Supporting Information for:

D-amino acids Modulate the Cellular Response of Enzymatic-Instructed Supramolecular Nanofibers of Small Peptides

Junfeng Shi, Xuewen Du, Dan Yuan, Jie Zhou, Ning Zhou, Yibing Huang[†], and Bing Xu*

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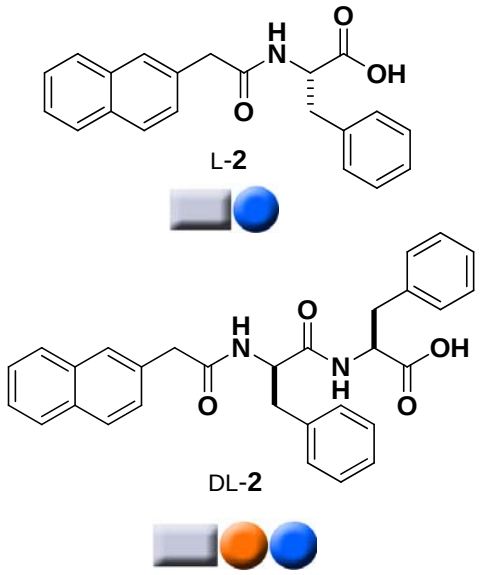


Figure S1. The chemical structure of L-**2** and DL-**2**.

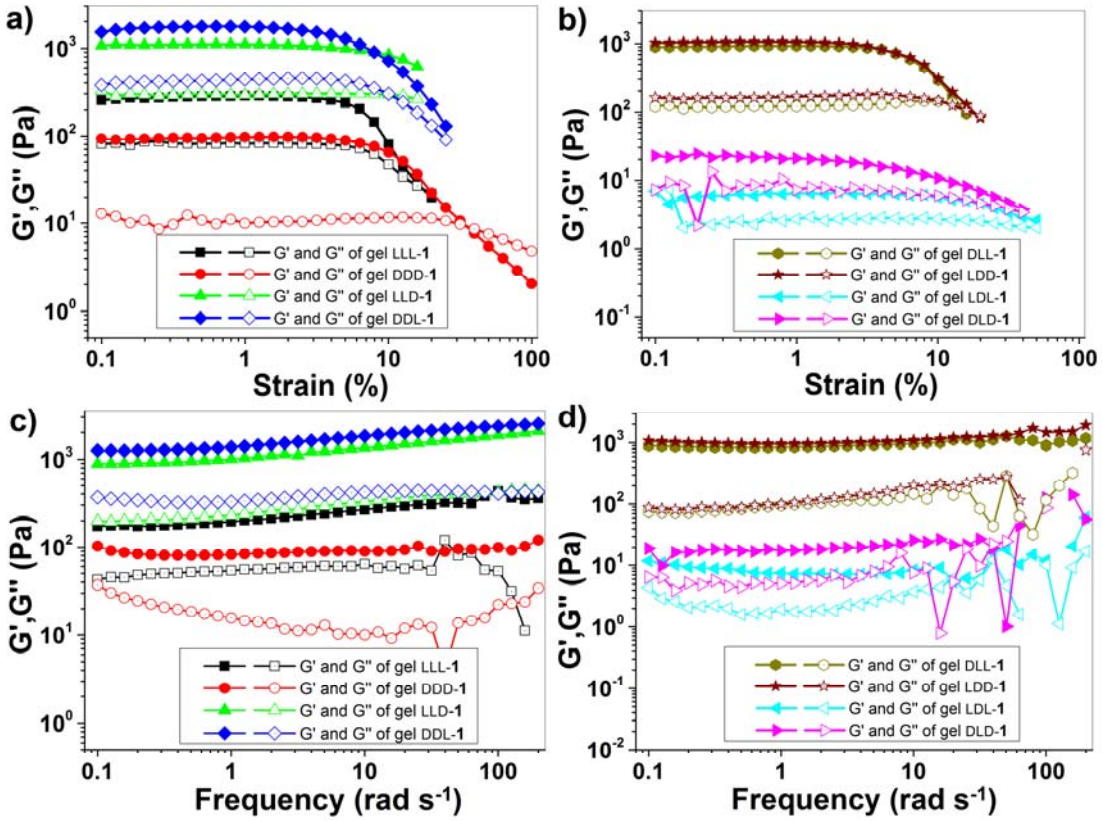


Figure S2. Rheological properties of hydrogels, Strain sweep (a,b); frequency sweep (c,d) of hydrogels prepared by adding the ALP (12.5 U/mL) to the solution of the corresponding precursors at the concentration of 0.6 wt %.

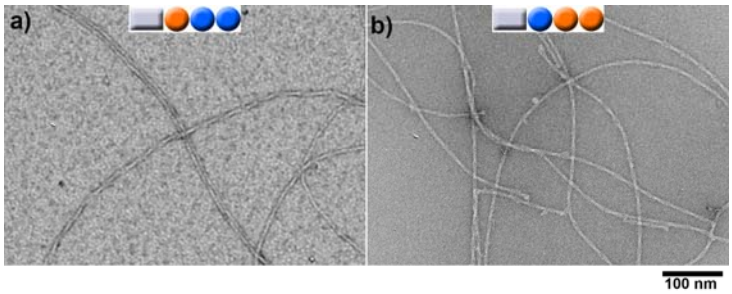


Figure S3. The TEM images of hydrogel of (A) DLL-**1** and (B) LDD-**1**.

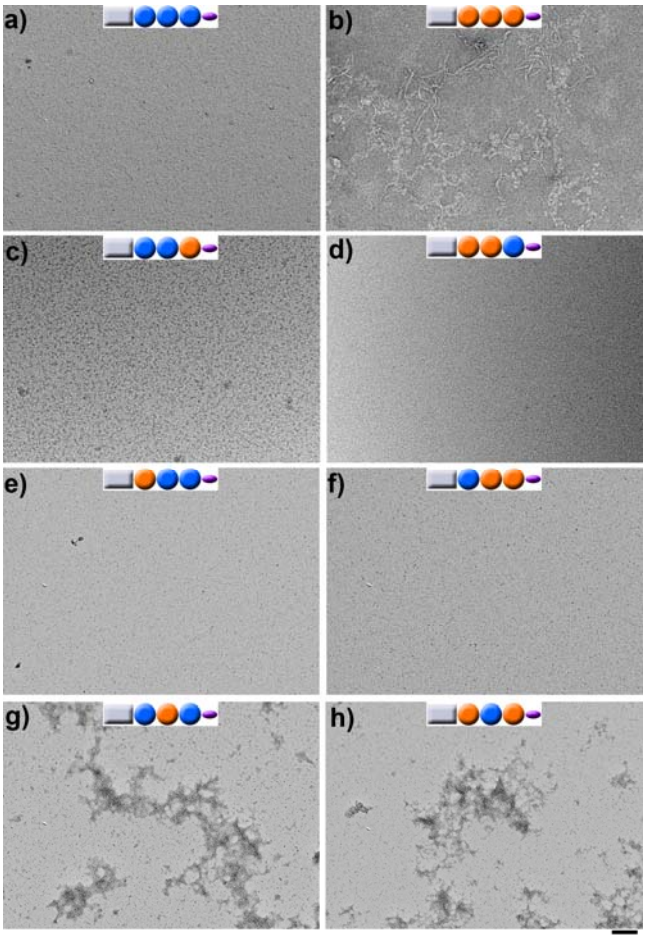


Figure S4. TEM images of precursors at the concentration of 500 μM. The compounds dissolve in the PBS buffer (pH 7.4), scale bar is 100 nm.

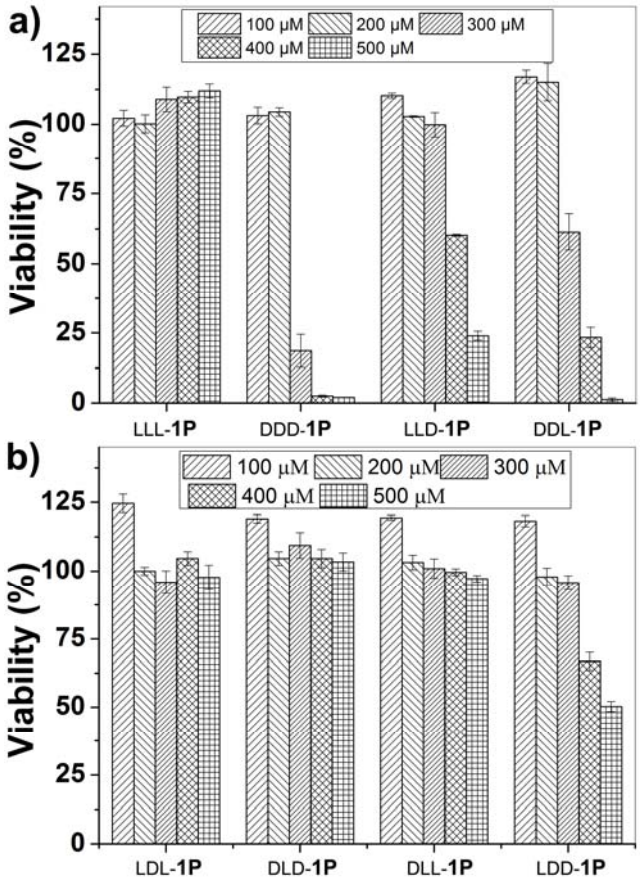


Figure S5. The viabilities of HeLa cells incubated with the precursors at concentrations from 100 μM to 500 μM for 48 h.

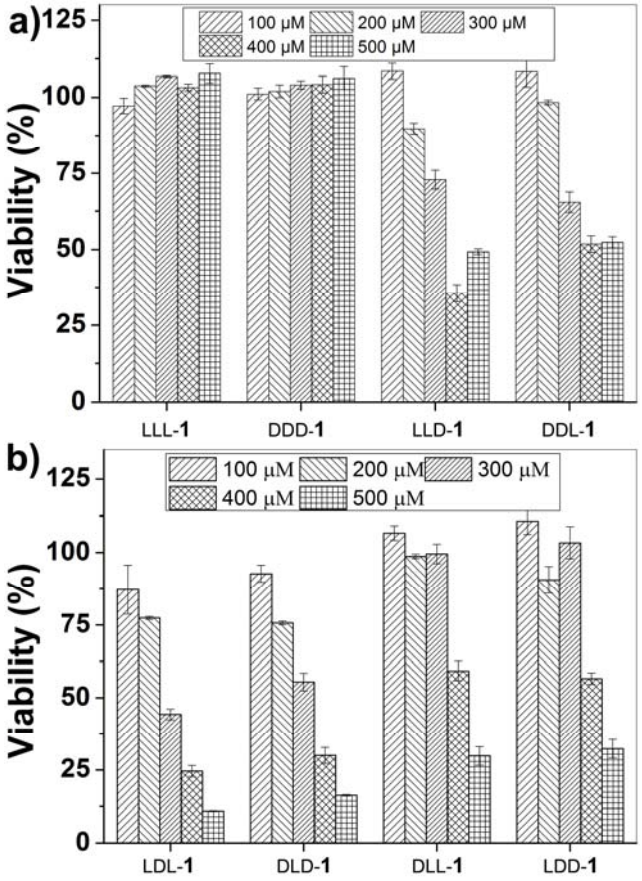


Figure S6. The viabilities of HeLa cells incubated with the hydrogelators at concentrations from 100 μM to 500 μM for 48 h

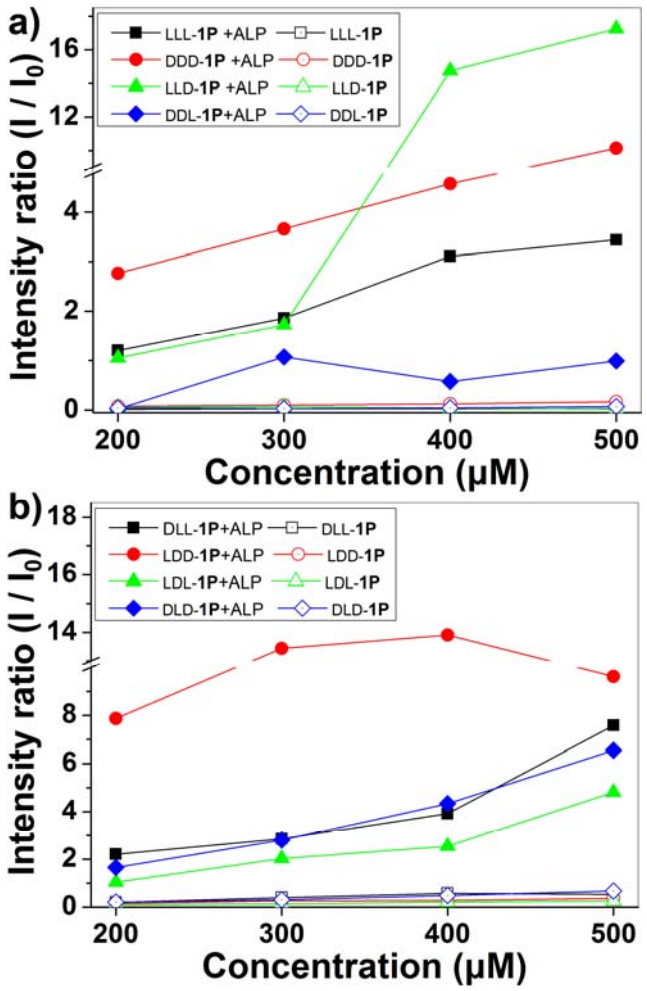


Figure S7. The intensity of static light scattering of the solution of precursors treated with (filled) and without (hollow) phosphatase (ALP) (condition: 0.5 U/mL of ALP, 24 h, room temperature). Only the light scattering signal at 30 degree was present.

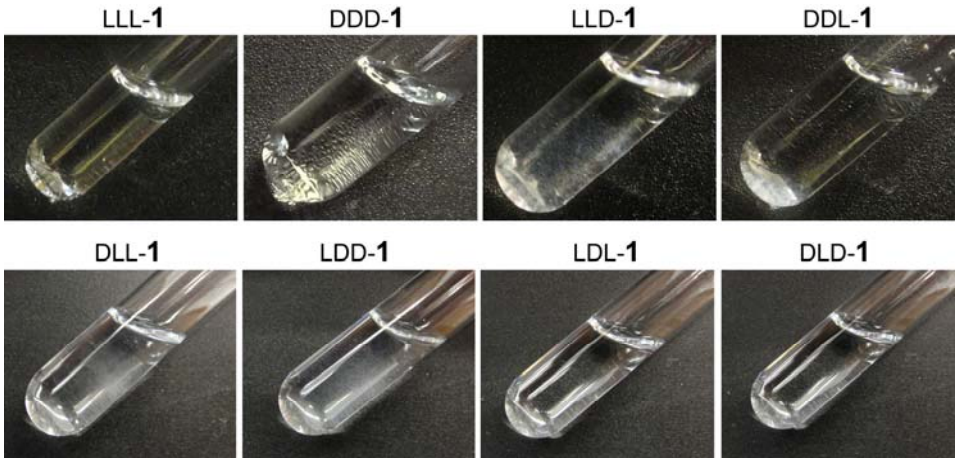


Figure S8**.** Optical images of the samples for light scattering Test (Condition see in Figure S7).

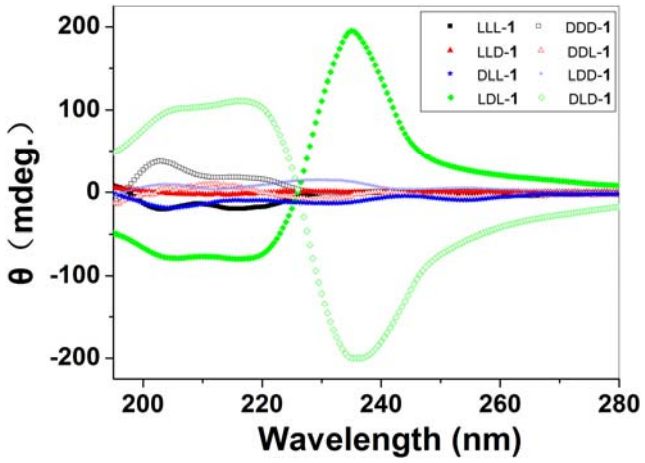


Figure S9. CD spectra of hydrogels, form upon addion of ALP to the solution of corresponding precusors at the concentration of 0.6 wt%.

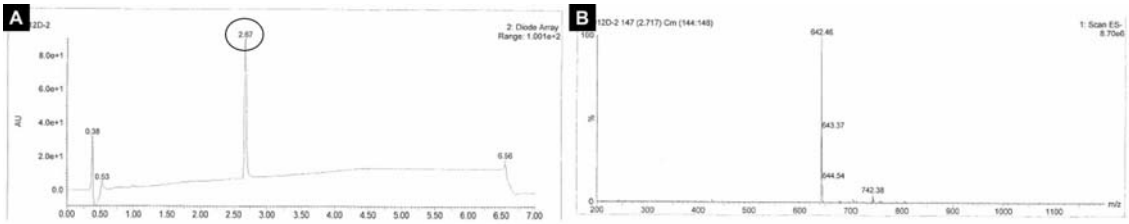


Figure S10. LC-MS result of cell culture medium of HeLa cell incubated with DDD-**1P** (300 μM) 24 hours at 37 ^{o}C (A) LC-spectrum of cell culture medium. We found the retention time of DDD-**1** was around 2.67 min. (B) The ESI^{-} mass spectrum of the peak marked 2.67 in (A) showed that is DDD-**1** an-ion (MW =643.73 Da)

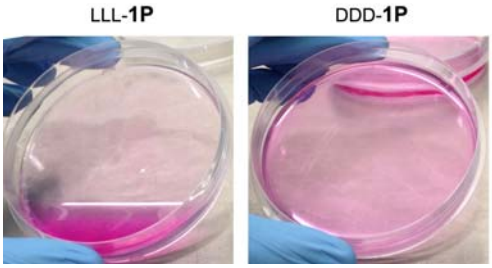


Figure S11. The optical images of HeLa cell incubated with precursors (300 μM) for 24h at 37 ^{o}C, sug-gesting that DDD-**1P** could form hydrogel in pericellular space.

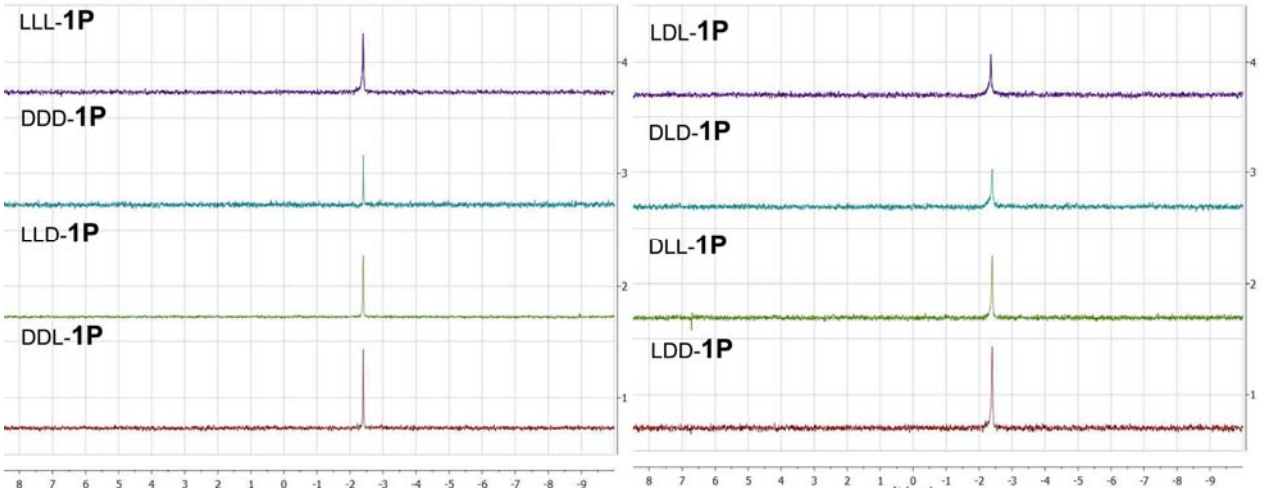


Figure S12. The ^{31}P-NMR spectra of the precursors.

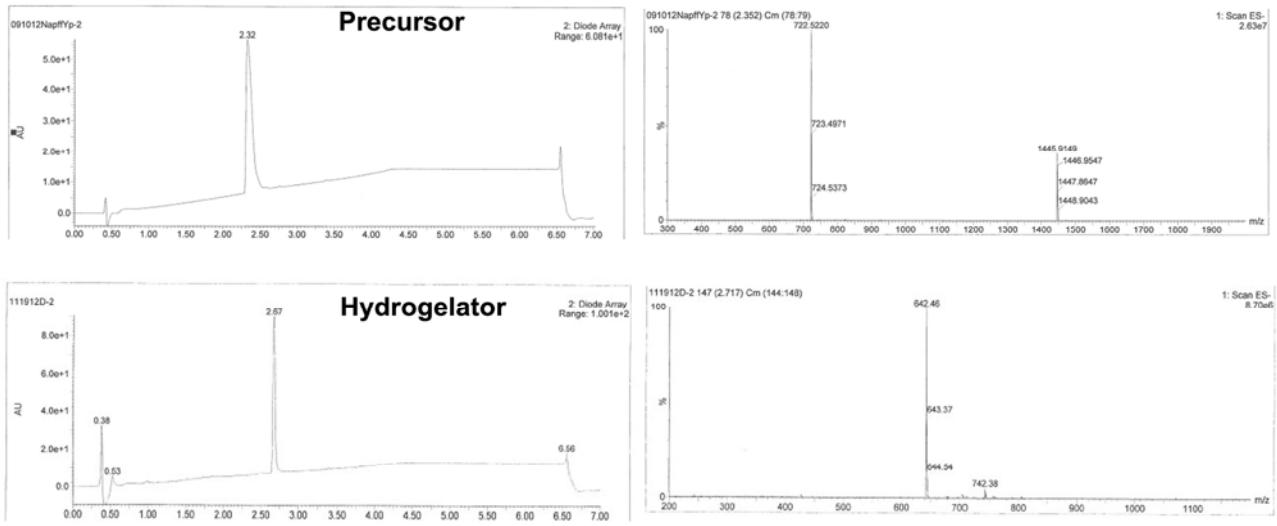


Figure S13. Representative LC-MS spectra of the precursor and corresponding hydrogelator

LC-MS (ESI):

LLL-**1P (**m/z): $C_{39}H_{38}N_3O_9P$, calc. 723.23; observed $(M+1)^+$ 724.41, $(M-1)^-$ 722.52.

LLL-**1 (**m/z): $C_{39}H_{37}N_3O_6$, calc. 643.27; observed $(M+1)^+$ 644.35, $(M-1)^-$ 642.46.

DDD-**1P (**m/z): $C_{39}H_{38}N_3O_9P$, calc. 723.23; observed $(M+1)^+$ 724.41, $(M-1)^-$ 722.52.

DDD-**1 (**m/z): $C_{39}H_{37}N_3O_6$, calc. 643.27; observed $(M+1)^+$ 644.35, $(M-1)^-$ 642.46.

LLD-**1P (**m/z): $C_{39}H_{38}N_3O_9P$, calc. 723.23; observed $(M+1)^+$ 724.41, $(M-1)^-$ 722.52.

LLD-**1 (**m/z): $C_{39}H_{37}N_3O_6$, calc. 643.27; observed $(M+1)^+$ 644.35, $(M-1)^-$ 642.46.

DDL-**1P (**m/z): $C_{39}H_{38}N_3O_9P$, calc. 723.23; observed $(M+1)^+$ 724.41, $(M-1)^-$ 722.52.

DDL-**1 (**m/z): $C_{39}H_{37}N_3O_6$, calc. 643.27; observed $(M+1)^+$ 644.35, $(M-1)^-$ 642.46.

LDL-**1P (**m/z): $C_{39}H_{38}N_3O_9P$, calc. 723.23; observed $(M+1)^+$ 724.41, $(M-1)^-$ 722.52.

LDL-**1 (**m/z): $C_{39}H_{37}N_3O_6$, calc. 643.27; observed $(M+1)^+$ 644.35, $(M-1)^-$ 642.46.

DLD-**1P (**m/z): $C_{39}H_{38}N_3O_9P$, calc. 723.23; observed $(M+1)^+$ 724.41, $(M-1)^-$ 722.52.

DLD-**1 (**m/z): $C_{39}H_{37}N_3O_6$, calc. 643.27; observed $(M+1)^+$ 644.35, $(M-1)^-$ 642.46.

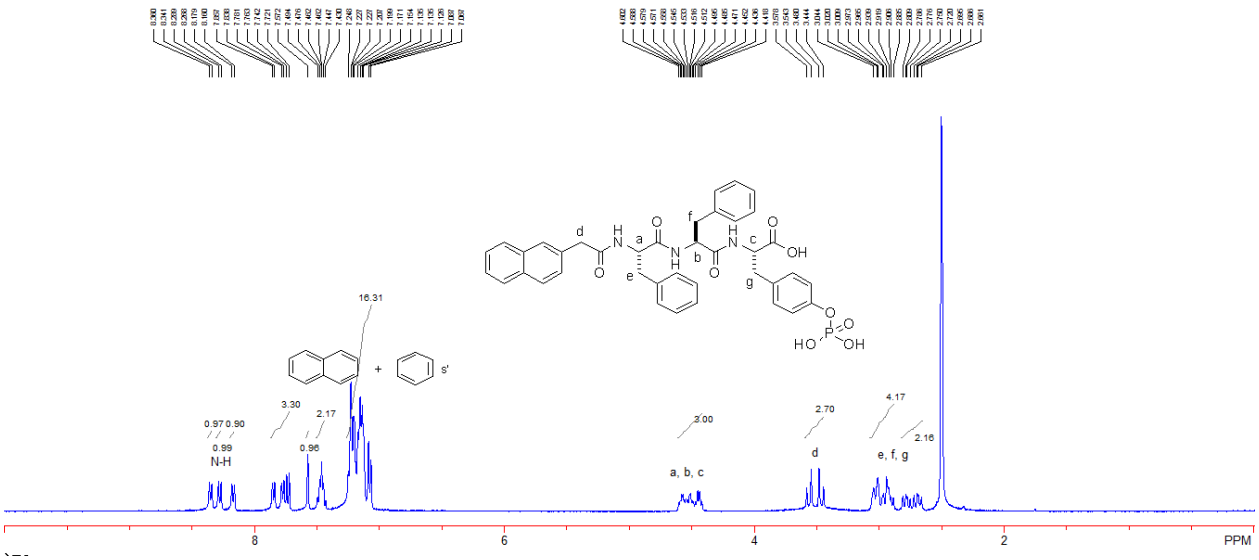
DLL-**1P (**m/z): $C_{39}H_{38}N_3O_9P$, calc. 723.23; observed $(M+1)^+$ 724.41, $(M-1)^-$ 722.52.

DLL-**1 (**m/z): $C_{39}H_{37}N_3O_6$, calc. 643.27; observed $(M+1)^+$ 644.35, $(M-1)^-$ 642.46.

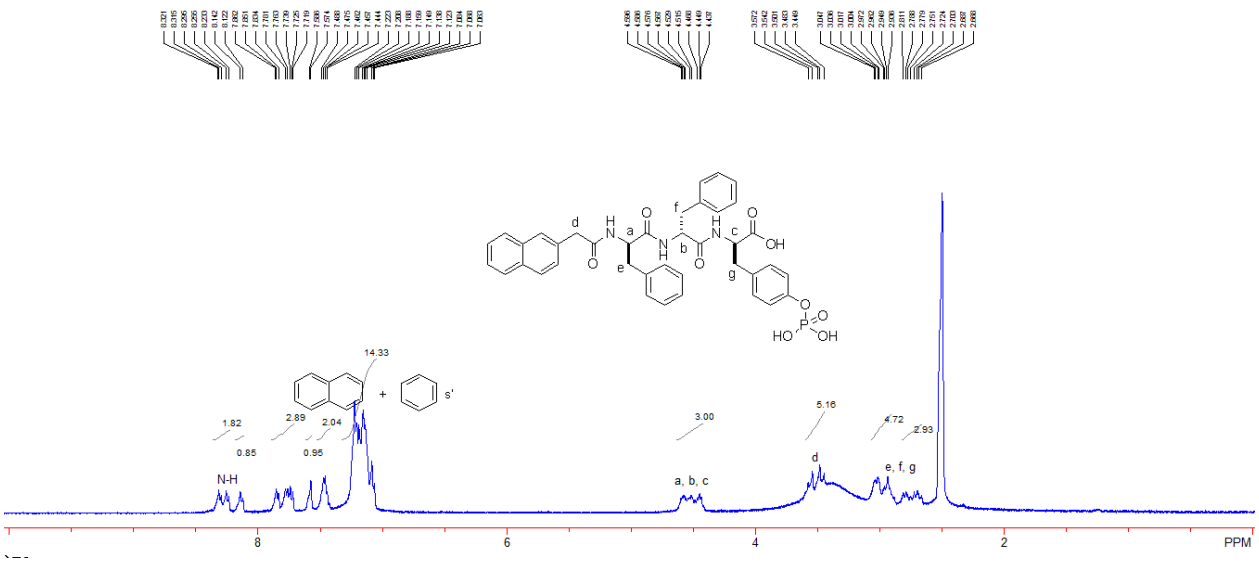
LDD-**1P (**m/z): $C_{39}H_{38}N_3O_9P$, calc. 723.23; observed $(M+1)^+$ 724.41, $(M-1)^-$ 722.52.

LDD-**1 (**m/z): $C_{39}H_{37}N_3O_6$, calc. 643.27; observed $(M+1)^+$ 644.35, $(M-1)^-$ 642.46.

a).The NMR spectra of precursors



NapFFYp-LLL-1P



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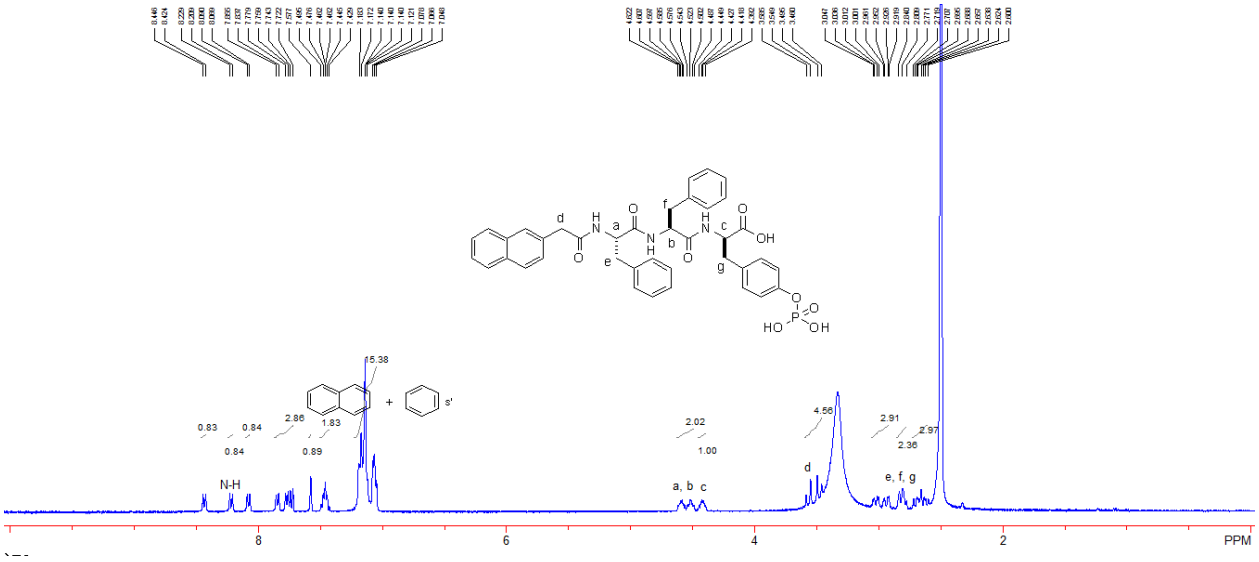

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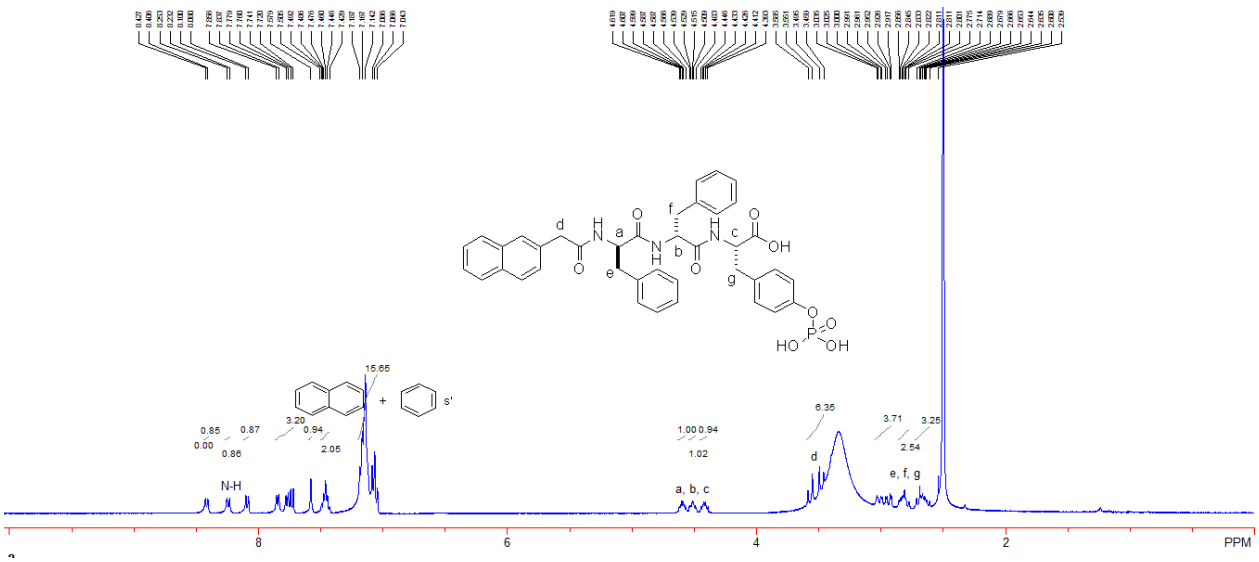

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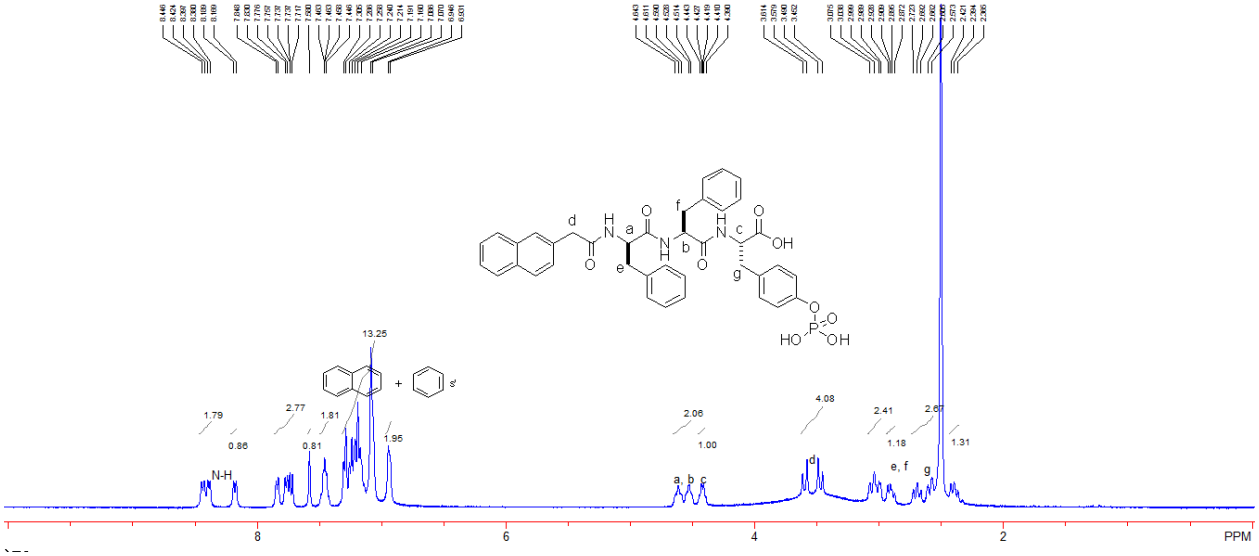

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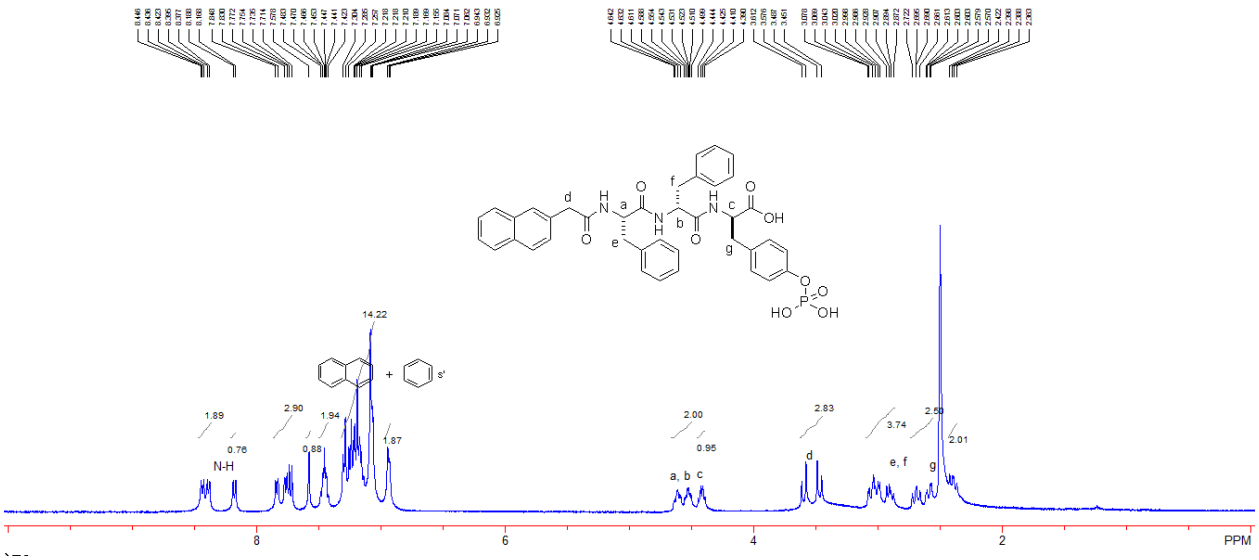

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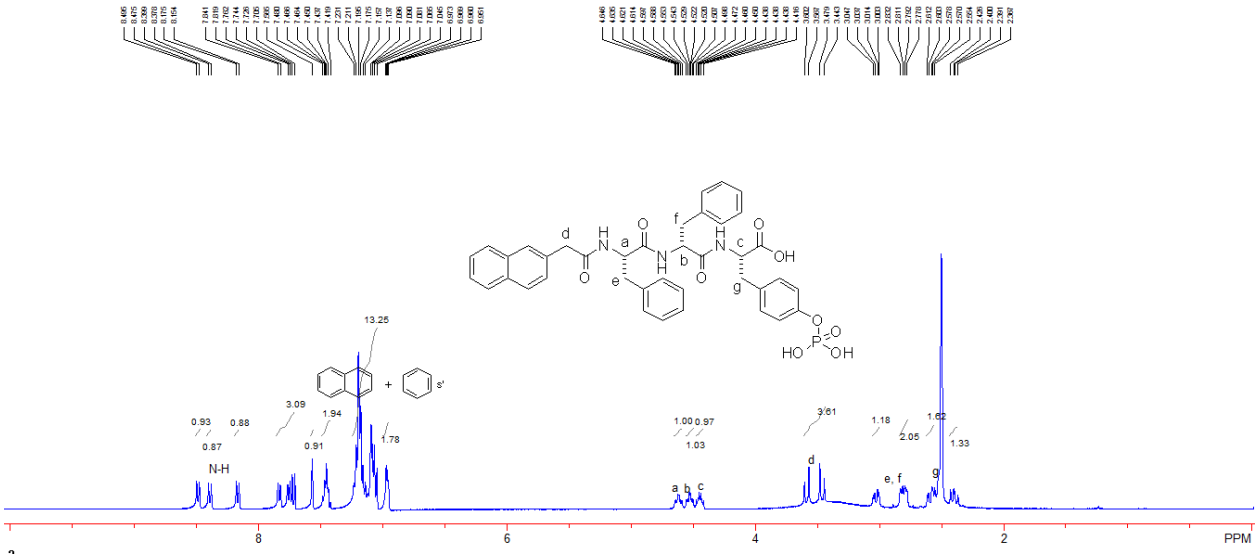

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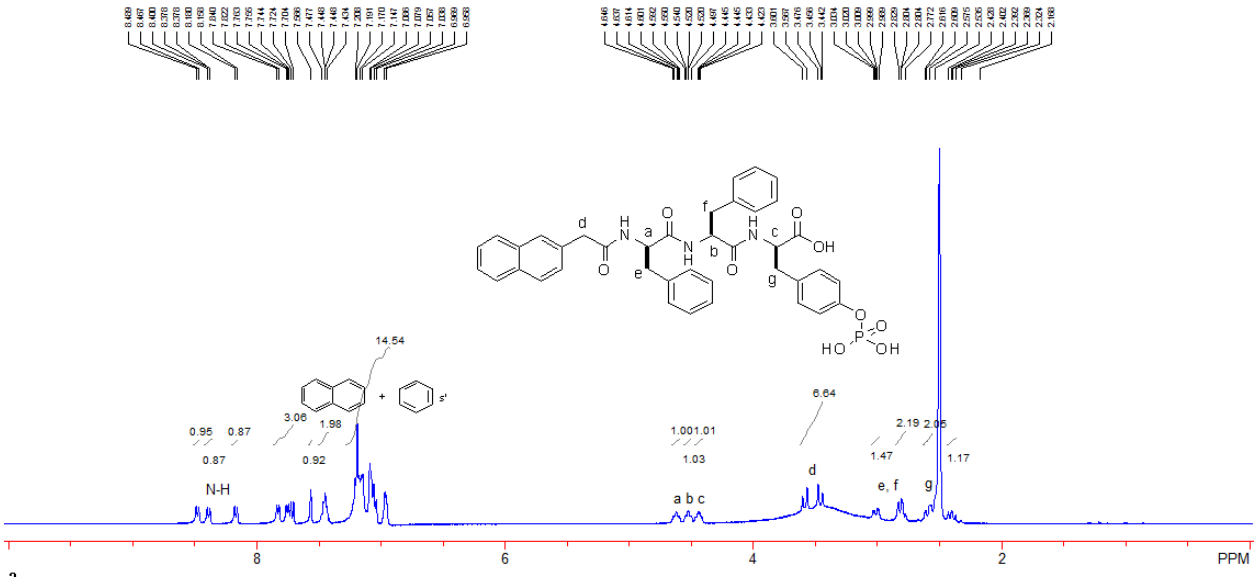
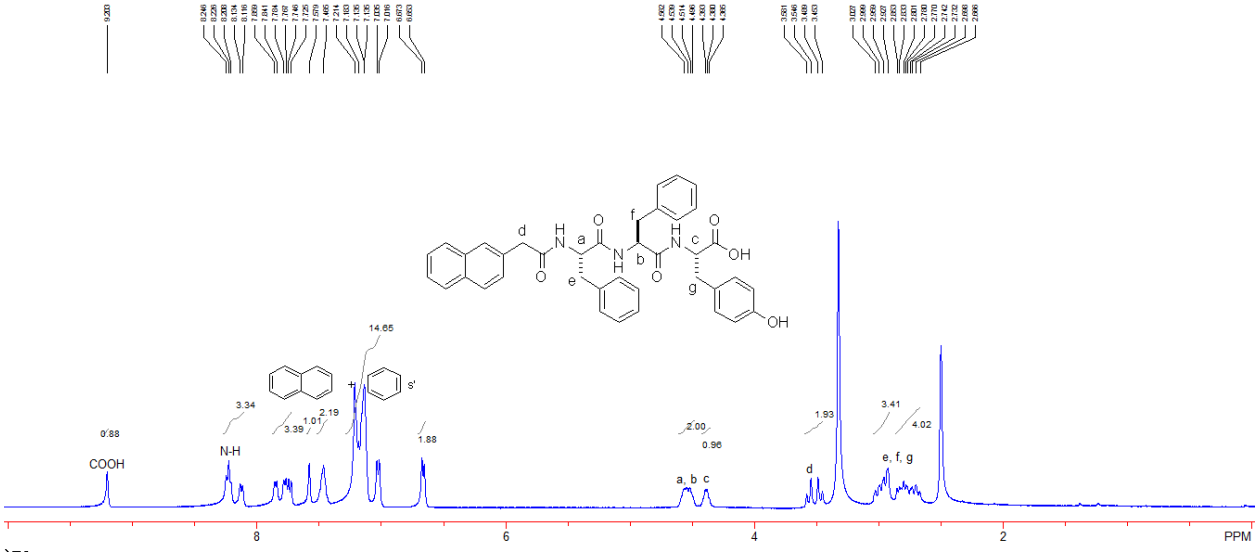


NapfFyp-DLD-1P

b).The NMR spectra of hydrogelators



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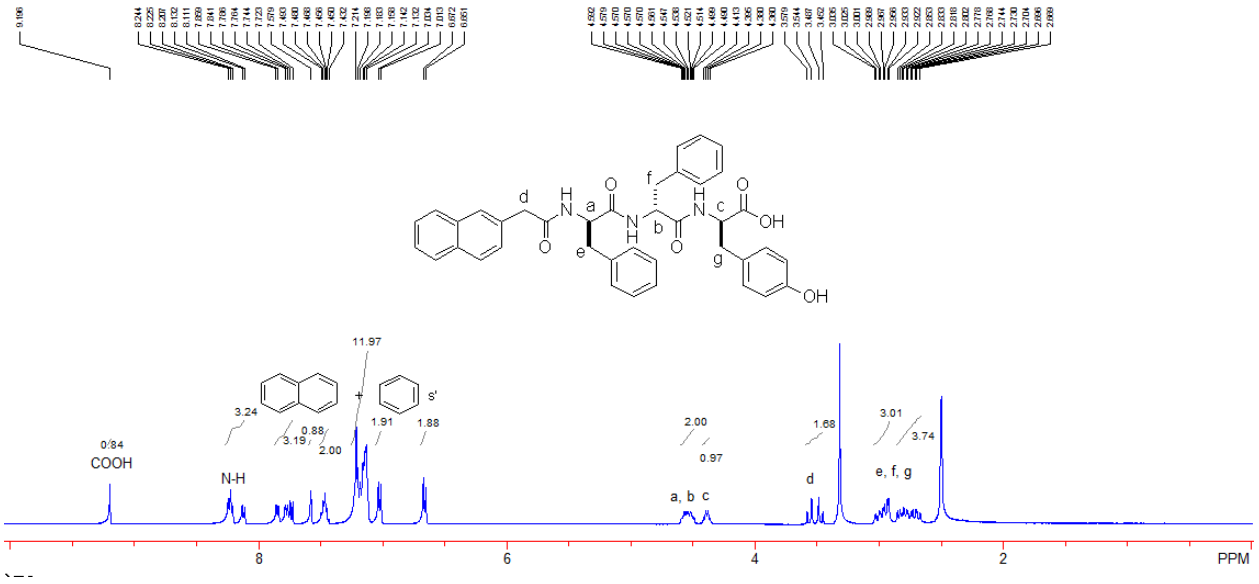

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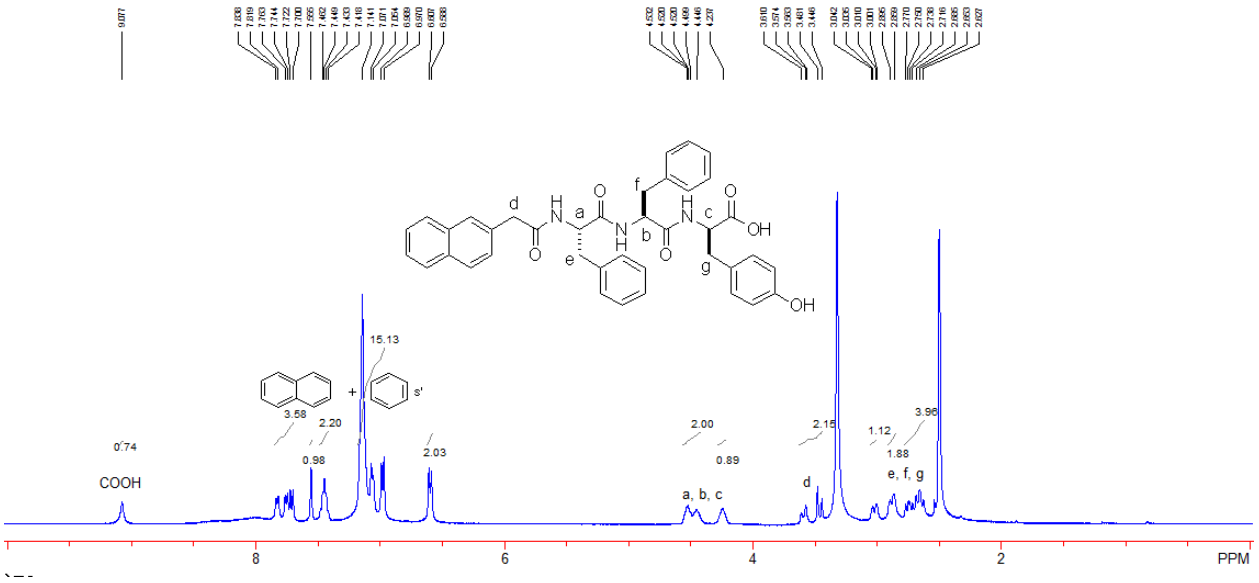
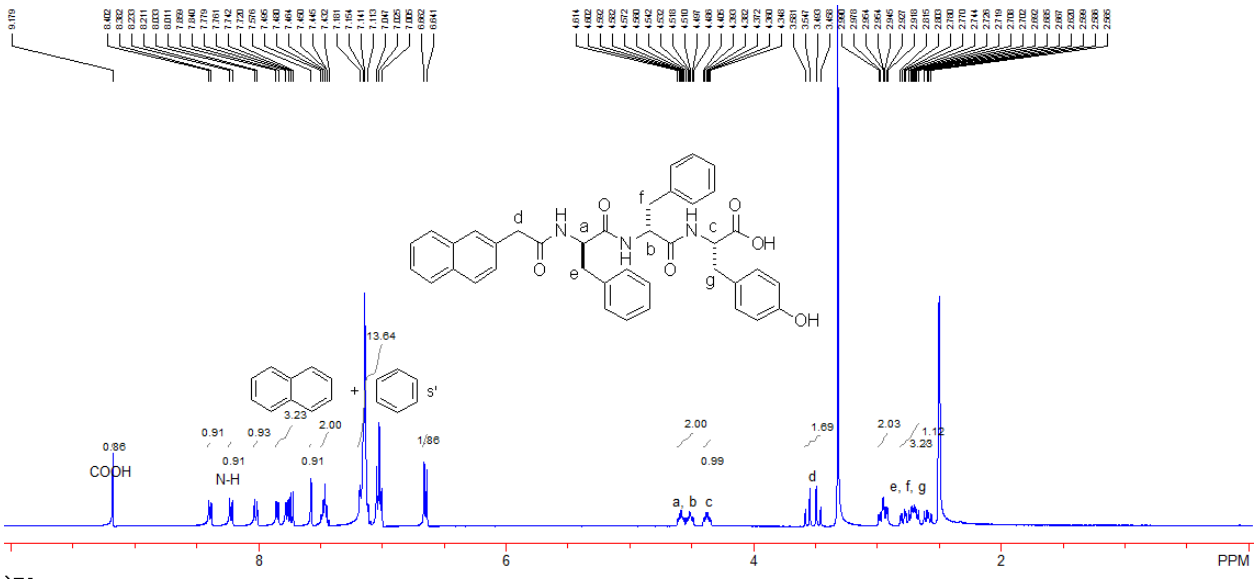


NapFFy-LLD-1



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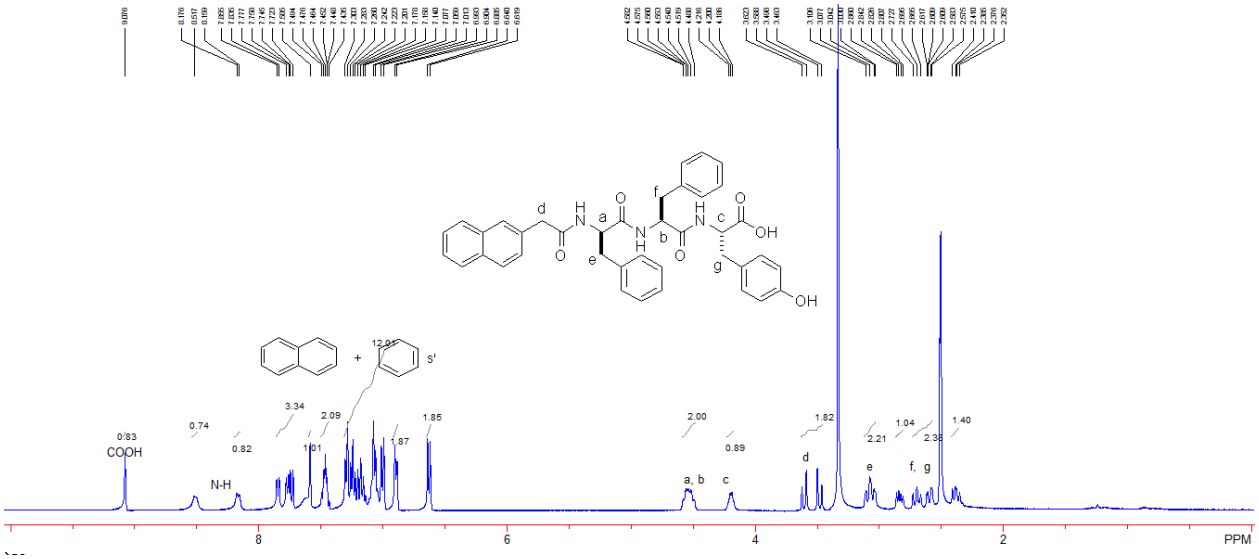

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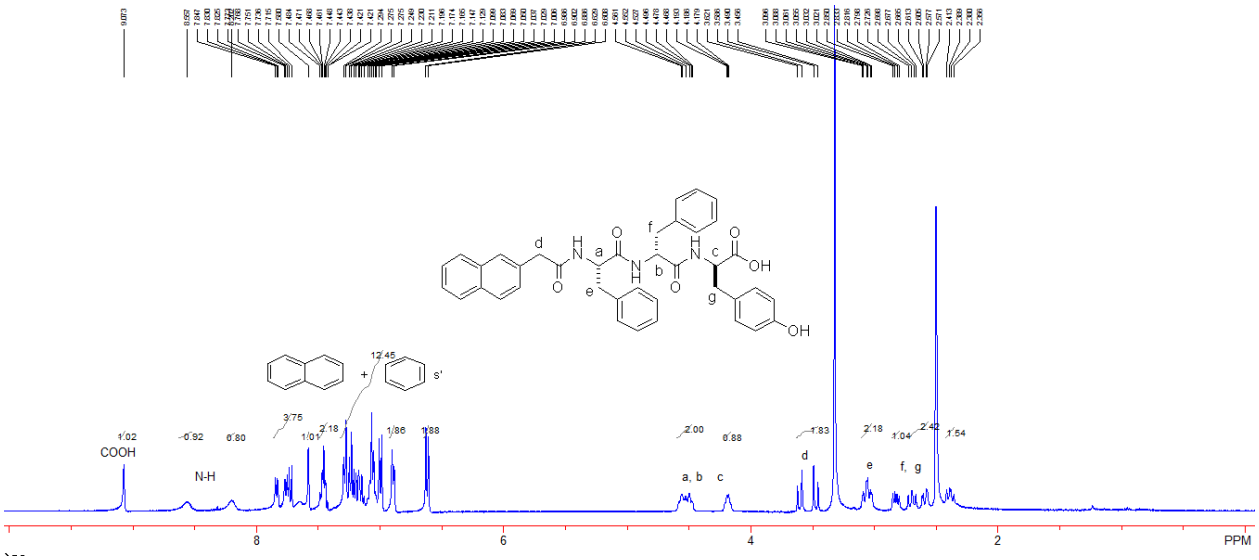

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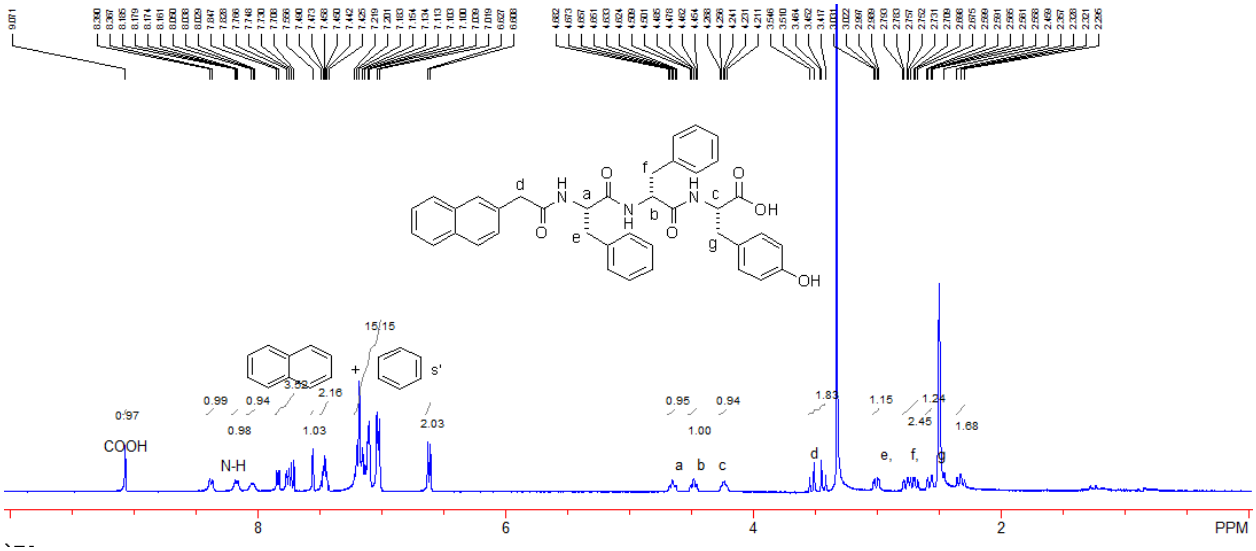

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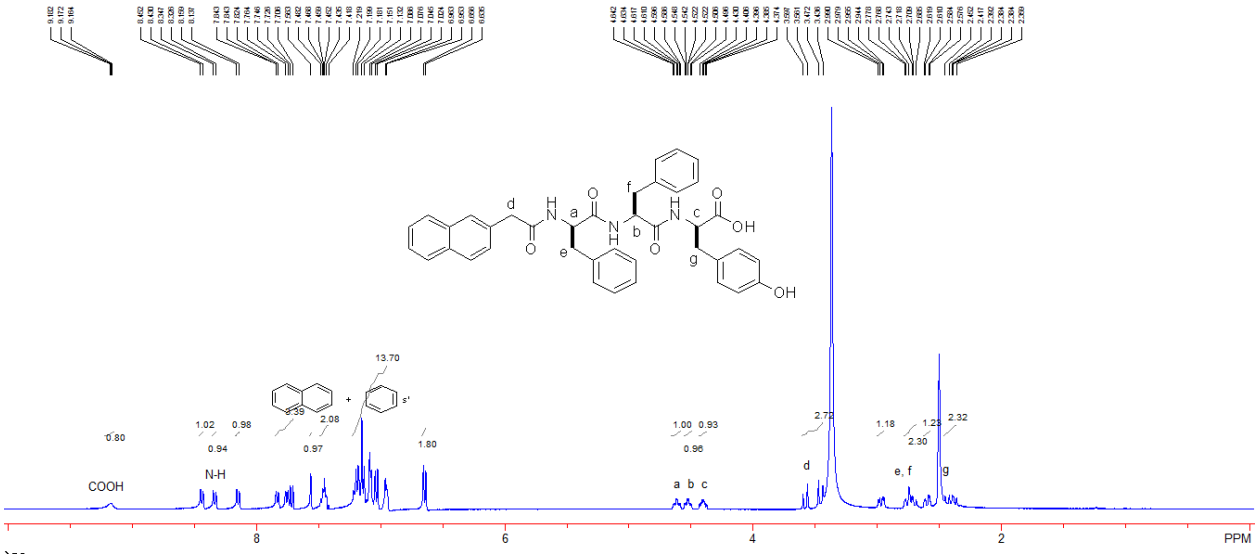
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c
g
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1.80
1.00
0.96
0.93
2.72
1.18
2.30
1.25
2.32
COOH
N-H
a b c
d
e, f
g
8
6
4
2
PPM

NapfFy-DLD-1