Supplementary Information

for

Potassium Ion-mediated Synthesis of Highly Water-soluble Dendritically Functionalized Melanin

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General Information

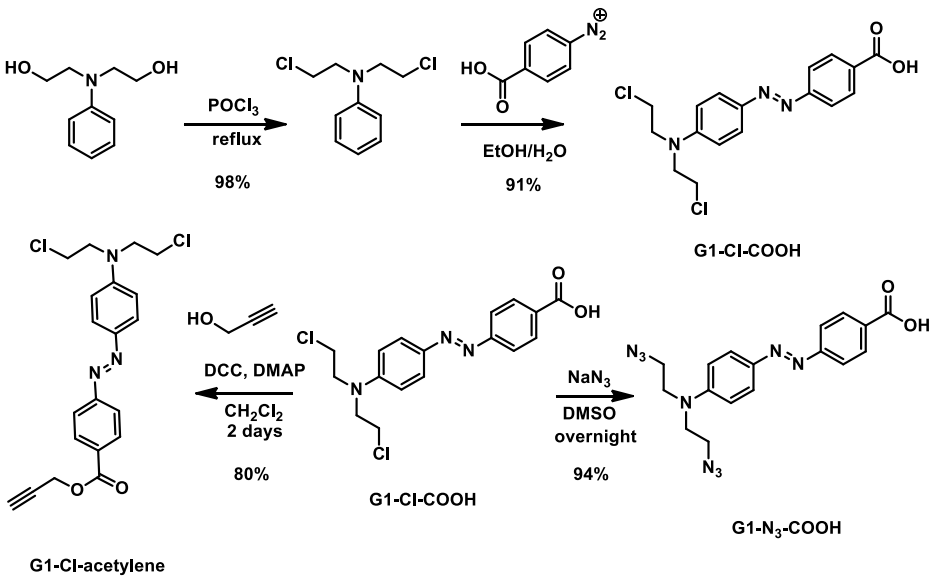
Melanin (from Sepia officinalis, M2649) was purchased from Aldrich and used without further purification. According to the X-Ray photoelectron spectroscopy (XPS) spectrum, only eumelanin is existed in bare melanin as the sulfur (S) signal is absent (Figure S17). Ethylene Glycol (99+%) was purchased from IL and used without further purification. Other chemicals were purchased from Aldrich and used without further purification. Reactions were performed under nitrogen unless otherwise stated. Deionized (DI) H_2O was obtained from Barnstead RO pure system. Chromatography purifications were performed on silica gel (SiO_2) with the indicated eluents. All other solvents and reagents were of reagent grade and used as received without purification. ^1H and ^{13}C NMR spectra for structural characterization were recorded with Bruker Avance 400 (^1H: 400 MHz; ^{13}C: 101 MHz) spectrometer at 297 K. All NMR samples were prepared in $CDCl_3$ unless otherwise stated. Spectra were calibrated internally ^1H: δ = 7.26 (solvent residual peak of $CDCl_3$); ^{13}C: δ = 77.16 ppm. Electrospray ionization (ESI) mass spectra were measured on a Bruker SolariX 9.4 T mass spectrometer using CH_3OH/CH_2Cl_2 (1:1) as solvent. Solubilities of dendritically functionalized melanins were determined by separately placing 10 mg of them into small vials (5 mL). Deionized water (100 μL) was added to the vial. The vial was shaked and then centrifuged for 1 min at 3000 rpm. This procedure (water addition and centrifugation) was repeated until all dendritically functionalized melanins were completely dissolved. The solubility of compounds was first inspected by naked eyes followed by analysis using UV/visible absorption spectrophotometer (Agilent Cary 100 UV-Visible spectrophotometer, 430 nm).

TOF-SIMS analyses were performed with an ULVAC-PHI Trift III System equipped with a 15 keV Ga^+ source. During analysis, the sample surface was bombarded with Ga^+ ions, and electrons from an electron gun were applied simultaneously onto the sample surface to avoid surface charging. All sputtered ion fragments were then collected and analyzed by a time-of-flight ion detector.

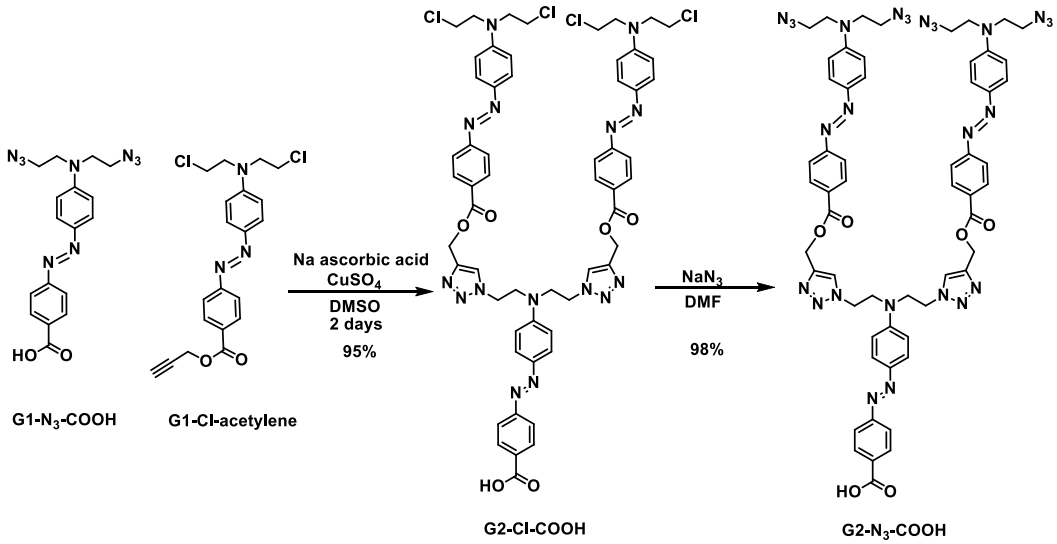
X-Ray photoelectron spectroscopy (XPS) was performed using a Sengyang SKL-12 spectrometer equipped with a VG CLAM 4 MCD electron energy analyzer and twin anode Mg Kα radiation (1253.6 eV) or Al Kα radiation (1496.3 eV) X-ray sources. Spectra were calibrated internally using carbon 1s peak (284.6 eV).

The photo-electrochemical measurements were performed by using a CHI 660D electrochemical workstation. The standard three-electrode cell with a Ag/AgCl electrode in saturated potassium chloride, a platinum foil (1.0 x 1.0 cm^{-2}) as a counter electrode, and TiO_2 nanotube arrays as working electrodes, was used. The electrolyte was 0.1 M Na_2SO_4. A 300 W xenon arc lamp was used as a radiation source and the average light intensity was about 100 mW cm^{-2}. The photocurrent responses under

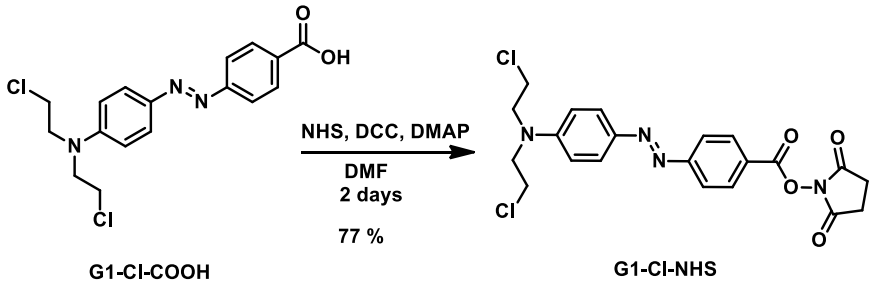
illumination of simulated sunlight (AM 1.5G) and visible light (400 nm cut-off filter) were analyzed.



Scheme S1. Preparation of azide (G1-N_3-COOH) and acetylene (G1-Cl-acetylene) precursors.



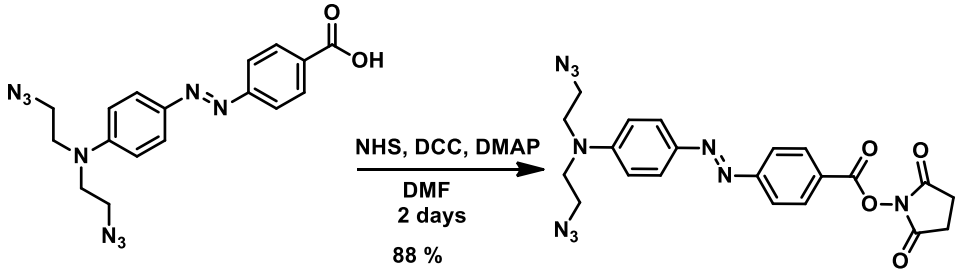
Scheme S2. Preparation of azide (G2-N_3-COOH) precursor.



G1-Cl-COOH G1-Cl-NHS

G1-Cl-NHS

G1-Cl-COOH (2.00 g, 5.48 mmol), *N,N′*-dicyclohexylcarbodiimide (4.85 g 23.5 mmol) and *N*-hydroxysuccinimide (1.3 g, 11.3 mmol) were dissolved in anhydrous CH_2Cl_2 (30 mL). Catalytic amount of 4-dimethylaminopyridine was added to the reaction mixture. The reaction was carried out for 2 d to achieve higher reaction yield and monitored by TLC. The insoluble solid was removed by filtration and the filtrate was dried by anhydrous $MgSO_4$. The mixture was filtrated and then concentrated in vacuo. The residue was purified by column chromatography with CH_2Cl_2 as the eluent. The product was an orange solid (1.95 g, 77%). ^{1}H NMR ($CDCl_3$): 2.93 (s, 4H, $COCH_2$), 3.71 (t, J = 7.0 Hz, 4H, NCH_2), 3.87 (t, J = 6.9 Hz, 4H, $ClCH_2$), 6.79 (d, J = 9.1 Hz, 2H, ArH), 7.93–7.97 (m, 4H, ArH), 8.25 (d, J = 8.6 Hz, 2H, ArH). ^{13}C NMR ($CDCl_3$): 25.9, 40.4, 53.6, 111.8, 122.7, 125.4, 126.2, 131.8, 144.8, 149.6, 156.9, 161.7, 169.4. HRMS (ESI): $C_{21}H_{21}Cl_2N_4O_4$ $[M+H]^+$ calcd: 463.09344, found: 463.09489 (error/ppm: 3.1).

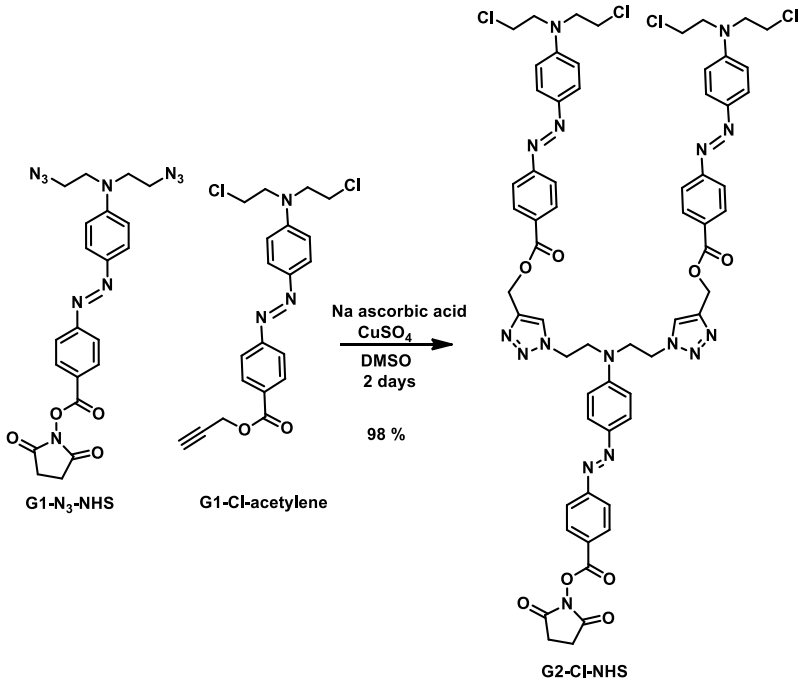


G1-N_3-COOH G1-N_3-NHS

G1-N_3-NHS

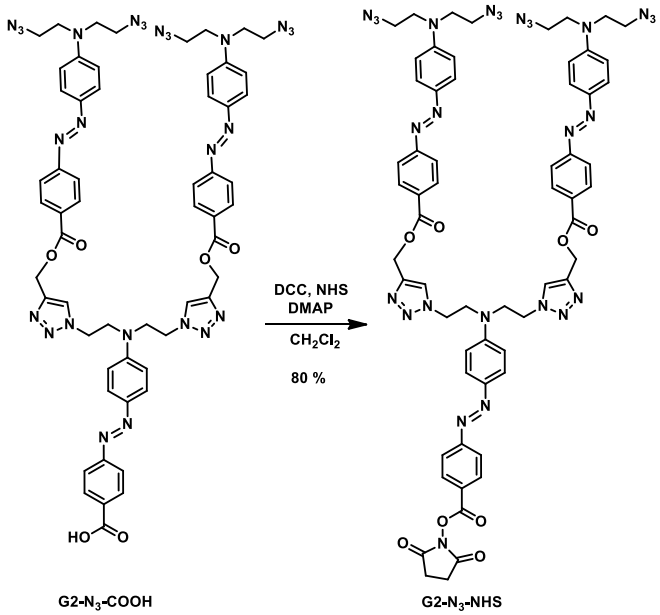
G1-N_3-COOH (3.23 g, 8.54 mmol), *N,N′*-dicyclohexylcarbodiimide (3.21 g, 15.6 mmol) and *N*-hydroxysuccinimide (2.00 g, 17.3 mmol) were dissolved in anhydrous CH_2Cl_2 (30 mL). Catalytic amount of 4-dimethylaminopyridine was added to the reaction mixture. The reaction was carried out for 2 d to achieve higher reaction yield and monitored by TLC. The insoluble solid was removed by filtration and the filtrate was dried by anhydrous $MgSO_4$. The mixture was filtrated and then concentrated in vacuo. The residue was purified by column chromatography with CH_2Cl_2 as the eluent. The product was an orange solid (3.60 g, 88%). ^{1}H NMR ($CDCl_3$): 2.92 (s, 4H, $COCH_2$), 3.57 (t, J = 6.2 Hz, 4H, N_3CH_2), 3.69 (t, J = 5.8 Hz, 4H, NCH_2), 6.80 (d, J =

9.2 Hz, 2H, ArH), 7.93 (d, J = 8.6 Hz, 4H, ArH), 8.24 (d, J = 8.6 Hz, 2H, ArH). ^{13}C NMR ($CDCl_3$): 25.8, 48.9, 50.9, 112.0, 122.7, 125.2, 126.2. 131.8. 144.6, 149.8, 156.9, 161.7, 169.4. HRMS (ESI): $C_{21}H_{21}N_{10}O_4$ $[M+H]^+$ calcd: 477.17418, found: 477.17468 (error/ppm: 1.1).



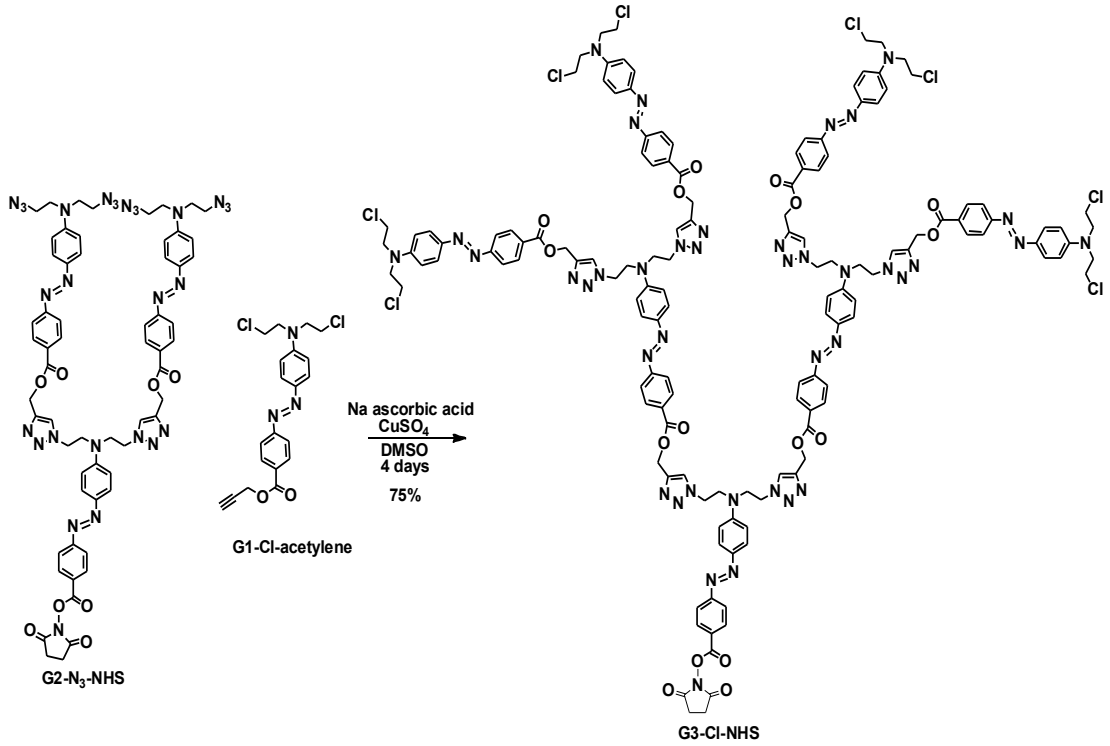
G2-Cl-NHS

G1-N_3-NHS (0.77 g, 1.6 mmol) and G1-Cl-acetylene (1.6 g, 4.0 mmol, 2.5 eqv.) were dissolved in DMSO (15 mL). Sodium ascorbic acid (0.17 g) and $CuSO_4 \cdot 5H_2O$ (0.09 g) was added to the reaction mixture. The reaction was carried out for 2 d and monitored by TLC. Shorter reaction will lead to incomplete reaction and a mixture of di- and mono- "clicked" product will be obtained. The reaction mixture was transferred into water and an orange solid was precipitated. The crude product was collected by filtration. Then, it was purified by column chromatography with $CHCl_3$, then gradient to ethyl acetate as the eluents. The product was an orange solid (2.0 g, 98%). ^{1}H NMR ($CDCl_3$): 2.93 (s, 4H, $COCH_2$), 3.69–3.77 (m, 12H, NCH_2CH_2), 3.85 (t, J = 6.8 Hz, 8H, $ClCH_2$) 4.46 (s, 4H, $COOCH_2$), 5.43 (s, 4H, NCH_2), 6.57 (d, J = 9.1 Hz, 1H, ArH), 6.66 (d, J = 8.8 Hz, 1H, ArH), 6.77 (d, J = 9.2 Hz, 4H, ArH), 7.54 (s, 2H, CH=C), 7.64–7.89 (m, 12H, ArH), 8.07 (d, J = 8.6 Hz, 4H, ArH), 8.16 (d, J = 8.4 Hz, 2H, ArH). ^{13}C NMR ($CDCl_3$): 25.8, 31.0, 40.3, 47.4, 53.5, 58.2, 111.7, 112.0, 122.3, 122.8, 125.4, 125.9, 130.0, 130.7, 131.7, 143.4, 144.6, 149.3, 155.9, 166.0, 169.4. HRMS (ESI): $C_{61}H_{59}Cl_4N_{16}O_8$ $[M+H]^+$ calcd: 1285.34363, found: 1285.34495 (error/ppm: 1.0).



G2-N_3-NHS

G1-N_3-COOH (1.45 g, 1.26 mmol), *N,N'*-dicyclohexylcarbodiimide (0.51 g, 2.48 mmol) and *N*-hydroxysuccinimide (0.27 g, 2.35 mmol) were dissolved in anhydrous CH_2Cl_2 (30 mL). Catalytic amount of 4-dimethylaminopyridine was added to the reaction mixture. The reaction was carried out for 2 d to achieve higher reaction yield and monitored by TLC. The insoluble solid was removed by filtration and the filtrate was dried with anhydrous $MgSO_4$. The mixture was filtrated and then concentrated in vacuo. The residue was purified by column chromatography with $CHCl_3$ as the eluent. The product was an orange solid (1.32 g, 80%). ^{1}H NMR ($CDCl_3$): 2.93 (s, 4H, $COCH_2$), 3.57 (t, J = 5.6 Hz, 8H, N_3CH_2), 3.67–3.74 (m, 12H, NCH_2CH_2), 4.45 (s, 4H, $COOCH_2$), 5.43 (s, 4H, NCH_2), 6.59 (d, J = 8.3 Hz, 2H, ArH), 6.79 (d, J = 8.5 Hz, 4H, ArH), 7.55 (s, 2H, CH=C), 7.77–7.90 (m, 12H, ArH), 8.07 (d, J = 8.1 Hz, 4H, ArH), 8.17 (d, J = 7.9 Hz, 2H, ArH). ^{13}C NMR ($CDCl_3$): 25.8, 47.3, 49.8, 50.8, 51.4, 58.1, 111.9, 122.3, 122.8, 125.3, 125.9, 126.1, 129.9, 130.7, 131.6, 143.4, 144.5, 144.9, 149.5, 155.8, 156.5, 161.6, 166.0, 169.4. HRMS (ESI): $C_{61}H_{59}N_{28}O_8$ $[M+H]^+$ calcd: 1311.50652, found: 1311.50840 (error/ppm: 1.4).



G3-Cl-NHS

G2-N_3-NHS (0.31 g, 0.24 mmol) and G1-Cl-acetylene (0.39 g, 0.97 mmol, 4.1 eqv.), were dissolved in DMSO (15 mL). Sodium ascorbic acid (0.17 g) and $CuSO_4 \cdot 5H_2O$ (0.09 g) was added to the reaction mixture. The reaction was carried out for 4 d and monitored by TLC. Shorter reaction will lead to incomplete reaction and a mixture of "clicked" product will be obtained. The reaction mixture was transferred into water and orange solid was precipitated. The crude product was collected by filtration. Then, it was purified by column chromatography with $CHCl_3$, then gradient to ethyl acetate as the eluents. The product was an orange solid (0.51 g, 75%). ^{1}H NMR ($CDCl_3$): 2.88 (s, 4H, $COCH_2$), 3.66–3.73 (m, 28H, NCH_2), 3.80–3.84 (m, 16H, $ClCH_2CH_2$), 4.43 (s, 12H, $COOCH_2$), 5.41 (s, 12H, NCH_2), 6.51 (d, J = 8.8 Hz, 2H, ArH), 6.63 (d, J = 8.7 Hz, 4H, ArH), 6.74 (d, J = 9.2 Hz, 8H, ArH), 7.57 (s, 6H, CH=C), 7.68–7.87 (m, 28H, ArH), 8.10 (d, J = 8.4 Hz, 14H, ArH). ^{13}C NMR ($CDCl_3$): 40.4, 47.5, 51.6, 53.6, 58.2, 111.8, 112.2, 122.4, 122.5, 125.4, 126.0, 130.1, 130.8, 144.7, 149.4, 155.9, 166.1. HRMS (ESI): $C_{141}H_{136}Cl_8N_{40}O_{16}$ $[M+2H]^{2+}$ calcd: 1464.92769, found: 1464.93071 (error/ppm: 2.1).

G1-Cl-NHS-melanin (by Et_3N)

G1-Cl-NHS (90 mg), melanin (31 mg) and Et_3N (0.5 mL) were dissolved in propylene carbonate (5 mL). The reaction mixture was heated to 60 °C with shaking for 2 weeks. Afterwards, the reaction mixture was centrifuged at 6000 rpm for 10 minutes. The supernatant was removed. The crude product was washed by acetone (or ethyl acetate) and collected by centrifugation. The product was dried under vacuum.

G2-Cl-NHS-melanin (by Et_3N)

The G2-Cl-NHS (110 mg), melanin (31 mg) and Et_3N (0.5 mL) were dissolved in propylene carbonate (5 mL). The reaction mixture was heated to 60 °C with shaking for 2 weeks. Afterwards, the reaction mixture was centrifuged at 6000 rpm for 10 minutes. The supernatant was removed. The crude product was washed by acetone (or ethyl acetate) and collected by centrifugation. The product was dried under vacuum.

G3-Cl-NHS-melanin (by Et_3N)

G3-Cl-NHS (150 mg), melanin (31 mg) and Et_3N (0.5 mL) were dissolved in propylene carbonate (5 mL). The reaction mixture was heated to 60 °C with shaking for 2 weeks. Afterwards, the reaction mixture was centrifuged at 6000 rpm for 10 minutes. The supernatant was removed. The crude product was washed by acetone (or ethyl acetate) and collected by centrifugation. The product was dried under vacuum.

G1-Cl-NHS-melanin (by K_2CO_3)

G1-Cl-NHS (83 mg), melanin (31 mg) and K_2CO_3 (0.2 g) were dissolved in propylene carbonate (5 mL). The reaction mixture was heated to 60 °C with shaking for 2 weeks. Afterwards, the reaction mixture was centrifuged at 6000 rpm for 10 minutes. The supernatant was removed. The crude product was repeatedly washed by acetone (or ethyl acetate) and collected by centrifugation until unreacted dendrons are completely removed. Then the crude product was dissolved in DI water to remove K_2CO_3 and the product was precipitated by adding EtOH and collected by centrifugation. The product was dried under vacuum.

G2-Cl-NHS-melanin (by K_2CO_3)

G2-Cl-NHS (110 mg), melanin (31 mg) and K_2CO_3 (0.2 g) were dissolved in propylene carbonate (5 mL). The reaction mixture was heated to 60 °C with shaking for 2 weeks. Afterwards, the reaction mixture was centrifuged at 6000 rpm for 10 minutes. The supernatant was removed. The crude product was repeatedly washed by acetone (or ethyl acetate) and collected by centrifugation until unreacted dendrons are completely removed. Then the crude product was dissolved in DI water to remove K_2CO_3 and the product was precipitated by adding EtOH and collected by centrifugation. The product was dried under vacuum.

G3-Cl-NHS-melanin (by K_2CO_3)

G3-Cl-NHS (145 mg), melanin (31 mg) and K_2CO_3 (0.2 g) were dissolved in propylene carbonate (5 mL). The reaction mixture was heated to 60 °C with shaking for 2 weeks. Afterwards, the reaction mixture was centrifuged at 6000 rpm for 10 minutes. The supernatant was removed. The crude product was repeatedly washed by acetone (or ethyl acetate) and collected by centrifugation until unreacted dendrons are completely removed. Then the crude product was dissolved in DI water to remove K_2CO_3 and the product was precipitated by adding EtOH and collected by centrifugation. The product was dried under vacuum.

Synthesis of TiO_2 nanotube arrays (TNAs)

TiO_2 nanotube arrays were fabricated by an anodic oxidation approach. Ethylene glycol (99+ %) with additions of 0.5 wt % of NH_4F and 2.5 wt % of DI water was used as electrolyte. Ti foil (2 cm x 3 cm) was used as a working electrode, and a platinum (1 cm x 1 cm) foil served as a counter electrode. Prior to anodization, Ti foils were washed with ethanol, acetone by sonication to remove the contamination, subsequently rinsed with DI water and dried in air. At room temperature, anodization is carried out by immersing a Ti foil in as-prepared electrolyte for 3 h at 60 V. Afterwards, the sample was removed from the electrochemical cell and washed with DI water. A subsequent heating at 400 °C for 1 h with a temperature increasing rate of 1 °C min^{-1} in air was applied to improve crystallization. The sample was cut into half for further use.

Fabrication of dendritically functionalized melanin onto TiO_2 nanotube arrays

Dip-casting: 10 mg of dendritically functionalized melanin (Et_3N treated) was dissolved in 10% ammonia solution. The TiO_2 nanotube arrays were immersed into the solution for overnight. Afterwards, the samples were collected and washed with DI water several times. Finally, the samples are dried in air.

EDC coupling: TiO_2 nanotube arrays were immersed into 2 wt% 3-aminopropyltrimethoxysilane (APTMS) in toluene for 30 min under sonication. Then, the amine-modified TiO_2 nanotube arrays were washed with EtOH and water. 2 mg of dendritically functionalized melanin (K_2CO_3 treated), 25 mg of ethyl(dimethylaminopropyl) carbodiimide (EDC), 10 mg *N*–hydroxysuccinimide (NHS) were dissolved in DI water. The amine-modified TiO_2 nanotube arrays were immersed into the reaction mixture for 30 min under sonication. The samples were collected and washed with DI water. The samples were dried in air.

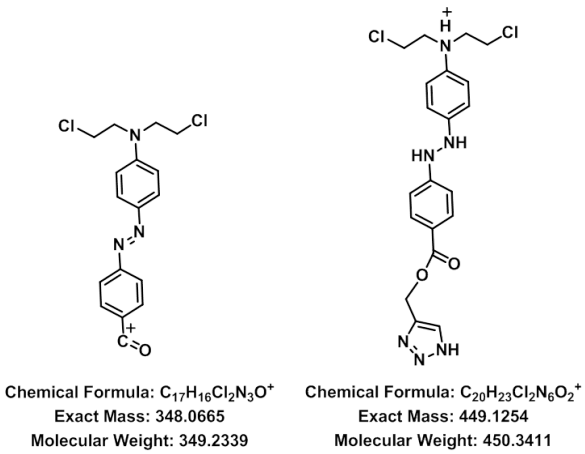


Figure S1. Proposed structure of azobenzene dendron at m/z 348 and 449.

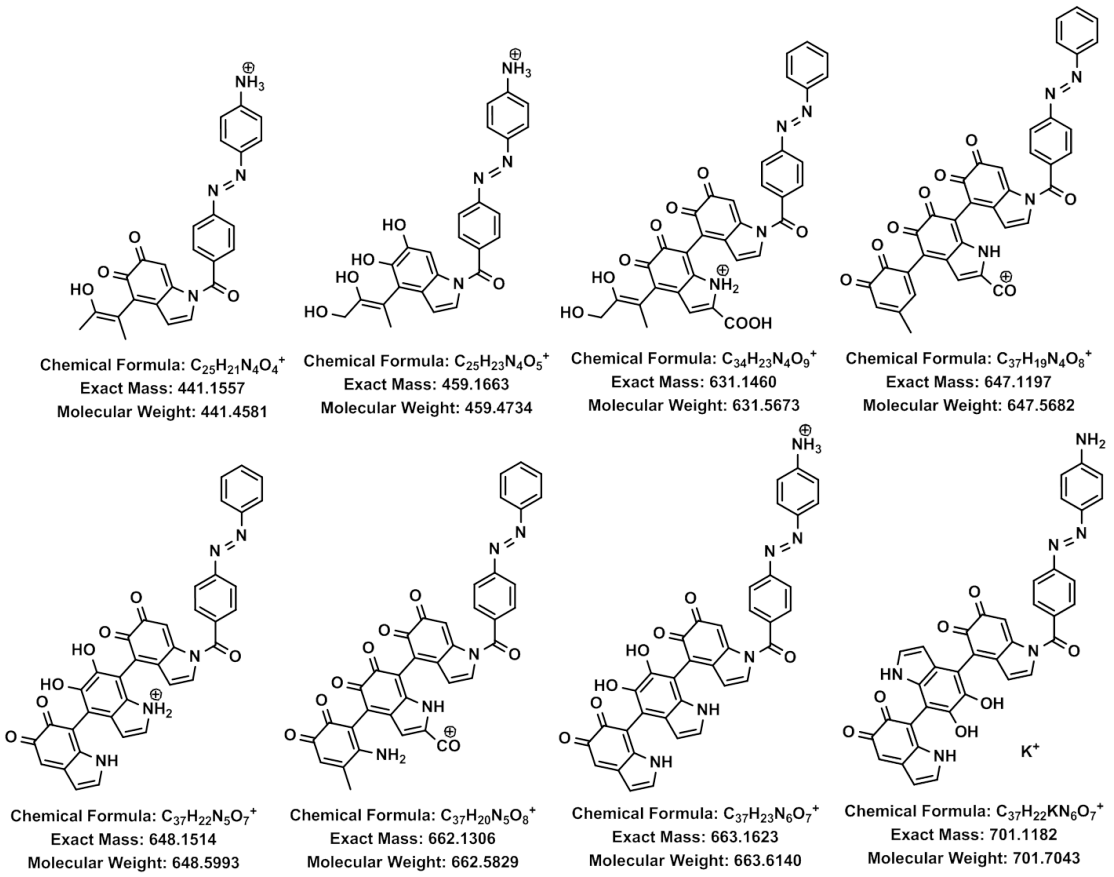


Figure S2. Proposed structure of dendritically functionalized melanin at m/z 441, 459, 631, 647, 648, 662, 663 and 701.

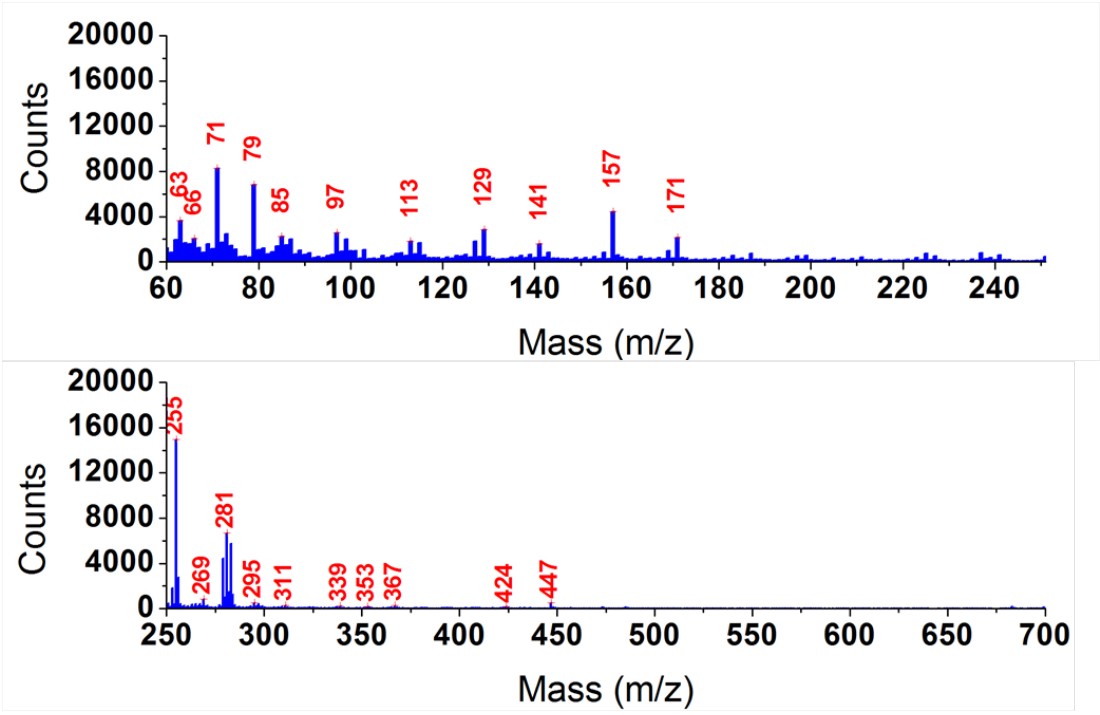


Figure S3. TOF-SIMS spectrum of melanin treated with potassium carbonate in propylene carbonate (control experiment).

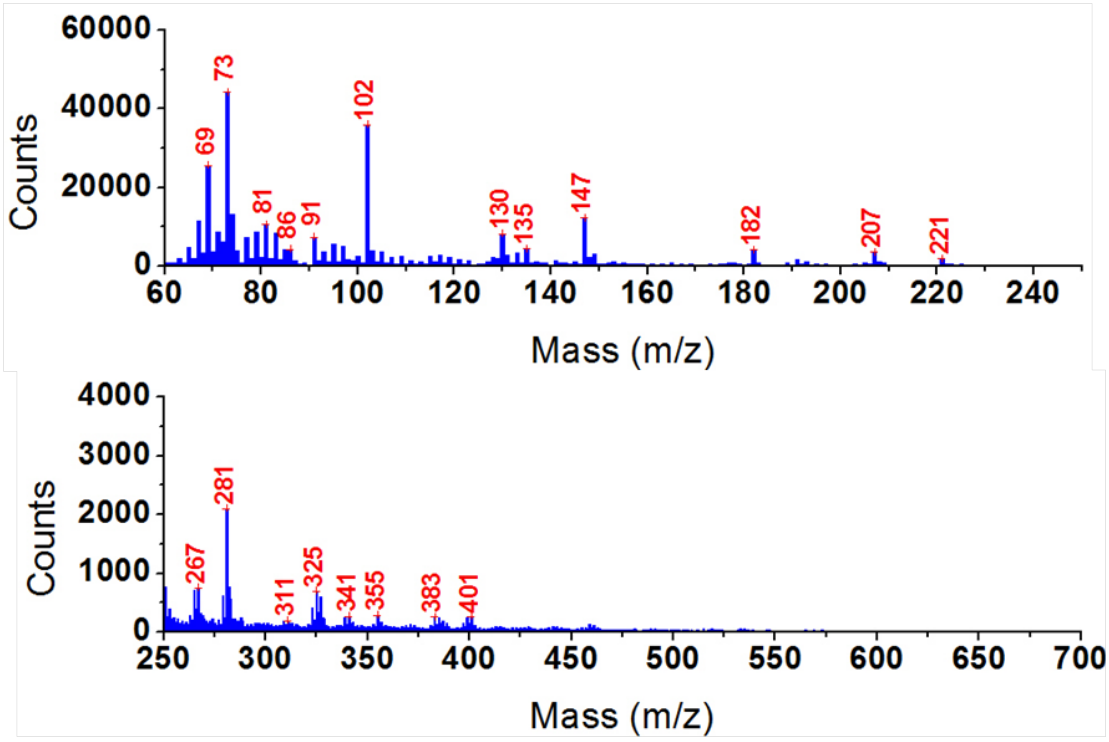


Figure S4. TOF-SIMS spectrum of melanin.

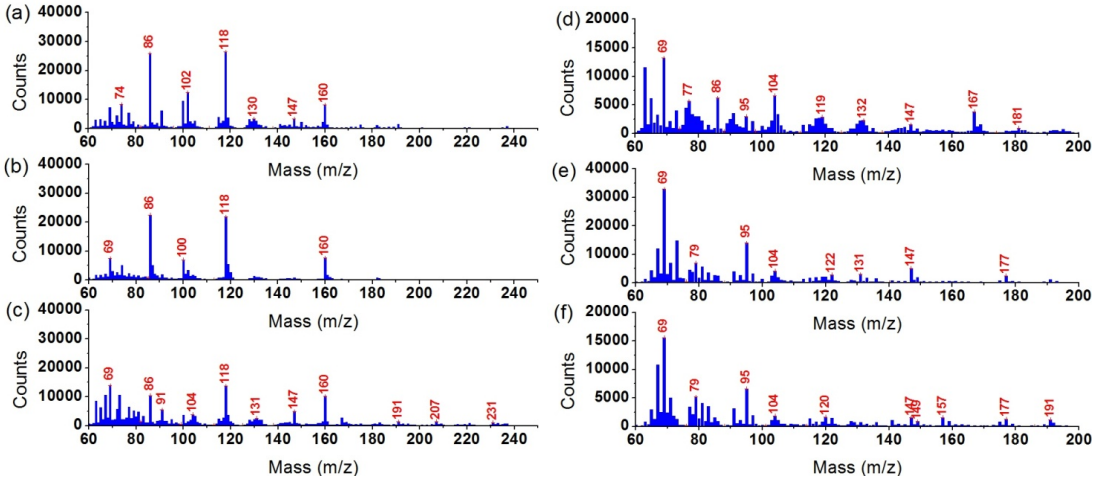


Figure S5. TOF-SIMS spectra at mass range (m/z = 60 to 200) of dendritically functionalized melanin, a) G1 treated with Et_3N, b) G2 treated with Et_3N, c) G3 treated with Et_3N, d) G1 treated with K_2CO_3, e) G2 treated with K_2CO_3, f) G3 treated with K_2CO_3.

Table S1. Summary of the solubility of K_2CO_3 treated dendritically functionalized melanin in water.

Sample	Solubility (g/L)
G3-melanin	23.2
G2-melanin	23.2
G1-melanin	10.4

Figure S6. Solubility of K_2CO_3 treated melanin (under the same conditions) in water.

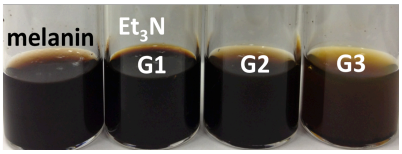


Figure S7. Solubility of Et_3N treated dendritically functionalized melanin in ammonia solution (10%).

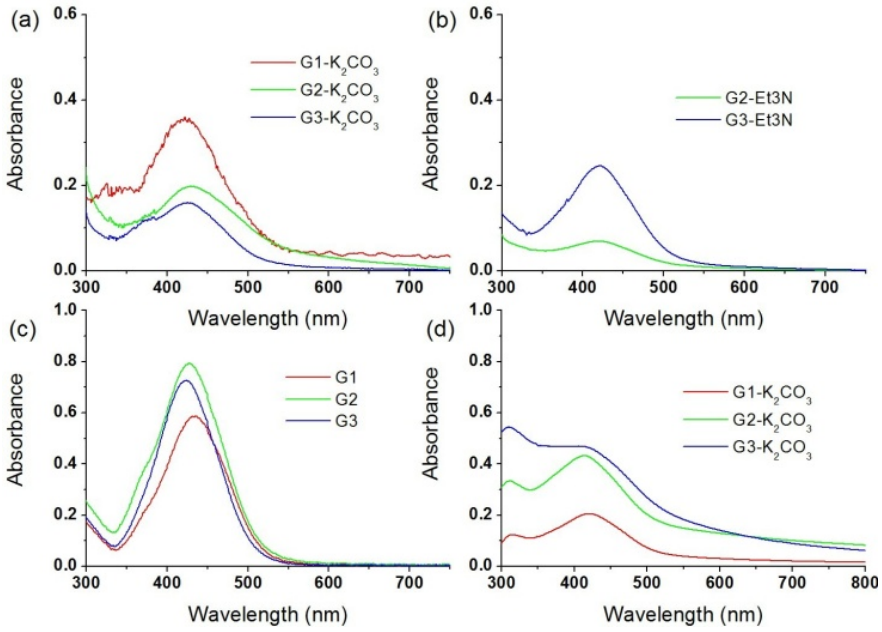


Figure S8. UV/Vis spectra of melanin and dendritic functionalized melanin in THF, (a) samples treated with K_2CO_3, (b) samples treated with Et_3N, (c) azobenzene dendrons, (d) solution of samples treated with K_2CO_3, then diluted in THF.

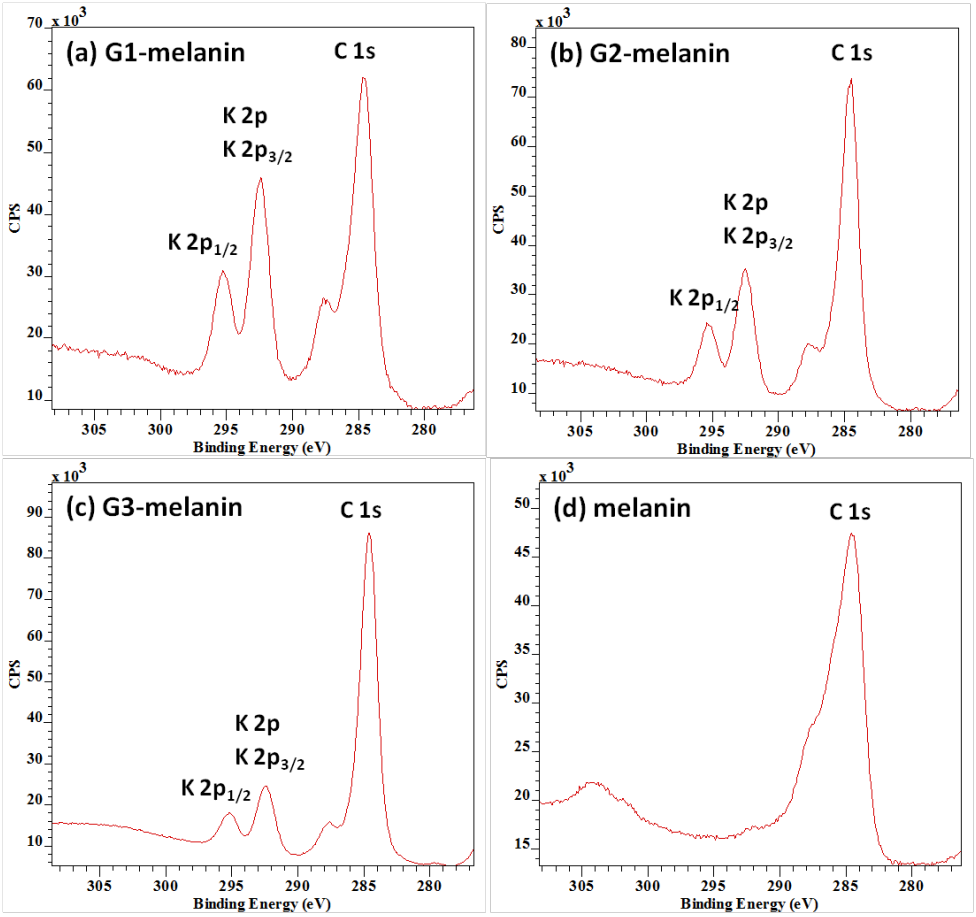


Figure S9. XPS spectra of melanin and dendritic functionalized melanin (treated with K_2CO_3), (a) G1-melanin, (b) G2-melanin, (c) G3-melanin, (d) melanin.

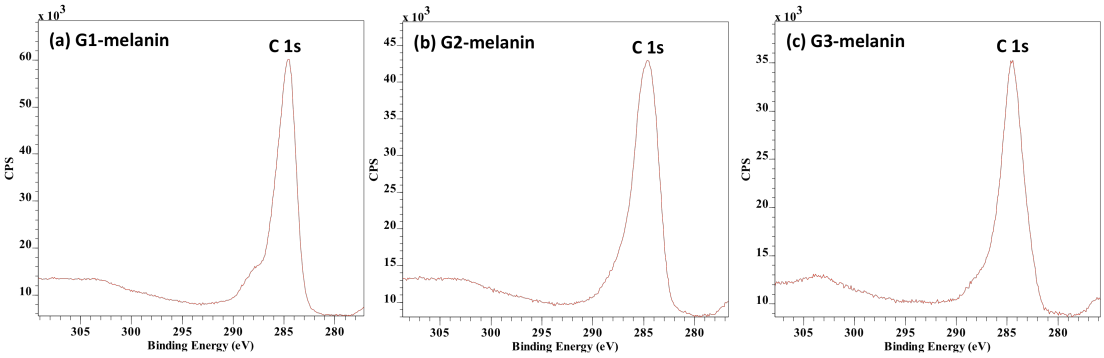


Figure S10. XPS spectra of dendritically functionalized melanins treated with Et_3N, (a) G1-melanin, (b) G2-melanin, (c) G3-melanin.

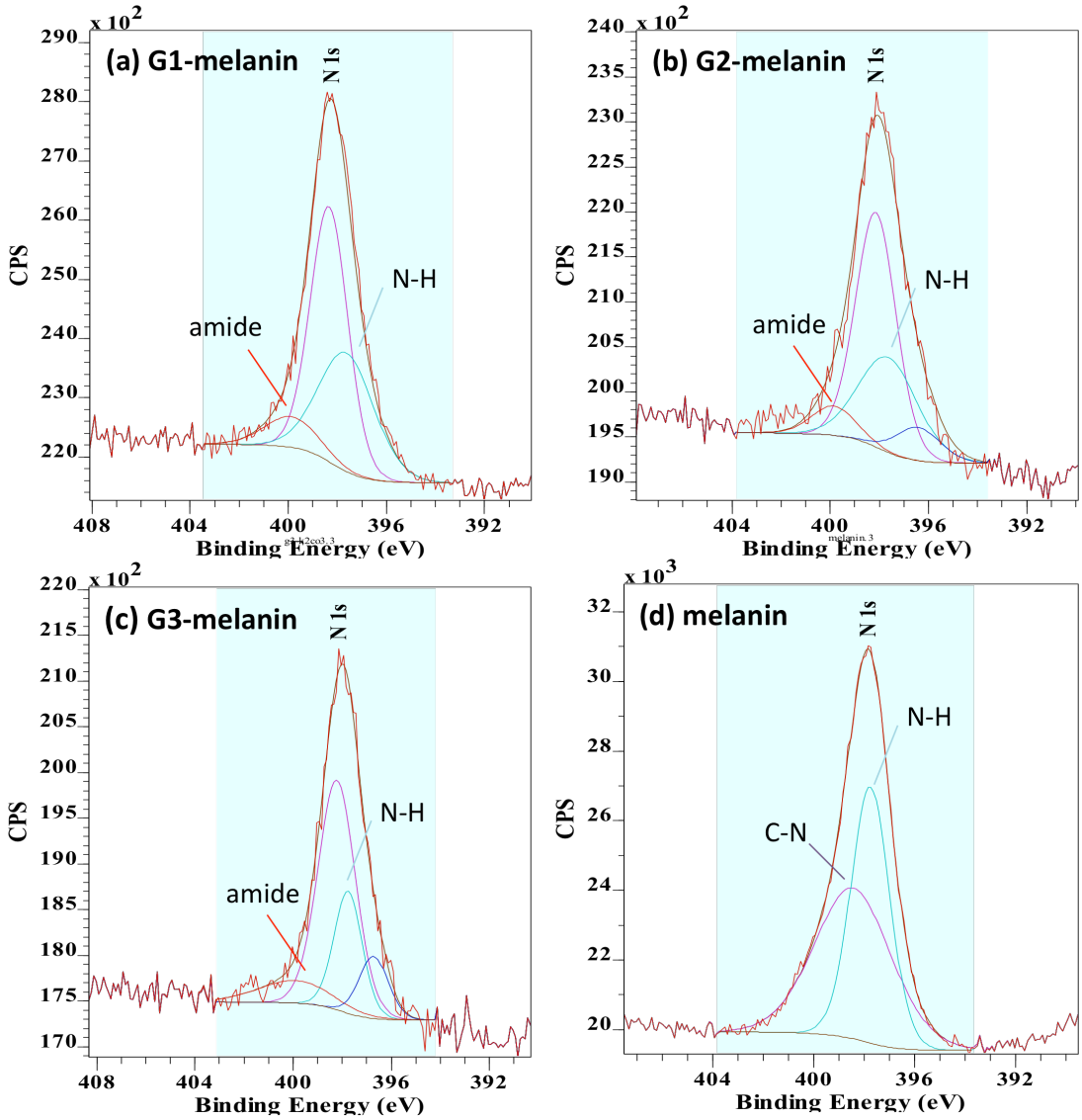


Figure S11. XPS spectra of N 1s of dendritically functionalized melanins treated with Et_3N, (a) G1-melanin, (b) G2-melanin, (c) G3-melanin.

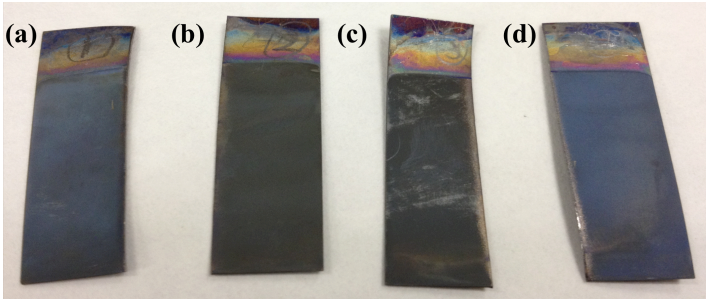


Figure S12. Appearances of the conjugated TiO_2 nanotube arrays (TNAs) by dendritically functionalized melanin treated with K_2CO_3, (a) G1-melanin, (b) G2-melanin, (c) G3-melanin, (d) control experiment (same conditions without dendritically functionalized melanin).

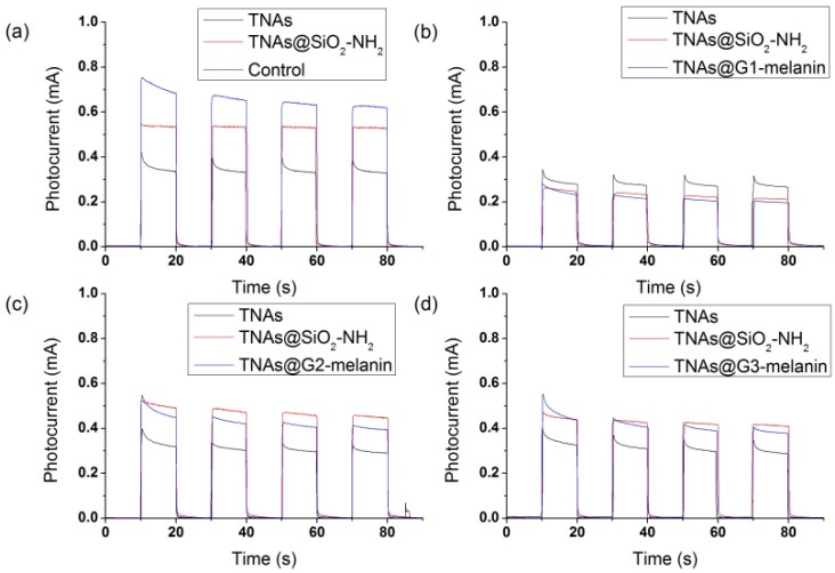


Figure S13. Photocurrent response of TiO2 nanotube arrays (TNAs) after amine-modification and EDC coupling by dendritically functionalized melanin (treated by K2CO3) under simulated sunlight illumination, (a) Control (same conditions without dendritically functionalized melanin), (b) G1-melanin, (c) G2-melanin, (d) G3-melanin.

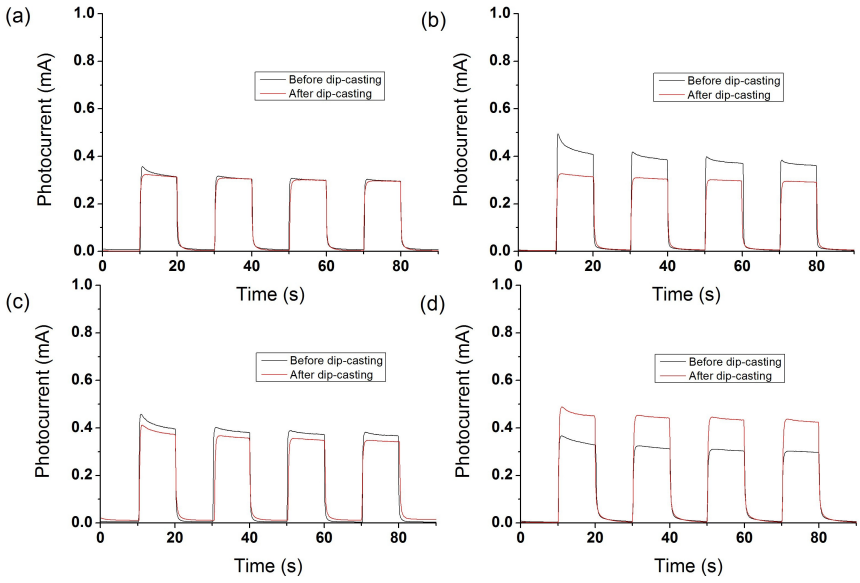


Figure S14. Photocurrent response of dip-casted TiO_2 nanotube arrays (TNAs) by dendritically functionalized melanin (treated with Et_3N) under simulated sunlight illumination, (a) G1-melanin, (b) G2-melanin, (c) G3-melanin, (d) melanin.

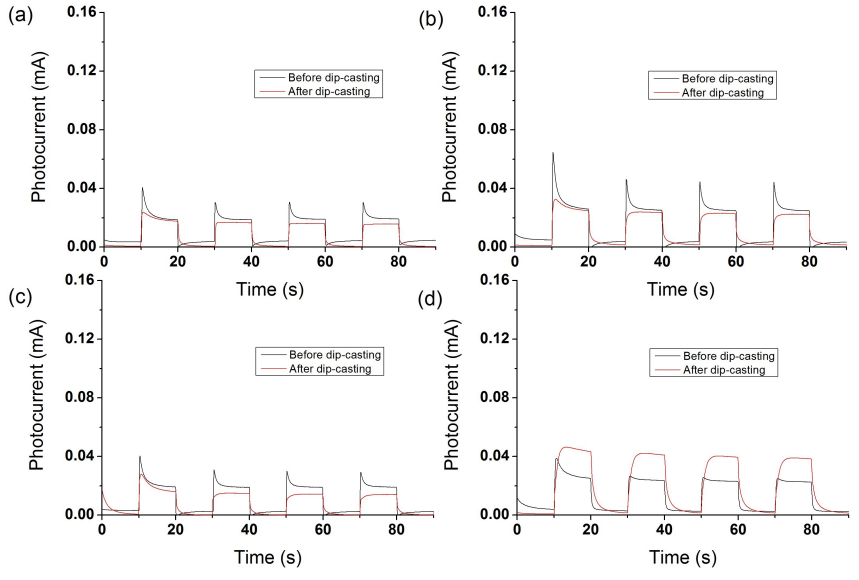


Figure S15. Photocurrent response of dip-casted TiO_2 nanotube arrays (TNAs) by dendritically functionalized melanin (treated with Et_3N) under visible light illumination, (a) G1-melanin, (b) G2-melanin, (c) G3-melanin, (d) melanin.

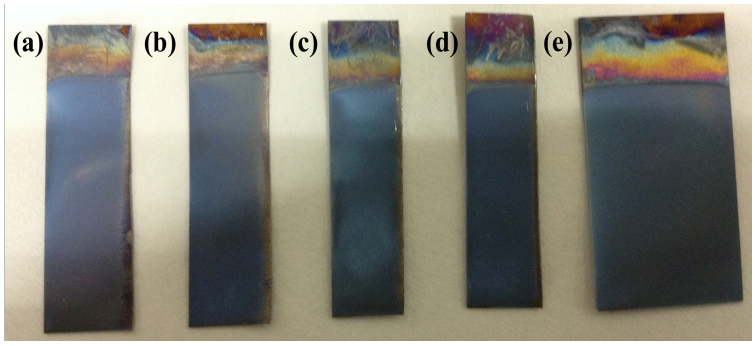


Figure S16. Appearances of the dip-casted TiO_2 nanotube arrays (TNAs) by dendritically functionalized melanin (treated with Et_3N), (a) G1-melanin, (b) G2-melanin, (c) G3-melanin, (d) melanin, (e) before dip-casting.

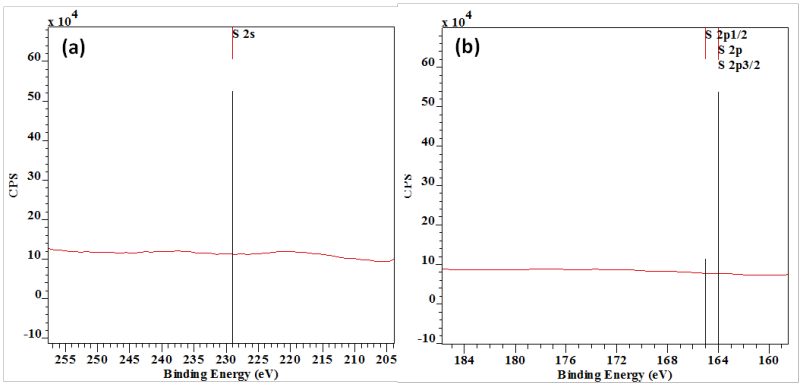


Figure S17. XPS spectra of bare melanin (a) binding energy from 205 to 255 eV, (b) binding energy from 160 to 184 eV.

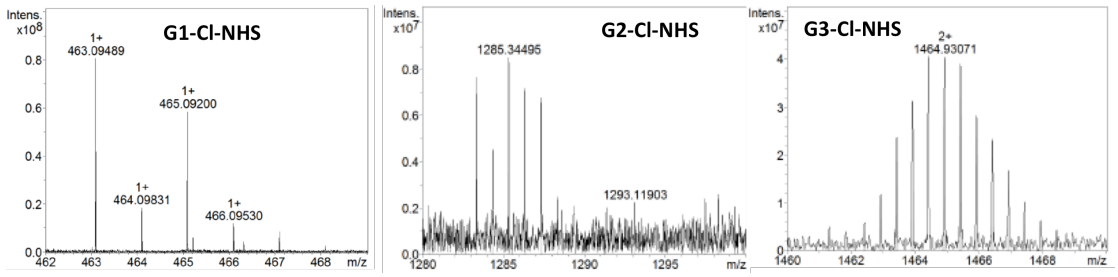
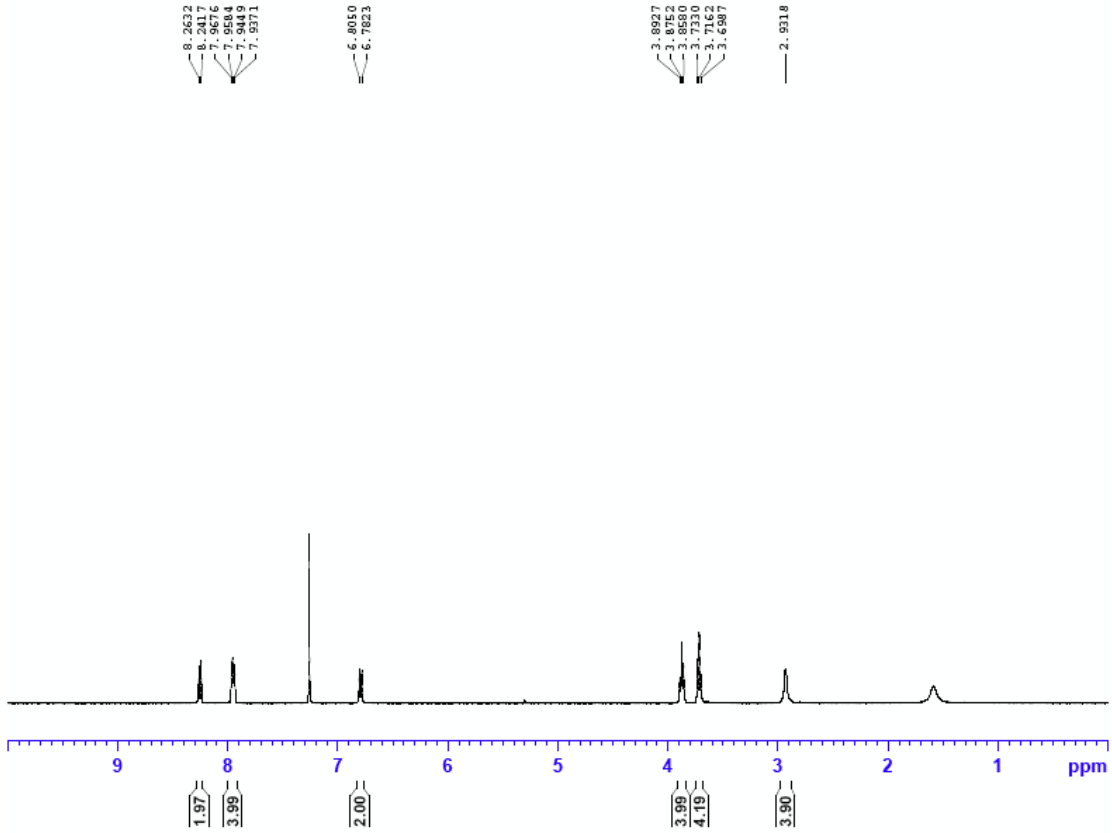
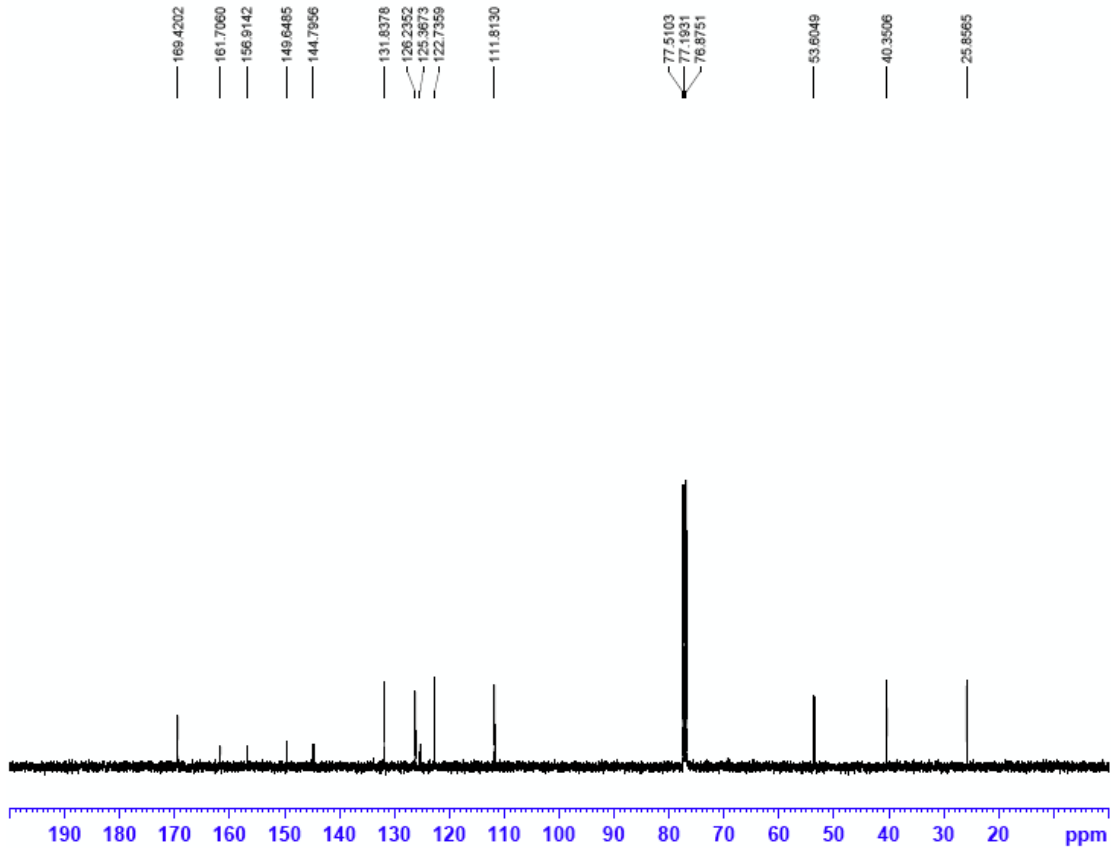


Figure S18. HR-MS spectrum of isotope patterns of G1-Cl-NHS, G2-Cl-NHS and G3-Cl-NHS dendron.

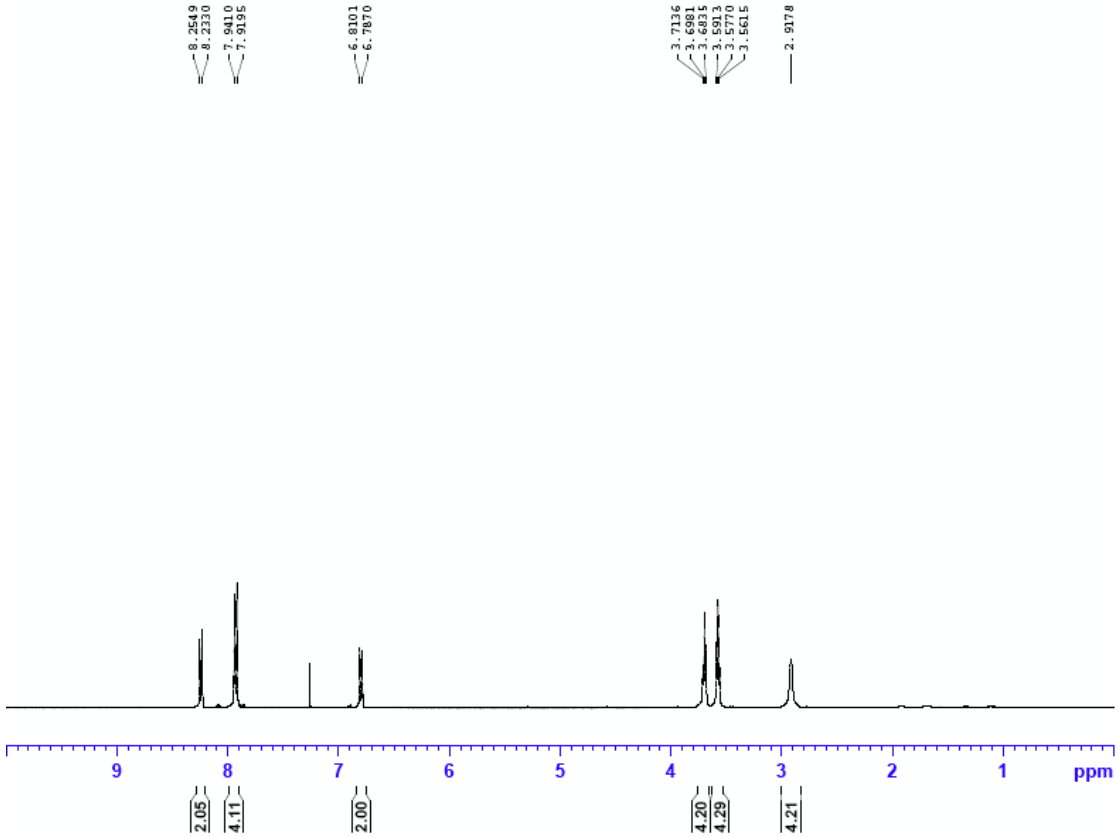
^{1}H NMR spectrum of G1-Cl-NHS



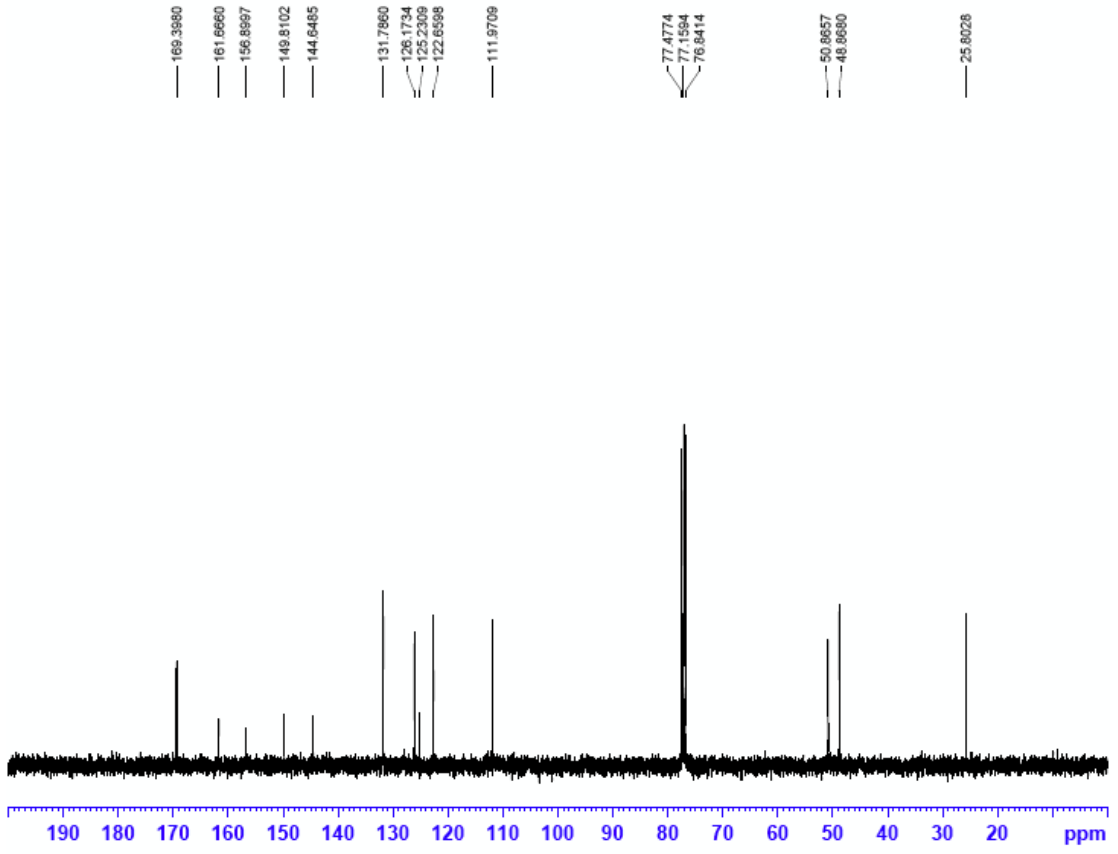
^{13}C NMR spectrum of G1-Cl-NHS



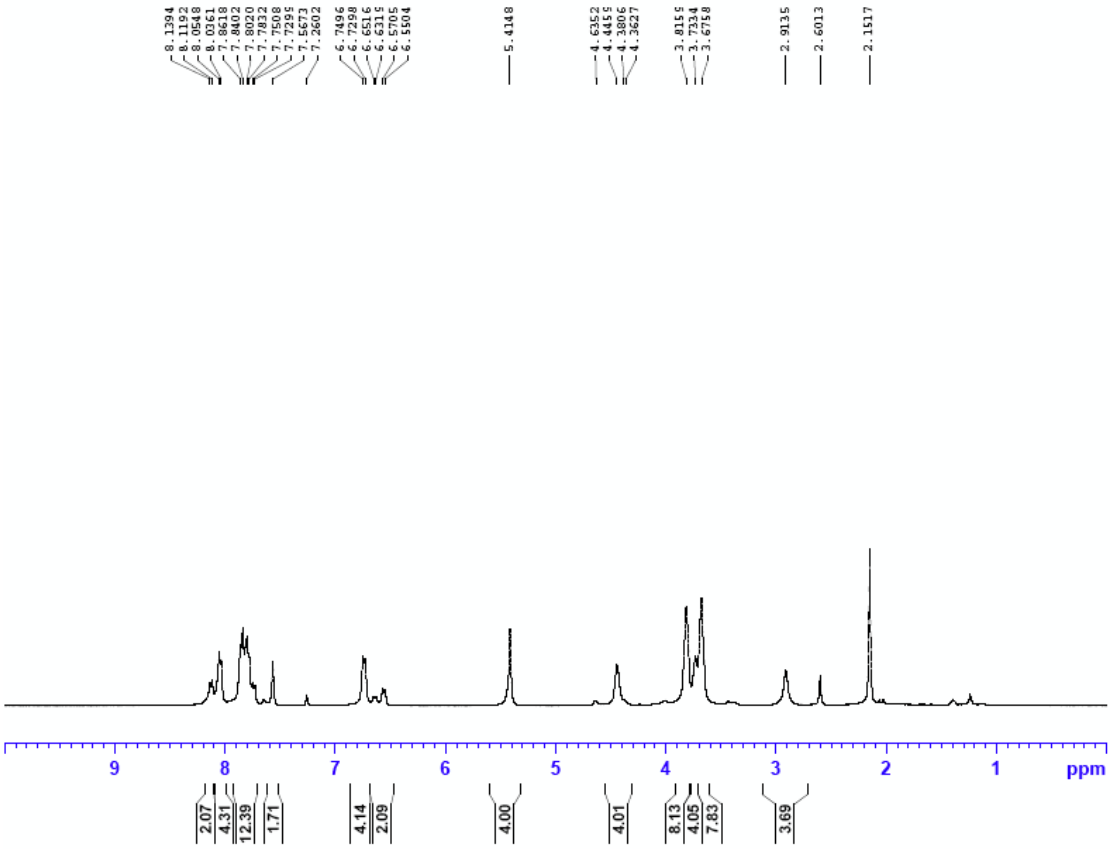
^{1}H NMR spectrum of G1-N_3-NHS



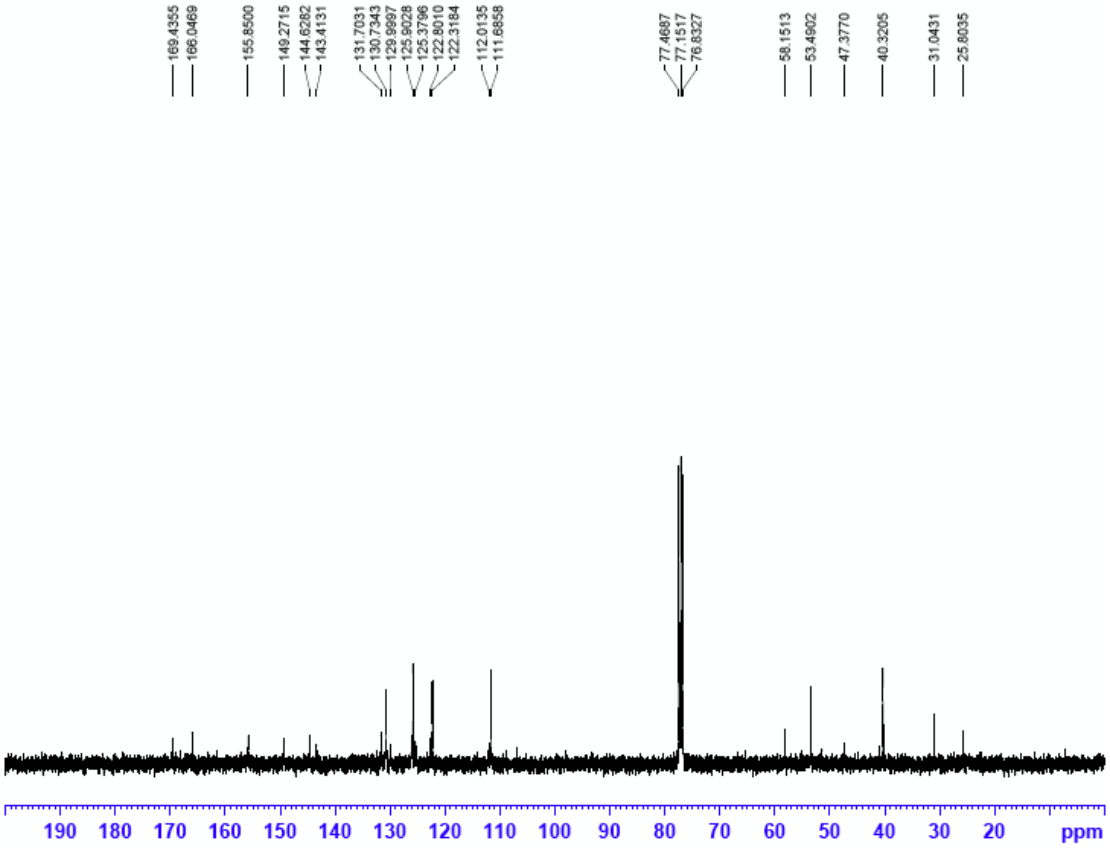
^{13}C NMR spectrum of G1-N_3-NHS



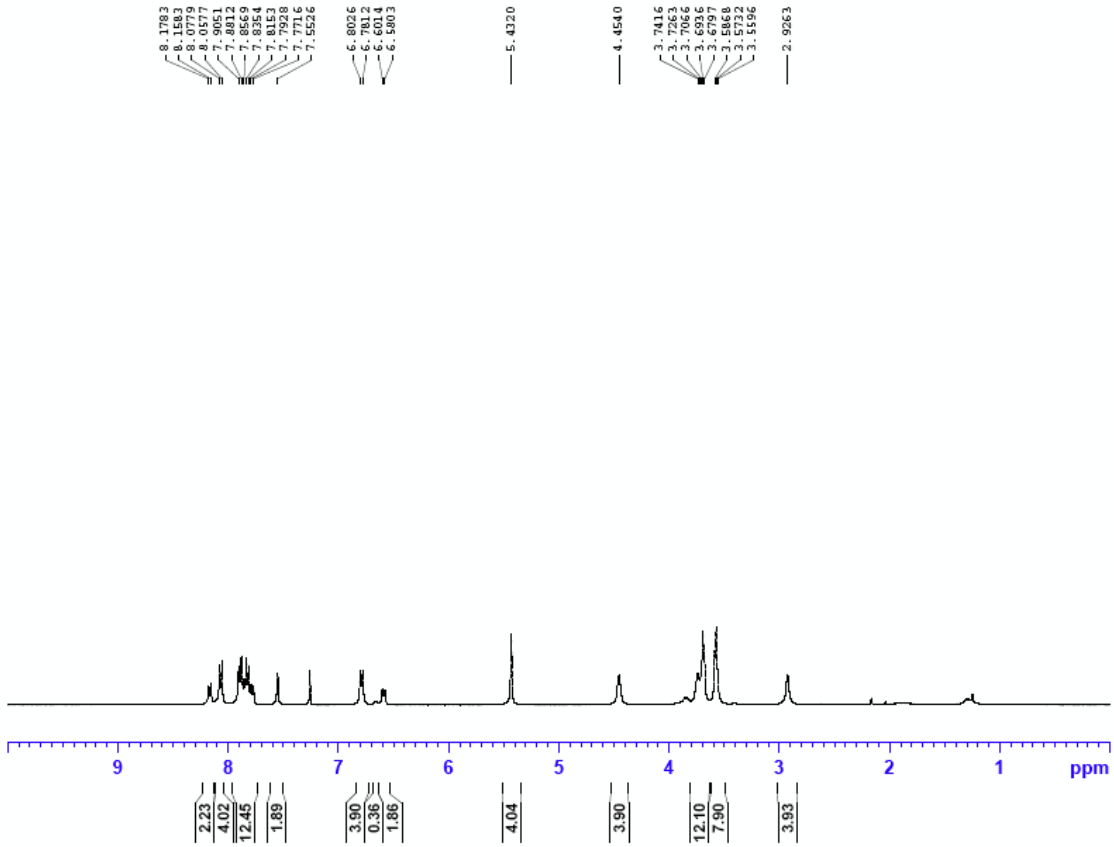
^{1}H NMR spectrum of G2-Cl-NHS



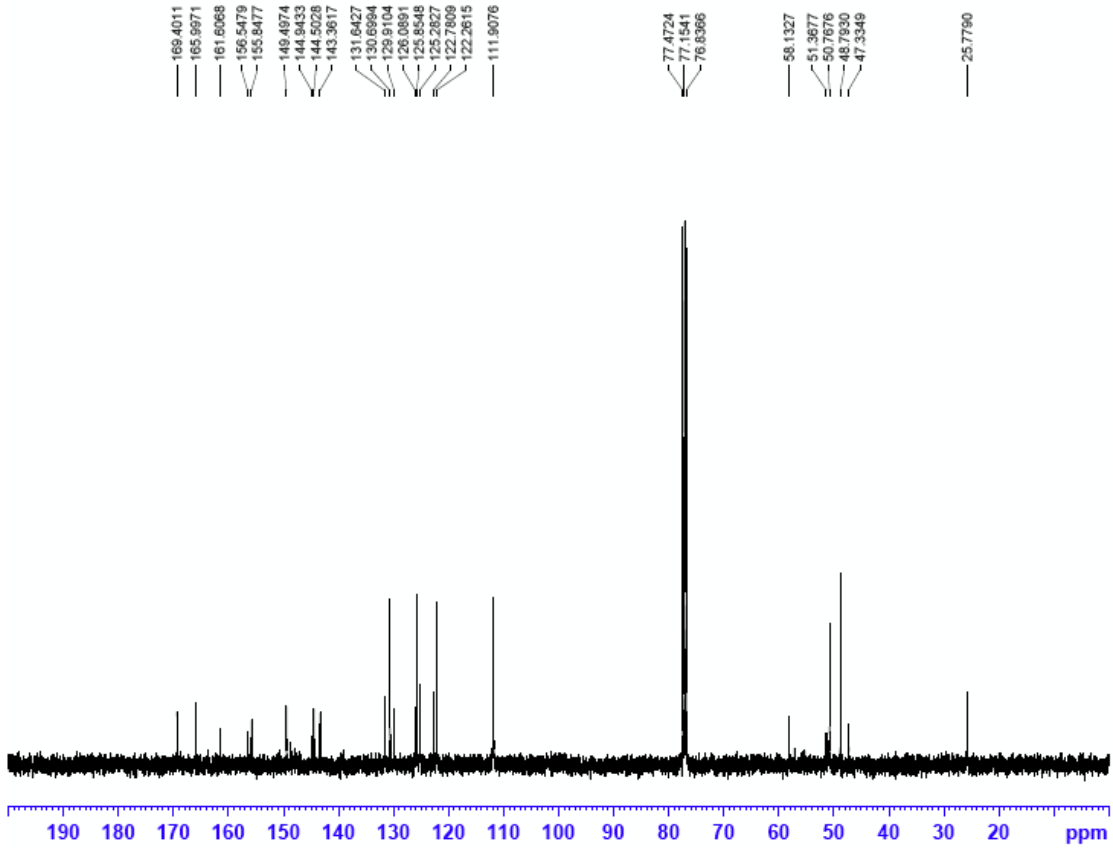
^{13}C NMR spectrum of G2-Cl-NHS



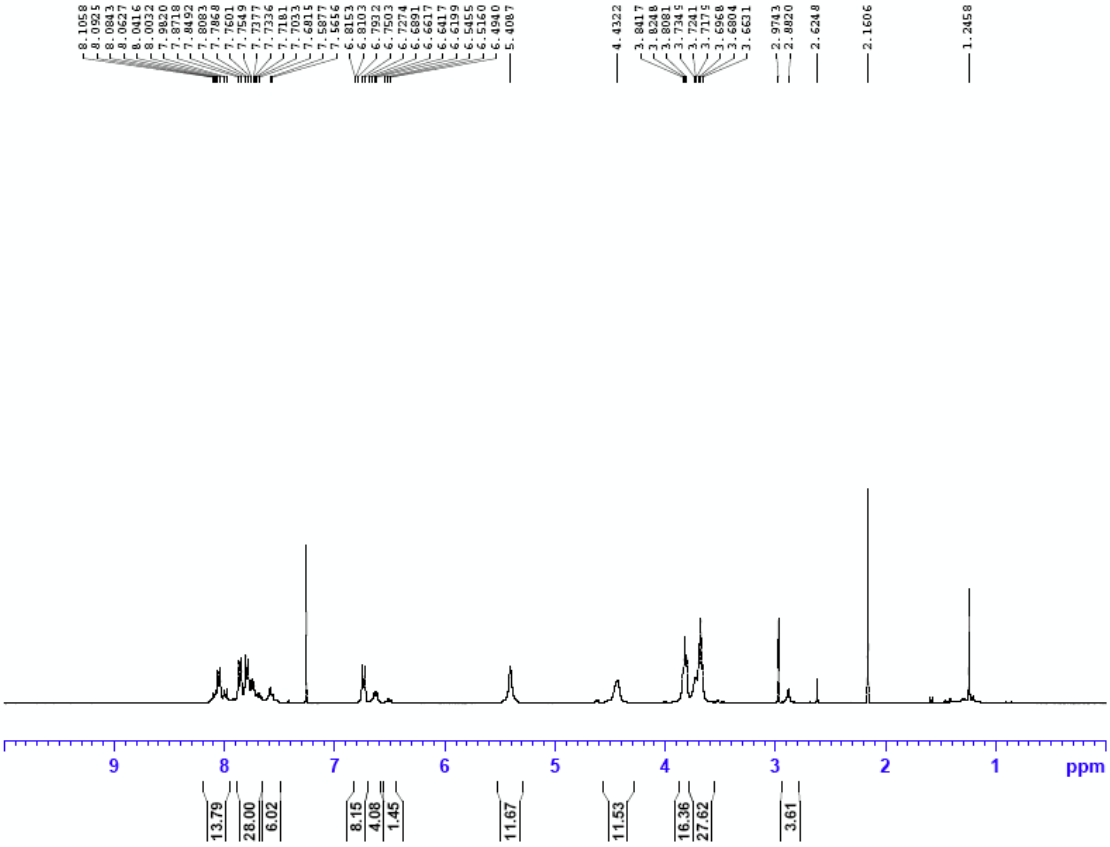
^{1}H NMR spectrum of G2-N_3-NHS



^{13}C NMR spectrum of G2-N_3-NHS



^{1}H NMR spectrum of G3-Cl-NHS



^{13}C NMR spectrum of G3-Cl-NHS