Supporting Information

Chimeric Enediynes as Potent Antivirals against Coronaviruses by Targeting a Conserved Free-Fatty-Acid Binding Site on the Spike Protein

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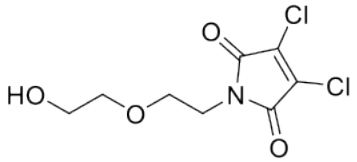
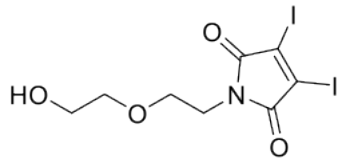
Synthesis



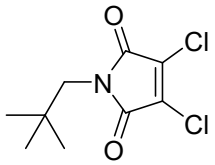
3,4-Dichloro-1-(2-(2-hydroxyethoxy)ethyl)-1H-pyrrole-2,5-dione **(1)**

This compound was synthesized according to a reported procedure with slight modification[1]. 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 separated by column chromatography on silica gel (EtOAc / hexane = 1:3) to give the product (4.72 g, 92%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.82 (t, J = 5.3 Hz, 2H), 3.71 – 3.65 (m, 4H), 3.57 (dd, J = 5.2, 3.8 Hz, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 163.2, 133.5, 72.3, 68.0, 61.9, 39.0. HRMS (ESI), m/z calcd. for $C_8H_9Cl_2NO_4$ $[M + Na]^+$: 275.9806; found 275.9809.



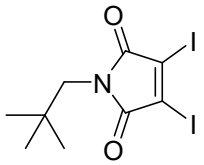
1-(2-(2-Hydroxyethoxy)ethyl)-3,4-diiodo-1H-pyrrole-2,5-dione **(2)**

This compound was synthesized following the procedure described in our previous work[2]. Briefly, a mixture of sodium iodide (8.35 g, 55.74 mmol) and **1** (4.72 g, 18.57 mmol) in acetonitrile (70 mL) was heated at 85 °C for 24 h. After removal of the solvent, the residue was purified by column chromatography on silica gel (EtOAc / hexane = 1:2) to give the product (7.0 g, 86%). ^{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.



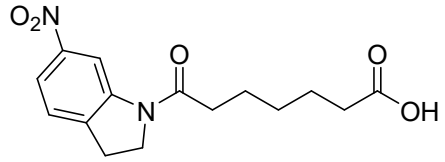
3,4-Dichloro-1-neopentyl-1H-pyrrole-2,5-dione **(3)**

This compound was synthesized following the procedure described in our previous work[2]. Briefly, dichloromaleic anhydride (7.95 g, 47.9 mmol) was dissolved in acetic acid (50 mL) with slow addition of neopentylamine (3.80 g, 43.55 mmol) at 0 °C, then the solution was heated at 120 °C for 18 h. After removal of the solvent, the crude residue was separated by column chromatography on silica gel (ethyl acetate / hexane = 1:19) to give the product (8.5 g, 82%).



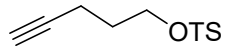
3,4-Diiodo-1-neopentyl-1H-pyrrole-2,5-dione **(4)**

This compound was synthesized following the procedure described in our previous work[2]. Briefly, a mixture of sodium iodide (16.89 g, 112.67 mmol) and **3** (6.65 g, 28.17 mmol) in acetonitrile (80 mL) was heated at 85 °C for 24 h. After removal of the solvent, the residue was purified by column chromatography on silica gel (ethyl acetate / hexane = 1:9) to give the product (10.69 g, 90%). ^{1}H NMR ($CDCl_3$) δ 3.43 (s, 2H), 0.92 (s, 9H).



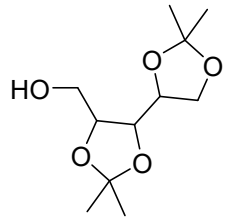
7-(6-Nitroindolin-1-yl)-7-oxoheptanoic acid **(5)**[3]

This compound was synthesized according to a reported procedure with slight modification[3]. Briefly, a mixture of heptaric acid (2 g, 13.6 mmol) in acetic anhydride (35 mL) was heated at 135 °C for 24 h under nitrogen. After removal of the volatile components, 6-nitroindoline (1.692 g, 10.31 mmol) and DCM (15 mL) were added and stirred overnight under nitrogen atmosphere at room temperature. After removal of the solvent, the crude residue was separated by column chromatography on silica gel (CH_3OH / DCM = 1:150) to give the product as a yellow solid (1.8 g, 57%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.98 (s, 1H), 8.80 (d, J = 2.4 Hz, 1H), 7.87 (dd, J = 8.2, 2.3 Hz, 1H), 7.46 (dt, J = 8.1, 1.2 Hz, 1H), 4.19 (t, J = 8.6 Hz, 2H), 3.24 (t, J = 8.6 Hz, 2H), 2.47 (t, J = 7.3 Hz, 2H), 2.22 (t, J = 7.4 Hz, 2H), 1.56 (dp, J = 32.8, 7.4 Hz, 4H), 1.38 – 1.30 (m, 2H).



Pent-4-yn-1-yl 4-methylbenzenesulfonate **(6)**[4]

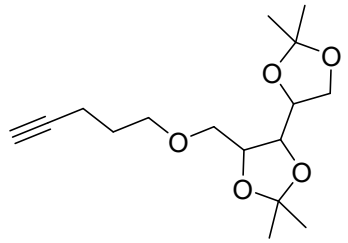
p-Toluenesulfonyl chloride (2.73 g, 14.3 mmol) was dissolved in dry DCM (35 mL) at 0 °C, 4-pentyn-1-ol (1 g, 11.9 mmol) and triethylamine (2.47 mL, 15.8 mmol) were then added dropwise. The mixture was stirred at room temperature for 20 h. After removal of the solvent, the residue was diluted with ether (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).



(2,2,2',2'-Tetramethyl-[4,4'-bi(1,3-dioxolan)]-5-yl)methanol **(7)**[5]

This compound was synthesized according to a reported procedure with slight modification[6]. To a suspension of xylitol (20 g, 131.45 mmol) in dry acetone (300 mL)

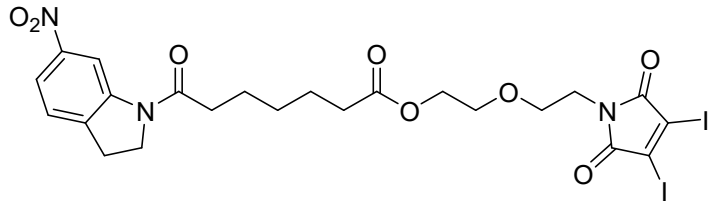
was added anhydrous copper sulfate (40 g, 250.6 mmol). After stirring at room temperature for 15 min, concentrated sulfuric acid (0.5 mL) was added dropwise. After the mixture was stirred at 40 °C for 3 days, it was filtered and the organic phase was collected. Calcium hydroxide (10 g, 134.97 mmol) was added to the filtrate, stirred at room temperature for 1 h. After removal of the volatile components, the residue was washed three times with saturated saline water and ethyl acetate, and the organic phase was dried and filtered and then the solvent was removed. Hexane (1000 mL) was added for recrystallization at -40 ° C. The mixture was filtered to give the product as a white solid (18.05 g, 59%).^{1}H NMR ($CDCl_3$) δ 4.23-3.96 (m, 4H), 3.90-3.77 (m, 2H), 3.67-3.58 (m, 1H), 1.46- 1.40 (m, 9H), 1.37 (m, 3H).



2,2,2',2'-Tetramethyl-5-((pent-4-yn-1-yloxy)methyl)-4,4'-bi(1,3-dioxolane) (**8**)

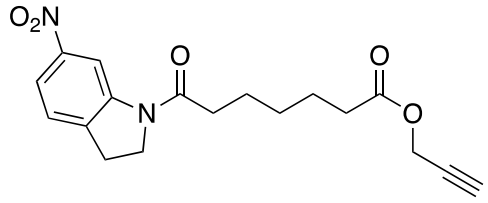
At 0 °C, compound 7 (0.581 g, 2.5 mmol) was dissolved in dry THF (15 mL) under a nitrogen atmosphere. Sodium hydride (NaH, 0.240 g, 10 mmol) was carefully added portionwise. *Caution: Sodium hydride is highly reactive, flammable, and may ignite upon contact with moisture. All operations involving NaH were carried out under an inert atmosphere with appropriate safety precautions in a well-ventilated fume hood.* The reaction mixture was stirred at room temperature for 1 h, after which compound 6 (1.192 g, 5 mmol) was added dropwise. The mixture was then heated to 50 °C and stirred for 36 h. The reaction was cautiously quenched by the slow addition of methanol (14 mL), followed by water (1 mL). The resulting mixture was stirred at 50 °C for an additional 24 h to ensure complete hydrolysis of residual compound 6. After removal of volatile components under reduced pressure, the residue was extracted with ethyl acetate and washed with water (3 ×). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated.The crude product was purified by column chromatography on silica gel (EtOAc/hexane = 1:5) to afford compound 8 as a colorless oil (430 mg, 58%). ^{1}H NMR ($CDCl_3$) δ 4.17 (td, 1H), 4.12 (m, 2H), 4.08 – 4.01 (m, 2H), 3.92 – 3.82 (m, 2H), 3.59 – 3.55 (m, 3H), 2.44 (s, 1H), 2.28 (td, J = 7.0, 2.7 Hz, 2H), 1.82 – 1.77 (m, 2H), 1.44 –1.37 (m, 12H). ^{13}C NMR ($CDCl_3$) δ 109.9, 109.8, 83.9,

78.6, 76.6, 75.8, 71.7, 70.2, 68.7, 65.8, 28.5, 27.2, 27.1, 26.4, 25.6, 15.3. HRMS (ESI), m/z calcd. for $C_{16}H_{26}O_5$ [M + Na]$^+$: 321.1672; found 321.1679.



2-(2-(3,4-Diiodo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethyl 7-(6-nitroindolin-1-yl)-7-oxoheptanoate **(9)**

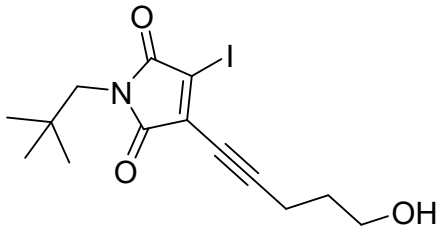
Compound **5** (2.22 g, 7.24 mmol) and DMAP (884 mg, 7.24 mmol) were added to dry DCM (10 mL). The solution was cooled down to 0 °C, and a solution of N,N'-dicyclohexyl carbodiimide (DCC, 1.5 g, 7.24 mmol) in DCM (5 mL) was added dropwise. After the mixture was stirred at 0 °C for 30 min, the solution of compound **2** (1.58 g, 3.62 mmol) in DCM (10 mL) was added dropwise. After the mixture was stirred at room temperature for 15 h, it was filtered and the organic phase was collected. The solvent was removed and the residue was separated and purified by column chromatography on silica gel (EtOAc / hexane/ DCM = 1:1:1) to give the product as a light yellow solid (1.07 g, 41%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.82 (d, J = 2.3 Hz, 1H), 7.88 (dd, J = 8.2, 2.3 Hz, 1H), 7.47 (d, J = 8.2 Hz, 1H), 4.20 (t, J = 8.6 Hz, 2H), 4.08 – 3.99 (m, 2H), 3.62 (t, J = 5.7 Hz, 2H), 3.57 – 3.54 (m, 2H), 3.52 (t, J = 5.7 Hz, 2H), 3.26 (t, J = 8.6 Hz, 2H), 2.48 (t, J = 7.3 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 1.58 (m, 4H), 1.39 – 1.31 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.8, 171.9, 167.1, 146.9, 144.0, 140.4, 125.3, 119.1, 118.6, 109.8, 67.8, 66.6, 62.7, 48.1, 34.6, 33.4, 28.1, 27.6, 24.3, 23.4. HRMS (ESI), m/z calcd. for $C_{23}H_{25}I_2N_3O_8Na$ [M + Na]$^+$: 747.9619, found 747.9630.



Prop-2-yn-1-yl 7-(6-nitroindolin-1-yl)-7-oxoheptanoate **(10)**

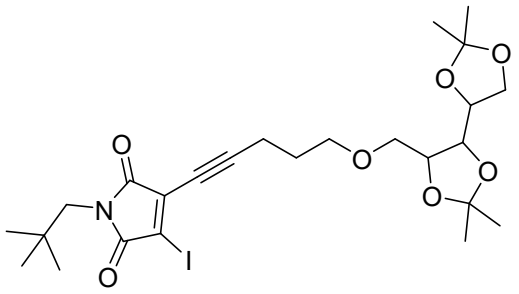
A mixture of compound **5** (2 g, 6.52 mmol), potassium carbonate (9 g, 65.12 mmol), and 3-bromoprop-1-yne (932 mg, 7.84 mmol) was dissolved in DMF (40 mL) and stirred at room temperature overnight. The reaction mixture was then filtered. The filtrate was sequentially washed three times with 1 M hydrochloric acid, 1 M sodium

bicarbonate solution, and saturated brine. The organic layer was collected, dried, and concentrated. The residue was separated and purified by column chromatography on silica gel (EtOAc / hexane = 1:1) to give the product as a colorless oil (984 mg, 46%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (d, J = 2.3 Hz, 1H), 7.88 (dd, J = 8.2, 2.3 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 4.68 (d, J = 2.5 Hz, 2H), 4.19 (t, J = 8.5 Hz, 2H), 3.52 (t, J = 2.4 Hz, 1H), 3.25 (t, J = 8.8 Hz, 2H), 2.46 (m, 2H), 2.33 (m, 2H), 1.59 (m, 4H), 1.36 (m, 2H).



3-(5-Hydroxypent-1-yn-1-yl)-4-iodo-1-neopentyl-1H-pyrrole-2,5-dione **(11)**

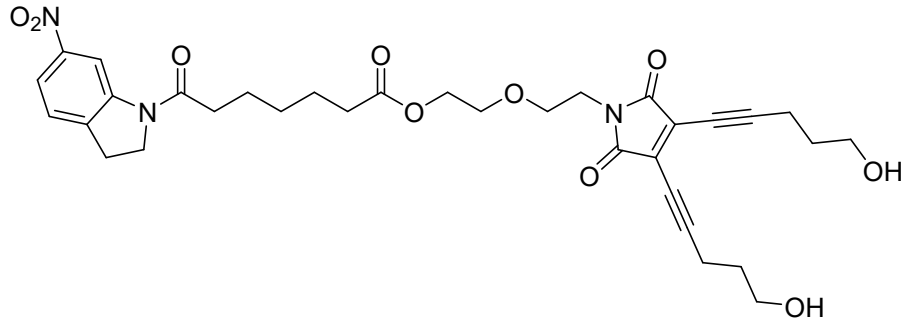
Under a nitrogen atmosphere, compound **4** (1.508 g, 3.6 mmol), NHC-Pd catalyst (163 mg, 0.24 mmol), CuI (183 mg, 0.96 mmol), and N,N-diisopropylethylamine (1.25 mL, 7.2 mmol) were successively added into a mixture of dry THF (8 mL) and toluene (16 mL). 4-Pentyn-1-ol (202 mg, 2.4 mmol) in THF (0.6 mL) was then added dropwise. The mixture was stirred at room temperature and monitored with TLC. After complete consumption of alkyne, the mixture was purified through column chromatograph on silica gel to give the desired compound. The residue was separated and purified by column chromatography on silica gel (EtOAc / hexane = 1:1) to give the product as a colorless oil (372 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.83 (t, J = 6.0 Hz, 2H), 3.37 (s, 2H), 2.67 (t, J = 7.0 Hz, 2H), 1.97 – 1.86 (m, 2H), 0.91 (s, 9H).



3-Iodo-1-neopentyl-4-(5-(2-(2,2,2',2'-tetramethyl-[4,4'-bi(1,3-dioxolan)]-5-yl)ethoxy)pent-1-yn-1-yl)-1H-pyrrole-2,5-dione **(12)**

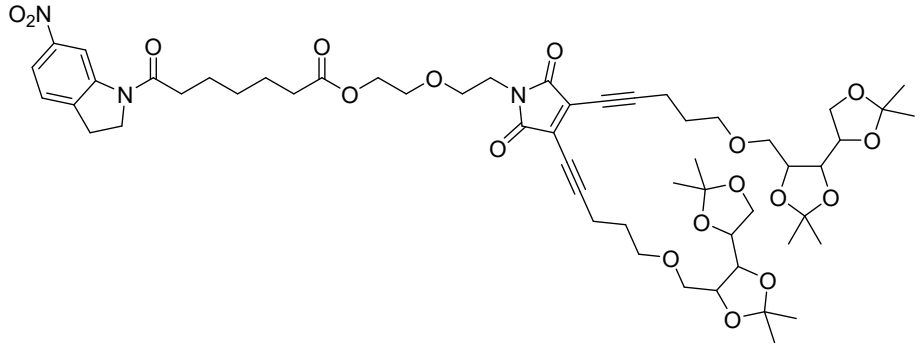
Under a nitrogen atmosphere, compound **4** (1.256 g, 3 mmol), NHC-Pd catalyst (136 mg, 0.2 mmol), CuI (152.4 mg, 0.8 mmol), and N,N-diisopropylethylamine (1.04 mL, 6 mmol) were successively added into a mixture of dry THF (8 mL) and toluene

(16 mL). The terminal alkyne (compound **8**) in THF (0.6 mL) was then added dropwise. The mixture was stirred at room temperature and monitored with TLC. After complete consumption of alkyne, the mixture was purified through column chromatograph on silica gel to give the desired compound. The residue was separated and purified by column chromatography on silica gel (EtOAc / hexane = 1:4) to give the product as a yellow oil (462 mg, 39%).^{1}H NMR (600 MHz, $CDCl_3$) δ 4.23 – 4.13 (m, 1H), 4.09 (m, 1H), 4.03 (m, 1H), 3.90 (m, 1H), 3.86 (m, 1H), 3.74 (m, 2H), 3.66 (t, J = 6.1 Hz, 2H), 3.63 – 3.57 (m, 2H), 3.37 (s, 2H), 2.64 (t, J = 7.0 Hz, 2H), 1.94 (m, 2H), 1.42 (s, 9H), 1.37 (s, 3H), 0.91 (s, 9H).



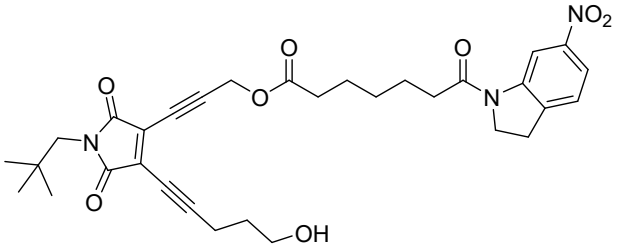
2-(2-(3,4-Bis(5-hydroxypent-1-yn-1-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethyl 7-(6-nitroindolin-1-yl)-7-oxoheptanoate **(EDY-1)**

Prepared from compound **9** and 4-pentyn-1-ol according to the **General Procedure A**, isolated in 34% yield as a yellow solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.80 (d, 1H), 7.87 (dd, 1H), 7.61 – 7.26 (m, 1H), 4.63 – 4.43 (m, 2H), 4.18 (t, *J* = 8.5 Hz, 2H), 4.06 – 4.00 (m, 1H), 3.97 (t, 2H), 3.89 – 3.84 (m, 1H), 3.54 – 3.42 (m, 6H), 2.66 – 2.60 (m, 2H), 2.46 (s, 4H), 2.28 – 2.23 (m, 2H), 2.04 – 1.95 (m, 2H), 1.85 – 1.72 (m, 2H), 1.69 (m, 2H), 1.63 – 1.51 (m, 4H), 1.34 (s, 2H), 1.20 – 1.14 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 173.0, 172.1, 166.9, 147.0, 144.1, 140.6, 128.1, 125.5, 118.8, 111.7, 109.9, 71.8, 68.0, 66.7, 62.9, 59.3, 55.1, 48.2, 34.8, 33.5, 31.1, 28.2, 27.7, 24.5, 23.6, 16.4. HRMS (ESI), m/z calcd. for $C_{33}H_{39}N_3O_{10}$ [M-H]$^{-}$: 636.2557, found 636.2556.



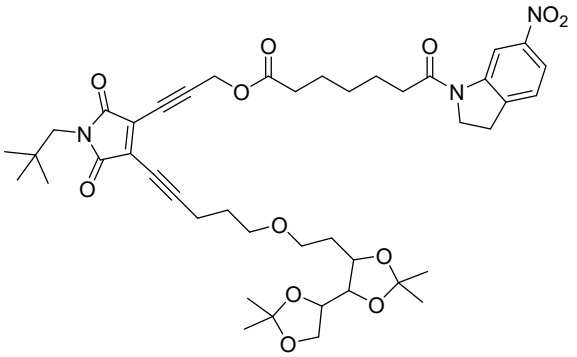
2-(2-(2,5-Dioxo-3,4-bis(5-(2-(2,2,2',2'-tetramethyl-[4,4'-bi(1,3-dioxolan)]-5-yl)ethoxy)pent-1-yn-1-yl)-2,5-dihydro-1H-pyrrol-1-yl)ethoxy)ethyl 7-(6-nitroindolin-1-yl)-7-oxoheptanoate **(EDY-2)**

Prepared from compounds **8** and **9** according to the **General Procedure A**, isolated in 54% yield as a yellow solid. ^{1}H NMR (600 MHz, DMSO) δ 7.89 (dd, *J* = 8.1, 1.9 Hz, 1H), 7.77 (dd, *J* = 8.3, 3.4 Hz, 1H), 7.51 – 7.40 (m, 1H), 4.19 (m, 2H), 4.16 – 4.09 (m, 4H), 4.09 – 4.03 (m, 2H), 3.99 (m, 4H), 3.81 (m, 2H), 3.69 (m, 2H), 3.61 – 3.50 (m, 6H), 3.49 (m, 1H), 3.25 (m, 3H), 2.68 – 2.59 (m, 4H), 2.49 – 2.43 (m, 2H), 2.37 (m, 2H), 2.28 (m, 2H), 1.86 (m, 2H), 1.78 (m, 2H), 1.64 – 1.51 (m, 4H), 1.38 – 1.21 (m, 24H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.8, 171.9, 167.2, 165.7, 146.9, 144.9, 144.0, 140.4, 132.2, 130.1, 127.6, 125.3, 118.6, 111.2, 110.9, 109.8, 108.7, 108.6, 77.3, 76.1, 74.7, 70.8, 68.9, 67.9, 66.5, 65.0, 62.7, 54.9, 33.3, 28.1, 27.7, 27.5, 26.9, 26.8, 26.0, 25.4, 24.3, 23.4, 21.0, 16.4. HRMS (ESI), m/z calcd. for $C_{55}H_{75}N_3O_{18}Na$ $[M + Na]^+$: 1088.4943 found 1088.4945.



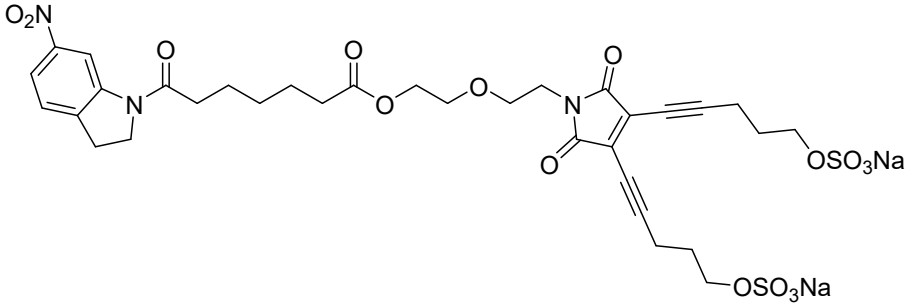
3-(4-(5-Hydroxypent-1-yn-1-yl)-1-neopentyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)prop-2-yn-1-yl 7-(6-nitroindolin-1-yl)-7-oxoheptanoate (**EDY-3**)

Prepared from compounds **11** and 4-pentyn-1-ol according to **General Procedure A**, isolated in 19% yield as a yellow-brown viscous oil. ^{1}H NMR (600 MHz, DMSO) δ 8.83 (s, 1H), 7.89 (dd, J = 8.2, 2.3 Hz, 1H), 7.47 (d, J = 8.1 Hz, 1H), 5.08 (s, 2H), 4.58 (t, J = 5.2 Hz, 1H), 4.19 (t, J = 8.6 Hz, 4H), 3.53 – 3.49 (m, 2H), 3.25 (t, J = 8.6 Hz, 2H), 3.18 (s, 2H), 2.65 (t, J = 7.1 Hz, 2H), 2.48 (m, 2H), 2.41 (t, J = 7.4 Hz, 2H), 1.73 – 1.67 (m, 2H), 1.61 (m, 4H), 1.41 – 1.32 (m, 2H), 0.83 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.3, 172.0, 167.4, 167.2, 147.0, 144.1, 140.5, 129.9, 126.2, 125.5, 118.8, 113.1, 109.9, 102.4, 59.9, 59.3, 52.2, 49.8, 48.2, 34.7, 33.2, 31.0, 28.1, 27.7, 24.4, 23.5, 20.9, 16.5, 14.2.



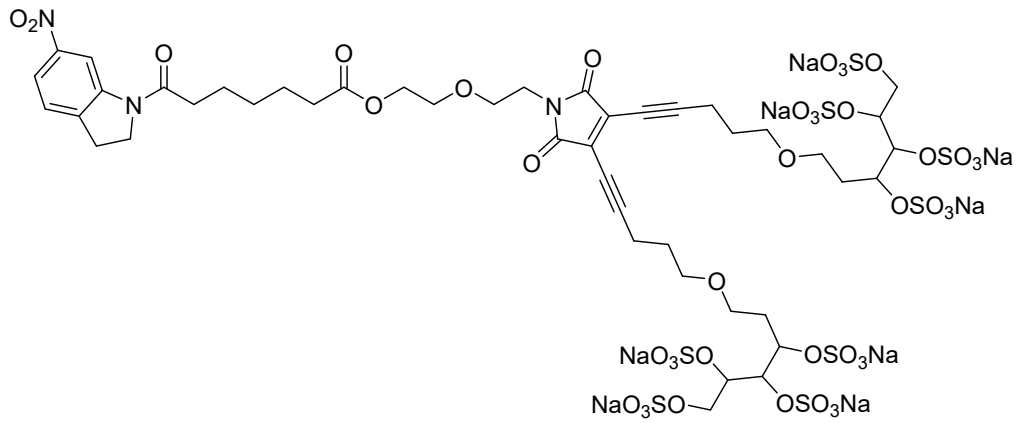
3-(1-Neopentyl-2,5-dioxo-4-(5-(2-(2,2,2',2'-tetramethyl-[4,4'-bi(1,3-dioxolan)]-5-yl)ethoxy)pent-1-yn-1-yl)-2,5-dihydro-1H-pyrrol-3-yl)prop-2-yn-1-yl 7-(6-nitroindolin-1-yl)-7-oxoheptanoate (**EDY-4**)

Prepared from compounds **10** and **12** according to **General Procedure A**, isolated in 36% yield as a yellow-brown viscous oil. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.82 (d, *J* = 2.3 Hz, 1H), 7.89 (dd, *J* = 8.2, 2.3 Hz, 1H), 7.47 (dd, *J* = 8.3, 1.2 Hz, 1H), 5.08 (s, 2H), 4.19 (t, *J* = 8.6 Hz, 2H), 4.12 (m, 1H), 4.01 – 3.97 (m, 2H), 3.81 (m, 1H), 3.69 (m, 1H), 3.56 (t, *J* = 6.1 Hz, 2H), 3.54 – 3.47 (m, 2H), 3.25 (t, *J* = 8.6 Hz, 2H), 3.18 (s, 2H), 2.67 (t, *J* = 7.0 Hz, 2H), 2.48 (t, *J* = 7.2 Hz, 2H), 2.41 (t, *J* = 7.4 Hz, 2H), 1.80 (m, 2H), 1.61 (m, 4H), 1.43 – 1.34 (m, 2H), 1.32 – 1.29 (m, 9H), 1.27 (s, 3H), 0.83 (s, 9H).



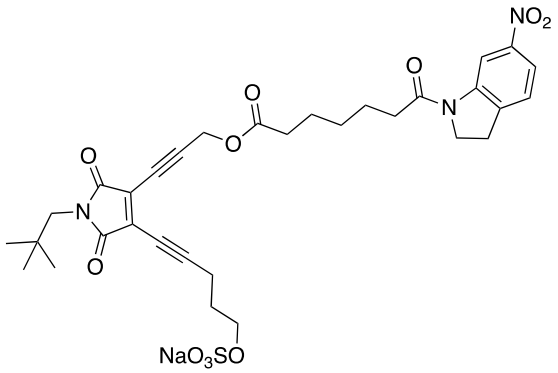
Sodium (1-(2-(2-((7-(6-nitroindolin-1-yl)-7-oxoheptanoyl)oxy)ethoxy)ethyl)-2,5-dioxo-2,5-dihydro-1H-pyrrole-3,4-diyl)bis(pent-4-yne-5,1-diyl) bis(sulfate) (**PROTEC-a**)

Prepared from **EDY-1** according to the **General Procedure C**, isolated in 46% yield after precipitation in diethyl ether as a yellow solid. ^{1}H NMR (600 MHz, D_2O) δ 8.51 (s, 1H), 7.95 – 7.85 (m, 1H), 7.43 – 7.37 (m, 1H), 4.31 – 4.11 (m, 6H), 4.10 – 3.95 (m, 4H), 3.50 (t, *J* = 6.9 Hz, 2H), 3.23 (m, 2H), 2.71 (t, *J* = 6.9 Hz, 2H), 2.53 (m, 2H), 2.47 – 2.24 (m, 2H), 2.01 (m, 4H), 1.86 (m, 4H), 1.77 – 1.57 (m, 4H), 1.54 – 1.41 (m, 2H). HRMS (ESI), m/z calcd. for $C_{33}H_{37}N_3NaO_{16}S_2$ [M-Na]$^-$: 818.1513 found 818.1514.



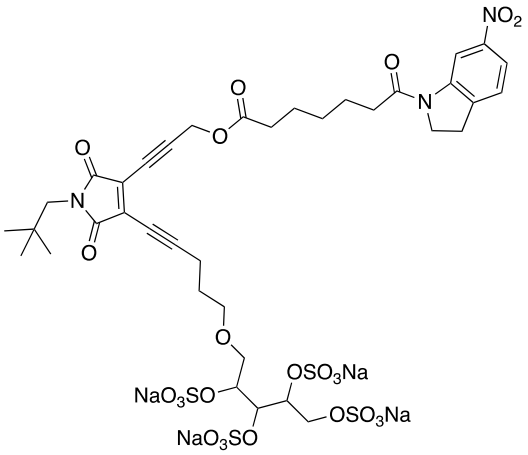
Sodium 6-((5-(1-(2-(2-((7-(6-nitroindolin-1-yl)-7-oxoheptanoyl)oxy)ethoxy)ethyl)-2,5-dioxo-4-(5-((3,4,5,6-tetrakis(sulfonatooxy)hexyl)oxy)pent-1-yn-1-yl)-2,5-dihydro-1H-pyrrol-3-yl)pent-4-yn-1-yl)oxy)hexane-1,2,3,4-tetrayl tetrakis(sulfate) (**PROTEC-b**)

Starting from **EDY-2**, hydrolyzed according to the **General Procedure B**, hypersulfated according to the **General Procedure C**, isolated as a yellow solid (quantitative). ^{1}H NMR (600 MHz, D_2O) δ 8.80 (s, 1H), 8.02 (s, 1H), 7.50 (s, 1H), 5.00 (s, 2H), 4.55 – 4.34 (m, 4H), 4.34 – 4.03 (m, 6H), 4.02 – 3.43 (m, 6H), 3.43 – 3.18 (m, 2H), 2.71 – 2.20 (m, 6H), 2.01 – 1.54 (m, 14H), 1.44 (m, 8H).



Sodium 5-(1-neopentyl-4-(5-((7-(6-nitroindolin-1-yl)-7-oxoheptanoyl)oxy)pent-1-yn-1-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)pent-4-yn-1-yl sulfate (**PROTEC-c**)

Prepared from **EDY-3** according to the **General Procedure C**, isolated as a yellow solid (quantitative). ^{1}H NMR (600 MHz, D_2O) δ 8.49 (s, 1H), 7.62 (s, 1H), 7.18 (s, 1H), 4.53 – 3.56 (m, 6H), 3.53 – 2.59 (m, 6H), 2.25 (m, 4H), 1.98 (m, 2H), 1.43 (m, 6H), 0.86 (s, 9H). HRMS (ESI), m/z calcd. for $C_{32}H_{36}N_3O_{11}S$ $[M\text{-}Na]^-$: 670.2071 found: 670.2070.



Sodium 5-((5-(1-neopentyl-4-(5-((7-(6-nitroindolin-1-yl)-7-oxoheptanoyl)oxy)pent-1-yn-1-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)pent-4-yn-1-yl)oxy)pentane-1,2,3,4-tetrayl tetrakis(sulfate) (**PROTEC-d**)

Starting from **EDY-4**, hydrolyzed according to the **General Procedure B**, hypersulfated according to the **General Procedure C**, isolated as a yellow solid (quantitative). ^{1}H NMR (600 MHz, D_2O) δ 8.50 (s, 1H), 7.65 (s, 1H), 7.19 (s, 1H), 5.00 (s, 2H), 4.56 – 3.97 (m, 6H), 3.97 – 3.46 (m, 6H), 3.44 – 2.94 (m, 5H), 2.59 – 2.08 (m, 6H), 2.08 – 1.71 (m, 4H), 1.70 – 1.50 (m, 4H), 1.50 – 1.10 (m, 6H), 0.82 (s, 9H).

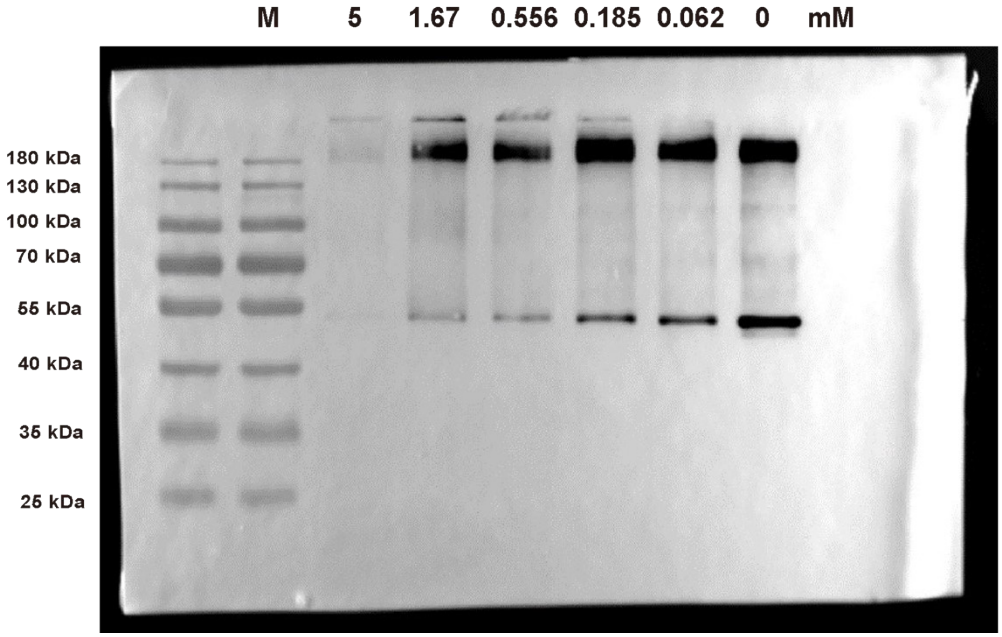


Figure S1. Western blot analysis of the SARS-CoV-2 spike protein after incubation with PROTEC-a at different concentrations. Notably, nucleocapsid protein degradation was also observed, which can be attributed to electrostatic interactions between the

positively charged nucleocapsid protein and PROTEC-a, enabling close proximity and subsequent radical-mediated cleavage.

Table S1. Experimental setup for virus–PROTEC mixtures in plaque assays.

Virus	PROTEC-a (Concentration in 1000 μL)	Working Concentration (PROTEC-a)	No.
1000 μL	2 mM	1 mM	1
1000 μL	200 μM	100 μM	2
1000 μL	20 μM	10 μM	3
1000 μL	2 μM	1 μM	4
1000 μL	200 nM	100 nM	5
1000 μL	20 nM	10 nM	6
1000 μL	0 nM (PBS) 1000 μL	0 nM	7

References:

1. S. V. Shelar and N. P. Argade, *Org. Biomol. Chem.*, 2021, **19**, 6160-6169.
2. M. Zhang, B. Li, H. Chen, H. Lu, H. Ma, X. Cheng, W. Wang, Y. Wang, Y. Ding and A. Hu, *J. Org. Chem.*, 2020, **85**, 9808-9819.
3. Q. Wang, F. H. Meng, Y. T. Xie, W. Wang, Y. M. Meng, L. J. Li, T. Liu, J. X. Qi, X. D. Ni, S. D. Zheng, J. H. Huang and N. Huang, *ACS Cent. Sci.*, 2023, **9**, 252–265.
4. K. Sun, Z. Ding, X. Jia, H. Cheng, Y. Li, Y. Wu, Z. Li, X. Huang, F. Pu, E. Li, G. Wang, W. Wang, Y. Ding, G. Wong, S. Chiu, J. Lan and A. Hu, *CCS Chem.*, 2024, **6**, 487-496.
5. P. Garg, S. Pandey, H. Seonwoo, S. Yeom, Y. H. Choung, C. S. Cho, P. H. Choung and J. Hoon Chung, *Chem. Commun.*, 2015, **51**, 3645-3648.
6. R. Zhang, X. Feng, R. Zhang, W. Shan, Z. Su, J. Mao, C. Wesdemiotis, J. Huang, X.-Y. Yan, T. Liu, T. Li, M. Huang, Z. Lin, A.-C. Shi and S. Z. D. Cheng, *Angew. Chem., Int. Ed.*, 2019, **58**, 11879-11885.