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Polyvalent Carbocyanine Molecular Beacons for Molecular Recognitions

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Synthetic dendrimers and other polyvalent compounds use synergistic multivalent interactions to amplify desired chemical or biological molecular recognitions.[1,2] Modification of their peripheral functional groups with fluorescent antennas provides a highly sensitive approach to track, visualize, and quantify different molecular interactions by optical methods. Whereas numerous fluorescent molecular beacons are conventionally used for these studies, background interference in heterogeneous media such as cells and tissues can be minimized by using probes that absorb light in the near-infrared (NIR) wavelengths between 700 and 900 nm.[3,4] Unfortunately, conjugating such multiple hydrophobic beacons at the outer sphere of polyvalent compounds may quench fluorescence, induce aggregation, disrupt bioactive conformations of adjacent small molecules, and destabilize the chromophore systems because of their exposure to harsh and metabolically active media. Therefore, an alternative strategy to overcome these limitations is to construct polyvalency on a fluorescent inner core.

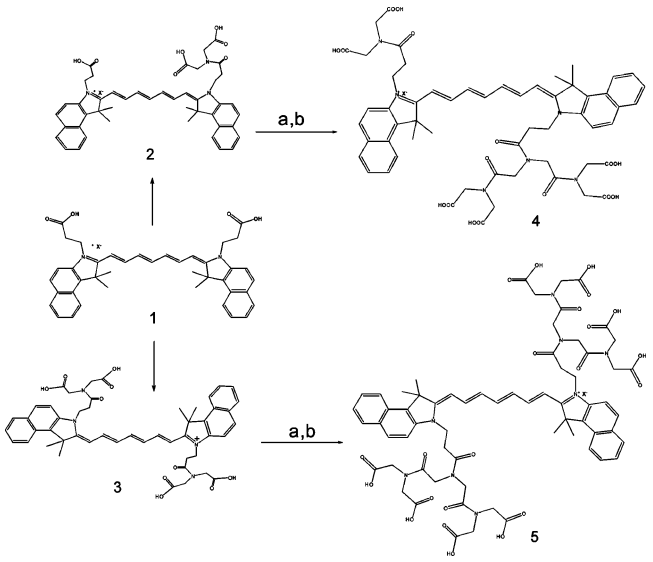
Currently, only nanoparticles and quantum dots provide inner core NIR chromophore system with outer core functional groups.[5−7] However, the biological applications of these particulates are limited by their potential to elicit immunogenic responses in vivo. Additionally, the constituents of quantum dots can be cytotoxic. Herein, we report the first polyvalent NIR carbocyanine-based molecular beacons, in which the carbocyanine moiety serves as not only the chromophore but also an inner core for constructing novel dendritic arrays of small molecules for molecular recognition studies.

On the basis of a recent report describing a convenient method to synthesize dicarboxylic acid-containing carbocyanine dye (cypate, **1**) from carboxyethyl benzoindole,[8] we used **1** as the central core for the polyvalent constructs. Thus, reaction of **1** with di-*tert*-butyl imino diacetate in the presence of EDCI/HOBt, followed by TFA-mediated deprotection, gave two second-generation polyvalent beacons (tri (**2**) and tetra (**3**) carboxylate-containing derivatives). Further reactions of **2** and **3** with di-*tert*-butyl imino diacetate in the presence of EDCI/HOBt and subsequent TFA-mediated deprotection afforded two third-generation derivatives (hexa (**4**) and octa (**5**) carboxylate-containing derivatives; Scheme 1). The compounds were purified and obtained in moderate to good yields as follows: **2** (33%), **3** (60%), **4** (30%), and **5** (30%).

To explore the potential of using the polyvalent molecular beacon for molecular recognition studies, we constructed a small library of dendritic arrays of glucosamine because synergistic effects of glycosidic clustering play important roles in carbohydrate-mediated interactions.[9] Accordingly, reaction of unprotected D-(+)-glucosamine with **1**−**5** in the presence of HBTU/HOBT/DIEA afforded a series of compounds including **6**−**11** in moderate yields (Chart 1).

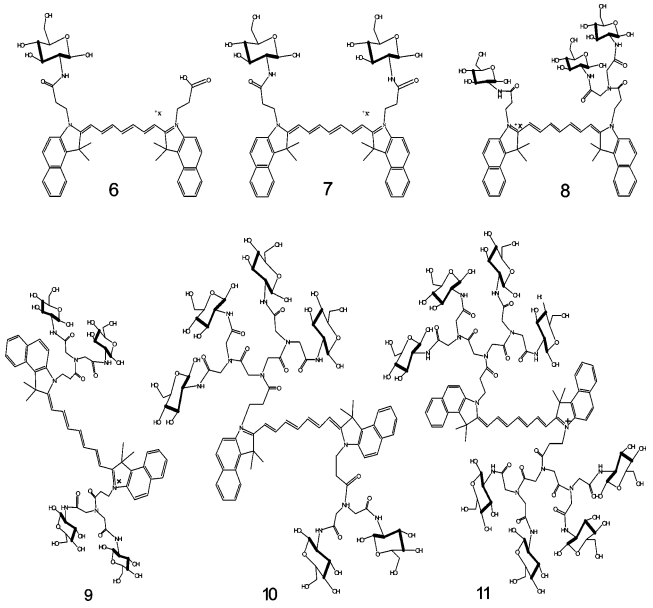
The absorption and emission spectra of all the compounds evaluated are similar, with their maxima centered at about 780 and 810 nm, respectively. Expectedly, the spectral properties of the dendritic arrays are also similar to the precursor polyvalent beacons because conjugation occurred at distal positions to the inner chromophore core, which is another advantage of the polyvalent

Scheme 1. Synthesis of Polycarboxylate-Containing Carbocyanine Fluorescent Probes[a]



[a] Reagents and conditions: (a) EDCI/HOBT/di-*tert*-butyl iminodiacetate/rt/5h (b) TFA.

Chart 1. Structures of Representative Dendritic Arrays of Glucosamine on an Inner NIR Carbocyanine Core



beacons. Figure 1 shows the normalized absorption and emission spectra of **5**. Interestingly, all the compounds have exceptionally high molar absorptivity in 20% DMSO/H_2O [ϵ_{max} mol^{-1} cm^{-1}: **1** (224 060), **2** (190 408), **3** (215 447), **4** (201 181), **5** (205 111), **6** (210 653), **7** (214 629), **8** (209 389), **9** (199 027), **10** (219 160), and

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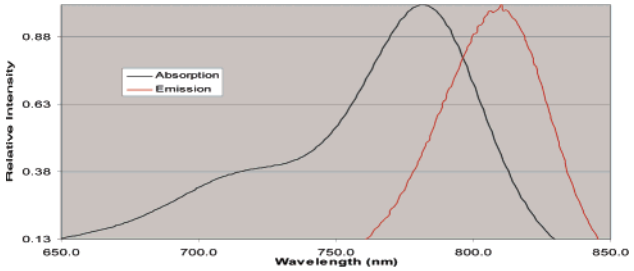

Figure 1. Spectral properties of a representative dendritic array of glucosamine **5** in 20% aqueous DMSO.

11 (207 156)]. The concentrations of solutions used for molar absorptivity determinations ranged from 0.3 to 8 μM.

The glycolytic pathway for energy production by cells requires delivery of glucose to the mitochondria, where it is phosphorylated by an enzyme called hexokinase.[10] Glucose transporters (GLUTs) are responsible for channeling the carbohydrate into cells. Because of their high energy needs, proliferating cells overexpress GLUTs to enhance glycolysis relative to normal cells. Consequently, radiolabeled glucose derivatives are used to detect cancers in humans.[11] Previous studies have shown that a fluorescent probe-labeled glucosamine derivative, 2-(*N*-(7-nitrobenz-2-oxa-1,3- diazol-4-yl)amino)-2-deoxyglucose (2-NBDG), is a viable nonradioactive method to monitor glucose transport and uptake in living cells.[12] Therefore, we evaluated the retention of representative polyvalent molecular beacons in a proliferating tumor model (CA20948) in nude mice.

Each tumor-bearing mouse received 0.1 μmol of the beacons/kg of body weight via tail vein injection. The in vivo and ex vivo distribution of the molecular beacons were monitored by NIR fluorescence imaging using two collimated solid 780-nm laser sources for excitation and a CCD camera equipped with 830-nm interference filter to capture the fluorescence emission.[4] An advantage of the optical method is that the blood clearance profile and the tumor uptake of the beacons can be monitored continuously and in real time. At 24 h post-injection, the nude mice were sacrificed. Aliquots of blood and some major organs (tumor, kidney, muscle, kidney, and liver) were harvested and washed with phosphate-buffered saline. The mean fluorescence count in each tissue was determined for each compound and normalized. Negative control studies show that the precursor dye **1** was not retained in the tumor but accumulated in the liver at about 1 h post-injection. On the contrary, the dendritic arrays of glucosamine were retained in the tumor up to 24 h post-injection (Figure 2). Uptake of **6** by the liver was slightly higher than uptake in the tumor and the kidneys. Evaluation of the retention rate and biodistribution suggests that compound **8** rapidly accumulated in the tumor and had the lowest overall uptake in nontarget tissues. These preliminary results also show that each compound can be used for different applications. For example, compound **9**, which is retained in blood for >24 h, could serve as a blood pool agent for monitoring blood flow and imaging blood vessels.

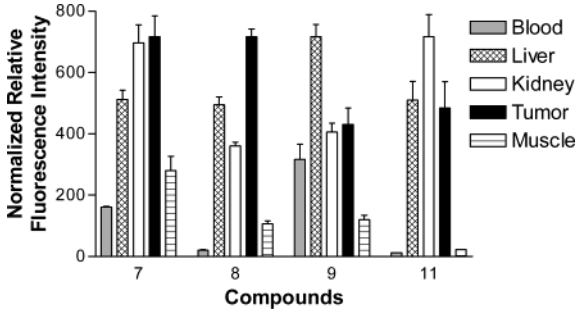

Figure 2. Biodistribution of dendritic arrays of glucosamine in CA20948 pancreatic tumor-bearing mice at 24 h post-injection of the probes.

In conclusion, we have described the synthesis of novel polyvalent molecular beacons that used a NIR carbocyanine as the inner core. On the basis of the synthetic strategy described above, higher generations of the polyvalent compounds are accessible. The strategic "encapsulation" of the chromophore system in the inner core prevents fluctuations in the spectral properties of the compounds. This improves data reproducibility and enables using the same excitation source and emission filter for the analyses of samples by optical methods. Our preliminary in vivo results showed enhanced uptake of the beacons in proliferating tumor cells, possibly mediated by GLUTs. Finally, the polyvalent structural framework could be used to amplify a variety of biological and chemical molecular recognition interactions.

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Supporting Information Available: Description of synthetic procedures, spectral characterization, and fluorescence imaging method. This material is available free of charge via the Internet at http://pubs.acs.org.

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