Article

Supporting Information: A Sialic Acid-Caged Generic Platform for Sialoengineering of Tumors with Artificial Immuno-Ligand

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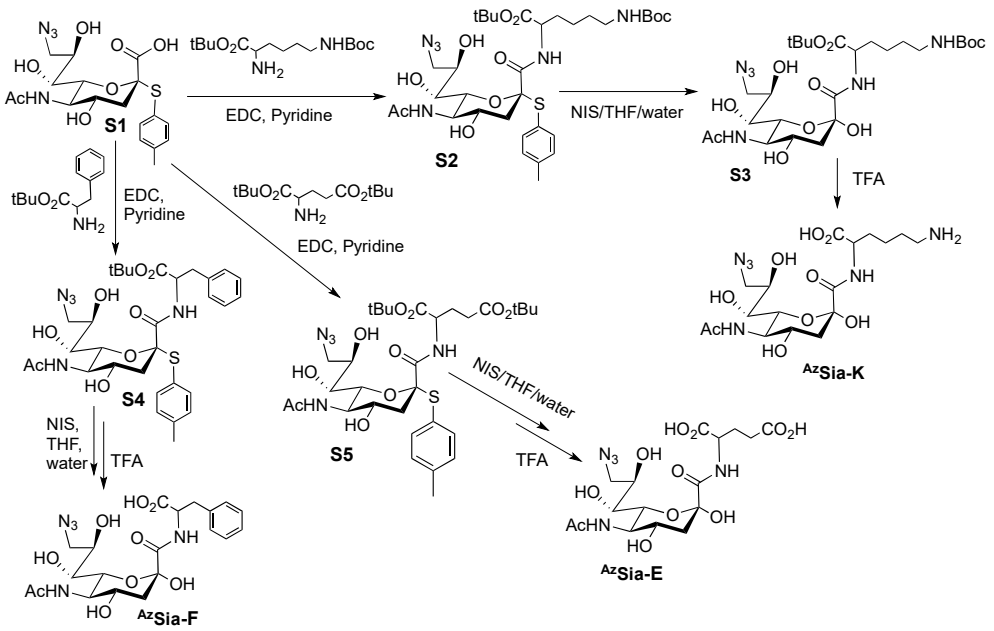
Materials and methods.

A549 (CCL-185™), B16-F10 (CRL-6475™), MCF7 (HTB-22™), HeLa (CCL-2™), Raw 264.7 (TIB-71 ™) and PC3 (CRL-1435 ™) cells were obtained from American Type Culture Collection (ATCC). All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, C11995500CP) containing 10% fetal bovine serum (Thermo, A3160901), 2 mM L-glutamine (Millipore, TMS-002-C), 100 IU penicillin (Gibco, 15140122), and 100 mg/mL streptomycin (cat# A610494-0050, Sangon) at 37°C in a humidified incubator under 5% CO_2, unless specified. Carboxypeptidase B was purchased from ACMEC (C54670). GEMSA was obtained Aladdin (G274649). CCK-8 Cell Counting Kit (A311-01) were purchased from vazyme. β-actin antibody (HRP-66009) and CPB antibody (12600-1-AP) were purchased from Proteintech. CCK-8 Cell Counting Kit (A311-01) were purchased from vazyme. ^{DBCO}FITC, 9-amino-sialic acid, compound **S1**, and *m*-(4-azidophenoxy)-benzoic acid were obstained from Chemedate (Nanjing). All other chemicals were obtained from thermofisher unless specified. Confocal fluorescence microscopic imaging was performed on Zeiss LSM 980 using the following filters: λex = 488 nm and λ_{em} = 499-553 nm for ^{DBCO}FITC. All the cells analysed by confocal microscopy were seeded in 35 mm glass-bottom cell culture dishes purchased from NEST, Wuxi. Flow cytometry analysis was performed on BD Fortessa. 10,000 Cells were gated and analysed under identical conditions. The data were processed by FlowJo V10. Graphs were generated by GraphPad Prism 8 software and Origin 9 software. Statistical analysis was performed with Prism software (GraphPad Software). Data are presented as the means ± SEM. Unpaired two-tailed Student's t-test was used to compare differences between treated groups and their paired controls. Differences in compared groups with P values lower than 0.05 were considered statistically significant. ns, non-significant; *P ≤ 0.05, **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001.

Synthesis of C1-caged Sia



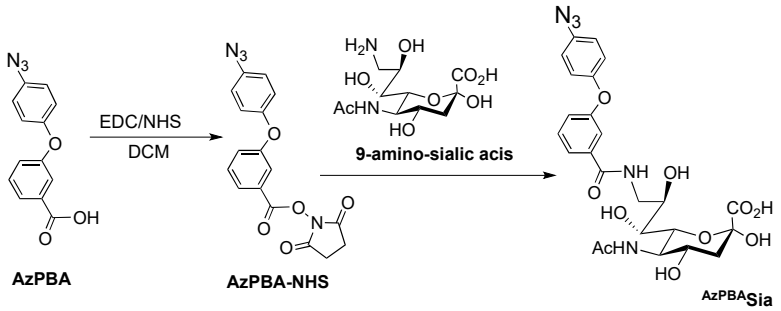
Scheme S1. Synthetic routes for ^{Az}Sia-K, ^{Az}Sia-F and ^{Az}Sia-E

Synthesis of ^{Az}Sia-K. To the solution of **S1** (1.20 g, 2.73 mmol) and N^6-[(1,1-dimethylethoxy)carbonyl]-L-lysine 1,1-dimethylethyl ester (989 mg, 3.28 mmol) in pyridine (40.0 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 1.05 g, 5.46 mmol). The mixture was stirred overnight and then concentrated in vacuo. The resulting residue was purified by silica gel chromatography (CH_2Cl_2/MeOH, 50:1) to afford **S2** as white solid in 70% yield (1.38 g). ^{1}H NMR (500 MHz, CD_3OD) δ 7.49 – 7.44 (m, 2H), 7.22 (d, *J* = 8.0 Hz, 2H), 3.99 (ddd, *J* = 9.3, 6.5, 2.6 Hz, 1H), 3.83–3.74 (m, 2H), 3.73 – 3.60 (m, 3H), 3.53 – 3.44 (m, 2H), 3.08–2.89 (m, 3H), 2.38 (s, 3H), 2.04 (s, 3H), 1.71 (dd, *J* = 12.7, 11.3 Hz, 1H), 1.47 (d, *J* = 2.6 Hz, 18H), 1.35 (dt, *J* = 18.0, 9.3 Hz, 3H), 1.29–1.19 (m, 1H), 1.03 – 0.85 (m, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 174.36, 171.89, 168.92, 157.04, 140.15, 136.55, 129.38, 126.21, 87.83, 81.51, 78.58, 75.88, 70.38, 69.45, 66.80, 54.25, 53.94, 52.40, 39.88, 39.26, 29.58, 29.06, 27.45, 26.91, 26.89, 22.76, 21.07, 20.12. MALDI-TOF MS calc'd for $C_{33}H_{52}N_6O_{10}S$ (M+Na) *m/z* 747.34, found 747.25.
To the solution of **S2** (1.00 g, 1.38 mmol) in tetrahydrofuran (THF, 20 mL) and water (2 mL) was added *N*-iodosuccinimide (NIS, 932 mg, 4.14 mmol). The mixture was stirred at room temperature for 10 min and then concentrated in vacuo. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH, 10:1), yielding **S3** as white solid (723 mg, 85%). MALDI-TOF MS calc'd for $C_{26}H_{46}N_6O_{11}$ (M+Na) *m/z* 641.31, found 641.46. The solution of **S3** (70 mg, 0.11 mmol) in trifluoroacetic acid (5 mL) was stirred at room temperature for 10 min and then concentrated in vacuo to afford ^{Az}Sia-K as a pale yellow solid (94%, 49 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 4.47 (dd, *J* = 9.5, 4.8 Hz, 1H), 4.12 (td, *J* = 10.6, 4.9 Hz, 1H), 4.08 – 3.98 (m, 1H), 3.93 (ddd, *J* = 9.2, 6.6, 2.6 Hz, 1H), 3.82 (t, *J* = 10.3 Hz, 1H), 3.67 – 3.51 (m, 2H), 3.44 (dd, *J* = 12.8, 6.6 Hz, 1H), 3.03 – 2.85 (m, 2H), 2.33 (dd, *J* = 12.8, 4.9 Hz, 1H), 2.06 (s, 3H), 2.05 – 1.96 (m, 1H), 1.83 (dtd, *J* = 14.4, 9.4, 5.4 Hz, 1H), 1.72 (dtd, *J* = 15.7, 9.0, 6.7 Hz, 2H), 1.67 – 1.57 (m, 1H), 1.50 (qdd, *J* = 15.8, 8.6, 5.3 Hz, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 173.99, 173.48, 172.33, 95.63, 70.71, 69.52, 69.47, 66.22, 54.17, 52.98, 51.78, 51.76, 40.88, 39.11, 30.52, 26.62, 22.56, 21.27. HRMS (ESI) calc'd for $C_{17}H_{30}N_6O_9$ (M+H) *m/z* = 463.2148, found 463.2151.

Synthesis of ^{Az}Sia-F. To the solution of **S1** (400 mg, 0.91 mmol) and L-phenylalanine tert-butyl ester (241 mg, 1.09 mmol) in pyridine (20.0 mL) was added EDC (349 mg, 1.82 mmol). The mixture was stirred overnight at room temperature then concentrated in vacuo. The residue was dissolved in DCM (50 mL). The organic solution was washed with 1 M HCl aqueous solution (40 mL), dried over anhydrous Na_2SO_4, and then concentrated. The crude was purified by silica gel chromatography (CH_2Cl_2/MeOH, 30:1), yielding **S4** as white solid (497 mg, 85%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.49 – 7.44 (m, 2H), 7.29 (dd, *J* = 8.1, 6.5 Hz, 2H), 7.24 (dd, *J* = 7.7, 2.9 Hz, 3H), 7.09 (dd, *J* = 7.0, 1.8 Hz, 2H), 3.99 (ddd, *J* = 9.0, 6.1, 2.6 Hz, 1H), 3.87 (dd, *J* = 10.7, 5.7 Hz, 1H), 3.80 – 3.69 (m, 2H), 3.65 (dd, *J* = 13.1, 2.6 Hz, 1H), 3.58 (ddd, *J* = 11.3, 9.4, 4.7 Hz, 1H), 3.52 (dd, *J* = 9.4, 1.8 Hz, 1H), 3.47 (dd, *J* = 13.1, 6.1 Hz, 1H), 2.95 (dd, *J* = 12.7, 4.6 Hz, 1H), 2.60 (dd, *J* = 13.6, 10.7 Hz, 1H), 2.32 (s, 3H), 2.26 (dd, *J* = 13.6, 5.8 Hz, 1H), 2.03 (s, 3H), 1.69 (dd, *J* = 12.7, 11.3 Hz, 1H), 1.18 (s, 9H). ^{13}C NMR (126 MHz, CD_3OD) δ 174.33, 171.31, 168.37, 140.25, 136.69, 136.49, 129.29, 128.89, 128.07, 126.52, 126.42, 88.14, 81.40, 75.90, 70.38, 69.26, 66.98, 55.58, 55.54, 53.93, 52.26, 39.55, 36.56, 26.60, 26.58, 21.12, 20.00, 19.97. MALDI-TOF MS calc'd for $C_{31}H_{41}N_5O_8S$ (M+Na) *m/z* 666.26, found 666.12. ^{Az}Sia-F was synthesized form **S4** (94%, 49 mg) using a procedure identical to ^{Az}Sia-K as aforementioned**.** ^{1}H NMR (500 MHz, CD_3OD) δ 7.37 – 7.24

(m, 4H), 7.22 (t, *J* = 7.0 Hz, 1H), 4.69 (dd, *J* = 9.7, 4.7 Hz, 1H), 4.11 – 3.97 (m, 2H), 3.89 (ddd, *J* = 9.2, 6.5, 2.6 Hz, 1H), 3.77 (t, *J* = 10.3 Hz, 1H), 3.64 – 3.47 (m, 2H), 3.44 (dd, *J* = 12.8, 6.6 Hz, 1H), 3.37 (s, 1H), 3.32 – 3.21 (m, 1H), 3.06 (dd, *J* = 13.9, 9.7 Hz, 1H), 2.11 – 2.07 (m, 1H), 2.05 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 173.95, 173.03, 171.88, 137.10, 129.05, 128.99, 128.90, 128.16, 126.49, 95.59, 70.72, 69.52, 69.40, 66.19, 54.19, 53.48, 52.90, 52.89, 48.15, 48.09, 47.98, 47.81, 47.64, 47.47, 47.34, 47.30, 47.13, 40.62, 36.66, 21.29. HRMS (ESI) calc'd for $C_{20}H_{27}N_5O_9$ (M+Na) *m/z* = 504.1701, found 504.1702.

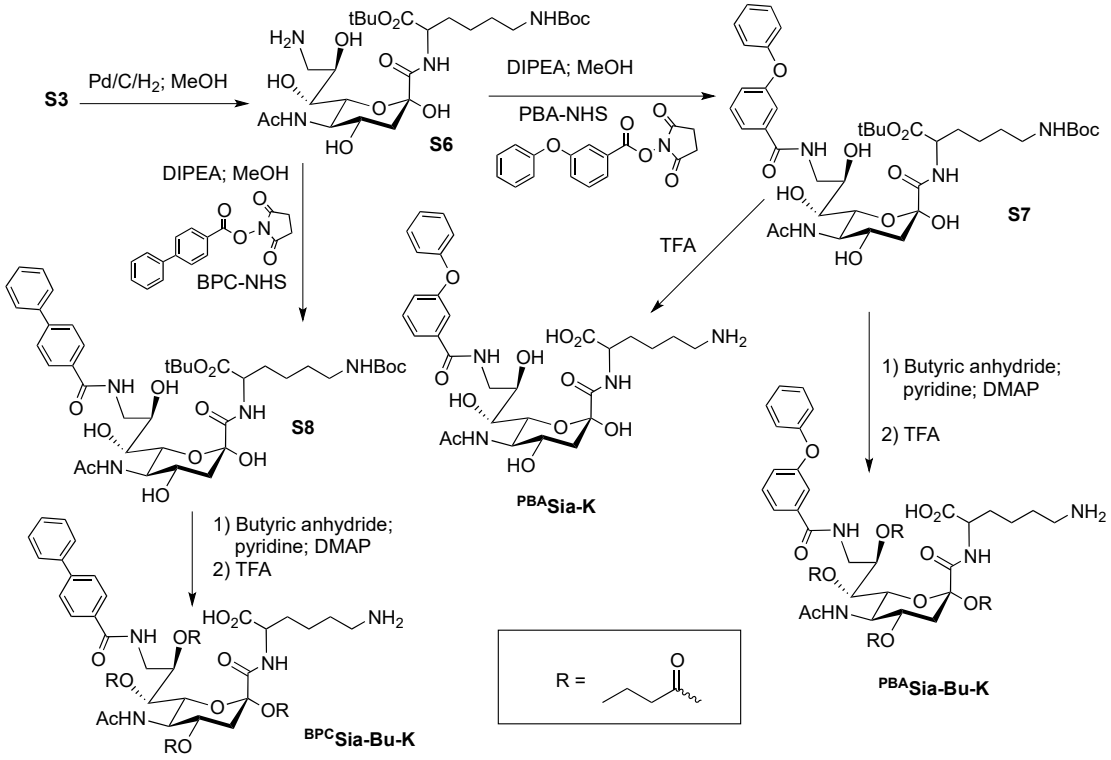
Synthesis of ^{Az}Sia-E. To the solution of **S1** (400 mg, 0.91 mmol) and L-glutamic acid di-tert-butyl ester hydrochloride (322 mg, 1.09 mmol) in pyridine (20.0 mL) was added EDC (349 mg, 1.82 mmol). The mixture was stirred overnight at room temperature and then concentrated in vacuo. The residue was trituated between DCM (50 ml) and 1 M HCl aqueous solution (40 mL). The organic phase was dried over anhydrous Na_2SO_4, and then concentrated. The crude residue was purified by silica gel chromatography (CH_2Cl_2/MeOH, 25:1) to afford **S5** as white solid (544 mg, 88%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.50 – 7.45 (m, 2H), 7.23 (d, *J* = 7.7 Hz, 2H), 3.98 (ddd, *J* = 9.5, 7.0, 2.6 Hz, 1H), 3.83 – 3.74 (m, 3H), 3.65 (ddd, *J* = 15.7, 12.1, 3.3 Hz, 2H), 3.51 – 3.45 (m, 1H), 3.39 (dt, *J* = 20.9, 6.1 Hz, 2H), 3.00 (dd, *J* = 12.7, 4.6 Hz, 1H), 2.40 (s, 3H), 2.05 (s, 3H), 1.86 – 1.69 (m, 4H), 1.49 (d, *J* = 9.5 Hz, 18H). ^{13}C NMR (126 MHz, CD_3OD) δ 174.40, 172.21, 171.43, 169.26, 140.38, 136.57, 129.43, 126.09, 87.67, 81.73, 80.49, 75.88, 70.34, 69.53, 66.82, 53.92, 53.62, 52.40, 48.12, 47.95, 47.78, 47.61, 47.61, 47.44, 47.27, 47.10, 39.92, 31.18, 27.03, 26.89, 25.25, 21.07, 20.21, 20.18. MALDI-TOF MS calc'd for $C_{31}H_{47}N_5O_{10}S$ (M+Na) *m/z* 704.29, found 704.20. ^{Az}Sia-E was prepared from **S5** (400 mg, 0.59 mmol) in an identical procedure as that of ^{Az}Sia-K**.** ^{1}H NMR (500 MHz, CD_3OD) δ 4.46 (td, *J* = 9.7, 4.9 Hz, 1H), 4.12 – 3.99 (m, 2H), 3.92 (dtd, *J* = 8.4, 6.1, 2.6 Hz, 1H), 3.89 – 3.77 (m, 1H), 3.59 – 3.51 (m, 1H), 3.50 (dd, *J* = 9.2, 1.6 Hz, 1H), 3.46 – 3.38 (m, 1H), 2.54 – 2.04 (m, 5H), 2.03 (s, 3H), 2.02 – 1.94 (m, 1H). ^{13}C NMR (126 MHz, CD_3OD) δ 175.08, 173.87, 173.28, 172.37, 95.64, 70.74, 69.57, 69.44, 66.34, 54.19, 52.94, 51.70, 40.85, 29.93, 26.12, 21.28. HRMS (ESI) calc'd for $C_{16}H_{25}N_5O_{11}$ (M+Na) *m/z* = 486.1448, found 486.1440.



Scheme S2. Synthetic route for ^{AzPBA}Sia

Synthesis of ^{AzPBA}Sia: EDC (1.13 g, 5.88 mmol) and N-hydroxy succinimide (NHS, 594 mg, 5.88 mmol) were added to a solution of m-(4-azidophenoxy)-benzoic acid (AzPBA) (1.00 g, 3.92 mmol) in CH_2Cl_2 (40.00 mL). The mixture was stirred at room temperature for 1 h. The reaction mixture was washed with 1 M aqueous HCl (50.00 mL) and saturated aqueous solution of sodium bicarbonate (50 mL). The organic phase was separated, dried over anhydrous Na_2SO4, and concentrated under reduced pressure to give AzPBA-NHS as a white

solid (966 mg) that was used directly. To the solution of 9-amino-sialic acid (100 mg, 0.30 mmol) in water (3 ml) was added **AzPBA-NHS** (211mg, 0.60 mmol) in dioxane (6 ml) with pH maintained between 8.0-9.0 with saturated sodium bicarbonate solution. The mixture was stirred in the dark for 24 h. The solvent was evaporated and the residue was purified by flash chromatography(CH2Cl2/MeOH, 2:1) to afford the product as a yellow solid **^{AzPBA}Sia** (99 mg, 61%).^{1}H NMR (500 MHz, CD_3OD) δ 7.51 (d, *J* = 7.9 Hz, 1H), 7.41 – 7.33 (m, 2H), 7.09 – 7.04 (m, 1H), 6.99 (dt, *J* = 13.0, 7.8 Hz, 4H), 3.99 (t, *J* = 8.5 Hz, 1H), 3.90 (d, *J* = 8.2 Hz, 1H), 3.86 – 3.79 (m, 1H), 3.60 (t, *J* = 15.1 Hz, 3H), 3.38 (d, *J* = 10.5 Hz, 3H), 3.33 – 3.28 (m, 1H), 2.15 – 2.07 (m, 1H), 1.93 (d, *J* = 29.5 Hz, 1H), 1.87 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 176.11, 173.00, 168.57, 157.69, 153.94, 136.20, 135.62, 129.80, 121.74, 121.22, 120.35, 120.17, 117.06, 96.36, 70.53, 70.00, 69.40, 62.89, 52.61, 43.48, 40.46, 21.44. HRMS (ESI) calc'd for $C_{24}H_{27}N_5O_{10}$ (M+Na) *m/z* = 568.1656, found 568.1652.



Scheme S3. Synthetic routes for ^{PBA}Sia-Bu-K, ^{BPC}Sia-Bu-K and ^{PBA}Sia-K

Synthesis of ^{PBA}Sia-K. To a solution of **S3** (600 mg, 0.97 mmol) in methanol (10 mL) was added Pd/C (60 mg, 10%). The mixture was stirred under hydrogen gas at room temperature overnight. After the reaction was complete, the catalyst was removed by filtration through diatomaceous earth. Removal of the solvent gave compound **S6** that was directly used without further purification (534 mg). HRMS (ESI) calc'd for $C_{26}H_{48}N_4O_{11}$ (M+H) *m/z* = 593.3398, found 593.3397. To a solution of m-phenoxybenzoic acid (2.00 g, 9.34 mmol) and EDC (3.59 g, 18.68 mmol) in dichloromethane (80 mL) was added NHS (1.89 g, 18.68 mmol). The mixture was stirred at room temperature for 3 h, and then washed swith 1 M hydrochloric acid (100 mL), and saturated brine (100 mL). The organic phase was then separated and dried over Na_2SO_4, and then concentrated to give crude PBA-NHS. To a solution of **S6** (400 mg, 0.68 mmol) in methanol (5 mL) was added PBA-NHS (422 mg, 1.36 mmol) and *N, N*-diisopropylethylamine (DIPEA, 262 mg, 2.04 mmol). The mixture was stirred overnight at

room temperature and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 25:1) to give **S7** (50%, 266 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 7.60 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.50 (t, *J* = 2.1 Hz, 1H), 7.47 (t, *J* = 8.0 Hz, 1H), 7.40 (ddq, *J* = 9.7, 4.3, 2.6 Hz, 2H), 7.20 – 7.13 (m, 2H), 7.06 – 7.00 (m, 2H), 4.29 (dd, *J* = 9.1, 5.2 Hz, 1H), 4.14 – 4.03 (m, 2H), 3.96 – 3.83 (m, 3H), 3.58 – 3.48 (m, 2H), 3.04 (tt, *J* = 7.9, 4.0 Hz, 2H), 2.31 (dd, *J* = 12.7, 4.9 Hz, 1H), 2.00 (s, 3H), 1.88 (ddt, *J* = 14.8, 11.2, 5.8 Hz, 1H), 1.75 (dtd, *J* = 14.1, 9.3, 5.3 Hz, 1H), 1.66 – 1.55 (m, 1H), 1.48 (s, 2H), 1.44 (s, 18H), 1.41 – 1.34 (m, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 173.66, 172.19, 171.28, 168.91, 157.65, 156.84, 136.09, 129.68, 123.50, 121.63, 118.70, 117.29, 95.66, 81.54, 78.46, 70.94, 70.43, 69.65, 66.34, 53.06, 52.94, 43.74, 40.95, 39.74, 30.77, 29.35, 27.43, 26.88, 22.93, 21.29. MALDI-TOF MS calc'd for $C_{39}H_{56}N_4O_{13}$ (M+Na) *m/z* 811.37, found 811.24.

The solution of **S7** (70 mg, 0.09 mmol) in trifluoroacetic acid (5 mL) was stirred at room temperature for 10 min, and then concentrated under reduced pressure. The residue was purified using Sep-Pak C18 column to afford ^{PBA}Sia-K (95%, 53 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 7.49 (d, *J* = 7.7 Hz, 1H), 7.40 – 7.33 (m, 2H), 7.29 (t, *J* = 7.9 Hz, 2H), 7.06 (ddd, *J* = 7.8, 4.9, 2.1 Hz, 2H), 6.92 (d, *J* = 8.1 Hz, 2H), 4.36 (dd, *J* = 9.5, 4.7 Hz, 1H), 4.00 (dd, *J* = 13.6, 10.2 Hz, 2H), 3.87 – 3.81 (m, 1H), 3.81 – 3.72 (m, 2H), 3.47 – 3.33 (m, 2H), 2.93 – 2.80 (m, 2H), 2.22 (dd, *J* = 12.8, 4.9 Hz, 1H), 1.90 (s, 3H), 1.76 – 1.67 (m, 1H), 1.66 – 1.46 (m, 4H), 1.39 (ddt, *J* = 13.2, 8.5, 4.9 Hz, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 173.79, 173.45, 172.35, 168.91, 157.73, 156.77, 136.05, 129.70, 123.56, 121.56, 118.77, 117.20, 95.67, 70.85, 70.30, 69.22, 66.29, 52.92, 51.75, 43.67, 40.72, 39.12, 30.47, 29.34, 26.60, 22.47, 21.31. HRMS (ESI) calc'd for $C_{30}H_{40}N_4O_{11}$ (M+H) *m/z* = 633.2772, found 633.2772.

Synthesis ^{PBA}Sia-Bu-K. To a solution of **S7** (140 mg, 0.18 mmol) in pyridine (5 mL) was added butyric anhydride (284 mg, 1.80 mmol) and then 4-dimethylaminopyridine (DMAP, 11 mg, 0.09 mmol). The mixture was stirred at room temperature for 24 h. The solution was concentrated under reduced pressure and the residue was dissolved in ethyl acetate (30 mL). The organic solution was washed sequentially with saturated $NaHCO_3$ (30 mL), 1 M HCl (30 mL), and saturated brine (30 mL). The organic phase was separated, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 50:1) to afford a pale yellow residue (76%, 144 mg). The resulting residue (100 mg, 0.09 mmol) was dissolved in trifluoroacetic acid (5 mL). The solution was stirred at room temperature for 10 min, and concentrated under reduced pressure. The residue was purified using Sep-Pak C18 column to afford ^{PBA}Sia-Bu-K (94%, 80 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 7.45 (d, *J* = 8.1 Hz, 1H), 7.38 – 7.26 (m, 4H), 7.12 – 6.99 (m, 2H), 6.93 (d, *J* = 7.9 Hz, 2H), 5.33 – 5.12 (m, 2H), 4.41 – 4.05 (m, 2H), 3.97 (t, *J* = 10.4 Hz, 1H), 3.85 – 3.70 (m, 1H), 3.50 (dd, *J* = 14.6, 8.4 Hz, 1H), 2.88 – 2.72 (m, 2H), 2.71 – 2.23 (m, 5H), 2.22 – 2.12 (m, 3H), 2.10 – 1.82 (m, 2H), 1.77 (d, *J* = 11.6 Hz, 3H), 1.74 – 1.66 (m, 2H), 1.65 – 1.43 (m, 10H), 1.42 – 1.25 (m, 3H), 1.21 (s, 2H), 0.98 – 0.69 (m, 12H). ^{13}C NMR (151 MHz, CD_3OD) δ 175.90, 173.14, 172.98, 172.87, 171.88, 170.90, 168.05, 167.07, 157.67, 156.79, 136.16, 129.73, 129.68, 123.49, 121.50, 121.38, 118.76, 117.07, 97.50, 72.42, 71.23, 68.32, 68.18, 54.11, 48.80, 39.18, 36.92, 35.55, 35.45, 35.38, 31.69, 29.38, 29.35, 29.33, 26.77, 21.74, 21.36, 18.05, 18.02, 17.82, 17.78, 17.74, 17.72, 17.70, 13.05, 12.78, 12.74, 12.70, 12.66, 12.64, 12.61, 12.54, 12.51. HRMS (ESI) calc'd for $C_{46}H_{64}N_4O_{15}$ (M+H) *m/z* = 913.4446, found 913.4447.

Synthesis of ^{BPC}Sia-Bu-K. To a solution of 4-phenylbenzoic acid (BPC, 2.00 g, 10.10 mmol) and EDC (3.88 g, 20.20 mmol) in DCM (80 mL) was added NHS (2.04 g, 20.20

mmol). The mixture was stirred at room temperature for 3 h, and then washed with 1 M hydrochloric acid solution (100 mL) and saturated brine solution (100 mL). The organic phase was collected, dried over anhydrous sodium sulfate, and then concentrated to give BPC-NHS. To a solution of BPC-NHS (148 mg, 0.50 mmol) in methanol (5 mL) was added **S6** (150 mg, 0.25 mmol) and DIPEA (97 mg, 0.75 mmol). The mixture was stirred at room temperature overnight and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: CH_2Cl_2/MeOH, 25:1) to give **S8** in 80% yield (156 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 7.99 – 7.92 (m, 2H), 7.78 – 7.66 (m, 4H), 7.48 (dd, *J* = 8.4, 6.9 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 1H), 4.29 (dd, *J* = 9.1, 5.2 Hz, 1H), 4.15 – 4.05 (m, 2H), 3.97 (ddd, *J* = 9.0, 7.2, 3.0 Hz, 1H), 3.94 – 3.86 (m, 2H), 3.72 (hept, *J* = 7.5, 7.1 Hz, 1H), 3.62 – 3.51 (m, 2H), 3.29 – 3.19 (m, 1H), 3.04 (td, *J* = 6.9, 2.1 Hz, 2H), 2.32 (dd, *J* = 12.7, 4.9 Hz, 1H), 2.01 (s, 3H), 1.88 (ddd, *J* = 15.0, 10.1, 5.6 Hz, 1H), 1.76 (dtd, *J* = 14.0, 9.3, 5.2 Hz, 1H), 1.64 (t, *J* = 12.0 Hz, 1H), 1.59 – 1.52 (m, 1H), 1.44 (d, *J* = 8.4 Hz, 18H), 1.34 – 1.27 (m, 1H). ^{13}C NMR (126 MHz, CD_3OD) δ 172.24, 171.27, 169.45, 144.40, 139.82, 132.72, 128.65, 127.73, 127.62, 126.73, 126.66, 95.71, 81.55, 78.46, 70.94, 70.37, 69.77, 66.38, 54.25, 52.94, 40.92, 39.76, 38.78, 30.77, 29.08, 27.42, 26.87, 21.29, 19.91. MALDI-TOF MS calc'd for $C_{39}H_{56}N_4O_{12}$ (M+Na) *m/z* 795.38, found 795.25.

To a solution of **S8** (100 mg, 0.13 mmol) in pyridine (5 mL) was added butyric anhydride (205 mg, 1.3 mmol) followed by DMAP (8 mg, 0.07 mmol). The mixture was stirred at room temperature for 24 h and then concentrated under reduced pressure. The residue was re-dissolved in ethyl acetate (30 mL). The organic phase was washed sequentially with saturated $NaHCO_3$ (20 mL), 1 M HCl (20 mL), and saturated brine (20 mL). The organic phase was separated, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/MeOH, 50:1) to afford a white solid compound (72%, 98 mg). The resultant solid (70 mg, 0.07 mmol) was dissolved in trifluoroacetic acid (5 mL). The solution was stirred at room temperature for 10 min. The mixture was concentrated under reduced pressure and the residue was purified using Sep-Pak C18 column to afford ^{BPC}Sia-Bu-K as pale yellow solid (95%, 57 mg). ^{1}H NMR (500 MHz, CD_3OD) δ 7.91 – 7.85 (m, 2H), 7.74 (dd, *J* = 8.5, 2.4 Hz, 2H), 7.71 – 7.65 (m, 2H), 7.49 (t, *J* = 7.6 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 1H), 5.42 (td, *J* = 6.0, 5.6, 2.3 Hz, 1H), 5.37 – 5.31 (m, 1H), 5.22 (td, *J* = 10.8, 4.7 Hz, 2H), 4.42 (ddd, *J* = 13.9, 9.4, 5.0 Hz, 1H), 4.26 (ddd, *J* = 13.7, 11.0, 2.1 Hz, 1H), 4.21 – 3.93 (m, 2H), 3.51 (dd, *J* = 14.4, 7.8 Hz, 1H), 2.94 (q, *J* = 7.4 Hz, 2H), 2.70 (td, *J* = 14.0, 13.5, 6.2 Hz, 1H), 2.55 – 2.25 (m, 9H), 1.87 (d, *J* = 6.7 Hz, 3H), 1.87 – 1.45 (m, 16H), 1.05 – 0.91 (m, 12H). ^{13}C NMR (126 MHz, CD_3OD) δ 173.34, 173.13, 172.94, 172.79, 171.94, 171.15, 168.61, 167.31, 144.48, 139.74, 132.74, 128.67, 127.79, 127.48, 126.72, 98.45, 72.56, 70.71, 68.44, 68.23, 51.86, 48.60, 40.44, 39.68, 39.24, 35.54, 30.74, 26.60, 22.33, 21.39, 18.03, 17.80, 17.75, 12.71, 12.61. HRMS (ESI) calc'd for $C_{46}H_{64}N_4O_{14}$ (M+H) *m/z* = 897.4497, found 897.4506.

Metabolic glycoengineering with ^{Az}Sia in different cell lines. A549, B16-F10, MCF-7, HeLa, RAW 264.7 and PC3 cells were cultivated in fresh DMEM or DMEM containing ^{Az}Sia (1 mM) for 24 h. Cells were washed three times with PBS and then further maintained in DMEM containing ^{DBCO}FITC （50 μM) for 2 h. The cells were washed with PBS and then imaged by confocal microscopy.

Metabolic glycoengineering with C-1 caged ^{Az}Sia A549, B16-F10, MCF-7, HeLa, RAW

264.7 and PC3 cells were cultivated in fresh DMEM or DMEM containing ^{Az}Sia-E (1 mM), ^{Az}Sia-F (1 mM), or ^{Az}Sia-K (1 mM) for 24 h, respectively. Cells were washed three times with PBS and then maintained in DMEM containing ^{DBCO}FITC（50 μM, 1 h). The cells were washed with PBS and then imaged by confocal microscopy.

In vitro hydrolysis of C1-caged Sia by Carboxypeptidase B: To the solution of ^{Az}Sia-K (1 mg/mL) in ammonium bicarbonate (50 mM) was added carboxypeptidase B to a final concentration of 1 mg/mL. The mixture was incubated at 37°C for 2 h and then analyzed by high-resolution mass spectrometry.

Involvement of carboxypeptidase B in MGE with C1-caged Sia. B16-F10 cells were cultivated in DMEM containing ^{Az}Sia-K (1 mM) and GEMSA (0, 50 μM) for 24 h. The cells were washed with for PBS three times, and then maintained in DMEM containing ^{DBCO}FITC (50 μM, 2 h). The cells were washed with PBS and then imaged by confocal fluorescence microscopy. B16-F10 cells treated with ^{DBCO}FITC alone were used as the control.

Western blotting analysis on Carboxypeptidase B in B16-F10 cells
A549, B16-F10, MCF-7, HeLa, and RAW 264.7 cells were individually seeded into 35 mm cell culture dishes such that they reached a density equivalent to 80% confluence after 24 h culturing, the cells were harvested and lysed and then subjected to immunoblot analysis using CPB antibody and β-actin antibody.

Metabolic glycoengineering with ^{AzPBA}Sia: B16-F10, HeLa cells were cultivated in DMEM spiked with 1 mM ^{Az}Sia-K or ^{AzPBA}Sia for 24 h, respectively. Cells were washed three times with PBS and then stained with ^{DBCO}FITC（50 μM, 1 h) before confocal microscopic analysis.

Cytotoxicity of caged Sia on B16-F10 cells: B16-F10 cells were incubated in a 96-well in 1640 containing ^{PBA}Sia-Bu-K, ^{PBA}Sia -K, or ^{BPC}Sia-Bu-K at concentrations of 0, 0.1, 0.5, 1, 2, 5, 10, 20, 50, 100, and 200 μM for 24 h at 37°C with 5% CO_2. The cells were washed with PBS for three times and then subjected to CCK-8 assay. The absorbance at 450 nm was measured to determine cell viability.

Effects of caged Sia on proliferation of B16-F10 cells in vitro: B16-F10 cells were seeded into 96-well plates at a density of 2,500 cells/well and cultured in RPMI 1640 medium supplemented with varying concentrations (0, 0.05, 0.1, 0.2, 0.5 and 1 mM) of ^{PBA}Sia-Bu-K, ^{PBA}Sia-K, or ^{BPC}Sia-Bu-K for 24 h. The cells were washed with PBS for 3 times, Cell proliferation was assessed at 0, 1, 2, 3, 4, and 5 days using the Cell Counting Kit-8 (CCK-8) assay, according to the manufacturer's instructions.

***In vivo* effect of caged-Sia on tumor growth**: C57BL/6 mice aged 6–8 weeks were injected subcutaneously with 5×10^5 B16-F10 cells on day 0. When the tumor foci reached 80–100 mm^3, the mice were injected intravenously with PBS or caged-Sia (11.0 or 33.0 μmol kg^{-1}) via the tail vein for 3 injections on days 7, 9, and 11, respectively. Tumor volume was calculated with the formula: Volume (mm^3) = 1/2 (length × width × height).[1] Tumors were dissected on day 14, photographed, and weighed. The changes in the body weight of the mice

were also monitored during this experiment.

Determination of $CD19^+$ B cells in the tumor. C57BL/6 mice were subcutaneously injected with 5×10^5 B16-F10 cells on day 0. The mice were injected intravenously with PBS or ^{PBA}Sia-Bu-K (33.0 μmol kg^{-1}) on days 7, 9 and 11, respectively. On day 12, the mice were euthanized, and the tumors were stripped out. Tumors in RPMI 1640 medium were ground with a glass tissue grinder homogenizer, and the cell homogenate was filtered with a 100 μm cell strainer to obtain a tumor cell suspension. After centrifugation of the cell suspension at 1200 rpm for 5 min at 4 ºC, the resulting cell pellet was washed twice with PBS and then treated with red blood cell lysis buffer for 2 min. The resulting cells were washed 3 times with PBS and stained with FITC-labeled CD19 antibody for 30 min on ice. After washing 3 times with FACS buffer (containing 1% FBS and 0.1% NaN_3), the cells were analyzed by flow cytometry. Each sample is performed in triplicate.

Reference

1. Tomayko, M.M., et al., *Determination of subcutaneous tumor size in athymic (nude) mice.* Cancer Chemother. Pharmacol., 1989. **24**(3): p. 148-154.

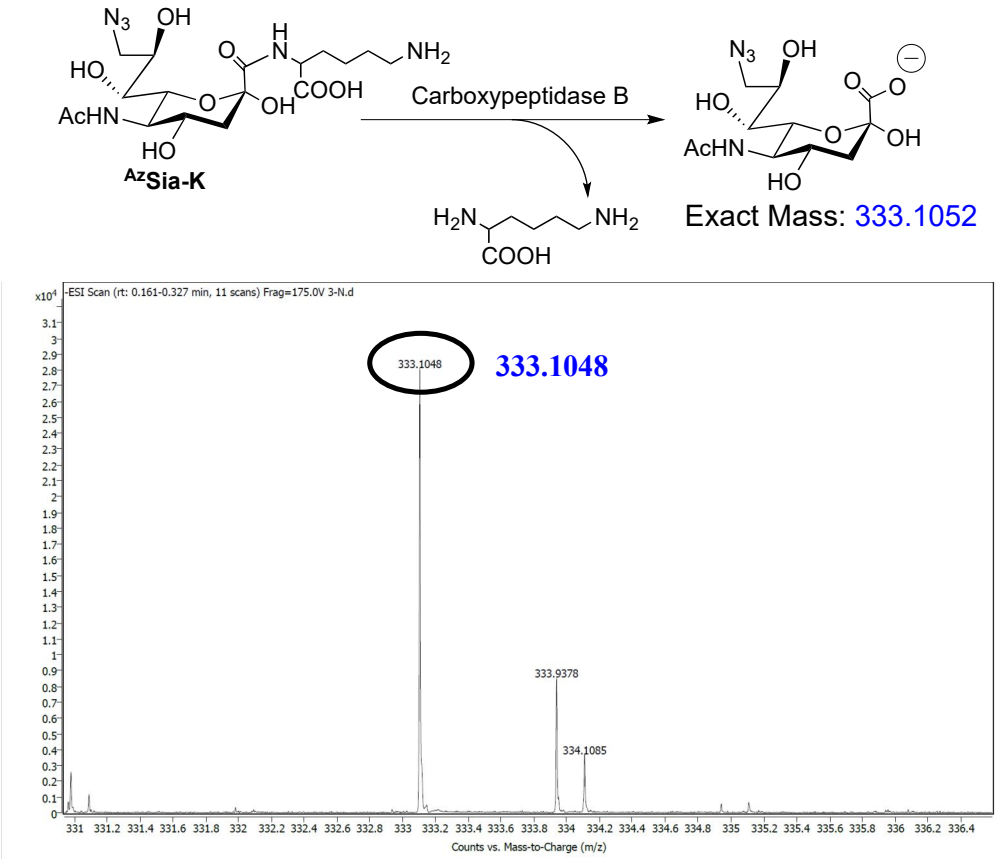


Figure S1. Carboxypeptidase B-catalysis triggered formation of ^{Az}Sia from ^{Az}Sia-K. The solution of ^{Az}Sia-K (1 mg/mL) and carboxypeptidase B (1 mg/mL) was maintained at 37 ºC for 2 h and then analyzed by high-resolution mass spectrometry.

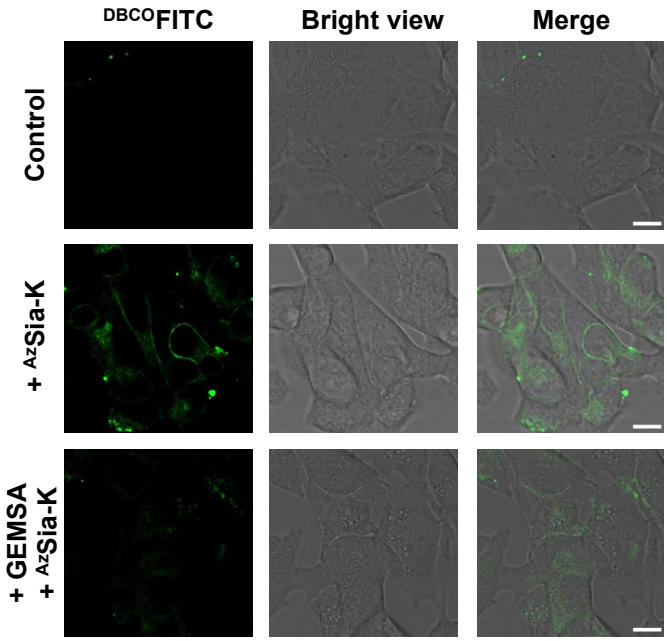


Figure S2. Carboxypeptidase B-dependent MGE with caged Sia. B16-F10 cells were cultivated in DMEM with ^{Az}Sia-K (1 mM) in the presence or absence of GEMSA (50 μM) for 24 h. The cells were washed with PBS, stained with ^{DBCO}FITC (50 μM, 2 h) and then imaged by confocal fluorescence microscopy. B16-F10 cells treated with ^{DBCO}FITC alone were used as the control. Scale bars: 10 μm.

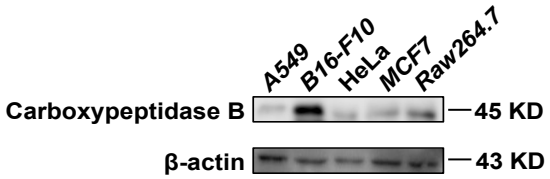


Figure S3. Western blot analysis on carboxypeptidase B in B16-F10 cells. B16-F10 cells, A549, HeLa, MCF7 and RAW264.7 cells were lysed and examined to immunoblot analysis using carboxypeptidase B (CPB) antibody and β-actin antibody.

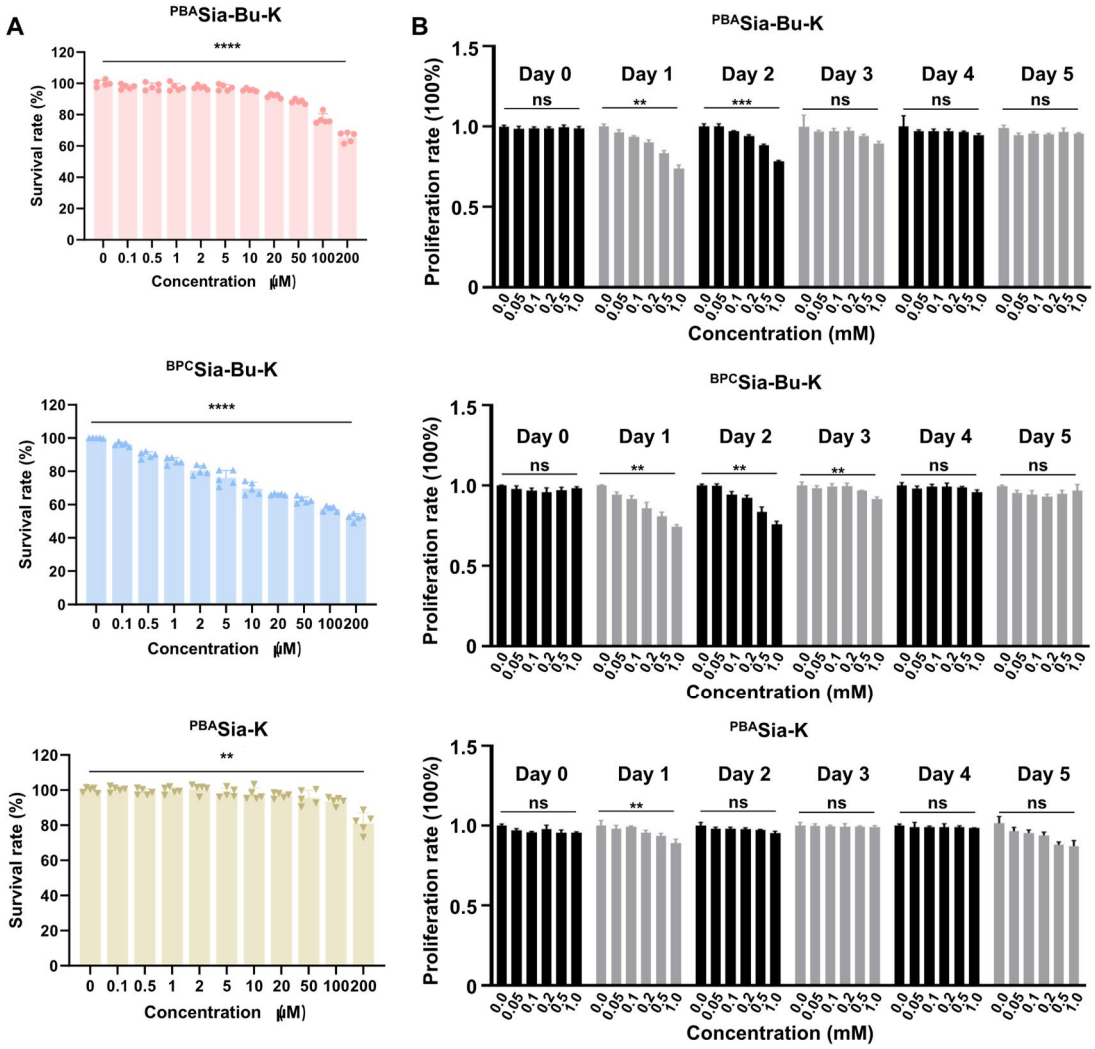


Figure S4. In vitro effects of C1-caged Sia on B16-F10 cells. (A) Cytotoxicity of Caged Sia. B16-F10 cells were cultured for 24 h with ^{PBA}Sia-Bu-K, ^{BPC}Sia-Bu-K or ^{PBA}Sia-K (0-200 μM), and then cultured in fresh DMEM for 0-48 h. The cell viability at 24 h post-incubation were determined as a function of probe concentration by cell count kit 8 assay (n=3). (B) Effecs of C1-caged Sia on cell proliferation. B16-F10 cells were cultured for 24 h with ^{PBA}Sia-Bu-K, ^{BPC}Sia-Bu-K or ^{PBA}Sia-K (0-1 mM) in DMEM. The cells were washed with PBS and then cultured in fresh 1640 medium for 0-5 days. The cell proliferation rate was determined by cell count kit 8 assay according to the manufacturer's guidelines (n=3). Survival rate and proliferation rate were quantified by GraphPad Prism 8 software. Data are presented as means ± SEM of three independent experiments. n = 3. ns, non-significant; **P ≤ < 0.01, ***P < 0.001, ****P < 0.0001. (t-test).

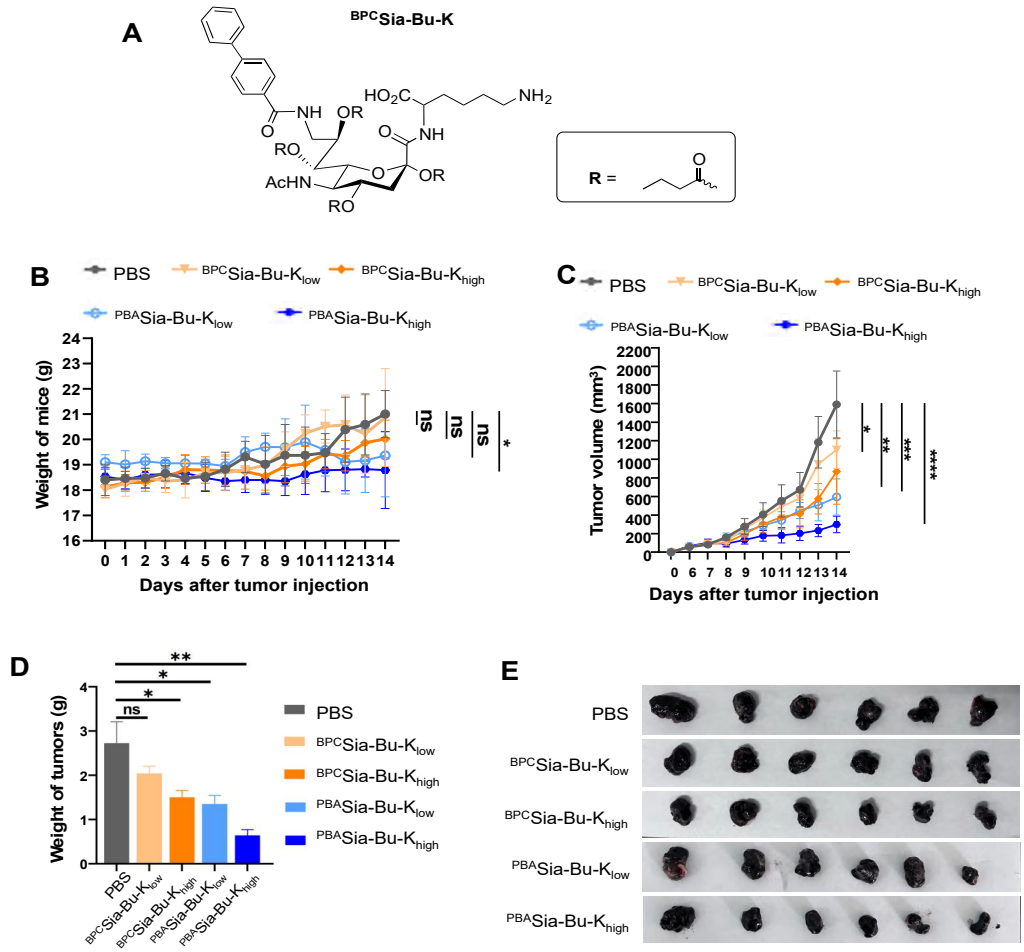


Figure S5. Evaluation of in vivo antitumor efficiency of caged ^{BPC}Sia. (A) Chemical structure of ^{BPC}Sia-Bu-K. Temporal changes on body weight (B) and tumor volume (C) of mice injected with caged Sia. C57BL/6 mice were subcutaneously inoculated with B16-F10 cells, and then treated with tail-veil injected PBS (100 μL), ^{BPC}Sia-Bu-K (11.0 or 33.0 μmol kg^{-1}) or ^{PBA}Sia-Bu-K (11.0 or 33.0 μmol kg^{-1}). The tumors were excised at 14th day postinjection and measured for mass analysis (D) and then pictured (E). Tumor weight or volume were quantified by GraphPad Prism 8 software. Data were presented as means ± SEM of a representative assay. n = 6. ns, non-significant; *P ≤ < 0.05, **P ≤ < 0.01, ***P < 0.001, ****P < 0.0001 (t-test).

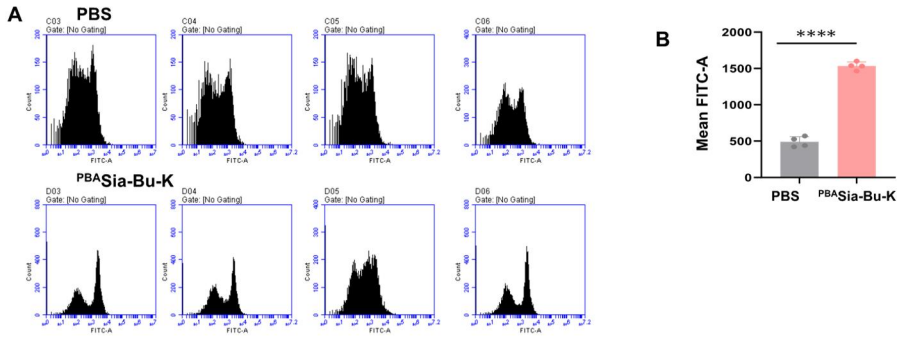


Figure S6. Evaluation on B cell leveles in B16-F10 tumors. Tumor-bearing mice were administered with PBS or ^{PBA}Sia-Bu-K evry other day for 3 times. The tumor were disected and gounded. The reluting cells were stained with FITC-labeled antibody specific for CD19. The cells were washed three times with PBS and then detected for cell surface fluorescence by flow cytometry. Data were quantified with GraphPad Prism 8 software. n = 10,000, mean ± SEM, n = 3. ****P < 0.0001 (t test).

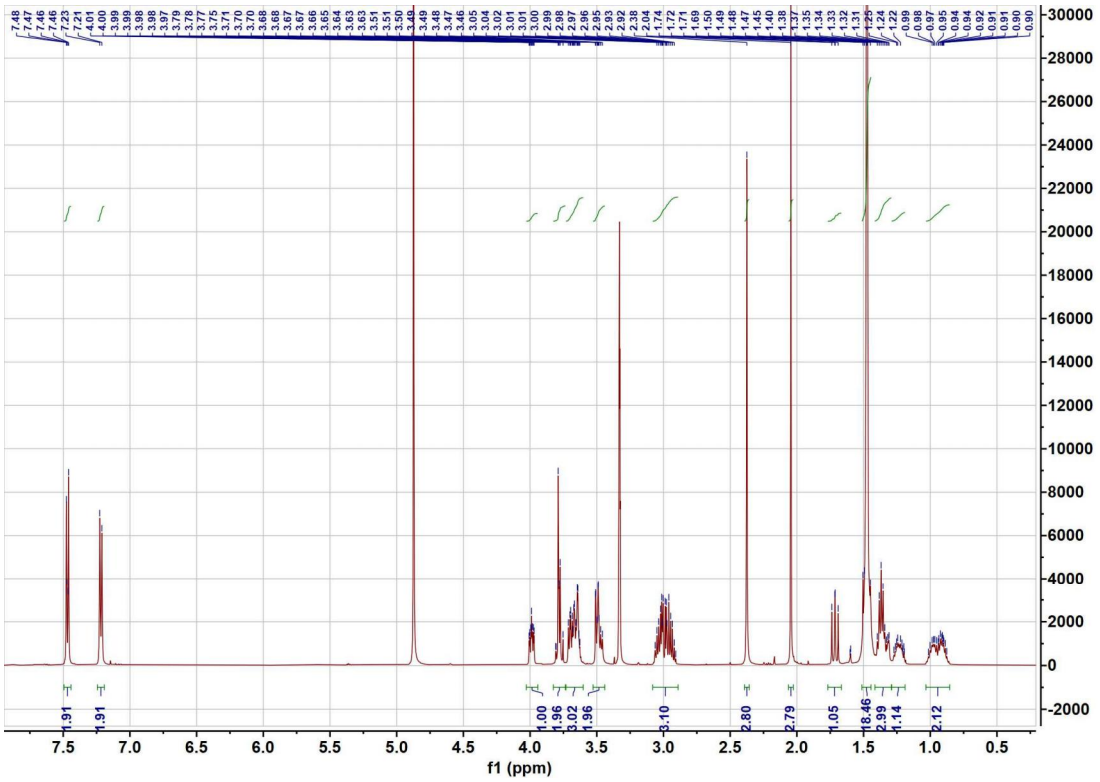


Figure S7. ^{1}H NMR spectrum of **S2**

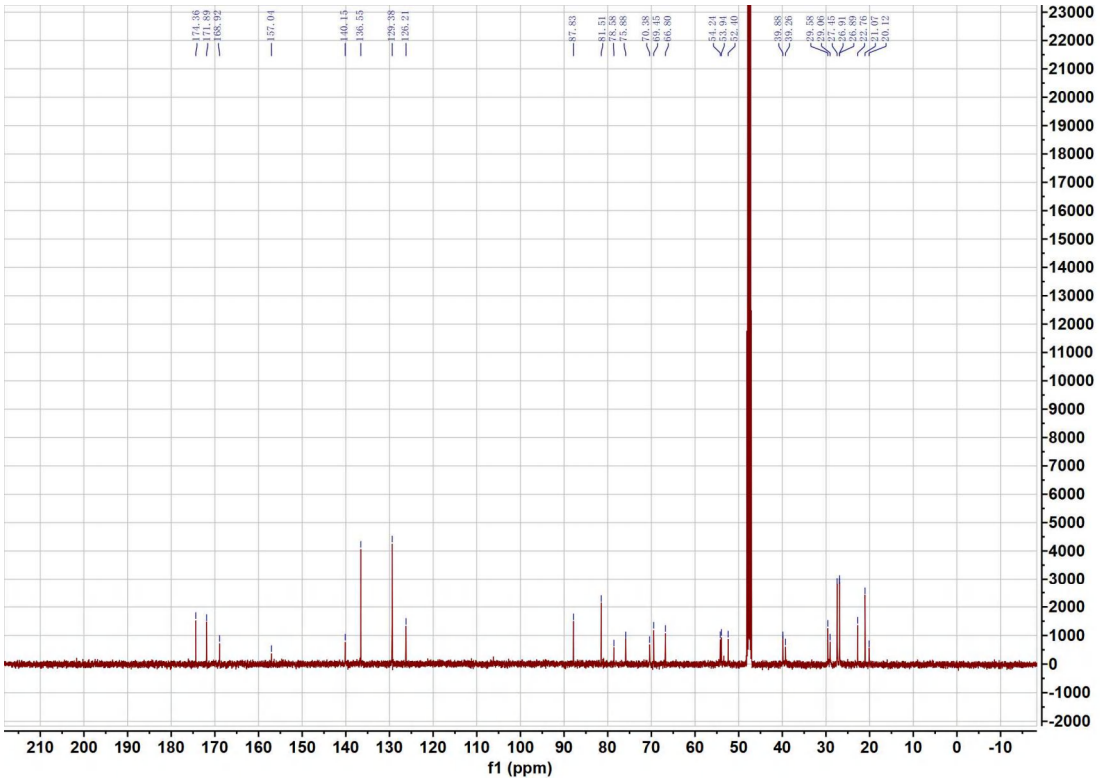


Figure S8. ^{13}C NMR spectrum of **S2**

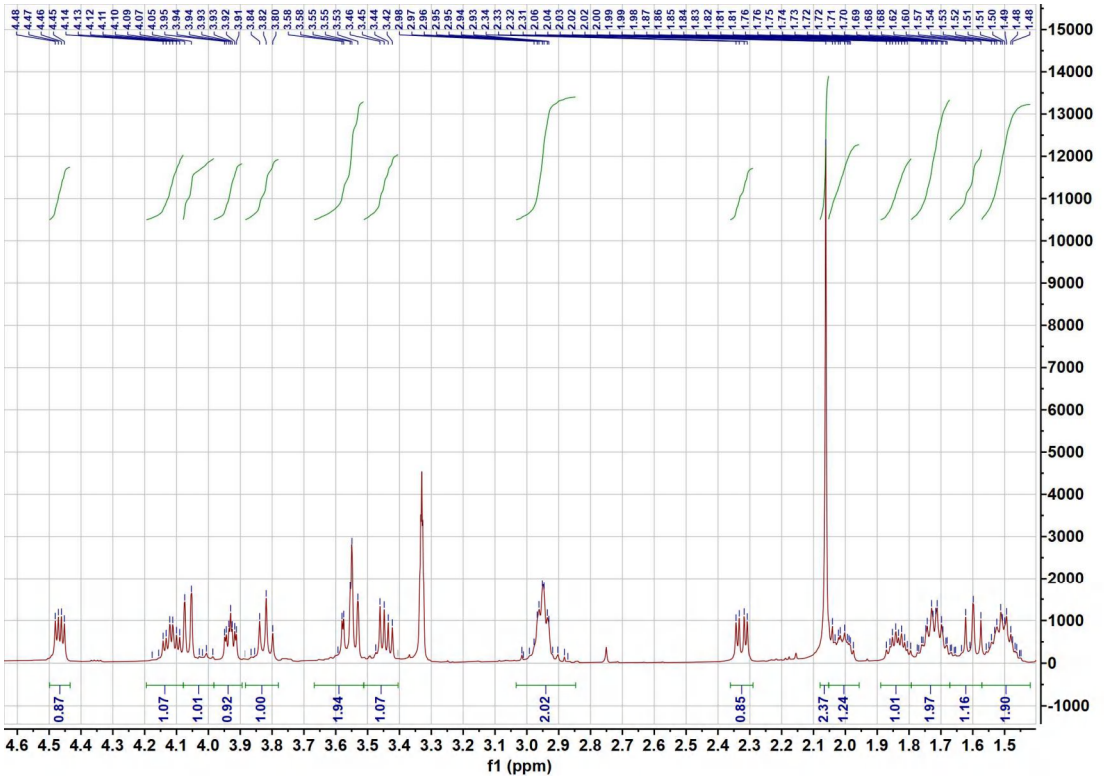


Figure S9. ^{1}H NMR spectrum of ^{Az}Sia-K

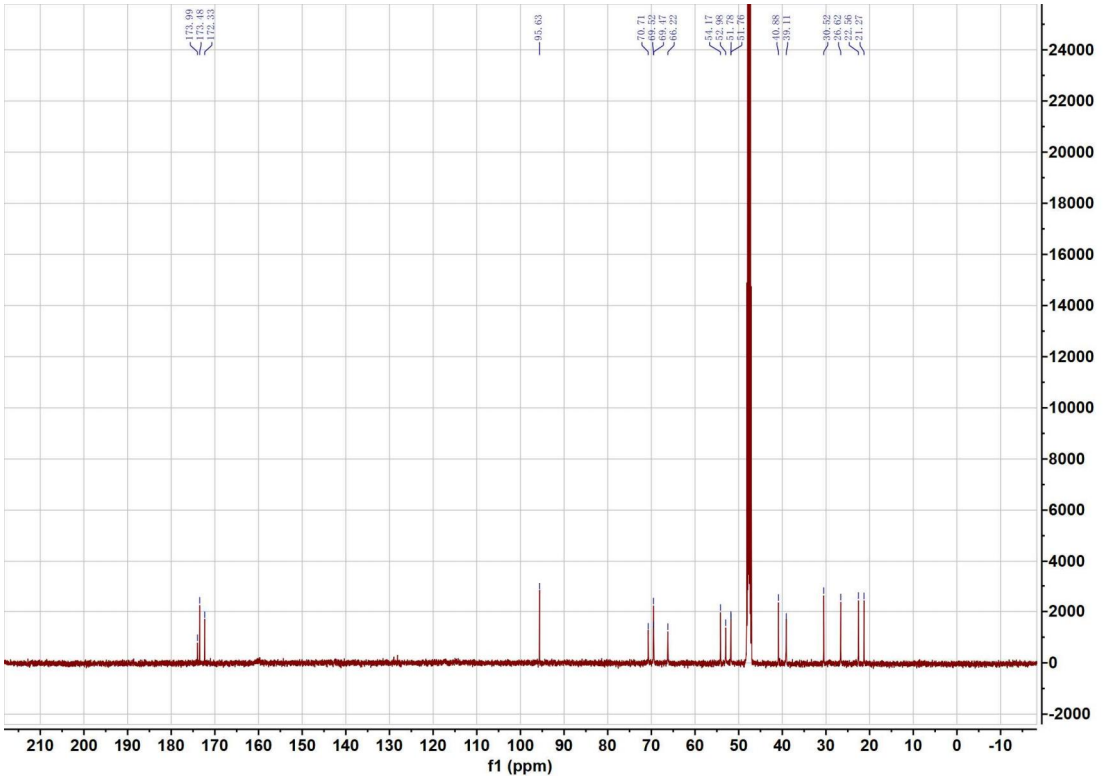


Figure S10. ^{13}C NMR spectrum of ^{Az}Sia-K

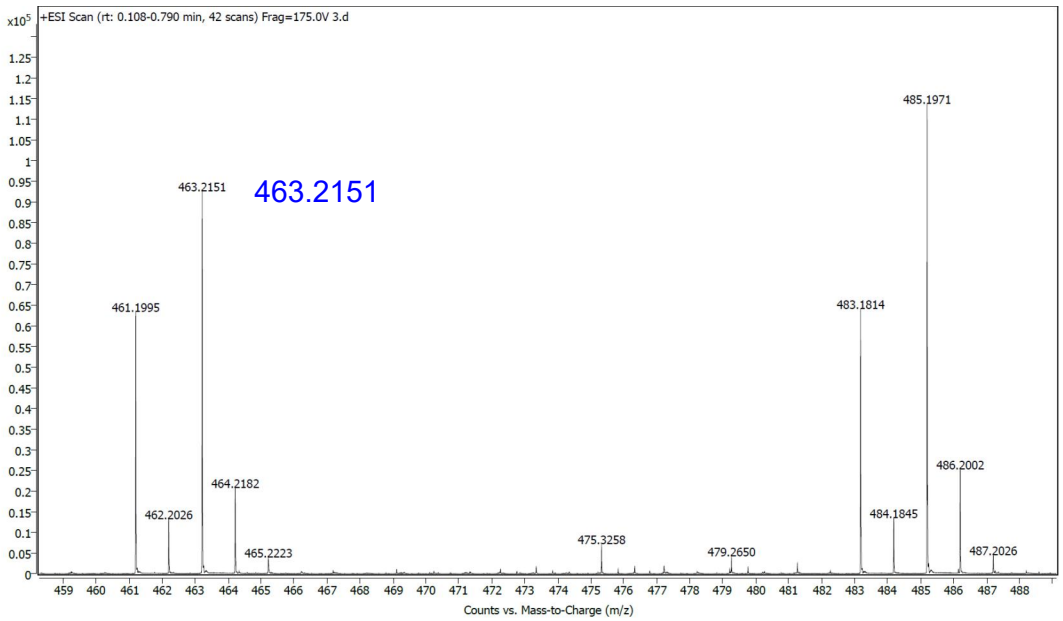


Figure S11. Mass spectrometry spectrum of ^{Az}Sia-K

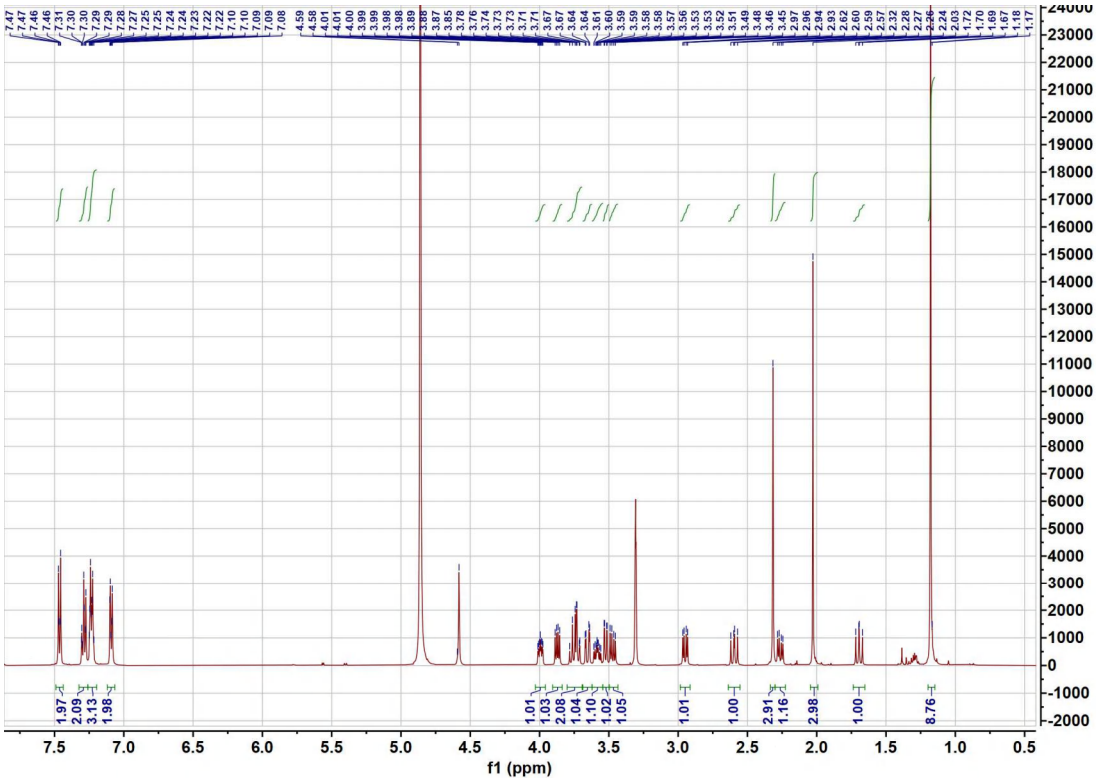


Figure S12. ^{1}H NMR spectrum of **S4**

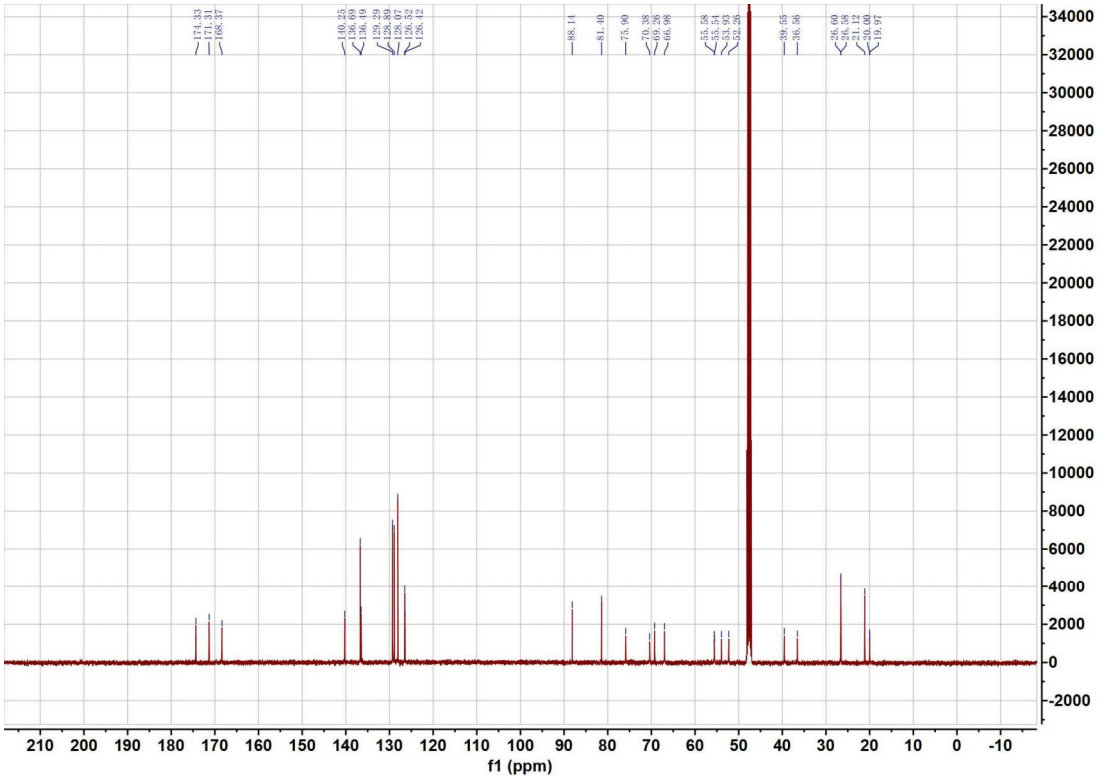


Figure S13. ^{13}C NMR spectrum of **S4**

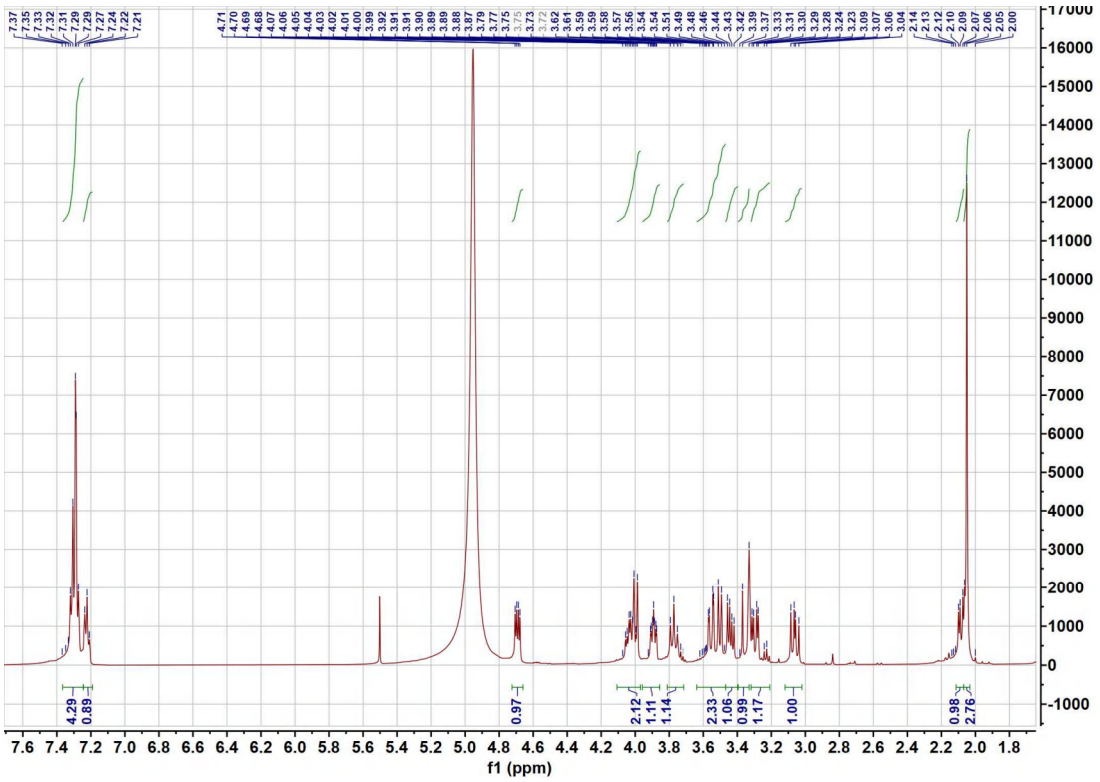


Figure S14. ^{1}H NMR spectrum of ^{Az}Sia-F

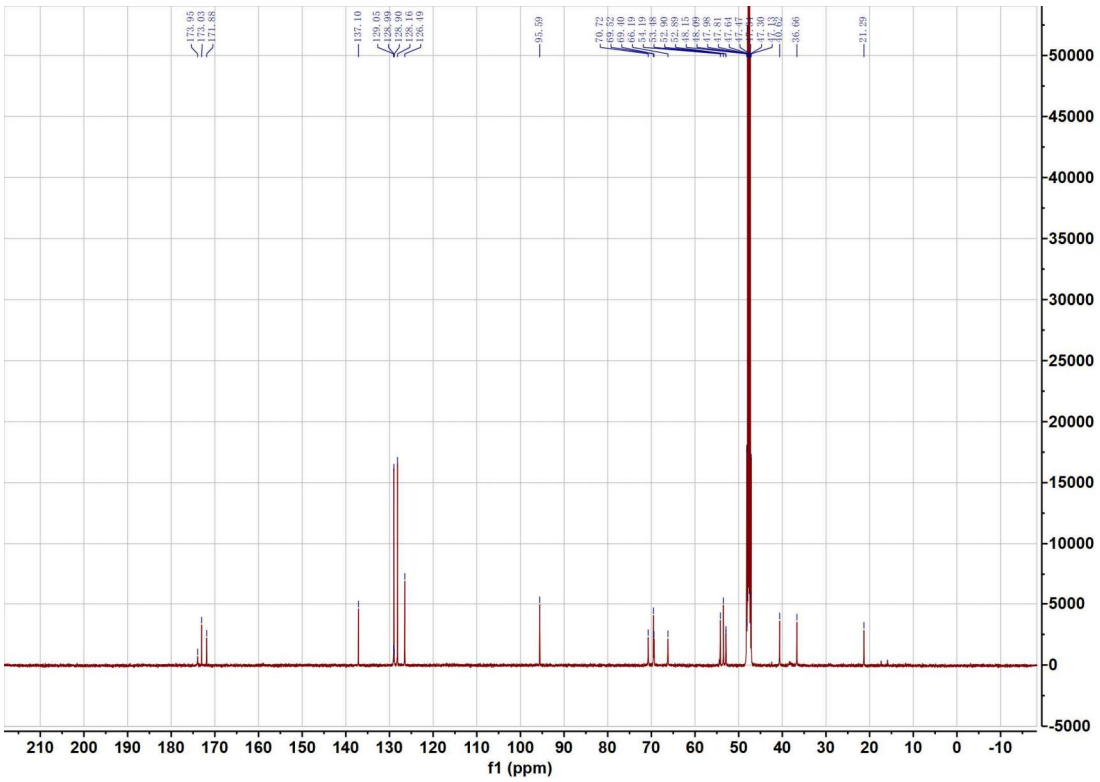


Figure S15. ^{13}C NMR spectrum of ^{Az}Sia-F

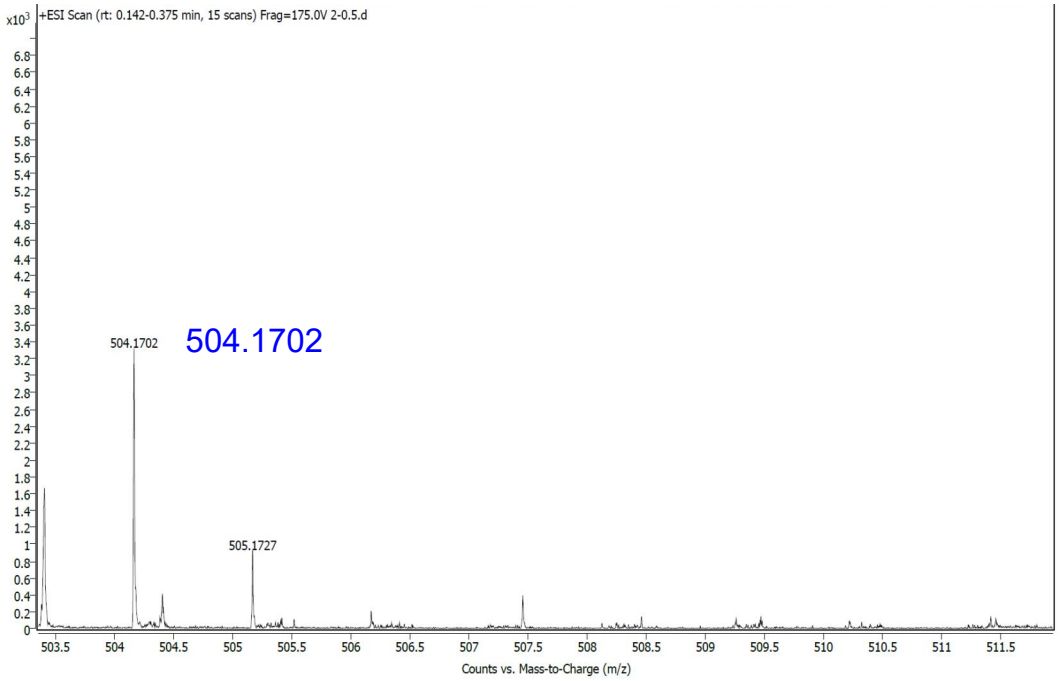


Figure S16. Mass spectrometry spectrum of ^{Az}Sia-F

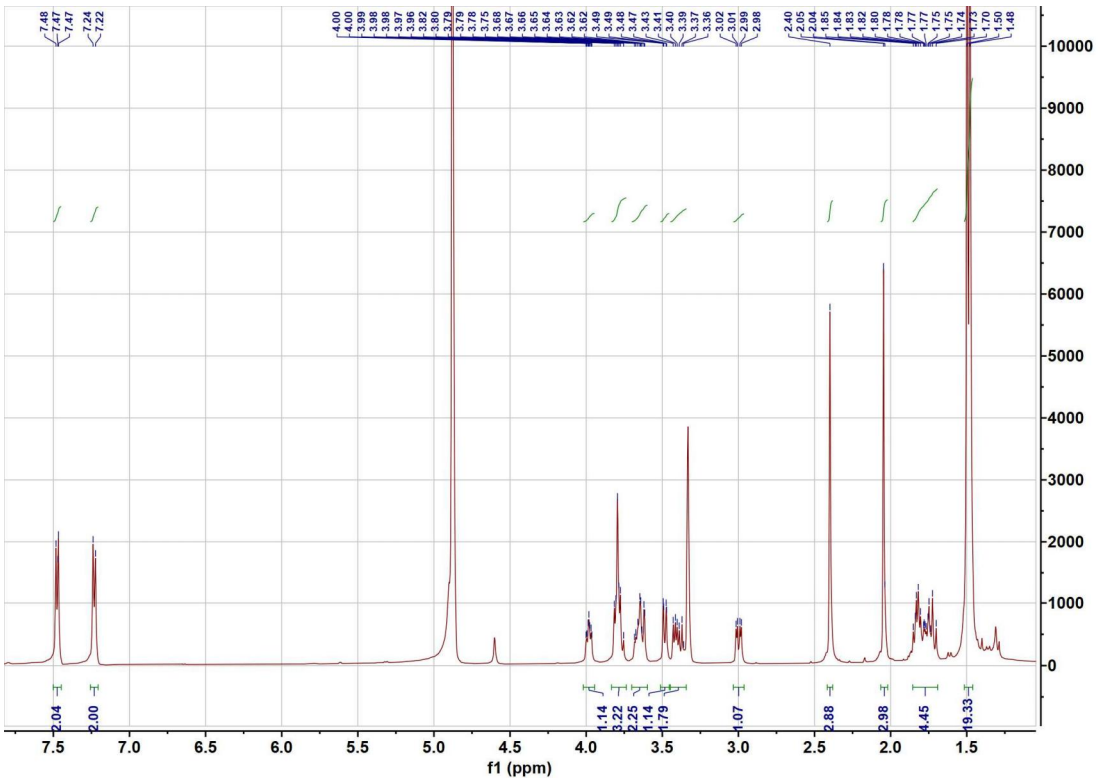


Figure S17. [1]H NMR spectrum of **S5**

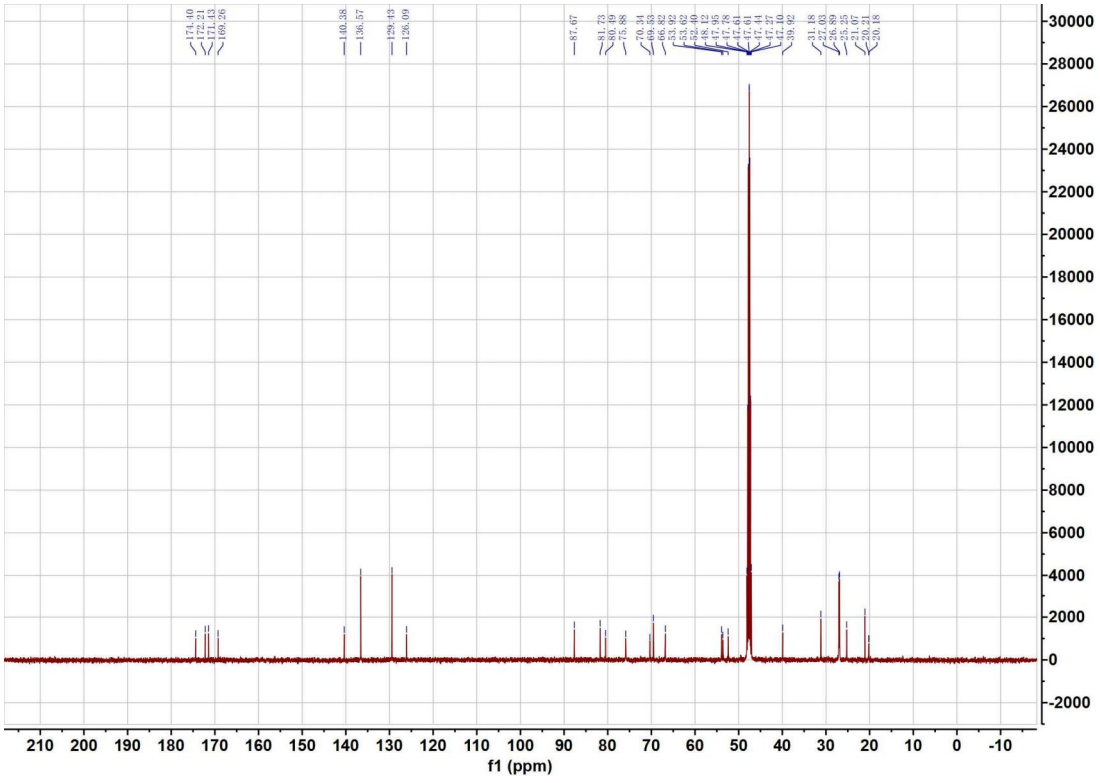


Figure S18. [13]C NMR spectrum of **S5**

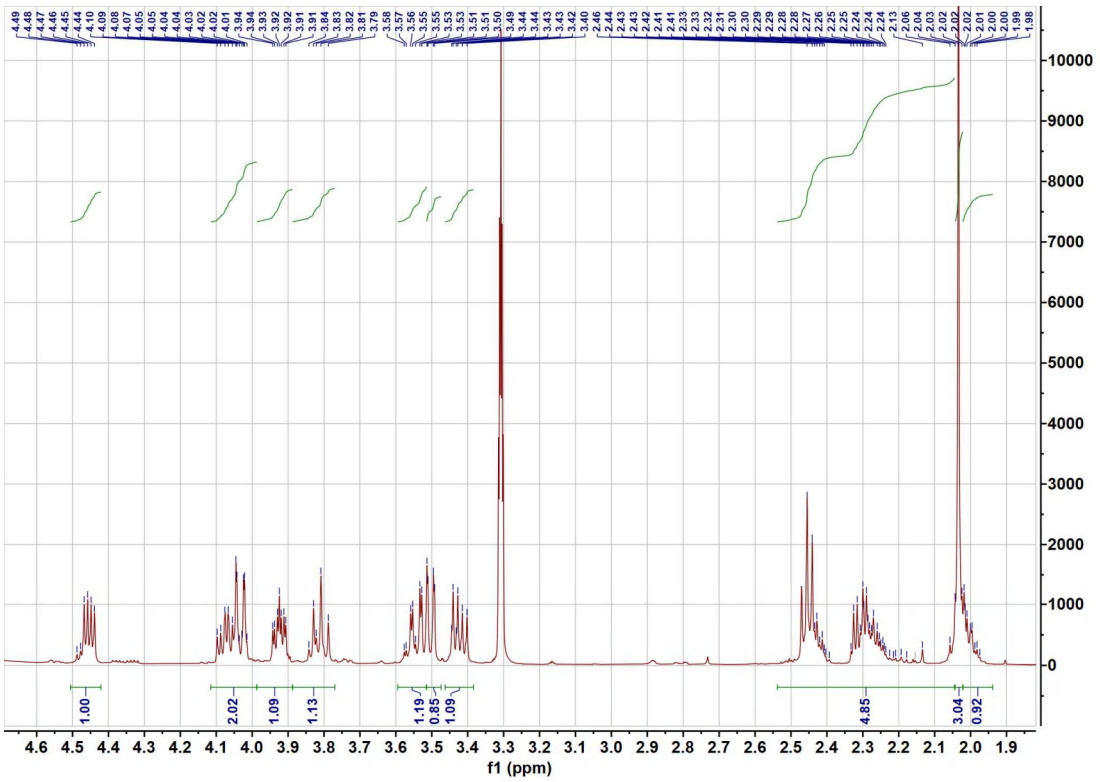


Figure S19. ^{1}H NMR spectrum of ^{Az}Sia-E

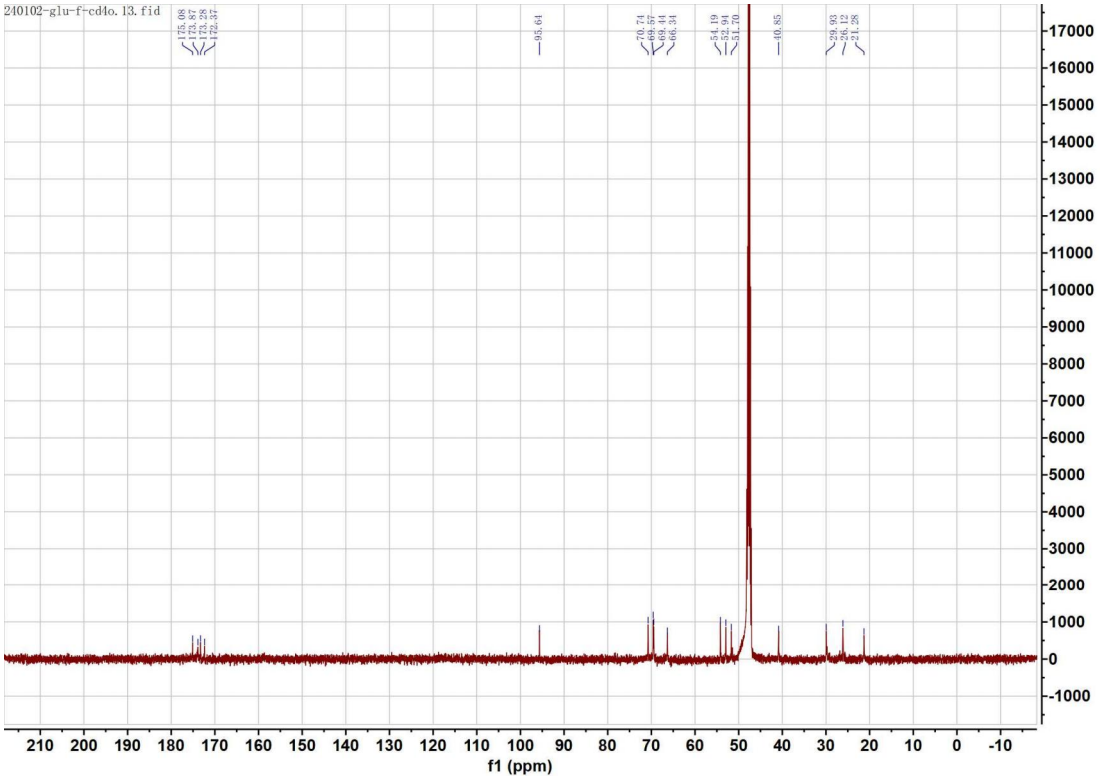


Figure S21. ^{13}C NMR spectrum of ^{Az}Sia-E

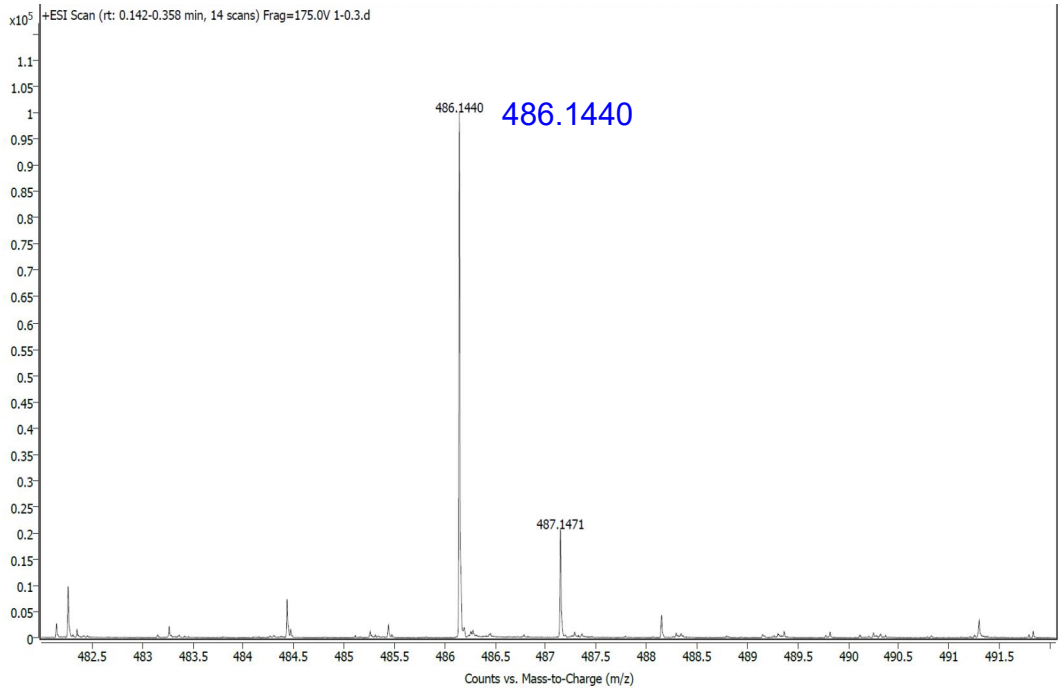


Figure S22. Mass spectrometry spectrum of ^{Az}Sia-E

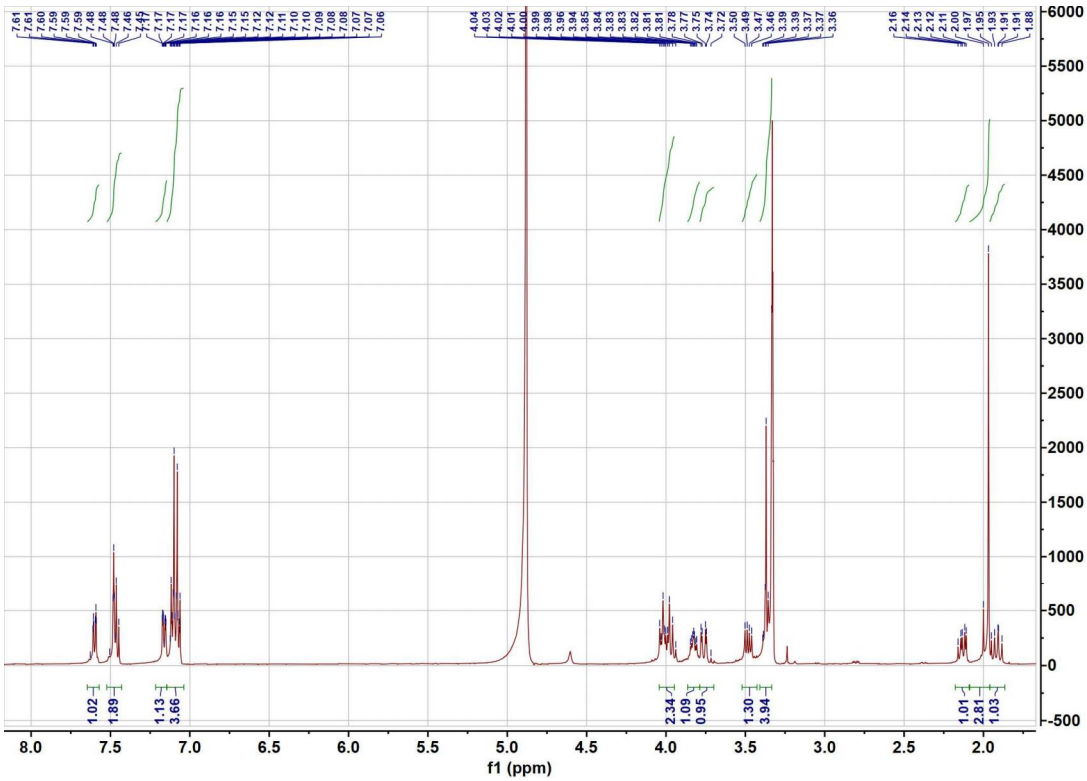


Figure S23. ^{1}H NMR spectrum of ^{AzPBA}Sia

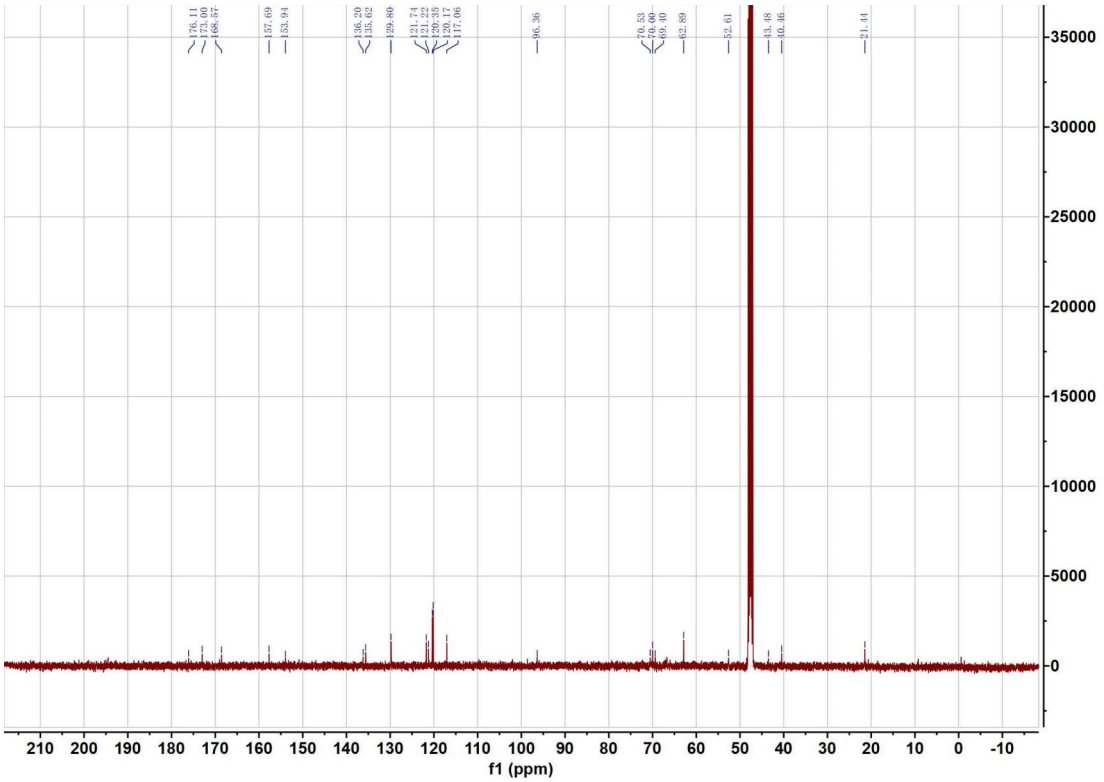


Figure S24. ^{13}C NMR spectrum of ^{AzPBA}Sia

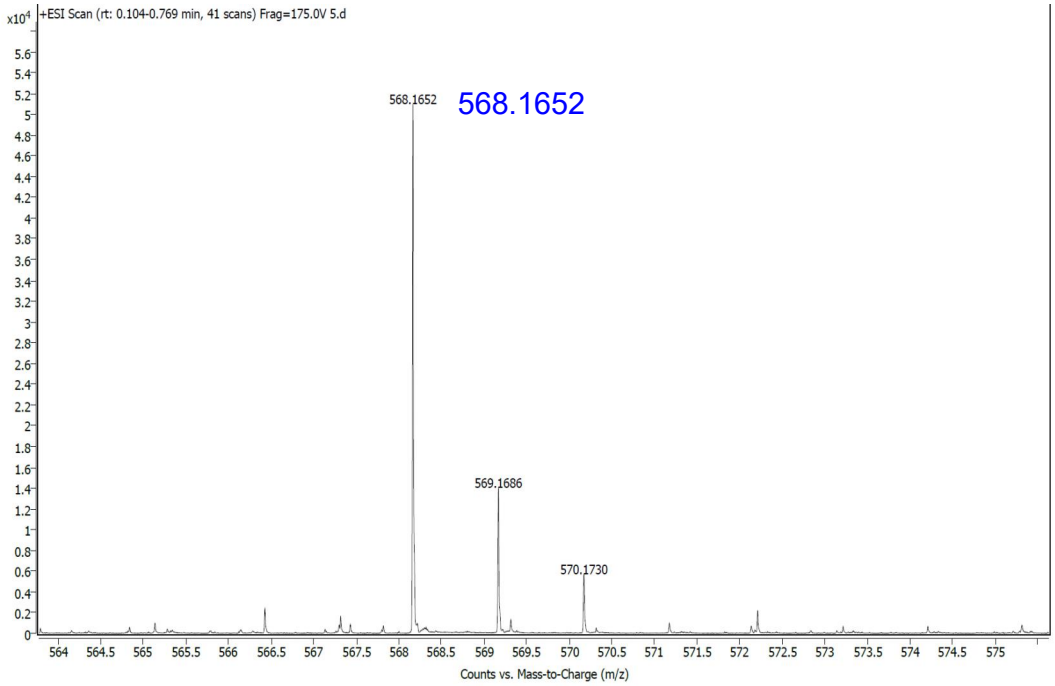


Figure S25. Mass spectrometry spectrum of ^{AzPBA}Sia

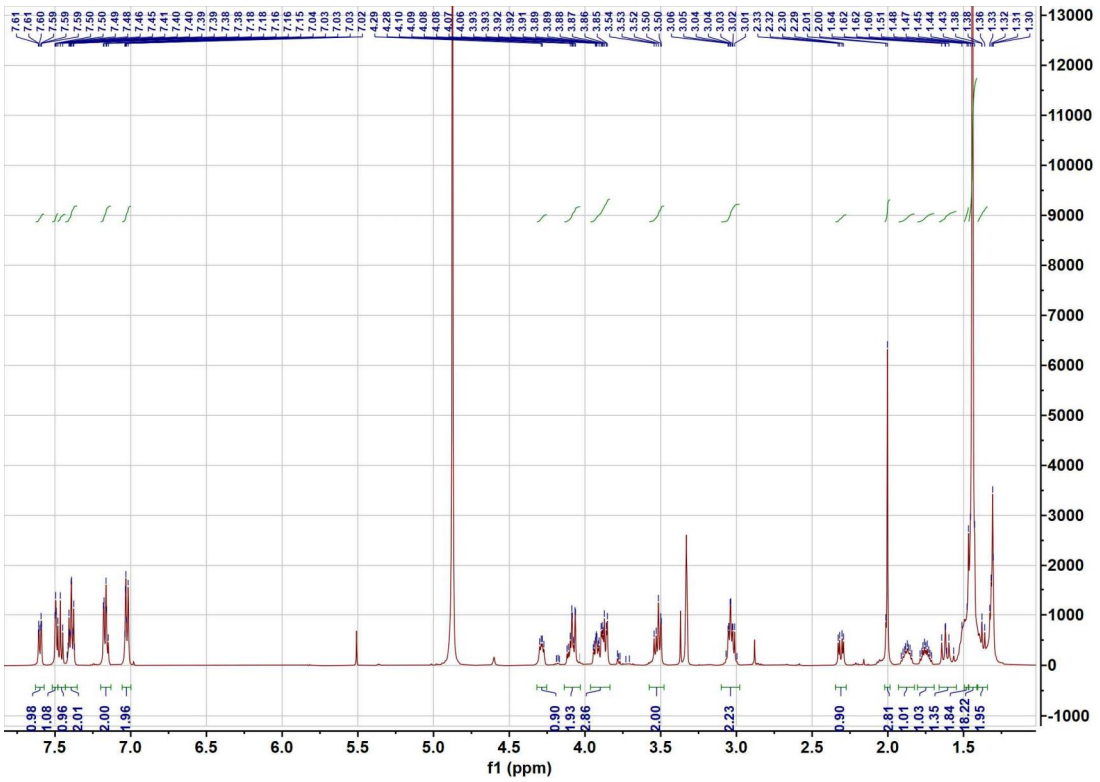


Figure S26. ^{1}H NMR spectrum of **S7**

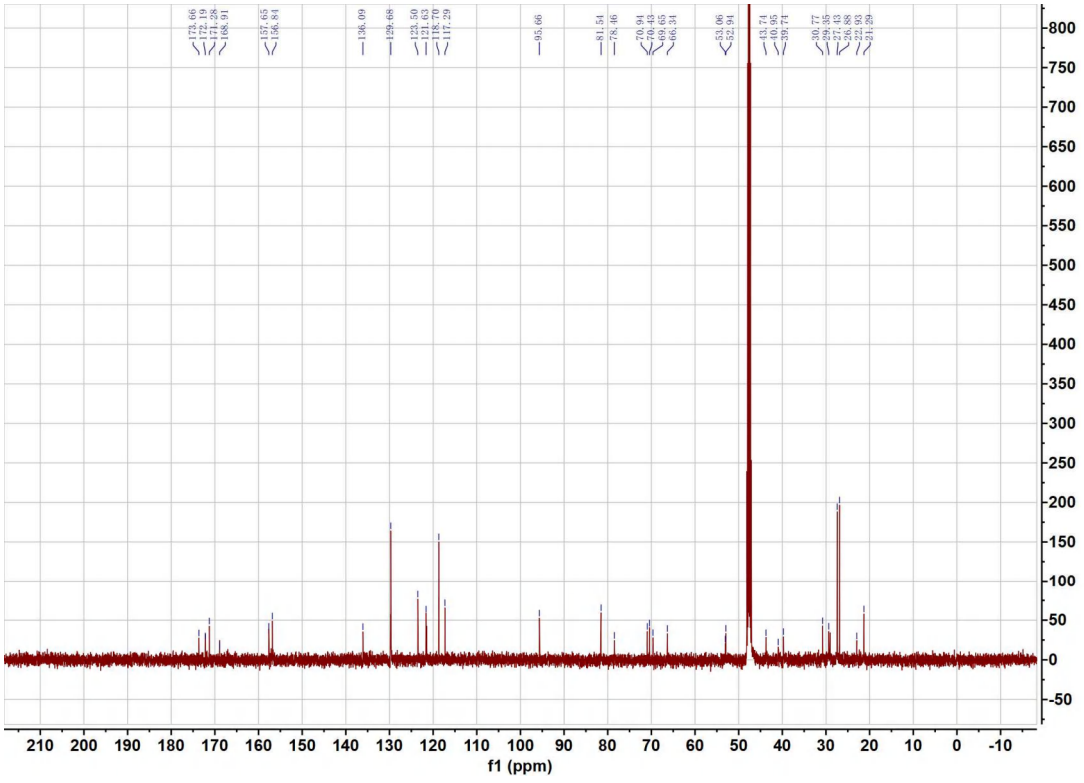


Figure S27. ^{13}C NMR spectrum of **S7**

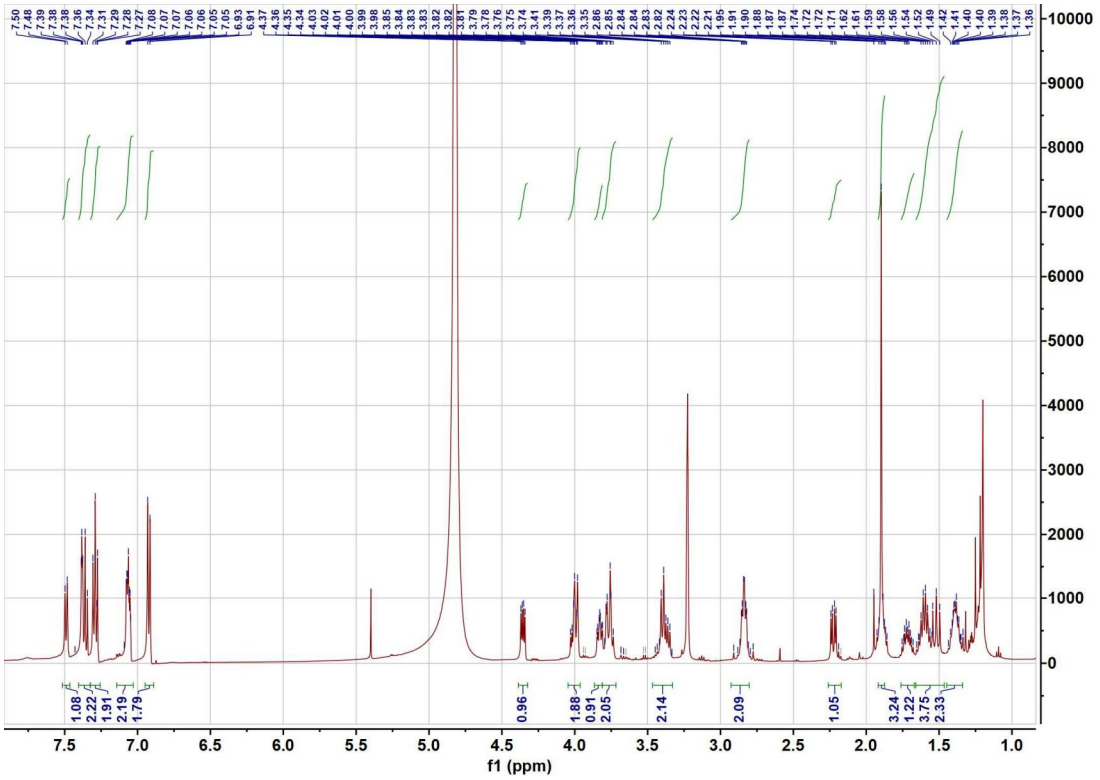


Figure S28. ^{1}H NMR spectrum of ^{PBA}Sia-K.

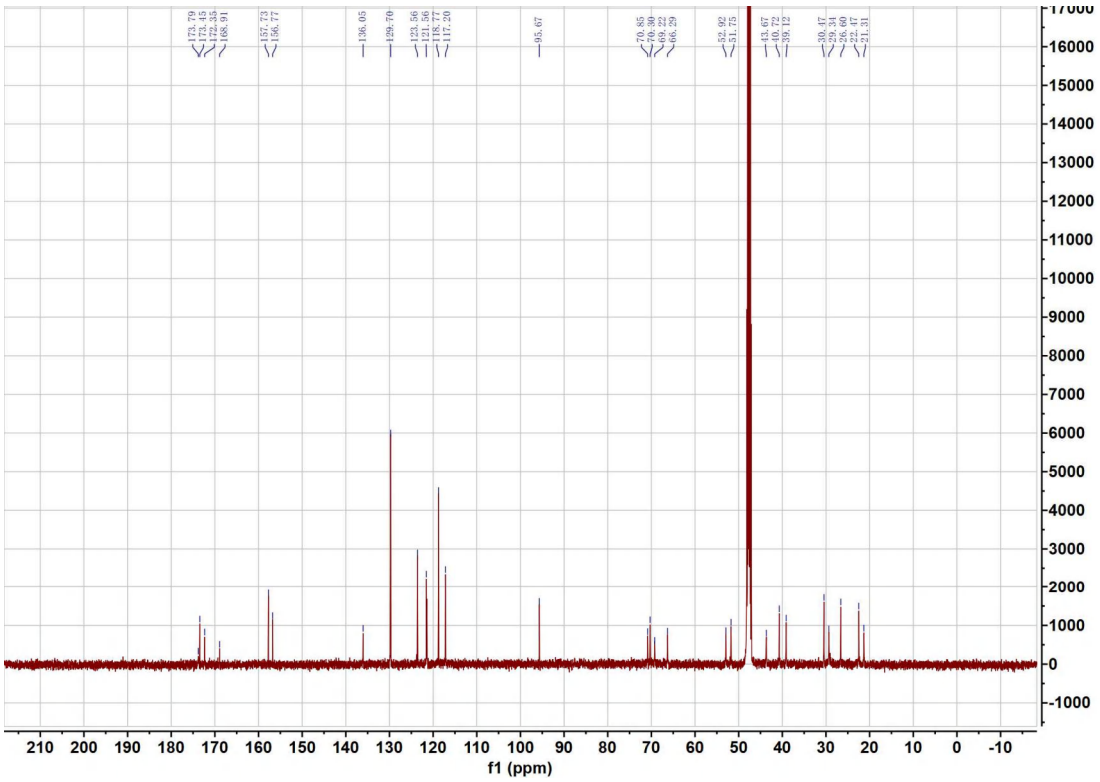


Figure S29. ^{13}C NMR spectrum of ^{PBA}Sia-K.

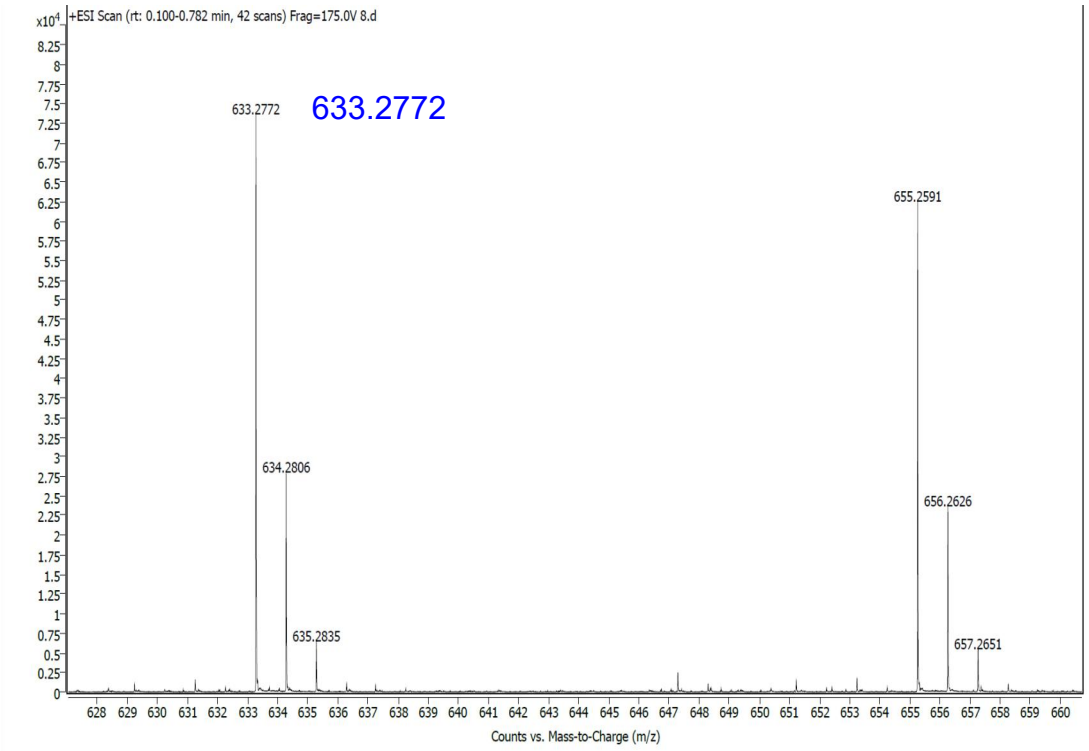


Figure S30. Mass spectrometry spectrum of ^{PBA}Sia-K.

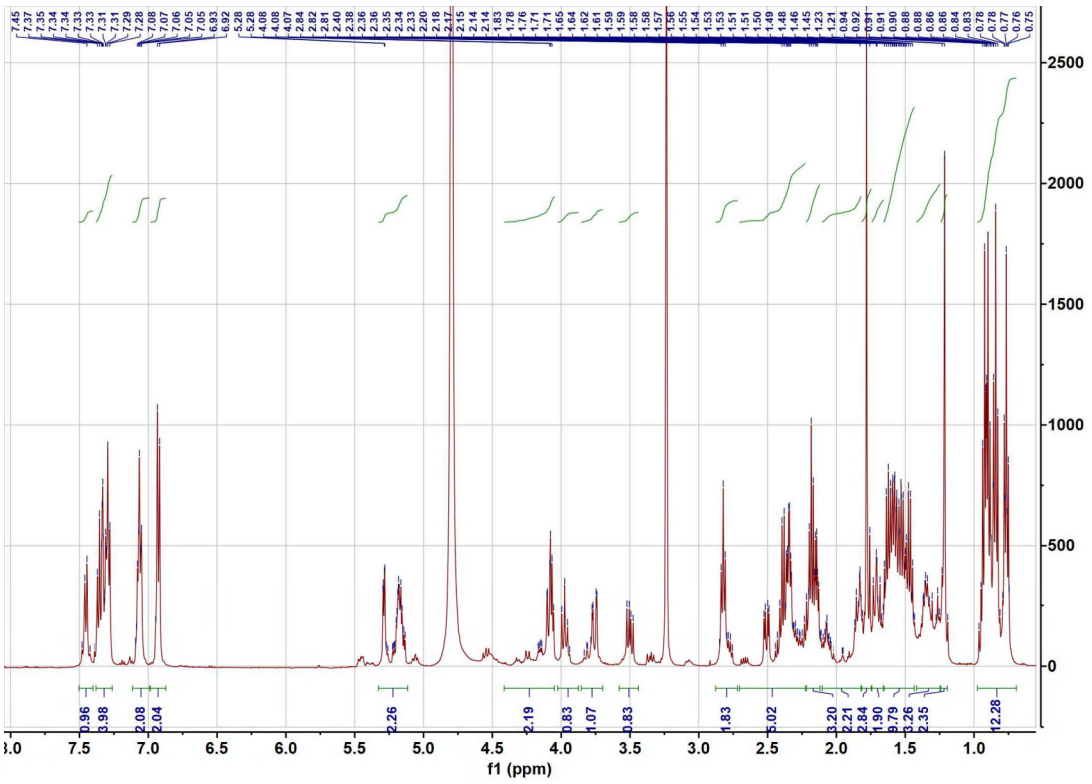


Figure S31. ^{1}H NMR spectrum of ^{PBA}Sia-Bu-K.

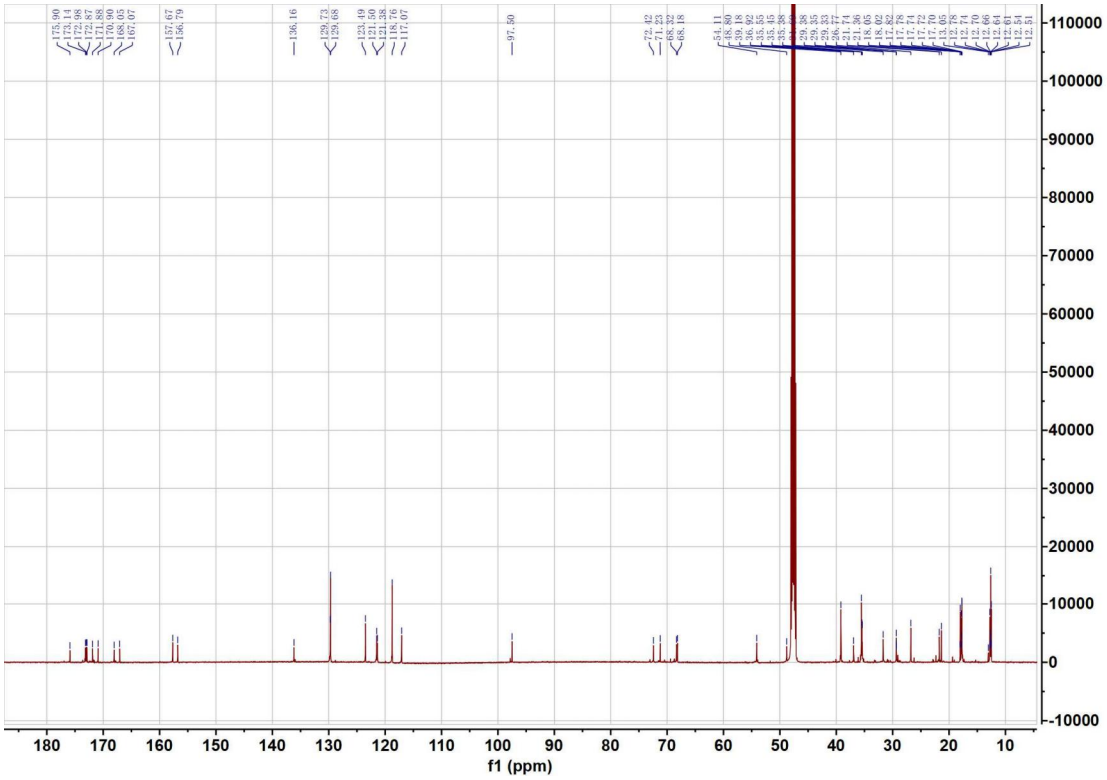


Figure S32. ^{13}C NMR spectrum of ^{PBA}Sia-Bu-K.

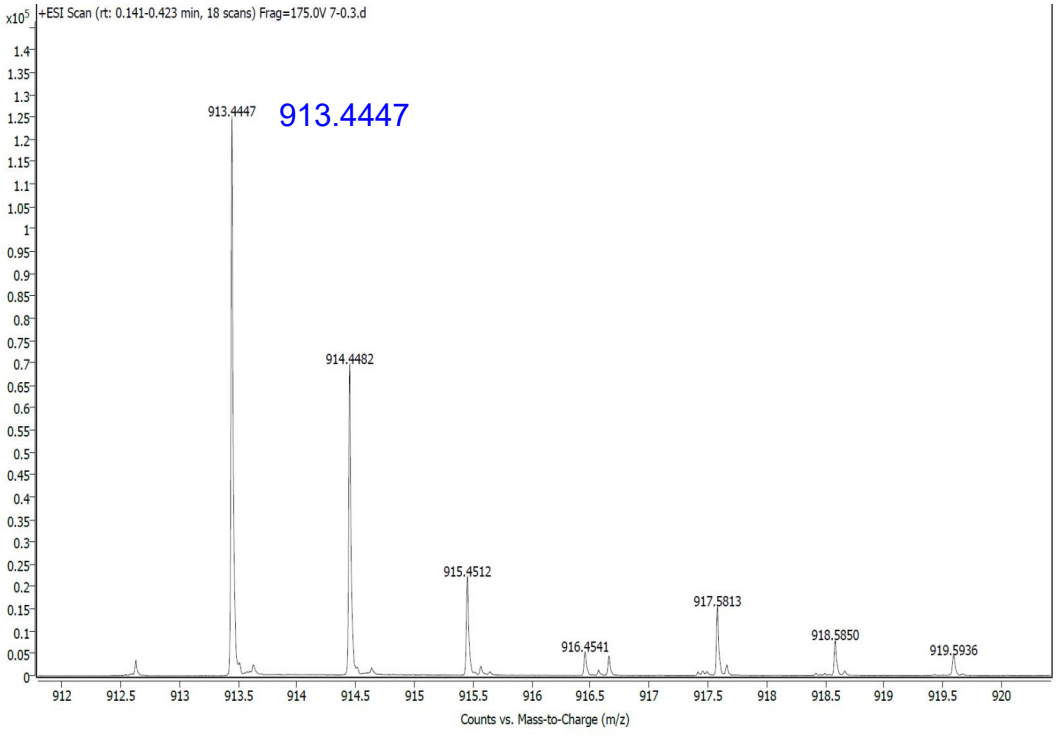


Figure S33. Mass spectrometry spectrum of ^{PBA}Sia-Bu-K.

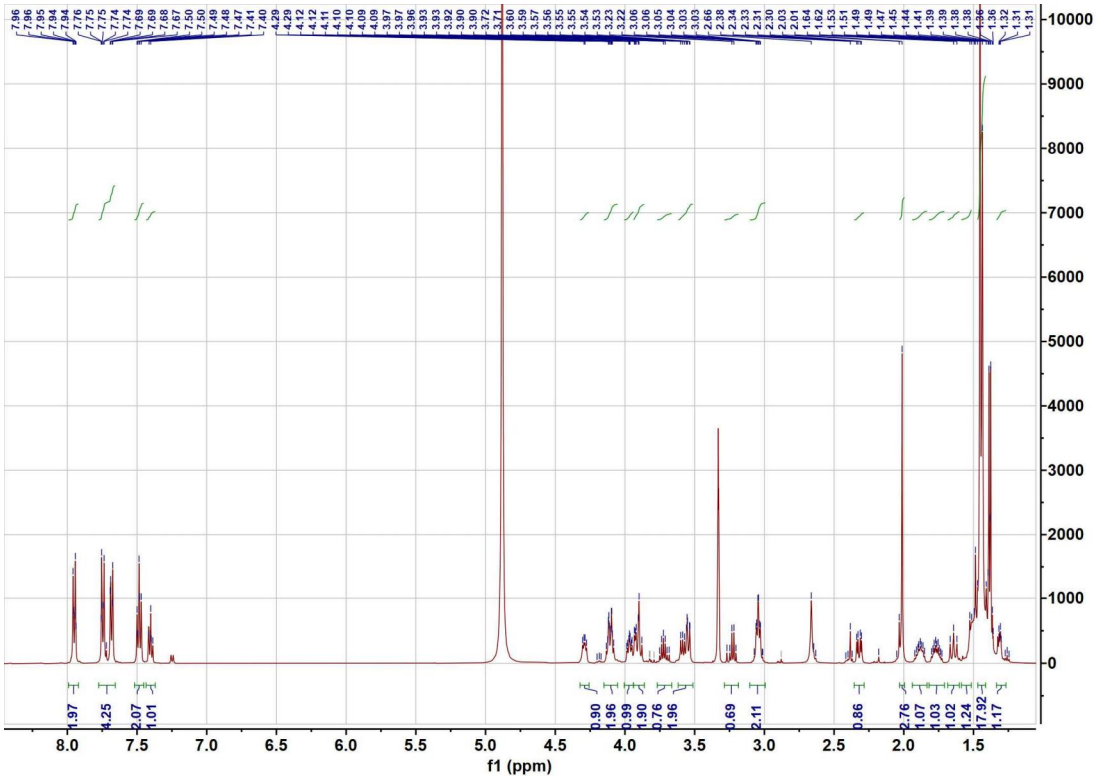


Figure S34. [1]H NMR spectrum of **S8**

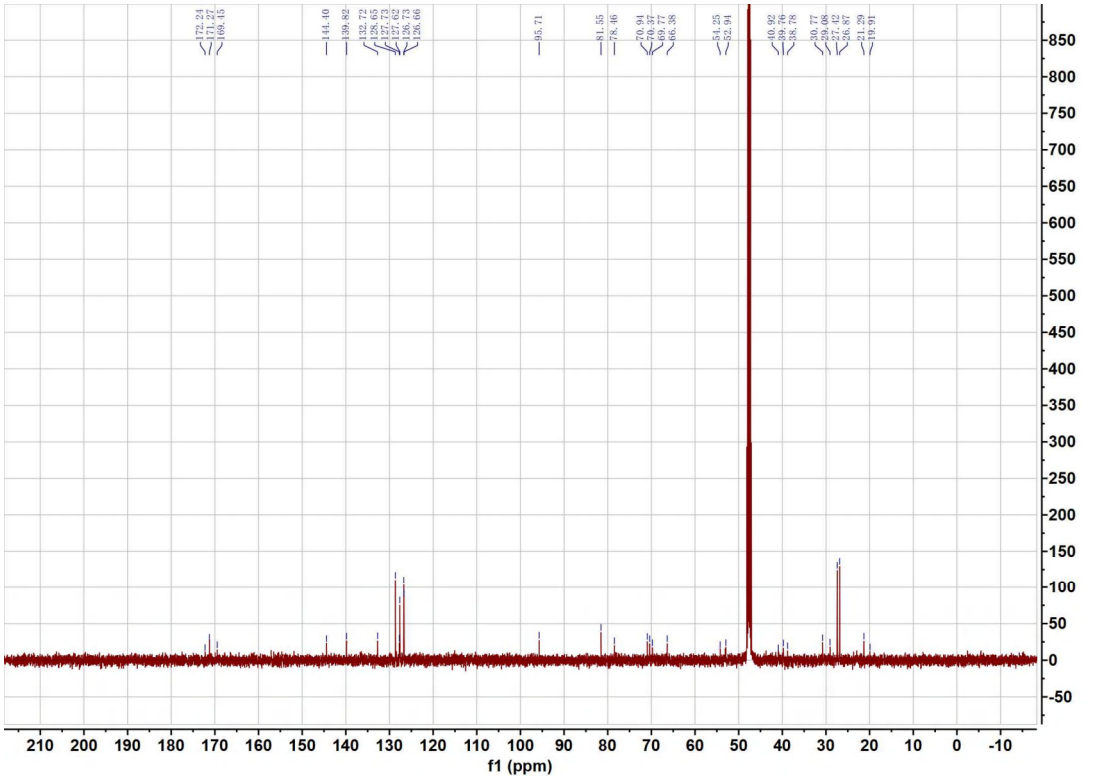


Figure S35. [13]C NMR spectrum of **S8**

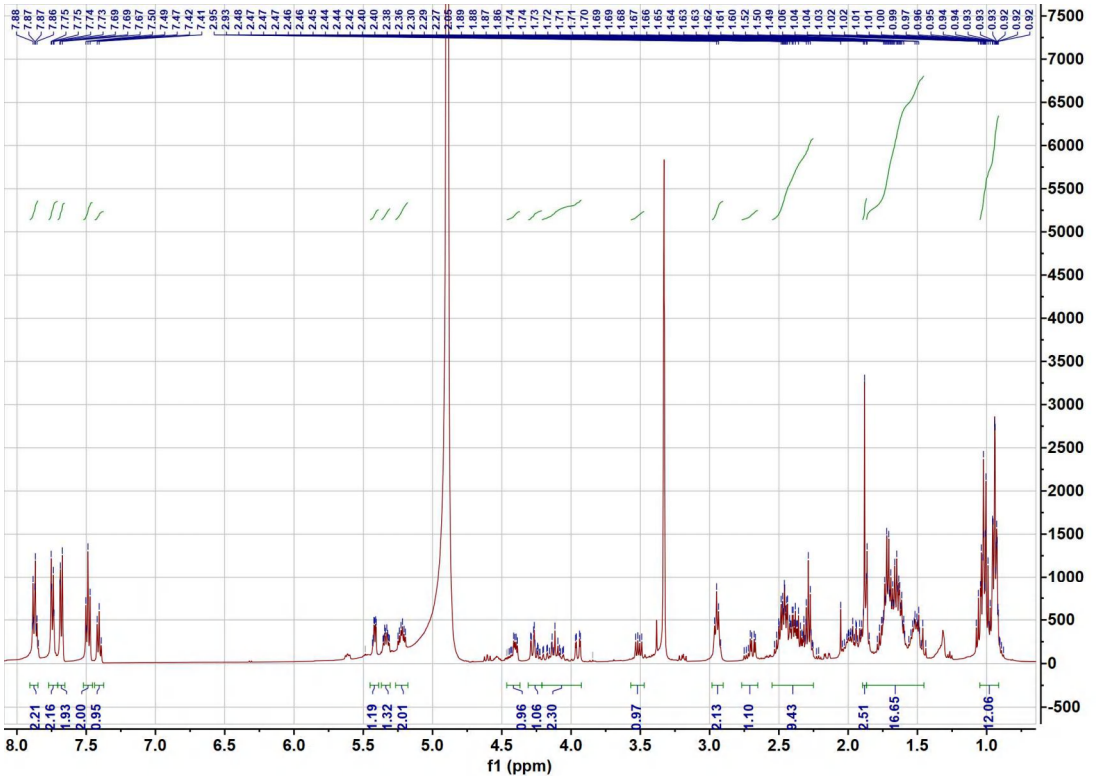


Figure S36. ^{1}H NMR spectrum of **^{BPC}Sia-Bu-K**

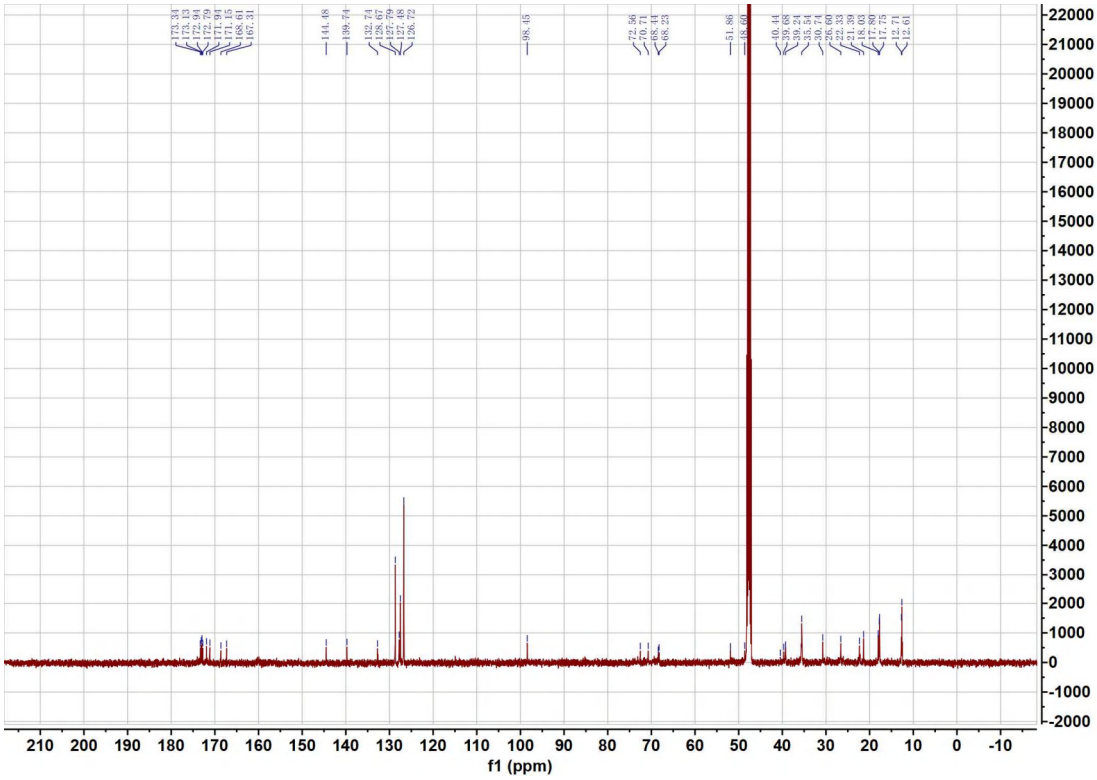


Figure S37. ^{13}C NMR spectrum of **^{BPC}Sia-Bu-K**

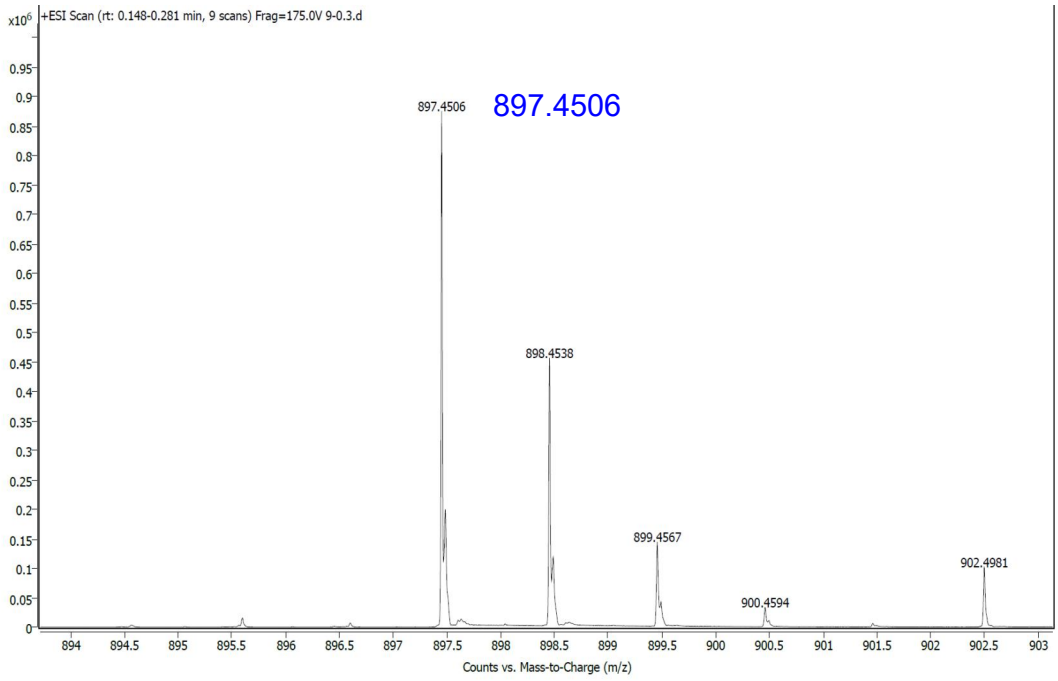


Figure S38. Mass spectrometry spectrum of ^{BPC}**Sia-Bu-K**