A New Synthetic Route to 4-Methylumbelliferyl-β-D-glucopyranosiduronic Acid (MUG)

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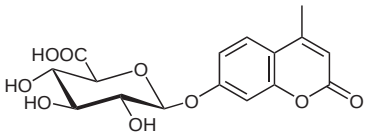
Abstract: A synthetic route to prepare 4-methylumbelliferyl-β-D-glucopyranosiduronic acid (MUG) from 4-methylumbelliferyl-β-D-glucopyranoside (MUGluc) was developed. The primary hydroxyl group in MUGluc was protected by tritylation followed by acetylation of secondary hydroxyls. The triphenylmethyl group was selectively removed by treatment with iodine-methanol in benzene and the free hydroxyl was transformed into the carboxylic acid by phase-transfer oxidation with sodium hypochlorite and TEMPO as catalyst. Finally, the acetate groups were removed by reaction with barium methoxide in methanol to afford the MUG with an overall yield of 37% from the MUGluc.

Key words: synthesis, MUG, 4-methylumbelliferyl-β-D-glucopyranoside, 4-methylumbelliferyl-β-D-glucopyranosiduronic acid

Substrates based on 4-methylumbelliferone (4-MU) have been extensively used for the detection of enzymes in microbiological diagnostics.[1,2] The facility of hydrolysis of these kind of compounds by a specific enzyme makes possible its detection by the fluorescence expressed by the 4-MU in the media.[3–5] The 4-methylumbelliferyl-β-D-glucopyranosiduronic acid (MUG, Figure 1) is a commercially available fluorogenic substrate (Biosynth, Fluka) widely used for quantitatively identifying and differentiating the presence of the Coliform group and specially *Escherichia coli* in water, food and other products for human consumption.[6–8]



MUG

Figure 1 Chemical structure of 4-methylumbelliferyl-β-D-glucopyranosiduronic acid (MUG)

From a synthetic point of view, there are two possible approaches of preparing MUG: (a) direct glycosylation of 4-MU or its salts with glucopyranuronic acid derivatives and (b) oxidation of primary hydroxyl group of the 4-methylumbelliferyl-β-D-glucopyranoside (MUGluc). However, there are no reports in the literature related with the first of both approaches, probably because of the instability of typical glucopyranuronic acid derivatives such as bromides or iodides. In effect, it is known that during the preparation of the umbelliferyl-β-D-glucopyranosiduronic acid (a MUG analogue) by reaction between methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide or iodide and umbelliferone, low yields (6–10%) of the conjugate are obtained.[9–11]

The only reported procedure in order to synthesize the MUG carries out the oxidation of primary hydroxyl group of the easily available MUGluc by oxygen gas in aqueous alkaline media using platinum as catalyst.[12] However, this approach has some drawbacks: high purity oxygen must be used to avoid catalyst poisoning, large charge of platinum catalyst is necessary (approximately 50% in weight related to MUGluc), ion-exchange column chromatography must be used to isolate the desired product and finally, large volumes of water have to be eliminated by freeze-drying and relatively low yields of MUG are commonly obtained (18–20%).

In order to overcome the previously cited drawbacks, we developed an alternative way for obtaining MUG (Scheme 1).

In the literature there is a report about an unsuccessful attempt of using this approach to synthesize 4-methylumbelliferyl α-L-idopyranosiduronic acid.[13] In that case, the work was interrupted after detecting the acyl migration and low yields during the elimination of the trityl group by treatment with hydrogen bromide in acetic acid.

In the present work, the primary hydroxyl group of MUGluc was selectively protected by reaction with trityl chloride in pyridine, followed by acetylation of the secondary hydroxyl groups with acetic anhydride to obtain compound **1** with good yield (73%). Although the hydrogen bromide in acetic acid is widely used in carbohydrate chemistry to remove selectively the trityl protecting group, this technique was not used because very short reaction times are required (below 1 min). As a consequence, the process is difficult to control and relatively low yields are obtained due the sensibility of the glycosidic linkage in acidic media. An attempt of effecting the deprotection using anhydrous cupric sulfate in refluxing

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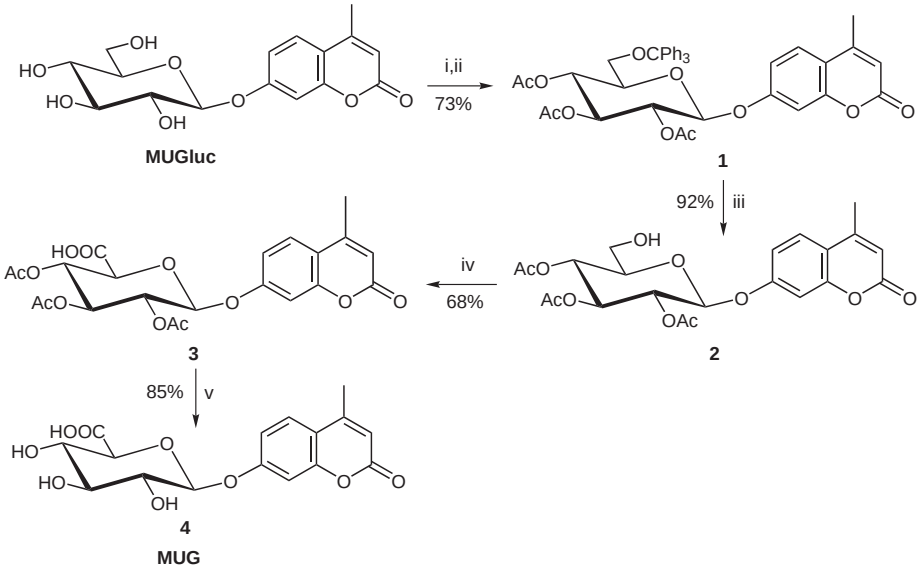

Scheme 1 Synthetic route to prepare MUG. *Reagents and conditions*: (i) Ph_3CCl, pyridine, 90 °C, 5 h; (ii) Ac_2O, r.t., 24 h; (iii) I_2, MeOH, benzene, 65 °C, 24 h; (iv) NaOCl, TEMPO, NaBr, $NaHCO_3$, Bu_4NBr, CH_2Cl_2–H_2O, 0 °C, 3 h; (v) $Ba(OMe)_2$, MeOH, 0–5 °C, 24 h.

benzene was made, according to the literature report.[14] However; no reaction was detected by TLC after 24 hours, a longer time than that reported (5 h). Moreover, the detritylation activity of several silica gels[15] is well documented since in the original work the reaction mixture was purified by column chromatography over silica gel. It is possible that the reaction observed in that case was promoted by this acidic absorbent and not by the cupric sulfate.

As a consequence, the detritylation step was performed using an iodine–methanol reagent.[16] This method has the advantage of being selective for the trityl group whereas the glycosidic linkage is stable to the reaction conditions. Detritylation probably occurs due to the in situ generation of acid traces (hydroiodic acid) produced by the oxidation of methanol by iodine. Thus, compound **2** was obtained with an excellent yield (92%) and good purity after removal of the triphenylmethanol (a by-product) with diisopropyl ether, 24 hours at 65 °C were required for the completion of the reaction and no acyl migration was detected.

Oxidation of the primary hydroxyl group in compound **2** to the corresponding acid was achieved by using a catalytic amount of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO), 5% aqueous sodium hypochlorite as oxidant and a basic medium generated by sodium hydrogencarbonate.[17] A biphasic system (dichloromethane–water), in the presence of tetrabutylammonium bromide (Bu_4NBr) as phase-transfer catalyst, was used to run the reaction because of the insolubility of **2** in water. Under these conditions, compound **3** was readily obtained (2–3 h) with an acceptable yield (68%) and good purity after acidification of the aqueous phase to pH 3, simultaneous extraction with dichloromethane and final solvent evaporation under reduced pressure.

The last step of this synthetic approach was the removal of acetate groups from secondary hydroxyls of **3**. It is well known that the hydrolysis of acetylated coumarin glycosides requires some attention, because a slight excess of base leads to the irreversible lactone opening.[18] If the alkali-metal salt of the glucuronide is desired, potassium or sodium hydroxide (or preferably the corresponding carbonates) in aqueous alcohols may be used. If the free glucuronic acid is required, it may be useful to carry out the deacetylation step by the Zemplen procedure (sodium methoxide–methanol), followed by treatment with an ion-exchange resin.[19]

Considering that the acetylated glycoside **3** has an acid group, it was decided to carry out the deacetylation with barium methoxide in methanol in order to simplify the final work-up for obtaining the MUG. It was necessary to add barium methoxide, not only to remove acetate groups but also to neutralize the acid function. Altough the formed barium salt of **3** is slightly soluble in methanol, the reaction was complete, as was demonstrated by TLC analysis.

The barium salt of the deacetylated product was also insoluble in methanol, but when the reaction mixture was treated with the calculated amount of sulfuric acid to remove barium all the obtained product become soluble in methanol and it was possible to quantitatively separate the barium sulfate to obtain MUG (**4**) in good yield (85%) and high purity, by removing the solvent under vacuum and crystallization from diethyl ether. It may be noted that during the process no lactone opening was observed.

In conclusion, the developed synthetic route is very useful to prepare MUG.[20] Despite of some synthetic steps more than the reported procedure, the overall yield obtained from MUGluc was 37%, it means almost twice the yield reported (18–20%) when this fluorogenic substrate was synthesized by oxidation with oxygen gas in the presence of platinum as catalyst.

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(20) **General Methods**
TLC was performed on precoated plates of silica gel GF-254 (Merck); EtOAc–$CHCl_3$–AcOH (8:6:1) and *n*-BuOH–H_2O–AcOH (5:1:1) were used as mobile phases. The chromatograms were visualized in a Camag UV/Vis lamp (λ = 254 and 360 nm). Compounds were also detected by spraying the plates with 5% H_2SO_4–EtOH reagent followed by heating. Melting points(mp) were determined using a Büchi capillary apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were registered on a Bruker AC 250F spectrometer for 250 MHz and 62.5 MHz, respectively, using $CDCl_3$, $CDCl_3$ + $MeOD_4$ or DMSO-d_6 as solvents and TMS as internal standard. Optical rotation of MUG was measured at 20 °C with an ADP220 Bellenghan + Stanley Ltd. apparatus. Elemental analysis was done in a Thermofinnigan FlashEA 1112 equipment.
4-Methylumbelliferyl-2,3,4-tri-*O*-acetyl-6-*O*-trityl-β-D-glucopyranoside (1)
Trityl chloride (4.25 g, 15.25 mmol) was added to a stirred suspension of 4-methylumbelliferyl-β-D-glucopyranoside (5.0 g, 14.78 mmol) in pyridine (11 mL). The mixture was heated at 90–100 °C for 5 h. After cooling to 0–5 °C, Ac_2O (7.5 mL, 79.34 mmol) was added and the mixture was left to stand overnight at r.t., poured into cold H_2O (225 mL) and stirred for 2 h. The resultant solid was filtered off, washed with cold H_2O (3 × 50 mL), vacuum-dried at 40 °C and recrystallized from EtOH to afford **1**. Yield 7.60 g (73%); mp 212–215 °C. ^{1}H NMR ($CDCl_3$): δ = 1.78 (3 H, s), 2.03 (3 H, s), 2.08 (3 H, s) 2.40 (3 H, s), 3.25 (2 H, m), 3.76 (1 H, m), 5.09–5.43 (4 H, m), 6.21 (1 H, s), 6.99–7.60 (18 H, m). ^{13}C NMR ($CDCl_3$): δ = 18.61, 20.31, 20.59, 20.60, 62.02, 68.40, 71.15, 72.78, 73.91, 86.89, 98.52, 104.40, 113.18, 113.74, 115.48, 125.72, 127.07 (3 C), 127.81 (6 C), 128.60 (6 C), 143.40 (3 C), 152.02, 154.83, 159.42, 160.76, 168.96, 169.28, 170.20. Anal. Calcd for $C_{41}H_{38}O_{12}$ (722.73): C, 68.14; H, 5.30. Found: C, 68.20; H, 5.17.
4-Methylumbelliferyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (2)
A solution of iodine (0.3 g, 1.18 mmol) in MeOH was added to a solution of compound **1** (1.8 g, 2.55 mmol) in benzene (18 mL) and the mixture was stirred at 65 °C for 24 h. The solvents were evaporated under vacuum and the residue was dissolved in EtOAc (60 mL), washed with 10% aq $Na_2S_2O_3$ (30 mL), followed by H_2O (3 × 10 mL), and dried over anhyd Na_2SO_4. The organic extract was evaporated to dryness under reduced pressure and the residue was stirred 2 h with diisopropyl ether (30 mL). The resulting solid was filtered off, washed with diisopropyl ether (3 × 5 mL) and vacuum-dried at 40 °C to afford **2**. Yield: 1.10 g (92%); mp 176–178 °C. ^{1}H NMR ($CDCl_3$): δ = 2.04 (6 H, s), 2.07 (3 H, s) 2.37 (3 H, s), 3.72 (3 H, m), 5.21 (4 H, m), 6.15 (1 H, s), 6.70–7.56 (3 H, m). ^{13}C NMR ($CDCl_3$): δ = 18.56, 20.52 (3 C), 60.91, 68.21, 71.04, 72.42, 74.65, 98.23, 103.95, 113.03, 113.61, 115.41, 125.75, 152.20, 154.70, 159.17, 160.81, 169.24, 170.06 (2 C). Anal. Calcd for $C_{22}H_{24}O_{12}$ (480.42): C, 55.00; H, 5.04. Found: C, 55.33; H, 5.20.
4-Methylumbelliferyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosiduronic Acid (3)
A sat. solution of aq $NaHCO_3$ (15 mL) and a 5% solution of NaOCl (19 mL) were added to a solution of **2** (1.25 g, 2.7 mmol), NaBr (0.06 g, 0.583 mmol), TBAB (0.06 g, 0.186 mmol), and TEMPO (0.06 g, 0.384 mmol) in a 1:1 mixture of CH_2Cl_2–H_2O (20 mL) at 0 °C. After 2–3 h the aqueous phase was separated and washed with CH_2Cl_2 (2 × 10 mL). The, CH_2Cl_2 (20 mL) was added; the mixture was cooled to 0–5 °C and adjusted to pH 3 with 10% HCl. The organic phase was separated, washed with H_2O (10 mL) and dried over anhyd Na_2SO_4. The solvent was evaporated to dryness under reduced pressure and the resulting solid was vacuum-dried to afford **3**. Yield 0.87 g (68%); mp 218–221 °C. ^{1}H NMR ($CDCl_3$ + $MeOD_4$): δ = 2.00 (6 H, s), 2.02 (3 H, s), 2.36 (3 H, s), 4.22 (1 H, m), 5.21–5.36 (4 H, m), 6.14 (1 H, s), 6.87–7.55 (3 H, m). ^{13}C NMR ($CDCl_3$ + $MeOD_4$): δ = 18.46, 20.37 (3 C), 68.86, 70.78, 71.92, 72.13, 98.11, 104.22, 112.80, 113.90, 115.50, 125.73, 152.70, 154.49, 159.09, 161.30, 167.94, 169.35, 169.69, 170.17. Anal. Calcd for $C_{22}H_{22}O_{13}$ (494.40): C, 53.45; H, 4.49. Found: C, 53.77; H, 5.03.
4-Methylumbelliferyl-β-D-glucopyranosiduronic Acid (MUG, 4).
A suspension of **3** (2.0 g, 4.18 mmol) in MeOH (30 mL) was stirred at 0–5 °C with a solution of $Ba(OMe)_2$ (0.503 g, 2.52 mmol) in MeOH (10 mL). After 24 h, concentrated H_2SO_4 (0.14 mL, 2.52 mmol) was added, the precipitated $BaSO_4$ was filtered off, the filtrate was evaporated to dryness under reduced pressure and the residue was crystallized with Et_2O (20 mL). The resultant solid was separated by filtration, washed with Et_2O (3 × 5 mL) and vacuum-dried to afford **4**. Yield 1.25 g (85%); mp 140–143 °C; $[\alpha]_D$ –116 (*c* 0.25, H_2O); {lit.[12] mp 139–145 °C; $[\alpha]_D$ –114 (*c* 0.25, H_2O)}. ^{1}H NMR (DMSO-d_6): δ = 2.40 (3 H, s), 3.23 (1 H, m), 3.56 (1 H, m), 4.98–5.19 (2 H, m), 5.38 (1 H, m), 6.22 (1 H, s), 6.99–7.75 (3 H, m). ^{13}C NMR (DMSO-d_6): δ = 17.93, 71.82, 72.96, 73.63, 76.43, 100.00, 103.29, 111.46, 113.43, 113.89, 126.14, 153.13, 154.22, 159.92, 160.23, 172.22.