Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

A Novel *"Pro-Sensitizer"* based Sensing of Enzymes using Tb(III) Luminescence in a Hydrogel matrix

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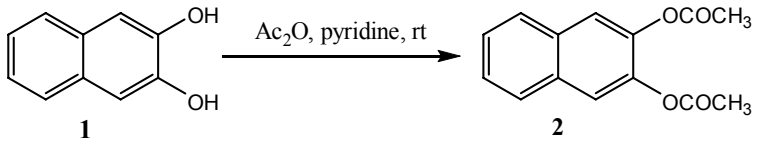
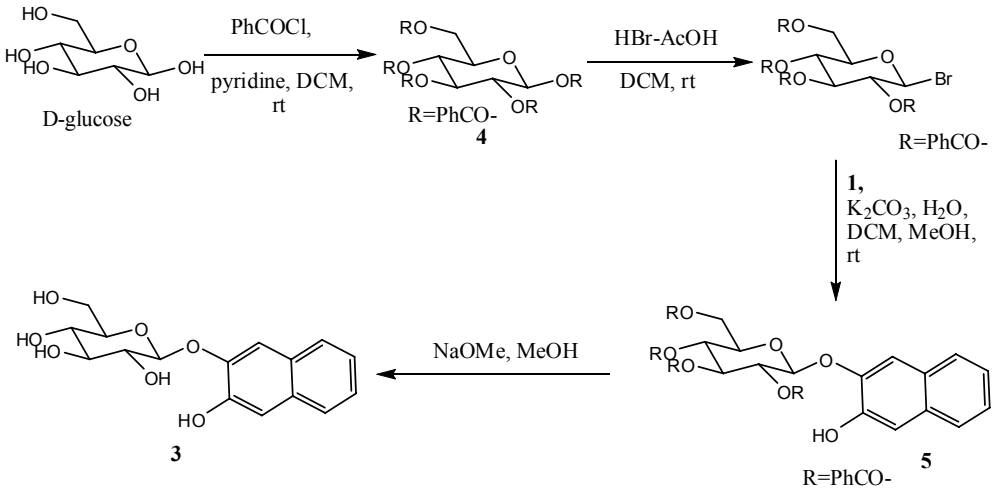
Synthesis:

Synthetic scheme 1:



Synthetic scheme 2:



Preparation of **2**:

2,3- Dihydroxynaphthalene (1 g, 6.24 mmol) was dissolved in a mixture of acetic anhydride (5ml, 49.02) and pyridine (2ml, 25.28 mmol). The reaction mixture was stirred at room temperature for 6 h. The resulting solution was diluted with chloroform (10 ml) and washed with water. The organic phase was concentrated under vacuum to obtain a white solid which was then recrystalised from ethanol to obtain **2** in 1.1g yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.34 (s, 6H), 7.47(dd, 2H), 7.65(s, 2H), 7.79(dd, 2H); ^{13}C NMR

(100 MHz, $CDCl_3$): δ 20.66, 120.87, 126.32, 127.42, 131.51, 140.86, 168.51; IR (KBr) ν 903, 1010, 1095, 1201, 1250, 1363, 1507, 1765; HRMS: observed (M+Na) 267.0632; calculated (M+Na) 267.0633. Anal. Calcd for $C_{14}H_{12}O_2$: C, 68.85; H, 4.95. Found: C, 68.79; H, 5.07

Preparation of **4**:

Benzoyl chloride (8 ml, 68.86 mmol) was added to a solution of pyridine (10 ml, 126.4 mmol) in dichloromethane (15 ml) at 0°C and stirred for 30 minutes. D-glucose (1 g, 5.55 mmol) was added to the mixture and stirred at room temp for 16 h. After removal of the solvent under vacuum, the residue was purified by column chromatography on silica gel using chloroform to afford **4** (2.5 g , 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.46-4.51(m, 1H), 4.63(d, 2H), 5.69 (dd, 1H), 5.87 (t, 1H), 6.32 (t, 1H), 6.86 (d, 1H), 7.26-7.57 (m, 14H), 7.66 (t, 1H), 7.88 (d, 4H), 7.95 (d, 2H), 8.03 (d, 2H), 8.17 (d, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 62.40, 68.76, 70.37, 70.42, 70.44, 89.99, 128.34, 128.38, 128.41, 128.49, 128.62, 128.75, 128.90, 129.46, 129.69, 129.74, 129.79, 129.84, 129.99, 130.11, 133.11, 133.33, 133.47, 133.57, 133.89, 164.37, 165.32, 165.87, 166.06; IR (KBr) ν 1022, 1094, 1269, 1601, 1734, 3465; HRMS: observed (M+Na) 723.1840; calculated (M+Na) 723.1842.

Preparation of **5**:

Compound **4** (1g, 1.42 mmol) was dissolved in dichloromethane (10 ml) and was cooled to 0°C. HBr (1.5 ml, in 33% acetic acid) was added drop wise to the reaction mixture and stirred at room temperature for 24 h. The reaction mixture was then diluted with chloroform (15 ml) and washed with saturated sodium bicarbonate solution. The organic phase was then dried under vacuum to obtain a brown solid (0.9g) which was dissolved in DCM (10 ml). Dihydroxynaphthalene (0.8 g, 4.99 mmol) was added to the solution followed by the addition of methanol (1.5 ml)and K_2CO_3 (0.36 g, 2.6 mmol, dissolved in 3ml of water). The reaction mixture was stirred for 72 h at room temperature. The reaction mixture was then diluted with chloroform (10 ml) and washed with water. The combined organic phase was dried under vacuum and purified by column chromatography on silica gel using chloroform to obtain **5** (0.9 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.83 (t, 1H), 4.54-4.58 (m, 1H), 4.77(dd, 1H), 5.46 (d, 1H), 5.72-5.83 (m, 2H), 6.11 (t, 1H), 6.23 (s, 1H), 7.16-7.21 (m, 2H), 7.26-7.62 (m, 15H), 7.88 (d, 2H), 7.96 (d, 2H), 8.00 (d, 2H), 8.08 (d, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 57.12, 63.09, 69.29, 72.03, 72.40, 72.92, 100.92, 102.03, 110.62, 111.40, 123.82, 125.22, 126.32, 126.81, 128.25, 128.39, 128.51, 128.54, 129.37, 129.70, 129.74, 129.77, 129.87, 129.95, 131.11, 133.20, 133.31, 133.48, 133.69, 133.79, 145.00, 145.92, 165.23, 165.66,

166.05, 166.31; IR (KBr) ν 1090, 1264, 1480, 1632, 1728, 3176, 3607. HRMS: observed (M+Na) 761.1996; calculated (M+Na) 761.1999. Anal. Calcd for $C_{44}H_{34}O_{11}$: C, 71.54; H, 4.64. Found: C, 71.93; H, 5.09.

Preparation of **3**:

Metallic sodium (27 mg) was carefully added to methanol (5 ml) at 0°C and the resulting solution was added to **5** (0.5 g, 0.67 mmol). The reaction mixture was stirred at room temperature for 24 h, vacuum dried and purified by column chromatography on silica gel using ethanol/chloroform (1:4) to afford 0.3 g of compound 4. ^{1}H NMR (400 MHz, D_2O): δ 3.54 (t, 1H), 3.65-3.74 (m, 3H), 3.78-3.83 (m, 1H), 3.99 (d, 1H), 5.25 (d, 1H), 7.28 (s, 1H), 7.35-7.44 (m, 2H), 7.51 (s, 1H), 7.73 (d, 1H), 7.78 (d, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 61.02, 69.94, 73.37, 76.10, 102.06, 109.93, 11.93, 123.07, 124.27, 125.44, 126.56, 128.77, 130.73, 146.26, 146.49; IR (KBr) ν 1072, 1258, 1612, 1695, 2352, 2918, 3442, 3684, 3812. HRMS: observed (M+Na) 345.0946; calculated (M+Na) 345.0950. Anal. Calcd for $C_{16}H_{18}O_{7,}$ H_2O: C, 56.46; H, 5.92. Found: C, 56.18; H, 6.11

General Informations:

The enzymes, lipase (from *Candida rugosa*), beta-lactumase and 2,3 dihydroxynaphthalene were obtained from Aldrich.

Preparation of the gel:

Fresh stocks were prepared by dissolving appropriate amounts of enzymes and pro-sensitizers in terbium acetate (10mM) and sodium cholate (30 mM) stock solutions respectively. Equal volumes of the two stock solutions were mixed at room temperature (~25 °C) followed by a mild sonication (for 10 sec) to obtain the gel samples.

Luminescence studies:

All luminescence studied were carried out in Cary Varian fluorescence spectrophotometer at a constant temperature of 25°C with 1mm path length quartz cells. For all the measurements, the gel samples were transferred to the corresponding cuvettes and the fluorescence intensity was measured at specified time intervals.

HPLC Analysis:

For HPLC Analysis several gel samples (200 µl each) were prepared (with the same composition of Terbium (III) acetate, Sodium cholate, Pro-sensitizer (DHN diacetate or DHN glucoside) and the enzyme) and incubated at 25°C. At specific time intervals, each gel was dissolved in 700 µl of HPLC grade methanol, followed by the addition of 100 µl of 5 % (W/V) acetic acid (in water). The solutions were then filtered through nylon membranes (0.45 µm) and HPLC analysis was carried out on a 25 sm C18 analytical column in 45:55 water/methanol.

Morphological studies:

It seemed relevant to explore the structural aspects of the gel platform in details to better understand the mechanism of enzyme sensing. For this purpose, the morphology of these gels was established by SEM and TEM techniques which revealed the network of nanofibers (Fig.1) of 20-30nm in width.

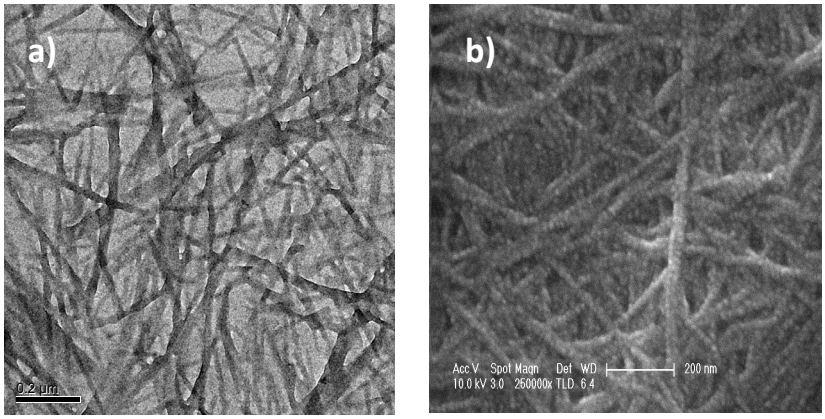


Figure 1. a) TEM (scale bar 0.2 µM) and b) SEM (scale bar 200nm) picture of Tb- cholate (5mM: 15mM) gel.

To assert the requirement of specific substrates for the sensing of a particular enzyme, control experiments were performed by swapping the substrates between the enzymes (Fig. 3) where neither lipase nor β–glucosidase was able to cause any enhancement of the lanthanide luminescence when their substrates were exchanged.

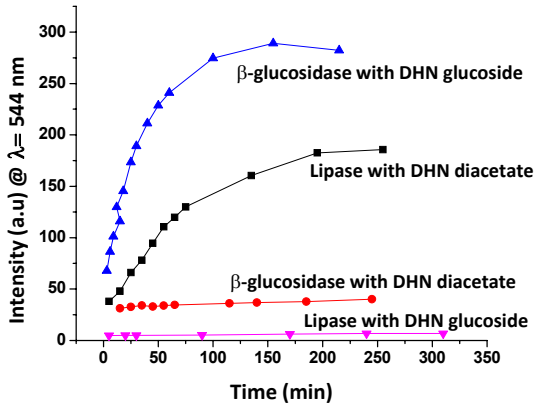


Figure 2. Control experiment with lipase (0.9 mg/mL) and β–glucosidase (0.7 mg/mL) with 33 μM of 2 and 0.37 mM of 3 in Tb:cholate gel sytem (5mM:15mM) at 25^0C.

To check the selectivity of the sensor, activity of beta-glucosidase was monitored in presence of another enzyme (Fig.3). Lysozyme which naturally coexists with beta glucosidase was chosen for this purpose. Results show that beta-glucosidase activity remains unhindered in presence of lysozyme.

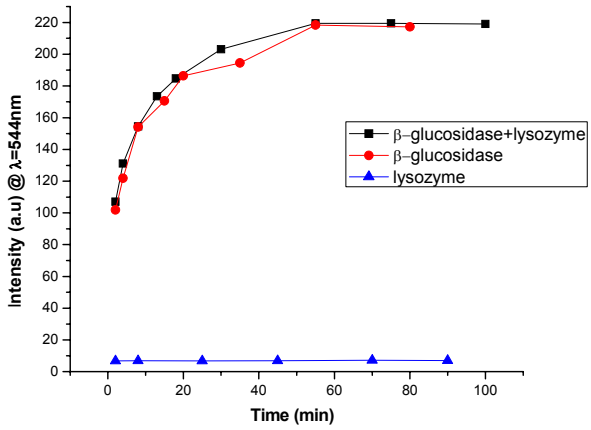


Figure 3. Control experiment with lysozyme (1.0 mg/mL) and β–glucosidase (0.9 mg/mL) with 0.43 mM of 3 in Tb:cholate gel sytem (5mM:15mM) at 25^0C

Enzyme sensing in *human Blood serum*:

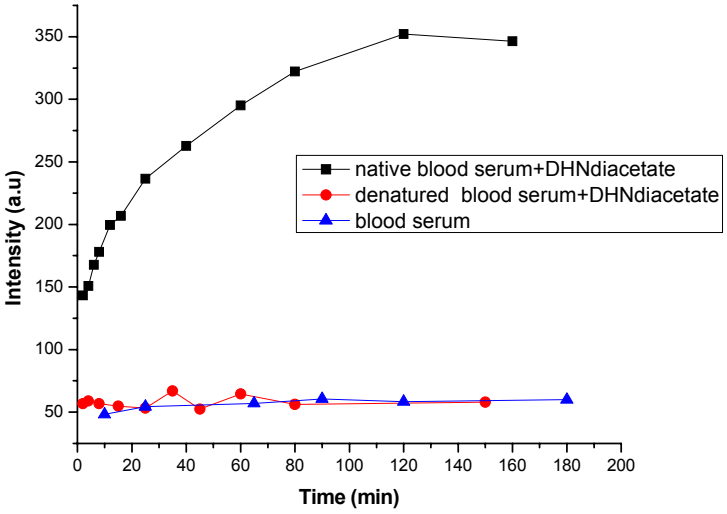


Figure 4. Test of lipase activity in Human blood serum (20 µL serum in 800 µL gel) with **2** (41 µM) in Tb:cholate gel sytem (5mM:15mM) at 25^0C.

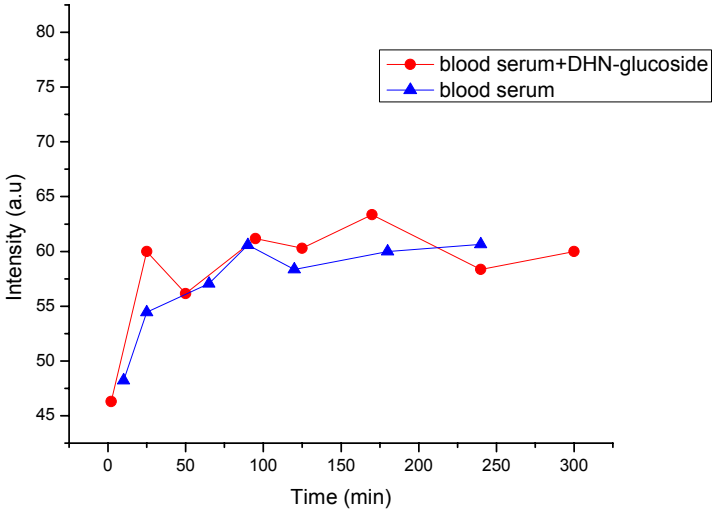
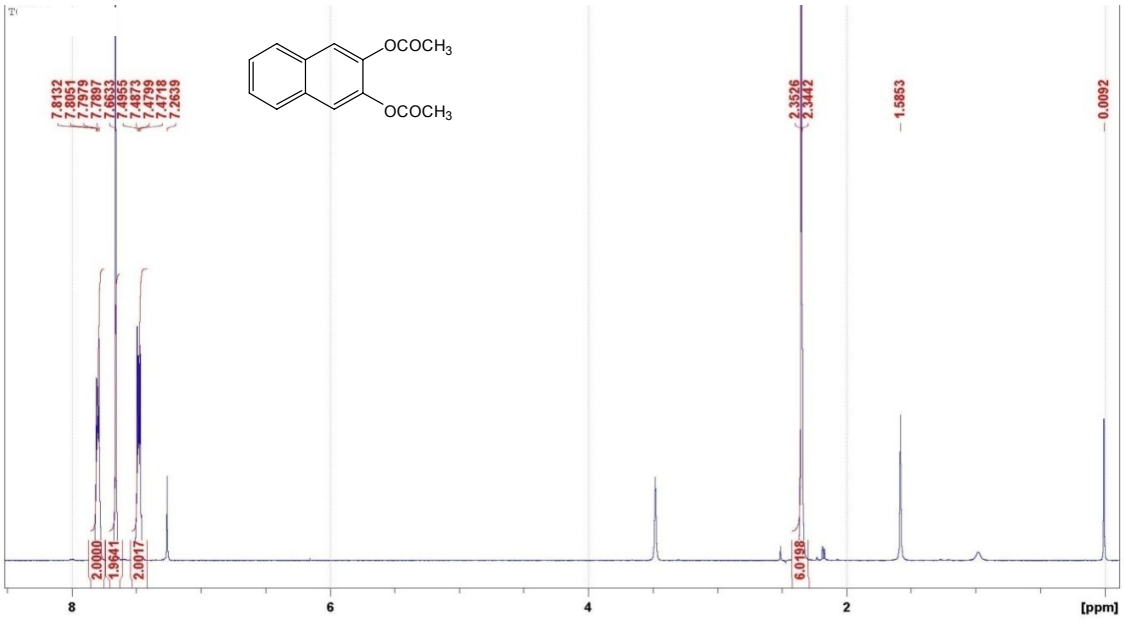


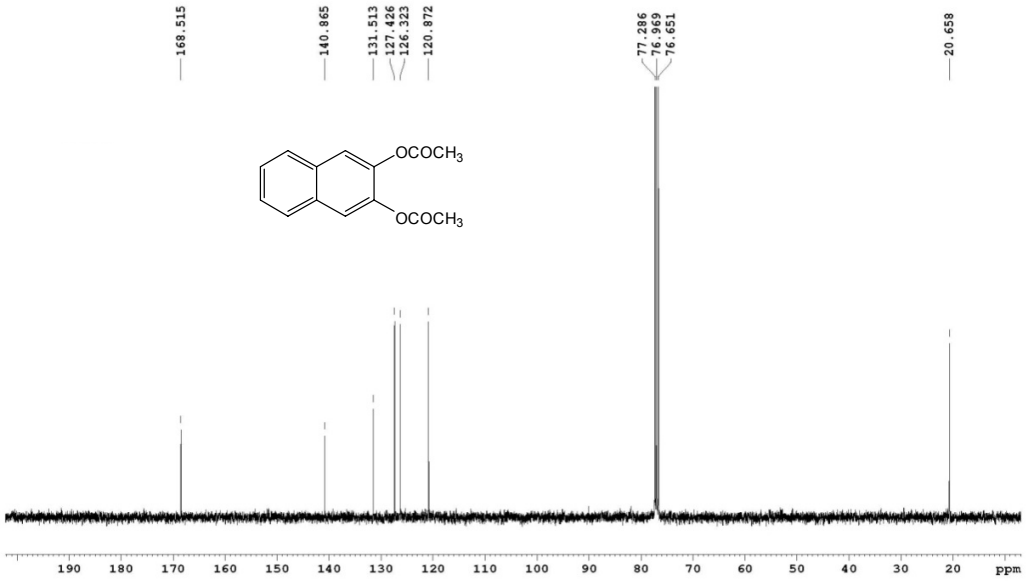
Figure 5. Test of β–glucosidase activity in Human blood serum (20 µL serum in 800 µL gel) with **3** (0.38 mM) in Tb:cholate gel sytem (5mM:15mM) at 25^0C.

NMR spectra:

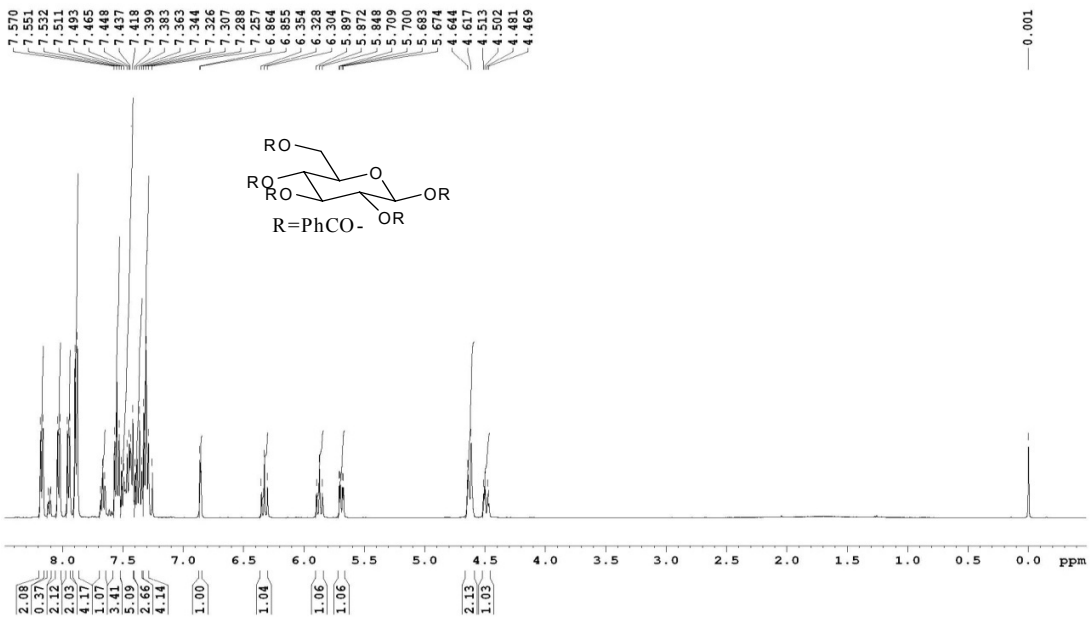
2 ^{1}H NMR (400 MHz, $CDCl_3$)



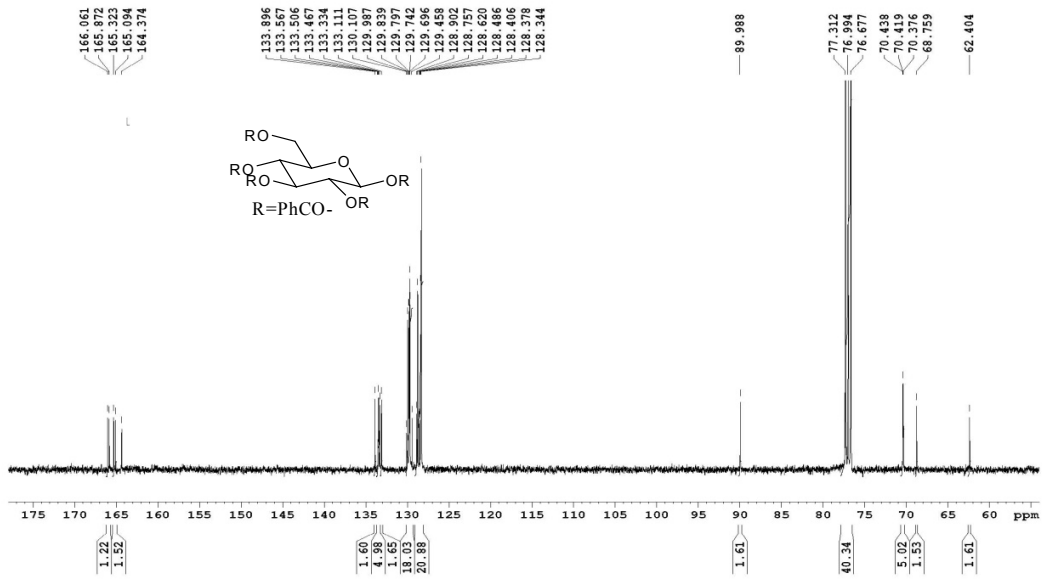
2 ^{13}C NMR (100 MHz, $CDCl_3$)



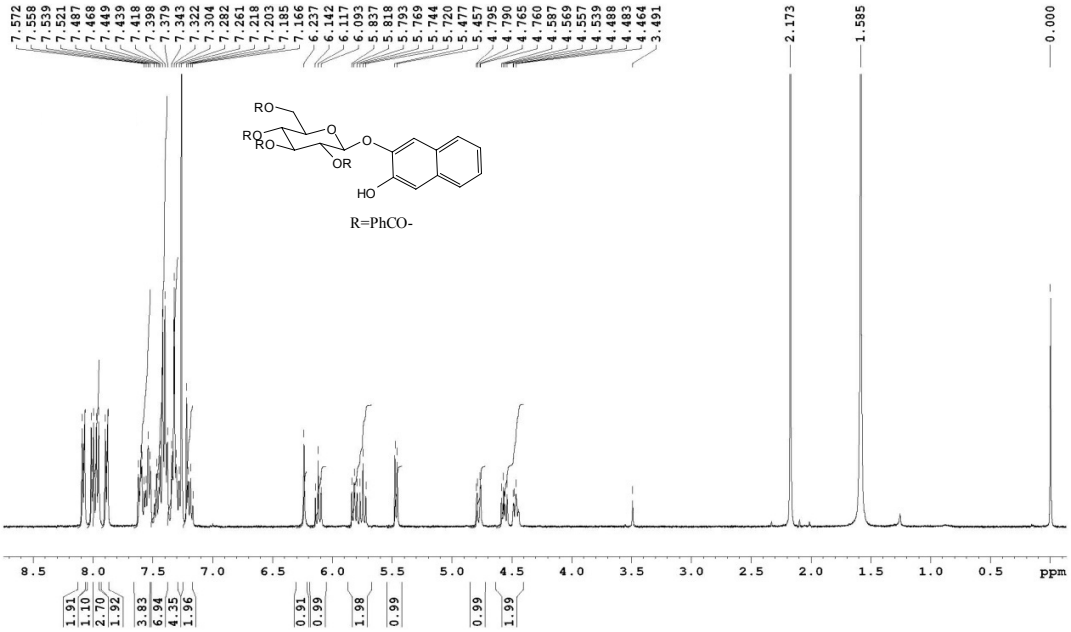
4 ^{1}H NMR (400 MHz, $CDCl_3$)



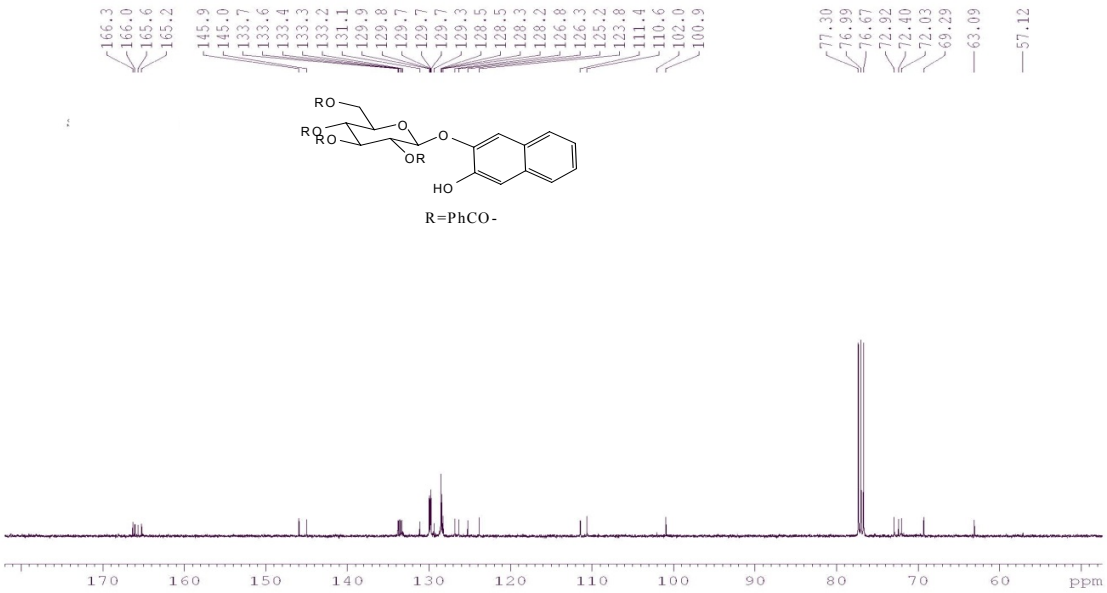
4 ^{13}C NMR (100 MHz, $CDCl_3$)



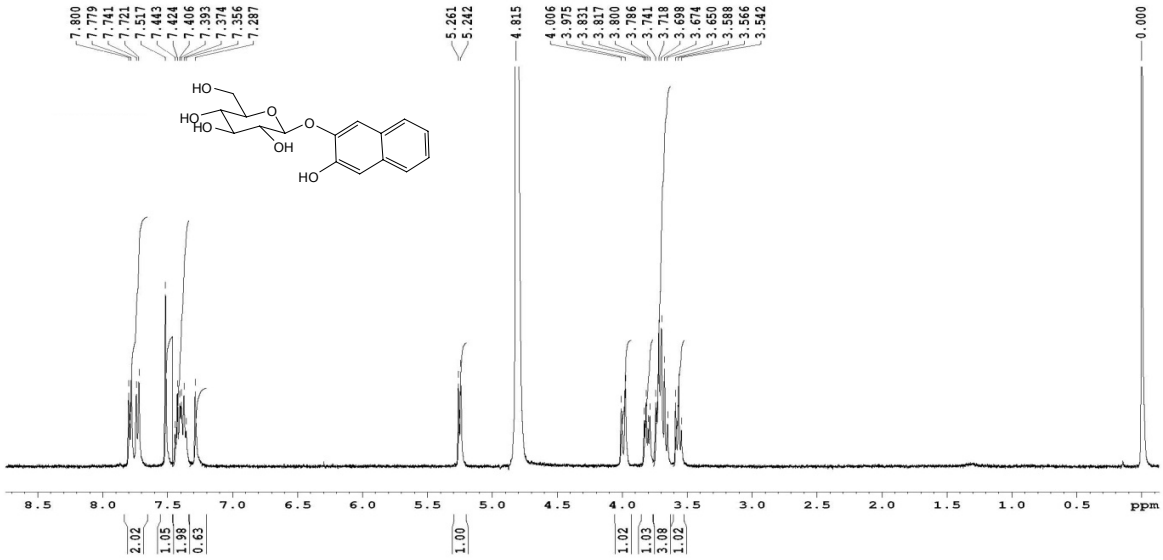
5 ^{1}H NMR (400 MHz, $CDCl_3$)



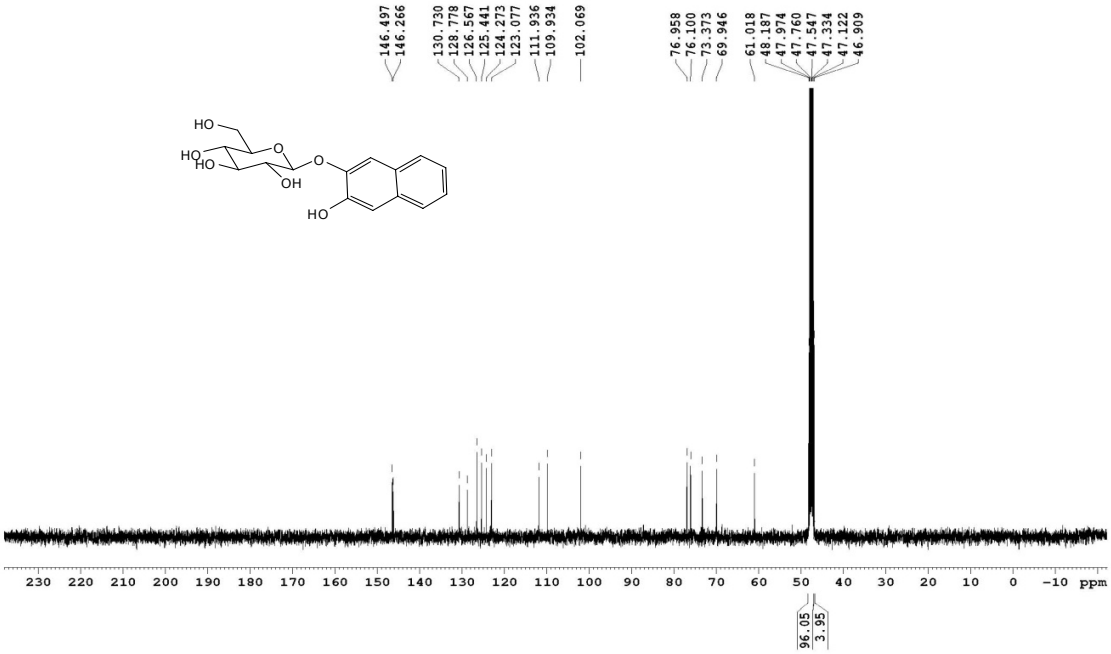
5 ^{13}C NMR (100 MHz, $CDCl_3$)



3 ^{1}H NMR (400 MHz, D_20)



3 ^{13}C NMR (100 MHz, CD_3OD)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47.547
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3.95