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Multidentate ^{18}F -polypegylated styrylpyridines as imaging agents for A β plaques in cerebral amyloid angiopathy (CAA)

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Abstract

Beta-amyloid plaques (A β plaques) in the brain are associated with cerebral amyloid angiopathy (CAA). Imaging agents that could target the A β plaques in the living human brain would be potentially valuable as biomarkers in patients with CAA. A new series of ^{18}F styrylpyridine derivatives with high molecular weights for selectively targeting A β plaques in the blood vessels of the brain, but excluded from the brain parenchyma is reported. The styrylpyridine derivatives, **8a–c**, display high binding affinities and specificity to A β plaques ($K_i = 2.87$ nM, 3.24 and 7.71 nM, respectively). *In vitro* autoradiography of [^{18}F]**8a** shows labeling of β -amyloid plaques associated with blood vessel walls in human brain sections of subjects with CAA, and also in the tissue of AD brain sections. The results suggest that [^{18}F]**8a** may be a useful PET imaging agent for selectively detecting A β plaques associated with cerebral vessels in the living human brain.

Keywords

PET imaging; Alzheimer's disease; cerebral blood vessels; β -amyloid plaque; cerebral amyloid angiopathy; autoradiography and in vivo biodistribution

Introduction

Cerebral amyloid angiopathy (CAA) is a common cause of stroke and dementia in older people. Patients typically present pathological symptoms associated with lobar intracranial macrohemorrhages or microbleeds. Postmortem studies suggest that there are distinctive morphological changes in the cerebral vessels including the deposition of β -amyloid (A β) peptide aggregates, of which A β peptides are the most common^{1,2}. The depositions are located in small to medium-sized cerebral and leptomeningeal arteries and less prominently in the walls of capillaries and veins. Similar to the deposition of A β in the parenchymal brain tissue in Alzheimer's disease (AD), the A β peptides form A β aggregates and neurofibrillary plaques are accumulated in the cerebral blood vessels. As the deposition of A β aggregates increases with time, they become hardened. These changes in the cerebral blood vessels lead to microbleeds^{3–6}. It has been estimated that CAA is present in 55 – 59%

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Supporting Information Available:

Method for in vitro binding assay and HPLC profiles of final tested compounds are included in the supporting material. This material is available free of charge via the Internet at <http://pubs.acs.org>.

of dementia patients. At least 30% of the dementia patients showed severe CAA, as compared to 7 – 24% in non-demented patients^{7,8}. In general, there is a very close, but not parallel, relationship between CAA and Alzheimer's disease (AD). They also share a set of common genetic risk factors. It is believed that there are specific mechanism(s), which put age-related cerebrovascular degeneration at a critical point in the pathogenesis of AD^{9,10}. Evidence collected from postmortem examination of brain tissues from CAA and AD patients found A β deposition in the parenchymal and cortical arteries of the brains of AD subjects, while in CAA patients the same deposition was visible predominantly in the blood vessels^{11,12}.

Imaging A β -plaques in the brain is generally accepted as a potential useful tool for studying the pathophysiology of neurodegenerative diseases associated with the formation of β -amyloid. One of the leading protocols is [¹¹C]PIB ([¹¹C]**1**) (Pittsburgh Compound B)/PET imaging of A β plaques in the living brain^{13–16}. Recently, several reports on [¹¹C]**1**/PET imaging in CAA and post-stroke patients suggested that A β depositions could be detected in the brain tissue as well as in the blood vessels^{6,13,17–19}. Since **1** is a small molecule, it readily penetrates the intact blood-brain barrier; therefore, [¹¹C]**1**/PET images of the A β deposition represents the map of total A β deposition in the brain, as well as in the cerebral vessels.

To improve the availability of PET tracers for routine clinical practice, several ¹⁸F A β -plaque imaging agents, including [¹⁸F]florbetapir f 18 ([¹⁸F]AV-45, [¹⁸F]**5**)^{20–24}, [¹⁸F]florbetaben f 18 ([¹⁸F]AV-1, BAY-94-9172, [¹⁸F]**4**)^{25–28}, [¹⁸F]flutemetamol f 18 ([¹⁸F]FPIB, GE067, [¹⁸F]**6**)^{29–33} and 2-(2-([¹⁸F]fluoro)-6-methylaminopyridin-3-yl)benzofuran-5-ol ([¹⁸F]AZD4694, [¹⁸F]**7**)³⁴ have been developed and found to be useful PET agents for targeting A β plaques in the brain. The phase III clinical trial for [¹⁸F]**5** has been completed²⁴, and this A β -plaque targeting imaging agent is currently under regulatory review for approval. Similar to [¹¹C]**1**, [¹⁸F]**5** labels all A β plaques, those deposited in the parenchymal of the brain, as well as those in the cerebrovascular vessels. In order to differentially diagnose the CAA related accumulation of A β plaques, a [¹⁸F] labeled A β -plaque imaging agent for specifically detecting A β deposition located on the wall of cerebral blood vessels is needed.

In order to be able to differentiate between A β plaques in the brain and those on the wall of cerebrovascular vessels, a series of [¹⁸F] imaging agents containing two styrylpyridine cores separated by long pegylated chains (n > 8) was prepared and tested. We reasoned that the “bivalent or trivalent ligands” containing two or three styrylpyridine binding cores would retain a high binding affinity towards the A β plaques due to the presence of multiple binding sites within the repeated β -sheet structure. It is probable that the multi-dentate styrylpyridine derivatives with the appropriate spacing between the binding moieties would display good binding to the A β aggregates, and that they would be unable to penetrate the intact blood-brain barrier (BBB) *in vivo* due to their high molecular weights, which exceed 600, a general cutoff point for a simple and neutral molecule to penetrate the BBB by diffusion²¹. Therefore, the “bidentate or tridentate ligands”, **8**, **9** and **10**, would likely bind only to the A β aggregates located outside of the blood-brain barrier on the vessel walls in CAA patients.

Reported herein is the synthesis and characterization of several A β aggregate-binding ligands, **8a**, **8b**, **8c**, **9** and **10**, which do not cross the intact blood brain barrier, and therefore may specifically target A β aggregates located on the cerebrovascular vessel walls outside of the BBB of CAA subjects.

Results

Chemical synthesis of bivalent and trivalent styrylpyridine derivatives, **8a**, **8b**, **8c**, **9** and **10**

The synthesis of the **5** derivatives, **8a**, **8b**, **8c**, **9** and **10**, with two (bivalent ligand) or three (trivalent ligand) styrylpyridine binding moieties tethered by a polyethylene glycol chain was successfully achieved as described in schemes 1, 2, 3 and 4. For the first series of bivalent ligands, compound **8**, the binding core, styrylpyridine, was attached with different chain lengths of polyethylene glycol (**8a**, **8b**, **8c**, $n = 4, 5$ or 6). The hydroxyl ends of the polyethylene glycol tethered with styrylpyridines were attached to the 1 and 3-hydroxy positions of a glycerol. The 2-hydroxyl group of the glycerol was linked to an ethylene glycol group leaving one free hydroxyl group, through which the fluorine atom was end-capped. The molecule has an achiral center and the hydroxyl groups of the glycerol provided a convenient way of stretching between the two styrylpyridine cores with poly-pegylated chains while leaving one more site for an additional ethylene glycol group for [^{18}F] labeling. We chose to use ether linkage (for **8a**, **8b**, **8c**) or amide linkage (for **9**) instead of carboxyl esters, in consideration of *in vivo* stability. However, the character of the linkage bond, ether vs amide, apparently has a dramatic effect on the *in vitro* binding affinity to the A β plaques, see discussion below. We also used “click chemistry” to explore the feasibility of constructing a tridentate ligand, **10**, in which three styrylpyridine cores would be included in one single molecule.

The intermediates **11** – **19**, **26** and **35** were readily prepared according to similar reported methods^{35–37}. Using the Williamson ether synthesis method, di-alcohol **19** and tosylates **15** were stitched together (Scheme 1) and the key bivalent intermediates **20a** – **c** were synthesized in good yields. Following a six-step transformation, fluoroethoxy group tethered bivalent styrylpyridine derivatives, **8a** – **c**, were obtained (Scheme 2). For the synthesis of the other bivalent styrylpyridine derivative, **9**, linked via amide bonds, the key intermediates, amine **32** and di-acid **30** were prepared first, followed by a 1-hydroxybenzotriazole hydrate/ *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (HOBt/EDCI) promoted coupling reaction. The intermediate **33** was employed as the starting material for fluorination, and subsequently the Boc protection group was removed to give **9** in an acceptable yield.

For preparation of a tri-dentate ligand we decided to use pentaerythritol as the backbone structure (Scheme 4). Three of the hydroxyl groups were reacted with propargyl bromide leaving one hydroxyl group for additional pegylation ($n = 3$). The end of the tri-glycolated hydroxyl group was capped with a fluorine atom. To assemble the targeted tridentate ligand, **10**, containing three 1,2,3-triazole rings, “click chemistry” between azide and alkyne was carried out. The desired intermediate **37** was first prepared through a simple three-step sequence. At this stage, copper-catalyzed “click chemistry”³⁸ was utilized to assemble the styrylpyridine polyethylene glycol-trimerized intermediates **38** and **39**. The final product, trivalent ligand **10**, was obtained by a simple acidic de-protection of intermediate **39**.

Two HPLC conditions were used to test the purity of the final compounds, **8a**, **8b**, **8c**, **9** and **10**. Using a Phenomenex Luna 5 μ C18 250 $\times$ 4.60 mm column, eluted with ACN/water, 8/2, 1 min/mL or a Phenomenex Luna 5 μ C18 250 $\times$ 4.60 mm column, eluted with MeOH/water, 9/1, 1 min/mL. All of the final tested samples showed a purity > 95%. The HPLC profiles are included in the supplemental information section.

In vitro binding studies of [^{18}F]**5** to A β -aggregates in the AD brain tissue homogenates

An *in vitro* binding assay was employed to measure the inhibition of [^{18}F]**5** binding to A β -aggregates in the AD brain tissue homogenates (see supplemental information). Inhibition constants (K_i , nM) of various agents against the binding of [^{18}F]**5** to A β -aggregates are

shown in Table 1. In addition, standard ligands, such as **1**, **4**, **5** and **6** were tested in the same binding assay (see Table 1). As expected, high binding affinities were obtained for the known compounds within the range of < 5 nM. The glycerol-based bivalent ligands, **8a**, **8b** and **8c**, displayed excellent binding affinities (K_i = 3.24, 7.71 and 3.86 nM, respectively). It appears that the chain length of the pegylation has no effect on the binding affinity. The K_i values (3 – 7 nM) were comparable when the chain length was 4, 5 or 6. However, the amide-based bivalent ligand, **9**, showed a significantly lower affinity, K_i = 71.2 nM. The amide linkage is apparently not suitable for designing this series of bivalent ligand. The trivalent ligand, **10**, also displayed a dramatically low binding affinity, K_i = 468 nM.

Radiolabeling of bivalent and trivalent styrylpyridine derivatives, [^{18}F]**8a**, **8b**, **8c**, **9** and **10**

The radiolabeling of the bidentate and tridentate ligands, **8a** – **c**, **9** and **10**, was successfully performed using the same protocol as reported for the preparation of [^{18}F]**5** (see scheme 5 and Fig. 3)²². The labeling yields (about 30% EOS) were comparable to that observed for [^{18}F]**5**, and the radiochemical purity was > 98%.

Autoradiography of postmortem brain tissue sections with [^{18}F]**5** and [^{18}F]**8a**

When confirmed CAA brain sections were incubated with [^{18}F]**8a**, the resulting autoradiogram clearly demonstrated a selective and highly dense labeling of the A β plaques of cerebral blood vessels with low labeling in the other areas of the brain sections (see Figure 4 upper panel). The labeling in the CAA brain sections was highly discrete. It is likely that in the CAA brain only the walls of cerebral blood vessels contained A β plaques; therefore the [^{18}F]**8a** only accumulated in these areas. For comparison, AD and healthy control (HC) brain sections were also labeled with [^{18}F]**5** or [^{18}F]**8a**. As expected, in the AD brain sections, which contained A β plaques in both the parenchymal tissue and the blood vessels, the sections were labeled intensely by both tracers. Due to a lack of A β plaques, both tracers showed very low labeling in the healthy control (HC) brain sections (Figure 4 lower panel).

To further confirm the nature of the A β plaque-labeling of CAA brain sections, a comparison experiment between autoradiography of [^{18}F]**8a** (Figure 5A and B) and fluorescent staining with thioflavin S (Figure 5C and D) was performed using neighboring CAA occipital brain sections. The results clearly indicate that the same labeling of the major blood vessels was observed with thioflavin S as with [^{18}F]**8a**. The data support the conclusion that [^{18}F]**8a** labeled A β plaques located on the major blood vessels in the CAA brain (Figure 5).

In vivo biodistribution in mice

After an iv injection in normal mice, [^{18}F]**8a**, **8b** and **8c** showed relatively low brain uptake ranging between 0.3 to 0.4 % dose/gram at 2 min (Table 2). In comparison to the brain uptake of [^{18}F]**5**, which showed 7.8 % dose/gram at 2 min, the bidentate ligands, [^{18}F]**8a**, **8b** and **8c**, clearly displayed a very low brain penetration. The low brain penetration of these new tracers would enable the selective labeling of A β plaques located on the major blood vessels in the CAA brain.

Discussion

Cerebral amyloid angiopathy (CAA) and Alzheimer's disease (AD) share common risk factors – mainly the accumulation of A β aggregates. It is generally believed that the pathology of CAA is associated with the deposit of β -amyloid on the walls of the cortical and leptomeningeal blood vessels. In addition to being a disease-defining pathological marker of AD, A β plaques are believed to play a significant role in the pathophysiological

mechanisms associated with cerebral amyloid angiopathy. The ability to use PET imaging to determine the location and concentration of A β aggregates would be useful for diagnostic purposes.

There are two major pre-requisites for imaging agents targeting A β plaques associated with cerebral blood vessel walls: 1. High binding affinity to A β plaques: To evaluate the feasibility of new ligands targeting the A β plaques of cerebral blood vessel walls suitable as CAA imaging agents, we employed an *in vitro* assay using AD brain tissue homogenates against the binding of [^{18}F]5 to measure the binding affinity. 2. Selective labeling of A β plaques on cerebral blood vessel walls and not in the cerebral parenchymal tissue: Preliminary testing of this requirement was based on biodistribution (brain uptake at 2 min after an iv injection) of [^{18}F] labeled ligands in normal mice. We reasoned that the inability of the new ligands to penetrate the intact blood-brain barrier (BBB) supported the conclusion that they would label only A β plaques on cerebral blood vessel walls, located outside of the BBB. It will be useful in the future to evaluate the distribution of A β plaque labeling in various transgenic mice reported recently^{1,39,40}. One important consideration is the total number of A β plaques on cerebral blood vessel walls, i.e. the B_{max} for these new ligands. Because humans are physically larger and have an increased number of A β plaques on their cerebral blood vessel walls, it may be possible to efficiently detect such differences.

We prepared the desired ligands by stitching together multiple styrylpyridine cores (selective core for binding A β aggregates) using pegylation chains with chain lengths of $n = 4, 5$ and 6 . It was assumed that A β aggregates composed of parallel β -sheets would likely contain repetitive and neighboring binding sites. These molecules were thus expected to show good binding to β -amyloid and to be excluded from the brain because of their large sizes.

Multidentate ligands have been utilized in various biological systems. Dimeric and multimeric ligands targeting $\alpha_v\beta_3$ -integrin binding sites have been very useful for tumor imaging^{41–47}. However, it is likely due to structural limitations of the parallel β -sheets of A β aggregates, as compared to that of the $\alpha_v\beta_3$ -integrin binding sites, that the structure-activity-relationship for binding would be very different. In this report, we have prepared and examined the multidentate A β aggregate-binding affinity of **8a**, **8b**, **8c**, **9** and **10**. Surprisingly, only the bidentate ligands **8a**, **8b** and **8c** showed the desired high binding affinity comparable to that of [^{18}F]5. The structural difference between the bidentate ligands **8a–c** and **9** was the linkage for the pegylated styrylpyridines, i.e. ether vs. amide linkage, which may have caused the big difference in binding affinity ($K_i = 3 - 7$ nM vs. 71 nM). The binding affinity of the tri-dentate ligand, **10**, also showed a dramatic reduction ($K_i = 468$ nM). This decrease may have been caused by changes in the geometry of the new ligand. These observations suggest that there are space limitations for multidentate ligands to insert themselves into β -amyloid and to bind to neighboring target sites.

The initial data from *in vitro* labeling of human brain sections also show that similar to [^{18}F]5, [^{18}F]8a is capable of labeling A β aggregates in human AD brain sections (Figure 4). It is important to note that the *in vitro* autoradiography of confirmed CAA brain sections showed successful labeling of major blood vessels by [^{18}F]8a and the labeling was confirmed by co-staining with thioflavin S (Figure 5). The results strongly suggest that it will be feasible to use [^{18}F]8a in imaging the accumulation of A β aggregates on the walls of major cerebral blood vessels in CAA patients. A buildup of A β aggregates in cerebral blood vessels also occurs in patients at risk of stroke and Alzheimer's disease¹⁹. CAA discriminating A β imaging agents, due to the location of A β aggregates on the wall of cerebral blood vessels, may provide selective diagnostic tools that would be useful in teasing out the accumulation of A β aggregates in different areas, which can be associated with

different clinical manifestations. Currently, it is assumed that the A β aggregates on the walls of major cerebral blood vessels in CAA are similar to those A β plaques in the brain (parenchymal). The new bidentate ligand, [^{18}F]**8a**, will likely bind to A β aggregates on the blood vessels in a similar binding mechanism as it would bind to A β plaques in the brain. Further studies will be required to confirm that this assumption is true.

One other key factor for consideration is the number of binding sites associated with A β plaques on the blood vessels walls. These may not be as high as those reported for AD brain $K_d = 3.72 \pm 0.30$ nM; $B_{\text{max}} = 8811 \pm 1643$ fmole/mg protein.²² However, the A β plaques located in the blood vessels may be more accessible and readily exposed to the CAA binding agent in the blood circulation. Therefore, the A β plaques in CAA may show higher apparent binding sites. This is a critical factor that warrants further investigation.

Conclusion

In summary, multi-dentate binding ligands targeting A β aggregates on cerebral vessels have been prepared and tested. They may provide useful tools for selective diagnosis of cerebral amyloid angiopathy (CAA), a significant risk factor in the older patient population.

Experimental

1. General

All reagents used for chemical synthesis were commercial products and were used without further purification. CD-1 mice (20 – 26 g) were used for the biodistribution studies. All protocols requiring the utilization of mice were reviewed and approved by the Institutional Animal Care and Use Committee (University of Pennsylvania and University of the Sciences in Philadelphia). Postmortem human samples were obtained from phase III clinical trials of [^{18}F]**5**. The synthesis of precursors and bidentate and tridentate styrylpyridine derivatives, **8a**, **8b**, **8c**, **9** and **10**, was accomplished by the following schemes (Scheme 1, 2, 3 and 4).

2-(2-Fluoroethoxy)-1,3-di((E)-2-(2-(2-(5-(4-(methylamino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)propane (8a)—A solution of **25a** (0.0338 g, 0.031 mmol) in 0.9 mL trifluoroacetic acid (TFA) and 0.1 mL dimethyl sulfide was stirred at rt for 1 h. The reaction mixture was evaporated in vacuo, and the residue was purified by FC (DCM/MeOH = 92.5/7.5) to give 0.024 g yellowish oil **8a** (yield: 87%) ^1H NMR (200 MHz, CDCl_3) δ : 8.14 (d, 2H, $J = 2.2$ Hz), 7.76 (dd, 2H, $J = 2.4$ Hz, $J = 8.8$ Hz), 7.35 (d, 4H, $J = 8.4$ Hz), 6.86 (d, 4H, $J = 8.0$ Hz), 6.77 (d, 2H, $J = 8.6$ Hz), 6.60 (d, 4H, $J = 8.6$ Hz), 4.65 (t, 1H, $J = 4.2$ Hz), 4.49 (t, 4H, $J = 4.8$ Hz), 4.41 (t, 1H, $J = 4.2$ Hz), 3.78–3.97 (m, 6H), 3.63–3.74 (m, 25H), 3.55–3.59 (m, 4H), 2.88 (s, 6H). ^{13}C NMR (200 MHz, CDCl_3) δ : 162.9, 149.3, 145.2, 135.3, 128.6, 127.8, 126.8, 120.7, 112.7, 111.5, 85.2, 81.8, 79.0, 71.82, 71.14, 70.9, 70.9, 70.8, 70.2, 70.0, 69.8, 65.5, 30.9. HRMS (ESI) calculated for $\text{C}_{49}\text{H}_{68}\text{FN}_4\text{O}_{11}$ (MH^+), 907.4869; found, 907.4870.

2-(2-Fluoroethoxy)-1,3-di((E)-2-(2-(2-(5-(4-(methylamino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)propane (8b)—Compound **8b** was prepared from **25b** (0.0650 g, 0.054 mmol) in 0.5 mL TFA and 0.05 mL dimethyl sulfide, with the same procedure described for compound **8a**. Compound **8b**: 0.050 g (yield: 93%): ^1H NMR (200 MHz, CDCl_3) δ : 8.14 (d, 2H, $J = 2.2$ Hz), 7.75 (dd, 2H, $J = 2.4$ Hz, $J = 8.6$ Hz), 7.34 (d, 4H, $J = 8.6$ Hz), 6.86 (d, 4H, $J = 8.0$ Hz), 6.76 (d, 2H, $J = 8.6$ Hz), 6.59 (d, 4H, $J = 8.6$ Hz), 4.65 (t, 1H, $J = 4.3$ Hz), 4.48 (t, 4H, $J = 4.8$ Hz), 4.41 (t, 1H, $J = 4.3$ Hz), 3.80–3.96 (m, 6H), 3.52–3.77 (m, 37H), 2.86 (s, 6H). ^{13}C NMR (200 MHz, CDCl_3) δ : 162.8, 149.2, 145.2, 135.3, 128.5, 127.8, 126.7, 120.6, 112.7, 111.4, 85.1, 81.8, 78.9, 71.8, 71.1, 70.8, 70.7, 70.2,

tert-Butyl methyl(4-vinylphenyl)carbamate (12)—To a suspension of NaH (60%, 0.600 g, 15 mmol) in 30 mL *N,N*-Dimethylformamide (DMF) was slowly added **11** (2.19 g, 10 mmol) in 20 mL DMF followed by iodomethane (CH₃I, 7.10 g, 50 mmol). After stirring at room temperature (rt) for 6 h, the reaction mixture was quenched with 40 mL sat. ammonium chloride (NH₄Cl) at 0 °C. The mixture was then extracted with 120 mL EtOAc. The organic layer was washed with H₂O (40 mL × 2) as well as brine (40 mL), dried by Na₂SO₄ and filtered. The filtrate was concentrated, and the residue was purified by FC (EtOAc/hexane = 2/8) to give 2.32 g white solid **12** (yield: 99%): ¹HNMR (200 MHz, CDCl₃) δ: 7.37 (d, 2H, *J* = 8.6 Hz), 7.20 (d, 2H, *J* = 8.6 Hz), 6.70 (dd, 1H, *J* = 11 Hz, *J* = 17.6 Hz), 5.72 (d, 1H, *J* = 17.6 Hz), 5.24 (d, 1H, *J* = 11 Hz), 3.26 (s, 3H), 1.46 (s, 9H).

2-(2-(2-(5-Iodopyridin-2-yloxy)ethoxy)ethoxy)ethanol (13a)—In a 20-mL Biotage microwave reaction vial, 2-Chloro-5-iodopyridine (2.39 g, 10 mmol), tetraethylene glycol (3.00 g, 20 mmol), cesium carbonate (Cs₂CO₃, 4.89 g, 15 mmol), and DMF (10 mL) were mixed and capped. After microwave heating at 150 °C for 55 min, the mixture was cooled to rt and diluted with 150 mL EtOAc and washed with H₂O (40 mL × 2) as well as brine (40 mL). The organic layer was dried by Na₂SO₄ and filtered. The filtrate was concentrated, and the residue was purified by FC (EtOAc/hexane = 7/3) to give 2.37 g white solid **4** (yield: 67%): ¹HNMR (200 MHz, CDCl₃) δ: 8.31 (d, 1H, *J* = 2.4 Hz), 7.79 (dd, 1H, *J* = 2.4 Hz, *J* = 8.6 Hz), 6.65 (d, 1H, *J* = 8.6 Hz), 4.45 (t, 2H, *J* = 4.8 Hz), 3.85 (t, 2H, *J* = 4.8 Hz), 3.60–3.76 (m, 8H).

2-(2-(2-(2-(5-Iodopyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethanol (13b)—Compound **13b** was prepared from 2-Chloro-5-iodopyridine (3.59 g, 15 mmol), triethylene glycol (5.83 g, 30 mmol), Cs₂CO₃ (7.34 g, 22.5 mmol), and DMF (10 mL), with the same procedure described for compound **13a**. Compound **13b**: 3.27 g (yield: 55%): ¹HNMR (200 MHz, CDCl₃) δ: 8.31 (d, 1H, *J* = 2.4 Hz), 7.78 (dd, 1H, *J* = 2.4 Hz, *J* = 8.6 Hz), 6.64 (d, 1H, *J* = 8.6 Hz), 4.45 (t, 2H, *J* = 4.8 Hz), 3.85 (t, 2H, *J* = 4.8 Hz), 3.60–3.74 (m, 12H).

(E)-tert-Butyl 4-(2-(6-(2-(2-(2-(2-(hydroxyethoxy)ethoxy)ethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)phenyl(methyl)carbamate (14a)—Compound **14a** was prepared from **12** (2.33 g, 10 mmol), **13b** (2.66 g, 6.7 mmol), K₂CO₃ (2.31 g, 16.75 mmol), tetrabutylammonium bromide (4.31 g, 13.4 mmol), Pd(OAc)₂ (0.0750 g, 0.34 mmol) and 50 mL DMF, with the same procedure described for compound **16**. Compound **14a**: 3.07 g (yield: 91%): ¹HNMR (200 MHz, CDCl₃) δ: 8.19 (d, 1H, *J* = 2.4 Hz), 7.80 (dd, 1H, *J* = 2.4 Hz, *J* = 8.6 Hz), 7.45 (d, 2H, *J* = 8.4 Hz), 7.23 (d, 2H, *J* = 8.6 Hz), 6.98 (s, 2H), 6.80 (d, 1H, *J* = 8.6 Hz), 4.51 (t, 2H, *J* = 4.8 Hz), 3.88 (t, 2H, *J* = 4.8 Hz), 3.60–3.78 (m, 12H), 3.28 (s, 3H), 2.56 (t, 1H, *J* = 6.0 Hz), 1.47 (s, 9H).

(E)-2-(2-(2-(2-(5-(4-(tert-Butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (15a)—To a solution of **14a** (3.07 g, 6.1 mmol) and triethylamine (3.03 g, 30 mmol) in 30 mL DCM was added *p*-toluenesulfonyl chloride (2.29 g, 12 mmol) and 4-(dimethylamino)pyridine (DMAP, 0.0732 g, 0.6 mmol) at 0 °C. The solution was allowed to warm to rt. After 50 min, the mixture was diluted with 80 mL DCM, washed with H₂O (30 mL × 2) as well as brine (30 mL). The organic layer was dried by Na₂SO₄ and concentrated to give an oil that was purified by FC (EtOAc/hexane = 1/1) to give 3.21 g white solid **15a** (80%): ¹HNMR (200 MHz, CDCl₃) δ: 8.19 (d, 1H, *J* = 2.4 Hz), 7.78–7.83 (m, 3H), 7.45 (d, 2H, *J* = 8.6 Hz), 7.34 (d, 2H, *J* = 8.4 Hz), 7.24 (d, 2H, *J* = 8.6 Hz), 6.98 (s, 2H), 6.80 (d, 1H, *J* = 8.6 Hz), 4.50 (t, 2H, *J* = 4.8 Hz), 4.17 (t, 2H, *J* = 4.8 Hz), 3.86 (t, 2H, *J* = 4.8 Hz), 3.63–3.72 (m, 10H), 3.28 (s, 3H), 2.45 (s, 3H), 1.47 (s, 9H).

(E)-14-(5-(4-(tert-Butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)-3,6,9,12-tetraoxatetradecyl 4-methylbenzenesulfonate (15b)—NaH (60% in mineral oil, 0.08 g, 2 mmol) was placed in a two-neck flask and washed with hexane. 4 mL DMF was added to form a suspension. A solution of **16** (0.459 g, 1 mmol) in 3 mL DMF was added dropwise at 0 °C. After stirring at rt for 30 min, the mixture was cooled to 0 °C, and a solution of diethylene glycol di(*p*-toluenesulfonate) (1.24 g, 3 mmol) in 3 mL DMF was added. The reaction mixture was stirred at rt overnight. The mixture was then poured into 10 mL cold 10% NaHCO₃, and extracted with DCM (20 mL × 2). The organic layer was washed with H₂O (10 mL × 2) and brine (10 mL), dried over Na₂SO₄, concentrated and purified by FC (DCM/MeOH = 98/2) to give 0.470 g clear oil **15b** (yield: 67%): ¹HNMR (200 MHz, CDCl₃) δ: 8.19 (d, 1H, *J* = 2.4 Hz), 7.78–7.83 (m, 3H), 7.45 (d, 2H, *J* = 8.6 Hz), 7.34 (d, 2H, *J* = 8.0 Hz), 7.24 (d, 2H, *J* = 8.6 Hz), 6.98 (s, 2H), 6.80 (d, 1H, *J* = 8.6 Hz), 4.50 (t, 2H, *J* = 4.8 Hz), 4.17 (t, 2H, *J* = 4.8 Hz), 3.7 (t, 2H, *J* = 4.8 Hz), 3.59–3.76 (m, 14H), 3.28 (s, 3H), 2.45 (s, 3H), 1.47 (s, 9H).

(E)-17-(5-(4-(tert-Butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (15c)—Compound **15c** was prepared from NaH (60% in mineral oil, 0.120 g, 3 mmol), **14a** (0.754 g, 1.5 mmol), diethylene glycol di(*p*-toluenesulfonate) (1.87 g, 4.5 mmol) and 15 mL DMF, with the same procedure described for compound **15b**. Compound **15c**: 0.759 g (yield: 68%): ¹HNMR (200 MHz, CDCl₃) δ: 8.19 (d, 1H, *J* = 2.4 Hz), 7.78–7.83 (m, 3H), 7.45 (d, 2H, *J* = 8.6 Hz), 7.34 (d, 2H, *J* = 8.0 Hz), 7.24 (d, 2H, *J* = 8.6 Hz), 6.98 (s, 2H), 6.80 (d, 1H, *J* = 8.6 Hz), 4.50 (t, 2H, *J* = 4.8 Hz), 4.17 (t, 2H, *J* = 4.8 Hz), 3.87 (t, 2H, *J* = 4.8 Hz), 3.59–3.75 (m, 18H), 3.28 (s, 3H), 2.45 (s, 3H), 1.47 (s, 9H).

(E)-tert-Butyl 4-(2-(6-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)phenyl(methyl)carbamate (16)—A mixture of **12** (0.710 g, 3.0 mmol), **13a** (0.706 g, 2.0 mmol), potassium carbonate (K₂CO₃, 0.690 g, 5.0 mmol), tetrabutylammonium bromide (1.29 g, 4.0 mmol), and palladium acetate (Pd(OAc)₂, 0.0224 g, 0.10 mmol) in 15 mL DMF was deoxygenated by purging into nitrogen for 15 min and then heated at 65 °C for 2 h. The mixture was cooled to RT, diluted with 80 mL EtOAc and washed with H₂O (20 mL × 2) as well as brine (20 mL). The organic layer was dried by Na₂SO₄ and filtered. The filtrate was concentrated, and the residue was purified by FC (EtOAc/hexane = 8/2) to give 0.753 g white solid **16** (yield: 82%): ¹HNMR (200 MHz, CDCl₃) δ: 8.19 (d, 1H, *J* = 2.4 Hz), 7.80 (dd, 1H, *J* = 2.4 Hz, *J* = 8.6 Hz), 7.45 (d, 2H, *J* = 8.4 Hz), 7.23 (d, 2H, *J* = 8.4 Hz), 6.98 (s, 2H), 6.80 (d, 1H, *J* = 8.6 Hz), 4.52 (t, 2H, *J* = 4.8 Hz), 3.89 (t, 2H, *J* = 4.8 Hz), 3.61–3.80 (m, 8H), 3.28 (s, 3H), 2.46 (t, 1H, *J* = 6.0 Hz), 1.47 (s, 9H).

2-(1,3-Dibromopropan-2-yloxy)-tetrahydro-2H-pyran (17)—To a solution of 1,3-dibromopropane-2-ol (2.18 g, 10 mmol) in 20 mL tetrahydrofuran (THF) was added pyridinium *p*-toluenesulfonate (PPTS, 0.25 g, 1 mmol) and 3,4-dihydro-2H-pyran (1.68 g, 20 mmol). After stirring at rt for 3 h, the mixture was diluted with 100 mL EtOAc and washed with H₂O (30 mL × 2) as well as brine (30 mL). The organic layer was dried by Na₂SO₄ and filtered. The filtrate was concentrated, and the residue was purified by FC (EtOAc/hexane = 5/95) to give 2.72 g clear oil **17** (yield: 90%): ¹HNMR (200 MHz, CDCl₃) δ: 4.78–4.82 (m, 1H), 3.89–4.06 (m, 2H), 3.51–3.74 (m, 5H), 1.55–1.85 (m, 6H).

2-(Tetrahydro-2H-pyran-2-yloxy)propane-1,3-diyl diacetate (18)—A mixture of **17** (3.83 g, 12.6 mmol), tetrabutylammonium acetate (11.4 g, 37.8 mmol), tetrabutylammonium iodide (0.923 g, 2.5 mmol) and K₂CO₃ (0.345 g, 2.5 mmol) in 20 mL anhydrous acetonitrile was stirred at rt overnight. The mixture was filtered and the filtrate was concentrated. The residue was purified by FC (EtOAc/hexane = 5/95) to give 2.81 g clear oil **18** (yield:

86%): ^1H NMR (200 MHz, CDCl_3) δ : 4.79–4.81 (m, 1H), 4.03–4.31 (m, 5H), 3.88–3.97 (m, 1H), 3.49–3.57 (m, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 1.55–1.84 (m, 6H).

2-(Tetrahydro-2H-pyran-2-yloxy)propane-1,3-diol (19)—A mixture of **18** (1.30 g, 5 mmol) and 25 mL ammonia solution (2.0 M in methanol) was stirred at rt overnight. The mixture was then concentrated in vacuo to afford 0.81 g yellow oil **19** (yield: 91%): ^1H NMR (200 MHz, CDCl_3) δ : 4.60–4.63 (m, 1H), 4.01–4.06 (m, 1H), 3.53–3.80 (m, 6H), 1.84–1.96 (m, 2H), 1.56–1.59 (m, 4H).

2-(1,3-Di((E)-2-(2-(2-(2-(5-(4-(tert-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-yloxy)-tetrahydro-2H-pyran (20a)—NaH (60% in mineral oil, 0.160 g, 4 mmol) was placed in a two-neck flask and washed with hexane. 5 mL DMF was added to form a suspension. A solution of **19** (0.176 g, 1 mmol) in 5 mL DMF was added dropwise at 0 °C. After stirring at rt for 30 min, the mixture was cooled to 0 °C, and a solution of **15a** (1.31 g, 2 mmol) in 3 mL DMF was added dropwise, and the reaction mixture was stirred at rt overnight. The mixture was then poured into 50 mL cold sat. NH_4Cl , and extracted with DCM (50 mL $\times$ 2). The organic layer was washed with H_2O (30 mL) and brine (30 mL), dried over Na_2SO_4 , concentrated and purified by FC (EtOAc: 100%) to give 0.835 g clear oil **20a** (yield: 73%). ^1H NMR (200 MHz, CDCl_3) δ : 8.19 (d, 2H, J = 2.4 Hz), 7.80 (dd, 2H, J = 2.4 Hz, J = 8.6 Hz), 7.45 (d, 4H, J = 8.4 Hz), 7.23 (d, 4H, J = 8.4 Hz), 6.97 (s, 4H), 6.80 (d, 2H, J = 8.6 Hz), 4.73–4.82 (m, 1H), 4.50 (t, 4H, J = 4.8 Hz), 3.85–4.02 (m, 6H), 3.50–3.71 (m, 29H), 3.28 (s, 6H), 1.56–1.96 (m, 6H), 1.47 (s, 18H).

2-(1,3-Di((E)-2-(2-(2-(2-(5-(4-(tert-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-yloxy)-tetrahydro-2H-pyran (20b)—Compound **20b** was prepared from NaH (60% in mineral oil, 0.052 g, 1.3 mmol), **19** (0.0581 g, 0.33 mmol) and **15b** (0.469 g, 0.67 mmol) in 8 mL DMF, with the same procedure described for compound **20a**. Compound **20b**: 0.310 g (yield: 76%): ^1H NMR (200 MHz, CDCl_3) δ : 8.19 (d, 2H, J = 2.4 Hz), 7.80 (dd, 2H, J = 2.4 Hz, J = 8.6 Hz), 7.45 (d, 4H, J = 8.4 Hz), 7.23 (d, 4H, J = 8.4 Hz), 6.97 (s, 4H), 6.80 (d, 2H, J = 8.6 Hz), 4.79–4.81 (m, 1H), 4.50 (t, 4H, J = 4.8 Hz), 3.84–4.05 (m, 6H), 3.62–3.71 (m, 37H), 3.28 (s, 6H), 1.56–1.96 (m, 6H), 1.47 (s, 18H).

2-(1,3-Di((E)-2-(2-(2-(2-(5-(4-(tert-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-yloxy)-tetrahydro-2H-pyran (20c)—Compound **20c** was prepared from NaH (60% in mineral oil, 0.080 g, 2 mmol), **19** (0.0851 g, 0.48 mmol) and **15c** (0.745 g, 1 mmol) in 10 mL DMF, with the same procedure described for compound **20a**. Compound **20c**: 0.522 g (yield: 82%): ^1H NMR (200 MHz, CDCl_3) δ : 8.19 (d, 2H, J = 2.4 Hz), 7.80 (dd, 2H, J = 2.4 Hz, J = 8.6 Hz), 7.45 (d, 4H, J = 8.4 Hz), 7.23 (d, 4H, J = 8.6 Hz), 6.97 (s, 4H), 6.80 (d, 2H, J = 8.6 Hz), 4.79–4.81 (m, 1H), 4.50 (t, 4H, J = 4.8 Hz), 3.85–4.05 (m, 6H), 3.45–3.71 (m, 45H), 3.28 (s, 6H), 1.56–1.96 (m, 6H), 1.47 (s, 18H).

1,3-Di((E)-2-(2-(2-(2-(5-(4-(tert-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-ol (21a)—A mixture of **20a** (0.841 g, 0.73 mmol) and PPTS (0.0183 g, 0.073 mmol) in 7 mL EtOH was stirred at 55 °C for 3 h. The mixture was concentrated, and the residue was purified by FC (EtOAc/MeOH = 9/1) to give 0.732 g colorless oil **21a** (yield: 94%): ^1H NMR (200 MHz, CDCl_3) δ : 8.19 (d, 2H, J =

2.4 Hz), 7.80 (dd, 2H, $J = 2.4$ Hz, $J = 8.6$ Hz), 7.45 (d, 4H, $J = 8.4$ Hz), 7.23 (d, 4H, $J = 8.6$ Hz), 6.97 (s, 4H), 6.80 (d, 2H, $J = 8.6$ Hz), 4.50 (t, 4H, $J = 4.8$ Hz), 3.84–4.05 (m, 6H), 3.45–3.80 (m, 28H), 3.28 (s, 6H), 1.47 (s, 18H).

1,3-Di((*E*)-2-(2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-ol (21b)—Compound **21b** was prepared from **20b** (0.295 g, 0.24 mmol) and PPTS (0.0060 g, 0.024 mmol) in 3 mL EtOH, with the same procedure described for compound **21a**. Compound **21b**: 0.177 g (yield: 62%): ¹HNMR (200 MHz, CDCl₃) δ: 8.19 (d, 2H, *J* = 2.4 Hz), 7.80 (dd, 2H, *J* = 2.4 Hz, *J* = 8.6 Hz), 7.45 (d, 4H, *J* = 8.4 Hz), 7.23 (d, 4H, *J* = 8.4 Hz), 6.97 (s, 4H), 6.80 (d, 2H, *J* = 8.6 Hz), 4.48–4.52 (m, 4H), 3.84–4.05 (m, 6H), 3.45–3.80 (m, 36H), 3.28 (s, 6H), 1.47 (s, 18H).

1,3-Di((*E*)-2-(2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-ol (21c)—
Compound **21b** was prepared from **20c** (0.524 g, 0.4 mmol) and PPTS (0.0100 g, 0.04 mmol) in 4 mL EtOH, with the same procedure described for compound **21a**. Compound **21c**: 0.340 g (yield: 69%): ¹HNMR (200 MHz, CDCl₃) δ: 8.19 (d, 2H, *J* = 2.4 Hz), 7.80 (dd, 2H, *J* = 2.4 Hz, *J* = 8.6 Hz), 7.45 (d, 4H, *J* = 8.4 Hz), 7.23 (d, 4H, *J* = 8.4 Hz), 6.97 (s, 4H), 6.80 (d, 2H, *J* = 8.6 Hz), 4.50 (t, 4H, *J* = 4.8 Hz), 3.84–4.05 (m, 6H), 3.45–3.80 (m, 44H), 3.28 (s, 6H), 1.47 (s, 18H).

2-(2-(1,3-Di((E)-2-(2-(2-(5-(4-(tert-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-yloxy)ethoxy)-tetrahydro-2H-pyran (22a)—NaH (60% in mineral oil, 0.0552 g, 1.38 mmol) was placed in a two-neck flask and washed with hexane. 4 mL DMF was added to form a suspension. A solution of **21a** (0.73 g, 0.69 mmol) in 3 mL DMF was added dropwise at 0 °C. After stirring at rt for 30 min, the mixture was cooled to 0 °C, and a solution of 2-(2-bromoethoxy)-tetrahydro-2H-pyran (0.288 g, 1.38 mmol) in 3 mL DMF was added dropwise. The reaction mixture was stirred at rt overnight. The mixture was then poured into 50 mL cold sat. NH₄Cl, and extracted with DCM (30 mL × 2). The organic layer was washed with H₂O (20 mL) and brine (20 mL), dried over Na₂SO₄, concentrated and purified by FC (EtOAc/MeOH = 98/2) to give 0.574 g colorless oil **22a** (yield: 70%). ¹HNMR (200 MHz, CDCl₃) δ: 8.19 (d, 2H, *J* = 2.0 Hz), 7.80 (dd, 2H, *J* = 2.4 Hz, *J* = 8.6 Hz), 7.45 (d, 4H, *J* = 8.4 Hz), 7.23 (d, 4H, *J* = 8.4 Hz), 6.97 (s, 4H), 6.80 (d, 2H, *J* = 8.6 Hz), 4.62–4.65 (m, 1H), 4.50 (t, 4H, *J* = 4.8 Hz), 3.55–3.89 (m, 39H), 3.28 (s, 6H), 1.56–1.96 (m, 6H), 1.47 (s, 18H).

2-(2-(1,3-Di((*E*)-2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-yloxy)ethoxy)-tetrahydro-2*H*-pyran (22b)—Compound **22b** was prepared from NaH (60% in mineral oil, 0.0180 g, 0.45 mmol), **21b** (0.172 g, 0.15 mmol) and 2-(2-bromoethoxy)-tetrahydro-2*H*-pyran (0.0627 g, 0.3 mmol) in 2.5 mL DMF, with the same procedure described for compound **22a**. Compound **22b**: 0.130 g (yield: 68%): ¹HNMR (200 MHz, CDCl₃) δ: ¹HNMR δ: 8.19 (d, 2H, *J* = 2.4 Hz), 7.80 (dd, 2H, *J* = 2.4 Hz, *J* = 8.6 Hz), 7.45 (d, 4H, *J* = 8.4 Hz), 7.23 (d, 4H, *J* = 8.4 Hz), 6.97 (s, 4H), 6.80 (d, 2H, *J* = 8.6 Hz), 4.62–4.65 (m, 1H), 4.50 (t, 4H, *J* = 4.8 Hz), 3.52–3.89 (m, 47H), 3.28 (s, 6H), 1.56–1.96 (m, 6H), 1.47 (s, 18H).

2-(2-(1,3-Di((*E*)-2-(2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-

ylxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-ylxy)ethoxy)-tetrahydro-2H-pyran (22c)—Compound **22c** was prepared from NaH (60% in mineral oil, 0.022 g, 0.55 mmol), **21c** (0.333 g, 0.27 mmol) and 2-(2-bromoethoxy)-tetrahydro-2H-pyran (0.115 g, 0.55 mmol) in 4 mL DMF, with the same procedure described for compound **22a**. Compound **22c**: 0.310 g (yield: 83%): $^1\text{H NMR}$ (200 MHz, CDCl_3) δ : $^1\text{H NMR}$ δ : 8.19 (d, 2H, $J = 2.4$ Hz), 7.80 (dd, 2H, $J = 2.4$ Hz, $J = 8.6$ Hz), 7.45 (d, 4H, $J = 8.4$ Hz), 7.23 (d, 4H, $J = 8.4$ Hz), 6.97 (s, 4H), 6.80 (d, 2H, $J = 8.6$ Hz), 4.62–4.65 (m, 1H), 4.50 (t, 4H, $J = 4.8$ Hz), 3.57–3.89 (m, 55H), 3.28 (s, 6H), 1.56–1.96 (m, 6H), 1.47 (s, 18H).

2-(1,3-Di((*E*)-2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-yloxy)ethanol (23a)—A mixture of **22a** (0.570 g, 0.48 mmol) and PPTS (0.0120 g, 0.048 mmol) in 6 mL EtOH was stirred at 55 °C for 6 h. The mixture was concentrated, and the residue was purified by FC (EtOAc/MeOH = 9/1) to give 0.477 g colorless oil **23a** (yield: 90%): ¹HNMR (200 MHz, CDCl₃) δ: 8.18 (d, 2H, *J* = 2.4 Hz), 7.79 (dd, 2H, *J* = 2.4 Hz, *J* = 8.6 Hz), 7.45 (d, 4H, *J* = 8.4 Hz), 7.23 (d, 4H, *J* = 8.6 Hz), 6.96 (s, 4H), 6.80 (d, 2H, *J* = 8.6 Hz), 4.49 (t, 4H, *J* = 4.8 Hz), 3.86 (t, 4H, *J* = 4.8 Hz), 3.64–3.79 (m, 29H), 3.49–3.58 (m, 4H), 3.27 (s, 6H), 1.47 (s, 18H).

2-(1,3-Di((*E*)-2-(2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-yloxy)ethanol (23b)—Compound **23b** was prepared from **22b** (0.128 g, 0.1 mmol) and PPTS (0.0025 g, 0.01 mmol) in 1 mL EtOH, with the same procedure described for compound **23a**. Compound **23b**: 0.116 g (yield: 98%): ¹HNMR (200 MHz, CDCl₃) δ: 8.19 (d, 2H, *J* = 2.2 Hz), 7.80 (dd, 2H, *J* = 2.6 Hz, *J* = 8.8 Hz), 7.45 (d, 4H, *J* = 8.6 Hz), 7.23 (d, 4H, *J* = 8.6 Hz), 6.97 (s, 4H), 6.80 (d, 2H, *J* = 8.6 Hz), 4.50 (t, 4H, *J* = 4.8 Hz), 3.87 (t, 4H, *J* = 4.8 Hz), 3.65–3.79 (m, 37H), 3.51–3.55 (m, 4H), 3.28 (s, 6H), 1.47 (s, 18H).

2-(1,3-Di((*E*)-2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-yloxy)ethanol (23c**)**—Compound **23c** was prepared from **22c** (0.314 g, 0.23 mmol) and PPTS (0.00577 g, 0.023 mmol) in 2 mL EtOH, with the same procedure described for compound **23a**. Compound **23c**: 0.235 g (yield: 80%); $^1\text{H NMR}$ (200 MHz, CDCl_3) δ : 8.19 (d, 2H, $J = 2.2$ Hz), 7.80 (dd, 2H, $J = 2.4$ Hz, $J = 8.8$ Hz), 7.45 (d, 4H, $J = 8.6$ Hz), 7.23 (d, 4H, $J = 8.6$ Hz), 6.97 (s, 4H), 6.80 (d, 2H, $J = 8.6$ Hz), 4.50 (t, 4H, $J = 4.8$ Hz), 3.87 (t, 4H, $J = 4.8$ Hz), 3.64–3.71 (m, 45H), 3.51–3.55 (m, 4H), 3.28 (s, 6H), 1.47 (s, 18H).

2-(1,3-Di((*E*)-2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-yloxy)ethyl-4-methylbenzene sulfonate (24a**)**—To a solution of **23a** (0.395 g, 0.36 mmol) and triethylamine (0.180 g, 1.79 mmol) in 5 mL DCM was added *p*-toluenesulfonyl chloride (0.135 g, 0.71 mmol) and DMAP (0.0044 g, 0.036 mmol) at 0 °C. The solution was allowed to warm to rt. After 1 h, the mixture was diluted with 30 mL DCM, washed with H₂O (10 mL × 2) as well as brine (10 mL). The organic layer was dried by Na₂SO₄ and concentrated to give an oil that was purified by FC (DCM/MeOH = 95/5) to give 0.444 g yellowish solid **24a** (yield: 97%): ¹HNMR (200 MHz, CDCl₃) δ: 8.18 (d, 2H, *J* = 2.0 Hz), 7.77–7.82 (m, 4H), 7.45 (d, 4H, *J* = 8.6 Hz), 7.33 (d, 2H, *J* = 8.0 Hz), 7.23 (d, 4H, *J* = 8.6 Hz), 6.97 (s, 4H), 6.79 (d, 2H, *J* = 8.6 Hz), 4.50 (t, 4H, *J* = 4.8 Hz), 4.15 (t, 2H, *J* = 4.8 Hz), 3.80–3.89 (m, 6H), 3.55–3.74 (m, 25H), 3.44–3.51 (m, 4H), 3.28 (s, 6H), 2.44 (s, 3H), 1.47 (s, 18H).

2-(1,3-Di((E)-2-(2-(2-(2-(5-(4-(tert-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-yloxy)ethyl-4-methylbenzene sulfonate (24b)—Compound **24b** was prepared from **23b** (0.125 g, 0.11 mmol), triethylamine (0.0525 g, 0.5 mmol), *p*-toluenesulfonyl chloride (0.0400 g, 0.21 mmol) and DMAP (0.0012 g, 0.01 mmol) in 2 mL DCM, with the same procedure described for compound **24a**. Compound **24b**: 0.134 g (yield: 96%): ¹HNMR (200 MHz, CDCl₃) δ: 8.19 (d, 2H, *J* = 2.0 Hz), 7.77–7.82 (m, 4H), 7.45 (d, 4H, *J* = 8.6 Hz), 7.34 (d, 2H, *J* = 8.4 Hz), 7.23 (d, 4H, *J* = 8.8 Hz), 6.97 (s, 4H), 6.79 (d, 2H, *J* = 8.6 Hz), 4.50 (t, 4H, *J* = 4.8 Hz), 4.14 (t, 2H, *J* = 4.8 Hz), 3.80–3.89 (m, 6H), 3.60–3.74 (m, 33H), 3.46–3.51 (m, 4H), 3.28 (s, 6H), 2.44 (s, 3H), 1.47 (s, 18H).

2-(1,3-Di((E)-2-(2-(2-(2-(5-(4-(tert-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propan-2-yloxy)ethyl-4-methylbenzenesulfonate (24c)—Compound **24c** was prepared from **23c** (0.230 g, 0.18 mmol), triethylamine (0.0909 g, 0.9 mmol), *p*-toluenesulfonyl chloride (0.0686 g, 0.36 mmol) and DMAP (0.0022 g, 0.018 mmol) in 3 mL DCM, with the same procedure described for compound **24a**. Compound **24c**: 0.240 g (yield: 98%): ¹HNMR (200 MHz, CDCl₃) δ: 8.18 (d, 2H, *J* = 2.2 Hz), 7.78–7.82 (m, 4H), 7.45 (d, 4H, *J* = 8.6 Hz), 7.34 (d, 2H, *J* = 8.0 Hz), 7.23 (d, 4H, *J* = 8.2 Hz), 6.97 (s, 4H), 6.79 (d, 2H, *J* = 8.6 Hz), 4.50 (t, 4H, *J* = 4.8 Hz), 4.14 (t, 2H, *J* = 4.8 Hz), 3.80–3.89 (m, 6H), 3.46–3.75 (m, 41H), 3.44–3.51 (m, 4H), 3.28 (s, 6H), 2.45 (s, 3H), 1.47 (s, 18H).

2-(2-Fluoroethoxy)-1,3-di((E)-2-(2-(2-(2-(5-(4-(tert-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy) propane (25a)—A mixture of **24a** (0.126 g, 0.1 mmol) and tetrabutylammonium fluoride (TBAF, 0.25 mL, 1.0 M in THF) in 1.5 mL THF was stirred at 70 °C overnight. The reaction mixture was evaporated in vacuo, and the residue was purified by FC (EtOAc/MeOH = 9/1) to give 0.054 g colorless oil **25a** (yield: 49%): ¹HNMR (200 MHz, CDCl₃) δ: 8.17 (d, 2H, *J* = 2.2 Hz), 7.78 (dd, 2H, *J* = 2.2 Hz, *J* = 8.6 Hz), 7.43 (d, 4H, *J* = 8.6 Hz), 7.22 (d, 4H, *J* = 8.6 Hz), 6.96 (s, 4H), 6.78 (d, 2H, *J* = 8.6 Hz), 4.64 (t, 1H, *J* = 4.2 Hz), 4.48 (t, 4H, *J* = 4.8 Hz), 4.40 (t, 1H, *J* = 4.2 Hz), 3.94 (t, 1H, *J* = 4.3 Hz), 3.85 (t, 4H, *J* = 4.8 Hz), 3.79 (t, 1H, *J* = 4.3 Hz), 3.62–3.73 (m, 25H), 3.54–3.58 (m, 4H), 3.26 (s, 6H), 1.46 (s, 18H).

2-(2-Fluoroethoxy)-1,3-di((E)-2-(2-(2-(2-(5-(4-(tert-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propane (25b)—Compound **25b** was prepared from **24b** (0.100 g, 0.074 mmol), TBAF (0.15 mL, 1.0 M in THF) in 1 mL THF, with the same procedure described for compound **25a**. Compound **25b**: 0.0726 g (yield: 82%): ¹HNMR (200 MHz, CDCl₃) δ: 8.18 (d, 2H, *J* = 2.2 Hz), 7.79 (dd, 2H, *J* = 2.4 Hz, *J* = 8.6 Hz), 7.45 (d, 4H, *J* = 8.6 Hz), 7.23 (d, 4H, *J* = 8.6 Hz), 6.97 (s, 4H), 6.79 (d, 2H, *J* = 8.6 Hz), 4.65 (t, 1H, *J* = 4.2 Hz), 4.50 (t, 4H, *J* = 4.8 Hz), 4.41 (t, 1H, *J* = 4.2 Hz), 3.95 (t, 1H, *J* = 4.3 Hz), 3.78–3.89 (m, 5H), 3.53–3.77 (m, 37H), 3.28 (s, 6H), 1.47 (s, 18H).

2-(2-Fluoroethoxy)-1,3-di((E)-2-(2-(2-(2-(5-(4-(tert-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)propane (25c)—Compound **25c** was prepared from **24c** (0.171 g, 0.12 mmol), TBAF (0.24 mL, 1.0 M in THF) in 2 mL THF, with the same procedure described for compound **25a**. Compound **25c**: 0.123 g (yield: 80%): ¹HNMR (200 MHz, CDCl₃) δ: 8.19 (d, 2H, *J* = 2.2 Hz), 7.80 (dd, 2H, *J* = 2.4 Hz, *J* =

8.6 Hz), 7.45 (d, 4H, $J = 8.6$ Hz), 7.23 (d, 4H, $J = 8.6$ Hz), 6.98 (s, 4H), 6.80 (d, 2H, $J = 8.6$ Hz), 4.65 (t, 1H, $J = 4.2$ Hz), 4.50 (t, 4H, $J = 4.8$ Hz), 4.42 (t, 1H, $J = 4.2$ Hz), 3.96 (t, 1H, $J = 4.3$ Hz), 3.78–3.89 (m, 5H), 3.53–3.77 (m, 45H), 3.28 (s, 6H), 1.47 (s, 18H).

Ethyl 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)acetate (26)—To a solution of tetraethylene glycol (3.02 g, 20 mmol) and boron trifluoride–diethyl ether (0.1 mL) in 40 mL DCM was slowly added ethyl diazoacetate (1.14 g, 10 mmol) in 10 mL DCM at 0 °C. The reaction mixture was stirred at rt for 2 h and then washed with H₂O (10 mL $\times$ 2) as well as brine (10 mL). The organic layer was dried by Na₂SO₄ and concentrated to give 1.19 g clear oil **26** (yield: 50%): ¹HNMR (200 MHz, CDCl₃) δ : 4.16–4.28 (m, 4H), 3.60–3.76 (m, 12H), 2.43 (t, 1H, $J = 6.2$ Hz), 1.30 (t, 3H, $J = 7.2$ Hz).

Ethyl 2-(2-(2-(2-bromoethoxy)ethoxy)ethoxy)acetate (27)—To a solution of triphenylphosphine (2.16 g, 8.25 mmol) and *N*-bromosuccinimide (1.47 g, 8.25 mmol) in 30 mL DCM was slowly added **26** (1.30 g, 5.5 mmol) in 20 mL DCM at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and then washed with H₂O (10 mL $\times$ 2) and brine (10 mL). The organic layer was dried by Na₂SO₄ and concentrated to give 1.39 g yellow oil **27** (yield: 85%): ¹HNMR (200 MHz, CDCl₃) δ : 4.16–4.28 (m, 4H), 3.68–3.86 (m, 10H), 3.48 (t, 2H, $J = 6.4$ Hz), 1.29 (t, 3H, $J = 7.2$ Hz).

2-(2-(2-(2-Bromoethoxy)ethoxy)ethoxy)acetic acid (28)—A solution of **27** (1.39 g, 4.6 mmol) in 15 mL sodium hydroxide (NaOH, 1 M)/THF (v/v, 1/1) was stirred at rt for 2 h. Hydrochloric acid (HCl, 0.9 mL) was then added to the mixture. The solvent was removed in vacuo and the residue was taken up in DCM. The mixture was filtered and the filtrate was concentrated to give 0.810 g clear oil **28** (yield: 65%): ¹HNMR (200 MHz, CDCl₃) δ : 4.17 (s, 2H), 3.75–3.88 (m, 10H), 3.52 (t, 2H, $J = 6.4$ Hz).

tert-Butyl 14-bromo-3-(2-tert-butoxy-2-oxoethyl)-4-oxo-6,9,12-trioxa-3-azatetradecan-1-oate (29)—To a solution of **28** (0.810 g, 2.9 mmol) in 15 mL DMF was added di-*tert*-butyl iminodiacetate (0.711 g, 2.9 mmol), 1-hydroxybenzotriazole hydrate (0.882 g, 5.22 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (1.0 g, 5.22 mmol) and *N,N*-diisopropylethylamine (0.674 g, 5.22 mmol). After stirring at rt overnight, the mixture was poured into 100 mL EtOAc and washed with H₂O (30 mL $\times$ 2) and brine (30 mL). The organic layer was dried by Na₂SO₄ and filtered. The filtrate was concentrated, and the residue was purified by FC (EtOAc/hexane = 1/1) to give 0.931 g clear oil **29** (yield: 64%): ¹HNMR (200 MHz, CDCl₃) δ : 4.24 (s, 2H), 4.14 (s, 2H), 4.05 (s, 2H), 3.64–3.86 (m, 10H), 3.48 (t, 2H, $J = 6.4$ Hz), 1.49 (s, 9H), 1.47 (s, 9H).

14-Bromo-3-(carboxymethyl)-4-oxo-6,9,12-trioxa-3-azatetradecan-1-oic acid (30)—A solution of **29** (0.157 g, 0.3 mmol) in 3 mL TFA/DCM (v/v, 1/1) was stirred at rt for 2 h. Removal of solvent gave clear oil. After several times of co-evaporation with methanol, 0.112 g **30** (yield: 97%) was given as a clear oil: ¹HNMR (200 MHz, CDCl₃) δ : 4.49 (s, 2H), 4.34 (s, 2H), 4.28 (s, 2H), 3.74–3.92 (m, 10H), 3.49 (t, 2H, $J = 6.0$ Hz).

(E)-tert-Butyl 4-(2-(6-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)phenyl(methyl)carbamate (31)—Sodium azide (0.566 g, 8.7 mmol) was added to a solution of **15a** (1.91 g, 2.9 mmol) in 20 mL DMF. After stirring at 60 °C for 3 h, the mixture was then poured into 100 mL EtOAc and washed with H₂O (30 mL $\times$ 2) as well as brine (30 mL). The organic layer was dried by Na₂SO₄ and filtered. The filtrate was concentrated, and the residue was purified by FC (EtOAc/hexane = 1/1) to give 1.33 g white solid **31** (yield: 87%): ¹HNMR (200 MHz, CDCl₃) δ : 8.20 (d, 1H, $J = 2.6$ Hz), 7.80 (dd, 1H, $J = 2.2$ Hz, $J = 8.6$ Hz), 7.45 (d, 2H, $J = 8.4$ Hz), 7.23 (d, 2H, $J = 8.6$ Hz), 6.98 (s, 2H), 6.81

(d, 1H, $J = 8.4$ Hz), 4.51 (t, 2H, $J = 4.8$ Hz), 3.88 (t, 2H, $J = 4.8$ Hz), 3.66–3.72 (m, 10H), 3.39 (t, 2H, $J = 4.8$ Hz), 3.28 (s, 3H), 1.47 (s, 9H).

(*E*)-*tert*-Butyl 4-(2-(6-(2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethoxy)ethoxy)pyridin-3-yl)vinyl)phenyl(methyl)carbamate (32)—Triphenylphosphine (0.472 g, 1.8 mmol) was added to a solution of **31** (0.379 g, 0.72 mmol) in 12.6 mL THF. After stirring at rt for 2h, 1.4 mL H₂O was added and the mixture was heated at 60 °C overnight. The reaction mixture was evaporated in vacuo, and the residue was purified by FC (DCM/MeOH/NH₄OH = 90/9/1) to give 0.360 g white solid **32** (yield: 100%): ¹HNMR (200 MHz, CDCl₃) δ: 8.19 (d, 1H, $J = 2.4$ Hz), 7.81 (dd, 1H, $J = 2.4$ Hz, $J = 8.6$ Hz), 7.45 (d, 2H, $J = 8.4$ Hz), 7.23 (d, 2H, $J = 8.6$ Hz), 6.98 (s, 2H), 6.81 (d, 1H, $J = 8.8$ Hz), 4.50 (t, 2H, $J = 4.8$ Hz), 3.88 (t, 2H, $J = 4.8$ Hz), 3.67–3.72 (m, 8H), 3.56 (t, 2H, $J = 5.2$ Hz), 3.28 (s, 3H), 2.890 (t, 2H, $J = 5.2$ Hz), 1.47 (s, 9H).

2-(2-(2-(2-Bromoethoxy)ethoxy)ethoxy)-*N,N*-bis(2-(((*E*)-2-(2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)amino)-2-oxoethyl)acetamide (33)—To a solution of **30** (0.116 g, 0.3 mmol) in 6 mL DCM was added *N,N'*-dicyclohexylcarbodiimide (0.247 g, 1.2 mmol), **32** (0.301 g, 0.6 mmol) and 4-(dimethylamino)pyridine (0.0073 g, 0.06 mmol). The mixture was stirred at rt for 3 h and filtered with celite. The filtrate was concentrated, and the residue was purified by FC (DCM/MeOH/NH₄OH = 95/5/0.5) to give 0.161 g clear oil **33** (yield: 40%): ¹HNMR (200 MHz, CDCl₃) δ: 8.19 (d, 2H, $J = 2.2$ Hz), 7.80 (dd, 2H, $J = 2.2$ Hz, $J = 8.6$ Hz), 7.45 (d, 4H, $J = 8.4$ Hz), 7.23 (d, 4H, $J = 8.4$ Hz), 6.97 (s, 4H), 6.79 (dd, 2H, $J = 3.4$ Hz, $J = 8.8$ Hz), 4.50 (t, 4H, $J = 4.8$ Hz), 4.18 (s, 2H), 4.12 (s, 2H), 3.97 (s, 2H), 3.78–3.86 (m, 6H), 3.58–3.70 (m, 28H), 3.44–3.50 (m, 6H), 3.28 (s, 6H), 1.47 (s, 18H).

***N,N*-Bis(2-(((*E*)-2-(2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yloxy)ethoxy)ethoxy)ethoxy)ethoxy)amino)-2-oxoethyl)-2-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)acetamide (34)**—A mixture of **33** (0.100 g, 0.074 mmol) and TBAF (0.15 mL, 1.0 M in THF) in 1 mL THF was stirred at 70 °C overnight. The reaction mixture was evaporated in vacuo, and the residue was purified by FC (DCM/MeOH/NH₄OH = 95/5/0.5) to give 0.045 g clear oil **34** (yield: 47%): ¹HNMR (200 MHz, CDCl₃) δ: 8.17 (d, 2H, $J = 2.2$ Hz), 7.79 (dd, 2H, $J = 2.4$ Hz, $J = 8.6$ Hz), 7.44 (d, 4H, $J = 8.6$ Hz), 7.22 (d, 4H, $J = 8.4$ Hz), 6.96 (s, 4H), 6.78 (dd, 2H, $J = 2.2$ Hz, $J = 8.6$ Hz), 4.67 (t, 1H, $J = 4.1$ Hz), 4.41–4.51 (m, 5H), 4.17 (s, 2H), 4.12 (s, 2H), 3.97 (s, 2H), 3.50–3.88 (m, 34H), 3.43–3.48 (m, 4H), 3.27 (s, 6H), 1.46 (s, 18H).

3-(Prop-2-ynyloxy)-2,2-bis((prop-2-ynyloxy)methyl)propan-1-ol (35)—An aqueous solution of NaOH (40 wt%, 10 mL) was added to a solution of pentaerythritol (2.00 g, 14.7 mmol) in 15 mL dimethylsulfoxide (DMSO). The solution was stirred at rt for 30 min. Propargyl bromide (97%, 9.8 mL, 110 mmol) was then added and the solution was kept at rt for an additional 10 h. The reaction mixture was then poured into 150 mL EtOAc and washed with H₂O (50 mL × 2) as well as brine (50 mL). The organic layer was dried by Na₂SO₄ and filtered. The filtrate was concentrated, and the residue was purified by FC (EtOAc/hexane = 1/4) to give 2.29 g yellowish oil **35** (yield: 62%): ¹HNMR (200 MHz, CDCl₃) δ: 4.13 (d, 6H, $J = 2.4$ Hz), 3.69 (d, 2H, $J = 6.4$ Hz), 3.56 (s, 6H), 2.43 (t, 3H, $J = 2.4$ Hz).

11,11-Bis((prop-2-ynyloxy)methyl)-3,6,9,13-tetraoxahexadec-15-ynyl 4-methylbenzenesulfonate (36)—NaH (60% in mineral oil, 0.120 g, 3.0 mmol) was

placed in a two-neck flask and washed with hexane. 6 mL DMF was added to form a suspension. A solution of **35** (0.500 g, 2.0 mmol) in 4 mL DMF was added dropwise at 0 °C. After stirring at rt for 30 min, the mixture was cooled to 0 °C, and a solution of triethylene glycol di(*p*-toluenesulfonate) (1.38 g, 3.0 mmol) in 3 mL DMF was added dropwise. After stirred at rt overnight, the mixture was poured into 50 mL cold sat. NH₄Cl, and extracted with DCM (30 mL × 2). The organic layer was washed with H₂O (20 mL) and brine (20 mL), dried over Na₂SO₄, concentrated and purified by FC (EtOAc/hexane = 3/7) to give 0.501 g colorless oil **36** (yield: 47%). ¹HNMR (200 MHz, CDCl₃) δ: 7.81 (d, 2H, *J* = 8.4 Hz), 7.35 (d, 2H, *J* = 8.4 Hz), 4.11–4.20 (m, 8H), 3.71 (t, 2H, *J* = 4.8 Hz), 3.58–3.60 (m, 8H), 3.52 (s, 6H), 3.45 (s, 2H), 2.46 (s, 3H), 2.41 (t, 3H, *J* = 2.4 Hz).

1-Fluoro-11,11-bis((prop-2-ynyloxy)methyl)-3,6,9,13-tetraoxahexadec-15-yne (37)—A mixture of **36** (0.200 g, 0.37 mmol) and TBAF (1.12 mL, 1.0 M in THF) in 4 mL THF was stirred at 70 °C overnight. The reaction mixture was evaporated in vacuo, and the residue was purified by FC (EtOAc/hexane = 3/7) to give 0.122 g clear oil **37** (yield: 86%): ¹HNMR (200 MHz, CDCl₃) δ: 4.69 (t, 1H, *J* = 4.2 Hz), 4.46 (t, 1H, *J* = 4.2 Hz), 4.13 (d, 6H, *J* = 2.4 Hz), 3.84 (t, 1H, *J* = 4.2 Hz), 3.67–3.71 (m, 5H), 3.59–3.64 (m, 4H), 3.54 (s, 6H), 3.47 (s, 2H), 2.41 (t, 3H, *J* = 2.4 Hz).

2-(2-(2-(3-(*E*)-(1-(2-(2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yl)oxy)ethoxy)ethoxy)ethoxy)ethyl)-1-*H*-1,2,3-triazol-4-yl)methoxy-2,2-bis((*E*)-(1-(2-(2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yl)oxy)ethoxy)ethoxy)ethoxy)ethyl)-1-*H*-1,2,3-triazol-4-yl)methoxymethyl)propoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (38)—A solution of **36** (0.0313 g, 0.058 mmol), **31** (0.0923 g, 0.175 mmol), sodium ascorbate (0.0174 g, 0.088 mmol) and copper sulfate (CuSO₄, 0.0028 g, 0.0175 mmol) in 2 mL *t*-BuOH/H₂O (v/v, 1/1) was stirred at rt for 4 h. The reaction mixture was then poured into 10 mL EtOAc and washed with H₂O (3 mL × 2) and brine (3 mL). The organic layer was dried by Na₂SO₄ and filtered. The filtrate was concentrated, and the residue was purified by FC (EtOAc/MeOH = 9/1) to give 0.110 g clear oil **38** (yield: 90%): ¹HNMR (200 MHz, CDCl₃) δ: 8.17 (d, 3H, *J* = 2.2 Hz), 7.76–7.82 (m, 5H), 7.71 (s, 3H), 7.45 (d, 6H, *J* = 8.6 Hz), 7.33 (d, 2H, *J* = 8.2 Hz), 7.23 (d, 6H, *J* = 8.6 Hz), 6.97 (s, 6H), 6.77 (d, 3H, *J* = 8.6 Hz), 4.46–4.59 (m, 18H), 4.11–4.16 (m, 2H), 3.83–3.90 (m, 12H), 3.61–3.71 (m, 26H), 3.38–3.56 (m, 16H), 3.28 (s, 9H), 2.43 (s, 3H), 1.47 (s, 27H).

1,3-Di(*E*)-(1-(2-(2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yl)oxy)ethoxy)ethoxy)ethoxy)ethyl)-1-*H*-1,2,3-triazol-4-yl)methoxy-2-(((*E*)-(1-(2-(2-(2-(2-(5-(4-(*tert*-butoxycarbonyl(methyl)amino)styryl)pyridin-2-yl)oxy)ethoxy)ethoxy)ethoxy)ethyl)-1-*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-((2-(2-fluoroethoxy)ethoxy)ethoxy)methyl)propane (39)—Compound **39** was prepared from **37** (0.0486 g, 0.126 mmol), **31** (0.201 g, 0.38 mmol), sodium ascorbate (0.0376 g, 0.19 mmol) and CuSO₄ (0.0061 g, 0.038 mmol) in 4 mL *t*-BuOH/H₂O (v/v, 1/1), with the same procedure described for compound **38**. Compound **39**: 0.208 g (yield: 84%): ¹HNMR (200 MHz, CDCl₃) δ: 8.17 (d, 3H, *J* = 2.4 Hz), 7.79 (dd, 3H, *J* = 2.4 Hz, *J* = 8.6 Hz), 7.71 (s, 3H), 7.45 (d, 6H, *J* = 8.6 Hz), 7.23 (d, 6H, *J* = 8.6 Hz), 6.97 (s, 6H), 6.77 (dd, 3H, *J* = 8.6 Hz), 4.65 (t, 1H, *J* = 4.2 Hz), 4.39–4.60 (m, 19H), 3.77–3.90 (m, 13H), 3.43–3.73 (m, 41H), 3.28 (s, 9H), 1.47 (s, 27H).

2. Radiosynthesis of [^{18}F]8a–c

Preparation of [^{18}F]8a–c was achieved by using a modified method reported previously (see Scheme 5)²⁷. Briefly, the ^{18}F -fluoride ions were trapped on an activated QMA anion exchange cartridge and eluted with 1 mL Kryptofix®222/ K_2CO_3 acetonitrile/water solution (22 mg Kryptofix®222/1.8 mg K_2CO_3 /0.84 mL ACN/0.16 mL water). The solution was azeotropically dried twice with acetonitrile at 110 °C under a stream of argon.

Approximately 1 mg of O-tosylated precursor (24a–c) was dissolved in 1 mL DMSO and added to the anhydrous Kryptofix/ K_2CO_3 /[^{18}F]fluoride. The reaction was heated for 10 minutes at 110 °C and allowed to cool at room temperature for 5 minutes. 1 mL of a 10% (~4 N) HCl solution was added and heated at 80 °C for 7 minutes. The reaction was cooled in an ice bath, neutralized with a NaOH solution and diluted with water to about 8 mL total volume. The mixture was pushed through an activated C4 mini column (Vydac® Bioselect 214SPE3000 Reversed-Phase C4), washed twice with 3 mL water and [^{18}F]8a–c was eluted with 1 mL ethanol (decay corrected radiochemical yields were 29%, 29% and 30%, respectively; radiochemical purity was > 98%). To ensure an accurate detection of free fluoride, other impurities and the desired products, [^{18}F]8a–c, two HPLC systems were employed: (a) Gemini C18 150 × 4.6 mm, acetonitrile/10 mM ammonium formate buffer 8/2 and flow rate of 1 mL/min and (b) Gemini C18 250 × 4.6 mm with a solvent gradient at a flow rate of 1 mL/min according to the following: 0 – 2 minutes 100% 10 mM ammonium formate buffer; 2 – 5 minutes ammonium formate buffer 100% to 30%, ACN 0% to 70%; 5 – 10 minutes ammonium formate buffer 30% to 0%, ACN 70% to 100%, 10 – 15 minutes ammonium formate buffer 0% to 100%, 15 – 18 minutes 100% ammonium formate buffer.

3. In vitro autoradiography of AD brain sections

Frozen brains from confirmed CAA, AD and control subjects were cut into 20 μm sections. The sections were incubated with the ^{18}F labeled tracer in 40% ethanol for 1 hour. The sections were then dipped in saturated Li_2CO_3 in 40% ethanol (2 min wash, twice) and washed with 40% ethanol (2 min wash, once) followed by rinsing with water for 30 seconds. After drying, the sections were exposed to Kodak Biomax MR film for 12 – 18 hours. After the film was developed, the images were digitized.

4. Thioflavin S staining

Brain sections were immersed in 10% neutral buffered formalin for 1 hr, then treated with 0.05% KMnO_4 and bleached in 0.2% $\text{K}_2\text{S}_2\text{O}_5$ /0.2% oxalic acid in order to quench autofluorescence. Quenched tissue sections were stained with 0.025% thioflavin S in 40% ethanol for 3 – 5 min. The sections were differentiated in 50% ethanol and viewed using a Nikon E800 Fluorescence microscope with a CCD digital camera.

5. In vivo biodistribution study in ICR mice

To test [^{18}F]8a–c as PET imaging agents for cerebral amyloid angiopathy, we first tested the biodistribution of these tracers in normal ICR mice (20 – 25 grams). A group of 5 – 6 mice were used for each time point of the biodistribution studies. After the mice were put under anesthesia with isoflurane (2 – 3%), 0.15 mL saline solution containing 925 KBq (25 μCi) of the tracer was injected via the lateral tail vein. The mice were sacrificed at 2 and 30 minutes post-injection by cardiac excision while under isoflurane anesthesia. The organs of interest were removed, weighed and the radioactivity was counted with a gamma counter (Packard Cobra). The percent dose per gram was calculated by a comparison of the tissue activity counts to counts of 1% of the initial dose. The initial dose was measured by aliquots of the injected material diluted 100 times and measured at the same time (0.5 min/sample, 80% efficiency).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

AD	Alzheimer's disease
Aβ	β -amyloid
CAA	cerebral amyloid angiopathy
APP	β -amyloid precursor protein
PET	positron emission tomography
PIB	Pittsburgh compound B
SB-13	4-N-methylamino-4'-hydroxystilbene
IMPY	6-[iodo-2-(4'-N,N-dimethylamino)-phenylimidazo[1,2-a]pyridine
[¹⁸F]AV-1, BAY-94-9172	[¹⁸ F]florbetaben f 18
[¹⁸F]AV-45	[¹⁸ F]florbetapir f 18
[¹⁸F]FPIB, GE067	[¹⁸ F]flutemetamol f 18
[¹⁸F]AZD4694	2-(2-([¹⁸ F]fluoro)-6-methylaminopyridin-3-yl)benzofuran-5-ol

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Figure 1.

The chemical structures and binding affinities of imaging agents targeting A β plaques in the brain are listed. All of these agents are small, neutral molecules showing high binding affinity and the ability to penetrate intact blood-brain barrier *in vivo* (previously published values ^{*22, **34}).

Figure 2.

Multi-dentate (bidentate and tridentate) ligands, **8a**, **8b**, **8c**, **9** and **10**, based on styrylpyridine cores aiming at multiple binding sites within the repeated β -sheet structure are shown. These novel ligands are designed to bind to the A β aggregates via a divalent or a trivalent attachment (binding) sites.

Figure 3. HPLC profiles of [^{18}F]**8a** and co-injected cold standard **8a** on a reversed phase Gemini C18 column (250×4.6 mm) with the following gradient and a flow rate of 1 mL/min: 0 – 2 minutes 100% ammonium formate buffer (10 mM); 2 – 5 minutes ammonium formate buffer 100% - 30%, ACN 0% – 70%; 5 – 10 minutes ammonium formate buffer 30% - 0%, ACN 70% – 100%; 10 – 15 minutes 0% – 100% ammonium formate buffer, 100% - 0% ACN; 15 – 18 minutes 100% ammonium formate buffer.

Figure 4.

In the upper panel the autoradiography of postmortem human brain sections of occipital samples from cerebral amyloid angiopathy (CAA) using the new bivalent ligand, $[^{18}\text{F}]\mathbf{8a}$, showed a highly distinctive labeling at the blood vessels. Other brain parenchymal regions displayed very little labeling, which suggests that there was a selective accumulation of A β plaques in the vessel walls in this CAA patient. The lower panel shows brain sections of AD and healthy control (HC) subjects using either $[^{18}\text{F}]\text{AV-45}$ ($[^{18}\text{F}]\mathbf{5}$) or $[^{18}\text{F}]\mathbf{8a}$. The AD brain section showed highly significant labeling of A β plaques, while both tracers showed little or no binding in the HC sections.

Figure 5.

Autoradiography of postmortem human brain samples of cerebral amyloid angiopathy (CAA) using [^{18}F]**8a** (A and B), which displayed excellent labeling of A β aggregates of the blood vessel walls. There was prominent labeling of major blood vessels only in the CAA brain sections. It is apparent that the parenchymal brain tissue had no A β plaques, therefore no labeling was observed. When the neighboring sections were labeled with thioflavin S (C and D), the fluorescent images displayed a matching staining of blood vessel walls comparable to the images from the autoradiography of [^{18}F]**8a**. The results strongly suggest that [^{18}F]**8a** labeled the A β aggregates of the blood vessel walls. The white bars represent a scale of 500 μm .

Scheme 1.

Reagent and conditions: (a) (Boc)₂O, H₂O, 35 °C; (b) NaH, CH₃I, DMF, rt; (c) triethylene glycol, CsCO₃, DMF, 150 °C; (d) K₂CO₃, Bu₄NBr, Pd(OAc)₂, DMF, 60 °C; (e) TsCl, Et₃N, DMAP, DCM, rt; (f) diethylene glycol ditosylate, NaH, DMF, rt; (g) DHP, PPTS, EtOH, rt; (h) K₂CO₃·Bu₄NI, Bu₄NOAc, ACN; (i) NH₃, CH₃OH.

Scheme 2.

Reagent and conditions: (a) NaH, 2 eq. **15**, DMF, rt; (b) PPTS, EtOH; (c) NaH, 2-(2-bromoethoxy)-tetrahydro-2H-pyran, DMF, rt; (d) TsCl, Et₃N, DMAP, DCM, rt; (e) TBAF, THP, 70°C; (f) TFA, rt.

Scheme 3.

Reagent and conditions: (a) $\text{N}_2\text{CHCO}_2\text{C}_2\text{H}_5$, BF_3 , Et_2O , DCM, 0 °C, (b) NBS PPh_3 , DCM, 0 °C, (c) NaOH, THF, rt; (d) HOBt, EDCI, DIPEA, ditert-butyl iminodiacetate, DMF rt; (e) TFA, rt; (f) NaN_3 , DMF, 60 °C; (g) PPh_3 , H_2O , THF, 60 °C; (h) DCC, DMAP, DCM, rt; (i) TBAF, THF, 70 °C.

Scheme 4.

Reagent and conditions: (a) 40% NaOH, DMSO, rt; (b) NaH, tri ethylene glycol di(*p*-toluenesulfonate), DMF, rt; (c) TBAF, THF, 70 °C; (e) sodium ascorbate, CuSO₄, *t*-BuOH, H₂O, rt; (f) TFA, rt.

Scheme 5. Reagent and conditions: (a) $\text{Na}[^{18}\text{F}]\text{fluoride}$, $\text{K}[2,2,2]/\text{K}_2\text{CO}_3$; (b) 6N HCl

Table 1

Inhibition constants (K_i , nM) of A β plaques targeting ligands against binding of [^{18}F]**5** to A β plaques in postmortem AD brain homogenates.

Compound Name	K_i (nM)	Compound Name	K_i (nM)
PIB, 1	0.87 ± 0.18	8a (n = 4)	$3.24 \pm .92$
SB-13, 2	3.18 ± 1.04	8b (n = 5)	7.71 ± 3.56
IMPY, 3	1.29 ± 0.46	8c (n = 6)	3.86 ± 1.05
florbetaben (AV-1), 4	2.22 ± 0.54	25a	> 1,000
florbetapir (AV-45), 5	2.87 ± 0.17	9 (n = 3)	71.2 ± 16.8
flutemetamol (3-FPIB), 6	0.74 ± 0.38	10	468

*22

Table 2

Comparison of the biodistributions of [^{18}F]8a, [^{18}F]8b, [^{18}F]8c and [^{18}F] 5 in normal mice (% dose/gram).

Organ	[^{18}F]8a		[^{18}F]5	
	2 min n = 6	30 min n = 6	2 min* n = 3	30 min* n = 3
Blood	4.77 $\pm$ 1.25	1.54 $\pm$ 0.12	3.14 $\pm$ 0.69	2.80 $\pm$ 0.44
Muscle	0.82 $\pm$ 0.16	1.21 $\pm$ 0.15	1.06 $\pm$ 0.39	1.78 $\pm$ 0.34
Kidney	8.13 $\pm$ 0.73	9.36 $\pm$ 0.42	10.9 $\pm$ 2.63	6.31 $\pm$ 0.58
Liver	21.2 $\pm$ 1.58	21.4 $\pm$ 2.24	21.5 $\pm$ 1.07	12.9 $\pm$ 0.72
Brain	0.40 $\pm$ 0.03	0.40 $\pm$ 0.05	7.77 $\pm$ 1.70	1.59 $\pm$ 0.22
Bone	1.30 $\pm$ 0.23	2.34 $\pm$ 0.80	1.43 $\pm$ 0.09	1.22 $\pm$ 0.17
Organ	[^{18}F]8b		[^{18}F]8c	
	2 min n = 6	30 min n = 6	2 min n = 6	30 min n = 6
Blood	2.55 $\pm$ 0.21	1.32 $\pm$ 0.04	3.12 $\pm$ 0.17	1.40 $\pm$ 0.10
Muscle	1.30 $\pm$ 0.70	1.62 $\pm$ 0.39	0.65 $\pm$ 0.22	1.29 $\pm$ 0.12
Kidney	14.9 $\pm$ 0.86	13.4 $\pm$ 1.84	13.7 $\pm$ 2.15	11.7 $\pm$ 0.57
Liver	33.5 $\pm$ 6.60	20.0 $\pm$ 6.48	30.2 $\pm$ 3.47	16.0 $\pm$ 1.91
Brain	0.40 $\pm$ 0.10	0.34 $\pm$ 0.07	0.29 $\pm$ 0.06	0.38 $\pm$ 0.04
Bone	1.64 $\pm$ 0.44	1.80 $\pm$ 0.57	1.06 $\pm$ 0.11	1.36 $\pm$ 0.12

About 925 KBq of each ligand in a saline solution was injected. Mice were sacrificed at 2 and 30 minutes post injection²².