*Supporting information*

# Self-assembled D-arginine derivates based on click chemical reaction for intracellular codelivery of antigen and adjuvant for potential immunotherapy

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## 1. Methods

### 1.1. Process of the synthesis of compound DR1

To a solution of D-arginine (180 mg, 1.04 mmol) in $H_2O$ was added p-nitrophenyl chloroformate-modified DBCO (200 mg, 0.52 mmol) and $Et_3N$ (288 μL, 2.08 mmol) in THF. The reaction solution was stirred at r. t. for overnight and then concentrated under reduced pressure. The residue was dissolved in EtOAc and washed with aq. HCl (pH = 2), sat. aq. NaCl, dried over $NaSO_4$ and concentrated in vacuo. Purification by flash column chromatography (EtOAc/$CH_3OH$, V/V = 3:1) afforded the compound as a yellow oil.

$^1$H-NMR (400 MHz, $CD_3OD$): δ 1.54-2.09 (m, 4H), 2.74-2.90 (dd, 1H), 3.13-3.31 (m, 3H), 4.04-4.25 (t, 1H), 5.34-5.48 (s, 1H), 7.20-7.69 (m, 8H). $^{13}$C-NMR (100 MHz, $CD_3OD$): δ 24.99, 29.88, 40.57, 45.55, 53.47, 76.68, 109.50, 112.49, 120.85, 123.54, 123.89, 125.49, 126.85, 126.96, 127.89, 129.63, 129.78, 151.01, 152.04, 156.12, 157.26. HR-MS (ESI) m/z: $[M + H]^+$ calcd 421.1831 found 421.1854.

### 1.2. Synthesis of compound 1a

6-aminodecan-1-ol (46.8 mg, 0.4 mmol) and $Et_3N$ (55 μL, 0.4 mmol) were added to a solution of 4-nitrophenyl chloroformate-modified DBCO (100 mg, 0.26 mmol) in $CH_2Cl_2$. The reaction mixture was stirred at room temperature for overnight and concentrated in vacuo. The residue was diluted with EtOAc (30 mL) and then washed with sat. aq. $Na_2CO_3$ (3 × 50 mL). the combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. Purified by flash column chromatography

(petroleum ether/ethyl acetate, V/V = 1:1) afforded the compound as a colorless oil. $^{1}$H-NMR (400 NMR, $CDCl_3$): δ 1.27-1.66 (m, 8H), 2.85-2.96 (dd, 1H), 3.06-3.34 (m, 3H), 3.54-3.70 (t, 2H), 5.40-5.58 (s, 1H), 7.26-7.52 (m, 8H). $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 25.33, 26.42, 29.98, 32.55, 40.96, 46.19, 62.75, 76.69, 109.98, 112.90, 121.29, 123.64, 125.94, 126.24, 127.02, 127.86, 128.02, 129.87, 151.00, 153.76, 155.41. FT-MS (ESI) m/z: $[M + H]^+$ calcd 364.18 found 364.44.

**1.3. Synthesis of compound 1b**

10-aminodecan-1-ol (69.2 mg, 0.4 mmol) and $Et_3N$ (55 μL, 0.4 mmol) were added to a solution of 4-nitrophenyl chloroformate-modified DBCO (100 mg, 0.26 mmol) in $CH_2Cl_2$. The reaction mixture was stirred at rt for 24 h. the subsequent treatment process of compound 1b was the same as that of compound 1a. $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 1.18-1.39 (m, 10H), 1.42-1.59 (m, 4H), 1.78-1.91 (m, 2H), 2.83-2.97 (dd, 1H), 3.10-3.25 (dd, 1H), 3.55-3.66 (t, 2H), 3.67-3.80 (t, 2H), 5.41-5.55 (s, 1H), 7.27-7.56 (m, 8H). $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 25.59, 26.72, 29.22, 29.35, 29.43, 29.47, 29.97, 32.74, 41.12, 46.21, 53.43, 60.40, 62.97, 109.94, 112.88, 121.28, 123.67, 123.80, 125.91, 126.21, 127.00, 127.86, 128.01, 129.88, 151.02, 152.19, 155.39. FT-MS (ESI) m/z: $[M + Na]^+$ cacld 442.25 found 442.49.

**1.4. Synthesis of compound 2a**

Compound 1a (200 mg, 0.54 mmol) was added into $CH_2Cl_2$, $Et_3N$ (88 uL, 0.64 mmol) and DMAP (78 mg, 0.64 mmol) were added. The mixture was stirred at room temperature for 10 min, and then 4-nitrophenyl carbonochloridate (128 mg, 0.64 mmol)

in $CH_2Cl_2$ was gently added dropwise. The resulting solution was allowed to stir at rt overnight. Then the solvent was removed under reduced pressure, and the crude product was purified through column chromatography with petroleum ether/EtOAc (V/V = 6:1) to afford the compound 2a. $^1$H-NMR (400 NMR, $CDCl_3$): δ 1.33-1.88 (m, 8H), 2.80-2.98 (dd, 1H), 3.03-3.33 (m, 3H), 4.16-4.37 (m, 2H), 5.44-5.58 (s, 1H), 7.26-7.56 (m, 10H), 8.19-8.36 (d, 2H). $^{13}$C-NMR (100 MHz, $CDCl_3$)：δ 25.32, 26.26, 28.39, 29.27, 29.90, 46.18, 69.38, 76.69, 109.91, 112.91, 121.32, 121.78, 123.59, 125.29, 125.97, 126.27, 127.05, 127.85, 128.02, 129.85, 145.32, 150.97, 151.61, 152.53, 155.42, 155.52. FT-MS (ESI) m/z: $[M + K]^+$ calcd 567.19 found 567.63.

**1.5. Synthesis of compound 2b**

Compound 1b (200 mg, 0.34 mmol) was added into $CH_2Cl_2$ in a round-bottom flask, $Et_3N$ (56.54 uL, 0.408 mmol) and DMAP (49.76 mg, 0.408 mmol) were added. Then the mixture was gently added dropwise a solution of 4-nitrophenyl carbonochloridate (82 mg, 0.408 mmol) in $CH_2Cl_2$ over a period of 30 min. the resulting solution was stirred at room temperature overnight and then concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (petroleum ether/ethyl acetate, V/V = 3:1) to afford compound 2b. $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.19-1.46 (m, 10H), 1.47-1.68 (m, 4H), 1.69-1.80 (m, 2H), 2.84-2.94 (m, 1H), 3.12-3.24 (m, 1H), 4.09-4.39 (m, 4H), 5.42-5.55 (s, 1H), 7.27-7.52 (m, 8H), 8.00-8.10 (d, 2H), 8.19-8.36 (d, 2H). $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 25.60, 26.70, 28.47, 29.12, 29.19, 29.36, 29.42, 29.91, 41.18, 46.10, 52.26, 69.67, 109.88, 112.93, 116.15, 121.27, 121.80, 123.59, 123.76, 125.28, 125.99, 126.32, 127.10, 127.14, 127.92, 128.05, 129.85, 139.72, 145.28,

150.89, 151.89, 152.55, 155.57, 155.83. FT-MS (ESI) m/z: $[M + Na]^+$ cacld 607.25 found 607.46.

**1.6. Process for the synthesis of compound DR2**

To the stirred compound 2a (200 mg, 0.38 mmol) was added D-arginine (132 mg, 0.76 mmol) and $Et_3N$ (300 μL, 1.52 mmol) in THF (20 mL). the mixture was allowed to stir at room temperature for 24 h. the solvent was removed in vacuo, then diluted with EtOAc and washed with aqueous HCl (pH = 2). The EtOAc phase was combined, dried over Na2SO4 and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography ($CH_3OH$/EtOAc, V/V = 1:2) to get the compound DR2. $^1$H-NMR (400 MHz, $CD_3OD$): δ 1.25-1.93 (m, 12H), 2.76-2.89 (dd, 1H), 3.00-3.27 (m, 5H), 3.93-4.18 (m, 3H), 5.36-5.46 (s, 1H), 7.21-7.64 (m, 8H). $^{13}$C-NMR (100 MHz, $CD_3OD$): δ 24.98, 25.16, 26.00, 28.63, 29.34, 40.29, 40.50, 45.74, 53.40, 64.64, 76.46, 109.59, 112.41, 120.97, 123.52, 125.49, 125.76, 126.92, 127.84, 127.93, 129.59, 151.02, 152.28, 156.62, 157.11, 157.63. FT-MS (ESI) m/z: $[M + H]^+$ calcd 564.27 found 564.69.

**1.7. Process for the synthesis of compound DR3**

To a stirred solution of compound 2b (200 mg, 0.34 mmol) was added D-arginine (120 mg, 0.68 mmol) and $Et_3N$ (188 μL, 1.36 mmol) in 10 mL THF/$H_2O$ (V/V = 1:1). The reaction mixture was stirred at room temperature for 24 h. after that, the solvent was concentrated under reduced pressure, then diluted with EtOAc and washed with aqueous HCl (pH = 2). The organic phase was combined, dried over $Na_2SO_4$ and

concentrated in vacuo. The crude product was purified by silica-gel column chromatography ($CH_3OH$/EtOAc, V/V = 1:2) to afford DR3. $^1$H-NMR (400 MHz, $CD_3OD$): δ 1.06-1.96 (m, 20H), 2.71-2.88 (dd, 1H), 3.01-3.27 (m, 5H), 3.93-4.16 (m, 3H), 5.35-5.48 (s, 1H), 7.20-7.63 (m, 8H). $^{13}$C-NMR (100 MHz, $CD_3OD$): δ 24.97, 25.42, 25.51, 26.40, 28.74, 28.94, 29.19, 29.47, 40.39, 40.54, 45.76, 52.56, 64.78, 76.37, 109.59, 112.39, 120.97, 123.52, 125.48, 125.75, 126.85, 127.82, 127.91, 129.59, 151.03, 152.32, 156.60, 157.13, 157.59. FT-MS (ESI) m/z: $[M + H]^+$ calcd 620.34 found 620.58.

**1.8. Synthesis of compound C2**

To a stirred solution of 1, 4, 7-triazonane (200 mg, 1.54 mmol) and $Cs_2CO_3$ (1.74 g, 5.38 mmol) in $CH_3CN$ (40 mL) was added dropwise a solution of 3-azidopropyl-1-triflate (1.25 g, 5.38 mmol) in dried $CH_3CN$. The reaction mixture was allowed to stir at rt for 8 h. then the resulting solution was filtered and concentrated under reduced pressure. The residue was dissolved in $CH_2Cl_2$ and washed with sat. aq. $NaHCO_3$. The $CH_2Cl_2$ phase was combined, dried over anhydrous $Na_2SO_4$, then filtered and concentrated in vacuo. The residue was purified by silica-gel column chromatography on silica with $CH_3OH/CH_2Cl_2$ (V/V = 1:10) to afford compound C2.

**1.9. Synthesis of compound C3[1]**

$Cs_2CO_3$ (1.9 g, 6 mmol) was added to a solution of cyclen (230 mg, 1.34 mmol) in $CH_3CN$. The mixture was added dropwise a solution of 3-azidopropyl-1-triflate (1.38 mg, 6 mmol) in dried $CH_3CN$. The resulting solution was stirred at rt for 8 h, then

filtered and concentrated in vacuo. The residue was dissolved in $CH_2Cl_2$ and washed with sat. aq. $NaHCO_3$. The $CH_2Cl_2$ phase was combined, dried over anhydrous $Na_2SO_4$. Then the solvent was removed and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography (methanol/dichloromethane, V/V = 1:10) to get compound C3. $^1$H-NMR (400 MHz, $CDCl_3$): δ1.60-1.84 (m, 8H), 1.88-2.31 (m, 8H), 2.49-3.08 (m, 16H), 3.45-3.69 (t, 8H). $^{13}$C-NMR (100 MHz, $CDCl_3$): δ21.86, 47.76, 49.15, 51.00. FT-MS (ESI) m/z: $[M + H]^+$ calcd 504.36 found 504.76.

**1.10. Antigen Loading Efficiency**

The equal 10 μg OVA (1 mg/mL) were added into the aqueous solution DR1, DR2 and DR3 (1 mg/mL) at the weight ratio of 1:2 (OVA/DR1-3, w/w, 1:2), respectively. Then, the crosslinker C1-C3 were respectively introduced into the above mixture solution DR1-3/OVA with the corresponding molar ratio (DR1-3/C1, molar/molar, 1:3; DR1-3/C2, molar/molar, 1:3; DR1-3/C3, molar/molar, 1:4). After incubation for 1 h, the supernatant was collected by centrifugation at 8000 rpm for 5 min, then measured for the amount of the unloaded OVA by BCA Protein Assay Kit according to the instruction. The antigen loading efficiency was calculated by the equation: Loading Efficiency = ((total weight of OVA – the weight of unloaded OVA)/the weight of nanoparticles) X 100

**1. 11. Binding with the CpG**

the combinatorial materials including DR1C1, DR2C1 and DR3C1 were screened and optimized for furtherly mixing with adjuvant CpG. The aqueous nanoparticles DR1C1/OVA, DR2C1/OVA and DR3C1/OVA were respectively added with CpG at

the weight ratio (DR1-3/OVA/CpG, w/w/w, 4/1/0.4). after incubation for 1 h, all the complex samples were analyzed by the agarose gel electrophoresis (4% agarose gel).

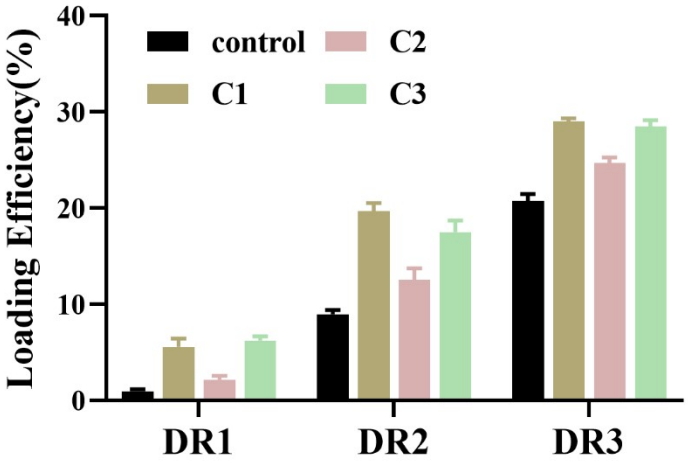


**Fig. S1.** The antigen OVA loading efficiency of the various combinatorial materials of DR1-3 and C1-3.

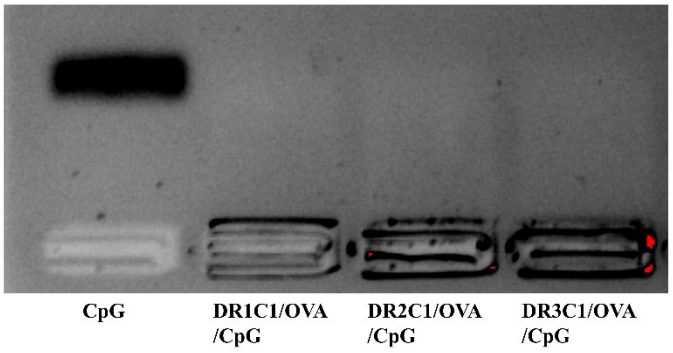


**Fig. S2.** The adjuvant CpG loading capacity of different nanoformualtions including DR1C1/OVA, DR2C1/OVA and DR3C1/OVA.

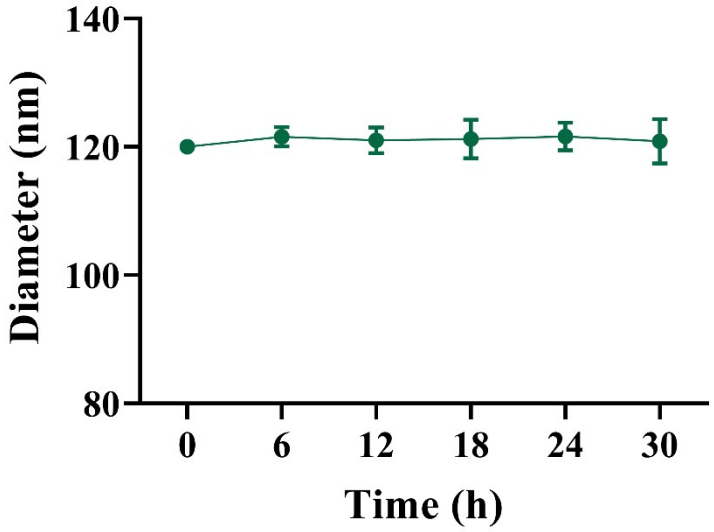


**Fig. S3.** The diameter size of self-assembled DR3C1 was measured for evaluating the stability of nanoparticles aqueous solution within 30 h.

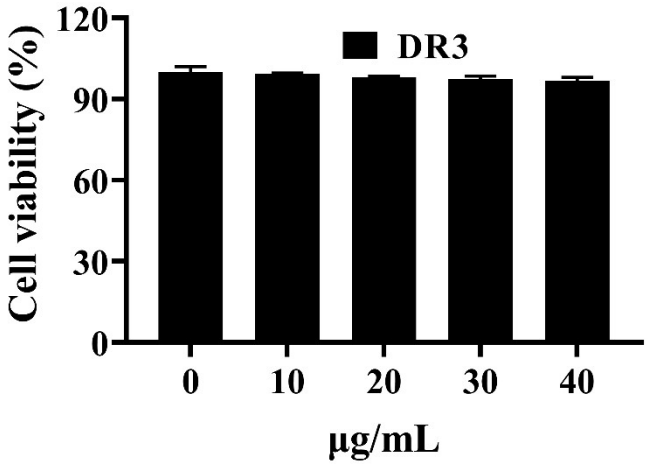


**Fig. S4.** Relative cellular viability of BMDCs after treatment with DR3 at different concentrations including 0 μg/mL, 10 μg/mL, 20 μg/mL, 30 μg/mL, 40 μg/mL.

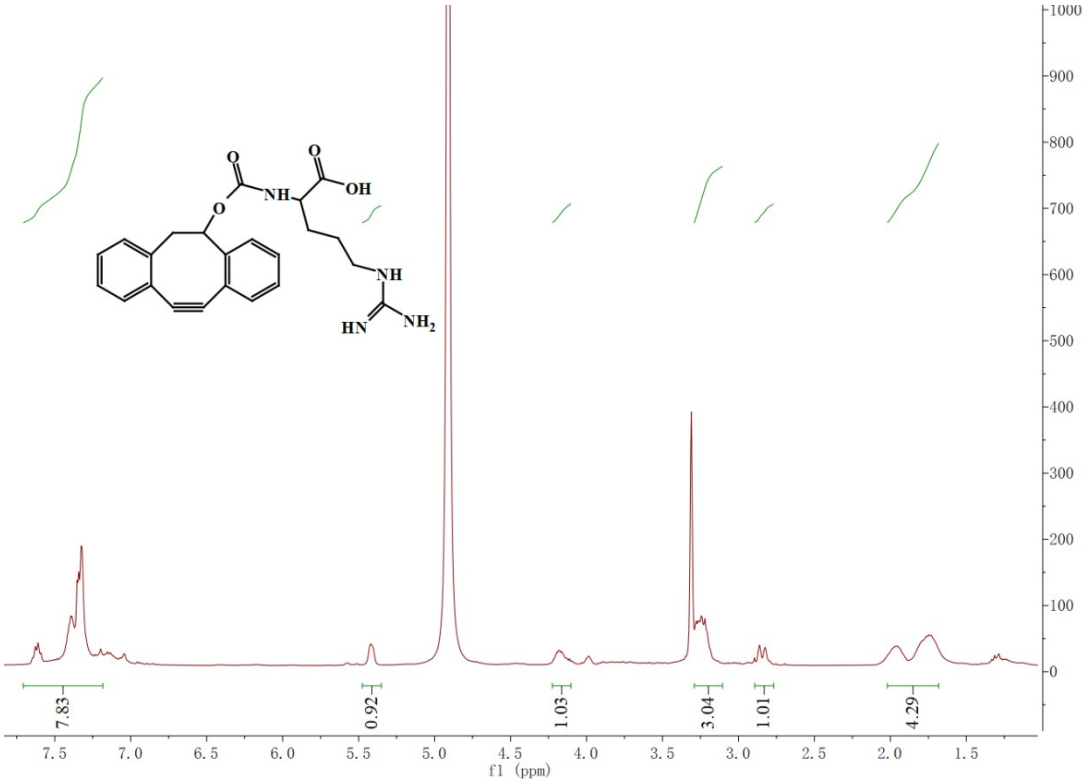


**Fig. S5.** $^{1}$H-NMR spectrum of DR1 (400 MHz, $CD_3OD$)

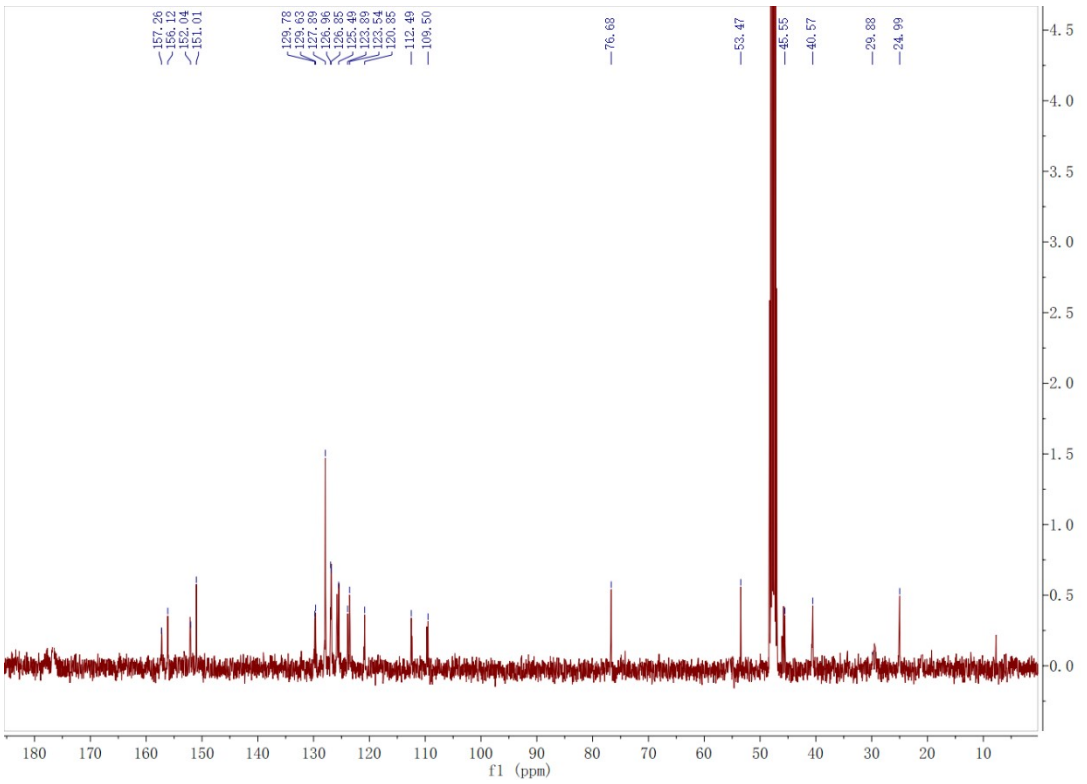


**Fig. S6.** $^{13}$C-NMR spectrum of DR1 (100 MHz, $CD_3OD$)

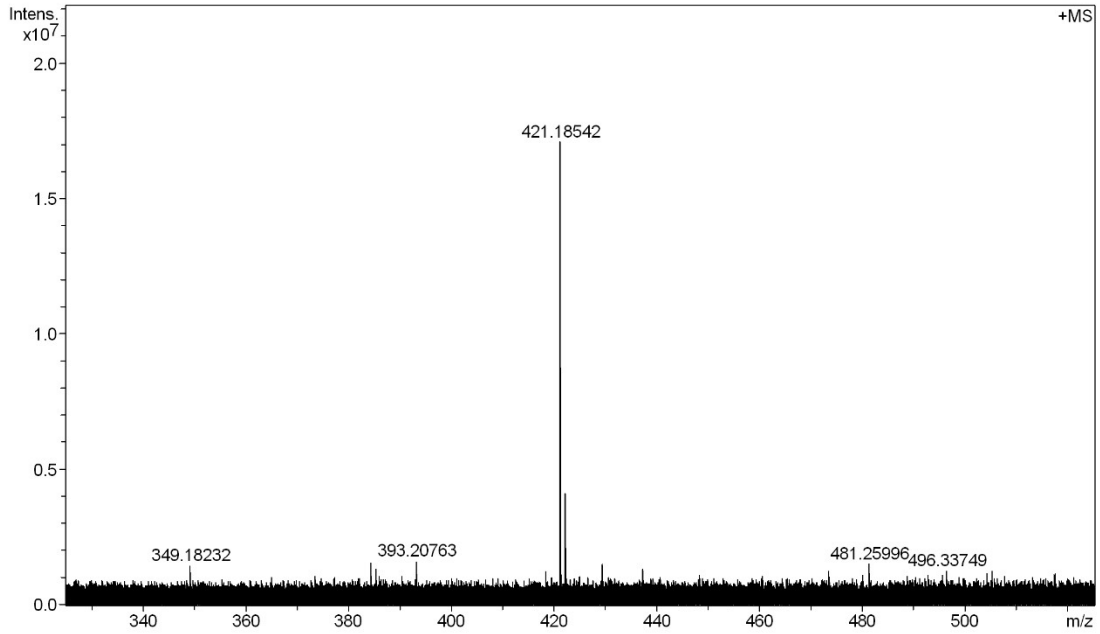


**Fig. S7.** The mass spectrum of DR1.

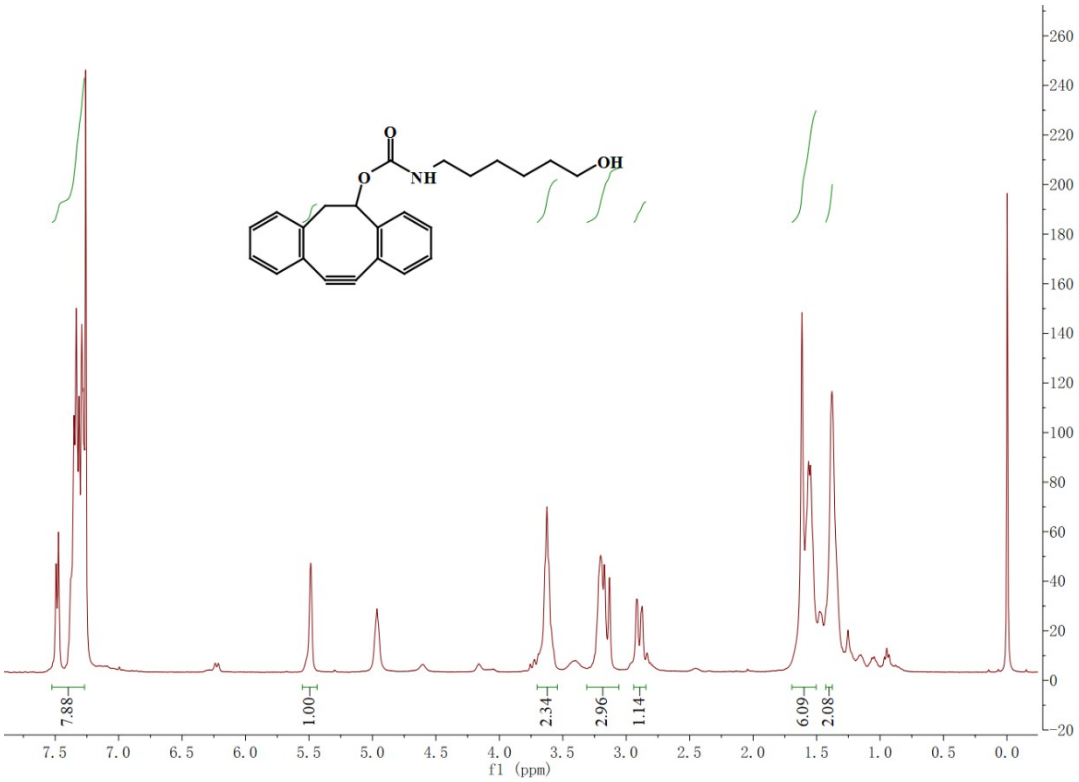


**Fig. S8.** $^{1}$H-NMR spectrum of compound 1a (400 MHz, $CDCl_3$)

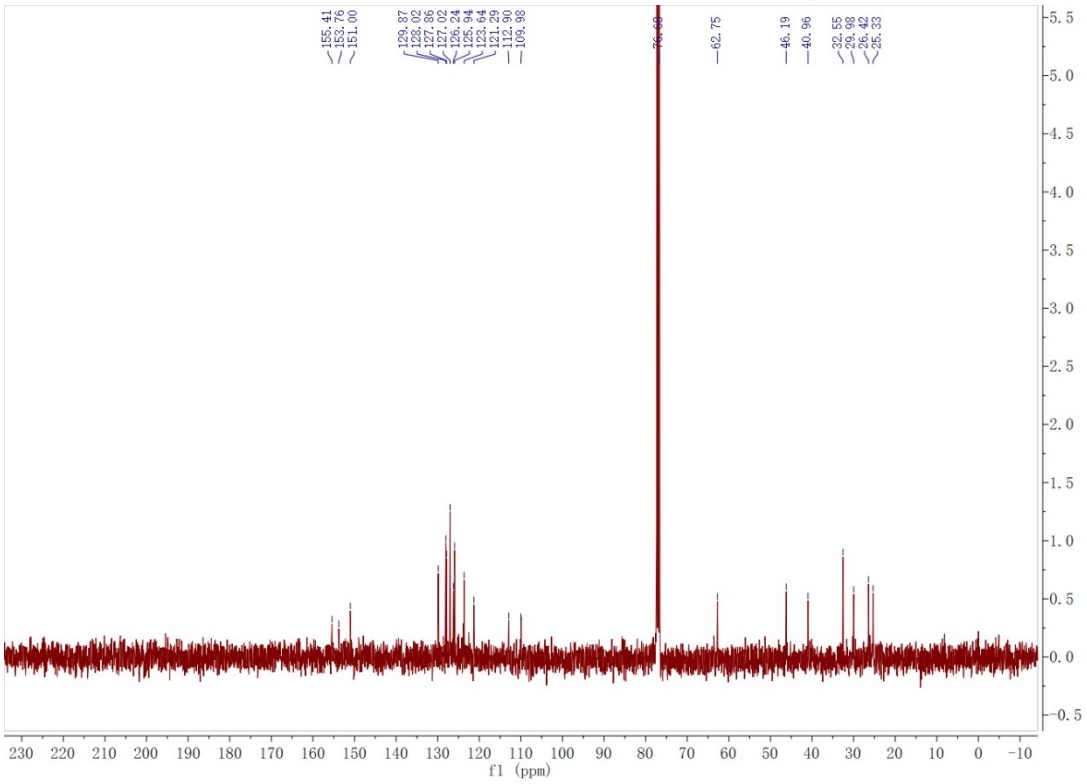


**Fig. S9.** $^{13}$C-NMR spectrum of compound 1a (100 MHz, $CDCl_3$).

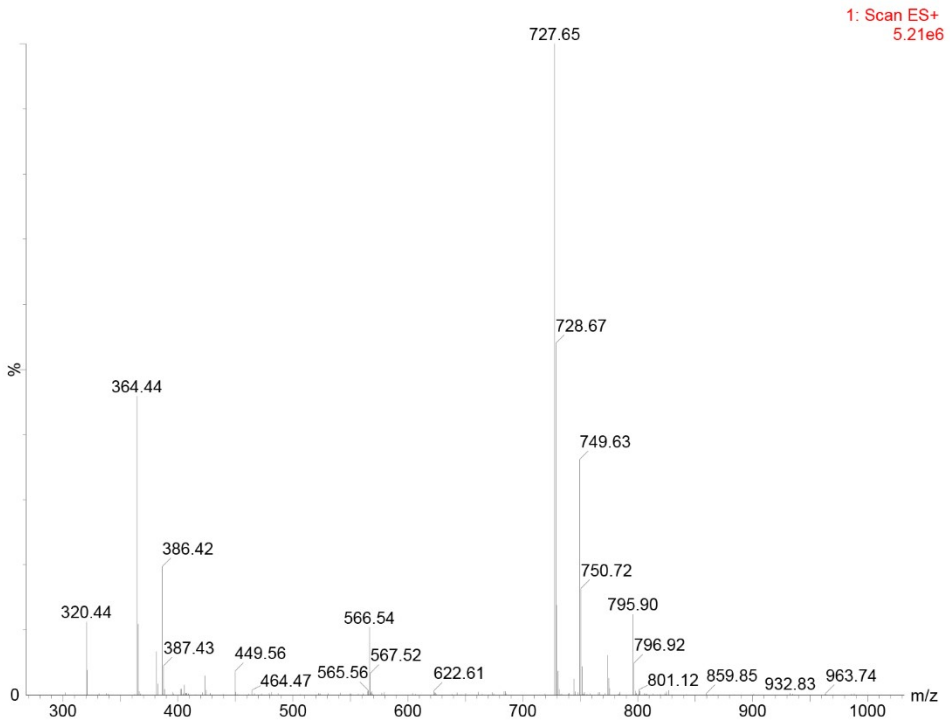


**Fig. S10.** The mass spectrum of compound 1a.

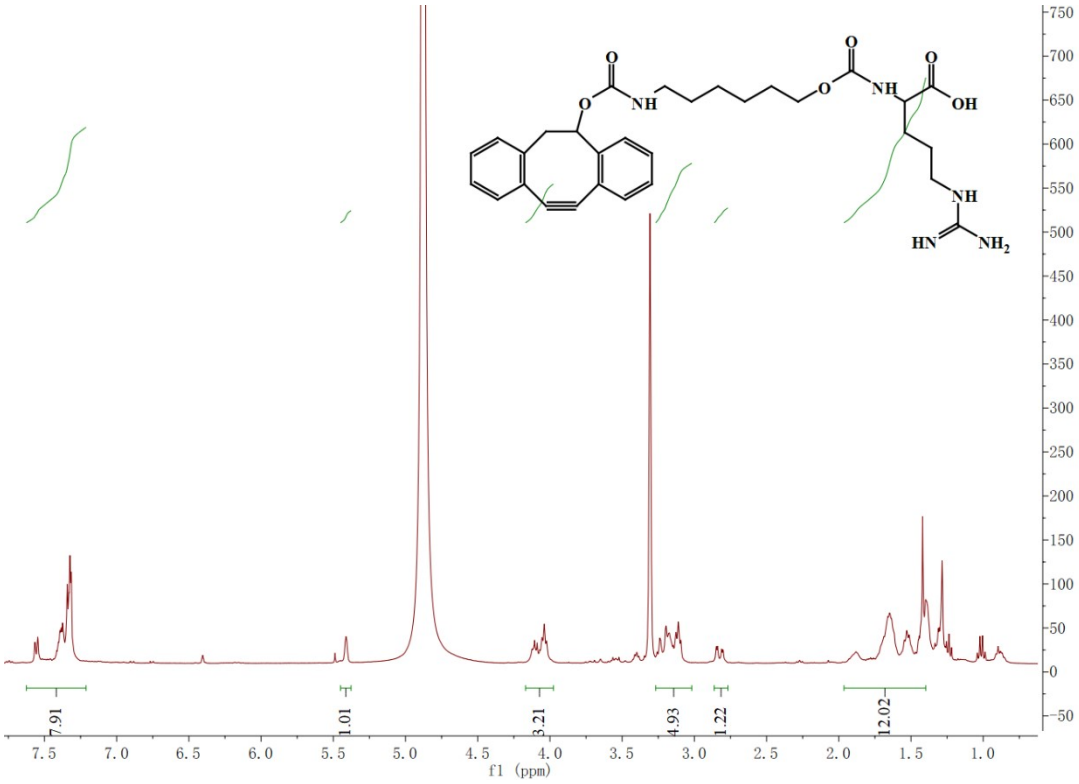


**Fig. S11.** $^{1}$H-NMR spectrum of DR2 (400 MHz, $CD_3OD$).

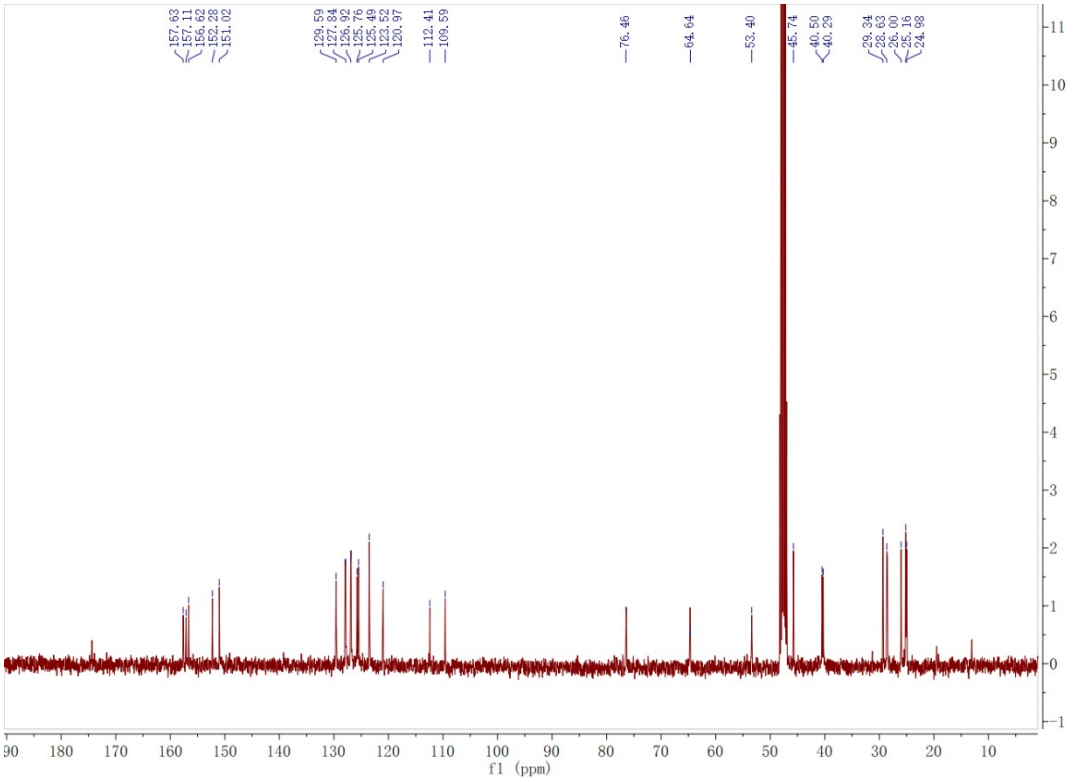


**Fig. S12.** $^{13}$C-NMR spectrum of DR2 (100 MHz, $CD_3OD$).

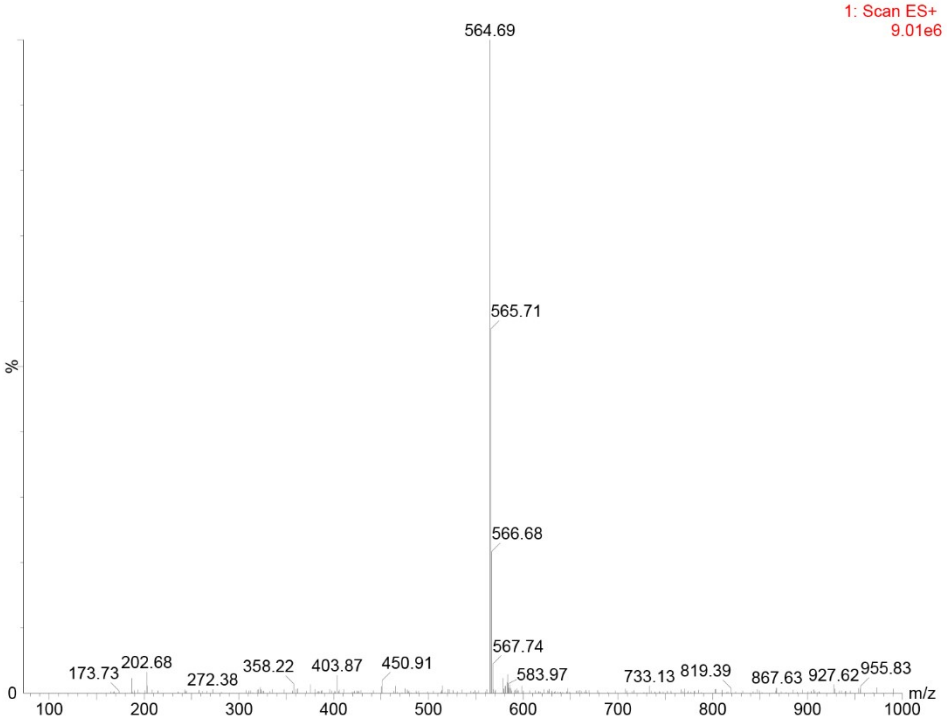


**Fig. S13.** The mass spectrum of DR2.

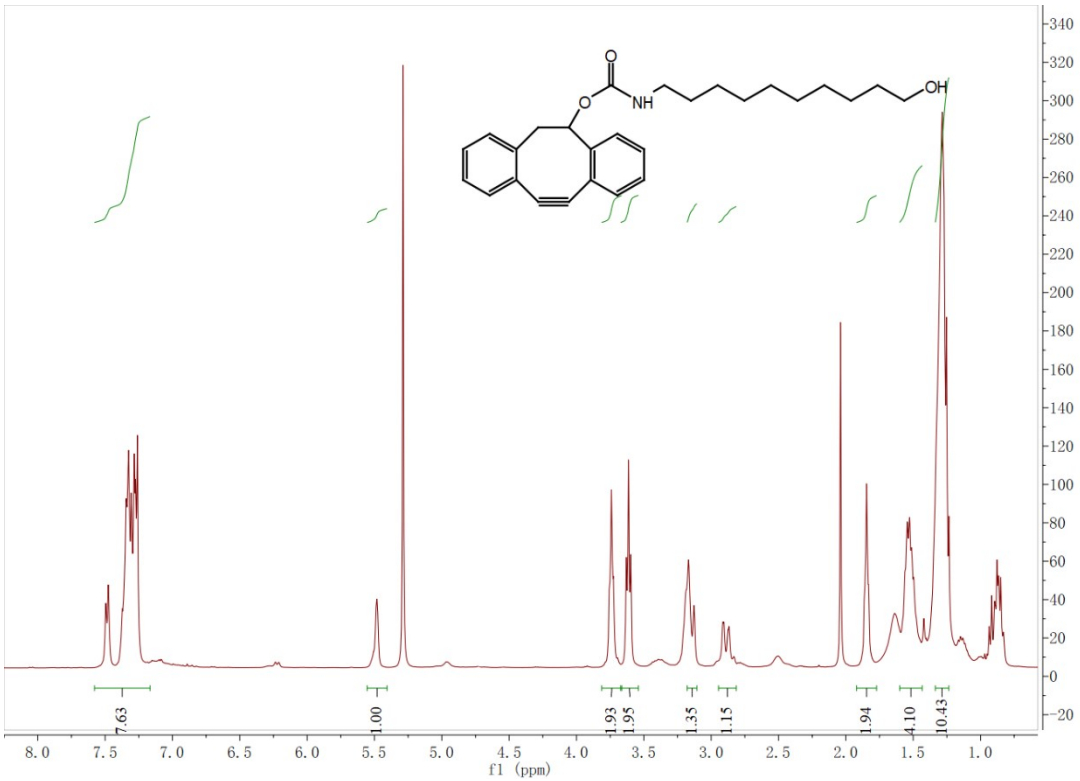


**Fig. S14.** $^{1}$H-NMR spectrum of compound 1b (400 MHz, $CDCl_3$).

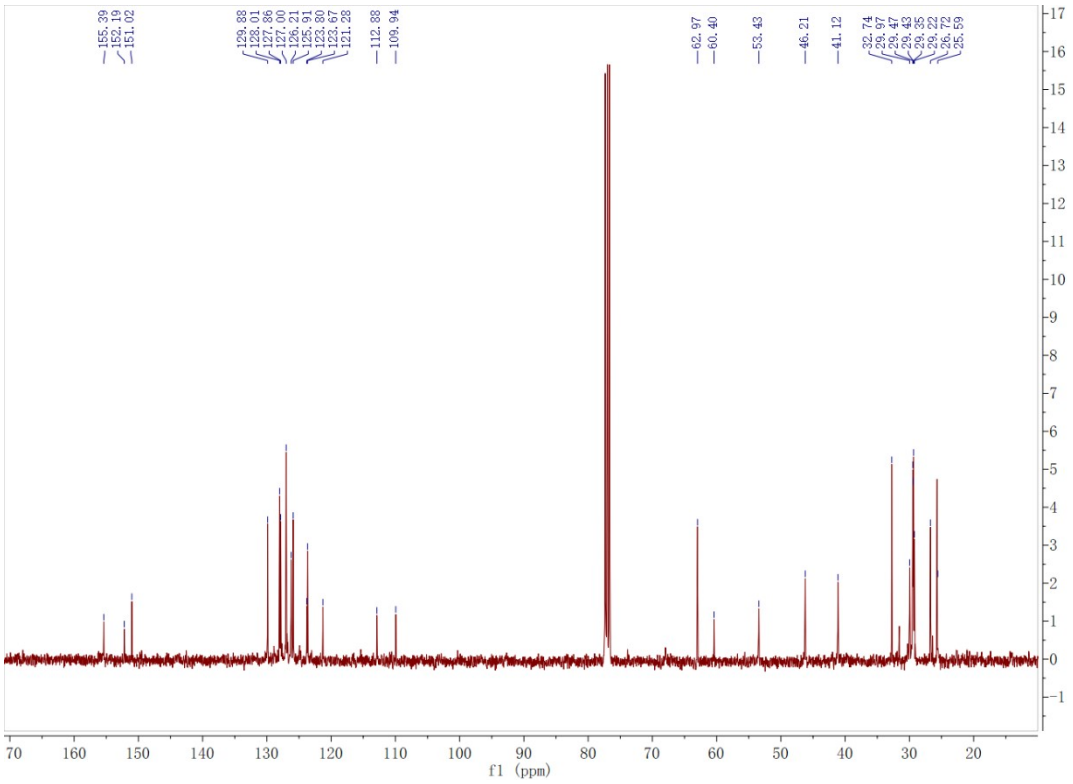


**Fig. S15.** $^{13}$C-NMR spectrum of compound 1b (100 MHz, $CDCl_3$).

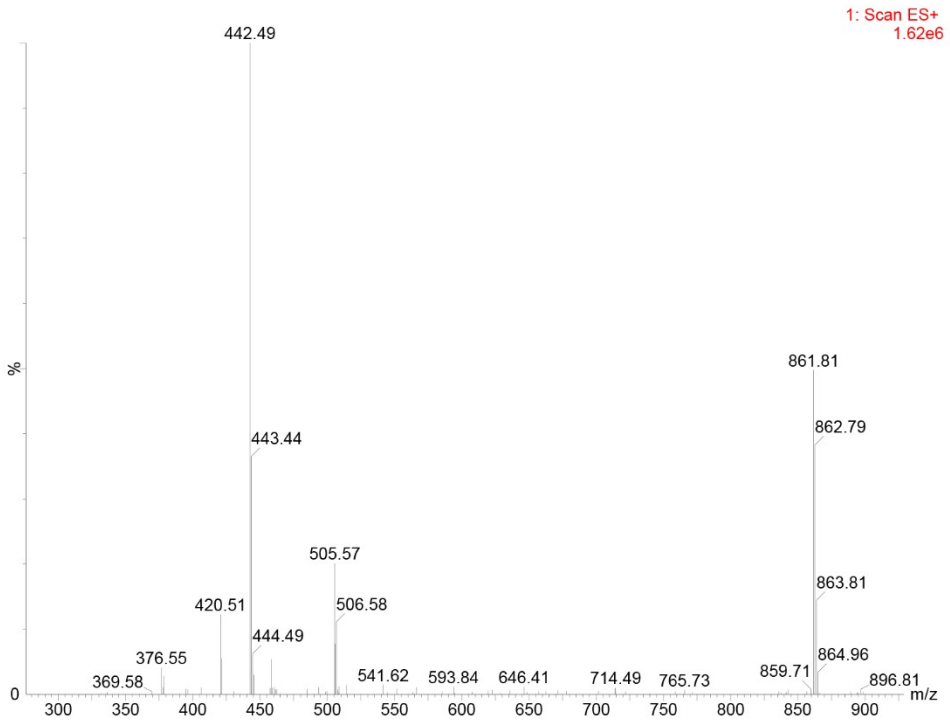


**Fig. S16.** The mass spectrum of compound 1b.

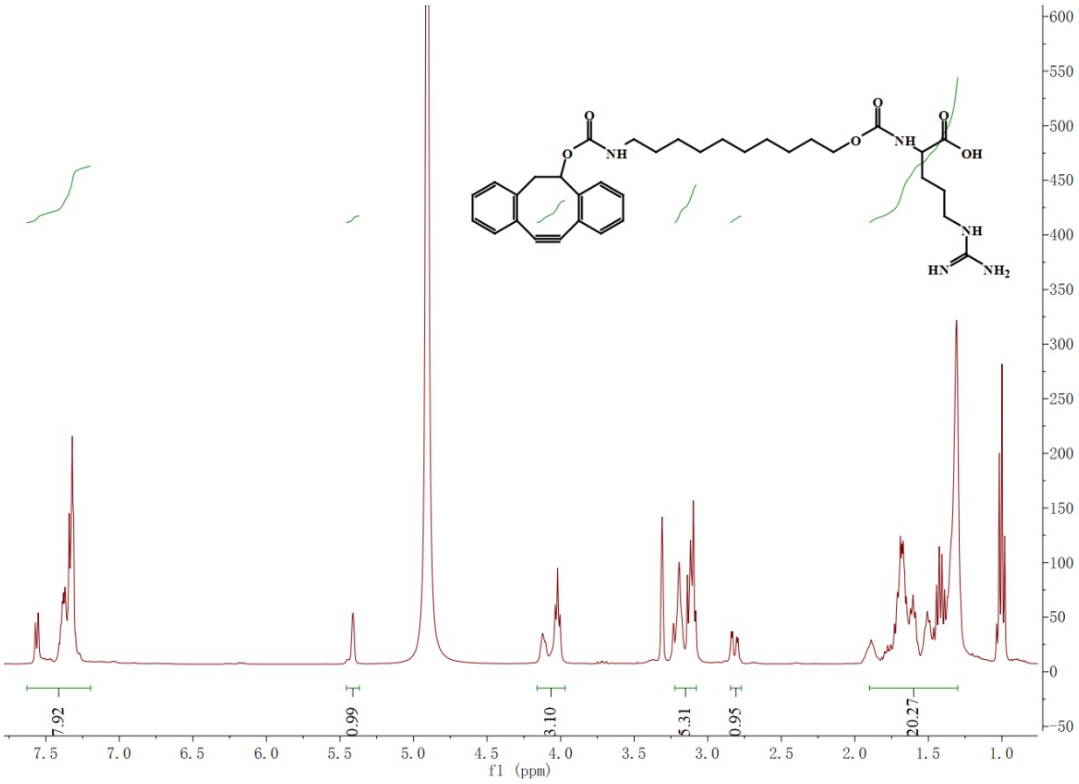


**Fig. S17.** $^{1}$H-NMR spectrum of DR3 (400 MHz, $CD_3OD$).

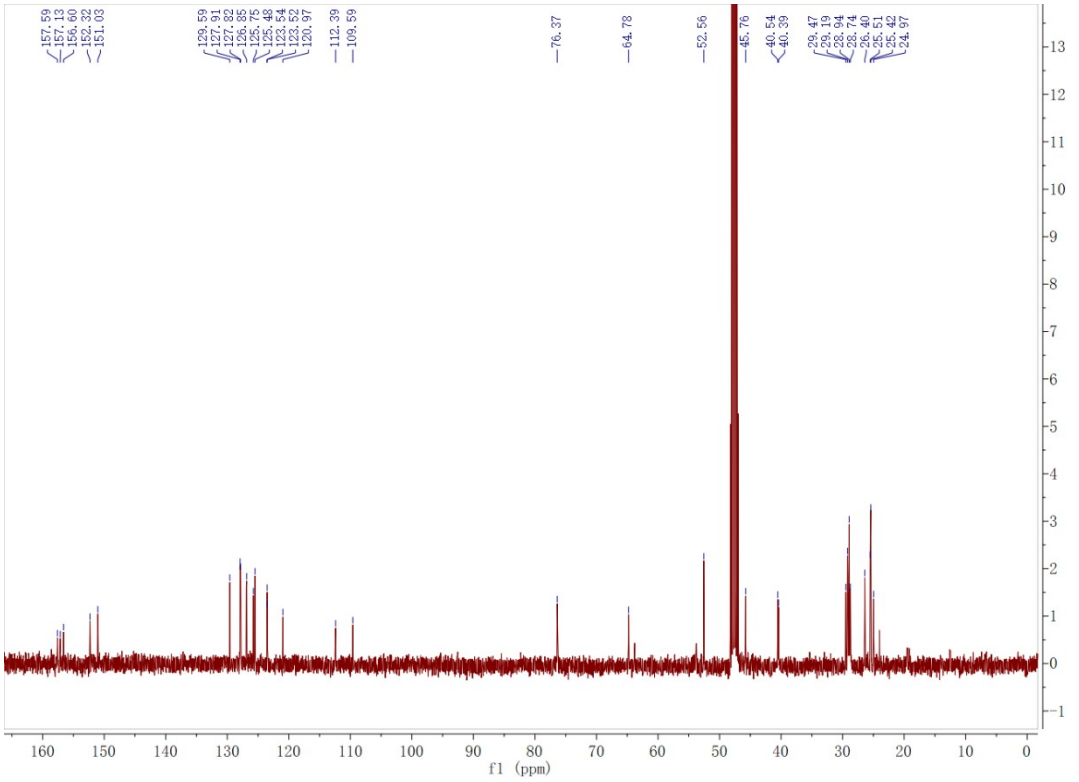


**Fig. S18.** $^{13}$C-NMR spectrum of DR3 (100 MHz, $CD_3OD$).

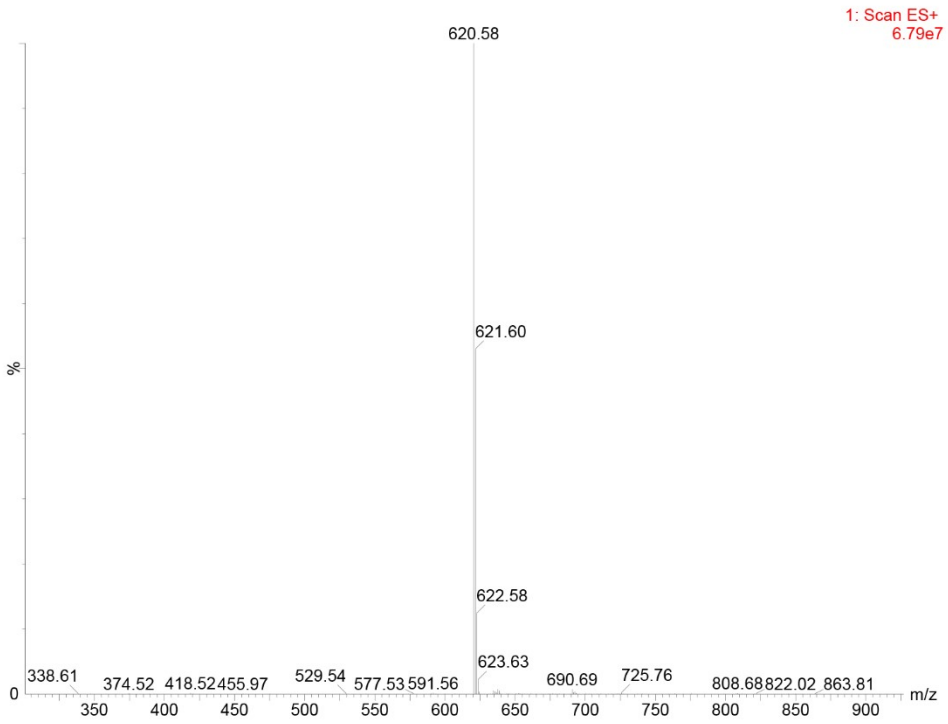


**Fig. S19.** The mass spectrum of DR3.

## References

1. X. He, L. Yang, H. Su, S. Lin, D. Qi, H. Chen, Y. Qu, L. Liu and X. Feng, *Journal of Controlled Release*, 2021, **337**, 306-316.