Syntheses of Two Potential Ligands for Tc-99m Labeling as Diagnosis Agents of Alzheimer's Disease†

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Two types of ligands-biphenyl and stilbene derivatives, which can be labeled with Tc-99m for the diagnosis of Alzheimer's disease (AD) have been synthesized successfully. The key steps in these two syntheses involved Suzuki reaction and Wittig reaction respectively. The new discovered debromination reaction may be expanded to the compounds with double or triple bond adjacent to the carbon atom bearing the bromine atoms. These types of syntheses provide a route to a series of biphenyl and stilbene derivatives that will benefit the search of new imaging agents for AD.

Keywords Alzheimer's disease, imaging agent, diagnosis, biphenyl, stilbene, Suzuki reaction, Wittig reaction

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease of the brain characterized by dementia, cognitive impairment and memory loss. It occurs commonly in aged people. Up to date, there is no effective methods for either diagnosis or therapeutic treatment. The longer the human life is, the more critical and urgent is to develop therapies and diagnostic tools for AD. Formation and accumulation of aggregates of β-amyloid (Aβ) peptides in the brain are the critical factors in the development and progression of AD. The fibrillar aggregates of amyloid peptides, A$\beta_{1\text{-}40}$ and A$\beta_{1\text{-}42}$ are found in senile plaques and cerebrovascular amyloid depositsin AD patients.[1] Various approches in trying to inhibit the production and reduce the extent of accumulation of fibrillar Aβ in the brain are currently being evaluated as potential therapies.[2-9] Early appraisal of clinical symptoms for diagnosis of AD is often difficult and unreliable. There is a need for *in vivo* imaging agents, which can specifically demonstrate the location and density of amyloid plaques in brain and will be useful for early detection or monitoring the progression and effectiveness for treatment of AD.[10]

Currently, there are two types of imaging modalities: one is Single Photon Emission Computed Tomography (SPECT) which uses the agents labeled with ^{99m}Tc ($T_{1/2}$, 6 h; 140 keV) or ^{123}I ($T_{1/2}$, 13 h; 159 keV), the other one is Positron Emission Tomography (PET) which uses agents labeled with ^{11}C ($T_{1/2}$, 20 min; 511 keV) or ^{18}F ($T_{1/2}$, 110 min, 511 keV). SPECT is more economic and PET needs more expensive cyclotron but images are usually much more clear. For good central nerve system (CNS) imaging agents, they must have good binding affinities for the target sites and they should readily pass through the blood-brain barrier (BBB).

Many attempts on developing imaging agents for amyloid-plaques based on Chrysamine-G(CG) (**1**) or Congo Red (**2**) were not successful.[11-20] Recently a new PET ligand, [^{18}F]FDDNP (**3**), for binding to tangles and plaques has been reported.[21,22] Using an *in vitro* fluorescent titration method, the binding affinity of FDDNP to Aβ aggregates was determined to be 0.4 nmol·L^{-1}. Preliminary studies in humans appear to suggest that [^{18}F]FDDNP showed a higher retention in regions of the brain suspected of having tangles and plaques and the PET images were consistent with autoradiography and staining of postmortem brain samples.[23] A neutral thioflavin (**4**) (benzothiazole) derivative, [^{3}H]BTA-1(**5**), was also reported[24-26] showing an excellent affinity (K_d = 3 nmol·L^{-1}) *in vitro* binding assay using A$\beta_{1\text{-}40}$ aggregates. When [^{11}H]BTA-1 was injected intravenously into mice, it showed an excellent brain penetration with an initial brain uptake at 2 min of 3% dose/organ. The C-11 labeled derivative, 6-OH-BTA-1, was shown to label the plaque successfully in AD patients recently.[26b] Two types of iodinated probes, styryl-

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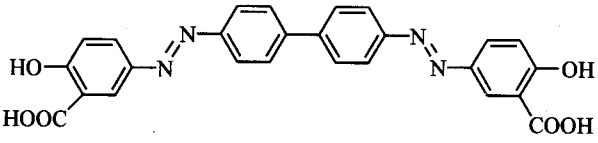
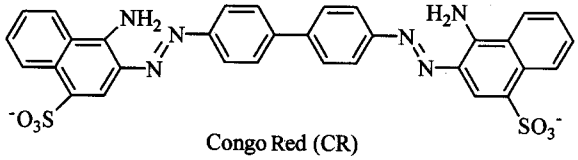
† Dedicated to Professor ZHOU Wei-Shan on the occasion of his 80th birthday.



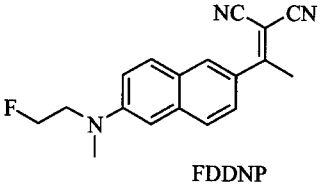
Chrysamine G (CG)

1



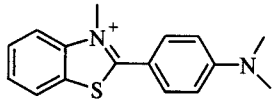
Congo Red (CR)

2



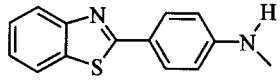
FDDNP

3



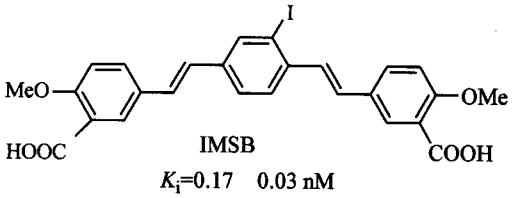
Thioflavin T

4



BTA-1

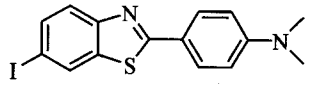
5



IMSB

K_i=0.17 0.03 nM

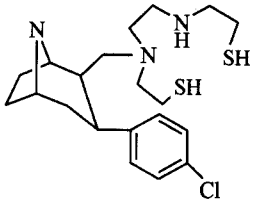
6



TZDM

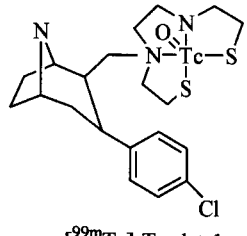
K_i=202 0.4 nM

7



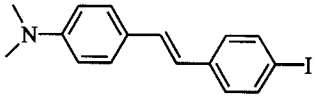
Trodat-1

8



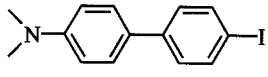
[^{99m}Tc]-Trodat-1

9



K_i = 2.0, 0.4 nM

10



K_i = 17.3 nM

11

benzene (IMSB) (**6**) and thioflavin derivative (TZDM) (**7**), were reported from our laboratory for binding to Aβ aggregates.[27,28] Excellent binding affinities (in nanomolar range) were observed for these iodinated ligands. Autoradiography studies using tissue sections from postmortem brains of AD patients demonstrated distinct binding of these probes to amyloid plaques.

Among the two isotopes used in SPECT mentioned above: ^{99m}Tc is more convenient (can be generated in the lab), less costly and much more safer than ^{123}I. Now Tc imaging agents have attracted more attention of radiopharmaceutical community. However there are also big challenges in the search of Tc ligands. It should add more functional groups such as N and S to the molecule in order to chelate with Tc atom. Thus the molecule will be bigger and heavier than the parent compound. Not like the iodine tracer, the biological properties on te Tc agents may be different from parent compounds. Will it maintain the aginity after adding Tc complex to the molecule? Will it go through BBB? All need to be answered in the process. Under the encouragement of successful development of the first ^{99m}Tc-labeled imaging agent ([^{99m}Tc]-Trodat-1) (**9**)

for Parkinson's disease in our group.[29] It is with great interest to continue to search the ^{99m}Tc-labeled imaging agents for Alzheimer's disease.

We recently found that stilbene[30] and biphenyl derivatives[31] such as **10** and **11** showed good binding affinities toward Aβ aggregates with the K_i values of 2.0 and 17.3 nmol·L^{-1} respectively. Based on these observations we have designed and synthesized two potential ligands for ^{99m}Tc labeling and the results are reported herein.

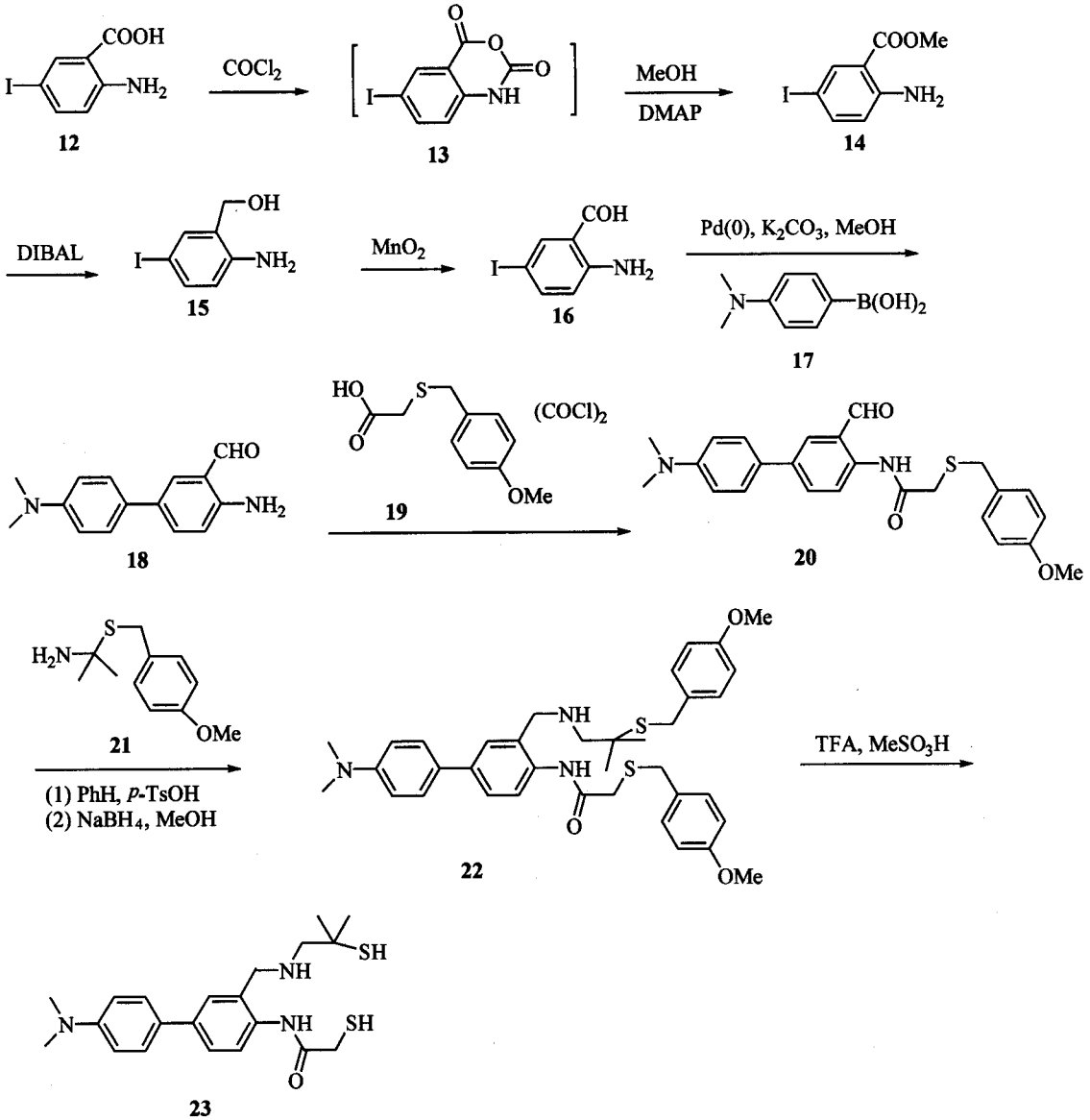
Results and discussion

Tc ligands normally contain two nitrogen and two sulfur atoms (N_2S_2) in the molecule such as Trodat-1 (**8**), so that it can chelate with Tc atom to form a complex. Usually adding gem dimethyl to the N_2S_2 moiety or making anilino amide increases the stability of the Tc complex. We designed two target compounds: **23** (with gem-dimethyl and carbonyl on N_2S_2 moiety) and **37** (with no gem-dimethyl and carbonyl on N_2S_2 moiety).

Synthesis of biphenyl ligand **23**

The synthesis of biphenyl ligand **23** with gem-dimethyl on one arm and amide on the other arm is shown in Scheme 1. There are one anilino amide moiety and one secondary amine in this molecule. As we know, amine could be obtained from amide by reduction. Could we selectively reduce the benzamide in the presence of anilino amide? In model studies (Scheme 2) for the selective reduction of diamide, the reductive products turned out to be monoamine-benzamide [from LAH ($LiAlH_4$) reduction] and diamine (from BH_3 reduction). Anilino amide did not survive in both cases. This selectivity is in contradiction to

Scheme 1



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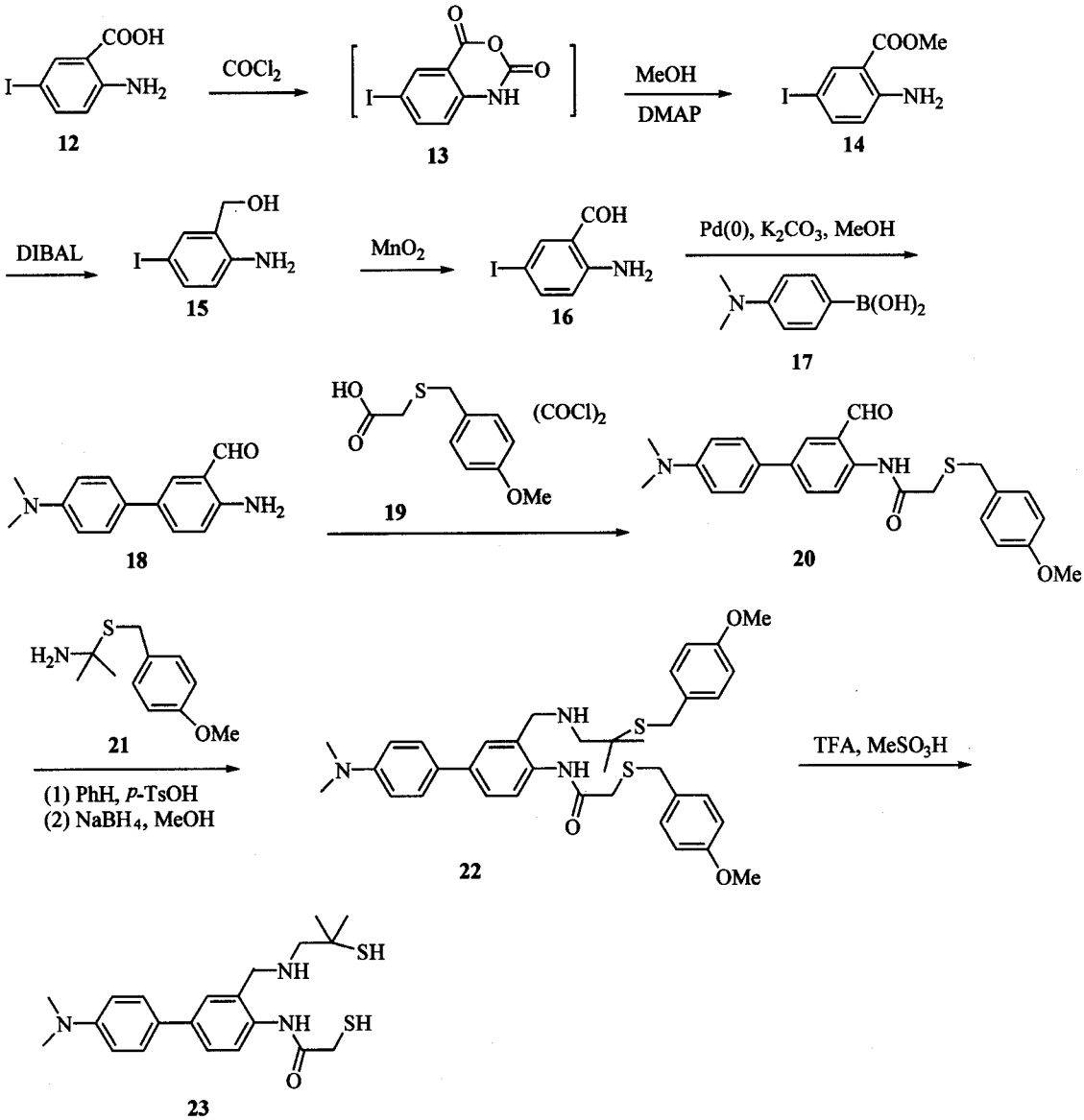
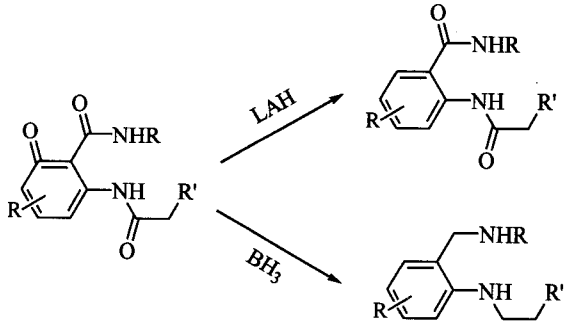


Scheme 2



what we are looking for. To avoid this unwanted selectivity, using any reducing agent which reduces the amide to amine should be prohibited or the anilino amide should be formed after benzylamine was formed. In the later case, the benzylamine group should be protected because it was more active than the amino group of aniline. Thus amino benzaldehyde **16** was designed and synthesized, in which the aldehyde moiety could be converted to secondary amine directly without going through benzamide stage. We started from 2-amino-5-iodobenzoic acid (**12**). Direct methylation of **12** by a common method, *i. e.* refluxing the acid in MeOH in the presence of sulfuric acid and even refluxing for a longer period (2 d) gave the ester in poor yield. According to the method reported by Vanut *et al.*[32] the acid firstly reacted with phosgene in dioxane to form the isatoic anhydride **13** as a solid intermediate followed by treating this intermediate with MeOH in DMF in the presence of DMAP. The desired amino ester was obtained in very good yield. Reduction of ester to alcohol by DIBAL followed by oxidation of the resulting alcohol with activated MnO_2 afforded aldehyde **16**. Suzuki reaction using this aldehyde and commercial available Suzuki reagent, 4-dimethylaminophenylboric acid (**17**), in MeOH in the presence of Pd(0) and potassium carbonate gave coupling compound **18**. Amidation of Suzuki reaction product **18** with the acyl chloride (obtained from 4-methoxybenzyl protected 2-thioacetic acid **19**[33] and oxalyl chloride) in methylene chloride in the presence of triethyl amine gave the anilino amide **20**. In this reaction, acyl chloride should be added to the solution containing the amine and Et_3N, otherwise much lower yield would be obtained. Reductive amination comprised stepwise reactions: (1) refluxing the aldehyde **20** with amine **21**[34] in the presence of *p*-TsOH afforded imine and (2) redution of imine by $NaBH_4$ in MeOH gave monoamide **22**. Finally deprotection of **22** with TFA-$MeSO_3H$ in the presence of anisol gave desired target compound **23**.

Synthesis of stilbene ligand **37**

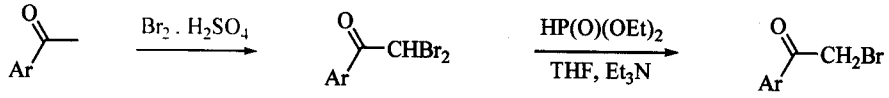
Wittig reaction is the most convenient and commonly used method to establish the stilbene skeleton. Scheme 3 shows the synthesis of stilbene type ligand **37**. First part is to make the Wittig reagent **27**: 3-nitro-4-bromo toluene **24** underwent bromination by NBS with benzoyl peroxide as the initiating agent in CCl_4. This reaction gave a mixture of dibromide **25** and monobromide **26** in 1∶1 ratio shown by NMR. The pure desired monobromide could be obtained after column separation, but it lost half of the material. Although there was no method found in the literature to convert the benzyl dibromide to monobromide, we noticed that Diwu *et al.*[35] reported that 2, 2-dibromo acetophenone can be converted to 2-monobromo acetophenone by using diethylphosphite in the presence of triethylamine (Scheme 4). Because the electronic effect of the double bond was similar to that of the carbonyl group, could we convert the dibromide adjacent to a double bond rather than a carbonyl group to monobromide? We applied this method to convert compound **25** to monobromide **26** successfully and finally **26** was obtained without separation of di- and monobromide after bromination. The total yield for 2 steps was 61% after final purification. This monobromide was heated with triethylphosphite to afford the Wittig reagent **27**. This reagent would give exclusive *E* isomer while triphenylphosphonim salt gave the mixture of *E* and *Z* isomers.[36]

Several conditions were tried for the Wittig reaction including NaOMe-MeOH, BuLi-THF and KO*t*Bu-DMF. The KO*t*Bu-DMF system gave the best result. It is worth mentioning that when the Wittig reagent, 4-*N*, *N*-dimethylaminobenzyl, diethylphosphonate, which was obtained from 4-*N*, *N*-dimethylaminobenzylbromide, reacted with 3-nitro-4-bromobanzaldehyde (Scheme 5), no desired product was obtained, but only the Wittig reagent was recovered. This result showed the nitro group on the benzene ring could change the activity of aldehyde dramatically owing to its strong electron withdrawing effect! Coupling of bromide **29** with 4-methoxybenzylthiol **30** using K_2CO_3 as base in DMF gave thioether **31**. Nitro group was converted to amino group by $SnCl_2$ to afford amine **32**. Because the aromatic amine is not as active as alkyl amine, chloro acetyl chloride was used to make the amide **33** at first. Coupling **33** with 2-(4-methoxybenzyl)thioethylamine **34** to afford the amide **35**. This anilio amide was easily reduced by LAH to afford **36**. The same procedure described above for synthesis of stilbene ligand was used to deprotect the sulfur protecting groups to give stilbene ligand **37**. These two ligands will be used to complex with ^{99m}Tc for biological testing. This research is underway.

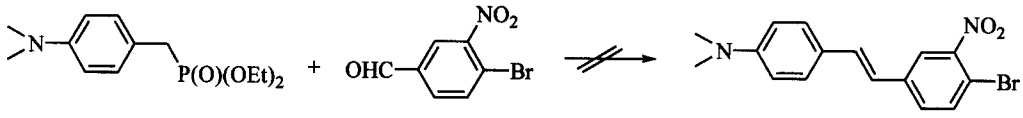
Summary

Two types of Tc-labeled imaging agent ligands: biphenyl and stilbene were synthesized for the future test. The key steps in these two syntheses involved Suzuki reaction and Wittig reaction respectively. Based on these two reactions, a series of ligands of theses two types of compounds could be synthesized. The activities of benzamide and anilino amide were different and the benzamide was more difficult to be reduced to amine by LAH and both

Scheme 4



Scheme 5



plates with a fluorescent indicator that was visualized with light at 254 nm. Flash 40 column was obtained from Biotage, Inc. ^{1}H NMR spectra were obtained on Bruker spectrometers (Bruker DPX 200). Chemical shifts are reported as δ values and referenced to $CDCl_3$ (δ 7.26 for ^{1}H). Coupling constants are reported in Hz. Mass spectrometry was performed by the Mass Spectrometry Center, University of Pennsylvania. Elemental analysis was performed by Elemental Analysis Center, University of Pennsylvania.

Methyl 2-amino-5-iodobenzoate (**14**)

To a solution of 2-amino-5-iodobenzoic acid (**12**) (5 g, 19 mmol) in 1,4-dioxane (25 mL) was added a solution of phosgene in toluene (20%, 28.5 mL, 57 mmol) dropwise at r.t. The resulting mixture was stired at 60 ℃ overnight. The precipitate (intermediate **13**) was collected by suction, and washed with dioxane. The solid was dissolved in DMF (25 mL), to which MeOH (2 mL) and DMAP (200 mg) were added successively. The mixture was stirred at 60 ℃ for 3 h. Solvent was removed and EtOAc (EA) was added. The solution was washed with brine, dried over Na_2SO_4, filtered and concentrated to give crude product which was purified by Flash 40 column (Hex:EA = 9:1 *V*:*V*) affording 4.52 g of pure product (85.8%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 3.86 (s, 3H), 5.76 (br, 2H), 6.45 (d, J = 8.7 Hz, 1H), 7.47 (dd, J = 8.7, 2.2 Hz, 1H), 8.13 (d, J = 2.2 Hz, 1H).

2-Amino-5-iodobenzylalcohol (**15**)

To a solution of **14** (100 mg, 0.36 mmol) in THF (3 mL) was added a solution of DIBAL (1.1 mL, 1 mol/L in hexane) dropwise at −30 ℃. The mixture was allowed to warm to r.t. and stirred for 3 h. MeOH was added to destroy the excess of DIBAL. EtOAc was added and the mixture was filtered. The filtrate was concentrated to give crude product which was purified by PTLC (CH_2Cl_2 : MeOH = 19:1 *V*:*V* as developing solvent) affording 41 mg of product (46%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 4.53 (s, 2H), 6.38 (d, J = 8.7 Hz, 1H), 7.29 (s, 1H), 7.32 (d, J = 8.7 Hz, 1H).

2-Amino-5-iodobenzaldehyde (**16**)

To a solution of **15** (770 mg, 3.1 mmol) in CH_2Cl_2 (50 mL) was added MnO_2 (860 mg, activated) and the resulting mixture was stirred at r.t. overnight. The solid was filtered and the filtration was concentrated and purified by column chromatography (Hex:EA = 9:1 *V*:*V*) to give 638 mg of product (83.2%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 6.08 (br, 2H), 6.39 (d, J = 8.7 Hz, 1H), 7.44 (dd, J = 8.7, 2.2 Hz, 1H), 7.68 (d, J = 2.2 Hz, 1 H), 9.71 (s, 1H).

4-Dimethylamino-3′-formyl-4′-amino-biphenyl (**18**)

A mixture of **16** (494 mg, 2 mmol), boric acid **17** (330 mg, 2 mmol), $Pd(Ph_3P)_4$ (960 mg, 0.8 mmol) and K_2CO_3 (552 mg, 4 mmol) in MeOH (20 mL) was stirred at 60 ℃ overnight. Water was added and the mixture was extracted with EA. The organic layer was dried, filtered and concentrated to give crude product which was purified by Flash 40 column (Hex:EA = 95:5 *V*:*V*) to give 384 mg of pure product (80%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 2.98 (s, 6H), 6.07 (br, 2H), 6.71 (d, J = 8.5 Hz, 1H), 6.81 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 8.5, 2H), 7.55 (dd, J = 8.5, 1.8 Hz, 1H), 7.65 (d, J = 1.8 Hz, 1H), 9.94 (s, 1H); IR (film) ν: 3467, 2884, 2761, 1660, 1587, 1482, 1172, 815, 711 cm^{-1}. Anal. cacld for $C_{15}H_{16}N_2O$: C 74.97, H 6.44, N 11.66; found C 74.62, H 6.50, N 11.51.

4-Dimethylamino-3′-formyl-4′-[2″-(p-methoxybenzylmercapto)-acetyl-]amino-biphenyl (**20**)

To a solution of acid **19** (106 mg, 0.5 mmol) in CH_2Cl_2 (5 mL) was added a solution of oxalyl chloride (2 mol/L in CH_2Cl_2) dropwise at r.t. followed by DMF (2 drops). The mixture was stirred at r.t. for 1 h. Solvent was removed. CH_2Cl_2 (5 mL) was added to the residue. The resulting solution was added to a solution of amine **18** (100 mg, 0.42 mmol) and Et_3N in CH_2Cl_2 (5 mL) dropwise and stirred for 2 h. Ice water was added. The organic layer was separated. The aqueous phase was extracted with CH_2Cl_2. The combined organic layers were dried, filtered and concentrated to give crude product which was purified

by PTLC (Hex:EA = 4:1 *V*:*V* as developing solvent) affording 47 mg of starting material amine **18** and 87 mg of product (90.1% based on consumed starting material). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 3.01 (s, 6H), 3.31 (s, 2H), 3.70 (s, 3H), 3.80 (s, 2H), 6.75 (d, J = 8.6 Hz, 2H), 6.78 (d, J = 8.6 Hz, 2H), 7.25 (d, J = 8.6 Hz, 2H), 7.51 (d, J = 8.6 Hz, 2H), 7.79 (dd, J = 8.6, 2.2 Hz, 1 H), 8.83 (d, J = 2.2 Hz, 1H), 8.72 (d, J = 8.6 Hz, 1H), 9.97 (s, 1H), 11.6 (s, 1H); IR (film) ν : 3301, 2892, 1669, 1612, 1509, 1243, 1177, 817 cm^{-1}; MS *m/z*: 435 (M^+ + 1). Anal. cacld for $C_{25}H_{26}N_2O_3S$: C 69.10, H 6.03, N 6.45; found C 69.46, H 5.66, N 6.86.

4-Dimethylamino-3'-[2''-(p-methoxybenzylmercapto-)-2'', 2''-gem-dimethyl-ethylamino-]methyl- 4'-[2''-(p-methoxybenzylmercapto-) acetyl-] amino-biphenyl (**22**)

A solution of **20** (50 mg, 0.12 mmol), amine **21** (60 mg, 0.24 mmol) and TsOH (30 mg) in benzene (5 mL) was refluxed with a Sean-Stark for 1 h. Solvent was removed. MeOH (10 mL) was added and the mixture was cooled to 0 ℃ in an ice water bath. $NaBH_4$ (50 mg) was added and the resulting mixture was stirred at r.t. for 1 h. Solvent was removed. Water was added and the mixture was made acidic with HCl (10%). The mixture was extracted with ether. The aqueous phase was made basic with concentrated NH_4OH and extracted with CH_2Cl_2. The combined organic layers were dried, filtered and concentrated to give crude product which was purified by PTLC (He:EA = 2:1 *V*:*V* as developing solvent) to give **28** mg of product (37.8%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.32 (s, 6H), 2.48 (s, 2H), 3.00 (s, 6H), 3.19 (s, 2H), 3.63 (s, 2H), 3.65 (s, 2H), 3.76 (s, 3H), 3.77 (s, 3H) 3.78 (s, 2H), 6.78—6.86 (m, 6H), 7.21—7.28 (m, 5H), 7.49 (d, J = 8.6 Hz, 3H), 8.18 (d, J = 8.6 Hz, 1H), 10.87 (s, 1H); IR (film) ν: 3300, 2956, 2832, 1681, 1606, 1515, 1249, 1031, 944, 613, 738 cm^{-1}. MS *m/z*: 644 (M^+ + 1). HRMS calcd for $C_{37}H_{45}N_3O_3S_2$ 643.9036, found 643.9034.

4-Dimethylamino-3'-(2''-mercapto-2'',2''-gem-dimethyl-ethylamino-) methyl-4'-(2''-mercapto-acetyl-) amino-biphenyl (**23**)

To a solution of **22** (28 mg, 0.04 mmol) and anisole (1 drop) in TFA (1 mL) was added $MeSO_3H$ (0.5 mL) dropwise at 0 ℃ in an ice-water bath. The mixture was stirred at r.t. for 1 h. Ice water was added and the mixture was extracted with ether. Aqueous phase was made basic with concentrated NH_4OH. The resulting mixture was extracted with mixed solvent (CH_2C_{l2}-MeOH = 9:1 *V*:*V*). The organic phase were dried, filtered, and concentrated to give crude pruduct which was purified by PTLC (CH_2Cl_2:MeOH = 9:1 *V*:*V* as developing solvent) affording 15 mg of product (85.7%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.40 (s, 6H), 2.67 (s, 2H), 2.99 (s, 6H), 3.40 (s, 2H), 3.96 (s, 2H), 8.79 (d, J = 8.6 Hz, 2H), 7.35 (s, 1H), 7.48 (d, J = 8.6 Hz, 3H), 8.17 (d, J = 8.5 Hz, 1H), 10.78 (s, 1H); IR (film) ν: 3387, 2954, 2849, 2792, 1677, 1606, 1509, 1359, 817, 736 cm^{-1}. Anal. cacld for $C_{21}H_{29}N_3OS_2 \cdot 3/4H_2O$: C 60.47, H 7.37, N 10.07; found C 60.39, H 7.54, N 9.87.

3-Nitro-4-bromo-benzylbromide (**26**)

To a solution of 3-nitro-4-bromo-toluene **24** (10.8 g, 50 mmol) in CCl_4 (100 mL) was added NBS (9.8 g, 55 mmol) followed by benzoyl peroxide (200 mg). The mixture was stirred under reflux overnight. The solvent was removed to give an oil which was a mixture of dibromide **25** and monobromide **26** shown by NMR in 1 : 1 ratio. This mixture was dissolved in THF (30 mL) without purification. A solution of diethylphosphite (2.7 mL) and Et_3N in THF (15 mL) was added dropwise at 0 ℃. The mixture was stirred at r.t. for 3 h. Solvent was removed and the residue was poured into ice water. The mixture was extracted with CH_2Cl_2. The organic phase was dried, filtered, concentrated to give crude product which was purified by Flash 40 column (Hex:EA = 95:5 *V*:*V*) affording 9.1 g of product (61.7% in 2 steps). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 4.45 (s, 2H), 7.46 (dd, J = 8.3, 2.1 Hz, 1H), 7.72 (d, J = 8.3 Hz, 1H), 7.87 (d, J = 2.1 Hz, 1H); IR (film) ν: 1602, 1538, 1477, 1355, 1334, 1224, 1031, 803 cm^{-1}. Anal. cacld for $C_7H_5Br_2NO_2$: C 28.51, H 1.71, N 4.75; found C 28.67, H 1.60, N 4.41.

Diethyl ,3-nitro-4-bromo-benzylphosphonate (**27**)

A mixture of bromide **26** (7.0 g, 23.7 mmol) and triethylphosphite (3.94 g, 23.7 mmol) was stirred at 160 ℃ for 4 h. The mixture was cooled to r.t. and purified by Flash 40 column (CH_2Cl_2 : MeOH = 97 : 3 *V* : *V*) to give 5.3 g of Wittig reagent (63.5%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.28 (t, J = 7.1 Hz, 6H), 2.15 (d, J = 21.8 Hz, 2H), 4.07 (pen, J = 7.4 Hz, 4H), 7.38 (td, J = 8.3, 2.2 Hz, 1H), 7.67 (d, J = 8.3 Hz, 1H), 7.77 (d, J = 2.2 Hz, 1H).

(*E*)*-4-Dimethylamino-3'-nitro-4'-bromo-stilbene* (**29**)

To a mixture of Wittig reagent **27** (2.9 g, 8.2 mmol) and 4-dimethylamino benzaldehyde **28** (1.23 g, 1 eq) in DMF (15 mL) was added solid KO*t*Bu (1.28 g, 1.5 eq.) in portions at r.t.. The mixture was stirred at r.t. overnight. Water was added and the mixture was filtered, washed with water and MeOH to give 1.94 g of product (68%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 3.01 (s, 6H), 6.70 (dt, J = 8.9, 2.5 Hz, 2H), 6.80 (d, J = 16.2 Hz, 1H), 7.11 (d, J = 16.2 Hz, 1H), 7.41 (dt, J = 8.9, 2.5 Hz, 2H), 7.46 (dd, J = 8.9, 2.1 Hz, 1H), 7.63 (d, J = 8.5 Hz, 1H), 7.89 (d, J = 2.1 Hz, 1H);

IR (film) ν: 3413, 1608, 1526, 1357, 1187, 815 cm^{-1}. Anal. cacld for $C_{16}H_{15}BrN_2O_2$: C 55.35, H 4.35, N 8.07; found C 55.22, H 4.57, N 8.30.

(E)-4-Dimethylamino-3'-nitro-4'-(p-methoxy-)benzylmercapto-stilbene (**31**)

A mixture of **29** (790 mg, 2.3 mmol), 4-methoxy-α-toluenethiol **30** (350 mg, 2.3 mmol) and K_2CO_3 (1.57 g, 11.5 mmol) in DMF (10 mL) was stirred at 90 ℃ overnight. The mixture was poured into ice water after cooling. The precipitate was collected by suction. The crude product was treated with the mixed solvent (EA:Hex = 1:2 $V:V$) and filtered, washed with the same solvent, dried to give 850 mg of pure product (90%). ^1H NMR ($CDCl_3$, 200 MHz) δ: 3.00 (s, 6H), 3.80 (s, 3H), 4.17 s, 2H), 6.71 (d, J = 8.9Hz, 2H), 6.83 (d, J = 16.2 Hz, 1H), 6.87 (d, J = 8.6 Hz, 2H), 7.10 (d, J = 16.2 Hz, 1H), 7.33 (d, J = 8.6 Hz, 2H), 7.38 (d, J = 8.6 Hz, 1H), 7.40 (d, J = 8.9 Hz, 2H), 7.59 (dd, J = 8.6, 2.0 Hz, 1H), 8.26 (d, J = 2.1 Hz, 1H); IR (film) ν: 2923, 1610, 1515, 1332, 1251, 1112, 962, 811 cm^{-1}. HRMS calcd for $C_{24}H_{24}N_2O_3S$ 420.1508, found 420.1501.

(E)-4-Dimethylamino-3'-amino-4'-(p-methoxy-)benzylmercapto-stilbene (**32**)

A mixture of **31** (240 mg, 0.57 mmol) and $SnCl_2$ (542 mg, 2.85 mmol) in mixed solvent (60 mL, EA:EtOH = 2:1 $V:V$) was stirred under reflux for 4 h. Ice water was added after the mixture was cooled down to r.t.. The resulting mixture was made basic with NaOH solution (40%) and extracted with ethyl acetate. The organic layer was dried, filtered and concentrated to give 202 mg of product which was clean enough shown by NMR to run the next reaction without further purification. ^1H NMR ($CDCl_3$, 200 MHz) δ: 2.99 (s, 6H), 3.79 (s, 3H), 3.87 (s, 2H), 6.71 (d, J = 8.8 Hz, 2H), 6.78 (d, J = 8.6 Hz, 2H), 6.79—6.89 (m, 3H), 7.02 (d, J = 16.2 Hz, 1H), 7.09 (d, J = 8.6 Hz, 2H), 7.17 (d, J = 8.6 Hz, 1H), 7.39 (d, J = 8.8 Hz, 2H); MS m/z: 391 ($M^+ + 1$).

(E)-4-Dimethylamino-3'-(2'-chloro-acetyl-)amino-4'-(p-methoxy-)benzylmer-capto-stilbene (**33**)

To a solution of **32** (200 mg, 0.51 mmol) and Et_3N (0.36 mL) in CH_2Cl_2 (20 mL) was added chloroacetylchloride (0.1 mL) dropwise at 0 ℃. The mixture was stirred at r.t. for 1 h. Water was added and the mixture was extracted with CH_2Cl_2. The organic phase was dried, filtered and concentrated to give crude product which was purified by PTLC (EA:Hex = 2:5 $V:V$ as developing solvent) affording 115 mg of product (43.1% in 2 steps) ^1H NMR ($CDCl_3$, 200 MHz) δ: 2.99 (s, 6H), 3.77 (s, 3H), 3.84 (s, 2H), 4.08 (s, 2H), 6.71(d, J = 8.8 Hz, 2H), 6.76 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 16.2 Hz, 1H), 6.99 (d, J = 8.6 Hz, 2H), 7.12 (d, J = 16.2 Hz, 1H), 7.19 (dd, J = 8.6, 1.7 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.42 (d, J = 8.8 Hz, 2H), 8.50 (d, J = 1.7 Hz, 1H), 9.48 (br, 1H).

(E)-4-Dimethylamino-3'-[2'-(2'''-p-methoxybenzylmercaptoethyl-)amino-acetyl-]amino-4'-(p-methoxy-)benzylmercapto-stilbene (**35**)

A mixture of **33** (115 mg, 0.25 mmol), p-methoxybenzylmercapto-ethylamine **34** (146 mg, 0.75 mmol), K_2CO_3 (170 mg, 1.25 mmol) and KI (40 mg) in DMF (2 mL) was stirred at r.t. overnight. Water was added and the mixture was extracted with CH_2Cl_2. The organic phase was dried, filtered and concentrated to give crude product which was purified by PTLC (EA:Hex = 2:3 $V:V$ as developing solvent) affording 105 mg of product (68%). ^1H NMR ($CDCl_3$, 200 MHz) δ: 2.63 (t, J = 5.6 Hz, 2H), 2.76 (t, J = 5.6 Hz, 2H), 2.98 (s, 6H), 3.29 (s, 2H), 3.68 (s, 2H), 3.75 (s, 3H), 3.78 (s, 3H), 3.81 (s, 2H), 6.71 (d, J = 8.3 Hz, 2H), 6.75 (d, J = 8.5 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 6.89 (d, J = 16.2 Hz, 1H), 6.99 (d, J = 8.6 Hz, 2H), 7.12 (d, J = 16.2 Hz, 1H), 7.13 (dd, J = 8.3, 2.0 Hz, 1H), 7.23 (d, J = 8.7 Hz, 2H), 7.33 (d, J = 8.1 Hz, 1H), 7.42 (d, J = 8.8 Hz, 2H), 8.62 (d, J = 2.0 Hz, 1H), 10.24 (s, 1H); IR (film) ν : 3392, 2923, 1679, 1608, 1509, 1247, 1133, 1027, 826 cm^{-1}. HRMS calcd for $C_{36}H_{41}N_3O_3S_2Na$ ($M^+ + Na$) 650.2487, found 650.2477.

(E)-4-Dimethylamino-3'-[2'-(2'''-p-methoxybenzylmer-capto-ethyl-)amino-ethyl-]amino-4'-(p-methoxy-)benzylmercapto-stilbene (**36**)

To a suspension of LAH (32 mg, 0.8 mmol) in THF (5 mL) was added a solution of **35** (105 mg, 0.17 mmol) in THF (10 mL) dropwise at r.t. The resulting mixture was stirred under reflux for 1 h. After the reaction mixture was cooled down to r.t. Water (0.05 mL), NaOH (10%, 0.05 mL) and water (0.15 mL) were added dropwise succesively. The resulting mixture was filtered and washed with mixed solvent (CH_2Cl_2:MeOH = 9:1 $V:V$). The filtrate was concentrated and the crude product was purified by PTLC (ethyl acetate as developing solvent) to give **44** mg of product (43%). ^1H NMR ($CDCl_3$, 200 MHz) δ: 2.61 (t, J = 6.0 Hz, 2H), 2.80 (t, J = 6.0 Hz, 2H), 2.83 (t, J = 6.0 Hz, 2H), 2.99 (s, 6H), 3.15—3.30 (m, 2H), 3.67 (s, 2H), 3.76 (s, 3H), 3.77 (s, 3H), 3.82 (s, 2H), 6.69—6.88 (m, 8H), 6.96 (d, J = 16.2 Hz, 1H), 7.07 (d, J = 8.6 Hz, 2H), 7.13 (d, J = 16.2 Hz, 1H), 7.22 (d, J = 8.3 Hz, 3H), 7.42 (d, J = 8.8 Hz, 2H); IR (film) ν : 3392, 2930, 1606, 1509, 1247, 1133, 1054, 816 cm^{-1}. HRMS calcd for $C_{36}H_{43}N_3O_2S_2$ 613.2797, found 613.2793.

(E)-4-Dimethylamino-3'-[2''-(2'''-mercapto-ethyl-)-amino-ethyl-]amino-4'-mercapto-stilbene (**37**)

The same procedure described above for preparation of compound **23** was employed. **37** was obtained in 48.5% of yield starting from **36** (44 mg, 0.07 mmol). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 2.98 (s, 6H), 2.90—3.45 (m, 8H), 6.34—7.61 (m, 9H); IR (film) ν: 3353, 2917, 1604, 1582, 1519, 1359, 958, 815, 732 cm^{-1}. HRMS calcd for $C_{20}H_{26}N_3S_2$ ($M^+ - 1$) 372.1568, found 372.1560.

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