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Multimodal formyl peptide receptor 1 targeted inflammation imaging probe: cFLFLF-MHI-DOTA

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ABSTRACT

Formyl peptide receptor 1 (FPR1) targeting multimodal probe cFLFLFK-MHI-DOTA for leukocyte based inflammation imaging is described. The compound consists of three domains, (a) cFLFLF peptide for FPR1 recognition and binding for activated leukocyte, (b) heptamethine cyanine dye (MHI) for near infrared fluorescence (NIRF) detection and imaging, and (c) metal chelator DOTA ligand that could form complex with a radiometal for nuclear (PET/SPECT) imaging or with a paramagnetic metal for MRI imaging. Detailed synthesis, characterization and in vitro evaluation are reported. The availability of dual mode inflammation imaging probe would allow in vivo gross level imaging of inflammation foci as well as ex vivo microscopic level cellular imaging for role played by innate immune cells in inflamed tissue.

Molecular imaging as a technique is epitome of observing both microscopic and macroscopic events in spatial temporal manner. However, due to inherent limitation imposed by individual modality, it is usually difficult to correlate the macroscopic in vivo imaging with cellular level microscopic observation. Therefore using molecular imaging in a single mode to understand complex dynamic biological process becomes highly challenging.[1,2] This is especially true in the case of inflammation imaging.[3,4] Inflammation imaging is one of the most sought after areas of research in the medical field because of its wide spread application in numerous pathophysiological conditions.[5,6]

Inflammation is considered as root cause of many disorders and diseases, especially in the acute inflammatory conditions such as myocardial infarction (cardiovascular disease—heart attack) or brain ischemic injury such as stroke. In other cases such as arthritis pain in joints as aseptic symptoms is results of, inflammation. Septic microbial infections lead to inflammatory response such as pneumonia and tuberculosis in the lungs. Therefore inflammatory biological condition is defined as a highly intricate dynamic phenomenon wherein variety of cells from innate immune system as well as local resident cells coordinates a response for self defense. The protection and healing of the injured cells/tissue from the abnormal stimulation caused by a physical, chemical, or biologic agent, including the local reactions is required for regular physiological functioning and survival. In response to these abnormal stress stimuli morphological changes in cells/tissue are ensued leading to cytokine release which results in making blood vessels leaky to have better supply of necessary ingredients for repair and removal of damaged cells from inflamed foci. In the process cellular infiltration of innate immune cells from circulation ensues in the affected areas with an ultimate aim for rescue, repair and healing.[7] The swelling, redness and generation of heat—raising local temperature are generally observed symptoms for inflammation. The whole process is well orchestrated, however, the controlled response from immune cells may turn into revolting situation only when persistent and sustained stress signal remains because of more damaged cells and debris leading to organ dysfunction. Thus inflammation is double edged sword and intricate response from many cellular components/cells makes it difficult to define and monitor the inflammatory dysfunction with high degree of specificity and selectivity from other physiologically active functions, and therefore defining and visualizing inflamed focal areas in vivo is not an easy task.

In the case of acute inflammation such as ischemic injury of heart, brain, lung and kidney as wells as in the case infection related injury it is now well established that the leukocytes particularly polymorphonuclear (PMNs) cells are the first responders.[8] Under normal circumstances these leukocytes are circulating blood as inactive cells but upon receiving stress signal in the form of

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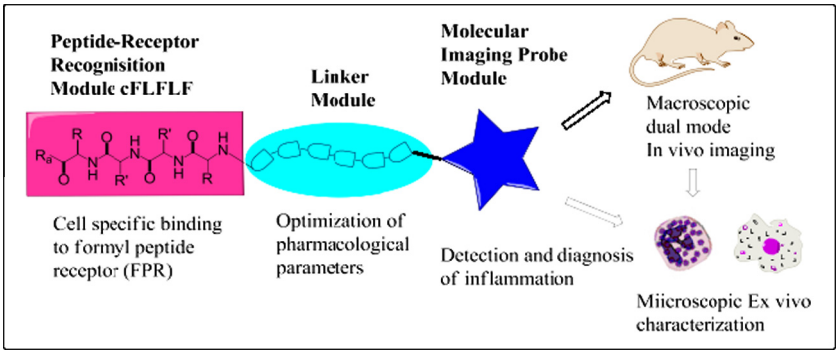


Figure 1. Schematic of multimodal peptide based molecular imaging probs.

chemotaxis or gradient created by formylated—peptidyl metabolites from microorganism. PMNs become activated, change their morphology and migrate to inflammation site for protecting self tissue for defense purpose along with killing the microorganism by generating reactive oxygen species.[9] It has been established now that the activated PMN overly express formyl peptide receptors (FPRs) on their surface for increased sensitivity towards recognizing the stress signal.[10] We and others have targeted FPR for imaging inflammation in variety of animal models mainly in single modality.[11–18] Faced with a daunting task of correlating the macroscopic imaging observations with microscopic cellular level details for qualitative and quantitative analysis of inflammation we designed a multi modal probe to address some of these issues.

The conceptual idea of the assembly is depicted in Figure 1. The FPR1 targeting domain, cFLFLF, is conjugated with a near infrared fluorophore, MHI-148, and a metal chelator, DOTA. The synthesis, characterization and in vitro preliminary data are described herein.

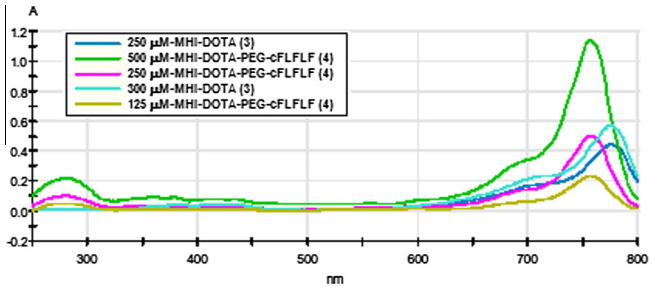
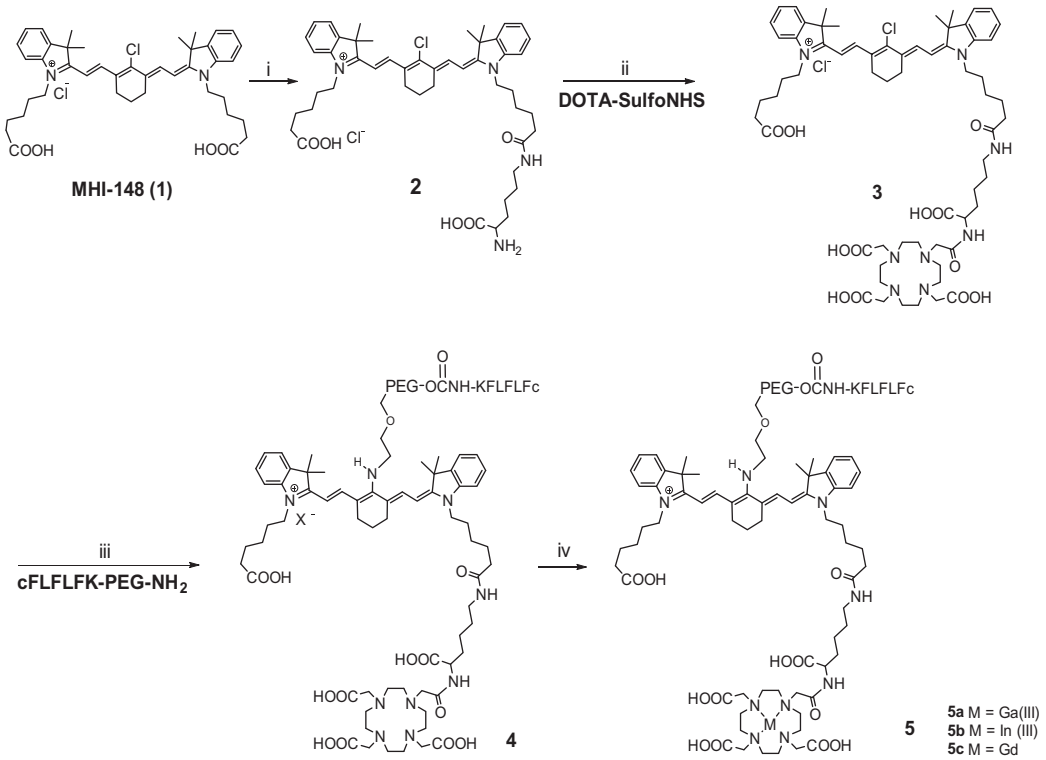


Figure 2. UV–vis spectra for compounds **3** and **4** at various concentrations.

The multimodal precursor probe **4** is synthesized in three straightforward chemical steps starting from heptamethine cyanine dye MHI-148 (**1**) as shown in Scheme 1. In the first step one



Scheme 1. Synthesis of multimodal cFLFLFK-PEG-MHI-DOTA probe: (i) (a) *N*-hydroxy succinamide, DCC, CH_2Cl_2, rt; (b) *N*-Boc lysine, borate buffer, pH 8.5, 4 °C, overnight; (c) TFA, 4 °C, 1 h; overall 68%; (ii) CH_3CN, borate buffer pH 8.5, 0 °C to rt, over night; 45%; (iii) CH_3CN/MeOH (3:1), heating, 60 °C, 2 h, 57%; (iv) metal complexation, for example, $GaCl_3$, quantitative.