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Facile, Efficient Conjugation of a Trifunctional Lanthanide Chelate to a Peripheral Benzodiazepine Receptor Ligand

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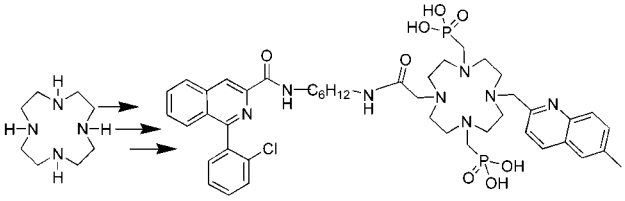
ABSTRACT



Receptor-mediated imaging and therapy of diseased tissue is rapidly gaining favor in the medical community. The synthesis and facile aqueous/organic coupling of a peripheral-type benzodiazepine receptor ligand to a cyclen-based fluorophore is described herein. The contrast agent QM-CTMC-PK11195, when chelated with lanthanides, produces bright luminescence and good MRI contrast and can potentially serve as an imaging and demarcation agent for certain types of cancers.

Benzodiazepines continue to be among the most highly prescribed central nervous system (CNS) drugs as a result of their pharmacological properties.[1] Overexpressed by certain types of cancerous tissue, the peripheral-type benzodiazepine receptor (PBR) is a class of binding sites for benzodiazepines. It has been well documented that this receptor also binds other types of small organic molecules (e.g., isoquinolines,[2] imidazopyridines,[3] and indole[4] derivatives) with very high affinity.

Receptor-mediated endocytosis can be employed as an attractive means of affording cell-selective targeting.[5,6] This process couples high transport capacity with ligand-specific cell targeting, yielding a methodology to efficiently deliver therapeutic and contrast agents to diseased tissue.[7] To this end, the ability to conjugate brightly luminescent fluorophores to biologically up-regulated substrates is a crucial step toward synthesizing contrast agents that clearly demarcate diseased tissue.[8] Recently, it has been shown that a PBR ligand conjugate is able to retain the biological activity of the native ligand.[1] In an analogous way, by conjugating a well-established tumor-specific ligand (PK-11195 analogue) to one of our unique trifunctional lanthanide chelates, it is believed that the resulting agent can be used for in vitro and in vivo visual imaging of certain cancer bearing tissues.[9]

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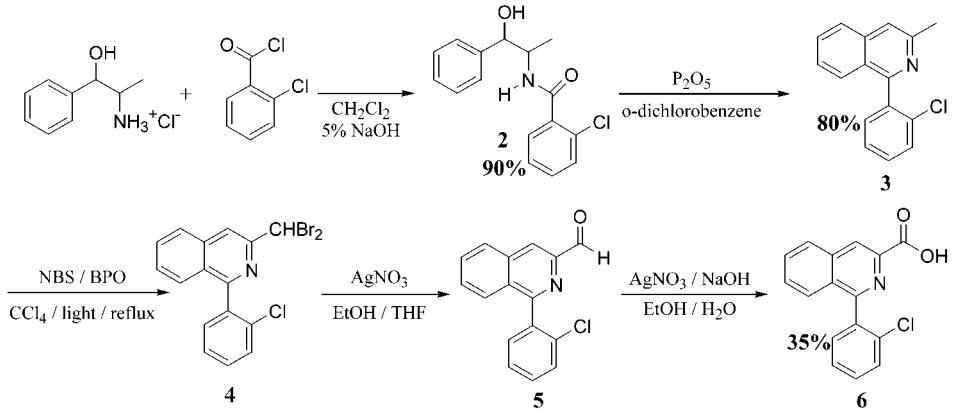
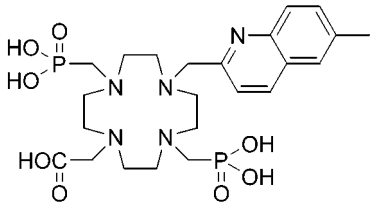
Scheme 1



Development of this new cell-specific, visual contrast agent is of paramount importance. Despite aggressive treatment strategies including surgical resection, irradiation, and chemotherapy, many cancer patients succumb to tumors (within weeks to months),[10,11] especially those of the intracranial region.[12−15] Current brain tumor imaging methods (detection of edema and the use of MRI)[16] are severely limited because they image the tumor indirectly and fail to fully demarcate the boundaries of disease.[17−19] Because clinical outcome is so closely linked to surgical resection, there is a critical need to develop new strategies for intraoperative imaging of brain cancer. The ideal brain cancer imaging modality would be tumor-specific, allowing infiltrating tumor margins to be well resolved, and provide real-time intraoperative fluorescence that correlates with anatomic imaging (MRI). We report methodology here to synthesize such an imaging agent.

Chart 1. Compound 1: Trifunctional Cyclen-Based Lanthanide Chelate, QM-CTMC



In previous work reported elsewhere, we have demonstrated that our novel class of nontoxic lanthanide chelates can be efficiently synthesized and provide significant in vivo contrast between normal and diseased (cancer) tissue, facilitating early detection.[20−22]

Recently we have reported a new trifunctional marker that exhibits bright fluorescence (ϕ ~0.25, Stokes shift ca. 300 nm), is chemically stable, and also has the necessary functionality to be conjugated to many types of biologics.[23]

Here, we report synthetic methodology used to prepare and conjugate a PK11195 analogue to our lanthanide chelate for use as a site-directed imaging agent. Our procedure is of particular interest because, by using a well-documented water-stable coupling agent (TSTU),[24] we are able to complete the conjugation under mixed aqueous/organic conditions, unlike typical anhydrous peptide couplings. This procedure will prove to be especially useful for the conjugation chemist whose substrate(s) are not fully soluble in the absence of an aqueous solvent component.

The first step toward the synthesis of the conjugate was to create the conjugable, trifunctional, macrocyclic ligand **1**. This molecule possesses two phosphonic acid pendant arms for strong chelating ability, an energy absorbing/transmitting quinoline chromophore, and a carboxylic acid

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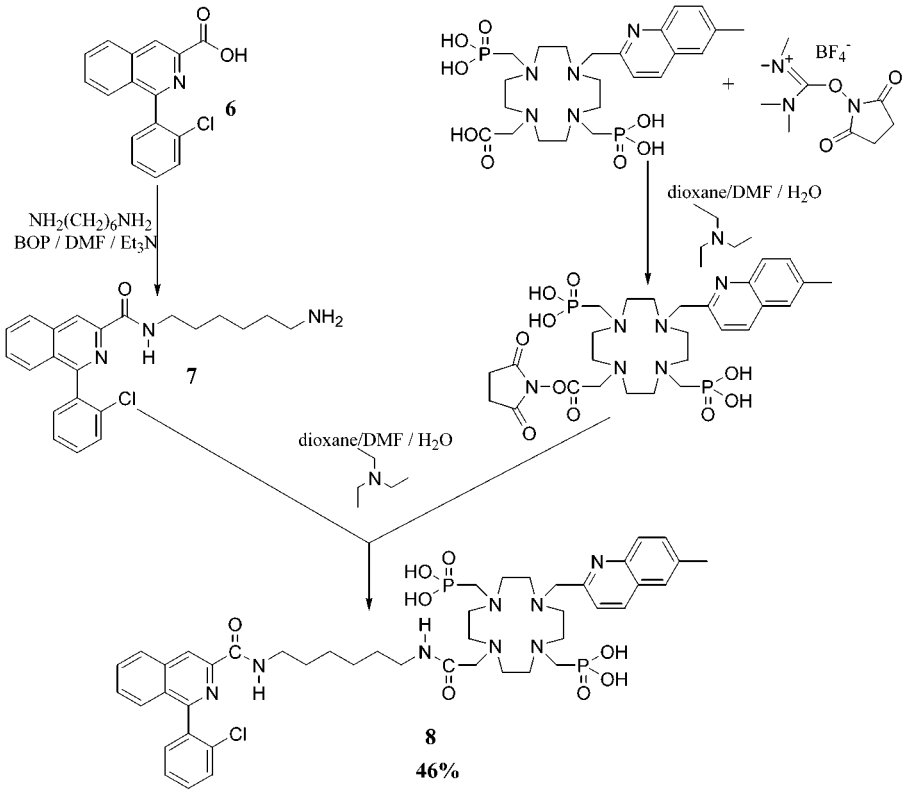
Scheme 2



residue for conjugation. When compound **1** is complexed with Eu^{3+}, the methylquinoline antenna absorbs light at 320 nm and the complex emits the characteristic lanthanide emission pattern from 580 to 700 nm. This large Stokes shift allows complete removal of the signal (fluorescence) from the background noise (excitation).

With **1** synthesized and characterized, we were ready to prepare the conjugable form of PK11195 (Scheme 1).

Initially guided by previously demonstrated methodology,[25] first compound **2** was synthesized by coupling racemic norephedrine with 2-chloro-benzoyl chloride in basic methylene chloride. The compound was easily purified by recrystallization in EtOH and obtained in high yield. Next, a condensation with P_2O_5 in *o*-dichlorobenzene at high temperature formed the isoquinoline ring, yielding compound **3**. Compound **3** was then brominated under free-radical conditions with 2 equiv of NBS, yielding the dibromo compound **4**. Next, **4** was hydrolyzed with the aid of $AgNO_3$ to the aldehyde **5**. Then the same reagent oxidized **5** to the carboxylic acid **6**. Finally, compound **6** is coupled with the hexanediamine linker in dry DMF, yielding a conjugable PK11195 analogue **7** (Scheme 2).

The final procedure in the synthesis of the target compound **8** was to conjugate the fluorophore **1** to the PBR ligand **7**. As seen in Scheme 2, this was accomplished under mixed aqueous/organic conditions utilizing the water-stable coupling reagent TSTU. This was exceedingly important when we discovered that **1** would not remain soluble throughout the course of reaction in organic solvents alone. Using this methodology, compound **8** was obtained pure in reasonably high yield.

Compound **8** is easily complexed at pH 6 with various lanthanides. An indication of complex stability, when Eu^{3+} is chelated with compound **8**, aqueous solutions are brightly luminescent upon excitation with a low energy TLC plate reader lamp (as one would expect from sensitized emission), and these solutions remain luminescent for at least 1 month. To demonstrate the typical luminescence of the conjugated-chelated complex, Figure 1 shows a *photograph* of a 1 μM

Figure 1. Photograph of 1 μM solution of Eu-QM-CTMC-PK11195.

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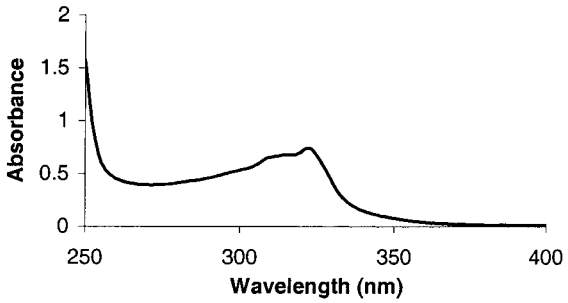

Figure 2. Absorbance spectrum of Eu-QM-CTMC-PK11195.

aqueous solution of Eu-QM-CTMC−PK11195 when illuminated by a TLC plate reader lamp. Note the stark contrast in color between the pink fluorescence (complex) and blue light reflected off the skin (tissue). Figures 2 and 3 show, respectively, the absorbance (antenna) and fluorescence (Eu^{3+}) spectra for the complex.

In summary, we have demonstrated that we can conjugate a PBR ligand to our class of exogenous fluorophores utilizing mixed aqueous/organic conditions, yielding a bioconjugate that could facilitate brain cancer imaging. In general, this procedure should also be applicable to the synthesis of other bioconjugates, particularly those requiring an aqueous environment to facilitate solubility.

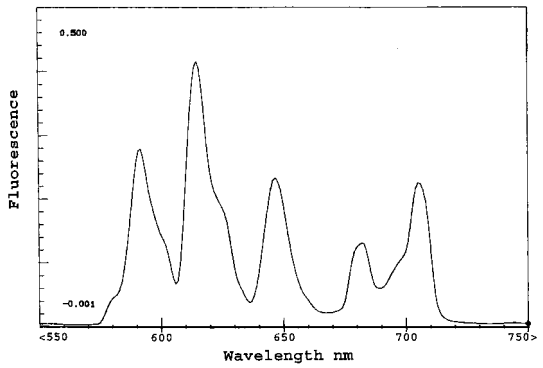

Figure 3. Fluorescence spectrum of Eu-QM-CTMC-PK11195 (excitation 320 nm).

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Supporting Information Available: Detailed experimental procedures and multinuclear NMR shifts and CHN/HRMS characterization is provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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