Tetrahedron Letters 49 (2008) 6876–6878

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Synthesis of 2-NBDLG, a fluorescent derivative of L-glucosamine; the antipode of D-glucose tracer 2-NBDG

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ARTICLE INFO

Article history:
Received 2 August 2008
Revised 12 September 2008
Accepted 16 September 2008
Available online 19 September 2008

Keywords:
L-Glucosamine
2-NBDG
2-NBDLG
GLUTs
SGLTs
Glucose uptake

ABSTRACT

2-[*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-L-glucose [2-NBDLG] (**2**) is a long-awaited control substance compensating the non-specific uptake of 2-NBDG (**1**), which has been widely used as a fluorescent tracer for monitoring D-glucose uptake into single, living cells. A new synthetic method of optically pure L-glucosamine, which is not available as a natural product, has been developed. The first and one-step synthesis of 2-NBDLG (**2**) from L-glucosamine is also described.

An essential sugar, D-glucose is one of the most important energy sources for the survival of various organisms, from *Escherichia coli* to mammals. Recent molecular techniques have revealed increasing numbers of glucose transporters such as GLUTs (glucose transporters) and SGLTs (sodium/glucose cotransporters) that may be located in particular sites of the plasma membrane.[1] In addition, translocation of some transporters in response to insulin stimulation has been documented.[2] Historically, glucose transport activity has been monitored by radiolabeled tracers, such as [^{14}C] 2-deoxy-D-glucose.[3] However, they cannot be used for time-lapse monitoring of glucose uptake at the single-cell level due to their poor spatial and temporal resolution.

In 1996, Matsuoka et al. developed a fluorescent D-glucose derivative, 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose [2-NBDG] (**1**) as shown in Figure 1, that allows a more sensitive measurement of glucose uptake in single-cell of living *E. coli*.[4] In 2000, Yamada et al. proved that 2-NBDG (**1**) is incorporated into mammalian cells through glucose transporters in a time, concentration, and temperature-dependent manner.[5]

So far 2-NBDG (**1**) has been successfully applied in various organisms by different groups.[6] Of particular interest is its application to the brain,[7] which utilizes glucose as a sole energy source, and to malignant tumor cells.[8] However, care should be taken in that the fluorescence intensity is an arbitrary measure and that the uptake of 2-NBDG (**1**) proceeds sometimes in seconds.

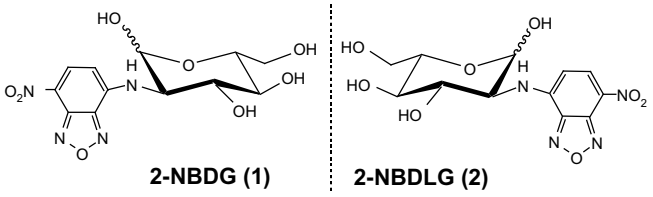

Figure 1. Structures of 2-NBDG (**1**) and 2-NBDLG (**2**).

Thus, quantification requires stability of the system as well as accurate procedures.[6]

Indeed, complete wash or removal of fluorescent 2-NBDG (**1**) from extracellular fluid and the cell-surface is difficult issue, particularly when applied to tissues consisting of heterogeneous cells showing divergent activity.[6] To overcome the difficulties, we selected 2-NBDLG (**2**), an enantiomer of 2-NBDG (**1**), as a control substrate for 2-NBDG (**1**). It is known that mammalian cells specifically incorporate D-, instead of L-isomer of glucose.[9] Thus, measurement of the difference in the fluorescence derived from 2-NBDG (**1**) and 2-NBDLG (**2**) would provide critical information on the net stereospecific uptake of D-glucose into single, living

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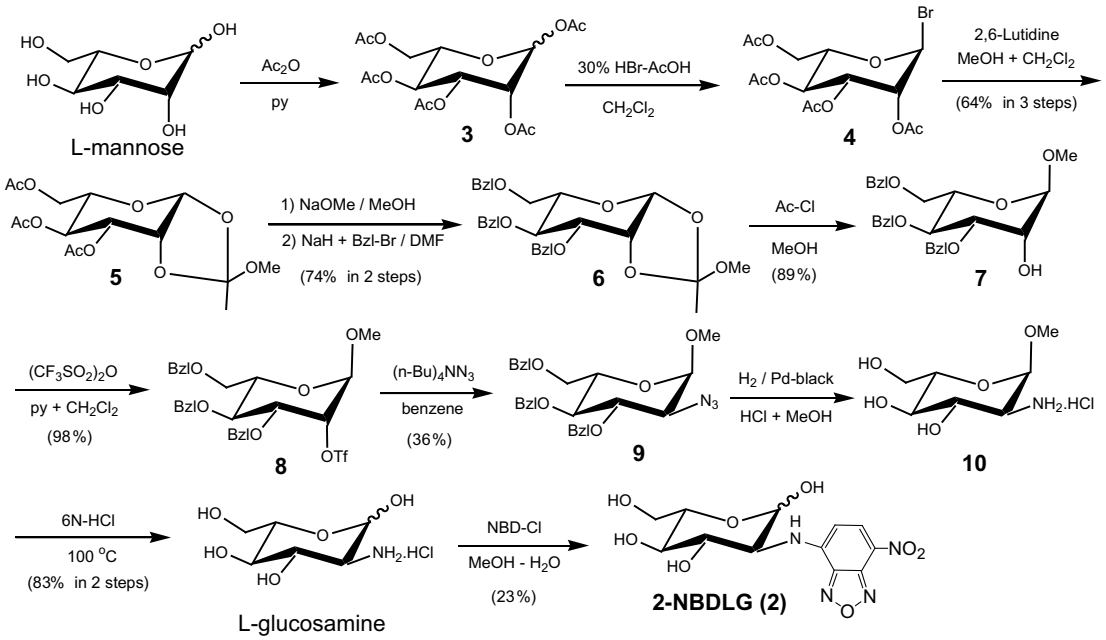
doi:10.1016/j.tetlet.2008.09.089



Scheme 1. Synthesis of L-glucosamine and 2-NBDLG (**2**).

cells, setting it apart from other factors such as non-specific uptake of the tracers and/or transporter-unrelated binding to the cellular surface that can be serious problems in some application.[10]

Although a few papers[11] on synthesis of L-glucosamine or its derivatives have been reported, a new synthetic method of L-glucosamine should be absolutely required in practical view of optical purity and preparative scale. Here, we describe the first synthesis of 2-NBDLG (**2**) as well as optically pure L-glucosamine in practical scale.

As shown in Scheme 1, L-glucosamine was synthesized from L-mannose in 10 steps. By the applications of Montgomery's method,[12] transformations of a starting material into the compound **7**[13] with one free hydroxyl group at C-2 position were carried out, namely, peracetylation, bromination at C-1 position, orthoester-formation, deacetylation, benzylation, and acidic methanolysis. The free 2-hydroxyl group in methyl glycoside **7** was sulfonylated with trifluoromethanesulfonic anhydride in the presence of pyridine to give the compound **8**.[14] By use of tetrabutylammonium azide[15] in benzene, the triflate **8** was converted to azide **9**, being accompanied by inversion of the configuration at C-2 position.[16] Catalytic hydrogenation of the azide **9** gave the primary amine **10**. Finally, the methyl glycoside was hydrolyzed with 6 N-HCl at 100 °C[17] to form the target compound. The ^{1}H NMR data[18] of synthetic L-glucosamine thus obtained were completely identical with those of commercially available D-glucosamine. On the other hand, optical purity of L-glucosamine was confirmed by the comparison of specific rotation with that of D-glucosamine.[19] Optically pure L-glucosamine thus obtained was coupled with 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD-Cl) to give 2-NBDLG (**2**).[20]

Use of 2-NBDG (**1**) has brought exciting implications including such as metabolic wave[7a] and intercellular transport of D-glucose and/or its phosphorylated form through gap junction.[21] Use of transporter-recognizable (D-isomer) and unrecognizable (L-isomer) fluorescent analogs combined with modern live-cell imaging techniques, such as real-time confocal microscopy, should provide valuable information on the ligand-transporter interactions. Bioassays by using 2-NBDLG (**2**) are currently under way. Those results will be shown elsewhere.

Acknowledgements

This research was supported by Science and Technology Incubation Program in Advanced Regions, Japan Science and Technology Agency.

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13. ^{1}H NMR data of the compound **7** (in $CDCl_3$, 400 MHz): δ 7.24–7.36 (m, 15H, Ph), δ 4.80 (d, 1H, J = 1.6 Hz, H-1), δ 4.69 (ABq, 2H, J = 11.9 Hz, CH_2-Ph), δ 4.67 (ABq, 2H, J = 11.3 Hz, CH_2-Ph), δ 4.60 (ABq, 2H, J = 12.4 Hz, CH_2-Ph), δ 4.03 (m, 1H, H-2), δ 3.70–3.88 (m, 5H, H-3, H-4, H-5, H-6a and H-6b), δ 3.37 (s, 3H, OMe), δ 2.49 (br d, 1H, J = 2.5 Hz, C2-OH).
14. ^{1}H NMR data of the compound **8** (in $CDCl_3$, 400 MHz): δ 7.10–7.38 (m, 15H, Ph), δ 5.11 (m, 1H, H-2), δ 4.90 (d, 1H, J = 1.9 Hz, H-1), δ 4.69 (ABq, 2H, J = 12.0 Hz, CH_2-Ph), δ 4.64 (ABq, 2H, J = 10.7 Hz, CH_2-Ph), δ 4.61 (ABq, 2H, J = 11.7 Hz, CH_2-Ph), δ 4.00 (dd, 1H, J = 2.9 and 8.9 Hz, H-3), δ 3.69–3.84 (m, 4H, H-4, H-5, H-6a and H-6b), δ 3.40 (s, 3H, OMe).
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16. ^{1}H NMR data of the compound **9** (in $CDCl_3$, 400 MHz): δ 7.15–7.38 (m, 15H, Ph), δ 4.87 (ABq, 2H, J = 12.4 Hz, CH_2-Ph), δ 4.83 (d, 1H, J = 3.5 Hz, H-1), δ 4.66 (ABq, 2H, J = 10.7 Hz, CH_2-Ph), δ 4.57 (ABq, 2H, J = 12.4 Hz, CH_2-Ph), δ 3.98 (dd, 1H, J = 8.9 and 10.2 Hz, H-3), δ 3.66–3.80 (m, 4H, H-4, H-5, H-6a, and H-6b), δ 3.45(dd, 1H, J = 3.5, 10.4 Hz, H-2), δ 3.43 (s, 3H, OMe).

Because of E2 elimination from the triflate **8** as a side reaction, the desired compound, azide **9** was obtained in low yield.
17. Under usual condition such as at 60 °C in 1 N-HCl compound **10** was very stable, and no hydrolysis occurred probably due to the amino group at C-2 position.
18. ^{1}H NMR data of L-glucosamine (in D_2O, 400 MHz): δ 5.36 (d, 0.6H, J = 3.5 Hz, H-1α), δ 4.85 (d, 0.4H, J = 8.3 Hz, H-1β), δ 3.36–3.84 (m, 5H, H-3α and 3β, H-4α and 4β, H-5α and 5β, H-6a α and β, and H-6b α and β), δ 3.21 (dd, 0.6H, J = 3.5, 10.6 Hz, H-2α), δ 2.92 (dd, 0.4H, J = 8.3, 10.6 Hz, H-2β). Anal. Calcd for $C_6H_{14}ClNO_5$: C, 33.42; H, 6.54; N, 6.50. Found: C, 33.31; H, 6.46; N, 6.36.
19. $[\alpha]_D$ at 20 °C (24 h after dissolving in water) synthetic L-glucosamine: −72.05 (c 1.0, H_2O) commercially available D-glucosamine: +72.20 (c 1.0, H_2O).
20. ^{1}H NMR data of 2-NBDLG (**2**) (in D_2O, 400 MHz): δ 8.52 (d, 1H, J = 9.1 Hz, H6′), δ 6.56 and δ 6.54 (d × 2, 0.5H × 2, J = 9.1 Hz and J = 9.1 Hz, H5′), δ5.38 (d, 0.5H, J = 2.8 Hz, H-1α), δ 4.89 (d, 0.5H, J = 8.1 Hz, H-1β), δ 3.50–4.02 (m, 6H, H-2α and 2β, H-3α and 3β, H-4α and 4β, H-5α and 5β, H-6a α and β, and H-6b α and β).
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