Bioorganic & Medicinal Chemistry Letters 28 (2018) 2741–2745

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

A chemically stable fluorescent marker of the ureter

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ARTICLE INFO

Article history:
Received 31 January 2018
Revised 18 February 2018
Accepted 19 February 2018
Available online 24 February 2018

Keywords:
Fluorophore synthesis
Near-IR fluorescence
Fluorescence-guided surgery

ABSTRACT

Surgical methods guided by exogenous fluorescent markers have the potential to define tissue types in real time. Small molecule dyes with efficient and selective renal clearance could enable visualization of the ureter during surgical procedures involving the abdomen and pelvis. These studies report the design and synthesis of a water soluble, net neutral C4′-*O*-alkyl heptamethine cyanine, Ureter-Label (UL)-766, with excellent properties for ureter visualization. This compound is accessed through a concise synthetic sequence involving an *N*- to *O*-transposition reaction that provides other inaccessible C4′-*O*-alkyl heptamethine cyanines. Unlike molecules containing a C4′-*O*-aryl substituent, which have also been used for ureter visualization, UL-766 is not reactive towards glutathione and the cellular proteome. In addition, rat models of abdominal surgery reveal that UL-766 undergoes efficient and nearly exclusive renal clearance *in vivo*. In total, this molecule represents a promising candidate for visualizing the ureter during a variety of surgical interventions.

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Despite remarkable progress in molecular medicine, surgical interventions are nearly always carried out using only visual, memory recall, and tactile cues. Adding insight through imaging is being explored with diverse modalities.[1] Fluorescence-guided surgical (FGS) methods provide real-time images using only relatively simple optical readouts.[2,3] These methods are progressing toward clinical use in a variety of disease contexts.[4] Fluorescent dyes, ideally with emission in the near-infrared (NIR) range (~700–900 nm), form the chemical component of these efforts.[3,5,6] Most clinical efforts use indocyanine green (ICG), which was FDA approved nearly 60 years ago.[7] To enable the broad adaptation of FGS, a new generation of dyes that address specific challenges in the field are needed.

The identification and precise dissection of critical structures is central to the surgical process. Unintended injury results in short and long-term complications, prolonged hospital stays and health care costs. Acute ureteral injury results from external trauma, open surgery, laparoscopy and endoscopic procedures. Nearly any abdominopelvic surgical procedure, whether gynecologic, obstetric, general surgical, or urologic can potentially injure the ureter. The incidence of ureter injury during abdominal and pelvic surgery has been reported to range from 0.5% to 10%.[6–8] An analysis of 13 studies concluded that the following procedures contribute to iatrogenic ureteral injuries: hysterectomy (54%), colorectal surgery (14%), pelvic procedures such as ovarian tumor removal (8%), transabdominal urethropexy (8%), and abdominal vascular surgery (6%).[9] The total incidence of ureteral injury after gynecologic surgery is reported to be 0.5–1.5%, and after abdominoperineal colon resection ranges from 0.3% to 5.7%.[9] The ureter is vulnerable to iatrogenic injuries because of its close proximity to vital visceral organs and it's 'hidden location' in the retroperitoneum of the abdomen and pelvis. Clear visualization during surgery through FGS could alleviate this significant morbidity. Prior work, including clinical trials, has centered on using methylene blue to illuminate the ureter during surgery.[8,9] However, this molecule does not absorb/emit in the NIR range, where light can maximally penetrate tissue, and also exhibits equal amounts of hepatic clearance. One study has reported the use of IR800-CW, though as described below, we observe significant competitive hepatic clearance rendering this molecule suboptimal for use in this context.[10] In these studies, we report the design, synthesis, and application of a novel net-neutral near-IR dye, which we term (Ureter-Label (UL)-766), that exhibits excellent chemical stability, is readily synthesized, and rapidly cleared through renal pathways. These characteristics

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https://doi.org/10.1016/j.bmcl.2018.02.040
0960-894X/Published by Elsevier Ltd.

make this compound an excellent candidate for use as a ureter marker in FGS.

The design of UL-766 was informed by several insights. Heptamethine cyanines are privileged molecules for *in vivo* optical imaging, with absorbance and emission maxima around 800 nm. Modifying substituents of the core cyanine scaffold provides a useful means to tune its biological properties.[11–14] We recently reported a novel group of cyanine fluorophores, exemplified by FNIR-774 (Fig. 1).[15] Synthetic access is enabled by an *N*- to *O*-rearrangement reaction sequence, providing a general approach to prepare otherwise inaccessible C4′-*O*-alkyl heptamethine cyanines.[15–18] Several prior studies examined the effect of cyanine polarity and functionality on biodistribution and clearance. In particular, Choi and others have shown that the attachment of polar functional groups, specifically quarternized trimethylalkylammoniums, can dramatically alter clearance pathways.[19–21] The resulting fluorophores, often as targeted conjugates, exhibit useful *in vivo* targeting and efficient renal clearance. While useful, these molecules contain phenols at the C4′-position, which may render them subject to covalent modification (see below).[15]

These studies report the discovery and characterization of UL-766. This molecule has triethylene glycol chains appended to the two indolenine nitrogens. This substituent, in combination with the aryl sulfonates, was anticipated to provide excellent water solubility. Our prior studies have suggested that installing a charged functional group proximate to the C4′-position of cyanine polyene serves to mitigate the formation of undesirable H-aggregates, a common issue with these molecules.[15,22,23] To introduce this charge, as well as to provide net-neutral molecules, we installed a trimethylalkyl-ammonium ether at the C4′ position. To examine the role of the alkyl linker, we have prepared both propyl and ethyl variants.

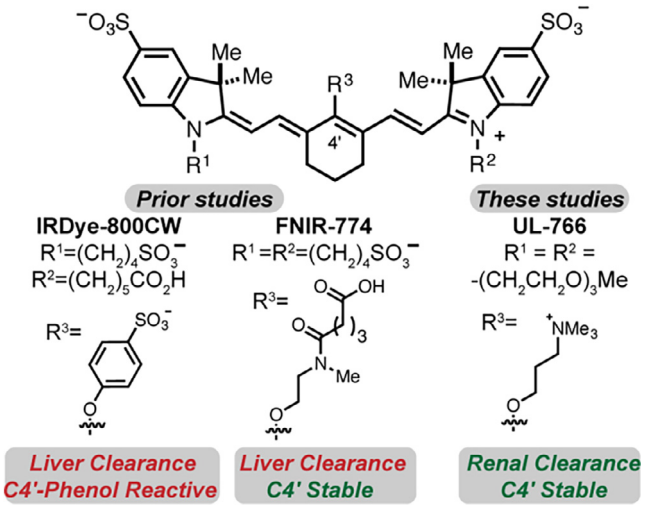


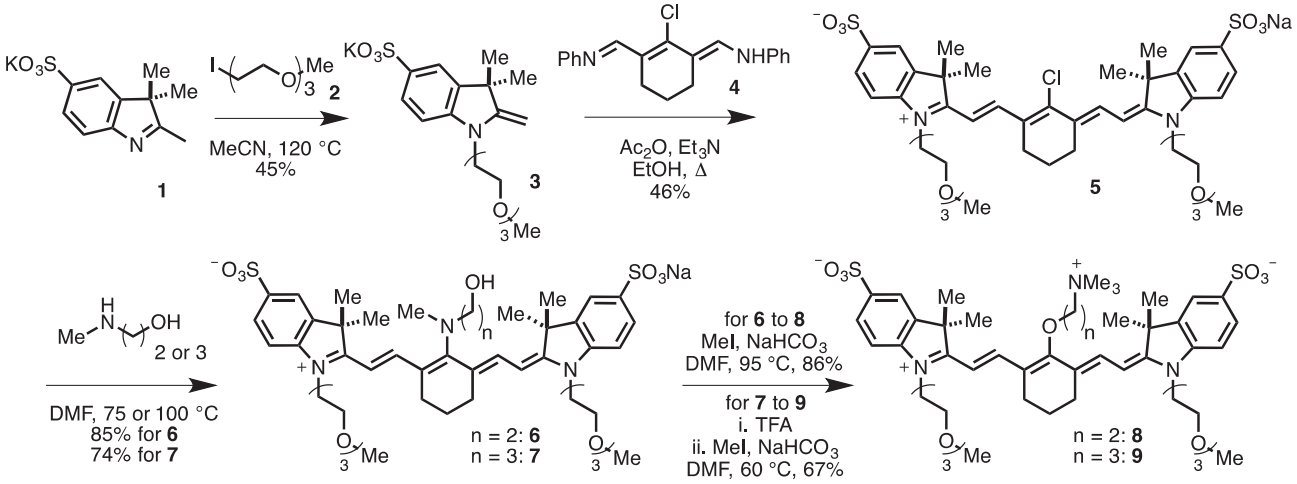
Fig. 1. Key prior cyanines, IRDye-800CW and FNIR-774, and the compound reported here, UL-766.

The synthesis of cyanines **8** and **9** is outlined in Scheme 1. The sequence commenced with alkylation of indolenine **1** with iodide **2** in MeCN at 120 °C to provide **3** in 45% yield. Conventional cyanine formation with **3** and commercial **4** in refluxing EtOH with Et_3N and Ac_2O affords **5** in 46% yield following reversed-phase purification. The ethyl congener could be accessed through a two-step sequence starting with *N*-methylethanolamine addition in DMF at 75 °C to afford **6** in 85% yield. The Smiles-type rearrangement of **6** proceeds as reported previously on a similar substrate using MeI, $NaHCO_3$ in DMF at 95 °C to provide **8** in 86% yield.[15] To access the propyl variant, we prepared **7** through *N*-methylpropanolamine addition to **5** in DMF at 100 °C in 74% yield. While direct rearrangement of **7** with the MeI, $NaHCO_3$ in DMF conditions was ineffective, we found that TFA treatment, solvent removal, followed by heating with excess MeI and $NaHCO_3$ in DMF provided **9** in 67% yield from **7**. In this instance, TFA treatment of **7** induces an —*N* to —*O* transposition (based on a bathochromic shift in the absorbance maxima) to provide a C4′-*O*-alkyl intermediate that then undergoes *N*-alkylation. Of note, the latter sequence has been applied on 0.5 g scale, which has provided sufficient material for extensive *in vivo* testing.

We investigated the spectroscopic properties of compounds **8** and **9**. As anticipated, these molecules have similar properties to related heptamethine cyanines, such as FNIR-774 (Table 1). Moreover, **8** and **9** display excellent water solubility of up to 5 mM in pH 7.4 PBS.

Table 1
Key spectroscopic properties of FNIR-774, **8**, and **9** (all measured in pH 7.4 PBS).

	$\lambda_{max(nm)}$	ε (M^{-1} cm^{-1})	$\lambda_{em(nm)}$	φ_F	Brightness ($\varepsilon \times \varphi_F$)
FNIR-774	774	204,000	789	0.10	21,000
8	774	255,000	796	0.067	17,000
9	766	229,000	789	0.095	22,000



Scheme 1. Synthesis of **8** and **9**.