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Syntheses of 2-NBDG analogues for monitoring stereoselective uptake of D-glucose

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ABSTRACT

2-NBDG is a widely used fluorescent tracer for monitoring D-glucose uptake into single living cells. However, 2-NBDG alone is not sufficient for monitoring the net stereoselective uptake of D-glucose, unless its possible non-stereoselective uptake is properly evaluated. L-Glucose derivatives, which emit fluorescence distinct from that of 2-NBDG, should provide valuable information on the stereoselective uptake, when used with 2-NBDG in combination. In the present study, we synthesized Texas Red (sulforhodamine 101 acid)-coupled and [2-(benz-2-oxa-1,3-diazol-4-yl)amino]-coupled 2-deoxy-D-glucose, referred to as [2-TRG] and [2-BDG], respectively. These derivatives showed emission wavelength longer and shorter than that of 2-NBDG, respectively. 2-TRLG, an antipode of 2-TRG, proved to be an effective tracer for evaluating the extent of non-stereoselective uptake of 2-NBDG when used simultaneously with 2-NBDG. On the other hand, 2-BDG exhibited very weak fluorescence, but the application of a novel cross coupling in the presence of a benzoxadiazole group may be useful for the future development of effective glucose tracers.

Measurements of the cellular uptake of D-glucose have contributed to broad spectra of research fields from functional brain mapping to cancer diagnosis.[1,2] To evaluate the glucose uptake activity, a variety of radio-labeled analogues of D-glucose, such as [^{14}C] 2-deoxy-D-glucose (2-DG)[3] and [^{18}F] fluoro-2-deoxy-D-glucose (2-FDG),[4] have been effectively used as tracers. However, a drawback of such tracers is their poor spatial and temporal resolution, and they can not monitor the glucose uptake in single, living cells in real time. 2-[*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose [2-NBDG (**1**)] (Fig. 1), a fluorescent analogue of D-glucose, provides more sensitive measurement of the glucose uptake.[5] 2-NBDG (**1**), which was originally designed to monitor the viability of *Escherichia coli* cells,[6] is taken up through mammalian glucose transporters (GLUTs) in a time, concentration and temperature-dependent manner with K_m values comparable to those reported for radio-labeled glucose tracers.[7] In the last decade, 2-NBDG (**1**) has been successfully applied to a wide variety of cells and organs, leading to new findings such as metabolic communication between astrocytes in the brain.[8–11] On the other hand, there was an increasing need for control substrates against 2-NBDG (**1**) to properly evaluate an occurrence of non-stereoselective uptake and membrane adsorption of 2-NBDG (**1**) in addition to its time-dependent degradation and extinction at the individual experimental condition used.[5]

To approach these issues, we synthesized transporter-unrecognizable (L-isomer) fluorescent tracers of glucose as control substrates for 2-NBDG (**1**),[12,13] because it is known that D- but not L-glucose binds to GLUTs.[14] Of these, 2-NBDLG (**2**) (Fig. 1), an enantiomer of 2-NBDG (**1**), is an ideal control substrate providing us with the valuable information on the net stereoselective uptake of D-glucose. However, the emission spectrum of 2-NBDLG (**2**), which is identical to that of 2-NBDG (**1**), makes it impossible to use 2-NBDLG (**2**) simultaneously with 2-NBDG (**1**). This is critical when examining if the stereoselective uptake of delicate cells such as neurons is preserved or not, because such cells often show gradually changing membrane states between healthy and dead especially during in vitro experiments.[15] Membrane-impermeable large molecules such as dextran-Texas Red may not properly monitor the stereoselectivity.[16] Indeed, it is known that severe metabolic stress induces an altered glucose transport prior to total

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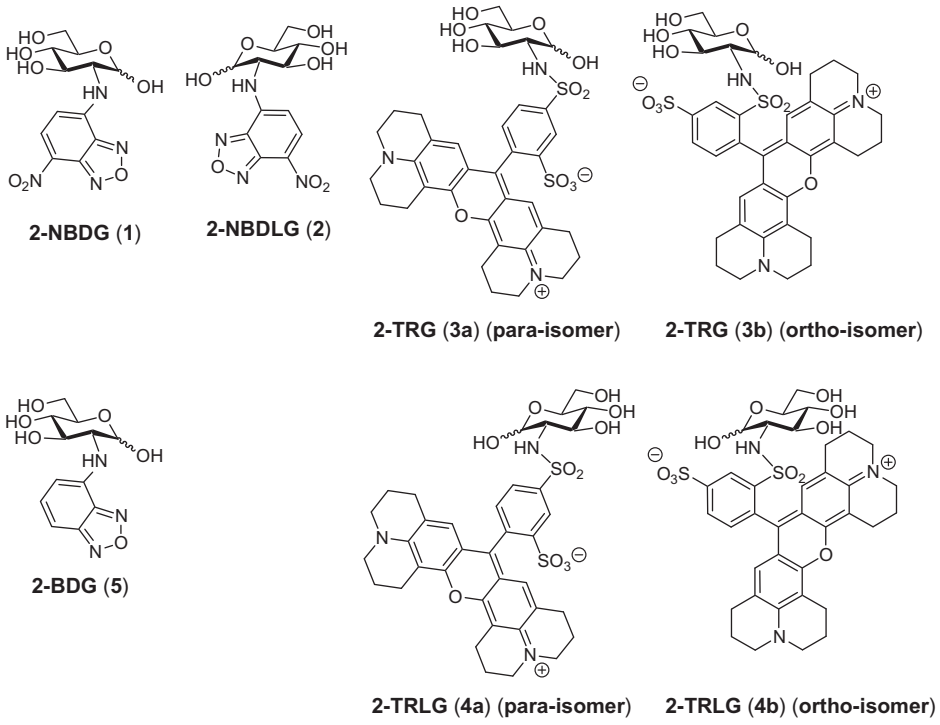
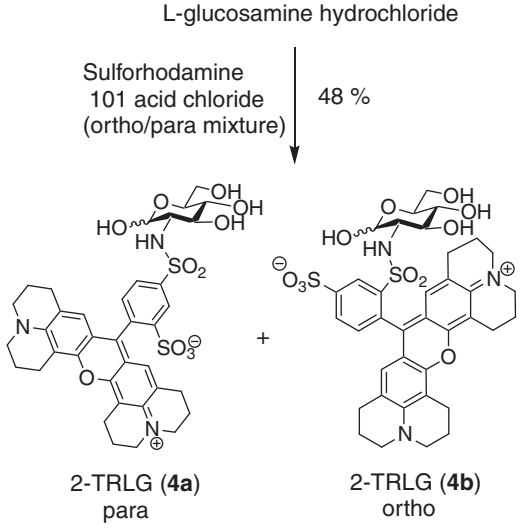


Figure 1. Structures of 2-NBDG (**1**) and its analogues.

disruption of the membrane integrity, possibly due to a rapid change in both protein expression and protein/lipid polarity in the plasma membrane.[15] Instead, L-glucose derivatives, which have wavelength distinct from that of 2-NBDG (**1**), might serve as reliable molecules informing the stereoselectivity status at the cost of their physicochemical properties that may not be identical to those of 2-NBDG (**1**).

2-Amino-2-deoxy-D-glucose derivative 2-TRG (**3**), which bears either para (**3a**) or ortho (**3b**) isomer of sulforhodamine 101 acid (Texas Red), was prepared from D-glucosamine hydrochloride with a mixture of *para*- and *ortho*-101-rhodamine sulfonyl chloride (Texas Red sulfonyl chloride) (Fig. 1).[17] Both para (**3a**) and ortho (**3b**) isomers emit strong red fluorescence with identical emission spectra (data not shown). Using L-glucosamine hydrochloride as a



Scheme 1. Synthesis of 2-TRLG (**4**) as a mixture of para (**4a**) and ortho (**4b**) isomers.

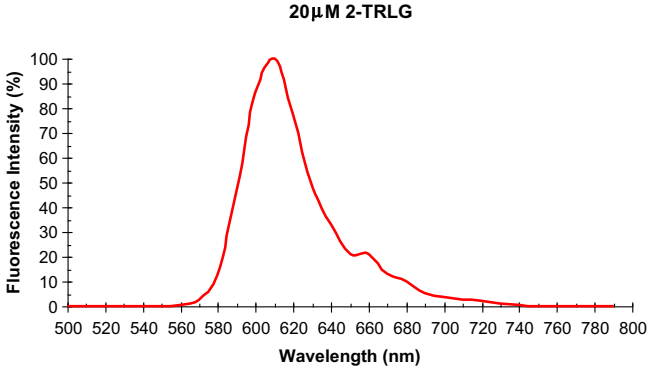


Figure 2A. Normalized emission spectrum of 2-TRLG (**4**) (in HEPES buffer, pH 7.35). Argon laser (488 nm) was used for excitation.

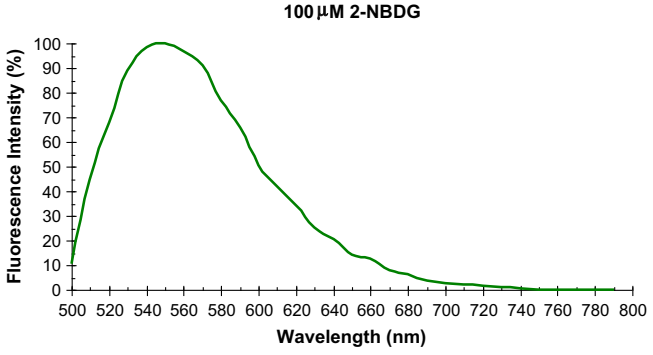


Figure 2B. Similar to 2A, but emission spectrum of 2-NBDG (**1**).