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Supporting Information

Insight into graphene oxide associated fluorogenic sensing of glycodye-lectin interactions

Ding-Kun Ji, Yue Zhang, Xiao-Peng He* and Guo-Rong Chen*

Key Laboratory for Advanced Materials & Institute of Fine Chemicals, East China University of Science and Technology (ECUST), 130 Meilong Rd., Shanghai 200237, PR China

Correspondence:

X.-P. He (xphe@ecust.edu.cn);

G.-R. Chen (mrs_guorongchen@ecust.edu.cn)

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S1. Additional figures

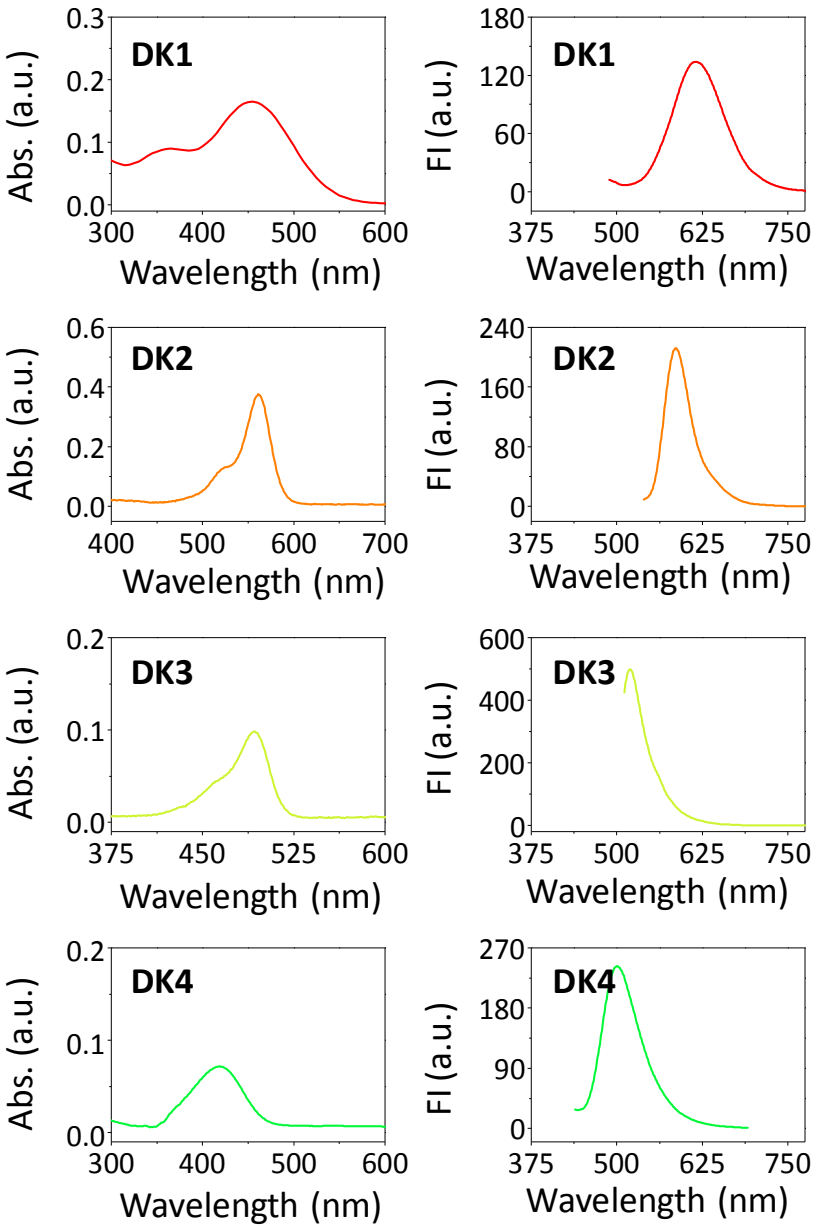


Figure S1 | UV absorbance (abs.) and fluoresence intensity (FI) of DK1, DK2, DK3 and DK4. All spectra were recorded in Tris-HCl buffer (0.01 M, pH 7.4) with a final compound concentration of 1 µM with excitation at 460, 520, 492 and 420 nm for **DK1** ($\lambda_{em/max}$ = 620 nm), **DK2** ($\lambda_{em/max}$ = 585 nm), **DK3** ($\lambda_{em/max}$ = 520 nm) and **DK4** ($\lambda_{em/max}$ = 500 nm), respectively.

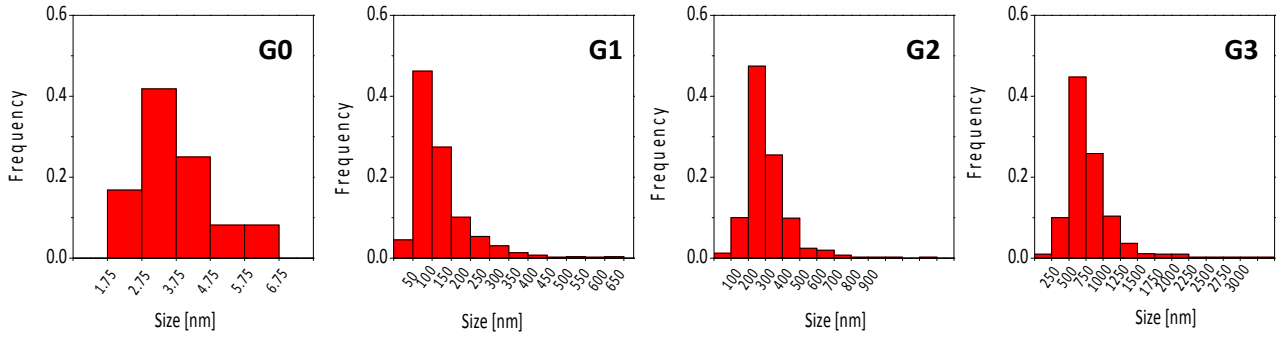


Figure S2 | Size distribution of G0-G4. The GO size distribution histograms were produced by counting over 1000 sheets in randomly selected AFM images for each sample.

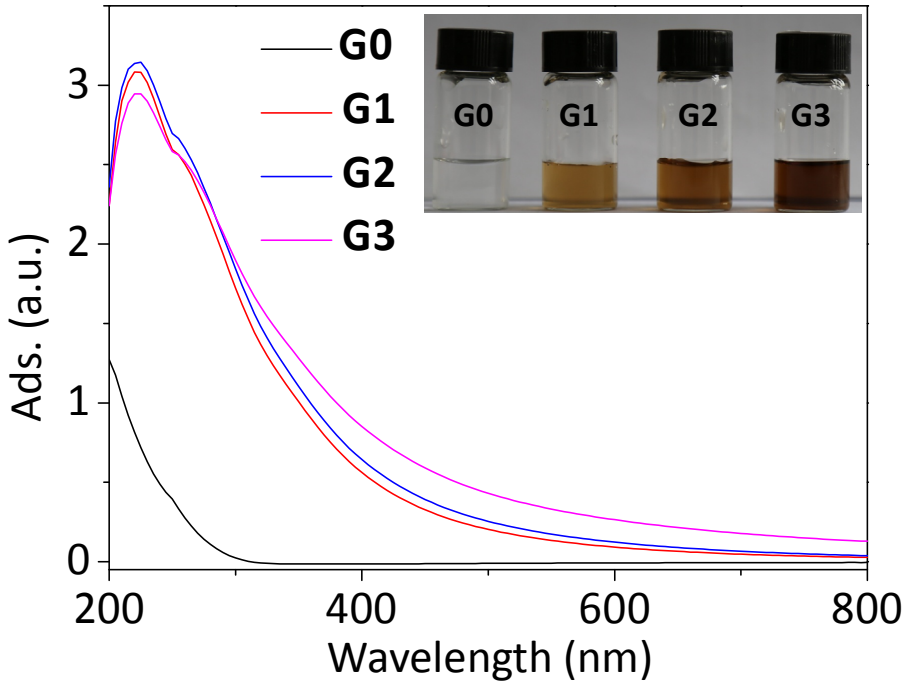


Figure S3 | Stacked UV absorbance (abs.) spectrum of G0-G4. All spectra were recorded in Tris-HCl buffer (0.01 M, pH 7.4) with a final compound concentration of 200 μg mL^{-1} (Inset: The aqueous solution of **G0**-**G3**).

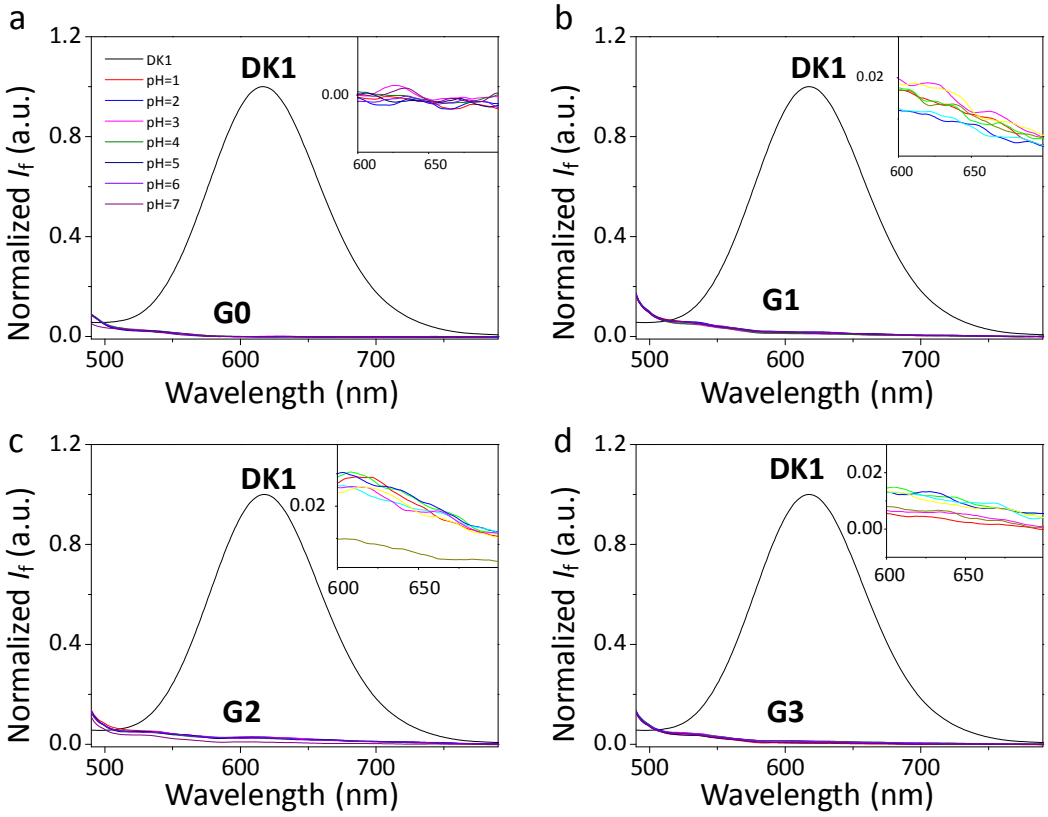


Figure S4 | Photoluminescence emission of G0-G4 with different pH. All spectra were recorded in Tris-HCl buffer (0.01 M, pH 1-7) using **DK1** as reference.

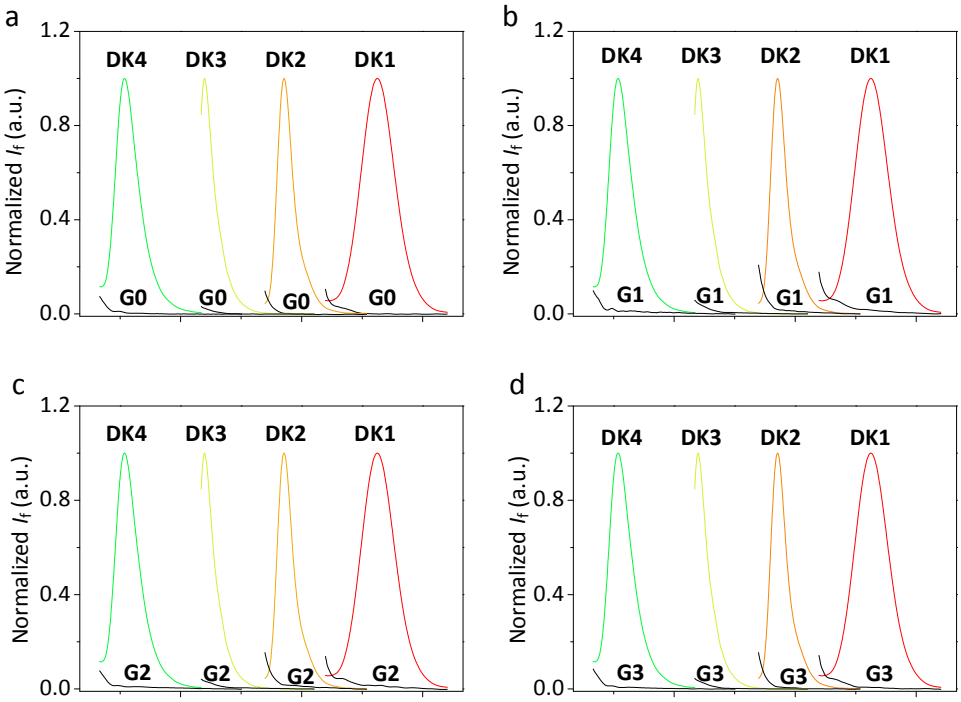


Figure S5 | Photoluminescence emission of G0-G4. All spectra were recorded in Tris-HCl buffer (0.01 M, pH 7.4) using **DK1-DK4** as reference.

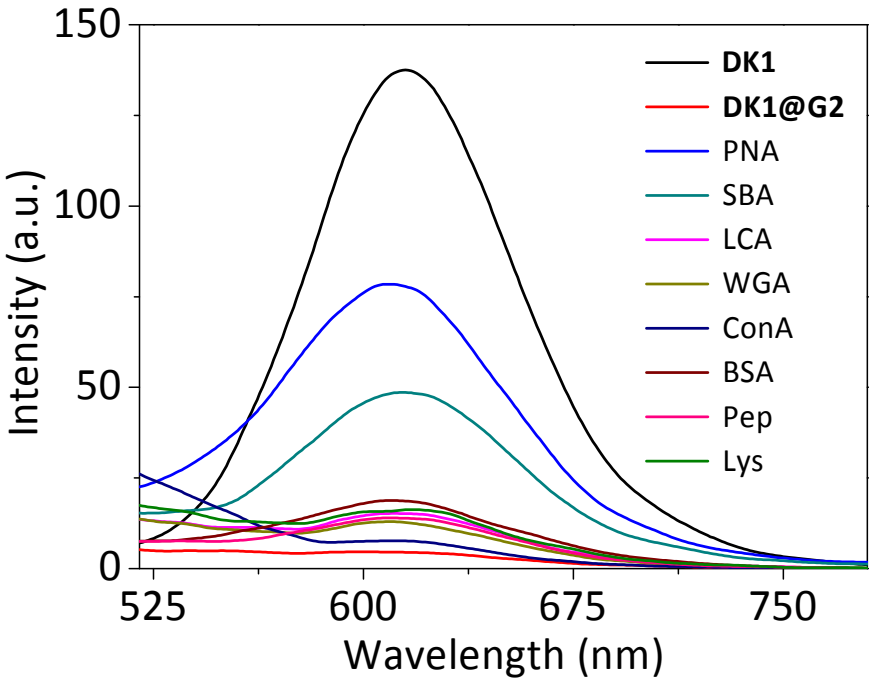


Figure S6 | Protein selectivity of DK1@G2. The fluorescence intensity of **DK1** (1 μM, $\lambda_{em/max}$ = 620 nm) without or with **G2** (35 μg mL^{-1}) in the absence and presence of different proteins (20 μM) in Tris-HCl buffer (0.01 M, pH 7.4) with excitation at 460 nm (PNA: peanut agglutinin, SBA: soyabean agglutinin, LCA: Lentil agglutinin, WGA: wheat germ agglutinin, Con A: Concanavalin A, BSA: bovine serum albumin, Pep: pepsin, and Lys: lysin).

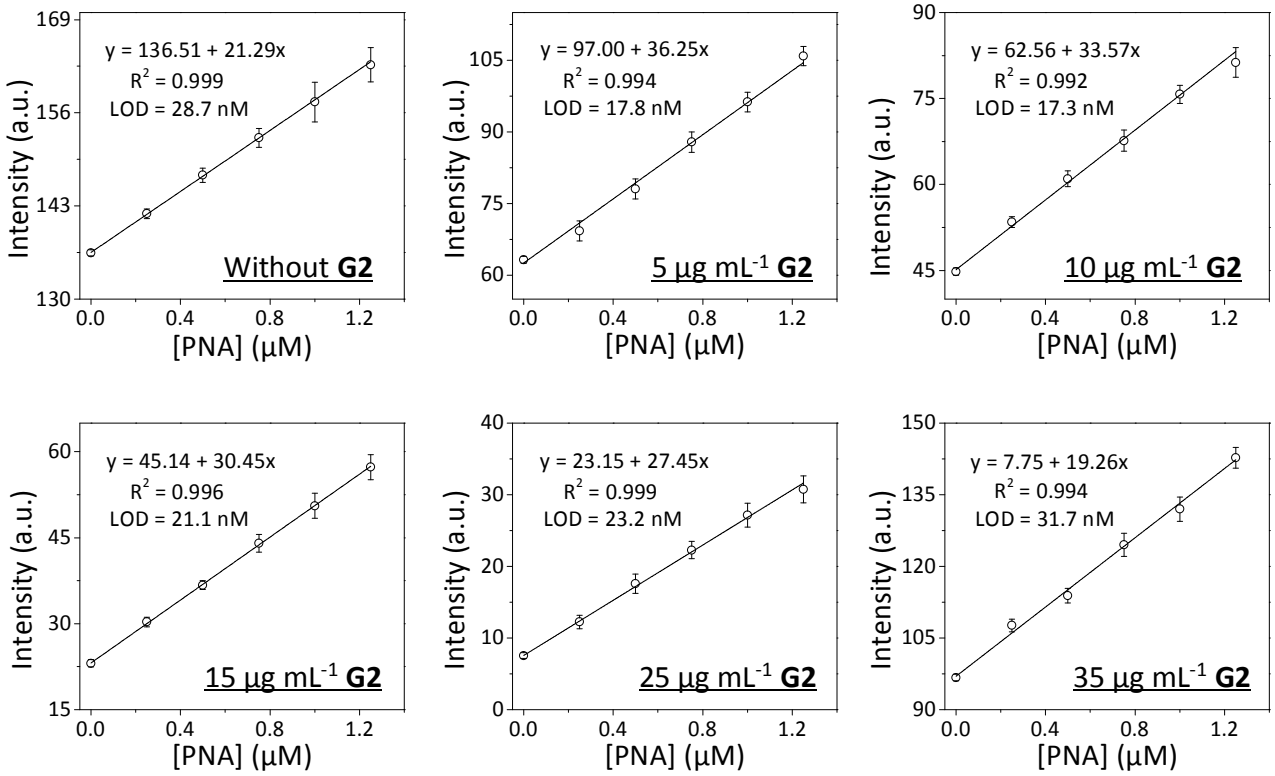


Figure S7 | Limit of detection (LOD) of DK1 in the absence and presence of G2 for PNA. The fluorescence intensity was plotted as a function of PNA concentration to determine the LOD of **DK1** (1 µM, $\lambda_{em/max}$ = 620 nm) in the absence and presence of increasing **G2** for PNA ($3\sigma_b/k$, where σ_b is the standard deviation of fluorescence in the absence of lectin) in Tris-HCl buffer (0.01 M, pH 7.4) with excitation at 460 nm.

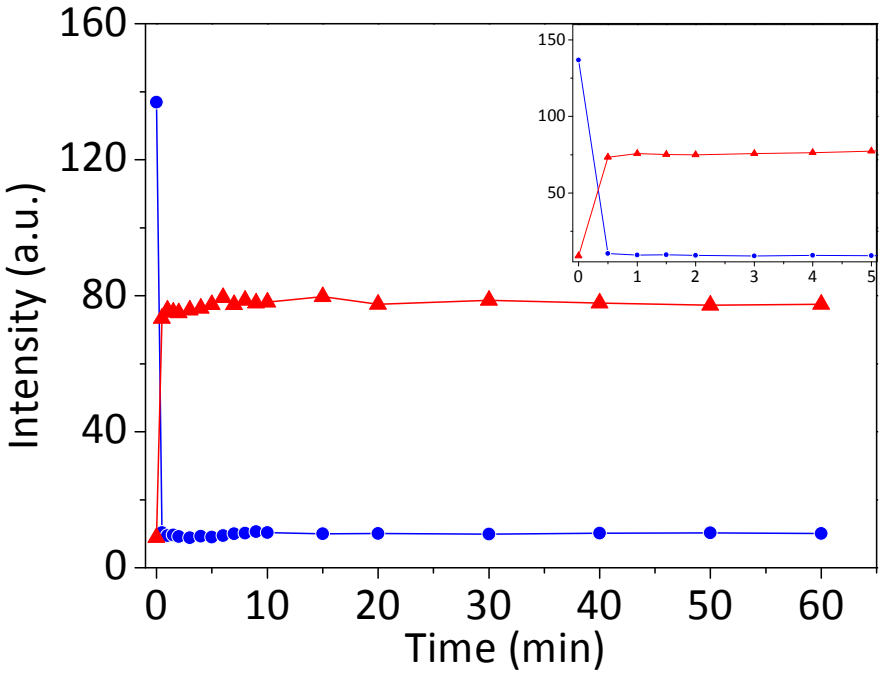
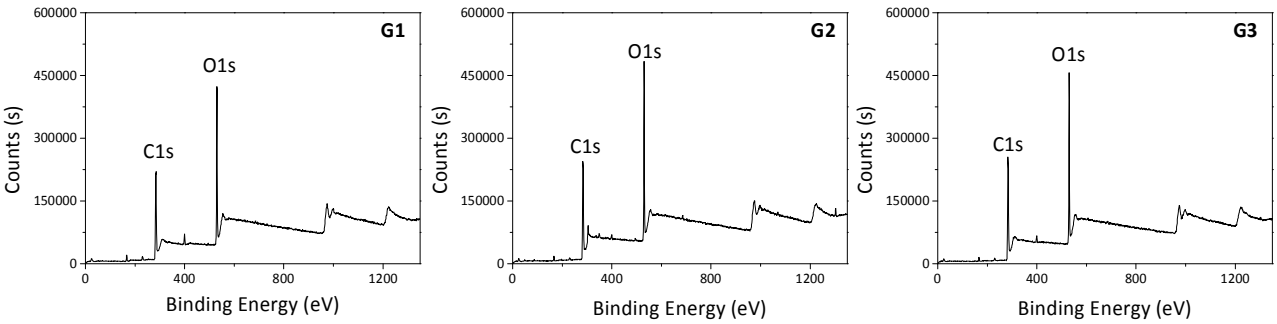


Figure S8 | Fluorescence stability assay. Plotting the fluorescence intensity of **DK1** (1 µM, $\lambda_{em/max}$ = 620 nm) in the presence of 35 µg mL^{-1} **G2** (blue curve) and **DK1@G2** (red curve) in the presence of 12.5 µM PNA as a function of time (0-1 h) in Tris-HCl buffer (0.01 M, pH 7.4) with excitation at 460 nm.



	O1s	C1s	O1s/C1s
G1	1259832	942924.1	1.33609
G2	1285267	1001367	1.283512
G3	1225584	1063060	1.152883

Figure S9 | XPS of G1-G3 for the determination of the O/C ratio of the materials.

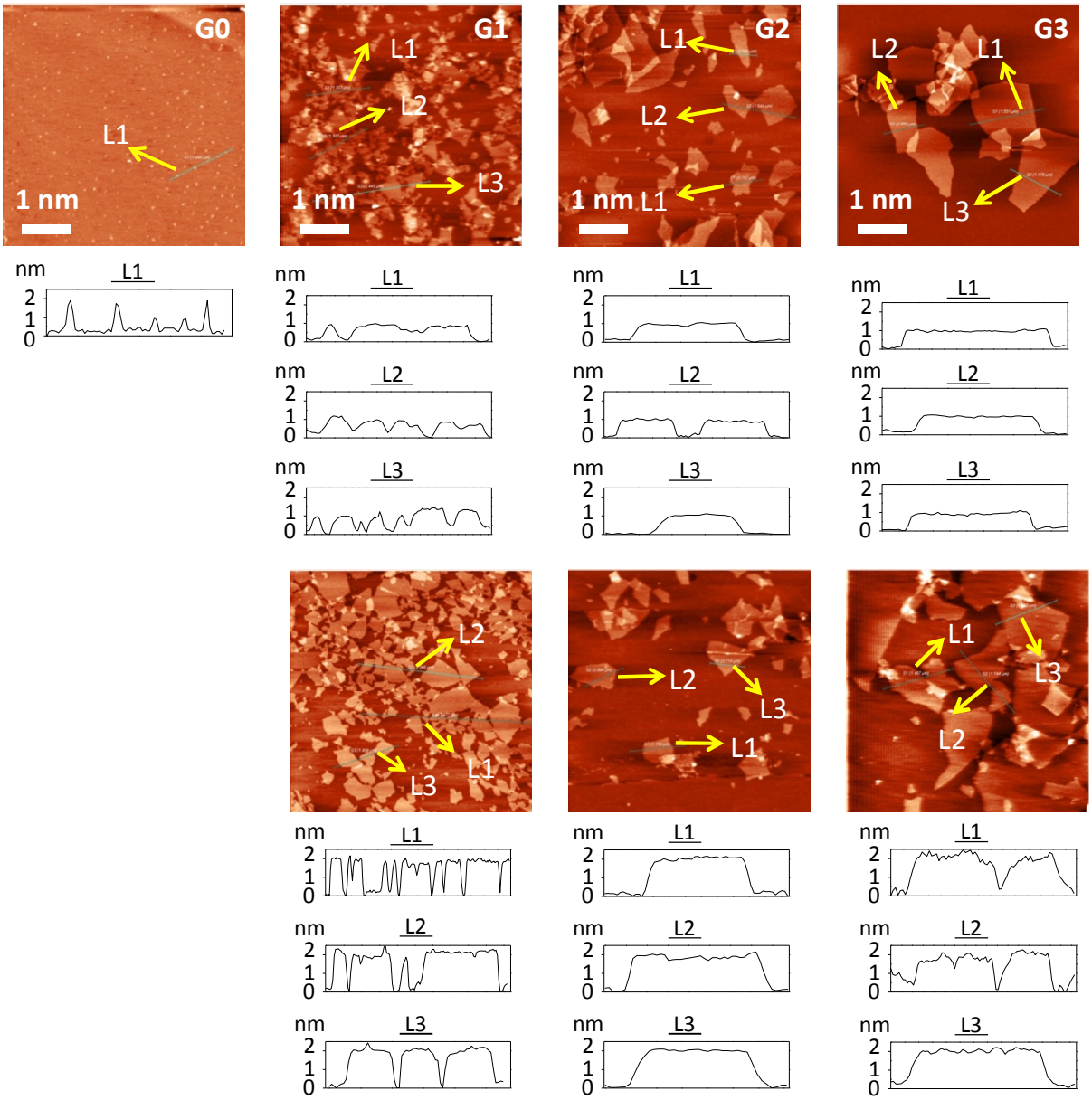


Figure S10 | Atomic force microscope images of GOs of different size. GO with different sizes show similar average heights (**G0**: 1.0-2.0 nm, **G1**: 1.0 nm, **G2**: 1.0 nm and **G3**: 1.2 nm), whereas complexation with **DK1** increases the average heights to **DK1@G1**: 1.9 nm, **DK1@G2**: 2.0 nm and **DK1@G3**: 1.9 nm, suggesting the stacking of **DK1** to the surface of GO.

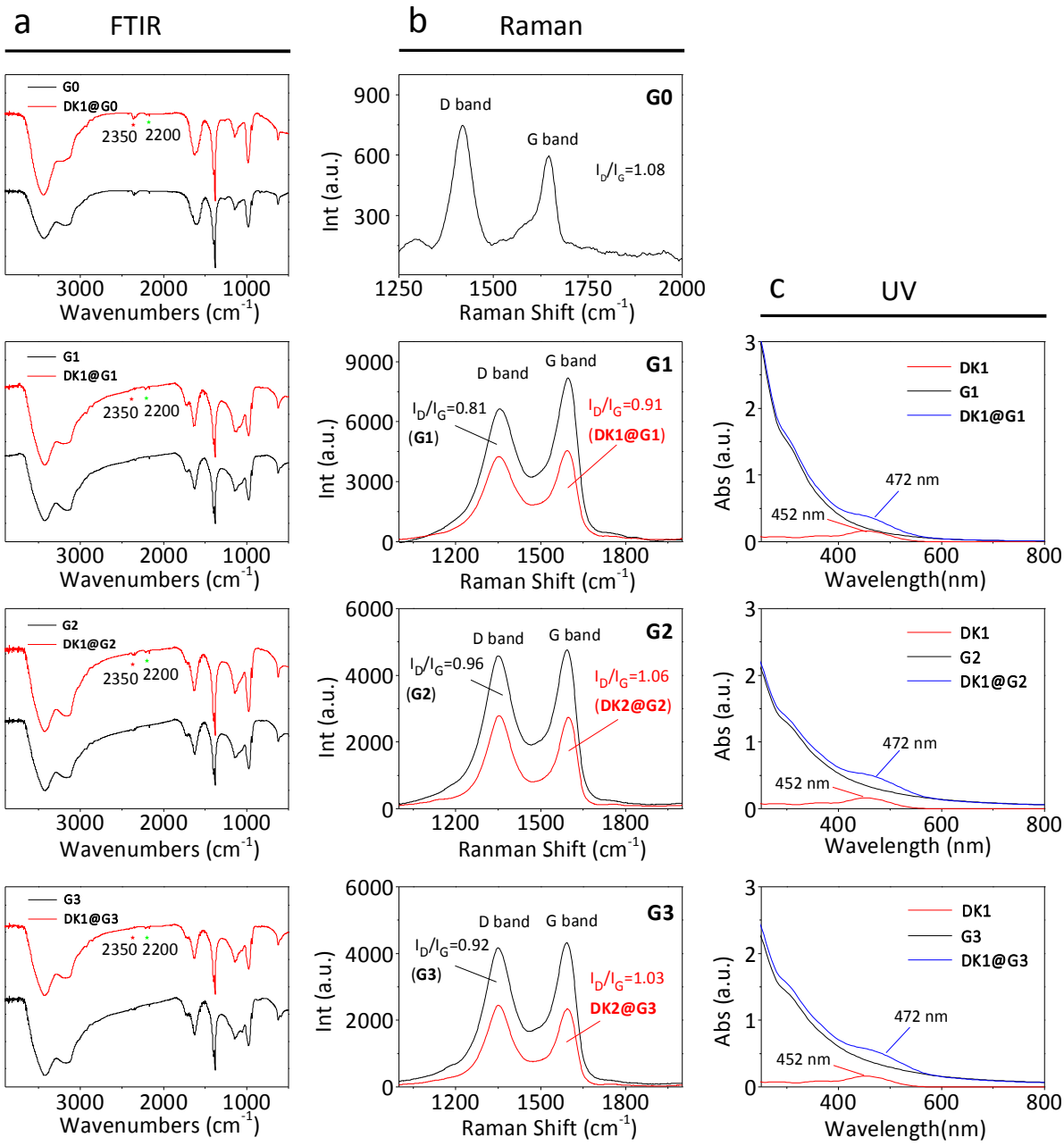


Figure S11 | Spectral characterization of the DK1@GO complex materials. (a) Fourier transmission infrared (FTIR) spectra of GOs in the absence and presence of **DK1** (peaks characteristic of π-stacking are observed at 2350 and 2200 nm for the material complexes). (b) Raman spectra of GOs in the absence and presence of **DK1** (the increased I_D/I_G ratios of the material composite suggest the stacking of the compound to GO). (c) UV spectra of **DK1**, GOs and **DK1**@GOs (red shifts characteristic of π-stacking are observed for the material composites).

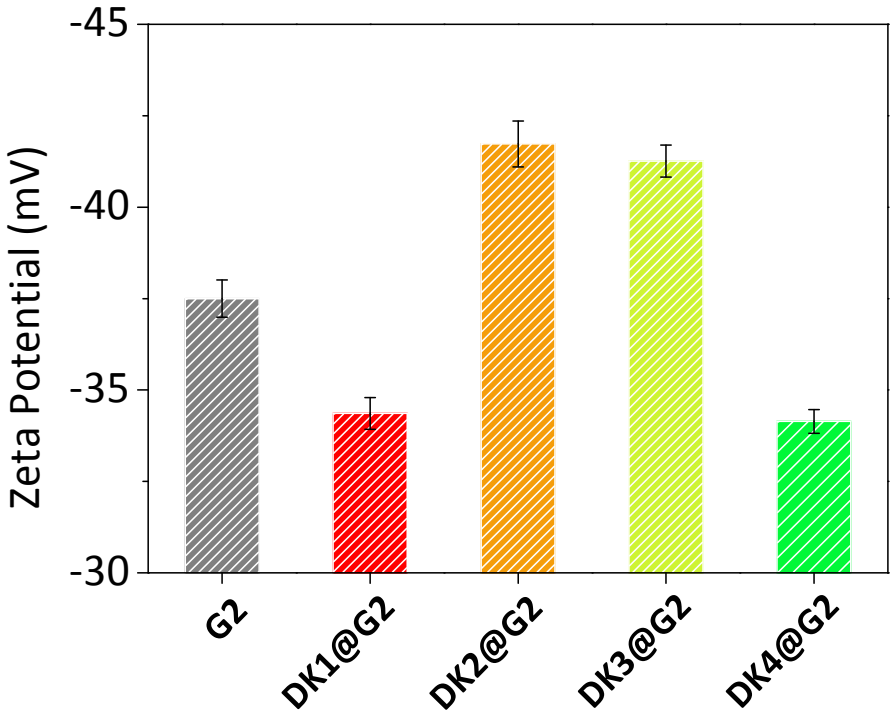


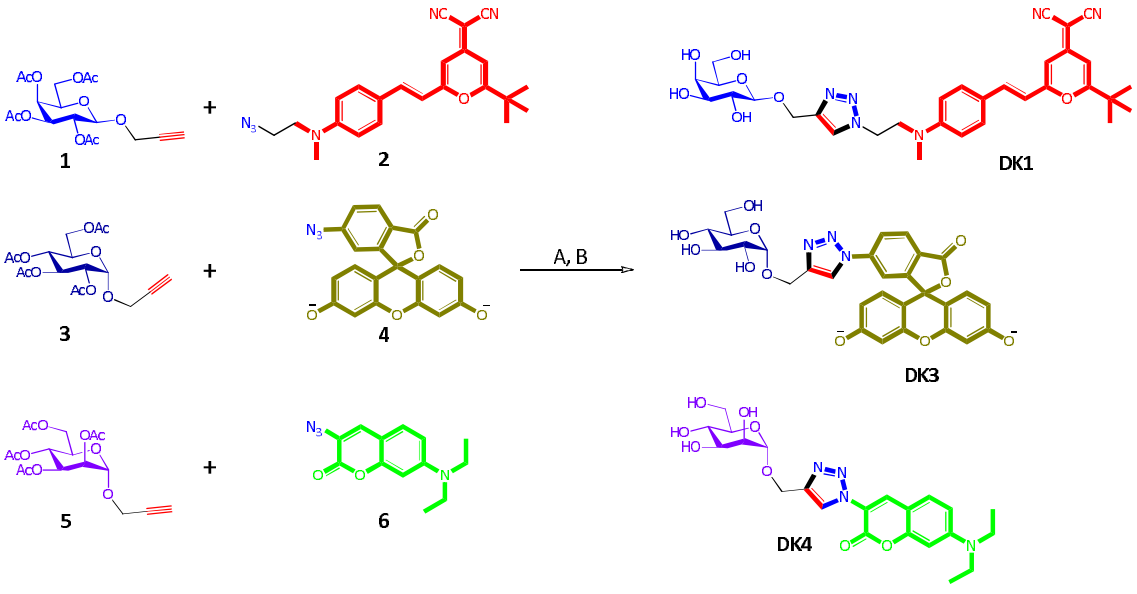
Figure S12 | Zeta potentials of G2 and G2 in composition with DK1-DK4.

S2. Additional experimental Section

General

^{1}H and ^{13}CNMR spectra were obtained with a Bruker Model AM 400 spectrometer (relative to tetramethylsilane (TMS)). High-resolution mass spectra were recorded using a Waters LCT Premier XE spectrometer. UV-vis spectra were performed on a Varian Cary 500 spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. Proteins were purchased from Sigma-Aldrich. The zeta (ζ) potentials of aqueous dispersions were measured by the use of a zetasizer nanosystem (Malvern Instruments). The X-ray photoelectron energy spectra (XPS) were performed using a ULVAC-PHI XPS spectrometer(Thermo Electron). Graphene oxide (2 mg/mL) was purchased from Xianfeng Nanotechnologies Co., Ltd (Nanjing, China). All chemicals used are of analytical grade. Ultrapure water was obtained from a Milli-Q integral Pure/Ultrapure Water Production unit.

Alkynyl O-glycosides (galactoside **1**[1], glucoside **3**[2] and mannoside **5**[3.4]) and azido fluorescence dyes (DCM **2**[5], fluorescein **4**[6] and coumarin **6**[7]) were prepared according to previous literature protocols. **DK2** has been prepared in a previous study[8].



Scheme S1 | **Synthesis of DK1, DK3 and DK4.** Conditions and Reagents: (A) $CuSO_4 \cdot 5H_2O$ and VcNa in CH_2Cl_2/H_2O (10/1, v/v) for **DK1** and **DK4**, and $[(CH_3CN)_4Cu]PF_6$ in Methanol/H_2O/Triethylamine (8/1/1,v/v/v) for **DK3**. (B) Methanol/H_2O/triethylamine (8/1/1, v/v/v).

S2.1. Synthesis of the glycosyl dyes

General procedure for the click reaction. For DK1 and DK4: To a vigorously stirring solution of sugar alkyne (1 equiv.) and azide (1.2 equiv.) in CH_2Cl_2/H_2O (10:1, v/v) were added cupric sulfate pentahydrate (2 equiv.) and sodium ascorbate (4 equiv.). The reaction was stirred at room temperature for 8 h. Then the mixture was extracted by dichloromethane. The combined organic layer was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration, the filtrate was concentrated in vacuum and used directly for the deacylation.

For DK3: Tetrakis(acetonitrile)copper(I) hexa-fluorophosph (0.5 equiv.) was added to a vigorously stirring solution of sugar alkyne (1 equiv.) and azide (1.2 equiv.) in methanol. The reaction was stirred at room temperature for 8 h. After removal of solvent, the residue was extracted by dichloromethane, washed with brine and then dried over anhydrous $MgSO_4$. After filtration, the filtrate was concentrated in vacuum and used directly for the deacylation.

General procedure for the deacylation. The acetyl intermediates were added to a solution mixture of methanol/H_2O/triethylamine(8/1/1, v/v/v), and the resulting mixture was stirred at rt for 12 h. After removal of solvent under reduced pressure, the crude product was purified by column chromatography on silica gel eluted with dichloromethane/methanol.

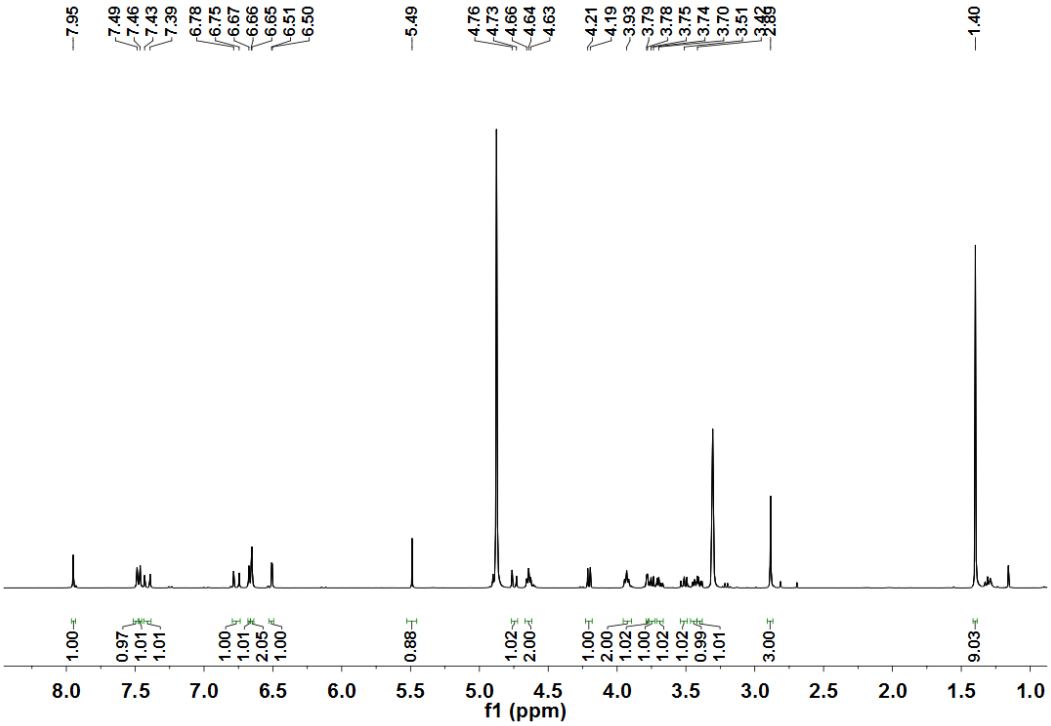
DK1. From **1** (150 mg , 0.39 mmol) and **2** (186 mg , 0.47 mmol), column chromatography (CH_2Cl_2/MeOH, 15/1, v/v) afforded **DK1** (204 mg, 85%) as a red solid. ^{1}H NMR (400 MHz, MeOD) δ 7.95 (s, 1H), 7.49 (s, 1H), 7.46 (s, 1H), 7.41 (d, J = 15.9 Hz, 1H), 6.76 (d, J = 15.9 Hz, 1H), 6.67 (s, 1H), 6.65 (d, J = 2.0 Hz, 2H), 6.51 (d, J = 2.0 Hz, 1H), 5.49 (s, 1H), 4.75 (d, J = 12.7 Hz, 1H), 4.64 (t, J = 5.9 Hz, 2H), 4.20 (d, J = 7.7 Hz, 1H), 3.93 (t, J = 5.8 Hz, 2H), 3.78 (d, J = 2.5 Hz, 1H), 3.77–3.73 (m, 1H), 3.69 (dd, J = 11.4, 5.0 Hz, 1H), 3.51 (dd, J = 9.7, 7.7 Hz, 1H), 3.46–3.43 (m, 1H), 3.43–3.37 (m, 1H), 2.89 (s, 3H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, MeOD) δ 173.9, 162.3, 158.6, 151.8, 146.0, 139.9, 131.1, 126.2, 124.6, 116.7, 116.6, 114.5, 113.1, 106.4, 103.9, 103.1, 76.8, 74.9, 72.5, 70.3, 62.8, 62.6, 57.4, 53.2, 38.6, 37.7, 30.8, 28.4. HR-ESI-MS (m/z): calcd for $C_{32}H_{39}N_6O_7$ 619.2880, found 619.2884. HPLC (t_R = 4.5 min over 15 min of 0.6 mL/min mobile phase (95% acetonitrile and 5% water), purity 96.4%).

DK3. From **3** (150 mg, 0.39 mmol) and **4** (174 mg, 0.47 mmol), column chromatography (CH_2Cl_2/MeOH, 10/1, v/v) afforded **DK3** (163 mg, 71%) as a yellow solid. ^{1}H NMR (400 MHz, D_2O) δ 8.54 (s, 1H), 8.16 (s, 1H), 7.78 (d, J = 8.1 Hz, 1H), 7.22 (d, J = 8.4 Hz, 1H), 7.04 (d, J = 8.8 Hz, 2H), 6.55 (d, J = 9.0 Hz, 2H), 6.50 (s, 2H), 5.02 (d, J = 3.3 Hz, 1H), 4.81 (dd, J = 31.1, 12.9 Hz, 2H), 3.75–3.66 (m, 3H), 3.65–3.59 (m, 1H), 3.53 (dd, J = 9.7, 3.2 Hz, 1H), 3.37 (t, J = 9.3 Hz, 1H); 13C NMR (100 MHz, DMSO-d_6, D_2O) δ 168.2, 162.7, 152.9, 145.2, 137.5, 130.7, 129.5, 126.8, 126.0, 122.8, 117.0, 114.6, 110.0, 102.4, 97.9, 72.8, 72.4, 71.4, 69.8, 60.6, 59.7, 45.8, 8.3. HR-ESI-MS (m/z): calcd for $C_{29}H_{26}N_3O_{11}$ 592.1567, found 592.1569. HPLC (t_R = 4.39 min over 15 min of 0.6 mL/min mobile phase (50% Methanol and 50% water), purity 97.6%).

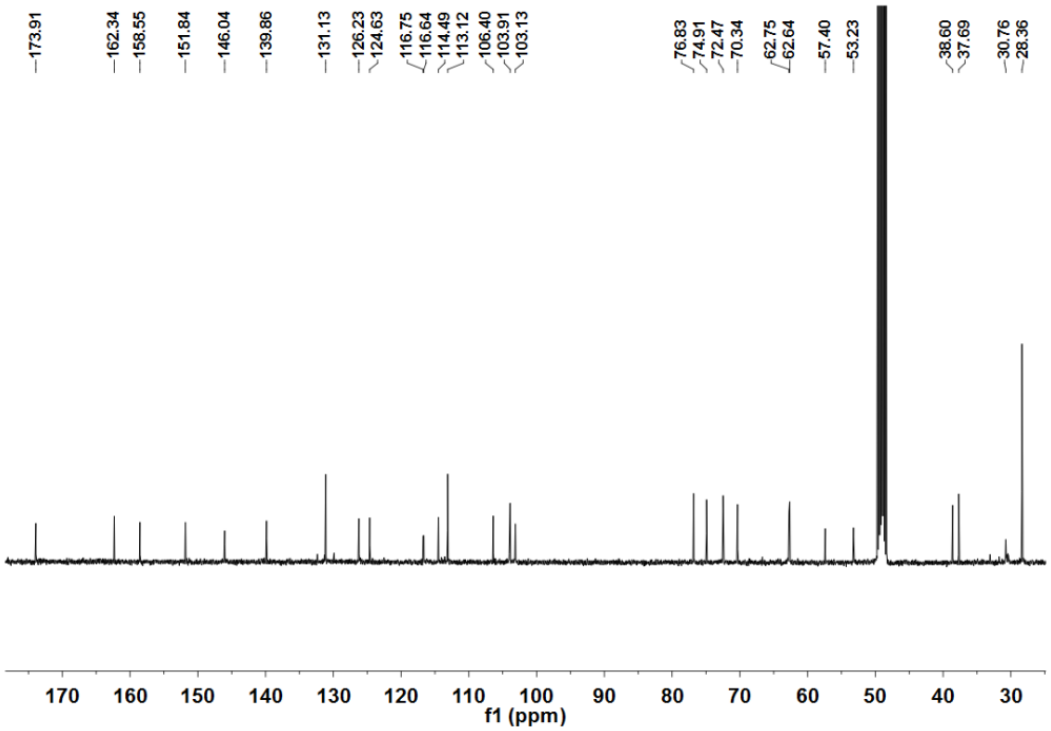
DK4. From **5** (150 mg , 0.39 mmol) and **6** (121 mg, 0.47 mmol), column chromatography (CH_2Cl_2/MeOH, 15/1, v/v) afforded **DK4** (139 mg, 75%) as an aquamarine solid. ^{1}H NMR (400 MHz, DMSO-d_6, D_2O) δ 8.50 (s, 1H), 8.37 (s, 1H), 7.62 (d, J = 9.0 Hz, 1H), 6.82 (dd, J = 9.0, 2.0 Hz, 1H), 6.63 (d, J = 1.7 Hz, 1H), 4.82 (s, 1H), 4.79 (d, J = 12.3 Hz, 1H), 4.64 (d, J = 12.3 Hz, 1H), 3.73 (d, J = 11.5 Hz, 1H), 3.66 (d, J = 2.9 Hz, 1H), 3.58–3.42 (m, 8H), 1.16 (t, J = 7.0 Hz, 6H); ^{13}C NMR (100MHz, DMSO-d_6, D_2O) δ 157.4, 156.1, 152.0, 143.9, 137.6, 131.1, 125.6, 116.3, 110.5, 106.7, 99.4, 96.6, 74.3, 71.1, 70.5, 67.2, 61.5, 59.3, 44.7, 12.6. HR-ESI-MS (m/z): calcd for $C_{22}H_{29}N_4O_8$ 477.1985, found 477.1939. HPLC (t_R= 4.29 min over 15 min of 0.6 mL/min mobile phase (50% Methanol and 50% water), purity >99%).

S3. Original NMR spectra of DK1

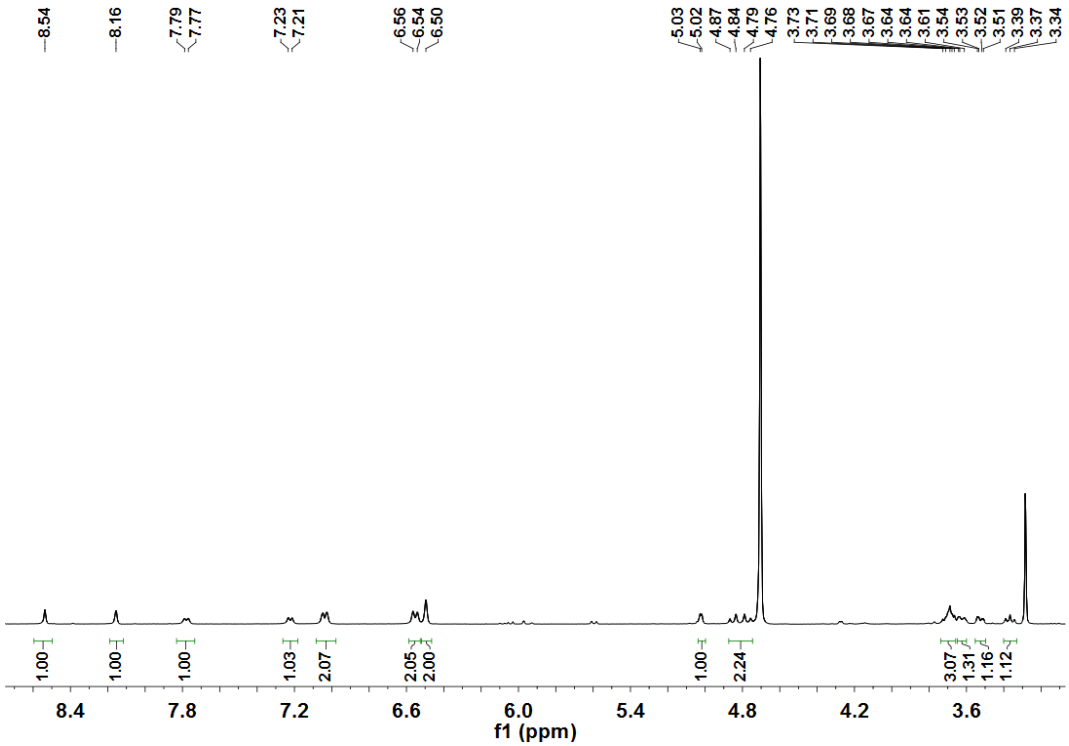
^{1}H NMR of **DK1**



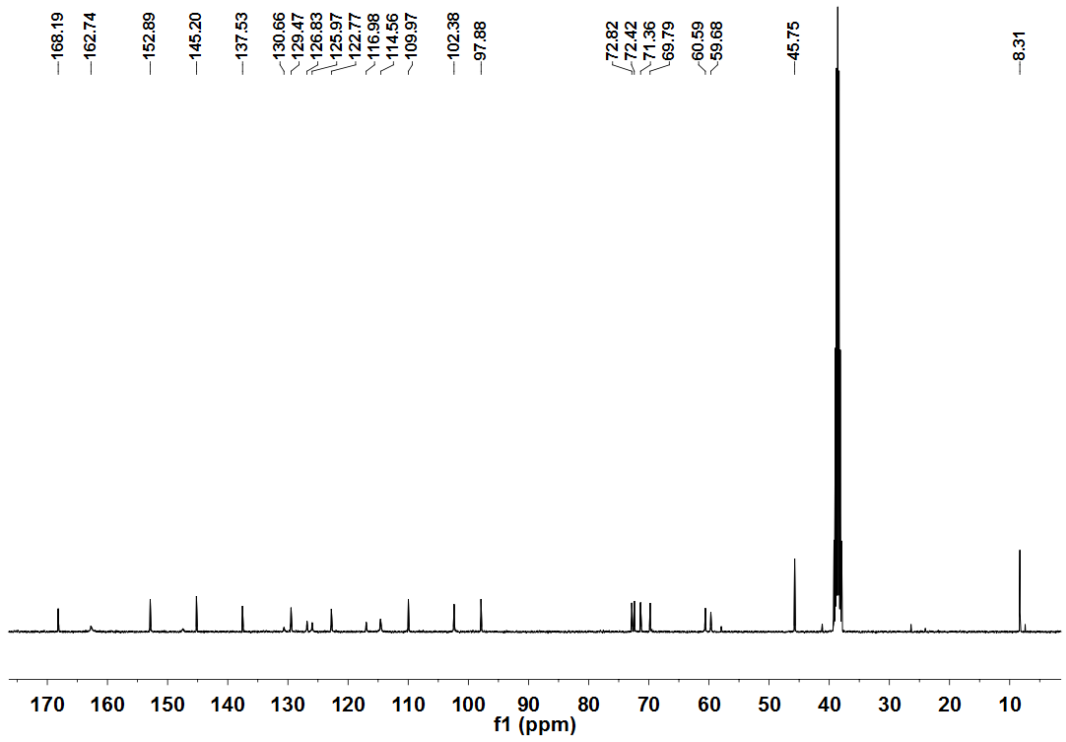
^{13}C NMR of **DK1**



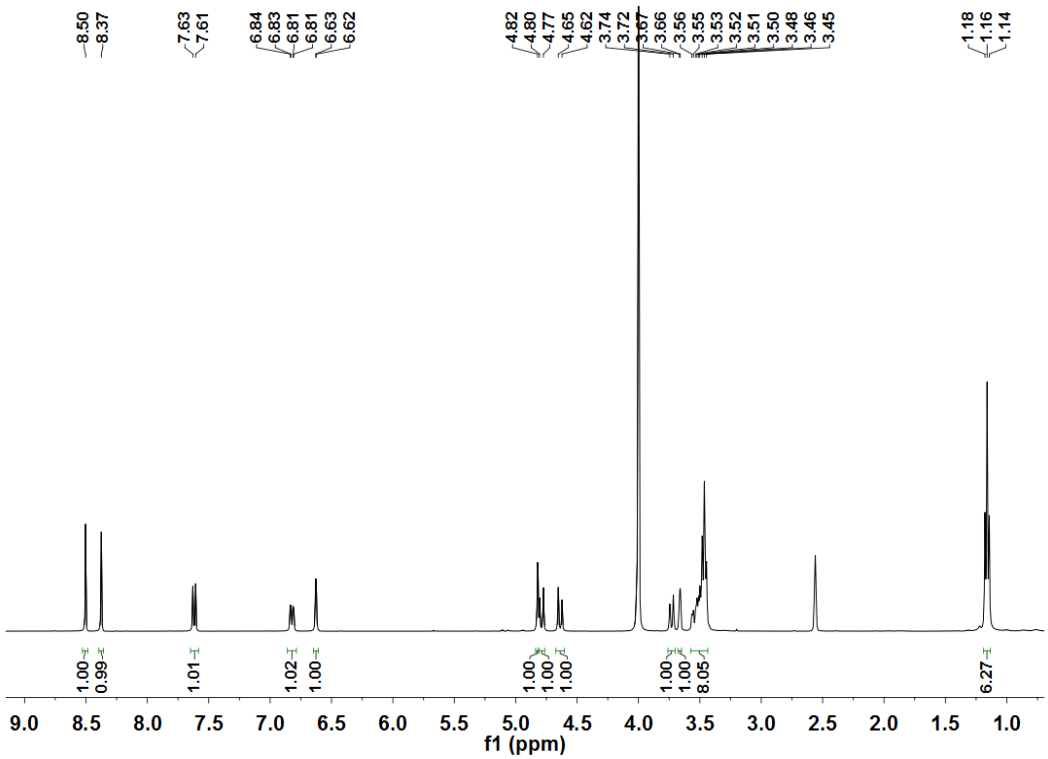
^{1}H NMR of **DK3**



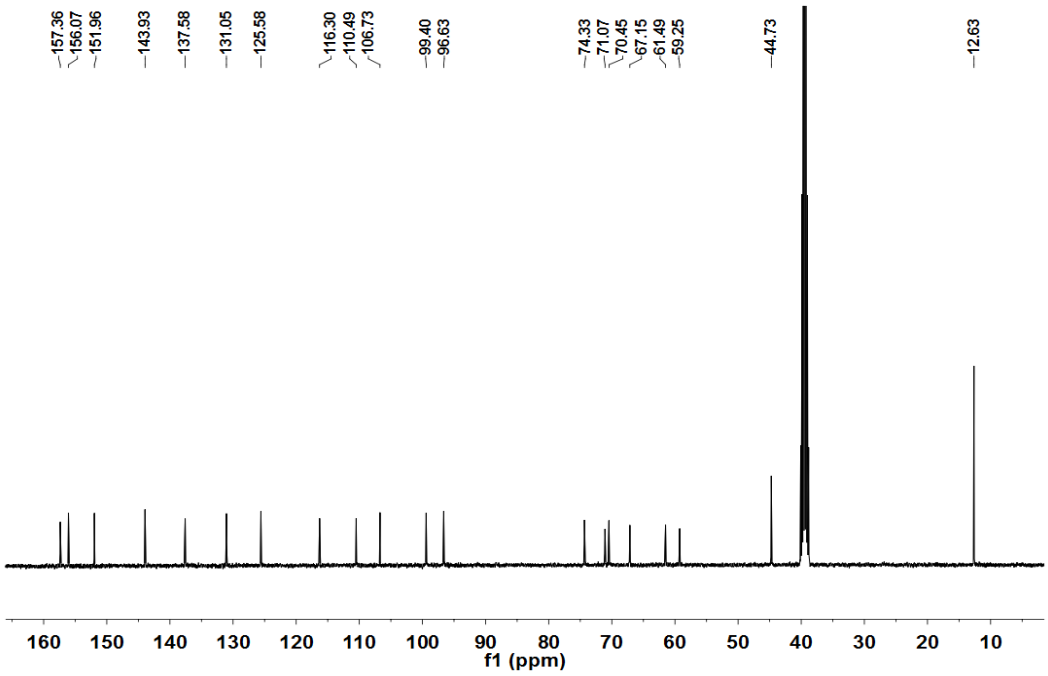
^{13}C NMR of **DK3**



^{1}H NMR of **DK4**



^{13}C NMR of **DK4**



S4. References

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