Synthesis of Fluorescent Probes of α-Monosaccharides

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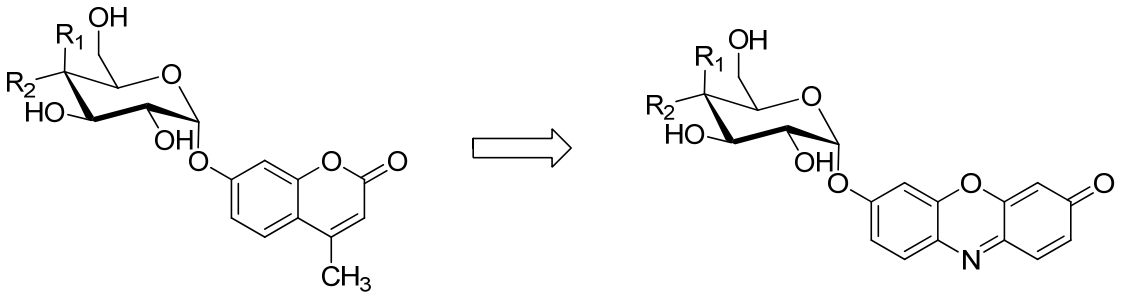
Background

Fluorogenic substrates are critical to the fluorogenic enzyme assays which are commonly used for screening of many small molecule libraries.[1] Multiple β-glucosidase and β-galactosidase fluorescent substrates are available using a variety of fluorophores such as umbelliferone, resorufin, fluorescein, coumarin, etc. However, for the α-glucosidase and α-galactosidase enzymes, only the 4-methylumbelliferone labeled substrates **1** and **2** are currently available commercially and these are sub-optimal substrates in compound screening assays compared to resorufin-labeled substrates. Therefore, two novel resorufin fluorescent probes **3** and **4** were designed and made for use in α-glucosidase and α-galactosidase enzyme assays. These two resorufin probes may be useful for high throughput screening assays using α-glucosidase and α-galactosidase in order to identify lead compounds for new therapies in various enzyme dysfunction conditions.



1 R_1 = H, R_2 = OH
2 R_1 = OH, R_2 = H

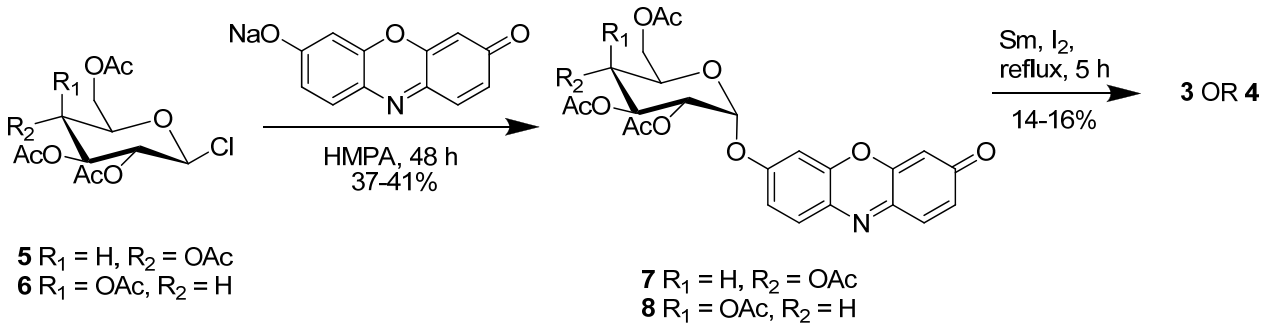
3 R_1 = H, R_2 = OH
4 R_1 = OH, R_2 = H

Figure 1. Structures of 4-methylumbelliferyl and resorufinyl-labeled α-D-glucopyranoside and α-D-galactopyranoside

Chemistry

The synthesis of the α-configuration of resorufin monosacchride is outlined in **Scheme 1** and includes two steps: an SN2 glycosidation and subsequent deprotection. Starting from the known compounds of tetraacetyl-β-chloro-glucose **5** or -galactose **6**,[2] glycosidation was performed in the presence of a two-equivalent excess of resorufin sodium salt in the aprotic solvent hexamethylphosphoric triamide (HMPA), which provided a mixture of the desired major α-configuration products **7** or **8** as well as the unwanted minor β-configuration products in a ratio of 2:1, in medium yields.[3] The α,β-configuration products could be separated by repeated flash chromatography, although deprotection of tetraacetyl- groups proved troublesome. We tried multiple basic or acid conditions including sodium methoxide, potassium carbonate, triethylamine and hydrochloric acid. All of these reagents gave complex mixtures of products which we weren't able to purify either by flash chromatography or by preparative HPLC. Finally, neutral conditions using samarium and iodine were employed for the successful cleavage of the four acetyl protecting groups of **7** and **8**.[4] This deprotection reaction mixture was purified by preparative HPLC to provide the final products **3** and **4** with good and sufficient quality (over 98% purity) for use in fluorogenic enzyme assays.

Scheme 1. Synthesis of resorufinyl-labeled α-D-glucopyranoside **(3)** and α-D-galactopyranoside **(4)**



Experimental

General. Thin-Layer Chromatography (TLC) analyses were performed with Analtech (Newark, DE) silica gel GHLF 0.25mm plates using UV and iodine detection. Flash chromatography was

performed on an Analogix Intelliflash Workstation with a SuperFlash Sepra Si 50 SF column. APCI mass spectrometry (APCI-MS) was performed on LC/MSD TrapXCl Agilent Technologies instrument. Analytical and preparative HPLC analysis was performed on Agilent 1200 series instrument equipped with multi-wavelength detector using an Agilent Eclipse XDB C18, 5.0 μm, 4.6 x 150 mm column and 21.2x50 mm column. Solvent A was 0.1% trifluoroacetic acied (TFA) in water, Solvent B was 0.1%TFA in acetonitrile (ACN), and a linear gradient of 5%B to 95%B over 12 min was used. ^{1}H-NMR spectra was recorded with a Varian spectrometer at 400MHz. Chemical shifts are reported in parts per million (δ) and are referenced to tetramethylsilane (TMS). All chemicals are commercially available from Aldrich.

Resorufinyl 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranoside (7). To a solution of 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl chloride (1.21 g, 3.0 mmol) in dry HMPA (12 mL) was added resorufin sodium salt (1.41 g, 6.0 mmol). The reaction was stirred for 48 h at room temperature. The mixture was diluted with ethyl acetate (EtOAc, 300 mL), washed with water (2 x 150 mL) and brine, and dried over sodium sulfate. After evaporation of the solvent, the residue was purified by column chromatography using a gradient of toluene:EtOAc (4:1 to 2:1) on silica gel to give **7** (670 mg, 41% yield) as an orange oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (d, 1H, J = 8.6 Hz), 7.42 (d, 1H, J = 10.0 Hz), 7.12 (dd, 1H, J = 2.5 Hz and 8.8 Hz), 7.09 (d, 1H, J = 2.5 Hz), 6.85 (dd, 1H, J = 2.0 Hz and 9.8 Hz), 6.33 (d, 1H, J = 2.2 Hz), 5.84 (d, 1H, J = 3.5 Hz), 5.70 (t, 1H, J = 10.0 Hz), 5.18 (t, 1H, J = 9.7 Hz), 5.08 (dd, 1H, J = 3.7 Hz and 10.4 Hz), 4.26 (dd, 1H, J = 5.2 Hz and 12.8 Hz), 4.09-4.04 (m, 2H), 2.09 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H). MS (*m/z*): 544 $(M+H)^+$.

Resorufinyl 2,3,4,6-Tetra-O-acetyl-α-D-galactopyranoside (8). 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl chloride (1.21 g, 3.0 mmol) and resorufin sodium salt (1.41 g, 6.0 mmol) were treated in the same manner as with the preparative procedure for **7** to provide **8 (**610 mg, 37% yield) as an orange oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.67 (d, 1H, J = 8.6 Hz), 7.35 (d, 1H, J = 9.8 Hz), 7.02 (dd, 1H, J = 2.6 Hz and 8.5 Hz), 7.00 (d, 1H, J = 2.3 Hz), 6.78 (dd, 1H, J = 2.0 Hz and 9.8 Hz), 6.29 (d, 1H, J = 2.0 Hz), 5.81 (d, 1H, J = 3.7 Hz), 5.49 (dd, 1H, J = 3.3 Hz and 10.6 Hz), 5.47(m, 1H), 5.24 (dd, 1H, J = 3.5 Hz and 10.6 Hz), 4.22 (t, 1H, J = 6.5 Hz), 4.04-4.02 (m, 2H), 2.11 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.88 (s, 3H). MS (*m/z*): 544 $(M+H)^+$.

Resorufinyl α-D-glucopyranoside (3). To a suspension of compound **7** (203 mg, 0.37 mmol) in a mixture of methanol-tetrahydrofuran (12 mL, 1:1 v/v) was added samarium (225 mg, 1.5 mmol) and iodine (380 mg, 1.5 mmol). The mixture was refluxed for 5 h. The solvents was removed in vacuo, and the residue was purified by preparative HPLC to give **3 (**19 mg, 14% yield) as an orange powder. ^{1}H NMR (DMSO-d_6/D_2O, 400 MHz) δ 7.76 (d, 1H, J = 8.6 Hz), 7.51 (d, 1H, J = 10.0 Hz), 7.19 (d, 1H, J = 2.5 Hz), 7.15 (dd, 1H, J = 2.5 Hz and 8.8 Hz), 6.78 (dd, 1H, J = 2.2 Hz and 9.8 Hz), 6.27 (d, 1H, J = 2.0 Hz), 5.59 (d, 1H, J = 3.7 Hz), 3.61 (dd, 1H, J = 8.9 Hz and 9.5 Hz), 3.50 (dd, 1H, J = 2.0 Hz and 12.1 Hz), 3.43 (t, 1H, J = 5.8 Hz), 3.41 (m, 1H), 3.35 (m, 1H), 3.18 (dd, 1H, J = 8.9 Hz and 9.9 Hz). MS (*m/z*): 376 $(M+H)^+$.

Resorufinyl α-D-galactopyranoside (4). Compound **8** (110 mg, 0.20 mmol) was treated in the same manner as with the preparative procedure for **3** to provide **4 (**12 mg, 16% yield) as an orange powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.79 (d, 1H, J = 8.8 Hz), 7.54 (d, 1H, J = 9.8 Hz), 7.21 (d, 1H, J = 2.5 Hz), 7.14 (dd, 1H, J = 2.6 Hz and 8.8 Hz), 6.80 (dd, 1H, J = 2.0 Hz and 9.8 Hz), 6.30 (d, 1H, J = 2.2 Hz), 5.68 (d, 1H, J = 3.3 Hz), 5.06 (d, 1H, J = 6.3 Hz), 4.84 (d, 1H, J = 5.5 Hz), 4.66 (d, 1H, J = 4.1 Hz), 4.56 (t, 1H, J = 5.7 Hz), 3.86-3.76 (m, 3H), 3.65 (t, 1H, J = 6.4 Hz), 3.53 (m, 1H), 3.39 (m, 1H). MS (*m/z*): 376 $(M+H)^+$.

References

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