Electronic Supplementary Information for

Supramolecular Nanoparticle Based on β-CD Modified Hyaluronic Acid for DNA Capsulation and Controlled Release

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Experimental Section

Material. All chemicals were reagent-grade unless noted otherwise. *β*-Cyclodextrin modified hyaluronic acid (**HACD**) was prepared according to the reported procedures[1-2], and the molecular weight of hyaluronic acid (HA) is 290 kDa. Dimethyl 5-hydroxyisophthalate, 1-adamantyl bromomethyl ketone, *N,N'*-dicyclohexyl-carbodiimide (DCC), N-hydroxysuccinimide (NHS), 3-dimethylaminopropylamine, and ethyl bromoacetate were purchased from commercial sources and used as received. Column chromatography was performed on 200–300 mesh silica gel.

Instruments. NMR spectra were recorded on a Bruker AV400 instrument. Mass spectra were performed on a Agilent 6520 Q-TOF LC/MS instrument. For the AFM measurements, a sample solution (0.1 mg/mL) was dropped onto newly clipped mica and air-dried, and the residue obtained was examined in tapping mode in the air under ambient conditions using a Veeco Nano IIIa Multimode AFM instrument. HR-TEM images were obtained on a Tecnai G^2 F20 microscope instrument operated at 200 kV. The samples were prepared by placing a drop of solution (0.1 mg/mL) onto a carbon-coated copper grid. The DLS experiments were performed on a Nano-ZS90 at λ = 636 nm at 25 °C. All DLS measurements were performed at the scattering angle of 90°.The zeta potentials were recorded on a Nano-ZS90 at 25 °C.

Synthesis of compound 1. Dimethyl 5-hydroxyisophthalate (1.05 g, 5 mmol) and 1-adamantyl bromomethyl ketone (1.03 g, 4 mmol) were dissolved in acetone (50 mL), and potassium carbonate (2.07 g, 15 mmol) was added. The mixture was

refluxed at 60 °C over night, and the reaction was monitored by TLC method. After cooling to room temperature, the solution was filtered, and the filtrate was dried under reduced pressure to remove the solvent. The residue was purified by column chromatography (silica gel, pure dichloromethane as eluent) to give compound **1** as white powder (1.5 g, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.73-1.81 (m, 6H, H of adamantane), 1.94-1,95 (m, 6H, H of adamantane), 2.10 (s, 3H, H of adamantane), 3.93 (s, 6H, H of methyl group), 4.95 (s, 2H, H of CH_2), 7.70 (d, J = 1.4 Hz, 2H, H of benzene), 8.28-8.29 ppm (t, J = 1.4 Hz, 1H, H of benzene); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 27.9, 36.5, 38.2, 45.8, 52.6, 68.8, 120.1, 123.7, 131.9, 158.2, 166.1, 208.3 ppm; ESI-MS: m/z: 404.2069 $[M + H]^+$.

Synthesis of compound 2. Compound **1** (410 mg, 1.06 mmol) was dissolved in THF (50 mL), and then 10 mL 10% potassium hydroxide aqueous solution was added. The mixture was refluxed at 70 °C for 12 h. After cooling to room temperature, the mixture was evaporated under reduced pressure to remove the solvent. The residue was re-dissolved in 40 mL water, and the pH value of the solution was adjusted to < 1 by adding 0.5 M HCl, quantities of precipitate appeared. The precipitate was filtered and washed with water for several times until the pH of the filtrate was adjusted to 7. Finally, the compound **2** was obtained as white solid (322 mg, 85% yield). ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ = 1.70 (m, 6H, H of adamantane), 1.87 (m, 6H, H of adamantane), 2.01 (s, 3H, H of adamantane), 5.26 (s, 2H, H of CH_2), 7.55 (s, 2H, H of benzene), 8.05 ppm (s, 1H, H of benzene); ^{13}C NMR (100 MHz, DMSO-d_6, TMS): δ = 27.3, 36.0, 37.0, 44.7, 68.9, 119.2, 122.4, 132.6, 158.1, 166.4, 208.9 ppm; ESI-MS:

m/*z*: 357.1347 $[M - H]^-$.

Synthesis of compound 3. Compound **2** (716.8 mg, 2 mmol), NHS (460.4 mg, 4 mmol), and DCC (824.7 mg, 4 mmol) were dissolved in THF (30 mL), and the mixture was stirred at room temperature for 24 h, quantities of white percipitate were produced. The mixture was filtered and the filtrate was evaporated under reduced pressure to remove the solvent. The residue was purified by column chromatography (silica gel, dichloromethane/methanol = 60 : 1 as eluent) to give compound **3** as white solid (1.1 g, 100% yield). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.72-1.80 (m, 6H, H of adamantane), 1.93 (m, 6H, H of adamantane), 2.09 (s, 3H, H of adamantane), 2.92 (s, 8H, H of succinimide), 4.99 (s, 2H, H of CH_2), 7.85 (s, 2H, H of benzene), 8.50 ppm (s, 1H, H of benzene); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 25.8, 27.8, 36.5, 38.1, 45.8, 69.0, 122.6, 125.1, 127.6, 158.8, 160.6, 168.9, 207.6 ppm; ESI-MS: *m*/*z*: 570.2078 $[M + NH_4]^+$.

Synthesis of compound 4. Compound **3** (1.15 g, 2.08 mmol), and 3-dimethylaminopropylamine (1.02 g, 10 mmol) were dissolved in dichloromethane (40 mL), and then triethylamine (1 mL) was added. The mixture was stirred at room temperature for 24 h. After that, the solution was washed by water (3 × 100 mL), and the organic layer was dried with anhydrate Na_2SO_4. The solvent was removed under the reduced pressure to obtained the compound **4** as yellow solid (944.2 mg, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 1.63-1.69 (m, 10H, H of adamantane and CH_2), 1.85 (s, 6H, H of adamantane), 2.00 (s, 3H, H of adamantane), 2.23 (s, 12H, H of methyl groups), 2.40-2.43 (t, J = 5.9 Hz, 4H, H of CH_2), 3.43-3.44 (m, 4H, H of

CH_2), 4.87 (s, 2H, H of CH_2), 7.36 (s, 2H, H of benzene), 8.61 ppm (s, 1H, H of benzene); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 25.3, 27.9, 36.5, 38.2, 40.2, 45.2, 58.8, 68.9, 116.2, 118.4, 136.3, 158.5, 166.1, 208.8 ppm; ESI-MS: *m*/*z*: 527.3600 $[M + H]^+$.

Synthesis of adamantane-bis(quaternary-N) conjugate (ADA2+, compound 5). Compound **4** (526.7 mg, 1 mmol) was dissolved in acetonitrile (15 mL), and ethyl bromoacetate (277 μL, 2.5 mmol) was added, the mixture was stirred at room temperature for 24 h. The solution was evaporated under reduced pressure to remove the solvent. After that, ether (50 mL) was added to the residue, and the white percipitate appeared immediately. The solution was centrifuged to removed the solvent, and the residue was washed by ether (2 × 50 mL) for another two times. Compound **5** was obtained as pale yellow solid (860 mg, 100% yield). ^1H NMR (400 MHz, D_2O, TMS): δ = 1.15-1.19 (t, 6H, *J* = 7.2 Hz, H of ethyl group), 1.66-1.77 (m, 6H, H of adamantane), 1.88 (m, 6H, H of adamantane), 2.02 (s, 3H, H of adamantane), 2.12 (m, 4H, H of CH_2), 3.27 (s, 12H, H of methyl group), 3.41-3.71 (m, 8H, H of ethyl group and CH_2), 4.14-4.15 (m, 4H, H of CH_2), 4.30 (s, 4H, H of CH_2 in ethyl acetate group), 5.19 (s, 2H, H of CH_2), 7.43 (s, 2H, H of benzene), 7.77 ppm (s, 1H, H of benzene); ^{13}C NMR (100 MHz, D_2O, TMS): δ = 13.0, 22.5, 27.4, 35.7, 36.4, 37.3, 45.6, 52.2, 60.9, 62.5, 63.4, 69.4, 116.7, 118.9, 135.4, 157.6, 165.0, 169.0, 214.6 ppm; ESI-MS: *m*/*z*: 350.2229 $[(M-2Br^-)/2]^+$; elemental analysis calcd (%) for $C_{38}H_{60}Br_2N_4O_8 \cdot H_2O$: C 51.94, H 7.11, N 6.38; found: C 51.91, H 7.36, N 6.81.

Preparation of ADA2+@HACD nanoparticles. HACD. (7.62 mg, 1.93×10^{-2}

μmol, containing 2.0 μmol β-CD units) and **ADA2+** (1.72 mg, 2.0 μmol) were dissolved in deionized aqueous solution/PBS (2 mL), and then the mixture was ultrasonicated for 5 min. The resulting **ADA2+@HACD** solution ([β-CD] = [**ADA2+**] = 1.0 mM) was stored at 4 °C.

Agarose gel electrophoresis experiments. Agarose gel electrophoresis experiments were performed in TAE buffer (0.04 M Tris, 0.02 M acetic acid, and 2.0 mM) ethylenediaminetetraacetic acid (EDTA)) at 25 °C. After samples loading and electrophoresis process, pDNA bands were stained by ethidium bromide (EB) solution and were visualized under UV light at 302 nm. The condensation and controlled release capability of **ADA2+** and **ADA2+@HACD** nanoparticles toward pDNA was measured by analyzing the electrophoretic mobility at different N/P ratios on agarose gel.

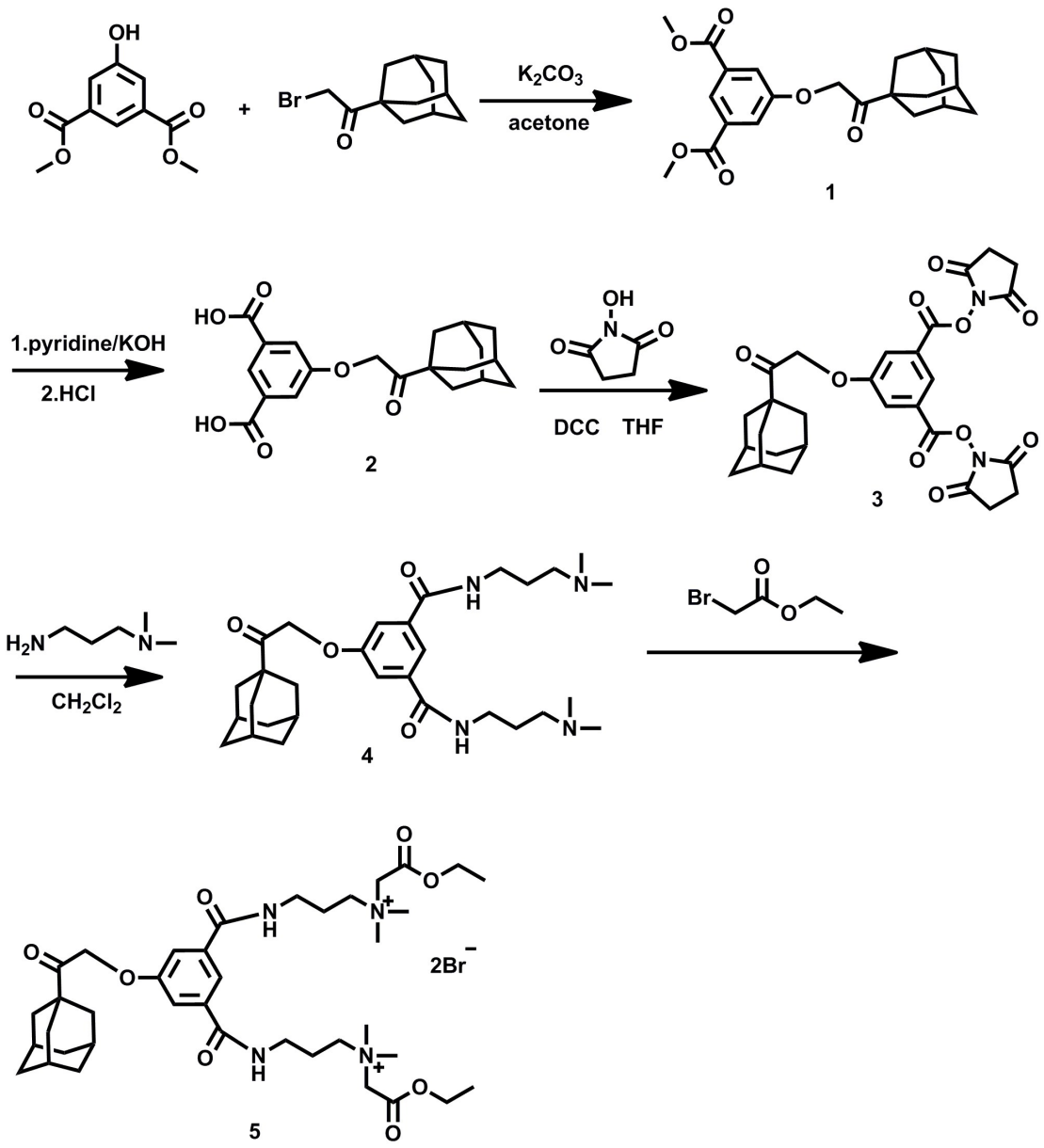

Figure S1. The synthetic routes of **ADA2+**.

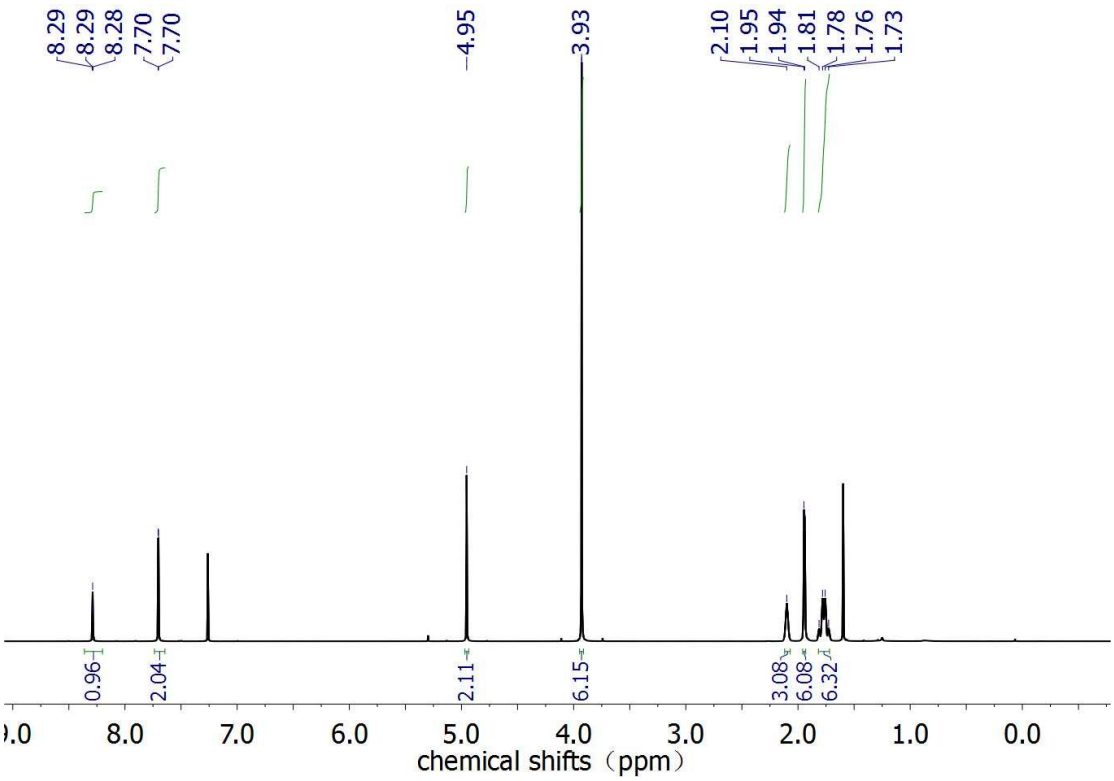

Figure S2. ^{1}H NMR (400 MHz) spectrum of compound **1** in $CDCl_3$ at 25 °C.

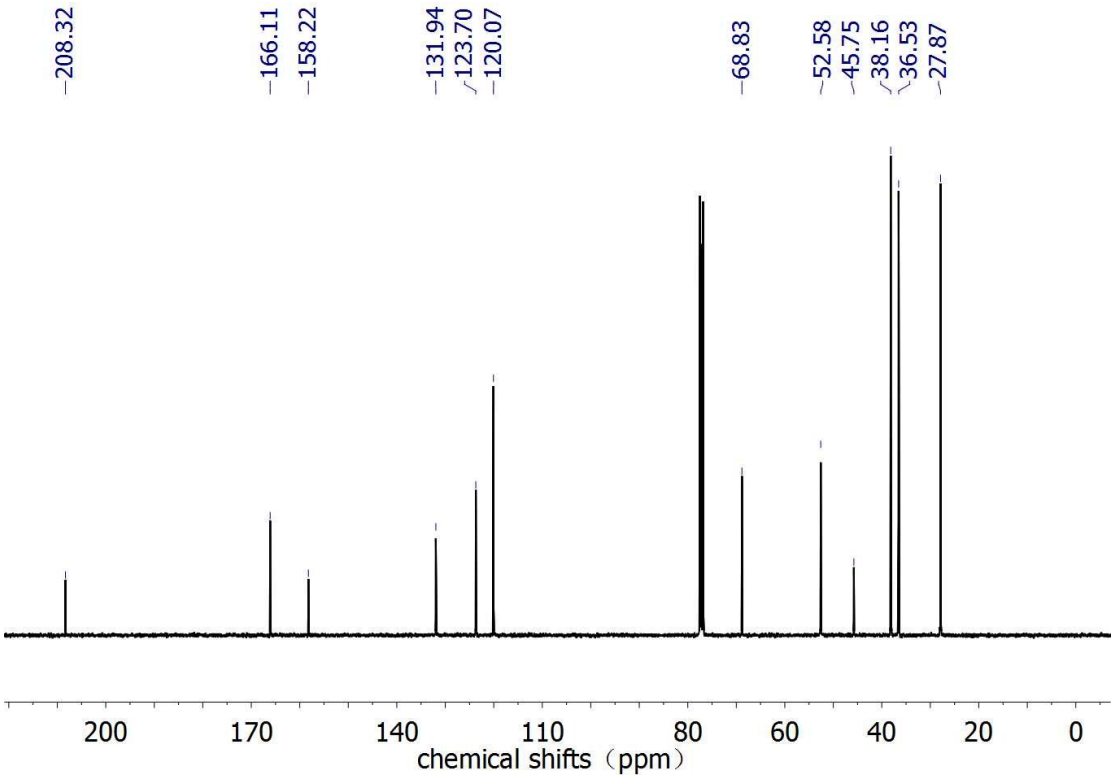

Figure S3. ^{13}C NMR (100 MHz) spectrum of compound **1** in $CDCl_3$ at 25 °C.

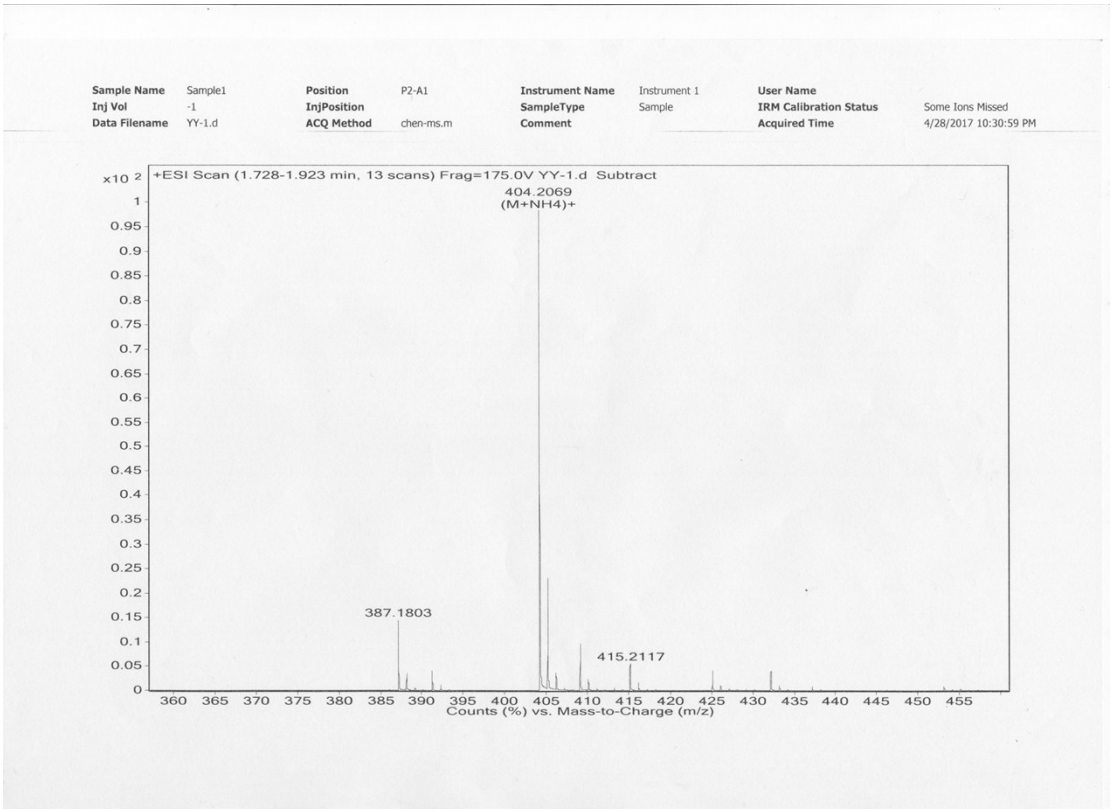

Figure S4. ESI mass spectrum of compound **1**.

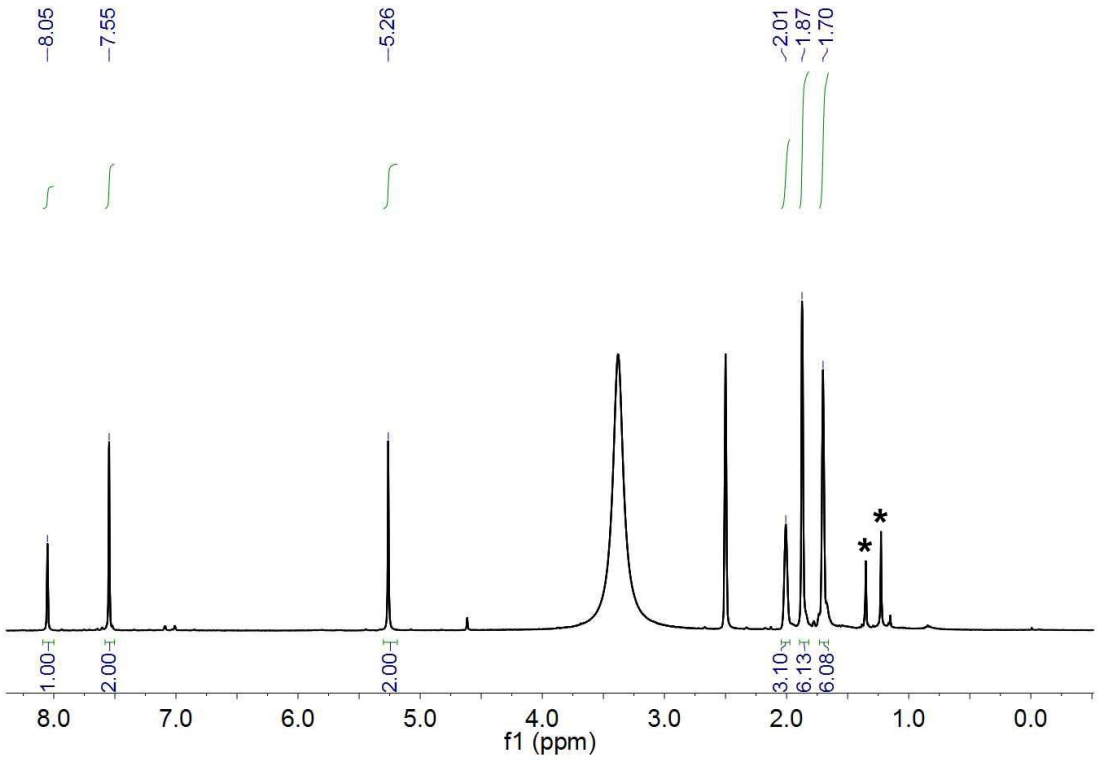

Figure S5. ^{1}H NMR (400 MHz) spectrum of compound **2** in DMSO-d^{6} at 25 °C. The asterisks indicate that these peaks belong to lubricating grease used in the reaction.

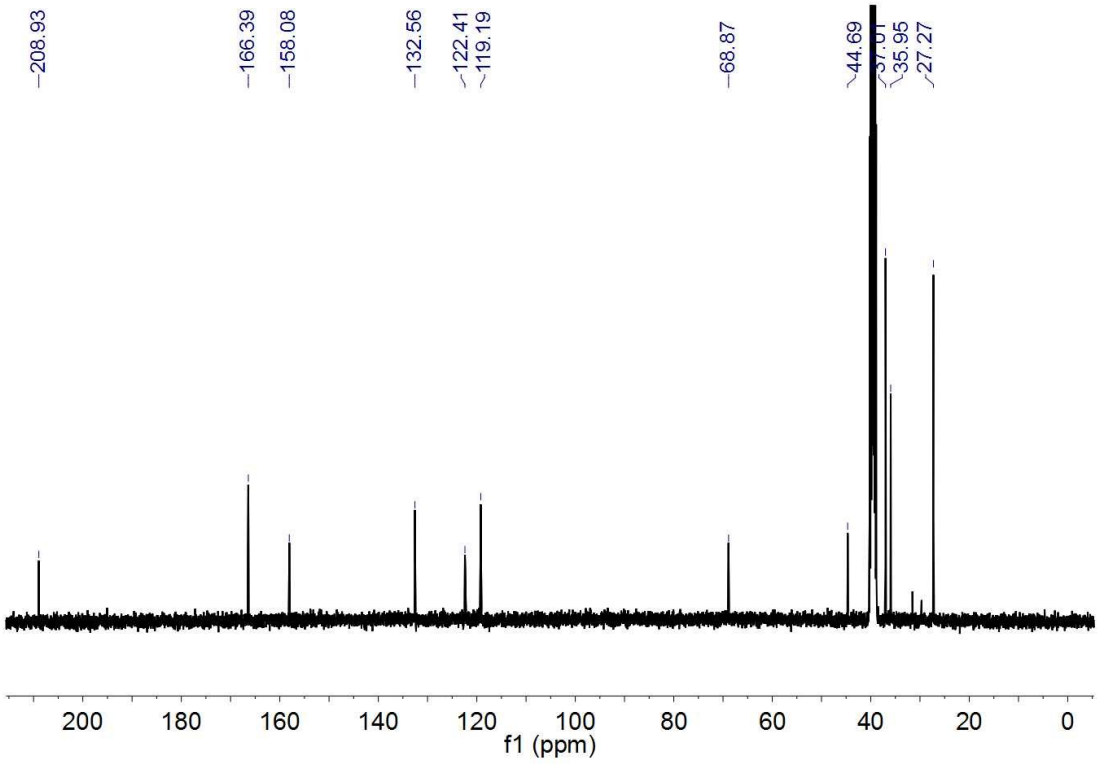

Figure S6. ^{13}C NMR (100 MHz) spectrum of compound **2** in DMSO-d^6 at 25 °C.

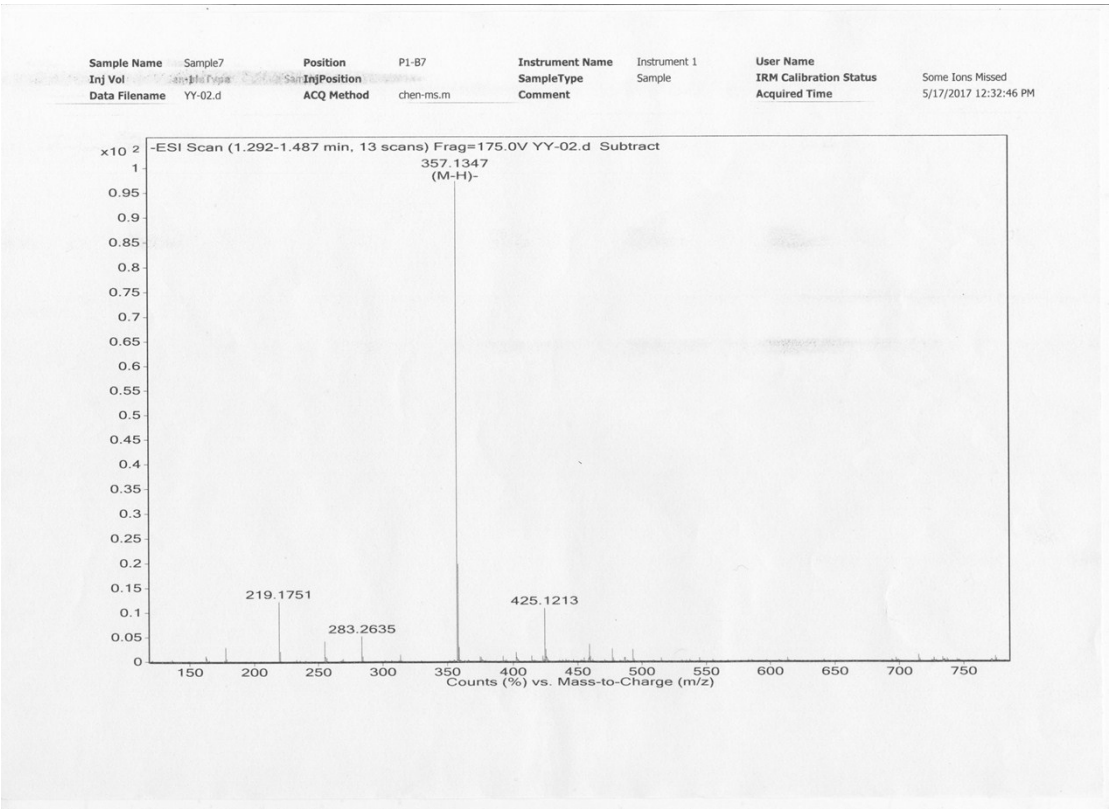

Figure S7. ESI mass spectrum of compound **2**.

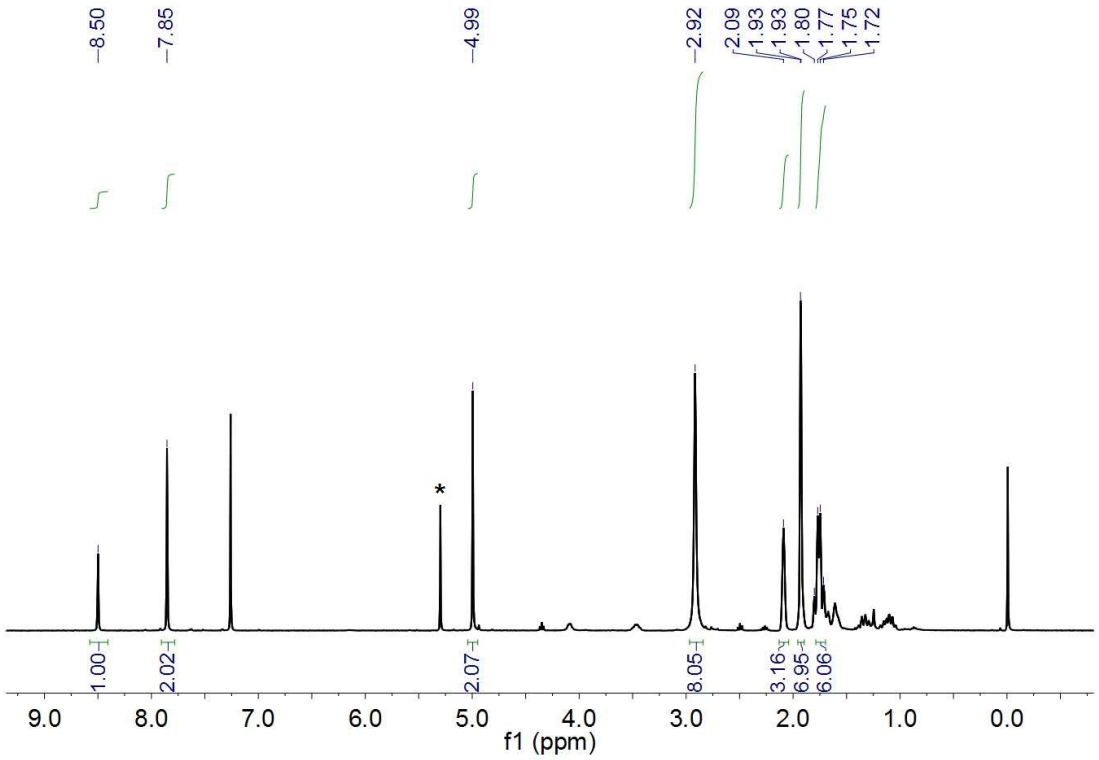

Figure S8. ^{1}H NMR (400 MHz) spectrum of compound **3** in $CDCl_3$ at 25 °C, the asterisk indicated that this peak belongs to un-removed CH_2Cl_2 from the eluent of column chromatography.

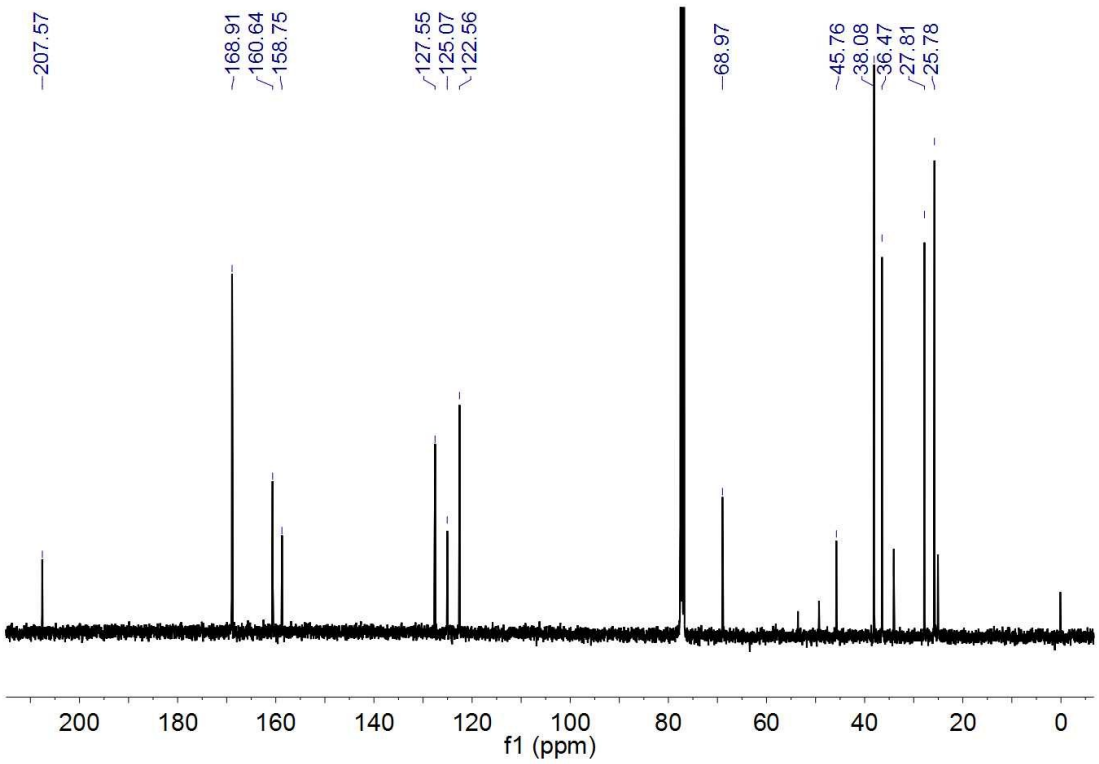

Figure S9. ^{13}C NMR (100 MHz) spectrum of compound **3** in $CDCl_3$ at 25 °C.

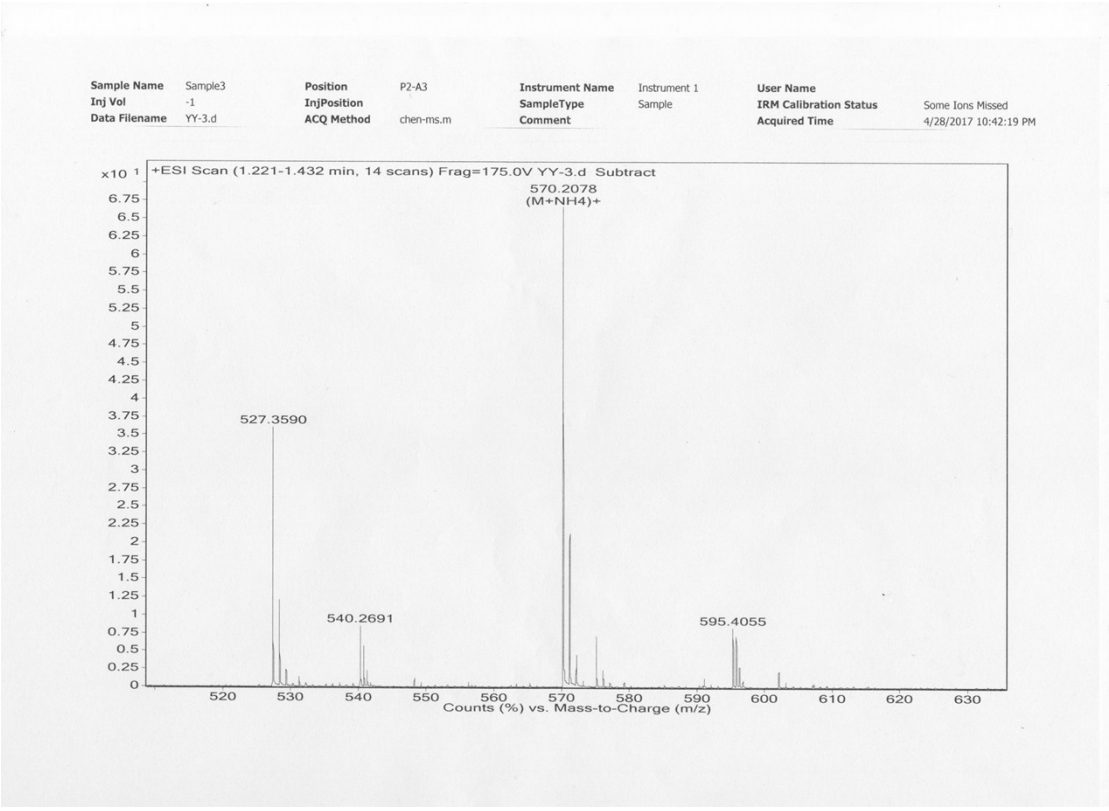

Figure S10. ESI mass spectrum of compound **3**.

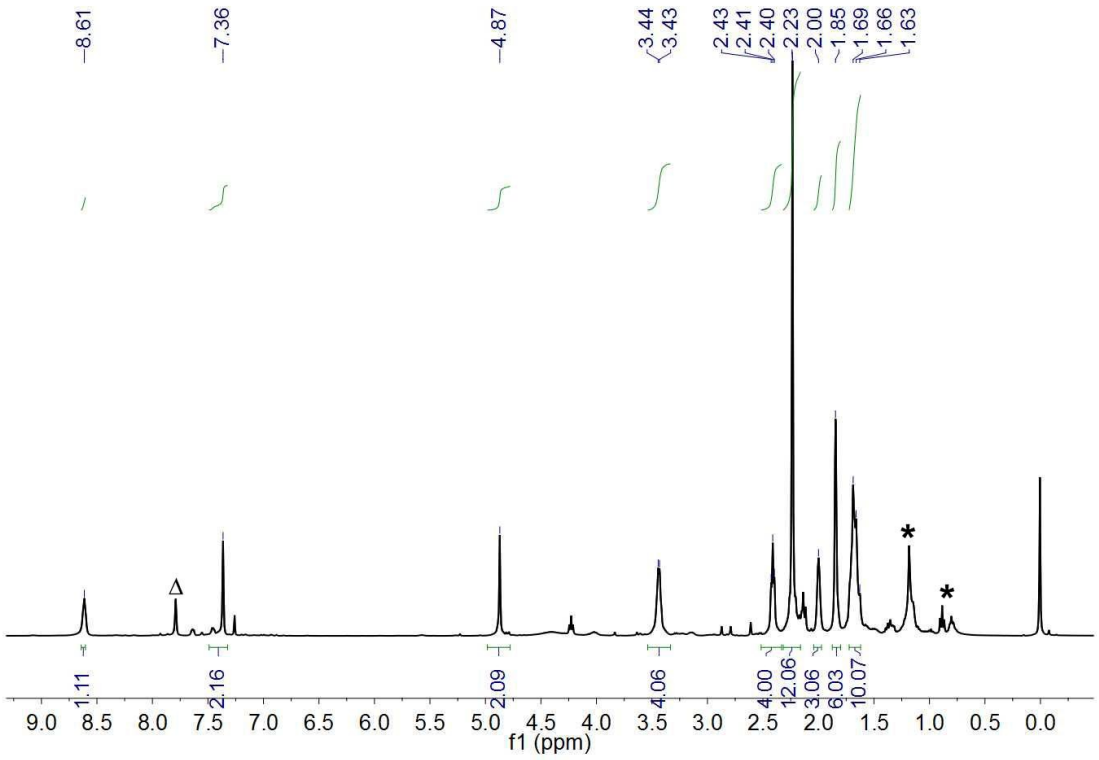

Figure S11. ^{1}H NMR (400 MHz) spectrum of compound **4** in $CDCl_3$ at 25 °C. The asterisks indicate that these peaks belong to lubricating grease used in the reaction; the triangle indicates the existence of thimbleful unreacted compound **3**.

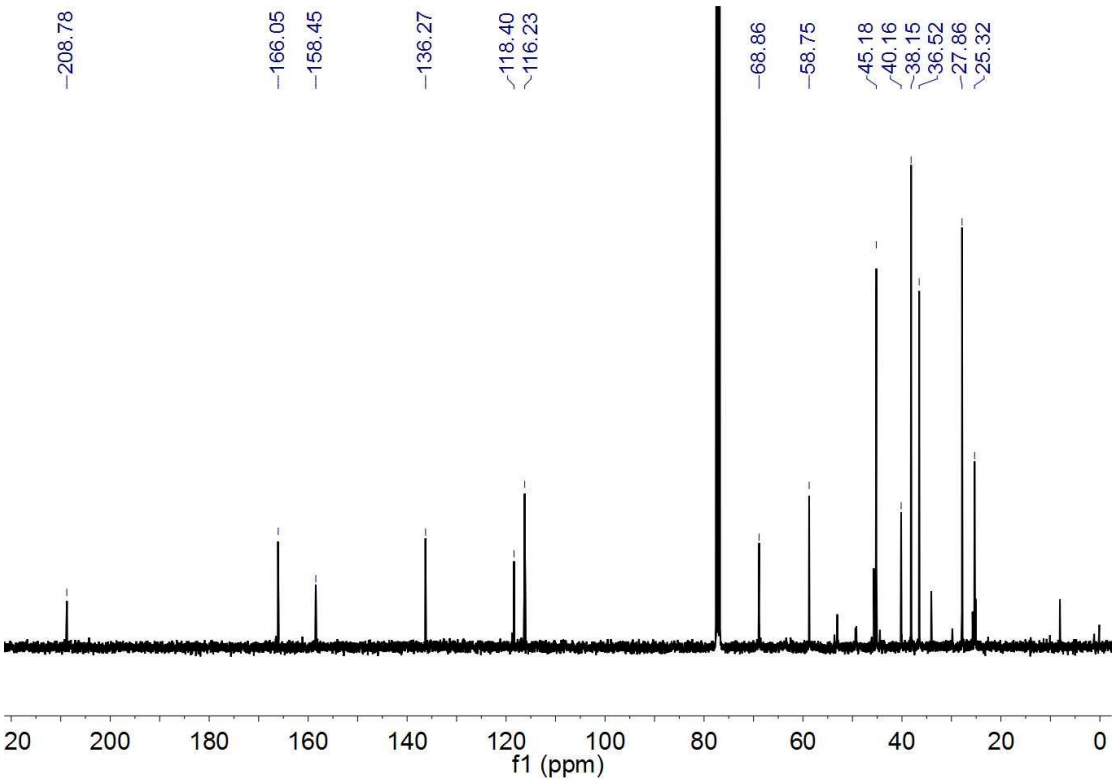

Figure S12. ^{13}C NMR (100 MHz) spectrum of compound **4** in $CDCl_3$ at 25 °C.

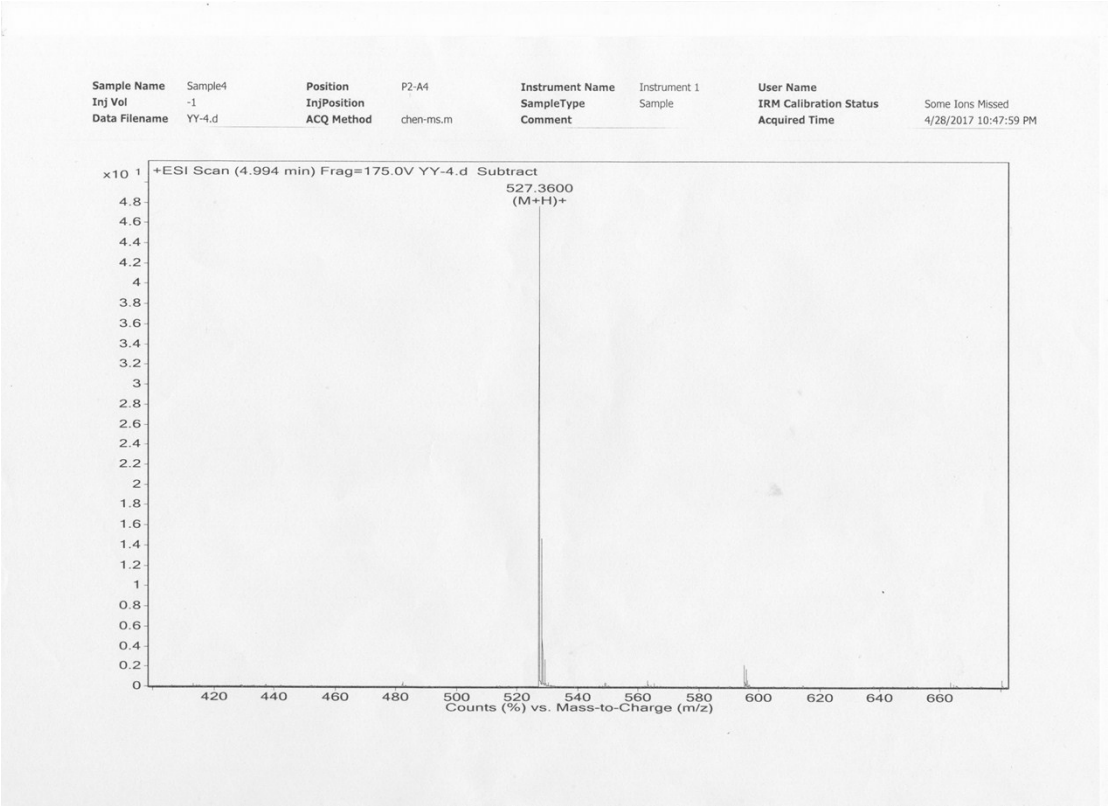

Figure S13. ESI mass spectrum of compound **4**.

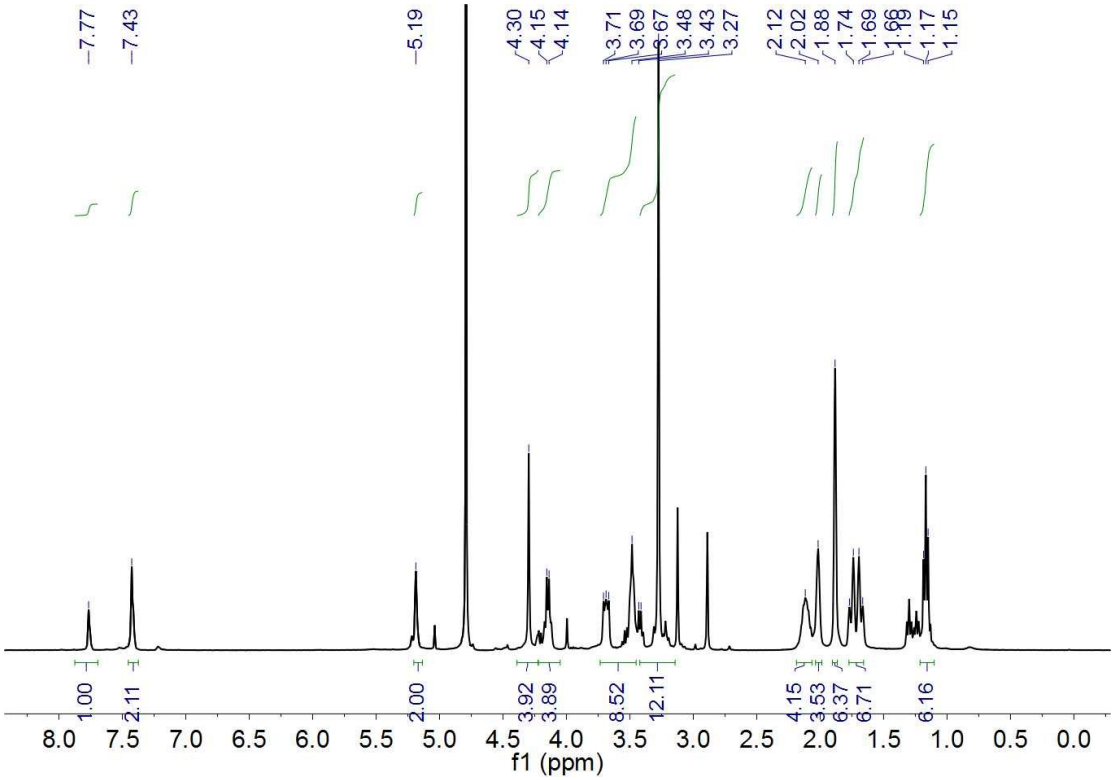

Figure S14. ^{1}H NMR (400 MHz) spectrum of compound **5** (**ADA2+**) in D_2O at 25 °C.

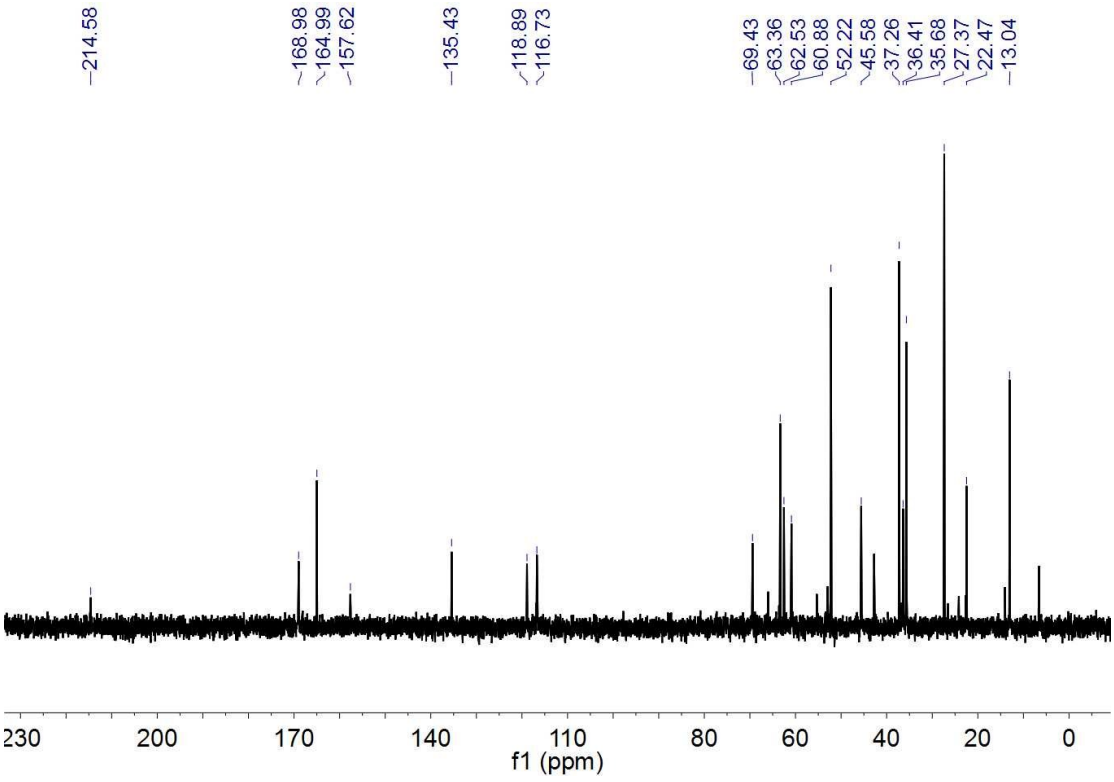

Figure S15. ^{13}C NMR (100 MHz) spectrum of compound **5** (**ADA2+**) in D_2O at 25 °C.

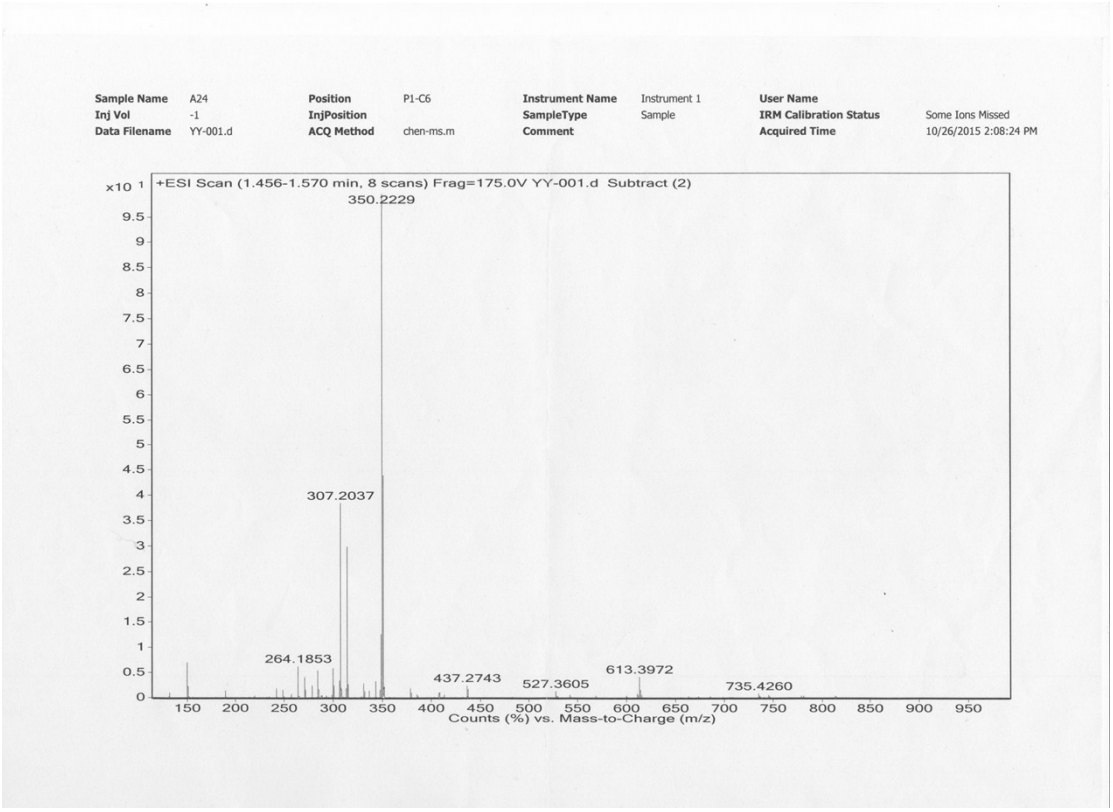

Figure S16. ESI mass spectrum of compound **5** (**ADA2+**).

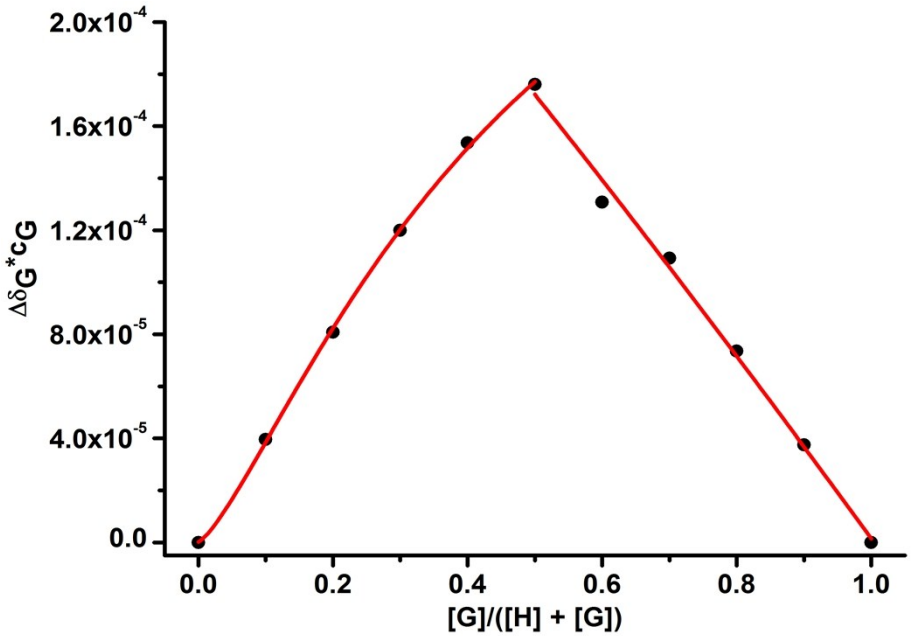

Figure S17. The Job's plot of **ADA2+** (G) and *β*-CD (H) in D_2O ([**ADA2+**] + [*β*-CD] = 2 mM) at 25 °C.

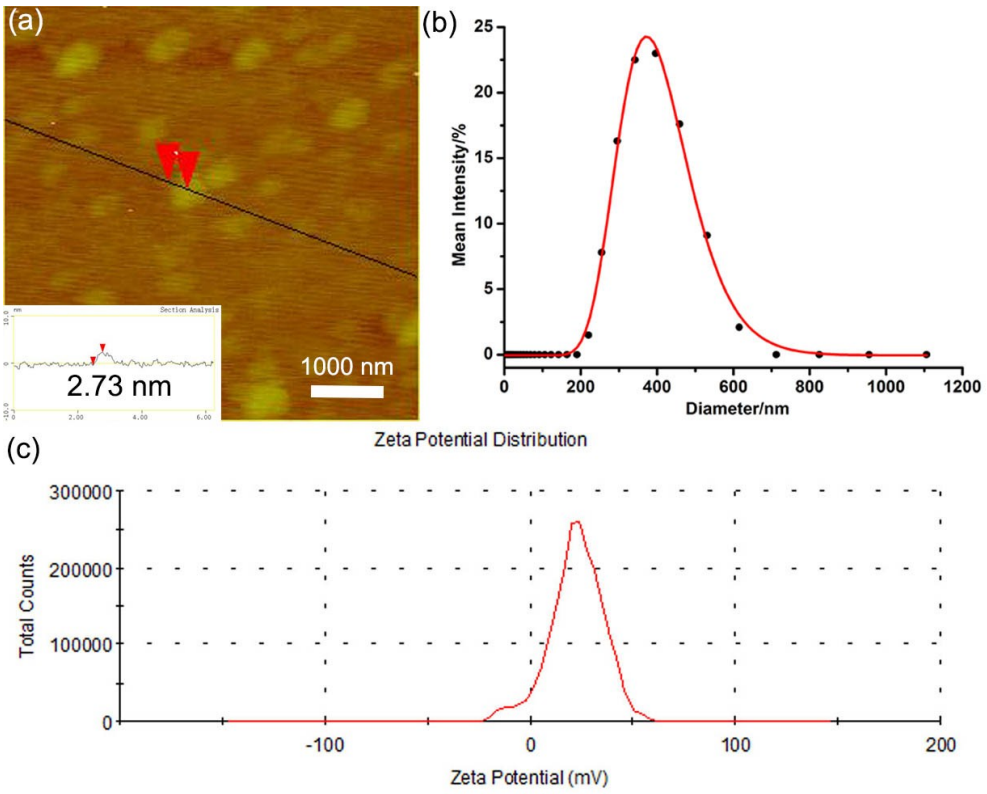

Figure S18. (a) AFM image of **ADA2+**, and (b) DLS and (c) *ζ* potential experiment results of **ADA2+** in deionized aqueous solution.

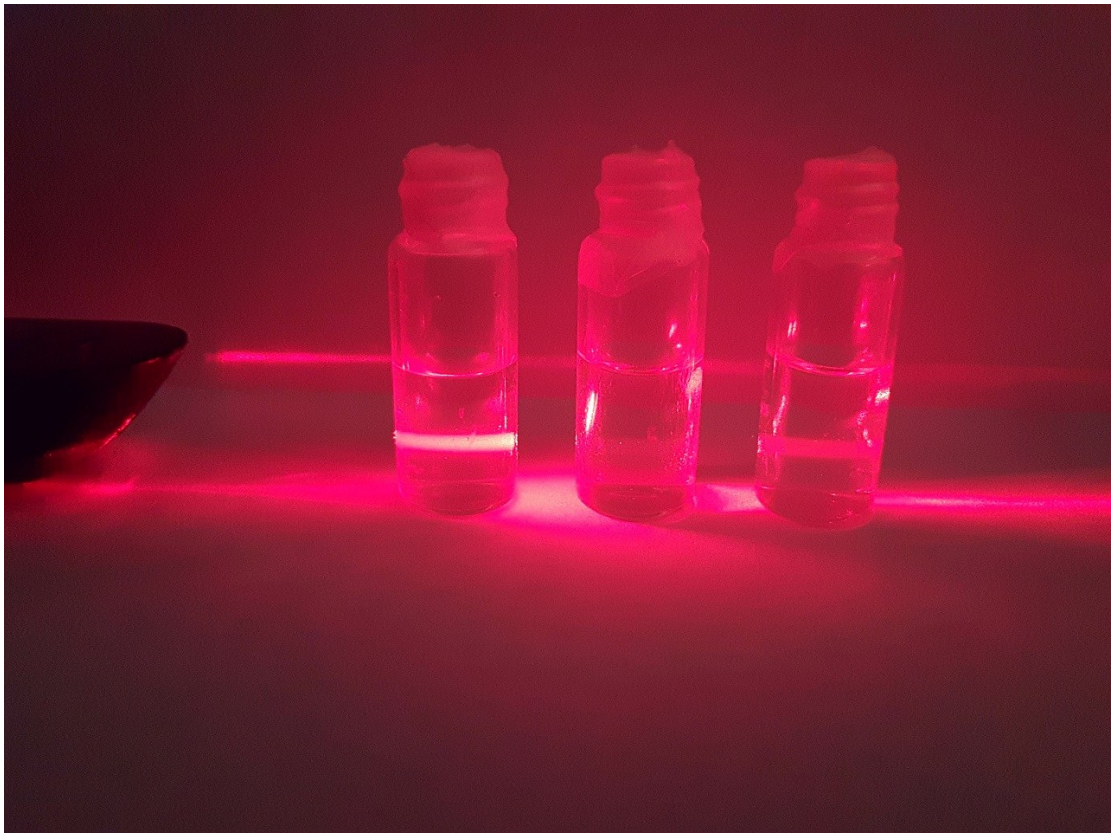

Figure S19. Tyndall effects of the aqueous solution of **ADA2+** (left), **HACD** (middle), and **ADA2+@HACD** nanoparticles (right).

Reference

1. Y.-H. Zhang, Y.-M. Zhang, Y. Yang, L.-X. Chen and Y. Liu, *Chem. Commun.* 2016, **52**, 6087.

2. Y. Yang, Y.-M. Zhang, Y. Chen, J.-T. Chen and Y. Liu, *J. Med. Chem.* 2013, **56**, 9725.