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Synthesis and application of clickable and biocompatible fluorescent glycosyl labels

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ABSTRACT

Two novel, biocompatible and highly stereoselective fluorescent glycosyl labels featuring azide groups have been successfully synthesized. The functional groups provide convenient routes for fluorescent glycosyl conjugates via click chemistry. Three fluorescent glycose-conjugates were synthesized. One fluorescent conjugate was used to trace the translocation in castor seedlings. The developed fluorescent labels for biomolecules are likely to provide an important method for investigating various biological events. We believe that the probes obtained are useful for studies on biological processes.

1. Introduction

Fluorescent labeling is a critical technique used to investigate a wide range of biological processes in cells and biochemistry [1–4]. Fluorescence allows easy and reliable qualitative and quantitative determination [5], and labeling with the use of fluorescent probes presents a good alternative to the use of radioactive compounds [6]. The technique can also provide real-time observation and determination of molecular locations. Given recent developments in carbohydrate research, fluorescent glycosyl labeling has received increased attention in various areas, such as monosaccharide transport [7], glucose uptake [8,9], polysaccharide mobility [10], glycoprotein analysis [11,12], carbohydrate–protein interaction [13,14], and DNA sequencing [15].

Many studies have used fluorophores, such as dansyl [16], anthraldehyde [17], and rhodamine derivatives [18], to label carbohydrate conjugates. 4-Nitrobenzo[*c*][1,2,5]oxadiazole (NBD), for example, was recently used as a fluorophore because of its small size and photophysical properties in aqueous media [19,20].

Azides play important roles in organic synthesis and biochemistry, such as protective groups for amines, and their reactive partners for tagging in biological system. Organic azides also involve in Cu^{I}-catalyzed Huisgen 1,3-dipolar cycloaddition (also known as “click chemistry”). The click reaction has been used as a synthetic tool for the preparation of a variety of carbohydrates containing molecular constructs [21]. It could also provide new options for selective labeling and biomolecular manipulation even within living cells. To the best of our knowledge, no reports on the synthesis of fluorescent glycosyl derivatives containing azides and their application in fluorescent labeling have yet been published. In this paper, we have successfully synthesized fluorescent glycosyl derivatives containing azido group, and report the preparation and basic photophysical properties and application of these molecules.

Abbreviations: AcNBDG, 1,3,4,6-tetra-O-acetyl-2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-β-D- glucoside; AcNBDGN3, 3,4,6-tri-O-acetyl-2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-β-D-glucopyranosyl azide; NBDGN3, 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-β-D-glucopyranosyl azide; $NBDGON_3$, β-D-Glucopyranoside, 2-azidoethyl 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy; NBDGTF, N-[3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)-phenyl]-4-[(trifluoromethyl)sulfinyl]- 1H-pyrazol-5-yl]-1-(2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-β-D- glucopyranosyl)-1H-1,2,3-triazole-4-methanamine; NBDGXCJ, 1-((1-(2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-β-D-glucopyranosyl)-1,2,3-triazol-4-yl)methyl)-4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile; NBDGLBQ, 2-((1-(2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-β-D-glucopyranosyl)-1,2,3-triazol-4-yl)methyl)-3-methyl-4-oxocyclopent-2-en-1-yl 3-(2,2-dichlorovinyl)-2,2-Dimethylcyclopropanecarboxylate.

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2. Experimental

2.1. General

Melting points were determined on an FP62 digital micro melting point apparatus; data were left uncorrected. NMR spectra were obtained on a Bruker AV-600 instrument. Deuterated solvents were obtained from Cambridge Isotope Laboratories (Andover, MA). CD_3OD and $CDCl_3$ solvent peaks (3.31 and 7.26 ppm for ^1H; 49.0 and 77.0 ppm for ^{13}C, respectively) were used as internal chemical shift references. ^1H NMR spectra are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), coupling constant(s) in Hertz, number of protons. The mass spectra (MS) of new compounds were obtained by a Waters ZQ 4000 with electrons pray ionization (ESI) or Bruker maXis 4G ESI-Q-TOF mass spectrometer. Data were reported as *m/z*. Analytical thin layer chromatography (TLC) was performed on silica gel GF254. Silica gel was used for column chromatography. Reagents and anhydrous solvents were used as purchased without further purification.

2.2. Spectroscopy analysis and Confocal Laser Scanning Microscope observation

Fluorescence spectra for all compounds were collected at 25 °C with F-4500 FL Spectrophotometer. The optimized parameters were as follows: EM Start WL: 200.0 nm; EM End WL: 800.0 nm; Scan speed: 2400 nm/min; EX Slit: 2.5 nm; EM Slit: 5.0 nm; PMT Voltage: 700 V. The presence of fluorescent conjugate in the roots of castor bean seedlings were analyzed using a Leica SP2 Confocal Laser Scanning Microscope (Leica, Heidelberg, Germany) fitted with an argon ion and two helium neon lasers. The beam splitter was DD488/543 (double dichroic, reflects at 488 and 543 nm). The fluorescence of specimens was excited at 488 nm and collected at 510–600 nm. Specimens were photographed using a combination of emitted light and transmitted light. The resultant images were overlaid in some cases to determine the location of the fluorescence signal, and thence the localization of conjugate. During image acquisition, each line was scanned eight times and averaged, and each image was scanned four times and averaged.

2.3. Plant materials

The roots of 6-day-old castor seedlings were immersed in the medium containing 1 mM MES-KOH, 0.5 mM $CaCl_2$ and 50 μM NBDGTF **(14)**, NBDGXCJ **(15)** and NBDGLBQ **(16)** at pH 5.5. After 3 h incubation, the root tips were excised and the upper parts of axial roots were sectioned for observation..

2.4. Synthesis

2.4.1. 1,3,4,6-tetra-O-acetyl-2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-β-D-glucoside (Scheme 1, **2**, AcNBDG)

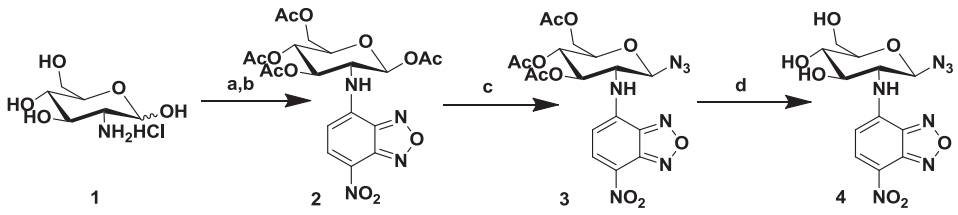
Glucosamine hydrochloride (216 mg, 1.0 mmol) and Et_3N (350 μL, 2.5 mmol) were suspended in dry methanol (10 mL) and the reaction mixture cooled to 0 °C on an ice bath. To the suspension was added 4-chloro-7-nitrobenzo[c][1,2,5]oxadiazole (NBD-Cl, 360 mg, 1.8 mmol) and then the reaction mixture was allowed at room temperature for another 18 h. Solvent was evaporated under reduced pressure and pyridine (3 mL) and acetic anhydride (1 mL) was added. The reaction mixture was kept at room temperature for 8 h and poured into ice water. The mixture was extracted with ethyl acetate and the organic layer was washed with 1 M HCl, sat. aq. $NaHCO_3$ and dried with Na_2SO_4. The solution was concentrated to give crude syrup which was purified by TLC (petroleum ether: ethyl acetate 3:1) to obtain compound **2**. Yield 48%; reddish brown solid; m.p. 114.6 °C; ^1H NMR (600 MHz, $CDCl_3$) δ 8.46 (d, 1H, J = 8.5 Hz, Ar–H), 6.41 (d, 1H, J = 8.9 Hz, Ar–H), 6.40 (d, 1H, J = 4.3 Hz, NH), 6.20 (d, 1H, J = 9.2 Hz, H-1), 5.53 (t-like, 1H, J = 9.7 Hz, H-3), 5.30 (t, 1H, J = 9.9 Hz, H-4), 4.39 (dd, 1H, J = 12.5, 4.0 Hz, H-6-a), 4.31 (t-like, 1H, J = 10.8 Hz, H-2), 4.14 (ddd, 1H, J = 10.2, 3.8, 2.1 Hz, H-5), 4.10 (dd, 1H, J = 12.3, 1.7 Hz, H-6-b), 2.26 (s, 3H, CH_3CO), 2.13 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.2, 170.6, 169.4, 168.6, 144.3, 143.7, 142.2, 135.5, 126.2, 100.1, 89.3, 71.3, 70.1, 67.4, 61.4, 55.6, 21.0, 20.8, 20.8, 20.7; ESI-MS, *m/z* 509.7[M−H]$^-$.

2.4.2. 3,4,6-tri-O-acetyl-2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-β-D-glucopyranosyl azide (Scheme 1, **3**, AcNBDGN$_3$)

Compound **2** (196 mg, 0.384 mmol) and azidotrimethylsilane (TMS-N_3, 56 μL, 0.423 mmol) were dissolved in anhydrous CH_2Cl_2 (5 mL) and $SnCl_4$ (45 μL, 0.384 mmol) was added dropwise. The resulting mixture was stirred at room temperature until TLC indicated the disappearance of starting material (about 2 h). The mixture was extracted with CH_2Cl_2 (3 × 20 mL) and then washed with $NaHCO_3$ (3 × 20 mL) and water (20 mL). The organic layer was dried (Na_2SO_4) and filtered, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (petroleum ether: ethyl acetate 2:1) to give **3**. Yield 75%; reddish brown solid; m.p. 172.5 °C; ^1H NMR (600 MHz, $CDCl_3$) 8.50 (d, 1H, J = 8.9 Hz, Ar–H), 6.53 (d, 1H, J = 8.9 Hz, Ar–H), 6.42 (d, 1H, J = 9.3 Hz, NH), 5.36 (t, 1H, J = 9.7 Hz, H-3), 5.24 (t, 1H, J = 9.7 Hz, H-4), 5.04 (d, 1H, J = 8.9 Hz, H-1), 4.40 (dd, 1H, J = 12.5, 4.5 Hz, H-6-a), 4.23 (dd, 1H, J = 12.5, 1.6 Hz, H-6-b), 3.93–3.97 (m, 1H, H-5), 3.90 (q, 1H, J = 9.36 Hz, H-2), 2.14 (s, 3H CH_3CO), 2.07 (s, 3H, CH_3CO), 1.94 (s, 3H CH_3CO); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.3, 170.8, 169.5, 144.5, 143.7, 142.8, 135.9, 125.9, 101.0, 89.9, 74.3, 73.0, 67.7, 61.7, 58.4, 20.9, 20.7, 20.7; ESI-MS, *m/z* 492.8 [M−H]$^-$.

2.4.3. 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-β-D-glucopyranosyl azide (Scheme 1, **4**, NBDGN$_3$)

Compound **3** (493 mg, 1 mmol) was added to a solution of sodium methoxide in dry MeOH (0.1 M, 5 mL). The resulting solution was stirred for 6 min at room temperature. The mixture was neutralized with Amberlite IR 120 (H^+) resin and filtered, and the filtrate was evaporated and the residue was purified by TLC (CH_2Cl_2: MeOH 12:1). Yield 88%; reddish brown solid; m.p. 87.5 °C; ^1H NMR (600 MHz, CD_3OD) δ 8.55 (d, 1H, J = 8.6 Hz, Ar–H), 6.60 (d, 1H, J = 8.6 Hz, Ar–H), 4.96 (d, 1H, J = 7.6 Hz, H-1), 3.97 (dd, 1H,



Scheme 1. Synthesis of fluorescent glycosyl label NBDGN$_3$. Reaction reagent and solvent: a. NBD-Cl, Et_3N, MeOH; b. pyridine, Ac_2O; c. $SnCl_4$, TMS-N_3, CH_2Cl_2; d NaOMe, MeOH.