Eu/Tb luminescence for alkaline phosphatase and β-galactosidase assay in hydrogels and on paper devices

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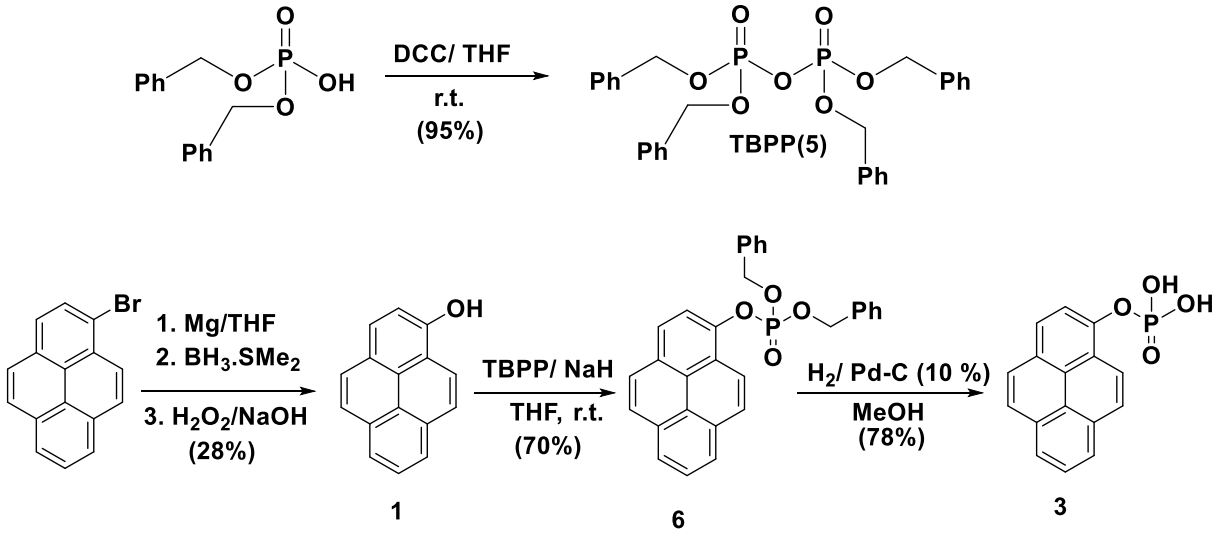
1. Materials and methods:

Tb-acetate, Eu-acetate, pyrene, 2,3-dihydroxynaphthalene, sodium cholate of commercial grades were purchased from Sigma Aldrich. Alkaline phosphatase from Bovine Intestinal Mucosa (10 DEA units/mg), β-Galactosidase from *Escherichia coli* (140 U/mg) and from *Aspergillus oryzae* (8 U/mg) were purchased from Sigma Aldrich. Whatman 3 and western blotting paper (thickness 0.83 mm) were purchased from Thermo scientific. Absorption spectra were recorded on a Perkin-Elmer Lambda 35 UV-Vis spectrometer and UV-3600 Shimadzu UV-Vis-NIR spectrometer. Time delayed emission for the gel samples were recorded on a Varian Cary Eclipse spectrometer in phosphorescence mode (delay time 0.2 ms, gate time 3.0 ms) and Varioskan® Flash Spectral Scanning Multimode Reader in TRF mode (delay time 0.2 ms, integration time 1.0 ms). SEM images of xerogel samples were recorded on a FEI Sirion XL30 FEG SEM instrument. AFM images of xerogel samples were recorded on the JPK Nanowizard II in tapping mode. SEM images of the gel coated paper were recorded in FEI ESEM Quanta 200 instrument. A Sony Cyber-Shot DSC-H70 16.1 MP Digital still Camera with 10x Wide-Angle Optical Zoom G Lens/ 3.0-inch LCD was used for capturing images under the UV lamp. ImageJ software was downloaded from "https://imagej.nih.gov/ij/" and was used on PC desktop for measuring the green/red color intensity of the discs. Time delayed emission of the gel coated discs were measured by Varioskan® Flash Spectral Scanning Multimode Reader in TRF mode (delay time 0.2 ms, integration time 1.0 ms). Milli Q water was used for all the measurements.

2. Synthesis of the "pro-sensitizer 3"



Scheme 1: Synthetic scheme for pro-sensitizer 3 from HP

Tetrabenzyl pyrophosphate (5):

N,*N*'-Dicyclohexylcarbodiimide (210 mg, 1.01 mmol) in dry THF (1.5 mL) was added to dibenzyl phosphate (504 mg, 1.81 mmol) in dry THF (6 mL). N,N'-dicyclohexylurea began to separate out immediately and after 1 h the reaction mixture was filtered and the colourless filtrate was collected. Solvent was removed in vacuum to obtain 5 (460mg, 95%) as a white solid. m.p. (Exp) 57- 60 ^{o}C (Lit 61-62 ^{o}C)[1]; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.32 (s, 20 H), 5.09 (8H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 128.73, 128.58, 128.09, 70.46, 70.43, 70.40.

Pyren-1-ol (1):[2]

Magnesium metal (146 mg, 6.08 mmol) and 1-bromopyrene (1 gm, 3.5 mmol) were taken in a two necked round bottomed flask fitted with a reflux condenser and septum under an argon atmosphere. Dry THF (5 mL) was added and the mixture was refluxed at 65 ^{o}C for 3 h. To the above mixture $BH_3.SMe_2$ was (400 µL, 4 mmol) was added and refluxed for 3h. The reaction mixture was cooled down to room temperature and the excess $BH_3.SMe_2$ was quenched by adding H_2O (1.5 mL). Aqueous sodium hydroxide (1.8 mL, 1M) solution was added and stirred for 5 min. 30% H_2O_2 (480 µL, 3.5 mmol) was added drop wise with ice cooling and stirred for 1.5 h. Conc. HCl (1 mL) and H_2O (3 mL) were added and the reaction mixture was extracted with ethyl acetate (2 × 20 mL), washed with brine and dried over Na_2SO_4. After removing the solvent in vacuum, the compound was purified by column chromatography on silica gel with 1-5% ethyl acetate/ pet ether to obtain 1 (214 mg, 28%) as a off white solid. ^{1}H MNR (400 MHz, $CDCl_3$) δ: 8.33 (d, *J*= 8 Hz, 1H), 7.91-8.10 (m, 7H), 7.46 (d, *J* = 5.6 Hz, 1H), 5.57 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 149.61, 131.56, 127.18, 126.53, 126.108, 125.44, 124.93, 124.42, 124.15, 120.44, 118.58, 112.96; IR (KBr, cm^{-1}): 3431, 1922, 1379, 1326, 1263, 1249, 1222, 1069, 1061, 1007, 831, 710; HRMS: calcd for $C_{16}H_{10}ONa$ [M+Na] 241.0631; observed $C_{16}H_{10}ONa$ [M+Na] 241.0629; MP 180-182 °C (lit. 179-181 °C).

Dibenzyl pyren-1-yl phosphate (6):

NaH (17 mg, 0.70 mmol) was added to 1-hydroxypyrene (70 mg, 0.32 mmol) in THF (10 mL) and stirred at room temperature. After 30 min tetrabenzyl pyrophosphate (**5**) (206 mg, 0.38 mmol) was added to the yellow coloured solution and stirring was continued for 12 h. The reaction mixture was filtered and the precipitate was washed with 5 mL of THF. The combined THF layer was collected and solvent was removed by rotary evaporation. The reaction mixture was dissolved in DCM (30 mL) and washed with

cold H_2O (20 mL), saturated $NaHCO_3$ solution (20 mL) and again with cold water (20 mL). The organic layer was dried over Na_2SO_4. After removal of solvent in vacuum the product was purified by column chromatography to obtained **6** (105 mg, 69%) as a wine red liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.23 (d, *J* = 9.2 Hz, 1H), 8.14-8.16 (m, 2H), 7.98-8.06 (m, 6H), 7.23-7.28 (m, 10H), 5.19 (dd, *J* = 21.2, 13.2, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 144.12, 144.05, 135.37, 135.30, 131.14, 131.03, 128.54, 128.49, 127.97, 127.87, 126.94, 126.83, 126.32, 125.63, 125.29, 125.05, 124.37, 122.43, 122.37, 120.59, 117.46, 117.43, 70.22, 70.17; IR (KBr, cm^{-1}): 2927, 2850, 1570, 1463, 1389, 1055, 1010, 948, 846. 811, 749, HRMS calcd for $C_{30}H_{23}O_4PNa$ [M+Na] 501.1232, observed $C_{30}H_{23}O_4PNa$ [M+Na] 501.1232

Pyren-1-yl dihydrogen phosphate (3):

10 % Pd on carbon (20 mg, 0.019) was added to the dibenzyl pyrenyl phosphate (**6**) (65 mg, 0.135) solution in MeOH. The reaction mixture was stirred for 2 h at slightly positive pressure of H_2 gas. The reaction mixture was filtered through celite. The filtrate was vacuum dried to obtain **3** as white solid (32 mg, 80 %). m.p. 171-173 °C; ^{1}H MNR (400 MHz, MeOH-d_4) δ: 8.54 (d, *J* = 9.2 Hz, 1H), 8.22 (d, *J* = 8.8 Hz, 1H), 7.96-8.01 (m, 3H), 7.89 (t, *J* = 9.2 Hz, 2H), 7.78-7.84 (m, 2H); ^{13}C NMR (100 MHz, MeOH-d_4) δ: 148.94, 148.87, 131.53, 131.46, 126.89, 125.96, 125.50, 125.42, 124.89, 124.65, 124.56, 123.64, 123.52, 122.35, 122.15, 117.23; ^{31}P NMR (161.9 MHz, MeOH-d_4): -0.757; IR (KBr, cm^{-1}): 3430, 2981, 2297, 1644, 1051, 1032, 1016, 717; HRMS: cald for $C_{16}H_{10}O_4PNa_2$ [M-H+ 2Na] 343.0115; observed $C_{16}H_{10}O_4PNa_2$ [M-H+ 2Na] 343.0112.

3. Synthesis of the "pro-sensitizer 4"

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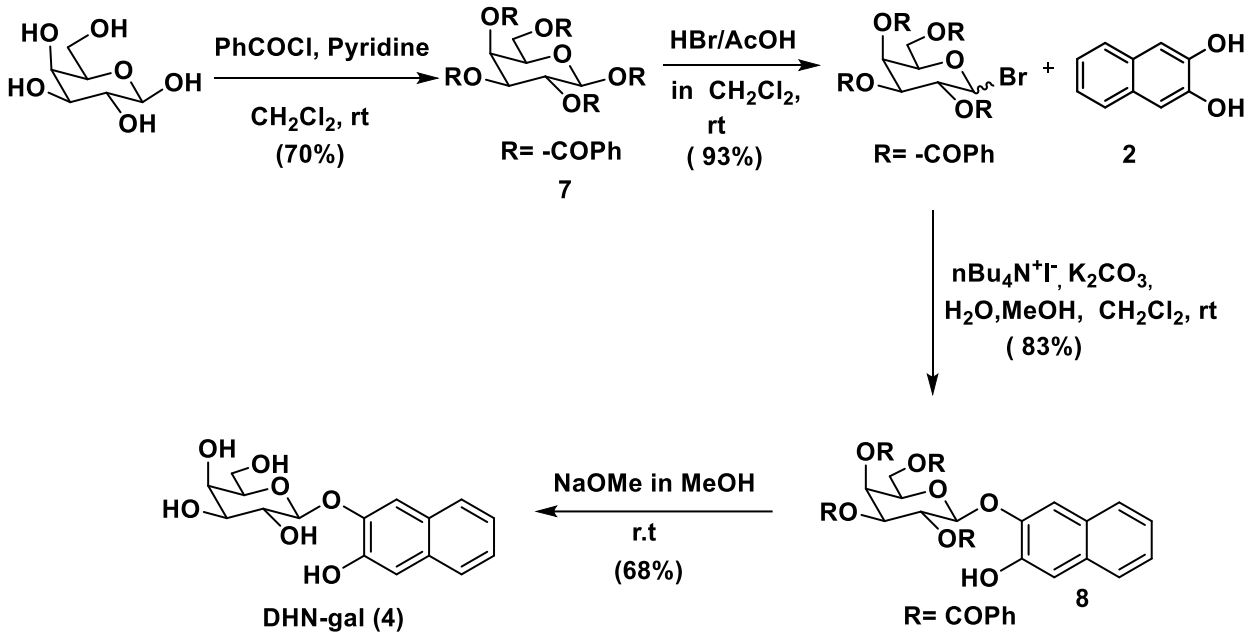


Scheme 2: Synthetic scheme for pro-sensitizer 4 from DHN

1,2,3,4,6-Penta-O-benzoyl-D-galactopyranoside (7):

Benzoyl chloride (2.7 mL, 23.2 mmol) was added to a solution of pyridine (2 mL, 25 mmol) in dichloromethane (5 mL) at 0°C and stirred for 30 min. D-galactose (0.5 gm, 2.77 mmol) was added to the mixture and stirred at room temp for 16 h. The reaction mixture was diluted with $CHCl_3$ (30 mL), washed with water (2 × 20 mL) and brine (20 mL). The organic layer was collected over Na_2SO_4 and after removal of the solvent under vacuum, the residue was purified by column chromatography on silica gel using chloroform to afford 1.16 gm of **7** (60 %) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.10- 8.13 (m, 4H), 7.96 (d, *J* = 7.2 Hz, 2H), 7.81-7.87 (m, 4H), 7.62-7.66 (m, 2H), 7.44-7.54 (m, 7H), 7.39 (t, *J* = 7.6 Hz, 2H), 7.26- 7.31 (m, 4H), 6.96 (d, *J* = 3.2 Hz, 1H), 6.19 (d, *J* = 2.4 Hz, 1H), 6.13 (dd, *J* = 10.8 Hz, 3.2 Hz, 1H), 6.03 (dd, *J* = 10.8 Hz, 3.6 Hz, 1H), 4.84 (t, *J* = 6.4 Hz, 1H), 4.64 (dd, *J* = 11.2 Hz, 6.4 Hz, 1H), 4.42 (dd, *J* = 11.2 Hz, 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 165.9, 165.7, 165.5, 165.5, 164.5, 133.9, 133.7, 133.5, 133.4, 133.2, 129.9, 129.9, 129.7, 129.3, 128.4, 128.3, 96.6, 69.4, 68.5, 68.4, 67.6, 61.8; IR (KBr, cm^{-1}): 2969, 1730, 1602, 1452, 1266, 1108, 1069, 1036, 863, 708; HRMS: Calcd for $C_{41}H_{32}O_{11}Na$ [M+Na] 723.1842, Observed $C_{41}H_{32}O_{11}Na$ [M+Na] 723.1842.

3-Hydroxy-2-naphthalenyl-2,3,4,6-tetra-O-benzoyl-β-D-galactopyranoside (8):

HBr (1.6 mL, 6.42 mmol, 33% in AcOH) was added dropwise to compound **7** (980 mg, 1.28 mmol) dissolved in DCM at 0 °C, stirred for 0.5 h and at room temperature for 3 h. The reaction mixture was diluted with dichloromethane (20 mL) and washed with cold water (20 mL) and cold saturated $NaHCO_3$ solution (10 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed by rotary evaporation to obtain a brownish white solid (860 mg, 96 %), which was then re-dissolved in DCM (12 mL). Dihydroxy naphthalene (2, 630.5 mg, 3.93 mmol) was added to the reaction mixture followed by the addition of K_2CO_3 (394 mg, 2.85 mmol, dissolved in 2 mL of water), MeOH (500 µL), tetra-n-butyl ammonium iodide (468 mg, 1.27 mmol). The reaction mixture was stirred at room temperature for 48 h. The reaction mixture was diluted with $CHCl_3$ (20 mL), washed with H_2O (20 mL) and Brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and after removal of solvent in vacuum, the product was purified by column chromatography to obtain 800 mg of **8** (83%) as white solid. m.p. 245-247 °C; ^{1}H MNR (400 MHz, $CDCl_3$) δ: 8.15 (d, *J* = 8 Hz, 2H), 8.10 (d, *J* = 8 Hz, 2H), 7.99 (d, *J* = 8Hz, 2H), 7.82 (d, *J* = 8 Hz,2H,),7.59-7.68 (m, 3H), 7.53 (t, *J* = 12 Hz, 3H), 7.40-7.47(m, 3H), 7.32-7.38 (m, 4H), 7.26-7.30 (m, 4H), 7.17-7.21 (m, 2H), 6.29 (s, 1H), 6.03-6.09 (m, 2H), 5.83-5.86 (m,1H), 5.45 (d, *J* = 4 Hz, 1H), 4.61-4.68 (m,3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.6, 166.0, 165.5, 145.9, 145.1, 133.8, 133.8, 133.5, 133.4, 131.1, 130.1, 129.9, 129.8, 129.8, 129.3, 128.7, 128.7, 128.6, 128.5, 128.5, 128.5, 128.3, 126.8, 126.3, 125.2, 123.8, 111.4, 110.6, 101.2, 72.2, 70.8, 70.3, 67.9, 62.5; IR (KBr, cm^{-1}): 3447, 2958, 1729, 1704, 1602, 1479, 1452, 1316, 1264, 1096, 1068, 865, 709; HRMS: Calcd for $C_{44}H_{34}O_{11}Na$ [M+Na] 761.1997, observed $C_{44}H_{34}O_{11}Na$ [M+Na] 761.1997;

3-Hydroxy-2-naphthalenyl-β-D-galactopyranoside (4):

Sodium metal (25.8 mg, 1.12 g-atom) was added to dry methanol (1.1 mL) at 0 °C to prepare methanolic sodium methoxide. The resulting solution was added to the solution of 8 (400 mg, 0.542 mmol) in methanol and stirred at room temperature for 24 h. The reaction mixture was vacuum dried and purified by silica gel column chromatography with 2-20% ethanol/chloroform to afford 120 mg of **4** (68 %) as white solid. m.p. 199-201 °C; ^{1}H MNR (400 MHz, MeOH-d_4) δ: 7.56 (d, *J* = 7.6 Hz, 1H), 7.59 (d, *J* = 9.6 Hz, 1H), 7.56 (s, 1H), 7.22-7.29 (m, 2H), 7.18 (s, 1H), 4.95 (d, *J* = 7.6 Hz, 1H), 3.92-3.90 (m, 2H), 3.76-3.88 (m, 3H), 3.64-3.68 (m, 1H), 3.31 (s, 1H); ^{13}C NMR (100 MHz, MeOH-d_4) δ: 146.6, 146.3, 130.8, 128.8, 126.6, 125.5, 124.3, 123.1, 112.2, 109.9, 102.8, 75.8, 73.0, 70.8, 68.8, 61.1; IR (KBr, cm^{-1}): 3420, 2922, 1384, 1255, 1170, 1048, 862, 750; HRMS: calcd for $C_{16}H_{18}O_7Na$ [M+Na] 345.0952, observed $C_{16}H_{18}O_7Na$ [M+Na] 345.0950.

4. Gel preparation procedure:

a. Alkaline phosphatase assay:

Fresh stocks were prepared by dissolving appropriate amounts of pyrene-1-phosphate **(3)** in sodium cholate (60 mM) and alkaline phosphatase in $MgCl_2$ (3 mM) solution. An aqueous Eu-acetate (10 mM) solution was also prepared. Gels were prepared by mixing these three stock solutions in 1:1:2 ratios with mild sonication (10-12 sec) at room temperature.

For inhibition studies, alkaline phosphatase solution (0.5 mg/mL) was pre-incubated with various concentrations of theophylline (**9**) and these solutions were used for checking the effect of inhibitor on ALP activity in the gels.

a. β-Galactosidase assay:

Fresh stocks were prepared by dissolving appropriate amounts of β-galactosidase and DHN-gal **(4)** in sodium cholate (30 mM) and Tb-acetate (10 mM) solutions, respectively. The Gels were prepared by mixing equal volume of both stock solutions followed by mild sonication (10-12 sec) at room temperature.

For inhibition studies, β-galactosidase solution (0.2 mg/mL) in Sodium cholate was pre-incubated with various concentration of the aza-sugar derivative (**10**) and then the gels were prepared with these solutions.

5. Preparation of the almond and lactase extract:

Almond (1.3 g) seeds were ground in a mortar and pestle. Distilled water (6 mL) was added to the powder taken in a vial. The mixture was shaken manually at an interval of 15 min while the sample was kept cold in a refrigerator. After 3 h the solid was removed by centrifugation and the filtrate was used for the detection and quantification of the β-galactosidase present in the extract.

Lactase tablet (Kirkland Signature Fast Acting Lactase) powder (4.98 mg) was suspended in water (4.98 mL) in an rb flask (5 mL) and stirred for 30 min. The supernatant was collected by centrifugation and used for detection and quantification of lactase present in the extract.

6. Procedure of luminescence Studies:

Most of the luminescence measurements were carried out in Varian Cary Eclipse fluorescence spectrometer at a constant temperature of 25 °C. For all the measurements, the gel samples were

transferred to 3 mm × 3 mm square quartz cuvettes and the luminescence was measured at specified time intervals with delay time of 0.2 ms, gate time of 3 ms.

For carrying out experiments in Varioskan plate reader, the gel samples (100 µL) were transferred into the 384 well plates and gel coated discs were transferred into the 96 well plate, luminescence was measured with delay time 0.2 ms, gate time 1 ms.

7. HPLC analysis procedure:

HPLC analysis of the gel samples were carried out at different time intervals. For this purpose several gel samples (200 µl) were prepared at the same composition of Na cholate, Tb-acetate, pro-sensitizer and enzyme. At certain time interval, each gel was dissolved in 700 µl HPLC grade methanol and 100 µl of 5% AcOH in water by mild sonication. The solutions were filtered through nylon membrane of 0.45 micron pore size and HPLC was carried out in 25 cm C18 analytical column with 55: 45 MeOH/H_2O compositions for β-galactosidase, 25:75 H_2O/MeOH compositions for alkaline phosphatase.

8. Time delayed excitation spectra of Eu-cholate gel without and with HP

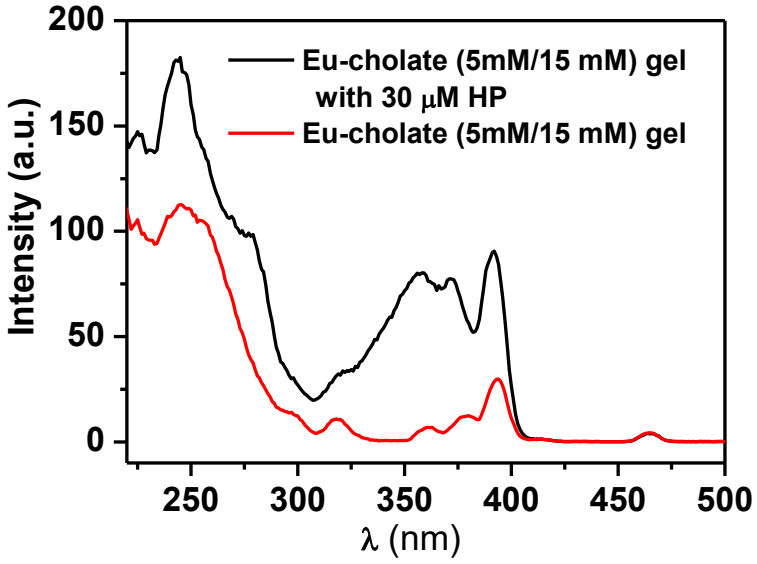


Figure S 1: Time delayed excitation spectra of Eu-cholate (5 mM/15 mM) gel for λ_{em} at 617 nm in absence and presence of HP (1).

9. Photophysical Characterizations of sensitizers 1 and 2, pro-sensitizers 3 and 4:

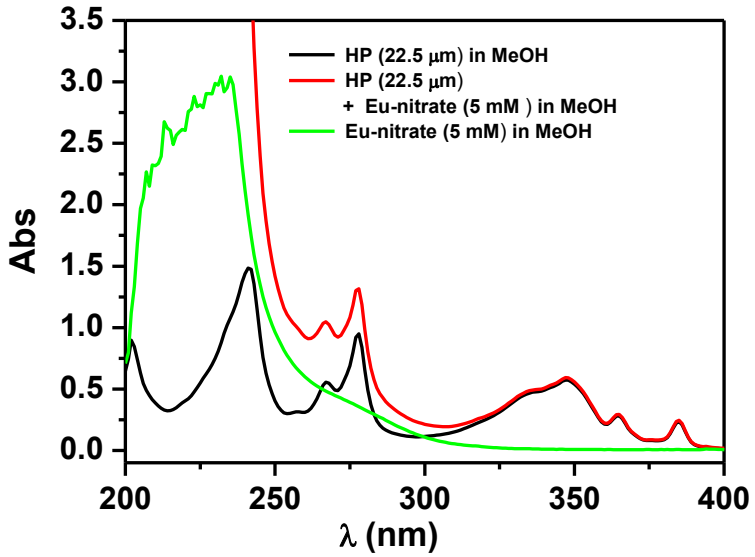


Figure S 2: Absorption spectra of HP (1) (black), Eu-nitrate (green) and mixed solutions of HP (1) and Eu-nitrate (red) in methanol.

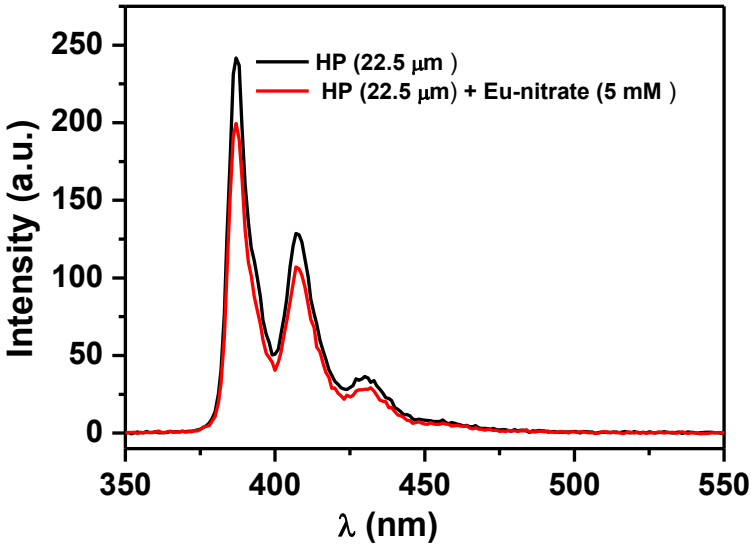


Figure S 3: Emission spectra of HP in methanol without (black) and with (red) Eu-nitrate (5 mM)

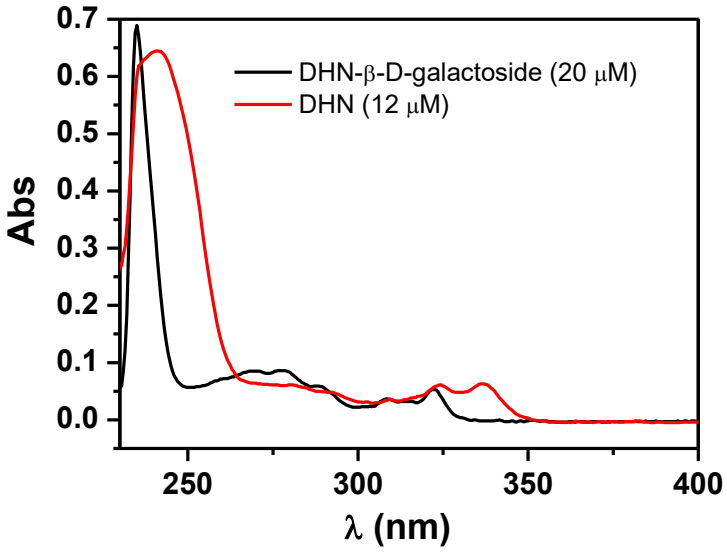


Figure S 4: Absorption spectra of DHN (2) and DHN-β-D-galactoside (4) in Tb acetate solution

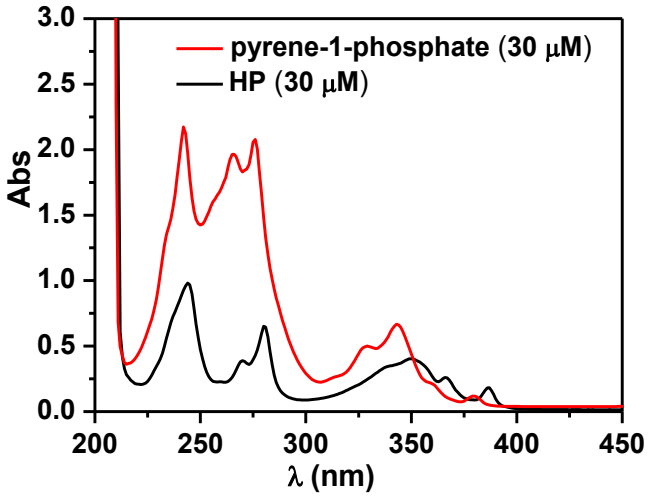


Figure S 5: Absorption spectra of HP (1) and pyrene-1-phosphate (3) in NaC solutions

10. Intensity enhancement at 617 nm and 545 nm normalized to one for ALP assay

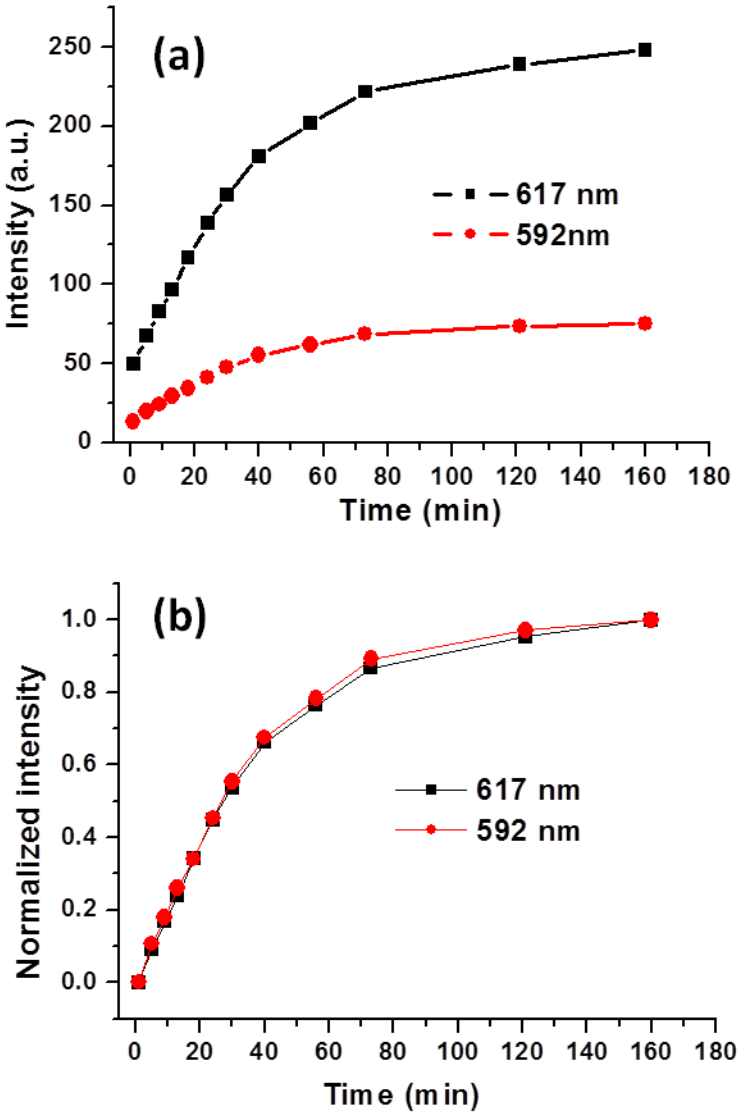


Figure S 6: (a) Increase in intensity as a function of time at λ_{em} 617 nm and 545 nm for ALP (0.26 mg /ml) assay with pro-sensitizer **3** (123 µM) doped Eu cholate (5mM:15mM) gel. (b) Intensity enhancement at λ_{em} 617 nm and 545 nm as a function of time for ALP (0.26 mg /ml) assay with maximum intensity normalized to 1.

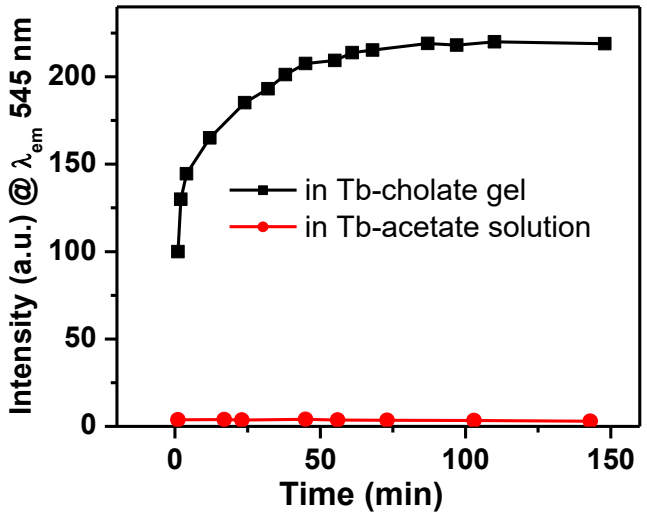


Figure S 7: comparative study of β-galactosidase (0.1 mg/mL) assay in DHN-β-D-galactoside (200 µM) doped Tb-Cholate hydrogel and DHN-β-D-galactoside (200 µM) containing Tb-acetate solution

11. Comparative study between 1-hydroxypyrene and pyrene-β-D-galactoside for sensitizing Eu(III) in Eu-cholate gel

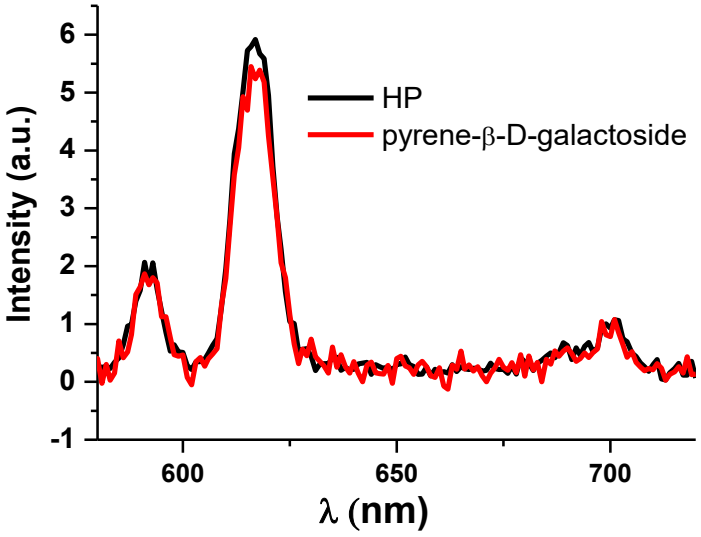


Figure S 8: Time delayed emission spectra of Eu-cholate hydrogel with HP (10 µM) and pyrene-β-D-galactoside (12 µM)

12. Determination of kinetic parameters for ALP assay:

ALP assay was done at various pyrene-1-phosphate (pro-sensitizer 3) concentrations in Eu-cholate gel to calculate initial rates.

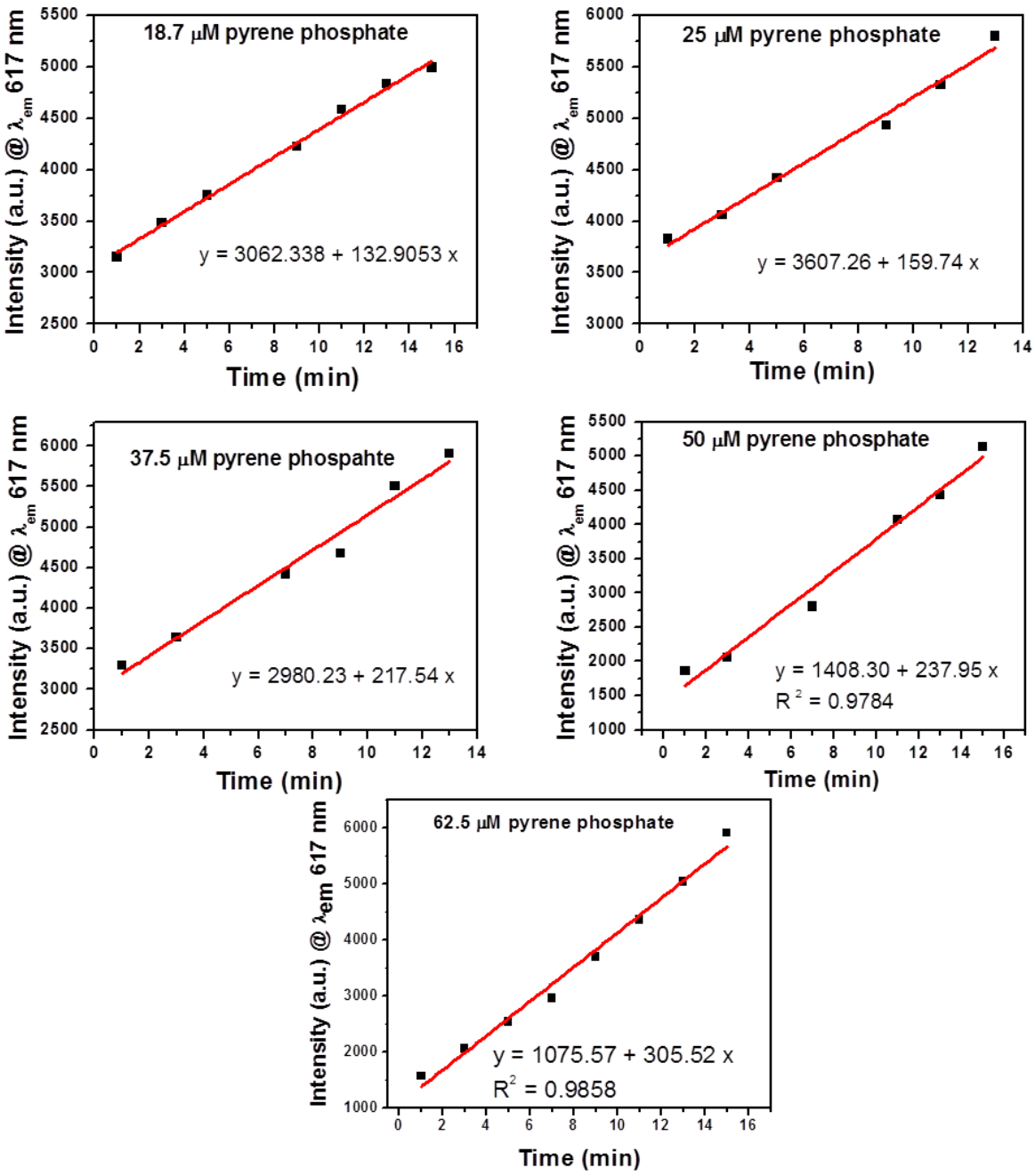


Figure S 9: Alkaline phosphatase (0.25 mg/mL) assay at varying concentration of pro-sensitizer **3** in Eu-cholate gel at 298K

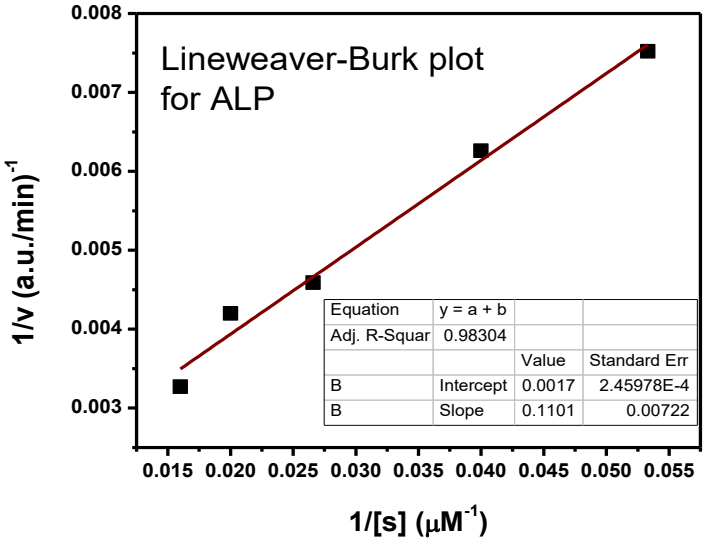


Figure S 10: Lineweaver-Burk plot for ALP assay with pro-sensitizer 3 in Eu-cholate gel at 298K

K_m = 65 µM, v_{max} = 150 nM/sec

a. Calibration plot for 1-HP doped Eu-cholate gels

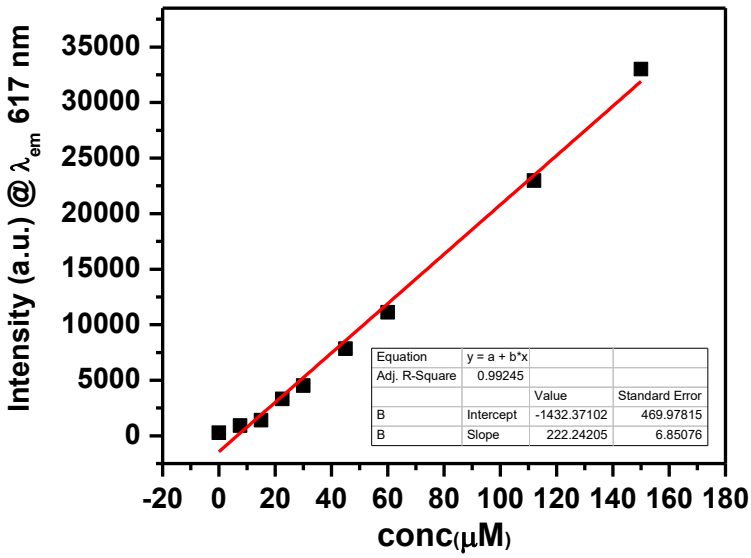


Figure S 11: Time delayed emission spectra for Eu-cholate gels with variable concentrations of HP

13. Determination of kinetic parameters for β-gal assay:

β-gal assay was done at various DHN-gal (pro-sensitizer 4) concentrations in Tb-cholate gel to calculate initial rates.

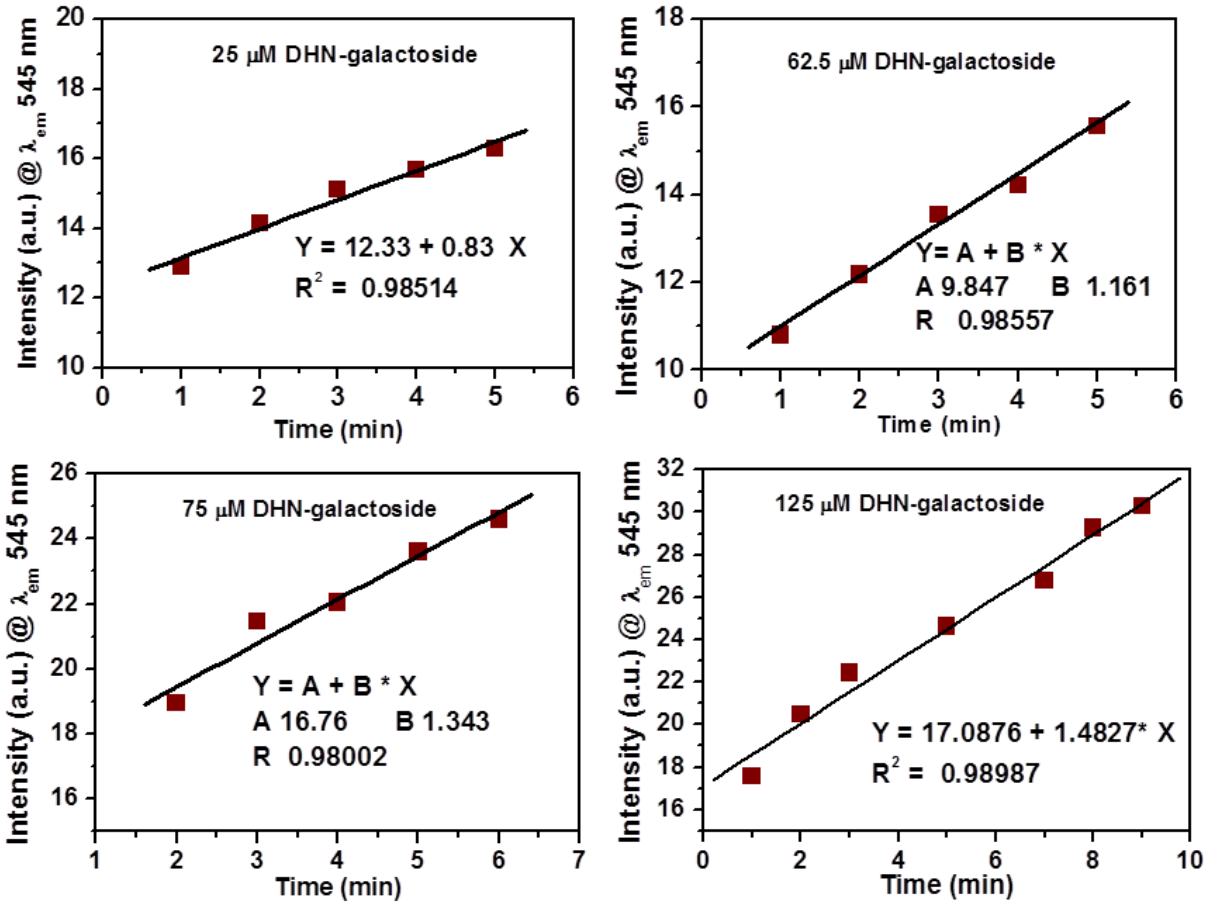


Figure S 12: β-Gal (0.1 mg/ mL) assay with various concentrations of pro-sensitizer **4** in Tb-cholate gel at 298 K

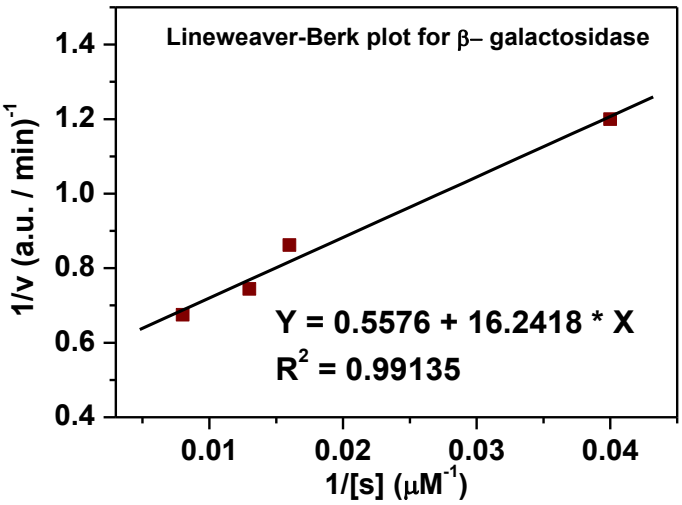


Figure S 13: Lineweaver-Burk plot for β-gal (0.1 mg/ mL) assay with pro-sensitizer 4 in Tb-cholate gel at 298 K.

K_m = 30 μM, v_{max} = 20 nM/sec

a. Calibration plot for DHN doped Tb-cholate gels in Varian Cary Eclipse spectrometer

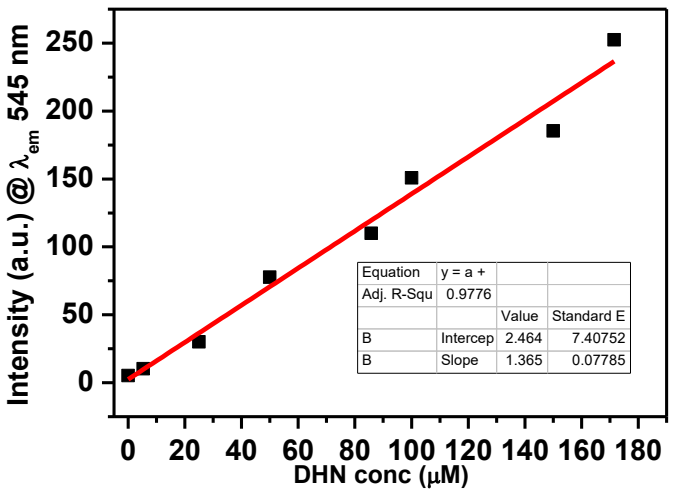


Figure S 14: Time delayed emission spectra of Tb-cholate gel with variable concentrations of DHN

14. Selectivity study and LoD determination for β-galactosidase

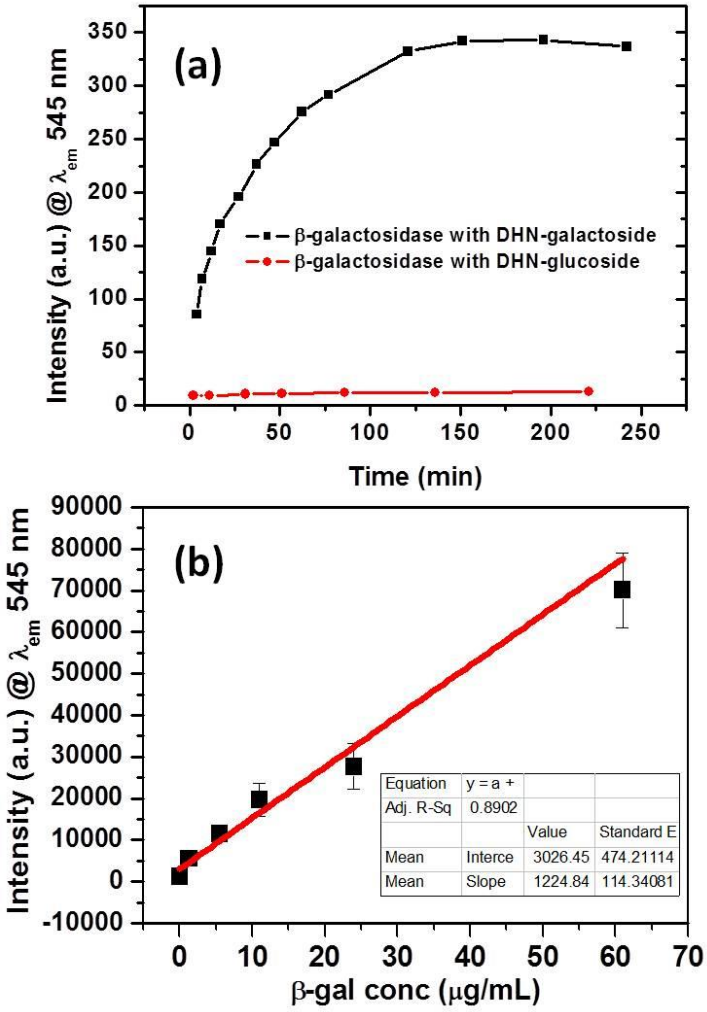


Figure S 15: (a) Time delayed emission of β-gal containing Tb-cholate gel doped with DHN-galactoside (4) and DHN-glucoside, (b) Time delayed emission for 4 doped Tb-cholate gel with variable concentrations of β-gal (n = 3).

15. β-galactosidase detection in almond extract

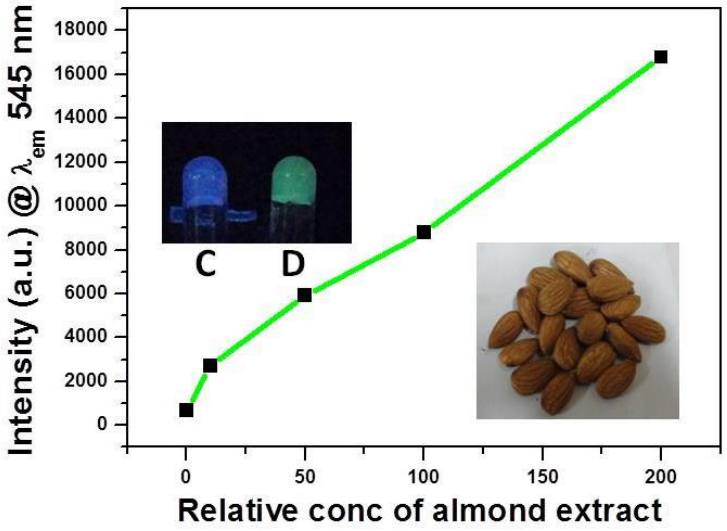


Figure S 16. Time delayed emission at λ_{em} 545 nm for DHN-galactoside containing Tb-cholate gel in presence of increasing concentration of almond extract. **Inset picture: C.** DHN-galactoside + Tb-cholate gel, **D.** 20 µl almond extract + DHN-galactoside + Tb-cholate gel

16. Kinetics study of almond β-gal in DHN-galactoside doped Tb-cholate gel:

Almond β-gal assay was done at various DHN-gal concentrations in Tb-cholate gel to calculate initial rates

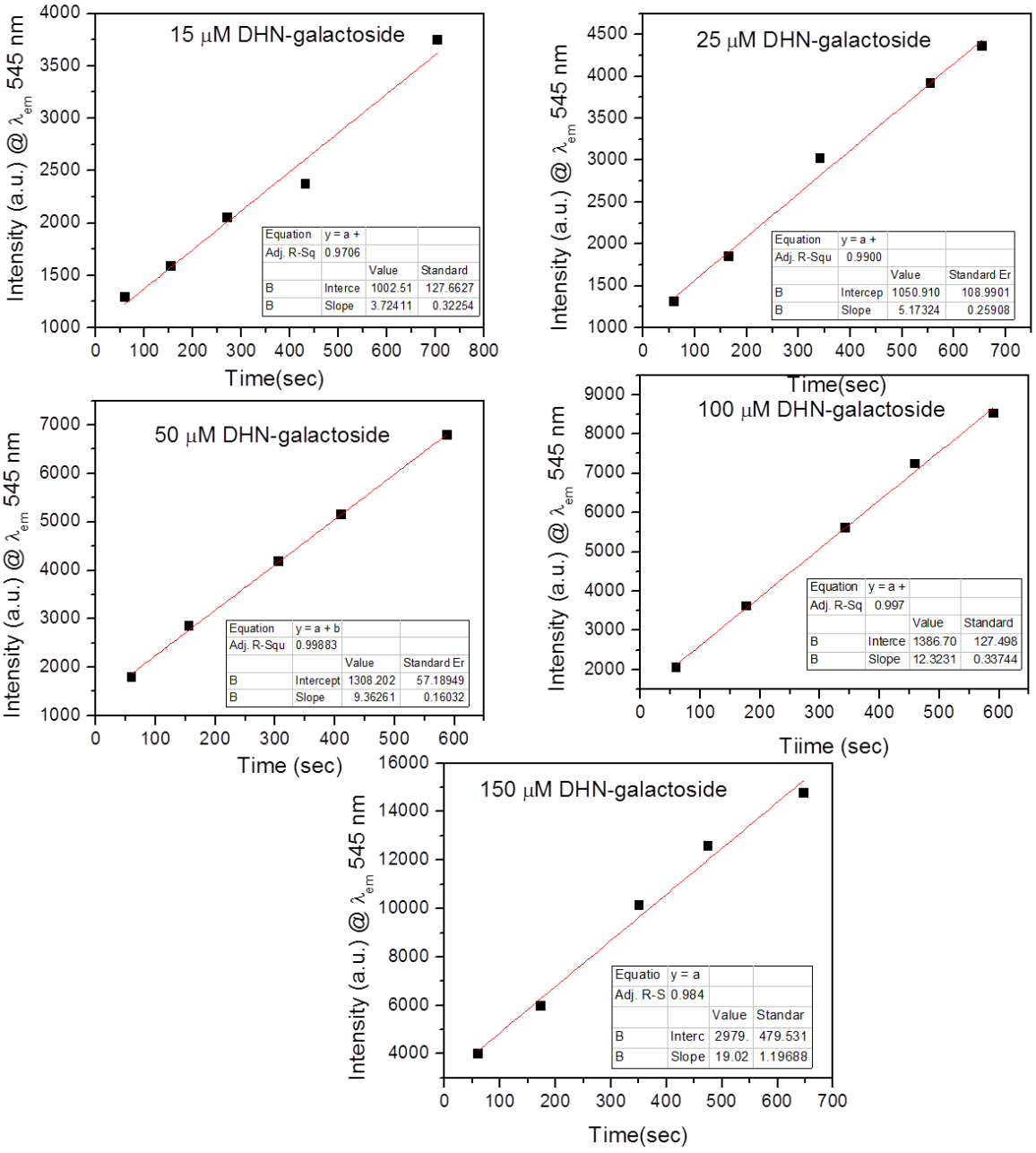


Figure S 17: Almond β-gal assay with various concentrations of pro-sensitizer **4** in Tb-cholate gel at 298 K

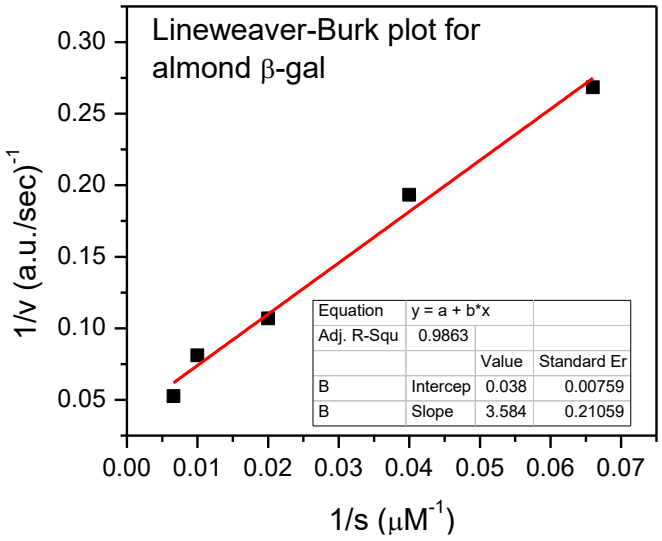


Figure S 18: Lineweaver-Burk plot for almond β-gal assay with pro-sensitizer 4 in Tb-cholate gel at 298 K.

K_m = 94 µM, v_{max} = 4.5 µM/min and almond extract contains 172 mU β-gal /mL

17. Kinetic study of lactase assay in DHN-gal doped Tb-cholate gel

Lactase assay was done with various DHN-galactoside concentrations in Tb-cholate gel to calculate initial rates

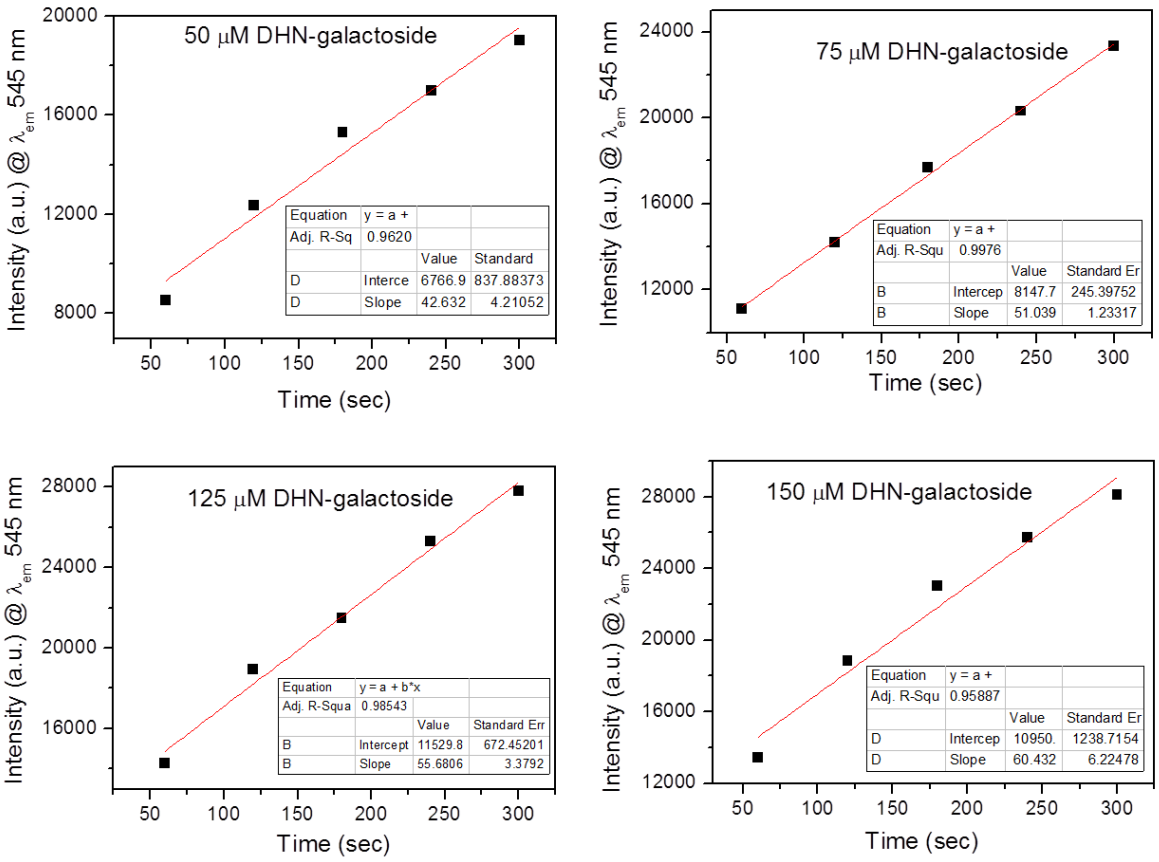


Figure S 19: Lactase assay with various concentrations of pro-sensitizer **4** in Tb-cholate gel at 298 K

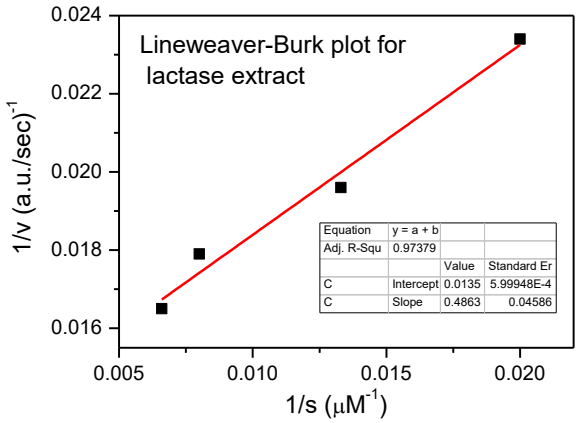


Figure S 20: Lineweaver-Burk plot for lactase assay with pro-sensitizer 4 in Tb-cholate gel at 298 K.

K_m = 36 µM, v_{max} = 14 µM/min and lactase extract contains 560 mU lactase /mL

a. Calibration plot for DHN doped Tb-cholate gels in plate reader instrument

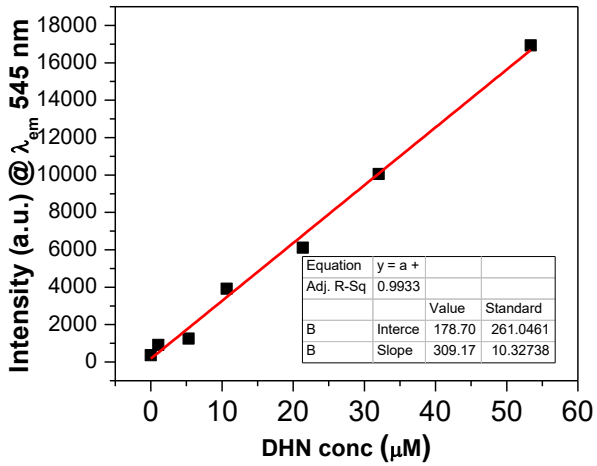


Figure S 21: Time delayed emission spectra of Tb-cholate gel with varying DHN concentration

18. AFM images of Tb-cholate xerogels without and with β-gal

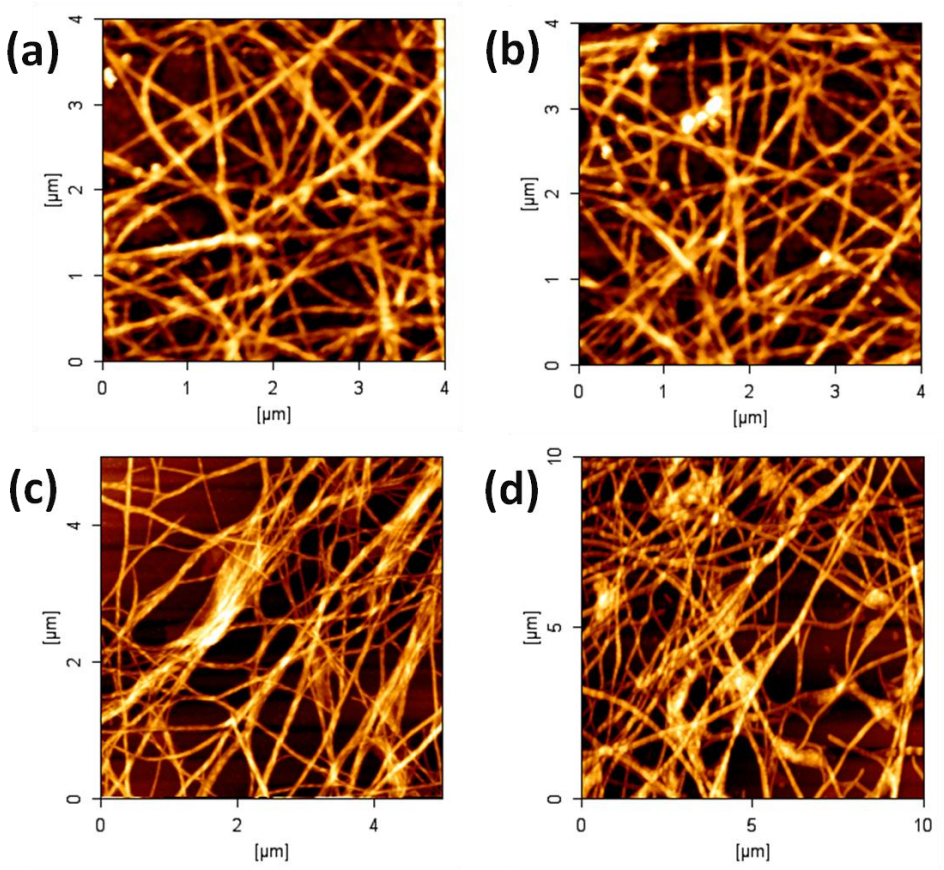


Figure S 22: AFM images of (a, b) Tb-cholate xerogel, (c, d) Tb-cholate xerogel doped with β-galactosidase.

19. SEM images of Eu-cholate xerogel

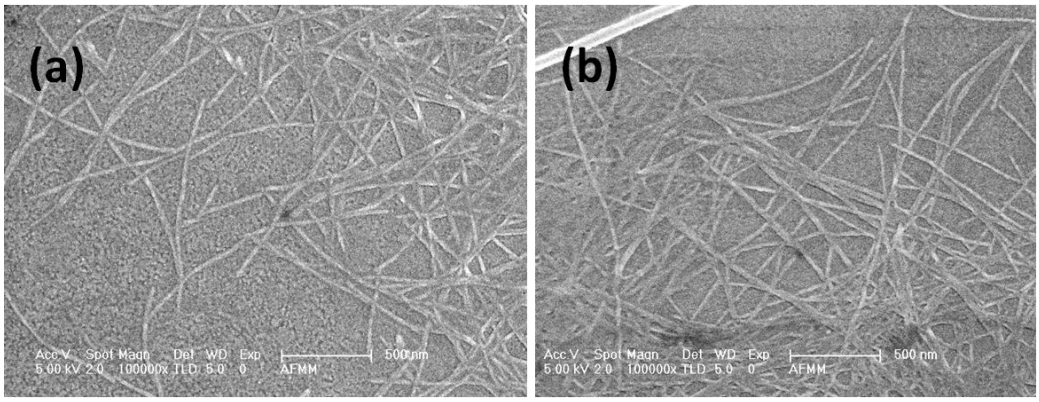


Figure S 23: SEM images of Eu-cholate xerogel.

20. SEM images of Tb-cholate xerogel

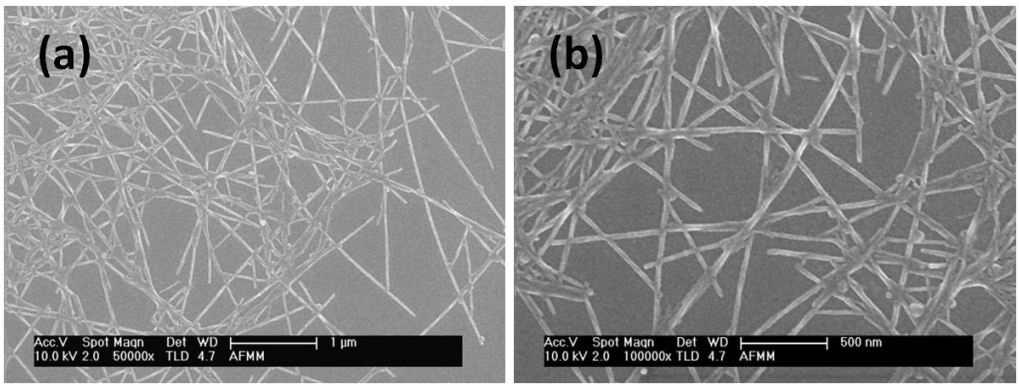


Figure S 24: SEM images of Tb-cholate xerogel.

21. SEM images of Western blotting paper before and after gel coating

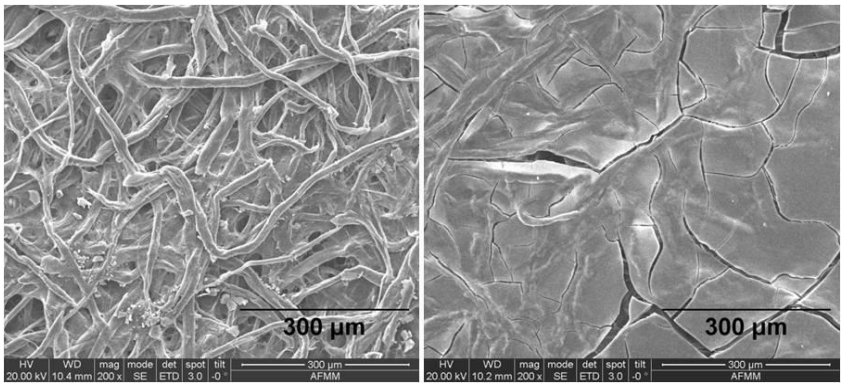


Figure S 25: SEM images of (a) uncoated, and (b) gel coated western blotting paper.

22. SEM images of Whatman filter paper before and after gel coating

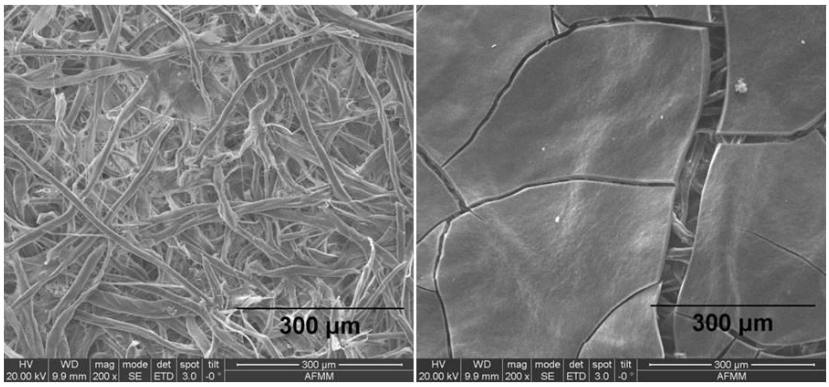


Figure S 26: SEM images of (a) uncoated, and (b) gel coated whatman 3 paper.

23. β-galactosidase detection on paper disc

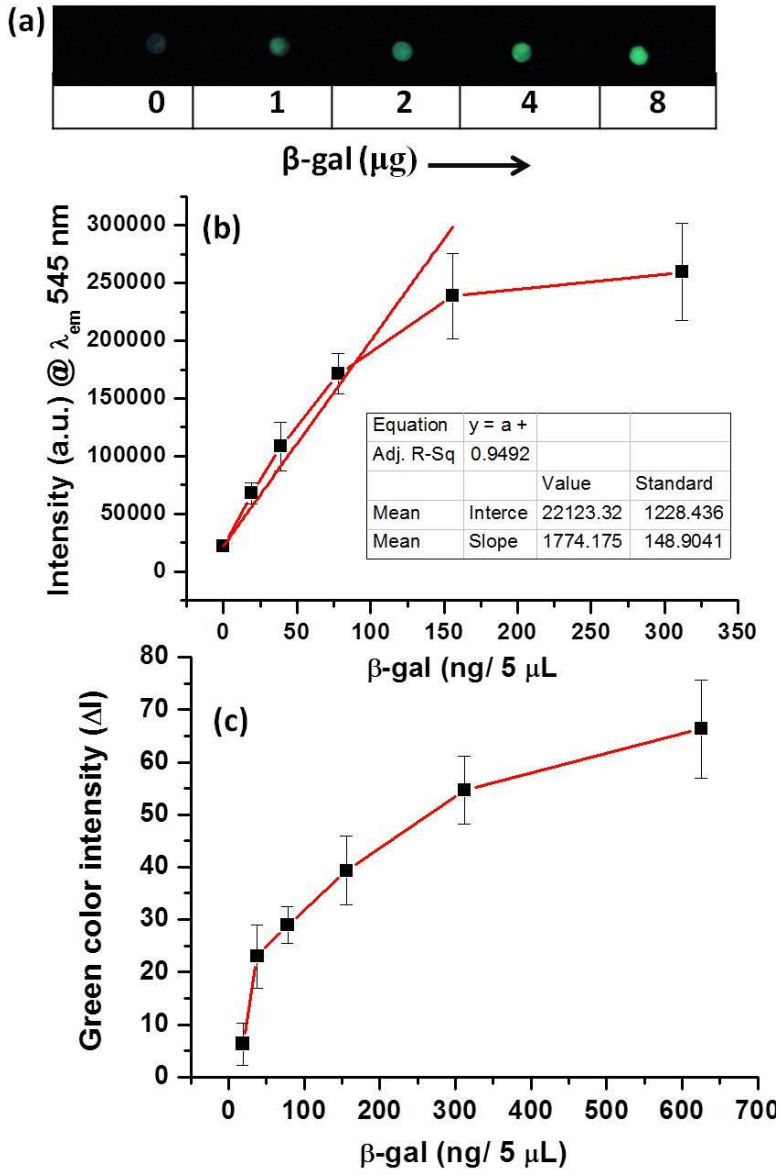


Figure S 27. (a) Image captured under 365 nm UV lamp for pro-sensitizer **4** containing Tb-cholate gel coated paper discs 60 min after adding an increasing concentration of β-gal. (b) Emission at λ_{em} 545 nm 27 min after adding variable concentrations of β-gal (n = 5). (c) Green intensity ($\Delta I = I_{Sample} - I_{Blank}$) 40 min after adding variable concentrations of β-gal (n = 3).

24. Emission change of the gel coated discs as a function of time after addition of alkaline phosphatase/β-galactosidase

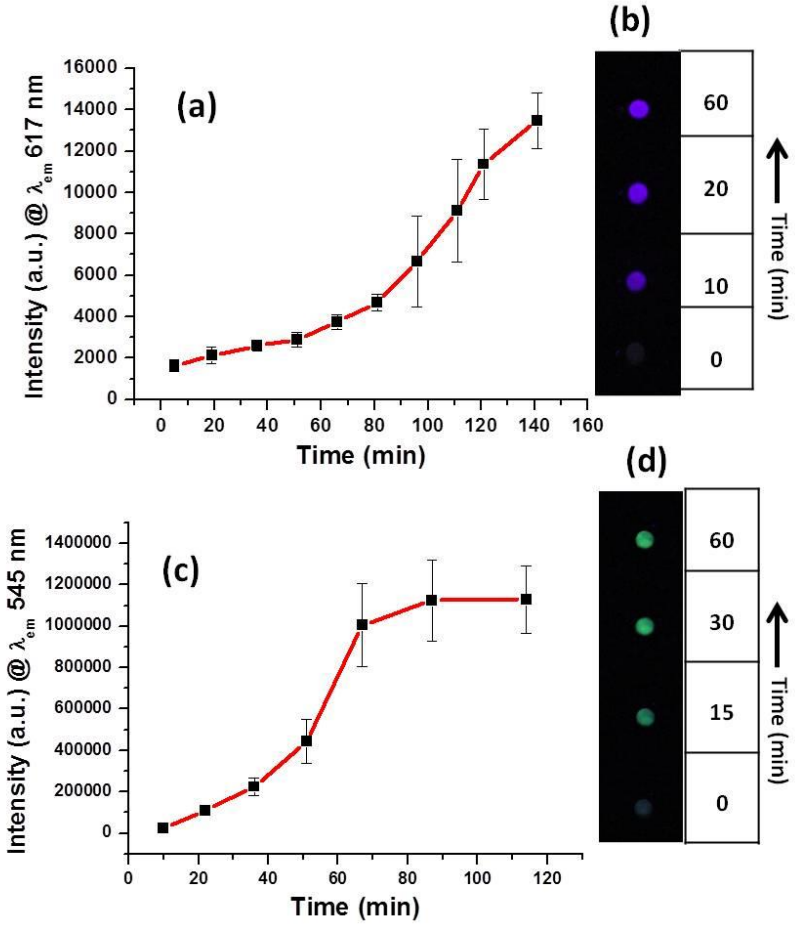
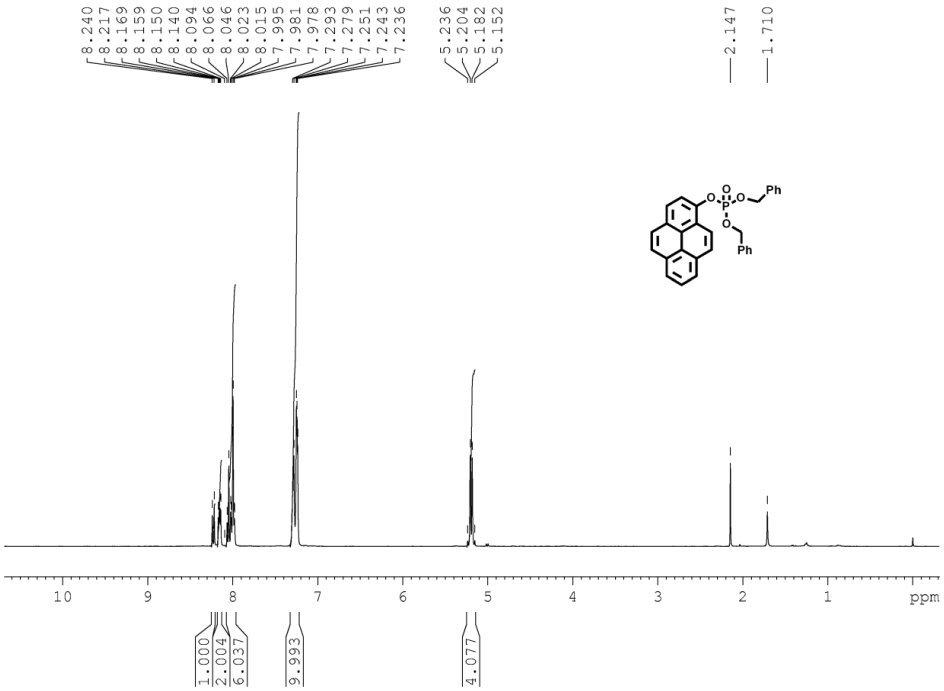


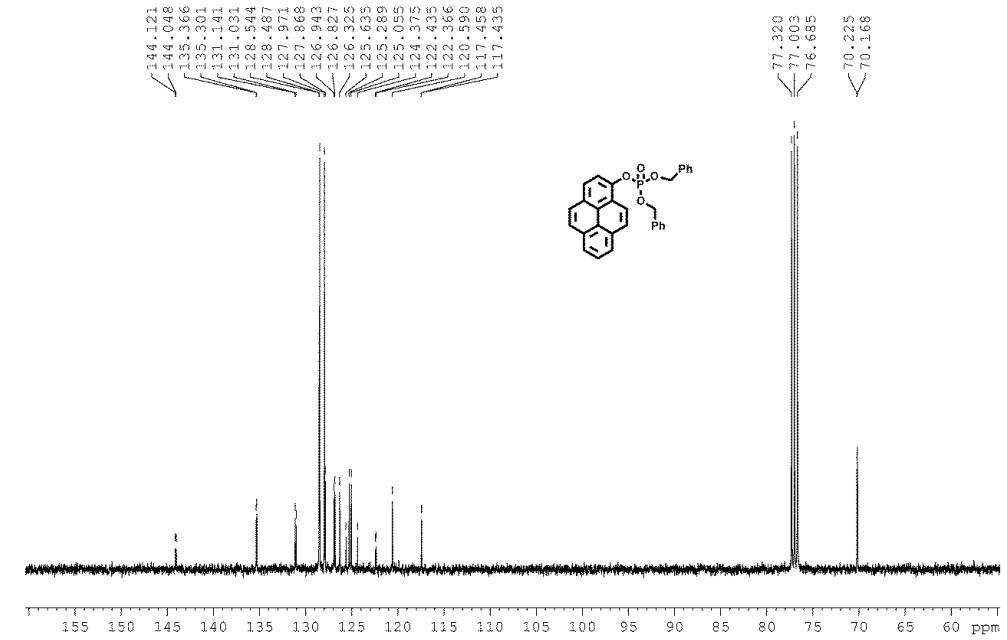
Figure S 28. (a) Intensity at λ_{em} 617 nm as a function of time (with 78 ng of ALP) (n = 4). (b) Images captured under 365 nm UV lamp for the pro-sensitizer 3 containing Eu cholate gel coated discs after adding 5 µg of ALP. (c) Intensity at λ_{em} 545 nm as a function of time (n = 5) (with 38 ng of β-gal). (d) Images captured under 365 nm UV lamp for pro-sensitizer 4 containing Tb cholate gel coated paper discs after adding 4 µg of β-gal,

25. NMR spectra:

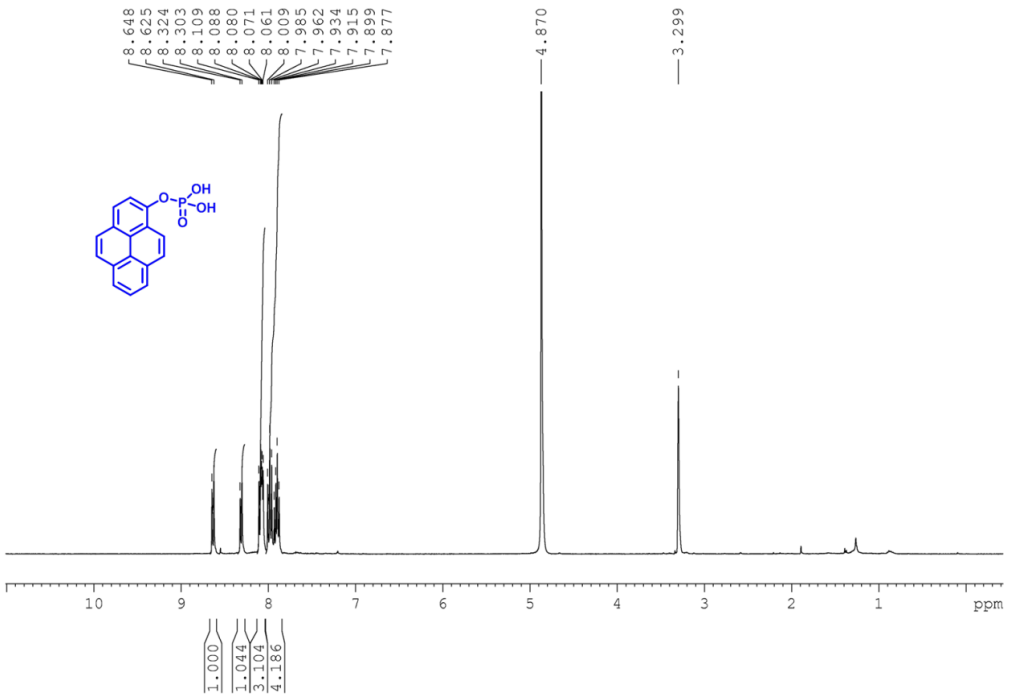
^{1}H NMR for compound **6** in $CDCl_3$



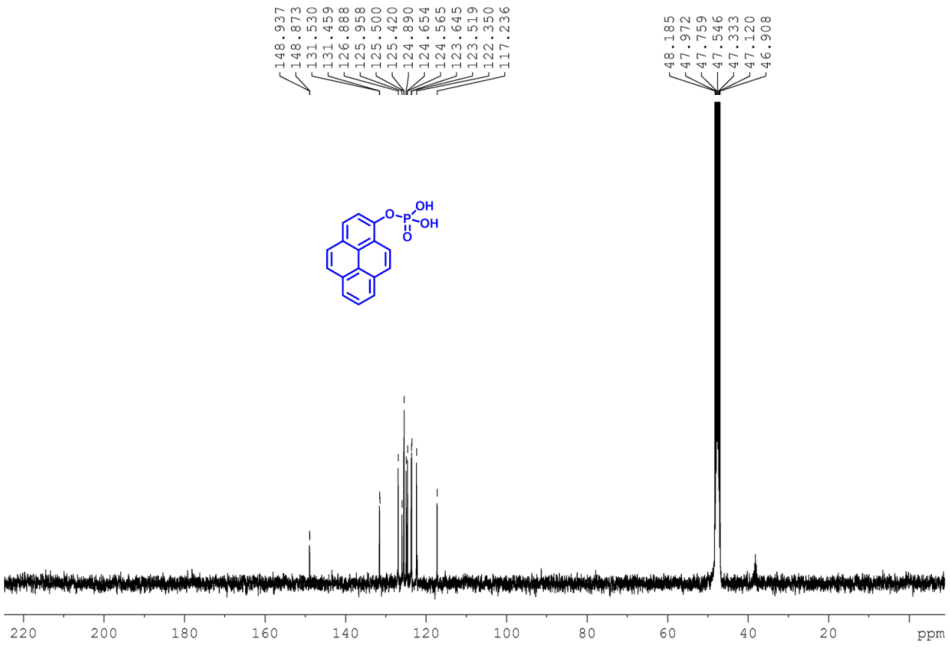
^{13}C NMR for compound **6** in $CDCl_3$



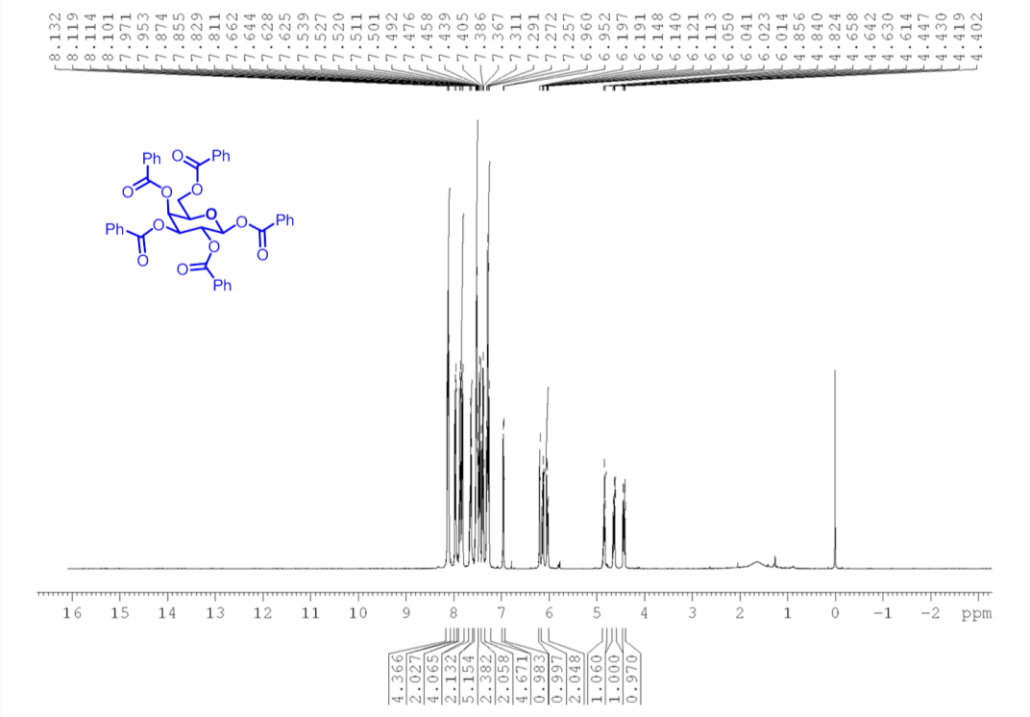
[1]HNMR for compound **3** in MeOH-d_4



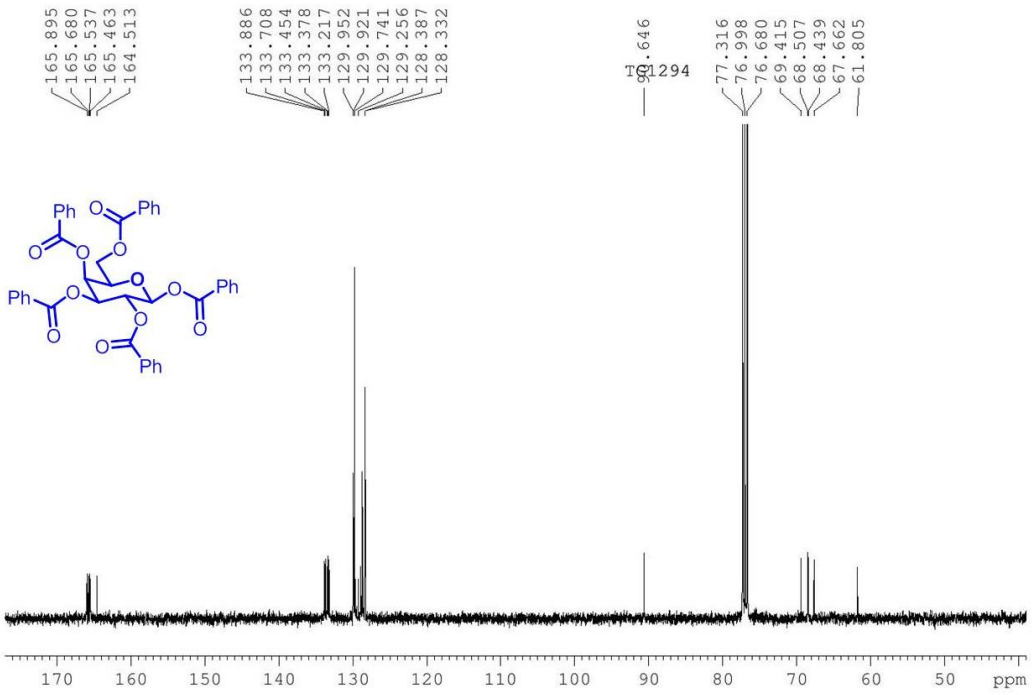
[13]C NMR for compound **3** in MeOH-d_4



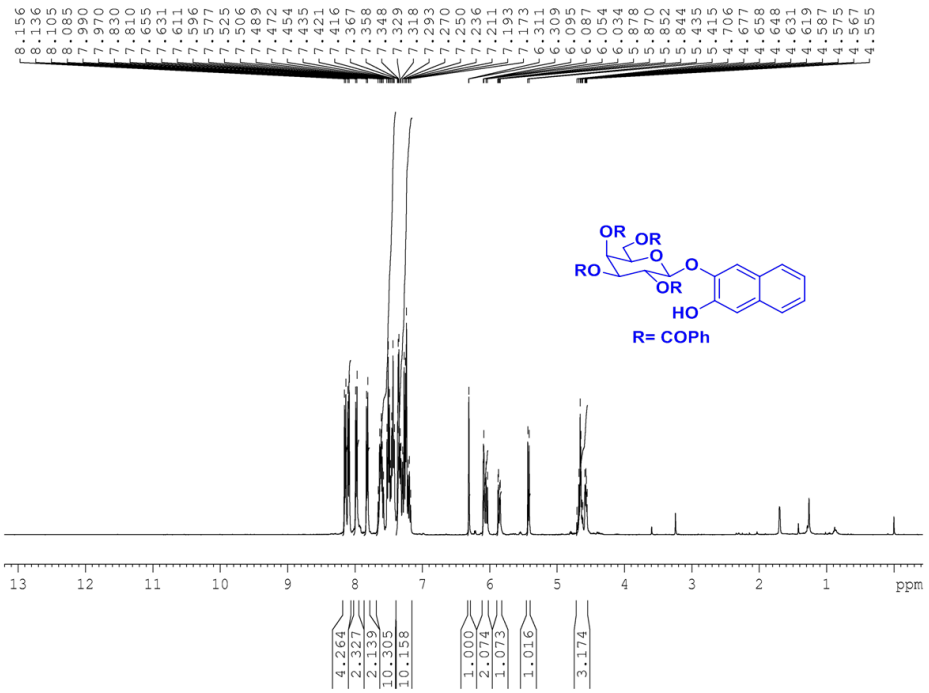
^{1}H NMR for compound **7** in $CDCl_3$



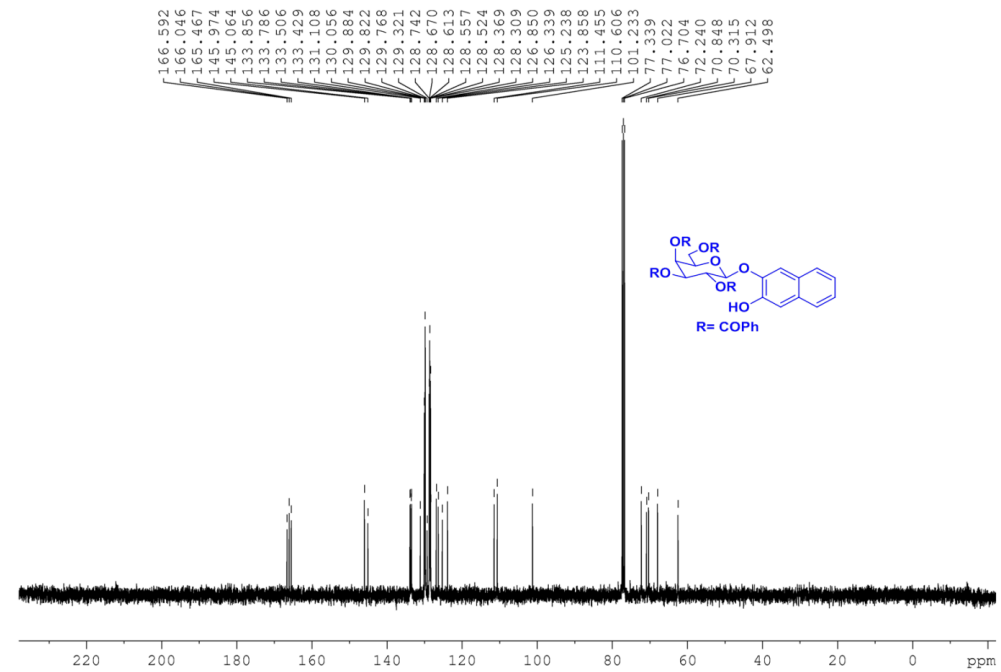
^{13}C NMR for compound **7** in $CDCl_3$



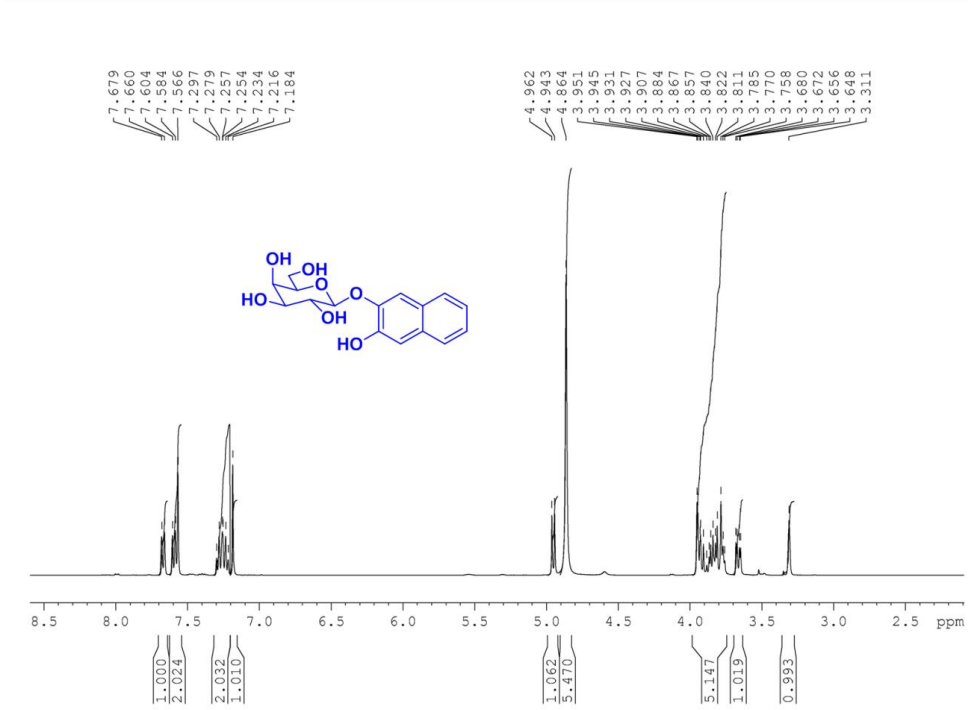
^{1}H NMR for compound **8** in $CDCl_3$



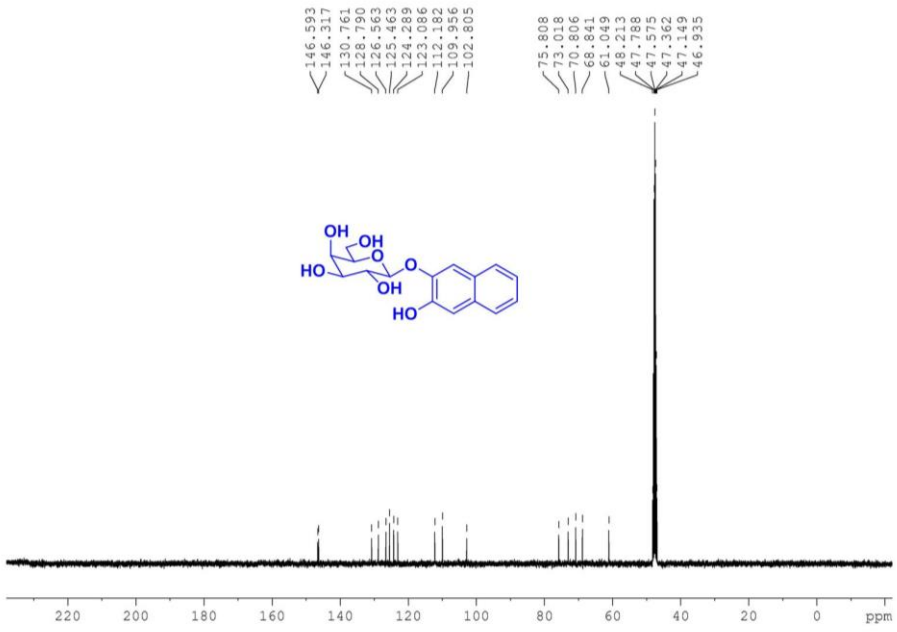
^{13}C NMR for compound **8** in $CDCl_3$



^{1}H NMR for compound **4** in MeOH-d_4



^{13}C NMR for compound **4** in MeOH-d_4



26. References:

[1] H. G. Khorana, A. R. Todd, *J. Chem. Soc.* **1953***,* 2257-2260.

[2] P. Babu, N. M. Sangeetha, P. Vijaykumar, U. Maitra, R.Kari, A. R Raju, *Chem. Eur. J.* **2003**, *9*, 1922-1932.