D-Amino Acids Boost the Selectivity and Confer Supramolecular Hydrogels of a Non-steroidal Anti-inflammatory Drug (NSAID)

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Supporting Information

Materials and Instruments.

All of the chemical reagents and solvents were used as received from the commercial sources without further purification unless otherwise noted. ^{1}H, ^{13}C, and ^{31}P NMR spectra were obtained on Varian Unity Inova 400. Chemical shifts are reported in δ (ppm) relative to the solvent residual peak (phosphoric acid for ^{31}P NMR). Coupling constants are reported in Hz with multiplicities denoted as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) and br (broad). The HeLa cell line (CCL2) was purchased from American Type Culture Collection. All of the medium were provided from Invitrogen. COX inhibitor screening assay kit (700100) was purchased from Cayman Chemical Company. Cytotoxicity test and COX inhibition tests were measured by DTX 880 Multimode Detector. Rheological data were measured on TA ARES G2 rheometer with 25 mm cone plate. TEM images were taken on Morgagni 268 transmission electron microscope. LC-MS was performed on a Waters Acouity ultra Performance LC with Waters MICRO-MASS detector.

Synthesis and Characterizations.

Solid-phase peptide synthesis (SPPS). All the hydrogelators were prepared by solid-phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin (100~200 mesh and 0.3~0.8 mmol/g) and *N*-Fmoc-protected amino acids with side chains properly protected by a *tert*-butyl (Fmoc-D-Tyr(tBu)-OH) or *tert*-butoxycarbonyl (Fmoc-D-Lys(Boc)-OH) group. Bubbled with nitrogen gas (N_2) in dry dichloromethane (DCM) for 20 minutes, the resin swelled and was washed with dry *N,N*-dimethylformamide (DMF) (3 x 3 mL). Then the first amino acid was loaded onto resin at its C-terminal by bubbling the resin in a DMF solution of Fmoc-protected amino acid (2 equiv.) and *N,N*-diisopropylethylamine (DIPEA) for 0.5 hour. After washed with DMF (3 x 3 mL), the resin was bubbled with the blocking solution (16:3:1 of DCM/MeOH/DIPEA) for 0.5 hour to deactivate the unreacted sites. Then the resins were treated with 20% piperidine (in DMF) for 0.5 hour to remove the protecting group, followed by coupling Fmoc-protected amino acid (2 equiv.) to the free amino group on the resin using DIPEA/*O*-

benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (2 equiv.) as the coupling reagent. These two steps were repeated to elongate the peptide chain, which were carried out by the standard Fmoc SPPS protocol. The resin was washed with DMF for 3~5 times after each step. At the final step, the peptide was cleaved with TFA (10 mL) for 2 hours and the resulted crude products were purified by reverse phase HPLC. For phosphate containing hydrogelators **2** and **4**, Fmoc-D-Try(PO_3H_2)-OH was prepared from D-Try and directly used in SPPS, which need longer coupling reaction time (1 hour).[1]

***N*-Hydroxysuccinimide assisted coupling reaction.** In addition to the solid-phase peptide synthesis, *N*-hydroxysuccinimide (NHS) assisted coupling reaction was also performed in the preparation of hydrogelators **5** and **6**. 115 mg (1.0 mmol) of *N*-hydroxysuccinimide (NHS) and 152 mg (1.2 mmol) of *N,N'*-diisopropylcarbodiimide (DIC) were added to a solution of 230 mg (1.0 mmol) of naproxen (Npx) in chloroform (10 mL). After the resulting mixture was stirred for 2 h at room temperature, filtration, evaporation, and recrystallization in ethanol were performed to give pure Npx-NHS ester. To an aqueous solution (6 mL) dissolving 405 mg (1.0 mmol) of the Fmoc-D-Lys-OH (pH was adjusted to 8.5 by Na_2CO_3), the acetone solution of Npx-NHS ester (6 mL) was added dropwise, and the resulting solution was stirred overnight at room temperature. The solution was concentrated by rotary evaporator until all the acetone was removed. 1 M of HCl was added to adjust the pH of the remaining aqueous solution to 3.0 and the resulting white precipitate was collected by filtration, followed by the purification with flash column. Then the pure product, Fmoc-D-Lys(Npx)-OH, was directly used in standard SPPS (described as above) with longer coupling reaction time (1 hour).

Naproxen-D-Phe-D-Phe (1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.27 (d, J = 7.7 Hz, 1H), 8.06 (d, J = 8.3 Hz, 1H), 7.71 (d, J = 9.0 Hz, 1H), 7.63 (d, J = 8.5 Hz, 1H), 7.57 (s, 1H), 7.31 – 7.18 (m, 7H), 7.13 (dd, J = 8.9, 2.3 Hz, 1H), 7.04 – 6.88 (m, 5H), 4.57 – 4.42 (m, 2H), 3.86 (s, 3H), 3.77 (q, J = 6.8 Hz, 1H), 3.15 – 2.84 (m, 3H), 2.70 (dd, J = 13.8, 9.7 Hz, 1H), 1.33 (d, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.03, 172.73, 171.13, 156.92, 137.44, 137.42, 136.91, 133.06, 129.16 (2C), 129.12 (2C), 129.07, 128.29, 128.19 (2C), 127.65 (2C), 126.45 (2C), 126.38, 125.92, 125.22, 118.37, 105.61, 55.14, 53.52, 53.45, 44.45, 37.38, 36.72, 17.81.

Naproxen-D-Phe-D-Phe-D-Tyr phosphate (2P). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (d, J = 7.5 Hz, 1H), 8.14 (d, J = 8.1 Hz, 1H), 8.05 (d, J = 8.3 Hz, 1H), 7.71 (d, J = 9.0 Hz, 1H), 7.63 (d, J = 8.5 Hz, 1H), 7.57 (s, 1H), 7.30 – 7.05 (m, 12H), 6.97 – 6.82 (m, 5H), 4.59 (dd, J = 12.7, 8.7 Hz, 1H), 4.51 – 4.40 (m, 2H), 3.86 (s, 3H), 3.77 (q, J = 6.8 Hz, 1H), 3.04 (dd, J = 13.8, 4.3 Hz, 2H), 2.93 (dd, J = 13.8, 8.1 Hz, 1H), 2.89 – 2.75 (m, 2H), 2.67 (dd, J = 13.5, 9.9 Hz, 1H), 1.32 (d, J = 6.9 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.05, 172.59, 171.08, 170.91, 156.92, 150.37, 137.61, 137.47, 136.95, 133.07, 132.57, 130.10 (2C), 129.30 (2C), 129.12 (3C), 128.30, 128.02 (2C), 127.61 (2C), 126.46 (2C), 126.28, 125.87, 125.28, 119.82, 119.78, 118.37, 105.61, 55.14, 53.66, 53.60, 53.55, 44.43, 37.62, 37.30, 35.97, 17.84; ^{31}P NMR (162 MHz, DMSO-d_6) δ -5.15.

Naproxen-D-Phe-D-Phe-D-Lys (3). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 – 7.96

(m, 3H), 7.70 (d, *J* = 8.6 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.56 (s, 1H), 7.36 – 7.04 (m, 8H), 7.04 – 6.66 (m, 5H), 4.58 (s, 1H), 4.43 (s, 1H), 4.15 (s, 1H), 3.86 (s, 3H), 3.77 (s, 1H), 3.04 (dd, *J* = 31.1, 11.0 Hz, 2H), 2.92 – 2.61 (m, 4H), 1.88 – 1.44 (m, 4H), 1.42 – 1.08 (m, 5H); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 173.15, 173.07, 170.96, 170.66, 156.93, 137.60, 137.53, 136.85, 133.07, 129.31 (2C), 129.04 (3C), 128.29, 128.03 (2C), 127.66 (2C), 126.41 (2C), 126.29, 125.91, 125.23, 118.39, 105.63, 55.14, 53.78, 53.65, 52.16, 44.49, 38.64, 37.58, 37.25, 30.80, 26.63, 22.27, 17.84.

Naproxen-D-Phe-D-Phe-D-Lys-D-Tyr phosphate (4P). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.20 – 8.04 (m, 4H), 7.88 (d, *J* = 8.1 Hz, 1H), 7.71 (d, *J* = 9.0 Hz, 1H), 7.63 (d, *J* = 8.6 Hz, 1H), 7.57 (s, 1H), 7.31 – 7.02 (m, 12H), 7.01 – 6.83 (m, 5H), 4.56 (dd, *J* = 12.4, 8.6 Hz, 1H), 4.42 (dd, *J* = 12.7, 9.0 Hz, 2H), 4.14 (dd, *J* = 14.2, 6.9 Hz, 1H), 3.86 (s, 3H), 3.78 (d, *J* = 7.0 Hz, 1H), 3.09 (d, *J* = 13.7 Hz, 2H), 2.92 (dd, *J* = 13.7, 10.6 Hz, 1H), 2.86 – 2.77 (m, 2H), 2.66 (dd, *J* = 13.7, 9.7 Hz, 1H), 2.54 (s, 2H), 1.47 – 1.28 (m, 7H), 1.11 – 0.93 (m, 2H); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 173.10, 172.78, 171.00, 170.97, 170.54, 156.92, 152.20, 137.71, 137.47, 136.91, 133.07, 130.66, 129.66 (2C), 129.29 (2C), 129.07 (3C), 128.29, 128.03 (2C), 127.63 (2C), 126.42 (2C), 126.24, 125.89, 125.28, 119.20, 119.16, 118.38, 105.63, 55.14, 53.75, 53.58, 53.07, 52.90, 44.49, 38.51, 37.32 (2C), 35.41, 31.97, 26.59, 22.01, 17.86; ^{31}P NMR (162 MHz, DMSO-*d*$_6$) δ -5.19.

D-Phe-D-Phe-D-Lys(naproxen) (5). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.71 (d, *J* = 7.8 Hz, 1H), 8.41 (d, *J* = 7.6 Hz, 1H), 8.00 (t, *J* = 5.3 Hz, 1H), 7.76 (d, *J* = 9.1 Hz, 1H), 7.73 (d, *J* = 8.7 Hz, 1H), 7.69 (s, 1H), 7.43 (d, *J* = 8.6 Hz, 1H), 7.33 – 7.16 (m, 11H), 7.13 (dd, *J* = 8.9, 2.4 Hz, 1H), 4.65 (dd, *J* = 12.5, 8.5 Hz, 1H), 4.18 (dd, *J* = 13.0, 8.1 Hz, 1H), 3.94 (s, 1H), 3.85 (s, 3H), 3.71 (dd, *J* = 13.9, 6.9 Hz, 1H), 3.15 – 2.93 (m, 4H), 2.92 – 2.78 (m, 2H), 1.73 (dd, *J* = 13.3, 5.6 Hz, 1H), 1.59 (dd, *J* = 13.8, 7.1 Hz, 1H), 1.39 (d, *J* = 7.0 Hz, 5H), 1.30 (dd, *J* = 14.0, 6.6 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 173.40, 173.23, 170.66, 168.55, 156.96, 137.53, 137.43, 135.07, 133.10, 129.57 (2C), 129.28 (3C), 129.06, 128.41, 128.37, 128.12 (2C), 127.00, 126.56, 126.45, 126.40, 125.20, 118.54, 105.68, 55.15, 53.92, 53.37, 51.94, 45.07, 38.42, 37.63, 37.30, 30.74, 28.75, 22.85, 18.63.

D-Phe-D-Phe-D-Lys(naproxen)-D-Tyr (6). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 9.22 (s, 1H), 8.70 (d, *J* = 7.9 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 8.10 (d, *J* = 7.4 Hz, 1H), 7.96 (d, *J* = 5.2 Hz, 1H), 7.75 (d, *J* = 9.4 Hz, 1H), 7.73 (d, *J* = 10.8 Hz, 1H), 7.69 (s, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.34 – 7.08 (m, 12H), 7.01 (d, *J* = 8.2 Hz, 2H), 6.65 (d, *J* = 8.1 Hz, 2H), 4.68 – 4.59 (m, 1H), 4.40 – 4.26 (m, 2H), 3.97 (s, 1H), 3.85 (s, 3H), 3.70 (d, *J* = 6.7 Hz, 1H), 3.16 – 2.66 (m, 8H), 1.72 – 1.57 (m, 1H), 1.57 – 1.44 (m, 1H), 1.38 (d, *J* = 6.8 Hz, 5H), 1.31 – 1.14 (m, 2H); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 173.20, 172.86, 171.35, 170.32, 168.07, 156.96, 155.95, 137.53, 137.48, 134.72, 133.09, 130.02 (2C), 129.60 (2C), 129.23 (2C), 129.07, 128.45 (2C), 128.36, 128.10 (2C), 127.36, 127.11, 126.57, 126.45, 126.35, 125.20, 118.56, 114.97 (2C), 105.67, 55.16, 53.99, 53.76, 53.10, 52.34, 45.06, 38.63, 37.57, 37.02, 35.89, 32.03, 28.91, 22.63, 18.67.

Naproxen-L-Phe-L-Phe (L-1). ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.24 (d, *J* = 7.5 Hz, 1H), 8.11 (d, *J* = 8.4 Hz, 1H), 7.72 (d, *J* = 9.1 Hz, 1H), 7.69 (d, *J* = 8.8 Hz, 1H), 7.63

(s, 1H), 7.34 (d, J = 8.3 Hz, 1H), 7.30 – 7.03 (m, 12H), 4.60 (s, 1H), 4.39 (dd, J = 13.1, 7.2 Hz, 1H), 3.85 (s, 3H), 3.74 (q, J = 6.8 Hz, 1H), 3.08 – 2.92 (m, 2H), 2.92 – 2.69 (m, 2H), 1.20 (d, J = 6.6 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.07, 172.65, 171.13, 156.93, 137.79, 137.24, 137.01, 133.08, 129.32 (2C), 129.07, 128.99 (2C), 128.31, 128.10 (2C), 127.88 (2C), 126.61, 126.44, 126.33, 126.16, 125.35, 118.43, 105.64, 55.56, 53.13, 53.33, 44.67, 37.74, 36.65, 18.69.

Naproxen-L-Phe-L-Phe-L-Tyr (L-2). 1H NMR (400 MHz, DMSO-d_6) δ 8.17 – 8.00 (m, 3H), 7.72 (d, J = 9.0 Hz, 1H), 7.68 (d, J = 8.6 Hz, 1H), 7.62 (s, 1H), 7.33 (d, J = 8.5 Hz, 1H), 7.29 – 6.96 (m, 14H), 6.64 (d, J = 8.3 Hz, 2H), 4.59 – 4.51 (m, 1H), 4.50 – 4.41 (m, 1H), 4.31 (dd, J = 12.9, 6.9 Hz, 1H), 3.84 (s, 3H), 3.72 (d, J = 6.9 Hz, 1H), 3.04 – 2.90 (m, 3H), 2.83 (dd, J = 13.8, 7.5 Hz, 1H), 2.78 – 2.64 (m, 2H), 1.18 (d, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.14, 173.07, 170.89, 170.51, 156.92, 155.85, 137.93, 137.56, 137.02, 133.08, 130.16 (2C), 129.33 (2C), 129.10 (3C), 128.33, 127.90 (2C), 127.85 (2C), 127.71, 126.62, 126.45, 126.09, 126.07, 125.36, 118.41, 114.91 (2C), 105.63, 55.13, 54.27, 53.81, 53.48, 44.68, 37.69, 37.55, 36.11, 18.72.

Naproxen-L-Phe-L-Phe-L-Lys (L-3). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (d, J = 7.7 Hz, 1H), 8.11 (d, J = 8.6 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 9.0 Hz, 1H), 7.69 (d, J = 8.6 Hz, 1H), 7.63 (s, 1H), 7.34 (d, J = 8.5 Hz, 1H), 7.27 – 7.02 (m, 12H), 4.57 – 4.46 (m, 2H), 4.18 (dd, J = 13.2, 8.3 Hz, 1H), 3.85 (s, 3H), 3.72 (q, J = 7.1 Hz, 1H), 2.98 (dd, J = 13.8, 3.9 Hz, 2H), 2.82 – 2.67 (m, 4H), 1.79 – 1.66 (m, 1H), 1.66 – 1.46 (m, 3H), 1.33 (dd, J = 15.1, 7.7 Hz, 2H), 1.20 (d, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.29, 173.23, 170.93, 170.86, 156.95, 137.87, 137.38, 136.91, 133.10, 129.25 (2C), 129.15 (2C), 129.12, 128.33, 127.94 (2C), 127.90 (2C), 126.59, 126.49, 126.16 (2C), 125.38, 118.43, 105.63, 55.14, 53.65, 53.53, 51.69, 44.74, 38.61, 37.55, 37.51, 30.48, 26.58, 22.32, 18.63.

Naproxen-L-Phe-L-Phe-L-Lys-L-Tyr (L-4). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22 (s, 1H), 8.13 – 7.99 (m, 4H), 7.73 (d, J = 9.0 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.63 (s, 1H), 7.33 (d, J = 8.5 Hz, 1H), 7.28 – 6.96 (m, 14H), 6.65 (d, J = 8.1 Hz, 2H), 4.50 (dd, J = 21.3, 12.7 Hz, 2H), 4.39 – 4.25 (m, 2H), 3.85 (s, 3H), 3.72 (d, J = 6.8 Hz, 1H), 3.02 – 2.89 (m, 3H), 2.86 – 2.64 (m, 5H), 1.61 (s, 1H), 1.55 – 1.43 (m, 3H), 1.26 (s, 2H), 1.19 (d, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.18, 172.87, 171.10, 170.95, 170.50, 156.93, 155.91, 137.82, 137.40, 136.89, 133.08, 130.01 (2C), 129.25 (2C), 129.09 (3C), 128.31, 127.90 (2C), 127.89 (2C), 127.44, 126.58, 126.48, 126.14, 126.11, 125.35, 118.43, 114.95 (2C), 105.62, 55.14, 53.89, 53.59 (2C), 52.09, 44.73, 38.64, 37.55, 37.40, 35.97, 31.62, 26.63, 21.92, 18.62.

COX Inhibitor Screening Assay. To study the drug efficacies of NSAID containing hydrogelators, here we used 'COX Fluorescent Inhibitor Screening Assay Kit' (700100; Cayman Chemical) to do *in vitro* inhibition assays for Npx and Npx containing hydrogelors **1**, **2**, **3**, **4**, **5**, and **6.** Each compound was tested by COX-1 (ovine) enzyme and COX-2 (human recombinant) enzyme separately in 96 well black assay plates. By utilizing the peroxidase component of COXs,[2] we monitored the reaction between PGG_2 and ADHP (10-acetyl-3,7-dihydroxyphenoxazine), which produced highly fluorescent compound resorufin. This resorufin can be easily analyzed by multimode detector with an

excitation wavelength of 530-540 nm and an emission wavelength of 585-595 nm. All the compounds are assayed in triplicate. After the combination of 10 μL of inhibitors, 10 μL of Heme solution, 10 μL of fluorometric substrate, 10 μL of enzyme (either COX-1 or COX-2), and 150 μL of assay buffer, we quickly add 10 μL of arachidonic acid solution to initiate the reaction. Then we read the plate exactly after two minutes incubation at room temperature. In this experiment, we also measure the blank data without enzyme and inhibitors, and the control data without inhibitors. The IC_{50} values of these hydrogelators were read from their activity curves (Figure S11), which were measured with 5 different concentrations of these hydrogelators (dissolved in DMSO).

Cytotoxicity. The HeLa cells in good condition were seeded into 96-well plate (2 x 10^5 cells/well) in 100 μL of MEM medium with 10% FBS. With 12 hours of incubation at 37 ^oC and 5% CO_2, the HeLa cells were attached to bottom of 96-well plate. Then the medium was replaced by another 100 μL of growth medium that contained serial diluents of our compounds and the cells were incubated at 37 ^oC and 5% CO_2 for additional 72 hours. The compounds were stocked at 10 μM in DMSO, followed by further dilutions with MEM medium. All of these serial diluents were adjusted to contain 0.5% of DMSO. To measure the proliferation of HeLa cell for 3 days, 10 μL of (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 0.5 mg/mL) was added every 24 hours, followed by adding 100 μL of 0.1% sodium dodecyl sulfate (SDS) 4 hours later. With medium as blank and untreated HeLa cell as control, we measure each concentration of these compounds in triplicate. Since the mitochondrial reductase in living cells reduced MTT to purple fomazan, the absorbance at 595 nm of the whole solution was finally measured by DTX 880 Multimode Detector. With the measurement of 5 different concentrations of these hydrogelators, their IC_{50} values were read from the activity curves for day 3 (Figure S13).

TEM sample preparation. The TEM images we reported in this paper were taken by negative staining technique. Carbon coated grids (400 mesh copper grids that had been coated with continuous thick carbon film ~35nm) were first glowed discharge just before use to increase their hydrophilicity. After sample solution placed onto the grid (3 μl, sufficient to cover the grid surface), the grid was rinsed by DI H_2O for 3 times (let the grid touch the water drop, with the sample-loaded surface facing the parafilm, then tilt the grid and gently absorb water from the edge of the grid using a filter paper sliver). Immediately after rinsing, the grid was stained by UA stain solution (2.0 % (w/v) uranyl acetate) for 3 times (let the grid touch the stain solution drop, with the sample-loaded surface facing the parafilm, then tilt the grid and gently absorb the stain solution from the edge of the grid using a filter paper sliver.) Then we allow the grid to dry in air and examine the grid as soon as possible. [3]

General procedure for anti-HIV drug release from hydrogels. To illustrate the *in vitro* release profile of hydrogelators, we prepared 100 μL of the hydrogels formed by **1** (0.8 wt%, pH 4.0), **2** (0.8 wt%, pH 7.6), **3** (0.8 wt%, pH 7.6), **4** (0.8 wt%, pH 7.6), **5** (0.8 wt%, pH 7.0), and **6** (0.8 wt%, pH 7.0). With the addition of PBS buffer (100 μL, pH 7.4) onto the surface of hydrogels, the gels were incubated at 37 ^oC for 24 hours. The release solutions (100 μL) were taken and refreshed at 0h, 2h, 4h, 8h, 12h, and 24h, which were

detected by analytical HPLC at 276 nm for the quantities of released Npx containing hydrogelators **1**, **2**, **3**, **4**, **5**, and **6**.

General procedure for hydrogel preparation. All the compounds were dissolved in de-ionized water. The pH of the solutions were adjusted by 1M of NaOH and 1M of HCl and measured by pH paper. Then the hydrogels were formed by changing the solutions to neutral or weekly acidic, or by adding enzymes (alkaline phosphatase) into a weakly basic (pH 7.6) solution. To prepare hydrogel of **1**, we first dissolved 4 mg of **1** in 0.5 mL of water at pH 9.0 with the aid of sonication and heating (70 ^{o}C), and then carefully adjusted the pH of solution to 4.0 at room temperature to afford stable hydrogel within seconds. For hydrogelators **3**, **5**, and **6**, we obtained transparent hydrogels by changing the temperature and pH. After dissolving 4 mg of hydrogelator **3**, **5**, and **6** in 0.5 mL of water at pH 9.0, we slowly neutralized the pH of the solutions to pH 7.6, 7.0 and 7.0 with the gently heating at 70 ^{o}C. The solution of **3** may afford hydrogel within 15 minutes at room temperature, while the hydrogel of **5** and **6** produce stable hydrogels at room temperature overnight. The phosphate containing hydrogelators **2P** and **4P** undergo enzymatic hydrogelation processes. After preparing 0.8 wt% of solutions of **2P** and **4P** at pH 7.6, we added 0.2 U/mL of alkaline phosphatase into the solutions to result in transparent hydrogel of **2** within 2 hours and hydrogel of **4** overnight.

Rheological measurement. Rheological tests were conducted on TA ARES G2 rheometer with TA Orchestrator Software. 25 mm cone-plate was used during the experiment. 0.2 mL of hydrogel sample was placed on the cone-plate.

(1) Dynamic strain sweep tests for the investigation of the system structures:
The measurement performs at the frequency of 6.28 rad/s (0.1 to 10 % strain, frequency =10 rads^{-1}, 10 points per decade). Sweep mode is "log" and temperature was carried out at 25 ^{o}C. The critical strain (γ_c) value was determined from the storage-strain profiles of the hydrogel sample. The strain applied to the hydrogel sample increased from 0.1 to 100 % (10 rad/s and 25 ^{o}C). Over a certain strain, a drop in the elastic modulus was observed, and the strain amplitude at which storage moduli just begins to decrease by 5 % from its maximum value was determined and taken as a measure of the critical strain of the hydrogels, which correspond to the breakdown of the cross-linked network in the hydrogel sample.

(2) Dynamic frequency sweep tests for the investigation of the viscoelastic properties:
The frequency ranges from 200 rad/s to 0.1 rad/s, depending on the viscoelastic properties of each sample. A suitable strain was used to ensure the linearity of dynamic viscoelasticity.

Table S1. The IC_{50} values for naproxen based hydrogelators inhibit COX-1 and COX-2 enzymes

IC_{50} / Hydrogel	COX-1 (μM)	COX-2 (μM)	Selectivity for COX-2[a]
Npx	4.8	6.8	0.7
1	853.8	487.7	1.8

2	273.7	68.8	4.0
3	383.5	143.2	2.7
4	428.9	31.7	13.5
5	476.3	132.2	3.6
6	367.3	36.7	10.0
L-1	985.7	715.0	1.4
L-2	294.0	143.5	2.0
L-3	706.4	313.1	2.3
L-4	114.83	38.0	3.0

[a]The selectivity for COX-2 enzyme is calculated by the equation IC_{50} of COX-1 / IC_{50} of COX-2.

Table S2. The critical gelation concentrations (CGC) of Npx containing hydrogelators.

	CGC (wt %)	pH	Optical image for Gels at cgc
1	0.2	4.0	
2[a]	0.2	7.6	
3	0.6	7.6	
4[a]	0.4	7.6	
5	0.8	7.0	
6	0.2	7.0	

[a]Hydrogels formed by treating with 0.2 U/mL of alkaline phosphatase.

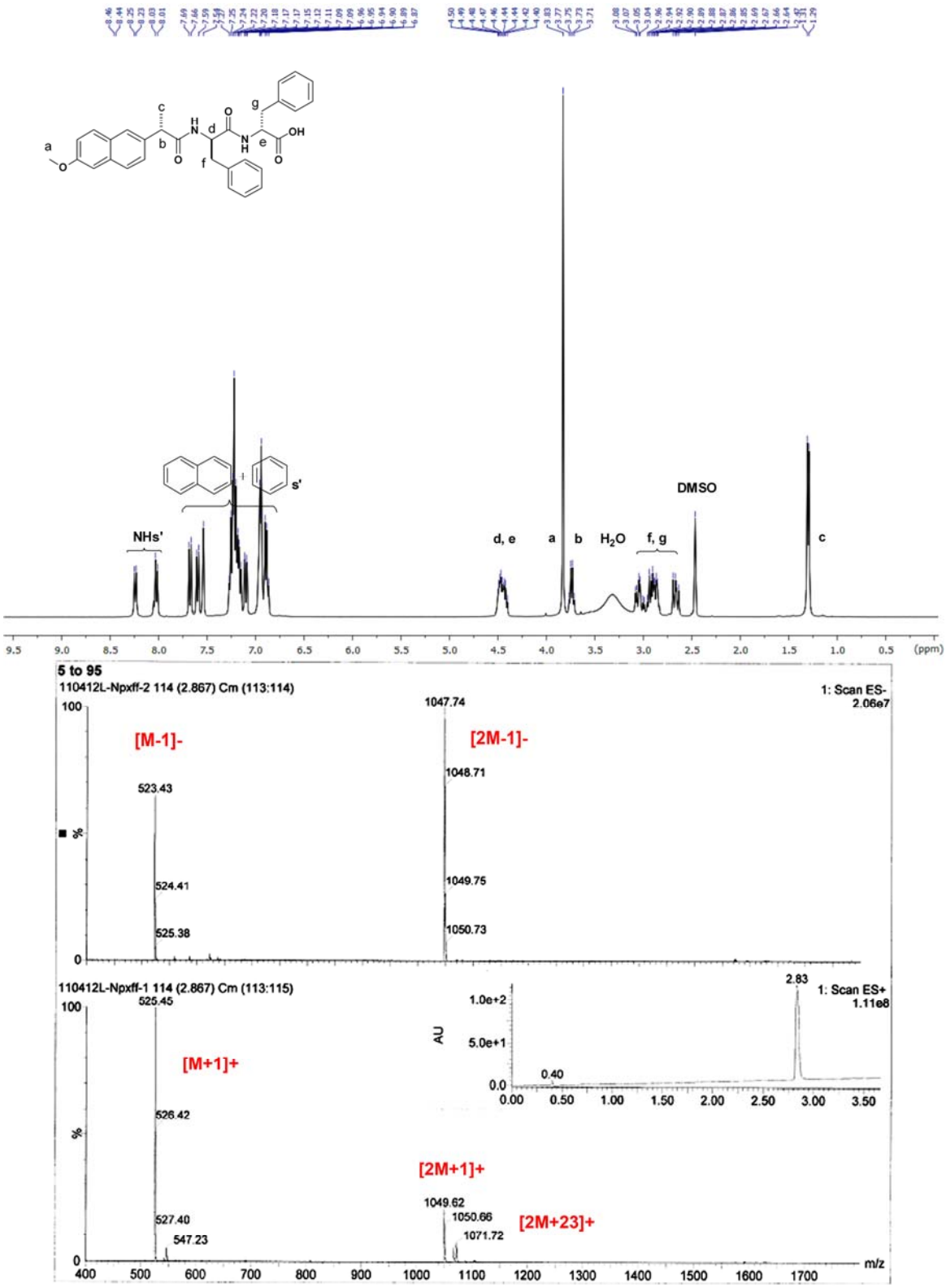

Figure S1. ^{1}H NMR spectrum of **1** in DMSO-d_6.

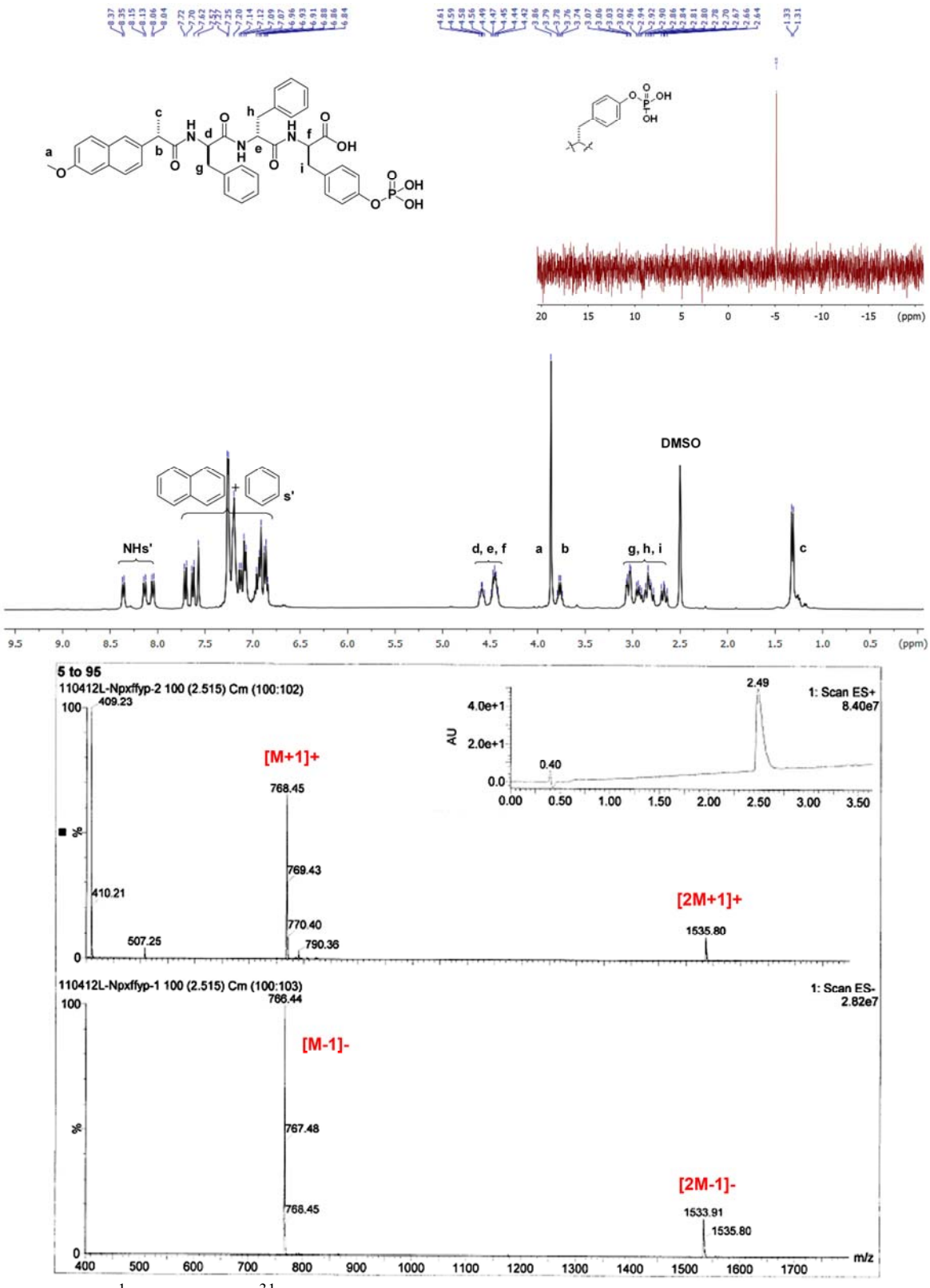

Figure S2. ^{1}H NMR and ^{31}P NMR (inset figure) spectra of **2P** in DMSO-d_6.

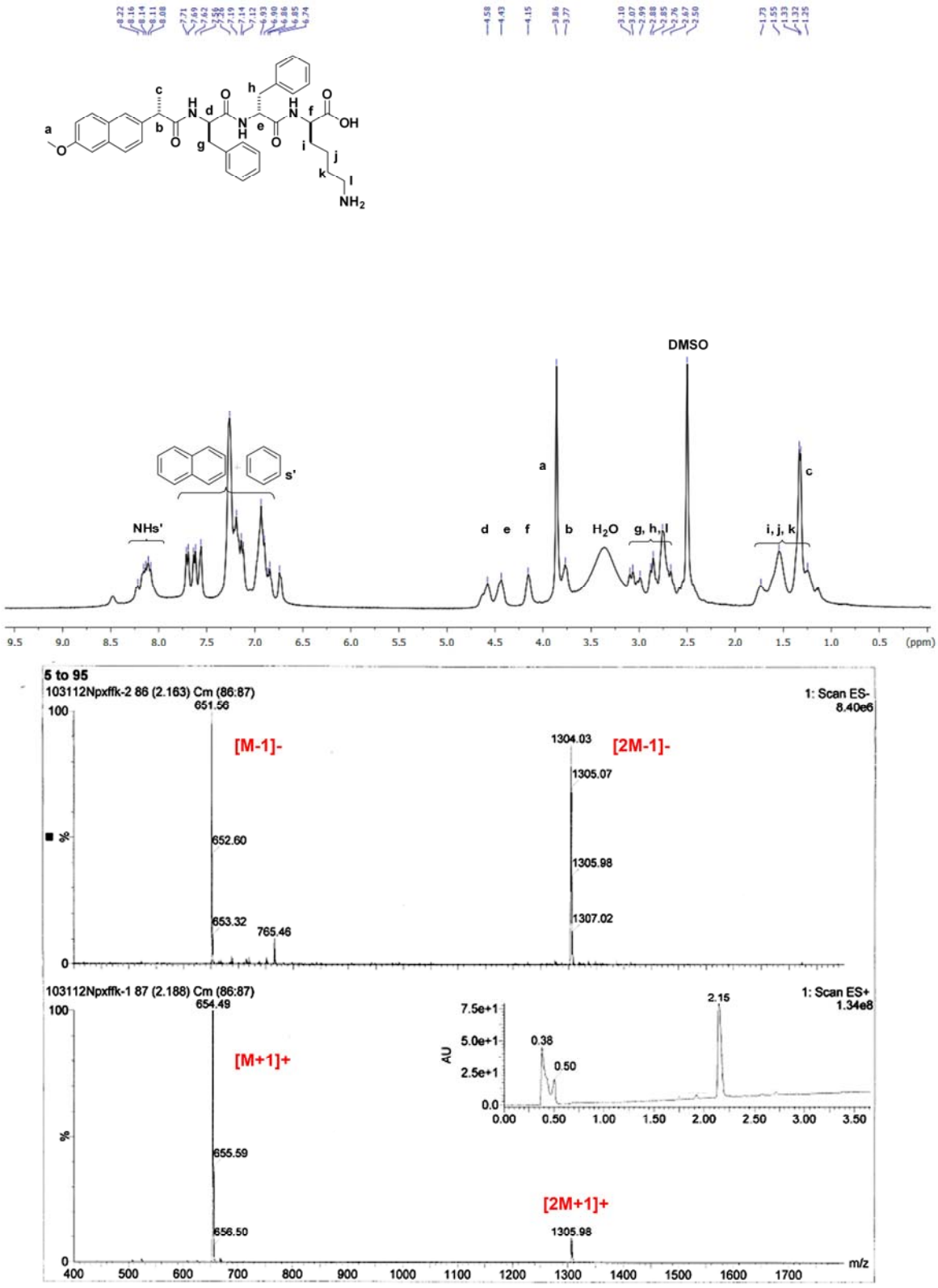

Figure S3. ^{1}H NMR spectrum of **3** in DMSO-d_6.

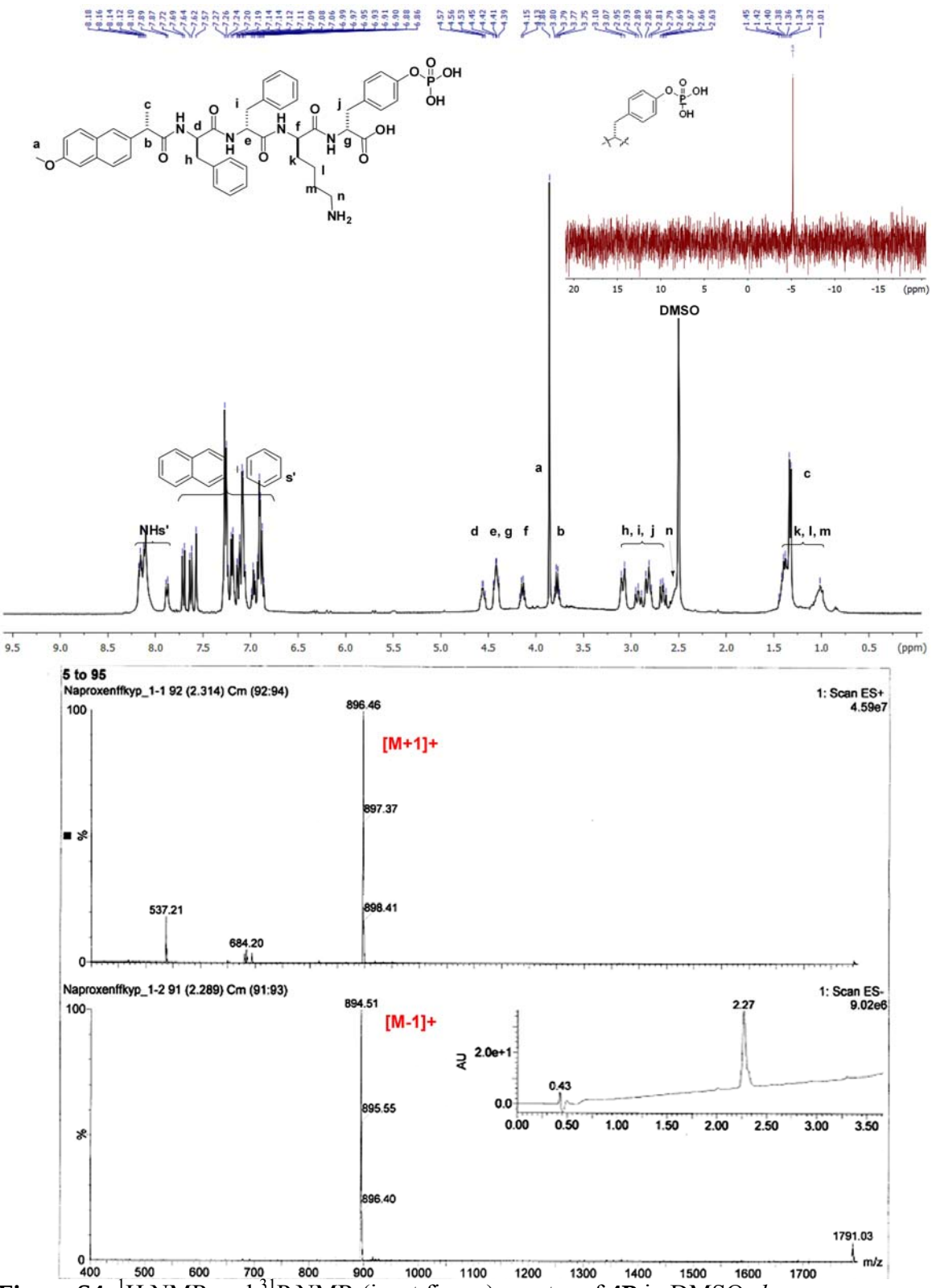

Figure S4. ^{1}H NMR and ^{31}P NMR (inset figure) spectra of **4P** in DMSO-d_6.

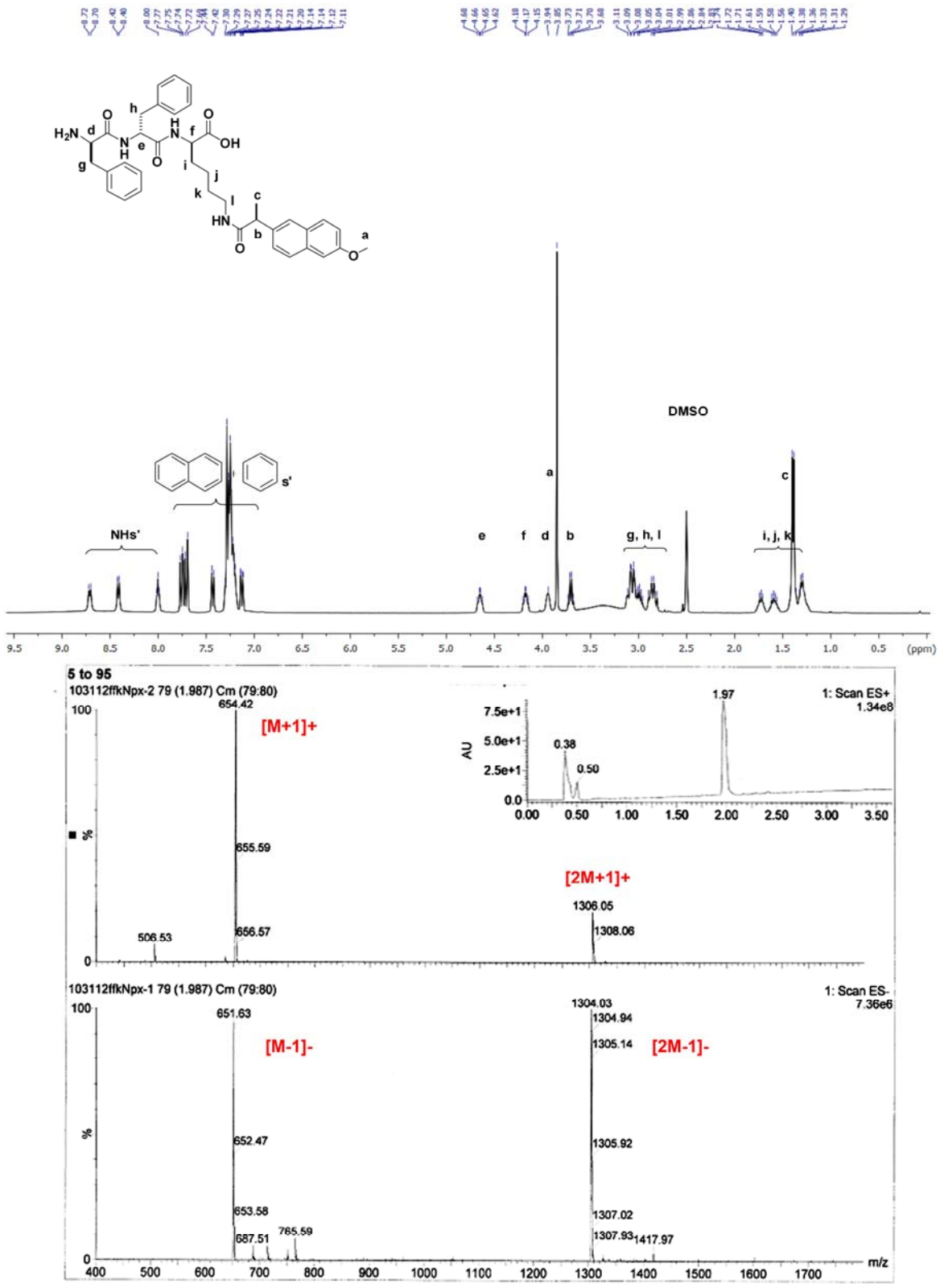

Figure S5. ^{1}H NMR spectrum of **5** in DMSO-d_6.

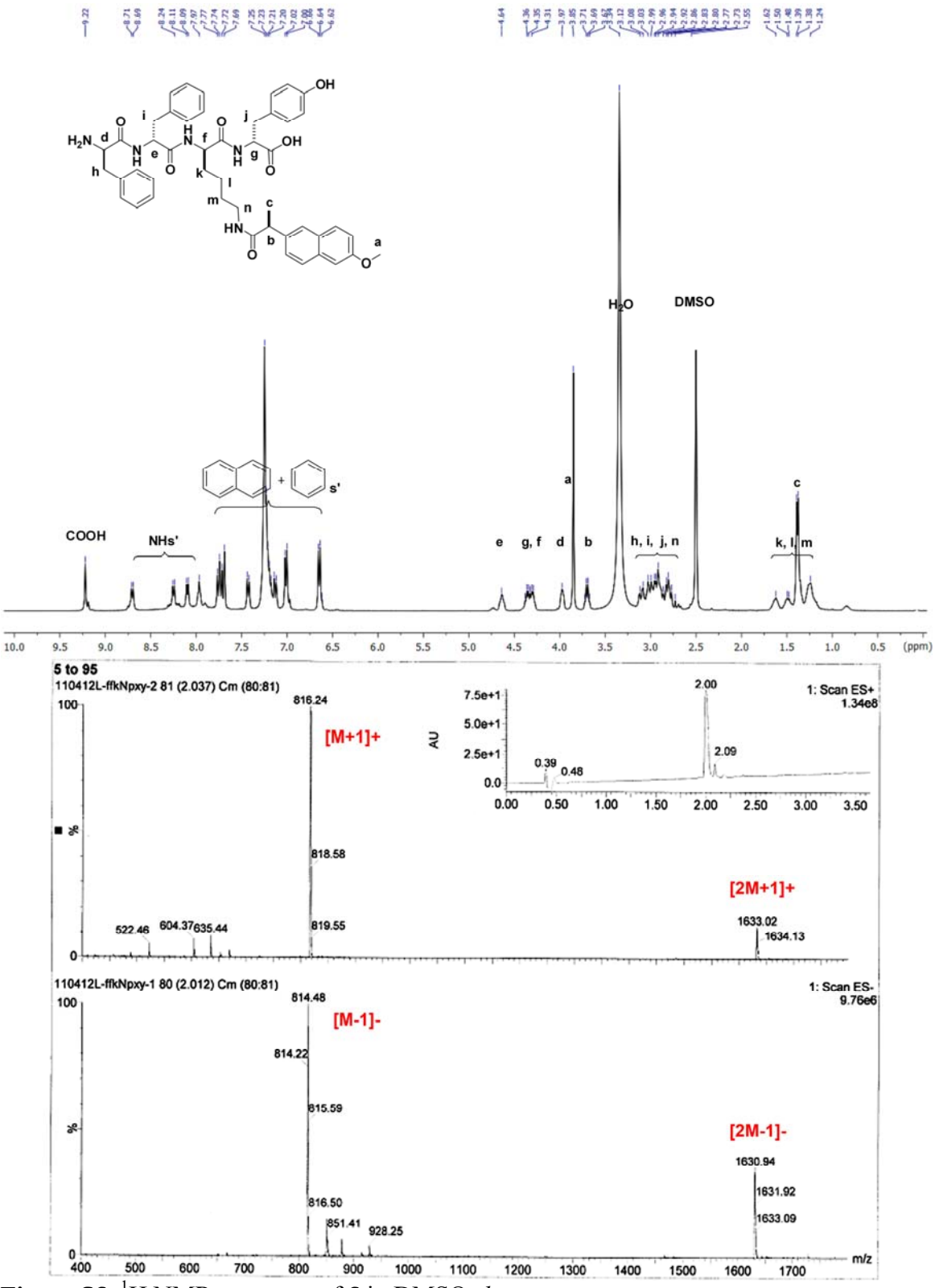

Figure S6. ^{1}H NMR spectrum of **6** in DMSO-d_6.

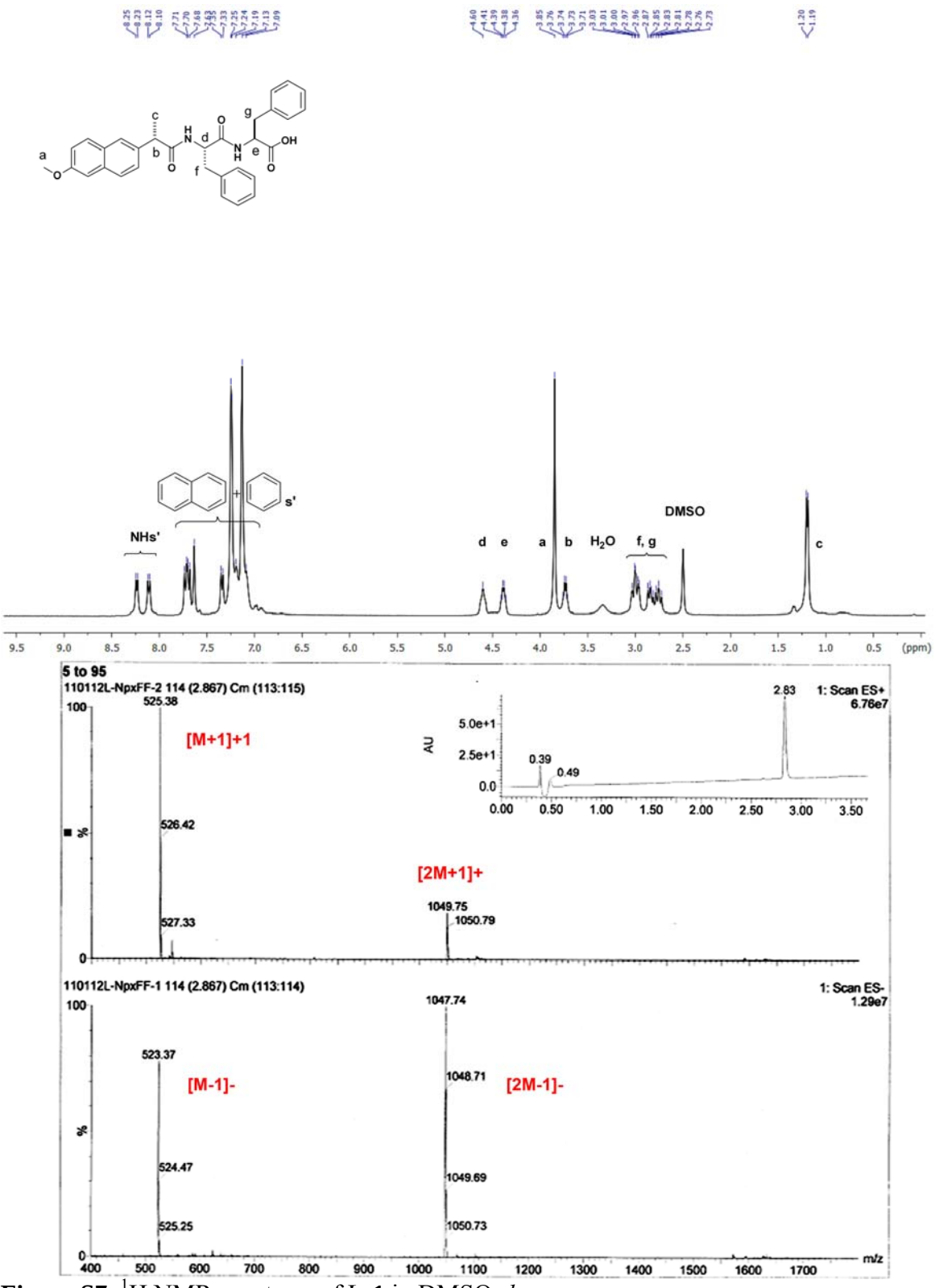

Figure S7. ^{1}H NMR spectrum of L-**1** in DMSO-d_6.

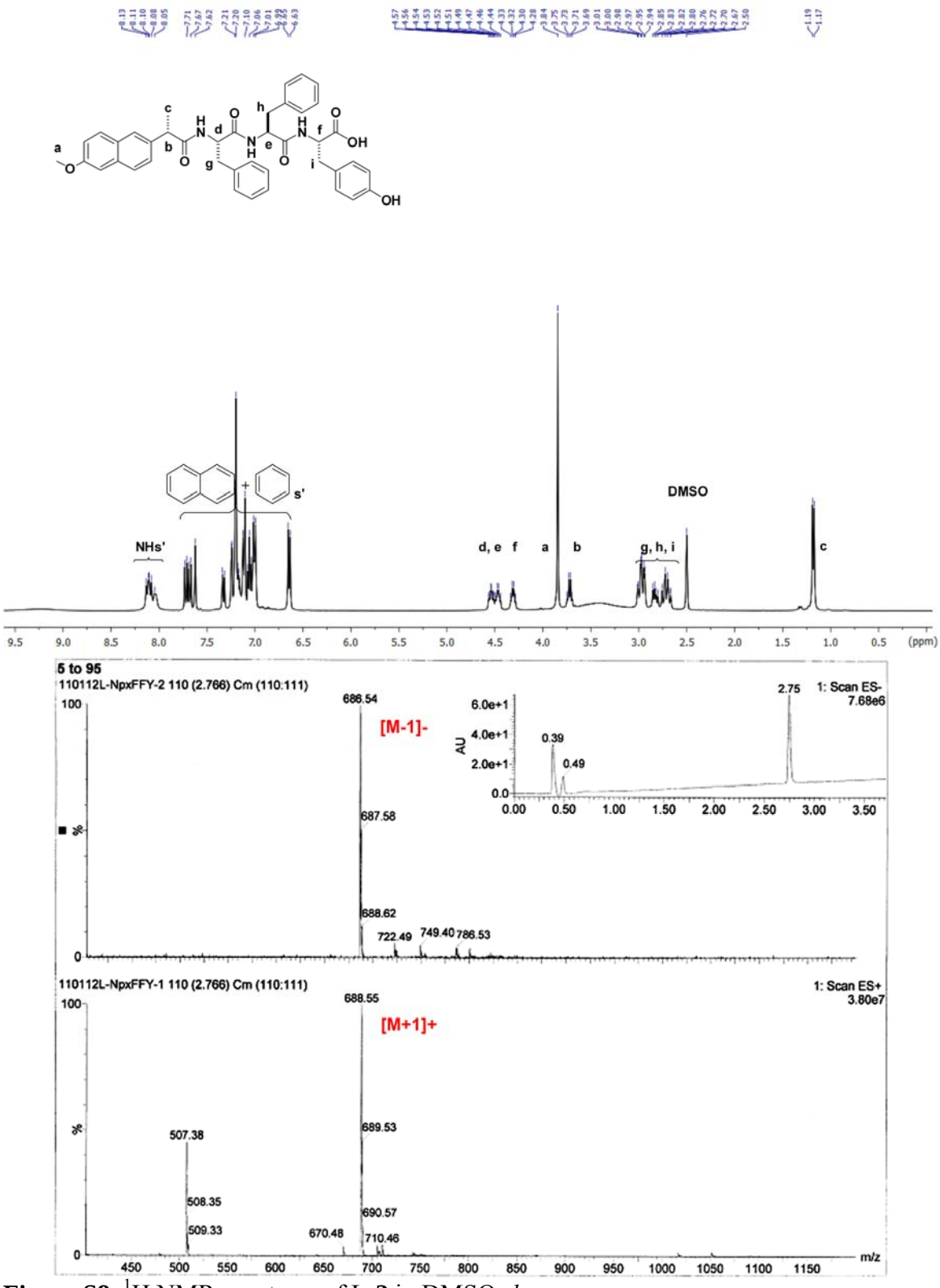

Figure S8. ^{1}H NMR spectrum of L-**2** in DMSO-d_6.

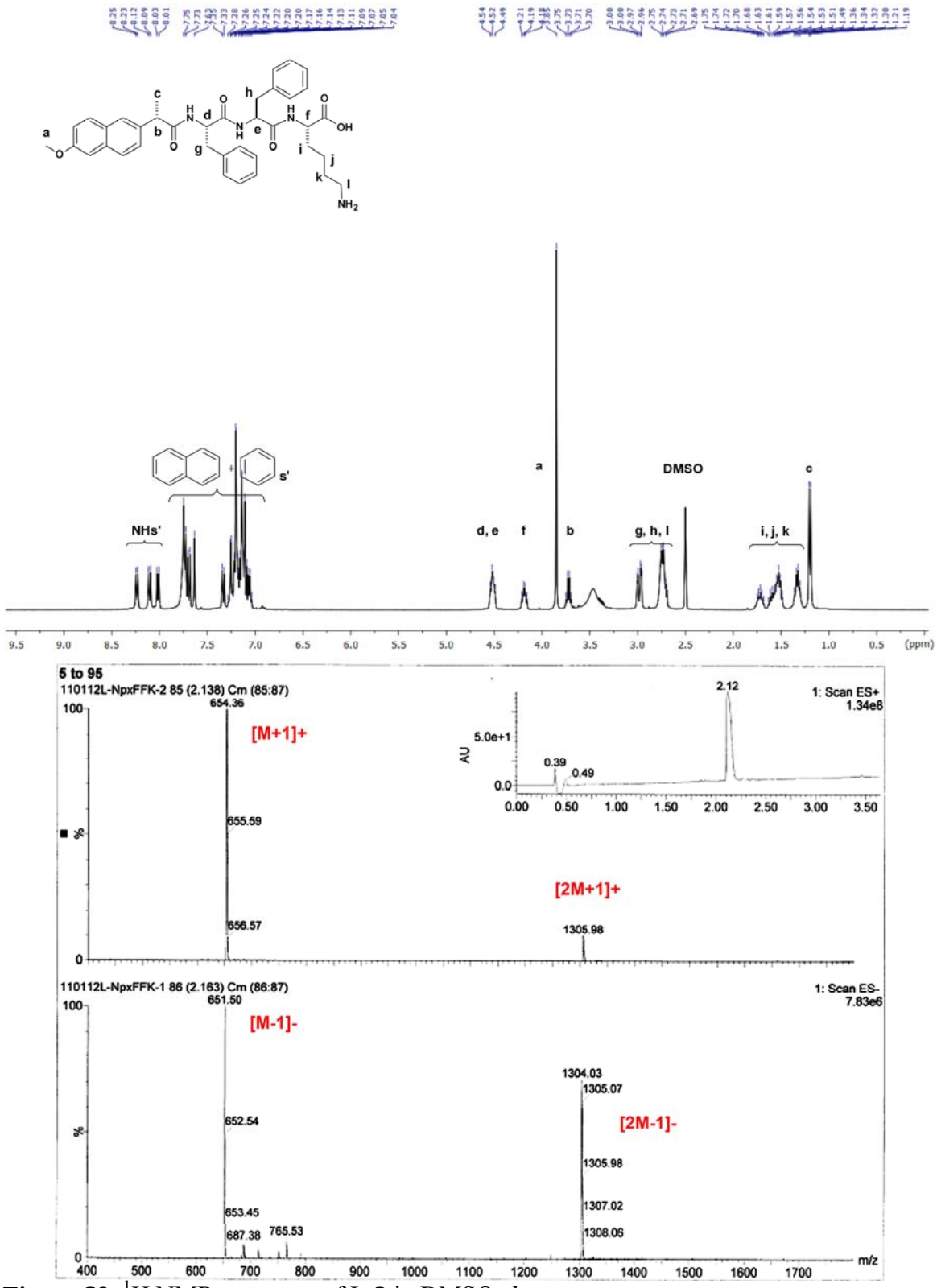

Figure S9. ^{1}H NMR spectrum of L-**3** in DMSO-d_6.

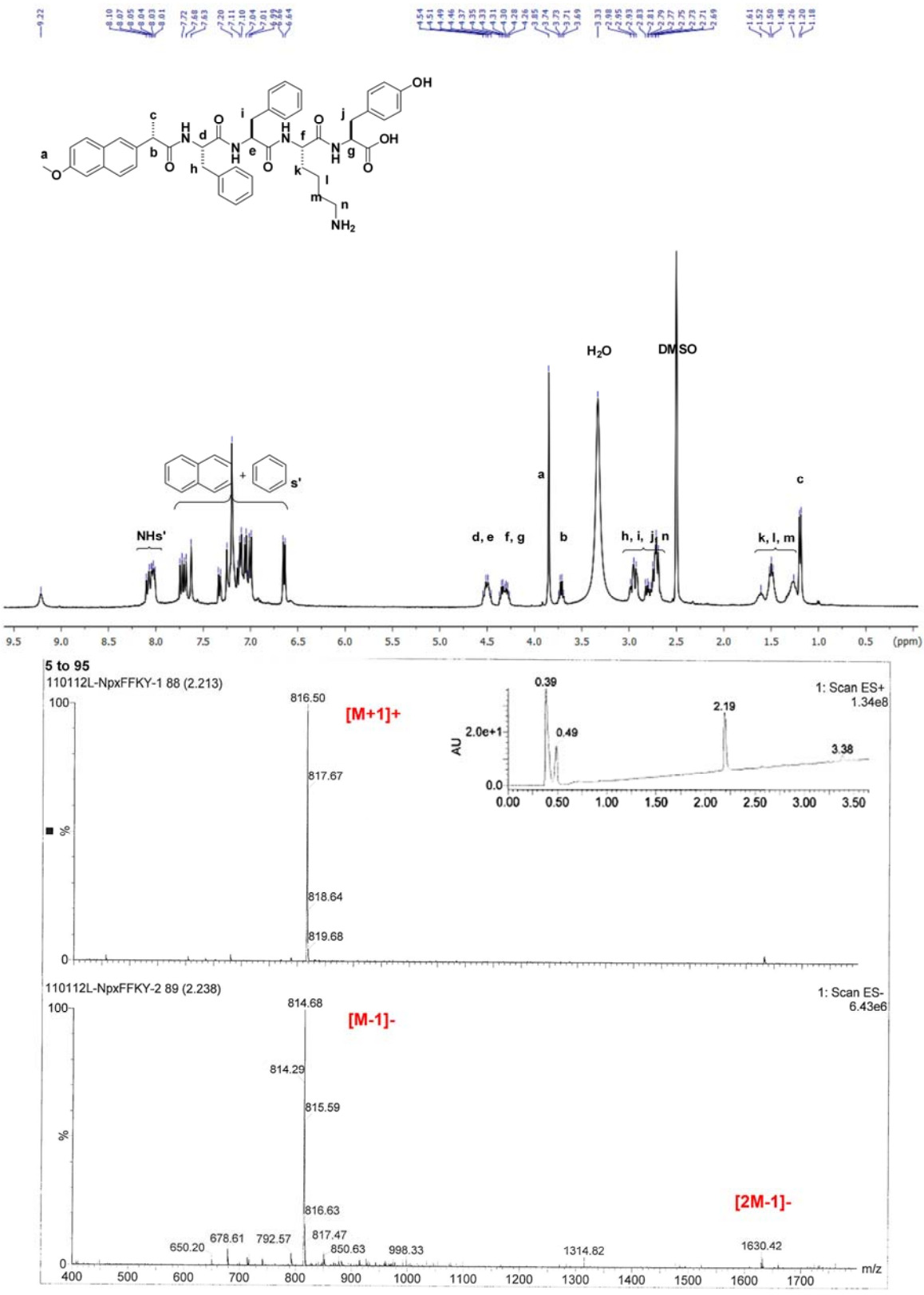

Figure S10. ^{1}H NMR spectrum of L-**4** in DMSO-d_6.

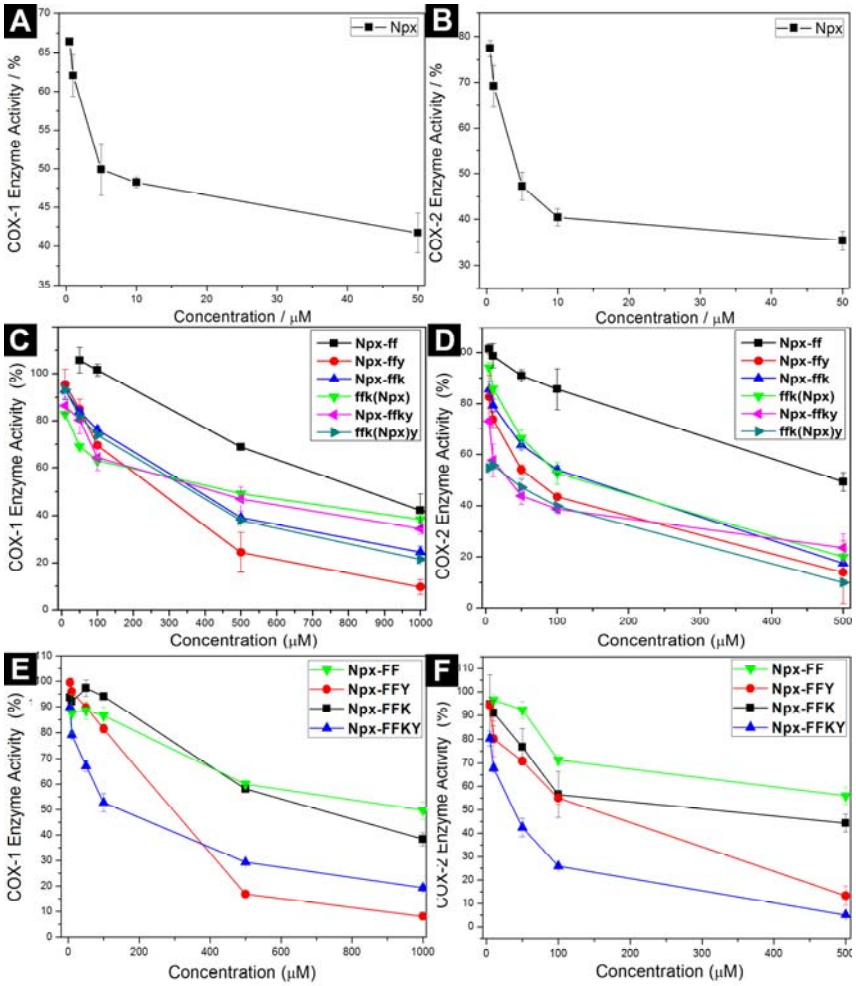

Figure S11. The COX-1 enzyme activity curves for (A) Npx, (C) D version hydrogelators **1**, **2**, **3**, **4**, **5**, and **6**, and (E) L version hydrogelators L-**1**, L-**2**, L-**3**, and L-**4**; The COX-2 enzyme activity curves for (B) Npx, and (D) D version hydrogelators **1**, **2**, **3**, **4**, **5**, and **6**, and (E) L version hydrogelators L-**1**, L-**2**, L-**3**, and L-**4.**

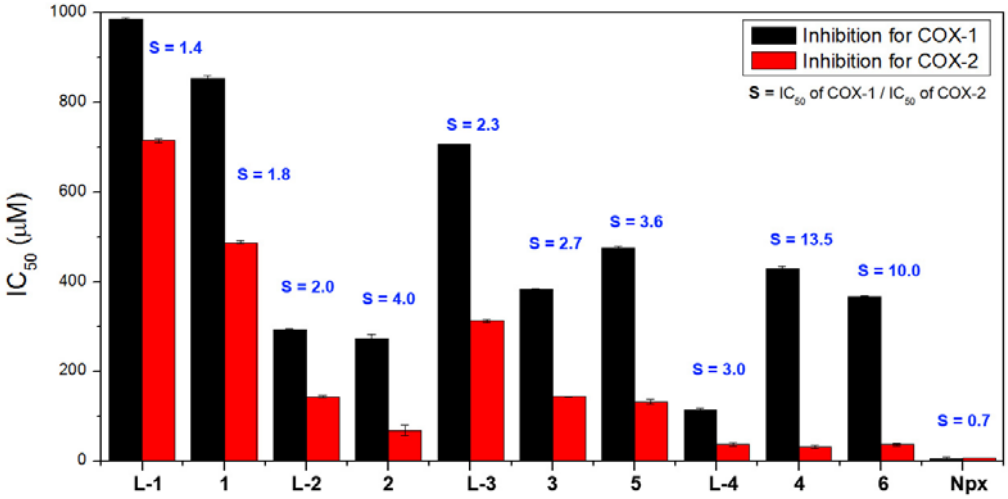

Figure S12. IC_{50} values of all these Npx containing hydrogelators.

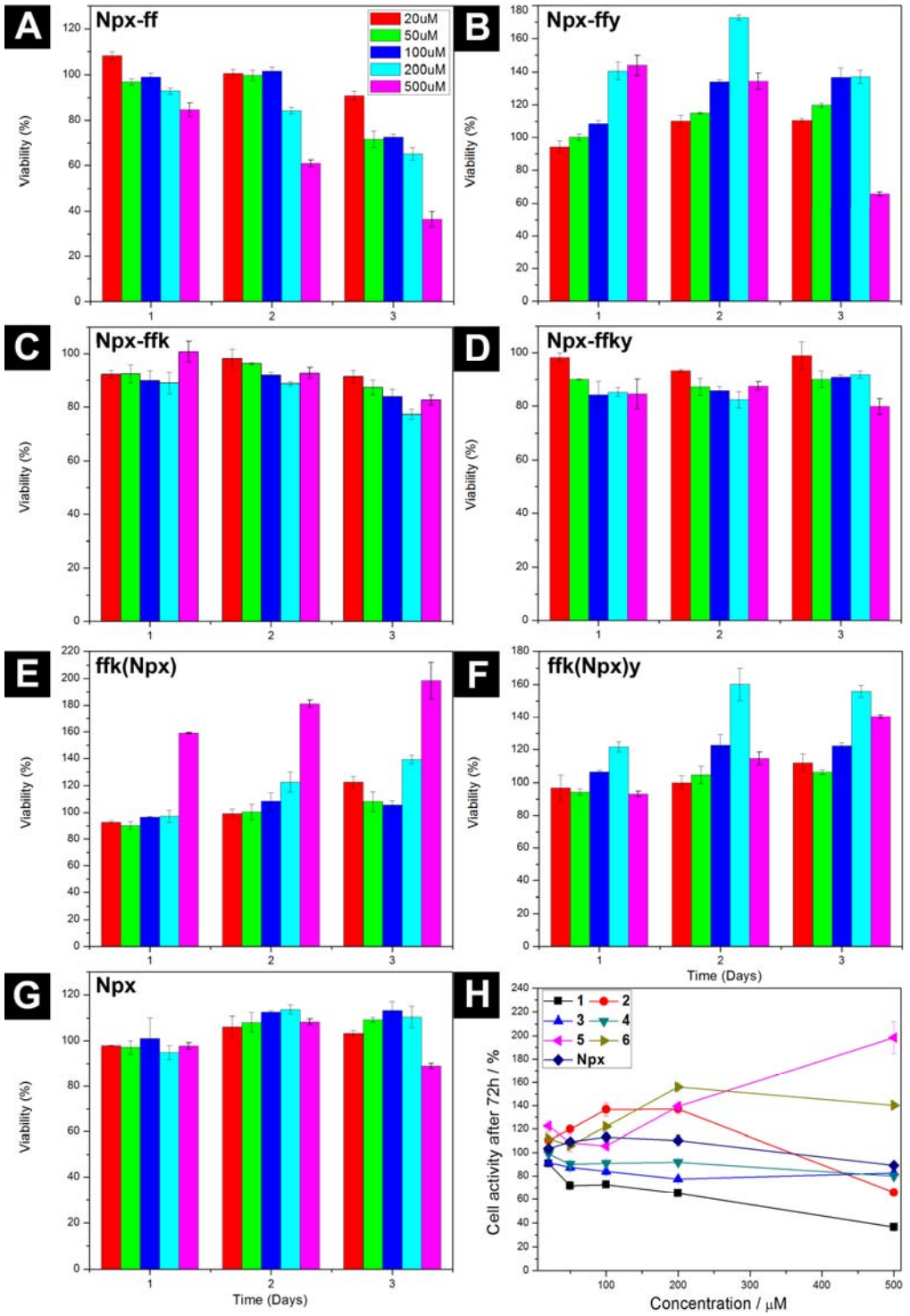

Figure S13. Cytotoxicity of (A) **1**, (B) **2**, (C) **3**, (D) **4**, (E) **5**, (F) **6** and (G) Npx treated with HeLa cells for 3 days; (H) the activity curves of HeLa cell after 72 hours.

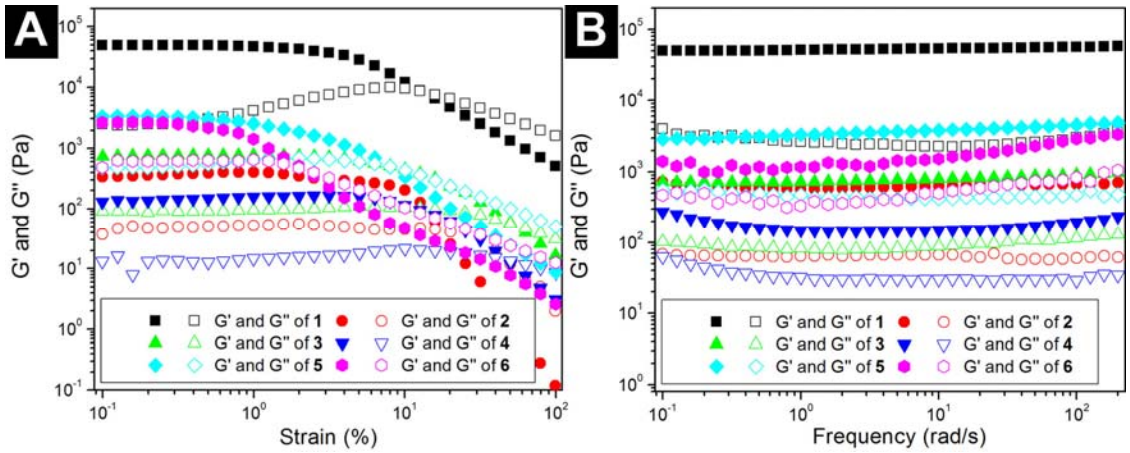

Figure S14. The strain (A) and frequency (B) dependence of dynamic storage modulus G' (solid) and loss modulus G'' (hollow) of Npx containing hydrogels of **1**, **2**, **3**, **4**, **5**, and **6**.

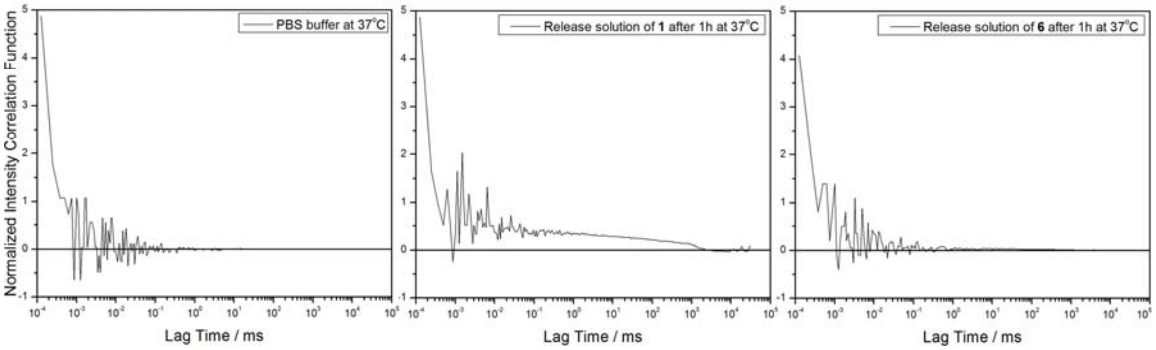

Figure S15. The dynamic light scattering data for the PBS buffer and the release solutions of **1** and **6** (at 90 degree, 37°C).

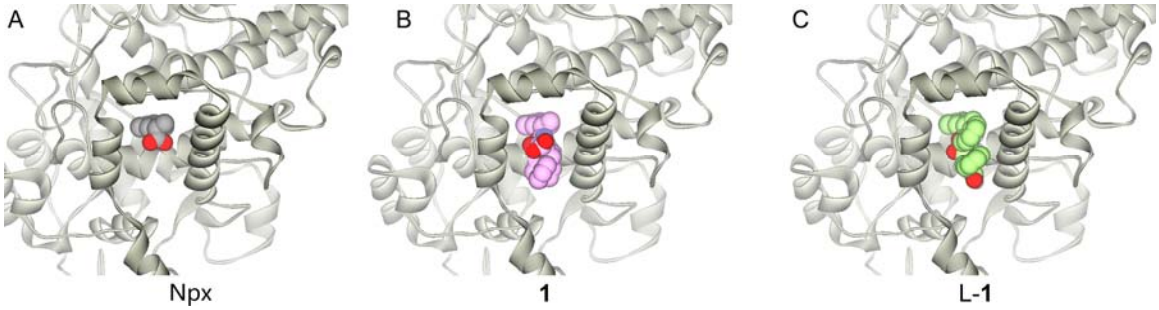

Figure S16. The docking of (A) Npx, (B) **1** and, (C) L-**1** in the binding pocket of COX-2.

[1] Ottinger, E. A.; Shekels, L. L.; Bernlohr, D. A.; Barany, G. *Biochemistry* **1993**, *32*, 4354.

[2] E. Cheong, K. Ivory, J. Doleman, M. L. Parker, M. Rhodes, I. T. Johnson, *Carcinogenesis* **2004**, *25*, 1945; J. A. Cordero, M. Camacho, R. Obach, J. Domenech, L. Vila, *Eur. J. Pharm. Biopharm.* **2001**, *51*, 135.

[3] M. A. Hayat (2000). *Principles and techniques of electron microscopy: biological applications*. Cambridge University Press. pp. 45–61.