Supporting Information

for

Flavonol-Based Fluorescent Indicator for Determination of β-Glucosidase Activity

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Experimental details for the preparation of compounds 1 and 3, corresponding characterization data and NMR spectra.

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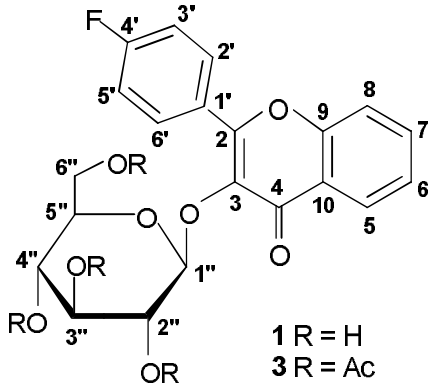
1. The atom numbering system for 4'-fluoroflavonol glucosides **1** and **3**:



2. Experimental Details

General methods

The IR spectra were recorded as Nujol mulls with a Bruker IFS 66 spectrophotometer. The ^{1}H and ^{13}C NMR spectra ($CDCl_3$ or CD_3OD, internal Me_4Si) were measured with a Bruker Avance III 500 (500.13/125.75 MHz) instrument (*J* values are given in Hz). Positive-ion mode MALDITOF mass spectra were obtained using a Bruker Biflex III spectrometer with 2,5-dihydroxybenzoic acid matrix. Elemental analysis was done on a Carlo Erba EA 1108 instrument. Thin-layer chromatography (TLC) was performed on the E. Merc Kieselgel 60 F-254 plates using the following eluent systems (v/v): A, 3:1 toluene-AcOEt; B, 5:1 toluene-AcOEt; C, 3:1 $CHCl_3$-MeOH; D, 2% i-PrOH in $CHCl_3$. Column chromatography was performed on MN Kieselgel 60 (< 0.08 mm) with one of the above listed eluent systems. Purity of all the samples used for enzymatic cleavage experiments were tested using reverse-phase HPLC method (purity > 95% at 254 nm).

4'-Fluoroflavonol β-D-glucopyranoside (1)

Approach 1

With separation of 4'-fluoroflavonol 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**3**)

Commercially available 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**4**) (305.5 mg, 0.74 mM) in $CHCl_3$ (6 mL) was added 4 times every 30 min with K_2CO_3 (51.1 mg, 0.37 mM) to a stirring suspension of 4'-fluoroflavonol (**2**) (95.2 mg, 0.37 mM), synthesized as reported previously,[1] K_2CO_3 (102.5 mg, 0.74 mM), TBAB (119.6 mg, 0.37 mM) in water (6 mL). The mixture was vigorously stirred at rt and utilization of flavonol **2** was controlled by TLC (solvent A). After 70 h the reaction mixture was diluted with DCM and washed several times with water. The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. Column chromatography of the crude product (solvent B) gave **3** as a white solid (157.1 mg, 60%). IR: ν_{max}/cm^{-1} 2953, 1752, 1658, 1619, 1510, 1468, 1415, 1388, 1371, 1228, 1204, 1042, 905, 851, 759, 622, 598. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 8.17 (1H, d, *J* 7.95, H5), 8.02 (1H, d, *J* 8.15, H2'), 8.01 (1H, d, *J* 7.95, H6'), 7.63 (1H, dd, *J* 7.15, *J* 8.4, H7), 7.47 (1H, d, *J* 8.45, H8), 7.36 (1H, dd, *J* 7.25, *J* 7.85, H6), 7.12 (2H, t, *J* 7.35, H3', H5'), 5.64 (1H, d, *J* 7.9, H1"), 5.22 (1H, t, *J* 9.3, H3"), 5.13 (1H, dd, *J* 7.95, *J* 9.3, H2"), 5.00 (1H, t, *J* 9.4, *J* 9.9, H4"), 3.94 (1H, dd, *J* 3.9, *J* 12.25, H6"$_A$), 3.84 (1H, d, *J* 12.25, H6"$_B$), 3.54 (1H, dd, *J* 2.5, *J* 10.0, H5"), 2.06, 1.95, 1.93, 1.81 (12H, 4s, 4 x OAc). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 173.9 (C4), 170.3, 170.0, 169.9, 169.5 (4 x C=O_{Ac}), 164.1 (1C, d, *J* 251, C4'), 156.4 (C2), 155.2 (C9), 136.1 (C3), 133.8 (C7), 131.5 (C2'), 131.4 (C6'), 128.2 (C1'), 125.7, 125.0 (C5, C6), 124.0 (C10), 118.1 (C8), 115.5, 115.3 (C3', C5'), 98.9 (C1"), 72.7 (C3"), 71.7 (C5"), 71.6 (C2"), 68.3 (C4"), 61.3 (C6"), 20.8, 20.6, 20.5, 20.4 (4 x CH_{3Ac}). MALDI-TOF-MS: *m/z* 587.2 (MH^+), 609.2 (MNa^+), 625.2 (MK^+).

1 I. E. Serdiuk, A. D. Roshal and J. Błażejowski, *Chem. Heterocycl. Compd.*, 2014, **50**, 396.

Next, 4'-fluoroflavonol 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**3**) (137.3 mg, 0.23 mmol) was dissolved in 0.1 M solution of NaOMe in abs MeOH (1.3 mL, 0.13 mM) and stirred at rt for 1 h. The end of deacetylation was detected by TLC (solvent C). The solution was then neutralized with Dowex-50Wx8 (H^+) ion-exchange resign and filtered. The filtrate was evaporated to yield **1** as a white solid (66.3 mg, 68%). IR: ν_{max}/cm^{-1} 3414, 2911, 1618, 1603, 1508, 1478, 1469, 1415, 1391, 1243, 1205, 1010, 905, 841, 759, 622. ^{1}H NMR (500 MHz, CD_3OD): δ_H 8.27 (1H, d, *J* 8.79, H2'), 8.26 (1H, d, *J* 8.79, H6'), 8.19 (1H, d, *J* 8.06, H5), 7.81 (1H, t, *J* 7.3, *J* 7.55, H7), 7.68 (1H, d, *J* 8.55, H8), 7.36 (1H, t, *J* 7.57, H6), 7.26 (2H, t, *J* 8.79, H3', H5'), 5.38 (1H, d, *J* 7.57, H1"), 3.69 (1H, dd, *J* 3.2, *J* 11.96, H6"$_A$), 3.52 (1H, d, *J* 5.37, *J* 11.96, H6"$_B$), 3.43 (2H, m, H2", H3"), 3.30 (1H, m, H4"), 3.21 (1H, ddd, *J* 2.2, *J* 5.61, *J* 9.79, H5"). ^{13}C NMR (125 MHz, CD_3OD): δ_C 176.5 (C4), 165.6 (1C, d, *J* 251, C4'), 158.2 (C2), 156.8 (C9), 138.2 (C3), 135.5 (C7), 133.1 (C2'), 133.0 (C6'), 128.5 (C1'), 126.4 (C5, C6), 124.9 (C10), 119.4 (C8), 116.3 (C3'), 116.1 (C5'), 103.6 (C1"), 78.5 (C5"), 78.1 (C3"), 75.7 (C2"), 71.3 (C4"), 62.5 (C6"). MALDI-TOF-MS: *m/z* 419.2 (MH^+), 441.2 (MNa^+), 457.2 (MK^+). Elemental analysis calcd (%) C_{21} $H_{19}F_1O_8$ (418.37): C 60.29, H 4.58; found: C 59.94, H 4.56.

Approach 2

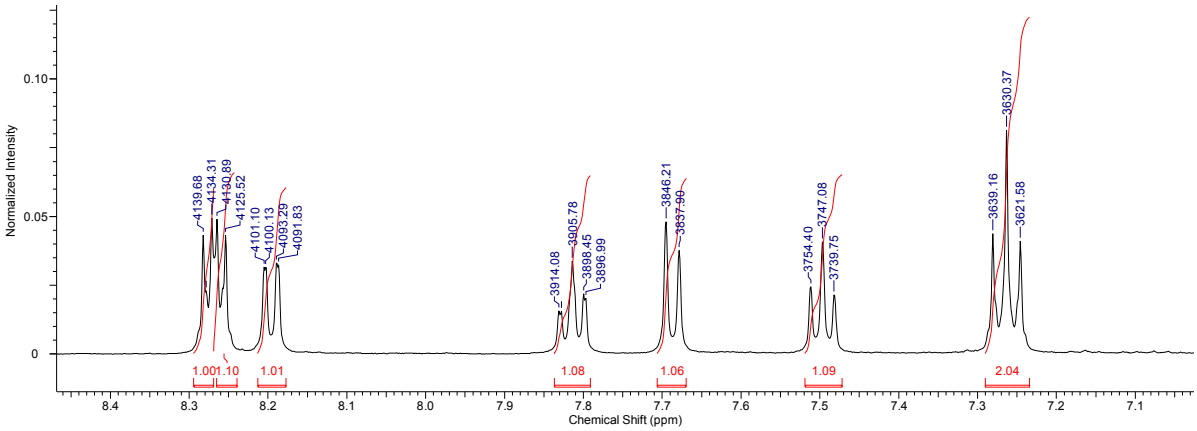
Without separation of 4'-fluoroflavonol 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**3**)

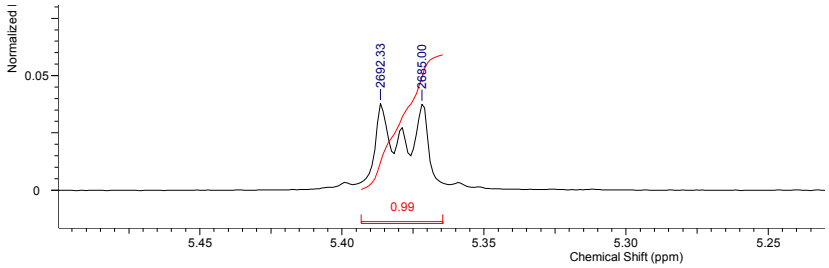
2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**4**) (1.6 mmol, 655 mg) in $CHCl_3$ was added 4 times in 10 h with K_2CO_3 (1.76 mmol, 242 mg) to a stirring suspension of 4'-fluroflavonol (**2**) (0.4 mmol, 102 mg), and TBAB (0.4 mmol, 128 mg) in water. The mixture was vigorously stirred at rt and utilization of flavonol **2** was controlled by TLC (solvent D). The mixture was diluted with $CHCl_3$ and washed several times with water. The organic layer was evaporated under reduced pressure and brought to deacetylation step without further purification. The obtained brownish oil was treated with 8 eq of K_2CO_3 in MeOH and stirred

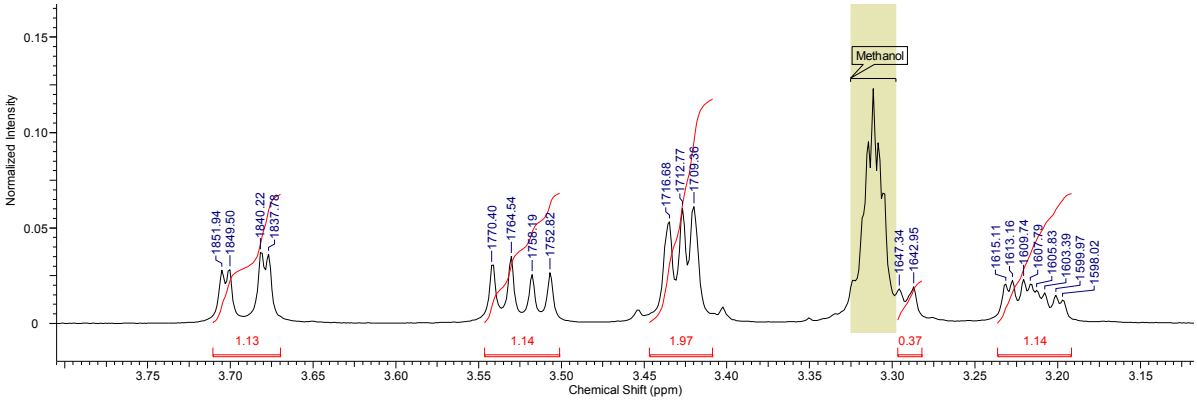
at rt 10 h. Methanol was evaporated under reduced pressure and obtained solid was treated with water, filtered, washed several times with water and dried to yield **1** (39%). All analyses are in agreement with those presented above.

3. NMR spectra for **1** and **3**

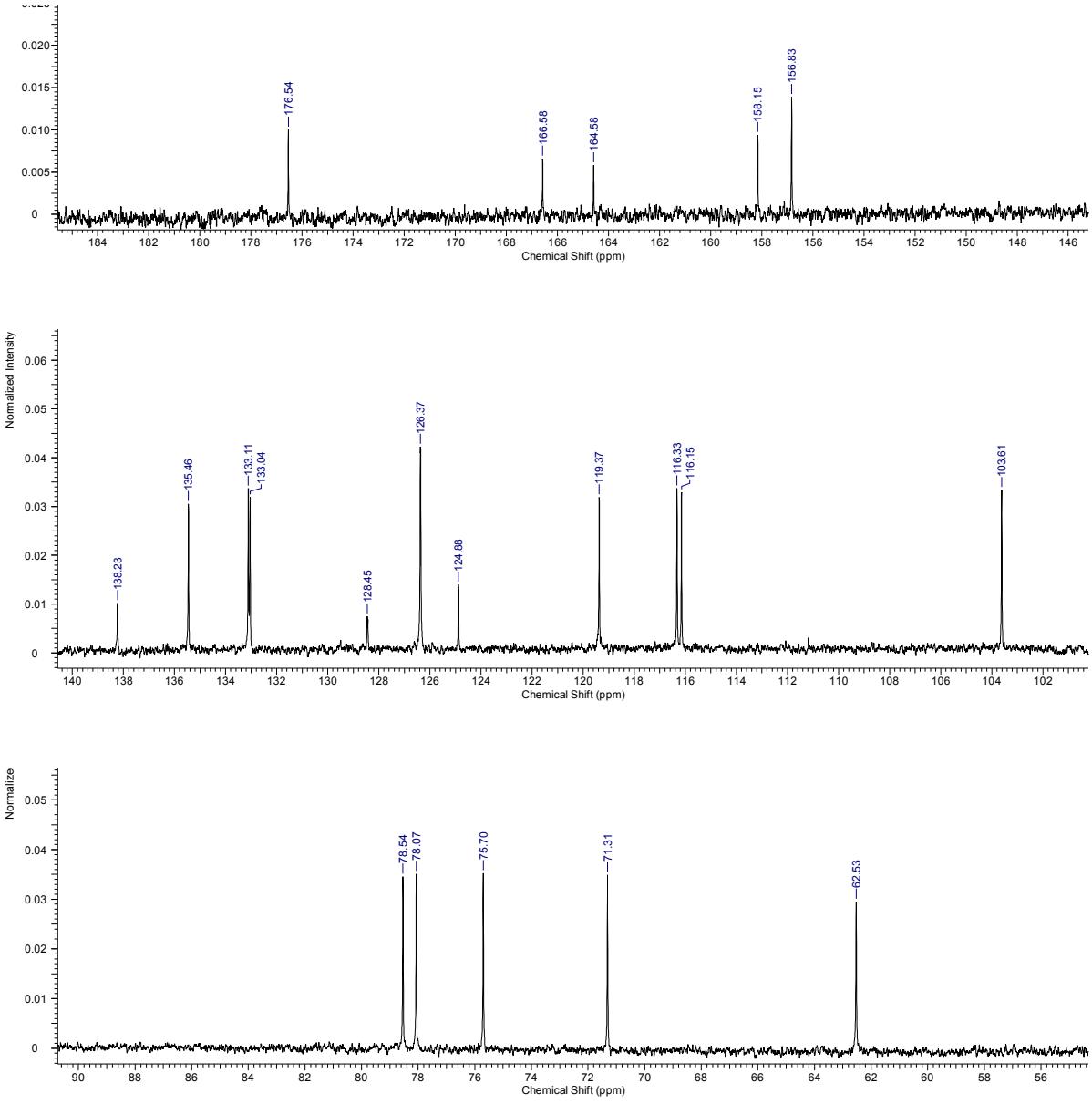
3.1. ^{1}H NMR spectrum of **1** (CD_3OD, 500 MHz)



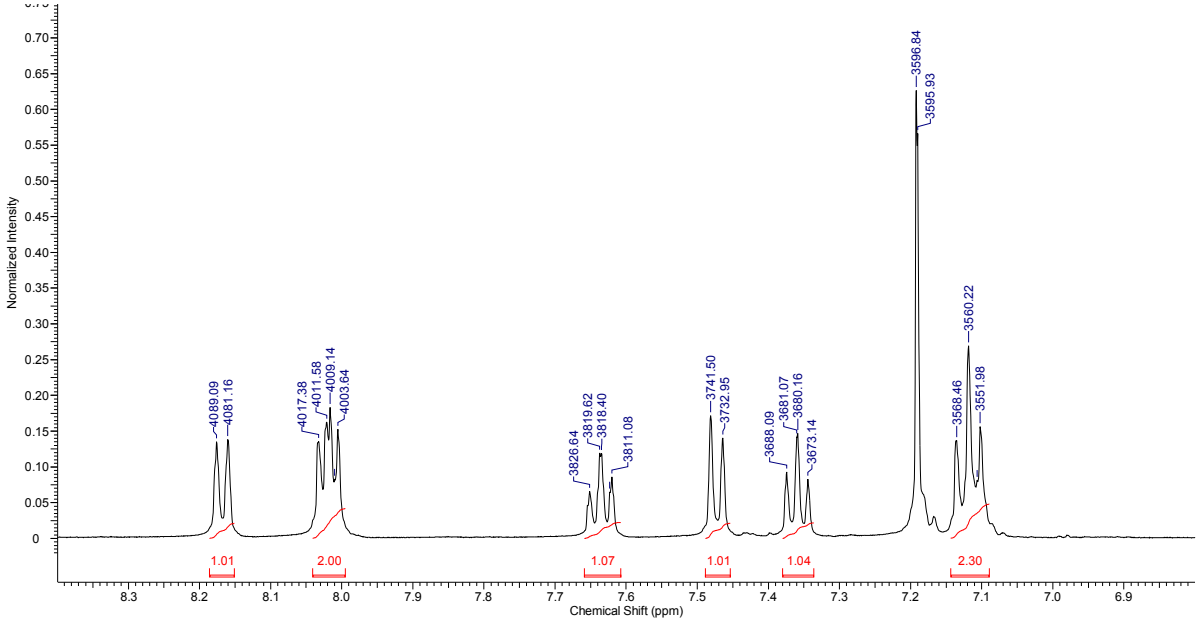


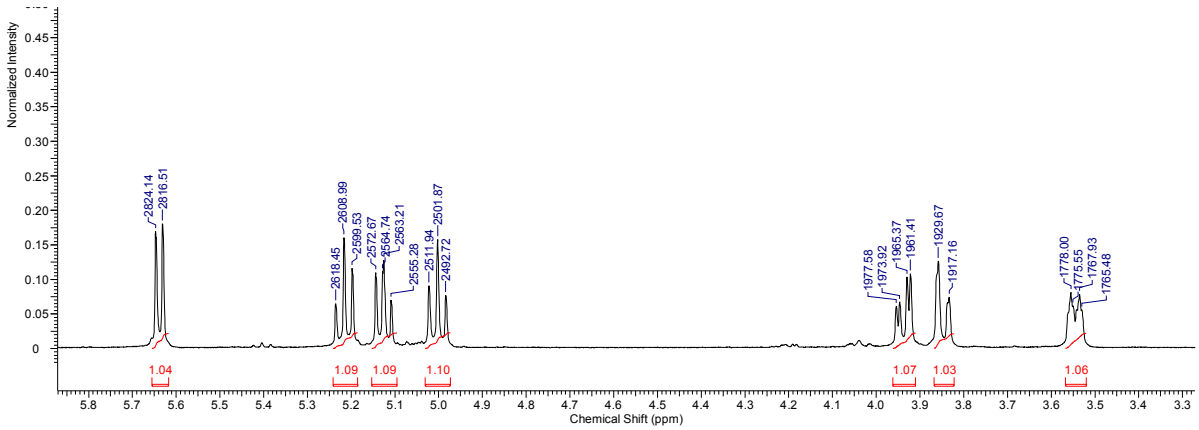


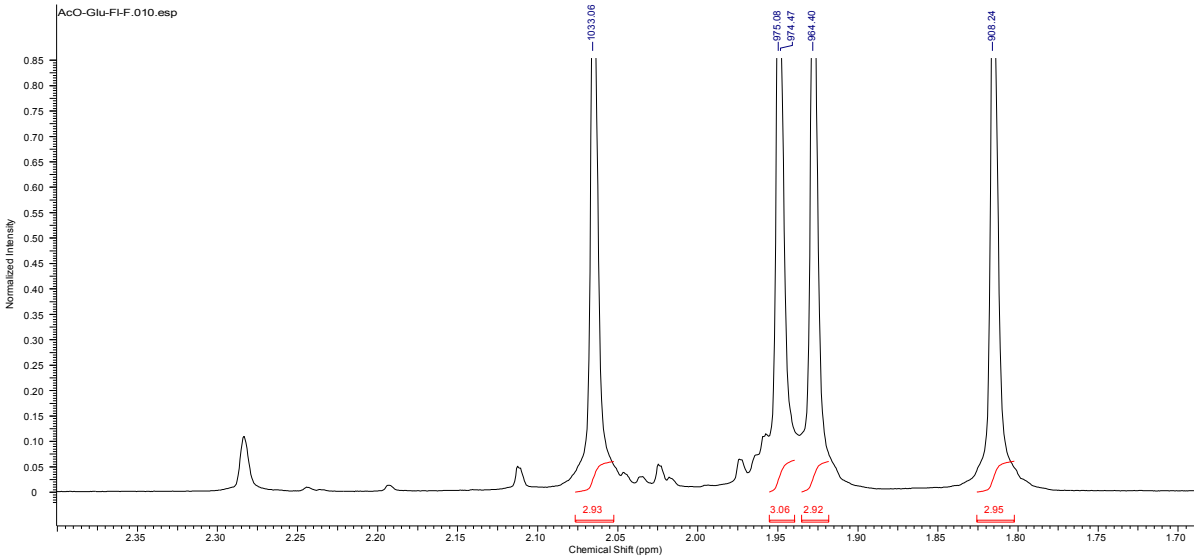
3.2. ^{13}C NMR spectrum of **1** (CD_3OD, 125 MHz)



3.3. ^{1}H NMR spectrum of **3** ($CDCl_3$, 500 MHz)







3.4. ^{13}C NMR spectrum of **3** ($CDCl_3$, 125 MHz)