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Invited feature article

Fluorescence phenomena in nerve-labeling styryl-type dyes

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ABSTRACT

Several classes of diversely substituted styryl type dyes have been synthesized with the goal of extending their expected fluorescent properties as much toward red as possible given the constraint that they maintain drug-like properties and retain high affinity binding to their biological target. We report on the synthesis, optical properties of a series of styryl dyes (*d1–d14*), and the anomalous photophysical behavior of several of these donor–acceptor pairs separated by long conjugated π-systems (*d7–d10*). We further describe an unusual dual emission behavior with two distinct ground state conformers which could be individually excited to locally excited (LE) and twisted intramolecular charge transfer (TICT) excited state in push–pull dye systems (*d7*, *d9* and *d10*). Additionally, unexpected emission behavior in dye systems *d7* and *d8* wherein the amino-derivative *d7* displayed a dual emission in polar medium while the *N*,*N*-dimethyl derivative *d8* and other methylated derivatives *d12–d14* showed only LE emission but did not show any TICT emission. Based on photophysical and nerve binding studies, we down selected compounds that exhibited the most robust fluorescent staining of nerve tissue sections. These dyes (*d7*, *d9*, and *d10)* were subsequently selected for *in vivo* fluorescence imaging studies in rodents using the small animal multispectral imaging instrument and the dual-mode laparoscopic instrument developed in-house.

1. Introduction

Nerves are often difficult to visualize during surgery, due to their intricacy and anatomical variations in their location [1]. As a result, unintended nerve injury has been recognized as an important cause of morbidity associated with several lifesaving surgical procedures such as prostatectomy, [2,3] thyroidectomy, [4,5] rhytidectomy [6], and breast cancer surgery [7]. The need for guidance during surgery has been recognized for about a decade, beginning with the evaluation of techniques employing pre-operative CT and MR imaging [8–11]. However, real time decision making has directed intraoperative identification efforts toward fluorescence imaging [12], including approaches relying on visualizing the nerves based on contrast agents designed to elicit selective nerve fluorescence during surgery, reviewed recently [13].

To facilitate clinical use, the ideal *in vivo* nerve contrast agent must be designed to cross the tight junctions of the blood nerve barrier, a crucial requirement for systemic injection. Thus, in addition to being fluorescent, the contrast agent should show high selectivity to a nerve target as well as exhibit properties of small molecules. The targeting moiety has to be inherently fluorescent because conjugating the targeting component to a fluorescent dye would significantly increase its molecular weight beyond the desirable range.

The styryl dye, BMB, (1,4-bis(*p*-aminostyryl)-2-methoxybenzene), developed to be a PET tracer for imaging myelin absorbs in the near-ultraviolet range and emits in the blue region [14]. While it bound with high affinity to myelin extracts from brain, BMB suffered from poor aqueous solubility. Moreover, intraoperative imaging at wavelengths optimal for BMB produced high background in non-target tissue because tissue autofluorescence was high in this region. Using BMB as a starting point, we sought to explore whether chemical modifications applied to bis-styryl dyes [15,16] would allow us to elaborate them into dyes having optical and biological properties suitable for their use in an intraoperative setting.

We have since reported on bis-styryl dyes (*d1*, *d7* and *d9*) for selectively targeting myelin basic protein (MBP), a major component of nerves [17–19], along with compact optical imaging instruments for open and minimal access surgeries [20,21]. Following intravenous injection, these dyes visualized central and peripheral nerves *in vivo* with high contrast relative to the surrounding muscle tissue, despite absorbing and emitting in the visible region. The affinity for MBP was maintained provided certain structural features were conserved [22]. We have shown

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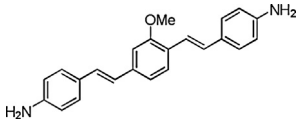
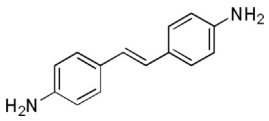
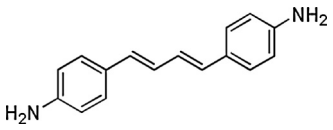
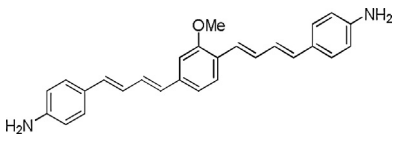
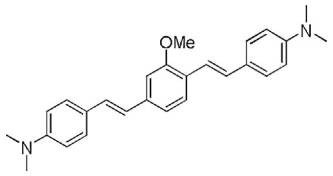
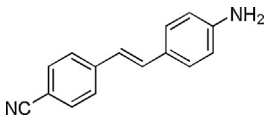
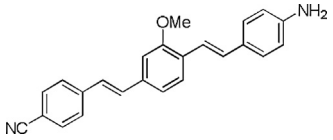
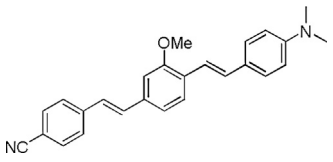
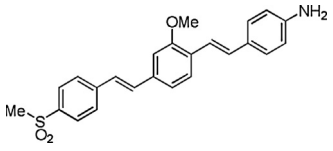
Table 1
Styryl and bis-styryl fluorophores in solution.

Name[a]	Chemical structure	λ_{Abs}[b], nm	λ_{Em}[c], nm (intensity)[d]
d1 (BMB)[a]		408	495 (1,06,800)
d2		362	414 (2,46,900)
d3		383	448 (84,300)
d4		400	575 (200)
d5		415	511 (93,300)
d6		385	522 (2800)
d7 (GE3082)[a]		417	491, 621[e] (1700;2520[e])
d8		412	518 (264)
d9 (GE3111)[a]		412	480, 624[e] (2250;4700[e])
d10 (GE3126)[a]		414	510, 618[e] (2100; 3970[e])