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Universal polyethylene glycol linkers for attaching receptor ligands to quantum dots

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Abstract—Biologically active small molecule derivatives that can be conjugated to quantum dots have the promise of revolutionizing fluorescent imaging in biology. In order to achieve this several technical hurdles have to be surmounted, one of which is non-specific adsorption of quantum dots to cell membranes. Pegylating quantum dots has been shown to eliminate non-specific binding. Consequently it is necessary to develop a universal synthetic methodology to attach small molecule ligands to polyethylene glycol. These pegylated small molecules may then be conjugated to the surfaces of quantum dots. Ideally this universal strategy should be adaptable and be applicable to PEG chains of varying lengths. This paper describes the development of one such methodology and the synthesis of a pegylated derivative of the known $5HT_2$ agonist 1-(2-aminopropyl)-2,5-dimethoxy benzene. This compound was tested and found to be an agonist for the $5HT_{2A}$ and $5HT_{2C}$ receptor having EC_{50} values of 250 and 50 nM, respectively.

Since their introduction into biological imaging in 1998[1,2] quantum dots have shown great promise as fluorescent imaging agents. Their unique fluorescent properties include narrow emission spectra, photostability, and high quantum yields making them superior to conventional organic fluorescent dyes.[3–9] To have biologically specificity the surfaces of quantum dots are modified with biologically active molecules such as proteins,[10,11] nucleic acids,[12–14] peptides,[15–18] small molecules,[19–23] and antibodies.[24–29] Studies have also reported their use in vitro[30] and in vivo. For example, quantum dots have been used as medical imaging agents to label sentinel lymph nodes thus eliminating the requirement for radioactive tracers.[31]

In order to use quantum dots as imaging agents in biology a number of physical properties are required. These include colloidal stability in saline, high quantum yields, and low non-specific adsorption to cellular membranes. A number of strategies have been developed to achieve these goals; these include encapsulating in micelles,[32] silinization,[33] encapsulation in amphiphilic polymers,[26] and encapsulation in proteins such as avidin and streptavidin.[26] Quantum dots encapsulated in modified amphiphilic polyacrylamide (AMP) are extensively used. This polymer has carboxylic acid functionalities that can be conjugated to ligands using coupling reagents such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC). We have reported that the non-specific adsorption of AMP dots to cellular membranes may be reduced by pegylating the surfaces of the AMP dots.[34]

In order to take advantage of this PEG/AMP strategy we have developed a synthetic methodology to synthesize PEG derivatives that may be attached to small organic molecules or peptides and subsequently conjugated to AMP dots. We have designed this methodology to be generic and applicable to a wide range of PEG lengths as we have reasoned that the length of the PEG chain may be important in ligand binding. In this letter we report the synthesis and characterization of a universal PEG linker. We demonstrate this strategy for 1-(2-aminopropyl)-2,5-dimethoxy benzene (**1**), a compound that has high affinity for $5HT_{2A}$ and $5HT_{2C}$ receptors.

In early work we synthesized a derivative of the known $5HT_{2A}$ agonist (**1**). An alkyl linker arm was attached to

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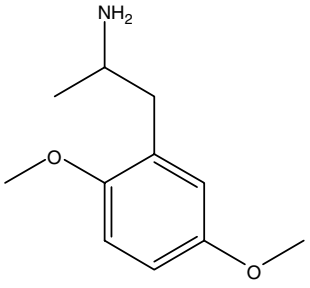
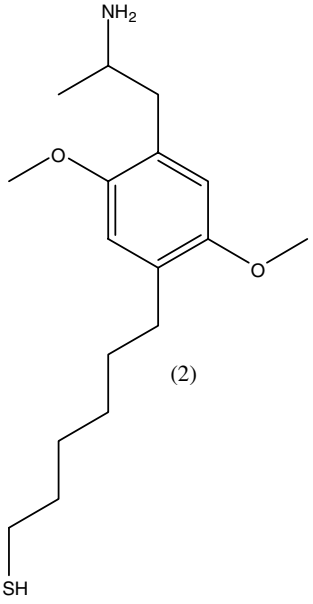


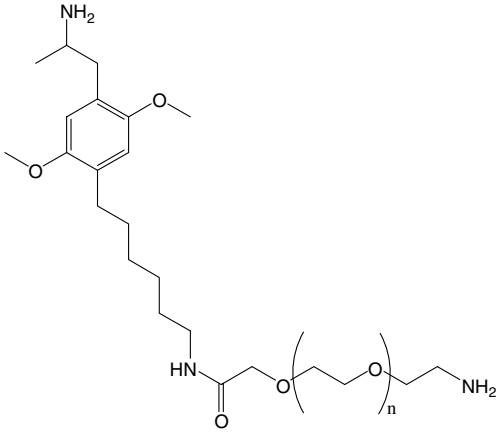
(1)



(2)

the aromatic ring resulting in 6-(2,5-dimethoxy-4-(2-aminopropyl)phenyl)hexylthiol (**2**). The ability of (**2**) to initiate the production of inositol pyrophosphate in stably transfected LLC cells was measured and found to be 88 nM for the $5HT_{2A}$ receptor. The synthetic route used to synthesize this ligand is described in an earlier publication.[35]

To reduce non-specific adsorption to cell membranes we have designed an analog of (**2**) that incorporates a PEG chain (**3**). This PEG chain is amino terminated and may be conjugated to the AMP surface of quantum dots.



(3)

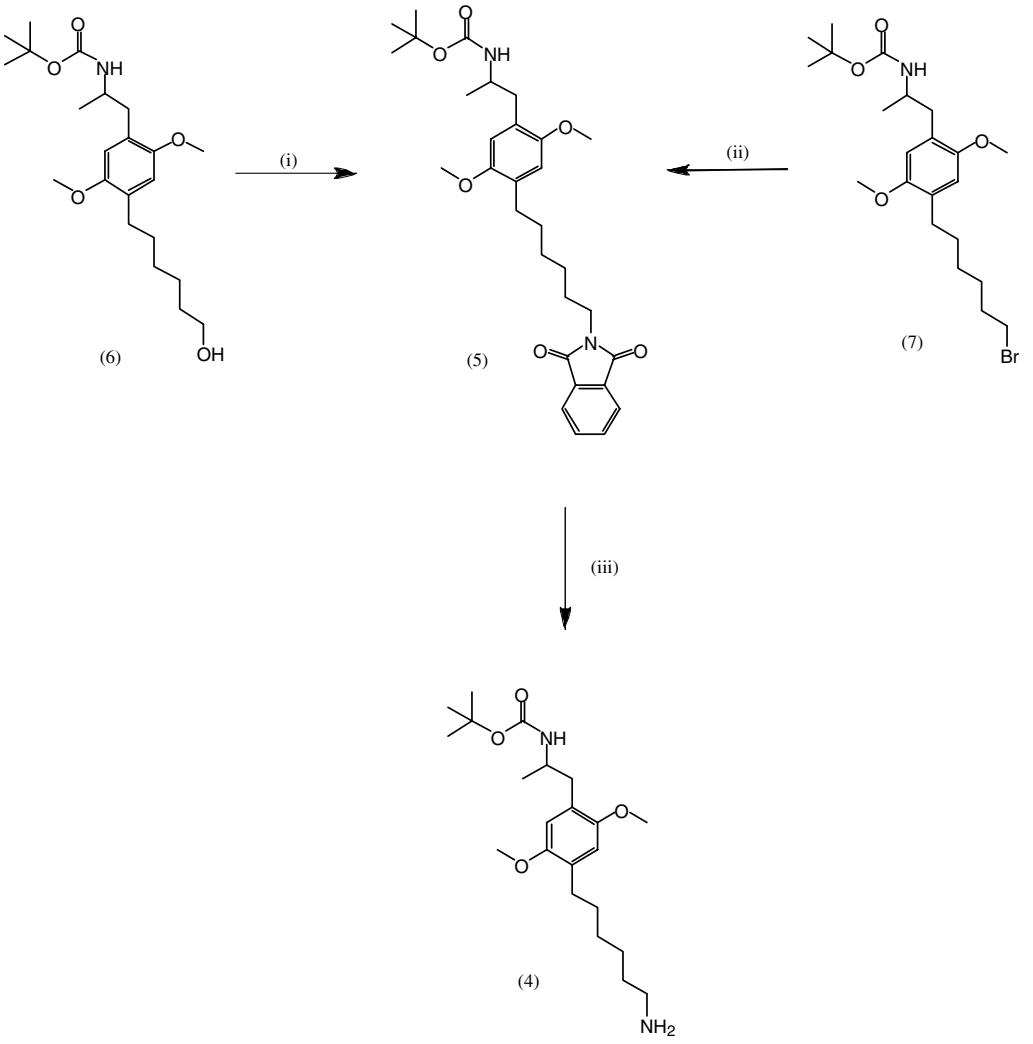
The synthetic methodology used to synthesize our functionalized PEGs and attach them to our ligand is outlined in Schemes 1–3. The synthesis of the *t*-Boc-protected $5HT_2$ agonist derivative is shown in Scheme 1.

tert-butyl 1-(4-(6-aminohexyl)-2,5-dimethoxyphenyl)-propan-2-ylcarbamate (**4**) was synthesized by converting the intermediate (**5**) to (**4**) in a 73% yield using hydrazine monohydrate. *tert*-Butyl 1-(4-(6-(1,3-dioxoisoindolin-2-yl)hexyl)-2,5-dimethoxyphenyl)propan-2-ylcarbamate (**5**) was synthesized using two different methods: in one method *tert*-butyl 1-(4-(6-hydroxyhexyl)-2,5-dimethoxyphenyl)propan-2-ylcarbamate (**6**) was converted to the intermediate (**5**) using a Mitsonubi reaction giving (**5**) in a 67% yield. Alternatively 6-(2,5-dimethoxy-4-(2-[*N*-(*tert*-butoxycarbonyl)aminopropyl]phenyl)hexylbromide (**7**) was reacted with potassium phthalimide giving (**5**) in a 40% yield. These compounds were characterized by ^1H NMR and ^{13}C NMR.

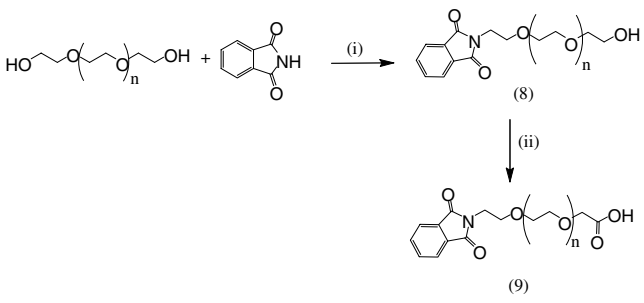
The polyethylene glycol linker arm was synthesized from a commercially available polyethylene glycol and the synthetic methodology used is shown in Scheme 2. All the pegylated derivatives were characterized by NMR and MALDI mass spectrometry. For our purposes we selected PEG 600 which was initially reacted with phthalimide using a Mitsonubi reaction giving the mono substituted product (**8**) in a 40% yield. The terminal hydroxyl group was oxidized using 70% nitric acid at 80 °C giving the desired carboxylic acid (**9**) in a 99% yield.

Compound **4** was coupled to the PEG chain and the methodology used is outlined in Scheme 3.

The monophthalimide derivative of the PEG 600 carboxylic acid (**9**) was coupled to 0.85 g of (**4**) using a DCC coupling in methylene chloride to give 1.1 g of the phthalimido protected PEG derivative of (10). The phthalimide was removed by dissolving (10) in ethanol and adding hydrazine monohydrate. This gave 0.85 g of (11). The *t*-Boc-protecting group was removed by stirring a solution containing 0.1 g of (11) dissolved in



Scheme 1. Reagents: (i) Phthalimide, DIAD, PPh_3; (ii) Potassium phthalimide; (iii) hydrazine hydrate.



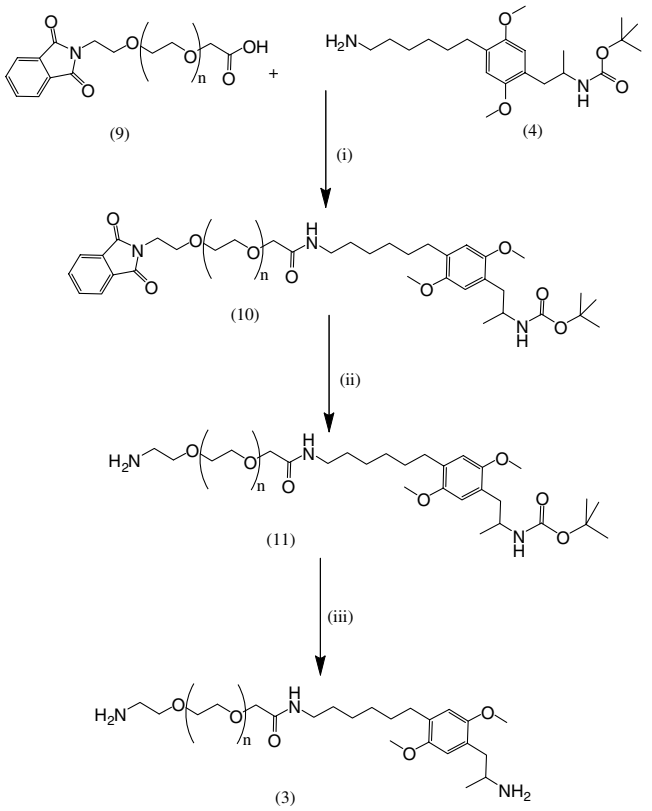
Scheme 2. Reagents: (i) PPh_3, DIAD; (ii) 70% HNO_3.

methylene chloride with trifluoroacetic acid resulting in 0.09 g of (III).

The MALDI spectrum of compound **3** is shown in Figure 1. The peaks shown indicate that there are different length PEG chains in the polydispersed PEG 600 conjugated to the agonist. As can be seen the predominant masses are 902.6, 946.6, and 990.7 which correspond to $n = 11$, 12, and 13. An AM1 optimization of an entirely *trans* isomer with $n = 12$ renders a total length from quantum dot attachment to ligand of ~46 Å, indicating that when the ligand is bound to the AMP dot the ligand is well removed from the AMP surface.[36] Shorter and longer PEG chains were also observed in MALDI however these were not the predominant species.

Compound **3** was tested for its ability to initiate Phosphoinositide hydrolysis, which was determined in NIH3T3 cell lines stably expressing 5-HT_{2A} or 5-HT_{2C} receptors as described by Barker et al.[37] Compound **3** stimulated phosphoinositide hydrolysis in NIH 3T3 cells expressing the rat 5-HT_{2A} receptor with an EC_{50} of 250 nM. In NIH 3T3 cells expressing the rat 5-HT_{2C} receptor, the EC_{50} of compound **3** was 50 nM. Thus, compound **3** demonstrates agonist properties at the 5-HT_{2A} and 5-HT_{2C} receptor.

One concern when conjugating positively charged small molecules to the surface of negatively charged AMP dots is the possibility of electrostatic interactions causing the ligand to fold over. We have studied similar systems where this was not any apparent problem. In one instance serotonin was conjugated to carboxylic acid coated dots through a short linker.[20] Electrostatic



Scheme 3. Reagents: (i) DCC, NHS; (ii) Hydrazine; (iii) trifluoroacetic acid.

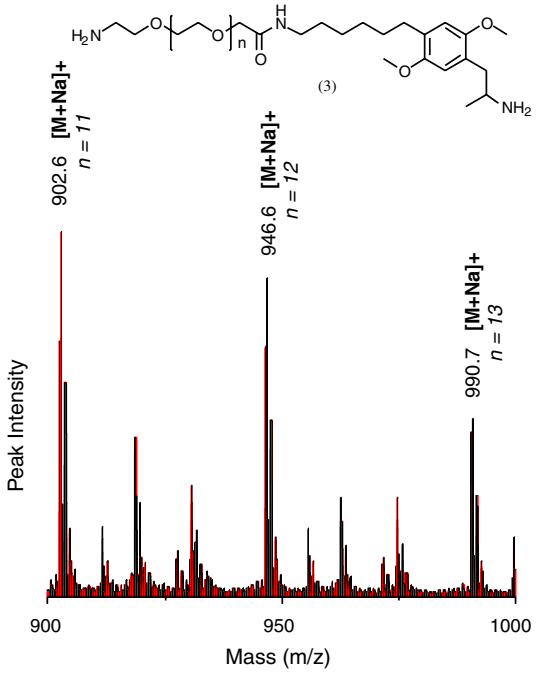


Figure 1. MALDI-TOF mass spectrum of compound **3**.

interactions between the ligand and the dots' surface may be likely due to the proximity of the serotonin to the surface of the dot, however the serotonin-conjugated nanocrystals were biologically active as determined by competitive uptake assay, electrophysiology, and fluorescent labeling. In general one might expect that a ligand that is further removed on a longer PEG should have weaker interactions with the dots' surface.

In conclusion we have developed a universal strategy for synthesizing amino-terminated PEGs that may be attached to agonists and antagonists and conjugated to the surfaces of AMP-coated quantum dots. This chemistry is generic and may be used with a wide range of PEG chain lengths and a number of different agonists or antagonists. When 1-(2-aminopropyl)-2,5-dimethoxy benzene was conjugated to PEG chains biological activity was retained and we hope to use such systems in future imaging applications.

Acknowledgments

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