Supporting Information

Practical Synthesis of Fluorogenic Enzyme Substrate 4-methylumbelliferyl-α-L-idopyranosiduronic acid

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General information

Moisture-sensitive reactions were carried out under an argon atmosphere, and glassware was flame-dried under high vacuum or in an oven. All solvents were distilled from appropriate drying agents prior to use. Unless otherwise specified, all the reagents were acquired from commercial and used except for special notification. Reaction progress was monitored by TLC performed on glass plates coated with silica gel F254. Samples were made visual by UV light at 254 nm or by staining with 5% H2SO4 in ethanol upon heating. Flash column chromatography was performed using silica gel 60 (230–400 mesh). Melting points were recorded on a BUCHI M-560 apparatus and are uncorrected. Optical rotation was measured on a Rudolph Autopol IV at a wavelength of 589 nm. IR spectra were acquired with Perkin-Elmer ONE FTIR spectrometers. 1H and 13C NMR spectra were recorded with a Bruker AV-400 spectrometer at 300K. The chemical shifts are reported in ppm relative to TMS as the internal standard (δ = 0.00) or the residual solvent signal (1H NMR: CDCl3, δ = 7.26; DMSO-d6, δ = 2.50; 13C NMR: CDCl3, δ = 77.16; DMSO-d6, δ = 39.52). Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). High resolution mass (HRMS) were acquired with AB Sciex TOF 4600 spectrometer.

Methyl 1-bromo-2,3,4-tri-O-acetyl-β-D-glucuronate (11)

A 500mL flask was charged with methyl 1,2,3,4-tetra-O-acetyl-β-D-glucuronate 10 (20.0 g, 53.2 mmol) and 33% HBr in acetic acid (200 mL). After addition, the flask was immediately sealed and the reaction mixture was stirred at 0 °C for 3 h. TLC detection showed the reaction was complete. The solution was diluted with CH2Cl2 (300 mL), washed with water (300 mL × 3), sat. NaHCO3 (300 mL × 3) and brine (300 mL × 3). The organic extracts were collected and dried over anhydrous MgSO4. After filtration, the filtrate was concentrated under reduced pressure to give compound 11 (20.1 g, yield: 95%).

Methyl 4-methylcoumarin-7-yl-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (8)

To a solution of compound 11 (20.0 g, 50.36 mmol) in anhydrous CH3CN was added 4-methylumbelliferone 3 (10.6 g, 60.43 mmol) and Ag2O (17.5 g, 75.53 mmol). The reaction mixture was stirred for 4 h at room temperature under argon atmosphere. TLC detection showed the reaction was complete. The mixture was concentrated under reduced pressure to remove CH3CN. The residue was dissolved in CH2Cl2 (350 mL), washed with water (300 mL × 3), sat. NaHCO3 (300 mL × 3), brine (300 mL × 3), and dried over anhydrous MgSO4. After
filtration, the filtrate was concentrated under reduced pressure. The remaining residue was recrystallized to afford compound 8 as a white powder solid (21.6 g, yield: 87%). m.p. 183–185 °C; [α]D25 -45.89 (c 0.073, CH2Cl2); 1H NMR (400 MHz, DMSO-d6) δ 7.73 (d, J = 8.8 Hz, 1H, H-5), 7.07 (t, J = 2.5 Hz, 1H, H-8), 7.00 (dd, J = 8.8, 2.5 Hz, 1H, H-6), 6.28 (s, 1H, H-3), 5.82 (d, J = 7.9 Hz, 1H, H-1'), 5.47 (t, J = 9.6 Hz, 1H, H-3'), 5.18 – 5.13 (m, 1H, H-2'), 5.10 (t, J = 9.7 Hz, 1H, H-4'), 4.78 (d, J = 9.9 Hz, 1H, H-5'), 3.64 (s, 3H, OCH3), 2.39 (s, 3H, 4-CH3), 2.02 (d, J = 6.5 Hz, 9H, OAc); 13C NMR (100 MHz, DMSO-d6) δ 169.5 (OAc), 169.3 (OAc), 169.0 (OAc), 167.0 (COOMe), 159.8 (C-2), 158.6 (C-7), 154.3 (C-9), 153.1 (C-4), 126.9 (C-5), 115.1 (C-10), 113.1 (C-6), 112.3 (C-3), 103.2 (C-8), 96.4 (C-1'), 71.1 (C-5'), 71.0 (C-3'), 70.3 (C-2'), 68.8 (C-4'), 52.6 (OCH3), 20.3 (C-2, OAc), 20.2 (OAc), 18.1 (4-CH3); HRMS (ESI+) m/z calcd for C23H25O12 [M+H]+: 493.1341; found 493.1346.

Methyl 4-methylcoumarin-7-yl-2,3,4-tri-O-acetyl-5-C-bromo-β-D-glucopyranuronate (7) and Methyl 4-bromomethylcoumarin-7-yl-2,3,4-tri-O-acetyl-5-C-bromo-β-D-glucopyranuronate (12)

To a solution of compound 8 (1.00 g, 2.03 mmol) in dry CCl4 (10 mL) was added slowly the solution of N-bromosuccinamide (NBS, 542 mg, 3.05 mmol) in dry CCl4 (10 mL) under the irradiation of tungsten lamp. The reaction mixture was refluxed for 2 h. TLC detection showed the reaction was complete. The reaction mixture was cooled and filtered. After filtration, the filtrate was diluted with CH2Cl2 (20 mL), washed with water (30 mL × 2), sat. NaHCO3 (30 mL × 2), brine (30 mL × 2). The organic extracts were collected and dried over MgSO4 and concentrated under reduced pressure. The residue was purified by flash-column chromatography to afford a mixture of 7 and 12 (1.03 g, 7:12 ≈ 1:1, yield: 83%).

Compound 7: m.p. 180–182 °C; [α]D25 -65.63 (c 0.064, CH2Cl2); 1H NMR (400 MHz, DMSO-d6) δ 7.80 (d, J = 8.7 Hz, 1H), 7.12 – 6.99 (m, 2H), 6.32 (s, 1H), 5.96 (d, J = 8.1 Hz, 1H), 5.67 (d, J = 9.1 Hz, 1H), 5.54 – 5.43 (m, 2H), 3.76 (s, 3H), 2.41 (s, 3H), 2.06 (s, 6H), 2.01 (s, 3H); 13C NMR (101 MHz, DMSO-d6) δ 169.3, 168.8, 168.3, 163.9, 159.6, 158.1, 154.1, 153.0, 127.1, 115.6, 112.9, 112.7, 103.5, 97.3, 89.8, 69.9, 69.0, 68.7, 54.1, 20.3, 20.2, 20.1, 18.0; HRMS (ESI+) m/z calcd for C23H23BrO12 [M+H]+: 571.0446; found 571.0451.

Compound 12: m.p. 90–92 °C; [α]D25 -58.86 (c 0.079, CH2Cl2); 1H NMR (400 MHz, DMSO-d6) δ 7.91 (dd, J = 8.8, 2.3 Hz, 1H), 7.19 – 7.05 (m, 2H), 6.66 (d, J = 2.4 Hz, 1H), 5.99 (dd, J = 8.1, 2.3 Hz, 1H), 5.68 (dd, J = 8.2, 2.3 Hz, 1H), 5.67 (d, J = 9.1 Hz, 1H); 13C NMR (101 MHz, DMSO-d6) δ 169.3, 168.8, 168.3, 163.9, 159.6, 158.1, 154.1, 153.0, 127.1, 115.6, 112.9, 112.7, 103.5, 97.3, 89.8, 69.9, 69.0, 68.7, 54.1, 20.3, 20.2, 20.1, 18.0; HRMS (ESI+) m/z calcd for C23H23BrO12 [M+H]+: 571.0446; found 571.0451.
= 9.2, 2.4 Hz, 1H), 5.55 (td, J = 8.9, 8.0, 2.4 Hz, 1H), 5.47 (td, J = 9.4, 2.3 Hz, 1H), 4.87 (d, J = 2.3 Hz, 2H), 2.06 (d, J = 2.3 Hz, 6H), 2.01 (d, J = 2.3 Hz, 3H); \textsuperscript{13}C NMR (101 MHz, DMSO-d\textsubscript{6}) δ 169.3, 168.8, 168.3, 163.9, 159.5, 158.3, 154.7, 150.8, 127.2, 114.1, 113.0, 112.9, 103.8, 97.2, 89.8, 69.9, 69.0, 68.7, 54.0, 27.8, 20.3, 20.2, 20.1; HRMS (ESI+) m/z calcd for C\textsubscript{23}H\textsubscript{23}Br\textsubscript{2}O\textsubscript{12}[M]+: 650.9530; found 650.9538.

Methyl 4-methylcoumarin-7-yl-2,3,4-tri-O-acetyl-\(\alpha\)-L-idopyranuronate (13)

\[
\text{MeO}_2C\text{CH}_3\text{O}_{\text{Ac}}\text{O}_{\text{Ac}}\text{O}_{\text{Br}}\text{O}_{\text{Ac}}\text{O}_{\text{Ac}}\text{Br} \xrightarrow{\text{Et}_3\text{B}, \text{Bu}_3\text{SnH}} \text{MeO}_2C\text{O}_{\text{Ac}}\text{O}_{\text{Ac}}\text{O}_{\text{Ac}}\text{O}_{\text{Ac}}\text{O}_{\text{Ac}}\text{O}_{\text{Ac}}\text{O}_{\text{Ac}}\text{O}_{\text{Ac}}\text{O}_{\text{Ac}}\text{O}_{\text{Ac}}
\]

To a solution of mixture of compound 7 and 12 (1.00 g) in dry CH\textsubscript{2}Cl\textsubscript{2} (50 mL) was added Bu\textsubscript{3}SnH (2.22 mL, 8.22 mmol) dropwise and Et\textsubscript{3}B (0.41 mL, 0.41 mmol, 1M sol. in hexane) in 1/3 portions every 15 min at -78 °C under argon atmosphere. After addition, the reaction mixture was heated to room temperature and stirred for 5 h. TLC detection showed the reaction was complete. The mixture solution was concentrated under reduced pressure. The residue was purified by column chromatography to afford little white solid powder 13 and major mixture of 13 and 8 (0.62 g, 13:8 ≈ 1:1, yield: 77%).

Compound 13: m.p. 89–91 °C; [\(\alpha\)]\textsubscript{D}\textsuperscript{25} -108.09 (c 0.068, CH\textsubscript{2}Cl\textsubscript{2}); \textsuperscript{1}H NMR (400 MHz, DMSO-d\textsubscript{6}) δ 7.75 (d, J = 8.8 Hz, 1H, H-5), 7.16 (s, 1H, H-8), 7.11 (d, J = 8.9 Hz, 1H, H-6), 6.28 (s, 1H, H-3), 6.02 (s, 1H, H-1'), 5.16 (s, 1H, H-3'), 5.04 (s, 2H, H-2', H-4'), 4.99 (s, 1H, H-5'), 3.66 (s, 3H, OCH\textsubscript{3}), 2.41 (s, 3H, 4-CH\textsubscript{3}), 2.16 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.05 (s, 3H, OAc); \textsuperscript{13}C NMR (101 MHz, DMSO-d\textsubscript{6}) δ 169.1(OAc), 169.0 (C\textsuperscript{1}, OAc), 167.3(COOMe), 159.8 (C-2), 158.1 (C-7), 154.2 (C-9), 153.1(C-4), 126.7 (C-5), 114.8 (C-10), 113.4 (C-6), 112.2 (C-3), 103.8 (C-8), 95.2 (C-1'), 67.5 (C-5'), 66.8(C-3'), 66.4(C-4'), 66.0(C-2'), 52.3 (OCH\textsubscript{3}), 20.5 (C\textsuperscript{1}, OAc), 20.3 (OAc), 18.0 (4-CH\textsubscript{3}); HRMS (ESI+) m/z calcd for C\textsubscript{23}H\textsubscript{25}O\textsubscript{12} [M+H]\textsuperscript{+}: 493.1341; found 493.1346.
Table 1 Optimization of radical reduction of 7 and 12

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<th>Temperature</th>
<th>Ratio of 13:8</th>
<th>Yield</th>
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<td>Ph₃SnH</td>
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<td>Toluene</td>
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<td>1:3</td>
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<td>Et₃B</td>
<td>DCM</td>
<td>-78 °C</td>
<td>1:1</td>
<td>77%</td>
</tr>
</tbody>
</table>

Methyl 4-methylcoumarin-7-yl-α-L-idopyranuronate (14) and Methyl 4-methylcoumarin-7-yl-β-D-glucopyranuronate (15)

A solution of mixture 13 and 8 (1.0 g, 2.03 mmol) in methanolic sodium (50 mg of sodium in 20 mL of dry methanol) was stirred at 0 °C for 0.5 h and then heated to room temperature for 4 h. TLC detection showed the reaction was complete. The reaction mixture was concentrated under reduced pressure and the residue was purified by flash-column chromatography to give compound 14 (358 mg, yield 48%) and compound 15 (330 mg, yield 44%).

Compound 14: m.p. 119–121°C; [α]₂⁰°° -111.73 (c 0.081, CH₃OH); ¹H NMR (400 MHz, DMSO-d₆) δ 7.73 (d, J = 8.5 Hz, 1H, H-5), 7.07-7.04 (m, 2H, H-8, H-6), 6.26 (s, 1H, H-3), 5.70 (d, J = 4.9 Hz, 1H, H-1'), 5.57 (d, J = 5.8 Hz, 1H, 2'-OH), 5.38-5.36 (m, 2H, 3'-OH, 4'-OH), 4.65 (d, J = 4.3 Hz, 1H, H-5'), 3.82-3.78 (m, 1H, H-4'), 3.67-3.63 (m, 4H, H-3', COOMe), 3.58 – 3.53 (m, 1H, H-2'), 2.40 (s, 3H, 4-CH₃); ¹³C NMR (101 MHz, DMSO-d₆) δ 170.0 (COOMe), 160.0 (C-2), 159.6 (C-7), 154.3(C-9), 153.2 (C-4), 126.6 (C-5), 114.2 (C-10), 113.3 (C-6), 111.8 (C-3), 103.3 (C-8), 99.0 (C-1'), 71.8 (C-5'), 71.4 (C-3'), 70.7 (C-2'), 70.0 (C-4'), 51.7
(OCH₃), 18.1 (4-CH₃); HRMS (ESI+) m/z calcld for C₁₇H₁₉O₉ [M+H]⁺: 367.1024; found 367.1029.

Compound 15: ¹H NMR (400 MHz, DMSO-d₆) δ 7.71 (d, J = 8.8 Hz, 1H, H-5), 7.09 (d, J = 2.3 Hz, 1H, H-8), 7.03 (dd, J = 8.9, 2.3 Hz, 1H, H-6), 6.25 (s, 1H, H-3), 5.60 (d, J = 4.2 Hz, 1H, H-1’), 5.50 (d, J = 5.4 Hz, 1H, H-5’), 5.38 – 5.34 (m, 1H, H-5’), 5.30 (d, J = 7.0 Hz, 1H, H-5’), 4.20 (d, J = 9.3 Hz, 1H, H-5’), 3.66 (s, 3H, H-5’), 3.46 – 3.27 (m, 5H, H-5’), 2.40 (s, 3H, H-5’); ¹³C NMR (101 MHz, DMSO-d₆) δ 169.1 (COOMe), 160.0 (C-2), 159.6 (C-7), 154.4 (C-9), 153.2 (C-4), 126.5 (C-5), 114.3 (C-10), 113.2 (C-6), 111.8 (C-3), 103.0 (C-8), 99.3 (C-1’), 75.5 (C-5’), 75.1 (C-3’), 72.8 (C-2’), 71.3 (C-4’), 51.9 (OCH₃), 18.1 (4-CH₃).

4-Methylcoumarin-7-yl-α-L-iduronic acid (1)

To a solution of compound 14 (40 mg, 109.2 µmol) in the mixture solution of THF (4 mL) and H₂O (0.4 mL) was added 65.52 µL of 2N aqueous NaOH solution at -10 °C. After addition, the reaction mixture was stirred at 0 °C for 3h and added Dowex-50WX8-200 ion-exchange resin to adjust the pH value to 7. After filtration, the filtrate was concentrated under reduced pressure and the residue was freeze-dried by freeze dryer to give compound 1 (36.4 mg, yield 95%). m.p. 205–207 °C; [α]D 38.3 (c 0.021, CH₃OH); ¹H NMR (400 MHz, DMSO-d₆) δ 7.87 (s 1H, H-8), 7.65 (d, J = 8.8 Hz, 1H, H-5), 7.34 (dd, J = 8.8, 2.4 Hz, 1H, H-6), 6.21 (s, 1H, H-3), 5.51 (d, J = 7.0 Hz, 1H, 2’-OH), 5.19 (d, J = 4.9 Hz, 1H, H-1’), 4.96 (d, J = 3.8 Hz, 1H, 3’-OH), 3.99 (d, J = 6.2 Hz, 1H, H-3’), 3.25 – 3.17 (m, 3H, H-2’, H-4’, H-5’), 2.40 (s, 3H, 4-CH₃); ¹³C NMR (101 MHz, DMSO-d₆) δ 172.1 (COOH), 160.8 (C-2), 160.4 (C-7), 154.6 (C-9), 153.4 (C-4), 126.0 (C-5), 113.6 (C x 2, C-10, C-6), 111.3 (C-3), 103.6 (C-8), 97.3 (C-1’), 74.4 (C-5’), 73.1 (C-3’), 72.5 (C-4’), 71.1 (C-2’), 18.1 (4-CH₃); HRMS (ESI+) m/z calcld for C₁₆H₁₇O₉ [M+H]⁺: 353.0867; found 353.0868.

¹H NMR (400 MHz, Methanol-d₄) δ 7.69 (d, J = 8.8 Hz, 1H, H-5), 7.49 – 7.47 (m, 1H, H-6), 7.44 (d, J = 2.4 Hz, 1H, H-8), 6.17 (s, 1H, H-3), 5.61 (d, J = 6.4 Hz, 1H, H-1’), 4.40 (d, J = 5.2 Hz, 1H, H-3’), 3.76 – 3.73 (m, 1H, H-2’), 3.65 (t, J = 8.1 Hz, 1H, H-4’), 3.52 (t, J = 7.1 Hz, 1H, H-5’), 2.45 (s, 3H, 4-CH₃). ¹³C NMR (101 MHz, Methanol-d₄) δ 176.2 (COOH), 163.5(C-2), 162.2 (C-7), 156.1 (C-9), 155.6 (C-4), 127.1 (C-5), 115.7 (C-10), 114.9 (C-6), 112.6 (C-3), 105.3 (C-8), 99.4 (C-1’), 74.6 (C-5’), 73.9(C-3’), 73.4(C-4’), 72.5(C-2’), 18.6 (4-CH₃).
$^1$H and $^{13}$C spectra of compound 8:
$^1$H and $^{13}$C spectra of compound 7:
$^1$H and $^{13}$C spectra of compound 12
$^1$H and $^{13}$C spectra of compound 13
$^1$H and $^{13}$C spectra of compound 14
$^1$H and $^{13}$C spectra of compound 15
$^1$H and $^{13}$C spectra of compound 1
$^1$H and $^{13}$C spectra of compound 1
HR-MS of compound 1: