Supplementary information

Siderophore-based Targeted Antibody Recruitment for Promoting Immune Responses towards Gram-negative Pathogens

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Supplementary Figures

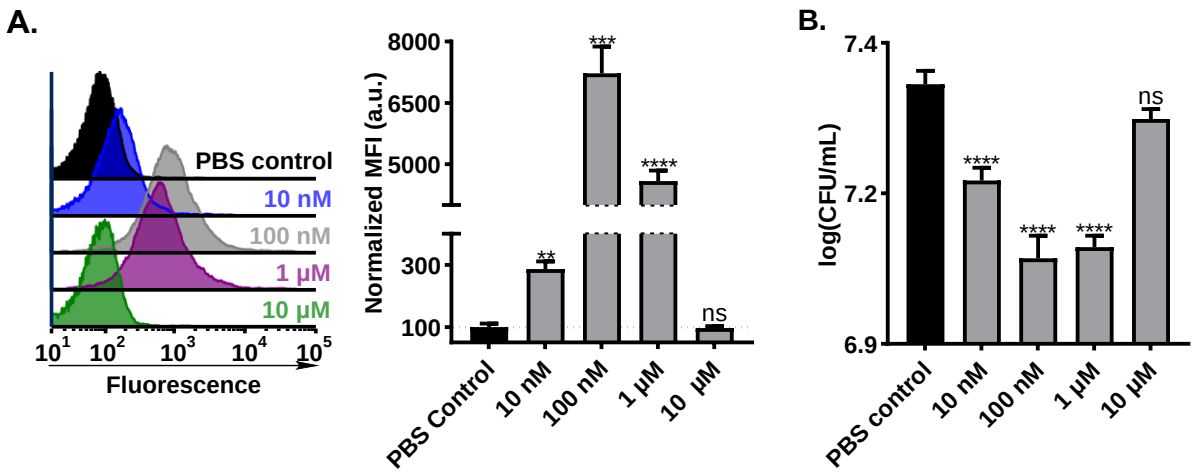

Figure S1. Determination of optimal GNP3 concentration for opsonization and complement-dependent cytotoxicity (CDC) against *E. coli* Δ*entA*.
The optimal **GNP3** concentration was evaluated in the presence of 0.02 mg/mL (ca. 130 nM) AF488-labeled or unlabeled anti-DNP antibody. (A) Opsonization activity was assessed via flow cytometry ($n = 3$), and (B) CDC activity was measured by determining colony-forming units (CFU/mL) ($n = 10$) at various **GNP3** concentrations. In CDC assays, 10% human complement serum (HCS) was added to provide complement components. Results indicate maximum **GNP3** activity for both opsonization and CDC at 100 nM. Data are presented as means ± standard error. Statistical significance was determined using two-tailed Student's *t*-tests (ns: not significant, *$p < 0.0332$, **$p < 0.0021$, ***$p < 0.0002$, ****$p < 0.0001$).

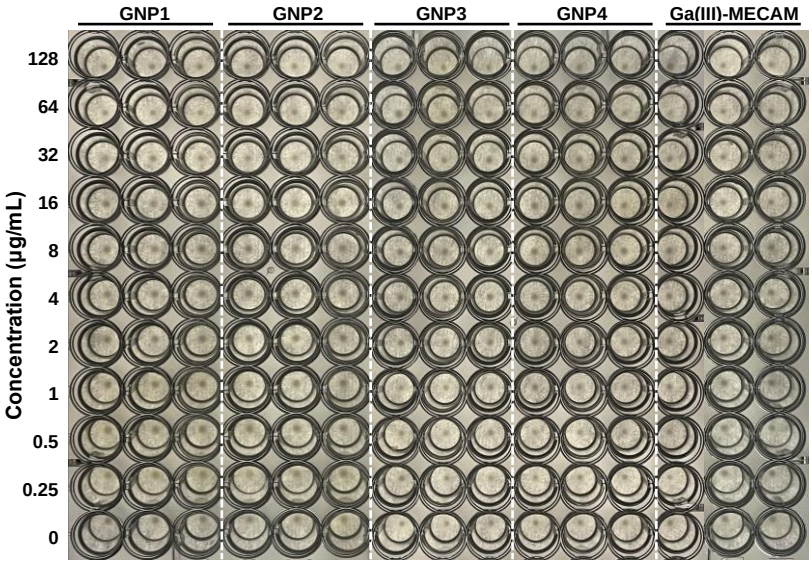

Figure S2. Cytotoxicity assessment of GNP1–4 against *E. coli* Δ*entA*.
The cytotoxicity of **GNP1**–**4** was evaluated using the minimal inhibitory concentration (MIC) determination protocol, following the Clinical & Laboratory Standards Institute (CLSI) Guidelines. Assays were conducted in iron-depleted, cation-adjusted Mueller-Hinton broth (ID-CAMHB) to induce overexpression of genes related to siderophore uptake. Each well of a 96-well U-bottom microplate was loaded with 50 µL of **GNP1**–**4** or Ga(III)-MECAM at the specified concentrations in ID-CAMHB, followed by 50 µL of starter culture prepared as described. The plate was sealed with Breath-Easy film (Diversified Biotech, Dedham, MA, USA) and incubated at 37 °C. After 24 h, microbial growth was monitored by visual inspection. All experiments were conducted in triplicate.

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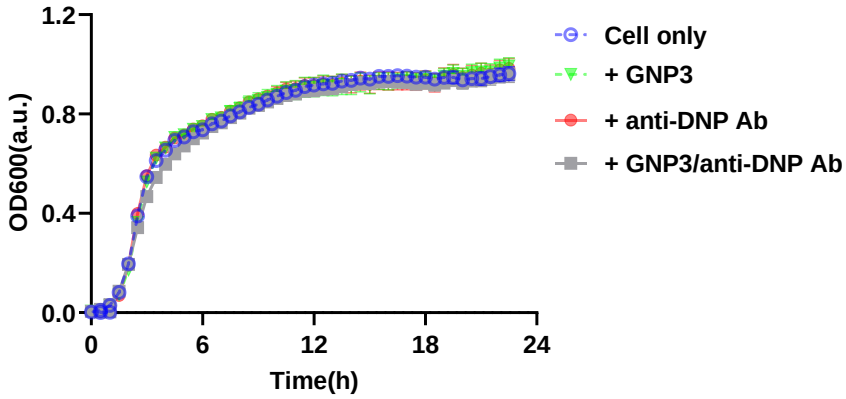


Figure S3. Impact of GNP3 and anti-DNP antibody on the *E. coli* Δ*entA* cellular growth.
E. coli Δ*entA* cells in LB media were treated with 100 nM **GNP3**, 0.02 mg/mL anti-DNP antibody, or both (total volume 100 µL) and incubated at 37 ºC. Growth was monitored by measuring absorbance at 600 nm over 24 h using Epoch 2 microplate reader (Biotek, Vermont, USA). Results indicate that neither **GNP3** nor anti-DNP antibody affect bacterial growth in the absence of HCS. Error bars indicate the standard error of the mean (n = 3).

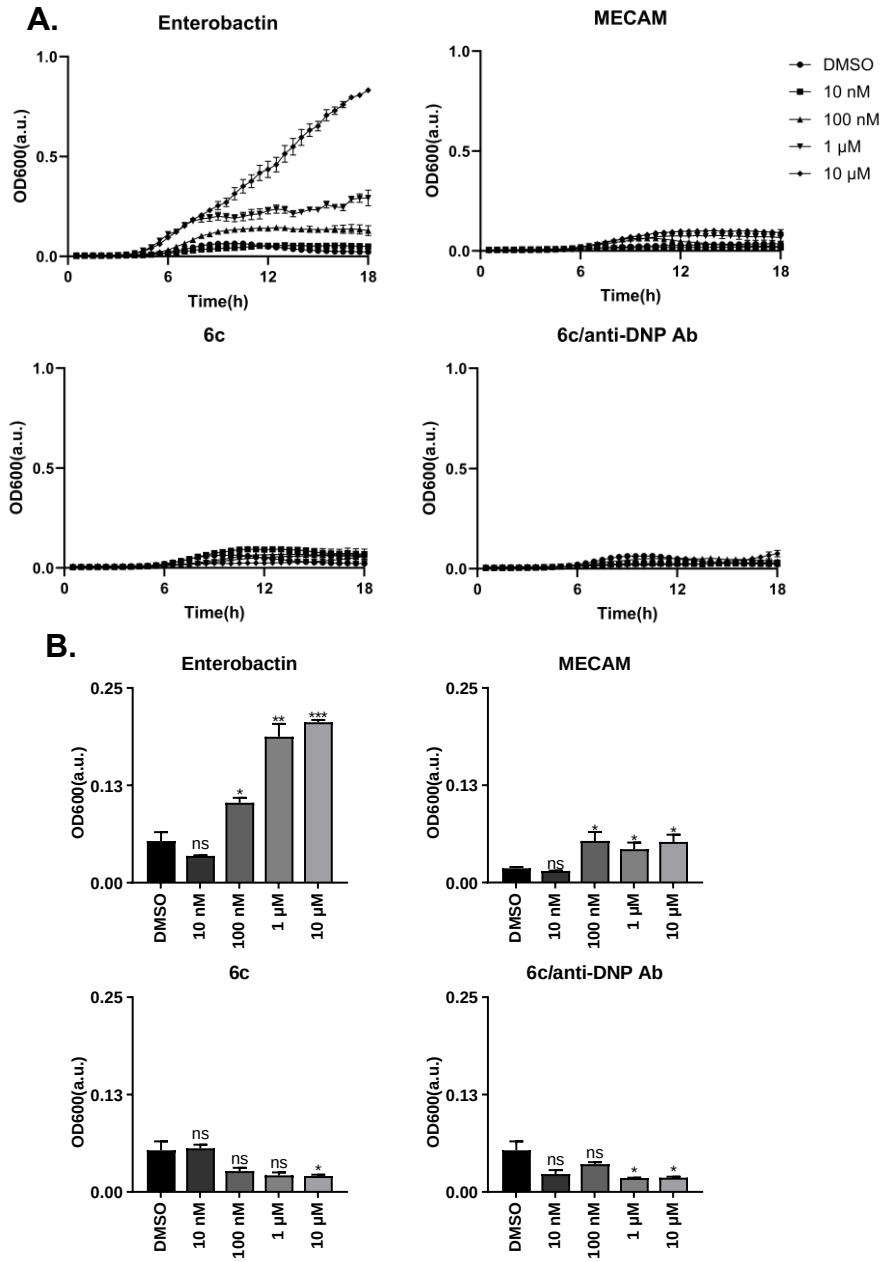


Figure S4. Evaluation of growth promoting activity of 6c, the *apo*-form of GNP3, on *E. coli* Δ*entA*.

The growth promoting activity of **6c** on *E. coli* Δ*entA* was assessed under iron deficient conditions over 18 h. *E. coli* Δ*entA* overnight cultures were suspended in 100 μL LB media containing 300 μM 2,2′-dipyridyl (DP) to achieve a cell density of 5×10^5 CFU/mL, along with specified compounds (enterobactin, MECAM, **6c**, or **6c**/0.02 mg/mL anti-DNP antibody) at different concentrations. The resulting mixtures were incubated at 37 ºC. (A) Growth curve monitored by measuring absorbance at 600 nm over 18 h using Epoch 2 microplate reader (Biotek, Vermont, USA). (B) Bar graphs showing the OD600 values measured at 8 h. Results indicate that the *apo*-form of **GNP3** and its combination with anti-DNP antibody do not exhibit growth promoting activity compared to enterobactin and MECAM. Error bars indicate the standard error of the mean ($n = 3$). Statistical significance was determined using two-tailed Student's *t*-tests (ns: not significant, *$p < 0.0332$, **$p < 0.0021$, ***$p < 0.0002$, ****$p < 0.0001$).

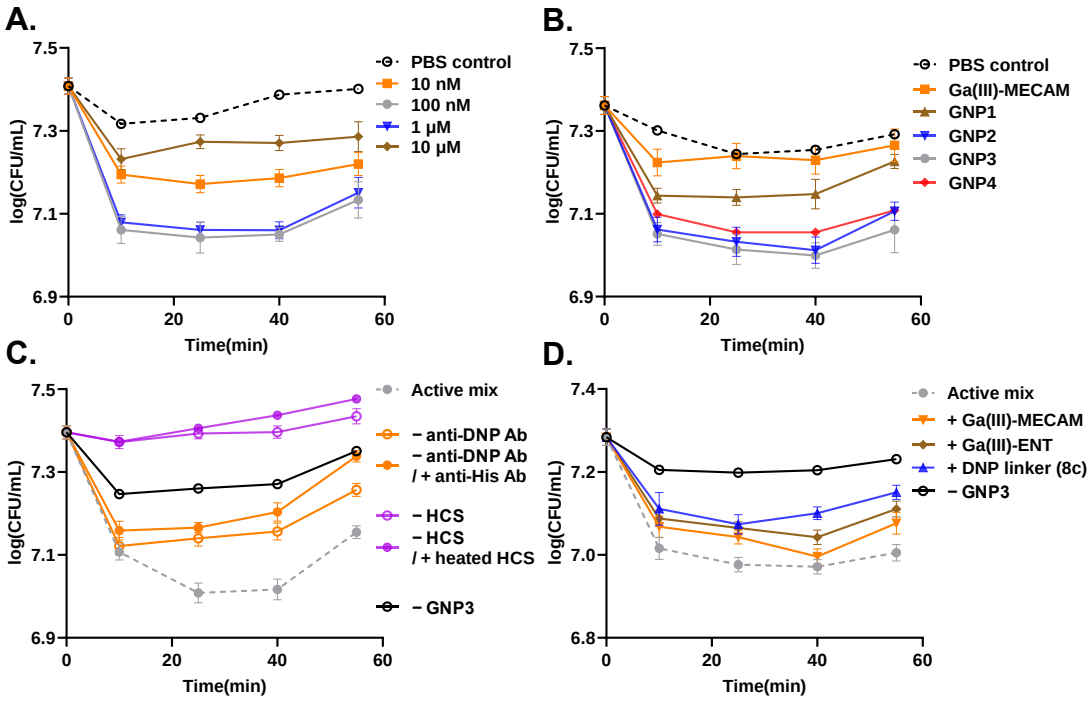


Figure S5. Time-course analysis of complement-dependent cytotoxicity (CDC) induced by MECAM-DNP conjugates, GNP1–4, against *E. coli* Δ*entA*.

The CDC response was evaluated over time in the presence of anti-DNP antibody and HCS. (A) Analysis of the CDC activity of **GNP3** across different concentrations (see Figure S1). (B) Comparison of CDC activity among **GNP1**–**4** and Ga(III)-MECAM (see Figure 2E). (C) Identification of key elements necessary for **GNP3**-mediated CDC induction (see Figure 2F). (D) Confirmation of the need for simultaneous binding of **GNP3** to both the siderophore receptor, FepA, and the anti-DNP antibody for effective CDC induction (see Figure 2G). Cell death was quantified by determining CFU/mL. Data are presented as means ± standard error (n = 10).

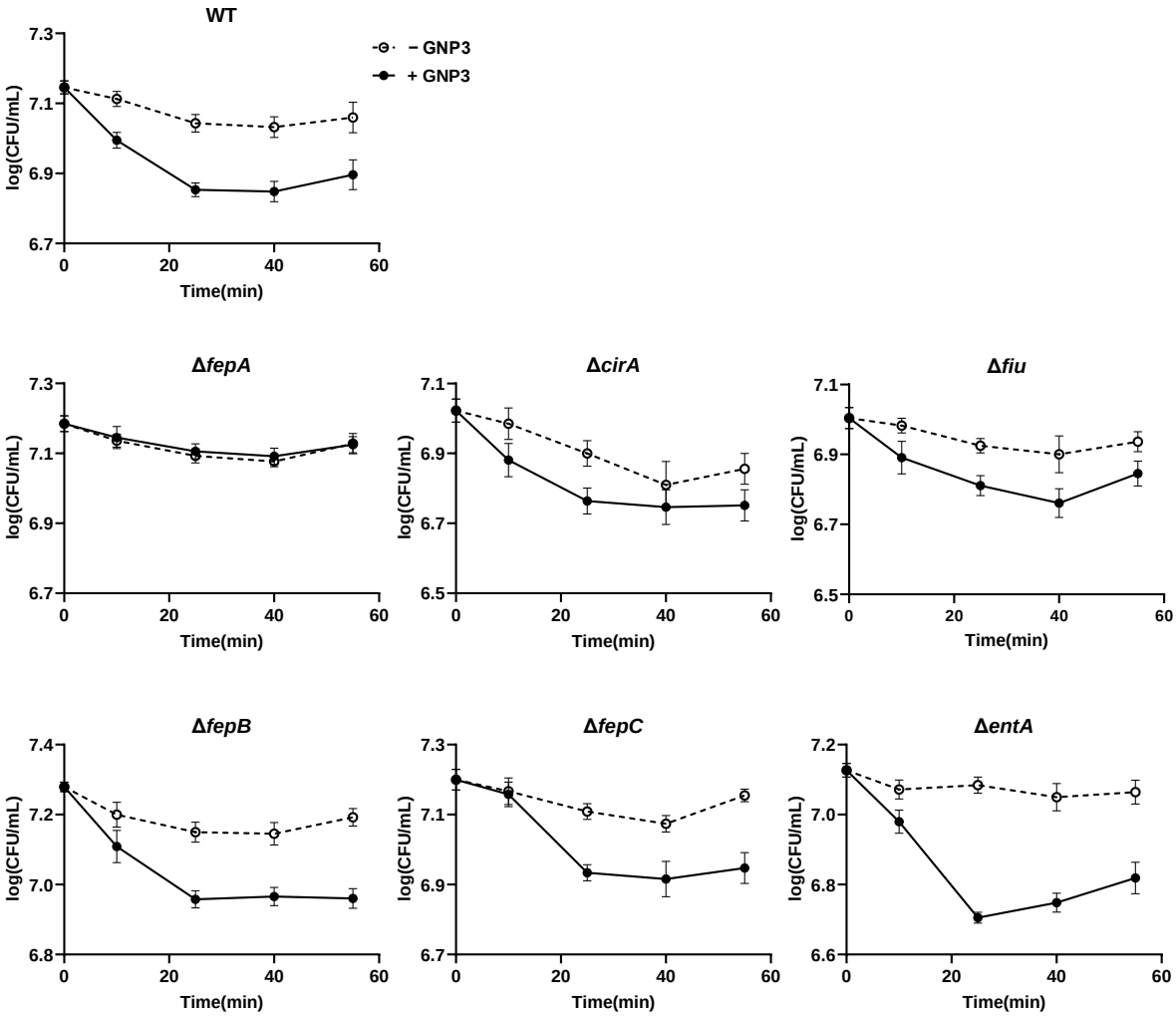


Figure S6. Time-course analysis of complement-dependent cytotoxicity (CDC) induced by GNP3 against various *E. coli* BW25113 mutants.

CDC activity was evaluated over time in both the presence and absence of **GNP3** (see Figure 2H). No significant difference in activity was observed in the Δ*fepA* mutant, indicating that **GNP3**-mediated CDC induction is dependent on the FepA receptor. Cell death was quantified by determining CFU/mL. Data are presented as means ± standard error (n = 10).

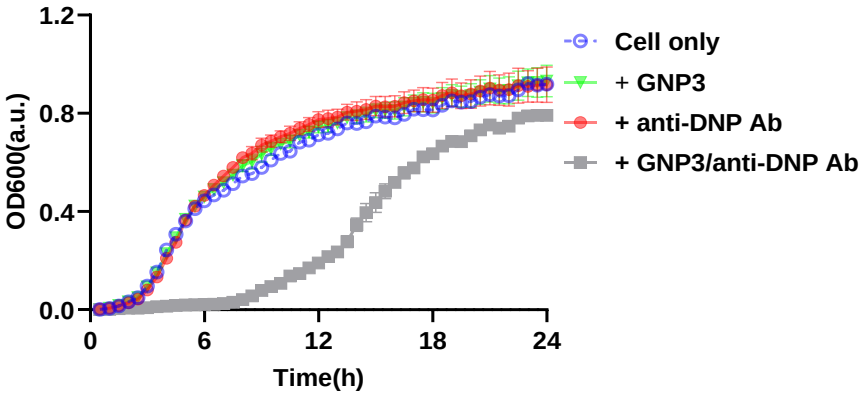


Figure S7. Impact of GNP3 and anti-DNP antibody on the *E. coli* Δ*entA* cellular growth in the presence of human complement serum over 24 h.

E. coli Δ*entA* cells in ID-CAMHB media containing 10% lysozyme depleted human complement serum were treated with 100 nM **GNP3**, 0.02 mg/mL anti-DNP antibody, or both (total volume 100 µL) and incubated at 37 ºC. Growth was monitored by measuring absorbance at 600 nm over 24 h using Epoch 2 microplate reader (Biotek, Vermont, USA). The observed apparent bacteriostatic activity of the **GNP3** and anti-DNP antibody combination is likely attributable to the inactivation of the complement components, which are presumed to mediate bactericidal activity, following the prolonged incubation. Error bars indicate the standard error of the mean (n = 3).

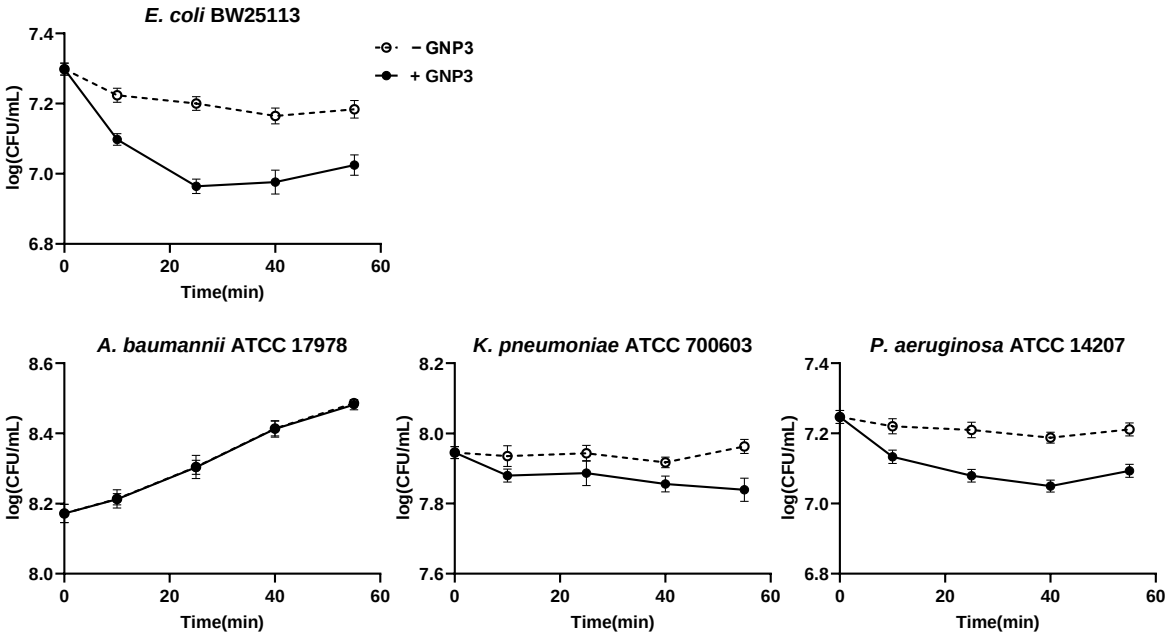


Figure S8. Time-course analysis of complement dependent cytotoxicity (CDC) induced by GNP3 against various Gram-negative bacteria beyond *E. coli*.

CDC activity was evaluated over time in both the presence and absence of **GNP3** for each bacterial species. Significant **GNP3** activity was observed against *K. pneumoniae* and *P. aeruginosa* (see Figure 3C). Cell death was quantified by determining CFU/mL. Data are presented as means ± standard error (n = 10).

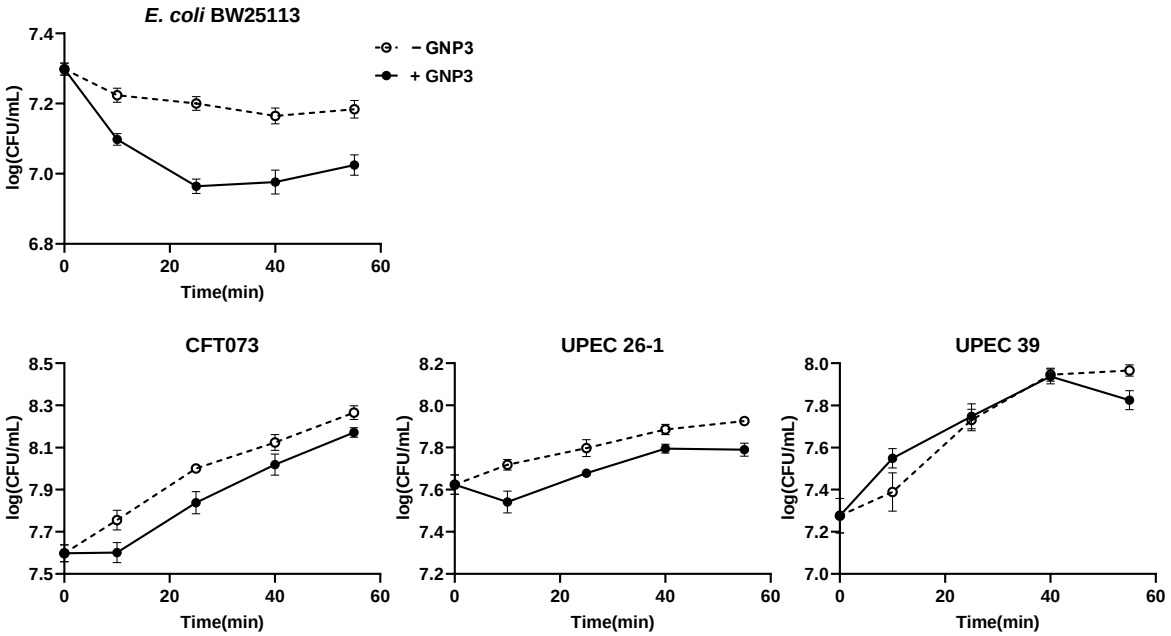


Figure S9. Time-course analysis of complement dependent cytotoxicity (CDC) induced by GNP3 against uropathogenic *E. coli* strains.

CDC activity was evaluated over time in both the presence and absence of **GNP3** for each bacterial species. Significant **GNP3** activity was observed against CFT073 and UPEC 26-1 strains (see Figure 3F). Cell death was quantified by determining CFU/mL. Data are presented as means ± standard error (n = 10).

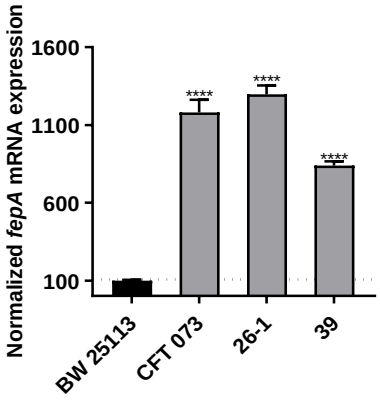


Figure S10. Relative *fepA* mRNA expression in BW25113 and uropathogenic *E. coli*. Data are presented as means ± standard error ($n = 4$). Statistical significance was determined using two-tailed Student's *t*-tests (ns: not significant, *$p < 0.0332$, **$p < 0.0021$, ***$p < 0.0002$, ****$p < 0.0001$).

BIOLOGICAL CHARACTERIZATION PROCEDURES

Strains and bacterial culture media

Escherichia coli BW25113 strains used in this study, wild-type, Δ*cirA*, Δ*fiu*, Δ*fepA*, Δ*fepB*, Δ*fepC*, and Δ*entA*, are parts of the Keio collection. They were obtained from National Institute of Genetics in Japan. *Acinetobacter baumannii* ATCC 17978, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 14207, and uropathogenic *E. coli* CFT073 ATCC 700928 were obtained from American Type Culture Collection (ATCC, Virginia, USA). Other uropathogenic *E. coli* strains 26-1 and 39 isolated from patients diagnosed with urinary tract infections were obtained from Chungnam National University Hospital, Korea.[3] Luria−Bertani broth (LB broth Miller, Cat# 244620) and cation-adjusted Mueller-Hinton II broth (CAMHB, Cat# 212322) were obtained from BD Difco (New Jersey, USA). The iron-deficient medium (ID-CAMHB) was prepared by treating CAMHB with Chelex 100 resin (Sigma-Aldrich, Missouri, USA, Cat# 95621).[4] All other reagents used in the following experiments were obtained from Sigma-Aldrich or Thermo Fisher Scientific (Massachusetts, USA) unless otherwise specified.

Fluorescence microscopic imaging of bacterial cells for opsonization assessment

A frozen stock of the indicated bacterium was streaked on the LB agar plate and overnight incubated at 37 °C. Then, a single colony was picked to inoculate into 5.0 mL of LB medium and grown overnight. The overnight bacterial cell culture was diluted 200-fold with 30 mL of a fresh LB medium. After a 2.5 h incubation at 37 °C to reach the log growth phase, the cells were harvested by centrifugation at 3000 *g*. Then, supernatant was removed, and the pellet was washed twice with cold phosphate buffered saline (PBS, pH 7.4) and resuspended in PBS to a concentration of 8.0×10^8 CFU/mL. The cell suspension was diluted in 50 μL of PBS to a concentration of 1.6×10^8 CFU/mL, and this diluted suspension was mixed with 50 μL of a solution containing the indicated MECAM-DNP conjugate or PBS along with Alexa Fluor 488 anti-DNP IgG (0.02 mg/mL, Invitrogen, Cat# A-11097) in PBS. The resulting mixture was incubated at 37 °C while protected from light. After 30 min, the cells were collected by centrifugation, washed twice with cold PBS, and resuspended in 5.0 μL cold PBS. An aliquot of 1.0 μL suspension was loaded onto a microscope slides of thickness approx.1 mm (Marienfeld, Lauda-Königshofen, Germany, Cat# 1000200), fixed with 1% agarose gel pad, and covered with a microscope cover glass (Marienfeld, Cat# 0101242). Then, the fluorescence images were captured using Axio Imager M1 upright microscopy (Zeiss, Oberkochen, Germany) with a 100× oil immersion lens. The images were processed using ImageJ software.

Flow cytometry analysis for opsonization assessment

A frozen stock of the indicated bacterium was streaked on the LB agar plate and overnight incubated at 37 °C. Then, a single colony was picked to inoculate into 5.0 mL of LB medium and grown overnight. The overnight bacterial cell culture was diluted 200-fold with 30 mL of a fresh LB medium. After a 2.5 h incubation at 37 °C to reach the log growth phase, the cells were harvested by centrifugation at 3000 *g*. Then, supernatant was removed, and the pellet was washed twice with cold phosphate buffered saline (PBS, pH 7.4) and resuspended in PBS to a concentration of 8.0×10^8 CFU/mL The cell suspension was diluted in 50 μL of PBS to a concentration of approximately 1.0×10^7–1.0×10^8 CFU/mL, and this diluted suspension was mixed with 50 μL of a solution containing the indicated

MECAM-DNP conjugate or PBS along with Alexa Fluor 488 anti-DNP IgG (0.02 mg/mL, Invitrogen, Cat# A-11097). The solution was incubated at 37 °C while protected from light. After 30 min, the cells were collected by centrifugation, washed twice with cold PBS, and resuspended in 100 μL of cold PBS. Then, the resulting suspension was subjected to the flow cytometry analysis (n = 3) using a BD Accuri C6 (BD Biosciences, New jersey, USA). The data was processed by FCS express 7 software (De Novo Software, California, USA).

Complement dependency cytotoxicity (CDC) assay

A frozen stock of the indicated bacterium was streaked on the LB agar plate and overnight incubated at 37 °C. Then, a single colony was picked to inoculate into 5.0 mL of LB medium and grown overnight. The overnight bacterial cell culture was diluted 200-fold with 30 mL of a fresh LB medium. After a 2.5 h incubation at 37 °C to reach the log growth phase, the cells were harvested by centrifugation at 3000 *g*. Then, supernatant was removed, and the pellet was washed twice with cold phosphate buffered saline (PBS, pH 7.4) and resuspended in PBS to a concentration of 8.0×10^8 CFU/mL. In a 96-well U-bottom microplate (Greiner Bio-One, Kremsmünster, Austria, Cat# 650161), 50 μL of a mixture containing 1.0×10^8 CFU/mL bacterial suspension, the indicated MECAM-DNP conjugate or PBS, 0.02 mg/mL anti-DNP IgG (Invitrogen, Cat# A-6430), and 10% lysozyme-depleted human complement serum (HCS) in PBS was added to each well. Lysozyme-depleted HCS was prepared by treating commercially available human complement serum (Sigma-Aldrich, Cat# S1764) with pre-washed bentonite.[5] After incubation, 10 μL aliquots were taken from each well at 10, 25, 40, and 55 min. These individual aliquots were serially diluted 10-fold in PBS and spotted onto LB agar plates. After overnight incubation at 37 ºC, the numbers of colonies were counted to determine the surviving bacterial population in CFU/mL (n = 10).

Phagocytosis assay & imaging

THP-1 cells, a human monocyte cell line, were cultured in RPMI 1640 medium (Welgene, Gyeongsan, Korea, Cat# LM011-01) with 10% fetal bovine serum (Welgene, Cat# S001-01) at 37 °C under 5% CO_2. Cells were passaged before reaching a density of 2×10^6 cells/mL. To induce differentiation into macrophages, THP-1 cell were seeded at a density of 2×10^5 cells/well in a 24-well microplate and treated with 100 nM phorbol-12-myristate-13-acetate, (PMA; Sigma-Aldrich, Missouri, USA, Cat# P1585) for 24 h.

For bacterial infection, a frozen stock of *E. coli* BW25113 was streaked on an LB agar plate and incubated at 37 ºC overnight. A single colony was picked, inoculated into LB medium, and cultured overnight. The overnight culture was diluted into fresh LB medium and incubated at 37 °C until reaching the log growth phase. The bacterial cells then were harvested by centrifugation and washed three times with Dulbecco's phosphate-buffered saline (DPBS, Welgene, Cat# LB001-02). A mixture of 200 μL of DPBS containing 9×10^6 CFU/mL *E. coli*, 100 nM **GNP3**, and 0.02 mg/mL anti-DNP IgG (Invitrogen, Cat# A-6430) was incubated at 37 °C for 30 min. After incubation, these treated bacteria were used to infect macrophage at a multiplicity of infection (MOI) of 50, and the resulting suspension was incubated at 37 ºC. At 25 min, 55 min, 2 h, and 6 h post-infection, the cells were harvested and washed three times with DPBS to remove extracellular bacteria. The number of intracellular bacteria phagocytosed by macrophages was determined by selectively lysing the macrophages with 0.1% Triton-X 100 (Sigma-Aldrich, Cat# X100-100ML) in sterile distilled water for 10 min. The resulting cell lysates were serially diluted 10-fold in DPBS, and 10 μL of each dilution

was plated on LB agar plates. After overnight incubation at 37 °C, colony-forming units (CFU/mL) were counted to determine the number of surviving bacteria (n = 10).

For visualization of intracellular phagocytosed bacteria, the infected macrophages prepared as described above were placed on glass coverslips. These cells were fixed with 4% paraformaldehyde (Electron Microscopy Sciences, Pennsylvania, USA, Cat# 15710) and permeabilized with 0.1% Triton-X 100. Actin was stained with Alexa Fluor 568-conjugated phalloidin (Thermo Fisher Scientific, Cat# A-12380), while the nuclei of both bacteria and cells were stained with DAPI (Invitrogen, Cat# 00-4959-52). Stained cells were visualized using a LSM 900 laser scanning confocal microscope (Zeiss, Oberkochen, Germany).

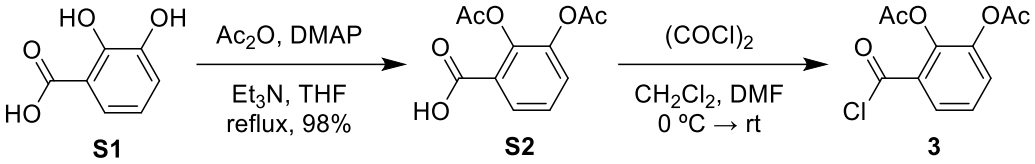
Quantitative reverse transcription PCR (qRT-PCR)

Total RNA from bacteria was extracted using the High Pure RNA isolation kit (Roche Applied Science, Mannheim, Germany, Cat# 11828665001) in accordance with the manufacturer's protocol. cDNA was amplified by reverse transcriptase premix (ElpisBio, Daejeon, Korea, Cat# EBT-1515). Quantitative PCR was performed on a CFX96 Real-time PCR detection system (Bio-Rad Hercules, California, USA) with cDNA, SYBR green (ElpisBio, Cat# EBT-1801), and primers (fepA forward: GTTAGCGAAACGGTGATGTG, fepA reverse: AGGTGAAGGTCGTTTGCATC, 16s rRNA forward: CGGGAACTCAAAGGAGACTG, 16S rRNA reverse: ACGACGCACTTTATGAGGTC). The expression level of the *fepA* gene was analyzed using the comparative quantitation cycle method using the 16S rRNA transcript level as the standard.

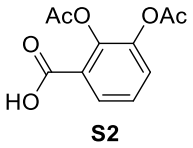
SYNTHETIC PROCEDURES

Materials and General Methods

Unless otherwise noted, all the reactions were carried out under nitrogen atmosphere with anhydrous solvents. Reactions were monitored by analytical thin-layer chromatography (TLC) which was carried out on pre-coated aluminum plates (Silica gel, F_{254}), and the product profiles were visualized by UV (254 nm) irradiation and staining with phosphomolybdic acid (PMA), ninhydrin, and/or potassium permanganate ($KMnO_4$) solution. Column chromatography was performed on silica gel (230-400 mesh). Analytical high performance liquid chromatography (HPLC) experiments were conducted using a Thermo Ultimate 3000 equipped with a diode array detector (commonly, the absorbance at 215 nm, 254 nm, and 280 nm was monitored). Acclaim 5 μm C18 120 Å column (Thermo Fisher scientific, Massachusetts, USA, 150 × 4.6 mm, Cat# 05941) were used for analysis. Reverse-phase preparative HPLC using an Agilent 1260 Infinity II Prep LC (Agilent Technologies, California, USA) equipped with an absorbance detector and the Agilent 5 prep-C18 column (50 × 21.1 mm, P/N 446905-702). Solvent A (sol. A) is 0.1% TFA in water and solvent B (sol. B) is 0.1% TFA in acetonitrile. The ^{1}H NMR and ^{13}C NMR data were recorded using Bruker Advance 500 (Bruker, Munich, Germany). Chemical shifts were reported in parts per million (ppm) relative to chloroform (^{1}H: 7.26 ppm, ^{13}C: 77.16 ppm), methanol (^{1}H: 3.31 ppm, ^{13}C: 49.00 ppm), dimethyl sulfoxide (^{1}H: 2.50 ppm, ^{13}C: 39.52 ppm), or tetramethylsilane (TMS, 0.00 ppm) and the coupling constants were reported in Herz (Hz). High-resolution mass spectra were collected using Bruker Compact QTOF, where the electrospray ionization method was employed for ionization. Methanol, tetrahydrofuran, dimethylformamide, and all other solvents were purchased from Sigma-Aldrich (Missouri, USA). Alfa-Aesar (Massachusetts, USA). All other chemicals were purchased from Sigma-Aldrich, Alfa-Aesar, TCI Chemicals (Japan), AK scientific (California, USA), or Daejung Chemicals & Metals (Republic of Korea), and they were used as received without further purification.

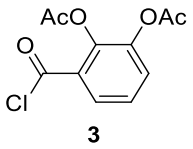


Scheme S1. Synthesis of compound **3**



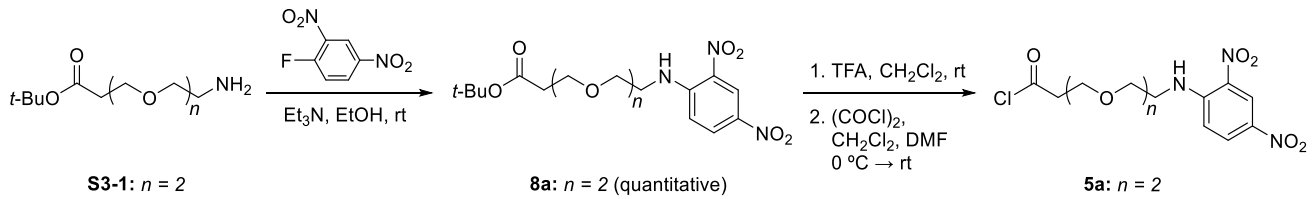
2,3-Diacetoxybenzoic acid (S2)

Compound **S2** (64.8 mmol, 98%) was synthesized from the 2,3-dihydroxybenzoic acid (**S1**) as previously reported.[1,2] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (dd, J = 7.9, 1.7 Hz, 1H), 7.44 (dd, J = 8.1, 1.7 Hz, 1H), 7.36 (t, J = 8.0 Hz, 1H), 2.34 (s, 3H), 2.32 (s, 3H).



3-(Chlorocarbonyl)-1,2-phenylene diacetate (3)

Compound **3** (12.5 mmol, quantitative yield) was synthesized from the 2,3-diacetoxybenzoic acid (**S2**) as previously reported.[1] The reaction was conducted, and the product was used for the preparation of compound **4** without further purification.

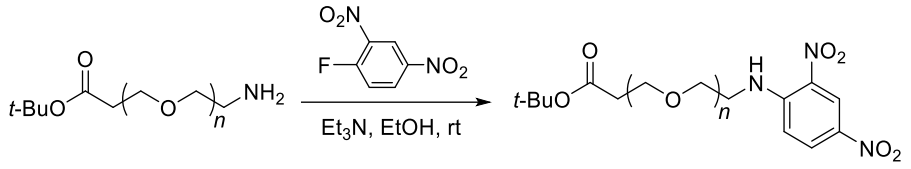


S3-1: *n* = *2*
S3-2: *n* = *3*
S3-3: *n* = *4*
S3-4: *n* = *5*

8a: *n* = *2* (quantitative)
8b: *n* = *3* (quantitative)
8c: *n* = *4* (98%)
8d: *n* = *5* (94%)

5a: *n* = *2*
5b: *n* = *3*
5c: *n* = *4*
5d: *n* = *5*

Scheme S2. Synthesis of compound **8a**–**8d** as well as **5a**–**5d**

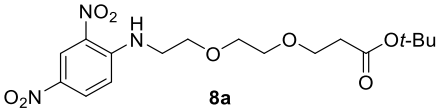


S3-1: *n* = *2*
S3-2: *n* = *3*
S3-3: *n* = *4*
S3-4: *n* = *5*

8a: *n* = *2* (quantitative)
8b: *n* = *3* (quantitative)
8c: *n* = *4* (98%)
8d: *n* = *5* (94%)

General Procedure for Synthesis of 8a–8d

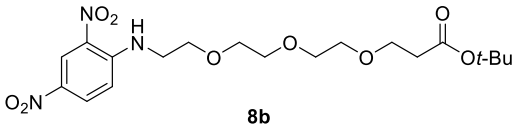
To a solution of 1-fluoro-2,4-dinitrobenzene (100 mg, 0.54 mmol) in ethanol (2.15 mL) was added triethylamine (109 mg, 1.07 mmol) at room temperature followed by **S3-1–4** (0.64 mmol). After stirring for 2 h, the solvent was removed under reduced pressure. The concentrated residue was diluted with ethyl acetate and acidified with 1 N hydrochloric acid aqueous solution. This mixture was extracted with ethyl acetate three times. The combined organic layers were then washed with a brine solution, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The resulting yellow oil was obtained without further purification. (0.51 – 0.54 mmol, 94.3% – quantitative yield).



8a

***tert*-butyl 3-(2-(2-((2,4-dinitrophenyl)amino)ethoxy)ethoxy)propanoate (8a)**

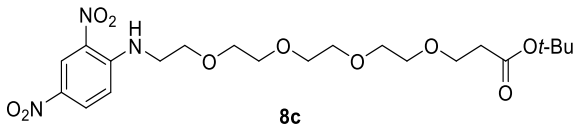
Compound **8a** (0.54 mmol, quantitative yield) was synthesized from **S3-1** by following the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (d, *J* = 2.6 Hz, 1H), 8.79 (s, 1H), 8.30 – 8.24 (m, 1H), 6.95 (d, *J* = 9.5 Hz, 1H), 3.83 (t, *J* = 5.3 Hz, 2H), 3.73 (t, *J* = 6.5 Hz, 2H), 3.71 – 3.68 (m, 2H), 3.67 – 3.64 (m, 2H), 3.59 (q, *J* = 5.2 Hz, 2H), 2.50 (t, *J* = 6.4 Hz, 2H), 1.44 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.95, 148.56, 136.22, 130.69, 130.34, 124.37, 114.18, 80.65, 70.83, 70.57, 68.75, 67.10, 43.44, 36.36, 28.19. HR-MS (ESI-TOF) *m/z*: calculated for $[C_{17}H_{25}N_3NaO_8]^+$ ($[M+Na]^+$) 422.1539, measured 422.1553.



8b

***tert*-butyl 3-(2-(2-(2-((2,4-dinitrophenyl)amino)ethoxy)ethoxy)ethoxy)propanoate (8b)**

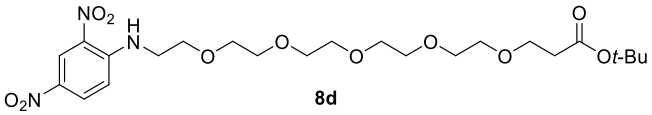
Compound **8b** (0.54 mmol, quantitative) was synthesized from **S3-2** by following the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (d, J = 2.7 Hz, 1H), 8.79 (s, 1H), 8.28 – 8.26 (m, 1H), 6.95 (d, J = 9.5 Hz, 1H), 3.83 (t, J = 5.3 Hz, 2H), 3.73 – 3.67 (m, 6H), 3.67 – 3.58 (m, 6H), 2.49 (t, J = 6.6 Hz, 2H), 1.44 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.96, 148.56, 136.20, 130.67, 130.31, 124.36, 114.21, 80.60, 70.88, 70.79, 70.74, 70.49, 68.74, 67.02, 43.42, 36.40, 28.19. HR-MS (ESI-TOF) *m/z*: calculated for $[C_{19}H_{29}N_3NaO_9]^+$ ([M+Na]$^+$) 466.1801, measured 466.1800.



8c

***tert*-butyl 1-((2,4-dinitrophenyl)amino)-3,6,9,12-tetraoxapentadecan-15-oate (8c)**

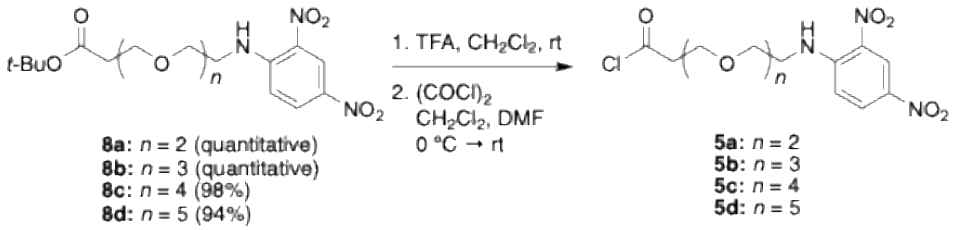
Compound **8c** (0.54 mmol, 98%) was synthesized from **S3-3** by following the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.14 (d, J = 2.7 Hz, 1H), 8.79 (s, 1H), 8.27 (dd, J = 9.5, 2.7 Hz, 1H), 6.96 (d, J = 9.5 Hz, 1H), 3.83 (t, J = 5.3 Hz, 2H), 3.72 – 3.57 (m, 16H), 2.49 (t, J = 6.6 Hz, 2H), 1.44 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.98, 148.55, 136.20, 130.66, 130.33, 124.37, 114.21, 80.62, 70.88, 70.80, 70.76, 70.71, 70.60, 70.47, 68.73, 67.00, 43.41, 36.39, 28.20. *m/z*: calculated for $[C_{21}H_{33}N_3NaO_{10}]^+$ ([M+Na]$^+$) 510.2064, measured 510.2068.



8d

***tert*-butyl 1-((2,4-dinitrophenyl)amino)-3,6,9,12,15-pentaoxaoctadecan-18-oate (8d)**

Compound **8d** (0.54 mmol, 94%) was synthesized from **S3-4** by following the general procedure described above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.14 (d, J = 2.7 Hz, 1H), 8.80 (s, 1H), 8.29 – 8.25 (m, 1H), 6.96 (d, J = 9.5 Hz, 1H), 3.83 (t, J = 5.2 Hz, 2H), 3.72 – 3.59 (m, 20H), 2.49 (t, J = 6.6 Hz, 2H), 1.44 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.97, 148.55, 136.19, 130.65, 130.32, 124.36, 114.21, 80.60, 70.88, 70.79, 70.75, 70.69, 70.67, 70.58, 70.46, 68.73, 67.00, 43.40, 36.38, 28.19. HR-MS (ESI-TOF) *m/z*: calculated for $[C_{23}H_{37}N_3NaO_{11}]^+$ ([M+Na]$^+$) 554.2326, measured 554.2322.

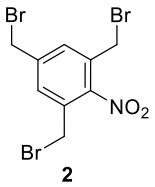


8a: n = 2 (quantitative)
8b: n = 3 (quantitative)
8c: n = 4 (98%)
8d: n = 5 (94%)

5a: n = 2
5b: n = 3
5c: n = 4
5d: n = 5

General Procedure for Synthesis of 5a–5d

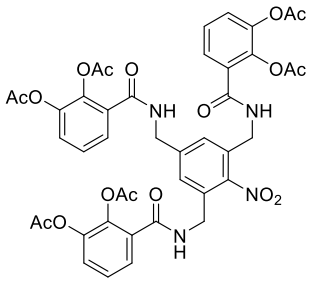
To a solution of compound **8a–8d** (0.33 mmol) in dichloromethane (2.64 mL) was added to trifluoroacetic acid (0.66 mL) dropwise. After stirring for 1 h, the solvent was evaporated under reduced pressure. The resulting residue was diluted with a co-solvent of dichloromethane and dimethylformamide (CH_2Cl_2:DMF = 99:1, 3.30 mL) and then treated with oxalyl chloride (0.66 mmol) at 0 ºC. The reaction mixture was slowly warmed to room temperature. After stirring for 2 h, the solvent was evaporated under reduced pressure, and the resulting yellow oil product (quantitative yield) was used without further purification.



2

1,3,5-tris(bromomethyl)-2-nitrobenzene (2)

Compound **2** (5.60 mmol, 91%) was synthesized from tris(bromomethyl)benzene (**1**) as previously reported.[1,2] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (s, 2H), 4.48 (s, 4H), 4.45 (s, 2H).

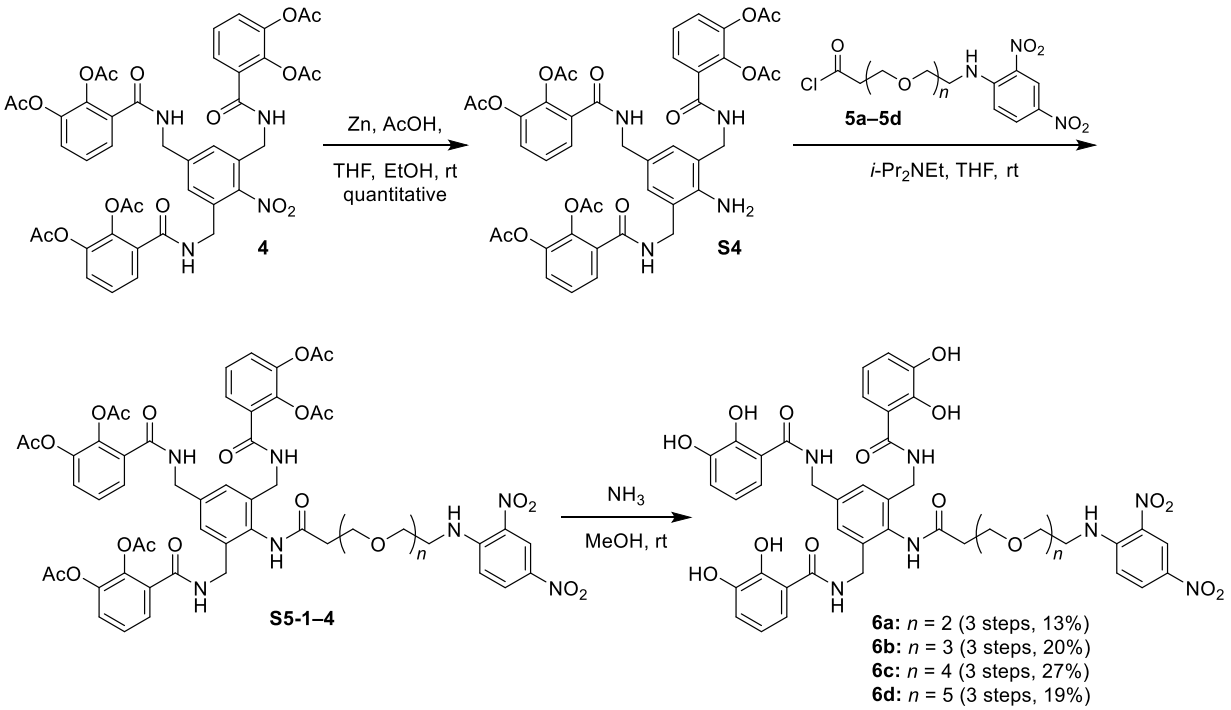


4

Nitro-MECAM-OAc (4)

Compound **4** was synthesized from compound **2** as previously reported[1] with minor procedural modifications. An aqueous solution of ammonia (30%, 70.0 mL) was added dropwise to a solution of **2** (3.01 g, 7.49 mmol) in tetrahydrofuran (40.0 mL) and ethanol (40.0 mL). The reaction was stirred overnight. After confirming that the reaction was completed by mass spectrometric analysis, all solvents and ammonia reagent were evaporated under reduced pressure. The resulting residue was then dissolved in water (0.1 M) and lyophilized. The crude lyophilized product was suspended in 20.0 mL aqueous sodium bicarbonate solution (0.5 M) and treated with compound **3** (663 mg, 2.58 mmol) dissolved in 1,4-dioxane (20.0 mL) dropwise over 15 min at 0 ºC. The reaction mixture gradually

became turbid upon addition of **3**. After the addition of **3** was completed, the solution was slowly warmed to room temperature. After stirring for 2 h, the reaction mixture was mixed with ice and diluted with ethyl acetate. After the organic layer was separated, the aqueous layer was further extracted with ethyl acetate three times. The combined organic layers were washed with saturated sodium bicarbonate solution followed by brine, and then dried over anhydrous magnesium sulfate. After concentrated under reduced pressure, the resulting crude reside was purified by flash column chromatography (SiO_2, CH_2Cl_2:EtOAc = 2:1 → 1:1) to afford the desired product **4** (213 mg, 0.25 mmol, 2-step 43% yield). ^{1}H NMR (500 MHz, DMSO) δ 9.06 (t, *J* = 6.1 Hz, 1H), 9.02 (t, *J* = 5.8 Hz, 2H), 7.47 (ddd, *J* = 9.0, 7.7, 1.6 Hz, 3H), 7.41 (s, 2H), 7.38 (ddd, *J* = 11.9, 8.1, 1.6 Hz, 3H), 7.32 (t, *J* = 7.9 Hz, 2H), 7.25 (t, *J* = 7.9 Hz, 1H), 4.45 (d, *J* = 5.9 Hz, 2H), 4.42 (d, *J* = 5.7 Hz, 4H), 2.28 (d, *J* = 1.0 Hz, 9H), 2.19 (s, 6H), 2.17 (s, 3H). ^{13}C NMR (125 MHz, DMSO) δ 168.21, 167.85, 167.82, 164.82, 164.77, 147.01, 142.83, 142.64, 140.18, 140.18, 131.40, 130.32, 130.10, 126.29, 126.15, 126.08, 125.99, 125.72, 125.58, 42.00, 38.76, 20.29, 20.16. HR-MS (ESI-TOF) *m/z*: calculated for $[C_{42}H_{38}N_4NaO_{17}]^+$ ($[M+Na]^+$) 893.2130, measured 893.2139.

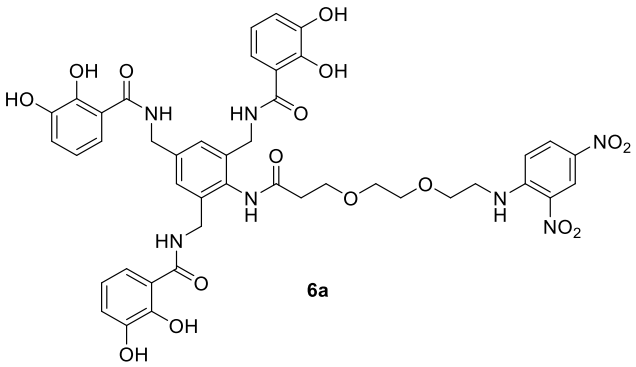


Scheme S3. Synthesis of compound **6a–6d**

General Procedure for Synthesis of 6a–6d

Compound **6a–6d** was synthesized from compound **4** as previously reported with minor procedural modifications.[1] Zinc dust (81.4 mg, 1.25 mmol) was added to a solution of compound **4** (72.3 mg, 83.0 μmol) in tetrahydrofuran (0.59 mL), ethanol (0.47 mL), and acetic acid (0.12 mL) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C and warmed to room temperature. After 20 min, the mixture was filtered over celite and the filter cake was rinsed with ethyl acetate. The combined organic extracts were washed with saturated sodium bicarbonate solution followed by brine, and then dried over anhydrous magnesium sulfate. After concentrated under reduced pressure, light brownish solid **S4** was obtained and used for the preparation of compound **S5-1–4** without further purification.

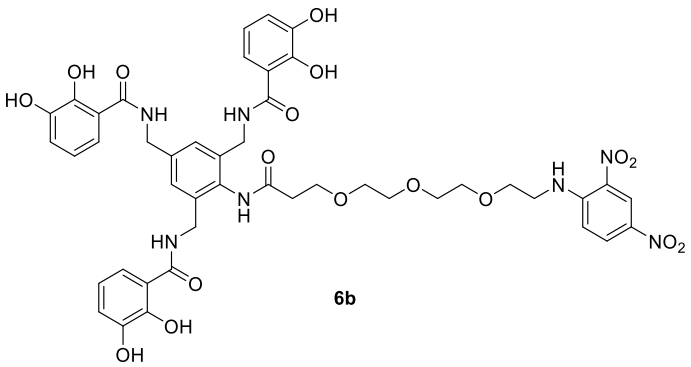
The solid was dissolved in tetrahydrofuran (0.83 mL), and this solution was treated with diisopropylethylamine (DIPEA, 0.25 mmol) followed by compound **5a–d** (0.66 mmol) dropwise at 0 ℃. The reaction mixture was slowly warmed to room temperature. After stirring for 1 h, the reaction was quenched by adding cold water, and the resulting suspension was extracted with ethyl acetate three times. The combined organic layers were washed with saturated sodium bicarbonate solution, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The resulting crude residue **S5-1–4** was immediately dissolved in a solution of 7 N ammonia in MeOH (0.83 mL) at 0 ℃ for global deprotection of *O*-acetates. The reaction mixture was slowly warmed to room temperature. After stirring for 4 h, the solvent was concentrated under reduced pressure and the resulting residue was purified by preparative HPLC (Isocratic elution at 38% sol. B over 40 min. Flow rate: 20 mL/min. Detection at 215 nm and 254 nm) to afford the desired products, **6a**–**6d**.



6a

N,N',N''-((2-(3-(2-(2-((2,4-dinitrophenyl)amino)ethoxy)ethoxy)propanamido)benzene-1,3,5-triyl)tris(methylene))tris(2,3-dihydroxybenzamide (6a)

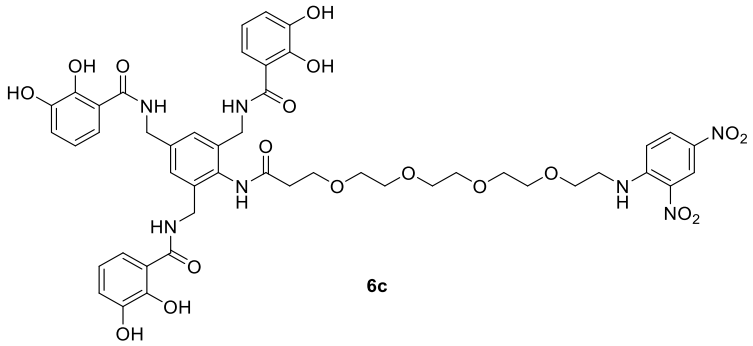
Compound **6a** (0.11 mmol, 13% yield, 3 steps) was synthesized from **4** using **5a** by following the general procedure described above. ^{1}H NMR (500 MHz, DMSO) δ 12.51 (s, 3H), 9.59 (s, 1H), 9.31 (t, *J* = 6.1 Hz, 1H), 9.11 (t, *J* = 6.1 Hz, 5H), 8.81 (d, *J* = 2.7 Hz, 1H), 8.77 (t, *J* = 5.6 Hz, 1H), 8.18 (dd, *J* = 9.6, 2.8 Hz, 1H), 7.25 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.19 (d, *J* = 8.9 Hz, 3H), 7.12 (d, *J* = 9.7 Hz, 1H), 6.89 (dd, *J* = 4.8, 1.4 Hz, 3H), 6.65 (t, *J* = 7.9 Hz, 2H), 6.60 (t, *J* = 7.9 Hz, 1H), 4.48 – 4.36 (m, 6H), 3.75 (t, *J* = 6.1 Hz, 2H), 3.65 (t, *J* = 5.5 Hz, 2H), 3.62 – 3.53 (m, 6H), 2.63 (t, *J* = 6.1 Hz, 2H). ^{13}C NMR (125 MHz, DMSO) δ 170.02, 169.73, 169.65, 149.58, 149.45, 148.18, 146.13, 146.09, 137.50, 135.93, 134.88, 132.14, 129.77, 129.59, 124.86, 123.44, 118.79, 117.96, 117.89, 117.19, 117.07, 115.25, 115.01, 114.88, 69.69, 69.63, 68.12, 67.03, 42.55, 42.12, 36.34. HR-MS (ESI-TOF) *m/z*: calculated for $[C_{43}H_{43}N_7NaO_{16}]^+$ ($[M+Na]^+$) 936.2664, measured 936.2676.



6b

N,N',N''-((2-(3-(2-(2-(2-((2,4dinitrophenyl)amino)ethoxy)ethoxy)ethoxy)propanamido)benzene-1,3,5-triyl)tris(methylene))tris(2,3-dihydroxybenzamide) (6b)

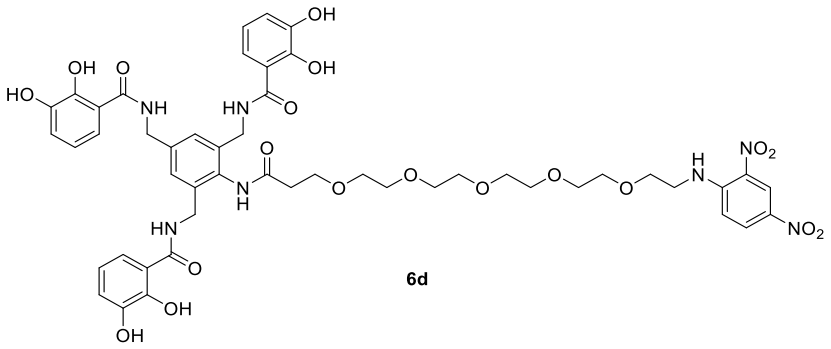
Compound **6b** (16 μmol, 20%, 3 steps) was synthesized from **4** using **5b** by following the general procedure described above. ^{1}H NMR (500 MHz, DMSO) δ 12.53 (d, *J* = 8.8 Hz, 3H), 9.59 (s, 1H), 9.31 (t, *J* = 6.0 Hz, 1H), 9.14 – 9.05 (m, 5H), 8.84 (d, *J* = 2.7 Hz, 1H), 8.78 (t, *J* = 5.4 Hz, 1H), 8.23 (dd, *J* = 9.6, 2.8 Hz, 1H), 7.27 (dd, *J* = 8.2, 1.4 Hz, 2H), 7.19 (m, 4H), 6.89 (dd, *J* = 11.1, 7.9 Hz, 3H), 6.66 (t, *J* = 7.9 Hz, 2H), 6.60 (t, *J* = 7.9 Hz, 1H), 4.46 – 4.38 (m, 6H), 3.73 (t, *J* = 6.1 Hz, 2H), 3.60 (t, *J* = 4.7 Hz, 2H), 3.58 (t, *J* = 5.0 Hz, 2H), 3.54 – 3.46 (m, 8H), 2.62 (t, *J* = 6.2 Hz, 2H). ^{13}C NMR (125 MHz, DMSO) δ 170.02, 169.73, 169.66, 149.57, 149.47, 148.26, 146.13, 146.09, 137.50, 135.94, 134.89, 132.15, 129.80, 129.63, 124.89, 123.46, 118.81, 117.97, 117.88, 117.20, 117.06, 115.45, 115.04, 114.88, 69.69, 69.66, 69.63, 69.58, 68.09, 66.99, 42.57, 42.12, 36.36. HR-MS (ESI-TOF) *m/z*: calculated for $[C_{45}H_{47}N_7NaO_{17}]^+$ ([M+Na]$^+$) 980.2926, measured 980.2927.



6c

N,N',N''-((2-(1-((2,4-dinitrophenyl)amino)-3,6,9,12-tetraoxapentadecan-15-amido)benzene-1,3,5-triyl)tris(methylene))tris(2,3-dihydroxybenzamide) (6c)

Compound **6c** (0.22 mmol, 27% yield, 3 steps) was synthesized from **4** using **5c** by following the general procedure described above. ^{1}H NMR (500 MHz, MeOD) δ 8.93 (d, *J* = 2.7 Hz, 1H), 8.17 (dd, *J* = 9.6, 2.7 Hz, 1H), 7.42 (s, 2H), 7.15 (ddd, *J* = 10.5, 8.1, 1.5 Hz, 3H), 6.98 (d, *J* = 9.6 Hz, 1H), 6.87 (m, 3H), 6.64 (td, *J* = 8.0, 4.7 Hz, 3H), 4.56 (s, 6H), 3.84 (t, *J* = 5.7 Hz, 2H), 3.63 – 3.55 (m, 5H), 3.52 – 3.41 (m, 12H), 3.40 – 3.36 (m, 2H), 2.72 (t, *J* = 5.7 Hz, 2H). ^{13}C NMR (125 MHz, DMSO) δ 170.00, 169.72, 169.67, 149.58, 149.49, 148.29, 146.13, 146.08, 137.49, 135.93, 134.86, 132.14, 129.80, 129.63, 124.85, 123.48, 118.81, 117.96, 117.87, 117.19, 117.05, 115.52, 115.01, 114.86, 69.69, 69.64, 69.60, 69.56, 68.14, 66.98, 42.59, 42.11, 36.34. HR-MS (ESI-TOF) *m/z*: calculated for $[C_{47}H_{51}N_7NaO_{18}]^+$ ([M+Na]$^+$) 1024.3188, measured 1024.3220.

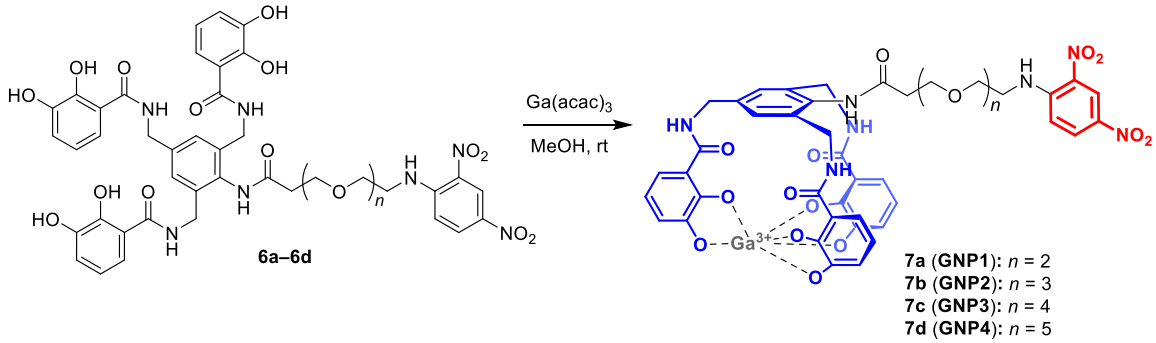


6d

N,N',N''-((2-(1-((2,4-dinitrophenyl)amino)-3,6,9,12,15-pentaoxaoctadecan-18-amido)benzene-

1,3,5-triyl)tris(methylene))tris(2,3-dihydroxybenzamide) (6d)

Compound **6d** (0.16 mmol, 19% yield, 3 steps) was synthesized from **4** using **5d** by following the general procedure described above. ^{1}H NMR (500 MHz, MeOD) δ 8.95 (d, J = 2.7 Hz, 1H), 8.17 (s, 1H), 7.42 (s, 2H), 7.17 (dd, J = 8.1, 1.4 Hz, 2H), 7.15 (dd, J = 8.1, 1.4 Hz, 1H), 7.05 (d, J = 9.6 Hz, 1H), 6.88 (dd, J = 7.8, 1.4 Hz, 3H), 6.65 (m, 3H), 4.56 (s, 6H), 3.86 – 3.78 (m, 3H), 3.68 (td, J = 5.2, 2.4 Hz, 4H), 3.62 – 3.50 (m, 12H), 3.45 (m, 7H), 3.35 (s, 2H), 2.72 (t, J = 5.6 Hz, 2H). ^{13}C NMR (125 MHz, DMSO) δ 170.02, 169.72, 169.67, 149.58, 149.49, 148.31, 146.13, 146.08, 137.49, 135.94, 134.88, 132.15, 129.80, 129.64, 124.88, 123.48, 118.82, 117.97, 117.88, 117.20, 117.06, 115.54, 115.03, 114.88, 69.73, 69.69, 69.66, 69.62, 69.57, 68.18, 66.99, 42.63, 42.12, 36.36. HR-MS (ESI-TOF) *m/z*: calculated for $[C_{49}H_{55}N_7NaO_{19}]^+$ ($[M+Na]^+$) 1068.345, measured 1068.3469.



Scheme S4. Synthesis of compound **7a–7d (GNP1–4)**

General procedure for Ga(III) complexation on 6a–6d to yield GNP1–4

To a solution of compound **6a–6d** (1 equiv.) in MeOH (1.0 mL/μmol) was added to Ga(III) acetylacetonate ($Ga(acac)_3$, 1.1 equiv.). After shaking for 1 h, the solvent was evaporated under reduced pressure. The resulting residue was resuspended in ether and centrifuged at 6000 *g* for 3 minutes. The supernatant was removed, and this process was repeated three times to remove residual $Ga(acac)_3$. Then, the resulting residue was completely dried under reduced pressure and was used for biological characterizations without further purification.

SUPPLEMENTARY REFERENCES

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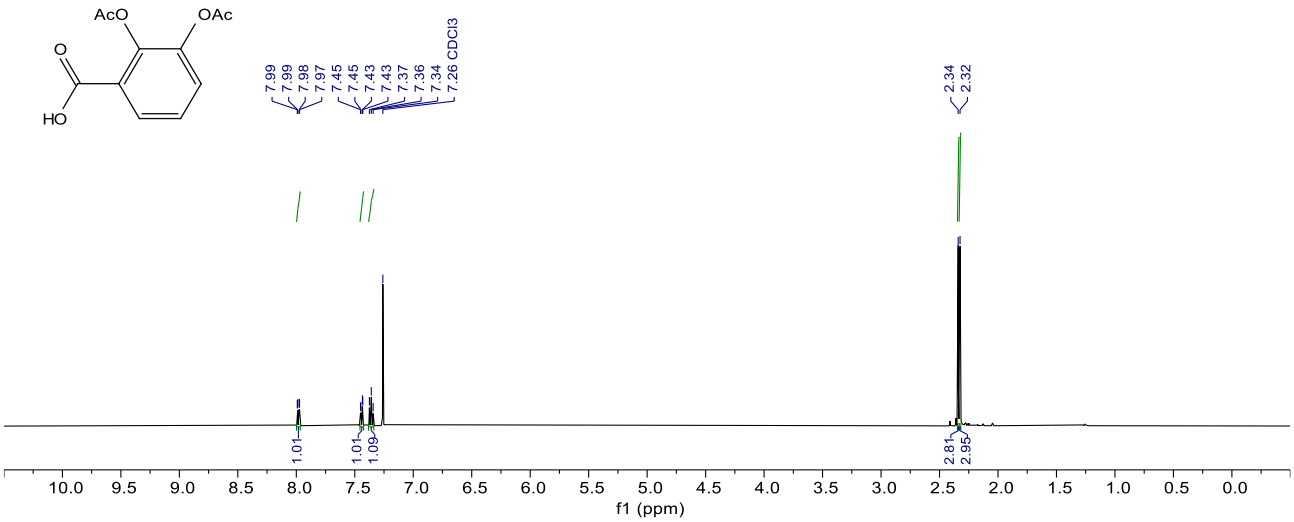
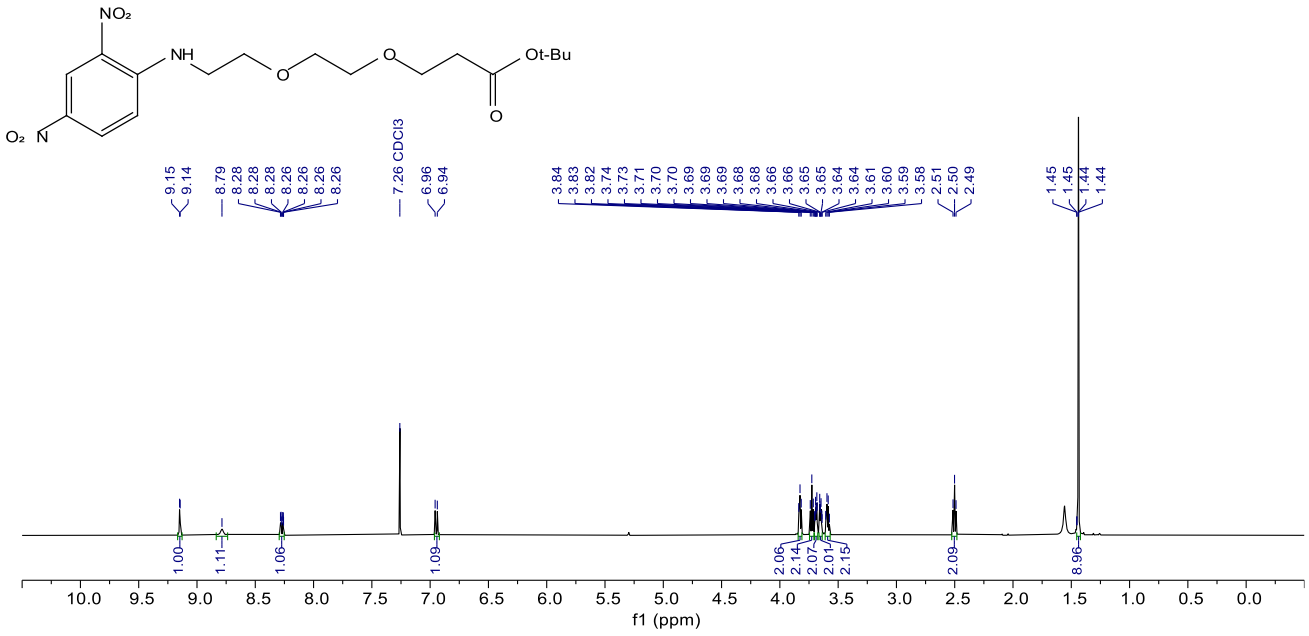
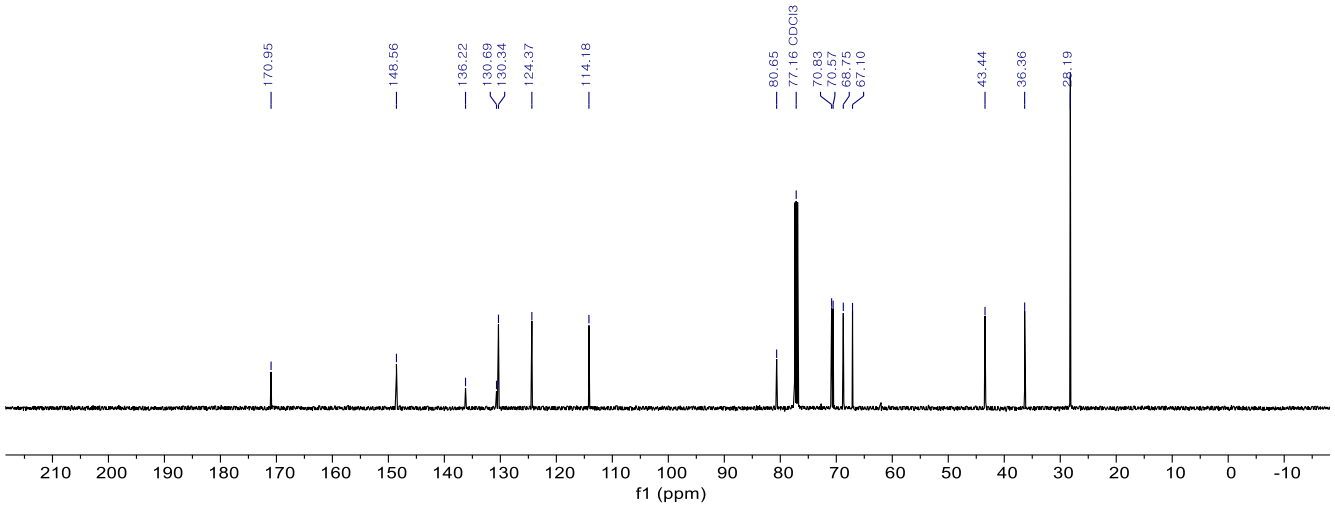
SPECTRAL DATA FOR COMPOUNDS



^{1}H NMR data of compound **S2** (500 MHz, $CDCl_3$)



^{1}H NMR data of compound **8a** (500 MHz, $CDCl_3$)



^{13}C NMR data of compound **8a** (125 MHz, $CDCl_3$)

Compound Spectrum SmartFormula Report

Analysis Info

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Sample Name	end method	Instrument	compact 8255754.20060
Comment			

Acquisition Parameter

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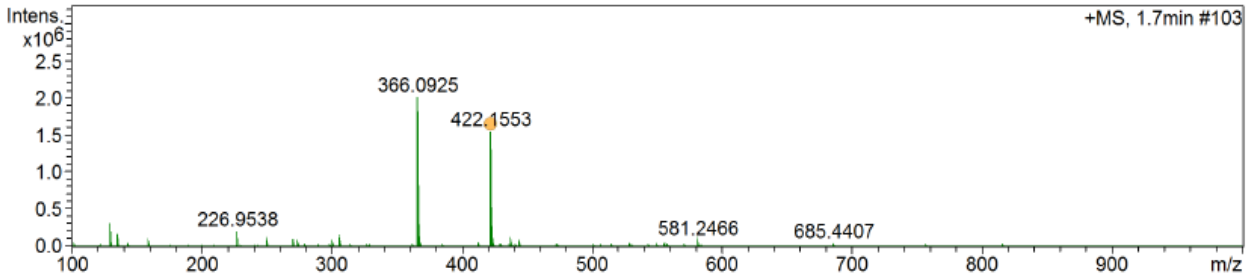
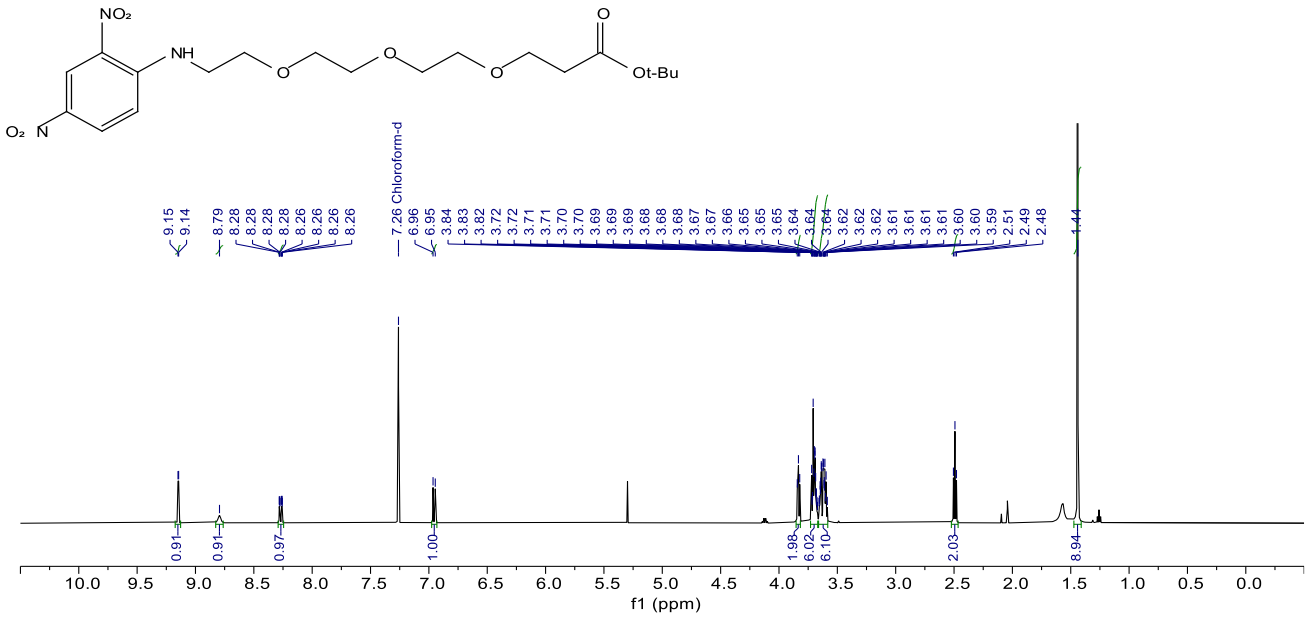
		Set Corona	0 nA	Set APCI Heater	0 °C

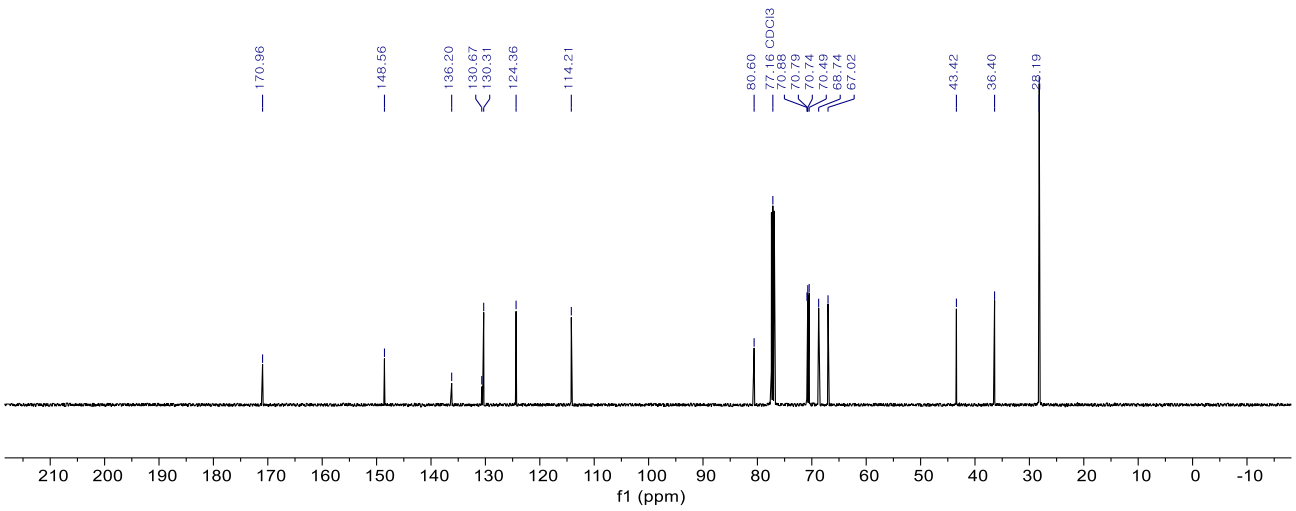
+MS, 1.7min #103



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e¯ Conf	N-Rule
422.1553	1	C17H25N3NaO8	422.1534	-4.6	4.1	1	100.00	6.5	even	ok



^{1}H NMR data of compound **8b** (500 MHz, $CDCl_3$)



^{3}C NMR data of compound **8b** (125 MHz, $CDCl_3$)

Compound Spectrum SmartFormula Report

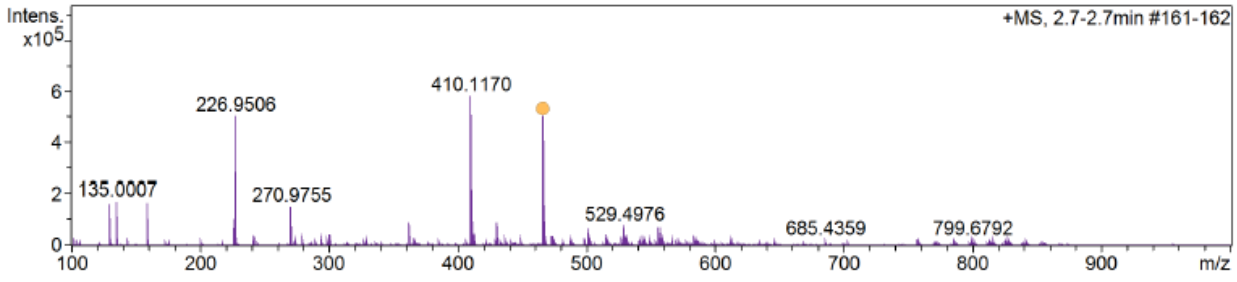
Analysis Info

Analysis Name	D:\Data\MCCB_Data\KSW\immuno modulator\8b.d	Acquisition Date	8/28/2024 12:04:14 PM
Method	end_method_neg.m	Operator	BDAL@DE
Sample Name	end method	Instrument	compact 8255754.20060
Comment			

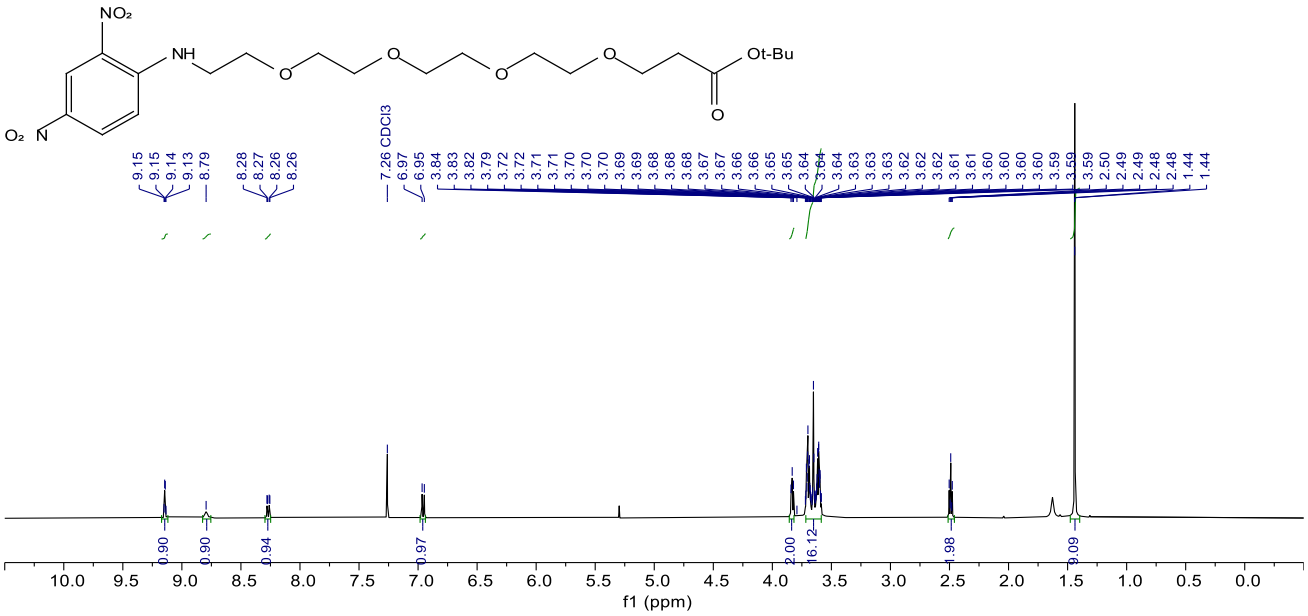
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.8 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

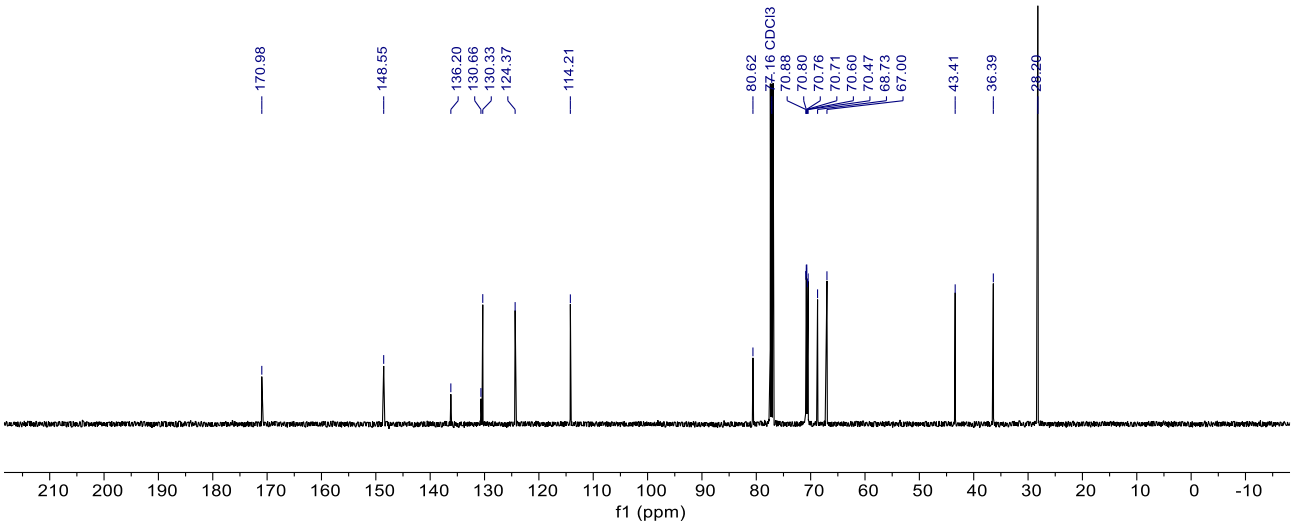
+MS, 2.7-2.7min #161-162



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
466.1800	1	C19H29N3NaO9	466.1796	-0.8	8.8	1	100.00	6.5	even	ok



^{1}H NMR data of compound **8c** (500 MHz, $CDCl_3$)



^{13}C NMR data of compound **8c** (125 MHz, $CDCl_3$)

Compound Spectrum SmartFormula Report

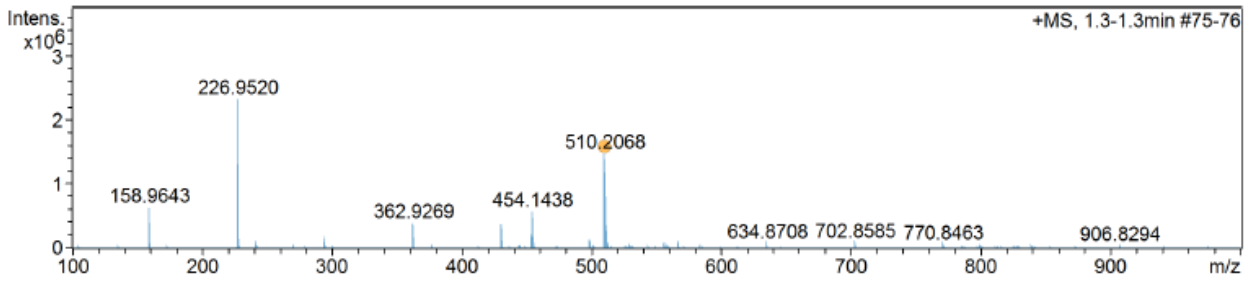
Analysis Info

Analysis Name	D:\Data\MCCB_Data\KSW\immuno modulator\8c-3.d	Acquisition Date	8/28/2024 12:46:38 PM
Method	end_method_neg.m	Operator	BDAL@DE
Sample Name	end method	Instrument	compact 8255754.20060
Comment			

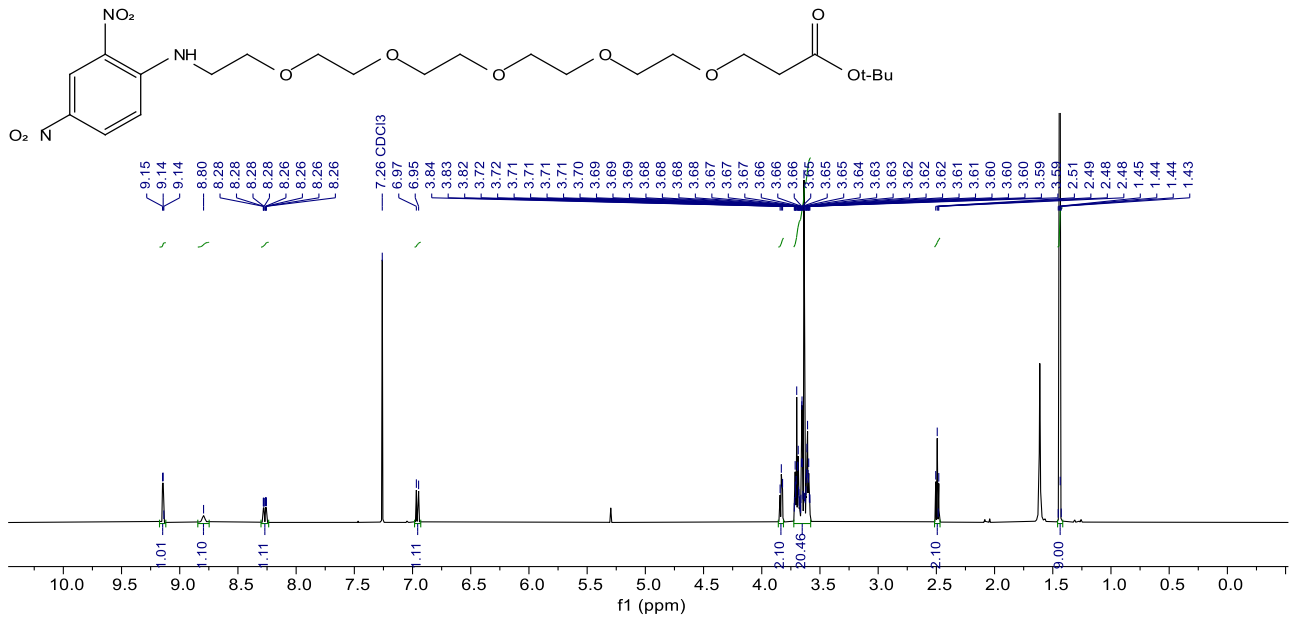
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.8 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

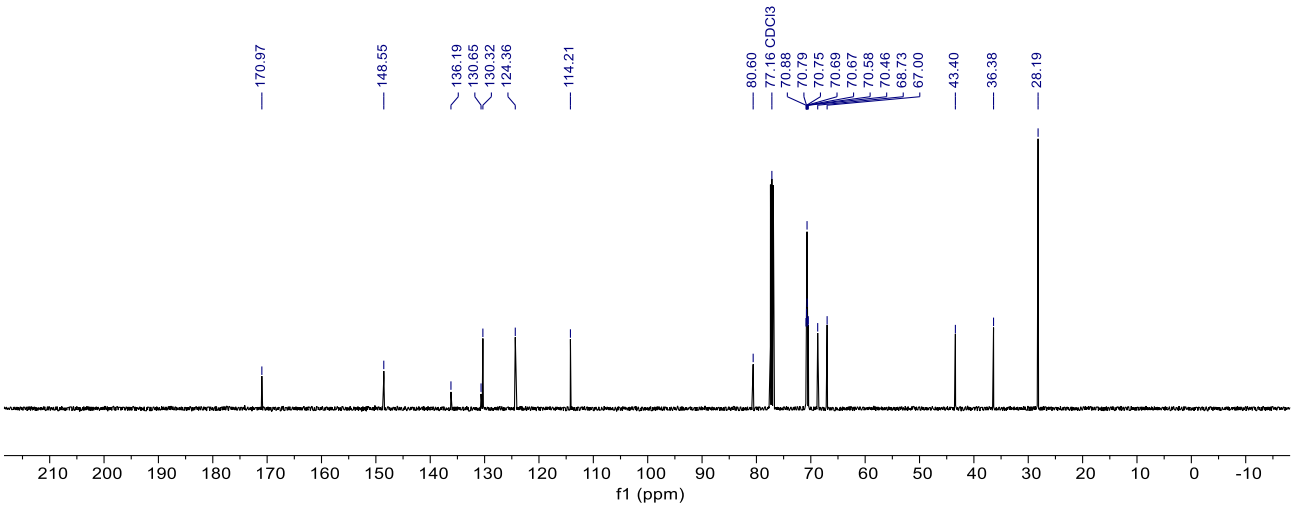
+MS, 1.3-1.3min #75-76



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
510.2068	1	C21H33N3NaO10	510.2058	-1.8	3.3	1	100.00	6.5	even	ok



^{1}H NMR data of compound **8d** (500 MHz, $CDCl_3$)



^{13}C NMR data of compound **8d** (125 MHz, $CDCl_3$)

Compound Spectrum SmartFormula Report

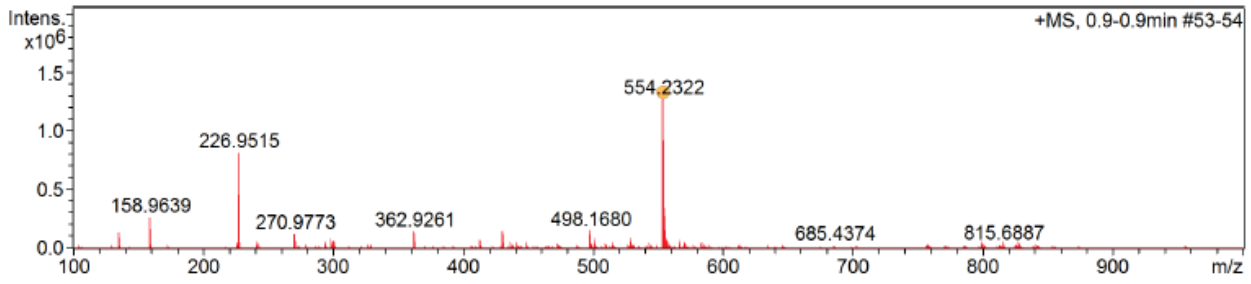
Analysis Info

Analysis Name	D:\Data\MCCB_Data\KSW\immuno modulator\8d.d	Acquisition Date	8/28/2024 12:53:12 PM
Method	end_method_neg.m	Operator	BDAL@DE
Sample Name	end method	Instrument	compact 8255754.20060
Comment			

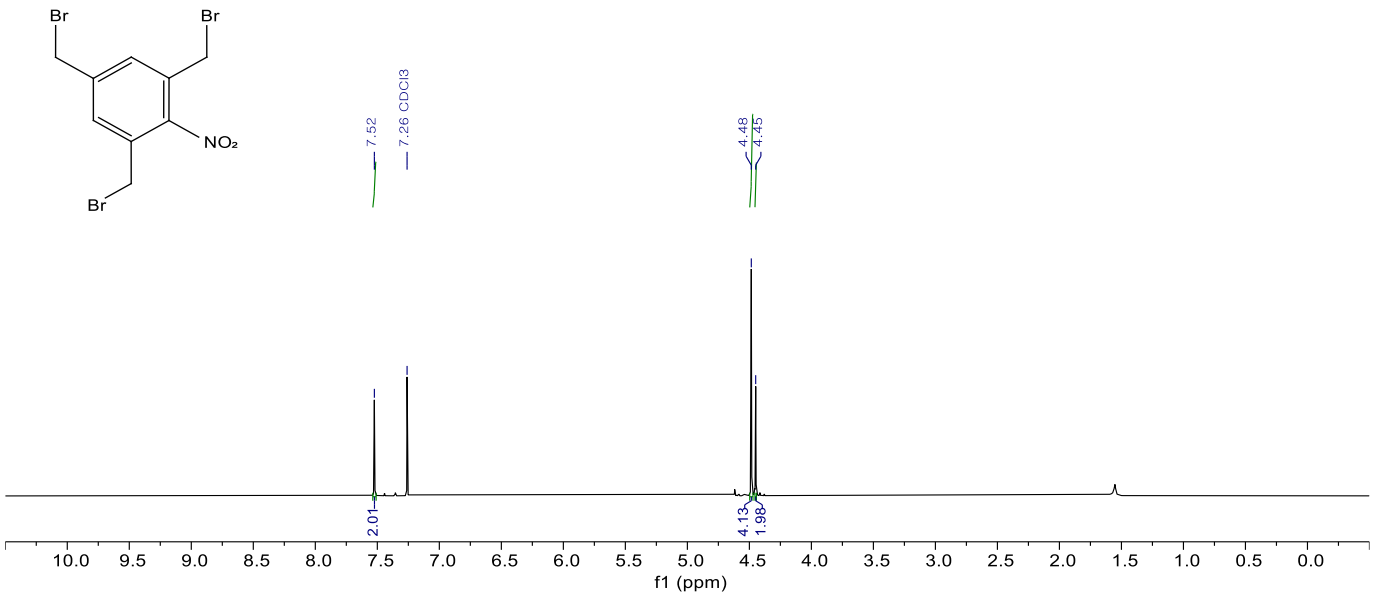
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.8 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1000 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

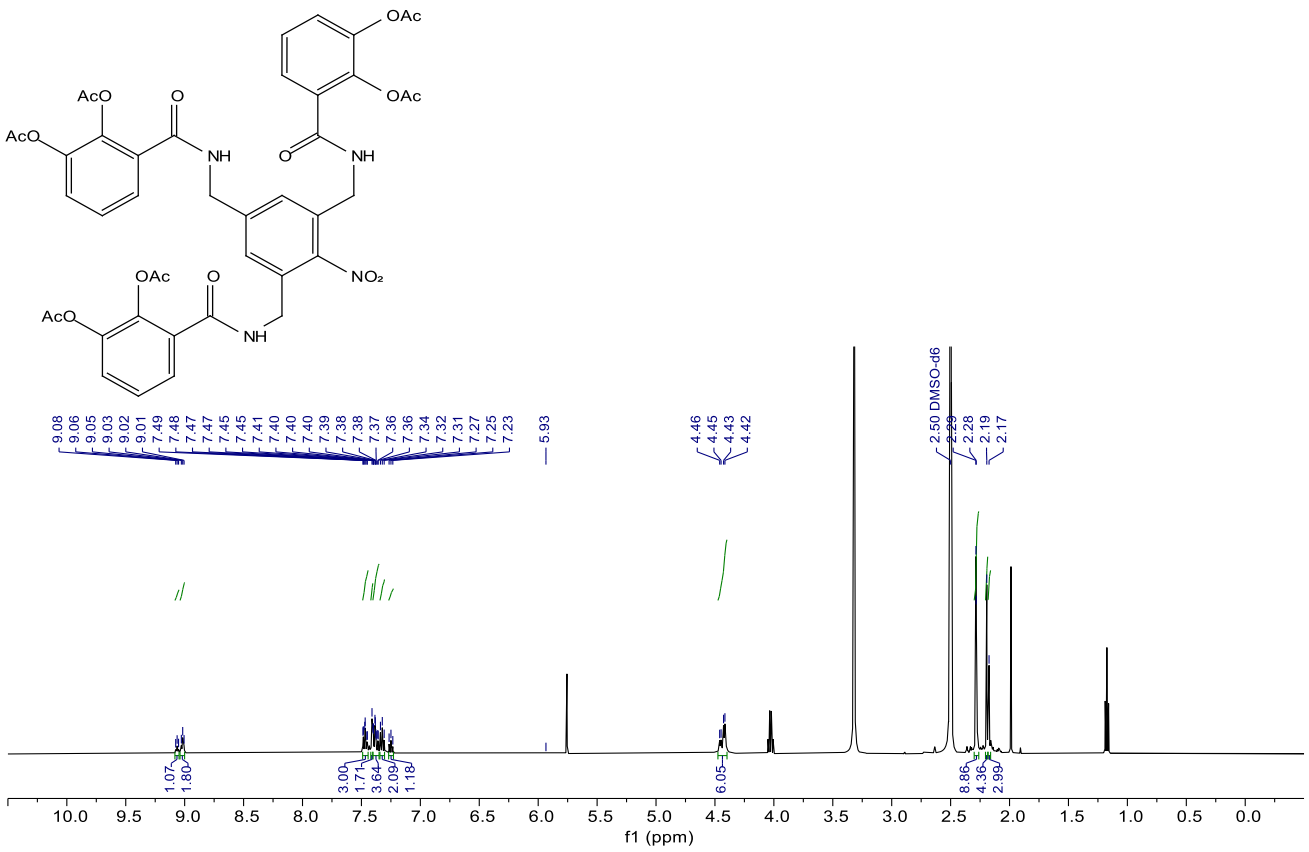
+MS, 0.9-0.9min #53-54



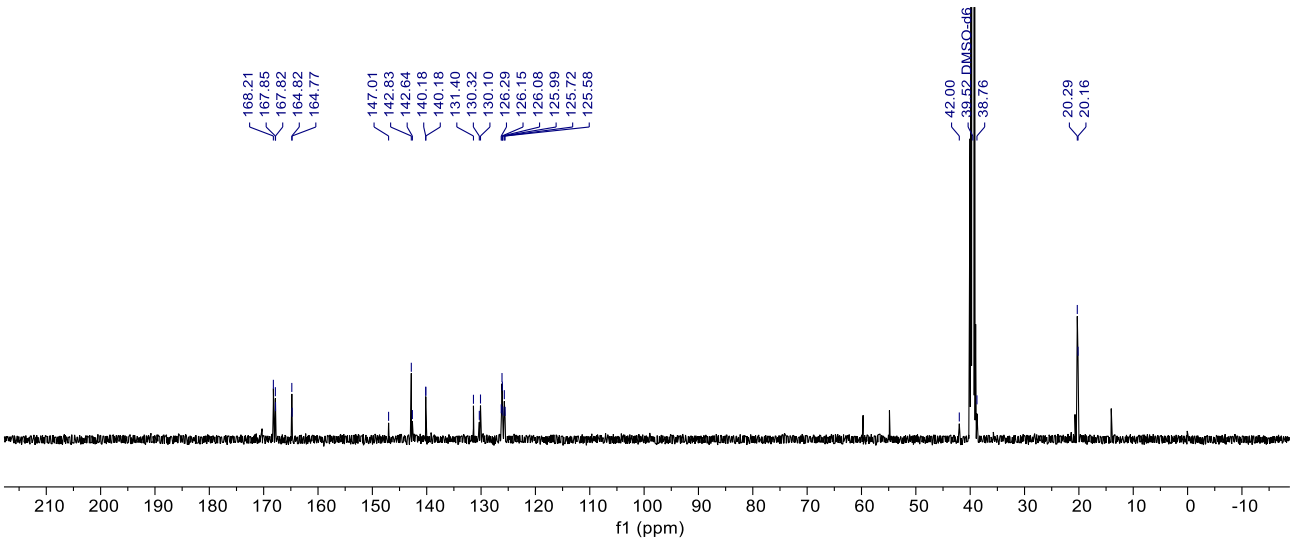
Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
554.2322	1	C23H37N3NaO11	554.2320	-0.3	5.0	1	100.00	6.5	even	ok



^{1}H NMR data of compound **2** (500 MHz, $CDCl_3$)



^{1}H NMR data of compound **4** (500 MHz, DMSO)



^{13}C NMR data of compound **4** (125 MHz, DMSO)

Compound Spectrum SmartFormula Report

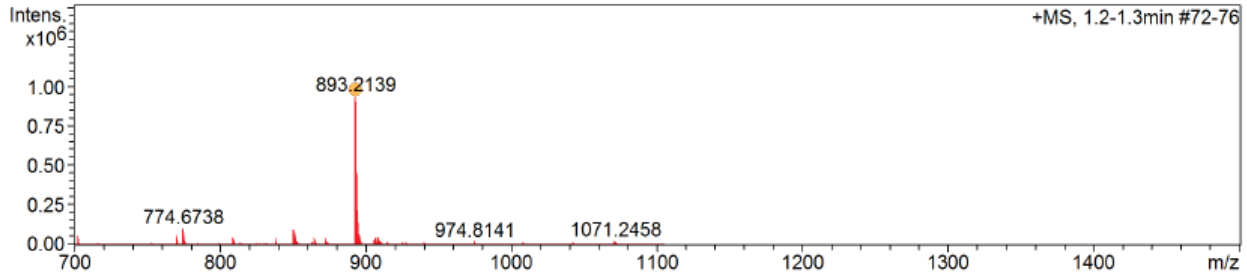
Analysis Info

Analysis Name	D:\Data\MCCB_Data\KSW\immuno modulator\4_ Nitro_MC5(Ac) pos.d	Acquisition Date	8/30/2024 4:00:30 PM
Method	end_method_neg.m	Operator	BDAL@DE
Sample Name	end method	Instrument	compact 8255754.20060
Comment			

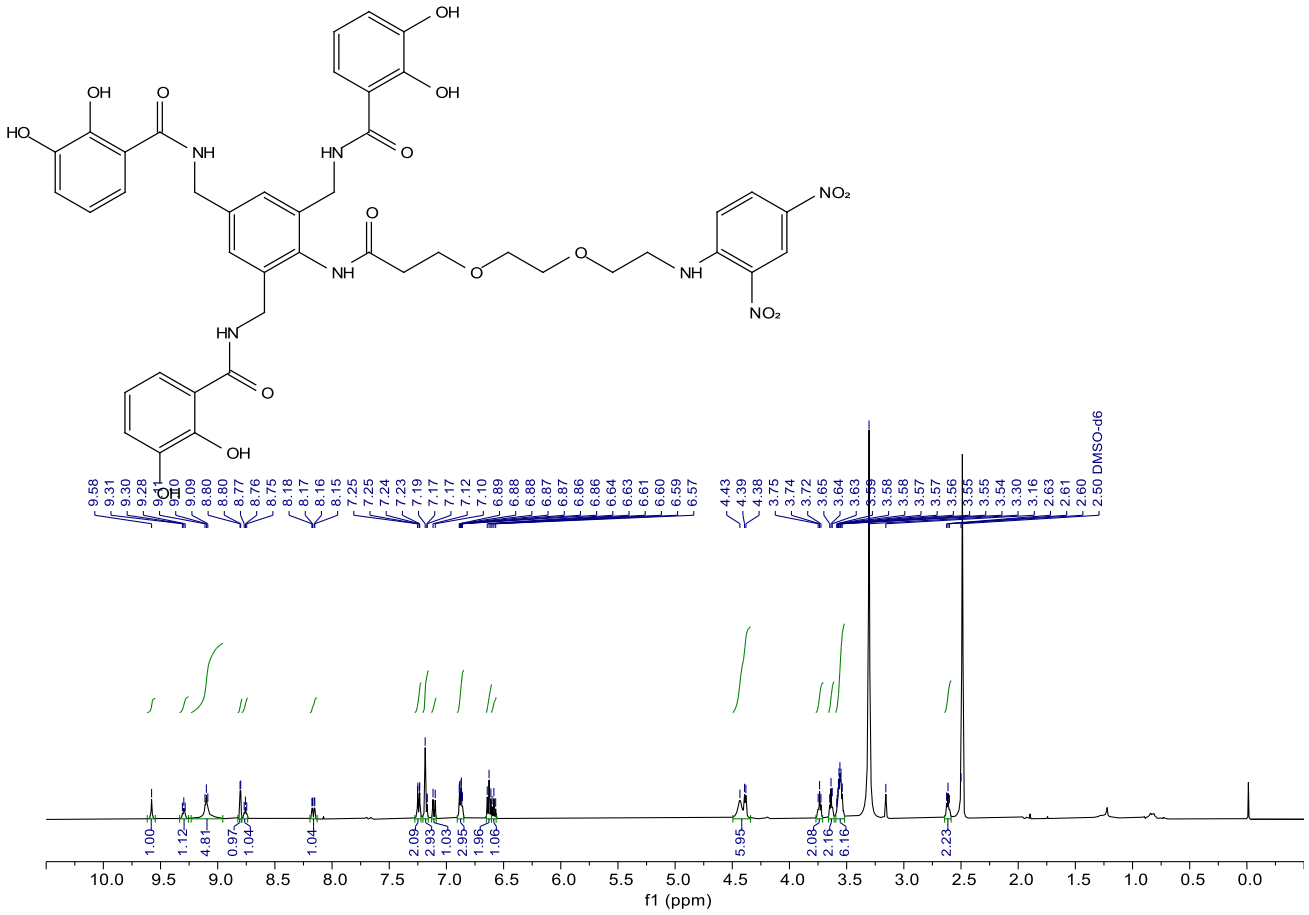
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.8 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	500 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1100 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

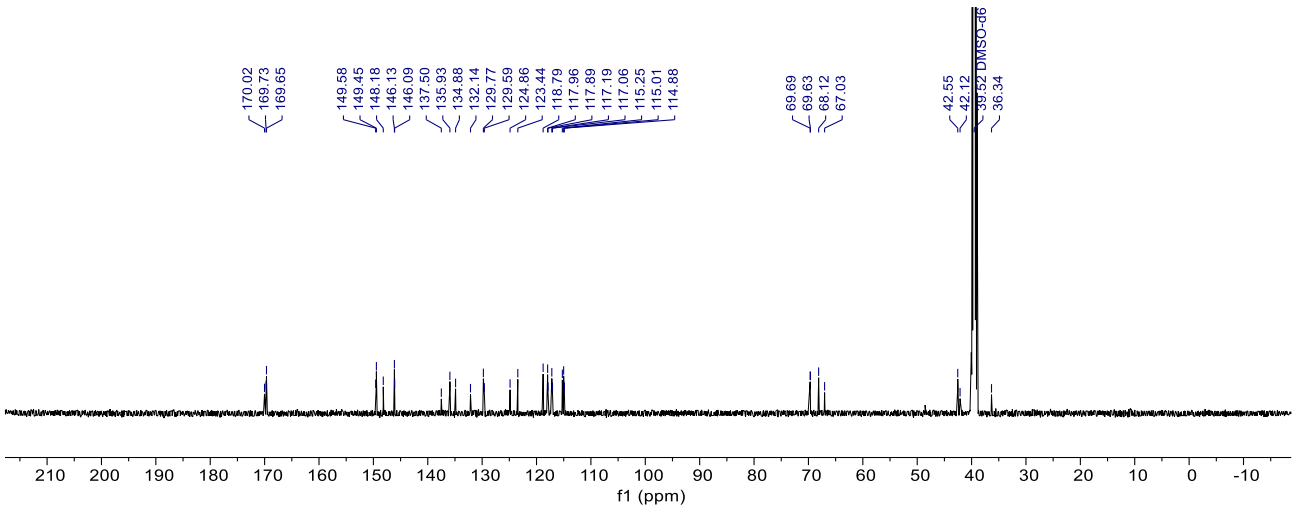
+MS, 1.2-1.3min #72-76



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e¯ Conf	N-Rule
893.2139	1	C42H38N4NaO17	893.2124	-1.7	8.7	1	100.00	25.5	even	ok



^{1}H NMR data of compound **6a** (500 MHz, DMSO)



^{13}C NMR data of compound **6a** (125 MHz, DMSO)

Compound Spectrum SmartFormula Report

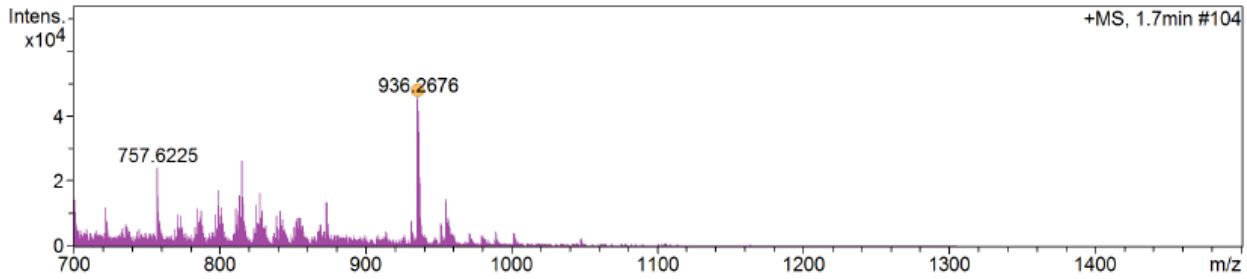
Analysis Info

Analysis Name	D:\Data\MCCB_Data\KSW\immuno modulator\6a.d	Acquisition Date	8/28/2024 1:03:29 PM
Method	end_method_neg.m	Operator	BDAL@DE
Sample Name	end method	Instrument	compact 8255754.20060
Comment			

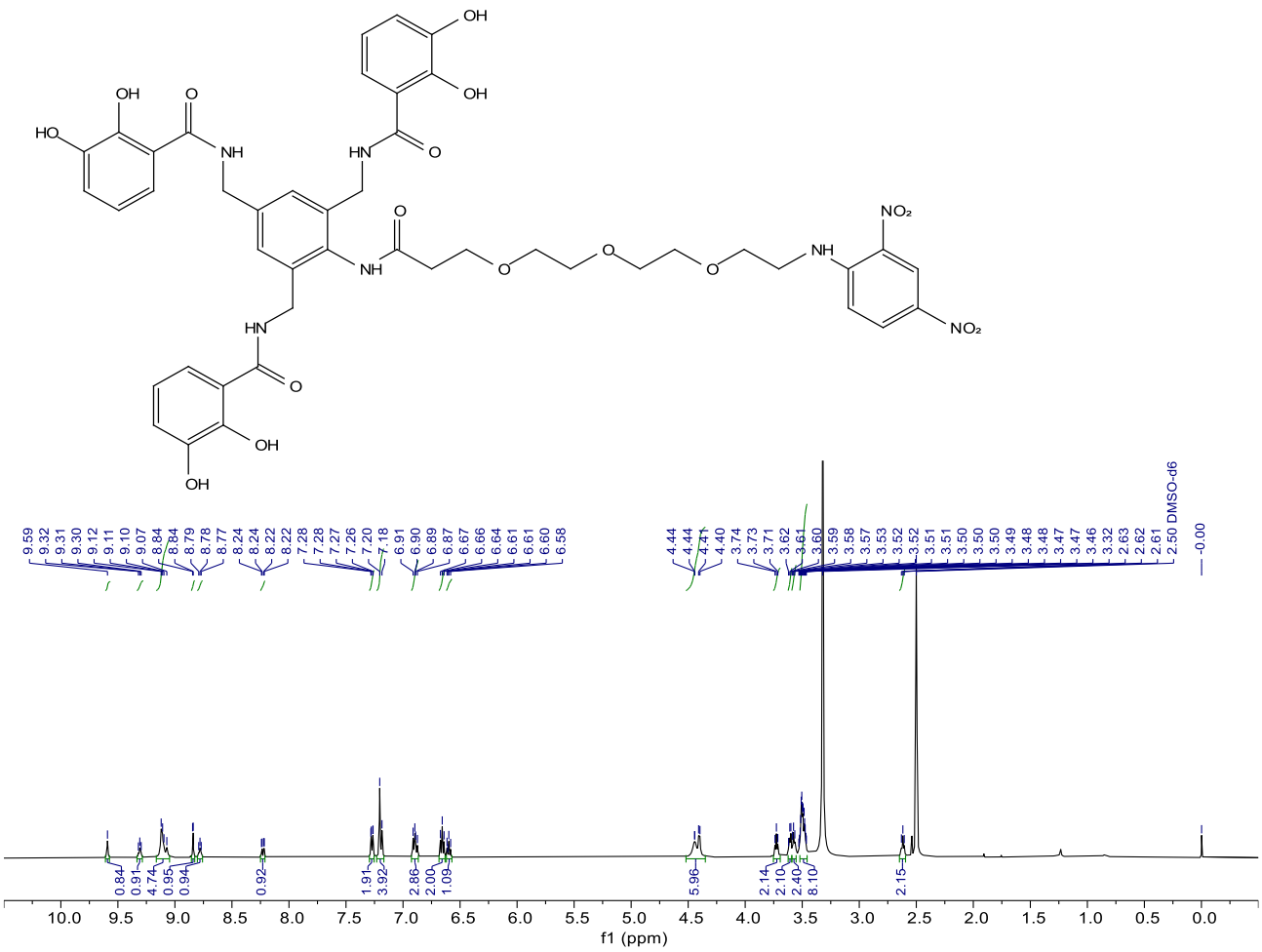
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.8 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	700 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1300 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

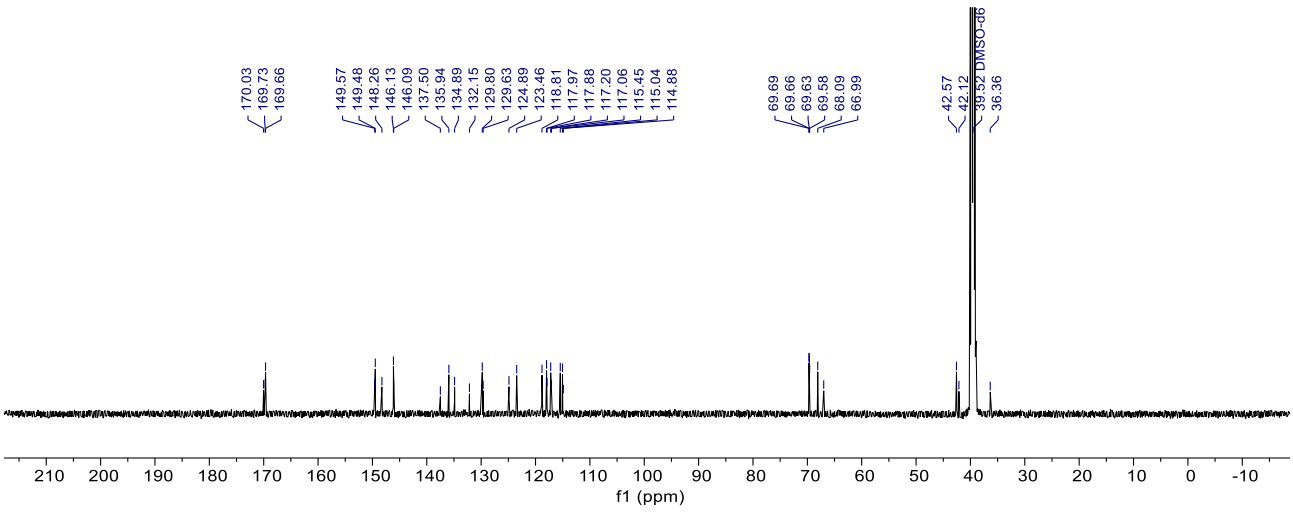
+MS, 1.7min #104



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e¯ Conf	N-Rule
936.2676	1	C43H43N7NaO16	936.2658	-1.9	24.7	1	100.00	25.5	even	ok
	2	C48H43N5NaO14	936.2699	2.4	41.4	2	57.13	29.5	even	ok



^{1}H NMR data of compound **6b** (500 MHz, DMSO)



^{13}C NMR data of compound **6b** (125 MHz, DMSO)

Compound Spectrum SmartFormula Report

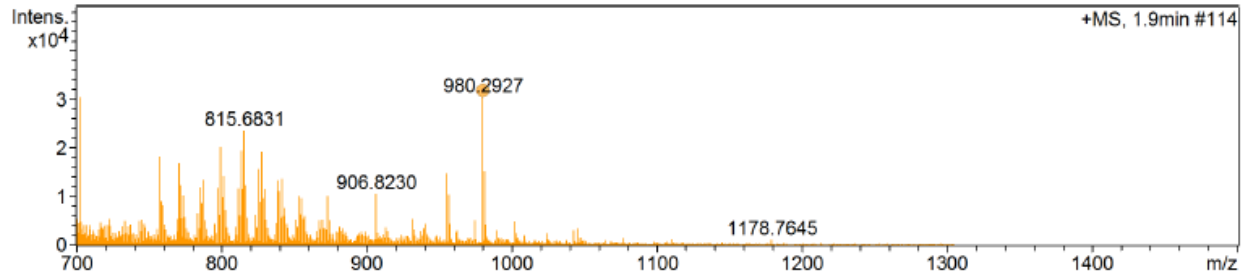
Analysis Info

Analysis Name	D:\Data\MCCB_Data\KSW\immuno modulator\6b.d	Acquisition Date	8/28/2024 1:39:47 PM
Method	end_method_neg.m	Operator	BDAL@DE
Sample Name	end method	Instrument	compact 8255754.20060
Comment			

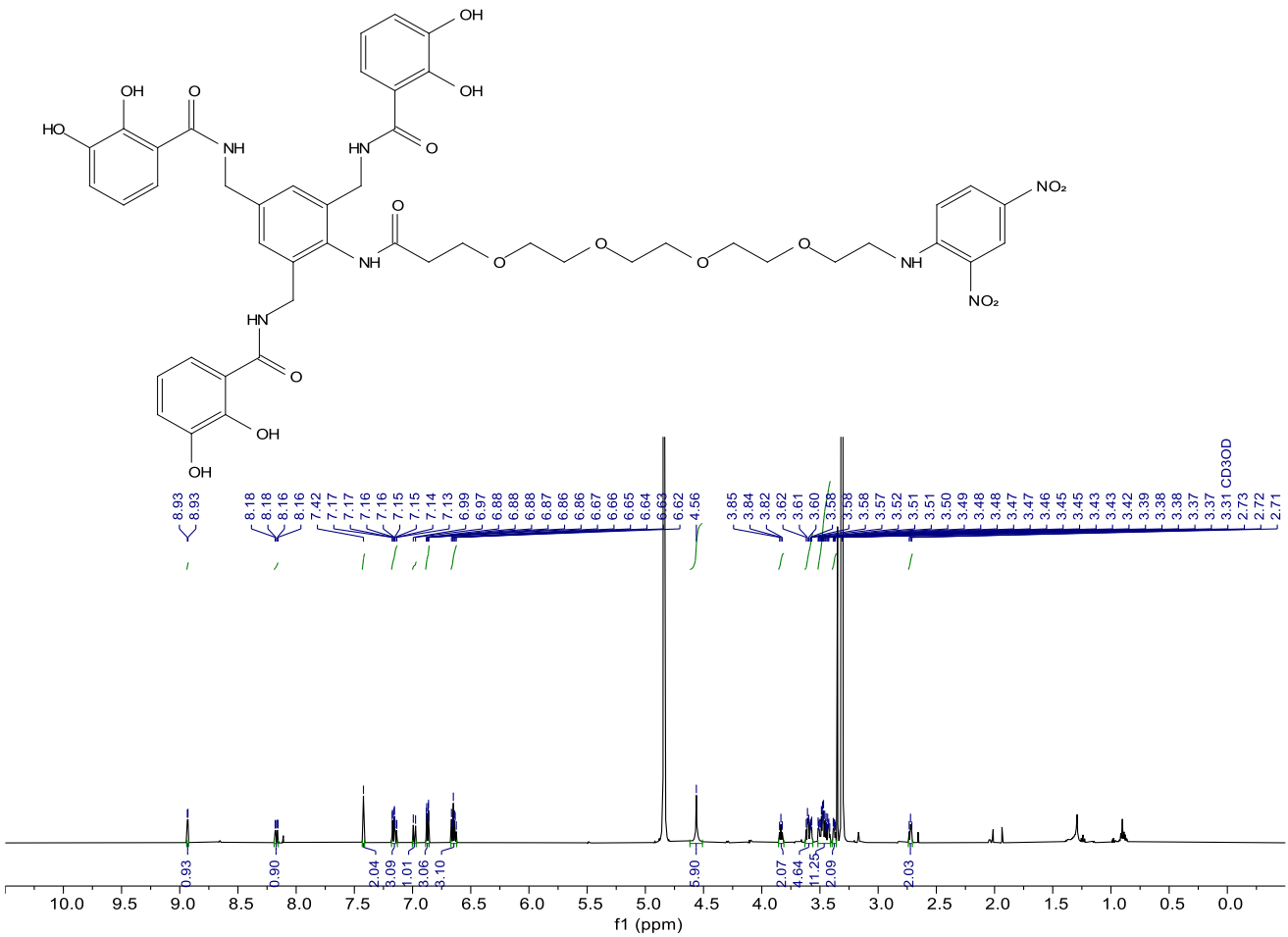
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.8 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	700 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1300 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

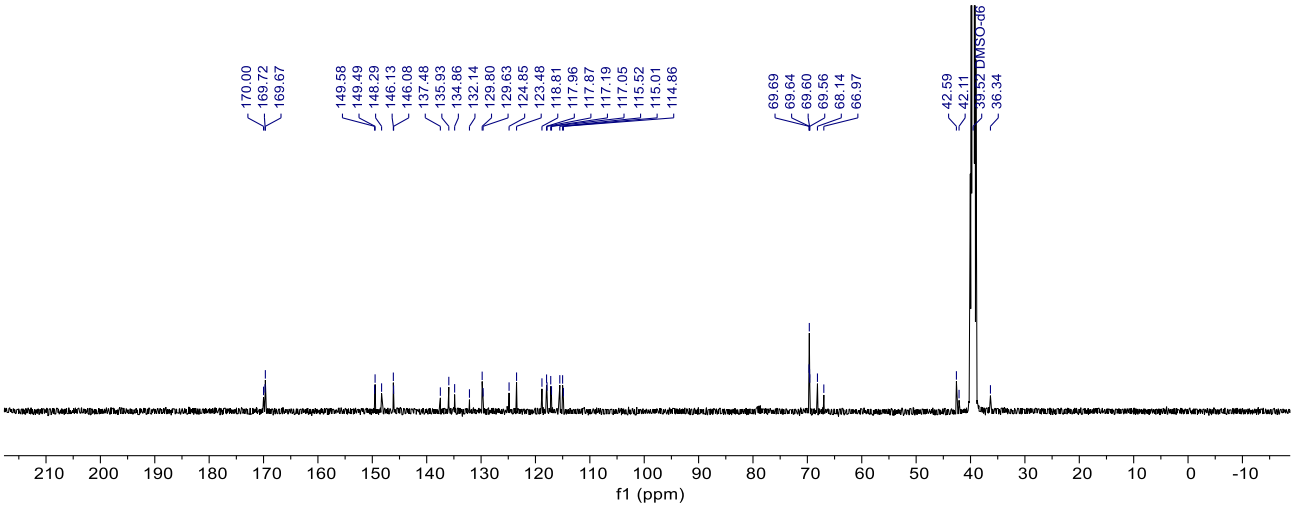
+MS, 1.9min #114



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e¯ Conf	N-Rule
980.2927	1	C45H47N7NaO17	980.2921	-0.6	19.3	1	100.00	25.5	even	ok



^{1}H NMR data of compound **6c** (500 MHz, MeOD)



^{13}C NMR data of compound **6c** (125 MHz, DMSO)

Compound Spectrum SmartFormula Report

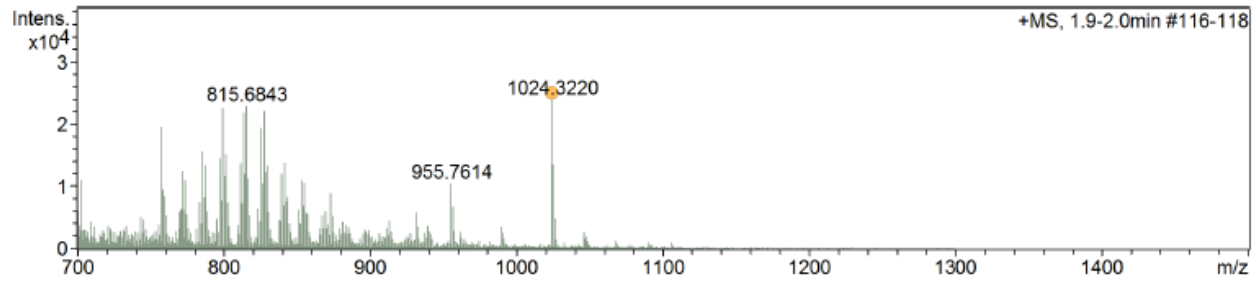
Analysis Info

Analysis Name	D:\Data\MCCB_Data\KSW\immuno modulator\6c-4.d	Acquisition Date	8/28/2024 3:15:00 PM
Method	end_method_neg.m	Operator	BDAL@DE
Sample Name	end method	Instrument	compact 8255754.20060
Comment			

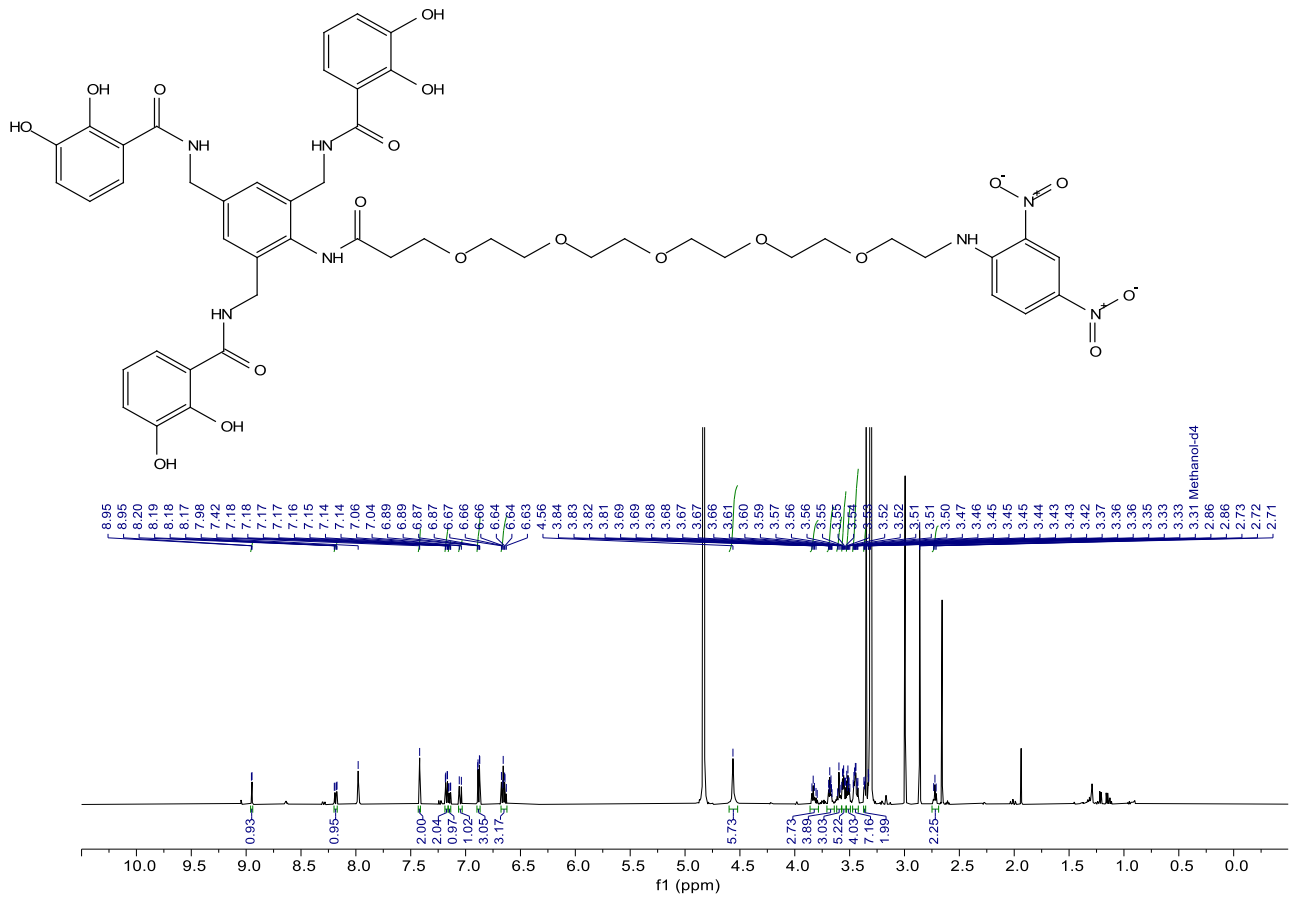
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.8 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	700 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1300 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

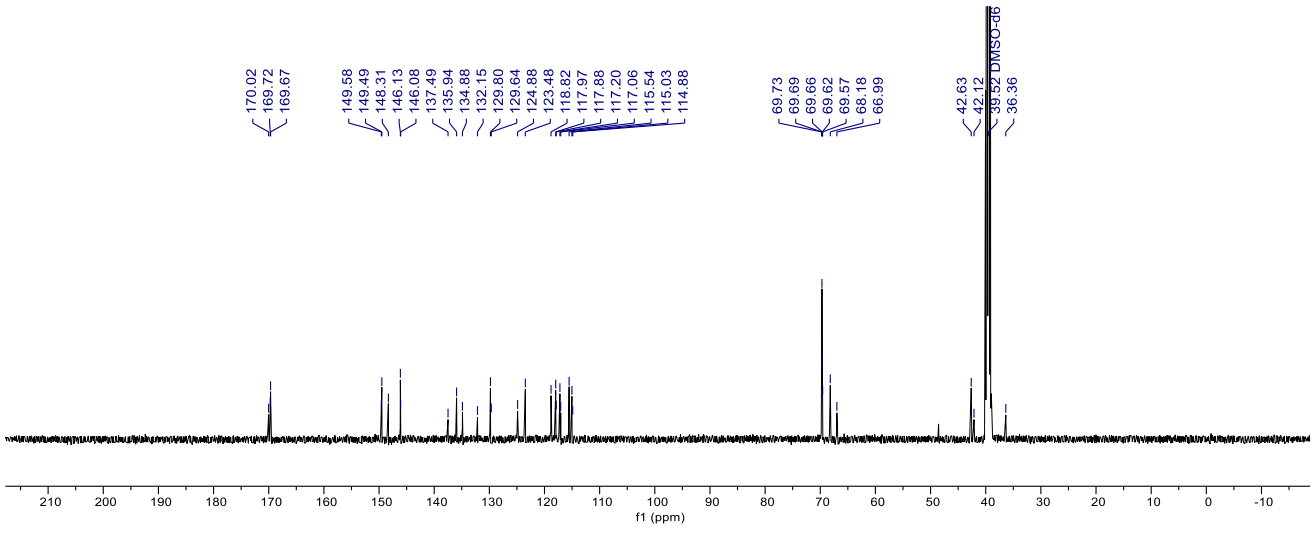
+MS, 1.9-2.0min #116-118



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
1024.3220	1	C47H51N7NaO18	1024.3183	-3.6	17.3	1	100.00	25.5	even	ok



^{1}H NMR data of compound **6d** (500 MHz, MeOD)



^{13}C NMR data of compound **6d** (125 MHz, DMSO)

Compound Spectrum SmartFormula Report

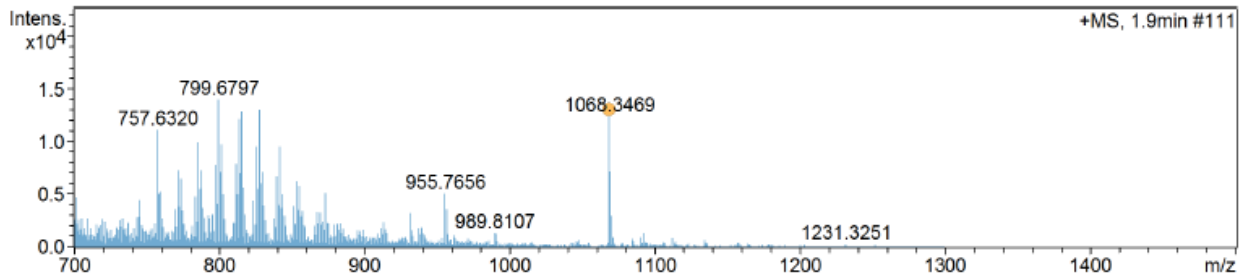
Analysis Info

Analysis Name	D:\Data\MCCB_Data\KSW\immuno modulator\6d.d	Acquisition Date	8/28/2024 3:27:29 PM
Method	end_method_neg.m	Operator	BDAL@DE
Sample Name	end method	Instrument	compact 8255754.20060
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.8 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	700 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	1300 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Source
		Set Corona	0 nA	Set APCI Heater	0 °C

+MS, 1.9min #111



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e¯ Conf	N-Rule
1068.3469	1	C49H55N7NaO19	1068.3445	-2.2	24.6	1	100.00	25.5	even	ok