Supporting Information

A π-Conjugated Hydrogel based on an Fmoc-Dipeptide Naphthalene Diimide Semiconductor.

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Experimental Section

General Methods. Fourier transform-infrared (FTIR) were performed on FTIR spectrometer (Thermo Nicolet, Madison, WI). Circular dichroism (CD) spectra were taken with an AVIV 202 CD spectrometer. Atomic force microscopy (AFM) was conducted in tapping mode. Matrix-assisted laser desorption ionization-time of flight MS (MALDI-TOF MS) spectrometry was performed using 2,5-dihydroxybenzoic acid as the matrix in tetrahydrofuran (THF). All fluorescence spectroscopy were performed in a Perkin-Elmer LS-50B using a cuvette with 1 mm or 1cm pass length at 25 ^{o}C. Transmission Electron Microscopy (TEM) was performed with Technai G2 Spirit instrument operating at 80 kV. Congo Red staining test was examined under polarized light with a Nikon Eclipse LV100 optical microscope connected to a digital camera (Digital Sight DS-2Mv). All reactions were performed under an argon or nitrogen atmosphere. ^{1}H NMR were recorded at 400 or 500 MHz and ^{13}C NMR spectra at 100 or 125 MHz on a Bruker DPX-400 or DPX-500 instrument as indicated. Dimethylformamide (DMF) was dried by distillation from $MgSO_4$. All water used for sample solutions was HPLC grade and passed through membrane filter (0.02 μm) before use.

Peptide Preparation

Fmoc-KK(NDI) was manually prepared using Fmoc/*t*-Bu solid-phase peptide synthesis on rink amide resin (loading 0.35mmol/g). Amide-coupling steps were accomplished with standard techniques for all amino acids: Fmoc-amino acid, 1,3-diisopropylcarbodiimide (DIC), and 1-hydroxybenzotriazole (HOBt) (500 mol% each relative to resin) in 1:1 DMF/DCM for 1 h. Piperidine (20%) in DMF was used for Fmoc removal.

The dipeptides were cleaved from the resin by the treatment with TFA/water/triethylsilane (95 / 2.5 / 2.5) at room temperature for 2 h. The crude peptides were precipitated with cold dimethylether and purified by reversed-phased HPLC on preparative Varian Dynamax C18 column eluting with a linear gradient of CH_3CN/water (30/70 to 100/0 over 50 minutes, 0.1 % TFA) and stored as lyophilized powers at 0 $^{\circ}$C. Peptide purity was assessed by analytical reverse-phase HPLC and identity confirmed using ESI mass spectrometry.

The Attachment of NDI on the Solid Support

The resin bearing peptides was treated with CH_2Cl_2/TFA (96/4) for 5 min to remove the lysine MTT protecting group followed by washing sequentially with CH_2Cl_2, DMF, DMF/DIPEA (95/5), and again with DMF. 2 ml DMF solution of monobutyl NDI (3 eq., Scheme S1) and DIPEA (4 eq) was added to the resin. The suspension was shaken for 30 min followed by the addition of HBTU/HOBt (0.4 M DMF solution, 3 eq). The reaction mixture was shaken for 12 h at room temperature and then filtered through a fritted syringe. The resin was washed thoroughly (3 x DMF, 3 x EtOH, 3 x CH_2Cl_2) and submitted for the next step.

Preparation of Hydrogel

The freeze-dried **Fmoc-KK(NDI)** sample (3 mg) was added to HPLC-grade water (200 μl) and the mixture was sonicated until all the solid was dissolved. The self-supporting hydrogel was usually formed within 6 h.

Dilution of Hydrogel for Characterization

The sample solutions for AFM, TEM, UV/Vis, Fluorescence, and CD studies were prepared by diluting the 1.5 wt% hydrogel into pure water. The solutions were stirred with a Vortex mixer and sonicated until homogeneous solutions were obtained. The solutions were kept at room temperature for 12 h before measurement.

Circular Dichroism (CD) Spectroscopy Measurement.

CD spectra were recorded on an AVIV 202 CD spectrometer under a nitrogen atmosphere. Experiments were performed in a quartz cell with a 1 cm or 1 mm path length over the range of 190-500 nm at 25 ^{o}C.

Fluorescence Spectroscopy Measurement

Aqueous **Fmoc-KK(NDI)** solutions (1 mM) were prepared by dilution from 10 mM solution and equilibrated for 24 h. The fluorescence emission spectra of NDI were measured with excitation at 330 nm.

Fourier Transform Infrared (FTIR) Spectroscopy Measurement

All FTIR spectra were collected on a Nicolet FTIR spectrometer at ambient temperature. The instrument was continuously purged with CO_2-free dry air. Spectra were recorded between 1700 and 1600 cm^{-1} at a resolution of 4 cm^{-1}, and a total of 64 scans were averaged. Samples for FTIR were dissolved in D_2O or TFE (about 10 mg/ml) and analyzed in a transmission cell having CaF_2 windows and a 0.25 μm path length.

Atomic Force Microscopy (AFM) Measurement.

The AFM images were collected on a NanoScope IIIa device at ambient temperature in tapping mode using silicon tips (NSC14/AIBS, MikroMasch). 10 μL of the sample solution (250 μM) was diluted 10 folds and then was place on freshly cleaved mica. After adsorption for 30 min under moist conditions, the excess solution was removed by absorption onto filter paper. The resultant substrates were rinsed with solvent (50 μl) twice to remove the loosely bound peptide and the samples were stored in a desiccator *in vacuo* for 1 h before imaging.

The scanning speed was at a line frequency of 1.0 Hz, and the original images were sampled at a resolution of 512 x 512 pixels.

Electron Microscopy Measurement – Negative Stain TEM

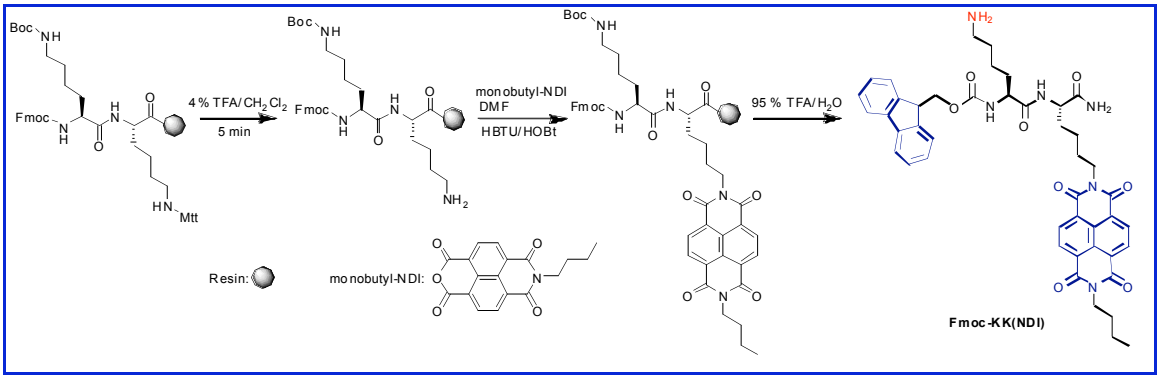
10 μL drops of dilute dipeptide hydrogel solution (250 μM) were applied to carbon-coated copper grid (Ted Pella, Inc.) for 2 min and. After removal the excess solution with filter paper, the grid was floated on 10 μL drops of 2 wt% uranyl acetate solution for negative stain for 2 min. The excess solution was removed by filter paper. The dried specimen was observed with Technai G2 Spirit instrument operating at 80 keV. The data were analyzed with Image pro software.

Congo Red Birefringence

Dipeptide hydrogel was placed on microscopic slides and left in air for drying for 20-30 min, and then stained with Congo Red solution (100 μM, 10% Ethanol) for 5 min. The stained gel was air-dried and visualized under polarized light with a Nikon Eclipse LV100 optical microscope connected to a digital camera (Digital Sight DS-2Mv).

X-ray Diffraction Measurement

Dipeptide samples were dissolved in water at a concentration of 15 mg/ml to produce hydrogels. The assemblies were isolated using Ultracentrifuge (80,000 rpm) for 2h. The bottom assemblies were collected and spread on power XRD sample holder. Powder XRD patterns were recorded on a Riguka powder diffractometer operating at 40 kV and 25 mA, using CuK_{α} radiation (λ = 1.5418 Å). Data were collected from 3^{o} to 50^{o} with a sampling interval of 0.01^{o} per step and a counting rate of 2 s per step.



Scheme S1. Synthesis of **Fmoc-KK(NDI)** using on-resin modification.

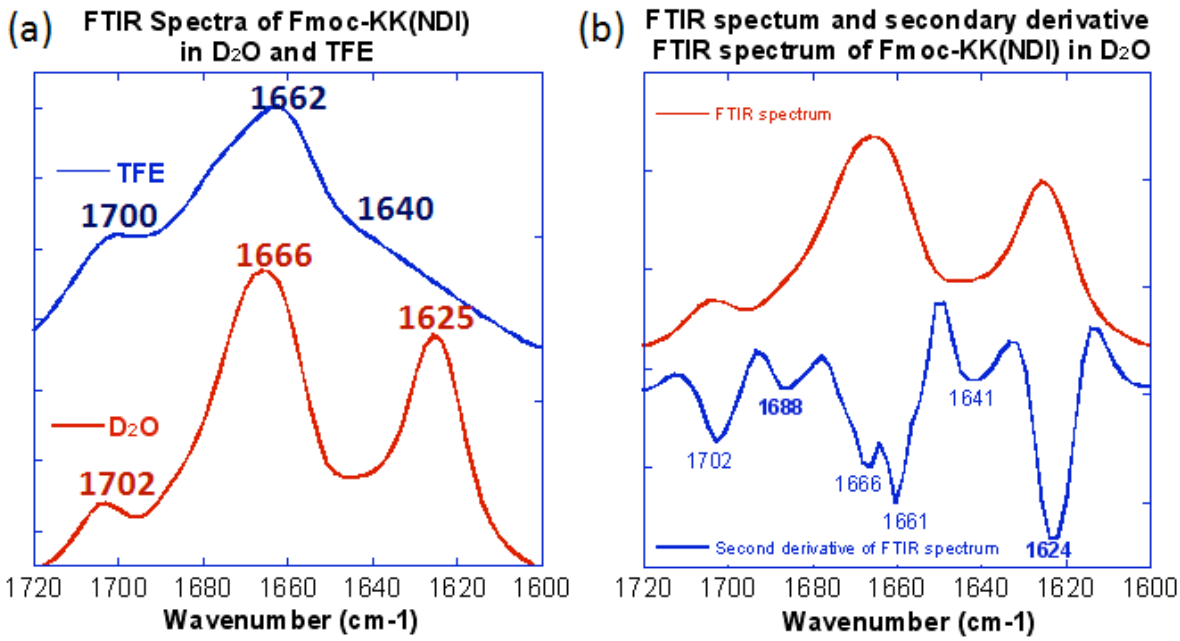


Figure S1. Secondary structure of Fmoc-KK(NDI).(a) FTIR spectra of Fmoc-KK(NDI) in TFE (10 mg/mL) and D_2O (10 mg/mL). (b) FTIR spectrum in H_2O and second derivative of FTIR spectrum in D_2O: absorbances at 1624 and 1688 cm^{-1} indicate the presence of antiparallal β -shhet stucture.

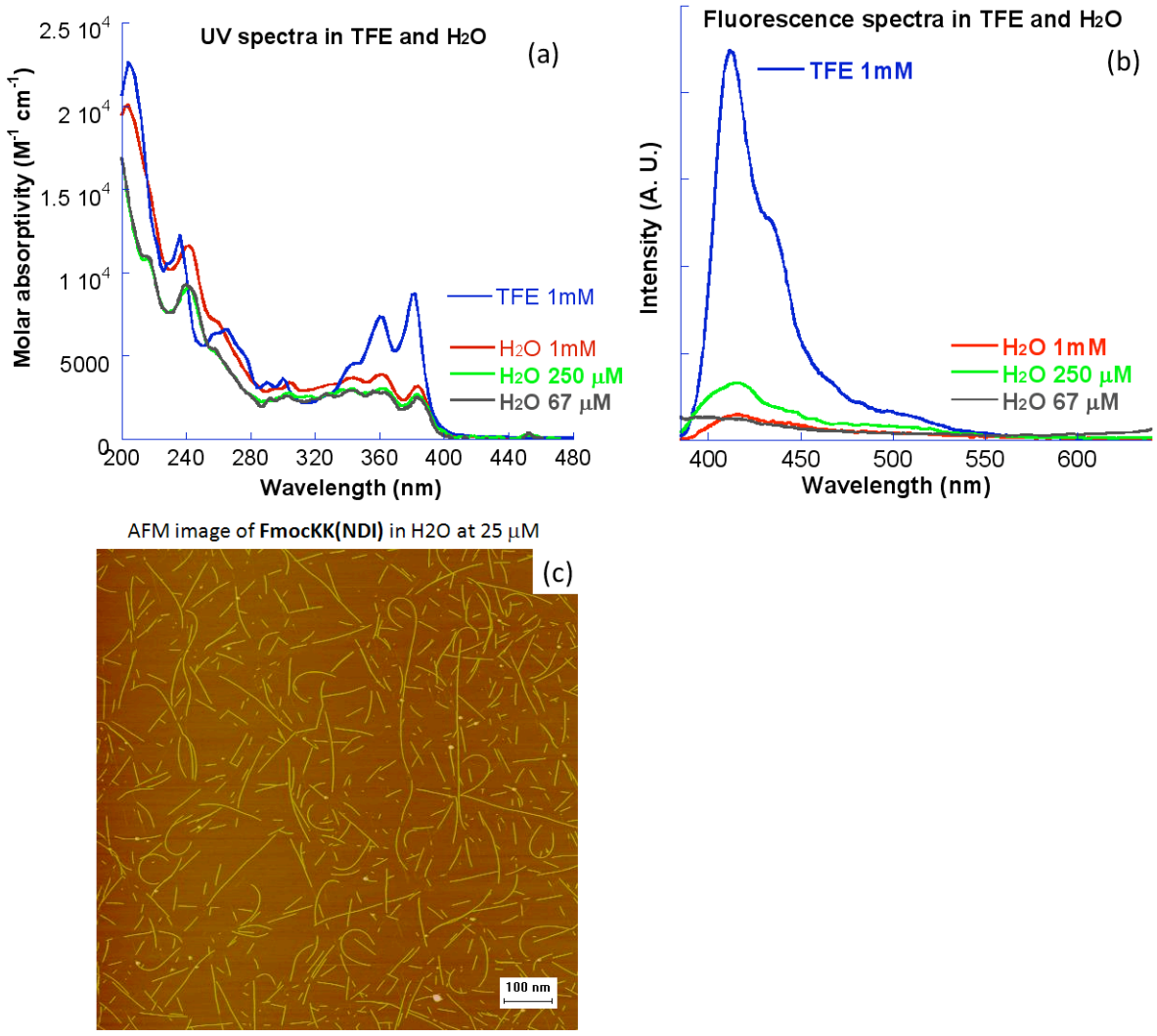


Figure S2. Concentration dependence of (a) UV/Vis and (b) Fluorescence spectra. (c) AFM image of Fmoc-KK(NDI) in H_2O at 25 µM, showing micrometer-long nanobelts.

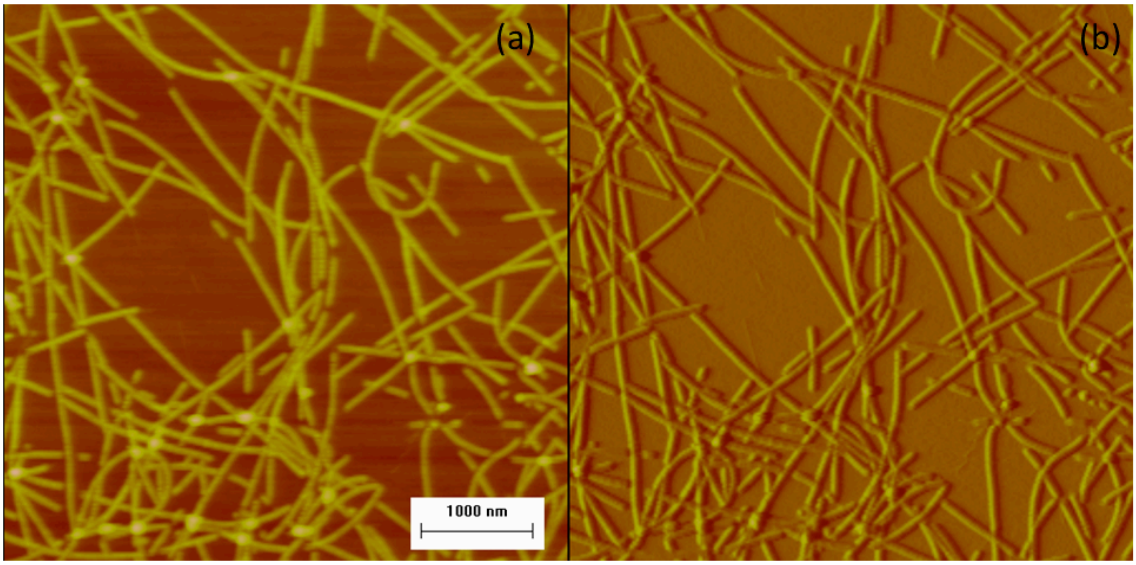


Figure S3. AFM image of dilute hydrogel showing the nanobelt networks. (a) Height image (b) Phase image.

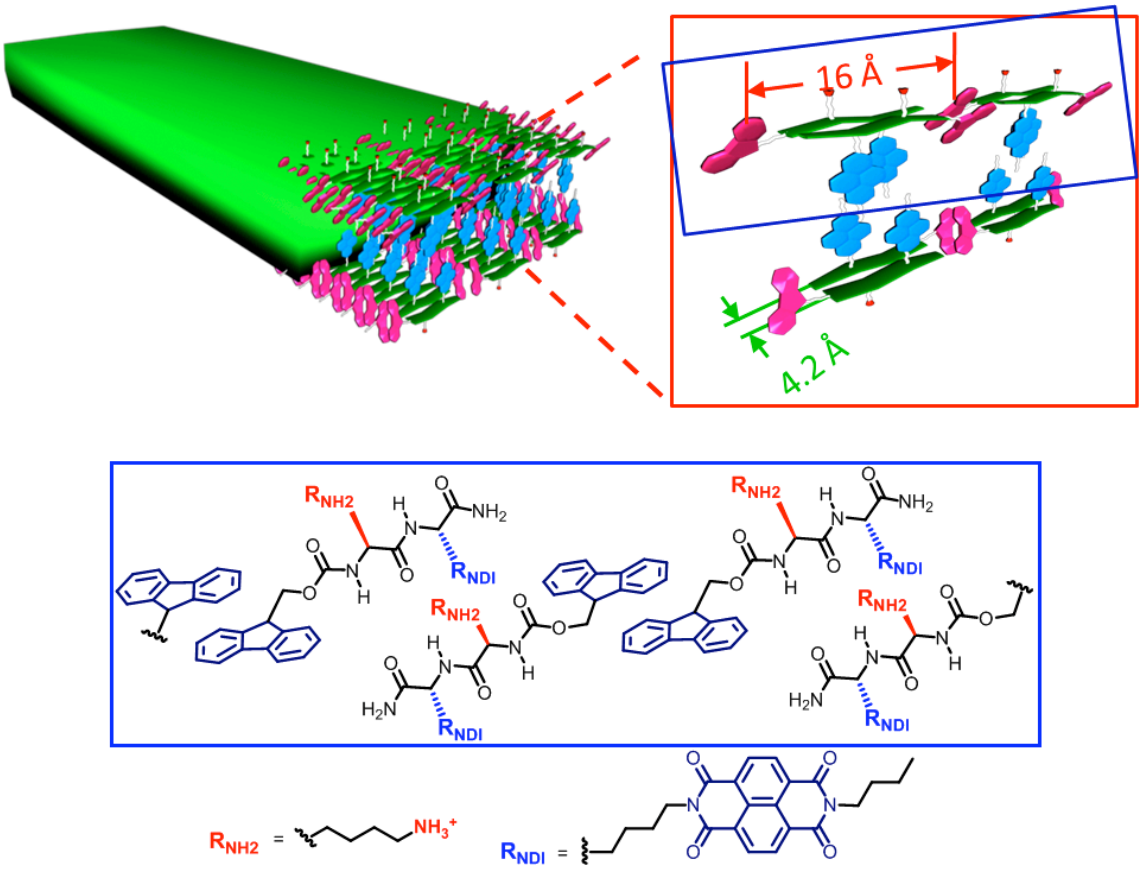
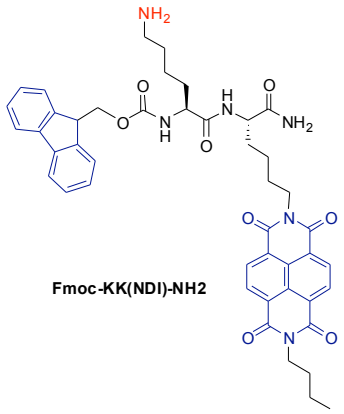


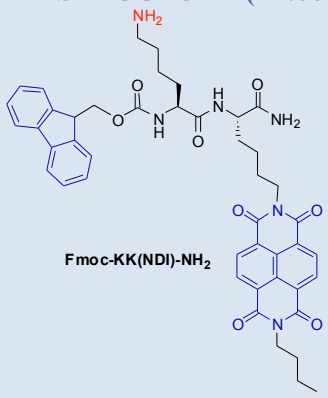
Figure S4. Schematic representation of the self-assembly of **Fmoc-KK(NDI)** into nanobelt. Powder X-ray diffraction study displays the strong reflections that correspond to the interstrand distance for β-sheet structures (4.2 Å) and the distance between the centers of the fluorenyl groups (16 Å). A molecular model was proposed based on the packing of antiparallel β-sheet of **Fmoc-KK(NDI)** (bottom).



Fmoc-KK(NDI)-NH2

Fmoc-KK(NDI)-NH_2: ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.09 (t, J = 6.8 Hz, 3H),1.22-1.45 (m, 6H), 1.47-1.81 (m, 10 H), 2.76 (m, 2H), 4.04 (m, 5H), 4.25 (m, 4H), 7.13 (s, 1H), 7.23 (m, 2H), 7.36 (m, 3H), 7.59 (d, J = 16.8 Hz, 1H), 7.62-7.75 (m, 5H), 7.85 (m, 3H), 8.66 (s, 4H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 13.67, 19.76, 22.29, 22.77, 26.55, 27.17, 29.52, 46.61, 52.15, 54.39, 120.01, 120.04, 125.15, 125.22, 126.05, 126.16, 126.21, 127.01, 127.54, 130.34, 140.63, 143.66, 143.82, 155.93, 162.47, 162.53, 173.41; IR (TFE) υ 3685, 3355, 2926, 2889, 1701, 1662, 1583, 1456, 1415, 1269, 1095, 947 cm^{-1}; ESI-MS calcd for $C_{45}H_{49}N_6O_8$ (M+H) 801.3606, found 801.6347.

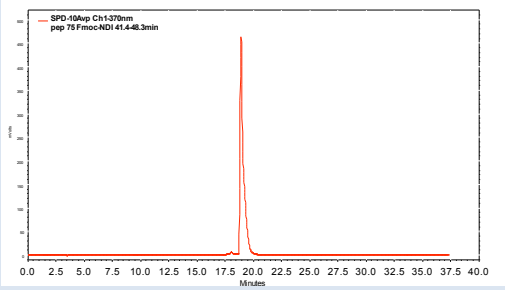
STRUCTURE (*Fmoc-KK(NDI)-NH$_2$*)



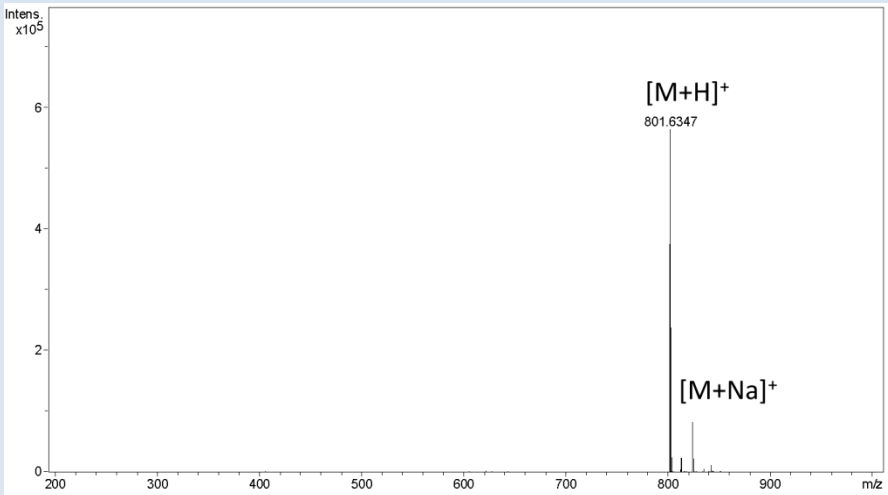
Fmoc-KK(NDI)-NH$_2$

Chemical Formula: $C_{45}H_{48}N_6O_8$
Exact Mass: 800.3534
Molecular Weight: 800.8980

HPLC TRACE



ESI-MASS



#	m/z	I
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3	801.6347	563087
4	802.6361	236798
5	803.6404	23577
6	811.7291	4247
7	811.8807	2922
8	812.3831	22006
9	812.5352	22043
10	823.6336	82221
11	824.6396	21776
12	834.4209	1478
13	834.6657	4984
14	839.6263	1292
15	841.6928	10235
16	842.7024	1805
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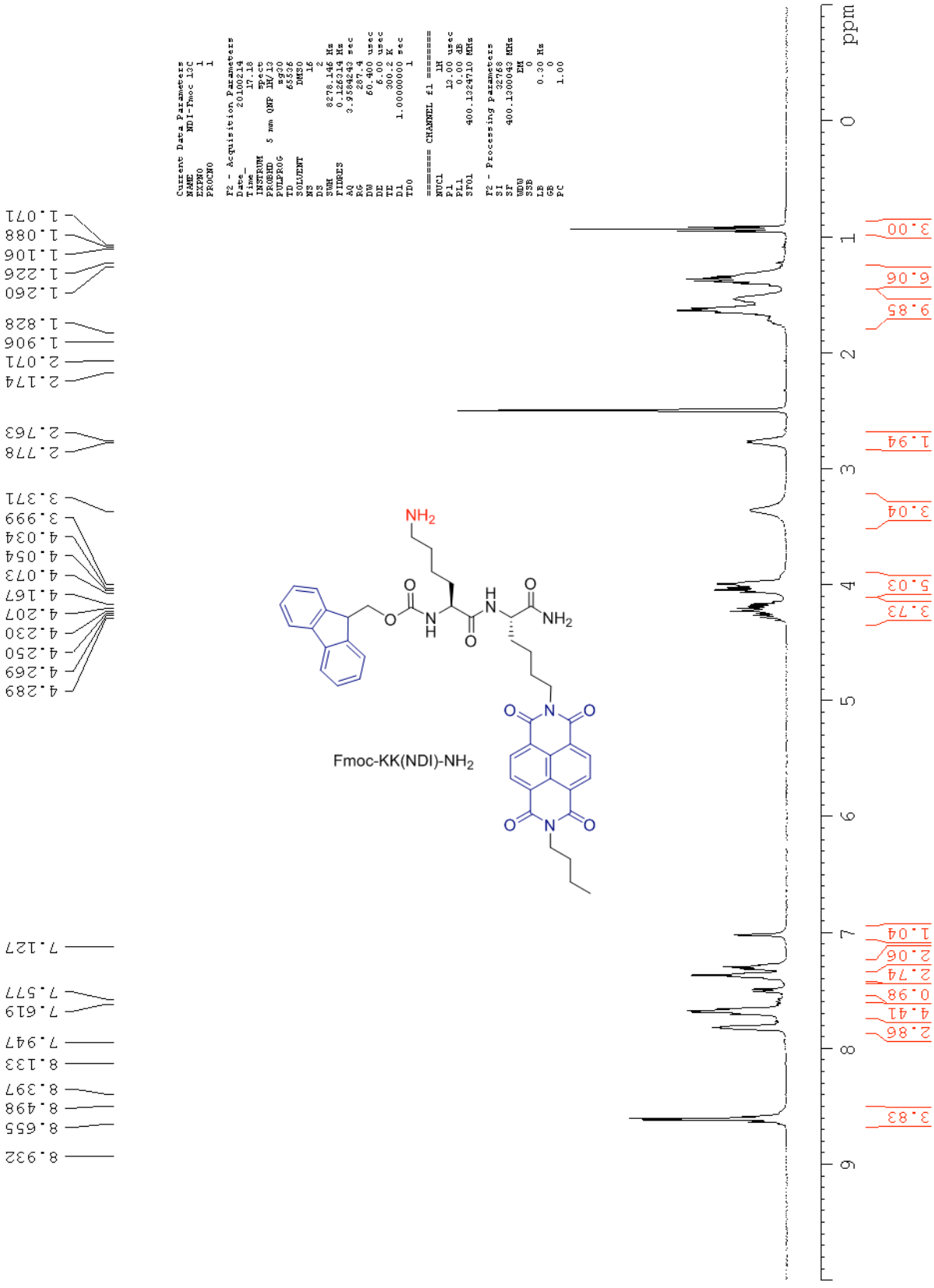
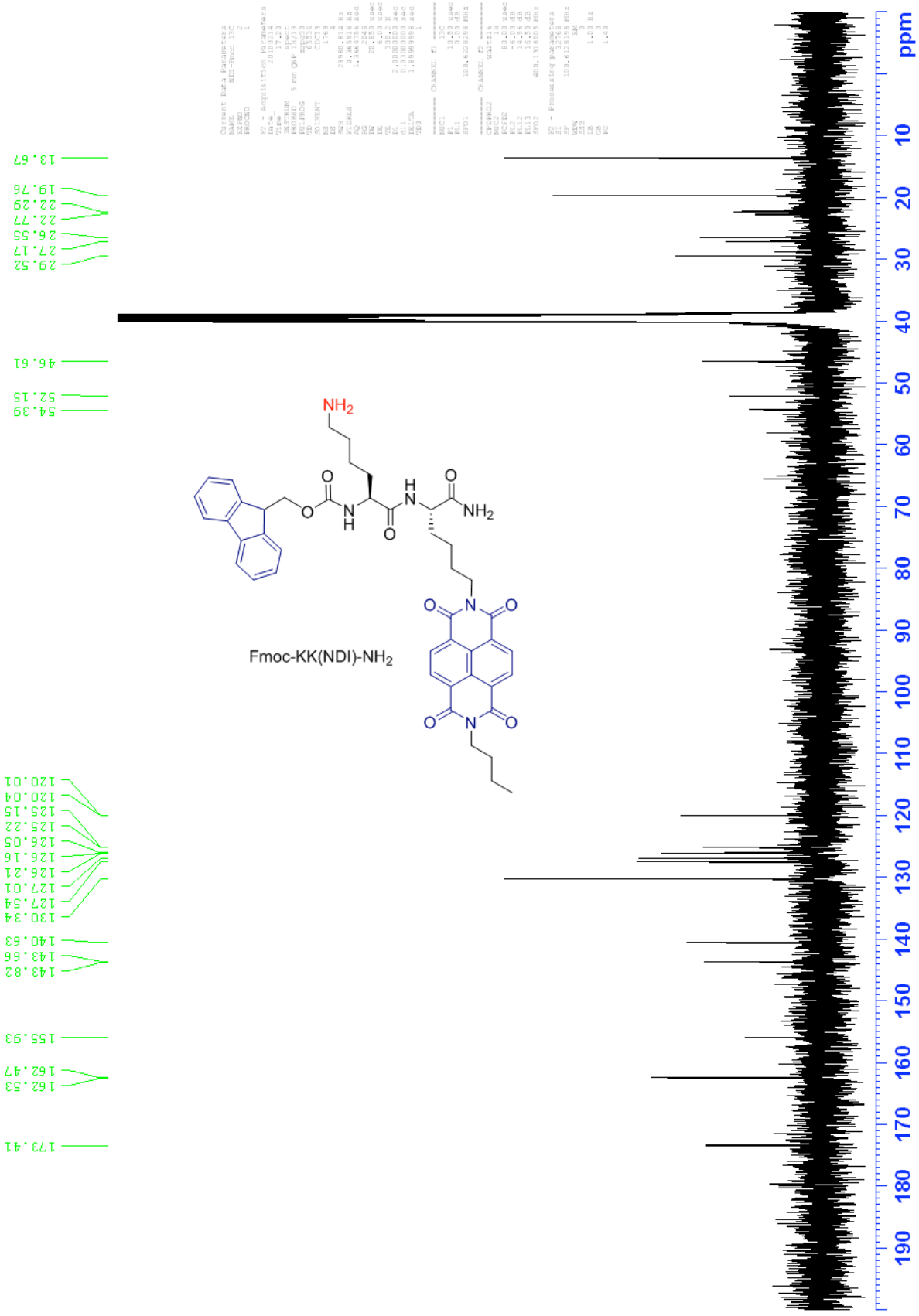

Fmoc-KK(NDI)-NH2
NH2
ppm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