Supporting Information for

Design and synthesis of enediyne chimeras for targeted degradation of PD-L1

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Materials and Methods for the Synthesis of Compounds

Materials

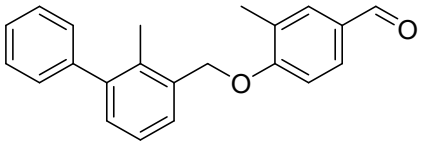
Toluene and tetrahydrofuran (THF) were dried using calcium hydride and distilled before use. Anhydrous N,N-dimethylformamide (DMF) with molecular sieves (Water $\leqslant$ 30 ppm) was provided by Energy Chemical. Dialysis bag MD31 (MW100-500) were obtained from Beijing Solarbio Science & Technology Co., Ltd. Other reagents were purchased at commercial grade and used without further purification. All the reactions were performed with dry Schlenk techniques under an atmosphere of nitrogen.

Methods

^{1}H NMR (400 MHz or 600 MHz) and ^{13}C NMR (100 MHz or 151 MHz) spectra were recorded on Bruker DRX-400 or DRX-600 instruments and chemical shifts (δ) are reported in ppm via the undeuterated solvent peaks (chloroform, methanol, dimethylsulfone and water) as the standard. Multiplicities were abbreviated as s for

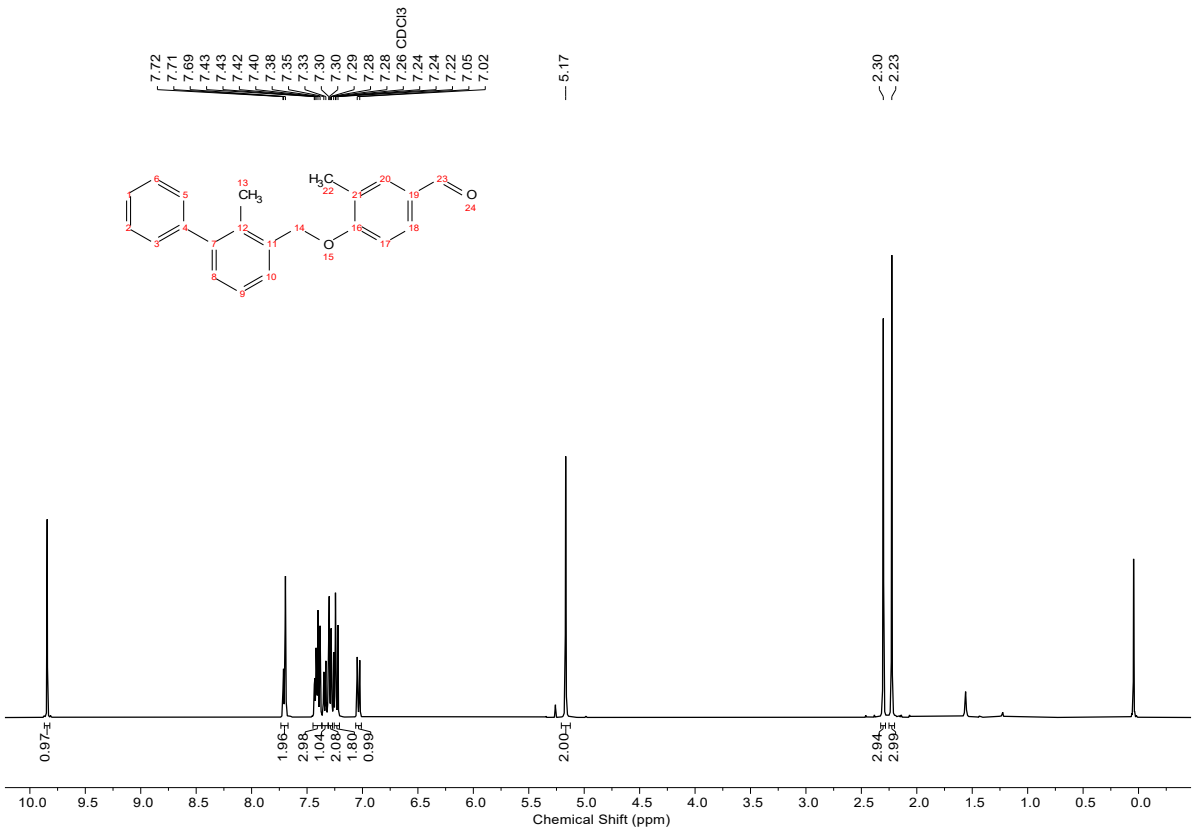
singlet, d for doublet, t for triplet and m for multiplet. Mass spectra was recorded on a Micromass LCTTM mass spectrometer using the ESI method.

Synthesis



3-methyl-4-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)benzaldehyde (**1**)

To an ice-cooled solution of 4-hydroxy-3-methylbenzaldehyde (1.36 g, 10.0 mmol), triphenylphosphine (2.88 g, 11.0 mmol), and 2-methyl-3-biphenylmethanol (1.98 g, 10.0 mmol) in THF (50 mL), diisopropyl azodicarboxylate (DIAD, 2.20 mL, 11.0 mmol) was added dropwise. The resulting solution was allowed to warm to room temperature and stirred for additional 24 h. The mixture was concentrated under reduced pressure. Purification by column chromatography on silica gel (10−15% EtOAc in hexane) yielded **1** as a white solid (2.13 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1H), 7.70 (d, *J* = 6.8 Hz, 2H), 7.45–7.37 (m, 3H), 7.34 (d, *J* = 7.3 Hz, 1H), 7.29 (dd, *J* = 8.2, 1.5 Hz, 2H), 7.25–7.21 (m, 2H), 7.04 (d, *J* = 9.0 Hz, 1H), 5.17 (s, 2H), 2.30 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (151 MHz, CDCl3) δ 191.3, 162.2, 143.1, 141.9, 134.9, 134.0, 131.8, 130.7, 130.3, 129.8, 129.5, 128.3, 128.1, 127.5, 127.1, 125.8, 111.0, 69.4, 16.6, 16.3. HRMS (ESI), m/z calcd. for $C_{22}H_{20}O_2$ $[M + H]^+$: 317.1542, found 317.1550.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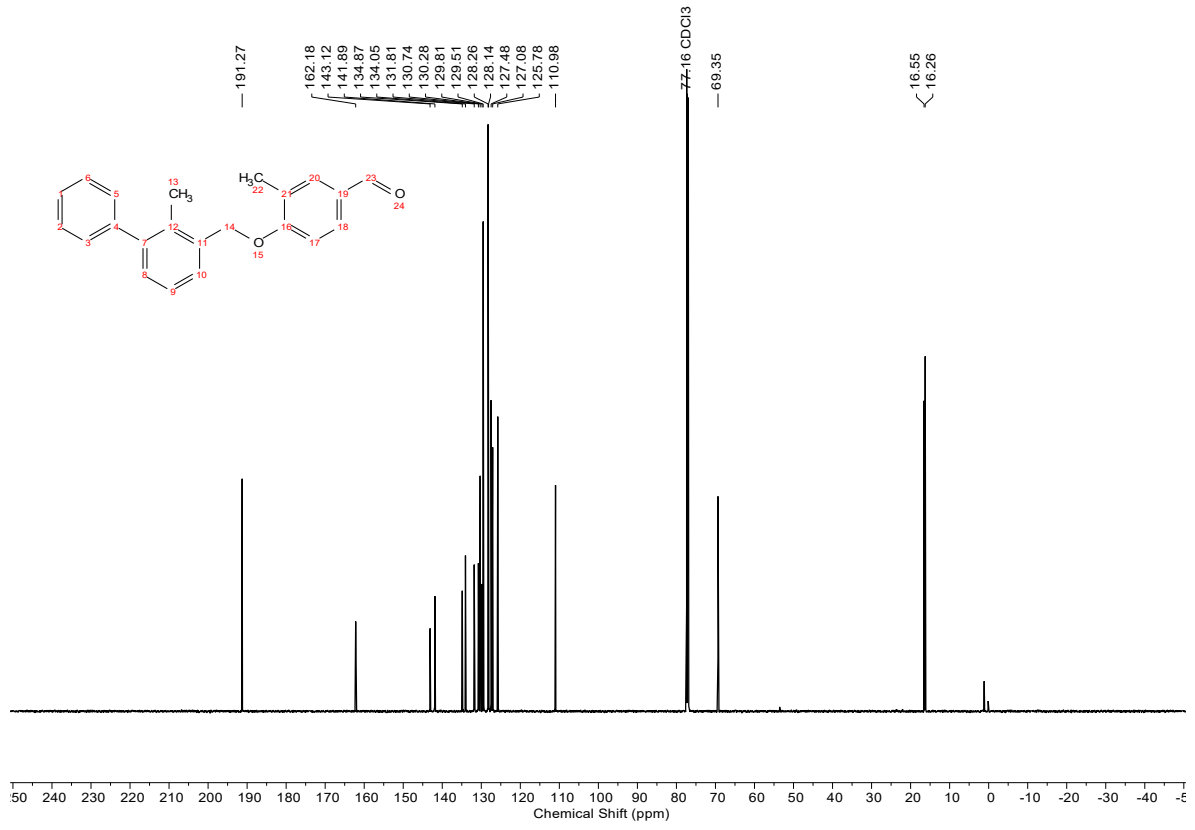
Chemical Shift (ppm)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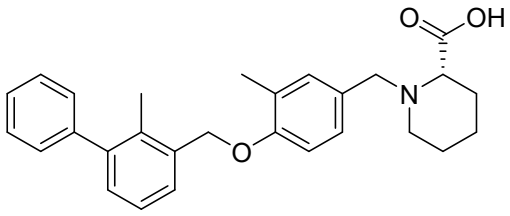
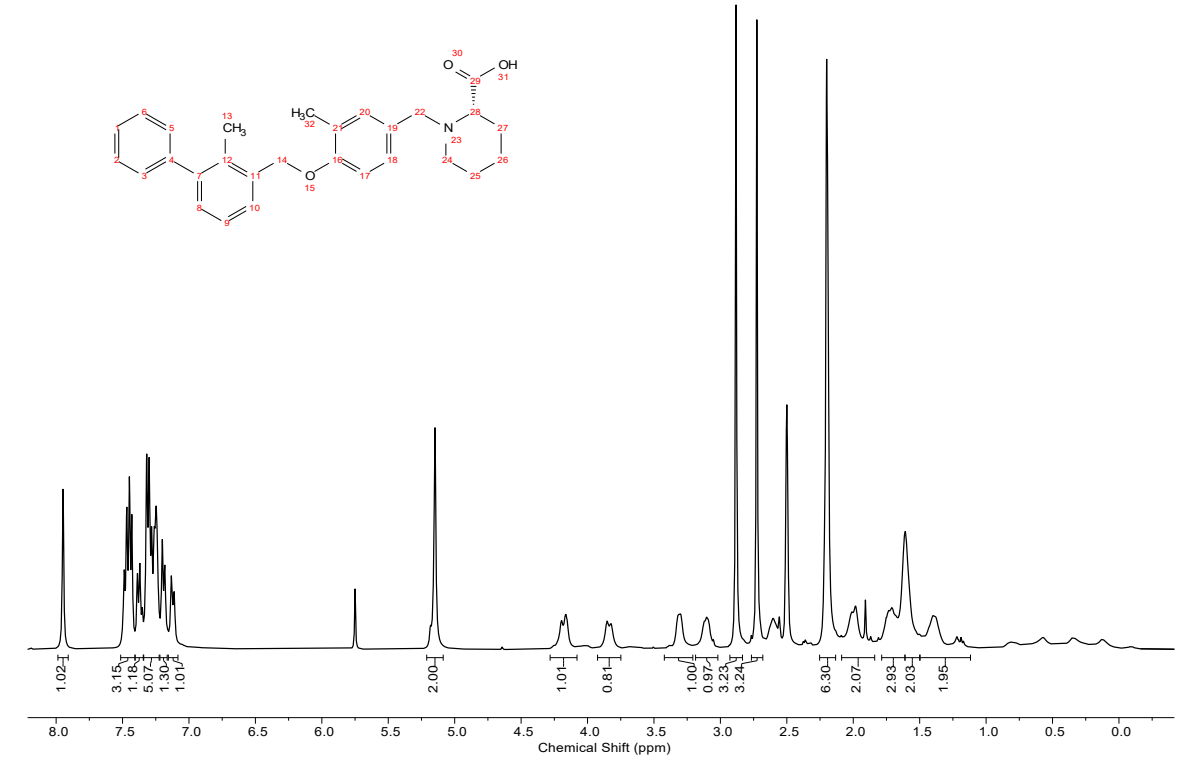


(*S*)-1-(3-methyl-4-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)benzyl)piperidine-2-carboxylic acid (**BMS-57**)

To a mixture of **1** (1.40 g, 4.4 mmol), L(-)-pipecolinic acid (1.14 g, 8.8 mmol) and glacial acetic acid (1.32 g, 22.0 mmol) in DMF (40 mL), sodium cyanoborohydride (1.38 g, 22.0 mmol) was added in one batch. Then the mixture was heated to 80 °C and stirred for 3 h. The volatiles were removed under reduced pressure. Purification of the residue by column chromatography on silica gel (5−10% methanol in DCM) yielded **BMS-57** (1.07 g, 57%) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.50–7.42 (m, 3H), 7.37 (t, *J* = 7.3 Hz, 1H), 7.33–7.25 (m, 3H), 7.23–7.16 (m, 3H), 7.08 (d, *J* = 8.2 Hz, 1H), 5.13 (s, 2H), 4.24 (d, *J* = 12.8 Hz, 1H), 3.64 (d, *J* = 12.8 Hz, 1H), 3.10–2.96 (m, 2H), 2.37 (m, 1H), 2.20 (s, 3H), 2.18 (s, 3H), 1.88 (m, 2H), 1.56 (m, 3H), 1.38–1.26 (m, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.0, 156.3, 142.2, 141.4, 135.9, 133.7, 132.4, 129.5, 129.2, 129.0, 128.3, 127.5, 127.0, 126.3, 125.7, 125.6, 111.4, 68.4, 65.0, 58.0, 49.4, 28.2, 23.4, 22.0, 16.2, 15.8. HRMS (ESI), m/z calcd. for $C_{28}H_{31}NO_3$ $[M - H]^-$: 428.2226, found 428.2227.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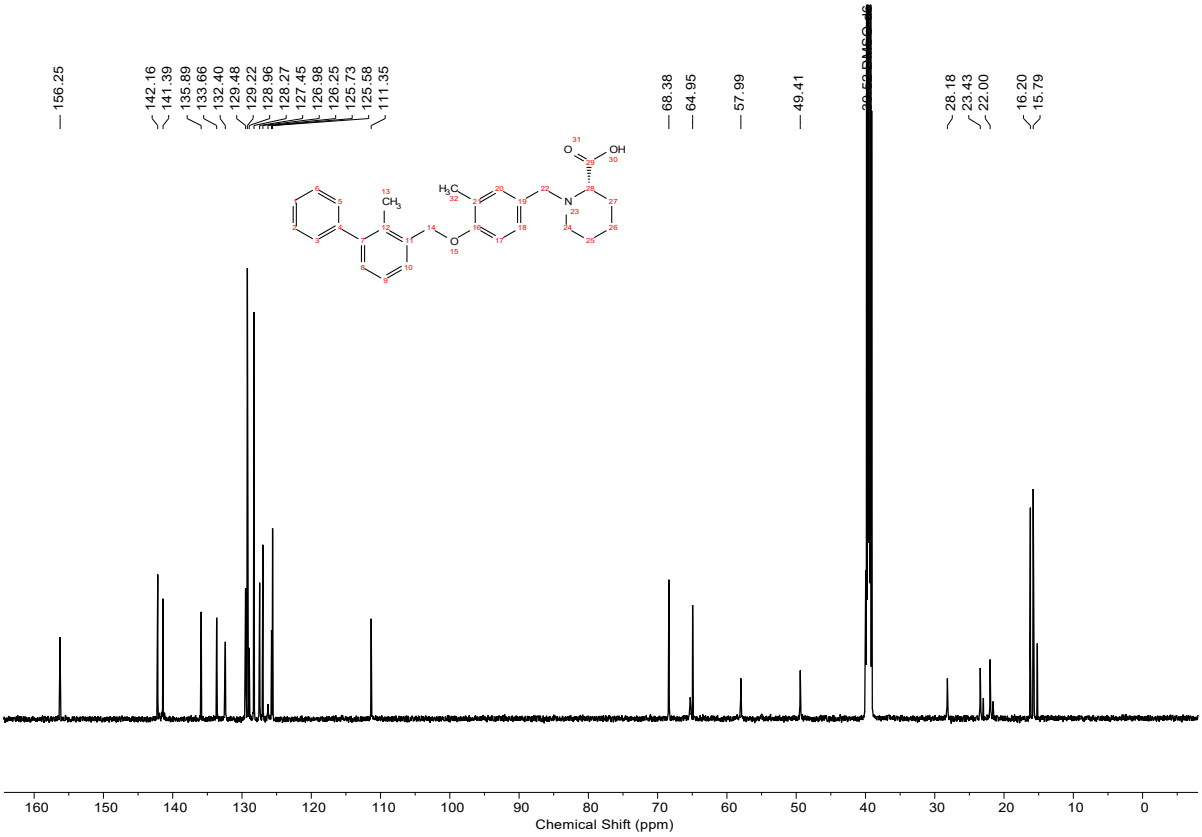
Chemical Shift (ppm)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16.20
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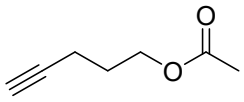
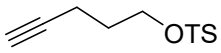
Chemical Shift (ppm)



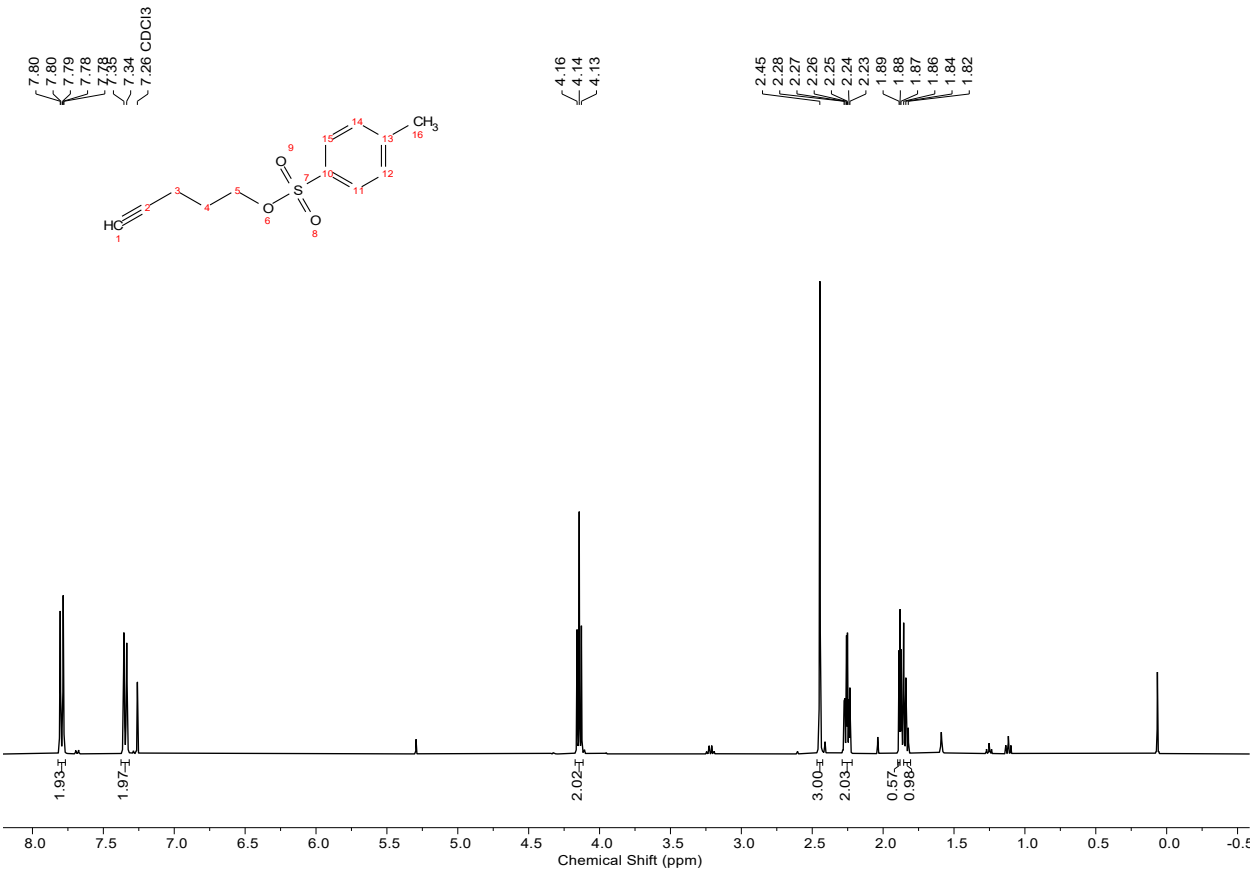
Pent-4-yn-1-yl acetate (**2**)

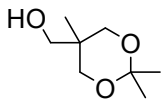
Compound **11** was synthesized according to a reported procedure[1]. Briefly, 4-pentyn-1-ol (5.05 g, 60 mmol) and triethylamine (16.8 mL, 120 mmol) were dissolved in DCM (200 mL). Acetyl chloride (7.06 g, 90 mmol) was added dropwise at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The reaction was quenched with H_2O (100 mL), extracted with DCM (3 × 200 mL). The combined organic layers were washed with brine (200 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography on silica gel (10−15% EtOAc in hexane) to afford **11** as a pale yellow oil (6.19 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.16 (t, *J* = 6.3 Hz, 2H), 2.28 (td, *J* = 7.1, 2.7 Hz, 2H), 2.04 (s, 3H), 1.96 (t, *J* = 2.6 Hz, 1H), 1.84 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.2, 83.1, 69.1, 63.1, 27.6, 21.0, 15.3.



Pent-4-yn-1-yl 4-methylbenzenesulfonate (**3**)[2]

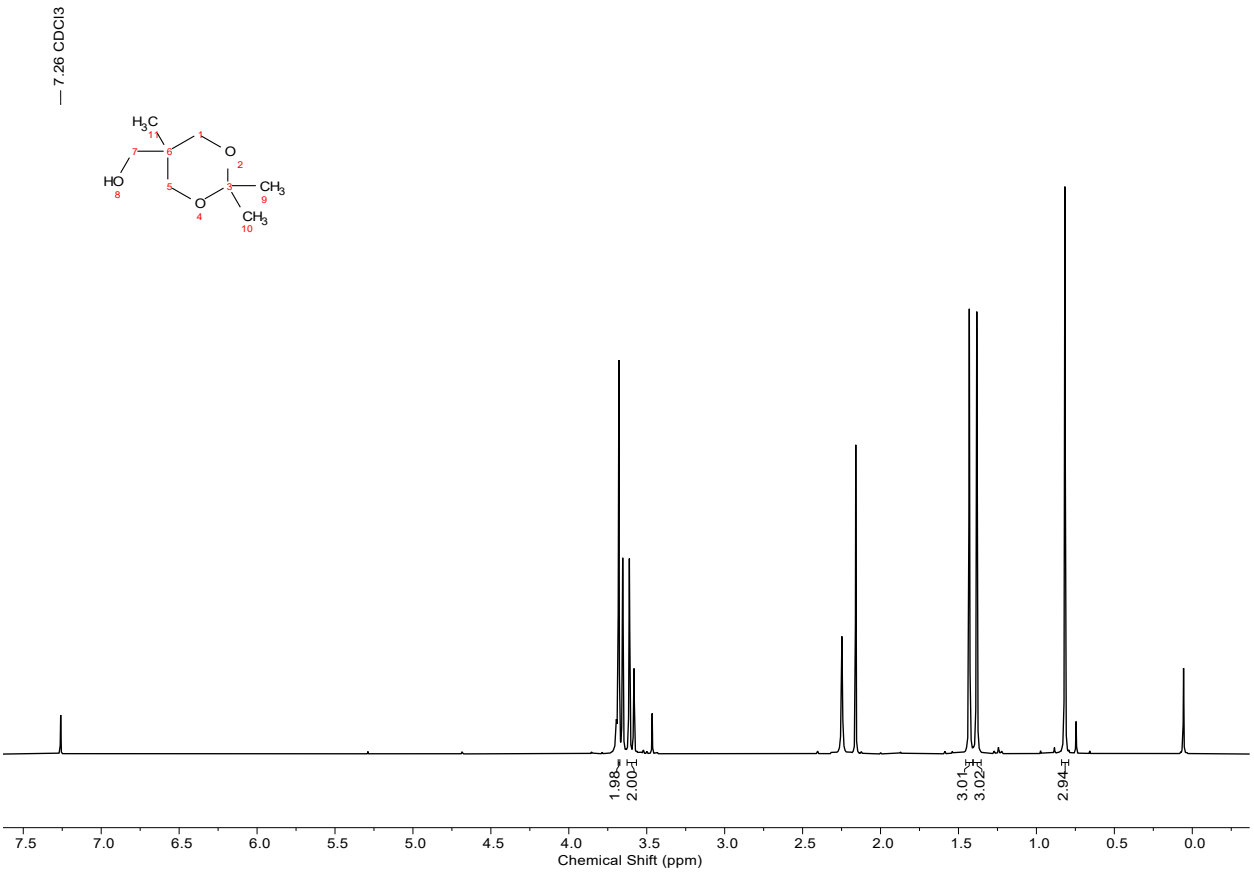
P-Toluenesulfonyl chloride (2.73 g, 14.3 mmol) was dissolved in dry DCM (35 mL) at 0 °C, 4-pentyn-1-ol (1 g, 11.9 mmol) and triethylamine (2.47 mL, 15.8 mmol) were then added dropwise. The mixture was stirred at room temperature for 20 h. After removal of the solvent, the residue was diluted with ether (100 mL) and filtered. The organic phase was collected and the solvent was removed. The residue was separated and purified by column chromatography on silica gel (EtOAc / hexane = 1:7) to give the product as a light yellow oil (1.37 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.2 Hz, 2H), 7.28 (d, *J* = 8.1 Hz, 2H), 4.08 (t, *J* = 6.1 Hz, 2H), 2.38 (s, 3H), 2.19 (td, *J* = 6.9, 2.6 Hz, 2H), 1.84–1.75 (m, 3H).

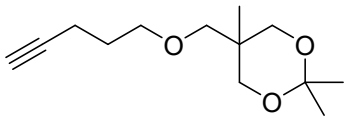




(2,2,5-Trimethyl-1,3-dioxan-5-yl)methanol (**4**)

Compound **7** was synthesized according to a reported procedure[3]. To a solution of tris(hydroxymethyl)ethane (6 g, 50 mmol) in acetone (50 mL), 2,2-dimethoxypropane (6.5 mL, 52 mmol) and p-toluenesulfonic acid monohydrate (133.3 mg, 0.70 mmol) was added. The mixture was stirred at room temperature for 4 h, then neutralized with potassium carbonate to a pH of 7. After filtration, the filtrate was concentrated under reduced pressure to afford **7** as a colorless oil with fine translucent crystals (7.60 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 (s, 2H), 3.63 (dd, *J* = 27.7, 10.9 Hz, 4H), 2.24 (s, 1H), 1.43 (s, 3H), 1.38 (s, 3H), 0.82 (s, 3H).

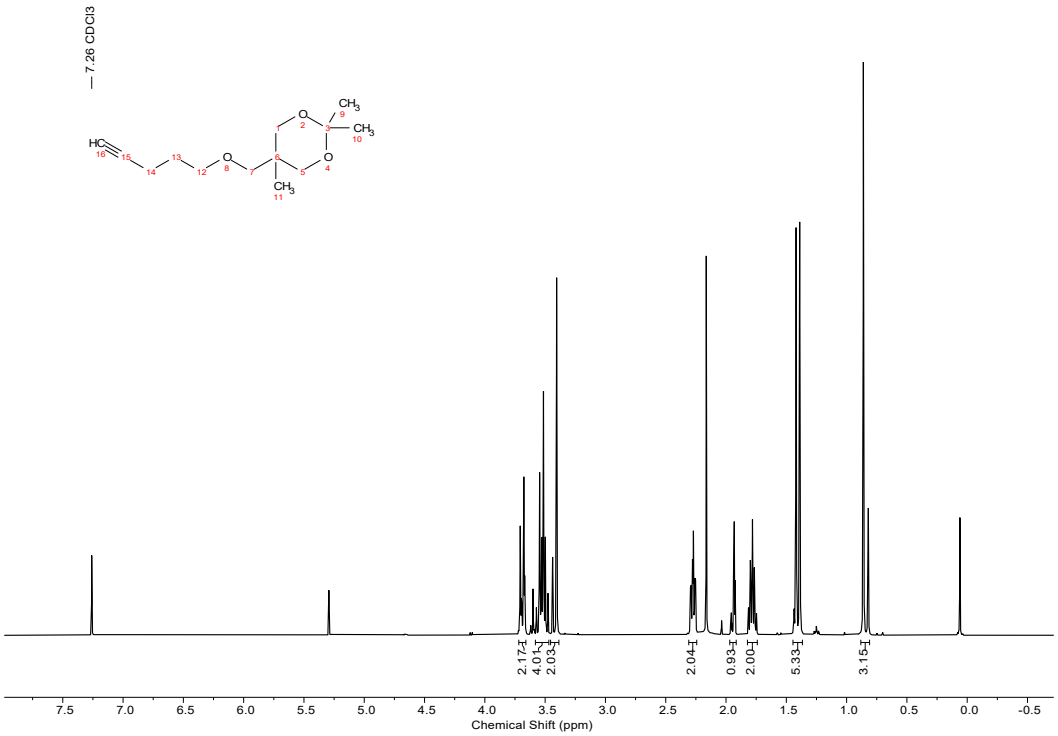


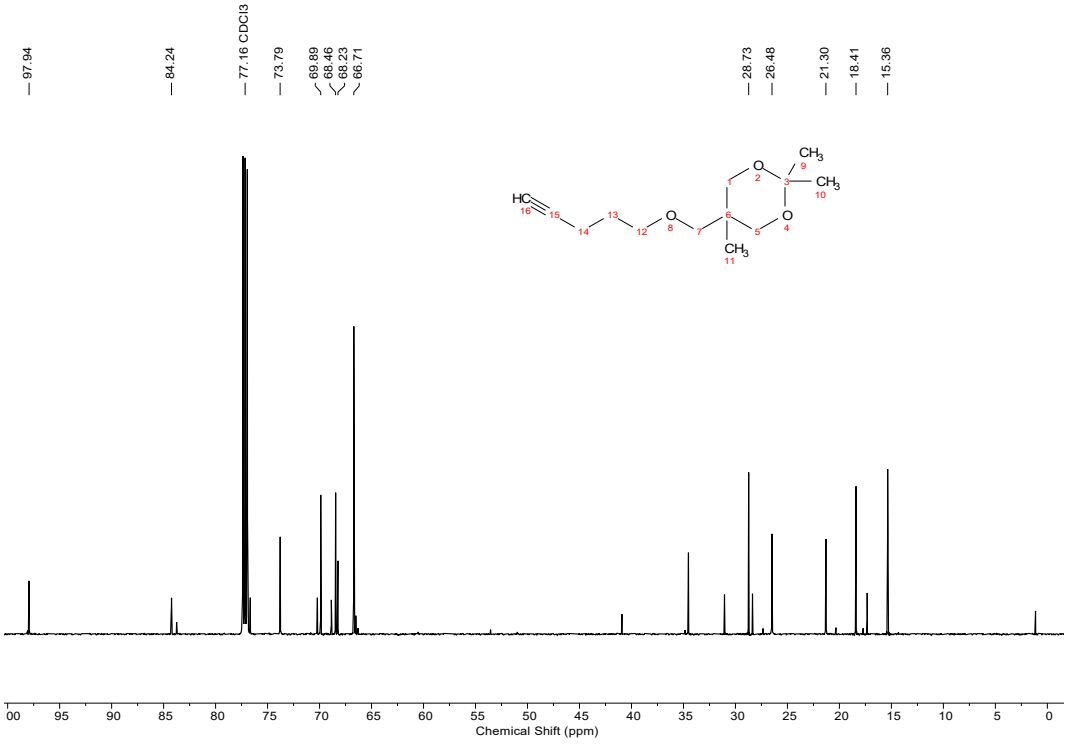


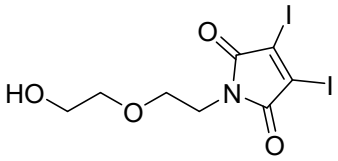
2,2,5-Trimethyl-5-((pent-4-yn-1-yloxy)methyl)-1,3-dioxane (**5**)

To a solution of **7** (1.10 g, 7.5 mmol) in dry THF (10 mL), NaH (60% in mineral oil, 600.0 mg, 15.0 mmol) was added in 3 batches. The mixture was stirred at room temperature for 40 min. **6** (1.20 g, 5.0 mmol) in THF (5 mL) was added slowly. The mixture was stirred at 30 °C for 24 h. After removal of THF under reduced pressure, the reaction was quenched with H_2O (15 mL), then extracted with EtOAc (100 mL). The organic phase was washed with saturated $NaHCO_3$ solution (100 mL) and brine (100 mL), then dried over Na_2SO_4. After concentration in vacuo, purification of the residue by column chromatography on silica gel (5−10% EtOAc in hexane) yielded **9** as a colorless oil (0.88 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.73–3.65 (m, 2H), 3.58–3.47 (m, 4H), 3.42 (m, 2H), 2.27 (td, *J* = 7.2, 2.6 Hz, 2H), 1.94 (m, 1H), 1.78 (m, 2H), 1.41 (s, 3H), 1.38 (s, 3H), 0.84 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 97.9, 84.2, 73.8, 69.9, 68.5, 68.2, 66.7, 28.7, 26.5, 21.3, 18.4, 15.4. HRMS (ESI), m/z calcd. for $C_{13}H_{22}O_3$ $[M + Na]^+$: 249.1467; found 249.1466.

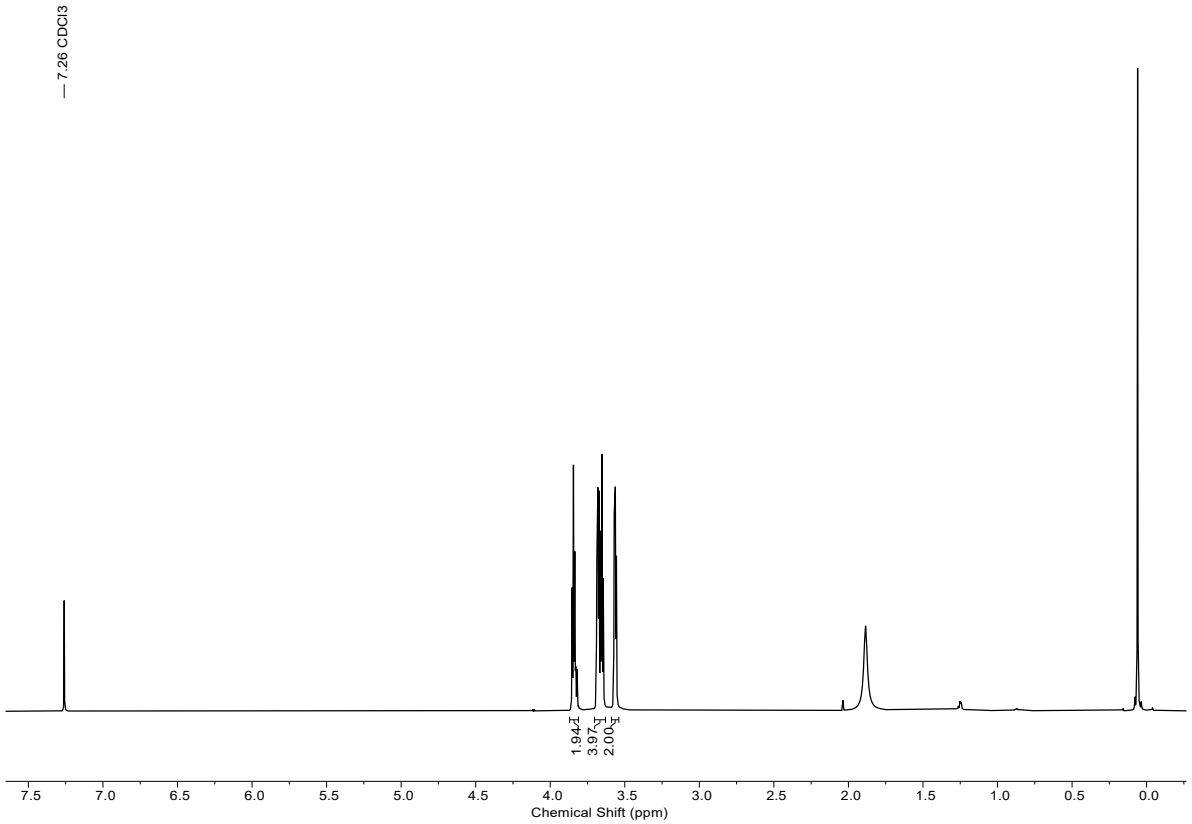


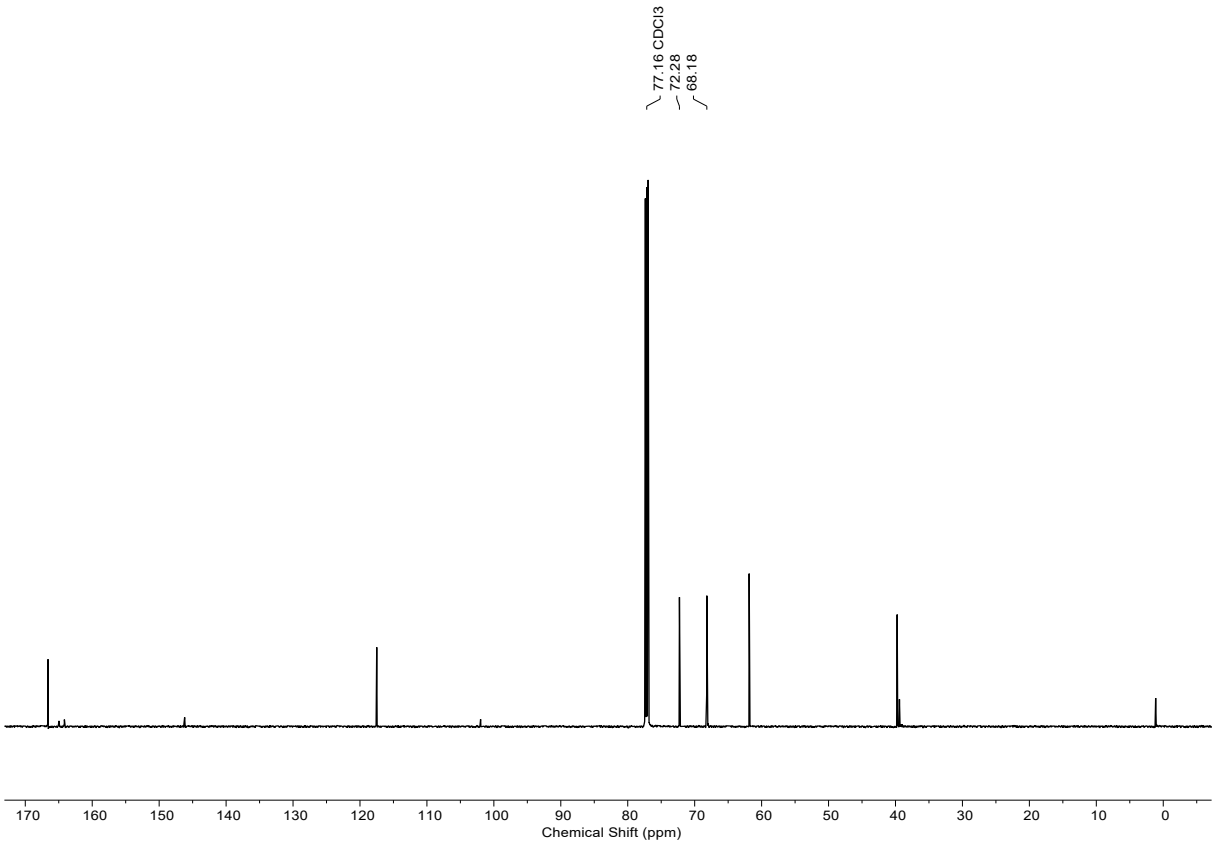


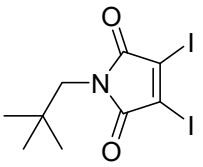


1-(2-(2-Hydroxyethoxy)ethyl)-3,4-diiodo-1*H*-pyrrole-2,5-dione (**6**)

At room temperature, dichloromaleic anhydride (3.34 g, 20 mmol) was dissolved in toluene (100 mL), followed by addition of diethylene glycol amine (2.10 g, 20 mmol), and then the solution was heated at 135 °C for 12 h. After removal of the solvent, the crude residue was dissolved in acetonitrile (70 mL) and sodium iodide (9.00 g, 60 mmol) was added. The mixture was heated at 90 °C overnight. After removal of the solvent, the residue was purified by column chromatography on silica gel (EtOAc / hexane = 1:2) to give the product (5.77 g, 66%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.79 (t, *J* = 5.4 Hz, 2H), 3.64–3.58 (m, 4H), 3.51 (t, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.5, 117.4, 72.1, 68.0, 61.6, 39.6. HRMS (ESI), m/z calcd. for $C_8H_9I_2NO_4$ $[M + Na]^+$: 459.8519; found 459.8520.







3,4-Diiodo-1-neopentyl-1*H*-pyrrole-2,5-dione (**7**)

This compound was synthesized following the procedure described in our previous work[4]. Briefly, dichloromaleic anhydride (3.34 g, 20 mmol) was dissolved in acetic acid (40 mL) with slow addition of neopentylamine (1.74 g, 20 mmol) at 0 °C, then the solution was heated at 50 °C for 24 h. After removal of the solvent, the crude residue was dissolved in acetonitrile (70 mL) and sodium iodide (9.00 g, 60 mmol) was added. The mixture was heated at 90 °C overnight. After removal of the solvent, the residue was purified by column chromatography on silica gel (EtOAc / hexane = 1:19) to give the product (5.87 g, 70%). ^{1}H NMR ($CDCl_3$) δ 3.43 (s, 2H), 0.92 (s, 9H).

General Procedure A for preparation of maleimide-fused enediynes

Compound **6** (218.5 mg, 0.50 mmol) or **7** (209.5 mg, 0.50 mmol), $Pd(PPh_3)_2Cl_2$ (35.1 mg, 0.05 mmol) and CuI (38.1 mg, 0.20 mmol) were placed in a 25 mL Schlenk flask. Under a nitrogen atmosphere, N,N-diisopropylethylamine (0.26 mL, 1.50 mmol), dry THF (1.5 mL) and dry toluene (4 mL) were successively added into the flask. The terminal alkyne(s) (1.50 mmol) in dry THF (0.5 mL) was then added. The mixture was subsequently freeze-thawed twice to remove oxygen, then stirred at room temperature overnight. After complete consumption of **6** or **7** monitored by TLC, the mixture was purified through column chromatography on silica gel to give the desired compound.

General Procedure B for preparation of enediynes linked with a PTG moiety through esterification

Carboxylic acid (1.1 equiv.), 2-methyl-6-nitrobenzoic anhydride (MNBA, 1.2 equiv.) and DMAP (0.25 equiv.) were added into a 25 mL Schlenk flask. Under a nitrogen atmosphere, N,N-diisopropylethylamine (4.0 equiv.) and corresponding enediyne (1.0 equiv.) in dry DCM (100 mM) were successively added into the flask, then nitrogen was pumped out and refilled twice. The mixture was stirred at room temperature overnight. After concentration *in vacuo*, the crude residue was purified through column chromatography on silica gel to give the desired compound.

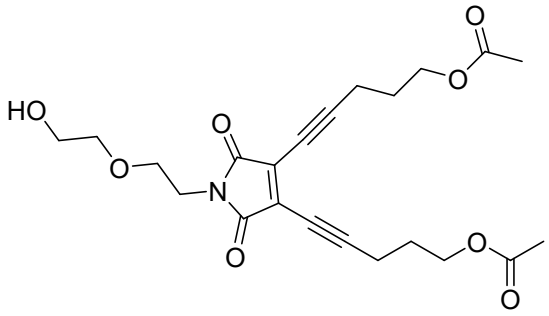
General Procedure C for removal of ketal mask groups in enediynes

Corresponding enediyne was dissolved in a mixed solvent of TFA/H_2O = 1/1 to form 10 mM solution. The mixture was stirred at room temperature and monitored by TLC for 3–4 h. After concentration *in vacuo*, the product obtained in quantitative yield was used without further purification for the next step.

General Procedure D for preparation of multi-sulfated enediynes

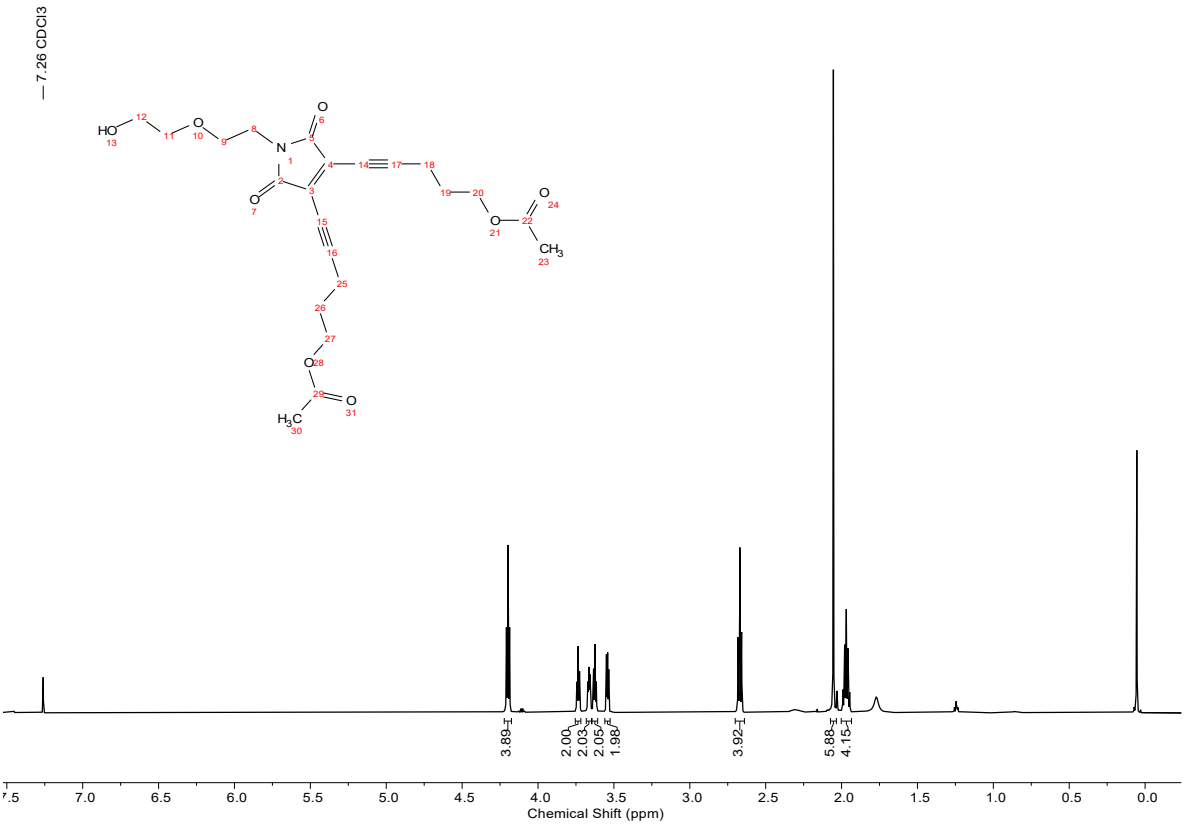
The multi-sulfation was conducted according to reported procedures[5, 6]. The enediyne with multi-hydroxyl groups (1 equiv.) obtained from the previous step was dissolved in anhydrous DMF (1 mL). The solution was cooled to 0 °C under an atmosphere of nitrogen, then treated dropwise with a solution of SO_3·DMF (5 equiv. per OH) in anhydrous DMF (1 mL). The resulting mixture was stirred for 1 h, then allowed to warm to room

temperature and stirred for an additional 2 h. Subsequently, the reaction flask was brought to -78 °C and quenched with aqueous sodium bicarbonate solution (5 equiv., 10% w/v) until bubbling had ceased (pH = 7). After removal of the volatile components under reduced pressure (in a water bath maintained below 30 °C), the residue was dissolved in ultrapure water (*ca.* 10 mL) and dialyzed in ultrapure water for 6 h (water changed at 0.5, 1, 2 and 4 h) using a dialysis bag to obtain a yellow transparent solution, which was then lyophilized to give a light-yellow powder in > 99% yield.



(1-(2-(2-Hydroxyethoxy)ethyl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl)bis(pent-4-yne-5,1-diyl) diacetate (**EDY-1**)

Prepared from **2** and **6** according to **General Procedure A**, isolated in 65% yield as a yellow-brown viscous oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.20 (t, *J* = 6.2 Hz, 4H), 3.74 (t, *J* = 5.4 Hz, 2H), 3.68–3.65 (m, 2H), 3.62 (t, *J* = 5.4 Hz, 2H), 3.54 (dd, *J* = 5.2, 3.8 Hz, 2H), 2.67 (t, *J* = 7.0 Hz, 4H), 2.05 (s, 6H), 1.97 (p, *J* = 6.7 Hz, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.1, 167.6, 128.6, 110.5, 72.3, 68.2, 62.9, 61.8, 38.4, 27.3, 21.0, 17.4. HRMS (ESI), m/z calcd. for $C_{22}H_{27}NO_8$ [M + Na]+: 456.1634, found 456.1633.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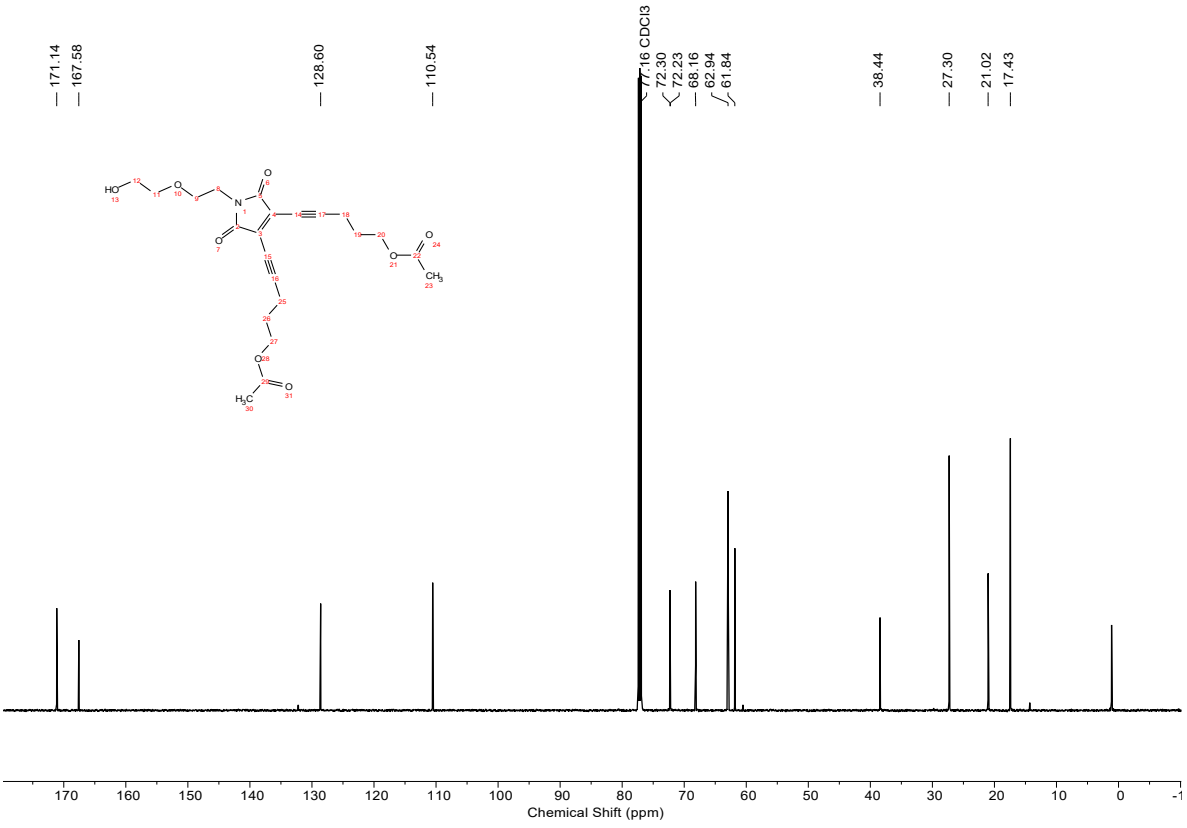
4.15
Chemical Shift (ppm)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77.16 CDCl3
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72.23
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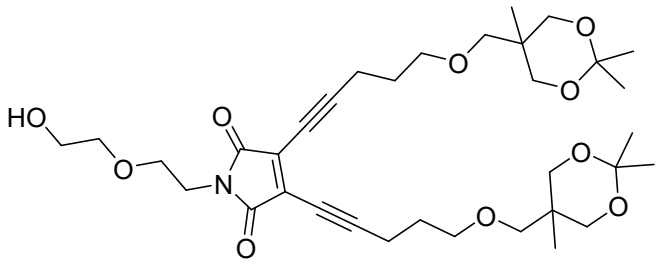
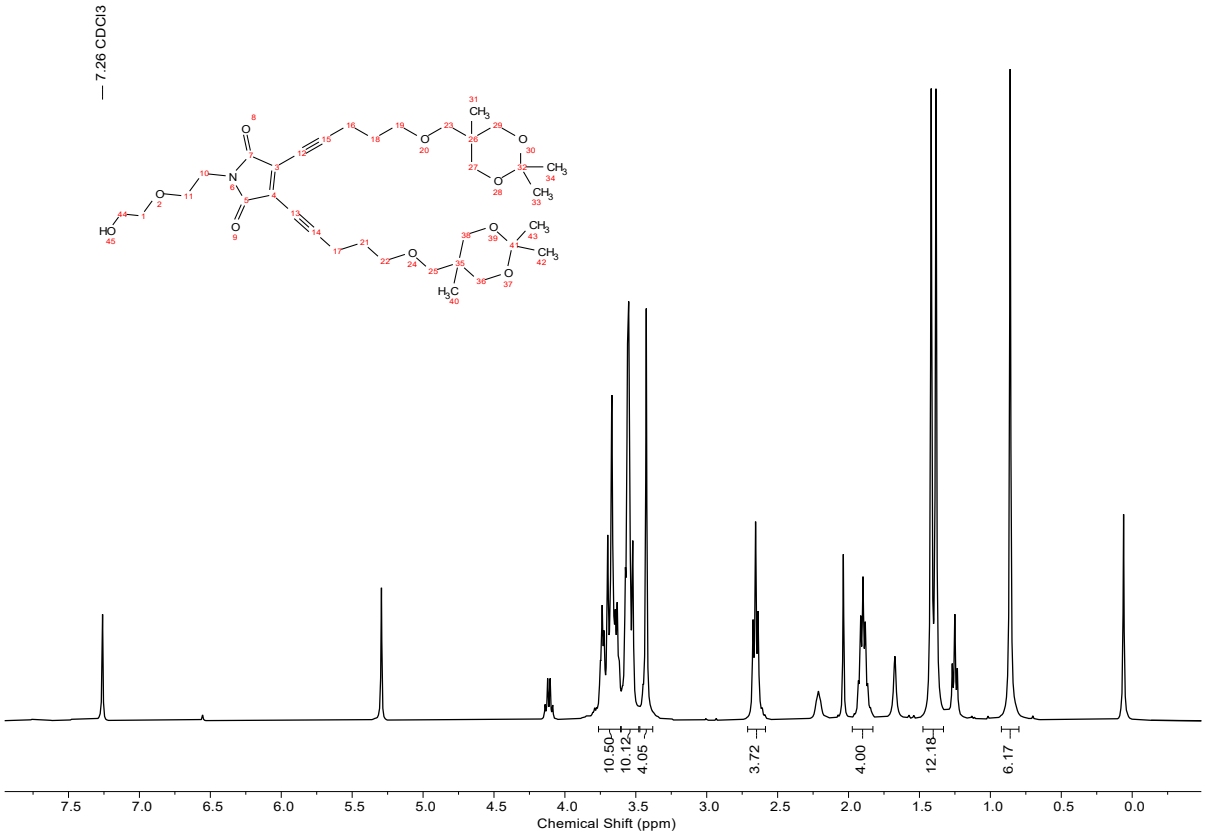
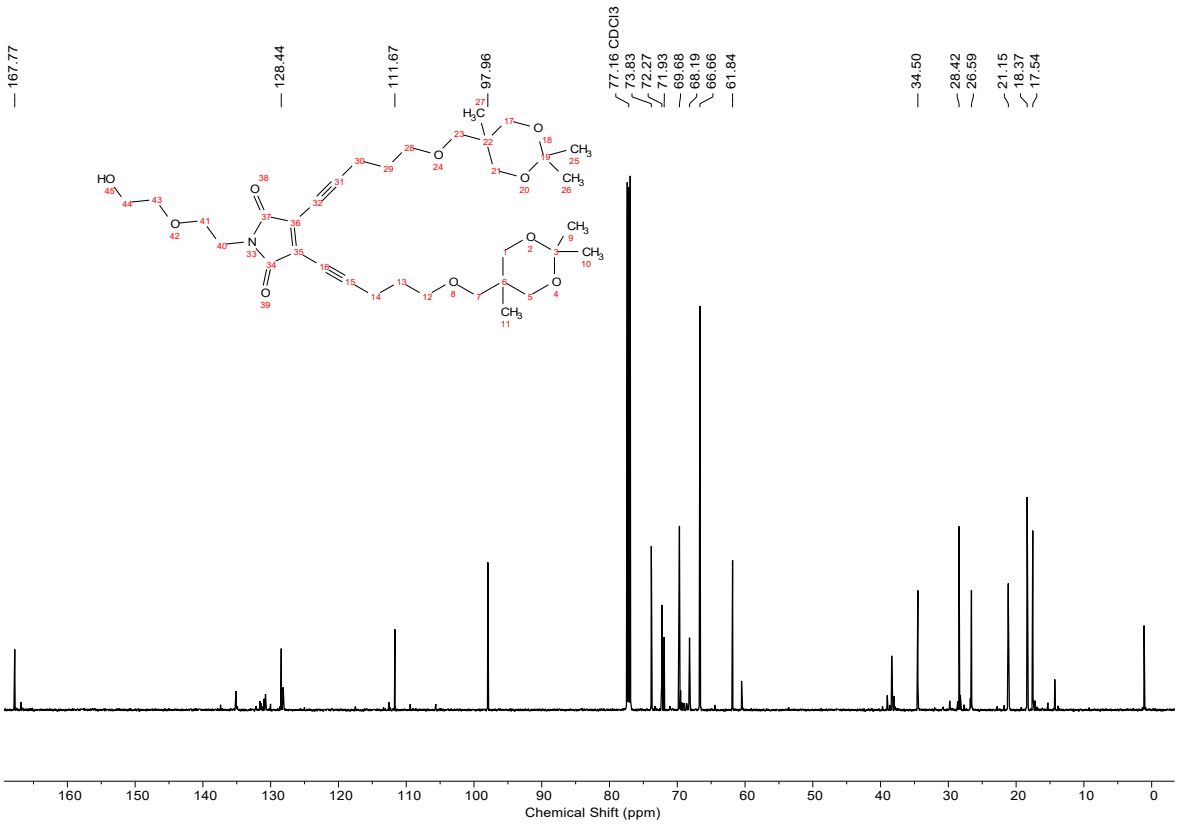
62.94
61.84
38.44
27.30
21.02
17.43
Chemical Shift (ppm)

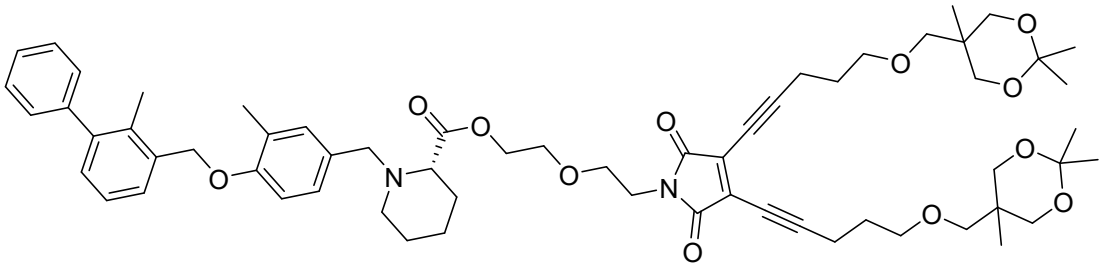


1-(2-(2-Hydroxyethoxy)ethyl)-3,4-bis(5-((2,2,5-trimethyl-1,3-dioxan-5-yl)methoxy)pent-1-yn-1-yl)-1*H*-pyrrole-2,5-dione (**EDY-2**)

Prepared from **5** and **6** according to **General Procedure A**, isolated in 60% yield as a yellow-brown viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.76–3.60 (m, 10H), 3.60–3.50 (m, 10H), 3.43 (s, 4H), 2.66 (t, *J* = 7.1 Hz, 4H), 1.90 (p, *J* = 6.7 Hz, 4H), 1.40 (d, *J* = 13.5 Hz, 12H), 0.86 (s, 6H). ^{13}C NMR (151 MHz, Chloroform-d) δ 167.8, 128.4, 111.7, 98.0, 73.8, 72.3, 71.9, 69.7, 68.2, 66.7, 61.8, 34.5, 28.4, 26.6, 21.2, 18.4, 17.5. HRMS (ESI), m/z calcd. for $C_{34}H_{51}NO_{10}$ [M + Na]$^{+}$: 656.3405, found 656.3416.



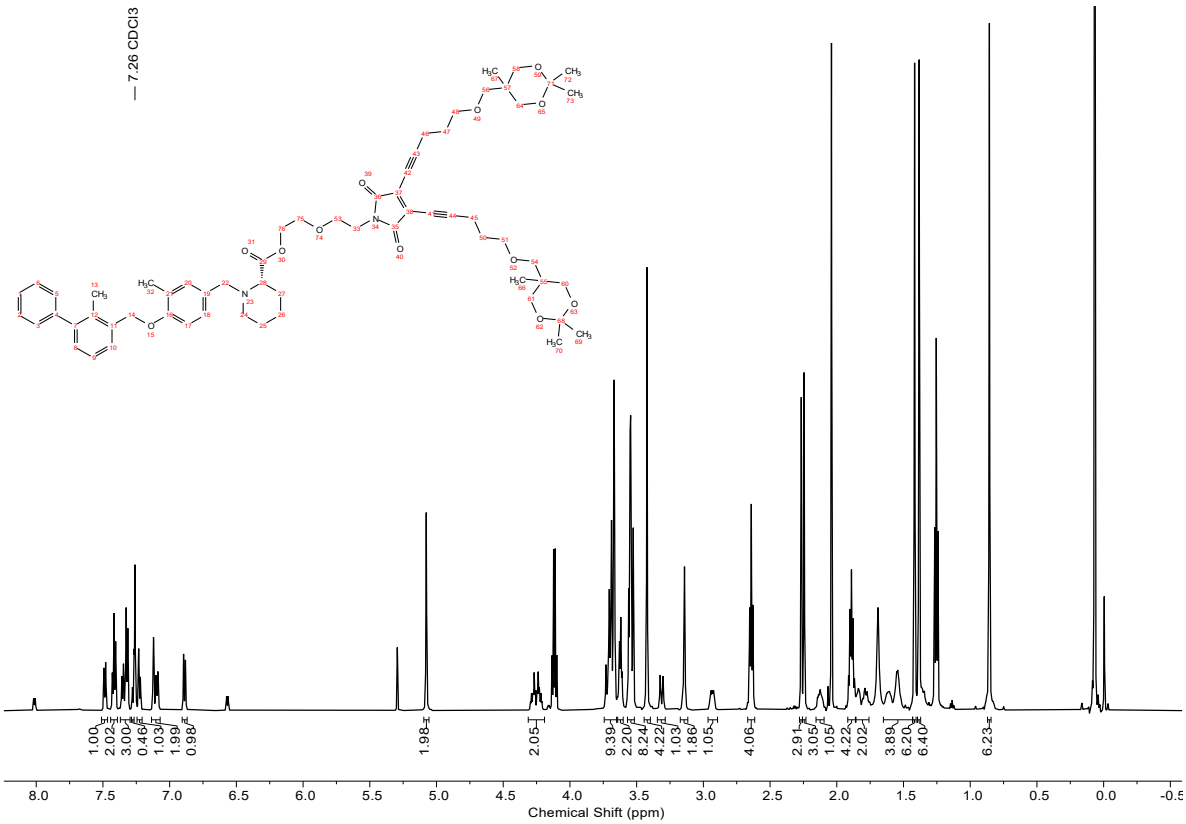


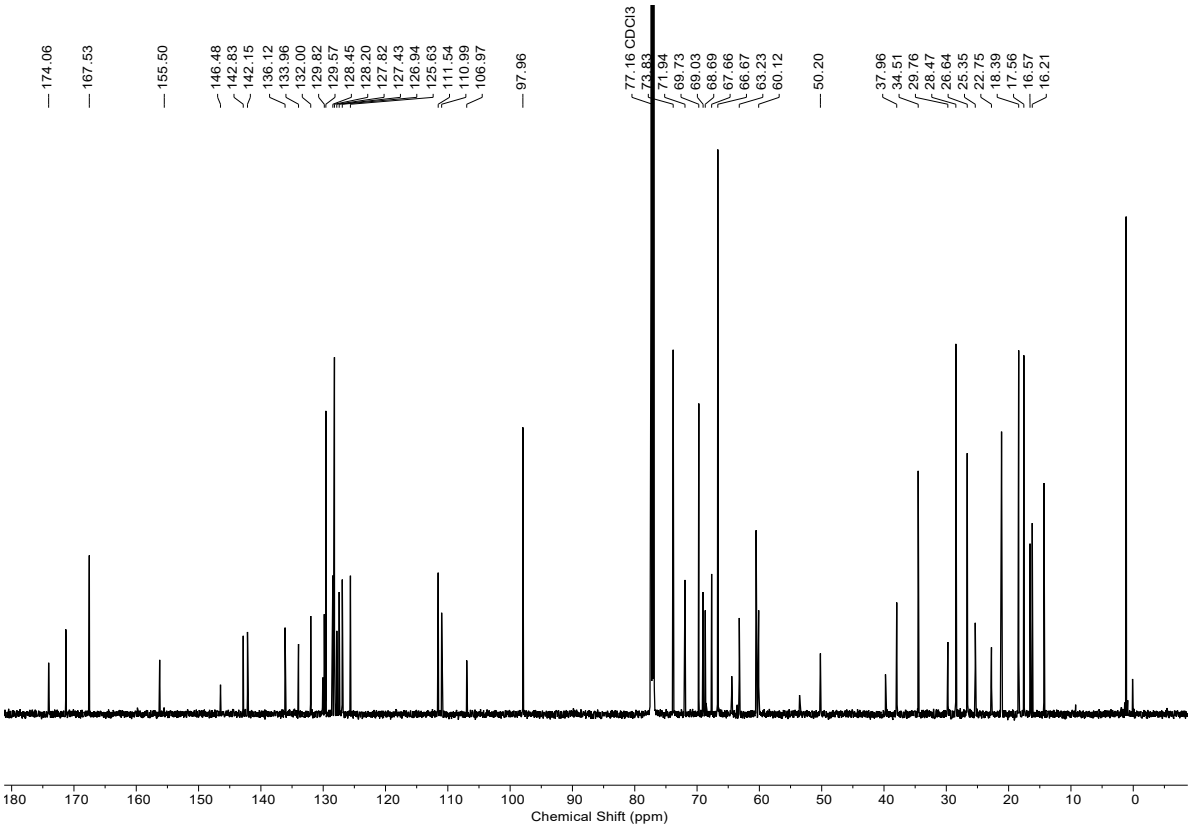


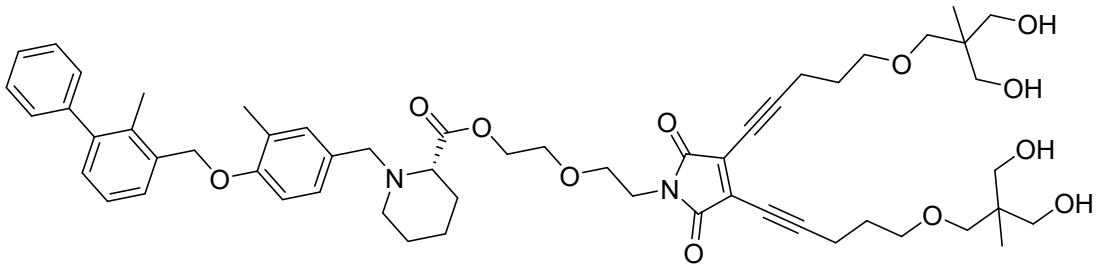
2-(2-(2,5-Dioxo-3,4-bis(5-((2,2,5-trimethyl-1,3-dioxan-5-yl)methoxy)pent-1-yn-1-yl)-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy)ethyl (S)-1-(3-methyl-4-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)benzyl)piperidine-2-carboxylate (**EDY-3**)

Prepared from **BMS-57** and **EDY-2** according to **General Procedure B**, isolated as a yellow-brown viscous oil (150 mg, 30%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49 (d, *J* = 7.5 Hz, 1H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.34 (m, 3H), 7.29–7.20 (m, 2H), 7.14–7.07 (m, 2H), 6.89 (d, *J* = 8.2 Hz, 1H), 5.08 (s, 2H), 4.31–4.20 (m, 2H), 3.74–3.65 (m, 9H), 3.62 (t, *J* = 5.8 Hz, 2H), 3.57–3.51 (m, 8H), 3.42 (s, 4H), 3.31 (d, *J* = 13.0 Hz, 1H), 3.16–3.13 (m, 2H), 2.93 (m, 1H), 2.64 (t, *J* = 7.2 Hz, 4H), 2.27 (s, 3H), 2.24 (s, 3H), 1.89 (m, 4H), 1.86–1.76 (m, 2H), 1.65–1.43 (m, 4H), 1.42 (s, 6H),

1.38 (s, 6H), 0.86 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.1, 167.5, 156.3, 146.5, 142.8, 136.1, 134.0, 132.0, 129.8, 129.6, 128.5, 128.2, 127.8, 127.4, 126.9, 125.6, 111.5, 111.0, 107.0, 98.0, 73.8, 71.9, 69.7, 69.0, 68.7, 67.7, 66.7, 63.2, 60.1, 50.2, 38.0, 34.5, 29.8, 28.5, 26.6, 25.4, 22.8, 18.4, 17.6, 16.6, 16.2. HRMS (ESI), m/z calcd. for $C_{62}H_{80}N_2O_{12}$ [M + H]+: 1045.5790, found 1045.5791.

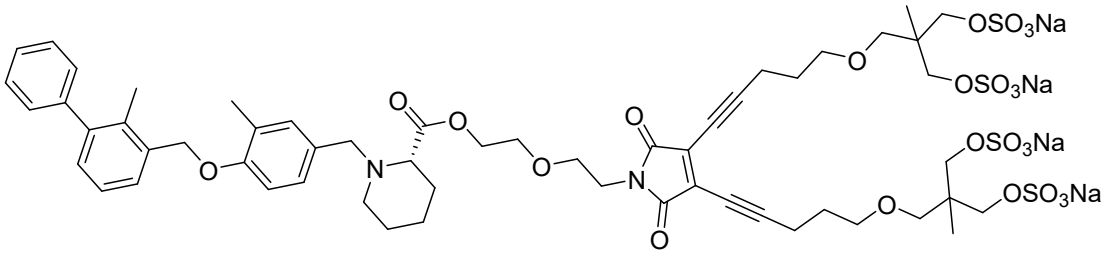






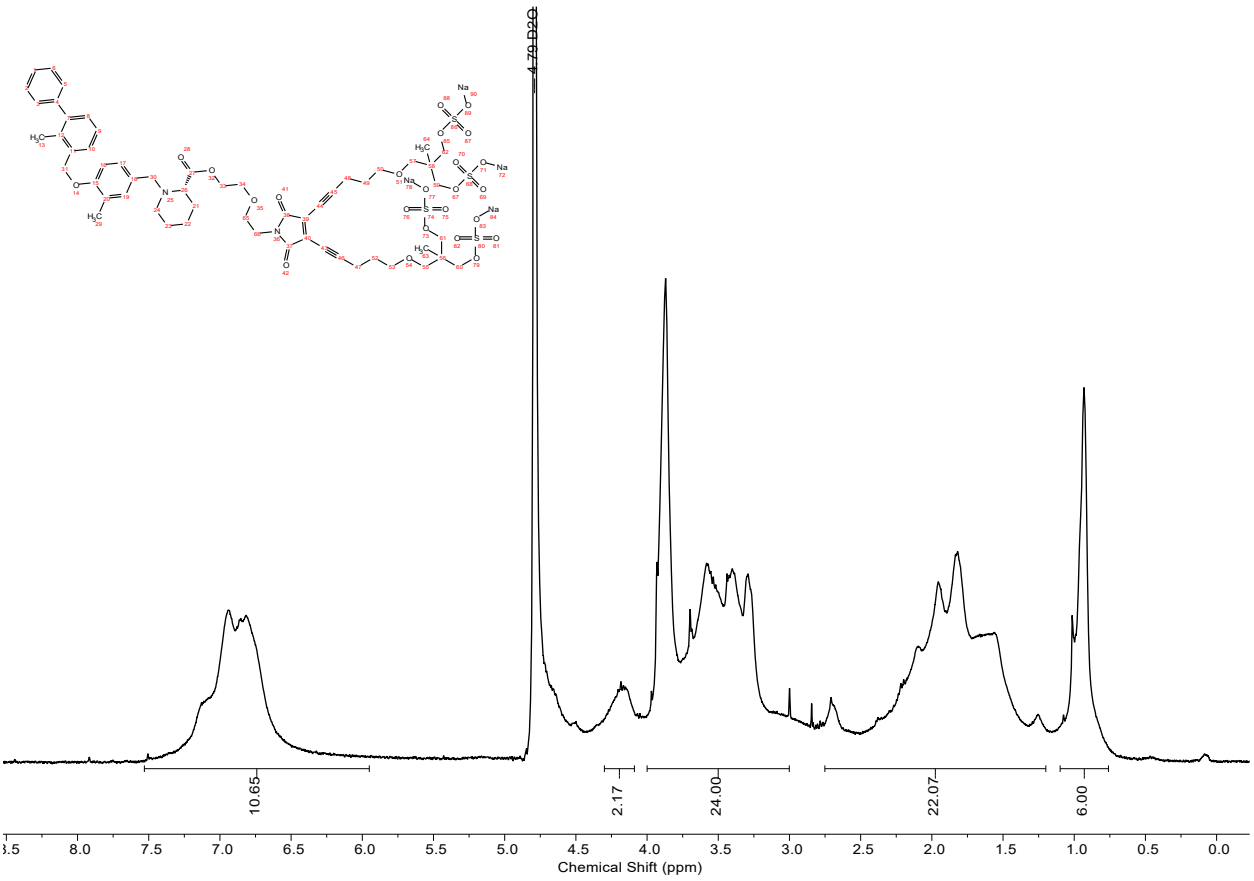
2-(2-(3,4-Bis(5-(3-hydroxy-2-(hydroxymethyl)-2-methylpropoxy)pent-1-yn-1-yl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethoxy)ethyl (S)-1-(3-methyl-4-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)benzyl)piperidine-2-carboxylate (**EDY-4**)

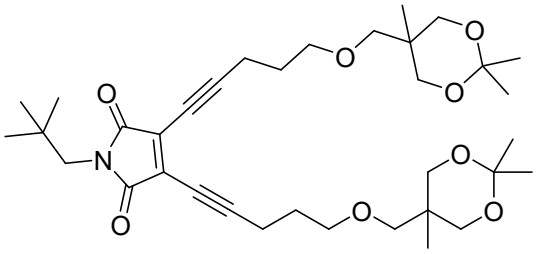
Prepared from **EDY-3** according to **General Procedure C**, as a yellow viscous oil (quantitative). HRMS (ESI), m/z calcd. for $C_{56}H_{72}N_2O_{12}$ $[M + H]^+$: 965.5164, found 965.5147.



Sodium (*S*)-((((1-(2-(2-((1-(3-methyl-4-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)benzyl)piperidine-2-carbonyl)oxy)ethoxy)ethyl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl)bis(pent-4-yne-5,1-diyl))bis(oxy))bis(methylene))bis(2-methylpropane-2,1,3-triyl) tetrakis(sulfate) (**Compound-1**)

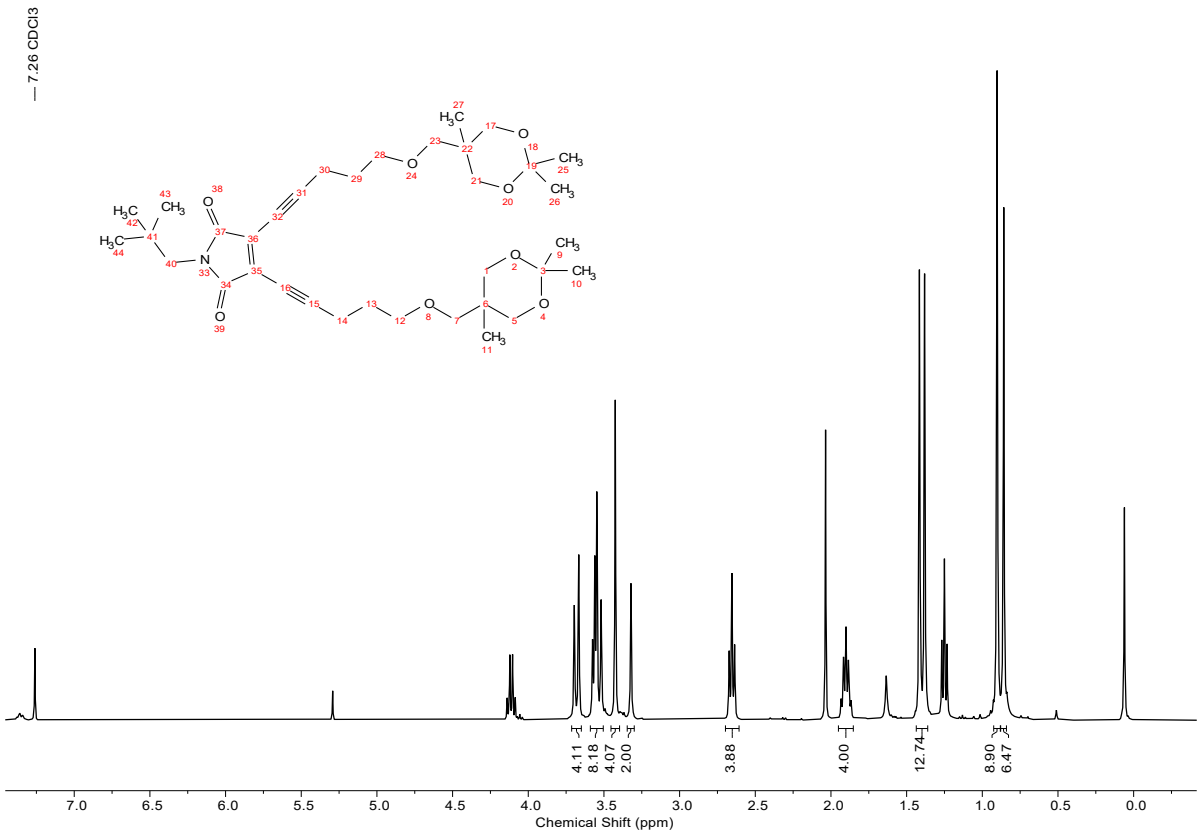
Prepared from **EDY-4** according to **General Procedure D**, isolated as a yellow solid (quantitative). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.53 – 5.95 (m, 11H), 4.17 (m, 2H), 4.00 – 3.00 (m, 24H), 2.75 – 1.20 (m, 22H), 0.93 (s, 6H).

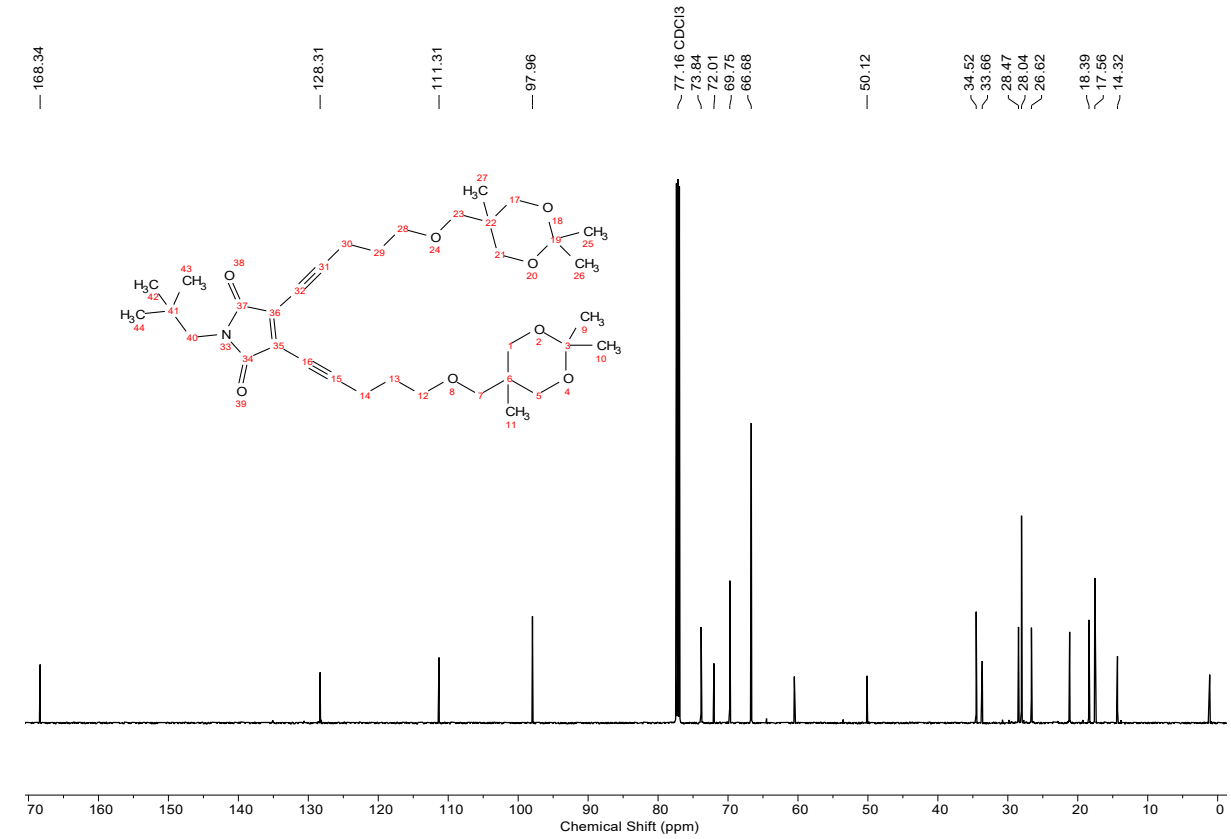


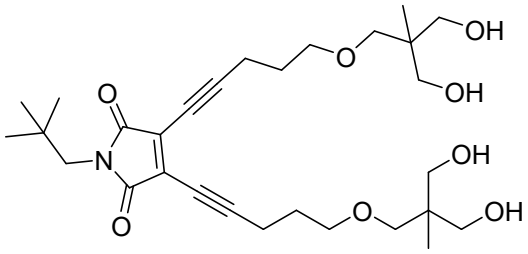


1-Neopentyl-3,4-bis(5-((2,2,5-trimethyl-1,3-dioxan-5-yl)methoxy)pent-1-yn-1-yl)-1*H*-pyrrole-2,5-dione (**EDY-5**)

Prepared compounds **5** and **7** according to **General Procedure A**, isolated in 63% yield as a yellow-brown viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 (m, 4H), 3.60–3.47 (m, 8H), 3.43 (s, 4H), 3.32 (s, 2H), 2.65 (t, *J* = 7.2 Hz, 4H), 1.90 (m, 4H), 1.42 (s, 6H), 1.38 (s, 6H), 0.90 (s, 9H), 0.86 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.3, 128.3, 111.3, 98.0, 73.8, 72.0, 69.8, 66.7, 50.1, 34.5, 33.7, 28.5, 28.0, 26.6, 18.4, 17.6, 14.3. HRMS (ESI), m/z calcd. for $C_{35}H_{53}NO_8$ [M + Na]+: 638.3669, found 638.3667.

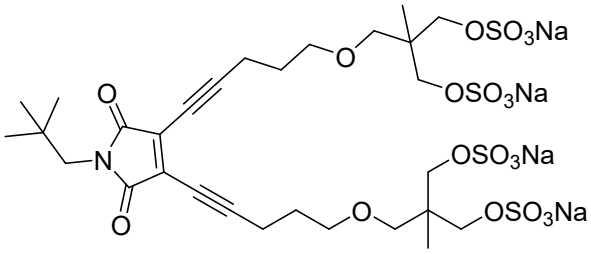






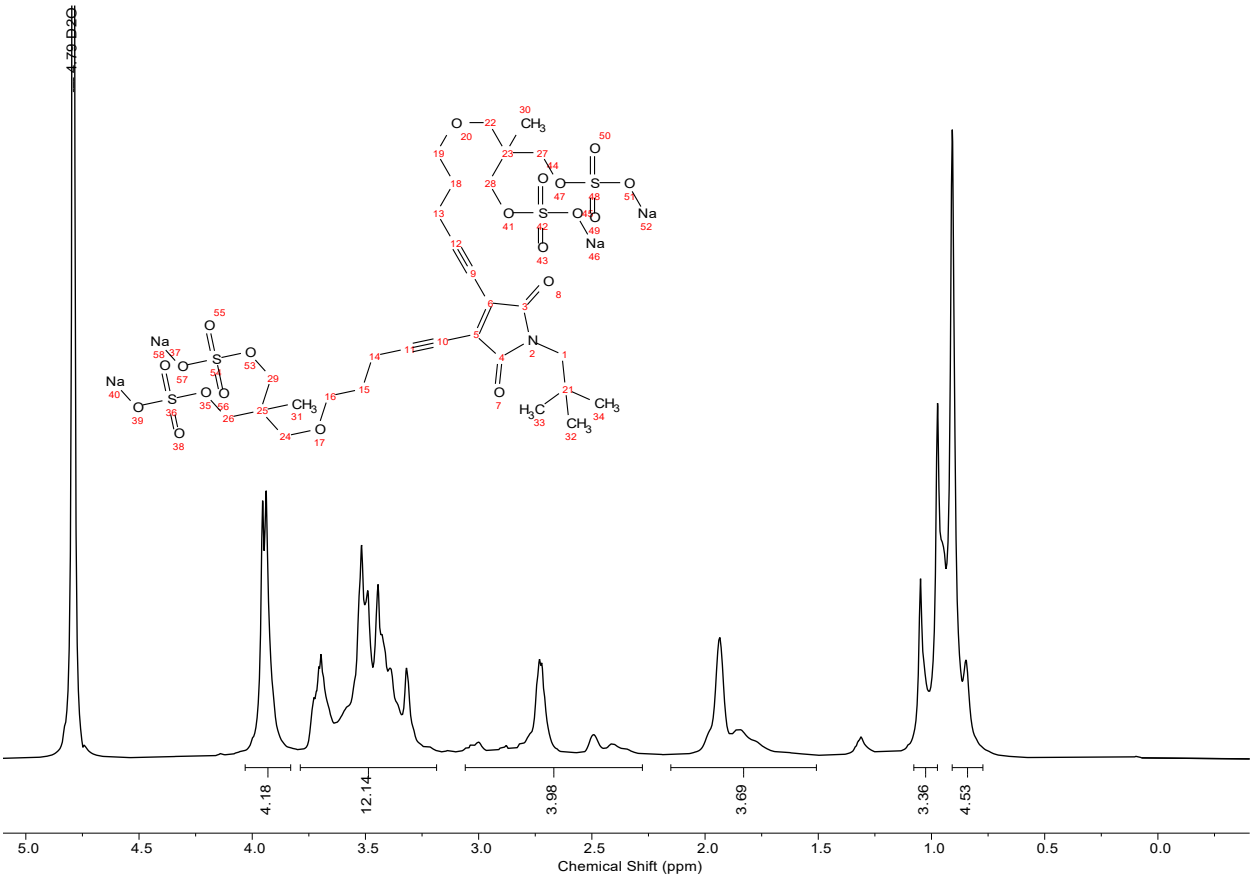
3,4-Bis(5-(3-hydroxy-2-(hydroxymethyl)-2-methylpropoxy)pent-1-yn-1-yl)-1-neopentyl-1*H*-pyrrole-2,5-dione (**EDY-6**)

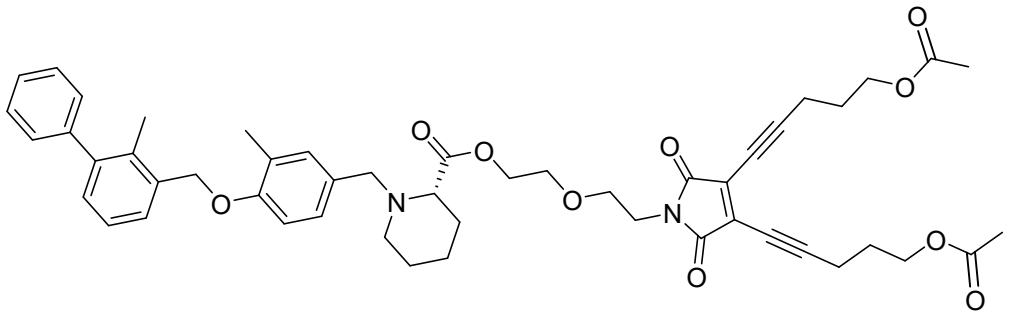
Prepared from **EDY-5** according to **General Procedure C**, as a yellow viscous oil (quantitative). HRMS (ESI), m/z calcd. for $C_{29}H_{45}NO_8$ $[M + H]^+$: 536.3218, found 536.3222.



Sodium ((((1-neopentyl-2,5-dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl)bis(pent-4-yne-5,1-diyl))bis(oxy))bis(methylene))bis(2-methylpropane-2,1,3-triyl) tetrakis(sulfate) (**C-a**)

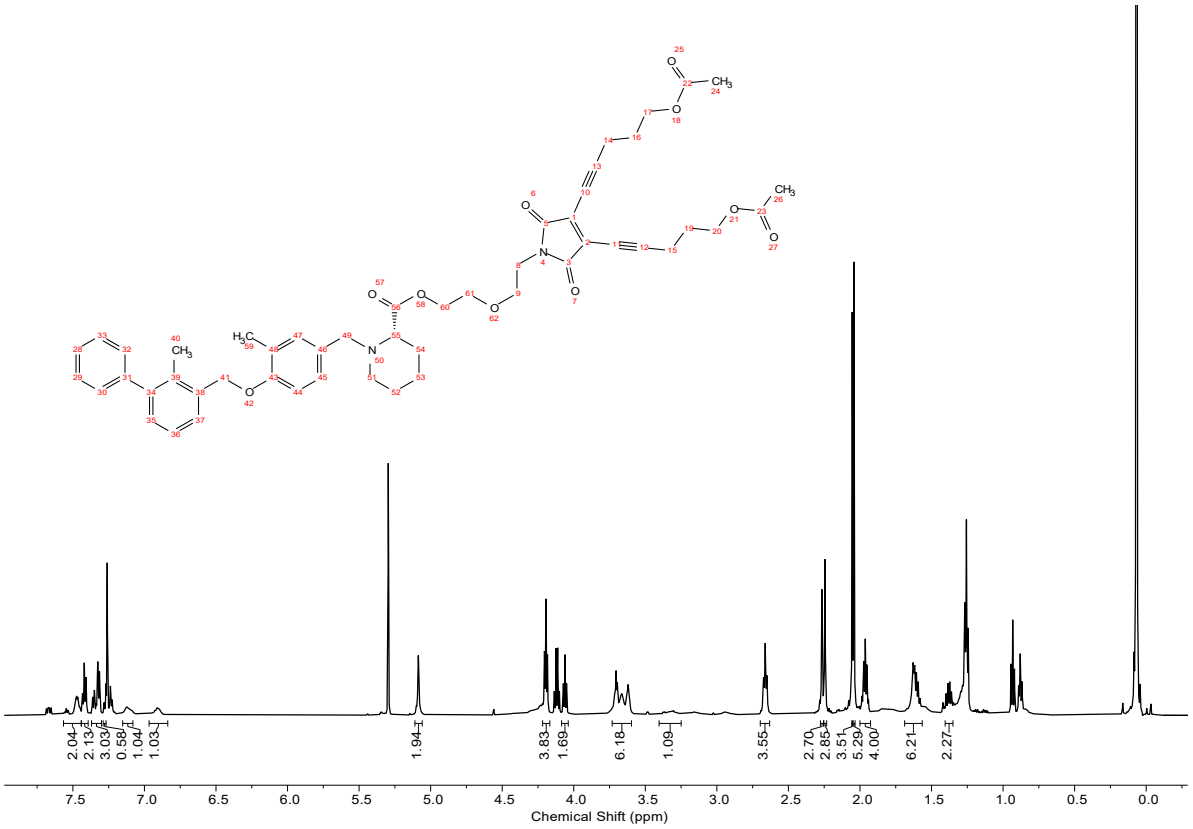
Prepared from **EDY-6** according to **General Procedure C**, isolated as a yellow solid (quantitative). ^{1}H NMR (600 MHz, Deuterium Oxide) δ 3.95 (d, J = 10.4 Hz, 4H), 3.79 – 3.19 (m, 12H), 3.06 – 2.28 (m, 4H), 1.90 (d, J = 48.2 Hz, 4H), 2.15 – 1.51 (m, 4H), 1.07 – 0.77 (m, 15H). HRMS (ESI), m/z calcd. for $C_{29}H_{41}NNa_4O_{20}S_4$ [M - Na]$^-$: 920.0803, found 920.0805.





(*S*)-(1-(2-(2-((1-(3-Methyl-4-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)benzyl)piperidine-2-carbonyl)oxy)ethoxy)ethyl)-2,5-dioxo-2,5-dihydro-1*H*-pyrrole-3,4-diyl)bis(pent-4-yne-5,1-diyl) diacetate (**C-b**)

Prepared from **BMS-57** and **EDY-1** according to **General Procedure B**, isolated as a yellow-brown viscous oil (168 mg, 28%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56–7.44 (m, 2H), 7.42 (m, 2H), 7.38–7.30 (m, 3H), 7.28 (t, *J* = 7.6 Hz, 1H), 7.25–7.22 (m, 1H), 7.16–7.06 (m, 1H), 6.98–6.86 (m, 1H), 5.08 (s, 2H), 4.19 (t, *J* = 6.3 Hz, 4H), 4.06 (t, *J* = 6.7 Hz, 2H), 3.72–3.59 (m, 6H), 3.39–3.25 (m, 1H), 2.66 (t, *J* = 7.1 Hz, 4H), 2.27 (s, 3H), 2.25 (s, 3H), 2.04 (s, 6H), 1.96 (m, 4H), 1.67–1.57 (m, 6H), 1.39–1.35 (m, 2H). ^{13}C NMR (151 MHz, Chloroform-d) δ 174.0, 171.0, 167.3, 156.2, 142.8, 142.1, 136.1, 134.0, 132.0, 130.0, 129.6, 128.6, 128.2, 127.8, 127.4, 126.9, 126.8, 125.6, 111.0, 110.4, 72.2, 69.0, 68.7, 67.6, 63.2, 62.9, 60.5, 60.1, 50.2, 38.0, 29.7, 27.3, 25.4, 22.7, 21.2, 21.0, 16.6, 14.3. HRMS (ESI), m/z calcd. for $C_{50}H_{56}N_2O_{10}$ $[M + H]^+$: 845.4013, found 845.4012.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2.19
3.03
0.58
1.04
1.03
1.94
3.83
1.69
6.18
1.09
3.55
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2.85
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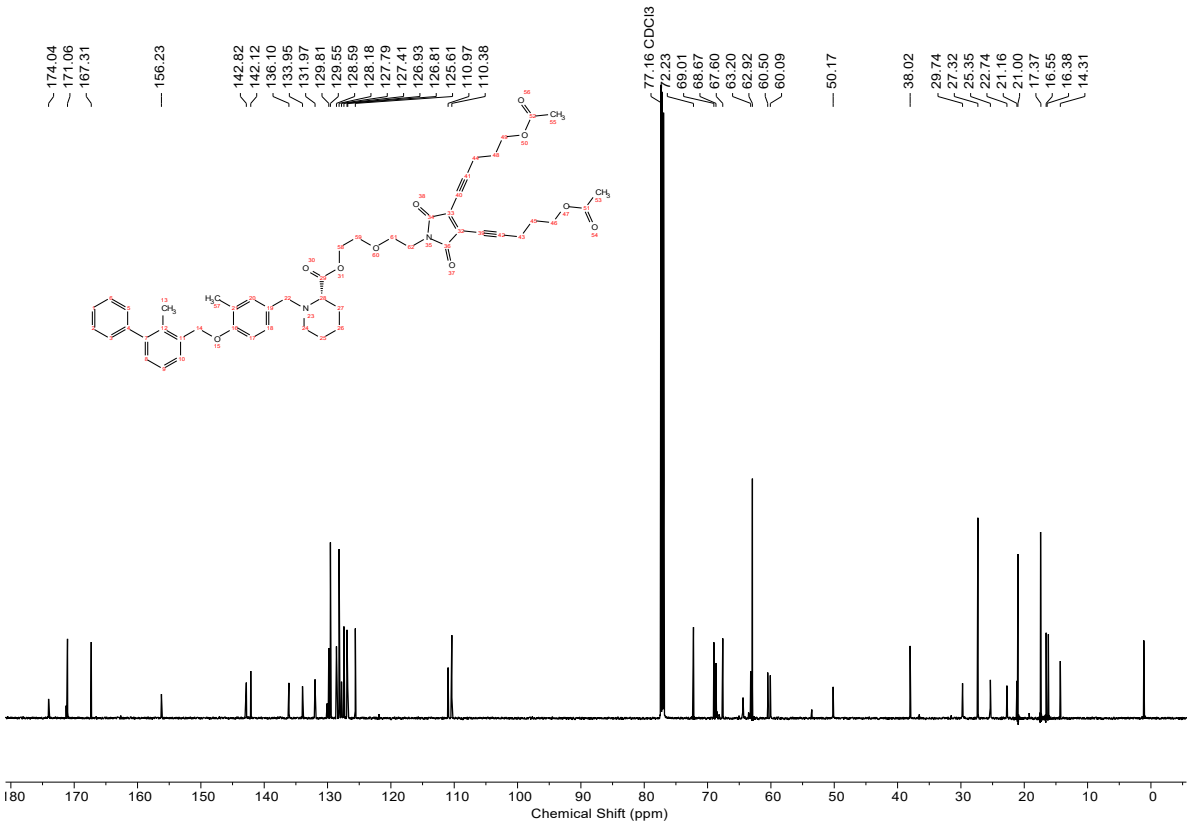
5.29
4.00
6.21
2.27
Chemical Shift (ppm)


174.04
171.06
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156.23
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142.12
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133.95
131.97
129.81
129.55
128.59
128.18
127.79
127.41
126.93
126.81
125.61
110.97
110.38
77.16 CDCl3
72.23
69.01
68.67
67.60
63.20
62.92
60.50
60.09
50.17
38.02
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25.35
22.74
21.16
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Chemical Shift (ppm)

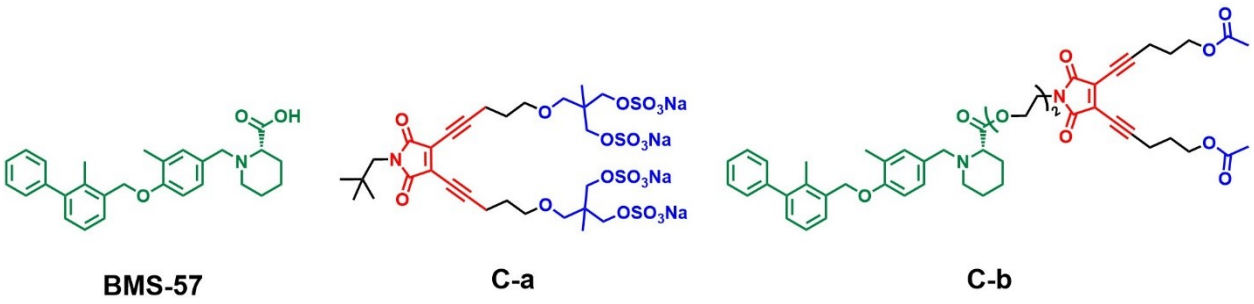

Fig. S1. Chemical structures of control compounds used in this study.

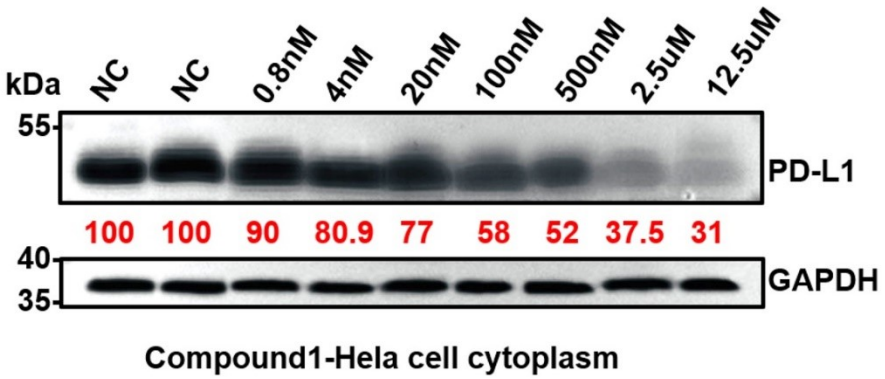

Fig. S2. The cytosolic PD-L1 levels in HeLa cells treated with Compound-1 at various concentrations for 48 hours.

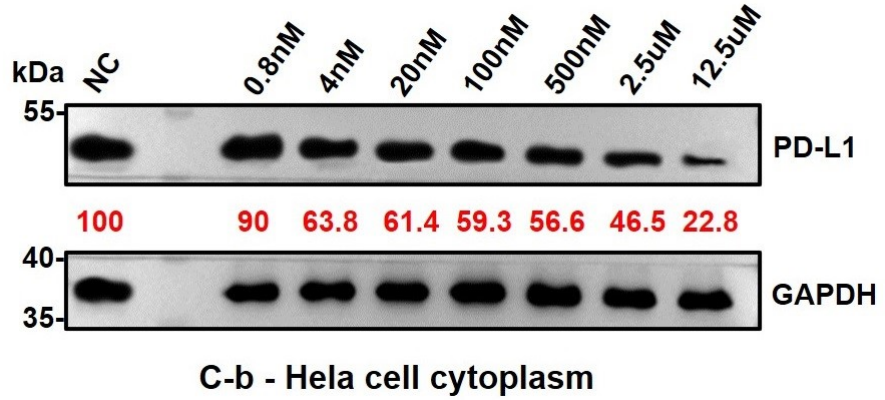

Fig. S3. The cytosolic PD-L1 levels in HeLa cells treated with C-b at various concentrations for 48 hours.

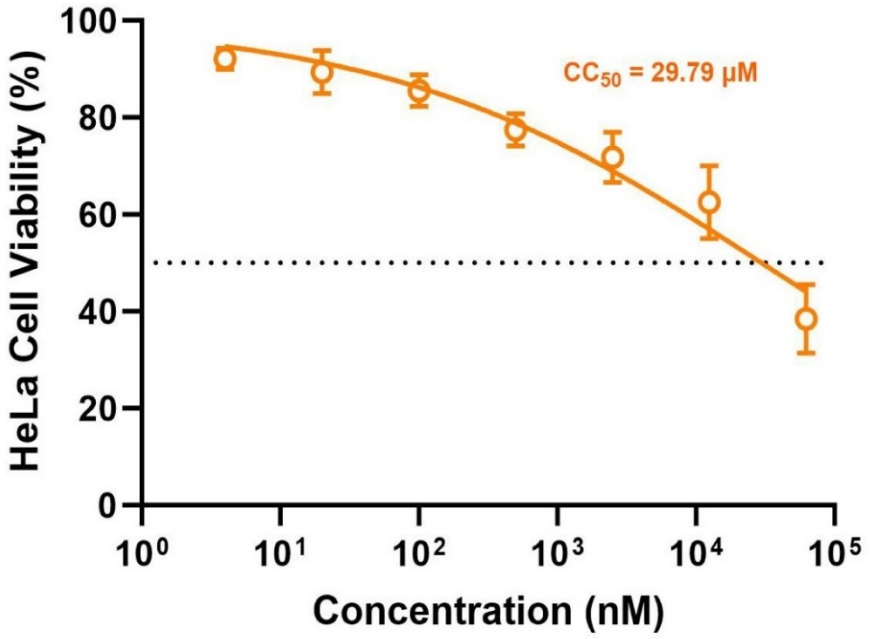

Fig. S4. HeLa cell growth inhibition activity of Compound-1.

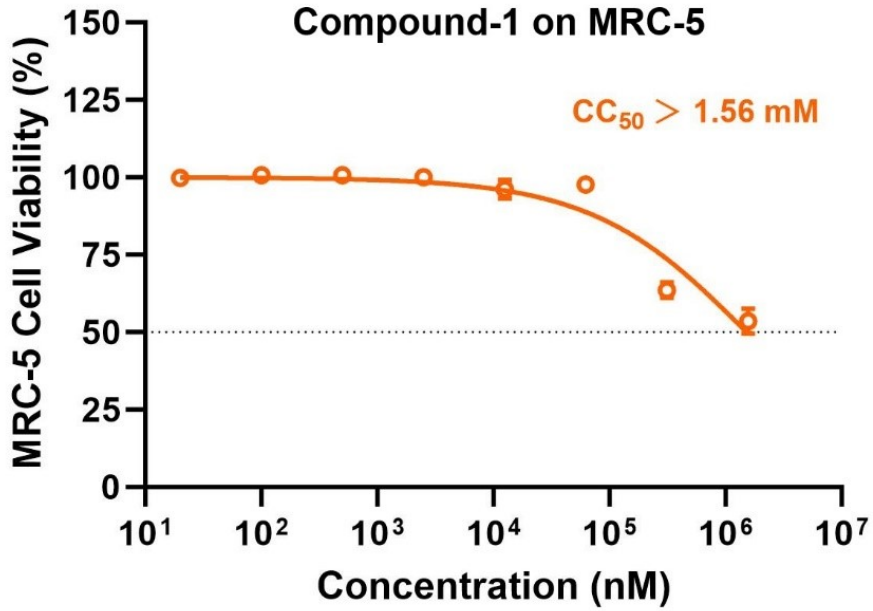

Fig. S5. MRC-5 cell growth inhibition activity of Compound-1.

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1. L. Tang, F. Yang, H. Cheng, C. Tan, C. C. Jin, H. F. Chen, Y. F. Huang, S. F. Zhang, W. H. Song and J. J. Tan, *Org. Lett.*, 2020, **22**, 8618-8623.
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