Construction of Rotaxanes and Their Biological Applications

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Abstract: Development of organelle targeting rotaxane-based fluorophores that can selectively stain specific intracellular compartments such as mitochondria, lysosome, Golgi apparatus, endoplasmic reticulum, etc., has become an emerging field of contemporary research. Nevertheless, due to the extremely intricate cellular milieu, rational control for selective targeting of cellular organelles using organic fluorescent probes has remained challenging. Among the intracellular organelles, mitochondria are unique and indispensable, playing the crucial role in regulating cellular protein homeostasis, oxidative metabolism, and intracellular redox balance. Unlike other cellular organelles, mitochondria are difficult to target due to their unusual double-layer membrane and extremely negative inner mitochondrial membrane (IMM) potential ($\Delta\Psi_m$ −150 to −180 mV for normal cell and $\Delta\Psi_m$ ~ −220 mV for cancer cell). Lysosomes are also a significant target for all malignant cells. Lysosomes are membrane-bound acidic organelles that have a pH value of 4-5. They include a range of enzymes that are responsible for the degradation of nucleic acids, fats, proteins, and so on. In my research work, I have utilized the microwave-assisted template-directed clipping reaction on low-loading 2-chlorotrityl chloride resin to create NIR unsymmetrical mechanically interlocked molecules. These squaraine rotaxane compounds have a high potential for selective targeting and NIR imaging of mitochondria. This is the first report of an NIR unsymmetrical 1,3,3-trimethyl indoline squaraine-rotaxane-based mitochondria targeting and staining agent. The rotaxane molecule conjugated with TPP^+ functionality might be useful for mitochondrial treatment with NIR imaging diagnostics. MW-assisted SPPS protocol is also used for the effective synthesis of RGDS peptide. Two N_3-RGDS peptides are conjugated at the terminal alkyne residue of the macrocycle

using CuAAC on the Wang resin to synthesize rotaxane/RGDS conjugates. RGDS peptide is incorporated to target the $\alpha_v\beta_3$ integrin which is overexpressed at the tumor site. A morpholine moiety is conjugated at the axle to selectively target the lysosomes. This dual targeting rotaxane has been used for live cancer cell specific active targeting followed by selective internalization and tracking of malignant lysosomes. I have also presented a multifunctional rotaxane molecule composed of a lipophilic TPP^+ for mitochondrial targeting and dopamine-containing catechol groups for the surface coating of the superparamagnetic Fe_3O_4 NPs. TPP^+ and dopamine residues are both attached to the axle of the NIR rotaxane molecule. Two carbohydrate moieties are attached at the tetralactam macrocycle to enhance the water solubility of MitoSQRot-DOPA. Click chemistry has been used to conjugate carbohydrates into the tetralactam macrocycle of the rotaxane. TBDMS-protected MitoSQRot-(Carb-$OH)_2$-DOPA is deprotected with TBAF/THF and capped with our synthesized magnetic nanoparticles to produce Fe_3O_4 NPs surface coated with the rotaxane molecules [MitoSQRot-(Carb-$OH)_2$-DOPA-Fe_3O_4]. Water-soluble rotaxane capped superparamagnetic Fe_3O_4 NPs could be a promising candidate for the targeted multimodal imaging applications.

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