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The design and synthesis of novel derivatives of the dopamine uptake inhibitors GBR 12909 and GBR 12935. High-affinity dopaminergic ligands for conjugation with highly fluorescent cadmium selenide/zinc sulfide core/shell nanocrystals

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Abstract—There is a growing demand for compounds with very high affinities for the dopamine transporter protein (DAT) that can be conjugated to fluorescent markers such as cadmium selenide/zinc sulfide core/shell nanocrystals. This paper describes the design and synthesis of two derivatives of the DAT antagonists GBR 12935 and GBR 12909. These compounds have a high biological affinity for DAT and may be conjugated to nanocrystals via a thiol linkage without a significant reduction in their biological activity. Such conjugates may be used in fluorescent imaging studies.

1. Introduction

Semiconductor nanocrystals, or 'quantum dots', are crystals with diameters on the 1–10 nm scale and consist of between a few hundred to several thousand atoms. These materials have the same crystal packing as the bulk material; however, their small size gives them unique physical properties.[1] For instance, when a cadmium selenide nanocrystal core is passivated with a shell of a wider band gap material such as zinc sulfide, highly fluorescent cadmium selenide/zinc sulfide core/shell nanocrystals are obtained. These core/shell nanocrystals have several unique optical properties.[2–4] They have fluorescent properties that are superior to organic dyes such as increased photo stability and brightness. This enables fluorescence experiments with core/shell nanocrystals to be performed over longer time-periods and with lower concentrations than can be achieved with conventional dyes. Additionally the fluorescence emission band is size tunable and narrow.[1] Small cores give rise to fluorescence at blue wavelengths while larger cores give rise to emission at red wavelengths. Their narrow emission bands enable several different sizes of core/shells to be used at once in multiplexing experiments.[5,6] Finally, the absorption of core/shell nanocrystals is a continuum above the band gap, enabling a single light source to be used for simultaneous excitation of all sizes of nanocrystals.

We are interested in using cadmium selenide/zinc sulfide core/shell nanocrystals as probes for integral membrane proteins involved in neural signaling. In order to make nanocrystals biologically active, a biologically active molecule must be attached to the surface of the nanocrystal without loosing its activity. Several groups have reported attaching proteins and antibodies to fluorescent nanocrystals and have demonstrated that these are biologically active.[5–8] Our approach is to attach small molecules such as drugs and neurotransmitters to nanocrystals. We have recently demonstrated that derivatives of serotonin may be attached to nanocrystals while maintaining activity.[9] Using these conjugates we have visualized serotonin transporter proteins on the surface of living HEK cells.[10] In addition to enabling dynamic imaging experiments in living tissue, drug-conjugated fluorescent nanocrystals could potentially be utilized in high throughput screening assays for drug discovery, cell sorting applications via flow cytometry, and in vivo imaging.

Changes in dopamine receptor distribution and in the dopamine transporter system have been shown to be important factors in a number of different disease states including Parkinson's disease, Huntington's chorea and schizophrenia.[11] Further, it has been shown that the dopamine transporter system may be responsible for the locomotor and reinforcing effects of cocaine.[12] The

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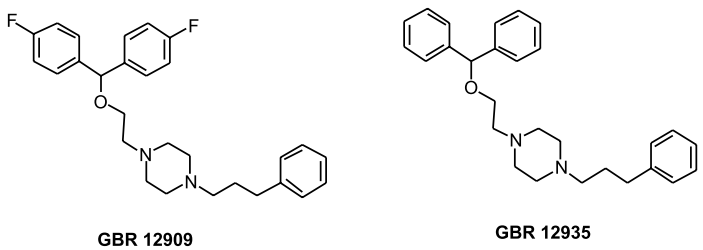


Figure 1. Two potent inhibitors of dopamine reuptake GBR 12909 and GBR 12935.

dopamine transporter complex (DAT) is a protein located on the presynaptic membranes of mesotelencephalic dopaminergic neurons. These neurons are located in the substantia nigra and ventral tegmentum in the midbrain and converge in the basal ganglia of the forebrain.[11] DAT is a member of the family of Na^+ Cl^- dependent transporters and transports dopamine via a coupled sodium chloride transport mechanism. We are developing high-affinity ligands for DAT that may be conjugated to cadmium selenide/zinc sulfide core/shell nanocrystals in order to image the localization and distribution of the dopamine transporter protein within neuronal cells. By imaging neurons containing DAT we hope to understand more about these disease states and the dopaminergic role in cocaine addiction.

Many different classes of compounds have been demonstrated to bind to DAT.[13–16] The DAT antagonists GBR 12909 and GBR 12935 (Fig. 1) have been extensively studied.[17–19] These compounds have been shown to have a high affinity for DAT and slowly dissociate from the transporter, resulting in a long duration of action.[20] The biological properties of these compounds make the GBR compounds ideal candidates for ligands that can be attached to fluorescent core/shell nanocrystals. They bind tightly to

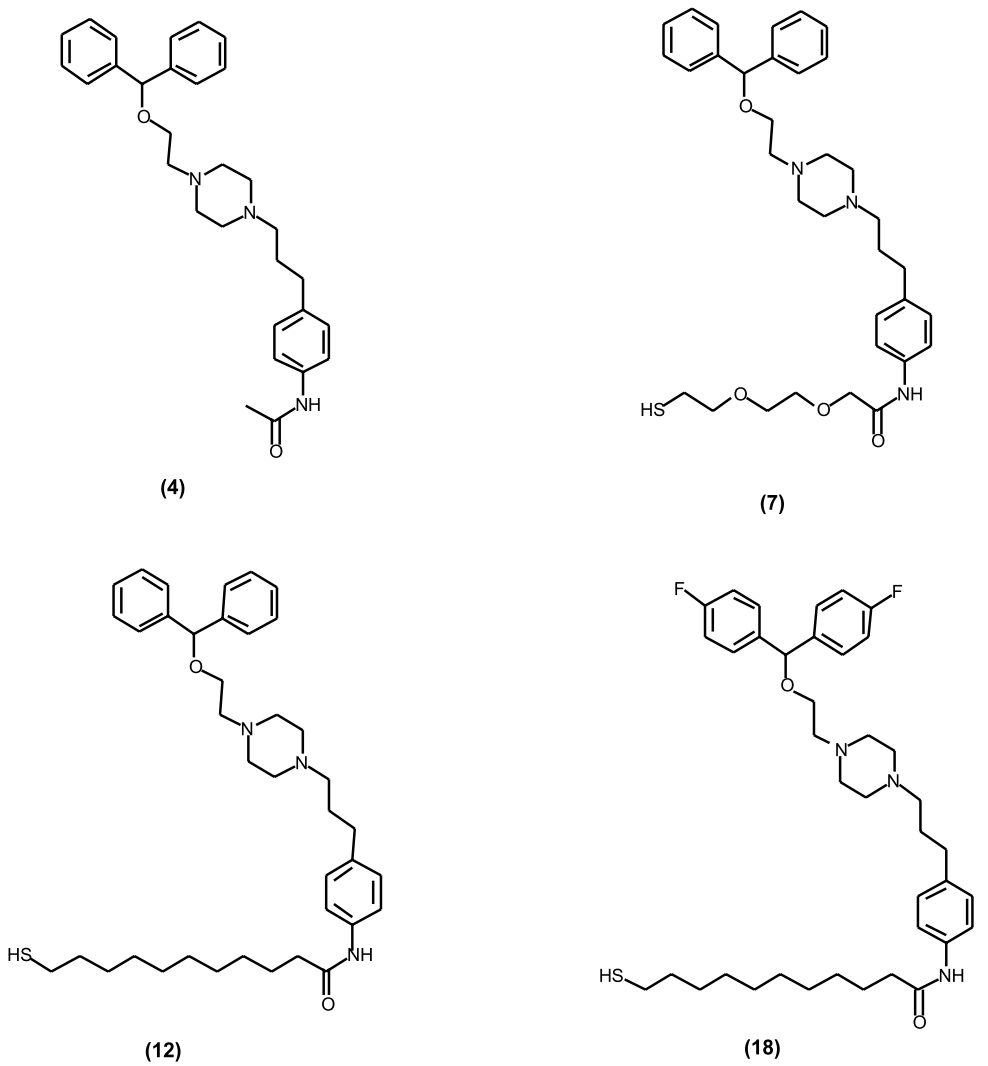


Figure 2. The DAT antagonists prepared in this study.

Table 1. The IC_{50} values obtained for the compounds used in this study (NA, not applicable)

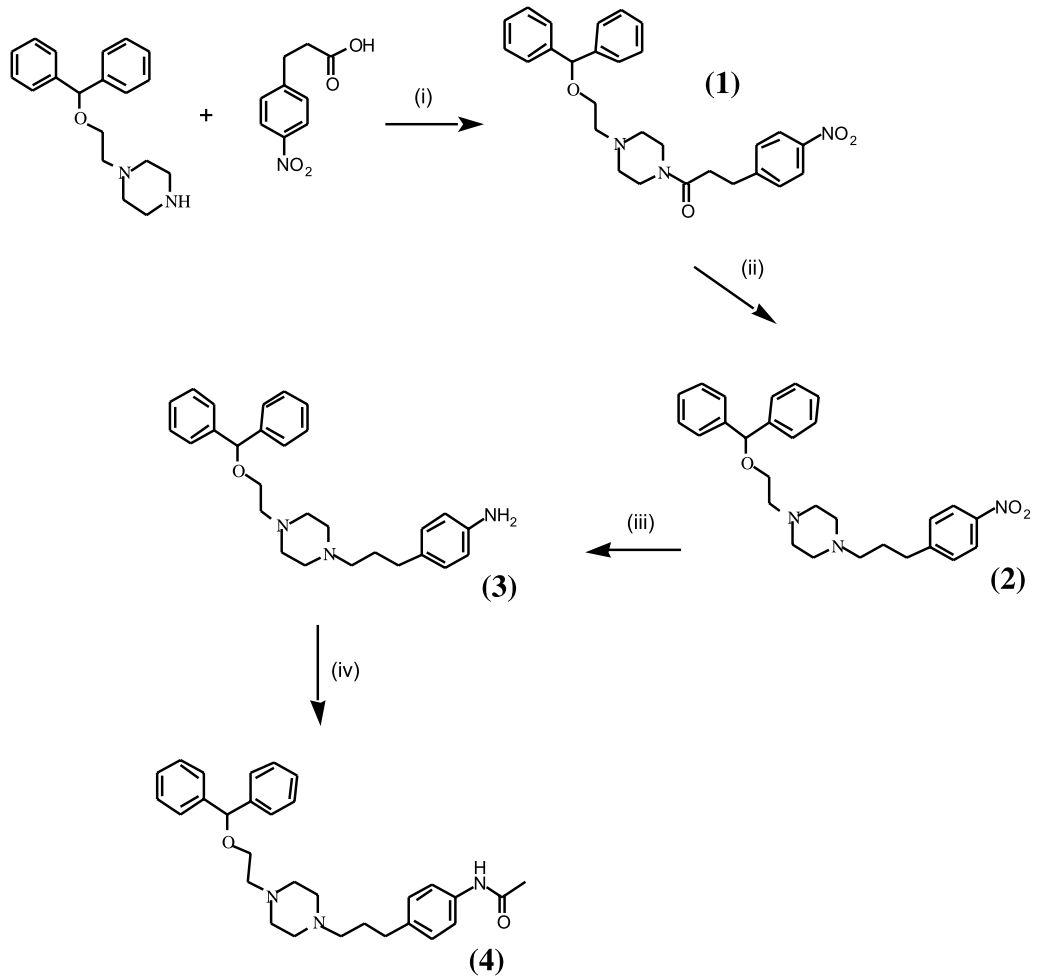
Compound no.	IC_{50} of free ligand (nM)	IC_{50} of bound ligand (nM)
4	30	NA
7	6000	NA
12	18	32
18	10	140

DAT and their IC_{50} values are in the nanomolar range. This should enable the development of fluorescence assays with high sensitivity and selectivity, with little or no binding to other transporter proteins, such as the serotonin transporter (SERT). In this paper we report the synthesis of analogues of GBR 12909 and GBR 12935 designed such that they can be attached to the surface of cadmium selenide/zinc sulfide core/shell nanocrystals.

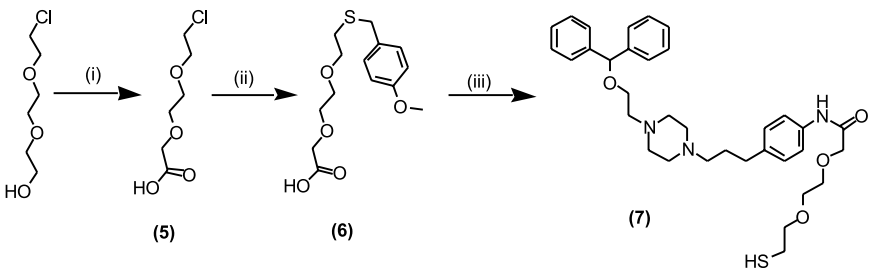
We require ligands that have a high affinity for DAT when bound to the nanocrystal and are inexpensive to synthesize from commercially available reagents. Structure activity relations for the GBR compounds[21–23] suggest bulky substituents at the *para* position on the phenyl propyl moiety are tolerated without a large reduction in biological activity.[24] Consequently, it was decided to attach a linker arm to this position. The other end of the linker arm is designed so that it can be attached to the surface of the nanocrystal. A thiol is used for the point of attachment to the nanocrystal, as thiols are known to bind tightly to the surfaces of cadmium selenide/zinc sulfide core/shell nanocrystals. It is hoped that the linker arm will reduce steric interactions between the drug and nanocrystal and the biological activity of the bound ligand will be comparable to that of the free ligand. Our synthetic sequence is designed so that the linker arm is attached to the derivative of GBR 12909 or GBR 12935 at the end of the synthetic scheme. Figure 2 shows the different ligands designed during the course of this study and their biological activities are summarized in Table 1.

2. Results and discussion

We considered many different methodologies for attaching the linker arm to the drug and concluded that an amide linkage would be the simplest way of attaching a linker arm. In order to ascertain whether or not an amide linkage would reduce biological activity we synthesized compound **4**. The synthesis of this compound is outlined in Scheme 1. In this



Scheme 1. (i) (a) Oxalyl chloride, DMF, (b) CH_2Cl_2, triethylamine, reflux 18 h, yield=99%; (ii) AlH_3, yield=68%; (iii) $SnCl_2$, yield=78.6%; (iv) acetyl chloride, triethylamine, CH_2Cl_2, yield=25%.

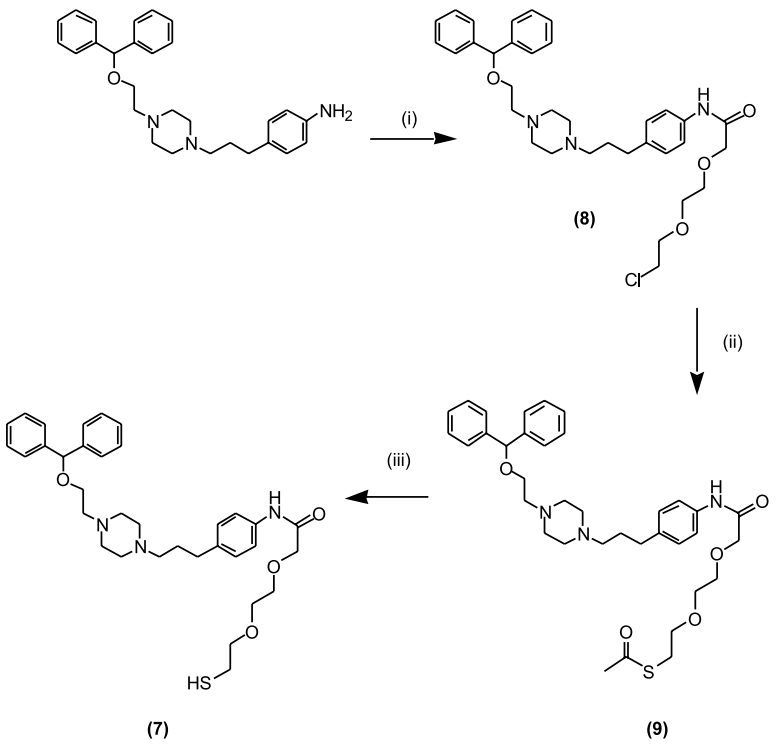


Scheme 2. (i) Chromium (IV) oxide, H_2SO_4, yield=93%; (ii) 4-methoxy-α-toluenethiol, sodium ethoxide, yield=94%; (iii) (a) oxalyl chloride, DMF, (b) triethylamine, (**6**), CH_2Cl_2, reflux 4 days, yield=28.9%.

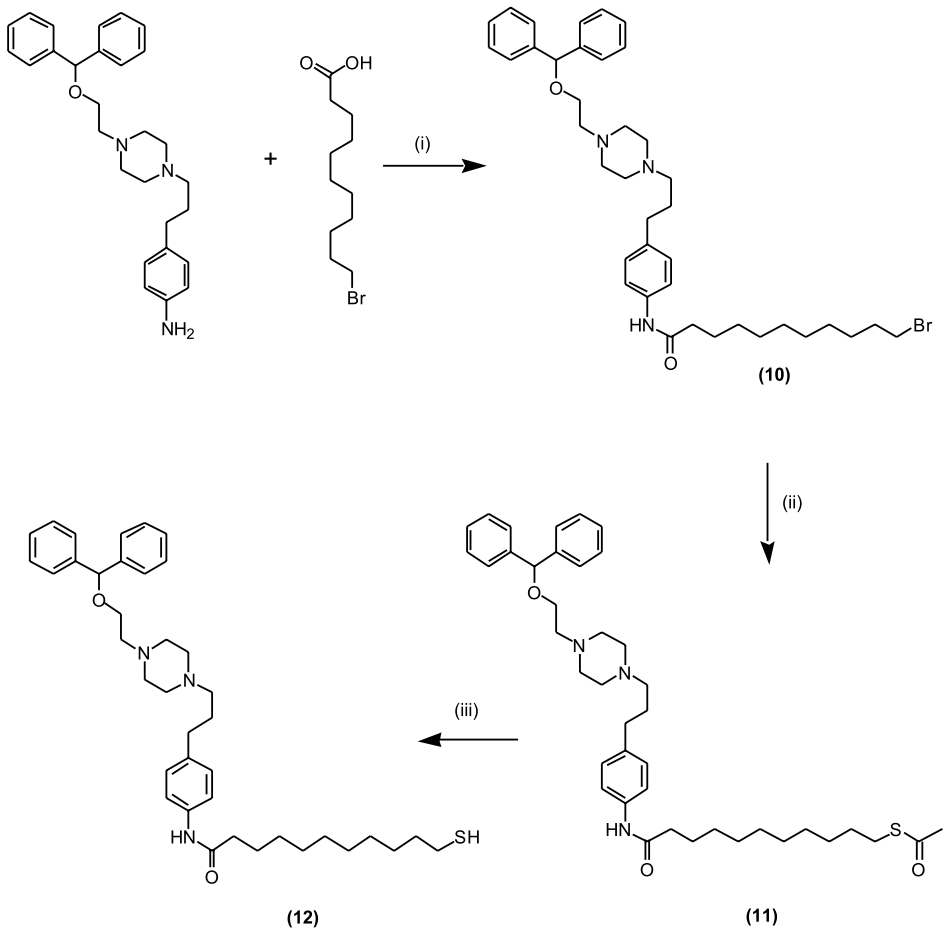
synthetic scheme, 1-[2-[bisphenylmethoxy]ethyl]piperazine was synthesized using the method described by Van Der Zee et al.[21] This was reacted with the acid chloride derivative of 4-nitrophenyl propionic acid, giving 1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-nitrophenyl)-1-oxopropyl)piperazine (**1**) in a 99% yield. The amide was reduced using alane–*N*,*N*-dimethylamine complex in THF[25] and 1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-nitrophenyl)propyl)piperazine (**2**) was obtained in a 68% yield. The *para*-nitro group was reduced using tin(II)chloride giving 1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-aminophenyl)propyl)piperazine (**3**) as a red oil in a 78.6% yield. Finally, the amine was acylated using acetyl chloride, giving *N*-(4-{3-[4-(2-benzhydryloxyethyl)-piperazin-1-yl]-propyl}-phenyl)-acetamide (**4**) in a 25% yield. Compound **4** was assayed against the transporter protein by using a competition assay with tritiated dopamine and was shown to have an IC_{50} of 30 nM. This compares favorably with the reported IC_{50} of 3.7 nM[26] for GBR 12935. Thus we concluded that an amide linkage attached to the GBR compounds would not be detrimental to biological activity.

Our first ligand, *N*-(4-(3-[4-(2-benzhydryloxyethyl)piperazine-1-yl]propyl)phenyl-2-[2-(2-mercaptoethoxy)ethoxy]-acetamide (**2**), was designed so that it is comprised of a polyethylene glycol chain that was attached to a derivative of GBR 12935. The other end of the polyethylene chain terminated in a thiol and was to be the point of attachment to the nanocrystal. A polyethylene glycol linker was selected as it was hoped that this would enhance the water solubility of the nanocrystal conjugate by interacting with solvated cations in a manner similar to crown ethers.

We developed two synthetic routes that can be used to attach generic polyethylene glycol linker arms to the GBR derivative, these are shown in Schemes 2 and 3. Both routes use 2-(2-(2-chloroethoxy)ethoxy)ethanoic acid (**5**), which was obtained in a 93% yield via a Jones oxidation[27] of 2-(2-(2-chloroethoxy)ethoxy)ethanol. In our first route (Route 1), the chlorine atom in 2-(2-(2-chloroethoxy)ethoxy)ethanoic acid was displaced with the sodium salt of 4-methoxybenzyl mercaptan by refluxing for 18 h in absolute ethanol. This



Scheme 3. (i) CDI, 2-(2-(2-chloroethoxy)ethoxy)ethanoic acid, THF, 18 h, yield=42%; (ii) potassium thioacetate, DMF, 18 h, yield=73%; (iii) ammonia, yield=94%.

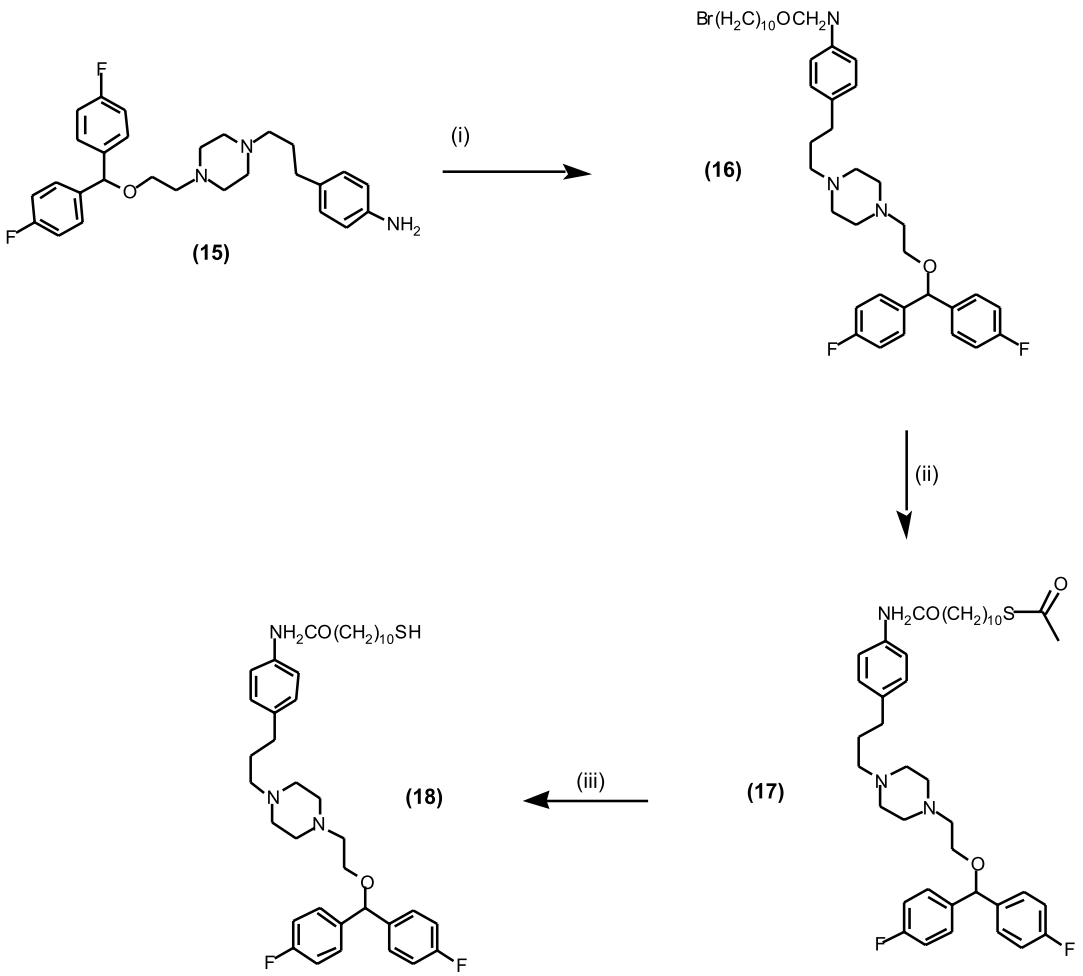


Scheme 4. (i) Method A: (a) $SOCl_2$, DMF, (b) Et_3N, CH_2Cl_2, 4 days 25°C, yield=23%. Method B: 11-bromoundecanoic acid, CDI, DMF, yield=28%; (ii) potassium thioacetate, DMF, 18 h, yield=31%; (iii) ammonia, yield=51%.

gave {2-[2-(4-methoxy-benzylsulfanyl)-ethoxy]-ethoxy}-acetic acid (**6**) in a 94% yield as a colorless oil. We decided to use a thiol protected with the 4-methoxybenzyl group, as it can be removed with several reagents, including mercury or silver salts, as well as by acid hydrolysis.[28] We considered using other protecting groups for the thiol, such as disulfides,[29,30] we rejected these as they required strong acids and high temperatures. We thought that the bisphenyl methyl ether in the GBR derivative might be cleaved under such conditions.

We discovered that when the acid chloride of compound **6** was added to 1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-aminophenyl)propyl)piperazine (**3**) and stirred at reflux in methylene chloride in the presence of triethylamine under argon for 4 days, **7** was produced in a 28.9% yield. Thus, we were able to couple the linker arm and deprotect the thiol in one pot. Whilst this route worked the percentage yield of final product was low. When we resynthesized the final product **7**, we observed that the reaction gave the product in a low to moderate yield intermittently. In order to overcome this variability we developed our second route (Route 2). In this route 2-(2-(2-chloroethoxy)ethoxy)ethanoic acid (**5**) was coupled to 1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-aminophenyl)propyl)piperazine (**3**) using CDI in THF. This gave *N*-(4-{3-[4-(2-benzhydryloxy-ethyl)piperazin-1-yl]-propyl}-phenyl)-2-[2-(2-chloro-ethoxy)-ethoxy]-acetamide (**8**) in a 42% yield as a clear oil. The chlorine atom in **8** was displaced using thioacetate to give thioacetic acid *S*-(2-{2-[(4-{3-[4-(2-benzhydryloxy-ethyl)-piperazin-1-yl]-propyl}phenylcarbomyl)-methoxy]-ethoxy}ethyl) ester (**9**) as a brown oil in a 73% yield. Compound **9** was hydrolyzed using methanolic ammonia to give the desired product (**7**) in a 94% yield.

The IC_{50} value for compound **7** was 6 μM. We hypothesized that this low biological activity may be due to the polyethylene glycol chain interacting with the transporter protein, thereby reducing the affinity of this ligand for DAT. Alternatively the conformation of the PEG linker may result in unfavorable steric interactions with the DAT protein. Such steric interactions could reduce the affinity of the compound for DAT. The ability of PEG to interact with cations may also reduce the affinity of this compound for the DAT protein by interacting with cations. Such complexes may have lower affinity for DAT if the compound is binding to region that carries a positive charge. As this ligand had too low an affinity for DAT to be used in a fluorescence assay system, it was decided to replace the polyethylene glycol chain with a simple alkyl chain. It has been shown that alkyl substituents in this region do not significantly reduce biological activity and we thought an analogue of



Scheme 5. (i) 11-Bromoundecanoic acid, CDI, DMF, yield=30%; (ii) potassium thioacetate, DMF, 18 h, yield=54%; (iii) ammonia, yield=51%.

GBR 12935 with a simple alkyl chain at this position might retain activity.

We decided to synthesize 11-mercaptoundecanoic acid (4-{3-[4-(2-benzhydryloxyethyl)-piperazin-1-yl]-propyl}-phenyl)-amide (**12**) as 11-bromoundecanoic acid is commercially available and inexpensive. The route we used to synthesize this ligand is shown in Scheme 4. Initially 1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-aminophenyl)propyl)-piperazine (**3**) was coupled to the acid chloride of 11-bromoundecanoic acid in methylene chloride at room temperature to give 11-bromoundecanoic acid (4-{3-[4-(2-benzhydryloxy-ethyl)-piperazine-1-yl]-propyl}-phenyl)-amide (**10**) in a 23% yield. We tried to increase this yield by coupling the 11-bromoundecanoic acid to **3** in dry DMF using CDI (Method B).[31] This increased the yield of **10** slightly to 28%. In the next step, we displaced the bromine atom in **10** using potassium thioacetate in dry DMF. This nucleophilic displacement gave thioacetic acid *S*-[10-(4-{3-[4-(2-benzhydryloxy-ethyl)-piperazin-1-yl]-propyl}-phenyl-carbamoyl)-decyl] ester (**11**) in a 31% yield. The thioacetate was hydrolyzed using ammonia dissolved in methanol under argon at room temperature to give compound **12** in a 51% yield. The biological activity of compound **12** was measured and it was found to have an IC_{50} of 18 nM. After conjugating compound (**12**) to cadmium selenide/zinc sulfide core/shell nanocrystals an IC_{50} of 32 nM was obtained. These results appear to confirm our original hypothesis that the biological activity of compound **7** is reduced due to unfavorable interactions with the DAT transporter and the polyethylene glycol chain.

The fluorinated derivative of GBR 12935, GBR 12909, has been shown to have a greater selectivity for DAT over the serotonin transporter (SERT) than GBR 12935.[32] Consequently we decided to synthesize a derivative of this ligand, as it may be of interest in future studies to compare the nanocrystal conjugates of the two different ligands in fluorescent biological assays. Our synthetic route is outlined in Scheme 5. 1-[2-[4,4′-Fluorobenzhydryloxy]ethyl]piperazine was synthesized using the method described by Buzas.[33] This was coupled to 4-nitrophenyl propionic acid via the acid chloride to give 1-[2-[4,4′-fluorobenzhydryloxy]-ethyl]-4-(3-(4-nitrophenyl)-1-oxopropyl)piperazine (**13**) in a 97% yield. Alane was used to reduce the resulting amide giving an 80% yield of 1-[2-[4,4′-fluorobenzhydryl-oxy]ethyl]-4-(3-(4-nitrophenyl)propyl)piperazine (**14**). Reduction of the nitro functionality in **14** was accomplished by refluxing **14** in ethanol with tin(II)chloride. 1-[2-[4,4′-Fluorobenzhydryloxy]ethyl]-4-(3-(4-aminophenyl)propyl)-piperazine (**15**) was obtained in a 92% yield. We coupled 11-bromoundecanoic acid to compound **15** using CDI,

giving 11-bromoundecanoic acid (4-{3-[4-(4,4′-fluorobenzhydryloxy-ethyl)-piperazine-1-yl]-propyl}-phenyl)-amide (**16**) in a 28% yield. The thioacetate was synthesized by reacting **16** with potassium thioacetate giving thioacetic acid *S*-[10-(4-{3-[4-(2-(4,4′-fluorobenzhydryloxy)-ethyl)-piperazin-1-yl}-propyl}-phenylcarbamoyl)-decyl] ester (**17**) in a 54% yield. Subsequent hydrolysis of **17** with ammonia resulted in a 51% yield of 11-mercapto-undecanoic acid (4-{3-[4-(2-(4,4′-fluorobenzhydryloxy)-ethyl)-piperazin-1-yl]-propyl}-phenyl)-amide (**18**). When **18** was assayed it was found to have an IC_{50} of 10 nM which compares favorably with GBR 12909 which has an IC_{50} 3.7 nM.[32] Once again this was attached to quantum dots and the IC_{50} for the conjugated dots was found to be 140 nM.

3. Conclusions

We have developed a synthetic methodology for two ligands with high affinity for the DAT transporter protein. We have shown that when these ligands are conjugated to cadmium selenide/zinc sulfide core/shell nanocrystals the biological activity of the ligand remains high. Such conjugates will be used for fluorescent imaging of neuronal cells in future studies.

4. Experimental

2-(2-(2-Chloroethoxy)ethoxy)ethanol, alane, 11-bromo-undecanoic acid, potassium thioacetate and tin(II)chloride were purchased from the Aldrich Chemical Company. 4-Nitrophenyl propionic acid was obtained by nitrating hydrocynamic acid and the melting point of the resulting product was consistent with commercially available 4-nitrophenyl propionic acid. Reagents were used as they were received. Cadmium selenide/zinc sulfide core/shell nanocrystals were provided by Quantum Dot Corporation. Thin-layer chromatography was carried out on precoated plates and the products were visualized using UV light. Column chromatography on silica refers to silica gel obtained from Scientific Adsorbents Inc. catalogue number 02826-25. All NMR data were obtained using $CDCl_3$ as a solvent unless otherwise stated. All NMR measurements were performed using a Brucker 300 MHz machine. Chemical shifts were measured relative to TMS and coupling constants are measured in Hz. Low resolution mass spectra (MS) were obtained from a Finnegan Thermo quest TSQ 7000 triple quadrapole LC-MS equipped with an API-1 electrospray ionization source (ES). High resolution MS were obtained either at the University of Notre Dame mass spectrometry facility using a JOEL GCmate employing FAB as the ionization method, or at Ohio State University using electrospray ionization (ESI) mass spectrometry. ESI analyses were performed with a 3-Tesla Finnigan FTMS-2000 Fourier Transform mass spectrometer. Samples were sprayed from a commercial Analytica electrospray ionization source, and then focused into the FTMS cell using a home-built set of ion optics. For ESI analysis, most compounds were sprayed from a micromolar concentration of the analyte in various solvent mixtures, such as tetrahydrofuran/CH_3OH, with added NaCl. This process generated the sodiated molecular ion (usually as the singly-charged species), denoted as $(M+Na)^+$. However, in some cases, acetic acid or trifluoroacetic acid was used to generate the protonated molecular ion $(M+H)^+$ instead. Electron impact (EI) ionization was performed with a Kratos MS-25, using 70 eV ionization conditions. Samples for elemental analysis were routinely dried at ca. 10 mm Hg. Elemental analysis was performed by Atlantic Microlabs, Georgia and the analysis is corrected for the presence of sodium and water when necessary. IR was performed using silver chloride plates with neat samples on a ATI Mattson Genesis Series FT IR machine.

4.1. Data for compounds

4.1.1. 1-[2-[Bisphenylmethoxy]ethyl]-4-(3-(4-nitrophenyl)-1-oxopropyl)piperazine (1). *para*-Nitrohydrocinnamic acid (2.8 g, 9.5 mmol) is added to dry toluene (100 mL), in a 250 mL round bottomed flask equipped with a stirrer and a reflux condenser. Oxalyl chloride (1 mL) was added, after which a catalytic quantity of dry DMF (2 drops) was also added. Then the mixture was stirred at room temperature for 2 h. The solvent was removed by evaporation and the crude acid chloride was dissolved in dry dichloromethane (100 mL). Dry triethylamine (10 mL) and 1-[2-[bisphenylmethoxy]ethyl]piperazine (1.84 g, 9.5 mmol) were dissolved in dry dichloromethane (50 mL) and added to the solution of *p*-nitrohydrocinnamyl chloride. The mixture was heated at reflux for 18 h under argon in a 250 mL round bottomed flask equipped with a stirrer and reflux condenser. Then the solvent was removed under reduced pressure and the product was purified using silica gel chromatography eluted with dichloromethane 96%–methanolic ammonia. The resulting yellow oil was converted to the yellow maleate salt by dissolving the base in diethyl ether (50 mL) and adding an ethereal solution of maleic acid. The resulting solid was collected by filtration and washed with diethyl ether (2×150 mL). The maleate was dried under reduced pressure, to yield 4.3 g (99%) of the product, mp 122–123°C. ^1H NMR ($CDCl_3$) δ 2.45 (t, J=4.9 Hz, 4H), 2.66–2.68 (m, 4H), 3.06 (t, J=7.6 Hz, 2H), 3.95 (t, J=4.9 Hz, 2H), 3.60–3.65 (m, 4H), 5.36 (s, 1H), 7.19–7.41 (m, 12H), 8.08–8.12 (d, 2H); ^{13}C NMR ($CDCl_3$) δ 30.7, 33.7, 41.5, 45.1, 41.5, 45.1, 52.9, 53.3, 57.5, 66.6, 83.6, 123.3, 126.6, 127.2, 128.1, 129.1, 141.9, 146.1, 149.2, 169.3; calculated for $C_{28}H_{31}N_3O_4$ *m/z* FAB (M+H) 474.2393; found 474.2398; IR (neat) CH_2 2821–2938 cm^{-1} (m), C═O 1641 cm^{-1} (s).

4.1.2. 1-[2-[Bisphenylmethoxy]ethyl]-4-(3-(4-nitrophenyl)-propyl)piperazine (2). 1-[2-[Bisphenylmethoxy]ethyl]-4-(3-(4-nitrophenyl)-1-oxopropyl)piperazine (5 g, 14.8 mmol) was dissolved in dry THF (100 mL) in a 250 mL round bottomed flask equipped with a stirrer and a reflux condenser. Alane in toluene (0.5 M, 59 mL) was added and stirred at room temperature for 30 min. The reaction was quenched with sodium hydroxide solution (10%, 200 mL) and the aqueous solution was extracted with diethyl ether (3×150 mL). It was dried over magnesium sulfate filtered and evaporated and the product was purified using silica gel chromatography eluted with a gradient system eluted with ethyl acetate 90%–methanol to ethyl acetate 87%–methanol 10%–triethylamine. This gave

3.35 g (68%) of the product as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.78 (t, J=6.5 Hz, 4H), 2.30 (t, J=7.1 Hz, 2H), 2.43 (br s, 4H), 2.53 (br s, 4H), 2.67 (t, J=2.9 Hz, 2H), 3.59 (t, J=3.0 Hz, 2H), 5.37 (s, 1H), 7.15–7.35 (m, 12H), 8.04–8.06 (d, 2H); ^{13}C NMR ($CDCl_3$) δ 27.7, 33.0, 52.8, 53.3, 57.0, 57.6, 66.6, 83.5, 123.1, 126.6, 127.0, 127.9, 128.8, 141.0, 145.8, 149.8; calculated for $C_{28}H_{33}N_3O_3$ m/z ESI (M+Na) 482.2420; found 482.2392; IR (neat) CH_2 2939–2809 cm^{-1} (m).

4.1.3. 1-[2-[Bisphenylmethoxy]ethyl]-4-(3-(4-aminophenyl)propyl)piperazine (3). 1-[2-[Bisphenylmethoxy]-ethyl]-4-(3-(4-nitrophenyl)-1-oxopropyl)piperazine (0.9 g, 2.8 mmol) was dissolved in absolute ethanol (10 mL) in a 100 mL round bottomed flask equipped with a stirrer and reflux condenser. Tin(II)chloride dihydrate (2.6 g) was added and the mixture was heated at reflux for 90 min. The solution was poured into crushed ice and a solution of sodium carbonate (5%) in water was added until a pH of 8 is obtained. The aqueous solution was extracted with ethyl acetate (3×200 mL) and this was dried over magnesium sulfate. The product was purified using column chromatography on silica gel eluted with ethylacetate 92%–methanol 5%–triethylamine, to give 0.66 g (78.6%) of the product as a pale yellow oil. The base was converted to the oxalate salt by adding oxalic acid (4 g) dissolved in methanol (50 mL) to a solution of the base dissolved in methanol (50 mL). The resulting salt was collected by filtration and washed with methanol (2×50 mL). After drying under reduced pressure the oxalate was obtained as a brown solid, mp 156–158°C. ^{1}H NMR ($CDCl_3$) δ 1.73 (t, J=6.2 Hz, 4H), 2.32 (t, J=6.2 Hz, 2H), 2.38–2.51 (m, 8H), 2.65 (t, J=5.9 Hz, 2H), 3.57 (t, J=5.9 Hz, 2H), 3.64 (br, NH_2), 5.35 (s, 1H), 6.51–6.54 (d, 2ArH), 6.91–6.94 (d, 2ArH), 7.18–7.34 (m, 10ArH); ^{13}C NMR ($CDCl_3$) δ 28.5, 32.5, 52.9, 53.3, 57.6, 60.0, 66.6, 83.5, 114.8, 126.7, 127.1, 127.7, 128.0, 131.5, 142.0, 144.1; calculated for $C_{28}H_{35}N_3O$ m/z FAB (M+H) 430.2858; found 430.2862; IR (neat) NH_2 3349 cm^{-1} (br s), CH_2 2809–2938 (m).

4.1.4. *N*-(4-{3-[4-(2-Benzhydryloxy-ethyl)-piperazin-1-yl]-propyl}-phenyl)-acetamide (4). 1-[2-[Bisphenylmethoxy]ethyl]-4-(3-(4-aminophenyl)propyl)piperazine (0.5 g, 1.2 mmol) was dissolved in dry dichloromethane. Triethylamine (1 mL) was added followed by acetyl chloride (1 mL). The mixture was stirred at room temperature overnight then evaporated under reduced pressure. The product was purified by column chromatography on silica gel eluted with methanol 4%–methylene chloride. This gave 0.14 g (25%) of the product as a pale brown oil. ^{1}H NMR ($CDCl_3$) δ 1.75–1.85 (m, 2H), 2.20 (s, 3H), 2.38 (t, J=7.9 Hz, 2H), 2.40–2.67 (m, 14H), 3.55 (t, J=5.7 Hz), 5.29 (s, 1H), 6.92 (d, 2ArH), 7.17–7.28 (m, 12ArH); ^{13}C NMR ($CDCl_3$) δ 26.9, 27.8, 33.1, 52.7, 53.0, 57.6, 57.6, 66.7, 83.9, 126.9, 127.4, 128.3, 128.4, 129.7, 137.1, 142.0, 142.6, 173.0; calculated for $C_{30}H_{37}N_3O_2$ m/z ESI (M+H) 472.2964; found 472.2973; IR (neat) C(C=O)NH 3249 cm^{-1} (br s), CH_2 2939–2816 (m), C=O 1676 cm^{-1} (s).

4.1.5. 2-(2-(2-Chloroethoxy)ethoxy)ethanoic acid (5). 2-(2-(2-Chloroethoxy)ethoxy)ethanol (1.69 g, 10 mmol) was dissolved in acetone (50 mL) and added dropwise to a solution of sulfuric acid (1.5 M, 60 mL) containing chromium VI oxide (5.79 g, 38 mmol) at 0°C. Upon complete addition of the alcohol the solution was allowed to warm to room temperature and stirred at room temperature for 18 h. Inorganic chromium salts were removed by filtration and the solution was concentrated under reduced pressure.[9] The crude product was extracted from the solution using dichloromethane (3×100 mL) and the combined extracts were dried over magnesium sulfate. After filtration and evaporation under reduced pressure the crude product was obtained as a colorless oil 1.7 g (93%). This was used without further purification. ^{1}H NMR ($CDCl_3$) δ 3.90–3.54 (m, 8H), 3.95 (s, 2H), 8.56 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 44.2, 69.9, 72.0, 72.5, 72.9, 175.8; calculated for $C_6H_{11}ClO_4$ m/z ESI (M+Na) 205.0238; found 205.0230; IR (neat) OH 3150 cm^{-1} (br s), CH_2 2920 (m), C=O 1737 cm^{-1} (s).

4.1.6. {2-[2-(4-Methoxybenzylsulfanyl)-ethoxy]-ethoxy}-acetic acid (6). Sodium (0.253 g, 1.1 mmol) was added to absolute ethanol (50 mL) at 0°C and stirred for 30 min. 4-Methoxy-α-toluenethiol (0.78 mL, 6 mmol) was added and this mixture was stirred at room temperature for 30 min. 2-(2-(2-Chloroethoxy)ethoxy)ethanoic acid (1 g, 5.5 mmol) was added and the mixture was heated at reflux for 18 h. The mixture was cooled to room temperature poured into distilled water (100 mL) and acidified with hydrochloric acid (2 M, 1×50 mL). The product was extracted with dichloromethane (2×100 mL) and the organic solution was dried over magnesium sulfate. After filtering the organic solution, it was evaporated under reduced pressure. The product was purified using column chromatography on silica eluted with a gradient system running from pure dichloromethane to dichloromethane 90%–methanol 10%. This gave 1.24 g (94%) of the product as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.58 (t, J=6.8 Hz, 2H), 3.54–3.59 (m, 4H), 3.67 (br s, 4H), 4.14 (s, 2H), 6.81 (d, 2ArH), 7.2 (d, 2ArH); ^{13}C NMR ($CDCl_3$) δ 29.6, 35.2, 54.7, 67.6, 69.4, 70.0, 70.2, 113.3, 129.6, 129.7, 157.9, 173.3; CHS ($0.5H_2O$) calculated for $C_{14}H_{20}O_5S$, C=54.35, H=6.84, S=10.36; found C=54.46, H=6.63, S=10.90; IR (neat) OH 3300–3000 cm^{-1} (br s), CH_2 2916–2837 (m), C=O 1739 cm^{-1} (s).

4.1.7. *N*-(4-(3-[4-(2-Benzhydryloxyethyl)piperazin-1-yl]-propyl)phenyl-2-[2-(2-mercaptoethoxy)ethoxy]acetamide (7). *Route 1*. 8-(4-Methoxybenzylthio)-3,6-dioxaoctanoic acid (0.6 g, 2.2 mmol) was dissolved in dry toluene (50 mL) under nitrogen in a 100 mL round bottomed flask equipped with a stirrer and a reflux condenser. Oxalyl chloride (0.5 mL) and a catalytic quantity of dimethyl formamide (1 drop) were added. The solution was stirred at room temperature for 2 h, then evaporated under reduced pressure. The resulting crude 8-(4-methoxybenzylthio)-3,6-dioxaoctonyl chloride was dissolved in dry dichloromethane (100 mL) in a 250 mL round bottomed flask equipped with a reflux condenser and a stirrer. 1-[2-[Bisphenylmethoxy]ethyl]-4-(3-(4-aminophenyl)propyl)-piperazine (0.66 g, 2.2 mmol) in dry tetrahydrofuran and triethylamine (5 mL) are added and the mixture was allowed to reflux under nitrogen for 4 days. Then the solvent was evaporated and the product was columned on silica eluted with ethyl acetate 93%–methanol 5%–triethylamine 2%.

The crude product was converted to the oxylate salt by dissolving it in methanol (50 mL) and adding oxalic acid (1 g) dissolved in methanol (20 mL). The resulting solid was left standing at room temperature for 18 h and removed by filtration. The oxalate salt was converted back to the base and the product was purified by column chromatography on silica eluted with a gradient system running from dichloromethane 90%–methanol 10% to dichloromethane 87%–methanol 10%–triethylamine 3%. This gave the product as a yellow oil; it was converted back to the oxylate salt, as described above and this was filtered and air dried to yield 0.28 g (28.9%) of the product as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 1.80 (t, J=9.0 Hz, 4H), 2.20 (s, 3H), 2.38 (t, J=8.0 Hz, 2H), 2.50–2.60 (m, 8H), 2.65 (t, J=5.7 Hz, 2H), 3.55 (t, J=5.8 Hz, 2H), 5.29 (s, 1H), 6.89–6.90 (d, 2ArH), 7.13–7.27 (m, 12ArH); ^{13}C NMR ($CDCl_3$) δ 26.9, 27.8, 33.1, 52.7, 53.0, 57.6, 57.6, 66.7, 83.9, 126.9, 127.4, 128.3, 128.4, 129.7, 137.1, 142.0, 142.6, 173.0; *m/z* ES (M+) 591.66 (low res); calculated for $C_{34}H_{45}N_3O_4S$ *m/z* ESI (M+Na) 614.3023; found 614.3070; IR (neat) C(=O)N–H 3300 cm^{-1} (br s), CH_2 2900–2750 cm^{-1} (m), C=O 1680 cm^{-1} (s).

Route 2. Thioacetic acid *S*-(2-{2-[(4-{3-[4-(2-benzhydryloxy-ethyl)-piperazin-1-yl]-propyl}phenylcarbomyl)-methoxy]-ethoxy}ethyl) ester (0.6 g, 0.9 mmol) was dissolved in methanolic ammonia (50 mL) and stirred under nitrogen at room temperature for 4 h. Then the solvent was removed by evaporation under reduced pressure and purified by column chromatography on silica eluted with methylene chloride 94%–methanol 5%–triethylamine 1%. This gave 0.5 g (94%) of the product as a pale brown oil.

4.1.8. *N*-(4-{3-[4-(2-Benzhydryloxy-ethyl)piperazin-1-yl]-propyl}-phenyl)-2-[2-(2-chloro-ethoxy)-ethoxy]-acetamide (8). 2-(2-(2-Chloroethoxy)ethoxy)ethanoic acid (0.8 g, 4.3 mmol) was dissolved in dry THF (10 mL) and carbonyl diimidazole (0.7 g, 4.3 mmol) was added. This mixture was stirred at room temperature for 5 min then 1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-aminophenyl)propyl)-piperazine (1.8 g, 4.3 mmol) dissolved in dry THF (10 mL) was added and the mixture was stirred at room temperature overnight. The THF was removed under reduced pressure and the product was purified via column chromatography on silica gel eluted with dichloromethane–methanol (5%). This yielded 1 g (42%) of the product as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.75–1.85 (m, 2H), 2.35 (t, J=8.2 Hz, 2H), 2.47–2.63(m, 12H), 2.69 (t, J=6.1 Hz, 2H), 3.61–3.80 (m, 8H), 4.10 (s, 2H), 5.4 (s, 1H), 7.15 (d, 2ArH), 7.23–7.37 (m, 10ArH), 7.54 (d, 2ArH), 8.67 (br s, NH); ^{13}C NMR ($CDCl_3$) 28.4, 32.9, 42.5, 52.9, 53.4, 57.6, 57.7, 66.7, 69.9, 70.3, 70.7, 71.1, 83.6, 119.8, 126.8, 127.2, 128.1, 128.6, 134.8, 138.2, 142.0, 167.6; IR (neat) C(=O)N–H 3360 cm^{-1} (br s), CH_2 2940–2813 cm^{-1} (m), C=O 1686 cm^{-1} (s); calculated for $C_{34}H_{44}ClN_3O_4$ *m/z* ESI (M+Na) 616.2913; found 161.2939.

4.1.9. Thioacetic acid *S*-(2-{2-[(4-{3-[4-(2-benzhydryloxy-ethyl)-piperazin-1-yl]-propyl}phenylcarbomyl)-methoxy]-ethoxy}ethyl) ester (9). *N*-(4-{3-[4-(2-Benzhydryloxy-ethyl)piperazin-1-yl]-propyl}-phenyl)-2-[2-(2-chloro-ethoxy)-ethoxy]-acetamide (1 g, 1.8 mmol) was dissolved in dry DMF (10 mL), potassium thioacetate (0.6 g, 5.2 mmol) and 4 Å molecular sieves (8 pellets) were added. The mixture was stirred at room temperature over night and filtered. Diethyl ether (100 mL) was added to the filtrate and it was washed with sodium bicarbonate solution (2 M, 1×50 mL). After drying the organic solution over magnesium sulfate the organic solvent was evaporated under reduced pressure and the product was purified by column chromatography on a silica column eluted with dichloromethane–methanol (5%). This gave 0.60 g (73%) of the product as a pale brown oil. ^{1}H NMR ($CDCl_3$) δ 1.72–1.82 (m, 2H), 2.25 (s, 3H), 2.30–2.60 (m, 15H), 2.66 (t, J=5.9 Hz, 2H), 3.10 (t, J=6.4 Hz, 2H), 3.56–3.62 (m, 8H), 4.06 (s, 2H), 5.36 (s, 1H), 7.10 (d, 2ArH), 7.12–7.34 (m, 10ArH), 7.44 (d, 2ArH), 8.70 (br s, NH);); ^{13}C NMR ($CDCl_3$) δ 28.2, 30.1, 32.7, 52.8, 53.2, 53.3, 57.4, 57.5, 66.6, 69.2, 69.3, 70.1, 70.6, 83.4, 119.6, 126.6, 127.0, 127.9, 128.4, 134.7, 137.9, 141.9, 167.4, 194.8; IR (neat) C(=O)N–H 3347 cm^{-1} (br s), CH_2 2940–2816 cm^{-1} (m), C=O 1690 cm^{-1} (s); calculated for $C_{36}H_{47}N_3O_5S$ *m/z* ESI (M+Na) 656.3129; found 656.3100.

4.1.10. 11-Bromoundecanoic acid (4-{3-[4-(2-benzhydryl-oxyethyl)-piperazin-1-yl]-propyl}-phenyl)-amide (10). *Method A*. 11-Bromoundecanoic acid (0.42 g, 1.6 mmol) was dissolved in dry dichloromethane (50 mL) and thionyl chloride (1 mL) was added. A catalytic quantity of dry dimethyl formamide (1 drop) was added and the mixture was heated at reflux for 30 min. The solvent was removed under reduced pressure and the acid chloride was dissolved in dry dichloromethane (20 mL). This solution was added dropwise to a methylene chloride solution containing 1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-aminophenyl)propyl)-piperazine (0.64 g, 1.5 mmol) and triethylamine (1 mL). The solution was stirred at room temperature for 4 days. Then the solvent was removed under reduced pressure and the product was purified on a silica column eluted with a gradient system running from dichloromethane to dichloromethane–methanol (5%). This yielded 0.19 g (23%) of the product as a pale yellow oil. ^{1}H NMR ($CDCl_3$) 1.15–1.4 (m, 12H), 1.50–1.62 (m, 6H), 2.19–2.55 (m, 14H), 2.59 (t, J=5.8 Hz, 2H), 3.29 (t, J=6.7 Hz, 2H), 3.52 (t, J=5.8 Hz, 2H), 5.28 (s, 1H), 6.99 (d, 2ArH), 7.11–7.26 (m, 10ArH), 7.34 (d, 2ArH), 7.63 (br s, NH); ^{13}C NMR ($CDCl_3$) δ 25.5, 28.0, 28.3, 28.5, 29.1, 29.2, 32.6, 32.9, 33.9, 37.4, 52.9, 53.3, 57.7, 66.7, 83.7, 119.8, 126.8, 127.2, 128.2, 128.6, 135.8, 137.6, 142.0, 171.4; calculated for $C_{39}H_{54}BrN_3O_2$ *m/z* FAB (M+H) 676.3478; found 676.3454; IR (neat) C(=O)N–H 3290 cm^{-1} (br s), CH_2 2920–2812 cm^{-1} (m), C=O 1676 cm^{-1} (s).

Method B. 11-Bromoundecanoic acid (1.06 g, 4 mmol) was dissolved in dry tetrahydrofuran (10 mL) and 1,1′-carbonyldiimidazole (0.65 g, 4 mmol) was added. The resulting solution was stirred at room temperature for 1 h, then 1-[2-[bisphenylmethoxy]ethyl]-4-(3-(4-aminophenyl)propyl)-piperazine (1.7 g, 4 mmol) was dissolved in dry tetrahydrofuran (10 mL). The solution was stirred at room temperature for 4 days, after which the solvent was removed under reduced pressure and the product was purified on a silica column eluted with a gradient system running from dichloromethane to dichloromethane–methanol (5%). This yielded 0.75 g (28%) of the product as a pale yellow oil.

4.1.11. Thioacetic acid *S*-[10-(4-{3-[4-(2-benzhydryloxy-ethyl)-piperazin-1-yl}-propyl}-phenylcarbamoyl)-decyl] ester (11). 11-Bromoundecanoic acid (4-{3-[4-(2-benzhydryloxy-ethyl)-piperazine-1-yl]-propyl}-phenyl)-amide (0.19 g, 0.35 mmol) was dissolved in dry dimethyl formamide (4 mL) and potassium thioacetate (0.08 g, 0.7 mmol) was added. The mixture was stirred under nitrogen for 48 h and then it was diluted with diethyl ether (100 mL). This was filtered and evaporated under reduced pressure. The product was purified by column chromatography on silica gel eluted with a gradient system running from dichloromethane to dichloromethane–methanol 5%. This gave 0.058 g (31%) of the product as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.20–1.45 (m, 12H), 1.50–52 (m, 2H), 1.55–1.71 (m, 4H), 2.27–2.35 (m, 5H), 2.44–2.61 (m, 15H), 2.66 (t, J=6.0 Hz, 2H), 2.85 (t, J=7.3 Hz, 2H), 3.59 (t, J=5.9 Hz, 2H), 5.36 (s, 1H), 7.09 (d, 2ArH), 7.18–7.35 (m, 8ArH), 7.41 (d, 2ArH), 7.73 (s, NH); ^{13}C NMR ($CDCl_3$) δ 25.5, 28.4, 28.6, 28.8, 29.0, 29.1, 29.1, 29.2, 29.3, 30.5, 32.9, 37.4, 53.0, 53.5, 57.8, 66.8, 83.7, 119.8, 126.9, 127.3, 128.1, 128.6, 135.8, 137.7, 142.1, 171.3, 195.9; calculated for $C_{41}H_{57}N_3O_3S$ *m/z* FAB (M+H) 672.4199; found 672.4178; IR (neat) C(=O)N–H 3300 cm^{-1} (br s), CH_2 2927–2812 cm^{-1} (m), C=O 1690 cm^{-1} (s).

4.1.12. 11-Mercaptoundecanoic acid (4-{3-[4-(2-benzhydryloxyethyl)-piperazin-1-yl]-propyl}-phenyl)-amide (12). Thioacetic acid *S*-[10-(4-{3-[4-(2-benzhydryloxy-ethyl)-piperazin-1-yl}-propyl}-phenylcarbamoyl)-decyl] ester (0.058 g, 0.11 mmol) was dissolved in degassed methanol (10 mL) and methanolic ammonia (10 mL) was added. The mixture was stirred at room temperature under nitrogen for 2 h and evaporated. The product was purified by column chromatography on silica gel eluted with a gradient system running from dichloromethane to dichloromethane–methanol 7%–triethylamine 3%. The base was obtained as a yellow oil and this was converted to the oxalate salt, by dissolving the base in methanol (50 mL) and adding a solution of oxalic acid (1 g) dissolved in methanol. The resulting oxalate salt precipitated from methanol. This was removed by filtration and dried under reduced pressure. This gave 30 mg (51%) of the product as a white solid. ^{1}H NMR ($CDCl_3$) δ 1.27–1.31 (m, 15H), 1.30–1.69 (m, 6H), 2.50–2.59 (m, 16H), 2.69 (t, J=6.0 Hz, 2H), 3.59 (t, J=6.0 Hz, 2H), 5.36 (s, 1H), 5.47 (br s, NH), 7.10 (d, 2ArH), 7.22–7.40 (m, 12ArH); ^{13}C NMR ($CDCl_3$) δ 24.5, 25.5, 28.1, 28.2, 28.9, 29.1, 29.2, 29.3, 29.3, 32.9, 33.9, 37.6, 52.7, 53.1, 57.5, 57.6, 55.7, 83.8, 119.9, 126.9, 127.3, 128.2, 128.7, 135.8, 137.7, 142.1, 171.3; calculated for $C_{39}H_{55}N_3O_2S$ *m/z* FAB (M+H) 630.4093; found 630.4085; CHN (0.75 H_2O) calculated C=72.80%, H=8.85%, N=6.53%; found C=72.96%, H=8.67%, N=6.41%; IR (neat) C(=O)N–H 3299 cm^{-1} (br s), CH_2 2926–2810 cm^{-1} (m), SH 2360 cm^{-1} (s), C=O 1659 cm^{-1} (s).

4.1.13. 1-[2-[4,4′-Fluorobenzhydryloxy]ethyl]-4-(3-(4-nitrophenyl)-1-oxopropyl)piperazine (13). *para*-Nitrohydrocinnamic acid (3 g, 15.4 mmol) was added to dry toluene (100 mL), in a 250 mL round bottomed flask equipped with a stirrer and a reflux condenser. Oxalyl chloride (2 mL) was added, after which a catalytic quantity of dry DMF (2 drops) was also added, and the mixture was stirred at room temperature for 2 h. The solvent was removed by evaporation, and the crude acid chloride was dissolved in dry dichloromethane (100 mL). Dry triethylamine (10 mL) and 1-[2-[4,4′-fluorobenzhydryloxy]ethyl]-piperazine (4.68 g, 15.4 mmol) was dissolved in dry dichloromethane (50 mL) and added to the solution of *p*-nitrohydrocinnamyl chloride. The mixture was heated at reflux for 18 h under argon in a 250 mL round bottomed flask equipped with a stirrer and reflux condenser. Then the solvent was removed under reduced pressure and the product is purified using silica gel chromatography eluted with dichloromethane 96%–methanol. 8.0 g (97%) of the product was obtained as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.37 (br s, 4H), 2.55–2.60 (m, 4H), 2.99 (t, J=7.5 Hz, 2H), 3.3 (t, J=4.6 Hz, 2H), 3.45 (t, J=5.6 Hz, 2H), 3.51 (t, J=4.9 Hz, 2H), 5.24 (s, 1H), 6.88–6.94 (m, 4ArH), 7.16–7.20 (m, 4ArH), 7.30 (d, 2ArH), 8.04 (d, 2ArH); ^{13}C NMR ($CDCl_3$) δ 30.8, 33.9, 41.6, 45.3, 53.1, 53.5, 57.6, 66.8, 82.5, 115.1, 115.4, 123.6, 128.4, 128.5, 129.3, 137.6, 137.7, 146.4, 149.3, 160.4, 163.7, 169.5; calculated for $C_{28}H_{29}F_2N_3O_4$ *m/z* ESI (M+Na) 532.2024; found 532.2057, (M+H) calculated 510.2204; found 510.2192; CHN (0.1H_2O) calculated C=65.71, H=5.69, N=8.22; found C=65.77, H=5.69, N=8.18; IR (neat) CH_2 2821–2939 cm^{-1} (m), C=O 1640 cm^{-1} (s).

4.1.14. 1-[2-[4,4′-Fluorobenzhydryloxy]ethyl]-4-(3-(4-nitrophenyl)propyl)piperazine (14). 1-[2-[4,4′-Fluorobenzhydryloxy]ethyl]-4-(3-(4-nitrophenyl)-1-oxopropyl)-piperazine (8 g, 15 mmol) was dissolved in dry THF (100 mL) in a 250 mL round bottomed flask equipped with a stirrer and a reflux condenser. Alane in toluene (0.5 M, 59 mL) was added and stirred at room temperature for 30 min. The reaction was quenched with sodium hydroxide solution (10%, 200 mL). The aqueous solution was extracted with diethyl ether (3×150 mL), dried over magnesium sulfate filtered and evaporated. The product was purified using silica gel chromatography eluted with methylene chloride 96%–methanol. This yielded 6.14 g (80%) of the product as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.79–1.88 (m, 2H), 2.35 (t, J=7.6 Hz, 2H), 2.46–22.54 (m, 8H), 2.67 (t, J=6.0 Hz, 2H), 2.74 (t, J=7.7 Hz, 2H), 3.57 (t, J=5.0 Hz, 2H), 5.34 (s, 1H), 6.97–7.02 (m, 4ArH), 7.26–7.35 (m, 6ArH), 8.13 (d, 2ArH); ^{13}C NMR ($CDCl_3$) δ 28.0, 33.4, 53.1, 53.5, 57.3, 57.7, 66.8, 82.4, 115.0, 115.3, 123.5, 128.4, 128.5, 129.1, 137.7, 137.8, 146.2, 150.0, 160.4, 163.6; calculated for $C_{28}H_{31}F_2N_3O_3$ *m/z* ESI (M+Na) 518.2231; found 518.2252, (M+H) calculated 496.2412; found 496.2442; CHN calculated C=67.86, H=6.31, N=8.48; found C=67.75, H=6.39, N=8.26; IR (neat) CH_2 2775–2941 cm^{-1} (m).

4.1.15. 1-[2-[4,4′-Fluorobenzhydryloxy]ethyl]-4-(3-(4-aminophenyl)propyl)piperazine (15). 1-[2-[4,4′-Fluorobenzhydryloxy]ethyl]-4-(3-(4-nitrophenyl)-1-oxopropyl)-piperazine (6.14 g, 12 mmol) was dissolved in absolute ethanol (10 mL) in a 100 mL round bottomed flask equipped with a stirrer and reflux condenser. Tin(II)chloride dihydrate (11.14 g) was added and the mixture was heated at reflux for 90 min. The solution was poured into crushed ice and a solution of sodium carbonate (5%) in water was added until a pH of 8 is obtained. The aqueous solution was extracted with ethyl acetate (3×200 mL) and this was dried over

magnesium sulfate. The product was purified using column chromatography on silica gel eluted with methylene chloride 96%–methanol. This gave 5.11 g (92%) of the product as a red oil. ^{1}H NMR ($CDCl_3$) δ 1.59–1.70 (m, 2H), 2.23 (t, J=8.0 Hz, 2H), 2.30–2.49 (m, 10H), 2.54 (t, J= 5.9 Hz, 2H), 3.45 (t, J=5.6 Hz, 4H), 5.21 (s, 1H), 6.47 (d, 2ArH), 6.83–6.90 (m, 6ArH), 7.13–7.18 (m, 4ArH); ^{13}C NMR ($CDCl_3$) δ 28.6, 32.6, 53.0, 53.4, 57.6, 57.8, 66.6, 82.2, 114.8, 114.9, 115.1, 128.4, 128.5, 128.9, 131.7, 137.6, 137.7, 144.1, 160.2, 163.5; calculated for $C_{28}H_{33}F_2N_3O$ *m/z* ESI (M+Na) 488.2489; found 488.2494, (M+H) calculated 466.2670; found 466.2677; CHN (0.25 H_2O) calculated C=71.53, H=7.08, N=8.94; found C=71.53, H=7.17, N=8.78; IR (neat) NH_2 3200–3400 cm^{-1} (m), CH_2 2775–2941 cm^{-1} (m).

4.1.16. 11-Bromoundecanoic acid (4-{3-[4-(4,4′-fluorobenzhydryloxy-ethyl)-piperazine-1-yl]-propyl}-phenyl)-amide (16). 11-Bromoundecanoic acid (0.65 g, 2.4 mmol) was dissolved in dry tetrahydrofuran (10 mL) and carbonyl diimidazole (0.4 g, 2.4 mmol) was added. The mixture was stirred at room temperature for 30 min. Then 1-[2-[4,4′-fluorobenzhydryloxy]ethyl]-4-(3-(4-aminophenyl)propyl)-piperazine (0.57 g, 1.2 mmol) in dry tetrahydrofuran (10 mL) was added. The solution was stirred at room temperature for 4 days, and then the solvent was removed under reduced pressure. The product was purified on a silica column eluted with dichloromethane–methanol (5%). This yielded 280 mg (30%) of the product as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.1–1.25 (m, 12H), 1.54–1.67 (m, 6H), 2.16–2.47 (m, 14H), 2.56 (t, J=5.8 Hz, 2H), 3.46 (t, J=6.0 Hz), 3.80 (t, J=6.9 Hz, 2H), 5.23 (s, 1H), 6.86–7.00 (m, 6ArH), 7.15–7.20 (m, 4ArH), 7.38 (d, 2ArH), 8.62 (br s, NH); ^{13}C NMR ($CDCl_3$) δ 25.4, 26.1, 28.2, 28.6, 30.0, 29.4, 30.7, 32.8, 37.2, 52.8, 53.2, 57.6, 57.7, 66.5, 82.3, 114.9, 115.2, 119.0, 119.8, 128.3, 128.4, 128.5, 136.0, 137.1, 137.5, 137.6, 160.4, 163.6, 171.5; calculated for $C_{39}H_{52}BrF_2N_3O_2$ *m/z* ESI (M+H) 712.3289; found 712.3261; IR (neat) N–H 3300–3400 cm^{-1} (br s), CH_2 2815–2928 cm^{-1} (m), C=O 1650 cm^{-1} (s).

4.1.17. Thioacetic acid *S*-[10-(4-{3-[4-(2-(4,4′-fluorobenzhydryloxy)-ethyl)-piperazin-1-yl}-propyl}-phenylcarbamoyl)-decyl] ester (17). 11-Bromoundecanoic acid (4-{3-[4-(2-(4,4′-fluorobenzhydryloxy)-ethyl)-piperazine-1-yl]-propyl}-phenyl)-amide (0.20 g, 0.28 mmol) was dissolved in dry dimethyl formamide (4 mL) and potassium thioacetate (0.065 g, 0.56 mmol) was added. The mixture was stirred under nitrogen for 48 h, and then it was diluted with diethyl ether (100 mL). This was filtered and evaporated under reduced pressure. The product was purified by column chromatography on silica gel eluted with dichloromethane–methanol 5%. This gave 100 mg (54%) of the product as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.2–1.35 (m, 12H), 1.44–1.50 (m, 2H), 1.60–1.78 (m, 4H), 221–2.28 (m, 5H), 2.32 (t, J=6.0 Hz, 2H), 2.40–2.53 (m, 6H), 2.32 (t, J=6.0 Hz, 2H), 2.78 (t, J= 5.5 Hz, 2H), 3.49 (t, J=4.4 Hz, 2H), 5.25 (s, 1H), 6.90–6.95 (m, 4ArH), 7.03 (d, 2ArH), 7.18–7.24 (m, 4ArH), 7.27 (s, NH), 7.35 (d, 2ArH); ^{13}C NMR ($CDCl_3$) δ 25.6, 28.4, 28.6, 28.9, 29.0, 29.1, 29.2, 29.2, 29.4, 33.0, 37.6, 53.0, 53.4, 57.6, 57.7, 66.7, 82.4, 115.0, 115.3, 119.8, 128.4, 128.5, 128.7, 135.8, 137.7, 137.8, 160.4, 163.7, 171.4, 196.1; calculated for $C_{41}H_{55}F_2N_3O_3S$ *m/z* ESI (M+Na) 730.3830; found 730.3795, (M+H) calculated 708.4010; found 708.3994; IR (neat) C(=O)N–H 3303 cm^{-1} (br s), CH_2 2929–2812 cm^{-1} (m), C=O 1689 cm^{-1} (s).

4.1.18. 11-Mercaptoundecanoic acid (4-{3-[4-(2-(4,4′-fluorobenzhydryloxy)-ethyl)-piperazin-1-yl]-propyl}-phenyl)-amide (18). Thioacetic acid *S*-[10-(4-{3-[4-(2-(4,4′fluorobenzhydryloxy)-ethyl)-piperazin-1-yl}-propyl}-phenylcarbamoyl)-decyl] ester (50 mg, 0.076 mmol) was dissolved in degassed methanol (10 mL) and methanolic ammonia (10 mL) was added. The mixture was stirred at room temperature under nitrogen for 2 h and evaporated. The product was purified by column chromatography on silica gel eluted with a gradient system of methylene chloride then methylene chloride (96%)–methanol, giving 24 mg (51%) as a red oil. ^{1}H NMR ($CDCl_3$) δ 1.21–1.35 (m, 15H), 1.54–1.63 (m, 4H), 1.83–1.86 (m, 2H), 2.28 (t, J=7.6 Hz, 2H), 2.44 (t, J=7.0 Hz, 2H), 2.52 (t, J=7.2 Hz, 2H), 2.58–2.66 (m, 10H), 3.51 (t, J=5.5 Hz), 5.25 (s, 1H), 6.89–6.93 (m, 4ArH), 7.02 (d, 2ArH), 7.16–7.28 (m, 4ArH), 7.39 (d, 2ArH), 7.61 (s, NH); ^{13}C NMR ($CDCl_3$) δ 25.64, 27.3, 28.4, 29.1, 29.2, 29.3, 29.4, 32.7, 37.7, 39.2, 52.3, 52.5, 57.4, 66.6, 82.6, 115.2, 115.5, 120.0, 128.5, 128.6, 128.7, 136.2, 137.6, 137.6, 160.5, 163.8, 171.6; calculated for $C_{39}H_{54}F_2N_3O_2S$ *m/z* ESI (M+H) 666.3905; found 666.3901; IR (neat) IR C(=O)N–H 3305 cm^{-1} (br s), CH_2 2928–2818 cm^{-1} (m), C=O 1687 cm^{-1} (s).

4.2. Nanocrystal ligand exchange methodology

The ligand exchanges were performed using a 1.9 μM solution of trioctylphosphine oxide (TOPO) coated cadmium selenide/zinc sulfide nanocrystals dissolved in hexanes. These dots had a fluorescence emission wavelength of 625.8 nm and an absorption maximum at 610 nm. 0.5 mL of this solution was added to 20 mL of methanol at which point the TOPO coated dots precipitated out of solution and were collected by centrifugation. The methanol was decanted and the dots were washed with another 20 mL of methanol. After centrifugation and decanting the methanol, the dots were dissolved in pyridine (0.5 mL). This solution was stirred at 60°C for 18 h. The solution was allowed to cool to room temperature and the dots were precipitated by adding hexanes (20 mL). This process was repeated three times and then the precipitated dots were dissolved in pyridine (0.5 mL). The concentration was determined using UV–vis spectroscopy based upon an extinction coefficient of 700000.[34] After measuring the concentration we were able to calculate the number of moles of dots present in the pyridine solution. Then we added 100 equiv. of ligand dissolved in 0.1 mL of methylene chloride and this mixture was stirred at 60°C for 2 h. After cooling to room temperature hexanes (20 mL) were added to the dots and the resultant precipitate was collected by centrifugation. The core/shells were washed with ethyl acetate (3×20 mL) to remove unbound ligand. Then they were dissolved in dimethyl formamide (0.5 mL) and thioacetic acid (0.5 mL) was added. The thioacetic acid was added as a co-solubility ligand in order to increase the water solubility of the nanocrystal ligand conjugate. This mixture was stirred at ambient temperature for 24 h and then potassium tertiary butoxide (1.8 g) dissolved in methanol

(20 mL) was added at 0°C. The solid was removed by centrifugation and excess potassium tertiary butoxide was removed by washing with methanol (2×20 mL). Then the solid was dried under reduced pressure and dissolved in DI water. It was kept at 4°C and used when required in biological assays.

Ligand coverage was determined using Rutherford Backscattering Spectroscopy (RBS) as previously described.[10,35] for the GBR 12909 derivative (compound **18**) conjugated to CdSe/ZnS core/shell nanocrystals. The fluorine atoms in **18** serve as a marker to quantify the coverage of the ligand, as N and O are always present in the RBS spectrum due to exposure of the substrate to air. In this experiment, samples were prepared by dropping 0.2 mL of a concentrated nanocrystal solution onto a 1 cm^2 graphite substrate and wicking off excess solution. RBS experiments were performed in a high vacuum chamber ($\sim 10^{-7}$ Torr) with a 1.8 MeV He ion beam at normal incidence. Scattered ions were collected on axis at 180° with a solid-state detector. To obtain the F and Zn ratios in order to determine ligand coverage, the individual peaks in the RBS spectrum are integrated and normalized by the square of their atomic numbers (the intensity of the peaks in the spectrum is proportional to the square of the atomic number of the element and its relative abundance). The core/shell nanocrystals have a 48 Å CdSe core and a 15 Å ZnS shell. To obtain the percentage ligand coverage from the Zn/F ratio, the core/shells are assumed to be spherical. In this way we find ~20 ligands on the surface of the nanocrystal. Addition of mercaptoacetic acid during the ligand exchange procedure does not displace the ligand from the surface of the nanocrystals, as verified by using RBS to analyze for F in the methanol washings.

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