Synthesis of a Glucocerebrosidase Fluorescent Substrate, C8-DCH-β-D-Glucose

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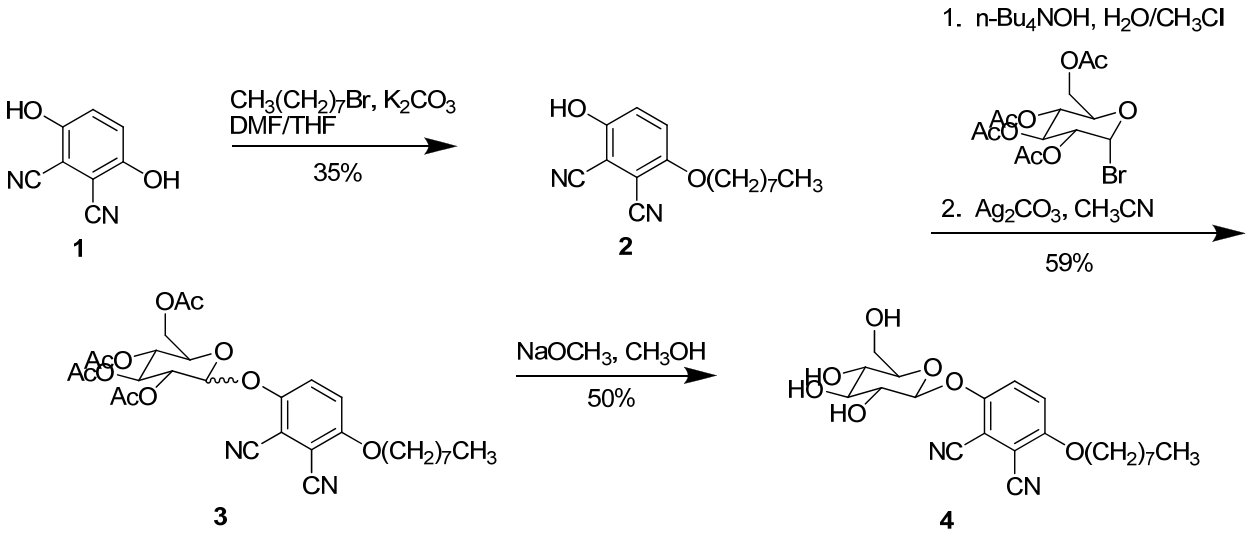
Background

The activity of the lysosomal enzyme glucocerebrosidase is reduced in patients with Gaucher disease. Fluorescent derivatives of glucocerebrosidase enzyme substrates may find use in the quantification of glucocerebrosidase activity in cells deficient in the enzyme and also for evaluation of possible treatments upon such diseased cells. A useful agent needs to be able to enter cells and then reach the lysosome. The C8-DCH-β-D-Glucose derivative has been shown to do that.

Chemistry

4-Octyl-2,3-dicyano-1,4-hydroquinoyl-β-glucopyranoside (C8-DCH-β-D-Glucose) (**4**) was synthesized in a similar manner to the originally reported methodology.[1] 2,3-Dicyanohydroquinone (**1**) was monoalkylated with 1-bromooctane in the presence of potassium carbonate to afford intermediate (**2**) in a 35% yield. After (**2**) was converted into its tetrabutylammonium salt, it was coupled with α-bromoglucose tetraacetate under Lewis acid catalysis using silver carbonate. We obtained the epimers of the sugar lipid product (**3**) in a 59% yield, in a ratio of β to α epimer of 2:1, as shown by ^{1}H NMR. The final product (**4**) was obtained enantiomerically pure in a 50% yield after hydrolysis of the tetraacetate ester and purification by flash chromatography.

Scheme 1. Synthesis of C8-DCH-β-D-Glucose.



Experimental

General. Silica gel 60 F254 TLC plates were from Merck. Flash chromatography was done on an Analogix Intelliflash Workstation with a SuperFlash Sepra Si 50 SF column. Mass spectral analyses were done on an Agilent 1200 Mass Spectrometer. ^{1}H-NMR were obtained on a Varian 400 MHz Spectrometer. Chemical shifts are reported in parts per million (δ) and are referenced to tetramethylsilane (TMS). All chemicals are commercially available from Aldrich.

2,3-Dicyano-4-(1-octyloxy)phenol (2). To a solution of 1-bromooctane (7.3 mL, 42 mmol) in anhydrous tetrahydrofuran (THF) (40 mL) was added a degassed suspension of potassium carbonate (50 g) in anhydrous DMF (400 mL) containing 2,3-dicyanohydroquinone (14.0 g, 87.5 mmol) over 1.5 h under an argon atmosphere. The resulting mixture was stirred for 24 hours at room temperature followed by filtration through Celite and neutralization using acetic acid. The filtrate was taken to dryness and the residue was dissolved in EtOAc (500 mL), washed with H_2O (50 mL), brine (50 mL) and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the residue purified by flash chromatography using a gradient of dichloromethane/methanol (50:1 to 30:1) to give product **2** in a yield of 8.32 g (35%) as a light grey solid. R_f = 0.57 (dichloromethane:methanol = 10:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.23 (d, 1H, J = 9.3 Hz), 7.14 (d, 1H, J = 9.4 Hz), 4.05 (d, 2H, J = 6.3 Hz), 1.83 (m, 2H), 1.48 (m, 2H), 1.38-1.22 (m, 8H), 0.89 (t, 3H, J = 6.5 Hz). MS (m/z): 271.1 $(M-H)^-$.

2,3-Dicyano-1-hydroxy-4-(1-octyloxy)phenyl-D-Glucopyranoside 2',3',4',6'-tetra-O-acetate (3). To a white suspension of compound **2** (2.04 g, 7.5 mmol) in water/chloroform (v/v, 1:3, 60 mL) was added a solution of 40% (wt/wt) aqueous tetrabutylammonium hydroxide (5.36 mL, 8.25 mmol). The white suspension became a yellow solution almost immediately. After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2x30 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and taken to dryness to afford a crude yellow solid. The crude solid (3.84 g, 7.5 mmol) was dissolved in anhydrous acetonitrile (300 mL) containing freshly dried 3Å molecular sieves (16 g) under an argon atmosphere. Silver carbonate (4.14 g, 15 mmol) and α-bromo glucose tetraacetate (6.615 g, 15 mmol) were added to this suspension. After stirring for 24 hours at room temperature, the suspension was filtered through Celite and the filtrate was taken to dryness. The resulting residue was purified by flash chromatography using a gradient of hexane/ethyl acetate (2:1 to 1:1) to give the product (**3**) 2.67g (59%) as a white solid. R_f = 0.50 (hexane:ethyl acetate = 1:2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.50 (d, 1H, *J* = 9.6 Hz), 7.13 (d, 1H, *J* = 9.6 Hz), 5.31-6.26 (m, 2H), 5.19 (m, 1H), 4.99 (m, 1H), 4.26-4.24 (m, 2H), 4.08 (t, 2H, *J* = 6.6 Hz), 3.82-3.77 (m, 2H), 2.15 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 4.05 (d, 2H, *J* = 6.3 Hz), 1.85 (m, 2H), 1.48 (m, 2H), 1.38-1.24 (m, 8H), 0.89 (t, 3H, *J* = 6.8 Hz).

4-Octyl-2,3-dicyano-1,4-hydroquinoyl-β-glucopyranoside (4). To a solution of compound **3** (2.46 g, 4.08 mmol) in MeOH (65 mL) was added sodium methoxide solution (4 mL, 2.0 mmol). A white solid began to precipitate within 5 minutes and the reaction was quenched with acetic acid (0.13 mL, 2.2 mmol) after 2 hours. The solvent was removed *in vacuo* and the resultant residue was purified by flash chromatography using a gradient of dichloromethane/methanol (8:1 to 4:1) to give the product 880 mg (50%) as a white solid. R_f = 0.27 (dichloromethane:methanol = 4:1). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.62 (d, 1H, *J* = 9.6 Hz), 7.45 (d, 1H, *J* = 9.6 Hz), 5.06 (d, 1H, *J* = 7.4 Hz), 4.15 (t, 2H, *J* = 6.4 Hz), 3.88 (dd, 1H, *J* = 2.2 Hz, *J* = 12.1 Hz), 3.68 (dd, 1H, *J* = 5.7 Hz, *J* = 12.1 Hz), 3.51 (m, 1H), 3.48-3.43 (m, 2H), 3.36 (m, 1H), 1.82 (m, 2H), 1.51 (m, 2H), 1.42-1.28 (m, 8H), 0.90 (t, 3H, *J* = 6.9 Hz). ^{13}C NMR (CD_3OD, 100 MHz) 156.9, 153.3, 122.8, 119.3, 113.0, 112.9, 105.6, 103.8, 101.3, 77.4, 76.9, 73.4, 70.2, 69.9, 61.3, 31.7, 29.2, 29.1, 28.8, 25.7, 22.5, 13.2. MS (m/z): 547.3 $(M+CF_3CO_2)^-$.

References

1. Miller SPF, French SA and Kaneski CR. Synthesis and characterization of a novel lysosomotropic enzyme substrate that fluoresces at intracellular pH. J. Org. Chem. 1991 56(1): 30-34. PMID