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Rhodamine analogs for molecular ruler applications

Yu-Hsuan Chu [a], Jorge O. Escobedo [a], Meiyan Jiang [b], Peter S. Steyger [b], Robert M. Strongin [a, *]

[a] Department of Chemistry, Portland State University, Portland, OR 97207, USA
[b] Oregon Hearing Research Center, Oregon Health & Science University, Portland, OR, USA

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ABSTRACT

A series of geometrically well-defined cationic fluorophores were designed based on molecular mechanics. They contain biaryl linkers to impart rigidity preventing intramolecular folding between a conjugated biomolecule and fluorophore. All probes have absorption and emission maxima within 20 nm from Texas Red, as predicted by TDDFT calculations and validated experimentally.

1. Introduction

Fluorescent probes are widely used for the detection of specific biomolecular pathways and cellular events. The objective of this study is to synthesize rigid, geometrically well-defined rhodamine derivatives (**1**–**4**, Fig. 1) to serve as the optical components of bioactive conjugates. A longer-term goal is to use drug-fluorophore conjugates to investigate pore dilation and the dimensions of ion channels involved in the cellular uptake of aminoglycosides, such as gentamicin (GT). This would lead to further understanding of the mechanism and potential prevention of gentamicin's serious ototoxic and nephrotoxic side effects.

Fluorescein, BODIPY and acridine dyes have been conjugated to aminoglycosides such as gentamicin and neomycin to study cellular uptake [1,2], HIV-RNA binding [3] and for use in fluorescent immunoassays [4]. The fluorescent conjugate of gentamicin and Texas Red (**1**-GT, Fig. 2) has been of great utility in the study of non-endocytotic aminoglycoside uptake and intracellular localization [5,6]. Texas Red succinimidyl ester, **1** (Fig. 1) is the fluorophore used for gentamicin conjugation because it imparts amphiphilic properties, cationic charge and enables the retention of drug bioactivity [6,7]. However, **1**-GT diameter, at first estimated to be 1.47 nm [8], is larger than the channel pore size (1.25 nm) of the hair cell stereocilia mechanoelectrical transduction (MET) channel that it permeates. This led researchers to conclude that either the MET channel pore size has been underestimated, or that the MET channel pore temporarily dilates upon **1**-GT entry [8]. The biophysical mechanisms associated with pore dilation are poorly understood [9].

Previously, we reported the synthesis of **2** possessing a rigid linker to address the propensity of the **1**-GT to quench in live cells [10]. Extending the length of the fluorophore as well as the rigidified linker may allow the conjugate to serve as a molecular ruler and enable to study the diameter and dilation properties of cationic channels. The synthesis of compounds **3** and **4** are described herein, as they embody systematically increased dimensions as compared to **1**, **2** and each other. They exhibit only minor changes in spectral properties compared to the parent structure **1**.

Molecular modeling was used as a tool to guide the design of the conjugatable fluorophores. Energy-minimized structures generated by molecular mechanics (MM2, SYBYL-X™ 2.0, Certara L. P.) showed that the sulfonamide group of **1** promotes folding of the structure, including interactions between the gentamicin and Texas-Red moieties. An energy-minimized structure of an unfolded

* Corresponding author. Tel.: +1 503 7259724.
E-mail address: strongin@pdx.edu (R.M. Strongin).

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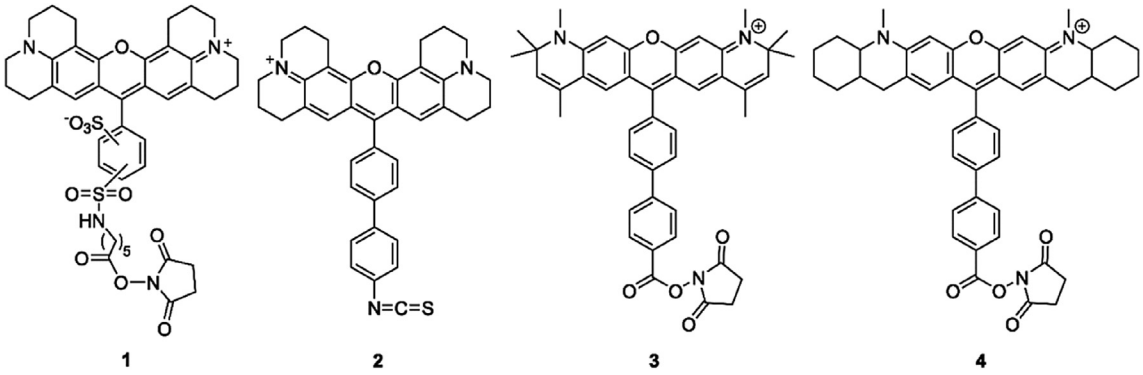

Fig. 1. Texas Red succinimidyl ester **1**, inflexible linker probe **2** and target fluorophores **3** and **4**.

conformer of **1**-GT may exhibit a maximal cross-sectional diameter of 1.35 nm (Fig. 2A) [10]. Although less than the 1.47 nm value reported by others, it also shows that **1**-GT is larger than non-selective cation channels on hair cells, such as the MET channel [11] and TRPV1 [12], which has an open pore size of ~1 nm [13,14] and increases upon agonist-induced pore dilation [15,16].

2. Experimental

2.1. General

All chemicals were used as received without further purification. ^{1}H NMR, ^{13}C NMR and were acquired on an ARX-400 Avance Bruker spectrometer. Chemical shifts (δ) are given in ppm. Mass spectra were obtained at the PSU Bioanalytical Mass Spectrometry Facility on a ThermoElectron LTQ-Orbitrap Discovery high resolution mass spectrometer coupled to an Accela HPLC system. Microwave synthetic procedures were performed in an Initiator™ microwave synthesizer (Biotage). Fluorescence measurements were carried out on a Cary Eclipse™ fluorescence spectrophotometer (Agilent Technologies). Absorption spectra were acquired with a Cary Eclipse™ 50 Bio UV–Vis spectrophotometer.

2.2. Synthesis

2.2.1. Synthesis of compound *6*

Compound **6** was synthesized using a modified procedure reported previously [17]. Under Ar, ytterbium(III) triflate (353 mg, 0.568 mmol) was added to a solution of *m*-anisidine (1 g, 8.12 mmol) in acetone (40 mL). The reaction mixture was stirred at rt for 24 h. Acetone was removed *in vacuo* and the residue dissolved in EtOAc, washed with H_2O, brine and dried over Na_2SO_4, filtered and the solvent evaporated under vacuum. The mixture was then separated by flash column chromatography on SiO_2 using

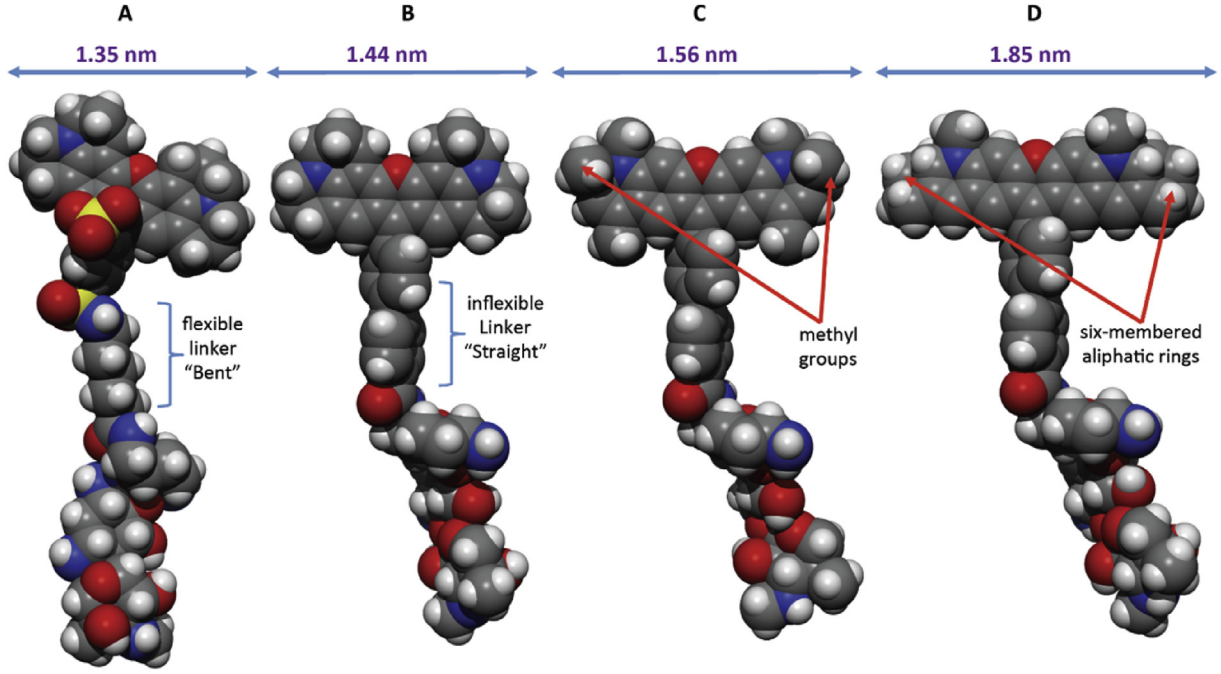

Fig. 2. Optimized geometries of GT conjugate analogues of TR. (A) **1**-GT; commercial Texas Red conjugated to GT. (B) **2**-GT; the SO_3 and SO_2 groups are removed and the alkyl chain is replaced by an inflexible *p,p'*-biphenylene linker. (C) **3**-GT; the inflexible linker is kept and four methyl groups are added at both ends of the fluorophore scaffold. (D) **4**-GT; the inflexible linker is kept and two six-membered aliphatic fused rings are added. Atom color codes: C = gray, H = white, N = blue, O = red, S = yellow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)