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Supplementary Materials for

Synthesis of Naphthoquinone-fused Enediyne Sugar Polysulfates for Nanomolar Inhibition of Coronavirus

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Contents

Materials and Characterization Methods

Tetrahydrofuran (THF) and dichloromethane (DCM) used in the experiment were pre-dried and redistilled, where THF was dried and treated with sodium sand, DCM was dried and treated with calcium hydride. Other solvents were pretreated with molecular sieve and redistilled. All other chemicals were obtained from commercial sources and used directly.

^{1}H NMR (400 MHz and 600 MHz) and ^{13}C NMR (600 MHz) spectroscopy were conducted on BRUKER AVANCE III 400 MHz and BRUKER ASCEND 600 MHz NMR spectrometers, and the deuterated solvents were deuterated chloroform ($CDCl_3$, δ_H = 7.26 ppm), deuterated methanol (CD_3OD, δ_H = 3.31 ppm) and deuterated water (D_2O, δ_H = 4.79 ppm).

High-resolution mass spectrometry (HR-MS) was conducted on a Xevo G2 TOF MS high-resolution mass spectrometer with an electrospray ionization (ESI) source whose positive and negative modes were determined by the substance properties.

Differential scanning calorimetry (DSC) was carried out with a Pyris Diamond thermal analysis workstation equipped with a model 822e DSC module under a constant nitrogen flow. The test conditions were from room temperature to 250 °C with a temperature increase rate of 10 °C/min.

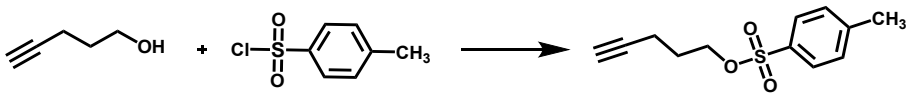
Electron paramagnetic resonance (EPR) measurements were performed with an X-band EMX-8/2.7C EPR spectrometer (Bruker). The solvent used for incubation was DMSO, and the free radical trapping agent was N-tert-butyl-α-phenylnitrone (PBN).

Synthesis of 1,4-diiodonaphthoquinone[1].

1-1

1,4-Dichloronaphthoquinone (4.0 g, 17.70 mmol) and sodium iodide (13.26 g, 88.4 mmol) were added into a 200 mL round-bottomed flask at room temperature, followed by the addition of 1,4-dioxane (60 mL) and N,N-dimethylformamide (DMF, 15 mL). The reaction was carried out under nitrogen atmosphere in an oil bath at 120 ˚C for 36 h. At the end of the reaction, the solvent was removed under vacuum and the residue was extracted with saturated saline (150 mL) and dichloromethane (DCM, 150 mL) for three times. The organic phase was collected and treated with anhydrous sodium sulfate, and the solvent was removed by with rotary evaporator. Finally, the residue was dried in a vacuum oven at 65 ˚C for 12 h, and a brown-red solid 1-1 (6.33 g, 87%) was obtained. ^{13}C NMR (600 MHz, $CDCl_3$) δ 176.6, 138.0, 134.3, 130.3, 128.9.

Synthesis of pentyl 4-alkyn-1-yl-4-methylbenzenesulfonate[2].



1-2

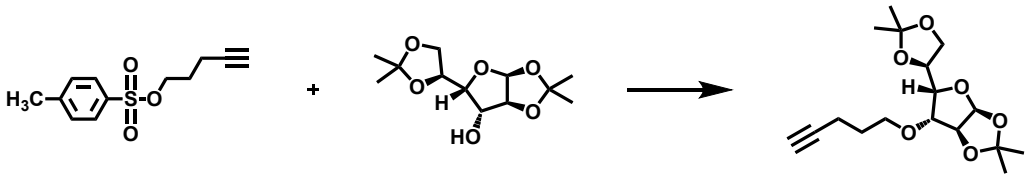
4-Toluenesulfonyl chloride (10.8 g, 57.10 mmol) was dissolved in DCM (90 mL) at room temperature and then placed in an ice-water bath. 4-Pentyn-1-ol (4.0 g, 47.58 mmol) and triethylamine (9.9 mL, 71.38 mmol) were successively added to the reaction system. After the

addition, the reaction mixture was slowly warmed up to room temperature for 24 h. The solvent was then removed, and ether (200 mL, in two portions) was added to the flask and the system became turbid. The obtained suspension was filtered and the filtrate was concentrated under vacuum. The residue was subjected to silica gel column chromatography (n-hexane: ethyl acetate = 5:1) to collect the light yellow oily product 1-2 (7.86 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 4.12 (d, J = 8.0 Hz, 2H), 4.13 (d, J = 8.0 Hz, 2H). 2H), 4.12 (t, J = 6.2 Hz, 2H), 2.43 (s, 3H), 2.22 (dd, J = 6.9, 2.7 Hz, 2H), 1.87 (t, J = 2.7 Hz, 1H), 1.85 - 1.79 (m, 2H).

General synthesis of protected hexose ether alkynes.

Bisacetone-protected hexose (1.95 g, 7.5 mmol) was dissolved in anhydrous tetrahydrofuran (THF, 20 mL) in a 100 mL Schlenk flask and then the system was placed in an ice-water bath under nitrogen with the addition of sodium hydride (0.72 g, 30 mmol) with the protective paraffin oil washed off in advance. The reaction mixture was warmed up to room temperature and stirred for 1 h. Then a solution of 1-2 (2.25 g, 9.0 mmol) in THF (15 mL) was added dropwise to the reaction system, and the reaction was carried out under nitrogen protection at 50 ˚C for 24 h. After the reaction was completed as indicated by thin layer chromatography (TLC) analysis, methanol (10 mL), water (5 mL) and saturated aqueous sodium hydroxide (5 mL) were added to the system, and the reaction was continued at 50 ˚C for another 24 h. The solvents in the system were removed by rotary evaporation, and then the residue was partitioned and extracted with saturated brine and ethyl acetate. The organic phase was dried with anhydrous sodium sulfate and concentrated by rotary evaporation, and finally the product was obtained by silica gel column chromatography (eluent: n-hexane: ethyl acetate).

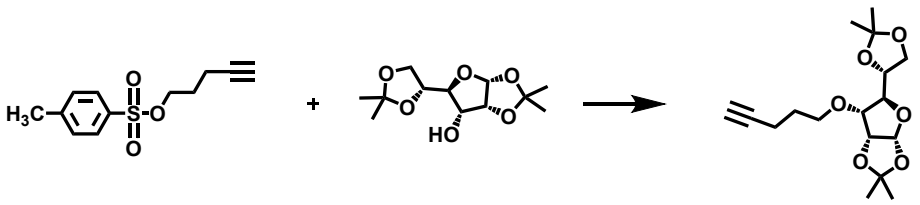
(3aR,5R,6S,6aR)-5-((R)-2,2-Dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-6-(pent-4-yn-1-hexyloxy)tetrahydrofuro[2,3-d][1,3]dioxolane.



1-2 1-3

Purification by silica gel column chromatography (eluent: n-hexane: ethyl acetate = 5:1) was carried out according to the general synthetic method, resulting in the yellow oily product 1-3 (1.13 g, 46%), which introduces a glucose structure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86 (d, J = 3.7 Hz, 1H), 4.55 (d, J = 3.7 Hz, 1H), 4.32 – 4.25 (m, 2H), 4.12 – 4.05 (m, 1H), 3.87 (d, J = 3.1 Hz, 1H), 3.71 (dd, J = 6.9, 5.4 Hz, 1H), 3.66 – 3.57 (m, 1H), 2.29 (td, J = 7.0, 2.7 Hz, 2H), 1.94 (t, J = 2.6 Hz, 1H), 1.84 – 1.70 (m, 2H), 1.49 (s, 3H), 1.42 (s, 3H), 1.35 (s, 3H), 1.31 (s, 3H)。 HR-MS (ESI), m/z calcd. for $C_{17}H_{26}O_6$, $[M + Na]^+$: 349.1627; found 349.1628.

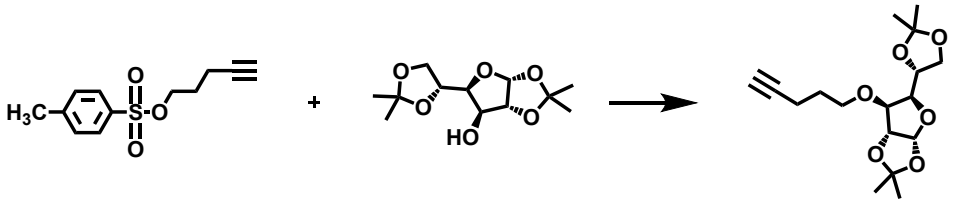
(3aR,5R,6R,6aR)-5-((R)-2,2-Dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-6-(pent-4-yn-1-hexyloxy)tetrahydrofuro[2,3-d][1,3]dioxolane.



1-2 1-4

Purification by silica gel column chromatography (eluent: n-hexane: ethyl acetate = 3:1) was carried out according to the general synthetic method, resulting in the yellow oily product 1-4 (0.79 g, 32%), which introduces an allulose structure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.76 (d, J = 3.7 Hz, 1H), 4.64 (t, J = 4.1 Hz, 1H), 4.38 (td, J = 7.0, 3.0 Hz, 1H), 4.05 – 3.93 (m, 3H), 3.84 – 3.71 (m, 2H), 3.64 – 3.52 (m, 1H), 2.31 (td, J = 7.6, 3.4 Hz, 2H), 1.92 (t, J = 2.6 Hz, 1H), 1.87 – 1.73 (m, 2H), 1.55 (s, 3H), 1.44 (s, 3H), 1.35 (d, J = 9.8 Hz, 6H). HR-MS (ESI), m/z calcd. for $C_{17}H_{26}O_6$, $[M + Na]^+$: 349.1627; found 349.1628.

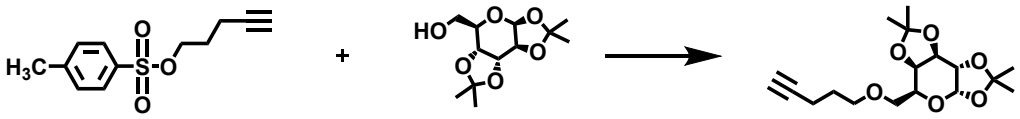
Synthesis of (3aR,5R,6S,6aR)-5-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-6-(pent-4-yn-1-hexyloxy)tetrahydrofuro[2,3-d][1,3]dioxolane



1-2 1-5

Purification by silica gel column chromatography (eluent: n-hexane: ethyl acetate = 1:1) was carried out according to the general synthetic method, resulting in the yellowish solid product 1-5 (1.65 g, 68%), which introduces the structure of gulose. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78 (d, J = 3.9 Hz, 1H), 4.69 – 4.58 (m, 2H), 4.16 – 3.97 (m, 3H), 3.81 – 3.71 (m, 1H), 3.59 – 3.47 (m, 2H), 2.28 (td, J = 7.0, 2.6 Hz, 2H), 1.95 (t, J = 2.7 Hz, 1H), 1.90 – 1.70 (m, 2H), 1.60 (s, 3H), 1.43 (s, 3H), 1.35 (d, J = 6.4 Hz, 6H). HRMS (ESI), m/z calcd. for $C_{17}H_{26}O_6$, $[M + Na]^+$: 349.1627; found 349.1628.

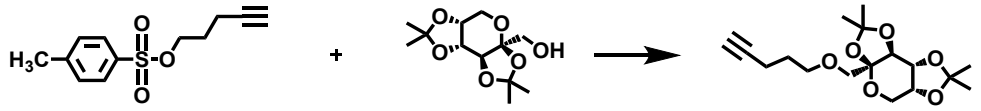
2,2,7,7-Tetramethyl-5-((pent-4-yn-1-yloxy)methyl)tetrahydro-5H-bis([1,3] dioxole)[4,5-b:4',5'-d]pyran.



1-2 1-6

Purification by silica gel column chromatography (eluent: n-hexane: ethyl acetate = 7:1) was carried out according to the general synthetic method, resulting in the yellow oily product 1-6 (1.30 g, 54%), which introduces a galactose structure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.52 (d, J = 5.0 Hz, 1H), 4.58 (dd, J = 7.9, 2.4 Hz, 1H), 4.29 (dd, J = 5.1, 2.4 Hz, 1H), 4.24 (dd, J = 7.9, 1.9 Hz, 1H), 3.95 (t, J = 5.4 Hz, 1H), 3.69 – 3.49 (m, 4H), 2.27 (td, J = 7.2, 2.6 Hz, 2H), 1.92 (t, J = 2.6 Hz, 1H), 1.78 (p, J = 6.7 Hz, 2H), 1.53 (s, 3H), 1.43 (s, 3H), 1.32 (d, J = 4.1 Hz, 6H). HR-MS (ESI), m/z calcd. for $C_{17}H_{26}O_6$, $[M + Na]^+$: 349.1627; found 349.1628.

2,2,7,7-Tetramethyl-3a-((pent-4-yn-1-yloxy)methyl)tetrahydro-5H-bis([1,3]dioxo[4,5-b:4',5'-d]pyrans.



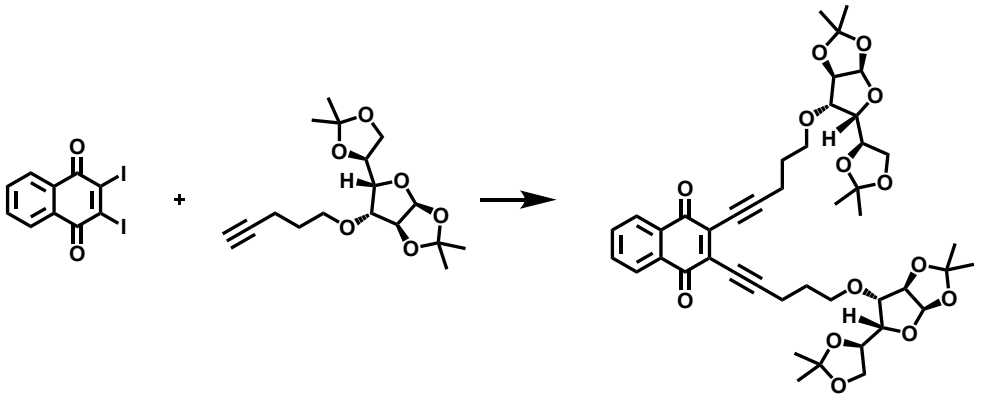
1-2 1-7

Purification by silica gel column chromatography (eluent: n-hexane: ethyl acetate = 7:1) was carried out according to the general synthetic method, resulting in the yellow oily product 1-7 (1.58 g, 65%), which introduces a fructose structure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.59 (dd, J = 7.9, 2.6 Hz, 1H), 4.38 (d, J = 2.6 Hz, 1H), 4.23 (d, J = 7.8 Hz, 1H), 3.95 – 3.86 (m, 1H), 3.72 (d, J = 13.8 Hz, 1H), 3.65 (dt, J = 9.5, 6.2 Hz, 1H), 3.61 – 3.49 (m, 3H), 2.28 (td, J = 7.2, 2.7 Hz, 2H), 1.94 (t, J = 2.6 Hz, 1H), 1.80 (p, J = 6.7 Hz, 2H), 1.53 (s, 3H), 1.47 (s, 3H), 1.42 (s, 3H), 1.34 (s, 3H)。 HRMS (ESI), m/z calcd. for $C_{17}H_{26}O_6$, $[M + Na]^+$: 349.1627; found 349.1628.

General procedure for the synthesis of enediynes (EDY-A~EDY-E)

Compound 1-1 (270.6 mg, 0.66 mmol), $PdCl_2(PPh_3)_2$ (46.3 mg, 0.06 mmol), and CuI (50.3 mg, 0.26 mmol) were added to a 25 mL Schlenk flask at room temperature and degassed for three times before adding DCM (6 mL), N,N-diisopropylethylamine (DIPEA, 0.3 mL), protected hexose ether alkyne (430.0 mg, 1.32 mmol), and dimethyl sulfoxide (DMSO, 3 mL). The reaction flask was for three times and stirred at room temperature under nitrogen protection and monitored with TLC until the complete consumption of the ether alkyne. The reaction mixture was rotary evaporated to remove most of the solvent, and the residue was extracted by washing with saturated saline and ethyl acetate. The organic phase was dried with anhydrous sodium sulfate, concentrated by rotary evaporation, and purified by silica gel column chromatography (eluent: n-hexane: ethyl acetate) to obtain the enediynes EDY-A~EDY-E.

EDY-A[3].

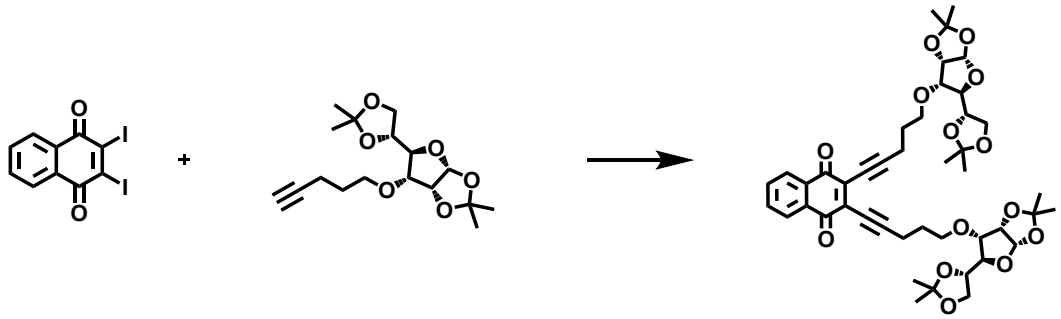


1-1 1-3 EDY-A

Following the general method for the synthesis of enediyne, purification by silica gel column chromatography (eluent: n-hexane: ethyl acetate = 1:2) resulted in the final enediyne EDY-A (222.8 mg, 42%), a brownish-red viscous liquid with the introduction of a glucose structure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, J = 5.8, 3.3 Hz, 2H), 7.74 (dd, J = 5.8, 3.3 Hz, 2H), 5.88 (d, J = 3.7 Hz, 2H), 4.66 (d, J = 3.7 Hz, 2H), 4.36 – 4.28 (m, 2H), 4.11 – 4.06 (m, 4H), 4.00 (dd, J = 8.5, 5.6 Hz, 2H), 3.91 (d, J = 3.1 Hz, 2H), 3.90 – 3.81 (m, 2H), 3.75 – 3.66 (m, 2H), 2.73 (q, J = 7.2 Hz, 4H), 2.04 – 1.82 (m, 4H), 1.49 (s, 6H), 1.42 (s, 6H), 1.35 (s, 6H), 1.31 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 181.11, 134.02, 131.84, 126.91, 111.76, 110.38, 108.98, 105.33, 82.36, 82.15, 81.14,

72.51, 68.25, 67.24, 28.35, 26.91, 26.80, 26.21, 25.39, 17.30. HR-MS (ESI), m/z calcd. for $C_{44}H_{54}O_{14}$, $[M + Na]^+$: 829.3411; found 829.3412.

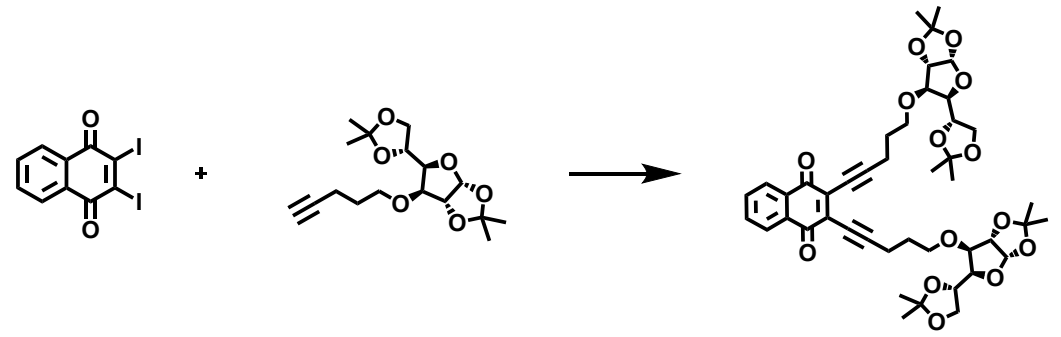
EDY-B.



1-1 **1-4** **EDY-B**

Following the general method for the synthesis of enediyne, purification by silica gel column chromatography (eluent: n-hexane: ethyl acetate = 1:2) resulted in the final enediyne EDY-C (269.0 mg, 50%), a brownish-yellow foamy solid symmetrical enediynes EDY-D (269 mg, 50%) with the introduction of an allosecose structure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, J = 5.8, 3.3 Hz, 2H), 7.74 (dd, J = 5.8, 3.3 Hz, 2H), 5.79 (d, J = 3.7 Hz, 2H), 4.74 (s, 2H), 4.40 (d, J = 3.0 Hz, 2H), 4.02 (dd, J = 7.0, 4.3 Hz, 6H), 3.93 – 3.85 (m, 4H), 3.81 – 3.70 (m, 4H), 2.76 (t, J = 7.0 Hz, 4H), 1.97 (dd, J = 13.3, 7.6 Hz, 4H), 1.57 (s, 6H), 1.44 (s, 6H), 1.35 (d, J = 3.8 Hz, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 179.22, 132.99, 130.84, 125.88, 111.88, 109.72, 108.70, 102.37, 77.92, 76.90, 76.76, 73.78, 67.66, 64.02, 27.64, 25.81, 25.59, 25.23, 24.10, 16.34. HR-MS (ESI), m/z calcd. for $C_{44}H_{54}O_{14}$, $[M + Na]^+$: 829.3411; found 829.3412.

EDY-C.

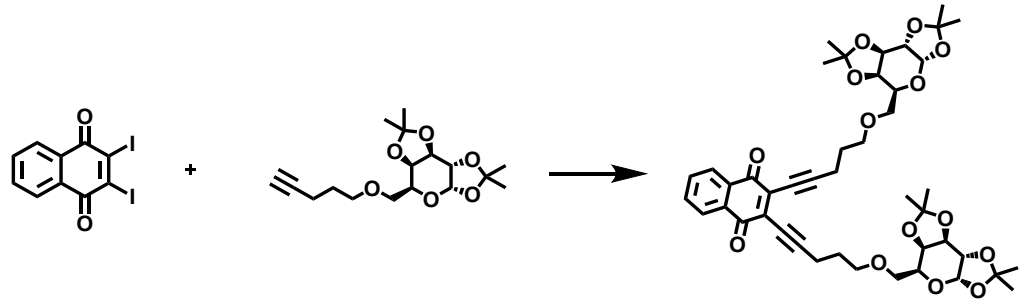


1-1 **1-5** **EDY-C**

Following the general method for the synthesis of enediyne, purification by silica gel column chromatography (eluent: n-hexane: ethyl acetate = 1:2) resulted in the final enediynes EDY-E (228.1 mg, 43%), a brownish-yellow foamy solid that introduces the structure of a gulose. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, J = 5.8, 3.3 Hz, 2H), 7.74 (dd, J = 5.8, 3.3 Hz, 2H), 5.80 (d, J = 3.9 Hz, 2H), 4.74 – 4.67 (m, 2H), 4.70 – 4.60 (m, 2H), 4.08 (d, J = 7.4 Hz, 3H), 3.94 – 3.85 (m, 2H), 3.79 – 3.65 (m, 2H), 3.63 – 3.56 (m, 2H), 2.72 (t, J = 6.9 Hz, 4H), 1.97 (dt, J = 18.7, 6.5 Hz, 4H), 1.62 (s, 6H), 1.43 (s, 6H), 1.35 (d, J = 8.4 Hz, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ179.61, 132.26, 130.83, 125.86, 114.50, 108.39, 107.55, 104.08, 81.62, 77.89, 77.01, 74.40, 67.94, 65.89, 27.42,

25.81, 25.75, 25.37, 24.28, 16.35. HR-MS (ESI), m/z calcd. for $C_{44}H_{54}O_{14}$, $[M + Na]^+$: 829.3411; found 829.3410.

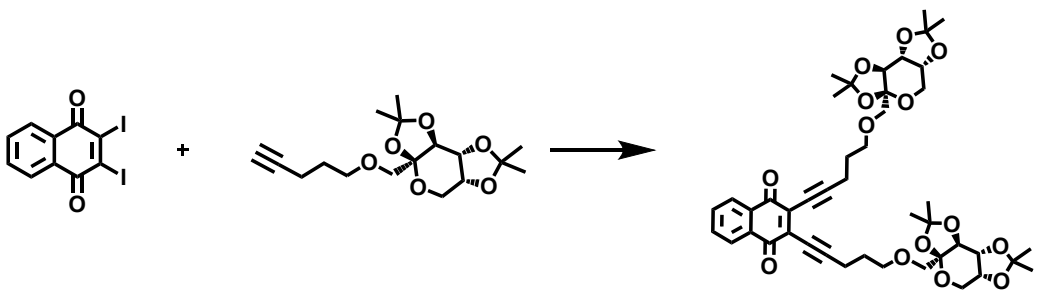
EDY-D.



1-1 **1-6** **EDY-D**

Following the general method for the synthesis of enediyne, purification by silica gel column chromatography (eluent: n-hexane: ethyl acetate = 3:2) resulted in the final enediyne EDY-B (217.5 mg, 41%), a brownish-red viscous liquid with an introduced galactose structure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 9.1 Hz, 2H), 7.73 (dd, J = 5.8, 3.3 Hz, 2H), 5.53 (d, J = 5.0 Hz, 2H), 4.59 (dd, J = 8.0, 2.4 Hz, 2H), 4.33 – 4.23 (m, 4H), 4.01 – 3.93 (m, 2H), 3.75 – 3.56 (m, 6H), 2.71 (t, J = 7.1 Hz, 4H), 1.94 (p, J = 6.9 Hz, 4H), 1.53 (s, 6H), 1.44 (s, 6H), 1.32 (d, J = 10.5 Hz, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 179.39, 133.94, 131.88, 126.78, 110.97, 109.38, 108.52, 96.96, 71.19, 70.64, 70.59, 69.66, 69.57, 66.73, 28.49, 26.10, 25.99, 24.93, 24.40, 17.41, 14.20. HR-MS (ESI), m/z calcd. for $C_{44}H_{54}O_{14}$, $[M + Na]^+$: 829.3411; found 829.3412.

EDY-E.



1-1 **1-7** **EDY-E**

Following the general method for the synthesis of enediyne, purification by silica gel column chromatography (eluent: n-hexane: ethyl acetate = 1:2) resulted in the final enediyne EDY-C (320.4 mg, 60%), a brownish-red viscous liquid that introduces a fructose structure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dd, J = 5.8, 3.3 Hz, 2H), 7.73 (dd, J = 5.8, 3.3 Hz, 2H), 4.58 (dd, J = 7.9, 2.6 Hz, 2H), 4.40 (d, J = 2.5 Hz, 2H), 4.22 (d, J = 8.0 Hz, 2H), 3.90 (dd, J = 13.0, 1.8 Hz, 2H), 3.80 – 3.65 (m, 6H), 3.58 (q, J = 10.4 Hz, 4H), 2.72 (t, J = 7.1 Hz, 4H), 2.02 – 1.90 (m, 4H), 1.53 (s, 6H), 1.45 (s, 6H), 1.43 (s, 6H), 1.30 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 181.81, 134.38, 134.05, 131.76, 126.91, 110.57, 108.90, 108.54, 102.62, 72.33, 71.03, 70.24, 70.06, 61.00, 28.43, 26.57, 25.90, 25.41, 24.00, 17.49. HR-MS (ESI), m/z calcd. for C44H54O14, $[M + Na]^+$: 829.3411; found 829.3413.

General procedure for the synthesis of naphthoquinone-fused enediyne sugar polysulfates.

1. Deprotection of bisacetone-protected enediynes: EDY-A (100 mg, 0.12 mmol) was added to a 25 mL round-bottomed flask, to which trifluoroacetic acid (3 mL) and water (3 mL) were added sequentially. Then the reaction was stirred for 4 h at room temperature, and the solvent was removed after the complete deprotection as indicated by TLC analysis. DCM was added to the mixture and removed with rotary evaporation. This procedure was repeated twice to give the deprotected enediynes.

2. Sulfation of deprotected enediyne: The deprotected enediynes obtained in the previous step was dissolved in 2 mL of anhydrous DMF, and then SO3 • DMF (735 mg, 4.80 mmol) in DMF (1 mL) was added dropwise into the reaction system. After 4 h of reaction at room temperature, aqueous solution of sodium bicarbonate (806 mg, 9.60 mmol) was added dropwise to the system under the condition of ice water bath to quench the excess of SO3 • DMF. Finally, the mixture was connected to a vacuum line remove all the volatile components at room temperature.

3. Post-treatment of enediyne sulfate: the residue obtained above was dissolved with water (3 mL) and transferred to a dialysis bag with a cut-off molecular weight of 300 D. The water outside of the dialysis bag was replaced with pure water in a 1 L beaker every 1 h for six times, till no sulfate could be detected outside of the dialysis bag with the addition of barium chloride. Finally, the solution in the dialysis bag was lyophilised to give the product.

Based on the general synthesis of naphthoquinone-fused enediyne sugar polysulfates, the yield was calculated as complete sulfation, resulting in EDY-1 (159.5 mg, 88%), EDY-2 (79.0 mg, 44%), EDY-3 (85.7 mg, 47%), EDY-4 (121.3 mg, 67%), and EDY-5 (125.9 mg. 69%).

Radical generating property of enediyne antivirals

A solution of N-tert-butyl-α-phenylnitrone (PBN, 100 mM) in DMSO was prepared in advance, then enediynes were added respectively to ensure their final concentration was 20 mM. The solution of PBN (100 mM) without addition of enediyne was used as the control. All samples were placed in a water bath at 37 °C and reacted for 24 h before the electron paramagnetic resonance (EPR) measurements. The EPR spectra were recorded with an X-band EMX-8/2.7C EPR spectrometer (Bruker, Germany).

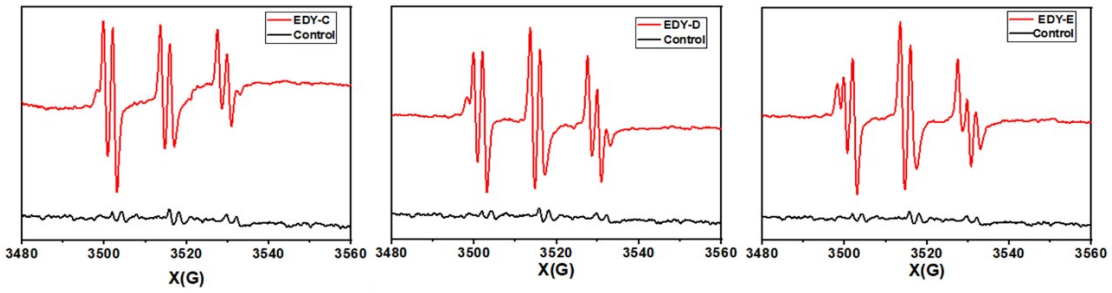


Figure S1. EPR spectra EDY-C, EDY-D and EDY-B in the presence of PBN.

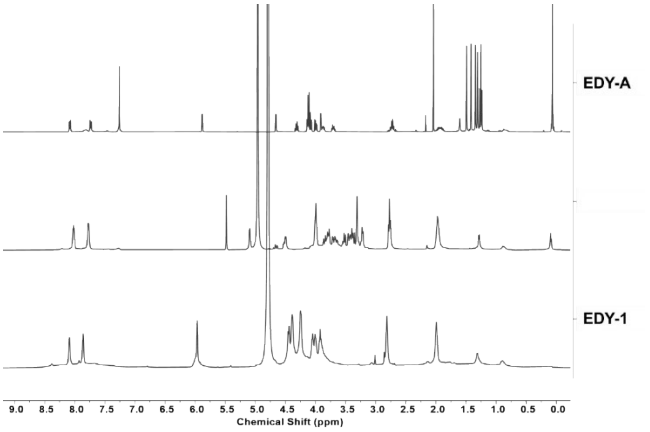


Fig S2. Comparison of the 1H-NMR spectra of EDY-A (in $CDCl_3$) and EDY-1 (in D_2O), with the ^{1}H-NMR spectrum of the deprotected intermediate of EDY-A (in CD_3OD) in the middle.

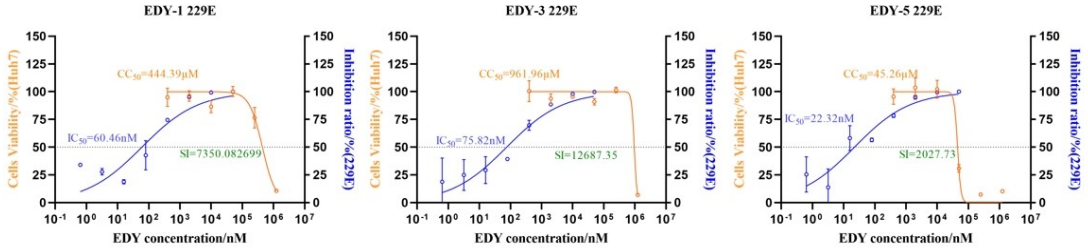


Fig S3. Antiviral performance and cytotoxicity of EDY-1, EDY-3 and EDY-5.

Antiviral activity of naphthoquinone-fused enediyne sugar polysulfates to seasonal coronavirus.

Seasonal coronavirus (HCoV-229E) viral stocks were diluted separately 1000 times by PBS to 200 PFUs/mL. The enediynes were serially diluted (5-fold) to different concentrations(100 μM, 20 μM, 4 μM, 800 nM, 160 nM, 32 nM, 6.4 nM, 1.28 nM and 0 nM) mixed with HCoV, and incubated at 37 °C for 1 h. To determine the viral titers, the mixtures were added to the monolayer Huh-7 respectively. The control group of viruses without the enediyne(0 nM) was also set. Each group was provided with three repeating wells. After an incubation at 32 °C for 1 h, the supernatant was removed, and culture medium (DMEM supplement with 2% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 1.2% Avicel) was layered on the cell. After 96 h culture, the layer was removed, and the cells were fixed with 4% polyformaldehyde and stained with 1% crystal violet. After staining at room temperature for 20 min, the plates were washed with running water to remove the dying liquid. The plaques were counted after the plates were dried at room temperature. The inhibition rate of enediyne against seasonal coronavirus was calculated as follows: (number of plaques in control group - number of plaques in experimental group)/number of plaques in control group × 100%.

Cytotoxicity assay of naphthoquinone-fused enediyne sugar polysulfates

The cytotoxicity of enediyne was tested based on cell counting kit-8 (CCK-8) (A311, Vazyme, China). The Huh-7 cells were seeded separately into the 96-well microplates (3×10^4 cells per well at 100 μL) and cultured at 37 °C overnight. The 5-fold diluted enediyne (from 1.25 mM to 400 nM) was added to the monolayer cells. Each enediyne dilution is conducted with 3 replicates. The preparation of the serum-free DMEM control and blank wells was conducted at the same time. After

being incubated at 37 °C for 24 h, the supernatant was removed and replaced with 100 μL DMEM. After 10 μL CCK-8 solution was added to each well, the cells were incubated at 37 °C for 1 h. The absorbance at a wavelength of 450 nm was detected with a microplate reader (Biotek Synergy H1, United States). The cell viability rate was calculated as (optical density (OD) value of experimental group - OD value of blank)/ (OD value of control group - OD value of blank) × 100%.

Hemolysis assay of naphthoquinone-fused enediyne sugar polysulfates

100 μL of the 2% rabbit erythrocytes (SenBeiJia, China) was added to each tube of the 8-Tube Strips. Then, 100 μL of serial diluted solution of EDY-2 (denoted as Z09 in this assay, 10, 5, 2.5, 1.25, 0.625, 0.3125 mM) was added to the corresponding tubes, including the control wells of positive (Trition X-100) and negative (PBS) controls. The tubes were gently inverted for several times and incubated at 37°C for 1 h. After incubation, the test tubes were centrifuged at 1,000 rpm for 1 min at room temperature for observation of hemolysis.

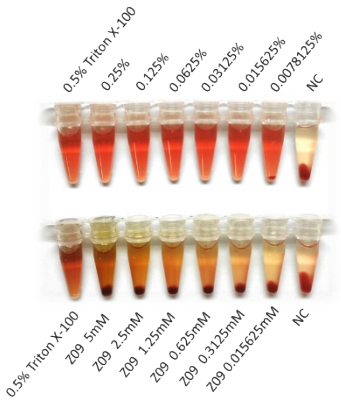


Fig S4. Hemolysis assay result of EDY-2.

NMR and high-resolution mass spectra of compounds

Fig. S5 ^{13}C NMR spectrum of 1-1 ($CDCl_3$)

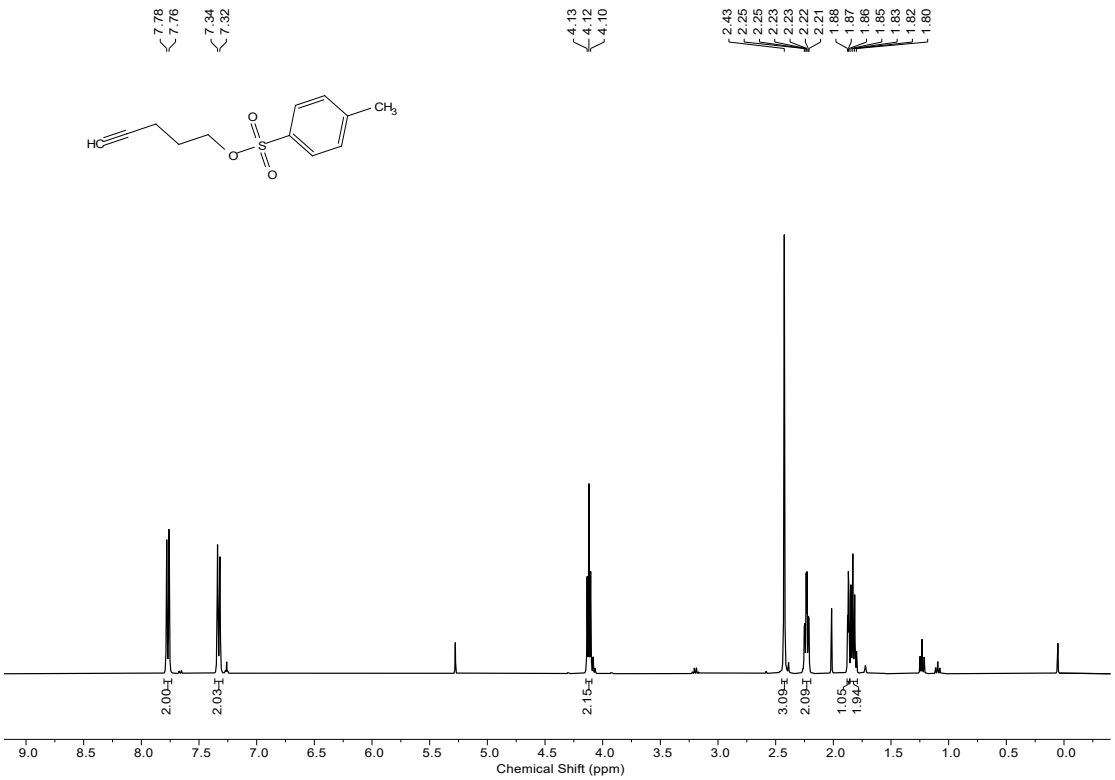


Fig. S6 ^{1}H NMR spectrum of 1-2 ($CDCl_3$)

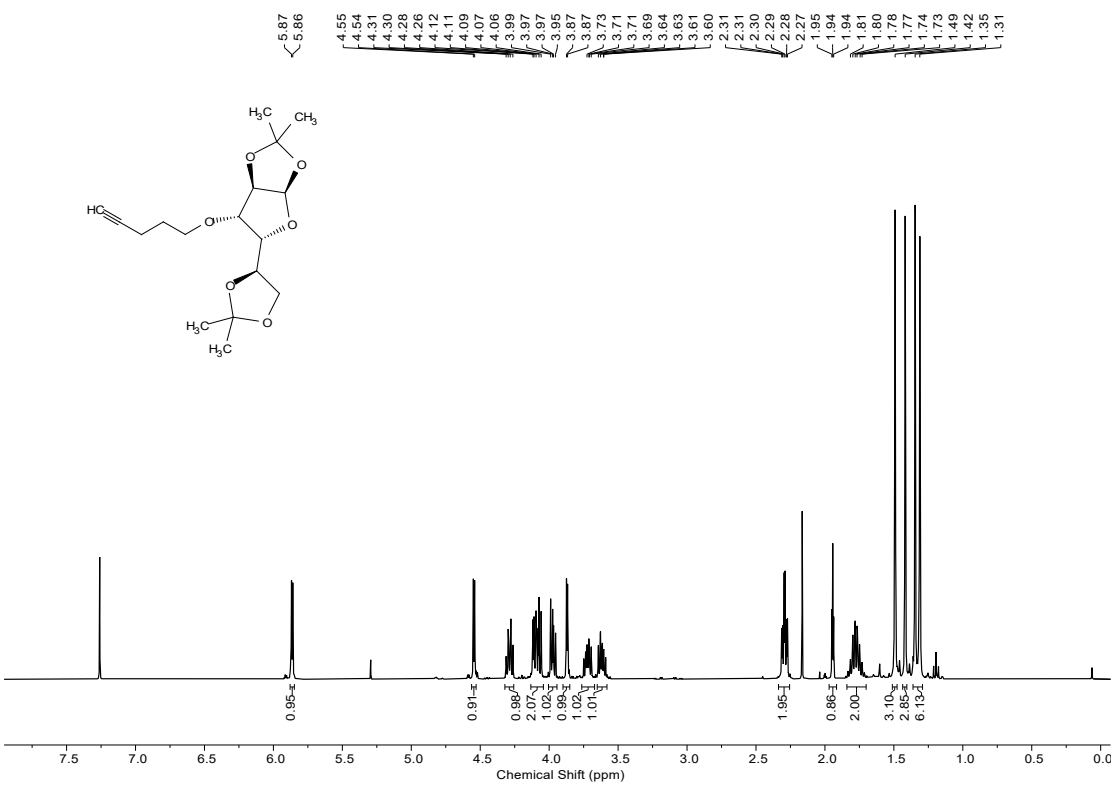


Fig. S7 ^{1}H NMR spectrum of 1-3 ($CDCl_3$)

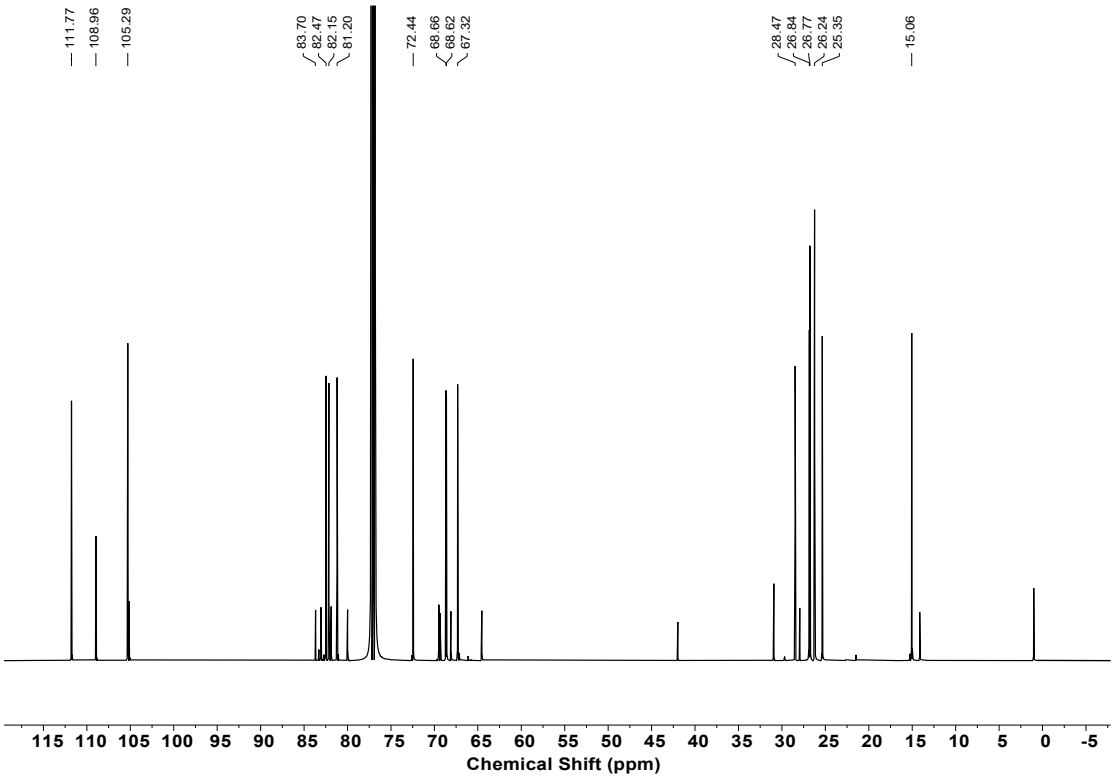


Fig. S8 ^{13}C NMR spectrum of 1-3 ($CDCl_3$)

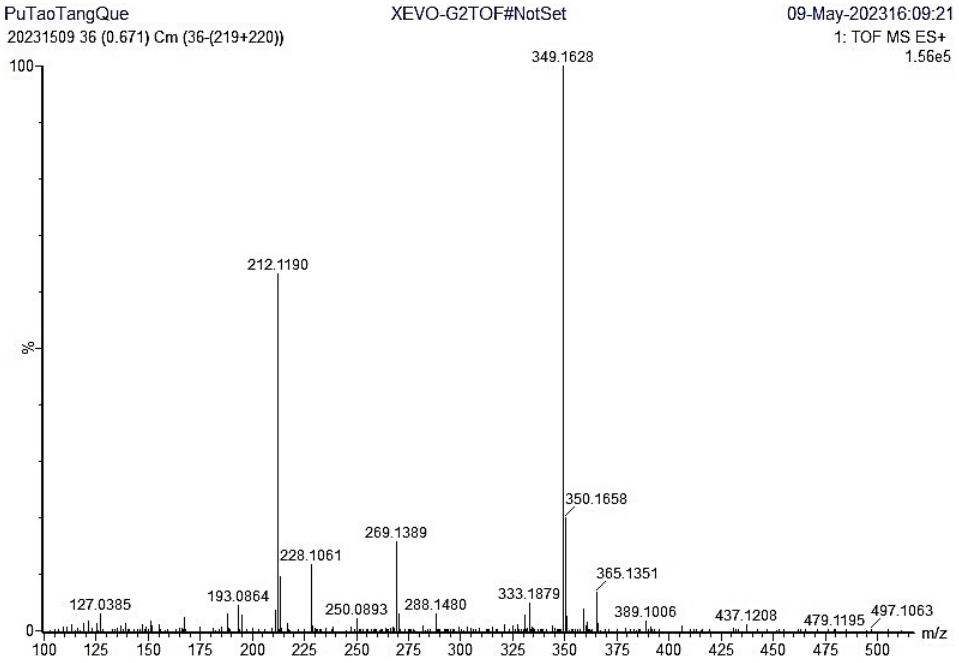


Fig. S9 High-resolution mass spectrum of 1-3

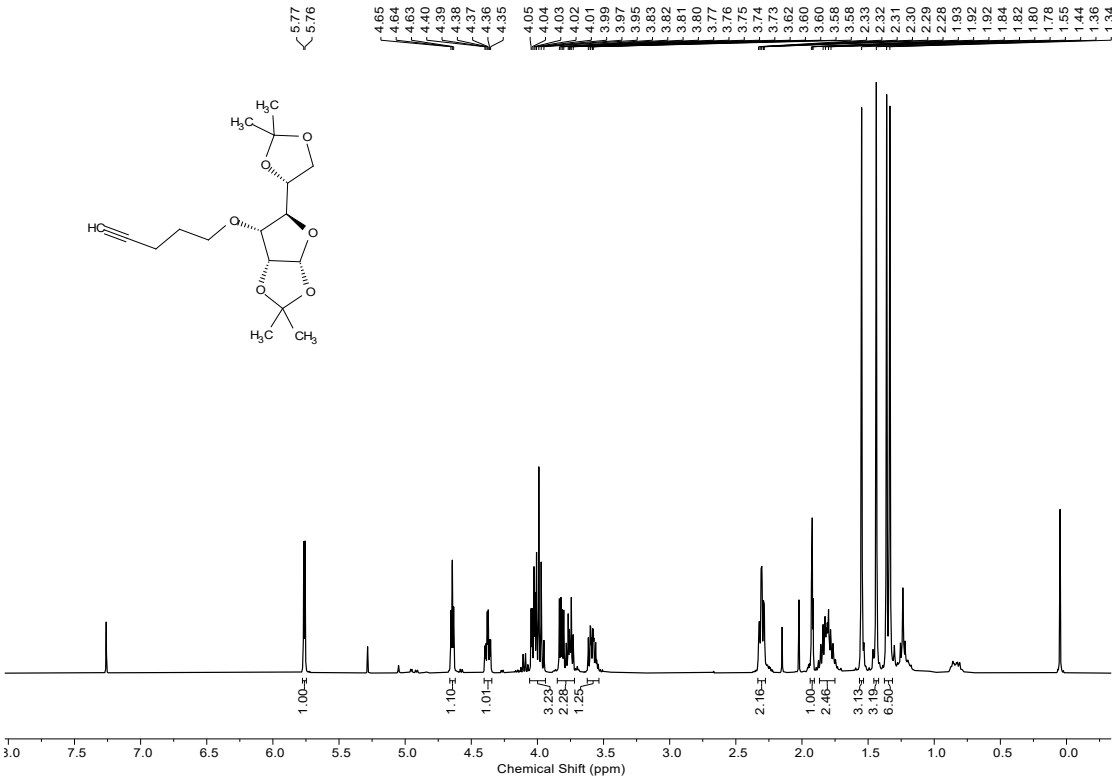


Fig. S10 ^{1}H NMR spectrum of 1-4 ($CDCl_3$)

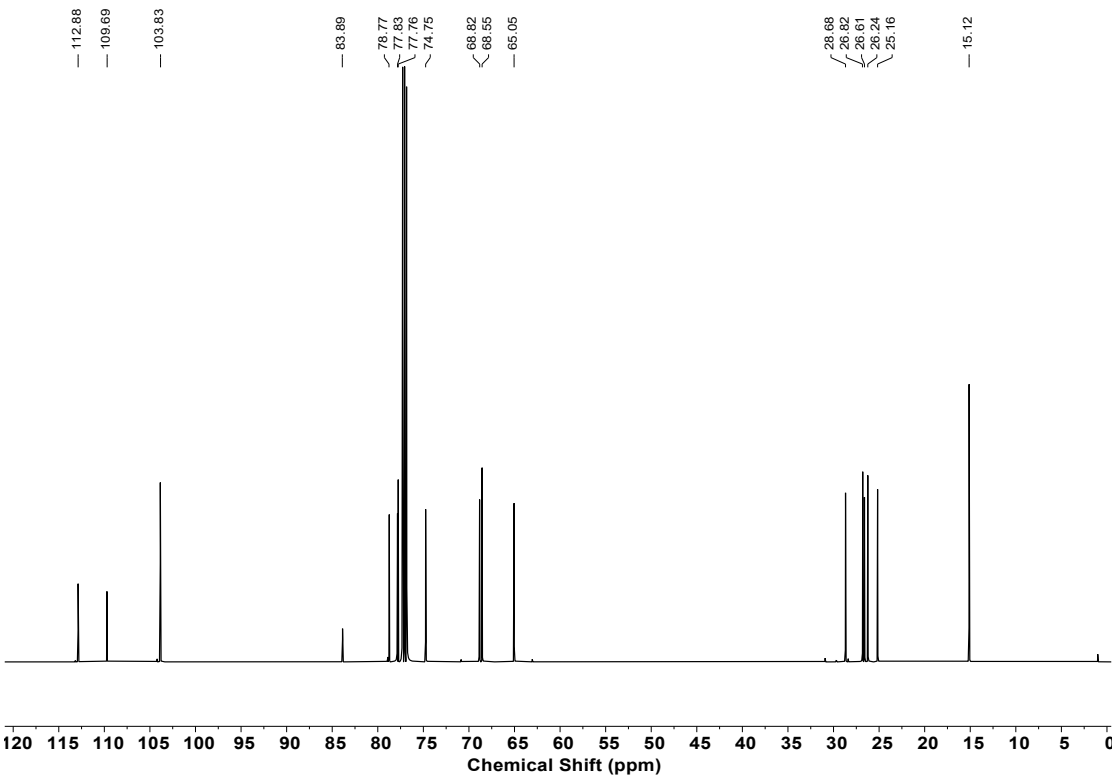


Fig. S11 ^{13}C NMR spectrum of 1-3 ($CDCl_3$)

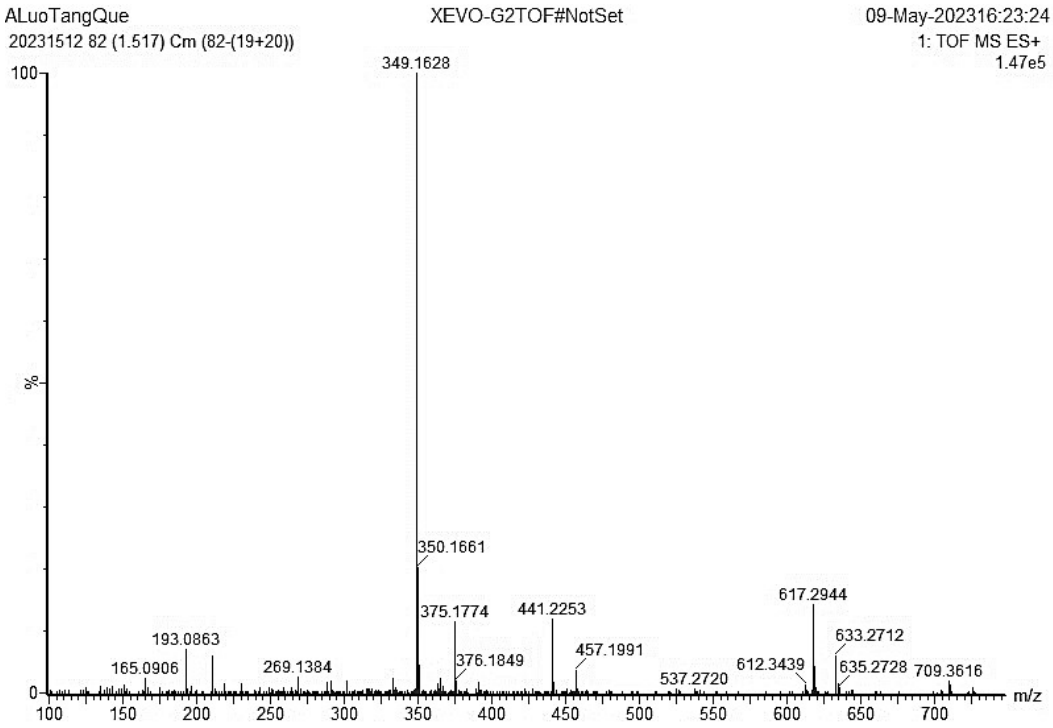


Fig. S12 High-resolution mass spectrum of 1-4

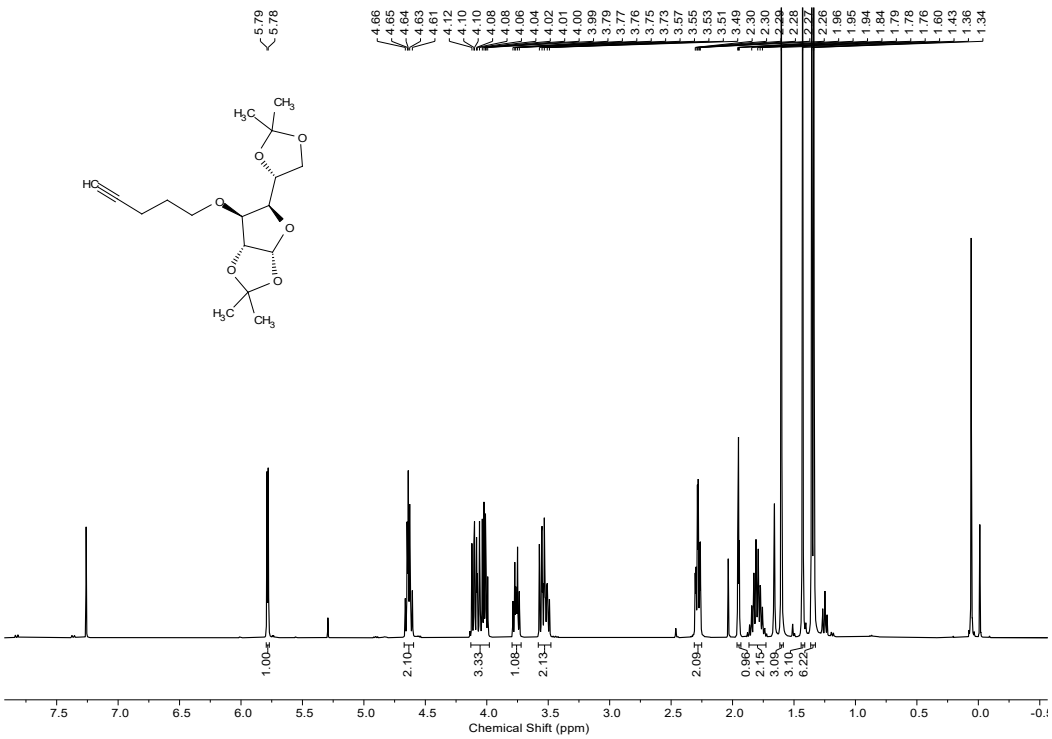


Fig. S13 ^{1}H NMR spectrum of 1-5 ($CDCl_3$)

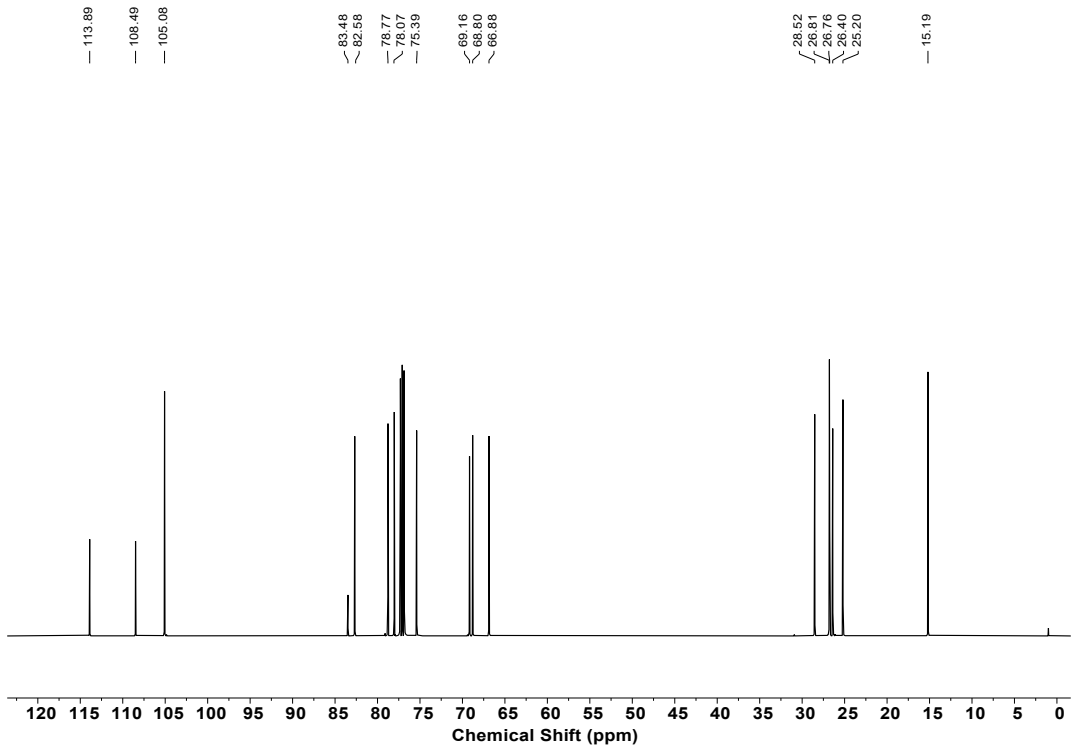


Fig. S14 ^{13}C NMR spectrum of 1-5 ($CDCl_3$)

Fig. S15 High-resolution mass spectrum of 1-5

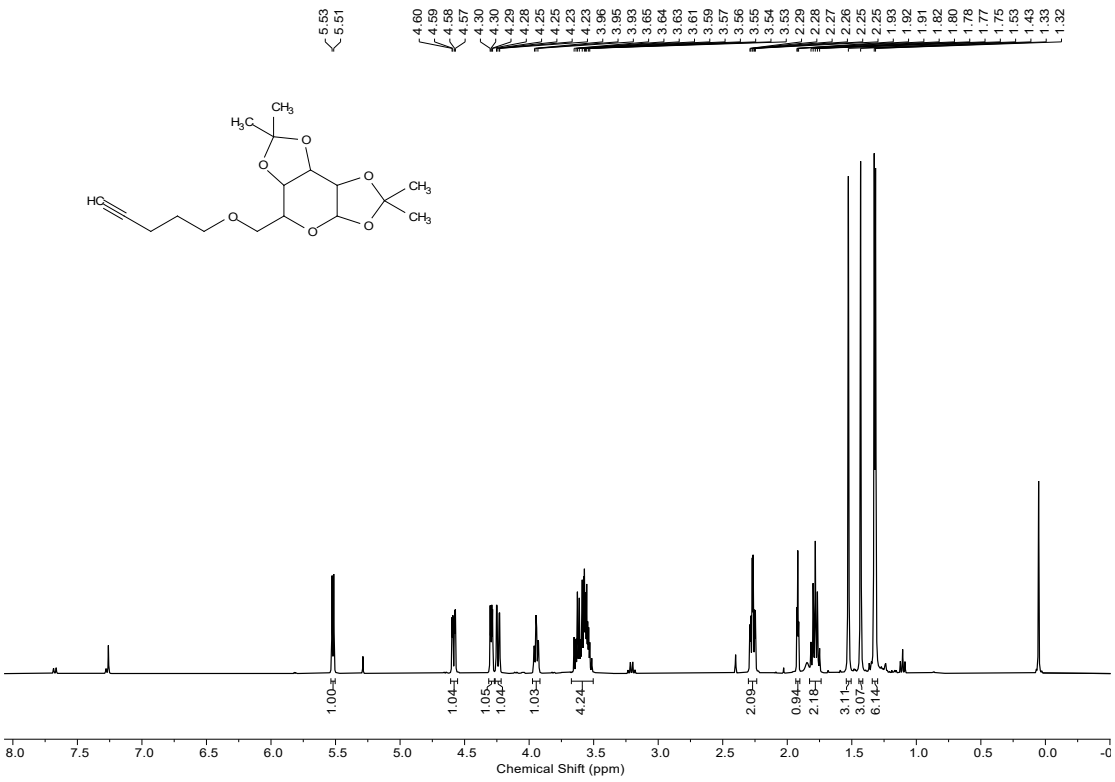


Fig. S16 ^{1}H NMR spectrum of 1-6 ($CDCl_3$)

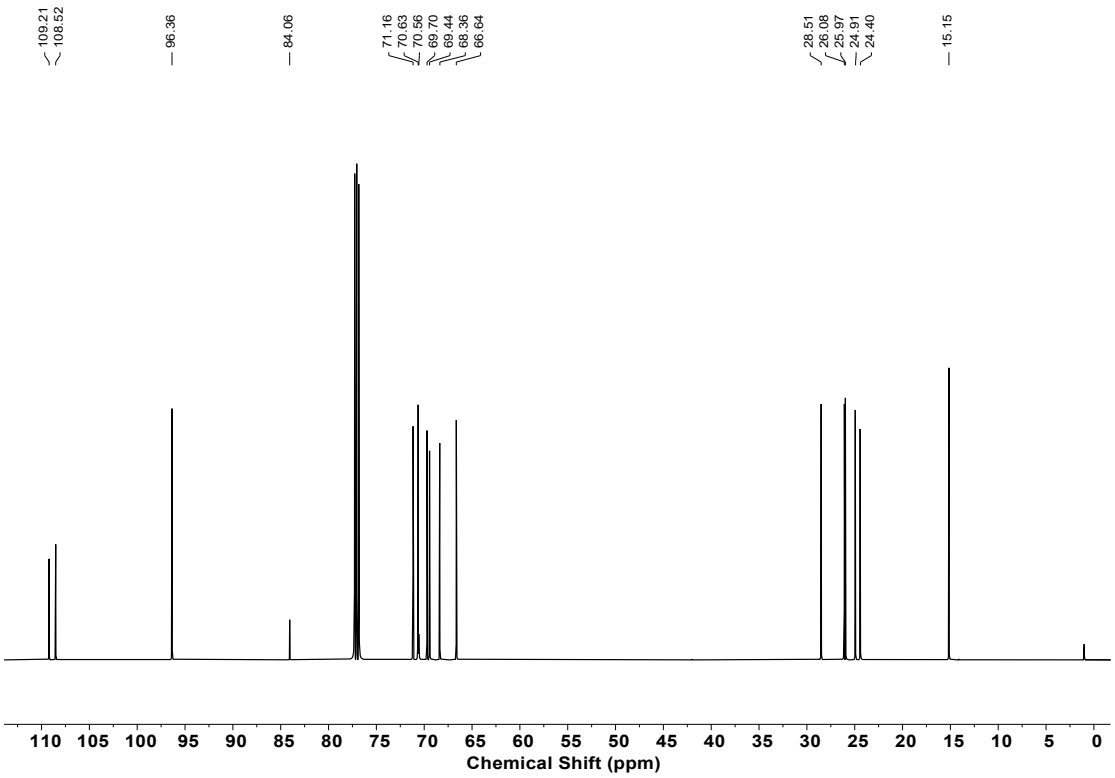


Fig. S17 ^{13}C NMR spectrum of 1-6 ($CDCl_3$)

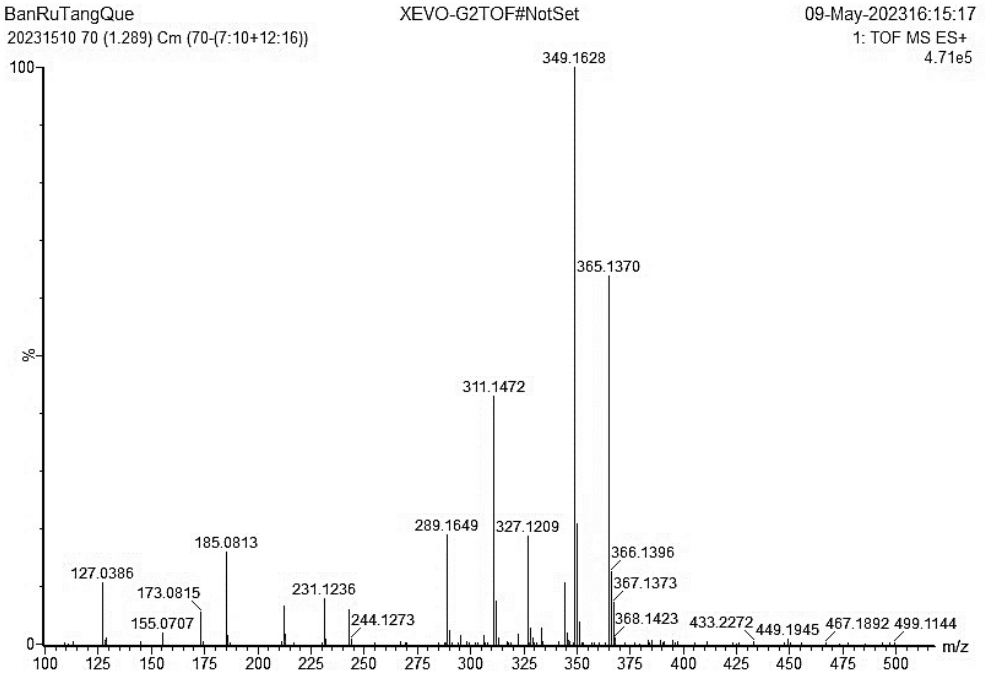


Fig. S18 High-resolution mass spectrum of 1-6

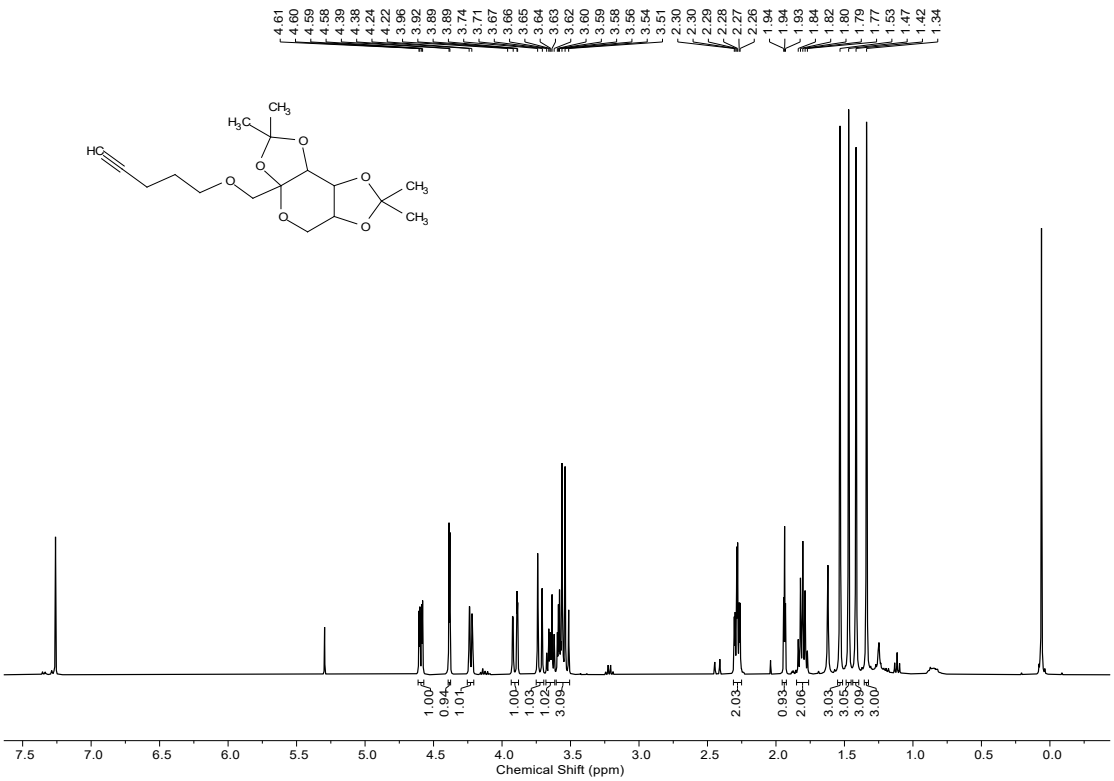


Fig. S19 ^{1}H NMR spectrum of 1-7 ($CDCl_3$)

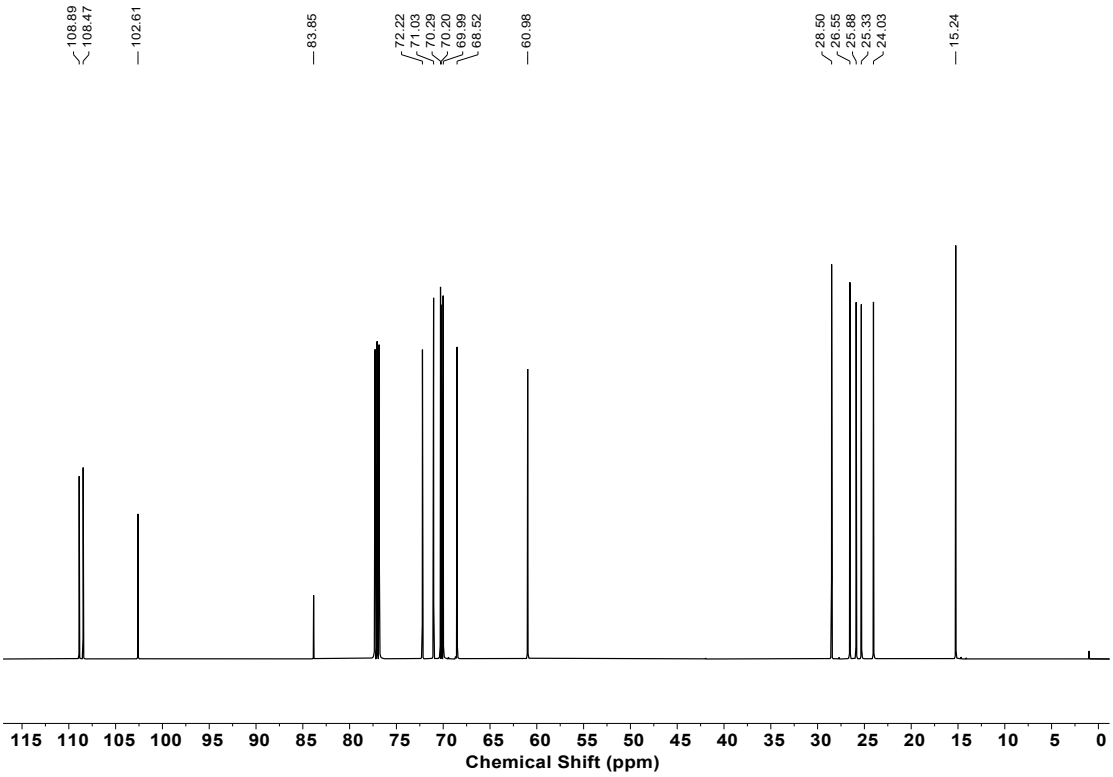


Fig. S20 ^{13}C NMR spectrum of 1-7 ($CDCl_3$)

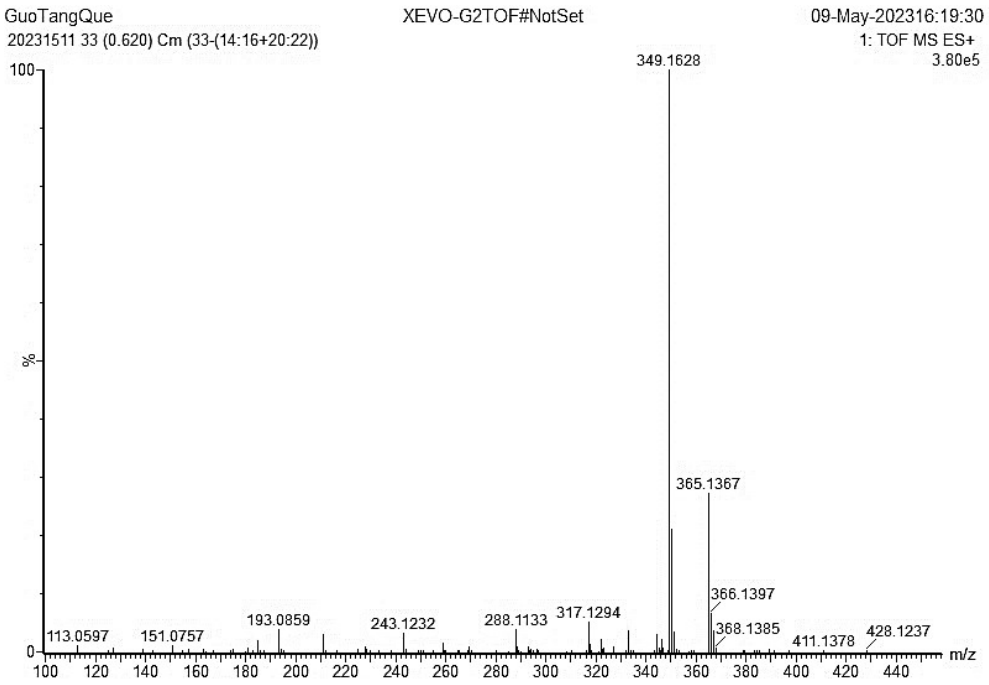


Fig. S21 High-resolution mass spectrum of 1-7

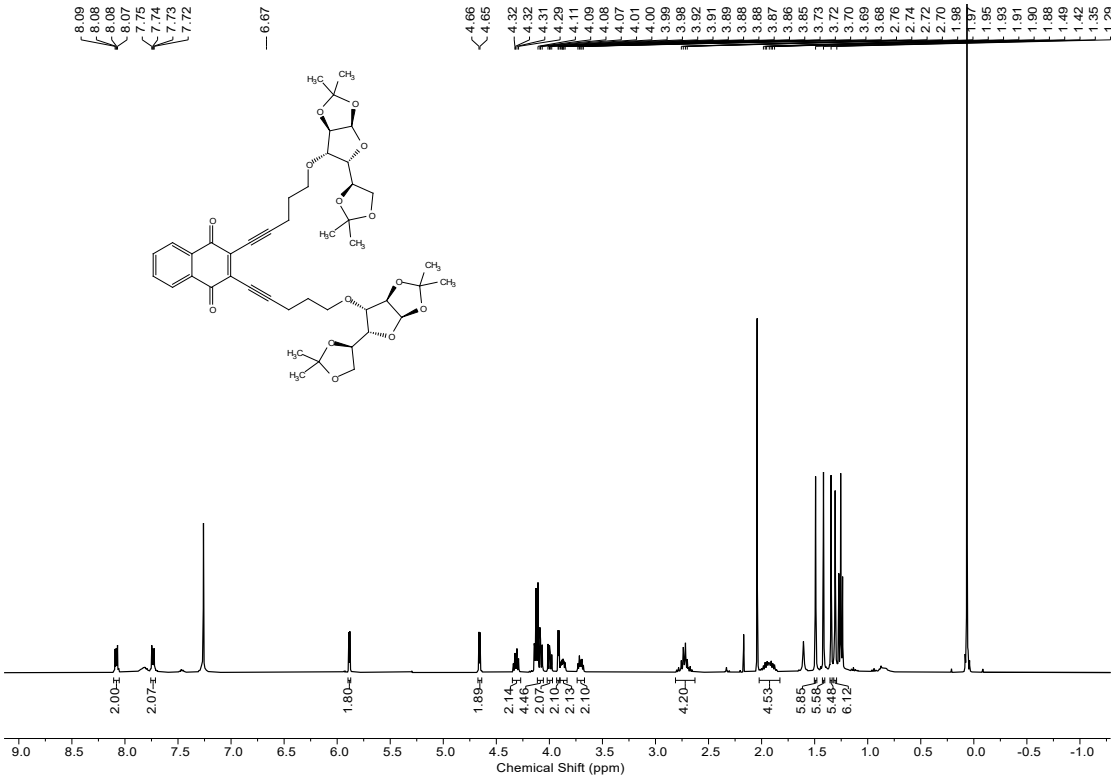


Fig. S22 ^{1}H NMR spectrum of EDY-A ($CDCl_3$)

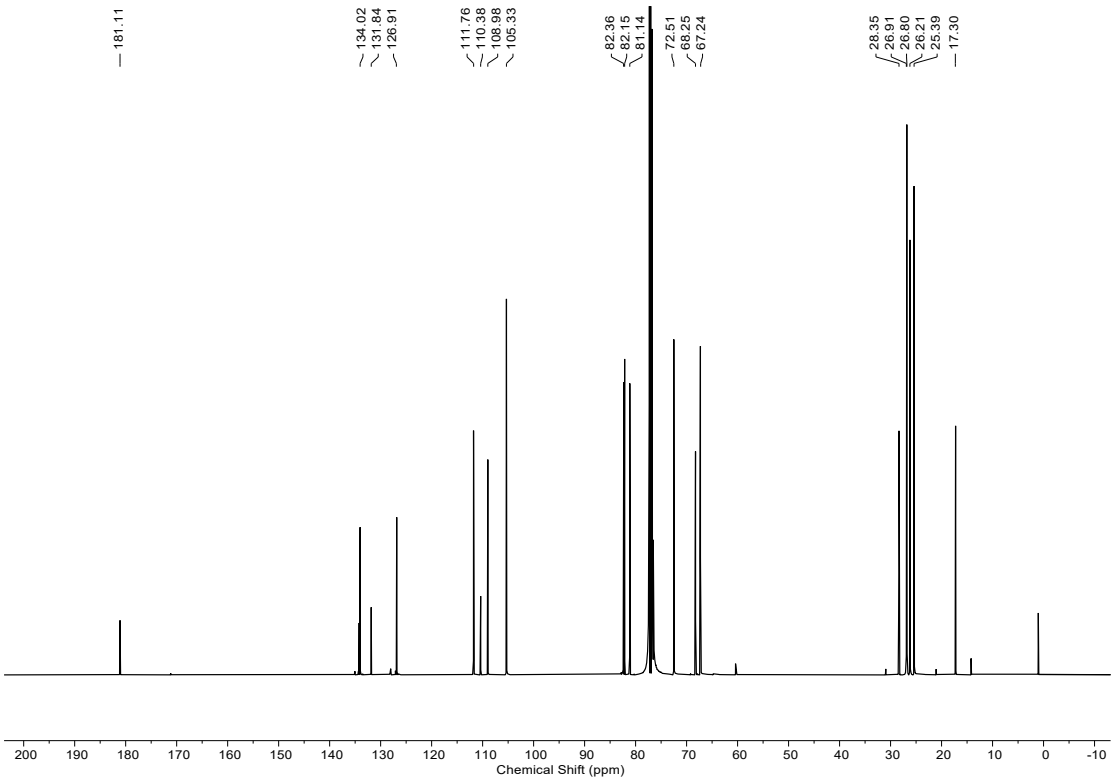


Fig. S23 ^{13}C NMR spectrum of EDY-A ($CDCl_3$)

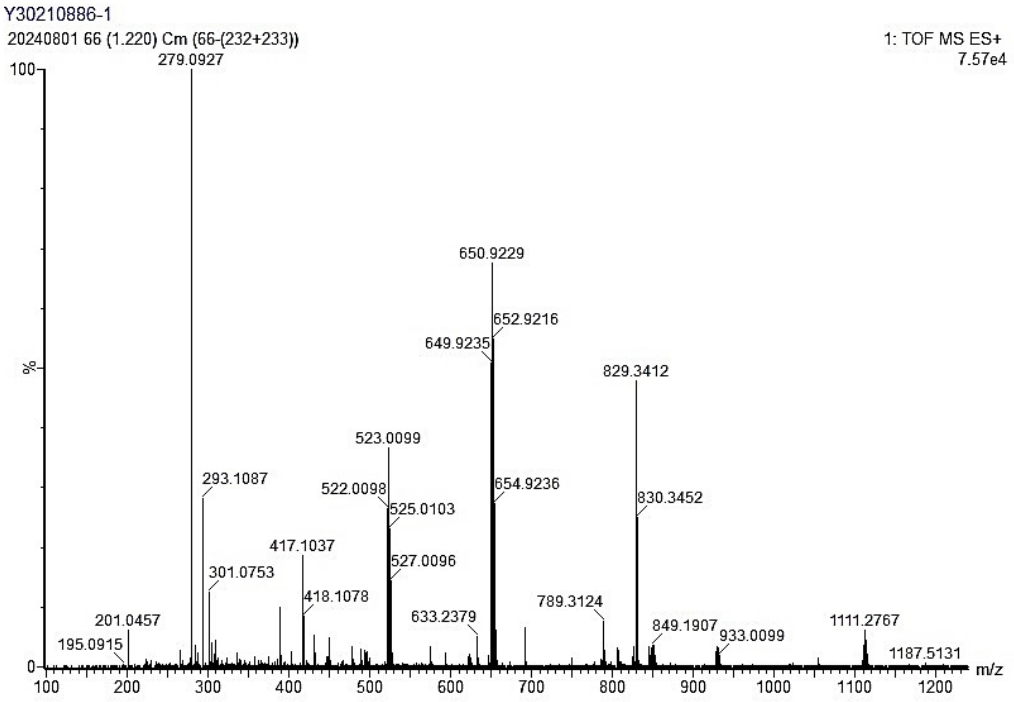


Fig. S24 High-resolution mass spectrum of EDY-A

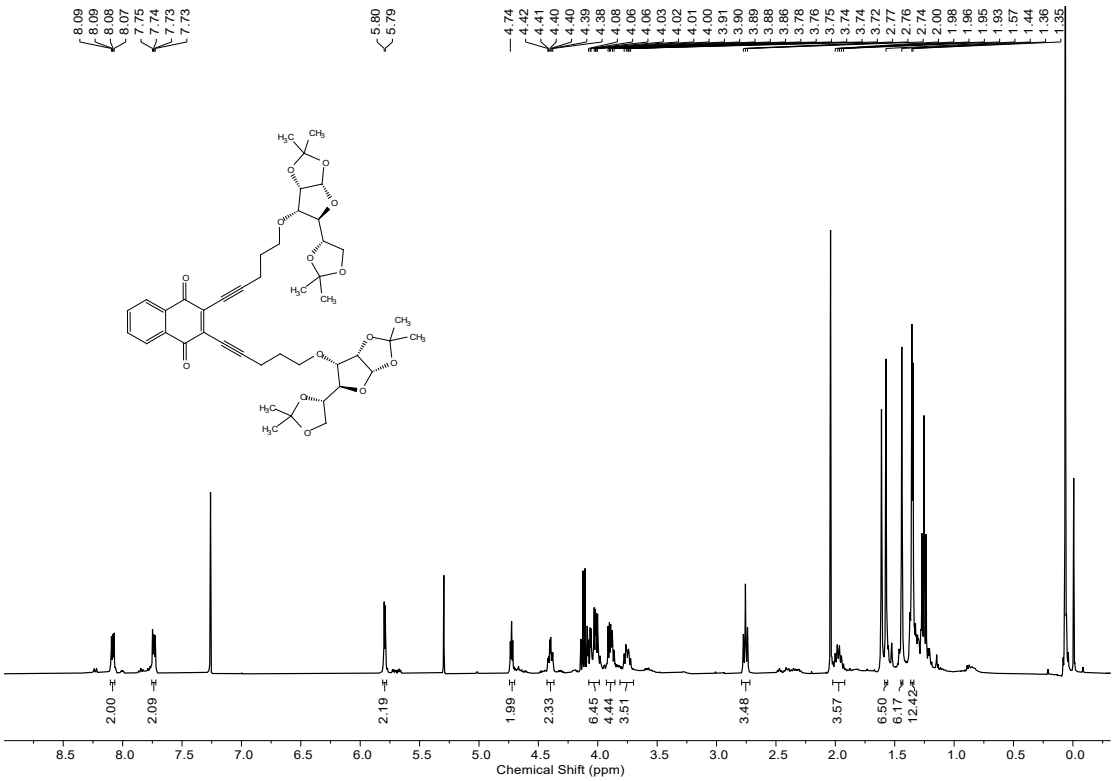


Fig. S25 ^{1}H NMR spectrum of EDY-B ($CDCl_3$)

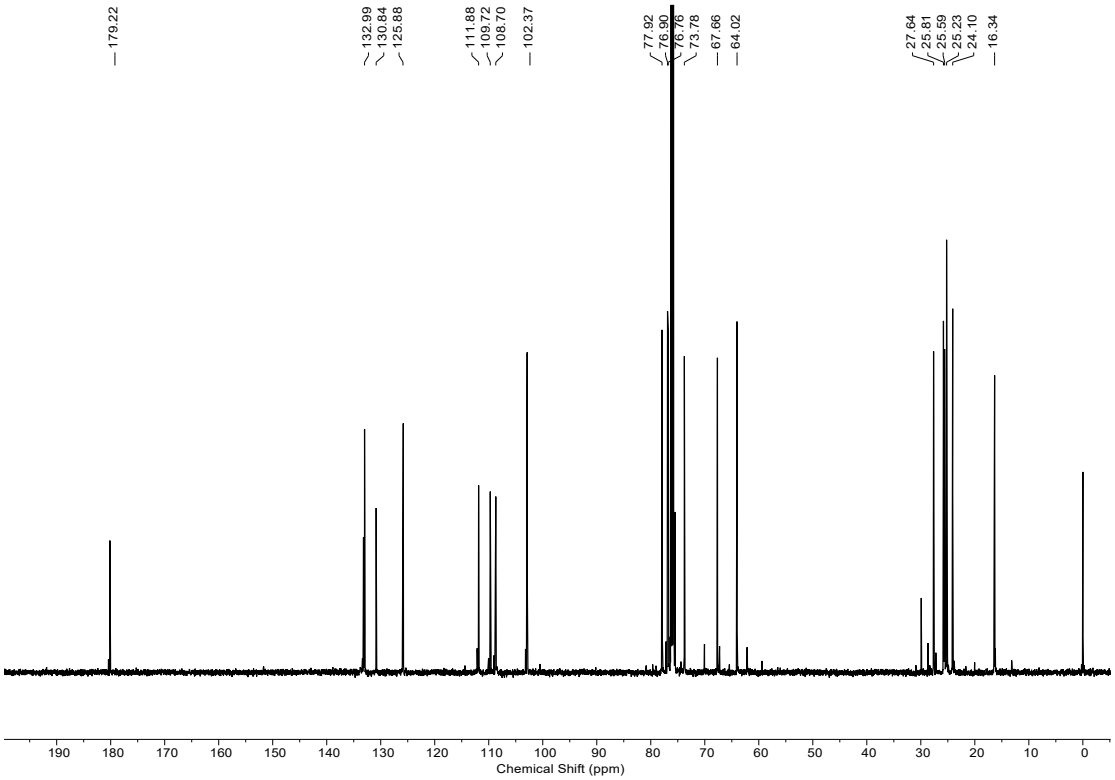


Fig. S26 ^{13}C NMR spectrum of EDY-B ($CDCl_3$)

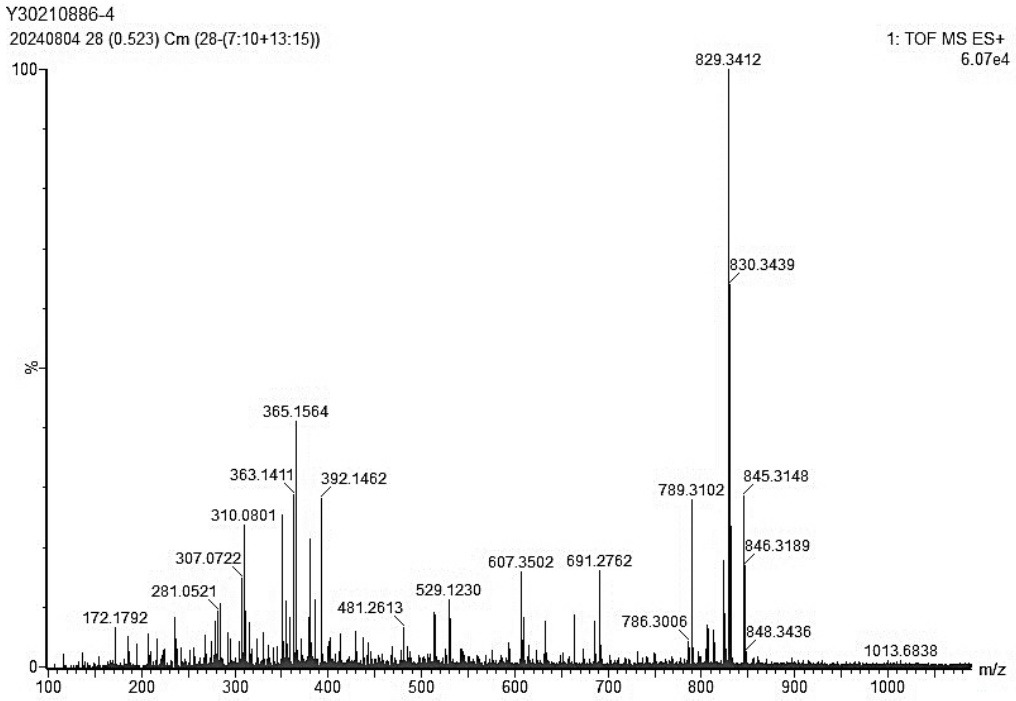


Fig. S27 High-resolution mass spectrum of EDY-B

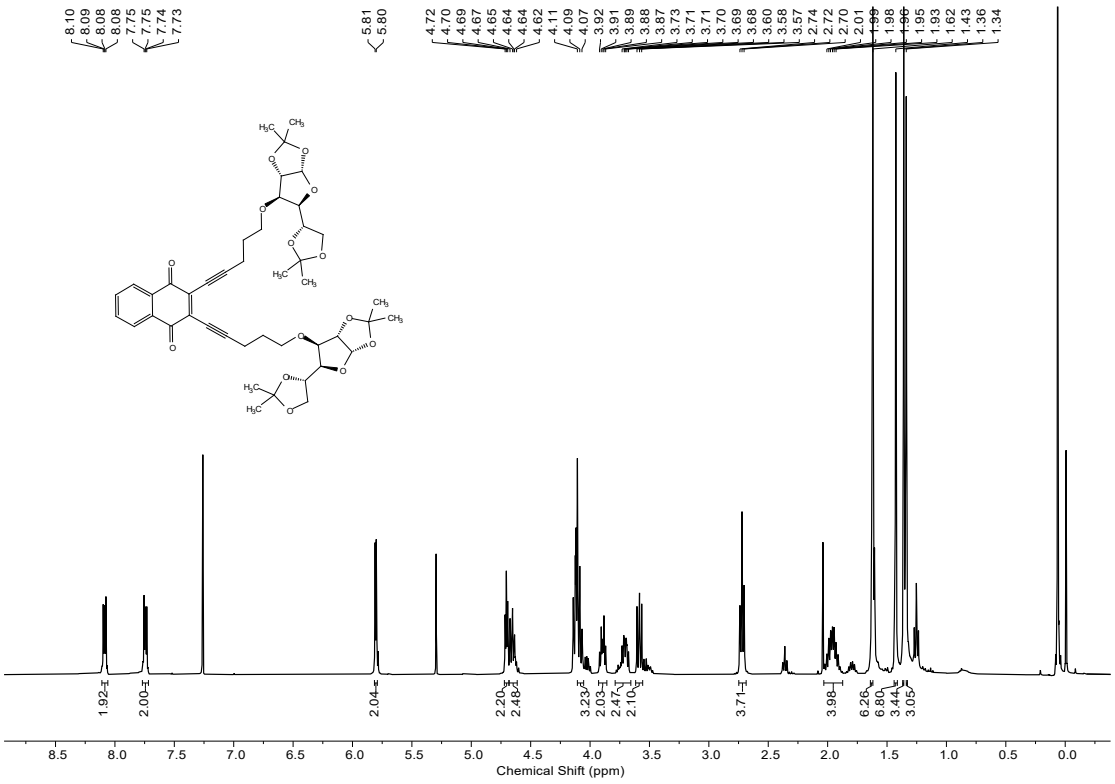


Fig. S28 ^{1}H NMR spectrum of EDY-C ($CDCl_3$)

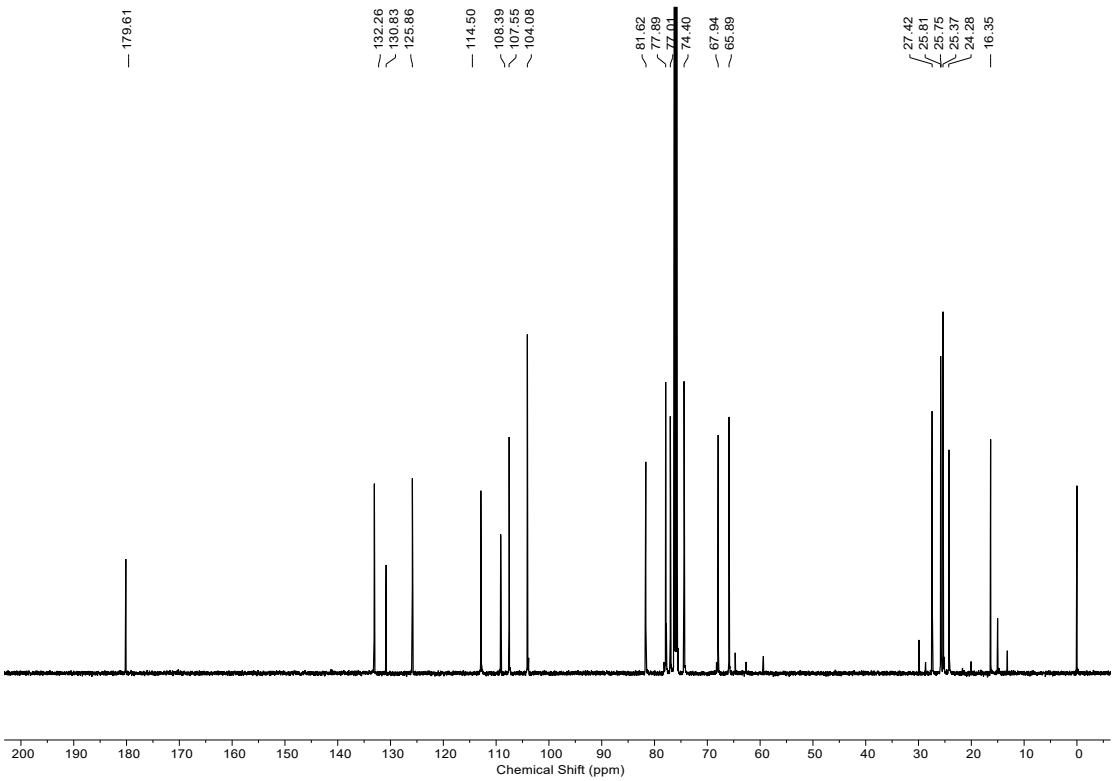


Fig. S29 ^{13}C NMR spectrum of EDY-C ($CDCl_3$)

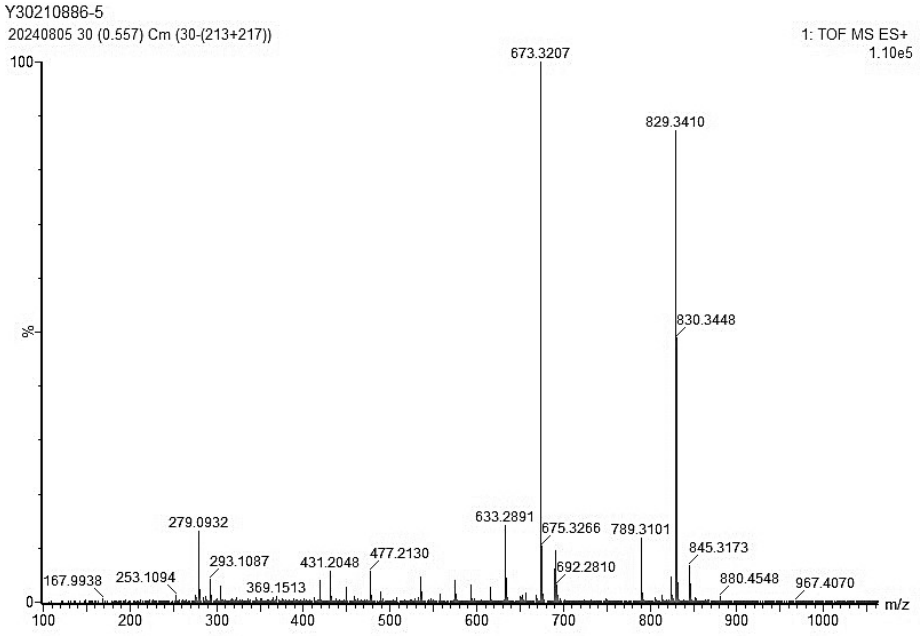


Fig. S30 High-resolution mass spectrum of EDY-C

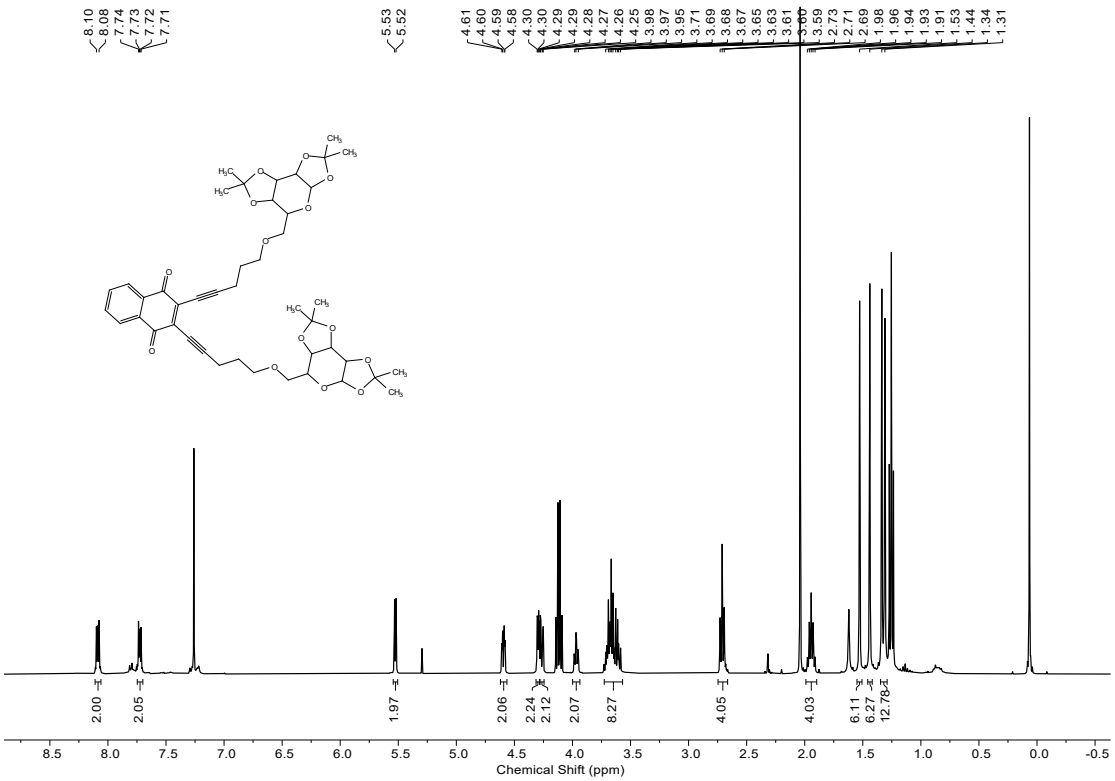


Fig. S31 ^{1}H NMR spectrum of EDY-D ($CDCl_3$)

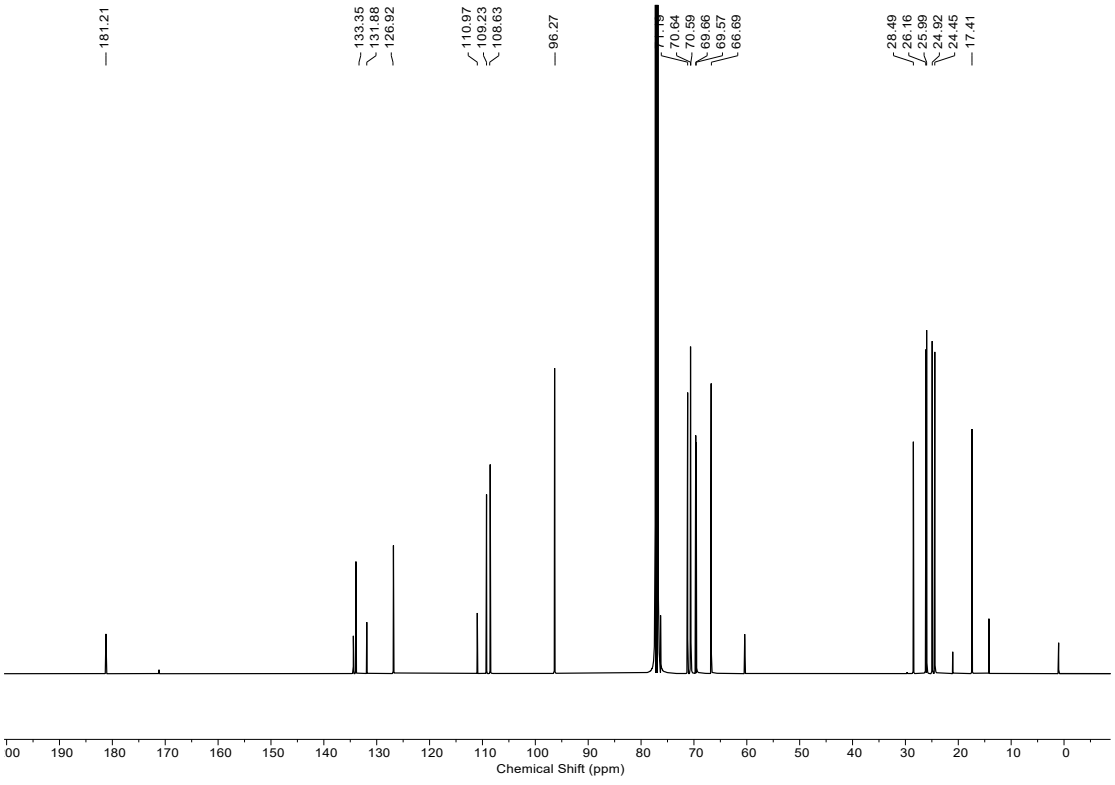


Fig. S32 ^{13}C NMR spectrum of EDY-D ($CDCl_3$)

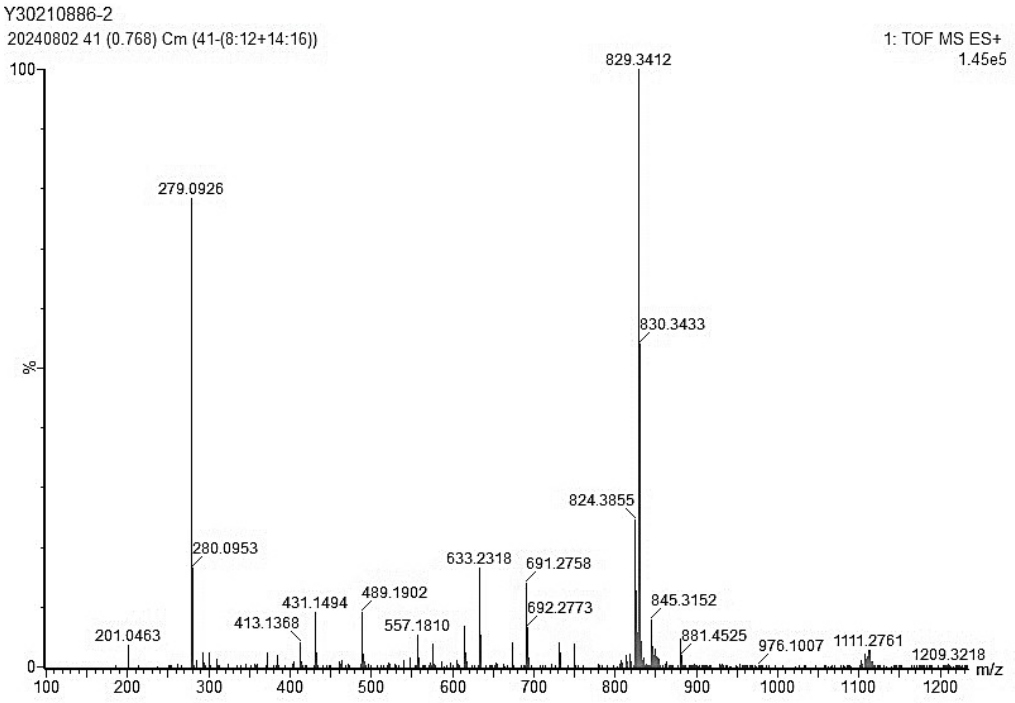


Fig. S33 High-resolution mass spectrum of EDY-D

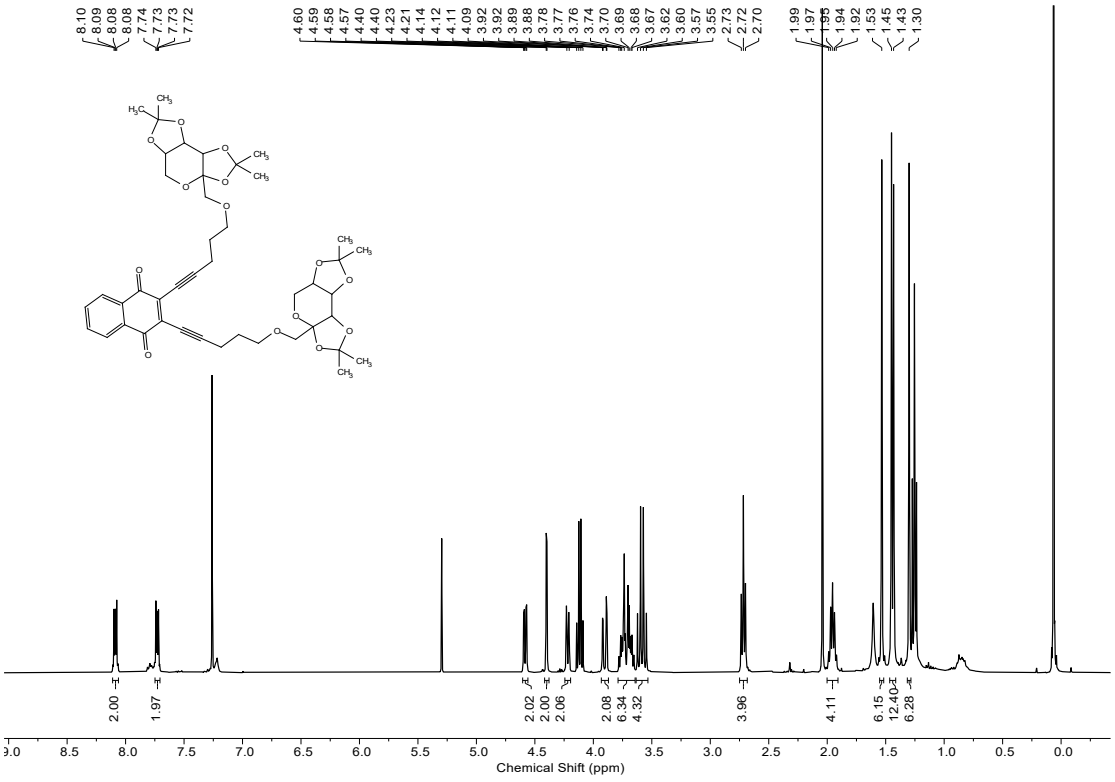


Fig. S34 ^{1}H NMR spectrum of EDY-E ($CDCl_3$)

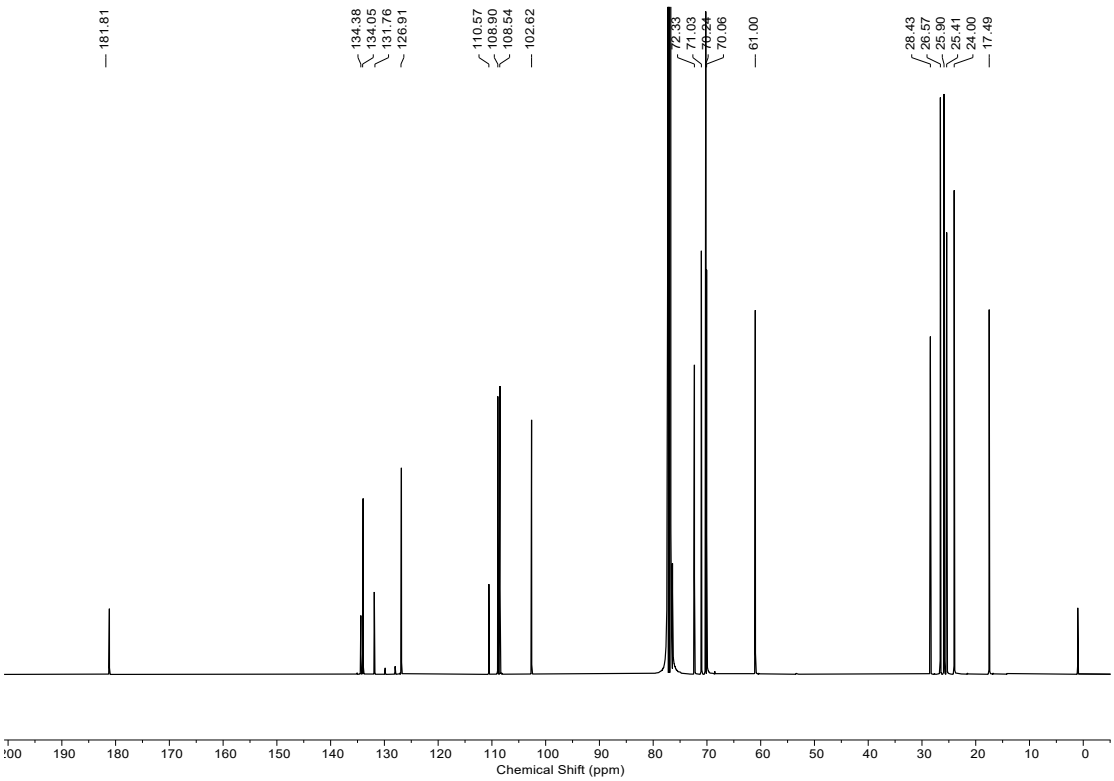


Fig. S35 ^{13}C NMR spectrum of EDY-E ($CDCl_3$)

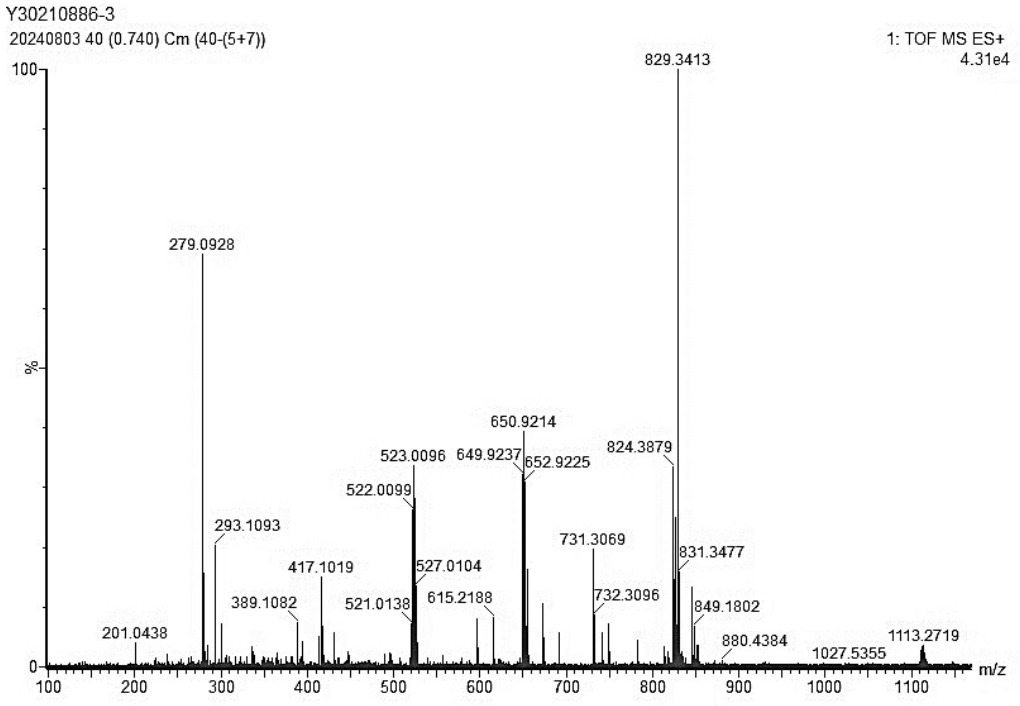


Fig. S36 High-resolution mass spectrum of EDY-E

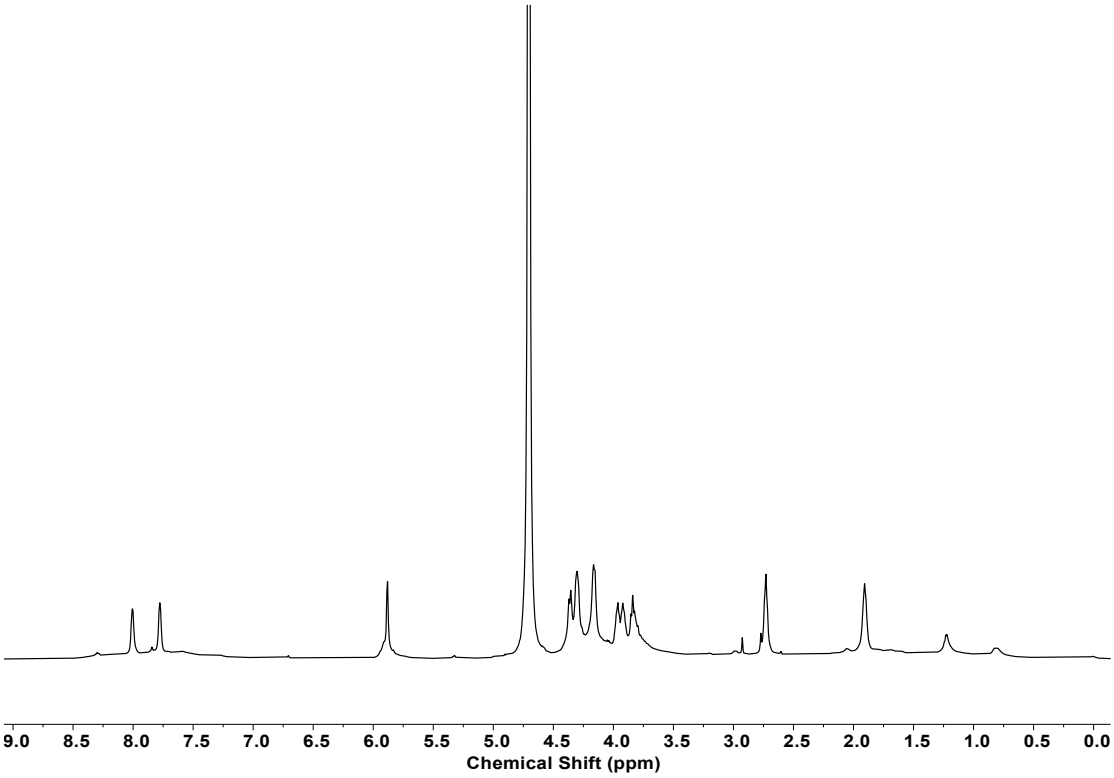


Fig. S37 ^{1}H NMR spectrum of EDY-1 (D_2O)

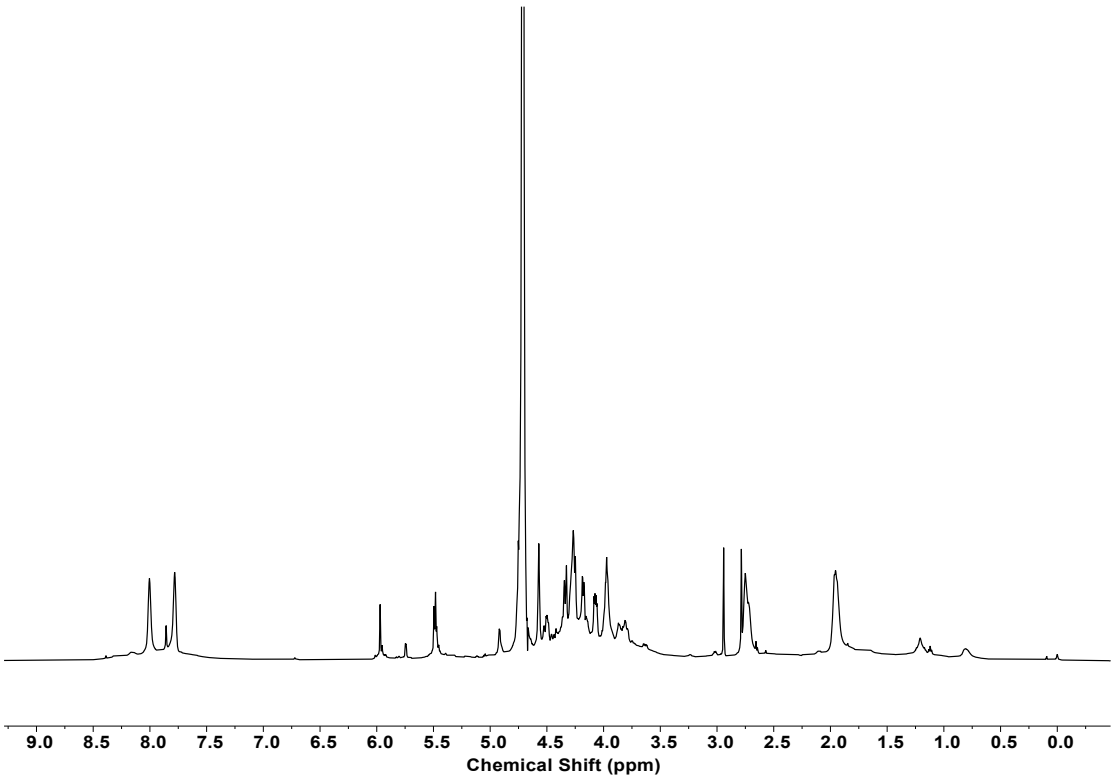


Fig. S38 ^{1}H NMR spectrum of EDY-2 (D_2O)

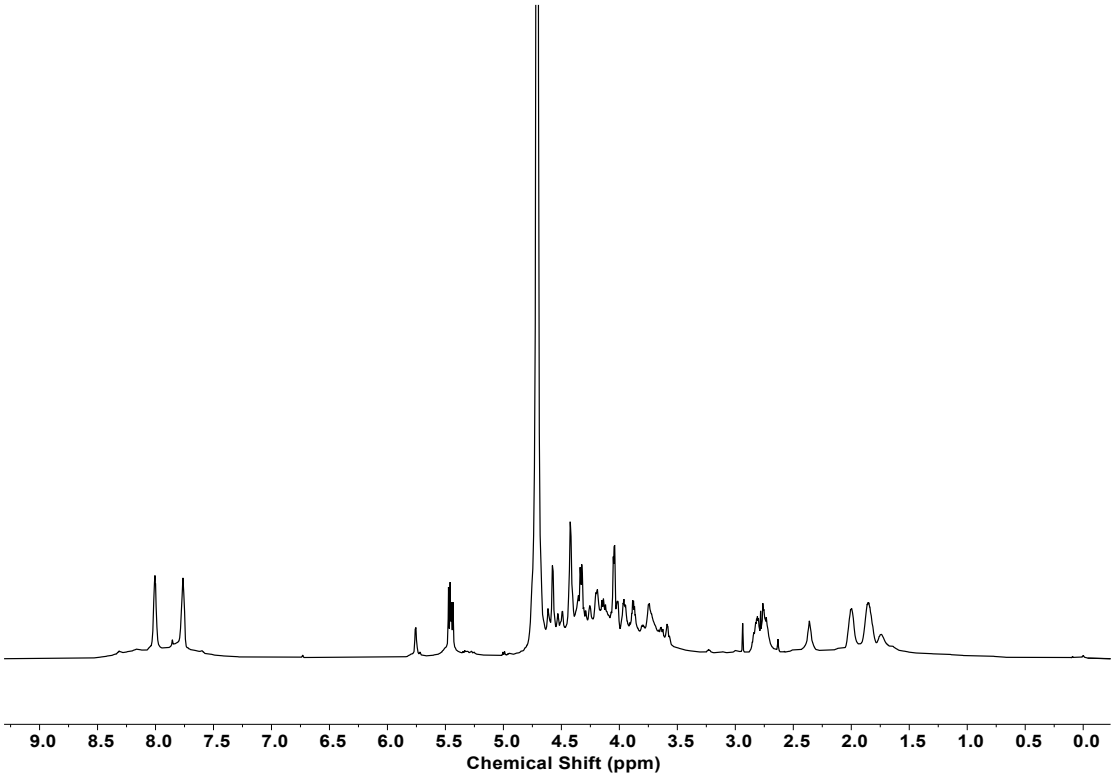


Fig. S39 ^{1}H NMR spectrum of EDY-3 (D_2O)

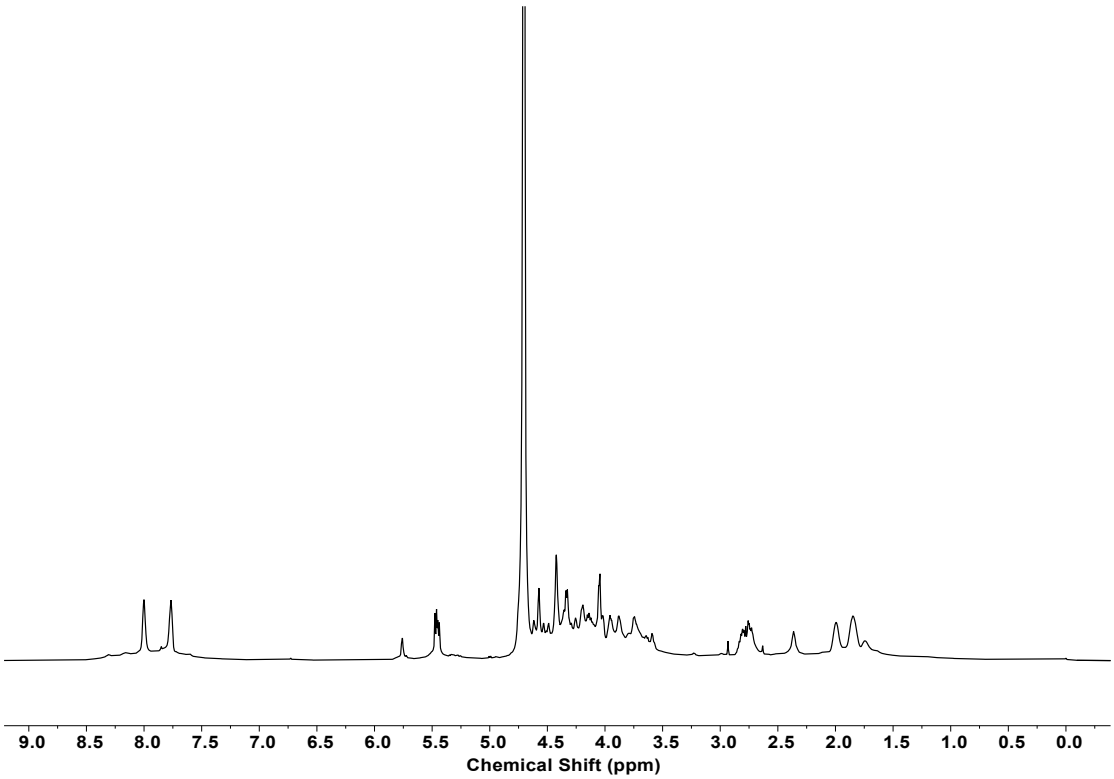


Fig. S40 ^{1}H NMR spectrum of EDY-4 (D_2O)

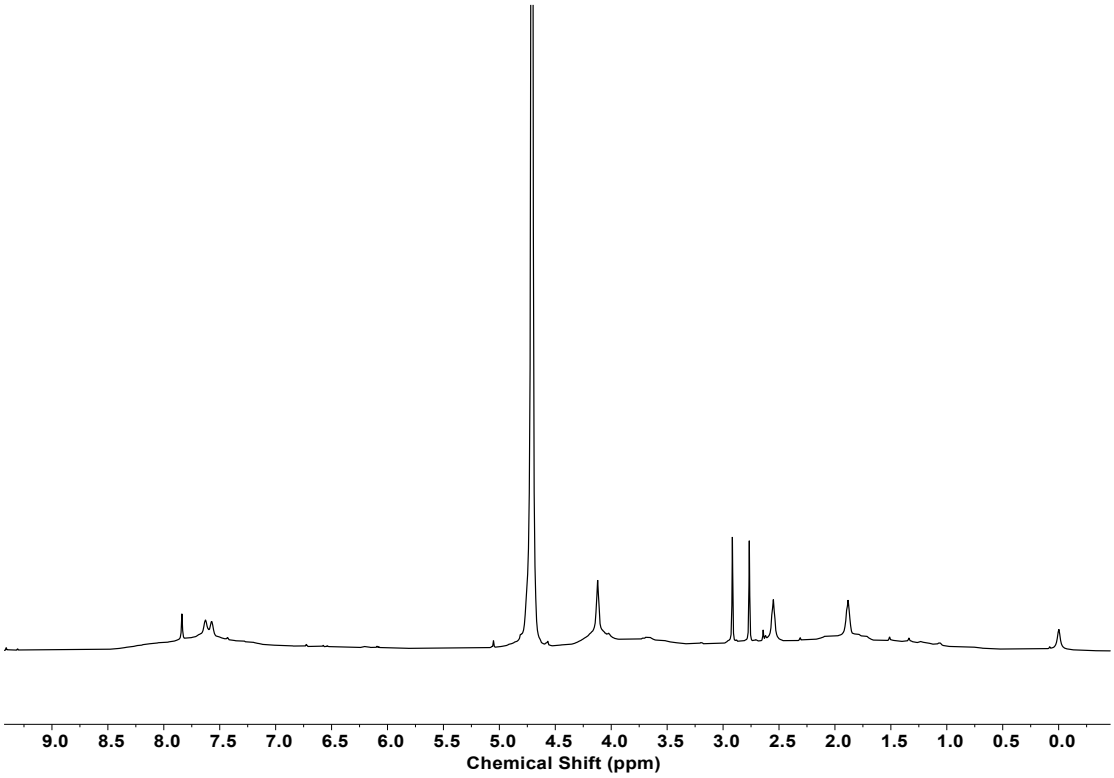


Fig. S41 ^{1}H NMR spectrum of EDY-5 (D_2O)

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3. H. Zheng, Y. Zeng, X. Huang, H. Zhang, F. Pu, H. Cheng, Z. Li, Y. Ding and A. Hu, *Asian J. Org. Chem.*, 2023, **12**, e202300306.