in AD.

Results and Discussion

Synthesis of bTVBTs, bTVBIs and tTBTs

As PBB3 (Figure 1B) was reported to display a selectivity towards tau aggregates in human AD brain tissue sections,^[17] this molecular scaffold was selected as a template to synthesize two classes of novel thiophene based compounds. Firstly, the pyridinyl-butadienyl-motif was replaced by a bi-thiophene-vinyl moiety rendering a set of compounds denoted bi-thiophene-vinyl-benzothiazoles/benzothiazoliums (bTVBTs) (Figure 1C). Compound bTVBT1 was achieved through a condensation reaction of 2-methylbenzothiazole and 5-bromo-2-thiophenecarboxaldehyde followed by a Suzuki coupling to attach the second thiophene moiety (Scheme 1a), whereas the benzothiazoliums variants were assembled by a condensation reaction of 3-ethyl-2-methylbenzothiazolium iodide and different bi-thiophene building blocks (Scheme 1b). These synthetic routes were utilized to synthesize five different bTVBT variants denoted bTVBT1, bTVBT2, bTVBT3, bTVBT4 and bTVBT5, respectively (Figure 1C). In addition, a compound, bTVBI1, having a benzimidazole moiety, 1-ethyl-1H-benzimidazole, instead of a benzothiazole/benzothiazolium attached to the bi-thiophene-vinyl moiety was synthesized in an analogous fashion as for the benzothiazoliums variants (Scheme 1c).

The second class of compounds (Figure 1D), denoted tert-thiophene- benzothiazoles (tTBTs) was achieved by replacing the pyridinyl-butadienyl-motif of PBB3 with different tert-thiophene moieties and this class of compounds was synthesized as outlined in scheme 2. Different brominated bi-thiophenes were attached to compound **7** by the same Suzuki coupling reaction used for the synthesis of bTVBT1. Overall, the synthetic schemes described above were utilized to generate a total of nine different thiophene based fluorescent ligands with different chemical composition (Figure 1).

Histological staining of AD brain tissue sections with bTVBTs, bTVBIs and tTBTs

As previously studies have shown that tau ligands display differential binding towards recombinant tau fibrils, tau deposits in transgenic mouse models or aggregated tau in human AD brain,^[30,31] it is essential to verify ligands that selectively detect tau pathology in AD towards human brain sections with AD pathology. Therefore, 100 nM of the ligands were applied for histological staining of human brain tissue sections with AD pathology (Figure 2 and 3). When stained in combination with an antibody (4G8) for A β pathology, bTVBT1, bTVBT2, bTVBT3, bTVBT4 and bTVBT5 showed no correlation with the antibody staining (Figure 2 and Supporting Information (SI), Figure S1). Instead, the staining pattern observed from the bTVBTs resembled classical AD tau pathology with NFTs, dystrophic neurites and neuropil threads. Thus, the bTVBT ligands appeared to stain aggregated species composed of tau, whereas staining of A β pathology was lacking. The bTVBTs selectivity towards aggregated tau species was also verified with co-staining experiments using the tau-specific antibody AT-100 (Figure 3). Clearly, all the bTVBTs labelled both immuno-positive NFTs, dystrophic neurites and neuropil threads. However, certain parts of individual NFTs or dystrophic neurites were intensively labelled with AT100 and displayed weak bTVBT fluorescence, whereas other parts of the same NFTs or dystrophic neurites showed the opposite pattern, namely strong bTVBT fluorescence and weak AT100 labeling. This discrepancy in fluorescence intensity is most likely a consequence of the different binding modes for AT100 and the bTVBTs. AT100 is recognizing an epitope (paired helical filaments phosphorylated at Thr 212 and Ser 214),^[32] whereas the bTVBTs are most likely interacting with the repetitive β -sheet structure composed of identical protofilaments comprising residues 306-378 of the tau protein.^[33] These different motifs are most likely displayed in a different fashion along the tau aggregates. In contrast to the tTVBTs, the benzimidazole variant, bTVBI1, displayed staining towards immuno-positive A β aggregates, as well as some weak staining of tau-like pathology (Figure 2 and SI, Figure S1). Hence, by replacing the benzothiazole/benzothiazolium moiety with a benzimidazole motif, the specificity towards tau pathology was abolished. Interestingly, an earlier study of PBB-like compounds showed that a benzimidazole derivative displayed different emission spectra when bound to A β or tau deposits, whereas the spectral difference was abolished when replacing the benzimidazole moiety with a benzothiazole,^[34] verifying that this rather minor chemical alteration could have an impact on the ligand/protein aggregate interaction.

Replacing the vinyl moiety in the bTVBTs with an additional thiophene unit also had a major influence on the ligands ability to detect A β and tau aggregates. When using 100 nM of the uncharged tTBTs, tTBT1 and tTBT3, staining of both immuno positive A β aggregates and tau-like pathology were lacking (Figure 2, SI Figure S2). In contrast, the negatively charged tTBT2, displayed staining of both A β and tau aggregates (Figure 2, SI Figure S1 and S2). Thus, similar to what has been reported earlier for oligothiophenes,^[24-29] anionic carboxyl groups along the tTBT backbone might be an essential chemical determinant for achieving high-affinity optical ligands towards protein aggregates. However, none of the tTBTs displayed selective staining towards tau pathology, signifying that the bTVBT molecular scaffold was superior for achieving optical ligands that selectively detect tau deposits in brain sections with AD pathology.

Optical characterization of bTVBTs bound to NFTs

In order to elucidate the tau selective staining of the bTVBTs in more detail, we next evaluated the photo-physical properties of the bTVBTs bound to tau deposits in comparison to the ligands dissolved in DMSO and further diluted in DMSO or PBS. Representative spectra from bTVBTs stained NFTs are shown in figure 4. All the bTVBTs displayed an emission spectrum with a maximum around 600 to 630 nm and an excitation spectrum with a maximum around 525 to 550 nm (Figure 4B and SI, Table S1), suggesting that all bTVBTs binds to tau deposits in a similar fashion. Furthermore, when analyzing the excitation- and emission profiles of the ligands in DMSO or PBS, the photo-physical behavior was strikingly different (Figure 4C and SI, Table S1). bTVBT1 exhibited a solvatochromatic behavior rendering a blue-shifted excitation spectrum, as well as a red-shifted emission spectrum, when switching solvent from DMSO to PBS, whereas the other bTVBTs showed similar spectra in both solvents. Notably, all the bTVBTs displayed a pronounced red-shift of the excitation maxima when bound to NFTs compared to the maxima obtained in the different solvents. Thus, upon interaction with the NFT, the bTVBTs adopt a distinct conformation that induces a specific optical signature from the ligands. Similar to what has been suggested for other amyloid ligands, such as thioflavin T (ThT),^[35-41] the molecular form of the bTVBTs that gives rise to the red-shifted excitation spectra upon binding to NFTs might be either monomeric or dimeric. For ThT, the monomer hypothesis is based on ThT behaving as a molecular rotor,^[35-38] while the dimer hypothesis is based on ThT forming an excimer.^[39-41] To achieve more molecular details regarding the conformation of the bTVBTs bound to NFTs, further photo-physical studies, as well as theoretical modeling are necessary and such experiments are ongoing. Overall, we conclude that upon interaction with NFTs, all the bTVBTs display distinct excitation- and emission spectra compared to when the ligands are dissolved in DMSO or PBS.

Comparison of the bTVBTs with q-FTAA-CN and PBB3

To further investigate the binding mode of the bTVBTs to tau deposits, tissue sections with AD pathology were simultaneously stained with 100 nM bTVBT2 and 100 nM of q-FTAA-CN (Figure 1). As previously reported,^[29] at this concentration (100 nM), q-FTAA-CN selectively labelled A β deposits. In addition, tau pathology could be identified by the spectral signature of bTVBT2 (Figure 5A-D). Thus, by applying two thiophene-based ligands, q-FTAA-CN and bTVBT2, specific optical assignment of the two pathological hallmarks of AD could be achieved.

Next we performed a similar staining experiment using 100 nM bTVBT and 100 nM of PBB3. However, as the previously reported^[17] *in vitro* fluorescence staining of AD brain sections with PBB3 was performed under different conditions (10⁻³ % ligands dissolved in 50% ethanol for 1 hour at room temperature) than for the bTVBTs, staining experiments with only 100 nM PBB3 in PBS pH 7.4 was first conducted. With this staining procedure, PBB3 evidently labelled both A β and tau deposits, as characteristic fluorescence was observed from both these aggregated species (SI, Figure S3). Due to these spectral properties of PBB3, bTVBT3 was selected as the thiophene-based ligand for being simultaneously used with PBB3. As shown in figure 5E, when using this combination of dyes, 100 nM PBB3 and 100 nM bTVBT3, A β deposits were visualized by the fluorescence

from PBB3, whereas tau deposits, NFTs, dystrophic neurites and neuropil threads displayed characteristic emission from bTVBT3. Thus, under these staining conditions, bTVBT3 showed a higher selectivity and most likely a higher affinity for tau deposits than PBB3, and seemingly these ligands also compete for the same binding site to the tau deposits. To verify the latter, tissue sections were first incubated in PBS or PBS with 1 μ M of PBB3 for 1 hour prior to staining with 10 nM of bTVBT3. Strikingly, in the section pre-incubated with PBB3, both A β and tau deposits exhibit strong PBB3 fluorescence, whereas no observable bTVBT3 fluorescence was obtained from the tau pathology (Figure 5F). In contrast, for the section pre-incubated in PBS, both NFTs, dystrophic neurites and neuropil threads displayed strong bTVBT3 fluorescence (Figure 5G). Hence, the tau staining of bTVBT3 was abolished by pre-incubating the sections with a 100-fold excess of PBB3, verifying that these two ligands share a similar mode of binding to tau aggregates.

As it was previously shown that q-FTAA-CN also stained NFTs when using micro-molar concentrations of ligand for histological staining,^[29] similar competition experiments as described above were also performed using q-FTAA-CN and bTVBT3. At this higher concentration, 1 μ M, q-FTAA-CN labelled both A β deposits and NFTs (Figure 5H). In addition, even though the section was pre-incubated with 1 μ M q-FTAA-CN, the characteristic bTVBT3 fluorescence from the NFTs could easily be observed from the q-FTAA-CN positive NFTs as well as from q-FTAA-CN negative dystrophic neurites (Figure 5H). Hence, q-FTAA-CN and bTVBT3 most likely have alternative modes of binding to tau aggregates. Previous studies have suggested that anionic oligothiophenes, including q-FTAA-CN, have a similar mode of binding to protein aggregates as Congo red and related analogues.^[29, 42] Anionic oligothiophenes efficiently displaced X-34, a Congo Red analogue, but not Pittsburgh compound B (PIB) from recombinant A β amyloid fibrils and Alzheimer's disease brain-derived A β .^[29] Overall, the experiments comparing the novel bTVBTs with the previously reported ligands PBB3^[17] and q-FTAA-CN^[29], showed that the bTVBTs bind to tau aggregates in as similar fashion as PBB3 and that the bTVBTs displayed a higher selectivity for tau pathology in AD brain compared to both PBB3 and q-FTAA-CN.

Conclusions

In conclusion, bi-thiophene-vinyl-benzothiazoles (bTVBTs) were identified as optical ligands for selective detection of tau pathology in human AD brain tissue sections and the superior functionality of these ligands compared to structurally related compounds could be assigned to distinct chemical motifs. We foresee that our findings will aid in the chemical design of ligands that can be utilized for exploring different aggregated morphotypes composed of tau, as well as for developing novel agents for clinical imaging of tau pathology in AD. Such ligands will also be vital for evaluating novel therapeutic strategies towards Alzheimer's disease.

Experimental Section

Full experimental details including additional characterization data and NMR spectra of new compounds, as well as supporting figures and tables are given in the Supporting Information.

Frozen brain tissue from a clinically and neuropathologically well-characterized case of AD was obtained from the Knight Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, Missouri, USA. Tissue was removed according to Washington University Local Ethics Committee guidelines and informed consent for brain donation was obtained from the next-of-kin. Frozen tissue from human AD brain was also purchased from Tissue Solutions Ltd, Glasgow, Scotland. Tissue Solutions Ltd confirmed that these human tissue samples have been collected with ethics committee approval and with permission to use these sections for research, and that all samples have been collected from donors following written consent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Our work is supported by the Swedish Foundation for Strategic Research, the Erling Persson Foundation and the Swedish Research Council. We would also like to thank Prof. David M. Holtzman and the Knight Alzheimer's Disease Research Center (NIH grants P50AG05681 and 5PO1-AG03991) Washington University, St. Louis, Missouri, USA), as well as CBD solutions for supplying us with human brain tissue.

References

1. Ross CA, Poirier MA. Nat Med. 2004; 10:10–17. [PubMed: 14702615]
2. Lockhart A. Drug Discovery Today. 2006; 11:1093–1099. [PubMed: 17129828]
3. Styren SD, Hamilton RL, Styren GC, Klunk WE. J Histochem Cytochem. 2000; 48:1223–1232. [PubMed: 10950879]
4. Kung HF, Lee CW, Zhuang ZP, Kung MP, Hou C, Plossl K. J Am Chem Soc. 2001; 123:12740–12741. [PubMed: 11741464]
5. Mathis CA, Wang Y, Holt DP, Huang GF, Debnath ML, Klunk WE. J Med Chem. 2003; 46:2740–2754. [PubMed: 12801237]
6. Nesterov EE, Skoch J, Hyman BT, Klunk WE, Bacskai BJ, Swager TM. Angew Chem Int Ed Engl. 2005; 44:5452–5456. [PubMed: 16059955]
7. Furumoto S, Okamura N, Iwata R, Yanai K, Arai H, Kudo Y. Curr Top Med Chem. 2007; 7:1773–1789. [PubMed: 17979786]
8. Nilsson KPR. FEBS Lett. 2009; 583:2593–2599. [PubMed: 19376114]
9. Ballatore C, Lee VMY, Trojanowski JQ. Nat Rev Neurosci. 2007; 8:663–672. [PubMed: 17684513]
10. Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, Bergström M, Savitcheva I, Huang GF, Estrada S, Ausen B, Debnath ML, Barletta J, Price JC, Sandell J, Lopresti BJ, Wall A, Koivisto P, Antoni G, Mathis CA, Långström B. Ann Neurol. 2004; 55:306–319. [PubMed: 14991808]
11. Kudo Y, Okamura N, Furumoto S, Tashiro M, Furukawa K, Maruyama M, Itoh M, Iwata R, Yanai K, Arai H. J Nucl Med. 2007; 48:553–561. [PubMed: 17401091]
12. Yang L, Rieves D, Ganley C. N Engl J Med. 2012; 367:885–887. [PubMed: 22931256]
13. Small GW, Kepe V, Ercolim LM, Siddarth P, Bookheimer SY, Miller KJ, Lavretsky H, Burggren AC, Cole GM, Vinters HV, Thompson PM, Huang SC, Satyamurthy N, Phelps ME, Barrio JR. N Eng J Med. 2006; 355:2652–2663.
14. Taghavi A, Nasir S, Pickhardt M, Haussen R Heyny-von, Mall G, Mandelkow E, Mandelkow EM, Schmidt B. J Alzheimers Dis. 2011; 27:835–843. [PubMed: 21891864]
15. Fodero-Tavoletti MT, Okamura N, Furumoto S, Mulligan RS, Connor AR, McLean CA, Cao D, Rigopoulos A, Cartwright GA, O'Keefe G, Gong S, Adlard PA, Barnham KJ, Rowe CC, Masters

- CL, Kudo Y, Cappai R, Yanai K, Villemagne VL. *Brain*. 2011; 134:1089–1100. [PubMed: 21436112]
16. Zhang W, Arteaga J, Cashion DK, Chen G, Gangadharmath U, Gomez LF, Kasi D, Lam C, Liang Q, Liu C, Mocharla VP, Mu F, Sinha A, Szardenings AK, Wang E, Walsh JC, Xia C, Yu C, Zhao T, Kolb HC. *J Alzheimers Dis*. 2012; 31:601–612. [PubMed: 22683529]
 17. Maruyama M, Shimada H, Suhara T, Shinotoh H, Ji B, Maeda J, Zhang MR, Trojanowski JQ, Lee VM, Ono M, Masamoto K, Takano H, Sahara N, Iwata N, Okamura N, Furumoto S, Kudo Y, Chang Q, Saido TC, Takashima A, Lewis J, Jang MK, Aoki I, Ito H, Higuchi M. *Neuron*. 2013; 79:1094–1108. [PubMed: 24050400]
 18. Xia CF, Arteaga J, Chen G, Gangadharmath U, Gomez LF, Kasi D, Lam C, Liang Q, Liu C, Mocharla VP, Mu F, Sinha A, Su H, Szardenings AK, Walsh JC, Wang E, Yu C, Zhang W, Zhao T, Kolb HC. *Alzheimers Dement*. 2013; 9:666–676. [PubMed: 23411393]
 19. Maarouf CL, Dausgs ID, Spina S, Vidal R, Kokjohn TA, Patton RL, Kalback WM, Luehrs DC, Walker DG, Castaño EM, Beach TG, Ghetti B, Roher AE. *Mol Neurodegener*. 2008; 3:20. [PubMed: 19021905]
 20. Levine H, Walker LC. *Neurobiol Aging*. 2010; 31:542–548. [PubMed: 18619711]
 21. Clavaguera F, Akatsu H, Fraser G, Crowther RA, Frank S, Hench J, Probst A, Winkler DT, Reichwald J, Staufenbiel M, Ghetti B, Goedert M, Tolnay M. *Proc Natl Acad Sci USA*. 2013; 110:9535–9540. [PubMed: 23690619]
 22. Lu JX, Qiang W, Yau WM, Schwieters CD, Meredith SC, Tycko R. *Cell*. 2013; 154:1257–1268. [PubMed: 24034249]
 23. Qiang W, Yau WM, Lu JX, Collinge J, Tycko R. *Nature*. 2017; 541:217–221. [PubMed: 28052060]
 24. Åslund A, Sigurdson CJ, Klingstedt T, Grathwohl S, Bolmont T, Dickstein DL, Glimsdal E, Prokop S, Lindgren M, Konradsson P, Holtzman DM, Hof PR, Heppner FL, Gandy S, Jucker M, Aguzzi A, Hammarström P, Nilsson KPR. *ACS Chem Biol*. 2009; 4:673–684. [PubMed: 19624097]
 25. Klingstedt T, Åslund A, Simon RA, Johansson LBG, Mason JJ, Nyström S, Hammarström P, Nilsson KPR. *Org Biomol Chem*. 2011; 9:8356–8370. [PubMed: 22051883]
 26. Shirani H, Linares M, Sigurdson C, Lindgren M, Norman P, Nilsson KPR. *Chemistry*. 2015; 21:15133–15137. [PubMed: 26388448]
 27. Klingstedt T, Shirani H, Åslund KOA, Cairns NJ, Sigurdson CJ, Goedert M, Nilsson KPR. *Chemistry*. 2013; 19:10179–10192. [PubMed: 23780508]
 28. Klingstedt T, Shirani H, Mahler J, Wegenast-Braun BM, Nyström S, Goedert M, Jucker M, Nilsson KPR. *Chemistry*. 2015; 21:9072–9082. [PubMed: 26013403]
 29. Bäck M, Appelqvist H, LeVine H, Nilsson KPR. *Chemistry*. 2016; 22:18335–18338. [PubMed: 27767229]
 30. Zhang W, Arteaga J, Cashion DK, Chen G, Gangadharmath U, Gomez LF, Kasi D, Lam C, Liang Q, Liu C, Mocharla VP, Mu F, Sinha A, Szardenings AK, Wang E, Walsh JC, Xia C, Yu C, Zhao T, Kolb HC. *J Alzheimers Dis*. 2012; 31:601–612. [PubMed: 22683529]
 31. Declercq L, Celen S, Lecina J, Ahamed M, Tousseyn T, Moechars D, Alcazar J, Ariza M, Fierens K, Bittelbergs A, Mariën J, Vandenberghe R, Andres IJ, Van Laere K, Verbruggen A, Bormans G. *Mol Imaging*. 2016; 15 pii: 1536012115624920.
 32. Zheng-Fischhöfer Q, Biernat J, Mandelkow EM, Illenberger S, Godemann R, Mandelkow E. *Eur J Biochem*. 1998; 252:542–552. [PubMed: 9546672]
 33. Fitzpatrick AWP, Falcon B, He S, Murzin AG, Murshudov G, Garringer HJ, Crowther RA, Ghetti B, Goedert M, Scheres SHW. *Nature*. 2017; 547:185–190. [PubMed: 28678775]
 34. Harada R, Okamura N, Furumoto S, Yoshikawa T, Arai H, Yanai K, Kudo Y. *Mol Imaging Biol*. 2014; 16:19–27. [PubMed: 23868612]
 35. Voropai ES, Samtsov MP, Kaplevskii KN, Maskevich A, Stepuro VI, Povarova OI, Kuznetsova IM, Turoverov KK, Fink AL, Uverskii VN. *J Appl Spectrosc*. 2003; 70:868–874.
 36. Lindgren M, Sörgjerd K, Hammarström P. *Biophys J*. 2005; 88:4200–4212. [PubMed: 15764666]
 37. Krebs MRH, Bromley EHC, Donald AM. *J Struct Biol*. 2005; 149:30–37. [PubMed: 15629655]

38. Stsiapura VI, Maskevich AA, Kuzmitsky VA, Turoverov KK, Kuznetsova IM. J Phys Chem A. 2007; 111:4829–4835. [PubMed: 17497763]
39. Retna Raj C, Ramaraj R. J Photochem Photobiol A. 1999; 122:39–46.
40. Groenning M, Norrman M, Flink JM, van de Weert M, Bukrinsky JT, Schluckebier G, Frokjaer S. J Struct Biol. 2007; 159:483–497. [PubMed: 17681791]
41. Groenning M, Olsen L, Weert M, Flink JM, Frokjaer S, Jørgensen FS. J Struct Biol. 2007; 158:358–369. [PubMed: 17289401]
42. Herrmann US, Schütz AK, Shirani H, Huang D, Saban D, Nuvolone M, Li B, Ballmer B, Åslund AK, Mason JJ, Rushing E, Budka H, Nyström S, Hammarström P, Böckmann A, Caflisch A, Meier BH, Nilsson KPR, Hornemann S, Aguzzi A. Sci Transl Med. 2015; 7:299ra123.

Figure 1. Chemical structure of fluorescent ligands for protein aggregates

(A) The chemical structure of q-FTAA-CN, a high-affinity ligand for A β deposits in human AD brain. (B) The chemical structure of PBB3, a high-affinity ligand for tau deposits in human AD brain. (C) Chemical structure of novel bi-thiophene-vinyl-benzothiazoles/benzimidazoles (bTVBTs/bTVBIs). (D) Chemical structure of novel tert-thiophene-benzothiazoles (tTBTs)

Figure 2. Fluorescence images of brain tissue sections with AD pathology stained with bTVBTs, bTVBIs and tTBTs

Frozen brain sections were fixed with ethanol and double stained with an antibody towards A β (4G8, red) and 100 nM of the respective ligand (green). Scale bar represent 50 μ m and white arrow-heads indicate auto-fluorescence from lipofuscin observed in the blue- and the green channel. bTVBT1, bTVBT2, bTVBT3, bTVBT4 and bTVBT5 showed no correlation with the antibody staining (red arrows) and the staining pattern observed for these ligands (green arrows) resembled NFTs, dystrophic neurites and neuropil threads. bTVBT1 showed overlap with the 4G8 staining (white arrows) as well as some minor staining from dystrophic neurites. tTBT2 displayed overlap with the 4G8 staining (white arrows) as well as staining

from tau-like pathology (green arrows). tTBT1 and tTBT3 did not display any substantial staining of any of the pathological aggregated species in AD brain.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3. Fluorescence images of brain tissue sections with AD pathology co-stained with bTVBTs and the hyperphosphorylated tau specific antibody AT100

Frozen brain sections were fixed with ethanol and double stained with an antibody towards tau (AT100, red) and 100 nM of the respective ligand (green). All the bTVBTs displayed overlap with the AT100 staining (white arrows), verifying that the ligands stained tau pathology in brain tissue sections with AD pathology. Scale bar represent 10 μ m.

Figure 4. Excitation and emission characteristics of the bTVBTs bound to NFTs or diluted in DMSO and PBS

(A) Fluorescence images of bTVBTs stained NFTs. The staining was performed using 100 nM bTVBT in PBS pH 7.4. Scale bar represents 10 μm. (B) Excitation- (blue) and emission (red) spectra from the bTVBTs bound to NFTs. (C) Excitation- (blue) and emission (red) spectra from bTVBT dissolved in DMSO and further diluted in DMSO (dotted line) or in PBS pH 7.4 (solid line) to a final concentration of 300 nM ligand.

Figure 5. Fluorescence images of brain tissue sections with AD pathology simultaneously stained with bTVBTs and q-FTAA-CN or PBB3

(A–C) Fluorescence spectral images of frozen brain sections with AD-pathology simultaneously stained with 100 nM q-FTAA-CN and 100 nM bTVBT2. q-FTAA-CN fluorescence (blue) was observed from Aβ deposits (white arrow-heads) whereas tau aggregates (white arrows) displayed bTVBT2 fluorescence (yellow-red). (D) Characteristic emission spectra (blue line) from q-FTAA-CN labelled Aβ deposits and bTVBT2 stained tau aggregates (yellow-red line). (E) Fluorescence images of frozen brain sections with AD-

pathology simultaneously stained with 100 nM PBB3 and 100 nM bTVBT3. PBB3 fluorescence (blue) was observed from A β deposits (white arrow-heads) whereas tau aggregates (white arrows) displayed bTVBT3 fluorescence (red). **(F)** Fluorescence images of frozen brain sections with AD-pathology pre-incubated with 1 μ M PBB3 prior to staining with 10 nM bTVBT3. PBB3 fluorescence (blue/green) was observed from both A β deposits (white arrow-heads) and tau aggregates (white arrows) whereas no bTVBT3 fluorescence was observed in the red channel. **(G)** Fluorescence images of frozen brain sections with AD-pathology stained with 10 nM bTVBT3. bTVBT3 fluorescence (red) was observed from the tau aggregates (white arrows). **(H)** Fluorescence images of frozen brain sections with AD-pathology pre-incubated with 1 μ M q-FTAA-CN prior to staining with 10 nM bTVBT3. q-FTAA-CN fluorescence (blue/green) was observed from both A β deposits (white arrow-heads) and tau aggregates whereas bTVBT3 fluorescence (red) was observed from the tau aggregates (white arrows). Scale bar represent 50 μ m (**A**) or 20 μ m (**B, C, E-H**) and green arrow-heads indicate auto-fluorescence from lipofuscin.

Scheme 1. Synthesis of bTVBTs and bTVBI1

Reagents and conditions: (a) (i) DMSO, KOH (2M), RT; (ii) 1,4-dioxane/MeOH, PEPPSI-IPr, K₂CO₃ 80 °C, 20 min. (b) (i) Pyridine, MeOH or MeOH/THF mixture, reflux. (c) (i) DMF, TMSCl, 110 °C, 2 h.

Scheme 2. Synthesis of tTBTs

Reagents and conditions: (i) 1,4-dioxane/MeOH, PEPPSI-IPr, K_2CO_3 , 80 °C, 20 min.; (ii) NaOH (1M), 1,4-dioxane, 70 °C, 5 h.