Electronic Supplementary Information (ESI)

Glucosamine Hydrochloride Functionalized Tetraphenylethylene: A Novel Fluorescent Probe for Alkaline Phosphatase Based on the Aggregation-Induced Emission

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Materials and Measurements

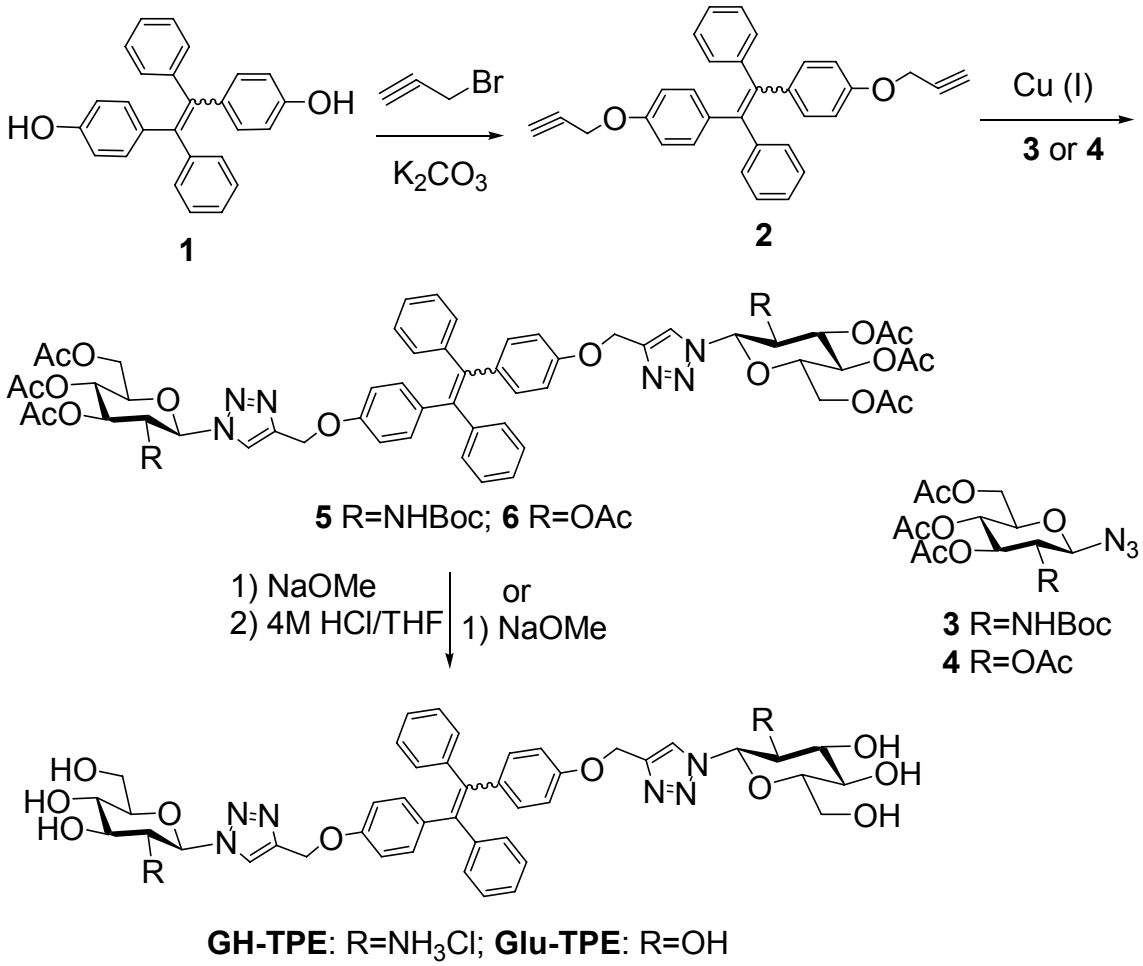
All chemical reagents were commercially available and used as received unless otherwise stated. Deionized water was purified with a Millipore Purification System (Milli-Q water). Alkaline phosphatase was purchased from Sigma-Aldrich Co. Dihydroxyl TPE (**1**)[1] and azido-functionalized sugar derivatives (**3** and **4**)[2] were prepared according to reported methods.

The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DMX400 NMR spectrometer. The optical rotations were measured with a JASCO DIP-1000 digital polarimeter. Mass spectra were recorded with a VG PLATFORM mass spectrometer

1 H. Tong, Y. N. Hong, Y. Q. Dong, M. Haeussler, Z. Li, J. W. Y. Lam, Y. P. Dong, H. H. Y. Sung, I. D. Williams and B. Z. Tang, *J. Phys. Chem. B*, 2007, **111**, 11817.

2 (a) Q. Chen, Q.-Y. Cheng, Y.-C. Zhao, B.-H. Han, *Macromol. Rapid Commun.*, 2009, **30**, 1651; (b) F. D. Tropper, F. O. Andersson, S. Braun, R. Roy, *Synthesis* **1992**, 618.

using the ESI(+) technique. Ultraviolet−visible (UV−vis) spectra were measured using a Perkin−Elmer Lamda 950 UV−vis−NIR spectrophotometer and quartz cells with 1 cm path length. The fluorescence spectra were measured in a conventional cell with 1 cm path length using a Perkin−Elmer LS55 luminescence spectrometer.



Propargylated TPE (2)

To the solution of compd **1** (2.98 g, 8.2 mmol) and propargyl bromide (2.2 mL, 20.1 mmol) in acetone (35 mL) was added anhyd K_2CO_3 (8.7 g, 62.9 mmol) and Bu_4NBr (37 mg, 0.115 mmol), the mixture was refluxed overnight. And then, the reacting mixture was cooled to room temperature and filtered. After concentration *in vacuo*,

the residue was purified with silica gel column chromatography (petroleum ether: ethyl acetate = 30:1) to give the desired product **2** (3.42 g, 95%) as yellow syrupe. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12-7.07 (m, 10H), 6.99 (m, 4H), 6.74 (m, 4H), 4.60 (m, 4H), 2.51 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.3, 144.2, 140.0, 137.4, 132.7, 131.6, 127.9, 126.5, 114.3, 78.8, 75.8, 55.9. ESI(+)-MS: calcd. for $C_{32}H_{24}O_2$: 440.5[M]; found 441.3 $[M+H]^+$.

Glucosamine TPE derivative 5

To the mixture of **2** (120 mg, 0.27 mol) and glucosamine azide derivative **3** (350 mg, 0.82 mol) in THF/water (2:1, 10 mL) was added ascorbate sodium (15 mg) and $CuSO_4$ (10 mg) as catalyst. After refluxed for 6 hours, the reaction mixture was cooled to room temperature and then extracted with ethyl acetate (2×25 mL). The combined organic layer was concentrated and then purified by silica gel chromatography (petroleum ether: ethyl acetate = 1:1) to yield **5** (329.6 mg, 96%) as a foamy solid. $[\alpha]_D^{25}$ −68^o (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (s, 2H), 7.11–6.99 (m, 10H), 6.91 (d, *J* = 8.8 Hz, 4H), 6.70 (dd, *J* = 8.56, 13.8 Hz, 4H), 5.98 (d, *J* = 10.2 Hz, 2H), 5.44 (t, *J* = 9.4 Hz, 2H), 5.21 (t, *J* = 9.72 Hz, 2H), 5.10–5.01 (m, 4H), 4.31–4.24 (m, 4H), 4.13–4.08 (m, 4H), 4.04–3.97 (m, 2H), 2.05(s, 18H), 1.24(s, 18H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 171.3, 170.7, 169.5, 156.9, 155.0, 144.3, 139.9, 137.0, 132.7, 131.5, 127.8, 126.4, 122.2, 114.0, 86.4, 80.8, 75.1, 72.3, 68.3, 61.8, 60.5, 54.8, 28.1, 21.1, 20.8, 20.7. ESI(+)-MS: calcd. for $C_{66}H_{76}N_8O_{20}$: 1301.3[M]; found 1324.5 $[M+Na]^+$. Elem. Anal. Calcd: C, 60.91; H, 5.89; Found: C, 60.78; H, 5.92.

Glucopyranosyl TPE derivative 6

Glucopyranosyl TPE derivative **6** was obtained from compound **2** and **4** using the same procedure for preparation of Glucosamine TPE derivative **5** as a foamy solid (92%). $[\alpha]_D^{25}$ −38 ° (*c* 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.86 (s, 2H), 7.11–6.99 (m, 10H), 6.94 (d, *J* = 8.0, 15.6 Hz, 4H), 6.71 (dd, *J* = 8.2, 12.8 Hz, 4H),

5.89 (d, J = 8.8 Hz, 2H), 5.48–5.39 (m, 4H), 5.24 (t, J =9.7 Hz, 2H), 5.13–5.10 (m, 4H), 4.30 (dd, J = 4.8, 12.4 Hz, 2H), 4.03–3.99 (m, 2H), 3.73–3.67 (m, 4H), 2.05 (s, 12H), 2.02 (s, 6H), 1.84 (s, 6H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 170.6, 170.0, 169.4, 169.0, 156.7, 145.1, 144.0, 139.8, 137.1, 132.6, 131.4, 127.8, 126.3, 121.2, 114.0, 85.8, 75.2, 72.8, 70.3, 67.8, 61.7, 60.5, 58.5, 20.8, 20.6, 20.2, 18.5. ESI(+)-MS: calcd. for $C_{60}H_{62}N_6O_{20}$: 1187.1[M]; found 1188.3 $[M+H]^+$.

GH-TPE

To a solution of compound **5** (55 mg, 42 μmol) in methanol (15 mL), 1 M sodium methoxide in methanol was added drop-wise until the pH = 10.The reaction mixture was stirred at room temperature overnight. After removal of the solvent under reduced pressure, the residue was dissolved in 5 mL THF and 10 mL aqueous HCl (4 M) solution, and then stirred for 24 h at room temperature. The solvent was removed and 10 mL of acetonitrile was added to precipitate the product as a light-yellow solid (38 mg, 90%) for GH-TPE. $[\alpha]_D^{25}$ –59 ^{o} (c 0.5, H_2O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61 (bs, 6H), 8.54 (S, 2H), 7.17–7.09 (m, 6H), 7.00–6.80 (m, 12H), 6.18 (d, J = 9.3 Hz, 2H), 5.07 (d, J = 9.3 Hz, 4H), 3.88–3.65 (m, 12H), 3.50–3.37 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.6, 143.7, 143.1, 139.4, 136.1, 132.0, 130.8, 127.9, 126.4, 125.2, 113.9, 83.5, 80.1, 72.7, 69.2, 60.9, 60.2, 54.3. ESI(+)-MS: calcd. for $C_{44}H_{50}Cl_2N_8O_{10}$: 921.8 [M]; found 886.5 $[M-Cl]^+$. Elem. Anal. Calcd: C, 57.33; H, 5.47; Found: C, 57.22; H, 5.45.

Glu-TPE

To a solution of compound **6** (60 mg) in methanol (15 mL), 1 M sodium methoxide in methanol was added drop-wise until the pH = 10. The reaction mixture was stirred at room temperature overnight. After neutralization with Amberlite IR120 acidic resin and filtration, the solvent was removed under reduced pressure to give the desired product (98%). $[\alpha]_D^{25}$ –50 ^{o} (c 0.5, H_2O); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.43 (s,

2H), 7.15–7.07 (m, 6H), 6.95 (d, J = 6.8 Hz, 4H), 6.91 (d, J = 8.8, 4H), 6.85 (d, J = 8.8 Hz, 4H), 5.54 (d, J = 9.2Hz, 2H), 5.06 (s, 4H), 3.76 (t, J = 8.8 Hz, 2H), 3.69 (d, J = 9.6 Hz, 2H), 3.45–3.37 (m, 6H), 3.22 (t, J = 8.8 Hz, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 156.6, 143.7, 142.5, 139.3, 136.0, 132.0, 130.8, 127.9, 126.3, 124.0, 113.9, 87.5, 80.0, 77.0, 72.1, 69.6, 60.8. ESI(+)-MS: calcd. for $C_{44}H_{46}N_6O_{12}$: 850.9 [M]; found 873.6 $[M+Na]^+$.

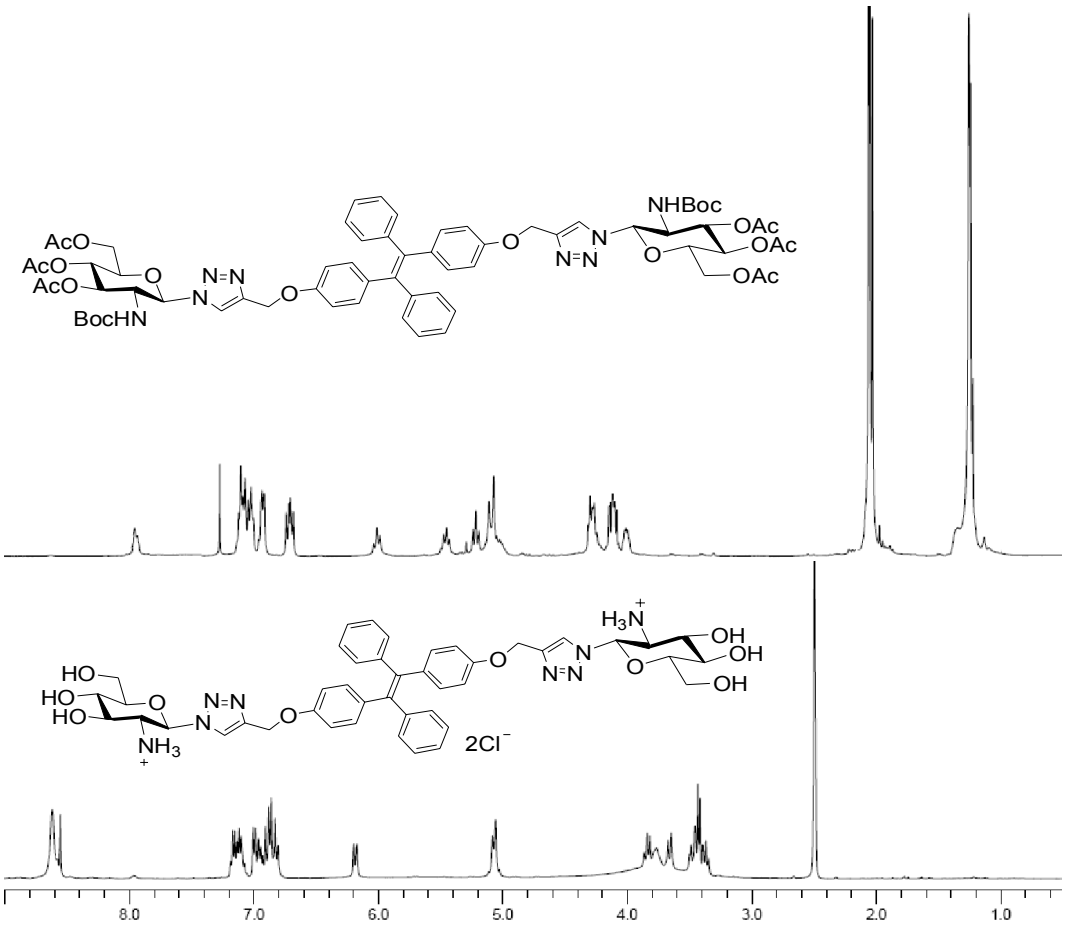


Figure S1. ^{1}H NMR of compd **5** and GH-TPE

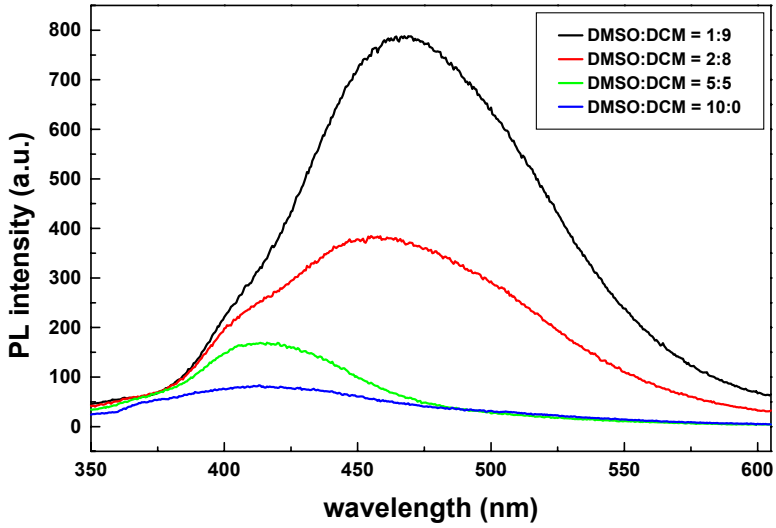


Figure S2. FL spectra for solutions of GH-TPE (90 μM) in DMSO and DCM–DMSO mixtures.

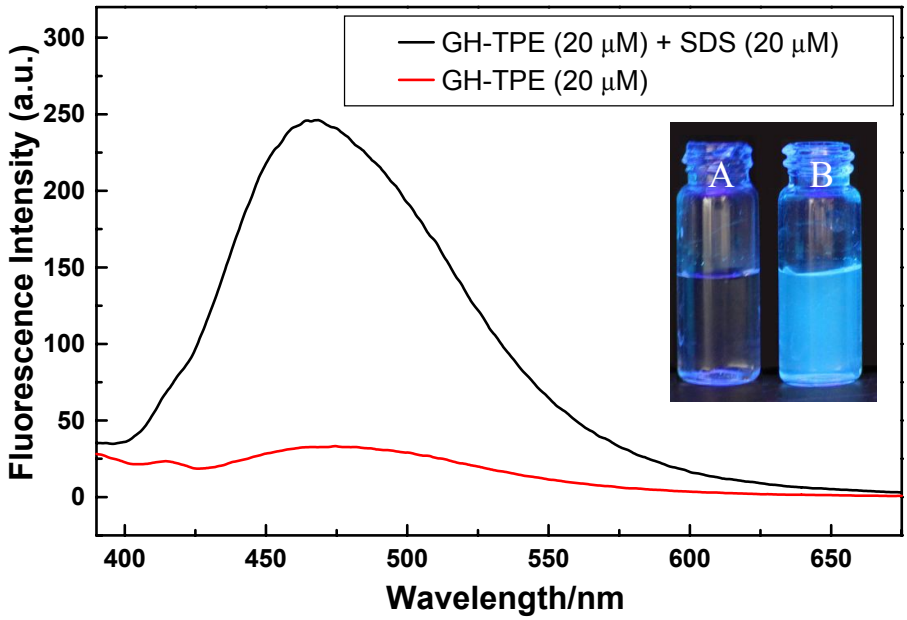


Figure S3. FL spectra of solutions of GH-TPE and GH-TPE/SDS complex in a phosphate buffer (pH 8.0). ; the inset displays the photos of the corresponding buffer solutions of GH-TPE (20 μM) in the absence (A) and presence (B) of SDS (20 μM) under UV light (365 nm) illumination.

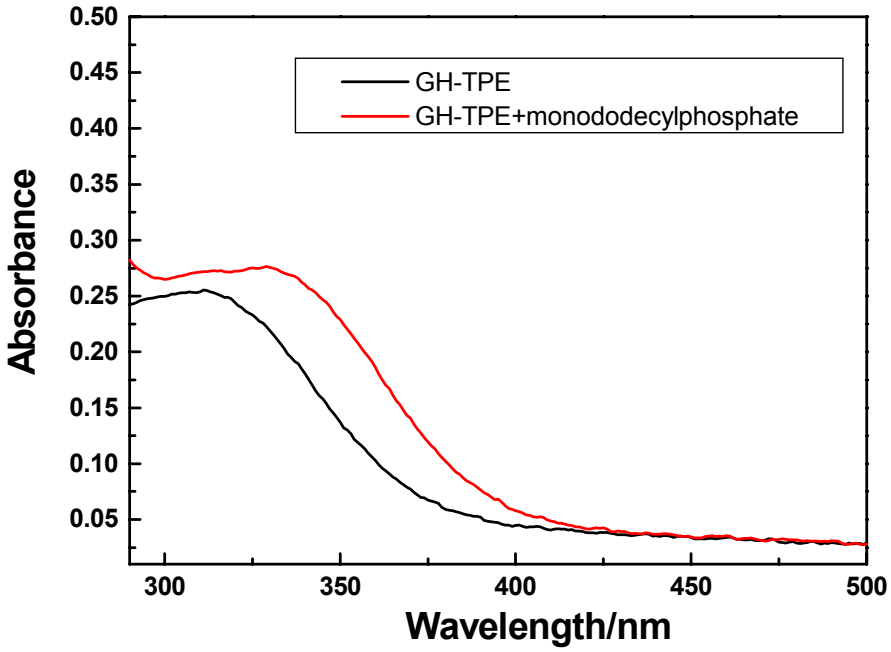


Figure S4. Absorption spectra of GH-TPE and GH-TPE/monododecylphosphate complex in a phosphate buffer (pH 8.0).

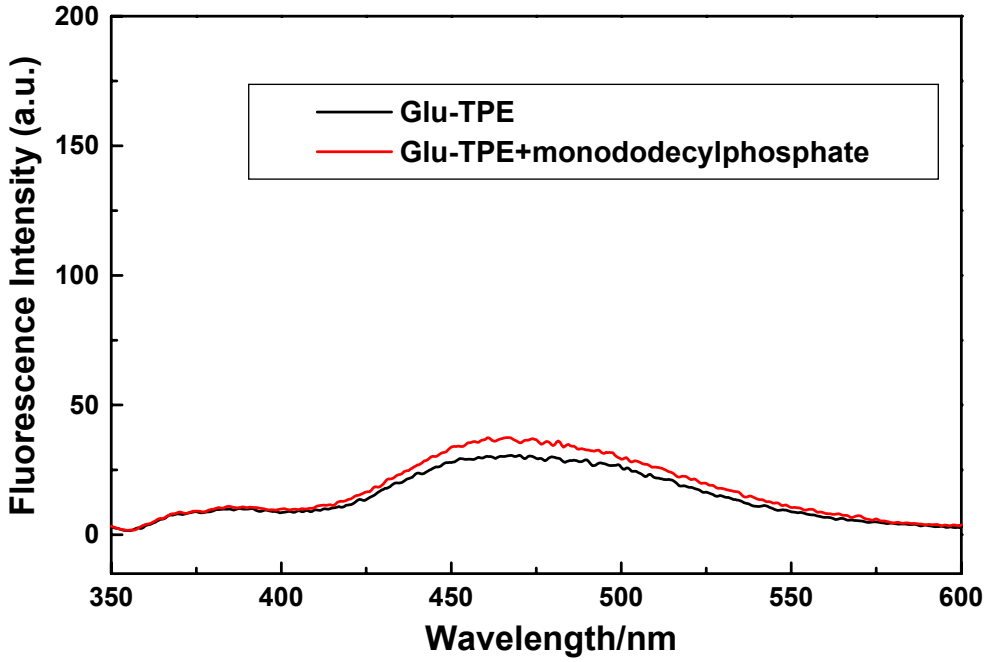
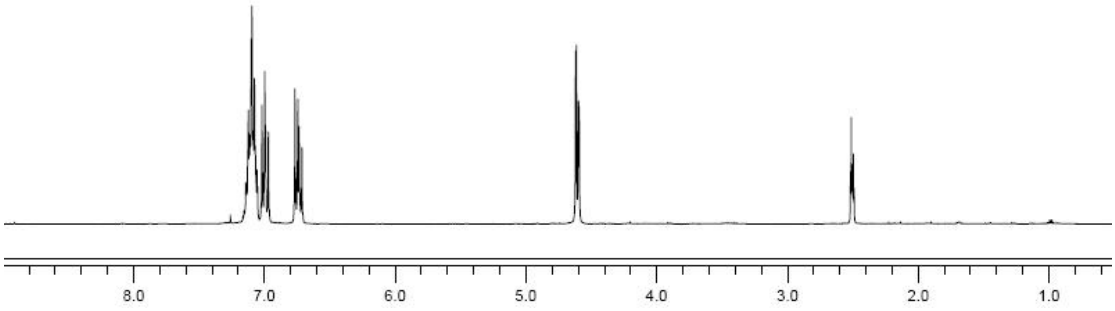
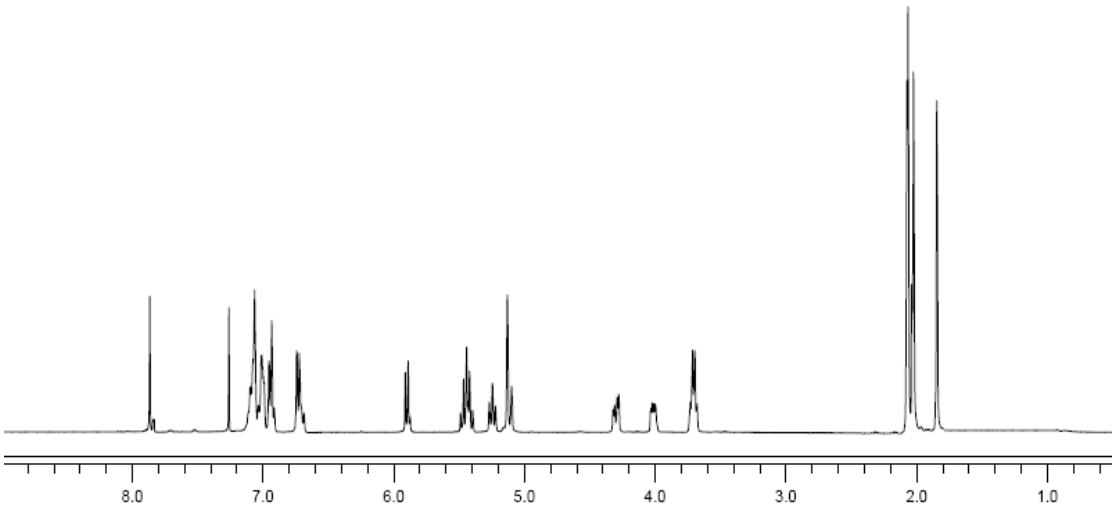


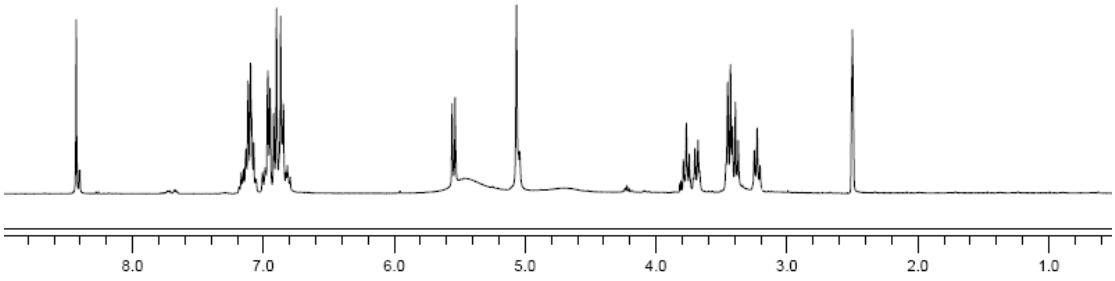
Figure S5. Fluorescence spectra of Glu-TPE [20 μM in PBS (10 mM) buffer solution, pH = 8.0] in the absence and presence of 30 μM monododecylphosphate.



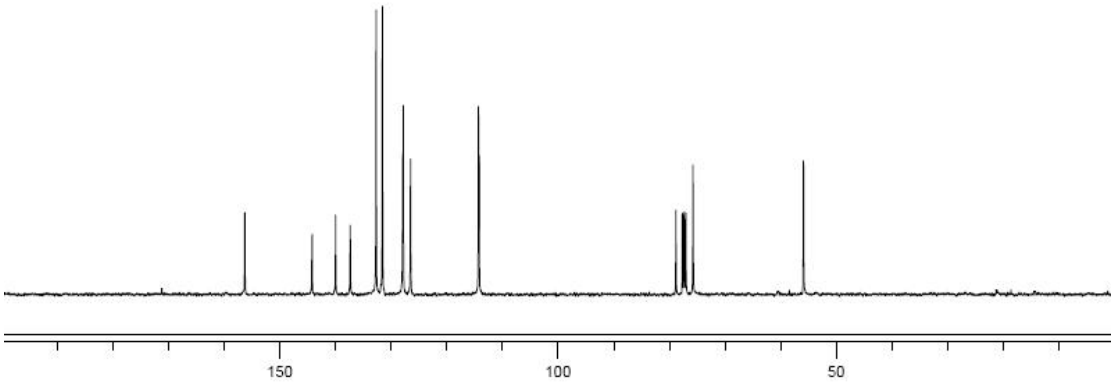
^{1}H NMR of compd **2**



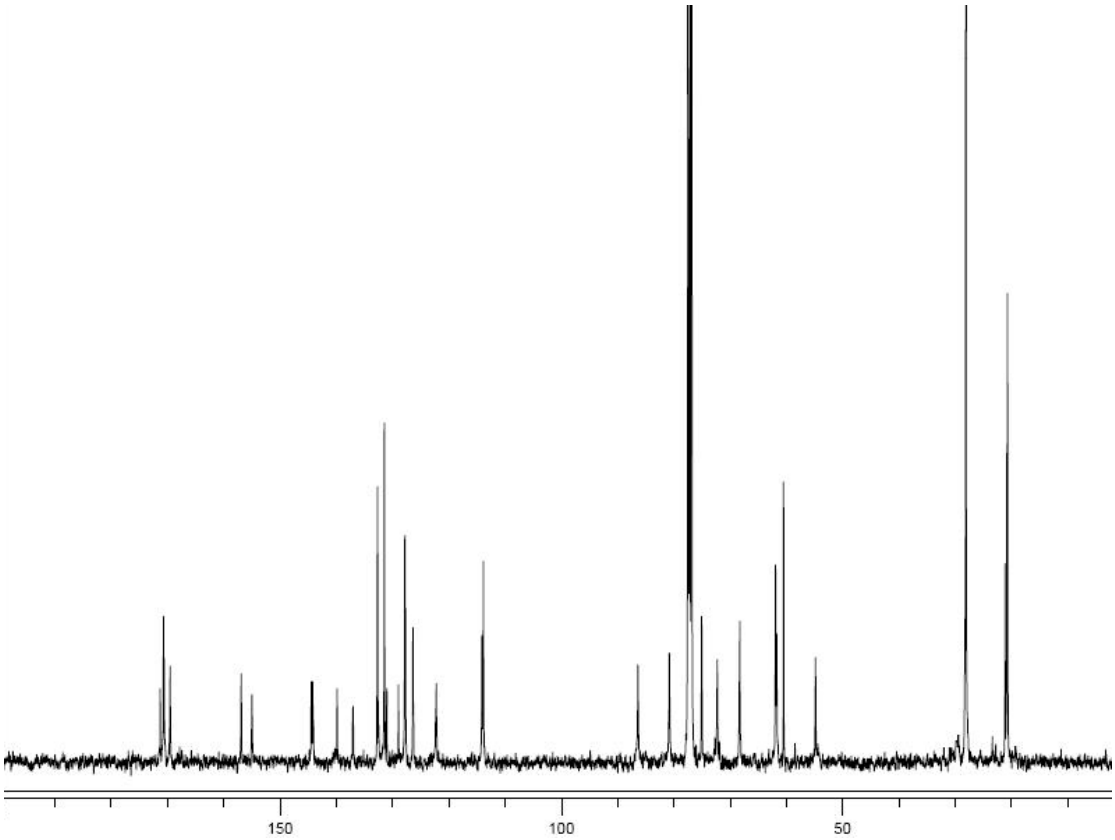
^{1}H NMR of compd **6**



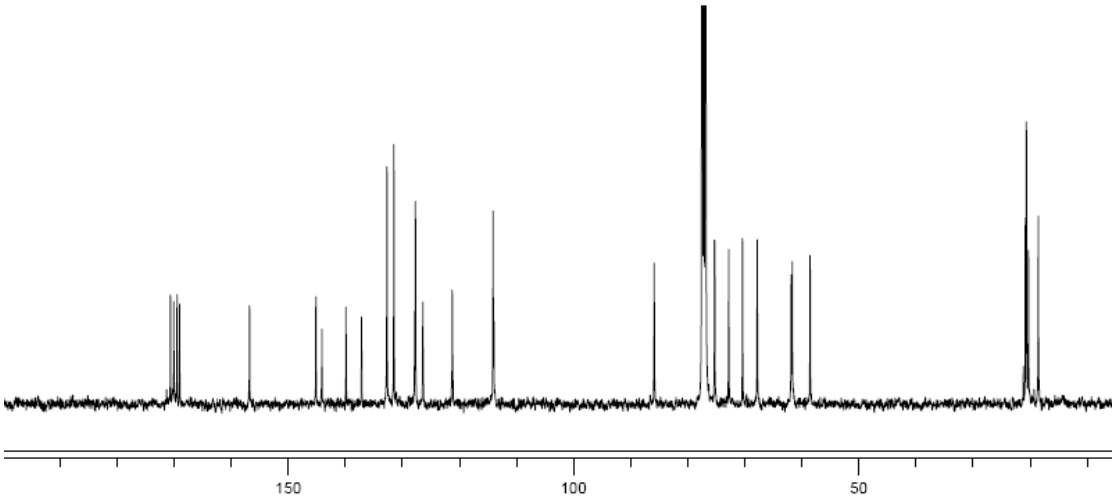
^{1}H NMR of **Glu-TPE**



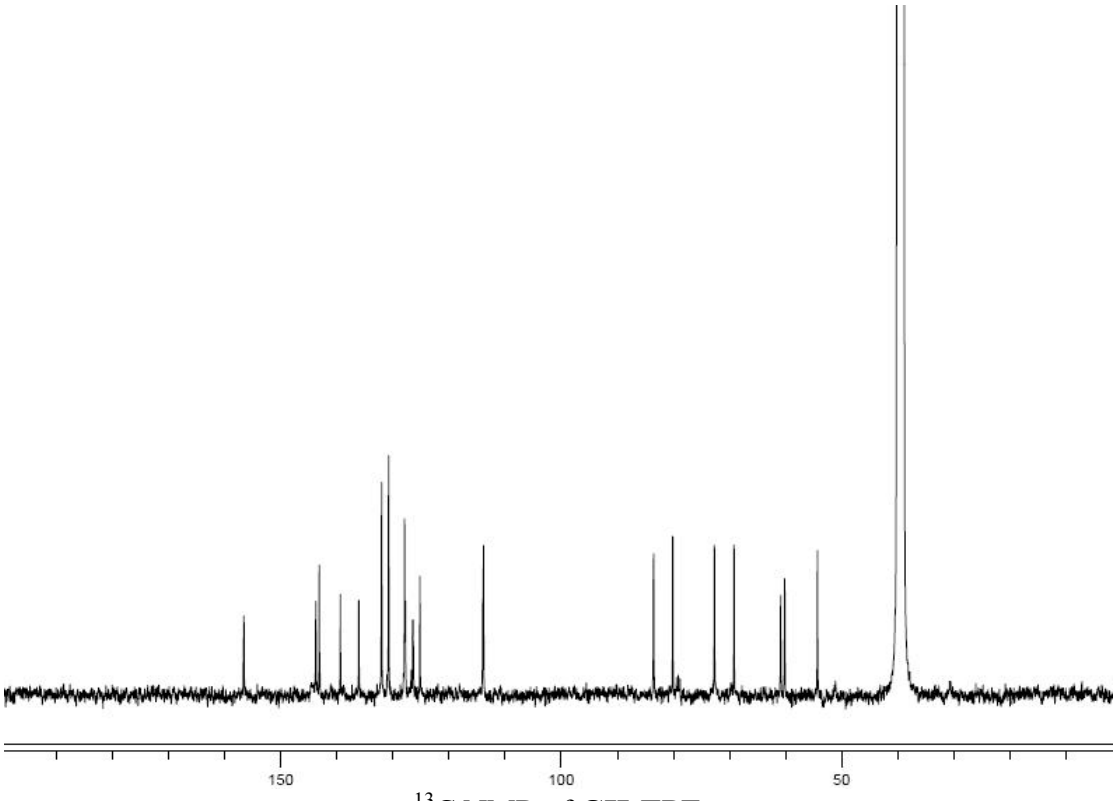
^{13}C NMR of compd **2**



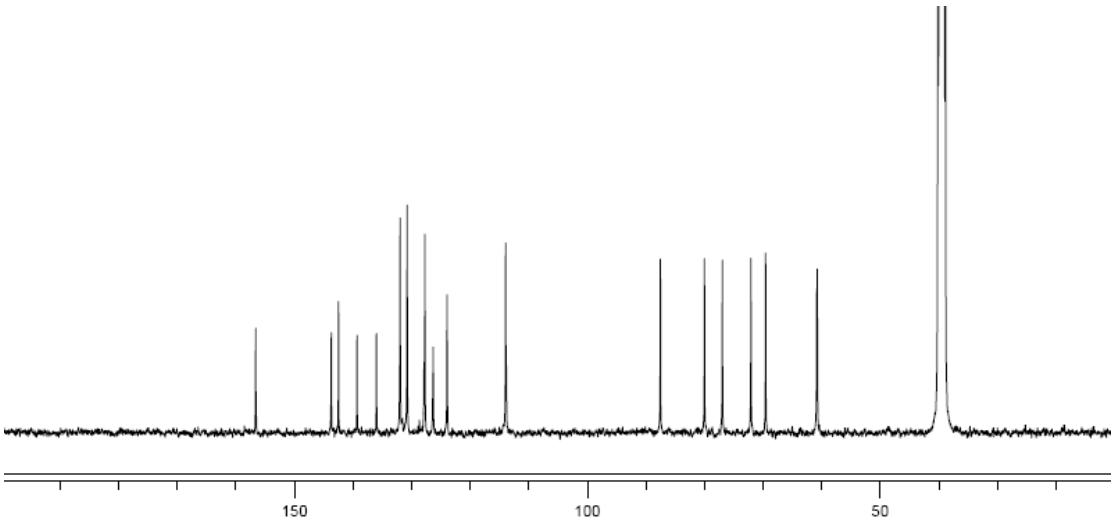
^{13}C NMR of compd **5**



^{13}C NMR of compd **6**



^{13}C NMR of **GH-TPE**



^{13}C NMR of **Glu-TPE**