Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Senescence-associated Sialidase Revealed by a Activatble Fluorescence-on Labeling Probe

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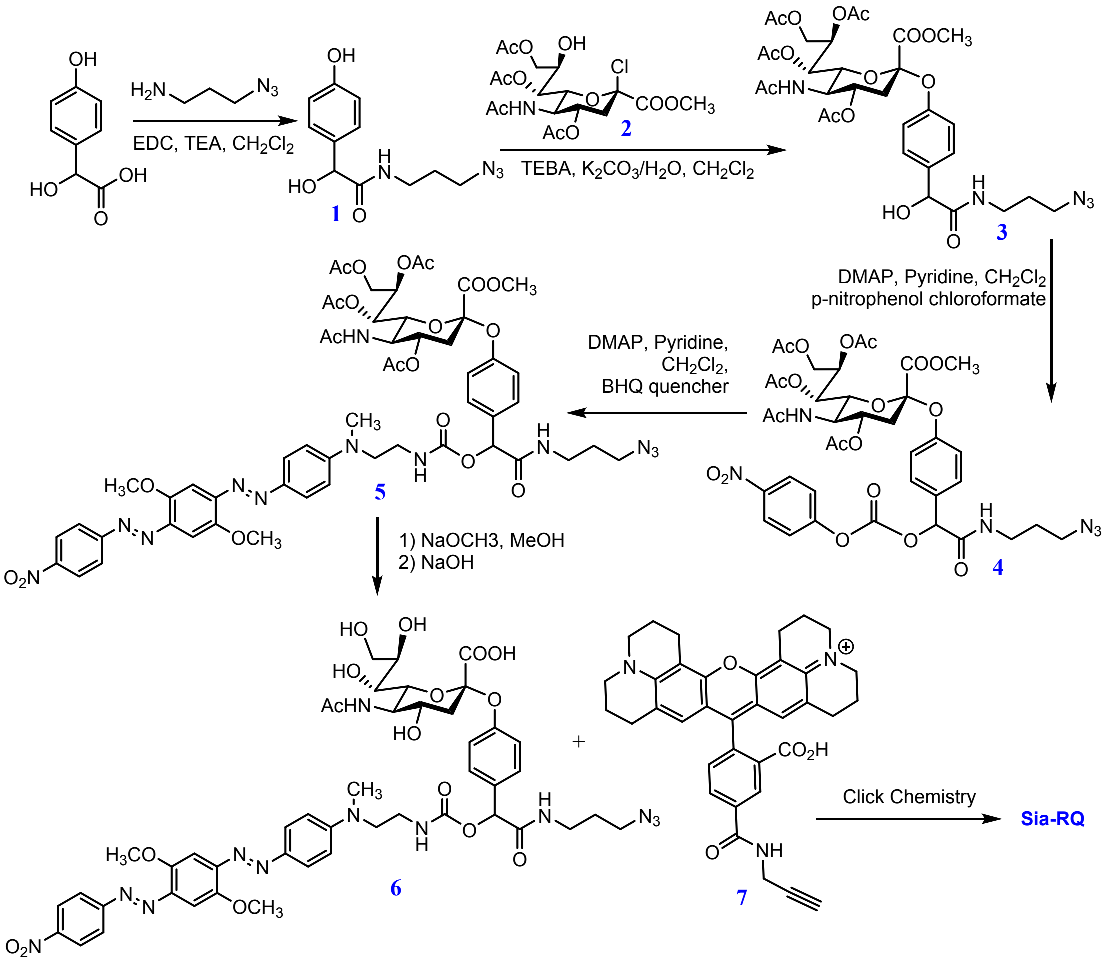
Experimental procedure

Synthesis of Sia-RQ



Scheme S1. Synthetic route of Sia-RQ.

4-Hydroxymandelic acid (35 g, 188.12 mmol) and 3-azidopropyl-1-amine (22.57 g, 225.74 mmol) were dissolved in CH_2Cl_2 (500 mL). To the solution were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 54.09 g, 282.18 mmol) and triethylamine (TEA, 57 g, 564.36 mmol). The reaction mixture was stirred overnight, and then concentrated under reduced pressure. The residue was purified by silica gel chromatography using petroleum ether (PE)/ethyl acetate PE/EtOAc (1:3) as the eluent to yield compound **1** (28g, 59%). MS (ES+) calc'd for $C_{11}H_{14}N_4NaO_3^+$ (M+Na) *m/z* 273.10, Found 273.09. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 9.31 (s, 1H), 8.00 (t, *J* = 6.0 Hz, 1H), 7.17 (d, *J* = 8.3 Hz, 2H), 6.69 (d, *J* = 8.6 Hz, 2H), 5.91 (d, *J* = 4.4 Hz, 1H), 4.77 (d, *J* = 4.3 Hz, 1H), 3.29 (t, *J* = 6.9 Hz, 2H), 3.13 (d, *J* = 6.4 Hz, 2H), 1.66 (p, *J* = 6.8 Hz, 2H). ^{13}C-NMR (126 MHz, DMSO) δ 173.15, 157.16, 132.25, 128.23, 115.12, 73.79, 48.90, 36.10, 28.94.

Compound **2** (47 g, 92.3 mmol), synthesized following a published procedure,[1] was dissolved in CH_2Cl_2 (500 mL). To the solution of compound 2 was added compound **1** (28 g, 111.96 mmol), triethylbenzylammonium chloride (TEBA, 4.22 g, 18.57 mmol) and K_2CO_3 (51.33 g, 371.36 mmol) in water (250 ml). The mixture was vigorously at room temperature for 5 h. The organic layer was washed with water, concentrated under reduced pressure and then chromatographed (Silica Gel, PE/EtOAc = 1:5) to yield compound **3** (40 g, 56%). MS (ES+) calc'd for $C_{31}H_{42}N_5O_{15}^+$ (M+H) *m/z* 724.27, Found 724.75. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.33 – 7.26 (m, 3H), 7.01 (dd, J = 8.3, 5.4 Hz, 2H), 6.74 (dt, J = 32.4, 6.0 Hz, 1H), 5.51 (dd, J = 14.5, 10.0 Hz, 1H), 5.33 (d, J = 8.6 Hz, 1H), 5.26 (ddd, J = 15.7, 5.7, 2.8 Hz, 1H), 5.00 – 4.87 (m, 2H), 4.47 – 4.19 (m, 3H), 4.18 – 3.97 (m, 3H), 3.70 (d, J = 7.5 Hz, 3H), 3.34 (ddt, J = 26.3, 15.1, 6.8 Hz, 5H), 2.75 (ddd, J = 17.5, 12.9, 4.7 Hz, 1H), 2.21 (td, J = 12.7, 3.0 Hz, 1H), 2.18 – 2.07 (m, 7H), 2.10 – 2.00 (m, 11H), 2.00 (s, 3H), 1.78 (td, J = 6.8, 4.6 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.60, 170.93, 170.86, 170.41, 170.38, 170.28, 170.09, 170.07, 168.32, 167.86, 153.73, 153.47, 135.48, 135.02, 128.19, 128.14, 121.24, 120.11, 99.76, 99.42, 77.30, 77.04, 76.79, 73.74, 73.59, 73.43, 73.13, 69.33, 69.16, 68.71, 68.63, 67.43, 67.29, 62.28, 62.15, 53.05, 52.94, 49.37, 49.34, 49.14, 49.11, 38.35, 38.23, 37.10, 37.08, 28.71, 28.66, 23.13, 20.98, 20.96, 20.83, 20.78, 20.76,

Compound **3** (20 g, 27.65 mmol), 4-nitrophenyl chloroformate (6.47 g, 33.18 mmol) and 4-dimethylaminopyridine (DMAP, 3.37 g, 27.65 mmol) were dissolved in CH_2Cl_2 (200 mL). To the solution was added pyridine (6.47 g, 82.95 mmol). The reaction mixture was stirred for 2 h, diluted with dichloromethane (100 ml), and then washed with hydrochloric acid (HCl, 1M, 200ml). The organic layer was concentrated under reduced pressure and then chromatographed (Silica Gel, PE/EtOAc = 1:3) to yield compound **4** (18 g, 73%). MS (ES+) calc'd for $C_{38}H_{45}N_6O_{19}^+$ (M+H) *m/z* 889.27, Found 889.13. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.28 (d, J = 8.9 Hz, 2H), 7.47 – 7.37 (m, 4H), 7.33 (dd, J = 8.9, 2.6 Hz, 1H), 7.10 (ddd, J = 13.1, 8.7, 6.3 Hz, 3H), 6.58 (td, J = 5.9, 3.6 Hz, 1H), 5.97 (d, J = 13.0 Hz, 1H), 5.42 (t, J = 9.1 Hz, 1H), 5.40 – 5.27 (m, 3H), 5.01 – 4.90 (m, 1H), 4.42 (dd, J = 42.8, 10.7, 1.8 Hz, 1H), 4.32 – 4.24 (m, 1H), 4.18 – 4.03 (m, 3H), 3.70 (s, 4H), 3.66 (s, 1H), 3.48 – 3.29 (m, 5H), 2.79 – 2.66 (m, 1H), 2.23 (td, J = 12.8, 4.6 Hz, 2H), 2.19 – 2.07 (m, 9H), 2.09 – 1.98 (m, 9H), 1.97 (q, J = 6.7 Hz, 1H), 1.91 (d, J = 2.8 Hz, 6H), 1.82 (q, J = 6.6 Hz, 2H), 1.26 (t, J = 7.1 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.89, 170.75, 170.65, 170.33, 170.22, 170.12, 169.99, 168.22, 167.88, 167.46, 167.40, 155.30, 155.08, 155.02, 154.85, 154.68, 151.33, 151.29, 145.54, 129.44, 129.22, 129.12, 129.07, 127.60, 127.55, 125.34, 121.72, 120.67, 119.89, 99.71, 99.45, 79.13, 79.12, 77.30, 77.05, 76.79, 73.45, 73.21, 68.94, 68.78, 68.61, 68.58, 68.54, 67.28, 67.18, 62.10, 53.15, 53.06, 49.37, 49.35, 49.30, 49.26, 38.38, 38.33, 38.28, 38.15, 37.43, 37.41, 28.53, 28.51, 26.92, 23.17, 21.00, 21.00, 20.83, 20.80, 20.75, 20.73, 14.18.

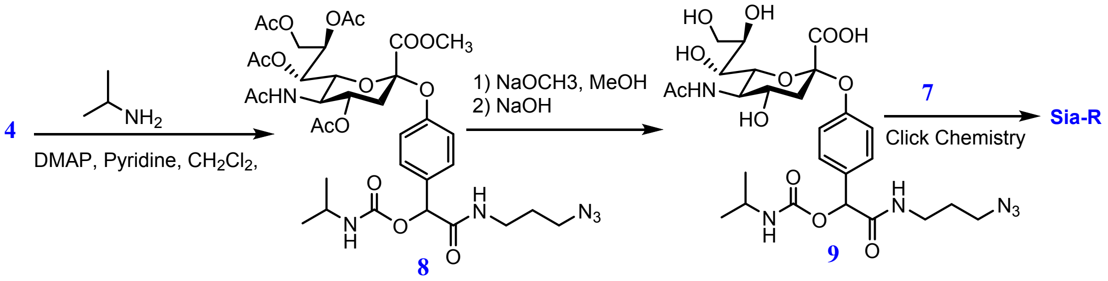
Compound **4** (3.06 g, 3.44 mmol), BHQ (1.33 g, 2.87 mmol) and DMAP (350 mg, 2.87 mmol) were dissolved in CH_2Cl_2 (200 mL). Pyridine (672 mg, 8.61 mmol) was added to the above reaction mixture. The reaction was stirred 3 h, concentrated under reduced pressure and then chromatographed (Silica Gel, EtoAc/CH_3OH = 30:1) to yield compound **5** (3.2 g, 92%). MS (ES+) calc'd for $C_{55}H_{65}N_{12}O_{20}^+$ (M+H) *m/z* 1213.44, Found 1213.53. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.36 (d, *J* = 8.6 Hz, 2H), 8.04 (d, *J* = 8.6 Hz, 2H), 7.92 (d, *J* = 8.7 Hz, 2H), 7.49 (s, 1H), 7.46 (s, 1H), 7.32 (dd, *J* = 8.4, 6.1 Hz, 2H), 7.04 (t, *J* = 8.6 Hz, 2H), 6.80 (d, *J* = 8.7 Hz, 2H), 6.39 (t, *J* = 6.1 Hz, 1H), 5.97 (d, *J* = 8.4 Hz, 1H), 5.40 – 5.24 (m, 4H), 4.95 (td, *J* = 11.4, 4.6 Hz, 1H), 4.41 (dd, *J* = 32.2, 10.9 Hz, 1H), 4.29 (ddd, *J* = 12.5, 7.2, 2.5 Hz, 1H), 4.16 – 4.04 (m, 7H), 4.04 (s, 3H), 3.67 (d, *J* = 5.3 Hz, 3H), 3.63 (s, 2H), 3.46 (q, *J* = 6.5 Hz, 2H), 3.41 – 3.24 (m, 4H), 3.10 (s, 3H), 2.70 (ddd, *J* = 13.1, 8.6, 4.6 Hz, 1H), 2.25 – 2.16 (m, 1H), 2.17 – 2.10 (m, 6H), 2.07 – 2.00 (m, 7H), 1.91 (s, 3H), 1.81 – 1.71 (m, 5H), 1.26 (t, *J* = 7.1 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.90, 170.36, 170.00, 169.11, 169.06, 168.66, 168.24, 167.95, 156.41, 154.16, 153.54, 151.98, 151.00, 148.37, 146.56, 144.73, 142.20, 128.90, 128.73, 126.13, 124.73, 123.53, 120.24, 119.66, 115.65, 111.51, 100.98, 100.08, 99.75, 99.46, 77.29, 77.03, 76.78, 75.50, 73.41, 73.18, 69.05, 68.89, 68.64, 68.60, 67.21, 62.04, 61.94, 60.41, 56.77, 53.14, 53.04, 49.25, 49.22, 38.87, 38.27, 38.19, 37.15, 36.24, 35.90, 28.55, 23.21, 21.05, 21.02, 20.84, 20.79, 20.77, 20.75, 14.19.

To the solution of compound **5** (2.6 g, 2.14 mmol) in anhydrous MeOH (30 mL) was added $NaOCH_3$ (5 mmol). The reaction mixture was stirred at room temperature until the reaction was completed as monitored by TLC, followed by addition of aqueous NaOH solution (2.4 M, 5 ml) The mixture was stirred at room temperature for 4 h, concentrated under reduced pressure and then chromatographed (Silica Gel, CH_2Cl_2/CH_3OH = 3:1) to yield compound **6** (1.83 g, 88%). MS (ES+) calc'd for $C_{46}H_{55}N_{12}O_{16}^+$ (M+H) *m/z* 1031.39, found 1031.38. ^{1}H NMR (850 MHz, DMSO-*d*$_6$) δ 8.57 – 8.38 (m, 3H), 8.33 – 8.23 (m, 1H), 8.06 (d, *J* = 8.5 Hz, 2H), 7.81 (d, *J* = 8.7 Hz, 2H), 7.69 – 7.57 (m, 1H), 7.43 (s, 1H), 7.37 (s, 1H), 7.25 – 7.08 (m, 4H), 6.88 (d, *J* = 8.8 Hz, 2H), 5.62 (d, *J* = 4.6 Hz, 1H), 4.00 (s, 3H), 3.95 (s, 3H), 3.70 – 3.57 (m, 5H), 3.54 (q, *J* = 6.4, 5.9 Hz, 2H), 3.33 – 3.21 (m, 6H), 3.21 – 3.16

(m, 2H), 3.16 – 3.12 (m, 3H), 3.07 (s, 3H), 2.83 – 2.76 (m, 1H), 1.89 (s, 3H), 1.63 (q, *J* = 13.2, 6.8 Hz, 2H), 1.54 (t, *J* = 11.4, 8.7, 4.2 Hz, 1H). ^{13}C NMR (214 MHz, DMSO) δ 172.99, 169.46, 156.16, 156.03, 153.52, 152.51, 150.79, 148.53, 146.57, 144.08, 141.81, 129.92, 128.32, 128.26, 126.18, 125.56, 123.96, 120.09, 119.98, 111.95, 102.46, 102.36, 101.12, 100.38, 78.88, 75.18, 74.05, 72.14, 67.32, 66.80, 63.73, 63.66, 56.91, 56.85, 53.69, 51.44, 49.04, 42.62, 40.33, 39.12, 38.22, 36.13, 33.37, 31.53, 28.83, 22.94, 20.92.

To the solution of compound **6** (200 mg, 0.20 mmol) and compound **7** (118 mg, 0.21 mmol) in tetrahydrofuran (THF, 5 mL) was added sodium ascorbate (20 mg, 0.10 mmol) and copper sulfate pentahydrate (14 mg, 0.05 mmol) in distilled water (1 ml). The mixture was maintained at room temperature for 12 h with stirring. After removal of the solvent, the residue was purified by silica gel column chromatography using CH_2Cl_2/CH_3OH = 1:3 as the eluent to yield **Sia-RQ** (250 mg, 78%). Then analyzed by HPLC and HRMS. MS (ES+) calc'd for $C_{82}H_{88}N_{15}O_{20}^{+}$ (M+H) *m/z* 1602.63, Found1603.13. HRMS (ESI) calc'd for $C_{82}H_{87}N_{15}Na_{2}O_{2}0^{2}$+ (M+2Na) *m/z* 823.80181, Found823.80360

Synthesis of Sia-R



Scheme S2. Synthetic route of Sia-R.

Isopropylamine (120 mg, 20.39 mmol), compound **4** (1.5 g, 17 mmol) and DMAP (146 mg, 17 mmol) were dissolved in CH_2Cl_2 (50 mL). To the solution was added pyridine (400 mg, 51 mmol). The reaction mixture was stirred for 3 h, concentrated under reduced pressure and then chromatographed (Silica Gel, PE/EtOAc = 1:4) to yield compound **8** (1.05 g, 78%). MS (ES+) calc'd for $C_{34}H_{47}N_{6}O_{16}^{+}$ (M+H) *m/z* 795.30, Found795.48.^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.42 – 7.27 (m, 2H), 7.09 – 6.99 (m, 2H), 6.56 – 6.42 (m, 1H), 5.94 (d, *J* = 5.0 Hz, 1H), 5.54 – 5.41 (m, 1H), 5.41 – 5.27 (m, 2H), 5.04 – 4.90 (m, 2H), 4.52 – 4.36 (m, 1H), 4.37 – 4.24 (m, 1H), 4.22 – 4.00 (m, 2H), 3.92 – 3.75 (m, 1H), 3.67 (d, *J* = 5.7 Hz, 3H), 3.46 – 3.27 (m, 4H), 2.81 – 2.64 (m, 1H), 2.29 – 2.16 (m, 1H), 2.19 – 1.84 (m, 19H), 1.86 – 1.73 (m, 2H), 1.30 – 1.11 (m, 7H).^{13}C NMR (126 MHz, $CDCl_3$) δ 171.59, 170.91, 170.74, 170.65, 170.31, 170.17, 170.13, 170.00, 154.05, 131.38, 131.20, 128.76, 128.58, 120.01, 119.59, 99.78, 99.54, 75.12, 75.09, 73.41, 73.24, 69.15, 69.04, 68.69, 67.34, 67.26, 62.01, 61.96, 60.40, 58.37, 53.09, 53.01, 49.39, 49.35, 49.23, 43.48, 38.18, 38.14, 37.01, 28.55, 23.17, 22.84, 20.99, 20.83, 20.77, 20.74, 20.73, 18.40, 14.18.

To the solution of compound **8** (1.05 g, 1.32 mmol) in anhydrous MeOH (5.0 mL) was added $NaOCH_3$ (0.4 mmol). The mixture was stirred at room temperature until the reaction was complete. Aqueous solution of NaOH (7M, 1 ml) was added to the solution, which was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure and chromatographed (Silica Gel, CH_2Cl_2/CH_3OH=3:1) to yield compound **9** (503 mg, 60%). MS (ES+) calc'd for $C_{26}H_{39}N_6O_{12}^+$ (M+H) *m/z* 627.26, Found627.13.^{1}H NMR (500 MHz, Methanol-*d*$_4$) δ 7.38 (d, *J* = 8.9 Hz, 2H), 7.23 (d, *J* = 8.4 Hz, 2H), 5.77 (s, 1H), 3.95 – 3.67 (m, 8H), 3.71 – 3.52 (m, 3H), 3.36 – 3.24 (m, 7H), 2.90 (d, *J* = 12.8, 3.6 Hz, 1H), 2.03 (s, 4H), 1.95 – 1.82 (m, 2H), 1.83 – 1.71 (m, 2H), 1.22 – 1.09 (m, 6H).^{13}C NMR (126 MHz, DMSO) δ 175.18, 173.05, 169.64, 155.97, 154.89, 146.07, 130.01, 128.31, 120.09, 119.98, 102.38, 85.61, 77.12, 74.88, 72.10, 69.37, 67.06, 63.64, 53.82, 49.04, 42.81, 42.59, 36.09, 28.83, 24.58, 22.98, 22.90, 22.84.

To a solution of compound **9** (100 mg, 0.16 mmol) and compound **7** obtained from Bioluminot, Xiamen (103 mg, 0.18 mmol) in THF (5 mL) was added sodium ascorbate (16 mg, 0.08 mmol) and copper sulfate pentahydrate (11 mg, 0.04 mmol) in distilled water (1 ml). The mixture was maintained at room temperature for 12 h with stirring. After removal of the solvent, the residue was purified by silica gel column chromatography using CH_2Cl_2/CH_3OH=1:3 as the eluent to yield **Sia-R** (150mg, 73%). HRMS (ES+) HRMS (ESI) calc'd for $C_{62}H_{72}N_9O_{16}^+$ (M+H) *m/z*1198.50915, Found1198.50927.

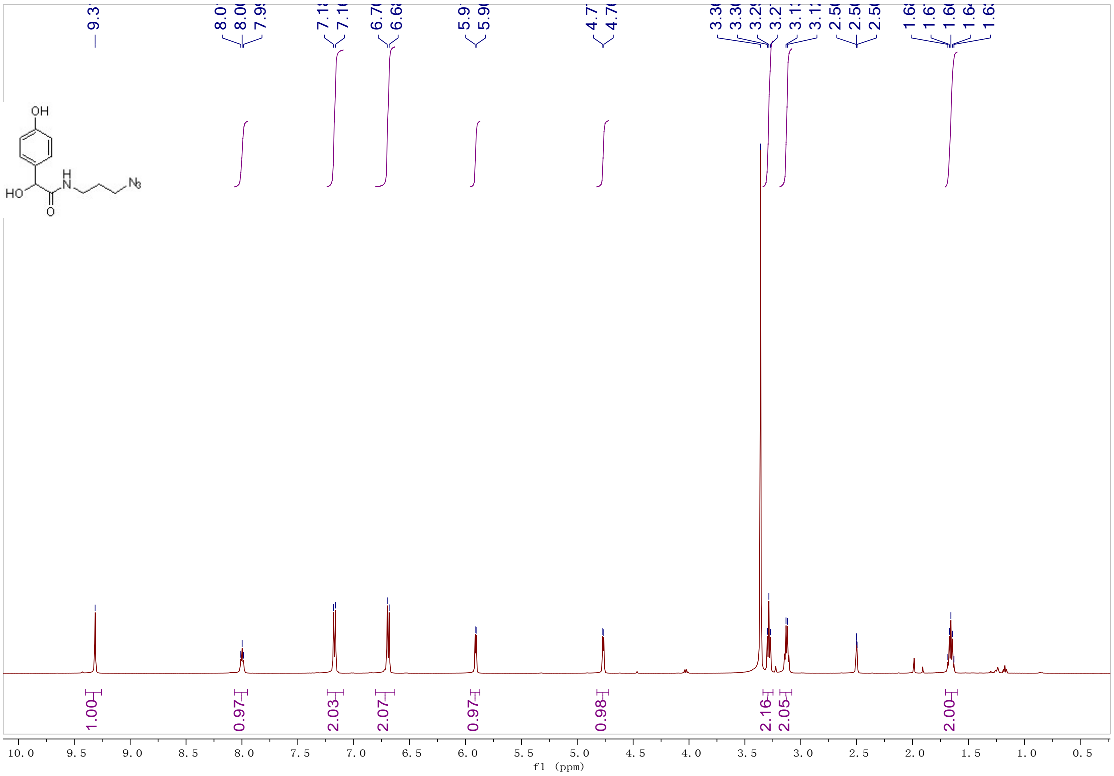


Figure S7. ^{1}H-NMR Spectrum of compound **1**

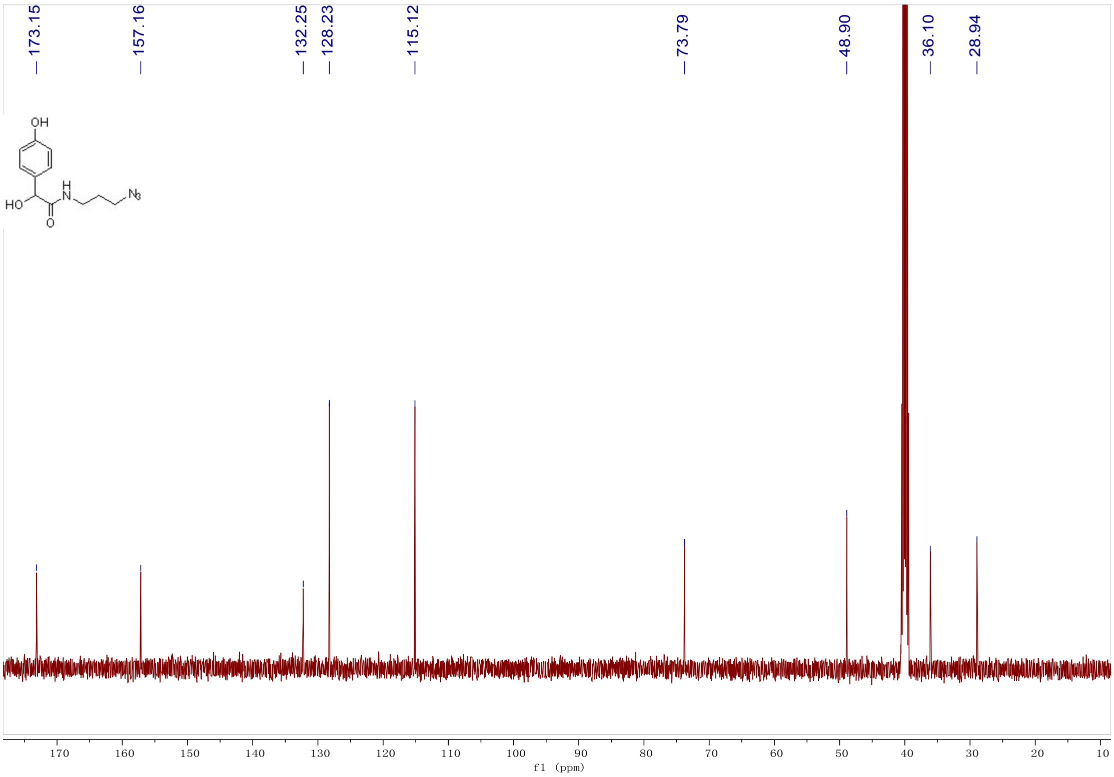


Figure S8. ^{13}C-NMR Spectrum of compound **1**

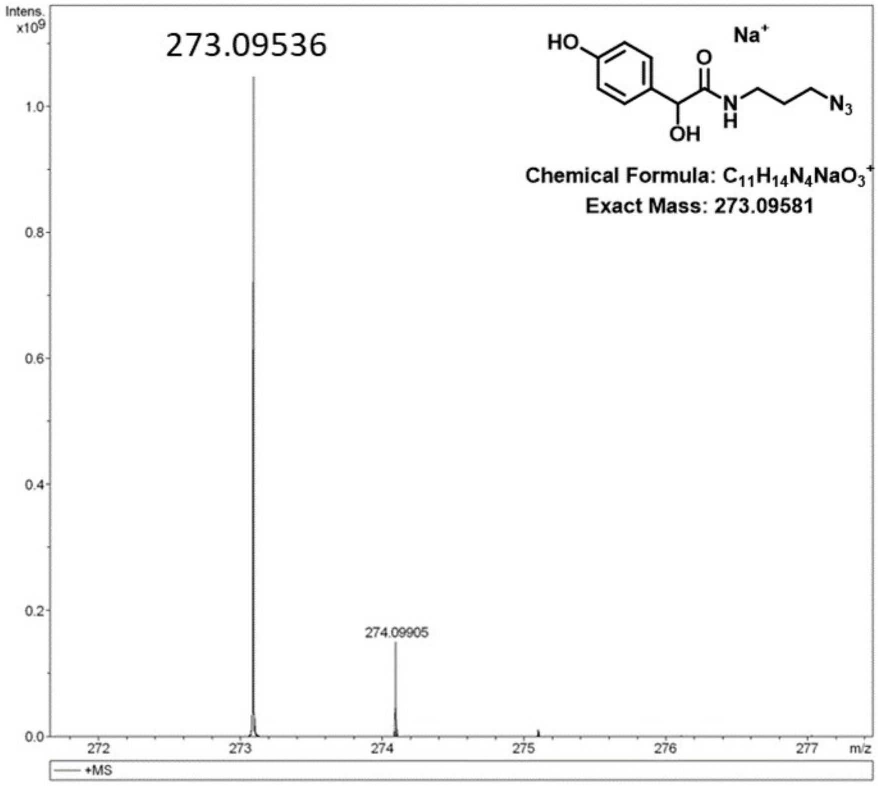


Figure S9. HRMS spectrum of compound **1**

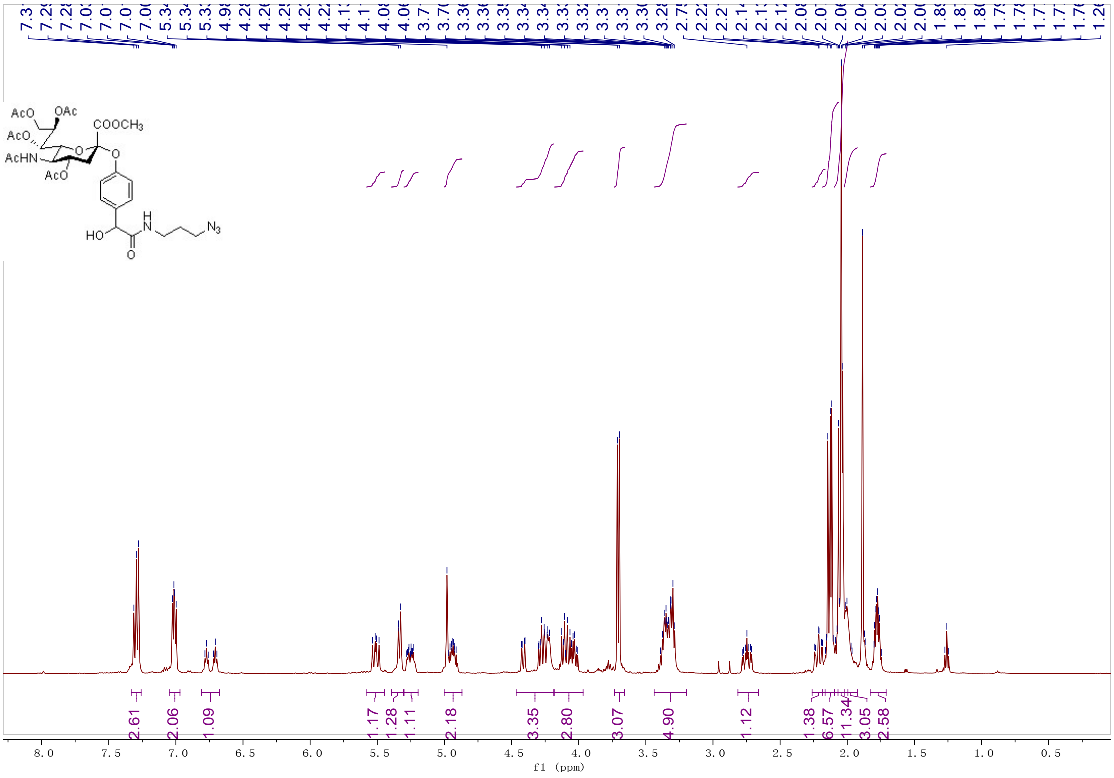


Figure S10. ^{1}H-NMR Spectrum of compound **3**

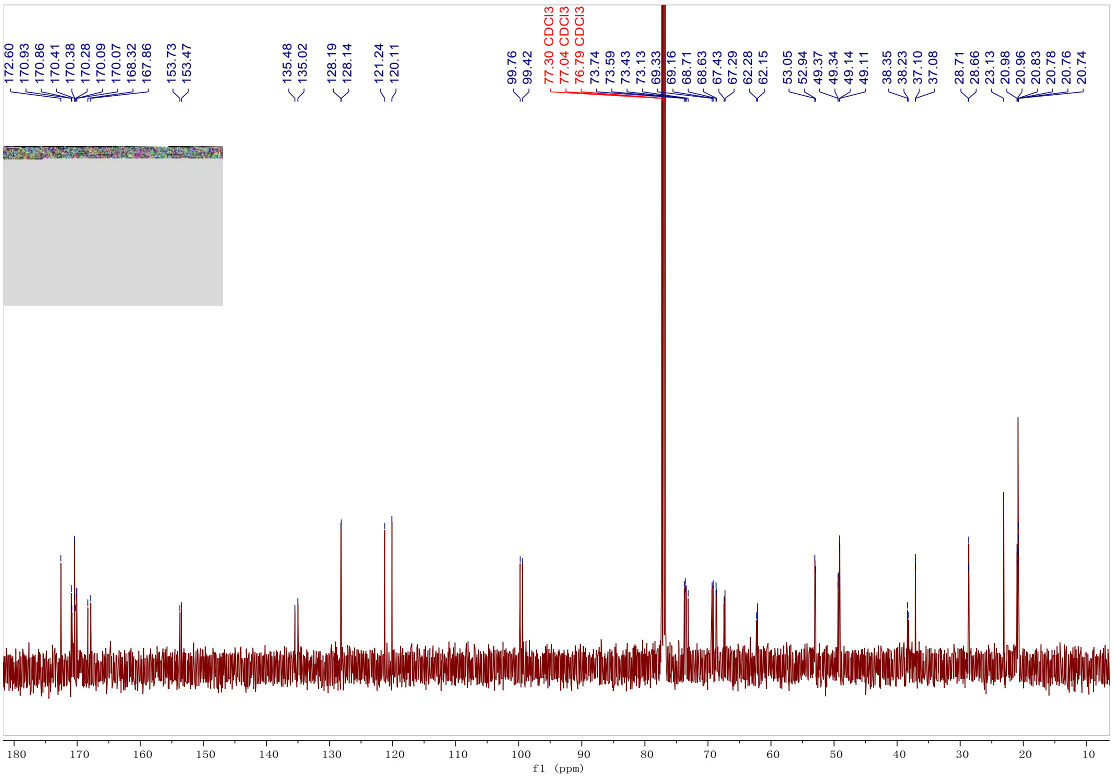


Figure S11. ^{13}C-NMR Spectrum of compound **3**

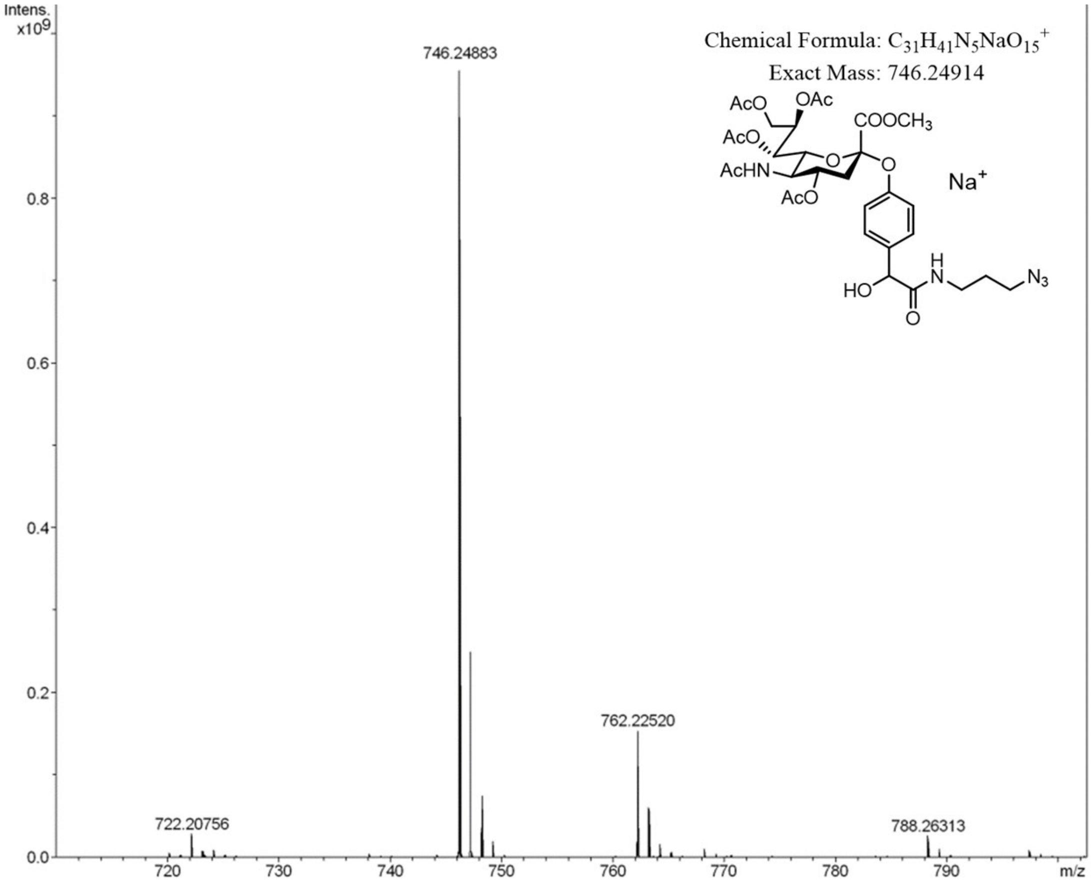


Figure S12. HRMS spectrum of compound **3**

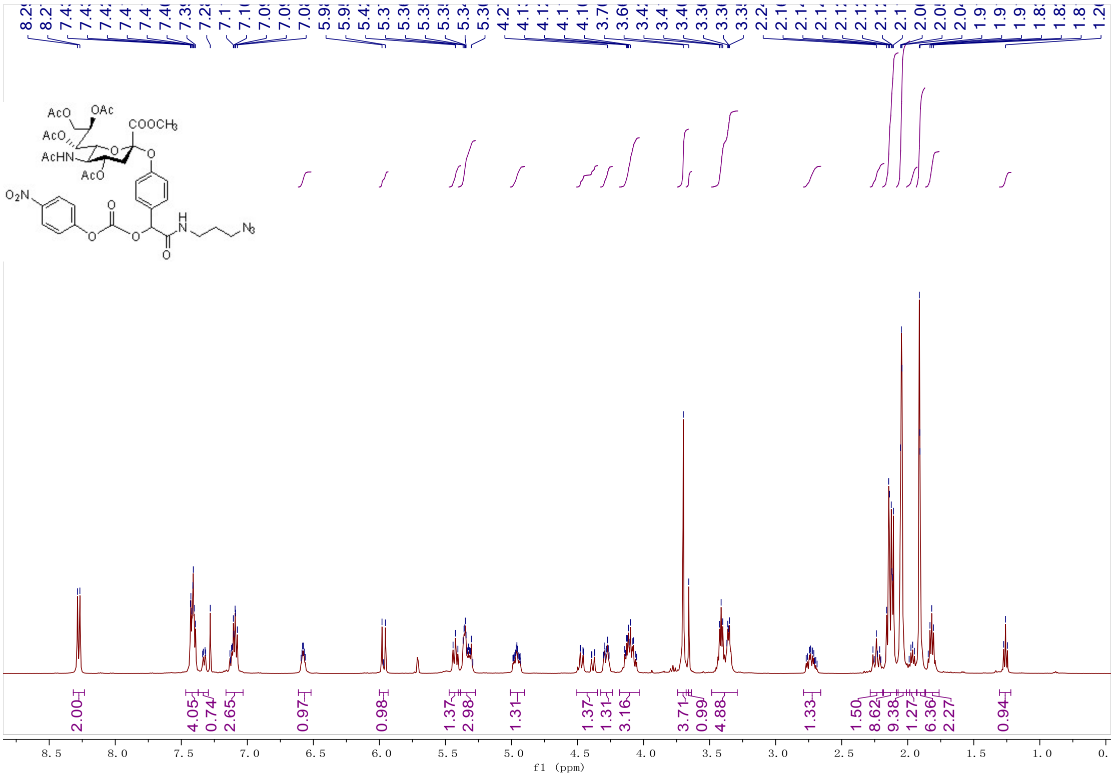


Figure S13. ^{1}H-NMR Spectrum of compound **4**

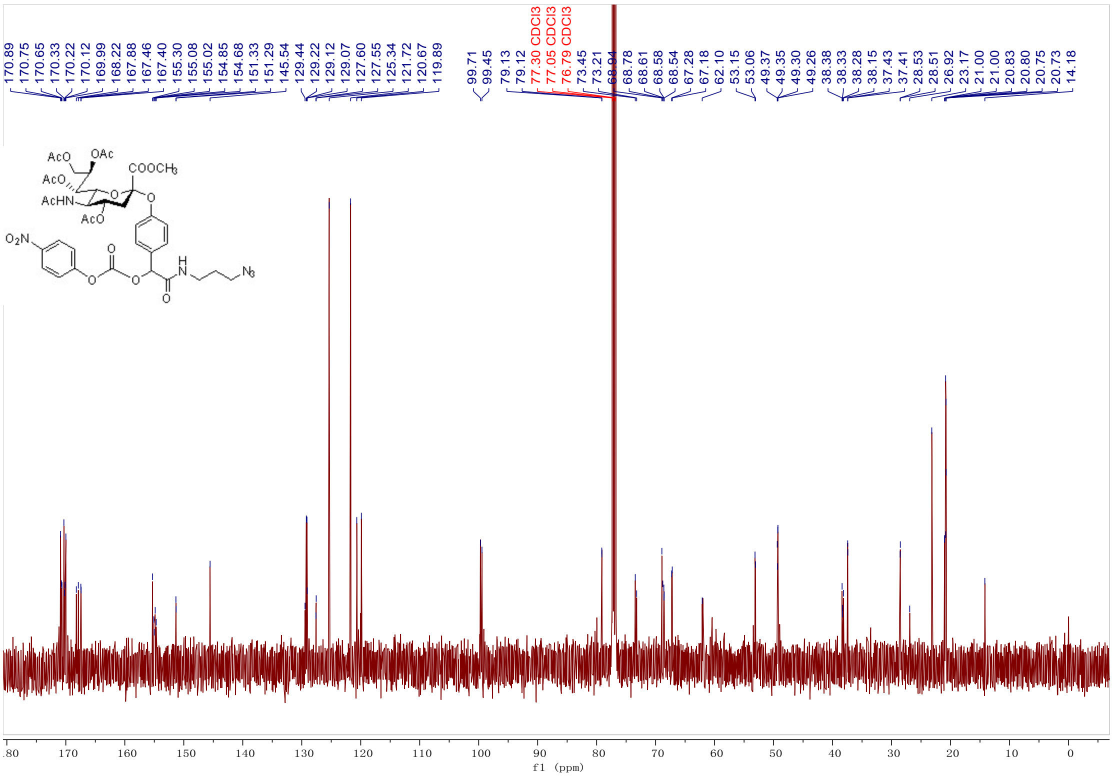


Figure S14. ^{13}C-NMR Spectrum of compound **4**

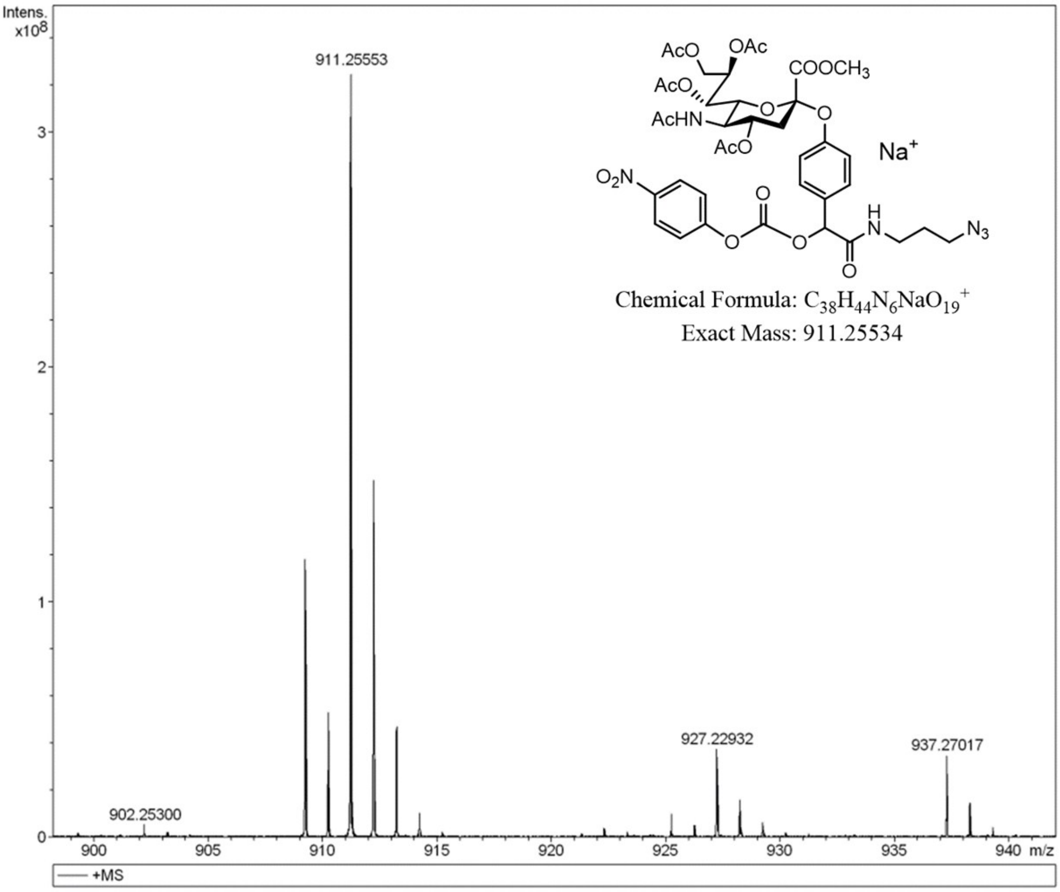


Figure S15. HRMS spectrum of compound **4**

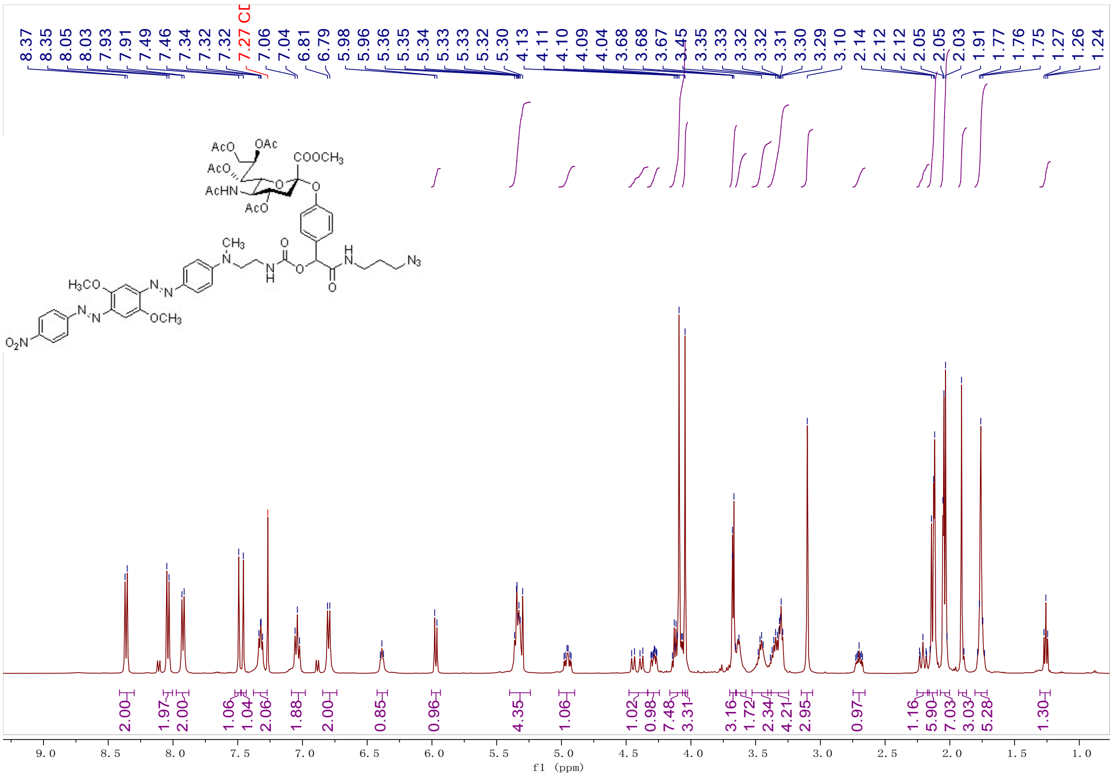


Figure S16. ^{1}H-NMR Spectrum of compound **5**

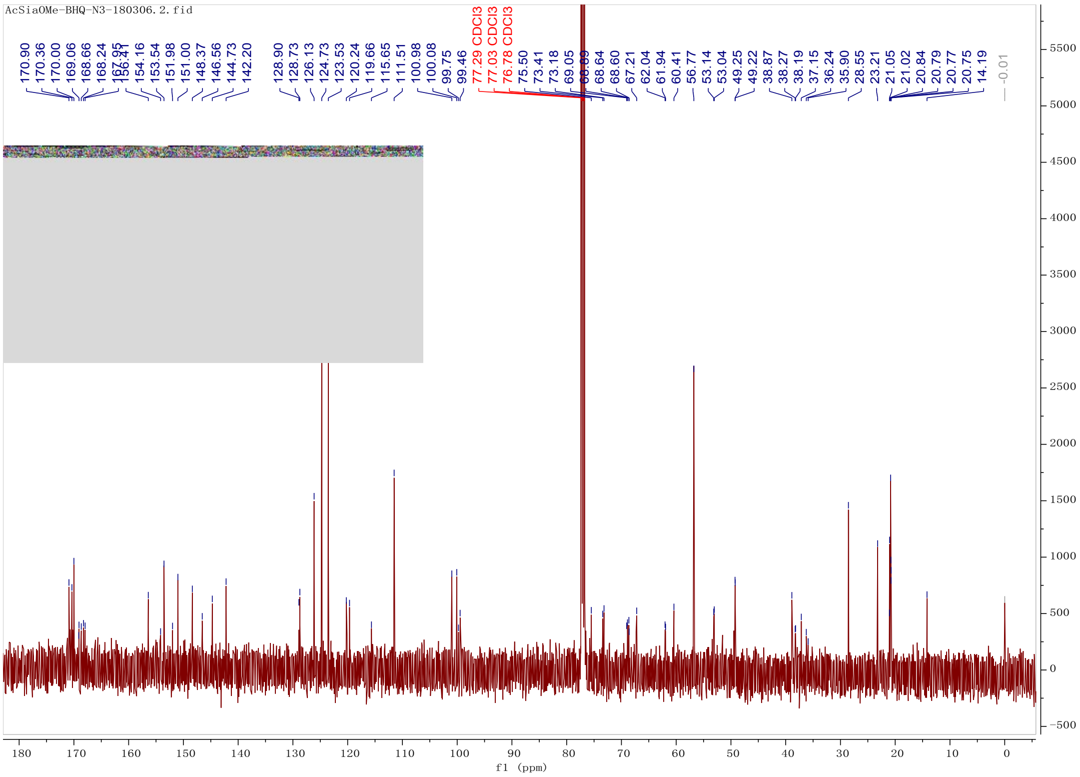


Figure S17. ^{13}C-NMR Spectrum of compound **5**

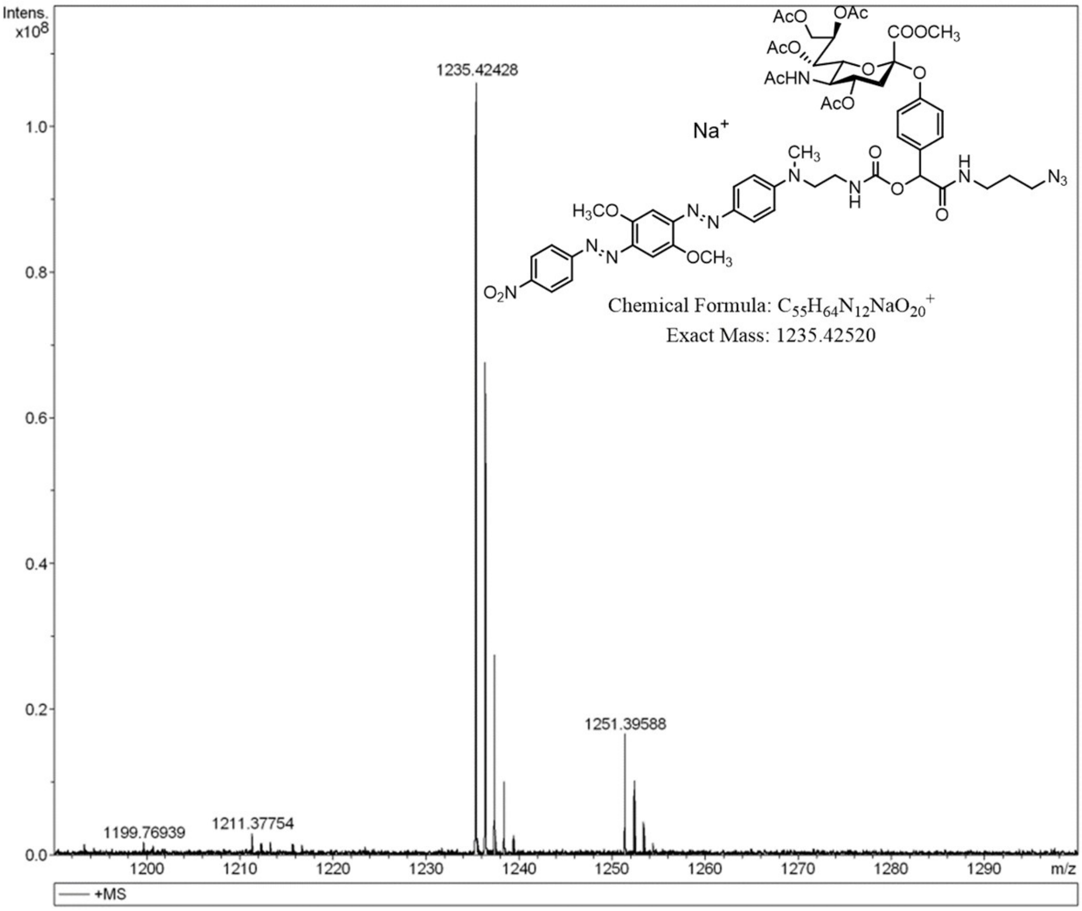


Figure S18. HRMS spectrum of compound **5**

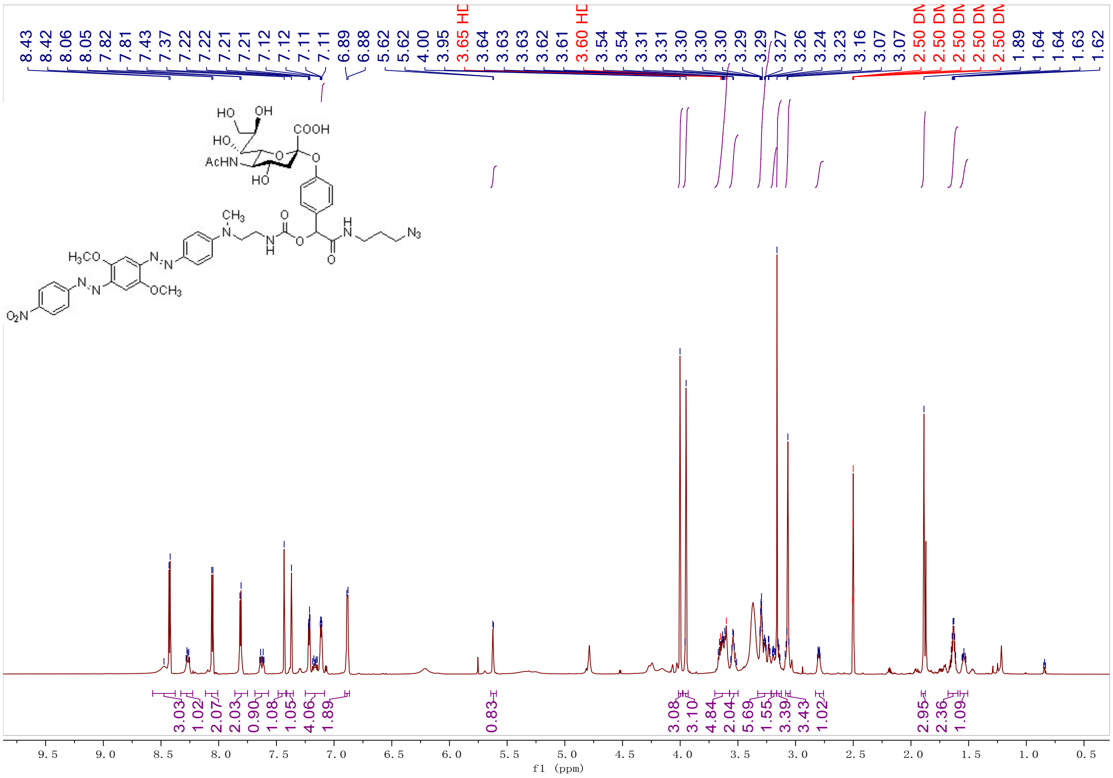


Figure S19. ^{1}H-NMR Spectrum of compound **6**

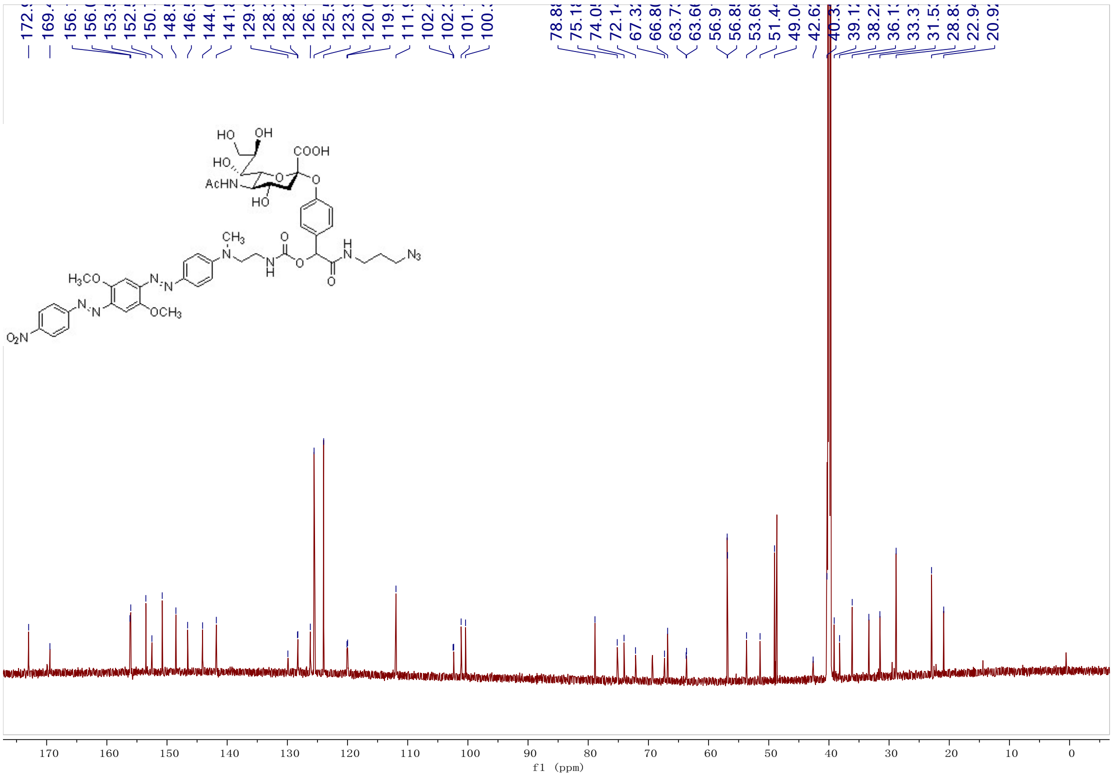


Figure S20. ^{13}C-NMR Spectrum of compound **6**

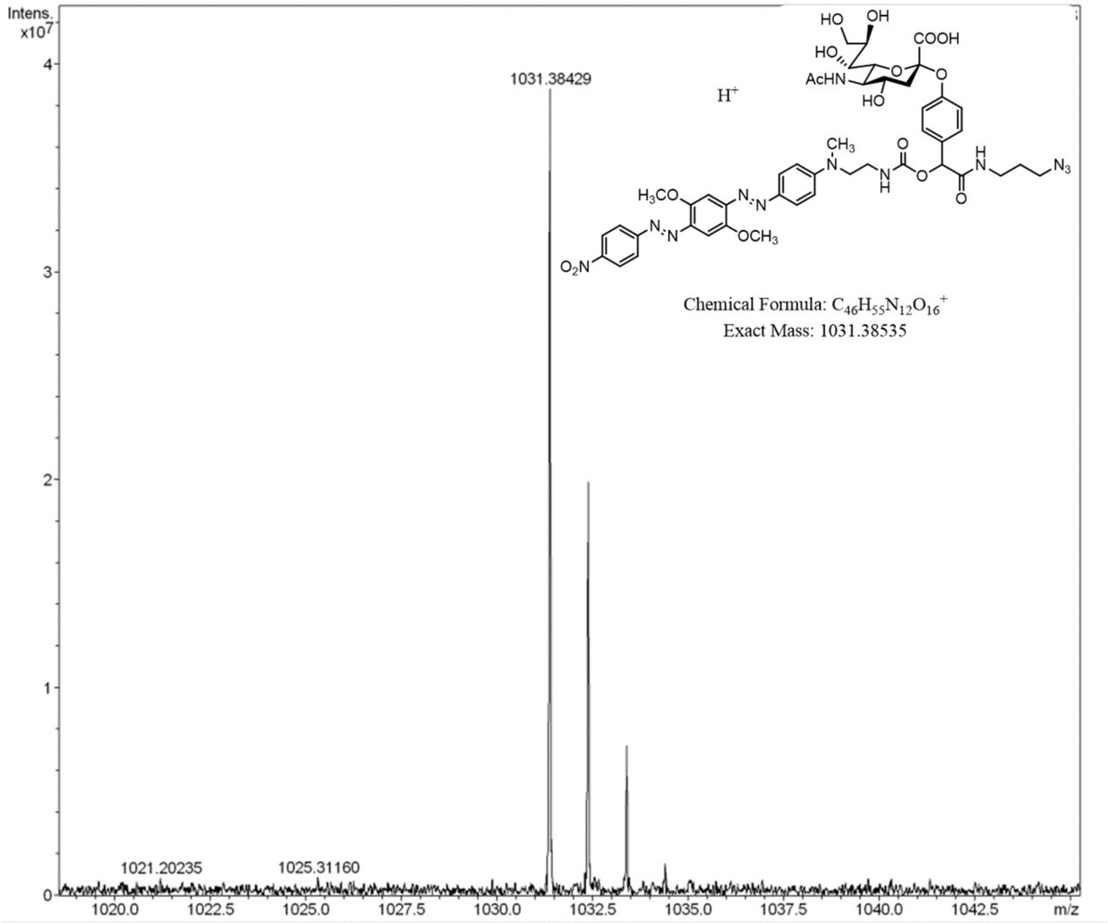


Figure S21. HRMS spectrum of compound **6**

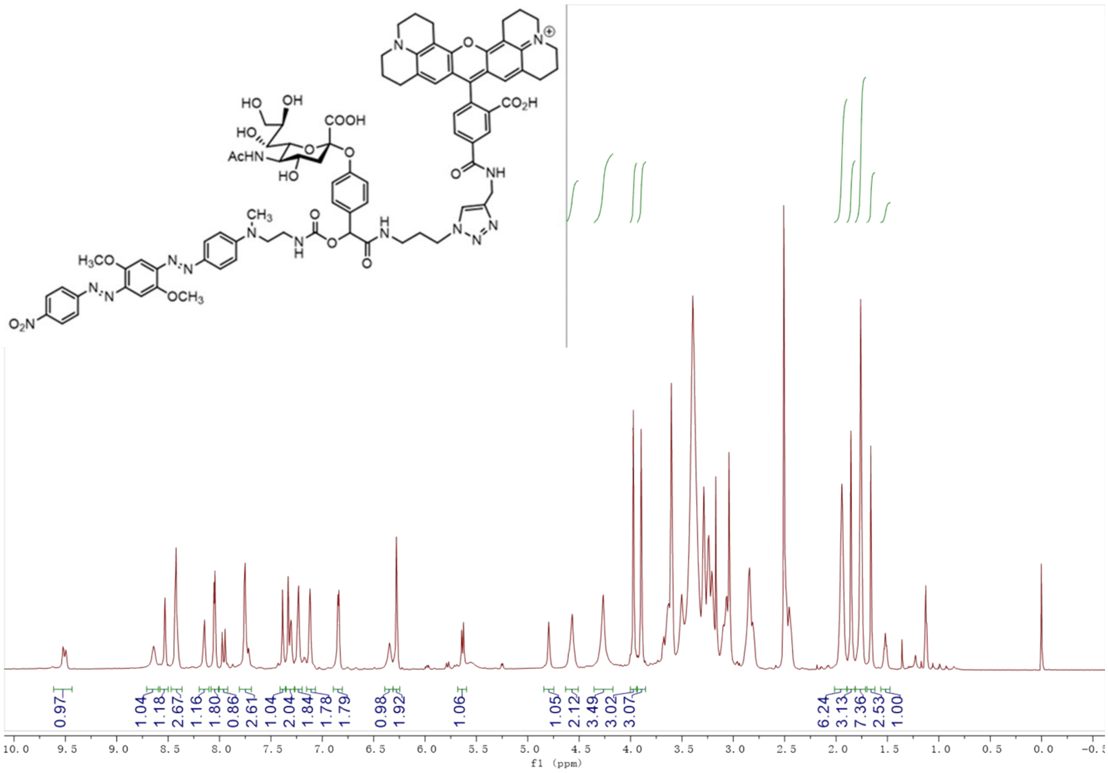


Figure S22. ^{1}H-NMR Spectrum of compound Sia-RQ.

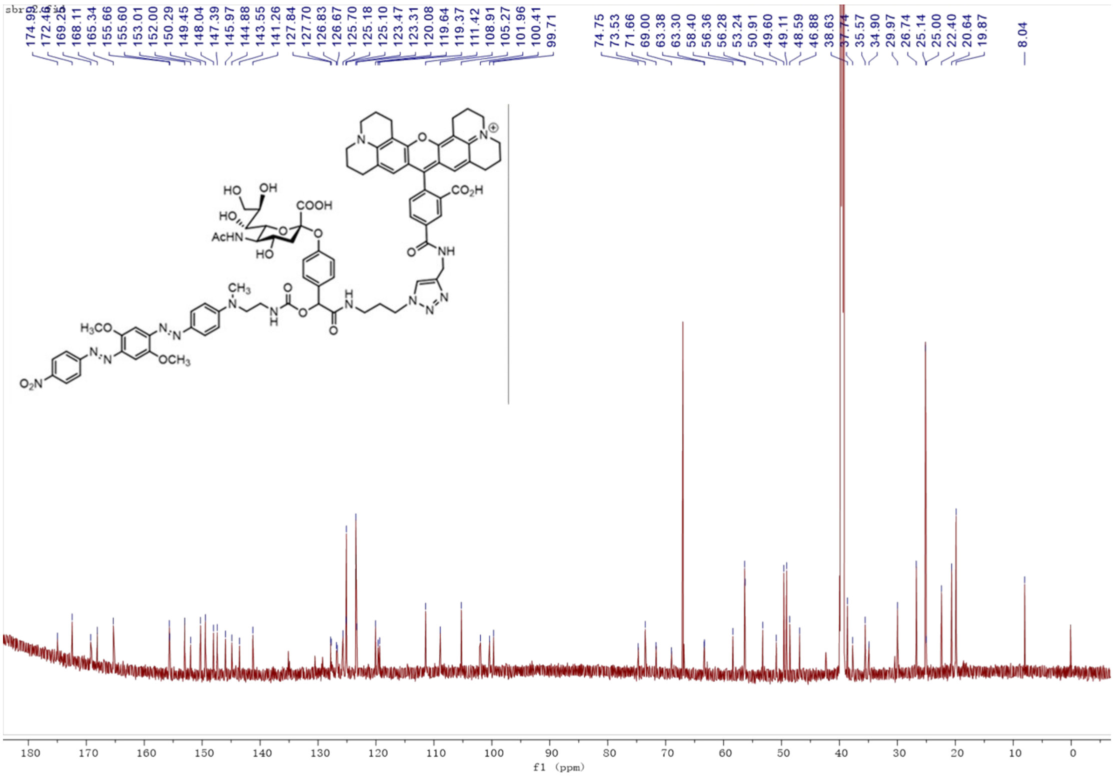


Figure S23. ^{13}C-NMR Spectrum of compound Sia-RQ

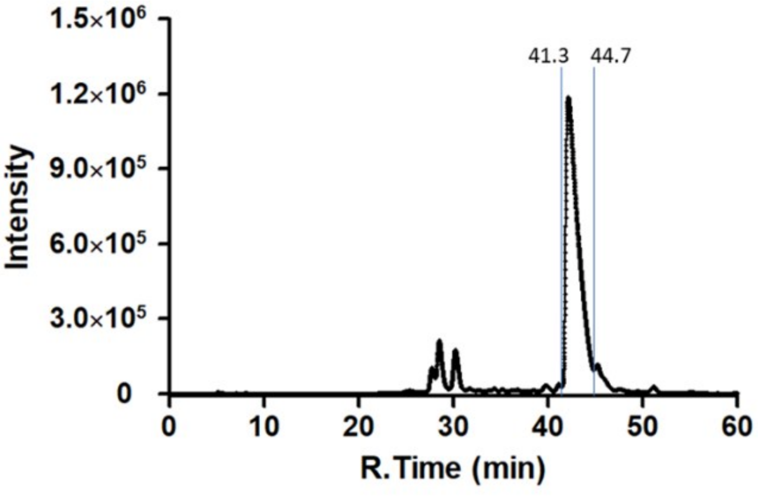


Figure S24. HPLC separation of Sia-RQ.

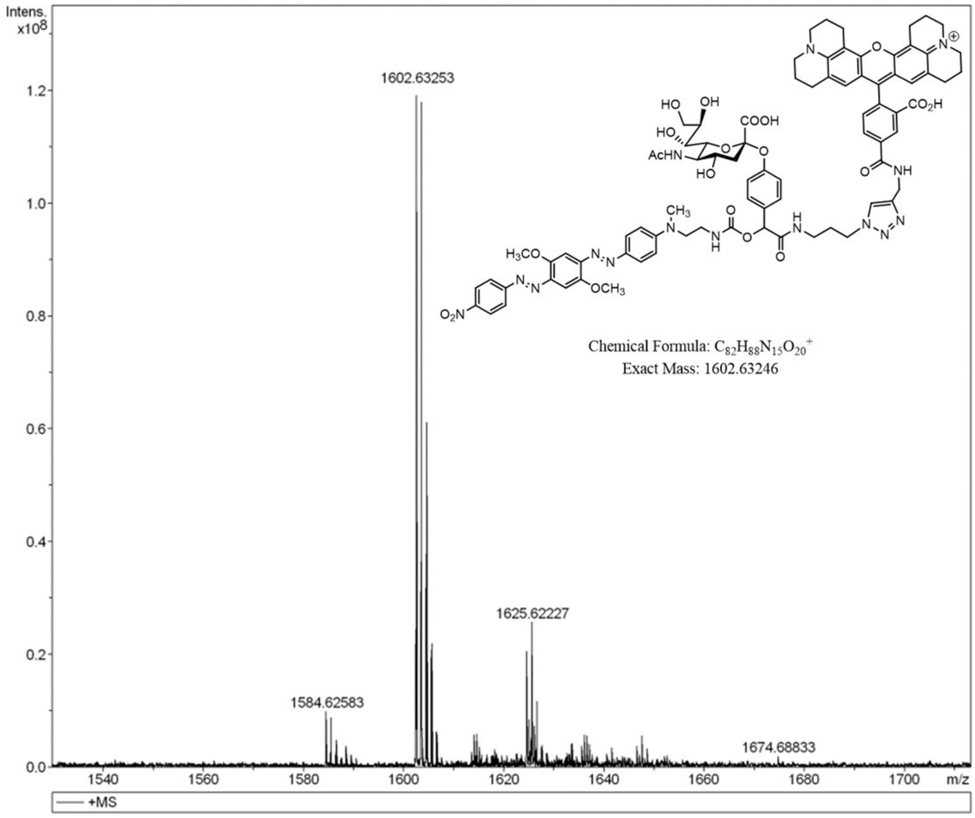


Figure S25. HRMS analysis of Sia-RQ.

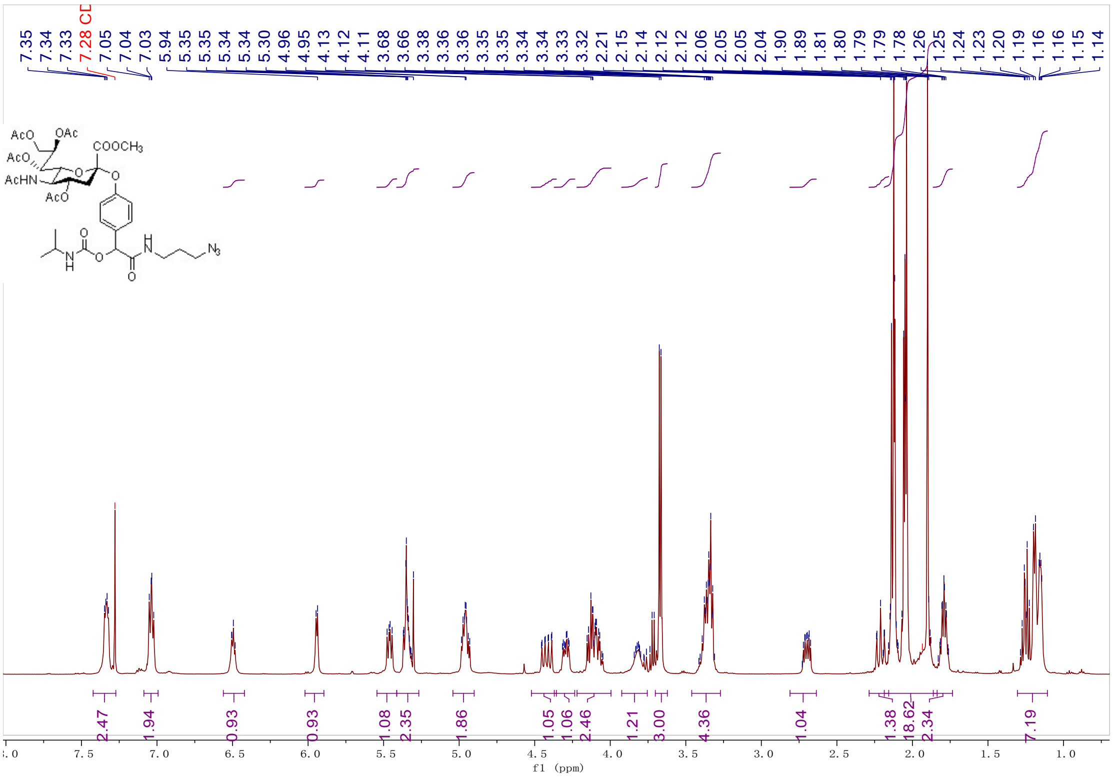


Figure S26. ^{1}H-NMR Spectrum of compound **8**

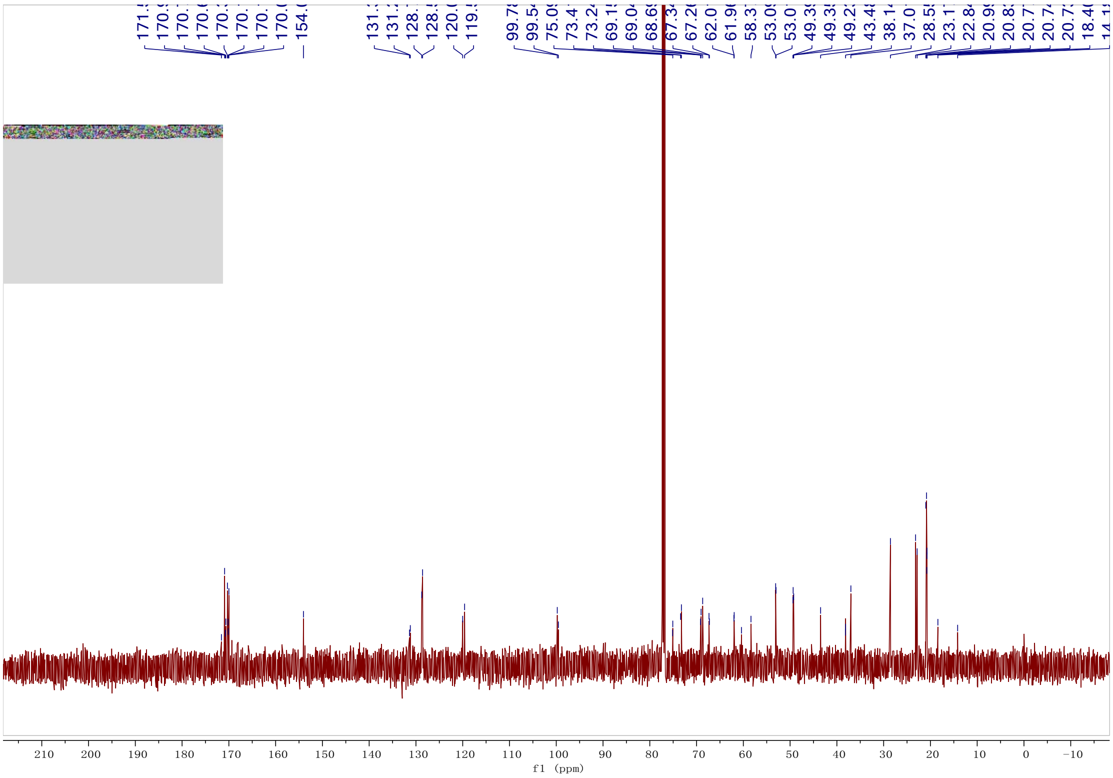


Figure S27. ^{13}C-NMR Spectrum of compound **8**

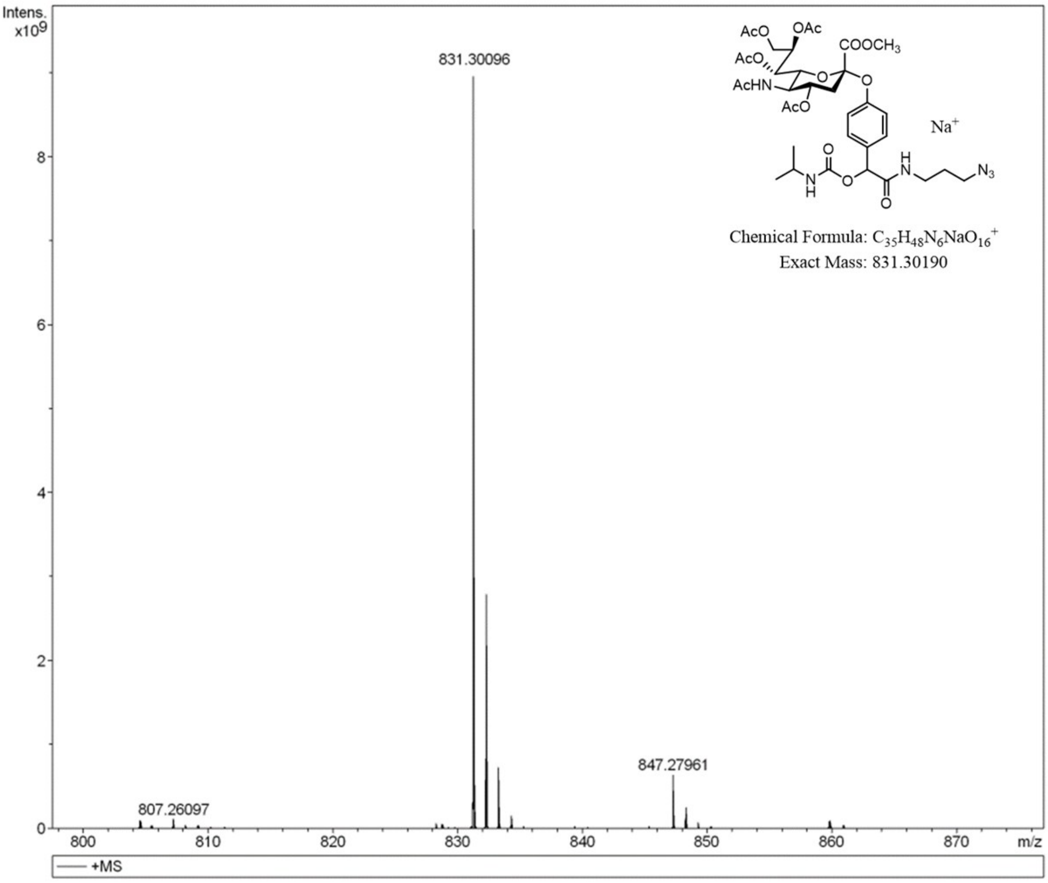


Figure S28. HRMS spectrum of compound **8**

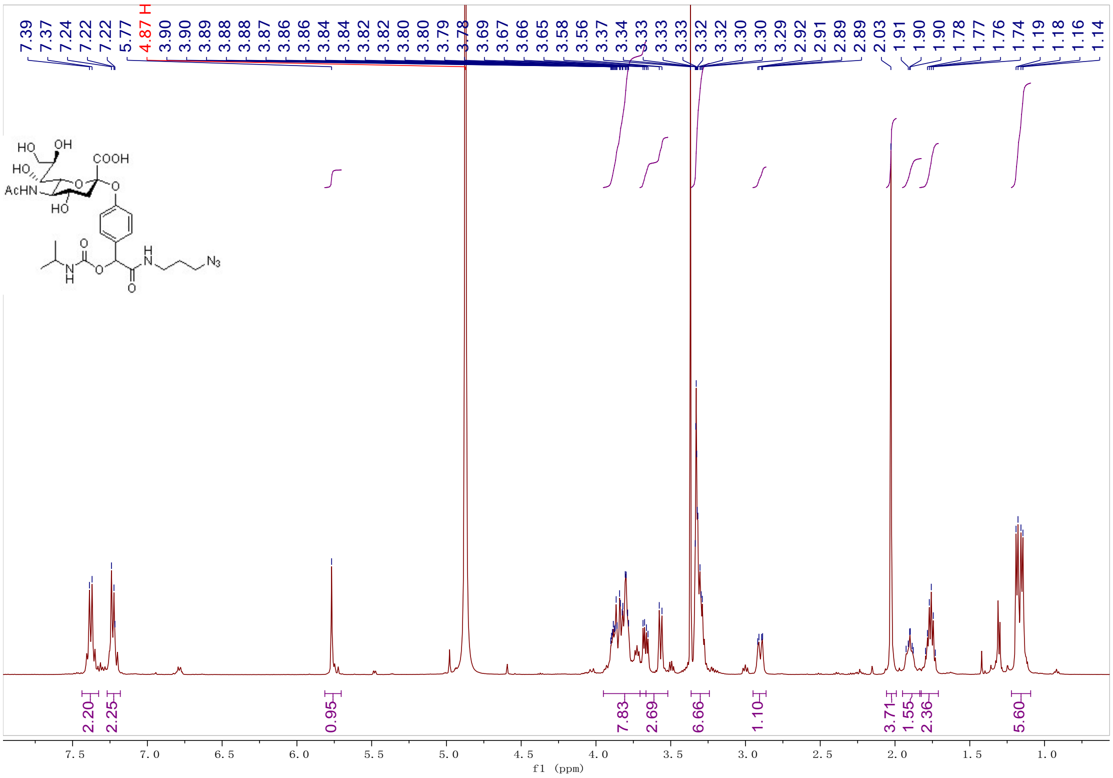


Figure S29. ^{1}H-NMR Spectrum of compound **9**

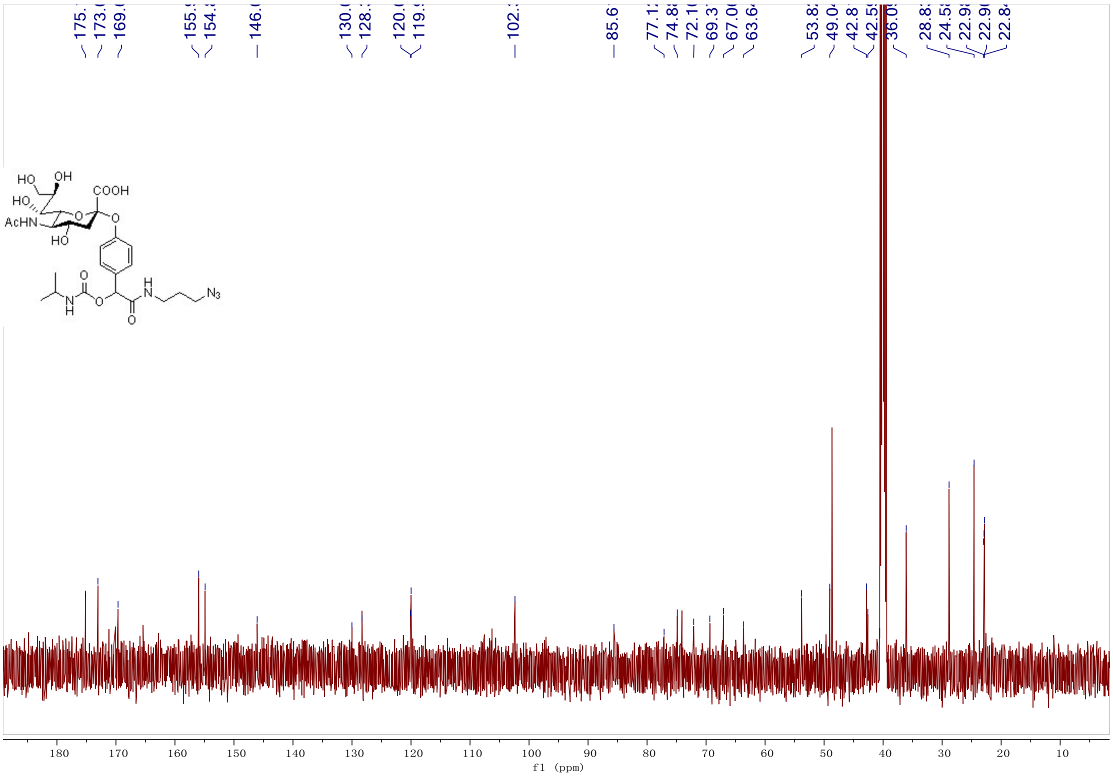
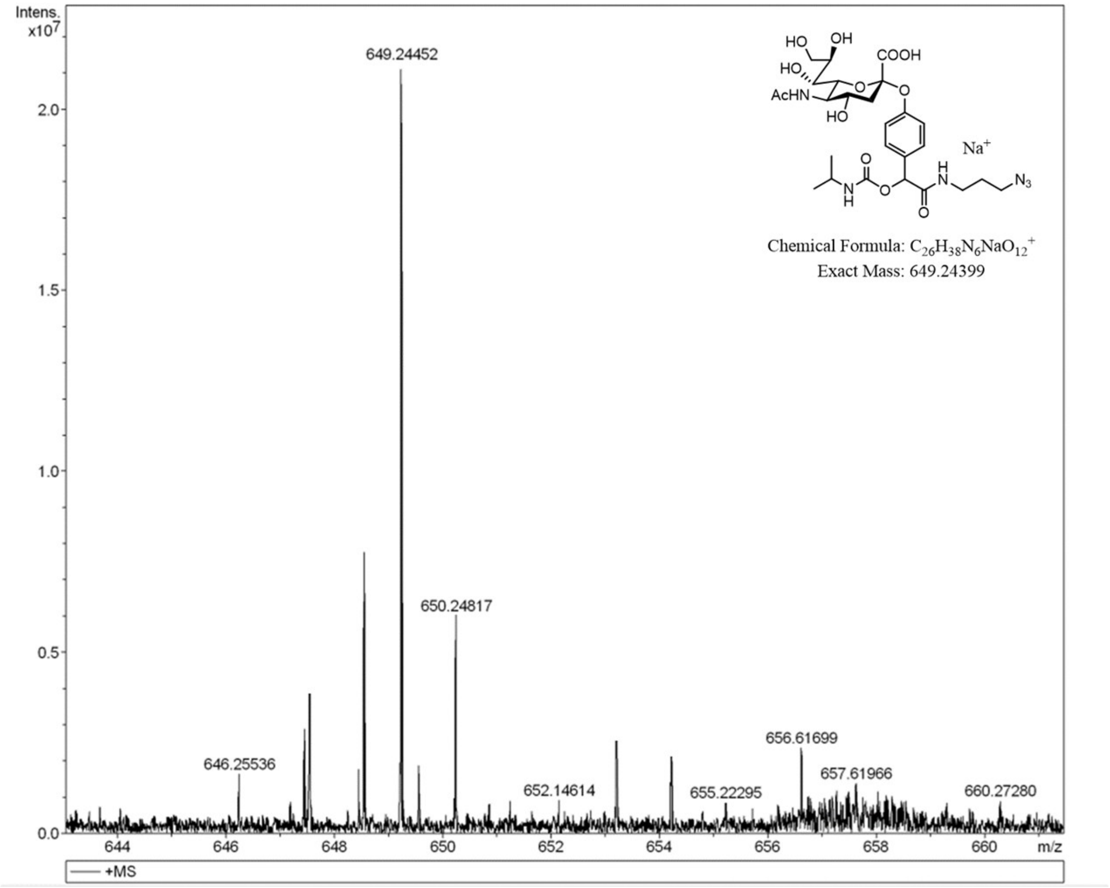


Figure S30. ^{13}C-NMR Spectrum of compound **9**



Chemical Formula: $C_{26}H_{38}N_6NaO_{12}^+$
Exact Mass: 649.24399

Figure S31. HRMS spectrum of compound **9**

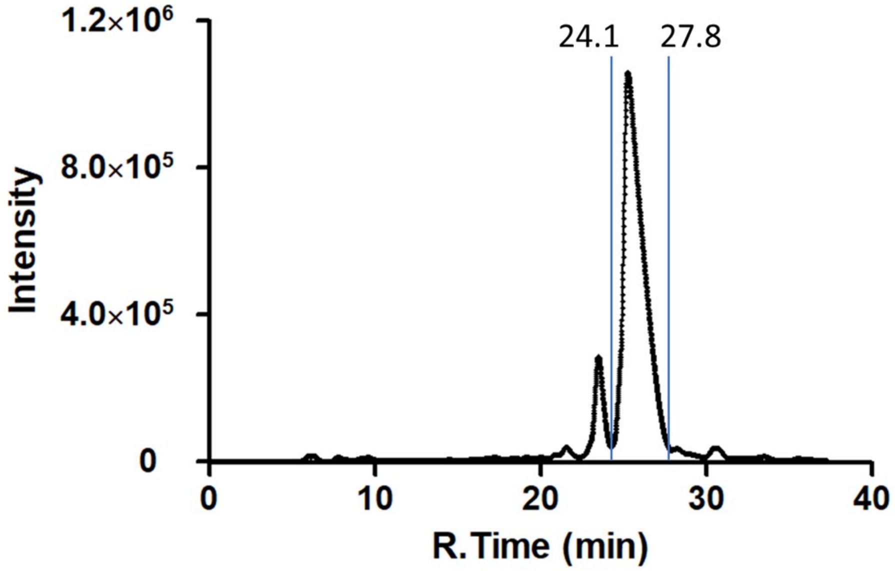


Figure S32. HPLC separation of **Sia-R**.

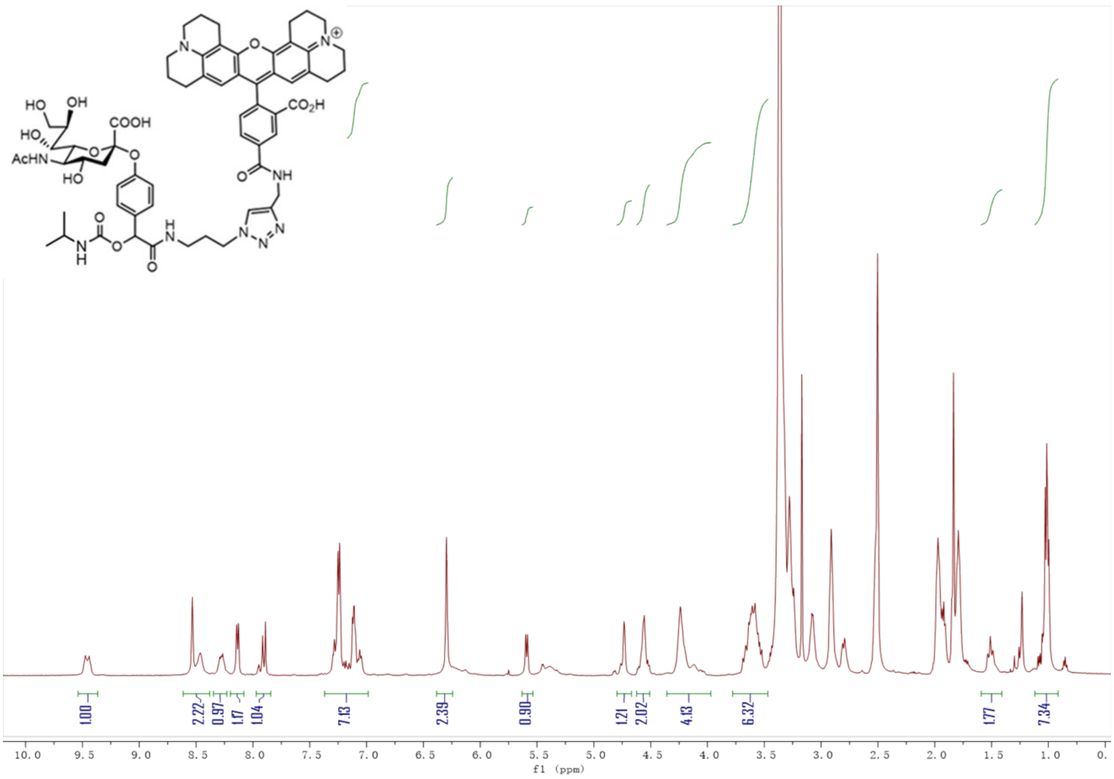


Figure S33. ^{1}H-NMR Spectrum of **Sia-R**

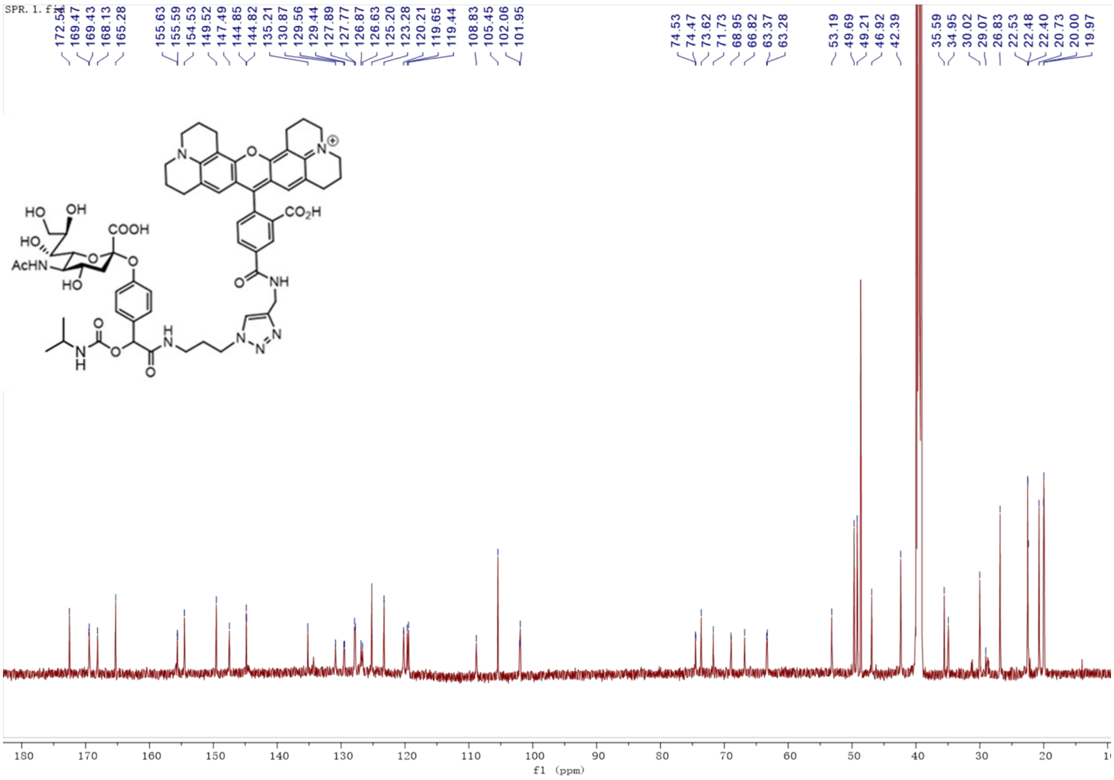


Figure S34. ^{13}C-NMR Spectrum of **Sia-R**

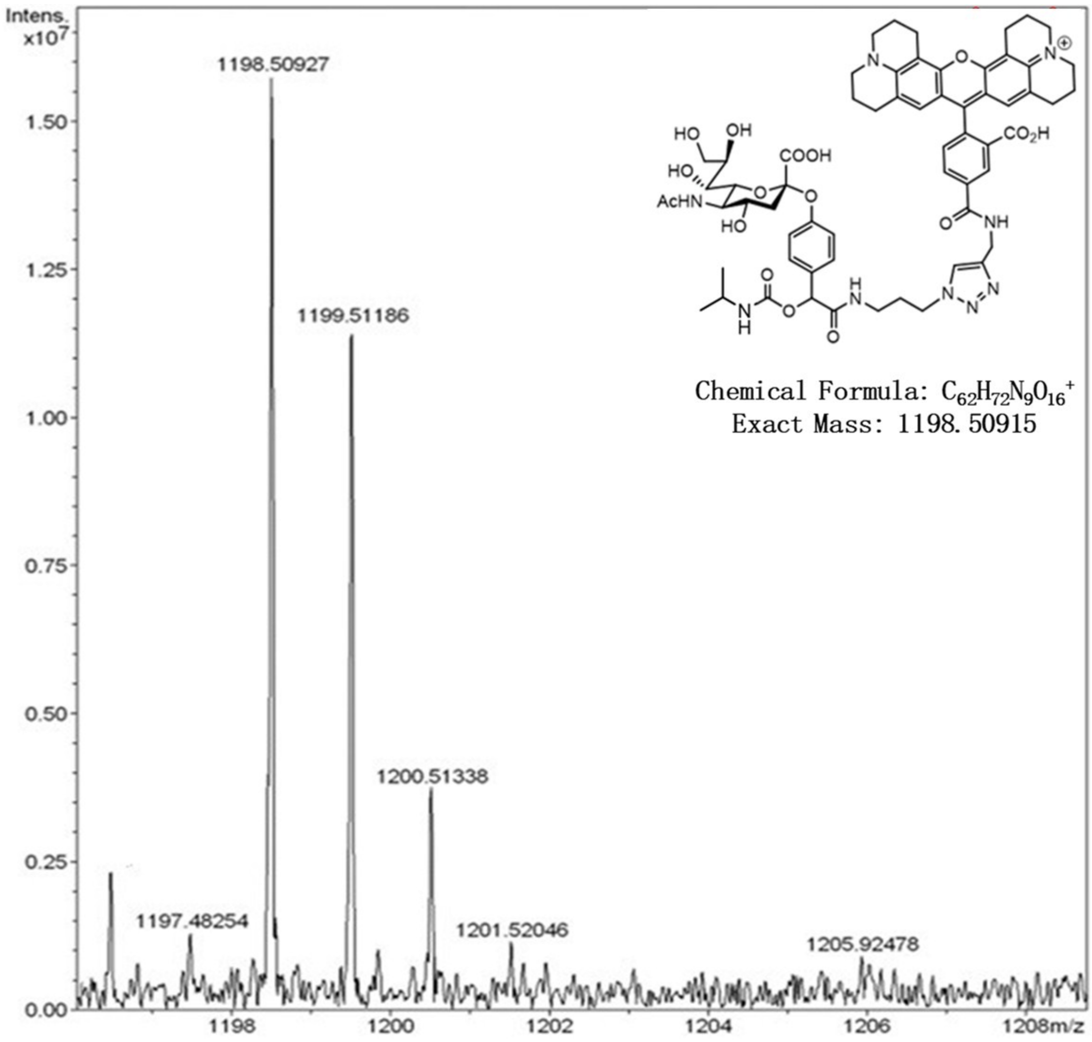


Figure S35. HRMS analysis of **Sia-R**.

Reference

1. Buchini, S. Gallat, F. Greig, I. R. Kim, J., Wakatsuki, S., Chavas, L. M. G., Withers, S. G. *Angew. Chem. Int. Ed*., 2014, **53**, 3382-3386